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STUDY OF PRE-NUCLEATION AND AGGREGATION IN PROTEIN WITH NOVEL OPTICAL METHODS LIKE DLS AND CDDLS.

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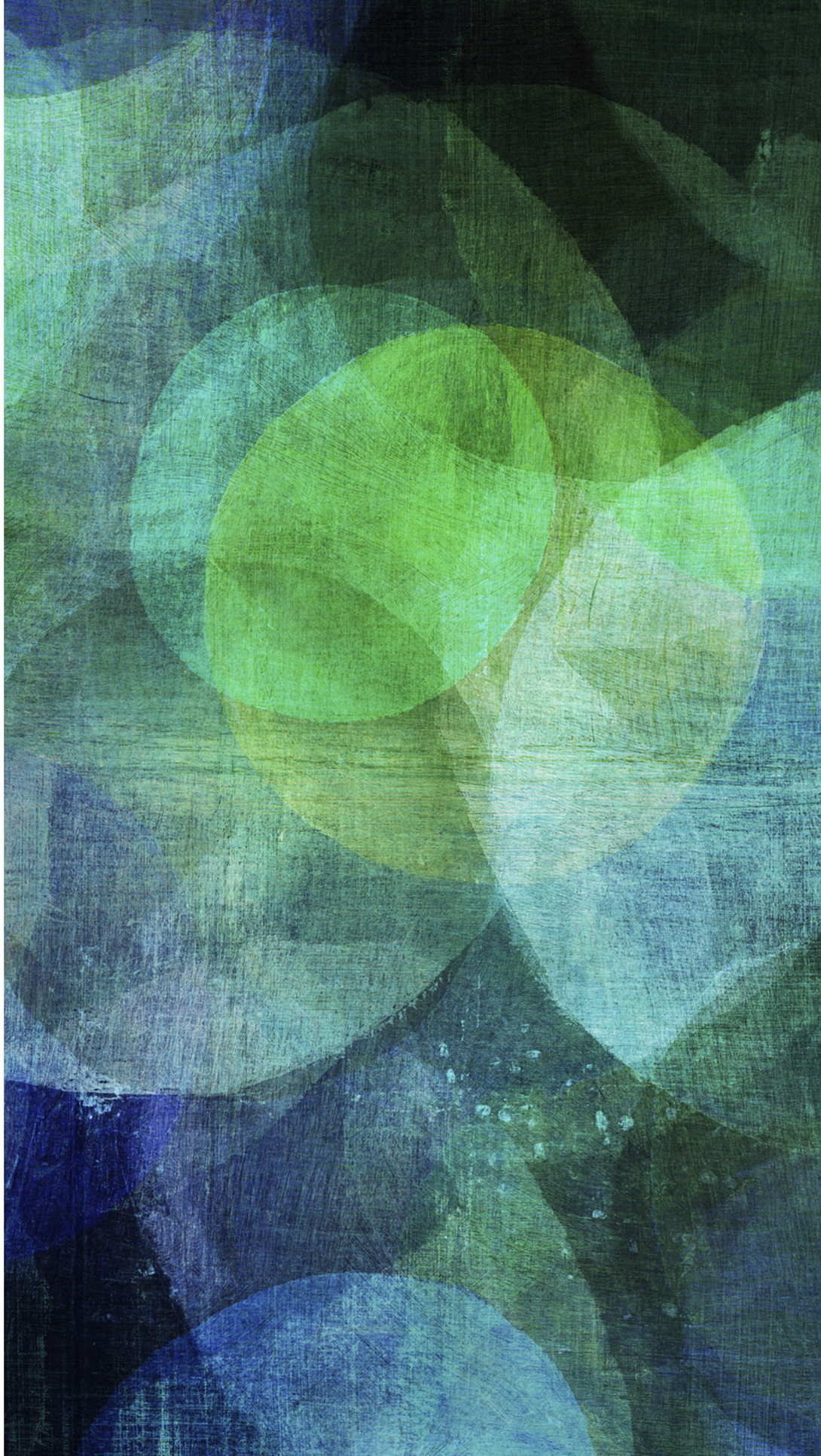
10th October 2018



MAIN TOPIC

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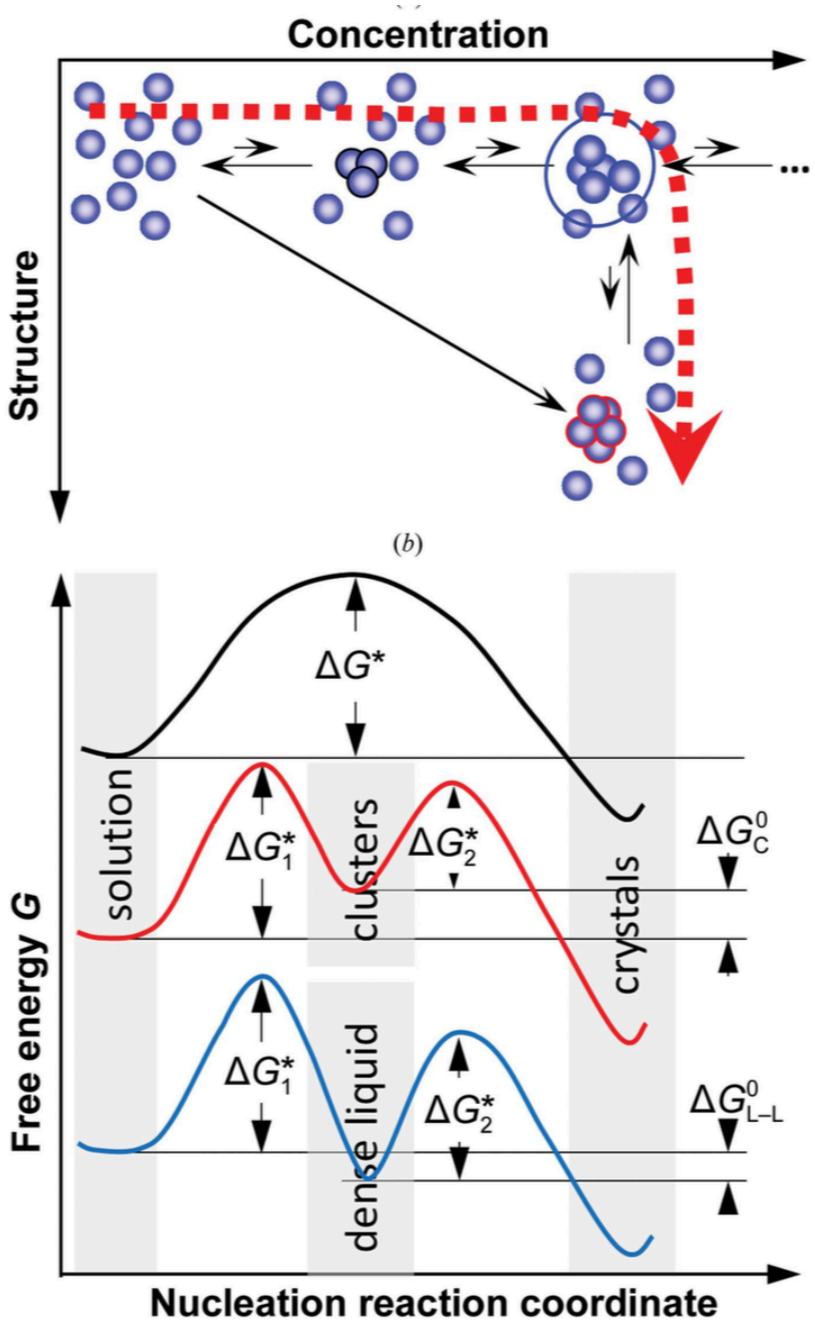
DO PROTEIN CRYSTALS NUCLEATE WITHIN DENSE LIQUID CLUSTERS?

D. Maes, M.A.C. Potenza et al. (2015)

Protein-dense liquid clusters are regions of high protein concentration that have been observed in solutions of several proteins. The typical cluster size varies from several tens to several hundreds of nanometres. According to the two-step mechanism of nucleation, the protein-rich clusters serve as locations for and precursors to the nucleation of protein crystals.

NUCLEATION: TWO-STEPS MECHANISM



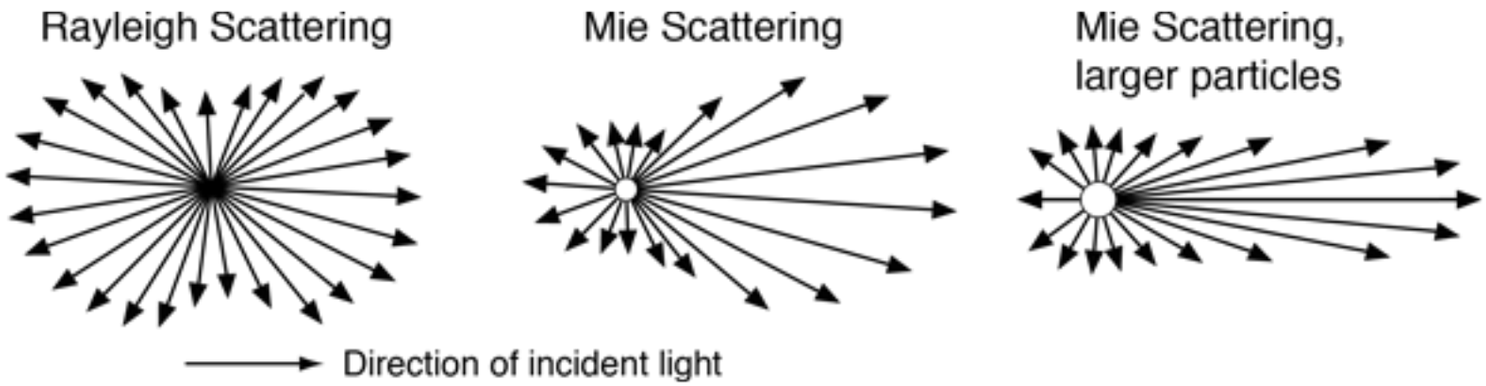
P. Vekilov, “The two-step mechanism of nucleation of crystals in solution” (2010)

The two-step mechanism of nucleation of crystals.

Top: Microscopic viewpoint in the (concentration, structure) plane; the thick dashed line highlights the two-step pathway and the diagonal solid arrow highlights direct nucleation.

Bottom: The free energy G along three possible nucleation pathways: direct nucleation, the two- step mechanism and crystals forming within macroscopic dense liquid, as seen by Vivare’s et al. (2005), following the Ostwald rule of stages (Ostwald, 1897).

WHY OPTICAL METHODS?



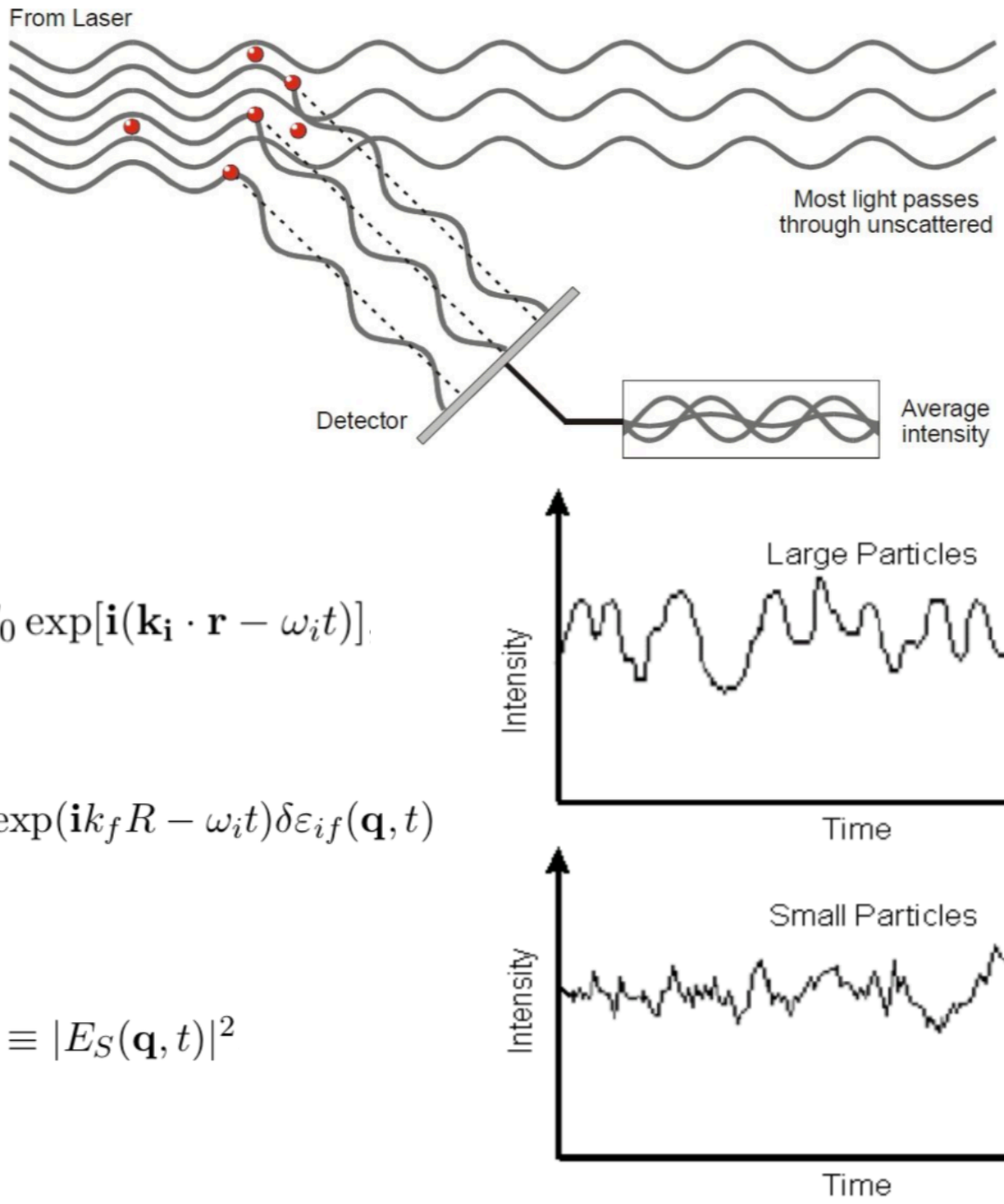
Direct access to optical properties of particles

- ✔ fast (statistically significant)
- ✔ non invasive
- ✔ continuous flow analysis
- ✔ field measurements

Indirect access to particle morphology

- → size (commonly investigated)
- → composition
- → shape, hence orientation
- → internal structure, surface properties

LIGHT SCATTERING



$$\mathbf{E_i}(\mathbf{r},t) = \hat{\mathbf{n}}_\mathbf{i} E_0 \exp[\mathbf{i}(\mathbf{k_i}\cdot\mathbf{r} - \omega_i t)]$$

$$E_S(\mathbf{q},t) = \frac{-k_f^2 E_0}{4\pi R\varepsilon_0}\exp(\mathbf{i}k_f R - \omega_i t)\delta\varepsilon_{if}(\mathbf{q},t)$$

$$I_S(\mathbf{q},t) \equiv |E_S(\mathbf{q},t)|^2$$

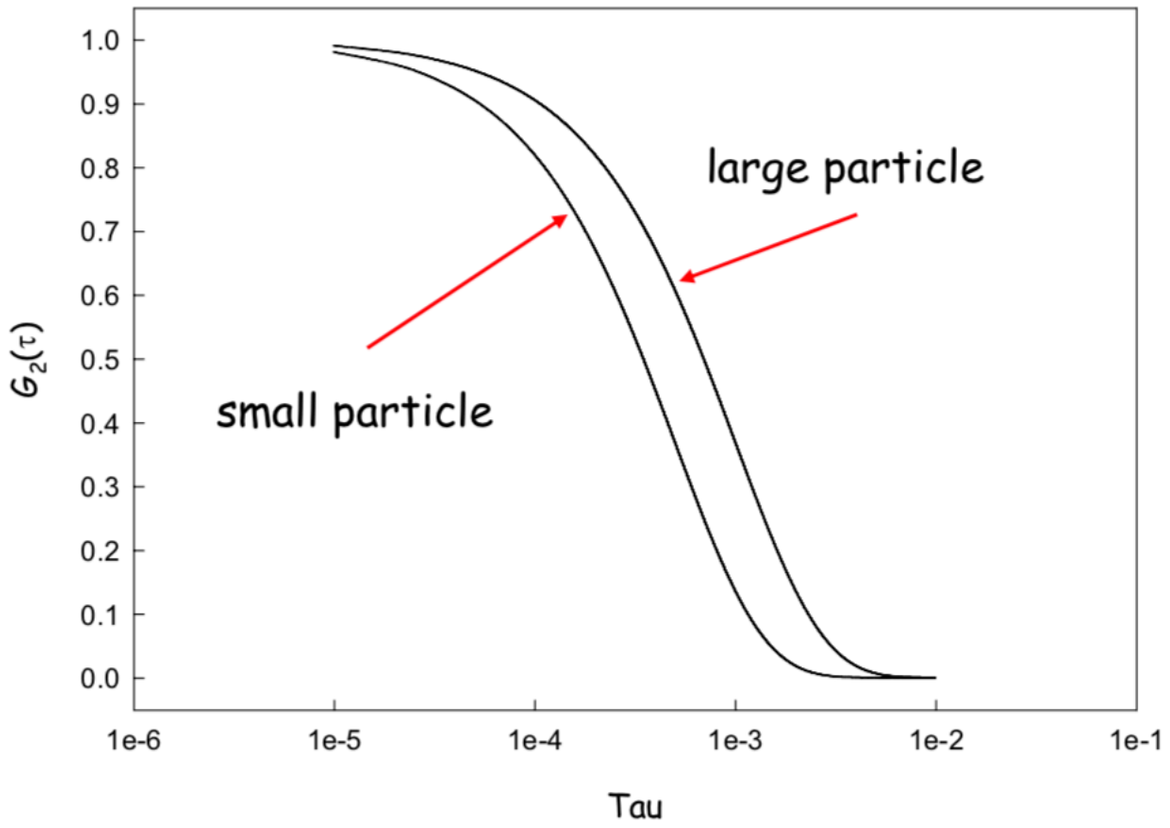
CORRELATION FUNCTION

$$G^{(2)}(q,\tau) = \langle I_S(\mathbf{q},0) I_S(\mathbf{q},\tau)\rangle \equiv \lim_{T\to\infty} \frac{1}{T}\int_0^T dt I_S(\mathbf{q},t) I_S(\mathbf{q},t+\tau)$$

$$g^{(1)}(q,\tau) \equiv \frac{\langle E_S^*(\mathbf{q},0) E_S(\mathbf{q},\tau)\rangle}{\langle I_S(\mathbf{q})\rangle} \qquad g^{(2)}(q,\tau) = A(1+\beta[g^{(1)}(q,\tau)]^2)$$

For a large number of mono-disperse particles in Brownian motion,
the correlation function is an exponential decaying function of the correlation time delay τ.

$$g^{(2)}(\tau) = A(1+\beta[e^{-2\Gamma\tau}])$$

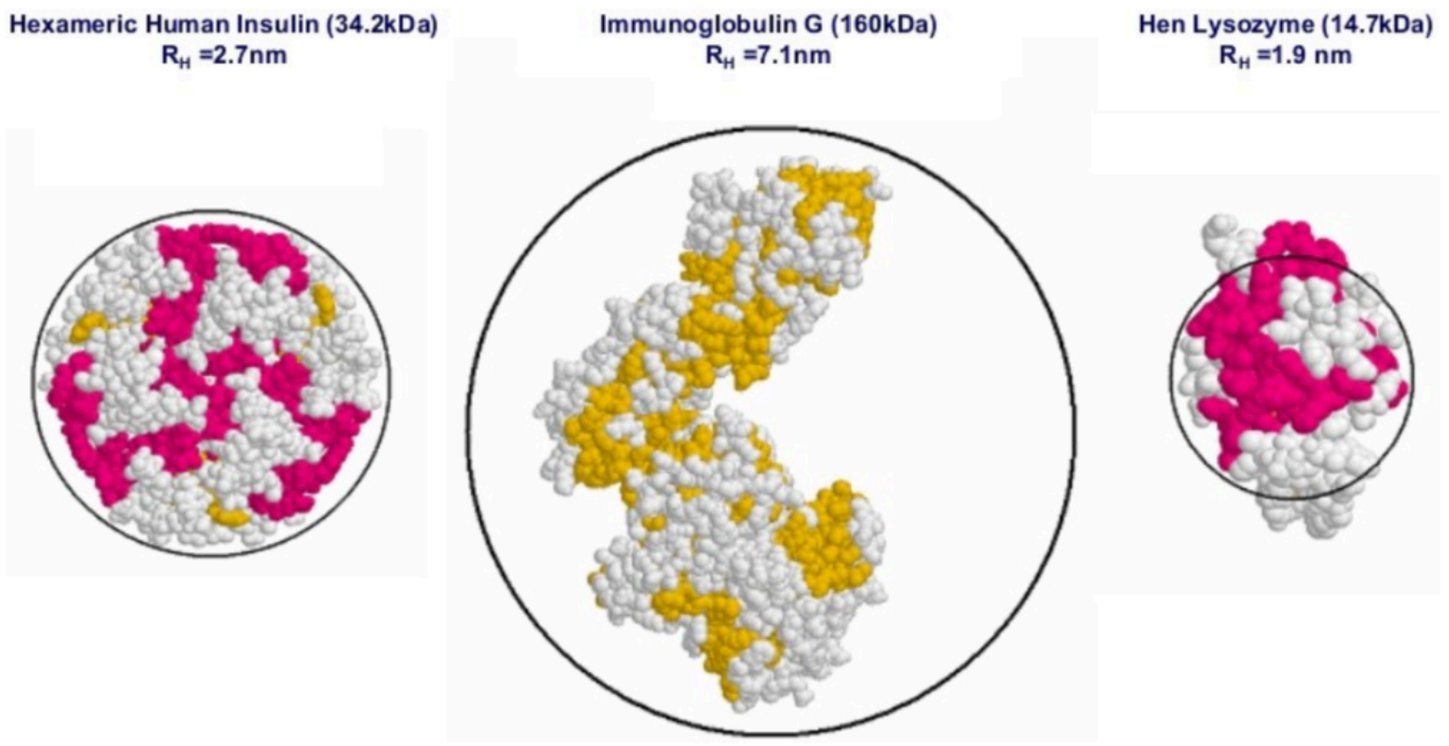


CORRELATION FUNCTION

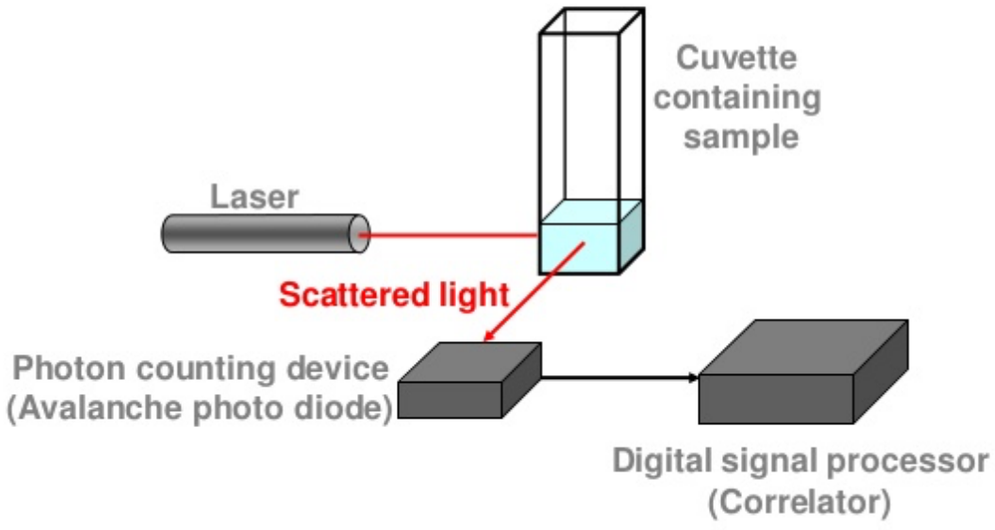
$$g^{(2)}(\tau) = A(1 + \beta[e^{-2\Gamma\tau}])$$

$$\Gamma = \tau_c^{-1} = q^2 D_0 \qquad\qquad R_H = \frac{k_B T}{6\pi\eta D_0}$$

The hydrodynamic diameter of a non-spherical particle is the diameter of a sphere that has the same translational diffusion speed as the particle.



DLS – DYNAMIC LIGHT SCATTERING



Dynamic light scattering refers to measurement and interpretation of light scattering data on a microsecond time scale.

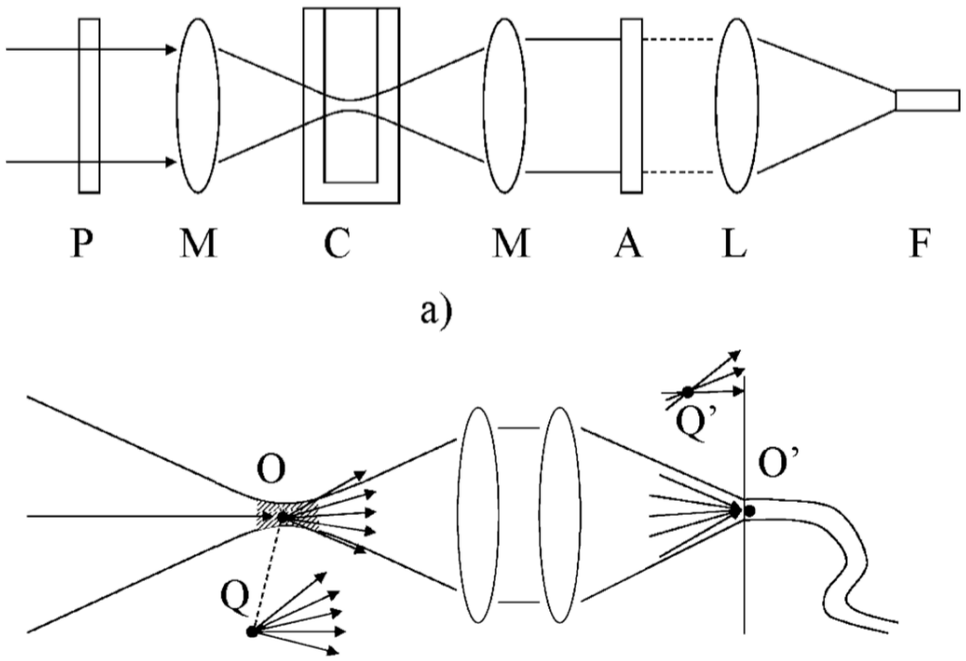
THE BENEFITS OF DLS

- Sample volumes are small, down to just a few micro-liters, making this an appealing technique for early stage research where valuable materials are involved.
- DLS is essentially very good at measuring particle size across the range ~ 0.1nm to ~ 10µm.
- It is possible to measure both dilute and turbid systems with the concentration range for analysis reaching down as low as 0.1ppm and up to 40% w/v.

CDDLS – COFOCAL DEPOLARIZED DYNAMIC LIGHT SCATTERING

Multiple scattering is when the scattered light from one particle is scattered by another before reaching the detector, and it compromises the accurate calculation of particle size in more concentrated samples.

To avoid them and to study not only translation diffusion, but also rotational diffusion, cDDLS has been developed.

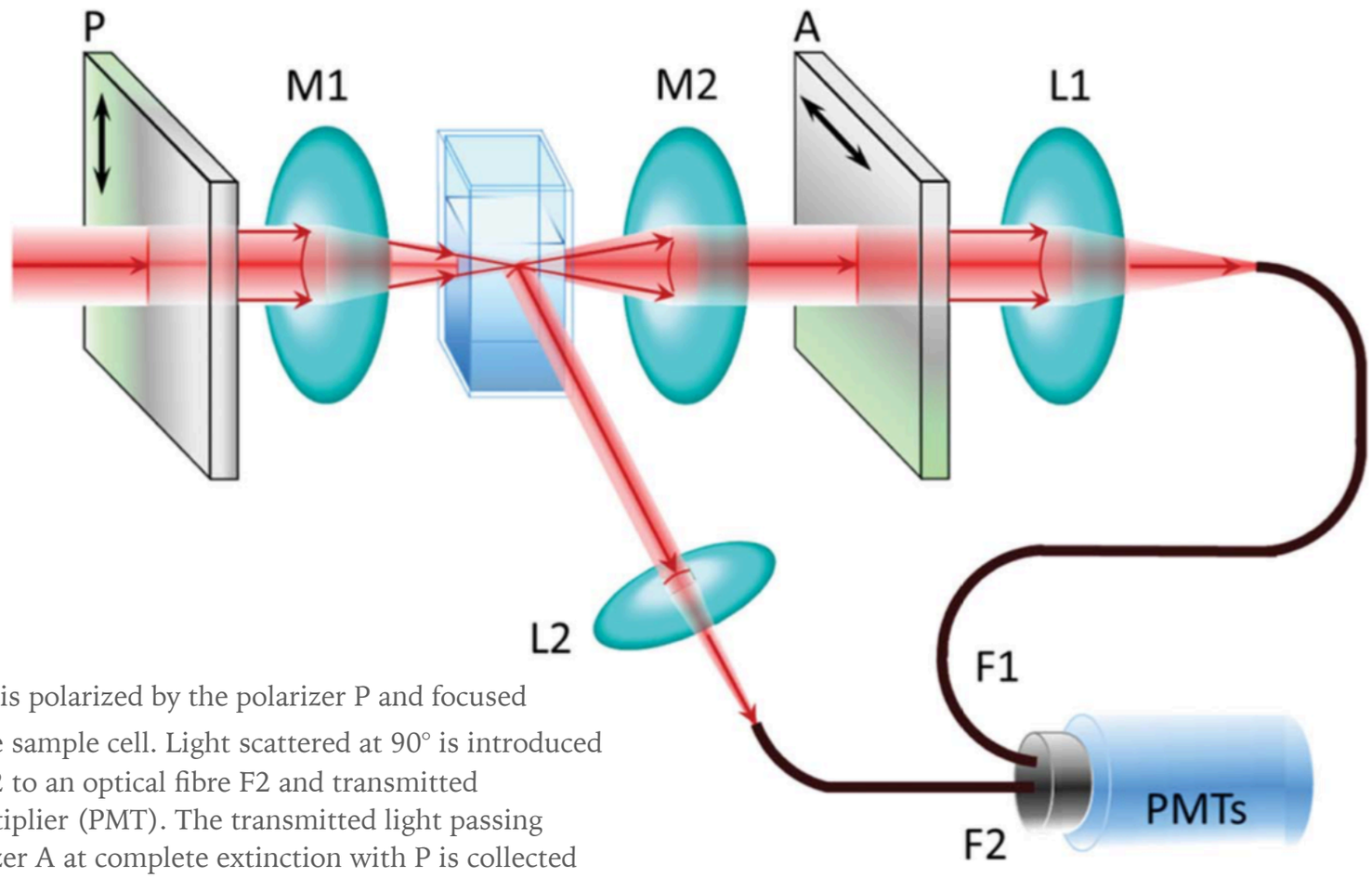


(P) polarizer; (M) micro- scope objectives (20×, NA = 0.3);
(C) cuvette; (A) analyzer; (L) focusing doublet; (F) single-mode fiber.
Potenza et al., Confocal zero-angle dynamic depolarized light scattering (2009)

The rotational Brownian motion randomly changes the orientation of the particles resulting in fluctuations in the depolarized scattered fields.

$$R^3 = \frac{3k_BT}{4\pi\eta}\tau$$

OUR INSTRUMENT: DLS + CDDLS



A laser beam is polarized by the polarizer P and focused by lens M1 into the sample cell. Light scattered at 90° is introduced by lens L2 to an optical fibre F2 and transmitted to a photomultiplier (PMT). The transmitted light passing through an analyzer A at complete extinction with P is collected by lens L1 in a confocal scheme and sent by optical fibre F1 to a second photomultiplier. Both PMTs are connected to correlators.

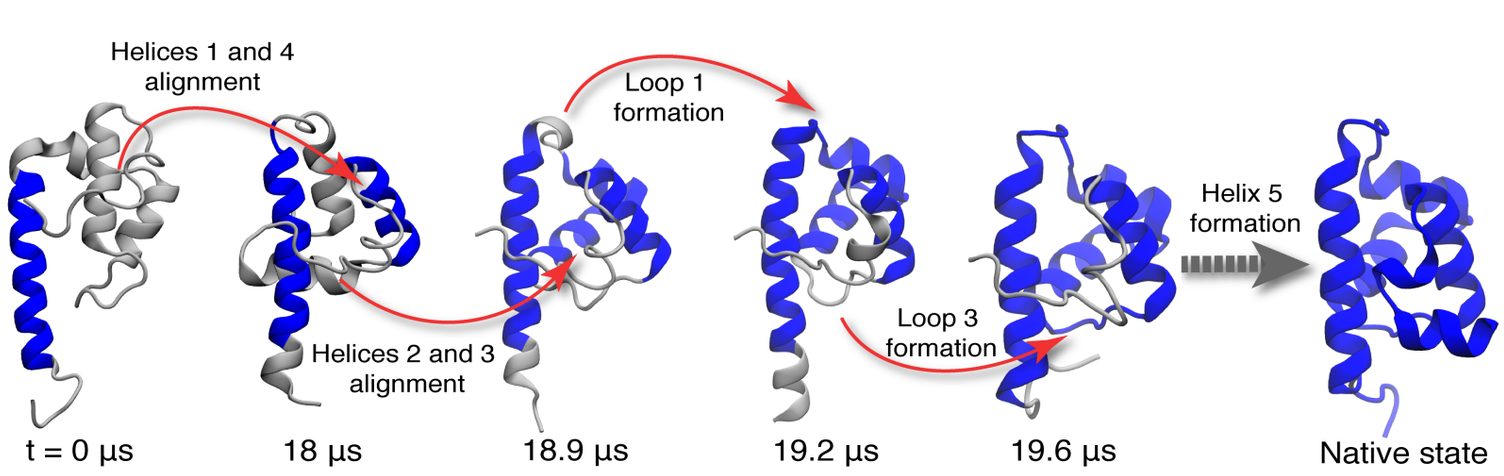
$$[g_2(t) - 1]^{1/2} = A \exp\left(-\frac{t}{\tau}\right) + b$$

$$[g_2(t) - 1]^{1/2} = \frac{A}{(2\pi)^{1/2}\sigma} \exp\left(\frac{-t^2}{2\sigma^2}\right) + b$$

$$[g_2(t) - 1]^{1/2} = A_1 \exp\left(-\frac{t}{\tau_1}\right) + A_2 \exp\left(-\frac{t}{\tau_2}\right) + b$$

PROTEINS PROPERTIES

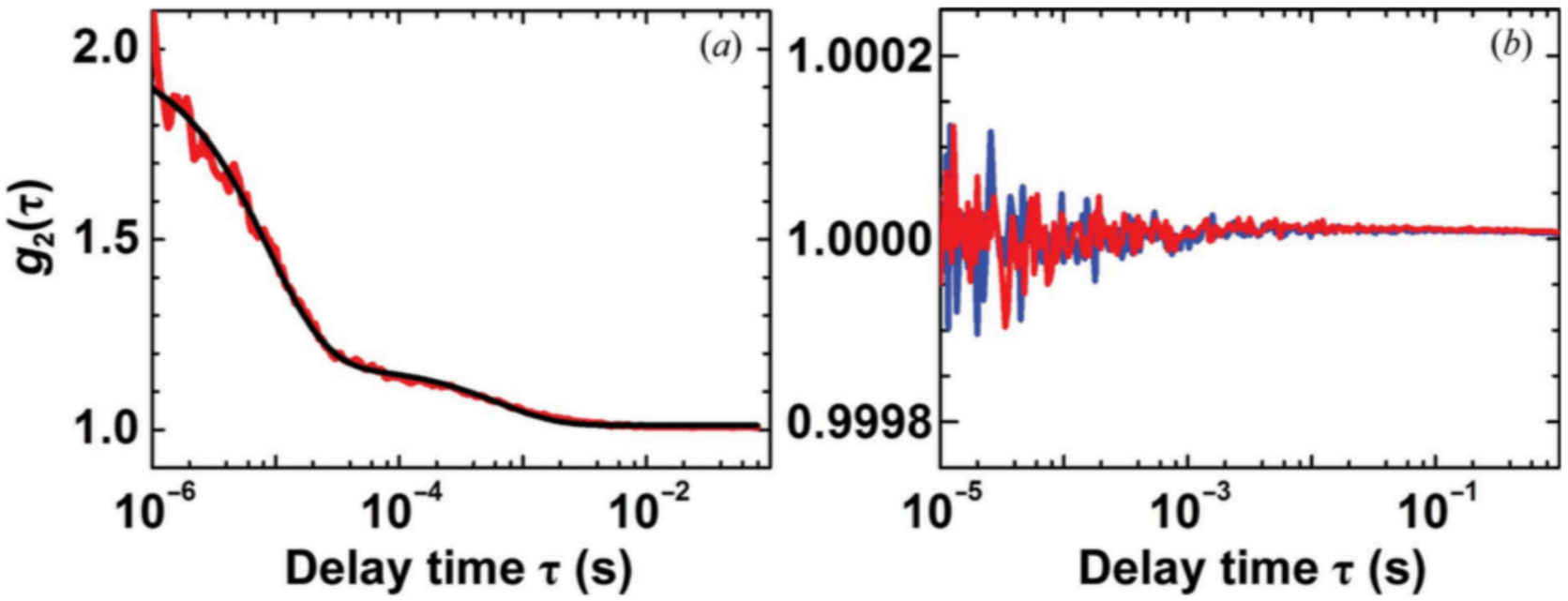
Proteins (also known as polypeptides) are organic compounds made of amino acids arranged in a linear chain and folded into various forms. Protein stability is a particularly relevant issue today in the pharmaceutical field and will continue to gain more importance as the number of therapeutic protein products in development increases.



During the development of a protein formulation, a combination of appropriate analytical methods must be used to detect subtle changes in the state of a protein to ensure the efficacy and safety. DLS and cDDLS measurements are two of these methods.

PROTEINS PROPERTIES MEASUREMENTS

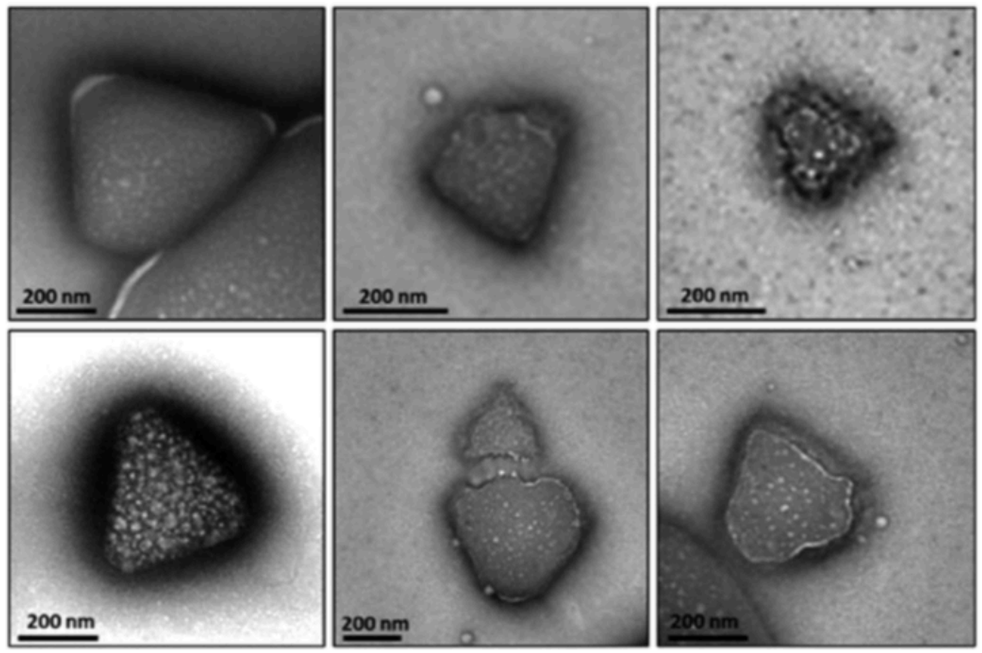
DLS and cDDLS are rapid, non-invasive techniques for determination of protein size. The size is calculated using the Stokes-Einstein equation. These techniques provide rapid access to size information for the characterization of proteins.



(a) A DLS intensity correlation function (red) of a lysozyme solution at 138 mg ml in 20 mM NaAc buffer pH 7.8 and the fitted function (black). (b) cDDLS intensity correlation functions of the same lysozyme solution (red) and water (blue).

CLUSTER: NUCLEATION PRECURSOR

Protein crystal nucleation is a central problem in biological crystallography and other areas of science, technology and medicine. Recent studies have demonstrated that protein crystal nuclei form within crucial precursors.



Schubert et al., “Real-Time Observation of Protein Dense Liquid Cluster Evolution during Nucleation in Protein Crystallization”

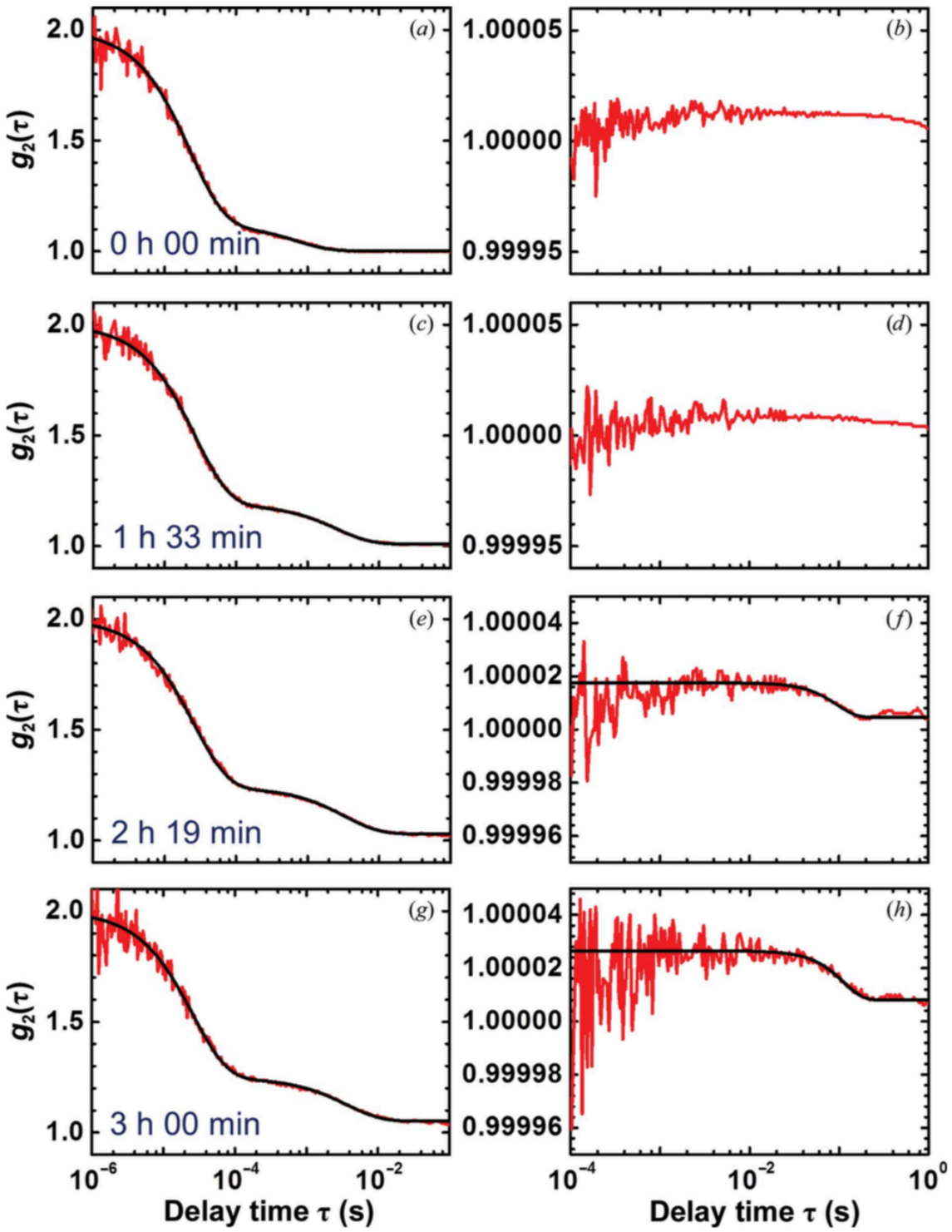
Protein-rich clusters that may be the nucleation precursors have been observed in solutions of several proteins: lysozyme, haemoglobin A and S, lumazine synthase, insulin and others. The size of the clusters typically varies from several tens to several hundreds of nanometres and their total volume fraction remains less than 10^{-3}.

LYSOZYME MEASUREMENTS – VUB RESULTS

- Lysozyme 35 mg/mL with NaCl 34 mg/mL, buffer NaAc pH 4.5

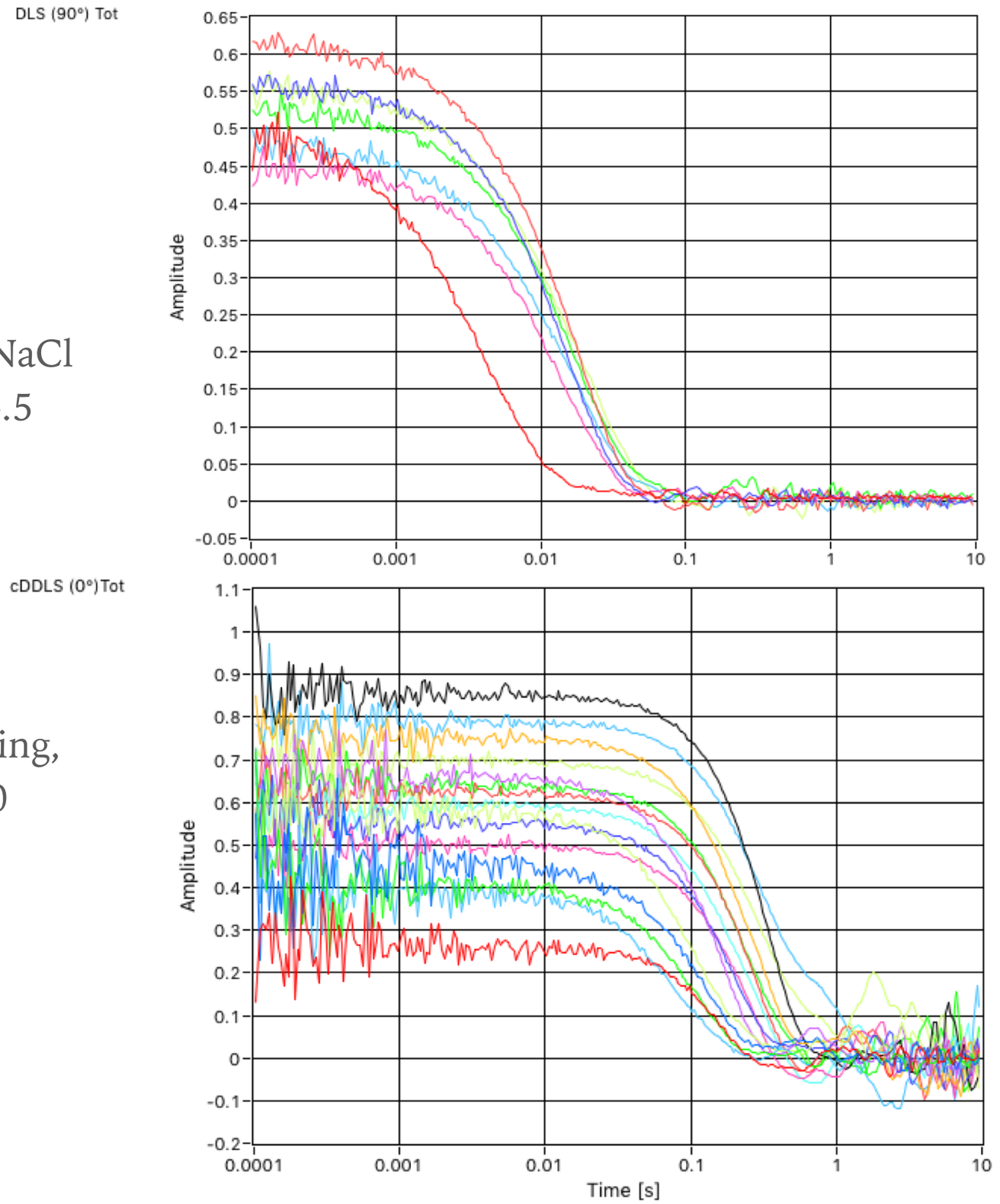
- Left column: DLS
  Fast mode: unchanged
  Slow mode: $r = at^{0.32}$

- Right column: cDDLS
  too less cluster

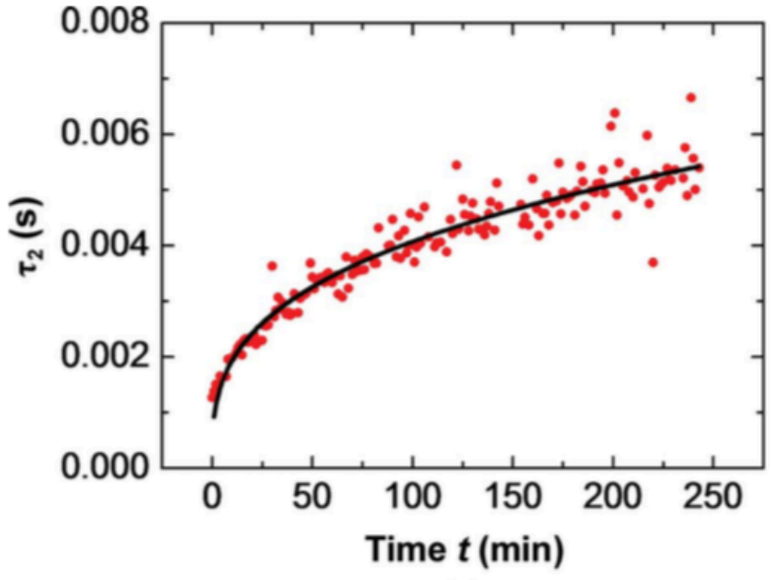


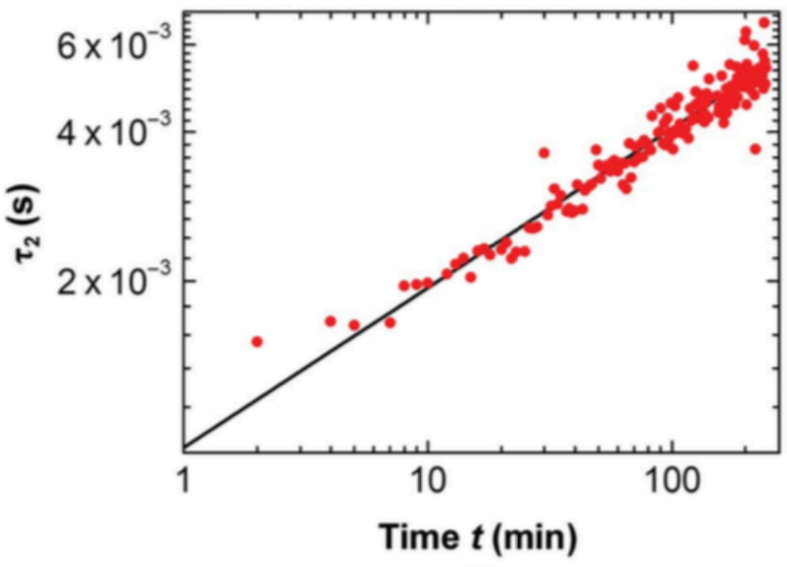
LYSOZYME MEASUREMENTS – OUR RESULTS

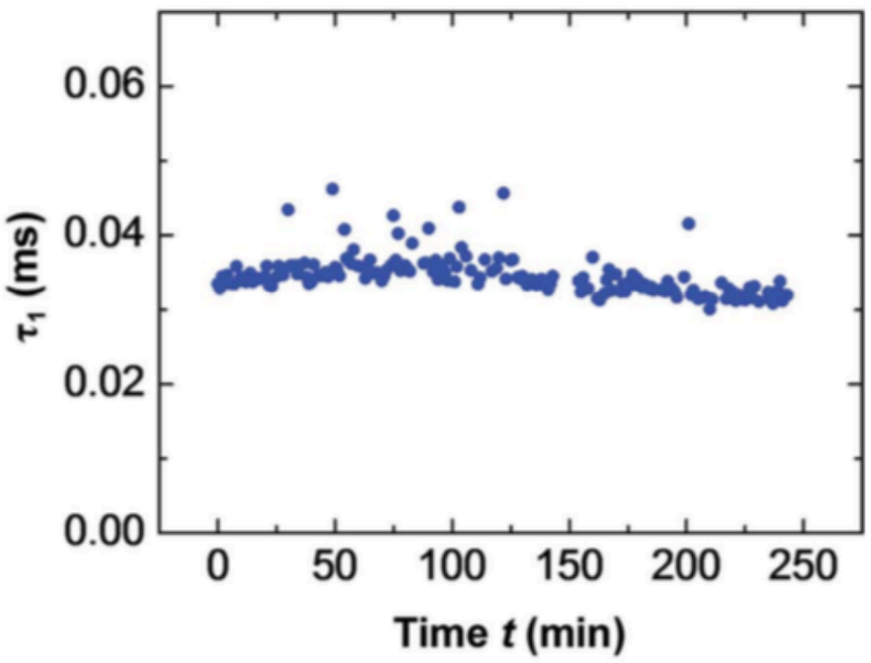
- Lysozyme 34 mg/mL with NaCl 50 mg/mL, buffer NaAc pH 4.5
- Top: DLS
  Fast mode: unchanged
  Slow mode: $r = at^{0.32}$
- Bottom: cDDLS
  Simple exponential at beginning, Gaussian exponential after 30 minutes.



LYSOZYME MEASUREMENTS – OUR RESULTS







HYPER GRAVITY EFFECT

Recent time resolved TEM demonstrated the existence of two types of pre-nucleation clusters. We propose to characterize both clusters family with DLS and cDDLS in the Large Diameter Centrifuge. Moreover, in hyper gravity the clusters that are already present in solution will sediment and can be excluded from the observed volume. This offers the opportunity to study the generation of new clusters

HYPERGRAVITY EFFECT: LDC

A Large Diameter Centrifuge (LDC) has been developed by ESA allowing the acquisition of measurement points in the range from 1 to 20 g in order to understand and describe the influence of gravity in systems. This instrument can provide a hypergravity environment for cells, plants and small animals, as well as physical science and technological experiments.

The LDC is part of the Life and Physical Sciences Instrumentation and Life Support Laboratory (LIS) at ESTEC (the Netherlands), dedicated to serve the science and technology user communities throughout Europe.

Thanks for your attention.