Following protein dynamics in real-time during crystallization

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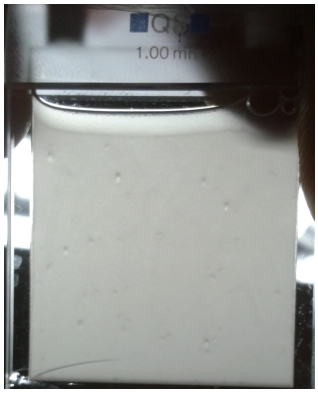
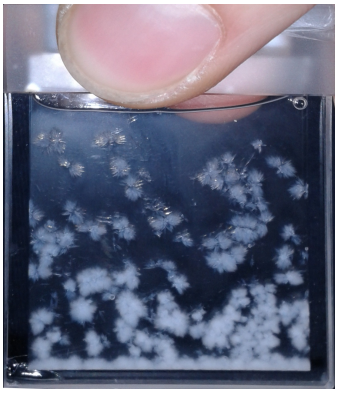
CONTENTS

I. PHOTOGRAPHS OF THE SAMPLE BEFORE AND AFTER THE CRYSTALLIZATION

In Figure S1 photos of the sample measured with NSE are shown before and after the crystallization process.



(a) Photo taken 16 hours after the sample preparation.



(b) Photo taken 58 hours after the sample preparation.

FIG. S1: Photos of the sample before and after protein crystallization.

II. COMPARISON OF TIME DEPENDENCIES OF DIFFERENT PARAMETERS

In Figure S2, the time dependence of the different fit results obtained from the QENS fits are presented. It is visible that both the fraction of the immobile proteins A_c as well as the parameters D and τ, describing the global diffusion as shown in Equation 3 in the main manuscript, follow, within the error-bars, the same time dependence.

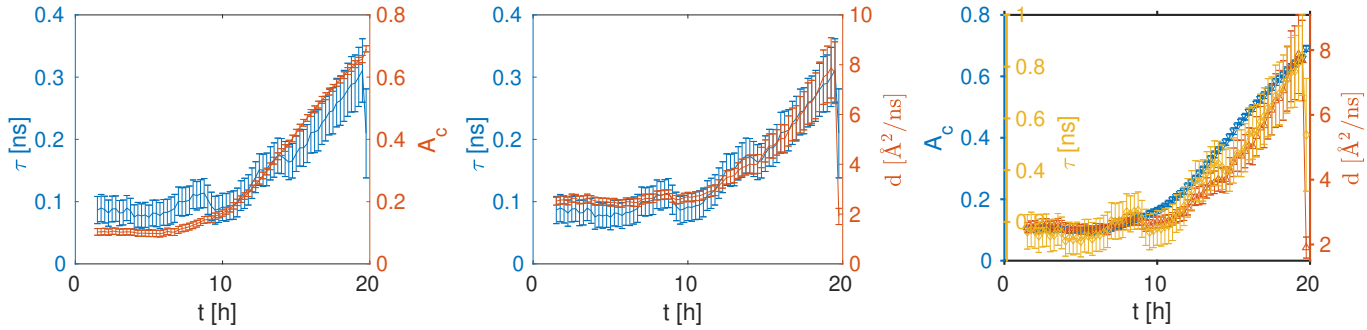


FIG. S2: Different combinations of two or three fit parameters with slightly rescaled and shifted Y-axes to compare their time dependencies.

III. COMPARISON OF DIFFERENT FIT ROUTINES

Two fitting approaches were presented in the manuscript. In the first approach, only the fraction of immobile proteins was fixed as a q-independent fit parameter, while the second approach directly fixed jump-diffusion as the model for the global dynamics. Figure S3 shows the results for the two different different approaches. The different subplots show the fit results for A_c, D or τ as a function of time. Figure S4 shows the time dependence of the parameters characterizing the EISF (see Equation 7 in the main manuscript) for the two different fit approaches. The color coding is the same as in Figure S3. No significant differences are seen between the two approaches and as a function of time.

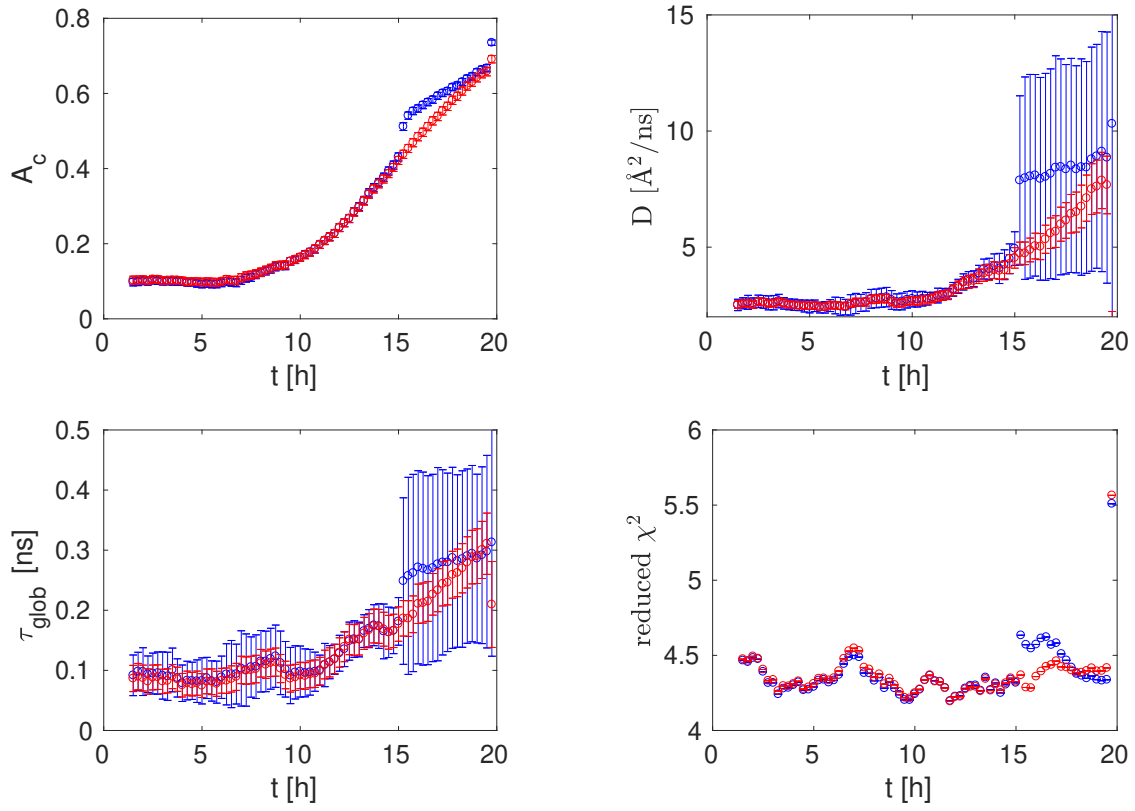


FIG. S3: Fit results obtained from the different fit routines. Blue points represent the fit results from the fits with only A_c as a q-independent fit parameter, the red points are obtained with D and τ as additional q-independent parameters.

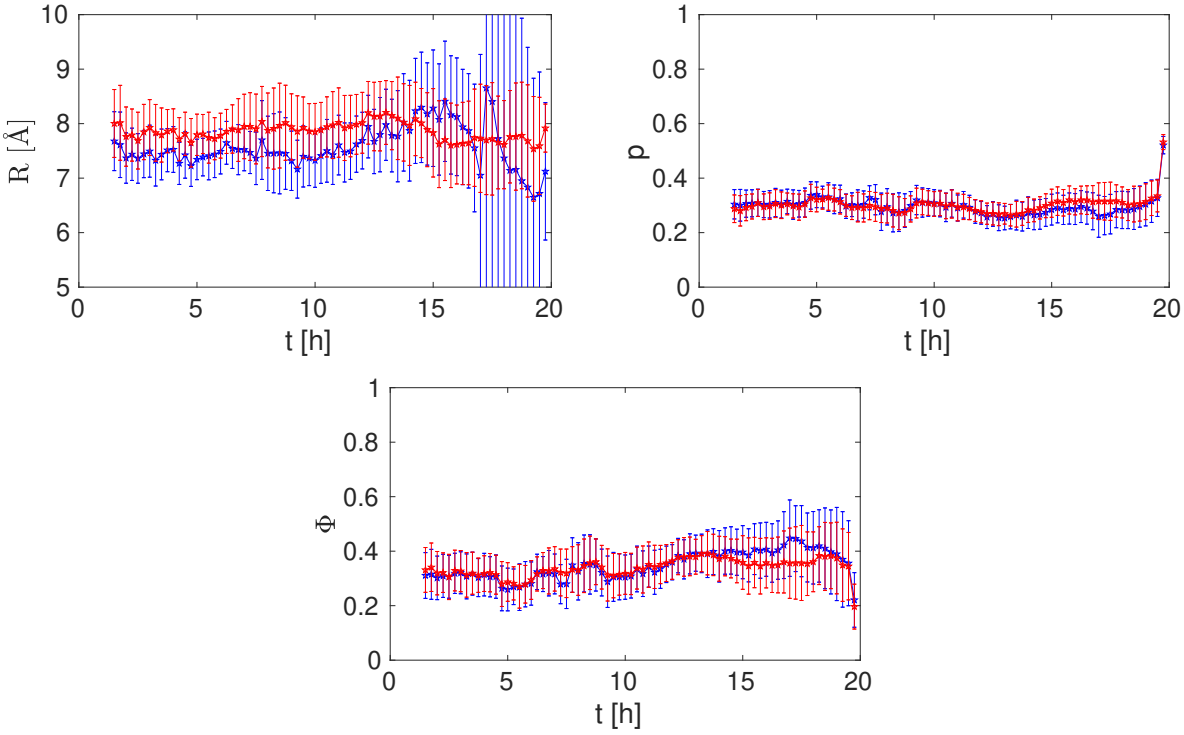


FIG. S4: Time dependence of the EISF fit parameters. Blue points represent the fit results from the fits with only A_c as q-independent fit parameter, the red points are obtained with D and τ as additional q-independent parameters.

IV. INFLUENCE OF FIXED INTERNAL DYNAMICS ON THE APPARENT GLOBAL DIFFUSION COEFFICIENT

In Figure S5 the time dependence of the apparent global diffusion coefficient is shown. For the fits with free internal dynamics (red symbols), the diffusion coefficient clearly exceeds the theoretical dilute limits for monomers (yellow dashed line) and dimers (brown dashed line). By fixing the width of the internal dynamics based on the first QENS-spectra measured (violet symbols), cross-talking can be avoided in the fit and the diffusion coefficients do not exceed the monomer limit anymore.

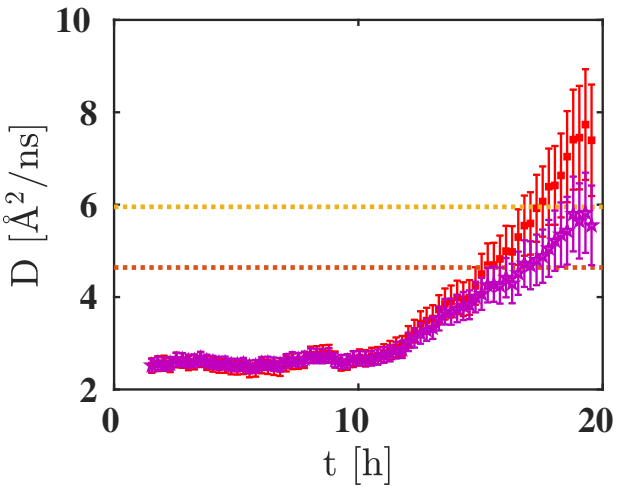


FIG. S5: Diffusion coefficients for fits with fixed (violet) and free internal dynamics contribution (red). The yellow and brown dashed lines represent the dilute limit for monomers and dimers, respectively.

V. COMPARISON WITH PURE PROTEIN SOLUTIONS

The fit model of Equation 2 to pure protein solutions without salt lead to A_c and τ being equal to zero within the errorbars. The diffusion coefficients obtained show the expected decrease due to crowding and cluster formation [1]. For different protein concentrations, the fit results are shown in Figure S6.

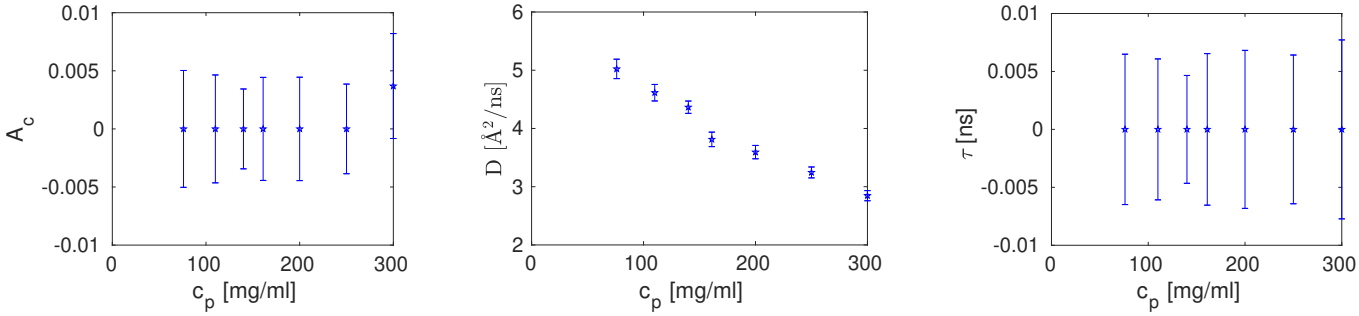


FIG. S6: Fit results of Equation 2 to pure protein solutions.

[1] M. K. Braun, M. Grimaldo, F. Roosen-Runge, I. Hoffmann, O. Czakkel, M. Sztucki, F. Zhang, F. Schreiber, and T. Seydel. Crowding-controlled cluster size in concentrated aqueous protein solutions: Structure, self- and collective diffusion. *The Journal of Physical Chemistry Letters*, 8(12):2590–2596, 2017.