

The Taming of the Shrew: Improving GPCR Expression and Stability

Andreas Plückthun

Biochemisches Institut, University of Zurich, Zürich, Switzerland

The G-protein coupled receptor (GPCR) family represents the largest and most diverse group of cell surface receptors. Due to their pharmacological relevance there is great interest in obtaining structural and functional data that could provide new leads in the search for selective therapeutic agents. Despite enormous efforts made, only very few structures of receptors have been determined. One major problem is that structural studies require fairly large amounts of purified, solubilized and, most importantly, stable receptor protein.

We developed a novel method for the directed evolution of integral membrane proteins in the inner membrane of *Escherichia coli*³. For a mammalian G-protein coupled receptor, we arrived at a sequence with an order-of-magnitude increase in functional expression that still retains the biochemical properties of wild type. This mutant also shows enhanced heterologous expression in eukaryotes (12-fold in *Pichia pastoris* and 3-fold in HEK293T cells) and greater stability when solubilized and purified, indicating that the biophysical properties of the protein had been under the pressure of selection. These improvements arise from multiple small contributions which would be difficult to assemble by rational design. These approaches may alleviate existing bottlenecks in structural studies of these targets by providing sufficient quantities of stable variants in defined conformational states.

To obtain well ordered crystals or even to allow crystal formation at all, one should also apply tools for the stabilization of solubilized receptors with specific binding proteins². In this respect we select specific binding Antibody Fab fragments and Designed Ankyrin Repeat Proteins (DARPs)¹ by phage display and ribosome display for GPCRs. By combining two different selection methods we already obtained several specific and high-affinity binding DARPs. This diversity of specific binding proteins can be of high value for the successful stabilization of solubilized GPCR and for the *in vitro* and *in vivo* characterization of the membrane protein.

References:

1. Binz, H. K., Amstutz, P., Kohl, A., Stumpp, M. T., Briand, C., Forrer, P., Grütter, M. G. and Plückthun, A., "High-affinity binders selected from designed ankyrin repeat protein libraries," *Nat. Biotechnol.* **22**: 575-582 (2004).
2. Huber, T., Steiner, D., Röthlisberger, D. and Plückthun, A., "*In vitro* selection and characterization of DARPs and Fab fragments for the co-crystallization

of membrane proteins: The Na(+)-citrate symporter CitS as an example," *J. Struct. Biol.* **159**: 206-221 (2007).

3. Sarkar, C. A., Dodevski, I., Kenig, M., Dudli, S., Mohr, A., Hermans, E. and Plückthun, A., "Directed evolution of a G-protein coupled receptor for expression level, stability, and binding selectivity," *Proc. Natl Acad. Sci USA* **105**: 14808-14813 (2008).