Supplementary Information

Optimizing the α_{1B}-Adrenergic Receptor for Solution NMR Studies

Matthias Schuster, Mattia Deluigi, Milica Pantić, Santiago Vacca, Christian Baumann, Daniel J. Scott, Andreas Plückthun, Oliver Zerbe

Supplementary Tables

Table S1: Mutations of the different mutants

α_{1B}-AR-#12 (6 mutations*)	**α_{1B}-AR-B1** (14 mutations*)	**Ballesteros and Weinstein** (according to GPCRdb †)
-	S95C	2.54x54
L105Q	-	2.64x63
-	I116T	3.23x23
-	V124M	3.31x31
S150Y	S150Y	ICL2
-	S168C	4.48x48
-	G183V	4.63x63
D191Y	D191Y	ECL2
E194G	E194V	ECL2
-	I255F	ICL3
T295M	T295M	6.36x36
-	V333L	7.38x37
F334L	F334L	7.39x38
-	P349L	7.54x54
-	S351F	7.56x56

* in the truncated version from S35 to G369
† for the exact numbering convention, see http://docs.gpcrdb.org/generic_numbering.html

Supplementary Figures

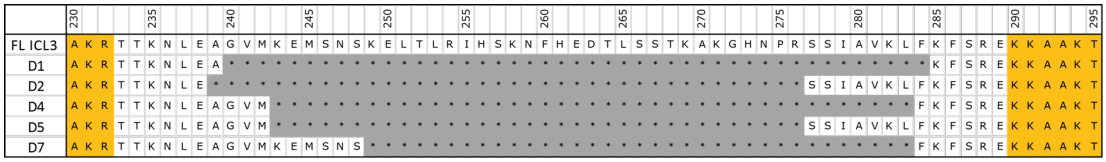

Figure S1. Amino acid sequence of the intracellular loop 3 (ICL3) deletions realized in the α1B-AR-B1 construct (Table S1).

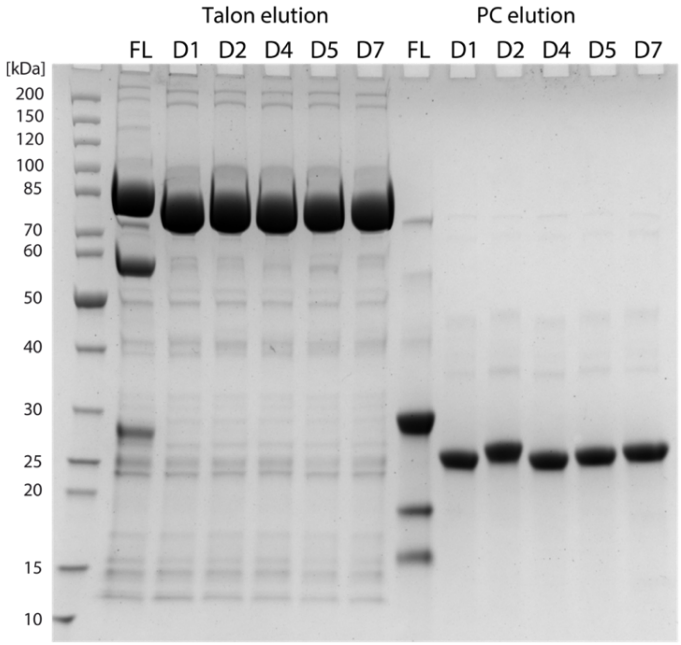

Figure S2. SDS-PAGE gel of the tested α_{1B}-AR-B1 receptor constructs with different ICL3 deletions.

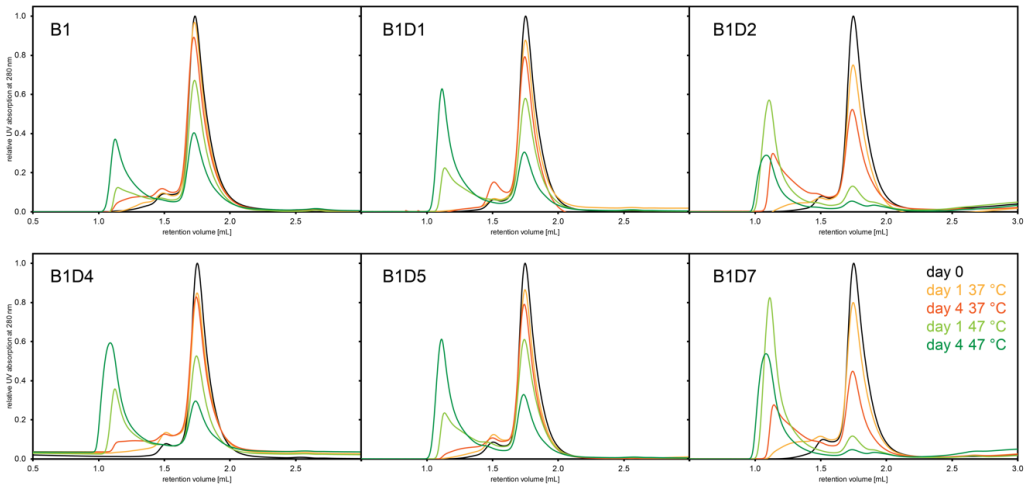

Figure S3. SEC thermostability assay of the B1 receptor constructs in DM/CHS with different ICL3 deletions.

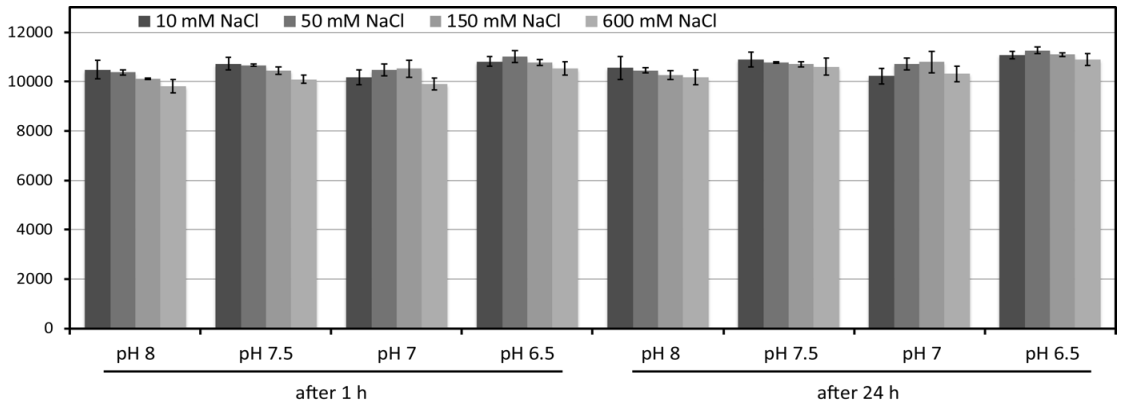

Figure S4. Measured fluorescence of BODIPY-FL-prazosin bound to α_{1B}-AR-#12-Avi in different buffer conditions after 1 h incubation at 4 °C and after 24 h incubation at 4 °C. Error bars indicate the standard deviation of the measured triplicates.

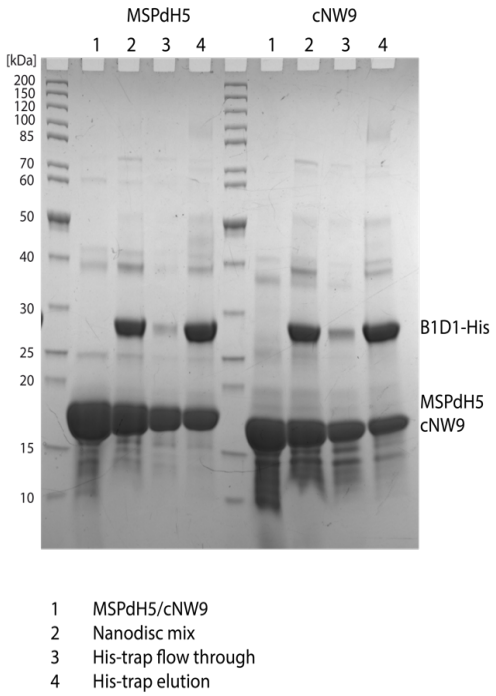

Figure S5. SDS-PAGE gel of the purification of α_{1B}-AR-B1D1-His in MSPΔH5 und cNW9 nanodiscs.

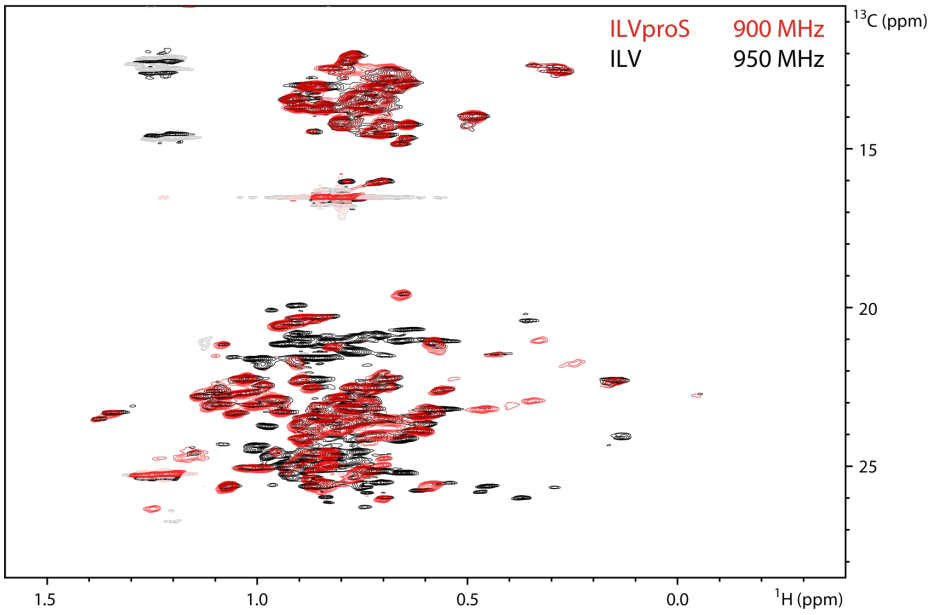

Figure S6. [^{13}C,^{1}H]-HMQC at 320 K of α_{1B}-AR-B1D1 (in black) with ILV U^{13}C precursor at 450 µM (NS=16, 1024*138 pts, constant time version) and α_{1B}-AR-B1D1 (in red) with ILVproS precursor at 400 µM (NS=8, 1024*300 pts)

Supplementary Methods

3C (or TEV) protease expression and purification

BL21(DE3) cells with the plasmid encoding the protease were grown in M9 media (with 2.5 g/L NH_4Cl and 10 g/L glucose) in a 5 L Biostat A bioreactor. Cells were induced at an OD_{600} of 2 with 0.5 mM IPTG (final concentration) and were grown for 16–20 h at 18 °C with DO set to 75 % and pH to 7.05. The cells were harvested by centrifugation (15 min, 5000 rpm, 4 °C) and 5 g aliquots were stored at −80 °C.
One aliquot of cells was resuspended by vortexing in 25 mL of precooled 3C buffer (40 mM HEPES pH 8 at 4 °C, 300 mM NaCl, 10 % (v/v) glycerol, 20 mM imidazole, 1 mM DTT). In another 20 mL of 3C buffer, 100 mg of lysozyme (2 mg/mL final), 2.5 mg of DNase and 250 µL of 1 M $MgCl_2$ were dissolved. Everything was mixed in a 100 mL flask and stirred for 15 min on ice. Subsequently, the suspension was sonicated with a Branson sonifier (40 % output, 1 s on, 3 s off) in an ice-water bath. The lysate was pelleted by centrifugation for 30 min at 4 °C and 18,000 rpm with a SS34 rotor. The supernatant was filtered with a 0.22 µm filter and loaded onto a 5 mL His-trap HP (GE) equilibrated with 3C buffer connected to an Äkta prime plus system at 4 °C. The column was washed with 15 % 3C elution buffer (40 mM HEPES pH 8 at 4 °C, 300 mM NaCl, 10 % (v/v) glycerol, 150 mM imidazole, 1 mM DTT) until a flat baseline at 280 nm was observed. The protein was eluted with 3C elution buffer and dialyzed with a 3.5 kDa dialysis membrane against 2 L of 3C dialysis buffer (10 mM HEPES pH 8 at 4 °C, 150 mM NaCl, 10 % (v/v) glycerol, 5 mM EDTA, 1 mM DTT) for 2–4 h, then against 5 L of 3C dialysis buffer overnight and again against 2 L of 3C storage buffer (10 mM HEPES pH 8 at 4 °C, 150 mM NaCl, 10 % (v/v) glycerol, 5 mM EDTA) for 4 h. The protein solution was filtered with a 0.22 µm filter to remove precipitates and the concentration was determined by $A_{280\ nm}$ using a Nanodrop instrument. The solution was diluted to 2.0 mg/mL with storage buffer and 1 mL aliquots were flash-frozen in liquid nitrogen and stored at −80 °C.

Expression and purification of MSPΔH5

MPSΔH5 containing a TEV-cleavable his-tag was expressed for 4 h in BL21(DE3) cells in TB media at 37°C with induction at OD_{600} = 2 in a 5 L Biostat A bioreactor. 20 g of cells were resuspended in 80 mL Lysis buffer (50 mM Tris pH 8 at 4 °C, 300 mM NaCl, 15 mM imidazole, 2 % Triton X-100, 5 mM $MgCl_2$, 2 mg/mL lysozyme, 0.06 mg/mL DNaseI). The solution was sonicated for 5 min (Branson sonifier, 40 % output, 1 s on, 3 s off) on ice. The lysate was cleared by centrifugation at 35000 × g for 30

min and the supernatant was loaded on an Äkta prime system at 4 °C equipped with three 5 mL HisTrap HP columns with 0.5 mL/min flow-rate. The columns were washed with 150 mL of each of the following buffers at 0.5 mL/min: Triton buffer (20 mM Tris pH 8 at 4 °C, 300 mM NaCl, 1 % Triton X-100), Cholate buffer (20 mM Tris pH 8 at 4°C, 300 mM NaCl, 50 mM Na-Cholate), MSP buffer (20 mM Tris pH 8 at 4°C, 300 mM NaCl), Wash buffer (20 mM Tris pH 8 at 4°C, 300 mM NaCl, 50 mM imidazole). The protein was eluted with MSP Elution buffer (20 mM Tris pH 8 at 4°C, 300 mM NaCl, 400 mM imidazole) and dialyzed against 2 L of Dialysis buffer (10 mM Tris pH 8, 150 mM NaCl) for 4 h in a 6–8 kDa membrane. The protein concentration was determined and TEV protease was added in a weight ratio of 1:50, following an overnight dialysis at room temperature against 4 L of Dialysis buffer with 0.5 mM DTT. The next day the solution was loaded on 3 x 5 mL HisTrap HP columns and the flow-through was collected and dialyzed overnight against Nanodisc buffer (20 mM Tris pH 8, 50 mM NaCl). The concentration was determined by $A_{280\ nm}$ using a Nanodrop instrument and aliquots with 2–5 mg/mL were stored at −80 °C. The usual yields were 300–600 mg.

Expression and purification of Sortase A

Sortase A, carrying a his-tag, was expressed in the same way as MPSΔH5. [60] A cell pellet of about 5 g was resuspended in 50 mL Lysis buffer (50 mM Tris pH 8 at 4 °C, 300 mM NaCl, 5 mM $MgCl_2$, 2 mg/mL lysozyme, 0.06 mg/mL DNaseI, 1 mM PMSF). The solution was sonicated for 5 min (Branson sonifier, 40 % output, 1 s on, 3 s off) on ice. The lysate was cleared by centrifugation at 35000 × g for 30 min and the supernatant was loaded on an Äkta prime system at 4 °C equipped with a 5 mL HisTrap HP column with 0.5 mL/min flow-rate. The column was washed with Buffer A (25 mM Tris pH 8 at 4 °C, 300 mM NaCl) and then with Buffer A + 8% Buffer B (25 mM Tris pH 8 at 4°C, 300 mM NaCl, 300 mM imidazole) until the baseline was flat. Sortase A was eluted with Buffer B and dialyzed overnight at 4 °C in Sortase Buffer (25 mM Tris pH 8 at 4°C, 150 mM NaCl). The concentration was determined by $A_{280\ nm}$ using a Nanodrop instrument and 1 mL aliquots were frozen in liquid nitrogen and stored at −80 °C.

Expression and purification of circularized Nanodisc precursor cNW9

The MSP precursor for the circularized Nanodiscs NW9, also carrying a TEV-cleavable his tag, was expressed and purified in the same way as the MSPΔH5 construct. After the TEV treatment the solution was stored at −80 °C. The circularization was performed (adapted from Nasr et al. [23]) in 0.5–1 L of 300 mM Tris pH 7.5 at 37 °C, 150 mM NaCl, 10 mM $CaCl_2$, with 10 μM NW9 and 5 μM Sortase A. The reaction was filtered (0.2 μm) and loaded on 2 x 5 mL HisTrap HP on an Äkta prime system at 4 °C. The flow-though containing the circularized NW9 was pooled and concentrated with an Amicon8200 (10 kDa

molecular weight cut-off) to 15 mL. The solution was further supplemented with 100 mM of Na-cholate (added as powder) and concentrated with an Amicon Ultra-15 (10 kDa molecular weight-cut off) to 1 mL. The monomeric cNW9 was further purified with multiple runs on a Superdex 200 Increase 10/300 with 20 mM Tris pH 7.5, 100 mM NaCl, 50 mM Na-cholate. The fractions containing the monomeric cNW9 were pooled and dialyzed against Nanodisc buffer and the concentration was measured by $A_{280\ nm}$ using a Nanodrop instrument. The protein was diluted or concentrated to 2–5 mg/mL, flash frozen in liquid nitrogen and stored at −80°C.

BioBeads regeneration

The BioBeads SM-2 could be regenerated. After use the BioBeads were stored in 70 % EtOH. To exchange the solution, the incubation with the new solution was performed in a bottle while slowly shaking at room temperature, and the washing was performed in a big Büchner funnel. The BioBeads were washed with H_2O, 1 M NaOH (1 h incubation), H_2O, 6 M guanidine (1 h incubation), H_2O and EtOH, and then 4 times with a hexane:isopropanol 3:2 (v/v) mixture (2 times 1 h, overnight and 1 h). Then the beads were washed with EtOH, very extensively with H_2O and with Nanodisc buffer containing 0.05 % NaN_3. The beads were stored at 4 °C until further use.

500 mL Trace Metals (1000x stock)

H_2O	475 mL
HCl 32 %	25 mL
$FeSO_4 \cdot 7\ H_2O$	3.5 g
$CaCl_2 \cdot 2\ H_2O$	92 mg
H_3BO_3	32 mg
$CoCl_2 \cdot 6\ H_2O$	9 mg
$CuCl_2 \cdot 2\ H_2O$	2 mg
$ZnCl_2$	170 mg
$Na_2MoO_4 \cdot 2\ H_2O$	302 mg
$MnCl_2 \cdot 4\ H_2O$	20 mg