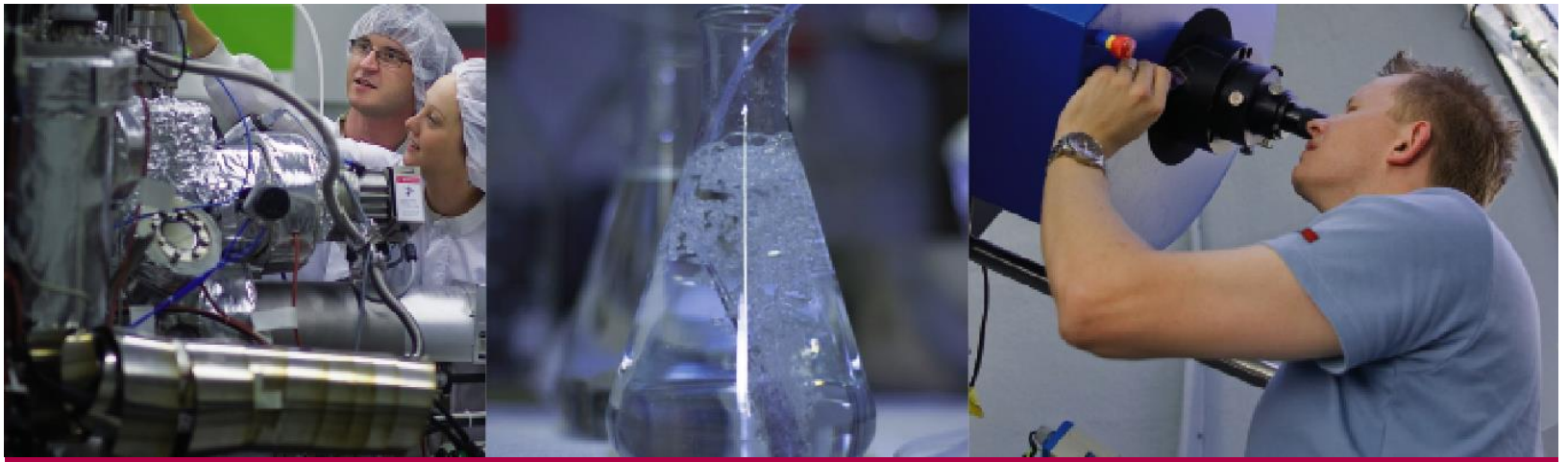
EBERHARD KARLS
UNIVERSITÄT
TÜBINGEN

FACULTY OF
SCIENCE
Institute of Applied Physics

NanoScience



Research in Biophysics

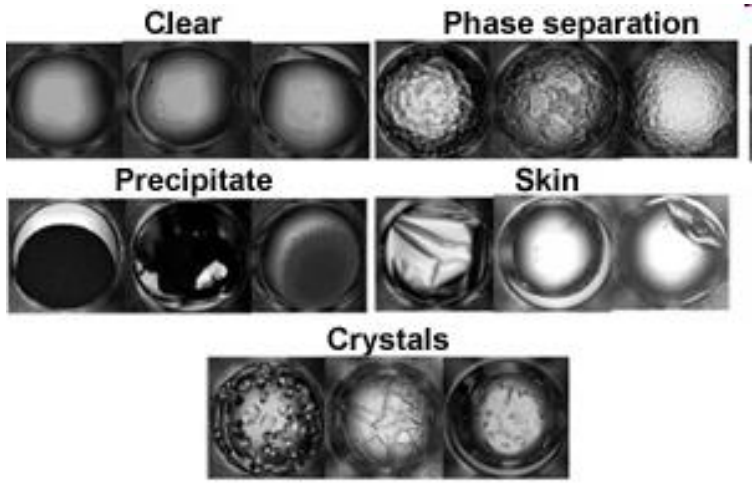
Towards Understanding of Protein Crystallization and Protein Condensation Related Diseases

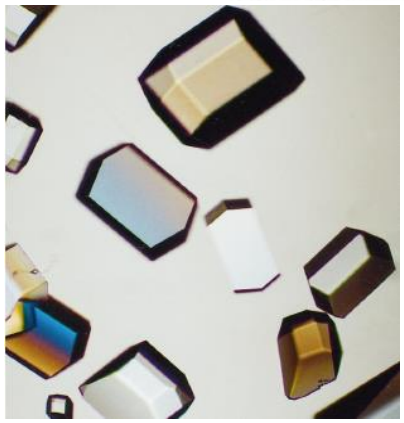
Institut für Angewandte Physik, Universität Tübingen

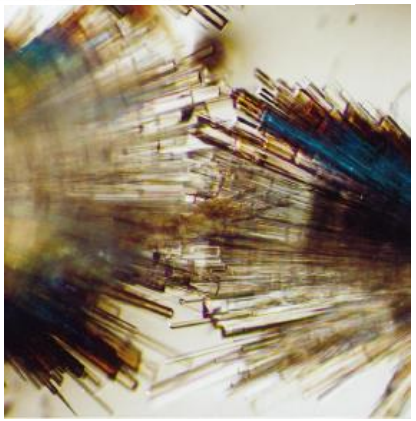
Outline

- **Introduction & Motivations**
- **Methods in our group**
- **"Magic" cations – Rich phase behavior in protein solutions induced by trivalent salt**
  - Nonclassical pathways of protein crystallization
  - Arrested phase transition
  - Static & dynamic properties of protein clusters
  - Tuning protein adsorption at interfaces
- **What you may contribute**

Introduction: Protein Crystallization







Protein crystallization is:

- un-predictable, trial-and-error method;
- bottle-neck of structural biology;
- less understanding of the interactions in the protein solutions.

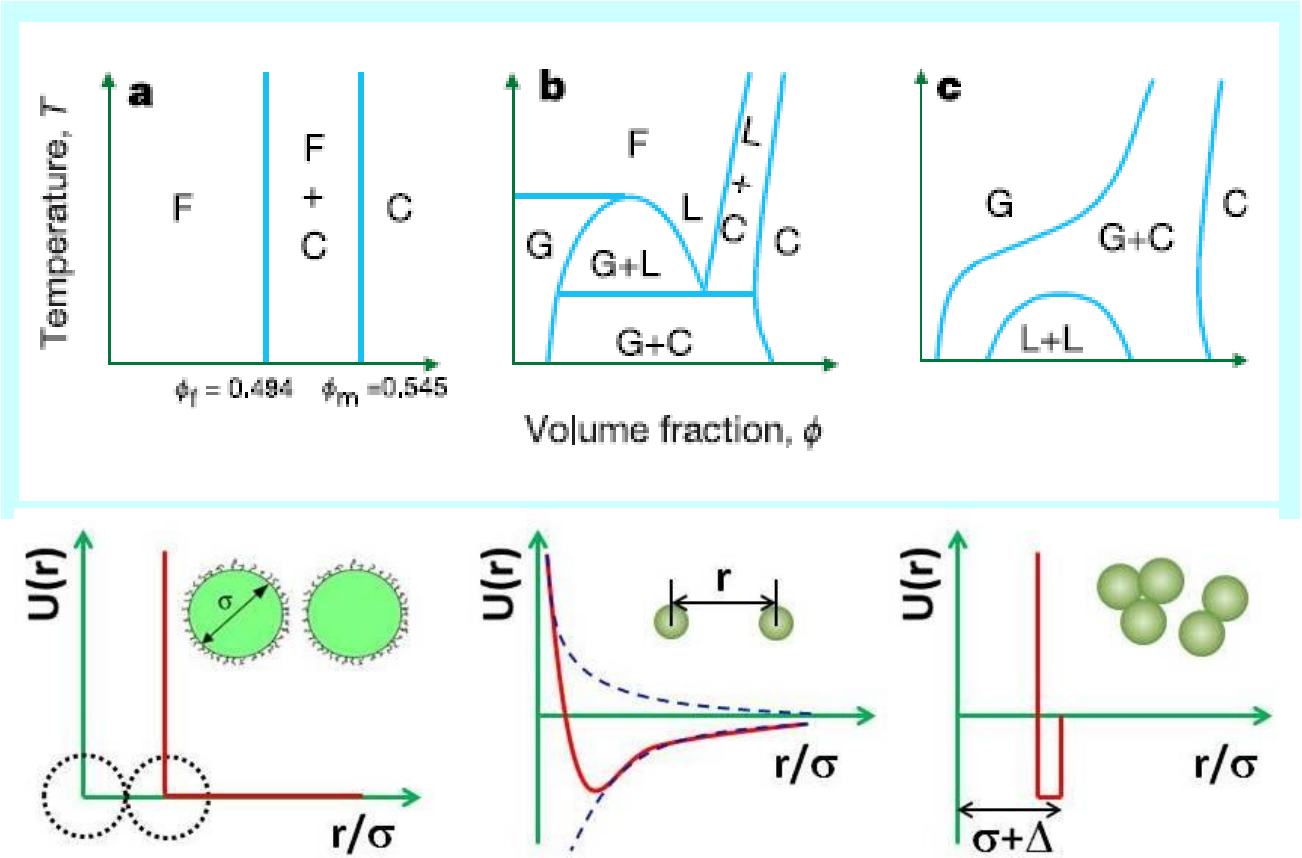
Velev, et al. *Biophys. J.* 1998, 75, 2682
http://www.chtsb.org/

Introduction: LLPS & Diseases

Left: LLPS of Normal Hemoglobins in red blood cell
Right: Sickle cell Hb polymerization from LLPS

Galkin, O. et al. PNAS 2000, PNAS. 2002, 99, 8479

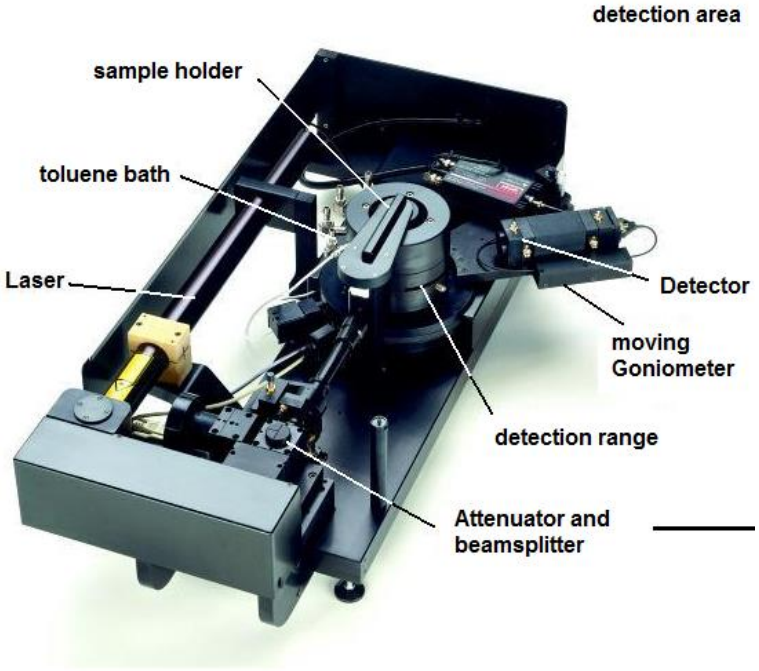
Typical phase diagrams



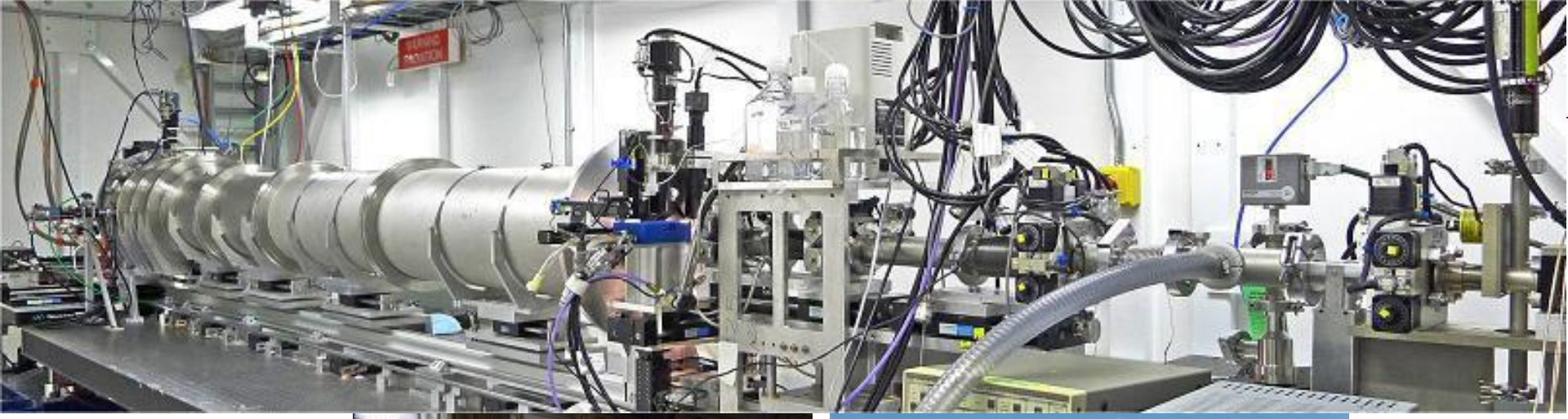
a. Hard sphere
b. Hard sphere with long-range attraction, $\Delta > 0.25\sigma$
c. Hard sphere with short-range attraction, $\Delta < 0.25\sigma$

Anderson, Nature 2002, 416, 811
Sciortino, F. Nature Mater.2003
Nägele 2005, Vekilov 2010

Methods in Our Lab

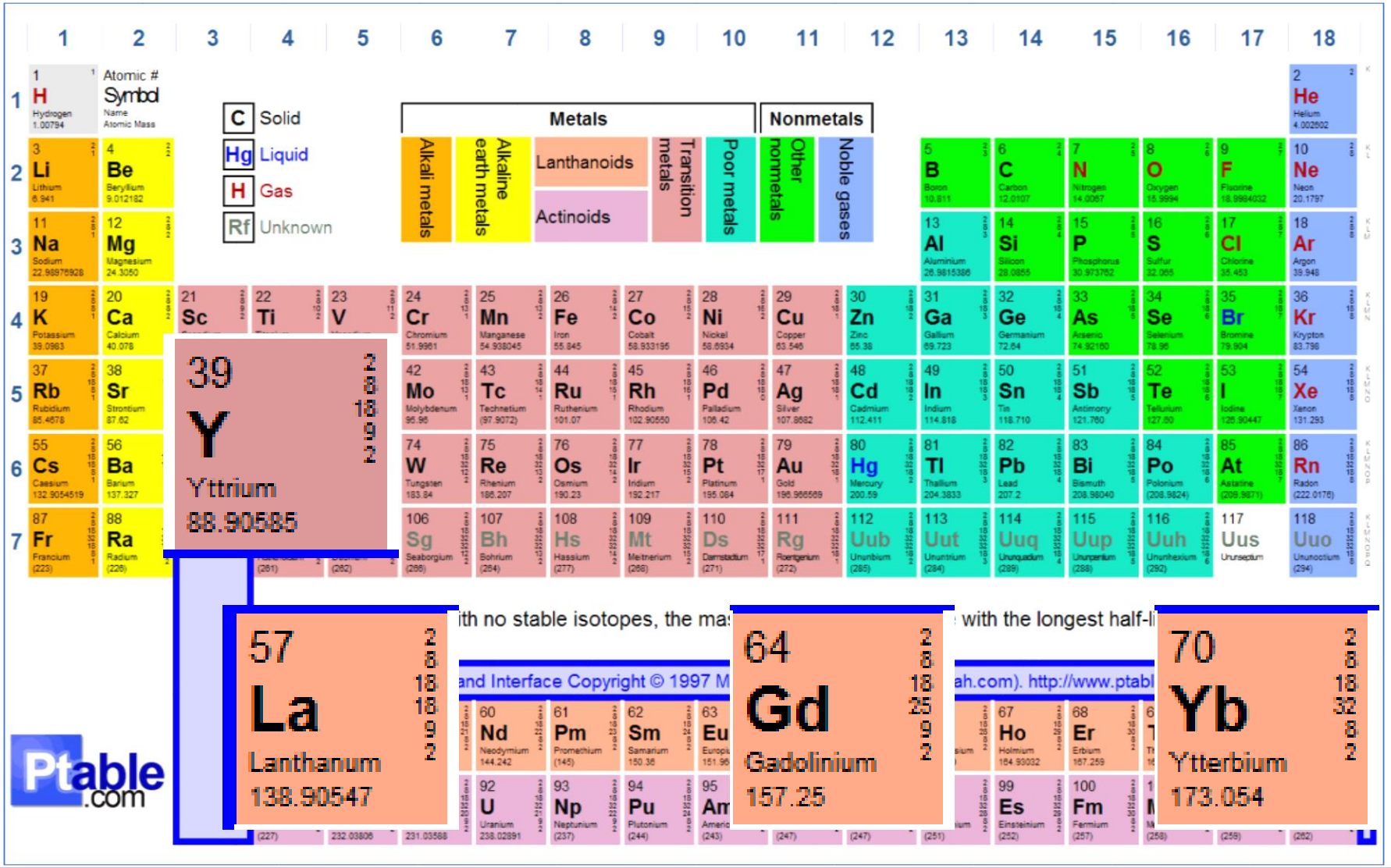


Multi-angle Static/Dynamic Light Scattering, and Small-Angle X-ray Scattering

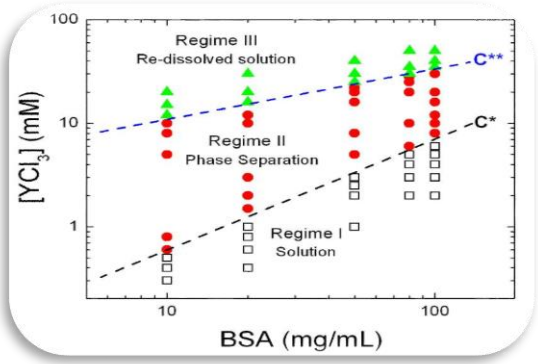


D11 at ILL

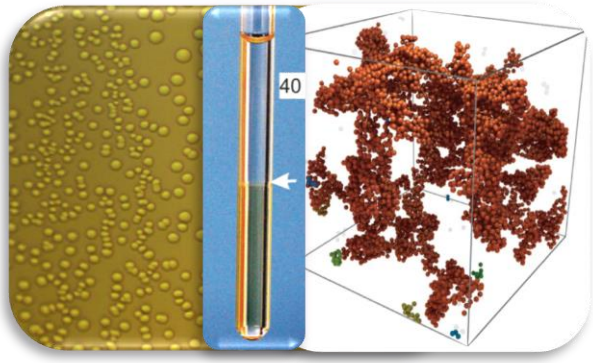
Metal ions with high co-ordination number



Tuning Interaction by Trivalent Ions

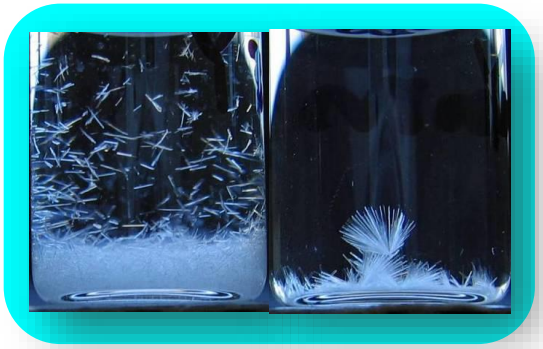


Reentrant condensation

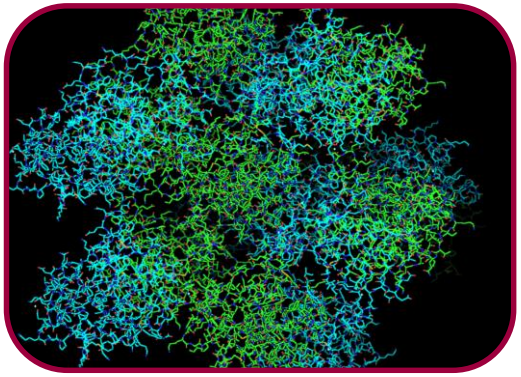


LLPS and Gelation

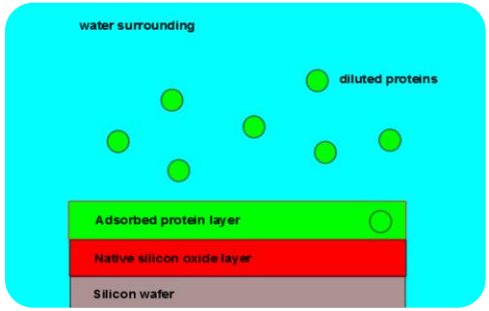
M^{3+}



Protein crystallization



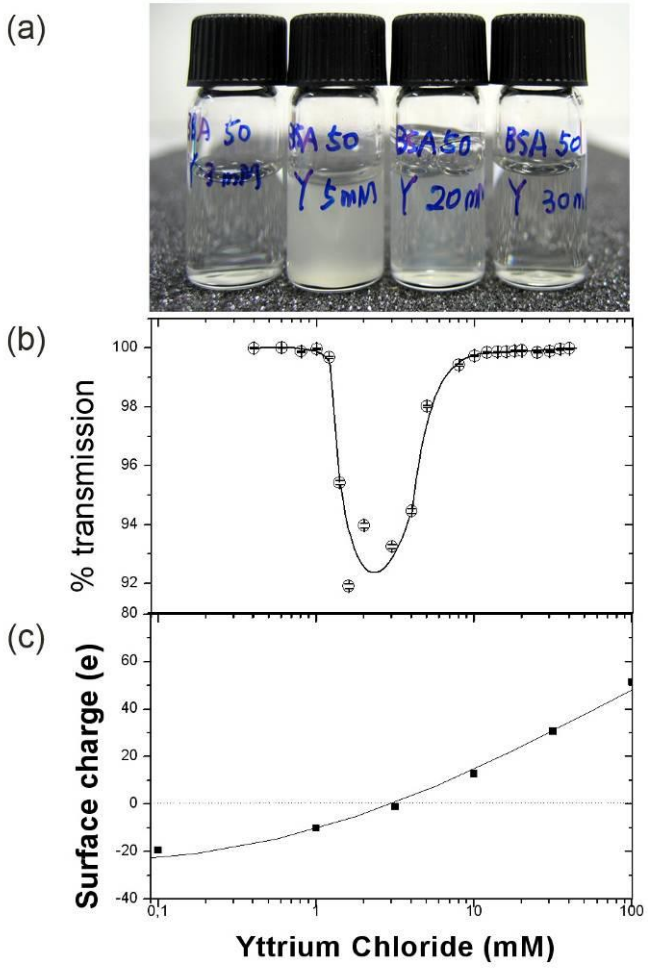
Static & Dynamic of Protein Clustering

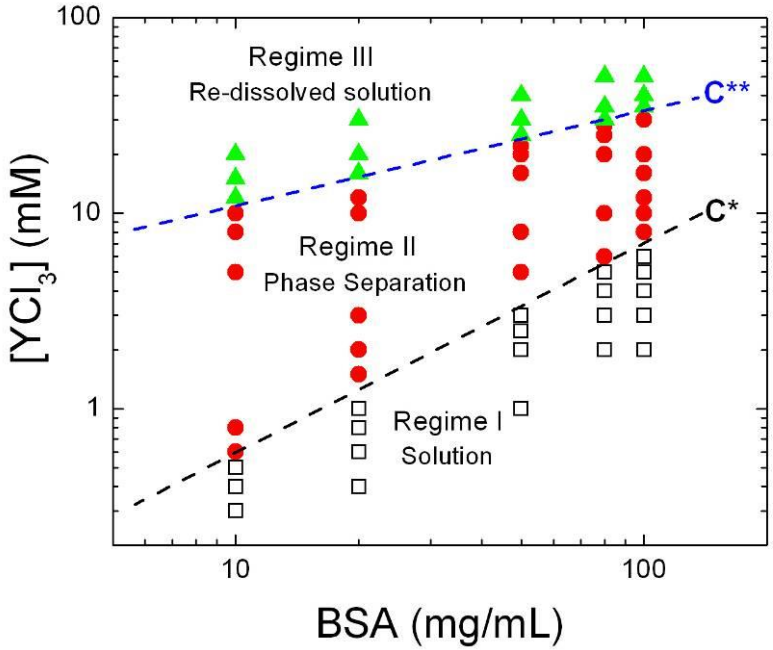


Protein adsorption

RC Induced by Multivalent Metal Ions

Reentrant Condensation

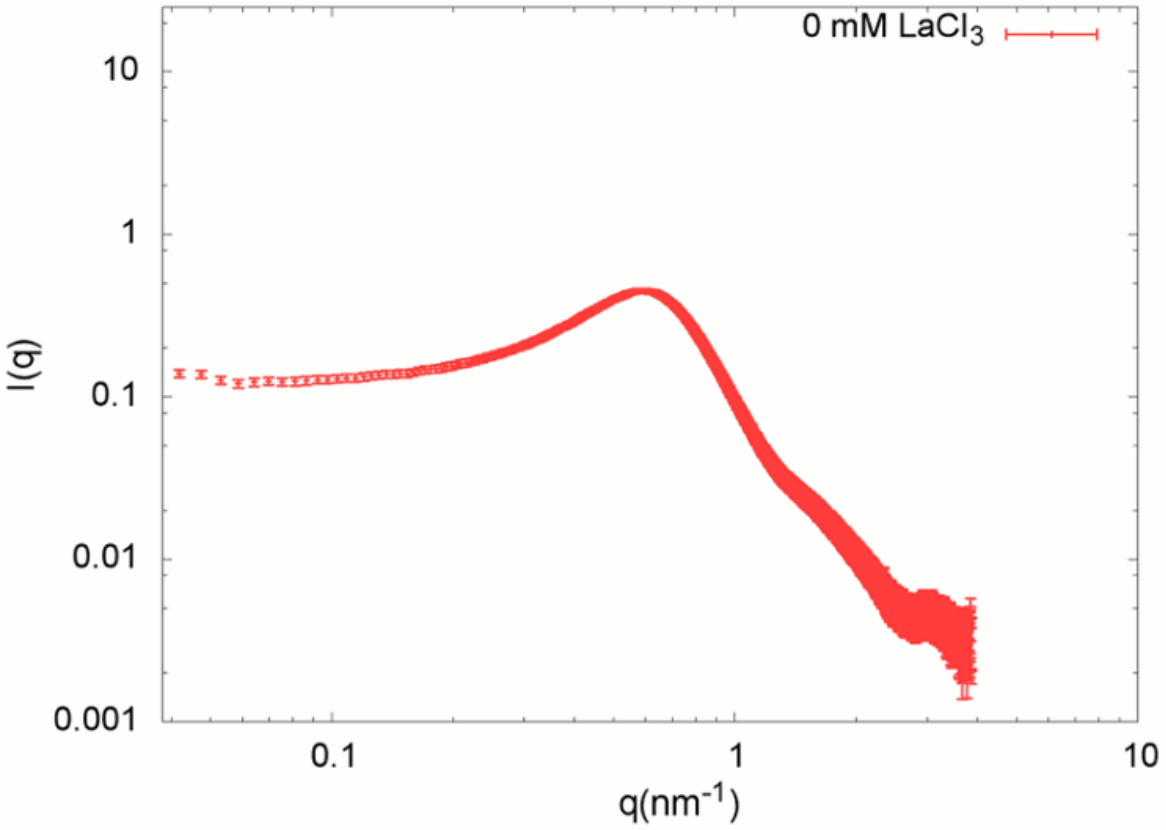


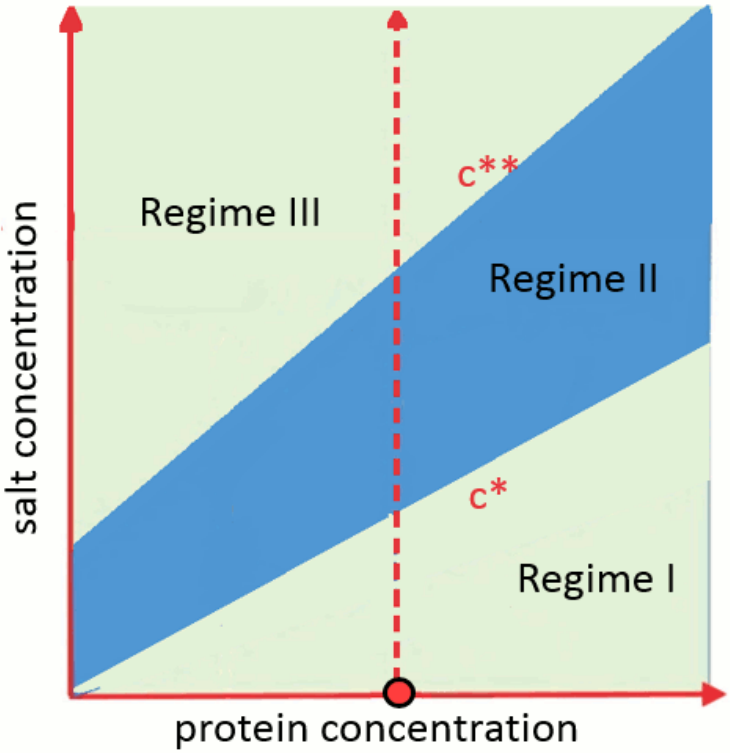


Bovine serum albumin (BSA), 583 amino acids in monomer; Mw 68 kDa, Rg 3.1 nm, pI 4.6, -11*e* at neutral pH

F. Zhang et al. *Phys. Rev. Lett.* **2008**, 101, 148101
F. Zhang et al. *J. Phys. Chem*. B **2007**. 111, 251

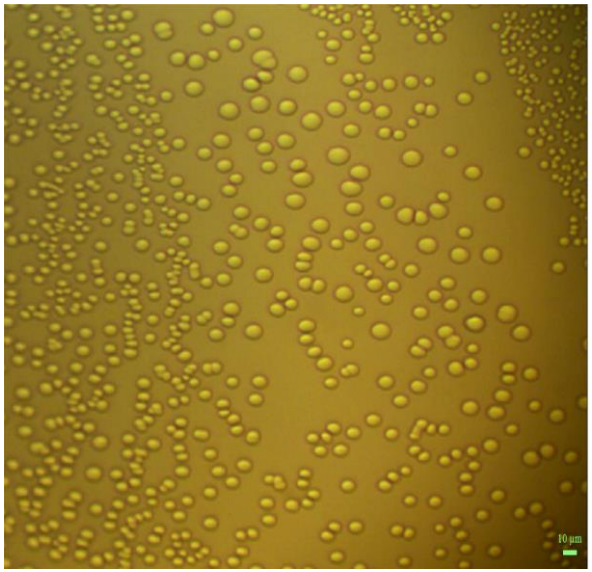
Reentrant Condensation

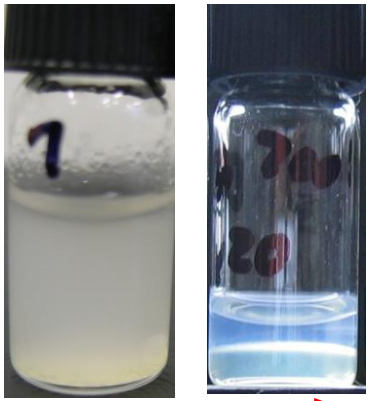


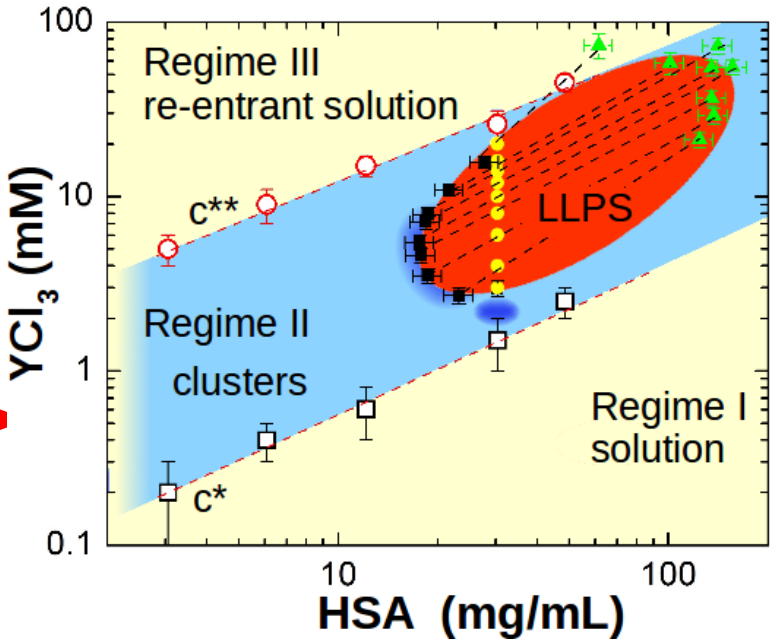


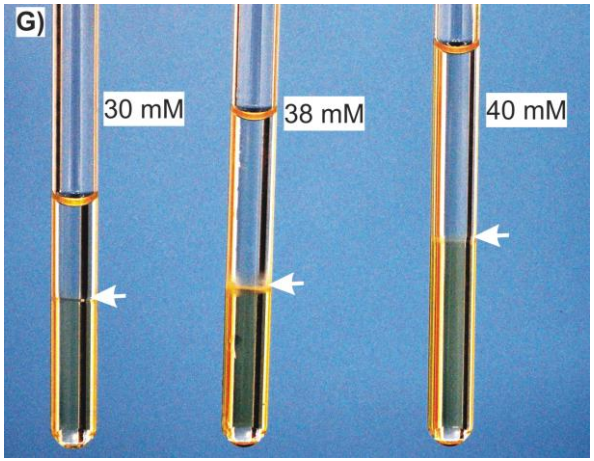
150 mg/ml BSA with different $LaCl_3$ protein concentrations

Liquid-Liquid Phase Separation





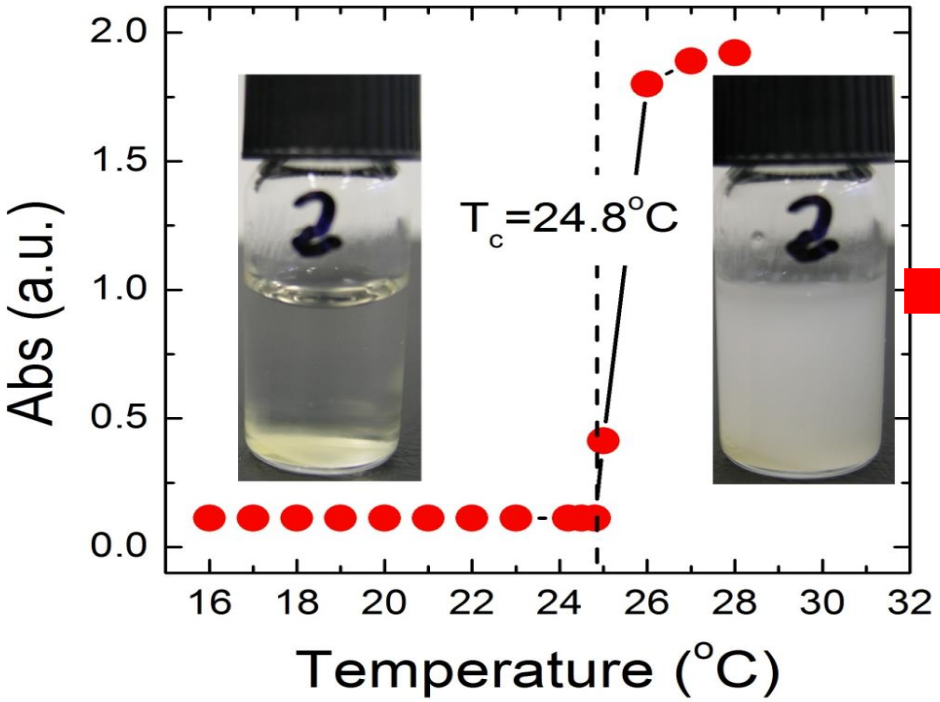


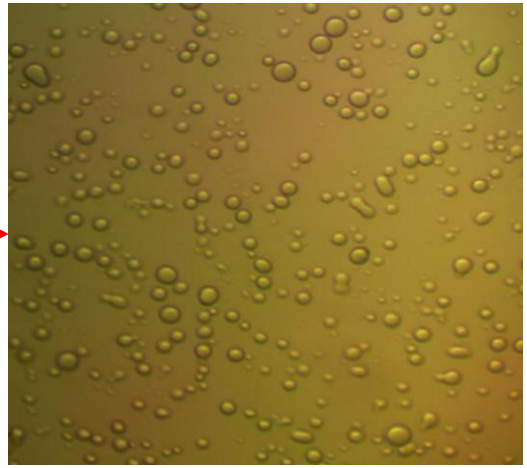


c_s^1 and c_p^1 was determined by X-ray and UV absorption, respectively and c_s^2 and c_p^2 was calculated from the volume of each phase and the initial c_s and c_p.

F. Zhang et al. *Soft Matter* **2012,** 8, 1313

Lower Critical Solution Temperature





Lower critical solution temperature (LCST)

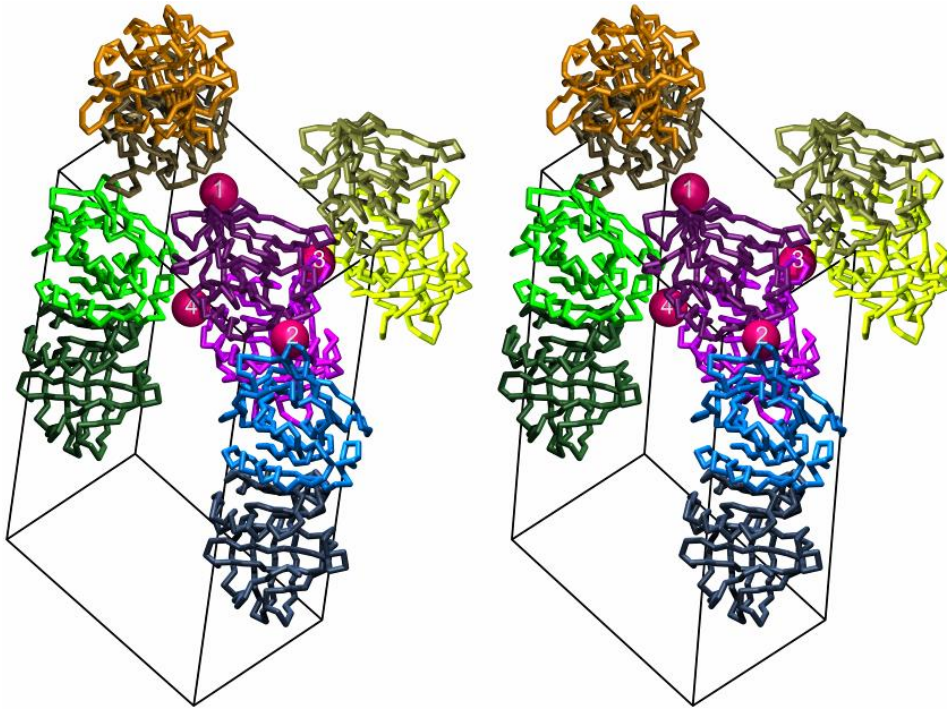
HSA30.3mg/mL with 4mM YCl_3

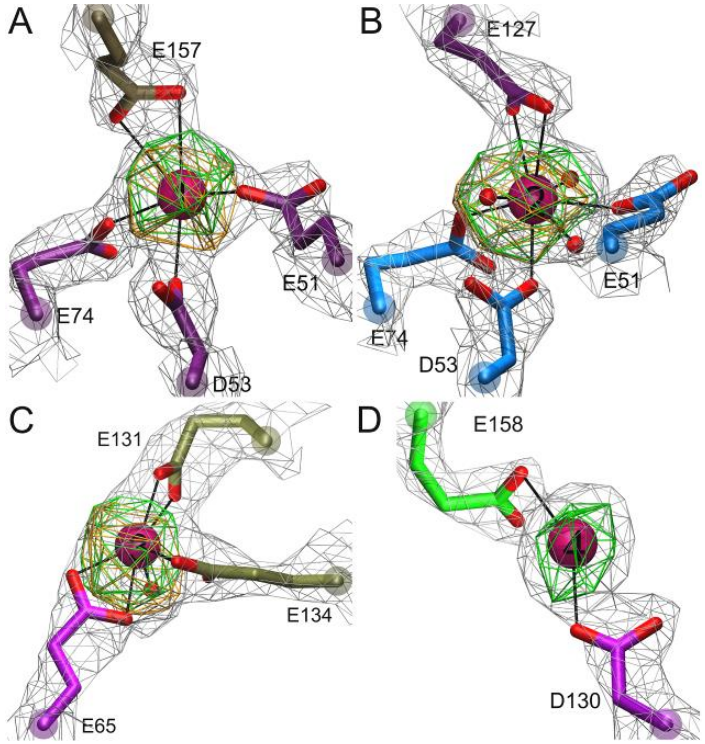
High Quality Single Crystals

- Crystals from a two-step process: BLG 6.7 mg/mL YCl_3 3.0 mM
- Crystals from a one-step process: BLG 6.7 mg/mL YCl_3 0.3 mM

Role of Metal Ion: Bridging

Dr. Zocher & Prof. T. Stehle *IFIB, Tübingen University*





- BLG_Y^{3+}: Orthorombic (new) structure $P2_12_12_1$
- Ion bridging – stable protein-protein contacts
- Serve to form the crystal lattice

F. Zhang et al. *J. Appl. Cryst.* **2011,** 44, 755

SCIENTIFIC REPORTS

OPEN

SUBJECT AREAS:
BIOLOGICAL PHYSICS
STATISTICAL PHYSICS
THERMODYNAMICS

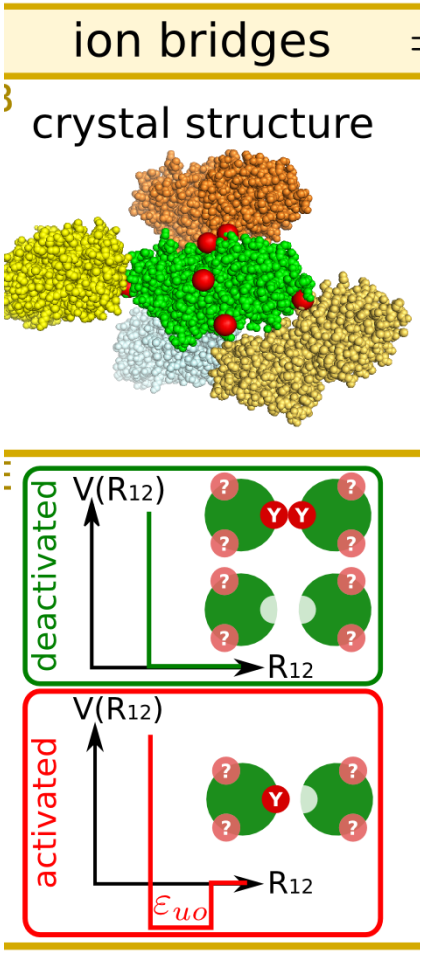
Ion-activated attractive patches as a mechanism for controlled protein interactions

Felix Roosen-Runge[1,2], Fajun Zhang[1], Frank Schreiber[1] & Roland Roth[3]

Theoretical Model

Can we predict the phase behavior?

Ion-Activated Patchy Model



F. Roosen-Runge, F. Zhang, F. Schreiber, R. Roth, *Scientific Reports* 2014, 4, 7016

JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY
Article
pubs.acs.org/JACS

Real-Time Observation of Nonclassical Protein Crystallization Kinetics

Andrea Sauter,[†] Felix Roosen-Runge,[‡] Fajun Zhang,*[,†] Gudrun Lotze,[∥] Robert M. J. Jacobs,[§] and Frank Schreiber[†]

Nonclassical pathways in protein crystallization

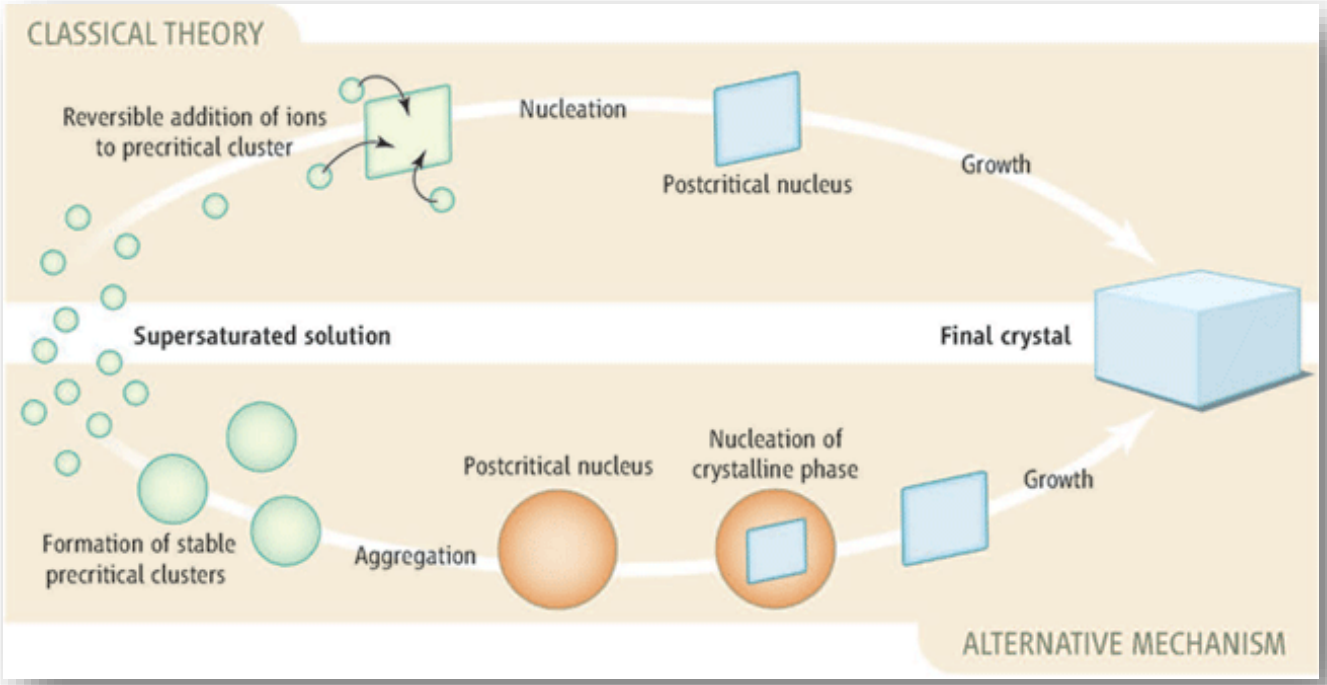


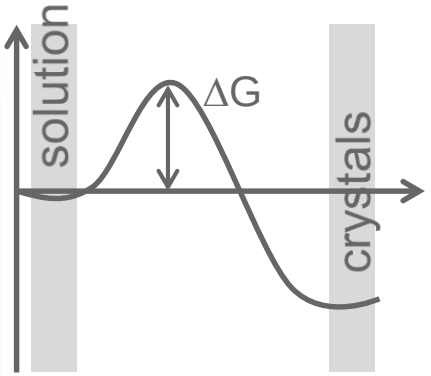
Master: Naman Jain

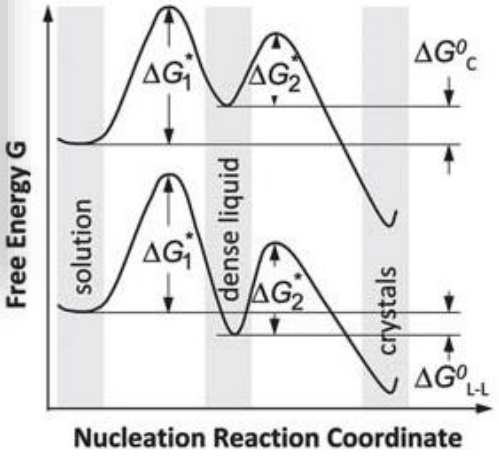
DFG funded PhD position 2017

Classical vs Nonclassical Pathways

$$\Delta G = -\frac{4}{3}\pi r^3 \Delta G_V + 4\pi r^2 \sigma$$

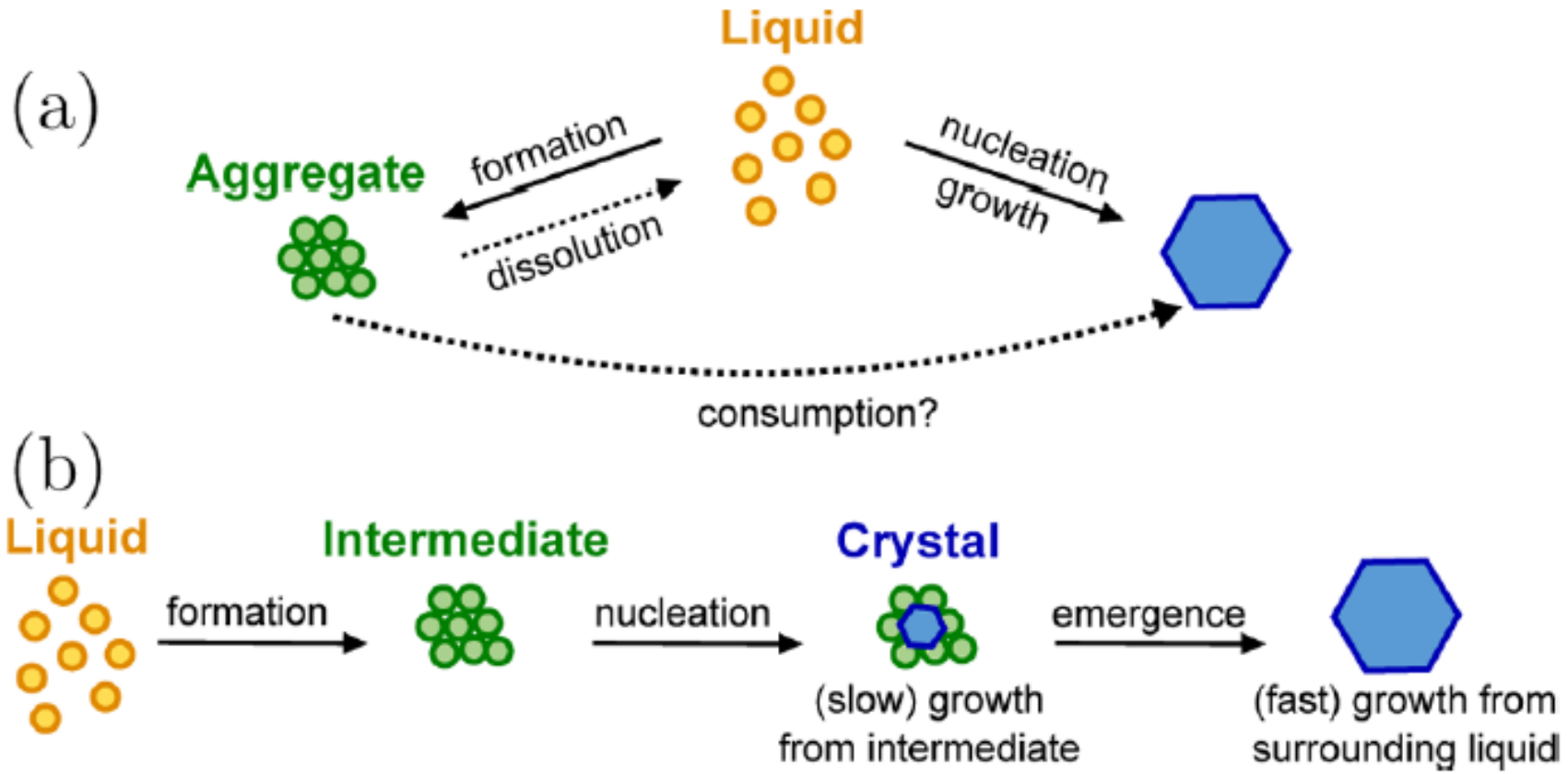




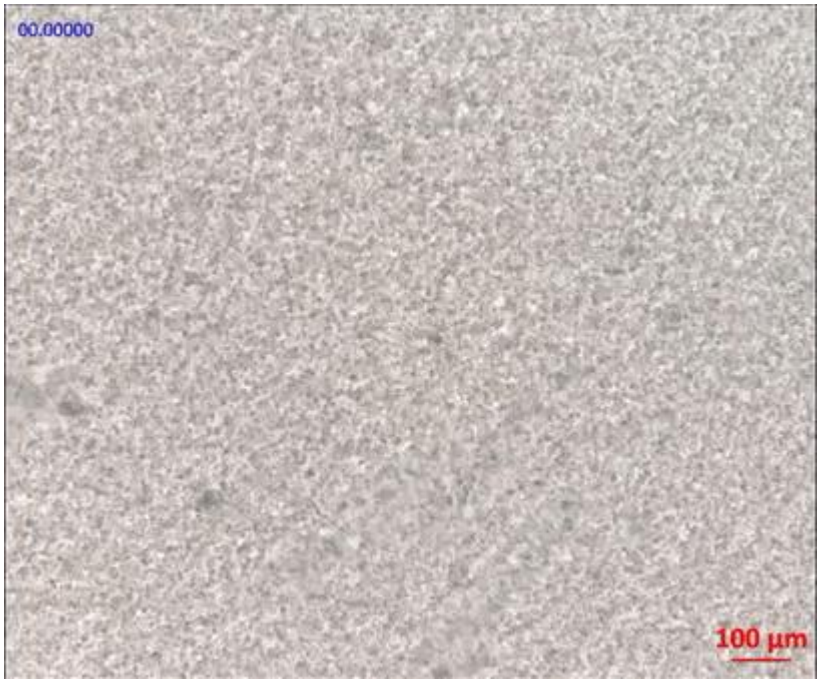


F.C. Meldrum, R. P. Sear, Science 2008,322,1802
P. G. Vekilov. Nanoscale 2010, 2, 2346

Open Question



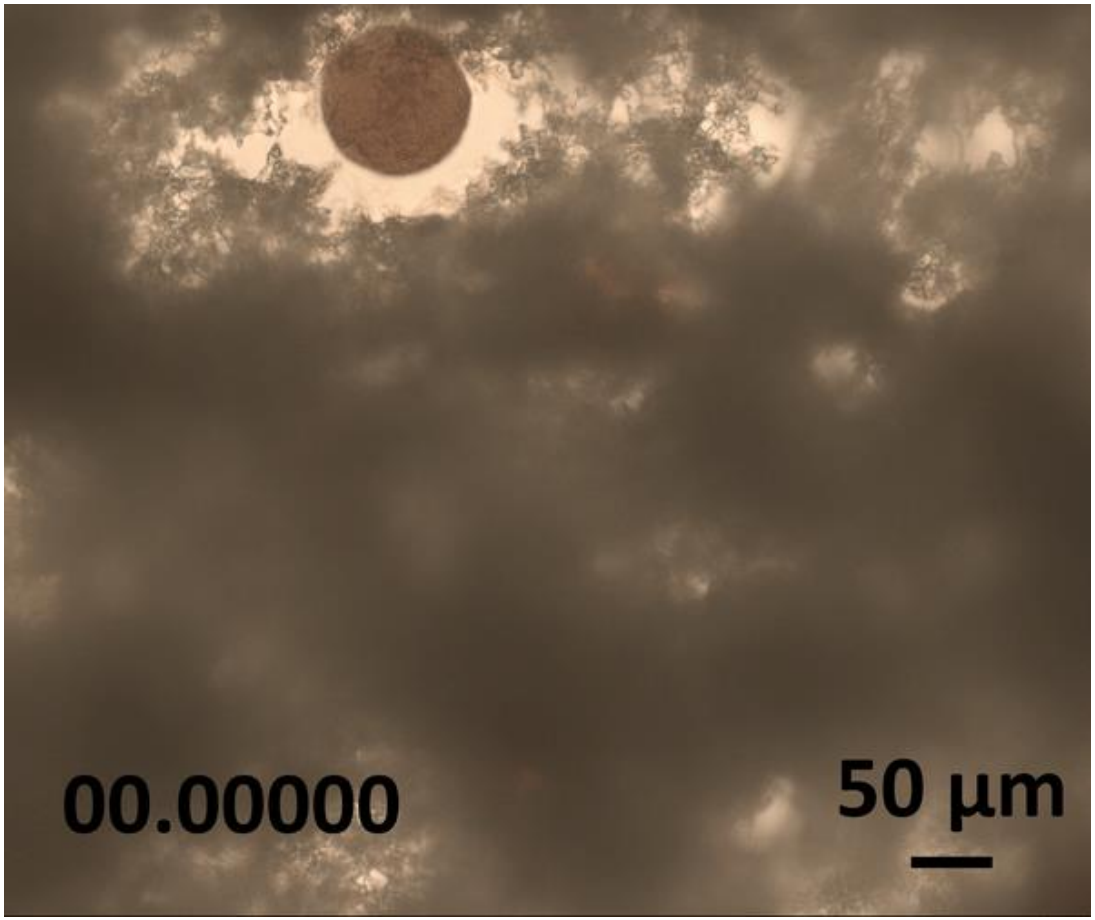
Real-Time Study: Optical Microscopy



BLG 33 mg/mL with $CdCl_2$ 19mM and 20mM, respectively.

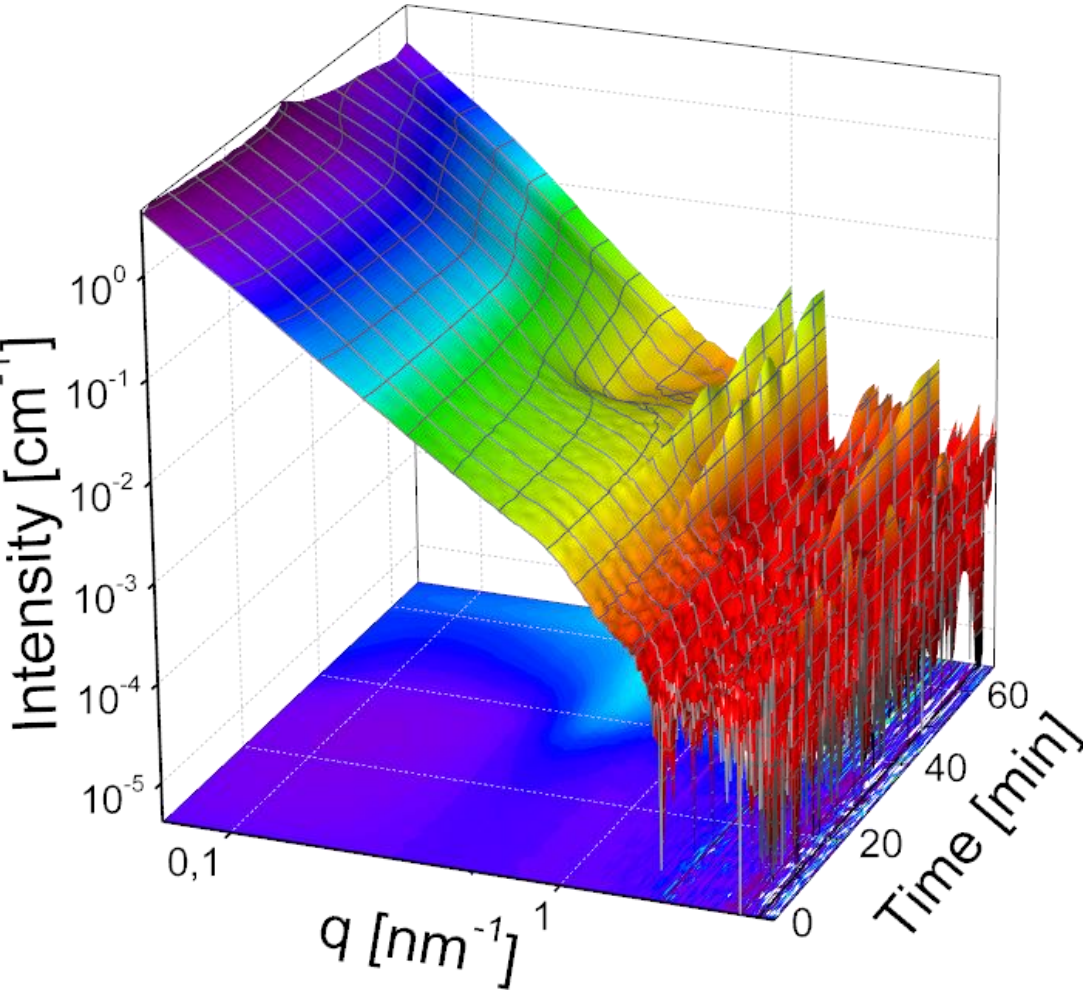
A. Sauter et al. *Faraday Discussion.* **2015**, 179, 41.

Real-Time Study: Optical Microscopy



BLG 20 mg/mL in D2O + 4mM YCl_3

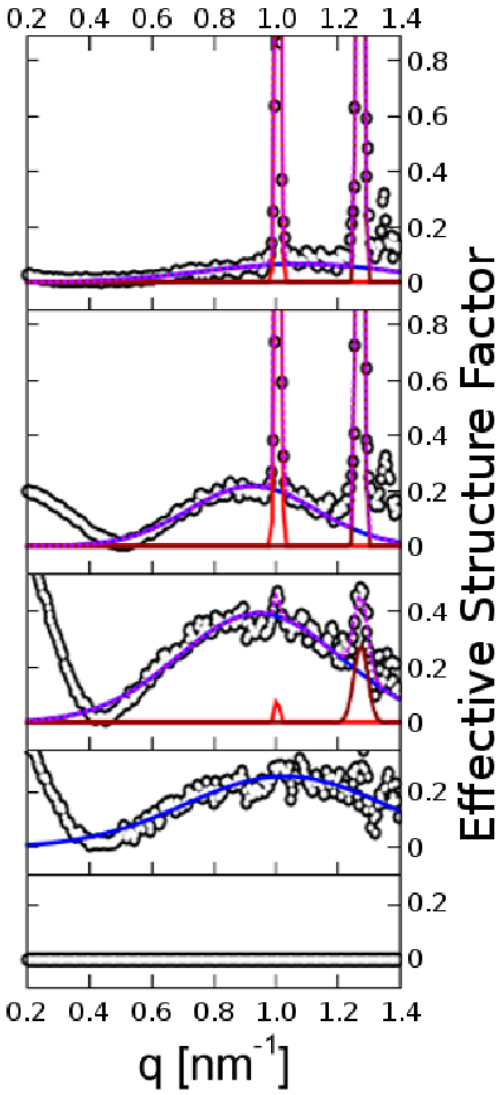
Real-Time SAXS

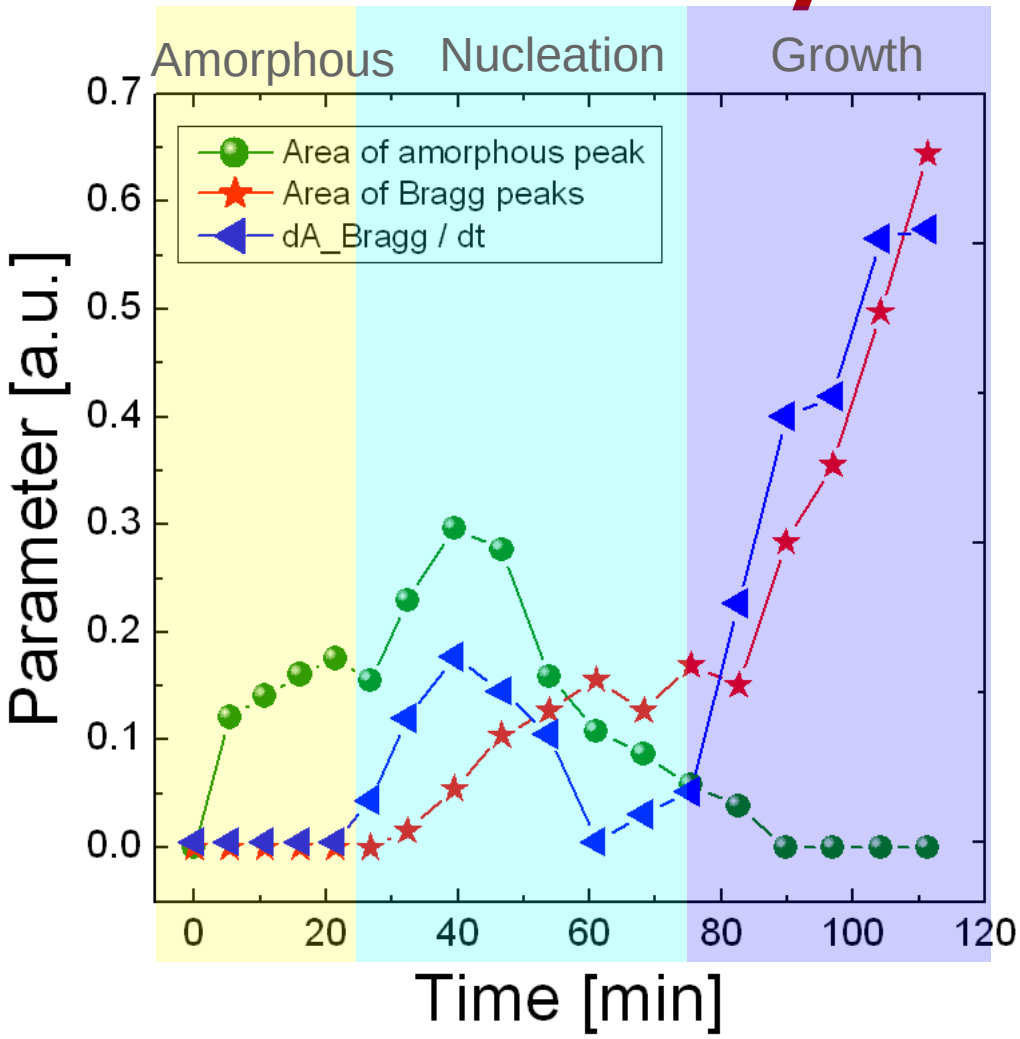


BLG 20 mg/mL + 15 mM $CdCl_2$

A. Sauter et al. *J. Am. Chem. Soc.* **2015**, 137, 1485

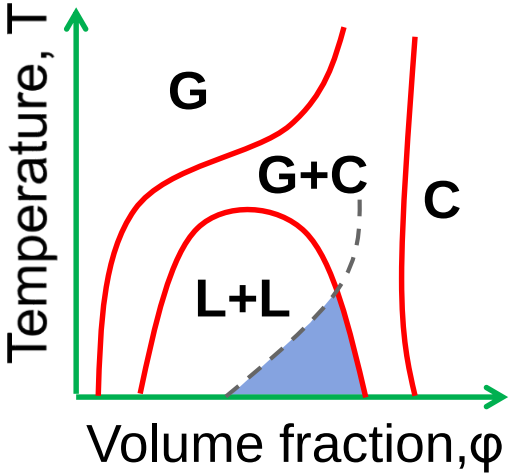
Growth Kinetics – Pathway



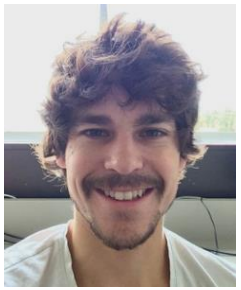


Kinetics of LLPS in Protein Solutions Studied by USAXS / VSANS

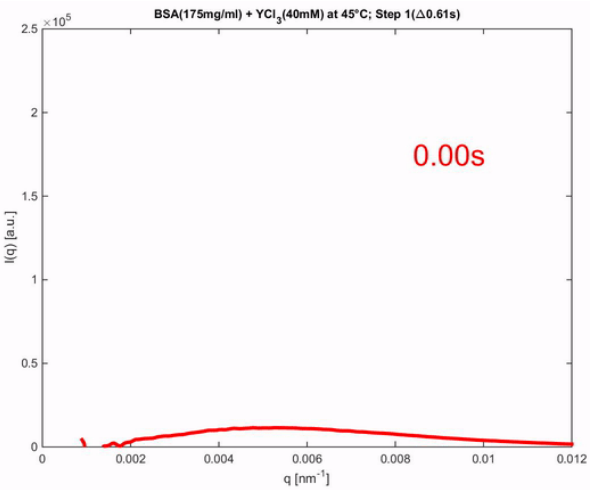
- LLPS – LCST, arrested phase transition
- Kinetics followed by USAXS and VSANS
- Dynamics by XPCS
- Smart materials



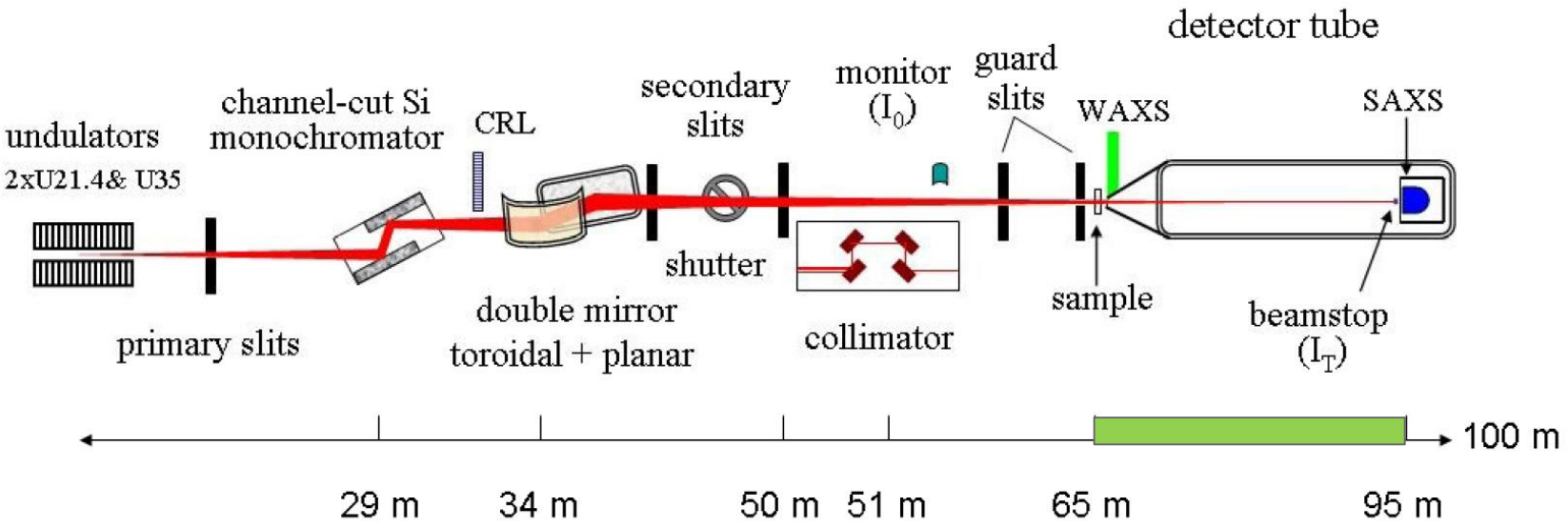





Master: Danylo Dyachok

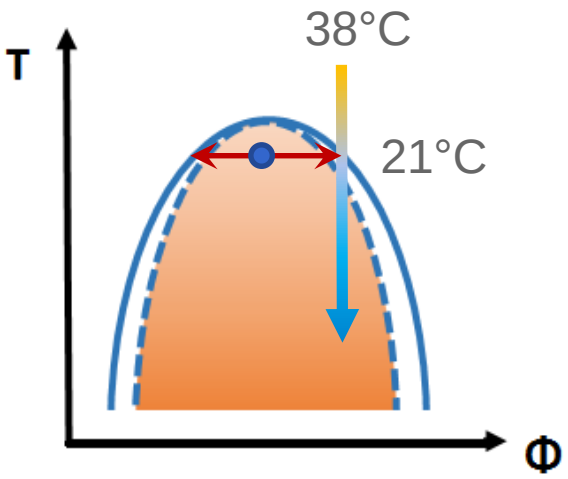


Ultra-Small Angle X-ray Scattering

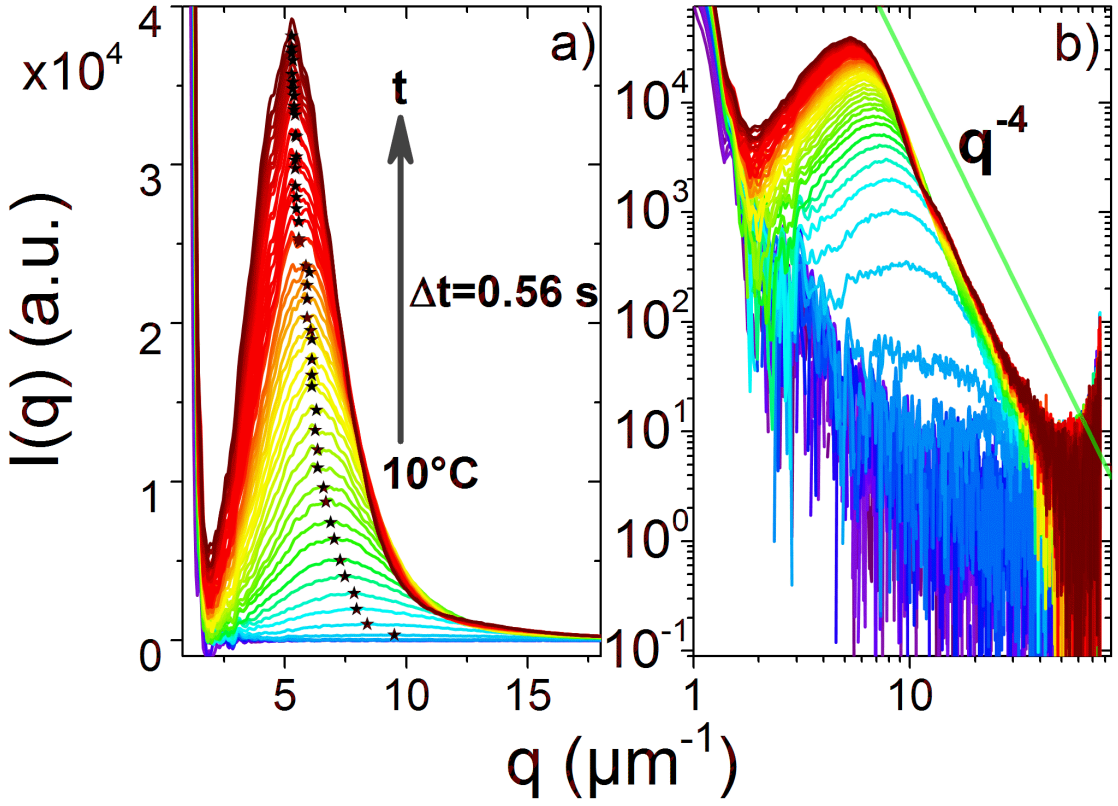


- **USAXS (ID02) at ESRF:**
  - The q_{min} is 9 x10^{-4} nm^{-1};
  - Temperature controlled by Linkam heating stage
  - Scan rate up to 3 Hz for early stage

Research in Biophysics



$$\xi = 2\pi/q^*$$



Initial composition: IgG 110 mg/ml
with PEG1k 9% w/v

IgG 220 mg/ml with PEG1k 3.8% w/v
Quench from 38°C to 10°C (early stage 30s)

S. Da Vela, *et al.* 2017 Submitted

Static & Dynamic Properties of Proteins in Solution

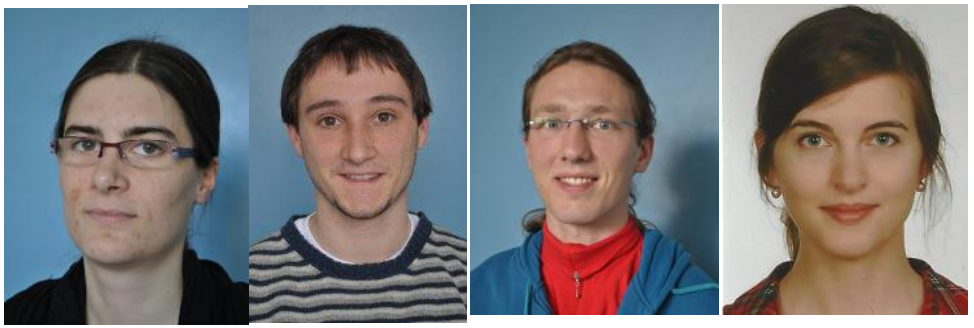
THE JOURNAL OF PHYSICAL CHEMISTRY Letters

Letter

pubs.acs.org/JPCL

Crowding-Controlled Cluster Size in Concentrated Aqueous Protein Solutions: Structure, Self- and Collective Diffusion

Michal K. Braun,[†] Marco Grimaldo,[†,‡] Felix Roosen-Runge,[*,‡,§] Ingo Hoffmann,[‡] Orsolya Czakkel,[‡] Michael Sztucki,[¶] Fajun Zhang,[*,†] Frank Schreiber,[†] and Tilo Seydel[*,‡]



Master: Anita Girelli

- Static: SAXS/SANS, SLS/DLS
- Dynamics: Neutron back scattering and spin-echo spectroscopy
- Collaboration with Dr. T. Seydel at ILL, Grenoble, France

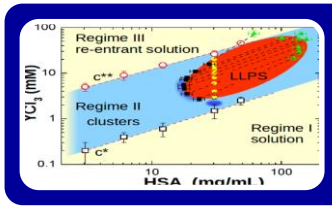
Tuning protein adsorption at interfaces

- Real – time study by ellipsometry
- Kinetics of protein adsorption with and without trivalent salts
- Contrast variation by neutron reflectivity
- Surface coating – OTS

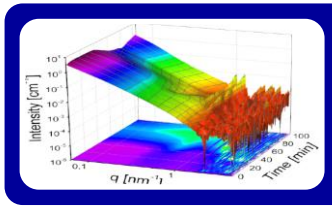
Collaboration with Dr. Robert J. Jacobs from Oxford University and Dr. Maximilian Skoda from STFC, ISIS, UK.

Masters: Simon P. Schoenberg, Amrita Das Gupta

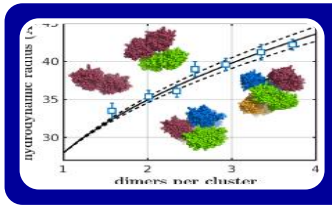
Summary



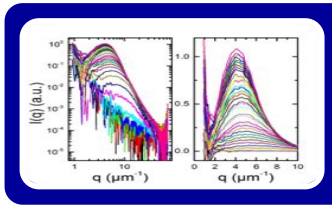
RC, LLPS and clustering induced by metal ions – DFG



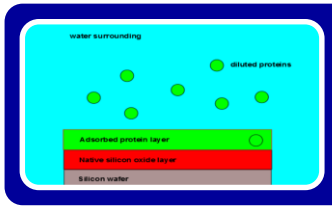
Nonclassical nucleation pathways in protein crystallization – DFG



Protein dynamics and cluster formation – DFG



Static and dynamic properties of antibodies in solution – DFG-ANR



Tuning protein adsorption at interfaces

What you may contribute:

- Characterization of proteins and nanoparticles by SAXS
- Following protein crystallization by optical microscopy and SAXS
- Characterization of protein interactions and cluster formation by DLS and SAXS
- Temperature sensitive SMART materials based on LCST phase behaviour of protein solutions.
- Tuning protein adsorption at interfaces: OTS coating, T, etc.
- Control parameters for LCST phase behaviour.
- Amyloid fibril formation in protein solution.

Welcome to join us!

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