



# Binding Specificities of Nanobody•Membrane Protein Complexes Obtained from Chemical Cross-Linking and High-Mass MALDI Mass Spectrometry

## Journal Article

### Author(s):

Köhler, Martin; Neff, Christoph; Perez, Camilo; Brunner, Cyrill; Pardon, Els; Steyaert, Jan; [Schneider, Gisbert](#) ; Locher, Kaspar P.; [Zenobi, Renato](#) 

### Publication date:

2018-04-17

### Permanent link:

<https://doi.org/10.3929/ethz-b-000255257>

### Rights / license:

[In Copyright - Non-Commercial Use Permitted](#)

### Originally published in:

Analytical Chemistry 90(08), <https://doi.org/10.1021/acs.analchem.8b00236>

### Funding acknowledgement:

166672 - Structural and mechanistic studies of components of bacterial protein N-glycosylation pathway and of vitamin B12 transport (SNF)

## SUPPORTING INFORMATION

### BINDING SPECIFICITIES OF NANOBODY•MEMBRANE PROTEIN COMPLEXES OBTAINED FROM CHEMICAL CROSS-LINKING AND HIGH-MASS MALDI MASS SPECTROMETRY

Martin Köhler,<sup>†</sup> Christoph Neff,<sup>†</sup> Camilo Perez,<sup>‡,§</sup> Cyrill Brunner,<sup>†</sup> Els Pardon,<sup>#,¶</sup> Jan Steyaert,<sup>#,¶</sup> Gisbert Schneider,<sup>†</sup> Kaspar P. Locher,<sup>‡</sup> and Renato Zenobi <sup>\*,†</sup>

<sup>†</sup>Department of Chemistry and Applied Biosciences, ETH Zürich, Vladimir-Prelog-Weg 3, 8093 Zurich, Switzerland

<sup>‡</sup>Institute of Molecular Biology and Biophysics, ETH Zürich, Otto-Stern-Weg 5, 8093 Zurich, Switzerland

<sup>#</sup>VIB-VUB Center for Structural Biology, VIB, Pleinlaan 2, 1050 Brussels, Belgium

<sup>¶</sup>Structural Biology Brussels, Vrije Universiteit Brussel, Pleinlaan 2, 1050 Brussels, Belgium

\*Correspondence and requests for materials should be addressed to R.Z. (email: zenobi@org.chem.ethz.ch). Tel.: +41 44 632 43 76. Fax: +41 44 632 2 92

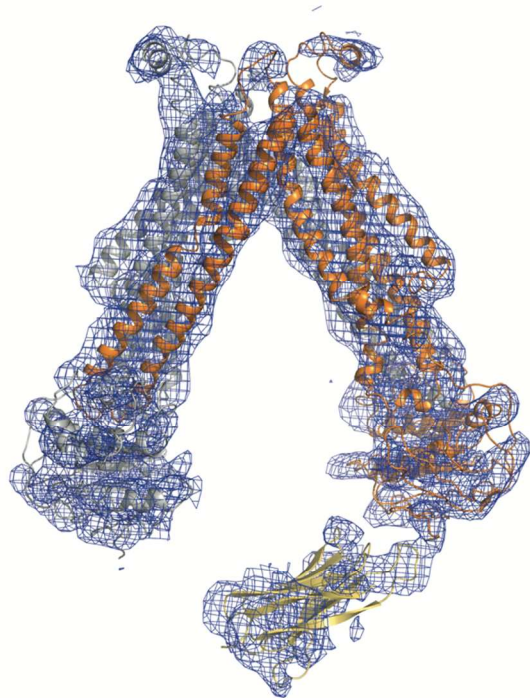
### TABLE OF CONTENTS

Supplementary Figure 1: 2Fo-Fc electron density map of PglK-E510Q•Nb93 complex

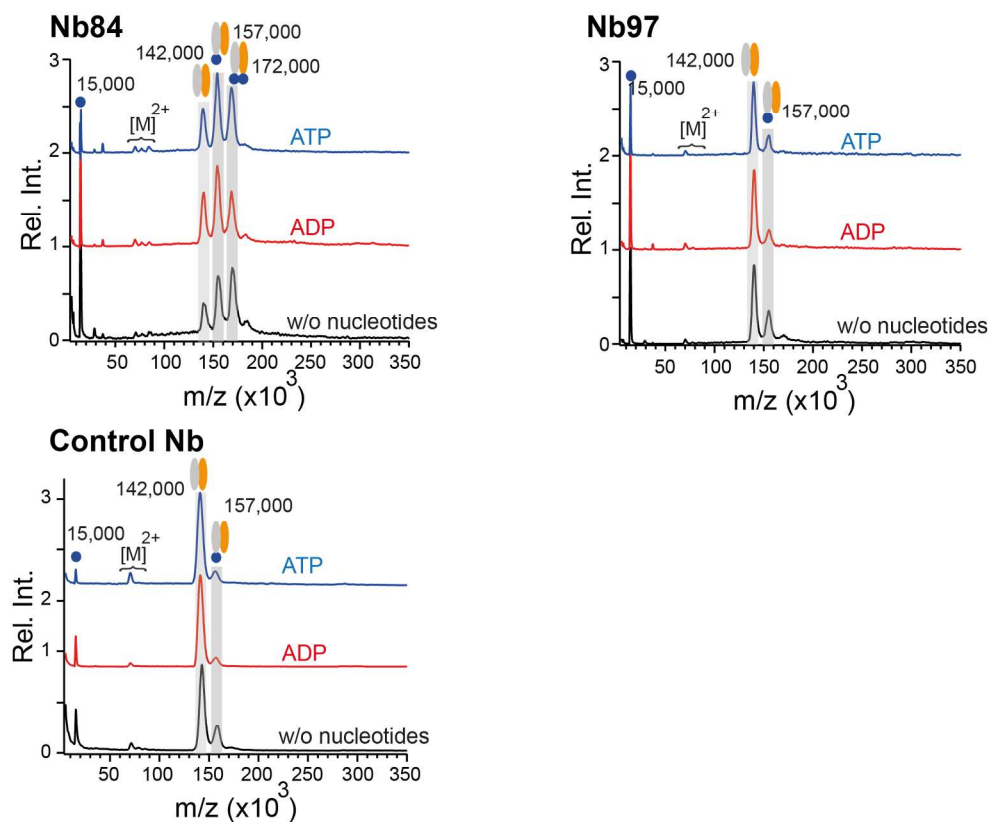
Supplementary Figure 2: High-mass MALDI MS spectra of Nbs and PglK in presence of nucleotides

Supplementary Table 1: Binding affinities of the Nbs investigated

Supplementary Table 2: Fitting parameters for MST experiment



**Supplementary Figure 1.** 2Fo-Fc electron density map of PglK-E510Q•Nb93 complex



**Supplementary Figure 2.** The complex formation of Nb84, 97 and control Nb were observed via MALDI-MS in presence of ADP or ATP, respectively (ADP red trace, ATP blue trace) and compared without nucleotides (black trace). No change in complex formation could be observed. Control Nb spectra were reproduced from Perez et al.<sup>5</sup>(reprinted by permission from Macmillan Publishers Ltd: SCIENTIFIC REPORTS, Perez, C.; Köhler, M.; Janser, D.; Pardon, E.; Steyaert, J.; Zenobi, R.; Locher, K. P., Scientific Reports 2017, 7, 46641. Copyright 2017).

**Supplementary Table 1. Binding affinities, signal-to-noise ratio and root mean square error of reported nanobodies against fluorescently labeled PglK. (-) indicates no fitting detected.**

| Nanobody   | Binding affinity $K_D$ [ $\mu$ M] | Signal-to-noise ratio | RSME   |
|------------|-----------------------------------|-----------------------|--------|
| Control Nb | -                                 | -                     | -      |
| Nb67       | -                                 | -                     | -      |
| Nb84       | $0.5 \pm 0.2$                     | 6.34                  | 263.58 |
| Nb87       | $1.0 \pm 0.5$                     | 8.54                  | 179.13 |
| Nb93       | $1.8 \pm 1.0$                     | 5.64                  | 324.94 |

**Supplementary Table 2. Fitting parameters for MST-experiment. (-) indicates no fitting detected.**

| Fitting parameter | Control Nb | Nb67   | Nb84   | Nb87   | Nb93   | Nb97   |
|-------------------|------------|--------|--------|--------|--------|--------|
| Bound             | -          | -      | 7634.5 | 6450.1 | 7429.8 | 5918.8 |
| Unbound           | -          | -      | 6074.2 | 5029.2 | 5749.2 | 4288.3 |
| $K_D$ [ $\mu$ M]  | -          | -      | 0.5    | 1.0    | 1.8    | 138.9  |
| $c_{tar}$         | 500 pM     | 500 pM | 500 pM | 500 pM | 500 pM | 500 pM |