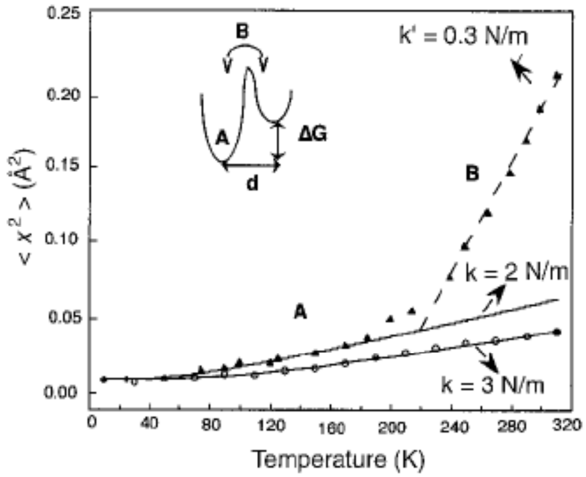
How Soft is a Protein? Force Constants Measured by Neutron Scattering

J. Zaccai, Science 2000 (Letter to the Editor)

The basic intention of this prize winning paper is to propose a simple method, how dynamic neutron scattering can be applied directly to solve biological problems: The mean square displacements play the role of a shortcut to biology. This is the low angle scattering approach to protein dynamics, where J. Zaccai has a lot of expertise. His method has several advantages: (1) it is easy to perform elastic scans versus the temperature and it requires little beam time. This means that you can easily flood the field with displacement papers (displacementology) performed with numerous biologically attractive molecules. (2) The analysis is deceptively simple, consisting of two linear fitting procedures, which avoids a complicated nonlinear spectral analysis: a) the slope of elastic intensity curves is determined by linear fits assuming a Gaussian Debey Waller factor, b) the temperature dependent displacements are approximated by linear regions (figure below), c) The resulting slope has an attractive physical meaning as a protein force constant. To make such suggestions is only possible if you have no idea of condensed matter physics or molecular dynamics. It is surprizing to me, how many MD people including the referees of major journals and a prize selection committee bought it inspite of drastic errors. The basic idea of using MSDs was taken from our publication in Nature (1989), where the protein dynamical transition was defined. I found it difficult to communicate with J. Zaccai about why I believe his method is too simple to yield relevant information. I never saw a Zaccai paper before it was published. He must have excluded me as a referee for more than 20 years, although he used my work in his numerous publications. Unfair competition?

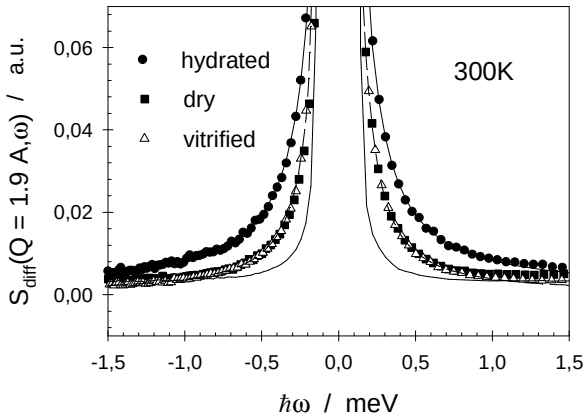
All conclusions in this Science paper are incorrect and were never corrected.



(1) in this figure Zaccai plots the myoglobin MSDs (triangles,1989), which are approximated by two slopes. This is quite incorrect, since we have demonstrated the existence of two transitions, which requires three slopes, vibrations (below 150 K), activated rotational transitions of side chains (>160 K) which were unified as the A regime, and the water coupled B regime above 240 K.

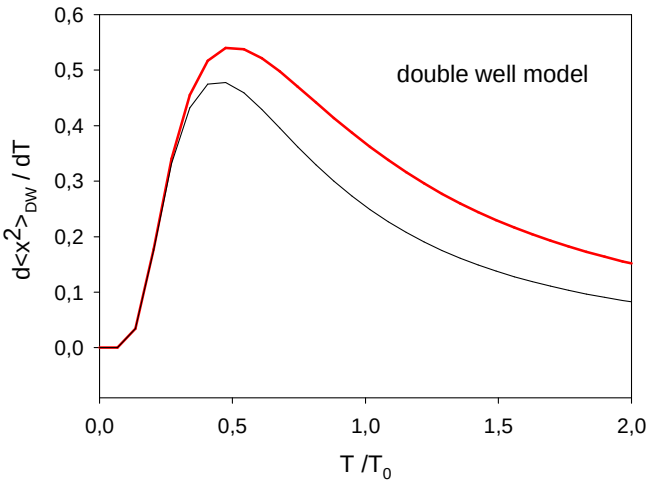
(2) The figure also shows new myoglobin data in a D-exchanged trehalose solvent. The conclusion is: all activated processes in the protein are arrested by the glassy solvent. We have repeated this experiment with D-exchanged and perdeuterated glassy glucose (Doster,

Settles BBA 2005). Result: Only the solvent coupled process, PDT above 240 K, is absent in the glass similar to the dry protein. The rotational transitions of side chains, which are resolved above 160 K are also active in the glassy state, they are independent of the solvent. Using D-exchanged protonated glucose and trehalose we could reproduce the Zaccai result. This means that in this experiment the protonated D-exchanged glassy trehalose dominates the cross section, this is trehalose not myoglobin. In our BBA paper (2005) we could assign the rotational transitions to methyl groups. With the incoherent structure factor of methyls and the T-dependent transition rate we could for the first time reproduce the experimental mean square displacements. Zaccai still believes his original explanation according to his lecture at a Les Houches seminar in 2013.



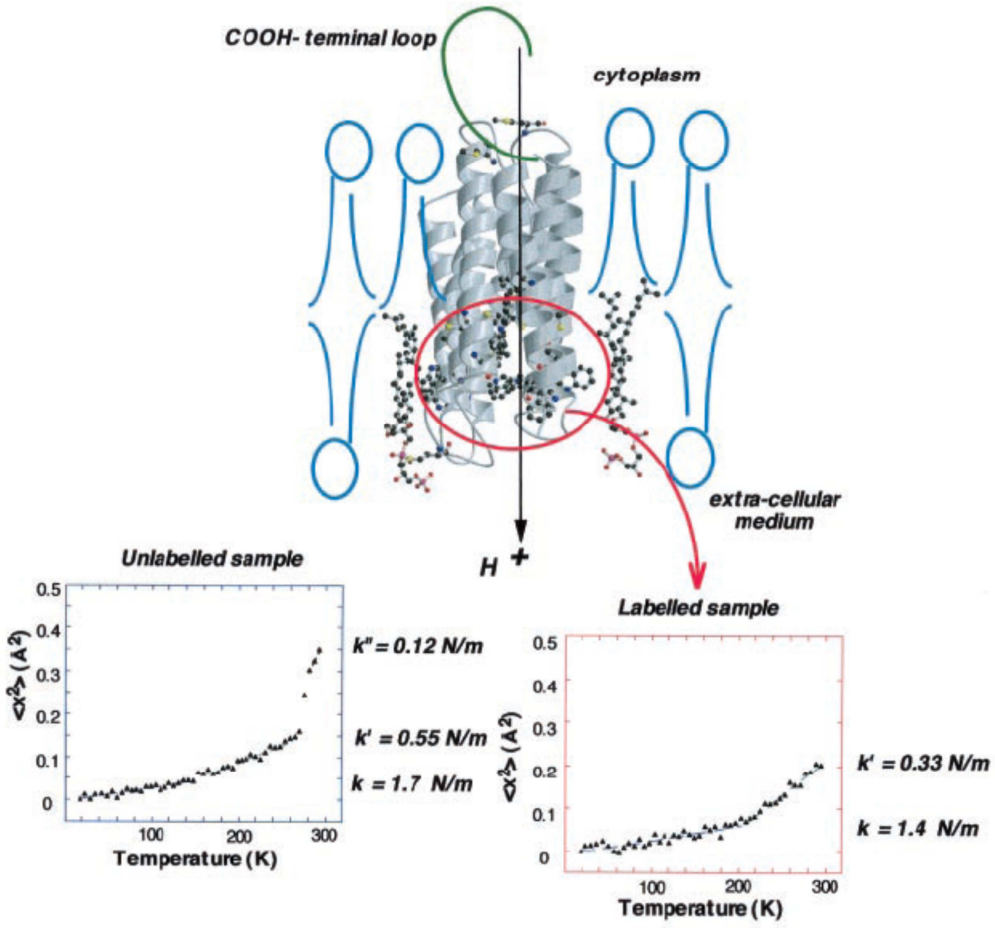
The figure compares IN6 TOF spectra of hydrated, dry and D-glucose vitrified myoglobin including the resolution. The dry and the vitrified protein spectra agree with each other showing significant quasi-elastic scattering due to methyl rotation (Doster, Settles BBA 2005).

(3) The force constant model strictly applies to a single harmonic oscillator but not to activated or diffusive processes as proposed in the first figure.
The figure below plots the MSD slope versus temperature for a symmetric and asymmetric (red) double well model (Doster, EBJ 2008, concepts and misconceptions of the PDT): The slope is never constant, it goes through a maximum and does not reflect a force constant. It is determined by an activation energy and entropy (Doster et al. JCP 2013, see review). The K' numbers above the PDT are cannot be called a resilence.



(4) The comparison of MSD of labelled and unlabelled BR does not show, that the BR core is less flexible than the "soft cytoplasmatic half". Instead the deuteration of the core of bacteriorhodopsin removed the protonated methyl groups, which no longer contribute to the displacements of the D-labelled sample. This interpretation was first given by us (Doster EBJ (2008)):….

isotopic substitution by CD_3. With perdeuterated bacteriorhodopsion the initial transition at 150–180 K is indeed absent (Zaccai 2000), which supports the above assignment.



Not until 2010 Wood et al. (JACS 1 32 4990) discussed the effect of methyl rotation in BR. The original interpretation of Zaccai was not withdrawn. Our first time methyl group analysis (Doster Settles BBA 2005) was of course not cited.

Result: MSD values derived from elastic neutron scattering cannot be used to derive protein force constants. What kind of dynamic information can be derived from an elastic scattering analysis was discussed recently by Doster et al. JCP (2013) 103, 45105.