istituto
dei sistemi
complessi

SAPIENZA
UNIVERSITÀ DI ROMA

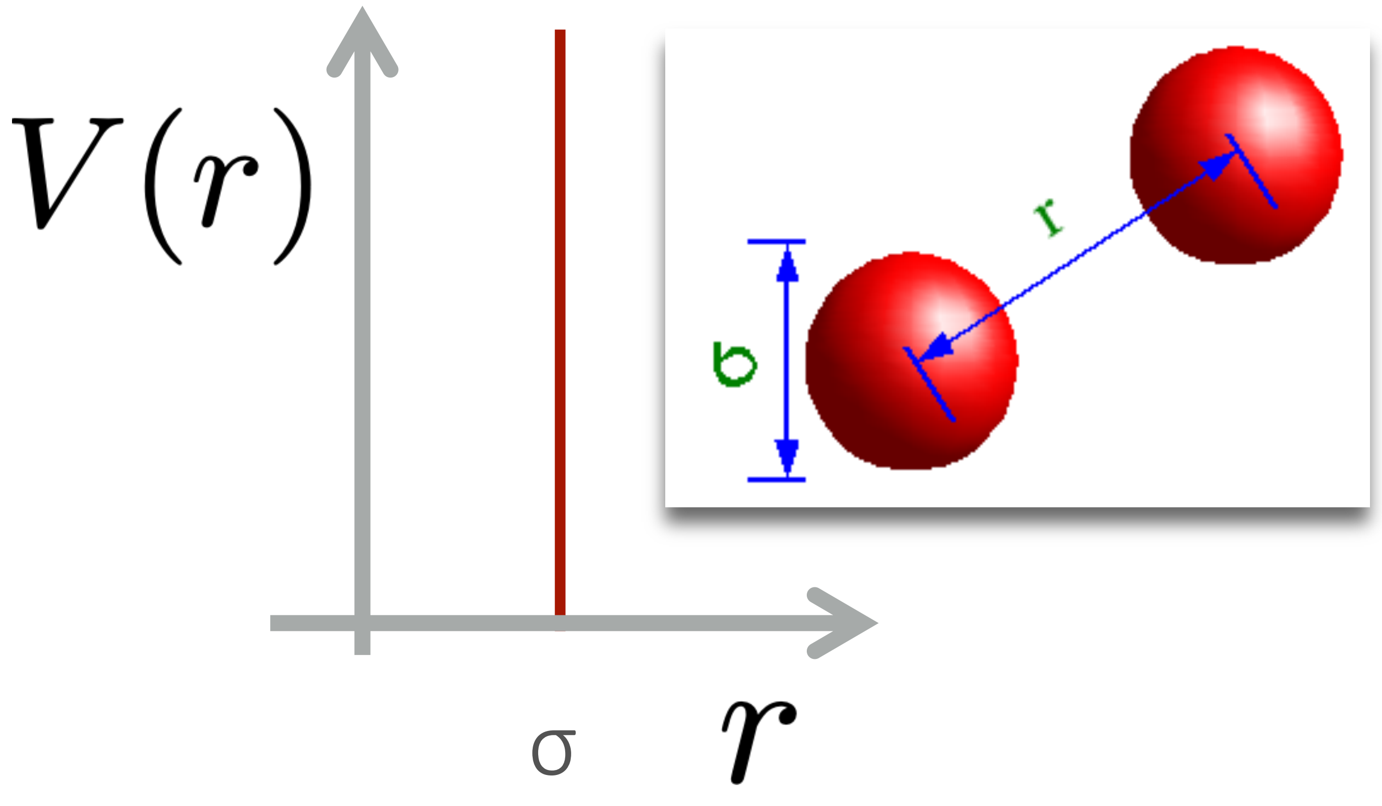
PROTEIN PHASE DIAGRAMS

Emanuela Zaccarelli

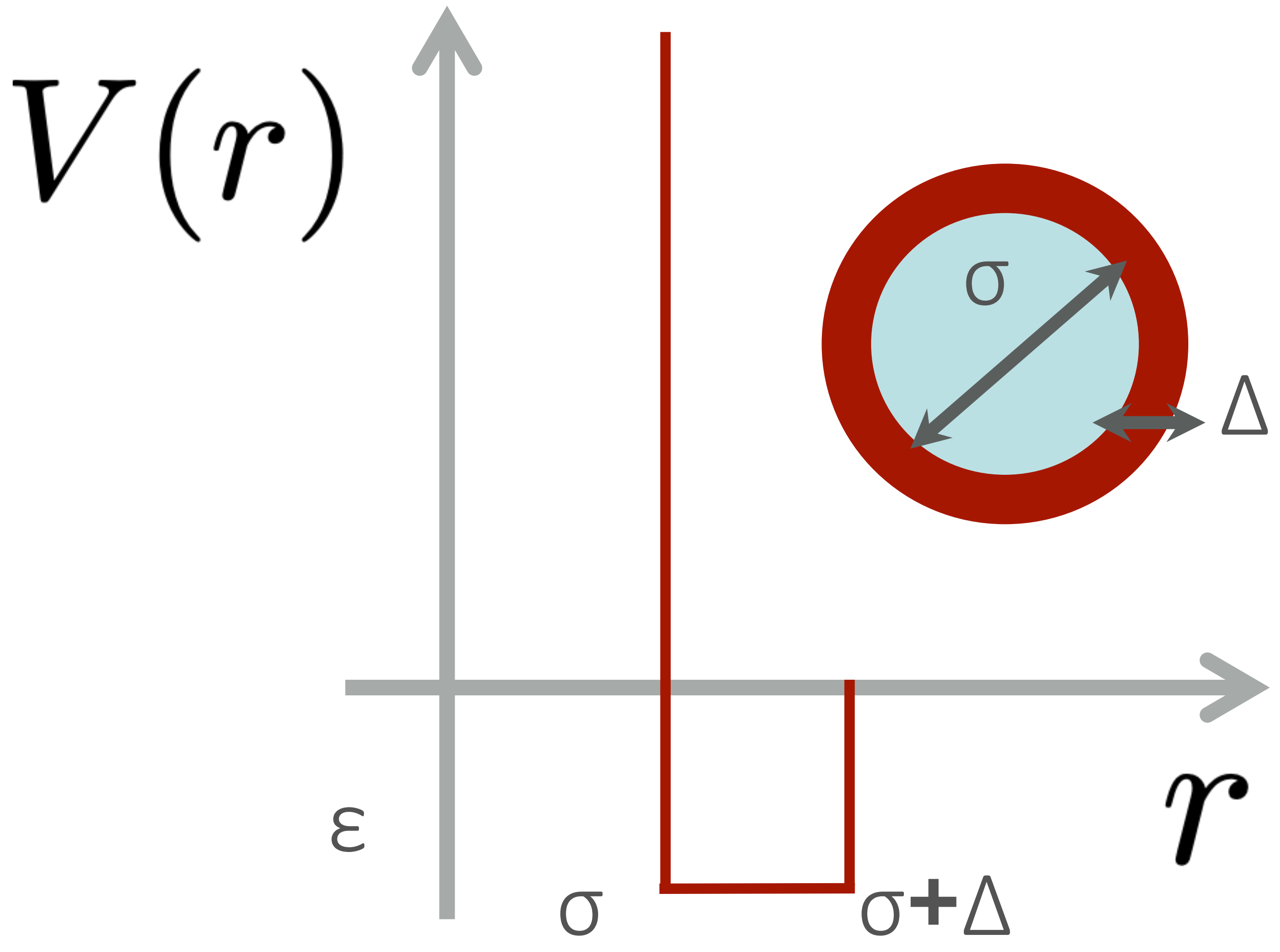
CNR Institute for Complex Systems and
Department of Physics, Sapienza University, Rome

erc

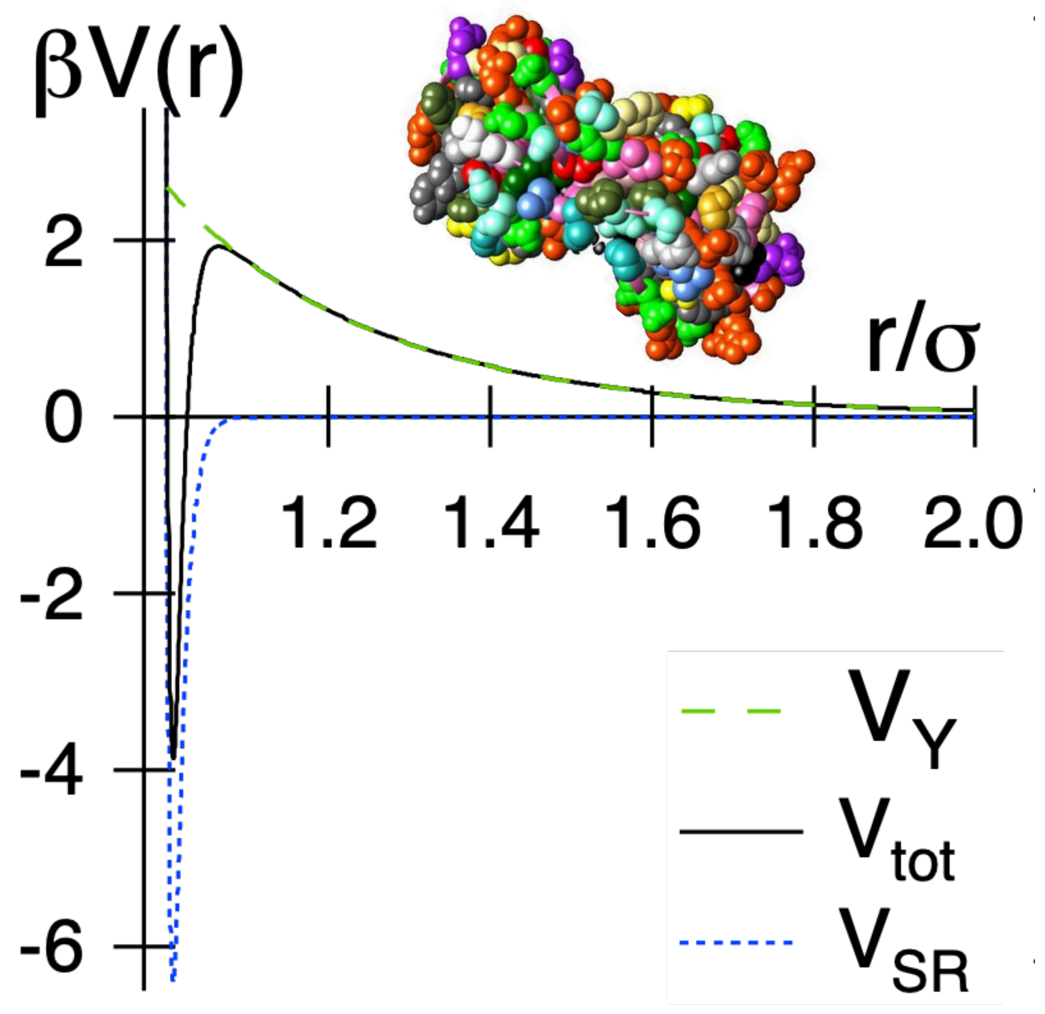
Soft Matter models I have enjoyed working with…



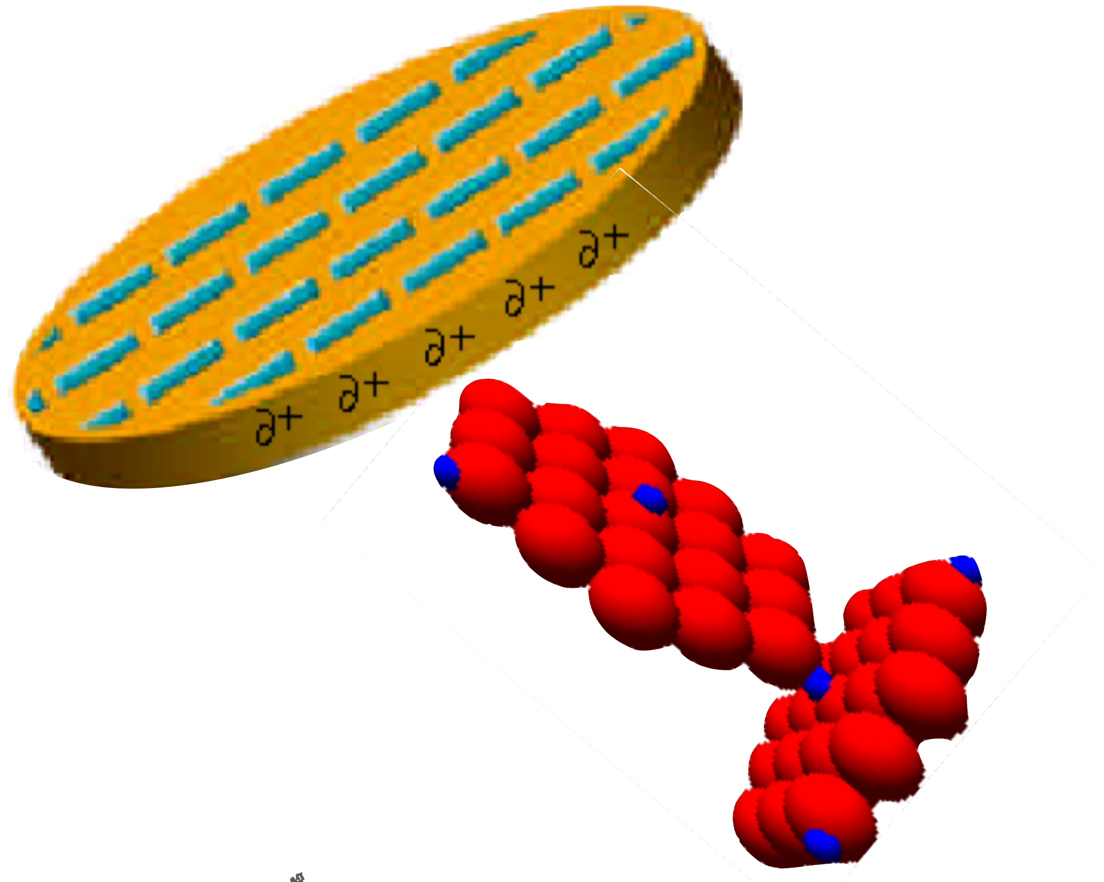
hard spheres



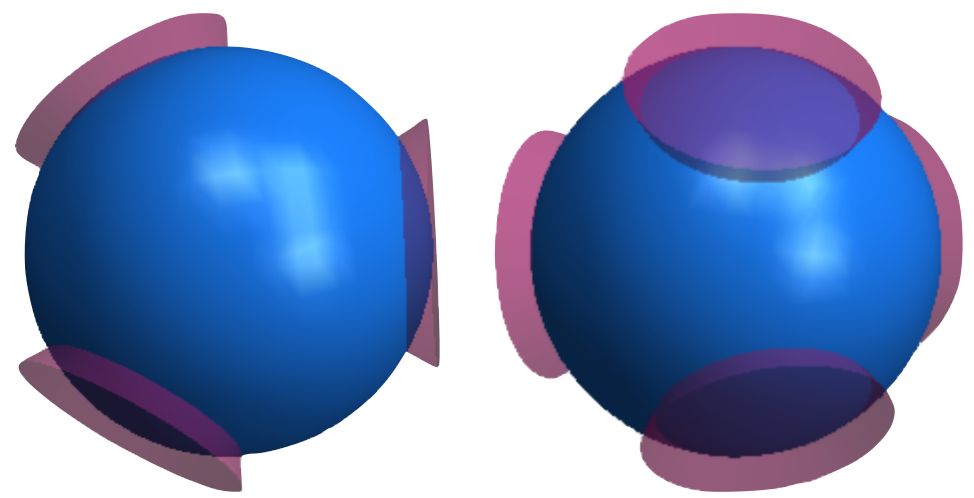
attractive colloids



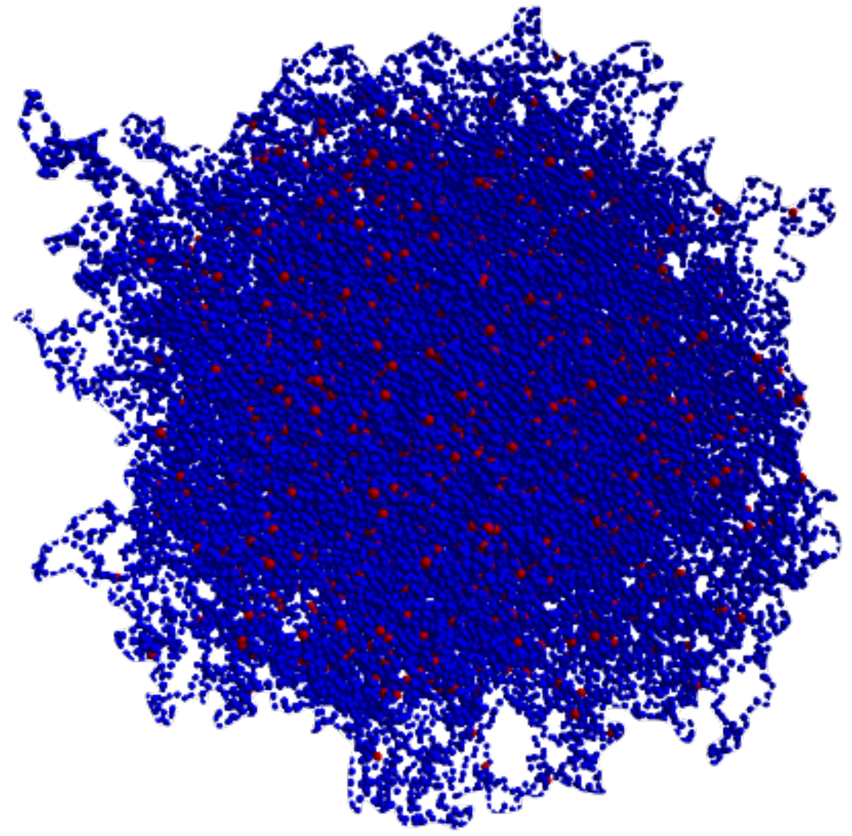
globular proteins



clays



patchy particles

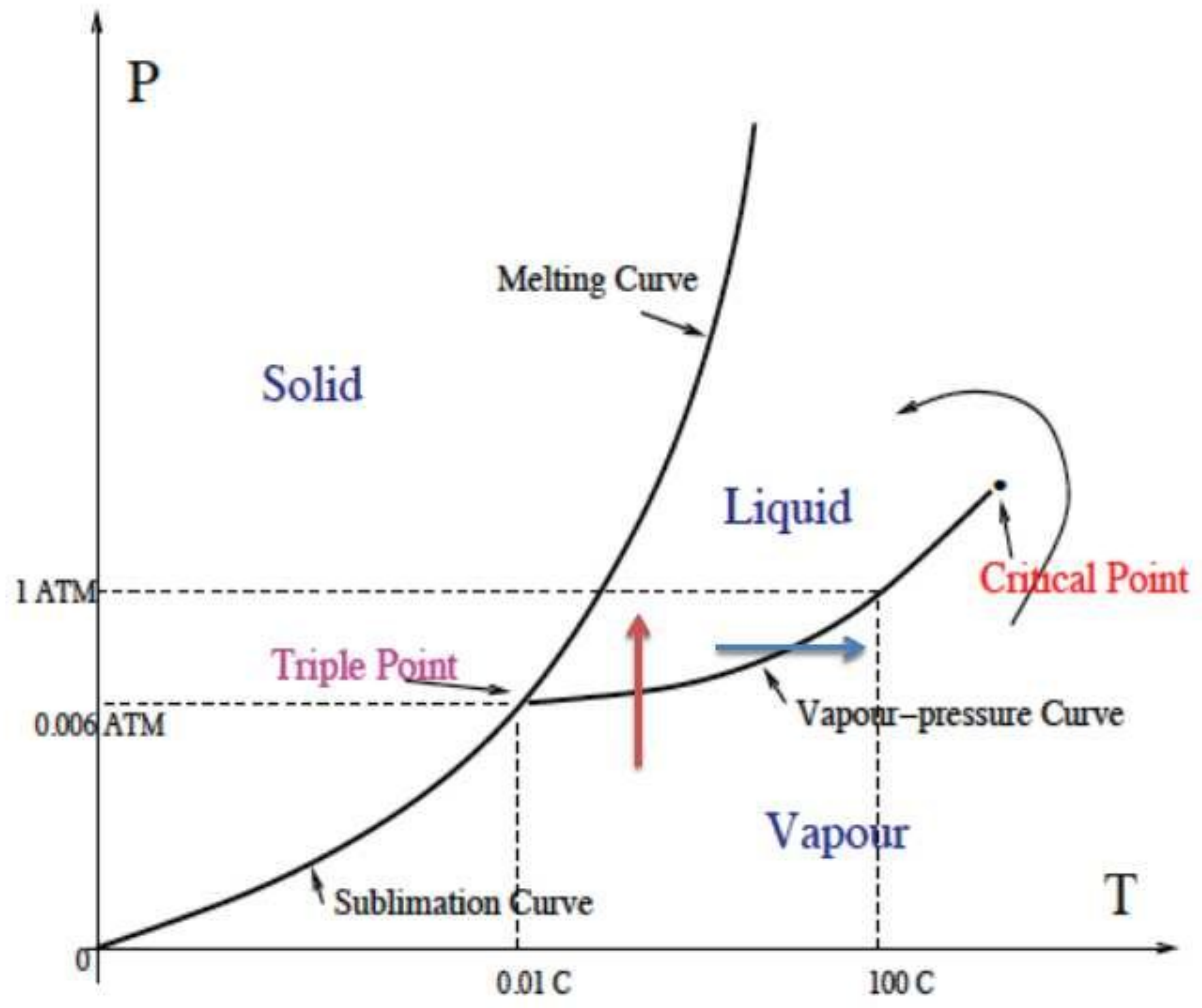


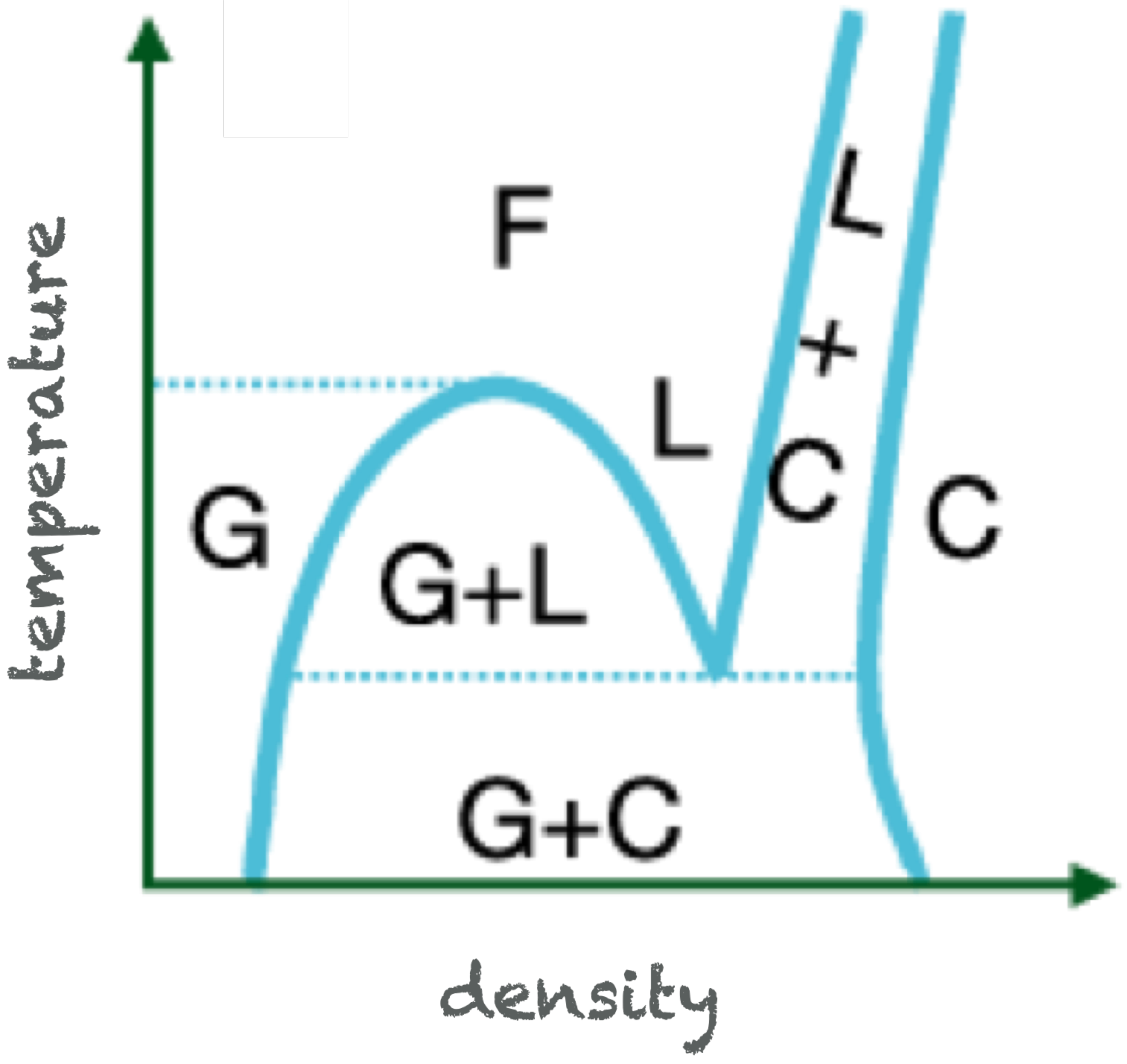
microgels, star polymers

Phase diagrams

maps of (thermodynamic) stability

atomic/molecular liquids

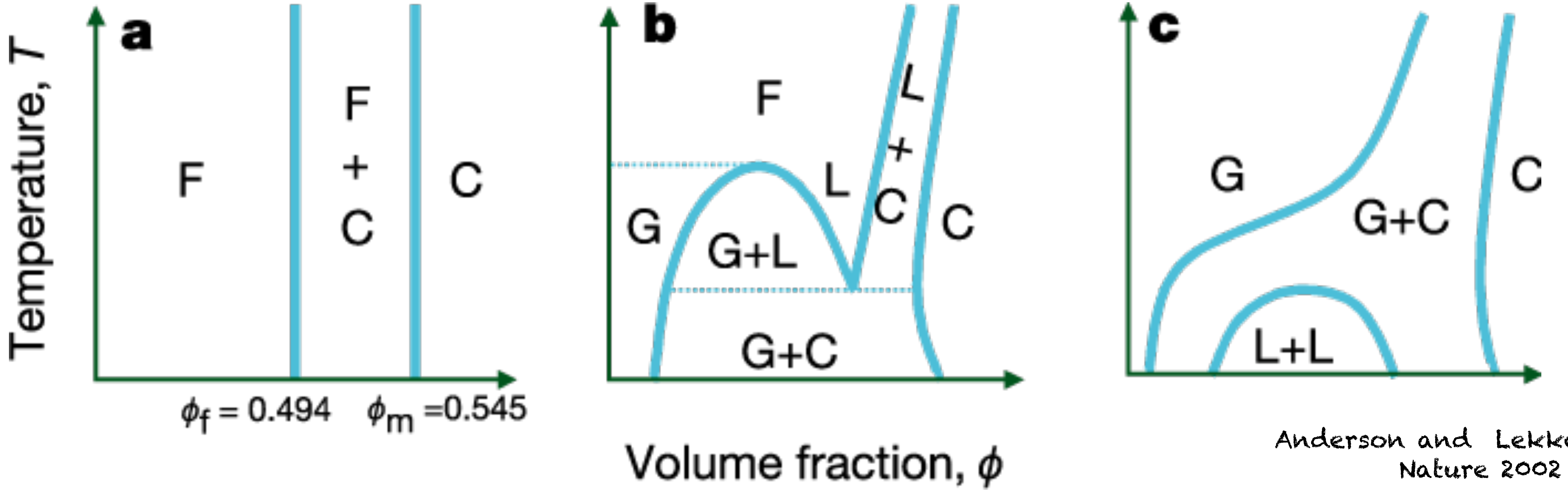




Colloidal phase diagrams

hard spheres

short-range attractions

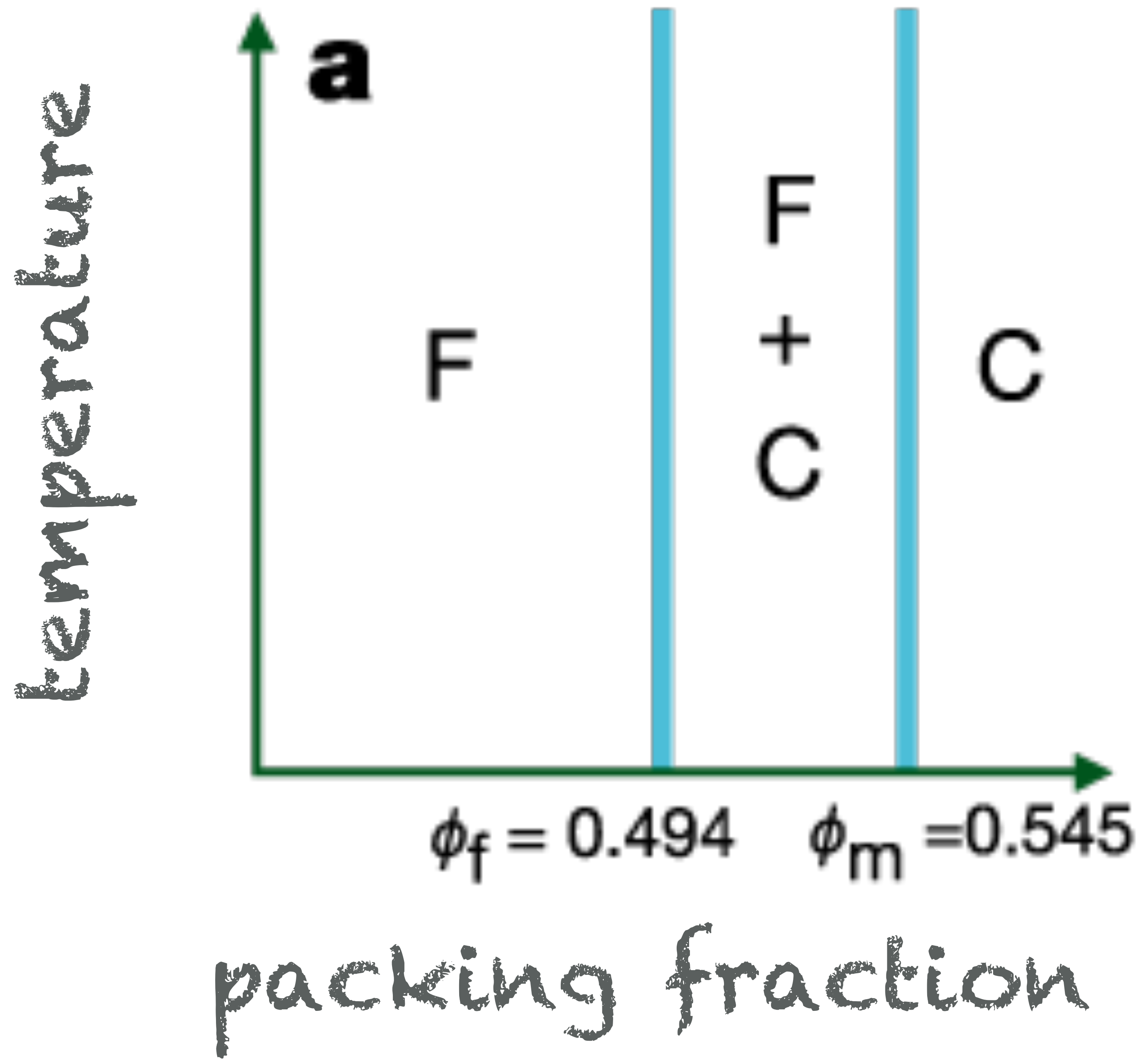


Anderson and Lekkerkerker
Nature 2002

(atomic/molecular liquids)

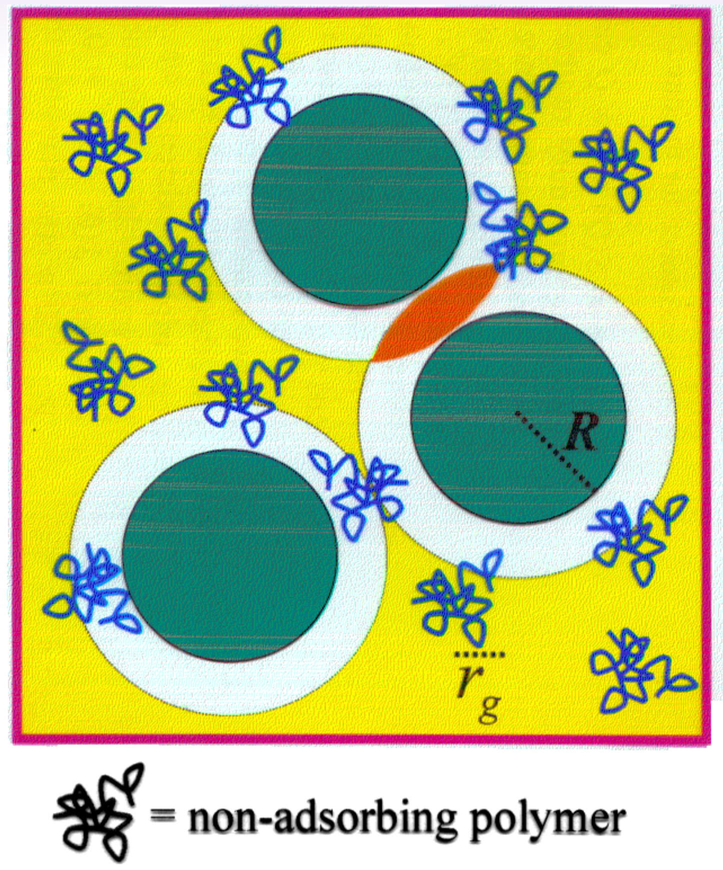
colloids with long-range attraction

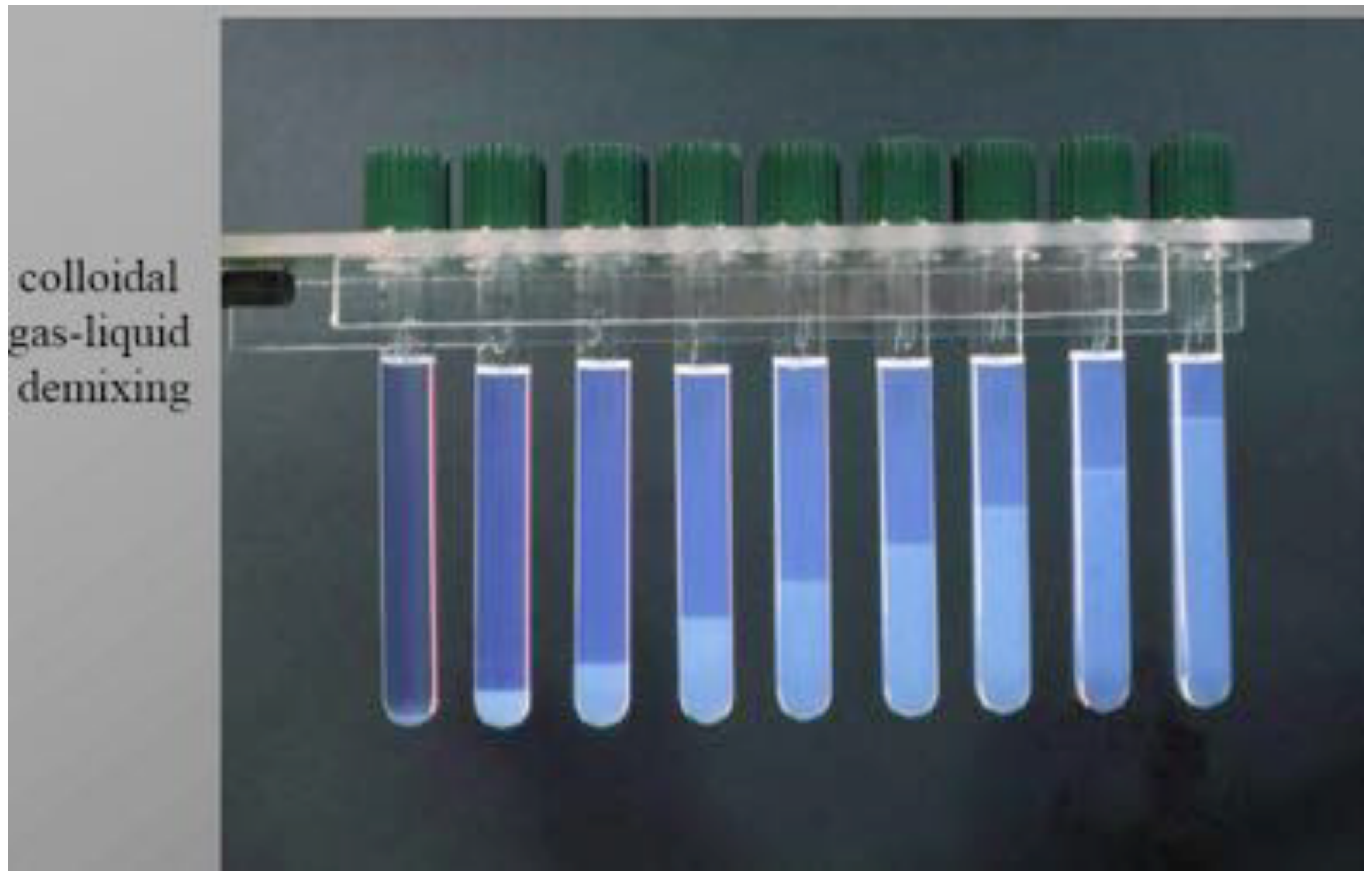
Hard spheres

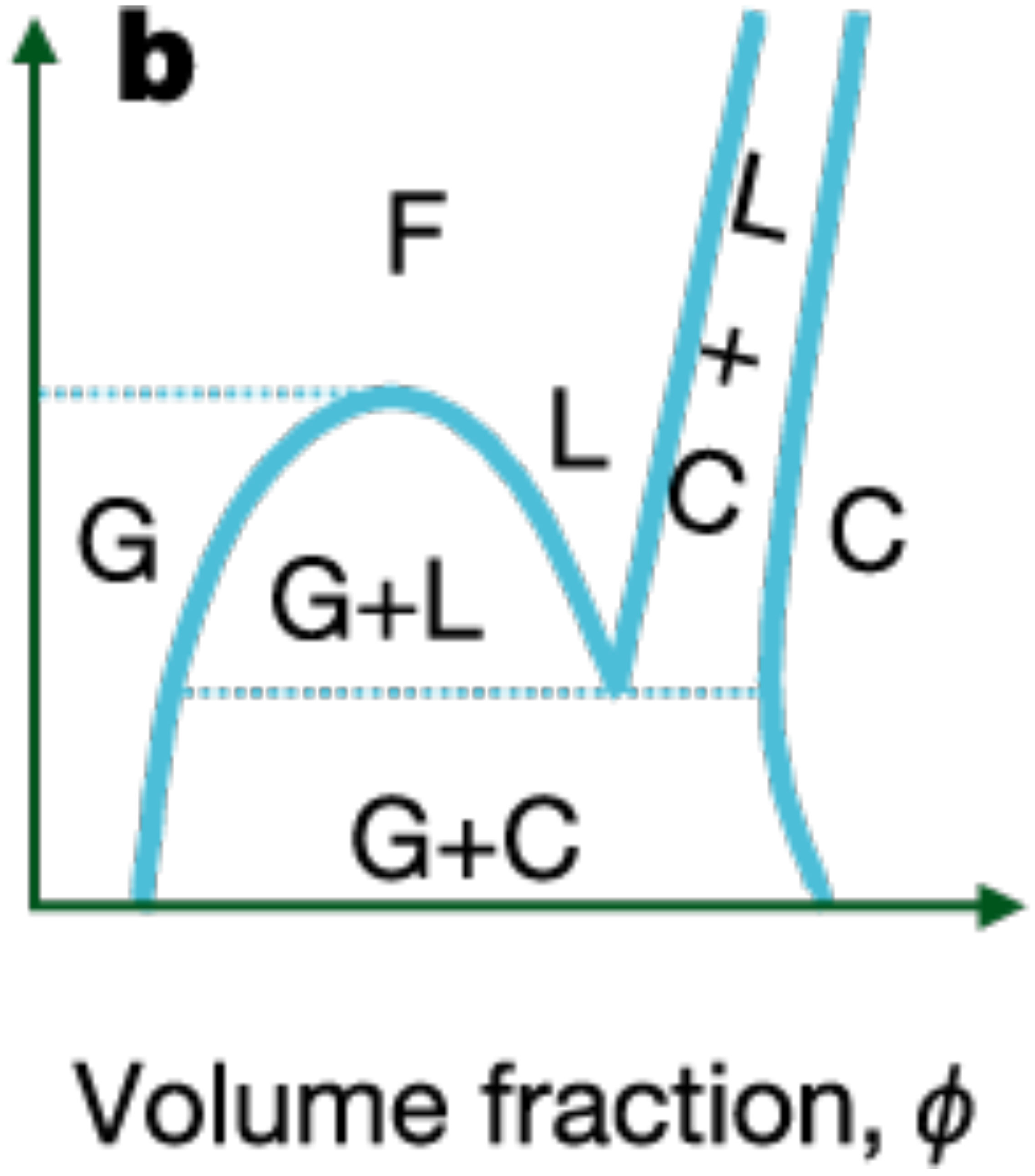


Peter Pusey & Bill Van Megen Nature 1986

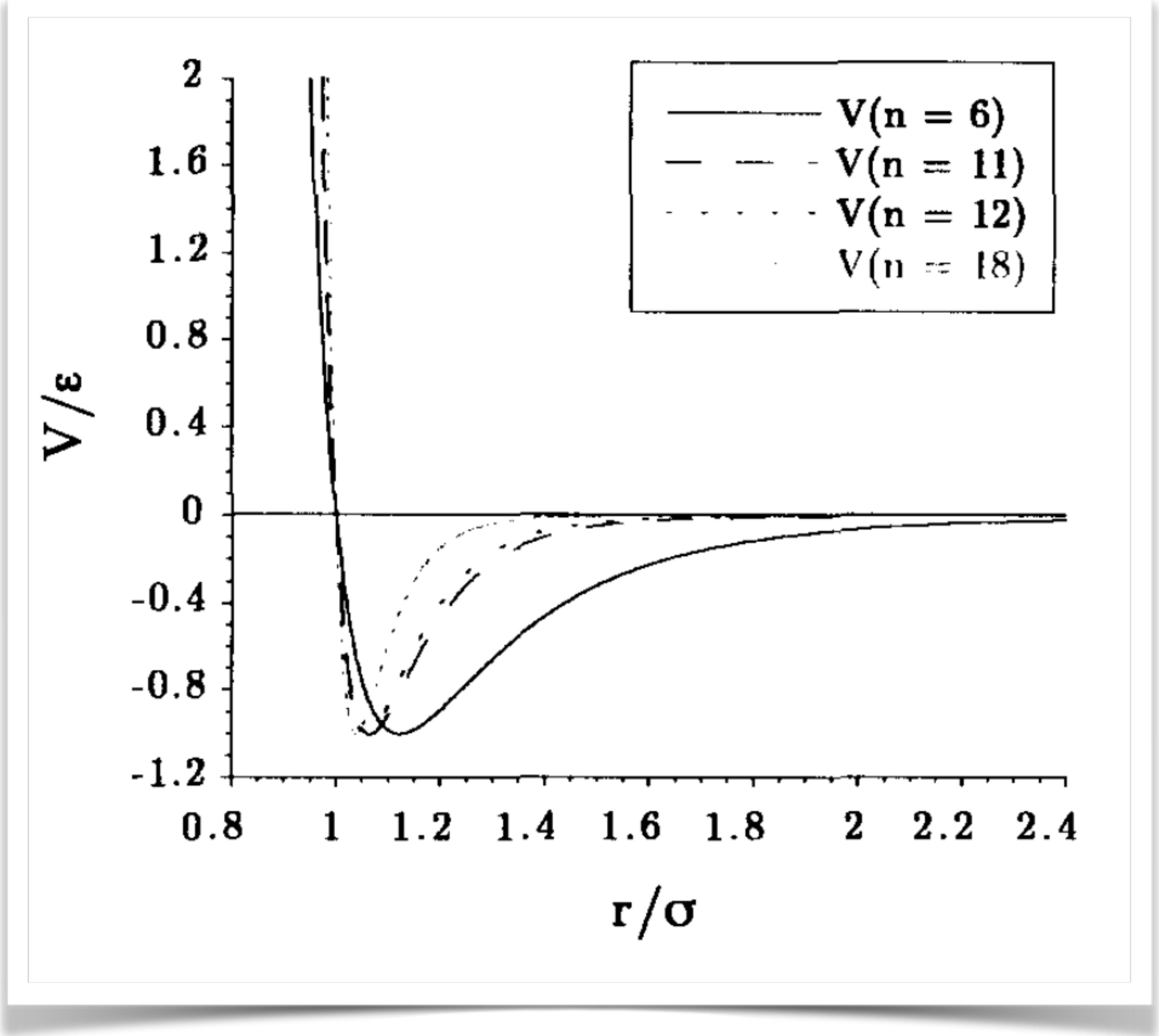
Attractive colloids

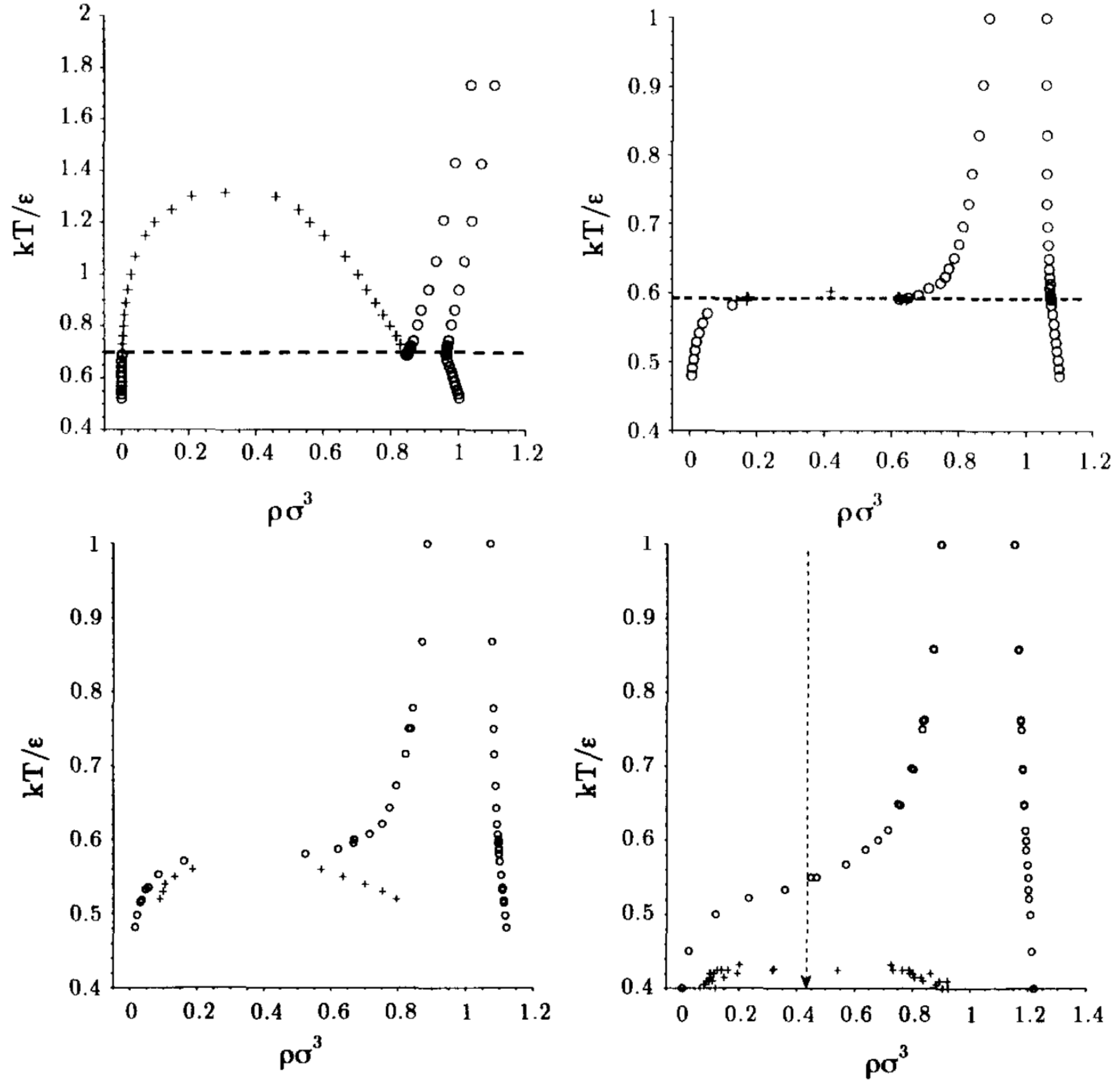






Effect of attraction range on phase diagram



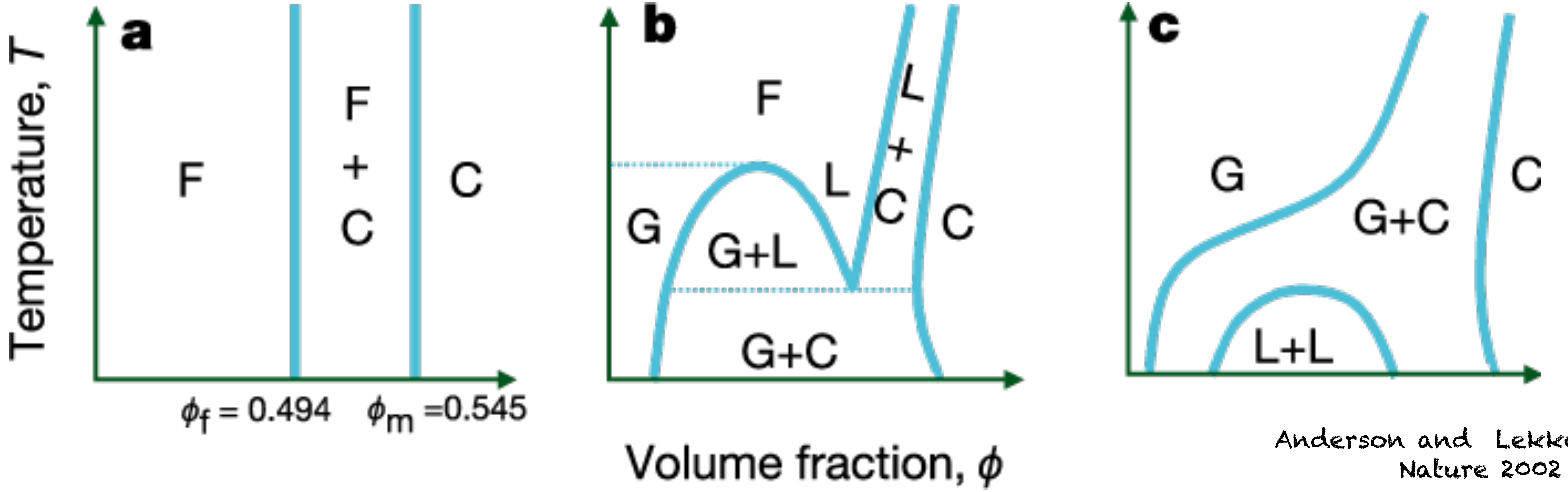


Vliegenthart and Lekkerkerker
Physica A 1999

Colloidal phase diagrams

hard spheres

short-range attractions



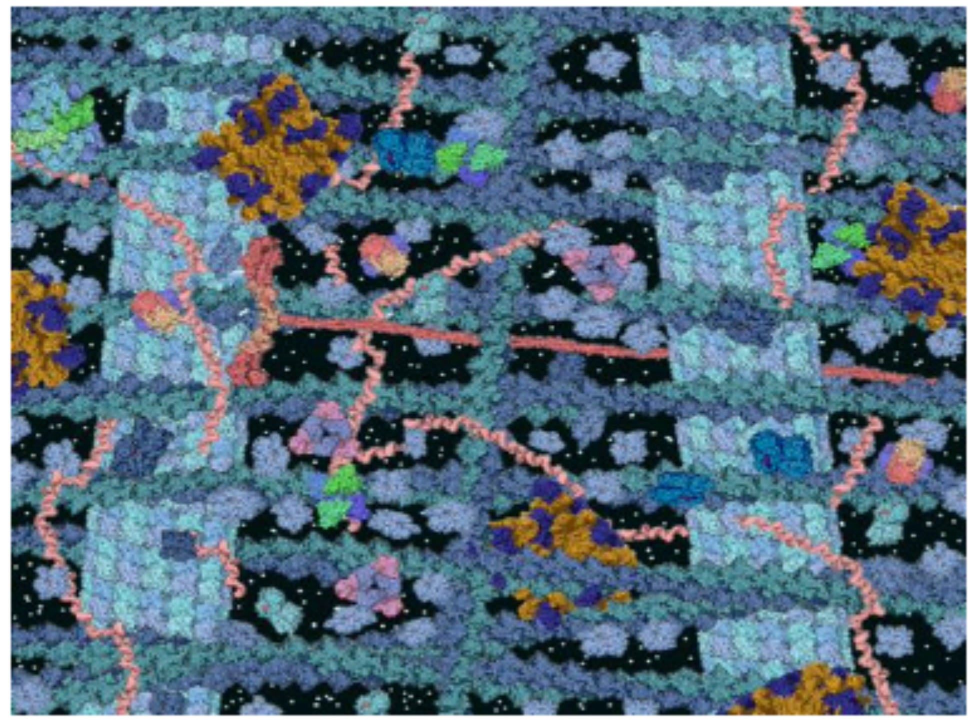
Anderson and Lekkerkerker
Nature 2002

(atomic/molecular liquids)

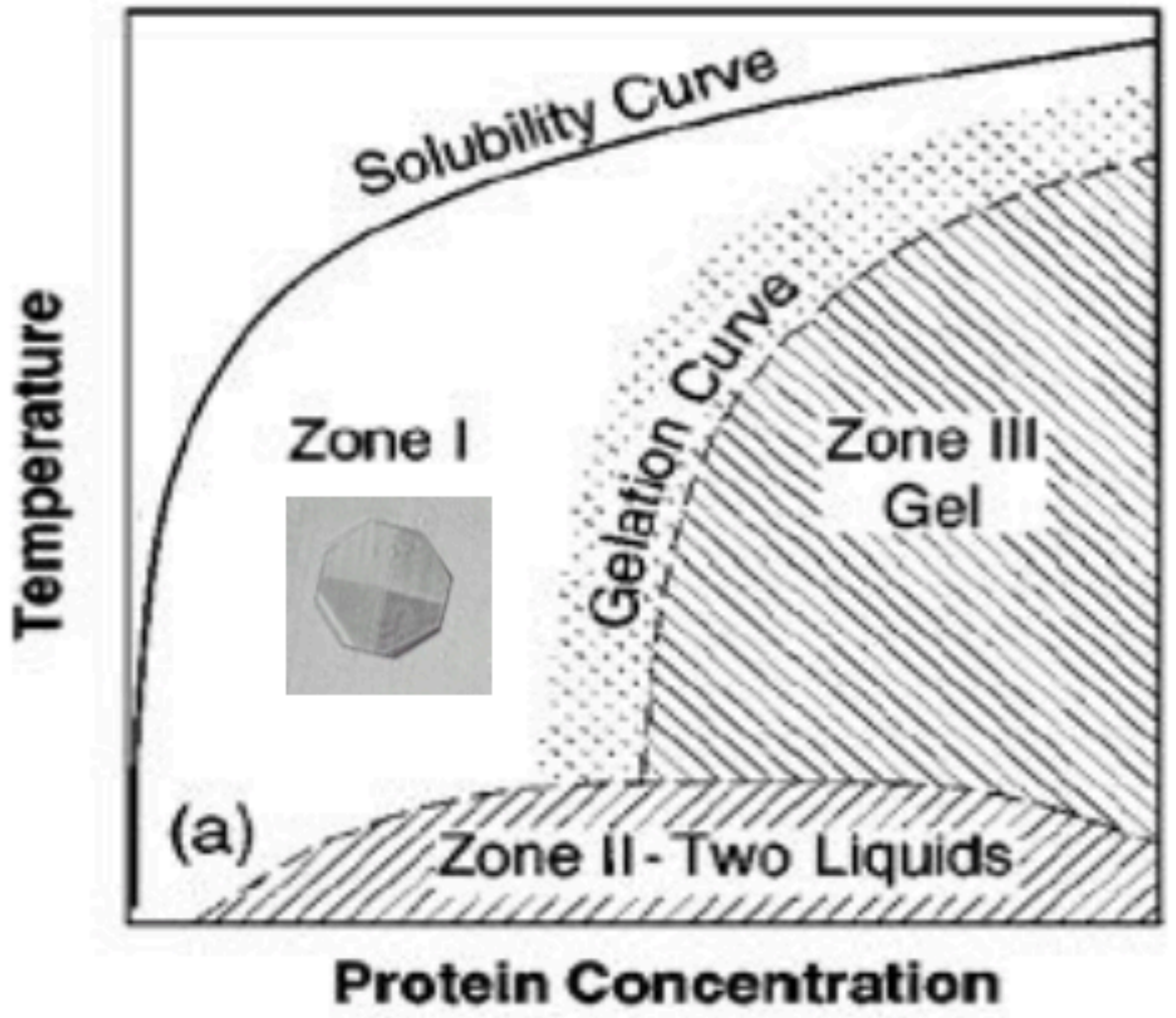
colloids with long-range attraction

Why are we interested in phase transitions in proteins?

Protein crowding and the cytosol stability

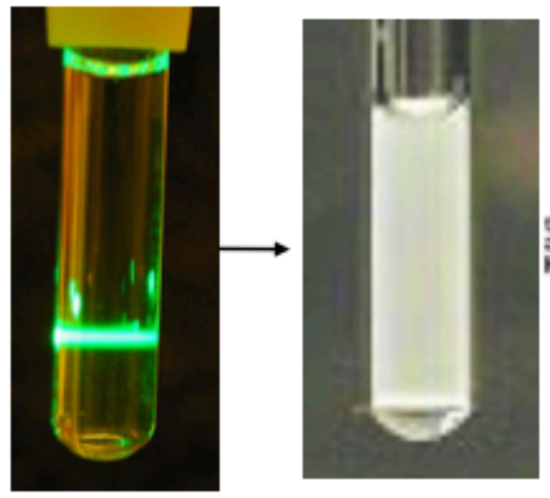


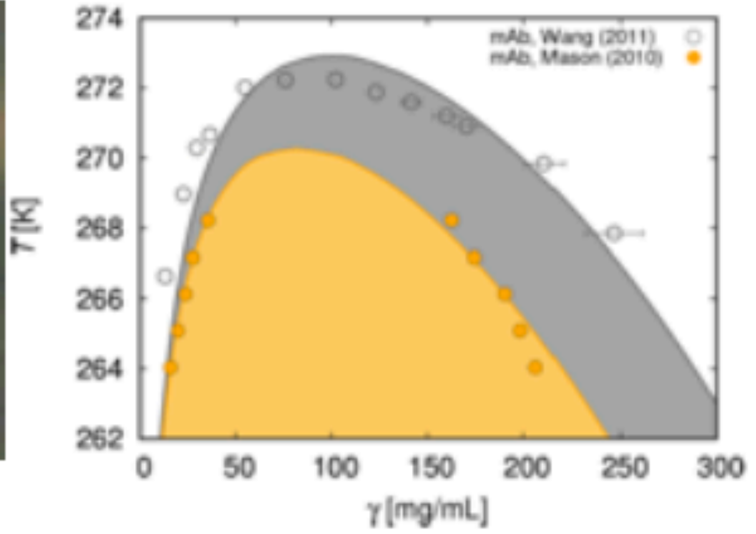
Optimal conditions for crystallization



Phase transitions in proteins

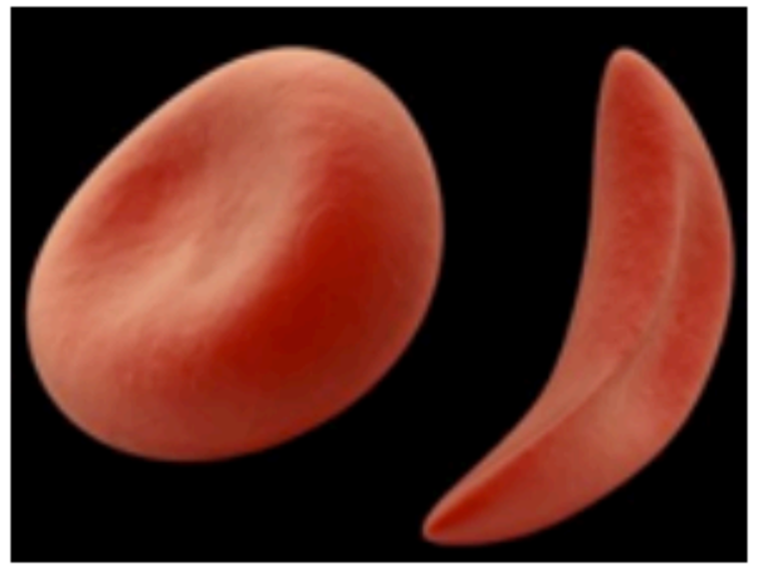
Concentrated formulations





Understanding protein condensation disease

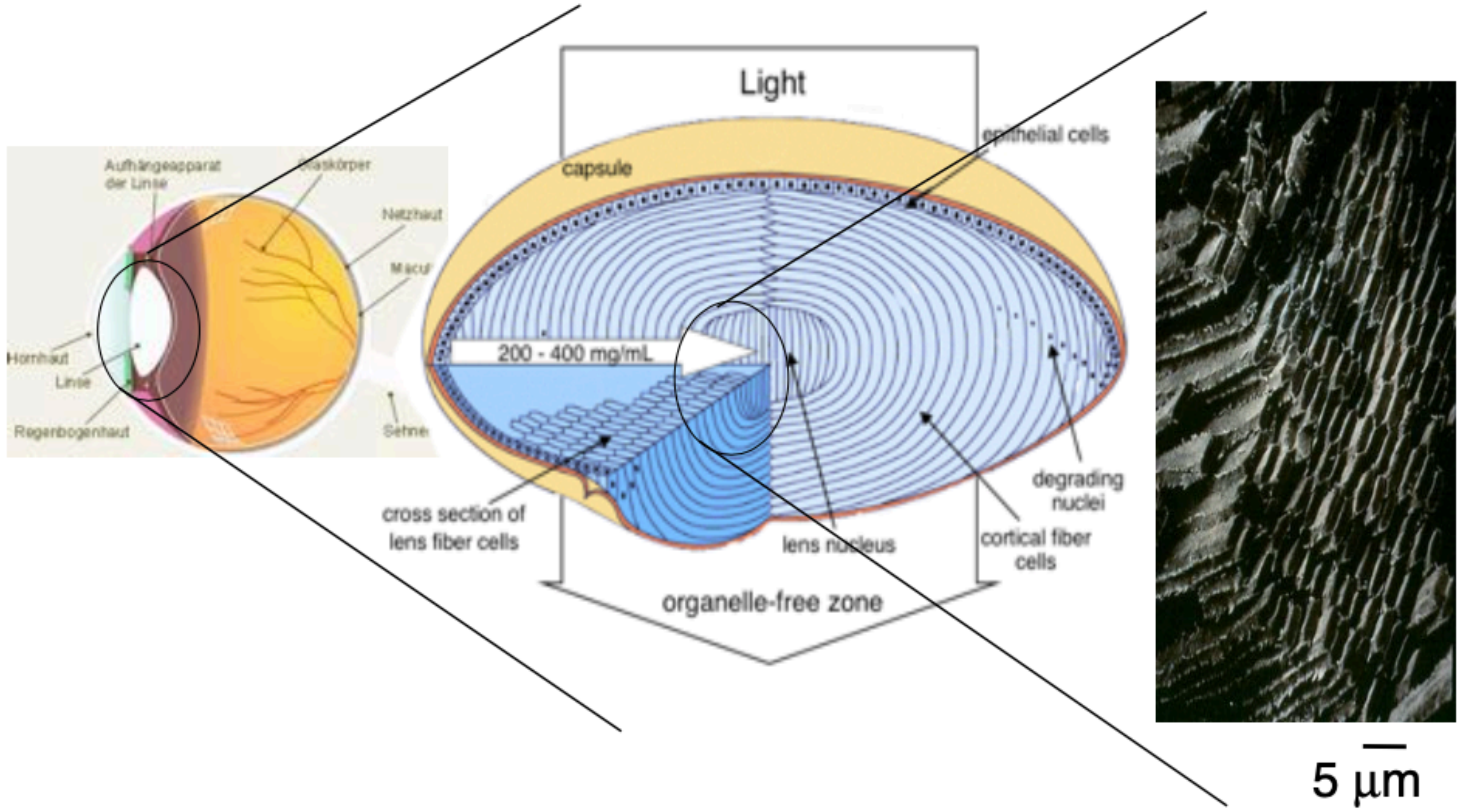
Cataract



Sickle Cell Disease

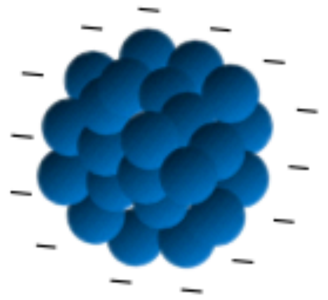
Peter Schurtenberger

The eye lens



The fiber cells consist of a highly concentrated protein solution:

Alpha-crystallins:



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

Beta-crystallins:

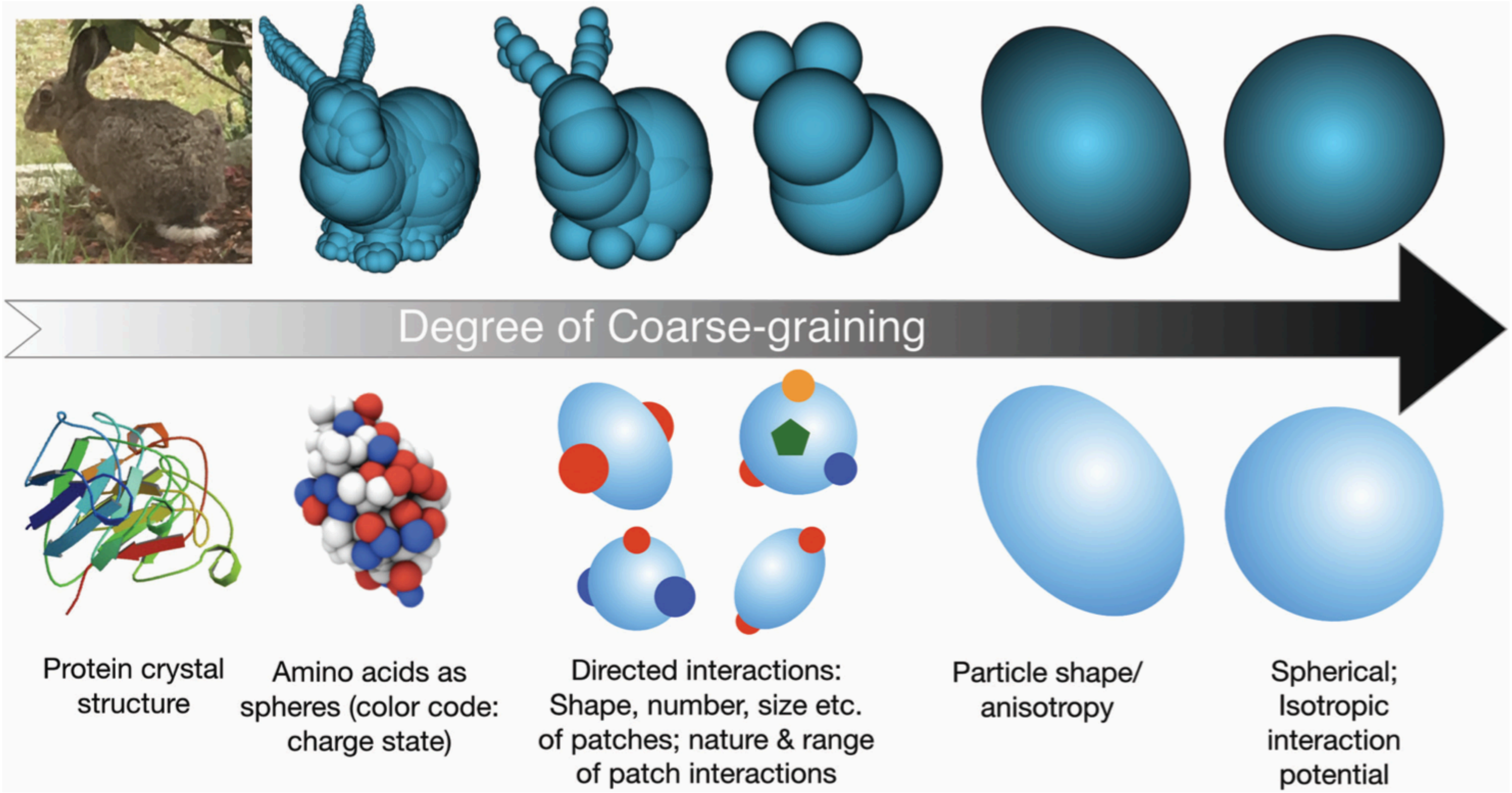
β_H ~ 200 kDa;
β_L ~ 50 kDa

Gamma-crystallins:

~ 20 kDa
specific volume:
~ 0.7 mL/g

Peter Schurtenberger

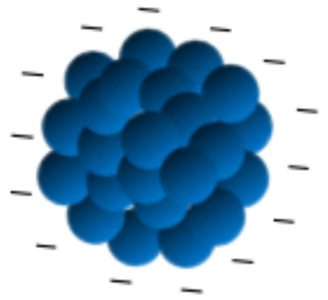
Coarse-graining approach



Anna Stradner & Peter Schurtenberger, review article, Soft Matter 2020

The eye lens

Alpha-crystallins:



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The eye lens

Biophysical *Journal*
Article

Biophysical Society

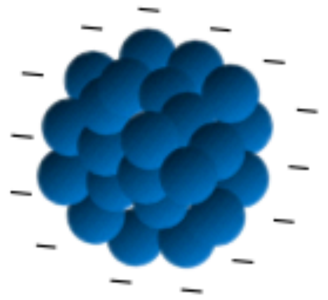
Crowding in the Eye Lens: Modeling the Multisubunit Protein β-Crystallin with a Colloidal Approach

Felix Roosen-Runge,[1,*] Alessandro Gulotta,[1] Saskia Bucciarelli,[1] Lucía Casal-Dujat,[1] Tommy Garting,[1] Nicholas Skar-Gislinge,[1] Marc Obiols-Rabasa,[1] Bela Farago,[2] Emanuela Zaccarelli,[3,4] Peter Schurtenberger,[1] and Anna Stradner[1,*]
[1]Division of Physical Chemistry, Lund University, Lund, Sweden; [2]Institut Laue-Langevin, Grenoble, France; [3]Institute for Complex Systems, National Research Council, Uos Sapienza, Rome, Italy; and [4]Department of Physics, Sapienza Università di Roma, Rome, Italy

ABSTRACT We present a multiscale characterization of aqueous solutions of the bovine eye lens protein β_H crystallin from dilute conditions up to dynamical arrest, combining dynamic light scattering, small-angle x-ray scattering, tracer-based micro-rheology, and neutron spin echo spectroscopy. We obtain a comprehensive explanation of the observed experimental signatures from a model of polydisperse hard spheres with additional weak attraction. In particular, the model predictions quantitatively describe the multiscale dynamical results from microscopic nanometer cage diffusion over mesoscopic micrometer gradient diffusion up to macroscopic viscosity. Based on a comparative discussion with results from other crystallin proteins, we suggest an interesting common pathway for dynamical arrest in all crystallin proteins, with potential implications for the understanding of crowding effects in the eye lens.

Biophysical Journal 2020

Alpha-crystallins:



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The eye lens

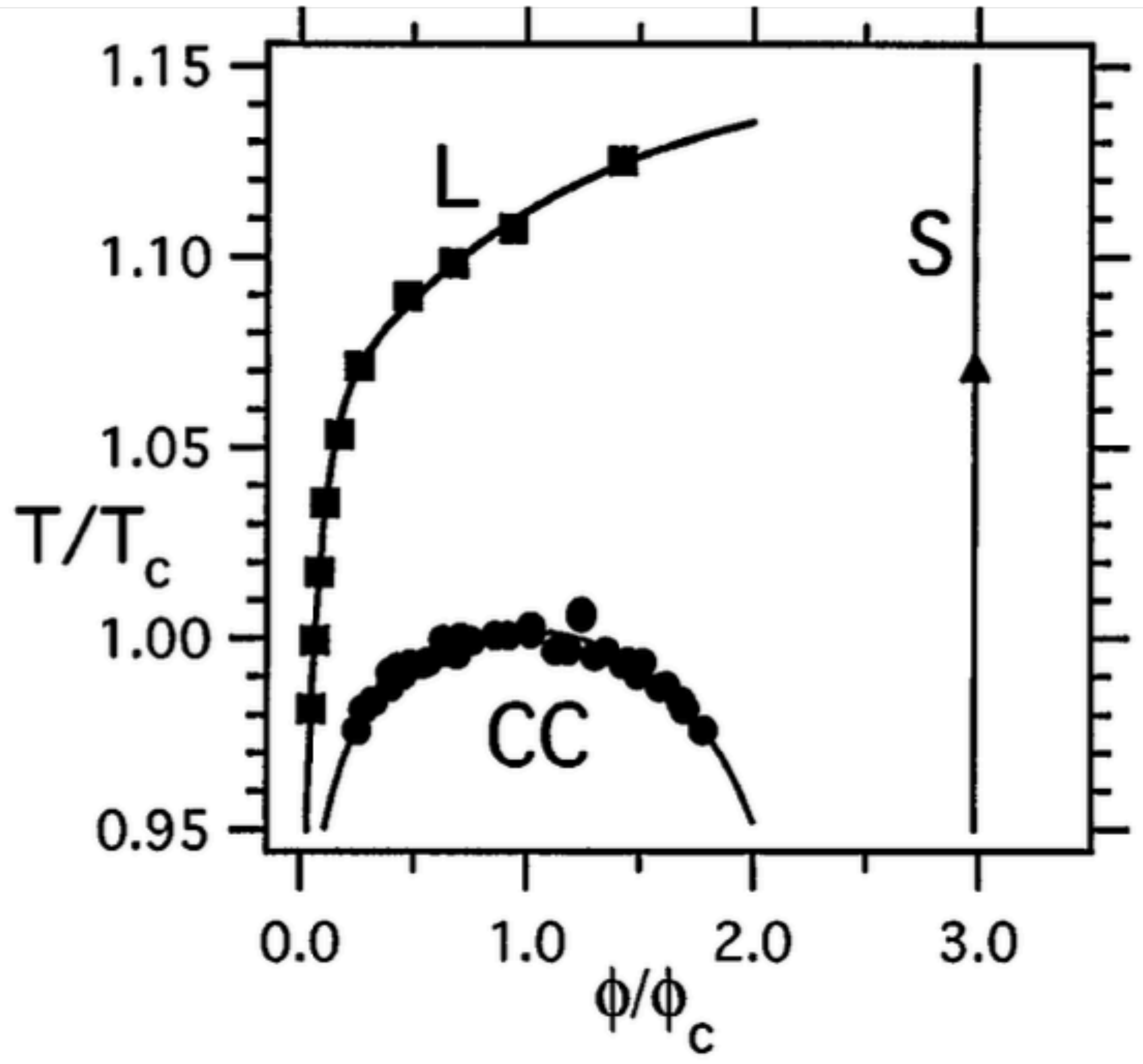
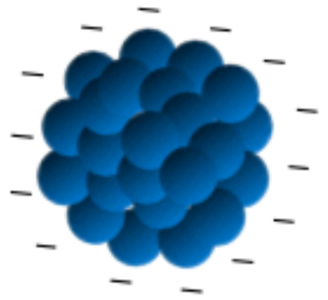


FIG. 1. The phase diagram of γ_{II}-crystallin [3–5]. The circles are points on the liquid-liquid coexistence curve (CC). The squares are points on the liquidus line (L). The triangle is a point on the solidus line (S). The lines are guides to the eye. The critical temperature is $T_c = 278.4$ K. The critical volume fraction is $\phi_c = 0.21$.

Asherie, Lomakin & Benedek PRL 1996

Alpha-crystallins:



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Colloidal approach to globular protein phase diagram: isotropic potentials

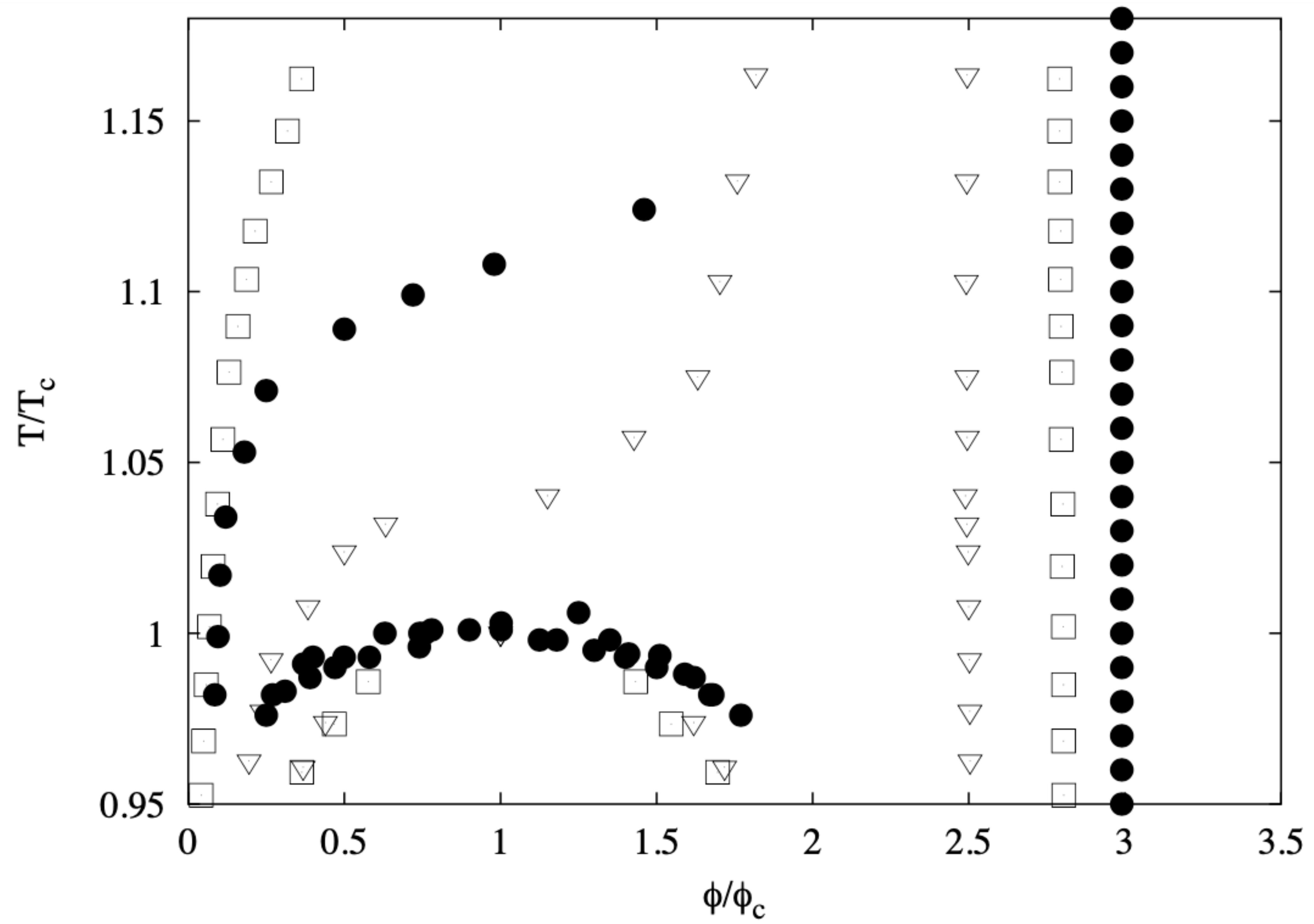
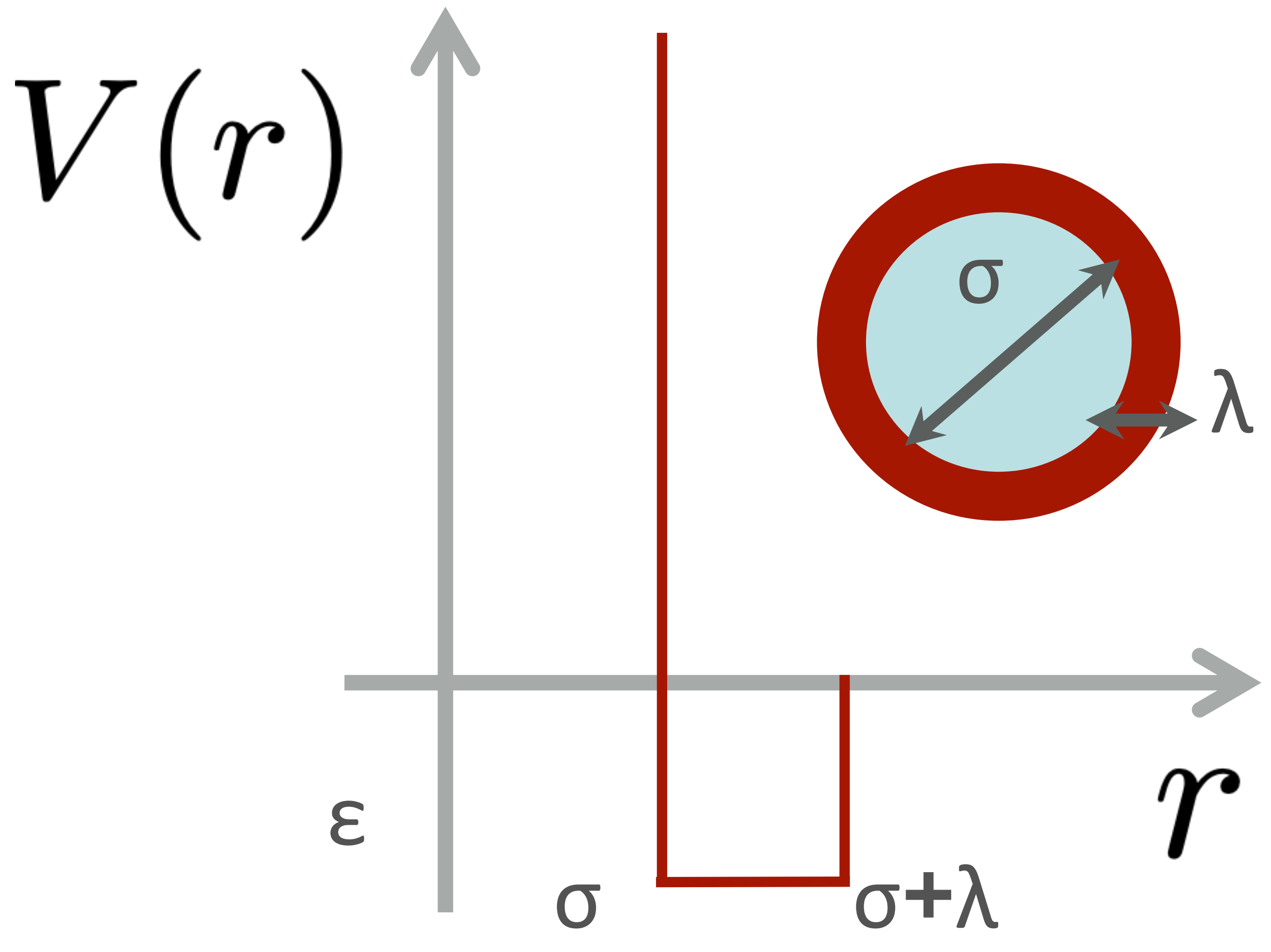


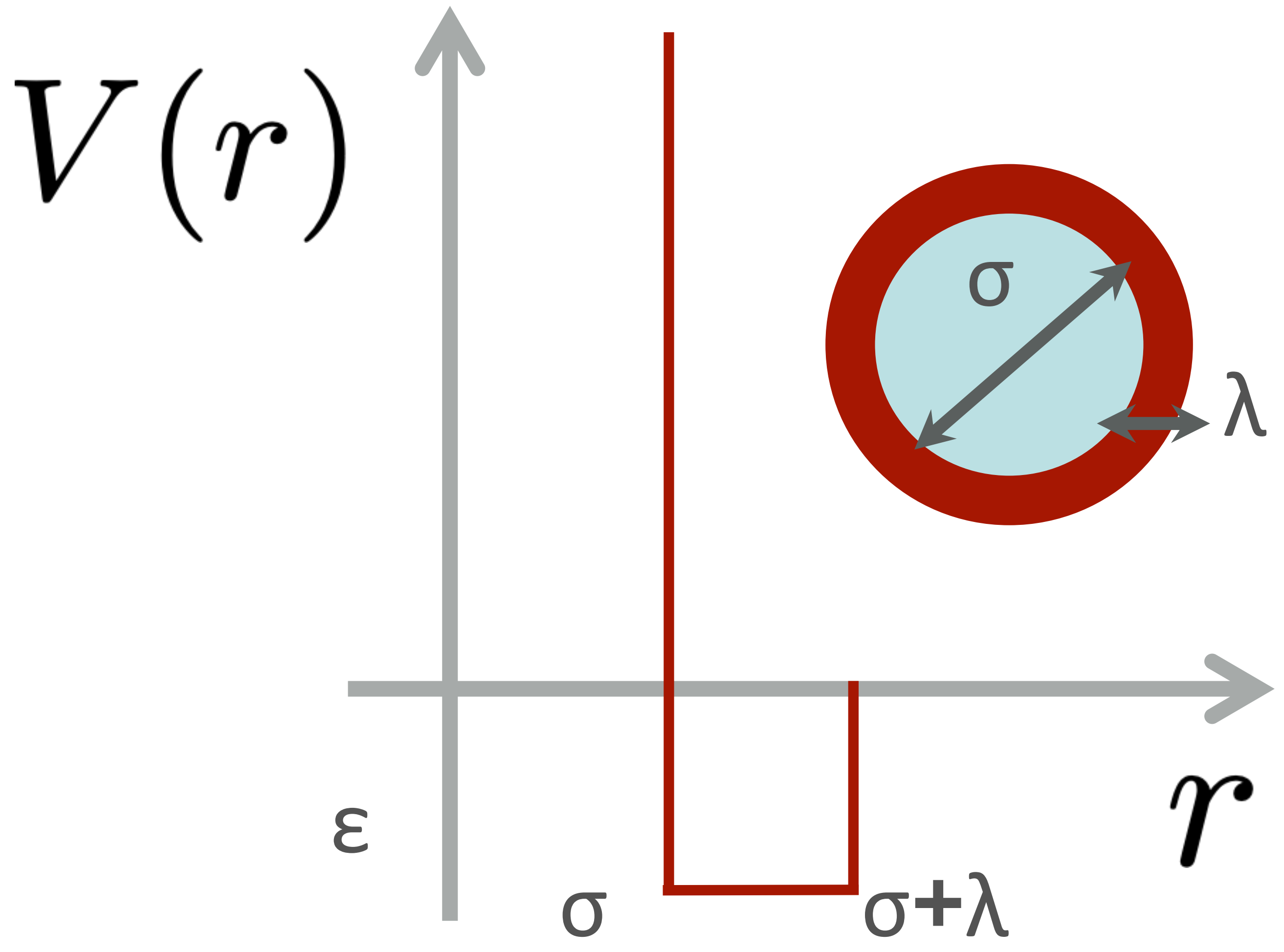
FIG. 8: Comparison of our Monte Carlo results for both $\lambda = 1.15$ (□) and $\lambda = 1.25$ (▽), respectively, to the gamma-II crystallin (•).

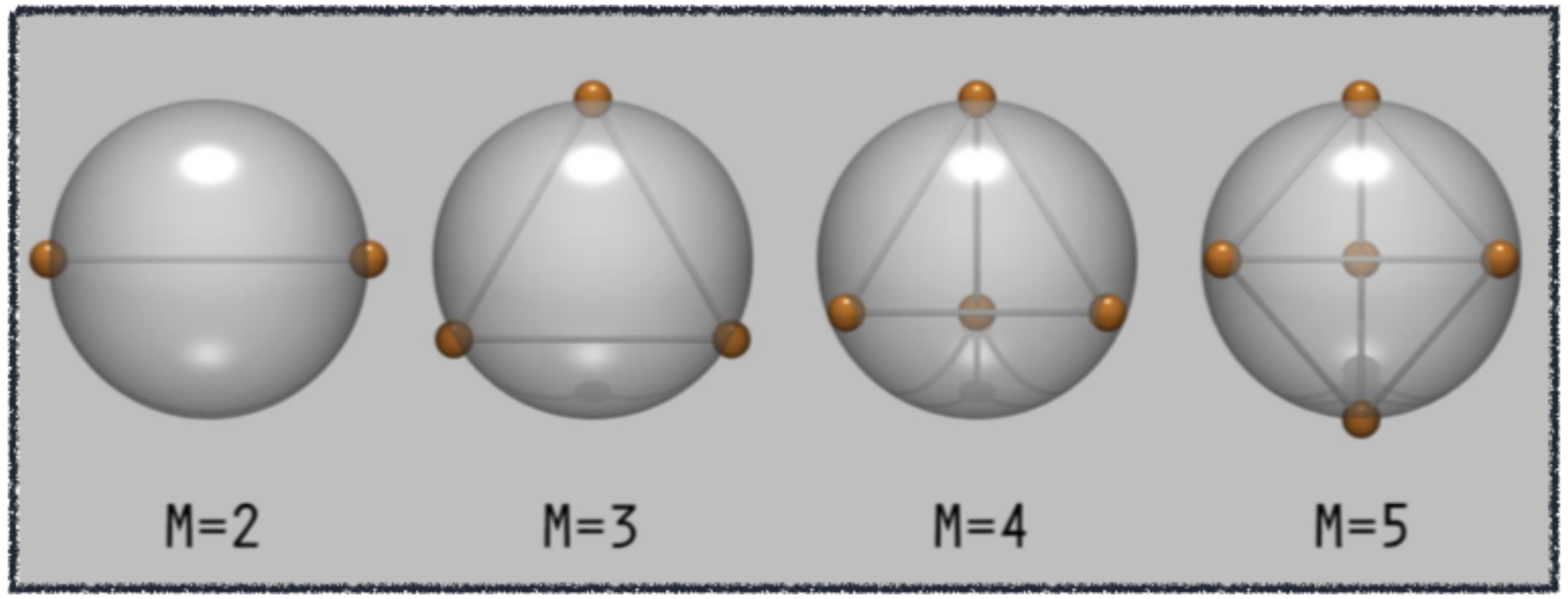
Pagan & Gunton J. Chem. Phys. 2005

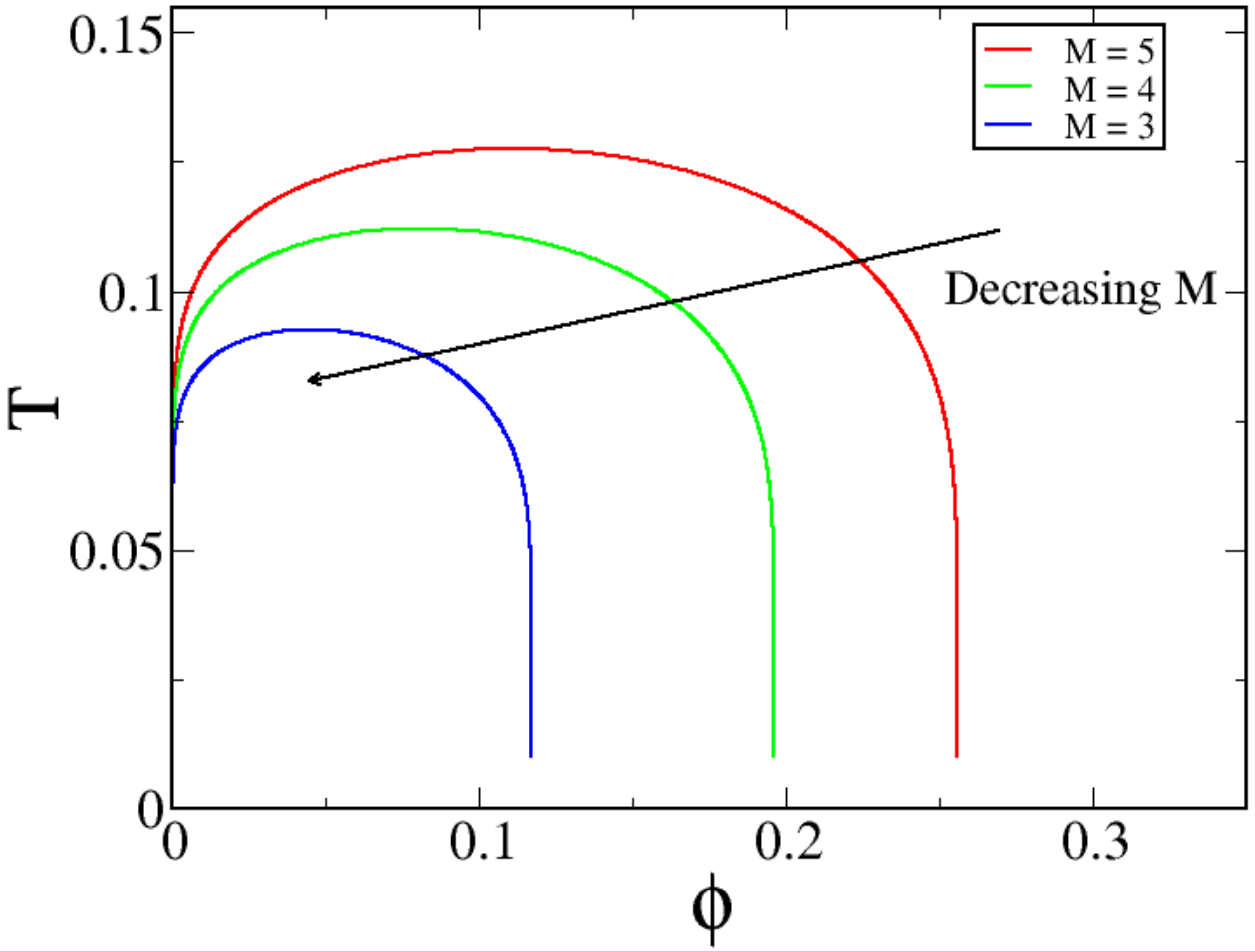


square-well potential

The need of anisotropic models: patchy models

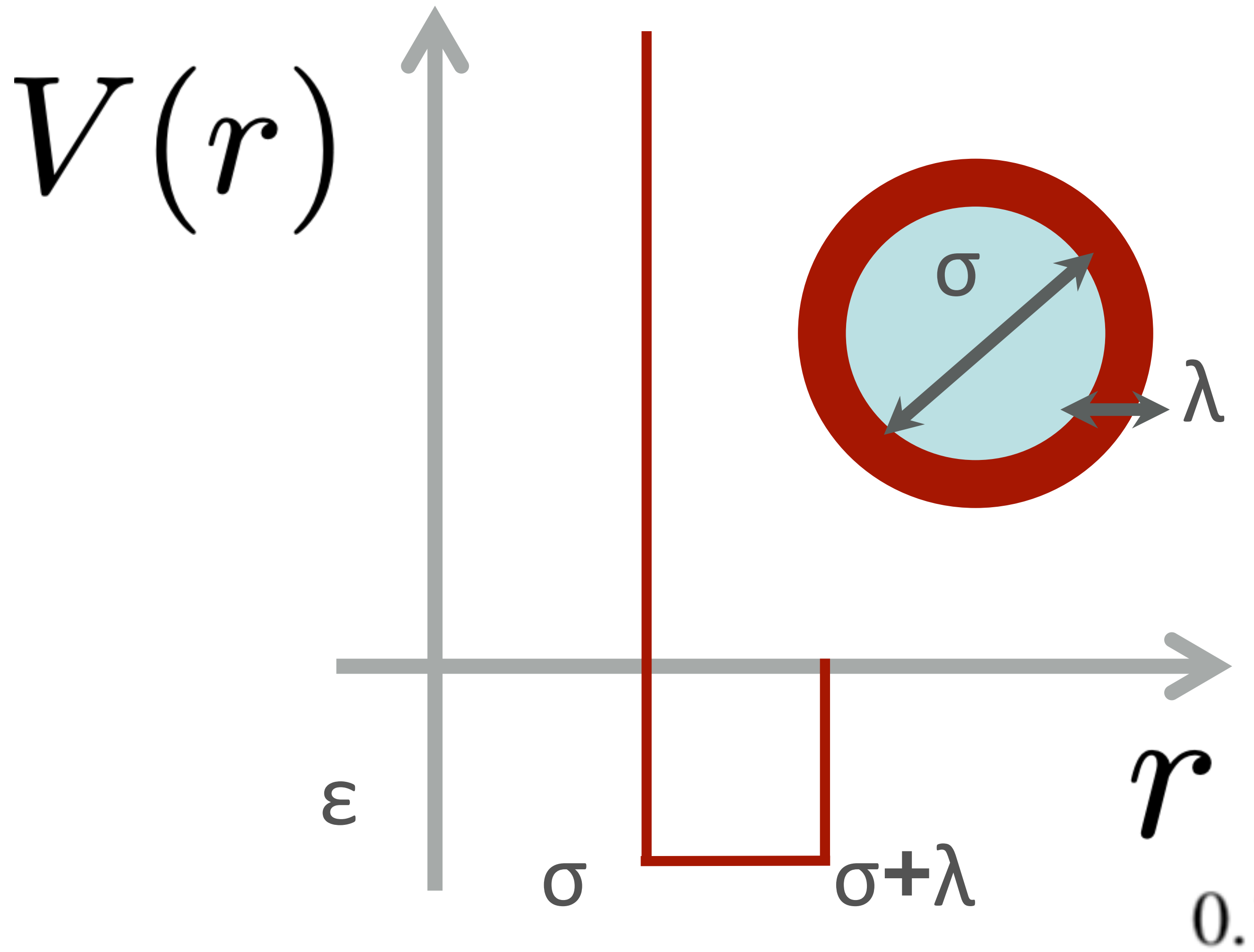


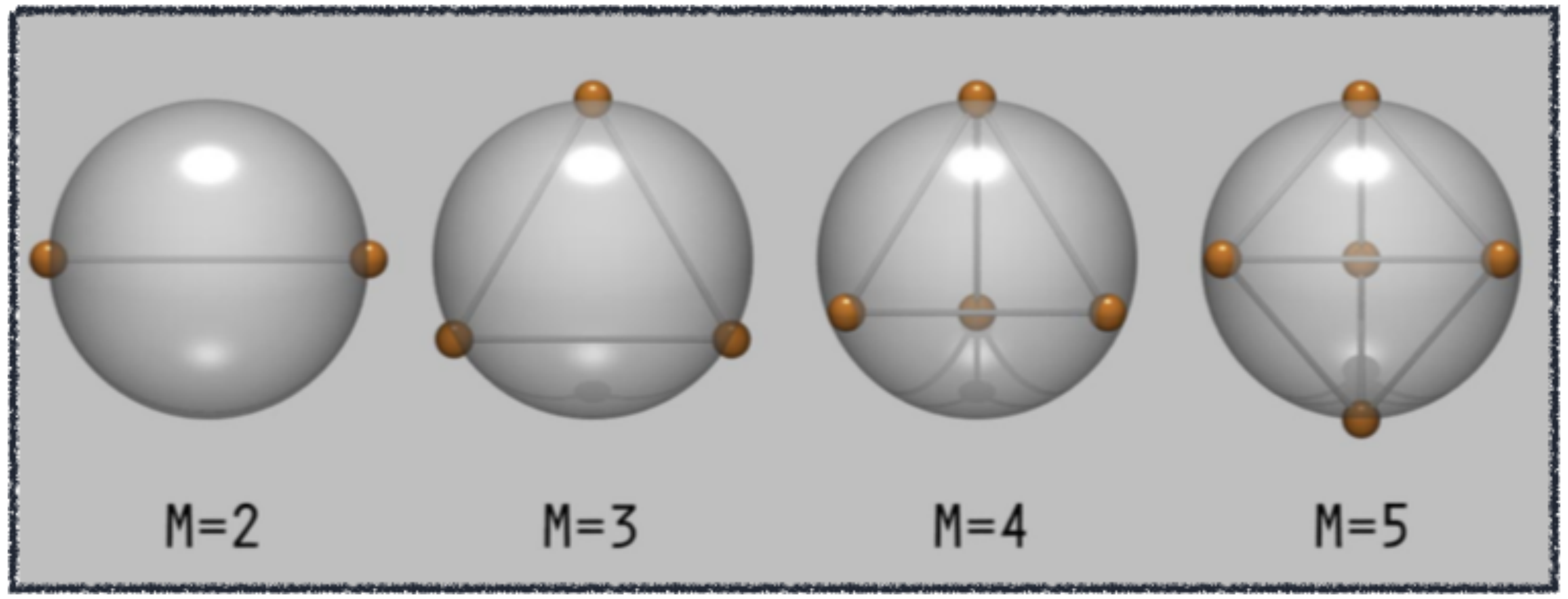


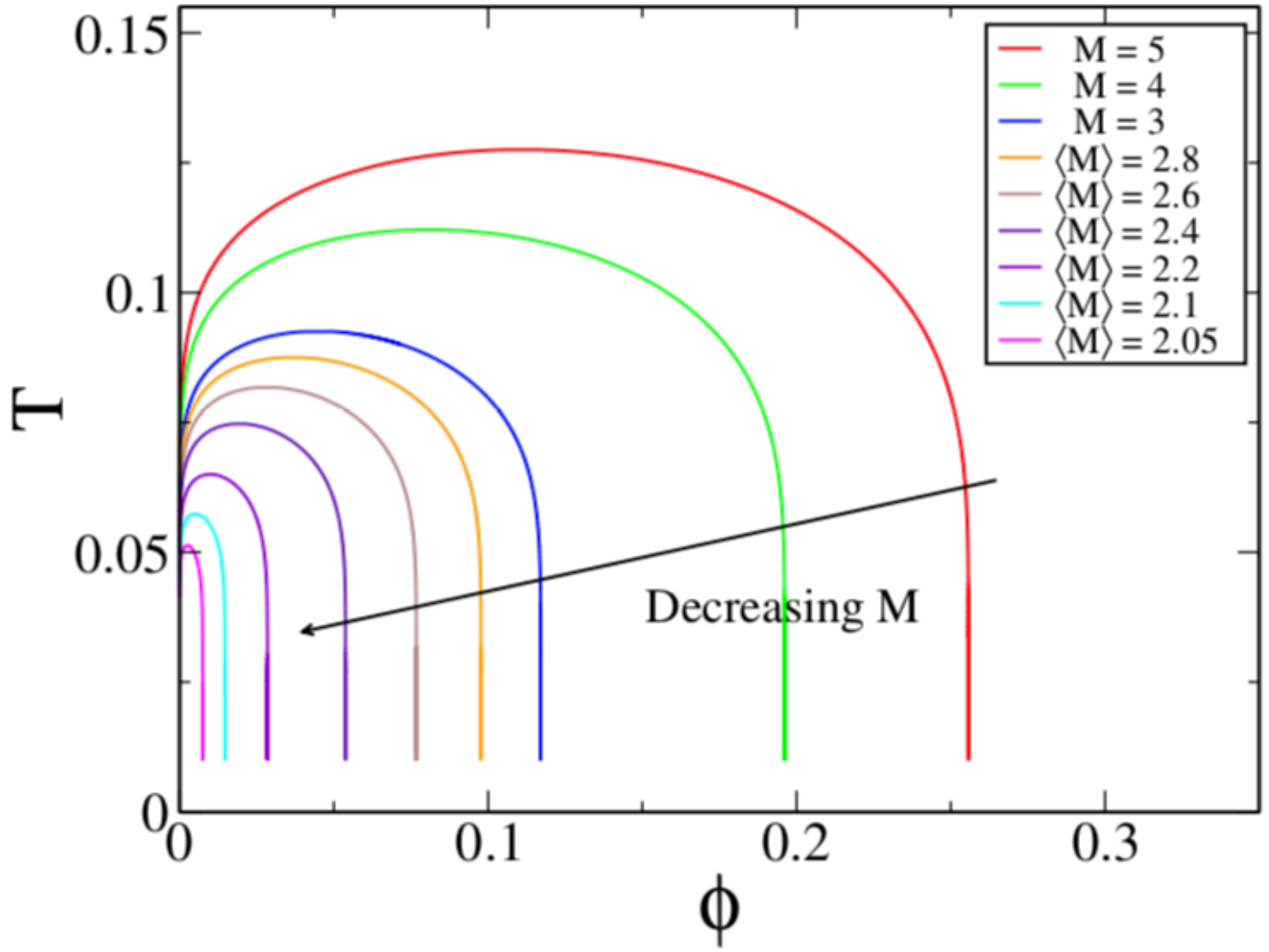


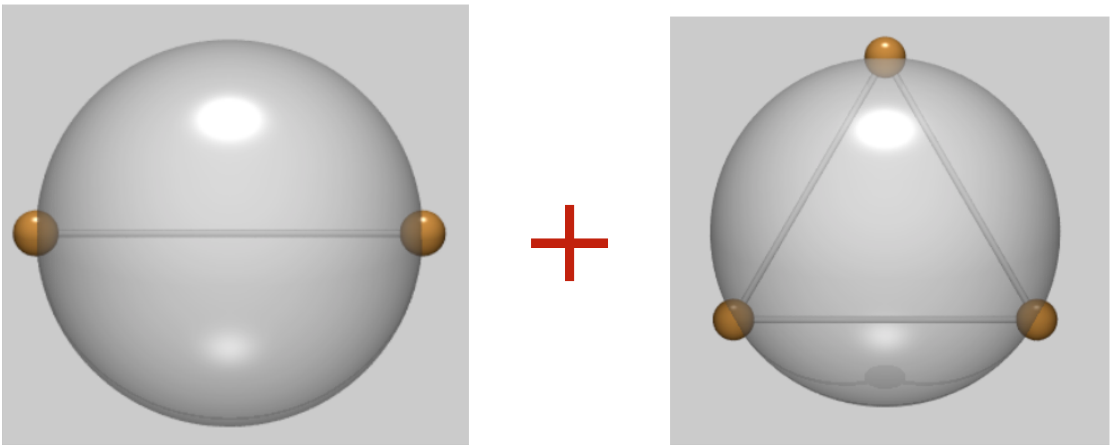
Bianchi, Largo, Tartaglia, EZ and Sciortino PRL (2006)

The need of anisotropic models: patchy models









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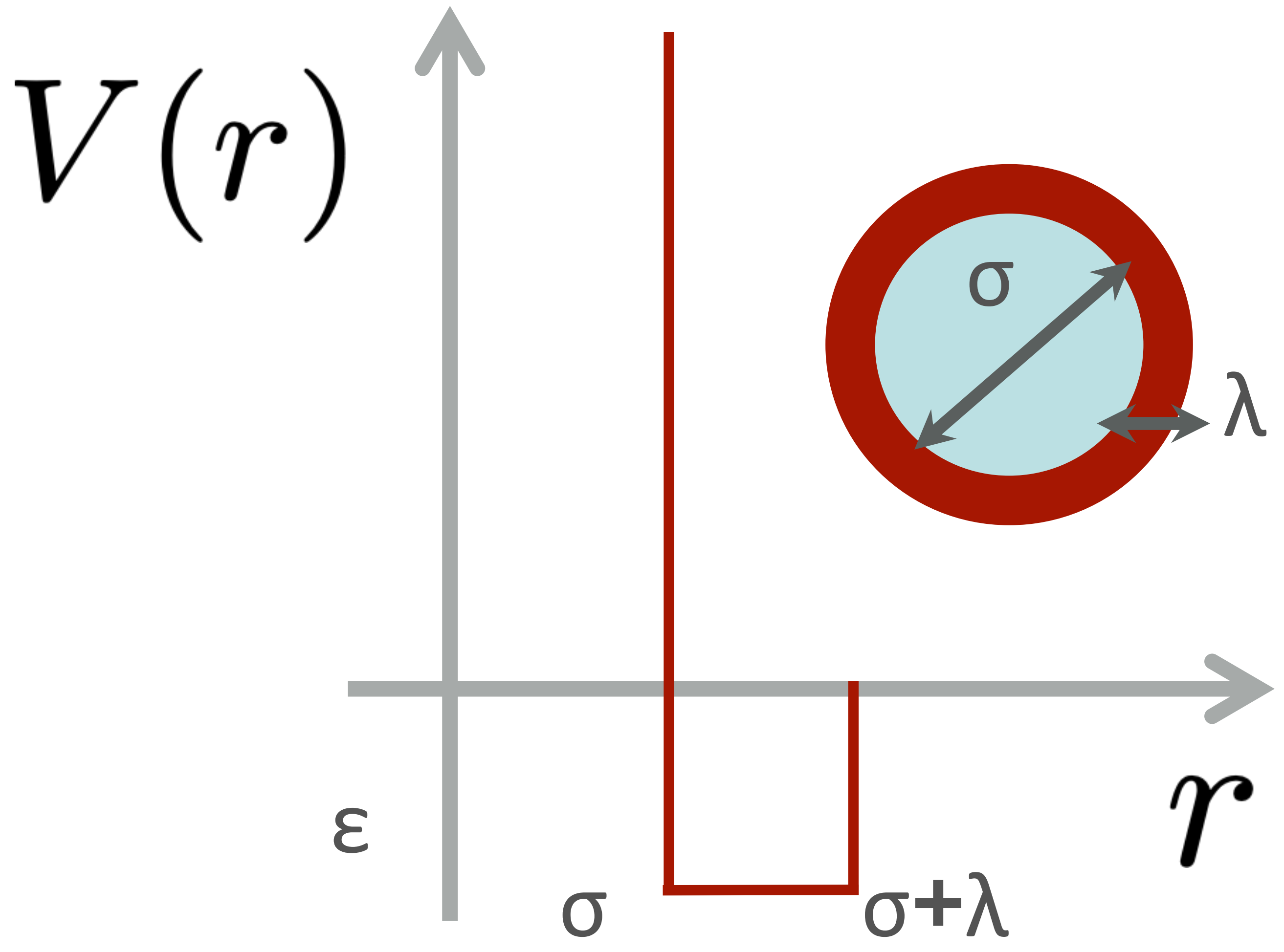

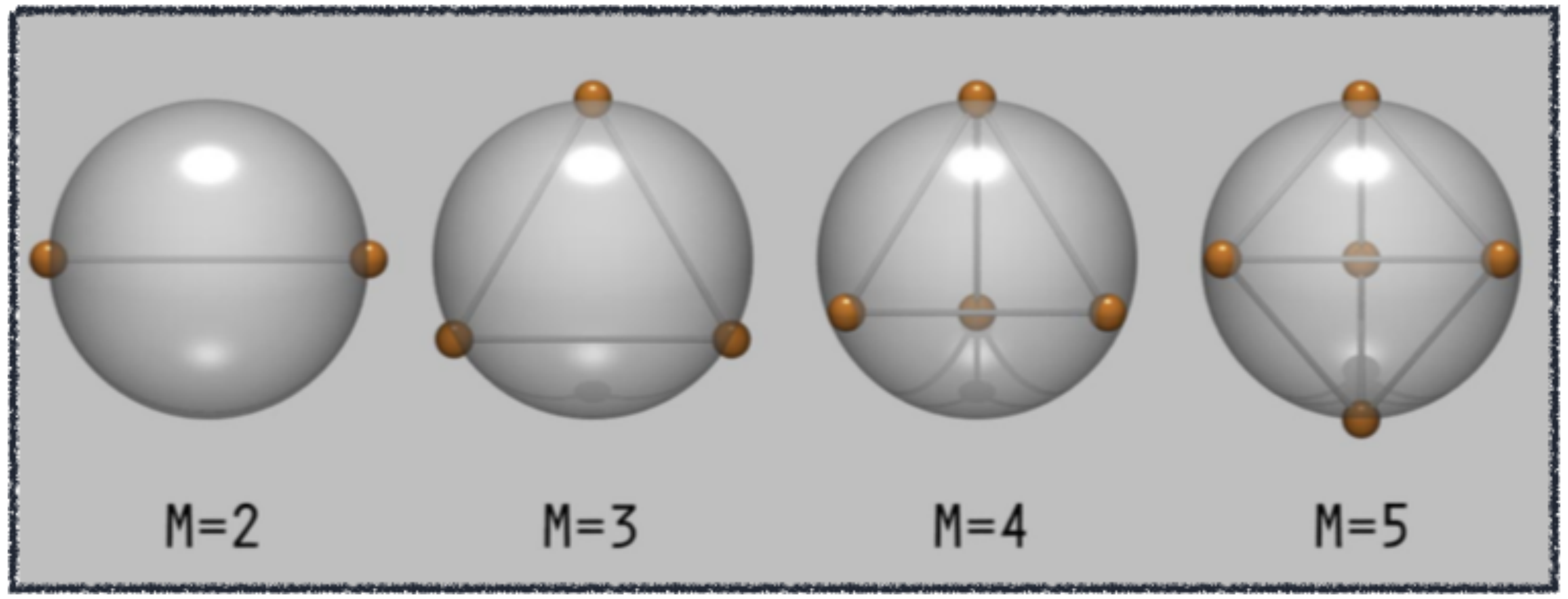

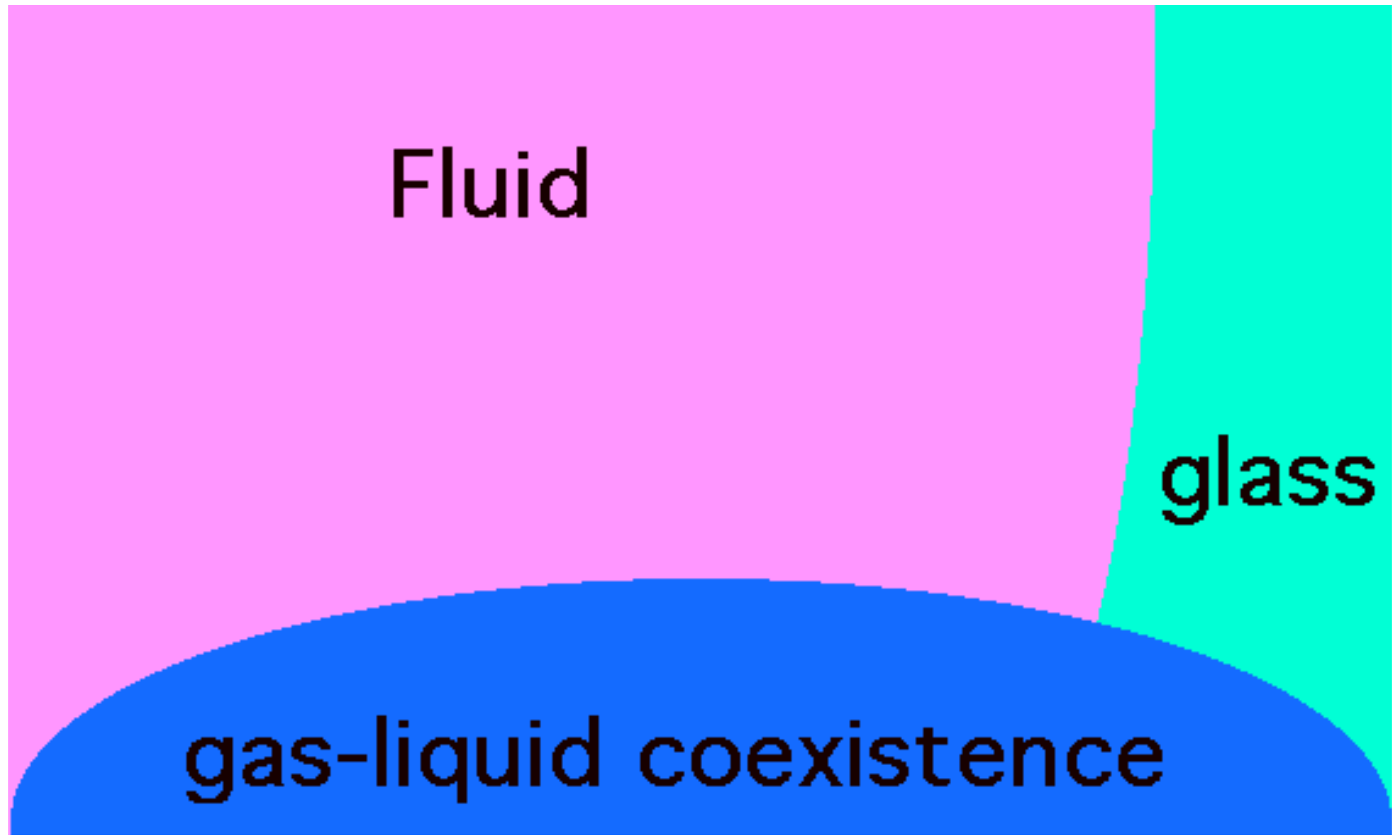

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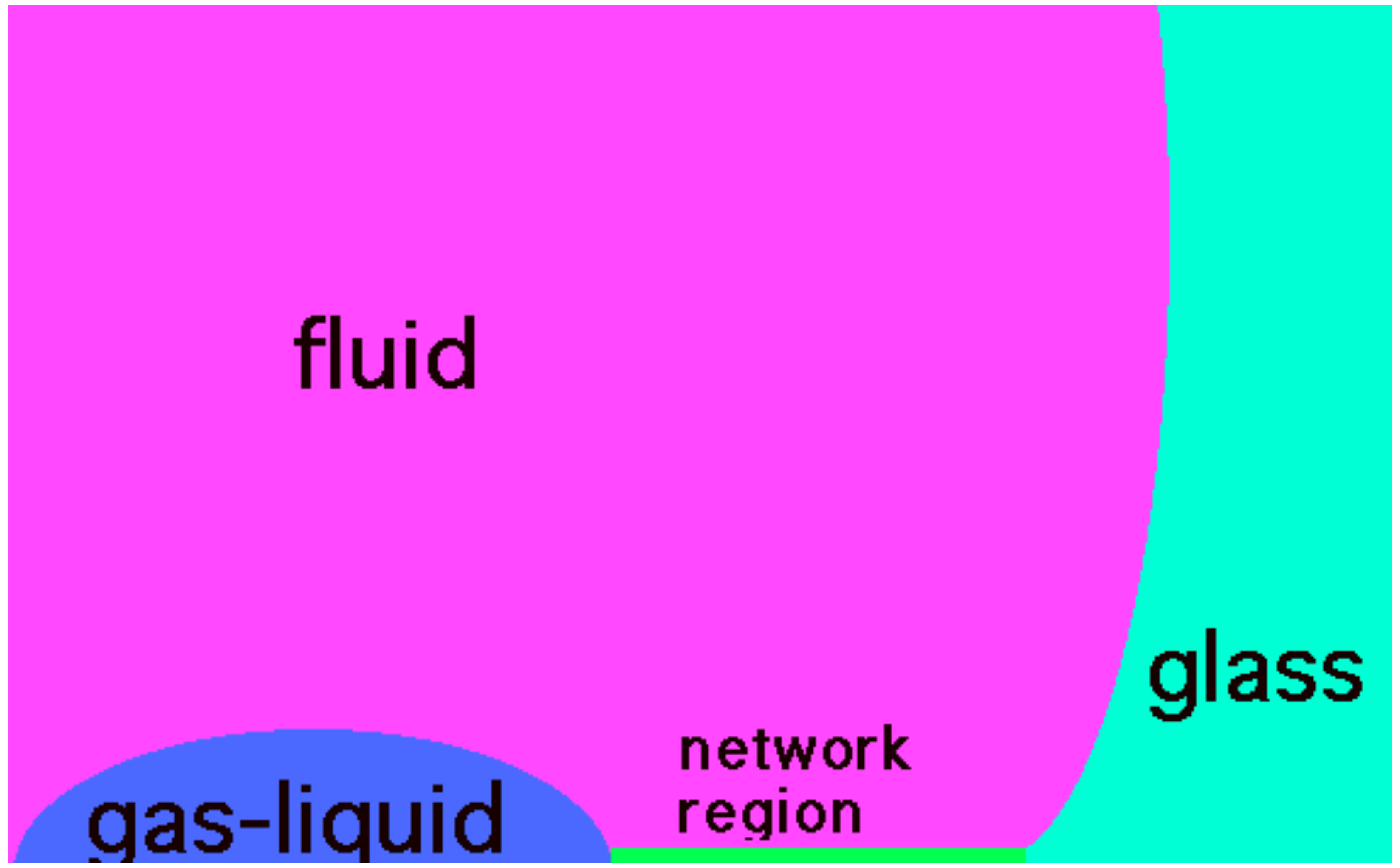
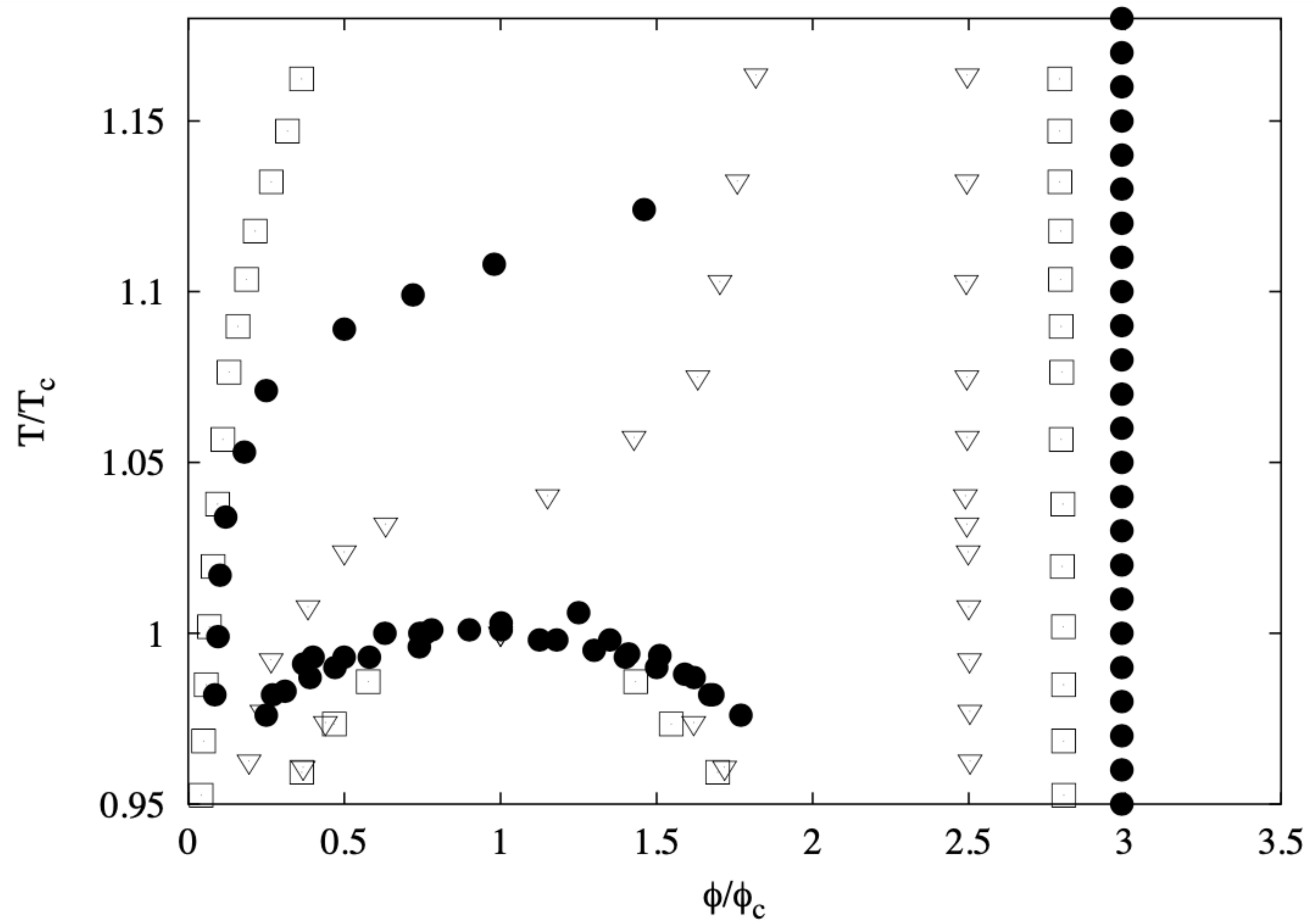


Bianchi, Largo, Tartaglia, EZ and Sciortino PRL (2006)

Back to proteins



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Pagan & Gunton J. Chem. Phys. 2005

square-well potential

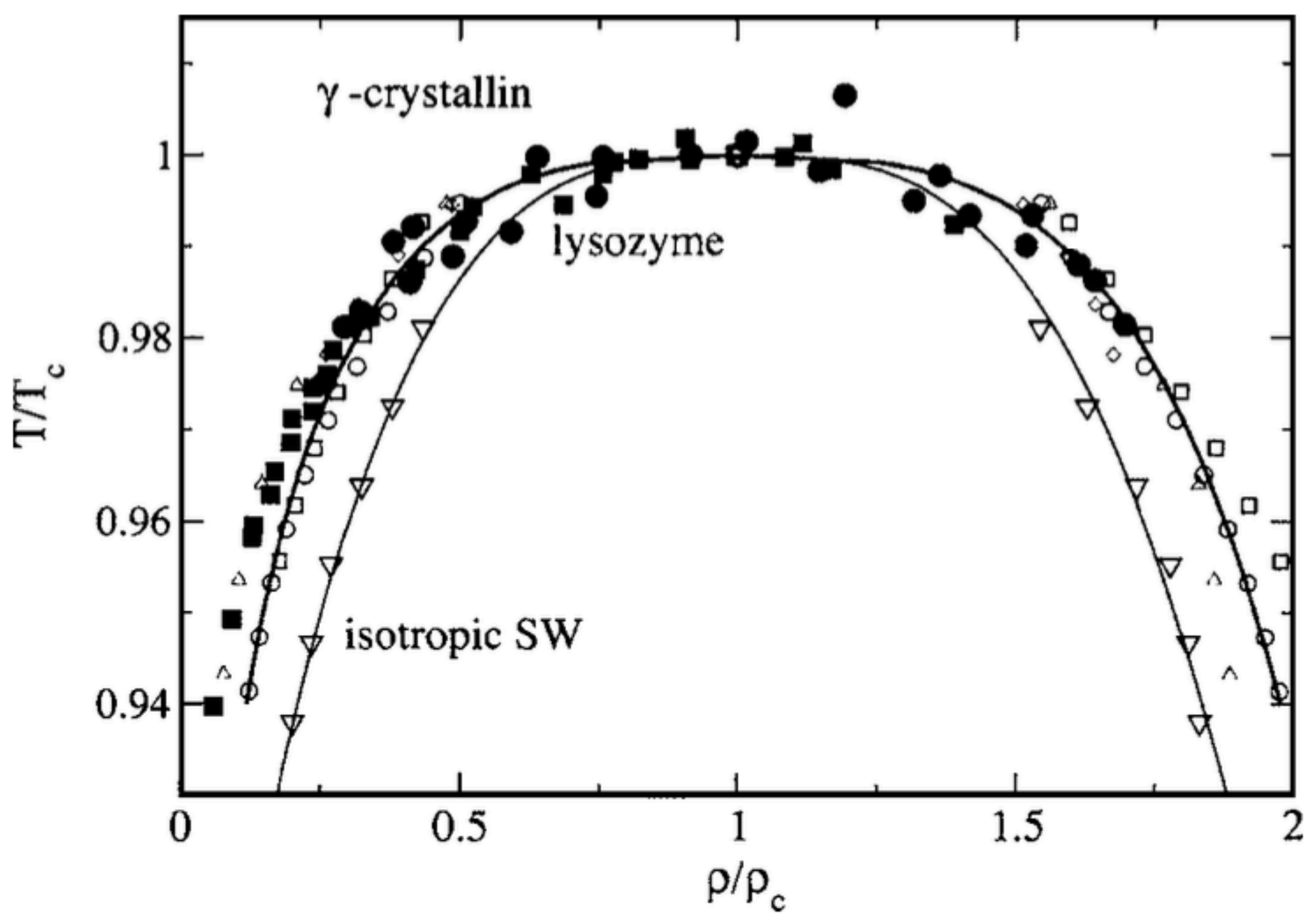


FIG. 4. Vapor-liquid coexistence curves in terms of the reduced temperatures T/T_c and the reduced density ρ/ρ_c for the studied patchy models: $M=4$ (open squares), $M=5$ (open circles), and $M=7$ (open diamonds). The isotropic only (Ref. 27) (inverted open triangles) and PMW (Ref. 16) (open triangles) data are shown for comparison. The experimental data for γ-crystallin (full circles) and lysozyme (full squares) are taken from Refs. 28 and 29, respectively. The lines are the fit to the standard critical scaling law used to describe coexistence curves.

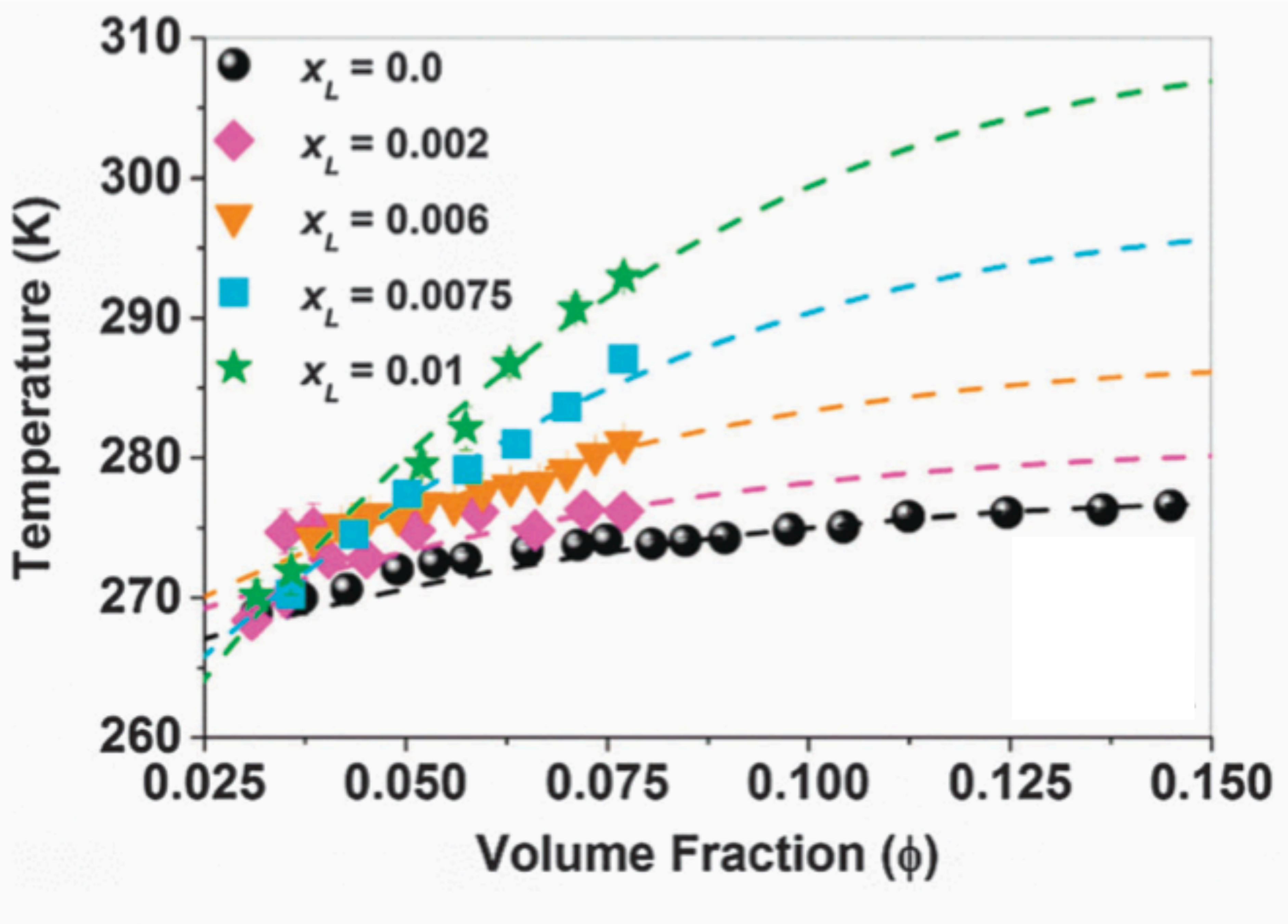
Liu, Kumar & Sciortino J. Chem. Phys. 2007

square-well + patchy

Single-point modification of protein interactions

How fluorescent labelling alters the solution behaviour of proteins

M. K. Quinn,[a] N. Gnan,[b] S. James,[a] A. Ninarello,[c] F. Sciortino,[bc] E. Zaccarelli[bc] and J. J. McManus*[a]



Phys. Chem. Chem. Phys. (2015)

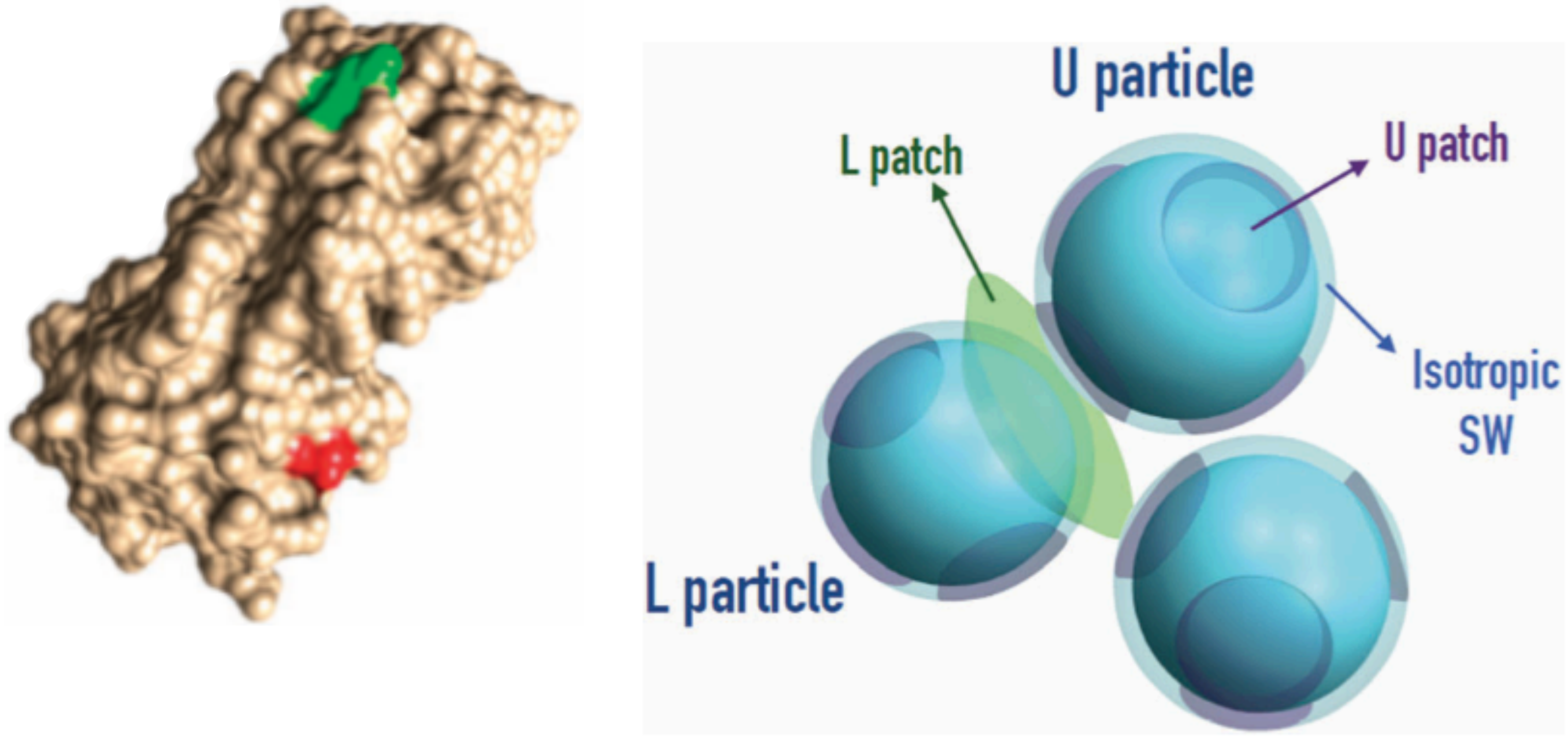
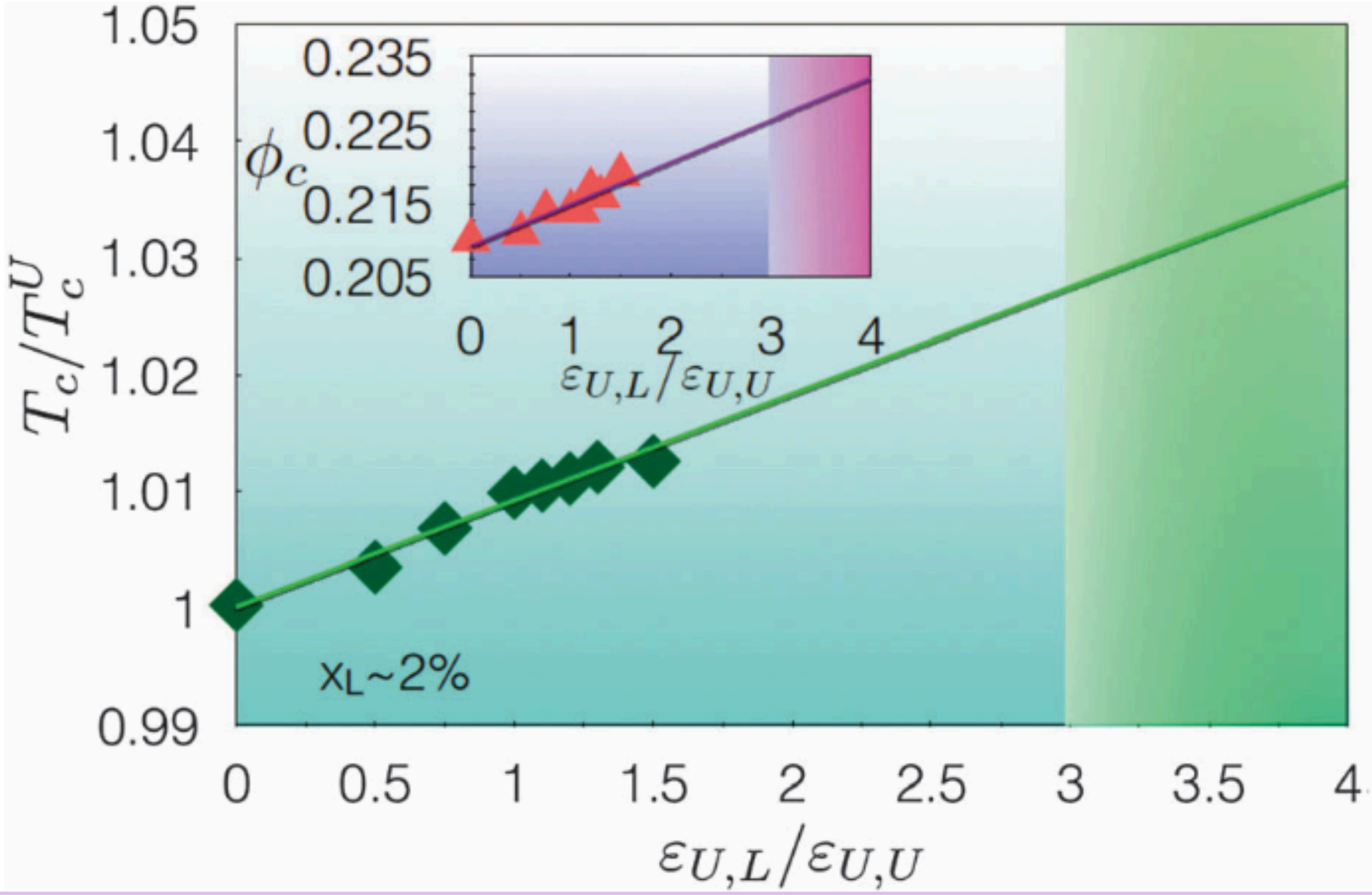
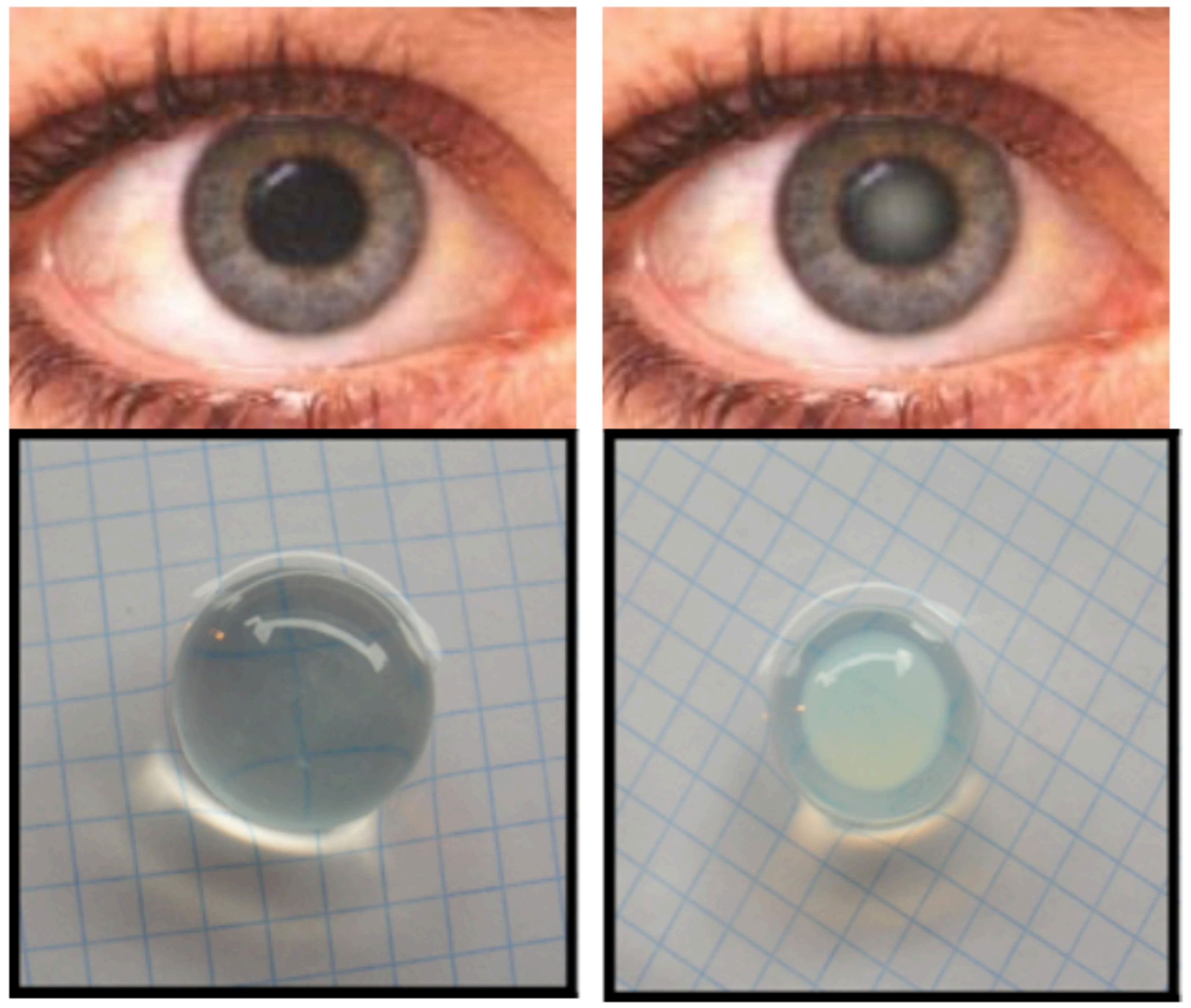


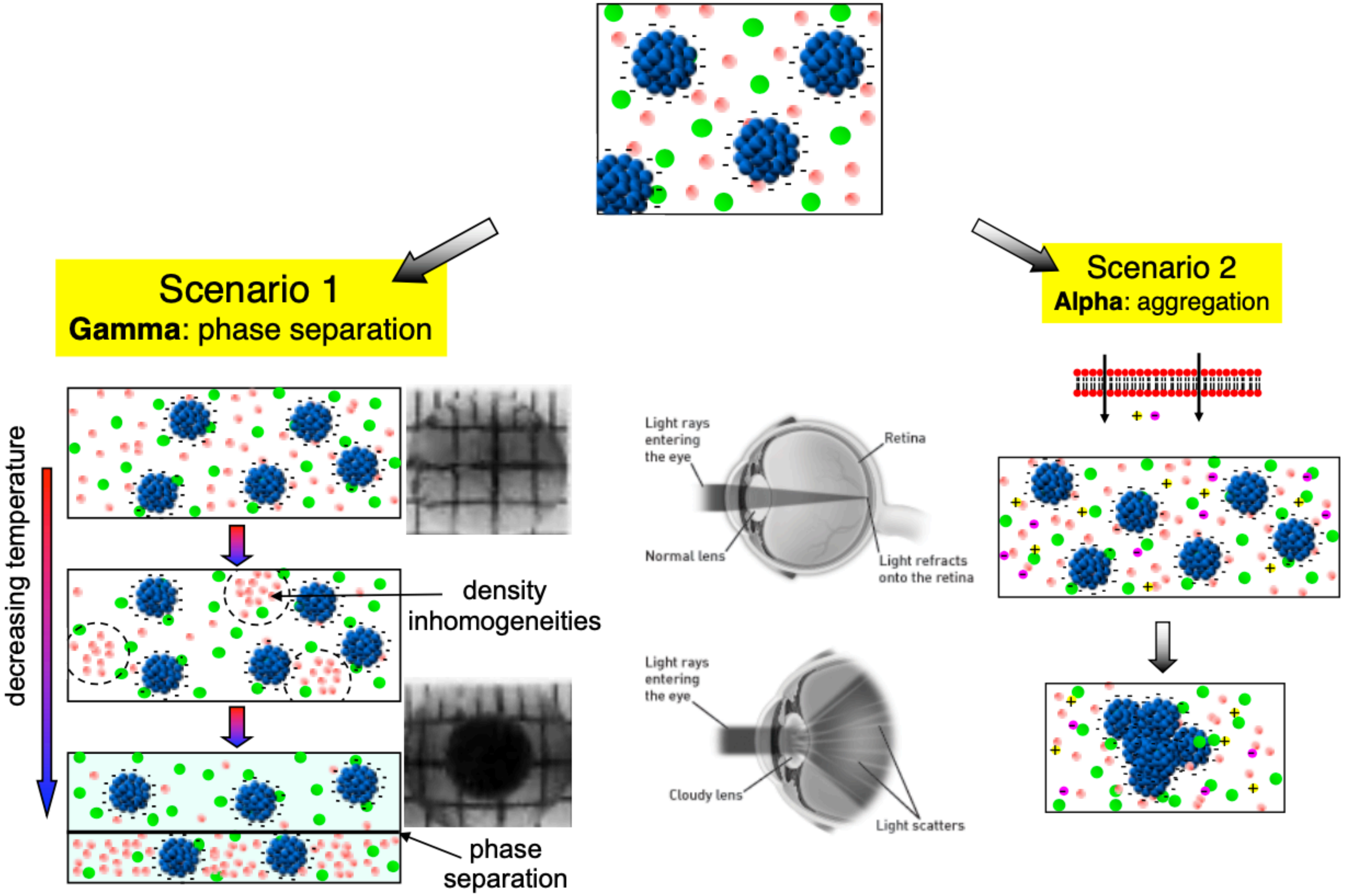
Fig. 1 Patchy particles used for modelling HGD proteins. Particles with four patches (U-patches) are unlabelled proteins (U-type), while the particle with green (wider) patch (L-patch) corresponds to a fluorescently labelled protein (L-type).



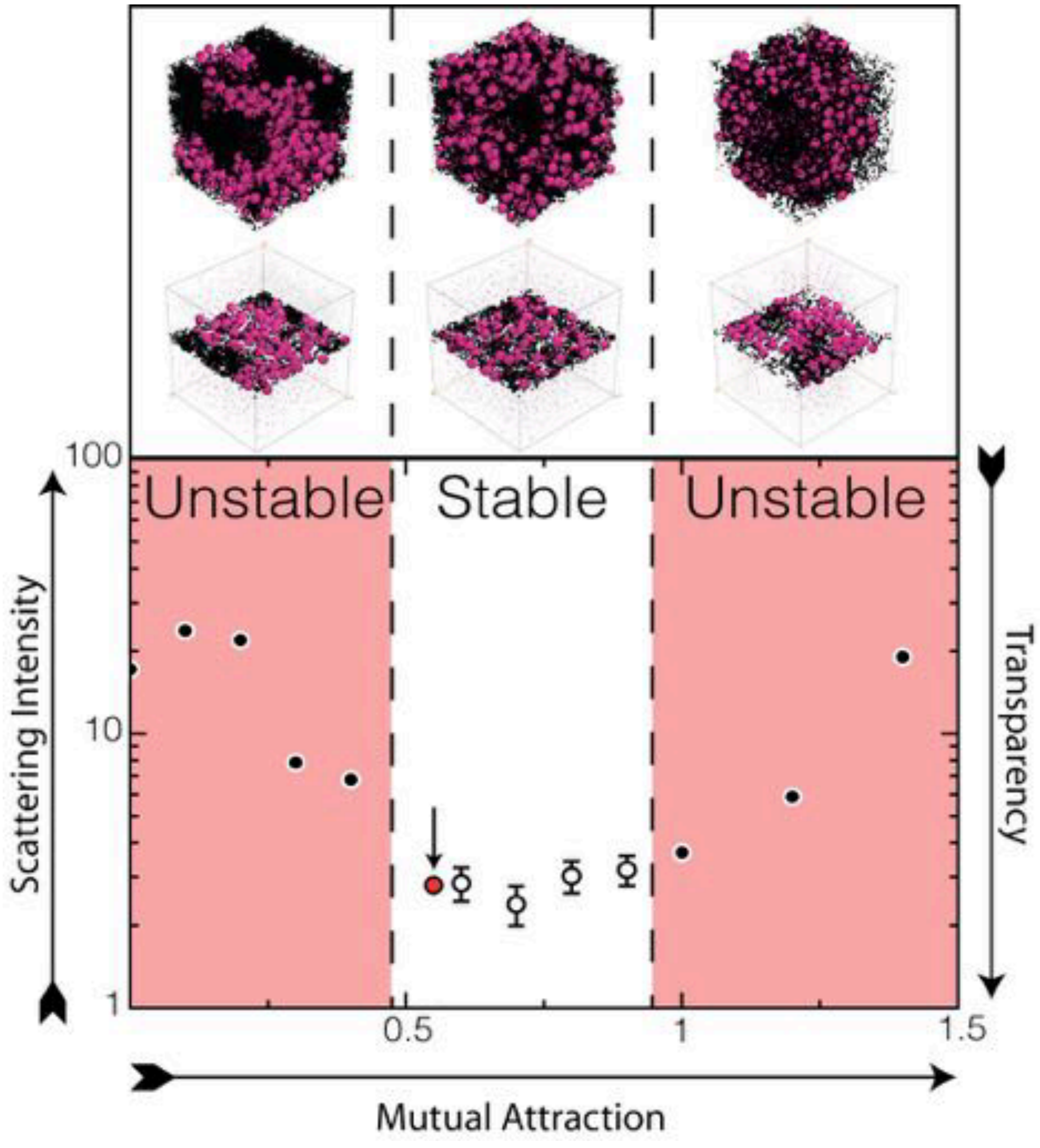
Back to the eye-lens

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

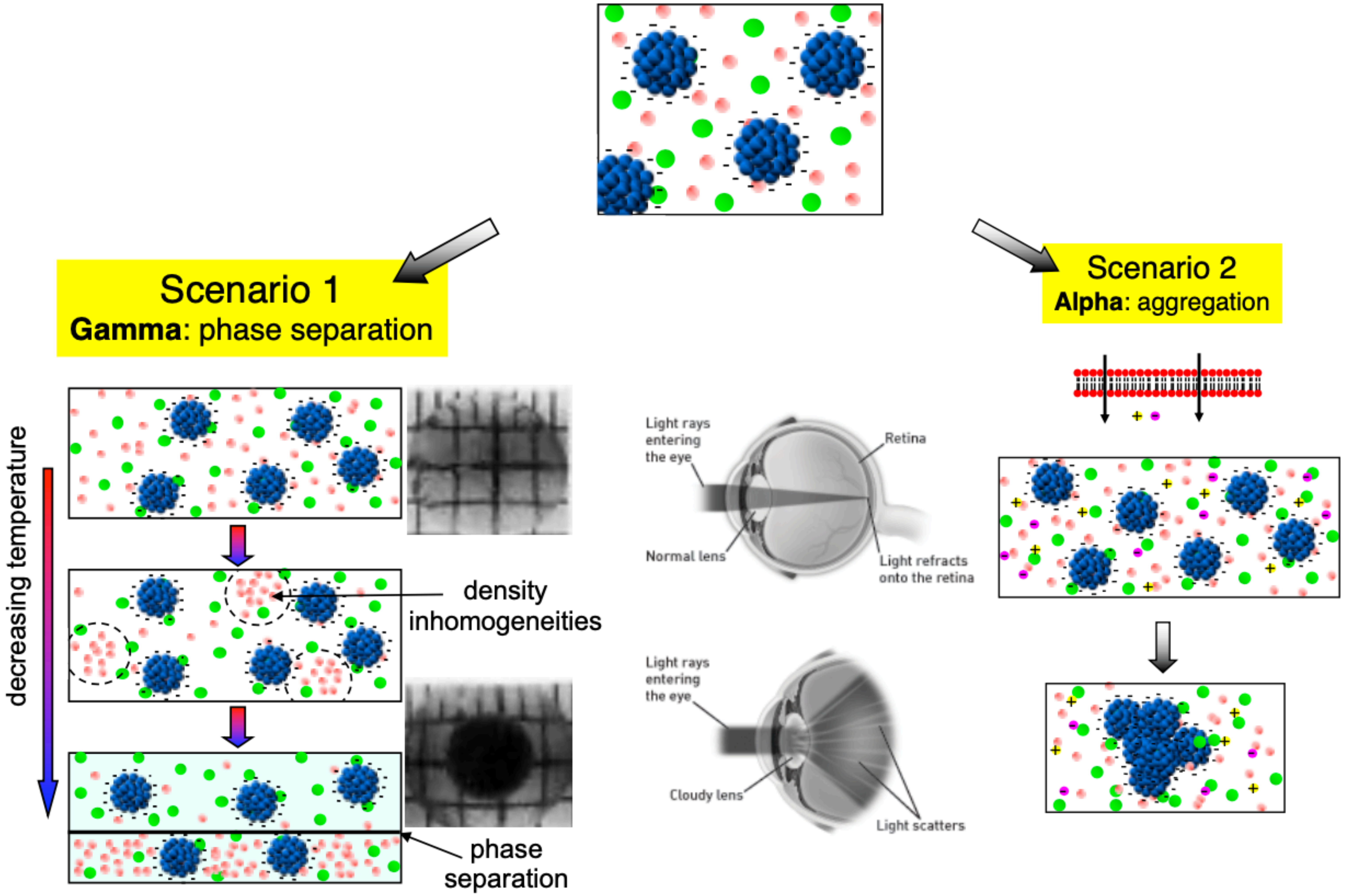




Back to the eye-lens

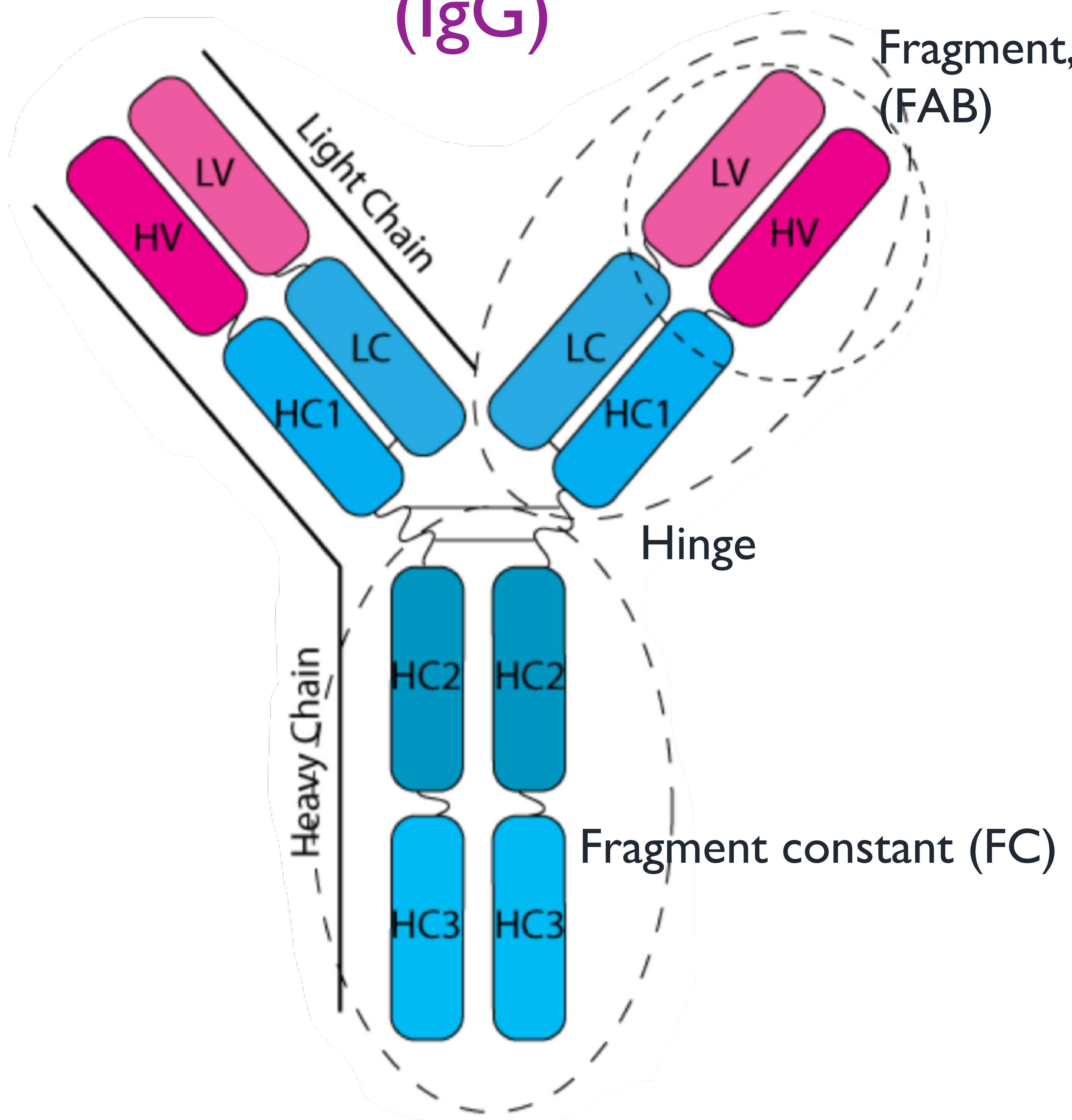


Stradner et al
Phys. Rev. Lett. 2007

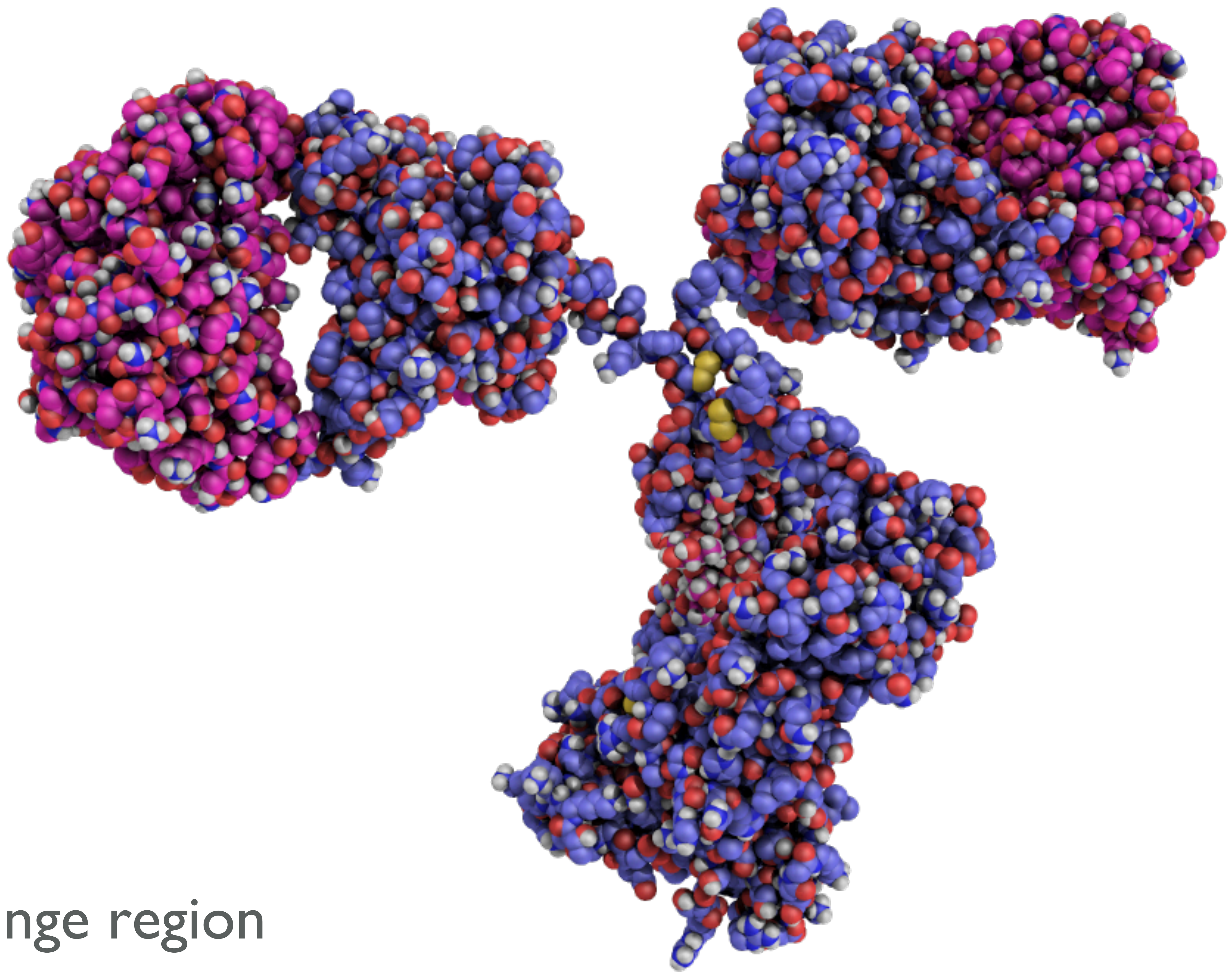


The last example: monoclonal antibodies

Immunoglobuline Gamma (IgG)

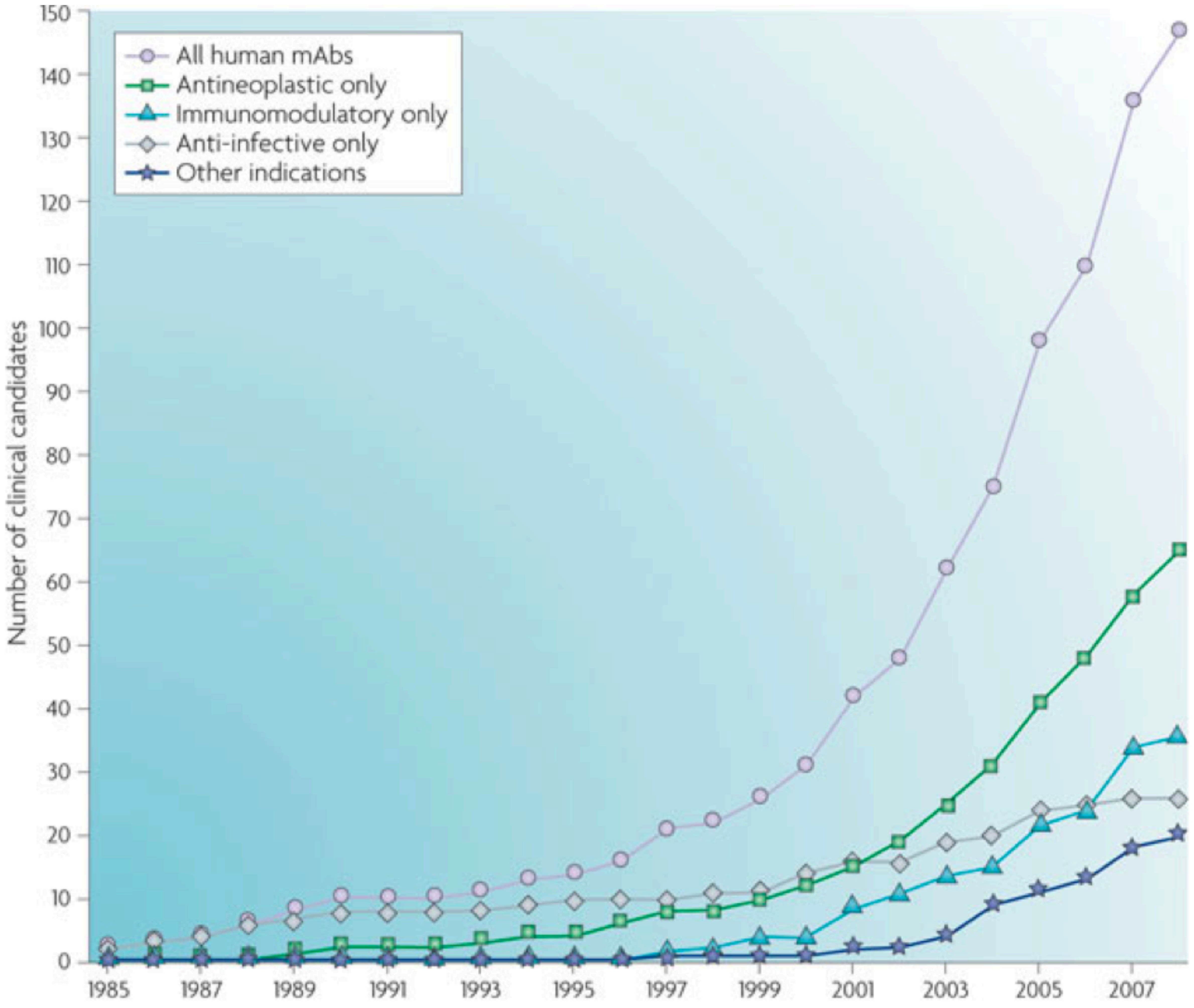


Antibodies are ***large*** proteins (~150 kDa) employed by the immune system for binding targets



three structured domains connected by a flexible hinge region

Antibodies as pharmaceutical drugs



Nature Reviews | Drug Discovery

widely investigated in biopharmaceutical industry due to

i) large flexibility in molecular recognition
ii) long half-life in the body
iii) possibility of humanization with low risk of immunogenicity

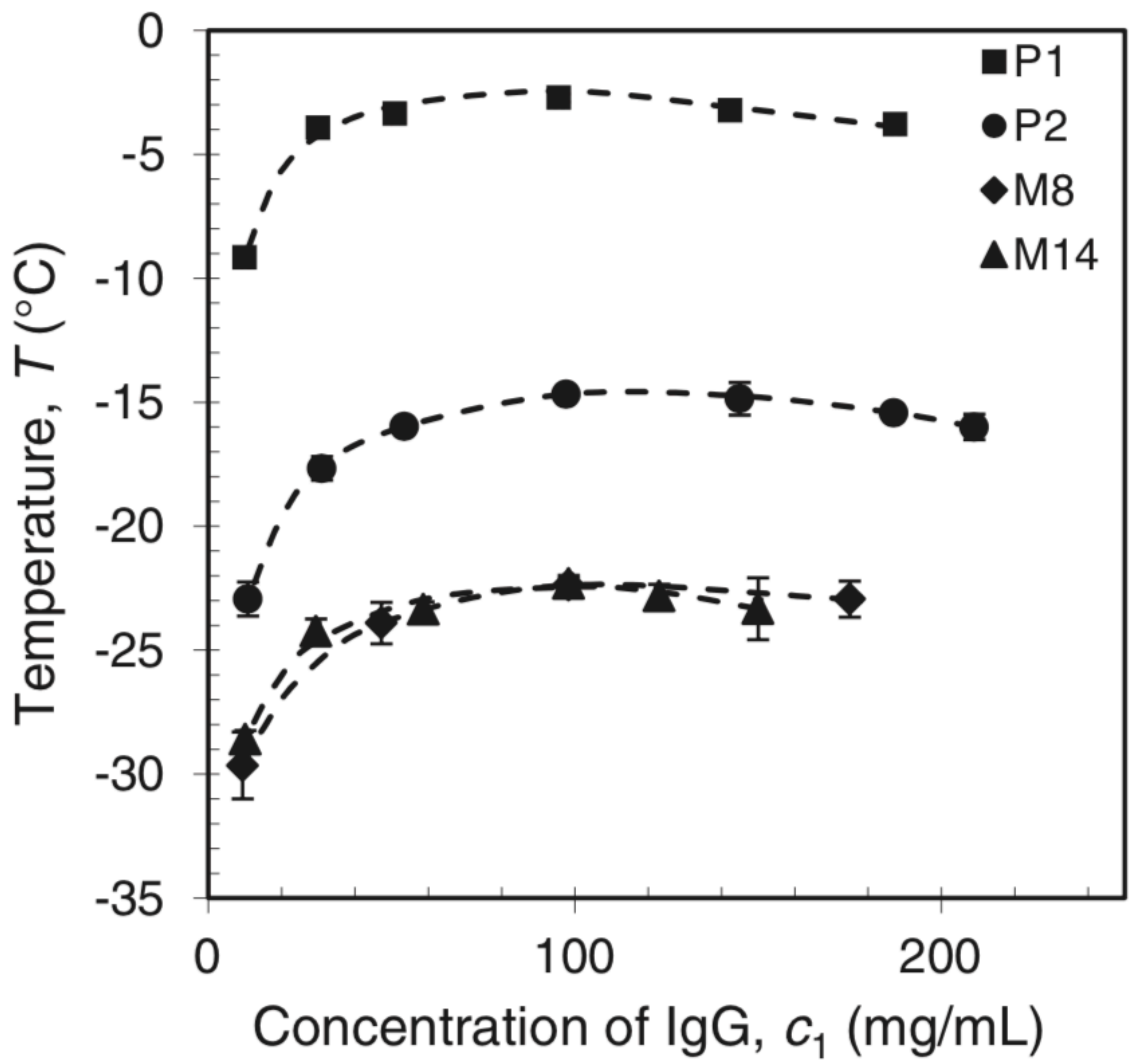
NEED FOR HIGH CONCENTRATION FORMULATIONS
> 100 MG/ML
OFTEN RESULTING IN TOO HIGH VISCOSITIES

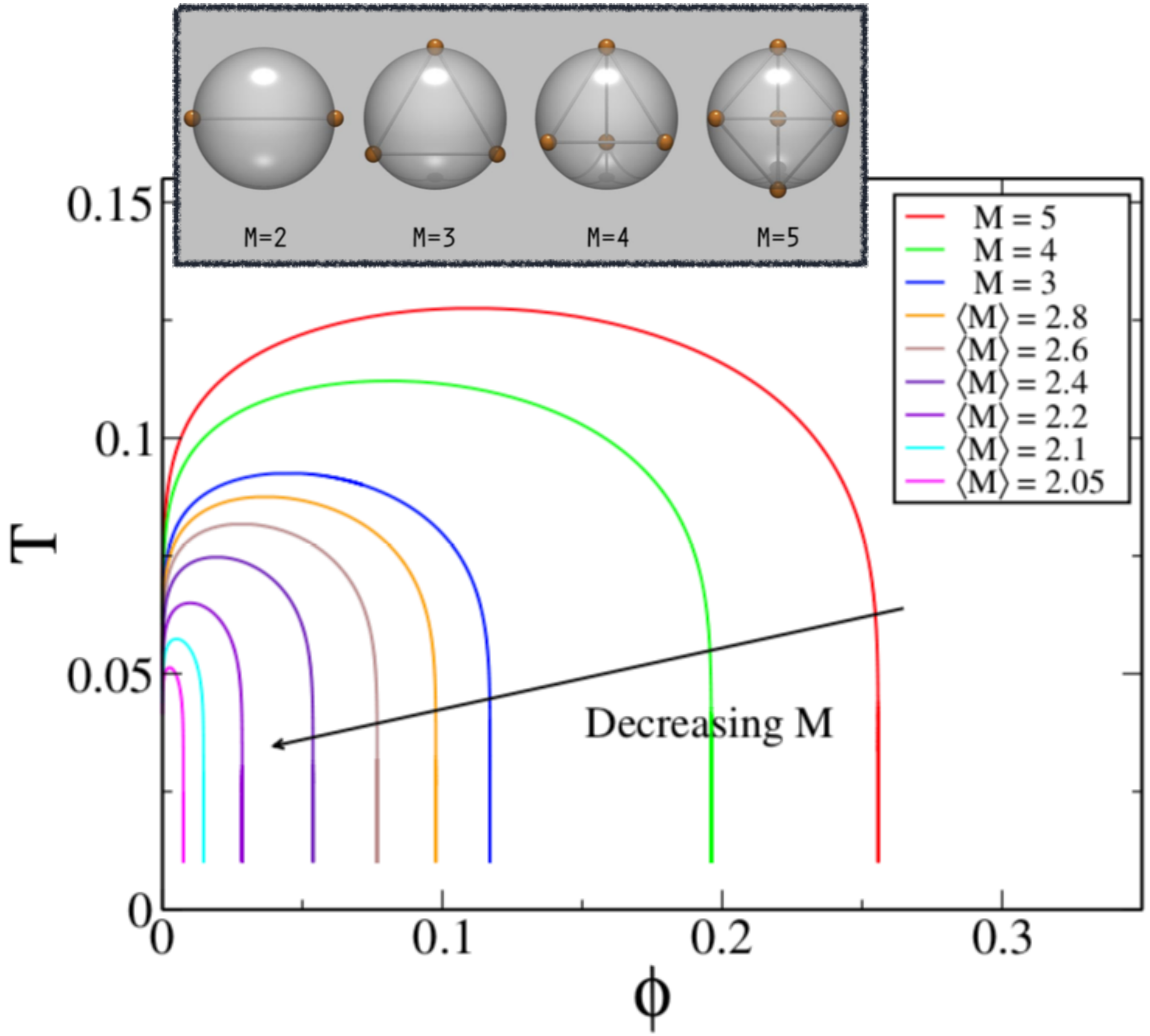
Nelson, Dhimolea and Reichert, Nature Reviews Drug Discovery 9, 767 (2010)

Phase separation of antibodies solutions

Phase transitions in human IgG solutions

Ying Wang, Aleksey Lomakin, Ramil F. Latypov, Jacob P. Laubach, Teru Hideshima, Paul G. Richardson, Nikhil C. Munshi, Kenneth C. Anderson, and George B. Benedek





exceptionally low critical volume fraction $\Phi \sim 0.07$

analogy with patchy particles

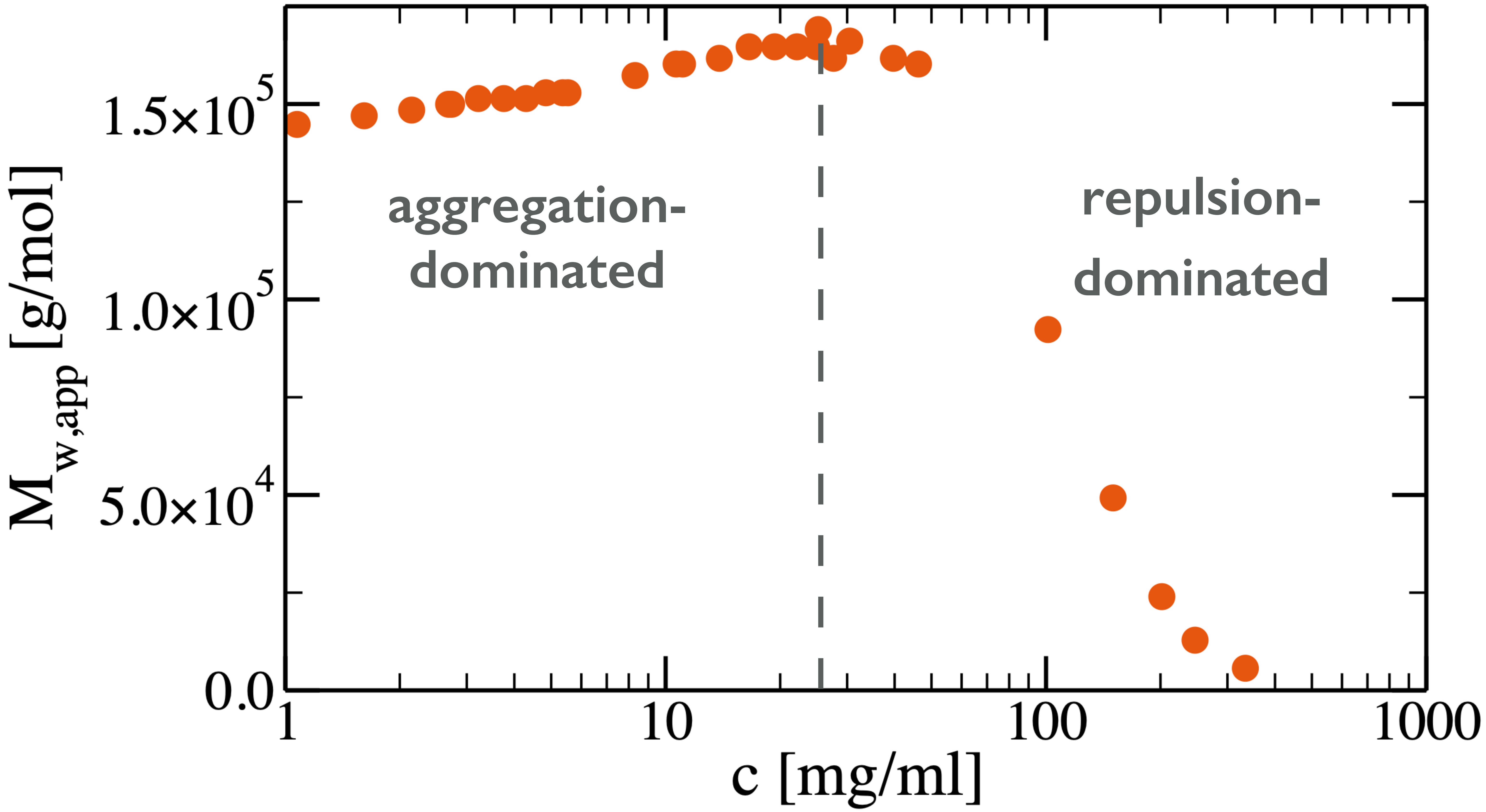
J. Chem. Phys. 139, 121904 (2013)

Bianchi, Largo, Tartaglia, EZ and F. Sciortino Phys. Rev. Lett. 97, 168301 (2006)

Experimental results for IGg4: Static light scattering

added 10mM NaCl, pH=6.5, T=25°C

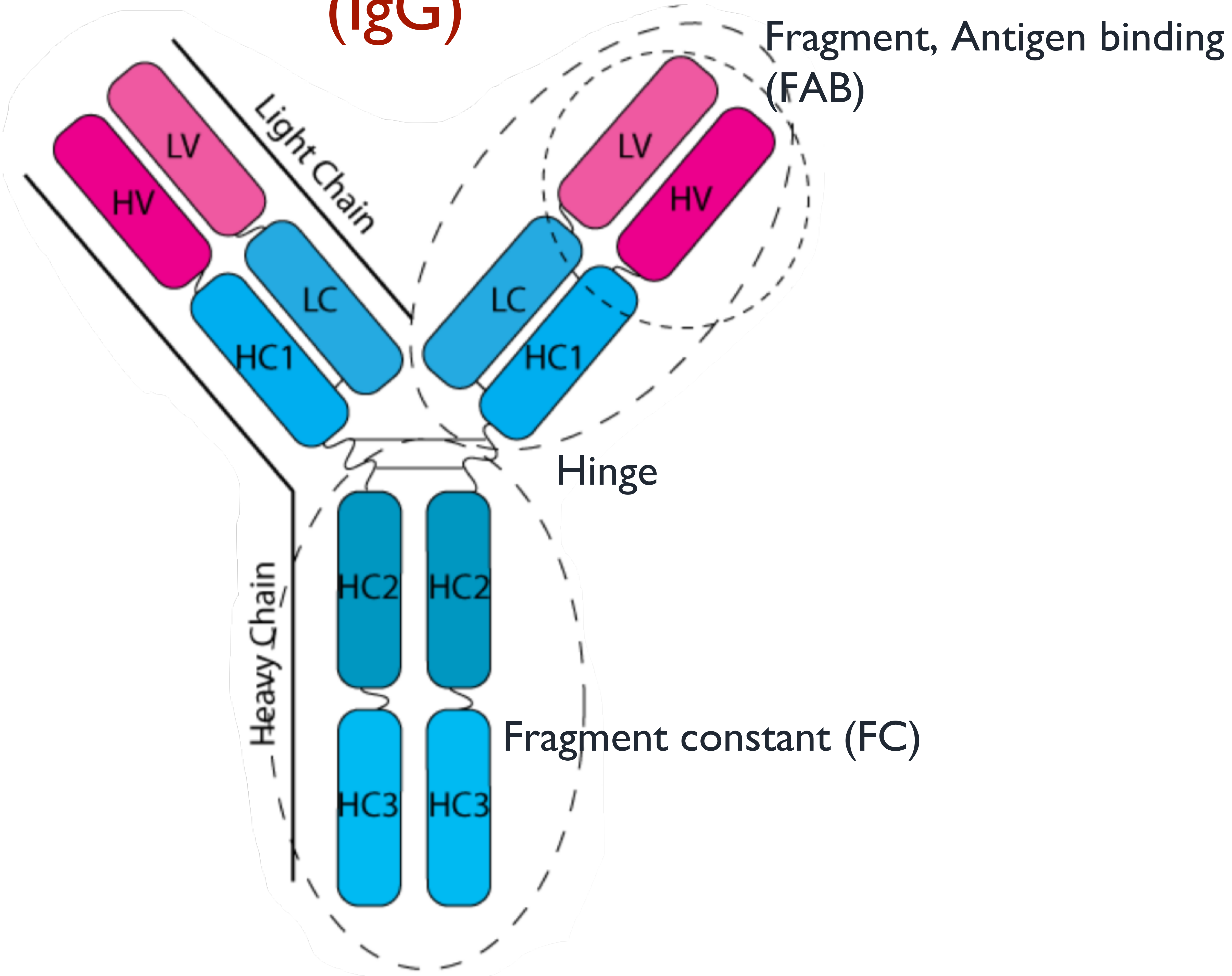
NO PHASE SEPARATION OBSERVED

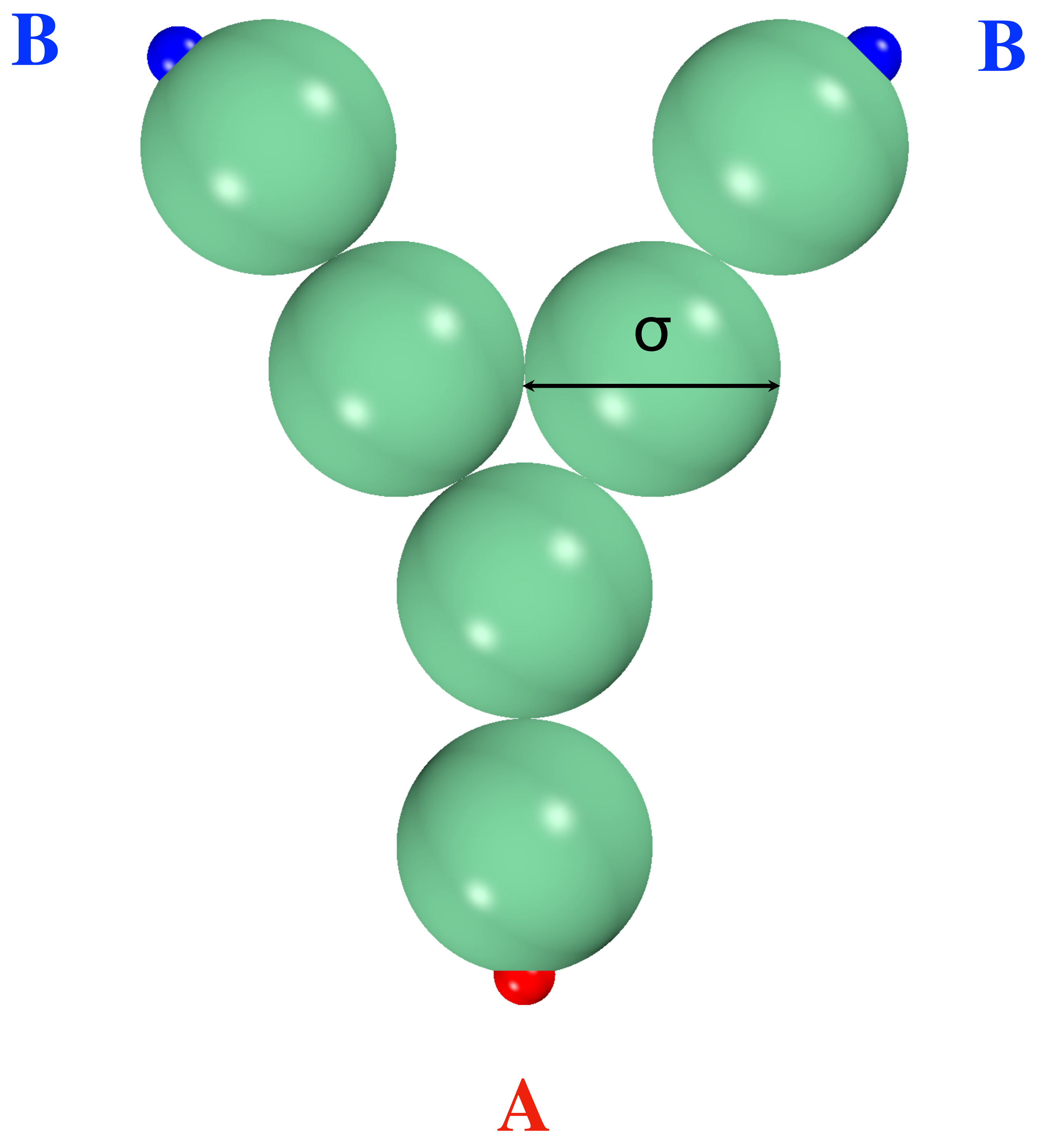


Apparent molecular weight shows a maximum at c ~ 25 mg/ml

A patchy model for antibodies

Immunoglobuline Gamma (IgG)





Main ingredients:

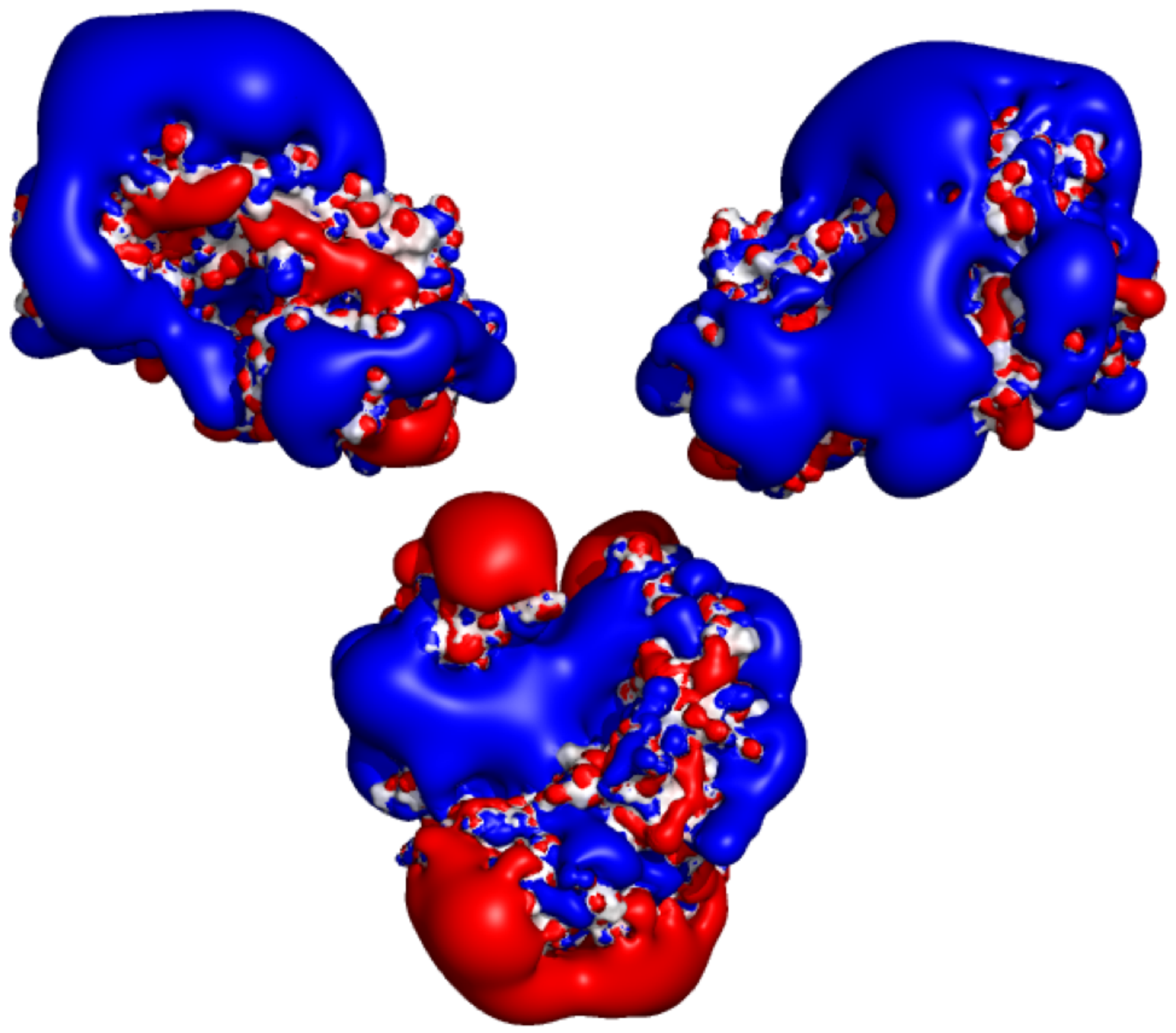
- 6 hard spheres in a symmetric Y-shape;
- rigid structure;
- decorated with three attractive patches:

2 patches of type A and 1 patch of type B

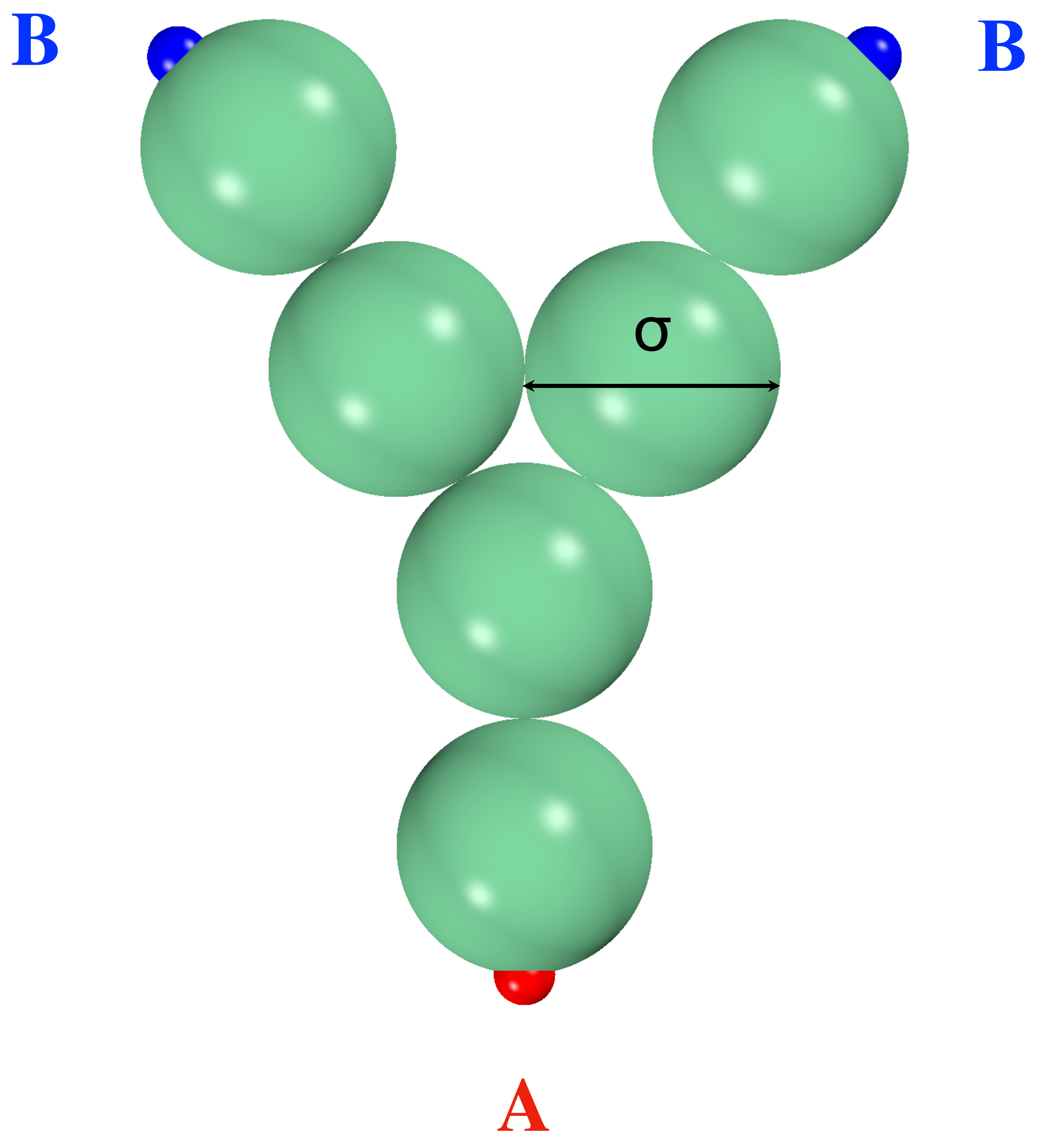
A patchy model for antibodies

electrostatic iso-surface calculations

positive arms



negative tail



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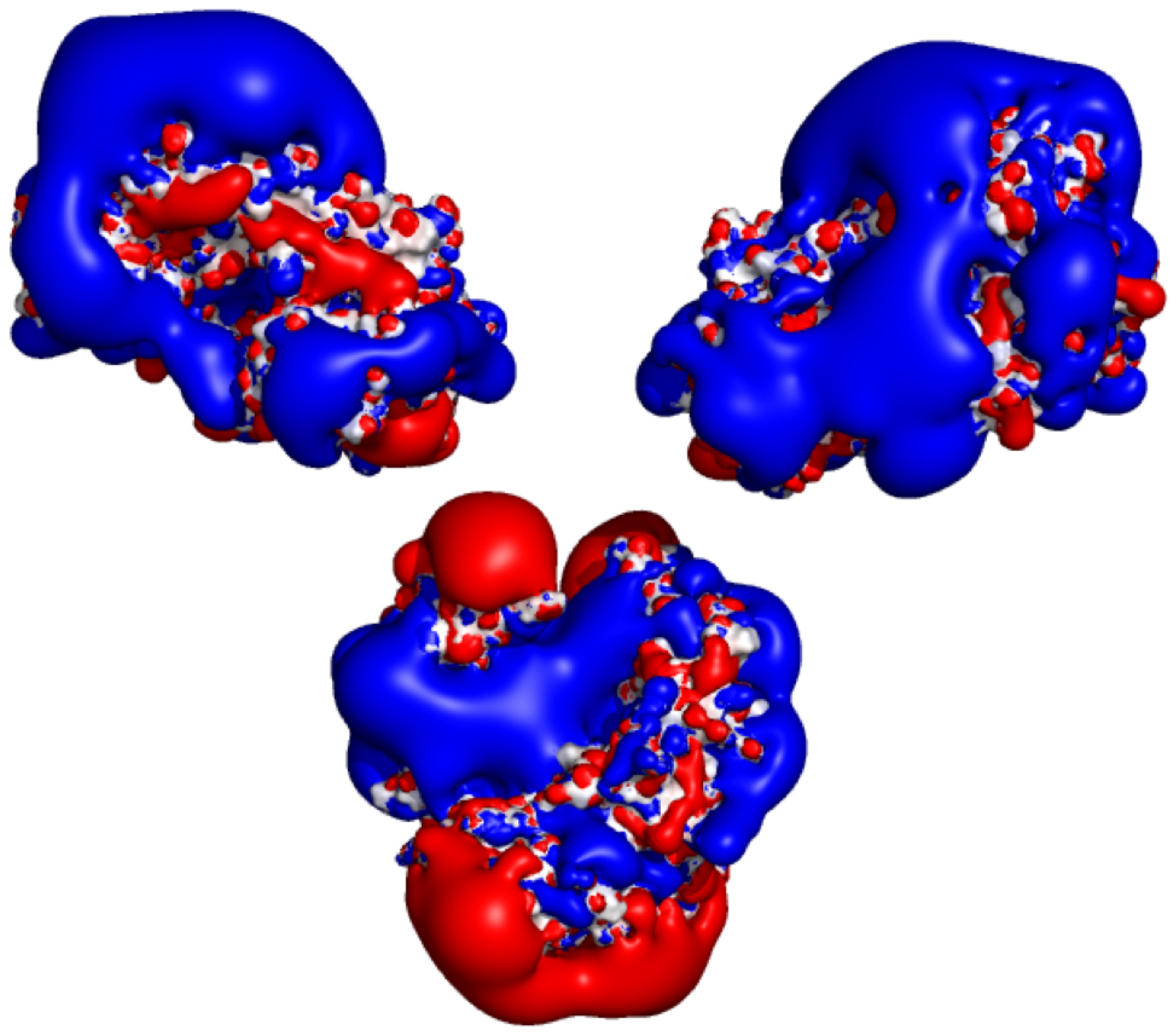

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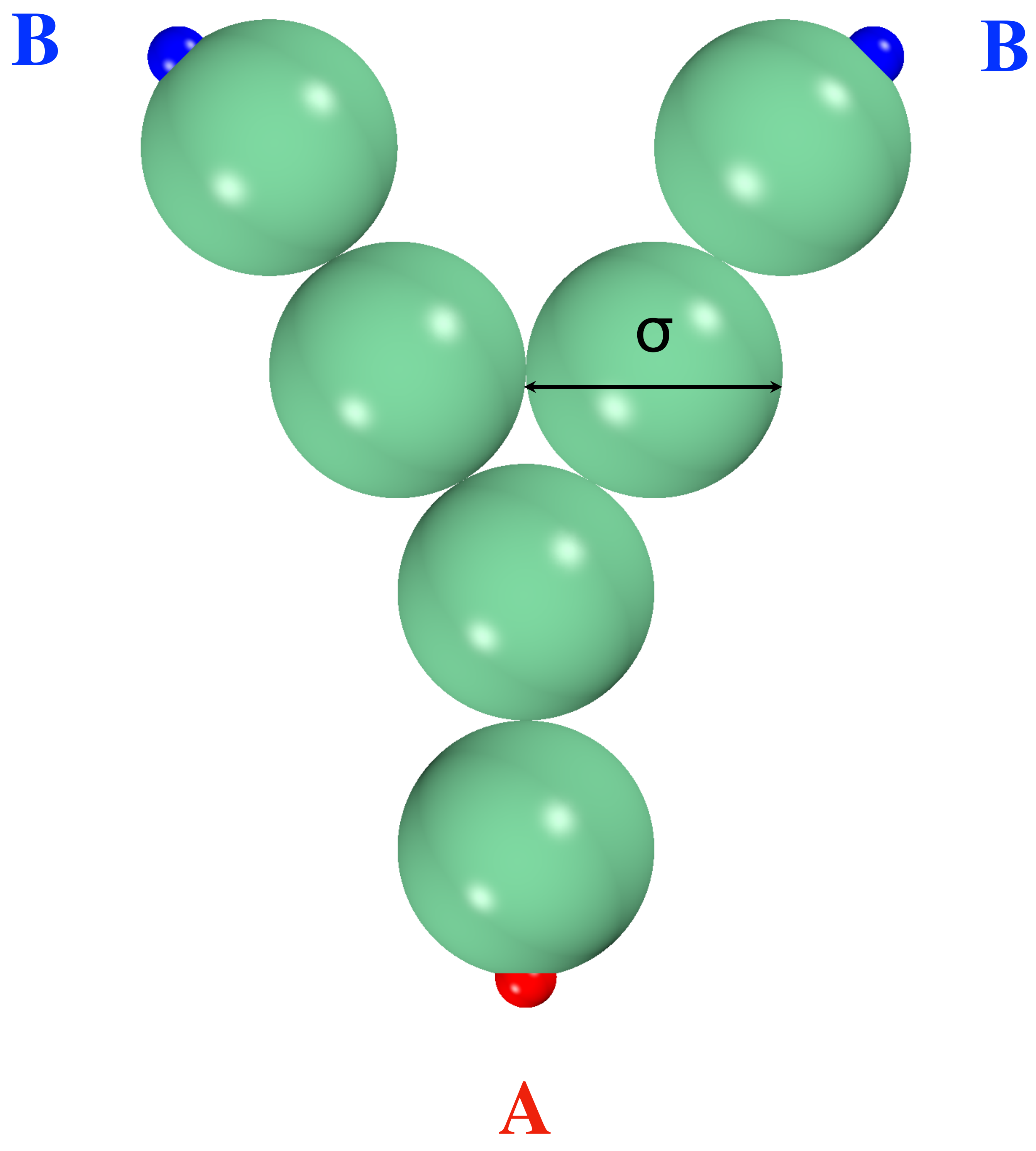


we consider only

AB (head-tail) bonds

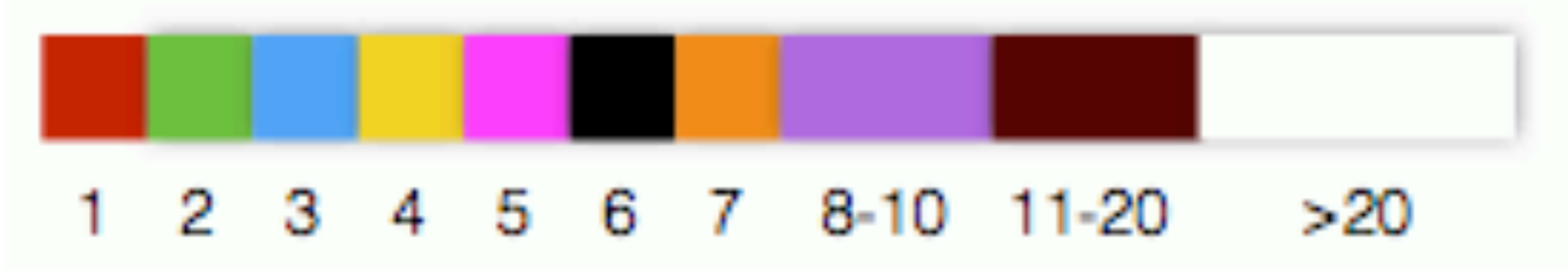
square-well attraction

fulfilling *one-bond-per-patch* condition

Monte Carlo Simulations

$k_B T/\varepsilon = 0.0775$

increasing concentration



cluster size colour bar

- finite-size clusters only
- weak aggregation also at very low temperatures
- no percolation is found; no phase separation

A patchy model for antibodies

Hyperbranched polymer model

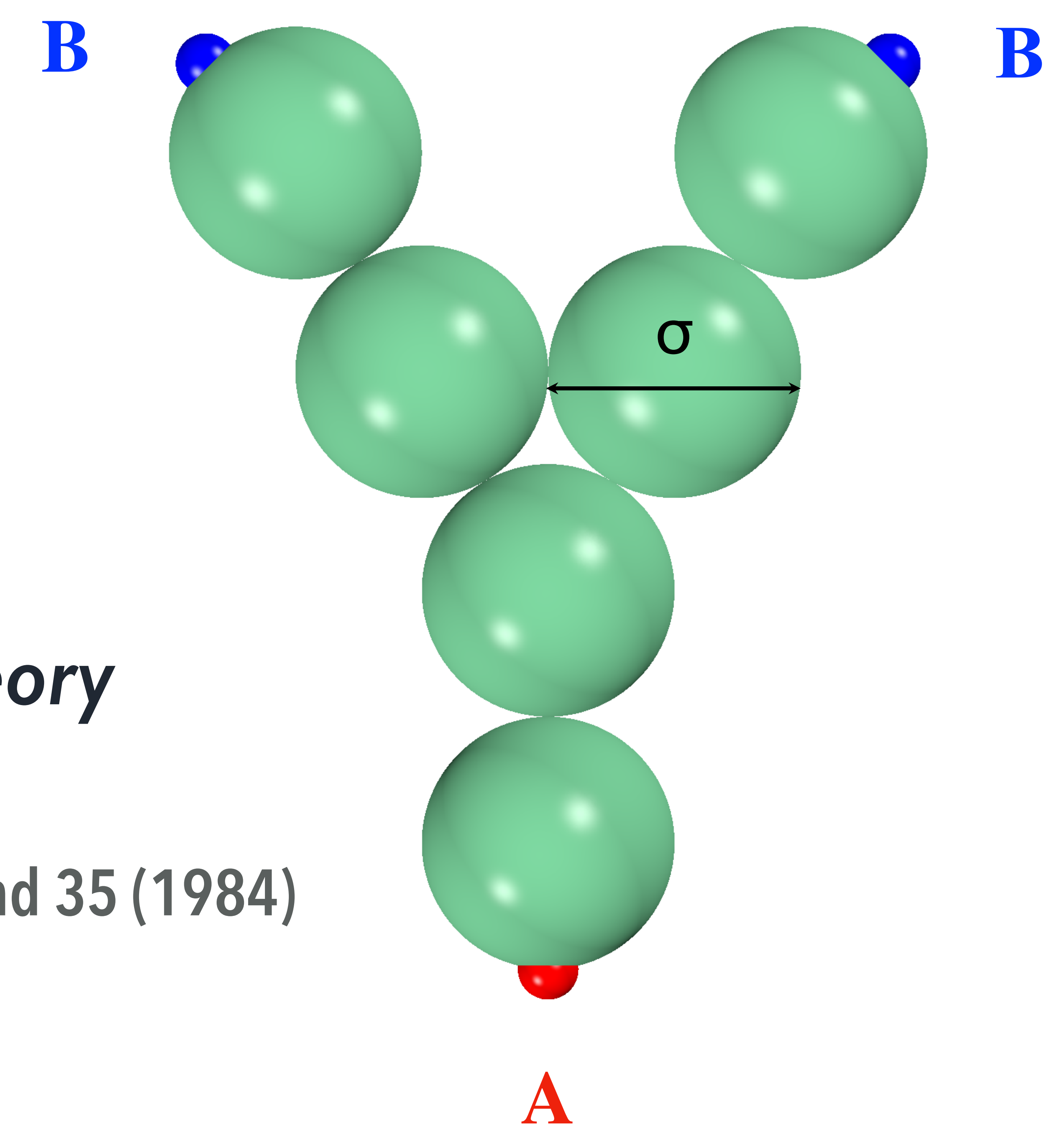
ANALYTICALLY SOLVABLE:

thermodynamic properties ⟶ *Wertheim theory*

M.S. Wertheim, J. Stat. Phys. 35, 19 and 35 (1984)

connectivity properties ⟶ ***hyperbranched polymers theory***

M. Rubinstein and R.H. Colby Polymer Physics (Oxford University Press), Oxford, 2003



AB (head-tail) bonds

square-well attraction

fulfilling *one-bond-per-patch* condition

Comparison between Wertheim theory and Monte Carlo Simulations

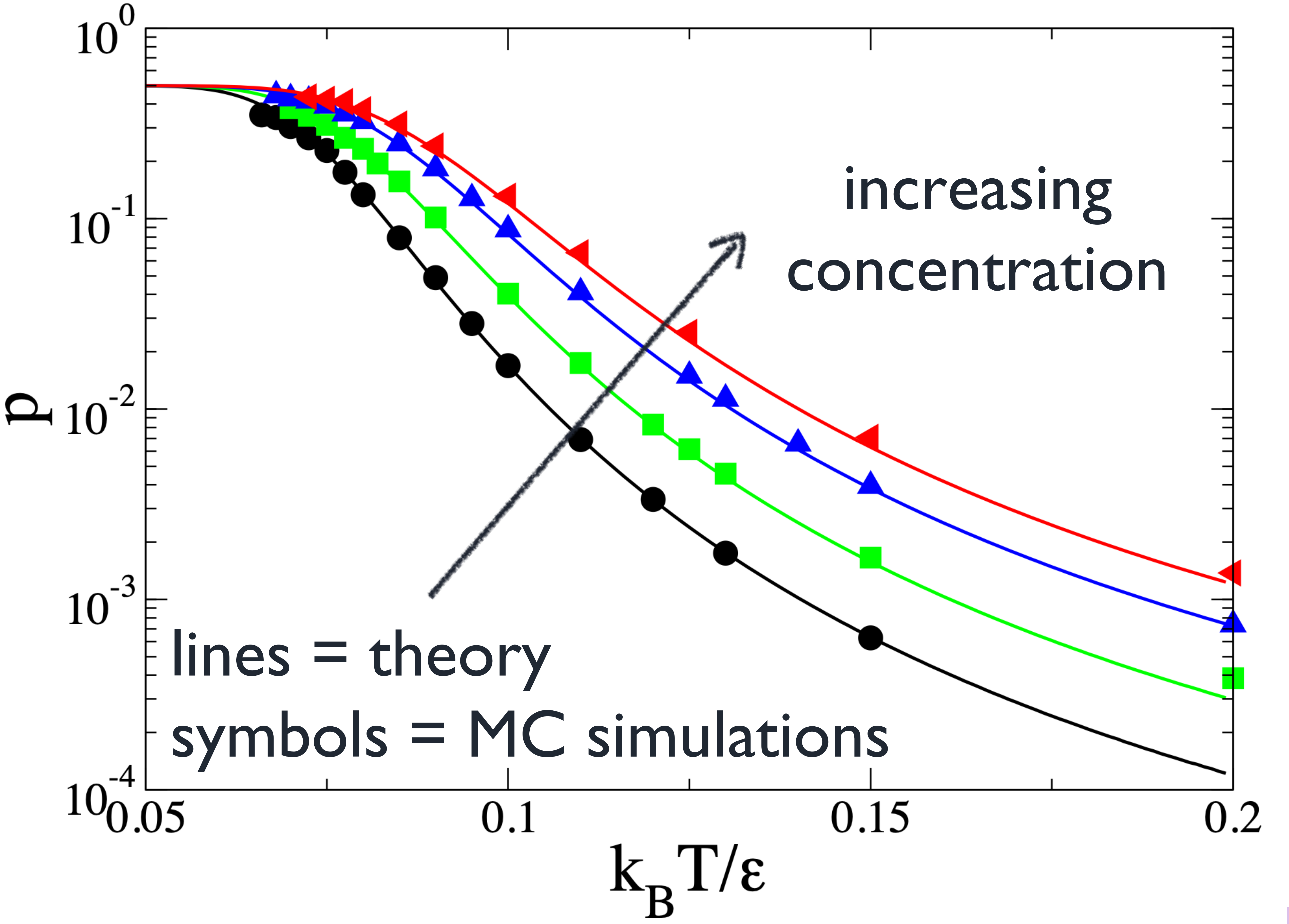
Thermodynamic perturbative approach

free energy $F = F_{HS} + F_b$ bond contribution

hard sphere reference term

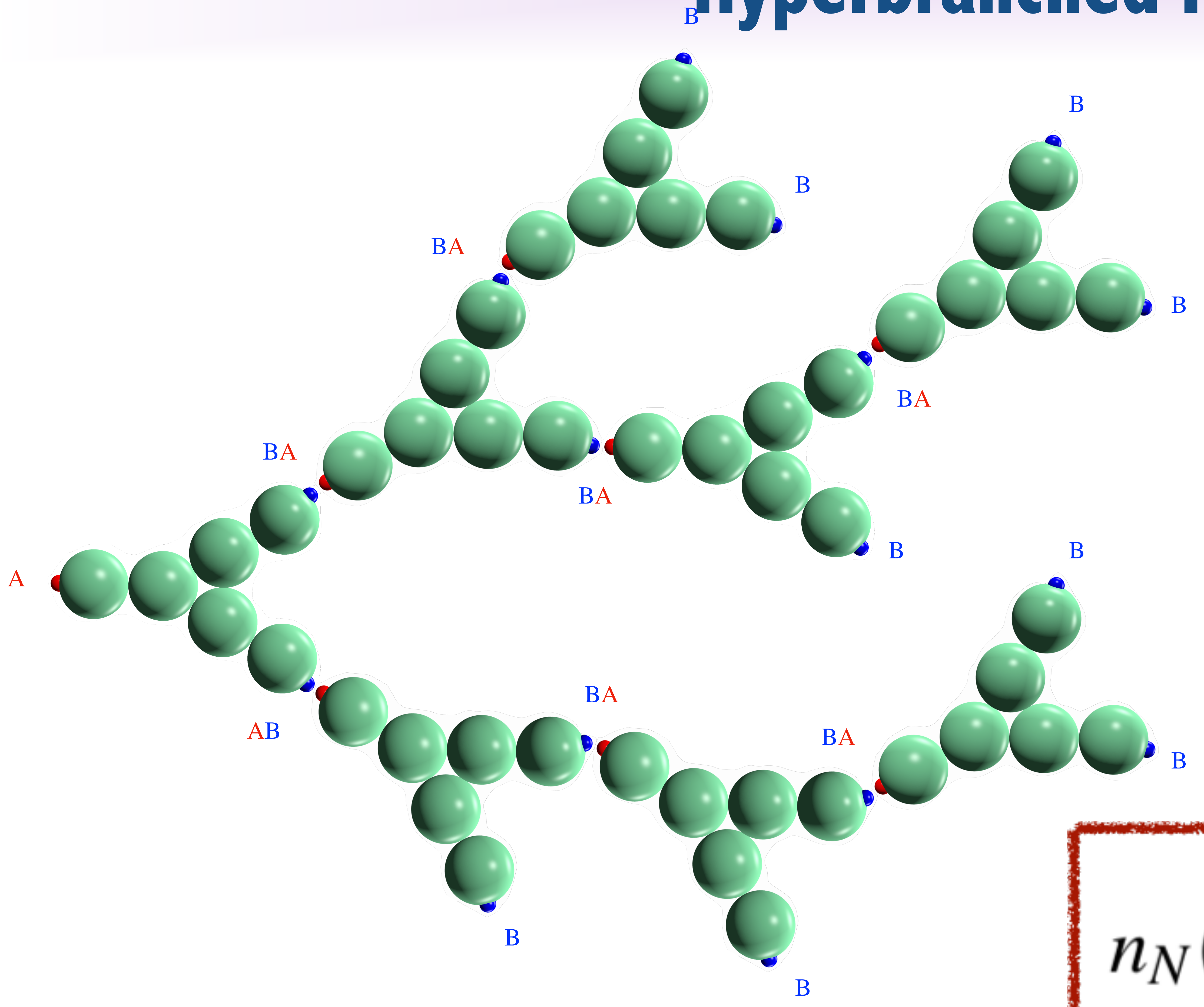
$$\beta \frac{F_b}{N} = 2 \ln X_B + \ln X_A - X_B - \frac{X_A}{2} + \frac{3}{2}$$

bond probability $p \equiv p_B = 1 - X_B$



very good agreement between theory and simulations at all temperatures and concentrations

Hyperbranched Polymers (Flory-Stockmayer)



AB_{f-1} with $f \geqslant 2$ and only AB bonds

Model for branching without gelation

cluster size distribution

$$n_N(p)) = \frac{[(f-1)N]!}{N![(f-2)N+1]!} p^{N-1}(1-p)^{(f-2)N+1}$$

for our IgG4 model

***f* = 2**

WE USE $p \equiv p_B$ FROM WERTHEIM THEORY

M. Rubinstein and R.H. Colby Polymer Physics (Oxford University Press), Oxford, 2003

Comparison with experiments: calculating S(0)

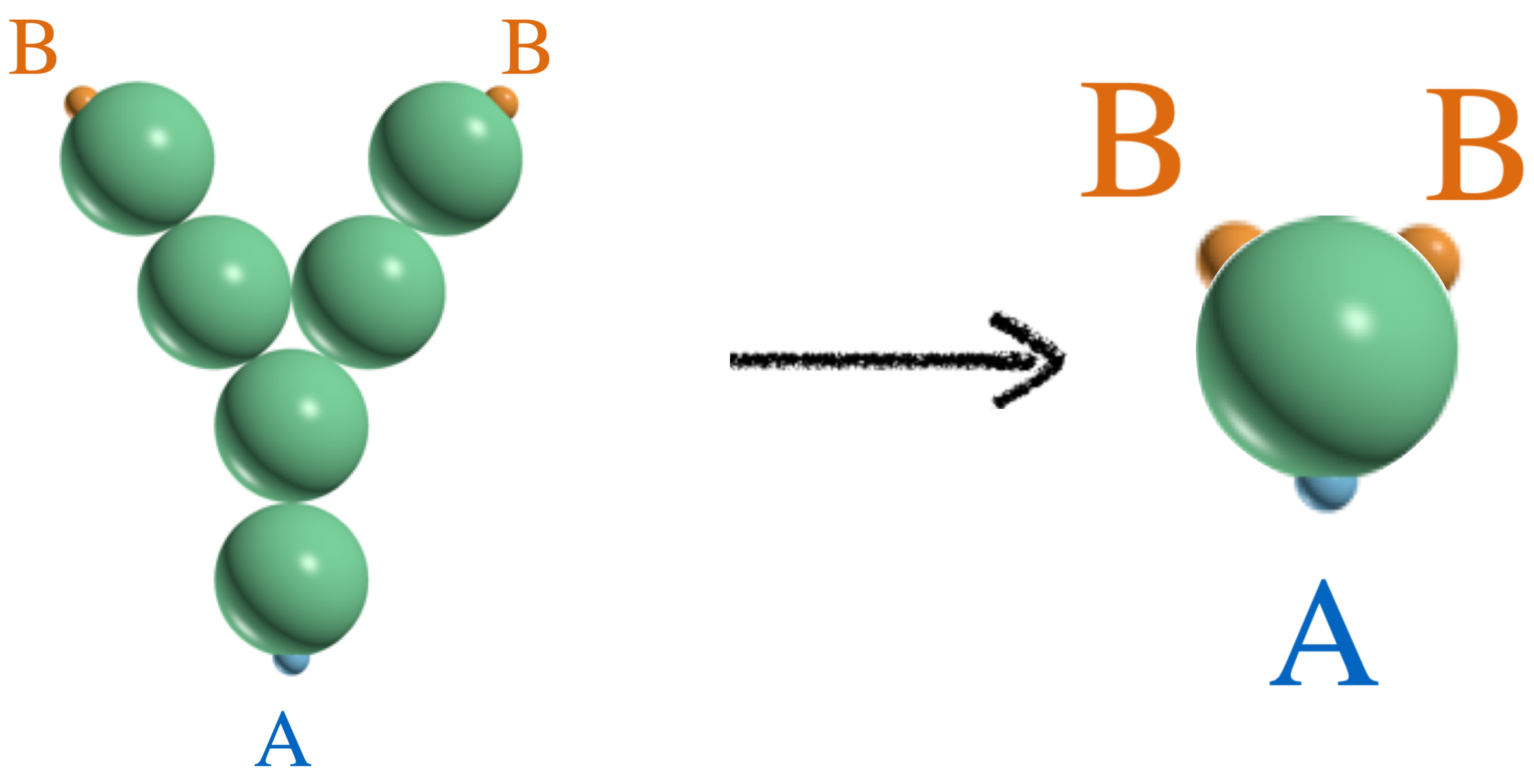
We use Wertheim theory to calculate $S(0) = \left(\frac{d\beta P}{d\rho}\right)^{-1} = \left(\frac{d\beta P_{HS}}{d\rho} + \frac{d\beta P_b}{d\rho}\right)^{-1}$

dependent on temperature and packing fraction of reference hard sphere system Φ_{HS}

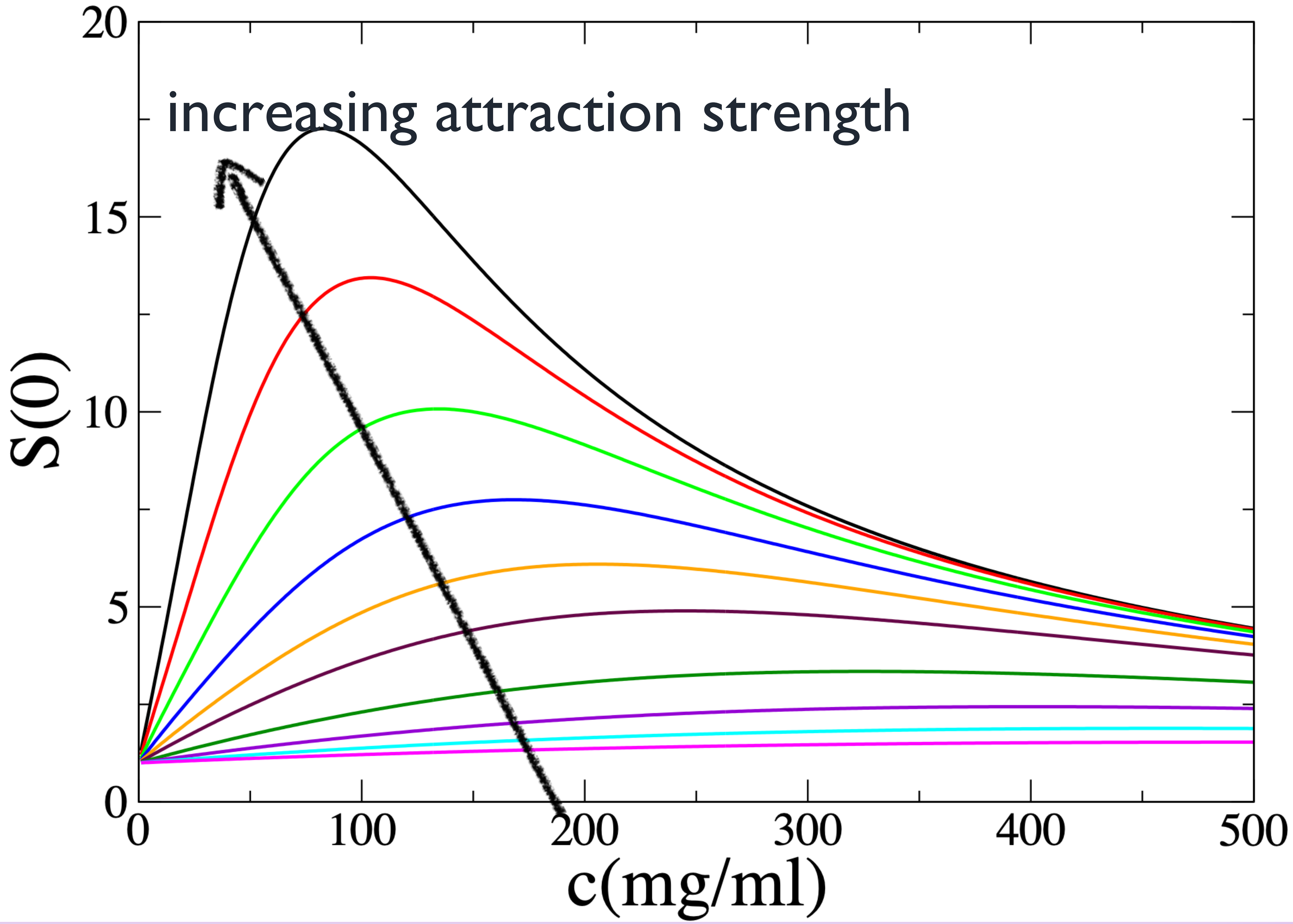
Carnahan-Starling

bond contribution

we use effective patchy spheres



which effective diameter?



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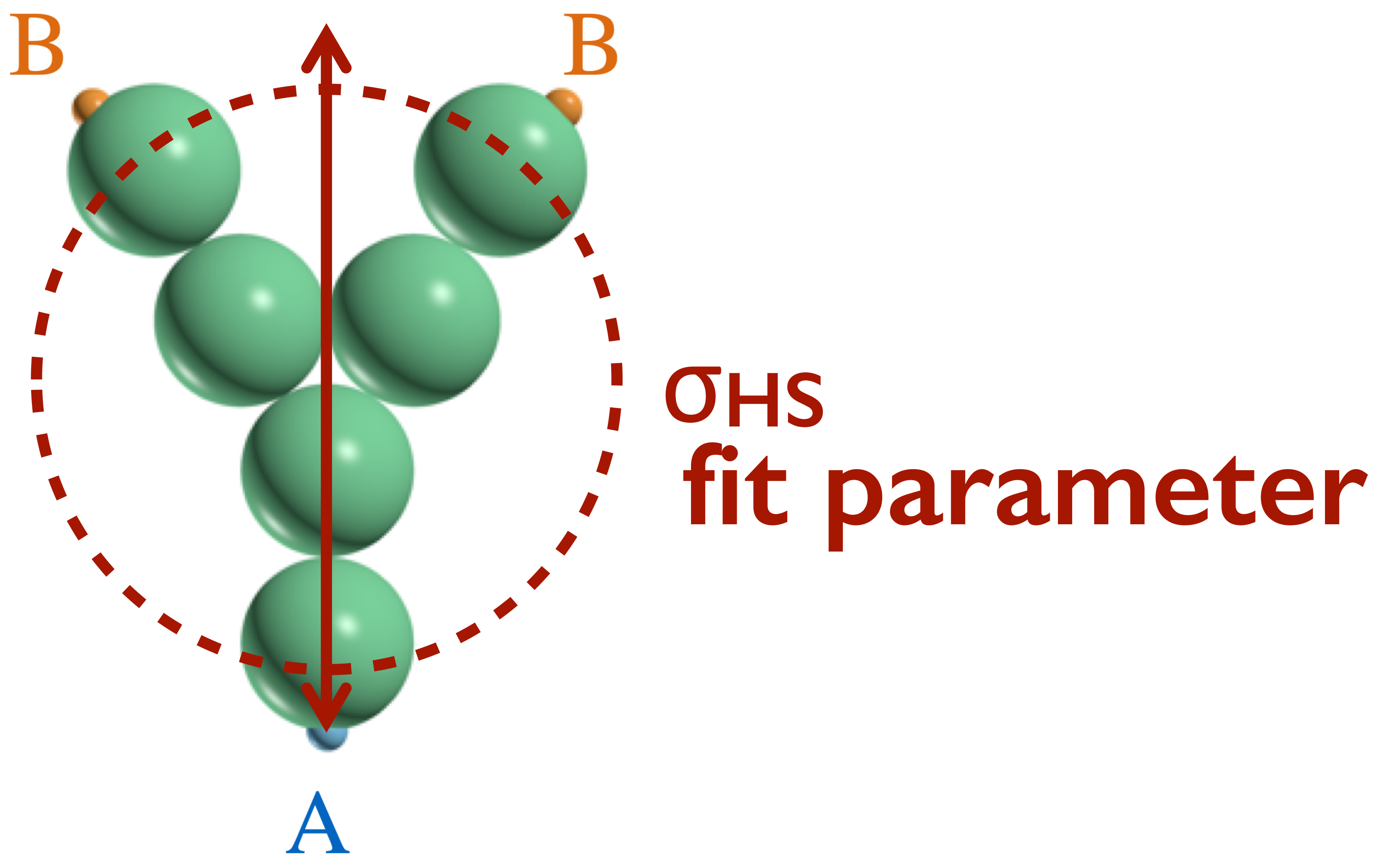
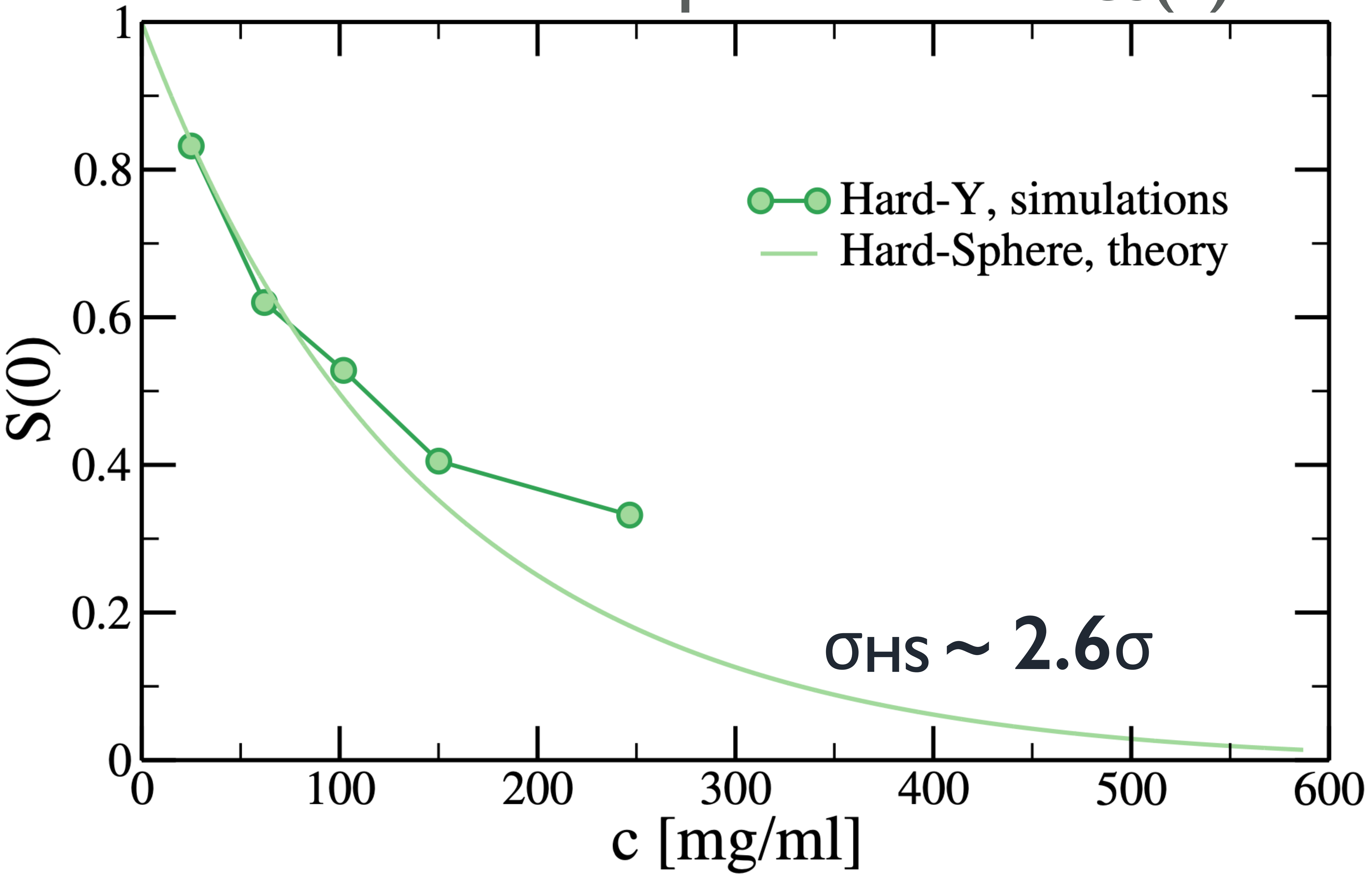
Carnahan-Starling — bond contribution

dependent on temperature and packing fraction of reference hard sphere system Φ_{HS}

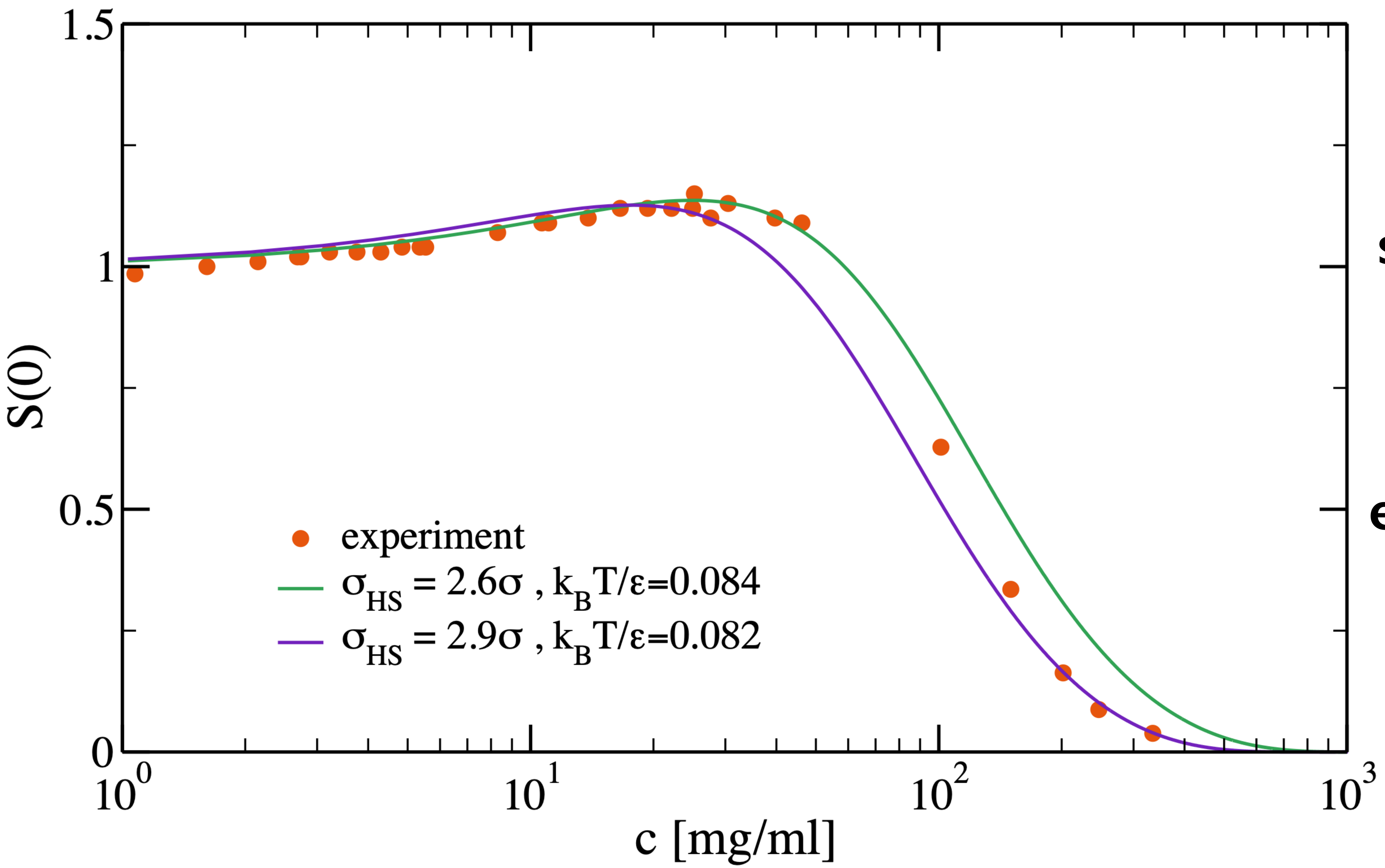


hard Y comparison with $S_{CS}(0)$



conversion to experimental units based on hydrodynamic radius

Comparison with experiments: calculating S(0)



bond attraction strength ~ 12 kBT

effective size much smaller than geometric Y size

quite good description of the data with the YAB patchy model

patchy interactions are not enough: **importance of the shape**

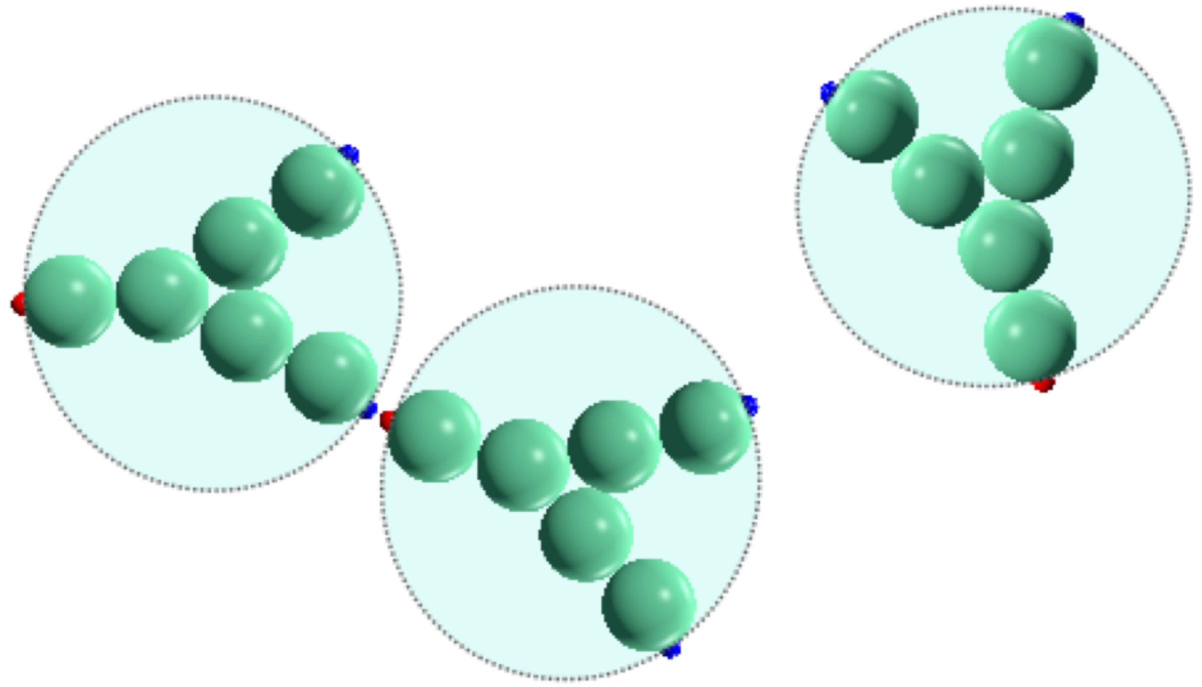
N. Skar-Gislinge, M. Ronti, T. Garting, C. Rischel, P. Schurtenberger, EZ and A. Stradner Molecular Pharmaceutics 16, 2394 (2019).

From static to dynamic properties

Coarse-graining strategy

Static Properties

building blocks: ***patchy monomers***



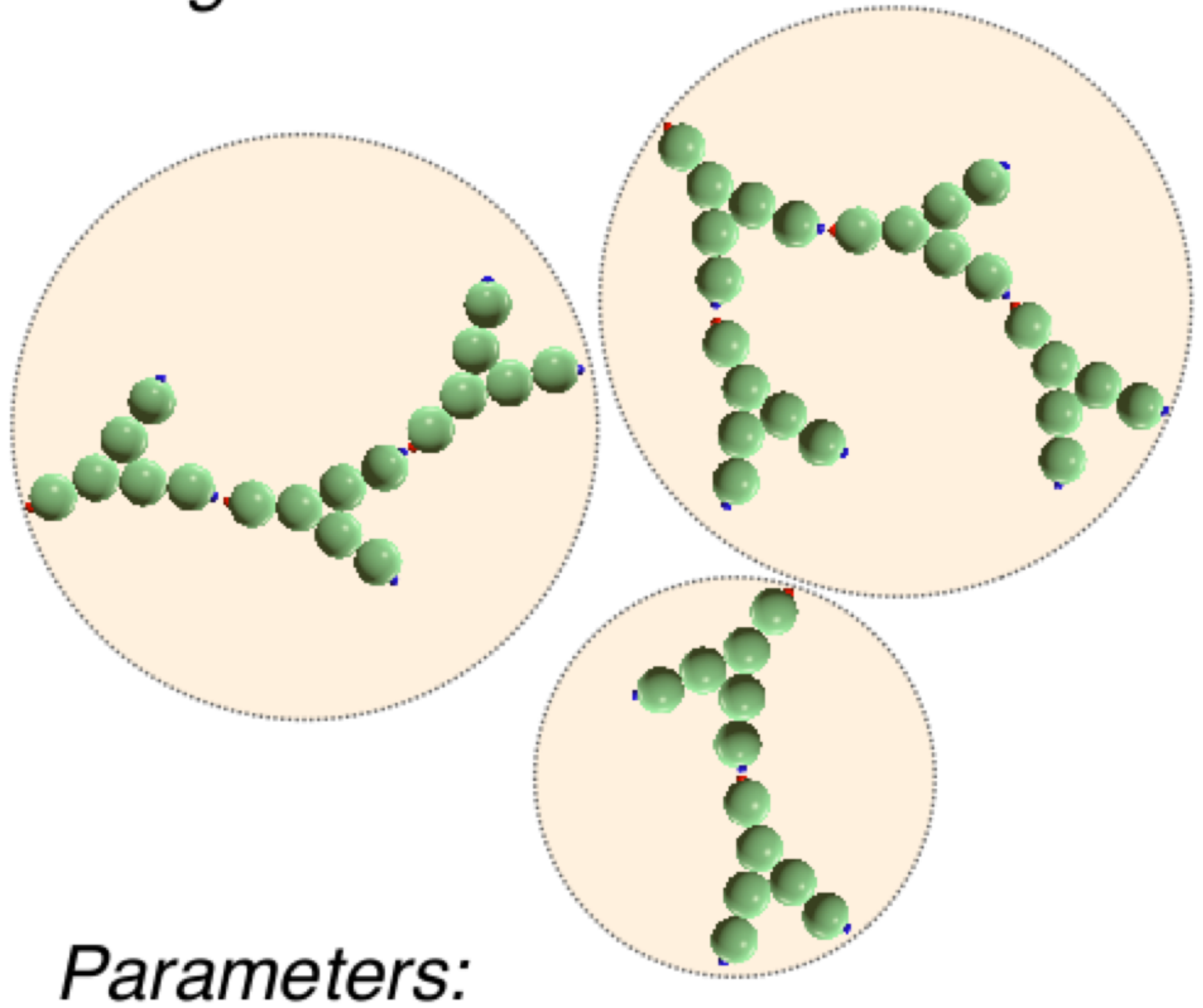
Parameters:

- Wertheim Theory: σ_{HS}, $k_B T/\varepsilon$
- Hyperbranched Polymer Theory: p

$\langle s \rangle_{w,\,app}$, $n(s)$

Dynamic Properties

building blocks: ***HS or SHS clusters***



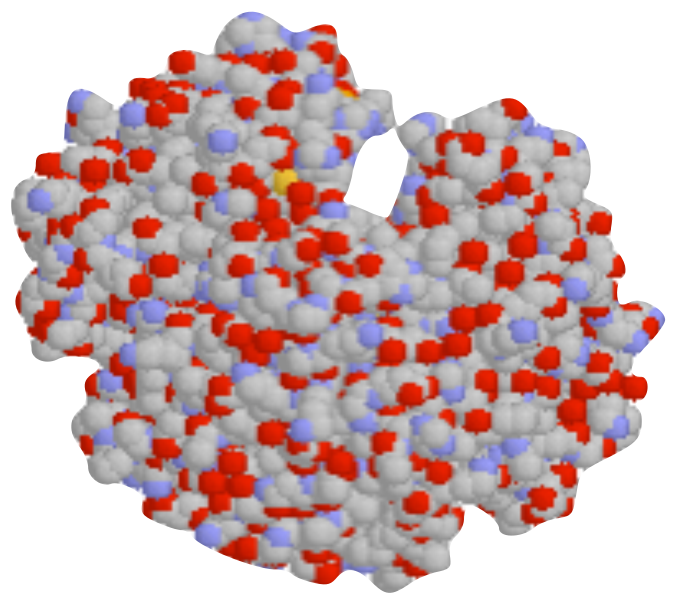
Parameters:

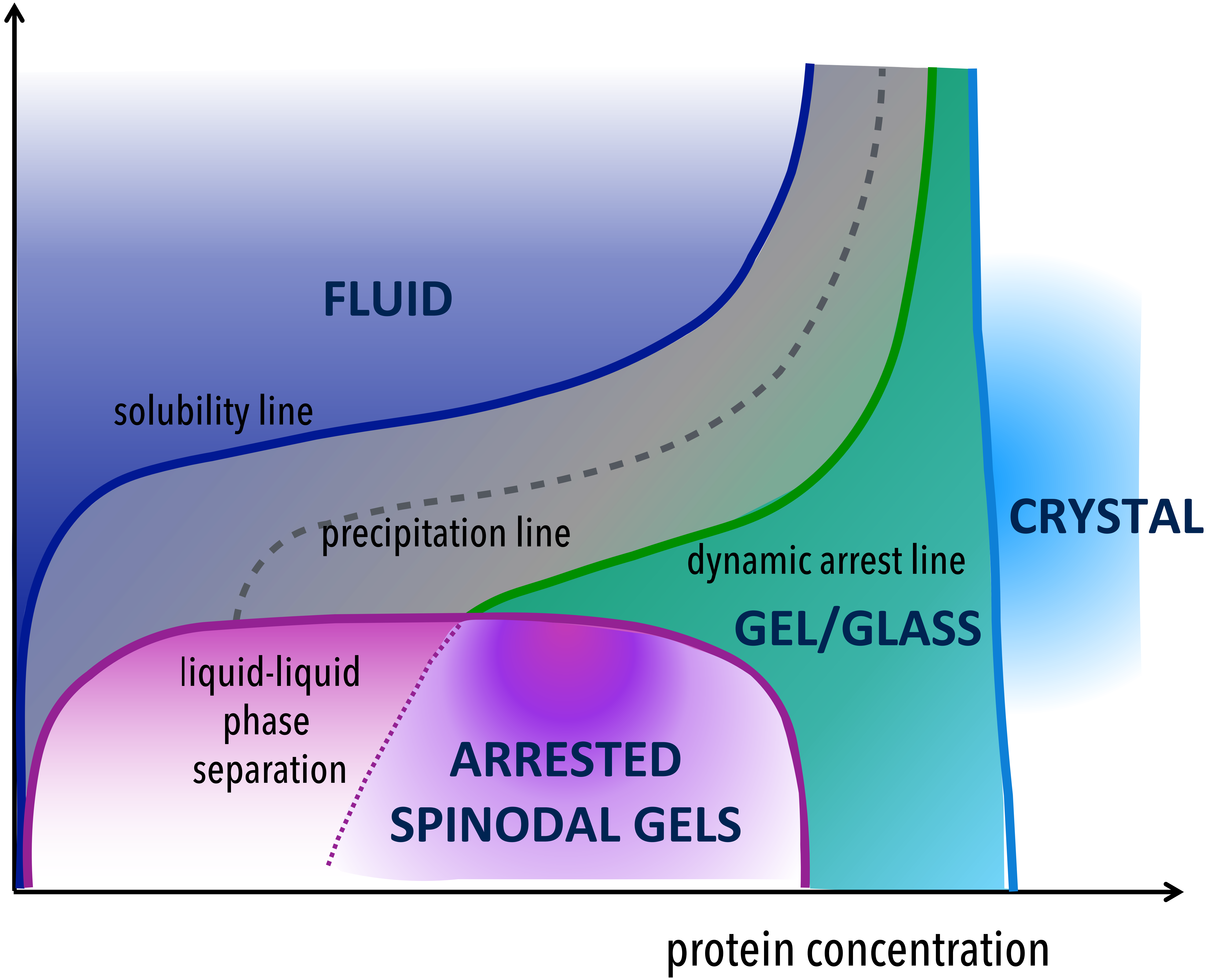
- HS Model: Φ_{HS} *of clusters*
- Sticky HS Model: τ

$\langle R_h \rangle_{z,\,app}$, η_r

N. Skar-Gislinge, M. Ronti, T. Garting, C. Rischel, P. Schurtenberger, EZ and A. Stradner Molecular Pharmaceutics 16, 2394 (2019).

Back to generic phase diagram: interplay with dynamics



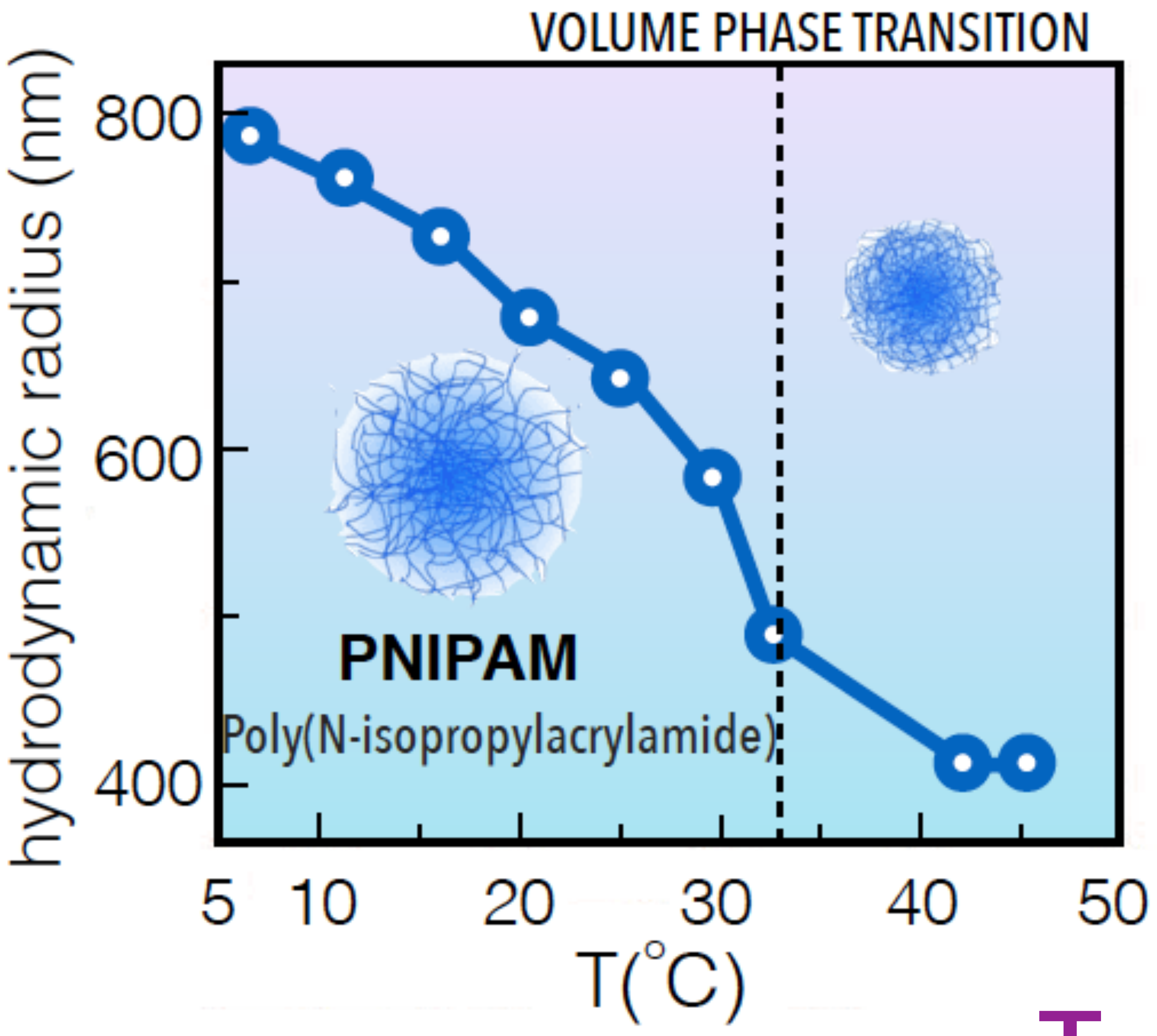


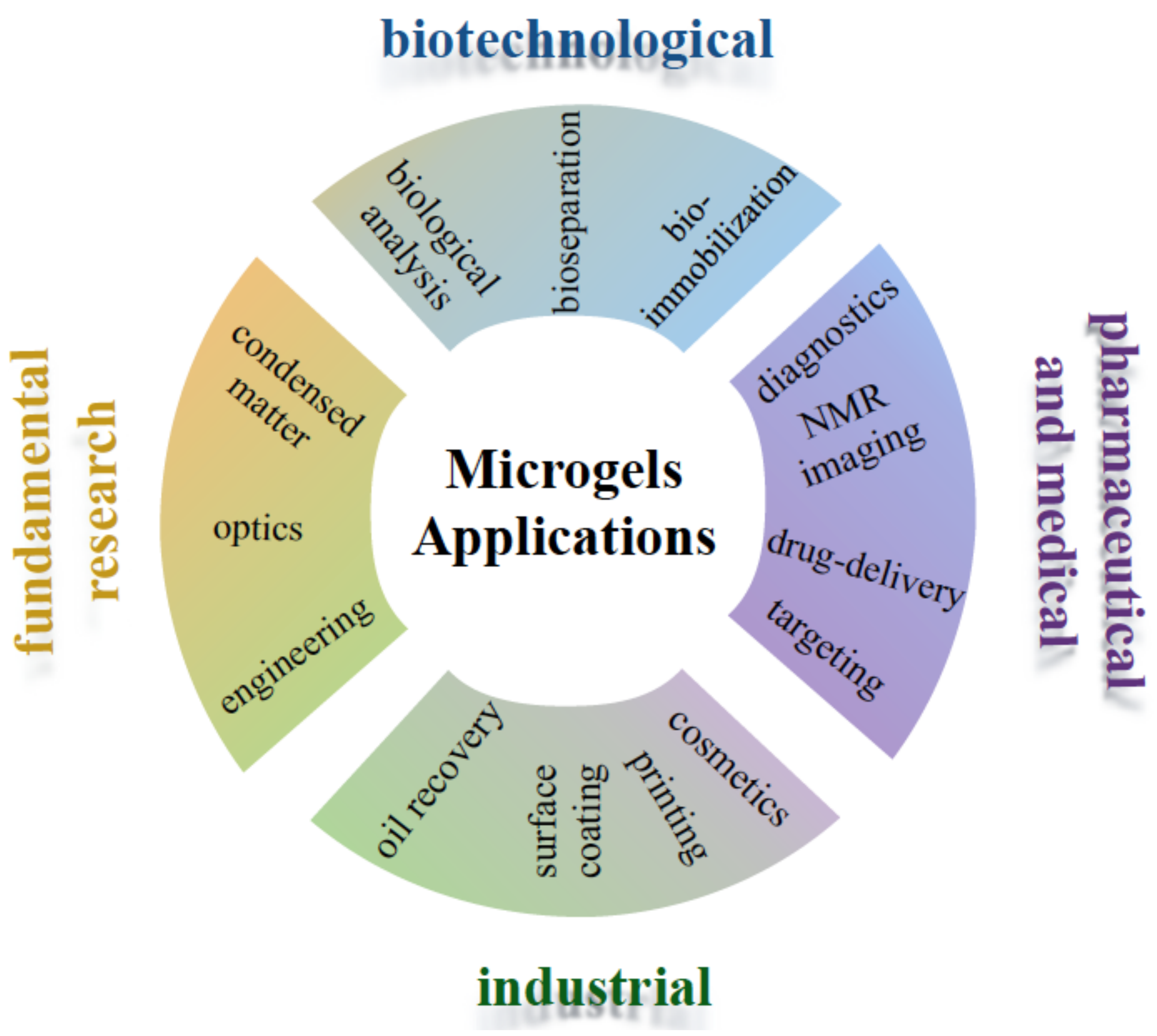
McManus, Charbonneau, EZ, Asherie protein self-assembly review 2016

Why microgels?

COLLOIDS MADE BY CROSS-LINKED POLYMER NETWORKS

- responsive
- easy to synthesize
- versatile for applications

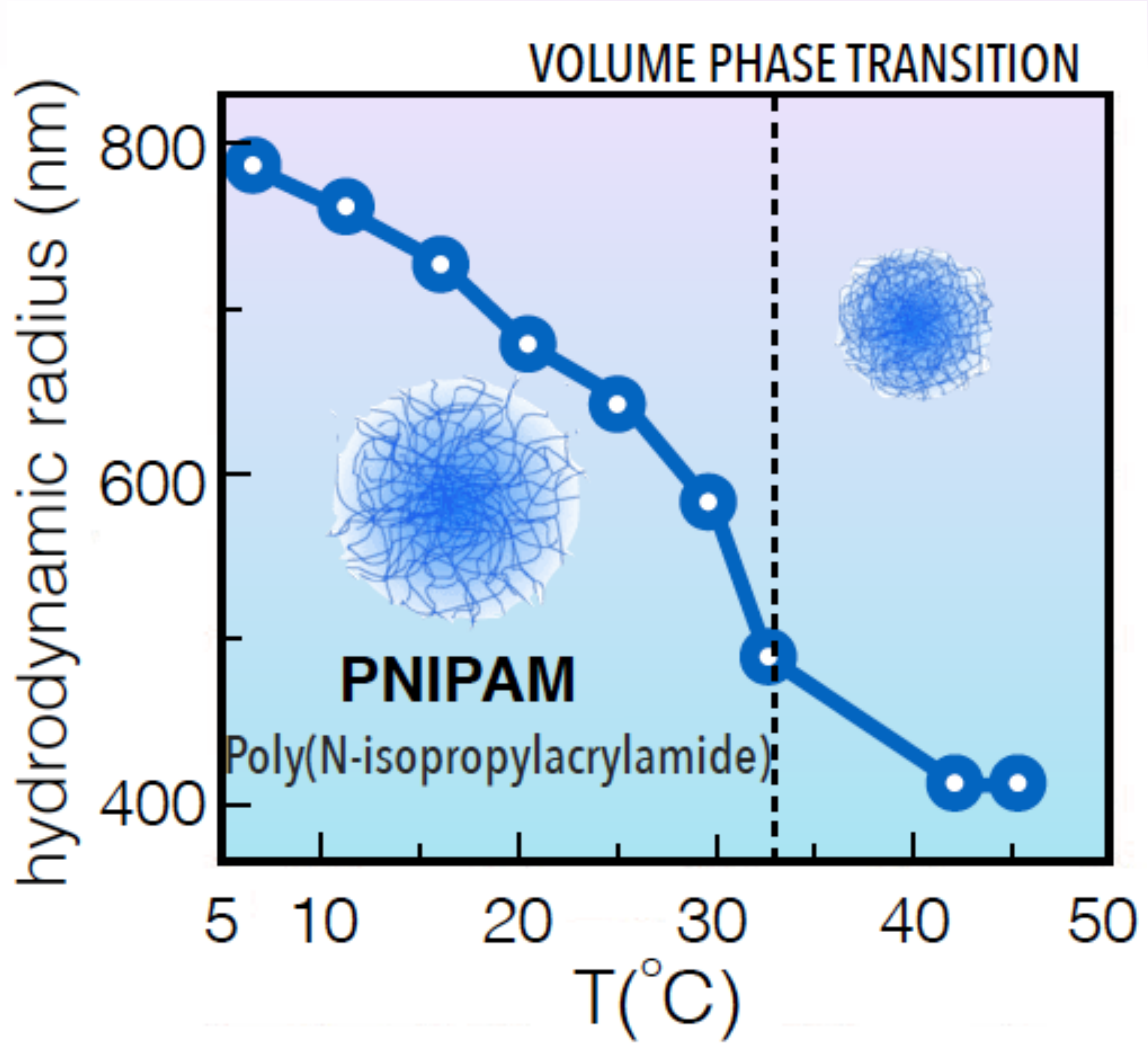


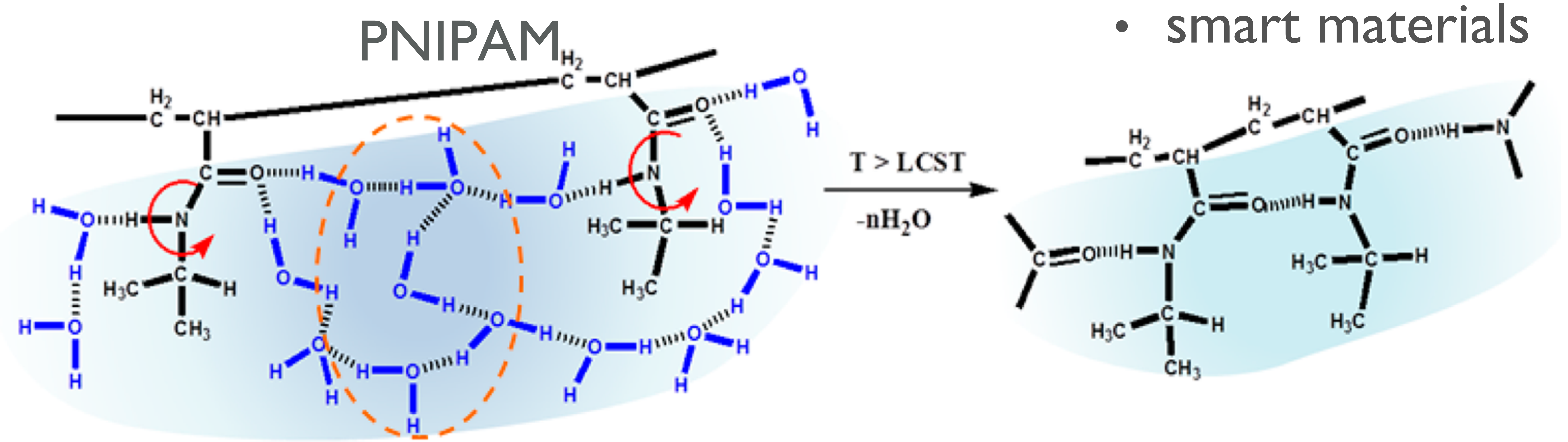


$T_{VPT} \sim 32°C$

Volume phase transition

- fully reversible
- smart materials

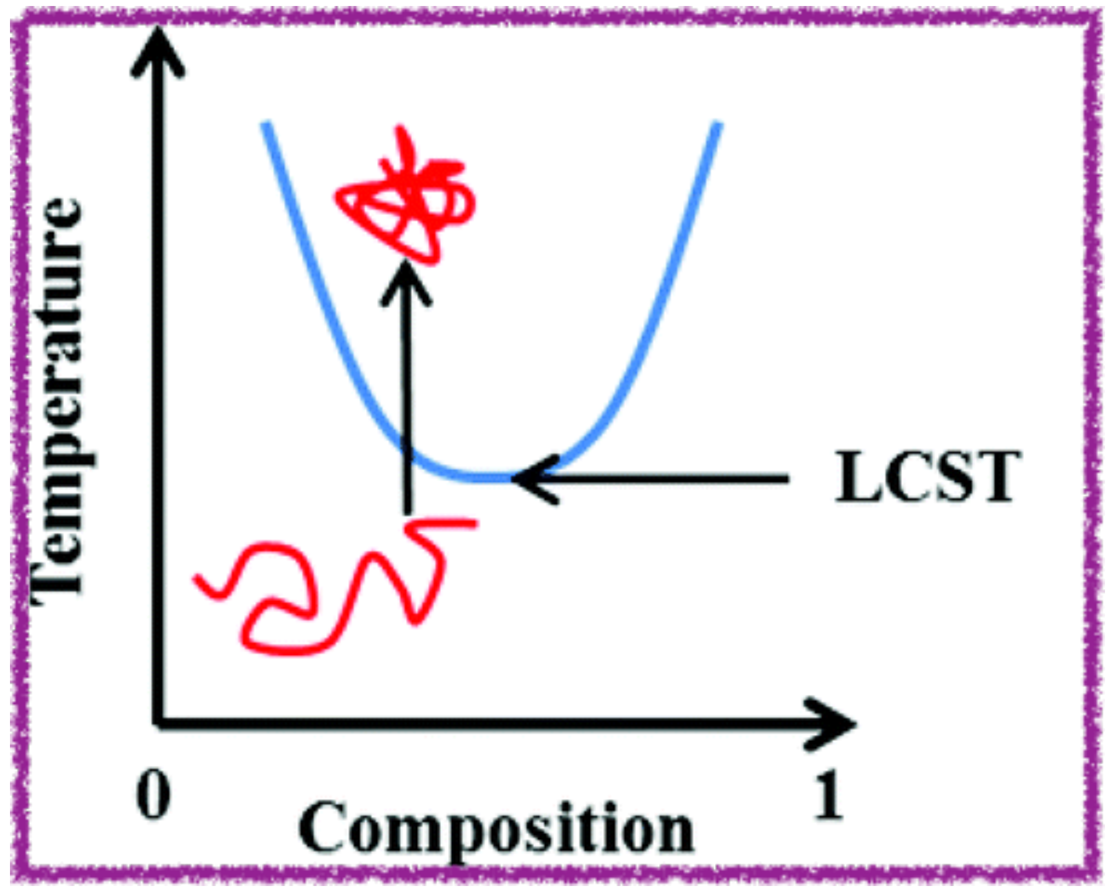


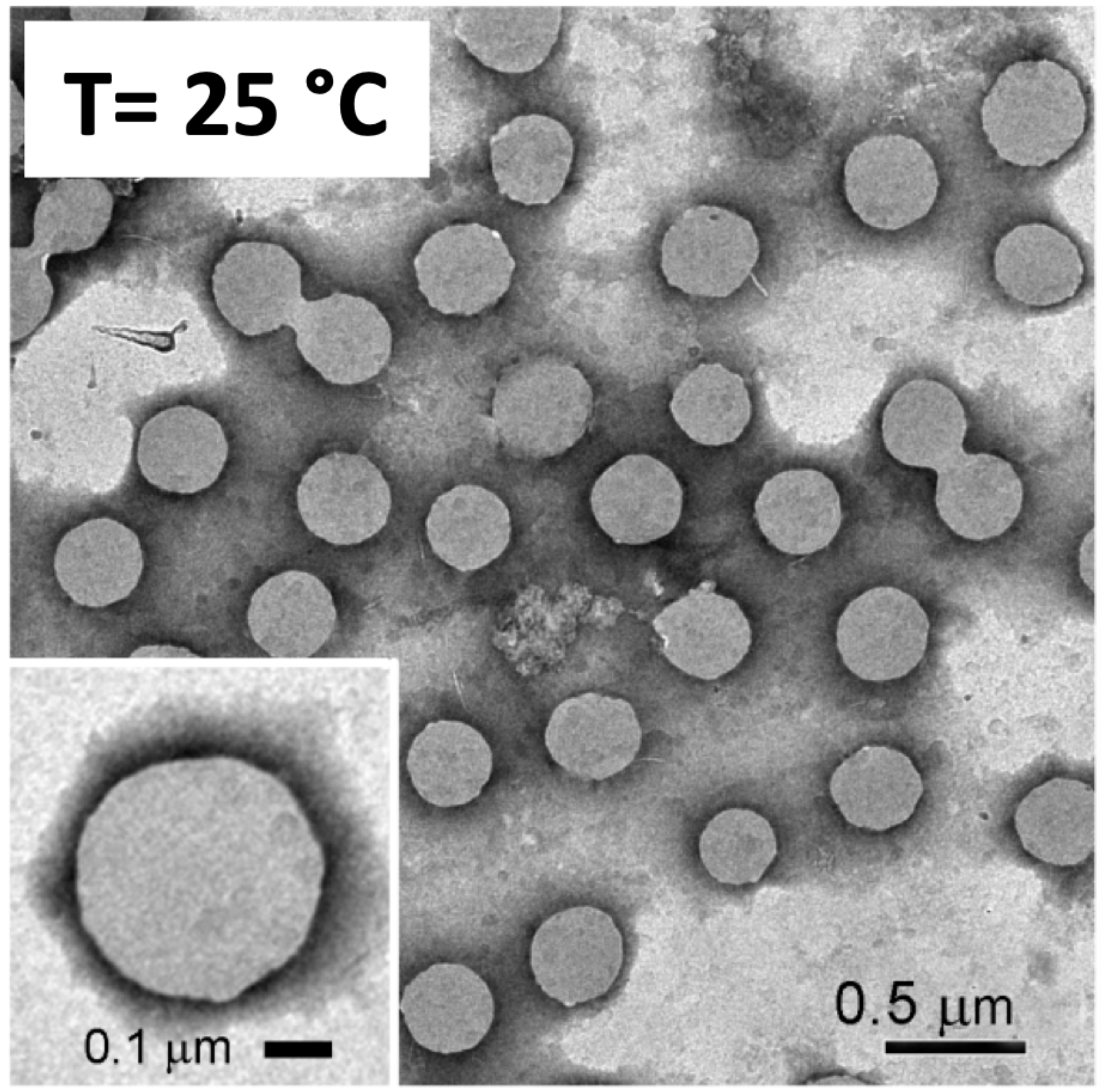


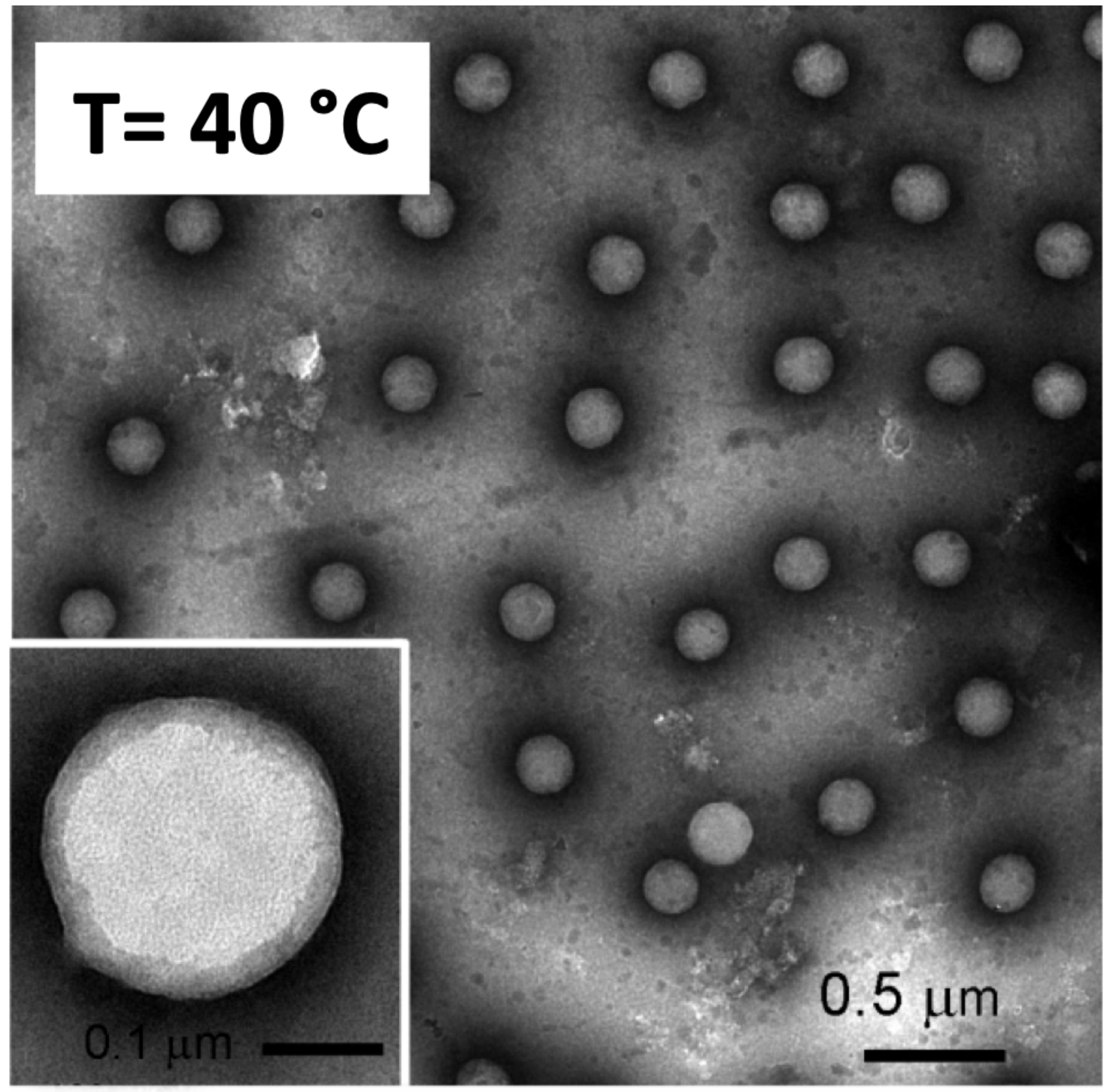
low T

high T

echo of the **COIL-TO-GLOBULE TRANSITION** in PNIPAM chains



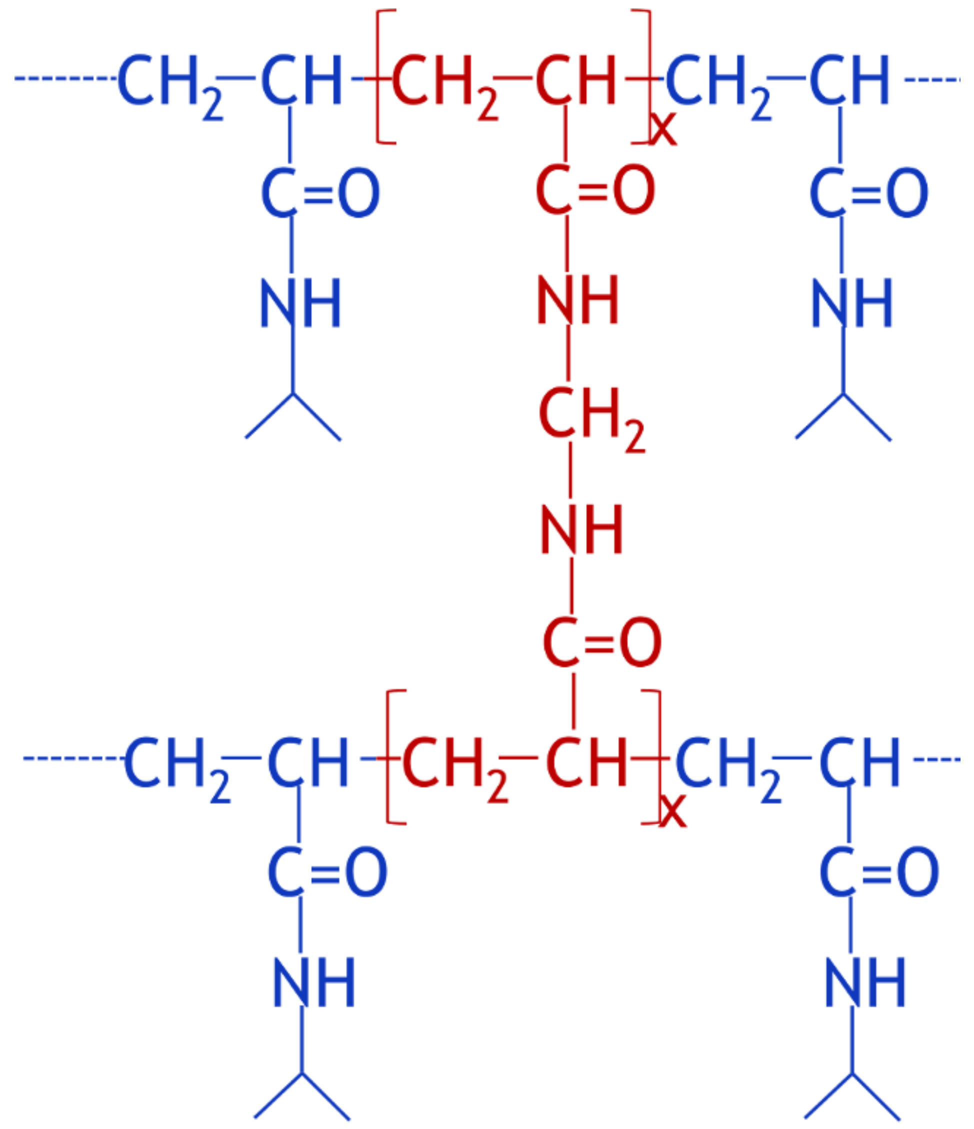




Truzzolillo et al *Soft Matter*, 2018

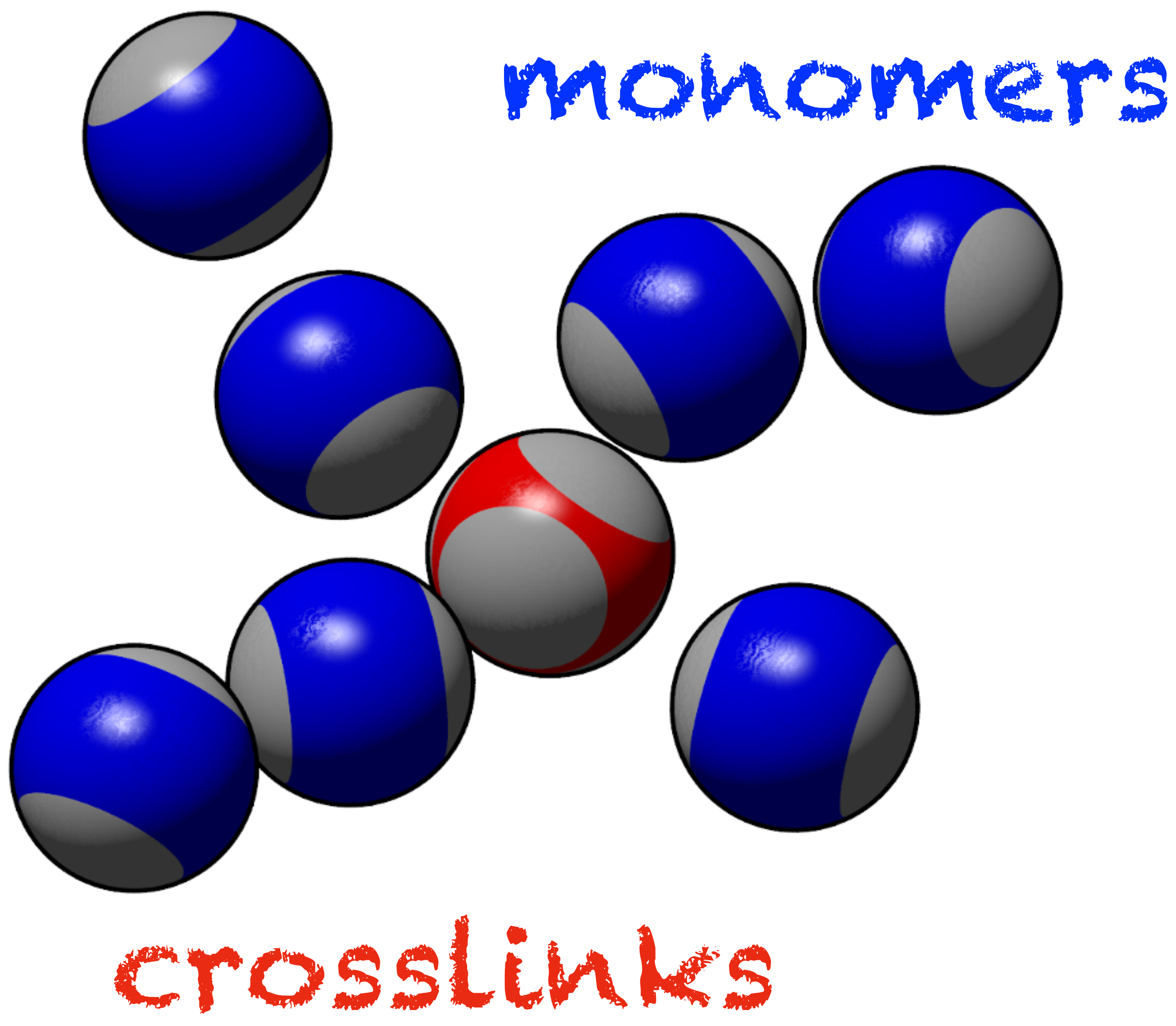
Microgels assembled from patchy particles

polymerization of
BIS crosslinkers
+ NIPAM monomers

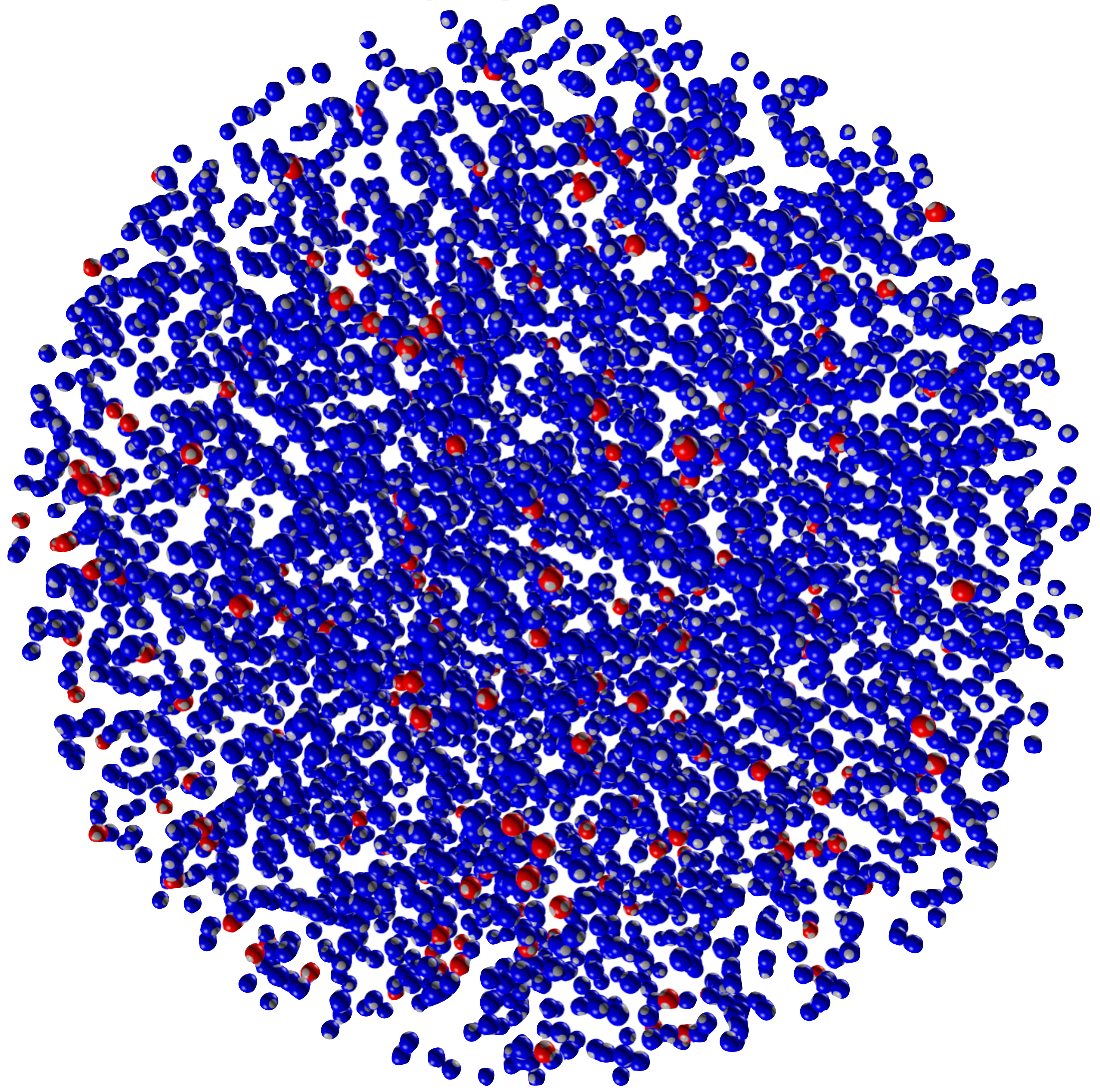


First step

self-assembly of
a binary mixture of
4-patch + 2-patch particles



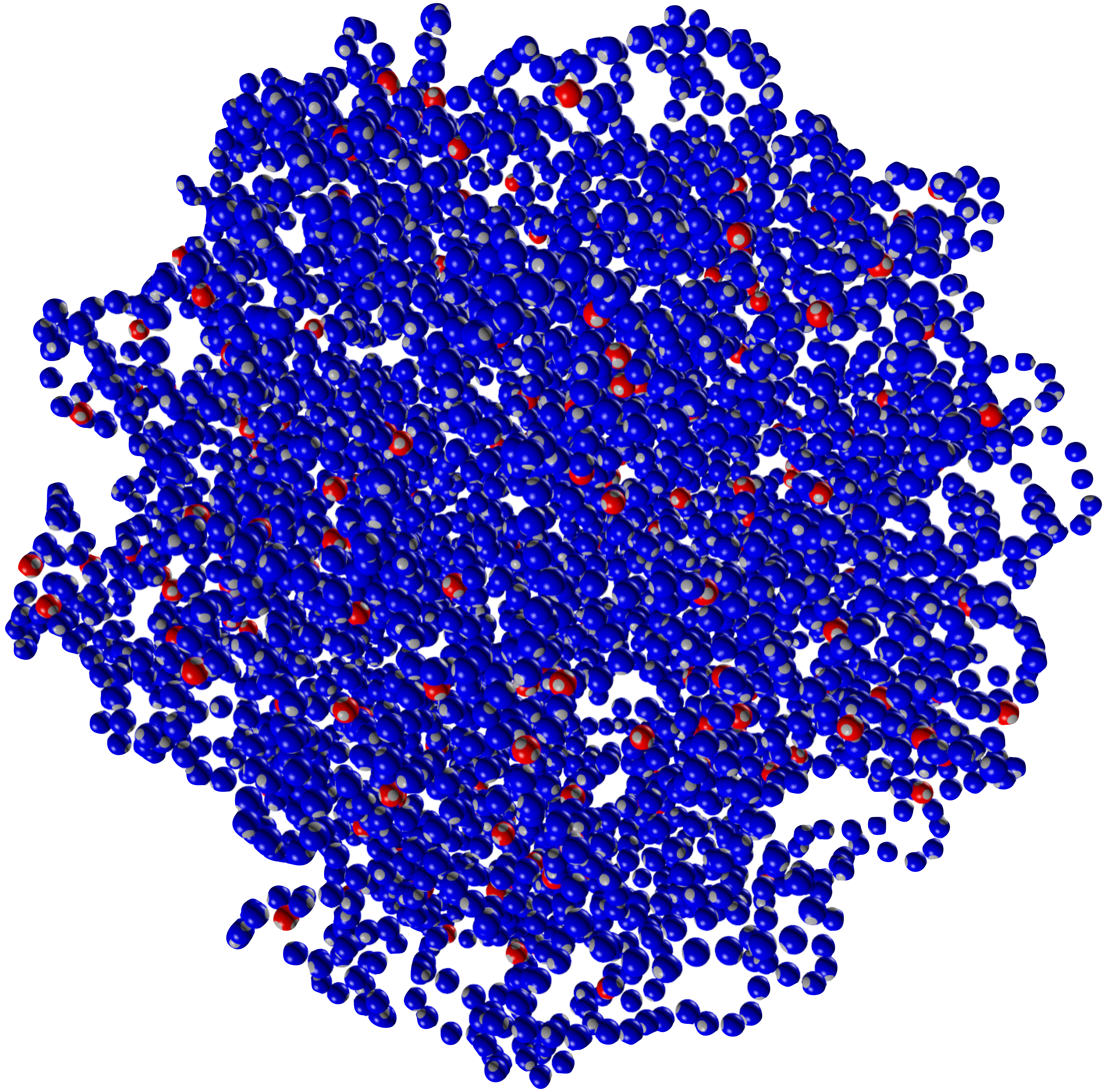
use a confining sphere of radius Z



how to assemble
fully-bonded networks?

N. Gnan, L. Rovigatti, M. Bergman, EZ Macromolecules 50, 8777 (2017)

Fully assembled network

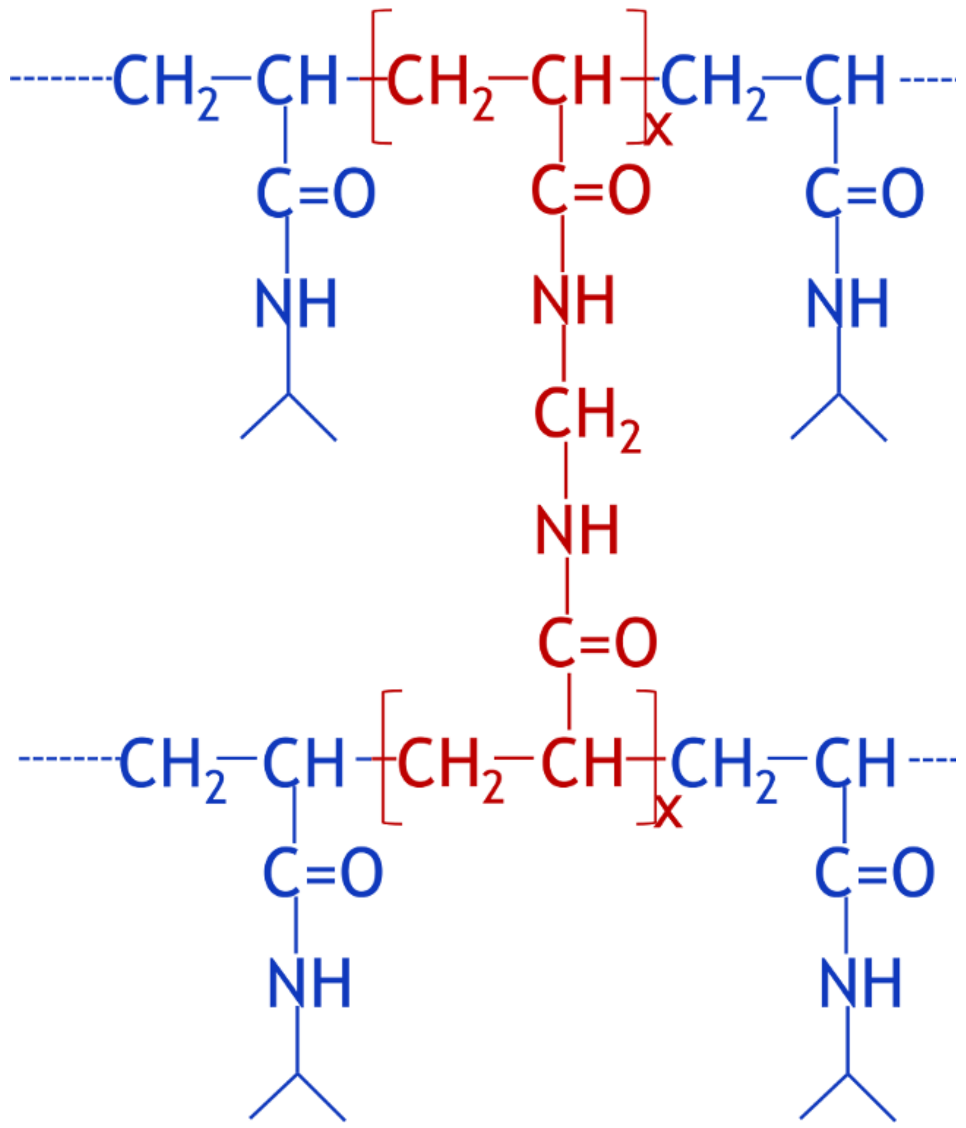


blue = monomers

red = crosslinks

Microgel assembly protocol

polymerization of
BIS crosslinkers
+ NIPAM monomers



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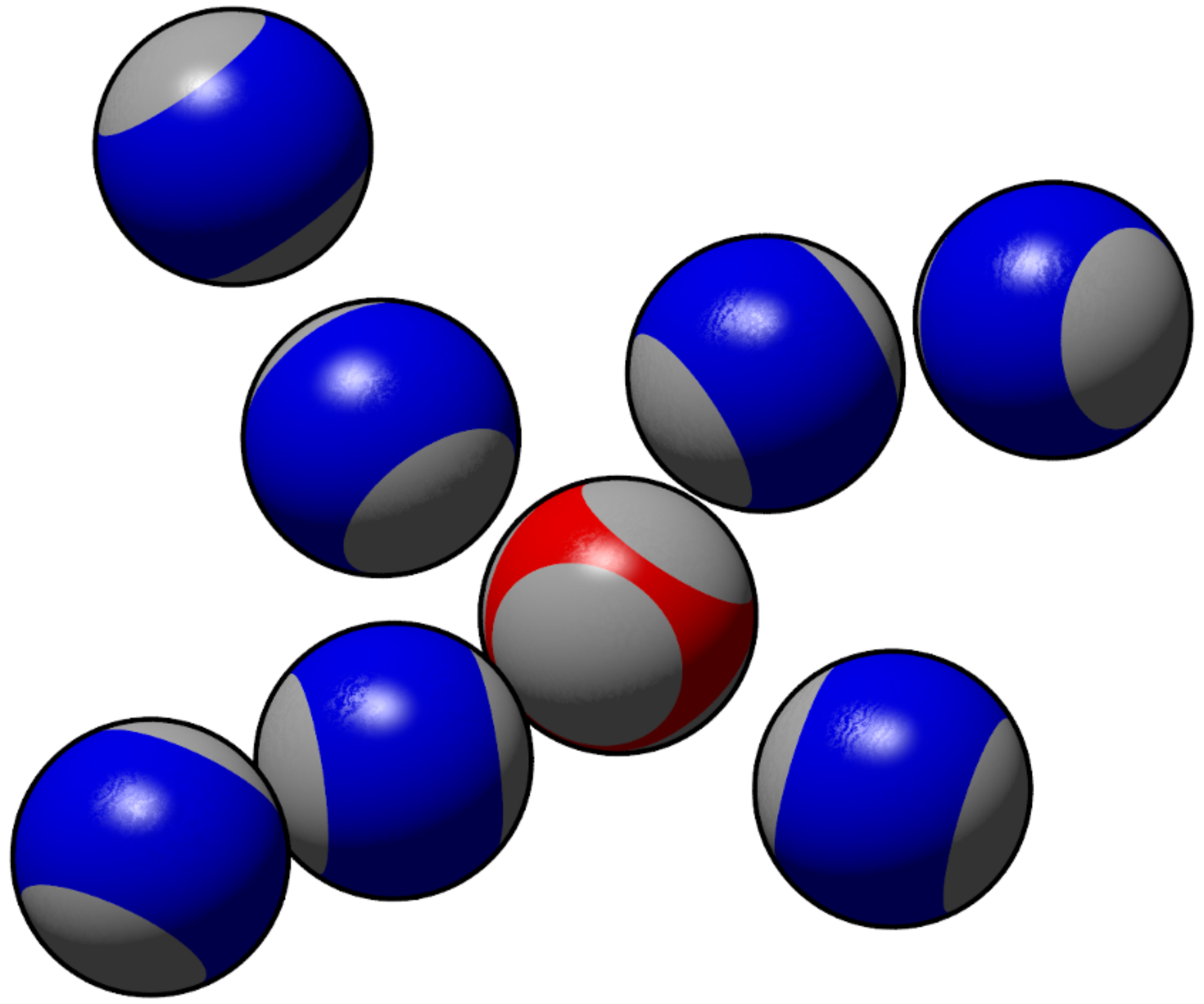
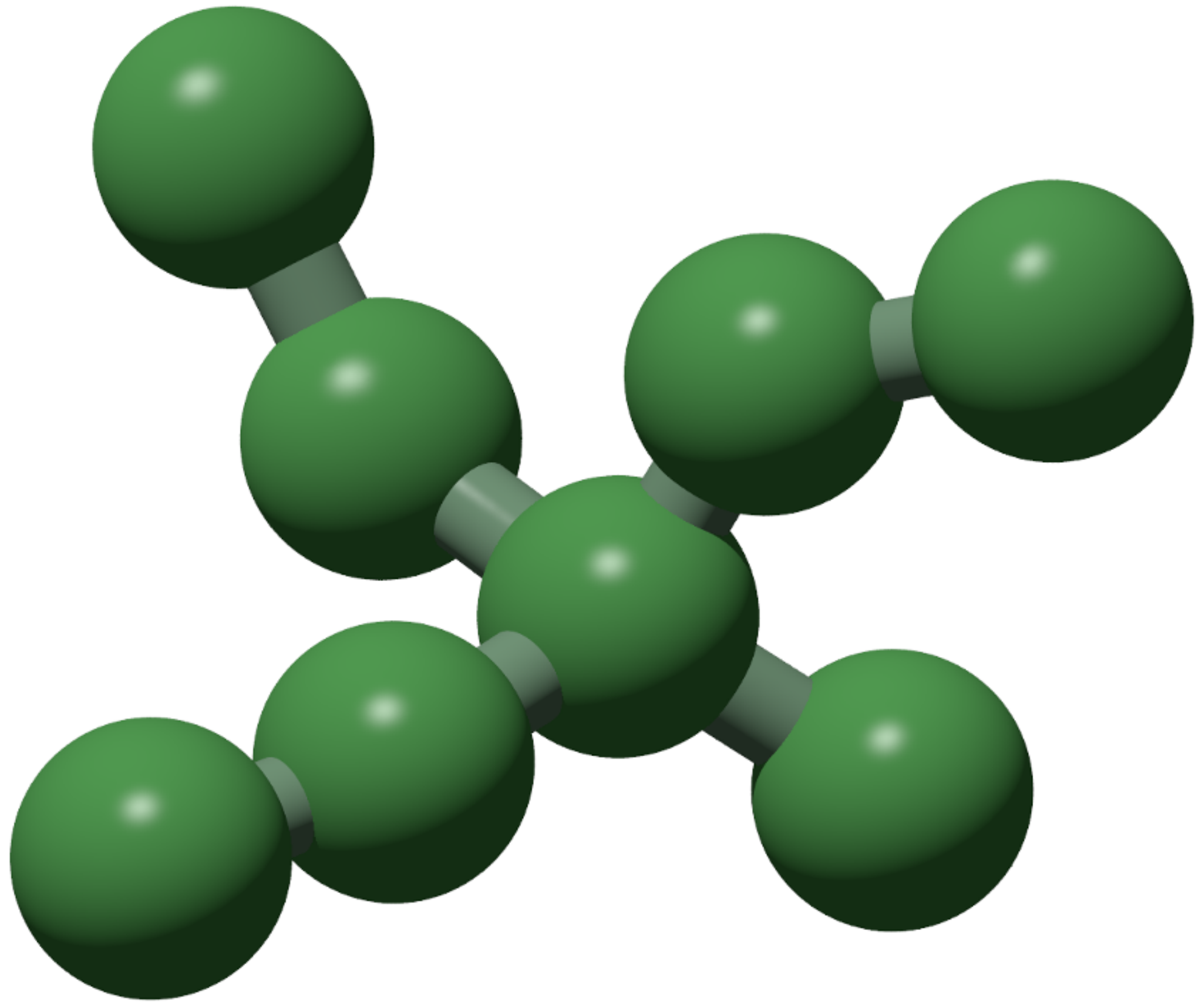


Second step →

polymer network



classical
bead-spring model

Grest & Kremer, Phys. Rev. A (1986)

$$V_{WCA}(r) = \begin{cases} 4\varepsilon\left[\left(\frac{\sigma}{r}\right)^{12} - \left(\frac{\sigma}{r}\right)^{6}\right] + \varepsilon & \text{if } r \le 2^{\frac{1}{6}}\sigma \\ 0 & \text{otherwise} \end{cases}$$

between all particles

$$V_{FENE}(r) = -\varepsilon k_F R_0^2 \ln\left(1 - \left(\frac{r}{R_0\sigma}\right)^2\right) \text{ if } r < R_0\sigma$$

between bonded particles

N. Gnan, L. Rovigatti, M. Bergman, EZ Macromolecules 50, 8777 (2017)

Results: assembled microgels — swollen regime

Swelling behaviour

We complement the bead-spring model adding a solvophobic term

Soddemman, Dunweg, Kremer Eur. Phys. J. E (2001)

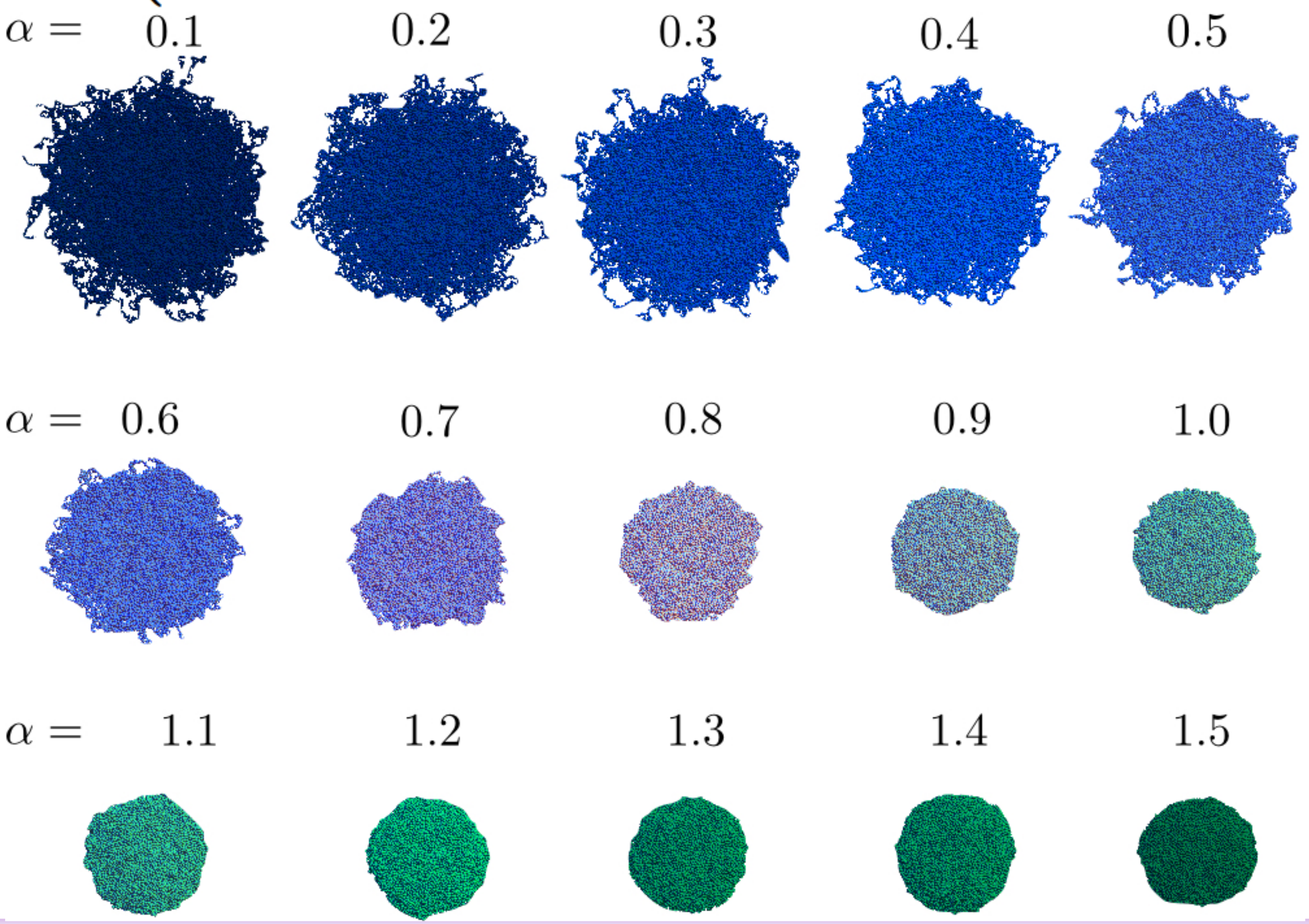
$$V_\alpha(r) = \begin{cases} -\varepsilon\alpha & \text{if} \quad r \le 2^{1/6}\sigma \\ \frac{1}{2}\alpha\varepsilon[\cos(\gamma(r/\sigma)^2 + \beta) - 1] & \text{if} \quad 2^{1/6}\sigma < r \le R_0\sigma \\ 0 & \text{otherwise} \end{cases}$$

$$\gamma = \pi(2.25 - 2^{1/3})^{-1}$$

$$\beta = 2\pi - 2.25\gamma$$

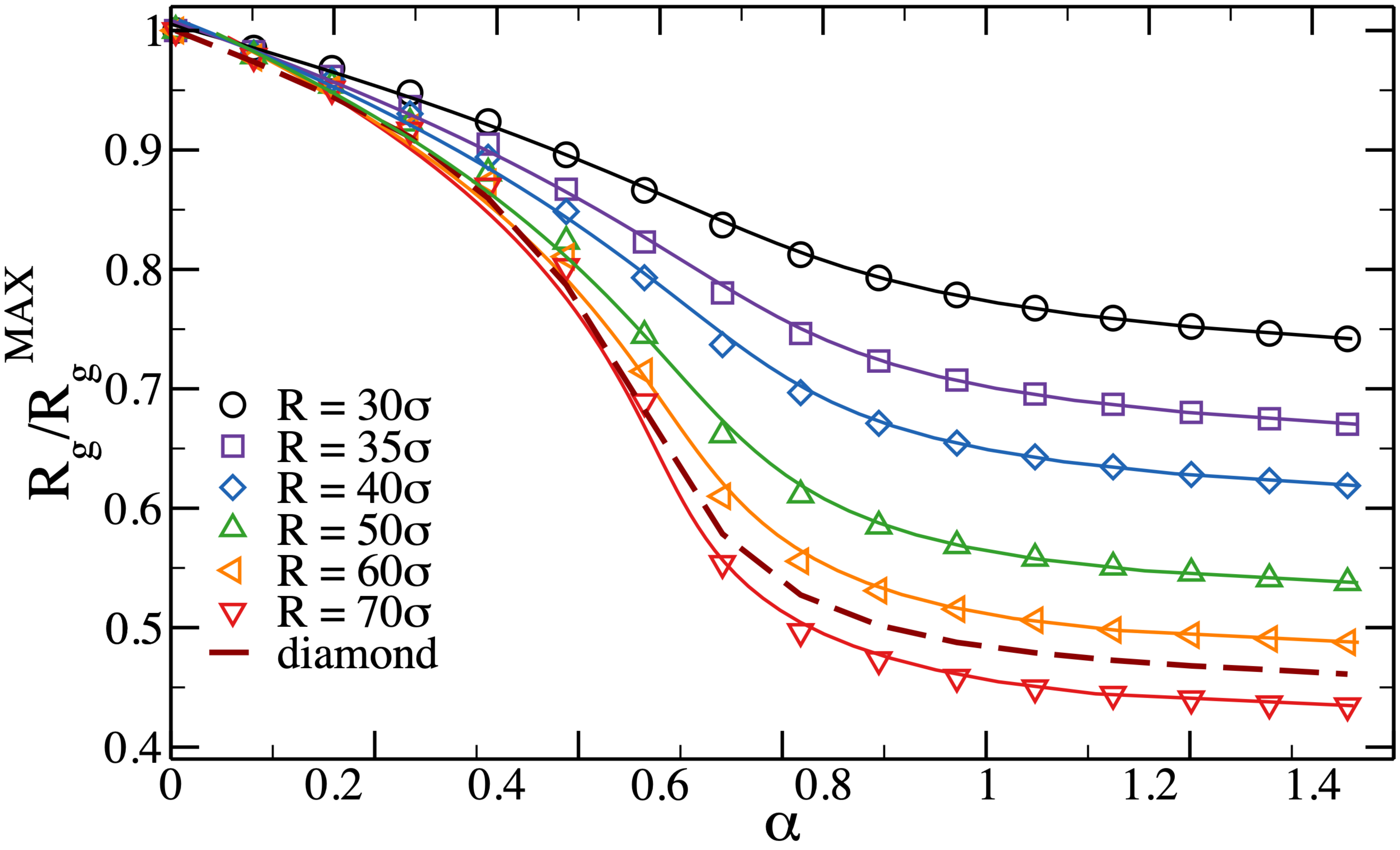
α : effective temperature

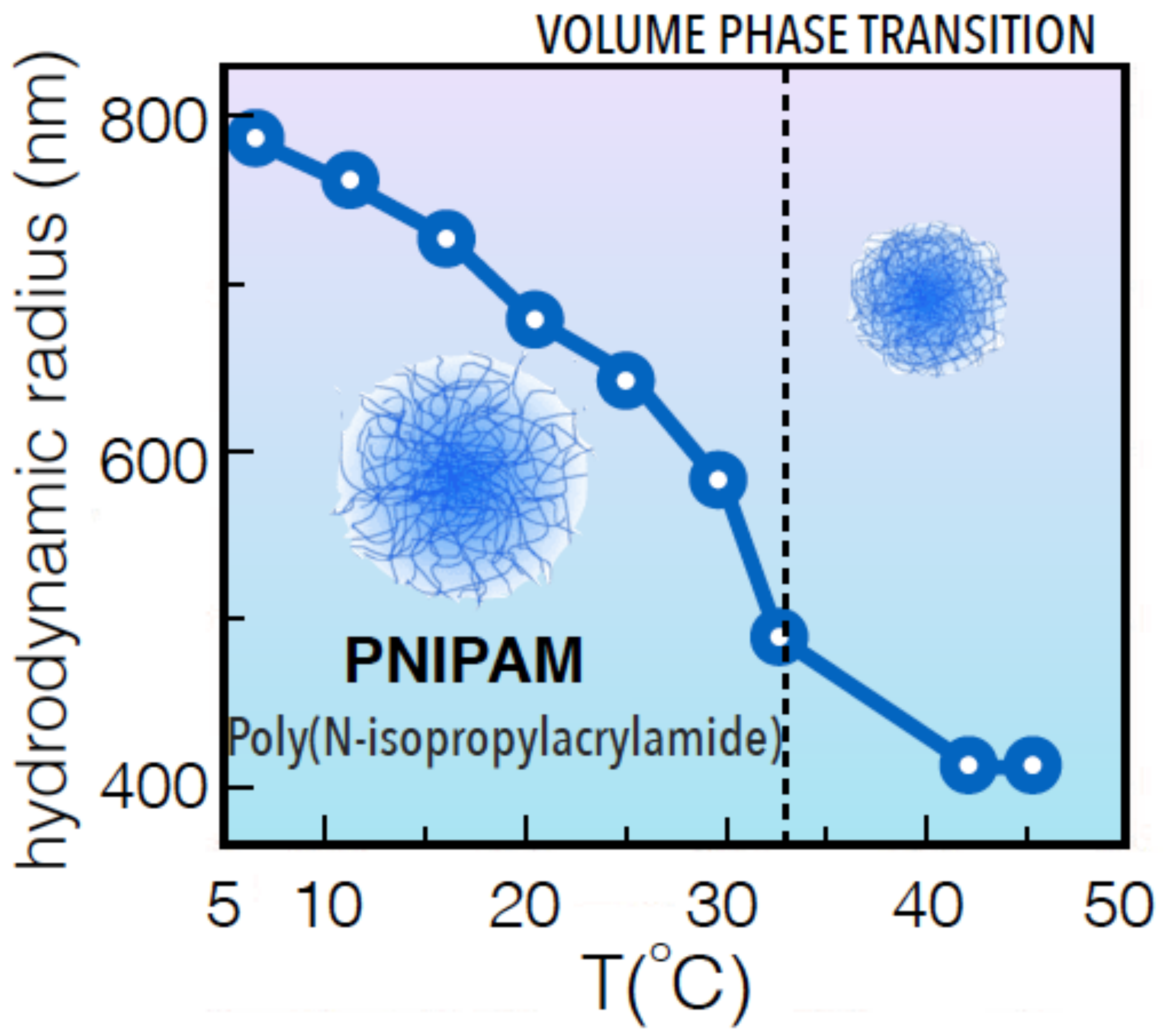
Volume Phase Transition (VPT)



Swelling behaviour

c=3.2%





A MULTISCALE APPROACH TO MICROGELS