The process of vision: Structure of ultrafast mammalian rhodopsin determined by time-resolved femtosecond crystallography

Vision starts by light hitting the back of our retina. Located in the rod cells of the retina, the protein rhodopsin absorbs a photon through its chromophore retinal. Rhodopsin, consisting of 348 amino acids and 7 α-helices folded inside the membrane of the rod discs, bears an 11-*cis* retinal located deep inside the core of the protein (Fig. 1(a)). The first rhodopsin structure in the resting state (dark state) was determined at the beginning of this millennium at SPring-8 [1], followed by two higher resolution structures [2,3]. The structure of rhodopsin, being a prototype of the highly druggable family of G protein-coupled receptors (GPCRs), in an activated state sheds light on some conserved features of the rest of the family. Numerous spectroscopy studies have shown that retinal binding proteins trigger their activity by the absorption of a photon followed by isomerization of the chromophore inducing the next steps of activation. The trigger is one of the fastest events in biology, happening in the femtosecond range for mammalian rhodopsin. The full intramolecular mechanism of rhodopsin activation is still unclear.

We studied the structures of early intermediates in the ultrafast picosecond range by capturing the conformation of rhodopsin 1, 10 and 100 ps after illumination, at room temperature, using serial crystallography in a pump-probe mode at SACLA and SwissFEL [4]. Rhodopsin microcrystals were initially optimized to grow small and in high density according to [2,3] but did not diffract to high enough resolution to refine the retinal conformation [5], at SACLA. A first hit (Fig. 1(b)) was found with low molecular-weight polyethylene glycol, often found to be successful with GPCRs. Crystal size optimization and large scale (microliters) production in lipidic cubic phase (LCP) (Fig. 1(c)) allowed in 2017 pilot diffraction tests at SACLA and LCLS suggesting the feasibility of time-resolved experiments.

In 2018, we eventually performed time-resolved femtosecond serial crystallography (TR-SFX) experiments at the SACLA and the SwissFEL. Rhodopsin microcrystals grown in the dark were delivered into the beam path of a pump laser at 480 nm, followed after exactly 100 ps by the X-ray pulse of the XFEL (Fig. 2(a)). Data were collected to a resolution beyond 2 Å under dim red light (Fig. 2(b)) at SACLA **BL3 EH2** (Fig. 2(c)). The pump laser at 480 nm, illuminated rhodopsin with a 100 fs-pulse and a repetition rate of 15 Hz. It was circularly polarized, had an orientation 90 degree to the XFEL beam and a focus size of 47–50 μm FWHM (80–85 μm $1/e^2$) (Fig. 2(a)).

We performed the control experiment that the ground state structures from cryo-crystallography studies [3] and from our SFX measurement were similar (rmsd less than 0.33 Å) [4]. Even in the two independent experiments, the electron density maps were quasi-identical and Fig. 3 displays the retinal chromophore binding pocket from the obtained rhodopsin structure. A minor but non-negligible domain of the rhodopsin crystal presenting a lattice translation was characterized and corrected before refinement [6].

The main signals obtained in the difference electron density (DED) maps after 100 ps were, as expected, associated with the isomerizing retinal event (Fig. 3(c)). Figure 3(d) shows the new bathorhodopsin model (in blue) at room temperature, 100 ps after illumination, built out of the extrapolated map (not shown) which displays a reconstructed electron density of photoactivated rhodopsin electron density taking into account the level of photoactivation. It shows a distorted (highly distorted at C7 and C14) all-*trans* retinal and gives insights into several features like the retinal isomerization in a "space-saving motion" inside the tight rhodopsin binding pocket; the compatibility with the mechanism of an aborted bicycle pedal isomerization mechanism with a large displacement of methyl C20 while the C19 keeps mostly in place (Fig. 3(d)); the observation of a displacement of the segment C8–C10 of the retinal polyene chain, inducing a displacement of the β-ionone ring towards a region of the receptor bundle typically involved in agonist interaction in the Class A receptors; the permanent

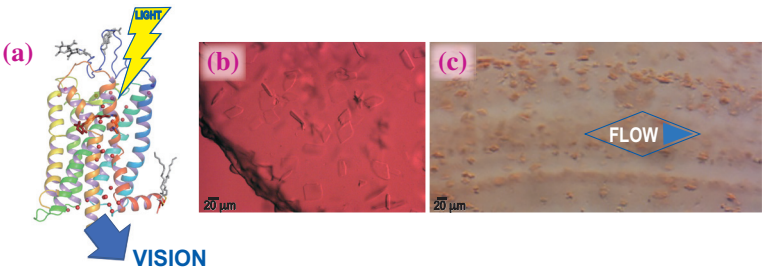

Fig. 1. Rhodopsin function, structure, and crystallization. **(a)** rhodopsin containing the red chromophore, 11 *cis*-retinal, which, absorbing a photon triggers the process of vision. **(b)** First rhodopsin crystal hit in an 80-nanoliters drop seen under dim red light. **(c)** Optimized rhodopsin crystals in microliters sample for TR-SFX.

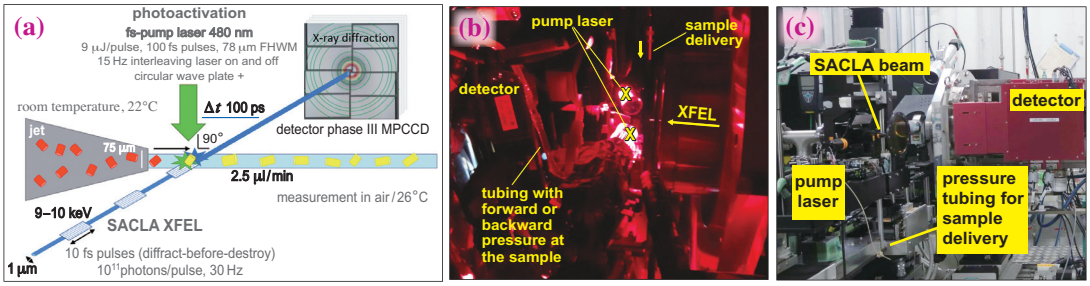

Fig. 2. TR-SFX experimental settings. **(a)** Overview of the experimental TR-SFX settings. **(b)** Picture of the TR-SFX stage in dim red light in an experiment having close settings (in the present experiment, we had only one pump laser output). **(c)** The sample stage of the SACLA BL3 EH2.

shift of the water molecule (Wat01) at the tip of the rotating methyl C20 (Fig. 3(c)) and belonging to the counterions (Glu 113 and Glu 181) H-bond network. It is interesting to observe that 1 ps after photoactivation (see details in [4]), more changes were observed than in the 100 ps time-delay. Several amino acids, even those a few helices turn away from the retinal, shifted of about half an angstrom in an anisotropic way towards the extracellular milieu, constituting a protein breathing motion due to the dissipation of excess energy. After 100 ps (Fig. 3 (c)), evanescent changes disappeared and the main rearrangements are located at the retinal and surrounding amino acids of the receptor bundle (Glu 122, Cys 110, Trp 265, Tyr 268) and the extracellular loop 2 (Cys 187, Tyr 191), highlighting the first amino acid involved in the first photoactivation step of vision. Molecular simulations and dynamics support our models.

This study does not only reveal the molecular trigger for vision and but investigate the early intramolecular mechanism in the process, that is, show the first molecular rearrangements after photon absorption [4]. More time-delays, from the nanosecond to the milliseconds are studied in order to follow the intramolecular mechanism of transduction of the light signal absorbed by retinal to the G protein partner transducin binding site located at the other end of the rhodopsin bundle, at the intracellular side of the rod cells.

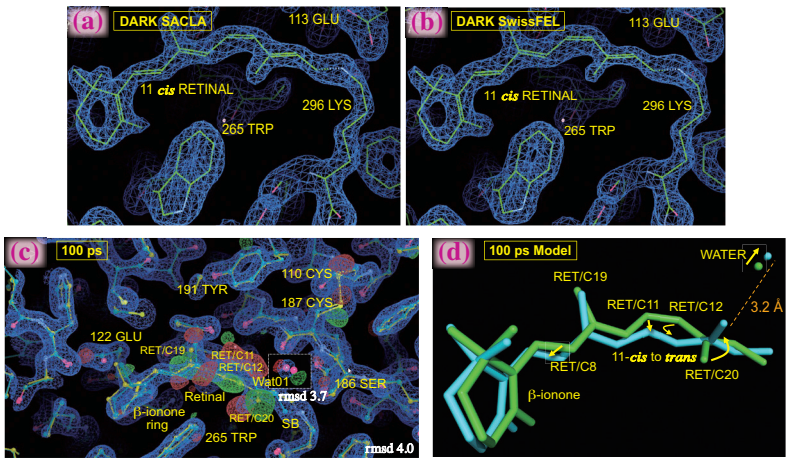

Fig. 3. Rhodopsin structures at the XFELs. **(a)** and **(b)** models of 11*cis*-retinal of the rhodopsin binding pocket (covalent Shiff base with Lys 296) in the dark state contoured with the $2F_O–F_C$ maps measured at SACLA and SwissFEL, respectively. **(c)** Difference map between 100 ps-illuminated state and the dark state shown at 4σ (for water Wat01 at 3.7σ). Colored with positive density in green and negative density in red. **(d)** Models of retinal from dark rhodopsin (in green) and 100 ps-photoactivated (cyan blue) with water Wat01 in a *spatio* temporal determination at the XFEL.

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References

[1] K. Palczewski *et al.*: Science **289** (2000) 739.
[2] T. Okada *et al.*: J. Mol. Biol. **342** (2004) 571.
[3] J. Li *et al.*: J. Mol. Biol. **343** (2004) 1409.
[4] *T. Gruhl, T. Weinert, M. J. Rodrigues, C. J. Milne, G. Ortolani, K. Nass, E. Nango, S. Sen, P. J. M. Johnson, C. Cirelli, A. Furrer, S. Mous, P. Skopintsev, D. James, F. Dworkowski, P. Båth, D. Kekilli, D. Ozerov, R. Tanaka, H. Glover, C. Bacellar, S. Brünle, C. M. Casadei, A. D. Diethelm, D. Gashi, G. Gotthard, R. Guixà-González, Y. Joti, V. Kabanova, G. Knopp, E. Lesca, P. Ma, I. Martiel, J. Mühle, S. Owada, F. Pamula, D. Sarabi, O. Tejero, C.-J. Tsai, N. Varma, A. Wach, S. Boutet, K. Tono, P. Nogly, X. Deupi, S. Iwata, R. Neutze, J. Standfuss, G. Schertler and V. Panneels: Nature **615** (2023) 939.*
[5] W. Wu *et al.*: Acta Crystallogr. F **71** (2015) 856.
[6] M. J. Rodrigues *et al.*: Acta Crystallogr. D **79** (2023) 224.