Molecular Engineering of Highly Magnetically Responsive Polymolecular Assemblies

A dissertation submitted to attain the degree of

DOCTOR OF SCIENCES of ETH ZURICH
(Dr. sc. ETH Zurich)

presented by

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Prof. Dr. Peter Johann Walde, Co-Examiner
Dr. Simon Kuster, Co-Examiner

2017
Arrête tes conneries et viens boire un verre de vin au jardin!

Elias Blatter
Acknowledgements

It was a great privilege and honor to be the third-generation of the Food Process Engineering (FPE) Lab’s bicelle team working on the SMhardBi project. It goes without saying that none of this would have been possible without the ground laying works of my predecessors. First and foremost, I would like to thank Prof. Dr.-Ing. Erich J. Windhab for bringing the magnetic world to FPE. On the chemical side, I wish to thank Dr. Simon Kuster for having built a top-notch synthetic laboratory. When it comes to paving the way for the SMhardBi project, Prof. Dr. Marianne Liebi is the chief engineer. Her birefringence setup and the strong network of multidisciplinary scientists she put in place were essential for the success of this project. Her top-quality work, publications and PhD thesis were a great source of inspiration, fueling my desire to live-up to the legacy. Today I hope that Master Bicelle Yoda will be proud of her Padawan.

A warm thank you to my examiners who have followed, supported and encouraged me throughout my time on the SMhardBi project. Prof. Dr.-Ing. Erich J. Windhab for taking me in, opening the doors to his lab, and for enabling research in a somewhat distant and multidisciplinary field. The open mindedness and richness of ideas emanating from our discussions were a strong source of inspiration. Prof. Peter J. Walde for introducing me to the fascinating and endless world of self-assembly science and research. The ideas and collaborations born from our discussions were essential for the successful outcome of this work. Dr. Simon Kuster for being an amazing supervisor, scientist, chemist, businessman, salesman, colleague, mentor, whiskey drinker, gardener, and friend. Last but not least, a warm thanks to Prof. Dr. Photographer Peter Fischer who always welcomed my craziness and inspired me to push science to the limits.

This next paragraph goes to my collaborators at the Paul Scherrer Institute (PSI, aka ‘Geek Disneyland’). Prof. Dr. Takashi Ishikawa for the high-quality time at the cryo-TEM, allowing us to bring the bicelles to life with spectacular images. Dr. Joachim Kohlbrecher for the sleepless nights at the SANS I beamline. Working on the SANS setup, living in a car, manipulating huge magnets, scattering neutrons in a giant pink tube, and discussing means of importing cheap cars from Poland were all part of an unforgettable experience. Moving away from PSI, I would like to thank Dr. Lukas D. Schuler for pursuing his passion for MD simulations.
Acknowledgements

of lipid systems with us and xirrus GmbH. His flexibility and rigorousness were highly appreciated. I would like to thank Prof. Dr. Lipidologist Andreas Zumbühl in Fribourg for the fruitful talks, the donation of synthetic lipids and for offering cubic liposomes to the world. I thank Dr. Marina Sturm for welcoming us at the Max Plank institute in Potsdam, enabling us to couple FT-IRRAS to the Langmuir trough.

Moving on to my colleagues at ETH, I would like to thank Dr. Sreenath Bolisetty and Dr. Jianguo Zhao for the fruitful collaboration, fun times in the lab and at PSI. A warm thanks to all my colleagues at the ETH Hönggerberg campus, especially Prof. Jan Vermant and his team for providing a rich platform for lipid membrane studies and a follow-up possibility for our project. My PhD experience would have been incomplete without the work done on Wormlike Micelles with Dr. Viviane Lutz-Bueno who introduced me to the field of rheology and X-ray scattering. Thank you Vivi for taking me aboard and for all the amazing work/social times spent together. It was an immense pleasure to supervise students and to tackle fundamental questions throughout our joint ventures. Finally, a huge thanks to Lukas Böni and the Hagfish team for offering me the unique opportunity to visit Norway and actively take part in marine rheo-biology. Lukas, my fellow office mate, it was always a pleasure to share a good bottle of wine with you, whilst discussing original ways to sell our science and save the world.

An enormous thank you goes to my dream-team and my guardian angels: Mirjam Baumgartner for joining me for both her Bachelor and Master thesis (yes she’s that crazy), Pernille Q. Reckey for coming all the way from Denmark to work on bicelles, Sarah Massabni for choosing the bicelle way of life in her Masters work on the SMhardBi project, Franziska Walker for dancing with wormlike micelles in the lab, Sandro Stucki for joining me for one last crazy beamtime at PSI, Arnel Hodzic and Dzana Durovic for choosing our labs for their traineeship and fighting hard together to achieve excellence. My beloved students, none of this would have been possible without you. I am eternally grateful for the amazing time spent together. It was an honor to work with you, offering me a priceless PhD experience. My friends it is now time to put the magnet into hibernation, give our CombiFlash Companion a well-deserved retirement, and kindly suggest Avanti lipids to find a new source of income.

I wish to warmly thank my loving family for supporting and believing in me throughout my studies. You offered me the richest of educations and, although I may have turned out a bit funny, you always did your best. Dr. Giga-Chef Linda Brütsch is obviously the greatest person... this entire page should be dedicate to her greatness, but for space-saving reasons we’ll leave it at that. Talking about family, I have to thank my extended family: the FPE team. You provided an amazing working environment, favoring scientific exchange combined with great
social events. I hope to have successfully played my part in maintaining the traditions. Thank you for all the beer, the mofas, the flat sharing experience, science week, the alternative exams and so much more!

Sorry for the long post, here is a rare image of a lanthanide-ion eating 1,2-dimyristoyl-sn-glycero-3-phosphopotatoe (Ln$^{3+}$-DMPP):
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## Notation

### Latin Letters

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<th>Unit</th>
<th>Meaning</th>
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<td>A₀</td>
<td>rad</td>
<td>PEM amplitude</td>
</tr>
<tr>
<td>a₀</td>
<td>nm²</td>
<td>surface area occupied by the polar headgroup in the assembly</td>
</tr>
<tr>
<td>Aᵢ</td>
<td>-</td>
<td>alignment factor</td>
</tr>
<tr>
<td>Aₑₓ</td>
<td>Å²</td>
<td>excess area per molecule</td>
</tr>
<tr>
<td>Aᵢ</td>
<td>Å²</td>
<td>mean measured area of the molecules in system i</td>
</tr>
<tr>
<td>B</td>
<td>T</td>
<td>magnetic field strength</td>
</tr>
<tr>
<td>B(S₁/₂)</td>
<td>T</td>
<td>magnetic field strength at which the order parameter S reaches one-half of its maximum value</td>
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<tr>
<td>CPP</td>
<td>-</td>
<td>critical packing parameter</td>
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<tr>
<td>D₇₅</td>
<td>nm</td>
<td>hydrodynamic diameter</td>
</tr>
<tr>
<td>d</td>
<td>nm</td>
<td>sample thickness</td>
</tr>
<tr>
<td>Eₘₐₙ</td>
<td>J</td>
<td>magnetic energy of the assembly</td>
</tr>
<tr>
<td>ΔEₘₐₙ</td>
<td>J</td>
<td>cumulative magnetic energy</td>
</tr>
<tr>
<td>ΔEₘₐₙ</td>
<td>J</td>
<td>magnetic energy of individual molecules in a magnetic field</td>
</tr>
<tr>
<td>G</td>
<td>J</td>
<td>Gibbs free energy</td>
</tr>
<tr>
<td>Gₘₑₙ</td>
<td>J</td>
<td>magnetic free energy</td>
</tr>
<tr>
<td>ΔGₘₑₙ</td>
<td>Jmol⁻¹</td>
<td>change in magnetic free energy</td>
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<tr>
<td>I</td>
<td>a.u.</td>
<td>neutron scattering intensity</td>
</tr>
<tr>
<td>I₁ω</td>
<td>Wm⁻²</td>
<td>first harmonic energy</td>
</tr>
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<td>I₂ω</td>
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<tr>
<td>J</td>
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<td>-</td>
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<td>kₜ</td>
<td>JK⁻¹</td>
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<tr>
<td>Lₙ</td>
<td>-</td>
<td>liquid-disordered phase of phospholipids</td>
</tr>
<tr>
<td>Lₙᵇ</td>
<td>-</td>
<td>solid-ordered phase of phospholipids</td>
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<tr>
<td>lₙ</td>
<td>nm</td>
<td>critical length of an amphiphile’s hydrophobic part</td>
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<tr>
<td>Ln³⁺</td>
<td>-</td>
<td>lanthanide ion</td>
</tr>
<tr>
<td>n</td>
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### Notation

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<td>(\Delta n')</td>
<td>-</td>
<td>birefringence signal</td>
</tr>
<tr>
<td>(\Delta n'_{\text{max}})</td>
<td>-</td>
<td>maximal achievable birefringence signal</td>
</tr>
<tr>
<td>(N_A)</td>
<td>mol(^{-1})</td>
<td>avogadro constant</td>
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<tr>
<td>(P_{\beta'})</td>
<td>-</td>
<td>ripple phase of phospholipids</td>
</tr>
<tr>
<td>(q)</td>
<td>nm(^{-1})</td>
<td>scattering vector</td>
</tr>
<tr>
<td>(R)</td>
<td>-</td>
<td>reflectance of the monolayer</td>
</tr>
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<td>(R_0)</td>
<td>-</td>
<td>reflectance of the subphase</td>
</tr>
<tr>
<td>(T)</td>
<td>°C</td>
<td>temperature</td>
</tr>
<tr>
<td>(T_m)</td>
<td>°C</td>
<td>phase transition temperature</td>
</tr>
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<td>(T_1)</td>
<td>ms</td>
<td>spin–lattice relaxation time</td>
</tr>
<tr>
<td>([tL])</td>
<td>mM</td>
<td>total lipid concentration</td>
</tr>
<tr>
<td>(S)</td>
<td>-</td>
<td>order parameter</td>
</tr>
<tr>
<td>(S_{\text{CD}})</td>
<td>-</td>
<td>deuterated chain order parameter</td>
</tr>
<tr>
<td>(v)</td>
<td>nm(^3)</td>
<td>hydrophobic chain volume of an amphiphile</td>
</tr>
<tr>
<td>(x_i)</td>
<td>-</td>
<td>mol fraction of compound i</td>
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### Greek Letters

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<tr>
<td>(\chi)</td>
<td>m(^3)</td>
<td>molecular magnetic susceptibility</td>
</tr>
<tr>
<td>(\chi_{\parallel})</td>
<td>m(^3)mol(^{-1})</td>
<td>molecular(volume) magnetic susceptibility parallel to the long molecular axis</td>
</tr>
<tr>
<td>(\chi_{\perp})</td>
<td>m(^3)mol(^{-1})</td>
<td>molecular(volume) magnetic susceptibility perpendicular to the long molecular axis</td>
</tr>
<tr>
<td>(\delta)</td>
<td>rad</td>
<td>retardation of polarized light in birefringence</td>
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<tr>
<td>(\delta)</td>
<td>ppm</td>
<td>chemical shifts in NMR</td>
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<tr>
<td>(\Delta \chi)</td>
<td>m(^3)mol(^{-1})</td>
<td>magnetic susceptibility anisotropy</td>
</tr>
<tr>
<td>(\lambda)</td>
<td>nm</td>
<td>laser wavelength</td>
</tr>
<tr>
<td>(\theta)</td>
<td>deg</td>
<td>angle between the long molecular axis and the magnetic field direction. Azimutal angle in SANS.</td>
</tr>
<tr>
<td>(\sigma)</td>
<td>-</td>
<td>standard deviation or width parameter of log-normal distribution</td>
</tr>
<tr>
<td>(\mu)</td>
<td>nm</td>
<td>radius of concentric hole in bicelles obtained from SANS fittings with a log-normal distribution</td>
</tr>
<tr>
<td>(\mu_0)</td>
<td>Hm(^{-1})</td>
<td>permeability of a vacuum</td>
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### Abbreviations

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<table>
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<th>Symbol</th>
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<tr>
<td>BOC</td>
<td>tert-butyloxycarbonyl protecting group</td>
</tr>
<tr>
<td>cryo-TEM</td>
<td>cryo transmission electron microscopy</td>
</tr>
<tr>
<td>Chol-OH</td>
<td>cholesterol</td>
</tr>
<tr>
<td>Chol-NH$_2$</td>
<td>aminocholesterol (3β-amino-5-cholestene)</td>
</tr>
<tr>
<td>Chol-C$_2$OC$_2$-NH$_2$</td>
<td>aminocholesterol conjugate, see Table 3.2</td>
</tr>
<tr>
<td>Chol-DTPA</td>
<td>lanthanide-ion steroid cholesterol conjugate</td>
</tr>
<tr>
<td>Chol-C$_n$-DTPA</td>
<td>lanthanide-ion chelating steroid conjugate with an apolar linker chain where n: 2, 5, or 6. See Table 3.2</td>
</tr>
<tr>
<td>Chol-C$_2$OC$_2$-DTPA</td>
<td>lanthanide-ion chelating steroid conjugate with a polar linker chain. See Table 3.2</td>
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<td>CMW</td>
<td>deuterated solvent mixture (CDCl$_3$/MeOD-d$_4$/D$_2$O 80:20:1)</td>
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<tr>
<td>DCC</td>
<td>N,N’-Dicyclohexylcarbodiimide</td>
</tr>
<tr>
<td>DCU</td>
<td>dicyclohexylurea</td>
</tr>
<tr>
<td>DIEA</td>
<td>N,N'-Diisopropylethylamine (Hünig’s base)</td>
</tr>
<tr>
<td>DLS</td>
<td>dynamic light scattering</td>
</tr>
<tr>
<td>DMAP</td>
<td>4-dimethylaminopyridine</td>
</tr>
<tr>
<td>DMF</td>
<td>dimethylformamide</td>
</tr>
<tr>
<td>DHPC</td>
<td>1,2-dihexanoyl-sn-glycero-3-phosphocholine</td>
</tr>
<tr>
<td>DLPC</td>
<td>1,2-dilauroyl-sn-glycero-3-phosphocholine</td>
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<tr>
<td>DMPC</td>
<td>1,2-dimyristoyl-sn-glycero-3-phosphocholine</td>
</tr>
<tr>
<td>DMPE</td>
<td>1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine</td>
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<td>DMPG</td>
<td>1,2-dimyristoyl-sn-glycero-3-phospho-(1’-rac-glycerol)</td>
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<td>1,2-dimyristoyl-3-trimethylammonium-propane</td>
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<td>DPPE-DTPA</td>
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<tr>
<td>DMPE-Glu-DTPA</td>
<td>lanthanide-ion chelating phospholipid, See Table 3.2</td>
</tr>
<tr>
<td>DTPAA</td>
<td>diethylenetriaminepentaaacetate dianhydride</td>
</tr>
<tr>
<td>Ext</td>
<td>extrusion</td>
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<tr>
<td>FT</td>
<td>freeze thawing cycles</td>
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<tr>
<td>FT-IR</td>
<td>fourier transform infrared spectroscopy</td>
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<td>FT-IRRAS</td>
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<tr>
<td>FPE</td>
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<td>FRP</td>
<td>free-radical polymerization</td>
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<td>GROMOS</td>
<td>force field for molecular dynamics simulation</td>
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<td>GmbH</td>
<td>gesellschaft mit beschränkter haftung</td>
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<td>H&amp;C</td>
<td>heating and cooling procedure</td>
</tr>
<tr>
<td>HBTU</td>
<td>coupling reagent used in solid phase peptide syn-</td>
</tr>
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<td></td>
<td>thesis</td>
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<td>HR-MS</td>
<td>high resolution mass spectroscopy</td>
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<td>LN$_2$</td>
<td>liquid nitrogen</td>
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<td>MD</td>
<td>molecular dynamics</td>
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<td>MsCl</td>
<td>methanesulfonyl chloride</td>
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<td>MM2</td>
<td>molecular modeling routine in vacuum</td>
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<tr>
<td>MRI</td>
<td>magnetic resonance imaging</td>
</tr>
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<td>NaCO$_3$ sat</td>
<td>saturated solution of sodium bicarbonate</td>
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<td>NMR</td>
<td>nuclear magnetic resonance</td>
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<td>NIBS</td>
<td>non-invasive backscatter technology</td>
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<td>POPC</td>
<td>1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine</td>
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<td>RDC</td>
<td>residual dipolar couplings</td>
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</table>
Abstract

Hydrogels offering on-demand tailorable optical properties are formidable smart materials. They offer promising perspectives in numerous applications including sensors and switches. They must provide a defined and readily measured response to environmental factors such as changes in temperature and exposure to magnetic fields. Magnetically responsive lanthanide-ion (Ln\(^{3+}\)) chelating bicelles are attractive active ingredients for the design of such systems. These bicelles deliver switchable anisotropy and orientation-dependent optical properties when aligned in a magnetic field and imbedded in gelatin. Although promising, these features were only demonstrated with one bicelle-gelatin pair: DPPC/Chol-OH/DPPE-DTPA/Ln\(^{3+}\) (molar ratio 16:4:5:5) bicelles in porcine gelatin. High magnetic field strengths of 8 T were required to achieve gels with a reasonable optical signature. With the goal of demonstrating the versatility and enhancing the viability of this optical hydrogel technology, a new generation of highly magnetically responsive and temperature resistant bicelles was engineered. Optical gels were achieved at commercially viable magnetic field strengths between 1 and 3 T. The gels were thermoreversible, displaying defined and encryptable optical signatures. The temperature-dependent nature of the gel’s properties, combined with the large degree of freedom in bicelle design, provides a rich toolbox for the development of modern temperature sensors and switches.

The reported fabrication procedures for Ln\(^{3+}\) chelating bicelles were tedious in comparison to other bicelle systems. This shortcoming reduced their attractiveness and hindered their potential for achieving a high degree of magnetic alignability. Therefore, simplified bicelle fabrication procedures were developed. Planar polymolecular assemblies in the size range of hundreds of nanometers were achieved delivering unprecedented gains in magnetic response. A 2-fold increase in alignment factor was achieved with DMPC/DMPE-DTPA/Tm\(^{3+}\) (molar ratio 4:1:1) systems and a 2.5-fold increase with DMPC/Chol-OH/DMPE-DTPA/Tm\(^{3+}\) (molar ratio 16:4:5:5) at 5 °C and 8 T. Heating above and cooling below the phase transition temperature T\(_m\) of the bilayer lipids was essential to guarantee successful formation of the polymolecular assemblies composed of DMPC/DMPE-DTPA/Ln\(^{3+}\) (molar ratio 4:1:1). Highly magnetically alignable DMPC/Chol-OH/DMPE-DTPA/Ln\(^{3+}\) (molar ratio 16:4:5:5) assemblies could be regenerated after storage for one month at −18 °C. The dimensions of DMPC/Chol-OH/DMPE-DTPA/Tm\(^{3+}\) (molar ratio 16:4:5:5) bicelles were readily tailored by extrusion through polycarbonate membranes with a fixed pore size at 60 °C. The dimensions of the bicelles were indirectly controlled by exploiting their thermoreversible transformation into vesicles. The simplicity and robustness of these procedures offers a new set of
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possibility for engineering of highly magnetically responsive Ln^{3+} chelating phospholipid polymolecular assemblies.

Strengthened from optimized fabrication procedures, the molecular composition of the bicelles was then addressed. The magnetic response is commonly enhanced by increasing the bicellar size or aggregate number \( n \) (i.e., the number of molecules capable of contributing to the assembly’s total magnetic energy \( E_{mag} \)). A novel approach employing Ln^{3+} chelating cholesterol conjugates (Chol-DTPA) was developed to expand on these possibilities. However, these techniques have reached their limits, being intrinsically bound to the region of the phase diagram guaranteeing bicelle formation. The magnetic alignability may only be further enhanced by selectively altering the magnetic susceptibility \( \Delta \chi \) of the bicelle. Such alternatives are lacking and call for further development. This goal was achieved by introducing aminocholesterol (Chol-NH\(_2\)) in the bicelle bilayer. A substantial increase in magnetic alignment was obtained without changing the bicelle’s dimensions. Previously reported degrees of alignment at 8 T and 5 °C could be matched with Chol-NH\(_2\) doped bicelles at field strengths as low as 4 T at 5 °C. Full alignment was reached at magnetic field strengths of 8 T, compared to 35 T required for the reference systems containing Chol-OH. A profound understanding of the origins behind the enhanced magnetic response was possible through a multiscale bottom-up comparative investigation of Chol-OH and Chol-NH\(_2\) mixed with DMPC. The investigation combined physico-chemical characterizations with molecular dynamics simulations. By altering the dynamics of the hydrophilic environment of the bicelle, Chol-NH\(_2\) changes the crystal field and angle of the phospholipid–lanthanide DMPE-DTPA/Tm\(^{3+}\) complex. These parameters largely determine the magnetic susceptibility \( \Delta \chi \) of the complex. Highly magnetically alignable DMPC/Chol-NH\(_2\)/DMPE-DTPA/Tm\(^{3+}\) (molar ratio 16:4:5:5) bicelles were achieved up to temperatures of 35 °C before a thermoreversible rearrangement into vesicles occurred. This thermal resistance, combined with a high magnetic response, offered the possibility of employing Chol-NH\(_2\) based bicelles to develop a new generation of switchable hydrogels with optical properties.

The extraordinary performance of Chol-NH\(_2\) bicelles illustrate the viability and importance of manipulating the magnetic susceptibility \( \Delta \chi \). Enlightened from these findings, a new Ln\(^{3+}\) chelating phospholipid with a glutamic acid backbone (DMPE-Glu-DTPA) was engineered and synthesized. The chelate polyhedron was specifically designed to alter \( \Delta \chi \). Planar asymmetric assemblies in the hundreds of nanometers in size were achieved delivering unprecedented magnetic alignments. The DMPE-Glu-DTPA/Ln\(^{3+}\) complex switched the \( \Delta \chi \), achieving perpendicular alignment for assemblies containing Dy\(^{3+}\) and parallel alignment for those containing Tm\(^{3+}\). Such a behavior has never been demonstrated for planar Ln\(^{3+}\) chelating polymolecular assemblies. The synthesized amphiphiles and the novel techniques for bicelle engineering developed herein offers perspectives going far beyond technological advances in smart hydrogels. Promising applications emerge in numerous and diverse fields including pharmaceutical technologies, structural characterization of membrane biomolecules by NMR spectroscopy, contrasting agents for magnetic resonance imaging, development of drug delivery platforms, and the magnetic control of surface properties and emulsions.
Zusammenfassung


Zusammenfassung


Die aussergewöhnliche Leistung von Chol-NH₂ Bicellen illustriert die Vielfältigkeit und Wichtigkeit der Manipulation der magnetischen Orientierbarkeit ∆χ. Basierend auf diesen Erkenntnissen konnte ein neues Ln³⁺ komplexbildendes Phospholipid mit Glutaminsäure Rückgrat (DMPE-Glu-DTPA) synthetisiert und konstruiert werden. Das polyhydratische Chelat wurde spezifisch zur Änderung von ∆χ entwickelt ohne stark von der geometrischen Struktur von DMPE-DTPA abzuweichen. Planare, asymmetrische Aggregate von Hunderten von Nanometern wurden generiert mit erstaunlicher magnetischer Ausrichtbarkeit. Der DMPE-Glu-DTPA/Ln³⁺ Komplex wechselte ∆χ was bei Dy³⁺ zu einer vertikalen und bei Tm³⁺ zu einer parallelen orientierung der Komplexe führte. Solche Opportunitäten würden bis anhin noch für kein planares Ln³⁺ chelatisierendes polyatomare Aggregat demonstriert. Die synthetisierten Amphiphile und neuartige Technik zur Bicellen Generierung bieten Optionen, welche weit über den tech-
1 Introduction

Bicelles are sub-micrometer sized disk-like polymolecular assemblies formed from amphiphiles in an aqueous solution. They are invaluable soft materials obtained from a multitude of lipid mixtures. These sub-micrometer sized disk-like polymolecular assemblies are typically composed of cylindrical lipids in the planar region and cone-like lipids covering the edge. The critical packing parameter \( CPP \) is commonly employed to characterize the geometry of amphiphilic molecules. Molecules with a cylindrical geometry, such as 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC), have a \( CPP \) of unity. Phospholipids with comparatively shorter hydrocarbon tails, such as 1,2-dihexanoyl-sn-glycero-3-phosphocholine (DHPC), or larger polar head groups, such as 1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine-diethylene triaminepentaacetate (DMPE-DTPA), are cone-like in geometry and have a \( CPP \) lower than unity. The bicelle’s dimensions is dictated by the ratio of cylindrical to cone-like phospholipids constituting the bilayer. Insertion of additional cone-like amphiphiles in the bilayer induces curvature resulting in smaller bicelles. Amphiphiles with an inverse cone-like geometry (\( CPP > 1 \)), such as cholesterol (Chol-OH), may be included in the bicelle’s bilayer to counteract the curvature and increase the size of the bicelle.

The physico-chemical and architectural properties of polymolecular assemblies are governed by numerous factors and forces. These include the nature of the surrounding hydrophilic environment, the lipids composing the bilayer and their concentration. The resulting large degree of tunability in terms of charge, size, magnetic alignability and thermal stability has largely contributed to their scientific importance. Although bicelles are widely used for the characterization of membrane integrated and associated proteins by NMR spectroscopy, numerous recent efforts aim to expand their field of application to other areas including pharmaceutical implementations or as building blocks for the smart soft-materials of tomorrow. The possibility of generating magnetically enhanced bicelles to create switchable anisotropy in optical gels has been demonstrated by M. Liebi et al. High magnetic field strengths of 8 T were required to deliver sufficiently aligned bicelles, necessary to induce the optical properties. Increasing the magnetic response of bicelle systems and their resistance to temperature is key to develop such novel technologies by reducing the required field strength to a commercially more affordable and practical range.

The magnetic response of bicelles is commonly enhanced by increasing their size and hence the number of molecules (or aggregate number \( n \)) capable of contributing to the cumulative magnetic energy \( E_{mag} \) of the bilayer. Nevertheless, this process involves iterative experimental approaches to optimize the total lipid content or ratio.
Such procedures are laborious and the largest achievable polymolecular assembly is intrinsically limited by the geometry of the lipids composing the bilayer. Optimizing the bicelle’s constituents and environment to enhance the assembly’s size does not eliminate the phase boundaries where the lipids self-assemble into other architectures than planar disks. Therefore, further means of selectively tuning the magnetic susceptibility $\Delta \chi$ of the bicelle without altering their size must be developed. A viable approach consists of altering the $\Delta \chi$ of the lipids composing the bilayer with paramagnetic lanthanide ions ($\text{Ln}^{3+}$). Incorporation of phospholipids capable of chelating $\text{Ln}^{3+}$, such as DMPE-DTPA, and other $\text{Ln}^{3+}$ chelates has been demonstrated in DMPC/DHPC bicelle systems. Metal-ion chelating phospholipids are highly beneficial for the study of membrane proteins by NMR spectroscopy as they speed-up the T1 relaxation of the embedded membrane proteins and considerably shorten measurement time. Bicelles composed of Chol-OH, DMPC and the phospholipid-lanthanide DMPE-DTPA/Ln$^{3+}$ complex, are admirable candidates for tailoring $E_{\text{mag}}$ through the magnetic susceptibility $\Delta \chi$. They strongly align in magnetic fields by chelating many Ln$^{3+}$ on the bilayer’s surface. These bicelles are highly tunable magnetically responsive soft materials, where chelation of different Ln$^{3+}$ further permits fine-tuning of the magnitude and direction of alignment in the presence of an external magnetic field. The introduction of Chol-OH or Ln$^{3+}$ chelating steroid conjugates (Chol-DTPA) in the bicelle’s bilayer leads to an increased magnetic alignment when compared to the steroid-free DMPC/DMPE-DTPA/Ln$^{3+}$ bicelle systems. This enhanced magnetic response originates from an increase in bicelle size. The $\Delta \chi$ is also affected by the presence of other molecules in the phospholipid bilayer. However, the contributions of the magnetic susceptibility $\Delta \chi$ and the aggregate number $n$ have not yet been decoupled.
Figure 1.1 S-PRO\textsuperscript{2} scheme for the molecular engineering of highly magnetically responsive polymolecular assemblies. The scheme is composed of three levels: the first level employs molecular engineering (process) to synthesize amphiphiles (structure) with a certain critical packing parameter $CPP$ and magnetic susceptibility $\Delta \chi$ (properties). The second level moves from the molecular scale to polymolecular assemblies. Specific fabrication protocols (process) were employed to generate bicelles (structure) with a given size, thermal stability and magnetic alignability (properties). In the third level, the polymolecular assemblies are incorporate into a gelatine network in an aligned state (process) for the production of smart gels (structure) delivering magnetically and thermally switchable optical properties (properties).
1 Introduction

Herein, a molecule engineering approach was developed to alter the $\Delta \chi$ of the bilayer, aiming towards tuning and enhancing the magnetic response of the polymolecular assemblies in an external magnetic field. $\Delta \chi$ was changed through the chemical structure of either the steroid dopants or the Ln$^{3+}$ chelating amphiphiles. This corresponds to the first level of the multidimensional three-step structure-process-properties (S-PRO$^2$) scheme in Figure 1.1. By designing and synthesizing the amphiphiles employed to dope or construct the bicelle assemblies (process), steroid derivatives such as aminocholesteryl (Chol-NH$_2$), Ln$^{3+}$ chelating steroid derivatives with various linker chain lengths and geometries (Chol-C$_n$-DTPA), and a novel Ln$^{3+}$ chelating phospholipid with an altered head group chemistry (DMPE-Glu-DTPA) were synthesized. The engineered properties are the molecular shape, defining the CPP of the amphiphile and the degree of curvature it induces when incorporated into the phospholipid bilayer. The $\Delta \chi$ of the bilayer composing amphiphiles is another important engineered property achieved by designing the chelator chemistry or the hydrophilic environment surrounding the complex, defining the magnetic energy it supplies to the polymolecular assembly.

In the second level of the S-PRO$^2$ scheme in Figure 1.1, the amphiphiles were mixed together and the bicelles were fabricated following a defined protocol (process). The fabrication protocols employed to evaluated the viability of the synthetic derivatives produced in the first level were the same as the previously developed protocols by M. Liebi,$^{30}$ offering a database of reference systems.$^{16,20,29,31}$ This protocol is similar to existing methods for the production of large unilamellar vesicles, involving freeze thawing cycles followed by multiple extrusion steps once the dry lipid film has been hydrated.$^{32–34}$ These procedures are far from optimal for the generation of maximally magnetically responsive polymolecular assemblies. Furthermore, they contradict the reported fabrication protocols of DHPC/DMPC bicelles, developed to favor the self-assembly of disk-like polymolecular assemblies.$^{13,35}$ Therefore, the possibility of forming large self-assembled polymolecular structures through removal of the extrusion step was investigated. The freeze thawing cycles were further replaced with a more gentle procedure involving heating and cooling cycles for the hydration of the dry lipid film. The proposed optimized fabrication procedures aim at considerably enhancing the magnetic response and thermal resistive properties of the polymolecular assemblies by altering their structure without changing the lipid composition. Furthermore, regeneration studies of the magnetically alignable polymolecular systems were undertaken to prolong their shelf-life and viability.

The unique properties of the polymolecular assemblies resulting from the two first levels of the S-PRO$^2$ scheme are valuable building blocks for the development of future soft materials and widen the frame of possibilities for the design of magnetically-switchable optical gels, introducing the third level of the scheme in Figure 1.1. In this uppermost level, the most promising systems developed in the lower levels are incorporated into a gelatin network (process). The structure of the gel is controlled with a combination of thermal treatments and exposure to an external magnetic field, delivering the required optical properties, proving the viability and increasing the versatility of the magnetically switchable optical gels.$^{20}$
2 Background

2.1 Lipids and Self-Assembly

Amphiphilic lipids, such as phospholipids, are essential molecular components of cell membranes. They generally consist of two hydrophobic alkyl tails and a hydrophilic head, linked together by a glycerol moiety. The phosphate group is readily modified by simple organic molecules such as choline. Phospholipids are amphiphilic and self-assemble when exposed to a polar aqueous environment. The hydrophobic effect is the major driving force of the self-assembly process as water molecules surrounding the lipid tails are released, increasing the entropy of the system. The polymolecular assemblies are further held together by diverse physico-chemical forces, whose strength vary based on the structure of the lipid, pH, temperature and the chemical composition of the aqueous phase. Van der Waals forces are strongly present between the hydrophobic tails of the phospholipids, whilst hydrogen bonding and electrostatic interactions dominate the headgroup region. Phospholipids are commonly employed in drug formulations to improve bio-availability, reduced toxicity and increase penetration of active ingredients.

The architecture of the polymolecular assemblies is defined by the geometry of the amphiphiles that compose them. The critical packing parameter $CPP$ is employed to characterize the molecular shape. It is calculated with $CPP = v/a_0l_c$ where $v$ is the hydrocarbon chain volume, $a_0$ the surface area occupied by the polar headgroup in the assembly and $l_c$ the critical length of the hydrophobic part. The $a_0$ corresponds to an energy minimum between repulsive and attractive forces acting between molecules in the assembly. The $CPP$ calculation is schematically presented in Figure 2.1 along with the possible geometries resulting from self-assembly. Cone-like molecules such as DHPC ($CPP < 1/3$) will preferentially form micellar structures. Cylindrical molecules such as DMPC ($CPP = 1$) will self-assemble into bilayers, an essential building block of mammalian cells. Planar bilayers seek to self-close into vesicles to avoid the energetically unfavorable exposure of the hydrophilic bilayer edges to the hydrophobic environment.

Phosphatidylycholine was the first identified phospholipid in 1847 in the egg yolk of chickens by Theodore Nicola Gobley. DHPC, DLPC DMPC, and DPPC are all phosphatidylycholines who differ in the length of their fully saturated alkyl tails. When assembled in a bilayer, phospholipids adopt different phases depending on temperature. In the solid-ordered phase $L_{β'}$, the lipid tails are in their all-trans conformation. This enables optimal packing, which maximises the van der Waals interactions resulting in
2 Background

Figure 2.1 The critical packing parameter $CPP$ describes the geometry of amphiphilic molecules such as phospholipids. The geometry of the amphiphiles defines the architecture of the resulting self-assembly structures. Figure adapted from M. Baumgartner. 

a gel phase where the lipids are locked in place. Upon heating above a given phase transition temperature $T_m$, the aliphatic tails of the phospholipids have enough energy to adopt gauche conformations. The increased disorder results in a liquid-disordered $L_\alpha$ phase where the lipids are free to move along the bilayer. The strength of the attractive van der Waals forces govern the phase behavior and largely determine the $T_m$. For example, DPPC, with its 16 carbons per alkyl tail, has a larger $T_m$ than DMPC that has 14 carbons. These additional two carbons per tail strengthen the van der Waals forces, resulting in an increase in $T_m$ from 24 to 41 °C.

The introduction of unsaturations in the phospholipid tails induces kinks, which hinder an effective packing, weaken the van der Waals forces, and ultimately reduce the $T_m$. POPC is a good example as it contains two different hydrophobic tails. One is composed of a fully saturated 16 carbon long chain and the second has 18 carbons with a double "$cis"$-bond in the middle of the chain. Although similar in length to DPPC, it has a $T_m$ of only -2 °C. In addition to hydrophobic interactions, physico-chemical interactions in the
2.1 Lipids and Self-Assembly

headgroup region such as hydrogen bonding also influence the $T_m$. Moreover, the phase behavior of lipid bilayers is often more complex, showing ripple phases at pre-transition temperatures whose structure depends on the thermal history of the bilayer.\textsuperscript{39–51} Synthetic lipids are required for systematically studying these phenomena.

Synthetic lipids are of high interest in numerous fields including biological research, pharmaceutical applications and the generation of smart soft materials. The ability to engineer the resulting formation of polymolecular assemblies is at the heart of their success. Eukaryote cells are surrounded by a lipid bilayer membrane. The lipid bilayer and the imbedded membrane proteins strongly influence how the cells interact with their surroundings. It is of capital importance to develop tools to study the constituents of cell membranes, understand them and unlock their full potential through biological engineering. The stepwise modification of natural lipids enables enhanced understanding of the complex physico-chemical phenomena occurring within the bilayer.\textsuperscript{52,53} For example, a change in the spatial orientation of the glycerol backbone from $sn$-1,2 to $sn$-1,3 increases the distance between the hydrophobic tails. This enables the interdigitation of the phospholipid membrane leaflets. The reduced sterical crowding in the headgroup region allows for a larger water incorporation in the hydrophilic region of the bilayer. This fact, in combination with the fully extended hydrocarbon tails permitting the optimal expression of van der Waals forces, results in an increased $T_m$ in interdigitated systems. Working with fully synthetic phospholipids permits for the construction of polymolecular assemblies with unique shapes and mechanical-sensitive properties.\textsuperscript{54} For example, vesicles made from artificial 1,3-diaminophospholipids are stable under static conditions and release their contents at elevated shear rates. This property arises from their lenticular shape, leading to preferential breaking points along the equator. Such technology is interesting for targeted drug delivery in constricted blood vessels of heart attack patients.\textsuperscript{55}

Mixing amphiphiles with different geometries results in unique self-assembly architectures. Perforated bilayers, bicelles, wormlike micelles and ribbons may be formed amongst other structures. Bicelles are sub-micrometre sized disk-like polymolecular assemblies formed from amphiphiles in an aqueous solution.\textsuperscript{2,4,8,56} They play an integral role in soft matter research and have been extensively studied since early descriptions in the 1980s.\textsuperscript{57,58} The nature of the lipids composing the bilayer, along with the composition of the surrounding hydrophilic environment, defines the properties of the bicelle. The resulting large degree of tunability in terms of charge, size, magnetic alignability and thermal stability has greatly contributed to their scientific importance. In the most characterized case, DMPC, with a packing parameter close to unity, constitutes the planar part of the bicelle. The regions of high curvature on the edge are covered by DHPC.\textsuperscript{7–9,59,60} The bicelle size is readily tailored by altering the composing lipid ratio or total concentration and the temperature.\textsuperscript{12,13,61,62} However, the DMPC/DHPC bicelles only exist in a defined range of temperatures and lipid ratios. Although the total lipid concentration influences the structure of the assembly to a lesser extent, the water-soluble nature of DHPC quickly becomes limiting at lower concentrations.\textsuperscript{60} Upon increasing the lipid ratio or the temperature, long slightly flattened cylindrical micelles
appear that eventually branch and form lamellar phases with holes.\textsuperscript{39,63} Lipid segregation is an important prerequisite for the formation of bicelles. However, it is not total as a small portion of the edge-composing lipids may also find themselves in the planar part of the disklike assembly. In DMPC/DHPC bicelles, the variation of miscibility of DHPC in the planar part of the bicelle mainly composed of DMPC acts as a driving force in the for mentioned structural transitions upon increasing temperature.\textsuperscript{14}

Further doping DMPC/DHPC bilayers with either anionic 1,2-dimyristoyl-\textit{sn}-3-phosphoglycerol (DMPG) or cationic 1,2-dimyristoyl-3-trimethylammoniumpropane (DMTAP) allows for tailoring of the bilayer’s charge.\textsuperscript{64} 1,2-dimyristoyl-\textit{sn}-glycerol-3-phospho-ethanolamine-diethylene triaminepentaacetate (DMPE-DTPA) is another common dopant that may be introduced in concentrations lower than 1% of the total lipid composition to chelate \textit{Ln}\textsuperscript{3+} on the bilayer’s surface. This enhances the magnetic alignability and permits control over the direction of alignment with respect to the field direction.\textsuperscript{23–25,65,66} P. Beck \textit{et al.} developed magnetically responsive and tunable bicelle systems by replacing DHPC with DMPE-DTPA, see Figure 2.2.\textsuperscript{28} DMPC/DMPE-DTPA/\textit{Ln}\textsuperscript{3+} (molar ratio 4:1:1) bicelles allows for the association of many more paramagnetic \textit{Ln}\textsuperscript{3+} on the bilayer’s surface. The resulting enhanced magnetic response coupled with the high tunability of the system was the main focus of this work. The large headgroup of the DMPE-DTPA/\textit{Ln}\textsuperscript{3+} complex gives it a cone-like geometry. Consequently, and analogously to DHPC, the phospholipid/\textit{Ln}\textsuperscript{3+} complex will preferentially assemble at the edge of the bicelle. A complete lipid segregation is unlikely and DMPE-DTPA/\textit{Ln}\textsuperscript{3+} will also be situated in the planar part of the bicelle.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{bicelle_diagram.png}
\caption{Schematic representation of DMPC/DMPE-DTPA/\textit{Ln}\textsuperscript{3+} (molar ratio 4:1:1, [tL] 15 mM) bicelles as reported by P. Beck \textit{et al.} and M. Liebi.\textsuperscript{28,30} Figure adapted from S. Isabettini \textit{et al.}\textsuperscript{67}}
\end{figure}
2.2 Cholesterol and Steroid Derivatives

Chol-OH is a weakly amphiphilic biomolecule of high importance in mammalian plasma membranes. It consists of a polar hydroxyl group and a bulky hydrophobic part composed of a rigid four ring system and a flexible side chain. The structural characteristics of Chol-OH are further depicted in Figure 2.3. Chol-OH plays an essential role in the self-assembly process of numerous multi-component systems. It is capable of substantially altering the physico-chemical properties of lipid-based assemblies. Membrane fluidity and permeability are predominantly affected, influencing numerous cellular processes. Chol-OH is employed in bicelle technology to harness the physico-chemical properties it delivers to the lipid bilayer. The clear transition between solid-ordered and liquid-disordered lipid phases vanishes upon incorporating Chol-OH in the bilayer. Instead, an intermediate liquid-ordered $L_\alpha$ phase appears. The presence of Chol-OH in the solid-ordered phase $L_{\beta'}$ of a bilayer weakens the van der Waals interactions between the hydrocarbon chains of the fatty acids and prevents lipid crystallisation. In the liquid-disordered phase $L_\alpha$, the hydrocarbon chains of the lipids interact with the rigid steroid backbone of Chol-OH and are partly immobilised, effectively increasing the degree of orientation and reducing molecular motion. This leads to a laterally condensed...
membrane with increased packing density, higher mechanical stability, reduced lipid flip-flop and lower permeability. Too much cholesterol will induce and excess of ordering, slowing down the diffusion of membrane proteins and increasing the bending modulus of the membrane.\textsuperscript{73} Chol-OH is also involved in numerous metabolic pathways involving vitamins and steroid hormones, membrane trafficking and cell signalling.\textsuperscript{68,73,74} In liposome drug delivery systems, Chol-OH is essential to enhance the liposome stability and control the release of the active compound.\textsuperscript{75} For example, the half-lives of encapsulated drugs in phosphocholine liposomes range from minutes to several hours upon the addition of Chol-OH to the bilayer.\textsuperscript{76}

The added order Chol-OH induces in the phospholipid bilayer strongly depends on temperature. The DMPC/Chol-OH bilayer has been extensively study and remains not fully understood. Experimentally obtained phase diagrams do not reach a consensus. A single experimental technique cannot grasp the full complexity and subtle structural changes occurring in the bilayer. It is only with the more recent combination of molecular dynamics simulations that more precise phase diagrams were proposed. Figure 2.4 presents part of the phase diagram obtained by F. De Meyer et al. with a coarse-grained model of the DMPC/Chol-OH bilayer employing hybrid dissipative particle dynamics (Monte Carlo method).\textsuperscript{72} At higher temperature, the membrane can accommodate a significant amount of Chol-OH without undergoing structural changes, remaining in a liquid-disordered phase $L_\alpha$. At lower temperatures, various phases are detected in which the lipid tails are more ordered, including liquid-ordered $L_\alpha$ and ripple phases $P_{\beta'}$. A more ordered structure allows the lipids to support Chol-OH by reducing the water hydrophobic contacts. In reality, the phase boundaries depicted in Figure 2.4 are much less defined due to the high complexity of the physico-chemical forces governing the bilayer. In this work, steroid contents of 16 mol\% were commonly employed in the polymolecular assemblies. The caption in Figure 2.4 shows a snapshot of the DMPC/Chol-OH bilayer at 5 °C obtained from our MD simulations discussed in chapter 6. The degree of order is not evident when looking at this snapshot and was thus always compared to simulation results of the same bilayer conducted at 30 °C, guarantying a liquid-disordered phase $L_\alpha$ as a reference.

Several models describe the molecular interactions of Chol-OH with phospholipids including the umbrella model,\textsuperscript{77,78} the condensed complex model,\textsuperscript{79} and the superlattice model.\textsuperscript{53,80} In the most popular umbrella model, the phospholipid headgroups are assumed to shield the hydrophobic steroid backbone of Chol-OH from unfavourable hydrophilic interactions.\textsuperscript{77} No single interaction model is sufficient to fully explain and consider all the subtle variables governing the physico-chemical properties of the bilayer. Van der Waals forces are responsible for the non-polar interactions occurring within the lipid bilayer. They predominantly dictate the structure and properties of the bilayer, including the position and condensing effect of Chol-OH.\textsuperscript{71,81–84} The hydrophilic interface of the bilayer membrane also plays an essential role in defining the properties of the system. Polar and/or charged species generate long-range electrostatic fields, whilst also participating in shorter ranged interactions such as hydrogen bonding.\textsuperscript{84} These interactions occur between lipids and water (hydration), among the lipids (dir-
2.2 Cholesterol and Steroid Derivatives

Figure 2.4 Simplified temperature-composition phase diagram of the DMPC/Chol-OH bilayer adapted from F. De Meyer et al. The boundaries between the different phases are not well defined and more complex liquid ordered phases appear at higher cholesterol contents. The snapshot of the DMPC/Chol-OH bilayer comes from the MD simulation discussed in Chapter 6, Figure 6.7. It is composed of 16 mol% Chol-OH at 5°C (highlighted in yellow), imitating the compositions employed in the steroid doped bicelles encountered throughout this work.

Most of the research conducted on synthetic or natural steroids has shown that their ordering and condensing abilities may singly correlated to the tilt of the steroid ring. The smaller the tilt, the stronger the ordering and condensing effect on the bilayer. Only ergosterol has been reported to be equally effective as Chol-OH for modifying the

ect hydrogen bonds and charged pairs), and indirectly between two lipids via water bridges. The hydroxyl group of Chol-OH may act as both a hydrogen bond donor and acceptor, while also participating in charge pairing. Consequently, it undergoes numerous types of interactions at the membrane interface. Most studies reveal that Chol-OH interacts with both water and the phosphatidylcholine carbonyl oxygen and phosphate oxygen. Hydrogen bonding plays a more dominant role in describing the physico-chemical properties in Chol-OH doped sphingolipids and diamidophospholipid bilayers. Both these lipids benefit from the presence of secondary amines (instead of the carbonyl groups found in phosphatidylcholine), resulting in a more polar environment favouring the formation of hydrogen bonds with Chol-OH. Sphingolipids contain both hydrogen bond donating and accepting groups, while the corresponding region in phosphatidylcholine contains only accepting groups.
physico-chemical properties of the bilayer. Chemical modifications of Chol-OH are of profound interest to harness the full potential of the molecule and tailor lipid-membrane properties. Synthetic derivatives of Chol-OH are exceptional biotechnological tools. Most synthetic strategies rely on modification of the polar hydroxyl group of Chol-OH. This polar headgroup provides control over the bilayer charge. For example, insertion of cholesterol sulphate in DMPC/DHPC bicelles prevents precipitation at lower temperatures and higher lipid concentrations allowing stabilisation of the aligned phase. Replacing the hydroxyl polar headgroup of Chol-OH with a primary amine in Chol-NH₂ allows for the presence of a pH value dependant positive charge in the lipid bilayer. Furthermore, cholesteryl chloroformate and Chol-NH₂ are common headgroup modified derivatives of Chol-OH. They are important starting blocks for the synthesis of more complex compounds. Chol-NH₂ may be synthesized from cholesterol following the three-step reaction protocol proposed by Q. Sun et al. Cholesteryl chloroformate is commercially available and readily reacts with primary amines to give a carbamate cholesterol derivative. The synthesis is usually performed in cooled anhydrous solvents and in the presence of catalytic amounts of base such as triethylamine, diisopropylethylamine (hunig’s base) or 4-dimethylaminopyridine (DMAP). This chemistry was employed for the synthesis of the Chol-C₂OC₂-NH₂ conjugate in Chapter 5 and 6 as well as for the synthesis of the Ln³⁺ chelating Chol-Cₙ-DTPA amphiphiles in Chapter 7.

Introducing 16 mol% Chol-OH in the DMPC/DMPE-DTPA/Ln³⁺ (molar ratio 4:1:1) lipid results in larger and more magnetically responsive DMPC/Chol-OH/DMPE-DTPA/Ln³⁺ (molar ratio 16:4:5:5) bicelles in Figure 2.5. Chol-OH, with its inversed cone-like geometry, acts as a spacer in the bilayer and counteracts the curvature induced by the DMPE-DTPA/Ln³⁺ species. Both an increase in aggregate number and a larger proportion of species with a high $\Delta \chi$ in the planar part of the bicelle result in an increase in $E_{mag}$. The presence of a liquid-ordered phase $L_o$ induced by Chol-OH in the bilayer further increases the thermal resistance of the bicelle. In the absence of Chol-OH, the thermoreversible collapse of the bicelles into vesicles occurs at the phase-transition temperature of the phospholipids. The compatibility of the steroid moiety of Chol-OH with phospholipid bilayers offers a fertile playground for further chemical modifications of the molecule. Therefore, a Ln³⁺ chelating cholesterol-diethylenetriaminepentaacetate compound (Chol-DTPA/Ln³⁺) was synthesized and introduced into the bicelle bilayer. The steroid backbone of Chol-OH was chemically bound to a DTPA moiety using the amino-acid lysine as a linker. The Chol-DTPA conjugate acts as an anchor for further doping of the lipid plane with paramagnetic Ln³⁺. Introduction of Chol-DTPA in the bilayer resulted in a substantial increase in bicelle size and magnetic alignability. The additional degrees of freedom induced by these specially designed multilipidic systems will be extensively described in Chapter 7 with the synthesis of novel Ln³⁺ chelating Chol-Cₙ-DTPA amphiphiles.
Figure 2.5 Schematic representation of a DMPC/Chol-OH/DMPE-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5, [tL] 15 mM) bicelle as first proposed by M. Liebi et al. in a 50 mM phosphate buffer at a pH value of 7.4. The bicelle diameter and bilayer thickness were obtained from reported SANS fittings. Figure adapted from S. Isabettini et al. 16
2.3 Magnetic Alignment of Ln$^{3+}$ Containing Polymolecular Assemblies

Every molecule subject to a magnetic field $B$ will experience forces arising from quantum mechanical or electromagnetic effects. In quantum theory, a particle possessing spin will have an associated magnetic moment, which interacts with the magnetic field. In electromagnetics, the orbital motion of electrons are influenced by Lorenz forces exerted on moving charges in a magnetic field. Analogously to the Gibbs free energy $G$ in thermodynamics, every molecule will have a magnetic free energy $G_m$ once exposed to a magnetic field. The response of the molecule to the magnetic field will aim towards lowering the $G_m$, resulting in a negative change in magnetic free energy.
2.3 Magnetic Alignment of $\text{Ln}^{3+}$ Containing Polymolecular Assemblies

\[ \Delta G_m = -\frac{1}{2\mu_0} \Delta \chi B^2 \]  

(2.1)

where $\mu_0$ is the permeability of a vacuum and $\Delta \chi$ is the change in magnetic susceptibility. The susceptibility $\chi$ is either negative or positive for diamagnetic or paramagnetic molecules, respectively. It largely depends on the chemical nature of the considered molecule. Both the nature and orientation of each bond relative to the magnetic field must be considered. For example, a $\Delta \chi$ of $-1.2021 \times 10^{-10}$ m$^3$mol$^{-1}$ and $-8.545 \times 10^{-10}$ m$^3$mol$^{-1}$ is reported for DMPC and DPPC, respectively. $^{91,92}$ Evidently, the magnitude of $\Delta \chi$ is largely affected by the alkyl chains of the phospholipid composed of 14 carbons in DMPC and 16 carbons in DPPC. The presence of unsaturated bonds in the tails of the phospholipids along with the lipid phase will also greatly influence the $\Delta \chi$. $^{91,93,94}$ Higher ordered lipid phases favoring the all trans configuration of the lipid chains offer the largest $\Delta \chi$.

$\Delta G_m$ is in the order of 1 Jmol$^{-1}$ at magnetic field strengths of 10 T. This remains three orders of magnitude smaller than thermal energy at room temperature dictated by $k_B T$. The magnetic energy of a single molecule is comparatively negligible and its effect may not be observed at technically feasible field strengths. In polymolecular assemblies, the individual magnetic energies of the molecules cumulate, resulting in anisotropic magnetic energies larger than the surrounding thermal energy. However, the assembly structure must provide a certain degree of order to ensure an effective cumulative effect. This is the case in phospholipid bilayers, where it’s propensity to align in a magnetic field $B$ depends on the difference $\Delta \chi = \chi_\parallel - \chi_\perp$ where $\chi_\parallel$ and $\chi_\perp$ correspond to the volume magnetic susceptibilities parallel and perpendicular to the long axes of the phospholipids composing the bilayer, respectively. The resulting magnetic energy associated to the bilayer is dependent on the magnetic field strength and orientation:

\[ E_{\text{mag}}(B, \theta) = -\frac{n\Delta \chi B^2}{2\mu_0 N_A \cos^2 \theta} \]  

(2.2)

where $n$ is the aggregate number corresponding to the number of molecules composing the assembly, $N_A$ is the avogadro constant and $\theta$ is the angle between the long molecular axis of the phospholipid (parallel to the alkyl chains) and the magnetic field direction. For $\Delta \chi < 0$, the positive $E_{\text{mag}}$ is minimized when the alkyl chains of the phospholipids align perpendicular to the magnetic field (i.e. the plane of the bilayer aligns parallel to the magnetic field). Consequently, phospholipid bicelles align parallel to the magnetic field. This is the case in the commonly employed DMPC/DHPC bicelles, whose magnetic response is readily tuned though its size (aggregate number $n$) by altering the lipid ratio or total concentration. $^{12,13}$

The $\Delta \chi$ may be further tuned by adding lanthanide ions ($\text{Ln}^{3+}$) that coordinate to the phosphodiester group of the phospholipids composing the bilayer. $^{24,90}$ $\text{Ln}^{3+}$ che-
2 Background

Ligating phospholipids, such as DMPE-DTPA, are commonly added to the bilayer as a means of reducing the paramagnetic shifts in membrane structural studies by NMR spectroscopy. The resulting Ln$^{3+}$ chelating species deliver high magnetic anisotropy, often one or two orders of magnitude larger than the corresponding organic systems. In addition to enhancing the negative $\Delta \chi$, Ln$^{3+}$ are capable of switching the sign of $\Delta \chi$ to positive values. The Ln$^{3+}$ may be classified in two distinct groups based on the sign of $\Delta \chi$. The first group contains Ce$^{3+}$, Pr$^{3+}$, Nd$^{3+}$, Sm$^{3+}$, Tb$^{3+}$, Dy$^{3+}$, and Ho$^{3+}$ ions, while the second group contains Eu$^{3+}$, Er$^{3+}$, Tm$^{3+}$, and Yb$^{3+}$ ions. Almost all experimental results obtained for Ln$^{3+}$ doped phospholipid bilayers and liquid crystals agree with a negative $\Delta \chi$ for the first group and a positive $\Delta \chi$ for the second. The inverse remains theoretically possible as long as two compounds not belonging to the same group always have opposite signs of $\Delta \chi$. For example, DMPC/DMPE-DTPA/Dy$^{3+}$ (molar ratio 4:1:1) bicelles orient parallel to the magnetic field direction, while the DMPC/DMPE-DTPA/Tm$^{3+}$ (molar ratio 4:1:1) counterparts align perpendicular. $^{29,30,90}$ Tm$^{3+}$ is capable of supplying a 155 times higher $\Delta \chi$ of opposite sign to a DMPC phospholipid. $^{24}$ The Bleaney theory suggests a strict regularity in the maximum magnetic anisotropy where Dy$^{3+}$ always provides the largest value. $^{96}$ However, this theory was partially dismantled by Mironov et al. who showed that $\Delta \chi$ strongly depends on the nature of the phospholipid/Ln$^{3+}$ complex. $^{97,98}$

Mironov et al. describe four contributions determining $\Delta \chi$ in their general theory on the magnetic anisotropy of lanthanide-containing metallomesogens, summarized in Figure 2.7.$^{97}$ The magnetic properties of Ln$^{3+}$ are determined by the electron state of the outer shell 4f electrons. $^{99}$ The first contribution considers the crystal field effects on the chelated Ln$^{3+}$. It defines the molecular magnetic anisotropy tensor, along with the values and direction of the molecular magnetic axis. The chemical nature of the ligands and the geometry of the complex split the levels of the ground J-multiplet into individual crystal field energies. The f-electrons of the complexed Ln$^{3+}$ define the crystal field, which partially removes the $2J + 1$ degenerate nature of the ground state. The second contribution considers the orientation of the long molecular axis with respect to the molecular magnetic axis. This molecular geometry will determine both the sign and magnitude of $\Delta \chi$. The last two contributions consider microscopic and macroscopic disorder effects. Microscopic disorder effects include geometric fluctuations resulting from the nonrigid nature of the Ln$^{3+}$ chelating molecules. Macroscopic disorder effects consider distortions of the ideal monodomain structure of the polymolecular assembly. For example, deviations from planarity in the bilayer will act to reduce $\Delta \chi$. For this reason, lipid vesicles are only weakly distorted by magnetic fields and may not align in the same way as planar disk-like bicelles. Both the microscopic and macroscopic disorder effects act to reduce the maximal value of $\Delta \chi$, but cannot change its sign. The first two contributions are the most important in defining the $\Delta \chi$ and are intrinsically linked to the molecular structure of the Ln$^{3+}$ chelating lipid. Therefore, bottom-up approaches are required for engineering of the $\Delta \chi$, ultimately defining the final magnetic response of the polymolecular assemblies. Such approaches involve starting from the molecular design of the Ln$^{3+}$ chelating lipids that are to be incorporated into the bilayer.
2.4 Quantifying the Magnetic Alignment of Polymolecular Assemblies

The structure of bicelles has been extensively studied with a broad range of characterization techniques. However, the alignment of bicelles exposed to a magnetic field has been quantified by using only NMR, SANS and birefringence experiments. Herein, a brief introduction on these three techniques is provided.

2.4.1 Nuclear Magnetic Resonance (NMR) Spectroscopy

Bicelles have been extensively characterized by NMR spectroscopy where they are essential tools for the study of membrane bound and associated biomolecules. This is especially true for the commonly employed DMPC/DHPC bicelle system. $^{31}$P- and $^2$H-NMR are widely employed to study the structure and dynamics of lipid phases. These experiments map out phase-diagrams of lipid mixtures in solution comparatively.
quickly.\textsuperscript{8,100} Once the region composed of bicelles identified, the spectra may be further analyzed to retrieve information on their degree of alignment. The $^2$H quadrupole splitting in $\text{D}_2\text{O}$ is a viable means of observing the phase behavior of bicelles. This splitting appears, and is proportional to, the degree of alignment of the bicelles.\textsuperscript{3,101,102} In $^{31}$P-NMR, aligned DMPC/DHPC bicelles display a spectrum composed of two peaks, arising from the chemically and magnetically distinct environments provided at the edge and the center of the assembly. The downfield peak corresponds to DHPC, whilst the upfield peak corresponds to DMPC. The ratio of integrated areas of the two peaks corresponds to the ratio of employed lipids.\textsuperscript{2,9,14,103,104} However, the $^{31}$P-NMR spectra of DMPC/DMPE-DTPA/Ln$^{3+}$ bicelles are far from being as straightforward as those found for DMPC/DHPC bicelles.\textsuperscript{28} The shift and broadening of the NMR peaks occurring in the presence of substantial amounts of Ln$^{3+}$ are serious limitations to the method.\textsuperscript{24,25,96} This is particularly problematic in membrane bound and associated structural studies where the lanthanides further seek and bind specific sites of the protein, altering their interactions with the membrane. Ln$^{3+}$ chelating phospholipids are a viable means of sequestering the ions, allowing to benefit from their high magnetic susceptibility whilst reducing paramagnetic shifts.\textsuperscript{24,25,65}

\subsection*{2.4.2 Birefringence}

Alternative tools to detect magnetically induced alignment of polymolecular assemblies in solution are desirable. Birefringence measurements are a viable and comparatively easy solution. Analogously to NMR based experiments, birefringence measurements also reveal substantial information on lipid rearrangements and lipid phases occurring in the bilayer. Moreover, geometric transformations occurring in the polymolecular assembly with changing environmental conditions such as temperature may be monitored.\textsuperscript{15,16,29,30} Numerous authors have relied on the birefringence signal $\Delta n'$ resulting from magnetically orientated structures to study various types of phospholipid systems including purple membrane suspensions, mechanically oriented multilayers, vesicles, and tubular aggregates.\textsuperscript{29,105–107} Birefringence measurements based on the phase modulation technique in a magnetic field is a viable method to detect orientation of bicelles.\textsuperscript{15,16,20,94} The possibility of investigating bicelles with birefringence in high magnetic fields up to 35 T was also demonstrated by M. Liebi \textit{et al.}.\textsuperscript{29}

When polarized light enters an anisotropic material, it will be refracted in an ordinary and extraordinary wave.\textsuperscript{30} The two waves have different velocities and are shifted in phase by a retardation $\delta$. The degree of retardation $\delta$ may be measured and converted into a birefringence signal $\Delta n'$ to quantify the degree of anisotropy in the material using:

$$\Delta n' = - \frac{\delta \lambda}{2\pi d}$$

(2.3)
2.4 Quantifying the Magnetic Alignment of Polymolecular Assemblies

where $\lambda$ is the wavelength of the laser and $d$ is the thickness of the sample. Phospholipids are optically anisotropic and their optical axis coincides with their long molecular axes, parallel to the hydrocarbon tails. No retardation is measured if the phospholipids are randomly orientated in solution. A retardation is detected when they are aligned parallel to each other in a bilayer. The birefringence signal $\Delta n'$ can be both positive or negative depending on the orientation of the molecule in the magnetic field, see Figure 2.8. Phospholipids aligned parallel to the x-axis will result in a negative $\Delta n'$, while those aligned along the z-axis result in a positive $\Delta n'$. No birefringence is observed when the optical axis coincides with the direction of light propagation as the phospholipid aligns parallel to the y-axis. Therefore, the absolute value of $\Delta n'$ obtained for phospholipids aligning perpendicular to the magnetic field is only half of the maximal achievable value $\Delta n'_{\text{max}}$ in case of full alignment. Species aligning parallel to the field may reach $\Delta n'_{\text{max}}$.

![Figure 2.8](image)

**Figure 2.8** Magnetic induced birefringence $\Delta n'$ measured depending on the orientation of the phospholipid in the magnetic field. Dashed lines indicate the optical axis of the molecule. The light is polarized at 45° and propagates in the y direction. The magnetic field $B$ is in the z direction. Schematic adapted from M. Liebi.\textsuperscript{30}

In the case of an isotropic colloidal suspension of bicelles, the orientation brought about by the arrangement of the phospholipids in the bilayer will be lost, zeroing the retardation $\delta$. The bicelles must also be aligned in order to orientate the optically active phospholipids in their bilayers, resulting in a retardation $\delta$ of the polarized light. Consequently, birefringence is a sensitive tool to quantify the magnetic alignability of bicelles. Bicelles aligned perpendicular to the magnetic field will yield a positive $\Delta n'$, while those aligned parallel will yield a negative $\Delta n'$. The sign depends on the alignment of the setup and may be checked with a reference sample.

2.4.3 Small Angle Neutron Scattering (SANS)

SANS experiments allow to quantify both the alignment of bicelles under a magnetic field and to obtain structural information by conducting experiments at 0 T.\textsuperscript{16,29,108,109} Aligned objects scatter neutrons anisotropically. Various calculations may be undertaken to quantify the anisotropy of the resulting 2D neutron scattering pattern and compute an alignment factor $A_f$. Herein, a $A_f$ was calculated based on the second
cosine Fourier coefficients of the normalized azimuthal intensity $I(\theta)$ according to Hongladarom et al.\textsuperscript{30,110}

\[
A_f = \frac{\Sigma I(\theta) \cos(2\theta)}{\Sigma I(\theta)}
\] (2.4)

The $A_f$ was computed in the q range between 0.3 and 0.4 nm\textsuperscript{-1} as shown in Figure 2.9. The $A_f$ theoretically ranges between -1 and 1. In practice, the bicelles already show signs of saturation or full alignment at absolute $A_f$ values of 0.9. The $A_f$ is positive when scattering is perpendicular to the magnetic field direction and negative when scattering is parallel, with $A_f = 0$ for isotropic scattering (no alignment). In terms of bicellar alignment, a positive $A_f$ means the bicelles align parallel and a negative $A_f$ perpendicular to the magnetic field direction.

![SANS pattern illustration](image)

**Figure 2.9** Schematic illustrating the calculation of the alignment factor $A_f$ from a 2D SANS pattern of an anisotropic sample aligned under a 8 T magnetic field. The q range between 0.3 and 0.4 nm\textsuperscript{-1} is shown with white circles on the 2D SANS pattern. The intensity as a function of azimutal angle $I(\theta)$ after averaging is plotted and employed to compute the $A_f$. Schematic adapted from M. Baumgartner.\textsuperscript{38}

The alignment factor, unlike the birefringence signal, is a result of the bulk bicellar alignment and is not dependent on the orientation of the phospholipids with respect to the incoming neutron beam. Therefore, the alignment factors obtained from SANS experiments allow to break down the birefringence signal obtained for the same sample into contributions arising from the bulk alignment of the bicine disks and the arrangement of the lipids in the bilayer. SANS experiments are strongly complementary to birefringence measurements and may highlight particular phase behaviors of the bilayer lipids and explain the mechanistic origins of changes in architecture of the polymolecular assemblies.
2.5 Bicelle Applications

Bicelles have been employed for decades in structural studies of membrane bound and associated biomolecules by NMR spectroscopy.\(^7,^8\) Both isotropically tumbling and magnetically aligned bicelles are valuable tools for such studies, justifying the need for tailorable systems.\(^2,^7,^9\) Highly magnetically alignable bicelles are mainly employed for solid-state NMR of proteins imbedded in the bilayer.\(^2,^7\) Globular proteins also feel a week orientational preference in the presence of aligned bicelles, allowing for their study by solid-state NMR. Molecules that are not free in their reorientation due to the presence of aligned materials such as bicelles will adopt a certain orientation. These weak alignments result in a small dipolar splitting in the Hz range, far smaller than the kHz range resulting from certain chemical bonds. For this reason, these couplings are referred to as residual dipolar couplings (RDC). RDCs hold essential information on both the molecular structure and the molecular dynamics of proteins. The protein extraction, purification and insertion in the bilayer of alignable bicelles are essential steps that must be undertaken in such structural studies. In fact, the first two steps are often the most difficult. When it comes to resuspending the proteins in the bilayer, standardized iterative protocols are based on altering the total lipid concentration and molar ratio until a suitable system is achieved.\(^12,^13\) The DMPC/DHPC bicelles are commonly applied for these structural studies due to their simplicity, availability and tailorability.

Numerous modifications with other constituents have been made to tune the DMPC/DHPC bicelles system, offering new possibility to the field.\(^8\) One of the most noticeable consists of doping the bicelles with as little as 1% of Ln\(^{3+}\) chelating phospholipids such as DMPE-DTPA/Ln\(^{3+}\). These Ln\(^{3+}\) chelating species deliver high magnetic anisotropy to the bilayer, often one or two orders of magnitude larger than the corresponding organic systems.\(^25\) Furthermore, Ln\(^{3+}\) are capable of switching the sign of \(\Delta \chi\) to positive values, resulting in an opposite direction of alignment of the bicelles in the magnetic field.\(^23,^24,^29,^98,^111\) In biomolecular membrane NMR studies, Ln\(^{3+}\) chelating phospholipids are a viable means of sequestering the Ln\(^{3+}\), allowing to benefit from their high \(\Delta \chi\), whilst reducing the paramagnetic shifts induced by the free ions.\(^24,^25,^65\) These metal-ion chelating phospholipids further speed-up the T1 relaxation of the embedded membrane proteins and considerably shorten measurement time.\(^27\) Broadening the temperature range in which magnetically alignable bicelles exist is another important parameter commonly addressed by modifying the constituents of the bilayer.\(^112\) This is particularly important as many of the studied proteins are heat sensitive and cannot withstand the lengthy measurement times imposed by NMR experiments. For example, doping DMPC/DHPC bicelles with charged amphiphiles such as cholesterol sulfate extends the temperature range in which they may be employed.\(^3\) Another possibility involves replacing the DHPC phospholipid with chemically engineered surfactants derived from cholic acid.\(^6\) The freedom in design offered by these synthetic amphiphiles permit the synthesis of polymerizable surfactants, also enabling to chemically lock the bicelles, delivering a high thermal and kinetic stability.\(^5\)

Bicelles may be composed of a multitude of lipid mixtures in diverse hydrophilic envir-
2 Background

...environments, each of which are capable of delivering unique properties. The resulting large degree of tunability in terms of charge, size, magnetic alignability and thermal stability has largely contributed to their scientific importance. The potential of DMPC/DMPE-DTPA/Ln\(^{3+}\) bicellar systems for biomolecular structural studies by NMR is high and remains to be explored. These systems present numerous advantages. Aside from the obvious high degree of tunability, they are also resistant to a wide range of temperatures (especially low temperatures), pH values and to dilution. The unique possibility of engineering their magnetic response through the magnetic susceptibility \(\Delta \chi\) revealed in this work, ascertains their potential for such studies and calls for further research.

Recent efforts have aimed at expanding the field of applications of bicelles. Synthetic lipids are essential tools for the development of innovative bicelle-based technologies. Inspired from the commonly employed DMPC/DHPC bicelle, the replacement of DMPC with polymerizable organoalkoxysilane lipids enables to chemically stabilize the assembly with a siloxane surface.\(^{113-115}\) These hybrid bicelles have been employed as pH-sensitive nanocarriers for hydrophobic drug delivery.\(^{17}\) The drug release rate is further modulated by incorporating PEGylated phospholipids in the partially silica-coated bicellar nanodisc.\(^{113}\) Remaining in the pharmaceutical field, the potential of bicelles for dermal treatments has been reviewed.\(^{116}\) The small dimensions of bicelles is ideal for penetrating the skin lamellae, delivering their functional properties where needed. For example, diclofenac (a known anti-inflammatory) was successfully delivered to the skin with the help of bicelles.\(^{117}\) The voltage gated potassium channel modulatory membrane protein KCNE3 was delivered into oocytes in functional form using bicelles.\(^{8,118}\) In the field of nanotechnology, bicelles were employed as temporary scaffolds for the directed covalent assembly of rigid polymeric nanodisks.\(^{19}\) The inhomogenous reaction environment offered by bicelles has been employed for the templated growth of platinum nanowheels.\(^{119}\) Most interestingly, M. Liebi et al. coupled the high magnetic response of DPPC/Chol-OH/DPPE-DTPA/Ln\(^{3+}\) (molar ratio 16:4:5:5) bicelles with their unique temperature dependent properties to deliver switchable anisotropy in optical gels.\(^{20}\) The bicelles were locked in a gelatin network in an aligned state, forming gel cubes exhibiting different spatial birefringence. This process is schematically represented in Figure 2.10. The novel bicelles developed in the frame of this work will be employed to enrich the toolbox for such applications.
2.5 Bicelle Applications

Figure 2.10 Schematic representation of DPPC/Chol-OH/DPPE-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5, [tL] 15 mM) bicelles being imbedded into a gelatin network in an magnetically aligned state. The process exploits the higher temperature resistance of DPPC based bicelle systems that thermo-reversibly collapses into vesicles above the phase transition temperature of DPPC at around 42 °C. The bicellar packing observed for these systems is not represented. Schematic adapted from M. Liebi.$^{30}$
3 Materials and Methods

3.1 General Information for Chemical Synthesis

Unless otherwise stated, all chemicals were purchased either from Sigma Aldrich, VWR, Merck or ABCR and employed without further purification. All reactions were conducted according to, or inspired from, published protocols. The reaction schemes, detailed protocols, and chemical characterization are available in chapter 10.

1,2-Dimyristoyl-sn-glycero-3-phosphocholine (DMPC) was purchased from COATSOME (NOF Corporation, JP) or CordenPharma (Plankstadt, DE). 1,2-Dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and 1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine were purchased from COATSOME (NOF Corporation, JP). The 1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine-N-diethyleneetriaminepentaacetic acid hexa-ammonium salt (DMPE-DTPA) and 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine-N-diethyleneetriaminepentaacetic acid hexa-ammonium salt (DPPE-DTPA) phospholipids were purchased from Avanti Polar Lipids (Alabaster, AL). All lipids were purchased in powdered form (99%) and were used without further purification. Cholesterol was purchased from Amresco (Ohio, USA). Anhydrous thulium(III) chloride (99.9%), ytterbium(III) chloride (99.9%), dysprosium(III) chloride (99.9%) were purchased from Sigma-Aldrich (Buchs, Switzerland). The chloroform (stabilized by ethanol) employed to make 10 mg/ml stock solutions of the amphiphiles and the methanol (99.8%) employed to make 10 mM stock solutions of the lanthanide salts were purchased from Sigma-Aldrich (Buchs, Switzerland). D$_2$O (99.9 atom % D) was purchased from ARMAR Chemicals (Döttingen, Switzerland). The chemical abbreviations, structures and IUPAC nomenclature of the amphiphiles are provided in Table 3.1
### Table 3.1 Chemical abbreviations, IUPAC nomenclature, and molecular structure of commercially available phospholipids, Ln$^{3+}$ chelating phospholipids and cholesterol.

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>IUPAC</th>
<th>Molecular Structure</th>
</tr>
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<tbody>
<tr>
<td>DMPC</td>
<td>1,2-dimyristoyl-sn-glycero-3-phosphocholine</td>
<td>![DMPC Diagram]</td>
</tr>
<tr>
<td>DPPC</td>
<td>1,2-dipalmitoyl-sn-glycero-3-phosphocholine</td>
<td>![DPPC Diagram]</td>
</tr>
<tr>
<td>DMPE-DTPA</td>
<td>1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine-N-diethylene-triaminepenta-acetic acid</td>
<td>![DMPE-DTPA Diagram]</td>
</tr>
<tr>
<td>DPPE-DTPA</td>
<td>1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine-N-diethylene-triaminepenta-acetic acid</td>
<td>![DPPE-DTPA Diagram]</td>
</tr>
<tr>
<td>Chol-OH</td>
<td>3β-cholest-5-en-3-ol</td>
<td>![Chol-OH Diagram]</td>
</tr>
</tbody>
</table>
The reactions were conducted in three necked round bottom flasks. They were monitored by analytical thin-layer chromatography (TLC) using silica gel baked on aluminium-foil with a fluorescent indicator 254 nm from RediSepTM or Merck. The TLCs were visualized under UV-light at 254 nm. They were further developed by staining with either an aqueous alkaline potassium permanganate solution or with a molybdenum phosphorus spray. Solvent evaporation was conducted on a Heidolph Laborota rotary evaporator with a bath temperature of 40 °C and an appropriate pressure. Flash chromatography was performed with a CombyFlash TeledyneISCO system from Companion using RediSepTM Normal phase disposable Columns of various sizes. Separation resolution was improved with 0.1% of 1,1,1,3,3,3-hexafluoro isopropanol purchased from Apollo scientific in the eluent. The yield was calculated based on the mass of the dried and purified compounds.

Nuclear magnetic resonance (NMR) spectra were recorded at room temperature in 5 mm broadband inverse probes on a Bruker spectrometer operating at 400 MHz for 1H-NMR and 100 MHz for 13C-NMR. Unless otherwise stated, deuterated chloroform (ARMAR) was used as a solvent. All NMR spectra were referenced to residual solvent signals. Some of the synthesized Ln3+ chelating amphiphiles were not sufficiently soluble in chloroform or showed self-assembly tendencies, reducing the resolution of the spectra. Therefore, a deuterated solvent mixture (CMW) was applied, which is composed of CDCl3, CD3OD and D2O in a ratio of 80:20:1. The mixture was calibrated to the methanol residual solvent signal at 4.07 ppm. For DMPE-Glu-DTPA, a drop of DCl was added to fully protonate the DTPA head group, effectively solubilizing the phospholipid. Data is reported as follows in the appendix: chemical shifts (δ) in parts per million (ppm), if possible identification according to the numeration in the drawn molecular structure, corresponding signal integral, multiplicity abbreviation (s: singlet, d: doublet, t: triplet, q: quartet, m: multiplet). Mass spectra were recorded on an ESI-HRMS from Thermo Scientific (exact) with direct injection dissolved in an appropriate concentration of methanol. Computed masses were based on single isotope masses for high-resolution spectra. Full calibration was conducted before measurements using the appropriate solutions for both positive and negative modes.

For simplicity, the synthesis protocol of the Ln3+ chelating steroid derivatives is described only for Chol-C25-DTPA. The procedure was analogous for the C2 and C6 carbon linkers, according to Rui et al. The additional synthesis of BOC-C2-O-C2 was required and prepared from 2,2’-oxybis(ethylamine) as described by Suzuki et al. Chol-C2OC2-NH2 was an intermediate product in the synthesis of Chol-C2OC2-DTPA. The synthesis of Chol-NH2 was conducted following the three-step reaction protocol proposed by Sun et al. Di-tert-butyl-2-bromoethyliminodiacetate (TBB) served as a precursor to the DTPA headgroup of DMPE-Glu-DTPA and was synthesized as described by Micklitsch et al. The synthesis of the Glu-DTPA precursor was inspired from Anelli et al. A Yamada coupling was employed to bind Glu-DTPA to DMPE with an amide bond, inspired from Gianella et al. followed by a final deprotection step in formic acid to give DMPE-Glu-DTPA as described by M. Liebi et al. The chemical abbreviations and structures of the synthesized amphiphiles are provided in Table 3.2.
### Table 3.2 Abbreviations and molecular structures of synthesized compounds.

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Molecular Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chol-NH₂</td>
<td><img src="image1" alt="Molecular Structure" /> Aminocholesterol 3β-amino-5-cholestene</td>
</tr>
<tr>
<td>Chol-C₂OC₂-NH₂</td>
<td><img src="image2" alt="Molecular Structure" /></td>
</tr>
<tr>
<td>Chol-Cₙ-DTPA</td>
<td><img src="image3" alt="Molecular Structure" /> (n: 2, 5, or 6)</td>
</tr>
<tr>
<td>Chol-C₂OC₂-DTPA</td>
<td><img src="image4" alt="Molecular Structure" /></td>
</tr>
<tr>
<td>DMPE-Glu-DTPA</td>
<td><img src="image5" alt="Molecular Structure" /></td>
</tr>
</tbody>
</table>
3.2 Bicelle Preparation

The bicelles were prepared as described previously and schematically illustrated in Figure 3.1. The mixture of amphiphiles were prepared from 10 mg/ml chloroform (lipids) or 10 mM methanol (lanthanides) stock solutions. A dry lipid film was obtained by removal of the solvents under vacuum. The dry lipid film was hydrated with a 50 mM phosphate buffer at a pH value of 7.4 at room temperature to obtain solutions with 15 mM total lipid concentration. An equivalent phosphate buffer was prepared in D$_2$O for SANS experiments. The samples were subject to five consecutive freeze-thawing cycles in liquid nitrogen before being extruded (Lipex Biomembranes, Vancouver, Canada) 10 times through 200 nm pores and another 10 times through 100 nm pores using polycarbonate membranes (Sterico, Dietikon, Switzerland). The bicelle samples were stored in the fridge and regenerated with heating and cooling cycles before any measurement. An in-depth study of these fabrication procedures and the development of novel protocols was undertaken in chapter 4.

![Figure 3.1](image_url) Schematic representation of the standard fabrication procedure of DMPC/DMPE-DTPA/Tm$^{3+}$ (molar ratio 4:1:1, [tL] 15 mM) or DMPC/Chol-OH/DMPE-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5, [tL] 15 mM) bicelles developed by M. Liebi. The extrusions were carried out at 40 and 60 °C for the Chol-OH free DMPC/DMPE-DTPA/Tm$^{3+}$ (molar ratio 4:1:1, [tL] 15 mM) and the DMPC/Chol-OH/DMPE-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5, [tL] 15 mM) bicelle systems, respectively. The samples were subject to freeze-thawing cycles (FT) followed by extrusion (Ext). Figure adapted from Isabettini et al.
3.3 Spectrophotometry

Spectrophotometry measurements were conducted at 400 nm on a Cary 100 UV-Vis Spectrophotometer (Agilent Tech.), equipped with an extended sample compartment containing a six by six multi-cell block Peltier element. Samples were kept at 5 °C for 5 min prior to starting the heat cycle experiment. A heat cycle consisted of ramping to 50 °C and back to 5 °C with a temperature rate of 1 °C/min. Changes in the transmitted intensity were expressed in terms of optical density with a cell path length of 1 cm. The 50 mM phosphate buffer was employed to correct the background.

3.4 Birefringence

Birefringence measurements of the samples exposed to a 5.5 T field were undertaken to quantify magnetic orientation and monitor changes in the architecture of the polymeric assemblies.29,105,126 The method and experimental setup was analogous to that described by M. Liebi.30 The sample was placed in a temperature-controlled quartz cuvette (Hellma, Germany). Light from a diode laser (Newport, Irvine, US) with a wavelength of 635 nm was polarized by two crossed linear polarizers (Newport, Irvine, US). A photoelastic modulator (PEM-90, Hinds Instruments) placed between the two polarizers was set to operate at 50 kHz. The PEM amplitude $A_0$ was set to 2.405 rad, making the DC component independent of birefringence. The sample cuvette was placed between the PEM and the second polarizer in the magnet operating at 5.5 T. A set of non-polarizing mirrors (Newport, Irvine, US) were employed to guide the laser light through the different elements and was finally directed onto a photo detector (Hinds Instruments, US). Two lock-in amplifiers (SR830, SRS, Sunnyvale, US) were employed for recording of the first $I_{ω1}$ and second $I_{ω2}$ harmonic of the AC signal used to evaluate the degree of retardation $δ$ of the polarized light with

$$δ = arctan \left( \frac{I_{ω1}J_2(A_0)}{I_{ω2}J_1(A_0)} \right) \quad (3.1)$$

where $J_1$ and $J_2$ are Bessel functions of the first kind, with $J_1(2.405) = 0.5191$ and $J_2(2.405) = 0.4317$. The retardation $δ$ was then converted into a birefringence signal $Δn'$ to quantify the degree of anisotropy in the material using

$$Δn' = -\frac{δλ}{2πd} \quad (3.2)$$

where $λ$ was the wavelength of the laser at 635 nm and $d$ is the thickness of the sample (10 mm). The birefringence signal $Δn'$ was normalized with the values obtained at 0 T and the temperature cycles were conducted using a gradient of 1 °C/min. The magnetic field strength at which the order parameter $S$ reaches one-half of its maximum value...
3.5 Cryo Transmission Electron Microscopy (Cryo-TEM)

Samples for cryo-TEM were prepared as described previously.\textsuperscript{16,30} The sample was suspended as a thin aqueous films on holey carbon grids (Quantifoil, Jena, Germany) before being flash-frozen in liquid ethane at 77 K using a cryoplunge 3 system (Gatan, USA). Samples were measured with JEM2200FS (JEOL, Japan) equipped with a field emission gun and an in-column energy filter (JEOL) operated at 200 kV. A 4096 x 4096 CMOS camera (F416, TVIPS, Germany) with 510 \( \mu \text{m} \) under focus was used to increase the contrast. High contrast particles on the micrographs are ice crystals resulting from the freezing procedure.

3.6 Dynamic Light Scattering (DLS)

DLS measurements were conducted at 5 °C on a Malvern Zetasizer Nano ZS equipped with a He-Ne laser fixed at 90° with non-invasive backscatter technology (NIBS).

3.7 Small Angle Neutron Scattering (SANS)

SANS measurements were conducted on the SANS-I beamline at PSI, Villigen, Switzerland. The detector distance was set at 2, 6, or 18 m with a neutron wavelength of 0.8 nm. A 2D \( ^3 \text{He} \) detector covered the q-range from 0.03 to 1.3 nm\(^{-1} \). Data was corrected for the blank cell, transmission, and detector efficiency. Radially averaged scattering curves were constructed from 2D neutron scattering patterns of samples measured at 0 T. The scattering curves were fitted with the SASfit software package.\textsuperscript{127} The disk-like bicelles were fitted with a form factor for Porod cylinders with an appropriate size distribution (delta, log-normal or fractal).\textsuperscript{15,16,30,128} The hydrodynamic radius was computed for comparison with DLS measurements.\textsuperscript{129} Bicelles with a hole were fitted with a form factor of a flat cylindrical shell.\textsuperscript{15,29,30} A log-normal distribution was used when needed to account for the size distribution of concentric holes. Vesicles were fitted with a form factor for spherical shells with a log-normal size distribution. Small angle neutron scattering patterns were also obtained under a 8 T magnetic field at a detector distance of 6 m to evaluate the degree of alignment of the polymolecular assemblies at 5 °C or with changing temperature either 0.1 or 1 °C/min. The degree of alignment of the bicelles was quantified by computing alignment factors \( A_f \) from the 2D SANS patterns.
3 Materials and Methods

3.8 Surface Pressure/Molecular Area Isotherms

The surface pressure/molecular area isotherms of DMPC/Chol-NH$_2$ and DMPC/Chol-OH monolayers were measured at the air/water interface on a KSV-NIMA ISR Langmuir trough of 533 cm$^2$ equipped with two moving barriers, a surface pressure microbalance and a platinum plate. The sub-phase consisted of a 50 mM phosphate buffer at a pH value of 7 prepared at room temperature from MilliQ water. The temperature was controlled with the integrated water cooling system of the trough connected to a water bath. 1 mg/ml stock solutions of DMPC/sterol in chloroform were prepared with sterol contents of either 0, 10, 16, 20, 30, 40 or 100 mol%. 50 µL were spread dropwise on the surface of the sub-phase when the temperature reached 5 °C using a 100 µL glass Hamilton syringe equipped with a metal needle with a diameter of 0.5 mm. The lipid-containing chloroform droplets were deposited on the sub-phase and never dropped from above to avoid lipidic material entering the sub-phase. For the same reason, the metal tip of the needle never touched the surface of the sub-phase. The monolayer was incubated for 15 min at a constant area of approximately 150 Å$^2$/molecule to ensure the solvent fully evaporated and a uniform lipid distribution remained. The monolayer was then compressed at a rate of 100 mm/min until a clear collapse of the monolayer was observed. All experiments were performed at least three times from fresh solutions and the average isotherms were calculated. The trough and the platinum plate were fully washed and dried with isopropanol, MilliQ water and phosphate buffer after each experiment.

3.9 Monolayer Studies with Infrared Reflection-Absorption Spectroscopy (FT-IRRAS)

The monolayer measurements with FT-IRRAS presented in chapter 6 were conducted at the Max-Planck-Institute of Colloids and Interfaces in Golm, Germany, as described by Tanasescu et al.$^{54}$ The setup consisted of a Vertex 70 FT-IR spectrometer (Bruker, Ettlingen, Germany) and a film balance (RK, Potsdam, Germany). The film balance was shielded from the surroundings in a plastic container (external air/water reflection unit XA-511, Bruker) and contained a sample trough with two movable barriers and a reference trough for measurements of the pure subphase. A shuttle technique enabled the fast recording of both the sample and reference spectra. The infrared light may be polarized in either the parallel (p) or perpendicular (s) direction via a KRS-5 wire grid.
polarizer. Reflectance-absorbance spectra were gained by using

$$- \log \left( \frac{R}{R_0} \right)$$

where \( R \) is the reflectance of the monolayer to be measured in the sample trough and \( R_0 \) is the reflectance of the same subphase in the reference trough. For each spectrum, 200 scans (s-polarized light) or 400 scans (p-polarized light) were recorded. The spectra obtained with s-polarized light and at an incidence angle of 40° were used for data analysis.

### 3.10 Molecular Dynamics (MD) Simulation

A MD simulation was conducted *in silico* for the DMPC/steroid and the DMPC/steroid/DMPE-DTPA/Tm\(^{3+}\) bilayers in chapter 6 in collaboration with L. Schuler from Xirrus GmbH. The simulation conditions were chosen to best imitate the bicelle environment with a 50 mM phosphate buffer at a pH value of 7, see Table 3.3. GROMOS force-field 54a7 parameters for DPPC headgroups were employed. Convenient charge group derivations for the phosphate groups were applied according to Junker et al.\(^{135}\) The Gromacs software package (version 4.5.x) was used on an Intel Xeon multicore machine in parallel.\(^{136}\) Typical conventions of GROMOS were followed including: leap-frog integration schemes with a time-step of 2 fs, Berendsen thermostat (\( \tau = 0.1 \) ps) and pressure coupling (\( \tau = 0.5 \) ps). The long-range electrostatics PME was applied with the split between real and reciprocal Fourier space set to 1.0 nm.\(^{137}\) Molecular structures were sketched in Avogadro,\(^{138}\) minimized and exported to MDL mol format. The topology was generated for all bonds, angles, exclusions, improper dihedrals and proper dihedral angles according to the force-field. The employed DMPC structure was proposed by Tieleman et al.\(^{139,140}\)

In a first step, the pure DMPC bilayer was equilibrated for 1 ns at 30 °C with both water and buffer molecules in the surroundings. The 20 steroid molecules were then introduced in the bilayer with one perturbation by slow growth from dummy atoms to the correct atom types. The primary amine headgroup of Chol-NH\(_2\) is expected to be mainly protonated at the studied pH,\(^{88,141,142}\) Protonated Lysine was employed as a basis for the simulation. 20 water molecules were replaced with Cl\(^-\) ions to act as counter-ions. The exact molecular composition of the simulated DMPC/steroid bilayer is presented in Table 3.3.

The simulation was performed for a total of 142 ns at 5 °C and 82 ns at 40 °C. The difference in simulation time between the two studied temperatures originates from the different speeds at which the modelled bilayers reach a steady state. The simulation was performed for another 30 ns at both temperatures to guaranty sufficient statistics. These last 30 ns of simulation were employed for computing the results and comparing the two steroid doped bilayers.
### 3 Materials and Methods

#### Table 3.3 Nature and number of the investigated molecules and ions in the DMPC/Chol-OH and DMPC/Chol-NH$_2$ MD simulations.

<table>
<thead>
<tr>
<th>Number of simulated Steroid in the DMPC/steroid bilayer molecules and ions</th>
<th>Chol-OH</th>
<th>Chol-NH$_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMPC</td>
<td>128</td>
<td>128</td>
</tr>
<tr>
<td>Chol-OH</td>
<td>20</td>
<td>-</td>
</tr>
<tr>
<td>Chol-NH$_2$</td>
<td>-</td>
<td>20</td>
</tr>
<tr>
<td>H$_2$PO$_4^-$</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>HPO$_4^{2-}$</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Na$^+$</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>H$_2$O</td>
<td>10415</td>
<td>10395</td>
</tr>
<tr>
<td>Cl$^-$</td>
<td>-</td>
<td>20</td>
</tr>
</tbody>
</table>

The DMPE-DTPA/Tm$^{3+}$ phospholipid-lanthanide complex was first modelled alone before incorporating it into the bilayer. Although the chemical structure of the phospholipid was readily constructed, the thulium ion was never applied in molecular simulation within the GROMOS force-field. Therefore, the Lennard-Jones parameters of the thulium ion were estimated. Thulium has been simulated in its ionic form Tm$^{3+} + 3$Cl$^-$ in a cubic box containing 3005 simple point charge (SPC) water molecules. The salt was included in a system imitating the final complex with DTPA$^{5-}$ and 5 NH$_4^+$ in a cubic box containing 2984 SPC-water molecules. The final lipid was then introduced as DMPE-DTPA$^{5-}$/Tm$^{3+}$ and its 5 NH$_4^+$ and 3 Cl$^-$ counter-ions with 2949 SPC-water molecules. The radial distribution and the coordination numbers were cross-checked to guarantee reasonable characteristics in the simulation. Although a convincing model was obtained with the salt and the simplified DTPA chelator, the high frequency of reorientation of the amide bond in the DMPE-DTPA molecule caused regular loss of contact with the thulium ion. For the purpose of this simulation, the stability of the DMPE-DTPA/Tm$^{3+}$ complex was artificially enhanced by introducing additional bonds between the ion and the DTPA ligands. These bonds were set to a length of 0.26 nm based on the energy minimization with Avogadro applying a universal force field and a force-constant of $6.0 \times 10^6$ kJ/mol$^{-1}$nm$^{-4}$. The chelator geometry was determined based on a simulated DTPA/Tm$^{3+}$ complex at an energy minimum. The coordination of the resulting DMPE-DTPA/Tm$^{3+}$ chelator was evaluated in aqueous solution before introducing it into the DMPC/steroid bilayers.

The DMPC/steroid/DMPE-DTPA/Tm$^{3+}$ bilayer simulations were conducted analogously to DMPC/steroid. A molar ratio of components of 16:4:5:5 was required to imitate the bicelle bilayer. However, DMPE-DTPA/Tm$^{3+}$ species are believed to accumulate at the edges of the bicelles. Since we aim to simulate the planar part of the bicelle, only six of the modelled DMPE-DTPA/Tm$^{3+}$ molecules were introduced into the bilayer. An additional 12 steroid molecules were introduced to guarantee a compar-
3.10 Molecular Dynamics (MD) Simulation

able phospholipid to steroid ratio. The exact molecular composition of the simulated DMPC/steroid/DMPE-DTPA/Tm$^{3+}$ bilayer is presented in Table 3.4.

**Table 3.4** Nature and number of the investigated molecules and ions in the DMPC/Chol-OH/DMPE-DTPA/Tm$^{3+}$ and DMPC/Chol-NH$_2$/DMPE-DTPA/Tm$^{3+}$ MD simulations.

<table>
<thead>
<tr>
<th>Number of simulated molecules and ions</th>
<th>Steroid in the DMPC/steroid/DMPE-DTPA/Tm$^{3+}$ bilayer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chol-OH</td>
</tr>
<tr>
<td>DMPC</td>
<td>128</td>
</tr>
<tr>
<td>Chol-OH</td>
<td>32</td>
</tr>
<tr>
<td>Chol-NH$_2$</td>
<td>-</td>
</tr>
<tr>
<td>H$_2$PO$_4^-$</td>
<td>6</td>
</tr>
<tr>
<td>HPO$_4^{2-}$</td>
<td>4</td>
</tr>
<tr>
<td>Na$^+$</td>
<td>14</td>
</tr>
<tr>
<td>H$_2$O</td>
<td>10397</td>
</tr>
<tr>
<td>Cl$^-$</td>
<td>18</td>
</tr>
<tr>
<td>NH$_4^+$</td>
<td>30</td>
</tr>
<tr>
<td>DMPE-DTPA$^{5-}$/Tm$^{3+}$</td>
<td>6</td>
</tr>
</tbody>
</table>
4 Fabrication Procedures for Highly Magnetically Responsive Assemblies

The reported fabrication procedure for DMPC/DMPE-DTPA/Ln$^{3+}$ bicelles is similar to existing protocols for the formation of large unilamellar vesicles. This process involves freeze thawing cycles followed by multiple extrusion steps once the dry lipid film has been hydrated as shown Figure 4.1 (top) and Figure 3.1 in the methods section. We explore the possibility of forming large self-assembled polymolecular structures through removal of the extrusion step. In a first step, we investigate the nature of the structures after hydration of the dry lipid film with consecutive freeze thawing cycles for...
two lipid systems: DMPC/DMPE-DTPA/Tm$^{3+}$ (molar ratio 4:1:1) and DMPC/Chol-OH/DMPE-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5). The necessity of freeze thawing will be questioned through the evaluation of a simplified procedure for the hydration of the dry lipid film of DMPC/Chol-OH/DMPE-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5). The procedure involves a combination of heating and cooling cycles from 5 to 60 °C at 1 °C/min. In a further step, the possibility of extruding the DMPC/Chol-OH/DMPE-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5) at 60 °C through polycarbonate membranes of various pore sizes.
will be investigated as a means of controlling the bicelle dimensions and hence their magnetic alignability. Through controlled extrusion conditions, we expand the available possibilities for tailoring the size and magnetic response of DMPC/DMPE-DTPA/Ln$^{3+}$ based bicelles. The success of this method relies on the unique thermo-reversible nature of these systems; forming vesicles when working above the phase transition temperature $T_m$ of the lipids composing the bilayer.\textsuperscript{16} Magnetically alignable bicelles are readily regenerated upon cooling below $T_m$ in a size range dictated by the vesicle precursors. This possibility is, for example, not applicable for the famous DMPC/DHPC bicelles, whose size and magnetic response is commonly tailored by changing the lipid ratio or total concentration.\textsuperscript{12,13,35} The adopted structure of the investigation is schematically summarized in Figure 4.1. The proposed optimized fabrication procedures contribute to the enrichment of the toolbox for intelligent bicelle design. Furthermore, we aim to considerably enhance the magnetic response of DMPC/DMPE-DTPA/Ln$^{3+}$ based soft materials and widen the frame of possibilities for future applications, moving onto the third level of the S-PRO\textsuperscript{2} scheme in Figure 1.1.

For the purpose of this chapter, the previously developed bicelle fabrication procedure presented in the methods section 3.2 was expanded as follows: the dry lipid film was rehydrated with 50 mM sodium phosphate buffer at a pH value of 7.5 following either the freeze thawing or the heating and cooling procedure. The freeze thawing procedure involved five consecutive freeze-thaw cycles composed of: freezing the sample in liquid nitrogen and heating it back to 60 °C. After the last freezing, the sample was left to warm up to room temperature. The heating and cooling procedure involved cooling the sample down to 5 °C after the phosphate buffer was added. The sample was then heated to 60 °C and cooled back to 5 °C with a temperature gradient of 1 °C/min. This process was repeated a second time and the samples were stored at room temperature before proceeding to further measurements. Two minutes of vortexing were applied when the sample reached the maximum and minimum temperature of the heating and cooling cycle, respectively. When required, the samples were extruded 10 times through a polycarbonate membrane with a define pore size. Membranes with diameters of 800, 400, 200 and 100 nm were employed. All extrusions were conducted at 60 °C to ensure the sample is fully composed of vesicles.\textsuperscript{16}
4 Fabrication Procedures for Highly Magnetically Responsive Assemblies

4.1 DMPC/DMPE-DTPA/Tm$^{3+}$ (molar ratio 4:1:1) assemblies

Removal of the extrusion steps in the fabrication procedure of DMPC/DMPE-DTPA/Tm$^{3+}$ (molar ratio 4:1:1) bicelles opens the possibility of generating larger and more alignable polymolecular assemblies. In a first stage, the attainable architectures were investigated by cryo-TEM and DLS. Their magnetic alignment was quantified and compared with the first generation of extruded bicelle systems. In order to further comprehend the origin of the polymolecular rearrangements occurring upon changing temperature and their impact on the sample’s magnetic alignment, complementary analytical methods were employed including SANS, birefringence, cryo-TEM, and spectrophotometry.

The cryo-TEM micrographs of a DMPC/DMPE-DTPA/Tm$^{3+}$ (molar ratio 4:1:1) sample at 5°C after freeze thawing in Figure 4.2A revealed a multitude of bicelles (red arrows) in the range of 20 – 70 nm in diameter along with larger species with folded and twisted sheet-like structures (blue arrows). The polymolecular assemblies are mainly composed of smaller structures with a mean hydrodynamic diameter $D_H$ of 68 nm as revealed by the number distribution obtained from DLS measurements of the sample at 5°C in Figure 4.2B. The intensity distribution confirms the presence of larger entities with a $D_H$ of 500 nm. In comparison, previously reported DMPC/DMPE-DTPA/Tm$^{3+}$ (molar ratio 4:1:1) bicelles displayed a $D_H$ of 34 nm. Therefore, removal of the extrusion step allows for the successful spontaneous formation of larger polymolecular assemblies that have the potential to deliver enhanced magnetic alignments.

SANS measurements at 5°C and under a 8 T magnetic field were employed to compute the alignment factor $A_f$. $A_f$ allows to quantify the sample’s alignment and ranges from -1 (scattering is parallel) to 0 for isotropic scattering. The DMPC/DMPE-DTPA/Tm$^{3+}$ (molar ratio 4:1:1) sample revealed an $A_f$ of -0.3, which is two-fold greater than the first generation of extruded bicelles. Nevertheless, the non-planar nature of the larger assembly structures hinders the individual contributions of the constituting lipid’s magnetic energy, reducing the overall magnetic energy of the assemblies and the sample’s magnetic alignment. The twists and folds present in their structure results in differing orientations of the composing lipid’s molecular axis. The cumulative magnetic energy of the composing lipids is reduced by the opposing directional components of the magnetic force acting on the individual lipids. Consequently, the magnetic alignment of the associated polymolecular assembly will be lower than that of a bicelle architecture with an equivalent planar surface area.
4.1 DMPC/DMPE-DTPA/Tm$^{3+}$ (molar ratio 4:1:1) assemblies

Regardless of the higher complexity of the non-extruded DMPC/DMPE-DTPA/Tm$^{3+}$ (molar ratio 4:1:1) systems, the change in alignment factor as a function of temperature under a 8 T magnetic field reveals a similar trend to what was observed in the previously extruded systems.$^{30}$ The results presented in Figure 4.3A show an enhanced $A_f$ between 10 and 20 °C before a thermo-reversible collapse of the magnetically alignable polymolecular assemblies into non-alignable vesicles occurs between 22 and 24 °C. This temperature range corresponds to the phase transition temperature $T_m$ of DMPC from the solid ordered to the liquid disordered phase. The characteristic evolution of the

Figure 4.2  A) Cryo-TEM micrographs of DMPC/DMPE-DTPA/Tm$^{3+}$ (molar ratio 4:1:1, [tL] 15 mM) at 5 °C after freeze thawing. Large sheet-like structures with folds and twists are indicated with blue arrows. They are surrounded by numerous smaller bicelles shown with red arrows. The scale bar represents 200 nm. B) Intensity (red) and number (black) distribution of the sample obtained from DLS at 5 °C. Figure adapted from S. Isabettini et al.$^{125}$
DMPC/DMPE-DTPA/Tm\(^{3+}\) (molar ratio 4:1:1) system’s alignment factor with temperature is dictated by the nature of the phospholipids composing the bilayer. Solely the magnitude of the alignment is influenced by the fabrication procedure. We further investigate the phenomena occurring in the polymolecular assembly’s bilayer and structure upon changes in temperature with cryo-TEM, birefringence, and spectrophotometry.

**Figure 4.3** A) Alignment factor \(A_f\) as a function of temperature on heating for DMPC/DMPE-DTPA/Tm\(^{3+}\) (molar ratio 4:1:1, [tL] 15 mM) after freeze thawing under a 8 T field. B) Birefringence signal as a function of temperature for the same sample under a 5.5 T field (filled lines) and absorbance as a function of temperature in the absence of a magnetic field (dashed lines). The data recorded on heating is shown in red and on cooling in blue. \(T_m\) corresponds to the phase transition temperature of DMPC.

The sample’s birefringence signal under a 5.5 T magnetic field was employed as a complementary method to monitor magnetic alignment.\(^{16,105}\) The signal was monitored during a heating and cooling cycle from 5 to 40 °C and back at a rate of 1 °C/min. Unlike for the alignment factor computed from SANS scattering patterns, birefringence is also sensitive to polymolecular rearrangements occurring in the bilayer.\(^{29}\) Therefore, the birefringence signal originating from changes in the sample’s magnetic alignment may be decoupled from the signal caused by molecular rearrangements in the bilayer. This is done by comparing how the \(A_f\) and birefringence signal evolved with temperature in Figure 4.3A and 4.3B, respectively. The birefringence results confirm the higher magnetic alignment at 5 °C with a value of 1.17x10\(^{-5}\), which is almost twice as strong as for the reported extruded systems.\(^{30}\) The zeroing of the birefringence signal above 24 °C supports the formation of non-alignable structures. This temperature corresponds to the \(T_m\) of DMPC, which acted as a trigger point for major rearrangements in the polymolecular assemblies. These transformations are thermo-reversible as alignable species are regenerated upon cooling below \(T_m\) where the birefringence signal follows the same trend as on heating. The distinct peaks occurring around \(T_m\) are characteristic of the DMPC/DMPE-DTPA/Tm\(^{3+}\) (molar ratio 4:1:1) assemblies. These peaks
cannot be caused by a sudden enhancement of the sample’s magnetic alignability as the A_f collapses in this temperature range. Instead, the peaks suggest the presence of a transition structure from the planar alignable polymolecular assemblies below T_m to the non-alignable structures present above T_m. The slow kinetics of the molecular rearrangements with respect to the applied heating and cooling rate of 1 °C/min could explain why the peaks are not overlapping. Nevertheless, both peaks start around the T_m of DMPC, suggesting that the bilayer lipids must have a certain degree of order to favor the formation of alignable polymolecular assemblies.

The flattening of the birefringence curve at around 10 °C in Figure 4.3A is correlated to the sudden enhanced sample alignment observed in SANS at the equivalent temperature in Figure 4.3B. Intermediate ripple phases occurring within the bilayer are proposed to be at the origin of this phenomenon. Spectrophotometry experiments were conducted in the absence of a magnetic field. A heating and cooling gradient of 1 °C/min was applied and the change in sample absorbance was monitored from 5 to 50 °C and back for a DMPC/DMPE-DTPA/Tm^3+ (molar ratio 4:1:1) sample after freeze thawing. To facilitate comparison with the birefringence results, the absorbance curves are also presented in Figure 4.3B with dashed lines. The large changes in absorbance occurring around the T_m of DMPC supports the presence of major polymolecular rearrangements in the sample. A variation in the composing lipid’s miscibility may be the driving force behind these structural changes. Such phenomena have been reported in DHPC/DMPC bicelles subject to higher temperatures where perforated and unperforated vesicles exist at 45 and 55 °C, respectively. The sudden collapse in absorbance upon heating the DMPC/DMPE-DTPA/Tm^3+ (molar ratio 4:1:1) bicelles could suggest an analogous process with the appearance of numerous perforations in the bilayer. Such structures are called Stomatosomes, identified in a number of amphiphilic lipid systems with fluid bilayers. They commonly induce similar drops in light absorbance. Therefore, stomatosomes could be possible transition structures around the T_m of DMPC. The simultaneous sudden increase in birefringence signal goes in line with the presence of these perforated structures that could enhance the form-birefringence as proposed by M. Liebi et al.29
Figure 4.4 A) Absorbance as a function of temperature in the absence of a magnetic field for DPPC/DPPE-DTPA/Tm$^{3+}$ (molar ratio 4:1:1, [tL] 15 mM) assemblies after freeze thawing. The data recorded on heating is shown in red and on cooling in blue at 1 °C/min. B) Birefringence signal of DPPC/DPPE-DTPA/Tm$^{3+}$ (molar ratio 4:1:1, [tL] 15 mM) assemblies as a function of temperature in a 5.5 T magnetic field. The heating and cooling cycle was conducted at 1 °C/min after the sample was coming out of the last freeze thawing step. Cryo-TEM micrographs of DPPC/DPPE-DTPA/Tm$^{3+}$ (molar ratio 4:1:1, [tL] 15 mM) assemblies produced by freeze thawing and a subsequent heating and cooling cycle, flash-frozen at C) 5 °C and D) 43 °C on heating. A multitude of complex assembly structures were distinguishable in the cryo-TEM micrograph in C) including large sheets, ribbons and partially folded planar assemblies. In the cryo-TEM micrograph D) vesicles were mainly observed along with an asymmetric vesicle with lumps in its shell-structure (red arrow) and a partially formed vesicle (blue arrow). The scale bars represent 200 nm.
4.2 DMPC/Chol-OH/DMPE-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5) assemblies

Building from our success in generating more alignable polymolecular assemblies with DMPC/DMPE-DTPA/Tm$^{3+}$ (molar ratio 4:1:1), we now introduce Chol-OH in the phospholipid bilayer to create larger and more magnetically responsive temperature-resistant assemblies. Chol-OH, with its inverse cone-like molecular geometry, acts to reduce curvature in the DMPC/DMPE-DTPA/Tm$^{3+}$ bilayer. This results in larger bicelles with enhanced magnetic alignability. The replacement of a clear transition temperature from the solid ordered to the liquid disordered phase allows DMPC/Chol-OH/DMPE-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5) bicelles to exist at temperatures well above the T$_m$ of DMPC. Magnetically alignable bicelles were reported at temperatures as high as 40 °C. To generate these materials, we further optimized the fabrication procedure by replacing the freeze thawing cycles with heating and cooling cycles in combination with vortexing. This procedure was directly inspired from commonly reported fabrication protocols of DHPC/DMPC bicelles and is expected to favor the self-assembly procedure. In a first stage, the polymolecular assemblies formed by
the DMPC/Chol-OH/DMPE-DTPA/Tm$^{3+}$ system are compared after hydration of the dry lipid film following either the freeze thawing or the heating and cooling procedure (1 °C/min). Once the most promising procedure identified, the system’s response to temperature is determined to identify the driving forces behind spontaneous formation of the polymolecular assemblies. The possibility of further tailoring their dimension by extrusion is addressed in a final stage.

4.2.1 Evaluation of the fabrication procedures for DMPC/Chol-OH/DMPE-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5)

The intensity and number distributions of the DMPC/Chol-OH/DMPE-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5) system produced by freeze thawing cycles were determined by DLS measurements at 5 °C and presented in Figure 4.5A. The sample reveals a bimodal distribution with a large population of smaller species in the 100 nm range. This size distribution is similar to the one observed for the cholesterol-free DMPC/DMPE-DTPA/Tm$^{3+}$ (molar ratio 4:1:1) system in Figure 4.2B. When applying the heating and cooling procedure, the bimodal distribution is replaced with a highly polydisperse distribution as shown in Figure 4.5B. The intensity distribution reveals an average hydrodynamic diameter $D_H$ of 712 nm and the number distribution suggests a population dominated by species in the size range of 220 nm. The two procedures result in contrasting populations of polymolecular assembly sizes. Nevertheless, they both result in unprecedented large structures, which foresees large gains in magnetic response.

The DMPC/Chol-OH/DMPE-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5) system’s $A_f$ is shown as a function of the magnetic field strength for both fabrication procedures in Figure 4.6A. The sample produced by the freeze thawing procedure reveals an $A_f$ of -0.9 at 8 T. In comparison, the reported extruded system had an $A_f$ of -0.35 at equivalent conditions. The same $A_f$ was achieved at magnetic field strengths below 2 T at 5 °C in the non-extruded system. Furthermore, the $A_f$ curve tends towards a plateau when reaching 8 T, indicating that the system was reaching full alignment. This saturation effect has only been observed for the former extruded sample by birefringence measurements at 33 T. When increasing the temperature to 40 °C, the DMPC/Chol-OH/DMPE-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5) sample produced by the freeze thawing procedure remained alignable as revealed by the red data points in Figure 4.6A. A maximum $A_f$ of -0.35 is achieved at 8 T and 40 °C. Such a degree of alignment is equivalent to what could be achieved with the former extruded system at 5 °C. The overall magnetic energy of the large polymolecular assemblies formed after the freeze thawing procedure is able to withstand the considerable amount of thermal energy acting against it at 40 °C and maintain a respectable magnetic response.

The DMPC/Chol-OH/DMPE-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5) sample produced by the heating and cooling procedure shows a similar behavior to the one produced by
freeze thawing as shown by the black data points in Figure 4.6A. However, the curve is shifted towards lower alignment factors with a maximum $A_f$ of -0.85 at 5 °C and 8 T. The DMPC/Chol-OH/DMPE-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5) sample produced by the freeze thawing procedure shows Bragg peaks on its 2D SANS scattering pattern at 8 T as indicated with white arrows in Figure 4.6B. These peaks appear already at low magnetic field strengths of 1 T and reach a maximum intensity at 2.5 T. They correspond to a characteristic length of 17.5 nm and may result from packing of the aligned polymolecular assemblies. Only part of the species are stacked based on the reported scatter patterns. Nevertheless, stacking increases the aggregate number $n$ that, in turn, enhances the cumulative magnetic energy of the assemblies and promotes a high magnetic response of the system. The sample produced by the heating and cooling procedure does not show any Bragg peaks as evidenced in Figure 4.6C. The absence of stacking may explain the weaker $A_f$ achieved by this sample when comparing to the one produced by freeze thawing.

When an additional heating and cooling cycle was applied to the DMPC/Chol-OH/DMPE-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5) sample prepared by freeze thawing, the $A_f$ fell and
**Figure 4.6** A) Alignment factor $A_f$ as a function of magnetic field strength for DMPC/Chol-OH/DMPE-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5, [tL] 15 mM) produced by either the freeze thawing (FT) procedure at 5 °C (blue squares) and at 40 °C (red triangles) or by the heating and cooling (H&C) procedure at 5 °C (black circles). 2D SANS scatter patterns of the sample at 5 °C and 8 T produced by B) the freeze thawing or C) the heating and cooling procedure. The Bragg peaks are shown with arrows in B. Figure partially adapted from S. Isabettini *et al.*

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**4 Fabrication Procedures for Highly Magnetically Responsive Assemblies**
matched the sample prepared by the heating and cooling procedure and the Bragg peaks disappeared. The sample had to be more closely monitored to comprehend the phenomena occurring during the heat treatment and hypothesize on the underlying mechanisms responsible for the spontaneous formation of the magnetically responsive species. This was achieved by monitoring the system as it came out of the last freezing cycle and was heated to 50 °C before cooling back to 5 °C. This was done in the absence of a magnetic field by cryo-TEM and in the presence of a 5.5 T field with birefringence measurements in Figure 4.7.

A DMPC/Chol-OH/DMPE-DTPA/Tm\(^{3+}\) (molar ratio 16:4:5:5) sample at 5 °C was studied by cryo-TEM in Figure 4.7A as it came out of the last freezing stage of the freeze thawing procedure. Long ribbon-like structures were observed. The sample was further heated to 50 °C and cooled back to 5 °C before again observed by cryo-TEM as shown in the micrographs of Figure 4.7B. After this temperature treatment, the sample was composed of a polydisperse population of bicelles in the size range of 200 nm in diameter. The heating and cooling cycle allowed for the generation of large bicelles, responsible for the strong alignment in the presence of an external magnetic field. The magnetic alignment of the sample was monitored by birefringence measurements under a 5.5 T field as it underwent two consecutive heating and cooling cycles coming out of the last freezing stage of the freeze thawing procedure. The results from the first and second cycles are presented in red and black respectively in Figure 4.7C. The birefringence signal increased two-fold at 5 °C after the first cycle. The corresponding increase in magnetic alignment may be attributed to the formation of large bicelles starting from the ribbon-like structures as revealed by the cryo-TEM micrographs in Figure 4.7A and 4.7B. The enhanced magnetic response of a bicelle structure when compared to a ribbon of equivalent surface area comes from less edge-area in the former. Consequently, more phospholipids are located in the planar region of the polymolecular assembly, capable of contributing to the cumulative magnetic energy \(E_{\text{mag}}\) of the bilayer. A distinct peak in the birefringence signal started to appear at 28 °C on heating, which marked a change in lipid arrangement in bilayer. This peak is similar to what was observed for the cholesterol-free DMPC/DMPE-DTPA/Tm\(^{3+}\) (molar ratio 4:1:1) system in Figure 4.3B when exceeding the \(T_m\) and moving into the liquid disordered state of the lipids. A similar mechanism could be responsible for the peak at 28 °C in the DMPC/Chol-OH/DMPE-DTPA/Tm\(^{3+}\) (molar ratio 16:4:5:5) system. This temperature corresponds to the phase boundary between the semi-ordered state and fully disordered state of a DMPC bilayers with 16 mol% Chol-OH.\(^72\) Upon further heating, the birefringence signal gradually decreased until all bicelles rearranged into vesicles at a temperature of 50 °C where all alignment was lost. In the second cycle, a clear hysteresis occurred in the birefringence signal upon heating and cooling. M. Liebi \textit{et al.} reported this phenomenon for DMPC/Chol-OH/DMPE-DTPA/Tm\(^{3+}\) (molar ratio 16:4:5:5) bicelles.\(^{16,29}\) The appearance of the hysteresis originated from large holes in the bicelle’s bilayer that form only upon heating. The peak in the birefringence signal at 28 °C remains visible in the hysteresis envelope. The birefringence signal evolves in the same way with temperature for both cooling cycles, suggesting that the determining parameter for lipid rearrangements responsible for the successfully formation of highly
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Figure 4.7 Cryo-TEM micrographs of DMPC/Chol-OH/DMPE-DTPA/Tm\(^{3+}\) (molar ratio 16:4:5:5, [tL] 15 mM) at 5 °C A) after melting out of the last freeze thawing cycle and B) after having been heated to 50 °C and cooled back to 5 °C. Long ribbon-like structures are evident in A), whereas bicelle structures are present in B). Each scale bar represents 200 nm. C) Birefringence signal as a function of temperature at 5.5 T of DMPC/Chol-OH/DMPE-DTPA/Tm\(^{3+}\) (molar ratio 16:4:5:5, [tL] 15 mM). Two consecutive heating and cooling cycles are reported. Heating is represented with full lines and cooling with dashed lines. In the first cycle (red lines), the sample melted out of the last freeze thawing cycle. Arrows indicate the direction of temperature change. The schematic representations of the polymolecular assembly geometries are shown where appropriate. The ribbons rearranged into vesicles upon heating and bicelles were formed when cooling back to 5 °C (first cycle, red lines). Bicelles with holes were a likely cause of the hysteresis on heating observed in the second cycle (black lines). Figure adapted from S. Isabettini et al.\(^{125}\)
4.2 DMPC/Chol-OH/DMPE-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5) assemblies

magnetically alignable structures occurs on heating. The movement of the lipids from a fully disordered state towards more order upon cooling allows the system to rearrange and form bicelles. Consequently, the freeze thawing procedure is not necessary and may be replaced with the proposed heating and cooling procedure. The latter is sufficient to guaranty the successful formation of highly magnetically responsive soft materials.

In order to ascertain the value of the procedure and increase the versatility of DMPC/Chol-OH/DMPE-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5) systems, we evaluated the possibility of regenerating the samples after one month of storage frozen at -18°C. The sample underwent the same treatment and behaved in the same way as the sample freshly coming out of the last freeze thawing cycle in Figure 4.7. Consequently, the proposed heating and cooling procedure was sufficient to guaranty both the successful formation and regeneration of the highly magnetically responsive species.

4.2.2 Tailoring the size and magnetic response of DMPC/Chol-OH/DMPE-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5) bicelles by extrusion

The highly alignable species discussed up until now could be useful tools for the study of membrane or membrane-associated proteins by NMR spectroscopy. However, due to the large diversity of experimental techniques in this field, bicelle constructions displaying either weak, partial or strong magnetic alignment are required. The ability of tailoring the bicelle dimensions is essential to determine their behavior in the presence of a magnetic field. We propose an alternative means of doing so with the DMPC/Chol-OH/DMPE-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5) systems based on extrusion. The success of this method relies on the system’s capacity of forming vesicles at temperatures above the phase transition temperature $T_m$ of the composing bilayer lipids. These vesicles were then repeatedly extruded through polycarbonate membranes of various dimensions in order to tailor their size. Bicelles of corresponding sizes were readily obtained by cooling the extruded vesicle suspension to temperatures below $T_m$ and exploiting the thermo-reversible nature of the polymolecular rearrangements.

The same DMPC/Chol-OH/DMPE-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5) sample was hydrated following the heating and cooling procedure and subsequently extruded 10 times at 60 °C through polycarbonate membranes of different pore sizes. After completing the extrusion, the hydrodynamic diameter $D_H$ of the bicelles was measured by DLS at 5 °C. Furthermore, their magnetic alignability was evaluated at 5 °C by computing the $A_f$ in SANS at 8 T and measuring the birefringence signal at 5.5 T as shown in Figure 4.8. The $D_H$ of the bicelles was reduced to 220, 190, 106 and 91 nm by successive extrusions through membranes with pore sizes of 800, 400, 200 and 100 nm respectively. The corresponding decrease in magnetic alignment is confirmed by the decreasing birefringence signal and the reduction in the absolute value of the $A_f$ as it approached zero. The results confirm the possibility of controlling the bicelle’s size and magnetic alignment through tailoring of the vesicles by extrusion at 60 °C and cooling back to 5 °C.
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**Figure 4.8** Alignment factor $A_f$ at 8 T (red circles) and birefringence signal (black squares) at 5.5 T of DMPC/Chol-OH/DMPE-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5, [tL] 15 mM) bicelles as a function of the hydrodynamic diameter $D_H$. All measurements were performed at 5 °C. The bicelles were formed by successive extrusions through membranes with pore sizes of 800, 400, 200 and 100 nm at 60 °C. Figure adapted from S. Isabettini et al.\textsuperscript{144}

4.3 Conclusion

Polymolecular assemblies displaying unprecedented magnetic response were successfully generated for both DMPC/DMPE-DTPA/Tm$^{3+}$ (molar ratio 4:1:1) and DMPC/Chol-OH/DMPE-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5) systems by following simplified fabrication procedures as summarized in Figure 4.9. Hydration of the dry lipid film by freeze thawing could be replaced by simple heating and cooling cycles from 5 to 60 °C. The added value of this simplified fabrication procedure is evident when comparing the magnetic alignment of the resulting polymolecular assemblies with those formed following the previously reported fabrication procedure in Figure 4.9B. The heating and cooling procedure allowed for a 2-fold increase in alignment factor for the DMPC/DMPE-DTPA/Tm$^{3+}$ (molar ratio 4:1:1) system and a 2.5-fold increase for the DMPC/Chol-OH/DMPE-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5) system at 5 °C and 8 T. The movement of the lipids to the liquid disordered and back to the solid ordered state induced major rearrangements of the polymolecular assemblies. Consequently, going above and below the phase transition temperature $T_m$ was the key parameter responsible for the successful formation of the highly magnetically responsive species. The proposed heating and cooling procedure was ideally suited for these purposes. DMPC/Chol-OH/DMPE-
4.3 Conclusion

Figure 4.9 A) Schematic representation of the polymolecular assemblies obtained by either the freeze thawing (FT) procedure, the heating and cooling (H&C) procedure, or extrusion (Ext) for both the DMPC/DMPE-DTPA/Tm$^{3+}$ (molar ratio 4:1:1, [tL] 15 mM) and DMPC/Chol-OH/DMPE-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5, [tL] 15 mM) systems. The drawings are not to scale. B) The alignment factor $A_f$ at 8 T and 5 °C of the samples prepared following the previously reported fabrication procedure involving freeze thawing cycles (FT) followed by extrusion (Ext) in red and following the new heating and cooling procedure (H&C) in black. Figure adapted from S. Isabettini et al.\textsuperscript{125}  
C) Schematic overview of the possible fabrication pathways available for the production of highly magnetically alignable Ln$^{3+}$ chelating polymolecular assemblies composed of either DMPC/DMPE-DTPA/Tm$^{3+}$ (molar ratio 4:1:1, [tL] 15 mM) or DMPC/Chol-OH/DMPE-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5, [tL] 15 mM). Although, the FT cycles are not obligatory for the effective hydration of the dry lipid film, they are comparatively faster when liquid nitrogen (LN$_2$) is available. H&C cycles are sufficient for either hydrating the dry lipid film, regenerating a sample coming out of the last freeze thawing step, or regenerating a sample kept frozen over a prolonged period of time. Maximally alignable polymolecular assemblies are achieved after the H&C cycles for both systems delivering different assembly architectures based on the lipid composition. The bicelle size and magnetic alignability may be further tailored downwards by extrusion through nanopore polycarbonate membranes. Figure adapted from S. Isabettini et al.\textsuperscript{144}
DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5) samples may be stored for prolonged periods of time in a frozen state, similarly to known bicelle systems. They were readily regenerated by following the same heating and cooling procedure initially employed to create them from a dried lipid film.

DMPC/DMPE-DTPA/Ln$^{3+}$ bicellar systems offer numerous advantages over the commonly employed DMPC/DHPC bicelles. They are more resistant to dilution, being composed of phospholipids with an equivalent chain length of 14 carbons (myristoyl tail). In DMPC/DHPC bicelle systems, DHPC is readily soluble in the aqueous environment and will migrate out of the polymolecular assemblies resulting in the loss of their structural integrity upon dilution. The ease in fabrication in combination with the enhanced magnetic response makes DMPC/DMPE-DTPA/Ln$^{3+}$ based materials attractive candidates for the study of membrane or membrane-associated proteins by NMR spectroscopy. The possibility of tailoring both the direction of alignment and the magnetic response emphasizes their versatility. The latter was readily achieved with the DMPC/Chol-OH/DMPE-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5) assemblies by extrusion through polycarbonate membranes with a fixed pore size at 60 °C. In these conditions, the bilayer lipids are in a liquid disordered state and the system self-assembles into vesicles that will reassemble into bicelles upon cooling back to 5 °C. The dimensions of the bicelles is indirectly controlled by templating on the dimensions of the vesicles. The resulting alignment factors are summarized in the last column of Figure 4.9A. The numerous advantages offered by these systems including their thermoreversible nature, their magnetic responses at field strengths as low as 1 T, and the freedom in design by introducing Chol-OH or altering the nature of the chelated lanthanide ion, opens doors for numerous alternative applications in the field of smart soft materials, namely for the fabrication of optical gels. Moreover, the potential of bicelles for dermal applications has recently been reviewed and the ability of tailoring DMPC/DMPE-DTPA/Ln$^{3+}$ bicelles through common processing methods, such as extrusion, offers viable pathways for industrial-scale integration of these unique systems.
5 Enhancing the Magnetic Susceptibility of Bicelles with Chol-NH$_2$


Increasing the magnetic response of bicelle systems is key to develop novel smart materials by reducing the required field strength to a commercially more affordable and practical range. The magnetic response of bicelles is commonly enhanced by increasing their size and hence the number of molecules capable of contributing to the cumulative magnetic energy ($E_{mag}$) of the bilayer. Nevertheless, this process involves iterative experimental approaches to optimize the total lipid content or ratio. Such procedures are laborious and the largest achievable polymolecular assembly is intrinsically limited by the geometry of the lipids composing the bilayer. Optimizing the bicelle’s constituents and environment to enhance the assembly’s size does not eliminate the phase boundaries where the lipids self-assemble into other architectures than planar disks. Therefore, it is necessary to develop further means of selectively tuning the magnetic susceptibility of the bicelle without altering their size.
A viable approach to achieve this aim consists of altering the magnetic susceptibility of the lipids composing the bilayer. Bicelles composed of Chol-OH, DMPC and the phospholipid-lanthanide DMPE-DTPA/Ln$^{3+}$ complex, are admirable candidates for tailoring E$_{mag}$ through magnetic susceptibility. They strongly align in magnetic fields by chelating many lanthanide ions on the bilayer’s surface. Chelation of different Ln$^{3+}$ further permits fine-tuning of the magnitude and direction of alignment in the presence of an external magnetic field. The introduction of Chol-OH or Ln$^{3+}$-chelating cholesterol derivatives such as Chol-C$_n$-DTPA (see chapter 7) in the bicelle’s bilayer leads to an increased magnetic alignment when compared to the sterol-free DMPC/DMPE-DTPA/Ln$^{3+}$ bicelle systems. This enhanced magnetic response originates from an increase in bicelle size. However, these techniques have reached their limits for enhancing the magnetic response of bicelles. Means of controlling the bicelle’s magnetic alignment by decoupling the contribution of the magnetic susceptibility to E$_{mag}$ with that of the assembly’s size are lacking and call for further development.

Herein, the magnetic susceptibility of lanthanide-chelating bicelles was selectively enhanced by replacing Chol-OH with 3$\beta$-amino-5-cholestene (Chol-NH$_2$) in the bilayer. The resulting DMPC/Chol-NH$_2$/DMPE-DTPA/Ln$^{3+}$ (molar ratio 16:4:5:5) bicelles delivered unprecedented magnetic alignments without changing in size. Previously reported degrees of alignment at 8 T and 5 °C could be matched with Chol-NH$_2$ doped bicelles at field strengths as low as 4 T. Full alignment was achieved at magnetic field strengths of 8 T. For comparison, the full alignment of the DMPC/Chol-OH/DMPE-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5) bicelles required magnetic field strengths of up to 35 T. The importance of the dopant’s molecular structure was further evaluated with an aminocholesterol conjugate (Chol-C$_2$OC$_2$-NH$_2$), offering the possibility of fine-tuning the bilayer’s magnetic susceptibility and the resulting bicelle’s magnetic alignment. Chol-C$_2$OC$_2$-NH$_2$ was chosen as the primary amine is separated from the steroid backbone by a linker, allowing it to interact differently in the hydrophilic environment.

### 5.1 Incorporating Chol-NH$_2$ and Chol-C$_2$OC$_2$-NH$_2$ in the phospholipid bilayer.

The bicelle samples were prepared as described in the methods section 3.2. They were stored at room temperature and measured within a week from their preparation. The presence of bicelles was confirmed for both systems containing either Chol-NH$_2$ or Chol-C$_2$OC$_2$-NH$_2$ by cryo-TEM, see Figure 5.1A and 5.1B, respectively. The polymolecular assemblies appear as black lines when viewed from side-on, proving their planar nature. The Cryo-TEM micrograph of the DMPC/Chol-NH$_2$/DMPE-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5) bicelle sample presented in Figure 5.1A are shown without artificial contrast enhancement in Figure 5.2A. The sample holder was tilted 65° in Figure 5.2B. The existence of planar bicelle structures is indicated with a red arrow in Figure 5.2A and 5.2B as
5.1 Incorporating Chol-NH$_2$ and Chol-C$_2$OC$_2$-NH$_2$ in the phospholipid bilayer.

the round bicelle seen from top-on view at 0° becomes oval-shaped when viewed at a 65° angle. Moreover, the appearance of bicelles seen from side-on view at 65° is shown with blue arrows in Figure 5.2B. The presence of bicelles is confirmed with another sample in the micrograph of Figure 5.2C measured at a 30° tilt angle. Dark patches are attributed to crystalline water resulting from the flash freezing procedure. The bicelles observed in cryo-TEM are much larger than the sterol-free DMPC/DMPE-DTPA/Tm$^{3+}$ systems and have a similar size to the DMPC/Chol-OH/DMPE-DTPA/Tm$^{3+}$ systems.$^{16,28}$ This supports the successful incorporation of Chol-C$_2$OC$_2$-NH$_2$ and Chol-NH$_2$ within the bicelle’s phospholipid bilayer. These findings were further investigated by birefringence measurements and SANS.

![Figure 5.1](image)

**Figure 5.1** Cryo-TEM micrographs of A) DMPC/Chol-NH$_2$/DMPE-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5, [tL] 15 mM) and B) DMPC/Chol-C$_2$OC$_2$-NH$_2$/DMPE-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5, [tL] 15 mM) flash frozen at 5 °C. Each scale bar represents 200 nm. The contrast of the bicelles in A) was artificially enhanced for clarity. Bicelles viewed top-on are indicated with a black arrow and with a white arrow from side-on. Additional cryo-TEM micrographs at various tilt angles are presented in Figure 5.2. Figure adapted from S. Isabettini et al.$^{90}$

The successful incorporation of Chol-NH$_2$ was confirmed by the disappearance of the transition temperature between the solid-ordered phase and the liquid-disordered phase occurring at 24 °C in steroid-free DMPC/DMPE-DTPA/Ln$^{3+}$ (molar ratio 4:1:1) bicelles.$^{28,84}$ Similarly to Chol-OH, Chol-NH$_2$ induced a liquid-ordered phase in the lipid bilayer. These phenomena were monitored by birefringence measurements of the DMPC/Chol-NH$_2$/DMPE-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5) bicelles under a 5.5 T field. The birefringence signal of the sample was monitored as it underwent a heating and cooling cycle from 5 to 55 °C and back at a rate of 1 °C/min in Figure 5.3. Steroid-free DMPC/DMPE-DTPA/Ln$^{3+}$ (molar ratio 4:1:1) bicelles undergo a thermo-reversible transition into magnetically non-alignable vesicles at the phase transition temperature of DMPC of 24 °C causing a zeroing of the birefringence signal.$^{30}$ When Chol-OH or cholesterol conjugates are present in the bicelle’s bilayer, the clear phase transition temperature of DMPC disappears as a liquid-ordered phase is induced. The bicelles become more resistant to higher temperatures.$^{15,16,20}$ This phenomenon is readily monitored as the collapse of the birefringence signal occurs at higher temperatures and serves as evidence that the Chol-OH or cholesterol conjugates are present in the bilayer. For the
Enhancing the Magnetic Susceptibility of Bicelles with Chol-NH$_2$

Figure 5.2 Cryo-TEM micrographs of DMPC/Chol-NH$_2$/DMPE-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5, [tL] 15 mM) flash frozen at 5 °C with a sample holder tilt angle of A) 0°, B) 65°, and C) 30°. The same sample is shown in A and B. Bicelles from top-on view are labeled with red arrows and from side-on view with blue arrows. The micrograph in C is from another sample. The scale bar represents 200 nm and no artificial contrast enhancement was applied. Dark particles are ice crystals resulting from the flash freezing procedure. Figure adapted from S. Isabettini et al. [90]

DMPC/Chol-NH$_2$/DMPE-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5) sample presented in Figure 5.3, the collapse of the signal occurs at 50 °C on heating and the regeneration at 40 °C on cooling. The existence of alignable species above 24 °C proves the incorporation of Chol-NH$_2$ in the bicelle’s bilayer. Moreover, the thermo-reversible collapse occurs in a similar range to previously reported DMPC/Chol-OH/DMPE-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5) bicelle systems. [2]

Results from fittings of radially averaged SANS curves in Figure 5.4 revealed a similar bicelle size for systems containing Chol-OH, Chol-NH$_2$, or Chol-C$_2$OC$_2$-NH$_2$. The fitting results are summarized in Table 5.1. This supports the observations made in the cryo-TEM micrographs. The similar bicelle dimensions may be explained by the fact that the replacement of the hydroxyl functional group of Chol-OH with a primary amine does not impact the molecule’s packing parameter. The introduction of an aliphatic chain -C$_2$OC$_2$- attached to the steroid backbone with a carbamate bond in Chol-C$_2$OC$_2$-NH$_2$ did not change the bicelle size. This result is in sharp contrast to
5.1 Incorporating Chol-NH$_2$ and Chol-C$_2$OC$_2$-NH$_2$ in the phospholipid bilayer.

Table 5.1 Magnetic alignment and size comparison of the steroid-free DMPC/DMPE-DTPA/Ln$^{3+}$ (molar ratio 4:1:1, [tL] 15 mM) bicelles and the DMPC/steroid/DMPE-DTPA/Ln$^{3+}$ (molar ratio 16:4:5:5, [tL] 15 mM) systems doped with either cholesterol (Chol-OH), 3β-amino-5-cholestene (aminocholesterol, Chol-NH$_2$) or the aminocholesterol conjugate (Chol-C$_2$OC$_2$-NH$_2$). Table adapted from S. Isabettini et al.\textsuperscript{90}

<table>
<thead>
<tr>
<th>Bilayer dopant$^a$</th>
<th>Ln$^{3+}$</th>
<th>Bicelle radius [nm]</th>
<th>$A_f^{\gamma}$ [-]</th>
<th>ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>Tm$^{3+}$</td>
<td>18</td>
<td>-0.14</td>
<td>28</td>
</tr>
<tr>
<td>Chol-OH</td>
<td>Tm$^{3+}$</td>
<td>60</td>
<td>-0.36</td>
<td>16</td>
</tr>
<tr>
<td>Chol-C$_2$OC$_2$-NH$_2$</td>
<td>Tm$^{3+}$</td>
<td>65$^b$</td>
<td>-0.68</td>
<td>-</td>
</tr>
<tr>
<td>Chol-NH$_2$</td>
<td>Tm$^{3+}$</td>
<td>61$^b$</td>
<td>-0.77</td>
<td>-</td>
</tr>
<tr>
<td>Chol-NH$_2$</td>
<td>Dy$^{3+}$</td>
<td>62$^b$</td>
<td>0.77</td>
<td>-</td>
</tr>
<tr>
<td>Chol-NH$_2$</td>
<td>Yb$^{3+}$</td>
<td>64$^b$</td>
<td>-0.56</td>
<td>-</td>
</tr>
</tbody>
</table>

$^a$DMPC/dopant/DMPE-DTPA/Ln$^{3+}$ (16:4:5:5, molar ratio), 15 mM total lipid concentration, 50 mM phosphate buffer, pH 7.4.

$^b$Calculated from radially averaged SANS curves fitted with a Porod cylinder model with a thickness of 4.6 nm and a LogNorm distribution with $\sigma = 0.5$. The average error on the bicelle radius amounts to ±4 nm.

$^c$Calculated from SANS 2D scattering patterns at 8 T and 5 °C. The average error on the alignment factors amounts to ±0.05.\textsuperscript{16}

the observed architectural diversity of polymolecular assemblies achieved by incorporating lanthanide-chelating cholesterol conjugates (Chol-C$_n$-DTPA/Ln$^{3+}$) in the bilayer discussed in chapter 7.\textsuperscript{15} In the absence of a large DTPA-Ln$^{3+}$ head group, the packing parameter is likely not sufficiently altered to cause any changes in bicelle architecture. Van der Waals forces between the hydrophobic steroid backbone and the aliphatic chains of the phospholipids govern the interactions of Chol-OH and its placement within the bilayer.\textsuperscript{1–6,15,16} Moreover, the polarity and flexibility of the -C$_2$OC$_2$- chain offers enough freedom for conformational changes, minimizing unfavorable interactions with the hydrophilic environment at the bicelles surface. Together, these aspects explain the comparable bicelle sizes obtained with both Chol-NH$_2$ and Chol-C$_2$OC$_2$-NH$_2$. Furthermore, the bicelle dimensions remain unchanged regardless of the applied paramagnetic lanthanide ion, analogously to DMPC/Chol-OH/DMPE-DTPA/Ln$^{3+}$ (molar ratio 16:4:5:5) bicelles.\textsuperscript{16,153}
5 Enhancing the Magnetic Susceptibility of Bicelles with Chol-NH$_2$

Figure 5.3 Birefringence signal as a function of temperature at 5.5 T of DMPC/Chol-NH$_2$/DMPE-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5, [Tl] 15 mM) bicelles. The sample was subject to a heating (red line) and cooling (blue line) cycle from 5 to 55 °C at 1 °C/min. Figure adapted from S. Isabettini et al.\textsuperscript{90}

5.2 Magnetic alignment of Chol-NH$_2$ and Chol-C$_2$OC$_2$-NH$_2$ doped bicelles.

The strength of the birefringence signal employed to confirm the successful incorporation of Chol-NH$_2$ in Figure 5.3 is correlated to the degree of alignment of the bicelles. The magnitude of the birefringence signal was two-fold greater for the DMPC/Chol-NH$_2$/DMPE-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5) bicelles when compared to the analogous reference system containing Chol-OH. This result implied a much larger magnetic alignability of the Chol-NH$_2$ doped bicelles. This result was of particular interest as the bicelle size was unaffected from the replacement of Chol-OH, implying that the number of molecules capable of contributing to the bilayer’s $E_{mag}$ was the same. Therefore, the change in magnetic response must result from a different magnetic susceptibility of the amphiphiles composing the bilayer. To confirm these findings, the magnetic alignment of the bicelles was further quantified for both the Chol-NH$_2$ or Chol-C$_2$OC$_2$-NH$_2$ steroid dopants by computation of alignment factors $A_f$ in SANS. The results were compared to the reference DMPC/Chol-OH/DMPE-DTPA/Ln$^{3+}$ (molar ratio 16:4:5:5) systems.\textsuperscript{16} Replacement of Chol-OH with Chol-NH$_2$ resulted in a six-fold or two-fold increase in alignment factor when comparing to the thulium-chelating Chol-OH free and doped
5.2 Magnetic alignment of Chol-NH$_2$ and Chol-C$_2$OC$_2$-NH$_2$ doped bicelles.

Figure 5.4 Radially averaged SANS curves (data points) and fittings (solid lines) of DMPC/Chol-NH$_2$/DMPE-DTPA/Ln$^{3+}$ (molar ratio 16:4:5:5, [tL] 15 mM) bicelles at 5 °C with Tm$^{3+}$ (red) Dy$^{3+}$ (blue) or Yb$^{3+}$ (green) and radially averaged SANS curve and fitting of DMPC/Chol-C$_2$OC$_2$-NH$_2$/DMPE-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5, [tL] 15 mM) bicelles at 5 °C (black). The SANS 2D scattering patterns were measured in the absence of any magnetic field. Figure adapted from S. Isabettini et al. 90

systems, respectively. The magnitude of the birefringence signal under a 5.5 T magnetic field at 5 °C was two-fold greater for the DMPC/Chol-NH$_2$/DMPE-DTPA/Tm$^{3+}$ bicelles when compared to the analogous reference system containing Chol-OH. 16 This result validates the observations made from SANS experiments as the strength of the birefringence signal is correlated to the degree of alignment of the bicelles. 29 The capacity of constructing highly magnetically responsive bicelles by replacing the hydroxyl group of Chol-OH with a primary amine was supported by the high alignment factor of -0.68 achieved by DMPC/Chol-C$_2$OC$_2$-NH$_2$/DMPE-DTPA/Ln$^{3+}$ (molar ratio 16:4:5:5) bicelles. However, the alignment factor remains smaller than for the Chol-NH$_2$ doped bicelles (see Table 5.1).
Figure 5.5 Alignment factor as a function of magnetic field strength for DMPC/Chol-NH$_2$/DMPE-DTPA/Ln$^{3+}$ (molar ratio 16:4:5:5, [tL] 15 mM) bicelles at 5 °C. The lanthanide ion (Ln$^{3+}$) was either Tm$^{3+}$ (black triangles), Dy$^{3+}$ (blue circles) or Yb$^{3+}$ (red squares). The alignment factor of the reference DMPC/Chol-OH/DMPE-DTPA/Ln$^{3+}$ (molar ratio 16:4:5:5, [tL] 15 mM) bicelle system is shown with open symbols for Tm$^{3+}$ (1) and Yb$^{3+}$ (2) at 5 °C and 8 T. The SANS 2D scattering patterns were measured at 8 T. The magnetic field B is directed upwards as indicated with an arrow. The alignment direction of the Dy$^{3+}$ and Tm$^{3+}$ bicelles is emphasized with a simplified schematic representation in blue and black, respectively. Figure adapted from S. Isabettini et al. 90

The superior magnetic alignment offered by incorporation of Chol-NH$_2$ or Chol-C$_2$OC$_2$-NH$_2$ in the bilayer was confirmed when working with different Ln$^{3+}$. The direction of alignment is determined by the sign of the magnetic anisotropy of the complexed Ln$^{3+}$. The sign of the magnetic anisotropy is governed by the orbital spin angular momenta of the complexed lanthanide and the orientation of the complex. 97,99,154
enhances the negative magnetic susceptibility anisotropy of the phospholipids, which intensifies the bicelle’s response to an external magnetic field. The disk’s plane will align parallel to the magnetic field, resulting in positive alignment factors. Tm$^{3+}$ and Yb$^{3+}$ convey a large positive magnetic anisotropy to the lipid domain causing the bicelle’s plane to align perpendicular to the magnetic field direction, resulting in negative alignment factors. Evidence of these two different alignment directions was provided by the 2D SANS scattering patterns in Figure 5.5. No alignment was observed with in the DMPC/Chol-OH/DMPE-DTPA/Yb$^{3+}$ (molar ratio 16:4:5:5) system under an 8 T magnetic field and at 5 °C. Replacement of Yb$^{3+}$ with Tm$^{3+}$ allowed for an increased alignment factor of -0.36, see Figure 5.5. Contrastingly, the corresponding Chol-NH$_2$ containing bicelles yield an alignment factor of -0.52 with Yb$^{3+}$ and -0.77 with Tm$^{3+}$ under equivalent conditions. The magnitude of the crystal field perturbation strongly depends on the chemical properties of the considered complex. The magnetic response of bicelles containing Tm$^{3+}$ is stronger to those with Yb$^{3+}$ owing to the smaller molar magnetic susceptibility of the latter. However, the magnetic susceptibility is largely influenced by the chemistry and geometry of the Ln$^{3+}$ chelate polyhedron which may be engineered as revealed in chapter 8. The curves presented in Figure 5.5 reach saturation when monitoring the evolution of the alignment factor with increasing magnetic field strength. A change in gradient is evident at 7 T for bicelle samples with Tm$^{3+}$ (Figure 5.5, black triangles) and at 4 T for samples containing Dy$^{3+}$ (Figure 5.5, blue circles). These results suggest that the two bicelle samples are reaching full alignment at 5 °C and at a magnetic field strengths as low as 8 T. Such a phenomenon was only reported with birefringence measurements for DMPC/Chol-OH/DMPE-DTPA/Ln$^{3+}$ (molar ratio 16:4:5:5) bicelles at 16 T and 32 T when working with Dy$^{3+}$ and Tm$^{3+}$, respectively.

The enhanced magnetic alignment must come from a change in magnetic susceptibility as the bicelle’s size remains unaltered. This originates from various effects proposed in the theory on magnetic anisotropy of lanthanide-containing metallomesogens by Mironov et al. (see Figure 2.7). The primary amine may influence the hydrophilic environment around the DTPA-Ln$^{3+}$ complex due to its enhanced capacity for hydrogen bonding in comparison to the hydroxyl group in Chol-OH. Since water acts as a 9th coordination site in the DTPA-Ln$^{3+}$ complex, the impact on the magnetic susceptibility could result from alterations in the crystal field energies due to the interaction of the lanthanide with the ligands. Moreover, the magnetic susceptibility might further be altered by two additional effects: 1) a change in orientation of the long molecular axis with respect to the magnetic axis or 2) ordering effects. The presence of charges or additional hydrogen bonding could influence both contributions, enhancing the magnetic susceptibility of the system. Such possibilities are conceivable as the primary amine of Chol-NH$_2$ may result in a stronger hydrogen bonding network similarly to sphingolipids. Furthermore, the amine moiety is mainly positively charged in the studied conditions at a pH value of 7.4. The -C$_2$OC$_2$- chain forces the primary amine to interact differently with neighboring bilayer amphiphiles when comparing the Chol-C$_2$OC$_2$-NH$_2$ to the Chol-NH$_2$ doped systems. The difference in magnetic alignment may again be attributed to a change in the system’s magnetic susceptibility as the bicelle’s size remained constant.
5 Enhancing the Magnetic Susceptibility of Bicelles with Chol-NH₂

5.3 Conclusion

DMPC/Chol-NH₂/DMPE-DTPA/Ln³⁺ systems supply numerous opportunities for tailoring the magnetic response of bicelles. Introducing a primary amine as polar head group of the steroid moiety allows for selective tuning of the bicelle’s magnetic susceptibility without changing their size or the nature of Ln³⁺. Full alignment was reached at field strengths of 8 T. Both the magnitude and the direction of magnetic alignment were adjusted through the chelated Ln³⁺. Further fine tuning of the bicelle’s magnetic susceptibility was achieved through chemical alterations of Chol-NH₂ as demonstrated with Chol-C₂OC₂-NH₂. Improving the magnetic alignment of bicelles is beneficial for NMR-based structural studies. In particular, the use of static solid state NMR experiments on aligned bilayers would greatly benefit from this technology. Furthermore, the proposed bicelle systems present maximal magnetic alignment at low temperatures, which goes in line with the requirements of NMR experiments. Analogously to cholesterol sulfate, the charged nature of Chol-NH₂ and Chol-C₂OC₂-NH₂ could be beneficial for stabilizing the aligned phase at low temperatures also in DMPC/DHPC bicelles. However, it is important to emphasize that these are non-natural compounds that may impede on the quality of the bicelle bilayer as a model system. The versatility in design of the presented materials offers the possibility to go beyond applications in the field of membrane protein characterization by NMR spectroscopy. In chapter 9, these novel bicelle systems are employed to generate magnetically switchable gels with novel optical properties. This application allows to move onto the third level of the S-PRO² scheme in Figure 1.1. However, the exact origins of the enhanced magnetic response are not clear. A more systematic study of the physico-chemical properties governing the bilayer and the molecular behaviour of the DMPE-DTPA/Ln³⁺ should be undertaken. Furthermore, the thermal behaviour of the Chol-NH₂ doped bicelles should be fully characterised to envisage imbedding them in an aligned state in a gelatine network. These two shortcomings are addressed in chapter 6 through a multiscale bottom-up comparative investigation of Chol-OH and Chol-NH₂ mixed with DMPC relying on complementary experimental and simulation based techniques.
6 Understanding the Magnetic Response of Chol-NH$_2$ Doped Bicelles


Chol-OH and its conjugates are powerful molecules for engineering the physico-chemical and magnetic properties of phospholipid bilayers in bicelles. Introduction of Chol-NH$_2$ in DMPC/Chol-NH$_2$/DMPE-DTPA/Ln$^{3+}$ (molar ratio 16:4:5:5) bicelles resulted in unprecedented high magnetic alignments by selectively tuning the magnetic susceptibility anisotropy $\Delta \chi$ of the bilayer as discussed in chapter 5. However, the reasons behind this enhanced magnetic susceptibility $\Delta \chi$ remain unclear and calls for further characterization of the physico-chemical properties of Chol-NH$_2$ doped lipid bilayer. Herein, a MD simulation was employed as a bottom-up approach to monitor the different contributions to the $\Delta \chi$. The effect of lipid order, the crystal field of the chelated Ln$^{3+}$, and the orientation of the long molecular axis of the DMPE-DTPA/Tm$^{3+}$ complex with respect to its magnetic axis were investigated to understand how the $\Delta \chi$ in the Chol-NH$_2$ doped bilayer was altered. Although this work is directed towards understanding the reasons behind the enhanced $\Delta \chi$ in DMPC/Chol-NH$_2$/DMPE-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5) bicelles, an initial characterization of simplified DMPC/Chol-NH$_2$ monolayers and bilayers was necessary before moving on to more representative models of the bicelles.

In order to characterise the physico-chemical phenomena governing the Chol-NH$_2$ doped bicelle system and understand the resulting high magnetic alignments, the Chol-NH$_2$ doped system was systematically compared to the well characterized Chol-OH doped system. The structural aspects of the bilayer amphiphiles are studied herein, corresponding to the first level of the S-PRO$^2$ scheme in Figure 1.1. The resulting properties (enhanced $\Delta \chi$) are better understood by investigating these structural aspects, enabling the development of future molecular engineering approaches. A multiscale bottom-up investigation was undertaken starting from simplified models of the lipid monolayer and bilayer before building up the complexity to best imitate the bicelle system as outlined in Figure 6.1. The physico-chemical interactions of Chol-NH$_2$ in DMPC bilayers has never
been characterized. However, M. Lönnfors et al. provide a detailed account of the interaction of Chol-NH$_2$ with phospholipids in other binary and ternary bilayer membranes.\textsuperscript{88} The characterization of simplified DMPC/Chol-NH$_2$ systems offers a preliminary understanding of the physico-chemical properties governing the DMPC phospholipid bilayers doped with Chol-NH$_2$ before moving on to the more complex bicelle systems.

In a first step, DMPC/Chol-NH$_2$ and DMPC/Chol-OH monolayers at the air/water interface and their surface pressure/molecular area isotherms on a Langmuir trough were studied (Figure 6.1A). The sub-phase was composed of a 50 mM phosphate buffer at a pH value of 7 and at 5 °C to imitate the hydrophilic environment of the bicelles when they display an optimal magnetic response. In a next step, a MD simulations was employed to move away from the monolayer and study a bilayer (Figure 6.1B), which was a more realistic representation of the bicelles. The introduction of the DMPE-DTPA/Tm$^{3+}$ complex was possible in the MD simulations. The Ln$^{3+}$-phospholipid complexes present in the planar part of the bicelle hold the key to understanding the magnetic response of the bicelles due to the large $\Delta \chi$ conveyed by the paramagnetic lanthanide ions. Therefore, a MD simulation of both the Chol-OH and Chol-NH$_2$ doped bilayers was employed to observe the parameters defining the $\Delta \chi$ (Figure 6.1C). These include the lipid order, the crystal field of the chelated Ln$^{3+}$ ion, and the orientation of the long molecular axis of the DMPE-DTPA/Tm$^{3+}$ complex with respect to its magnetic axis. The investigation is concluded by monitoring the thermal behavior of DMPC/Chol-NH$_2$/DMPE-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5) bicelles by SANS. The structure of the bicelles at different temperatures were characterized and their magnetic alignment quantified. The different physico-chemical forces governing the bicelle properties were understood by comparing to the well characterized DMPC/Chol-OH/DMPE-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5) bicelles (Figure 6.1D). These temperature studies expand the versatility of the Chol-NH$_2$ doped bicelles by demonstrating their ability to remain in a highly-aligned state at higher temperatures. Understanding the thermal behavior of these polymolecular assemblies is of capital importance to envisage their application as building blocks for the magnetically responsive soft materials of tomorrow.
Figure 6.1 Schematic representation of the undertaken multiscale bottom-up comparative investigation of Chol-NH$_2$ and Chol-OH mixed with DMPC. The physico-chemical forces governing the two steroid doped systems were first identified and studied with simplified models involving monolayers at the air/water interface (A), followed by bilayers in a molecular dynamics (MD) simulation (B). A more representative model of the bicelle bilayer was achieved by introducing the DMPE-DTPA/Tm$^{3+}$ complex (C). In a final step, the thermal resistance and magnetic alignability of the DMPC/Chol-NH$_2$/DMPE-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5) bicelles was investigated and compared to the analogous Chol-OH doped bicelles (D). By characterizing the physico-chemical properties governing these amphiphilic systems, this study aims at understanding how Chol-NH$_2$ alters the magnetic susceptibility anisotropy $\Delta \chi$ of Ln$^{3+}$ chelating bicelles, resulting in a high magnetic response. Figure adapted from S. Isabettini et al.$^{152}$
6.1 DMPC/sterol monolayer

DMPC/Chol-NH$_2$ and DMPC/Chol-OH monolayers isotherms were investigated with varying sterol contents at the air/water interface in Figure 6.2A and 6.2B, respectively. This model system allowed for a direct comparison between the two sterol molecules and their respective interactions with DMPC.$^{157}$ Chol-OH readily mixes with phospholipids with an aliphatic tail length between 14 and 18 carbons.$^{158}$ The Langmuir trough technique was employed and the surface pressure/molecular area isotherms of the DMPC/sterol monolayers were recorded at 5 °C with a 50 mM phosphate buffer sub-phase at a pH value of 7. These conditions were chosen to best imitate the bicelle environment when displaying an optimal magnetic response.

![Figure 6.2](image)

**Figure 6.2** Surface pressure/molecular area isotherms of A) DMPC/Chol-OH and B) DMPC/Chol-NH$_2$ monolayers on a 50 mM phosphate buffer sub-phase at 5 °C and with a pH value of 7. The sterol contents were either 0, 16, 20, 30, 40, or 100 mol%. Figure adapted from S. Isabettini et al. $^{152}$

The appearance of a plateau at 71 Å$^2$ for the pure DMPC monolayer was induced by the transition from a liquid-expanded to a liquid-condensed phase when working below the phase transition temperature of the lipid. The isotherms of the sterol compounds displayed a very different behavior. Condensed sterol domains grow until a critical area was reached and a steep lift-off into the condensed phase occurs. Both Chol-NH$_2$ and Chol-OH resulted in similar isotherms with a lift-off at about 43 Å$^2$/molecule, in line with previous findings.$^{54,157}$

The effect of adding 10 mol% sterol in the DMPC monolayer was immediately visible as the lift-off point of the isotherms was shifted towards lower areas per molecule. When Chol-OH was employed, the plateau occurring in the pure DMPC monolayer disappeared. This finding is consistent with the ordering effect induced by Chol-OH in phospholipid systems.$^{72,157,159,160}$ The disappearance of the phase transition was not as clear for the DMPC/Chol-NH$_2$ system where a kink in the isotherm remained visible.
at about 65 Å²/molecule. At higher steroid contents of 30 and 40 mol%, the isotherms look very similar to those of the pure steroid molecules. The monolayer properties seem dominated by the presence of the steroid lipids. Although these steroid contents are not comparable to those of the bicelle systems, the isotherms emphasize the differences induced by the presence of primary amine in Chol-NH₂ instead of a hydroxyl group in Chol-OH.

The appearance of a more ordered lipid phase with Chol-NH₂ was confirmed from the similar evolution of the isotherms compared to the Chol-OH doped systems with increasing steroid contents. A similar behavior was expected for Chol-NH₂ that possesses the same steroid backbone as Chol-OH. Moreover, these findings go in line with the observations of M. Lönnfors et al.⁸⁸ where Chol-NH₂ was shown to form liquid-ordered phases in bilayers composed of other saturated lipids, such as palmitoyl sphingomyelin and DPPC. However, the isotherms remained markedly different regardless of the similar trend upon increasing steroid contents, implying that different physico-chemical forces govern the properties of the Chol-OH and Chol-NH₂ doped systems.

To facilitate comparison between the two systems, the excess area per molecule $A_{ex}$ was computed with

$$A_{ex} = A_{DMPC/steroid} - (x_{DMPC}A_{DMPC} + x_{steroid}A_{steroid})$$  \hspace{1cm} (6.1)

where $A_{DMPC/steroid}$ is the mean measured area of the molecules in the DMPC/steroid monolayer at a fixed surface pressure.⁵⁴ $A_{DMPC}$ and $A_{steroid}$ are the areas of the individual molecules as evaluated from the single-component monolayers. $x_{DMPC}$ and $x_{steroid}$ are the molar fractions of the respective components. Consequently, $A_{ex} = 0$ in the scenario where the lipids mix ideally and no steroid-induced condensation occurs. The excess area per molecule was plotted as a function of steroid content at different surface pressures in Figure 6.3.

Both steroid compounds condensed the monolayer as evidenced by the negative $A_{ex}$ values in Figure 6.3. This condensation effect was marked at lower surface pressures where the lipid tails are more prone to organise in the presence of Chol-OH.⁵⁴ At 10 mol% steroid contents, Chol-NH₂ had a stronger condensation effect than Chol-OH on the lipid monolayer as evidenced by the larger excess area per molecule $A_{ex}$ obtained with the former. However, the simultaneous unclear disappearance of the phase transition observed in the 10 mol% DMPC/Chol-NH₂ isotherm (see the kink at 65 Å²/molecule in Figure 6.2B) suggests complex phase behaviours, which may not be fully appreciated only based on these findings. Contrastingly, Chol-OH had a larger impact on the physico-chemical forces governing the monolayer at higher contents of 16 and 20 mol%. The larger $A_{ex}$ of Chol-NH₂ with respect to Chol-OH suggests a smaller induced condensation effect of the former. The appearance of a minimum in $A_{ex}$ at steroid contents between 30 and 40 mol% has been associated to the umbrella effect where phospholipid headgroups shield Chol-OH from the bulk water phase. Nevertheless, numerous interaction models exist and the complexity of phospholipid-steroid interactions may not be solely understood in this manner.⁵³ At these larger steroid contents, Chol-NH₂
Figure 6.3 Excess area per molecule $A_{ex}$ as a function of steroid content evaluated at 5, 10, 15, and 25 mN/m. The monolayers were measured with a 50 mM phosphate buffer sub-phase at 5 °C and with a pH value of 7. Filled circles represent data points measured from Chol-OH doped monolayers and open squares from Chol-NH$_2$ doped monolayers. A standard error of ±0.8 Å$^2$ was observed in the excess area and is not shown on the figure for clarity. Figure adapted from S. Isabetinni et al.\textsuperscript{152}

had a larger condensation effect than Chol-OH as evidenced by the more negative $A_{ex}$ values of the former in Figure 6.3. The opposite effect was observed at lower steroid contents of 16 and 20 mol%. It is possible that the physical interactions resulting from headgroup interactions between Chol-NH$_2$ and neighbouring lipids only comes into play at higher steroid contents where it becomes a more dominant component. The replacement of the hydroxyl headgroup of Chol-OH with a positively charged primary amine certainly induces large changes in the already complex phase-diagram of Chol-OH doped phospholipid bilayers.\textsuperscript{71,72,159,160}

The monolayers were further studied by coupling Fourier transform infrared reflection-absorption spectroscopy (FT-IRRAS) to the Langmuir trough experiment. IR measurements allow to directly measure the chemical interactions occurring in the monolayer. The technique monitors the evolution of reflectance-absorbance bands oc-
Figure 6.4 FT-IRRA spectrum of a 30 mol% DMPC/Chol-NH$_2$ monolayer at 5 °C and a surface pressure of 25 mN prepared on phosphate buffer at pH 7 (blue) or on D$_2$O (red) as subphases. The amide I and II, phosphate and C-H stretching regions of the IRRA spectra are labeled and indicated in grey boxes. Peaks at 2340 cm$^{-1}$ and 2360 cm$^{-1}$ are caused by CO$_2$ in the atmosphere. The D-O stretch of D$_2$O and the H-O stretch of H$_2$O are visible at 2600 cm$^{-1}$ and 3600 cm$^{-1}$, respectively. The spectra was obtained with s-polarized light at an incidence angle of 40° and was baseline corrected. Figure adapted from S. Massabni.\textsuperscript{161}

occurring at wave numbers defined by the motion of the chemical bonds of the amphiphiles composing the monolayer. These bands may be monitored as a function of surface pressure, revealing important information on the lipid structure in the monolayer.\textsuperscript{54,162–165} More specifically, this study aimed at monitoring the C-H stretch regions of the phospholipids to complement the findings obtained with the surface pressure isotherms in Figure 6.3. The intensity of the C-H bands at 2854 and 2926 cm$^{-1}$ increases with surface pressure. Moreover, the bands shift to 2850 and 2920 cm$^{-1}$, respectively when molecular packing increases, giving an indication on the degree of order in the monolayers.\textsuperscript{162} Important information on head group interactions including hydration state
and the occurrence of hydrogen bonding may be further monitored through the amide and phosphate stretching regions.\textsuperscript{162,165} The experiments were performed with phosphate buffer at a pH value of 7 as subphase in order to reproduce the natural bicelle environment. Several additional measurements were performed with D$_2$O as subphase to account for the probable interference of the buffer with the phosphate peaks expected from the sample. Moreover, the amide bands are known to appear more clearly in the absence of H$_2$O. The full IR-spectra of a 30 mol\% DMPC/Chol-NH$_2$ monolayer once on phosphate buffer and once on D$_2$O are shown in Figure 6.4.

**Figure 6.5** C-H stretching bands of the FT-IRRRA spectra of DMPC/Chol-NH$_2$ monolayers with A) 16 mol\% and B) 30 mol\% steroid content at different surface pressures. Phosphate buffer at a pH value of 7 served as a subphase and the spectra were recorded with s-polarized light with an incidence angle of 40°. Figure adapted from S. Isabettini \textit{et al.}\textsuperscript{152}
No significant peaks were observed in the phosphate or amide region for both subphases presented in Figure 6.4 and in all measured samples. However, the C-H stretching regions were easily distinguished and employed for monitoring the degree of order on a molecular level for the rest of this study. A zoom on the C-H bands of the FT-IRRA spectra obtained for DMPC/Chol-NH$_2$ monolayers at 16 and 30 mol% at different surface pressure are shown in Figure 6.5A and 6.5B, respectively. The IR signal intensity increased with the surface pressure due to increasing monolayer compression. These results were in line with the excess area results presented in Figure 6.3. An evident shift in wavenumber occurs from 2924 to 2920 cm$^{-1}$ and from 2854 to 2850 cm$^{-1}$ as molecular packing and order increased in the monolayers. The surface pressure at which the shift takes place coincided with the phase transition from the liquid disordered to the solid ordered phase.$^{165}$ The peak is always at 2850 cm$^{-1}$ for all measured surface pressures in the 30 mol% DMPC/Chol-NH$_2$ monolayer, demonstrating the more dominant ordering effect of Chol-NH$_2$ at such high steroid contents. The phase transition is a marked feature of the DMPC monolayer, which is only observed at lower steroid contents of 16 mol%. The 10 mol% DMPC/Chol-OH monolayer revealed a peak shift from 2924 cm$^{-1}$ to the more ordered 2920 cm$^{-1}$ between 10 and 15 mN/m. In the corresponding 10 mol% Chol-NH$_2$/DMPC monolayer, this shift occurred earlier between 5 and 10 mN/m (results not shown). This result confirmed the stronger condensing effect of Chol-NH$_2$ at 10 mol% steroid contents in the DMPC monolayer.

The maximum intensity of the 2924 or 2920 cm$^{-1}$ band was plotted as a function of surface pressure for the studied steroid contents in Figure 6.6. This facilitates the comparison with the excess area results in Figure 6.3, which coincided perfectly. All the maximum FT-IRRA signals originating from the Chol-NH$_2$/DMPC monolayers at 10 and 30 mol% steroid contents are larger than those obtained in the Chol-OH/DMPC monolayers. This confirmed the larger condensation effect of Chol-NH$_2$ at these steroid contents, also observed in the excess area data in Figure 6.3. However, at 16 mol% steroid content, the IR signal of the Chol-OH containing monolayer is larger than that of the Chol-NH$_2$ counterpart at surface pressures above 5 mN/m, indicating a larger condensation effect of the former. The 16 mol% steroid content is of particular interest as it imitates the content employed in the bicelles. Since the membrane pressure of the bicelle bilayer is certainly above 5 mN/m, the Chol-OH doped assemblies will have a more ordered and condensed bilayer than the Chol-NH$_2$ doped counterparts. This physico-chemical property may be altered by changing the steroid content as confirmed by both the excess area experiments on the macroscale and the FT-IRRAS results on the molecular scale.
Figure 6.6 Maximum intensity of the 2924 or 2920 cm$^{-1}$ C-H band as a function of surface pressure for the DMPC/Chol-OH and DMPC/Chol-NH$_2$ monolayers at steroid contents of A) 0, B) 10, C) 16, and D) 30 mol%. The data was obtained by subtracting the peak maxima of the respective systems from the baseline measurement. Figure adapted from S. Isabettini et al.\textsuperscript{152}
6.2 Molecular dynamics (MD) simulation of the DMPC/steroid bilayer

A MD simulation of the DMPC/Chol-NH$_2$ and the DMPC/Chol-OH bilayer was undertaken to complement the findings of the monolayer study and serve as another simplified model of the bicelle systems. The simulation conditions were chosen to best imitate the bicelle environment. The bilayer consisted of 128 DMPC phospholipids with 16 mol% steroid (20 molecules). The hydrophilic surroundings of the bicelle was composed of a 50 mM sodium phosphate buffer at a pH value of 7. The average number of hydrogen bonds per time frame to surrounding water molecules and DMPC are presented for both steroids at 30 and 5 °C in Table 6.1. Chol-NH$_2$ showed double the amount of hydrogen bonds to DMPC and one third more with water molecules when compared to Chol-OH, regardless of the temperature. At a pH value of 7, the primary amine of Chol-NH$_2$ is mainly protonated. The increased extent of hydrogen bonding with Chol-NH$_2$ was expected when compared to Chol-OH that only contains one hydrogen atom in its hydroxyl group.

<table>
<thead>
<tr>
<th>Steroid</th>
<th>Temperature [°C]</th>
<th>Average hydrogen bonds per time frame to</th>
</tr>
</thead>
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<tr>
<td></td>
<td></td>
<td>H$_2$O</td>
</tr>
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<td>Chol-OH</td>
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</tr>
<tr>
<td></td>
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<td>20.3</td>
</tr>
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<td>32.8</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>32.5</td>
</tr>
</tbody>
</table>

The simulation revealed a considerably larger diffusion coefficient for Chol-NH$_2$ than Chol-OH at 5 °C. Moreover, the snapshot of the DMPC/Chol-NH$_2$ bilayer taken at the end of the simulation at 5 °C in Figure 6.7A revealed that three out of the twenty Chol-NH$_2$ molecules do not seek further contact with the DMPC bilayer. They remain in solution at the surface of the bilayer. This was not the case for Chol-OH that fully integrated into the bilayer, see Figure 6.7B. A lower ordering effect could explain the larger condensation effect of Chol-OH over Chol-NH$_2$ at 16 mol% steroid contents observed in the monolayer study. The deuterated chain order parameters -$S_{CD}$ were evaluated from the simulated trajectories and are presented for both steroid doped bilayers in Figure 6.8. In the case of Chol-OH, a clear increase in order was observed when cooling from 30 to 5 °C as the curves shift upwards towards larger -$S_{CD}$ values. These results are consistent with the simulations of De Meyer et al. where DMPC bilayers containing 16 mol% Chol-OH move from semi-ordered phases to a complete
liquid-disordered phase at temperatures above 28 °C. In the case of Chol-NH$_2$, no change in order or in phase behavior occurred from 5 to 30 °C. The number of molecules per unit area was computed for the two bilayers at 5 °C. The DMPC/Chol-NH$_2$ bilayer revealed a value of 50 Å$^2$/molecule compared to 48 Å$^2$/molecule for the DMPC/Chol-OH bilayer. The larger condensation effect of Chol-OH at 5 °C goes in line with the computed larger order in the bilayer. Furthermore, these simulation results support the findings where Chol-OH induced a larger condensation effect than Chol-NH$_2$ in the 16 mol% DMPC/steroid monolayer at the air/water interface, see Figure 6.3.

**Figure 6.7** Snapshot of the simulated A) DMPC/Chol-NH$_2$ and B) DMPC/Chol-OH bilayers at 5 °C. Atoms and bonds constituting the DMPC phospholipids are represented by a wireframe model and the steroids with a space-filling model at their van der Waals radii, water is omitted. Carbon atoms are shown in black, oxygen in red, nitrogen in blue, phosphorus in purple, sodium in teal and chlorine in green. The bilayer consists of 128 DMPC phospholipids with 20 steroid (16 mol%) molecules in a 50 mM sodium phosphate buffer at a pH value of 7. Figure adapted from Isabettini et al.}$^{152}$
6.3 MD simulation of the DMPC/steroid/DMPE-DTPA/Tm$^{3+}$ bilayer

Six DMPE-DTPA/Tm$^{3+}$ molecules were incorporated into the DMPC/steroid bilayer to truly simulate a portion of the bicelle. The aim being to understand the reported large gains in magnetic response upon replacement of Chol-OH with Chol-NH$_2$ in DMPC/steroid/DMPE-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5) bicelles. A three-step analysis of the bilayer was undertaken focusing on the factors capable of altering $\Delta\chi$ as described in the general theory by Mironov et al. In the first step (i), the possibility that an increase in order could explain the enhanced magnetic response of the Chol-NH$_2$ doped bilayer was questioned. In a second step (ii), the crystal field of the chelated lanthanide ion was analysed. In a third and final step (iii), the possible change in orientation of the long molecular axis with respect to its magnetic axis in the DMPE-DTPA/Tm$^{3+}$ complex was evaluated.

(i) The degree of order in the two steroid doped systems was compared. Bilayers with a higher degree of order were expected to enhance the total magnetic energy of the bicelle. More order coincides with reduced fluctuations arising from the nonrigidity of phospholipid-lanthanide species within the bilayer. Consequently, the microscopic disorder contribution was reduced, as proposed in the general theory of magnetic anisotropy
of lanthanide-containing liquid crystals by Mironov et al.\textsuperscript{97} The \(S_{CD}\) was computed for the myristoyl tails of the lipids in the respective bilayer systems. For DMPC, the values were analogous to those obtained in the DMPC/steroid bilayer. The \(S_{CD}\) parameters of the DMPE-DTPA/Tm\textsuperscript{3+} phospholipids revealed a higher degree of order with Chol-OH in the bilayer than with Chol-NH\(_2\). To further evaluate the order in the two systems, a cluster analysis was undertaken according to Daura \textit{et al.}\textsuperscript{166} The results did not allow for any noticeable difference between the two systems. Therefore, the larger degree of magnetic alignment in DMPC/Chol-NH\(_2\)/DMPE-DTPA/Tm\textsuperscript{3+} (molar ratio 16:4:5:5) bicelles may not be attributed to a difference in order within the bilayer.

(ii) It was necessary to take a closer look at the phospholipid-lanthanide complex and consider other contributions that define the \(\Delta \chi\) to explain the observed large differences in magnetic response of the steroid doped bicelles. The analysis revealed that Chol-NH\(_2\) underwent significantly more hydrogen bonding interactions with its surroundings than Chol-OH. It interacted to a greater extent with DMPC, DMPE-DTPA/Tm\textsuperscript{3+}, and the phosphate buffer. These results confirm those observed in the DMPC/steroid simulation in Table 6.1. Chol-NH\(_2\) has the ability to influence the hydrophilic environment of the bilayer to a larger extent than Chol-OH. Surrounding water molecules act as the 9\textsuperscript{th} coordination site of thulium in the DTPA complex.\textsuperscript{153} This will define the crystal field of the chelated lanthanide, which in turn determines the \(\Delta \chi\) of the molecule. Therefore, the cumulative occupation time of the 9\textsuperscript{th} coordination site for the two DMPC/steroid/DMPE-DTPA/Tm\textsuperscript{3+} systems was analysed in Table 6.2. As expected, water molecules account for most of the occupation time. Nevertheless, a clear difference was observed as the coordination to oxygen from neighbouring DMPC phospholipids was preferred in the Chol-NH\(_2\) doped bilayer. The oxygen from the carboxylic group in the ester bond of the phospholipids acted as a ligand. Consequently, Chol-NH\(_2\) influences the pool of molecules available to act as a 9\textsuperscript{th} coordination site in the DTPA/Tm\textsuperscript{3+} complex by altering the dynamics of the hydrophilic environment of the bilayer. The resulting differences in the crystal field and \(\Delta \chi\) offers an element of explanation to the contrasting magnetic properties of the steroid doped bicelles.
Table 6.2 Cumulative occupation times of the 9th coordination site in the DTPA/Tm\(^{3+}\) complex. The occupation times were evaluated over the entire 30 ns trajectory and averaged over all six DMPE-DTPA/Tm\(^{3+}\) molecules in either the Chol-OH or Chol-NH\(_2\) bilayers. Oxygen atoms from water or from the carboxylic group in the ester bond of neighbouring phospholipids may act as a 9th ligand. In the case of the Chol-NH\(_2\) doped bilayer, one of the six DMPE-DTPA/Tm\(^{3+}\) molecule’s headgroup adopted a closed conformation resulting in an intramolecular 9th ligand (also on the oxygen from the carboxylic group in the chelator head). Table adapted from S. Isabettini et al.\(^{152}\)

<table>
<thead>
<tr>
<th></th>
<th>Chol-OH</th>
<th>Chol-NH(_2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMPC</td>
<td>15.6%</td>
<td>37.1%</td>
</tr>
<tr>
<td>DMPE-DTPA</td>
<td>-</td>
<td>0.3%</td>
</tr>
<tr>
<td>phosphate buffer</td>
<td>16.7%</td>
<td>-</td>
</tr>
<tr>
<td>H(_2)O</td>
<td>67.7%</td>
<td>62.6%</td>
</tr>
</tbody>
</table>

(iii) The orientation of the myristoyl tails with respect to the chelator headgroup was analyzed for all the DMPE-DTPA/Tm\(^{3+}\) molecules in the bilayer. The myristoyl tails define the long molecular axis. The crystal field of the DTPA/Tm\(^{3+}\) complex defines the principal magnetic axis. The orientation of the long molecular axis with respect to the principal magnetic axis determines the sign and magnitude of the magnetic susceptibility \(\Delta \chi\). This corresponds to the second contribution in the general theory of magnetic anisotropy of lanthanide-containing liquid crystals by Mironov et al.\(^{97}\) For example, rotations of rod-like molecules around their long molecular axis reduces the \(\Delta \chi\) of the molecule.\(^{97}\) Although the exact position of the principal magnetic axis was not computed, a simplified interpretation using various identified angles in the DMPE-DTPA/Tm\(^{3+}\) molecule is proposed. Phosphorus or thulium atoms were employed as a hinge for computing the angles shown in Figure 6.9A and 6.9B, respectively. The Tm\(^{3+}\)-P-CH\(_3\) angles shown in Figure 6.9A were measured for both lipid tails and averaged for each molecule. Analogously, the two P-Tm\(^{3+}\)-O angles shown in Figure 6.9B were averaged for each molecule. Both angles were averaged over all six DMPE-DTPA/Tm\(^{3+}\) molecules and the resulting difference between the two DMPC/steroid/DMPE-DTPA/Tm\(^{3+}\) (molar ratio 16:4:5:5) systems are presented in Table 6.3.

The results from the computed angles in Table 6.3 revealed a contrasting orientation of the phospholipid tails with respect to the chelator headgroup in the two steroid doped bilayer systems. Consequently, the different hydrophilic environment around the bilayer resulting from the replacement of Chol-OH with Chol-NH\(_2\) induced a change in the molecular geometry of the Tm\(^{3+}\) chelating phospholipids. The \(\Delta \chi\) of the DMPE-DTPA/Tm\(^{3+}\) molecule was altered through a change in orientation of the long molecular axis with respect to the principal magnetic axis. Final snap-shots of the simulated DMPC/Chol-NH\(_2\)/DMPE-DTPA/Tm\(^{3+}\) and DMPC/Chol-OH/ DMPE-DTPA/Tm\(^{3+}\) bilayers at 5 °C are presented in Figure 6.10. These results offer another element of explanation to the contrasting magnetic properties of the steroid doped bicelles.\(^{90}\)
Figure 6.9 Ball and stick molecular representation of the DMPE-DTPA/Tm$^{3+}$ molecules. Red atoms correspond to oxygen, orange phosphorus, grey carbon, blue nitrogen and green thulium. The hydrogen atoms are not represented for clarity. A) The angles formed by the positions of the thulium ion (Tm$^{3+}$), the phosphorus atom (P) and the last carbon of each of the myristoyl tails ($^1$CH$_3$ and $^2$CH$_3$) were evaluated. B) The angles formed by the position of the phosphorus atom (P), the thulium ion (Tm$^{3+}$), and two of the furthest deprotonated carboxylic acid oxygen atoms acting as ligands in the DTPA/Tm$^{3+}$ complex (O$^1$ and O$^8$) were evaluated. In both cases, the calculated angles are shown in green and yellow shades and where averaged for each DMPE-DTPA/Tm$^{3+}$ molecule. The hydrogen atoms on the myristoyl tails are not represented in the united-atom model for clarity. Figure adapted from S. Isabettini et al.\textsuperscript{152}
Table 6.3 Average angles of the DMPE-DTPA/Tm\(^{3+}\) molecules incorporated in either the Chol-OH or Chol-NH\(_2\) doped DMPC/steroid/DMPE-DTPA/Tm\(^{3+}\) (molar ratio 16:4:5:5) bilayers. Two different angles were calculated employing either phosphorus (P) or the complexed Tm\(^{3+}\)) as a hinge. The angles are schematically represented in Figure 6.9 and were averaged over all six of the DMPE-DTPA/Tm\(^{3+}\) molecules in the respective steroid doped bilayers. CH\(_3\) corresponds to the terminal carbon in the myristoyl tails and O to the deprotonated carboxylic acid oxygen atoms acting as ligands in the DTPA/Tm\(^{3+}\) complex. Table adapted from S. Isabettini et al.\(^{152}\)

<table>
<thead>
<tr>
<th>Steroid in the DMPC/steroid/DMPE-DTPA/Tm(^{3+}) bilayer</th>
<th>Average angle</th>
<th>Tm(^{3+})-P-CH(_3)</th>
<th>P-Tm(^{3+})-O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chol-OH</td>
<td>132°</td>
<td>96°</td>
<td></td>
</tr>
<tr>
<td>Chol-NH(_2)</td>
<td>108°</td>
<td>105°</td>
<td></td>
</tr>
</tbody>
</table>

Figure 6.10 Snapshot of the simulated DMPC/Chol-NH\(_2\)/DMPE-DTPA/Tm\(^{3+}\) (left) and DMPC/Chol-OH/DMPE-DTPA/Tm\(^{3+}\) (right) bilayers at 5 °C. Atoms and bonds constituting the amphiphiles are represented by a wireframe model and the ionic species with a space-filling model at their van der Waals radii, water is omitted. The steroid molecules are bolded in black and the DMPE-DTPA/Tm\(^{3+}\) lipids in yellow. The bilayer consists of 128 DMPC phospholipids, 32 steroid (16 mol%) molecules and 6 DMPE-DTPA/Tm\(^{3+}\) complexes in a 50 mM sodium phosphate buffer at a pH value of 7. A more detailed accounting of the bilayer constituents is available in Table 3.4. Figure adapted from S. Isabettini et al.\(^{152}\)
6.4 Thermal behavior of DMPC/Chol-NH₂/DMPE-DTPA/Tm³⁺ bicelles

Chol-OH increases the thermal resistance of DMPC/DMPE-DTPA/Tm³⁺ bicelle systems by inducing a liquid-ordered phase in the bilayer. In the absence of Chol-OH, the thermoreversible collapse of the bicelles into vesicles occurs at the phase-transition temperature of DMPC at 24 °C. A similar behavior was expected for Chol-NH₂ that removed the phase-transition temperature of pure DMPC bilayers and induced a type of liquid-ordered state of its own. However, the lack of a clear phase transition revealed from the molecular dynamic simulation makes it difficult to predict the behavior of DMPC/Chol-NH₂/DMPE-DTPA/Tm³⁺ (molar ratio 16:4:5:5) bicelles with changing temperature. Therefore, the structure and alignment of DMPC/Chol-NH₂/DMPE-DTPA/Tm³⁺ (molar ratio 16:4:5:5) bicelles was monitored upon heating and cooling in Figure 6.11A and 6.11B, respectively.

The radially averaged SANS curves in Figure 6.11A were recorded in the absence of a magnetic field to obtain structural information on the DMPC/Chol-NH₂/DMPE-DTPA/Tm³⁺ (molar ratio 16:4:5:5) sample at various temperatures. The data obtained at 5 °C was fitted with a Porod cylinder model with a log-normal size distribution (σ = 0.5). The bilayer thickness was 4.6 nm and the disk radius 61 nm, in line with literature and the findings of chapter 5. Moreover, the scattering intensity decays with q⁻², which is characteristic of planar disk-like structures. Upon heating, the dimension of the bicelle remained unchanged as the SANS curves at 10, 20, and 30 °C could be fitted with the same model. A clear difference was observed at 40 °C with the appearance of a kink at a q-value of 0.08 nm⁻¹. This phenomenon is consistent with the appearance of vesicles. The data was fitted with a form factor for spherical shells with a thickness of 4.6 nm and a log-normal distribution with μ = 43 nm and σ = 0.26. The unchanged bilayer thickness, when compared to the sample at lower temperatures, was consistent with the results from MD simulation showing no marked difference in the phase behavior of the bilayer. Similarly to Chol-OH doped systems, the bicelles reform upon cooling as evidenced by the SANS curve recorded at 20 °C in Figure 6.11A. The data could be fitted using the same Porod cylinder model and log-normal distribution employed for the fitting at 20 °C on heating.
6.4 Thermal behavior of DMPC/Chol-NH$_2$/DMPE-DTPA/Tm$^{3+}$ bicelles

Figure 6.11 A) Radially averaged SANS curves of DMPC/Chol-NH$_2$/DMPE-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5, [tL] 15 mM) at 5, 10, 20, 30 and 40 °C on heating and 20 °C on cooling. The curves were shifted vertically for clarity. The $q^{-2}$ decay of the scattering intensity is typical of disk-like structures and is shown with a red line. B) Alignment factor $A_f$ as a function of temperature of DMPC/Chol-NH$_2$/DMPE-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5, [tL] 15 mM) measured at 1.0 °C/min (circles) from 4 to 42 °C and at 0.1 °C/min (triangles) from 25 to 37 °C. The heating and cooling cycles are presented in red and blue, respectively. The highest degree of alignment of the bicelles was recorded at 4 °C with an $A_f$ of -0.77. Figure adapted from Isabettini et al. 152
The alignment factor $A_f$ of the DMPC/Chol-NH$_2$/DMPE-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5) sample was monitored as a function of temperature under a 8 T magnetic field in Figure 6.11B. The highest alignment was observed at 5 °C with an $A_f$ value of -0.78, in line with the finds from chapter 5. The alignment weakens with increasing temperature as the $A_f$ values decrease. The lower alignment may be explained by the increasing thermal energy of the solution, opposing the magnetic energy of the bicelle. A sharp collapse in the $A_f$ was observed upon heating at 35 °C, corresponding to the collapse of bicelles into vesicles. However, the bicelles do not regenerate at the same temperature upon cooling. The $A_f$ values only increase again at temperatures below 28 °C. The alignment was fully retrieved when cooling back to 5 °C. DMPC/Chol-OH/DMPE-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5) bicelles display a similar hysteresis effect where complex lipid rearrangements induce very different polymolecular assemblies upon heating from bicelles to vesicles than upon cooling from vesicles to bicelles.$^{16,29}$

To ensure that the hysteresis was not caused by the slow kinetics of rearrangement of the polymolecular assemblies, the bicelle-to-vesicle collapse was interrupted upon heating at an $A_f$ value of -0.1 by introducing a gentler cooling cycle of 0.1 °C/min in Figure 6.11B (blue empty triangles). The fall in alignment immediately stopped and remained constant until the limiting temperature of 28 °C was reached and the bicelles start to regenerate. Here again the regeneration process could be immediately interrupted at an $A_f$ value of -0.3 by applying a gentler heating cycle of 0.1 °C/min (red filled triangles). These results show the highly-responsive nature of the DMPC/Chol-NH$_2$/DMPE-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5) system, where the degree of alignment is readily tuned by thermal means within the hysteresis region. Previously reported DMPC/Chol-OH/DMPE-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5) bicelles provided only weak alignments above 5 °C, emphasizing the viability of moving toward Chol-NH$_2$ doped systems when working at higher temperatures.

The possibility of further fine tuning the magnetic susceptibility anisotropy $\Delta \chi$ of the bilayer was demonstrated by employing an aminocholesterol conjugate, namely Chol-C$_2$OC$_2$-NH$_2$. The thermal behavior of the magnetic alignment of DMPC/Chol-C$_2$OC$_2$-NH$_2$/DMPE-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5) bicelles was characterized in Figure 6.12. The $A_f$ were always lower than those of the corresponding DMPC/Chol-NH$_2$/DMPE-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5) system, in line with the findings in chapter 5. Both bicelle systems were governed by analogous physico-chemical properties, displaying a hysteresis upon heating and cooling. However, the collapse and regeneration of the magnetic alignment was shifted towards lower temperatures. The molecular engineered aminocholesterol derivatives allowed for both the fine tuning of the magnetic response and the thermal properties of the bicelle systems.
6.4 Thermal behavior of DMPC/Chol-NH₂/DMPE-DTPA/Tm⁢³⁺ bicelles

Figure 6.12 Alignment factor $A_f$ as a function of temperature of DMPC/Chol-C₂OC₂-NH₂/DMPE-DTPA/Tm³⁺ (molar ratio 16:4:5:5, [tL] 15 mM) compared to DMPC/Chol-NH₂/DMPE-DTPA/Tm³⁺ (molar ratio 16:4:5:5, [tL] 15 mM) measured at 1.0 °C/min. The heating and cooling cycles are presented in red and blue, respectively.
6.5 Conclusion

Replacement of Chol-OH with Chol-NH$_2$ in DMPC/steroid/DMPE-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5) bicelles enhances the magnetic energy of the lipid bilayer and results in unprecedented gains in magnetic alignability. Since the dimension of the bicelles remained unchanged, the increase in magnetic energy must be the result of an enhanced magnetic susceptibility anisotropy $\Delta\chi$ of the Ln$^{3+}$ chelating phospholipid DMPE-DTPA/Tm$^{3+}$.

The underlying mechanisms behind the magnetic response were investigated by comparing the physico-chemical forces governing a Chol-NH$_2$ and a Chol-OH doped DMPC monolayer, bilayer and bicelle as outlined in Figure 6.13.

<table>
<thead>
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<th>Characterization Technique</th>
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<th>Cholesterol Chol-OH 16 mol%</th>
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<td>Monolayer Condensation</td>
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<td>Lipid Chain Order</td>
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<tr>
<td>C MD Study of the Bilayer</td>
<td>DMPC/steroid/DMPE-DTPA/Tm$^{3+}$</td>
<td>Extent of Hydrogen Bonding</td>
<td>+</td>
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</tr>
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<td>D SANS Study of Magnetically Alignable Bicelles</td>
<td>DMPC/steroid/DMPE-DTPA/Tm$^{3+}$</td>
<td>Bicelle Magnetic Alignability at 5 °C</td>
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<td>Temperature Resistance of Bicellar Alignment</td>
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Figure 6.13 The characterization techniques employed in the proposed multiscale bottom-up comparative investigation of Chol-NH$_2$ and Chol-OH mixed with DMPC are presented in the first column. They are labeled from A to D, analogously to the introductory Figure 6.1. The steroid content was 16 mol% to best imitate the composition of the bicelle. The identified and characterized key physico-chemical properties are presented in the second column. The impact of Chol-NH$_2$ and Chol-OH on these properties is presented with a + (property is influenced) or – (property is not influenced) sign in the last two columns. The relative degree of influence induced by the respective steroids is stressed with multiple + signs. Figure adapted from Isabettini et al.$^{152}$
6.5 Conclusion

The results from MD simulation were in good agreement and complementary to experimental data obtained from different methods including a model monolayer study and a SANS study of the bicelles. The combined monolayer study (Figure 6.13A) and MD bilayer simulation (Figure 6.13B) of the simplified Chol-NH$_2$/DMPC and Chol-OH/DMPC systems revealed that Chol-NH$_2$ underwent significantly more hydrogen bonding interactions with neighboring DMPC lipids. Furthermore, Chol-OH displayed a larger condensation effect and an enhanced degree of order in the lipid monolayers at steroid contents of 16 and 20 mol% and at 5 °C. This effect was inverted at higher steroid contents, where Chol-NH$_2$ becomes more dominant and induced a larger condensation effect. The MD simulation of the DMPC/Chol-NH$_2$/DMPE-DTPA/Tm$^{3+}$ bilayer (Figure 6.13C) demonstrated how the hydrophilic environment was altered around the headgroup region of the DMPE-DTPA/Tm$^{3+}$ lanthanide-phospholipid complex. The positively charged primary amine headgroup of Chol-NH$_2$ influenced the pool of molecules available to act as a 9th coordination site in the DTPA/Tm$^{3+}$ complex. This may result in alterations of the crystal field of the molecule. The enhanced $\Delta \chi$ could also result from a change in the angle between the principal magnetic axis and the long molecular axis in DMPE-DTPA/Tm$^{3+}$. The introduction of Chol-NH$_2$ allowed for an enhanced thermal resistance of the DMPC/Chol-NH$_2$/DMPE-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5) bicelles (Figure 6.13D). Large $A_f$ of -0.6 were achieved up to 35 °C upon heating before the bicelles collapsed into vesicles. The bicelles could be regenerated upon cooling below 28 °C. The thermoreversible nature of these systems combined with their high magnetic response makes them valuable candidates for the development of future smart soft-materials. For example, DPPC/Chol-OH/DPPE-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5) bicelles have been employed to generate switchable anisotropy in optical gels. The additional thermal resistance offered by Chol-NH$_2$ opens the possibility to work with the shorter DMPC lipid, whilst simultaneously benefiting from the enhanced magnetic response of the DMPC/Chol-NH$_2$/DMPE-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5) bicelles. Moreover, the existence of a temperature-alignment hysteresis, combined with the rapid response of the system to temperature change, allows for a fine-tuning of the degree of alignment of the colloidal system. These aspects will be exploited in chapter 9, moving on to the third level of the S-PRO$^2$ scheme in Figure 1.1.
7 Bicelle Design with Ln\(^{3+}\) Chelating Cholesterol Conjugates.

The content of this chapter has been partially published by Isabettini, S.; Liebi, M.; Kohlbrecher, J.; Ishikawa, T.; Windhab, E. J.; Fischer, P.; Walde, P.; Kuster, S. in "Tailoring Bicelle Morphology and Thermal Stability with Lanthanide-Chelating Cholesterol Conjugates." *Langmuir* 2016, **32**, 9005–9014.\(^{15}\)

The single change in chemical nature of the hydroxyl polar head group of Chol-OH into a primary amine in Chol-NH\(_2\) delivered unprecedented gains in magnetic response by selectively tuning the magnetic susceptibility of the bicellar bilayer \(\Delta \chi\). With further synthetic modification, it is possible to attach a Ln\(^{3+}\) chelating DTPA moiety directly onto the steroid backbone (see chapter 10). The steroid moiety acts as an anchor to supply more paramagnetic Ln\(^{3+}\) to the planar part of the bicelle, enabling an enhanced magnetic response. This possibility was first demonstrated by M. Liebi *et al.* through the synthesis and incorporation of cholesterol-diethylenetriaminepentaacetate (Chol-DTPA) within the bicelle bilayer.\(^{31}\) The steroid rings were chemically bound to DTPA with an amino-acid lysine as a linker, delivering a substantial gain in bicelle size and magnetic alignment. However, the presence of a large DTPA/Ln\(^{3+}\) head group considerably changes the geometry or critical packing parameter (CPP) of the Chol-DTPA amphiphile. The inversed cone-like nature of Chol-OH is replaced with a cone-like geometry in the Chol-DTPA/Ln\(^{3+}\) complex. Therefore, these amphiphilic additives permit tailoring of the bicelle dimensions, whilst simultaneously benefitting from an enhanced thermal stability of the bilayer (caused by the liquid-ordered phase) and a stronger magnetic response (caused by the association of many more Ln\(^{3+}\) at the surface of the bicelle). The additional degrees of freedom induced by these specially designed multiphospholipidic systems calls for further investigation.

Engineering of polymolecular assemblies is only feasible by understanding the chemical and physical forces that govern both the hydrophilic and hydrophobic interactions between the individual molecules that compose them. Minor changes on the molecular level have the potential to induce very different packing behaviors and result in unique polymolecular species.\(^{167}\) In this chapter, we explore the morphology of bicelles resulting from the incorporation of a series of newly synthesized Chol-C\(_n\)-DTPA and Chol-C\(_2\)OC\(_2\)-DTPA compounds presented in Figure 7.1. These Ln\(^{3+}\) chelating cholesterol conjugates were synthesized by multistep reactions involving cholesteryl chloroformate...
(precursor of the sterol moiety labeled chol), a diamine-bearing linker (precursor of \( C_n \) and \( C_2-O-C_2 \)) and DTPA bisanhydride (precursor of the DTPA-chelator moiety).\(^\text{120}\)
The detailed synthesis procedure and molecular characterization is available in chapter 7. The range of attainable architectures was first revealed by cryo-TEM. Based on the observed geometries, an appropriate form factor for fitting of the considered sample’s radially averaged SANS curve was chosen, delivering a statistically more relevant description of the system. Bicelle geometries were achieved through the combined addition of Chol-OH and Chol-DTPA conjugates to a DMPC/DMPE-DTPA/Tm\(^{3+} \) bilayer. The number of carbon atoms in the linker offered control over the bicelle’s hydrodynamic radius. Polarity was further introduced in the carbon chain of the linker to identify the forces responsible for the formation of specifically sized bicelles. The resulting magnetic alignability of the assemblies was characterized by birefringence experiments. In an ultimate step, the thermal resistance of these novel polymolecular assemblies was highlighted. The present study aimed at expanding the toolbox for intelligent design of bicelle structures on a molecular level, covering the first and second layers of the S-PRO\(^2 \) scheme in Figure 1.1.

**Figure 7.1** Molecular structures of the synthesized and employed Chol-\( C_n \)-DTPA (\( n: 2, 5, 6 \)) and Chol-C\(_2\)O\(_2\)-DTPA compounds. Detailed synthesis protocols are available in chapter 10. Figure adapted from S. Isabbettini et al.\(^{15} \)
7.1 DMPC/Chol-C\textsubscript{n}-DTPA/DMPE-DTPA/Tm\textsuperscript{3+} (molar ratio 16:4:5:9)

Figure 7.2 Cryo-TEM micrographs of DMPC/Chol-C\textsubscript{2}-DTPA/DMPE-DTPA/Tm\textsuperscript{3+} (molar ratio 16:4:5:9, [tL] 15 mM) flash frozen at A) 5 °C and at B) 40 °C. C) Cryo-TEM micrographs of DMPC/Chol-C\textsubscript{6}-DTPA/DMPE-DTPA/Tm\textsuperscript{3+} (molar ratio 16:4:5:9, [tL] 15 mM) flash frozen at 5 °C. If not specified, the sample holder tilt angle was at 0°. Each scale bar represents 200 nm. Figure adapted from M. Liebi and S. Isabettini et al.\textsuperscript{15,30}

Chol-C\textsubscript{2}-DTPA and Chol-C\textsubscript{6}-DTPA were integrated within the phospholipid bilayers composed of DMPC and DMPE-DTPA/Tm\textsuperscript{3+} at a total lipid concentration of 15 mM. The resulting polymolecular assembly structures were investigated by cryo-TEM and DLS. In this subsection, all samples mentioned were composed of DMPC/Chol-C\textsubscript{n}-DTPA/DMPE-DTPA/Tm\textsuperscript{3+} (molar ratio 16:4:5:9). The linker length \( n \) was varied and the samples will be referred to through the Chol-C\textsubscript{n}-DTPA molecule name for simplicity.

For the Chol-C\textsubscript{2}-DTPA system, cryo-TEM micrographs revealed that long cylindrical assemblies were preferentially formed over bicelles at both investigated temperatures in Figure 7.2A and 7.2B. Tilting the sample holder by 30° showed assemblies with the same thickness and contrast as in the non-tilted view in Figure 7.2B, supporting the existence of a round cross-section in these structures. Cryo-TEM micrographs of samples containing Chol-C\textsubscript{6}-DTPA kept at 5 °C prior to flash freezing revealed the presence of small bicelles and several long and thin ribbon-like assemblies in Figure 7.2C. DLS measurements supported the presence of bicelles with a hydrodynamic radius of 18.3 nm at 5 °C. Both Chol-DTPA molecules induced polymolecular assembly structures with a higher degree of curvature than bicelles.
7.2 DMPC/Chol-OH/Chol-C_n-DTPA/DMPE-DTPA/Tm^{3+} (molar ratio 16:2:2:5:7)

In order to reduce the curvature resulting from the incorporation of the Chol-DTPA conjugates and benefit from the molecule’s potential for generating magnetically alignable bicelle structures, 50% of the Chol-C_n-DTPA was replaced with Chol-OH. The resulting polymolecular assembly structures were investigated by Cryo-TEM, DLS and SANS. In this subsection, all samples mentioned were composed of DMPC/Chol-OH/Chol-C_n-DTPA/DMPE-DTPA/Tm^{3+} (molar ratio 16:2:2:5:7). The linker length n was varied and the samples will be referred to through the Chol-C_n-DTPA molecule name for simplicity.

For the mixture containing Chol-C_2-DTPA, bicelles were observed in both edge-on and face-on view at 5 °C in Figure 7.3A. Holes within the bicelle disk appeared as white spots on the micrographs. Bright patches may be distinguished on some of the polymolecular assemblies presented in Figure 7.3A. Small holes were found to a larger extent at 40 °C as emphasized with arrows in Figure 7.3B. By introducing cholesterol, bicelles formed pref-
7.2 DMPC/Chol-OH/Chol-C₅-DTPA/DMPE-DTPA/Tm³⁺ (molar ratio 16:2:2:5:7)

Differentially over the cylindrical assemblies. Chol-OH, with its inverted cone-like molecular geometry, acted against the curvature induced by the Chol-DTPA conjugates. The presence of Chol-C₅-DTPA also induced curvature, resulting in smaller assembly structures. DLS measurements revealed an average hydrodynamic radius of 47 nm at 5 °C, which is 15% smaller than bicelles containing only cholesterol. The existence of several non-disk shaped assemblies containing multiple small holes within the bilayer was evidenced by the lighter spots in the cryo-TEM micrograph of Figure 7.3C. The appearance of perforations within the bilayer may have resulted from higher local concentrations of the cone-like lipids. Such a possibility is conceivable when considering the diversity of forces induced by the coexistence of four structurally distinct amphiphiles in the bilayer.

Systems containing Chol-C₆-DTPA revealed long ribbon-like assemblies together with bicelles with concentric holes as shown in Figure 7.3D. The part of the ribbon indicated with a tilted arrow in Figure 7.3D appeared in face-on view and in edge-on view respectively if the sample holder was tilted from 0° to 30°. Ribbon-like structures increase the edge area of the assemblies when comparing to bicelles of equivalent surface area. The replacement of Chol-C₆-DTPA with 50% of Chol-OH was not sufficient to counterbalance the curvature-inducing nature of the Chol-C₆-DTPA molecule. Nevertheless, an increased assembly size was evident from Cryo-TEM micrographs and confirmed by DLS measurements as the hydrodynamic radius was, with 40.9 nm, about twice as big as in the mixture without Chol-OH.

The existence of perforations within the bilayer of bicelles containing Chol-C₂-DTPA was supported by SANS measurements at 5 °C presented in Figure 7.4A. The best fit was achieved with a form factor describing a disk with a concentric hole of radius 13.7 nm surrounded by a rim of 56.7 nm. The total radius of 70.4 nm was in good agreement with the hydrodynamic radius of 71.5 nm obtained from DLS measurements at 5 °C. The geometric diversity of bicelles containing Chol-C₅-DTPA was confirmed by SANS measurements in Figure 7.4B. The radial averaged scatter curve of the sample at 5 °C was fitted with a form factor for flat cylinder disks. A log-normal size distribution proved ineffective for fitting of the data. Instead, a fractal distribution was used to account for the large size disparity that was particularly pronounced in favor of smaller species. A bilayer thickness of 4.8 nm resulted from the fitting procedure and was in good agreement with literature. Furthermore, the computed hydrodynamic radius of 47 nm was in agreement with the DLS results. The scattering intensity of both samples decays with q⁻², which is characteristic of planar disklike structures. The intensity was multiplied by q² and plotted as a function of the scattering vector q in Figure 7.4C. This emphasizes scattering resulting from other geometric considerations. The proposed contrasting nature in the bicelles formed from the Chol-C₂-DTPA and Chol-C₅-DTPA systems is confirmed by the observable difference in these SANS curves.
Figure 7.4 Radially averaged SANS curve [x] and fitting [red solid line] of A) DMPC/Chol/Chol-C_2-DTPA/DMPE-DTPA/Tm^{3+} and B) DMPC/Chol/Chol-C_5-DTPA/DMPE-DTPA/Tm^{3+} (molar ratio 16:2:2:5:7, [tL] 15 mM) measured at 5 °C. The respective form factors employed for the fittings are schematically represented. The geometric difference between the two samples is emphasized in the SANS curves in caption C) where the intensity I was multiplied by the square of the scattering vector q^2. Figure adapted from S. Isabettini et al. 15

7.3 Molecular modeling of Chol-C_n-DTPA/Tm^{3+}

Chol-C_2-DTPA and Chol-C_6-DTPA complexed with Tm^{3+} were geometrically optimized with a molecular modeling routine MM2. The energetic ground configuration of the molecules was identified to further speculate on reasonable conformational changes that could occur in the bilayer’s environment. This simplified picture offered a basis to understand the observed polymolecular architectures without proceeding to costly and time-consuming molecular dynamics simulations. The amount of thermal-induced conformational changes and electrostatic forces that the molecules would be exposed to are not considered in this first step. However, by observing interactions already occurring within the molecule in a simplified environment, sites prone to interact with the surroundings are identified. A realistic average molecular geometry for the Chol-DTPA conjugates was constructed based on the range of conformations they may adopt. The results are presented and schematically analyzed in Figure 7.5A.
When comparing Chol-C\textsubscript{2}-DTPA and Chol-C\textsubscript{6}-DTPA, it was evident that the length of the carbon chain of the linker had a significant impact on the geometry of the molecules. The all-trans conformation of the hydrocarbon chain suggested by MM2 calculations is highly unlikely in the bicelle environment. Different conformations induced by the surrounding environment’s thermal energy or electrostatic forces are highly conceivable.
7 Bicelle Design with Ln$^{3+}$ Chelating Cholesterol Conjugates.

The low energy barrier for rotation around carbon-carbon single bonds essentially allows free rotation about their axis in the temperature range of this study. Van der Waals interactions between adjacent lipids in the bilayer would remove this conformational freedom from the aliphatic chains of the phospholipids that are in the solid-ordered phase. The carbon chains forming the linkers would not benefit from such stabilizing interactions as they are exposed to the unfavorable hydrophilic environment of the bilayer and have freedom to undergo conformational changes. The resulting molecular reorientations deviate from the fully extended zigzag arrangement and lead to a larger effective head group size, which attributes a cone-like geometry to the amphiphilic Chol-DTPA conjugate. Moreover, the amount of different conformations that the six carbon long linker may adopt is considerably larger than for the two carbon counterpart. The added degrees of freedom of the Chol-C$_6$-DTPA head group further reduces the CPP of the amphiphile. Consequently, the tendency of forming polymolecular architectures with an enhanced degree of curvature is highly probable. Moreover, MM2 calculations revealed the possibility of forming a hydrogen bond stabilized ring structure between the carbamate and the amide bond closest to the DTPA moiety in Chol-C$_2$-DTPA. This ring formation could further reduce the length of the spacer and limit the molecule’s conformation freedom, forcing it to assume a cone-like geometry (CPP<1).

7.4 Correlation between the linker length of Chol-C$_n$-DTPA and bicelle geometry

In order to test the hypothesis that arose from molecular modeling, the order parameter $S$ of the polymolecular assemblies was measured at higher magnetic field strengths of 33 T. Such high magnetic field strengths allow full saturation of alignment of the bicelle samples to be reached. These conditions permit the determination of the order parameter $S$ and quantitative data analysis as discussed by Liebi et al.$^{29}$ For bicelle systems containing Tm$^{3+}$, the maximum value of $S$ is 1. The magnetic field strength $B$ at which the order parameter reaches half of its maximum value ($S_{1/2} = 0.5$) is readily computed and directly related to the product $n\Delta\chi$ of the number of molecules $n$ in one bicelle and the molar magnetic susceptibility anisotropy $\Delta\chi$. This results in $B(S_{1/2})$, which is a direct measurement of the magnetic alignability of the bicelles. The magnetic field required to reach half the order parameter $B(S_{1/2})$ is reported in Figure 7.5B. The lower $B(S_{1/2})$, the stronger the bicelle alignment in the magnetic field. The increase in $B(S_{1/2})$ when moving from 5 to 50 $^\circ$C may be explained by higher thermal energy that must be surpassed by the magnetic energy of the bicelles for alignment to occur.$^{106}$ An increase in magnetic alignability upon replacement of 50% of the Chol-C$_6$-DTPA with cholesterol was observed as the $B(S_{1/2})$ values dropped when comparing the filled and empty circles in Figure 7.5B. The enhanced alignment goes in line with the aforementioned increase in bicelle size. Moreover, samples containing Chol-C$_2$-DTPA exhibited considerably lower $B(S_{1/2})$ values, confirming its superior capacity of creating
7.4 Correlation between the linker length of Chol-C\textsubscript{n}-DTPA and bicelle geometry

larger and more alignable assembly structures when comparing to the six-carbon long linker Chol-C\textsubscript{6}-DTPA compound.

![Figure 7.6](image)

**Figure 7.6** Hydrodynamic radius $R_H$ of the bicelles as determined by DLS (black circles) and their birefringence signal at 5 °C and 5.5 T (red squares) as a function of the number of carbon atoms, $n$, in the Chol-C\textsubscript{n}-DTPA linker. Filled markers correspond to the mixtures with cholesterol: DMPC/Chol-OH/Chol-C\textsubscript{n}-DTPA/DMPE-DTPA/Tm\textsuperscript{3+} (molar ratio 16:2:2:5:7, [tL] 15 mM). Empty markers are from a DMPC/Chol-C\textsubscript{2}-DTPA/DMPE-DTPA/Tm\textsuperscript{3+} (molar ratio 16:4:5:9, [tL] 15 mM) sample. A schematic representation of the birefringence experiment is presented on the right hand side. The polarized laser shines through the sample cuvette exposed to an external magnetic field $B$. A sample composed of magnetically aligned bicelles will result in a strong birefringence signal (top zoom), whilst weakly aligned bicelles do not (bottom zoom). Figure adapted from S. Isabettini et al.\textsuperscript{15}

These results were further supported by comparing the hydrodynamic radius and magnetic alignability of bicelles at 5.5 T containing Chol-C\textsubscript{n}-DTPA with $n$: 2, 5, and 6 at 5 °C. The results of birefringence measurements were plotted as a function of the number of carbon atoms in the linker in Figure 7.6. There exists an inverse correlation between the number of carbon atoms in the linker separating the steroid backbone to the chelator moiety and the subsequent mean bicelle radius as revealed by DLS measurements. The enhanced birefringence signal with increasing radius of the polymolecular assemblies supported the existence of this trend. Larger magnetic alignability was correlated to the increased cumulative contribution of the magnetic orientation energy $E_{mag}$ of individual molecules in the bicelle when it increased in size.

The results suggested that curvature increased within the polymolecular assemblies with increasing number of carbons in the linker chain. The interactions of Chol-OH with
neighboring phospholipids in the bilayer are dominated by van der Waals forces between the hydrophobic steroid backbone and the aliphatic chains of the phospholipids. The position of Chol-OH within the bilayer is fixed and majorly dictated by these forces.\textsuperscript{71,83} Therefore, increasing the linker chain length would tend to push out the DTPA-chelator head group into the hydrophilic environment surrounding the bicelle. The degree of freedom that the amphiphile’s head group has to adopt different conformations increases with the number of atoms in the linker. This induces an increase in effective head group size, resulting in smaller packing parameters as the molecules take on more cone-like geometries and generate curvature in the final polymolecular assemblies. These results go in line with the hypothesized schematic based on MM2 calculation disclosed in Figure 7.5A.

To further support these affirmations, Chol-C\textsubscript{2}OC\textsubscript{2}-DTPA was synthesized and incorporated in the bilayer. This molecule differs from Chol-C\textsubscript{5}-DTPA in that the carbon atom in the middle of the linker chain was replaced with an oxygen atom as shown in Figure 7.1. By introducing polarity in the carbon chain of the linker, the ability of the hydrophilic environment to stabilize the linker structure and hence limit its conformational degrees of freedom was investigated.

Cryo-TEM micrographs of the sample at 5 °C presented in Figure 7.7A supported the existence of bicelles upon the incorporation of Chol-C\textsubscript{2}OC\textsubscript{2}-DTPA. These bicelle structures were similar to those obtained with Chol-C\textsubscript{5}-DTPA revealed in Figure 7.3. The presence of Chol-C\textsubscript{2}OC\textsubscript{2}-DTPA in the bilayer further yielded systems with comparable alignability as those with Chol-C\textsubscript{5}-DTPA, evidenced by their similar birefringence signal: 3.31 × 10\textsuperscript{-6} for the Chol-C\textsubscript{5}-DTPA containing bicelles compared to 3.42 × 10\textsuperscript{-6} for the Chol-C\textsubscript{2}OC\textsubscript{2}-DTPA system at 5 °C. The similarity between the bicelle architectures resulting from these two compounds was further confirmed by SANS measurements. The radially averaged SANS curve of the DMPC/Chol-OH/Chol-C\textsubscript{2}OC\textsubscript{2}-DTPA/DMPE-DTPA/Tm\textsuperscript{3+} (molar ratio 16:2:2:5:7) sample at 5 °C in Figure 7.7B yielded a computed hydrodynamic radius of 48 nm. This was in strong agreement with a mean hydrodynamic radius of 49 nm as measured in DLS. These values were analogous to those obtained for bicelle systems containing Chol-C\textsubscript{5}-DTPA. Both amphiphiles interacted in similar ways with the neighboring components in the bilayer, leading to complementary polymolecular structures. The introduction of polarity within the linker’s chain did not permit control over the bicelle’s size; the linker’s chain length and its resulting freedom for conformational change is the determining parameter.
7.4 Correlation between the linker length of Chol-$C_n$-DTPA and bicelle geometry

Figure 7.7 DMPC/Chol-OH/Chol-$C_2OC_2$-DTPA/DMPE-DTPA/Tm$^{3+}$ (molar ratio 16:2:2:5:7, [tL] 15 mM) at 5 °C A) Cryo-TEM micrograph of the flash-frozen sample with a sample holder tilt angle at 0°, the scale bar represents 200 nm and B) radially averaged SANS curve fitted with a form factor for flat cylinder disks. Figure adapted from S. Isabettini et al.\textsuperscript{15}
7.5 Thermal resistance of bicelles containing Chol-C<sub>n</sub>-DTPA

DMPC/Chol-OH/DMPE-DTPA/Ln<sup>3+</sup> (molar ratio 16:4:5:5) bicelles are capable of withstanding temperatures as high as 40 °C due to the induced liquid-ordered phase in the bilayer. Without Chol-OH, the thermo-reversible collapse into vesicles would occur at the phase transition temperature of DMPC at 24 °C when the lipids move from the solid-ordered to the liquid-disordered phase. With both Chol-OH and Chol-DTPA conjugates in the bilayer, the temperature range in which disk-like assemblies exist was enhanced.

The thermal resistance of the multilipidic systems was explored with DMPC/Chol-OH/Chol-C<sub>5</sub>-DTPA/DMPE-DTPA/Tm<sup>3+</sup> (molar ratio 16:2:2:5:7) by heating the samples to temperatures above 5 °C. The appearance of a large concentric hole within the bicelles was observed at 25 °C in the Cryo-TEM micrographs presented in Figure 7.8A. The similarity between this Chol-C<sub>5</sub>-DTPA system and the Chol-C<sub>2</sub>OC<sub>2</sub>-DTPA system in DMPC/Chol-OH/Chol-C<sub>2</sub>OC<sub>2</sub>-DTPA/DMPE-DTPA/Tm<sup>3+</sup> was also evident at 25 °C in the Cryo-TEM micrograph in Figure 7.8B. Analogously, large concentric holes appeared within the bicelles. The transformation was noticeable by SANS for the system containing Chol-C<sub>5</sub>-DTPA in Figure 7.8C where a change in gradient appeared at a q value of 2.5 for 10 °C and, more pronounced, at 25 °C. These curves confirmed the appearance of a disk with a concentric hole as the data at 25 °C was fitted with a form factor of a flat cylindrical shell of thickness 15 nm and a core radius with a log-normal distribution of µ = 15 nm and σ = 0.5. The presence of this architecture was also evident at 10 °C where SANS fittings yielded the equivalent parameters except for a larger shell of 20 nm. Furthermore, the radial averaged scatter curves for bicelles containing Chol-C<sub>2</sub>OC<sub>2</sub>-DTPA in Figure 7.8D showed the same trends as for Chol-C<sub>5</sub>-DTPA along the studied temperature range. The SANS curve at 25 °C was fitted with a form factor of a flat cylindrical shell of thickness 13 nm and a core radius with a log-normal distribution of µ = 19 nm and σ = 0.5. The appearance of a concentric hole in the bilayer was previously reported for DMPC/Chol-OH/DMPE-DTPA/Tm<sup>3+</sup> bicelles at an equivalent temperature range upon heating. This suggests that the mechanics of lipid rearrangement within the bilayer remained highly influenced by cholesterol-phospholipid interactions. The formation of a single hole at the core of the bicelle could be a result of coalescence of the smaller perforations observed at lower temperatures. Under certain conditions of bilayer line tension, such a phenomenon is common and has been investigated by Monte Carlo simulation.

Chol-C<sub>2</sub>-DTPA and Chol-C<sub>5</sub>-DTPA in a 1:1 mixture with Chol-OH in the phospholipid bilayer resulted in bicelle structures at 40 °C as evidenced in the Cryo-TEM micrographs in Figure 7.3B and in Figure 7.8A, respectively. The planar nature of the polymolecular assembly was observed from side-on view where they appear as a black line on the micrographs. The system containing Chol-C<sub>2</sub>OC<sub>2</sub>-DTPA continued to yield polymolecular...
7.5 Thermal resistance of bicelles containing Chol-\(C_n\)-DTPA

Figure 7.8 Cryo-TEM micrograph of A) DMPC/Chol-OH/Chol-C\(_5\)DTPA/DMPE-DTPA/Tm\(^{3+}\) (molar ratio 16:2:2:5:7, [tL] 15 mM) and B) DMPC/Chol-OH/Chol-C\(_2\)OC\(_2\)-DTPA/DMPE-DTPA/Tm\(^{3+}\) (molar ratio 16:2:2:5:7, [tL] 15 mM) flash-frozen at 25 °C with a sample holder tilt angle at 0°, the scale bars represent 200 nm. Radially averaged SANS curve of C) DMPC/Chol-OH/Chol-C\(_5\)DTPA/DMPE-DTPA/Tm\(^{3+}\) (molar ratio 16:2:2:5:7, [tL] 15 mM) at 10 and 25 °C, and of D) DMPC/Chol-OH/Chol-C\(_2\)OC\(_2\)-DTPA/DMPE-DTPA/Tm\(^{3+}\) (molar ratio 16:2:2:5:7, [tL] 15 mM) at 13 and 25 °C. A form factor for a disk with a concentric hole was employed in all fittings. Figure adapted from S. Isabettini et al.\(^{15}\)

Assemblies of similar architecture to those containing Chol-C\(_5\)-DTPA at 40 °C as evidenced in the Cryo-TEM micrograph in Figure 7.9B. The ability of forming stable bicelles at temperatures as high as 40 °C was highlighted by the SANS data in Figure 7.9C. Unlike previously reported DMPC/DMPE-DTPA/Tm\(^{3+}\) bicelle systems, the collapse into vesicles did not occur at this temperature as there was no evidence of a characteristic
Figure 7.9 Cryo-TEM micrographs of A) DMPC/Chol-OH/Chol-C₅-DTPA/DMPE-DTPA/Tm³⁺ (molar ratio 16:2:5:7, [tL] 15 mM), B) DMPC/Chol-OH/Chol-C₂OC₂-DTPA/DMPE-DTPA/Tm³⁺ (molar ratio 16:2:5:7, [tL] 15 mM) flash-frozen at 40 °C with a sample holder tilt angle at 0°, the scale bar represents 200 nm and C) the radially averaged SANS curves of the sample at 40 °C. Figure adapted from S. Isabettni et al.¹⁵

kink in the q-range of 0.2 nm⁻¹ for both the Chol-C₅-DTPA and Chol-C₂OC₂-DTPA systems.¹⁶,²⁸ However, the SANS results do not discard the possible existence of small perforations in the bilayer. The cryo-TEM micrographs revealed mainly planar assemblies (see Figure 7.9A and 7.9B), concentric holes were not observed at 40 °C. The introduction of Chol-OH and Chol-DTPA conjugates within the bilayer enhanced the thermal stability of the polymolecular assemblie by exploiting the stabilization effect.
induced by the steroid backbone whilst simultaneously benefiting from the added functionality of an engineered polar head group. The combined possibility of fine-tuning the Chol-DTPA conjugate’s molecular structure to generate desired polymolecular assembly architectures in a specific temperature range results in a unique engineering toolbox for lanthanide-ion doped, magnetically responsive soft materials.

7.6 Conclusion

Design of DMPC/DMPE-DTPA/Tm$^{3+}$ based bicelle systems with Chol-DTPA conjugates demands for careful consideration of the molecular structure of the linker unit employed. The number of atoms in the linker chain was a key parameter to control the adopted geometry of the molecule and to tailor the degree of curvature introduced in the polymolecular assembly’s geometry. Adding atoms in the linker chain resulted in a larger effective head group size by an increased degree of freedom for adopting different conformations. The introduction of a polar component with an ether bond within the linker structure of Chol-C$_2$O$_2$DTPA was not sufficient to stabilize the head group through a reduction of repulsive hydrophobic interactions with the surrounding environment. The inverse correlation established between the number of carbon atoms in the linker and bicelle radius provides guidelines for tailoring the polymolecular assembly’s geometry with Chol-DTPA conjugates following a simple, packing parameter based, model. Short linker structures, such as in Chol-C$_2$-DTPA, were required to guaranty the largest and most magnetically alignable bicelle structures. The ideas conveyed in this work could provide essential building blocks towards a more systematic approach for the design of optimal bicelle architectures. The resulting degree of control could, for example, be most welcome for the study of bilayer-associated molecules by NMR spectroscopy where the magnetic alignment of the employed bicelles must be carefully
tailored based on the nature of the study. Although this is commonly achieved by controlling the bicelle’s size, through the ratio of the composing phospholipids and the overall lipid concentration, working with Chol-DTPA conjugates offers alternative possibilities. Furthermore, the possibility of forming stable bicelle structures at temperatures equivalent to physiological conditions up to 40 °C is a valuable feature offered by these cholesterol-based bilayer-doping compounds. Tuning temperature-resistance of self-assembled polymolecular assemblies is of great interest in numerous applications including drug-delivery for dermal applications, and the development of smart optical gels. Furthermore, the synthesized Chol-DTPA conjugates alone may find use as contrast-enhancing agents in magnetic resonance imaging. Their ability to integrate into phospholipid bilayers is of particular interest for such studies.
8 Switching the Magnetic Susceptibility with DMPE-Glu-DTPA


The magnetic alignability of bicelles is readily tuned by changing their size or the $\Delta \chi$ of the bilayer lipids. The former technique is intrinsically bound to the region of the phase diagram guaranteeing the formation of bicelles. Methods aiming towards manipulating the $\Delta \chi$ of the bilayer are comparatively more robust, flexible and lacking. Both the magnitude and direction of alignment may be tailored through the chelated paramagnetic Ln$^{3+}$. The Ln$^{3+}$ may be classified in two distinct groups based on the sign of $\Delta \chi$. The first group noticeably includes dysprosium (Dy$^{3+}$) and the second group thulium (Tm$^{3+}$) and ytterbium (Yb$^{3+}$). Almost all experimental results obtained for Ln$^{3+}$ doped phospholipid bilayers and liquid crystals agree with a negative $\Delta \chi$ for the first group and a positive $\Delta \chi$ for the second. Consequently, DMPC/DMPE-DTPA/Dy$^{3+}$ (molar ratio 4:1:1) bicelles orient parallel to the magnetic field direction, while the Tm$^{3+}$ chelating counterparts align perpendicular to the field. However, the reverse remains possible, namely where Dy$^{3+}$ chelating species have a positive $\Delta \chi$ and Tm$^{3+}$ or Yb$^{3+}$ deliver a negative $\Delta \chi$. The only requirement being that two Ln$^{3+}$ belonging to opposite groups always have opposite signs of $\Delta \chi$. The $\Delta \chi$ of the Ln$^{3+}$ chelating phospholipids is determined by four contributions according to the general theory on the magnetic anisotropy of Ln$^{3+}$ containing liquid crystals proposed by Mironov et al. These contributions are discussed in full detail in chapter 2.3. The first two contributions define the $\Delta \chi$ and are intrinsically linked to the molecular structure of the Ln$^{3+}$ chelating lipid. Therefore, bottom-up synthetic approaches starting from the molecular structure of the Ln$^{3+}$ chelating amphiphiles are required for engineering of the $\Delta \chi$, ultimately defining the final magnetic response of the assemblies.

The possibility of selectively tuning the $\Delta \chi$ of DMPC/DMPE-DTPA/Ln$^{3+}$ bicelles by doping the bilayer with Chol-NH$_2$ was demonstrated in chapters 5 and 6. These results highlight the importance and the tailoring power offered by engineering the
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Δχ of the bilayer through molecular design. Herein, the Δχ was tuned by chemically engineering the Ln³⁺ chelating moiety bound to DMPE. A new Ln³⁺ chelating phospholipid, DMPE-Glu-DTPA, was specifically synthesized for this purpose. The chemical structure of the DMPE-Glu-DTPA phospholipid was first designed towards achieving a different Δχ than the commercially available DMPE-DTPA/Ln³⁺ complex. In a second step, DMPE-DTPA was replaced with the new phospholipid to generate DMPC/DMPE-Glu-DTPA/Ln³⁺ (molar ratio 4:1:1) assemblies. The system was characterized by cryo-TEM, DLS and SANS before the magnetic response was evaluated by monitoring the birefringence signal under a 5.5 T magnetic field and computing alignment factors in SANS under a 8 T magnetic field. The typicity of the magnetic response resulting from the engineered DMPE-Glu-DTPA phospholipid validated the viability of our approach for altering the Δχ. The influence of temperature, lipid content, and phosphate buffer on the assembly structure and magnetic was evaluated. Fabrication considerations were addressed and the effect of introducing Chol-OH to the bilayer was studied. The samples were characterized and compared to previously studied systems made from DMPE-DTPA, achieving a more profound understanding of the system’s magnetic response.

8.1 Design of a new Ln³⁺ chelating phospholipid – DMPE-Glu-DTPA

The Ln³⁺ chelating DMPE-Glu-DTPA/Ln³⁺ complex was specifically designed to alter the Δχ, with respect to that offered by the commercially available DMPE-DTPA/Ln³⁺ complex. Moreover, a similar molecular geometry than DMPE-DTPA/Ln³⁺ was required in DMPE-Glu-DTPA/Ln³⁺ to maintain the possibility of forming planar and magnetically alignable assemblies when mixed with DMPC. The molecular structures of the two Ln³⁺ chelating phospholipids are presented in Table 3.2. When compared to DMPE-DTPA, DMPE-Glu-DTPA offers an additional carboxylic acid, which may act...
8.1 Design of a new Ln\(^{3+}\) chelating phospholipid – DMPE-Glu-DTPA

as a 9\(^{th}\) coordination site for the Ln\(^{3+}\). The chemical structure of the DTPA headgroup in DMPE-Glu-DTPA is different from that of DTPA in DMPE-DTPA due to the chiral center of the glutamic acid employed as a backbone. This altered chelate geometry will impact the splitting of the ground J-multiplet levels into individual crystal field energies, defining the crystal field of the Ln\(^{3+}\)-phospholipid complex.

The potential of the proposed head-group molecular geometry for enhancing the magnetic alignment of bicelles was initially revealed by Liebi et al.\(^{31}\) The DMPE-DTPA phospholipid chelates Ln\(^{3+}\) in a distorted tricapped trigonal prismatic molecular geometry (or distorted mono capped square antiprismatic). The Ln\(^{3+}\) coordinates eight out of nine ligands with DTPA and one with water, see Figure 2.5. In DMPE-Glu-DTPA, the crystal field may be more defined as the additional carboxylic acid acts as a 9\(^{th}\) coordination site, which is chemically bound to the chelator structure as shown in Figure 8.1. Contrastingly for DMPE-DTPA, the water molecule is constantly exchanged in the surrounding hydrophilic environment.\(^{153}\) The carboxylic oxygen of the amide bond linking the DTPA moiety to the phospholipid in DMPE-DTPA is involved in the complexation of the Ln\(^{3+}\). Two additional carbon atoms separate this oxygen (\(^{1}O\) in Figure 8.1A) from Ln\(^{3+}\) in DMPE-Glu-DTPA. Consequently, the DMPE-Glu-DTPA/Ln\(^{3+}\) complex results in a 7-membered ring composed of the DTPA backbone shown in blue in Figure 8.1A. The open structure shown in Figure 8.1B is energetically more favourable and a more realistic structure of the DMPE-Glu-DTPA/Ln\(^{3+}\) complex. Water would act as a 9\(^{th}\) ligand, analogously to the DMPE-DTPA/Ln\(^{3+}\) complex.\(^{153,170–172}\) The carboxylic oxygen of the amide bond is probably replaced by the additional carboxylic acid available in DMPE-Glu-DTPA, being geometrically closer to the Ln\(^{3+}\). The molecular configuration of DMPE-Glu-DTPA/Ln\(^{3+}\) presented in Figure 8.1A would result in a lower CPP in the bilayer when compared to the open structure in Figure 8.1B. Consequently, the closed configuration would induce more curvature and reduce the size of the assemblies.\(^{15}\)

The DMPE-Glu-DTPA/Tm\(^{3+}\) complex was geometrically optimized using a molecular modelling MM2 routine. The energetic ground configuration of the molecule was identified to hypothesize on reasonable conformational changes that could occur in the bilayer’s environment. Although highly simplified and unable of considering the physicochemical properties governing a phospholipid bilayer, this model offers a cost effective and fast preliminary structural evaluation of the Ln\(^{3+}\)-phospholipid complex. Sites prone to undergo intramolecular interactions were identified by monitoring the geometric behavior of the molecule in a simplified environment. The all-trans conformation of the atomic chain separating the DTPA moiety from the phospholipid backbone presented in Figure 8.1B is highly unlikely in the bicelle environment. This is analogous to the findings reported for Ln\(^{3+}\) chelating Chol-DTPA in chapter 7.\(^{15}\) Numerous conformational changes may arise from thermal energy or electrostatic forces in the surrounding environment. This conformational freedom is not present in the aliphatic chains of the phospholipids that are limited by van der Waals interactions in the solid-ordered bilayer. The MM2 calculations in vacuum revealed a more realistic conformation of the DMPE-Glu-DTPA/Tm\(^{3+}\) complex involving the possible formation of an 8-membered
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Figure 8.1 Molecular structures of DMPE-Glu-DTPA/Ln$^{3+}$ phospholipid-lanthanide ion (Ln$^{3+}$) complexes with either A) oxygen (O$^1$) or B) water as a 9th ligand. In comparison, the DMPE-DTPA phospholipid employs both oxygen (O$^1$) and water to chelate Ln$^{3+}$ (see Figure 2.5).$^{90,153,170–172}$ Two additional carbon atoms separate oxygen (O$^1$) from Ln$^{3+}$ in DMPE-Glu-DTPA compared to DMPE-DTPA. Consequently, the complex in A) results in a 7-membered ring composed of the DTPA backbone shown in blue and the Ln$^{3+}$. The open structure in B) is energetically more favourable, suggesting that water is a more likely 9th ligand.$^{153}$ The oxygen (O$^1$) is probably replaced by the additional 5th carboxylic acid available in DMPE-Glu-DTPA, which is geometrically closer to the Ln$^{3+}$. However, the all-trans conformation presented in B) is unlikely as revealed by the 3D model of DMPE-Glu-DTPA gained by an MM2 geometry optimization shown in C). Carbon atoms are in gray, hydrogen atoms in white, oxygen atoms in red, loan electron pairs in light red, nitrogen atoms blue, Tm$^{3+}$ in gold, and phosphorus in purple. The possible formation of an 8-membered ring stabilized by a hydrogen bond (dotted line) is highlighted in yellow. The hydrogen bond occurs between the polar hydrogen (H$^2$) on the amide bond and the loan electron pair of the carboxylic oxygen (O$^5$) shown in red in B). Figure adapted from S. Isabetanni et al.$^{67}$
ring stabilized by a hydrogen bond as shown in Figure 8.1B and 8.1C. It must be stressed that the results of these MM2 calculations offer a highly simplified model that must not be taken as the true average representation of the DMPE-Glu-DTPA/Tm$^{3+}$ complex in the phospholipid bilayer. A more robust molecular dynamics simulation taking into account the chemical species and forces in the surrounding environment would be necessary to offer a more realistic representation.

**8.2 DMPC/DMPE-Glu-DTPA/Ln$^{3+}$ (molar ratio 4:1:1) assembly structure**

The lanthanide ion (Ln$^{3+}$) chelating DMPE-Glu-DTPA/Ln$^{3+}$ complex was specifically engineered to have a similar geometry or CPP than the commercially available DMPE-DTPA/Ln$^{3+}$ complex to favour the formation of magnetically responsive assemblies when mixed with DMPC.$^{10,11}$ Therefore, as a first step, DMPE-DTPA in DMPC/DMPE-DTPA/Ln$^{3+}$ (molar ratio 4:1:1) bicelles was replaced with the newly synthesized DMPE-Glu-DTPA phospholipid. The optimal lipid molar ratio of 4:1:1, total lipid concentration, sample preparation, buffer concentration and pH value of 7.4 were maintained.$^{108}$ This maximized the chances of obtaining magnetically alignable species, while allowing for a direct comparison with the reference system. The resulting DMPC/DMPE-Glu-DTPA/Ln$^{3+}$ (molar ratio 4:1:1) system was characterized by cryo-TEM, DLS and SANS at 5 °C in the absence of a magnetic field. Assemblies containing either Tm$^{3+}$, Dy$^{3+}$, or Yb$^{3+}$ were investigated.

The DMPC/DMPE-Glu-DTPA/Ln$^{3+}$ (molar ratio 4:1:1) lipid mixture resulted in planar structures as evidenced by the cryo-TEM micrographs in Figure 8.2. DLS measurements of the DMPC/DMPE-Glu-DTPA/Ln$^{3+}$ (molar ratio 4:1:1) systems with chelated Tm$^{3+}$ or Dy$^{3+}$ at 5 °C revealed assemblies with a hydrodynamic radius of 132 and 107 nm, respectively. The polydisperse nature of the species was confirmed with a standard deviation of 0.2 regardless of the chelated Ln$^{3+}$. The polymolecular assemblies were asymmetric in shape and ripples were observed as periodic darker stripes from top-on view in the cryo-TEM micrographs, see Figure 8.2. The assemblies were longer in the direction of the ripples. The ripples were more evident when looking at the partially folded assemblies in Figure 8.2B (white arrows). Moreover, an assembly observed from side-on view appeared as a dark sinusoidal line in Figure 8.2B (black arrow). The thickness of the dark line observed from side-on view suggested the assemblies were composed of a single bilayer, justifying their low contrast when viewed from top-on in the cryo-TEM micrographs.

The presence of ripples was observed previously in both, DMPC/DMPE-DTPA/ Ln$^{3+}$ (molar ratio 4:1:1) and DMPC/DHPC bicelle systems.$^{16,63,108}$ Similar ripple phases were also reported in vesicles composed of various types of single or mixed phospholipids.$^{40,41}$ The ripple phase adopts a certain structure depending on the nature of the constituting bilayer phospholipids and the surrounding environment.$^{42,44}$ This structure is classified
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Figure 8.2 Cryo-TEM micrographs of DMPC/DMPE-Glu-DTPA/Ln\(^{3+}\) (molar ratio 4:1:1) with chelated A) Dy\(^{3+}\) and B) Tm\(^{3+}\) flash-frozen at 5 °C. The total lipid concentration [tL] was 15 mM in a 50 mM phosphate buffer at a pH value of 7.4. Ripples appear as regular darker lines on the planar surface of the polynuclear assemblies. The white arrows point at partially folded assemblies. The black arrow points at an assembly seen from side-on view. This structure was further lightened and surrounded by a white box for clarity. The scale bars represent 200 nm. High contrast particles are ice crystals and the carbon walls of the lacey microgrid appear as high contrast areas. C) Schematic summary of the obtained DMPC/DMPE-Glu-DTPA/Ln\(^{3+}\) (molar ratio 4:1:1) assemblies represented by sheets with waves and the reference bicelle system resulting from DMPC/DMPE-DTPA/Ln\(^{3+}\) (molar ratio 4:1:1). These schematics are not to scale. Figure adapted from S. Isabettini et al.\(^{67}\)

as either symmetric or asymmetric (sawtooth).\(^{44-47,173}\) The ripples in DMPC/DMPE-Glu-DTPA/Ln\(^{3+}\) (molar ratio 4:1:1) assemblies had a repeat distance between 10-15 nm evaluated from top-on view in the cryo-TEM micrographs in Figure 8.2. This is consistent with the asymmetric ripple structure of DMPC bilayers.\(^{47,173}\) Although presumably the dominant structure, the sinusoidal shape of the assembly seen from side-on in Figure 8.2B (black arrow) displayed a repeat distance of about 30 nm, in line with the reported symmetric geometry of the DMPC ripple phase.\(^{45,173}\) The thermal history of the bilayer determines the predominating ripple structure.\(^{45,48}\) The symmetric phase was reported to be metastable, eventually converting to the more stable asymmetric geometry.\(^{49}\) However, the bilipidic nature of the studied DMPC/DMPE-Glu-DTPA/Ln\(^{3+}\) (molar ratio 4:1:1) system must be stressed, where the exact structure of the ripple phase has never been characterized. Furthermore, these ripples appeared at 5 °C, far from the reported temperature range of pure DMPC bilayers around 18 °C.\(^{47}\)

The morphology of the DMPC/DMPE-Glu-DTPA/Ln\(^{3+}\) (molar ratio 4:1:1) samples were very different to those obtained with the reference DMPC/DMPE-DTPA/
8.2 DMPC/DMPE-Glu-DTPA/Ln\(^{3+}\) (molar ratio 4:1:1) assembly structure

Ln\(^{3+}\) (molar ratio 4:1:1) system as illustrated in Figure 8.2C. The later consists of small bicelles in the size range of 20 nm in radius.\(^{108}\) DMPE-Glu-DTPA/Ln\(^{3+}\) induced less curvature in the bilayer, resulting in larger polymolecular assemblies. Consequently, DMPE-Glu-DTPA/Ln\(^{3+}\) had a CPP closer to unity, when compared to DMPE-DTPA/Ln\(^{3+}\). This finding supports the open structure of the DMPE-Glu-DTPA/Ln\(^{3+}\) complex proposed in Figure 8.2B and 8.2C. The presence of a ripple phase may have enabled a more homogenous mixing of the curvature-inducing DMPE-Glu-DTPA/Ln\(^{3+}\) species with DMPC. Analogously to DMPC/DMPE-DTPA/Ln\(^{3+}\) systems, the DMPE-Glu-DTPA/Ln\(^{3+}\) complex is likely not restricted to the edge-area of the bilayer. This fact, in combination with the large assembly dimensions, foresees high alignments in the presence of an external magnetic field.

![Figure 8.3 Radially averaged SANS curves (data points) and corresponding fits (solid lines) of DMPC/DMPE-Glu-DTPA/Ln\(^{3+}\) (molar ratio 4:1:1) chelating Yb\(^{3+}\) (black), Tm\(^{3+}\) (red) or Dy\(^{3+}\) (blue) at 5 °C. The total lipid concentration [tL] was 15 mM in a 50 mM phosphate buffer at a pH value of 7.4. All samples were fitted with a form factor for Porod cylinders with a lognormal distribution. The disk thickness was 4.2 nm with an average radius of 95 nm and standard deviation \(\sigma : 0.25\) for all three Ln\(^{3+}\). The curves were shifted vertically for clarity. The scattering intensity decays with \(q^{-2}\). Figure adapted from S. Isabettini et al.\(^{67}\).](image)

Radially averaged SANS curves were obtained for the DMPC/DMPE-Glu-DTPA/ Ln\(^{3+}\) (molar ratio 4:1:1) assemblies with chelated Tm\(^{3+}\), Dy\(^{3+}\) or Yb\(^{3+}\) at 5 °C, see Figure 8.3.
The decay of the scattering intensity with $q^{-2}$ confirms the existence of phospholipid bilayers. All samples could be fitted with a form factor for Porod cylinders. An average radius of 95 nm was obtained for the samples regardless of the chelated Ln$^{3+}$. This size range supported the observations made from the cryo-TEM micrographs in Figure 8.2 and the DLS results for the species chelating Tm$^{3+}$ and Dy$^{3+}$. Similarly to the reported DMPE-DTPA/Ln$^{3+}$ systems, the size of the DMPC/DMPE-Glu-DTPA/Ln$^{3+}$ (molar ratio 4:1:1) assemblies was not influenced by the nature of the chelated Ln$^{3+}$. The similar ionic radius of the Ln$^{3+}$ does not induce a change in the packing parameter of the Ln$^{3+}$-phospholipid complex. The polydisperse nature of the assemblies was further confirmed as a log normal distribution was necessary to fit the data with an average standard deviation of 0.25. The fittings revealed a bilayer thickness of 4.2 nm for all the samples. This goes in line with reported dimensions for DMPC bilayers in the solid-ordered phase and confirms the assemblies were composed of a single bilayer.

### 8.3 Magnetic alignment of DMPC/DMPE-Glu-DTPA/Ln$^{3+}$ assemblies

DMPC/DMPE-Glu-DTPA/Ln$^{3+}$ assemblies were an order of magnitude large than the reference DMPC/DMPE-DTPA/Ln$^{3+}$ bicelles. An enhanced magnetic response was expected as more molecules are capable of contributing to the overall magnetic energy of the assembly. The replacement of DMPE-DTPA with DMPE-Glu-DTPA resulted in a 2.5-fold increase in the absolute value of the alignment factor $A_f$ computed from 2D SANS scattering patterns of DMPC/DMPE-DTPA/Tm$^{3+}$ (molar ratio 4:1:1) and DMPC/DMPE-Glu-DTPA/Tm$^{3+}$ (molar ratio 4:1:1) at 5 °C under a 8 T magnetic field, see Figure 8.4A. However, the sign of the $A_f$ went from negative to positive, implying a switch in alignment direction. This inversion also occurred in the Dy$^{3+}$ chelating DMPC/DMPE-Glu-DTPA/Dy$^{3+}$ (molar ratio 4:1:1) assemblies, yielding negative $A_f$ values. A positive $A_f$ corresponds to species aligned parallel to the magnetic field direction, whereas a negative $A_f$ implies a perpendicular alignment. The 2D SANS scattering patterns from which the alignment factors were computed at 5 °C and 8 T confirmed the inverted alignment as presented in Figure 8.4B. This phenomenon originates from an inversion in the sign of $\Delta \chi$ of the Ln$^{3+}$-phospholipid complex imbedded in the bilayer. The results suggest a positive $\Delta \chi$ and a negative $\Delta \chi$ for the Dy$^{3+}$ and Tm$^{3+}$ chelating polymolecular assemblies, respectively.

This switch in $\Delta \chi$ and alignment direction is theoretically possible based on the general theory proposed by Mironov et al. However, the DMPC/DMPE-Glu-DTPA/Ln$^{3+}$ lipid systems are the first experimental report of such an inversion for planar bicellar assemblies. A switched anisotropy has been observed in Ln$^{3+}$ loaded paramagnetic liposomes by D. Castelli et al. The findings were explained by the special insertion characteristics and geometry of the employed amphiphilic Ln$^{3+}$ complex in the
8.3 Magnetic alignment of DMPC/DMPE-Glu-DTPA/Ln$^{3+}$ assemblies

**Figure 8.4** A) Alignment factor $A_f$ as a function of the magnetic field strength $B$ of DMPC/DMPE-Glu-DTPA/Ln$^{3+}$ (molar ratio 4:1:1) polymolecular assemblies chelating either Tm$^{3+}$ (red), Dy$^{3+}$ (black), or Yb$^{3+}$ (blue) during ramping from 0 to 8 T at 5 °C. The total lipid concentration [$tL$] was 15 mM in a 50 mM phosphate buffer at a pH value of 7.4. The dotted line represents the $A_f$ range of the reference DMPC/DMPE-DTPA/Tm$^{3+}$ (molar ratio 4:1:1) bicelles. B) 2D SANS scattering patterns of the Tm$^{3+}$ and Dy$^{3+}$ chelating samples on the right and left, respectively. The magnetic field direction $B$ is pointing upwards as shown with a white arrow. Anisotropy parallel to the magnetic field direction implies a perpendicular alignment of the assemblies, while anisotropy perpendicular to the magnetic field direction implies a parallel alignment. The sign of $\Delta \chi$ of the assemblies is either positive (green, $+$) or negative (red, $-$). Figure adapted from S. Isabettini et al.\textsuperscript{67}
The engineered DMPE-Glu-DTPA/Ln$^{3+}$ complex may have acted in a similar way. The magnetic properties of Ln$^{3+}$ are determined by the electron state of the outer shell 4f electrons. The chemical nature of the ligands and the geometry of the complex split the levels of the ground J-multiplet into individual crystal field energies. Moreover, the orientation of the long molecular axis with respect to the molecular magnetic axis, determines both, the sign and magnitude of $\Delta \chi$. Ln$^{3+}$ is a non-paramagnetic lanthanide ion as it does not contain any 4f electrons. Nevertheless, DMPC/DMPE-Glu-DTPA/La$^{3+}$ (molar ratio 4:1:1) assemblies yielded an $A_f$ of 0.1 at 8 T and 5 °C (results not shown). This weak parallel alignment was expected and originates from the intrinsic negative $\Delta \chi$ of the bilayer phospholipids. The switched alignment direction only occurred when paramagnetic Ln$^{3+}$ were employed.

The switch in $\Delta \chi$ and the high degree of alignment offered by the DMPE-Glu-DTPA/Ln$^{3+}$ complex was confirmed when chelating Yb$^{3+}$. DMPC/DMPE-Glu-DTPA/Yb$^{3+}$ (molar ratio 4:1:1) assemblies delivered an alignment factor of 0.46 at 5 °C and 8 T. Chelation of Yb$^{3+}$ resulted in a negative $\Delta \chi$ and a parallel alignment of the assemblies with respect to the magnetic field direction. Yb$^{3+}$ belongs to the same group of lanthanides as Tm$^{3+}$, effectively influencing the $\Delta \chi$ in the same direction. However, the Yb$^{3+}$ chelating species were more alignable than the Tm$^{3+}$ chelating counterparts as evidenced by the larger alignment factors in Figure 8.4. Since the size of the DMPC/DMPE-Glu-DTPA/Ln$^{3+}$ assemblies was independent of the employed lanthanide ion, the differences in magnetic alignability must come from an altered $\Delta \chi$ of the bilayer lipids. Chelation of Tm$^{3+}$, compared to Yb$^{3+}$, usually results in a stronger magnetic response owing to its larger intrinsic molar magnetic susceptibility. However, the magnitude of the crystal field perturbation defining the $\Delta \chi$ strongly depends on the type of coordination polyhedron and the nature of the complexed Ln$^{3+}$. These results confirm the unique magnetic properties offered by the DMPE-Glu-DTPA/Ln$^{3+}$ complex. Engineering the chemical structure of the Ln$^{3+}$ chelating lipids constitutes a viable means of tailoring the magnetic response of assemblies.
8.4 Temperature-induced assembly transformations

Temperature has a profound effect on the structure of bicelles. In the case of DMPC/DMPE-DTPA/Ln$^{3+}$ (molar ratio 4:1:1), a collapse of the bicelles into vesicles occurs when heating above the phase transition temperature $T_m$ of DMPC at 24 °C. The resulting vesicles are not alignable in the presence of a magnetic field. However, the bicelles are readily regenerated upon cooling below 24 °C. Herein, temperature induced transformations occurring in DMPC/DMPE-Glu-DTPA/Ln$^{3+}$ (molar ratio 4:1:1) assemblies were evaluated to make parallels with the DMPC/DMPE-DTPA/Ln$^{3+}$ reference systems. This study was important to validate our molecular engineering approach consisting of imitating the geometry of the DMPE-DTPA lipid to guaranty the successful formation of magnetically responsive species. The newly synthesized Ln$^{3+}$ chelating DMPE-Glu-DTPA phospholipid was expected to show a similar thermo-reversible behavior when mixed with DMPC as its 14-carbon long myristoyl chain remained unaltered.

The cryo-TEM micrograph in Figure 8.5A was obtained when heating a DMPC/DMPE-Glu-DTPA/Tm$^{3+}$ (molar ratio 4:1:1) sample from 5 to 23 °C, close to the $T_m$ of DMPC. The micrograph revealed a coexistence of vesicles (white arrows) and planar assemblies. The ripples remained visible as regular striped patterns. They were no longer visible when the lipids moved into the liquid-disordered phase and assembled into vesicles. The micrograph captured the point of collapse of the planar assemblies into vesicles. Asymmetric vesicles or partially folded planar assemblies were observed as species with darker edges (bilayer seen from top-view) in Figure 8.5A (black arrows). The magnetic alignability of DMPC/DMPE-Glu-DTPA/Ln$^{3+}$ (molar ratio 4:1:1) samples chelating either Dy$^{3+}$ or Tm$^{3+}$ ions were further monitored upon heating and cooling back to 5 °C at 1 °C/min in Figure 8.5B and 8.5C. The inversion in alignment direction was confirmed over the entire temperature range for both Ln$^{3+}$.

The bicelles collapsed into vesicles above the $T_m$ of DMPC at 24 °C, resulting in a loss of alignment evidenced by a zeroing of the $A_f$ and the birefringence signal. These transformations were thermo-reversible as the magnetically aligned species were regenerated upon cooling to 5 °C, analogously to the reference DMPC/DMPE-DTPA/Ln$^{3+}$ (molar ratio 4:1:1) systems. These findings confirmed our hypothesis and demonstrated the viability of the proposed molecular engineering approach to deliver novel Ln$^{3+}$ chelating lipids capable of delivering assemblies with a tuned magnetic response.
Figure 8.5 A) Cryo-TEM micrographs of DMPC/DMPE-Glu-DTPA/Tm$^{3+}$ (molar ratio 4:1:1) flash-frozen at 23 °C on heating from 5 °C. The total lipid concentration [tL] was 15 mM in a 50 mM phosphate buffer at a pH value of 7.4. Ripples appear as regular darker lines on the planar surface of the assemblies. The white arrows point at vesicles coexisting in the sample. The black arrows points at unfinished asymmetric vesicles or partially folded planar assemblies. The micrograph captures the point of collapse of the planar assemblies into vesicles when approaching the phase transition temperature $T_m$ of DMPC at 24 °C. The scale bar represents 200 nm. High contrast particles are ice crystals and the carbon walls of the lacey microgrid appears as a high contrast area. B) Alignment factors $A_f$ at 8 T and C) birefringence signal at 5.5 T of DMPC/DMPE-Glu-DTPA/Ln$^{3+}$ (molar ratio 4:1:1) with Tm$^{3+}$ and Dy$^{3+}$ as a function of temperature on heating and cooling at 1 °C/min. Figure adapted from S. Isabettini et al.\textsuperscript{67}
8.5 Optimising the magnetic response of DMPC/DMPE-Glu-DTPA/Ln\(^{3+}\) assemblies

The dimensions and magnetic response of the DMPC/DMPE-Glu-DTPA/Ln\(^{3+}\) assemblies were optimized by altering the lipid molar ratio of the constituting lipids. This technique is commonly employed to tailor the magnetic alignment in DMPC/DHPC bicelles through their size\(^{12,13}\). The lipid molar ratio is also crucial in DMPC/DMPE-DTPA/Ln\(^{3+}\) systems to generate highly magnetically alignable bicelles\(^{16,30}\). Therefore, the magnetic alignment achieved at different molar ratios of DMPC/DMPE-Glu-DTPA/Tm\(^{3+}\) of 7:1:1, 5:1:1, 3:1:1, and 3:2:2 was monitored by the birefringence signal and compared to the 4:1:1 system as a reference, see Figure 8.6.

**Figure 8.6** A) Evolution of the birefringence signal under a 5.5 T magnetic field as a function of temperature. DMPC/DMPE-Glu-DTPA/Tm\(^{3+}\) (molar ratio 4:1:1) was compared to different molar ratios of 7:1:1 and 3:1:1. The total lipid concentration \([tL]\) was 15 mM in a 50 mM phosphate buffer at a pH value of 7.4. B) Evolution of the alignment factor \(A_f\) of DMPC/DMPE-Glu-DTPA/Tm\(^{3+}\) molar ratio 4:1:1 and 3:1:1 under an 8 T magnetic field as a function of temperature. All heating and cooling was conducted at 1 °C/min. Figure adapted from S. Isabettini et al.\(^{67}\)

DMPC/DMPE-Glu-DTPA/Tm\(^{3+}\) (molar ratio 3:2:2) showed no signs of alignment. The cone-like nature of the DMPE-Glu-DTPA/Tm\(^{3+}\) complex may have induced too much curvature in the bilayer at such a high lipid content. The resulting possible formation of small bicelles or even micellar assemblies could explain the absence of a magnetic response. All the other investigated samples switched the alignment direction when exposed to an external magnetic field. These findings confirm the capacity of the engineered Ln\(^{3+}\) chelating DMPE-Glu-DTPA phospholipid to provide a dramatically different
Δχ. Both DMPC/DMPE-Glu-DTPA/Tm$^{3+}$ samples with a molar ratio of 7:1:1 and 5:1:1 revealed similar weak birefringence signals. However, the signal followed an analogous trend to that of the reference DMPC/DMPE-Glu-DTPA/Tm$^{3+}$ (molar ratio 4:1:1) system with changing temperature, see Figure 8.6A. A characteristic thermoreversible collapse of the birefringence signal occurred above the phase transition temperature $T_m$ of DMPC at 24 °C. Therefore, the weak birefringence signal probably originated from the alignment of similar assembly structures in the magnetic field. However, the lipid content remained too low to effectively form highly alignable species. DMPC/DMPE-Glu-DTPA/Tm$^{3+}$ (molar ratio 3:1:1) delivered a stronger birefringence signal than the reference system, see Figure 8.6. The higher magnetic alignability was confirmed by SANS measurements at 5 °C and 8 T, revealing an alignment factor $A_f$ of 0.47, up from 0.36 obtained with the reference DMPC/DMPE-Glu-DTPA/Tm$^{3+}$ (molar ratio 4:1:1) system. Analogously to the birefringence results, larger alignment factors $A_f$ were obtained over the studied temperature range on heating and cooling, see Figure 8.6B. The structure of these polymeric assemblies was further studied by cryo-TEM and SANS to identify the origins of the enhanced magnetic response.

![Figure 8.7](image.png)

**Figure 8.7** A) Cryo-TEM micrographs of DMPC/DMPE-Glu-DTPA/Tm$^{3+}$ (molar ratio 3:1:1, [tL] 15 mM) flash-frozen at 5 °C. Ripples appear as regular darker lines on the planar surface of the assemblies. The scale bar represents 200 nm. High contrast particles are ice crystals resulting from the freezing procedure. B) Radially averaged SANS curves (data points) and corresponding fits (solid lines) at 5 °C of DMPC/DMPE-Glu-DTPA/Tm$^{3+}$ molar ratio 4:1:1 (black) and 3:1:1 (red), [tL] 15 mM. All samples were fitted with a form factor for Porod cylinders with a lognormal distribution. The disk thickness was 4.2 nm with an average radius and standard deviation of 94 nm and $\sigma : 0.23$ for the molar ratio 4:1:1 and 105 nm and $\sigma : 0.22$ for the molar ratio 3:1:1. The curves were shifted vertically for clarity. Figure adapted from S. Isabettini et al.67
The cryo-TEM micrograph of DMPC/DMPE-Glu-DTPA/Tm$^{3+}$ (molar ratio 3:1:1) flash frozen at 5 °C in Figure 8.7A revealed similar asymmetric polymolecular assemblies with ripples as those observed in the reference DMPC/DMPE-Glu-DTPA/Tm$^{3+}$ (molar ratio 4:1:1) in Figure 8.7. The assemblies were polydisperse in size and the higher magnetic alignment could originate from larger species. These findings were confirmed from the radially averaged SANS curve of the sample at 5 °C in the absence of a magnetic field in Figure 8.7B. The data was fitted with a form factor for Porod cylinders and a lognormal distribution. The bilayer thickness was 4.2 nm, the radius 105 nm, and the standard deviation $\sigma$: 0.22. These findings confirm that the DMPC/DMPE-Glu-DTPA/Tm$^{3+}$ (molar ratio 3:1:1) assemblies were on average larger than the reference DMPC/DMPE-Glu-DTPA/Tm$^{3+}$ (molar ratio 4:1:1) species. Therefore, the enhanced magnetic response of the former originates from their larger size, having more bilayer lipids contributing to the overall magnetic energy of the polymolecular assembly. Such lipid ratios did not allow the formation of magnetically alignable bicelles in the reference DMPC/DMPE-DTPA/Ln$^{3+}$ system due to increased curvature in the bilayer. This finding again supports the larger CPP of DMPE-Glu-DTPA/Ln$^{3+}$ compared to DMPE-DTPA/Ln$^{3+}$ when mixed with DMPC and the open molecular structure of the complex proposed in Figure 8.1B and 8.1C.

8.6 Phosphate buffer for an optimal magnetic response

Working with a 50 mM phosphate buffer at a pH value of 7.4 was essential to guaranty the optimal formation of DMPC/DMPE-DTPA/Ln$^{3+}$ bicellar systems. Controlling the pH is necessary to compensate the acidic nature of the lanthanide salts and enable optimal chelation of the Ln$^{3+}$. The phosphate buffer dictates the electrostatic forces surrounding the polymolecular assemblies, influencing their architecture. Many structural phenomena observed on assemblies made from a phospholipid bilayer are induced by the buffer. This is particularly true for the appearance of ripples. Herein, the polymolecular assemblies resulting from DMPC/DMPE-Glu-DTPA/Tm$^{3+}$ (molar ratio 4:1:1) in water and their magnetic alignability are evaluated. The pH was adjusted to a value of 7.4 by titration with 1 M NaOH. Analogously to bicelles made with the DMPE-DTPA/Ln$^{3+}$ complex, the assemblies made with DMPE-Glu-DTPA/Ln$^{3+}$ were sensitive to low pH values where the engineered Glu-DTPA head group of the phospholipid was protonated and no longer able to chelate Ln$^{3+}$.

Similar asymmetric assemblies were obtained for the of DMPC/DMPE-Glu-DTPA/Tm$^{3+}$ (molar ratio 4:1:1) sample produced in D$_2$O as evidenced by the cryo-TEM micrograph in Figure 8.8A. The existence of ripples was not jeopardized by the absence of phosphate buffer. The radially averaged SANS curves in Figure 8.8B confirmed that the structure of the assemblies was not majorly affected when working in D$_2$O. Polydisperse species with an average radius of 95 nm were obtained, analogously to the system produced in
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Figure 8.8 A) Cryo-TEM micrographs of DMPC/DMPE-Glu-DTPA/Tm\(^{3+}\) (molar ratio 4:1:1) in D\(_2\)O flash-frozen at 5 °C. The total lipid concentration \([tL]\) was 15 mM. Ripples appear as regular darker lines on the planar surface of the polymolecular assemblies. The scale bar represents 200 nm. High contrast particles are ice crystals resulting from the freezing procedure. B) Radially averaged SANS curves (data points) and corresponding fits (solid lines) at 5 °C of DMPC/DMPE-Glu-DTPA/Tm\(^{3+}\) (molar ratio 4:1:1, \([tL]\) 15 mM) produced in 50 mM phosphate buffer (black) and in D\(_2\)O (red). All samples were fitted with a form factor for Porod cylinders with a lognormal distribution. The disk thickness was 4.2 nm with an average radius and standard deviation of 94 nm and \(\sigma : 0.23\) for the sample in phosphate buffer and 95 nm and \(\sigma : 0.27\) for the sample in D\(_2\)O. The curves were shifted vertically for clarity. Figure adapted from S. Isabettini et al.\(^67\)

Phosphate buffer. These findings were further confirmed by DLS measurements where a hydrodynamic radius of 99 nm was obtained. Although the structural integrity of the assemblies was maintained when working in D\(_2\)O, the magnetic alignability was reduced. The alignment direction remained inverse and a thermoreversible collapse into magnetically non-alignable vesicles occurred at the phase transition temperature of DMPC at 24 °C. The phosphate buffer plays an integral role in guarantying the high magnetic response of DMPC/DMPE-Glu-DTPA/Ln\(^{3+}\).
8.7 Fabrication considerations for DMPC/DMPE-Glu-DTPA/Ln$^{3+}$ assemblies

The DMPC/DMPE-Glu-DTPA/Ln$^{3+}$ systems discussed in this chapter were produced following the same fabrication procedure: The dry lipid film was hydrated with 50 mM phosphate buffer at a pH value of 7.4 and 5 freeze-thaw cycles in liquid nitrogen. The resulting suspension was extruded 10 times through polycarbonate membranes with a pore diameter of 200 nm at 40 °C for Chol-OH free samples and 60 °C for samples containing Chol-OH. These protocols were directly inspired from those employed for the fabrication of DMPC/DMPE-DTPA/Ln$^{3+}$ based bicelle systems outlined in chapter 3.2. The findings of chapter 4 highlight the importance of fabrication procedures to generate highly responsive polymolecular assemblies. The DMPC/DMPE-Glu-DTPA/Ln$^{3+}$ systems showed an analogous behavior by thermo-reversibly forming vesicles above the phase transition temperature $T_m$ of DMPC. Therefore, their assembly size may also be indirectly tailored by extruding the vesicle precursors.

The polymolecular assemblies obtained after the freeze thawing cycles were characterized by cryo-TEM in Figure 8.9A and by computing the radially averaged SANS curve in Figure 8.9B for a sample of DMPC/DMPE-Glu-DTPA/Tm$^{3+}$ (molar ratio 4:1:1) at 5 °C. The cryo-TEM micrograph revealed a multitude of polydisperse assembly structures including folded planar assemblies, vesicles and multilamellar vesicles. The sample was also composed of similar asymmetric planar assemblies with ripples observed after extrusion (results not shown). The chaotic nature of the sample was confirmed by the SANS measurements in Figure 8.9B. Although the scattering intensity decayed with $q^{-2}$, confirming the existence of polymolecular assemblies made from a phospholipid bilayer, the data could not be fitted by any reasonable single geometry. The magnetic alignability of the sample was considerably hindered by the presence of vesicles structures, evidenced by the weak alignment factor $A_f$ of 0.15 obtained at 5 °C and 8 T. The extrusion process is necessary to guaranty the formation of the more alignable planar DMPC/DMPE-Glu-DTPA/Tm$^{3+}$ (molar ratio 4:1:1) species.

The sample was further extruded 10 times through a polycarbonate membrane with a pore diameter of 200 nm and another 10 times through a membrane with a pore diameter of 100 nm. The birefringence signal was monitored on heating and cooling (1 °C/min) and compared to the reference extruded through 200 nm pores in Figure 8.10. The maximum birefringence signal obtained for the fully extruded sample was lower than that obtained for the reference sample extruded only through 200 nm pores. Since the birefringence signal is directly related to the magnetic alignment of the polymolecular assemblies, the lower magnetic alignment of the fully extruded sample likely originates from a smaller assembly size. Smaller assemblies have less lipids contributing to the cumulative magnetic energy of the assembly. The smaller size originates from the smaller precursor vesicles obtained by further extrusion through 100 nm pores. Analogously to
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DMPC/DMPE-DTPA/Ln$^{3+}$ systems, extrusions may be applied for tailoring the size and magnetic response of DMPC/DMPE-Glu-DTPA/Ln$^{3+}$ assemblies.

**Figure 8.9** A) Cryo-TEM micrographs of DMPC/DMPE-Glu-DTPA/Tm$^{3+}$ (molar ratio 4:1:1) after 5 freeze thawing (FT) cycles, flash-frozen at 5 °C. The total lipid concentration [tL] was 15 mM in a 50 mM phosphate buffer at a pH value of 7.4. A multitude of asymmetric structures are observed including vesicles and multilamellar vesicles (top) and large, partially folded assemblies (bottom). The scale bar represents 200 nm and is valid for both the top and bottom part of the composite image. High contrast particles are ice crystals resulting from the freezing procedure. B) Radially averaged SANS curves at 5 °C of DMPC/DMPE-Glu-DTPA/Tm$^{3+}$ (molar ratio 4:1:1, [tL] 15 mM) after the 5 FT cycles (red) and after an additional 10 extrusions through a polycarbonate membrane with a pore diameter of 200 nm. Figure adapted from S. Isabettini et al.⁶⁷
Figure 8.10 Evolution of the birefringence signal under a 5.5 T magnetic field as a function of temperature on heating and cooling (1 °C/min) for DMPC/DMPE-Glu-DTPA/Tm$^{3+}$ (molar ratio 4:1:1) after 10 extrusions through a polycarbonate membrane with a pore diameter of 200 nm (red and dark blue) and another 10 extrusions through a membrane with a pore diameter of 100 nm (magenta and blue). The total lipid concentration [tL] was 15 mM in a 50 mM phosphate buffer at a pH value of 7.4. Figure adapted from S. Isabettini et al.\textsuperscript{67}
8.8 DMPC/Chol-OH/DMPE-Glu-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5) assemblies

The introduction of Chol-OH within the phospholipid bilayer eliminates the ripple phases by introducing a liquid-ordered phase.\textsuperscript{50,51} This property was exploited previously in DMPC/Chol-OH/DMPE-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5) bicelles, delivering a two-fold increase in magnetic alignment when compared to the Chol-OH-free DMPC/DMPE-DTPA/Tm$^{3+}$ (molar ratio 4:1:1) system at 5 °C and 8 T.\textsuperscript{16} Analogously, we introduced 16 mol% Chol-OH in the phospholipid bilayer to produce DMPC/Chol-OH/DMPE-Glu-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5) assemblies. The aim was to eliminate the ripples and assess the resulting impact on the magnetic alignment. In a first step, the change in assembly architecture induced by Chol-OH was evaluated by cryo-TEM and SANS experiments at 5 °C. In a second step, the resulting magnetic alignment was quantified by computation of the alignment factor in SANS at 8 T and 5 °C. Moreover, the magnetic alignment was monitored as a function of temperature by birefringence measurements at 5.5 T. The cryo-TEM micrograph of a DMPC/Chol-OH/DMPE-Glu-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5) sample at 5 °C in Figure 8.11A revealed planar symmetric bicelles. The previously observed asymmetric species (see Figure 8.2) were no longer visible. Chol-OH effectively removed the ripples in the phospholipid bilayer. The radially averaged SANS curve of the sample at 5 °C was obtained, see Figure 8.12. The Chol-OH doped sample had a similar size range than the DMPC/DMPE-Glu-DTPA/Tm$^{3+}$ assemblies and was less polydisperse with a standard deviation of the log normal distribution of 0.11, down from 0.23. The symmetric nature of the bicelles may explain a more monodisperse sample. The fittings revealed an increased bilayer thickness from 4.2 nm to 4.6 nm upon the introduction of Chol-OH in the bilayer. This result is consistent with Chol-OH doped DMPC bilayers in the liquid-ordered phase and supports the successful integration of Chol-OH in the bilayer.\textsuperscript{16,71,90,174}

The DMPC/Chol-OH/DMPE-Glu-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5) assemblies resulted in a $A_f$ of 0.29 at 5 °C and 8 T. This was 30% smaller than the $A_f$ obtained for the DMPC/DMPE-Glu-DTPA/Tm$^{3+}$ (molar ratio 4:1:1) assemblies. The fall in magnetic alignment was confirmed from the lower birefringence signal obtained for the Chol-OH doped species in Figure 8.11B. The birefringence signal remained lower over the studied temperature range on both heating and cooling. These results suggest that the ripple phase plays an essential role in guarantying the maximal magnetic alignment of DMPE-Glu-DTPA/Ln$^{3+}$ based polymolecular assemblies. However, the ripple phase is not responsible for the inversed magnetic alignment as the sign of the $A_f$ and the birefringence signal remained switched compared to the reference DMPE-DTPA/Ln$^{3+}$ based systems. The unique magnetic properties of the assemblies resulted from the chemical nature of the designed and synthesized Ln$^{3+}$ chelating DMPE-Glu-DTPA phospholipid. These findings prove the viability of our engineering approach to tune $\Delta \chi$ of the molecules composing the bilayer and the magnetic response of the resulting assemblies.

DMPC/Chol-OH/DMPE-Glu-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5) bicelles collapse into
8.8 DMPC/Chol-OH/DMPE-Glu-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5) assemblies

Figure 8.11 A) Cryo-TEM micrograph of DMPC/Chol-OH/DMPE-Glu-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5) flash-frozen at 5 °C. The total lipid concentration [tL] was 15 mM in a 50 mM phosphate buffer at a pH value of 7.4. The scale bar represents 200 nm. High contrast particles are ice crystals and the carbon walls of the lacy microgrid appear as high contrast areas. B) Evolution of the birefringence signal under a 5.5 T magnetic field as a function of temperature. DMPC/Chol-OH/DMPE-Glu-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5, [tL] 15 mM) was compared to DMPC/DMPE-Glu-DTPA/Tm$^{3+}$ (molar ratio 4:1:1, [tL] 15 mM). The signal was monitored both on heating and cooling at 1 °C/min. Figure adapted from S. Isabettini et al.$^{67}$

vesicles upon heating as evidenced by the zeroing of the birefringence signal at higher temperatures. The process remains thermo-reversible as the birefringence signal is regenerated upon cooling. However, the liquid-ordered phase resulting from the inclusion of cholesterol pushed the reversible collapse towards higher temperatures. The existence of a hysteresis on heating and cooling suggested a very different mechanism of lipid
Figure 8.12 Radially averaged SANS curves and corresponding fit of DMPC/DMPE-Glu-DTPA/Tm$_{3+}$ (molar ratio 4:1:1) and DMPC/Chol-OH/DMPE-Glu-DTPA/Tm$_{3+}$ (molar ratio 16:4:5:5) at 5°C. The total lipid concentration [tL] was 15 mM in a 50 mM phosphate buffer at a pH value of 7.4. The data for the Chol-OH doped assemblies was fitted with a form factor for Porod cylinders and a log normal distribution. The average radius was 104 nm, the bilayer thickness was 4.6 nm, and the standard deviation was 0.11. The curves were shifted vertically for clarity. Figure adapted from S. Isabettini et al.\textsuperscript{67} 

rearrangement within the bilayer and temperature-induced changes in assembly architecture. These findings were in line with those observed in the reference DMPC/Chol-OH/DMPE-DTPA/Tm$_{3+}$ (molar ratio 16:4:5:5) bicelles.\textsuperscript{16} Similarly, Chol-OH and possibly cholesterol conjugates may be employed to further tune the thermal resistance and magnetic properties of DMPE-Glu-DTPA/Ln$_{3+}$ systems.\textsuperscript{15,31,90}
8.9 Conclusion

The molecular engineering approach employed for the design and synthesis of the \( \text{Ln}^{3+} \) chelating DMPE-Glu-DTPA phospholipid proved to significantly alter \( \Delta \chi \). DMPE-Glu-DTPA was a viable alternative to DMPE-DTPA, allowing the formation of magnetically responsive DMPC/DMPE-Glu-DTPA/Ln\(^{3+}\) (molar ratio 4:1:1) assemblies. Using glutamic acid as a backbone for the \( \text{Ln}^{3+} \) chelating head group resulted in a very different geometry of the coordination polyhedron offering a fifth carboxylic acid moiety for complexation. In addition to the nature of the chelated \( \text{Ln}^{3+} \) ion, the chemical and geometrical characteristics of DMPE-Glu-DTPA defined a new set of crystal field energies, resulting in a unique \( \Delta \chi \). The DMPE-Glu-DTPA/Ln\(^{3+}\) complex inverted the \( \Delta \chi \), achieving perpendicular alignment of assemblies containing \( \text{Dy}^{3+} \) and parallel alignment for those containing \( \text{Tm}^{3+} \) and \( \text{Yb}^{3+} \) see Figure 8.13. Such a possibly has never been demonstrated for planar or bicellar systems.\(^{23-25,65,95-98,105,169}\) Offering promising opportunities for the proposed \( \text{Ln}^{3+} \) chelating DMPE-Glu-DTPA phospholipid. It may be complementary to the known and commercially available DMPE-DTPA. The possibility of tuning, enhancing and inverting the magnetic properties of phospholipid assemblies shown herein may find use in NMR structural studies of biomolecules.\(^{2,7-9,23-25,65,95}\) DMPE-Glu-DTPA complexed to copper could possibly speed-up the T1 relaxation of the embedded membrane proteins and shorten measurement time.\(^{27}\) In magnetic resonance imaging (MRI), \( \text{Ln}^{3+} \) chelating moieties chemically bound to biological molecules such a Chol-OH and phospholipids are important contrast agents.\(^{153,170-172}\) The field of MRI is in constant search of new contrast agents providing unique changes in relaxation rates of neighbouring water molecules for specific applications.\(^{155}\) The DMPE-Glu-DTPA/Ln\(^{3+}\) complex may be interesting for such applications. Moreover, the Glu-DTPA head group may readily be associated to other biomolecules such as Chol-OH or Phosphatidylethanolamines.\(^{123}\) Such degrees of freedom in chemical synthesis are essential to fully exploit the properties of the Glu-DTPA/Ln\(^{3+}\) moiety in, for example, the design of recombinant high-density lipoprotein nanoparticles for MRI studies of the liver.\(^{120,175}\) \( \text{Ln}^{3+} \)-based nanomaterials capable of emitting visible light when excited by NIR light represent another growing field of biomedicine and imaging in which \( \text{Ln}^{3+} \) chelating compounds such as DMPE-Glu-DTPA could be useful.\(^{176}\) The possibility of altering the \( \Delta \chi \) of these compounds and achieving very different magnetic responses could further pave the way for developments in the field of magnetic biosensing.\(^{177,178}\) DMPC/Chol-OH/DMPE-Glu-DTPA/Ln\(^{3+}\) (molar ratio 16:4:5:5) bicelles conserved their inverted \( \Delta \chi \), whilst benefiting from an enhanced thermal resistance offered by the liquid ordered phase induced by Chol-OH in the phospholipid bilayer. This enhanced thermal stability may find use in numerous applications including drug delivery for dermal applications,\(^{113,116}\) protein characterization by NMR spectroscopy,\(^{3,7,8,112}\) and for the development of smart optical gels.\(^{15,16,20,90}\) DMPE-Glu-DTPA, coupled with the more general tuning of \( \Delta \chi \) of \( \text{Ln}^{3+} \) chelating molecules from the molecular level, are additional and important tools for the vast and ever-growing field of soft material science and engineering.

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Figure 8.13 Schematic representations of the studied polymolecular assemblies aligned with respect to the magnetic field direction B (black arrow). The inversed alignment achieved with the engineered DMPE-Glu-DTPA/Ln$^{3+}$ complex is illustrated for Dy$^{3+}$ (blue) and Tm$^{3+}$ (red). The maximal alignment factors $A_f$ obtained at 5 °C and 8 T are provided. The DMPC/DMPE-Glu-DTPA/Ln$^{3+}$ (molar ratio 4:1:1, [tL] 15 mM) system revealed asymmetric assemblies with ripples in the cryo-TEM micrographs of Figure 8.2, which are represented here as sheets with waves. The bicelles obtained for the reference system DMPC/DMPE-DTPA/Ln$^{3+}$ (molar ratio 4:1:1, [tL] 15 mM) are represented as disks. The sign of $\Delta \chi$ of the assemblies is either positive (green, +) or negative (red, -). Assemblies aligning parallel to the magnetic field direction have the freedom to rotate without experiencing any change in potential energy as shown with a curved arrow. $^{20,29}$ Assemblies aligning perpendicular to the field direction do not possess this additional degree of freedom. Consequently, they may reach full alignment at sufficiently high magnetic field strengths. $^{29}$ Figure adapted from S. Isabettini et al. $^{67}$
9 Development of Novel Smart Optical Hydrogels

Smart hydrogels are formidable soft smart materials that may be engineered to selectively respond to a trigger or stimuli, giving rise to a multitude of abrupt changes in physical properties and functionality. These systems provide countless engineering possibilities, interesting for a wide range of applications comprising nanoscale templates, biomedical devices, drug delivery, tissue engineering and sensor development. Magnetic fields are important external stimuli owing to their commercial availability and non-invasive nature. Numerous smart hydrogels have been engineered to respond to external magnetic fields by associating magnetic nanoparticles to the gel network. Polymolecular assemblies such as micelles, bicelles and liposomes are additional building blocks for the design of modern smart hydrogel systems. Magnetically responsive bicelles imbedded in gelatin deliver switchable anisotropy and orientation-dependent optical properties. These phenomena were only reported for a single bicelle-gelatin pair: DPPC/Chol-OH/DPPE-DTPA/Ln\(^{3+}\) (molar ratio 16:4:5:5) bicelles in porcine gelatin. The system’s ability to deliver a high degree of anisotropy was bound to the depletion mechanism occurring in the vicinity of the gelatin aggregates, causing bicelle stacking. Moreover, high magnetic field strengths of 8 T were required to deliver gels with a reasonable optical activity. Herein, we demonstrate the versatility and enhance the viability of this technology by incorporating the findings from the previous chapters to achieve novel smart optical hydrogels, moving to the third and uppermost level of the S-PRO\(^2\) scheme in Figure 1.1.
9 Development of Novel Smart Optical Hydrogels

A four-step investigation is proposed: first, the DPPC/Chol-OH/DPPE-DTPA/ Ln$^{3+}$ (molar ratio 16:4:5:5) bicelles were replaced with DMPC/Chol-NH$_2$/DMPE-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5) bicelles in 10% (w/w) porcine gelatin. Building up from the findings of chapter 5 and 6; the presence of Chol-NH$_2$ in the bilayer significantly enhances the magnetic response of the bicelles by selectively altering the magnetic susceptibility of the Ln$^{3+}$-chelating phospholipids.$^{67}$ Moreover, the presence of a steroid compound generates a liquid-ordered state in the phospholipid bilayer, enabling the magnetically alignable bicelles to exist at temperatures as high as 35 °C.$^{152}$ In a second step, the magnetic response of the DMPC/Chol-NH$_2$/DMPE-DTPA/Ln$^{3+}$ (molar ratio 16:4:5:5) bicelles was greatly enhanced employing the novel fabrication procedures revealed in chapter 4.$^{125}$ The largest and most magnetically responsive species were required to generate gels with a strong optical signature at magnetic field strengths lower than 3 T. The alignment direction of the imbedded bicelles was tailored by altering the nature of the chelated Ln$^{3+}$. In a third step, the porcine gelatin was replaced by cold-water fish gelatin to ascertain the versatility of the proposed technology. The different amino acid profile of cold-water fish gelatin enables working at lower temperatures before gelling occurs.$^{198–200}$ Since the thermal energy of the solution acts against bicelle alignment, lower temperatures are beneficial.$^{16,30,111}$ In a fourth and final step, the additional degrees of freedom offered by the DMPC/Chol-NH$_2$/DMPE-DTPA/Ln$^{3+}$ (molar ratio 16:4:5:5) system in cold-water fish gelatin was employed to gel bicelles that simultaneously align in two directions. The resulting unique optical gel properties were investigated as a function of the temperature history. Systems with highly specific optical signatures were developed, offering promising perspectives for the development of modern temperature sensors and switches.

9.1 Chol-NH$_2$ bicelles in porcine gelatin

DMPC/Chol-NH$_2$/DMPE-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5) bicelles are highly alignable in magnetic fields and deliver a formidable thermal resistance.$^{90,152}$ The high magnetic response originates from the altered magnetic susceptibility of the bilayer phospholipids in the presence of Chol-NH$_2$. Moreover, the liquid ordered phase induced by the presence of the steroid molecule gives rise to the high thermal resistance of the bicelles.$^{15,16,84,152,159}$ These systems are alignable above the gelling temperature of porcine gelatin of 22 °C on cooling at 1 °C/min. Therefore, the bicelles may be entrapped in an aligned state in the gelatin network, fulfilling the requirements for delivering optical properties to the gel. The highly tunable magnetic properties of DMPC-based systems makes them promising candidates and viable alternatives to the DPPC/Chol-OH/DPPE-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5) bicelles proposed by M. Liebi et al. for delivering switchable anisotropy in optical gels.$^{20}$ This possibility was evaluated by replacing the reference DPPC-based bicelle systems with DMPC/Chol-NH$_2$/DMPE-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5). The samples were prepared following the same protocols described by M. Liebi et al. (see chapter 3.2) to guaranty comparability.$^{16,20,30}$ 10% w/w porcine gelatin was subsequently dissolved in the bicelle solution.
Figure 9.1 Alignment factor $A_f$ as a function of temperature of DMPC/Chol-NH$_2$/DMPE-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5) bicelles in 10% (w/w) porcine gelatin. The bicelles were prepared in a 50 mM phosphate buffer at a pH value of 7.4 and a [tL] of 15 mM from a dry lipid film by consecutive freeze-thawing cycles followed by extrusion through membranes with a pore diameter of 200 and 100 nm at 60 °C. The sample was subject to heating and cooling cycles at 1 °C/min either in the absence of a magnetic field or at 8 T. Key positions along the temperature cycle are labelled from A to F and a corresponding schematic of the polymolecular assemblies is provided. Vesicles are represented with circles and the planar bicelles as lines. Bicelle alignment is shown assuming a vertical magnetic field direction. Light blue represents the liquid state of the surrounding solution and dark blue corresponds to the gelled state. In A, isotropic bicelles were immobilized in the gelatin matrix at 0 T and 5 °C. In B, vesicles coexisted with bicelles in a liquid solution at 0 T and 45 °C. In C, the solution mainly consisted of aligned bicelles at 8 T and 34 °C. In D, the solution gelled and entrapped the aligned bicelles at 8 T and 22 °C. In E, the bicelles remained aligned as the field was ramped down from 8 to 0 T at 5 °C. F corresponds the same state as B at 0 T and 45 °C, stressing the thermoreversible nature of the process.

at 60 °C and the sample was stored in a gelled state at 5 °C before measurement. The magnetic alignment of the bicelles as a function of temperature was evaluated through computation of alignment factors $A_f$ in SANS at 8 T and the resulting optical properties were monitored by birefringence at 5.5 T.

The $A_f$ obtained from the 2D neutron scattering patterns as a function of temperature for DMPC/Chol-NH$_2$/DMPE-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5) bicelles in 10% (w/w) porcine gelatin are presented in Figure 9.1. The sample was first heated from 5 to 45 °C at 1 °C/min in the absence of a magnetic field (Figure 9.1, point A to B). The gelatin melted at 33 °C, freeing the bicelles into solution. At high temperatures of 45
°C (Figure 9.1, point B), most of the bicelles collapsed into non-alignable vesicles. The bicelle-to-vesicles transformation is thermoreversible and originates from the disordered liquid state of the bilayer lipids (see chapter 4).\textsuperscript{125} The magnetic field was ramped up to 8 T at 45 °C causing the $A_f$ to rise from 0 to -0.30. The anisotropy arised from partial stacking of the bicelles as evidenced by the appearance of Bragg peaks in the 2D neutron scattering pattern in Figure 9.2A. An analogous phenomenon was reported in the reference DPPC/Chol-OH/DPPE-DTPA/Tm\textsuperscript{3+} (molar ratio 16:4:5:5) bicelles in gelatin.\textsuperscript{20} The stacking probably originates from the thermodynamic depletion interactions occurring between the bicelles and the gelatin molecules.\textsuperscript{20,30} Stacking promotes the magnetic aligning by enhancing the aggregation number $n$. The bicelle stacks have a larger cumulative magnetic orientation energy, allowing them to overcome the high thermal energy of the solution at 45 °C and align.

The $A_f$ increased with decreasing temperature at a constant field strength of 8 T (Figure 9.1). This result is attributed to two phenomena: the thermal energy acting against the alignment is reduced and the alignable bicelles are gradually regenerated from non-alignable vesicles upon cooling.\textsuperscript{16,30,125} The bicelle species remained partially stacked and the anisotropy strengthened upon cooling to 34 °C (Figure 9.1, point C). The $A_f$ rose at an increasing rate from 34 °C, reaching a peak value of -0.78 at 30 °C before decreasing again to -0.67 moments before gelling at 26 °C. This result is attributed to the disappearance of the Bragg peaks in this temperature window, causing a change in shape of the anisotropic 2D neutron scattering patterns from which the $A_f$ values are computed, see Figure 9.2A. The bicelles were no longer stacked when gelled. This result is in sharp contrast to the reference DPPC/Chol-OH/DPPE-DTPA/Tm\textsuperscript{3+} (molar ratio 16:4:5:5) system that remained stacked in the gel. Although the stacking behavior enabled alignment at high temperatures, it was the naturally high magnetic alignability of DMPC/Chol-NH\textsubscript{2}/DMPE-DTPA/Tm\textsuperscript{3+} (molar ratio 16:4:5:5) bicelles that delivered a high degree of anisotropy moments before gelling. This ultimately guarantees the gel’s optical properties and stresses the importance of working with intrinsically highly magnetically responsive polymolecular assemblies for generating switchable anisotropy in optical gels.

The $A_f$ was stable as the anisotropic 2D neutron scattering pattern remained unchanged upon further cooling. The bicelles were fixed into position by the gel until the target temperature of 5 °C was reached (Figure 9.1, point E). The $A_f$ was unchanged as the magnetic field was ramped down at 5 °C, proving the successful gelling of the aligned bicelles. Although these systems did not benefit from an enhanced alignment due to stacking, a high $A_f$ value of -0.70 at 5 °C and 0 T was achieved. The optical properties of the gel may be dismantled upon heating above 33 °C, where the bicelles are freed from the gelatin network and returned to an isotropic state in the absence of an external magnetic field (Figure 9.1, point E to F). The process was thermoreversible and the bicelles may be re-aligned and gelled starting from point B of Figure 9.1.

The SANS experiments in Figure 9.1 demonstrate the possibility of imbedding the DMPC/Chol-NH\textsubscript{2}/DMPE-DTPA/Tm\textsuperscript{3+} (molar ratio 16:4:5:5) bicelles in 10% (w/w) porcine gelatin in a highly aligned state in the absence of any stacking. However, the
Figure 9.2 A) 2D neutron scattering patterns of DMPC/Chol-NH$_2$/DMPE-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5, [tL] 15 mM) bicelles in 10% (w/w) porcine gelatin upon cooling at 8 T from 45 to 26 °C. The solution is liquid under these conditions as gelling only occurs at 22 °C on cooling. Bragg peaks were apparent in the scattering patterns at 45, 35 and 30 °C and disappeared at 26 °C. B) Birefringence signal of the sample as a function of temperature. The solution was cooled from 60 to 5 °C at 1 °C/min under a 5.5 T magnetic field (blue line). The noise from 60 to 35 °C was caused by the highly turbid nature of the solution. The solution was transparent when gelled as evidenced by the spectrophotometry measurements in Figure 9.3. The bicelles were successfully entrapped in the gel in an aligned state as the birefringence signal remained unchanged when ramping the field down to 0 T at 5 °C, offering the gel optical properties. The sample was subsequently heated from 5 to 60 °C at 1 °C/min (red line) and the birefringence signal collapsed to zero as the gel melted at 33 °C and the bicelles returned to an isotropic state before forming vesicles.
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optical properties remain to be demonstrated. Analogously to what was done for the reference bicelle system by M. Liebi et al., the anisotropy for electromagnetic wave transmission was assessed by monitoring the birefringence signal as a function of temperature in Figure 9.2B. The birefringence signal is proportional to the degree of alignment of the bicelles, acting as a complementary means of quantifying anisotropy alongside the \( A_f \) values computed from SANS.\(^{15,16,20,29,30,67,90,105,125}\) A cooling cycle from 60 to 5 °C at 1 °C/min was applied under a 5.5 T field to gel the bicelles in an aligned state. The high turbidity of the solution did not permit monitoring of the birefringence signal from 60 to 35 °C as evidenced by the noisy signal in Figure 9.2B and the spectrophotometry results in Figure 9.3. However, the system became transparent in the pre-gelling and gelled state below 35 °C and the birefringence signal could be monitored. The peak in birefringence signal between 35 and 25 °C was analogous to that observed in the \( A_f \) values in SANS in Figure 9.1. It may be attributed to the disappearance of bicelle stacking before complete gelation. The birefringence signal remained unchanged when the magnetic field was ramped down to 0 T at 5 °C. Consequently, the bicelles were successfully gelled in an aligned state. The gel had an optical signature, supporting the affirmations made in the discussion of the \( A_f \) results in Figure 9.1. Subsequent heating to 45 °C at 0 T removed the optical activity as the gel melted and the bicelles returned to an isotropic state above 33 °C. This was evidenced by a zeroing of the birefringence signal and the reappearance of a turbid solution as supported by spectrophotometry measurements in Figure 9.3.

Gels with defined optical characteristics may be generated by working at various field strengths and gelling the bicelles at different degrees of alignment. The magnetic response and alignment direction may also be tailored with the chelated Ln\(^{3+}\).\(^{23,29,30,65,90}\) These possibilities will be demonstrated along with other variables, including the nature of the gelatin, which define the optical properties of the resulting gel. The multitude of available variables gives rise to a large degree of freedom in design, offering the possibility of generating gels with uniquely different optical properties.
Figure 9.3 Optical density of DMPC/Chol-NH$_2$/DMPE-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5, [tL] 15 mM) bicelles in 10% (w/w) porcine gelatin as a function of temperature measured at a wavelength of 400 nm on heating and cooling at 1 °C/min. The hysteresis was caused by the difference in gelling and melting temperature of the porcine gelatin upon cooling or heating.
9.2 Enhancing the magnetic response of Chol-NH$_2$ bicelles in porcine gelatin

Figure 9.4 Alignment factor $A_f$ as a function of temperature of A) DMPC/Chol-NH$_2$/DMPE-DTPA/Tm$^{3+}$ and B) DMPC/Chol-NH$_2$/DMPE-DTPA/Dy$^{3+}$ (molar ratio 16:4:5:5, [tL] 15 mM) bicelles in 10% (w/w) porcine gelatin. The bicelles were extruded through membranes with a pore diameter of 800 nm at 60 °C. The sample was subject to heating and cooling cycles at 1 °C/min either in the absence of a horizontal magnetic field (white arrow) or at 8 T. Key positions along the temperature cycle are labelled from A to F analogously to Figure 9.1 to facilitate comparison. 2D neutron scattering patterns of the samples were recorded upon cooling at 8 T from 45 to 5 °C. The Tm$^{3+}$ chelating bicelles aligned perpendicular to the field direction (parallel neutron scattering pattern), while the Dy$^{3+}$ chelating bicelles aligned parallel (perpendicular neutron scattering pattern). Bragg peaks were apparent in the Tm$^{3+}$ chelating system. No Bragg peaks were observed in the corresponding Dy$^{3+}$ chelating system. The bicelles showed no signs of stacking when gelled, regardless of the Ln$^{3+}$.

The gels produced by DMPC/Chol-NH$_2$/DMPE-DTPA/Tm$^{3+}$ (molar ratio 16:4:5 :5) bicelles in 10% (w/w) porcine gelatin required a strong magnetic field strength of 8 T to obtain respectable optical properties. In order to envisage future applications, the magnetic response of these systems must be enhanced, aiming to achieve anisotropy at magnetic field strengths ranging from 1 T (permanent magnets) to 3 T (commercially available electromagnets). In a first step, the magnetic response of the bicelles
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was enhanced by increasing their size with an altered fabrication procedure. Larger bicelles contain more lipids capable of contributing to the cumulative magnetic energy $E_{mag}$ (increased aggregate number $n$) of the bilayer, resulting in more alignable species.$^{12,13,16,61,125}$ The samples were extruded 10 times through membranes with a pore diameter of 800 nm at 60 °C instead of 200 and 100 nm. The solution was composed of vesicles at 60 °C, whose size was tailored by the extrusion process.$^{16,152}$ Bicelles were subsequently regenerated upon cooling to 5 °C and their dimensions were dictated by the vesicle precursors as described in chapter 4.$^{125}$ In a second step, the lanthanide ion was changed to Dy$^{3+}$, which has a larger and switched molar magnetic susceptibility $\Delta \chi$. $^{23,29,30,65,90}$ The negative $\Delta \chi$ of Dy$^{3+}$ adds on to the naturally negative $\Delta \chi$ of the bilayer phospholipids, enhancing the contribution of the individual lipids to the total $E_{mag}$ of the bilayer. Consequently, a higher magnetic response is expected at lower magnetic field strengths.$^{29}$ The switched $\Delta \chi$ of Dy$^{3+}$ resulted in a parallel bicelle alignment to the magnetic field, instead of perpendicular with Tm$^{3+}$. This altered alignment direction offers additional orientation-dependent optical properties to the gels.$^{30}$

The $A_f$ of DMPC/Chol-NH$_2$/DMPE-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5) bicelles in 10% (w/w) porcine gelatin fabricated by extrusion through pores with a diameter of 800 nm in Figure 9.4A evolved analogously with temperature to the corresponding sample extruded through 200 and 100 nm pores in Figure 9.1. The larger bicelle species successfully induced a high degree of alignment with an $A_f$ of -0.75 at 45 °C and 8 T, up from the -0.30 achieved with the previous sample in Figure 9.1. The alignment was aided by bicelle stacking as evidenced by the appearance of Bragg peaks in the 2D neutron scattering pattern at 45 °C and 8 T in Figure 9.4A. However, the 2D neutron scattering pattern at 35 °C and 8 T in Figure 9.4A showed no evidence of Bragg peaks. Therefore, stacking was lost upon cooling from 34 °C onwards. The $A_f$ gradually fell until it was fixed by gelling at a value of -0.60 (see Figure 9.4A, point D). There was no minimum in $A_f$ between 34 and 26 °C, unlike what was observed in the sample composed of smaller bicelles in Figure 9.1. The loss of alignment on gelling could be attributed to the larger bicelle size, whose alignment is likely disturbed by the formation of the gelatin network.$^{201–203}$ Nevertheless, the system was successfully gelled in an anisotropic state when ramping the magnetic field down to 0 T at 5 °C (see Figure 9.4A, point E), delivering optical properties to the gel.

A high $A_f$ of +0.70 was also achieved at 45 °C and 8 T when working with DMPC/Chol-NH$_2$/DMPE-DTPA/Dy$^{3+}$ (molar ratio 16:4:5:5) bicelles in 10% (w/w) porcine gelatin fabricated by extrusion through pores with a diameter of 800 nm in Figure 9.4B. The evolution of the $A_f$ with temperature was analogous to that of the Tm$^{3+}$ chelating sample in Figure 9.4A. However, the $A_f$ was switched to positive values due to the opposite alignment direction of the Dy$^{3+}$ chelating bicelles. The inversed alignment direction is confirmed by the perpendicular scattering of neutrons with respect to the magnetic field direction in Figure 9.4B. Unlike both Tm$^{3+}$ chelating bicelles in Figure 9.1 and 9.4A, the Dy$^{3+}$ chelating counterparts do not show any sign of stacking as evidenced by the absence of Bragg peaks in the neutron scattering patterns. Polymolecular assemblies aligning parallel to the magnetic field direction are free to rotate around the
Figure 9.5 Alignment factor $A_f$ as a function of temperature of DMPC/Chol-NH$_2$/DMPE-DTPA/Dy$^{3+}$ (molar ratio 16:4:5:5, [tL] 15 mM) bicelles in 10% (w/w) porcine gelatin. The bicelles were extruded through membranes with a pore diameter of 800 nm at 60 °C. The sample was subject to heating and cooling cycles at 1 °C/min either with or in the absence of a magnetic field, at 1 T or at 2 T. The bicelles showed no signs of stacking and were successfully gelled in an anisotropic state at 5 °C, yielding an $A_f$ of +0.10 and +0.25 at 1 and 2 T, respectively.

This added degree of freedom could make it energetically unfavorable for the bicelles to stack, which could explain the absence of Bragg peaks in the Dy$^{3+}$ chelating bicelles.

The high degree of magnetic alignment achieved with DMPC/Chol-NH$_2$/DMPE-DTPA/Dy$^{3+}$ (molar ratio 16:4:5:5) bicelles in 10% (w/w) porcine gelatin was exploited to deliver anisotropic optical gels at lower magnetic field strengths. The gels were prepared following the same heating and cooling protocols under a 1 and 2 T field, delivering $A_f$ values of +0.1 and +0.25, respectively (see Figure 9.5). The bicelles successfully delivered optical anisotropy to the gel at commercially viable magnetic field strengths.
9.3 Gelling aligned Chol-NH₂ bicelles in fish gelatin

Magnetically aligned DMPC/Chol-NH₂/DMPE-DTPA/Tm³⁺ (molar ratio 16:4:5:5) bicelles were imbedded in gelatin from cold-water fish skin instead of porcine gelatin. The samples were extruded 10 times through membranes with a pore diameter of 800 nm at 60 °C to guarantee optimal magnetic response. Fish gelatin has a lower concentration of imino acids (proline and hydroxyproline) than porcine gelatin, resulting in lower melting and gelling temperatures. The super-helix structure of gelatin gels is stabilized by steric restrictions imposed by pyrrolidine rings of the imino acids and by hydrogen bonding between amino acid residues. These physicochemical forces are responsible for stabilizing the ordered conformation of the gelatin gel.

The fish gelatin does not gel as effectively as porcine gelatin, requiring lower temperatures and sufficient time to develop a stable network. Therefore, the gelatin content was increased to 30 % (w/w), ensuring gel formation within 30 minutes at 5 °C. The lowered gelling temperature is strategic to achieve maximal alignment of the imbedded bicelles. The thermal energy of the solution is reduced, allowing the assemblies to reach higher degrees of alignment. Different gelling media demonstrates the versatility and enhances the viability of the proposed technology. The enriched toolbox widens the tailoring possibilities for developing temperature-dependent optical gel properties, paving the way for the soft-material temperature sensors of tomorrow.

The evolution of the $A_f$ with temperature of the DMPC/Chol-NH₂/DMPE-DTPA/Tm³⁺ (molar ratio 16:4:5:5) bicelles in 30% (w/w) fish gelatin in Figure 9.6A was very different than the corresponding system in 10% (w/w) porcine gelatin revealed in Figure 9.4A. The 2D neutron scattering patterns showed no evidence of Bragg peaks throughout the cooling process from 45 to 5 °C at 8 T. Consequently, the bicelles did not benefit from an enhanced alignment due to stacking, achieving a weak $A_f$ value of -0.10 at 45 °C and 8 T (see Figure 9.6A, point II). However, the slow gelling kinetics of the fish gelatin at 5 °C provided sufficient time for the regeneration of alignable bicelles from non-alignable vesicles upon cooling at 8 T. A strong regeneration occurred below 28 °C (see Figure 9.6A, point III), in line with previous results from SANS studies of DMPC/Chol-NH₂/DMPE-DTPA/Tm³⁺ (molar ratio 16:4:5:5) bicelles. An equivalent increase in birefringence signal occurred upon cooling (see Figure 9.6B, point III). An alignment factor of -0.48 and a birefringence signal of $2.5 \times 10^{-5}$ was achieved at 5 °C after ramping down the magnetic field, delivering optical properties to the gel (see Figure 9.6A and 9.6B, point IV). These results prove the possibility of generating optical properties in different gelatin systems. The different degree of bicellar alignment in fish gelatin offers a unique optical signature to the gel.

The optical properties of the gel obtained from anisotropic DMPC/Chol-NH₂/DMPE-DTPA/Tm³⁺ (molar ratio 16:4:5:5) bicelles entrapped in 30% (w/w) fish gelatin were lost upon heating to 15 °C at 0 T as the gel melts, see Figure 9.6. The optical properties were only lost above 33 °C in the analogous system made from porcine gelatin, see Figure 139.
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Figure 9.6 A) Alignment factor $A_f$ as a function of temperature at 8 T of DMPC/Chol-NH$_2$/DMPE-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5, [tL] 15 mM) bicelles in 30% (w/w) fish gelatin. B) Birefringence signal as a function of temperature at 5.5 T of DMPC/Chol-NH$_2$/DMPE-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5, [tL] 15 mM) bicelles in 30% (w/w) fish gelatin. The bicelles were by extrusion at 60 °C through membranes with a pore diameter of 800 nm. The samples were subject to heating and cooling cycles at 1 °C/min either with or without an external magnetic field. Key positions along the temperature cycle are labelled in roman numerals from I to V. The magnetic field was ramped up from point I to II, causing alignment of the bicelles coexisting in solution with non-alignable vesicles. The 2D neutron scattering patterns showed no evidence of Bragg peaks. Both the $A_f$ and birefringence signal rapidly increased on cooling at point III as the alignable bicelles were fully regenerated from the vesicles. The bicelles were successfully entrapped in the gel in an aligned state as both, the $A_f$ and the birefringence signal remained unchanged when ramping the field down to 0 T at point IV. The system was thermoreversible as the gel was melted upon heating to point V in the absence of a magnetic field and the bicelles returned to an isotropic state in solution.

9.4. This temperature-induced loss of optical properties may be interesting for tracking the temperature history of sensitive goods. For example, a solid gel cube composed of DMPC/Chol-NH$_2$/DMPE-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5) bicelles aligned at 8 T and gelled at 5 °C in fish gelatin 30% (w/w) could be placed in the packing material of a product as it is being transported from one location to the other. If the gel cube is no longer birefringent upon arrival, the temperature history of the product was not respected during shipping. The optical properties of the gel effectively act as a temperature sensor. The thermoreversible nature of these materials make them reusable when regenerated with a heating and cooling cycle in the presence of an external magnetic field. Further tuning of the gel’s thermal-resistance with additives could increase the temperature window for applications. The melting point of the gel and the corresponding loss of optical properties could be tuned. However, the impact of additives on
the bicelle alignment and the stability of the gel’s optical properties over time would need to be evaluated.

9.4 Gelling Chol-NH$_2$ bicelles simultaneously aligned in two directions

The possibility of imbedding bicelles simultaneously aligning parallel and perpendicular to the magnetic field direction in a single solution was evaluated to deliver gels with unique 3D optical properties. In a first stage, DMPC/Chol-NH$_2$/DMPE-DTPA/Ln$^{3+}$ (molar ratio 16:4:5:5) bicelles chelating either Dy$^{3+}$ or Tm$^{3+}$ were mixed 50/50 (v/v) at 5 °C in the absence of gelatin. The two bicelle samples were separately prepared in a 50 mM phosphate buffer at a pH value of 7.4 and a total lipid concentration of 15 mM. The dry lipid film was hydrated by consecutive freeze thawing cycles followed by extrusion through membranes with a pore diameter of 200 and 100 nm at 60 °C. The sample’s alignment was monitored as a function of temperature by SANS and birefringence measurements at 8 T and 5.5 T, respectively in Figure 9.7. This preliminary study was required to first understand the alignment behavior of the mixture of bicelles. The optimal conditions to imbed the mixture of bicelles in a state of maximal alignment in a gelatin network could then be identified.

The species chelating Dy$^{3+}$ aligned parallel to the magnetic field direction, resulting in a perpendicular anisotropy in the 2D neutron scattering pattern. Those chelating Tm$^{3+}$ aligned perpendicularly (parallel anisotropy). The neutron scattering patterns superimposed resulting in the cross observed in Figure 9.7, point I. Conclusively, the mixture of DMPC/Chol-NH$_2$/DMPE-DTPA/Ln$^{3+}$ (molar ratio 16:4:5:5) bicelles chelating either Dy$^{3+}$ or Tm$^{3+}$ mixed at 5 °C successfully resulted in species simultaneously aligning perpendicular and parallel to the magnetic field direction. This mixture of aligned states had an overall negative birefringence signal of -3.1x10$^{-6}$ at 5 °C in Figure 9.7B due to the more aligned Dy$^{3+}$ chelating bicelles. This bidirectional alignment was not thermoreversible. After undergoing a heating and cooling cycle, the superimposed 2D neutron scattering pattern was lost (see Figure 9.7A, point III) and the birefringence signal was altered to -7.5x10$^{-6}$ (see Figure 9.7B, point III). The same birefringence signal was obtained after a second heating and cooling cycle, proving the system reached an equilibrium and the initial bidirectional alignment was irreversibly lost.

The two bicelle species associated when forming vesicles upon heating. The bilayer lipids had sufficient freedom to diffuse and mix when the solution was heated to 45 °C. Consequently, the regenerated bicelles upon cooling contained both DMPE-DTPA species chelating Dy$^{3+}$ and Tm$^{3+}$ in their bilayer. Since Dy$^{3+}$ has an intrinsically larger molar magnetic susceptibility than Tm$^{3+}$, the resulting bicelles aligned parallel to the magnetic field direction. This was evidenced by the perpendicular scattering of neutrons in Figure 9.7A point III. Furthermore, the 2D neutron scattering pattern was broader
Figure 9.7 A) 2D neutron scattering patterns with corresponding schematic representations of the polynuclear assemblies existing in solution. Vesicles are represented by circles and bicelles as flat lines. Species exclusively chelating Tm$^{3+}$ are shown in red, while those chelating Dy$^{3+}$ are black. Assemblies simultaneously chelating Tm$^{3+}$ and Dy$^{3+}$ are shown in green. I: a 50/50 (v/v) mixture of Tm$^{3+}$ and Dy$^{3+}$ chelating DMPC/Chol-NH$_2$/DMPE-DTPA/Ln$^{3+}$ (molar ratio 16:4:5:5, [tL] 15 mM) bicelles was prepared at 5 °C and exposed to a 8 T horizontal magnetic field (white arrow). The two bicelle samples were separately prepared and extruded through membranes with a pore diameter of 200 and 100 nm at 60 °C. The Tm$^{3+}$ chelating bicelles aligned perpendicular to the magnetic field direction, while the Dy$^{3+}$ chelating bicelles simultaneously aligned parallel. The resulting neutron scattering patterns superimposed, resulting in a cross pattern. II: The sample was heated to 45 °C causing the bicelles to collapse into non-alignable vesicles before being regenerated when cooled back to 5 °C in III. The sample no longer contained species aligning in both directions as the resulting vertical 2D neutron scattering pattern was caused by species aligning parallel to the magnetic field direction. B) The same experiment was conducted whilst monitoring the birefringence signal at 5.5 T over two consecutive heating and cooling cycles at 1 °C/min. The key positions identified in SANS are labelled with the corresponding roman numerals.

than that obtained with the previously reported reference DMPC/Chol-NH$_2$/DMPE-DTPA/Dy$^{3+}$ (molar ratio 16:4:5:5) system, indicating the presence of less alignable bicelles. The Tm$^{3+}$ chelating DMPE-DTPA phospholipids reduced the cumulative magnetic energy of the bilayer by bringing a positive contribution to the predominantly negative system dominated by Dy$^{3+}$. The birefringence results supported this finding as the reference bicelles chelating only Dy$^{3+}$ delivered a stronger birefringence signal of -1.3x10^-5 at 5 °C and 5.5 T, see Figure 9.8.

The results obtained in Figure 9.7 indicated that bicelles chelating opposite Ln$^{3+}$ must be mixed at low temperatures to obtain bidirectional alignment. The gelling temperature range of porcine gelatin would require mixing of the species at temperatures above...
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Figure 9.8 Birefringence signal as a function of temperature of DMPC/Chol-NH$_2$/DMPE-DTPA/Dy$^{3+}$ (molar ratio 16:4:5:5, [tL] 15 mM) bicelles (dotted lines) and a 50/50 (v/v) mixture of Tm$^{3+}$ and Dy$^{3+}$ chelating DMPC/Chol-NH$_2$/DMPE-DTPA/Ln$^{3+}$ (molar ratio 16:4:5:5, [tL] 15 mM) bicelles (filled lines). Both samples were subject to a heating and cooling cycle from 5 to 60 °C and back prior to the measurement.

33 °C, where the solution remains sufficiently liquid. However, this temperature is too close to the point of collapse of the bicelles into vesicles, leading to an irreversible mixing of the DMPE-DTPA/Ln$^{3+}$ species in the bilayer. Therefore, cold-water fish gelatin was employed to gel the aligned bicelles and benefit from the resulting optical properties. The DMPC/Chol-NH$_2$/DMPE-DTPA/Ln$^{3+}$ (molar ratio 16:4:5:5) bicelles chelating either Dy$^{3+}$ or Tm$^{3+}$ were extruded through membranes with a pore diameter of 800 nm to guarantee the formation of maximally alignable species. The two samples were mixed 50/50 (v/v) with 30% (w/w) fish gelatin at 19 °C and cooled down to 5 °C in a 2 mm thick quartz cuvette. The resulting gel was placed in the neutron beam for monitoring of the $A_f$ as a function of temperature as described in Figure 9.9.

The bicelles aligned under a 8 T magnetic field when the fish gelatin melted at 15 °C in Figure 9.9 from point A to B. The sample was composed of species simultaneously and strongly aligning parallel and perpendicular to the magnetic field direction as evidenced by the marked cross in the 2D neutron scattering pattern in Figure 9.9. These results were analogous to those observed in the absence of gelatin and with smaller
Figure 9.9 Alignment factor $A_f$ as a function of temperature of DMPC/Chol-NH$_2$/DMPE-DTPA/Ln$^{3+}$ (molar ratio 16:4:5:5; [tL] 15 mM) bicelles chelating either Dy$^{3+}$ or Tm$^{3+}$ mixed 50/50 (v/v) with 30% (w/w) fish gelatin at 19 °C and cooled down to 5 °C (point A). The bicelles were extruded through membranes with a pore diameter of 800 nm at 60 °C. The sample was subject to heating and cooling cycles at 1 °C/min either in the absence of a magnetic field or at 8 T. Key positions along the temperature cycle are labelled from A to F and the corresponding snapshots of the 2D neutron scattering patterns are provided. The neutron scattering patterns of bicelles simultaneously aligning parallel and perpendicular superimpose and resulted in a cross from points B to C. Bicelles in solution are schematically represented as flat lines at points B and F. Species exclusively chelating Tm$^{3+}$ are shown in red, while those chelating Dy$^{3+}$ are black. Assemblies simultaneously chelating Tm$^{3+}$ and Dy$^{3+}$ are shown in green. Bicelle alignment is represented assuming a horizontal magnetic field direction.

DMPC/Chol-NH$_2$/DMPE-DTPA/Ln$^{3+}$ (molar ratio 16:4:5:5) bicelles in the neutron scattering pattern in Figure 9.7A, point I. Similarly to the birefringence signal, the low $A_f$ of -0.18 obtained in Figure 9.9 originated from the opposite alignment direction of the Dy$^{3+}$ and Tm$^{3+}$ chelating bicelles mixed in solution. The individual alignment factors act against each other and the obtained overall $A_f$ of the sample is low. This should not be misinterpreted as weak alignment. This simultaneous opposite aligned state of the bicelles was successfully gelled upon cooling back to 5 °C and switching off the magnetic field in Figure 9.9, point C.

Unlike the previous systems, the gel’s optical signature was not thermoreversible if heated above a certain temperature. The bilayer lipids diffuse and irreversibly mix upon melting of the gel, and subsequent vesicle formation, on heating from 5 °C (Figure 9.9, point C) to 45 °C (Figure 9.9, point D). The regenerated bicelles when cooling to 5 °C at 8 T were dominated by the Dy$^{3+}$, yielding positive alignment factors. These
results supported those obtained in the gelatin-free system in Figure 9.7. Analogously, the maximal $A_f$ of $+0.38$ at $5 \, ^\circ C$ in Figure 9.9, point F was smaller than that expected from bicelles of equivalent dimensions composed solely of Dy$^{3+}$. Nevertheless, we demonstrate the possibility of forming a smart hydrogel delivering two distinctly different optical properties using the same starting material. The optical properties were readily controlled by the combined action of temperature and magnetic fields.

9.5 Conclusion

The combined high magnetic response and thermal stability of DMPC/Chol-NH$_2$/DMPE-DTPA/Ln$^{3+}$ (molar ratio 16:4:5:5) bicelles allowed for the formation of novel smart hydrogels with defined optical signatures. The optical properties originated from the aligned state of the bicelles imbedded in the gel, resulting in an anisotropic transfer of electromagnetic waves yielding different spatial birefringence. The interaction of the Chol-NH$_2$ based bicelles with the gelatin network was markedly different when compared to the previously described DPPC-based systems. DMPC/Chol-NH$_2$/DMPE-DTPA/Ln$^{3+}$ (molar ratio 16:4:5:5) bicelles were not stacked in the gelatin matrix, providing a unique optical signature. The viability of these bicelles for the development of smart optical gels was demonstrated by enhancing their magnetic response, delivering anisotropy at commercially viable magnetic field strengths of 1 and 2 T. The enhanced magnetic alignability was achieved by increasing the bicellar size through optimized fabrication procedures. The versatility of the technology was extended by replacing the porcine gelatin with cold-water fish gelatin, demonstrating the possibility of employing different gels with defined gelling properties. The lower gelling temperature of cold-water fish gelatin allowed to gel a mixture of bicelles chelating either Tm$^{3+}$ or Dy$^{3+}$, which simultaneously aligned parallel and perpendicular to the magnetic field direction. The optical signature of the gel was defined by the degree of alignment of the imbedded bicelles. Every system offered a unique optical signature, tailored by the magnetic field strength or the nature of the bicelles. The temperature history of the gels influenced their optical properties, effectively acting as sensors. These smart hydrogels could find use in tracking of temperature-sensitive goods. The birefringence signal of an optical gel cube transported alongside the product may be monitored before, during and upon arrival, guaranteeing a respected cold chain. Furthermore, the large panel of achievable and well-defined gel optical properties acts as a bar-code. These materials are reusable owing to their thermoreversible nature. They are readily regenerated with a heating and cooling cycle in the presence of an external magnetic field. The gels may contain more than one distinct optical property when imbedding bicelles chelating different Ln$^{3+}$. The resulting optical characteristics could be reversibly switched upon heating above a given temperature. This feature enhances the degrees of freedom for engineering optical gel properties, providing a rich toolbox for future development of modern temperature sensors and switches.
10 Synthesis and Characterization

10.1 Synthesis of 3β-cholest-5-en-3-amine (Aminocholesterol, Chol-NH₂)

Step 1: 50 mmol of cholesterol (Amresco, USA) was dissolved in 250 ml of anhydrous dichloromethane and cooled to 4 °C. 100 mmol of Hönig’s base (DIEA) was added to the cholesterol solution followed by the dropwise addition of 55 mmol of methanesulfonyl chloride (MsCl) in 50 ml of anhydrous dichloromethane. The reaction ran for 3 hours at 4 °C and was monitored by TLC (heptane/ethyl acetate 4:1). The solvents were removed under vacuum and the residue dissolved in 15 ml of dichloromethane. The intermediate product (3β-cholest-5-en-3-ol methanesulfonate) was precipitated with 150 ml of methanol and recrystallized in methanol to achieve a pure compound with 94% yield.

Step 2: 43 mmol of trimethylsilyl azide (TMSN₃) and 77 mmol of boron trifluoride etherate were added to a solution of 38 mmol of 3β-cholest-5-en-3-ol methanesulfonate (product from step 1) in 200 ml of anhydrous dichloromethane. The reaction ran for 2 hours at 22 °C before it was quenched with 200 ml of a saturated sodium bicarbonate solution (NaCO₃ sat) in water. The organic layer was recovered and the aqueous layer was extracted three times with 100 ml of methyl tert-butyl ether. The organic layers were combined and washed with 200 ml of deionized water, dried over sodium sulfate, and concentrated under vacuum to afford the crude intermediate product. The compound was purified by flash chromatography (heptane/ethyl acetate 9:1) to afford 3β-azido-5-cholestene with 78% yield.
Step 3: 12 mmol of \(3\beta\)-azido-5-cholestene (product from step 2) was dissolved in 80 ml of anhydrous diethyl ether at 4 °C. 18 mmol of LiAlH\(_4\) were subsequently added in four equal portions over 30 min. The reaction was gradually heated to 22 °C and stirred for an additional 2 hours. The reaction was monitored by TLC (heptane/ethyl acetate 2:1). The mixture was subsequently cooled to 4 °C and quenched by dropwise addition of 10 ml of cold deionized water. The resulting solution was poured over an ice bath and the organic layer was recovered. The aqueous layer was extracted twice with 40 ml of ethyl acetate. The organic layers were combined, washed with 100 ml of a saturated NaCl solution and 100 ml of deionized water, dried over sodium sulfate, and concentrated under vacuum. The recovered solid was further recrystallized in acetonitrile to afford pure \(3\beta\)-cholest-5-en-3-amine with 63% yield. \textbf{HR-MS(+)}: calculated for C\(_{27}\)H\(_{48}\)N \([\text{M+H}]^+\): 386.3787. Found: 386.3781. \(\Delta m/z\): 1.5 ppm. \textbf{\(^1\)H-NMR} (400 MHz, CDCl\(_3\)) \(\delta\): 5.32 (d, 1H, H-6), 2.61 (m, 1H), 0.85-2.16 (m, 43H, cholesterol), 0.67 (s, 3H, cholesterol) ppm. \textbf{\(^13\)C-NMR} (100 MHz, CDCl\(_3\)/MeOD-d\(_4\) 4:1) \(\delta\): 140.69, 120.97, 56.53, 55.93, 51.25, 49.98, 42.03, 41.67, 39.54, 39.25 37.74, 36.21, 35.93, 35.56, 31.62, 31.57, 31.15, 27.96, 27.71, 23.98, 23.57, 22.39, 22.13, 20.73, 18.96, 18.34, 11.48 ppm.
10.2 Synthesis of the Chol-C\textsubscript{2}OC\textsubscript{2}-NH\textsubscript{2} aminocholesterol conjugate

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure10.2}
\caption{Three-step reaction protocol for the synthesis of the Chol-C\textsubscript{2}OC\textsubscript{2}-NH\textsubscript{2} aminocholesterol conjugate, an intermediate in the synthesis of Chol-C\textsubscript{2}OC\textsubscript{2}-DTPA proposed by Isabettini \textit{et al.}^{15}}
\end{figure}

\textit{Step 1:} 48 mmol of 2,2'-Oxybis(ethylamine) was dissolved in 500 ml of methanol (dried over 3 Å molecular sieves) at 0 °C. A 150 ml solution of THF (dried over 4 Å molecular sieves) containing 43 mmol of di-\textit{tert}-butyl dicarbonate was subsequently added drop wise. The reaction mixture was heated to room temperature and stirred for 38 hours. The residue after solvent removal was purified by flash chromatography with
isocratic CHCl$_3$/EtOH (1:1) and, subsequently, isocratic NH$_4$OH/EtOH/CHCl$_3$ (2:5:5). The product (C$_2$OC$_2$-BOC) yield was 57%. HR-MS(+) calculated for C$_{9}$H$_{21}$N$_{2}$O$_{3}$ [M+H]$^+$: 205.1547. Found: 205.1545. $\Delta m/z$: 1.0 ppm.

**1H-NMR** (400 MHz, CDCl$_3$) $\delta$: 4.99 (s, 1H, NH), 3.48 – 3.52 (m, 4H, CH$_2$-O-CH$_2$), 3.31 (m, 2H, H$_2$N-CH$_2$), 2.88 (t, 2H, CH$_2$-NH-COO...), 2.39 (s, 2H, H$_2$N-CH$_2$), 1.44 (s, 9H, Boc-N) ppm.

**Step 2:** 10.4 mmol of C$_2$OC$_2$-BOC (product from step 1) was dissolved in 85 ml of chloroform and added dropwise to a 50 ml solution containing 11.7 mmol of cholesteryl chloroformate in anhydrous, amylene stabalized chloroform. The reaction was stirred 24 hours at room temperature and monitored by TLC (heptane/ethyl acetate 3:1). The reaction vessel was periodically flushed with nitrogen to remove the produced HCl gas. 70 ml of a 5% NaHCO$_3$ solution was used to quench the reaction. Two extractions with 140 ml of a saturated NaHCO$_3$ solution and a last extraction with 140 ml of deionized water. The recovered organic layer was dried over sodium sulfate before solvent removal under vacuum. The recovered crude product was further purified by flash chromatography using heptane/ethyl acetate 3:1. The product (Chol-C$_2$OC$_2$-BOC) yield was 87%. HR-MS(+) calculated for C$_{37}$H$_{64}$N$_{2}$O$_{5}$Na [M+Na]$^+$: 639.4707. Found: 639.4701. $\Delta m/z$: 0.9 ppm.

**1H-NMR** (400 MHz, CDCl$_3$) $\delta$: 5.37 (d, 1H, H-6), 4.79 - 5.10 (s, 2H, NH), 4.49 (dt, 1H, H-3), 3.49 – 3.52 (m, 4H, CH$_2$-O-CH$_2$), 3.30 – 3.36 (m, 4H, N-CH$_2$...-CH$_2$-N), 1.45 (s, 9H, Boc-N), 0.67-2.35 (m, 44H, cholesterol) ppm.

**Step 3:** 6.2 mmol of Chol-C$_2$OC$_2$-BOC (product from step 2) was dissolved in 150 ml of anhydrous ethanol. 16.7 mmol of acetyl chloride was added dropwise to the ice-cooled mixture. The reaction was stirred for 24 hours and monitored by TLC (heptane/ethyl acetate 2:1). An additional 10% of acetyl chloride (1.53 mmol) was added and the reaction left for another 24 hours to react at room temperature and another 24 hours at 40 °C. The solvent was removed under vacuum and the crude product was purified by crystallization in ethanol and acetonitrile. The Chol-C$_2$OC$_2$-NH$_2$ product yield was 74%. HR-MS(+) calculated for C$_{32}$H$_{57}$N$_{2}$O$_{3}$ [M+H]$^+$: 517.4364. Found: 517.4360. $\Delta m/z$: 0.8 ppm. **1H-NMR** (400 MHz, CDCl$_3$) $\delta$: 8.39 (s, 3H, NH$_3$) 5.36 (d, 1H, H-6), 4.46 (dt, 1H, H-3), 3.55 – 3.76 (m, 4H, -CH$_2$-O-CH$_2$), 3.24 – 3.35 (m, 4H, N-CH$_2$...-CH$_2$-N), 0.67-2.33 (m, 44H, cholesterol) ppm. **13C-NMR** (100 MHz, CDCl$_3$/MeOD-d$_4$ 4:1) $\delta$: 156.75, 139.32, 122.02, 74.07, 69.84, 56.28, 55.78, 49.63, 41.86, 42.70, 40.03, 39.33, 39.20, 39.09, 38.11, 36.58, 36.09, 35.78, 35.40, 31.43, 27.81, 27.69, 27.53, 23.84, 23.46, 22.31, 22.06, 20.62, 18.80, 18.25, 11.38 ppm.
10.3 Synthesis of Chol-C\textsubscript{n}-DTPA

### Preparation of BOC-C\textsubscript{2}-O-C\textsubscript{2}

2,2'-Oxybis(ethylamine) (5 g, 48 mmol) was dissolved in 500 ml of methanol (dried over 3 Å molecular sieves) at 0 °C. A 150 ml solution of THF (dried over 4 Å molecular sieves) containing di-tert-butyl dicarbonate (9.4 g, 43 mmol) was subsequently added drop wise. The reaction mixture was gradually heated to room temperature and stirred for 38 hours. Solvents were removed under vacuum before proceeding to flash chromatography. An isocratic phase of CHCl\textsubscript{3}/EtOH (1:1) was initially applied to remove the di-bocylated side product before switching to an NH\textsubscript{4}OH/EtOH/CHCl\textsubscript{3} (2:5:5) system. TLC was employed to monitor the product formation, which has an $R_f$ of 0.35 when using the aforementioned solvent system. The product yield was 57%. HR-MS(\(+\)): calculated for C\textsubscript{9}H\textsubscript{21}N\textsubscript{2}O\textsubscript{3} [M+H]\(+\): 205.1547. Found: 205.1545. $\Delta m/z$: 1.0 ppm.

### Preparation of BOC-C\textsubscript{n}-Chol

#### BOC-C\textsubscript{5}-Chol

N-Boc-1,5-diaminopentane (2.11 g, 10.4 mmol) dissolved in 85 ml of chloroform was added drop wise in a 50 ml solution of cholesteryl chloroformate (5.25 g, 11.7 mmol) in chloroform (amylene stabilized, dried over 4 Å). The reaction was stirred 24 hours at room temperature. TLC with a heptane/ethyl acetate solvent system 3:1 was used to monitor the reaction. The product has an $R_f$ of 0.26. The reaction vessel was periodically flushed with nitrogen to remove the produced HCl gas. 70 ml of a 5% NaHCO\textsubscript{3} solution was used to quench the reaction followed by two subsequent extractions with 140 ml of a saturated NaHCO\textsubscript{3} solution. A final extraction was executed with 140 ml of deionized water and the recovered organic layer was dried before proceeding to solvent removal under vacuum. The recovered crude product was further purified by flash chromatography using heptane/ethyl acetate 3:1. The product yield was 63%. HR-MS(\(+\)): calculated for C\textsubscript{38}H\textsubscript{66}N\textsubscript{2}O\textsubscript{4}Na [M+Na]\(+\): 637.4915. Found: 637.4907. $\Delta m/z$: 1.2 ppm. $^1$H-NMR (400 MHz, CDCl\textsubscript{3}) $\delta$: 5.35 (d, 1H, H-6), 4.55 - 4.66 (s, 2H, NH), 4.48 (dt, 1H, H-3), 3.09 – 3.15 (m, 4H, N-CH\textsubscript{2}-...-CH\textsubscript{2}-N), 1.28 (s, 9H, Boc-N), 0.67-2.37 (m, 44H, cholesterol) ppm.

#### BOC-C\textsubscript{2}-Chol

Synthesized following the same protocol as for BOC-C\textsubscript{5}-Chol. Yield: 65% HR-MS(\(+\)): calculated for C\textsubscript{35}H\textsubscript{60}N\textsubscript{2}O\textsubscript{4}Na [M+Na]\(+\): 595.4445. Found: 595.4435. $\Delta m/z$: 1.7 ppm. $^1$H-NMR (400 MHz, CMW) $\delta$: 5.84 - 5.32 (m, NH), 5.25 (d, 1H, H-6), 4.33(dt, 1H, H-3), 3.88 - 3.72 (s, NH), 3.11 - 2.95 (m, 4H, N-CH\textsubscript{2}-CH\textsubscript{2}-CH\textsubscript{2}-N), 1.28 (s, 9H, Boc-N), 0.53-2.14 (m, 44H, cholesterol) ppm.

#### BOC-C\textsubscript{6}-Chol

Synthesized following the same protocol as for BOC-C\textsubscript{5}-Chol. Yield: 65% HR-MS(\(+\)): calculated for C\textsubscript{35}H\textsubscript{60}N\textsubscript{2}O\textsubscript{4}Na [M+Na]\(+\): 595.4445. Found: 595.4435. $\Delta m/z$: 1.7 ppm. $^1$H-NMR (400 MHz, CMW) $\delta$: 5.84 - 5.32 (m, NH), 5.25 (d, 1H, H-6), 4.33(dt, 1H, H-3), 3.88 - 3.72 (s, NH), 3.11 - 2.95 (m, 4H, N-CH\textsubscript{2}-CH\textsubscript{2}-CH\textsubscript{2}-N), 1.28 (s, 9H, Boc-N), 0.53-2.14 (m, 44H, cholesterol) ppm.

BOC-C\textsubscript{6}-Chol
Synthesis and Characterization

Synthesized following the same protocol as for BOC-C₅-Chol.

**Yield**: 84%. **HR-MS(+)**: calculated for C₃₉H₆₈N₂O₄Na [M+Na]^+: 651.5071. Found: 651.5069. \( \Delta m/z: 0.3 \text{ ppm} \). **¹H-NMR** (400 MHz, CDCl₃) \( \delta \): 5.37 (dt, 1H, H-6), 4.71 - 4.58 (br, 1H, NH), 4.58 - 4.36 (m, 2H, H-3, NH), 3.25 - 2.95 (m, 4H, N-CH₂-)CH₂-N), 1.32 – 1.59 (m, 8H, -(CH₂)₄-), 1.45 (s, 9H, Boc-N), 0.67-2.37 (m, 44H, cholesterol) ppm.

**BOC-C₂-O-C₂-Chol**

Synthesized following the same protocol as for BOC-C₅-Chol.

**Yield**: 87% **HR-MS(+)**: calculated for C₃₇H₆₄N₂O₅Na [M+Na]^+: 639.4707. Found: 639.4701. \( \Delta m/z: 0.9 \text{ ppm} \). **¹H-NMR** (400 MHz, CDCl₃) \( \delta \): 5.37 (d, 1H, H-6), 4.79 - 5.10 (s, 2H, NH), 4.49 (dt, 1H, H-3), 3.49 – 3.52 (m, 4H, CH₂-O-CH₂), 3.30 – 3.36 (m, 4H, N-CH₂-)CH₂-N), 1.45 (s, 9H, Boc-N), 0.67-2.35 (m, 44H, cholesterol) ppm.

**Preparation of Cₙ-Chol**

**C₅-Chol**

BOC-C₅-Chol (3.8 g, 6.2 mmol) was dissolved in 150 ml of bone dry ethanol (3 Å molecular sieves). Heating up to 40 °C before cooling back down to 0 °C was necessary to fully dissolve the compound. Acetyl chloride (1.31 g, 16.7 mmol) was added drop wise to the ice-cooled mixture. The reaction was stirred for 24 hours and monitored by TLC with heptane/ethyl acetate 2:1. An additional 10% of acetyl chloride (0.12 g, 1.53 mmol) was added and the reaction left for another 24 hours to react at room temperature. To enhance conversion further, the temperature was increased to 40 °C and the reaction was stirred for an additional 24 hours. The solvent was removed under vacuum and the crude product was purified by crystallization in ethanol and acetonitrile. The product yield was 72%. **HR-MS(+)**: calculated for C₃₃H₅₉N₂O₂ [M+H]^+: 515.4571. Found: 515.4574. \( \Delta m/z: 0.6 \text{ ppm} \). **¹H-NMR** (400 MHz, CDCl₃) \( \delta \): 8.22 (s, 3H, NH₃+), 5.35 (d, 1H, H-6), 4.48 (dt, 1H, H-3), 3.16 (t, 2H, CH₂-NH-), 3.03 (s, 2H, +H₃N-CH₂⁻), 1.61 – 1.93 (m, 6H, CH₂-CH₂-CH₂), 0.6-2.4 (m, 44H, cholesterol) ppm.

**C₂-Chol**

Synthesized following the same protocol as for C₅-Chol.

**Yield**: 46%. **HR-MS(+)**: calculated for C₃₀H₅₃N₂O₂ [M+H]^+: 473.4102. Found: 473.4094. \( \Delta m/z: 1.7 \text{ ppm} \). **¹H-NMR** (400 MHz, CDCl₃): \( \delta \): 8.40 - 7.76 (br, 3H, NH), 7.08 - 6.20 (m+br, 1H, NH), 5.52 - 5.17 (br, 1H, H-6), 4.72 - 4.20 (br, 1H, H-3), 3.79 - 3.37 (m+br, 2H, CH₂-NH-), 3.37 - 3.01 (m+br, 2H, H₂N-CH₂⁻), 0.6-2.4 (m, 44H, cholesterol) ppm.

**C₆-Chol**

Synthesized following the same protocol as for C₅-Chol.
10.3 Synthesis of Chol-C<sub>n</sub>-DTPA

**Yield:** 63%. **HR-MS(+):** calculated for C<sub>34</sub>H<sub>61</sub>N<sub>2</sub>O<sub>2</sub> [M+H]<sup>+</sup>: 529.4728. Found: 529.4722. \( \Delta m/z : 1.1 \) ppm. **1H-NMR** (400 MHz, MeOD-d<sub>4</sub>): \( \delta : 8.19 \) (s, 3H, NH<sub>+</sub>3), 5.90 - 5.20 (m, 1H, H-6), 4.58 - 3.98 (m, 1H, H-3), 3.20 - 3.05 (m, 2H, -CH<sub>2</sub>-NH-), 3.03 - 2.81 (m, 2H, H<sub>2</sub>N-CH<sub>2</sub>-), 1.59 – 1.91 (m, 8H, CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-), 0.6-2.4 (m, 44H, cholesterol) ppm.

**C<sub>2</sub>-O-C<sub>2</sub>-Chol**

Synthesized following the same protocol as for C<sub>5</sub>-Chol.

**Yield:** 74%. **HR-MS(+):** calculated for C<sub>32</sub>H<sub>57</sub>N<sub>2</sub>O<sub>3</sub> [M+H]<sup>+</sup>: 517.4364. Found: 517.4360. \( \Delta m/z : 0.8 \) ppm. **1H-NMR** (400 MHz, CDCl<sub>3</sub>): \( \delta : 8.39 \) (s, 3H, NH<sub>+</sub>3), 5.36 (d, 1H, H-6), 4.46 (dt, 1H, H-3), 3.55 – 3.76 (m, 4H, -CH<sub>2</sub>-O-CH<sub>2</sub>-), 3.24 – 3.35 (m, 4H, N-CH<sub>2</sub>-...-CH<sub>2</sub>-N), 0.67-2.33 (m, 44H, cholesterol) ppm.

**Preparation of Chol-C<sub>n</sub>-DTPA**

**Chol-C<sub>5</sub>-DTPA**

Diethylenetriaminepentaacetate dianyhydride (DTPAA) (10.5 g, 29.3 mmol) was dissolved in 180 ml of dimethylformamide (dried over 4 Å molecular sieves) and triethylamine (dried over 4 Å molecular sieves and distilled, 8 mL). The mixture was stirred for two hours at 50 °C. 3x 100 ml of toluene (bone dry over 4 Å molecular sieves) was used to further dehumidify the C<sub>5</sub>-Chol (3 g, 5.83 mmol), which was subsequently removed under vacuum. The resulting gel-like product was dissolved in 40 ml of dimethylformamide (dried over 4 Å molecular sieves) and added to the reaction mixture. The mixture was stirred for 3 hours at 50 °C and then cooled down to room temperature. The reaction was monitored by TLC with the solvent system CHCl<sub>3</sub>/MeOH/H<sub>2</sub>O/HCOOH (65:25:4:1). Under continuous stirring (1 hour), 40 ml of water was added to quench the reaction. 250 ml of 1 M HCl was subsequently added to the reaction mixture. Three extractions were conducted with 500 ml of a 4:1 mixture of chloroform/methanol. The combined organic layers were dried and filtered. The residue was washed with chloroform and the recovered filtrate solvents were removed under vacuum. The retrieved solid was dissolved in a small amount of the 4:1 chloroform/methanol solvent system and acetonitrile was added in sufficient amounts to cause the compound to precipitate out of the solution. The resulting flocs were recovered by filtration before being further purified by flash chromatography. An isocratic phase was employed consisting of CHCl<sub>3</sub>/MeOH/H<sub>2</sub>O/HCOOH (65:25:4:1). The product yield was 12%. **HR-MS(+):** calculated for C<sub>47</sub>H<sub>80</sub>N<sub>5</sub>O<sub>11</sub> [M+H]<sup>+</sup>: 890.5849. Found: 890.5848. \( \Delta m/z : 0.45 \) ppm. **HR-MS(-):** calculated for C<sub>47</sub>H<sub>78</sub>N<sub>5</sub>O<sub>11</sub> [M-H]<sup>−</sup>: 888.5703. Found: 888.5712 \( \Delta m/z : -0.9 \) ppm. **1H-NMR** (400 MHz, CMW): The likely existence of polymolecular aggregate structures during measurement reduces the resolution of the spectra. Splitting on the up field peaks of cholesterol suggests the formation of aligned species in the timeframe of measurement. The acquired spectra confirms the successful synthesis of the Chol-C<sub>5</sub>-DTPA compound by the characteristic cholesterol peaks: \( \delta : 5.36 \) (m, 1H, H-6), 4.42 (m, 1H, H-3), 0.60 - 2.40 (m, 44H) ppm. In combination with the appearance of the DTPA head group associated hydrogen atoms from 3.00 to 3.60 ppm.
Chol-C$_2$-DTPA

Synthesized following the same protocol as for Chol-C$_5$-DTPA.

**Yield:** 9%. **HR-MS(-):** calculated for C$_{44}$H$_{72}$N$_5$O$_{11}$ [M-H]$^-$: 846.5223. Found: 846.5238. $\Delta m/z$: -1.8 ppm. **$^1$H-NMR** (400 MHz, CMW): $\delta$: 5.31 - 5.12 (br, 1H, H-6), 4.35 - 4.17 (br, 1H, H-3), 4.12 - 2.49 (m+br, 22H), 2.29 - 1.98 (m, 2H), 1.96 - 1.56 (m, 5H), 1.57 - 0.61 (m, 33H), 0.60 - 0.41 (s, 3H, H-18) ppm. Due to aggregation and probably also partially alignment in the magnetic field the spectrum showed low resolution and the peaks were very broad.

Chol-C$_6$-DTPA

Synthesized following the same protocol as for Chol-C$_5$-DTPA.

**Yield:** 9%. **HR-MS(-):** calculated for C$_{48}$H$_{80}$N$_5$O$_{11}$ [M-H]$^-$: 902.5849. Found: 902.5869. $\Delta m/z$: -2.2 ppm. **$^1$H-NMR** (400 MHz, CMW): $\delta$: 5.27 - 5.09 (br, 1H, H-6), 4.07 - 3.67 (m+br, 1H, H-3), 3.62 - 2.47 (m, 22H), 2.33 - 1.95 (m, 2H), 1.90 - 1.56 (m, 5H), 1.47 - 0.57 (m, 41H), 0.53 - 0.45 (br, 3H, H-18) ppm. Due to aggregation and probably also partial alignment in the magnetic field the spectrum showed low resolution and the peaks were very broad.

Chol-C$_2$-O-C$_2$-DTPA

Synthesized following the same protocol as for Chol-C$_5$-DTPA.

**Yield:** 13%. **HR-MS(+):** calculated for C$_{46}$H$_{78}$N$_5$O$_{12}$ [M+H]$^+$: 892.5642. Found: 892.5643. $\Delta m/z$: -0.11 ppm. **HR-MS(-):** calculated for C$_{46}$H$_{76}$N$_5$O$_{12}$ [M-H]$^-$: 890.5496. Found: 890.5500 $\Delta m/z$: -0.5 ppm. **$^1$H-NMR** (400 MHz, CMW): The likely existence of polymolecular aggregate structures during measurement reduces the resolution of the spectra. Splitting on the up field peaks of cholesterol suggests the formation of aligned species in the timeframe of measurement. The acquired spectra confirms the successful synthesis of the Chol-C$_2$-O-C$_2$-DTPA compound by the characteristic cholesterol peaks: $\delta$: 5.30 (m, 1H, H-6), 4.38 (m, 1H, H-3), 0.61 - 2.26 (m, 44H) ppm. In combination with the appearance of the DTPA head group associated hydrogen atoms from 3.40 to 4.00 ppm.
10.3 Synthesis of Chol-C\textsubscript{n}-DTPA

Figure 10.3 Synthetic pathway for the preparation of Chol-C\textsubscript{n}-DTPA. 

- **i**: synthesis of BOC-C\textsubscript{2}-O-C\textsubscript{2} as described by Suzuki et al.\textsuperscript{121} 
- **ii**: condensation reaction to form BOC-C\textsubscript{n}-DTPA. 
- **iii**: cleavage of the protecting group. 
- **iv**: synthesis of Chol-C\textsubscript{n}-DTPA according to Rui et al.\textsuperscript{120} 

The synthetic pathway for Chol-C\textsubscript{2}-O-C\textsubscript{2}-DTPA is analogous to that of Chol-C\textsubscript{n}-DTPA presented here.
10.4 Synthesis of DMPE-Glu-DTPA

Di-tert-butyl-2-bromoethyliminodiacetate (TBB)

This synthesis was performed following previously reported protocols.\textsuperscript{1,2} Tert-butyl bromoacetate (58.515 g, 300 mmol) and potassium bicarbonate (33.37 g, 333 mmol) were dissolved in 600 ml of anhydrous DMF. The mixture was cooled in an ice bath and ethanolamine (8.14 g, 133 mmol) was added progressively over 10 minutes. The reaction mixture was left to warm up to room temperature and stirred for 24 hours. The reaction volume was reduced to 1/10th of its original volume by removal of DMF under vacuum. 300 ml of a saturated solution of sodium bicarbonate was subsequently added before extracting three times with 400 ml of tert-butyl methyl ether (MTBE). The organic fractions were individually washed twice with a saturated sodium carbonate solution and once with water. The organic fractions were combined, dried over anhydrous sodium sulfate and the solvent removed under vacuum to recover a yellow oil residue.

The residue was dissolved in 400 ml of CH\textsubscript{2}Cl\textsubscript{2} and triphenylphosphine (38.5 g, 147 mmol) was added at room temperature under stirring. The mixture was cooled down to 5 °C and placed under an Argon atmosphere. N-bromosuccinimide (26.1 g, 147 mmol) was added portion wise over 10 minutes. The reaction mixture was further stirred for 2 hours at 5 °C before the solvent was removed under vacuum. 300 ml of MTBE was added to the resulting oil, causing the formation of a solid. The solid was further extracted with MTBE and the resulting organic phases were combined. After removal of the MTBE under vacuum, the recovered orange oil was purified by flash chromatography using ethyl acetate/heptane (1:9) ramping up from pure heptane. The product was a colorless liquid at room temperature with a yield of 72\%. HR-MS(\textsuperscript{-}): calculated for C\textsubscript{14}H\textsubscript{27}NO\textsubscript{4}Br [M-H]: 352.11180. Found: 352.11141. ∆m/z: 1.1 ppm. \textsuperscript{1}H-NMR (400 MHz, CDCl\textsubscript{3}) δ: 3.47 (s, 4H, -C\textsubscript{6}H\textsubscript{4}-N-C\textsubscript{6}H\textsubscript{4}-), 3.42 (t, 2H, N-CH\textsubscript{2}-C\textsubscript{6}H\textsubscript{2}-Br), 3.13 (t, 2H, N-C\textsubscript{6}H\textsubscript{2}-CH\textsubscript{2}-Br), 1.44 (s, 18H, BOC) ppm. \textsuperscript{13}C-NMR (100 MHz, CDCl\textsubscript{3}) δ: 170.54, 81.46, 56.59, 30.28, 28.27 ppm.

protected Glu-DTPA (p-Glu-DTPA)

1 M phosphate buffer (150 ml, pH 8) was added to a solution of H-Glu(OBzl)-O\textsubscript{t}Bu-HCl (6.611 g, 20 mmol, Christof Senn Laboratories) and tert-butyl N-(2-bromoethyl)iminodiacetate (17.000 g, 48 mmol) in 80 ml of acetonitrile. The reaction mixture was vigorously stirred for 2 hours before the aqueous phase was replaced with 100 ml of fresh buffer. The reaction mixture was further stirred for 48 hours and the organic phase was recovered and dried under vacuum. The residue was dissolved in 150 ml of dichloromethane, washed twice with 20 ml of water, dried over sodium sulfate, and the solvent removed under vacuum. The compound was purified by flash chromatography using ethyl acetate/heptane (1:4) ramping up from pure heptane. The product was a slightly yellow tainted oil with a yield of 55\%. HR-MS(\textsuperscript{+}): calculated for C\textsubscript{44}H\textsubscript{74}N\textsubscript{3}O\textsubscript{12} [M+H]\textsuperscript{+}: 836.5267. Found: 836.5269. ∆m/z : 0.2 ppm and for C\textsubscript{44}H\textsubscript{73}N\textsubscript{3}O\textsubscript{12}Na [M+Na]\textsuperscript{+}: 858.5087. Found: 858.5082. ∆m/z : 0.6 ppm. \textsuperscript{1}H-NMR (400 MHz, CDCl\textsubscript{3}) δ: 7.28 – 7.38 (m, 5H, Ph), 5.10 (s, 2H, Ph-CH\textsubscript{2}-O-), 3.41 (s, 8H, -N-CH\textsubscript{2}-CO-), 3.34
10.4 Synthesis of DMPE-Glu-DTPA

(m, 1H, -CO-CH₂-CH₂-CH⁻), 2.67-2.79 (m, 8H, -N-CH₂-CH₂-N⁻), 2.38-2.55 (m, 2H, -CO-CH₂-CH₂-CH⁻), 1.79-2.05 (m + m, 1H + 1H, -CO-CH₂-CH₂-CH⁻), 1.44-1.45 (s + s, 45H, BOC) ppm. \(^{13}\)C-NMR (100 MHz, CDCl₃) \(\delta\): 171.88, 170.36, 170.26, 135.99, 128.31, 128.16, 127.99, 127.91, 80.68, 80.55, 80.43, 65.85, 63.16, 55.82, 53.61, 50.01, 30.68, 28.11, 28.06, 27.98, 24.73 ppm.

Glu-DTPA

0.104 g of Palladium (5%) on activated carbon were added to a 20 ml methanol solution containing p-Glu-DTPA (1.778 g, 2.2 mmol). 1 ml of acetic acid and 1 ml of water were added and the suspension was stirred for 2 hours under a hydrogen atmosphere at 20 °C. The reaction was monitored by TLC with ethanol/chloroform (1:15) where the product had an \(R_f\) of 0.3 and the reactant had an \(R_f\) of 0.8. The reaction mixture was filtered through Celite Hyflo Supercell (0.45 \(\mu\)m). The filter waters were further purified by flash chromatography using isocratic ethanol/chloroform (1:15). The product was a colorless oil with a yield of 98%. HR-MS(+) calculated for C\(_{37}\)H\(_{68}\)N\(_3\)O\(_{12}\) [M+H]\(^+\): 746.4798. Found: 746.4804. ∆m/\(z\): 0.8 ppm. \(^1\)H-NMR (400 MHz, CDCl₃) \(\delta\): 3.55 (m, 1H, -CO-CH₂-CH₂-CH⁻), 3.34 (s, 8H, -N-CH₂-CO⁻), 2.67-2.81 (m, 8H, -N-CH₂-CH₂-N⁻), 2.37-2.54 (m, 2H, -CO-CH₂-CH₂-CH⁻), 1.74-1.97 (m + m, 1H + 1H, -CO-CH₂-CH₂-CH⁻), 1.37-1.38 (s + s, 45H, BOC) ppm. \(^{13}\)C-NMR (100 MHz, CDCl₃) \(\delta\): 177.14, 170.44, 81.36, 80.97, 63.76, 55.73, 53.18, 49.85, 32.01, 28.23, 28.13, 24.48 ppm.

protected DMPE-Glu-DTPA (p-DMPE-Glu-DTPA)

A Yamada reaction was employed to couple the Glu-DTPA to DMPE with an amide bond.\(^4\)\(^5\) Glu-DTPA (4.26 g, 5.7 mmol) was dried three times by a toluene distillation and dissolved in 40 ml of anhydrous DMF. N,N-Diisopropylethylamine (2.96 g, 22.8 mmol) and HBTU (2.014 g, 5.3 mmol) were subsequently added. The reaction mixture was stirred for 30 minutes and monitored by TLC with ethanol/chloroform (1:10). 60 ml of anhydrous chloroform containing DMPE (3.56 g, 5.6 mmol, COATSOME) was added to the reaction mixture, which was placed under nitrogen atmosphere and further stirred for 5 hours at room temperature. The reaction was monitored by TLC with ethanol/chloroform (1:7). Unreacted solids were filtered out of the reaction mixture before adding 500 ml of chloroform. The mixture was washed twice with 150 ml of 0.5 M citric acid, once with 200 ml of saturated sodium chloride solution, and once with 200 ml of water. The organic fraction was dried over anhydrous sodium sulfate and the solvents removed under vacuum. The oily residue was purified by flash chromatography using isocratic ethanol/chloroform (1:7). The product was a slightly yellow oil with a yield of 41 %. HR-MS(-) calculated for C\(_{70}\)H\(_{130}\)N\(_4\)O\(_{19}\)P [M-H]⁻: 1361.90724. Found: 1361.9066. ∆m/\(z\): 0.5 ppm. \(^1\)H-NMR (400 MHz, CDCl₃) \(\delta\): 5.13 (m, 1H, DMPE), 4.35-4.32 (m, 1H, DMPE), 4.11-4.06 (m, 1H, DMPE), 3.90 (m, 4H, DMPE), 3.64-3.59 (q, 1H, Glu-DTPA), 3.55-2.40 (m, 24H, DMPE and Glu-DTPA), 2.30 (m, 2H, Glu-DTPA), 2.23-2.18 (m, 4H, DMPE), 1.94 (m, 2H, Glu-DTPA), 1.52 (m, 4H, DMPE), 1.40 (s, 45H, BOC from Glu-DTPA), 1.19 (m, 40H, DMPE), 0.82 (t, 6H, terminal CH₃ from myristoyl tails of DMPE) ppm. \(^{13}\)C-NMR (100 MHz, CDCl₃) \(\delta\): 173.02, 172.89,
DMPE-Glu-DTPA

1 g of p-DMPE-Glu-DTPA from was dried with three toluene distillations before 20 ml of formic acid was added and the mixture heated to 50 °C. The reaction was placed under nitrogen atmosphere and stirred for 24 hours. The reaction mixture became cloudy and could be monitored by TLC with ammonium hydroxide/ethanol/chloroform (2:5:5). The formic acid was removed under vacuum and the residue dissolved in a mixture of methanol/chloroform (1:4) and crystalized by the addition of acetonitrile. The recovered yellow powder was purified by flash chromatography starting with isocratic methanol/chloroform (1:4) for 3 column volumes. The solvent system was then switched to ammonium hydroxide/ethanol/chloroform (2:5:5) to recover the product as a white powder. The product was re-crystallized with methanol/chloroform (1:4) and acetonitrile for additional purity. The product was stored under Argon in the freezer and a yield of 30 % was obtained. HR-MS(+) calculated for C_{50}H_{92}N_{4}O_{19}P [M+H]^+: 1083.60879. Found: 1083.6082. ∆m/z : 0.5 ppm and for C_{50}H_{91}N_{4}O_{19}PNa [M+Na]^+: 1105.59073. Found: 1105.5895. ∆m/z : 1.1 ppm. HR-MS(-): calculated for C_{50}H_{90}N_{4}O_{19}P [M-H]^-: 1081.59424. Found: 1081.5947. ∆m/z : 0.4 ppm. DMPE-Glu-DTPA was not soluble in chloroform and had to be dissolved in CMW (deuterated solvent mixture composed of CDCl₃, CD₃OD and D₂O in a ratio of 80:20:1) in an acidic environment provided by a drop of DCl for ^1H and ^13C-NMR. The likely existence of polymolecular assemblies during measurement and the presence of five carboxylic acids considerably reduces the resolution of the spectra. Nevertheless, the obtained spectra confirm the successful synthesis of the compound by the characteristic phospholipid and headgroup peaks. The mixture was calibrated on the chloroform residual solvent signal at 7.26 ppm for ^1H-NMR and 77.16 ppm for ^13C-NMR. ^1H-NMR (400 MHz, CMW + a drop of DCl) δ: 5.45 (m, 10H, DMPE and Glu-DTPA), 5.18 (m, 1H, DMPE), 3.0-4.5 (m, 21H, DMPE and Glu-DTPA), 2.55 (m, 2H, Glu-DTPA), 2.26 (m, 4H, DMPE), 2.11 (m, 2H, Glu-DTPA), 1.54 (m, 4H, DMPE), 1.22 (m, 40H, DMPE), 0.81 (t, 6H, terminal CH₃ from myristoyl tails of DMPE) ppm. ^13C-NMR (100 MHz, CMW + a drop of DCl) δ: 174.83, 173.89, 173.49, 167.75, 69.75, 65.69, 65.07, 62.26, 55.42, 53.44, 46.97, 39.95, 37.46, 34.18, 34.07, 32.77, 31.91, 29.68, 29.57, 29.35, 29.15, 27.10, 24.87, 22.66, 19.72, 14.05 ppm.
10.4 Synthesis of DMPE-Glu-DTPA

Figure 10.4 Synthetic pathway for the preparation of DMPE-Glu-DTPA. *i and ii:* synthesis of di-tert-butyl-2-bromoethyliminodiacetate (TBB) as described by Micklitsch et al.\textsuperscript{122} *iii:* synthesis of p-Glu-DTPA and *iv:* Glu-DTPA inspired from Anelli et al.\textsuperscript{123} *v:* Yamada coupling for the synthesis of p-DMPE-Glu-DTPA inspired from Gianella et al.\textsuperscript{124} *vi:* deprotection in formic acid for the synthesis of DMPE-Glu-DTPA as described by M. Liebi et al.\textsuperscript{31}
11 Conclusion and Outlook

The molecular engineering of highly magnetically responsive polynuclear assemblies was demonstrated employing a multidimensional S-PRO$^2$ approach described in Figure 1.1. On the first level, the molecular components of the phospholipid bilayer were engineered to fit specific geometric considerations and deliver a defined magnetic susceptibility $\Delta \chi$ to the assemblies. Three amphiphile systems were proposed: Chol-NH$_2$, Chol-C$_n$-DTPA, and DMPE-Glu-DTPA. Chol-NH$_2$ and its conjugates described in chapters 5 and 6 delivered unprecedented magnetic alignments of the Ln$^{3+}$ chelating assemblies by selectively tuning the magnetic susceptibility $\Delta \chi$ of the bilayer. The Chol-C$_n$-DTPA conjugates revealed in chapter 7 combined the magnetic alignment enhancing capacities of the Ln$^{3+}$ chelating amphiphiles with geometric considerations to construct highly tailorable assembly morphologies with enhanced thermal stability. Finally, the novel DMPE-Glu-DTPA phospholipid presented in chapter 8 offered an unrivalled control over the magnetic susceptibility $\Delta \chi$, delivering unique assembly architectures with unparalleled magnetic properties.

The high potential for applications of the for mentioned amphiphiles, namely for structural studies of biomolecules by NMR spectroscopy and as contrast enhancing agents in MRI, calls for further investigations.

Moving to the second level of the S-PRO$^2$ scheme in Figure 1.1, simplified fabrication procedures were developed to enhance the versatility of the Ln$^{3+}$ chelating assemblies and demonstrate their viability by proposing regeneration possibilities after prolonged sample storage. The full potential of DMPC/DMPE-DTPA/Ln$^{3+}$ (molar ratio 1:1:1) and DMPC/Chol-OH/DMPE-DTPA/Ln$^{3+}$ (molar ratio 16:4:5:5) assemblies was unleashed, achieving unprecedented magnetic responses. Extrusion through membranes of various pore dimensions offers a unique processing-based technique to tailor the size and magnetic response of bicelles by exploiting their thermo-reversible capacity to transform into vesicles.

The new materials developed throughout this work have considerably increased the versatility of the magnetically switchable optical gels proposed by M. Liebi et al.$^{20,30}$ Moving onto the third and final level of the S-PRO$^2$ scheme (see Figure 1.1), gels offering unprecedented optical activity were generated in a highly tunable way with DMPC-based bicellar assemblies. The magnetic response of the imbedded bicelles was sufficiently enhanced to reach a respectable optical activity at 2 T, within the range of commercially affordable electromagnets. Magnetic alignments at magnetic field strengths as low as 1 T were recorded, in the range of permanent magnets, bringing such technologies one step closer to applications. These smart hydrogels could be employed in packing materials of temperature sensitive foods or pharmaceutical goods as a means of guarantying
the temperature history of the merchandise during transported or prolonged storage. These systems deliver defined optical signatures based on the degree of anisotropy of the imbedded bicelles. A wide range of signals may be obtained, allowing to trace the thermal history of a complex network of goods, functioning analogously to a bar-code. Moreover, the thermoreversible nature of the gels allows to move away from ecologically unviable single-use platforms. Only micro-sized temperature sensors could deliver an equivalent degree of traceability.

However, the physical nature of the forces governing the self-assembly process of the magnetically responsive bicelles are unable to withstand large changes in environmental conditions. Chemically cross-linking of the bilayer components would be necessary to truly enhance the stability and viability of these materials for the development of tomorrow’s smart soft materials. This shortcoming may be tackled by cross-linking the hydrophobic core of the bilayer, or by generating a cross-linked shell at the polar head group level, or with a combination of both.

Conducting free-radical polymerization (FRP) reactions within the phospholipid bilayer was addressed by numerous authors as a means of enhancing the stability of the poly-molecular assemblies.\cite{206,207} Reports of successful attempts were often doubtful, contradictory and of limited reproducibility. It wasn’t until the works of M. Jung \textit{et al.} that the mechanism of FRP within the bilayer was revealed, illustrating the underlying limitations of the strategy. Instead of forming a plastic shell around the vesicle, neckless or parachute-like structures were obtained where the polymer aggregated as a single or multiple beads within the bilayer.\cite{208,209} This phase separation between the polymer and bilayer occurred regardless of the chemical nature of the lipids composing the bilayer, the monomer and initiator, and the reaction conditions. A monomer diffusion mechanism was identified as being the cause of this phenomena.\cite{210,211} At first the monomer is uniformly distributed in the bilayer. On initiation, the nucleation sites attract more monomer and growing oligomers, as they are probably more soluble therein than in the aliphatic vesicle bilayer. As a consequence, a polymer/monomer microenvironment develops where propagation preferentially takes place. Fast migration of monomers and oligomers in the surfactant matrix supports the polymerization in one nucleus, simultaneously inducing a depletion of monomer in the rest of the bilayer. After these findings, it was evident that the lipids composing the bilayer had to contain polymer-izable moieties that could react with a cross-linking monomer to generate chemically enforced vesicles.\cite{212,213} Although the nucleation of small polymer beads could not be fully prevented, the presence of polymerizable lipids allowed for the creation of multiple nucleation sites, reducing the likelihood of monomers diffusing freely in the bilayer before reacting. Pinkhassik \textit{et al.} demonstrated the possibility of employing DMPC/DHPC bicelles as temporary scaffolds for the synthesis of rigid nanodiscs through polymerization of styrene in the bilayer.\cite{19,214} At first this result may sound incompatible with the monomer diffusion mechanism. However, the reduced reaction volume in bicelle bilayers, combined with specifically chosen monomer concentrations, resulted in the successful synthesis of the claimed nanodiscs.\cite{214,215}

Numerous phospholipids containing polymerizable moieties were synthesized and their
ability to form bicelles was tested within the scope of this work. However, only vesicles or micellar structures were achieved when mixed with the Ln$^{3+}$ chelating phospholipids. The introduction of polymerizable groups caused kinks in the hydrophobic lipid tails, weakening the van der Waals forces and hindering the formation of a solid-ordered phase. Positioning of the polymerizable moieties at the terminal position of the acyl chains was not sufficient to generate a solid-ordered phase in a reasonable temperature range. In the liquid-disordered phase, the phospholipids are mixed and cannot form the planar magnetically alignable assemblies. Furthermore, the synthesis of polymerizable phospholipids bearing a head group capable of complexing Ln$^{3+}$ is not trivial. These polymerizable phospholipids would need the same hydrophobic tails as the lipids they are mixed with to maximize the chances of bicelle formation. This necessity was shown in the DMPC/DMPE-DTPA/Ln$^{3+}$ and DPPC/DPPE-DTPA/Ln$^{3+}$ based systems described by M. Liebi et al. and the DMPC/DMPE-Glu-DTPA/Ln$^{3+}$ system described in chapter 8. However, a certain degree of mixture is possible as demonstrated in DPPC/Chol-OH/DMPE-DTPA/Tm$^{3+}$ (molar ratio 16:4:5:5) bicelle systems. Here again the presence of Chol-OH is essential to supply the necessary order in the bilayer to guaranty lipid segregation and bicelle formation. Synthetically modified Chol-OH bearing polymerizable moieties on their aliphatic tails could also aid bilayer ordering when working with polymerizable phospholipids and their Ln$^{3+}$ chelating counterparts.

Recently developed 1,3-diamidophospholipids are promising candidates for the formation of novel bicelle systems. Alignable assemblies were achieved when mixing them with DMPE-DTPA/Ln$^{3+}$ compounds. The versatility in synthesis and the unique physico-chemical properties of 1,3-diamidophospholipids present numerous advantages for bicelle design. However, the existence of the magnetically responsive species remains intrinsically bound to the degree of order in the bilayer permitting an effective phase separation. Another possibility to surpass this bottleneck would be to work with chemically engineered surfactants derived from cholic acid as proposed by Matsui et al. These cholic acid derivatives cover the edge of the bilayer when the lipids are in the liquid disordered state, permitting the formation of magnetically alignable and stable bicelles. Furthermore, polymerizable derivatives of the cholic acids were successfully synthesized allowing to chemically lock the bicelles and deliver high thermal and kinetic stability.

Another possibility to chemically stabilize polymolecular assemblies involves the synthesis of a siloxane shell on the surface of the bilayer. This approach was demonstrated in vesicles and bicelles employing synthetic lipids bearing a polymerizable triethyloxysilane group. This group may undergo silanization by a combination of the sol-gel reaction and subsequent polymerization. In the scope of this project, the hydroxyl group of Chol-OH was chemically modified to carry a triethyloxysilane functional group. The resulting steroid derivative may be employed to further tune the properties of these synthetic bicelles. However, incorporation of the amphiphile in DMPC/DMPE-DTPA/Ln$^{3+}$ systems quickly revealed the challenges arising from this strategy. The appearance of charged species during the polymerization process, combined with the
altered geometry of the steroid derivative, resulted in the destruction of the magnetically responsive assemblies. Reports on cerosome preparations highlight the importance of pH for an effective polymerization, resulting in stabilized assembly structures.\textsuperscript{115,227} Acidic conditions are preferred as the hydrolysis of the triethoxysilane groups occurs in a step-wise manner on every molecule. Such conditions are not viable for polymolecular assemblies composed of Ln\textsuperscript{3+} chelating phospholipids where the carboxylic groups responsible for chelation would be protonated, resulting in the destruction of the magnetically responsive assemblies.\textsuperscript{30} The polymerization reaction is also catalyzed under basic conditions where the assemblies are stable. However, the sol-gel reaction is chaotic and heterogeneous as some molecules are preferentially hydrolyzed, whilst others remain untouched. Unlike under acidic conditions, the first hydrolysis step is not the fastest step and the reaction may end before condensation begins.\textsuperscript{228}

The results from chapters 5, 6 and 8 highlight the importance of the magnetic susceptibility $\Delta \chi$ of the individual bilayer phospholipids to generate an optimal magnetic response in the polymolecular assemblies. Altering the chemistry of these lipids through the generation of a cross-linked polymer network will affect the $\Delta \chi$ in unpredictable ways. This is especially true in the polar region of the membrane that is particularly sensitive to a change in chemistry, as revealed with the unprecedented gains in magnetic response achieved by simply replacing the hydroxyl group of Chol-OH with a primary amine in Chol-NH$_2$ in chapter 5.\textsuperscript{90} Considering the complexity and low predictability of such an approach, it may be more strategic to move towards other materials than phospholipids. For example, block co-polymer assembly systems or cellulose could offer a defined, covalently bonded structure that may be further chemically modified to associate with paramagnetic lanthanide ions.\textsuperscript{229–234} Working instead with synthetically modified cellulose capable of binding iron nanoparticles results in highly magnetically responsive systems that could act as building blocks for the smart soft materials of tomorrow.\textsuperscript{235} Such cellulose based systems could be imbedded into powdered food products and aid in the regeneration of functionality during hydration by hierarchically guiding the structure formation in the presence of an external magnetic field. The presence of iron nanoparticles would simultaneously fortify the foods, answering consumer demand for tailored nutrition.\textsuperscript{236}

Ln\textsuperscript{3+} chelating lipids are interesting functional molecules, which may find applications outside of the scope of polymolecular assembly engineering. In MRI, Ln\textsuperscript{3+} chelating moieties chemically bound to biological molecules such a Chol-OH and phospholipids are important contrast agents.\textsuperscript{153,170–172} The field of MRI is in constant search of new contrast agents providing unique changes in relaxation rates of neighbouring water molecules for specific applications.\textsuperscript{153} Ln\textsuperscript{3+}-based nanomaterials capable of emitting visible light when excited by NIR light represent another growing field of biomedicine and imaging in which Ln\textsuperscript{3+} chelating compounds would be useful.\textsuperscript{176} The possibility of altering the $\Delta \chi$ of these compounds, achieving very different magnetic responses could further pave the way for developments in magnetic biosensing.\textsuperscript{177,178} Surfactant molecules may be chemically modified to associate with Ln\textsuperscript{3+}, analogously to phospholipids and steroids.\textsuperscript{237} These surfactants can form magnetic emulsions, where the liquid
surface properties are controlled through the application of a magnetic field.\textsuperscript{238–241} This is a valuable added degree of freedom for the engineering of emulsion-based products. Moreover, these surfactants should be capable of forming magnetically responsive self assemblies of their own. Wormlike micellar aggregates are a known outcome of surfactant self-assembly, delivering solutions with impressive viscoelastic properties.\textsuperscript{242} These properties are of high interest for numerous industrial applications and are often used as model systems for soft matter physics. Further tailoring of the viscoelastic properties of solutions composed of wormlike micellar aggregates with magnetic fields would be a valuable contribution to the field.\textsuperscript{243–245}
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