Supplementary Information for

A generic selection system for improved expression and thermostability of G proteincoupled receptors by directed evolution.

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Supplementary figures



Supplementary Figure S1 | Selections of the NTR1-D03SLN library based only on GFP fluorescence.

To test whether C-terminal fusion of GFP is sufficient to select for highly expressing receptor variants from a randomized library, the synthetic library NTR1-D03SLN encompassing approx. 8.5×10^8 variants of NTR1 (also used in the other experiments of this study) was expressed with a C-terminal sfGFP fusion in *E. coli* and four rounds of selection by FACS were performed. (a) Gating scheme for receptor selection based only on GFP fluorescence. A gate was set to sort the top 1% of GFP-fluorescent cells. (b) Flow cytometry histogram plots for GFP of selection rounds 1 to 4 (coloured open traces) in comparison to the naïve library (black traces filled in grey). They show a strong shift of the GFP signal after the first selection round, which further increases during the following rounds. Gating was only for GFP. After selection round 4, ten clones were isolated and analysed by Sanger sequencing. In all isolated clones, the receptor cassette was deleted whereby the GFP reading frame was fused directly to the promoter.



Supplementary Figure S2 | Optimization of MG-2p labelling.

(a) FADA-NTR1-TM86V was expressed in *E. coli*, and 2×10^7 cells were labelled for 1 to 4 h with 1 µM MG-2p in 1× PBS, 2.5× PBS, 5× PBS, PBS-E or TKCI, respectively. MG-2p fluorescence was measured by flow cytometry. Labelling time-dependent MG-2p fluorescence for each buffer is shown as superimposed histogram plots. (b) FADA-sfGFP was expressed in *E. coli* and 2×10^7 cells were labelled for 4 h with 1 µM MG-2p in 1× PBS, 2.5× PBS, 5× PBS, PBS-E or TKCI, respectively. MG-2p fluorescence was measured by flow cytometry. MG-2p fluorescence of FADA-sfGFP in each buffer in comparison to unlabelled cells is shown as superimposed histogram plots. (c) For saturation binding, *E. coli* cells expressing FADA-NTR1-TM86V were labelled for 4 h with 0 to 1 µM MG-2p in 5× PBS, and unspecific binding was obtained from cells expressing NTR1-TM86V lacking the FADA module. MG-2p fluorescence was measured by flow cytometry and is given as mean fluorescence intensities (MFI) ± S.E.M. of three independent experiments. MG-2p affinity was obtained by a non-linear regression fit, giving an K_a^{app} of 121 ± 2.4 nM.

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Supplementary Figure S3 | Comprehensive sequence analysis from MG-2p selection round 6 of the NTR1-D03SLN library in comparison to previously stabilized NTR1 variants.

Mutations of each clone are indicated, and the corresponding wild-type amino acids are given on top of the mutation data. The positions of the mutations are indicated by structural regions, Ballesteros-Weinstein numbering, and sequential amino acid numbering. The frequency of each sequence relative to all sequenced clones is given at the end.

References

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