Efficient production of functional $\beta_1$-adrenergic receptor in *E. coli* for structural studies

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Supporting Information
Figure S1. Quantification of TS-β1AR E. coli expression and purification by gel electrophoresis and western blotting. Western blotting against the histidine tag was used for quantifying the early steps of membrane solubilization and purification (panel A), in which the TS-β1AR band is not distinguishable due to the presence of other proteins. For the later steps (after nickel affinity chromatography), protein bands were quantified from an SDS-page gel (panel B). Details of the purification steps, sample preparation, and quantification using the program ImageJ are described in Materials and Methods. The labels indicate: “not induced”: E.coli cells before induction of protein expression, “induced”: E.coli cells after induction of protein expression by addition of IPTG, “supernatant”: supernatant of cell harvesting step, “insoluble urea”: insoluble fraction after membrane solubilization resuspended with buffer containing 7M urea, “solubilized membrane”: membrane fraction solubilized with detergents (DDM, CHAPS and CHS), “NiNTA FT”: flow through from nickel column, “NiNTA W1-W5”: wash fractions from nickel column, “PreSc, 4h and 12h”: Cleavage reaction of fusion protein with 3C PreScission protease after 4 and 12 h, respectively, “SPFF FT”: flow through from the fast flow cation exchange column (SPFF), “SPFF W”: wash fraction from SPFF column, “SPFF top peak”: center fraction of the absorption (280 nm) peak from the SPFF column, “empty”: empty lane, “SPFF full peak”: pool of all fractions of the TS-β1AR peak in the SPFF, “ALAC FT”: flow through from the alprenolol
affinity column (ALAC), “ALAC W”: wash fraction from ALAC column, “ALAC elution”: pool of fractions within the peak corresponding to the TS-β₁AR in the ALAC, “SPHP FT”: flow through from the high-performance cation exchange column (SPHP), “SPHP top peak”: fraction with the higher absorption (280 nm) from SPHP column, “SPHP full peak”: pool of fractions within the peak corresponding to the TS-β₁AR in the SPHP column.

The western blot of the 3C reaction sample (panel A) shows the 3C PreScission protease and the cleaved thioredoxin (identified in the figure), but not the maltose-binding protein or the TS-β₁AR bands because these two proteins do not carry a poly-histidine tag. The band of the fusion protein after the nickel affinity purification is marked by a green rectangle. The band for the TS-β₁AR monomer in the SDS-page gel (panel B) after the last purification step is marked by a blue rectangle. Very small amounts of higher TS-β₁AR oligomers are also visible in the concentrated ‘top peak’ fraction.

(C) Final purification step using high-performance cation exchange chromatography (SPHP). The elution profile of the 280 nm absorption of TS-β₁AR is shown in blue. The center of the TS-β₁AR peak elutes at a conductivity (red line) of about 40 mS/cm. SDS-page analyses of the ‘full peak’ and ‘top peak’ from the SPHP elution are shown in panel B.
**Figure S2.** Comparison of $^1$H-$^{15}$N TROSY spectra of $^{15}$N-valine-labeled TS-$\beta_1$AR obtained from *E. coli* (blue) or insect cells (red) in complex with the antagonist cyanopindolol. Additional peaks and a relative weakening of valine resonances in the *E. coli* receptor spectra are the result of efficient metabolic scrambling in *E. coli* to alanine, isoleucine, leucine, glutamate and other branched amino acids. Both spectra were recorded for ~45 hours at 304 K on samples containing 165 µM (*E. coli*) or 150 µM (insect cell) TS-$\beta_1$AR, 1 mM cyanopindolol, 20 mM Tris, 1 mM EDTA, 100 mM NaCl, 37 mM DM, 0.02% NaN$_3$, 5% D$_2$O, pH 7.5.