Cryo-EM and x-ray structures of signalling complexes of rhodopsin shedding light on the G protein selectivity of GPCRs

Gebhard F.X. Schertler

Head of Biology and Chemistry Division, Paul Scherrer Institut, 5232 Villigen PSI, Switzerland and Professor for Structural Biology ETH Zürich D-BIOL

One of the largest membrane protein families in eukaryotes are G protein-coupled receptors (GPCRs). GPCRs modulate cell physiology by activating diverse intracellular transducers, prominently heterotrimeric G proteins. The recent surge in structural data has expanded our understanding of GPCR-mediated signal transduction. However, many aspects, including the existence of transient interactions, remain elusive. We present the cryo-EM structure of the light-sensitive GPCR rhodopsin in complex with heterotrimeric Gi. Our density map reveals the receptor C-terminal tail bound to the Gβ subunit of the G protein, providing a structural foundation for the role of the C-terminal tail in GPCR signaling, and of Gβ as scaffold for recruiting Gα subunits and G protein-receptor kinases. By comparing available complexes, we found a small set of common anchoring points that are G protein-subtype specific. Taken together, our structure and analysis provide new structural basis for the molecular events of the GPCR signaling pathway.

Selective coupling of GPCRs to specific Gα-protein subtypes is critical to transform extracellular signals, carried by natural ligands and clinical drugs, into cellular responses. At the center of this transduction event lies the formation of a signaling complex between the receptor and G protein. We report the crystal structure of light-sensitive GPCR rhodopsin bound to an engineered mini-Go protein. The conformation of the receptor is identical to all previous structures of active rhodopsin, including the complex with arrestin. Thus, rhodopsin seems to adopt predominantly one thermodynamically stable active conformation, effectively acting like a “structural switch,” allowing for maximum efficiency in the visual system. Furthermore, our analysis of the well-defined GPCR–G protein interface suggests that the precise position of the carboxyl-terminal “hook-like” element of the G protein, its four last residues, relative to the TM7/helix 8 (H8) joint of the receptor is a significant determinant in selective G protein activation.

In another project, we explore the use of light-sensitive G protein-coupled receptors (GPCRs) – opsins– for the development of new optogenetic tools to control cellular signalling processes using light: opto-GPCRs. In a first stage we identified several new opsins capable of GPCR pathways. We extensively characterized the most promising candidate opsins biochemically in cellular assays and finally in vivo. We developed the basis for engineering bistable opsins towards more effective optogenetic tools. For this, we determined the first structure of a recombinant invertebrate rhodopsin, carried out a detailed study of the chromophore binding site with advanced biophysical methods. We were able to compare in detail monostable and bistable visual pigments. The bistable pigments in several aspects are closer to the ligand binding pharmacologically relevant family A GPCRs. In a successful engineering attempt, we were able to identify mutations that shift the wavelengths of an invertebrate rhodopsin towards the infrared. This is important for the penetration of the light into tissues. The engineered opto-GPCRs are an important alternative to the channel opsins related optogenetic tools and they have a wide range of applications that is not restricted to neurons.

C. Tsai, J. Marino, R. Adaixo, F. Pamula, J. Muehle, S. Maeda, T. Flock, N. Taylor, I. Mohammed, H. Matile, R. Dawson, X. Deupi, H. Stahlberg, G. Schertler: Cryo-EM structure of the rhodopsin-Gαi-βγ complex reveals binding of the rhodopsin C-terminal tail to the gβ subunit. eLife 8 (2019)

CJ. Tsai, F. Pamula, R. Nehmé, J. Mühle, T. Weinert, T. Flock, P Nogly, P. Edwards, B. Carpenter, T. Gruhl, P. Ma, X. Deupi, J. Standfuss, C. Tate, G. Schertler: Crystal structure of rhodopsin in complex with a mini-Go sheds light on the principles of G protein selectivity. Science advances 4 (9), eaat7052 (2018)

N. Varma, E. Mutt, 1, J. Mühle, V. Panneels, A. Terakita, X. Deupi, P. Nogly, G.F.X. Schertler, E. Lesca: Crystal structure of a light-sensitive bistable class A GPCR. Proceedings of the National Academy of Sciences, 201902192 (2019)

T. Nagata, M. Koyanagi, H. Tsukamoto, E. Mutt, GFX. Schertler, X. Deupi, A. Terakita: The counterion–retinylidene Schiff base interaction of an invertebrate rhodopsin rearranges upon light activation. Communications biology 2 (1), 180 (2019)

E. Gerrard, E. Mutt, T. Nagata, M. Koyanagi, T. Flock, E. Lesca, G. F. X. Schertler, A. Terakita, X. Deupi, R. Lucas: Convergent evolution of tertiary structure in rhodopsin visual proteins from vertebrates and box jellyfish. Proceedings of the National Academy of Sciences (USA) 115(24):6201-6206 (2018)

D. Ehrenberg, N. Varma, X. Deupi, M. Koyanagi, A. Terakita, G.F.X. Schertler, J. Heberle, E. Lesca: The two-photon reversible reaction of the bistable jumping spider rhodopsin-1. Biophysical journal 116 (7), 1248-1258 (2019)