Formation of Dynamic Clusters in Concentrated Protein Solution

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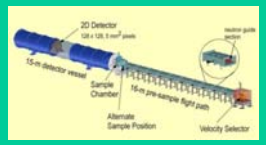
Abstract

In lysozyme solution, it has been shown that the competition of a short-ranged attraction and long-ranged Coulomb interaction results in the formation of clusters, as indicated by a so-called cluster peak in the structure factor revealed by SANS. This result has recently been questioned by a joint study of SANS/SAXS at the same experiment conditions, which claims the absence of equilibrium protein clusters. We present a combined study of lysozyme solutions by SANS and neutron spin-echo (NSE), which simultaneously investigates the structural and dynamical properties of the system at the nanometer length scale. Our results indicate that at relatively low concentration, the monomer or dimer dominate the short-time dynamics while at large concentrations, dynamic protein clusters form in solution.

SANS instruments at NCNR.



Probed length scale: ~1 nm to ~ 500 nm

Why Neutrons?

- Unlike X-ray scattering, neutron scattering is sensitive to low charge elements, such as hydrogen.
- Appropriate energy (probing the key motions of atoms).
- Good penetrating capability.
- Isotope labeling (selectively observing structures).

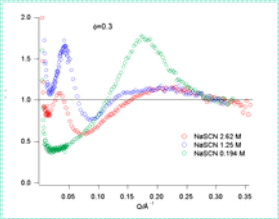
NSE (Neutron Spin Echo)

Dynamic range: a few ps to 100 ns!

Motivations

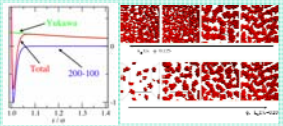
- Protein aggregations/nucleation are responsible for many diseases, such as cataracts, Alzheimer's disease, and Parkinson's disease.
- Protein crystallization.
- Controlled self-assembly and spontaneous patterning by tuning interaction potentials.

1. Protein Clusters



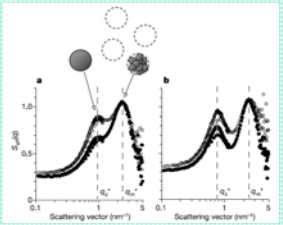
When Cytochome C protein forms a gel by adding excessive amount of salt, its SANS results show a surprising extra peak (cluster peak) at a Q value much smaller than that of the protein-protein correlation peak (monomer peak) indicating the formation of clusters in protein gel. (Q is the scattering wave vector.)

B. Lonetti et al, Phys. Chem. Chem. Phys. **6**, 1388, (2004)



Computer simulation demonstrates that the competition of a short-range attraction and a relatively long-range repulsion is responsible for the formation of colloidal clusters which can led to the gelation of a system.

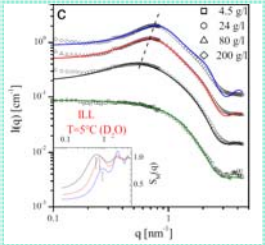
Sciortino et al, Phys. Rev. Lett. **93**, 055701, (2004)



Interestingly, even in lysozyme solution, the inter-particle structure factor also shows an extra peak (cluster peak) at small Q value. The appearance of this peak is interpreted due to the formation of equilibrium clusters in a solution. Some interesting features of the clusters have been claimed:

- The position of the cluster peak, Q_c, is independent of concentration, i.e., the number density of clusters remains constant at different concentrations.
- The aggregation number for each cluster is small.
- The clusters are long-lived.

A. Stradner et al, Nature, **432**,492 (2004)

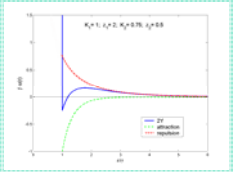


By combining SANS and SAXS study, the experiments by Shukla et al. have had different conclusions for lysozyme protein clusters in solution at the same experiment conditions.

- Q_c is dependent on concentration.
- There are NO equilibrium clusters. The system consists of "largely repulsive individual lysozyme molecules".

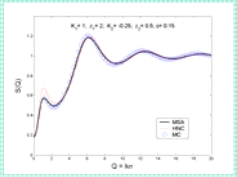
A. Shukla et al, PNAS, **105**,5075 (2008)

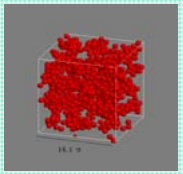
2. Understanding Structure Information



$$\beta u(r) = \begin{cases} \infty, & 0 < r \le 1 \\ -K_1 \frac{e^{-z_1(r-1)}}{r} + K_2 \frac{e^{-z_2(r-1)}}{r}, & r > 1 \end{cases}$$

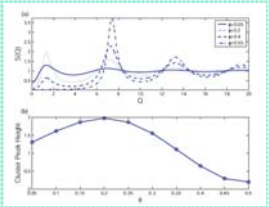
Two Yukawa Model: One Yukawa term is used to simulate the short-range attraction and another one is used to simulate the long-range repulsion.





The inter-particle structure factor of a system interacting with a two-Yukawa potential is calculated by solving OZ equation using different closures. When the strength of the short-range attraction is small, both MSA and HNC closures work reasonably good when comparing to simulation data.

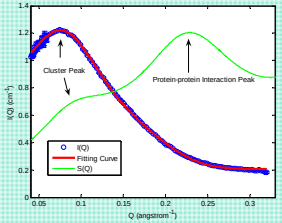
M.Broccio, D.Costa, Y.Liu, S.H. Chen, JCP 124, 084501 (2006)



Y. Liu, W. R. Chen, S. H. Chen, JCP, 122, 044507 (2005)

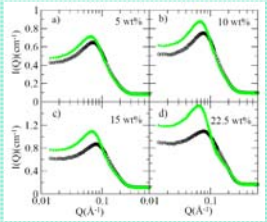
The cluster peak position is independent of volume fraction in a wide range of volume fraction if the potential parameter is kept as constant. (K_1=10,z_1=10,K_2=−1,z_2=0.5) The invariance of the peak position is not a necessary condition for the formation of clusters. The appearance of this peak is not a sufficient condition of protein cluster formation either.

SANS experiments were performed at NCNR and ILL. When decreasing temperature, the cluster peak shifts slightly to the smaller Q value. (Samples were prepared by dissolving the lysozyme proteins in 20 mM HEPES buffer.)

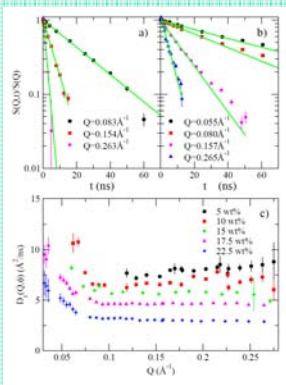


S. H. Chen, M. Broccio, Y. Liu, E. Fratini, P. Baglioni, J. Appl. Cryst., 40, s321 (2007)

The fitting of the SANS curve of 20 % lysozyme solution at room temperature using two-Yukawa model shows a very good agreement. The short-range attraction strength is about 4 k_BT when the HNC closure is used.



3. Understanding Dynamic Information



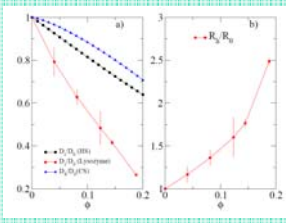
NSE experiments were performed at NCNR (NG5) and ILL (IN15).

NSE measures the normalized intermediate scattering functions (ISF), $S(Q,t)/S(Q)$.

At the short time limit, $S(Q,t)/S(Q) = exp(-Q^2D_c(Q)t)$, where Q is the scattering wave vector and t is the correlation function. $D_c(Q)$ is the collective diffusion coefficient and can be formulated as $D_0H(Q)/S(Q)$, where D_0 is the diffusion coefficient at infinite dilution and $H(Q)$ is the hydrodynamic function.

At the short-time limit, $S(Q,t)/S(Q)$ can be fitted with one exponential function nicely. The extracted collective coefficient as a function of Q and concentration is shown.

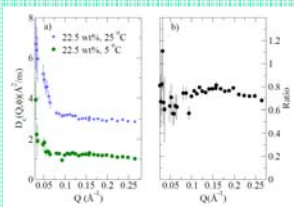
The near constant value at high Q of $D_c(Q)$ implies that there is no characteristic inter-protein dynamics at the time range probed.



The asymptotic value of $D_c(Q)$ at large Q is a good approximation of the self diffusion coefficient , D_s, of protein or protein clusters at the short-time limit.

D_s decreases much faster as a function of protein volume fraction, Φ, compared with that of a hard sphere (HS) system and a charge-stabilized (CS) colloidal system.

The estimated hydrodynamic radius based on the generalized Einstein-Stokes relation shows a monotonic increase of the cluster size.
At 5 wt%, $R_h/R_0 \approx 1.2$ indicating that the majority of proteins remain in a monomer or dimer state while at 22.5 wt%, $R_h/R_0 \approx 2.5$ indicating the progressive formation of large dynamic clusters.



When decreasing the temperature, $D_c(Q)$ remains constant at $Q > Q_c$.

$R_h/R_0 \approx 3.6$ at 5 °C.

The ratio of D_c/D_0 between 5 °C and 25 °C seems to have a minimum around 0.06 Å^{-1} indicating that at 5 °C, there might be additional slowing down of dynamics at the length scale corresponding to the cluster peak position.

L. Porcar, P. Falus, W. R. Chen, A. Faraone, E. Fratini, K. Hong, P. Baglioni, Y. Liu, *J. Phys. Chem. Lett.* 1, 126 (2010).