Dynamical heterogeneity in a cooked egg

Microscopic dynamics of a heat-induced protein gel revealed using coherent X-ray scattering

Protein gelation is a fundamental topic in food chemistry as well as in condensed-matter physics. Gelation, as a result of heat-induced denaturation, leads to a three-dimensional network structure through the formation of disulfide cross-links and hydrogen bonds [1]. The properties of such gels, which form through a non-equilibrium process, are closely related to the microscopic dynamics of the network. Thus, understanding the microscopic dynamics of a gel at the lengthscales of the network structures is of interest in the fields concerning gelation of colloids, polymers and proteins.

Reorganisation of the microscopic structures which are under stress in a protein gel network can lead to heterogeneity in its relaxation dynamics. Understanding such reorganisation requires the understanding of the structure and dynamics of protein gels on a broad range of lengthscales and timescales, ranging from single proteins (nanometres) up to the network mesh size (micrometres), and milliseconds up to hundreds of seconds. However, the studies on the dynamic properties of thermal gels of proteins, so far, have only focused on the understanding of internal or short-time processes [2,3].

We have demonstrated the applicability of the sophisticated state-of-the-art technique low-dose X-ray photon correlation spectroscopy (XPCS) in ultra-small-angle X-ray scattering (USAXS) geometry on a real protein gel [4,5]. In this experiment, a series of time-resolved scattering patterns is recorded using an area detector. Each of these scattering patterns is divided into different wavevector modulus (q) regions and the intensity autocorrelation functions from each region are calculated using

$$g_2(q,t) = \frac{\langle I(t_1)I(t+t_1)\rangle}{\langle I(t_1)\rangle^2},$$

where $I(t_1)$ is the scattered intensity at a measurement time t_1 and <...> indicates the average over the measurement time t_1 and the pixels within the wavevector modulus range $q\pm\delta q$. This intensity autocorrelation function can be described by the Kohlrausch-Williams-Watts (KWW) function [4,5]

$$g_2(q,t) = 1 + \beta \exp[-2\left(\frac{t}{\tau}\right)^{\gamma}],$$

where τ is the characteristic relaxation time of the system and γ is the stretching exponent, reflecting the deviations from the exponential behaviour.
In the case of a non-equilibrium system, it is common to calculate the two-time correlation function given by

$$C_I(q,t_1,t_2) = \frac{\langle I_p(q,t_1)I_p(q,t_2)\rangle_{pixels}}{\langle I_p(q,t_1)\rangle_{pixels}\langle I_p(q,t_2)\rangle_{pixels}},$$

where I_p is the intensity at the pixel p and $<...>_{pixels}$ indicates an average over the pixels within the wavevector modulus range $q\pm\delta q$. The lines perpendicular to the diagonal of all the two-time correlation plots can be extracted to obtain the intensity correlation functions, $g_2(q, t)$.

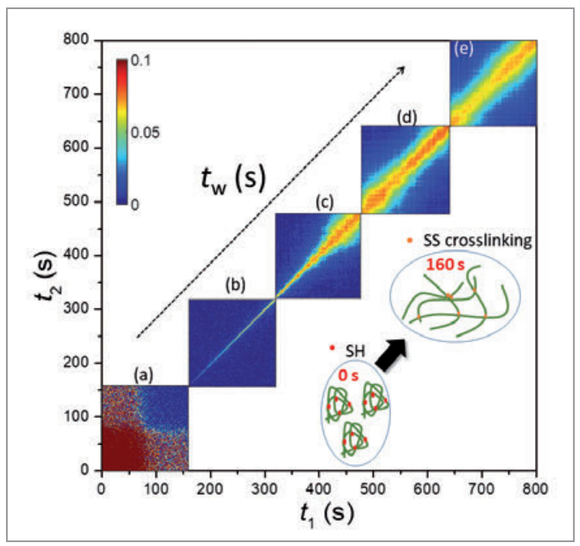

Figure 1

Two-time correlation functions collected at 80 °C (at $q \approx 0.01\ nm^{-1}$) in five time intervals after reaching the temperature: a) 0–160 s, b) 160–320 s, c) 320–480 s, d) 480–640 s, e) 640–800 s. Temporal fluctuations can be observed in Fig. d-e. Inset schematic shows the native state of the proteins before denaturation (0 s after heating at 80 °C) and after unfolding due to heat denaturation (160 s after heating at 80 °C).

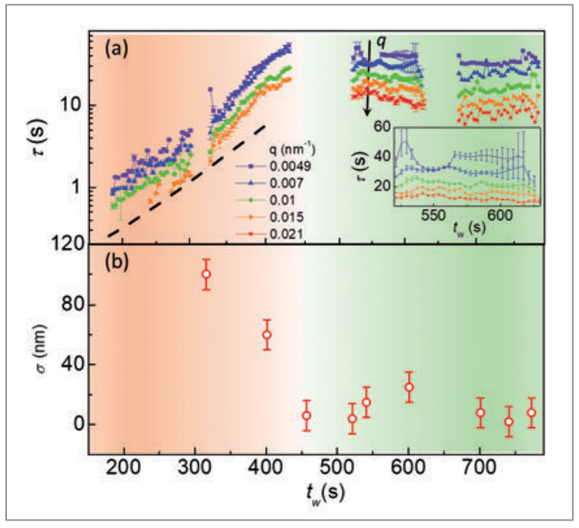

Figure 2

a) Evolution of the relaxation time τ as a function of t_w at 80 °C at different q as indicated in the legend. The inset shows the data within the time window $t_w \approx$ 480–640 s on a linear scale to visualise the temporal fluctuations. The dashed black line represents an exponential growth function. b) σ as a function of t_w showing a reduction with waiting time.

Using this technique, we followed the simultaneous evolution of the microscopic dynamics on lengthscales of the network mesh size and the structural evolution corresponding to the gelation kinetics of a hen egg white.
XPCS experiments were performed at the beamline P10 of PETRA III, using an X-ray wavelength of 1.54 Å. During the gelation, we performed five consecutive XPCS runs, each run corresponding to 160 s, at different fresh sample spots. The corresponding two-time correlation functions are depicted in Figs. 1a-e, and allow us to follow the evolution throughout the entire measurement time. The corresponding growth kinetics of the network structure show that, under the chosen conditions, the network structure evolution is remarkably well separated from the dynamics, i.e. the dynamics are observable only after 160 s (the bottom left corner of Fig. 1b) when the major part of the structural evolution is complete. The microscopic dynamics are observed to be hyper-diffusive and a pronounced slowing-down with time t_w is observed in Fig. 1. The stress-activated dynamics exhibit an exponential rise of the relaxation time (ageing) and a subsequent steady-state ballistic motion displaying significant temporal heterogeneity (Fig. 2a). The lengthscale (inversely proportional to the wavevector modulus q) dependence of the dynamics is used to calculate the spatial extension σ of the decorrelation events using the method described in [6]. Figure 2b depicts that σ decreases from 100 nm to a few nanometres upon ageing, accompanied by a lowering of the degree of dynamical heterogeneity as a result of the discrete rearrangement events in the gel. These dynamical events are such that they do not change the structure of the gel.

Our investigation paves the way for future studies of dynamics following protein gelation, aggregation, liquid–liquid phase separation, as well as other phase transitions on lengthscales from nanometres to microns. Thus, the established framework in this research has profound implications both for the food industry and the fundamental study of phase transitions of various soft-matter systems.

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