



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

## Journal Article

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## SUPPORTING INFORMATION

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

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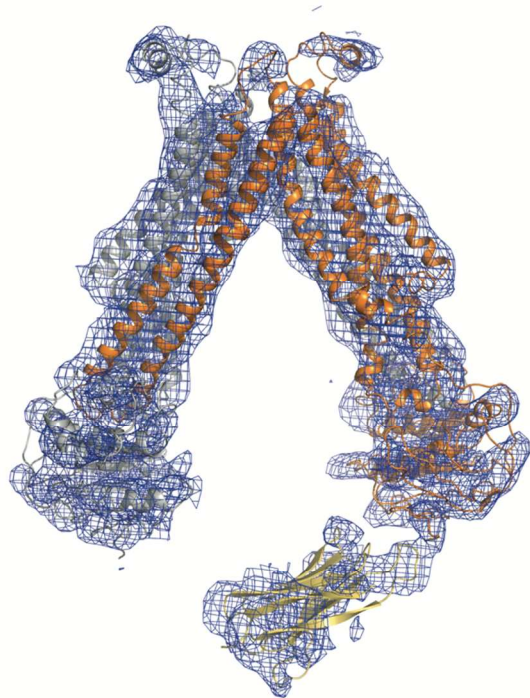
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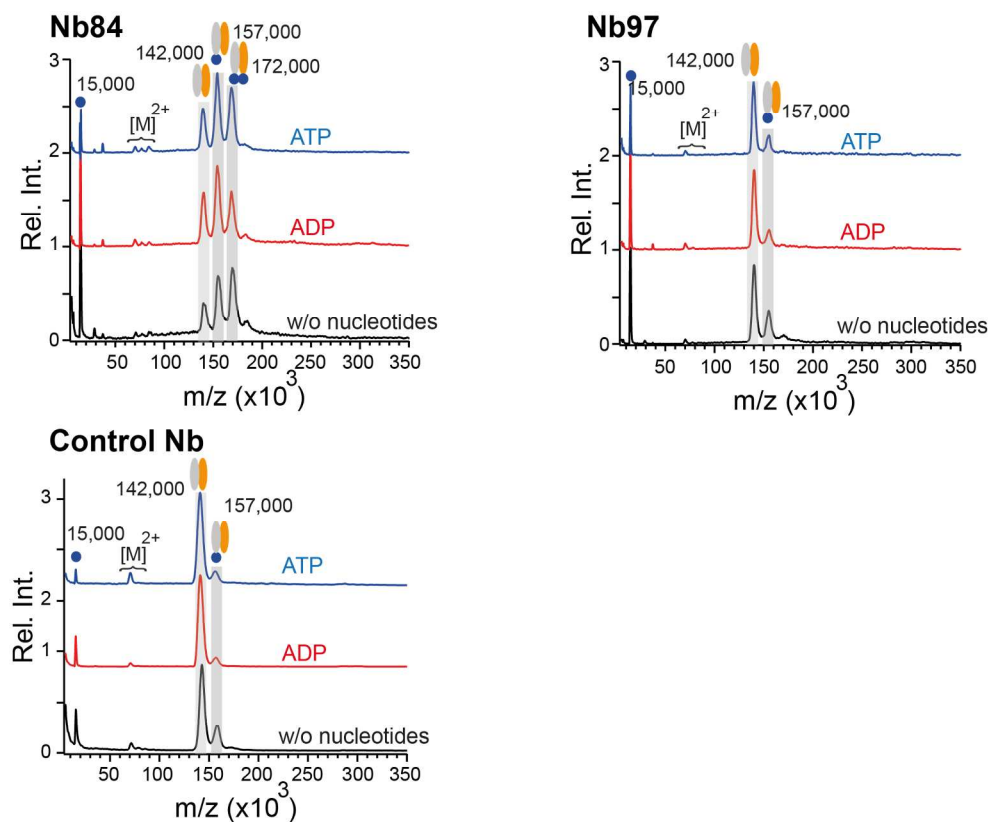
Supplementary Figure 2: High-mass MALDI MS spectra of Nbs and PglK in presence of nucleotides

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