ESRF	**Experiment title:** Resolving hydrated protein dynamics in supercooled water	**Experiment number**: SC5055
Beamline: ID02	**Date of experiment**: from: 29/01/2021 to: 31/01/2021	**Date of report**: March 5, 2021
Shifts: 9	**Local contact(s)**: Thomas Zinn, Narayanan Theyencheri	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

PERAKIS Foivos, Stockholm University Department of Physics
BIN Maddalena, Stockholm University Department of Physics
REISER Mario, Stockholm University Department of Physics
BERKOWICZ Sharon, Stockholm University Department of Physics
DAS Sudipta, Stockholm University Department of Physics
LEHMKUEHLER Felix, Laboratory DESY Hasylab at DESY
MOELLER Johannes, Laboratory European XFEL MID Group
GUTT Christian, Laboratory University of Siegen Department of Physics

Report:

The aim of experiment SC5055 was to investigate the protein dynamic transition by measuring the dynamics of hydrated proteins by means of X-ray Photon Correlation Spectroscopy (XPCS) from ambient conditions to the deeply supercooled regime. The protein dynamic transition is known to occur for various biological systems at T≈220 K below which the proteins lose their biological function. The causal relation of this transition to the strong-to-fragile transition which occurs in supercooled water at similar temperature is under discussion in the literature. Thus, novel experimental insights are necessary to resolve this controversy.

During the experiment at ID02 (Jan 2021) we studied hydrated lysozyme (Sigma L6876) powder with various hydration levels (h = 0.2, 0.25, 0.3, 0.35, 0.47) as well as lysozyme solvated in water/glycerol mixtures (200 mg/ml in 1:1 and 2:3 water/glycerol mixtures), at temperatures ranging from 293 down to 173 K. The hydration of the samples was performed in our home-lab using a dedicated hydration chamber which allowed the control of humidity and temperature, based on peltier cooling. The hydrated powders as well as protein solutions were chosen to prevent freezing upon supercooling based on our in-house pre-characterization. The samples were shipped to ESRF before the beamtime.

First, we examined the static scattering signal covering both the SAXS and WAXS regions with hydrated Lysozyme powders with different hydration levels as labeled in the legend (Fig. 1 (left)) and with Lysozyme solutions (Fig. 1 (right)). Measurements in this wide range of momentum transfers were enabled by using two detectors. In Fig. 1 (left), the low-q region exhibits strong contribution from powder scattering, the scattering signal at higher q-values reflects the protein arrangement and the influence of the hydration

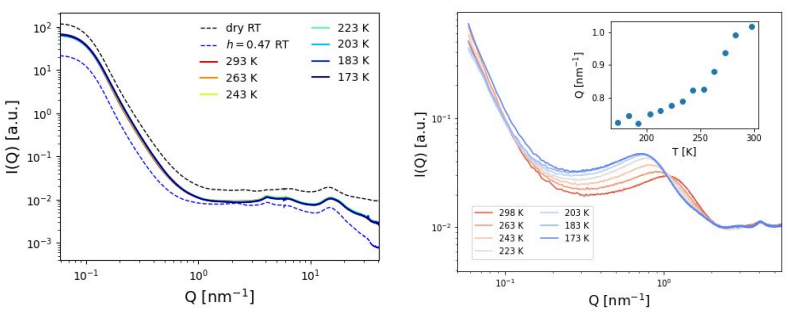

Figure 1: Left: Static scattering signal of hydrated Lysozyme powder with different hydration levels (h=0.47, h=0.35, dry). For Lysozyme powder with h=0.35, WAXS/SAXS shown as a function of temperature from 293K down to 173K (solid lines). **Right**: Temperature dependence of the static scattering signal measured with Lysozyme solution in (2:3) water/glycerol mixture (200mg/ml). The inset shows the peak position over temperature.

layer. The peaks at ≈5-6 nm^{-1} reflect the protein packing arrangements and secondary structures, while the peak at ≈15nm^{-1} arises due to the interatomic distances and contains contributions of hydration water. The protein-protein interaction is influenced by the water/glycerol mixture evident in the peak at ≈1 nm^{-1} that shifts towards lower q upon cooling (Fig. 1 right) and the increased scattering intensity indicating an increasing inter-protein distance as a function of decreasing temperature. The more tetrahedral water network at lower temperatures could explain the increased distance between the proteins.

We acquired time series of speckle patterns under various conditions to study dynamics by means of XPCS. Thorough analysis of these data is currently in progress. Special attention is put on examining the scattering patterns and masking out artifacts which is crucial for accurate quantitative analysis of the results. Intensity autocorrelation functions are calculated and modeled by $g_2(\tau, q) = 1 + \beta \exp\{-2(\Gamma_0(q)\tau)^{\alpha}\}$. $\Gamma_0(q)$ is the dispersion relation and α is the KWW exponent. The data shown here have been normalized to the speckle contrast β (30% in USAXS). Figure 2 displays correlation functions measured with hydrated (h=0.35) Lysozyme powder. The measurements were conducted below the beam damage threshold which is evident in the two-time correlation function (Fig. 2(a) inset) that does not show any indication of radiation induced dynamics. Interestingly, the hydrated powders seem less prone to radiation damage than the dry proteins (not shown here, see proposal 88266). The correlation functions of hydrated powders exhibit compressed exponential shape ($\alpha \approx 1.75$) and the dispersion relation (Fig. 2(b)) increases linearly with momentum transfer, which indicates directional dynamics.

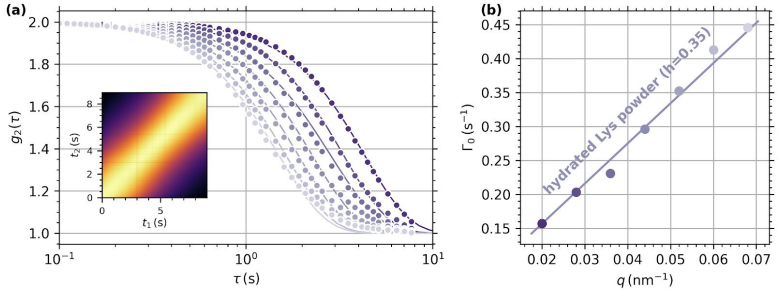

Figure 2: (a) XPCS correlation functions measured with hydrated (h=0.35) Lysozyme powder for different momentum transfers from q=0.02 nm^{-1} to 0.07 nm^{-1}. The inset shows a two-time correlation function at q=0.02nm^{-1}. The correlation functions exhibit a KWW exponent of $\alpha \approx 1.75$. **(b)** Dispersion relations calculated from the correlation function in (a). The solid line is a guide to the eye.

In contrast to isotropic dynamics in case of the hydrated powder, we find that the dry Lysozyme powder exhibits anisotropic dynamics (Fig. 3). Dynamics parallel to the direction of gravity $\vec{g}$ can be disentangled from a perpendicular component by selecting the corresponding $\vec{q}$-regions on the detector. As a preliminary result, we can deduce that the dynamics in vertical direction ($\vec{q} \parallel \vec{g}$) indicated in red are faster than the horizontal component, which is evident in the higher decay rate Γ_0, and show strong directional characteristics with a KWW exponent of $\alpha = 1.8$. A possible explanation could be that the grains in the dry powder are more prone to move in this direction when voids in the powder collapse. The horizontal component (blue) can be described by a KWW exponent that decreases with q (Fig. 3(d)). This indicates that at higher momentum transfers the contribution of grain dynamics is reduced and the correlation functions start to reflect the protein dynamics more clearly.

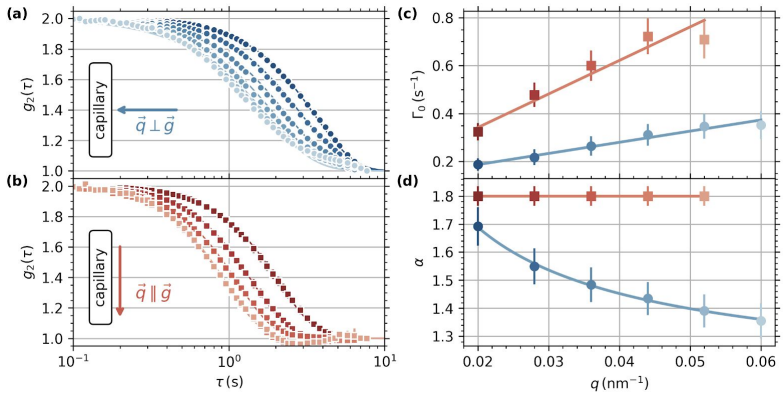

Figure 3: Anisotropic dynamics: correlation functions of dry Lysozyme powder shown for momentum transfers from q=0.02 nm^{-1} to 0.06 nm^{-1}. The analysis was performed selecting ROIs corresponding to momentum transfer directions $\vec{q}$ perpendicular **(a)** and parallel **(b)** to the direction of gravity $\vec{g}$. **(c)** Dispersion relations $\Gamma_0(q)$ calculated for both directions from the correlation functions shown in (a) and (b). **(d)** Corresponding KWW exponents α for both directions. The solid lines in (c) and (d) are guides to the eye. The $\vec{q} \parallel \vec{g}$ data are shown as red squares the $\vec{q} \perp \vec{g}$ data as blue circles.

We would like to emphasize again that the presented results are very preliminary since only one month passed since the beamtime. A lot of data under various conditions were acquired despite the fact that SC5055 was a remote experiment. We are grateful for the help of Thomas Zinn and Narayanan Theyencheri who made this experiment possible and contributed with their expertise in many discussions. The results presented here show the feasibility of the experiment in the low-q regime and contain indications of interesting phenomena in hydrated proteins. While the low-q region is dominated by the grain scattering, the contribution of single protein dynamics is increased at higher momentum transfers. Therefore, we aim to continue this study at ID10 which is optimized for XPCS measurements in the desired q-range (see proposal 88266).