Supporting information

# High-Affinity Binding of Monomeric but Not Oligomeric Amyloid-β to Ganglioside GM1 Containing Nanodiscs

*Maren Thomaier*[1,2], *Lothar Gremer*[1,2], *Christina Dammers*[1], *Judith Fabig*[1], *Philipp Neudecker*[1,2], *Dieter Willbold*[1,2]*

[1]Institute of Complex Systems (ICS-6), Structural Biochemistry, Forschungszentrum Jülich, 52425, Jülich, Germany.

[2]Institut für Physikalische Biologie, Heinrich-Heine-Universität Düsseldorf, 40225, Düsseldorf, Germany.

**Table S1.** Amino acid sequence of biotinylated Aβ fragments and biotinylated myc peptide.

| Peptide | Amino acid sequence | Corresponding amino acids of the Aβ sequence |
|---|---|---|
| **Aβ(1-15)** | Biotin-DAEFRHDSGYEVHHQ | 1-15 |
| **Aβ(6-20)** | Biotin-HDSGYEVHHQKLVFF | 6-20 |
| **Aβ(11-25)** | Biotin-EVHHQKLVFFAEDVG | 11-25 |
| **Aβ(16-30)** | Biotin-KLVFFAEDVGSNKGA | 16-30 |
| **Aβ(21-35)** | Biotin-AEDVGSNKGAIIGLM | 21-35 |
| **Aβ(26-40)** | Biotin-SNKGAIIGLMVGGVV | 26-40 |
| **Aβ(28-42)** | Biotin-KGAIIGLMVGGVVIA | 28-42 |
| **Myc** | GEQKLISEEDLGK-Biotin | - |

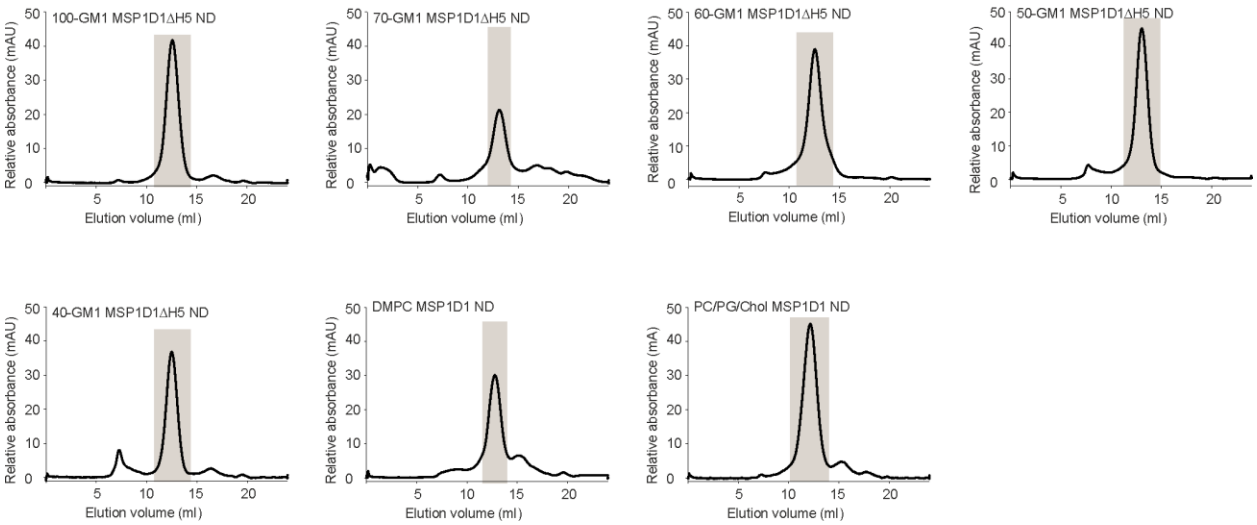


**Figure S1**. Size exclusion chromatograms of seven different nanodiscs (NDs). Details of ND compositions are given in table 2 of the main text. The chromatography was performed on a Superdex200 100/300 GL column with a flow rate of 0.5 ml/min. Shown is the recorded relative absorbance at a wavelength of 280 nm. The void volume of the column is 8 ml corresponding to the first peak containing high molecular weight aggregates (*1*). NDs elute in the second peak with an elution volume of 12.3 to 12.6 ml (boxed in grey). Free MSP1D1 eluted at 15 ml, free MSP1D1ΔH5 eluted at 16 ml and other residual impurities from the MSP purification eluted between 17 and 20 ml.

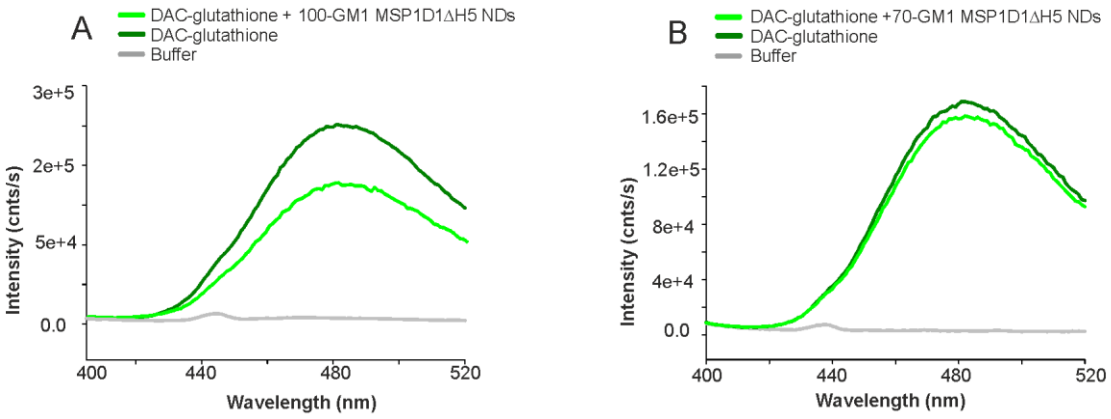


**Figure S2**. Analysis of DAC-glutathione binding to NDs by fluorescence spectroscopy. The absence of any fluorescence enhancement of DAC-glutathione upon ND addition indicated the absence of any interaction. The reduction of the fluorescence intensity in A results from dilution effects. Emission spectra (400 to 520 nm) were recorded with an excitation wavelength of 380 nm at 37 °C. Grey: emission spectrum of buffer (30 mM $KH_2PO_4/K_2HPO_4$, pH 7.2, 0.5 mM EDTA); dark green: emission spectrum of DAC-glutathione; light green: emission spectrum of the highest concentration (175 nM) of 100-GM1 MSP1D1ΔH5 NDs (A) or of the highest concentration (203 nM) of 70-GM1 MSP1D1ΔH5 NDs added to DAC-glutathione (B).

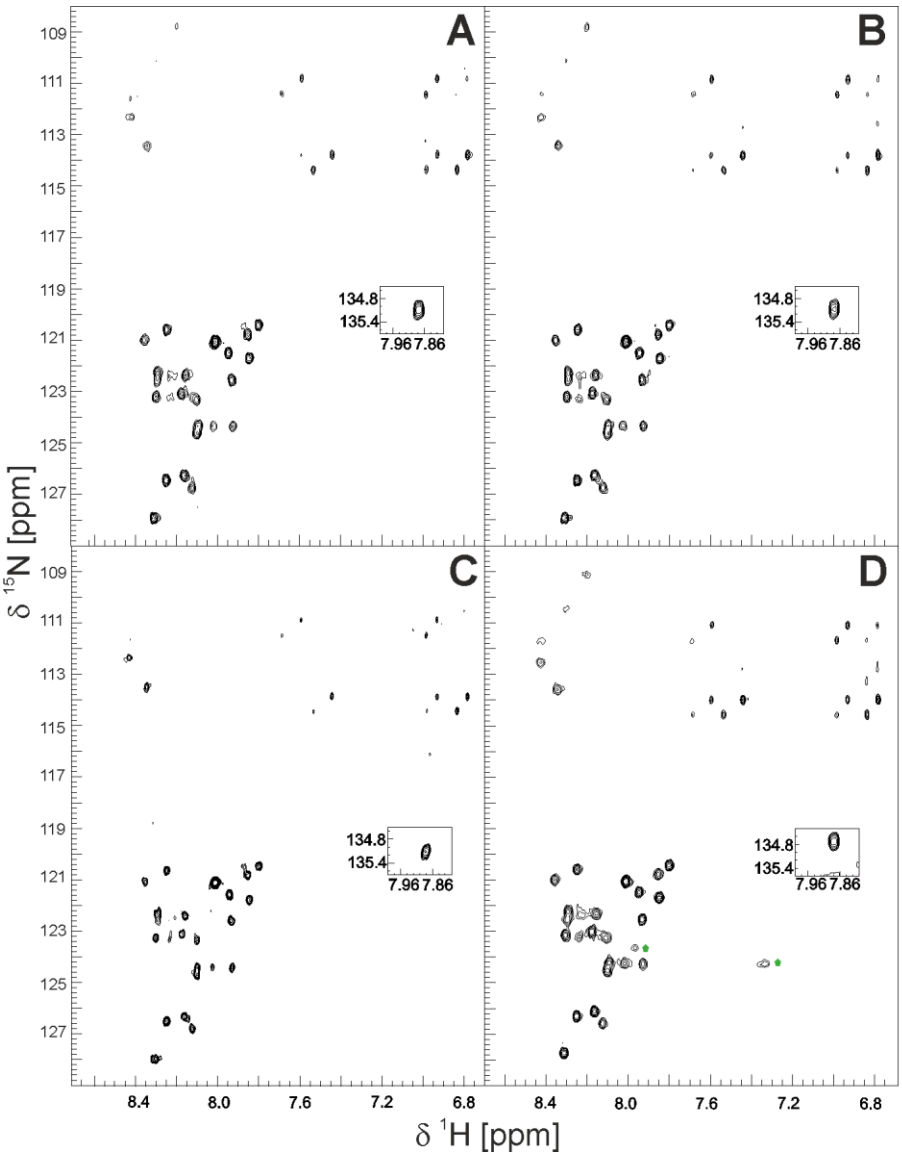


**Figure S3.** 2D-[$^{1}$H,$^{15}$N]-TROSY (*2*) spectra of [U-$^{15}$N]-Aβ(1-42) in the absence or presence of NDs. All four spectra were recorded at a magnetic field corresponding to a proton resonance frequency of 600 MHz at 25 °C in 50 mM NaCl, 10 mM $NaH_2PO_4/Na_2HPO_4$, pH 7.4. A) 25 μM [U-$^{15}$N]-Aβ(1-42) B) 50 μM [U-$^{15}$N]-Aβ(1-42) in the presence of DMPC MSP1D1 NDs (28 μM) C) 50 μM [*U*-$^{15}$N]-Aβ(1-42) in the presence of PC/PG/Chol MSP1D1 NDs (50 μM) D) 56 μM [*U*-$^{15}$N]-Aβ(1-42) in the presence of 70-GM1 MSP1D1 NDs (62 μM). Green pentagons mark the peaks originating from ganglioside GM1.

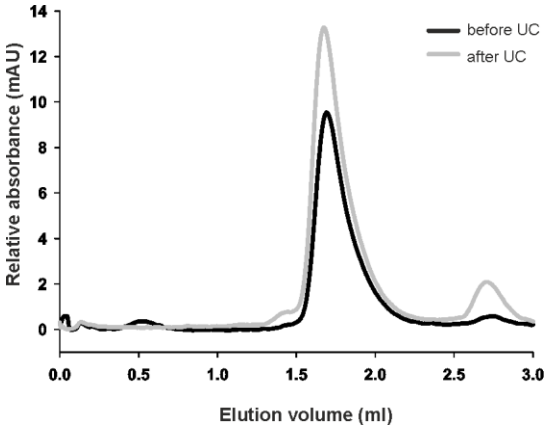


**Figure S4.** Analytical size exclusion chromatography of NDs. Size exclusion chromatograms of 100-GM1 MSP1D1ΔH5 NDs with bound Aβ(1-42) before (black) and after (grey) concentration by ultracentrifugation are shown. The chromatography was performed on a Superdex 200 Increase 5/15 column with a flow rate of 0.3 ml/min (30 mM $KH_2PO_4/K_2HPO_4$, pH 7.2, 0.5 mM EDTA). Shown is the recorded relative absorbance at a wavelength of 280 nm. NDs eluted at an elution volume of 1.65 ml.

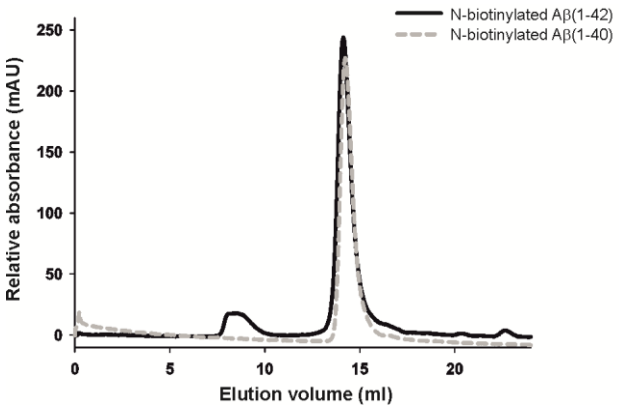


**Figure S5.** Preparation of monomeric N-biotinylated Aβ(1-40) and Aβ(1-42) by size exclusion chromatography. The chromatography was performed on a Superdex 75 100/300 GL column with a flow rate of 0.5 ml/min (30 mM $KH_2PO_4/K_2HPO_4$, pH 7.2, 0.5 mM EDTA). Shown is the recorded relative absorbance at a wavelength of 214 nm. The void volume of the column is 8 ml corresponding to the first peak visible in the N-terminal biotinylated Aβ(1-42) sample (black curve) and contains aggregated Aβ(1-42). Monomeric N-terminal biotinylated Aβ eluted in the second peak at an elution volume of 14 ml.

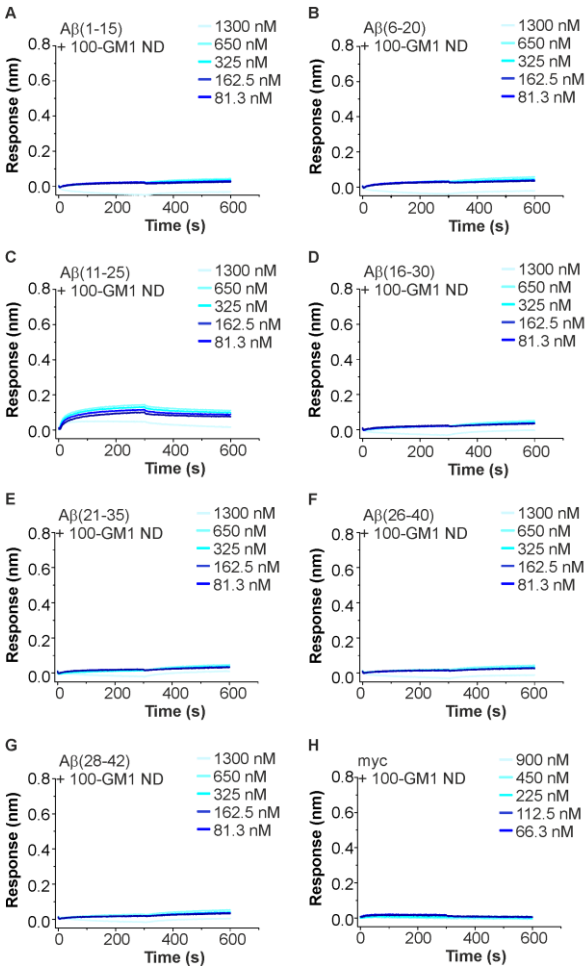


**Figure S6.** Biolayer interferometry sensorgrams testing 100-GM1 ND-Aβ fragment interactions (A-G) and 100-GM1 ND-myc interactions (H). Biotinylated peptides were coupled to streptavidin sensor tip surfaces used for BLI. 100-GM1 NDs in the indicated concentrations were used as analytes. The given sensorgrams (showing association and dissociation phases of 300 s) are representative examples from at least two independent experiments. Amino acid sequences of the Aβ fragments and myc are given in Table S1.

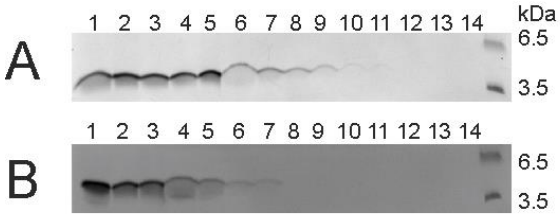


**Figure S7.** Analysis of Aβ(1-42) (A) and Aβ(1-40) (B) aggregate size distributions derived by density gradient centrifugation (DGC). The mixtures of oligomeric Aβ species (*3*) were prepared by incubation of monomeric N-terminal biotinylated Aβ and monomeric non-biotinylated Aβ in a molar ratio of 1:10, with subsequent separation by DGC and fractionation. DGC fractions were analysed for Aβ by Tris-Tricine SDS-PAGE and silver staining.

1. Bayburt, T. H., and Sligar, S. G. (2010) Membrane protein assembly into Nanodiscs, *FEBS letters 584*, 1721-1727.
2. Nietlispach, D. (2005) Suppression of anti-TROSY lines in a sensitivity enhanced gradient selection TROSY scheme, *Journal of biomolecular NMR 31*, 161-166.
3. Brener, O., Dunkelmann, T., Gremer, L., van Groen, T., Mirecka, E. A., Kadish, I., Willuweit, A., Kutzsche, J., Jurgens, D., Rudolph, S., Tusche, M., Bongen, P., Pietruszka, J., Oesterhelt, F., Langen, K. J., Demuth, H. U., Janssen, A., Hoyer, W., Funke, S. A., Nagel-Steger, L., and Willbold, D. (2015) QIAD assay for quantitating a compound's efficacy in elimination of toxic Abeta oligomers, *Scientific reports 5*, 13222.