Supporting Information

Dynamics and rigidity in an intrinsically disordered protein, β-casein

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Brillouin light scattering data

Polarized Brillouin scattering measurements were performed in the same conditions described in ref.[1]. The backscattered light was analyzed by a Sandercock type (3+3)-pass tandem Fabry-Pérot interferometer with incident light of λ=532nm and 4 mm mirror separation. Measurements were taken at 298K and 170K on the same samples used for the depolarized light scattering experiments. Each spectrum was collected for 1-2 hours.

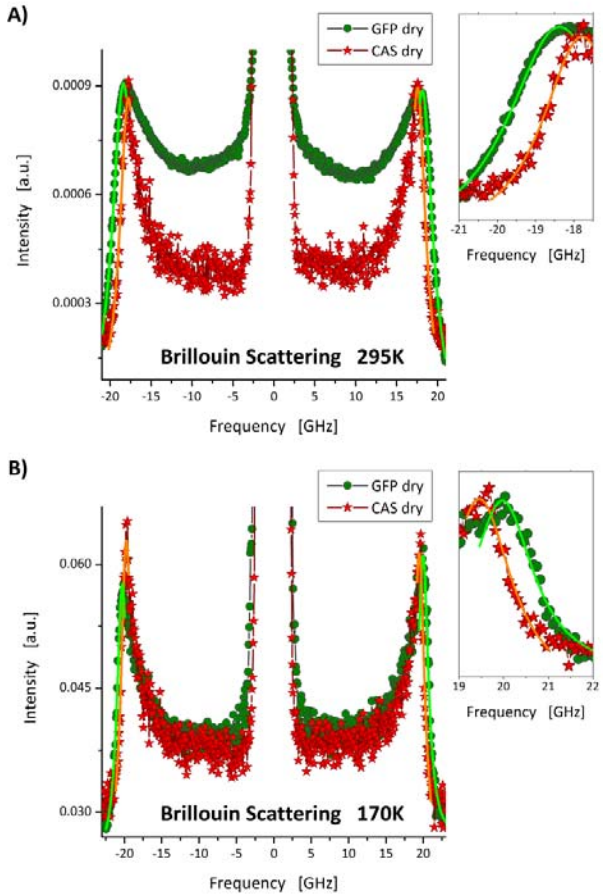

Figure S1. Brillouin spectra (symbols) of dehydrated β-casein (red stars) and green fluorescent protein (green circles) at 295K (A) and 170K (B). Continuous lines represent the DHO fit (orange for CAS, light green for GFP). The inserts show that at both temperatures the Brillouin peaks of CAS are located at smaller frequencies relative to GFP.

To fit the Brillouin peaks we used the damped harmonic oscillator (DHO) model:

$$\mathrm{I}(\nu) = A \cdot \frac{\Gamma_L \cdot \Omega_L}{\left(\nu^2 - \Omega_L^2\right)^2 + (\Gamma_L \cdot \nu)^2} + y_0 \qquad (1)$$

where Ω_L is the frequency and Γ_L the width of the longitudinal modes, A and y_0 are free parameters. For each spectrum both the energy loss and the energy gain peaks were fitted and their parameters averaged.[2]

Fig. S1 shows the Brillouin spectra collected for dry CAS and GFP at 298K (Fig. S1A) and 170K (Fig. S1B) together with the obtained fits.

The Ω_L values obtained from the curve fitting procedure were used to calculate the longitudinal sound velocity c_L, according to the equation:

$$c_L = \frac{\Omega_L \lambda}{2n} \qquad (2),$$

where n is the refractive index of the medium, and λ is the incident wavelength.

The Young's modulus, E, was then extracted assuming elastic isotropy, as in Tachibana et al. [3], and applying the relation[4] ,

$$E = \frac{(1+\sigma)\cdot(1-2\sigma)\cdot M_L}{(1-\sigma)} \qquad (3),$$

where σ is Poisson's ratio and M_L the longitudinal modulus, that can be expressed as;

$$M_L = \rho c_L^2 \qquad (4),$$

with ρ density of the system.

The density values used for the calculations on the dry proteins at 298K were 1.02 g/cm^3 for CAS [5] and 1.32 g/cm^3 for GFP [1]; the Poisson's ratio was assumed to be 0.33 [1, 6].

At 298K the dry CAS powder shows smaller values of c_L (3198 m/s) and E (~7.0 GPa) compared to the GFP ones (3300 m/s and 9.7 GPa).

Therefore, on the hundreds of nanometers length scale probed by Brillouin spectroscopy CAS is softer than GFP. This result is in agreement with the rigidity information obtained in the boson peak region (few nanometers length scale) and with our analysis of the picosecond dynamics of the two proteins.

Curve Fitting of QENS spectra of CASEIN samples: LINE WIDTHS and EISF(Q).

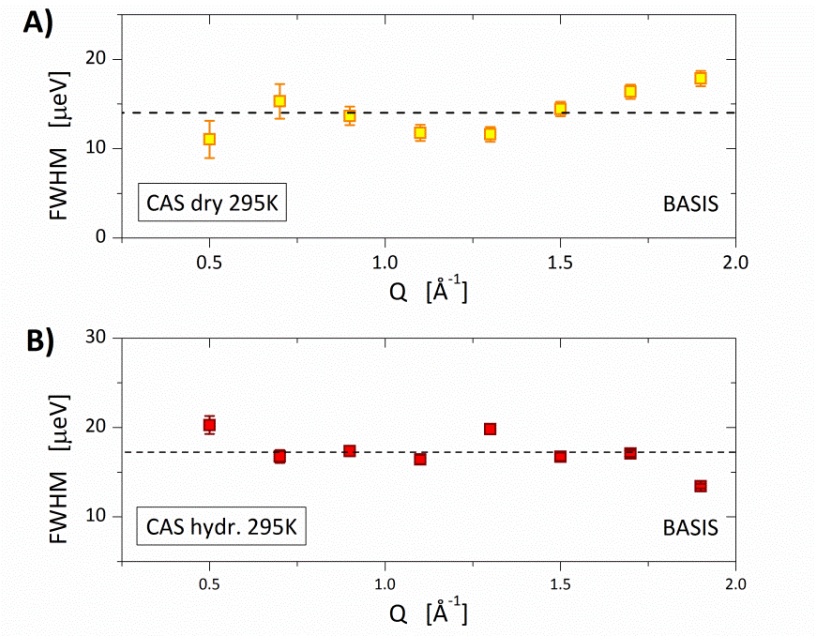

Figure S2. Line widths from the fitting of the quasielastic spectra of dry (**A**) and hydrated (**B**) CAS to calculate EISF(Q) for Fig. 3. The fit assumes elastic line and a single Lorentzian. BASIS spectrometer (~300ps); T=295K.
Analogous plots for GFP samples at 280K can be found in ref. [7]

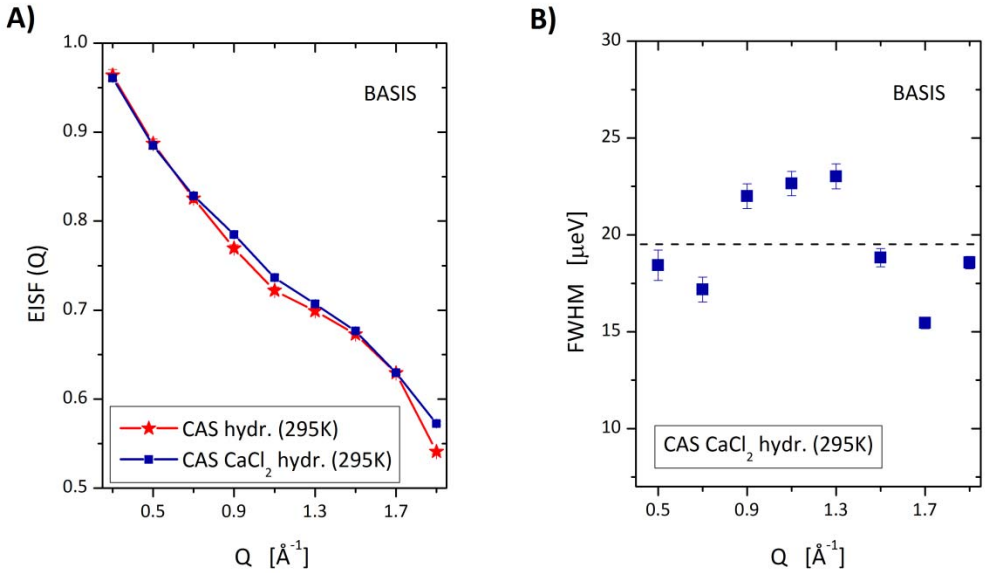

Figure S3. A) EISF(Q) for CAS and CAS-$CaCl_2$ in hydrated state, measured on BASIS spectrometer (~300ps) at 295K. **B)** Line widths from the fitting of the quasielastic spectra of hydrated CAS-$CaCl_2$ to calculate EISF(Q).

Literature data on casein: characteristic frequencies and assignments of different secondary structures from vibrational spectroscopies.

Table S1. Assignment of characteristic frequencies for the amide I and II band components of β-casein.

Peak	Peak wavenumber [cm^{-1}]				
	α-helix	β-structure	extended	unordered (loops)	turns
Amide I	1655 [a]	1658 [b]	1620 [b] – 1623 [a*]	1643 [b]	1668 [a]
	1660 [a]		1626 – 1631 [a,b*]	1646 [a]	1675 [a]
	1658 [b]		1637 [a] – 1639 [b*]	1658 [b]	1683 [a]
	1652 [b]				1691 [a]
					1697 [b]
Amide II	1540 [a]		1525 [a]	1548 [a]	1556 [a]
			1532 [a]		1564 [a]
					1571 [a]
					1578 [a]

*a 8 b9; *these elements might also include some contributions from stranded β- sheet9*

$CaCl_2$ is a hygroscopic salt and the capture of humidity from the atmosphere results in a doublet around 1625 cm^{-1} (combination with bending of water) (Fig. 4, in the article). This phenomenon has already been observed [10-11].

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