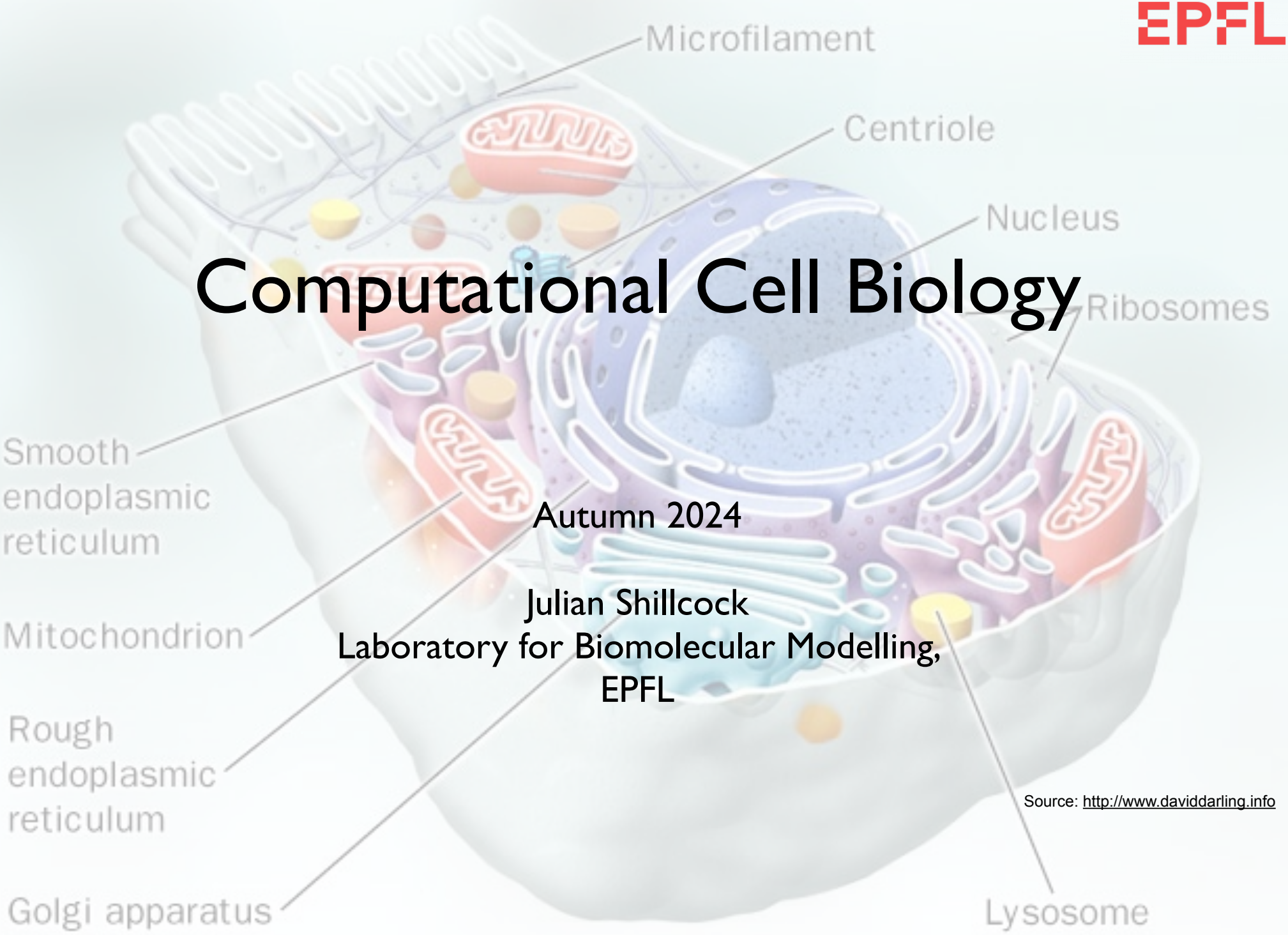
Computational Cell Biology



Source: http://www.daviddarling.info

Autumn 2024

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Core Concepts

Reversible phase transitions are a sign of health

Irreversibility is a sign of disease

Liquid-liquid phase separation of proteins is used by cells to create compositional gradients (gradients are life!) that localise functions

e.g., RNA translation, DNA repair, synapse formation, measles virus to reproduce, etc.

But flexible polymers are not hard spheres

Many types of phase transition

What is the *size* of a fluctuating polymer?

NMR
Size exclusion chromatography
(DLS - not enough data)

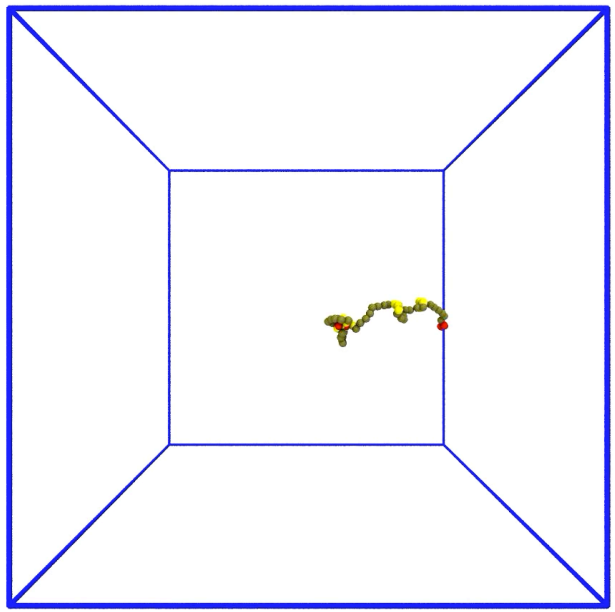
Diffusion coefficient

Hydrodynamic radius

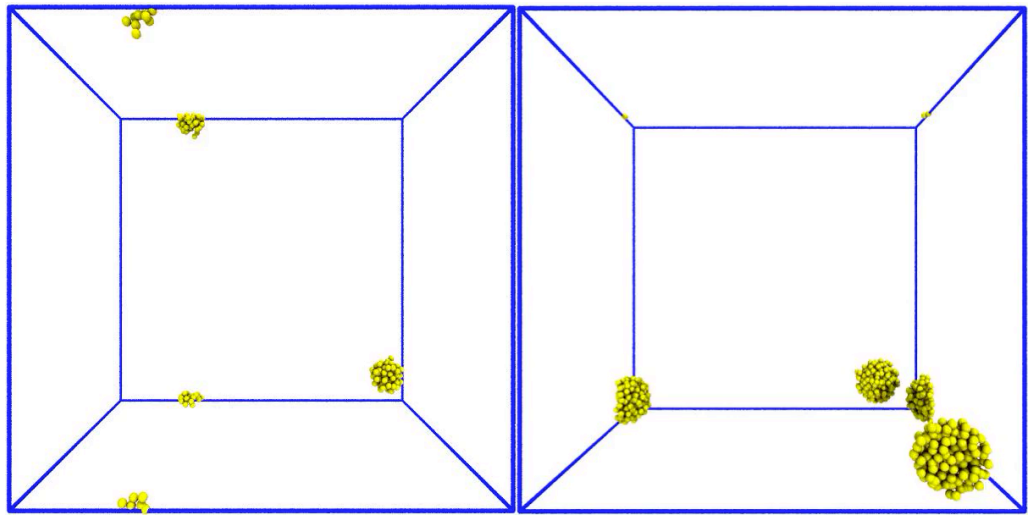
A simple way to assess structure in a disordered protein is to measure its hydrodynamic radius (R_h). The R_h is the radius of an idealized sphere that would diffuse at the same rate as the molecule of interest, and is based on the Stokes-Einstein relation in Eq. 1, where k_B is the Boltzmann constant, T is the temperature, η is the viscosity, and D is the translational diffusion coefficient. Thus, although the R_h is not a true measure of the radius of a nonglobular protein, as its diffusion is related to its nonspherical shape, it is very useful as a simple measure of compaction in disordered proteins.

$$R_h = \frac{k_B T}{6\pi\eta D}. \quad (1)$$

Warning this is not going to be trivial



Marsh and Forman-Kay, Biophys. J 98:2383 (2010)

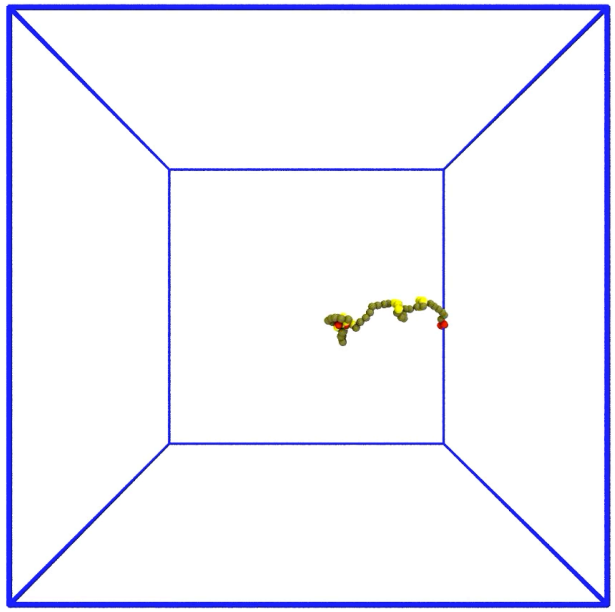


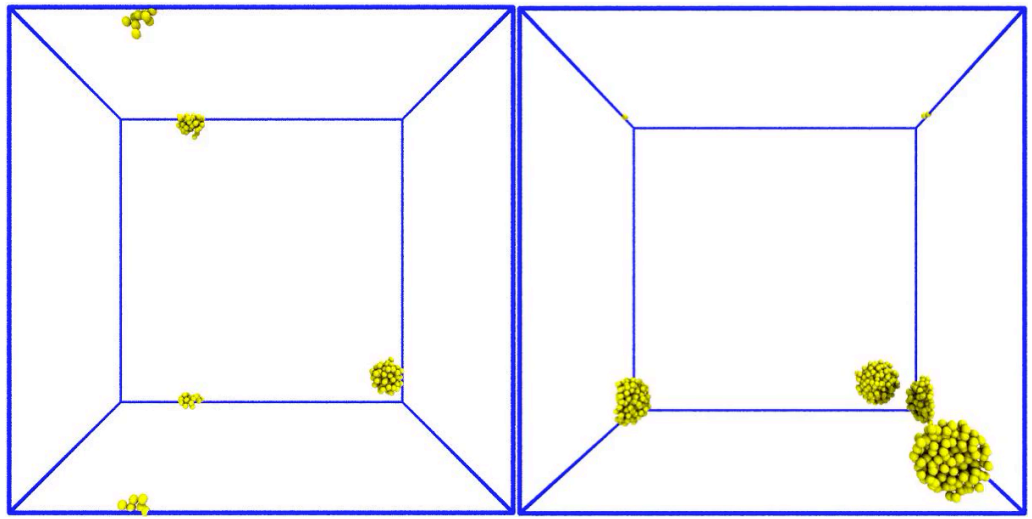
Think - Pair - Share

Q. Do polymers/IDPs diffuse like hard spheres? 5 mins.

a) what is diffusion? (see Lecture 6)
b) how do hard spheres diffuse?
c) compared to a hard sphere, do you expect a polymer with the same Rg as the sphere to diffuse:

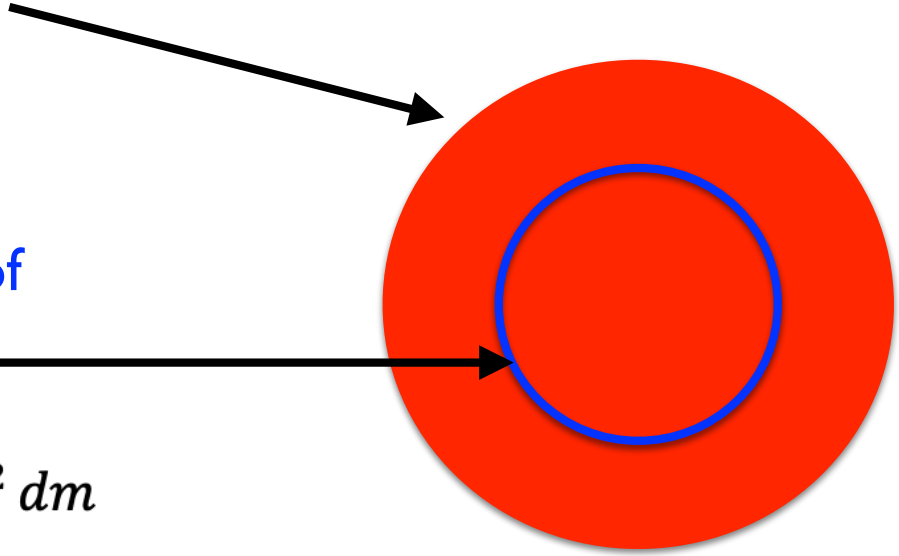
Answer	Votes	Why?
Faster		
Same		
Slower		





Hard sphere

R_h = actual sphere radius



R_g ~ distribution of mass in space

$$M R_g^2 = \int r(m)^2 \, dm$$

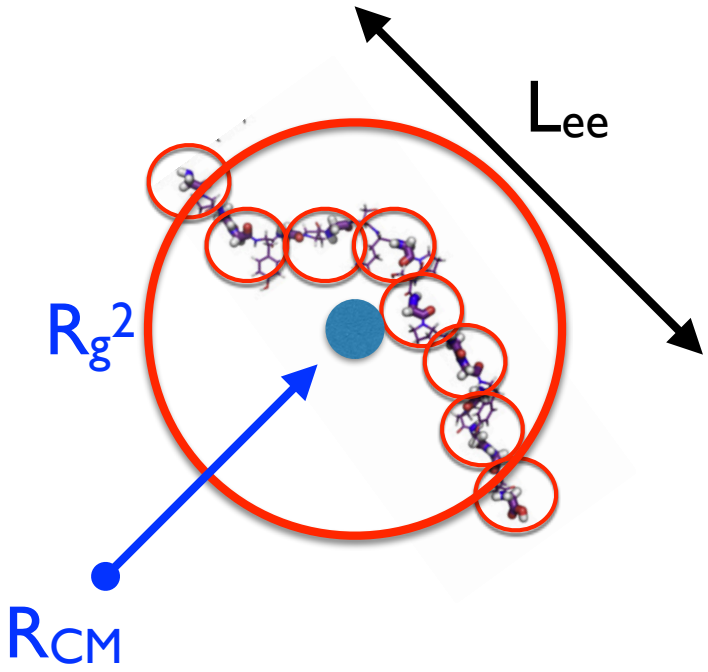
e.g.,

Spherical shell: $R_g^2 = R^2$

Solid sphere: $R_g^2 = 3/5 \, R^2$ which is smaller than for the shell because the interior mass pulls Rg to smaller values

Classical polymer physics gives us different "sizes" for a polymer:

L_{ee} = End-to-end length
R_g = Radius of gyration
R_h = hydrodynamic radius (equivalent diffusing sphere)



and different kinds of polymer model:

Size	Lee^2	v	Rg^2 / Lee^2	R_g/R_h
Ideal	$a^2 N^{2v}$	0.5	1/6	1.5045[1]
SAW	$a^2 N^{2v}$	0.6	1/6.254	1.591[2]

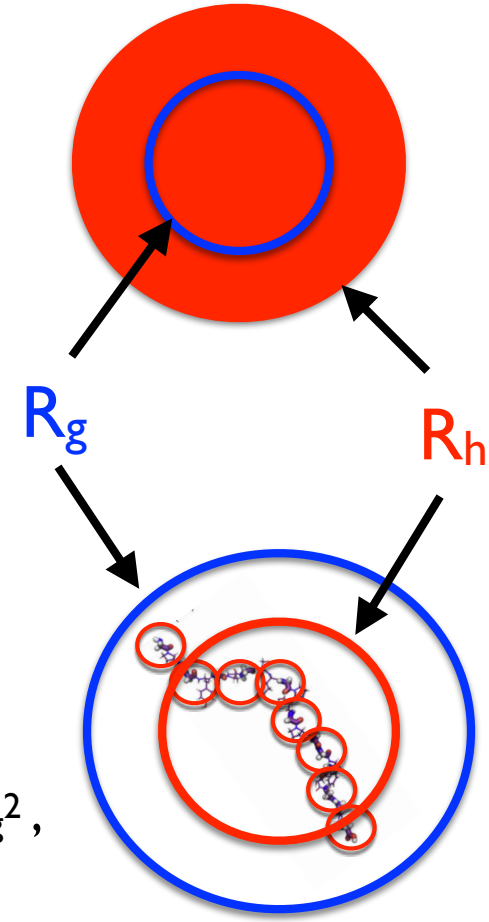
[1] Dunweg et al. J. Chem. Phys. 117:914 (2002)
[2] Clisby et al. Phys. Rev. E 94:052102 (2016)

Recall from Lecture 2: *Centre of mass* of a polymer (with N monomers): $\mathbf{R_{cm}} = 1/N \sum \mathbf{R}_i$

Radius of gyration of a polymer: $\mathbf{R_g^2} = 1/N \sum_{i \neq j} (\mathbf{R}_i - \mathbf{R}_{cm})^2 = 1/2N^2 \sum \mathbf{R}_{ij}^2$ $\quad \mathbf{R}_{ij} = \mathbf{R}_i - \mathbf{R}_j$

A fluctuating polymer with size R_g does ***not*** diffuse as if it occupied a spherical volume like a hard sphere with the same R_g

	Rg / Rh	From
Sphere	$\sqrt{3/5} \sim 0.775$	$R_g^2 = 3/5\ R^2$
Ideal polymer	1.5045	Dunweg et al., J. Chem. Phys. 117:914 (2002)
SAW polymer	1.591	Clisby et al., Phys. Rev. E 94:052102 (2016)



While a hard sphere diffuses with a hydrodynamic radius $R_h = 5/3\ R_g^2$, a polymer diffuses with an $R_h < R_g$, so it diffuses faster than the equivalent sized sphere.

$$R_h = \frac{k_B T}{6\pi\eta D}.$$

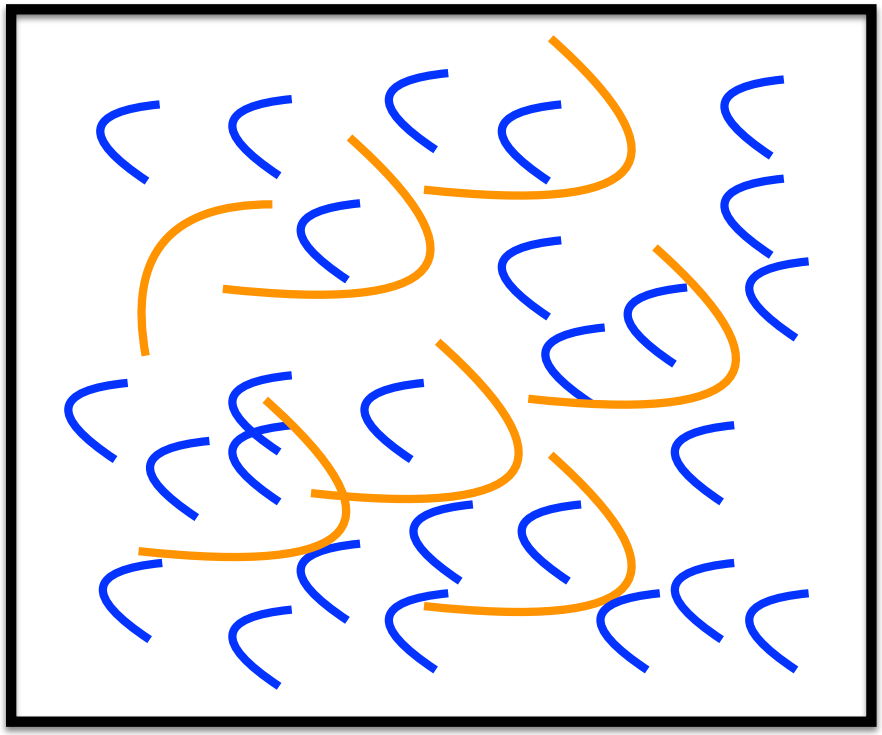
Experimentalists beware

Polymer phase separation

Consider a mixture of a polymer in a solvent (which may be another polymer):

Do they mix? Do they phase separate?

We can construct a thermodynamic theory of the mixture that predicts a phase separation as as function of the polymer/solvent interactions.



Assume: composition, V, T are constant.

Helmholtz free energy is:

$$F = U - TS$$

U ~ energetic interaction between polymers
S ~ number of configurations of polymers/solvent

How do we find U and S? Just as the entropic spring's behaviour was dominated by the largest number of microstates (bond flips), the polymer mixture's behaviour is dominated by the most likely "number" of interactions

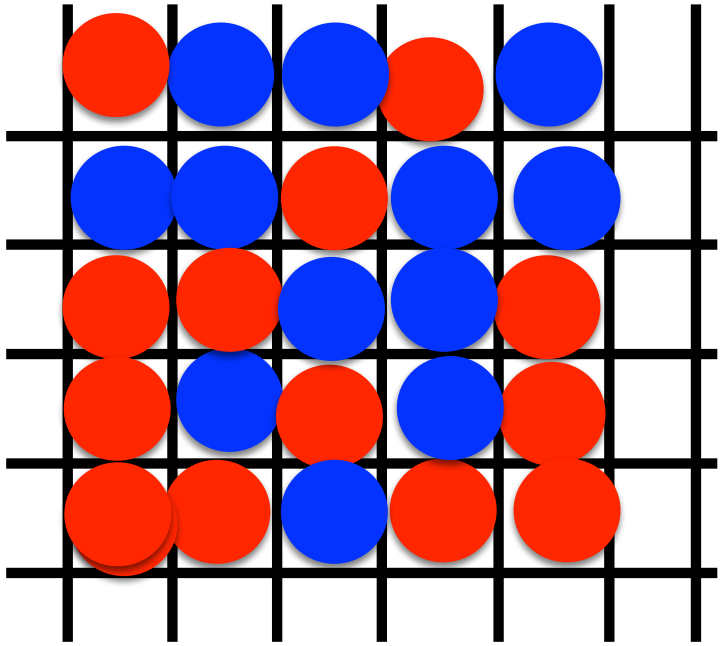
Calculation

Consider a lattice with N sites that is filled with **monomers** such that

n1 monomers of type 1

n2 monomers of type 2

and N = n1 + n2



How many ways $\Omega(n_1, n_2, N)$ are there of placing n1 (blue) monomers and n2 (red) monomers on the lattice?

Express the result in terms of the volume fractions $\phi_1 = n_1 / N$ and $\phi_2 = n_2 / N$

$$\ln \Omega(\phi_1, \phi_2) = ?$$

Entropic spring from Lecture 8
Think - Pair - Share
5 mins

The free energy of an entropic spring is a 1dimensional model:

$$F(L/L_0) = k_BT\ (\ L_0/2a\ )(\ (1 - x)\ \ln(1 - x) + (1 + x)\ \ln(1 + x) - 2\ \ln 2\ )$$

and $x = L/L_0$

And Flory-Huggins is a 2-dimensional lattice model:

$$(\ \ln \Omega\ )/N\ = -\phi\ \ln(\phi) - (1 - \phi)\ \ln(1 - \phi)$$

where $\phi = n_1/N$

But we can show (letting $\phi = (1 - x)/2$)

$$-\phi\ \ln(\phi) - (1 - \phi)\ \ln(1 - \phi) = 1/2(\ (1 - x)\ \ln(1 - x) + (1 + x)\ \ln(1 + x) - 2\ \ln 2\ )$$

Question: why do these two models have the same free energy?

Flory-Huggins theory of polymer mixtures

Let species 1 be a polymer with N monomers, and volume fraction ϕ_1 and species 2 a monomeric solvent with volume fraction $\phi_2 = 1 - \phi_1$.

The essence of the Flory Huggins theory is based on two points:

- the connectivity of the polymers is *ignored* when placing their monomers on the lattice
- the translational entropy of the polymers is reduced by a factor 1/N

U = energetic interactions among monomers and solvent proportional to their volume fractions $\sim \phi (1 - \phi)$

S = translational entropy of the polymers and solvent

Flory-Huggins theory of polymer mixtures

Why this form?

$$\beta F = (\phi/N)\ln(\phi) + (1 - \phi)\ln(1 - \phi) + \chi\,\phi\,(1 - \phi)$$

polymer entropy

solvent entropy

entropy favours mixing

energy favours separating if $\chi > 0$

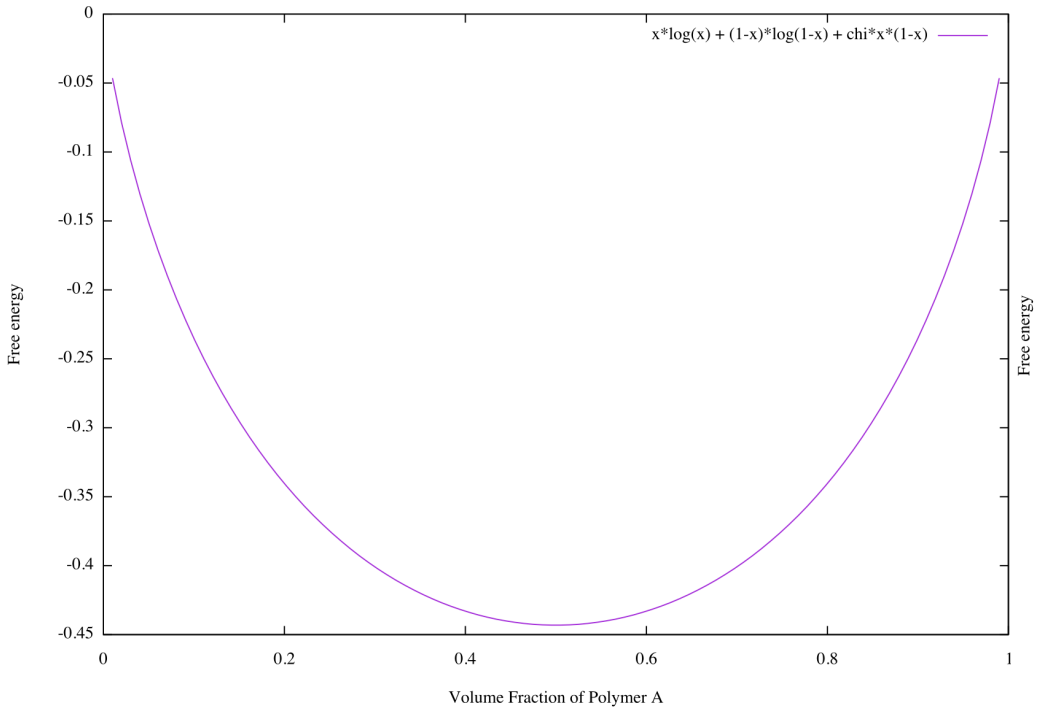
Plot F for several χ

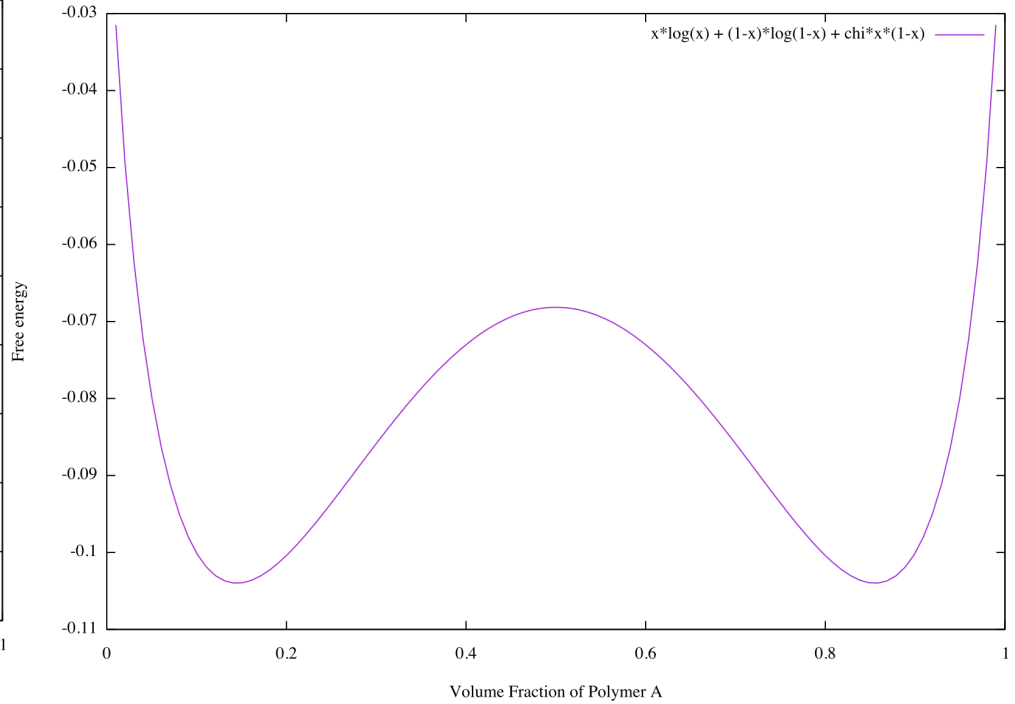
Notes

1) The first term is usually negligible because polymers have N >> 1 (PEG has a molecular weight ~ 10,000 Da or more)
2) Polymers have very low translational entropy compared to solvent
3) Energetic term: every monomer in the polymers interacts with the solvent, a small, repulsive χ increases the energetic term very rapidly with polymer length
4) Temperature dependence of $\chi(T) \sim A + B / T$ and values of A, B are tabulated for different polymer mixtures.

FH theory predicts a phase transition as the parameter χ increases

$$\beta F = (\phi_1/N_1)\ln(\phi_1) + (1 - \phi_1)\ln(1 - \phi_1) + \chi\,\phi_1(1 - \phi_1)$$





χ = 1 χ = 2.5

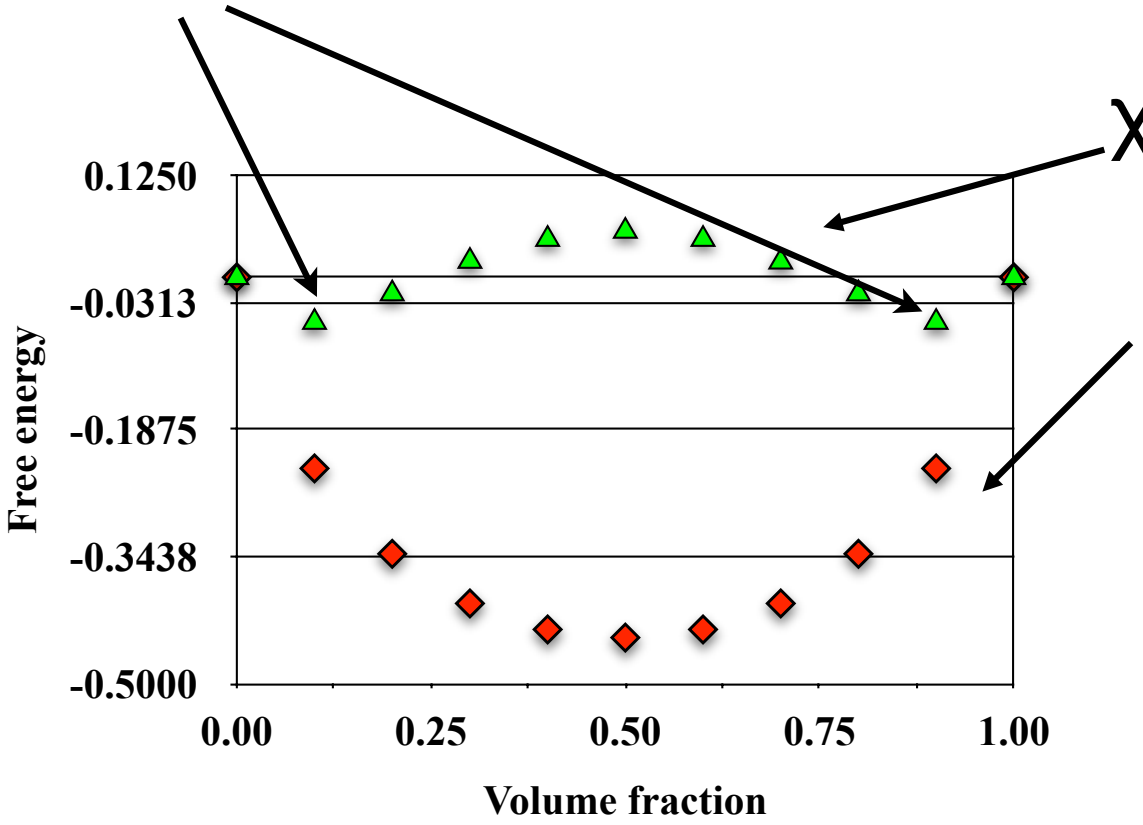
As the monomer-monomer repulsion increases, the homogeneous state becomes unstable to breaking up into two separate phases: one enriched in one polymer and the other enriched in the other polymer/solvent

Flory-Huggins phase separation

Minimising βF with respect to ϕ_A, ϕ_B predicts phase separation for mixing parameters satisfying

$$\chi > \chi_C = 0.5.(1/\mathrm{sqrt}(N_A) + 1/\mathrm{sqrt}(N_B))^2$$

The condition $\partial F/\partial \phi_A = 0$ (with $N_1 = N_2$) leads to $\chi N_1 = \log((1-\phi_1)/\phi_1)/(1-2\phi_1)$



If $N_1 = N_2 = 1$ then $\chi_c = 2$

For general $N_1 = N_2 = N$

$\chi_c = 2/N$

and for long polymers, χ_c is small, which is why polymers usually don't mix well.

Flory-Huggins/DPD equivalence

Groot and Warren (1997) found a correspondence between the soft DPD fluid and the Flory-Huggins theory of polymer mixtures.

$$\beta F_V = \rho_A/N_A \ln(\rho_A) + \rho_B/N_B \ln(\rho_B) - \rho_A/N_A - \rho_B/N_B + \beta\alpha(a_{AA}\,\rho_A^{\,2} + 2a_{AB}\,\rho_A\,\rho_B + a_{BB}\,\rho_B^{\,2})$$

where $\beta = 1/k_BT$, $\alpha \sim 0.1$ from simulations

ρ_i = Number density of particles of type i ($N_A = N_B = 1$)

$a_{AA} = a_{BB}$ = like-particle conservative force parameter

a_{AB} = unlike-particle conservative force parameter

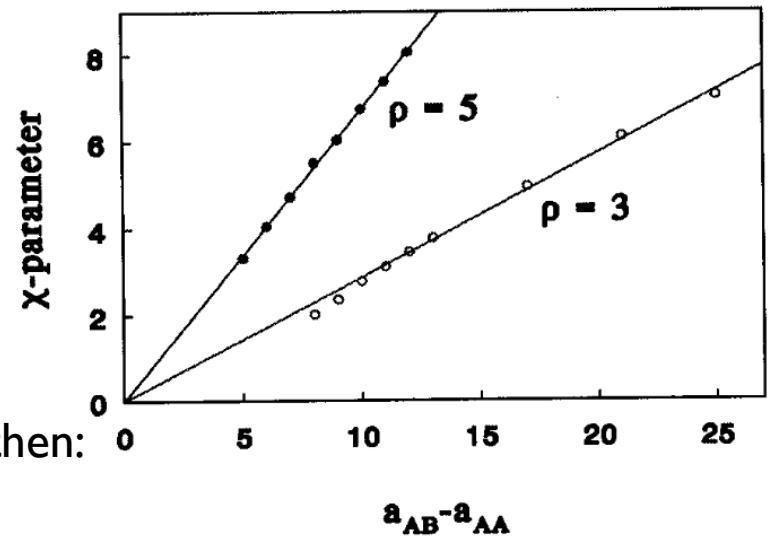


Fig. 7 in Groot and Warren, 1997

Now let $x = \rho_A/(\rho_A + \rho_B)$ and assume that $\rho_A + \rho_B \sim$ constant then:

$$\beta F_V \sim x/N_A \ln(x) + (1-x)/N_B \ln(1-x) + \chi\, x\,(1-x) + \text{const.}$$

yielding the relation: $\chi = 2\,\beta\alpha(a_{AB} - a_{AA})(\rho_A+\rho_B)$, between the Flory-Huggins parameter and the relative DPD cross interaction

$a_{AB} - a_{AA}$. As χ is known from experiment this allows DPD to be calibrated for polymer mixtures.

Can Flory Huggins theory explain LLPS?

When does an homogeneous polymer mixture become unstable to phase separation?

⇒ Translational entropy favours mixing

Problem: polymers interact along their whole length

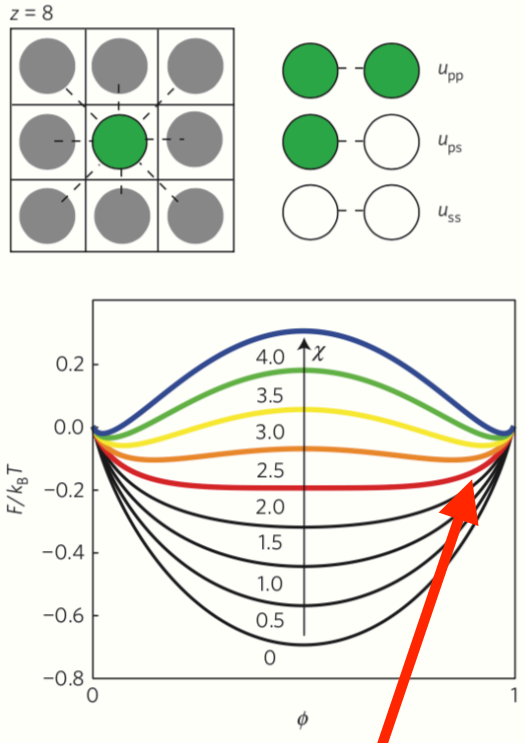
⇒ Monomers repulsion favours separation if $\chi > \chi_c$

Problem: dense phase is too dense

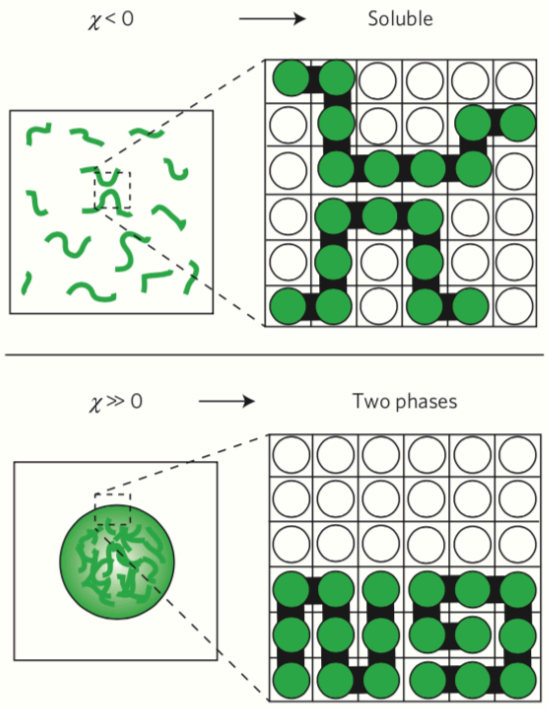
$$\frac{F}{k_B T} = \frac{\phi}{N}\ln\phi + (1-\phi)\ln(1-\phi) + \chi\phi(1-\phi) \quad (1)$$

In equation (1), the first two terms represent the mean-field entropy of mixing per lattice site and the third term represents the energy of mixing per lattice site. The Flory χ parameter quantifies the balance between chain–chain and chain–solvent interactions, and is written as:

$$\chi = \frac{z}{k_B T}\left[u_{ps} - \frac{1}{2}(u_{pp} + u_{ss})\right] \quad (2)$$



Look at the density of condensed phase



Brangwynne et al.
Nature Physics 11:899 (2015)

Phase separated droplets of FUS are ~ 65% solvent by volume
(Murthy et al. Nature. Struc. Mol. Biol. 26:637 (2019)

We now have two approaches to understanding why IDPs might form biomolecular condensates:

- experimental/atomistic modelling: residues define the forces between IDPs that control whether they phase separate and their material properties

- coarse-grained modelling: generic properties of polymers with sticky sites show a phase transition from dilute to concentrated

So, let's make a NEW theory about

liquid phase separation of IDPs

Reduce an IDP to its simplest form: a semi-flexible polymer with sticky end-caps (*telechelic*)

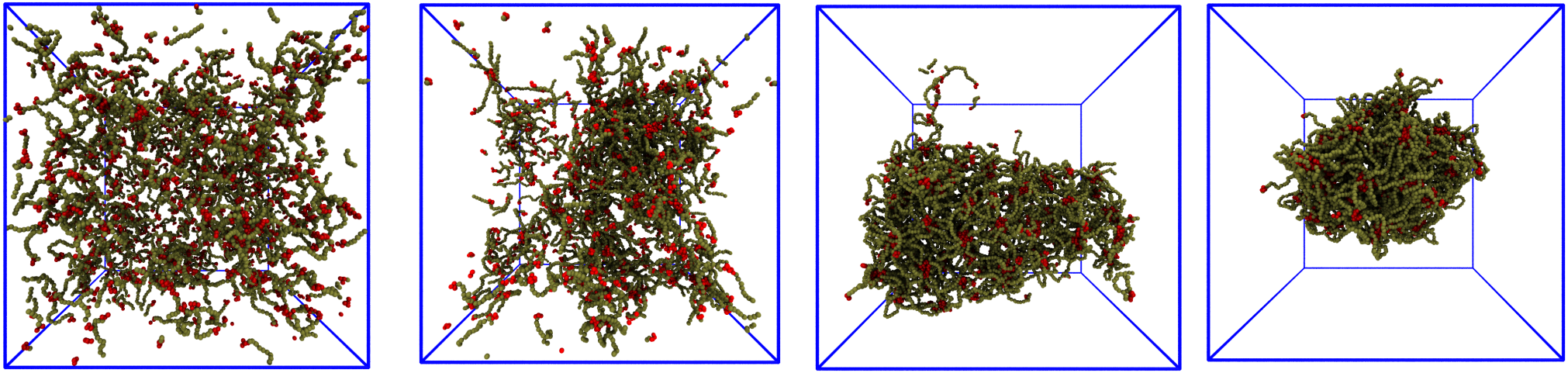
Still many parameters: molecular weight, backbone stiffness, end-cap affinity, concentration …

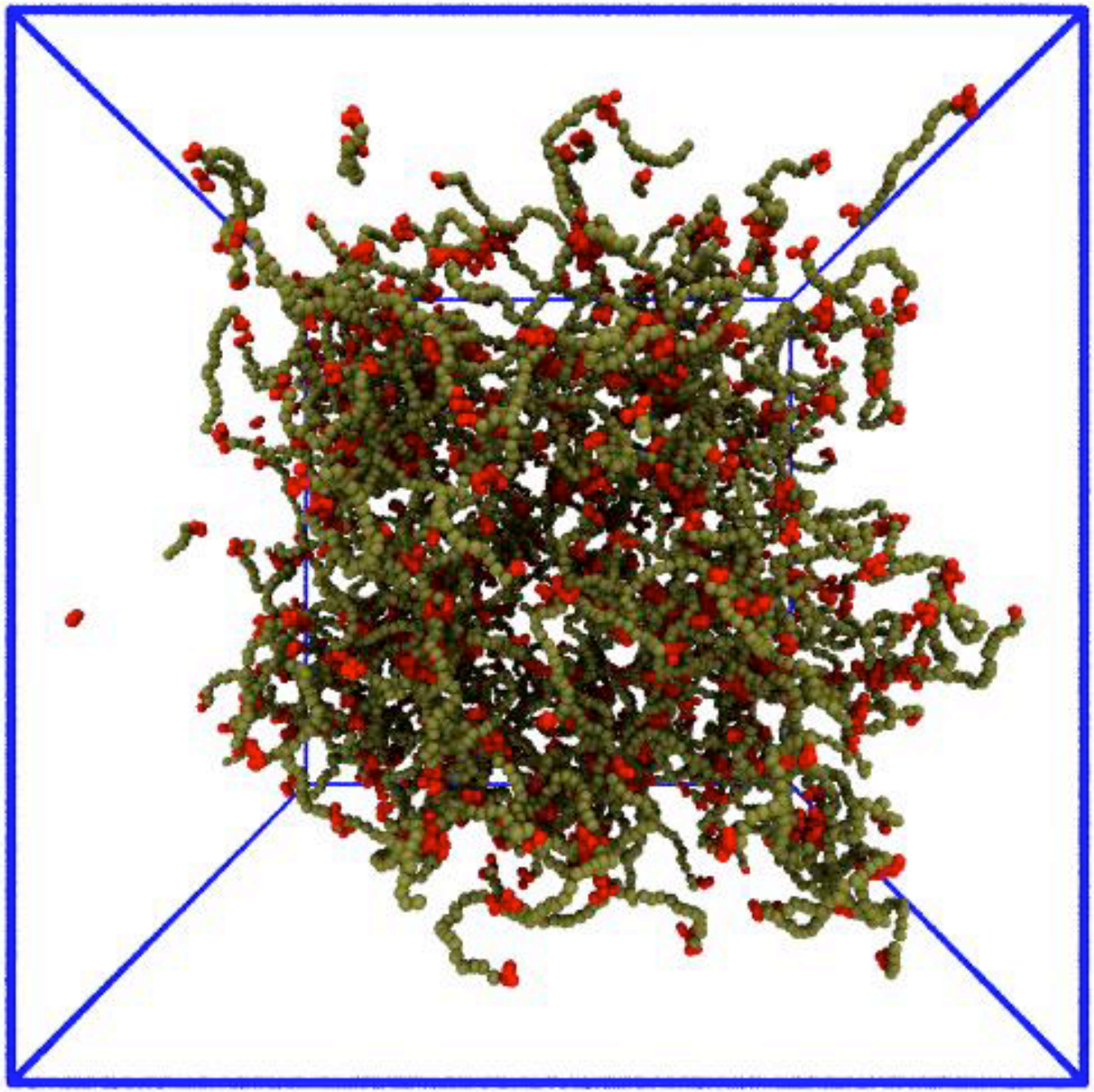
Choose two

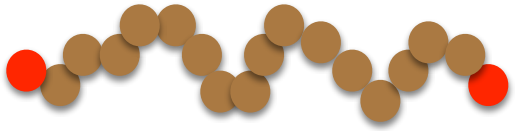
A) Backbone length (molecular weight) = 16, 24, 32, … beads

B) Dimensionless end-cap affinity = [0, 1]; where 0 = no affinity and 1 = "very strong" affinity (defined in terms of the conservative interactions between end-caps and water)

N = 634 hydrophilic polymers (*FH doesn't apply*) with increasing affinity →







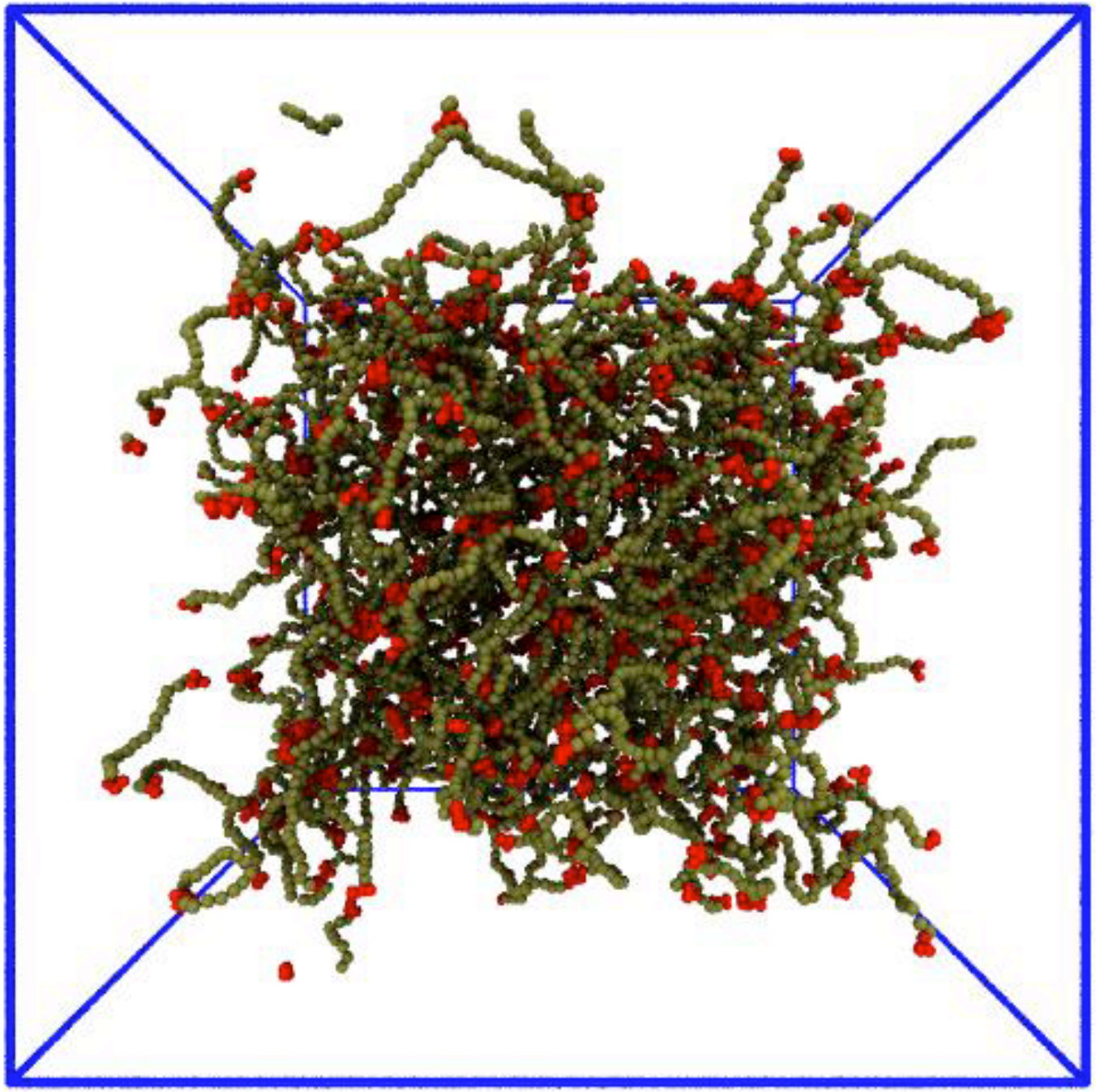
Mol. architecture: E - B_{16} - E

B is hydrophilic backbone
E is a hydrophilic binding site

Solvent is invisible for clarity

Assembly

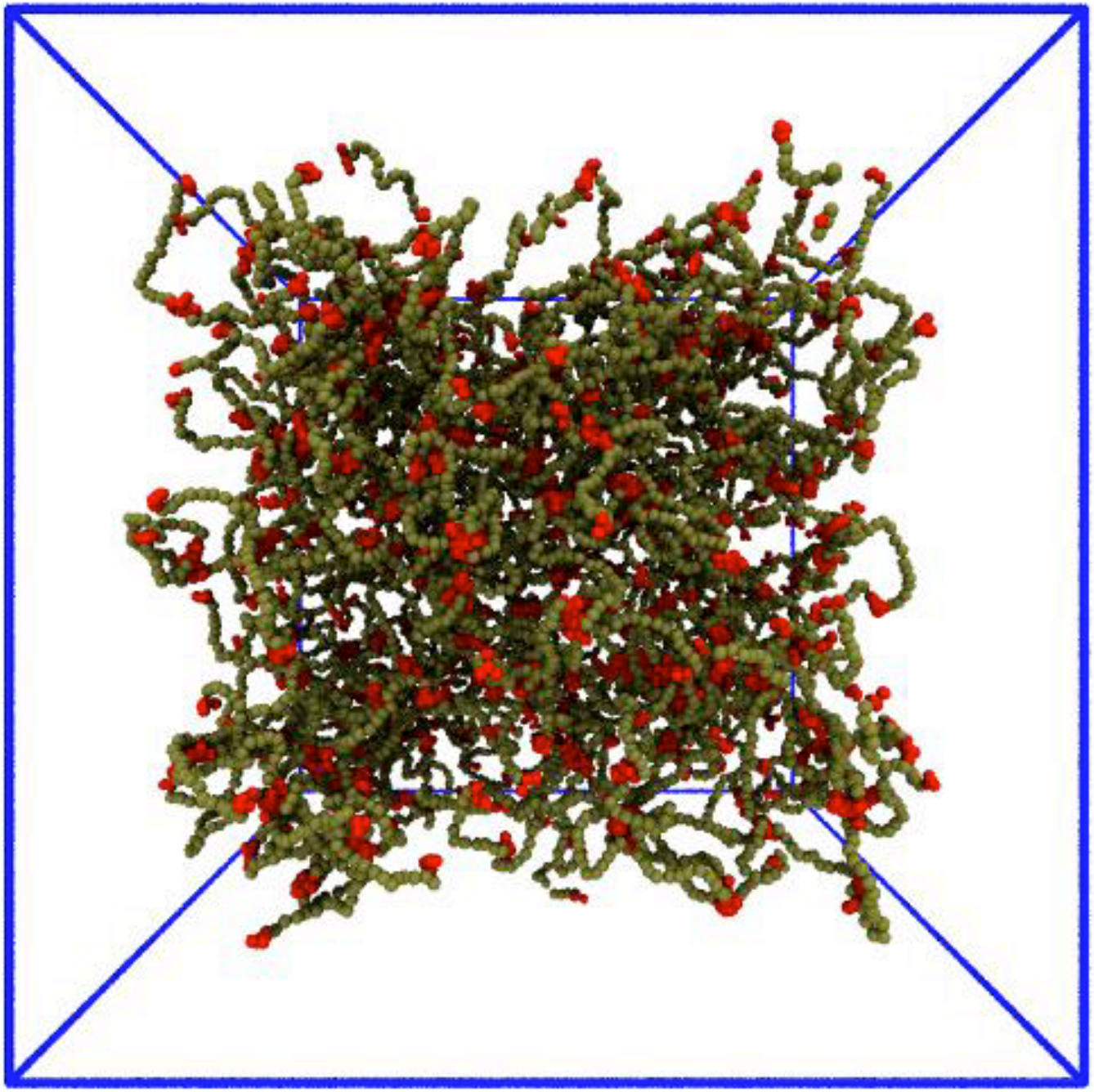
Box = 50 nm
N = 634
Affinity ε ~ 0.68 or *weak*



Mol. architecture: E - B_{16} - E

Assembly

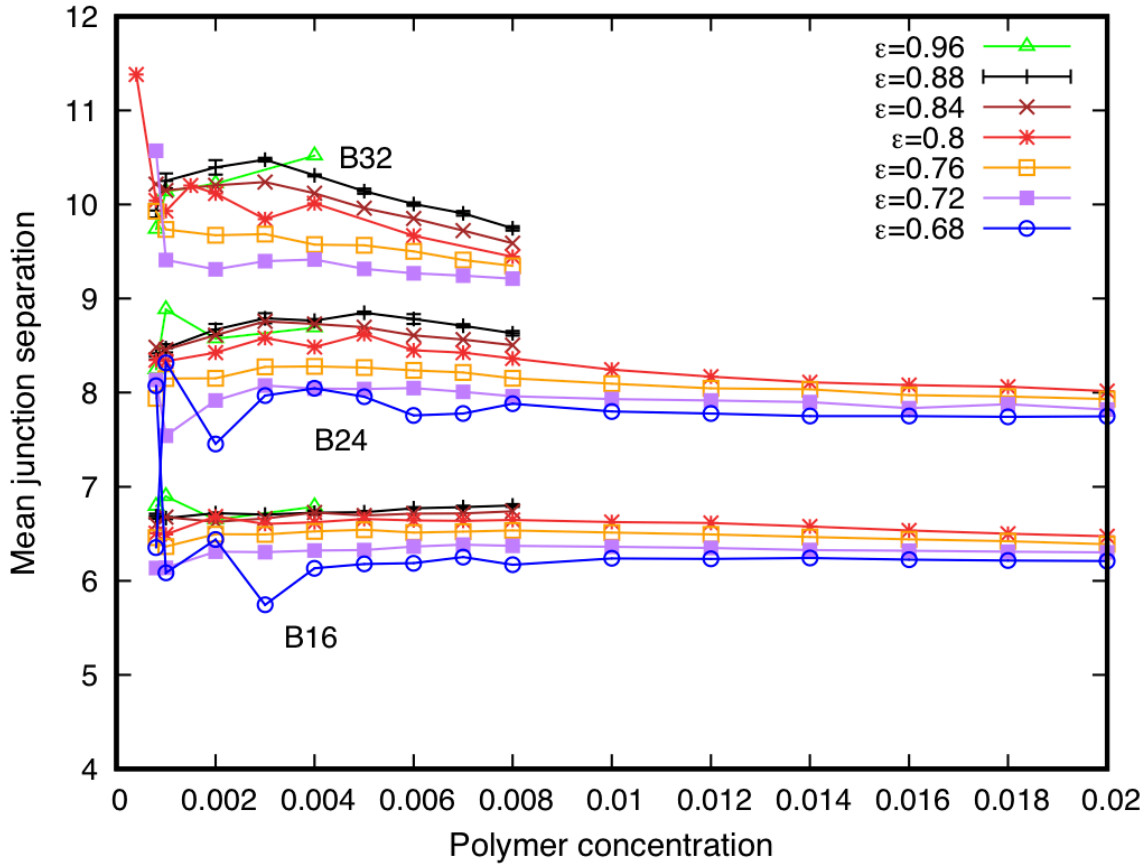
Box = 50 nm
N = 634
Affinity ε ~ 0.76 or *threshold*

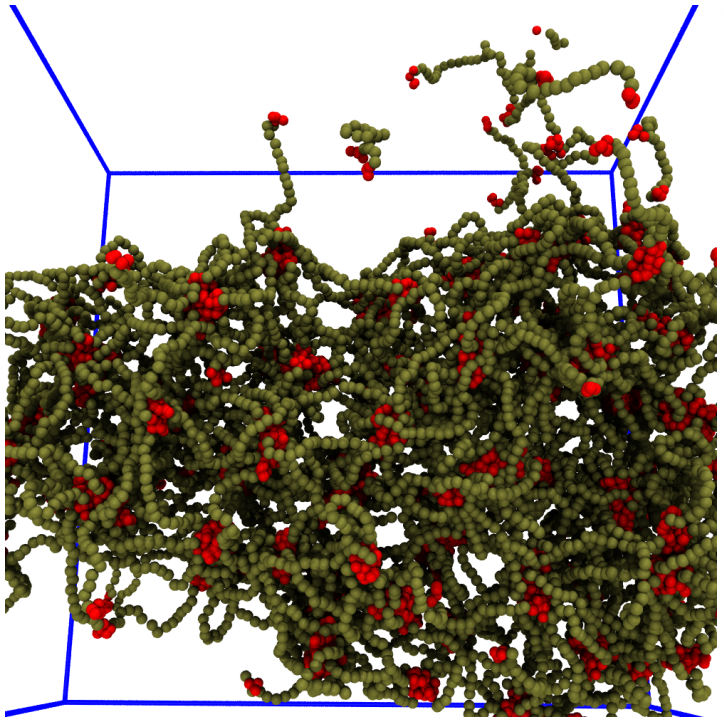


Mol. architecture: E - B_{16} - E

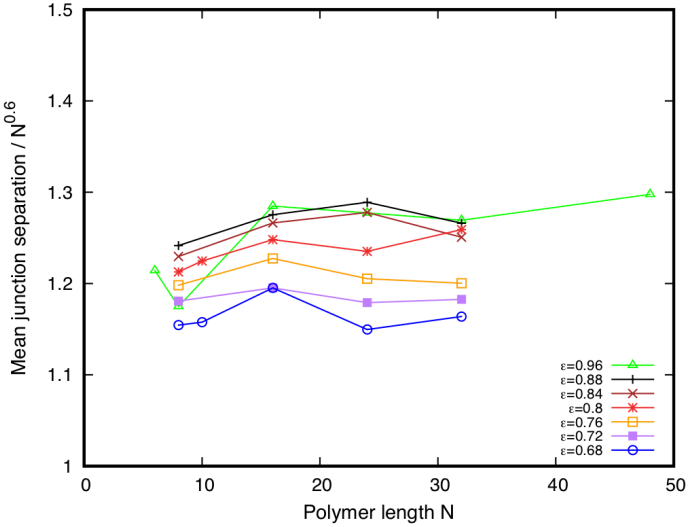
Assembly

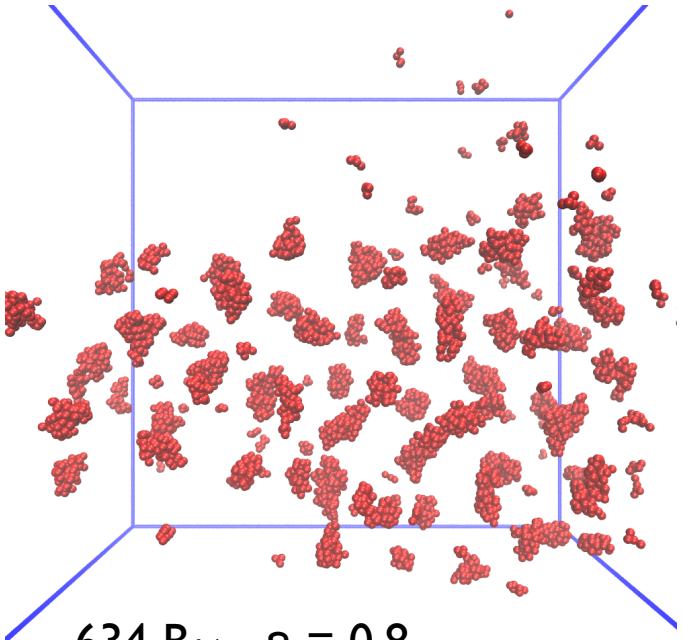
Box = 50 nm
N = 634
Affinity ε ~ 0.8 or *strong*





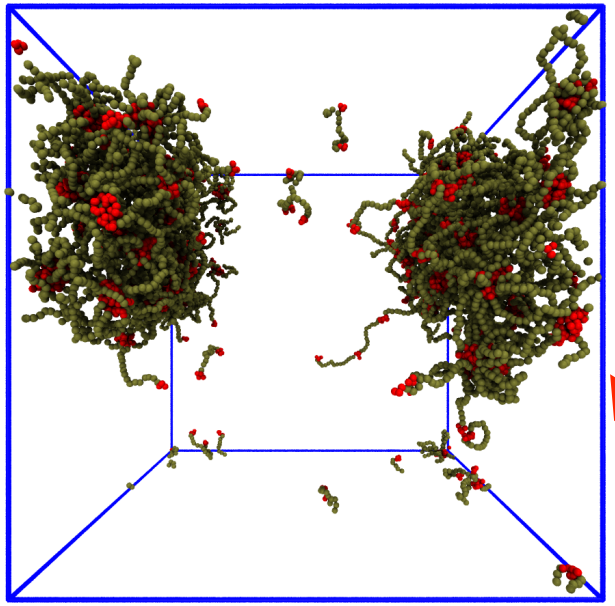
Separation is *independent* of concentration and (almost) affinity, and scales as a SAW with backbone length: $\langle R \rangle \sim N^{0.6}$

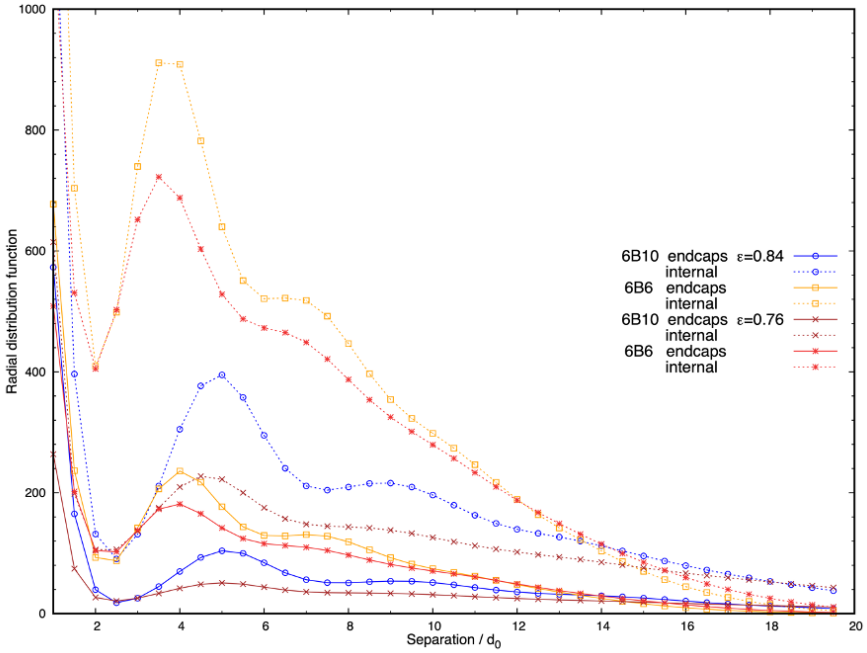




634 B_{16} $\varepsilon = 0.8$

Node separation is modulated by the binding site location not affinity



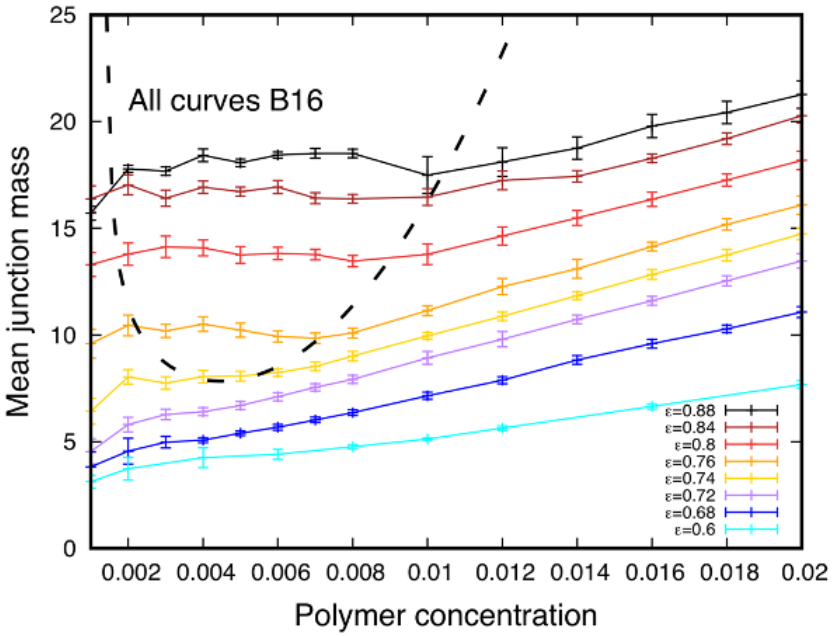


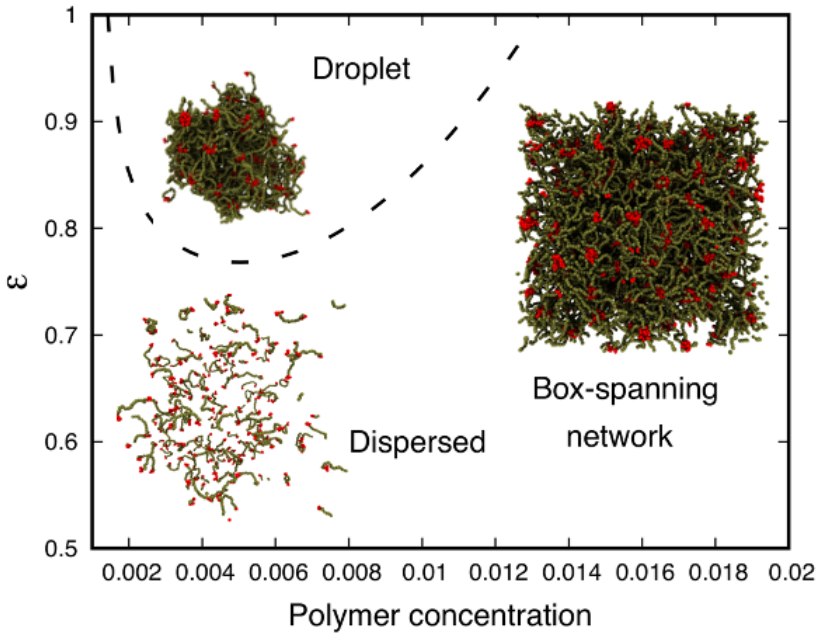
RDF of the nodes (red beads)
Increasing binding site sepn - peaks move apart
(yellow to blue; red to brown)

Reducing affinity - no change in peaks
(yellow to red; blue to brown)

Node separation controls porosity of the dense phase ~ diffusion of biochemical reactants

Phase diagram

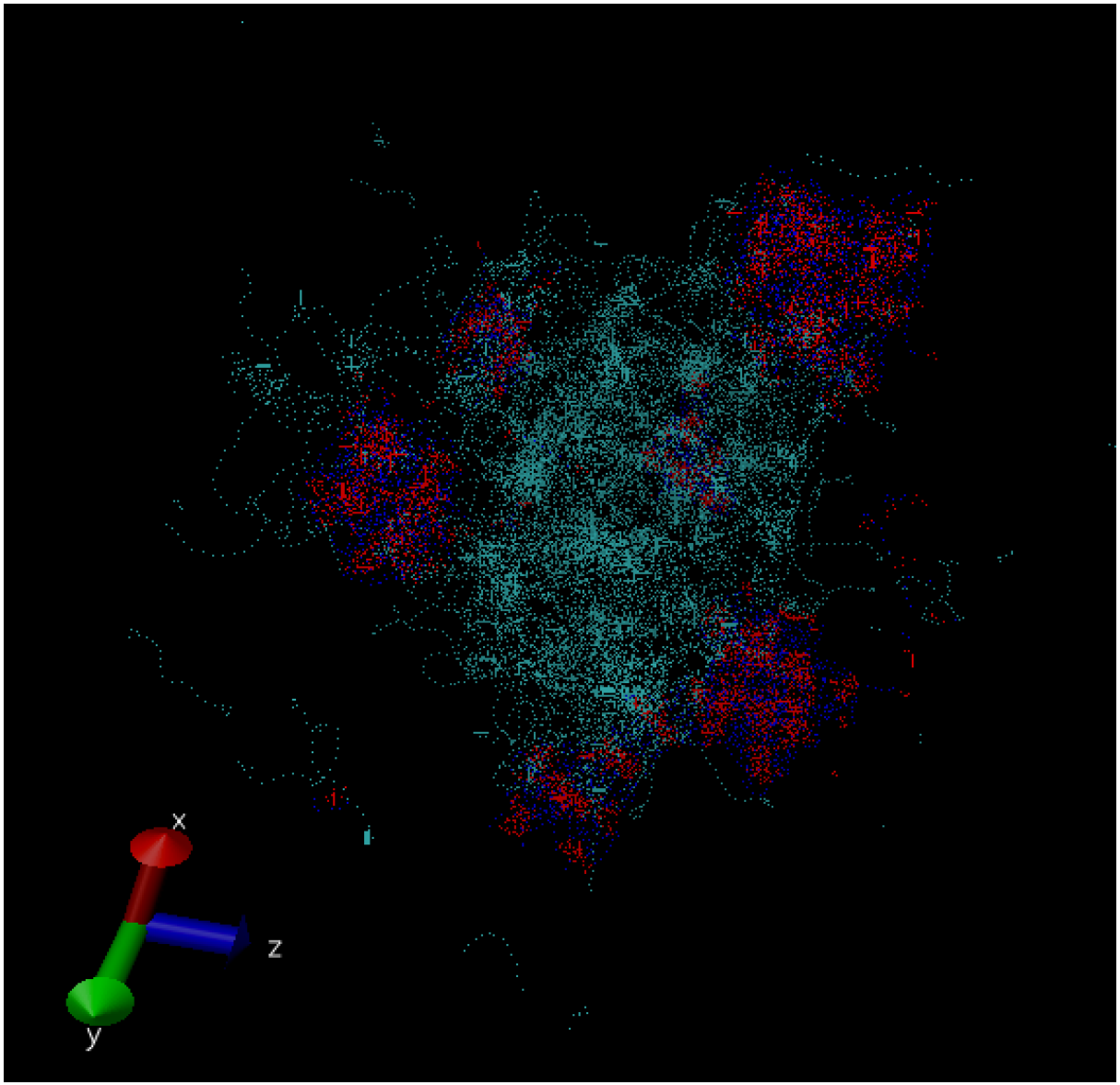




- R*eversible binding* + *entropy of fluctuations* lowers the free energy of the condensed phase below the dispersed phase
- Condensed phase has a spatial structure, low density, and mass distribution not predicted by Flory-Huggins theory
  - Spatial structure is controlled by binding site **separation**
  - Junction mass is modulated by binding site **affinity**
- Network porosity may functionally modulate diffusion and interaction of other proteins, and could be controlled by activating/deactivating interaction sites

Shillcock et al., Soft Matter 16:6413 (2020)

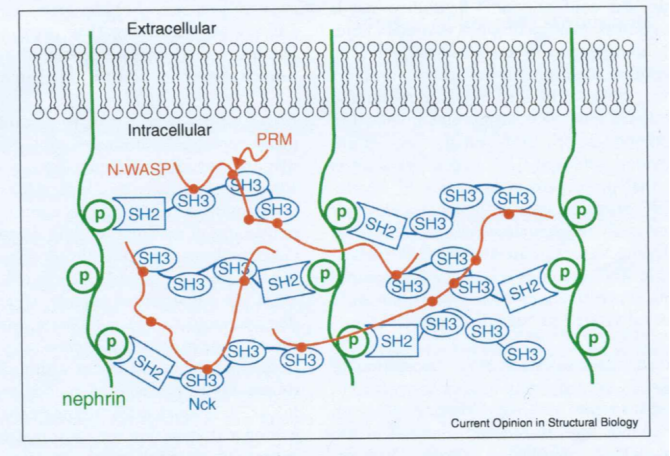
Going further …



This is an open field

- Droplets have many functions, but their structure is obscure
- Lots of experimental results, but little theory (see papers on moodle)
- Need better experiments on structure and dynamics
- Need better theories that predict observed properties

Biomolecular condensates often assemble at membranes in signalling networks … and tight junctions



Chong and Forman-Kay,
Curr. Op. Struct. Biol. 41:180 (2016)

Role of protein phase separation in the assembly of tight junctions

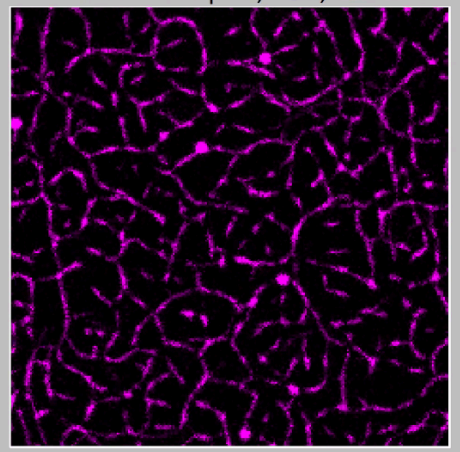
Oliver Beutel[a], Riccardo Maraspini[a], Alf Honigmann[a]

[a] Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany

https://phasage.eu/phasage-conference-1/

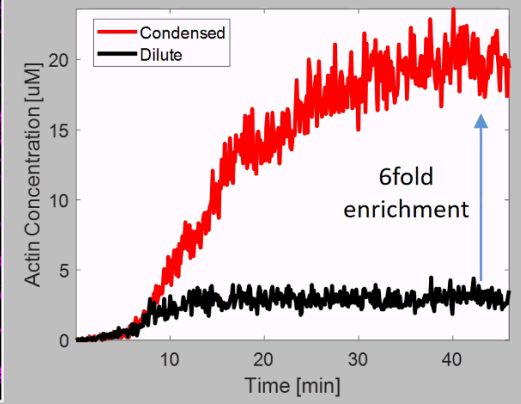
Activity: ZO1/Receptor condensates drive actin polymerization

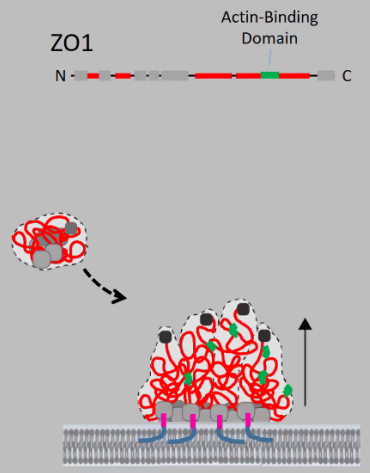
CLDN2-Receptor, ZO1, G-Actin



30min (10s per Frame)

5 µm

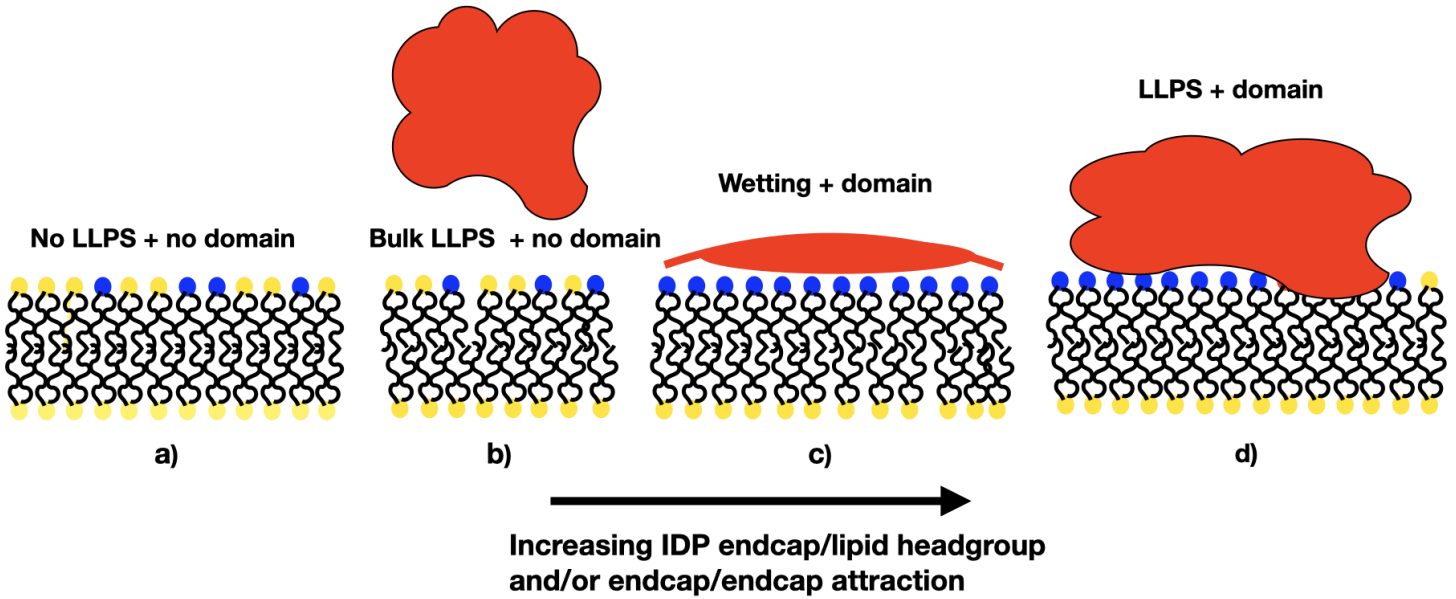




- Actin is enriched in ZO1/Receptor clusters ($K_p \approx 6$)
- Actin polymerizes out of clusters and forms network

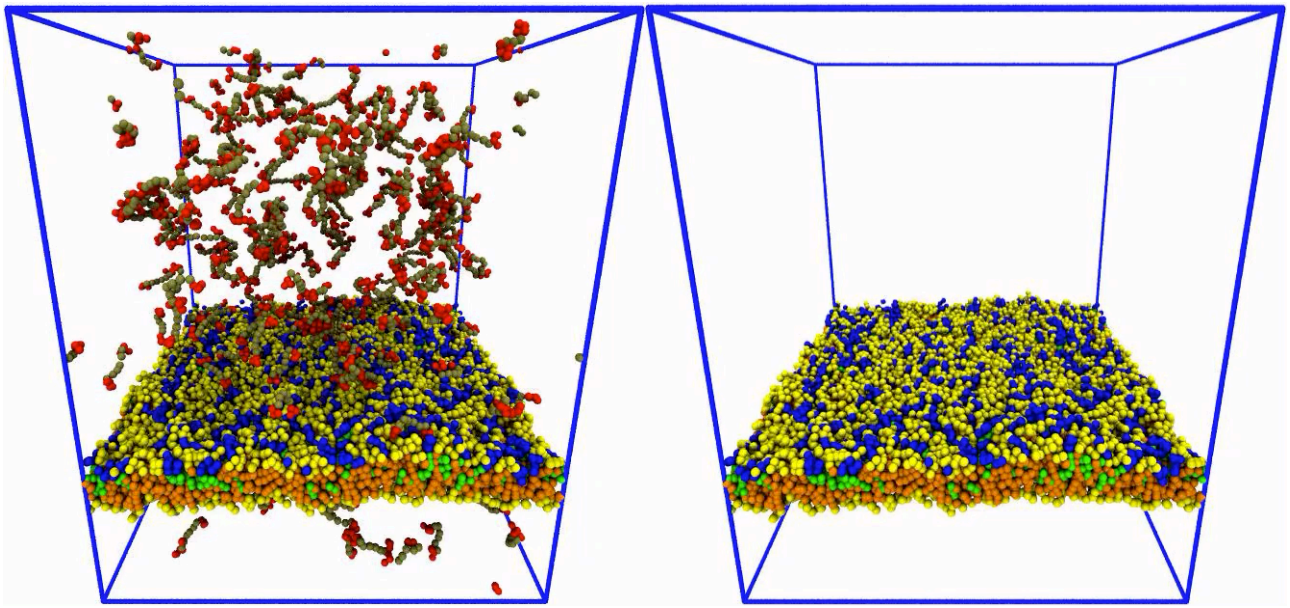
Sun et al, *in preparation*

Can phase separation of bulk IDPs create membrane domains?



- No IDP-membrane attraction at Time = 0; droplet forms in bulk
- Turn on at ~ 0.1 T_{max} ; adsorption occurs
- Turn off at ~ 0.8 T_{max}; IDPs desorb and forms droplet

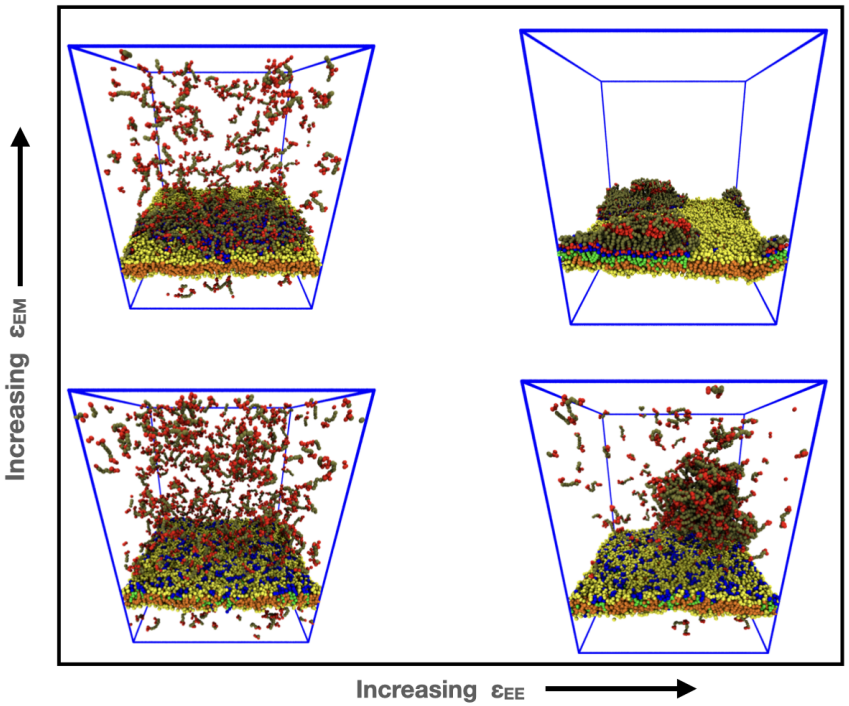
Adsorption reduces bulk conc. so droplet dissolves

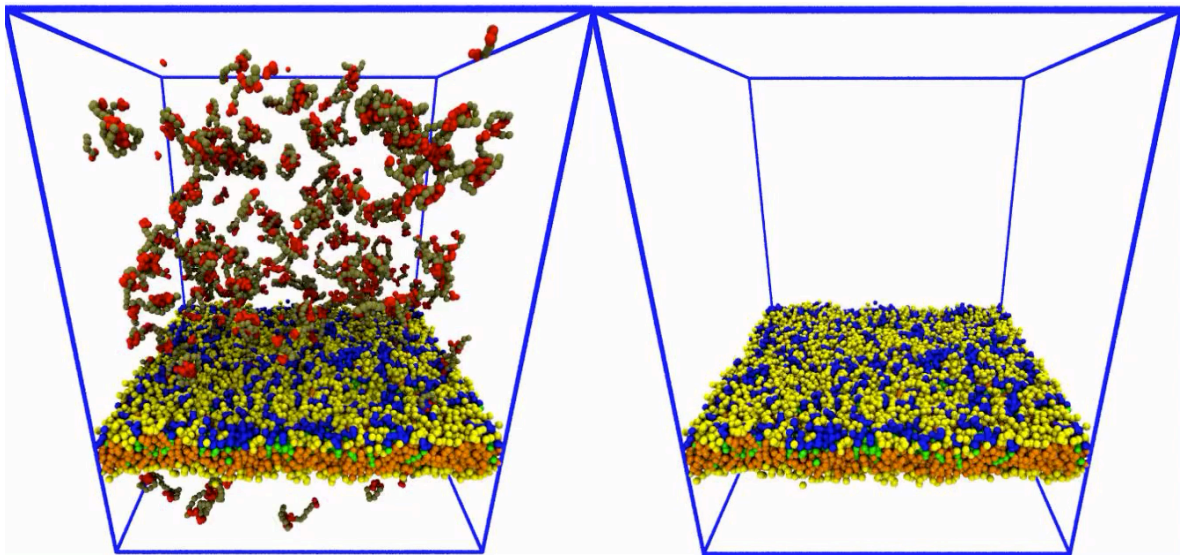


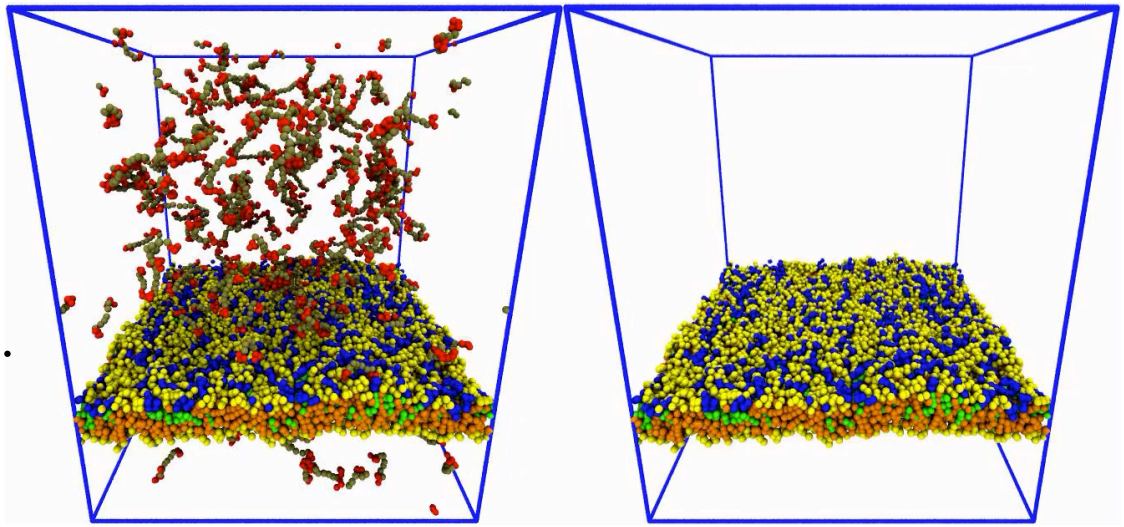
Morphology diagram in the endcap-endcap / endcap-membrane plane

Wetting

Domain formation by LLPS



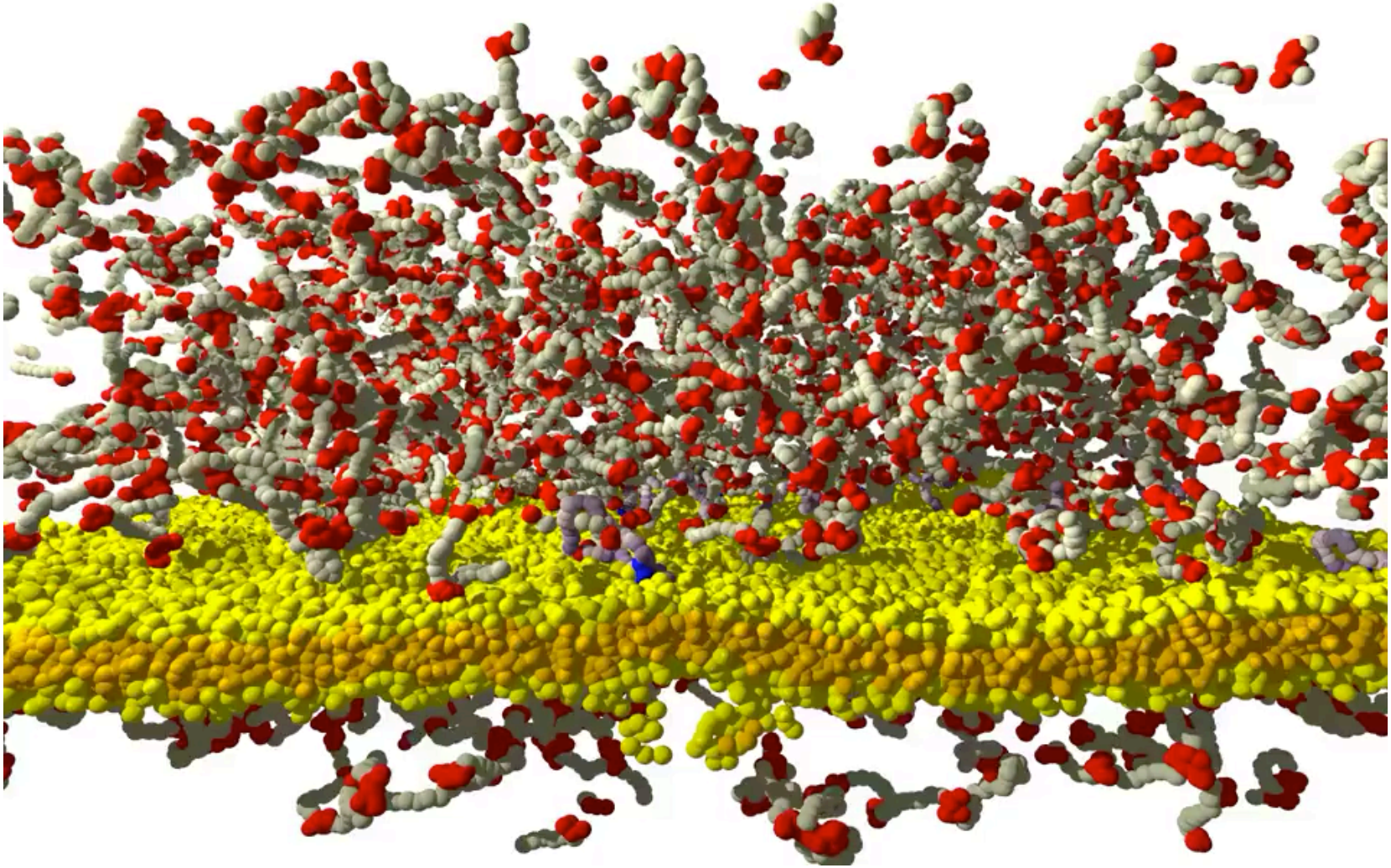




Independent

Bulk LLPS

Adsorption reduces bulk conc.
so droplet dissolves



System size

100 x 100 x 48 nm^3
14837 lipids + 24 colipid/linkers
2477 telechelic polymers

Romeo and Juliet droplets

Summary of lecture

Flory-Huggins theory predicts a phase transition in polymer mixtures because *on average* the monomers are constantly repelling each other between species (the chi - χ - parameter) - it's a Mean Field Theory.

DPD and Flory Huggins share a theoretical basis but simulations keep fluctuations

Assumptions of Flory-Huggins theory are wrong (not all monomers repel, conformational fluctuations are important not averaged over)

DPD is a good technique for exploring LLPS because:

a) polymers are *weakly* interacting and fluctuate strongly

b) they are in the fluid phase

c) Material properties/structure of the dense phase probably don't depend on atomic details, cp Van der Waals theory of liquid-gas phase transition.

Summary of course

EPFL

Biophysics is:

mapping (complex) biological processes onto (simpler) physical ones to reveal the principles underlying the biology
(random walks, membrane surfaces, polymer droplets)

hiding the complexity of biology within models/simulations
(packing parameter for lipids, pore creation/growth parameters, RW with binding sites for IDPs)

Equipartition theorem, random walks, diffusion, membrane-mediated forces, entropic spring, Flory-Huggins theory, LLPS

Building models based on:

what is important? energy, entropy, shape, flexibility, barrier, fluctuations, …

what is ignorable? detailed chemistry, initial conditions, diffusion, …

Break
10 minutes

As you know, all courses receive an in-depth evaluation each semester. The in-depth evaluation survey for your course BIOENG-455_SA24/25 has just been opened to students and will remain available until 12.01.2025 23:59:00.

The report will be available to you once the exam period has ended, on 11 February 2025.

The student feedback will be more useful to you if the response rate is higher and so we recommend that, if possible, you dedicate 5 minutes at the beginning or end of a class for students to complete the survey.
The evaluations are accessible via the moodle. To access them, students have to:
Log onto moodle and stay on the moodle home page (dashboard, not the course page).Click on the arrow to the top right of the screen which will reveal a block that contains the entitled "In-depth evaluation" tile (please note: all evaluations will be together in the evaluation tile on the moodle home page, and not separate in each course moodle page). Students can then select your course and complete the feedback.

Students will also be able to access the course evaluations via the EPFL Campus App. We hope this will make the surveys more accessible and so help you to increase the response rate.
Teachers can access evaluations in the same location on the moodle home page. You will be able to:
Access the response rate while the evaluation is open (Moodle - nouveau plugin). Access the report from 11 February 2025.

If you share teaching responsibilities for this course, please inform your colleagues since, in order to avoid many teachers getting multiple emails, only one teacher per course has received this notification.

In-depth evaluation of BIOENG 455
5 mins

Comme vous le savez, tous les cours font l'objet d'une évaluation approfondie chaque semestre. L'enquête d'évaluation approfondie pour votre cours BIOENG-455_SA24/25 vient d'être ouverte aux étudiant·es et restera disponible jusqu'au 12.01.2025 23:59:00.

Le rapport sera disponible une fois la période d'examen terminée, le 11 février 2025.

Les commentaires des étudiant·es vous seront plus utiles si le taux de réponse est élevé et nous vous recommandons donc, si possible, de consacrer 5 minutes au début ou à la fin d'un cours pour qu'ils et elles puissent répondre à l'enquête.
Les évaluations sont accessibles via la page d'accueil de moodle. Pour y accéder, les étudiant·es doivent :
Se connecter à moodle et rester sur la page d'accueil (tableau de bord, pas la page du cours).Cliquer sur la flèche en haut à droite de l'écran qui fera apparaître un bloc contenant la tuile intitulée "Évaluation approfondie" (veuillez noter que toutes les évaluations seront regroupées dans la tuile d'évaluation sur la page d'accueil de moodle, et non pas séparées dans chaque page moodle de cours).Les étudiant·es peuvent alors sélectionner votre cours et compléter le feedback.

Les étudiant·es peuvent également accéder aux évaluations de cours via l'application PocketCampus. Nous espérons que cela rendra les enquêtes plus accessibles et vous aidera ainsi à augmenter le taux de réponse.

Les enseignant·es pouvez accéder aux évaluations au même endroit sur la page d'accueil de moodle (Tableau de bord):
Accéder au taux de réponse pendant que l'évaluation est ouverte (Moodle - nouveau plugin). Accéder au rapport dès le 11 février 2025.

Si vous partagez les responsabilités d'enseignement pour ce cours, veuillez en informer vos collègues car, afin d'éviter que de nombreux enseignants reçoivent plusieurs courriels, seul un·e enseignant·e par cours a reçu cette notification.

Cordialement,

Centre d'appui à l'enseignement (CAPE).
Remarque: Il s'agit d'un e-mail généré automatiquement. N'hésitez pas à contacter le conseiller pédagogique de votre section si vous avez des questions.

In-depth evaluation of BIOENG 455
5 mins.

Comments	Pro	Contra
Lectures/ contents		
Marking/Tests		
Organisation		
Workload		
Overall		

Comments: