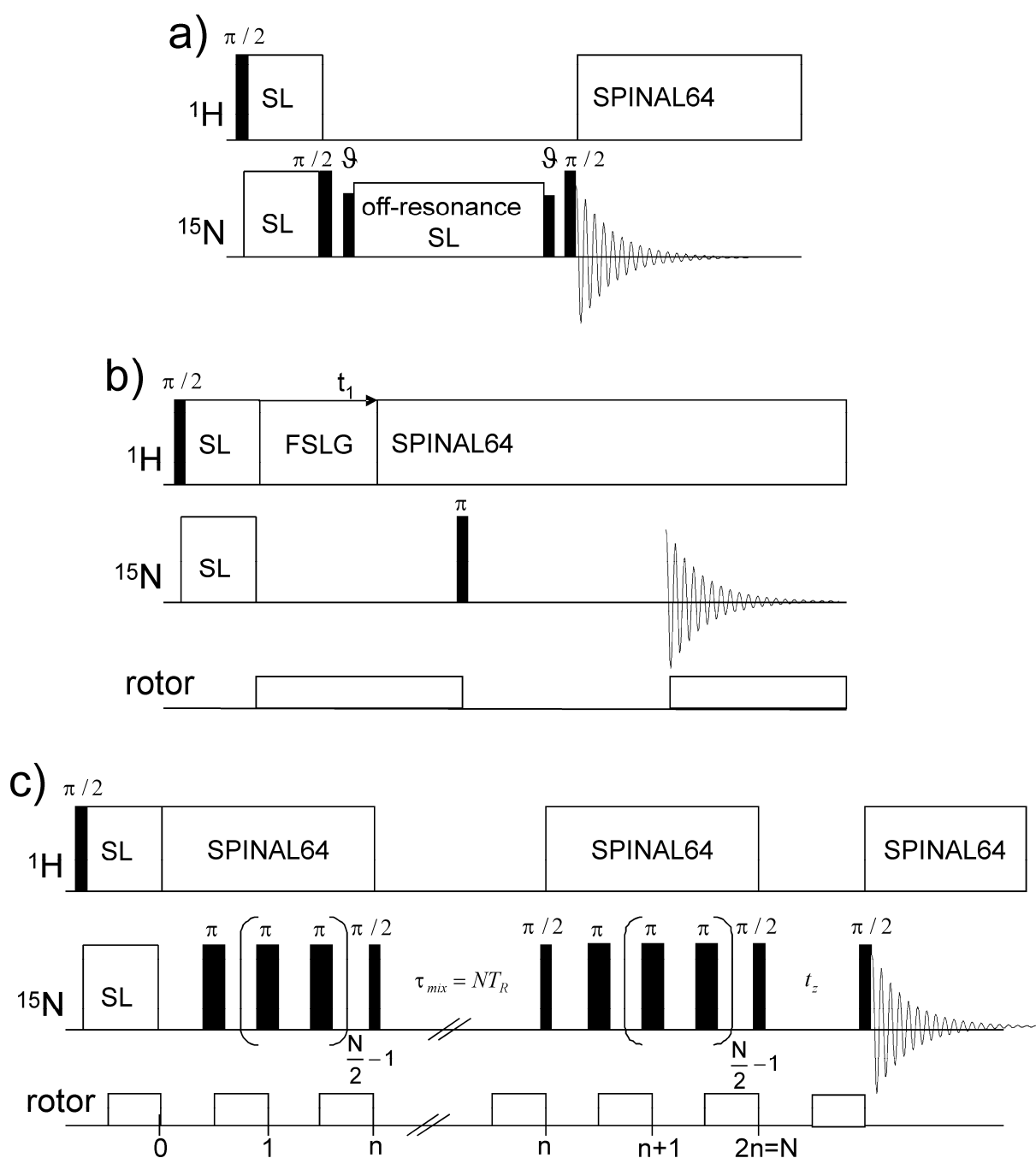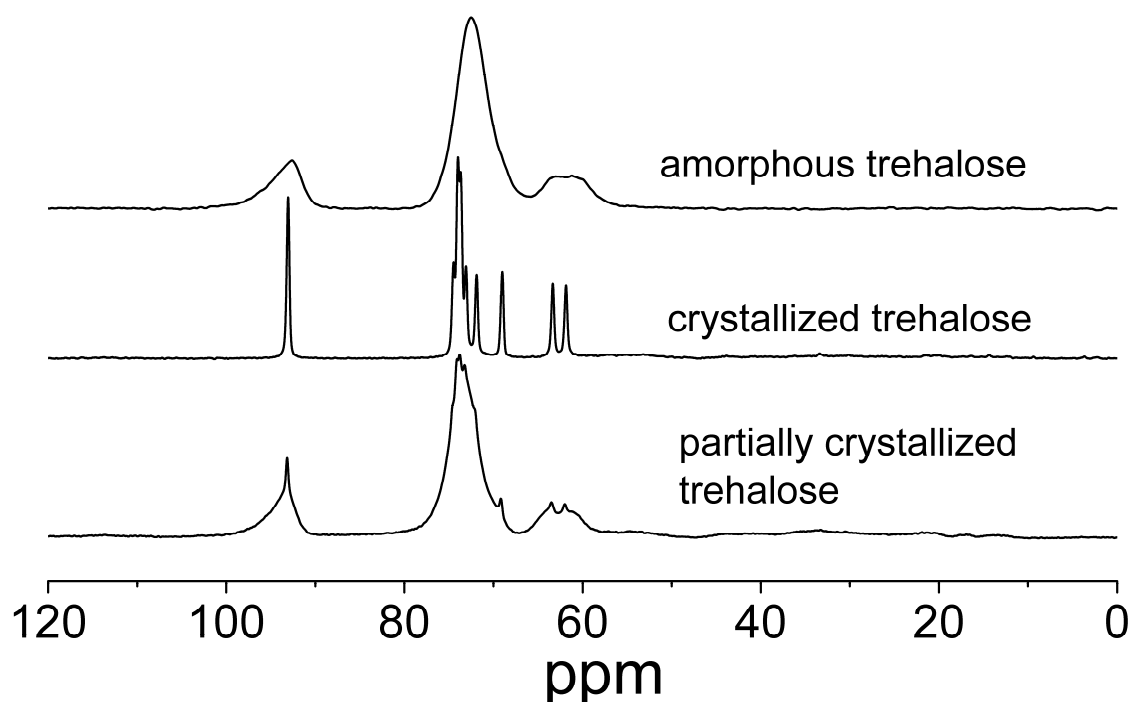Electronic Supplementary Information
for the article

The trehalose coating effect on the internal protein dynamics

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1) Schematic drawings of the $T_{1\rho}$ (a), DIPSHIFT (b) and CODEX (c) pulse sequences.

2) ^{13}C spectra of trehalose

Natural abundance ^{13}C spectra of trehalose in amorphous (dry), crystallized and partially crystallized states. The bottom spectrum (partially crystallized trehalose) was recorded on the Csp-trehalose sample at h=4% after performing a set of the relaxation experiments. Since the amount of the protein in this sample is much less than that of trehalose, the protein signal is hardly visible.

3) The simulation of the DIPSHIFT curves

The simulation of the DIPSHIFT data is based on the previous calculations of FID for the CSA (Herzfeld, Berger, J. Chem. Phys. 73, 6021, 1980; Hong et al., J. Phys. Chem. B, 106, 7355, 2002) and dipolar coupling (Schmidt-Rohr and Spiess, Multidimensional Solid-State NMR and Polymers, Academic Press, 1994; Olejniczak et al., J. Chem. Phys., 81, 4804, 1984). The DIPSHIFT curves (i.e. the signal intensity as a function of the variable time t_1, see Fig. 1) for the carbons in the CH and CH_2 groups can be calculated as:

$$\text{CH:} \qquad \frac{I(t_1)}{I(0)} = \left\langle \cos \Phi \right\rangle_{\alpha,\beta,\gamma} \qquad (1a)$$

$$CH_2: \quad \frac{I(t_1)}{I(0)} = \left\langle \cos \Phi_1 \right\rangle_{\alpha,\beta,\gamma} \cdot \left\langle \cos \Phi_2 \right\rangle_{\alpha,\beta,\gamma} = \frac{1}{2} \left\langle \cos(\Phi_1 - \Phi_2) \right\rangle_{\alpha,\beta,\gamma} + \frac{1}{2} \left\langle \cos(\Phi_1 + \Phi_2) \right\rangle_{\alpha,\beta,\gamma} \quad (1b)$$

where the square brackets define the powder averaging over the Euler angles α, β and γ and Φ is the phase of the magnetization acquired over the precession during time t_1:

$$\Phi = \int_0^{t_1} D_{ZZ}^{LF}(t)dt \qquad (2)$$

where $D_{ZZ}^{LF}(t)$ is the ZZ-component of the dipolar tensor in the laboratory frame. The difference between CH and CH_2 cases is due to the fact that in the CH_2 group one obviously has to take into account two dipolar interactions, thus Φ_1 and Φ_2 in Eq. (1b) are the phases acquired due to the interactions to two different protons in the CH_2 group. The NH DIPSHIFT data analysis is of course analogous to that of CH.

The dipolar coupling tensor in the Principal Axis system (PAS) has a form:

$$D^{PAS} = d_{CH} \begin{pmatrix} -\dfrac{1}{2} & 0 & 0 \\ 0 & -\dfrac{1}{2} & 0 \\ 0 & 0 & 1 \end{pmatrix} \tag{3}$$

where d_{CH} is a dipolar coupling constant. To determine D_{ZZ} in the laboratory frame, one has to perform a set of frame transformations: PAS → static rotor frame (SRF) → rotating rotor frame (RRF) → laboratory frame (LF).

The first transformation corresponds to the alignment of the C-H axis along the magic angle axis:

$$D^{SRF} = R_Z^{-1}(\gamma)R_Y^{-1}(\beta)R_Z^{-1}(\alpha)D^{PAS}R_Z(\alpha)R_Y(\beta)R_Z(\gamma) \tag{4}$$

where $R_Z(\gamma)$ is the rotation matrix corresponding to the rotation γ around axis Z on the Euler angle γ. Note that since the dipolar coupling tensor is axially symmetric, the rotation on the angle α does not change the tensor and is not in fact necessary. The rotation of the rotor is taken into account as

$$D^{RRF} = R_Z^{-1}(\omega_{MAS}t)D^{SRF}R_Z(\omega_{MAS}t) \tag{5}$$

where ω_{MAS} is the circular MAS frequency. At the end, the dipolar tensor in the laboratory frame is:

$$D^{LF} = R_Y^{-1}(\beta_m)D^{RRF}R_Y(\beta_m) \tag{6}$$

where β_m is the magic angle.

For the CH_2 group, the PAS is chosen in such a way so that Z axis of this system is directed along bisector of the angle H-C-H. Thus, the first transformation in this case is aligning the first or the second C-H vector along PAS Z-axis:

$$D^{PAS\pm} = R_Y^{-1}(\pm\beta_{CH})D^{PAS}R_Y(\pm\beta_{CH}) \tag{7}$$

where β_{CH} is the half-angle of the $\angle$ H-C-H (i.e. 54.5°). Sign "$\pm$" corresponds either to the first or to the second C-H bonds. All other subsequent transformations correspond to the CH-group, Eqs. (4-6). Note, that in the transformation PAS$\pm$ → SRF (Eq. 4) one has to take into

account the rotation around Euler angle α since after transformation (7), the dipolar tensor becomes not axially symmetric.

At the end, one may obtain the following expression for the ZZ-component of the dipolar tensor in the LF.
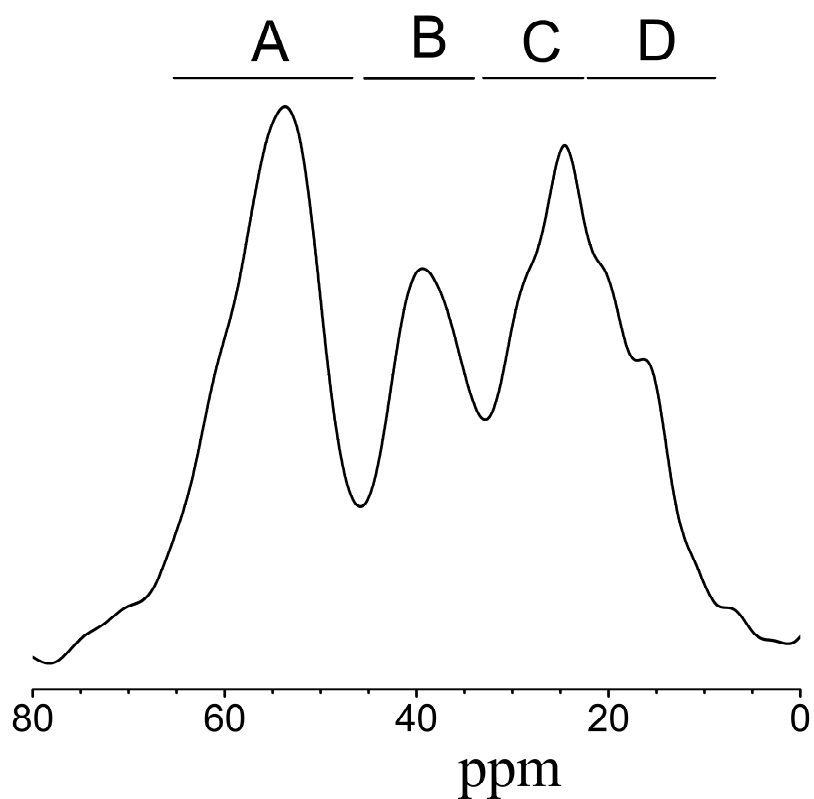
CH group:

$$D_{ZZ}^{LF}(t)=d_{CH}\left[-\frac{1}{2}\left(\cos^2\beta-1\right)\cos\left(2\omega_{MAS}t+2\gamma\right)-\sqrt{2}\sin\beta\cos\beta\cos\left(\omega_{MAS}t+\gamma\right)\right] \tag{8}$$

CH$_2$ group:

$$\begin{aligned}D_{ZZ\pm}^{LF}(t)=d_{CH}&\left[\left(\frac{1}{6}\cos2\alpha\cdot\left(\cos^2\beta+1\right)\pm\frac{\sqrt{2}}{3}\cos\alpha\cdot\sin\beta\cdot\cos\beta\right)\cos\left(2\omega_{MAS}t+2\gamma\right)+\right.\\&\left(-\frac{2}{3}\cos\alpha\cdot\sin\alpha\cdot\cos\beta\mp\frac{\sqrt{2}}{3}\sin\alpha\cdot\sin\beta\right)\sin\left(2\omega_{MAS}t+2\gamma\right)+\\&\left(\frac{\sqrt{2}}{3}\cos2\alpha\cdot\cos\beta\cdot\sin\beta\mp\frac{2}{3}\cos\alpha\cdot\cos2\beta\right)\cos\left(\omega_{MAS}t+\gamma\right)+\\&\left.\left(-\frac{2\sqrt{2}}{3}\cos\alpha\cdot\sin\alpha\cdot\sin\beta\pm\frac{2}{3}\sin\alpha\cdot\cos\beta\right)\sin\left(\omega_{MAS}t+\gamma\right)\right]\end{aligned} \tag{9}$$

Then, substituting Eqs. (8) and (9) into Eq. (2) and performing the powder averaging one finally obtains the signal intensity as a function of t_1.

4) Natural abundance 13C experiments on hen egg white lysozyme

Aliphatic domain of the natural abundance ^{13}C spectrum of dry lysozyme. Spectral regions A to D mark the used integration regions for the data analysis of the experiments done with lysozyme (see the details in Krushelnitsky et al., J. Phys. Chem. B, 2009, 113, 10022). Band A corresponds to CH carbons, band B to CH_2 carbons, band C to a mixture of CH_2 and CH_3 carbons and band D mainly to CH_3 carbons.
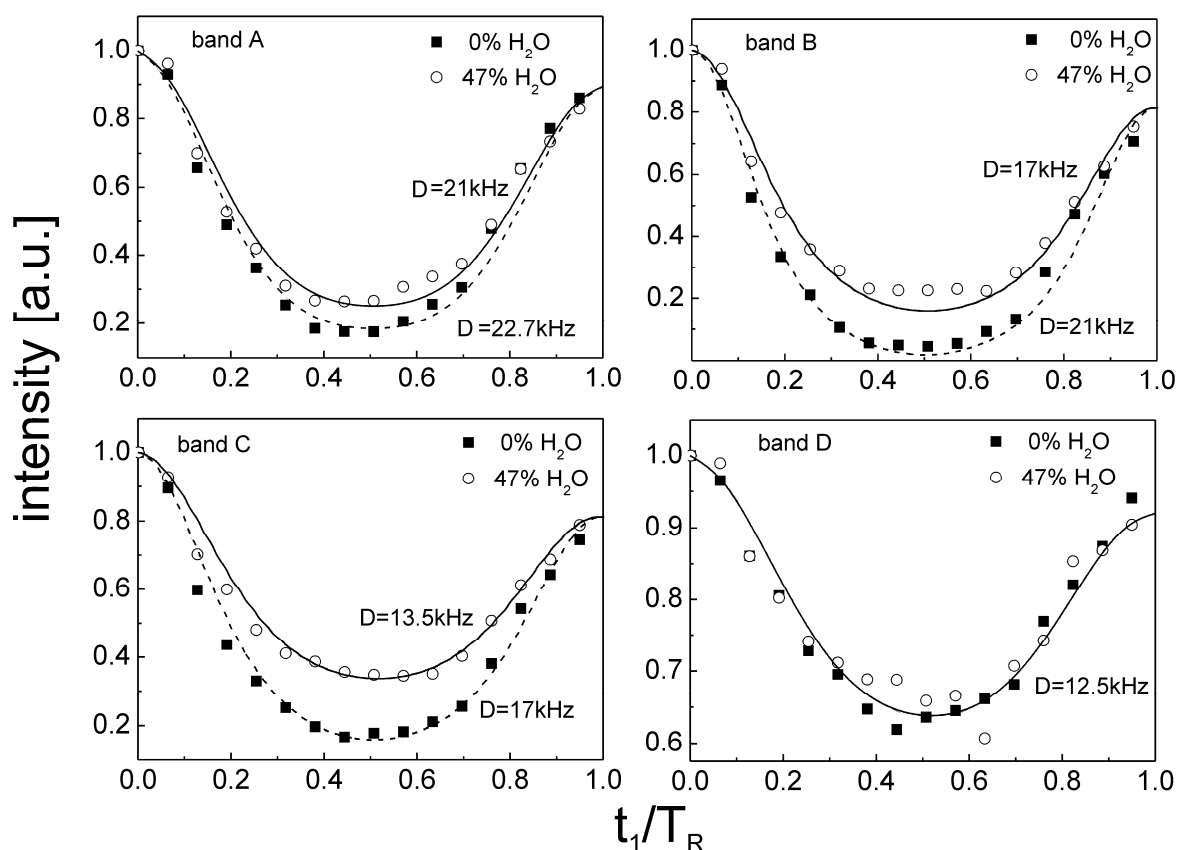
^{13}C DIPSHIFT experiments performed for lysozyme at 0% and 47% of hydration. Data are analyzed for the different spectral regions shown in the figure above. Solid and dashed lines represent simulated DIPSHIFT curves for the dry and hydrated samples, respectively. Dipolar couplings for CH$_2$ groups (bands B and C) correspond to the dipolar interaction per one bond. Dipolar coupling for the band D does not depend on the hydration since this band corresponds to the methyl groups: it has been already shown that the motion of the methyl groups does not depend on hydartion (Roh et al., Biophys. J., 2006, 91, 2573; Krushelnitsky et al., J. Phys. Chem. B, 2009, 113, 10022).