

Supplementary Information:

Secondary structure and rigidity in model proteins

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Table S1: Refractive index of proteins measured in our experiments. A comparison with literature values is also provided.

<i>n</i>	dry	wet	literature
GFP	1.473	1.475	1.34 -1.46 ¹
LYS	1.472	1.484	1.48 ² 1.51 ³ 1.538 – 1.575 ⁴ 1.45 ⁵
BSA	1.480	1.487	1.582 ⁶ 1.4 ⁷
MYO	1.478	1.483	1.549 ⁶
<i>proteins</i>			1.35-1.55 ⁸ 1.45 ⁵

In agreement with terahertz (THz) time domain spectroscopy data reported for Cytochrome C samples⁹ the refractive index of hydrated proteins is slightly higher than that of the dry system. The variation of *n* with temperature can be neglected^{10, 11}

Table S2. Density values used to calculate the Young’s modulus of hydrated samples. Variation of density of protein crystals as a function of hydration and temperature (literature data) used to calculate density of dry samples and the temperature dependence of ρ .

ρ	ρ wet [g/cm ³]	% $\Delta\rho$ dry to wet	% $\Delta\rho$ T _r (room) to T _c (cryogenic)
GFP	1.220	7.3%	T _r =300K, T _c =100K -4.5% ¹²
LYS	1.227 ¹³	(298K) 7.3% ¹³	T _r =298K, T _c =100K -1.8% ¹⁴
BSA	1.170 ¹⁵	7.3%	T _r =300K, T _c =100K -1.8% ¹⁶
MYO	0.976 ¹⁷	7.3%	T _r =277K, T _c =77K -2.9% ¹⁷

Table S3 Poisson’s ratio σ reported in literature for different proteins

protein	technique	σ
Tetragonal lysozyme	Brillouin spectroscopy	0.33 as observed in many polymers ^{3, 18}
Tetragonal lysozyme	Ultrasonic pulse-echo	0.37 ¹³
Collagen	Brillouin spectroscopy	II 0.42 ; $\perp$ 0.26 ¹⁹
lysozyme	Derived from X-Ray	0.25 ²⁰
Tetragonal lysozyme	Ultrasonic pulse-echo	0.42 ²¹
Lysozyme, haemoglobin, myoglobin	ultrasound	0.47 ²²

Table S4 Literature data at room temperature (~295K) for longitudinal sound velocity c_L and Young's modulus E of biological systems with different secondary structures. c_L or E values for dry and hydrated proteins are reported in separated columns. Data in each column refer to samples with comparable hydration levels. For the hydrated samples the hydration ratio h =g water/g protein (similar to our samples) is given in parenthesis.

protein	Secondary structure	technique	c_L ,[m/s]		Young's modulus E [GPa]	
			DRY	HYDR.	DRY	HYDR.
Bombyx mori silk (dry, in oil)	30% pleated β -sheet	Brillouin scattering	4700 ¹⁹		30.9 ¹⁹	
β -Keratin 28% from feather rachis (dry in oil)	β structure	Brillouin scattering	3710 ¹⁹		19.3 ¹⁹	
β -lactoglobulin	β barrel	X-ray scattering		2900 $\pm$ 160 (h=0.5) 2860 $\pm$ 110 ²³ (h=1)		
Maltose Binding Protein (MBP)	α/β	Brillouin neutron scattering	3780 $\pm$ 130 ²⁴			
Tetragonal lysozyme	α/β	Ultrasonic pulse-echo method	[110] v1 3097 [001] v4 3149 ²¹	v1 2070 $\pm$ 34 v4 2001 $\pm$ 33 ²¹ (h=0.47)	7.25 ²¹	3.21 ²¹ (h=0.47)
Triclinic lysozyme	α/β	Resonance method			[011] 10 $\pm$ 1.1 [01 $\bar{1}$] 11.2 $\pm$ 1.3 ²⁵	
monoclinic P2, lysozyme						[010] 2.5-3.5 ²⁶ (h=0.3)
Ribonuclease crystal	α/β	Ultrasound		1784 $\pm$ 72 ²² (h~0.5)		
α -Keratin 25% from Porcupine quill (dry in oil)	α -helix	Brillouin scattering	3660 ¹⁹		18.8 ¹⁹	
Cytochrome C	α -helix	X-ray scattering	3458 $\pm$ 42 ²⁷			
Paramyosin from Molluscan catch muscle	supercoiled α -helix	Brillouin scattering	3530 ²⁸		17.4 ²⁸	
Hemoglobin crystal	α -helix	Ultrasound ²² / Resonance method ²⁵		1828 ²² (h~0.5)		[110] or [$\bar{1}$ 10] 5 $\pm$ 1 ²⁵ (h=0.3)
myoglobin monoclinic	α -helix	Resonance method				[001] 3.6 $\pm$ 1.3 ²⁵ (h=0.3)
Whale myoglobin amorphous films	α -helix	Resonance method				2-3.6 ²⁶ (h=0.3)

Morozov et al.²⁶ reported also measurements of E at low temperature for hydrated samples of amorphous whale myoglobin and cross-linked BSA amorphous films. Their results are in agreement with our estimates: at 150K MYO systems (E =7.8 GPa) are softer than the BSA (E =13.2 GPa) ones.

Table S5 Number of HBs per residue in the secondary structural units for MYO, BSA (helices) as well as for GFP (β -barrel)

	Number of residues in helices	Total HBs in helices	Number of HBs/residues in helices
BSA	450	375	0.83
MYO	123	102.5	0.83
	Number of residues in β -barrel	Total HBs in β -barrel	Number of HBs/residues in β -barrel
GFP	121	109 ²⁹	0.9

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