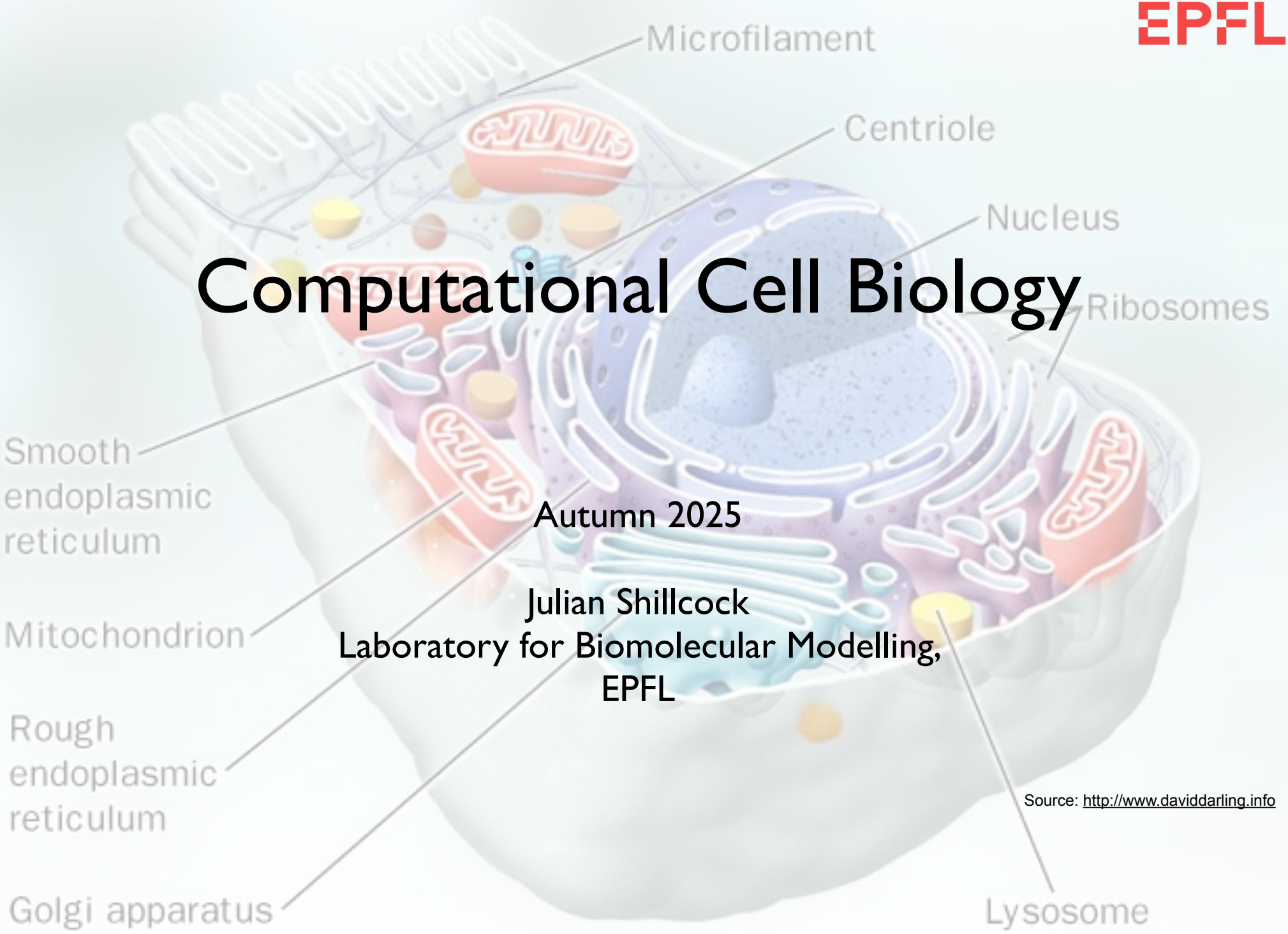
Computational Cell Biology



Source: http://www.daviddarling.info

Autumn 2025

Julian Shillcock
Laboratory for Biomolecular Modelling,
EPFL

What do the following have to do with this course?

the 19th century English poet William Blake

the 19th century Russian writer Dostoyevsky

the American rock band The Doors

Blake

"If the doors of perception were cleansed, man would see everything as it really is: infinite. For man has closed himself up till he sees all things through the narrow chinks of his cavern."

Dostoyevsky

"If the world were rational, nothing would happen."

The Doors

Blake —> Aldous Huxley's book about experience of mescaline "The Doors of Perception" —> band's name

Computer simulations are *rational* instruments of exploration

they don't contain what is out there in Nature

only what is in our mental model

(if you extend this to all of science, it becomes a controversial statement …)

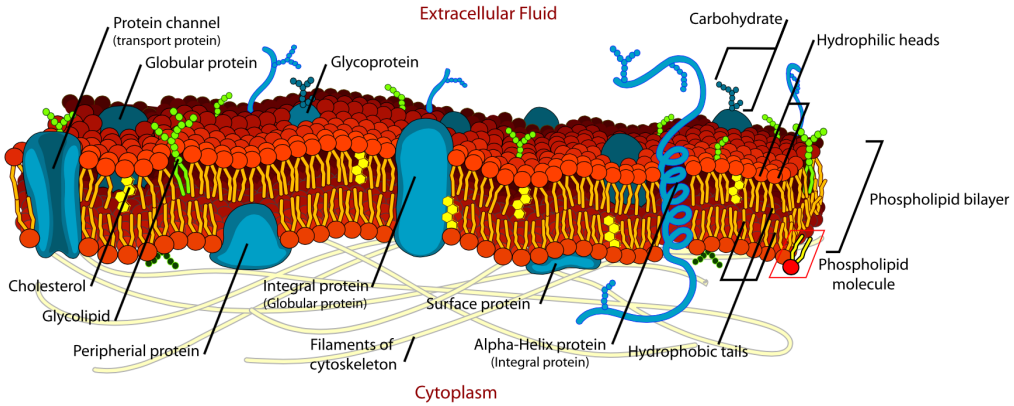
For ***some*** purposes, ***some*** details are enough to predict an experiment

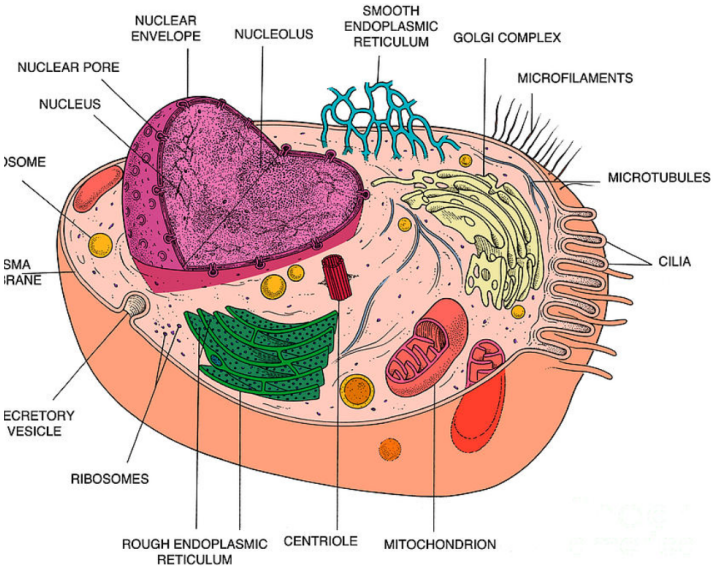
Computer simulations embody our assumptions about a part of the world in a way that we can test:

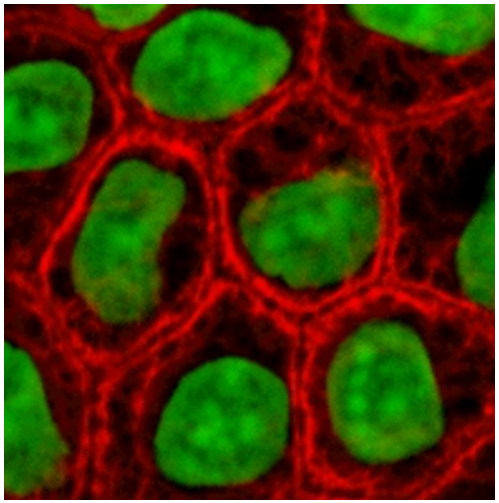
e.g., Disordered protein ~ polymer ~ random walk $\asymp \langle R_{ee}^2 \rangle = N\, a^2$

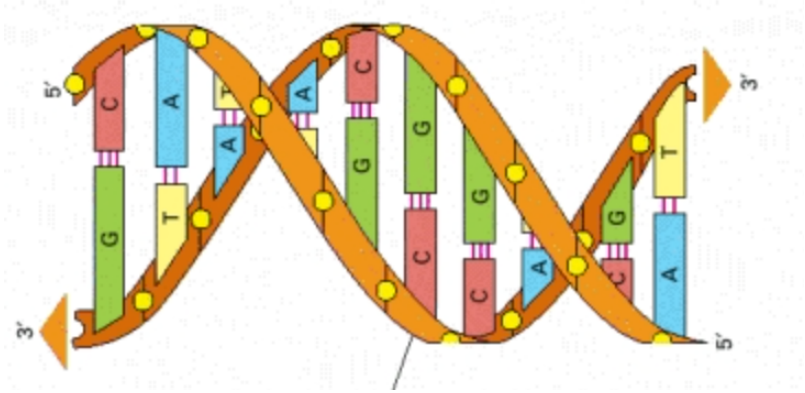
What is your picture of a cell?

It is probably static, based on textbook figures:









These misrepresent the reality - they ignore the **Equipartition Theorem** - everything is moving - it costs energy to keep things still; what are the important degrees of freedom?

Core Concepts

Our mental picture of a cell and cellular functions is probably inaccurate

What must we take into account in understanding cell biology?

Today:

Phenomenon of Liquid-liquid phase separation of proteins is used by cells to create compositional gradients that localise biochemical functions

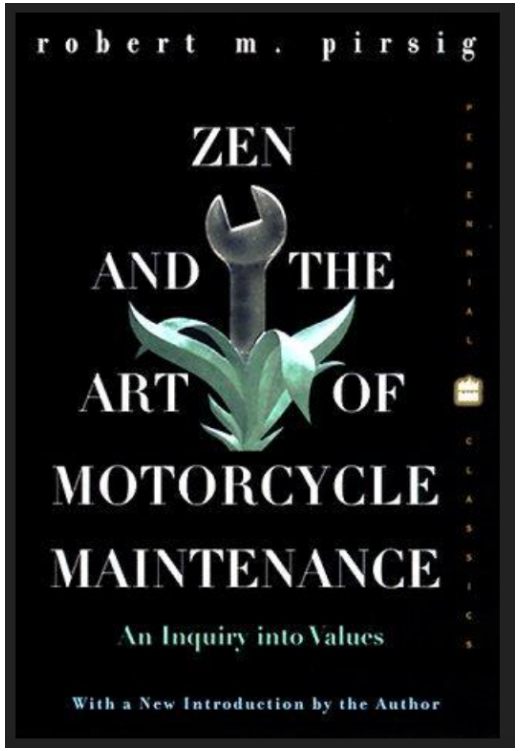
e.g., RNA translation, DNA repair, synapse formation, measles virus to reproduce, etc.

gradients are life!

name some

...

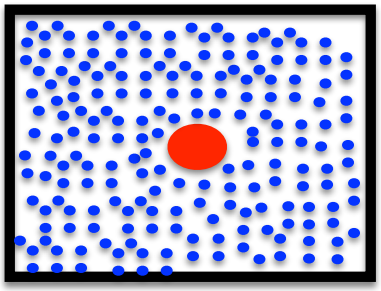
How do we *cut* into a cell



Robert M Pirsig

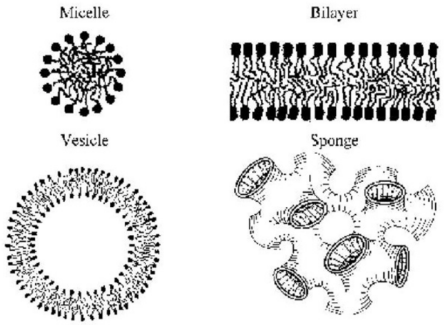
Analytic: $(P + aN^2/V^2)(V - Nb) = Nk_BT$

Numerical: $x(t + dt) \sim x(t) + v(t) * dt + O(dt^2) + \ldots$

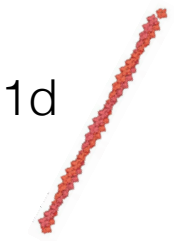


Simulation

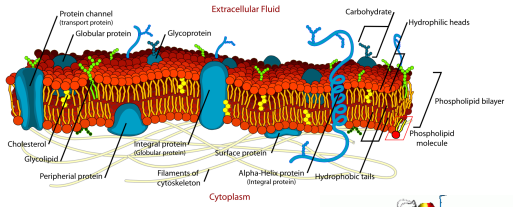
Phase



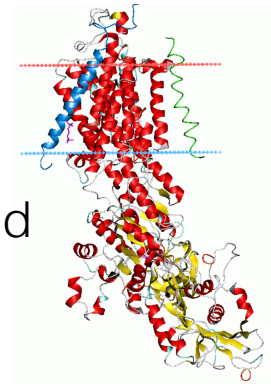
Dimensionality



$\langle X^2 \rangle = 2\,d\,D\,t$

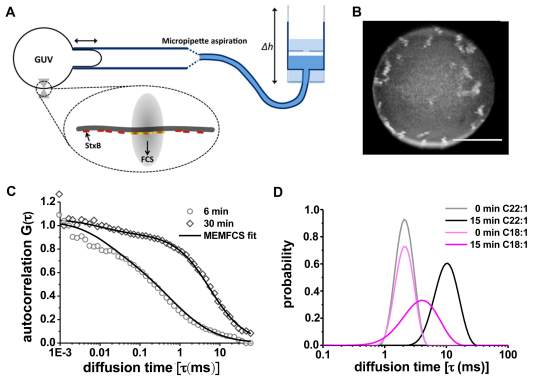


2d



3d

Analysis



Experimental

or length scale,
time scale,
energy scale,
…

What's behind the surface?

Axis: Analysis - Q&A

Biology - experiments, the ground truth, this is what's ***there*** in Nature.

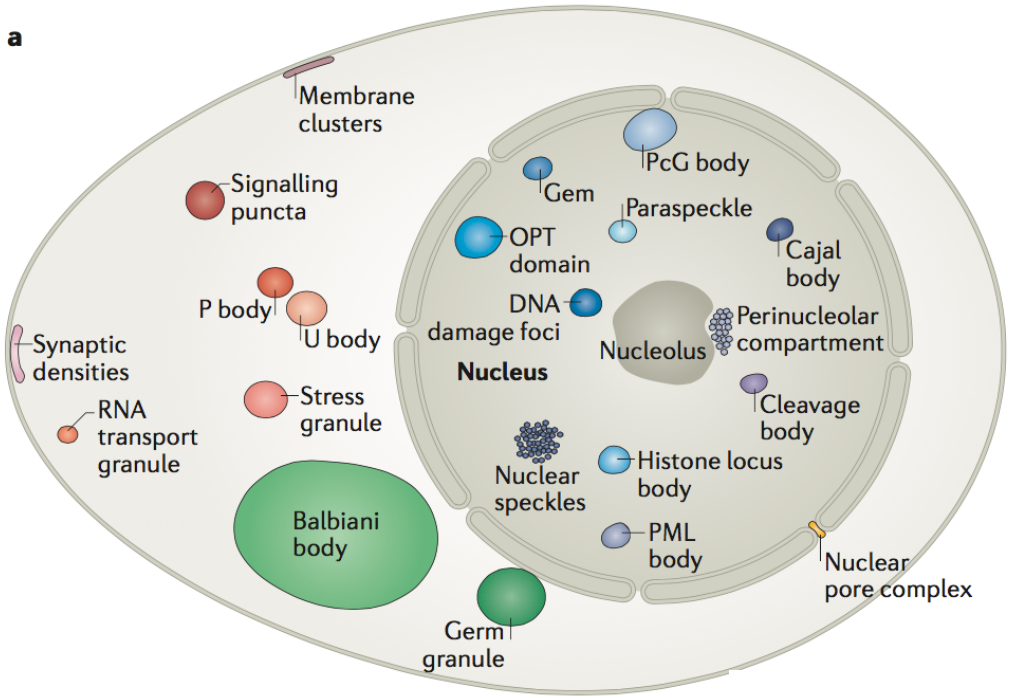
But it's complicated, limited by technique (may kill the cell or freeze it), space or time resolution, and we usually need simpler systems to learn from.

Mathematical - this is how we measure things, predict simple structures and motions. Usually too simple or too hard.

Simulation - gives us virtually unlimited access to any measurable quantity in a system, complete time evolution, what-if experiments but still very expensive in computer and real time for biological-size systems.

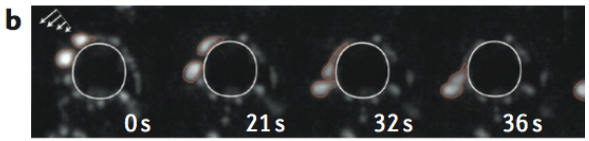
We can get behind the surface of the experiments

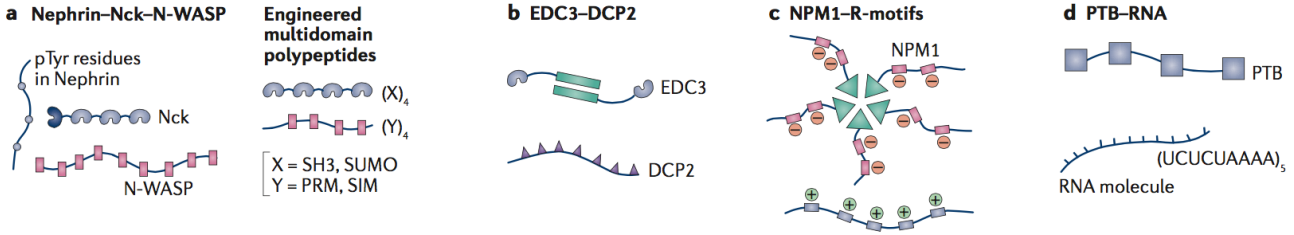
Biomolecular condensates are a new (*bizarre*) phase of cellular matter - *how do we understand them?*



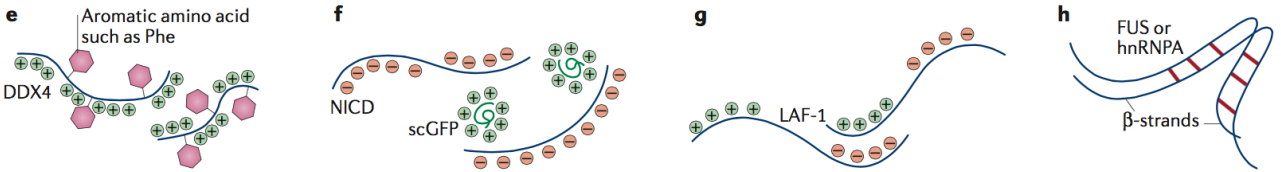
Formed of long, flexible proteins (Intrinsically-Disordered Proteins) that have ~ no secondary structure in solution

phase separate via multiple, distributed, weak binding sites

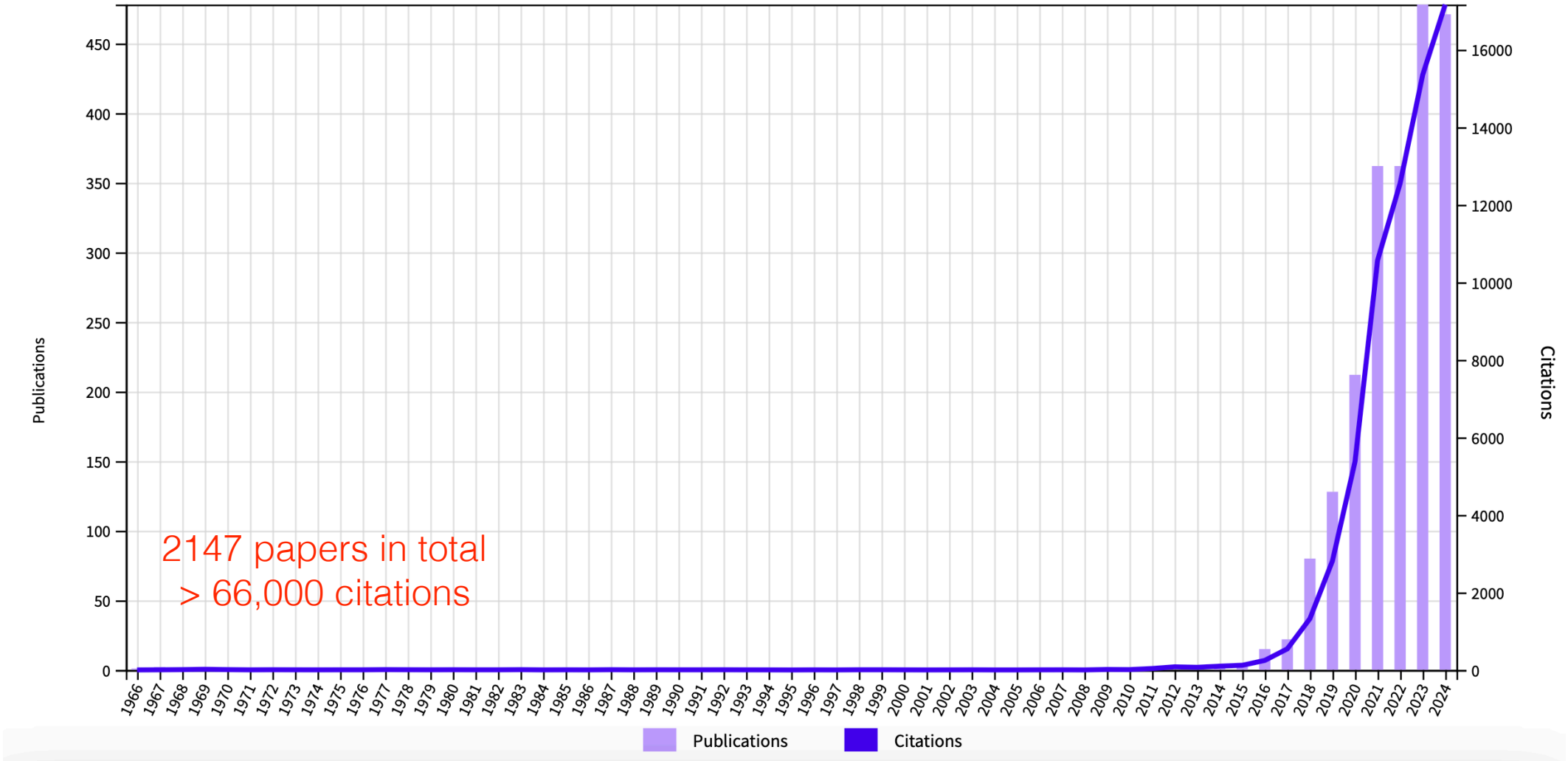




S. F. Banani et al. Biomolecular condensates: organizers of cellular biochemistry, Nat Rev. Mol. Cell Biol. 18:285 (2017)



papers on biomolecular condensates/membraneless organelles



Source: Web of Science, Clarivate

What is your picture of the *Central Dogma?*

DNA —> RNA —> Protein

How do we think about this process?

RNA polymerase *walks* along DNA *transcribing* it into mRNA which is used by ribosomes to make proteins….

But … it turns out …

Nuclear proteins exert ***forces*** on chromatin that influences DNA transcription; the mechanical properties of DNA and chromatin become vital for transcription.

Phase separation of proteins pulls together distant regions of chromatin and excludes other, non-targetted region, they help regulate transcription; so the *physical properties* of chromatin are used to guide transcription.

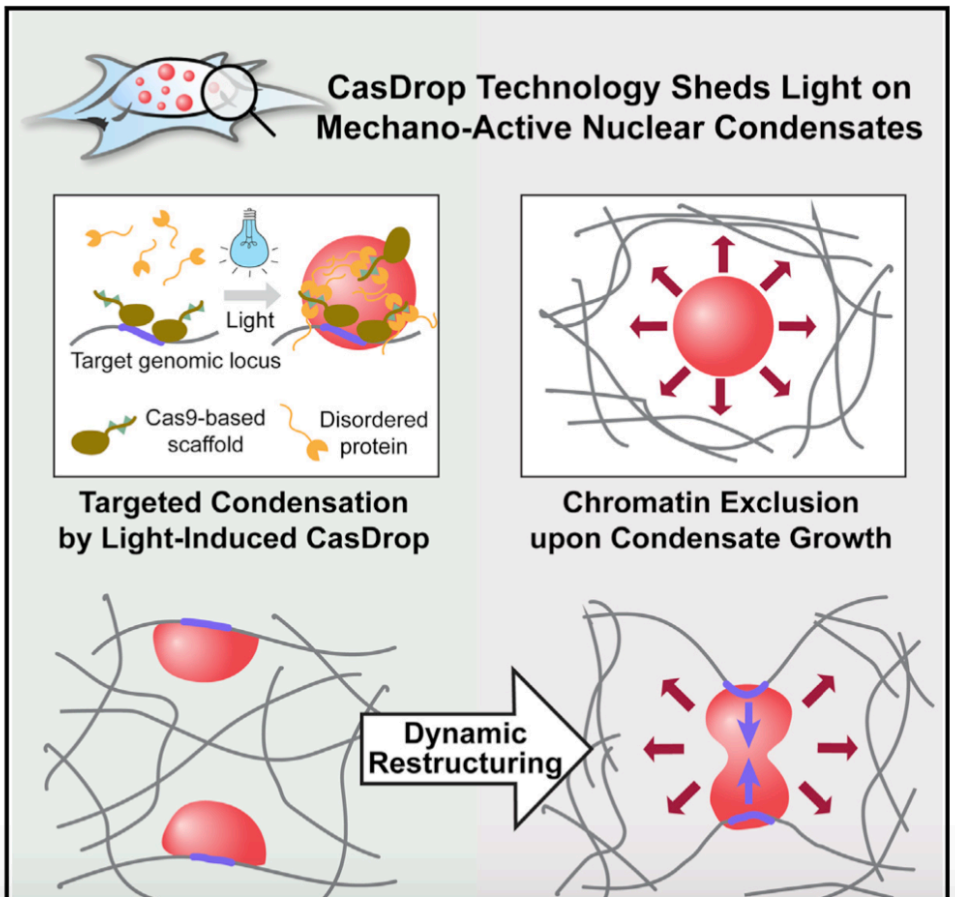
… recall bacterium using rigidity of STxB toxin to generate a membrane-mediated force that drives clustering and invagination

Cell

EPFL

Liquid Nuclear Condensates Mechanically Sense and Restructure the Genome

Graphical Abstract



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In Brief

Nuclear condensates physically pull in targeted genomic loci while excluding non-targeted regions of the neighboring genome.

Y. Shin et al. Cell 175:1481 (2018)

Received: 3 May 2019 | Revised: 3 October 2019 | Accepted: 6 October 2019 | Uncorrected manuscript published: 13 October 2019
DOI: 10.1111/tra.12704

REVIEW

Traffic WILEY

Biomolecular condensates in neurodegeneration and cancer

Stephanie Spannl[1] | Maria Tereshchenko[1] | Giovanni J. Mastromarco[1] | Sean J. Ihn[1] | Hyun O. Lee[1,2]

Protein phase separation and its role in tumorigenesis

Shan Jiang[1], Johan Bourghardt Fagman[2], Changyan Chen[3], Simon Alberti[4]*, Beidong Liu[1,5]*

Abstract Cancer is a disease characterized by uncontrolled cell proliferation, but the precise pathological mechanisms underlying tumorigenesis often remain to be elucidated. In recent years, condensates formed by phase separation have emerged as a new principle governing the organization and functional regulation of cells. Increasing evidence links cancer-related mutations to aberrantly altered condensate assembly, suggesting that condensates play a key role in tumorigenesis. In this review, we summarize and discuss the latest progress on the formation, regulation, and function of condensates. Special emphasis is given to emerging evidence regarding the link between condensates and the initiation and progression of cancers.

Jiang *et al.* eLife 2020;9:e60264. DOI: https://doi.org/10.7554/eLife.60264

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*[,∥]

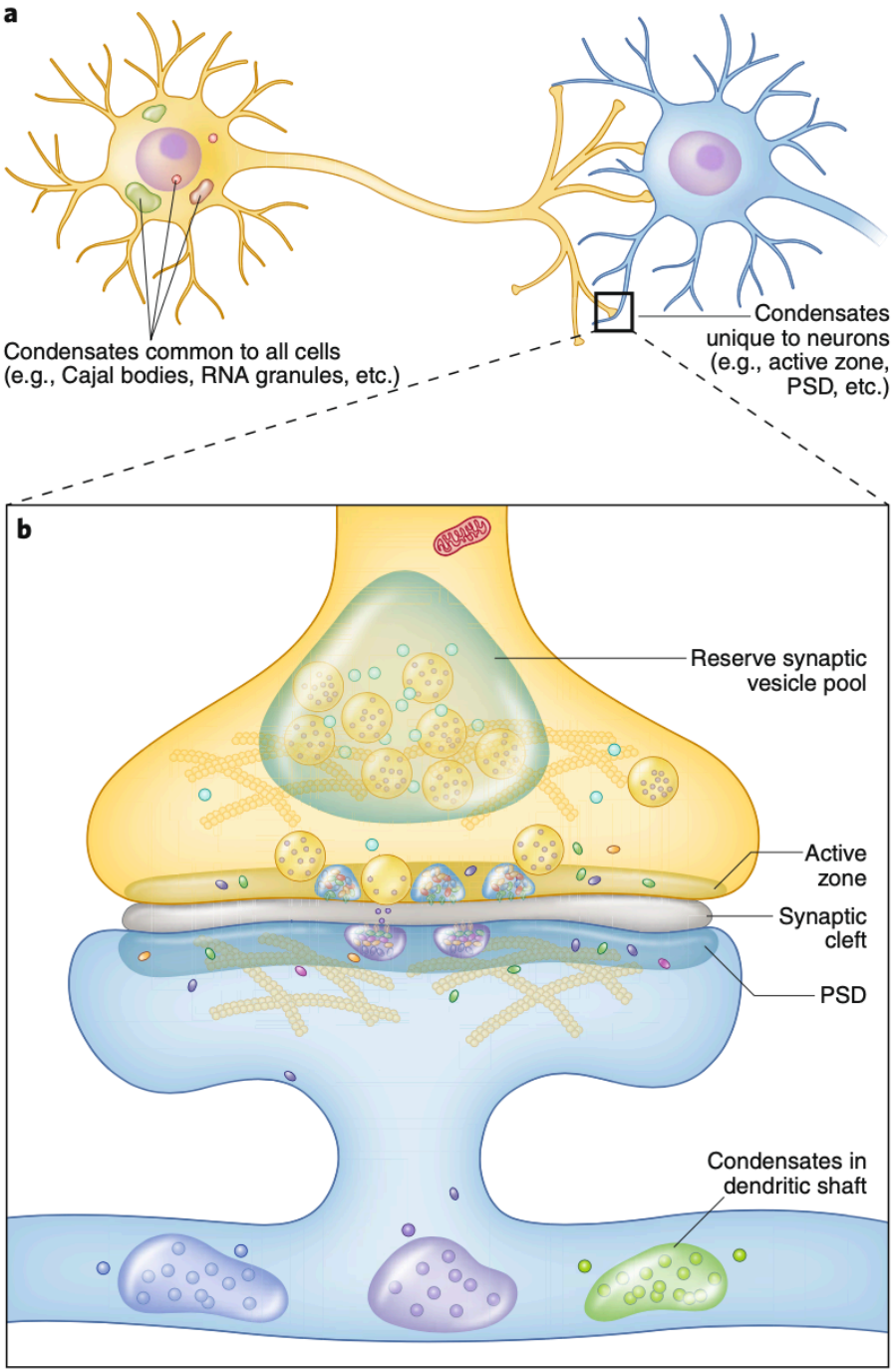
ABSTRACT: The two hallmarks of Alzheimer's disease (AD) are the presence of neurofibrillary tangles (NFT) made of aggregates of the hyperphosphorylated tau protein and of amyloid plaques composed of amyloid-β (Aβ) peptides, primarily Aβ1−40 and Aβ1−42. Targeting the production, aggregation, and toxicity 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)

Phase separation in neurons:

- in the nucleus and cytoplasm[1]
- in axons where it clusters vesicles[2]
- in dendrites where receptors aggregate and bind neurotransmitter[3]



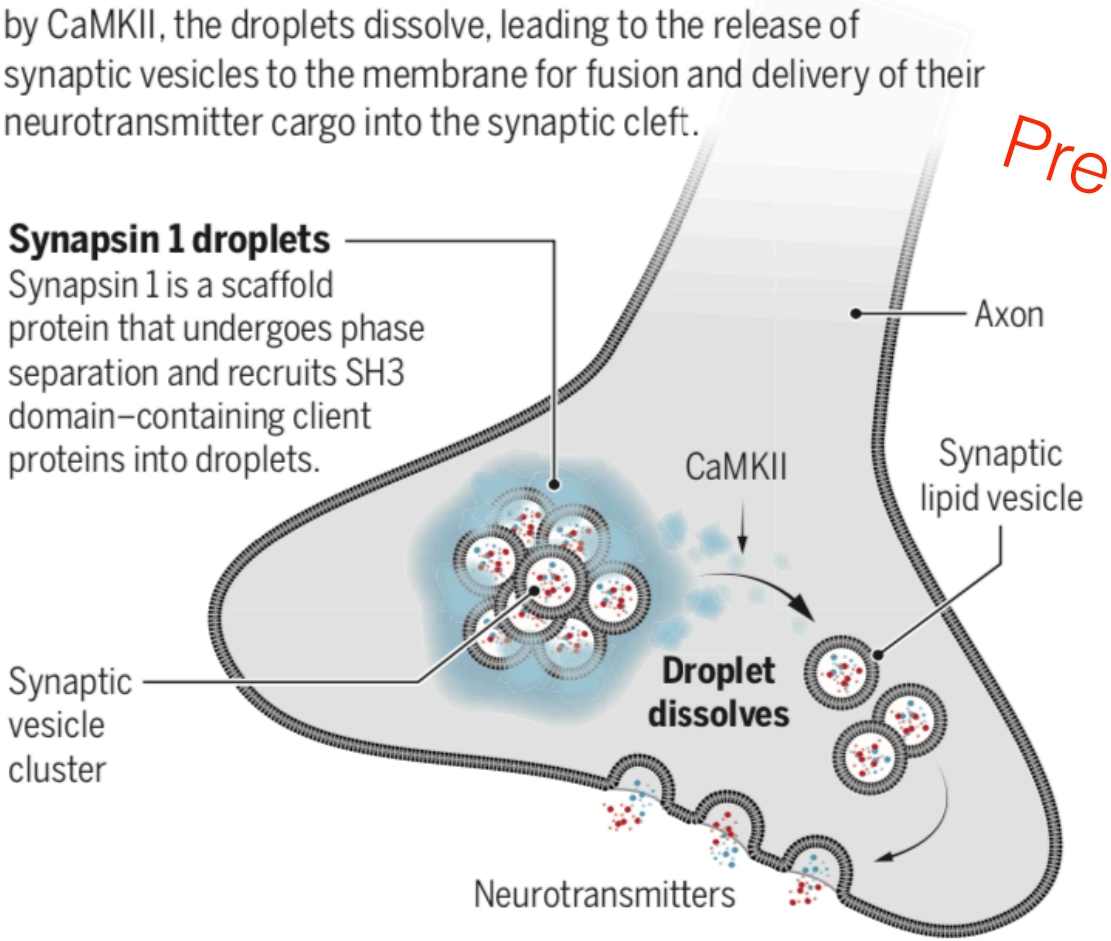
1 Alberti and Hyman, Bioessays 38:959 (2016)

2 Milovanovic et al. Science 361: 604 (2018)
Wu et al. Molecular Cell 73:971 (2019)

3 Chen et al. Nature Neuroscience 23:301 (2020)

Clustering of synaptic vesicles

Synaptic lipid vesicles are proposed to be clustered together by partitioning into synapsin 1 droplets. Upon synapsin 1 phosphorylation by CaMKII, the droplets dissolve, leading to the release of synaptic vesicles to the membrane for fusion and delivery of their neurotransmitter cargo into the synaptic cleft.

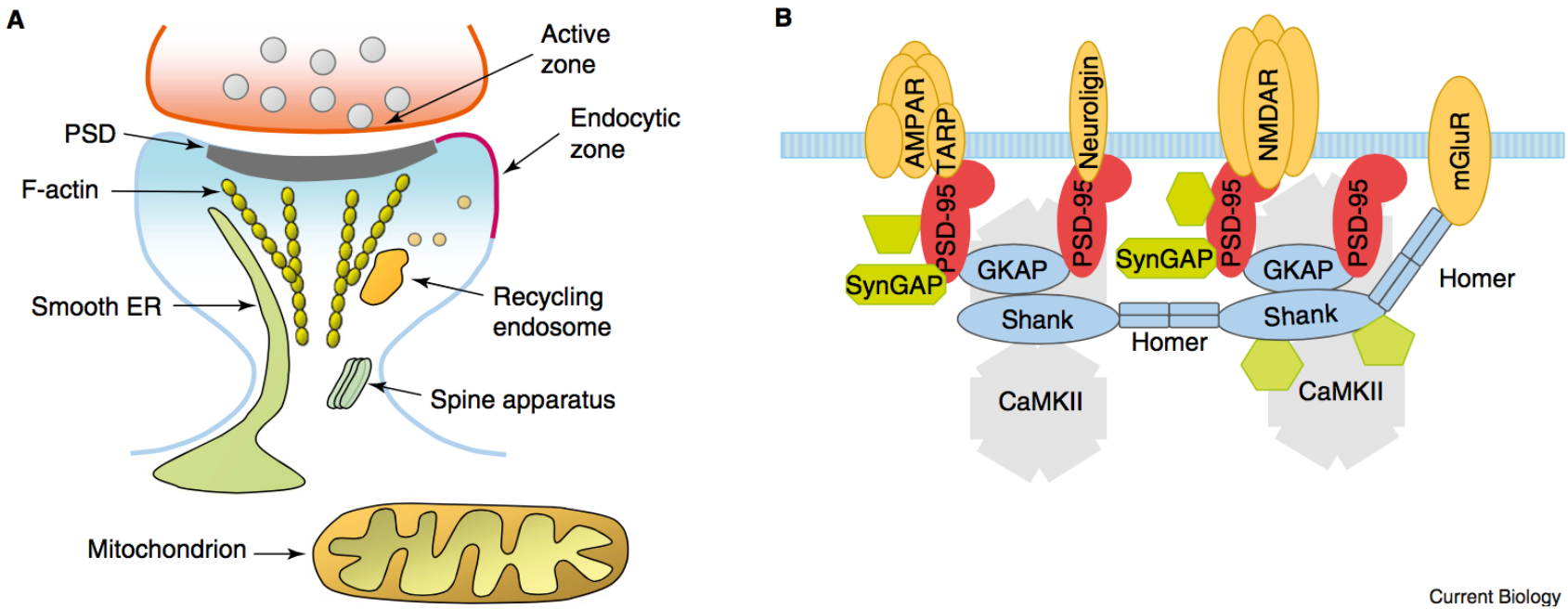


Pre-synaptic phase separation

Boczek and Alberti, Science 361:548 2018

Synapses connect neurons

Current Biology Vol 19 No 17
R724

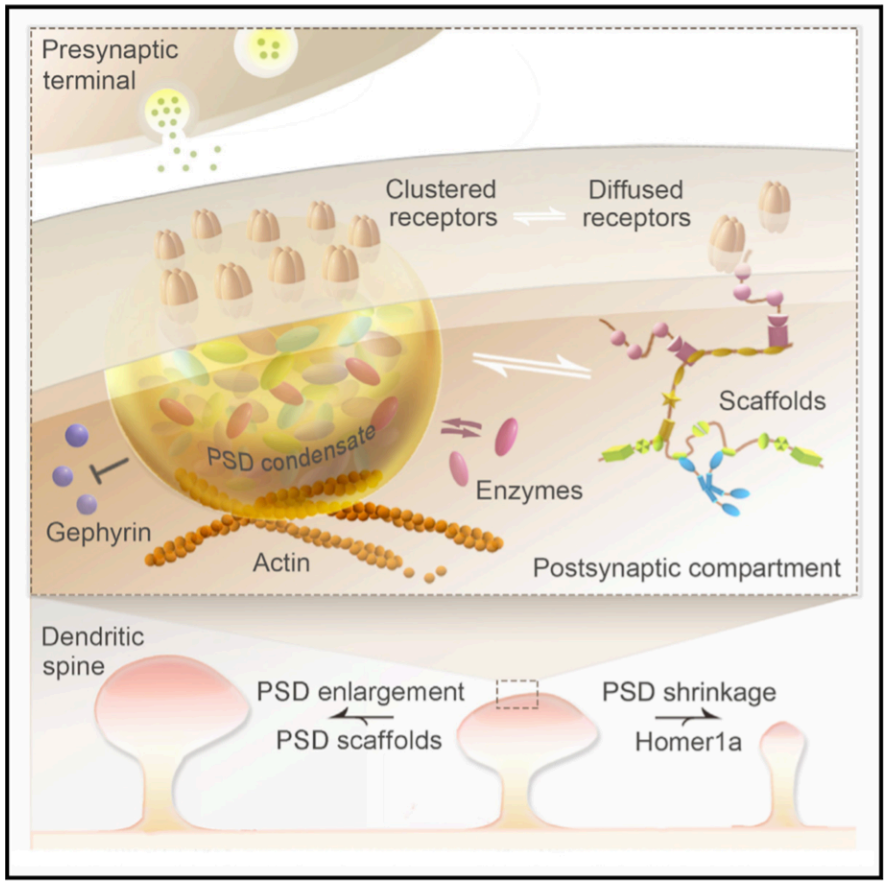


PSD is ~100s nm wide x 30 - 50 nm thick, membraneless organelle containing proteins:

Major components:
- ~ 5600 - CamKII family
- ~ 400 - PSD-95 family (PSD-95, PSD-93, SAP97, SAP102)
- ~ 360 - SynGAP
- \+ lesser numbers of SAPAP, Shank, Homer, AMPA, NMDA receptor subunits.

PSD transduces the electrical signal from the pre-synaptic axon into electrical and biochemical changes in the post-synaptic dendrite; involved with learning and dsyfunction leads to mental disorders

Post-synaptic phase separation



- Biochemical reconstitution reveals PSD assembly formation via phase separation
- The ePSD condensates cluster NMDA receptor and promote actin bundle formation
- The ePSD condensates selectively enrich SynGAP and actively exclude gephyrin
- The ePSD condensates can be modulated by activity-dependent protein modifications

Zeng et al. Cell 174:1172 2018

Zeng et al. construct an artificial synapse from 4 proteins that is able to selectively recruit/exclude other proteins and activate actin filament growth

Purpose?

Why could droplets of IDPs be useful to the cell?

- Compartmentalise biochemistry/sequestering/temporary protection
- Separate form from function (no need to encode "droplet forming" for all proteins)
- Are reversible unlike oil/water or lipid membrane but like water/ice
- Form/dissolve "freely" via diffusion and so reduce energy consumption (ATP)

(cp. Water H-bond network ⇒ Hydrophobic effect ⇒ Self-assembly of lipid membranes

Packing frustration of sat / unsat lipid / cholesterol in membrane ⇒ liquid ordered phase)

Do **thermodynamic** properties of proteins as polymers - not their sequence specificity - drive droplet formation?

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

Liquid phase condensation in cell physiology and disease,
Shin and Brangwynne, Science 357:1253 (2017)

Functional implications of Intracellular Phase Transitions,
Holehouse and Pappu, Biochemistry DOI: 10.1021/acs.biochem.7b01136 (2018)

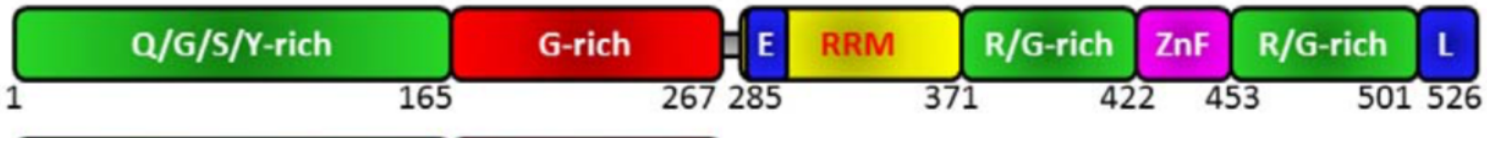
Intrinsically-Disordered Proteins

IDPs are common but behave differently to folded proteins - human proteome has 30 - 50% disordered residues (mainly E, K, P, Q, S) *

- IDPs are long, flexible chains of amino acids (just like proteins that fold)
- Contain regions or domains of low complexity (repeated amino acids)
- No unique folded state/no average shape/no mean atom coordinates
- Mechanically floppy, fluctuate due to random thermal motion
- Multiple weak, non-specific binding sites
- Transform in disease/ageing into rigid fibrils

* C. J. Oldfield and A. K. Dunker, Ann. Rev. Biochem. 83:553 (2014)

Fused in Sarcoma (FUS)



Low complexity NTD

Full-length FUS phase separates

FUS LC phase separates

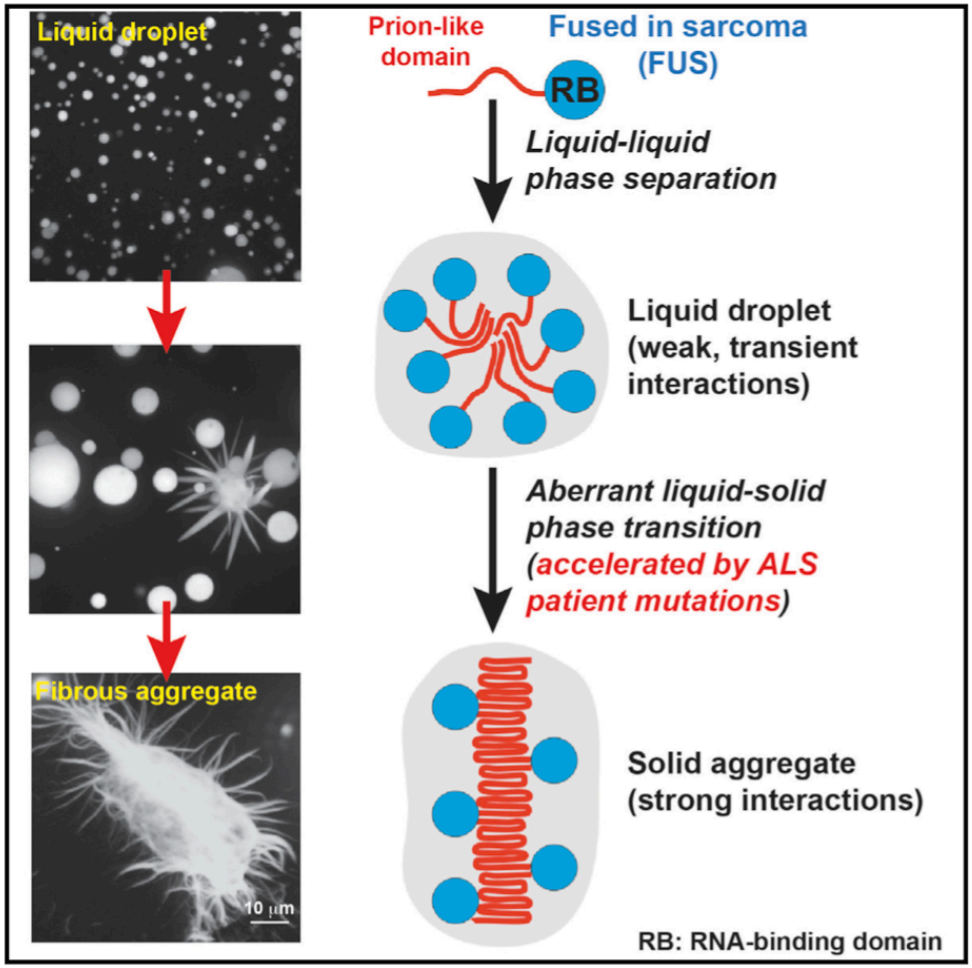
FUS \ LC does not separate

Soluble FUS undergoes LLPS into droplets

FUS droplets make pathological transitions to a rigid state in disease

Reversible = healthy
Irreversible = disease

How do proteins make this phase transition?
Why do so many proteins do it?



A. Patel et al., Cell, 162:1066 (2015)

Experimental observations of biomolecular condensates

Little/no secondary structure in condensed phase; IDP interactions are transient, weak, *multivalent*
(Burke et al. Mol. Cell. 60:231 2015)

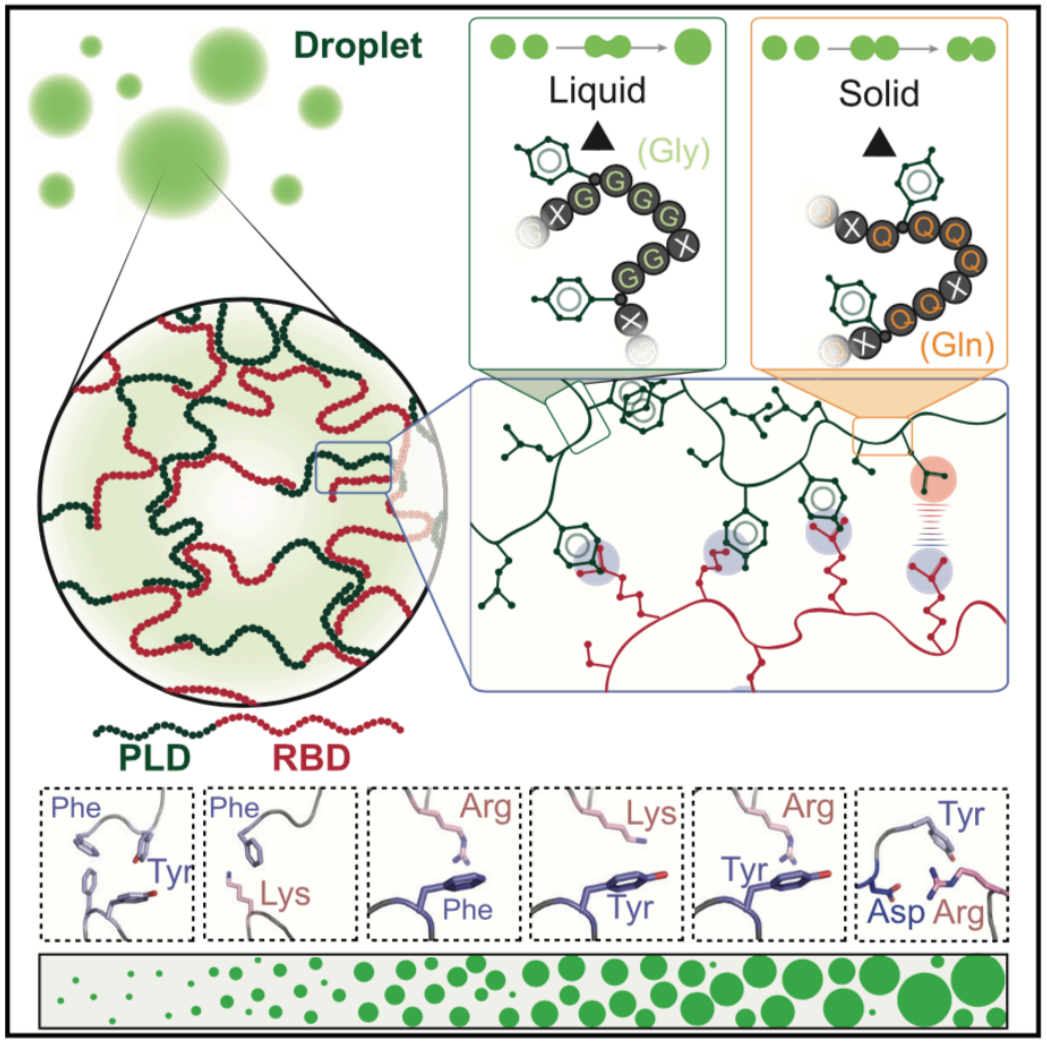
Translational diffusion is much slower (x10-100) than in bulk (Burke, 2015)

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

Disordered NTD and CTD are required for FUS LLPS

Saturation concentration decreases with increasing Arg-Tyr content

(Wang et al. Cell 174:1 (2018)



J. Wang et al., Cell, 174:688 (2018)

How can we understand the phase separation of IDPs?

build a model …

Simulations embody a theory

What could be driving long, flexible IDPs to aggregate?

Obviously - their interactions - but these interactions are weak - How do they give rise to phase separation?

Consider the range of inter-molecular forces

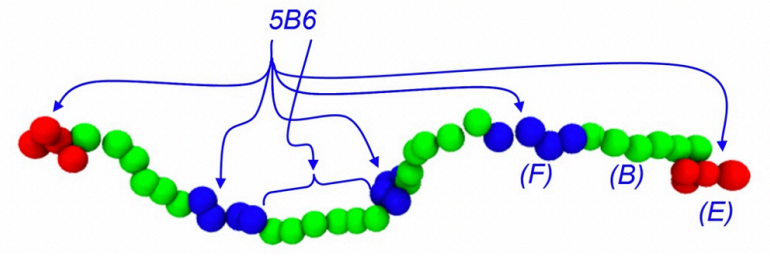
Ideal Gas - molecules only have position and velocity (KE) ⇒ fills available volume - no forces

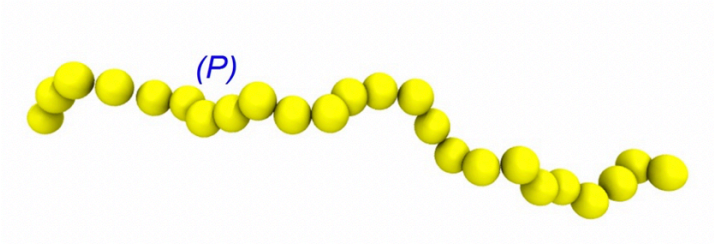
Van der Waals gas - position, KE, PE ⇒ fills available volume unless temperature is reduced when it undergoes a phase transition into liquid - attractive forces

Liquid crystal - translational and orientational freedom at low density, but loses orientational freedom at high density - repulsive/steric forces, entropy transition

Flexible polymers - mix unless repulsive ⇒ Flory Huggins phase separation into dense phase

Flexible polymer mixture with binding sites - trans. / orient. freedom unless binding ⇒ phase separation into droplet without strong repulsion?





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

$(48\ nm)^3$
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)

Oil molecules with sticky patches?

The IDPs are NOT strongly repelled from water (few hydrophobic residues), but they form membraneless organelles with a small surface tension.

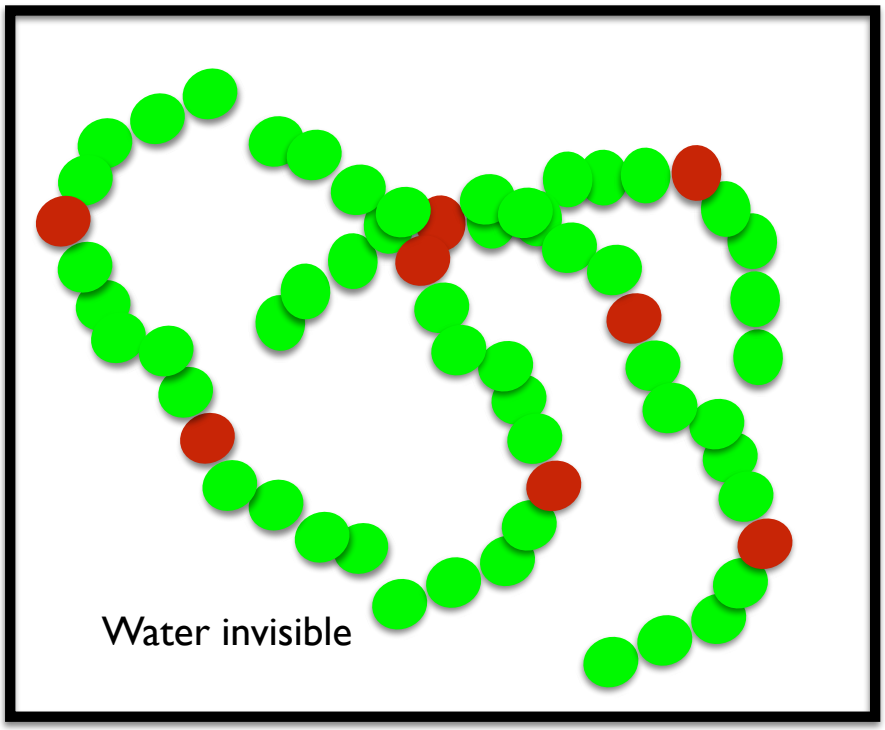
BUT IDPs have multiple, weakly-attractive binding sites.

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

This suggests we might model them as a phantom chain …. see Lecture 2

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

with *sticky* patches.



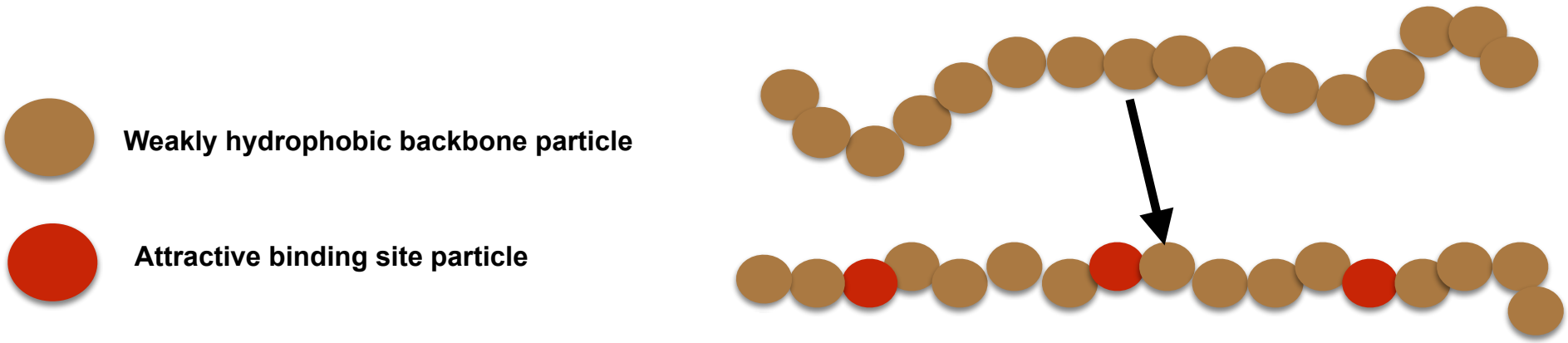
IDPs are not (very) hydrophobic, so we model them as hydrophilic polymers with sticky patches

Think - Pair - Share
5 mins.

Consider a polymer solution in which weakly hydrophobic polymers are immersed in water, but have no specific attraction for each other. Hydrophobicity drives them to form a droplet.

Is it possible for the droplet to *expand* if the polymers have *attractive* binding sites between each other?

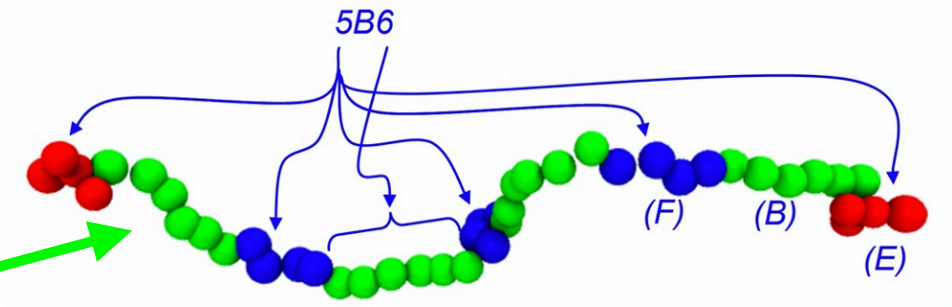
or, can sticky polymers form larger droplets than non-sticky ones?



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



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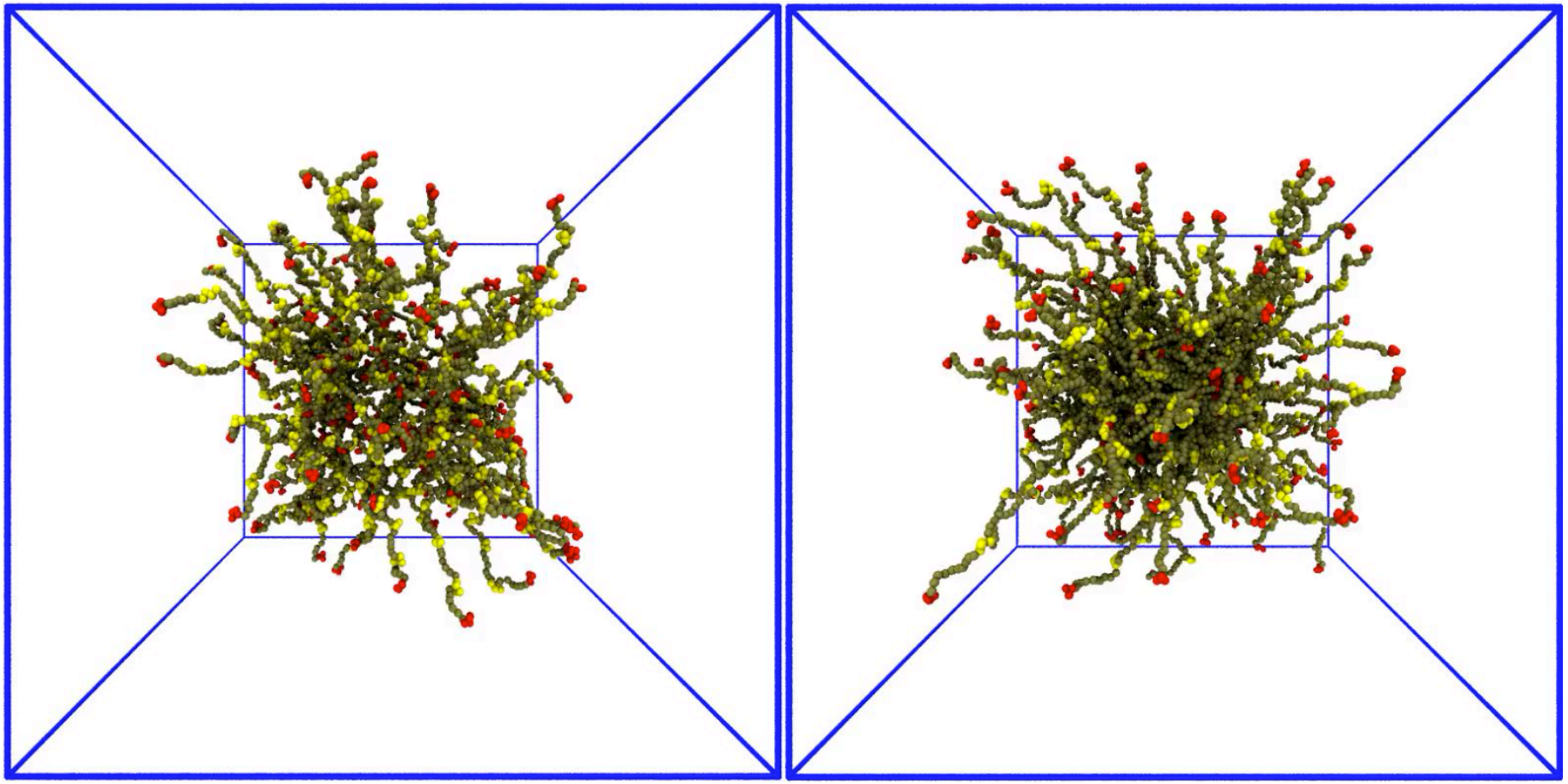
6B10



Model IDPs swell in dense phase

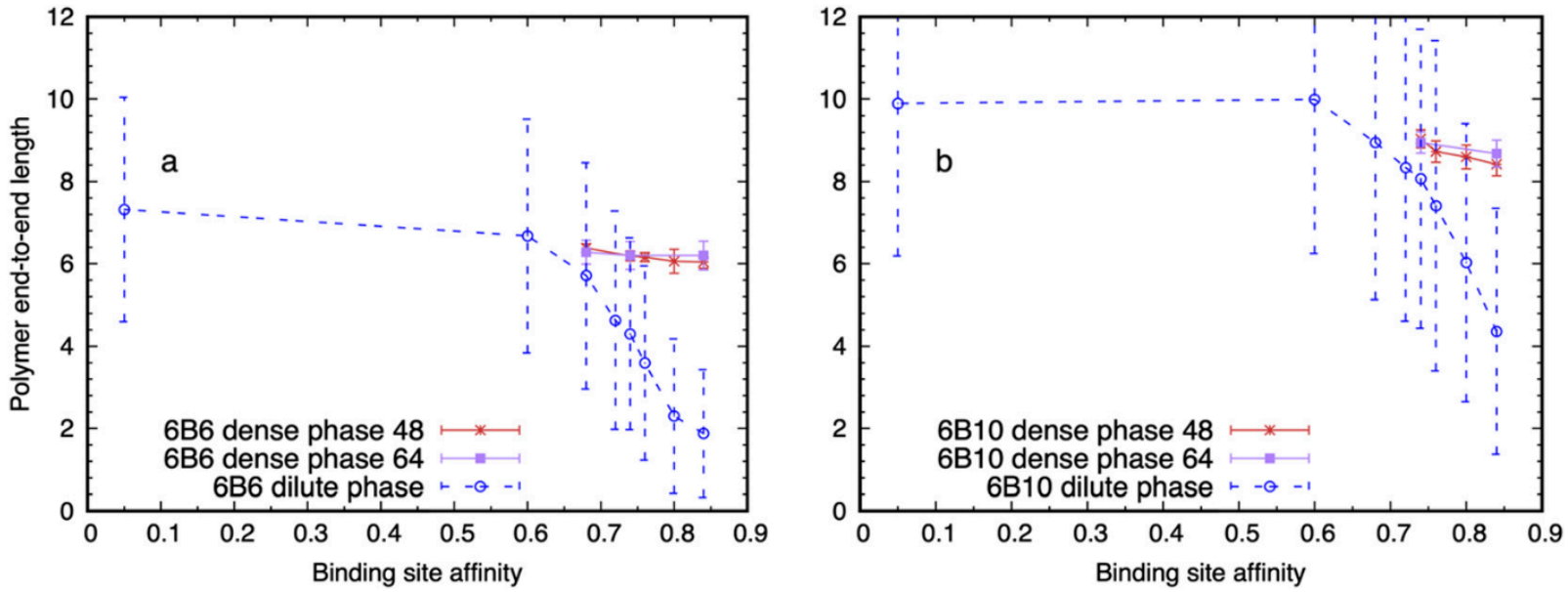
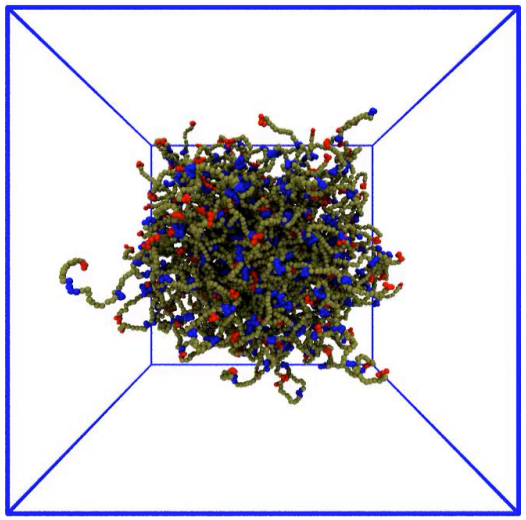


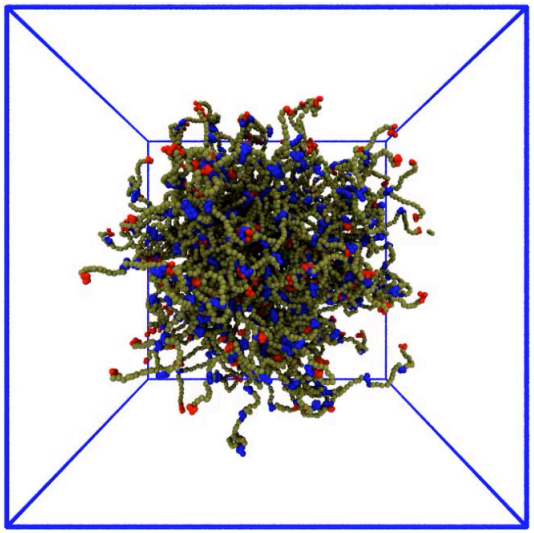
Fig. 4 (a) Variation of the end-to-end length (L_{ee}) of a polymer of type 6B6 in the dilute phase (dashed blue curve) and dense phase (red and purple curves for box sizes 48 and 64 respectively) with the binding site affinity in the range $\varepsilon = 0-0.84$. (b) Same as (a) for a polymer of type 6B10. Note that the dense phases are only stable for affinities starting at the first data point shown, which occurs when the polymer's mean size in the dilute phase drops below that in the dense phase. Error bars are the standard deviation of the distributions, illustrating the large fluctuations in the dilute phase and much smaller fluctuations in the dense phase. The zero affinity points have been displaced along the abscissa for clarity.

Shillcock et al. Soft Matter 18:6674 (2022)

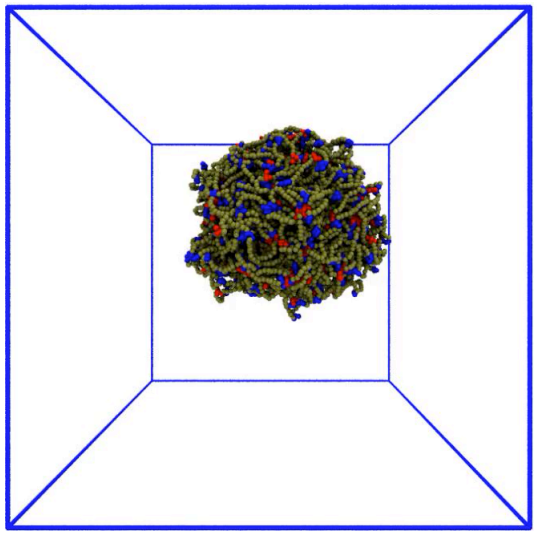
For weak affinity, phase separation is assisted by crowding



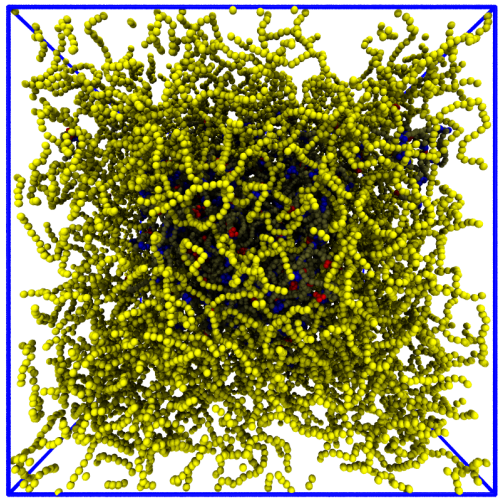
No crowder,
190 6B10 IDPs

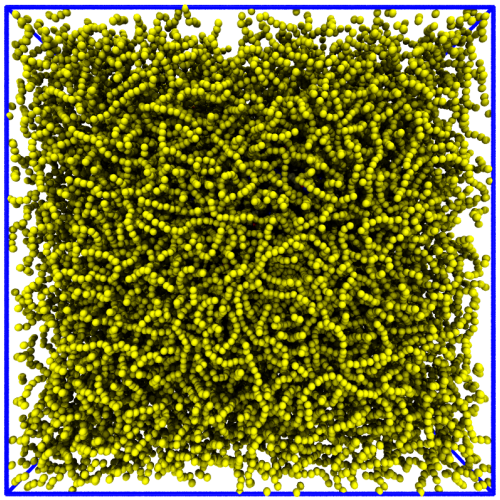


361 crowders P48



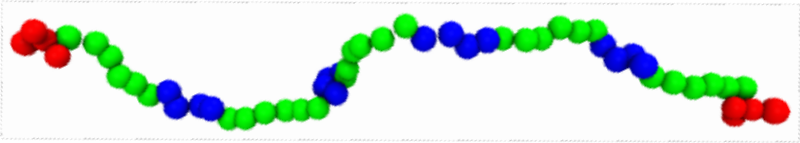
1071 crowders P48



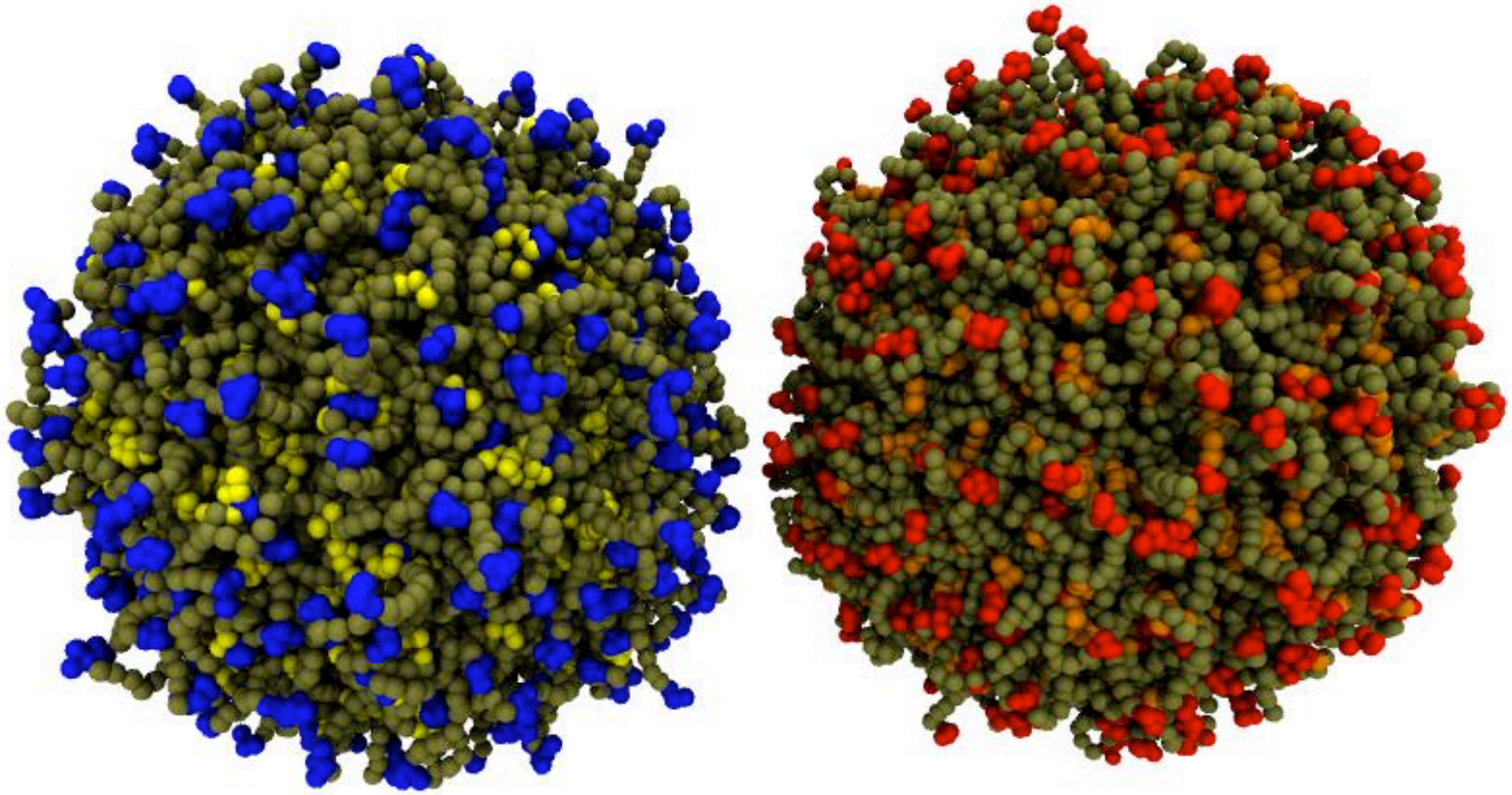


(not movies)

We can simulate sticky IDPs



EPFL



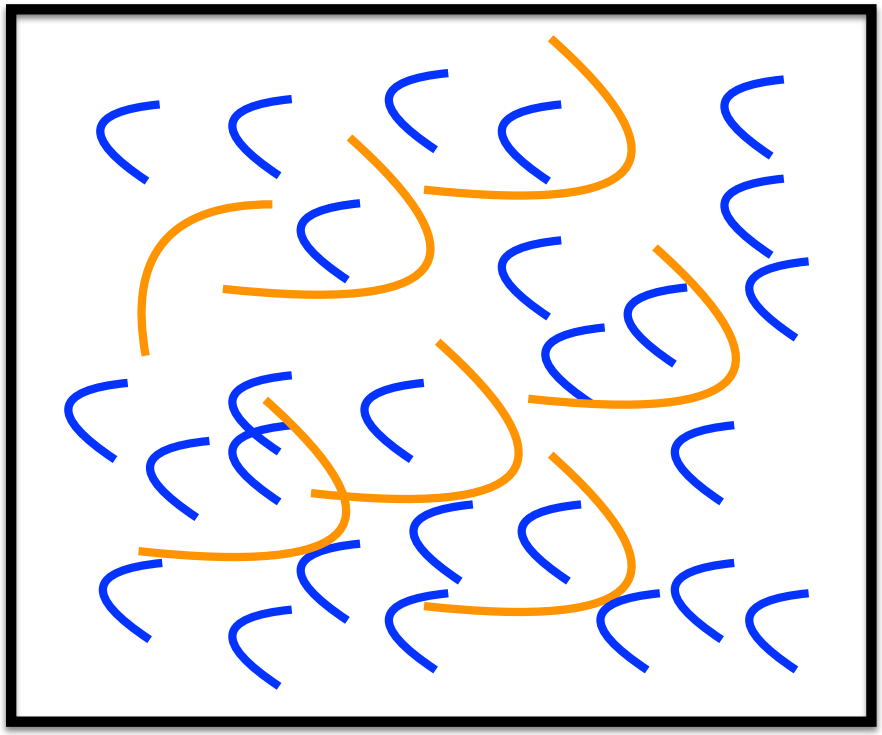
But what does it tell us?

Polymer phase separation

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

Do they mix? Do they phase separate?

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



Assume: composition, V, T are constant.

Helmholtz free energy is:

$$F = U - TS$$

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

How do we find U and S? *Next week … Flory Huggins theory of polymer mixtures*

Summary of lecture

Simulations provide a rational view of a fundamentally mysterious Nature; (they have finite size, finite resolution, approximations, …)

They let us peer behind experiments and manipulate our mental models to generate predictions, test what-if scenarios

Asking the right questions is as important as finding the answers, and more important than answering the wrong questions

Liquid-liquid phase transitions are increasingly recognised as an important cellular mechanism for homeostasis, synapse function, disease, measles, …

There are probably more new phases and mechanisms that Nature uses in maintaining living cells

Break
10 minutes

Deadlines

Read the Project tips document (and example reports) on today's moodle - style matters

In the final lecture on 17th December: each group gives a brief (~5-15 min.) presentation description of your project (with a few slides to get the basic aim across, but don't need to have final results) so we can all share in the work of the others.

Is everyone here on 17th December???

Each person in a group has to write their own report, but you can share graphs/ figures/data.

Final acceptance date for reports: Friday, 17th January 2026 (end of day)

Final grade submission date: 9th February 2026