Protein dynamics of bacteriorhodopsin probed by photon echo and transient absorption spectroscopy

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The outcome of a photochemical reaction in the condensed phase depends on the nature of the reacting chromophore and its environment, which may for instance be a liquid, glass or a protein. In liquids, solvation dynamics has been well characterized, primarily through the use of time-resolved fluorescence and photon echo techniques, combined with molecular dynamics (MD) simulations [1]. Two solvation components are often observed: a fast inertial response, occurring on a timescale less than 100 fs, followed by a slower, diffusive component on a picosecond timescale. The inertial response often accounts for half the total reorganization energy or more, and entails independent, underdamped motion of the solvent molecules in their native potential wells. The diffusive component represents the reorientation of the solvent molecules into a new equilibrium, whereby the nuclei may hop between different potential wells. It involves collective motions of the solvent molecules, like librations and large rotations. It is thought that the diffusive solvent rearrangement plays an important role in chemical reactivity, as it may reduce reaction barriers and stabilize reaction intermediates [2].

Bacteriorhodopsin (bR) is an efficient light-driven proton pump which shows a trans-cis isomerization reaction of its retinal chromophore upon absorption. The protein environment of retinal in bR not only serves to exert the light-triggered biological function, but also actively catalyzes the isomerization of retinal [3]. Despite the protein's defining role on bR's photochemistry, the protein solvation dynamics, i.e., the response of the protein matrix to photoexcitation of retinal, has largely remained unknown.

![Graph A](image1)

**Fig. 1A**: 3PEPS profile of bR taken at 568 nm (circles); **B**: TA profile of bR excited at 605 nm, probed at 870 nm (circles); **C**: same as B for the D85S mutant. Solid lines denote the result of an MBO simulation as described in the text.

To assess the protein solvation dynamics of bR, we have performed a three-pulse echo peak shift (3PEPS) study. Fig. 1A shows a 3PEPS measurement on bR recorded at 568 nm (circles). The peak shift profile is directly related to the transition frequency correlation function, M(t), and hence provides a direct probe of the time scale on which bR loses memory of its original transition frequency [1]. Moreover, unlike other techniques like time-resolved fluorescence, transient absorption (TA) and transient grating (TG), the peak shift is insensitive to electronic lifetime effects [1]. The peak shift of bR has a low initial value \( \tau_0 \) of 10.5 fs, indicative of a large reorganization energy \( \lambda \). The peak shift decays rapidly, within 80 fs, to a terminal value of 0.5 fs. After this, no discernible relaxations occur up to a population time of 1 ps. The 3PEPS results thus denote that the diffusive, picosecond solvation timescale is completely absent in bR's solvation dynamics.

Figure 1B shows a TA measurement on bR probed at 870 nm on excitation at 606 nm (circles). At this wavelength, stimulated emission (SE) from the excited state to the ground state at the far red edge of the emission
spectrum is observed and thus the dynamic Stokes shift is monitored. The TA signal at 870 nm shows a rapid rise of the SE, followed by a decay in ~300 fs. The shape of the TA signal during the first ~200 fs is non-exponential and weak oscillations can be discerned. The phenomenon that the SE from BR rises very rapidly has been well documented [3] and is consistent with the rapid decay of the peak shift. Fig. 1C shows the TA signal in the D85S mutant at 870 nm on excitation at 600 nm. Again, a rapid rise of the SE is observed. In contrast to BR, the SE hardly decays during the first picosecond as a result of a 10-fold increased excited state lifetime. It could be argued that in BR, evolution of the SE due to 'diffusive' type protein solvation components on a sub-ps to ps timescale could go unnoticed owing to the short excited state lifetime of 300 fs. The increased lifetime of the D85S mutant provides for an extended detection window, and any such evolution would be revealed. However, no slow rise components are observed, which indicates that protein solvation in the D85S mutant does not occur on a >100 ps timescale.

Fig. 2. A: absorption and fluorescence line shapes of BR (solid lines), result of the MBO simulation (dashed lines), B: same as A for the D85S mutant. The measured absorption and fluorescence spectra were divided by u and v respectively to obtain the line shapes.

To quantitatively account for the excited state properties of BR, the data are simulated in the framework of the multimode Brownian oscillator (MBO) model [4]. We describe BR's line shapes and nonlinear spectroscopic features with a minimal two-component model, consisting of a Gaussian protein relaxation with a decay of 50 fs and a λ of 1430 cm⁻¹ (BR) or 1060 cm⁻¹ (D85S mutant), and the intramolecular modes of retinal derived directly from resonance Raman spectroscopy [5], accounting for 1100 cm⁻¹ of the total λ. The static absorption and fluorescence line shapes of BR and the D85S mutant, in particular the Stokes shifts, are well reproduced with these parameters, as shown in Fig. 2. The results of the MBO simulation for the 3PEPS and TA measurements are shown as solid lines in Figs 1A, B and C. The agreement with the experimental data is satisfactory, and we conclude that this minimal nonreactive model is sufficient to reproduce the essential properties of the linear and nonlinear signals in BR.

The Gaussian component can, in analogy with solvation dynamics in liquids, be related to inelastic motions of the individual groups of the protein scaffolding. Our results strongly indicate that after the inertial relaxation, no diffusion-type motions take place up to a few ps in BR. The absence of such diffusive motion is very likely due to the covalently constrained, polypeptide nature of the protein. The sub-ps to ps region is the time scale on which retinal's isomerization takes place, and our results thus imply that during this time, the protein's nuclei do not restructure themselves in a significant way. The idea that such a protein rearrangement would take place in BR to actively stabilize a transition state which enables the high speed, efficiency and selectivity of the isomerization photo-reaction thus seems to be unlikely.

Our results are in close agreement with MD simulations on BR, which indicated that after retinal excitation, which is accompanied by a significant charge relocalization along the polypeptide backbone, the protein exhibits an extensive dielectric relaxation on a 100 fs timescale representing an energy change of ~1700 cm⁻¹ [6]. The MD simulations showed that the relaxations involve relatively small motions of many nuclei up to relatively long distances from the retinal chromophore. The protein conformation was distorted only minimally due to large activation barriers, in agreement with our finding that diffusive protein relaxations do not occur.