Phase separation in protein solutions

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Phase diagrams and phase transitions

a phase diagram

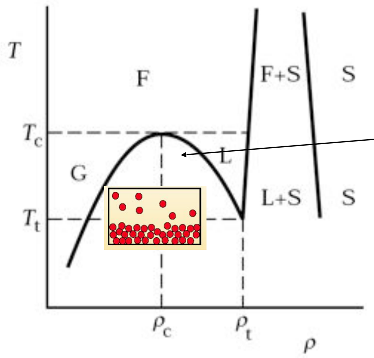
What is it?

'a map that presents the domains of stability of phases and their combinations........

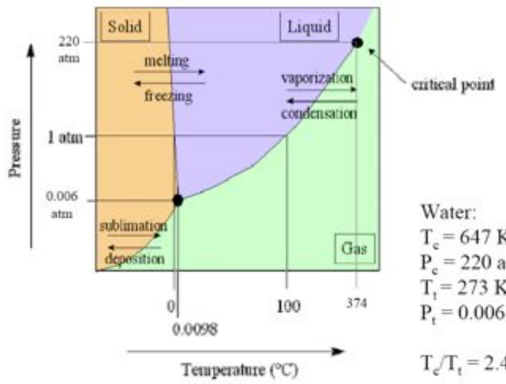
....reading the map tells you

1. What phases are present
2. The states of those phases
3. The relative quantities of each phase'

Typical phase diagram of an atomic fluid at constant P



Phase diagram H_2O



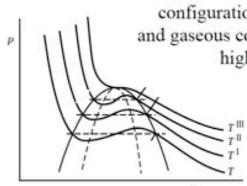
Water:
$T_c = 647$ K
$P_c = 220$ atm
$T_t = 273$ K
$P_t = 0.006$ atm

$T_c/T_t = 2.4$

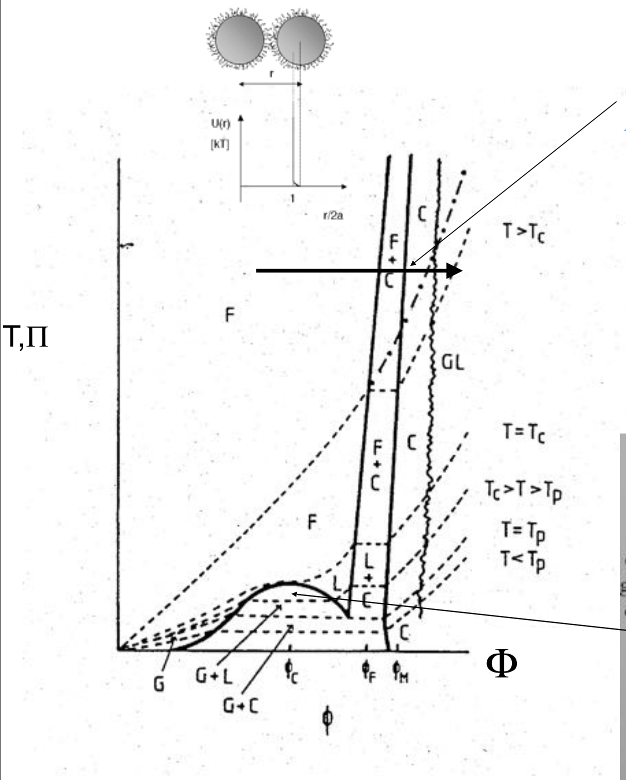
Condensation Gas-Liquid

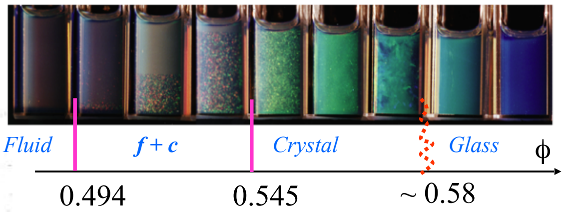
Essential mechanism of **condensation**:

competition between liquid configurations : low U
and gaseous configurations: high S

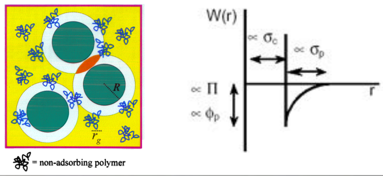


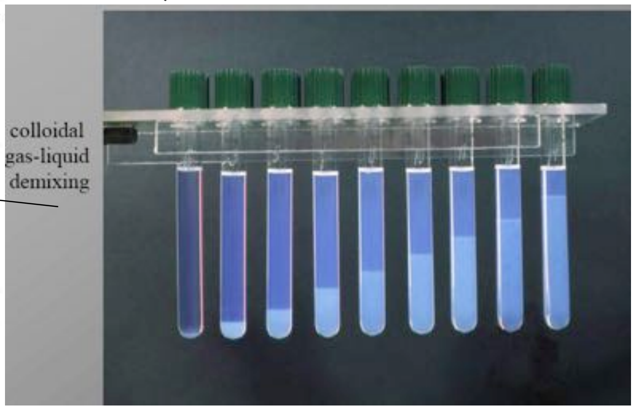
Colloid phase diagrams





Depletion attraction





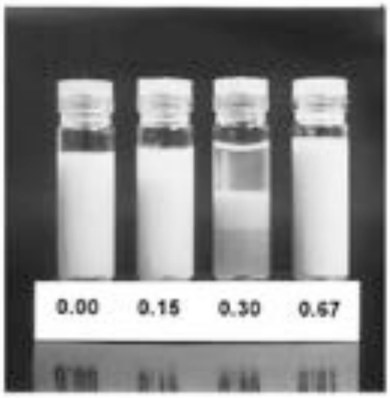
Different types of phases in colloidal dispersions

Demixed HEC/PS mixtures

Colloidal gas

Colloidal liquid

Colloidal solid



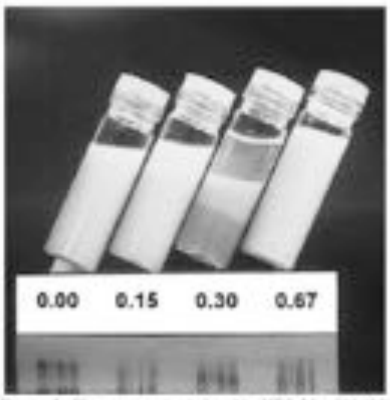
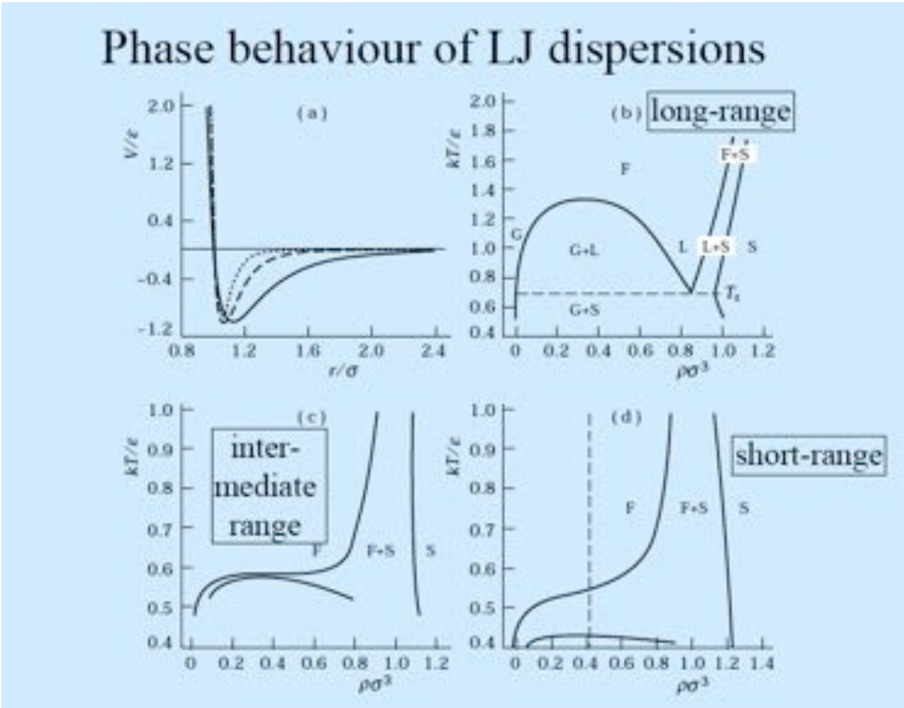


Figure 1. Phase separation induced by HEC (M = 300 000) in a 67 nm polystyrene latex suspension stabilized with F108. Concentration (% w/v) of HEC as shown.

M. A. Faers[*,†] and P. F. Luckham[†]

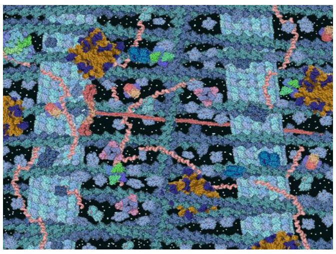
Langmuir 1997, *13*, 2922−2931

The influence of the range of attraction in colloids

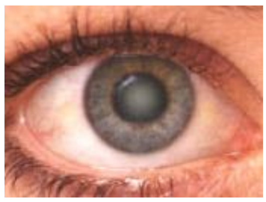


Why bother about phase transitions for proteins?

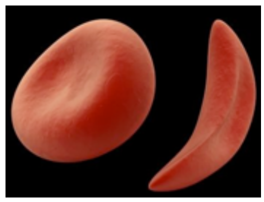
Protein crowding and the cytosol stability



Understanding protein condensation disease



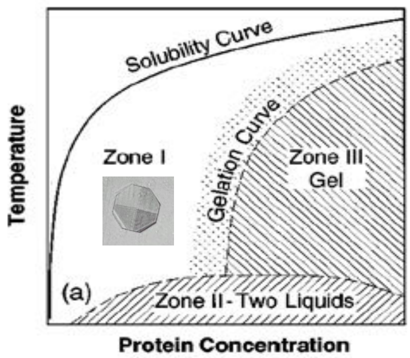
Cataract



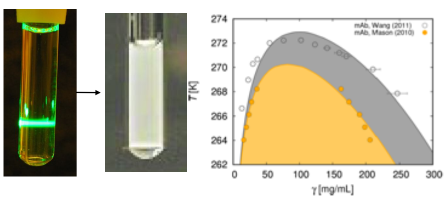
Sickle Cell Disease

Phase transitions in proteins

Optimal conditions for crystallization

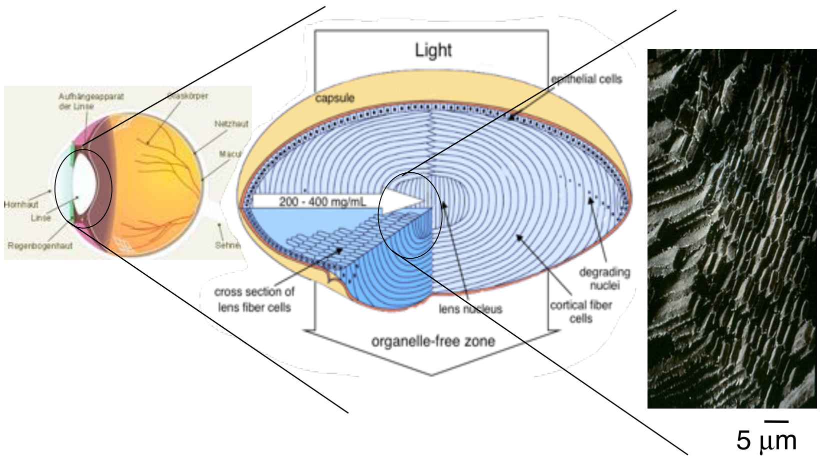


Concentrated formulations



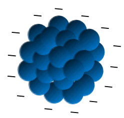
The eye lens - an unusual cytosol of relatively low complexity

The fiber cells consist of a highly concentrated protein solution:



5 µm

Alpha-crystallins:



~ 800 kDa
specific volume:
~ 1.5 - 1.7 mL/g

Beta-crystallins:

β_H ~ 200 kDa;
β_L ~ 50 kDa

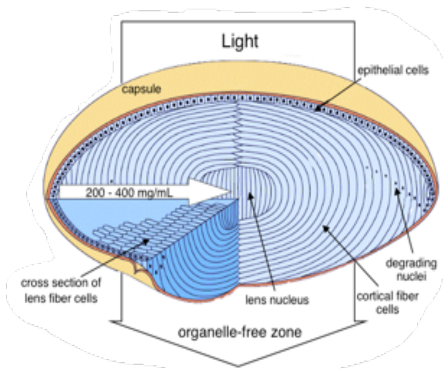
Gamma-crystallins:

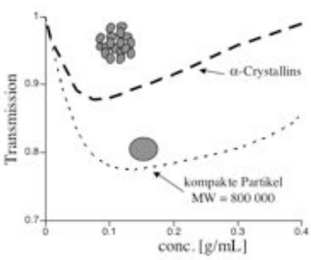
~ 20 kDa
specific volume:
~ 0.7 mL/g

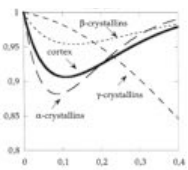
Why has nature chosen such a complex design?

Main lens requirements:

- high transparency
- high index of refraction
- gradient in index of refraction
- flexibility

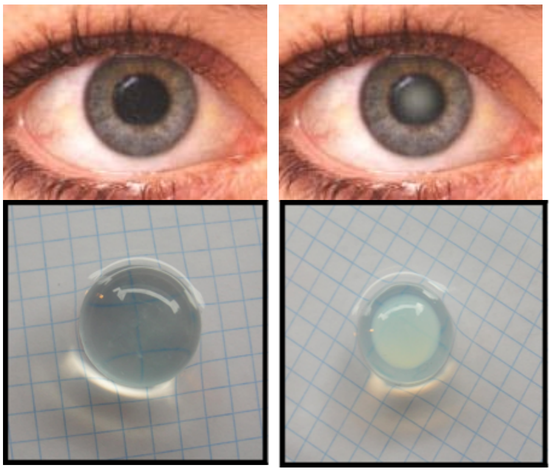




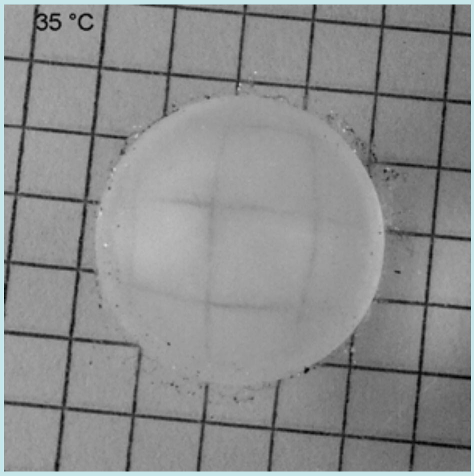


A. Tardieu, IJBM 1998

Cataract: protein condensation disease, where protein aggregation and phase separation lead to a *clouding of the eye lens*; cataract is still the leading cause of blindness worldwide



Example of “cold cataract”



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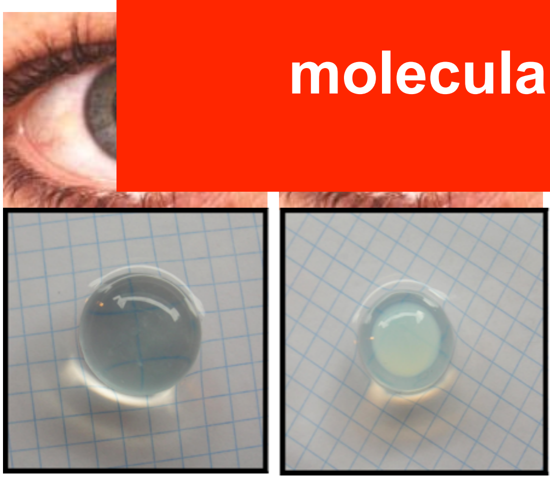
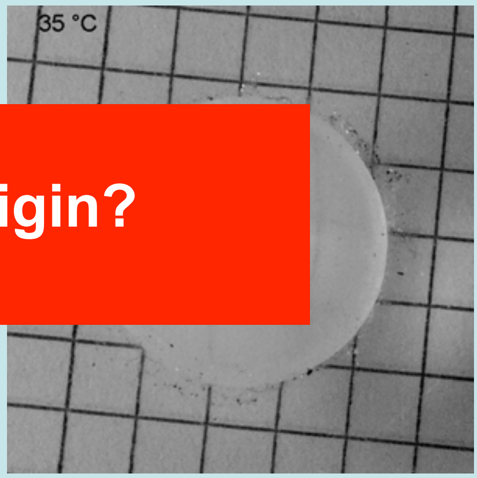
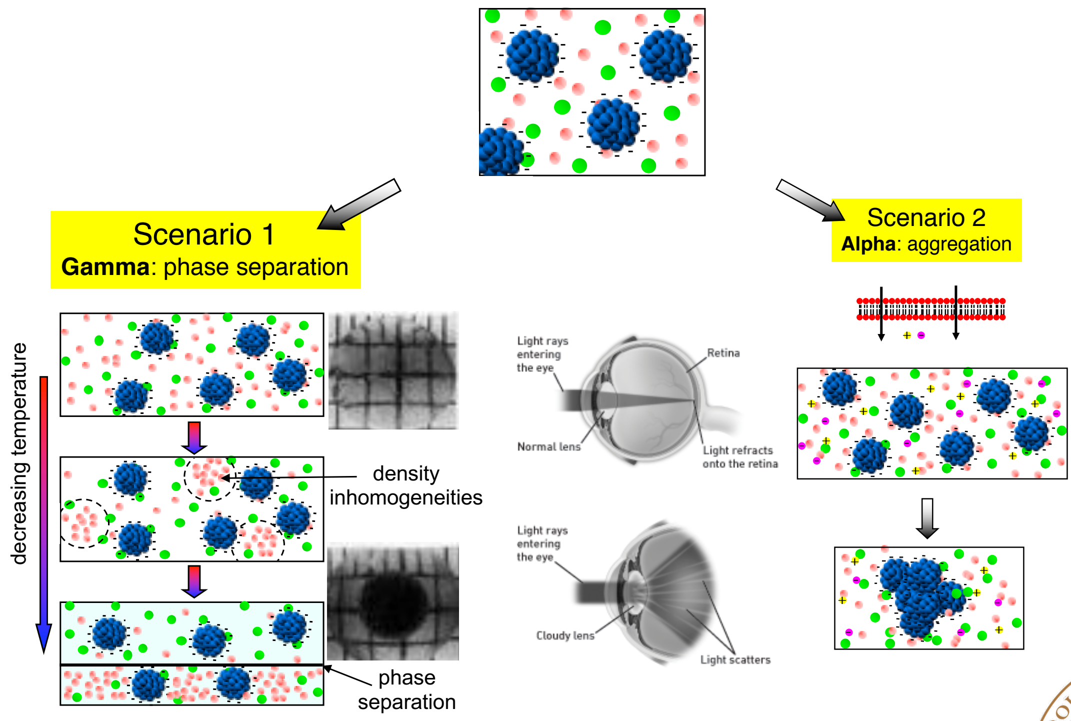


molecular origin?

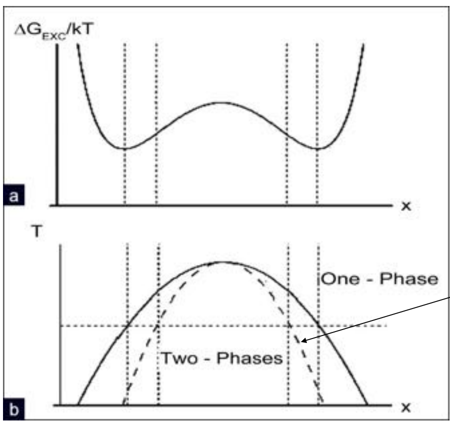
Example of “cold cataract”



Main scenarios for cataract formation



How do we test for the existence of g-l (or l-l) phase separation in protein solutions



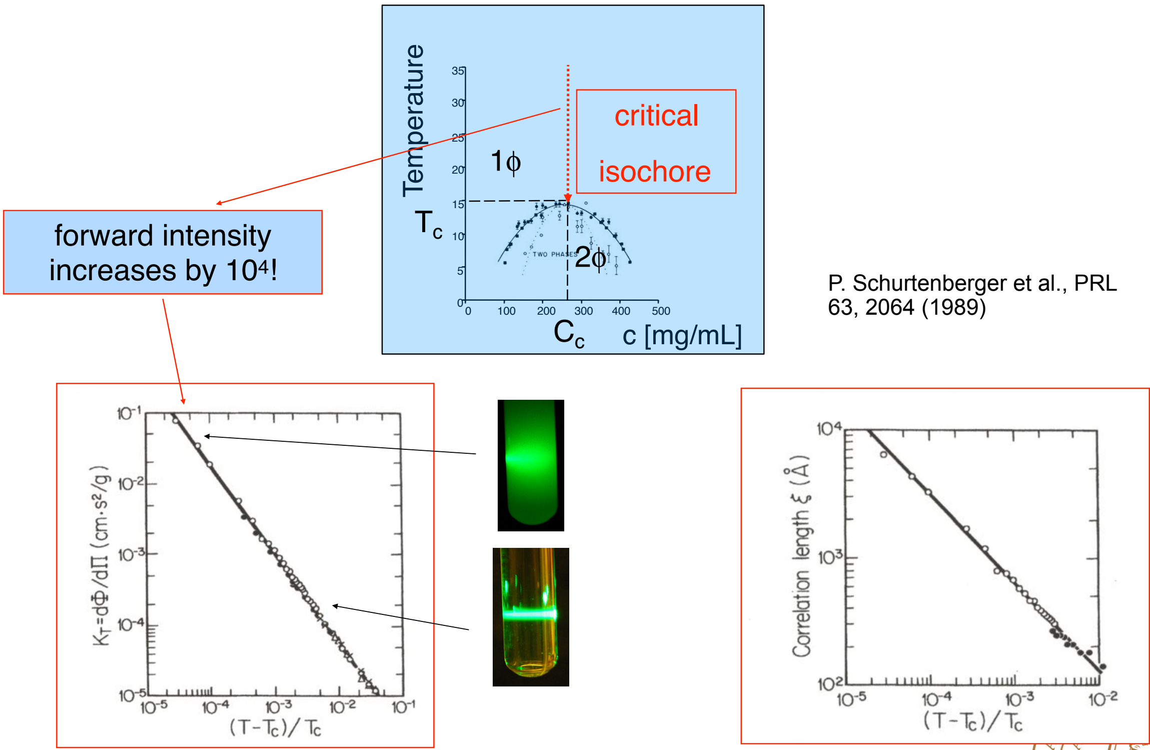
Scattering

intensity diverges

Determining critical point and spinodal from scattering:

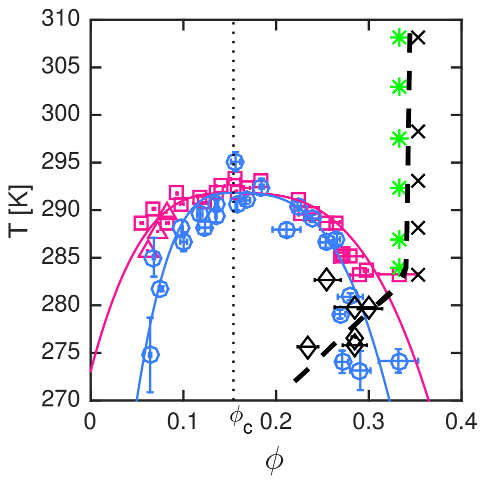
$$S(0) = \rho k_B T \kappa_T \quad \text{where} \quad \kappa_T = -\frac{1}{V}\left(\frac{\partial V}{\partial P}\right)_T = \frac{1}{V}\left(\frac{\partial^2 V}{\partial^2 F}\right)_T$$

Existence of critical phenomena in γ-crystallin solutions

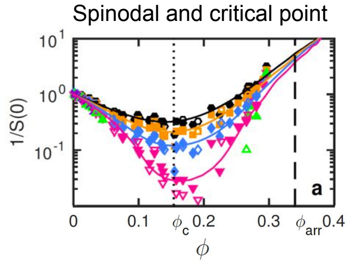


P. Schurtenberger et al., PRL 63, 2064 (1989)

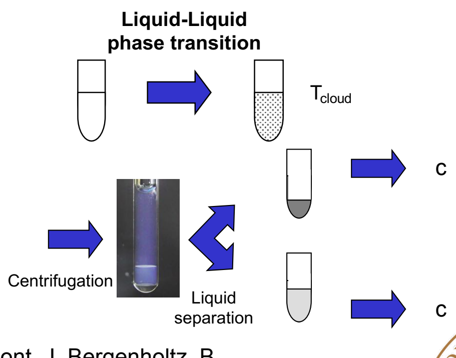
Determining the complete coexistence curve (binodal) and spinodal lines



Spinodal and critical point

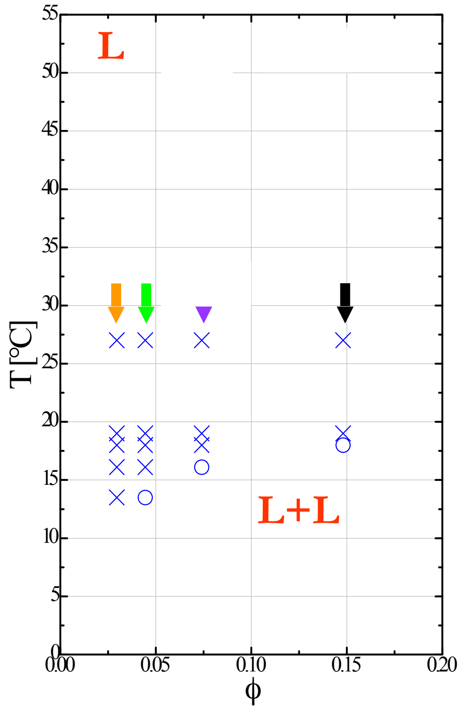


Binodal: cloud point and macroscopic phase separation

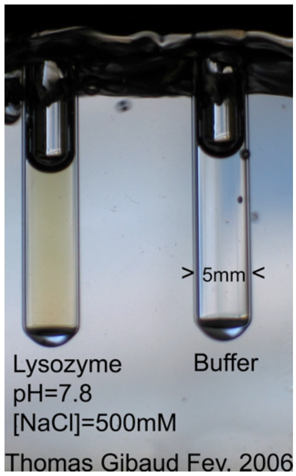


S. Bucciarelli, L. Casal-Dujat, C. De Michele, F. Sciortino, J. Dhont, J. Bergenholtz, B. Farago, P. Schurtenberger, and A. Stradner, J. Phys. Chem. Lett. 6, 4470 (2015)

Determining the complete coexistence curve (binodal) and spinodal lines - lysozyme



Phase separation



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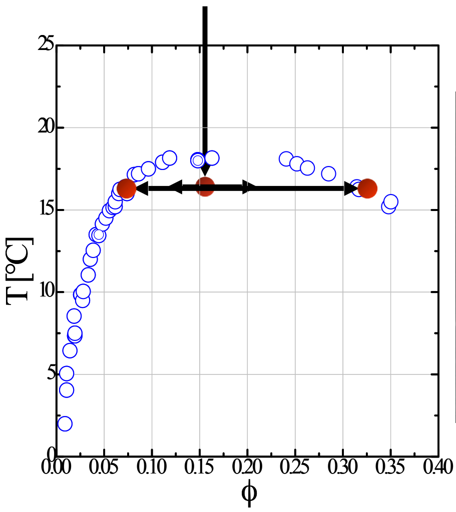
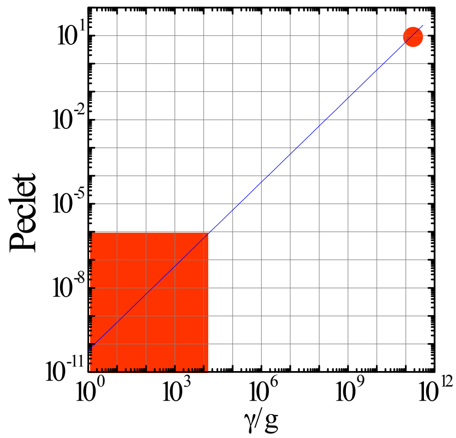


Sedimentation *versus* Diffusion

$$\begin{cases} v_{sed} = \dfrac{2}{9}\dfrac{\Delta\rho\gamma r^2}{\eta} \\ D = \dfrac{kT}{6\pi\eta r} \end{cases} \Rightarrow Peclet = \frac{2a.v_{sed}}{D} = \frac{8\pi a^4 \Delta\rho\gamma_{max}}{3kT}$$

$NB: \gamma_{centri} \in [1g; 25000g]$



Determining the complete coexistence curve (binodal) and spinodal lines - lysozyme

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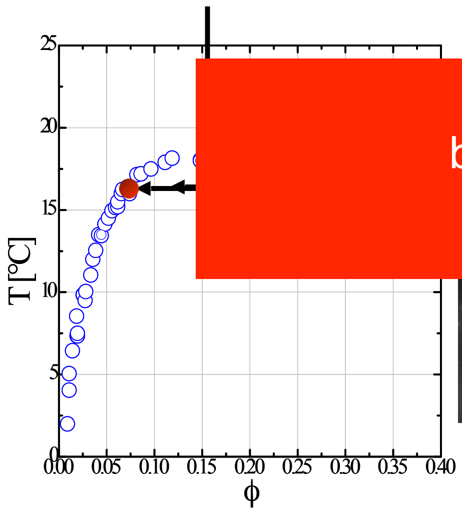
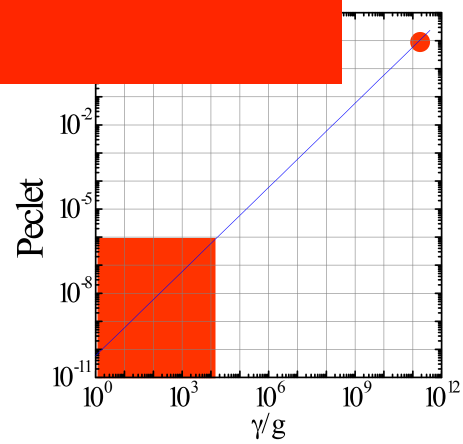
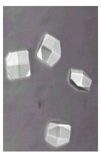


but that is not all!

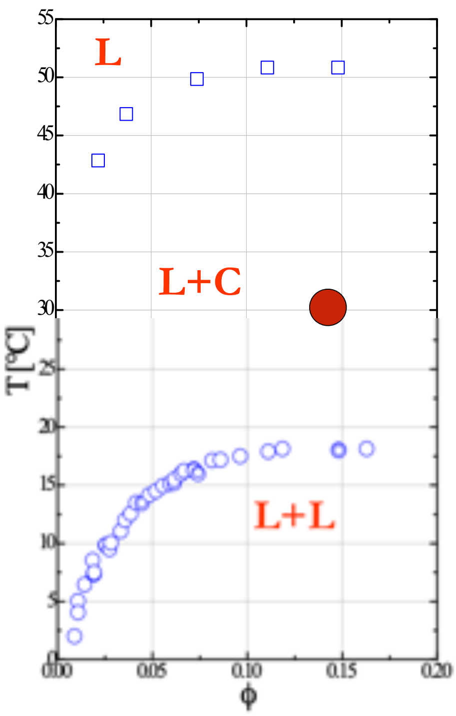


l-l phase separation is metastable - crystal formation

Crystal melting

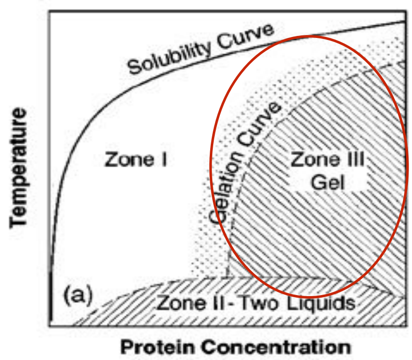


crystals form



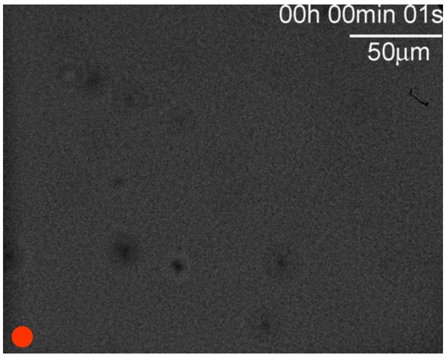
Muschol & Rosenberger, J. Chem. Phys. 1997

Generic phase diagram for globular proteins:



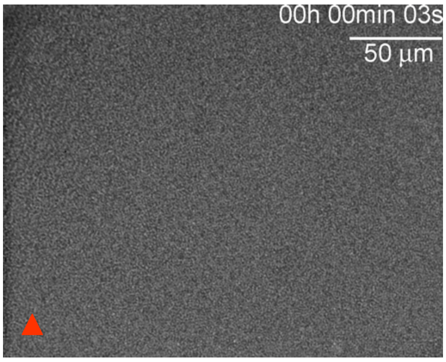
Optimal region for protein crystallization: Zone I outside cross-hatched region

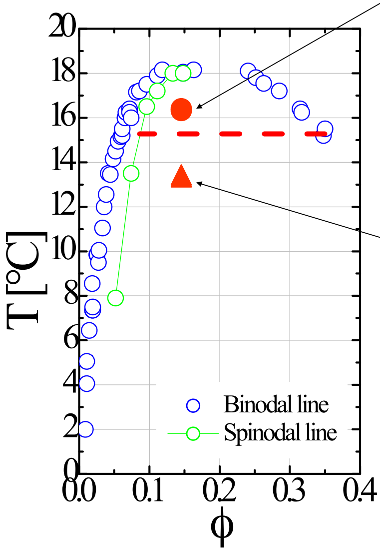
Existence of non-equilibrium arrested states - lysozyme

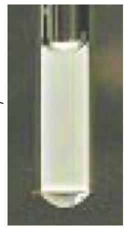


Spinodal decomposition

Kinetic arrest

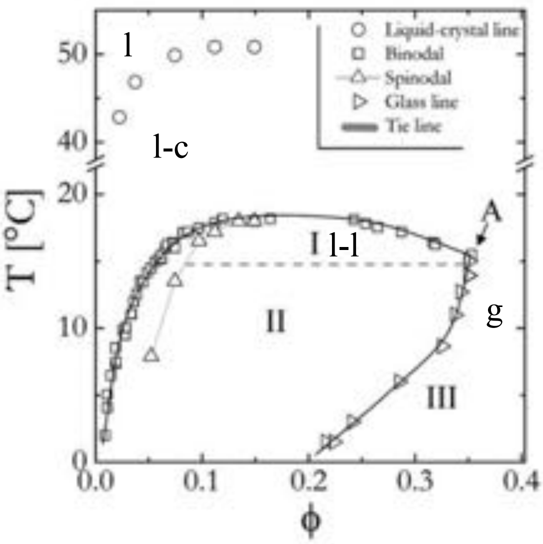


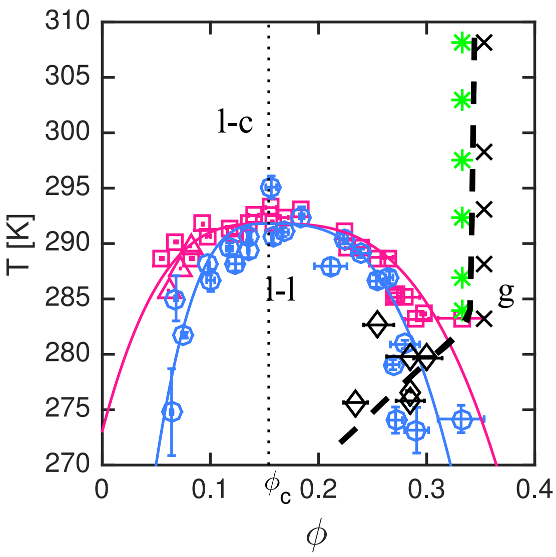




arrested spinodal decomposition

Existence of non-equilibrium arrested states - lysozyme and γ-crystallin

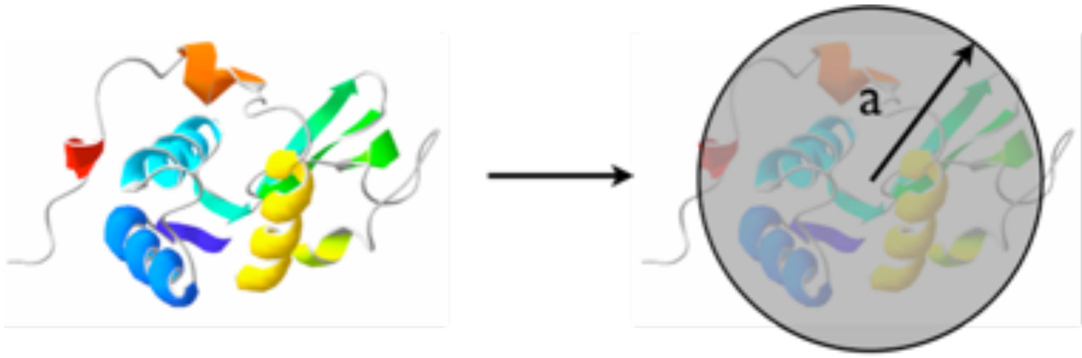




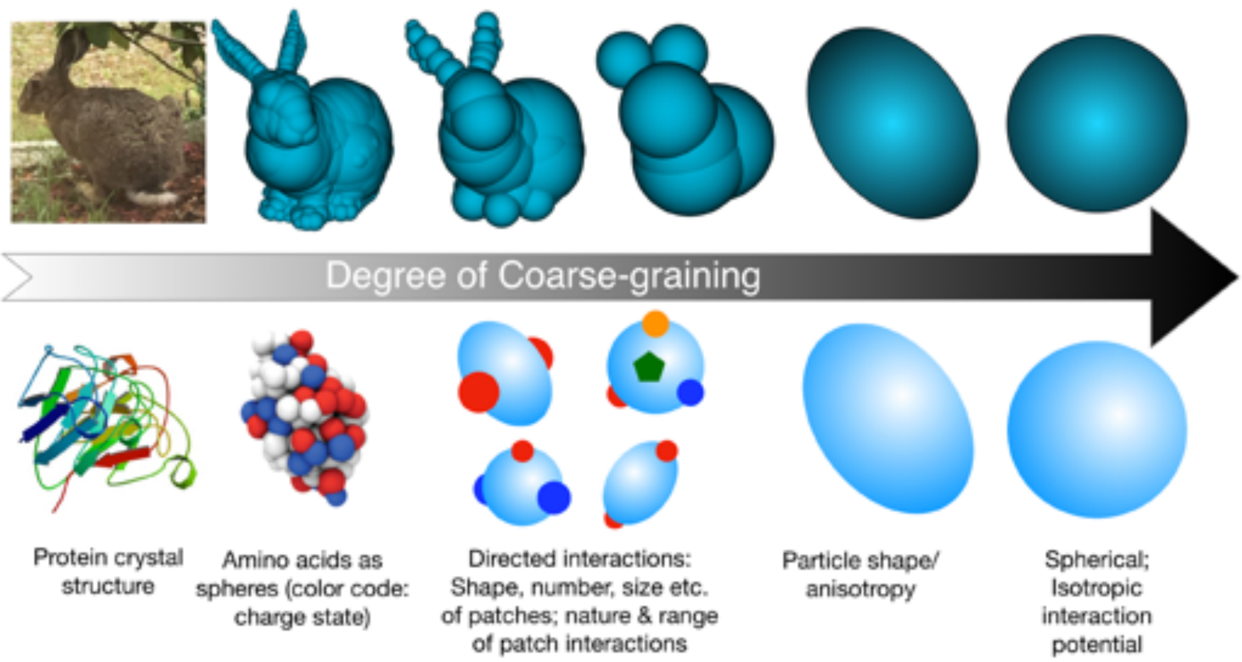
How can we understand protein phase behavior: a coarse-graining approach

Let's take a spherical horse... The colloid-protein analogy & the physicist's view of the protein lysozyme

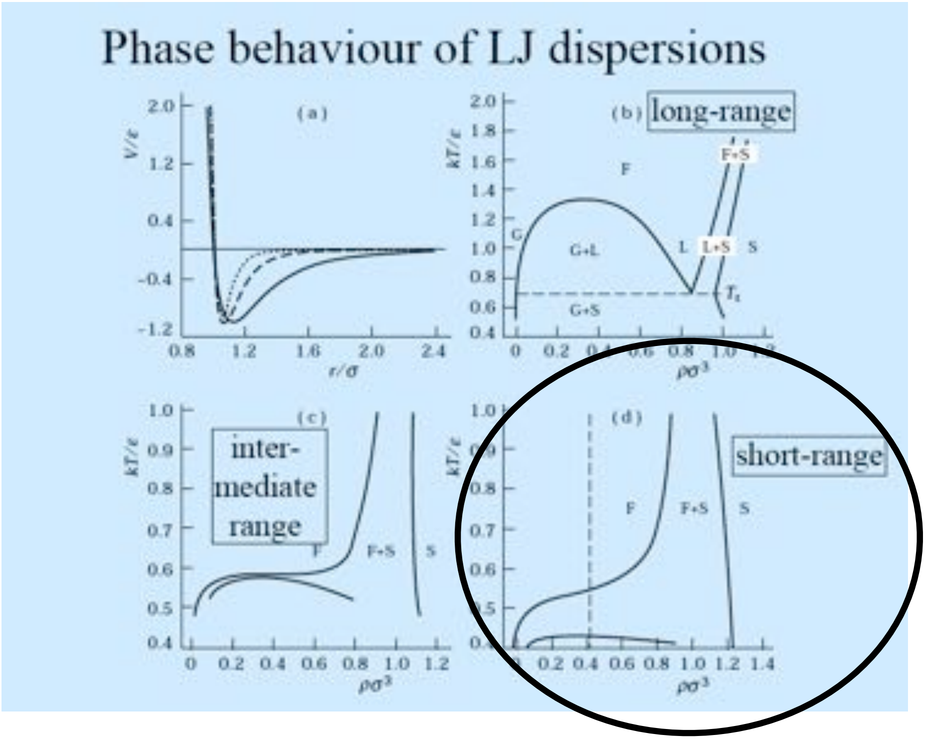
this corresponds to this



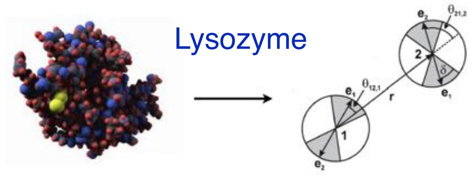
Protein modeled as colloid with effective pair interaction potential that depends on temperature and pH

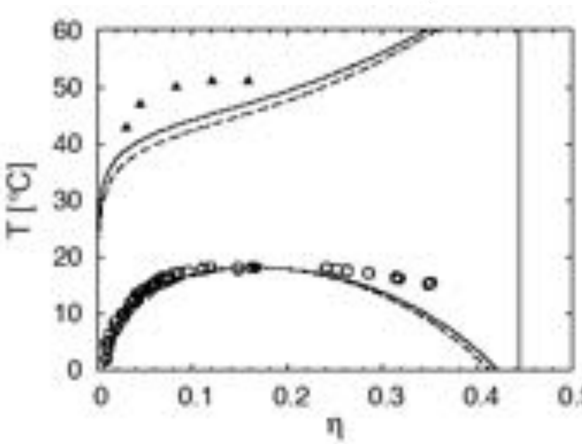


The influence of the range of attraction in colloids

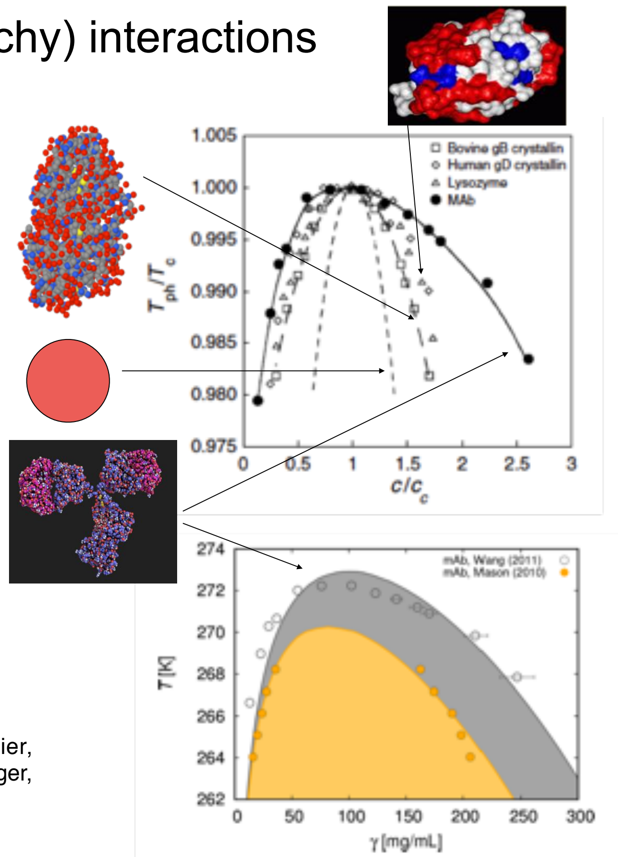


Importance of anisotropic (patchy) interactions

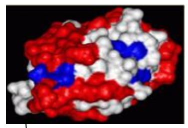


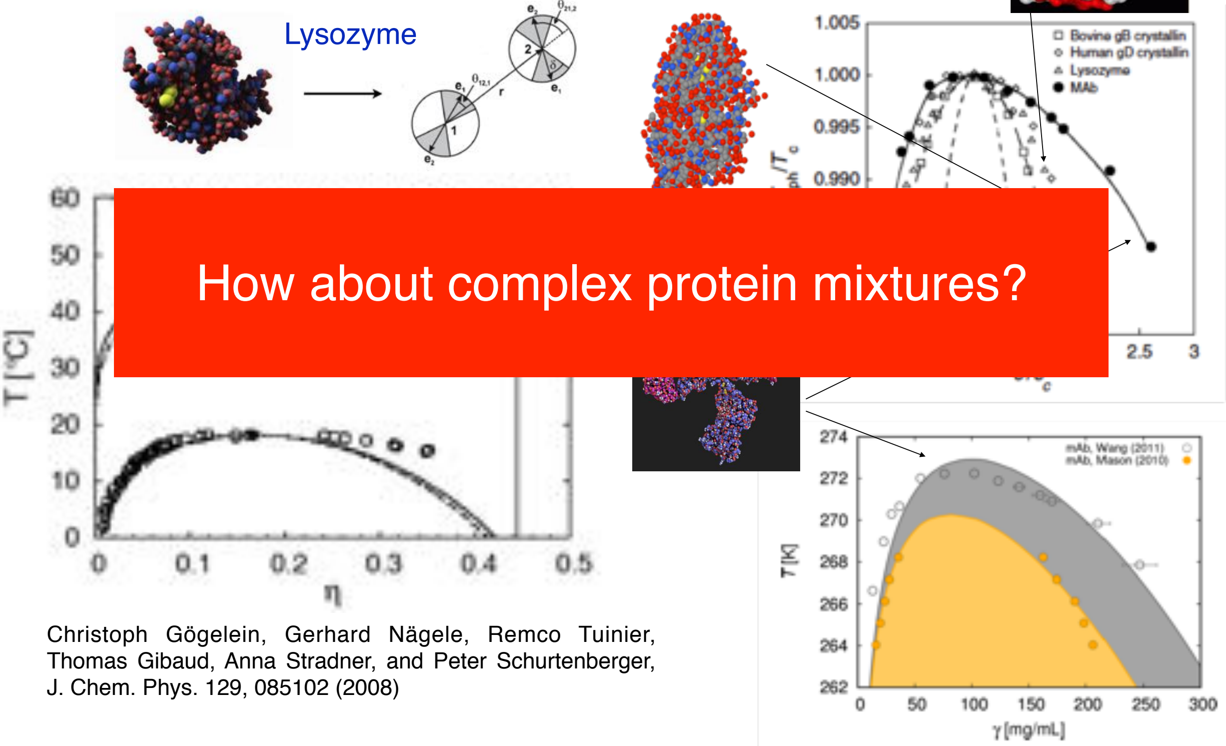


Christoph Gögelein, Gerhard Nägele, Remco Tuinier, Thomas Gibaud, Anna Stradner, and Peter Schurtenberger, J. Chem. Phys. 129, 085102 (2008)



Importance of anisotropic (patchy) interactions

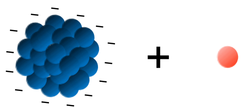




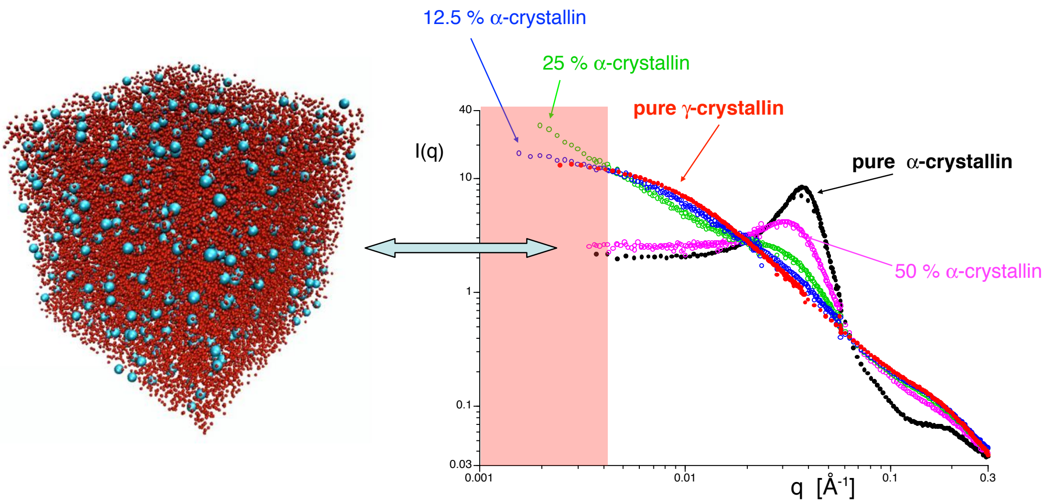
How about complex protein mixtures?

Christoph Gögelein, Gerhard Nägele, Remco Tuinier, Thomas Gibaud, Anna Stradner, and Peter Schurtenberger, J. Chem. Phys. 129, 085102 (2008)

Back to lens proteins - binary mixtures



Neutron scattering and MD computer simulations on concentrated mixtures of alpha und gamma crystallins (c_{tot} = 230 - 260 mg/ml)

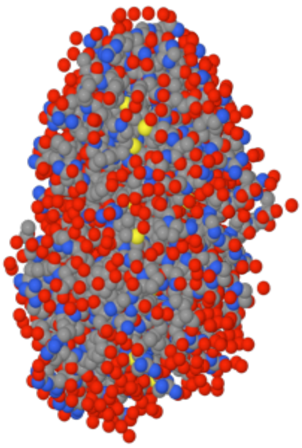


A.Stradner, N. Dorsaz, G. Thurston, G. Foffi, P. Schurtenberger, PRL (2007)

Computer simulations

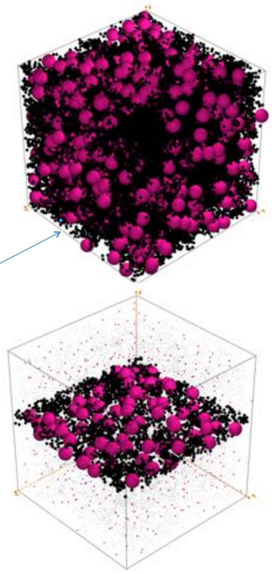
Coarse Grained simuations

All atomistic simulations



N~ 2000 Atoms (PDB: 1H4A)

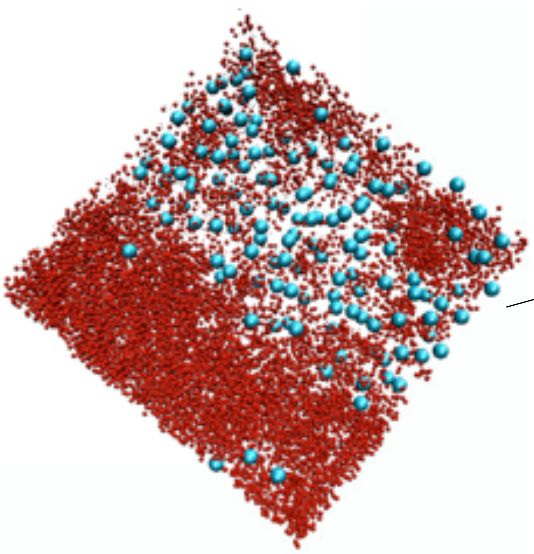
KEY STEP!!!

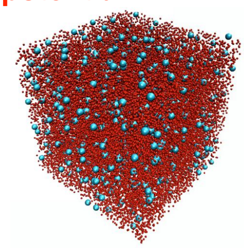


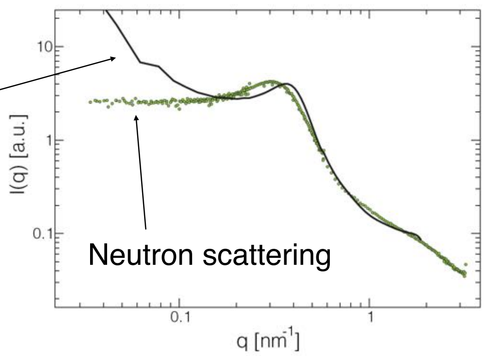
N~ 32k/64k Particles

Assessing α-γ interactions

Simulations: Gamma and Alpha **interact via hard sphere potential**

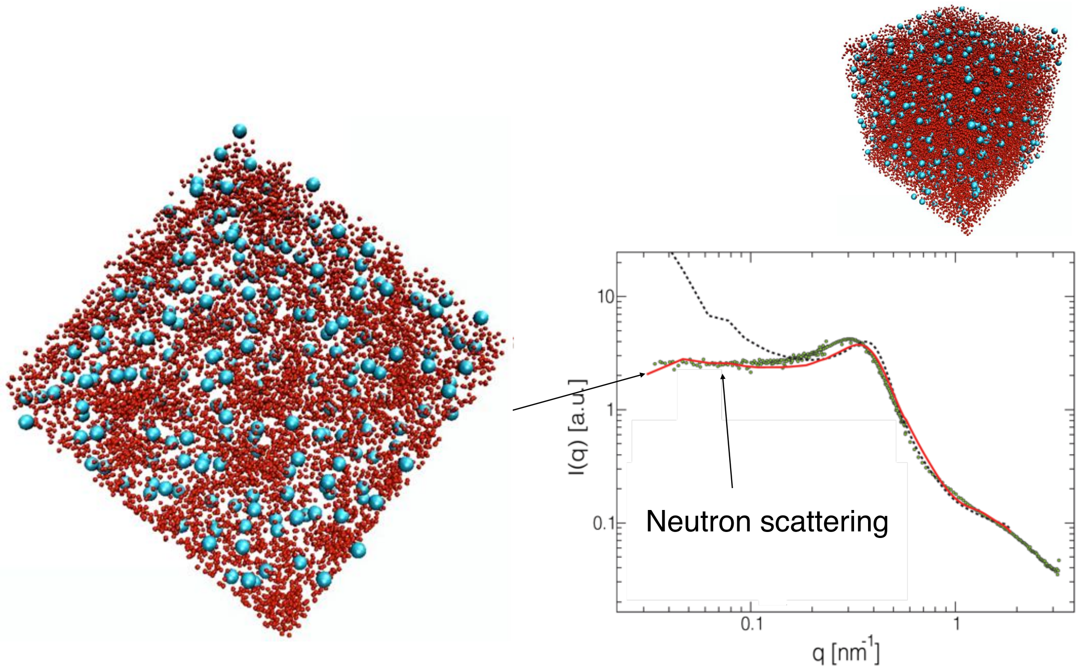




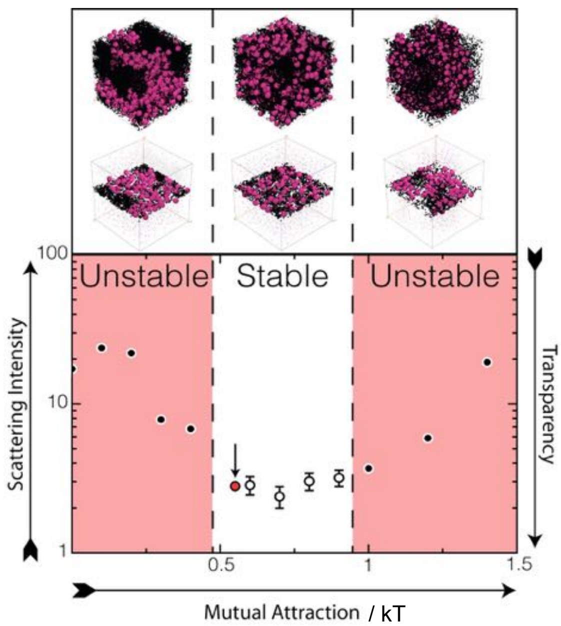


Assuming additional weak mutual attraction

Simulations: Gamma and Alpha **interact via hard sphere + weak attraction**

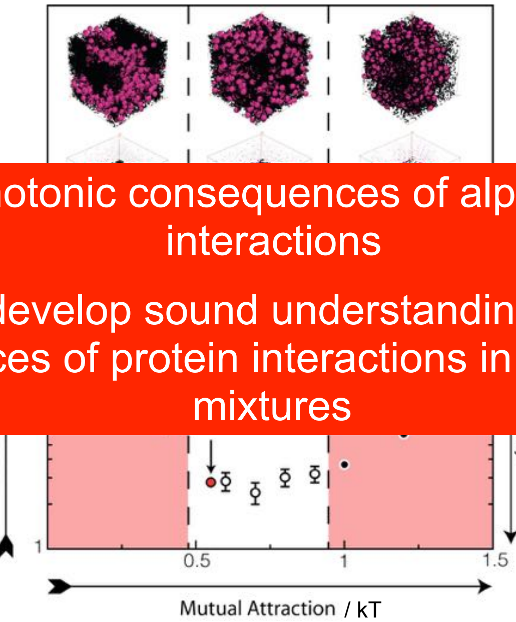


A first important result



A.Stradner, N. Dorsaz, G. Thurston, G. Foffi, P. Schurtenberger, PRL (2007)

A first important result

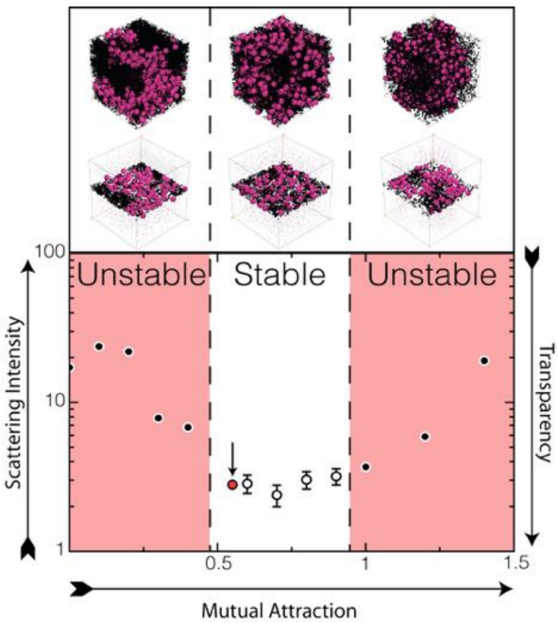


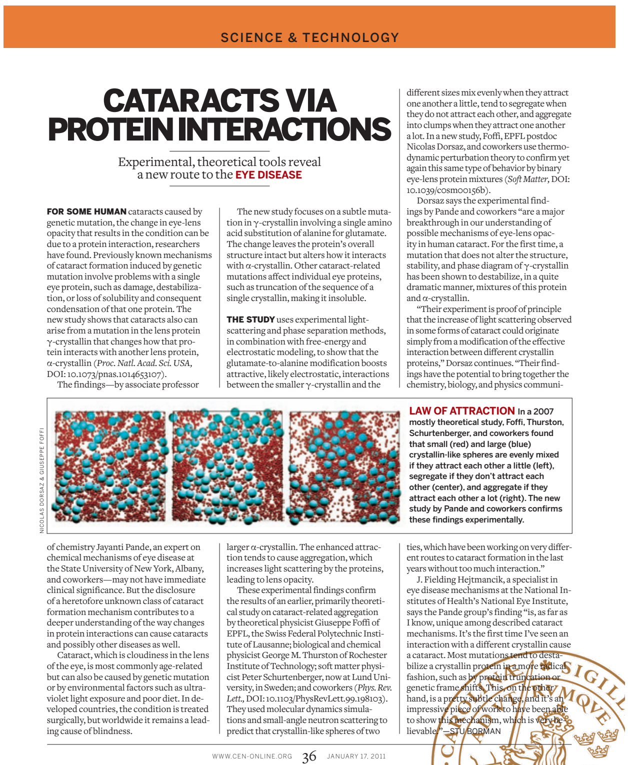
Non-monotonic consequences of alpha-gamma interactions

➪ need to develop sound understanding of ACTUAL consequences of protein interactions in concentrated mixtures

A.Stradner, N. Dorsaz, G. Thurston, G. Foffi, P. Schurtenberger, PRL (2007)

A first important result - stability through mutual attractions





SCIENCE & TECHNOLOGY

CATARACTS VIA PROTEIN INTERACTIONS

Experimental, theoretical tools reveal a new route to the EYE DISEASE

FOR SOME HUMAN cataracts caused by genetic mutation, the change in eye-lens opacity that results in the condition can be due to a protein interaction, researchers have found. Previously known mechanisms of cataract formation induced by genetic mutation involve problems with a single eye protein, such as damage, destabilization, or loss of solubility and consequent condensation of that one protein. The new study shows that cataracts also can arise from a mutation in the lens protein γ-crystallin that changes how that protein interacts with another lens protein, α-crystallin (*Proc. Natl. Acad. Sci. USA*, DOI: 10.1073/pnas.1014653107).

The findings—by associate professor of chemistry Jayanti Pande, an expert on chemical mechanisms of eye disease at the State University of New York, Albany, and coworkers—may not have immediate clinical significance. But the disclosure of a heretofore unknown class of cataract formation mechanism contributes to a deeper understanding of the way changes in protein interactions can cause cataracts and possibly other diseases as well.

Cataract, which is cloudiness in the lens of the eye, is most commonly age-related but can also be caused by genetic mutation or by environmental factors such as ultraviolet light exposure and poor diet. In developed countries, the condition is treated surgically, but worldwide it remains a leading cause of blindness.

The new study focuses on a subtle mutation in γ-crystallin involving a single amino acid substitution of alanine for glutamate. The change leaves the protein's overall structure intact but alters how it interacts with α-crystallin. Other cataract-related mutations affect individual eye proteins, such as truncation of the sequence of a single crystallin, making it insoluble.

THE STUDY uses experimental light-scattering and phase separation methods, in combination with free-energy and electrostatic modeling, to show that the glutamate-to-alanine modification boosts attractive, likely electrostatic, interactions between the smaller γ-crystallin and the larger α-crystallin. The enhanced attraction tends to cause aggregation, which increases light scattering by the proteins, leading to lens opacity.

These experimental findings confirm the results of an earlier, primarily theoretical study on cataract-related aggregation by theoretical physicist Giuseppe Foffi of EPFL, the Swiss Federal Polytechnic Institute of Lausanne; biological and chemical physicist George M. Thurston of Rochester Institute of Technology; soft matter physicist Peter Schurtenberger, now at Lund University, in Sweden; and coworkers (*Phys. Rev. Lett.*, DOI: 10.1103/PhysRevLett.99.198103). They used molecular dynamics simulations and small-angle neutron scattering to predict that crystallin-like spheres of two different sizes mix evenly when they attract one another a little, tend to segregate when they do not attract each other, and aggregate into clumps when they attract one another a lot. In a new study, Foffi, EPFL postdoc Nicolas Dorsaz, and coworkers use thermodynamic perturbation theory to confirm yet again this same type of behavior by binary eye-lens protein mixtures (*Soft Matter*, DOI: 10.1039/c0sm00156b).

Dorsaz says the experimental findings by Pande and coworkers "are a major breakthrough in our understanding of possible mechanisms of eye-lens opacity in human cataract. For the first time, a mutation that does not alter the structure, stability, and phase diagram of γ-crystallin has been shown to destabilize, in a quite dramatic manner, mixtures of this protein and α-crystallin.

"Their experiment is proof of principle that the increase of light scattering observed in some forms of cataract could originate simply from a modification of the effective interaction between different crystallin proteins," Dorsaz continues. "Their findings have the potential to bring together the chemistry, biology, and physics communities, which have been working on very different routes to cataract formation in the last years without too much interaction."

J. Fielding Hejtmancik, a specialist in eye disease mechanisms at the National Institutes of Health's National Eye Institute, says the Pande group's finding "is, as far as I know, unique among described cataract mechanisms. It's the first time I've seen an interaction with a different crystallin cause a cataract. Most mutations tend to destabilize a crystallin protein in a more radical fashion, such as by protein truncation or genetic frame shifts. This, on the other hand, is a pretty subtle change, and it's an impressive piece of work to have been able to show this mechanism, which is very believable."—STU BORMAN

NICOLAS DORSAZ & GIUSEPPE FOFFI

LAW OF ATTRACTION In a 2007 mostly theoretical study, Foffi, Thurston, Schurtenberger, and coworkers found that small (red) and large (blue) crystallin-like spheres are evenly mixed if they attract each other a little (left), segregate if they don't attract each other (center), and aggregate if they attract each other a lot (right). The new study by Pande and coworkers confirms these findings experimentally.

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A.Stradner, N. Dorsaz, G. Thurston, G. Foffi, P. Schurtenberger, PRL (2007)

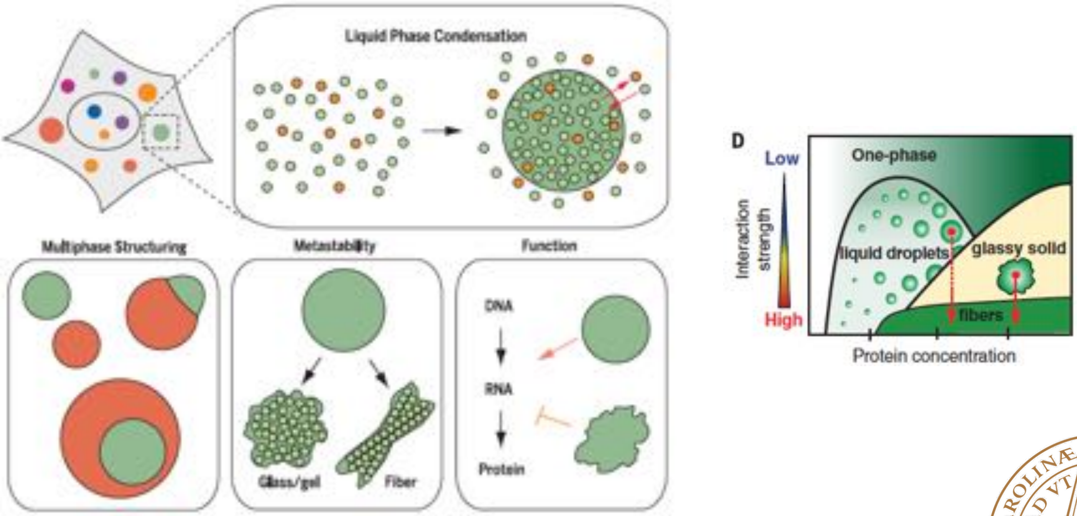
New hot topic:

REVIEW SUMMARY

CELLULAR BIOPHYSICS

Liquid phase condensation in cell physiology and disease

Yongdae Shin and Clifford P. Brangwynne*



Conclusions

- Phase separation ubiquitous in protein solutions and cells
- Play key role in a number of diseases
- Important role in the development of modern high concentration biopharmaceutical formulations
- Membraneless organelles require rethinking of the basis of the stability of the cytosol and the role of intrinsically disordered proteins
- Proteins can be successfully modeled as (patchy) colloids to understand phase behavior
- But: need to incorporate molecular viewpoint, treat multicomponent systems, existence of non-equilibrium (solid-like) states