Supplementary Information

Quasi-elastic Neutron Scattering Reveals Ligand-induced Protein Dynamics of G-protein–coupled Receptor

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SAMPLE PREPARATION

Rhodopsin was extracted and purified[1] from bovine rhodopsin disk membranes (RDMs) using a detergent 3-[(3 Cholamidopropyl) dimethylammonio]-1 propanesulfonate) (CHAPS), which is crucial for membrane protein sample preparation[2]. A powdered membrane protein sample containing 73 % (w/w) of photochemically functional bovine rhodopsin and 27 % (w/w) of CHAPS was used for the neutron scattering experiment. A total of about 600 mg of the powdered rhodopsin was used to prepare a dark-state sample, and the photo-bleached ligand-free apoprotein opsin sample. Each of the samples was hydrated with ^2H_2O ($h \approx 0.27$), and enclosed in aluminum foil to prevent exposure to light. Finally, each of the samples was inserted in rectangular aluminum sample holder for the neutron scattering experiment.

NEUTRON SCATTERING EXPERIMENT

The neutron scattering experiment was performed with the near-backscattering spectrometer BASIS at the Spallation Neutron Source (SNS) at Oak Ridge National Laboratory (ORNL)[3]. The BASIS measurements had an energy resolution of 3.4 μeV (HWHM for Q-averaged resolution value). The data analysis was conducted in the dynamic range of ±100 μeV in the energy domain. The analysis in the time domain used the data collected from −120 to +520 μeV. EINS data were obtained by monitoring the elastic intensity determined by the integration over a ±3.4 μeV 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.

DATA REDUCTION AND ANALYSIS

Calculation of mean-squared displacements (MSDs). The mean-squared displacement $\langle x^2(T)\rangle$ of hydrogen atoms in the samples is calculated from elastic incoherent neutron scattering (EINS) measurements[4-5]. Experiments were carried out from 20–300 K, which covers the dynamic transition temperature range in proteins. The EINS data collected from the samples are assumed to be predominantly from the motion of the hydrogen atoms in the protein. The MSD is calculated from the linear fit of the logarithm of the dynamic incoherent scattering function $S_m(Q,\omega=0)$ versus Q^2 using the following formulae:

$$S_{\mathrm{m}}(Q,\omega=0)=\exp(-Q^2\langle x^2(T)\rangle) \qquad (S1)$$

$$\ln S_{\mathrm{m}}(Q,\omega=0)=-Q^2\langle x^2(T)\rangle \qquad (S2)$$

Furthermore, $S_{\mathrm{m}}(Q,\omega=0)$ is calculated from the ratio of the temperature-dependent elastic intensity $I_{\mathrm{elastic}}(Q,\omega=0)$ and elastic intensity at the lowest measured temperature, which is given by:

$$S(Q,\omega=0)=\frac{I_{\mathrm{elastic}}(Q,T,\omega=0)}{I_{\mathrm{elastic}}(Q,T\sim 0,\omega=0)} \qquad (S3)$$

Energy domain data analysis. In a QENS experiment, the measured dynamic incoherent scattering function $S_m(Q,\omega)$ of hydrogen atoms in a protein molecule is a combination of elastic and quasi-elastic components and can be evaluated using the following expression:

$$S_{\mathrm{m}}(Q,\omega)=\left[A_0(Q)\delta(\omega)+\sum_i^N A_i(Q)L_i(Q,\omega)+B(Q)\right]\otimes R(Q,\omega) \qquad (S4)$$

Here $A_0(Q)$and $A_i(Q)$ are the fractions of elastic and quasi-elastic components, respectively, δ(ω) is the Dirac delta function at zero energy transfer representing the elastic component, $B(Q)$ is the background, $R(Q,\omega)$ is the resolution function, and ⊗ is the convolution operation. The

elastic component $A_0(Q)$ contains the information on the length scale of the diffusing entity (hydrogen atoms in this case), whereas the quasi-elastic component provides information on the time scale of the diffusive motion of hydrogen atoms. The quasi-elastic components are represented by two Lorentzians (L_1 and L_2) that correspond to the dynamics of hydrogen atoms in the CHAPS and proteins in the protein-CHAPS samples respectively[6], and are given by the following expressions,

$$L_1 = \frac{1}{\pi} \frac{\Gamma_1(Q,T)}{\omega^2 + \Gamma_1^2(Q,T)} \tag{S5}$$

$$L_2 = \frac{1}{\pi} \frac{\Gamma_2(Q,T)}{\omega^2 + \Gamma_2^2(Q,T)} \tag{S6}$$

where Γ_1 and Γ_2 are the half width at half maximum (HWHM) corresponding to H-atom motions in the CHAPS and proteins, respectively.

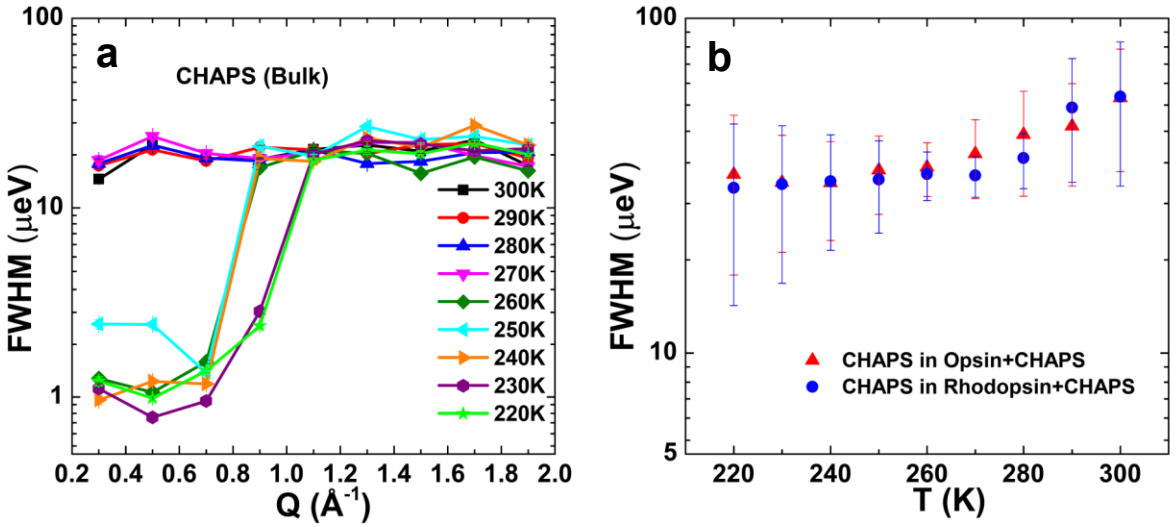

Figure S1. Full-width at half-maximum (FWHM) of Lorentzian ($2\Gamma_1$) of CHAPS component in different samples. (a) Dynamics of CHAPS in bulk CHAPS sample as functions of Q at different temperatures ranging from 220 K to 300 K. (b) FWHM of CHAPS component in protein-CHAPS samples averaged over all Q-values, corresponding to decoupled CHAPS dynamics in these samples at different temperatures. Note that rhodopsin and opsin correspond to the dark-state and the ligand-free apoprotein obtained after light exposure, respectively.

The QENS data were fitted with the peak analysis software PAN in the Data Analysis and Visualization Environment (DAVE) package developed by NIST Center of Neutron Research (NCNR)[7]. Data in the energy domain were fitted in a symmetric energy range of ±110 μeV. The full-width at half-maximum (FWHM, $2\Gamma_1$) obtained by fitting the data from the bulk CHAPS sample with one Lorentzian (one quasi-elastic component representing CHAPS dynamics solely) is plotted in Fig. S1a. It can be clearly observed that above a temperature of T = 260 K, there is no Q- and temperature-dependence of the FWHM of CHAPS. On the other hand, the CHAPS dynamics was studied in the protein-CHAPS complex samples. As explained above, one of the two Lorentzians (L_1) represents the dynamics of the hydrogen atoms in CHAPS. The values of the FWHM of CHAPS averaged over all the Q-values ($2\Gamma_1$) are plotted as a function of temperature, as shown in Fig. S1b. Comparing to Fig. S1a, the FWHM values of CHAPS in protein-CHAPS samples are very close to that in bulk CHAPS sample, implying that the CHAPS motions are similar with or without the proteins, i.e. the presence of protein does not significantly alter the dynamics of CHAPS that can be detected in our QENS experiments. Therefore, when fitting the QENS data from the protein-CHAPS samples with two Lorentzians analysis, the FWHM ($2\Gamma_1$) of CHAPS can be determined according to the results shown in Fig. S1b, and the FWHM ($2\Gamma_2$) corresponding to the protein dynamics can be evaluated as a free parameter after the FWHM of CHAPS is fixed. The FWHM ($2\Gamma_2$) values of dark-state rhodopsin and opsin obtained in this way are plotted in Fig. S2a,b respectively, which demonstrate a clear Q- and T-dependence.

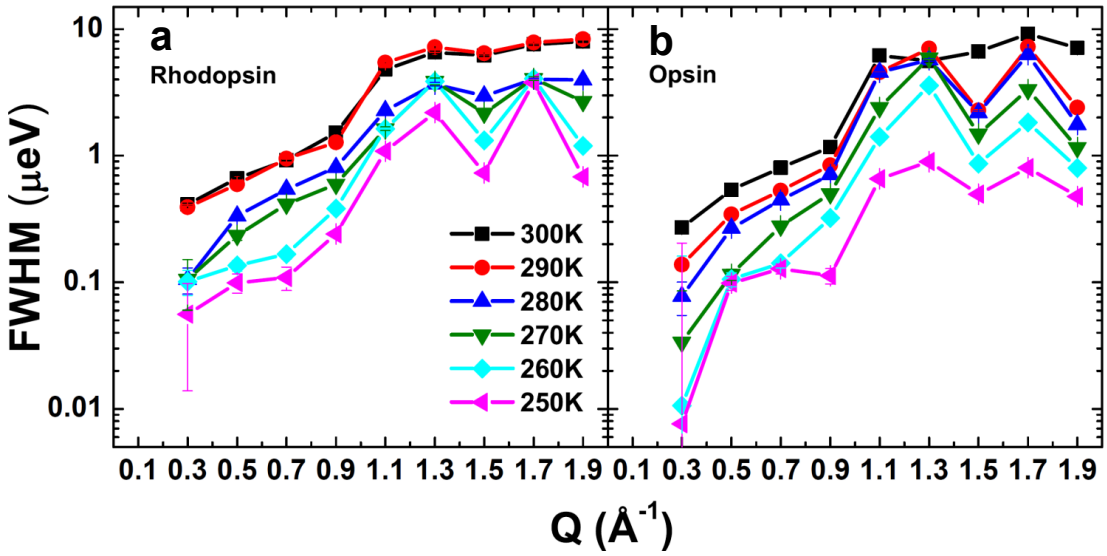

Figure S2. FWHM of Lorentzian ($2\Gamma_2$) of (a) dark-state rhodopsin and (b) opsin as functions of Q at all measured temperatures.

Time domain analysis. For the time domain data analysis, the detergent CHAPS contribution was subtracted from the protein-detergent complex, using the pure CHAPS data. Knowing the molar ratio of the detergent (CHAPS) to protein (rhodopsin or opsin) (~27:1), first we estimated their molecular weight ratio in the complex, which was 0.41:1 in both the cases. Since the total weight of the sample (protein-detergent complex) used for the neutron scattering experiment was known, it was possible to separately estimate the weight of the protein (rhodopsin or opsin) and the detergent (CHAPS) in each experiment. Assuming the protein and the detergent behave independently, one can then eliminate the detergent (CHAPS) contribution to the QENS signal. The QENS signal from CHAPS was multiplied by an appropriate factor based on the amount of CHAPS in the respective protein-detergent samples. Hence, the contribution from the protein dynamic structure factor $S_{\text{protein}}(Q,\omega)$ was calculated for each case. The intermediate scattering

function, $I(Q,t)$ of the reduced $S_{\mathrm{protein}}(Q,\omega)$ is then calculated using Eq. S7. The $I(Q,t)$ represents the relaxation process of mainly hydrogen atoms present in the sample.

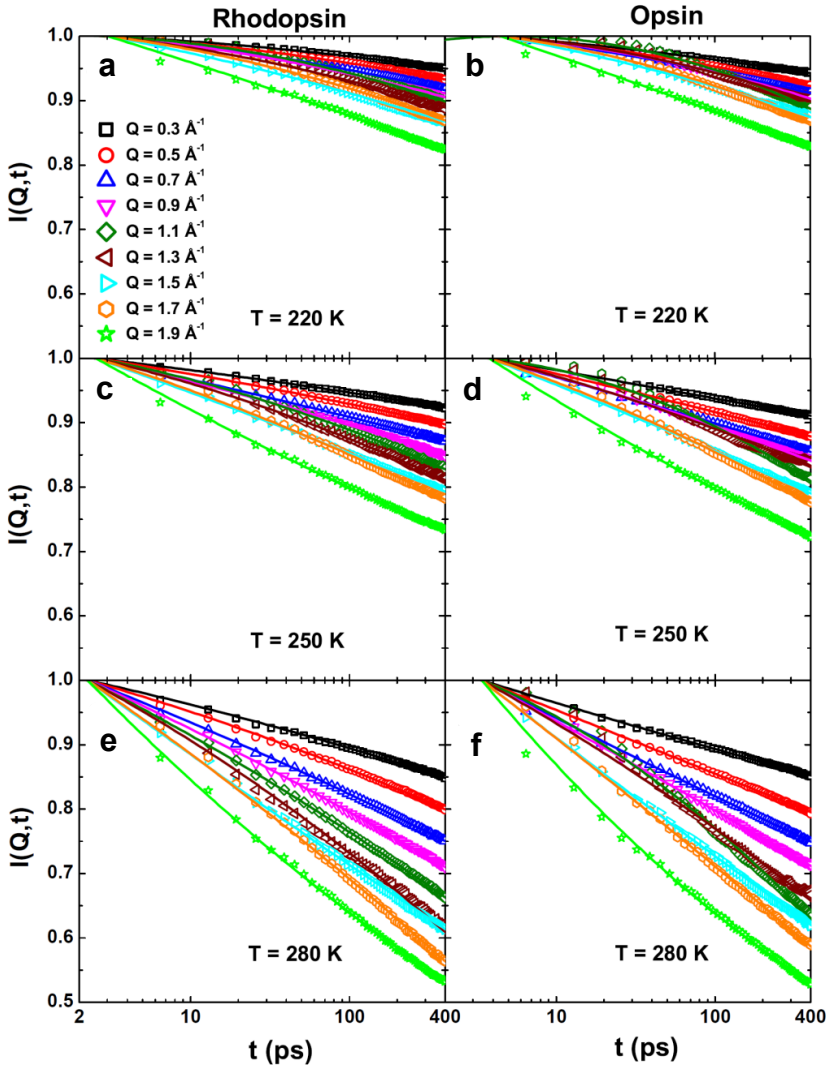

Figure S3. Comparison of intermediate scattering function (ISF) denoted by *I(Q,t)* for dark-state rhodopsin and opsin at temperatures T = 260 K, 280 K, and 300 K for Q-values from 0.3 Å^{-1} to 1.9 Å^{-1} with a step of 0.2 Å^{-1}. The solid lines in the panels are the fitted values to ISF with logarithmic decay model for the β-relaxation region of protein dynamics at the corresponding Q-values and temperatures.

The QENS measurement gives a convolution of the incoherent dynamic scattering function $S_{\mathrm{m}}(Q,\omega)$ of H-atoms in the sample with the energy resolution function *R(Q,ω)* of the

instrument. The inverse Fourier transformation ($\mathcal{F}^{-1}$) of $S_{\mathrm{m}}(Q,\omega)$ yields the intermediate scattering function (ISF) denoted by $I(Q,t)$ of the measured spectra in the time domain, which is calculated as:

$$I(Q,t) = \frac{\mathcal{F}^{-1}[S_{\mathrm{m}}(Q,\omega)]}{\mathcal{F}^{-1}[R(Q,\omega)]} \qquad (S7)$$

One should note that $I(Q,t)$ is one of the most essential parameters to be derived from QENS experiment, which quantifies the time dependence of the relaxation of hydrogen atom dynamics. The time range in the β-relaxation regime (1–400 ps) is much shorter than α-relaxation time range. Hence, it can be fitted with;

$$I(Q,t) = f(Q,T) - H_1(Q,T)\ln\left[t/\tau_\beta(T)\right] + H_2(Q,T)\ln^2\left[t/\tau_\beta(T)\right] \qquad (S8)$$

which is derived from the mode-coupling theory (MCT) for glass-forming liquids[4]. Figure S3 shows the decay of the intermediate scattering function (ISF) of hydrogen atoms in rhodopsin and opsin at T = 260 K, 280 K, and 300 K respectively at a series of Q-values. In Eq. S8 the characteristic β-relaxation time is τ_β and $f(Q,t) = \exp(-A(T)Q^2)$ is the temperature-dependent prefactor, proportional to the Debye-Waller factor for small Q-values. The Q- and T-dependent first-and second-order logarithmic decay parameters $H_1(Q,T)$ and $H_2(Q,T)$ respectively, can be written as:

$$H_1(Q,T) = h_1(Q)B_1(T) \qquad (S9)$$

$$H_2(Q,T) = h_1(Q)B_2(T) \qquad (S10)$$

Here $h_1(Q)$ is a power law of Q for small Q-values, while $B_1(T)$ and $B_2(T)$ are temperature-dependent fitting parameters. The direct relationship between $H_1(Q,T)$ and Q is given by $H_1(Q,T) = B_1(T)Q^\beta$, where β is the exponent of Q, which takes the value ~1-2, and $B_1(T)$ is a temperature-dependent parameter. The temperature dependences of the first-order logarithmic-

decay parameter $H_1(Q,T)$ obtained from the fits for different Q-values for rhodopsin and opsin, are shown in Fig. 3c,d in the main text respectively. The corresponding values of $B_1(T)$ are shown in Fig. S4.

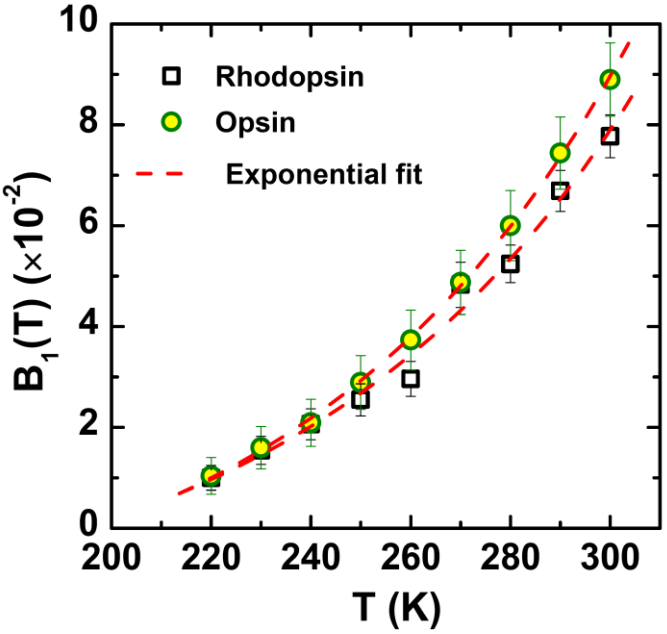

Figure S4. Plot of $B_1(T)$ as a function of temperature for dark-state rhodopsin and opsin fitted with exponential function.

REFERENCES

(1) Struts, A. V.; Chawla, U.; Perera, S. M.; Brown, M. F. Investigation of Rhodopsin Dynamics in its Signaling State by Solid-State Deuterium NMR Spectroscopy. *Methods Mol. Biol.* **2015,** *1271*, 133-58.

(2) Aveldaño, M. I. Phospholipid Solubilization during Detergent Extraction of Rhodopsin from Photoreceptor Disk Membranes. *Arch. Biochem. Biophys.* **1995,** *324*, 331-343.

(3) Mamontov, E.; Herwig, K. W. A Time-of-Flight Backscattering Spectrometer at the Spallation Neutron Source, BASIS. *Rev. Sci. Instrum.* **2011,** *82*, 085109.

(4) Chu, X.-Q.; Lagi, M.; Mamontov, E.; Fratini, E.; Baglioni, P.; Chen, S.-H. Experimental Evidence of Logarithmic Relaxation in Single-Particle Dynamics of Hydrated Protein Molecules. *Soft Matter* **2010,** *6*, 2623-2627.

(5) Chen, S. H.; Mallamace, F.; Chu, X. Q.; Kim, C.; Lagi, M.; Faraone, A.; Fratini, E.; Baglioni, P. Neutron Scattering Studies of Dynamic Crossover Phenomena in a Coupled System of Biopolymer and its Hydration Water. *JPCS* **2009,** *177*.

(6) Chu, X.-Q.; Mamontov, E.; O'Neill, H.; Zhang, Q. Apparent Decoupling of the Dynamics of a Protein from the Dynamics of its Aqueous Solvent. *J. Phys. Chem. Lett.* **2012,** *3*, 380-385.

(7) Azuah, R. T.; Kneller, L. R.; Qiu, Y. DAVE: A Comprehensive Software Suite for the Reduction, Visualization, and Analysis of Low Energy Neutron Spectroscopic Data. *J. Res. Natl. Inst. Stan.* **2009,** *114*, 341-358.