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Elastic resolution spectroscopy: a method to study molecular motions in small biological samples

W. Doster[a,*], M. Diehl[a], W. Petry[a], M. Ferrand[b]

[a] *Fakultät für Physik E13, Technische Universität München, 85747 Garching, Germany*
[b] *Institut Laue–Langevin, F- 38042 Grenoble Cedex 9, France*

Abstract

Few biological molecules are available in quantities, 200–400 mg, that are required for quasi-elastic neutron scattering analysis. We show that the time correlation function of molecular motions can be extracted with much less material using only the strong scattering intensity at zero energy transfer. The time axis is introduced by varying the width of the instrumental resolution function, which is achieved using multiple-chopper time-of-flight spectrometers. The elastic intensity decreases with increasing energy resolution due to molecular motions in the sample. The technical feasibility of ERS is demonstrated with 23 mg of hydrated myoglobin in comparison with the standard approach.

Keywords: Neutron instruments; Time of flight; Resolution; Protein dynamics

1. Introduction

Quasi-elastic neutron scattering experiments have revealed the nature of fast intra-molecular motions of proteins and of protein–water dynamics [1–4]. Moreover, such data provide important information to assess the validity of computer simulations of proteins [5,6]. There are however two major obstacles to such studies: the difficulty to discriminate between different types of molecular motion and the excessive amount of material that is necessary to obtain high quality inelastic data. Therefore only a few models systems, such as myoglobin or purple membrane, could be studied in detail in the past. To overcome both problems we propose to focus on the elastic intensity of the scattering spectrum, which is typically by a factor of 100–1000 stronger than the quasi-elastic contribution at low energy transfer (Fig. 3 and Ref. [7]). The elastic intensity versus momentum exchange $\hbar Q$, allows to classify molecular motions according to their geometry [8]. Moreover the elastic intensity refers to a characteristic time, t_{res}, defined by the energy resolution, ΔE, of the instrument. Fig. 1 shows an example, suggesting that the intensity at zero energy transfer $S_{el}(Q, t_{res})$ approaches the value of the intermediate scattering function $I(Q, t)$ at $t = t_{res} \approx 13$ ps.

Thus, by changing the energy resolution of the instrument and thus t_{res}, the elastic intensity probes the approximate shape of the time correlation function according to: $I(Q, t_{res} = 2\hbar/\Delta E) \propto S(Q, E = 0, \Delta E)$ as shown also in Fig. 2. Multiple-chopper time of flight spectrometers allow to vary the resolution continuously within at least two orders in magnitude [9,10].

*Corresponding author. Tel.: +49-89-289-12456.
E-mail address: wdoster@ph.tum.de (W. Doster).

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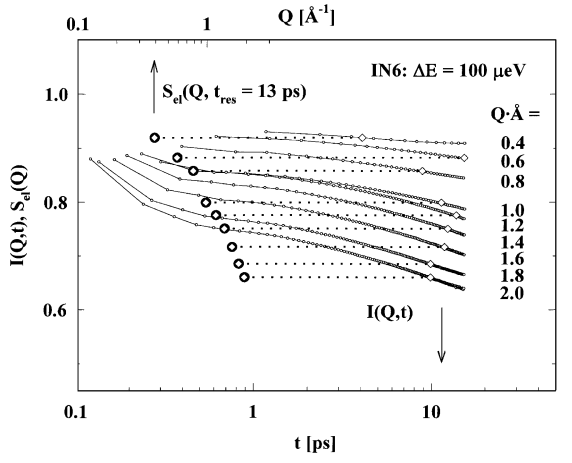

Fig. 1. Intensity at zero energy transfer $S_{el}(Q)$ versus Q and $I(Q, t)$ versus time of D_2O-hydrated myoglobin (400 mg). $I(Q, t)$ represents the resolution-deconvoluted Fourier transform of the dynamical structure factor $S(Q, E)$, measured using the time of flight spectrometer IN6 (ILL, Grenoble), $\Delta E \approx 100\,\mu$eV [12].

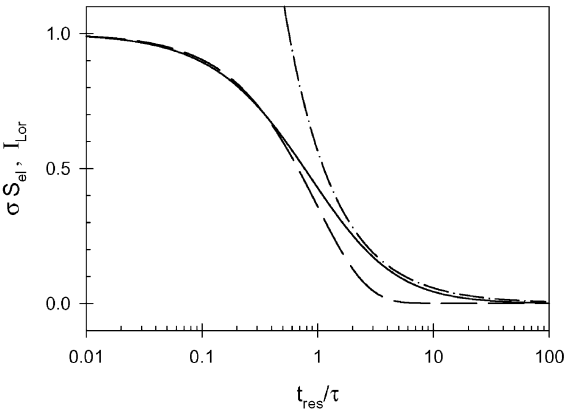

Fig. 2. $\sqrt{(2\pi)}\sigma\, S_{el}$ of Eq. (2) (full line), assuming EISF = 0, is shown in comparison with an exponential decay (dashed) reflecting the intermediate scattering function $I_{Lor}(t_{res}/\tau)$. Also shown is the long time asymptote (dashed-dot) of Eq. (3).

2. Theoretical background

Denoting the instrumental resolution function by $R(Q, E, \Delta E)$ and the scattering function of the sample by $S(Q, E)$, the elastic intensity is given by convolution of the two functions at $E = 0$:

$$S_{el}(Q, E=0, \Delta E) = \int_{-\infty}^{\infty} R(Q, E', \Delta E) \times S(Q, E')\,dE'. \quad (1)$$

Assuming a Lorentzian spectrum, $S_{Lor}(Q, E, \tau)$, and a Gaussian resolution function of width σ allows us to derive the following expression for the intensity at $E = 0$:

$$S'_{el}(Q, \sigma, \tau) = \exp(-2W)\left[\mathrm{EISF}(Q) + (1 - \mathrm{EISF}(Q)).\right.$$
$$\left.\times \mathrm{erfc}\left(\frac{\hbar}{\sqrt{2}\sigma\tau}\right)\exp\left(\frac{\hbar^2}{2\sigma^2\tau^2}\right)\right] \quad (2)$$

with $S'_{el} = \sqrt{(2\pi)}\sigma S_{el}$. The first term specifies the Debye–Waller factor. The EISF(Q) denotes the elastic fraction of the spectrum and erfc(x) is the complementary error function. τ denotes the correlation time of the molecular process and σ is the Gaussian width of the energy resolution function. The full width at half maximum ΔE_G and σ are related by $\Delta E_G = 2\sigma\sqrt{(2\ln 2)}$. The corresponding width of the time window is then $t_{res} = 2\hbar/\Delta E_G$. Fig. 2 shows S'_{el} of Eq. (2) in comparison with an assumed exponential decay of the intermediate scattering function, $I_{Lor}(t/\tau)$. Eq. (2) represents a reasonable approximation to $I(t)$ covering the initial 50% of the decay. At high resolution the elastic peak vanishes and the decay becomes proportional to $1/t_{res}$, the amplitude of a Lorentzian at $E = 0$. The asymptotic expansion of Eq. (2) yields:

$$\exp(2W)S'_{el}\left(\frac{t_{res}}{\tau} \gg 1\right) \approx \mathrm{EISF}(Q) + (1 - \mathrm{EISF}(Q))\frac{1}{\sqrt{\pi \ln 2}}\frac{\tau}{t_{res}}. \quad (3)$$

3. Experimental details

To investigate the technical feasibility, we have performed a first ERS experiment using the time of flight spectrometer IN5 at the ILL. The resolution function of a multiple-chopper TOF instrument is effectively Gaussian due to the convolution of triangular resolution functions of the individual choppers. Its width can be varied continuously

according to: $\Delta E_G = (273400/\lambda^3 S)$ meV Å^3 rpm (IN5) [11].

In our experiment the chopper speed, S, was varied between 5000 and 20 000 rpm in combination with the incident neutron wavelength, namely $\lambda = 5, 8, 12, 16$ Å. The resulting resolution range was $\Delta E_G = 3.3$–$440\,\mu$eV, covering about two decades. Since only the elastic intensity is of interest, one can further increase the signal by running the chopper, which prevents frame overlap, in phase with the frame setting choppers (ratio 1 instead of 2 or 3). Fig. 3 shows the time of flight spectrum of a typical protein sample obtained at ratio 1. The TOF channels represent the neutron flight time from sample to detector. No frame overlap at $E = 0$ is observed at 5000 rpm. The peak above channel 400 reflects the fast neutrons of the following frame, the slow tail of this spectrum is seen below channel 150. At 10 000 rpm the overlap is significant at $E = 0$. The figure also shows that a scaled overlap spectrum obtained at 5000 rpm can be used to correct for this effect at 10 000 rpm.

We studied two samples of D_2O-hydrated myoglobin (0.34 g/g): A reference sample (400 mg) and a small sample containing 23 mg of protein for the ERS experiment. The reference sample covered the entire cross-section of the standard IN5 sample holder, resulting in a scattering power of 10%. The small sample distributed in a similar manner would scatter less than 0.5% of the incident intensity. This number is comparable in magnitude with the 0.5–1% scattering from the aluminium sample holder. To improve the signal to noise ratio in the case of the small sample, we restricted the sample area to cover only 1 cm^2 of the beam, which yields a scattering power of 8%. The empty can was measured at all wavelengths and chopper speeds to ensure that this component dominates the background scattering independent of the set-up. With 23 mg of myoglobin the background contribution to the scattering intensity at $E = 0$ was about 0.5–2%. The resulting errors in the determination of the elastic intensity from the sample, using fits to Gaussian functions, are of the same magnitude. A Gaussian line was fit to the data to determine the intensity at zero energy transfer. To derive this parameter one does not require the exact shape of the central line or a fit of the wings. The duration of runs at a given set-up was between 1 h at 5000 rpm and 14 h at 16 Å and 20 000 rpm. The complete experiment including empty can and low temperature measurements took 6 days of beam time. The data were normalised using measurements at 150 K, in the absence of quasi-elastic scattering, and accounting for the temperature dependence of the Debye–Waller factor.

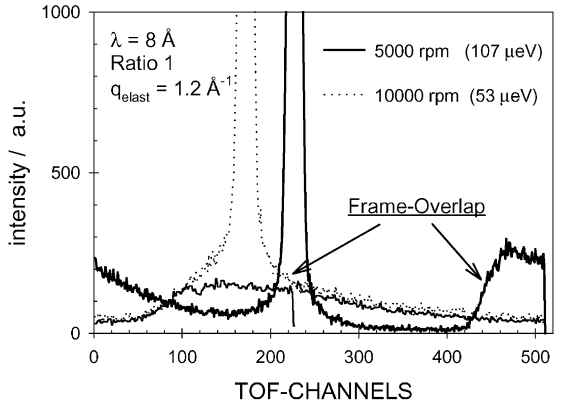

Fig. 3. Time of flight spectra of myoglobin at 5000 and 10 000 rpm chopper speed, without correcting for frame overlap. The frame selecting and the frame overlap choppers have the same frequency, ratio 1.

4. Results and discussion

Fig. 4 displays the first results of an ERS experiment with 23 mg of myoglobin. This amount appears to be sufficient to produce data of reasonable quality covering 3–400 ps. Shorter times could be reached using shorter wavelengths (3 Å). The figure also compares a Fourier transformed frequency spectrum of the 400 mg sample at $Q = 0.85$ Å^{-1} with the ERS result. The model function of Eq. (2) does not account for the data at long times, suggesting a non-exponential decay of the intermediate scattering function. A two-component analysis however leads to reasonable fits with two well separated correlation times of 10 ($\pm$ 3) and 350 ($\pm$ 50) ps. To restrict the number of fitting parameters, we use that

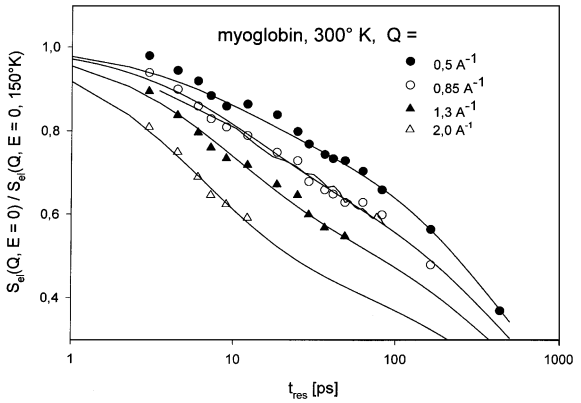

Fig. 4. Normalised elastic scattering intensity of hydrated myoglobin (23 mg) versus the reciprocal instrumental resolution at various Q values. The full lines represent fits to a two component spectrum according to Eq. (2). Also shown for $Q = 0.85\,\text{Å}^{-1}$ is the intermediate scattering function derived from a time of flight spectrum of the 400 mg sample.

the characteristic time of localised motions should be nearly independent of Q [13]. Moreover the EISF of the second component was set to zero. These results are compatible with previous studies of hydrated myoglobin using conventional time-of-flight and back-scattering spectroscopy [1,13]. The fast component reflects rotational transitions in the side-chain methyl groups and in the heavy atom dihedral angles. The second component is associated with water-induced motions of a more collective nature. This component is missing at low hydration.

The neutron cross-section of native proteins is dominated by the strong incoherent contribution of the hydrogens. A promising application of inelastic neutron scattering involves the labelling of particular regions of a protein using partial or full deuteration. This will reduce the incoherent part and will thus decrease the total cross-section further. Our method could be used to compensate for the corresponding loss in signal. Further applications using weakly scattering samples could aim at obtaining data, which are not contaminated by multiple scattering.

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