Dynamics of different functional parts of bacteriorhodopsin, H-^{2}H labeling and neutron scattering by V. Reat, H. Patzelt, M. Ferrand, C. Pfister, Oesterhelt, G. Zaccai , PNAS (1998) 95, 4970

Abstract
In contrast to the protein globally, the thermal motions of the labeled atoms were found to be shielded from solvent melting effects at 260 K. Above this temperature, the labeled groups appear as more rigid than the rest of the protein, with a significantly smaller mean square amplitude of motion. These experimental results quantify the dynamical heterogeneity of BR (which meets the functional requirements of global f lexibility), on the one hand, to allow large conformational changes in the molecule and of a more rigid region in the protein, on the other, to control stereo-specific selection of retinal conformations.

Comment by Wolfgang Doster at Bioneutron.de

The conclusions of this "seminal" dynamics paper, derived from elastic scattering only, are essentially wrong. The main problem is their incomplete understanding of neutron scattering data at the time. For dry and hydrated myoglobin, after performing complete spectral analysis, we derived two dynamic components (Doster Nature 1989, Doster, Setttles BBA 2005):

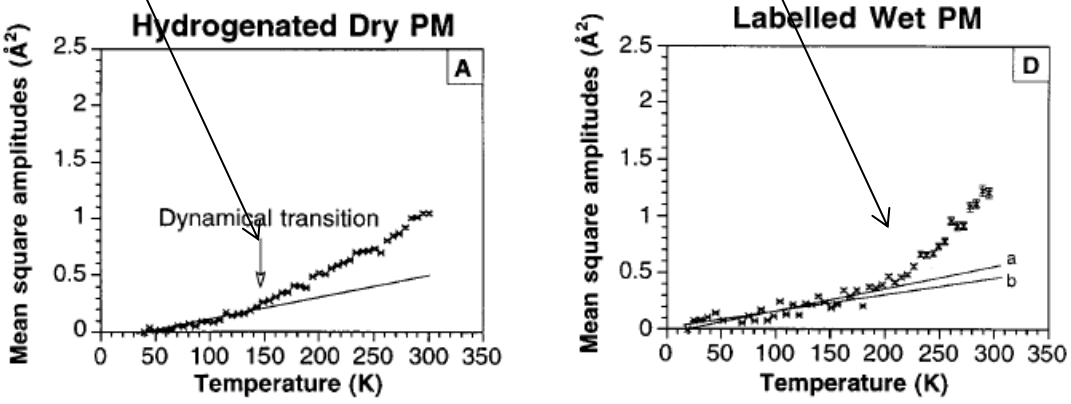
(1) rotational transitions of side chains (methyl groups), onset of "anharmonic" displacements around 160-180 K for IN13 and

(2) Gaussian small scale water-assisted motions, which emerge at 240 K above vibrational level (IN13), the protein dynamical transition (PDT).

For their BR sample, the authors compare dry protonated BR, which only shows the onset due to methyl group rotation (1). In the labelled sample BR, all methyl groups were deuterated except some amino acids inside the core, which were protonated, but díd not contain methyl groups. Thus only the water-assisted PDT (2) transition occurs but not (1). The celebrated difference thus originates from methyl groups contributing to the displacements or not, which **has nothing to do with dynamic heterogeneity** as postulated by the authors.

1 (not a dynamical transition) (dynamical transition)

2



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Some technical remarks:

1) The authors do not understand the neutron scattering technique and how dynamic information is derived. They use the analogy to small angle static scattering:

"The similarity is expressed in equ. 1 which is analogous to the Guiner approximation in classical small angle scattering…

$$ln(I(Q)el) = A - 1/6 \langle u^2 \rangle Q^2 \quad (1)$$

where I(Q,el) is the EINS as a function of the scattering vector Q.., $\langle u^2 \rangle$ is the mean square amplitude describing the spatial extent of ***the atomic "blurs"*** *and A is a constant. Note that we followed the definition given by Smith, which refers to the full amplitude of the motion. It differs by a factor of 2 from the definition of Ferrand et al in the first EINS study of BR which referred to displacements from the average atomic positions (in the harmonic approximation)."*

The atomic blurs is actually in the mind of the authors including Jeremy Smith, which gives an incorrect Lamb-Mössbauer factor in his QRB article in 1991 (see comment). Equ. 1 is not a matter of definition, the displacements are determined as averaged projections on the wave vector of the type $\langle (Q\Delta \mathbf{r})^2 \rangle$**.** For powder samples and incoherent scattering only relative displacements survive the averaging, there is no blur.

2) Their understanding of the PDT (protein dynamical transition) is primitive and wrong:

The first EINS measurements on a protein were performed on myoglobin (13). Plotting $\langle u^2 \rangle$ vs. T (absolute temperature) allowed these authors to investigate the nature of protein motions. A classical harmonic regime at low temperatures was described, where $\langle u^2 \rangle$ is proportional to T and extrapolates to close to $T = 0$. The dynamical transition was shown by a break in the line, leading to an anharmonic regime with a steeper increase in $\langle u^2 \rangle$ vs. temperature. Protein motions in the higher temperature regime can be modeled in various ways (13, 30). For example, the dynamical transition can be related to a simple interpretation within the conformational substate (CS) model (33). Each protein molecule, at low temperatures, is trapped in one of many possible conformational substate potential wells, where it vibrates harmonically. At the dynamical transition temperature, it can acquire sufficient activation energy to move anharmonically between conformational substate potential wells. In hydrated myoglobin, a dynamical transition was observed at ≈180 K that could be accounted for

The PDT is identified with any deviation of MSD from linearity. In Doster et al. 1989, the PDT transition temperature was spotted at 240 K , resolving motions coupled to hydration water. The method to capture dynamics by adjusting straight lines to elastic scattering data is still used today in 2014. Further analysis in 2018 (see preprint, and “are proteins heterogeneous? “

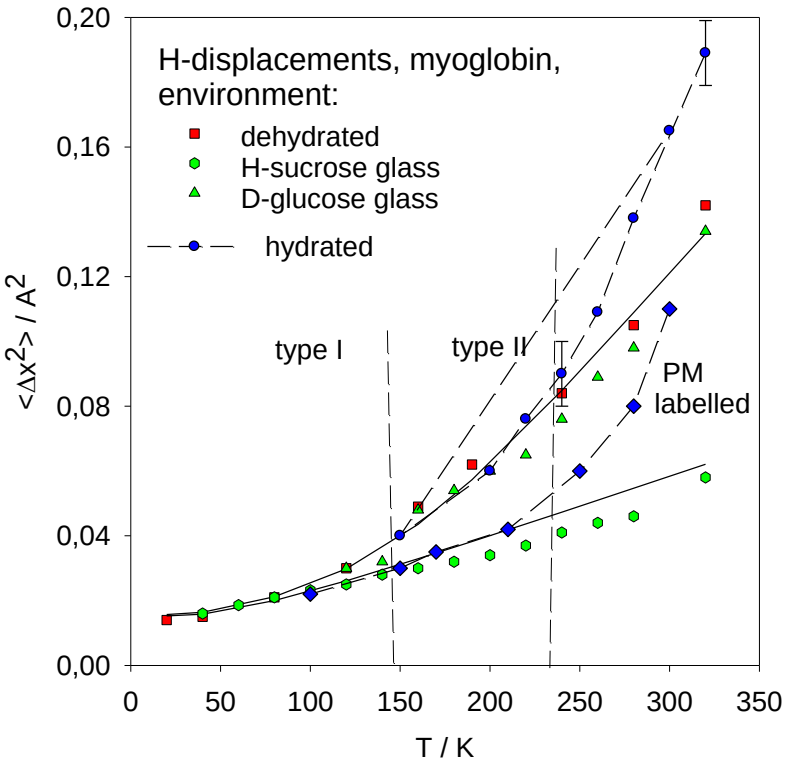

Fig. 1: mean square displacements (IN13, τ_{res} = 140 ps) of myoglobin in various environments: dehydrated (red squares), hydrated (blue circles), green triangles: per-deuterated glucose glass, green circles: D/ H-sucrose glass[10,2], blue diamonds: H-labelled per-deuterated PM fragments[10], line: calculated f vibrational displacements[16]. The onset temperatures at 150 K (I) and 240 K (II) are indicated. (PM = purple membrane)

Surprisingly, dehydrated myoglobin exhibits nearly the same MSD temperature dependence as myoglobin in the deuterated glass[3]. The type I transition at 150 K is thus attributed to the onset of protein internal motions, which are not sensitive to changes of the protein environment. By contrast, with D_2O- hydrated myoglobin, two transition temperatures at 150 K (type I) and 240 K (type II) are recorded, suggesting two well separated molecular processes. Since the second transition (type II) does not occur without water, these motions were assigned to protein displacements related to a wet protein surface[1,3]. Since the scattering fraction of hydration water (D_2O) amounts to less than 5 %, type II motions characterize the indirect effect of hydration on structural fluctuations. Experiments performed with "wet" per-deuterated purple membrane fragments yield similar but noisier MSD scans with two transitions at the same temperatures as hydrated myoglobin. By contrast, if the per-deuterated fragments are specifically labelled with protonated, but methyl-free residues, the type I transition is missing, although type II at 240 K is still occurs[10]. This implies, that type I displacements reflect mostly the methyl side chains[3,4]. Then Type II by contrast may involve prominently polar residues near the surface. But an indirect effect of hydration on the mobility of nonpolar, non-methyl side chains cannot be excluded.