Structural Biology of GPCRs: The impact of NMR and x-ray Free Electron Lasers on Membrane Protein Dynamics

Gebhard F.X. Schertler

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

Our collaborative team has successfully introduced N^{15}-labeled valine in a conformationally stabilized beta 1 adrenergic receptor and used the labeled positions for evaluating backbone dynamics of this GPCR. We were able to see heterogeneous responses across the receptor after binding a number of agonists and antagonists. But, remarkably, we were able to pick up a homogenous response reflecting the signaling transmission within the receptor in a region distant from the ligand binding site. In addition, we were able to observe changes of loop dynamics in the ligand entrance channel of the adrenergic receptor. With these experiments, and a sophisticated network analysis of known active and inactive GPCR structures, our team was able to outline a detailed allosteric mechanism of GPCR activation.

Free-electron lasers are X-ray sources with unprecedented peak brilliance. Switzerland, in a bold move, has decided to build its own Free Electron laser at the Paul Scherrer Institute (PSI). The facility has taken up operation. In an international team, we have applied FELs in the U.S. and Japan to explore serial crystallography for membrane proteins. Using bacteriorhodopsin as a model system, we were able to trigger its photo cycle in lipidic cubic phase, and we have also observed reaction intermediates at room temperature. We can observe already after 16 ns disordering of a water cluster that is directly hydrogen bonded to the Schiff base. The difference densities are further increasing over time. This change is an important part of the mechanism that modulates the affinity of the proton to the Schiff base nitrogen. Later structural changes raise the pKa of Asp85 to the point where it spontaneously accepts a proton from the Schiff base. This is a key discovery in understanding how the proton pump bacteriorhodopsin is able to pump protons against a proton gradient. Using the exceptional free electron laser facilities at LCLS and SACLA, our consortium has measured a first molecular movie of structural changes in a membrane protein.

Membrane protein structural biology using X-ray free electron lasers
Current opinion in structural biology 33, 115-125 (2015)
A three-dimensional movie of structural changes in bacteriorhodopsin
Science 354 (6319), 1552-1557 (2016)
Lipidic cubic phase injector is a viable crystal delivery system for time-resolved serial crystallography
Nature Communications 7, 12314 (2016)
Lipidic cubic phase serial millisecond crystallography using synchrotron radiation
IUCrJ 2 (2), 168-176 (2015)
Molecular signatures of G-protein-coupled receptors
Nature 494 (7436), 185-194 (2013)
Diverse activation pathways in class A GPCRs converge near the G-protein-coupling region
Nature 536 (7617), 484-487 (2016)
Probing G alpha i1 protein activation at single-amino acid resolution
Nature structural & molecular biology 22 (9), 686-694 (2015)
Backbone NMR reveals allosteric signal transduction networks in the β1-adrenergic receptor
Nature 530 (7589), 237-241 (2016)