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Molecular dynamics of hydrated proteins

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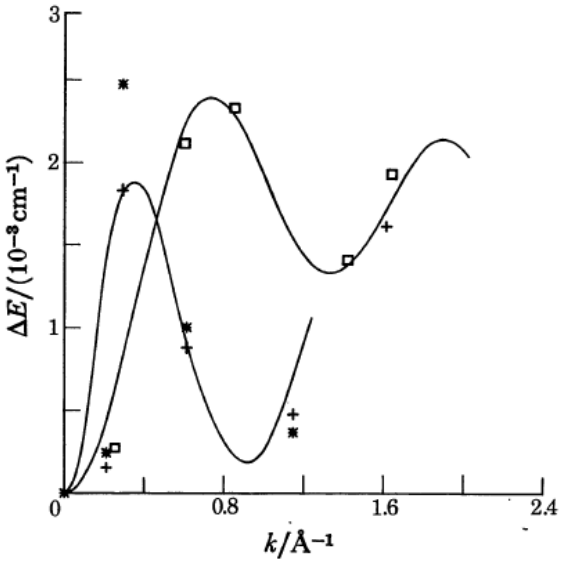
Comment to: Molecular dynamics of hydrated proteins by W. Doster

It is now quite popular to study water on top of perdeuterated proteins (see later comments). Although this sounds logical, there are serveral traps to be avoided. This experiment was first performed in 1980 with D-phycocyanin (CPC) by H. D. Middendorf, a pionier of applying dynamic neutron scattering to biological molecules. The same sample was used later by M.C. Bellissent and S. Chen. The striking result of his IN 10 back-scattering experiment was a water correlation time (linewidth ΔE) oscillating with momentum exchange k (figure)!!



C-PC linewidth, open sqares: hydrated 90%, crosses, full squares: low and intermediate hydration.

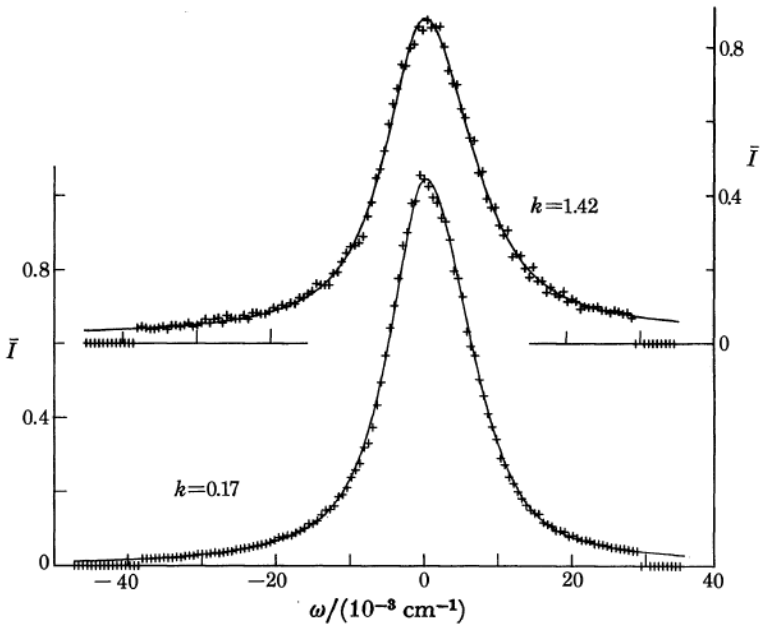
This result would imply transitions of water molecules between distinct sites of a regular arrangement on the protein surface.

These data were never reproduced in later studies, but also no corrections were ever published. In fact, Middendorf discusses the same results uncritically in a Review paper 16 years later: Physica B 226 (1996)116 entitled "Neutron Scattering Studies of Biological Water". The term "biological water" is just a catch word and has no sound scientific basis. Because of the title, it is used as a basic reference in review articles such as on "confined water" (Cerveny et al. Chem. Rev. (2015) oct 13), thus a discussion still matters.

In the context of my own experiments with H_2O-CPC I became interested in the question, what went wrong in this study. This problem seems still relevant to current investigations. I came across a number of disturbing deficiencies:

(1) The oscillating linewidth is seen for low, intermediate and full hydration. I would expect to see just the resolution at low hydration.
(2) The quasi-elastic broadening is ten times smaller than the instrumental resolution, about 0,01 cm^{-1}.
(3) This variation in linewidth with k by a factor of two should be easily visible in the spectra. But only two spectra (fully hydrated) are shown for k = 0,17 and 1,42 A^{-1} .

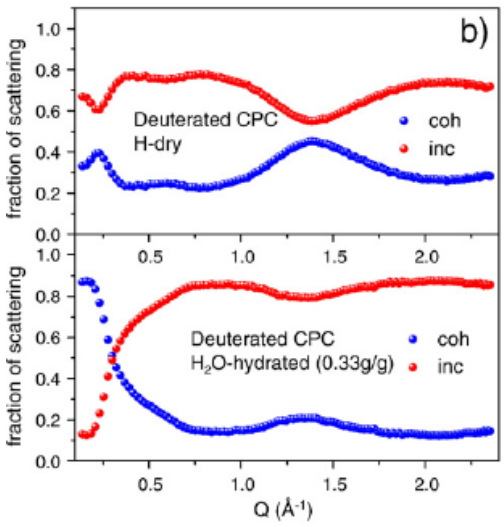
the two spectra of fully hydrated CPC stacks at two k values:



The low k spectrum is somewhat narrower, but not much, considering the factor of ~10 difference in k, which would be a factor of 100 in linewidth for a k^2 dependence in the case of regular diffusion. I performed single Lorentzian fits with selected data points, which works remarkably well at both k-values in contrast to a Gaussian shape. The respective widths are 0,012 cm^{-1} at low k and 0,014 (± 0,0003) cm^{-1} at k = 1.42. These numbers are identical with the given resolutions determined from the dry sample. Thus there is no line-broadening at all! In the linewidth plot, the ΔE at low k is 0,00025 cm^{-1} and 0,002 at k = 1.6. These numbers are not evident from the spectra, which exhibit a much larger width. How do you get a width of 0,00025 cm^{-1} with a resolution of 0,012, which is not shown in the figure? The authors resort to wet-dry "difference broadenings".

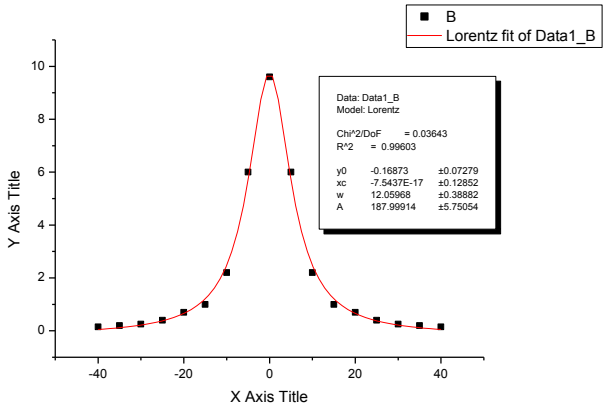
(4) By varying k from 0.17 to 1.6 one has an intricate interplay of coherent and incoherent scattering (*Polarisation analysis of dry and hydrated C-PC, Gaspar et al. BBA 1804*

(2010) 76): At k = 0,17, the scattering is about 90 % coherent, while at 0.8 it is almost 90 % incoherent. The incoherent water signal will thus emerge from nothing with increasing k to nearly 100 %.

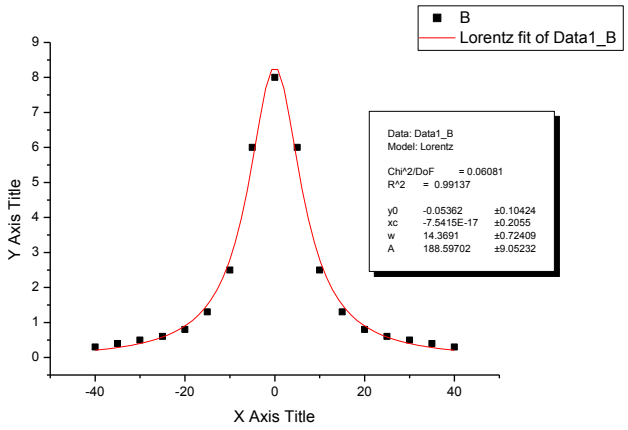


(5) This implies that the spectrum at k = 0.17 (hydrated) should be identical with the nearly Gaussian resolution function of the dry protein. By contrast at k = 1.6 it should be dominated by the resolved Lorentzian spectrum of hydration water. However from my preliminary fits I conclude that the lineshapes are Lorentzian at high and low k with similar linewidths of 12 and 14 cm^{-1}! Moreover, the spectral areas as determined by the fits are precisely identical!! We are thus looking at the same quasi-elastic Lorentzian process, whose linewidth changes only slightly with k, irrespective of the drastic change in cross-section. This is almost impossible! The only process that comes to my mind is multiple scattering, which produces a nearly k-indpendent spectrum averaging over all k (Cusack, Doster, BJ (1990), Settles/Doster in Biological Macromolecular Dynamics (1996)). For 600 mg protein hydrated with 200 mg H_2O (stacks) multiple scattering would play a significant role. One could conclude that the oscillation just outlines the error bar implicit in this data analysis.

(6) *Lorentzian Fits of hydrated CPC spectra at k = 0,17 and 1,47 A^{-1}*:



(7)



Conclusion:

The relevance of multiple scattering and a discussion of the coherent and incoherent cross sections should play a more important role in serious publications.