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Coupling microfluidics and SAXS to study the whole crystallization process

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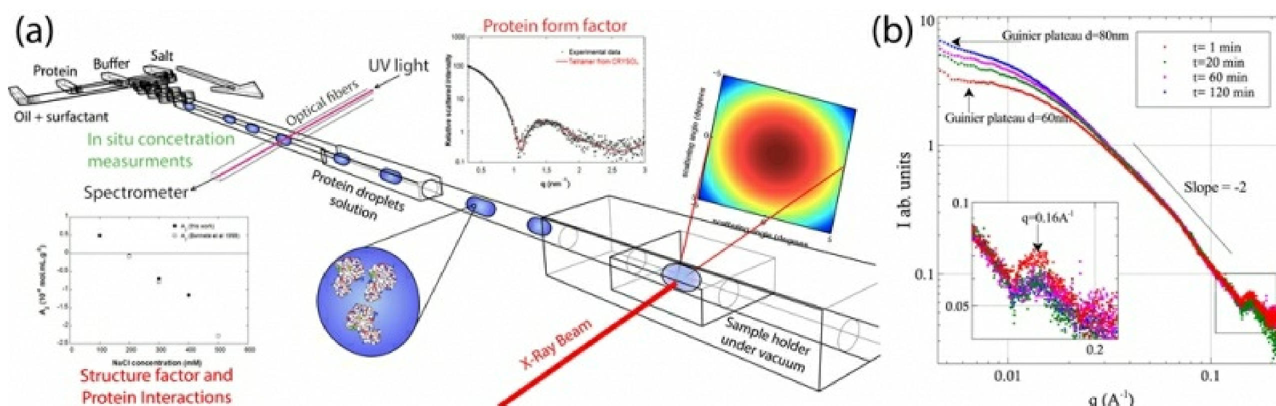
Small-Angle X-Ray Scattering is a particularly suitable technique to characterize structure and form factors of colloidal systems in solution and therefore to probe nanometer-scale structures. The combination of microfluidics and SAXS provides a powerful tool to investigate phase transitions at different molecular levels and relevant timescales. In droplet based microfluidics, the sample is compartmentalized inside droplets, each droplet acting as a microreactor in which the operating conditions can be finely tuned without cross-contamination. Several microfluidic platforms were developed in different BioSAXS beamlines (BM29 at ESRF and SWING at Soleil) to probe protein interactions in solution and phase transitions for nucleation and phase transition studies. The developed microfluidic systems generate droplets containing proteins, crystallization agents and buffer, carried by an inert fluorinated oil. A huge number of crystallization conditions can be screened with a small amount of biological material just by changing the flow rates of stock solutions. In the microfluidic platform developed on BM29 (fig. 1a), the droplets are passed from the chip into the sample holder for exposure to the X-ray beam. The droplet flow is synchronized with SAXS measurements to probe protein form and structure factors while minimizing radiation damage. To this end, the experimental setup was used to successfully determine the form factor of three proteins (rasburicase, lysozyme and glucose isomerase). For instance, the experimentally obtained scattering curves of rasburicase fit well with their scattering curves determined from the crystal structure proving the structural stability of the protein in droplets and the absence of radiation damage. Subsequently, weak interactions of lysozyme solutions were studied as a function of protein and salt concentrations. The obtained second virial coefficients values were found to be in good agreement with data previously reported in literature [1] but using only a few milligrams of protein [2]. In the microfluidic platform developed on SWING the microfluidic chips were directly inserted in the beam. In this setup, tens of droplets are stored in a capillary trap, allowing to follow the temporal evolution of the supersaturated lysozyme solution inside the droplets. The results obtained in this setup enable to demonstrate the existence of equilibrium clusters prior to crystal nucleation. As shown in Figure 1b, the SAXS signal of supersaturated lysozyme solution exhibits some important features: a correlation peak appears at q=0.16A-1 and a Guinier plateau, starting around q=0.01A-1, is observed. The size of this clusters range from 60nm at the beginning of the experiment to 80nm at the end. The broad peak obtained at 0.16A-1 corresponds to a correlation length of 3.9nm compatible with one dimension of the crystal lattice of tetragonal lysozyme. Finally, the slope of -2 in the intermediate q region shows that the clusters are not spherical. These observations suggest a 2-setp nucleation process: first quickly after the temperature quench, large clusters are formed. In these clusters, the lysozyme molecules are in a disordered state but at a distance compatible with those of crystal structure. Afterwards, the formation of the crystal might be due to the reorganization of molecule within these clusters.

[1] Bonneté et al. (1999). Journal of Crystal Growth 196(2),403-414
[2] Nhat Pham, et al. (2017). Analytical Chemistry 89 (4), 2282-2287



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