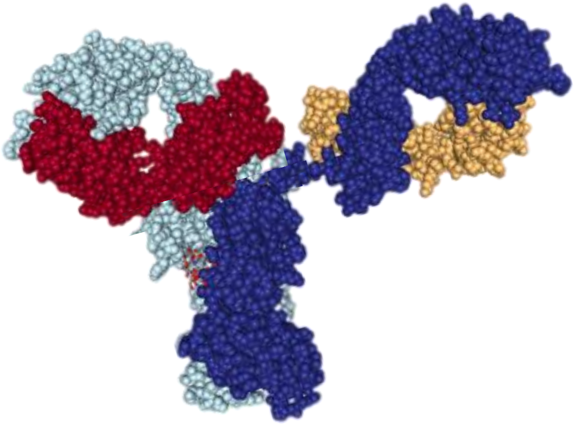
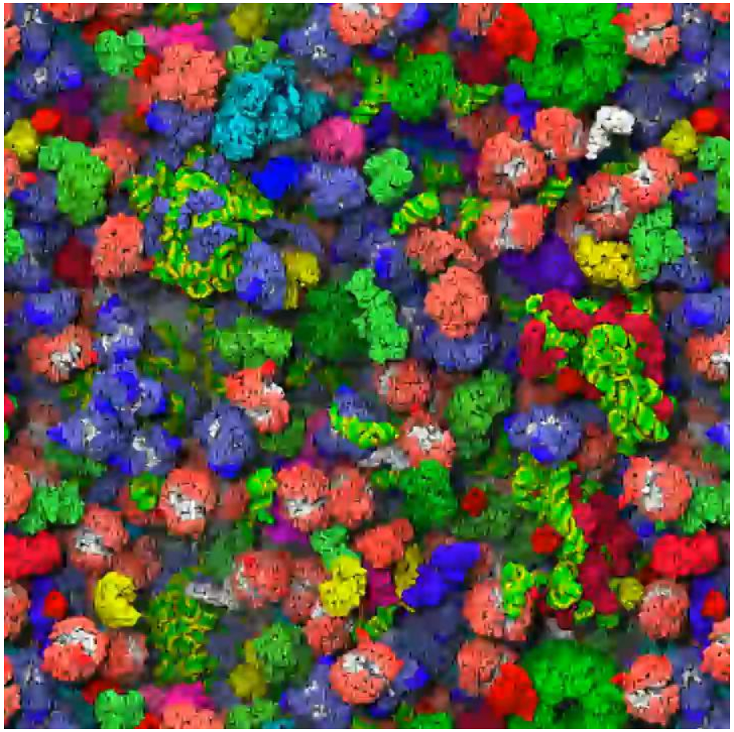
Connecting dynamics and phase behavior of proteins: The neutron perspective (and beyond)

Frank Schreiber
http://www.soft-matter.uni-tuebingen.de

A. Girelli, N. Begam, A. Ragulskaya, S. Chandran, F. Zhang, R. Roth, M. Oettel (Tübingen)
H. Rahmann, C. Gutt (Siegen), J. Möller, M. Reiser, F. Westermeier, M. Sprung (Hamburg), F. Roosen-Runge (Malmö)
T. Seydel, C. Beck, O. Matsarskaia, K. Pounot, I. Mosca, T. Forsyth, H. Lopez, J.-L. Barrat, T. Narayanan (Grenoble)
R. Jacobs (Oxford), M. Skoda (Rutherford)
et al.

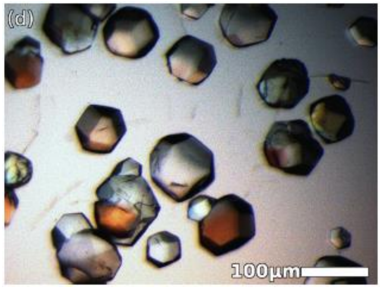


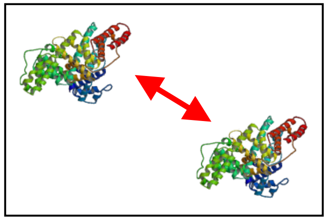


πάντα ῥεῖ
From individual proteins to ensembles

Financial support from BMBF, DFG, ILL, DAAD, AvH, Studienstiftung, etc. is gratefully acknowledged.

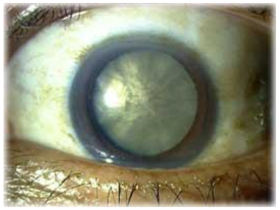
Interactions and Aggregation



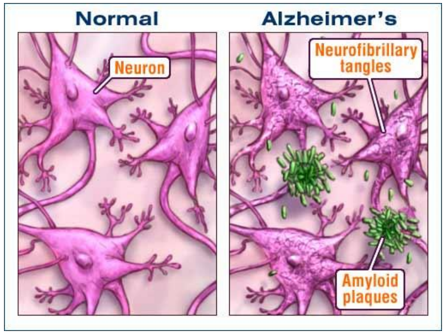


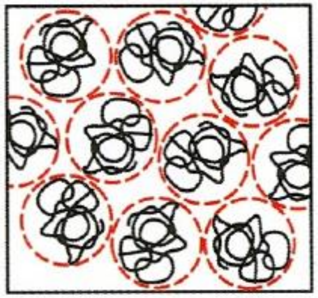
- understanding crystallization (structural biology!)

- understanding phase behavior (stability of pharmaceuticals)



- eye as an example:
  - inside: avoid cataract (LLPS)
  - outside: avoid sticking and infection





- aggregation-related diseases
  (Alzheimer; Kreutzfeld-Jacob; Parkinson)

- *in vivo*: ionic cloud required (salt)

- crowding (high concentration in cells)
  (key to understanding of kinetics etc.; Ellis et al.)

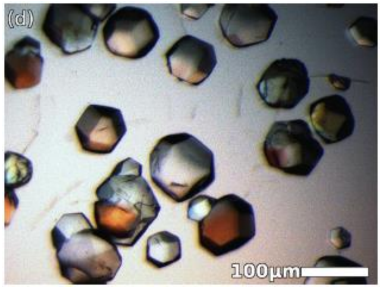
- ‘extreme conditions’
  - highly concentrated solutions (molecular crowding)
  - high salt concentrations
  - high temperatures (denaturing and network formation)

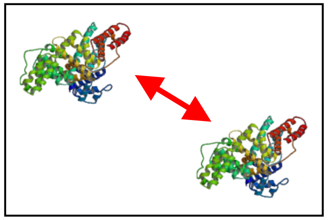
- note the impact of interfaces (container walls, sensors, implants, …)
  - adsorption effects
  - nucleation effects
    (see Fries et al., PRL 2017; Sci. Rep. 2020)

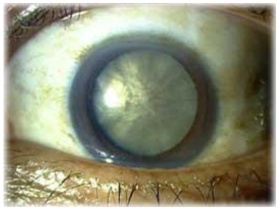
Nafisa Begam et al., PRL 2021
New Scientist 2021

Interactions and Aggregation

- understanding crystallization (structural biology!)
- understanding phase behavior (stability of pharmaceuticals)
- eye as an example
- ...





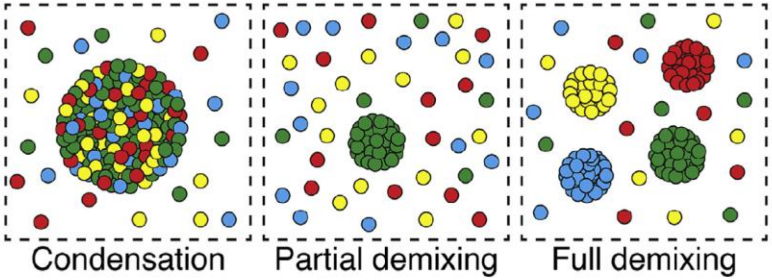


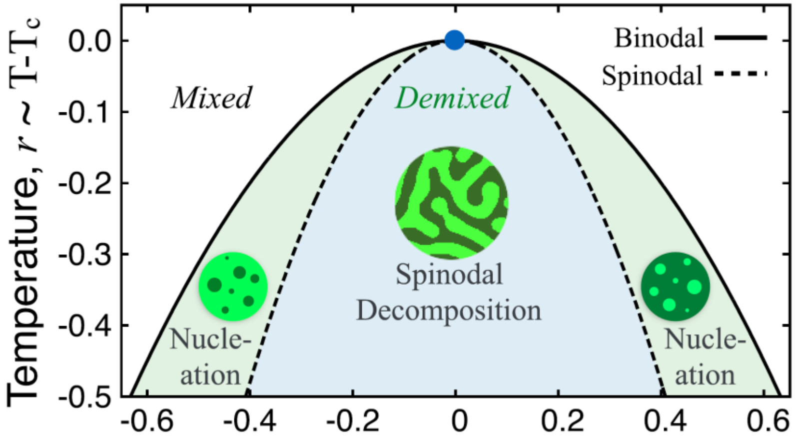
Theory ?

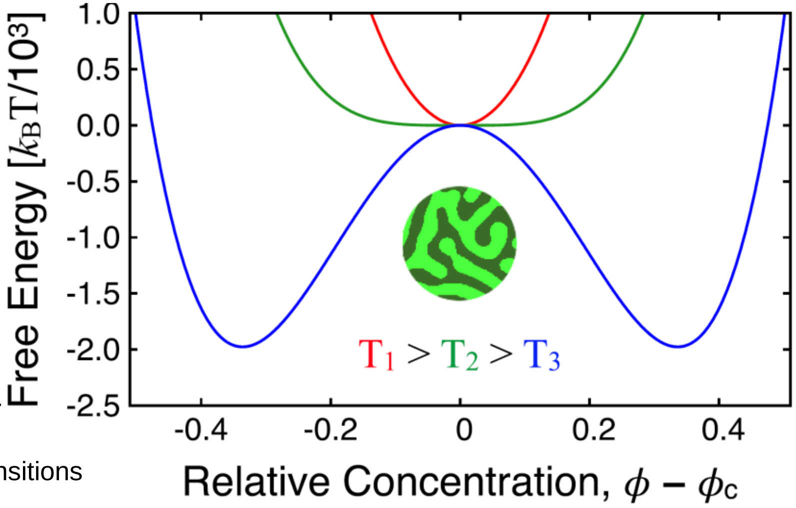
Free energy of a multi-component system

$$f_{\mathrm{RS}} = \sum_{i=1}^{N} \left[\phi_i \ln(\phi_i) + \sum_{j=1 \neq i}^{N} \frac{\chi_{ij}}{2} \phi_i \phi_j + \sum_{j=1}^{N} \frac{K_{ij}}{2} (\nabla \phi_i) \cdot (\nabla \phi_j) \right]$$

entropy interactions interfaces



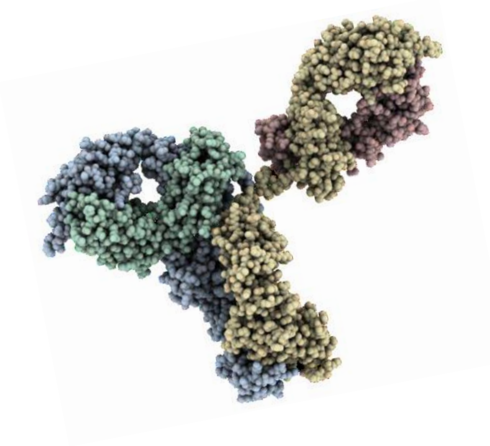




Berry et al., Rep. Prog. Phys. 81 (2018) 046601
Physical principles of intracellular organization via active and passive phase transitions

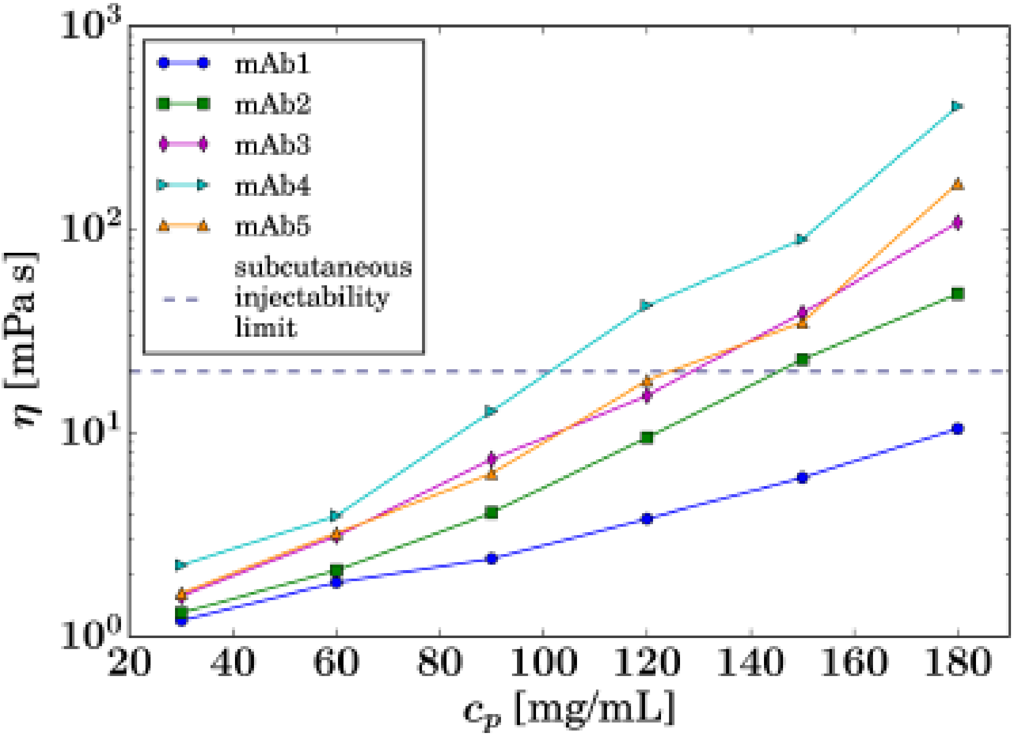
Protein Dynamics: Going beyond “Static“ Phase Behavior

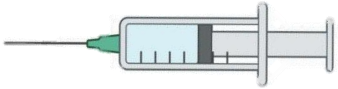
Protein Dynamics: Macroscopic Viscosity η



Viscosity η has to stay below threshold for injection !

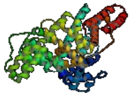
Viscosity η





Diffusion D ~ 1 / η
(Stokes-Einstein)

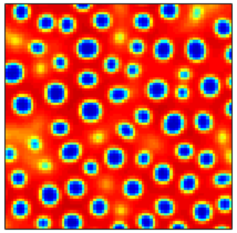
See also talk 16:15
by Ilaria Mosca



Protein data bank, DOI: 10.2210/pdb1hzh/pdb

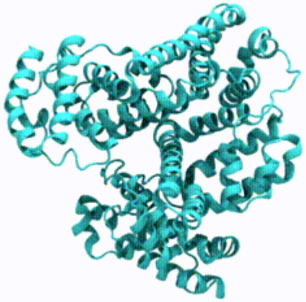
Anita Girelli et al., PRL 2021
Stefano Da Vela et al., Soft Matter 2017, 13, 8756
Ilaria Mosca et al., in preparation

Protein Dynamics: Types of Motion

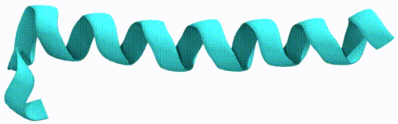


diffusion;
collective
motion

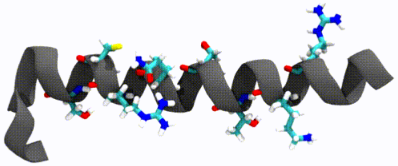
If you want to understand function,
you have to understand structure
and dynamics!



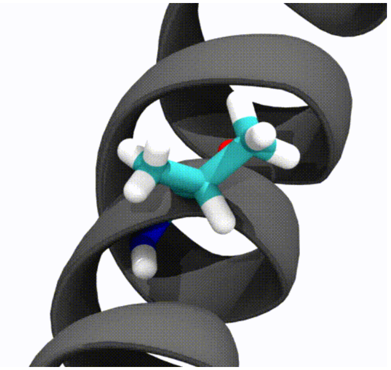
entire protein



backbone



sidechains



methyls

larger units
slower motion
(different regimes for
time and length scales!)

Grimaldo et al., Q. Rev. Biophys. 2019, 52, e7, 1–63
see also various papers by JCNS / Delaware / NIST groups

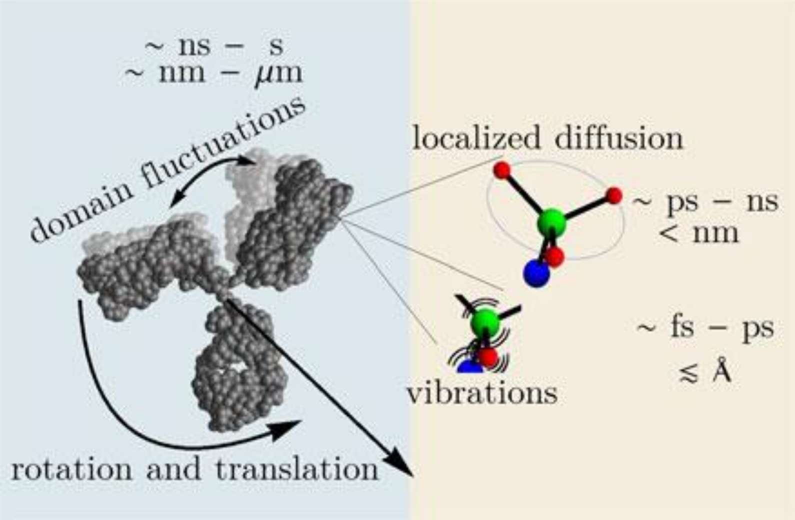
simulations by Kevin Pounot

Protein Dynamics: Control Parameters

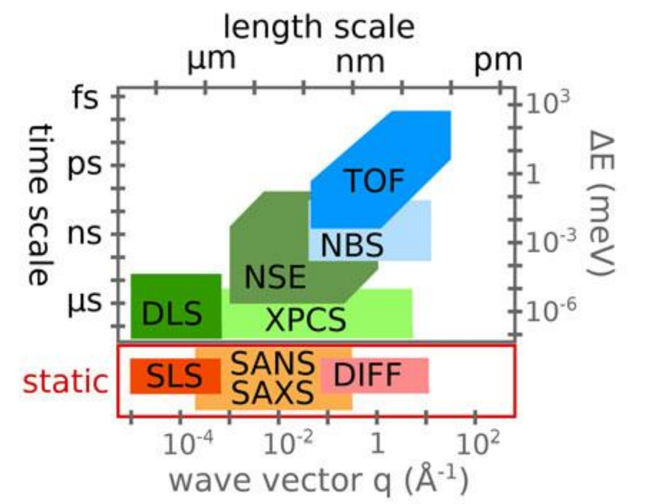
Protein Dynamics

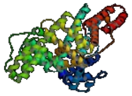
→ Effect of c_p ((self-)crowding) ?
→ Effect of c_s (Y^{3+} ions near c*) ?
→ Effect of lysate (natural crowding) ?
→ Effect of crystallization (“dynamics during kinetics“) ?
→ Effect of *T* (denaturation / gel-like network) ?

Generally complex task
with many different time and length scales



Requires advanced instrumentation
for different time and length scales



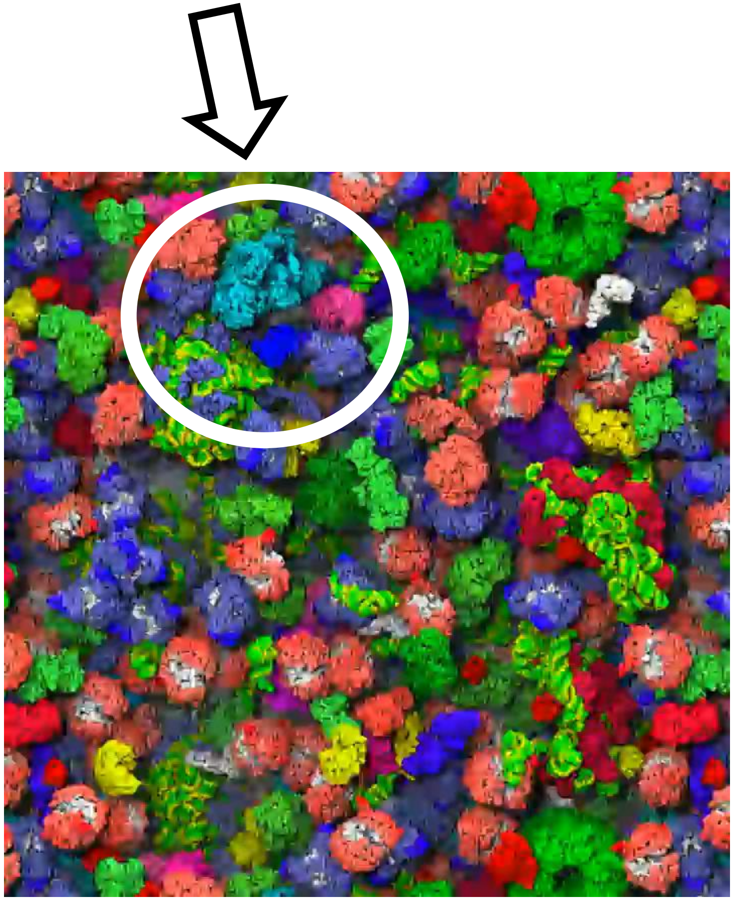


Dynamics of proteins in solution
M. Grimaldo et al., Quarterly Reviews of Biophysics (2019)

Protein Dynamics: Crowding

Now targeted deuteration of environment

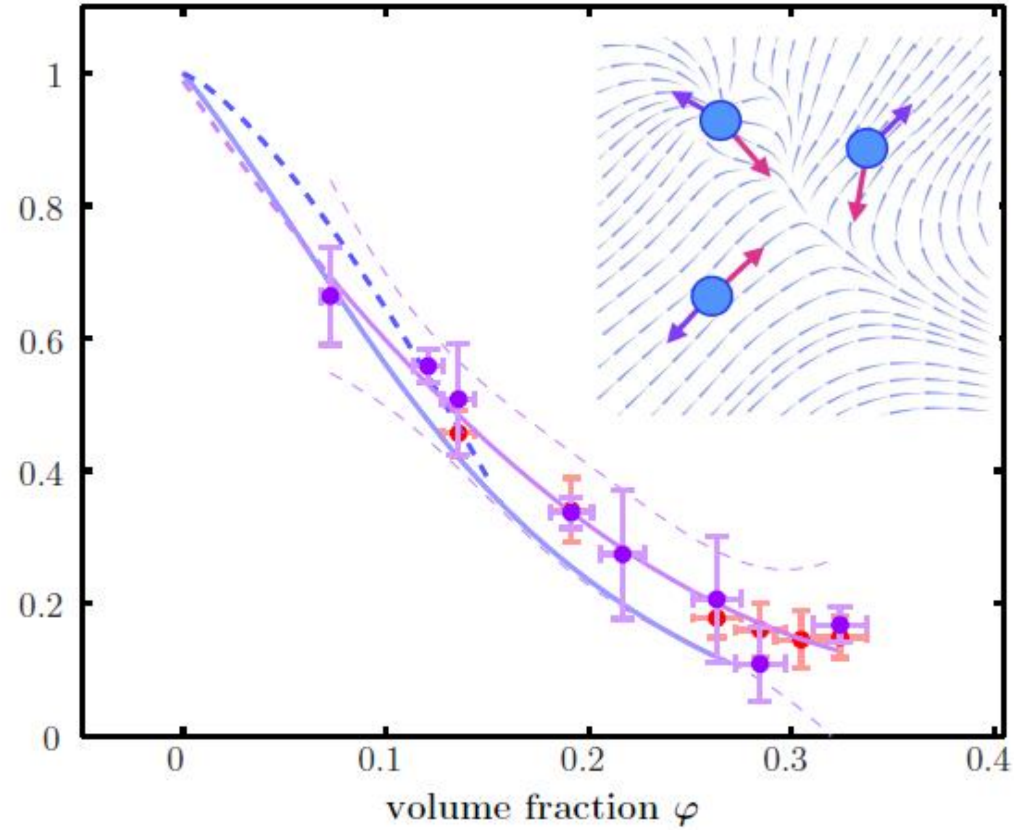
→ Only proteins of interest visible



Self-diffusion probed by QENS

→ Hydrodynamic interactions important
   0.3 – 5.0 ns probed are short time diffusion
   no direct interactions

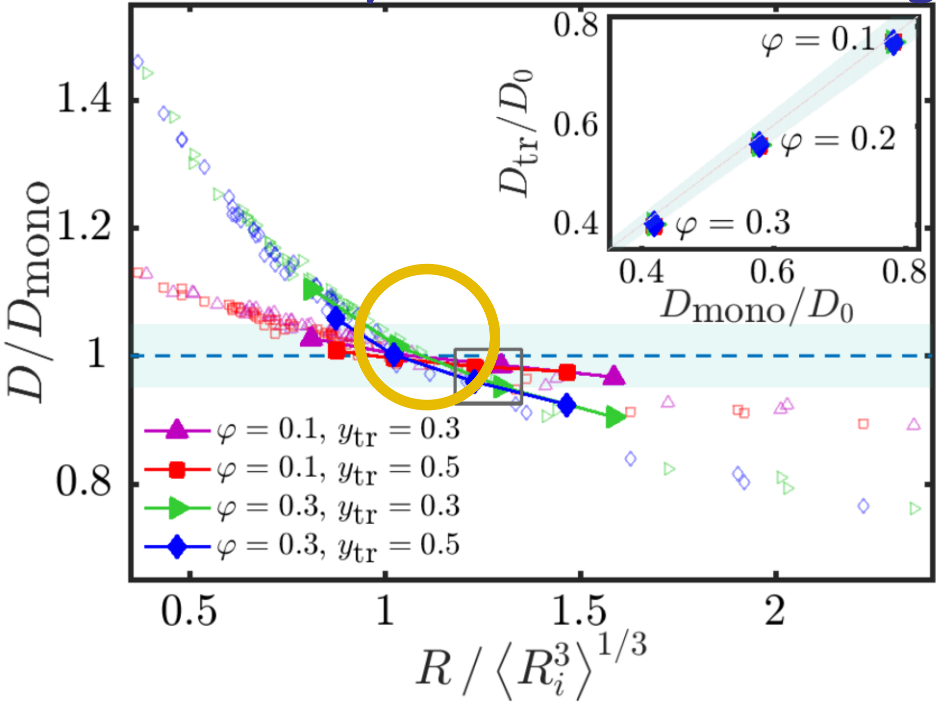
→ Slower for higher protein concentration

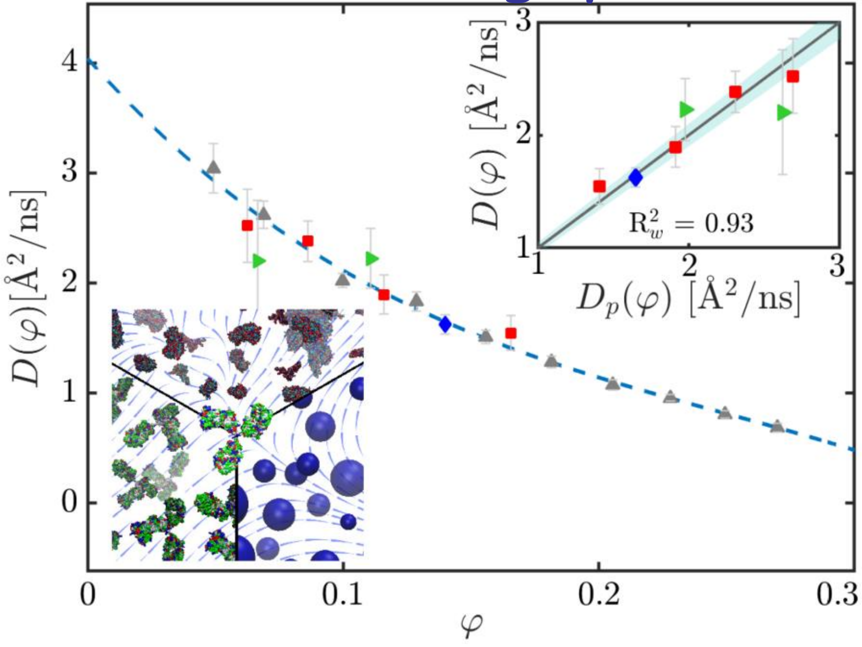


McGuffee & Elcock, Diffusion, Crowding & Protein Stability in a Dynamic Molecular Model of the Bacterial Cytoplasm. PLOS Computational Biology 6(3): e1000694 (2010)

F. Roosen-Runge et al., PNAS 108 (2011) 11815
M. Grimaldo et al., Quart. Rev. Biophys. 52 (2019), e7, 1

Protein Dynamics: Crowding (now natural, using lysate)





Simulated diffusion of spheres as a function of their radius in the Ig-lysate mixture normalized by the diffusion of with self-crowding.

Filled symbols: D of tracer of size R

(R (=1.3) of Ig or other hypothetical R).

Empty symbols: D of the lysate for $R_{tr} = R_{Ig}$.

The rectangle refer to tracers with $R_{tr} = R_{Ig}$

(only incidentally similar to self-crowding “near 1”).

Measured diffusion as a function of the total volume fraction, i.e. the volume fraction of Ig and lysate combined (T = 293K).

Key conclusion:

Data for lysate-crowding follow self-crowding.

Important contributions by theory group H. Lopez / J.-L. Barrat / M. Oettel
and D-lab T. Forsyth et al. (deuterated lysate, i.e. “cell components“)

M. Grimaldo et al., J. Phys. Chem. Lett. 10 (2019), 1709

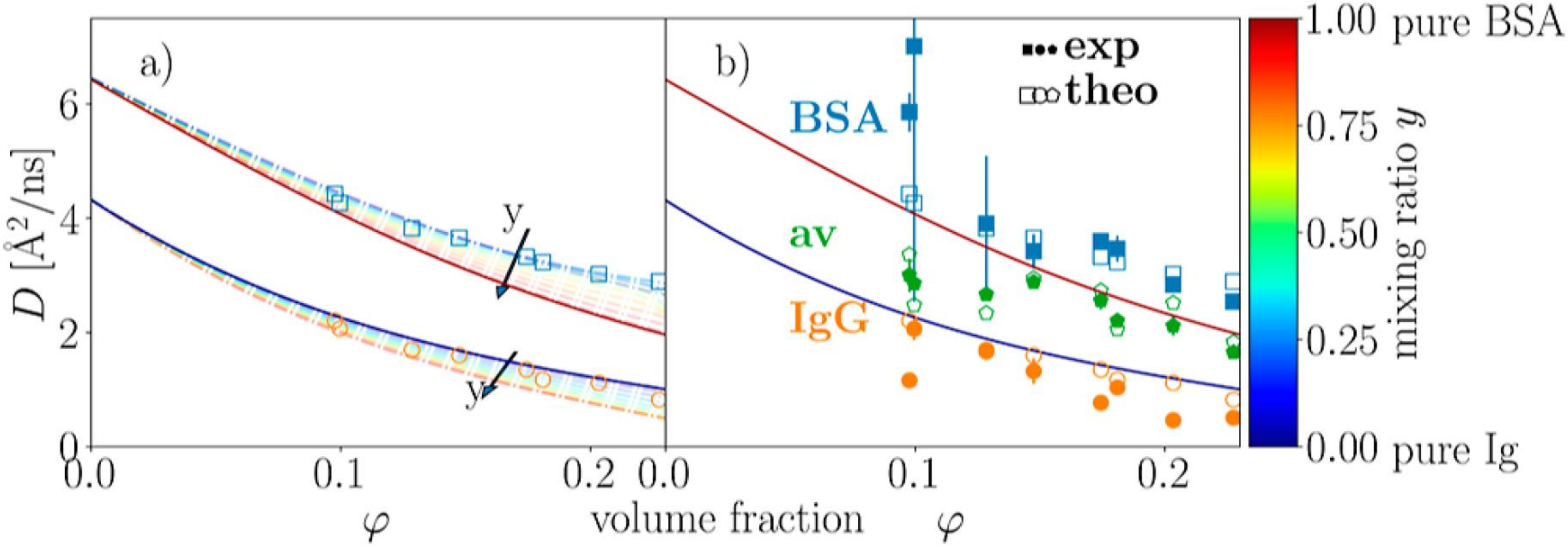
Protein Dynamics: Crowding in bidisperse systems

BSA and IgG mimicking polydispersity in biology

→ Diffusion under crowding conditions similar

→ Diffusion with mixing consistent (experiment and theory derived from effective hard spheres)

→ Diffusion is not simply the average

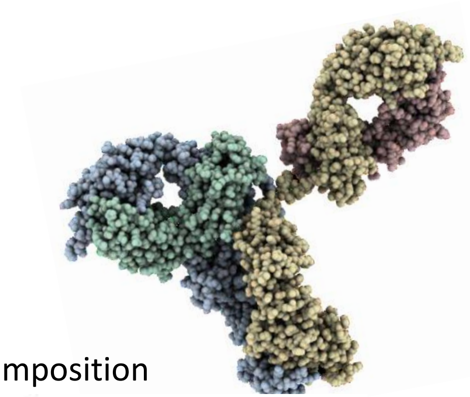


C. Beck et al., J. Phys. Chem. B (2022)

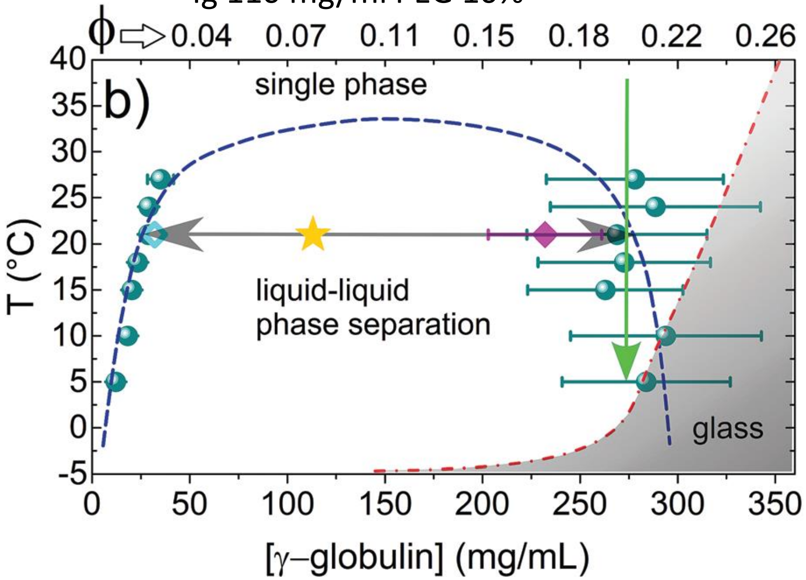
Protein Dynamics upon Phase Separation

Protein Dynamics upon Phase Separation

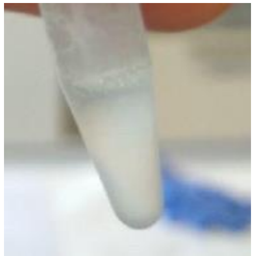
Protein Ig ("γ-globulin"), i.e. an antibody
in water, with polyethylene glycol (PEG1000)
to increase attractions through depletion



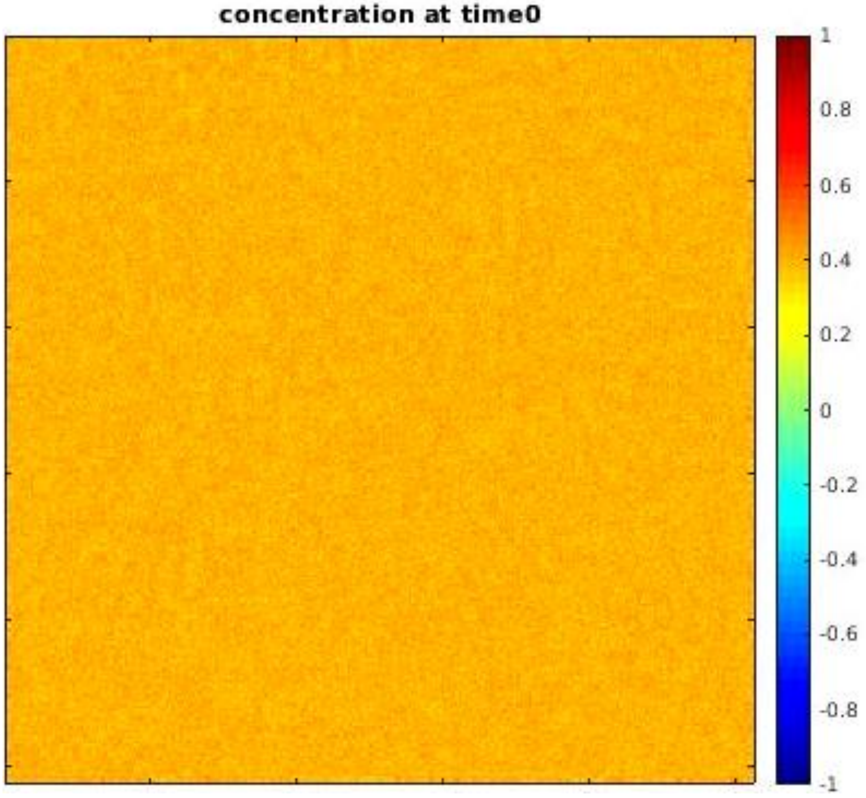
Ig 110 mg/ml PEG 10%



Da Vela et al., *Soft Matter* 2017, 13, 8756-8765



Simulation of a spinodal decomposition

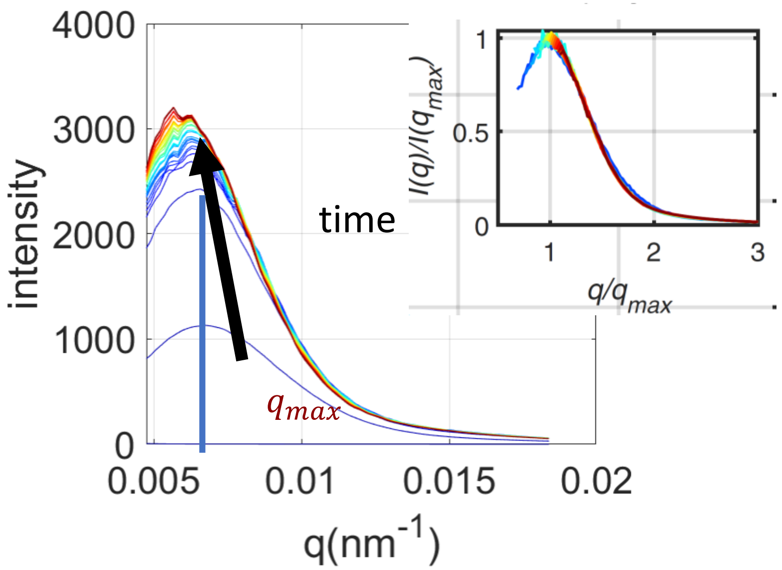


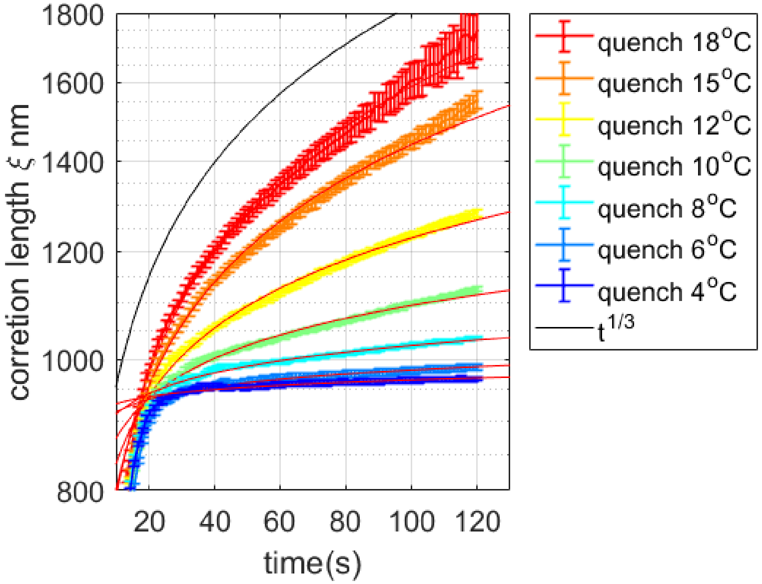
Anita Girelli et al., PRL 2021
Stefano Da Vela et al., Soft Matter 2017, 13, 8756

Protein Dynamics upon Phase Separation

Kinetics of a spinodal decomposition (small angle scattering)

$$\xi = \frac{2\pi}{q_{max}}$$

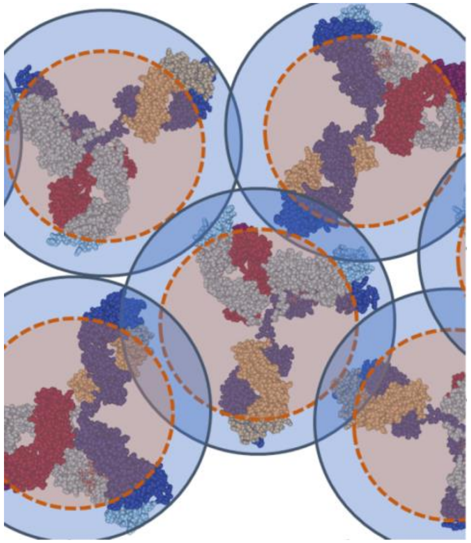




Anita Girelli et al., PRL 2021
Stefano Da Vela et al., Soft Matter 2017, 13, 8756

Protein Dynamics upon Phase Separation

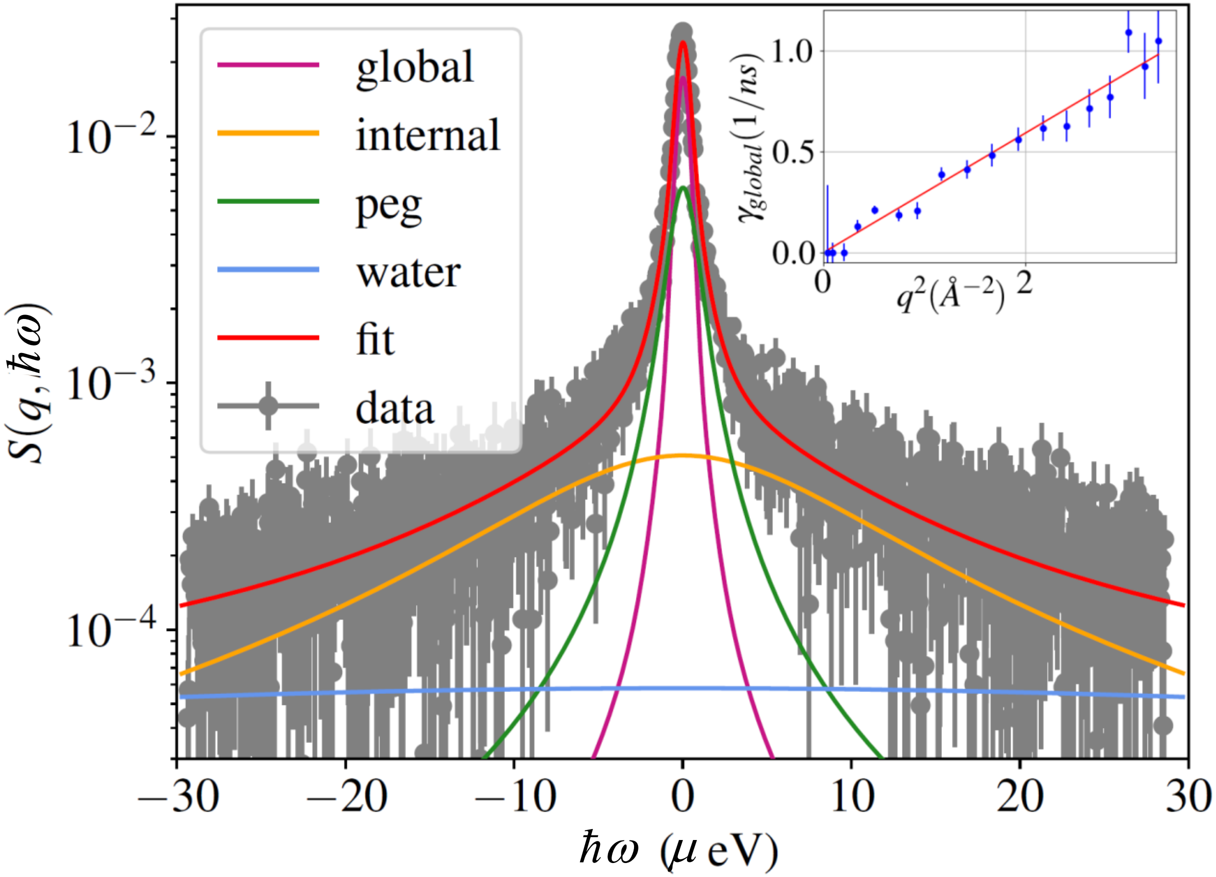
First focus on the dense phase



Anita Girelli et al., PRL 2021
Anita Girelli et al., Mol. Pharmaceutics 2021

Protein Dynamics upon Phase Separation

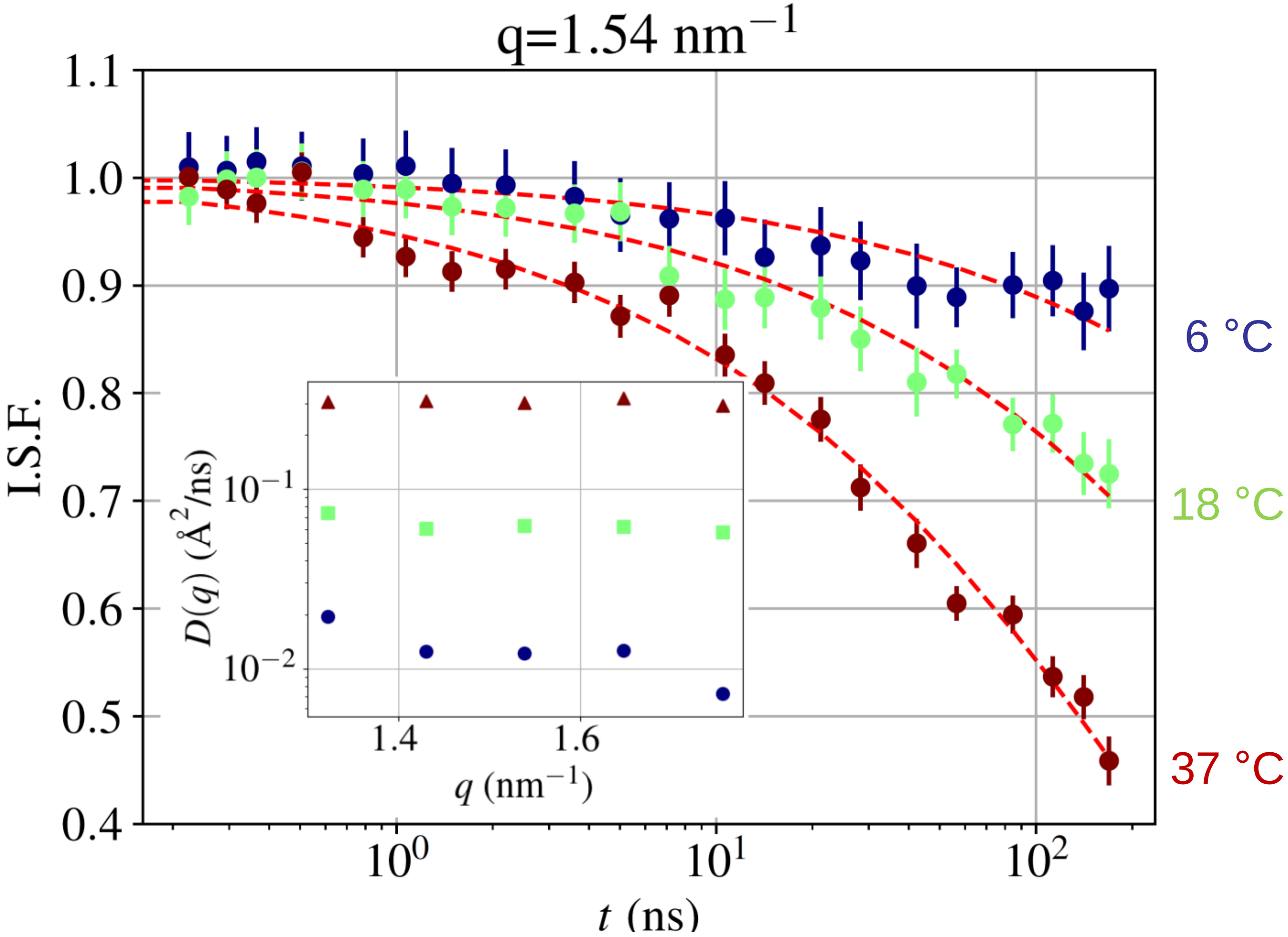
NBS (IN16B) to determine effective diffusion parameter in the dense phase



Anita Girelli et al., PRL 2021
Anita Girelli et al., Mol. Pharmaceutics 2021

Protein Dynamics upon Phase Separation

NSE (IN15) to determine effective diffusion parameter in the dense phase



Anita Girelli et al., PRL 2021
Anita Girelli et al., Mol. Pharmaceutics 2021

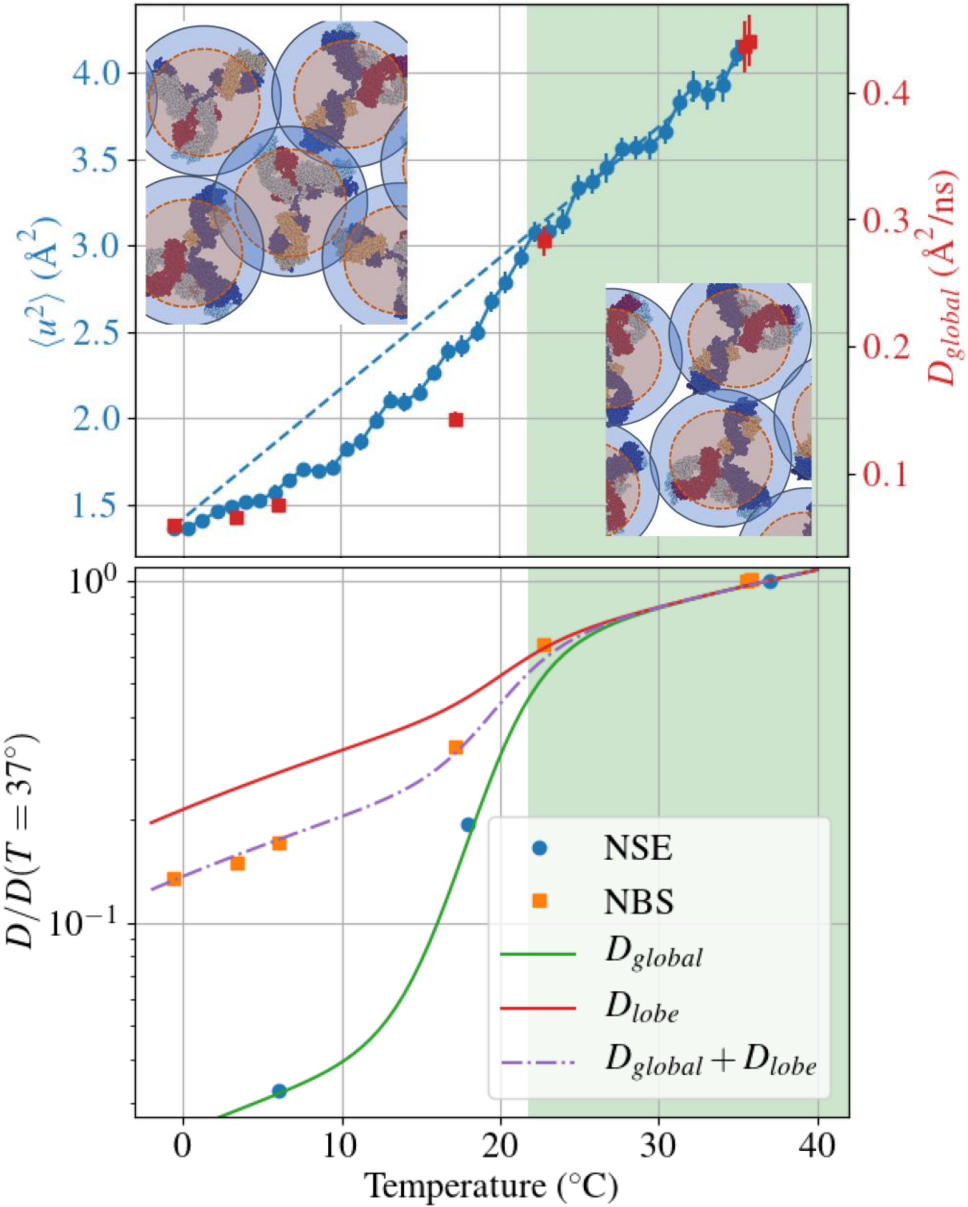
Protein Dynamics upon Phase Separation

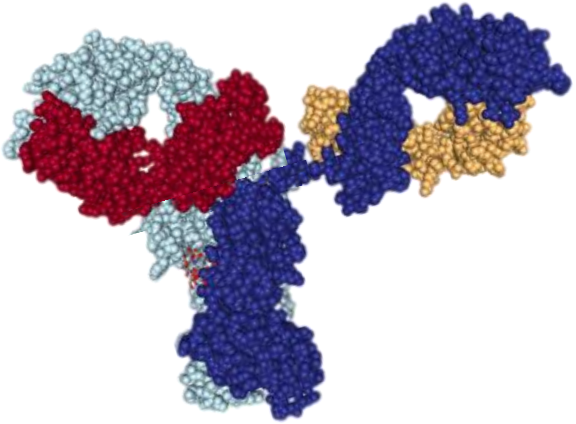
From comparison NSE and NBS:

→ *D* slows down with *T*

→ difference NSE vs NBS suggests *local* motion preserved (different length scales; long for NSE, more local for NBS):

→ i.e. antibody flexibility in principle preserved even under crowded conditions





Anita Girelli et al., PRL 2021
Anita Girelli et al., Mol. Pharmaceutics 2021

Bonus Track

Protein Dynamics: Time and Length Scales

Reiser et al., Nature Comm 2022

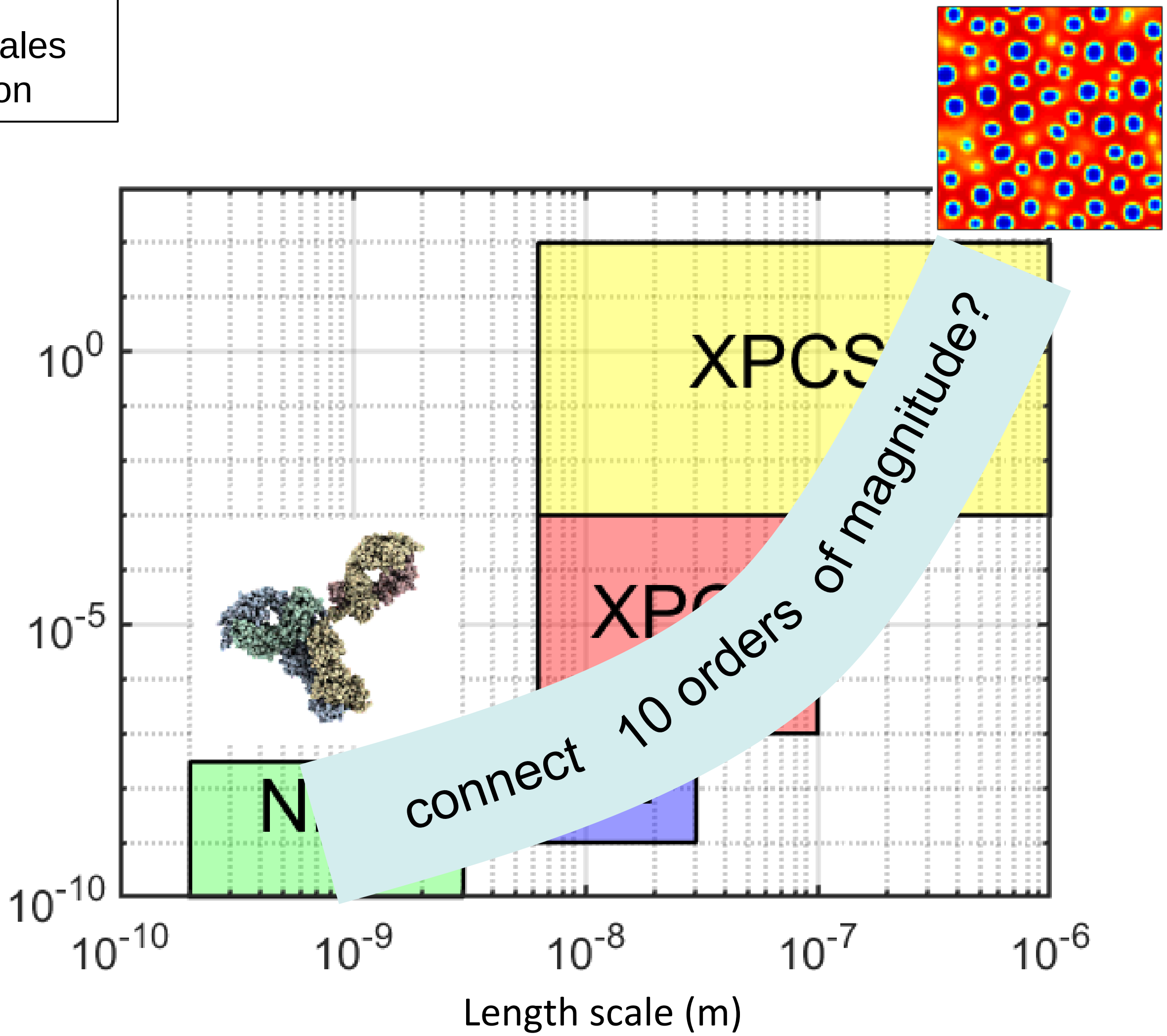
Grimaldo et al., Q. Rev. Biophys. 2019, 52, e7, 1–63

Girelli et al., PRL 2021

Protein Dynamics: Time and Length Scales

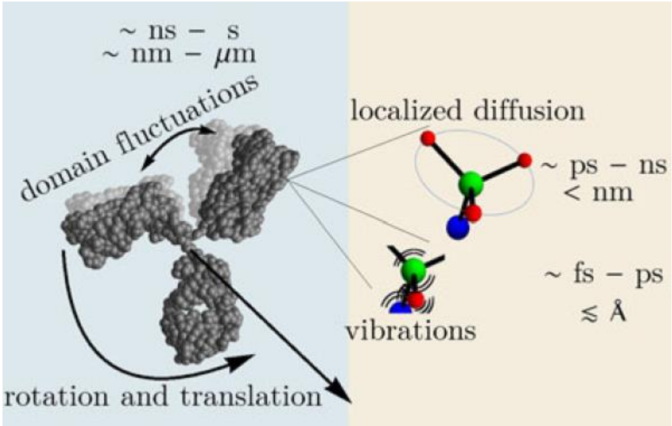
Generally complex task
many different time and length scales
requiring advanced instrumentation

Dynamics of domains (~ μm)



connect 10 orders of magnitude?

Dynamics at molecular level (~nm)



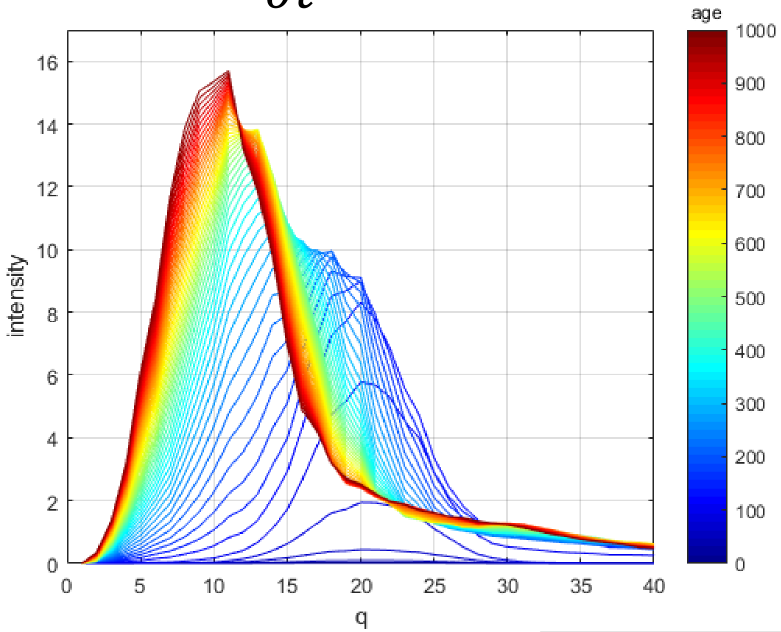
Reiser et al., Nature Comm 2022

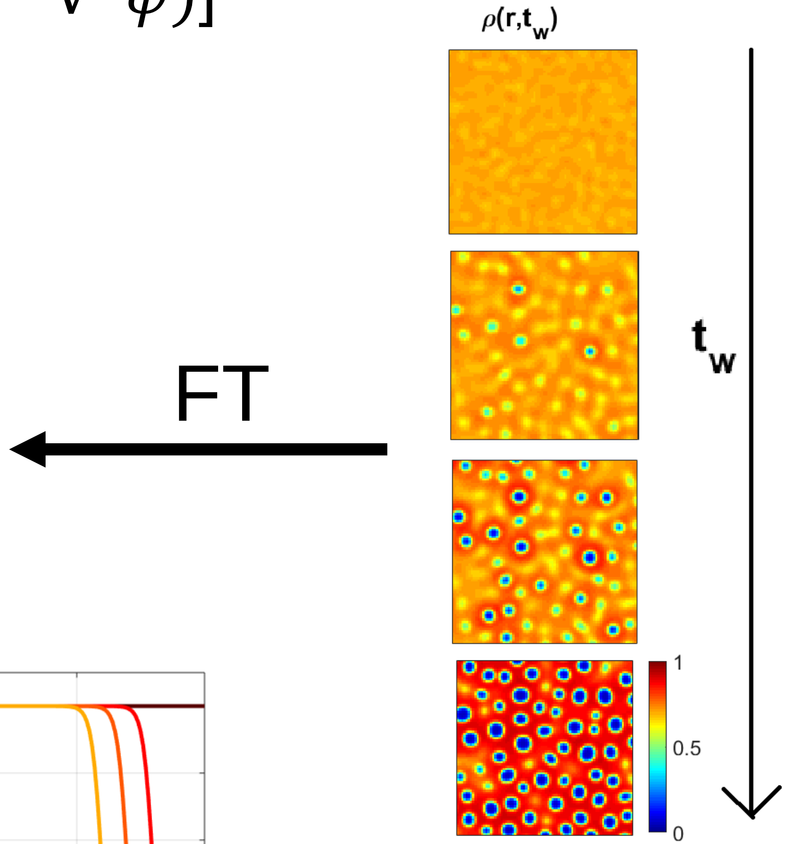
Grimaldo et al., Q. Rev. Biophys. 2019, 52, e7, 1–63
Girelli et al., PRL 2021

Protein Dynamics upon Phase Separation

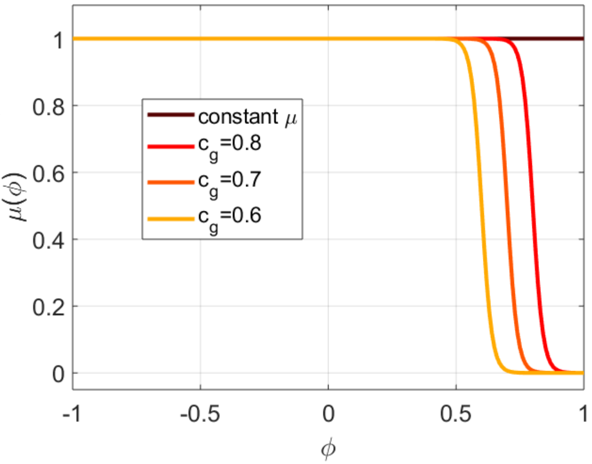
Simulations based on Cahn-Hilliard equation

$$\frac{\partial\psi}{\partial t} = \nabla[\mu\nabla(\epsilon\psi - \psi^3 - \nabla^2\psi)]$$





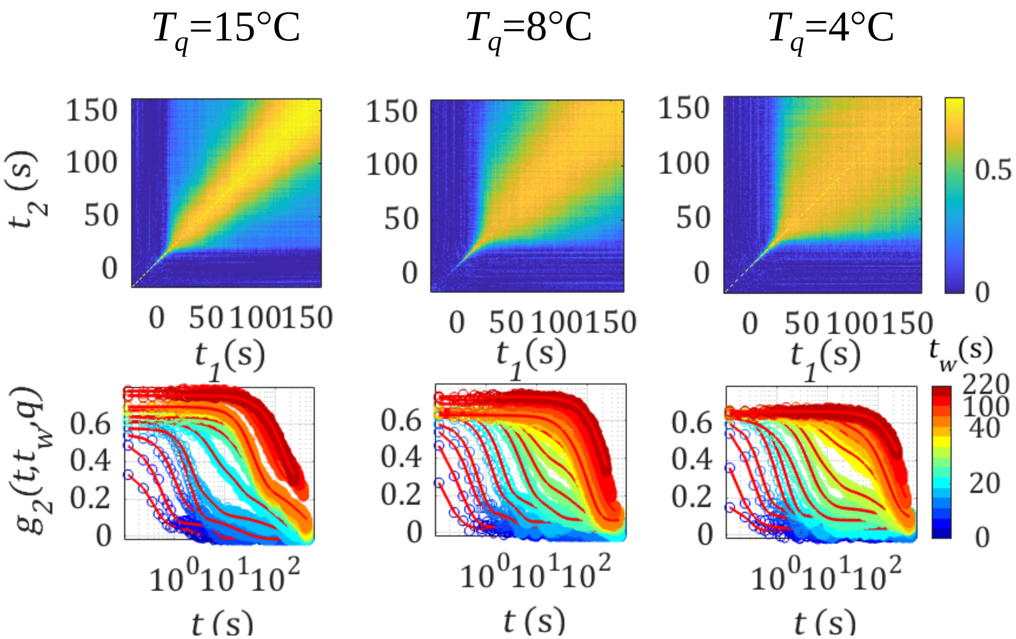
Mobility parameter μ depends on concentration ϕ

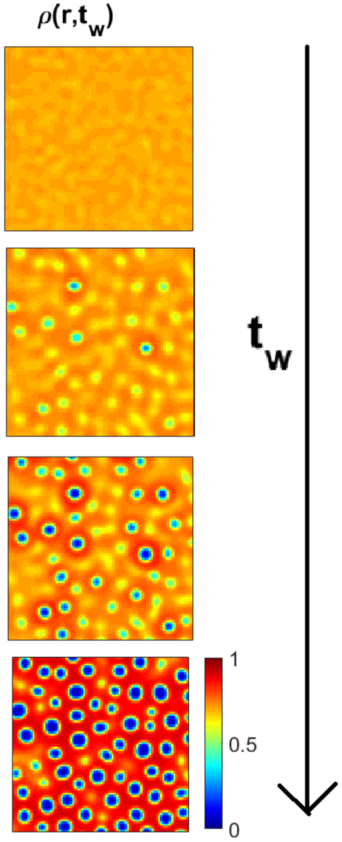


Anita Girelli et al., PRL 2021

Protein Dynamics upon Phase Separation

XPCS data @ $q = 0.005\mathrm{nm}^{-1}$



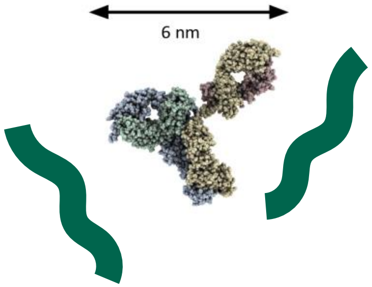


- Cahn-Hilliard simulations of phase separation
- Kinetics at low T reproduced with strong difference in mobility between phases
- Dynamics reproduced only partially (viscoelastic effects?)

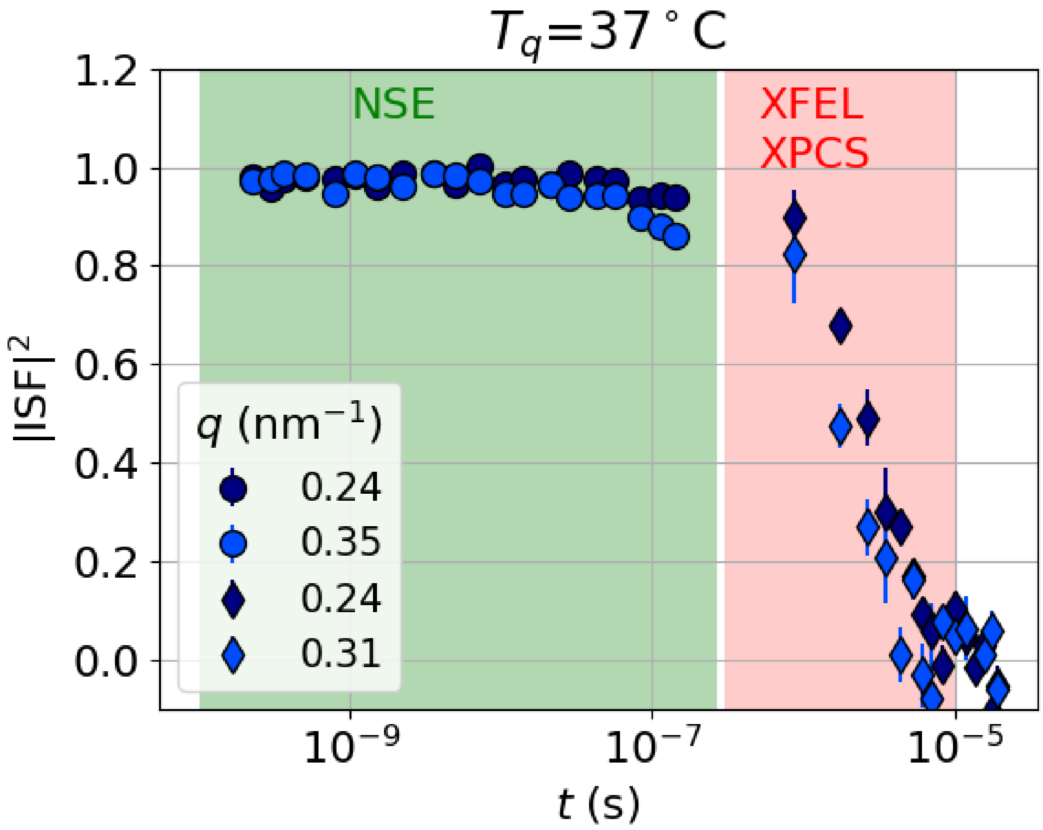
Anita Girelli et al., PRL 2021

Bonus Track

Bonus Track: Connecting NSE with XPCS



now in one-phase region at high T
for the same *q* for XPCS and NSE!



Connecting NSE with XPCS works

- Dynamics resolved with NSE and XFEL-XPCS
- Consistent with Brownian motion

Anita Girelli et al., PRL 2021
Mario Reiser et al., Nature Comm. 2022

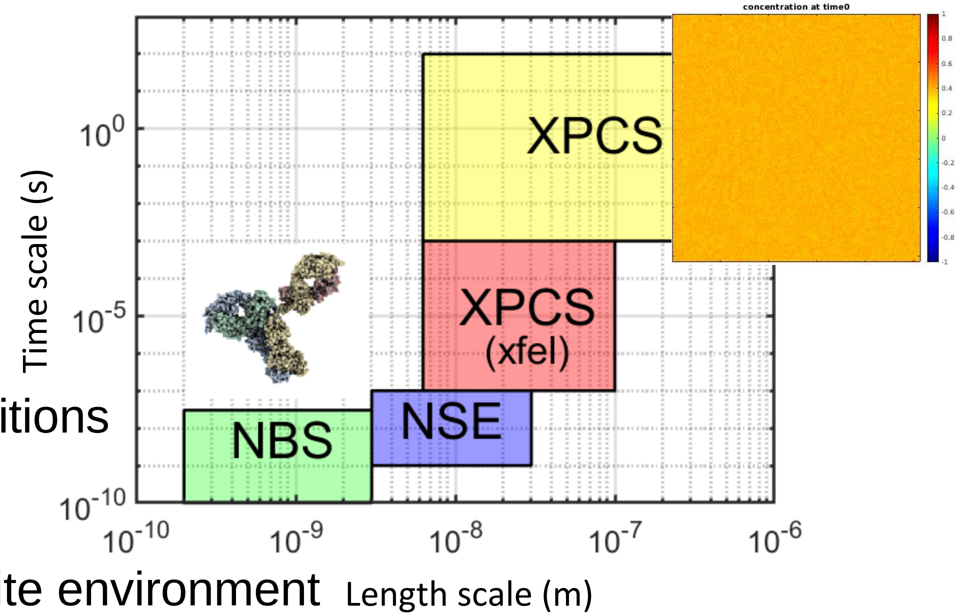
Conclusions

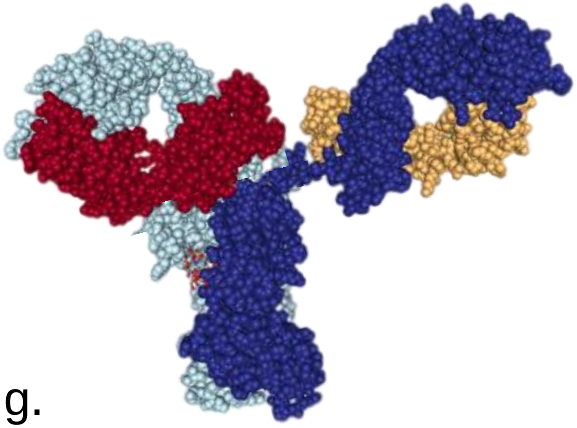
- For a holistic understanding of complex protein solutions, study structure *and* dynamics

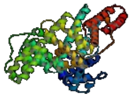
- Dependence on environment and conditions

- Specific example of IgG/PEG
  - Antibody flexibility preserved despite environment
  - Difference in NBS and NSE
  - Short-time self-diffusion not strongly affected by arrest of the phase separation
  - Phase separation described by Cahn-Hilliard with difference in mobility of the two phases
  - Phase separation kinetics and dynamics followable "over 10 orders of magnitude" ?

- For complete picture, need more than one technique, e.g. SANS / NBS / NSE / XPCS / rheo etc.
- SANS & QENS
- Need NSE @ ESS !







Felix Roosen-Runge et al., PNAS 2011
Marco Grimaldo et al., Q. Rev. Biophys. 2019
Mario Reiser et al., Nature Comm. 2022

Nafisa Begam et al., PRL 2021
Anita Girelli et al., PRL 2021
Anita Girelli et al., Mol. Pharmaceutics 2021