Prospects for XPCS on biophysical systems

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Outline

- Science Prospects for Dynamics in Biophysics using XPCS
- Dynamics of Protein suspensions: some current experiments
- Dynamics of Membranes: survey of results
- Prospects and Challenges for an MBA

Biomaterials are Soft-Matter

- Thermal fluctuations at room temperature create significant structural modifications
  - Protein shape fluctuations
  - Membrane bending modes
  - Fluctuations of filaments

Diffusion is Central to the function of Biomaterials

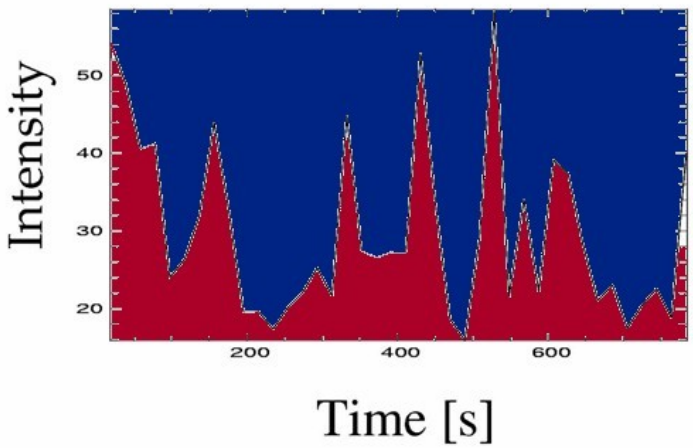
- At the cellular level, diffusive processes play a central role in transport. Diffusion is crucial in cellular signaling, regulating reaction rates, transmission of neurotransmitters accros synaptic gaps in nerve cells.
- Active transport processes depend partially on diffusion. For example active motor protein transport on the cytoskeleton.

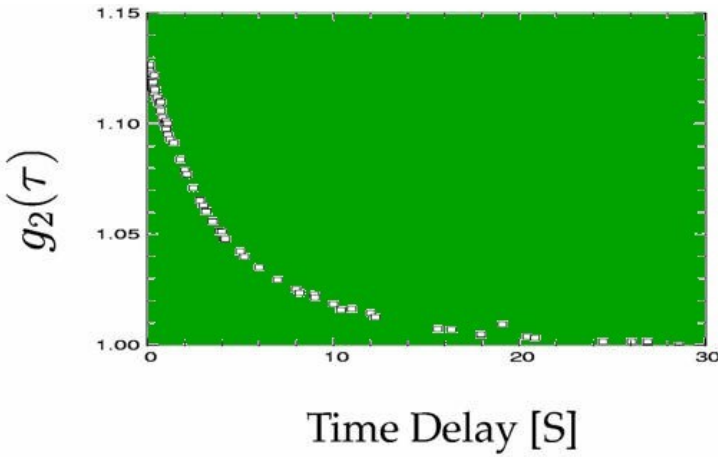
Self-Assembly

- Self assembly can depend on avoiding equilibrium states by following kinetic preferred paths. A detailed understanding of dynamics at the cellular level is thus crucial to understanding self-assembly mechanisms.
- Understanding protein crystallization kinetics could aid in studies of protein structure.
- Avoiding crystallization can also be important, for example in preventing cataract formation.

X-ray photon correlation spectroscopy as a probe of dynamics

Time correlation function $$g_2(\tau) = \langle I(t) I(t+\tau) \rangle / \langle I \rangle^2$$

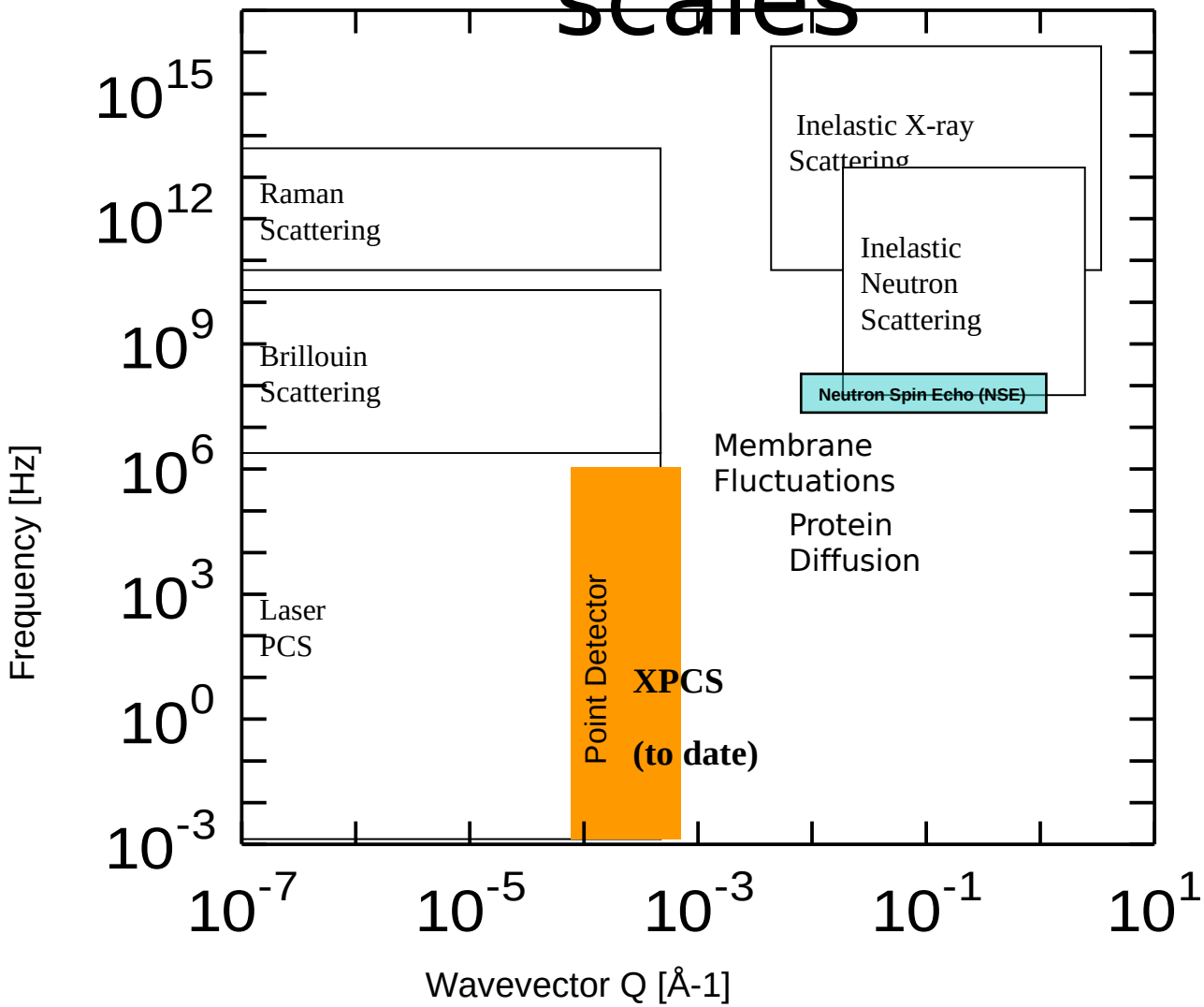




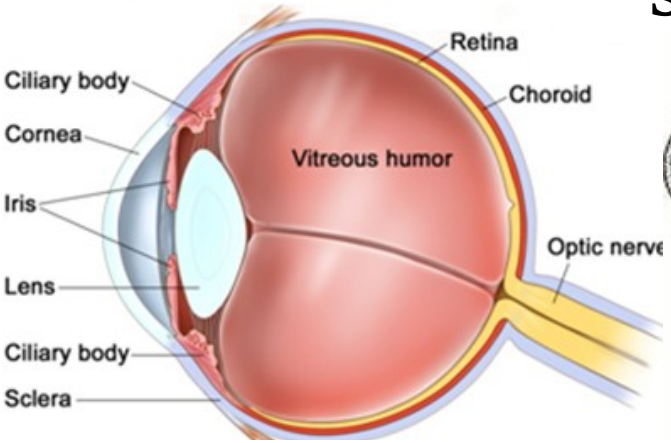
$$g_2(Q,\tau) = 1 + \beta f(Q,\tau)^2$$

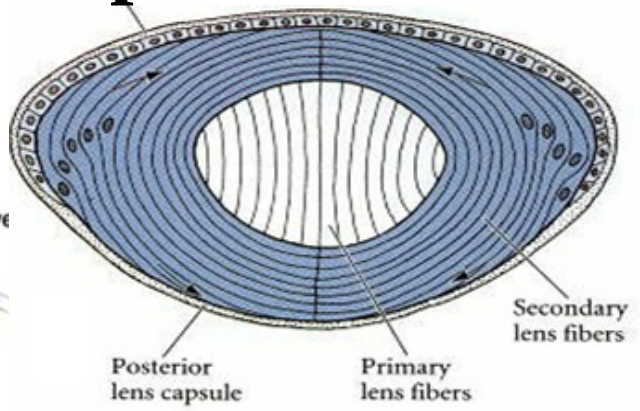
For Brownian Motion $$f(Q,\tau) = e^{-DQ^2\tau}$$

XPCS time and length scales



Diffusive dynamics of concentrated alpha crystallin suspensions





Bovine eye-lens

Oligomeric structure (Diameter ~15 nm)

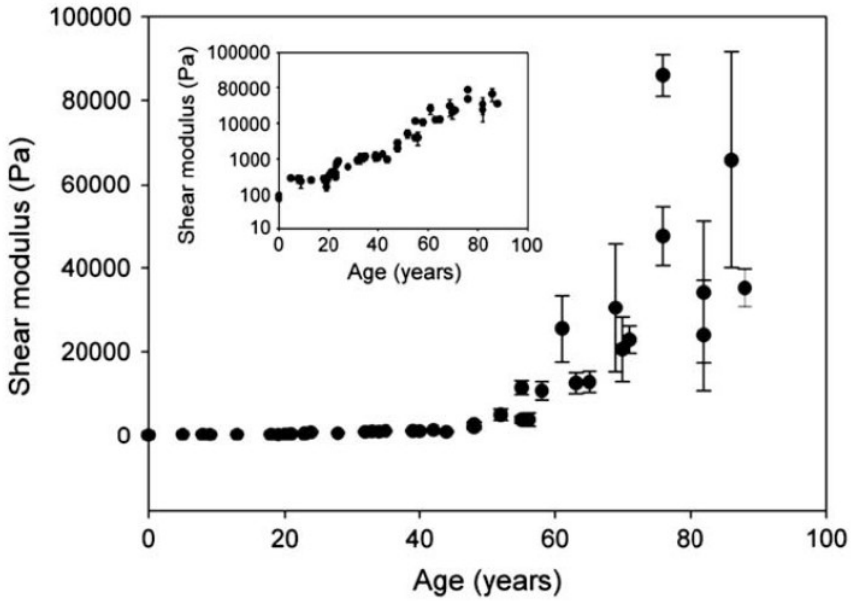
- The crystallin family of proteins determine the index refraction of the eye lens. Proteins within the central cortex of the eye are never replaced from birth.
- Alpha-crystallin is the largest and most prevalent of these proteins. In addition to forming the index of the lens, it also plays a crucial role in maintaining the transparency of the eye lens by sequestering damaged proteins.

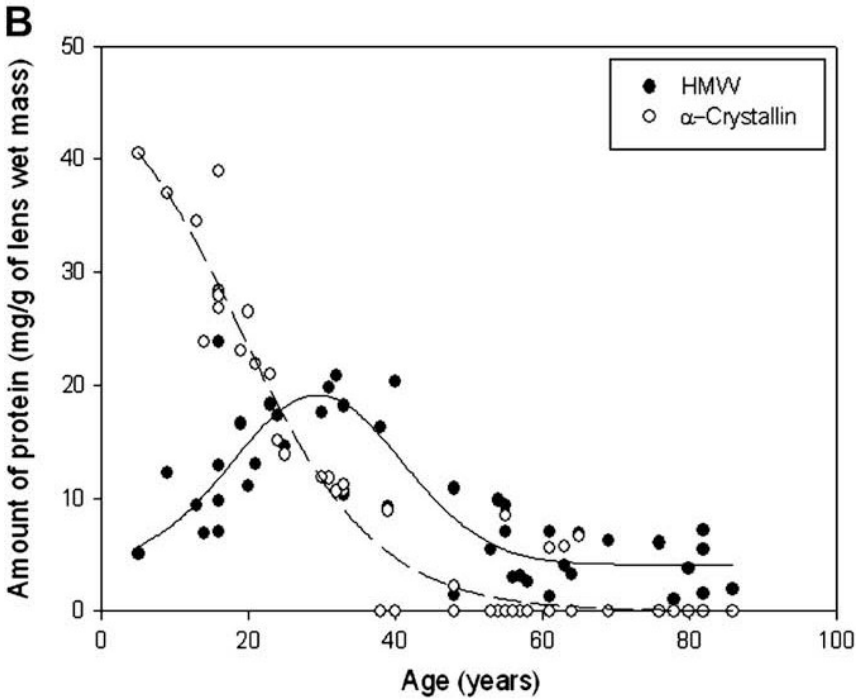
Crystallins and Eye Disease

There are two major diseases related to the aging of the eye-lens, cataract and

- Cataract: Opacification of the eye-lens. Can be caused by phase separation or crystallization of the eye-lens components.
- Presbyopia: Loss of flexibility in the eye lens.

Changes in alpha crystallin content is correlated to changes in lens elasticity



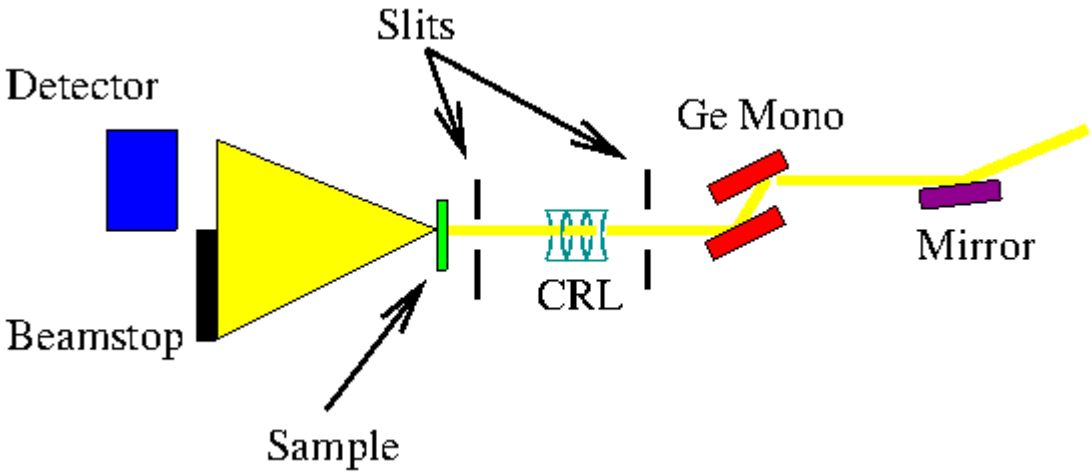


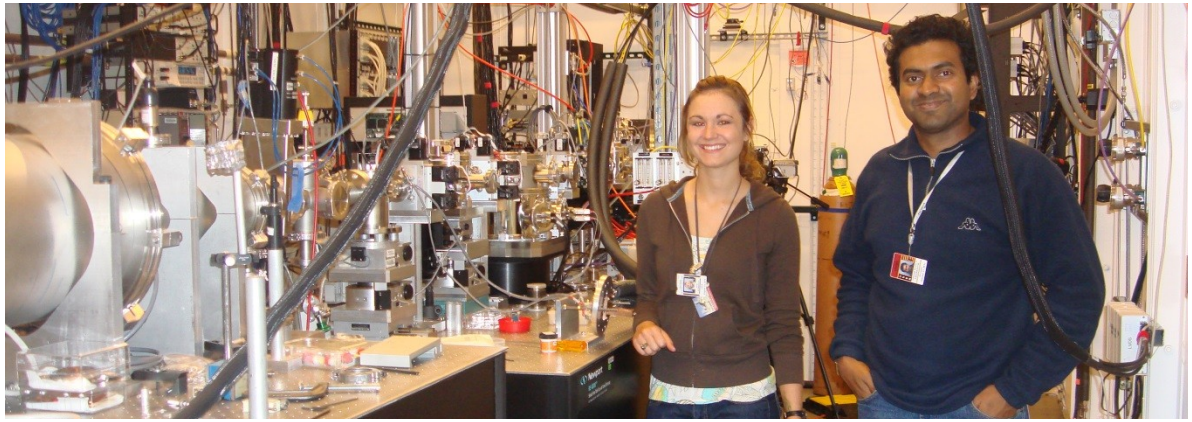
R. J. Truscott, Experimental Eye Research **88**, 241 (2009).

Motivations

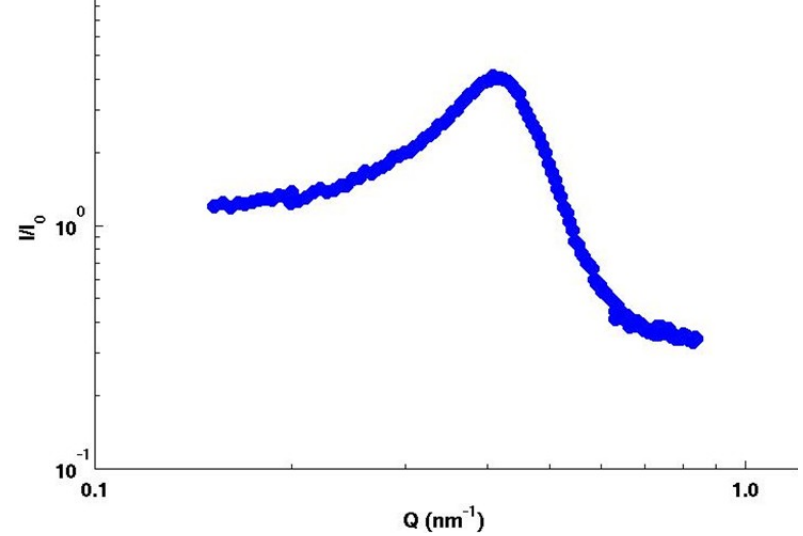
- The concentration of proteins in the eye can be up to 70% by weight. Why don’t they crystallize or phase separate? Has nature evolved these proteins to be glassy?
- Can dynamical studies of the crystallins provide new insight into interactions between these proteins?
- How does aging modify the dynamics of concentrated suspensions?
- How does the dynamics of mixtures of alpha, beta and gamma crystallins differ from single component suspensions?

Measurements at 8-ID-I at the Advanced Photon Source





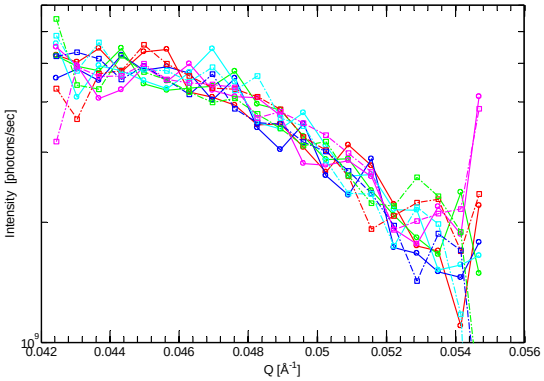
Damage Studies
I(Q) at peak in scattering



For XPCS measurements total sample exposures kept below 1s per spot

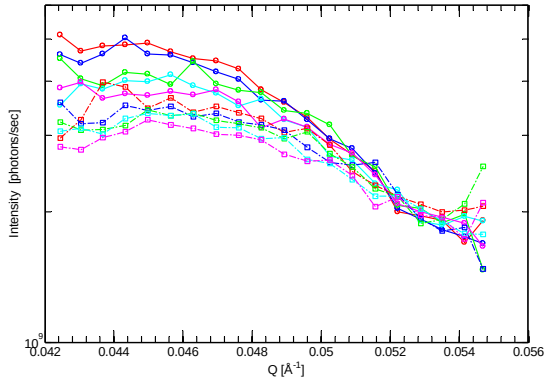
10 s total exposure

1 s intervals



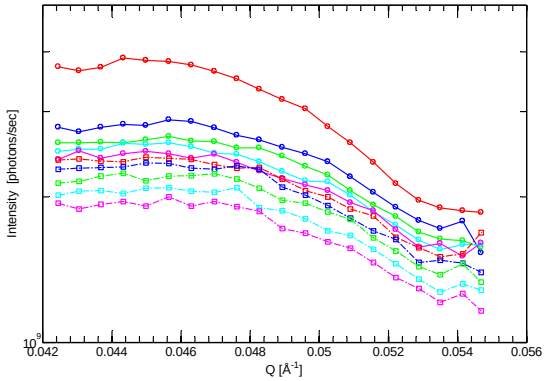
100 s total exposure

10 s intervals

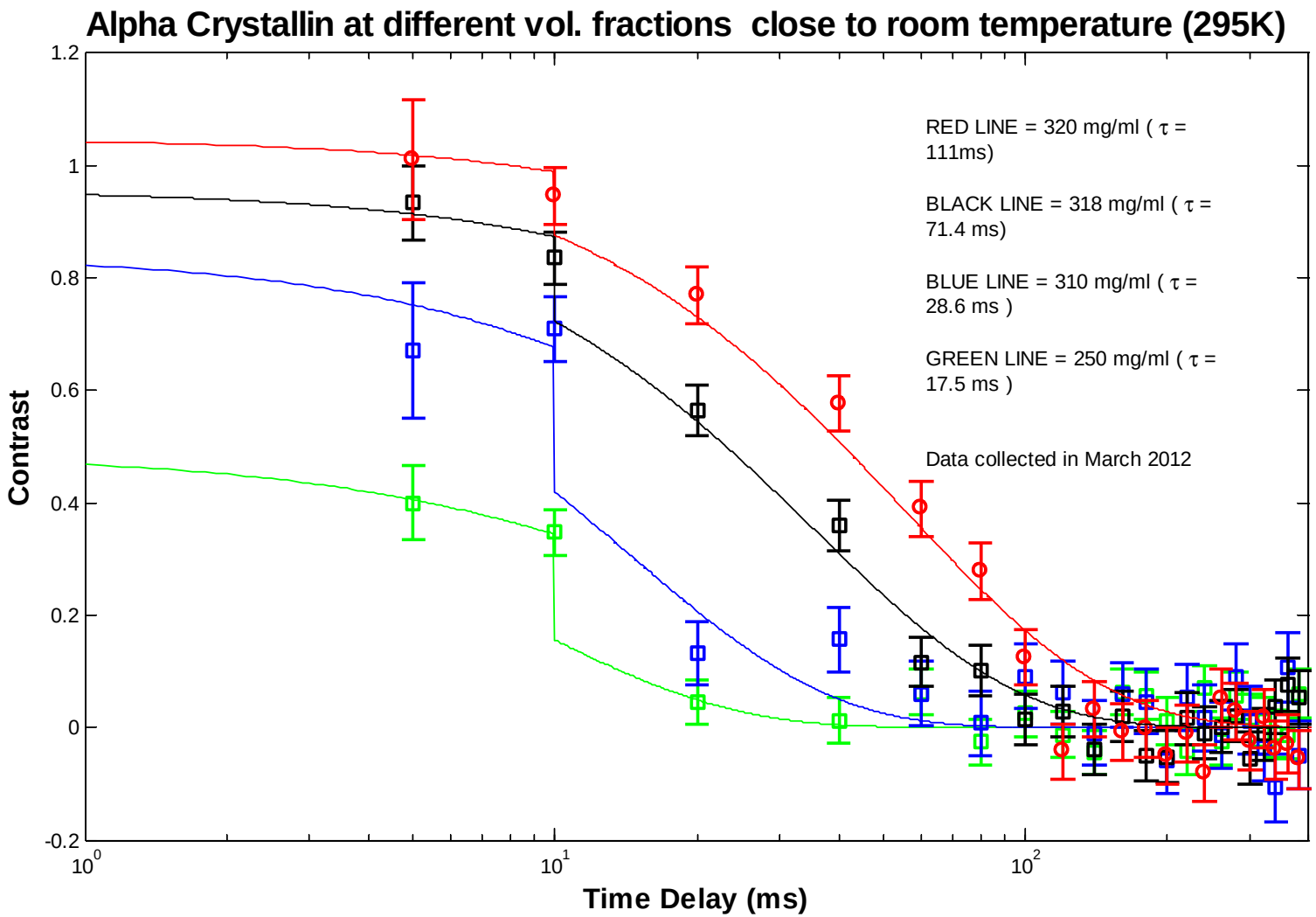


1000 s total exposure

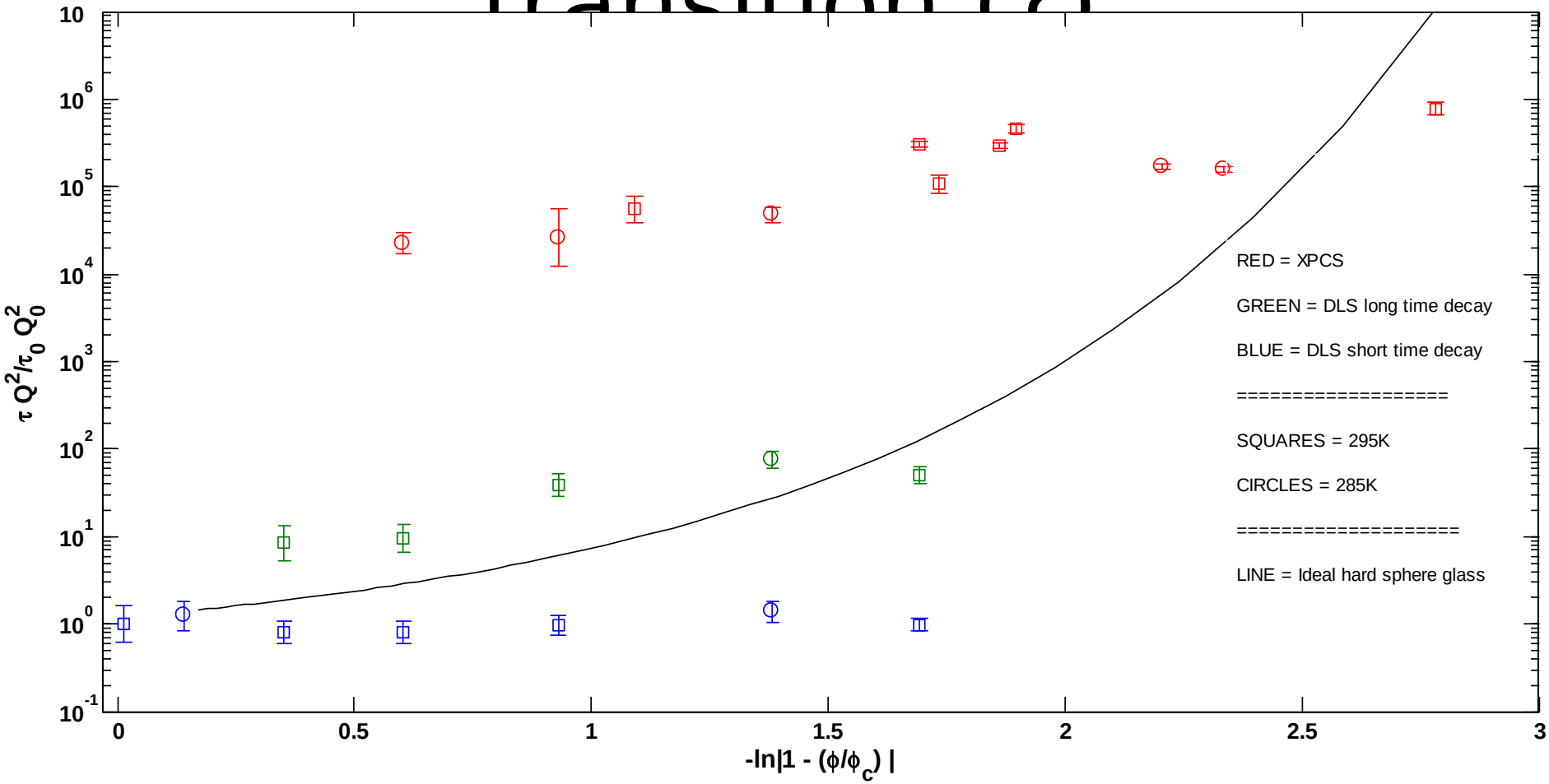
100 s intervals



Combined Speckle Visibility and XPCS Measurements



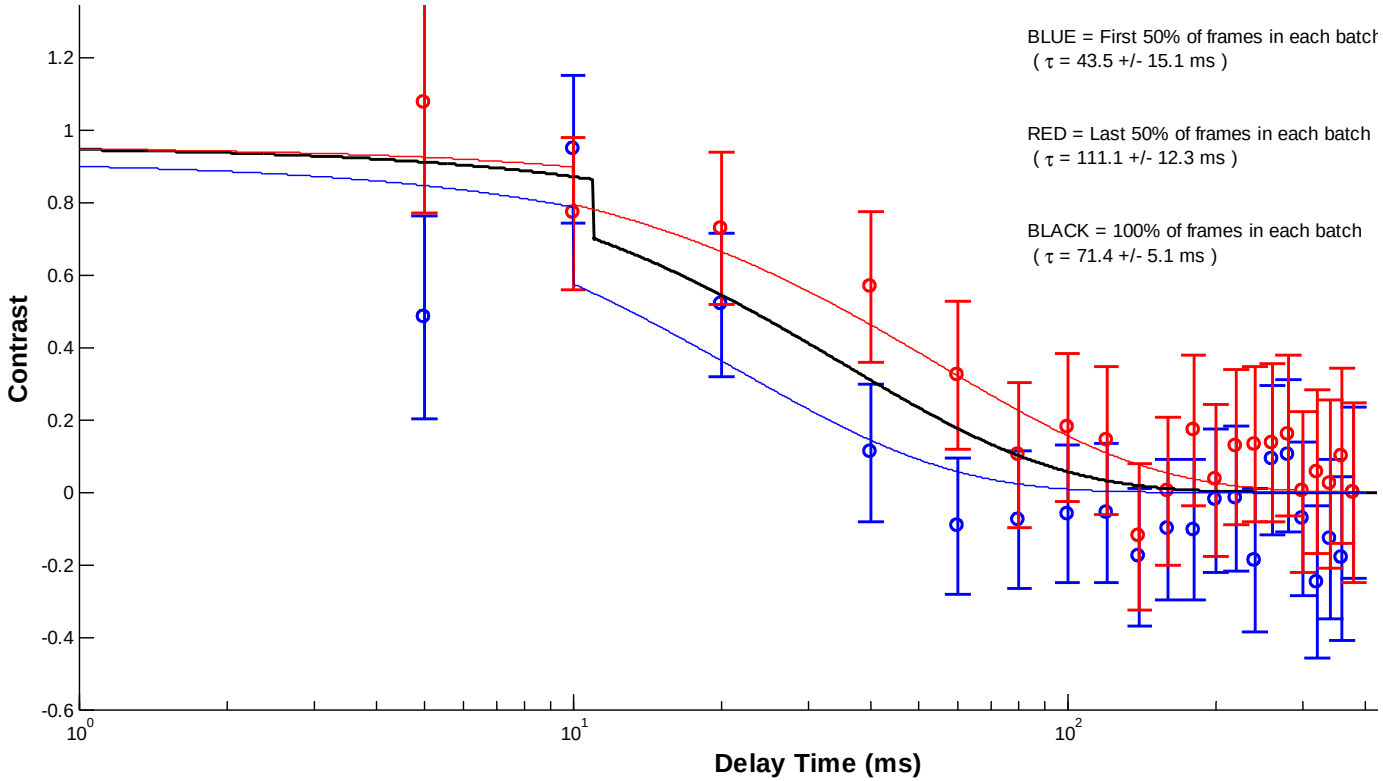
Approach to the glass transition (2)



Viscosity near a glass transition is given by Doolittle equation

$\eta = C \exp[D\varphi/(\varphi - \varphi 0)]$

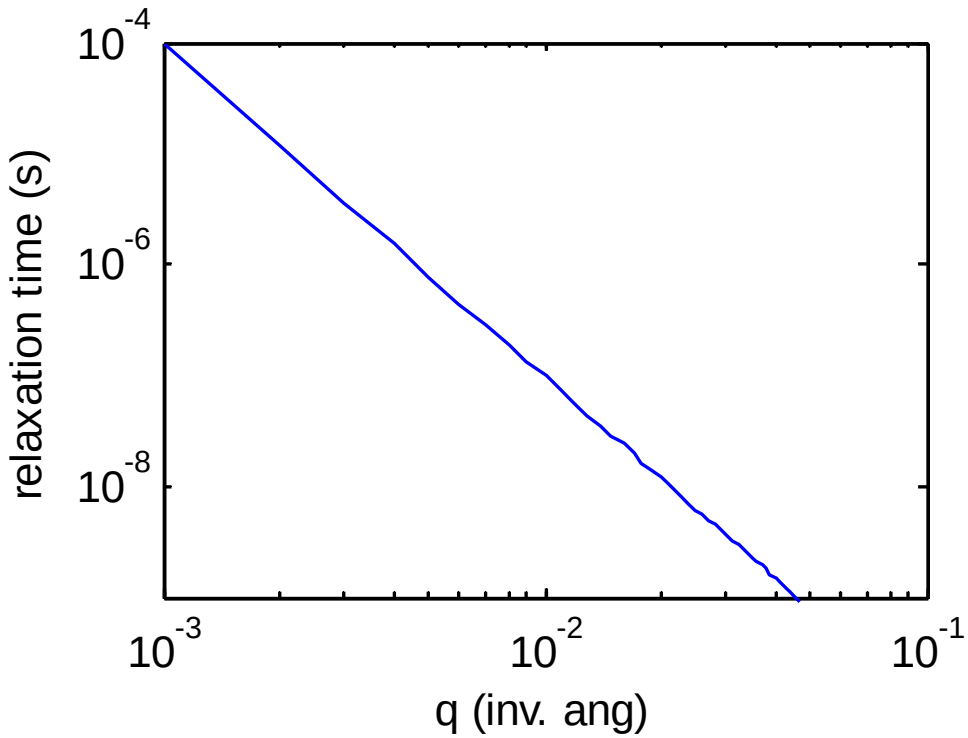
There appears to be x-ray damage visible in the dynamics at a significantly lower threshold than visible for static scattering. Damage threshold appears to be < 105 Gy



XPCS from Biomimetic Membranes

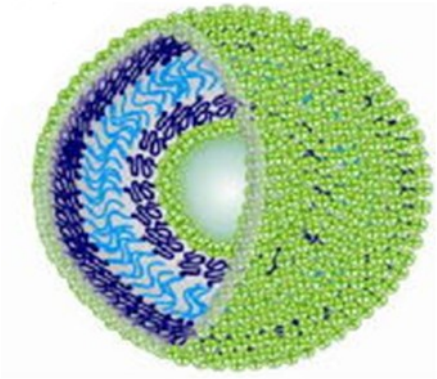
- Thermal fluctuations in membranes yield structural parameters such as bending moduli.
- In multi-component membranes thermal shape fluctuations can couple to composition fluctuations, changing the biochemistry of the membrane.
- No XPCS measurements of Phospholipid Membranes, but there have been a few successful measurements on related systems.

Typical Dynamics of a free Membrane



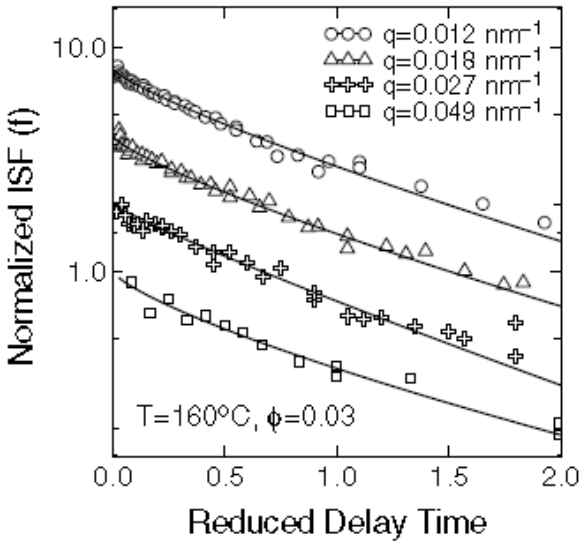
$$\tau \approx \frac{4\eta}{\kappa q^3}$$

- Falus, Borthwick and Mochrie, PRL, 2005.



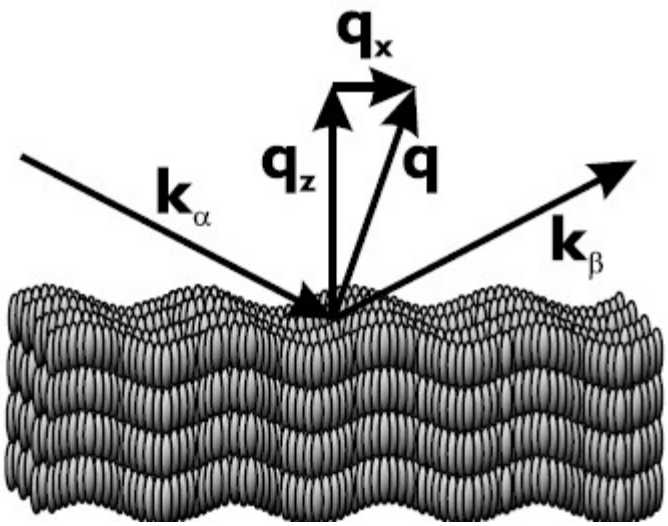
O'Reily et. al , Chem Comm 2010

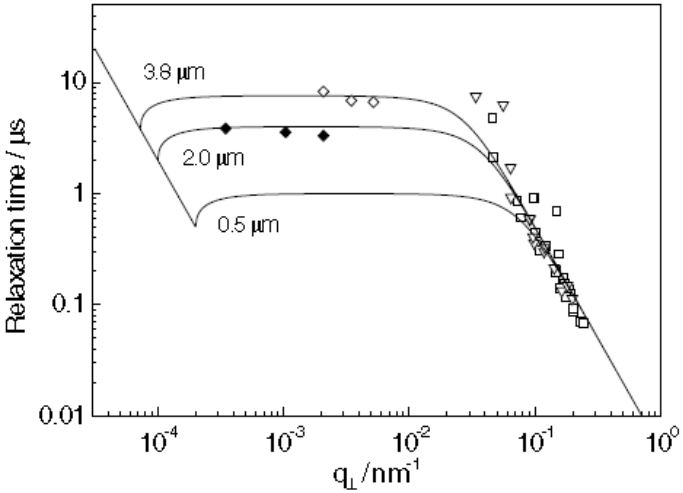
Measure dynamics from vesicle (L3) phase of di-block copolymers. Find stretched exponential relaxation $g_{2(\tau)} \sim e^{-(\Gamma\tau)^{\alpha}}$. Stretching exponent $\alpha \approx \frac{2}{3}$ consistent with theory for free membrane plaquettes.



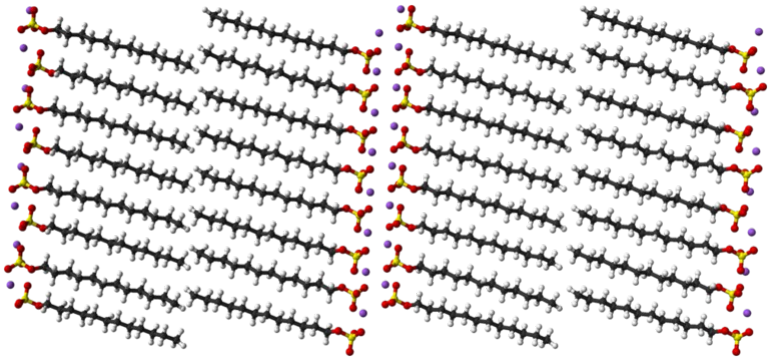
- Sikharulidze et. al. (PRL 2003) studied the dynamics of lyotropic liquid crystals 4O.8 and FPP.

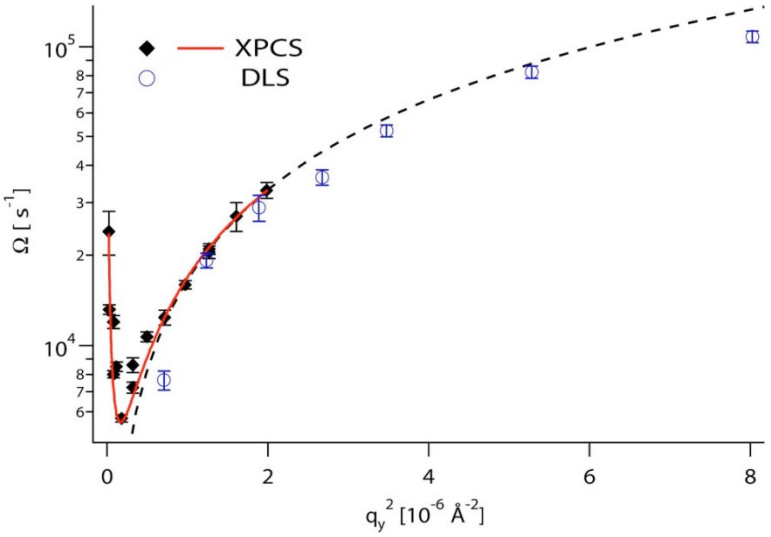
- They could crossover the XPCS measurements with neutron spin echo at small q. Found good agreement with continuum theory for smectic liquid crystals.





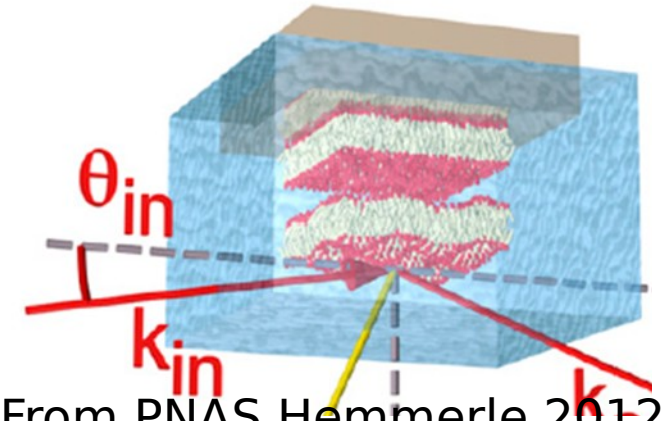
- Constantin et. al (PRE 2006) Studied the dynamics of lyotropic liquid crystal SDS/pentanol/H2O
- They could crossover the XPCS measurements with dynamic light scattering at low Q.
- Obtain material parameters for the lyotropic phase (e.g. bending moduli) and get good agreement with theory.





Challenges that remain for biomembranes

- Dynamics are fast (microseconds) need sufficient flux.
- Would like to look at uncoupled layers. Either need high q measurements, or measurements on single layers.
- Can make single layers suspended across apertures, floating above a surface or at air water interface, but need more flux to measure scattering.
- Damage is a serious issue (more about this later)



From PNAS Hemmerle 2012

Challenges for a MBA

- Need enough flux to measure fast dynamics
- Need to solve x-ray damage problems
- Need detectors which can keep up with the flux.

Signal to Noise Ratio

Falus et. al. JSR 2006

- Assume that the scattered intensity within a region of q-space is $\langle I \rangle$ and that this region is divided into N_{pix}pixels each of which is matched to the solid angle of a speckle. Then

$$R_{sn} = \langle I \rangle \beta \sqrt{\frac{T\tau}{g_2 N_{pix}}}\,.$$

- Note that this assumes that your camera has the correct resolution and spans the whole relevant portion of q-space. This is not the current situation with cameras, but is would be the ideal situation.

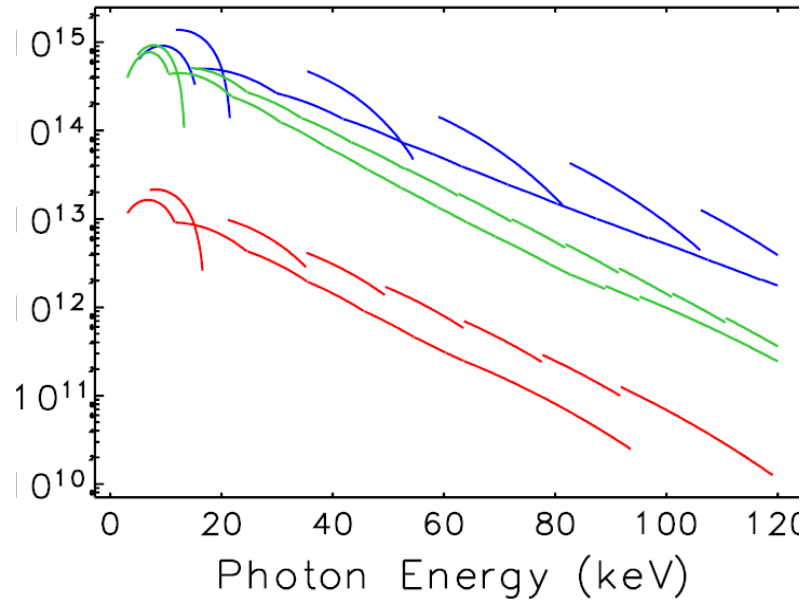
Minimum Correlation Time

Falus et. al. JSR 2006

- Solve signal to noise equation for τ

$$\tau = \frac{R_{sn}^2}{\langle I \rangle^2 \beta^2} \left(\frac{g_2 N_{pix}}{T} \right).$$

- A 50x increase in $\langle I \rangle$ leads to 2500x increase in time resolution.
- Current measurements can probe ms, MBA should be able to probe sub microsecond



Can the flux be used?

- Biophysical systems are very susceptible to damage and already at or beyond the damage limit. Will an MBA be useful with these materials?

- Increase coherent fraction of beam (less damage for fixed R_{sn}.
- Go to higher energy (see next slide)
- Flow sample

Will higher energies reduce damage?

- Absorption E^{-3}
- Damage E
- $\frac{d\sigma}{d\Omega}$ (no energy dependence)
- At fixed Q resolution $\frac{d^2Q}{d\Omega} \sim E^{-2}$

- For fixed sample volume and fixed Q resolution, the only gain is a wider range of Q, but not better R_{sn} at each Q.
- One can measure thicker samples, this gains as E^3
- To take advantage of thick samples one needs longitudinal coherence $\xi_l = \frac{\lambda^2}{\Delta\lambda}$. Thus need narrow energy bandwidth.

Flowing the Sample

- Fastest dynamics $\sim \langle I \rangle^2$ but damage $\sim \langle I \rangle$.
- If you are able to flow sample (or alternately move sample cell) studying faster dynamics should actually *reduce* damage!
- Slowest time that can be measured $\tau \approx h/v$ with h the beam height.
- To measure slower dynamics may need to either attenuate beam, or better, move off focus.

Detectors(!)

- This feasibility analysis assumes that area detectors exist which can capture the full scattering and read out the data as fast as the time scale of interest.
- Such detectors do not currently exist.
- Detectors are expensive long-term projects, but they are less expensive than synchrotrons, and shorter term!

Conclusions

- A MBA upgrade would provide enough coherent flux to move accessible times from milliseconds to microseconds in biomaterials.
- Will allow study of membranes, proteins, filaments in aqueous solution.
- Damage will be a challenge but there are good strategies which can be employed.

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