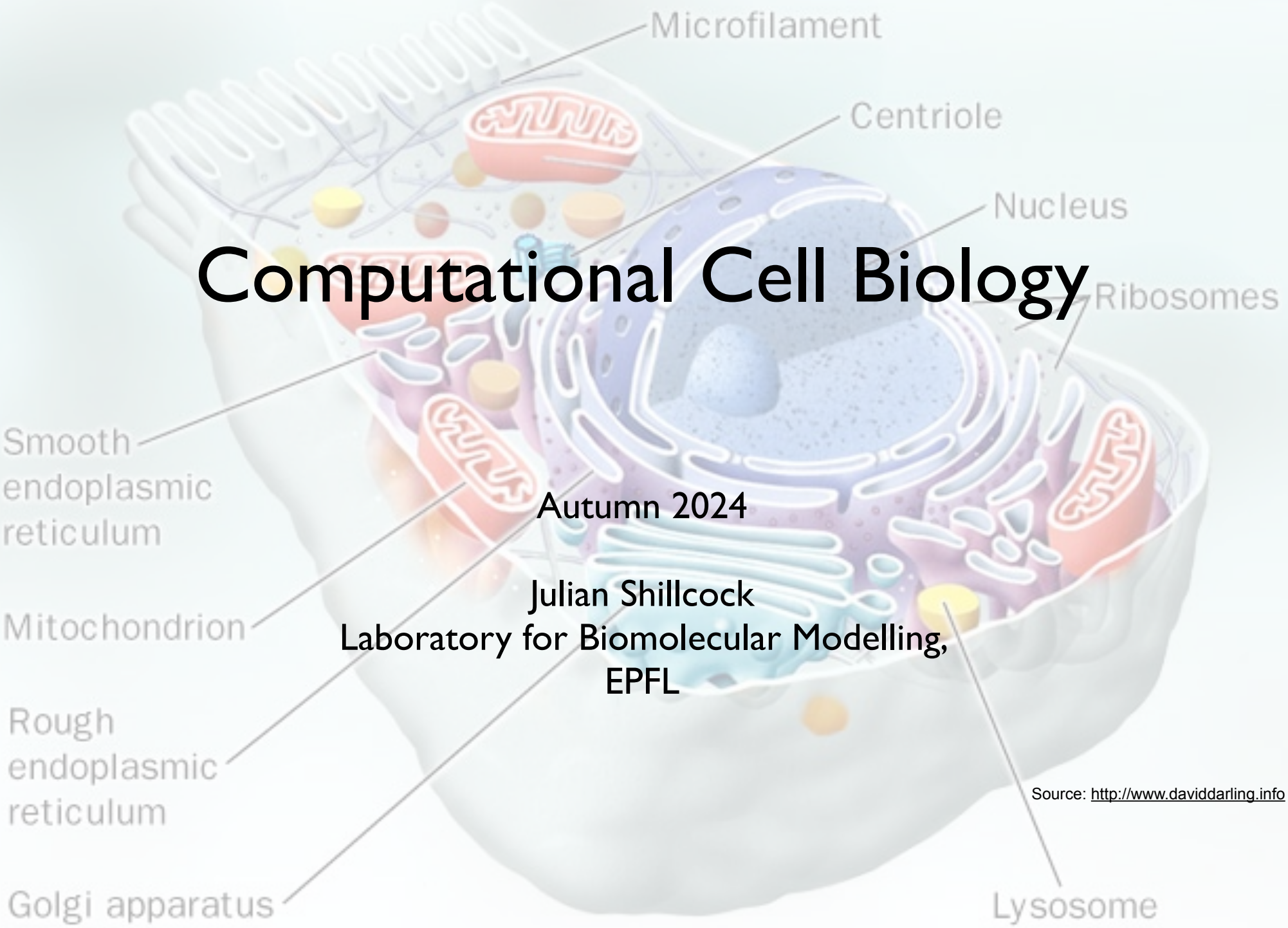
Computational Cell Biology



Autumn 2024

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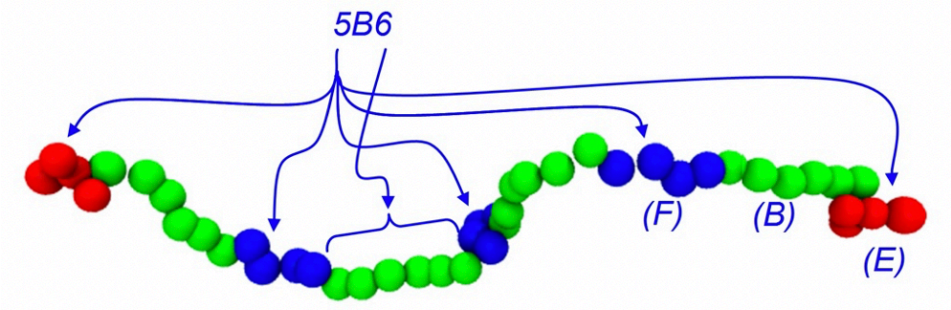
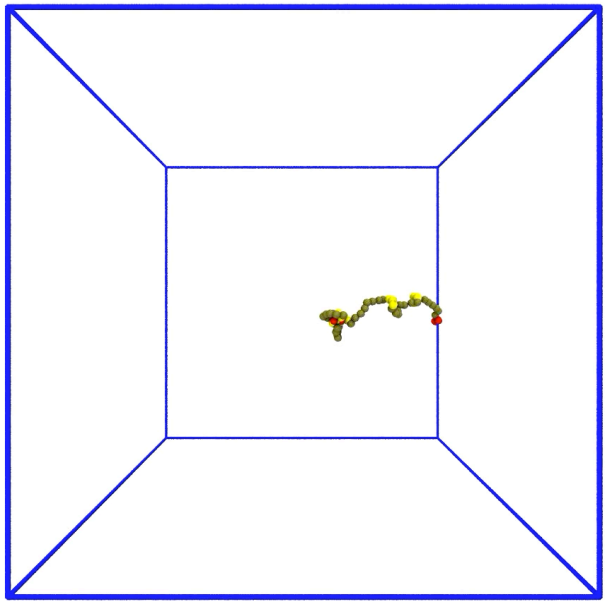
Source: http://www.daviddarling.info

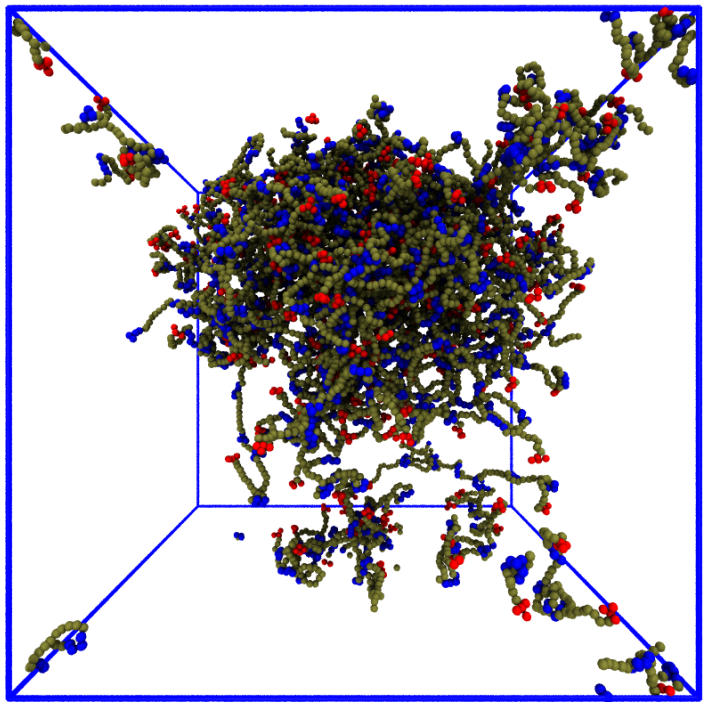
Question

Consider a polymer with only repulsive interactions fluctuating in water. Now consider one with discrete, sticky parts (red and blue below) that attract each other, and consider **many** sticky polymers soluble in water.

Q. Is it possible for the sticky polymer's Lee to increase with increasing stickiness? so it is bigger/longer than its non-sticky counterpart?







Core Concepts

Sometimes you have to be sneaky to get your way (indirect forces)

Divide and conquer (compartments)

Noise can be useful… it can do work

(Polymers can be quite weird)

Forces and compartmentalisation

How to classify forces relevant in cells? Direct versus Indirect

Direct: Gravity, Coulomb, H-bonds, covalent and ionic bonds

- Operate between two atoms "independently" of others ⇒ pairwise additive
- Typically short-ranged (not gravity! or Coulomb force in a vacuum)

Indirect: Hydrophobic effect, Depletion, Capillary forces, Membrane-mediated, Fluctuation-induced forces

- Arise between many molecules simultaneously, strongly affected by other objects ⇒ NOT pairwise additive
- Can be long ranged (~ 1/R)
- Proportional to temperature (so pressure is *entropic*… PV = RT)

Direct forces

Gravitational force between two point masses:

$F = G M_1 M_2 / R^2$ $\quad G = 6.67\ 10^{-11}\ N.m^2/Kg^2$

Nearly negligible for cells, but they can sink under gravity

Coulomb force between charges in vacuum

$F = k Q_1 Q_2 / R^2$ $\quad k = 1 / 4\pi\varepsilon_0 = 9\ 10^9\ N.m^2 / C^2$

in a material ε_0 is replaced by $\varepsilon_0\,\varepsilon$, where ε is the relative permittivity of the material, and makes a big difference between water ($\varepsilon \sim 80$) and oil ($\varepsilon \sim 1$).

Bare force is usually screened out by ions under physiological conditions

Screened Coulomb force

$F = e^{-\kappa R} / R$ $\quad \kappa^{-1}$ = screening length ~ 1 nm in physiol. conditions

$F_G / F_C \sim G M_1 M_2 / k Q_1 Q_2 \sim 10^{-10}\ (10^{-27})^2 / 10^{10}\ (10^{-19})^2 \sim 10^{-36}$

so gravitational force between bare ions is utterly negligible.

Chemical and physical bonds

Bond Type	Strength (kJ/Mol)	Strength (k_BT)	Length (nm)	Description
Covalent	500	200	0.154 (C-C)	Shared outer e^-
Ionic	~ 880	~ 355	0.276 (NaCl nn)	e^- donated/summed
Hydrogen	10 - 40	4 - 16	~ 0.176 (O…H)	small H - electroneg. atom
"Van der Waals"	~ 1	~ 0.4	~ $1/R^6$	fluctuating induced dipoles

NB 1 kJ/Mol ~ 0.4 k_BT per particle

J. Israelachvili, Intermolecular and Surface Forces, Academic Press, 2nd ed. London 1992.

If Strength ~ k_BT, a force has no effect as thermal noise overwhelms it:

- Covalent and ionic bonds cannot be broken just by (room) temperature
- H-bonds may be broken by a large fluctuation
- VDW must occur as many bonds to have any effect

Limitations of direct forces

Bare electrostatic forces are usually not important in cells because:

- short-ranged/screened
- cell is not a vacuum, water, ions, membranes reduce the forces
- ionic crystals don't form inside cells (but in blood, high concentrations can lead to crystallisation, e.g., uric acid leads to gout)

Exceptions: ion channels, binding pocket where particles are very close, actin/microtubule filaments which are rigidly-connected monomers

For large aggregates or materials >> atomic size, direct forces appear as **material constants**, e.g., surface tension, stiffness, compressibility, bending modulus, etc., that must be determined by measurements.

These material properties then give rise to **indirect forces** that are mediated by the material or environment.

Indirect forces

Indirect forces originate in Coulomb's law (electrostatics) but involve many weak interactions rather than one strong interaction between two points.

This makes them hard to calculate, and almost impossible to guess their functional form or even their sign, except that they $\propto$ Temp

Importantly: indirect forces are free, i.e., they arise from thermodynamics, not from the specific atomic structure of the interacting molecules. Such *generic* forces are everywhere in a cell. (cp. polymer $R_{ee}^2 \sim Na^2$ does not depend on monomer type).

Entropic forces	**Membrane-mediated forces**
hydrophobic effect of oily chains in water	curvature force
depletion (molecular crowding)	composition mismatch
fluctuation-induced forces	thickness mismatch

Equipartition theorem

If we regard a thermodynamic system as composed of molecules, the question arises how its internal energy is distributed among all the molecules?

An important result is the **Principle of Equipartition of Energy:**

" Each additive, quadratic degree of freedom in the Hamiltonian of a system *in equilibrium* contributes 1/2 $k_B T$ to its internal energy. The energy is shared among all *accessible* degrees of freedom of the system: if the temperature is such that some degrees of freedom cannot be excited they do not contribute to the internal energy. "

(Blackboard calculation)

A consequence of this theorem is:

We have to do work on a macroscopic object to move it - all motion tends to cease as energy is lost to friction, heat, etc.

We have to do work on a microscopic object to STOP it moving - all molecules tend to move continually, bouncing off each other and sharing their energy. In a fluid, this motion can be observed and is called Brownian motion (cp. vesicle on slide 12)

Equipartition theorem

Let $H(\{x\})$ be the Hamiltonian of a system with dof $\{x\} = x_1, x_2, x_3, \ldots x_N$

The expected value of an Observable $A(\{x\})$ is defined by:

$$\langle A \rangle = \int A(\{x\})\, e^{-\beta H(\{x\})}\, dx_1 \ldots dx_N \,/\, Z$$

$$Z = \int e^{-\beta H(\{x\})}\, dx_1 \ldots dx_N$$

If the following two conditions on H hold:

1) H is additive in some coordinate x_1: $H(\{x\}) = H_1(x_1) + H'(x_2 \ldots x_N)$
2) H_1 is quadratic in x_1: $H_1(x_1) \sim \alpha x_1^2$

Then the mean value of $\langle H_1 \rangle$ is:

$$\langle H_1 \rangle = 1/2\, k_B T$$

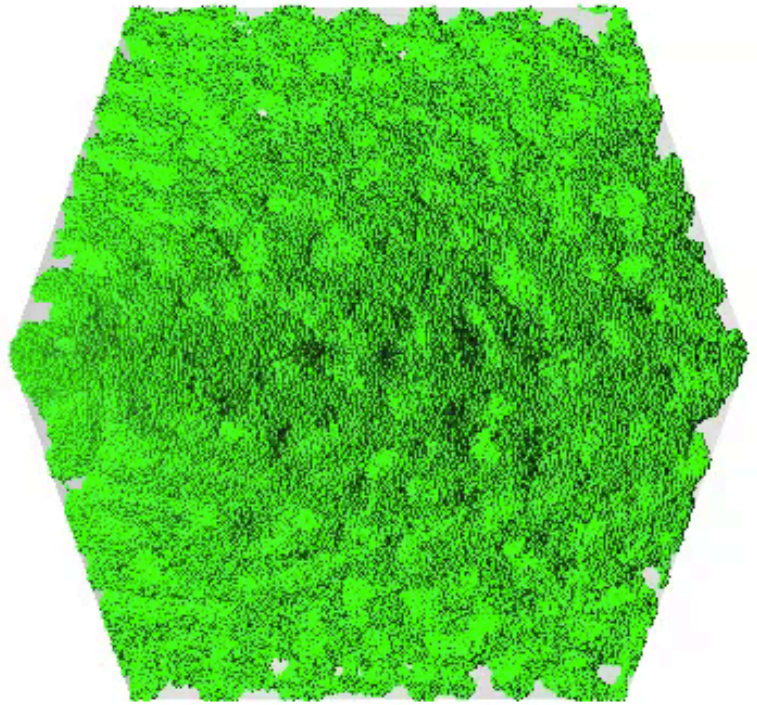
Hydrophobic effect

Water - an homogenous liquid with no bulk structure beyond ~ 1nm, that possesses the unique property of a hydrogen bonded network between the H_2O molecules.

This network has a huge entropy as the molecules continually make and break H-bonds (Eq. Thm again …)

The hydrophobic effect is the name for the prevention of H-bonding between water by the presence of non-polar molecules that drives them to aggregate in one place.

C. Tanford, The Hydrophobic Effect, Wiley, New York 1980



NB. Movies produced from DPD simulations and visualized with Povray and Quicktime. Water in the simulation box is invisible for clarity.

Mix oil and water ⇒ droplets appear by phase separation and create a bounding surface between the phases.

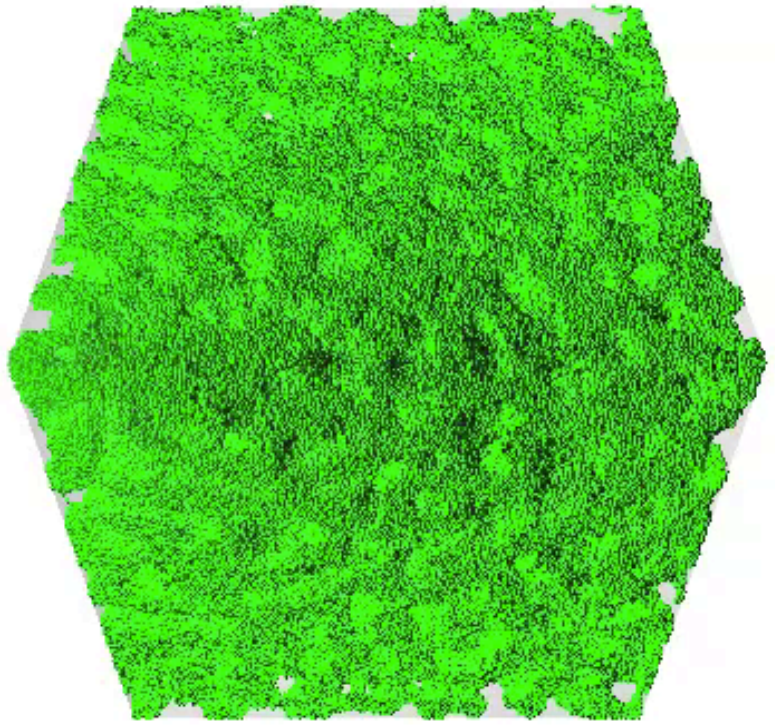
Q. How could you reduce or eliminate the planar interface between oil and water?

3 mins.

Why does it form?

What could you do to change the free energy of the interface?

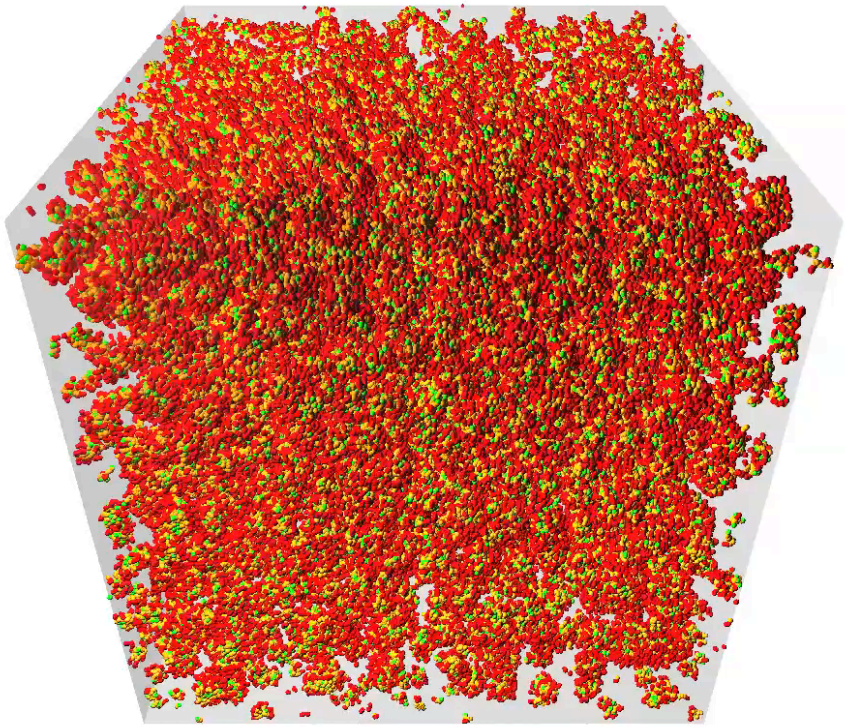
What chemical could you add?

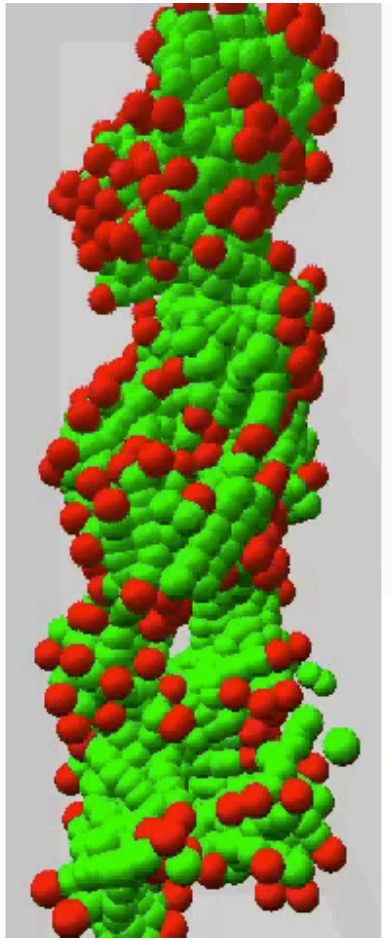


Amphiphiles form complex aggregates in water

Mix lipids and water ⇒ interfaces and *compartments* appear:

Compartments can support gradients, and gradients can be used to do work, e.g., ion concentrations can differ across the neuronal plasma membrane.





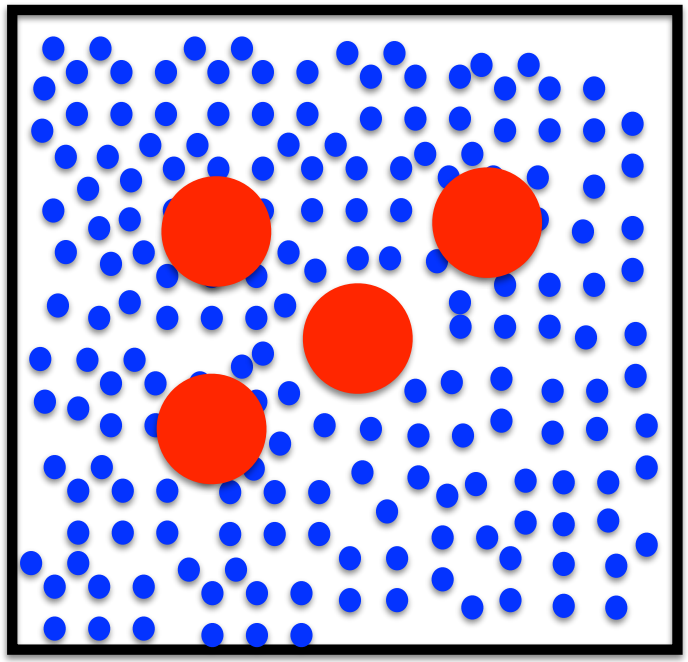
Type of aggregate is encoded in lipid's molecular shape: no external control is needed: we expect that simulations will be useful in predicting lipid phases if we can just capture their amphiphilic nature.

Depletion forces

Depletion forces arise when some entities restrict the freedom of others to move (or fluctuate).

Large solute particles (red) exclude the smaller solvent molecules (blue) from around them.

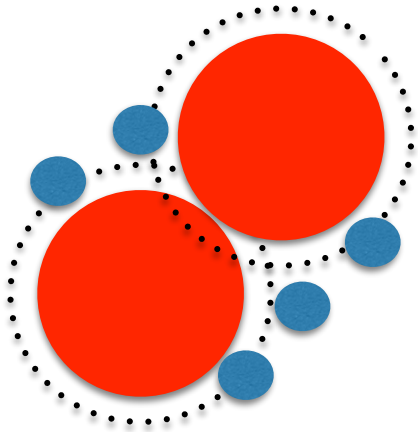
If the red ones cluster, some volume is freed up, and there is more space for the solvent to move around, hence the entropy increases.



Not to scale

Depletion forces don't only arise in bulk solvent, they also arise within membranes (see later).

They are independent of the type of entity (atom, molecule, nanoparticle, membrane) and only require the molecules to be repulsive at short range (steric repulsion) and mobile.

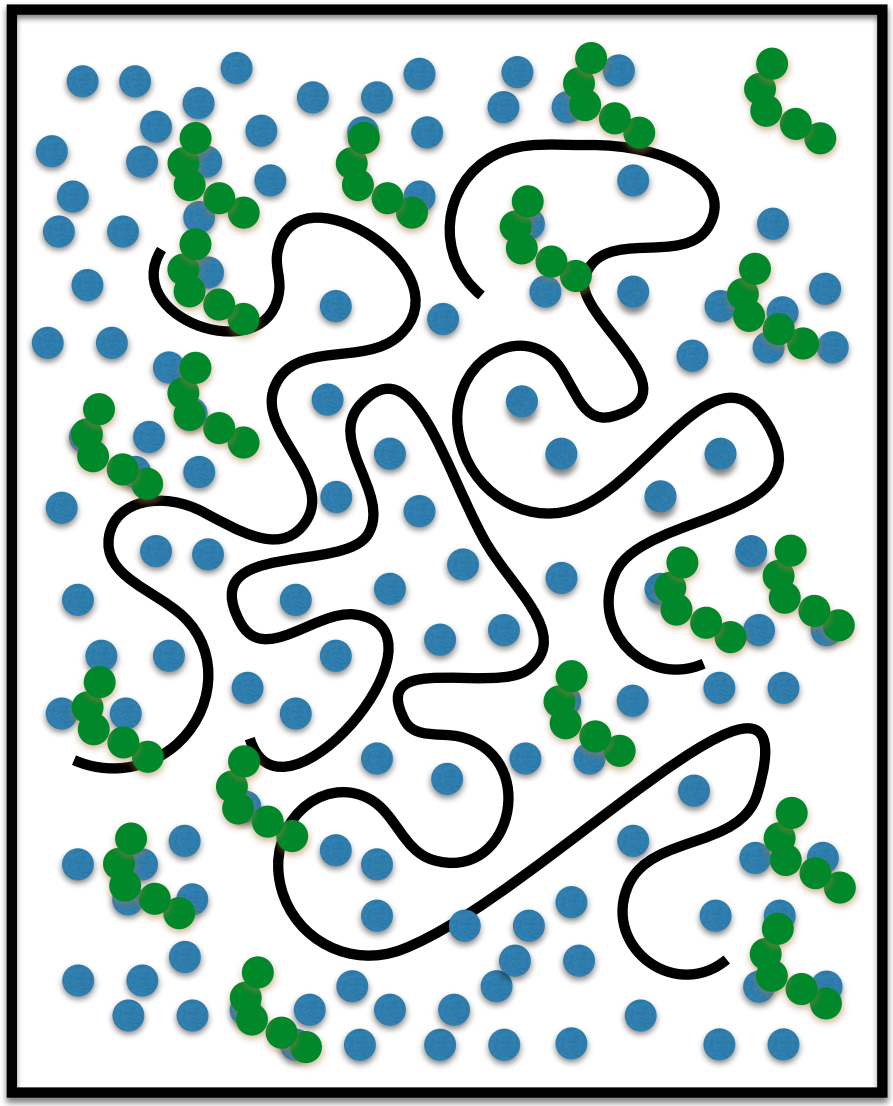


Molecular crowding

Consider a dilute solution of long polymers in water in the presence of concentrated short polymers.

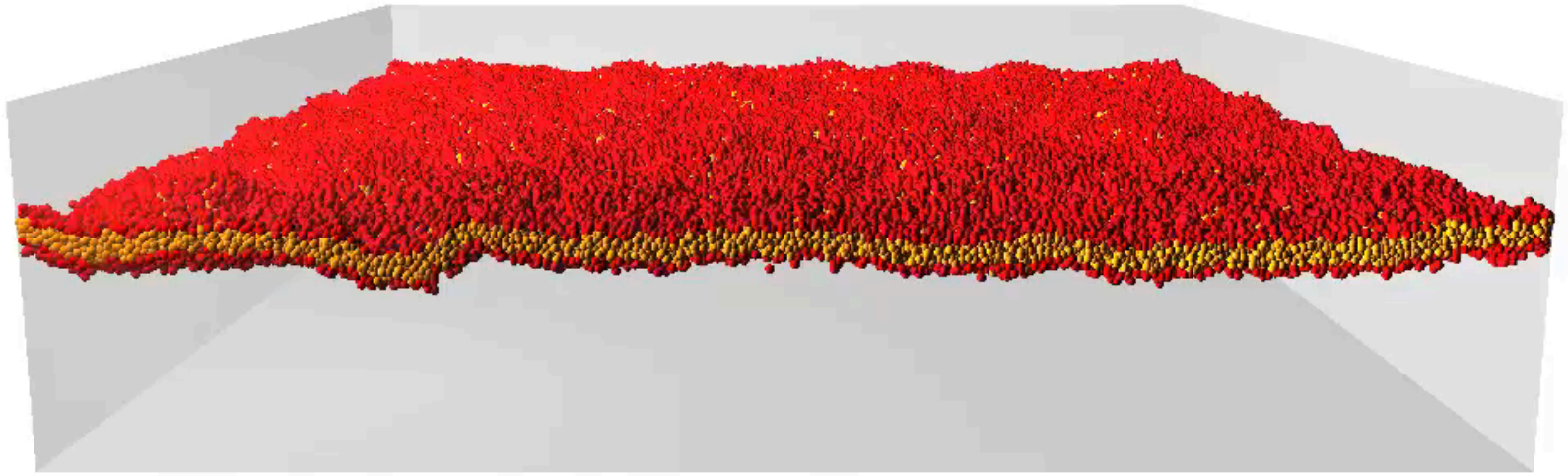
If the two polymer types are sterically repulsive, the long ones restrict the motion and shape fluctuations of the short ones.

This leads to a force on the long ones that squeezes them into a region to maximise the entropy of the short ones.



Membrane fluctuation force

The thermal fluctuations of a lipid membrane give rise to a repulsive force on an object or material that approaches and suppresses its fluctuations: this would lower its entropy and therefore is opposed.

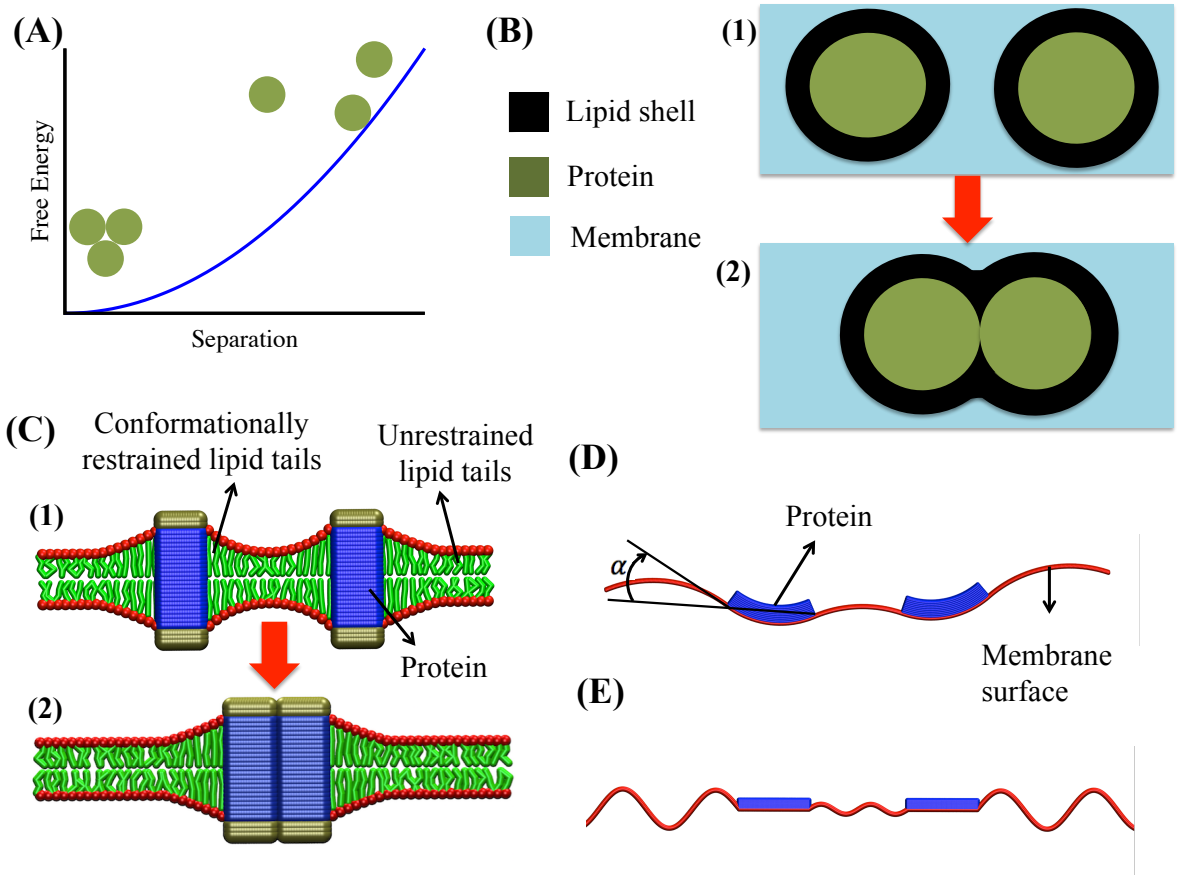


What about forces on small objects in, or adsorbed to, a membrane?

This is a whole new set of indirect forces.

Indirect forces: membrane-mediated

Unlike bare forces, membrane-mediated forces arise when two (or more) proteins/nanoparticles adsorb to/embed in a membrane and perturb its state.



Johannes et al. Trends Cell Biol. 2018

A) All operate by lowering the total free energy of membrane+proteins

B) Capillary force/line tension

C) Depletion force

D) Curvature force

E) Fluctuation-induced force (Lecture 11)

What does a cell do with these forces?

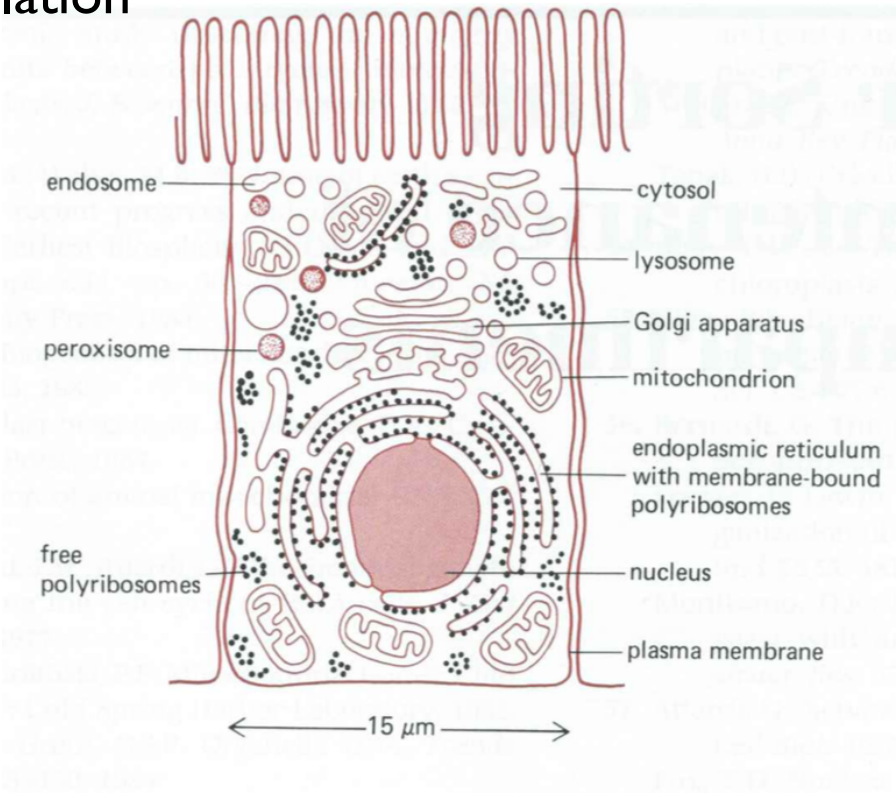
- Bring materials together / push them apart
- Make compartments
- Bacteria use them to lyse cells (Lecture 11)

Why compartmentalise?

Compartments allow spatial segregation of function: reactions can be kept apart, acidic environment in one place/neutral in another, gradients can form, ATP made, DNA transcribed in nucleus, etc.

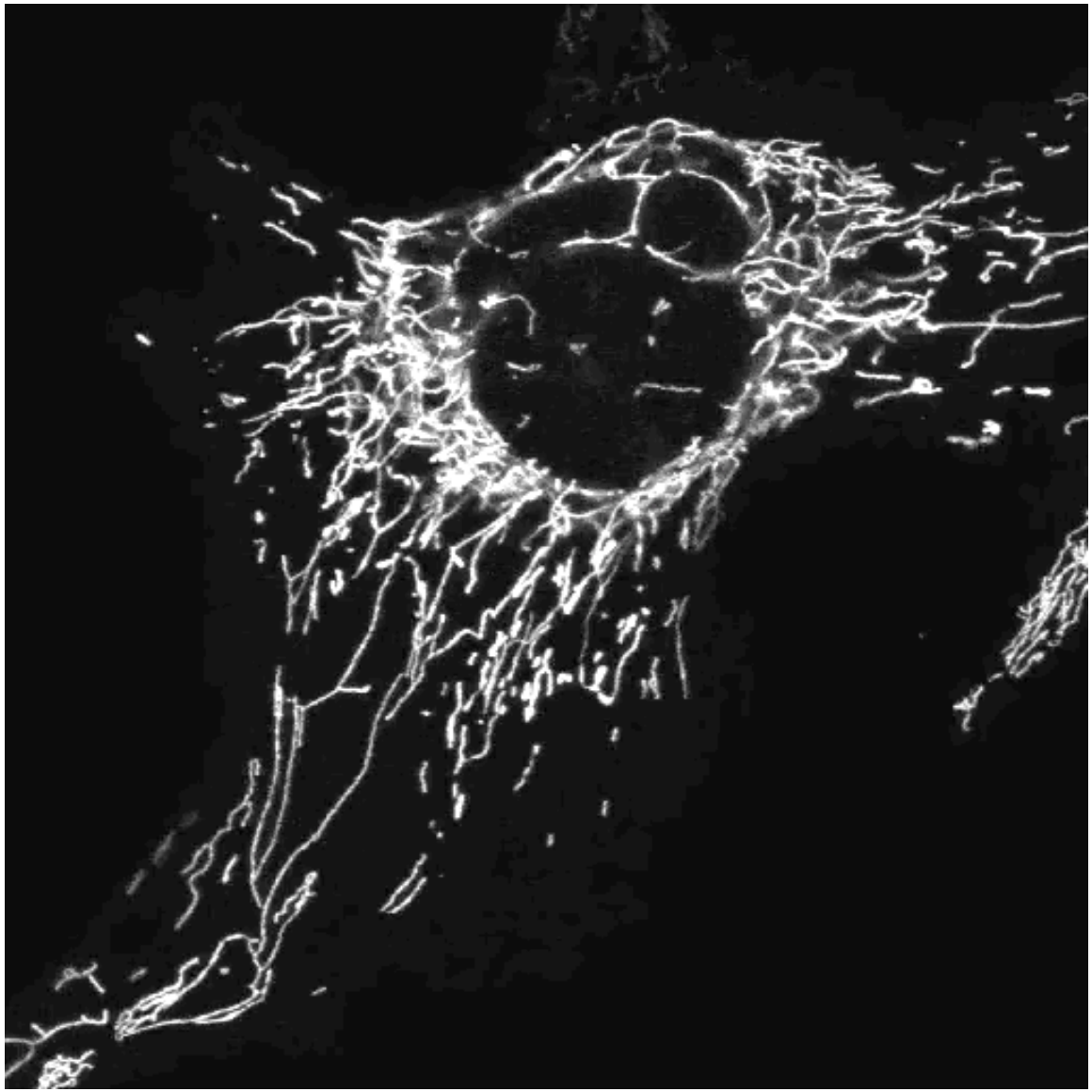
- Nucleus - separates transcription from translation
- Rough ER - ribosomes synthesize proteins
- Smooth ER - lipid synthesis
- Golgi - sorting proteins for destination
- Mitochondria - e^- transport, H^+ gradient
- Endosome - imports material into cell

Alberts and Bray, Fig. 8.1



and lipid membrane-bound compartments come ***for free*** because of the hydrophobic effect.

Membrane-bound organelles are not static



David Chan, Caltech

Fusing and fission of mitochondria

Self-assembly is a consequence of Equipartition Thm.

- Self-assembled structures form because they minimise a system's free energy by **phase separating** rather than staying mixed
- The cell uses self-assembly because it's **free**
- It's free because of the Equipartition Theorem

The total energy of a molecular system is continually redistributed among all its atoms and molecules by random thermal motion; this allows the system to eventually discover its state of lowest free energy.

Membrane-bound compartments self-assemble from dispersed lipids because of the hydrophobic effect

- Are there other inter-molecular forces that stabilise aggregates or compartments?
- Are all cellular compartments bounded by lipid membranes?

Short opinion poll

All models are wrong but some are useful

Who has heard this before?

Do you agree with it?

What is the difference between "wrong" and "inaccurate" in the context of science?

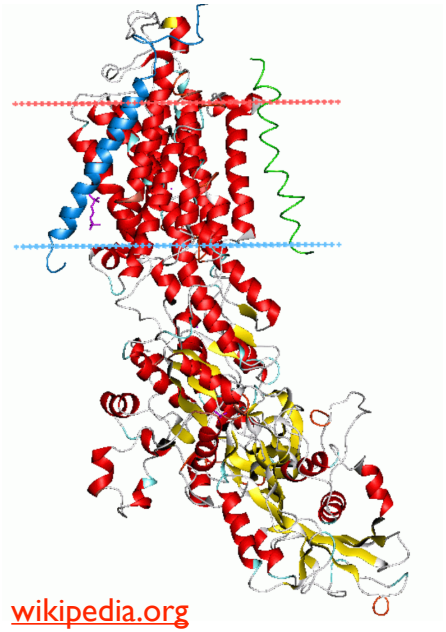
Opposite extremes of protein models

Folded protein

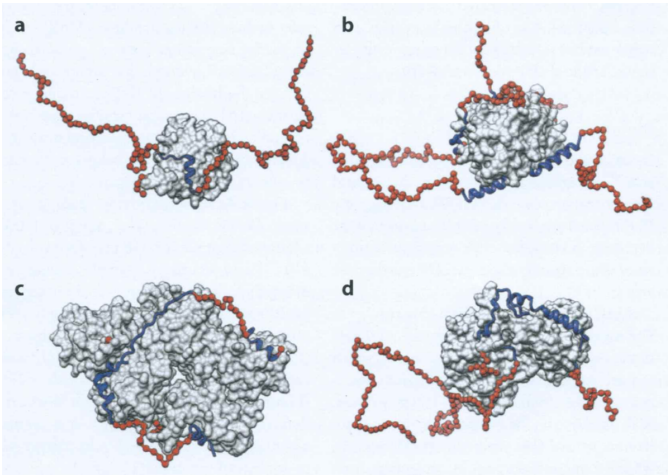
- Unique folded state
- Lowest energy (energy dominated)
- Precise shape
- Precise functions
- Disrupted by single aa mutation
- No model, need the actual protein

Intrinsically Disordered protein

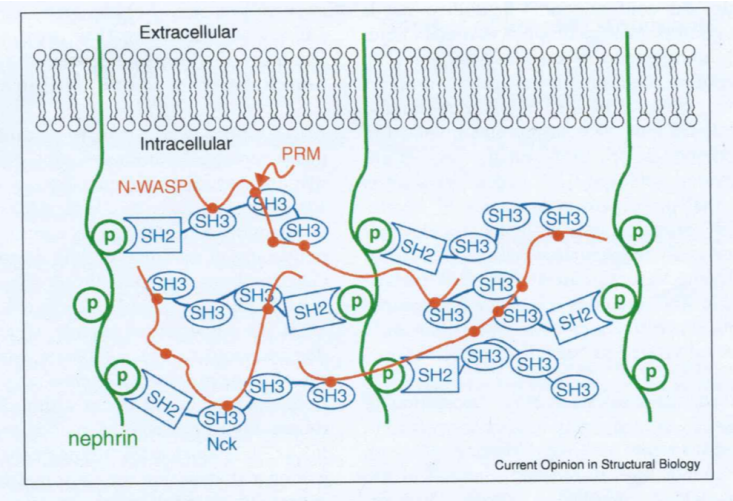
- No unique folded state
- Many conformations of similar energy (entropy dominated)
- Generic binding via multiple, weak sites
- Sequence not conserved but properties are
- Can fold/unfold on binding
- Model it as a phantom chain?



wikipedia.org



Oldfield and Dunker
Ann. Rev. Biochem.l. 83:553 (2014)



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

Can we make a mathematical model of IDPs?

Relevant facts:

- an IDP protein is a long, flexible polymer with multiple, weak binding sites
- aggregate into spherical droplets with a (small) surface tension
- droplets have low density, not densely packed like oil droplets

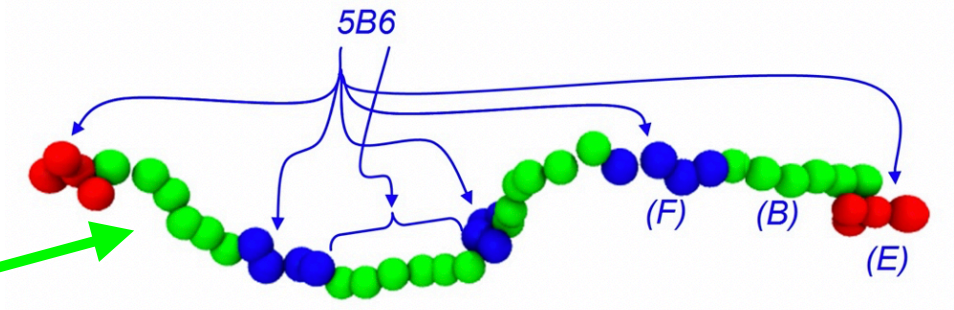
Relevant questions:

- How does a polymer's average size in solution scale with its molecular weight?
- At what concentration do proteins in solution "notice" each other?
- What is their shape in solution and in the aggregate (if they form one)?
- What is the shape of the aggregate?
- What are typical energies of the proteins' interactions?
- What is their entropy?
- Which free energy should we consider?

Phase separation depends on IDP molecular structure, e.g., moving sticky sites apart weakens it at constant affinity

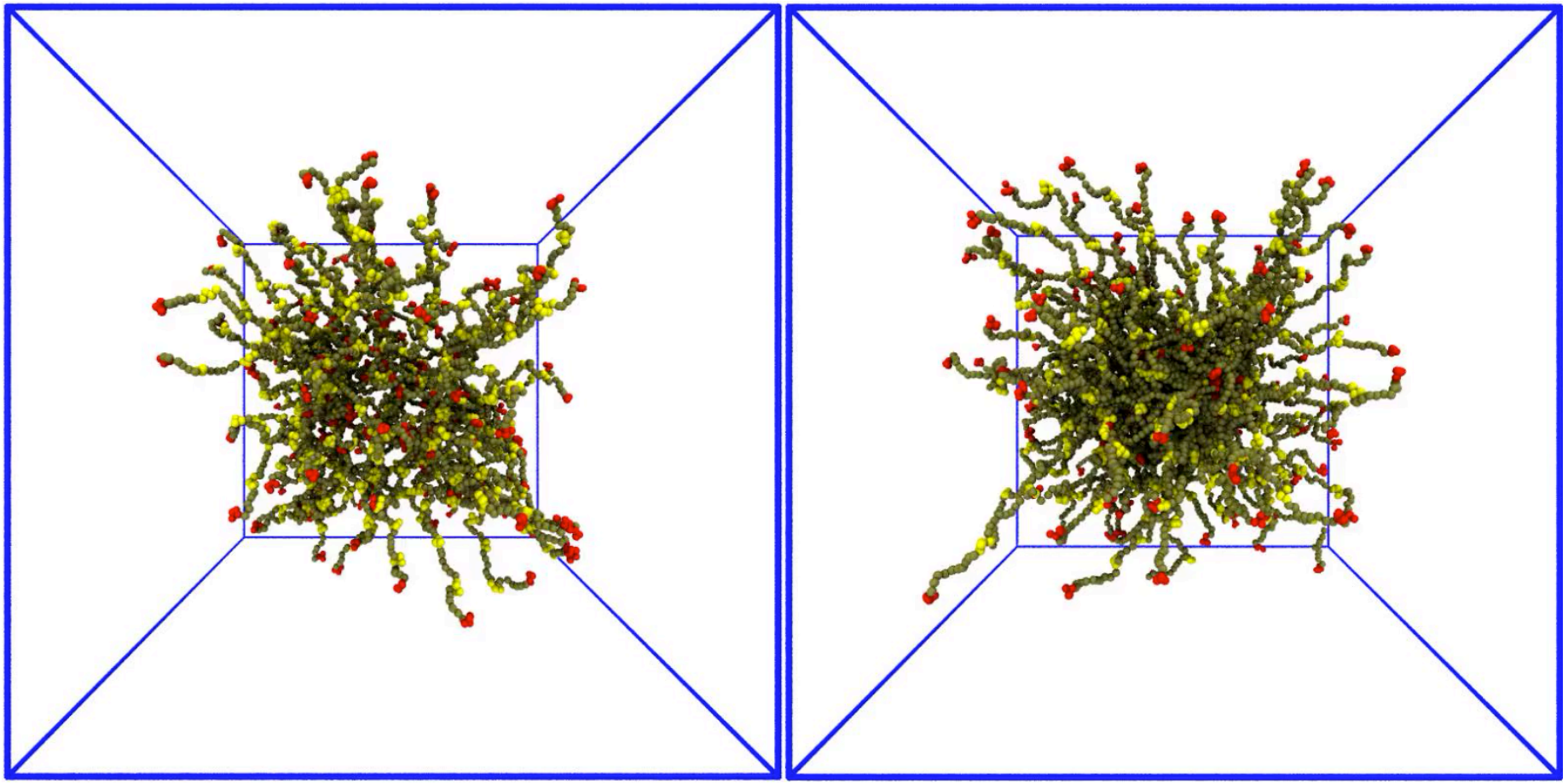
IDP - semi-flexible polymer with multiple sticky sites (e.g., Tyrosine)

Solvent - water, invisible

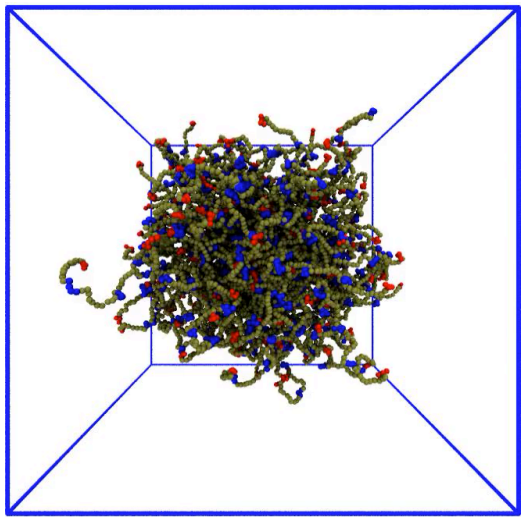


6B6

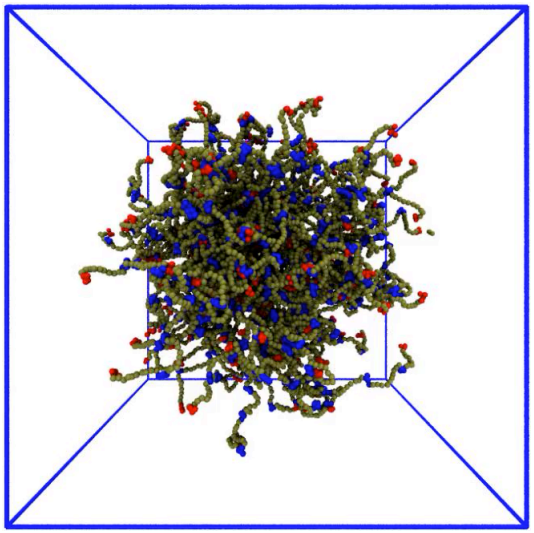
6B10



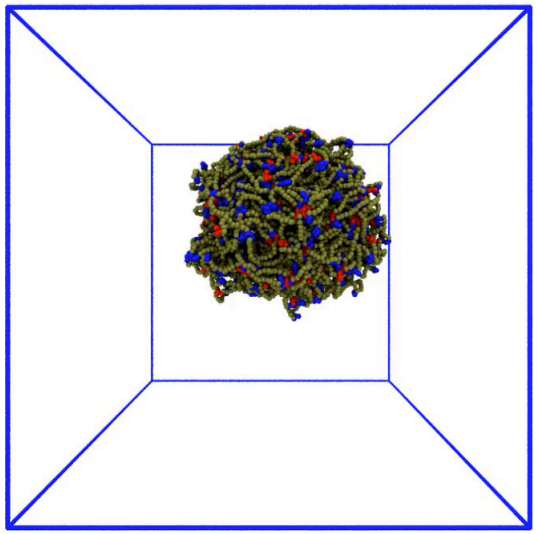
For weak affinity, phase separation is assisted by crowding



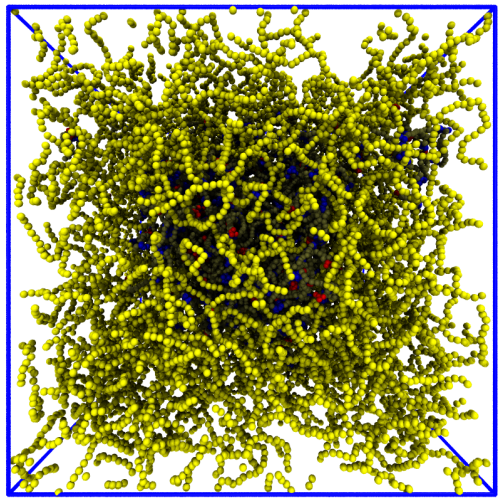
No crowder,
190 6B10 IDPs

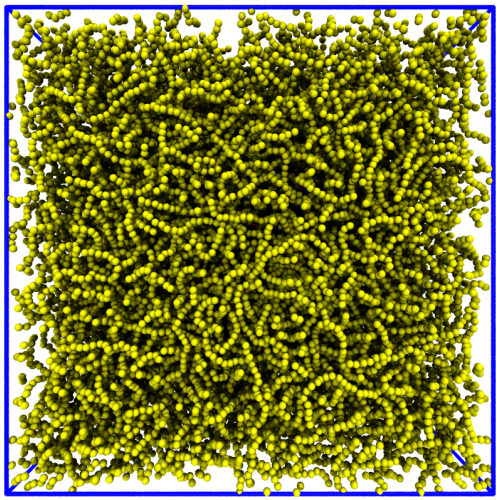


361 crowders P48

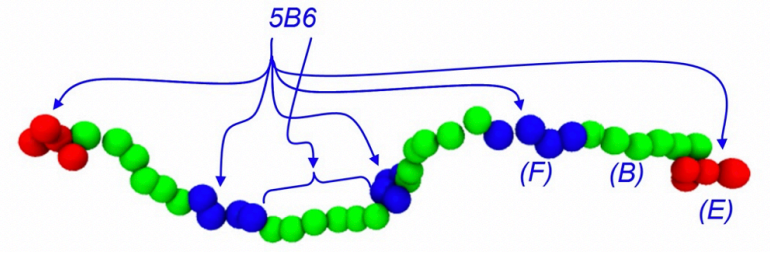


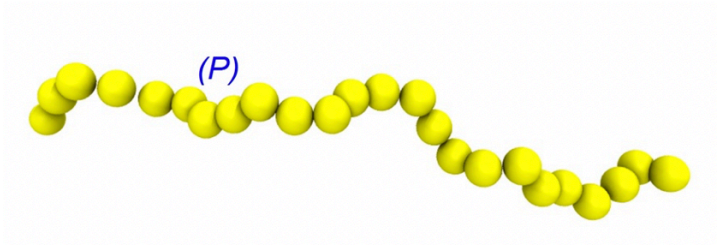
1071 crowders P48





(not movies)





How do we explore the parameter space of this model?

Too many parameters …

… and it's very slow

We have > 10 parameters for 1 IDP and 1 Crowder molecule:

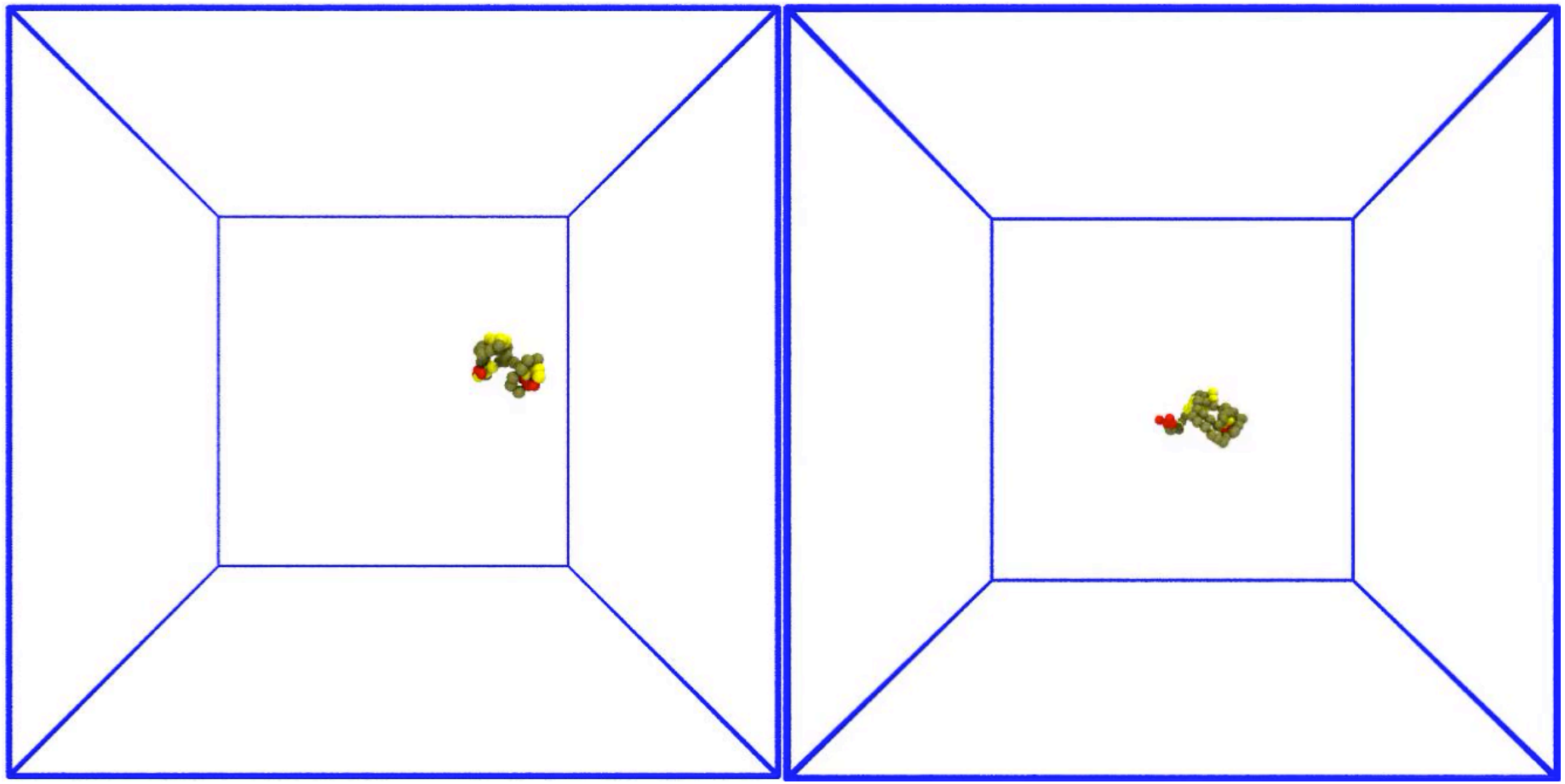
2 x molecular weights
2 x backbone stiffnesses
2 concentrations

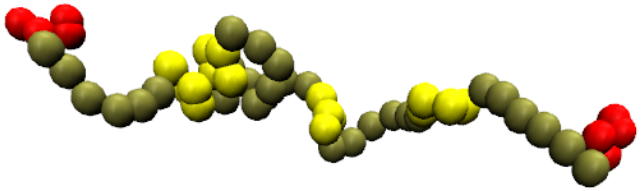
and placement of binding sites on IDP

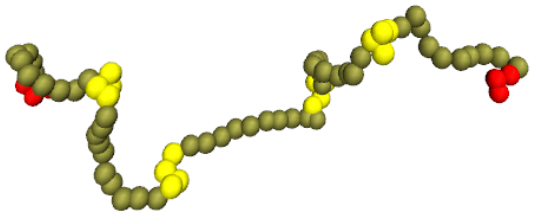
$(48\ \text{nm})^3$
331,723 particles
10^6 timesteps
takes 7 days on 1 core

Simplify to: equal mol. weights (FUS LC, 16.7 kDa), same stiffness; 6 sticky sites with same affinity and equal spacing.

Still leaves 2 concentrations, IDP self-attraction (a_{EE}) and IDP/crowder repulsion (a_{EE} = 80)



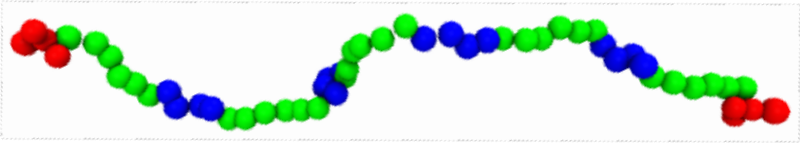


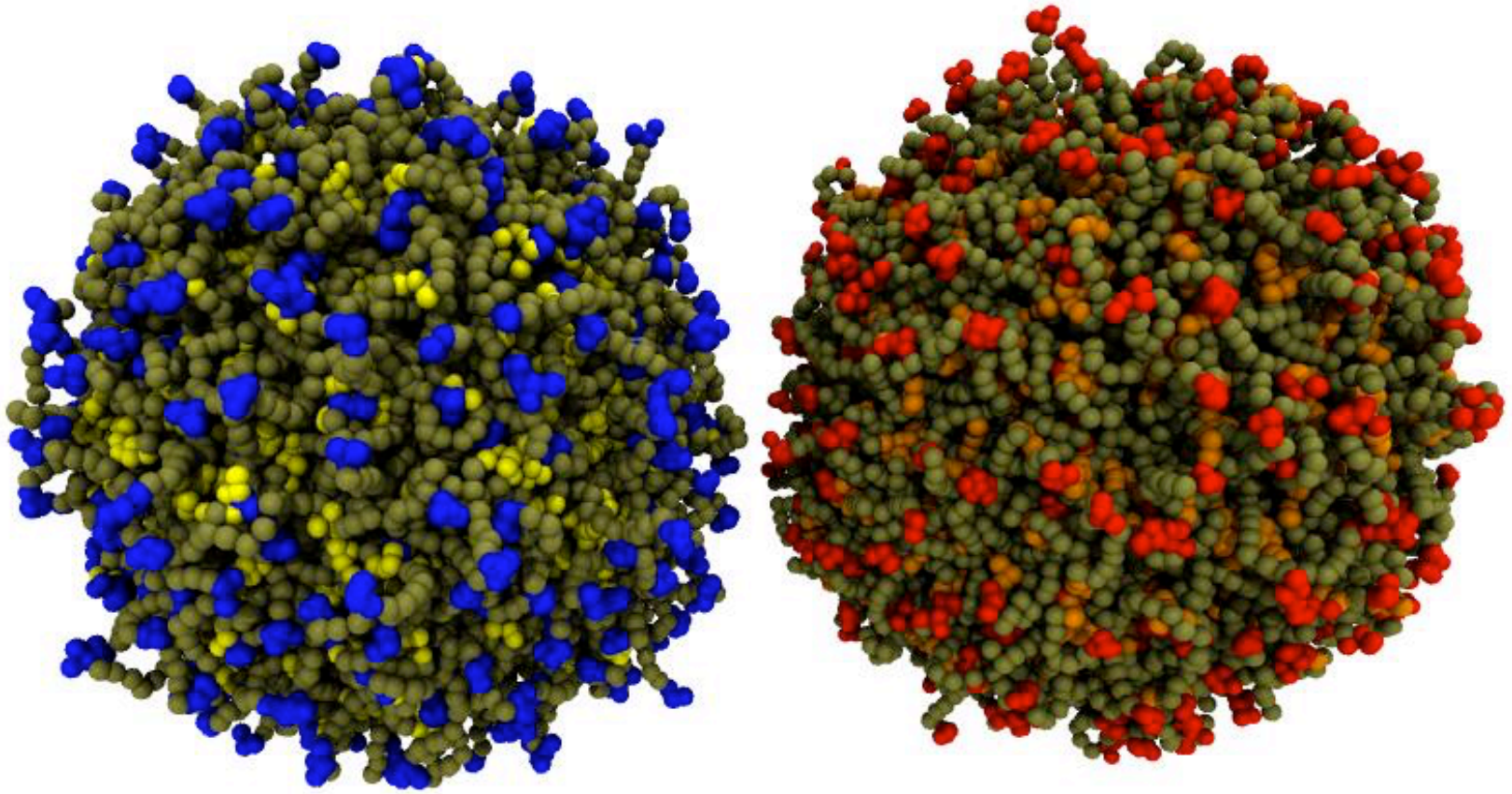


6 sticky sites, spacing 6 beads

6 sticky sites, spacing 10 beads

We can simulate sticky IDPs





But what does it tell us?
We need a theory.

Start with a simple model of an oil droplet

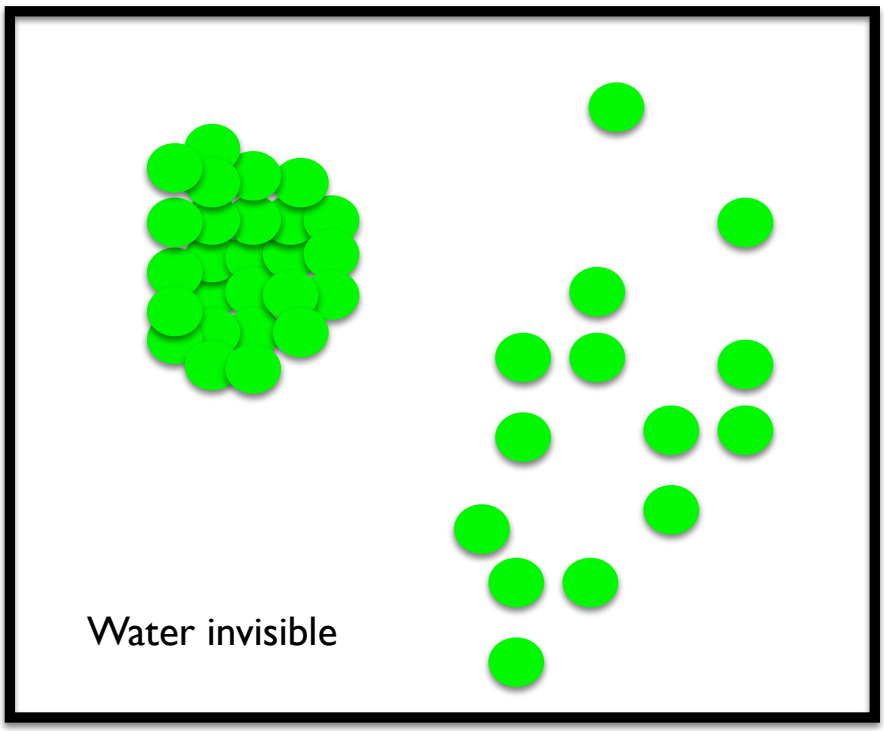
Why do oil molecules aggregate?

Each oil molecule is repelled from water by a *short-ranged* effective force: the hydrophobic effect.

How can we model this?

When oil molecules aggregate into a sphere, they reduce their energy by a term proportional to the volume (γ), but still have a repulsive surface energy proportional to the surface area (σ):

$$U(R) \sim 4\pi\sigma R^2 - 4/3\pi\gamma R^3$$



What preferred size droplets form?

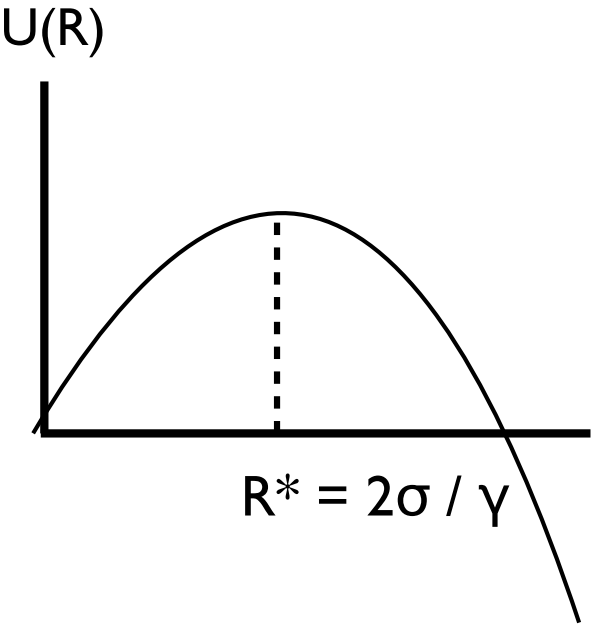
(Blackboard plot of U(R), find dU/dR, asymptotes, zeroes, …)

Oil and water phase separate

$U(R) \sim 4\pi\sigma R^2 - 4/3\pi\gamma R^3$

$dU/dR = 8\pi\sigma R - 4\pi\gamma R^2 = 0$

So, the energy has a peak at droplet size R*



If the ratio(σ/γ) is high, the barrier is large and at large radius but it is always energetically favourable to increase the droplet size.

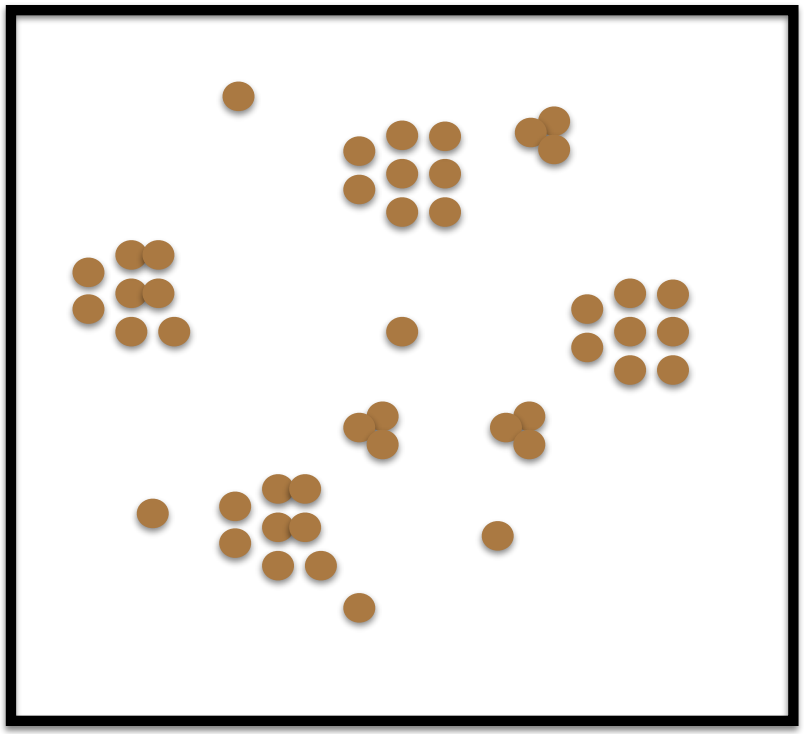
There is no equilibrium droplet size: smaller ones will break up while a larger one would grow without bound ⇒ phase separation.

Can we find out how the droplets grow with time?

Oil droplet growth

Consider N_0 oil molecules initially, uniformly dispersed in a closed box of bulk water with volume $V = L^3$.

We intuitively expect the oil to form droplets that coalesce and grow in time, but how precisely do the radius and mass change with time?



Assumptions

1) energy cost of the interface drives droplets spherical
2) at any time, all droplets have same mean radius
3) droplets of radius R diffuse with a coefficient that is given by the Stokes-Einstein relation: $D = k_B T/6\pi\eta R$ according to $\langle X^2 \rangle \sim 6\ D\ t$

Oil droplet growth

Let N(t) = mean number of droplets at time t
R(t) = mean radius of droplets "

$$\langle \text{vol/droplet} \rangle \sim L^3 / N(t)$$

$$\langle \text{separation} \rangle \sim L / N(t)^{1/3}$$

Is this reasonable?

Droplets diffuse around and grow by coalescing when they touch, and we assume that the coalesence time is short compared to the diffusion time.

$$(L / N^{1/3}(t))^2 \sim 6 (k_B T / 6\pi\eta R(t)) \, . \, t$$

But the number of oil molecules is constant, so:

$N(t).4\pi R(t)^3/3 = \text{constant}$ or $N(t) \sim 1 / R(t)^3$

$\pi\eta R(t) L^2 / k_B T = N(t)^{2/3}.t \sim t / R(t)^2$

$$R(t) \sim (k_B T / \pi\eta L^2) \, t^{1/3}$$

$$M(t) \sim R(t)^3 \sim t$$

Think - Pair - Share

The radius and mass of an oil droplet grow with time like:

$$R(t) \sim (k_B T / \pi \eta L^2)\, t^{1/3}$$

$$M(t) \sim R(t)^3 \sim t$$

This is NOT an equilibrium thermodynamic description

Q. Why not? 3 mins.

Is this a good model of IDPs forming membraneless organelles?

No - IDPs are NOT hydrophobic like oil - they are soluble in water

Aggregation must be driven by something else

From Oil to IDPs

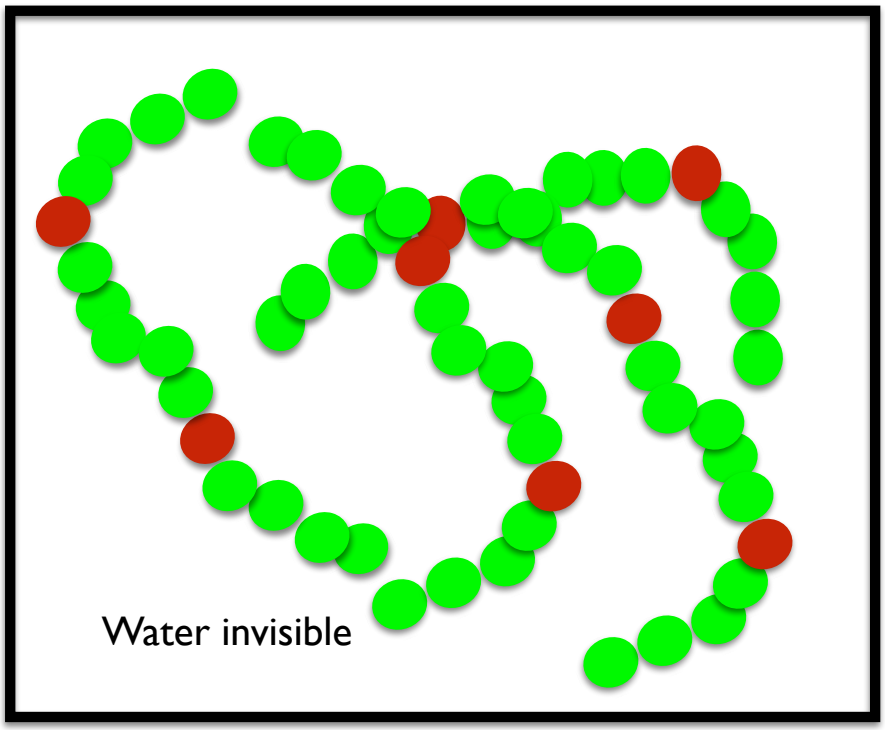
Some IDPs are NOT strongly repelled from water (few charged and hydrophobic residues), but membraneless organelles do have a small surface tension.

BUT they have **weakly-attractive** binding sites. We must modify the terms in the energy equation U(R).

An important property of IDPs is their conformational **entropy** - they are constantly fluctuating in position.

This suggests we could model them as a phantom chain (from last week)

$\langle R_{ee}^2 \rangle = Na^2$



Question

Oil molecules are long flexible polymers too, why didn't we take their fluctuations into account?

Building a model of a membraneless organelle

We assume:

Single disordered protein = a phantom chain with n monomers of size a (/nm): $\langle R_{ee} \rangle = an^{\nu}$ (ν is a constant that controls how $\langle R_{ee} \rangle$ varies with n: $\nu = 0.5$ for phantom chain but can be different for more complex models). NB I'm using n not N as the latter is the number of polymers in the next slide.

Aggregated proteins = **spherical** droplet of radius R with a surface tension σ (J/m^2)

Why?

- Experiments show they are spherical
- Experiments show droplets are fluid (FRAP)
- Non-zero surface tension from experiments
- Volume of the system does not change whether polymers are dispersed or aggregated, so pressure is not important (is this accurate?)

We use the Helmholtz free energy: $F = U - TS$ as volume and temperature are constant.

Can we make a model like the oil droplet one?

Model predictions

U = surface energy of spherical aggregate = surface tension x area

P(R) = prob. of polymer with R_{ee} having a size of R ~ exp($-(R - R_{ee})^2 / 2R_{ee}^2$) - why?

The Helmholtz free energy of N polymers in a sphere of radius R is then:

$F = U - TS = 4\pi \sigma R^2 - Nk_BT \log P(R)$

$= 4\pi \sigma R^2 + Nk_BT (R - R_{ee})^2 / 2R_{ee}^2$

Minimising this gives:

$$R = R_{ee} / (1 + 8\pi \sigma R_{ee}^2 / Nk_BT)$$

What does this look like? Is it dimensionally correct?

Is it correct? Have we made hidden assumptions? We've ignored the binding sites.

Better model - include binding energy

U = surface energy of spherical aggregate = surface tension x area

P(R) = prob. of polymer with R_{ee} having a size of R ~ $\exp(-(R - R_{ee})^2 / 2R_{ee}^2)$

Add the binding energy of M binding sites per polymer: $-\varepsilon$

The Helmholtz free energy is now:

$F = U - TS = 4\pi \sigma R^2 - N M(R) \varepsilon / 2 - N k_B T \log P(R)$

But how many binding sites M do the IDPs make?

And how does M depend on the aggregate's radius?

Do stretched polymers expose more binding sites or do they find it harder to bind due to increased shape fluctuations?

Problem rapidly gets hard to solve analytically

But a simulation of N polymers with binding sites can answer these questions

Summary

Sometimes you have to be sneaky to get your way (indirect origin of forces in a cell)

- Indirect forces are more important for cells than direct/bare Coulomb forces
- Indirect forces do **not** depend on **atomic details/chemistry** but arise from the **material** properties of aggregates/membranes/solutes
- Cells use thermal motion to move things, create compartments, create forces
- Noise is a continual source of energy for fluctuations that can exert forces (equipartition theorem)

Divide and conquer

- Cell is very crowded, bad for biochemistry
- Compartments allow specialisation, protection, separation;
  they permit gradients like the H+ in mitochondria that is used for ATP
- Membraneless organelles have no bounding lipid membrane; they form because they minimise the free energy of their constituents, *cp.* lipids forming a membrane due to hydrophobic effect

Break

10 mins.

Practise test for Test 1 on 2nd October will be on moodle this week; the actual test will be on the following topics, and last 1 hour at the start of the class.

- Scales, ratios and dimensional analysis in the cell
- Know the tables of lengths from lecture 1
- Biological polymers, polymer models, Kuhn length
- Types of indirect forces in the cell, their origin and effects
- Statement of the equipartition theorem

- Send me title/group for JC paper before weekend please

Exercise

Goal 1: How to organise the output files from many simulations?

- Create a new sub-directory for each exercise (no spaces in path)

  e.g., ~/BIOENG455/ex1, …

- Use descriptive extensions, e.g., p001 = polymer run 1,

  dmpci.es001 = entropic spring run 001, or dmpci.es10, dmpci.es20, etc.

  where force = 10, 20

Goal 2: Measure the Lee of a single polymer in water

- Description of dmpci.nnn input file
- Measure Lee from the dmpcas file and plot it against the number of monomers in the polymer

Goal 3: Apply a force to the ends of the polymer and measure the F(L) curve of this *entropic* spring.

Is a DPD polymer a phantom chain?

EPFL

To Do:

1. Create a new directory to hold the runs, e.g., ~/BIOENG455/Exercise-2
2. Copy the dmpci file that contains a *single* polymer of the form B_{10} (B_{10} = B-B-B- …. 10 times. This is more easily entered as: " (B (8 B) B) " Note the first and last beads in a polymer's shape must be a simple bead name.
3. Run a simulation in a 20^3 box for 10,000 steps, sample every 10 steps
4. Plot the time series of the polymer's end-to-end length from the dmpchs file. Note R_{ee} is not an average, but an instantaneous value, but it should approach a stationary value over time, this is the average value $\sqrt{\langle R_{ee}^2 \rangle} \sim N^{1/2}$ to be plotted. This value appears in the dmpcas file as well.
5. Each group use different lengths (how do you sensibly choose the length? what happens if the polymer is longer than the box?)
6. Is it a phantom chain? Does $\sqrt{\langle R_{ee}^2 \rangle} \sim N^{1/2}$

Polymer in water input file

```
dpd

Title   " Water/PEG "
Date    19/09/18
Comment " ~1 polymer in water simulation. How does the polymer's end-to-end length fluctuate?   "


State   random


    Bead  W
    0.5
    25
    4.5

    Bead  B
    0.5
    25    25
    4.5   4.5

    Bond  B B 128.0 0.5


Polymer Water    0.999  " (W) "
Polymer PEG      0.001  " (B B B B B B B B B B) "


Box          10  10  10       1  1  1
Density      3
Temp         1
RNGSeed      -33145
Lambda       0.5
Step         0.02
Time         10000
SamplePeriod     10
AnalysisPeriod   5000
DensityPeriod    10000
DisplayPeriod    100
RestartPeriod    10000
Grid         1  1  1


Command ToggleBeadDisplay            1    W
Command SetCurrentStateCamera        1    0.5 -1.0 -0.5  0.5 0.5 0.5
Command SetCurrentStateDefaultFormat 1    Paraview
```

If $a_{BW} = a_{WW}$ then B is neutral towards water

If $a_{BW} > a_{WW}$ then B will be repelled from water. In DPD, larger conservative parameter means greater repulsion.

If $a_{BW} < a_{WW}$ then the B beads be solvated by water.

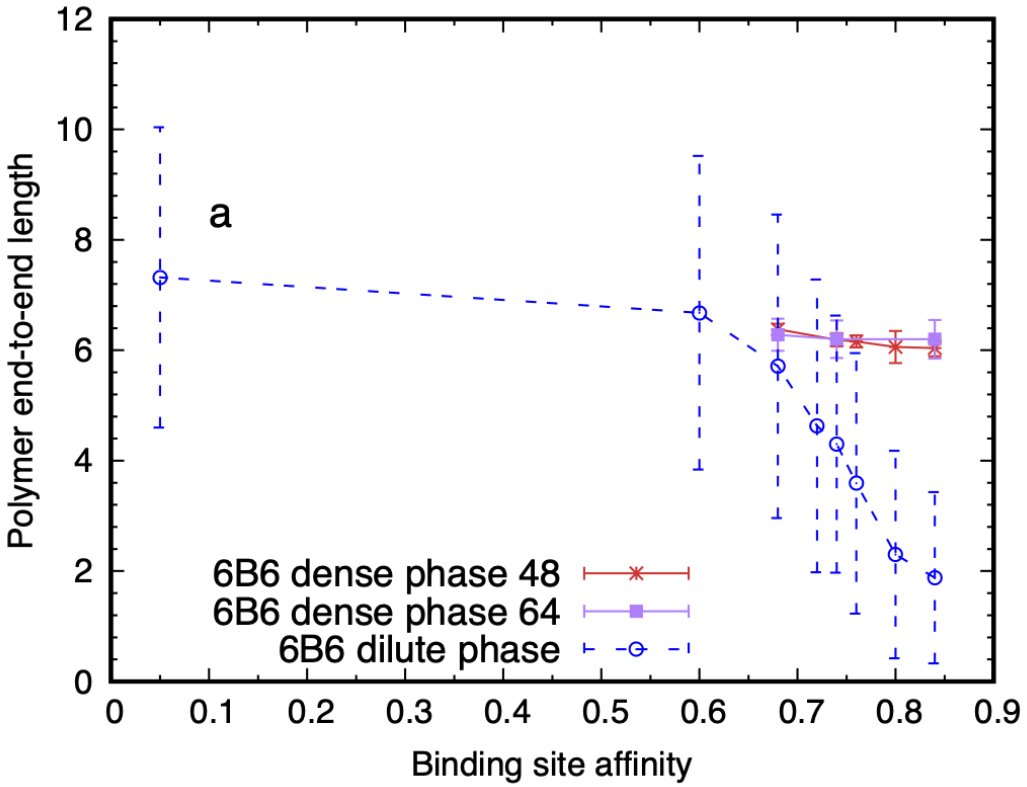
If a polymer type contains more than one bead there must be a bond to connect them: these are Hookean springs that need the names of the beads, the spring constant and the unstretched length.

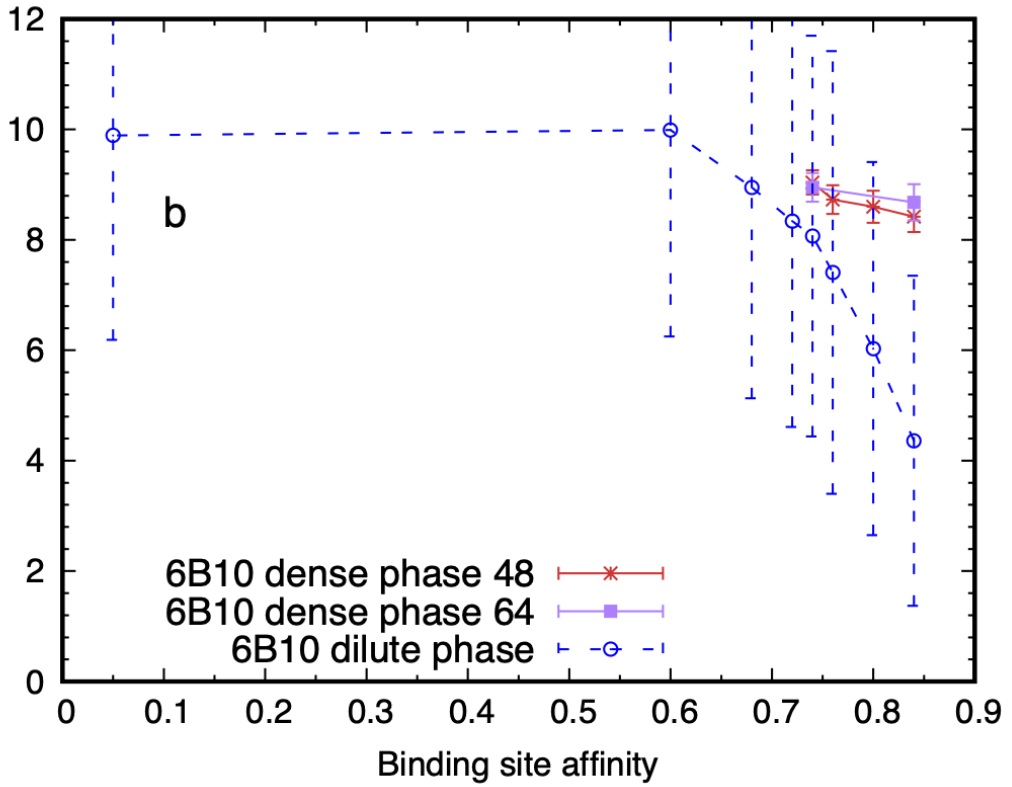
Improving the measurements?

Questions to ponder:

1. Can you improve the statistics by having many polymers in the simulation box? What could go wrong with this idea?
2. What if the polymer has sticky parts that like to bind to themselves; how does this affect $<\sqrt{R_{ee}^2}>$? How can we implement this.
3. What if the polymer is slightly hydrophobic: how does this affect $<\sqrt{R_{ee}^2}>$?

Answer to the question is … Yes





The dashed blue curves show Lee for a single polymer with 6 sticky sites separated by 6 (left) or 10 (right) backbone beads. The sticky site affinity increases from 0 to a high value.

The horizontal curves show the same Lee for polymers in a condensed phase.

Info on homework exercises

There are ~ 10 possible homework exercises, and you must hand in one of them in Week 7 (1st November) and Week 12 (6th December). I have put a description on moodle and will go through some of them in the exercise periods.

Once we have finished the entropic spring simulation to illustrate how to use the DPD code, the exercise period can be used to work on the homework problems, Journal club talks, and projects.

You may use the **simulation homework exercises** as a starting point for the semester project, but you should extend them significantly. The project report should cover:

- Explain the question you want to answer with simulations
- Identify sources of error (systematic and statistical)
- Describe the results
- Conclusions, possible future work

Sample reports are on moodle.

Homework Ex. 1 Entropic spring

Simulation of an entropic spring - apply a stretching force to both ends of a single, long polymer in a DPD simulation and measure the end-end length as a function of the force (it probably has to be a very small force).

Then invert it to get F(L) and plot it including error bars of the statistical errors. How do you convert results to physical units?

Now make a fraction of the beads sticky (so that the polymer tends to stick to itself) and see how this changes the F(L) curve. You will need to vary the number of sticky beads to find an interesting regime (too few and nothing will happen, too many and the polymer will just stick together in a tight ball). Interesting means that the system shows some unusual, non-linear behaviour.

Needs commands in DPD to solve - see Section 8 of the User Guide.

Equipart. Thm. and Muscles

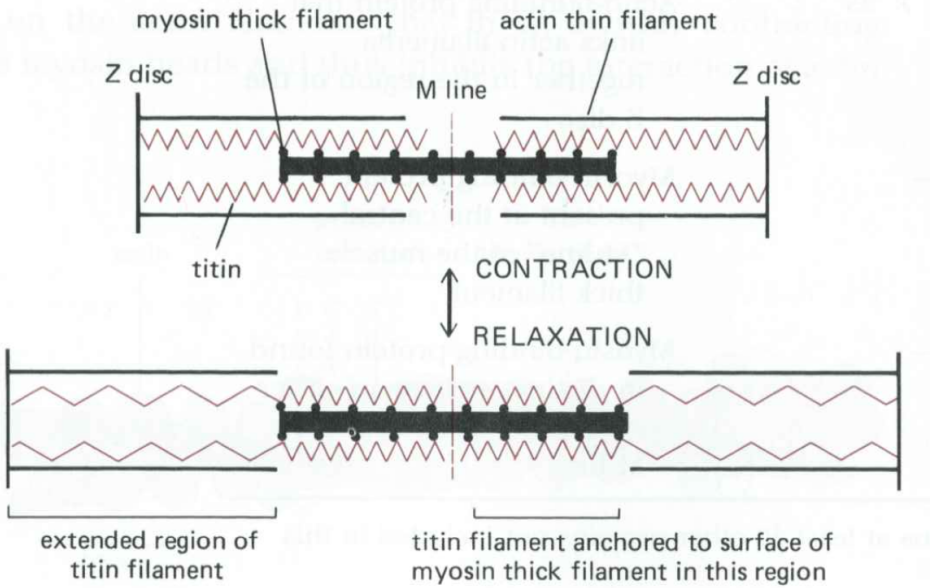
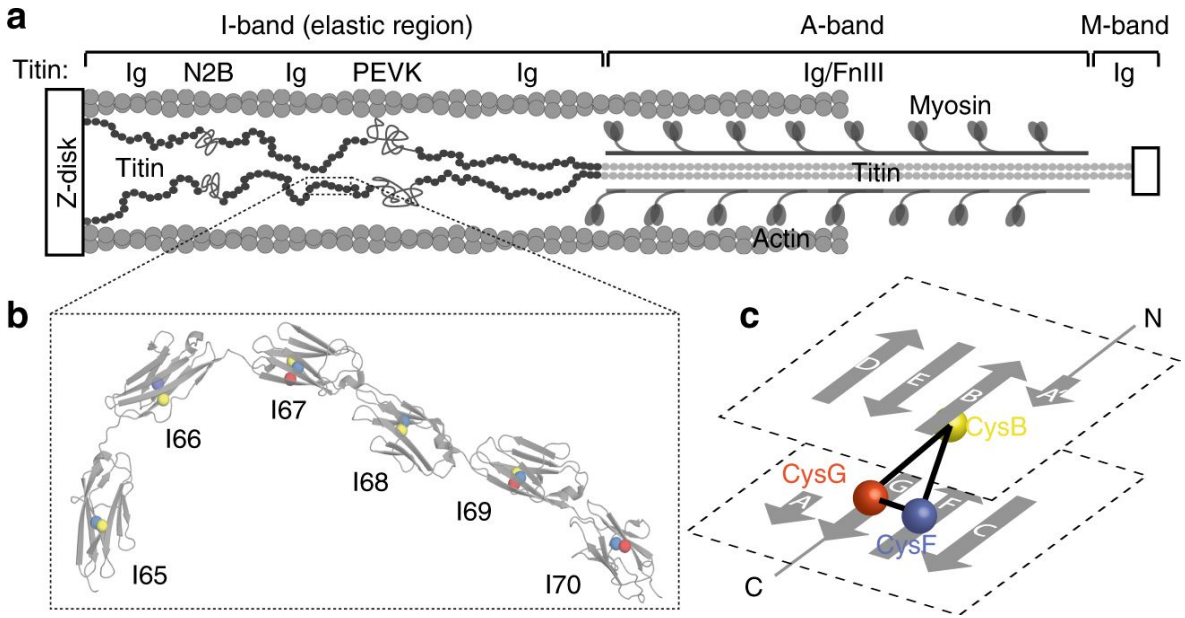


Fig.. 11-21 Mol. Biol. Cell, B Alberts et al.

Titin has many folded domains that unbind on stretching; each unbound domain contributes many k_BT to the internal energy

Not every atom matters, but there are many *blobs* that are harmonic ~ 1/2 k_BT.



Single-molecule spring constant experiments

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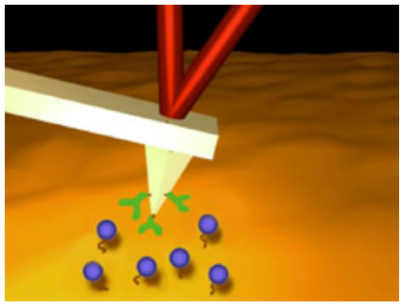
Share:

spring constants of single molecule and molecular complexe

Direct measurement of the spring constants of single molecule and molecular complexe



Recently we have proposed a new method of direct and continuous measurement of the spring constant of single molecule or molecular complex (see Chtcheglova et al. 2003a). To that end the standard Force Spectroscopy technique with functionalized tips and samples is combined with a small dithering of the tip (fig. 1) The change of the dithering amplitude as a function of the pulling force is measured using a lock-in amplifier in order to extract the spring constant of the complex.

The potentialities of this method have been illustrated for the experiments with single bovine serum albumin (BSA) – its polyclonal antibody (Ab – BSA), fibrinogen – fibrinogen complexes and avidin – biotin. Example of experimental curves obtained for BSA – Ab BSA are presented in Fig. 2: lower curves are standard quasi static AFM pulling off curves, while

RESEARCH INTERESTS

- AFM-based Single Molecule Force Spectroscopy
- Protein interaction studied with the Atomic Force Microscope
- Cryo-AFM, Atomic Force Microscope at low temperature
- Static and dynamic properties of DNA knots
- Direct measurement of the spring constants of single molecules and molecular complex
- Topoismerase II activity and its interaction with DNA
- Single Molecule Fluorescence Resonance Energy Transfer Scanning Near-field Optical Microscopy (FRET SNOM)

CONTACTS

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https://lpmv.epfl.ch/facilities/page-47572-en-html/page-48534-en-html/

```
Bead   W
       0.5
       25
       4.5

Bead   B
       0.5
       25     25
       4.5    4.5

Bead   BH
       0.5
       25    25    25
       4.5   4.5   4.5

Bead   BT
       0.5
       25    25    25    25
       4.5   4.5   4.5   4.5



Bond   BH  B 128     0.5
Bond   BT  B 128     0.5
Bond   B  B  128     0.5

Polymer Water    0.99995   " (W) "
Polymer Spring   0.00005   " (BH (14 B) BT) "

Box          30  15  15        1  1  1
Density      3
Temp         1
RNGSeed      -999
Lambda       0.5
Step         0.01
Time         6000
SamplePeriod      10
AnalysisPeriod    2000
DensityPeriod     6000
DisplayPeriod     100
RestartPeriod     6000
Grid         1  1  1
```

Input file: dmpci.f1 on moodle for today

Simulating an entropic spring under tension

We create command targets for the two ends of a molecule and apply equal and opposite forces to stretch it.

```
Command  SelectBeadTypeInSimBox 1  head  BH
Command  SelectBeadTypeInSimBox 1  tail  BT

Command  Comment  1000  // Apply a constant force to the first and last beads in
the +X and –X directions  //

Command ConstantForceOnTarget        1000  head  fh   1 0 0   5.0
Command ConstantForceOnTarget        1000  tail  ft   1 0 0   -5.0


Command  Comment  5000  // Delete the applied forces  //

Command RemoveCommandTargetActivity  5000   fh
Command RemoveCommandTargetActivity  5000   ft
```

Measuring the strain

To Do:

1. Pick a box size of 30 x 15 x 15; adjust the number fractions to have 1 polymer of type (BH (14 B) BT), i.e., distinct head and tail beads so they can be selected.
2. Turn force on at T = 1000 steps. How long should you keep it on?
3. How can you measure the extension?
4. Next, change the backbone to contain a new bead type that is "sticky". Try (BH B B B S S S S B B B BT), and give S the same interactions as B except for its self interaction that is reduced to make it sticky. Vary the number of S beads until you find a value that makes an observable difference.

Questions to answer

What is the stress/strain relation F(L) for the "molecular spring"?

Does it have different regimes for F(L) under different tensions? Why?

With sticky beads there are two new parameters: the number of sticky beads and their self-interaction. How can you reasonably select values for these?

dmpcas file contains time-averaged observables

Typically there are 2 columns: mean and standard deviation

dmpcas.ex1 has <Lee> averaged between 2000 - 4000 steps

```
Time = 4000
Temperature
1.0017449      0.0051323594

Pressure
23.671181      0.081945563

...

BB bond length
0.58796179     0.020986014

Water EE distance
0              0

Spring EE distance
6.4922776      0.6952996
```

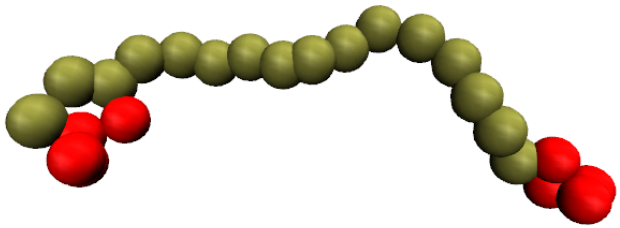
Use the time period while force is applied, which is at the end time of the period

This is the mean and std. dev. of polymer end-to-end length

Ex. 2 Tethered FRET experiment

FRET measures the separation of two parts of a molecule by attaching two halves of a fluorescent group to the two parts of a molecule and measuring the amount of fluorescence.

Here we measure the distribution of the separation of the two ends of a linear polymer as the attraction between them is varied.



Create a single polymer

```
Polymer Water   0.99998 " (W) "
Polymer Rod     0.00002  " (E E (16 B) E E)  "
```

Measure its radial distribution function averaged over time

```
Command SavePolymerBeadRDF                10000 4 60 15 Rod  E
```

How do the endcap binding strength and polymer stiffness affect the RDF?

1) Choose a length, e.g., 20 beads, (E-E-(16 B)-E-E) and create one polymer in the box

2) Choose a weak BondPair potential for the backbone, e.g., $k_3 = 2\ k_BT$

3) Start with no attraction ($a_{EE} = 25 = a_{WW}$) and gradually increase it by reducing a_{EE} in steps of 5 down to $a_{EE} = 5$.

4) Use the SavePolymerBeadRDF command to save the radial distribution function of the endcap beads to a file (see the User Guide for the arguments):

Command SavePolymerBeadRDF 5000 3 60 15 Rod E

5) Plot the RDF of the endcaps for several values of a_{EE} including $a_{EE} = 25$ as baseline.

6) For a given value of the a_{EE}, increase the backbone bondpair parameter k3 to make it stiffer; how does the RDF change now?

Ex. 3 Polymer in a good/bad solvent

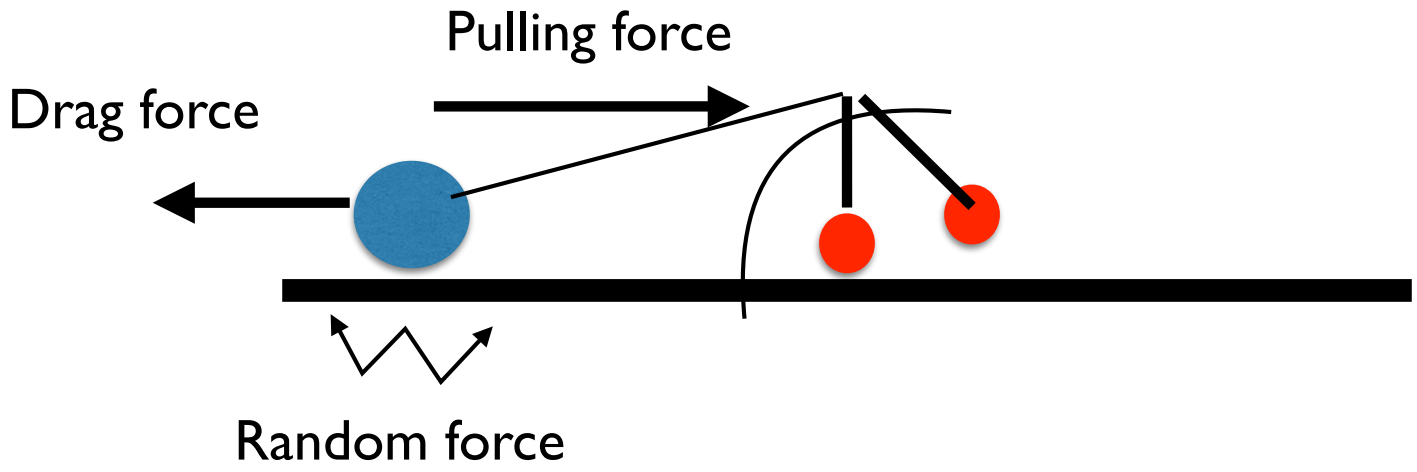
A polymer in a good solvent swells while one in a bad solvent collapses into a dense ball.

1) Create a single homopolymer (as in Ex. 2) of length 20, and set a_{BW} = 10. This represents a **good** solvent as the backbone wants to be solvated.
2) Plot its end-to-end length using the time series from the dmpchs file, make sure it is equilibrated (how?) and find the equilibrium value.
3) Then repeat the simulation and cary the conservative interaction between the polymer beads and the solvent from attractive (aBW =10), through neutral (aBW = 25) to repulsive (aBW >> 25) in steps of 10.
4) Plot the √<Lee2> from the dmpcas file against a_{BW}.

Is there a sharp transition between an extended state and a collapsed state as aBW is made increasingly repulsive?

Ex. 4 Langevin equation and nanoparticle

Suppose a molecular motor pulls a vesicle along an actin filament: we can use a Langevin equation to model this or do a DPD simulation.



We model this as a rigid sphere being pulled in a straight line by a constant force while also subject to a (Stokes) drag force and a random force.

Pulling a nanoparticle in water

Because the vesicle moves slowly - no acceleration - there are two parameters: magnitude of the viscous drag force, and magnitude of the (random) thermal fluctuation force.

Friction coefficient is: $\gamma = 6\pi\eta a$ (from macroscopic Stokes law for drag on a sphere)

We also need a value for σ/γ. Recall from the DPD random force equation: $\sigma^2 = 2\gamma k_B T$ which leads to:

$$x(t + dt) = x(t) + F_{const}.dt/\gamma + \sqrt{D}.\, N(0, 1).\sqrt{dt}$$

where $D = 2k_B T/\gamma$. If we want to relate the results to a physical experiment, we would have to set appropriate values for the solvent viscosity η, nanoparticle size a, and temperature.

N(0,1) = Gaussian distributed random number with zero mean 0 and unit variance. But we will use a uniform random number in the interval (-1/2, 1/2).

NB make sure it is normalised to unity, i.e., use 2*coefficient*RN in (-1/2, 1/2).

Pulling a nanoparticle in DPD

```
dpd

Title   " Pulling a nanoparticle in water "
Date    27/10/18
Comment " Create a spherical nanoparticle then apply a constant force to it.  "

State   random

Bead  W
      0.5
      25
      4.5

Polymer Water  1  " (W) "

Box       48  12  12      1  1  1
Density     3
Temp        1
RNGSeed     -21114
Lambda      0.5
Step        0.01
Time        2000
SamplePeriod     10
AnalysisPeriod   2000
DensityPeriod    2000
DisplayPeriod    20
RestartPeriod    2000
Grid        1  1  1



Command ToggleBeadDisplay            1    W
Command SetCurrentStateCamera        1    0.5 -4.0 0.5  0.5 0.5 0.5
Command SetCurrentStateDefaultFormat 1    Paraview

Command  Comment  1  // Create a solid spherical nanoparticle of radius 2.0 //


Command  SelectPolymerTypeHeadInSphere   100  ball1   Water    0.25 0.5 0.5  0.0 2.0
Command  PolymerisePolymersInTarget      100  ball1   12  1.5  1.0  256 0.0
Command  SetTargetDisplayId              100  ball1   1

Command  SelectPolymerTypeHeadInSphere   100  ball2   Water    0.75 0.5 0.5  0.0 2.0
Command  PolymerisePolymersInTarget      100  ball2   12  1.5  1.0  256 0.0
Command  SetTargetDisplayId              100  ball2   2

Command  Comment  100  // Apply a constant force along the +X axis to the NP  //

Command ConstantForceOnTarget             100  ball1  fz   1 0 0   5.0

Command RemoveCommandTargetActivity       1000  fz
```

Simple RNG in C


```
#define IA 16807
#define IM 2147483647
#define AM (1.0/IM)
#define IQ 127773
#define IR 2836
#define MASK 123459876

float ran0(long *idum)
"Minimal" random number generator of Park and Miller. Returns a uniform random deviate
between 0.0 and 1.0. Set or reset idum to any integer value (except the unlikely value MASK)
to initialize the sequence; idum must not be altered between calls for successive deviates in
a sequence.
{
    long k;
    float ans;

    *idum ^= MASK;                    XORing with MASK allows use of zero and other
    k=(*idum)/IQ;                         simple bit patterns for idum.
    *idum=IA*(*idum-k*IQ)-IR*k;       Compute idum=(IA*idum) % IM without over-
    if (*idum < 0) *idum += IM;           flows by Schrage's method.
    ans=AM*(*idum);                   Convert idum to a floating result.
    *idum ^= MASK;                    Unmask before return.
    return ans;
}
```

The period of ran0 is $2^{31} - 2 \approx 2.1 \times 10^9$. A peculiarity of generators of the form (7.1.2) is that the value 0 must never be allowed as the initial seed — it perpetuates itself — and it never occurs for any nonzero initial seed. Experience has shown that users always manage to call random number generators with the seed idum=0. That is why ran0 performs its exclusive-or with an arbitrary constant both on entry and exit. If you are the first user in history to be proof against human error, you can remove the two lines with the $\wedge$ operation.

Numerical Recipes in C: The Art of Scientific Computing
W. H. Press et al. Cambridge University Press, 2nd ed. 1992

Better RNG in C

ÉCOLE POLYTECHNIQUE FÉDÉRALE DE LAUSANNE

```
#define MBIG 1000000000
#define MSEED 161803398
#define MZ 0
#define FAC (1.0/MBIG)
```

According to Knuth, any large MBIG, and any smaller (but still large) MSEED can be substituted for the above values.

```
float ran3(long *idum)
```

Returns a uniform random deviate between 0.0 and 1.0. Set idum to any negative value to initialize or reinitialize the sequence.

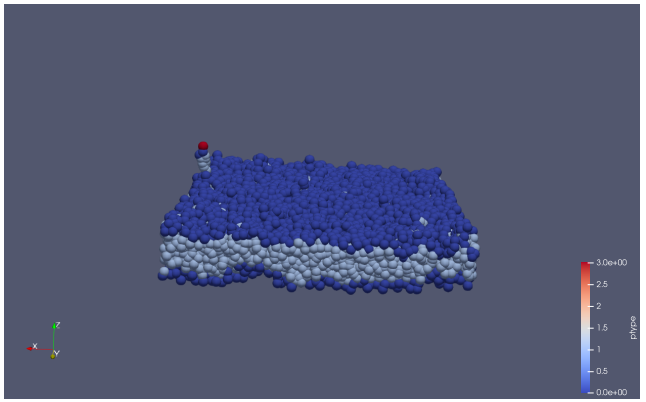
```
{
    static int inext,inextp;
    static long ma[56];                 The value 56 (range ma[1..55]) is special and
    static int iff=0;                       should not be modified; see Knuth.
    long mj,mk;
    int i,ii,k;

    if (*idum < 0 || iff == 0) {        Initialization.
        iff=1;
        mj=MSEED-(*idum < 0 ? -*idum : *idum);      Initialize ma[55] using the seed
        mj %= MBIG;                                     idum and the large number
        ma[55]=mj;                                      MSEED.
        mk=1;
        for (i=1;i<=54;i++) {           Now initialize the rest of the table,
            ii=(21*i) % 55;             in a slightly random order,
            ma[ii]=mk;                  with numbers that are not especially random.
            mk=mj-mk;
            if (mk < MZ) mk += MBIG;
            mj=ma[ii];
        }
        for (k=1;k<=4;k++)              We randomize them by "warming up the gener-
            for (i=1;i<=55;i++) {           ator."
                ma[i] -= ma[1+(i+30) % 55];
                if (ma[i] < MZ) ma[i] += MBIG;
            }
        inext=0;                        Prepare indices for our first generated number.
        inextp=31;                      The constant 31 is special; see Knuth.
        *idum=1;
    }
    Here is where we start, except on initialization.
    if (++inext == 56) inext=1;         Increment inext and inextp, wrapping around
    if (++inextp == 56) inextp=1;           56 to 1.
    mj=ma[inext]-ma[inextp];            Generate a new random number subtractively.
    if (mj < MZ) mj += MBIG;            Be sure that it is in range.
    ma[inext]=mj;                       Store it,
    return mj*FAC;                      and output the derived uniform deviate.
}
```

Numerical Recipes in C: The Art of Scientific Computing
W. H. Press et al. Cambridge University Press, 2nd ed. 1992

Ex. 5 Molecular force spectroscopy 1

Select a single lipid:

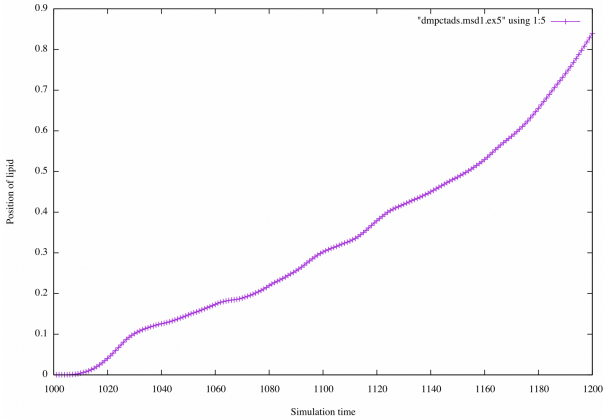
```
Polymer Water   0.978967   " (W) "
Polymer Lipid   0.021013   " (H H (* (T T T T)) H T T T T) "
Polymer Lipid1  0.00002    " (H1 H (* (T T T T)) H T T T T) "
```



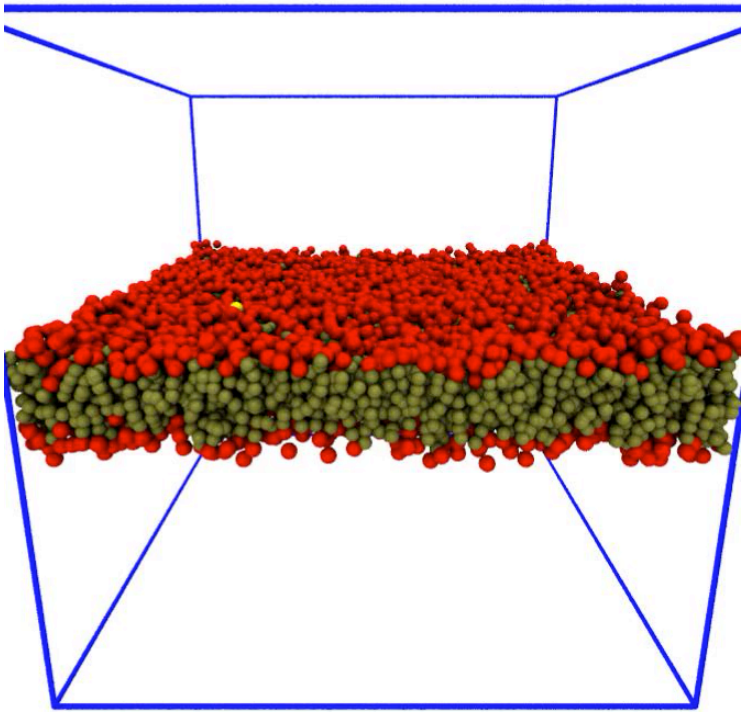
apply a pulling force normal to membrane:

```
Command Comment   1 // Following commands apply a force upwards to the single  lipid with the H1 bead  //

Command SelectBeadTypeInSimBox        1  head  H1

Command ConstantForceOnTarget         100  head  fh   0 0 1   20.0

Command RemoveCommandTargetActivity   1000   fh
```

measure position of lipid and work done on it

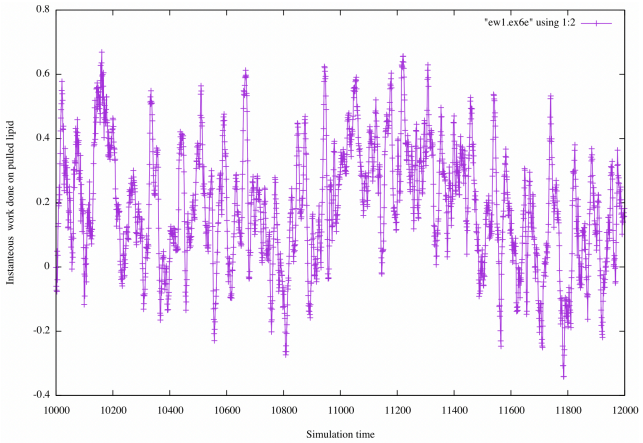


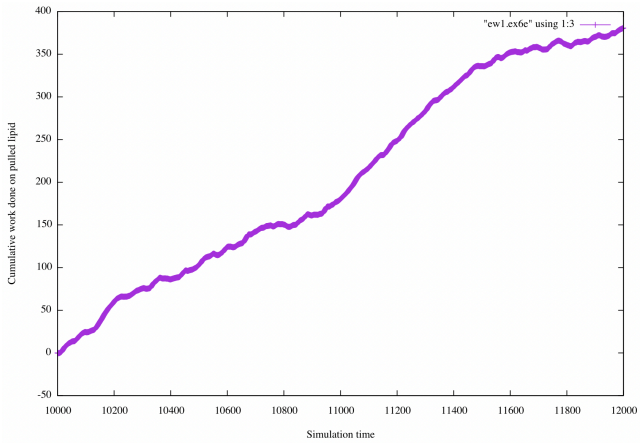
Lipid position in z direction

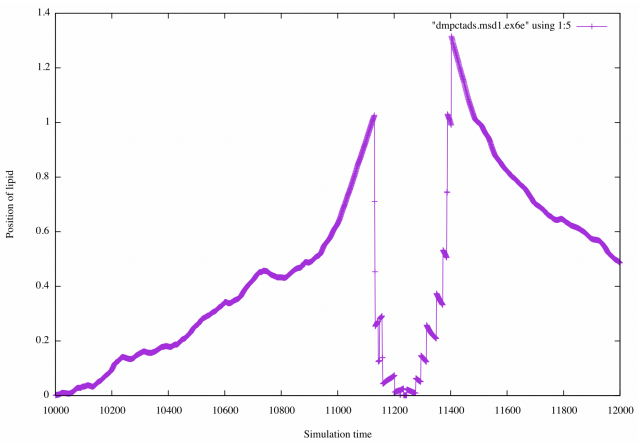


Ex. 6 Molecular force spectroscopy 2

Apply a force to a single IDP in a droplet and extract it and measure the work done against the force

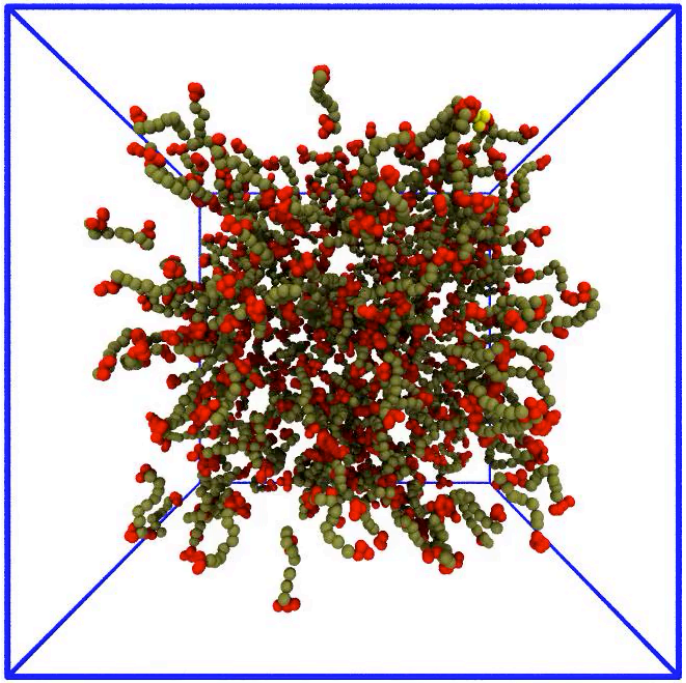






Work done

Position



see dmpci.ex6e

Ex. 7 Thermodynamic model of IDP droplet

Recall from Lecture 3, a model of the self-assembly of polymers with sticky binding sites:

U = surface energy of spherical aggregate = surface tension x area

P(R) = prob. of polymer with R_{ee} having a size of R ~ $\exp(-(R - R_{ee})^2 / 2R_{ee}^2)$

Add the binding energy of M binding sites per polymer ($-\varepsilon$) and let M vary with droplet size; M has an upper limit of M_{max} per polymer.

The Helmholtz free energy is now:

$F = U - TS = 4\pi \sigma R^2 - N M(R) \varepsilon / 2 - N k_B T \log P(R)$

How does M depend on the aggregate's radius? Do stretched polymers expose more binding sites or do they find it harder to bind due to increased shape fluctuations?

Assume a functional form for M, minimise the free energy, and find how the radius varies with the parameters σ, ε, M_{max}, and T.

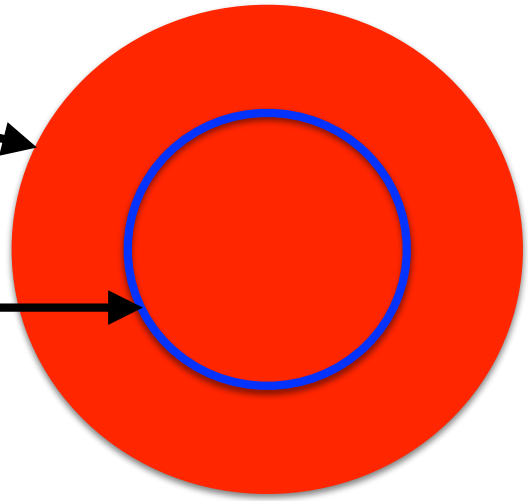
Check the dimensions make sense.

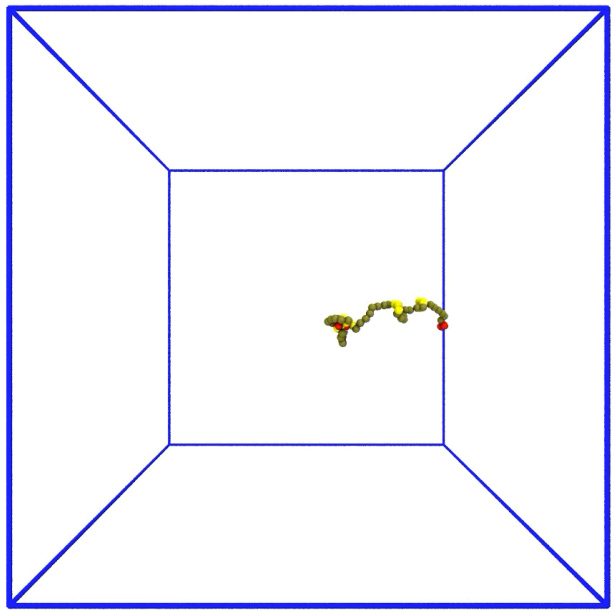
Ex. 9 Compare the diffusion of a hard sphere and a fluctuating polymer

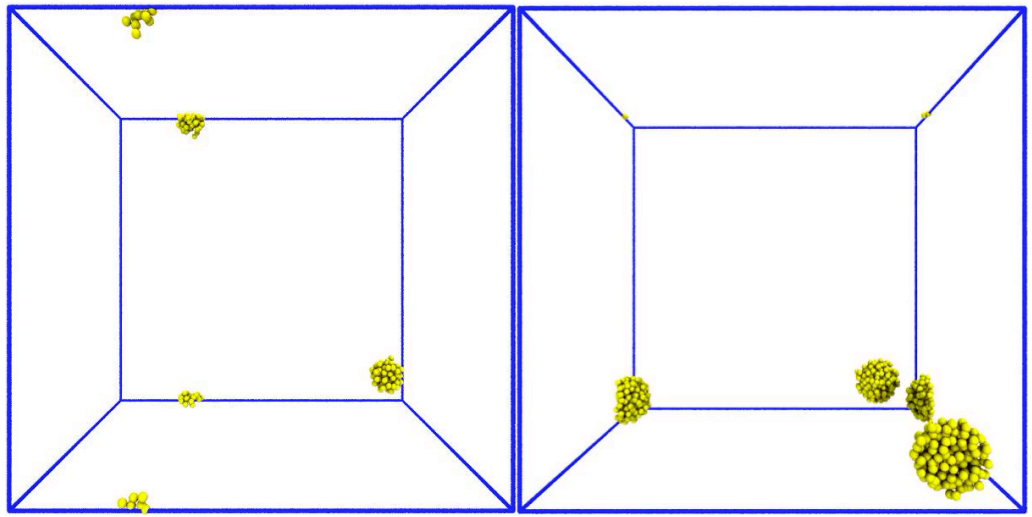
R_h = actual sphere radius

R_g ~ distribution of mass in space

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

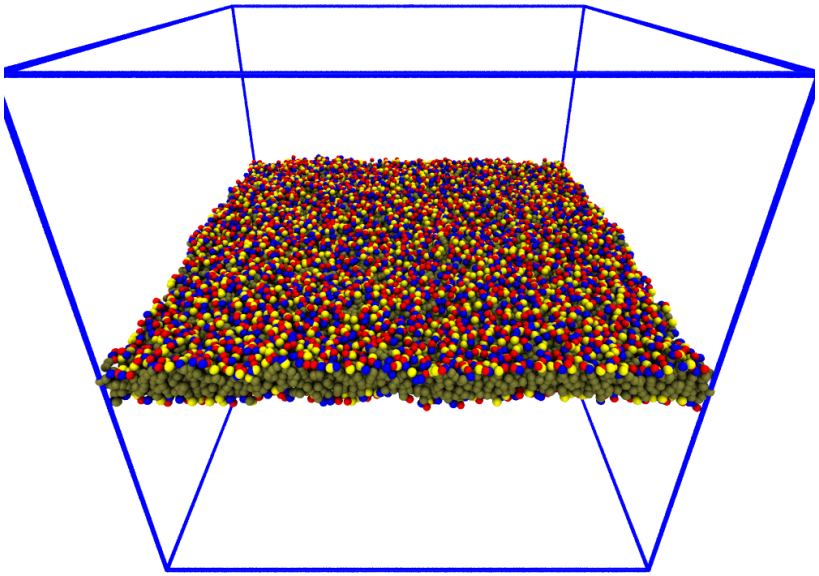




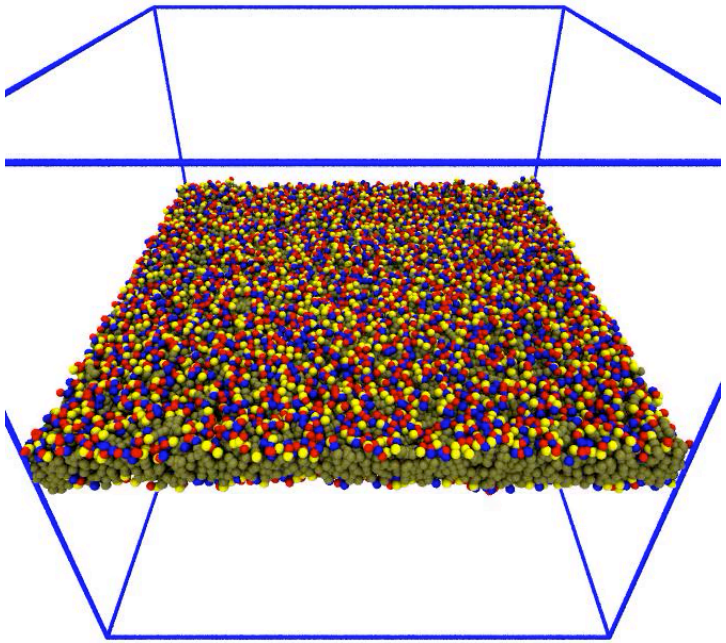


Ex. 10 Effect of charged headgroups on lipid bilayer stability

Simulate a stable membrane with a lipid whose headgroup can be charged, then set a screened Coulomb repulsion between the headgroups on a fraction of the lipids (from 1 down to ?) and observe the effect on the membrane.



No charge



Charged

```
Polymer Water  0.98787   " (W) "
Polymer Lipid  0.01213   " (PO4 (* (COH T T T T)) NHO T T T) "

Command ChargeBeadByType        10000    1  2.8  2.0
```

Ex. 11 Effect of cutting off a lipid tail on lipid bilayer stability

Create a stable membrane using two lipid types with same molecular architecture but different headgroup beads (and variable number fraction of minority lipid):

```
Polymer Water          0.9821   " (W) "
Polymer Lipid          0.0158   " (PO4 (* (COH T T T T)) NHO T T T) "
Polymer SphingoLipid   0.0021   " (CPO4 (* (COH T T T T)) NHO T T T) "
```

Once membrane is stable, cut off one of the tails (here the first one in Sphingolipid) by setting the bond strength to zero:

```
Command Comment  1000 // Cut off one tail of the lipid  //

Command SelectPolymerTypeInSimBox    10000  sphingo  SphingoLipid
Command SetBondStrengthInTarget      10000  sphingo  CPO4COH   0.0  0.5
```