The riddle of the Gaspar TOF experiment: comparison of casein with compact folded proteins

In a comprehensive study (25) Gaspar et al. compare three globular proteins and three unfolded caseins in different states, dry, hydrated and in solution. They applied the method of neutron time of flight spectroscopy (TOF). With the samples in solution, the conditions were chosen to stabilize a low degree of association. Two distinct spectral components were identified: The broad component displayed a q-independent line width, corresponding to a 5 ps time scale and was assigned to methyl rotational transitions. The linewidth of the second narrow component varied with q^2 and was thus assigned to translational diffusion. This linewidth is reproduced from ref. (25) for myoglobin and α and β casein in fig. 15. The authors identified as the main difference an offset in the diffusion line width $\Gamma_{diff}(q \rightarrow 0)$ at low q of 3 - 4,5 μeV in all three casein samples. For the globular proteins the offset was near 1 to 1,5 μeV. "The presence of this offset appears to suggest the presence of other structural relaxation processes on the time scale of global diffusion". The question of limited resolution, ~ 30 μeV, was mentioned, and back-scattering experiments were suggested. Perez et al. (57) have shown for the first time, that diffusion broadenings of a few μeV observed with protein solutions can be discerned from a ten time broader central line (fig. 15). The discrepancy observed with the offset between compact and unfolded proteins motivated the present study.

With protein solutions, much less for hydrated samples, diffusion broadening of the central line however reduces the apparent instrumental resolution. It is thus more involved than with hydrated samples to deduce the spectral broadening Γ_{relax} of slow internal relaxation processes relative to q^2D:

$$\Gamma_{diff}(q) = q^2 D_s + \Gamma_{relax} \quad (16)$$

For incoherent neutron scattering the q-dependent spectral linewidth of global diffusion, equ.(16), constitutes the main method to deduce slow structural relaxation processes from the spectrum. D_s denotes the q-independent self- or tracer diffusion coefficient.

With coherent neutron scattering an additional option exists: One derives large scale molecular motions from a q-dependent collective diffusion coefficient $D_c(q)$. The dynamic deviation from the rigid body diffusion coefficient, caused by normal modes, is most pronounced in the vicinity of the maximum of the solution structure factor S(q) (55,56). The predicted effects of interdomain motions on $D_c(q)$ are small and have to be discriminated from other effects due to protein interactions at high concentrations (23).

In the case of β-casein, the slow global structural relaxation process (GSR) observed with coherent scattering, is displayed in fig. 5 directly in the time domain. The respective q-values range above the maximum of the structure factor in the incoherent region, implying self-diffusion. The slow process is thus not the result of dynamic deviations in $D_s(q)$.

Turning back to incoherent scattering, we now investigate, whether the offset in fig. 15 reflects indeed the "fast process" observed with NBS as assigned in fig. 13.

For this purpose TOF data recorded in the frequency domain (25) were transformed to the time domain (26), deconvoluted from the resolution function, by numerical Fourier transformation at constant q. The resulting intermediate scattering functions of myoglobin

and β-casein in solution are displayed in fig. 16 at the lowest q = 0,6 A^{-1}, where the relaxation contribution dominates. The intermediate scattering function of β-casein decays faster than for myoglobin in spite of a larger diffusion coefficient of the latter. A two-component analysis accounting for methyl rotation and global diffusion was performed according to:

$$I_s(q,t) = (1- A_{met}(q) + A_{met}(q)) \exp(-t/\tau_{met})) \exp(-t/\tau_{diff}(q)) \qquad (17)$$

$A_{met}(q)$ denotes the elastic fraction of methyl rotation, τ_{met} denotes the respective rotational correlation time and $\tau_{diff} = 1/\Gamma_{diff}(q)$ is the relaxation time of global diffusion, including the structural relaxation. Two kinds of fits were performed with and without accounting for the offset in fig. 15. If no offset was assumed, τ_{diff}^{-1} was fixed at q^2D_s, while including the offset implies equ.(16). Then the additional relaxation process leads to a faster longtime relaxation.

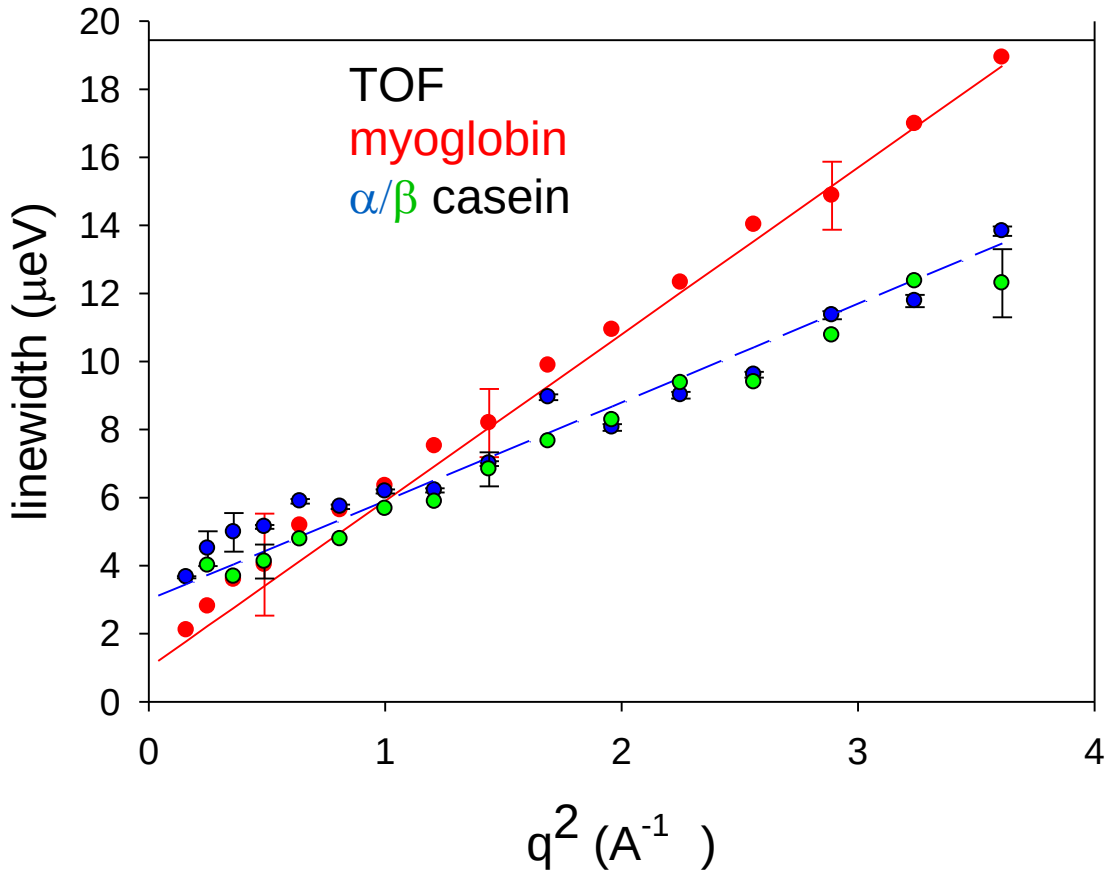

Fig. 15: linewidth of the diffusion spectral component of the TOF spectrum versus momentum exchange squared: myoglobin, α and β casein in solution at low degree of association (ref. 25)

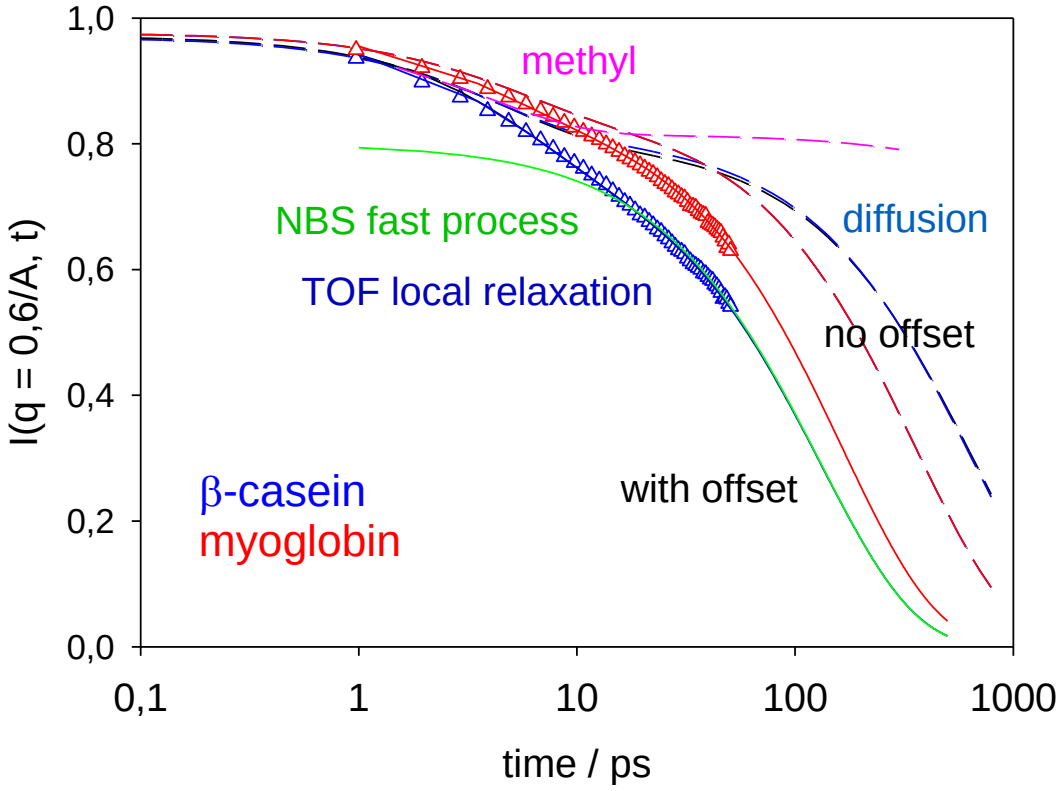

Fig. 16: incoherent intermediate scattering functions of myoglobin and β-casein (TOF and NBS)

Table 2: TOF time domain fits including the offset in comparison with the NBS fast process

		q	A_{met}	τ_{met} ps	$\tau_{diff}(q = 0,6\ A^{-1})$ ps
β-casein	TOF	0,6	0,19	5,2 (± 0,2)	130 (± 10)
	NBS	fast process			130
myoglobin	TOF	0,6	0,11	5,2	165 (± 20)
	NBS	fast process			150

It is obvious, that without offset, the data cannot be explained even inside the limited available time range. With offset the fits are perfect. The parameters of the free fit to equ.(17) are shown in Table 2. As already noticed by Gaspar et al. (25), the amplitude of the methyl rotation is larger with the disordered β-casein than for the compact myoglobin, while the correlation times are similar. The correlation time for diffusion-relaxation at low q amounts to 130 ps in the case of β -casein. A very similar value was obtained for the fast or local structural relaxation by backscattering (fig. 13). At this low q, the line-width is dominated by the off-set. This result confirms our assignment of the TOF offset to the fast structural relaxation process observed with back-scattering. In previous work with hydrated globular proteins a similar structural relaxation process was recorded on a 150 ps time scale as shown in fig. 14 (manuscript), which is also confirmed by the TOF analysis. Thus the “fast process” (I would prefer now “local structural relaxation LSR) occurs with both types of protein

structures, compact and unfolded. But with compact proteins, the correlation time is longer. This is the reason, why the offset in fig. 15 is smaller for compact proteins compared to casein. The riddle of the Gaspar process is solved. Of course the TOF data just provide an “indication” of an internal relaxation due to limited resolution. Similarly, the deviation of the collective diffusion coefficient versus q from a rigid body behaviour is also just an indication, most of which cannot be confirmed in the time domain.