Supplemental Material
for

A Cleavable Ligand Column for the Rapid Isolation of Large Quantities of Homogeneous and Functional Neurotensin Receptor 1 Variants from *E. coli*

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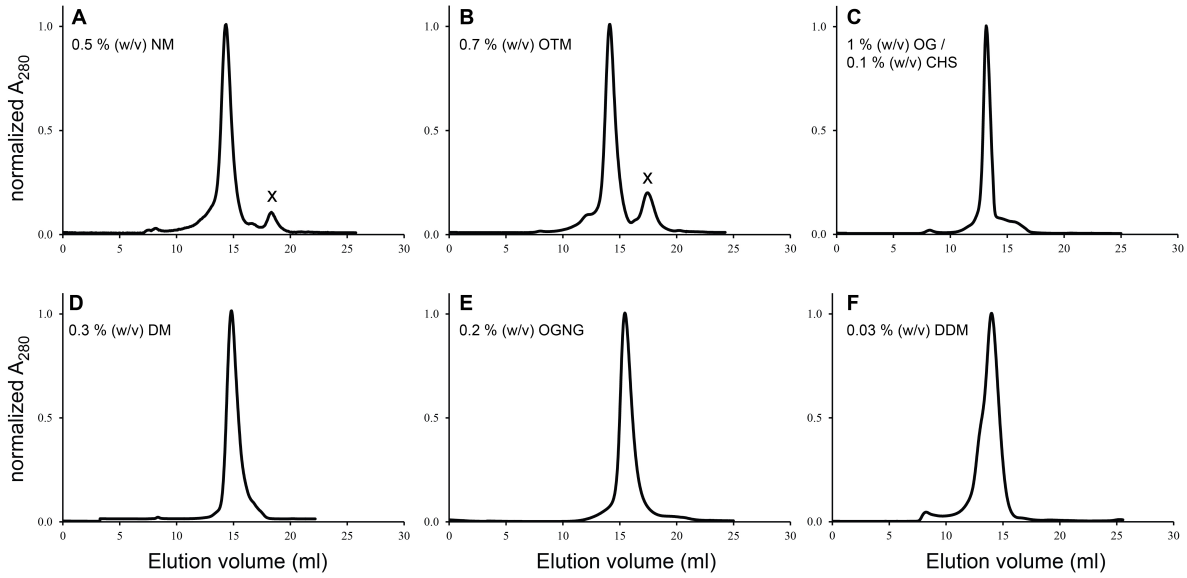

Fig. S1. Compilation of semi-quantitative SEC runs (S200 10/300 GL) using various detergents. This figure may guide future in vitro experiments (e.g. optimization of NMR spectra), as it provides an overview of detergent conditions that allow reasonably homogeneous preparations of evolved NTR1 variants. The chromatograms represent three different receptor variants, which were purified for various purposes (purification optimization, crystallography, NMR, MALS). All shown chromatograms represent purification procedures that were carried out using the agonist-complex purification strategy described in the main text. The exchange from DM to a detergent of choice was performed on the pD-NT ligand column and the detergent was kept constant in all subsequent buffers. Since the illustrated receptor preparations were not performed in parallel and on different FPLC systems, no conclusions from small differences in running behaviors can be drawn.
(A, B) Early purification optimization trials on NTR1-C7E02 (precursor of NTR1-TM86V [1]. "X" denotes an absorbance peak, which is due to residual amounts of TrxA, as verified by SDS-PAGE (data not shown). The cleaved fusion protein was not entirely removed during these purification attempts, due to an inefficient wash step on the SP Sepharose column. The inefficiency of TrxA removal resulted from the fact that the SP wash buffer was kept at pH 7 here, as the optimal pH of 7.7 (current protocol) was identified only at a later time point in process development. (C, D) Large-scale purification of NTR1-TM86V. (E, F) Large-scale purification of NTR1-HTGH4. Note that this variant may exhibit a tendency for dimerization under these conditions in DDM at high concentrations (shoulder at around 12.5 ml elution volume). NM: n-nonyl-β-D-maltopyranoside, OTM: n-octyl-β-D-thiomaltopyranoside, OG: n-octyl-β-D-glucopyranoside, DM: n-decyl-β-D-maltopyranoside, OGNG: octyl glucose neopentyl glycol, DDM: n-dodecyl-β-D-maltopyranoside.

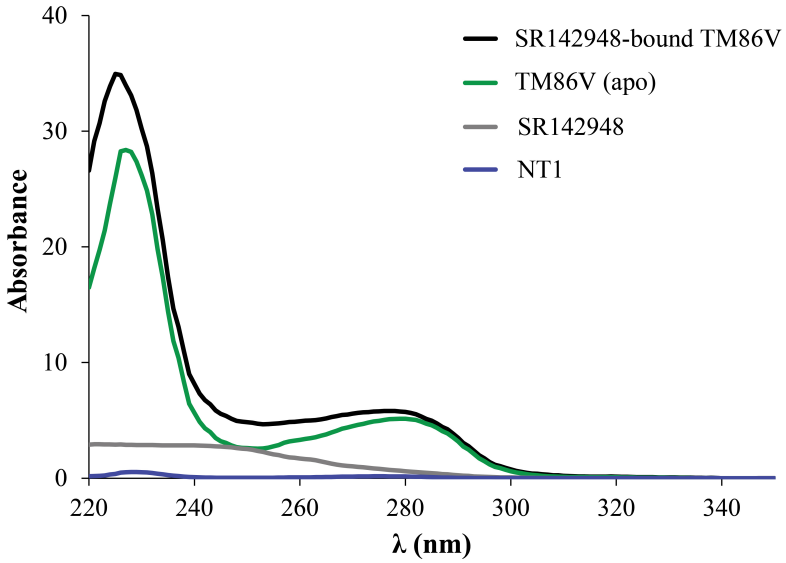

Fig. S2. Absorbance spectra of purified TM86V, antagonist SR142948 and agonist NT1 (GGRRPYIL). All spectra were either recorded at 100 μM (SR142948) or scaled to the same concentration using estimated extinction coefficients (ProtParam online tool from EXPASY). Note that the spectrum of TM86V (apo) was determined by measuring the absorbance of purified NT1-bound TM86V followed by subtraction of the NT1 spectrum. The difference in absorbance between TM86V (apo) and SR142848-bound TM86V corresponds well to the antagonist absorbance, thus clearly confirming that the receptor can be purified in the antagonist-bound state using the described protocol. This finding is expected, since TM86V can be competed from the pD-NT-P10A column by excess antagonist (Fig. 6) and also because competition efficiency is dependent on the position of the alanine substitution in NT8-13 (Fig. 5).

Reference

1. P. Egloff, M. Hillenbrand, C. Klenk, A. Batyuk, P. Heine, S. Balada, K.M. Schlinkmann, D.J. Scott, M. Schütz, A. Plückthun, *Proc. Natl. Acad. Sci. USA* **111** (2014) E655-662.