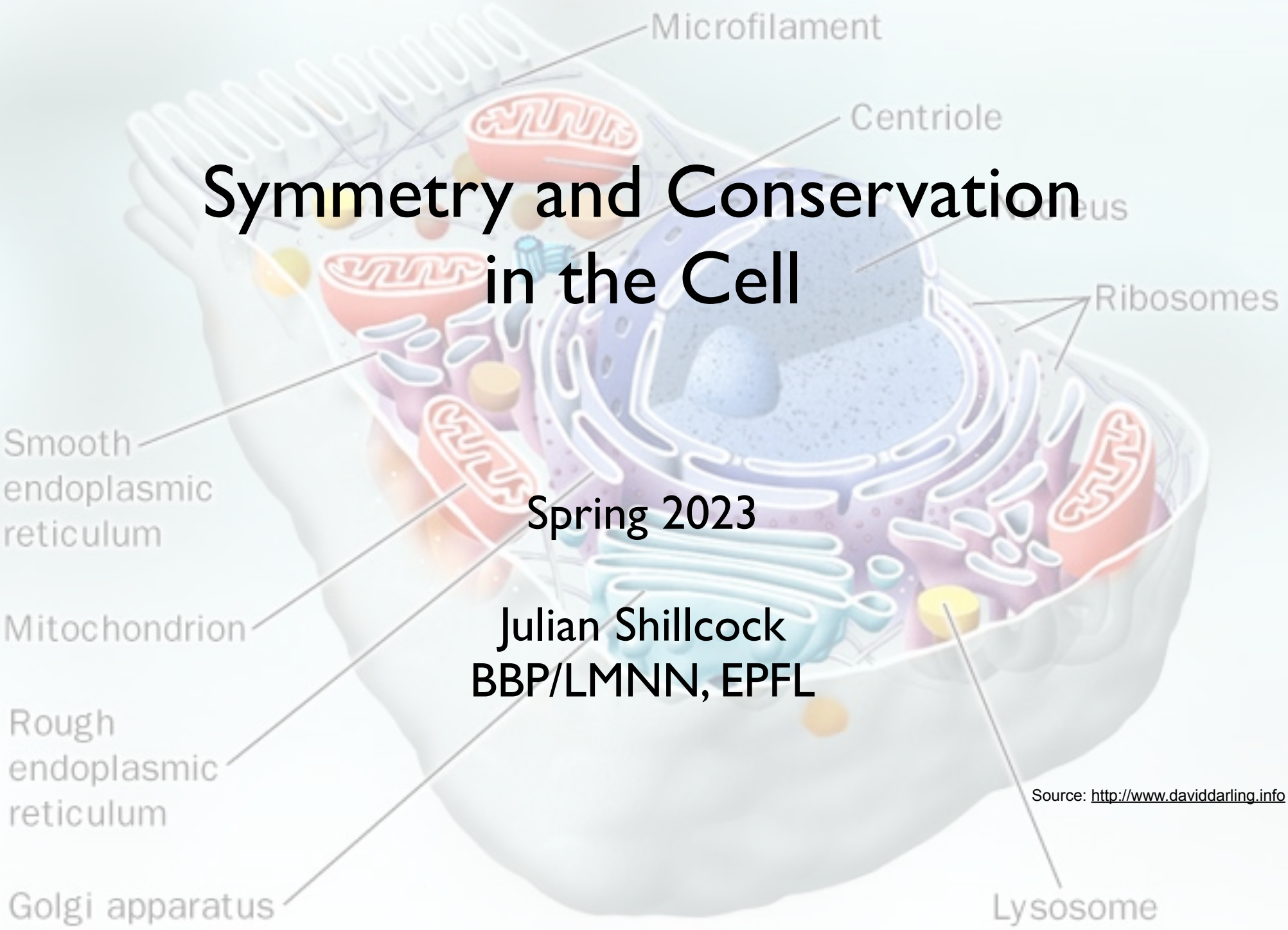
Symmetry and Conservation in the Cell

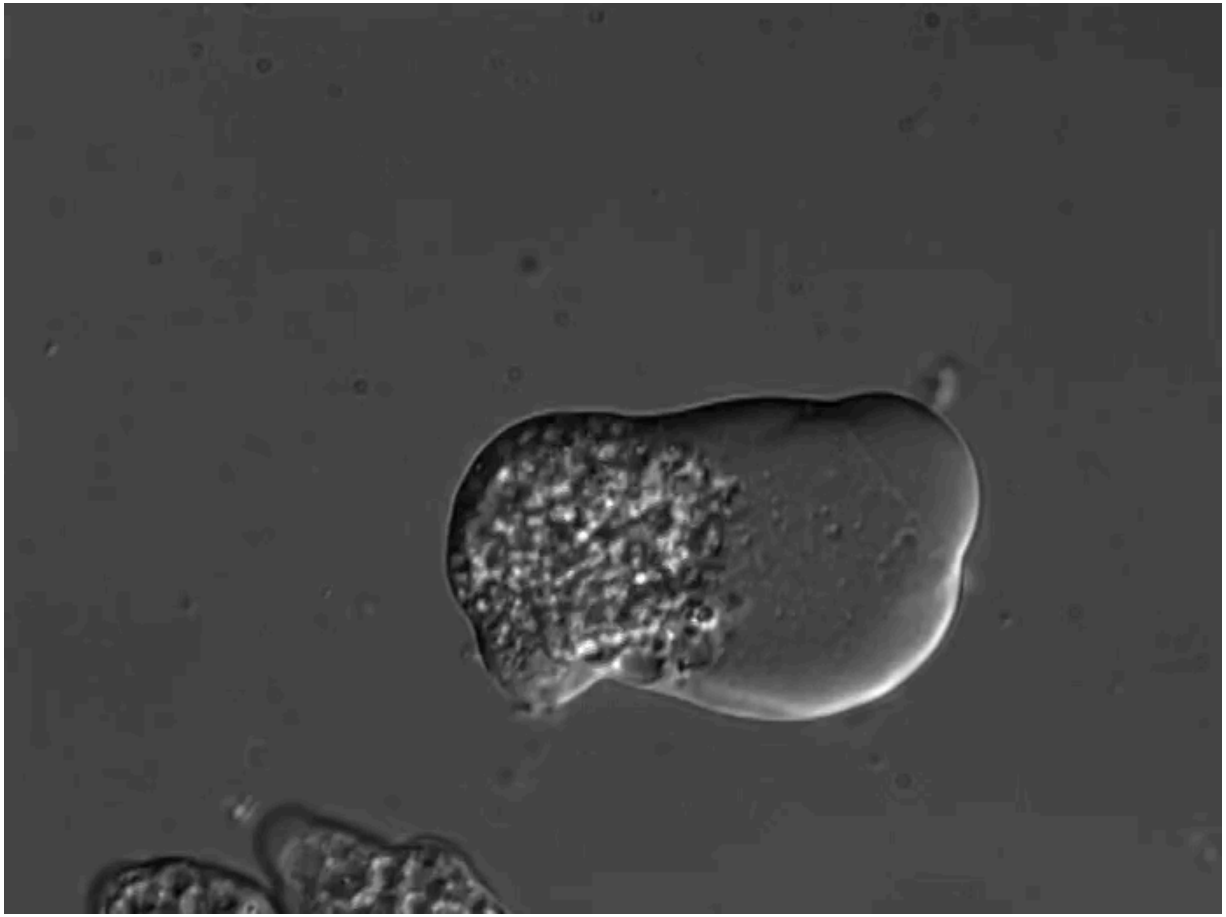


Spring 2023

Julian Shillcock
BBP/LMNN, EPFL

Source: http://www.daviddarling.info

Is the cell a machine?



The Roberto Stock group at IBt-UNAM

Entamoeba histolytica - anaerobic protozoan

Differential interference microscopy, 5x speedup

hyaline - clear cytosol

vesicle-filled - granular cytosol

leading edge - lobopod

How can we build a model of this?

We need to know **what** is there, and **how** does it behave?

Q. Is this cell diffusing?

If it is a machine, it's not like a car

EPFL

Common design principles of artificial machines:

- whole is made of precisely-arranged parts that execute ***stable/periodic*** functions ***independently*** of the others (chassis, wheels, transmission, engine, electrics, washer, windscreen, seats, etc. : if brakes fail, the lights still work)
- almost nothing is in equilibrium (by design)
- functions are ***independent*** of the environment (temperature, pressure, etc.)

Common design principles of cells:

- cellular cytosol, proteins, all molecules continually move and *interact* (diffusion, filaments assemble/disassemble, mechanical forces, gradients drive flow)
- many functions operate close to equilibrium because leaving equilibrium is expensive *
- cellular functions often *require randomness* derived from environment (T, P), e.g., diffusion; they are *strongly coupled* to their (changing) environment internally and externally

* cell is often said to be non-eq., but it uses eq. for stability, e.g., [ATP]

Course Overview

- "A day in the life of a cell" - overview of biophysics of a cell (**1 lecture**)
- Random walks, polymers and filaments (**3 lectures**)
- Molecular forces, thermodynamics and self-assembly (**3 lectures**)
- Computer simulations of cellular dynamics (**3 lectures**)
- Membranes and self-assembly (**3 lectures**)
- How bacteria harness symmetry in a cell (**1 lecture, project presentations)**

After this course you will be able to:

1) identify the important physical parameters relevant to a specific biological structure/ function in a cell

2) build computational models of cellular structure/function to explore *what-if* scenarios

3) identify a simulation technique useful to simulate selected cellular dynamics

This course in three sentences

EPFL

- Life is a consequence of the equipartition theorem

- Self-assembly, symmetry and dimension control cellular dynamics

- What is important? energy, entropy, shape, flexibility, fluctuations, …

What is ignorable? detailed chemistry, initial conditions, fluctuations, …

Marking Scheme

- 3 homework derivations of important results (20%, lectures 3, 8, 12)
- 2 homework DPD simulation exercises (20%, due by lectures 6, 10)
- 1 Journal club presentation (10%, lectures 4) - ~ 10-15 minutes, see next slide
- Semester project using the provided DPD code (50%, end semester)

  > 8 page report written as a scientific paper (intro, method, results, conclusion references); ~15 minute presentation on your results
- Choose the simulation topic from examples in the course or your choice (check with me for suitability first)

You **may** collaborate on all elements and **should** collaborate on simulation/projects to share compute resources: but each person has to speak/submit a report/project.

Week	Topics	Marked event due
1	What is biophysics?	
2	Brownian motion, Langevin equation	
3	Random walks in a potential; observer changes system	Derivation 1 due
4	Polymers in the cell, RW models of polymers	JC or later?
5	Forces, compartments, IDPs	
6	Thermodynamics	Homework simulation 1 due
7	Thermodynamics, phase transitions, Flory-Huggins theory	
8	Anatomy of a simulation	Derivation 2 due
9	Coarse-grained simulations, DPD	
10	Other cg simulations, Monte Carlo simulations, RW in phase space	Homework simulation 2 due
11	Membranes on different scales	
12	Amphiphile self-assembly	Derivation 3 due
13	Membranes as random surfaces, pores and fusion	
14	Bacteria and symmetry	Project presentation and report

One JC selected on one of these papers:

S. Alberti and A. A. Hyman Are aberrant phase transitions a driver of cellular aging?
Bioessays ***38***:959 (2016)

C. Brangwynne et al., Polymer physics of intracellular phase transitions,
Nature Physics **11**:899 (2015)

R. Groot and P. Warren, Dissipative Particle Dynamics: bridging the gap between atomistic and mesoscopic simulation,
J. Chem. Phys. **107**:4423 (1997)

M. Edidin, Lipids on the Frontier: a Century of Cell-Membrane Bilayers,
Nature Reviews Mol. Cell. Biol. **4**:414 (2003)

A. Klosin et al., Phase separation provides a mechanism to reduce noise in cells,
Science **367**:464-468 (2020)

A. Musacchio, On the role of phase separation on the biogenesis of membraneless compartments.
EMBO Journal 41:e100952 (2022)

or choose one related to the course material (check with me first please).

Today's Lecture

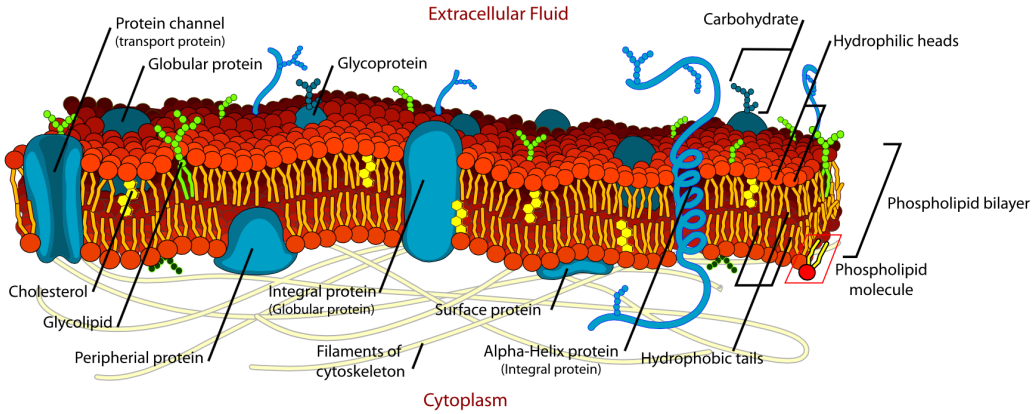
- What is your background?
  - Biology
  - Physics/Chemistry/Engineering
  - Mathematics
  - Computing
  - Simulations: which ones?
- Overview of cellular structure
- How do you build a cell? Oil, amphiphiles, proteins
- A day in the life of a cell: intuition and natural scales in a cell
- Symmetry and phase transitions in a cell, membraneless organelles
- Computer simulations
- Downloading the DPD code (Windows x - Mac x - linux x)
- Exercise 1: Measuring the equation of state of DPD water

Overview of cellular structure

Lipids self-assemble into many types of aggregate:

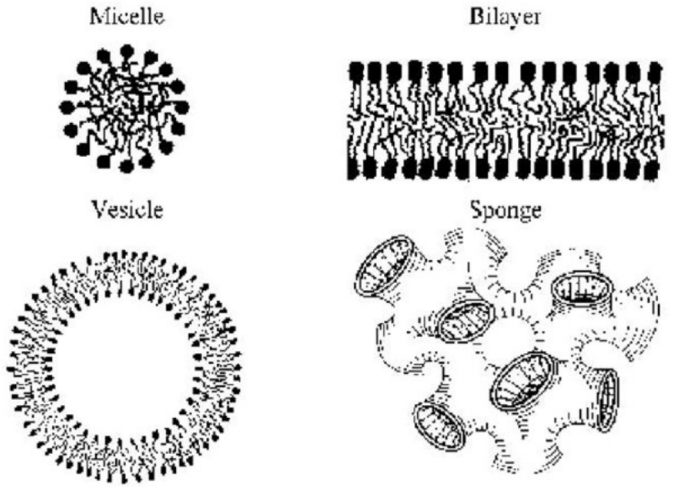
micelles, bilayers, vesicles, continuous phases (plumber's nightmare)

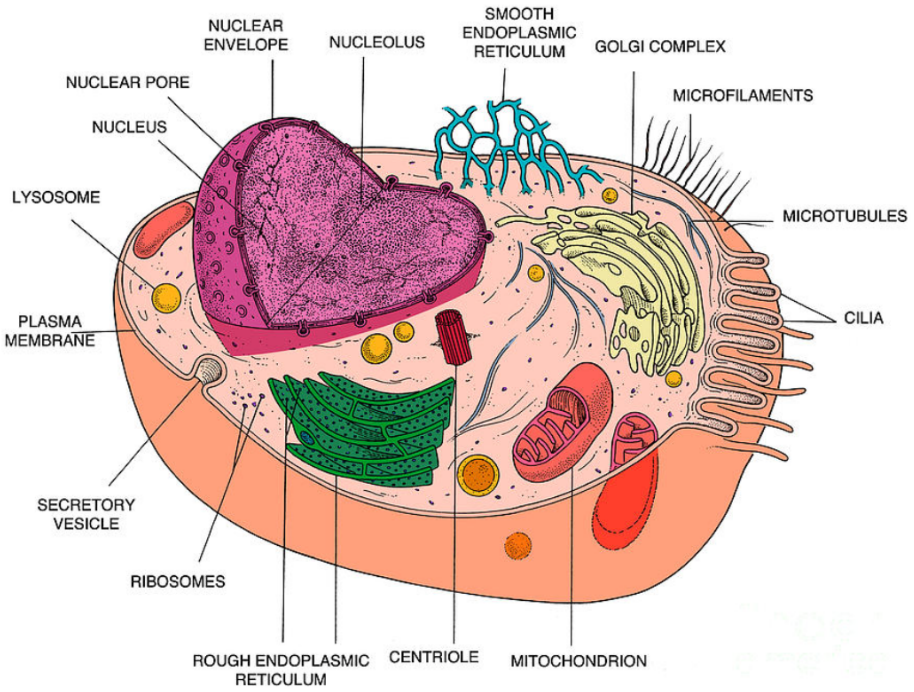
Whereas oil/water completely phase separate, lipid aggregates have a preferred area per lipid which leads to a selected finite size (vesicles and membranes)



Proteins - once the internal environment is distinct from the external, gradients across membranes used to generate energy and drive localised biochemical reactions; proteins create and maintain gradients

DNA ⇒ growth and reproduction





What is biophysics?

A.V. Hill, "the employment of physical instruments in a biological laboratory does not make one a biophysicist," rather it is "the study of biological function, organization, and structure by physical and physicochemical ideas and methods"

"Biophysics, as a distinct discipline, can be traced to a "gang of four": Emil du Bois-Reymond, Ernst von Brücke, Hermann von Helmholtz, and Carl Ludwig"

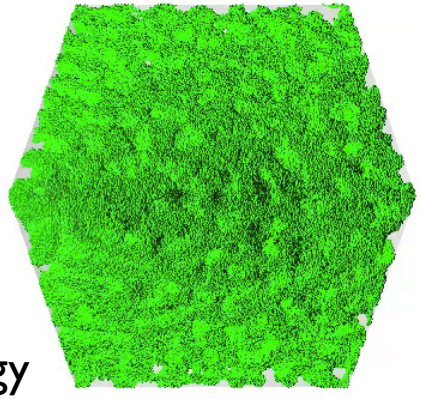
"the focus on the importance of providing a quantitative, theoretically based, analysis of the problem under study…"

"This issue provides examples of how one can use the power of the biophysical approach—the methods and analysis, the emphasis on quantitation, and the conceptual approach to problem solving—to understand important questions related to both normal and abnormal biological function, including human disease."

O. Andersen, Biophysical Journal 110:E01-E03 (8 March, 2016)

How do you build a cell?

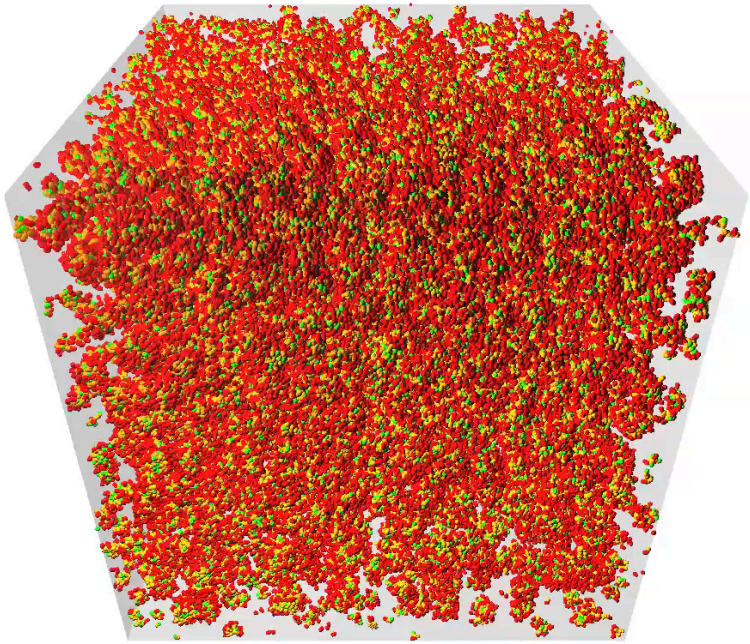
Water - an homogenous, uniform phase
no structure beyond ~ 1nm

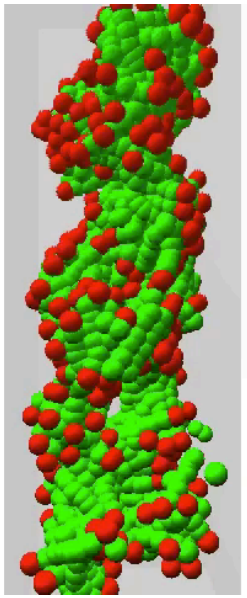


A cell is 70% water but clearly not only water

Mix oil and water ⇒ phase separation as

minimising interfacial area lowers total free energy





Mix amphiphiles like lipids and water
⇒ interfaces and *containers* appear:

compartments support gradients,
and can be used to do work

NB. Movies produced from DPD simulations and visualized with Povray and Quicktime. Water in the simulation box is invisible for clarity. Details of DPD simulations will be given in later lectures.

A day in the life of a cell - intuition

What is wrong with intuition?

Macroscopic intuition does not always apply at the molecular scale

> Physics is different at different length scales. By this I do not mean that the fundamentals of physics are different. Ultimately, all phenomena must be explained by the same sets of equations. What I mean is that the way things behave is different, because different aspects of physics are important. We all have an intuitive notion of physics. It is not that we know the equations; but if we throw a ball, if we make a splash in a bucket of water, if we watch someone fall over, then we know in an intuitive way how things are going to unfold. This is the kind of physics that it is important to get right in putting together a video game; when we cannot get our car round a bend and it flies off we expect it to behave in a realistic way, and the programmers go to some lengths to make sure that this happens. They do not do this by making every atom in the car obey Schrödinger's equation, instead they use a series of approximations that are correct for the scale that they are working at. When we change the scale the necessary approximations have to change too.

Ch. 4, Soft Machines: nanotechnology and life, R. A. L. Jones, Oxford University Press, 2004

A day in the life of a cell - intuition

EPFL

Examples of bad intuition:

1) Cell is round, so there must be a pressure difference? $\Delta p = 2\gamma/R$

No, if the plasma membrane were surface-tension controlled, like a bubble, there would be a tension, but there are a fixed number of lipid molecules in the PM with a preferred area per molecule that keeps the total membrane area constant (Lecture 9,10)

Red blood cells have a zoo of shapes that are controlled by a few parameters such as enclosed volume, Area/Volume ratio, number of molecules in the PM leaflets, etc.

2) Molecules diffuse freely in cytoplasm: $\langle X^2 \rangle = 6DT$

No, diffusion is not free in a cell: the environment is too crowded; nothing is more than ~50 nm from a membrane/wall; sub-diffusion is common (Lecture 5)

M. J. Saxton, Biophys. J. 66:394 (1994),
D. Ridgway et al. Biophys. J. 94:3748 (2008)

A cell is far from equilibrium, it extracts energy from "sugar" to do work while subject to random forces; concepts like symmetry, conservation, locality, minimising energy use, etc, help in constructing models that reproduce ***some*** of the properties of the cell, and we can do computer experiments on these simpler systems.

Goal: create simpler analogies for the cell that allow us to make accurate if qualitative predictions.

A day in the life of a cell - natural scales

What are some natural scales are in a cell?

In order to think quantitatively about a complex system, we need to be able to compare the complex thing with a simpler, better understood thing., e.g, if we say that a cell is a machine - what is a machine?

Qin = energy in, Qout = energy out, W = work done, Conservation of energy Qin = Qout + W

Efficiency = W/Qin = 1 - Qout/Qin < 1

Consider important parameters for a car:

top speed
boot capacity
time to accelerate to 100 mph
mpg (litre/100 km)
emissions in gms per km

There are constraints on a cell's dynamics due to the following:

Constant temperature drives random thermal motion, soft or fluid materials, small forces, large fluctuations, need to minimise energy use, etc.

A day in the life of a cell - natural scales

EPFL

Length - molecular size, area per molecule in a membrane, radius of gyration, density,…

Force/Energy - Surface tension (N/m), Membrane bending modulus (J or k_BT,)
Voltage difference across PM (80 mV/4 nm ~ 10**7 V/m cp lightning 10**8 V)

Time - Compared to diffusion in water, in the PM, of directed motor transport, neurite growth, ATP production, etc.

Thermal motion - how fast do molecules diffuse and cells move? compare diffusion to ballistic motion of billiard balls

Forces - compare ES force or covalent bond strength to lipid in a membrane; surface tension of water to hydrophobic effect of lipid molecule

Homework - natural scales

Before the 2nd lecture: calculate the following quantities from Mass, Length and Time scales given (or the table); and write what the answer tells you about a cell or the process:

Ex. 1 Cell diameter/membrane thickness (~ 2 * lipid end-to-end length)

Ex. 2 How many vesicles would fit into a single cell? i.e., have the same volume

Ex 3. What is the ratio of the area of all the vesicles in Ex. 2 to the plasma membrane area? How does this value compare to the experimental result that the PM is 2% of all membranes in a cell?

Ex. 4 How long does a lipid take to diffuse its own diameter in the PM due to thermal motion?
Assume D ~ 1 micron^2/sec, and area per lipid ~ 0.7 nm^2

Ex. 5 How long does an Na ion with Q = +e take to move through a passive ion channel in the PM under the applied electric field of the membrane?

Assume V_{mem} = 65 mV and d_{mem} = 5 nm, mass of Na = $3.8.10^{-26}$ Kg

What is its speed at the end?

Is relativity important for ion channels? (Relativity is important if $(v/c)^2$ is not negligible)

How long would it take to diffuse across without the electric field?

A day in the life of a cell - Length

Name	Value	Units	Reference
GUV diameter	50	micron (μm)	Mouritsen
RBC diameter	7.5	μm	Guyton
Mammalian cell diameter	20	μm	Alberts
Nuclear diameter	6	μm	Alberts
Lysosome diameter	0.5	μm	Alberts
Synaptic vesicle diam.	60	nanometre (nm)	Alberts
Plasma Membrane thickness	~ 4 (composition?)	nm	Israelachvili
Phospholipid length	~ 2 (lipid?)	nm	Israelachvili
Area per lipid	~ 0.7 (lipid?)	nm^2	Israelachvili
Microtubule width	25	nm	Alberts
Intermediate filament	10	nm	Alberts
Actin filament width	7	nm	Alberts
Tubulin mononer	~5	nm	Alberts
Actin monomer	~5	nm	Alberts
PM area/Total mem. area	0.02	-	Alberts
C-C bond length in lipid tail	0.154 + 0.126*n	nm	Israelachvili

A day in the life of a cell - time/speed

Name	Value	Units	Reference
Cell division/mitosis time	~30	minutes	Guyton
Vesicle fusion time	~20	ms	Domanska
Clathrin-coated pit formation	~60	sec	Weigel
Actin filament growth rate	3	mono/μM·sec	Fujiwara
Myosin V motor speed	200	nm/sec	book.bionumbers.org
Water diffusion in bulk	2300	μm^2/sec	Wraight
Water diffusion in gA channel	200	μm^2/sec	Wraight
Lipid diffusion in membrane	0.1 - 10	μm^2/sec	Gaede
Lipid flip-flop across membranee	10^2 - 10^5	sec	Israelachvili
Lipid chain equilibration	~1	ns	Roberts

A day in the life of a cell - Energy/force

Name	Value	Units	Reference
k_BT	4.1e-21 J at 300 K ~ 4 pN.nm 1 kJ/mol ~ 0.4 k_BT/molecule	Joules	-
Covalent bond energy	500	kJ/mol	Israelachvili
H-bond energy	20	kJ/mol	Israelachvili
Van der Waals "bond" energy	1	kJ/mol	Israelachvili
Denature a fusion protein	~200	pN	Yersin
Membrane stretch mod. (DMPC)	240 ~ 50 k_BT/nm^2	mN/m	Rawicz
Membrane bending mod. (DMPC)	$0.56.10^{-19}$ J ~ 13.5 k_BT	J	Rawicz
Water-air surface tension	70	mJ/m^2	Wikipedia
Water-oil surface tension	50	mJ/m^2	Israelachvili

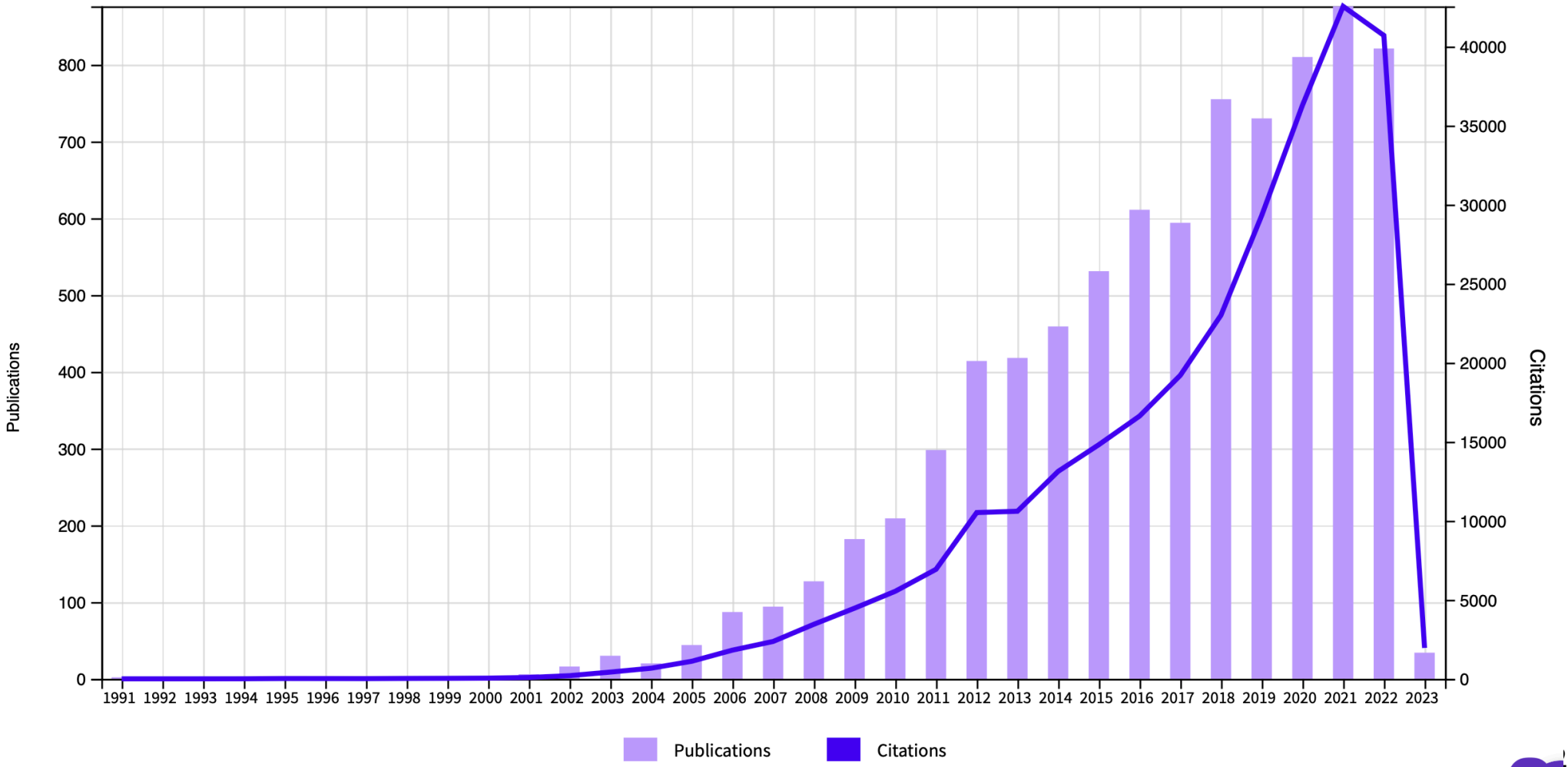
References for useful numbers table

1) O. Mouritsen, **Life as a Matter of Fat** (Springer 2005)
2) A. Guyton, **Textbook of Medical Physiology,** 8th ed. (Harcourt, Brace and Co. 1991)
3) B. Alberts, D. Bray, J. Lewis, M. Raff, K. Roberts, and J. D. Watson, **Molecular Biology of the Cell**, 2nd ed. (Garland Science, NewYork, 1989)
4) J. Israelachvili, **Intermolecular and Surface Forces** (Academic Press, 1992)
5) M. Domanska et al. **J. Biol. Chem**. 284:32158 (2009)
6) A. Weigel et al. **PNAS** E4591 (published online Nov. 11 ,2013)
7) http://bionumbers.hms.harvard.edu/default.aspx
8) A. Yersin et al. **PNAS** 100:8736 (2003)
9) W. Rawicz et al., **Biophys. J.** 79:328 (2000)
10) H. Gaede an K. Gawrisch, **Biophys. J.** 85:1734 (2003)
11) I. Fujiwara et al. **PNAS** 104:8827 (2007)
12) M. Roberts and A. Redfield, **JACS** 126:13765 (2004)
13) C. Wraight, **Biochim. Biophys. Acta.** 1757:886 (2006)
14) http://book.bionumbers.org/how-fast-do-molecular-motors-move-on-cytoskeletal-filaments/

Quote of the Day

disorder can do things structure can't

A. Keith Dunker, Indiana Univ.



Neurodegenerative diseases

Are aberrant phase transitions a driver of cellular aging?

Simon Alberti and Anthony A. Hyman**

Bioessays 38: 959–968, © 2016

ACS Chemical Neuroscience
Viewpoint
pubs.acs.org/chemneuro

Why Is Research on Amyloid-β Failing to Give New Drugs for Alzheimer's Disease?

DOI: 10.1021/acschemneuro.7b00188
ACS Chem. Neurosci. 2017, 8, 1435–1437

Andrew J. Doig,[†] Maria P. del Castillo-Frias,[†] Olivia Berthoumieu,[‡,§] Bogdan Tarus,[∥] Jessica Nasica-Labouze,[∥] Fabio Sterpone,[∥] Phuong H. Nguyen,[∥] Nigel M. Hooper,[⊥] Peter Faller,[‡,§] and Philippe Derreumaux*[,∥]

ABSTRACT: The two hallmarks of Alzheimer's disease (AD) are the presence of neurofibrillary tangles (NFT) made of aggregates of the hyperphosphorylated tau protein and of amyloid plaques composed of amyloid-β (Aβ) peptides, primarily Aβ1−40 and Aβ1−42. Targeting the production, aggregation, and toxicity of Aβ with small molecule drugs or antibodies is an active area of AD research due to the general acceptance of the amyloid cascade hypothesis, but thus far all drugs targeting Aβ have failed. From a review of the recent literature and our own experience based on in vitro, in silico, and in vivo studies, we present some reasons to explain this repetitive failure.

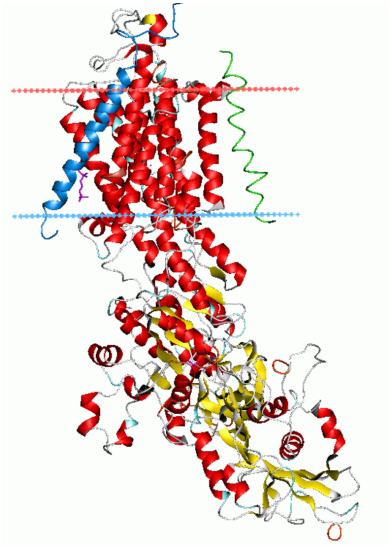
KEYWORDS: *Amyloid-β, Alzheimer's disease, in vitro and in vivo studies, computer simulations, drugs*

There's a BIG problem in drug development for AD, PD, ALS (motor neuron disease)

Not all proteins fold to function

Folded proteins

- Unique folded state
- Lowest energy (energy dominated)
- Precise shape
- Precise functions
- Disrupted by single aa mutation
- Enriched in catalysis, ion transport, binding

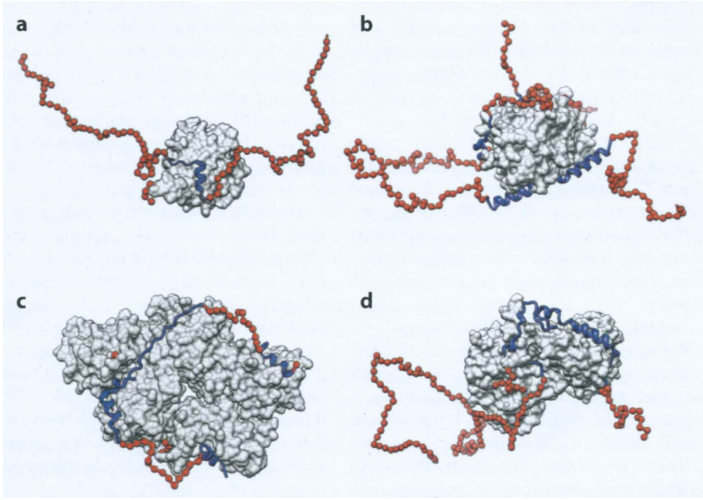


Na K ATPase

wikipedia.org

Intrinsically Disordered Proteins

- 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
- Enriched in signalling and regulation
- 30-50% of all protein sequences

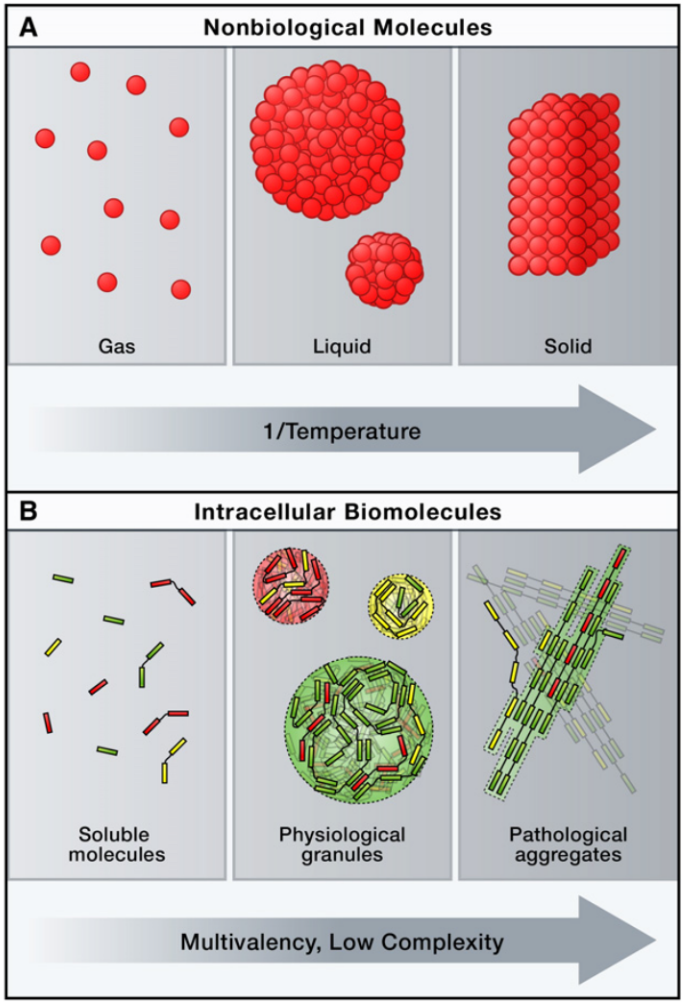


Oldfield and Dunker
Ann. Rev. Biochem.l. 83:553 (2014)

How do we go beyond analogies?

Water - Ice

Soluble protein - Droplet



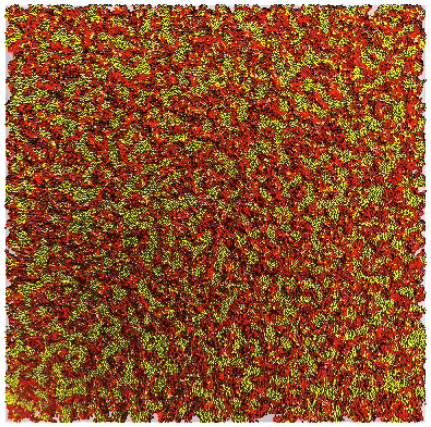
Weber and Brangwynne, Cell **149**:1188 (2012)

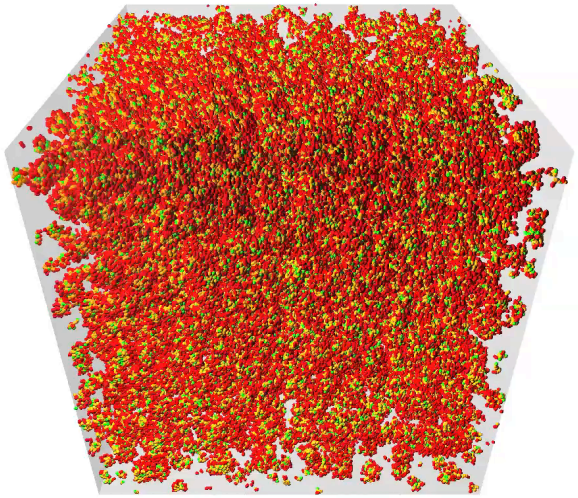
- In vitro experiments suggests a reversible *equilibrium* process (sometimes…)
- Flexible protein properties *independent* of the atomistic structure of their monomers (many proteins with no seq. similarity form droplets): are they random walks/phantom chains?
- Polymers have conformational fluctuations (high entropy) suggesting entropy may be important (Chatteraj et al. BJ 2019 show that **freely-jointed chain** polymers do NOT form droplets in Langevin sims.)

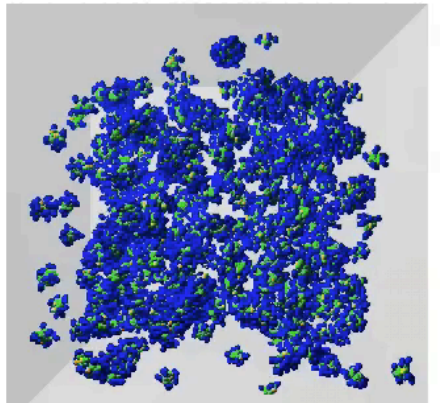
What is the effect of the interaction range on self-assembled structures?

Short → Long

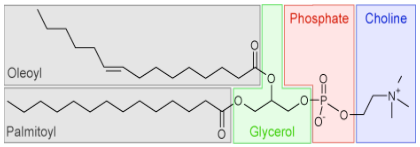
Ideal gas	van der Waals gas	Oil Water	Lipids Water	Polymers

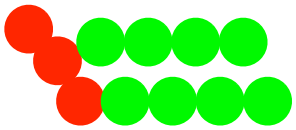


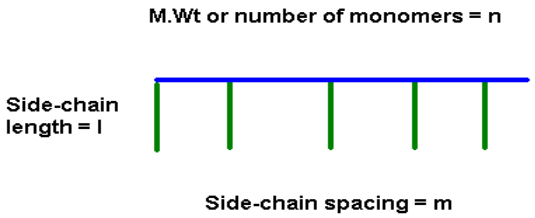




Yellow = oil
Red = tracer
Water invisible

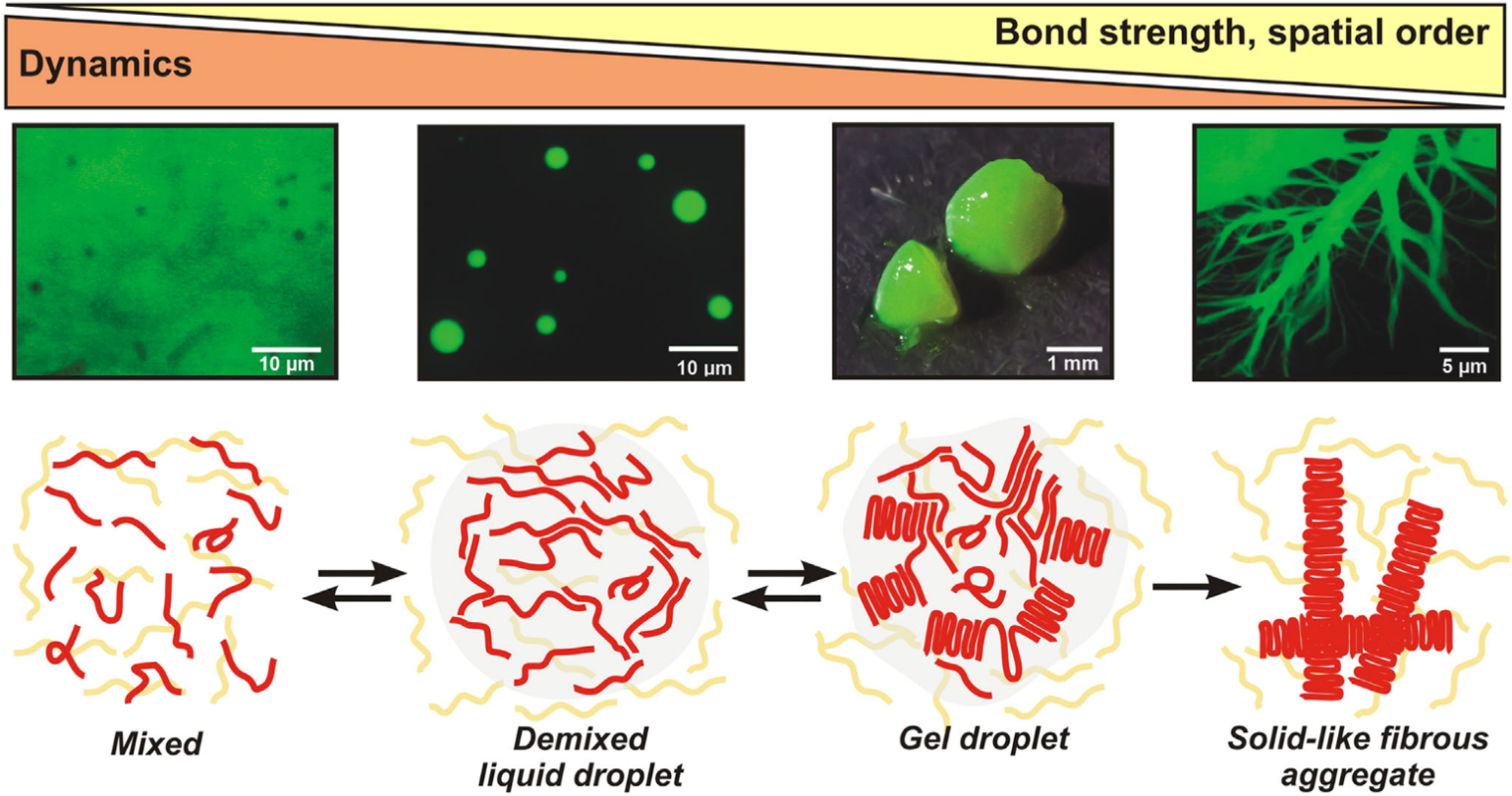






JC Shillcock, Langmuir 28:541-547 (2012)

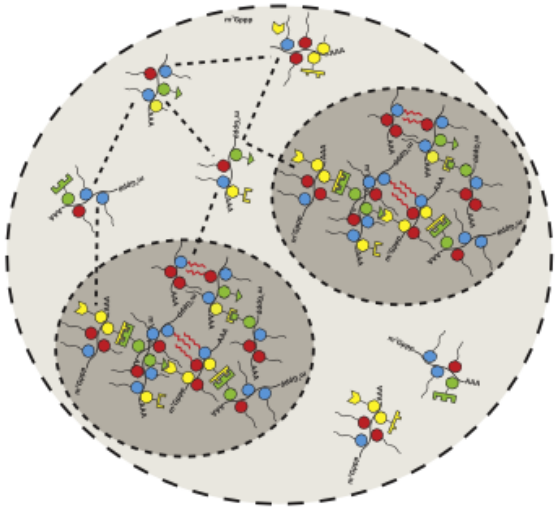
Oily tails of lipids want to be *segregated* from water and headgroups want to be *solvated* but they are bonded together - frustration; lipids and comb polymers self-assemble into a variety of structures



Single component droplet -

Alberti and Hyman,
Bioessays 38:959 (2016)

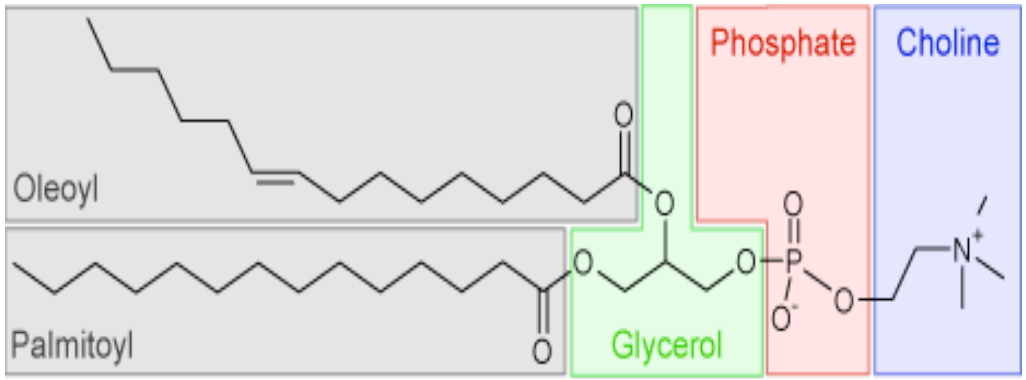
Multi-component droplets also exist -
Protter and Parker,
Trends Cell Biol.26:668 (2016)



Mature biphasic stress granule

What's special about lipids?

A number of the examples in this course will be related to phospholipids.



Lipids are usually seen as just a bounding surface to cells, and a "sea" for proteins to float in.

But they have important active roles too:

Signalling - endocannabinoids

Disease - lipid-binding proteins - PLAs (inflammation), ApoE (Alzheimer's), alpha synuclein, PUFAs (Parkinson's)

Synaptic dynamics - modify ion channel dynamics, receptor currents, shaping tubes disks, etc.

B. Davletov and C. Montecucco, Lipid function at synapses. Curr. Op. Neurobiol. 20:543 (2010)

Lipid-related diseases are fatal

seen. The infantile and juvenile forms are inherited as recessive traits, appearing mo
often in Jewish families. Patients may show xanthomas, pigmentation, hepatosplen
megaly, lymphadenopathy, and mental retardation. Pancytopenia is common. Diagno
sis may be made by tissue biopsy and confirmed by enzyme assay. Absence of th
sphingomyelin-cleaving enzyme can be demonstrated in both biopsy specimens an
tissue culture. Serum lipids usually are normal. **Treatment** at present is supportiv
there is no specific therapy.

Fabry's Disease
(Angiokeratoma Corporis Diffusum Universale; α-Galactosidase Deficiency)

*A rare, familial, sex-linked disorder of lipid metabolism in which glycolipid (galacto
galactosylglucosyl ceramide) accumulates in many tissues.* The metabolic abnormality
due to the absence of the lysosomal enzyme α-galactosidase A needed for the norm
catabolism of trihexosyl ceramide. Clinical recognition in males results from characte
istic skin lesions (angiokeratomas) over the lower trunk. Patients may have co
opacities, febrile episodes, and burning pain in the extremities. Death results fro
renal failure, or cardiac or cerebral complications of hypertension or other vascul
disease. Heterozygous females may exhibit the disorder in an attenuated form and a
most likely to show corneal opacities. Enzymatic replacement of the deficient enzy
by transfusion is being explored and may have potential therapeutic value. Treatme
is otherwise supportive, especially during periods of pain and fever.

Wolman's Disease
(Acid Cholesteryl Ester Hydrolase Deficiency)

*A familial autosomal recessive disease characterized by hepatosplenomegaly, steat
rhea, and adrenal calcification manifested in the first weeks of life.* Large amounts
neutral lipids, particularly cholesteryl esters and glycerides, accumulate in the bo
tissues. Deficiency of an acid lipase has been described. There is no specific therap
and death usually occurs by 6 mo of age.

Cholesteryl Ester Storage Disease

*An extremely rare familial autosomal recessive disease characterized by hepatom
and accumulation of cholesteryl esters and triglycerides mainly in lysosomes in the li
spleen, lymph nodes, and other tissues.* Hyperbetalipoproteinemia is common and p
mature atherosclerosis may be severe. A deficiency in cholesteryl ester hydrolase
been described. Patients may be asymptomatic. Diagnosis is made by liver biop
There is no treatment.

Cerebrotendinous Xanthomatosis
(van Bogaert's Disease)

*A rare recessive familial disorder characterized by progressive ataxia, dementia
racts, and tendon xanthomas.* Cholestanol (dihydrocholesterol), which is usually h
detectable in the body, is found in increased concentrations in the nervous sy
lungs, blood, and xanthomas. The underlying defect involves a deficiency of a he
enzyme that catalyzes the 24S hydroxylation of an intermediate sterol in the bile
synthetic pathway. Though plasma cholesterol levels are usually low or normal, pr
ture atherosclerosis also occurs. Disability is progressive, though often not manif
until after age 30. Treatment with chenodiol (0.5 to 1.5 gm/day), which inhibits
mal bile acid synthesis, reduces plasma cholesterol and may prevent further pro
sion of the disease.

β-Sitosterolemia and Xanthomatosis

A rare recessive familial disease characterized by the accumulation of plant sterols in the blood and tissues and by the occurrence of tendon and tuberous xanthomas, premature atherosclerosis, and abnormal RBCs. Increased intestinal absorption of dietary β-sitosterol has been demonstrated. Treatment consists in reducing the intake of foods rich in plant sterols (such as vegetable oils), and administering cholestyramine resin to promote sterol excretion.

Refsum's Syndrome
(Phytanic Acid Storage Disease)

A rare recessive familial disorder of phytanic acid metabolism characterized clinically by peripheral neuropathy, cerebellar ataxia, retinitis pigmentosa, and bone and skin changes. The disorder is due to a deficiency of phytanic acid hydroxylase, an enzyme that metabolizes phytanic acid. It is associated with marked accumulation of phytanic acid in the plasma and tissues. (See also Table 128–1 in Ch. 128.) A diet deficient in phytanic acid ("chlorophyll free") is beneficial. Serial plasmapheresis may help keep plasma phytanic acid levels down.

Other Lipidoses

Several rare inheritable lipidoses have been demonstrated using sophisticated techniques of tissue culture and enzyme analysis. The more common ones are described.

Tay-Sachs disease (G_{M2} gangliosidosis) is characterized by very early onset, progressive retardation in development, paralysis, dementia, blindness, cherry red retinal spots, and death by age 3 or 4. This recessive disorder is most common in families of Eastern European Jewish origin and is caused by deficiency of the enzyme hexosaminidase A, resulting in accumulation of gangliosides (complex sphyngolipids) in the brain. An infantile disorder often fatal by age 2 is **generalized (G_{M1}) gangliosidosis** in which the ganglioside G_{M1} accumulates in the nervous system. In **sulfatide lipidosis (metachromatic leukodystrophy)** there is a deficiency of the enzyme cerebroside sulfatase, causing metachromatic lipids to accumulate in the white matter of the CNS, peripheral nerves, kidney, spleen, and other visceral organs. It is characterized by progressive paralysis and dementia usually beginning before age 2 and fatal by age 10. **Galactosyl ceramide lipidosis,** also known as **Krabbe's disease** or **globoid leukodystrophy,** is a fatal infantile disorder characterized by progressive retardation, paralysis, blindness, deafness, and pseudobulbar palsy. This familial condition is secondary to a deficiency of galactocerebroside β-galactosidase. Diagnosis of these disorders may be made *prenatally* from amniotic fluid. No specific therapy is known.

ANOMALIES IN KIDNEY TRANSPORT
(See in Chs. 155 and 187)

86. AMYLOIDOSIS

Accumulation in the tissues of the fibrillar protein amyloid usually in amounts sufficient to impair normal function.

Pathophysiology and Classification

The cause of amyloid production and its deposition in tissues is unknown. Immunologic derangements have been implicated—B cell activation, T cell suppression, mac-

Merck Manual, 15th ed. https://en.wikipedia.org/wiki/Merck_Manual_of_Diagnosis_and_Therapy

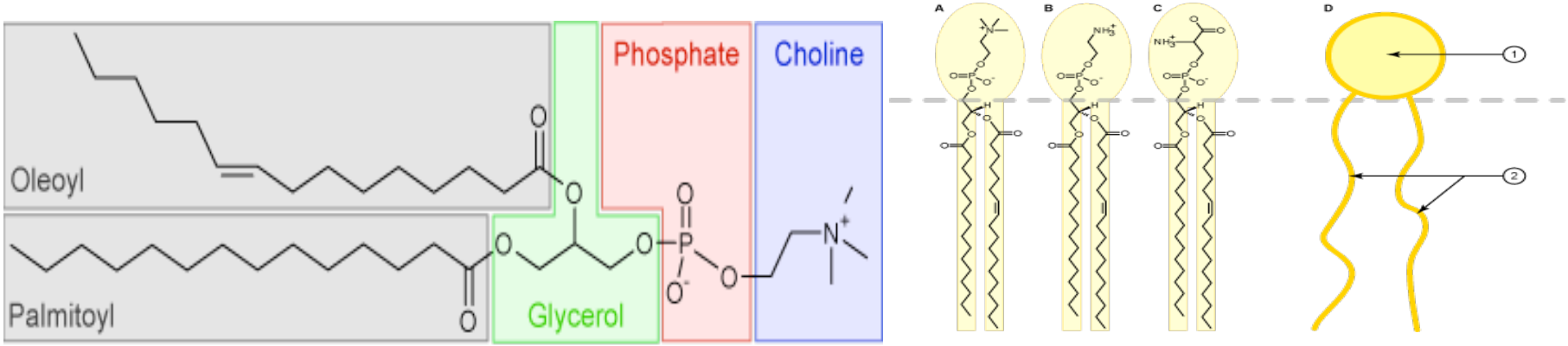
Symmetry

Symmetry may be built into a structure like lipids/membranes/proteins or appear or disappear at a *phase transition* because it minimises the energy cost of the new phase arising out of the old one,

e.g., when water freezes to ice, the crystal structure exhibits a symmetry derived from the bonds formed by the water molecules; even though oil is a polymer, it forms spherical drops - why?

Phospholipids are amphiphiles = polar headgroup + hydrocarbon tail(s)

Hydrophobic effect = oily chains disrupt the bulk water's H-bonding network, lowering the entropy and contributing to the oil-water repulsion that leads to phase separation. So the self-assembled aggregates actually have HIGHER entropy than the dispersed phase



Israelachvili devised a *packing parameter* to capture the "shape" of lipids in the fluid phase:

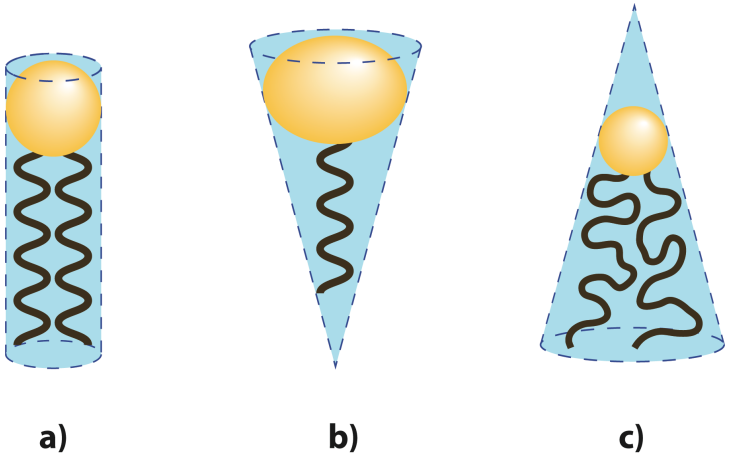
$$p = v / a_0.l_c$$

v = equilibrium volume of the molecule (depends on environment, temperature, etc!)

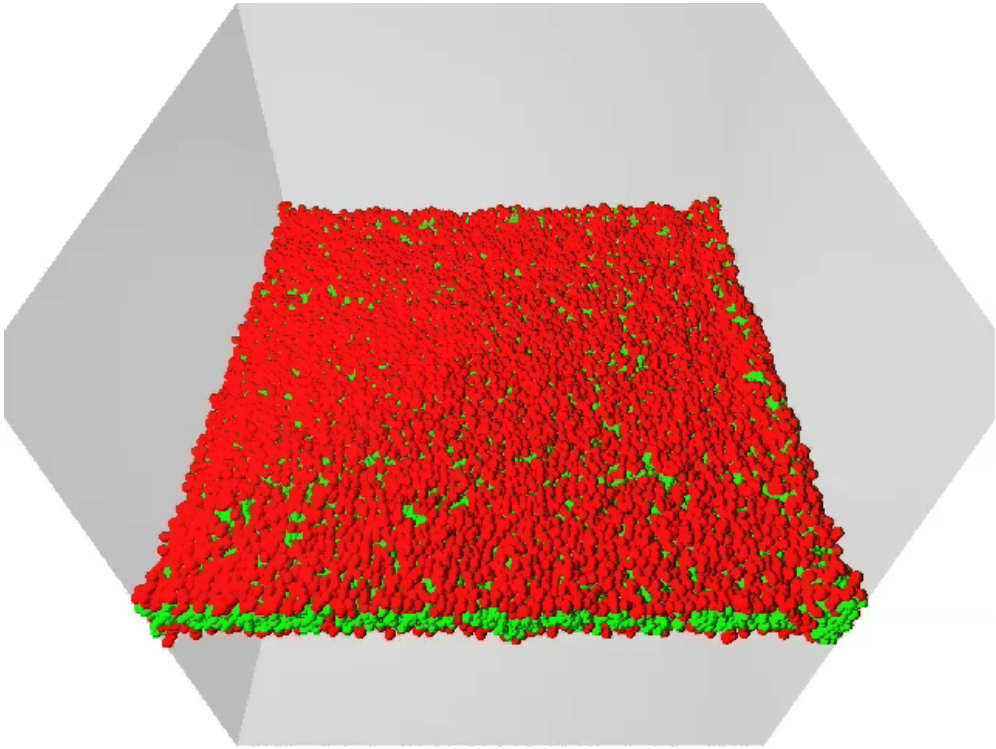
a_0 = equilibrium cross-sectional area of the molecule (" ")

l_c = maximum extension of the hydrocarbon chains

J. Israelachvili, Intermolecular and Surface Forces, (1992)

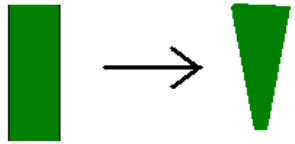


p ~ 1 p < 1/3 p > 1



e.g., modifying the ionic conditions changes effective headgroup area, or a phospholipase enzyme (e.g., PLA 2) may cut off one tail releasing a fatty acid and lysolipid

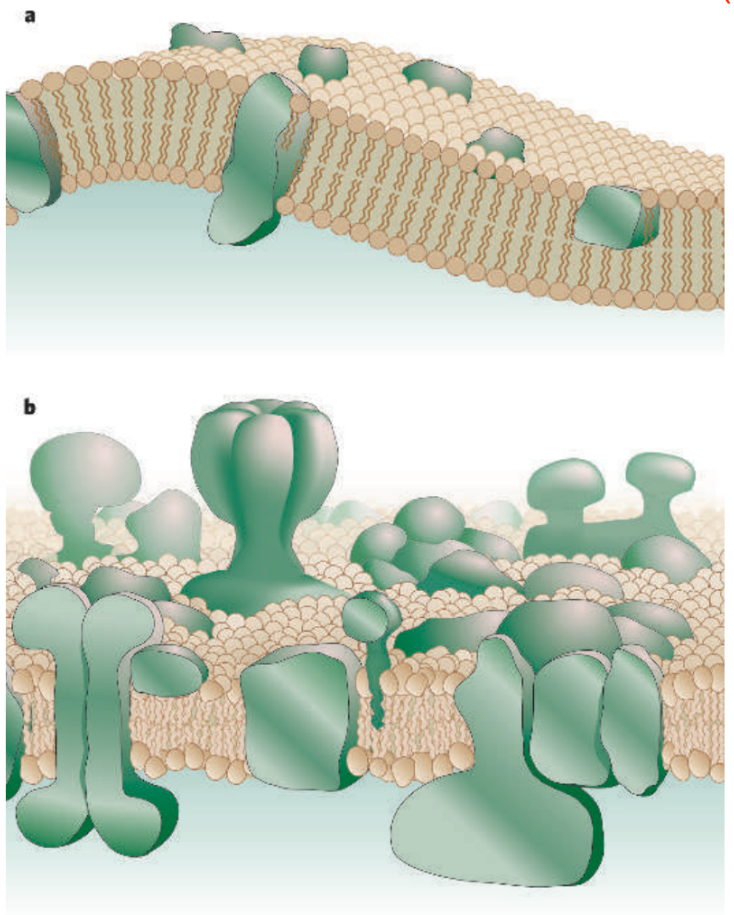
Initially-tensionless membrane 5538 lipids
40 nm x 40 nm
$C_0 = 0 \rightarrow > 0$



Fluid mosaic model of the PM

The Fluid Mosaic model of Singer and Nicholson (1972) says that 1) the PM is a fluid bilayer of lipids in which 2) proteins diffuse freely at low concentration and 3) have a width that matches the hydrophobic thickness of the lipid bilayer, 4) the proteins are monomeric and 5) the lipid surface is exposed to the external environment.

Singer SJ and Nicolson GL Science 175:720-731 (1972)



This picture has to be updated:

- >100s of lipid types, not randomly mixed; creates domains or *rafts*
- proteins are oligomers and heteromers, large exterior parts cover the PM, distort bilayer thickness to match transmembrane part
- diffusion of lipids and proteins not always free

Engelman DM Membranes are more mosaic than fluid Nature 438:578-580 (2005)

Why do computer simulations?

EPFL

- Experiments are too complicated and theories are too simple
- Simulating a model captures what we *think* are the important aspects of an experiment, and allows us to ignore irrelevant aspects. If we later find out it is incomplete, we can look for and add the missing properties
- We have almost complete control over all aspects of the simulation; so we can do thought experiments like turning ES off or changing pressure or temperature
- Even if a model is inaccurate, it can reveal discrepancies or assumptions in our thinking that can be valuable; and sometimes a less accurate answer obtained quickly is better than an accurate one that takes a long time
- We can visualize a simulation in ways not possible for experiments

If we are interested in molecules, we **must** keep fine details (e.g., bond vibrations) , but if we are interested in behaviour on microns or larger length scales we **cannot** use atomistic Molecular Dynamics.

What are the important degrees of freedom?
What are their dynamics?
What do we want to measure?

What is a simulation?

A simulation is not:

- analytically solving a differential equation or pde - e.g., ballistics versus weather
- quadratures - e.g., calculating a Fourier transform of electron density vs. P.F. $Z(\{\mathbf{x}\})$

What is a simulation?

"a computer *experiment* of the behaviour of a *model* of a physical system in which matter is replaced by mathematical constructs that interact in ways that mimic the interactions in the physical system, and where the model's evolution generates states corresponding to those of the real system."

Caveat

We never simulate a real system, but only a model of a real system; we first have to construct a model and second adapt it for calculation on a computer.

Computer simulations and a cell

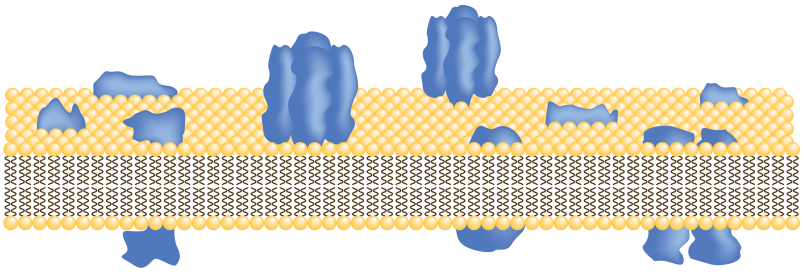
Example: What is the plasma membrane?

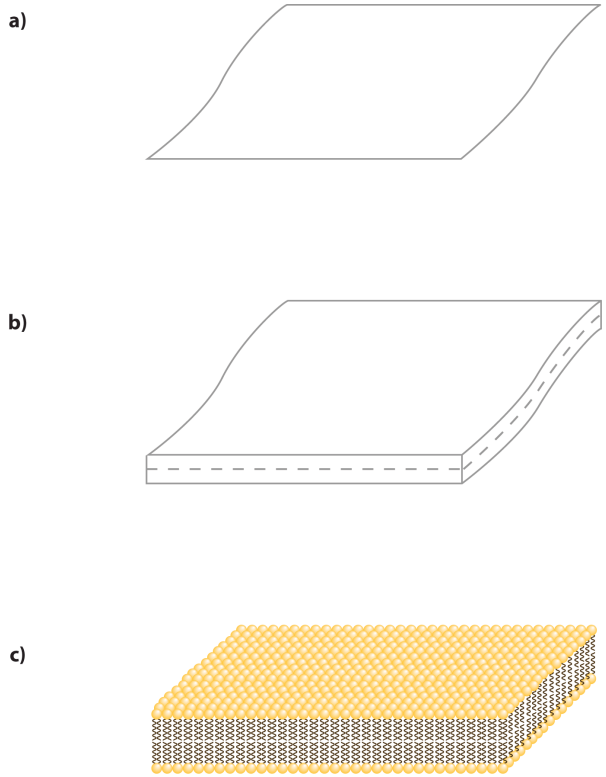
Solid or fluid?

Tense sheet or floppy liquid?

Infinitesimally thin monolayer or bilayer?

Proteins inside or outside?





Shillcock, Figs. 1, 2, Ch. 26 in Biomolecular Simulations,
ed. L. Monticelli and E. Salonen, Methods in Mol. Biol. 924, Humana Press 2012

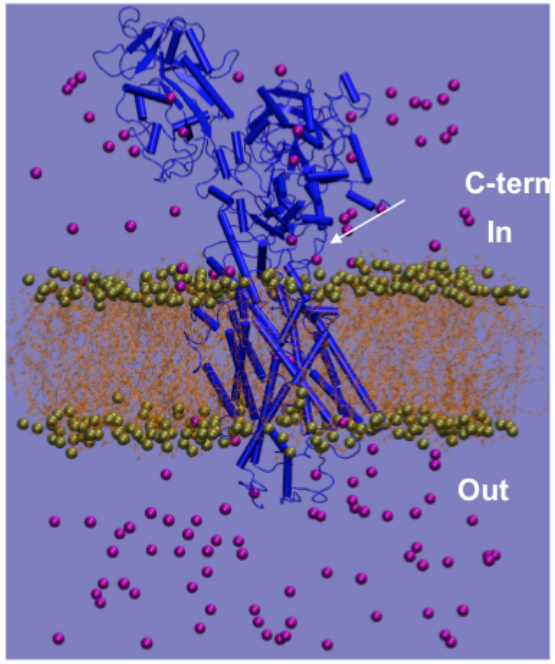
The PM is symmetric in that the lipids have translational symmetry but asymmetric in that it has different lipids in one leaflet than the other; lipids have rotational symmetry because they can rotate, but not up-down symmetry because flip-flop is very slow, etc.

Particle-based simulations

Atomistic Molecular Dynamics of membrane protein

Solving Newton's second law to evolve the positions and velocities of the system in time and space

- N ~ 170000 atoms
  - 337 POPC Berger lipids
  - Protein
  - ~ 45000 water
  - Counterions and electrolyte
- NPT ensemble, GROMACS
- Temperature: 310 K
- 115 x 115 x 160 Å
- Time step: 2 x 10^{-15} s

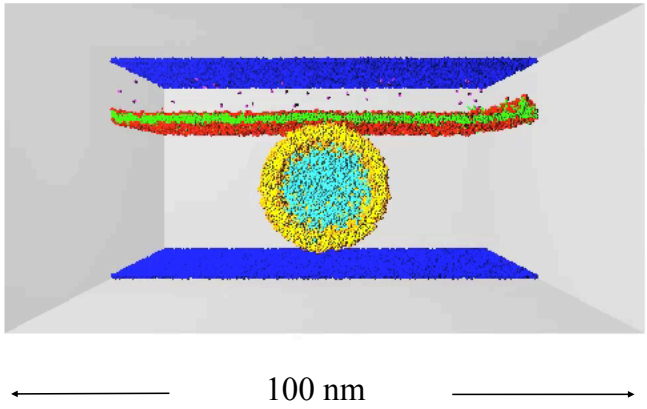


Computing time: 54 cpu years, ~ 80 simulations
6-8 ns a day on 64 cpus for ~ 200,000 atoms

NB. Special HW allows ms simulations of 1 protein in days, Shaw et al Science, 330:341-346 (2010)

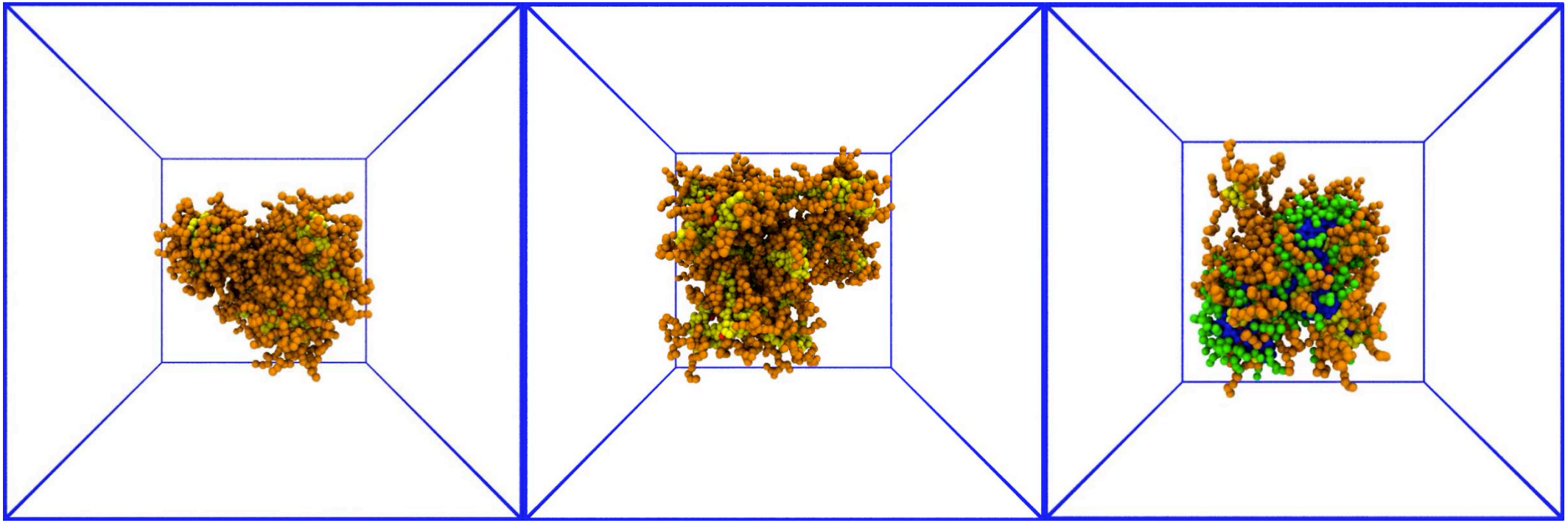
Himanshu Khandalia, U. Southern Denmark

Dissipative Particle Dynamics of vesicle fusion



$(100\ nm)^3$ is a large computational volume for a single processor (roughly the volume that can be simulated by DPD at a rate of 10 µs per 64 processor-days) and produces 10 GB of coordinate data per snapshot for visualization

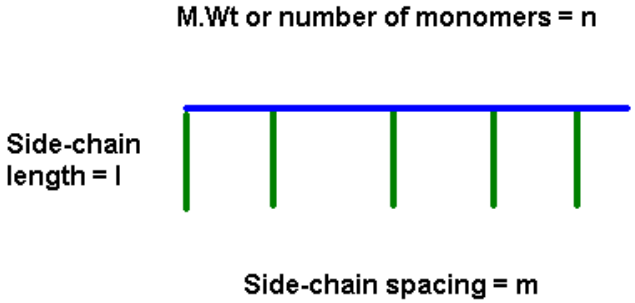
Dissipative particle dynamics



strongly hydrophobic backbone

weakly hydrophobic backbone

strong hydrophobic backbones; long/short side-chains



Comb polymers

We can predict aggregates larger than molecular scale, material properties, structure evolution, from "small" changes in molecular properties

Break

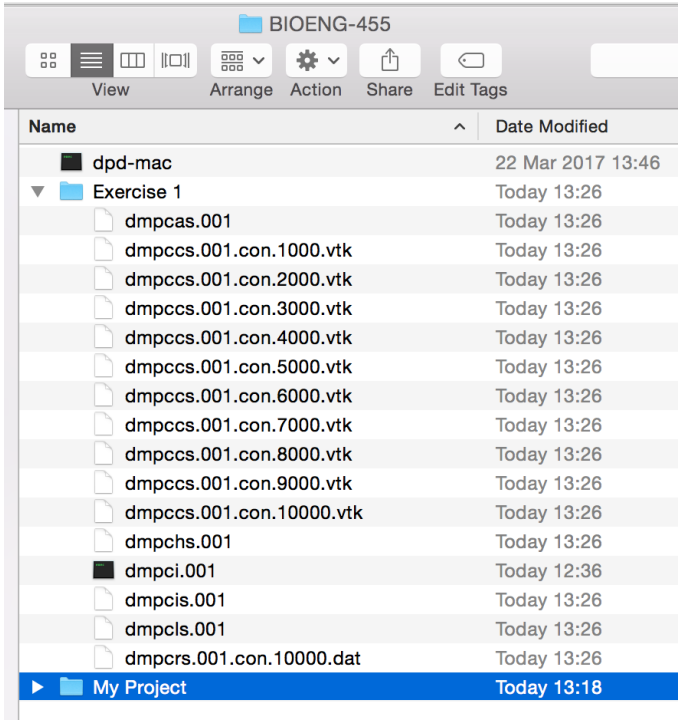
10 mins.

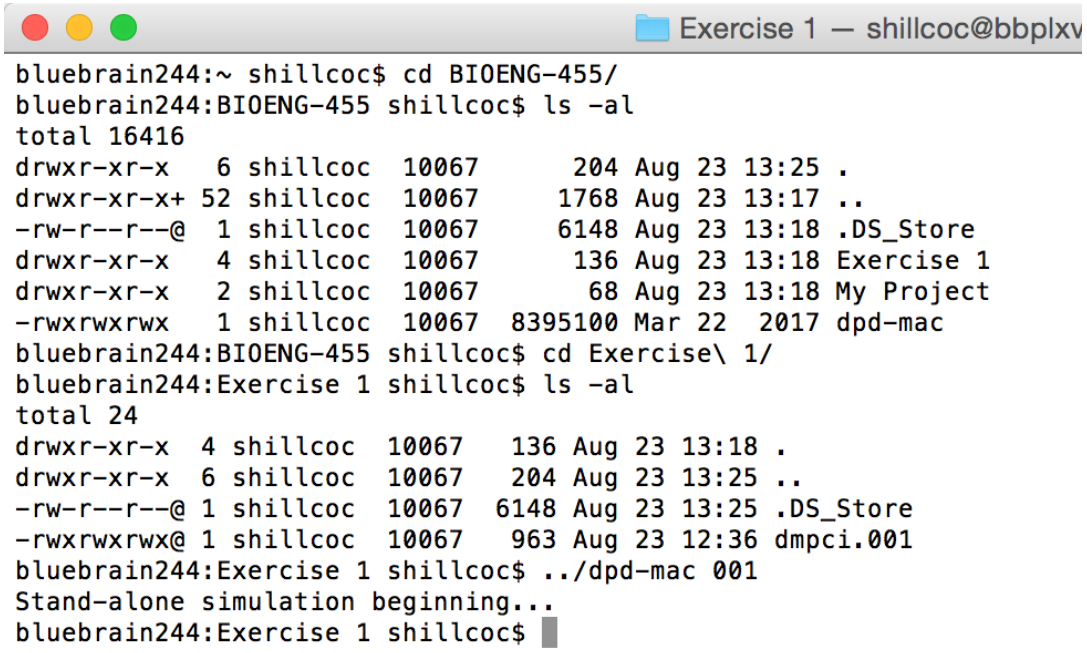
Break / Exercise

- Install DPD simulation code and get user guide
- Install Paraview or VMD for visualisation of simulations
- Check you can run the sample input file on your laptop
- How will you plot graphs?

Download and run the DPD code

- Download the executable (Linux, Mac, Windows) from moodle and the User Guide (or get the source code from github: https://github.com/Osprey-DPD/osprey-dpd and compile it for your platform)
- Create a directory structure to hold the runs, e.g., ~/BIO-692/Exercise 1
- Download the sample input file to your laptop (dmpci.ex1) and run it
- Enter execution command in the directory containing the input file





Exercise

Goal of this exercise is to calculate the equation of state of a single component fluid (water) from DPD simulations.

- Download the DPD code for your platform, and the input file dmpci.001
- Understand the structure of the input file (dmpci) and output files
- Simulate pure water for a range of densities and extract the *equilibrium* temperature and pressure against time from the dmpchs file

Equation of state of DPD water

The ideal gas EOS is: $pV = N k_B T$

For a real gas, it can be written as a Virial expansion ($\rho = N/V$ = density)

$$p = \rho k_B T(1 + B_2(T)\,\rho + B_3(T)\,\rho^2 + \dots)$$

where B_2, B_3 are independent of density.

What is the EOS for "DPD water"?

$$p = p(a_{ij}, \rho, T)$$

p = Pressure
a_{ij} = Conservative interaction parameter
ρ = Bead density
T = Temperature

dmpci.nnn input file

```
dpd

Title   " Water "
Date    19/09/18
Comment " Pure water simulation. Measuring the pressure as a function of the bead
          density parameter (Density 3) allows the equation of state to be determined.
          Ignore the first analysis period (1 - 5000 timesteps) to allow the system
          to equilibrate and then take the value from the second period (5001 - 10000).
|
         Note. If you edit the title above or this comment there must be at least
         one space between the quotes and the text. Blank lines are allowed.   "
|
State   random


Bead  W
      0.5
      25
      4.5


Polymer Water    1.0   " (W) "


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    1000
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
```

The runId "nnn" can only contain letters, numbers, - and _

Title, Date and description of run - there MUST be space between text and " "

Initial state type

Bead type definitions
(Name, radius, cons. int., diss. int.)

Polymer (or molecule) type definitions
(Name, number fraction, shape) - note spaces between shape and " "

dmpci.nnn input file

```
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     1000
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
```

Simulation box size/CNT cell size

Bead density, Temperature

RNG Seed and "lambda parameter"

Integration step size

No of time steps, sampling period, etc

Grid size for analysis

Commands to change display

Commands must be time-ordered

Equation of state for DPD water

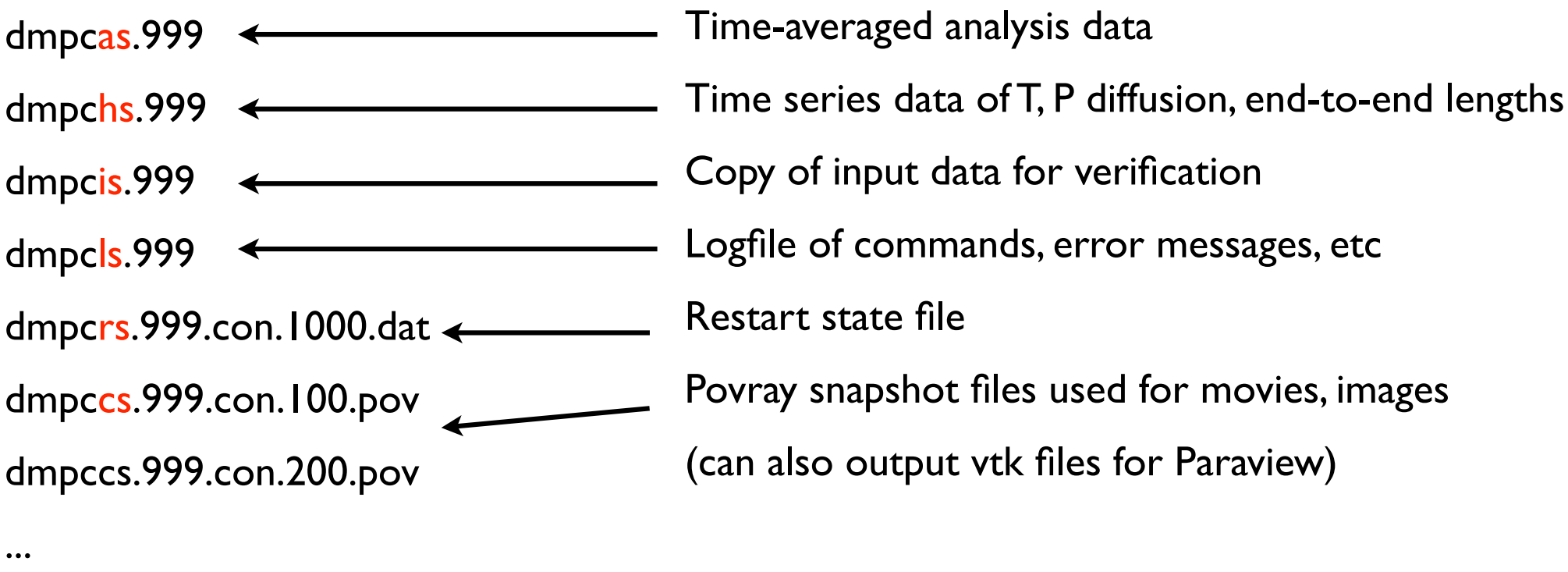
EPFL

To Do:

1. Run a simulation of pure water in a 10^3 box for 10^4 steps
2. Set T = 1, a_{ij} = 25
3. Sample every 100 steps, set DisplayPeriod = 1000 to check progress
4. Vary bead density ρ from 3 - 25 and note the resulting temperature and pressure in the dmpcas file
5. Plot P(ρ) and try to find an expression to fit the curve, extract $B_2(T)$.
6. What is the DPD EOS for water?
7. (Optional: vary aww between 25-100 and repeat steps 4-6.)

Output files

The code produces a set of output files: they all start with "dmpc" and have a suffix identifying the data they contain and the same extension as the input file.



Files produced repeatedly (display and restart states) have time encoded in their names.

When is a simulation equilibrated?

EPFL

History State File - dmpchs.nnn

The History file contains information on the time evolution of observables; we use this to determine if the simulation is in equilibrium, unstable, or if numerical errors are large.

To Do:

1. Run a simulation of pure water in a 10^3 box for 10,000 steps, sample every 10 steps
2. Plot time series of temperature and pressure from the dmpchs file
3. Why are there large fluctuations initially?
4. Increase the temperature to 2 or 3 and repeat. Compare the fluctuations in the observables with the previous case (NB. too high a temperature will destabilise the simulation)

Exercise - Time Series Data

Log State File - dmpcls.nnn

- Sequence of time-ordered information, warning, error messages.
- Shows results of commands executed during a run

History State File - dmpchs.nnn

- Time series data of observables saved at a frequency of: *SamplePeriod*
- Time, Temperature, Pressure, (ignore 4-6), Bead diffusion, Polymer end-end length
- One diffusion column for each bead type defined in input file
- One end-to-end length column for each polymer defined in input file (head and tail beads are defined as first and last in the polymer's shape string)

1000	1.24793	23.9731	0	0	0	0.0394071	0.0461911	0.0210613	0	7.13659
2000	1.01469	23.1952	0	0	0	0.170226	0.084775	0.0428191	0	5.83192
3000	1.01307	23.1768	0	0	0	0.212399	0.0842294	0.0470196	0	5.73367
4000	1.01176	23.1712	0	0	0	0.233601	0.0791722	0.0466596	0	5.66858
5000	1.01094	23.1694	0	0	0	0.246302	0.0741255	0.0450801	0	5.71554

T, P are in dmpcas.nnn file

Use second analysis period

Mean

Std. dev.

Ignore these for now

Mean / Std. dev

```
Time = 10000
Temperature
1.0113735      0.01671942

Pressure
23.741893      0.15968666

CM Mom
-2.4872872e-17 3.3823041e-17
-7.1290559e-17 2.896698e-17
-1.2226453e-17 4.3828009e-17
9.5172672e-17  2.6393749e-17

CM Pos
4.9993607       0.017085057
4.9994945       0.019554523
5.000931        0.018034628
8.6601654       0.01997787

Stress
20.6903        -0.00642093   0.00504999    0.224169      0.12813       0.131096
-0.00642093    20.7182       0.00368064    0.12813       0.205656      0.121857
0.00504999     0.00368064    20.7148       0.131096      0.121857      0.214418

Stress Spherical
0              0             0             0             0             0
0              0             0             0             0             0
0              0             0             0             0             0

Inertia
66.6698        -24.9941      -25.0036      0.294344      0.153215      0.143393
-24.9941       66.6687       -25.0047      0.153215      0.258729      0.157127
-25.0036       -25.0047      66.6526       0.143393      0.157127      0.285485

Bond Length
0               0

Water EE distance
0               0
```

Density is in the dmpci.nnn file

```
23
24 Box          10  10  10       1  1  1
25 Density      6
26 Temp         1
27 RNGSeed      -26784
28 Lambda       0.5
29 Step         0.02
30 Time         10000
31 SamplePeriod     10
32 AnalysisPeriod   5000
33 DensityPeriod    10000
34 DisplayPeriod    1000
35 RestartPeriod    10000
36 Grid         1  1  1
37
38
39 Command ToggleBeadDisplay              1    W
40 Command SetCurrentStateCamera          1    0.5 -1.0 -0.5  0.5 0.5 0.5
41 Command SetCurrentStateDefaultFormat   1    Paraview
42
43
```

Note that whereas the numerical value of the density is on the same line as the keyword "Density", the temperature and pressure are on the *next line*!

Script to automate plotting the DPD EOS

EPFL

Goal

For this exercise, we'll extract the temperature and pressure from the **dmpcas** file and the bead density from the **dmpci** file, to allow the equation of state to be plotted.

Write a script to extract data from a set of DPD simulation files in the same directory and combine the values in a single file for easy analysis and plotting.

Steps

1. Generate required output files by doing the dpd runs
2. Put all the dmpcas and dmpci files are in one directory
3. Write a script to iterate over the **dmpcas** files, search for the keywords **Temperature**, **Pressure** at a specified time and extract the corresponding values; then iterate over the **dmpci** files and extract the **Density** value and write the numerical values (ρ, T, P) in 3 columns to a new file ordered by the density.
4. Plot the pressure against density and find the equation of state.

Visualising a simulation

The **dmpccs.nnn.con.ttt.pov** (or.vtk) files contain snapshots of the simulation state (x, y, z coordinates and an integer identifying the type of the beads). These can be exported in povray (*.pov) or Paraview (*.vtk) format.

NB. Add the command "ToggleBeadDisplay 1 W" to the dmpci file to make the water invisible otherwise you won't see anything.

Two options for making images and movies:

- Paraview allows import of a sequence of *.vtk files to make movies.
- Or convert the *.pov files into *.gro and *.xtc files, and use VMD to view them (ask me for the script).

The dmpccs files can also be used for off-line analysis that requires particle coordinates as they are written in plain ascii text.

Free visualization software

PovRay is an open-source, ray-tracing programme that allows a scene to be drawn with a fixed camera angle and view

http://povray.org/download/

Advantages: easy to use, high scene details (not very useful for simulations)

Disadvantages: only single snapshots possible, movies require other software, e.g., Quicktime, have to compile it manually on OS X

Paraview is am open-source data analysis and visualization programme

http://www.paraview.org

Advantages: 3d images viewable from any angle, multiple rendering options, movie making, native version for OS X

Disadvantages: harder to use

DPD code exports in both formats

VMD is another option - beautiful graphics/movies, but harder to use

1) Go to: https://www.ks.uiuc.edu/Development/Download/download.cgi

2) Select your platform (linux, Mac OS X, windows)

3) Create a user/pw and agree to license

4) Get VMD bundle and install it

5) Get from moodle

6) Get make-gro.sh, pov2vmd.sh, xtc.zip, and rod9.vmd files from moodle

7) Unzip xtc.zip, and compile povtoxtc on your platform with one of the following commands:

linux: compile.sh

mac OS X: comac.sh

8) Place the resulting pov2xtc executable in your path along with make-gro.sh and pov2vmd.sh from step 6

9) Given a bunch of dmpccs.runid.* files, convert them to runid.gro, runid.xtc with the command:

NB The number of time-steps between snapshots is hardwired to 1000

make-gro.sh. runid 1

10) Invoke vmd on them:

vmd -e ~/path-to-scripts/rod9.vmd runid.gro