Structure, Hydration, and Lubricity of Surface-Attached Dextran

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Chapter 6: Hydration and conformation in dextran and PEG brushes

3.5.1 Effect of L/2R_g ................................................................. 38
3.5.2 Effect of load .................................................................. 39
3.6 Conclusions ...................................................................... 40
3.7 Supporting data: calibration of lateral-force measurements .................................................................. 41
3.8 References ........................................................................ 44

Chapter 5: Impact of chain morphology on the lubricity of surface-grafted polysaccharides ......... 64
5.1 Abstract............................................................................ 64
5.2 Introduction ...................................................................... 65
5.3 Materials and methods .......................................................... 67
5.3.1 Synthesis of poly(allylamine)-graft-perfluorophenylazide (PAAm-g-PFPA) ......................... 67
5.3.2 Substrate preparation and dextran attachment................................................................. 67
5.3.3 Characterization by variable-angle spectroscopic ellipsometry (VASE) ......................... 68
5.3.4 Atomic force microscopy measurements ................................................................. 69
5.4 Results and discussion ........................................................... 70
5.4.1 Gradient fabrication ........................................................... 70
5.4.2 Friction measurements ......................................................... 74
5.5 Conclusions ...................................................................... 79
5.6 Supplementary information ...................................................... 79
5.7 References ........................................................................ 81

Chapter 6: Hydration and conformation in dextran and PEG brushes ........................................... 87
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.1</td>
<td>Abstract</td>
<td>87</td>
</tr>
<tr>
<td>6.2</td>
<td>Introduction</td>
<td>87</td>
</tr>
<tr>
<td>6.3</td>
<td>Materials and methods</td>
<td>89</td>
</tr>
<tr>
<td>6.3.1</td>
<td>Poly(L-lysine)-<em>graft</em>-dextran (PLL-<em>g</em>-dextran) and poly(L-lysine)-<em>graft</em>-poly(ethylene glycol) (PLL-<em>g</em>-PEG)</td>
<td>89</td>
</tr>
<tr>
<td>6.3.2</td>
<td>Transmission interference adsorption sensing (TInAS)</td>
<td>90</td>
</tr>
<tr>
<td>6.3.3</td>
<td>Coarse-grained dextran simulations</td>
<td>90</td>
</tr>
<tr>
<td>6.3.4</td>
<td>ATR crystal preparation and brush attachment</td>
<td>91</td>
</tr>
<tr>
<td>6.4</td>
<td>Results and discussion</td>
<td>92</td>
</tr>
<tr>
<td>6.4.1</td>
<td>TInAS thickness and refractive index measurements of PLL-<em>g</em>-dex</td>
<td>92</td>
</tr>
<tr>
<td>6.4.2</td>
<td>Simulations</td>
<td>94</td>
</tr>
<tr>
<td>6.4.3</td>
<td>ATR-IR</td>
<td>98</td>
</tr>
<tr>
<td>6.5</td>
<td>Conclusions</td>
<td>101</td>
</tr>
<tr>
<td>6.6</td>
<td>Supporting information</td>
<td>102</td>
</tr>
<tr>
<td>6.7</td>
<td>References</td>
<td>104</td>
</tr>
</tbody>
</table>

**Chapter 7: Thesis Summary and Outlook** ................................................................. 110
Tribosystems found in nature consist of biomolecule-coated surfaces interacting under aqueous conditions, often operating in the boundary-lubrication regime. Highly glycosylated biomolecules play a key role in lending these tribosystems their characteristically low friction and wear rates. While synthetic biomimetic coatings such as polymer brushes have shown promise in achieving ultralow friction and wear, the specific role of sugars in the friction of natural glycoproteins and synthetic polysaccharide brushes is not yet understood. This work aims to determine the influence of structure and conformation on the hydration and lubricity of dextran-based brushes and brush-like films. Colloidal-probe friction-force measurements of dextran brush-density gradients showed friction coefficients which varied over several orders of magnitude with applied load as well as chain density, as inter-chain hydrogen bonding lent the polysaccharide brush resistance against collapse, yet exacerbated friction forces upon collapse or interpenetration with a brush on the opposing surface. The addition of kosmotropic solutes was shown to reduce the load-bearing capacity of dextran brushes, disrupting the inter-chain hydrogen bonding and moving the transition to the high-friction regime to lower applied loads. An alternative grafting system was used to produce disordered, brush-like dextran films with higher chain densities, which showed less extreme friction behavior and an inverted influence of chain density on friction at high loads compared with dextran brushes, highlighting the importance of the ordered chain conformation in both resisting collapse and sticking upon collapse. The hydration of dextran brushes and the conformation dependence on chain density was also investigated, showing key differences in chain stiffness and equilibrium height compared to conventional polymer brushes. These results cumulatively enhance our understanding of potential mechanisms in biolubrication, showing the importance of control of film structure and environmental parameters, such as loading and salt concentration, in achieving the desired friction regime.
Zusammenfassung

Salzkonzentration zu kontrollieren, wenn ein gewünschter Reibungsbereich erreicht werden soll.
Acknowledgments

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Chapter 1: Introduction

1.1 Aqueous lubrication in nature

Look around you! You don’t have to look far: the surfaces of your eyeballs consist of epithelial cells bearing a layer of mucins, which extend out into a protein-rich tear film. When you blink, your upper eyelid slides along this surface at about 1 cm/s, applying a pressure of 1-5 kPa onto the cornea – a tribological process in the boundary-lubrication regime, which mechanically wipes the surface of the eye while renewing the tear film. But you don’t even have to look that far: blood cells are being transported around your retinas through vessels and capillaries, which in some cases are narrower than the diameter of a single red blood cell. Erythrocytes are physically deformed into plug shapes as they squeeze through the narrowest capillaries, lubricated by the surrounding blood plasma and by the carbohydrate-rich glycocalyx coating the capillary walls.

The aqueous lubrication mechanisms found in these and other natural tribosystems consist of complex, mutually interacting biological structures. The characteristic low-friction and low-wear behavior of oil-based lubricants operating in the fluid-film and elastrohydrodynamic lubrication regimes are difficult to achieve with aqueous lubricants alone, due to their relatively low viscosity and tendency to be squeezed out of the contact area. Natural tribosystems have evolved to meet the challenge by deploying various lubricant additives, such as hyaluronan, and utilizing surfaces of hierarchical composition bearing glycosylated biomolecules, such as lubricin, which both help to trap the water film under pressure and directly reduce friction upon direct contact under hydrated conditions. The combination forms a tribosystem that operates in the boundary regime but with friction and wear rates resembling fluid film or elastrohydrodynamic lubrication. Understanding the physical phenomena that these systems exploit is useful for industries where aqueous lubrication is a necessity (including biomedical, civil and aquatic engineering) or a desirable non-toxic, environmentally friendly alternative to oil-based lubrication (including food and pharmaceutical engineering). While existing systems fall short of true biomimicry, synthetic aqueous lubrication solutions have come into use both as model systems for study and in a variety of applications.
Glycoproteins, which consist of a protein bearing multiple saccharide chains, are an important class of natural lubricant additives. The hydrophilic sugars serve to trap large amounts of water under pressure, and elaborate glycosylation mechanisms produce glycans of controlled, heterogeneous composition, which gives fine control over the length, stiffness, and chemical activity of the chains, and thereby their role in controlling aqueous lubrication.

1.2 Polysaccharides in aqueous solutions

The behavior of polysaccharides in water is closely interrelated with their hydration and their inter-chain interactions. Glucose is extremely water-soluble, chemically resembling a water cluster, aside from small hydrophobic patches on the two carbohydrate ring faces. Despite this, many glucans form insoluble structures by virtue of rigid glycosidic linkages and coordinated inter-chain hydrogen bonding, which can cooperate to emphasize these hydrophobic regions. Amylose, a principle component of starch, consists of α(1→4) glycosidic linkages between glucose monomers, and is generally found in a tight helix structure, which is largely insoluble in water. Similar to the amylose helix is cyclodextrin, which has a relatively hydrophobic inner surface that can trap guest molecules. Cellulose, which consists of β(1→4) glycosidic linkages, also forms insoluble fibers. In contrast, dextran is a naturally occurring glucan with high water solubility. The orientation and degrees of freedom conferred by its relatively flexible α(1→6) linkages hinder the formation of stable lamellar structures by the chains.

Dextran is not completely flexible, however, with a persistence length exceeding 2.75 nm. Nor is it completely free of ordered association, sometimes aggregating to form flakes in aqueous solutions. Inter-chain associations have been shown in solutions of high dextran concentrations, and evidence of crystallinity in dextran solutions has been shown following treatment with ethanol. As with any polymer, these associations depend on chain length, polydispersity, and the presence of side groups. Dextran exhibits a certain degree of branching (about 5%), with predominantly (1→3) side chains, about 85% of which are one or two units long and 15% of which are long (30+ units).

1.3 Polymer brushes

The term “polymer brush” describes a system of polymer chains end-attached to a surface. Conventional usage of the term implies the presence of one or a few polymer species with a
suitably low polydispersity, in contrast to the diversity of the extracellular membrane, for example. The chain length must generally exceed the radius of gyration ($R_g$) of the species in the medium under study, in contrast to a self-assembled monolayer. Finally, the polymer chains must be attached with sufficient density for inter-chain interactions to force them into an extended conformation, stretching away from the surface, in the presence of a good solvent. Systems that satisfy only the latter criteria can be termed “brush-like”. Conversely, systems that satisfy all but the last criteria, such as low-density end-attached polymer coatings or adsorbed complex molecules such as GAGs, may also be described as “brush-like”.

Polymer and polyelectrolyte brushes in an aqueous environment have been shown to exhibit ultra-low friction under sliding conditions, which has been attributed to brush resistance to interpenetration, as well as hydration of the films.\textsuperscript{13-16} The mechanisms that govern interfacial sliding of solvated polymer brush-covered surfaces create a unique system distinct from classical tribological or rheological pictures. The polymer chains, forced into an extended conformation by interaction with their neighbors and the solvent, resist compression by a counterface or penetration by asperities or an opposing polymer brush, while maintaining a fluid layer at the interface even under moderate compression.\textsuperscript{17} At low loads, the friction between brush-coated surfaces is below the detection limit of laboratory techniques.\textsuperscript{17} With increasing load, the lubricity of opposing brush-coated surface is reduced stepwise as different mechanisms come into play.\textsuperscript{18,19}

There are several routes to forming a polymer brush on a surface. A “grafting-to” approach typically exposes the surface to a solution containing fully formed polymer chains with reactive ends, which diffuse to the surface and attach there. This route is versatile with respect to chain chemistry, and allows the chains to be characterized prior to adsorption. When incorporating long chains (relative to their $R_g$), grafting-to brushes tend to suffer from low chain densities, since the brush layer itself forms a barrier against chain diffusion to the surface. The alternative “grafting-from” approach exposes the surface to a solution of monomers, and polymerization is initiated at the surface. This route can yield the highest chain densities, but is least versatile with respect to chain chemistry. Specifically, polysaccharide chains such as dextran must be synthesized enzymatically, and so cannot be produced by polymerization from a monomer solution.
One approach to increasing chain density without limiting chain selection is the “graft copolymer” approach, where multiple chains are grafted to a polymer backbone in solution, and the resulting copolymer is then adsorbed onto the surface. This route is the most versatile with respect to chain chemistry, can be characterized prior to adsorption, and can also yield high brush densities compared with most other grafting-to routes. One predominant example of this is poly(L-lysine)-graft-poly(ethylene glycol) (PLL-g-PEG),\textsuperscript{20-23} a bottlebrush copolymer consisting of PEG chains grafted onto the backbone of PLL, a polycation bearing positively charged amine residues. The resulting graft copolymers are highly hydrophilic, and the positive charges of the ungrafted amines cause the backbone to spontaneously adsorb onto negatively charged surfaces, such as metal oxides or mica, while the PEG side chains extend outwards into solution. The appropriate choice of backbone length, side chain length, and ratio of grafted to ungrafted lysine residues ensure that the molecules graft with sufficient density for the side-chains to form a brush. The adsorbed density can be controlled by varying the immersion time, and density gradients on a surface can be produced using a computerized dipping system. A similar molecule bearing polysaccharide side chains, PLL-g-dextran,\textsuperscript{24-27} is the primary object of this study.

Another route to achieve yet higher density, at further expense of brush quality, is to spin-coat a reactive surface with polymer chains and then immobilize them to the surface, producing a polydisperse loop-train-tail structure. One example of this method utilizes the graft copolymer poly(allylamine)-graft-perfluorophenylazide (PAAm-g-PFPA),\textsuperscript{28,29} in which another polycationic backbone, PAAm, is partially functionalized with PFPA groups. The unreacted positive residues spontaneously adsorb onto negatively charged surfaces. After adsorption of PAAm-g-PFPA onto a surface, a solution containing the desired polymer (dextran, in the case of this study) is spin-coated onto a surface and dried. Then, a UV light or elevated temperature is used to activate the azides, which bind randomly to the polymer (by the hydroxyl residues in the case of dextran). The resulting chain density can be significantly higher than those achieved in the case of graft copolymers.\textsuperscript{29} However, the polymers are unlikely to be end-attached, and in fact may be attached in multiple places, forming loops and tails.
1.4 Scope of the thesis

The aim of this project is to determine how the structure and environment of surface-attached dextran systems influence their hydration and lubricity. The ultimate goals are to gain insight into the lubricating properties of polysaccharides brushes, thereby helping to illuminate the behavior of their natural counterparts.

In two preceding projects, the lubricity and hydration of surface-attached polysaccharide systems in aqueous conditions were investigated. In one project, the copolymer PLL-g-dextran was synthesized and adsorbed onto substrates to form dextran brushes, and the lubricity and non-fouling properties of these were compared to those of PLL-g-PEG. In the other project, different glycoproteins from synovial fluid were adsorbed onto surfaces and studied. Those systems were also shown to reduce friction. Both projects also studied the hydration of their respective systems, with the finding that the systems were highly hydrated but that higher hydration was not necessarily correlated with lower friction. The combined results indicated that the lubricity and hydration of surface-attached polysaccharides were worthy of further study, which was the origin of the present project.

This thesis focuses on dextran-based brushes, which are a desirable model system because of their relatively well-defined composition and conformation. Friction studies of PLL-g-dextran brushes explore the influence of chain density and applied load, as well as the role of additives that enhance or impair hydrogen bonding between the chains. The influence of dextran chain density and brush conformation is further explored by comparison to a PAAm-g-PFPA-dextran system. The hydration of PLL-g-dextran brushes is investigated by different experimental methods as well as via computer simulations. At the end of the project, a better understanding of what governs the lubricity of polysaccharide brushes and films under boundary conditions could be gained.

1.5 References


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Chapter 2: Experimental Methods

This chapter introduces the experimental methods used in this work. As each subsequent chapter has its own complete Methods and Materials section, only overviews and remarks of select methods are discussed here.

2.1 Optical characterization of thin films

2.1.1 Principles and models

Measurements of the mass of an adsorbed layer on a substrate can be carried out by optical means. Light is guided to the layer, and the resulting interaction (transmission or reflection) induces a change in the phase, amplitude and/or polarization of the light. These parameters are measured (directly or indirectly) by the instrument, and fit to an optical model suitable for the geometry of the substrate and the nature of the light, to yield the thickness and/or refractive index of the layer. These values can then be related to the sample composition to determine the surface concentration of polymer. In the case of a multilayer system, each layer must be measured separately if one wishes to distinguish them.

In the case of a truly homogeneous layer of constant density and known refractive index, the optical thickness represents the actual thickness of the layer, from which a mass logically follows. However, the effect on light is much more strongly correlated with the amount of material present than its spatial organization, so for heterogeneous substances that can swell or collapse spontaneously, such as polymer brushes and hydrogels, the optical thickness correlates more with the adsorbate mass than with its true thickness. An empirical linear correlation between the density and refractive index of a medium was first reported by Gladstone and Dale in 1863, and later that finding was reformulated by de Feijter et al. to pertain to thin films: 

$$M = D \frac{n - n_0}{dn/dc}$$

with $M$ being the mass per surface area of polymer, $D$ the thickness of the polymer, $n$ the refractive index of the polymer, $n_0$ the refractive index of the medium, and $dn/dc$ the refractive index increment of the polymer in the medium.

In a liquid medium, the refractive index increment $dn/dc$ is chosen by simply measuring the refractive index for different polymer concentrations in solution and fitting the results to a line. In the case of polymer films measured in air, determining the refractive index increment
is less straightforward, since polymers cannot be easily uniformly distributed through bulk air. Therefore, various workarounds must be employed to calculate the adsorbed mass directly from measurements in air.\textsuperscript{3} For example, the optical mass may be calculated from a measurement in liquid, and then divided by the thickness of a measurement in air.

In optical measurements of films on the order of 10nm or less, the values for thickness and refractive index are coupled, and the difficulty of resolving their independent values varies depending on the experimental setup. Therefore, one of the two parameters (usually the refractive index) may have to be assumed, and the absolute value that results from the fitting must be treated with that fact in mind. Therefore the calculated thickness assuming this refractive index cannot be thought of as a physical thickness, but as representing some combination of thickness and refractive index of the film. In fact for the optical mass models described above, when measuring films in air the choice of refractive index is not critical, as the Gladstone-Dale relation implies $\frac{\Delta D}{D_{\text{true}}} = \frac{\Delta n}{n_{\text{true}}-1}$. So if the actual dry refractive index were actually 1.4 instead of 1.45, this would correspond to an 11% error in the measured thickness or mass.

One unfortunately common mistake is to perform an optical measurement of a thin film, and the corresponding data analysis, using a default refractive-index setting, calculate the optical mass with the de Feijter equation assuming the same refractive index, and then later decide that a different refractive index may be more suitable for the film, changing the value in the de Feijter equation to recalculate the mass without redoing the data analysis of the original optical data with the new refractive index assumption. One must keep in mind that the thickness has not been measured directly, but rather calculated from the optical parameters measured using the assumed refractive index – and therefore must be recalculated if one wishes to use a different refractive index.

\textbf{2.1.2 Variable-angle spectroscopic ellipsometry (VASE)}

Ellipsometry is the measurement of phase and amplitude shifts in polarized light upon reflection from a set of interfaces. Specifically, the complex ratio of the reflection coefficients of the different light polarizations is measured as the light interacts with a multilayered substrate,\textsuperscript{4} as shown in Figure 2.1. Spectroscopic ellipsometry also measures the dependence of these parameters on the wavelength of light. VASE is typically performed on silicon wafers under ambient lab conditions, in air or inside a liquid cell.
Figure 2.1 Representation of polarized light reflecting off of a biomolecule-coated silicon wafer. The polarized light beams are attenuated and phase shifted by interaction with the three-layered sample.

2.1.3 Optical waveguide lightmode spectroscopy (OWLS)
Optical waveguide lightmode spectroscopy is an optical biosensing technique that monitors the grating-assisted in-coupling of a He-Ne laser into the planar waveguide.\(^5\) Total internal reflection guides the light to the end of the waveguiding layer, where it is detected by a photodiode. The critical angle of reflection is related to the change in refractive index near the waveguide surface upon adsorption of molecules. From this information, and the refractive index increment (dn/dc), the mass and refractive index of the adsorbed layer are calculated by the software. The optical waveguide chip is composed of Si\(_{0.75}\)Ti\(_{0.25}\)O\(_2\) on glass, presumably with < 10 nm RMS roughness.

2.1.4 Transmission interference adsorption sensing (TInAS)
Transmission interferometric adsorption sensing (TInAS) is an optical biosensing technique that monitors interference fringes formed when light is transmitted through a film adsorbed onto a special multilayered TInAS sensor (8 nm RMS roughness with some 10-100 nm grains), as shown in Figure 2.2. Measurements are made in real time with a sensitivity on the order of 1 ng/cm\(^2\) (when converted to adsorbed density) and a temporal resolution below 1 s.\(^6\) The wavelengths of the interference peaks are related to the optical thicknesses of the layer, and the default software measures this thickness using an assumed refractive index. However, the TInAS-FIT software package can use the peak shapes to also fit the refractive index.\(^7\)
While the TInAS may be measured in air, in practice it is more suited to measurements in liquid, as the polymer may be adsorbed onto the surface in situ without disturbing the setup from the baseline measurement, and subsequent drying induces instrumental drift, which is problematic for such thin films.

Figure 2.2 Illustration of the operating principle of TInAS. White light passes through a custom multilayer sensor, and multiple partial reflections interfere to produce modulation of the transmitted light intensity with wavelength.

2.2 Measurement of friction with the atomic force microscope

Atomic force microscopy (AFM) is widely used for topographic imaging and force spectroscopy, and may also be used for nanotribological studies via a technique called lateral force microscopy. A probe, in this case consisting of a borosilicate colloidal sphere glued to the end of a cantilever, is scanned laterally along a surface, and the normal and lateral deflections are measured independently. Given the known properties of the cantilever, these can in principle be calibrated to measure normal and lateral (friction) force in real time, although not all AFMs allow for the quantitative calibration of lateral forces. The lateral force is obtained by scanning the probe perpendicularly to the cantilever and recording the TMR (Trace Minus Retrace in Volts) value. The TMR value is proportional to the friction force and minimizes the contributions to the lateral force from non-friction sources, but both the
friction force $F_f$ and local coefficient of friction $\mu = \frac{dF_f}{dF_L}$, $F_L$ being the applied load, are given in arbitrary units. Useful friction comparison may nevertheless be obtained by making measurements on a heterogeneous sample in a single run with the same tip and cantilever, the resulting values being directly comparable with each other. Calibration of the deflections (normal or lateral) is achieved by measuring the spring constant of the cantilever (prior to colloid attachment!) as well as the ratio of photodiode response in Volts to deflection of the cantilever in meters (at the time of measurement!). This is commonly known as the InvOLS value for normal force measurements.

An important consideration during measurement is the positioning of the laser spot on the cantilever. The laser spot typically has a large size compared to the width of the cantilever, and some of the light is lost when focusing near the tip of the cantilever. The measurement sensitivity (inverse of the InvOLS) depends on the strength of the laser signal measured by the photodiode (the sum) as well as the distance from the end of the deflecting cantilever, as shown in Figure 2.3.
Figure 2.3 Factors influencing the measurement sensitivity. Focusing the laser spot too near the cantilever tip will reduce the intensity, while positioning it too far away will reduce the influence of cantilever bending.

Varying the scan size is not likely to make an experimental difference in non-wearing experiments unless the scan size is reduced to sizes on the order of the contact area with the probe, which can change the nature of the test. In contrast, varying the scan rate is likely to change the measured friction by sampling dissipative forces on a different timescale. Varying the normal force may influence the contact area, and therefore change the influence of the probe morphology (roughness) on the experiment. Varying the probe size can have similar effects, as colloidal spheres of different sizes tend to have different surface roughnesses. An important consideration when selecting the cantilever and colloidal probe is to minimize in-plane bending, which is an alternative mechanical response to lateral forces that, unlike twisting, cannot be measured by the laser/photodiode setup. Larger probes and shorter cantilevers are optimal for minimizing the ratio of in-plane bending to twisting in response to friction forces.

2.3 Remarks on computer simulations

While computer simulations become increasingly widespread in the study of biomolecules, there often exists an unfortunate divide between experimentalists and modelers. In the interest of making the topic more accessible to experimentalists, it should be pointed out that, in most cases, the accurate determination of the equilibrium state of a complex molecule is a far simpler task than modeling physically accurate kinetic processes. The kinetics of a ball rolling down a hill can be difficult to predict correctly if one attempts to model terrain roughness, friction, sticky soil, and air resistance. In contrast, determining that it must eventually end up at the bottom of the hill can be as simple as a couple of potential energy calculations and a ballpark estimation of dissipative forces. Therefore the distinction between studies of equilibrium conformations and studies of kinetic processes should be kept in mind when trying to understand any simulation work.

The other difficulty inherent to course-grained simulations is the selection of useful parameterization of the forces in question. The work described in Chapter 6 used a course-
grained model of dextran in water from the literature, which parameterized all non-neighboring interactions using the standard Morse equation – therefore neglecting many finer aspects of hydrogen bonding, such as directional heterogeneity or cooperative effects.\textsuperscript{10} While this was deemed sufficiently accurate to determine the overall brush structure, such course graining inevitably imposes limits on the fidelity of the resulting system, which should be kept in mind during any discussion or comparison to other findings.

\section{2.4 References}


Chapter 3: Load-induced transitions in the lubricity of adsorbed poly(L-lysine)-g-dextran as a function of polysaccharide chain density

This work was published in 2011 in ACS Applied Materials and Interfaces. The supplementary materials have been added to the end of this chapter. The major part of this work was done by myself and Dr. Kenneth Rosenberg working together, with some initial friction measurements made solely by him and some final friction measurements and calculations made solely by myself. Dr. Rowena Crockett and Prof. Nicholas D. Spencer supervised the work, contributed in discussions about the project steps, and corrected the manuscript.

3.1 Abstract

Chain-density gradients of poly(L-lysine)-graft-dextran (PLL-g-dex), a synthetic comblike copolymer with a poly(L-lysine) backbone grafted with dextran side chains, were fabricated on an oxidized silicon substrate. The influence of the changing dextran chain density along the gradient on the local coefficient of friction was investigated via colloidal-probe lateral force microscopy, as shown in Figure 3.1. Both in composition and structure, PLL-g-dex shares many similarities with bottlebrush biomolecules present in natural lubricating systems, while having the advantage of being well-characterized in terms of both architecture and adsorption behavior on negatively charged oxide surfaces. The results indicate that the transition of the dextran chain density from the mushroom into the brush regime coincides with a sharp reduction in friction at low loads. Above a critical load, the friction increases by more than an order of magnitude, likely signaling a pressure-induced change in the brush conformation at the contact area and a corresponding change in the mechanism of sliding. The onset of this higher-friction regime is moved to higher loads as the chain density of the film is increased. While in the low-load (and low-friction) regime, increased chain density leads to lower friction, in the high-load (high-friction) regime, increased chain density was found to lead to higher friction.
Figure 3.1 Gradients of PLL-g-dextran were fabricated on silicon wafers by controlled dipping, and the influence of the changing dextran chain density on the local coefficient of friction was investigated via colloidal-probe lateral force microscopy.

3.2 Introduction

The molecular architecture and structure of bottle-brush polymers such as poly(l-lysine)-graft-poly(ethylene glycol) (PLL-g-PEG), and their use in modifying surface properties, have been the subject of several recent studies.\textsuperscript{1-5} In particular, these polymers are attractive friction-reduction additives in an aqueous environment, because they tend to adsorb onto surfaces to form dense brushlike layers, in which the highly solvated nature of the extending polymer chain is known to correlate well with effective lubrication.\textsuperscript{3,4,6-8} Recently, the lubrication properties of poly(l-lysine)-graft-dextran (PLL-g-dex) with a variety of grafting ratios and dextran molecular weights under aqueous conditions were also studied both on the macro- and the nanoscale.\textsuperscript{9,10} It was shown that PLL-g-dex lubricates metal oxide surfaces very efficiently, and readily forms high-density polymer brush layers with properties dependent on the molecular weight of the dextran side chains.

Dextran is a naturally occurring polysaccharide consisting of an α(1→6)-linked glucan with branches attached to the 3-positions of the straight-chain glucose units. PLL-g-dex is formed by grafting dextran chains onto the backbone of poly(l-lysine), a polycation that spontaneously adsorbs onto negatively charged surfaces, such as metal oxides, at multiple points via the ungrafted, primary amine groups, as shown in Figure 3.2. The resulting graft copolymer, PLL-g-dex, is highly soluble in water, and, when bound to a substrate at
sufficient density, effectively renders the surface nonfouling, similarly to other dextran-containing surfaces.\textsuperscript{9,11-15} The overall stability of PLL-\textit{g}-dex, similar to that of PLL-\textit{g}-PEG, is a feature that biomedical applications can readily exploit, for example as a gene-delivery vector.\textsuperscript{16,17} In contrast to PEG, however, dextran is not prone to undesirable oxidation reactions and possesses multiple reactive sites, enabling further functionalization and chemical modification.

![Figure 3.2 Structure of PLL-\textit{g}-dextran copolymer with grafting ratio $k + 1$.](image)

An important characteristic of PLL-\textit{g}-dex that it shares with virtually all lubricious biomolecules is the presence of oligosaccharide side chains. Many studies have focused on the lubricating properties of glycoproteins, including lubricin and naturally occurring polysaccharides on a variety of substrates and under various environmental conditions.\textsuperscript{18} Low friction and wear are normally associated with the complex mixtures that constitute natural lubricious layers, which often consist of highly hydrated glycoproteins, such as proteoglycans and mucins.\textsuperscript{18} A common difficulty in interpreting these measurements lies in the complexity of the structure and composition of the glycoproteins. The topological complexity of the molecules, and the subtle differences in the electrostatic and hydrophobic interactions that govern the adsorption properties, lead to an uncertainty in adsorbed conformation. This presents a challenge when attempting to determine the features contributing to lubricity. In
PLL-g-dex, the composition and structure are much simpler than in glycoproteins, and thus it constitutes a biomimetic system that maintains the critical features important for lubricity.

In the present study, we examine the relationship between the density of dextran chains and the frictional properties of the resulting system. Although a great deal is known regarding the structure of polymer films and their interactions, much less is understood regarding how changes in polymer conformation and, as a consequence, film mechanical properties, influence friction.19-21 The conformation of surface-attached polymer chains is dependent on the mean spacing between neighboring chains $L$ and the radius of gyration, $R_g$. When $L >> 2R_g$, the non-overlapping, isolated chains are restricted in conformation only by the surface, and may assume a pancake- or mushroom-shaped conformation, depending on the interaction of the polymer with the surface. At the other extreme, for $L < 2R_g$ (the brush regime), the interchain steric repulsive interactions cause the chains to assume more extended conformations. The use of a gradient to generate gradual changes in polymer chain density is convenient for producing the full range of conformations. Gradients, in general, are a powerful technique in studying parametric dependency, as evidenced by their use in a variety of applications, including wetting22,23 and cell-adhesion24 studies. For example, using a grafting-density gradient of poly(acrylamide) (PAAm) on silica, Genzer and co-workers measured the mushroom-to-brush transition point (0.065 chains/nm$^2$), based on height measurements obtained by ellipsometry, and correlated variations in chain density with wetting properties.25

3.3 Materials and Methods

3.3.1 Poly(L-lysine)-graft-dextran (PLL-g-dex)
The PLL-g-dex copolymer used in these experiments was synthesized as described previously.9,10 Briefly, dextran (dextran T5, 5.2 kDa, polydispersity 1.8, Pharmacosmos A/S, Denmark) undergoes reductive amination with poly(L-lysine)-HBr (20 kDa, polydispersity 1.1, Sigma-Aldrich, Switzerland). The solvent for the reaction was a sodium borate buffer (0.1 M, pH 8.5, 0.4 M NaCl). The terminal dextran aldehyde group was first reacted with the primary amine groups of PLL to form a Schiff base, which was subsequently reduced by sodium cyanoborohydride (NaBH$_3$CN). The resultant PLL-g-dex graft copolymer was separated from the unreacted materials through filtration with ultracentrifugation. The specific ratio of lysine monomers to dextran chains is defined as the grafting ratio and can be
controlled during synthesis by adjusting the reactant ratio.\textsuperscript{26,27} The grafting ratio was determined through \textsuperscript{1}H-NMR spectroscopy of PLL-g-dex in D\textsubscript{2}O and elemental analysis (EA).

The particular copolymer studied here was PLL(20)-g[5.3]-dex(5), where 20 kDa is the molar mass of PLL including the Br\textsuperscript{-} counterions, 5.3 is the grafting ratio, and 5 kDa is the weight-average molecular weight (M\textsubscript{w}) of the dextran. On average, dex(5) consists of 32 monomer units, which assuming each monomer is 0.7 nm long corresponds to a fully stretched chain length of 22 nm.

### 3.3.2 Gradient preparation

Bare silicon wafers of 2 cm × 1 cm were cleaned by sonicating twice in toluene, then twice in 2-propanol, each for five minutes. Next, the substrates were exposed to O\textsubscript{2} plasma for two minutes (Plasma Cleaner/Sterilizer, PDC-32G instrument, Harrick, Ossining, NY, USA). The thickness of the surface SiO\textsubscript{2} layer was measured by ellipsometry (described below), after which each substrate was immediately dipped at a controlled rate into a 0.02 mg/mL solution of PLL-g-dex in 1 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) at pH 7.4.\textsuperscript{28} After removal from the PLL-g-dex HEPES solution, the substrate was first rinsed with polymer-free HEPES buffer to remove any weakly bound or nonadsorbed PLL-g-dex, and then copiously rinsed with ultrapure water (Milli-Q Gradient A10, Millipore SA, Molsheim, France) and finally dried under N\textsubscript{2}. The final PLL-g-dex thicknesses were then measured in 2 mm increments along the substrate by ellipsometry.

Both SiO\textsubscript{2} and PLL-g-dex film thicknesses were measured in ambient air by ellipsometry using a M-2000 V spectroscopic ellipsometer (J.A. Woollam Co., Inc., Lincoln, NE, USA) at 65°, 70°, and 75° at wavelengths between 370-1000 nm. The variable-angle spectroscopic ellipsometry (VASE) spectra were analyzed using WVASE32 software. The “dry” thickness of PLL-g-dex was obtained by fitting the VASE spectra to a multilayer model based on the optical properties of a generalized Cauchy layer (A = 1.45, B = 0.01, C = 0).\textsuperscript{29} The results of ellipsometric characterization of a PLL-g-dex surface-chemical gradient are shown in Figure 3.3A. The dry thickness of the copolymer layer ranges from below the experimental scatter of SiO\textsubscript{2} thickness (0.1 Å) at the bare end to 16 ± 1 Å, corresponding to the maximum thickness for this particular PLL-g-dex architecture.
The adsorbed mass of PLL-g-dex onto a standard Si$_{0.75}$Ti$_{0.25}$O$_2$ waveguide in buffer solution was measured via optical waveguide lightmode spectroscopy (OWLS), an optical biosensing technique which monitors the grating-assisted in-coupling of a He–Ne laser into the planar waveguide. Total internal reflection guides the light to the end of the waveguiding layer, where it is detected by a photodiode. The critical angle of reflection is related to the change in refractive index near the waveguide surface upon adsorption of molecules. From this information, and the refractive index increment ($dn/dc$), the mass and refractive index of the adsorbed layer are calculated$^{30-32}$ by the software. The refractive index increment of PLL-g-dex was taken to be 0.131.$^9$

Prior to the adsorption experiment, the optical waveguide chip (standard: Si$_{0.75}$Ti$_{0.25}$O$_2$ on glass, Microvacuum, Budapest, Hungary) was cleaned by ultrasonication in 0.1 M HCl for 10 min, rinsed with ultrapure water, ultrasonicated in 2-propanol for 10 min, rinsed again with ultrapure water, dried under nitrogen, and cleaned in a UV/ozone cleaner (UV/Clean, model 135500, Boeckel industries Inc., Feasterville, PA) for 30 min. The cleaned waveguide was placed into the OWLS flow cell and equilibrated by exposing to HEPES buffer solution overnight, in order to obtain a stable baseline. The waveguide was then exposed to a polymer solution (0.015 mg/mL in a similar HEPES buffer) under constant flow for at least 30 min, resulting in the formation of a polymer adlayer.

### 3.3.3 Atomic force microscope measurements

The AFM experiments were conducted in contact mode using a Dimension 3000 AFM (Nanoscope III controller, Digital Instruments, Santa Barbara, CA, USA) and a MFP-3D AFM (Asylum Research, Santa Barbara, CA, USA). The probes were commercially available V-shaped Si$_3$N$_4$ cantilevers (NP-S20, nominal normal spring constant 0.58 N/m, Veeco,
Plainview, NY, USA) and rectangular Si cantilevers (CSC12A, nominal normal spring constant 0.95 N/m, Mikromasch, Tallinn, Estonia).

A borosilicate colloidal sphere (Kromasil®, Eka Chemicals AB, Bohus, Sweden; radius ≈ 3 μm) was glued to the end of the cantilever with UV-curable epoxy (Norland Optical Adhesive #61 or #63, Norland, Cranbury, NJ, USA). The entire assembly was then irradiated with UV light for 30 min and allowed to sit for at least 1 day to further strengthen the adhesion between the colloidal sphere and the cantilever. The nanotribological experiments described here include measurements taken first with a bare, uncoated SiO₂ colloid probe and then with a PLL-g-dex brush-like layer attached to the same colloidal sphere by the same method as used for the gradient preparation, and with sufficiently long exposure to solution to ensure maximum possible brush density (according to the gradient and OWLS kinetics measurements).

Friction measurements were obtained by scanning the probe perpendicularly to the major axis of the cantilever and recording the TMR (Trace Minus Retrace in mV) value. The TMR value is directly proportional to the friction force and minimizes the contributions to the lateral force from non-friction sources.33 Each bare colloid was first slid against the uncoated part of the gradient until the coefficient of friction reached a constant value. This “running in” step was done to check the quality of the tip and to ensure that any transient colloidal wear or damage could not affect the measured friction. To test for any such changes as well as material transfer from the PLL-g-dex gradient, the bare surface was scanned intermittently to ensure the friction response remained constant. Additionally, the gradient was measured in a non-sequential order to minimize possible effects arising from drifting of force sensitivity. All scanning distances were 1 μm with velocities of 1 μm/sec, unless otherwise noted. The dependence of scanning distance on lateral force was investigated and showed that for lengths over 100 nm, the lateral force remained constant.

The normal and lateral forces were calibrated as described in the Supporting Information. Briefly, the cantilever spring constants were calibrated from the power spectral density of the thermal noise fluctuations34 and by the method of Sader and coworkers.35 The lateral force measurements of the rectangular cantilevers were calibrated using the test probe method described by Carpick and coworkers,36 and the measurements of the V-shaped cantilevers were scaled to the resulting curve. Rectangular cantilevers were chosen that approached the
normal spring constant of the V-shaped cantilevers while minimizing errors due to in-plane bending.\textsuperscript{37}

Following the nanotribological measurements with an uncoated SiO\textsubscript{2} colloid, the entire tip-cantilever assembly was gently lifted off the gradient surface and immediately exposed to a 0.02 mg/mL PLL-g-dex solution in 1 mM HEPES to form a brush layer on the colloid, as predicted both by the OWLS and ellipsometry measurements. After 30 minutes, the AFM cantilever was thoroughly rinsed with polymer-free HEPES buffer and placed back into contact with the same gradient substrate to resume the friction experiments.

### 3.4 Results

#### 3.4.1 Measurement of $L/2R_g$

In order to understand how lubrication varies with dextran chain density and conformation, the parameter $L/2R_g$ is calculated along the gradient. Depending on the specific technique used, literature values of $R_g$ of 5 kDa dextran range from 1.9 nm (dynamic light scattering)\textsuperscript{38} to 2.4 nm (fluorescence correlation spectroscopy).\textsuperscript{39} Because the focus of this study is on the relative, rather than absolute, change in $L/2R_g$, the value of $R_g$ throughout this paper is simply fixed at 1.9 nm. The only effect of setting $R_g$ to 2.4 nm would be to shift all $L/2R_g$ values by about 25% in the direction of lower chain density. Two independent methods to measure $L$ are discussed here. First, from the “dry” PLL-g-dex thickness $h$ (Figure 3.3A), the grafting density of dextran chains $\sigma \approx 1/L^2$ can be found from $\sigma = h\rho N_A/M_N$, where $\rho$ is the bulk polymer mass density, $N_A$ is Avogadro’s number, and $M_N$ is the polymer molecular weight.\textsuperscript{40} Previous adsorption results of PLL-g-dex on SiO\textsubscript{2} substrates measured with optical waveguide lightmode spectroscopy (OWLS) give the maximum amount of adsorbed PLL-g-dex as 290 ± 16 ng/cm\textsuperscript{2}.\textsuperscript{9,10} Finally, because $h$ is proportional to $\sigma = 1/L^2$ from the above relationship, $L$ can be calculated at each point along the gradient where $h$ is known. These data points are shown in Figure 3.3B. Although one end of the substrate was not exposed to PLL-g-dex solution, $L$ remains finite because of the experimental uncertainty in the ellipsometric measurement, as mentioned in Materials and Methods.

A separate technique to calculate $L$ utilizes the kinetic adsorption data obtained from OWLS data to map the time exposed to PLL-g-dex solution directly to adsorbed polymer mass. In this method, there is no assumption in relating dextran chain density to “dry” thickness.
Instead, the adsorption time is known for each position along the gradient, which is then mapped to the OWLS adsorption curve to generate PLL-g-dex mass as a function of position. The data from this method is shown as a dashed line in Figure 3.3B, in excellent agreement with the analytical model described above.

### 3.4.2 Friction measurements

Figure 3.4 shows three representative “friction-load” curves measured with a bare, uncoated SiO$_2$ colloid probe at different values of $L/2R_g$ along a PLL-g-dex gradient. The friction-load curves measured with a coated SiO$_2$ sphere show a similar shape to those in Figure 3.4 (not shown). The lateral force ($F_L$) increases as a function of normal force ($F_N$) and initially displays linear behavior, as seen in the figure inset. However, upon increasing the load past a critical value, the linear relationship ceases to be valid as the friction, $\mu = dF_L/dF_N$, rapidly increases for each of the curves shown. Continued ramping up of load again results in a constant, but higher, friction value. This transition was not found to occur when the bare silica colloid was sliding against a bare silicon wafer, which yielded a stable friction coefficient of 0.22 across the entire load range, in excellent agreement with literature.\textsuperscript{41} The coefficients of friction of both regimes (termed “low load” and “high load”) were recorded along the gradient, i.e., at different values of $L/2R_g$, and are shown in Figure 3.5. The initial slope (low load) for both uncoated and coated colloid is displayed in Figure 3.5A while both slopes (low and high load) are shown in Figure 3.5B. Although the coated colloid exhibits a lower friction coefficient at low load, it gives a much higher friction coefficient than the uncoated colloid at high load. The transition in normal load between the low and high friction regimes, defined as $F_T$, is shown in Figure 3.6.
Figure 3.4 Set of typical friction vs. load curves (A) of a bare SiO$_2$ colloidal probe sliding on a PLL-g-dex-coated SiO$_2$ gradient for $L/2R_g = 2.9$ (◊), $L/2R_g = 1.2$ (■), and $L/2R_g = 0.6$ (▲), with (B) showing the same data zoomed in on the region up to $F_L = 50$ nN.
Figure 3.5 Coefficient of friction plotted against $L/2R_g$ (A) in both low and high load ($F_L > 100$ nN) and (B) just low load ($F_L < 50$ nN) regimes. The squares represent a bare SiO$_2$ colloid at low load (empty) and high load (filled). The circles represent a PLL-g-dex-coated SiO$_2$ colloid at low load (grey) and high load (black).
Figure 3.6 Characteristic load at which the friction starts to deviate from linearity, denoted as the transition load, is shown as a function of $L/2R_g$ along the gradient with uncoated colloid sphere (open) and PLL-$g$-dex-coated colloid sphere (filled).

3.5 Discussion

3.5.1 Effect of $L/2R_g$

Polymer-brush layers at a tribological interface are known to cause a significant reduction in the friction coefficient.\textsuperscript{2-4,42} Essentially, the long-range steric interaction arising from the osmotic repulsion at high chain density provides a suitable boundary film to reduce, if not eliminate entirely, direct contact of opposing surfaces.\textsuperscript{43} Therefore, it is not surprising that, over the moderate pressures generally studied (in the megapascal range or lower), the lower the value of $L/2R_g$, or, equivalently, the higher the dextran chain density, the lower the coefficient of friction. For $L > 2R_g$, the coefficient of friction is large and relatively independent of dextran density. In this non-brush regime, the rather inhomogeneous distribution of dextran chains along the surface is an ineffective barrier against the external load. In contrast, when $L < 2R_g$, the higher density produces a more homogeneous brush layer.
that can give rise to a greater steric repulsion. With increasing dextran density, the thickness of the film layer is expected to grow, allowing for greater physical separation for a given load between sliding surfaces. Moreover, as a result of the increasing density of dextran chains, the total amount of associated water within the layer also increases.\textsuperscript{9,10} These combined factors of increased physical separation and increased film hydration are instrumental in rapidly lowering the coefficient of friction as the chain density transitions along the gradient from the bare end until the maximum brush density. However, even sub-optimal coverage still functions as an effective barrier layer between the colloid and bare substrate, as evidenced by the roughly twofold friction reduction near $L \sim 2R_g$. This reduction in friction, even at partial coverage, is consistent with other published results indicating that modifying the interfacial properties of solid substrates with adsorbed polymers, not necessarily in the brush regime, still achieves improved lubricity.\textsuperscript{44-47}

For identical values of $L/2R_g$, the friction coefficient with the brush-coated colloid is decreased with respect to the uncoated colloid at low loads (Figure 3.5A). Furthermore, it is apparent that the friction coefficient is highly dependent on the interfacial properties of the sliding and stationary surfaces individually. Specifically, with the uncoated SiO$_2$ colloid sliding against the uncompressed brush, the friction is much lower than when the brush-coated SiO$_2$ colloid is sliding against a nearly bare (i.e., very high $L/2R_g$) surface, when both are at low load. Here, even though both systems consist of similar surfaces within the tribocontact, the surface histories are very different. Presumably the continual shearing of the brush-coated colloid against the relatively bare substrate has a wearing effect on the brush, in comparison with the uncoated colloid sliding across the uncompressed, previously unperturbed brush.

### 3.5.2 Effect of load

The tribological response of the PLL-$g$-dex gradient to external load exhibits two distinct linear regimes over the loading range studied (Figure 3.4). At low loads, the lateral force increases linearly up to a transition load $F_T$ (Figure 3.4 inset), after which it increases with a higher slope. From Figure 3.6, the value of $F_T$ measured along the gradient with an uncoated SiO$_2$ colloidal sphere remains virtually constant as $L/2R_g$ is decreased, with a value around 60 nN, until $L/2R_g$ reaches unity, $F_T$ nearly doubling by $L/2R_g = 0.6$. The similarity between the dependence of $F_T$ and friction coefficient on $L/2R_g$ is most likely not coincidental—that is, the stiffer and more hydrated PLL-$g$-dex caused by the more densely packed dextran chains leads
to both a decrease in friction and an increase in $F_T$. As expected, with the brush attached to the colloid, the ability of the polymer film within the tribocontact to support an external load is increased compared with a bare colloid. In other words, the presence of the brushlike layer on the sliding colloid helps to maintain a lower friction coefficient before the friction abruptly rises, above $F_T$. For both the coated and uncoated colloidal sphere, as the dextran chain density on the gradient increases towards full brush, $F_T$ rises in response to the growing steric repulsion of the PLL-g-dex layer.

Interestingly, although this transition in the friction–load curve has been observed in a variety of polymer brush systems, both neutral and charged, the literature provides no answers as to a possible mechanism. A similar transition in friction with increasing brush compression, caused by increasing load, has also been predicted for entangled polymer brush systems, in contrast to nonentangled systems. Although the dextran chains studied are likely too short and stiff to experience entanglement, their interchain hydrogen bonding can have a comparable effect. The transition load represents the tipping point of an energy balance between brush compression and brush parting or interpenetration, for the bare and brush-coated colloidal spheres, respectively. If interchain hydrogen bonds, which scale with brush density, are assumed to behave comparably to rapidly forming entanglements, they serve as a hindrance to this transition by resisting the parting of the brush. Additionally, the friction of the brush-on-brush system at high loads may be particularly exacerbated by the formation and disruption of new hydrogen bonds between the chains of the interpenetrating brushes. A simplified Hertzian analysis shows that the contact pressures in the ‘low’ regime are still far higher than those commonly achieved in physiological conditions. For $F_L \approx 10$ nN, with a colloid radius of $\sim 3.5 \mu m$, the maximum pressure reaches $\sim 50$ MPa. For comparison, the typical contact pressures seen in joints are normally only 10% of this value, and many lubricated physiological tribosystems have far lower contact pressures (e.g., in the eye).

### 3.6 Conclusions

The lubricating properties of a density gradient of PLL-g-dex copolymer were investigated by means of colloidal-probe lateral force microscopy. The dominant parameter determining the coefficient of friction was the relative spacing ($L/2R_g$) of the surface-grafted dextran chains. Furthermore, the nanotribological analysis showed that the friction coefficient along the gradient is highly load dependent. At high contact loads, the lubrication behavior is very
different compared to that at low loads, both for brush-brush and brush-surface contact. Although higher polysaccharide chain densities lower friction more effectively at low loads, they actually increase friction in the high-load regime compared to lower chain densities.

Although this study highlights potential design limits in the use of polysaccharide brushes, it also opens new potential toward high-friction applications of polymer coatings. Although this study only examined the effect of dextran chain density, other architectural parameters may be critical in determining the overall friction, including grafting ratio and dextran molecular weight. These initial studies should prove useful in better designing and tuning PLL-g-dex and related copolymers for specific water-based lubrication systems.

3.7 Supporting data: calibration of lateral-force measurements

The normal force spring constant (nN/nm) of each V-shaped cantilever was calibrated from the power spectral density of the thermal noise fluctuations in air using the Asylum MFP-3D. The V-shaped cantilevers were then mounted on the Veeco Dimension for lateral force measurements, which were obtained by scanning the probe perpendicularly to the cantilever and recording the TMR (Trace Minus Retrace in mV) value, which is directly proportional to the friction force. Since all experiments are measured within a gradient in a single run with the same tip and cantilever, the values from the V-shaped cantilevers are directly comparable with each other, even in the absence of calibration. In order to facilitate comparison with other studies, calibration was performed (and additional data obtained) by repeating selected measurements using rectangular cantilevers mounted on the MFP-3D. For these cantilevers, the normal and lateral force spring constants were calibrated by measuring the thermal-noise fluctuations of the tipless cantilever in air, then obtaining the cantilever dimensions from SEM images and using the Sader method to obtain the spring constants. The sphere was subsequently attached and a full range of friction measurements performed according to the same procedure as with the V-shaped cantilevers. The lateral sensitivity of the rectangular cantilevers was inferred by comparison with another cantilever using the test probe method described by Carpick and coworkers, yielding absolute values for both the friction force $F_t$ and local coefficient of friction $\mu = dF_t/dF_L$, $F_L$ being the applied load. The test probe calibration method itself includes correcting factors for differences in cantilever dimensions, colloidal sphere size, normal and lateral spring constant of the cantilever, and spot intensity.
between the experimental and reference setups. Additionally, all the rectangular cantilevers used for the calibration and measurement were produced in a single batch from the same wafer, so that any errors stemming from non-ideal behavior of the cantilevers or unaccounted factors, such as variations in the reflectivity of the cantilever top coatings, would be consistent across all measurements.

Finally, all the uncalibrated lateral force measurements from the V-shaped cantilever were multiplied by a single scaling factor (mV/nN) to match the curve of the calibrated measurements. The scaled results are shown in Figure 3.7. While the calibration proved highly effective within the range relevant to the low-friction regime, extrapolation to the high-friction regime inherently leads to a higher error, due to the non-linearity involved in the twisting of the V-shaped cantilever. The friction results in the high-friction regime should therefore be regarded as being a) semiquantitative and b) potentially an underestimate of the true values.
Figure 3.7 The coefficient of friction of (A) a bare SiO$_2$ colloid and (B) a brush-coated SiO$_2$ colloid against a gradient substrate, plotted against L/2R$_g$ in the low-friction regime. The filled points represent fully calibrated data measured using rectangular cantilevers, while the empty points represent scaled data measured using V-shaped cantilevers. Error bars are shown in Figure 3.5.
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Chapter 4: Influence of solutes on hydration and lubricity of dextran brushes

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4.1 Abstract

The characteristic lubricity and non-fouling behavior of polymer brushes is critically dependent on the solvation of the polymer chains, as well as the chain-chain interactions. Dextran brushes have shown promise as non-toxic aqueous lubricant films, and are similar in composition to natural lubricating systems, while their comparative simplicity allows for controlled preparation and fine characterization. This project entails measuring the solvation and lubricity of dextran brushes in the presence of additives which modify the inter-chain hydrogen bonding. The thickness and refractive index of the film were measured during adsorption of the brush layer onto a silica substrate and the subsequent immersion in solutions of potassium sulfate and α,α-trehalose. We also studied the lubricity of the system as a function of normal loading using colloidal-probe AFM. Both solutes are shown to have a minimal effect on the hydration of the brush while significantly reducing the brush lubricity, indicating that inter-chain hydrogen bonding supports the load-bearing capacity of polysaccharide brushes.

4.2 Introduction

Polymer brushes have shown great promise as lubricious surface coatings for aqueous tribosystems. Composed of densely packed end-attached polymer chains, their high degree of solvation and load-bearing capacity act to reduce friction and wear, even in boundary-lubrication conditions, where the hydrodynamic fluid film alone would be insufficient to maintain separation between opposing asperities. When the brush is comprised of non-toxic polymers, such as PEG or dextran, the resulting system has great potential in the fields of food and drug processing, biomedical engineering, and microfluidics.
The study of polymer brush-mediated aqueous tribosystems is also interesting because they share some common properties with naturally occurring lubricious systems, such as the glycocalyx, articular cartilage joints, and slippery surfaces such as the tongue, which are comprised of densely arranged oligosaccharide-bearing biomolecule-coated surfaces separated by aqueous lubricants such as blood plasma, synovial fluid, or mucus. The complexity and sensitivity of these systems render the study of their mechanisms of lubrication highly challenging. Polymer- and especially polysaccharide-based brushes represent simpler, homogeneous systems that capture some of the critical aspects of natural lubrication.

Dextran is a naturally occurring glucan with high water solubility. The orientation and degrees of freedom conferred by its (1→6) linkage hinder the formation of stable lamellar structures by the relatively flexible chains, making it suitable for the formation of brushes. Poly(L-lysine)-graft-dextran (PLL-g-dex) is a bottlebrush copolymer formed by grafting some of the primary amine groups of a poly(L-lysine) backbone to the terminal aldehydes of dextran chains via reductive amination. The unreacted amines maintain a positive charge in aqueous solution at neutral pH, and the backbone will spontaneously adsorb onto negatively charged surfaces at multiple locations, while the uncharged, hydrophilic dextran chains extend into solution. On silicon oxide surfaces in water, these molecules adsorb spontaneously with sufficient density that the dextran inter-chain spacing is significantly lower than its radius of gyration, forcing the chains into a brush conformation.

Dextran brushes have been shown to have non-fouling properties and to be highly lubricious at low loads. Above a critical load, however, the friction increases sharply. This critical load is attributed to the point at which the adjacent chains of the brush are forced apart, disrupting the hydrogen bonds, which may then stick to the counterface. Tribosystems composed of opposing dextran brushes show lower friction at low loads, a higher critical load, and higher friction above the critical load than dextran brushes against bare silica. Similar behavior can be seen in models of brush-brush interaction where the chains are long and flexible enough to entangle. This is not possible for the shorter, stiffer dextran chains studied, but the inter-chain hydrogen bonds are hypothesized to cause this similar behavior.

In order to better understand the role of inter-chain hydrogen bonding in polysaccharide brushes, we measured the lubricity and swelling of the brush in the presence of solutes that influence hydrogen bonding. Potassium sulfate is an ionic kosmotrope that can screen...
electrostatic interactions and reduce hydrogen bonding by enhancing dextran’s affinity to water.\textsuperscript{15,16} Mono- and disaccharides, such as glucose and α,α-trehalose, can also reduce inter-chain cohesion by acting as "zippers”, as they bridge dextran chains and their mobility allows increased freedom to restructure the hydrogen bonds.\textsuperscript{17,18} These and similar low-molecular-weight saccharides and polyols are often used as a plasticizers in polysaccharide melts. Trehalose is extremely hydrophilic, with a hydration shell four times as large of that of glucose, and a strong kosmotrope, making it a powerful bioprotectant against plant dehydration.\textsuperscript{19} The friction, and especially the transition load, of the dextran brush in the presence of these solutes should be a strong indicator of their effects on the inter-chain hydrogen bonding.

### 4.3 Experimental

#### 4.3.1 PLL-g-dextran brushes

The PLL-g-dex copolymer synthesis via reductive amination has been described previously.\textsuperscript{8,9} Dextran (dextran T5, 5.2 kDa, polydispersity 1.8, Pharmacosmos A/S, Denmark) was mixed with poly(L-lysine)-HBr (20 kDa, polydispersity 1.1, Sigma-Aldrich, Switzerland) in a sodium borate buffer (0.1 M, pH 8.5, 0.4 M NaCl). The terminal aldehydes of the dextran chains formed a Schiff base with the primary amines of the PLL, which was then reduced with sodium cyanoborohydride. The product was separated from the reactants by filtration with ultracentrifugation. The grafting ratio of lysine monomers to dextran chains was determined to be 5.3 via $^1$H-NMR in $D_2$O and elemental analysis.

#### 4.3.2 Optical measurements of brush thickness and refractive index

Optical waveguide lightmode spectrometry (OWLS, Microvacuum, Hungary) was used to monitor the adsorption of the brush and subsequent exposure to trehalose solution in real time. The standard Si$_{0.75}$Ti$_{0.25}$O$_2$ chip was ultrasonicated in COBAS (Roche, Switzerland) for 10 min, rinsed in ultrapure water, ultrasonicated in 2-propanol, dried in a nitrogen stream, and cleaned in a UV/ozone cleaner (Bioforce Nanosciences, Iowa, USA) for 30 min. The cleaned waveguide was incubated in ultrapure water in the flow cell overnight to achieve a stable baseline. The solution of 0.02 g/L PLL-g-dex in ultrapure water was then introduced, and the
sample allowed to stabilize until the change was less than the instrument experimental drift, then rinsed with ultrapure water, allowed to stabilize again, and finally exposed to the 5.5 mM trehalose solution and allowed to stabilize. The thickness and refractive index given by the instrument were used to calculate the optical mass with the de Feijter equation,\(^{23}\) assuming a refractive index increment \(dn/dc\) of 0.131 cm\(^3\)/g.\(^{8,9}\) As the experiment is performed in aqueous conditions, the water associated with the polymer layer is not included in the measured optical mass, referred to as the “dry mass”.

Transmission interference adsorption spectroscopy (TInAS)\(^{20,21}\) was used to monitor the adsorption of the brush and subsequent exposure to \(\text{K}_2\text{SO}_4\) solution in real time. A standard TInAS sensor, consisting of glass coated with 25 nm Al and 3 μm SiO\(_2\), was ultrasonicated twice for 5 minutes in toluene, then twice for 5 minutes in 2-propanol, then dried with nitrogen and exposed to oxygen plasma (Plasma Cleaner/Sterilizer PDC-32G, Harrick, NY, USA) for 2 min. The cleaned sensor was incubated in ultrapure water overnight to achieve a stable baseline. Then, the solution of 0.02 g/L PLL-g-dex in ultrapure water was introduced, and the sample allowed to stabilize until the change was less than the instrument experimental drift, then rinsed with ultrapure water, allowed to stabilize again, and finally exposed to the 10 mM \(\text{K}_2\text{SO}_4\) solution and allowed to stabilize. The dry optical mass was calculated from the obtained thickness and refractive index in the same way as with the OWLS.

Variable angle spectroscopic ellipsometry (VASE, J. A. Woolam Co, Inc., NE, USA) was used to check the adsorption of the brush onto the silicon wafers used for the friction measurements. Bare wafers were cleaned by the same procedure as the TInAS sensors, then rinsed in ultrapure water and dried with nitrogen. The thickness of the native oxide was measured in multiple places at wavelengths 370-1000 nm at an incident angle of 70°. The thickness variation over the 1 cm\(^2\) wafers was less than 0.5 Å. The substrates were then exposed for different lengths of time to the PLL-g-dex solutions, rinsed in ultrapure water, and dried with nitrogen. The dextran brush thickness was then measured in several places under the same conditions, and also showed variation over the wafers of less than 0.5 Å. In contrast to OWLS and TInAS, the VASE measurement is performed in air, rather than in aqueous solution, and therefore also includes any water associated with the film. Additionally, the effective refractive index of the film is not measured by the VASE and must be assumed. For these reasons, the measured thickness does not agree quantitatively with TInAS/OWLS measurements, and the adsorbed dry mass cannot be directly calculated.
However, in previous work, the VASE thickness (assuming an effective refractive index of 1.45) was found to scale linearly with the TInAS/OWLS dry mass. Therefore the VASE thickness was multiplied by the scaling proportion to determine the dry optical mass on the wafers, and thereby the L/2R_g values, of the dextran layers, with resulting values of L/2R_g ranging from 1.04 to 0.5.

### 4.3.3 Colloidal-probe lateral force measurements

The friction measurements were conducted using an MFP-3D AFM (Asylum Research, California, USA). Borosilicate colloidal spheres (Kromasil, Eka Chemicals AB, Sweden; radius ≈ 8 μm) were glued to tipless rectangular cantilevers (CSC12, Mikromasch, Estonia) with UV-curable epoxy (Norland Optical Adhesive 63, Norland, NJ, USA). The glue was cured for 15 minutes under UV and then allowed to set overnight at 50°C. Prior to attaching the sphere, the normal and lateral spring constants of the sphere were calibrated from the power spectral density of the thermal noise fluctuations and the method of Sader et al. using the measured cantilever dimensions.

Each bare colloid was first slid against a bare substrate to ensure that no transient effects, such as initial colloidal wear or initial laser drift, could influence the measurements. Friction measurements were made in contact mode by sliding the probe perpendicular to the cantilever axis over a distance of 1 μm at a speed of 1 μm/s. The trace minus retrace (TMR) signal was recorded, which is directly proportional to the friction force. Fifteen curves were taken for each load, after which the position on the sample was shifted slightly to avoid any sample wear and the load increased for the next step. To obtain the friction force, the TMR signal was multiplied by the calibration constant determined using the test probe method of Carpick et al. The measurements shown in Figure 4.1 were made in a single session without disturbing the cantilever or laser, and likewise for Figure 4.2, so the calibration does not affect their relative values, but only facilitates the comparison between the two datasets, shown in Figure 4.3, and with other studies.

### 4.4 Results and discussion

#### 4.4.1 Brush solvation

The sensors of two different optical instruments, the optical waveguide lightmode spectroscopy (OWLS) and the transmission interference adsorption sensor (TInAS), were
exposed to a solution of PLL-\textit{g}-dex(5 kDa) in ultrapure water, and the thickness and refractive index of the film were measured in real time during adsorption as the brush was formed. The adsorbed mass of the polymer on the surface, not including any associated water (the “dry mass”), were calculated from the thickness and refractive index. The two instruments generally show excellent agreement, in this case reporting the maximum adsorbed mass of $306 \pm 15 \text{ ng/cm}^2$, corresponding to an average inter-chain distance $L$ of 1.9 nm or a grafting density $L/2R_g$ of 0.5, taking the radius of gyration $R_g$ to also be 1.9 nm.\textsuperscript{22} The measurements indicate a mean solvated brush thickness of 7.1 nm with a refractive index of 1.42, although these values have a higher mutual uncertainty than their combined optical mass.\textsuperscript{21}

After adsorption had slowed down to within the drift of the instruments, the samples were first rinsed with ultrapure water and then exposed to a solution of either 10 mM potassium sulfate or 5.5 mM trehalose in ultrapure water. The brush showed no measurable change in thickness or refractive index in the presence of either solute, indicating that the solvent quality did not significantly decrease, which would cause the brush to collapse, nor did the solute cause any measurable desorption of the copolymer.

\subsection*{4.4.2 Aqueous lubricity}

Silicon wafers were incubated in the same PLL-\textit{g}-dex solution for varying times to achieve different chain densities. They were subsequently rinsed and dried, and the thickness of the film measured with variable angle spectroscopic ellipsometry (VASE) to determine the adsorbed mass and the homogeneity of the films. Finally, the friction force was measured as a function of load by means of lateral force microscopy, between the brushes and a borosilicate colloidal sphere glued to the cantilever tip. Measurements were performed in ultrapure water and subsequently in the appropriate solution.

\subsection*{4.4.3 Influence of grafting density}

Figure 4.1 shows the friction response as a function of applied load of PLL-\textit{g}-dex films of different densities. In good agreement with an earlier study,\textsuperscript{10} the local coefficient of friction, $\mu = \text{dF}_{\text{lat}}/\text{dF}_{\text{norm}}$, is lowest at low loads, but increases sharply above a critical load. Higher dextran grafting density results in lower friction at low loads and a higher critical load. This critical load is the value at which the applied load partially overcomes the inter-chain bonds and steric forces that force the chains into a brush configuration. Above this load, phenomena
such as plowing and dragging overcome the tendency of the brush to maintain a lubricious aqueous film at the surface, resulting in sharply increased friction.

![Image of friction force measurements](image)

**Figure 4.1** Friction force measurements of a colloidal probe sliding on a silicon wafer coated with PLL-\(g\)-dextran at a grafting density of \(L/2R_g = 1.04\) (dotted line), \(L/2R_g = 0.68\) (dashed line), and \(L/2R_g = 0.5\) (solid lines), in ultrapure water (\(\odot\)) and in 5.5 mM trehalose (\(\blacktriangle\)).

### 4.4.3 Influence of solutes

The friction response of the densest dextran brush in the presence of 5.5 mM trehalose solution was similar to that in ultrapure water at the lowest loads, but continuously increased with increasing load, exhibiting a less well-defined critical load (Figure 4.1). At the highest normal force measured in the presence of trehalose, the friction force was around double that in ultrapure water. This is consistent with our prediction that the trehalose acts as a plasticizer for the dextran chains, easing their separation and thus causing a reduction in load-bearing capacity.

The influence of potassium sulfate on the friction of the densest dextran brush is similar to that of trehalose, as shown in Figures 4.2 and 4.3. The friction force and local friction coefficient are similar to the response in ultrapure water at the lowest applied loads, but
Diverge at higher loads. A similar trend is already apparent in Figure 4.2, which shows the local coefficient of friction diverging more rapidly from the initial value with increasing salt concentrations. Figure 4.3 also shows that the dense brush in the presence of solutes exhibits a higher critical load than a sparser brush in water with similar friction values. This difference may reflect the contribution to the load-bearing capacity of the brush made by steric forces, which are only weakly affected by the presence of solutes.

**Figure 4.2** Friction force measurements of a colloidal probe sliding on a silicon wafer coated with PLL-g-dextran at a grafting density of \( \frac{L}{2R_g} = 0.5 \) in ultrapure water (◊), 2 mM \( K_2SO_4 \) (○), and 10 mM \( K_2SO_4 \) (●).
Figure 4.3 Data from Figures 4.1 and 4.2 overlaid for comparison. The friction response of the dense dextran brush in the presence of solutes (filled shapes) is comparable to the response of sparser dextran films in ultrapure water (broken lines).

4.5 Conclusions

We have demonstrated that the lubricity of dextran brushes can be influenced by solutes that reduce the strength of the inter-chain hydrogen bonding, without influencing the height or density of the brush in the absence of load. In particular, the solutes reduce the ability of the dextran brush to support high loads, while having less effect on the friction at low loads than a corresponding reduction in brush density, illustrating the relative contributions of inter-chain hydrogen bonding and steric forces to the lubricity of the brush as a function of applied load. This has implications for the model of physiological lubrication by saccharide-bearing, brush-like systems, which usually operate in the presence of much higher salt concentrations. In biological systems, the presence of other species that enhance the hydrogen bonding or directly cross-link the chains may serve to offset the failure of inter-chain hydrogen bonding under those conditions. Similar techniques might be used to extend the lubricious regime of
the dextran brush to higher loads, or to tailor the inter-chain interactions for further applications such as trapping and then releasing biomolecules.

4.6 References


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Chapter 5: Impact of chain morphology on the lubricity of surface-grafted polysaccharides

This work is from a manuscript in preparation to be submitted to an upcoming special issue of RSC Advances. The supplementary materials have been added to the end of this chapter. The work described was done by myself. Dr. Rowena Crocke and Prof. Nicholas D. Spencer supervised the work, contributed in discussions about the project steps, and corrected the manuscript.

5.1 Abstract

Surface-density gradients of dextran have been fabricated on silicon wafers by means of an intermediate azide-terminated monolayer, which binds to random segments of the dextran chains to form a complex loop-train-tail structure. Friction-force microscopy was employed to understand the relative contributions of chain density and other parameters on the tribological behavior of the immobilized chains. The results are contrasted with similar investigations performed with density gradients of poly(L-lysine)-graft-dextran, a bottlebrush copolymer that adsorbs onto silica to form a well-characterized dextran brush. Both systems exhibit friction coefficients that vary over more than an order of magnitude with applied load, with a sharp transition from low- to high-friction regimes occurring upon increasing load. The brush architecture exhibited more extreme friction coefficients than the loop-train-tail architecture, lubricating better at low loads while exhibiting higher friction at high loads, despite involving less than a third of the amount of dextran (on a monomer basis) in comparison to the loop-train-tail system. The coefficient of friction at high loads decreased with increasing dextran surface density in the loop-train-tail system, while the opposite was true for the polymer brush. The surface density required to forestall the pressure-induced transition to high friction was also significantly higher for the loop-train-tail system than for the brush system. These results illustrate the influence of brush regularity on resistance to collapse under applied load, but also its role in exacerbating friction forces.
5.2 Introduction

Surfaces bearing densely packed, end-grafted neutral or charged polymer chains in good solvents are extraordinarily resistant to the intrusion of foreign bodies, leading to their characteristic low-friction and non-fouling behavior.\(^1\,^2\) Particularly interesting are brushes composed of hydrophilic biocompatible polymers, such as poly(ethylene glycol), which have been the subject of many investigations as non-fouling surfaces, aqueous lubricants, and so-called “stealth coatings” against undesired immune-system response.\(^3\,^4\) These characteristics, observable over a wide range of chain chemistries and architectures, originate from the steric hindrance presented by the chains and their associated solvent molecules, as well as the osmotic pressure induced by the strong interactions of the chains with the solvent.\(^7\) The architecture of polymer-brush-mediated aqueous tribosystems also bears a striking similarity to that of naturally occurring lubricious systems, including the glycocalyx on the inner walls of capillaries,\(^8\) the surface of articular cartilage,\(^9\) and slippery surfaces such as the eye and tongue.\(^10\) Such natural systems typically consist of oligosaccharide-bearing surfaces in aqueous environments. A characteristic feature of oligo- and polysaccharide chains is the presence of hydroxyl groups, which can directly engage in hydrogen bonding with adjacent chains, in contrast to polymers such as poly(ethylene glycol), for which water intercalation is necessary to bridge between chains. Hydroxyl groups also facilitate selective functionalization of the chains to produce films with complex architectures by the addition of reactive groups, branching, or crosslinking. Synthetic polysaccharide brushes and thin films have been produced by various means, including covalent grafting to silica surfaces,\(^11\) Langmuir-Blodgett films of block copolymers,\(^12\) and electrostatic adsorption of graft copolymers.\(^13,\,14\)

In order to better understand the roles of the film architecture and inter-chain hydrogen bonding in polysaccharide films, we compared the friction behavior of two different dextran-based architectures. Dextran is a naturally occurring hydrophilic polysaccharide consisting of D-glucose monomers connected by α-1,6 glycosidic linkages, which confer a relatively high degree of flexibility and prevent the formation of stable crystalline structures. PLL-g-dextran consists of dextran chains end-grafted to some of the pendant lysine groups of poly(L-lysine).\(^13,\,16\) The unreacted lysine groups are positively charged under aqueous conditions at physiological pH, and thus facilitate spontaneous adsorption of the PLL-g-dextran molecule from solution onto negatively charged surfaces, such as silicon dioxide, with sufficient chain density to force the hydrophilic dextran chains into a brush conformation, as shown in Figure...
5.1. PLL-g-dextran brushes in water are highly lubricious against a hard counterface at low loads, but the friction increases sharply above a critical load.\textsuperscript{17,18} When the counterface consists of a dextran brush, lower friction is obtained at low loads, the critical load is higher, and friction is higher above the critical load.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figures-figure5.png}
\caption{Chemical structures of poly(L-lysine)-\textit{graft}-dextran (PLL-g-dextran) (A) and poly(allylamine)-\textit{graft}-perfluorophenylazide-dextran (PAAm-g-PFPA-dextran) (B), and the corresponding expected polymer architectures, when attached to silicon oxide substrates.}
\end{figure}

A high-coverage, PLL-g-dextran-functionalized surface probably represents the highest dextran brush density achievable via grafting-to means, with adsorption eventually limited by steric hindrance of the adsorbed brush, and largely independent of the number of unreacted...
lysine “feet” or the dextran or PLL chain lengths.\textsuperscript{13,14,19} Since there is no readily available route to achieve higher dextran surface densities in a uniform brush, an alternative method of attaching dextran to the surface has been pursued. In the present study, substrates were coated with the adhesion promoter, poly(allylamine)-\textit{graft}-perfluorophenylazide (PAAm-g-PFPA), an azide-bearing graft copolymer, and chain-density gradients of dextran chains were subsequently fabricated, being attached at random points to form a complex loop-train-tail structure, as shown in Figure 5.1. The influence of the chain density and applied load on the friction coefficient of the resulting film was investigated, and the results contrasted to the behavior of PLL-g-dextran brushes.

5.3 Materials and methods

5.3.1 Synthesis of poly(allylamine)-\textit{graft}-perfluorophenylazide (PAAm-g-PFPA)

Poly(allylamine)-\textit{graft}-perfluorophenylazide (PAAm-g-PFPA) was synthesized as previously described.\textsuperscript{20} Briefly, poly(allylamine hydrochloride) (6.33 mg, 6.8x10^{-6} mol monomer, 15 kDa, Sigma) and excess potassium carbonate (15.82 mg, 1.1x10^{-4} mol, Merck) were dissolved in 1.3 mL of water (Milli-Q Gradient A10, Millipore SA, Molsheim, France), and a solution of N-hydroxysuccinimide-perfluorophenylazide (NHS-PFPA, 5.62 mg, 1.7x10^{-5} mol, SuSoS AG, Dübendorf, Switzerland) in 1.3 mL of ethanol (Merck) was slowly added. The resulting solution was stirred overnight and then diluted to 100 mL with a 3:2 (v/v) ethanol/water mixture, yielding a polymer concentration of 0.1 g/L. All solutions were kept in the dark to avoid activation of the azide groups. The grafting ratio, defined as the number of allylamine monomers divided by the number of grafted PFPA monomers, is observed to be 8 or 9.\textsuperscript{20}

5.3.2 Substrate preparation and dextran attachment

Bare 2 cm x 2 cm silicon wafers were cleaned by sonication twice for five minutes in toluene, then twice for five minutes in 2-propanol, after which they were exposed to O\textsubscript{2} plasma for two minutes (Plasma Cleaner/Sterilizer, PDC-32G instrument, Harrick, Ossining, NY, USA). The surface SiO\textsubscript{2} layer was characterized by ellipsometry, as described below, with none of the wafers exhibiting more than 0.1 nm variation, after which the wafers were immediately immersed in a 0.1 g/L solution of PAAm-g-PFPA. After thirty minutes of immersion, the
substrates were rinsed and then sonicated for 5 minutes in a 3:2 (v/v) ethanol/water mixture, then rinsed with ultrapure water and dried with nitrogen. The PAAm-g-PFPA thickness was then measured by ellipsometry (described below).

Following this, the wafers were placed on a spin coater, covered in a solution of 25 g/L dextran (dextran T200, 2 MDa, Pharmacosmos A/S, Denmark) in water, and then spun at 4000 rpm for 40 s, then 5000 rpm for 10 s. The wafers were allowed to dry for 15 minutes, then exposed to UV light (Philips TUV 11W, peak wavelength 254 nm) at a distance of 10 cm. Dextran gradients were produced by varying the UV exposure time (from 3.5 s to 240 s) along the wafer using a moving shutter, programmed to evenly space the desired exposure durations in discrete steps along the wafer. Following the UV exposure, the wafers were immersed overnight in ultrapure water to remove excess non-attached polymer, then rinsed with water, dried with nitrogen, measured by ellipsometry, and then stored in a dark prior to friction measurements.

Subsequent UV illumination in ambient air or in air with 100% relative humidity, with the aim of activating any remaining azides, was found to influence neither the thickness nor the friction measurements, within experimental error.

5.3.3 Characterization by variable-angle spectroscopic ellipsometry (VASE)

Thickness measurements of the substrate SiO$_2$ layer, the adsorbed PAAm-g-PFPA layer, and the attached dextran film were performed in air using an M-2000 variable angle spectroscopic ellipsometer (VASE, J.A. Woollam Co., Inc., Lincoln, NE, USA) at a reflection angle of 70° and over the wavelength range of 370-994 nm. The spectra were analyzed using WVASE32 software, using a multilayer model of Si/SiO$_2$ and top films with the optical properties of a Cauchy layer, as detailed in the Supporting Information. The surface density of each layer can be calculated from the measured thickness and the bulk density of PAAm-g-PFPA and dextran. Ellipsometric measurements on the gradient in water were also made using a liquid cell, and the results evaluated using the Bruggeman effective-medium approximation, whereby the top layer was treated as a mixture of dextran (n=1.51) and water (n=1.333), and the layer thickness and dextran fraction were both fitted. The multilayer models for the dry and wet systems are shown in the Supporting Information.
5.3.4 Atomic force microscopy measurements

The AFM experiments were conducted in contact mode by means of a MFP-3D AFM (Asylum Research, Santa Barbara, CA, USA) with gold-coated rectangular Si cantilevers (CSC12B, nominal normal spring constant 1.75 N/m, Mikromasch, Tallinn, Estonia), chosen to match the loading range of interest, while maintaining sensitivity and minimizing errors due to in-plane bending. A borosilicate colloidal sphere (Kromasil, Eka Chemicals AB, Bohus, Sweden; radius ≈ 8 μm) was glued to the end of the cantilever with UV-curable epoxy. (Norland Optical Adhesive #63, Norland, Cranbury, NJ, USA) The entire assembly was then irradiated with UV light for 30 minutes and left for a full day prior to use, to further strengthen the adhesion between the colloidal sphere and the cantilever.

Friction measurements were obtained by scanning the probe perpendicularly to the major axis of the cantilever and recording the TMR (Trace Minus Retrace in mV) value. The TMR value is directly proportional to the friction force and minimizes the contributions to the lateral force from non-friction sources. The gradient was characterized by measuring points in a random order, in order to minimize possible effects arising from gradual drift. All scans were 1 μm in length with velocities of 1 μm/sec. The dependence of lateral force on scanning distance was investigated and it was shown that for scans longer than 100 nm, the lateral force remained constant.

The normal and lateral cantilever spring constants were calibrated prior to sphere attachment from the power spectral density of the thermal noise fluctuations and by the method of Sader and coworkers. The lateral force measurements of the rectangular cantilevers were calibrated using the test-probe method described by Carpick and coworkers, yielding absolute values for both the lateral force and the local coefficient of friction (the ratio of lateral to normal force). The test-probe calibration method itself includes correcting factors for differences in cantilever dimensions, colloidal sphere size, normal and lateral spring constant of the cantilever, and spot intensity between the experimental and reference setups. All experiments were measured within a gradient in a single run with the same tip and cantilever. Additionally, all the rectangular cantilevers used for the calibration and measurement were produced in a single batch from the same wafer, so that any errors stemming from non-ideal behavior of the cantilevers or unaccounted factors, such as variations in the reflectivity of the cantilever top coatings, would be consistent across all measurements.
Following nanotribological measurements with an uncoated borosilicate colloidal probe, the entire tip-cantilever assembly was gently lifted off the gradient surface and immediately exposed to a 0.02 mg/mL PLL-g-dextran (synthesized from previous work)\textsuperscript{13,14} solution to form a dextran brush layer on the probe surface. After 30 minutes, the assembly was thoroughly rinsed with water and placed back into contact with the same gradient substrate to resume the friction experiments.

5.4 Results and discussion

5.4.1 Gradient fabrication

The VASE dry thickness of the PAAm-g-PFPA layer was $1.7 \pm 0.1$ nm, and the additional dry thickness of the attached dextran film reached 6 nm at the dense end of the gradient, corresponding to a density of 36 dextran monomers per nm$^2$, or 343 nm$^2$ per 2MDa dextran chain. The kinetics of the UV-activated attachment of dextran to the substrate are seen to approximate a logarithmic curve until reaching saturation after about one minute, as shown in Figure 5.2. The aqueous VASE measurements indicate a relatively constant (water + dextran) film thickness of $124 \pm 5.4$ nm, independent of the UV irradiation time, while the fraction of dextran within the film increased with increasing UV irradiation, as shown in Figure 5.3. Films produced with the shorter irradiation times could not be reproducibly measured in the wet state due to weak signal.
Figure 5.2 Surface density of dextran in PAAm-g-PFPA-dextran films, calculated from VASE thickness measurements, plotted against UV exposure time.
Figure 5.3 Thickness (A) and mass fraction (B) of the dextran/water layer associated with the PFPA-coated substrate, measured by VASE in a water-filled liquid cell and calculated using the Bruggeman mean-field approximation, plotted against the dextran surface density. Mass fraction is also plotted against UV exposure time (C) for comparison of the kinetics with those in Figure 5.2.

Comparing the surface densities of dextran and PAAm-g-PFPA calculated from these VASE measurements at the densest end of the gradient, and taking the experimental grafting ratio of 9 allylamines to each PFPA, we obtain 542 PFPA units per 2 MDa dextran chain, or 56.9 dextran monomers per PFPA unit, with 1.58 PFPA units per nm$^2$. What fraction of these PFPA units is actually bound to a dextran is unknown. However, since the kinetics are roughly logarithmic until the saturation point, and neither the wet height nor the friction changes appreciably upon further UV irradiation, we conjecture that relatively few PFPA units are bound to the dextran chains, and attempt to estimate the number of attachment points from the measured values.

Assuming that the chain is attached to the PFPA layer at equidistant points along the chain, and that these can be treated as single attachments (therefore neglecting trains), then the film can be treated as a “brush” consisting of two end tails and a number of loops in the middle,
with the spacing between points such that the tail and loop lengths are equal, as shown in the Supporting Information. For example, if a 2 MDa chain is bound at two points, then that chain can be treated as two 500 kDa tails and a 1MDa loop, which approximates to four equally spaced 500 kDa end-attached chains. If these effective 500 kDa chains are swollen in liquid to their bulk hydrodynamic radius, the theoretical film thickness can be calculated from the measured dextran surface density, and compared to the measured value. Replacing the single-point attachments with short trains would not significantly influence the result. The best agreement with the measured wet thickness of the film was achieved when the 2 MDa chain was bound at three points, yielding two 333 kDa tails and two 666 kDa loops, which if equated to an effective brush of six 333 kDa chains would have a theoretical film thickness of 125.5 nm at the densest end of the gradient. While there are numerous sources of error in this calculation, it supports the hypothesis that each chain is bound by only a few PFPA units. A uniform 333 kDa brush of the measured density would have an L/2R\textsubscript{g} value of 0.21, taking R\textsubscript{g} to be 19 nm.

5.4.2 Friction measurements

The friction coefficient of the PAAm-g-PFPA-dextran films is highly load dependent, with all but the sparsest films exhibiting a low-friction regime at low loads. A transition to a high-friction regime occurs above a critical normal load, as shown in Figure 5.4. This behavior is similar to that observed in PLL-g-dextran brushes, and Figures 5.5 and 5.6 show the results from that study alongside the results of the PAAm-g-PFPA-dextran findings for comparison. Figure 5.5 shows the friction coefficient in the low- and high-load regimes plotted against the dextran monomer density, while Figure 5.6 shows the critical normal load at which the friction increases, also plotted against the dextran monomer density. All measurements were performed against a bare borosilicate sphere as well as a PLL-g-dextran-coated sphere.
Figure 5.4 Typical friction vs. load curves of a colloidal probe sliding on a PFPA-dex-coated wafer exposed to UV for 5 seconds (yielding a coverage of 9 dextran monomers/nm$^2$, white), 20 seconds (24 monomers/nm$^2$, grey), and 120 seconds (35 monomers/nm$^2$, black). Fits of the linear parts of each series, excluding the non-linear intermediate points, are extrapolated to their intersecting point, the x-coordinate thereof representing the transition load.
Figure 5.5 Coefficient of friction of PLL-g-dextran\textsuperscript{17} (black and white) and PAAm-g-PFPA-dextran (blue) plotted against surface density of dextran monomers in both low and high load
regimes (A) and just the low load regimes (B). The squares represent a bare borosilicate sphere at low load (white or light blue) and high load (black or dark blue). The circles represent a PLL-g-dextran coated borosilicate sphere at low load (white or light blue) and high load (black or dark blue).

**Figure 5.6** Critical load associated with the transition from low to high friction regimes of PLL-g-dextran (grey) and PAAm-g-PFPA-dextran (blue) plotted against surface density of dextran monomers. The squares represent a bare borosilicate sphere, while the circles represent a PLL-g-dextran coated borosilicate sphere.

The PAAm-g-PFPA-dextran system, consisting of a more disordered loop-train-tail architecture, showed a higher friction coefficient at low loads compared to the brush formed by PLL-g-dextran, despite the greater wet film thickness and much higher surface monomer density. However, the disordered system showed a lower friction coefficient at high loads compared to the brush system, with the coefficient of friction decreasing as the dextran density increased, while the opposite was true for the brush. The transition from low to high friction regimes also occurred at much higher dextran surface monomer concentrations in the disordered case than for the brush, indicating that the disordered system is less able to support
high loads. Finally, the impact of dextran-coating the borosilicate sphere is opposite in the high-friction regime, with the dextran coating exacerbating friction in the brush case while reducing it in the disordered case.

The significance of the brush conformation has been demonstrated previously by collapsing brushes with minimal changes to the solvent,\textsuperscript{31} which dramatically changes the mechanical properties of the film. In the absence of significant hydrodynamic forces, longer, more disordered systems will significantly interpenetrate and interact under even low loads. Brush interpenetration increases friction even when inter-chain interactions from opposing substrates, such as tangling or hydrogen bonding, are neglected.\textsuperscript{32} Tangling is also known to increase friction coefficient between polymer brushes,\textsuperscript{33-36} although tangling is not possible for the 5 kDa dextran chains, since they are too short and stiff. However, the interpenetration of chains under high loads, with the subsequent formation of inter-chain hydrogen bonds, nevertheless creates high friction between the surfaces.\textsuperscript{17} The impact of interpenetration, entanglement and hydrogen bonding together, as in the case of the dextran loop-train-tail system, is more complex. While hydrogen bonding between chains on the same substrate may inhibit entanglement with chains of the counterface at low loads, the application of higher loads and lateral forces does ultimately yield increased friction, which is controlled by inter-chain association as well as tangling.\textsuperscript{37}

The combination of increased chain entanglement and hydrogen bonding between the counterfaces can explain the differences between the disordered, thicker dextran system and the brush system. The friction coefficient of the brush is lower at low loads, due to its density and the lack of tip interpenetration, thanks to the inter-chain hydrogen bonding holding the chain ends in place. As the load increases, the ordered brush system is more capable of resisting this interpenetration than the disordered system, which more rapidly becomes enmeshed with the counterface. However, the ordering and hydrogen bonding show the opposite effect once interpenetration is achieved, with interactions between opposing surfaces of ordered chains creating much more resistance than is the case for the longer, disordered conformation, where the chains are freer to rearrange themselves.

While these findings are significant in the field of brush and thin-film design, they are most pertinent to the case of more complex architectures, in which gels and brushes may be combined in various ways to achieve tailored properties. For example, it has been shown that
short polymer brushes on top of gels reduce their friction, while long polymer brushes increase it,\(^3\) a phenomenon that seems likely to be related to the differences outlined here.

## 5.5 Conclusions

The lubricating properties of a dextran surface-density gradient, attached at random intervals to the surface by means of an intermediate azide-bearing monolayer, were investigated by means of colloidal-probe lateral force microscopy. The results were contrasted with previous work involving density gradients of end-attached dextran (a polymer brush density gradient). Both systems showed highly load-dependent friction, with the randomly attached dextran chains exhibiting less extreme differences than the polymer-brush case. For the randomly attached system, significantly higher surface concentrations of dextran were necessary to reach the same critical load (marking the transition from low to high friction regimes). This suggests that the polymer-brush architecture significantly enhanced the load-bearing properties of the dextran film. Increasing the density of the disordered dextran film reduced the friction coefficient at high loads, while the opposite was true for the polymer brush. These findings demonstrate that the brush architecture is critical in resisting collapse under applied load, but is susceptible to inter-chain interactions between the surfaces, which increase friction once the load has exceeded a critical threshold. This highlights the tradeoff involved in designing film architectures to maximize layer thickness and lubricity under extreme loads or to aim for an ideal brush with ultra-low friction at low and moderate loads. Future work on heterogeneous architectures may allow tailored films that exhibit the appropriate mixture of properties, depending on the expected loading of the film.

## 5.6 Supplementary information

<table>
<thead>
<tr>
<th>Liquid VASE Fit Layers</th>
<th>Thickness</th>
<th>Refractive Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dextran</td>
<td>Measured</td>
<td>(1.51 + 0.01/\lambda^2)</td>
</tr>
<tr>
<td>PAAm-g-PFPA</td>
<td>Measured before spin-coating</td>
<td>(1.45 + 0.01/\lambda^2)</td>
</tr>
<tr>
<td>Silicon oxide</td>
<td>Measured before adsorption</td>
<td>Built in to software</td>
</tr>
<tr>
<td>Silicon substrate</td>
<td>Infinite</td>
<td>Built in to software</td>
</tr>
</tbody>
</table>
Table 5.1 VASE multilayer model used to fit the dry measurements. As the refractive index formula indicates, the PAAm-g-PFPA and dextran layers are treated as Cauchy films.

<table>
<thead>
<tr>
<th>Liquid VASE Fit Layers</th>
<th>Thickness</th>
<th>Refractive Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambient water</td>
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<tr>
<td>Dextran/water mixture (Bruggeman effective medium)</td>
<td>Measured</td>
<td>Measured mix of 1.51 and 1.333</td>
</tr>
<tr>
<td>PAAm-g-PFPA</td>
<td>Measured dry before spin-coating</td>
<td>1.45 + 0.01/λ²</td>
</tr>
<tr>
<td>Silicon oxide</td>
<td>Measured dry before adsorption</td>
<td>Built in to software</td>
</tr>
<tr>
<td>Silicon substrate</td>
<td>Infinite</td>
<td>Built in to software</td>
</tr>
</tbody>
</table>

Table 5.2 VASE multilayer model used to fit the liquid-cell measurements. As the refractive index formula indicates, the PAAm-g-PFPA layer is treated as a Cauchy film.
Figure 5.7 Schematic representation of the model used to calculate probable dextran attachment density. The 2 MDa dextran chain is attached to the substrate at either one, two, or three points, spaced such that the end and loop lengths are equal. These are then treated as discrete end-attached chains, and the height of the polymer brush of corresponding chain length and surface density is then calculated assuming that the chains are swollen to their hydrodynamic volume.

5.7 References


3. Gregory L. Kenausis, Janos Vörös, Donald L. Elbert, Ningping Huang, Rolf Hofer, Laurence Ruiz-Taylor, Marcus Textor, Jeffrey A. Hubbell and Nicholas D. Spencer, “Poly(L-lysine)-g-Poly(ethylene glycol) Layers on Metal Oxide Surfaces: Attachment Mechanism and Effects of Polymer Architecture on Resistance to Protein Adsorption”, Journal of Physical Chemistry B, 2000, 10.1021/jp993359m

4. Ning-Ping Huang, Roger Michel, Janos Voros, Marcus Textor, Rolf Hofer, Antonella Rossi, Donald L. Elbert, Jeffrey A. Hubbell and Nicholas D. Spencer, “Poly(L-lysine)-g-poly(ethylene glycol) Layers on Metal Oxide Surfaces: Surface-Analytical Characterization and Resistance to Serum and Fibrinogen Adsorption”, Langmuir, 2001, 10.1021/la000736+


force microscope cantilever spring constants”, Nanotechnology, 2006, 10.1088/0957-4484/17/9/010


Chapter 6: Hydration and conformation in dextran and PEG brushes

This work is from a manuscript in preparation. The ATR-IR experiments on PLL-g-dextran and the related discussion were done by myself and Dr. Venkataraman Nagayanallur working together, while the ATR-IR experiments on PLL-g-PEG were done solely by him. He also contributed to the manuscript text and to discussions about the project. The rest of the described work was done by myself. Dr. Rowena Crockett and Prof. Nicholas D. Spencer supervised the work, contributed in discussions about the project steps, and corrected the manuscript. Dr. Patrick Ilg also gave valuable suggestions concerning the simulation study.

6.1 Abstract

The hydrated conformation of dextran brushes at different chain densities was investigated via optical-thickness measurements and course-grained molecular dynamics simulations. The dextran conformation was controlled to a large degree by inter-chain interactions, with the brush height being only weakly dependent on brush grafting density, in contrast to the scaling law for ideal brushes in good solvent. To compare this behavior to the influence of hydration on chain conformation, the variation of PEG and dextran brush structure under dry, humid and wet conditions was studied via ATR-IR. Significant shifts in the C-O-C stretching bands were observed in PEG brushes with changing humidity, indicative of conformational changes due to the presence of water, while no corresponding shifts were observed in dextran. These observations of the differing roles of water and structure in PEG and dextran brushes are useful in interpreting their differing properties, as reported in the literature.

6.2 Introduction

Surfaces bearing densely packed polymers in good solvents are protected to an extraordinary degree from adsorbates and foreign bodies. The steric barrier presented by the polymer chains and their associated solvent molecules, as well as the osmotic pressure induced by the favorability of interactions with the solvent, lend them characteristic low-friction and nonfouling behavior, which can be observed over different chain chemistries and architectures.\(^1\)\(^2\) Polymer brushes composed of biocompatible polymers such as poly(ethylene
glycol) (PEG) are of interest as biocompatible and environmentally friendly non-fouling surfaces, aqueous lubricants, and “stealth coatings” against immune system response.\(^3\)\(^-\)\(^5\) Polysaccharide brushes and thin films have also been produced,\(^6\)\(^-\)\(^8\) many incorporating dextran – a naturally-occurring glucan consisting of \(\beta(1\rightarrow6)\) glycosidic linkages, which confer a relatively high degree of flexibility and inhibit the formation of stable crystalline structures. PLL-\(g\)-dextran consists of dextran chains end-grafted to some of the pendant lysine groups of poly(L-lysine).\(^9\)\(^-\)\(^12\) The remaining unreacted lysine groups are positively charged under aqueous conditions, and thus the molecule adsorbs spontaneously onto negatively charged surfaces, such as silicon dioxide, with sufficient chain density to force the hydrophilic dextran chains into a brush conformation. PLL-\(g\)-dextran-based brushes behave similarly to PLL-\(g\)-PEG in terms of adsorption behavior, fouling resistance, and lubricity under low loads in aqueous conditions.\(^11\)\(^,\)\(^12\) However, the ability of the dextran chains to hydrogen bond together influences the inter-chain interactions, as well as the hydration, of the brush.

A widely cited model\(^13\) describes a helical conformation of PEG within a cage of water, with the coincidence of the matching structures of PEG and the hydration cage lending high solubility to the chemically amphiphilic polymer.\(^14\) Similar behavior has been observed in PEG brushes, as discussed by Heuberger \textit{et al.} and references therein.\(^15\) In contrast, glucose is chemically akin to a water cluster, aside from the small hydrophobic patches on the two carbohydrate ring faces, although the crystalline structure of certain glucans, such as cellulose or amylose inhibits their solubility, and even dextran can form insoluble agglomerates in water in some cases.\(^16\)\(^-\)\(^18\) In other words, PEG is chemically amphiphilic but structurally water-soluble, while glucans are chemically hydrophilic but can be structurally water-insoluble. PEG brushes also show decreasing hydration on a per-monomer basis with increasing chain density,\(^19\) whereas dextran brushes exhibit no correlation between hydration and chain density.\(^20\)

Infrared spectroscopy is a sensitive tool to determine molecular structure and chain conformation of polymers. Several structural investigations of polysaccharides, including dextran, in their bulk phase as well as the changes occurring in solutions have been probed using IR spectroscopy.\(^21\)\(^,\)\(^22\) However, structural investigations of polysaccharides grafted to surfaces and the conformational changes occurring upon hydration/dehydration have, to the best of our knowledge, not been reported.
Computer simulations can aid in the understanding of dextran chain conformation and solvation, but atomistic simulations of brushes with explicit water are too computationally intensive to study over useful timescales. Therefore, a coarse-grained force field was used to represent the dextran and water molecules. The M3B model was first developed for 1→4-linked oligosaccharides in water, and subsequently modified to represent 1→6-linked dextran chains. The model treats each dextran monomer as three beads corresponding to carbons 1, 4, and 6, with water molecules represented by a single bead. Intermolecular bonding is limited to two-body Morse functions, although the model parameterization was performed using atomistic simulations that did include hydrogen bonding.

6.3 Materials and methods

6.3.1 Poly(L-lysine)-graft-dextran (PLL-g-dextran) and poly(L-lysine)-graft-poly(ethylene glycol) (PLL-g-PEG)

The poly(L-lysine)-graft-dextran (PLL-g-dextran) copolymer used in these experiments was synthesized during previous work. Briefly, dextran (dextran T5, 5.2 kDa, polydispersity 1.8, Pharma-cosmos A/S, Denmark) undergoes reductive amination with poly(L-lysine)-HBr (20 kDa, polydispersity 1.1, Sigma-Aldrich, Switzerland) in a sodium borate buffer (0.1 M, pH 8.5, 0.4 M NaCl). The terminal dextran aldehyde group reacts with the primary amine groups of PLL to form a Schiff base, which is subsequently reduced by sodium cyanoborohydride (NaBH₃CN). The resultant PLL-g-dextran graft copolymer is separated from the unreacted materials via ultracentrifugation. The grafting ratio of lysine monomers to dextran chains can be controlled during synthesis by adjusting the reactant ratio, and was subsequently determined through ¹H-NMR spectroscopy of PLL-g-dextran in D₂O and elemental analysis. The particular copolymer studied here was PLL(20)-g[5.3]-dextran(5), where 20 kDa is the molar mass of PLL including the Br⁻ counterions, 5.3 is the grafting ratio, and 5 kDa is the weight-average molecular weight (M₆) of the dextran. On average, these dextran chains consist of 32 monomer units, which, assuming each monomer to be 0.7 nm long, corresponds to a fully stretched chain length of 22.3 nm.

The poly(L-lysine)-graft-poly(ethylene glycol) (PLL-g-PEG) copolymer used in these experiments was obtained from SuSoS (SuSoS AG, Dübendorf, Switzerland). The particular copolymer studied here was PLL(20)-g(3.5)-PEG(2), where 20 kDa is the molar mass of PLL
including the Br⁻ counterions, 3.5 is the grafting ratio, and 2 kDa is the weight-average molecular weight (M_w) of the PEG chains. On average, these PEG chains consist of 45 monomer units, which, assuming each monomer to be 0.36 nm long, corresponds to a fully stretched chain length of 16.3 nm.

6.3.2 Transmission interference adsorption sensing (TInAS)

Transmission interference adsorption sensing (TInAS) is an optical biosensing technique that monitors interference fringes formed when light is transmitted through a film adsorbed onto a special multilayered TInAS sensor. The wavelengths of the interference peaks are correlated to the optical thickness of the adsorbed layer, while the peak shapes may be used to measure the refractive index. In a typical TInAS experiment, an SiO₂-coated sensor is exposed on one side to a solution containing the desired adsorbate, and the optical thickness of the deposited film is measured in real time, with a sensitivity on the order of 1 ng/cm² (when converted to adsorbed density) and a temporal resolution below 1 s. All spectra were fitted using both the standard TInAS software and the TInAS-FIT software package. From these data, the film’s optical mass may be calculated using the de Feijter method.

TInAS sensors were produced by sputter coating flat, clean glass substrates with 25 nm Al, followed by 2-3 μm SiO₂, resulting in a surface with ~8 nm RMS roughness. Prior to use, these sensors were cleaned by sonicating twice in toluene, then twice in 2-propanol, each for five minutes. Afterwards, the sensors were exposed to O₂ plasma for two minutes (Plasma Cleaner/Sterilizer, PDC-32G instrument, Harrick, Ossining, NY, USA). After mounting the sensors in the flow cell, the baseline and adsorption measurements were performed in ultrapure water. A polymer concentration of 0.02 g/L was used for adsorption, with a continuous flow rate of 20 μL/min to replenish the solution without affecting adsorption kinetics.

6.3.3 Coarse-grained dextran simulations

The goal of the simulation was to determine the equilibrium conformation of the hydrated dextran brush at varying adsorbed densities. To this end, a coarse-grained force field was used to represent the dextran and water molecules. Unfortunately, due to the conformational constraints imposed by the triangular geometry of the coarse-grained dextran chains, equilibrated polymer conformations are difficult to achieve. Starting from straight
conformations, molecular dynamics simulations were performed, following an annealing procedure to equilibrate the system.

Molecular dynamics simulations in this study were performed in the NVT ensemble, with the equations of motion being integrated using the Verlet algorithm and a time step of 1.0 fs. All simulations were performed with LAMMPS. The system temperature was set at 300 K and controlled with the Nose-Hoover thermostat. The simulation floor and ceiling were bounded by Lennard-Jones walls, while the lateral dimensions were periodic. The simulation consisted of six chains, each 32 monomers long, with the anchoring monomers fixed in place 3.5 Å above the minimum of the simulation floor wall. The simulation box height was set to 100 Å for the sparser chains and 120 Å for the densest chains – values that were determined using trial runs to be sufficient to prevent the ceiling from playing a role in the brush equilibration. The lateral dimensions of the box were set by the desired chain density of the simulation. The dextran monomers were placed with random x and y coordinates, with the z-coordinate increasing regularly from the anchoring end at 3.5 Å up to a maximum value of 35 Å, 65 Å, or 95 Å, in order to sample different starting heights. Water molecules were randomly placed throughout the cell to achieve a density of one particle per 33 cubic Ångstroms. Energy minimization using the Polak-Ribiere conjugate gradient algorithm was first performed on these randomized positions to achieve a random, but more physically reasonable, initial condition, following which the annealing procedure was applied. The system was heated from 300 K to 500 K over 0.1 ns, then cooled back down to 300 K over another 0.1 ns, after which another energy minimization was performed. This procedure was repeated for 2-4 ns. After each energy minimization, the system energy and the average chain height were monitored to ensure that a steady state was reached within the first half of the total simulation time.

To validate this protocol, a separate series of simulations was performed to measure the R_g of a single dextran chain in explicit water. A single, unattached chain of 5 kDa or 35 kDa was simulated in a fully periodic box according to the same procedure as described above, at the end of which the R_g of the molecule was calculated. The results for both chain lengths were in excellent agreement with experimental measurements of dextran R_g.

6.3.4 ATR crystal preparation and brush attachment

ATR-IR spectra were measured on a Nicolet 5700 FTIR spectrometer equipped with a liquid-nitrogen-cooled MCT-A detector and multiple-internal-reflection accessory (Perkin-Elmer)
mounted with a liquid cell. 52x20x2mm 45° trapezoidal germanium ATR prisms (Crystran, Dorset, UK) were employed for the measurement of the ATR spectra of PLL-g-dextran and PLL-g-PEG adsorbed on the native oxide surface of the Ge crystal. Spectra of the adsorbed polymer were measured both in a dry and a wet state by referencing to a freshly plasma-cleaned Ge ATR crystal and a clean crystal in contact with ultrapure (MilliQ) water, respectively. Measurements at different relative humidities were performed using a custom-built, two-way flow system.

For the measurements of PLL-g-dextran, the crystal was cleaned by sonication in COBAS cleaner (Roche), followed by ultrapure water. Next, both sides of the crystal were exposed to O₂ plasma for one minute (Plasma Cleaner/Sterilizer, PDC-32G instrument, Harrick, Ossining, NY, USA). The thickness of the surface oxide layer was measured by ellipsometry (described below), following which the crystal was exposed to a 0.1 M solution of PLL-g-dextran for 30 minutes, and then rinsed with ultrapure water and dried under N₂. The final PLL-g-dextran thickness on the surface was then measured by ellipsometry.

Both the germanium oxide layer and PLL-g-dextran film thicknesses were measured in ambient air by ellipsometry using a M-2000 V spectroscopic ellipsometer (J.A. Woollam Co., Inc., Lincoln, NE, USA) in reflection at 70° between 370-800 nm. The variable-angle spectroscopic ellipsometry (VASE) spectra were analyzed using WVASE32 software. The thickness of PLL-g-dextran was obtained by fitting to a multilayer model, consisting of a bottom layer of germanium (using the built-in optical parameters), a layer of germanium oxide with refractive index of 1.65 and the thickness measured prior to adsorption, and a generalized Cauchy layer (A = 1.45, B = 0.01, C = 0).

6.4 Results and discussion

6.4.1 TInAS thickness and refractive index measurements of PLL-g-dex

TInAS sensors were exposed to an 0.02 M solution of PLL-g-dextran(5 kDa) in ultrapure water, and the spectra were measured in real time as the brush was formed. The spectra were then interpreted with the standard TInAS software, which measures the thickness of the top film assuming a refractive index of 1.45, as well as the TInAS-FIT software package, which measures both the thickness and the refractive index of the film using a different algorithm.
The two methods showed excellent agreement, reporting a maximum adsorbed mass of $306 \pm 15$ ng/cm$^2$. The TInAS-FIT thickness and refractive index values have a higher mutual uncertainty than their combined optical mass, and this uncertainty is reduced over the measurement with increasing film thickness.$^{26}$

The measurements indicated that after adsorption had slowed down to within the drift of the instrument, the hydrated brush had a mean thickness of $5.6 \pm 0.4$ nm and a refractive index of $1.41 \pm 0.2$. A representative measurement is shown in Figure 6.1, with the calculated optical mass of the same experiment shown in the Supporting Information. The final thickness of the film was achieved within the first minute of adsorption, with the refractive index increasing much more slowly, achieving its ultimate value only after approximately ten minutes.

![Figure 6.1](image)

**Figure 6.1** Simultaneous thickness (blue) and refractive index (red) measurements of PLL-g-dextran adsorption kinetics in the TInAS. The final film thickness is rapidly achieved, while the refractive index grows more gradually.

The TInAS measurements showed that the PLL-g-dextran brush reaches its maximum thickness very early in the adsorption curve, when the adsorbed chain density is still low. This implies that the chain height is not significantly influenced by the packing, and the dextran chains maintain their shape in solution instead of adopting a mushroom-like conformation in the absence of steric forces from neighboring chains to force them into an extended conformation. The ultimate adsorbed mass of $306$ ng/cm$^2$ corresponds to an average
inter-chain distance $L$ of 1.9 nm, or a brush parameter $L/2R_g$ of 0.5, taking the radius of gyration $R_g$ to also be 1.9 nm. Comparing this value to the simulation results below (Figure 6.2) shows a relatively sharp increase in chain stretching if this grafting density is exceeded, which would likely also correspond to significant steric hindrance to further polymer adsorption.

### 6.4.2 Simulations

Coarse-grained simulations of dextran brushes in explicit water were used to obtain brush heights at varying chain densities and with different initial simulation heights. Figure 6.2 shows these heights plotted against the chain grafting density and against $L/2R_g$, the inter-chain distance divided by the radius of gyration of 5 kDa dextran, taken to be 1.9 nm. Initial brush heights of 3 nm, 6 nm, and 9 nm are shown for each brush density. The brush height was defined as the $z$-coordinate bounding 90% of the dextran monomers. A visual representation of this is shown in the Supporting Information.

The dextran brush height is seen to increase with increasing chain density. The simulation initial conditions also have a statistically significant, but comparatively minor, influence on the brush height. Averaging the different initial conditions at each chain density yields a curve which is best fit by a power law with the scaling exponent of 0.234, much lower than the expected value of $1/3$ for a good solvent (significantly different with $>99\%$ confidence, as detailed in the Supporting Information). The simulations show an average thickness of ~5.8 nm at the maximum experimental brush density of $L/2R_g = 0.5$, in reasonably good agreement with the thickness measured in TInAS.
Figure 6.2 (A) Equilibrium dextran brush height vs. chain density, obtained from coarse-grained simulations using the modified M3B model. Starting brush heights of 3 nm (blue diamonds), 6 nm (red squares), and 9 nm (green triangles) were used. The brush height was defined as the z-coordinate bounding 90% of the dextran monomers. A power law best fit is shown (solid line) corresponding to a scaling exponent of 0.234, with a 95% confidence
interval (dotted lines), corresponding to an exponent range of 0.175-0.283. (B) The same data plotted against L/2R_g.

Density profiles of the dextran monomers as well as the surrounding water were obtained from the simulations. A representative plot is shown in Figure 6.3. In every case, the water concentration increased and the dextran density decreased with distance from the surface, aside from binning artifacts. The binning interval used for all plots was 0.4 nm. The dextran density profiles of each brush density, averaged over all initial conditions, are shown in Figure 6.4. The density profiles of the sparser brushes are typical for moderately stretched brushes,\textsuperscript{32-34} while the densest brushes exhibit more slab-like conformations.

![Figure 6.3](image-url)

**Figure 6.3** Representative density profiles of a dextran brush (solid line) and the surrounding water (dashed line), showing an increase in water concentration with decreasing dextran concentration.
The simulation results also show a relatively weak relationship between the inter-chain spacing and the equilibrium brush height. Theoretical and experimental studies show that brushes in good solvents tend to grow proportionally to the cube root of the chain density in a good solvent, and to higher powers with lesser solvent quality or with higher brush densities, due to the increased interaction between monomers. In contrast, the best fit to the simulation results has an exponent of only 0.24, and a fit with an exponent of 0.3 or higher is excluded at the 99% confidence level. One possible cause of a weak dependence of dextran brush height on chain density is chain stiffness. SAXS measurements showed that dextran has a persistence length of over 2.75 nm. The ATR-IR spectroscopic data also shows that the PEG chains of the PLL-g-PEG adsorbed on the surface are more sensitive to the presence of water molecules in the surrounding medium (both in vapor phase or in liquid) than the corresponding dextran chains in PLL-g-dextran adsorbed on the surface, as further described below. It should be noted that studies of the scaling behavior of PEG brushes did not mention any discrepancies from theory.
Another possible factor contributing to the relatively constant conformation of dextran brushes is inter-chain hydrogen bonding. The combination of inter-chain hydrogen bonding and chain stiffness can cause the chains to cluster and extend further than the steric hindrance of crowding alone would cause. Dextran brushes have been shown to resist collapse and spreading under applied load and shear, while interpenetration of opposing dextran brushes under shear leads to extremely high friction due to hydrogen bonding between the opposing chains.\(^{40}\) The compressibility and shear moduli of 5 kDa dextran brushes in aqueous solutions are significantly higher than those of 5 kDa PEG brushes as measured in SFA\(^{41}\) and QCM,\(^{42}\) respectively, despite the higher solubility of dextran than PEG in water. Hydrogen bonding also causes short-chain dextran to form insoluble agglomerates in solution, which are resistant even to aggressive sonication and heating.\(^{16-18}\)

### 6.4.3 ATR-IR

VASE measurements of PLL-g-dextran adsorbed onto the Ge ATR crystal yielded an optical layer thickness of 0.74 ± 0.23 nm. The lower thickness compared to PLL-g-PEG(2) in other studies (e.g. 2 ± 0.28 nm on Nb\(_2\)O\(_3\))\(^{43}\) can be due to the lower charge density or the lower stability of germanium oxide, compared to silica or niobia, which can impact adsorption of the PLL moieties, as well as possibly changing in optical thickness during the adsorption process compared to the prior baseline measurement. This variability was acceptable given that the aim of this work was to qualitatively understand the differences between PEG and dextran behavior, rather than to quantitatively compare PEG or dextran spectra under different conditions.

Figures 6.5 and 6.6 display representative ATR-IR spectra of PLL-g-dextran and PLL-g-PEG adsorbed on the native oxide layer of a Ge ATR crystal, respectively. Characteristic bands of the dextran moieties are clearly distinguishable in the spectra in Figure 6.5. A comparison of the powder spectrum of dextran and that of PLL-g-dextran adsorbed on Ge over the entire spectral range (4000-900 cm\(^{-1}\)) is given in the supporting information.

The spectra in Figures 6.5 and 6.6 exhibit two sets of peaks that are readily assignable to CH\(_2\) bending modes and C-O, C-C stretching modes. The peaks due to the CH\(_2\) bending modes are not well resolved in the spectra of dextran, in comparison to PEG. However, the C-O-C stretching region of dextran exhibits several well-resolved peaks in contrast to that of PEG. This qualitative difference in spectra between PEG and dextran is understandable considering
the several distinct C-O, C-C linkages of the sugar units compared to PEG with only one type of C-C and C-O bonds.

**Figure 6.5** ATR-IR spectra of PLL-g-dextran adsorbed onto a native oxide layer of Ge ATR crystal measured dry, humid and immersed in water. C-O-C stretching bands (combination of C-O and C-C stretching modes) characteristic of different positions within the dextran molecule are clearly distinguishable.
Figure 6.6 ATR-IR spectra of PLL-g-PEG adsorbed onto a native oxide layer of Ge ATR crystal measured dry, humid and immersed in water. CH$_2$ bending modes and C-O-C stretching bands (combination of C-O and C-C stretching modes) characteristic of the PEG chains are clearly distinguishable.

Apart from the qualitative differences in the spectra of dextran and PEG, a comparison of the spectra of the adsorbed polymers measured in dry, humid and immersed in water reveal some further details. The spectra of PLL-g-dextran measured under dry (red) and humid (blue) conditions are essentially identical. No change in either the positions or the intensities of the C-O-C stretching bands are observable. The spectrum of adsorbed PLL-g-dextran measured in water also shows a few minor changes. The C-O-C stretching band at around 1018 cm$^{-1}$ in the dry/humid condition is shifted to 1014 cm$^{-1}$ in the spectrum measured in water. The intensity of this band, relative to the band at 1040 cm$^{-1}$, increases in the wet condition in comparison to the dry/humid state. These changes in the spectral intensity and positions are most likely due to the conformational changes happening to the dextran chains upon
hydration. However, the spectra of PLL-g-PEG measured under similar conditions exhibit more significant changes in the positions of the conformationally sensitive C-O-C stretching bands.

In the case of PLL-g-PEG (Figure 6.6), appreciable changes in the position of the C-O-C stretching bands are already visible between the dry and humid conditions. The C-O-C stretching band at 1103 cm\(^{-1}\) in the dry state (red) shifts to 1095 cm\(^{-1}\) (blue) under humid (RH90\%) conditions and further to 1087 cm\(^{-1}\) (black) in the spectrum measured in water. These results are in general agreement with the previously published results on oligo- or poly-ethylene glycol self-assembled monolayers (SAM). For example, Skoda et al. have observed shifts of the order of 17 cm\(^{-1}\) in the position of C-O-C stretching bands for oligo-(ethylene glycol) terminated alkanethiol SAMs on gold, measured in air and in water by means of polarization-modulation IR spectroscopy.\(^{44}\) These changes in peak positions are related to the penetration of water molecules into the adsorbed polymer and the hydrogen bonding of the water molecules to the ether linkages, causing the vibrational frequencies to shift to lower wavenumbers.

### 6.5 Conclusions

The hydrated conformation of dextran and PEG brushes were investigated by means of optical-thickness measurements, computer simulations, and ATR-IR measurements. The IR results showed that PEG conformation is influenced by the presence of water, with the C-O-C stretching bands shifting in position between dry, humid, and wet conditions, unlike dextran, which exhibits no major band shifts. The optical measurements and computer simulations suggested that the dextran-brush conformation is instead strongly influenced by inter-chain interactions. Optical measurements showed that brush height was largely independent of grafting density over the experimental range, while the simulation results, which reached higher grafting densities, showed a smaller increase in height than predicted by the scaling law for ideal brushes in good solvent. These findings are in agreement with literature regarding the presence of an equilibrium hydrated structure for PEG brushes, which if partially dried induces measurable conformation changes in the PEG chains, as well as the significant inter-chain hydrogen bonding in dextran brushes, which is responsible for their characteristic resistance to collapse under applied load and shear.
6.6 Supporting information

**Figure 6.7** Optical mass calculated from the simultaneous thickness and refractive index measurements shown in Figure 6.1, using the de Feijter equation shown below. For dextran in water, the refractive index increment $dn/dc$ is taken to be 0.15 cm$^3$/g.

$$Mass = \frac{n_{adsorbate} - n_{medium}}{dn/dc} \cdot Thickness$$
**Figure 6.8** Equilibrium dextran brush density profile for inter-chain spacings of 1 nm (blue), 1.5 nm (red), 2 nm (green), 3 nm (purple), and 4 nm (orange). The dotted lines indicate the $z$-coordinate bounding the lower 90% of dextran monomers, which is taken as the brush height for the purpose of this study.

**Simulation statistics**

Statistical tests were performed to ascertain whether the exponent of a power-law fit differed significantly from the hypothetical value of $1/3$, separately for each set of initial conditions (starting heights of 3, 6, or 9 nm), taking into account the standard error of each measurement as shown in Figure 6.4. The results are shown in Table 6.1.

<table>
<thead>
<tr>
<th>Initial Height, nm</th>
<th>Scaling Exponent Best Fit</th>
<th>99% Confidence Interval</th>
<th>$R^2$</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>0.2416</td>
<td>0.1850 to 0.2982</td>
<td>0.8665</td>
<td>0.0002</td>
</tr>
<tr>
<td>6</td>
<td>0.2463</td>
<td>0.1997 to 0.2928</td>
<td>0.9093</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>9</td>
<td>0.2144</td>
<td>0.1611 to 0.2677</td>
<td>0.863</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

**Table 6.1** Statistical tests of the scaling exponent of the data shown in Figure 6.2.

**Figure 6.9** Representative IR spectra comparing the spectra of pure dextran (black), PLL-g-dextran (red) measured as KBr pellets along with that of the PLL-g-dextran adsorbed onto a
Ge ATR crystal surface (blue). The spectra in the fingerprint region, sensitive to the structural changes of the dextran chains (1350-950 cm\(^{-1}\)), are considered in detail in Figure 6.5.

### 6.7 References


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This project was motivated by the observation of ultralow friction and wear in biological tribosystems, and in hydrophilic polymer brushes under aqueous conditions. The scope of the thesis was to understand the impact of incorporating sugar-bearing chains into synthetic brushes and thin films, thereby mimicking some of the mechanisms of natural lubrication.

In the first part of the project, surface-density gradients of dextran-bearing bottlebrush copolymers were fabricated and the friction response measured using colloidal-probe atomic force microscopy in aqueous solution. The results showed that the lubricity of dextran brushes varies over several orders of magnitude and is dependent on both the chain density and the applied load, with a characteristic transition between ultra-low- and high-friction regimes manifesting above a certain applied load, which was also found to depend on chain density. The explanation for this phenomenon was that inter-chain hydrogen bonding lent the polysaccharide brush resistance against collapse, yet exacerbated friction forces upon collapse and subsequent dragging on the counterface or interpenetration with a brush on the opposing surface. This behavior is comparable to that of neutral brushes of sufficient length and flexibility to entangle with the opposing brush. While the transition to high friction under higher applied loads would seem detrimental to natural tribosystems or their future biomimetic counterparts, the transition to the high friction regime occurs at much higher pressures than are encountered in natural tribosystems, such as joints. Applications of related materials could either avoid these high loads, or else use them to deliberately switch to high-friction behavior at a certain applied load, which is tunable via the chain grafting density.

Upon discovery of this behavior, a new set of aqueous friction experiments on dextran brushes was performed with kosmotropic additives in solution, in order to measure the effect of a reduction in the strength of the inter-chain hydrogen bonding. The addition of moderate amounts of potassium sulfate and trehalose in solution was found to shift the friction transition to lower applied loads, while having less effect on the friction at low loads than a corresponding reduction in brush density. This illustrated the relative contributions of inter-chain hydrogen bonding and steric forces to the lubricity of the brush under different load regimes. Since biological tribosystems of saccharide-bearing, brush-like molecules usually operate in the presence of much higher salt concentrations, it is suggested that the presence of other species that enhance the hydrogen bonding, or directly cross-link the chains, must serve
to offset the failure of inter-chain hydrogen bonding under those conditions. Similar techniques could be used to extend the lubricious regime of the dextran brush to higher loads, or to selectively inhibit the resistance to collapse of the film in order to create a heterogeneous film.

These findings led to a new line of investigation, to determine whether the thickness of the layer and the brush conformation of the chains were influencing the hydrogen bonding, and thereby the characteristic friction response of the system. To that end, a different set of dextran-based brush-like films with higher chain densities, but less well-defined structure, was synthesized and characterized. Dextran chains were attached at random intervals to the surface by means of an intermediate UV-activated azide-bearing monolayer, and a moving shutter was used to produce dextran surface density gradients. The results confirmed that the brush conformation has a unique effect on friction, with the disordered chains displaying less extreme friction behavior, exhibiting lower friction with increasing chain density but also showing much weaker resistance to collapse on a per-monomer basis than the brush. For the randomly attached system, significantly higher surface concentrations of dextran were necessary to reach the same critical load (marking the transition from low to high friction regimes) as in the original dextran brush, suggesting that the ordering of the polymer-brush architecture significantly contributed the load-bearing properties of the dextran film. Increasing the density of the disordered dextran film reduced the friction coefficient at high loads, while the opposite was true for the polymer brush. Future brush architectures should therefore aim to increase the film thickness and chain density without sacrificing the chain ordering associated with the relatively uniform end-attached brushes – although this is a task easier said than done.

Since the brush conformation was seen to play such an important role in friction compared to less ordered hydrogen-bonding systems, the next goal was to understand the hydrated conformation of the dextran chains. Computer simulations of coarse-grained dextran in explicit water were used to study the equilibrium conformation of the brush, while optical kinetics measurements were used to monitor the change in height and brush density during the adsorption process. These experiments showed that the stiffness of dextran chains, combined with the hydrogen bonding between chains, results in more extended brush conformation than predicted by the scaling law for ideal brushes in good solvent. To compare this behavior to the influence of hydration on chain conformation, the variation of PEG and dextran brush structure under dry, humid and wet conditions was also studied via ATR-IR.
Significant shifts in the C-O-C stretching bands were observed in PEG brushes with changing humidity, indicative of conformational changes due to the presence of water. In contrast, no corresponding shifts were observed in dextran, showing that the presence of water is secondary to the inter-chain hydrogen bonding achieved by ordered chains in determining brush structure, a factor which might explain the resistance to applied load exhibited by the ordered brush in comparison to the disordered chains.

The findings of this work paint an interesting picture of polysaccharide brush lubrication. The high availability, low toxicity and potential for extensive functionalization of polysaccharides makes them exciting candidates for various industrial applications. The potential of achieving ultra-low friction comparable to the best polymer brushes is present, but overloading the polysaccharides, adding the wrong solutes or producing less regular brushes can quickly change the situation for the worse. This implies that the use of comparable lubrication mechanisms in nature must avoid or mitigate these potential pitfalls, for example by limiting the operating pressure of tribosystems such as joints, exerting fine control over glycan structure, stiffness and interactions, and/or physical or chemical crosslinking to strengthen inter-chain interactions against the constant presence of high salt and other solute concentrations. These insights will hopefully guide future studies of natural biolubrication, as well as assist in the development of synthetic biomimetic lubricants.

Future work on heterogeneous architectures may allow for tailored films that exhibit the appropriate mixture of properties, depending on the expected loading of the film. For example, partial chemical crosslinking of dextran chains, using diisocyanates or another active crosslinker, may lend increased load-bearing properties to the brush. It is possible that even a small fraction of crosslinked chains might lead to a significant improvement in resistance to spreading and interpenetration without reducing the hydration and lubricity of the film, just as elastomers use a deliberately light degree of crosslinking to achieve their unique properties. This method might also increase the brush resistance to collapse in the presence of kosmotropes.

Brushes with heterogeneous chain compositions might also be synthesized using a grafting-from method, in which parts of the chains are composed of hydrogen-bonding monomers such as polyols, while other regions consist of a different monomer designed to bind water without directly hydrogen bonding, such as PEG. Such a system might exhibit low friction
even under high applied loads, and the “dual-action” mode of hydration and lubrication may also reduce the friction in the high-load regime, for example in the presence of kosmotropes.

Ultimately, it is possible to imagine surfaces with heterogeneous composition, bearing polysaccharide chains of different length, stiffness, degrees of crosslinking, and hydration. They could be capable of either sliding past or trapping particles or biomolecules, perhaps with some cyclodextrin-like side groups, or of achieving a tailored level of friction with different counterfaces, sometimes entangling like Velcro and sometimes sliding like Crisco, in response to the functionality of the opposing surface. While such surfaces are still far from becoming a reality from a synthetic perspective, they have long been commonplace in the eyes of Nature.