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Quasi-elastic neutron scattering reveals ligand-induced protein dynamics of a G-protein–coupled receptor

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Abstract

Light activation of the visual G-protein-coupled receptor rhodopsin leads to significant structural fluctuations of the protein embedded within the membrane. Enhancement of protein dynamics upon stimulation of the GPCR yields activation of the cognate G-protein (transducin) that initiates biological signaling. Although X-ray crystallographic analysis reveals static structures, changes in protein dynamics are the key to understanding the activation mechanism. Here we show how the integral membrane protein mobility is regulated by the retinal cofactor of the visual GPCR rhodopsin using both elastic and quasi-elastic neutron scattering. Our quasi-elastic neutron scattering (QENS) experiments revealed a logarithmic-like relaxation of the hydrogen-atom dynamics in the *Rhodopsin* family A GPCRs, as only observed for globular proteins previously. Application of mode-coupling theory (MCT) as originally developed for glass-forming liquids to our QENS analysis reveals the picosecond–nanosecond dynamics in the β -relaxation region crucial to protein function. Our novel powdered GPCR preparation method together with the QENS technique allowed us to uncover subtle changes in protein dynamics regulated by the retinal cofactor of rhodopsin. For the ligand-free opsin apoprotein versus the dark-state rhodopsin, removal of the retinal cofactor increases the relaxation time in the β -relaxation regime (ps–ns), evincing greater protein flexibility. Because opsin is structurally similar to active metarhodopsin-II, which catalytically activates transducin, the cofactor plays a pivotal role in regulating the protein dynamics required for GPCR function.

Keywords

rhodopsin; GPCR; neutron scattering; energy landscape; protein dynamics

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Introduction

Protein dynamics (1, 2) are the key to understanding the biological activities of pharmacologically important proteins such as G-protein-coupled receptors (GPCRs) (3–6). The conformational fluctuations of the protein upon extracellular stimulation lead to the activation of GPCRs in a cellular membrane lipid environment. X-ray crystallographic experiments (7) and recent time-resolved wide-angle X-ray scattering (WAXS) studies (8) conducted on the prototypical visual GPCR rhodopsin have revealed valuable information about the conformational changes that occur during activation. However, thus far little information is available regarding how the internal dynamics evolve during GPCR function (9). In this report, for the first time, we use quasi-elastic neutron scattering (QENS) technique to study changes in a GPCR mobility upon activation, using rhodopsin as a prototype.

Rhodopsin is a class A GPCR responsible for vision under dim-light conditions in vertebrates. It is the canonical prototype of the *Rhodopsin* family of GPCRs (5). The chromophore 11-*cis*-retinal locks the rhodopsin in the inactive dark state (10), and acts as an inverse-agonist by preventing the interaction with its cognate G-protein (transducin). Upon photon absorption, the 11-*cis*-retinal isomerizes to all-*trans*, yielding rearrangement of the protein conformation due to two protonation switches (11). The photoisomerization of retinal occurs within 200 fs, causing rhodopsin to undergo a series of multi-scale transitions (12, 13). Currently, X-ray crystal structures are available for rhodopsin in the dark state (14, 15), as well as several freeze-trapped photointermediates (7, 16), including the ligand-free opsin apoprotein. In addition to solid-state NMR methods (9, 12), site-directed spin labeling (SDSL) has been extensively applied to study rhodopsin. (17). Here we compared the protein dynamics of the dark-state rhodopsin to those of ligand-free opsin, which is structurally similar to active metarhodopsin-II. Both elastic and quasi-elastic neutron scattering (18) were utilized, with the aim of studying the functional protein dynamics that lead to transducin activation (12).

The intrinsic fluctuations of protein structures are due to a large number of conformational substates represented by a hierarchical (rough) energy landscape (EL) (19, 20), as discussed for globular proteins by Frauenfelder *et al.* (2). Notably, the protein dynamics (1, 21) encompass a broad range of time scales, ranging from local motions (ps-ns) to collective domain motions (ns- μ s) (1, 20). In analogy with glass-forming liquids, the short time dynamics (β -relaxations) include small amplitude local motions (e.g. side chains and methyl group rotations) whereas the long-time dynamics (α -relaxations) are due to collective protein motions of larger amplitude. To date mainly globular proteins such as myoglobin (2) and lysozyme (22) have been studied with this approach. Here we experimentally proved this concept is also valid for membrane proteins such as GPCRs and apply this concept in explaining the ligand-binding mechanisms of GPCR rhodopsin upon photoactivation.

In this paper, we exploited advances in quasi-elastic neutron scattering (QENS) technique to probe the effect of the retinal cofactor on the dynamics of rhodopsin in the β -relaxation time range (ps-ns) crucial for its activation. QENS can be used to study the relaxational dynamics of hydrogen atoms within the protein molecule including vibrations, relaxations and

rotational motions (18, 23). By combining a new powdered GPCR-detergent complex preparation with QENS technique, we are able to address the role of the cofactor of GPCRs like rhodopsin in the functional protein dynamics. Our QENS experiments probed the hydrogen atom dynamics in the β -relaxation range for the dark-state rhodopsin and ligand-free opsin state, thus revealing new information about the role of protein internal motions in the GPCR activation. Specifically, we discovered that the local relaxational dynamics in the opsin apoprotein is slower versus dark-state rhodopsin, which corresponds to a greater flexibility (more degree of freedom for protein movement) due to removal of the retinal cofactor. Because opsin is structurally similar to the metarhodopsin-II active state, our study shows how the retinal cofactor (24, 25) regulates the protein dynamics in the actual signaling mechanism. Our findings suggest the intrinsic dynamics of the protein are unlocked by the light-induced isomerization of the 11-*cis* retinal cofactor of visual rhodopsin required for interaction of the GPCR with its cognate G-protein (transducin).

Results and Discussion

Powdered functional rhodopsin

Quasi-elastic neutron scattering (QENS) technique provide insights into differences in the hydrogen-atom dynamics in dark-state rhodopsin versus the ligand-free apoprotein opsin. In the present QENS experiments, we use powdered GPCR to investigate the dynamics of rhodopsin versus the ligand-free opsin apoprotein. The powdered GPCR-detergent complex preparation removes water from the samples completely, and enables the subsequent controlled hydration with D₂O by isopiestic transfer through the vapor phase. Hydration with D₂O allowed us to isolate the incoherent neutron scattering signal from the non-exchangeable hydrogen atoms in the protein, which have much larger incoherent scattering cross-section as compared to any other atoms in the sample, such as deuterons, carbons and oxygens.

We chose 3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) detergent with an aggregation number of 10 to have the minimum detergent/protein ratio in the samples. In this way, the majority of the signal recorded is due to the protein dynamics, and the correction for the presence of the detergent is minimal during the subsequent data reduction and analysis. The RDM were solubilized in CHAPS detergent, and purified using a zinc-extraction method (26). The rhodopsin purified in CHAPS had a purity (A_{280}/A_{500} ratio) of 1.8 and a yield of about 60%. The nominal amount of CHAPS detergent in the sample was about 25% (w/w), and was adjusted using ultracentrifugal filters of 30-kDa molecular weight cut-off.

UV-visible spectroscopic characterization of the powdered samples (after rehydrating with D₂O) showed that the photochemical functionality of rhodopsin remained unaffected by lyophilization (Fig. 1). The 500-nm peak observed for the rehydrated powdered rhodopsin-CHAPS samples indicated that the rhodopsin is still in the dark state, and that the photoillumination yielded a mixture of inactive Meta-I and active Meta-II. This is surprisingly true for both powdered rhodopsin disk membranes and rhodopsin solubilized in CHAPS detergent (Fig. 1 and Fig. S2). Moreover, far-UV circular dichroism (CD) spectra acquired for the redissolved powdered rhodopsin-CHAPS and powdered opsin-CHAPS

samples revealed that the helicity remained (Fig. 1 inset). The UV-visible characterization (after rehydrating) of the powdered samples shows that even one year after sample preparation (stored at -20°C), the proteins are still photochemically functional. This new method of preparing functional powders of rhodopsin may be applied to other GPCRs, thus demonstrating the proof of concept. Therefore we are safe to use these powdered GPCR samples in elastic and quasi-elastic neutron scattering experiments to determine how the dynamics of change during GPCR activation. Our experiment is the first application of the QENS techniques to such powdered samples.

Elastic neutron scattering shows mean-square displacements of hydrogen atoms of rhodopsin and opsin are independent of retinal

First, we employ elastic incoherent neutron scattering (EINS) (27, 28) to determine whether the dissociation of the retinal ligand from rhodopsin affects the protein flexibility (Fig. 2). The mean square displacement (MSD), denoted by $\langle x^2(T) \rangle$, is traditionally used as the index of “softness” or flexibility of globular proteins (29). It can be calculated from the EINS intensities by applying a Gaussian approximation to the Debye-Waller factor, which is valid for small Q -values (30): $S_H(Q, T, \omega = 0) = \exp(-Q^2 \langle x^2(T) \rangle)$. Here, $S_H(Q, T, \omega = 0)$ is calculated from the ratio of temperature-dependent elastic intensity, $I_{\text{elastic}}(Q, T, \omega = 0)$ and elastic intensity at the lowest measured temperature, $I_{\text{elastic}}(Q, T \sim 0, \omega = 0)$. The slope of the logarithm of $S_H(Q, T, \omega = 0)$ versus Q^2 yields the MSD $\langle x^2(T) \rangle$ (see SI for details).

The calculated MSDs are plotted as a function of temperature in Fig. 2, for both dark-state rhodopsin and the ligand-free apoprotein, opsin. According to the plot, for rhodopsin versus opsin there is no major difference in hydrogen-atom MSDs of the samples within the measured temperature range. Notably, there is a sudden increase in the slope of the MSDs above the so-called dynamic transition temperature (31) of $T_D \approx 220\text{ K}$, indicating an onset of rapid thermal fluctuations of the substates in both rhodopsin and opsin. This dynamical transition in hydrated proteins reveals the change in motion of the protein groups from harmonic to anharmonic behavior. Above T_D sufficient energy is acquired to move anharmonically among the various substate potential wells. At this point, we can conclude that above $T \approx 220\text{ K}$ the membrane protein rhodopsin attains the conformational flexibility required to perform its biological function, which is cofactor-independent. In the following sections, we describe how the cofactor-dependent hydrogen dynamics are studied using the QENS technique.

Energy domain analysis shows the average hydrogen-atom motions are slower in opsin compared to rhodopsin

Next, we conducted QENS measurements on D_2O -hydrated ($h \sim 0.27$) dark-state rhodopsin and ligand-free apoprotein opsin, at temperatures ranging from $T = 220\text{ K}$ to 300 K , with Q ranging from 0.3 – $1.9\text{ \AA}^{-1}$. The measured QENS spectra for both rhodopsin and opsin are illustrated in Figs. 3a–b, respectively, at nine different temperatures and momentum transfer $Q = 1.1\text{ \AA}^{-1}$. The measured QENS intensity, i.e. the self-dynamic incoherent scattering factor $S_m(Q, \omega)$, shows increase in quasi-elastic broadening with temperature, indicating faster ps–ns diffusive motions in the hydrogen atoms within protein molecules. In QENS, the elastic component (central peak) originates from the immobile atoms within the

experimental energy (or time) window, and the quasi-elastic components (broadenings from the elastic central peak, or the resolution functions) are due to the spatial motion of the mobile atoms (see SI for details).

Using a model-independent analysis, we are able to decouple the motions of the detergent (CHAPS) and protein (32). Figs. 3c–d demonstrate the analysis of the measured $S_m(Q, \omega)$ as a superposition of a Dirac delta function, two Lorentzians (L_1 and L_2), and a linear background convoluted with resolution, within the energy transfer range $\pm 110 \mu\text{eV}$ for rhodopsin and opsin, respectively. The full-width at half-maximum (FWHM, 2Γ) of the Lorentzians provides information about the motions of hydrogen atoms within the samples. According to the analysis in Fig. 3c–d, the FWHM of L_1 ($2\Gamma_1$) is much broader than the FWHM of L_2 ($2\Gamma_2$) and is Q -independent (details are shown in Figs. S3 and S4 in the SI), with the values very close to the FWHM values extracted from the analysis of the QENS data of the pure CHAPS sample. By contrast, the FWHM of L_2 ($2\Gamma_2$) is much narrower and Q -dependent comparing to that of L_1 ($2\Gamma_1$). Thus we can confidently attribute the faster CHAPS dynamics to L_1 and the slower protein dynamics to the L_2 component. Using this model-free analysis, we can then readily separate the dynamics of rhodopsin and the detergent CHAPS by the decoupling approach which has been successfully applied in the analysis of previous QENS data (32, 33).

The energy domain analysis is summarized in Fig. 3e, where we plot the relaxation time τ for diffusive motion of the hydrogen atoms of rhodopsin and opsin versus Q at temperatures between $T = 260 \text{ K}$ and 300 K . The relaxation time τ was calculated using the relation, $\tau = \hbar/2\Gamma_2$, corresponding to the diffusive motion. Our results show that the diffusive motion of the hydrogen-atoms is slower in opsin (reflected in larger τ values) compared to that of rhodopsin at all measured Q s and temperatures (Fig. 3e). In the Q range from $0.5 \text{ \AA}^{-1}$ to $0.9 \text{ \AA}^{-1}$, the relaxation time of both states decreases with Q , due to diffusive motion of H-atoms. However, in the Q range from $1.1 \text{ \AA}^{-1}$ to $1.9 \text{ \AA}^{-1}$, the relaxation time reaches its minimum value and is barely Q -dependent, indicating that the motion in the protein is localized in the length scale $\sim 6 \text{ \AA}$. The analysis in energy domain gives us the first impression that the diffusive motion of the hydrogen-atoms is slower in opsin compared to rhodopsin, and provide a firm foundation to extend to the analysis in time domain, as we describe below.

Time domain analysis using mode-coupling theory reveals β -relaxation is slower in opsin versus rhodopsin

To further investigate the differences in hydrogen-atom motion in dark-state rhodopsin and the ligand-free apoprotein opsin, we evaluated the relaxation dynamics in the real-time domain. The inverse Fourier transform of the measured QENS data in energy domain yields the intermediate scattering function (ISF), denoted by $I(Q, t)$ of the measured spectra in the time domain, as described in the SI Materials and Methods. In QENS measurements, the ISF represents the single-particle correlation function of hydrogen atoms within the biomolecules. It is the essential function to describe the relaxation dynamics in biomolecules and can be directly connected to theoretical calculations and molecular dynamics (MD) simulations. In our analysis, the contribution of the detergent intensity was subtracted before the Fourier transformation, according to our energy domain analysis (34) (see SI for details).

The ISF $I(Q, t)$ of H-atoms in rhodopsin and opsin is plotted at the physiological temperature $T = 300$ K in Fig. 4 at a series of Q -values. Further analysis at different temperatures $T = 260, 280$ K, and 300 K are shown in the SI Fig. S5.

From theoretical prediction, protein dynamics at different timescales can be approximately divided into three groups (20, 35): (i) a short-lived Gaussian-like ballistic region due to vibrations; (ii) Fast dynamics in the β -relaxation region (ps-ns) governed by a logarithmic decay; followed by (iii) slow dynamics in the α -relaxation region (μ s-ms) governed by a stretched-exponential decay. The correlation between dynamics and biological activity has been demonstrated on the μ s–ms timescale, but fluctuations at the atomic level are much faster than this (19, 36, 37). Our experimental results correspond to the β -relaxation region within the time window of ps to ns. Upon increasing temperature, the protein local dynamics become faster in both rhodopsin and opsin. Furthermore, there is a striking Q -dependence, showing the relaxation process varies within the different length scales in the sample (from Å upto nm). Notably, rhodopsin and opsin (both membrane proteins) demonstrate the characteristic logarithmic-like decay of the ISF in the β -relaxation regime, previously observed only in aqueous soluble globular proteins (22, 38, 39).

Having observed the logarithmic decay in the ISFs of both rhodopsin and opsin, we applied mode-coupling theory (MCT) to fathom the differences in β -relaxation (10–400 ps) dynamics for opsin versus rhodopsin. The MCT was originally developed to describe the complex dynamics in glass-forming liquids (40–42), but has been successfully used in predicting the logarithmic-like decay in β -relaxation dynamics of globular proteins and other biopolymers (22, 38, 39, 43). The ISFs with logarithmic-like decay behavior can be fitted with an asymptotic expression derived from the MCT:

$$I(Q, t) = f(Q, T) - H_1(Q, T) \ln[t/\tau_\beta(T)] + H_2(Q, T) \ln^2[t/\tau_\beta(T)] \quad (1)$$

Here $\tau_\beta(T)$ is the characteristic β -relaxation time, and $f(Q, T) = \exp[-A(T)Q^2]$ is related to the Debye-Waller factor for small Q -values. The quantities $H_1(Q, T)$ and $H_2(Q, T)$ are the Q - and T -dependent first- and second-order logarithmic decay parameters, respectively. The fitting parameter $H_1(Q, T)$, shown in Figure 4 (c) and (d) for rhodopsin and opsin respectively, is qualitatively understood as the slope of the decay, or the power of the decay, and can be expressed as a power law in Q as given by, $H_1(Q, T) = B_1(T)Q^\beta$ where the exponent can take the value $\beta \sim 1$ – 2 and $B_1(T)$ is a temperature-dependent parameter.

In Fig. 5a, the opsin and the dark-state rhodopsin crystallographic structures (14) are compared. The opsin* crystal structure resembles the more open metarhodopsin-II active structure due to tilting of transmembrane helices H5 and H6 away from the H1–H4 core. One could expect slower dynamics in opsin, because it is the more open conformation. The characteristic β -relaxation time (τ_β) values from fitting the ISF (Eq. 1) are summarized in Fig. 5b. Notably, we observed longer β -relaxation times (τ_β) for temperatures ranging from 220 K to 300 K, which suggests the ligand-free opsin structure is more flexible versus the dark-state rhodopsin. In the temperature range of 220–300 K, the τ_β values of both

rhodopsin and opsin follow an Arrhenius behavior: $\tau_{\beta} = \tau_0 \exp(E_a/k_B T)$, where E_a is the average activation energy.

Ligand-induced changes in dynamics of rhodopsin versus ligand-free opsin, and its implication of protein flexibility in visual signaling

Protein flexibility and ligand binding is coupled to each other, and are usually described by different biophysical models (44). In figure 5c, we plot a schematic free energy landscape (EL) model representing the rhodopsin activation process. The black curve models the free energy of ligand-binding rhodopsin. The red curve represents the free energy of ligand-free opsin. The free energy differences between different states contains many contributions, including the direct protein-ligand interactions, hydrophobic association and the conformational and vibrational entropy of the rhodopsin and all-*trans* retinal. The hierarchical ELs are reflected by the small fluctuations in the curves. This schematic picture explains the mechanisms of rhodopsin conformational change during the photo-activation.

One of the features of complex systems (35, 42) is highly non-exponential relaxation, which describes the energy landscape (EL) due to the many conformational substates with similar energies. The different basins of the EL give us a framework for understanding the conformational changes during a reaction, such as GPCR activation of the cognate G-protein. Because the fluctuations are thermally driven, temperature plays a major role (31). At sufficiently low temperature, the individual protein molecules are trapped in various potential wells, where they undergo harmonic vibrations due to the conformational substates (18). On the other hand, the higher energy barrier in opsin results in longer relaxation time in the ligand free opsin which is shown in figure 5b.

According to our experimental results, the dark-state rhodopsin structure is more flexible (8). Following dissociation of all-*trans* retinal from the Schiff-base linkage, opsin is structurally similar to the active metarhodopsin-II (45). Using QENS, we found that in the presence of 11-*cis* retinal, the protein has larger relaxation time which enables it to sample more conformational substates. The cofactor helps to maintain in an energetically less favorable conformation (frustration). Upon photoactivation, the 11-*cis* retinal isomerizes to all-*trans*, yielding greater flexibility of the protein than in ground-state rhodopsin. The increase in protein flexibility is crucial to subsequent binding and catalytic activation of the cognate G-protein (transducin), and is due to lack of stabilizing interactions between the retinal chromophore and the secondary structures involving the receptor binding pocket. The stabilizing forces in opsin are weaker compared to the dark-state rhodopsin, giving an increase in flexibility, consistent with an ensemble-activation mechanism of the visual GPCR rhodopsin (25).

The influences of both temperature (46) and hydration (18, 21, 47) then allow one to further address the EL in terms of a hierarchical organization (2). As the all-*trans* retinal binds to the rhodopsin, the water molecules in the solvent shell surrounding the hydrophobic moieties of the ligand and binding site will be released to the bulk solvent and gain entropy, thereby the free energy of the dark-state rhodopsin is lower than opsin (44). In addition, when binding to small but solvent-accessible hydrophobic cavities of rhodopsin, the disordered water molecules have a density much lower than the bulk water density, therefore will

increase the solvent free energy. Increased hydration upon light activation is fully consistent with recent MD simulations (48).

Conclusion

In summary, The QENS data were analyzed in the energy domain by a model-independent analysis, and in the time domain by the mode-coupling theory (MCT), as originally formulated to describe the complex dynamics in glass-forming liquids (42). Significantly, MCT predicts the logarithmic decay phenomenon observed for globular proteins (49). We report for the first time the logarithmic-like decay for a membrane protein. From both energy and time domain analysis of the QENS data, we observed that the opsin dynamics are significantly slower at the lower Q -values compared to rhodopsin, suggesting the opsin apoprotein is relatively adopting more conformational substates (10). Moreover, we show how the cofactor influences the dynamics in the activation mechanism of a canonical prototype for the *Rhodopsin* (Family A) GPCRs.

We further use a schematic free energy model to explain our findings, which support the notion that the flexibility of the protein structure increases in the absence of the retinal cofactor. This increase is important for the interaction between the rhodopsin GPCR and its cognate G-protein, yielding catalytic activation of transducin. Our results are consistent with regulation of protein structural dynamics by the retinal cofactor of rhodopsin. Light causes isomerization of 11-*cis* retinal, which unlocks the intrinsic dynamics of the dark-state rhodopsin that are pivotal for the activation mechanism. Our findings pave the road to study the crucial dynamics of other biologically important membrane proteins in the GPCR superfamily. An important question remaining for future research is whether active metarhodopsin-II yields results consistent with greater flexibility of the protein structure as compared to the apoprotein opsin due to the presence of all-*trans* retinal.

Materials and Methods

Sample preparation

Rhodopsin was extracted and purified from bovine rhodopsin disk membranes (RDMs) as described (see supporting information). For the first time, a powdered membrane protein sample containing 72 % (w/w) of photochemically functional bovine rhodopsin and 28 % (w/w) of CHAPS (3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate)) detergent was successfully prepared. About 600 mg of the powdered rhodopsin was used to prepare a dark-state sample, and the ligand-free apoprotein opsin sample. The opsin was prepared by photobleaching the dark-state sample with a locally constructed 515-nm LED light source. Far-UV circular dichroism (CD) spectra collected for dark-state rhodopsin and opsin samples confirmed that the helicity is conserved during the lyophilization (SI). Thereafter, each of the samples was hydrated with $^2\text{H}_2\text{O}$ ($h \sim 0.27$), and enclosed in aluminum foil to prevent exposure to light. Finally, each of the samples was inserted in rectangular aluminum sample holder for neutron scattering experiments.

Neutron Scattering Experiments

The neutron scattering experiments were performed with the near-backscattering spectrometer BASIS (50) at the Spallation Neutron Source (SNS) at Oak Ridge National Laboratory (ORNL). The BASIS measurements had an energy resolution of $3.4 \mu\text{eV}$ (HWHM for Q -averaged resolution value) and an extended dynamic range of -120 to $+520 \mu\text{eV}$, compared to the more common $\pm 100 \mu\text{eV}$ range. EINS data were obtained by monitoring the elastic intensity determined by the integration over a $\pm 3.4 \mu\text{eV}$ interval (HWHM of the elastic peak). The QENS data were collected over the range 220 – 300 K, and at 10 K to characterize the sample-specific energy resolution of the spectrometer. Further details are available in the SI.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Significance

We report a quasi-elastic neutron scattering study of the activation of rhodopsin as a G-protein-coupled receptor prototype. For the first time, we produced powdered functional rhodopsin in minimum detergent for neutron scattering study. Our studies revealed significant differences in the dynamics of the dark-state rhodopsin versus the ligand-free apoprotein, opsin. These differences can be attributed to the influence of the covalently bound retinal ligand on the intrinsic protein dynamics. Moreover, a generic free energy model is used to explain the GPCR dynamics upon light activation/ligand binding, which can be further applied to other GPCR systems.

Fig. 1. Powdered rhodopsin-CHAPS detergent complex retains its photochemical functionality and native helicity. UV-visible spectra for rhodopsin-CHAPS complex after lyophilization for dark state, light activated state, and when bleached with opsin. Inset: Far-UV circular dichroism (CD) spectra for re-solubilized powdered rhodopsin and opsin at pH 6.9 and $T=288$ K.

Fig. 2.

Mean-square hydrogen atom displacements of rhodopsin and its ligand free apoprotein opsin are nearly identical and both shows a dynamical transition $T_D \sim 220$ K. Atomic mean-square displacements (MSDs) of H-atoms in dark-state rhodopsin (teal circles) and ligand-free opsin (open green circles) are shown as functions of temperature. The inset shows the elastic incoherent neutron scattering (EINS) intensities for dark-state rhodopsin and opsin respectively.

Fig. 3.

Ligand-free opsin apoprotein shows slower hydrogen-atom dynamics compared to the dark-state rhodopsin. Left Panels: QENS spectra for dark-state rhodopsin and ligand-free opsin samples. (a, b) Normalized dynamic incoherent scattering function, $S_m(Q, \omega)$ from two samples respectively, at $Q = 1.1 \text{ \AA}^{-1}$ from 220 K to 300 K along with resolution. (c, d) Analysis of the QENS spectra at $Q = 1.1 \text{ \AA}^{-1}$ and $T = 300 \text{ K}$, showing elastic scattering component (delta function shown as dark yellow line), quasi-elastic scattering components (two Lorentzians indicated by cyan line and magenta line), background (blue line), and the fitted curves (red line). (e) Comparison of the relaxation time (τ) of dark-state rhodopsin and ligand-free opsin as a function of Q for $T = 260 \text{ K}$ to 300 K in 10 K steps.

Fig. 4.

Mode-coupling theory (MCT) analysis of QENS data in the time domain. (a) and (b) intermediate scattering function $I(Q,t)$ of dark-state rhodopsin and opsin at $T = 300$ K at Q -values from 0.3 $\text{\AA}^{-1}$ to 1.9 $\text{\AA}^{-1}$ with 0.2 $\text{\AA}^{-1}$ step. Solid lines are fits to ISF with a logarithmic decay model for β -relaxation region of protein dynamics at various Q -values. (c) and (d) The first order logarithmic decay parameter $H_1(Q,T)$ as a function of Q for dark-state rhodopsin and opsin respectively.

Fig. 5.

A schematic free energy model for the rhodopsin activation process. (a) Opsin* crystal structure (PDB code 3CAP, yellow) is overlaid on dark state rhodopsin crystal structure (PDB code 1U19, green). Activation of rhodopsin involves disruption of two ionic locks, between Glu¹¹³ and the retinylidene protonated Schiff base (PSB), and Glu¹³⁴ and Arg¹³⁵ of the ERY motif (11) (b) Arrhenius plot of characteristic β -relaxation time (τ_β) as a function of inverse temperature for dark-state rhodopsin and opsin. Solid green circles represent τ_β of opsin, open black squares denote dark state rhodopsin, and dashed red and black lines represent the τ_β values fitted with the Arrhenius law. The activation energy (E_β) of the

atomic fluctuations for dark-state rhodopsin and ligand-free opsin apoprotein are 22 ± 2 meV and 23 ± 1 meV, respectively. (c) Schematic free energy model representing the rhodopsin activation process. The black curve displays the free energy of ligand-binding rhodopsin, as a function of arbitrary conformational coordinates. The red curve represents the free energy of ligand-free opsin. The free energy differences between different states contains many contributions, including the direct protein-ligand interactions, hydrophobic association and the conformational and vibrational entropy of the rhodopsin and retinal.