Biomolecular condensates: a new paradigm for subcellular organization?

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GSK Core Course Lecture
November 17th, 2025

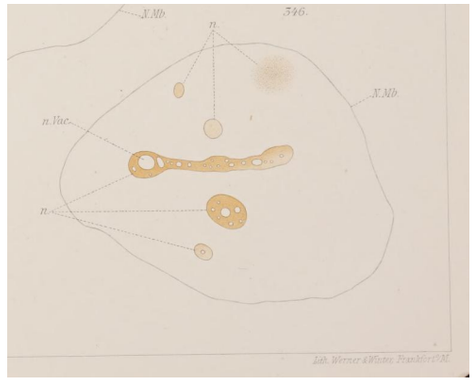
Key lessons to take away from today

When new paradigms run up against old dogma

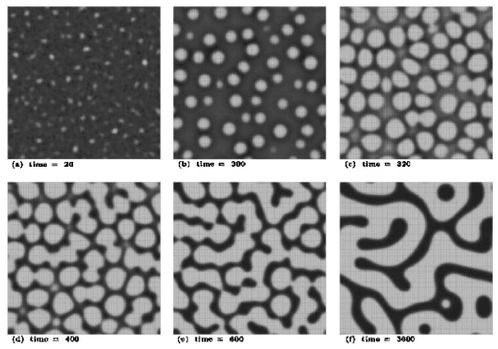
I. What can we learn from physical models of phase separation as it applies to condensate function?
II. What are the limitations of these models?

Balanced viewpoint of the field

Discovery of nucleoli, canal bodies, germ granules…
1800s



Nucleoli show liquid features
TSH Montgomery
1898



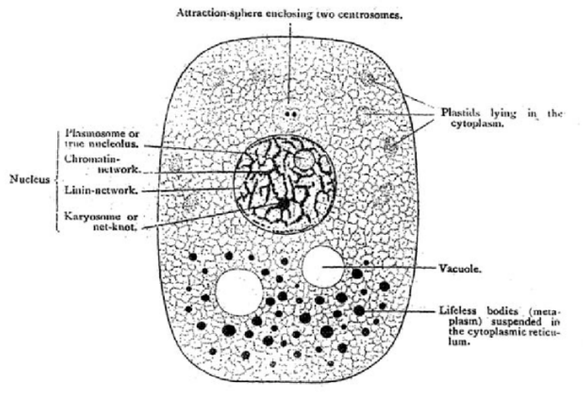
Polymer chemistry established as a field
Staudinger, Huggins, Flory….
1940s

Nucleoli show features of LLPS
Brangwynne
2011

Explosion of LLPS claims
2012-2019

Critical re-evaluation of the field
2019-2023

1899
EB Wilson, A Kossel and O Bütschli
Cells are collections of liquid droplets



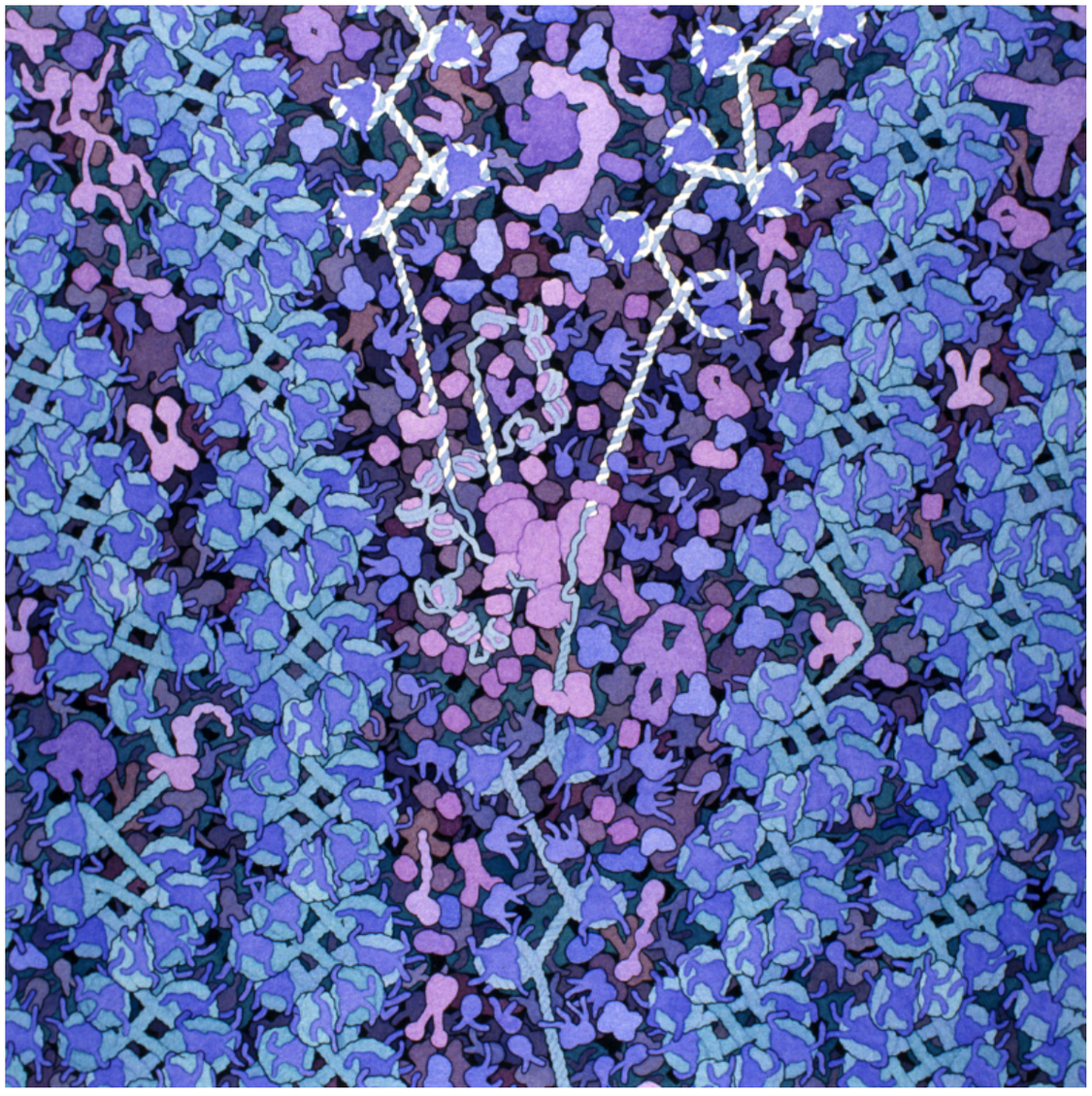
1924
AJ Oparin
Coacervate model for origin of life

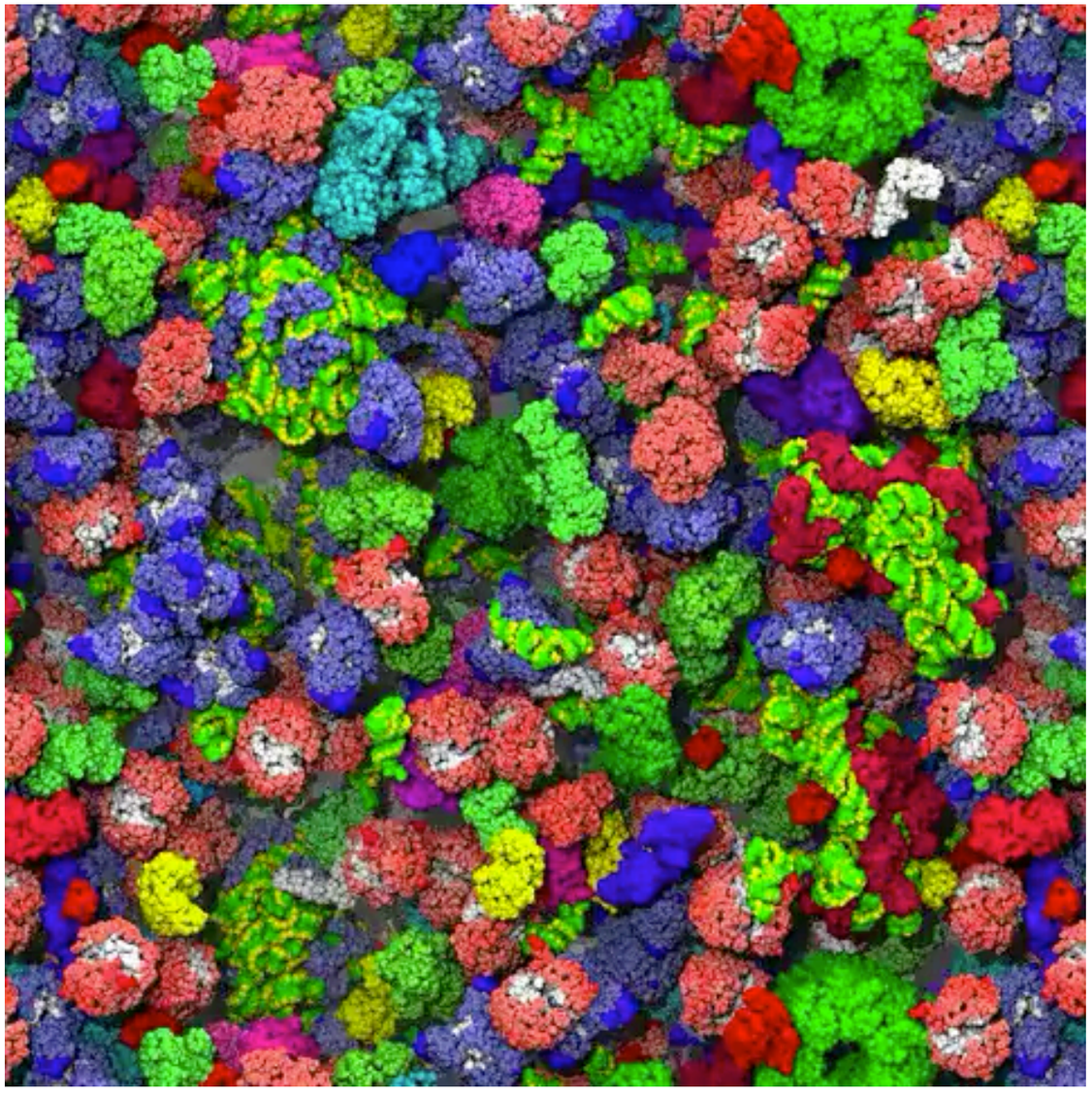
2009
Brangwynne
P-Granules form through LLPS

2012
Rosen
Multivalency drives LLPS

2019
McSwiggen
Hallmarks of LLPS MAY NOT be LLPS

The cell is immensely crowded

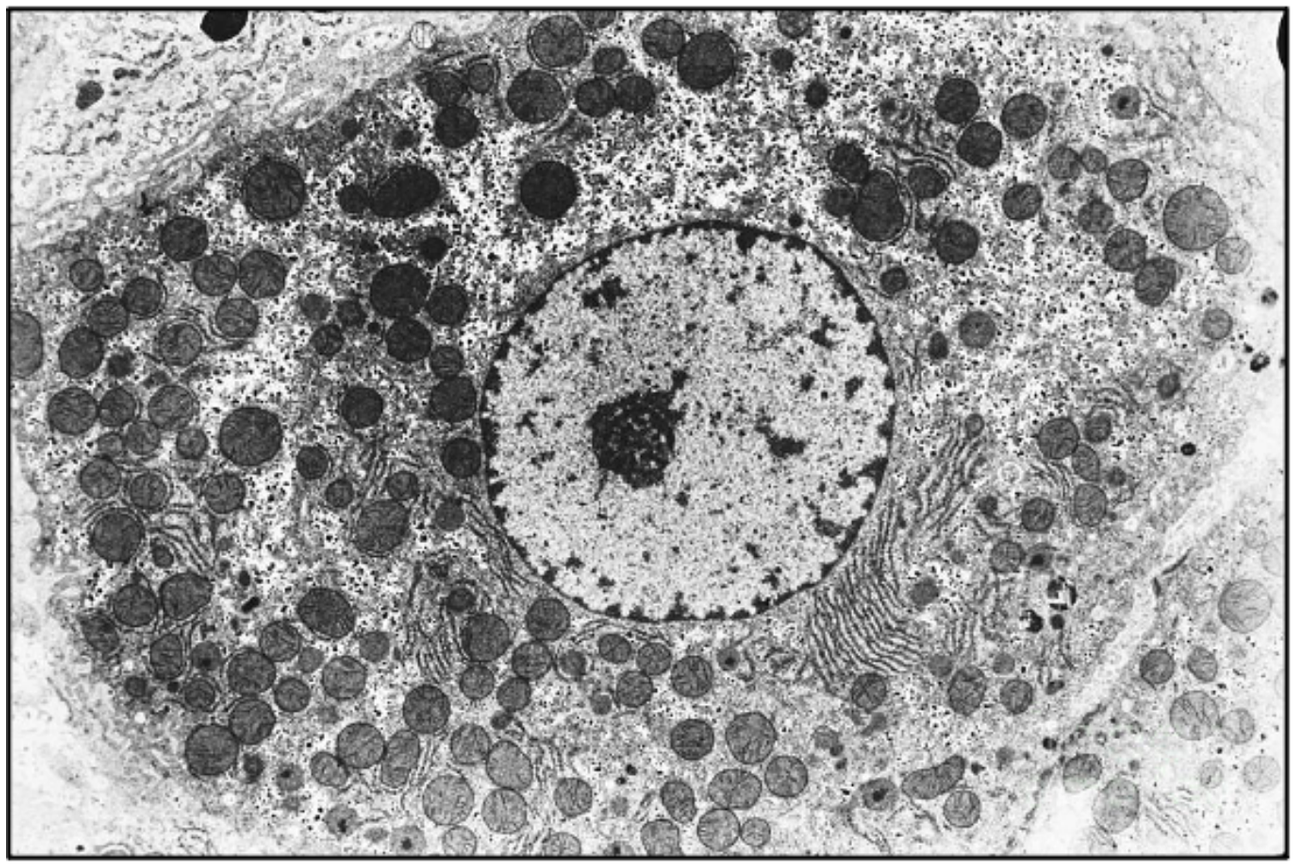




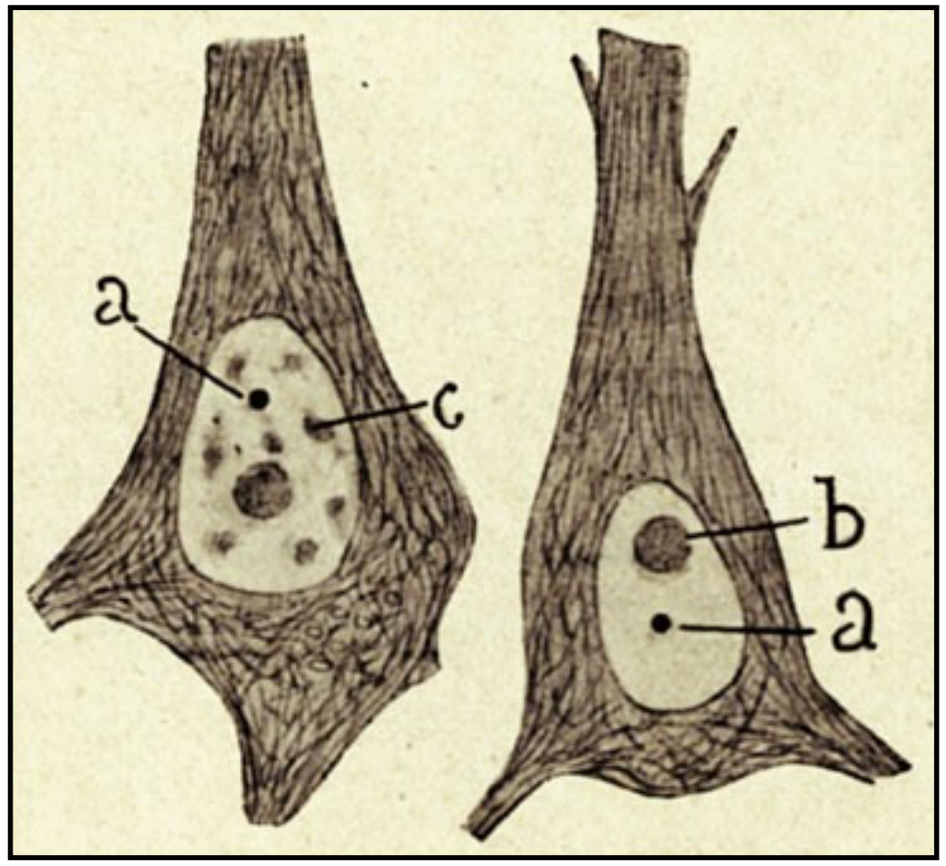
David Goodsell; McGuffey and Elcock 2010 PMID: 20221255

Cellular mechanisms for organization

Membrane-bound organelles



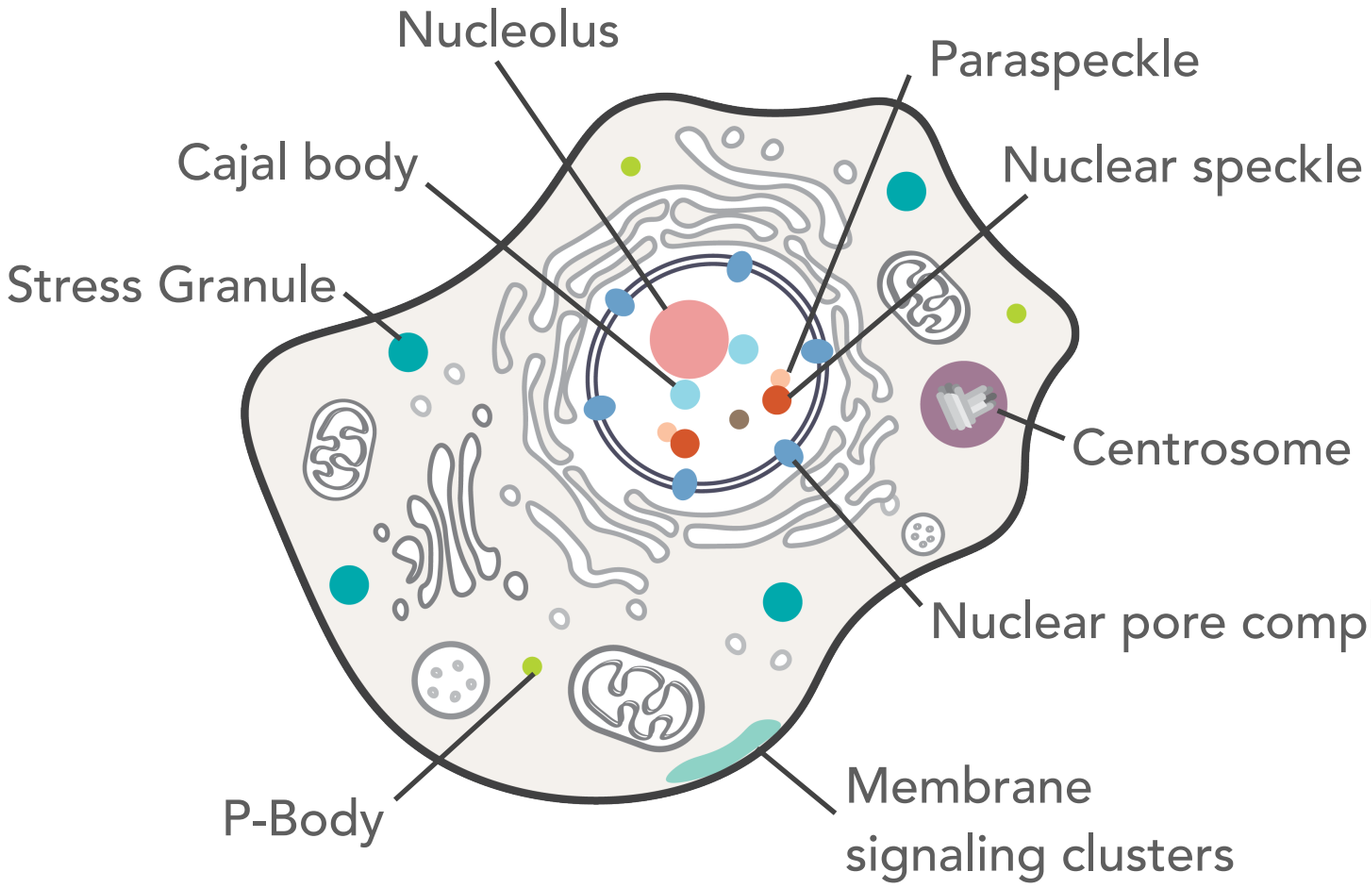
Membraneless organelles



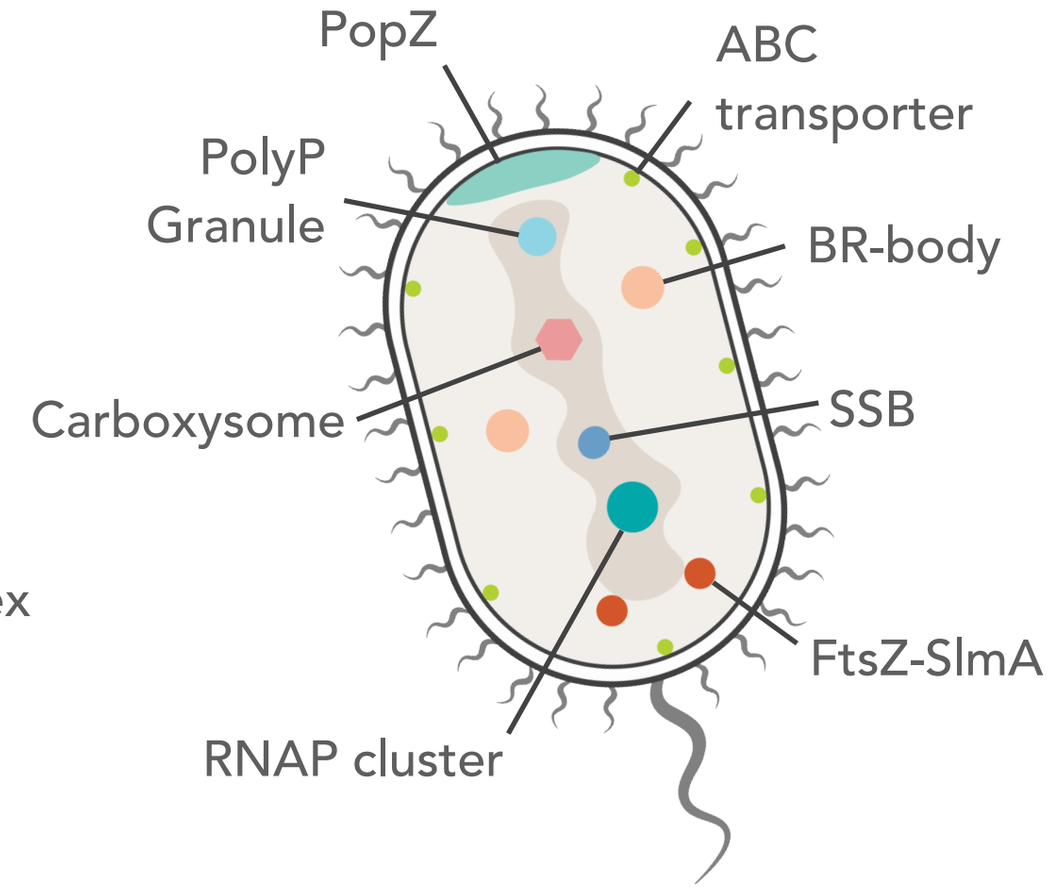
Santiago Ramón y Cajal, 19th c.

Biomolecular condensates are ubiquitous

Eukaryotes



Prokaryotes



How do you make an organelle without a membrane?

II. Discovery of phase separation

A new perspective to an old problem

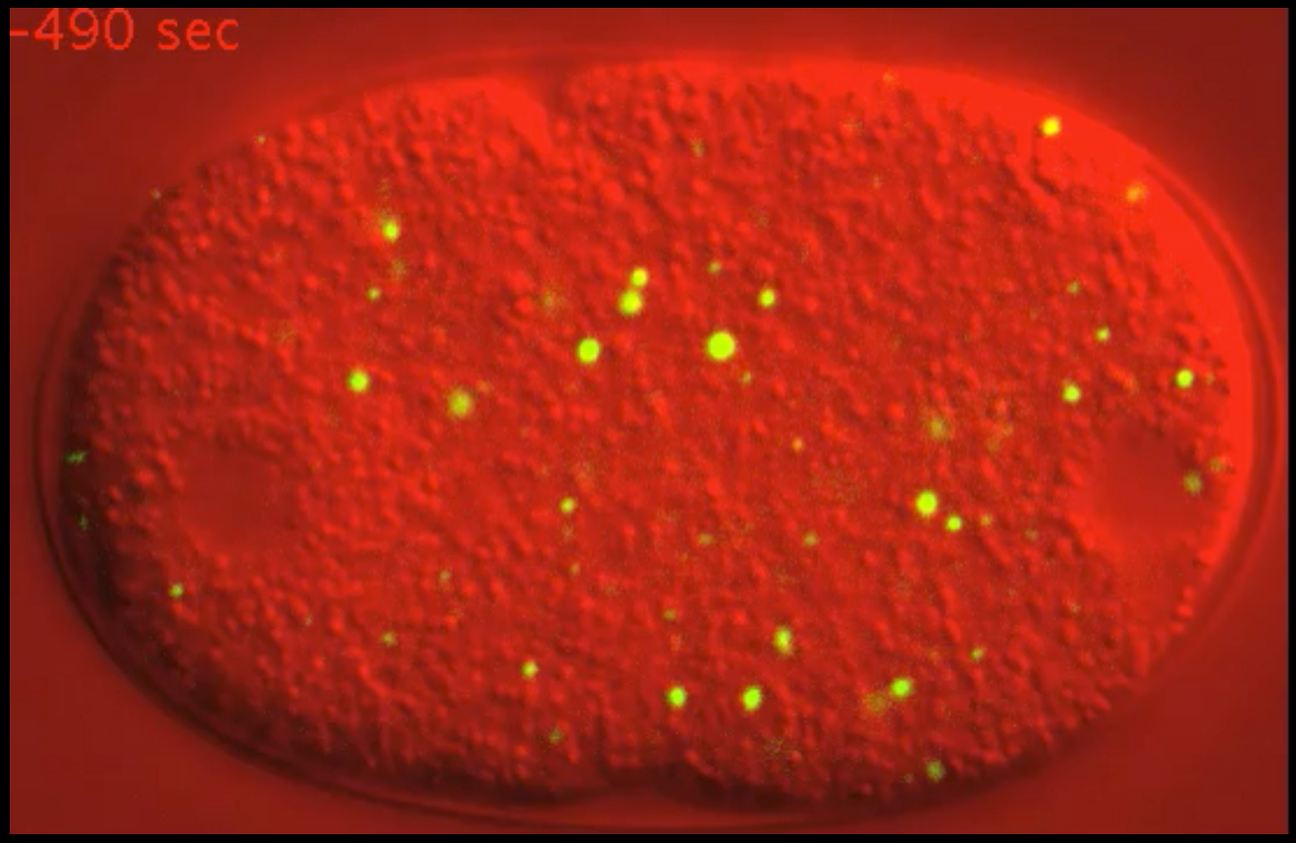
Cliff Brangwynne, PhD (Princeton)

- Material science major
- PhD in soft matter physics
- Postdoc with Tony Hyman

Can we use a materials science lens to understand cellular materials and organization?

2009: A mystery and a paradigm shift

P-Granules

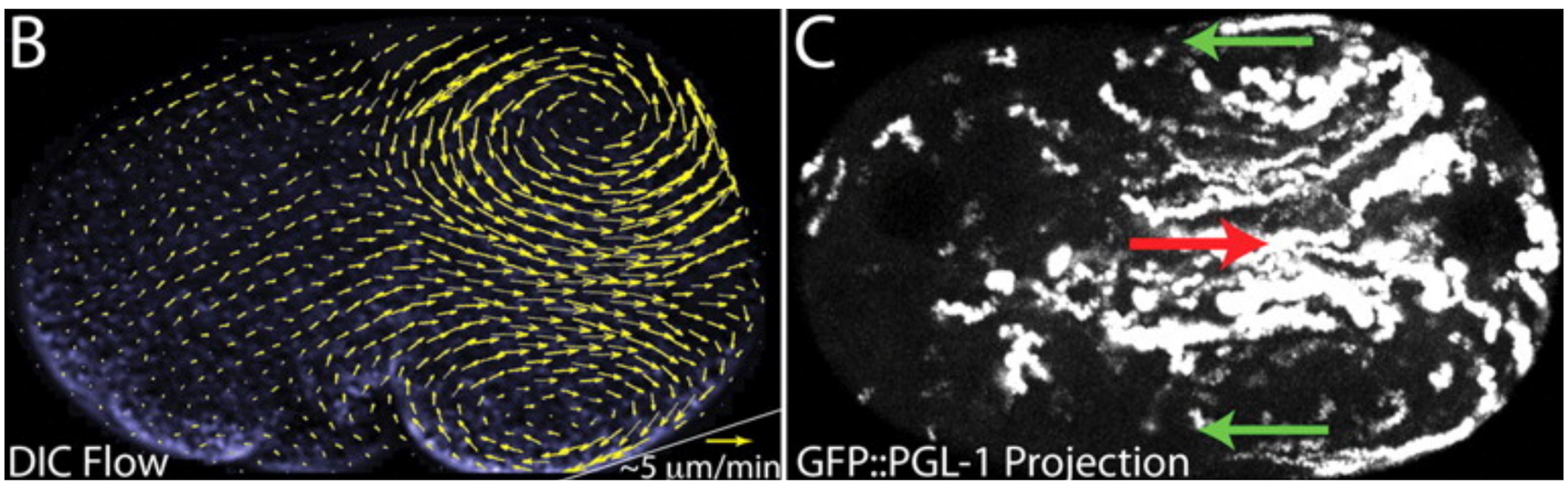


What gives rise to posterior localization of the P-granule?

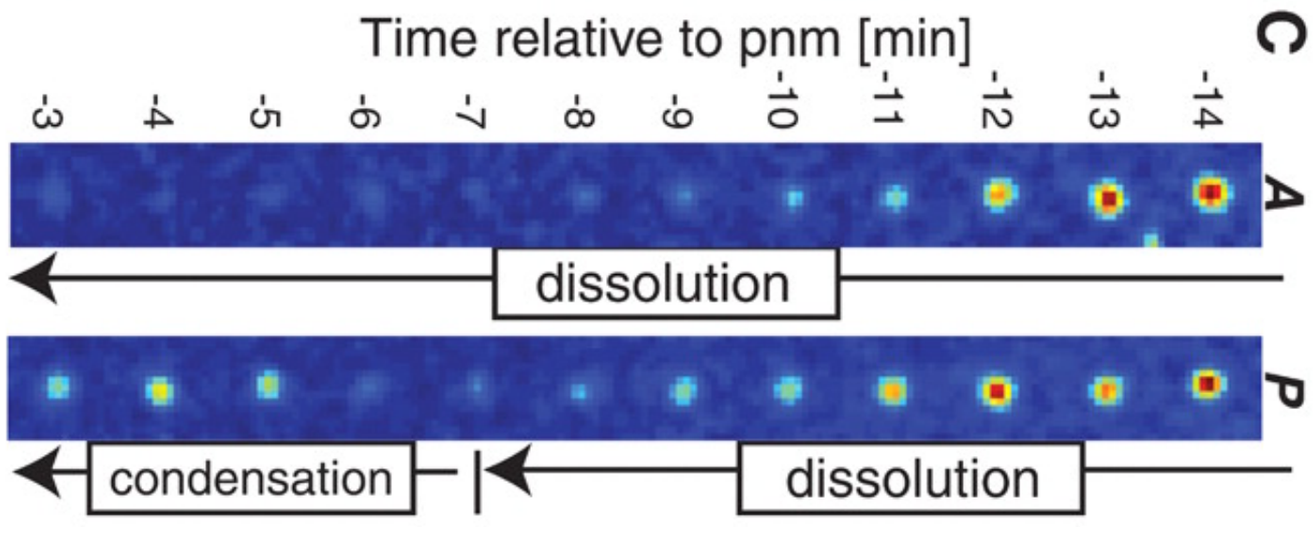
Brangwynne et al. and Hyman, 2009, PMID: 19460965

What gives rise to P-granule relocalization?

Hypothesis 1: Migration of existing P-granules by cytoplasmic flow



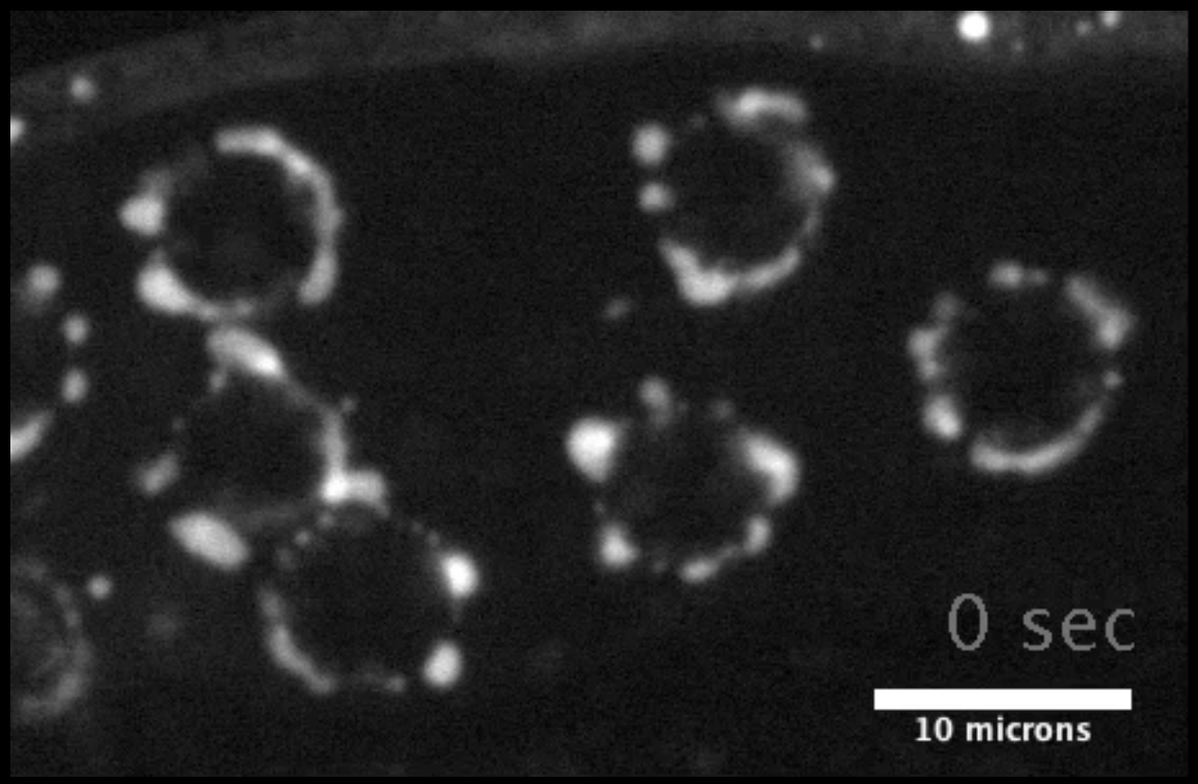
Hypothesis 2: Disassembly or degradation of anterior P-granules



Brangwynne et al. and Hyman, 2009, PMID: 19460965

P-granules exhibit liquid-like behaviors

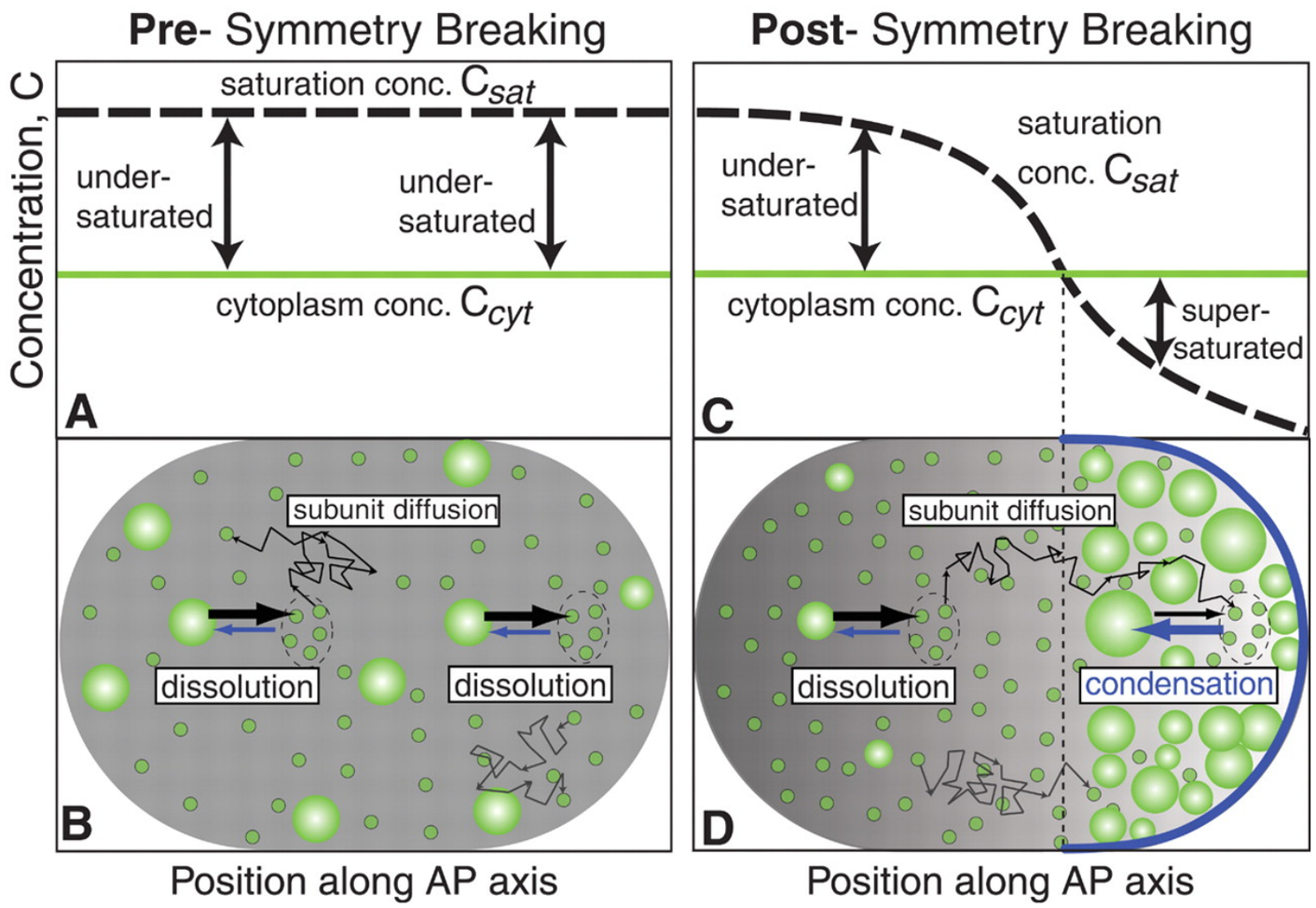
P-Granules



Direction of Flow

Brangwynne et al. and Hyman, 2009, PMID: 19460965

Controlled phase separation gives rise to P-granule relocalization

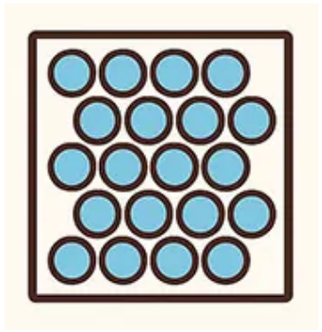


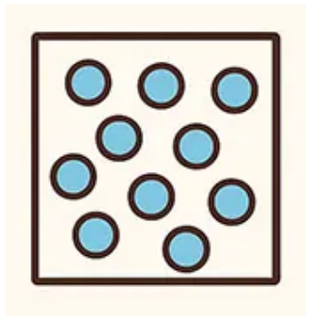
Big take home: allows for dynamic, responsive assemblies!

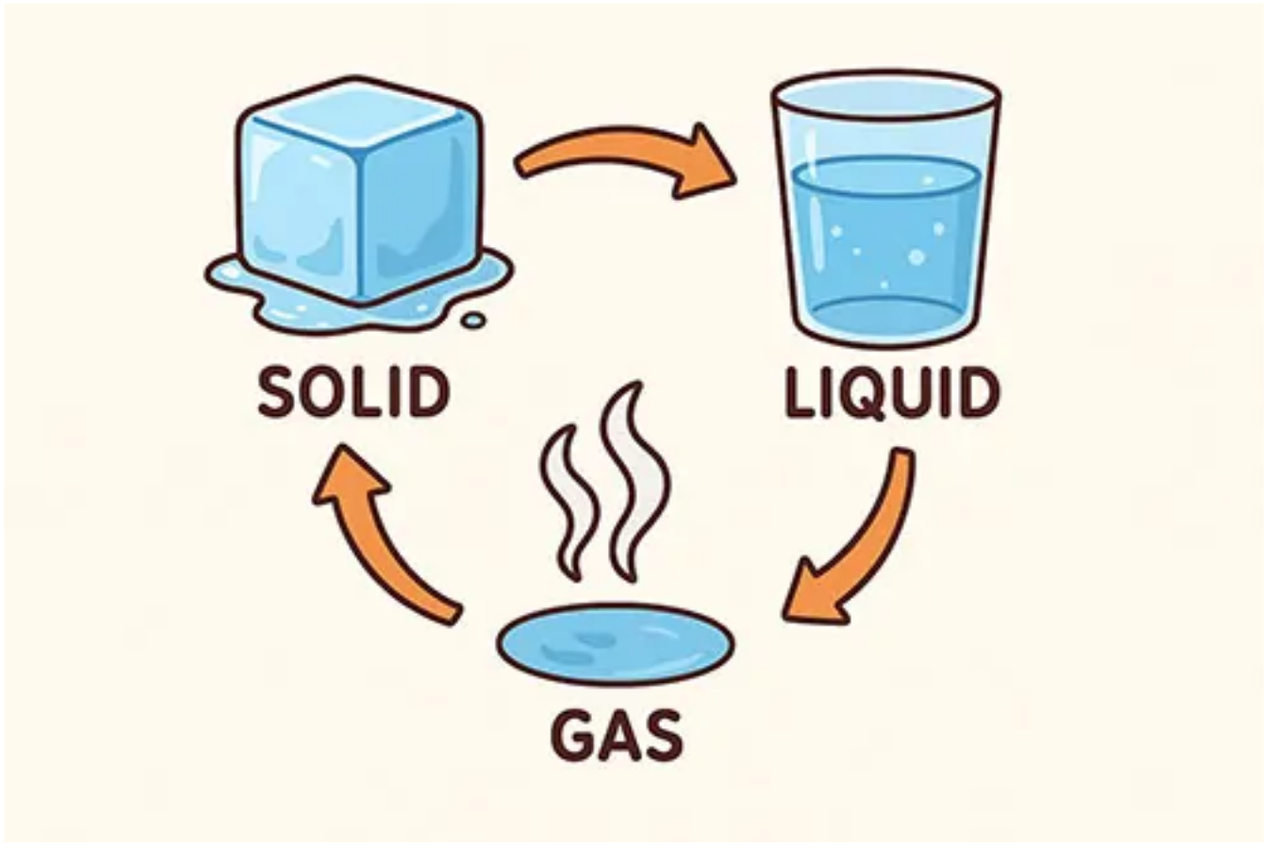
Brangwynne et al. and Hyman, 2009, PMID: 19460965

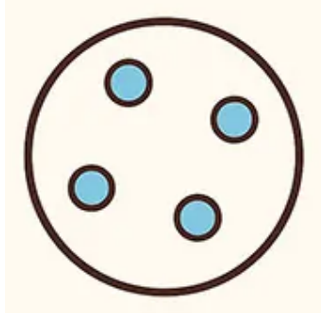
III. Physical principles of phase separation

Phase separation is a type of phase transition

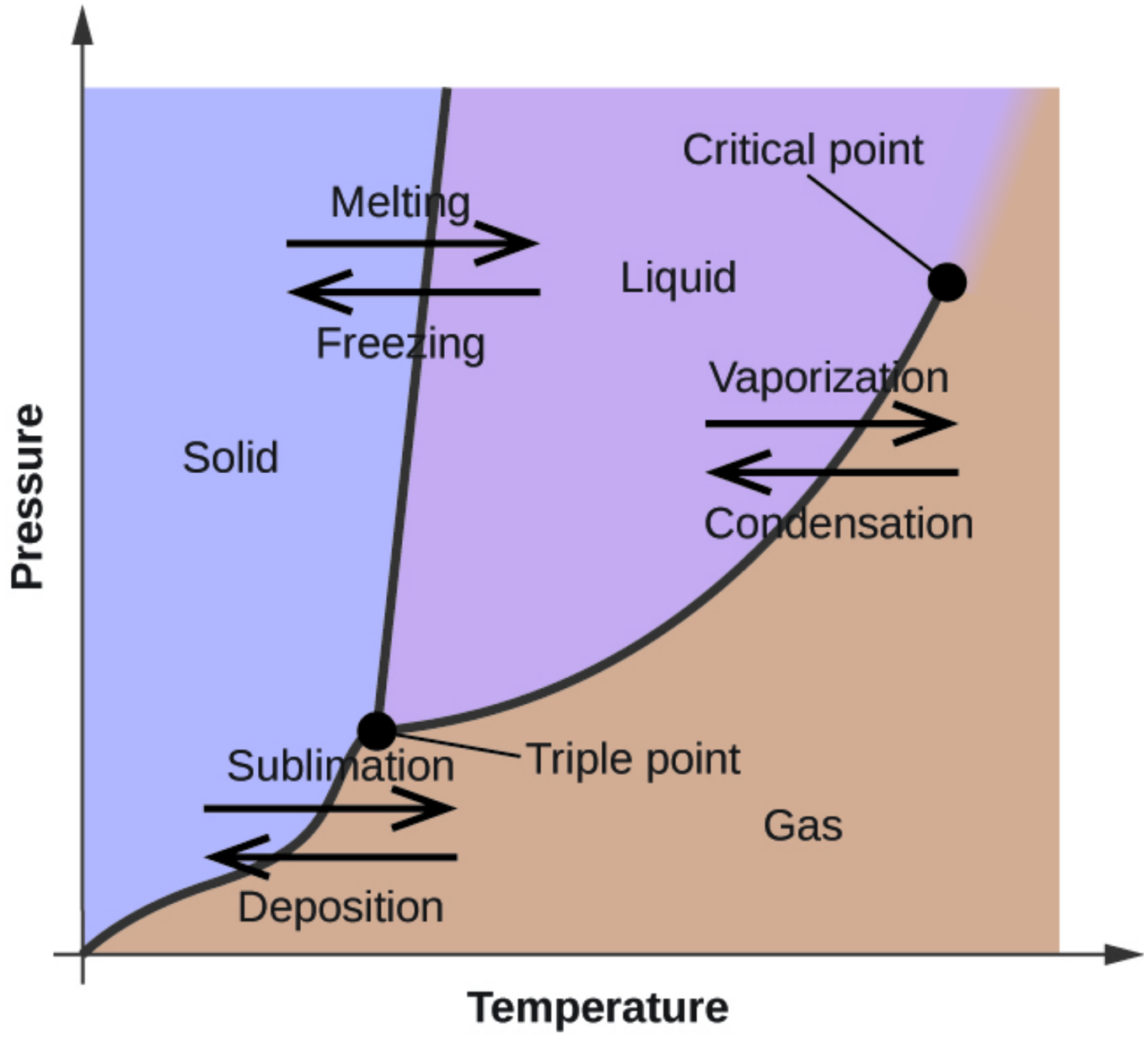








Phase diagrams describe physical state of matter in different conditions



What is Liquid-Liquid Phase Separation (LLPS)?

Phase transition

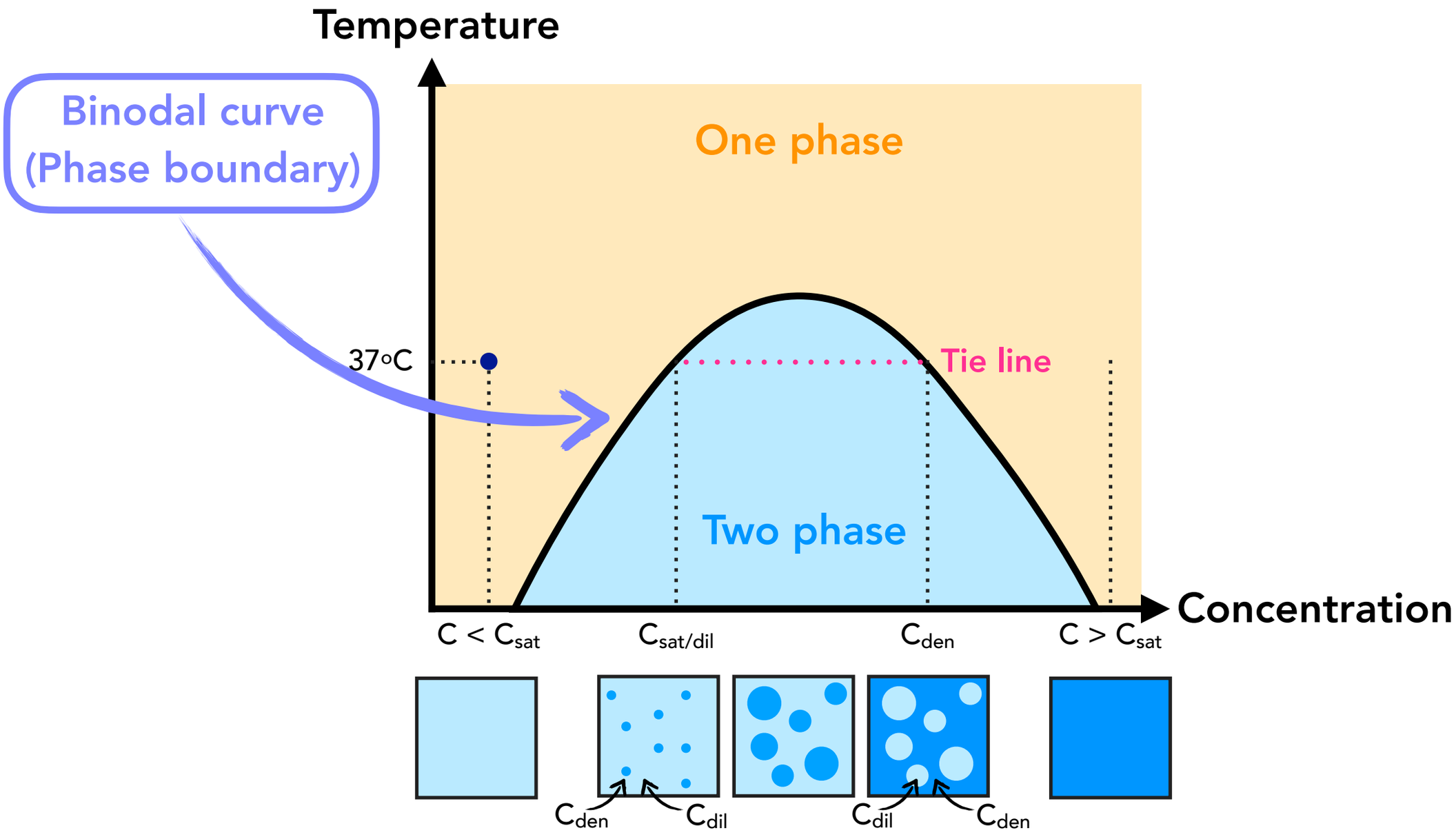
Liquid

Solid

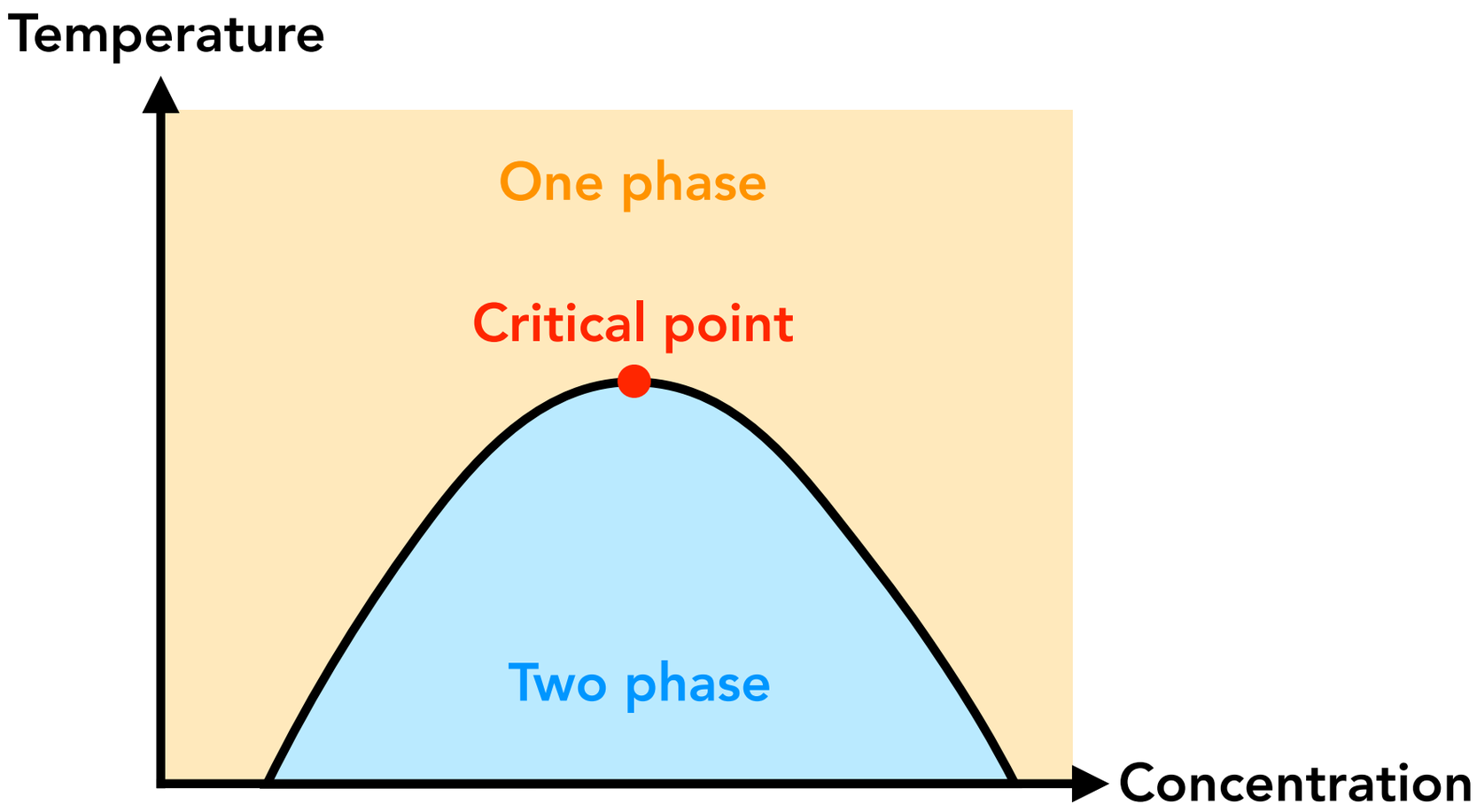
Phase *separation*

Liquid

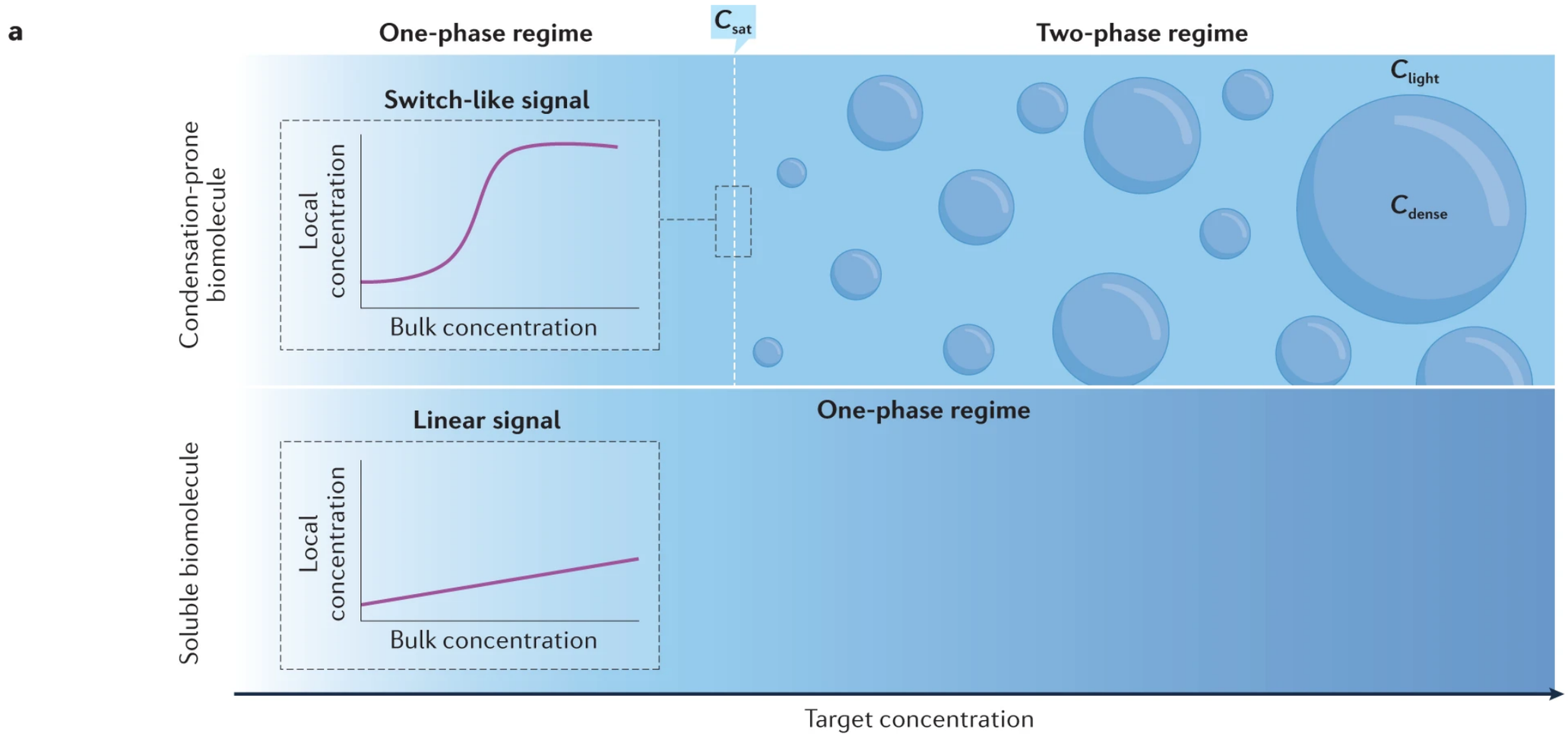
Phase diagrams also describe phase separation



Critical point: point past which PS will not occur

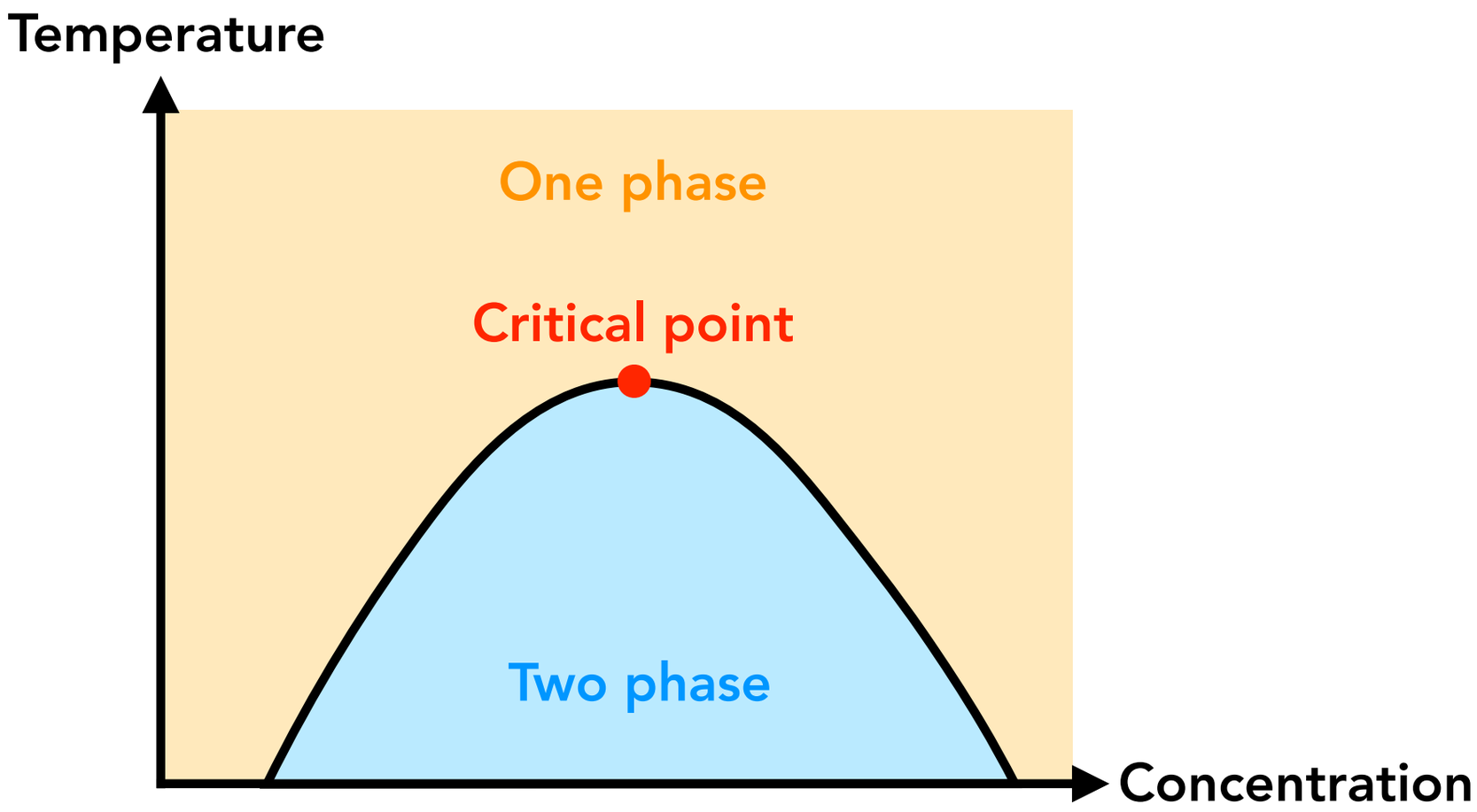


Allows for switch-like signals



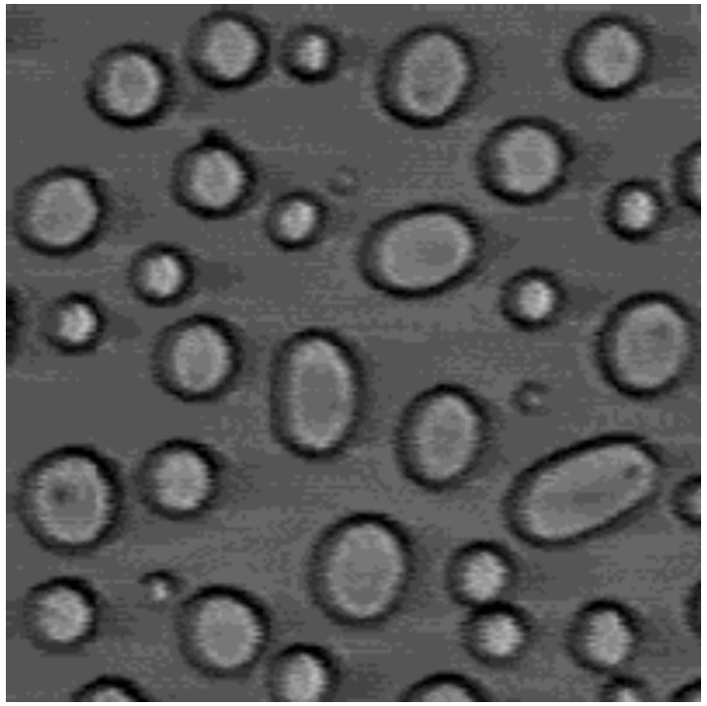
Mitrea et al. 2022, PMID: 35974095

Where might switch-like regulation occur?



A note on size: Coarsening or Ostwald Ripening

- While total volume for phases is determined by phase diagram, the size of an individual condensate can change over time
- Small condensates - less stable
- Over time, larger condensates are favored



Stavans lab, Weizmann Institute

Phase diagram: what are the key take-aways?

- Sharp concentration threshold- switch-like behavior
- Equilibrium partitioning: concentration is maintained in dilute and in dense (buffering), volumes change with concentration changes
- Reversible when crossing binodal
- Critical point provides regulation point, defines fundamental physical properties of system
- Sensitive to environment (pH, temperature, ion concentration, etc.)

Free energy minimization drives phase separation

(Flory-Huggins theory)

Free energy minimization drives phase separation

$$\Delta G = \Delta H - T\Delta S$$

What contributes to ΔH?

- Affinity between molecules (binding energy)
- Favorable interactions lower ΔH —> promote phase separation

What contributes to ΔS?

- Mixing entropy
  - Phase separation reduces mixing entropy
- Hydrophobic effect
  - Phase separation can release ordered water

Quick understanding check:

$$\Delta G = \Delta H - T\Delta S$$

If a protein phase separates at low temperature, is this system enthalpically or entropically driven?

Of note: gets much more complicated in biology!

Many components

- Multidimensional phase diagrams
- Complex (and often non-fixed) C_{sat}

Active processes

- ATP-dependent processes
- Remodeling and/or PTMs
- Active transport

A note: many different kinds of phase transitions

- Liquid-gel, liquid-solid, etc.
- Percolation, sol-gel: forms system-spanning network
- Phase separation coupled to percolation (PSCP): percolation in dense phase
- Coacervation: a type of LLPS driven by electrostatics

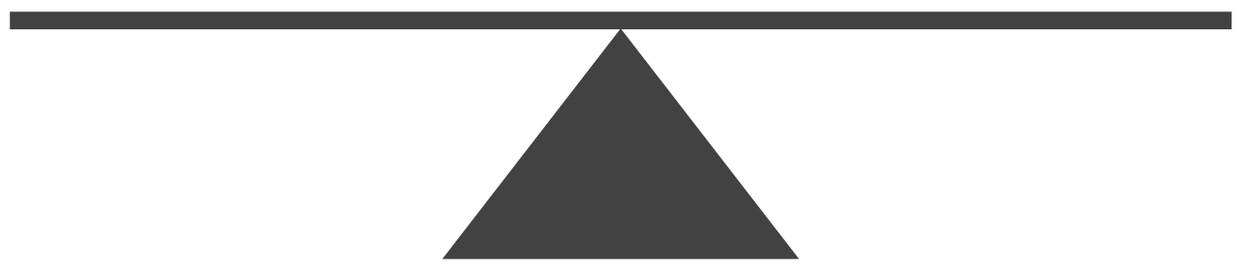
Constantly refining!

IV. Molecular drivers of phase separation

What kind of interactions drive phase separation?

Strong

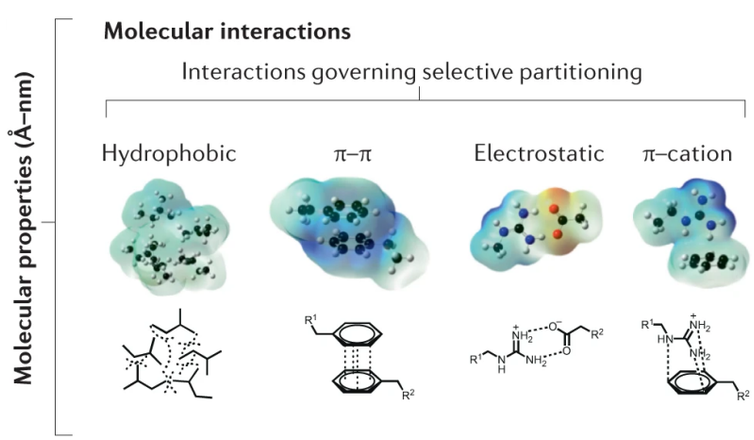
Dynamic



What kind of interactions drive phase separation?

Low-affinity

- Strong enough to come together; not too strong to trap low mobility states



Also: Domain-domain
Base pairing

Multivalent

- Allows for avidity and cooperatively
- Collective strength of many weak binding events

Oligomerization domains
Modular chains
IDRs

Typically: IDRs or multivalent modular proteins (or combined)

Mitrea et al. 2022, PMID: 35974095

What kind of interactions drive phase separation?

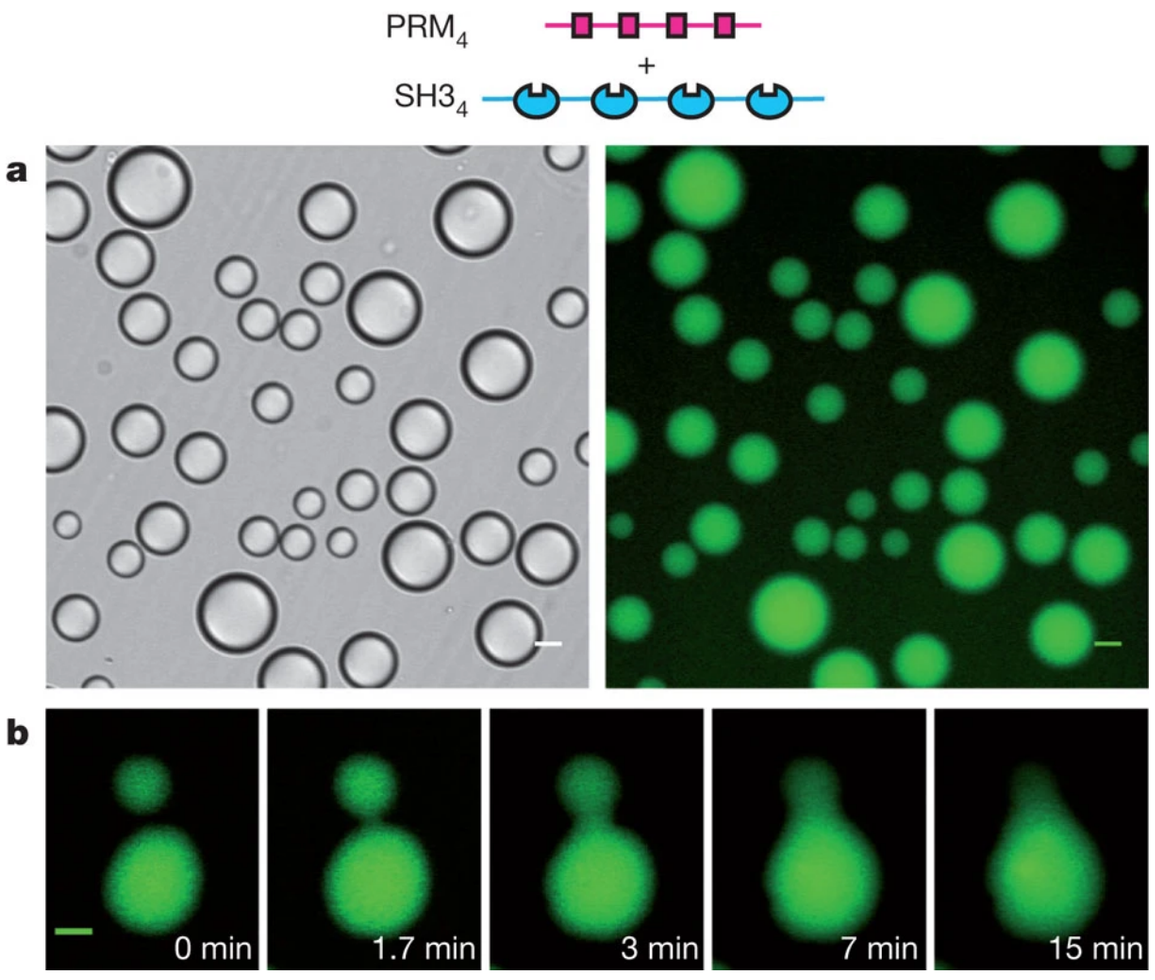
Michael Rosen, PhD (UTSW, previously MSKCC)

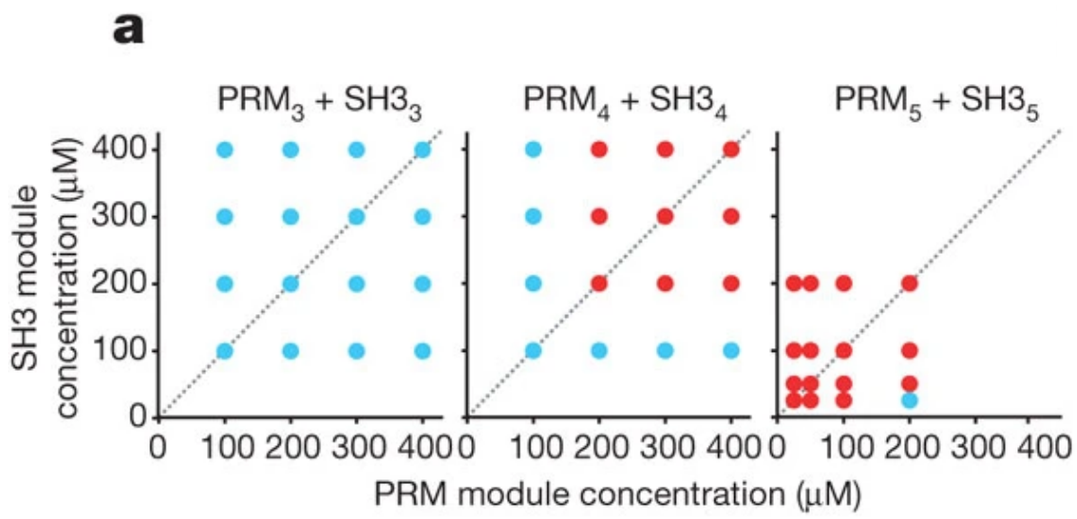
- Structural biologist
- Laid foundations for molecular features that drive phase separation

Can we use a biochemical lens to uncover the molecular drivers of phase separation?

An example: modular multivalency drives phase separation

PRM-SH3 interactions

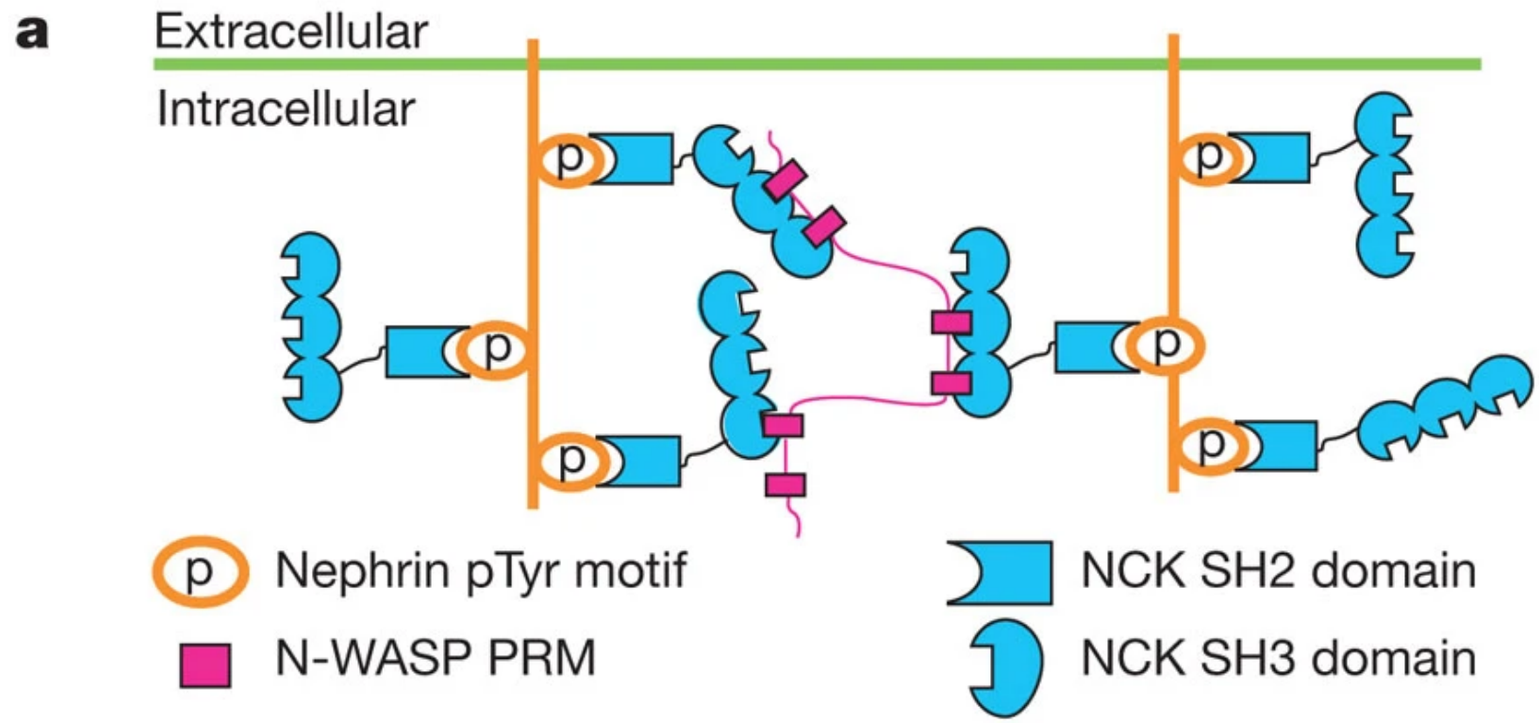




Li et al. and Rosen, 2012, PMID: 22398450

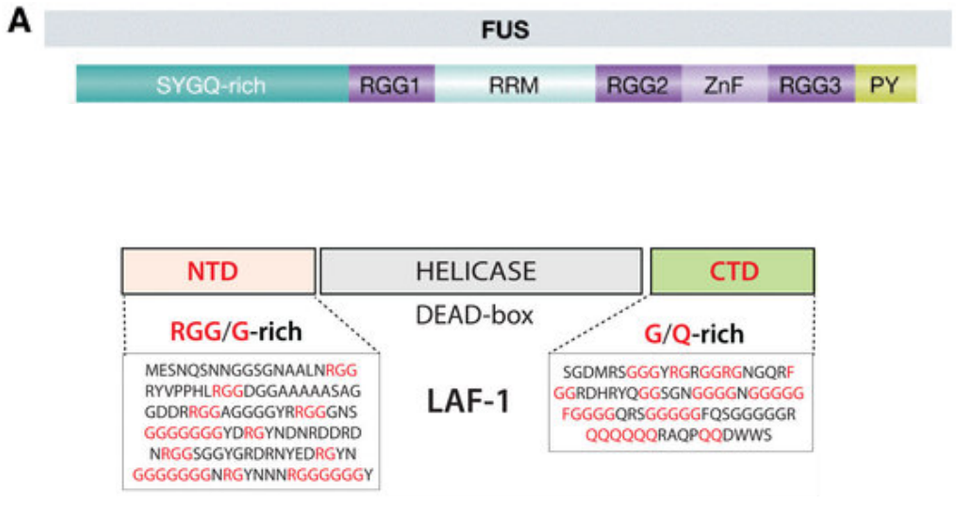
An example: modular multivalency drives phase separation

Regulated multivalency



Li et al. and Rosen, 2012, PMID: 22398450

Another source of dynamic multivalent interactions: IDRS



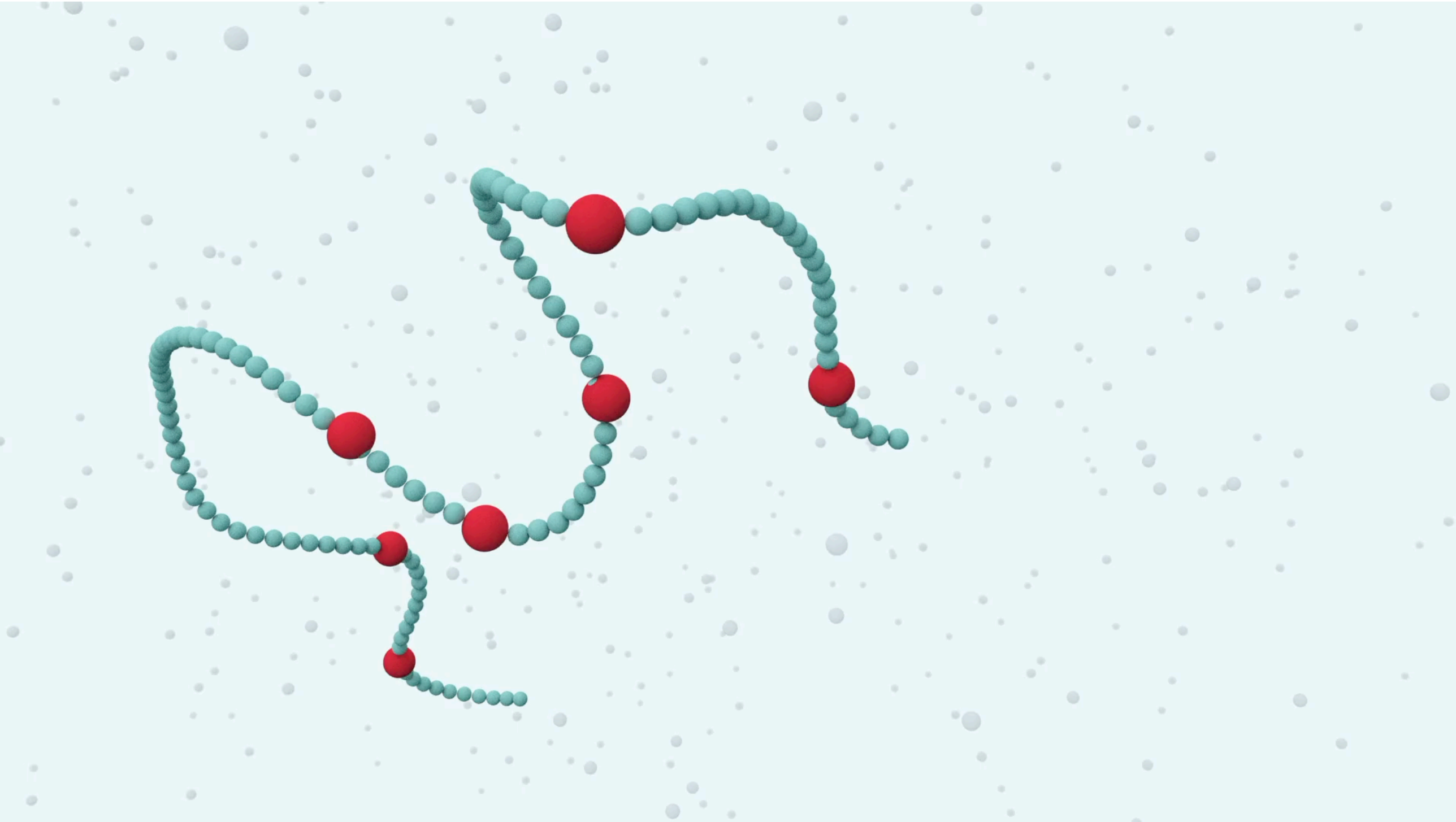
FUS:
Y repeats in LCD - Hydrophobic, cation-pi
R repeats in RGG - cation-pi

LAF-1:
R/Q repeats: electrostatic

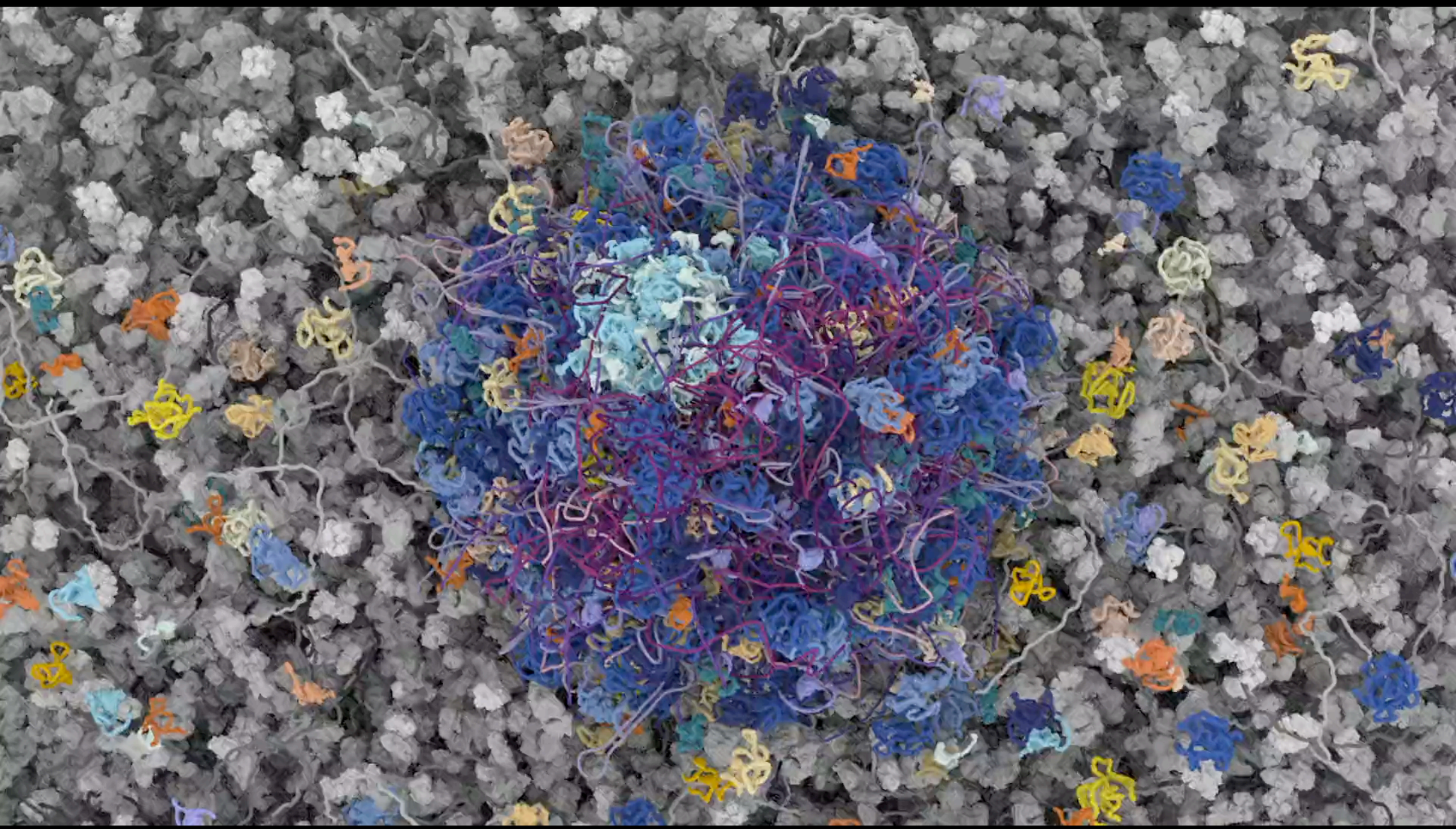
TNRC6B:
W repeats: hydrophobic, pi-pi

Patel et al. and Alberti, 2015, PMID: 26317470
Kim and Myong, 2016, PMID: 27546789
Sheu-Gruttadauria and MacRae, 2018,PMID: 29576456

Stickers and spacers model



Biological condensates are complex: composition



Biological condensates are complex!

- Condensates are compositionally complex
- Condensates can be multiphase
- Condensates may not be equilibrium systems (active matter)

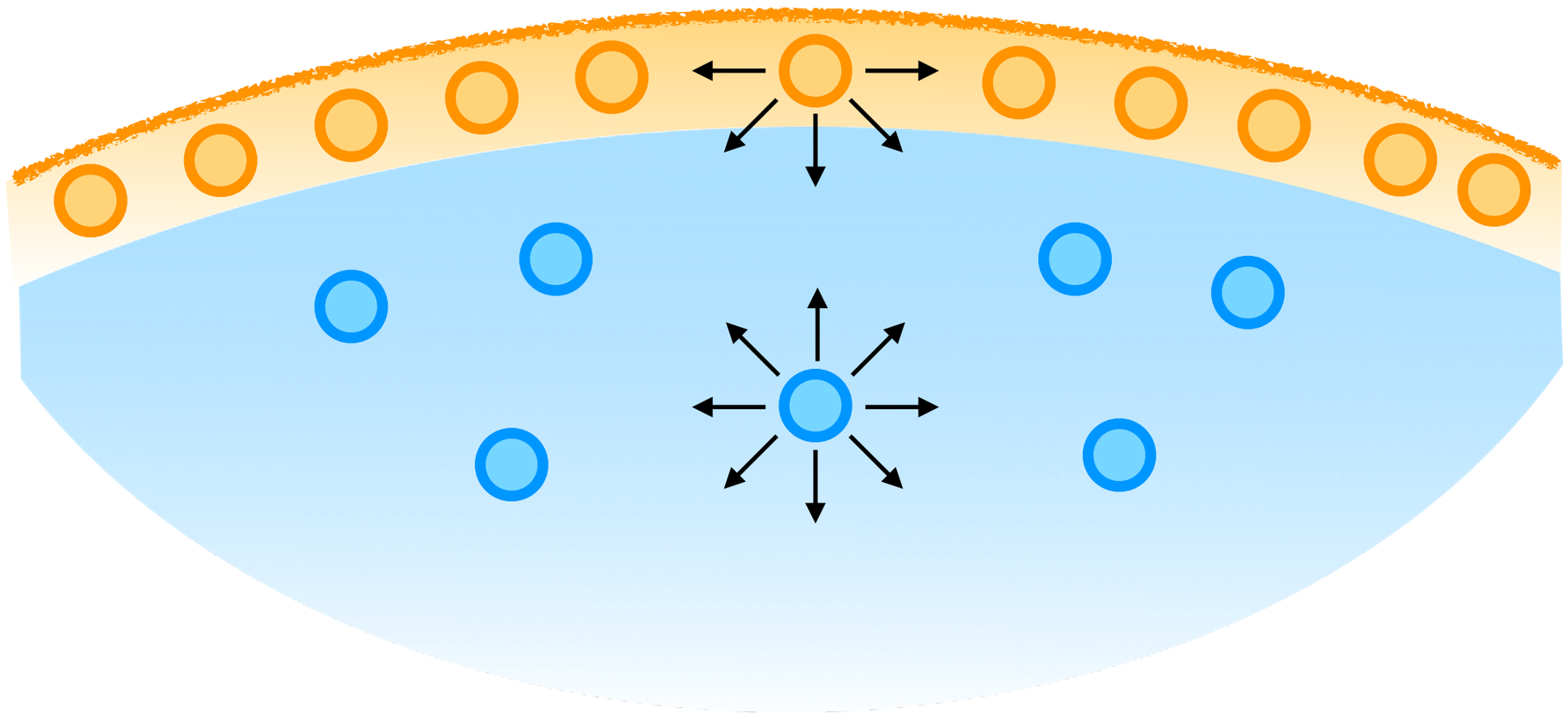
What can we learn from simple phase separation models in the face of such complexity?

Recap: molecular interactions that drive phase separation

- Low affinity, multivalent
- Can be modular (beads on string) or IDRs, or combination (very common)
- Stickers and spacers model provides framework for describing phase separating molecules (IDRs or modular)
- Clients and scaffolds model explains composition

Physical emergent features of LLPS

Interfacial tension (surface tension)



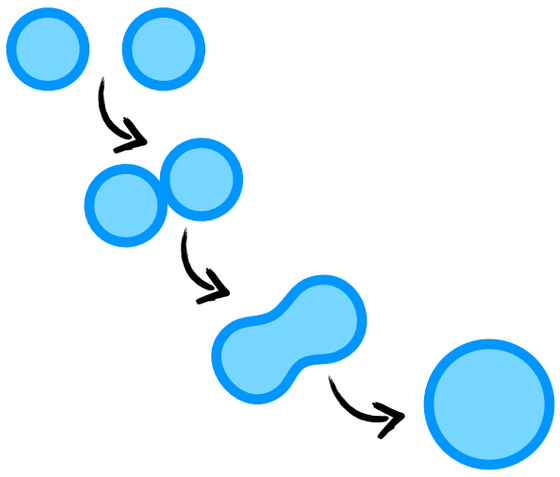
Impact:

- Higher surface tension = more spherical shape
- Influences how interacts with environment (wetting, exchange across boundary)

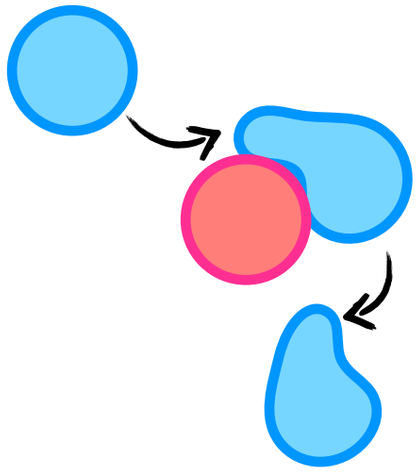
Physical emergent features of LLPS

Liquid-like behaviors

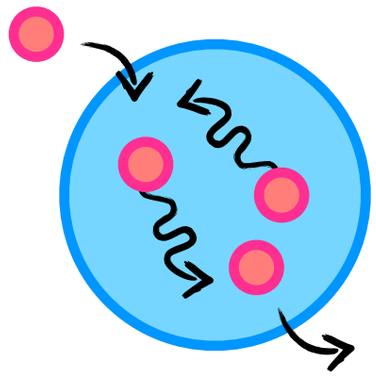
Fusion



Deformation



Dynamic exchange



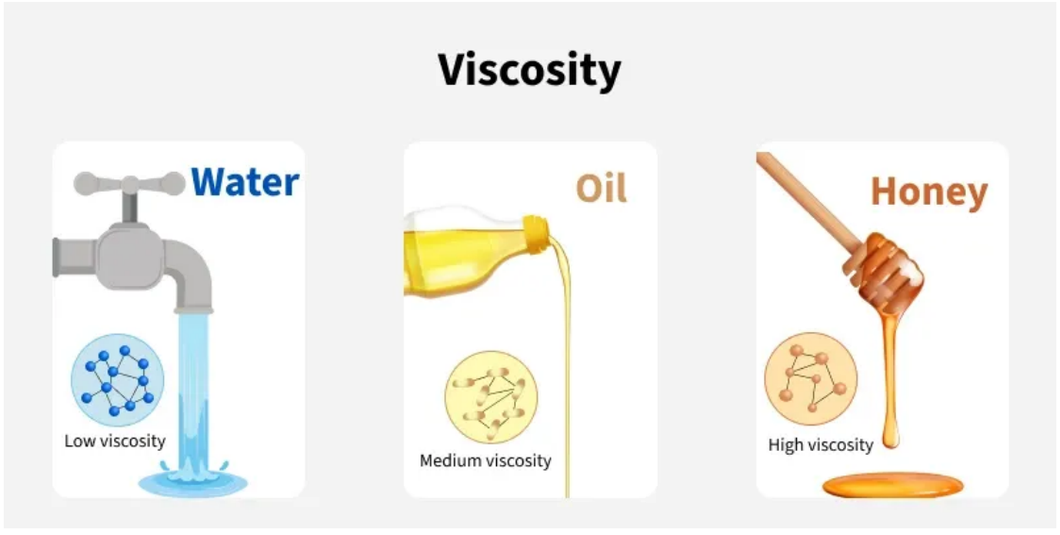
Impact:

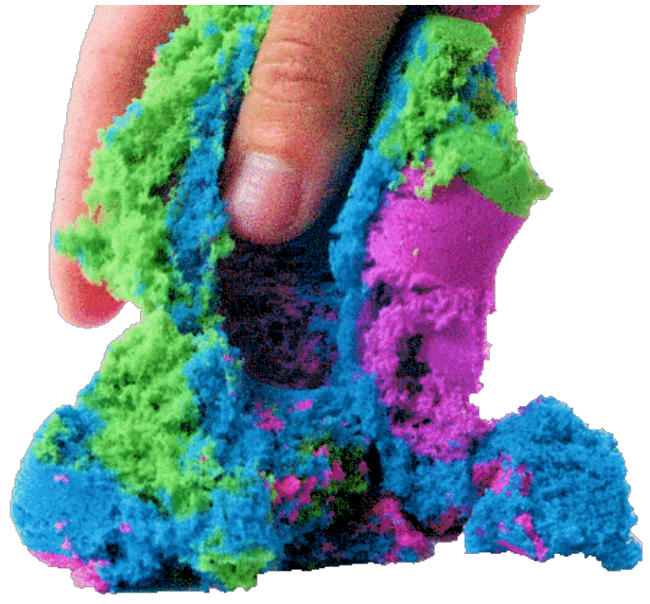
- Responsive (on molecular and mesoscale)
- Can grow

Physical emergent features of phase separation

Range of material properties

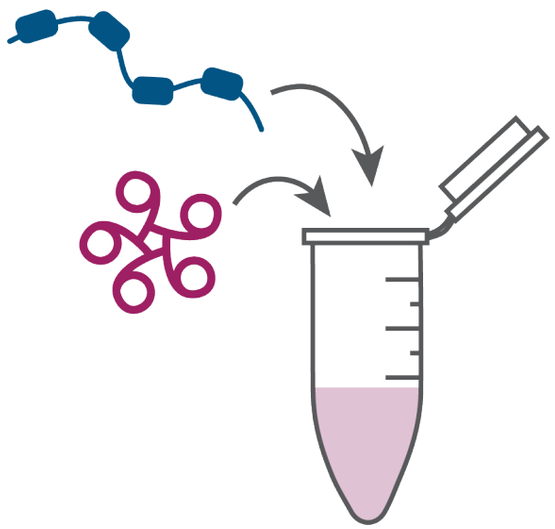
Viscosity/viscoelasticity/elasticity





V. How do we study condensate properties?

In vitro



Purified proteins/RNAs
Reconstitution of activity

↓

Molecular grammar
of representative players

Cannot capture complexity

(Somewhere in between)

Optogenetics
In-cell reconstitution

In cell

Quantitative microscopy
Label scaffolds/constituents
Proteomics/transcriptomics

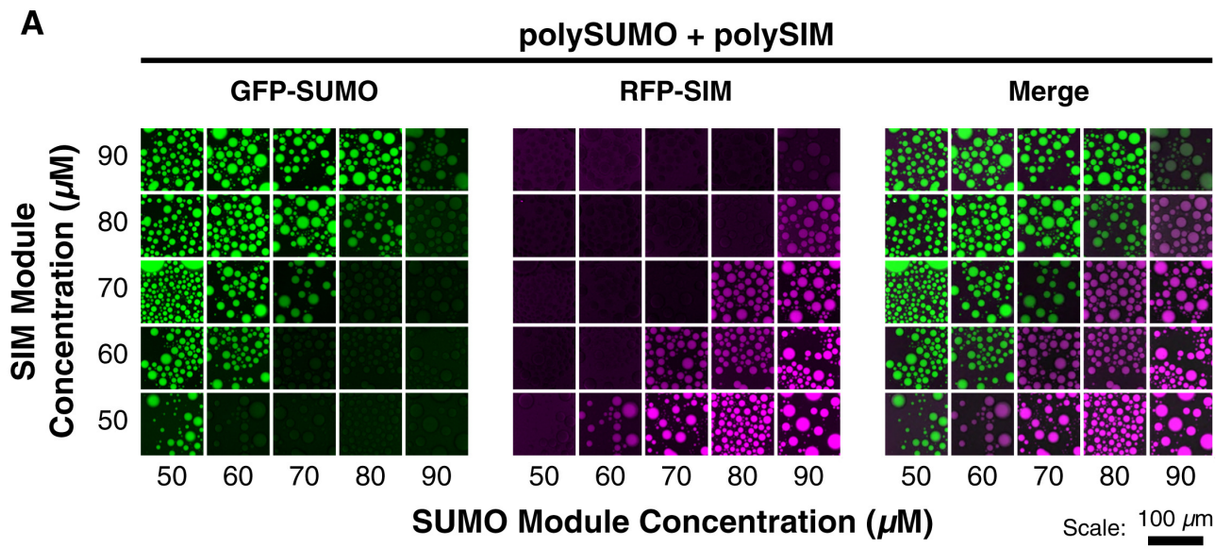
↓

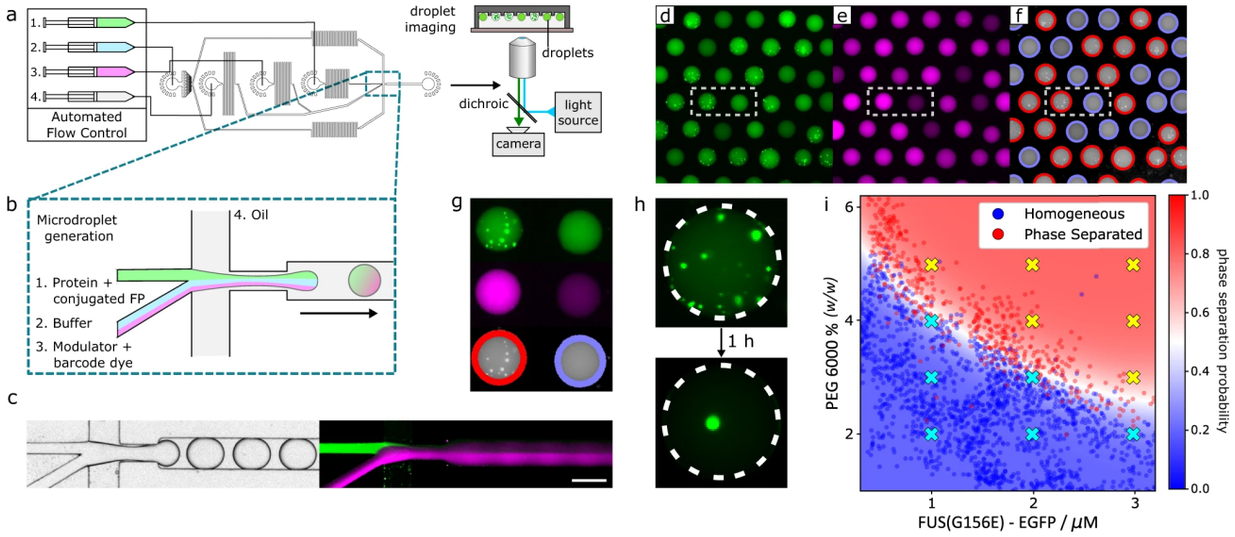
Behaviors
of complex systems

Difficult to interpret

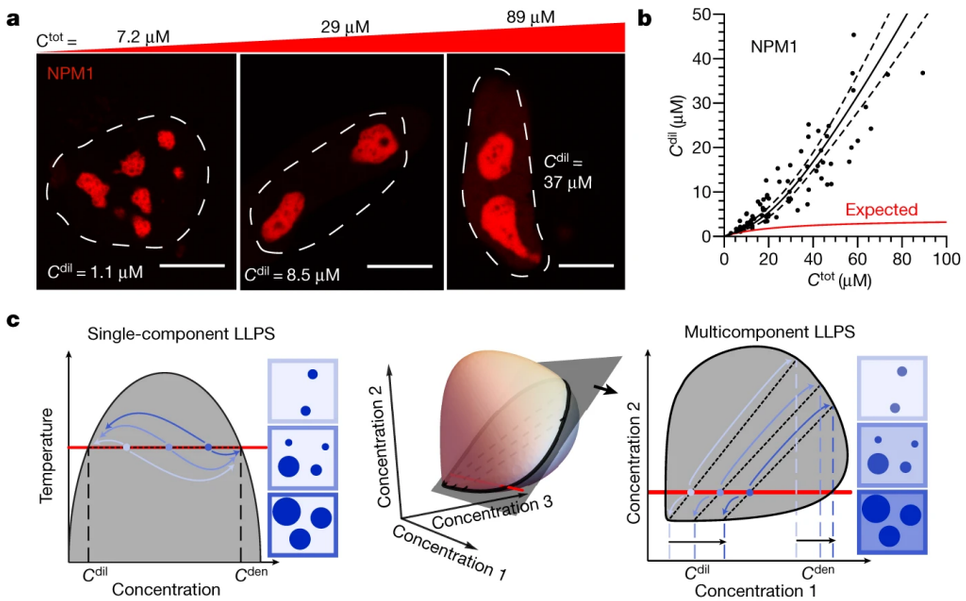
Phase diagrams and C_{sat}

In vitro





In vivo



Measures:

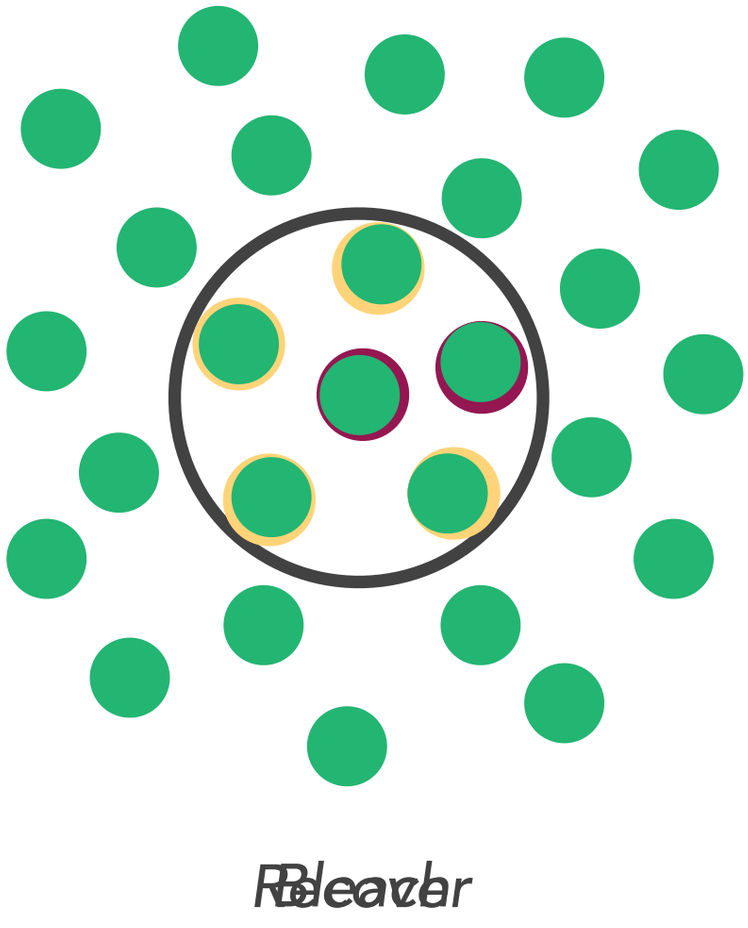
Thermodynamics

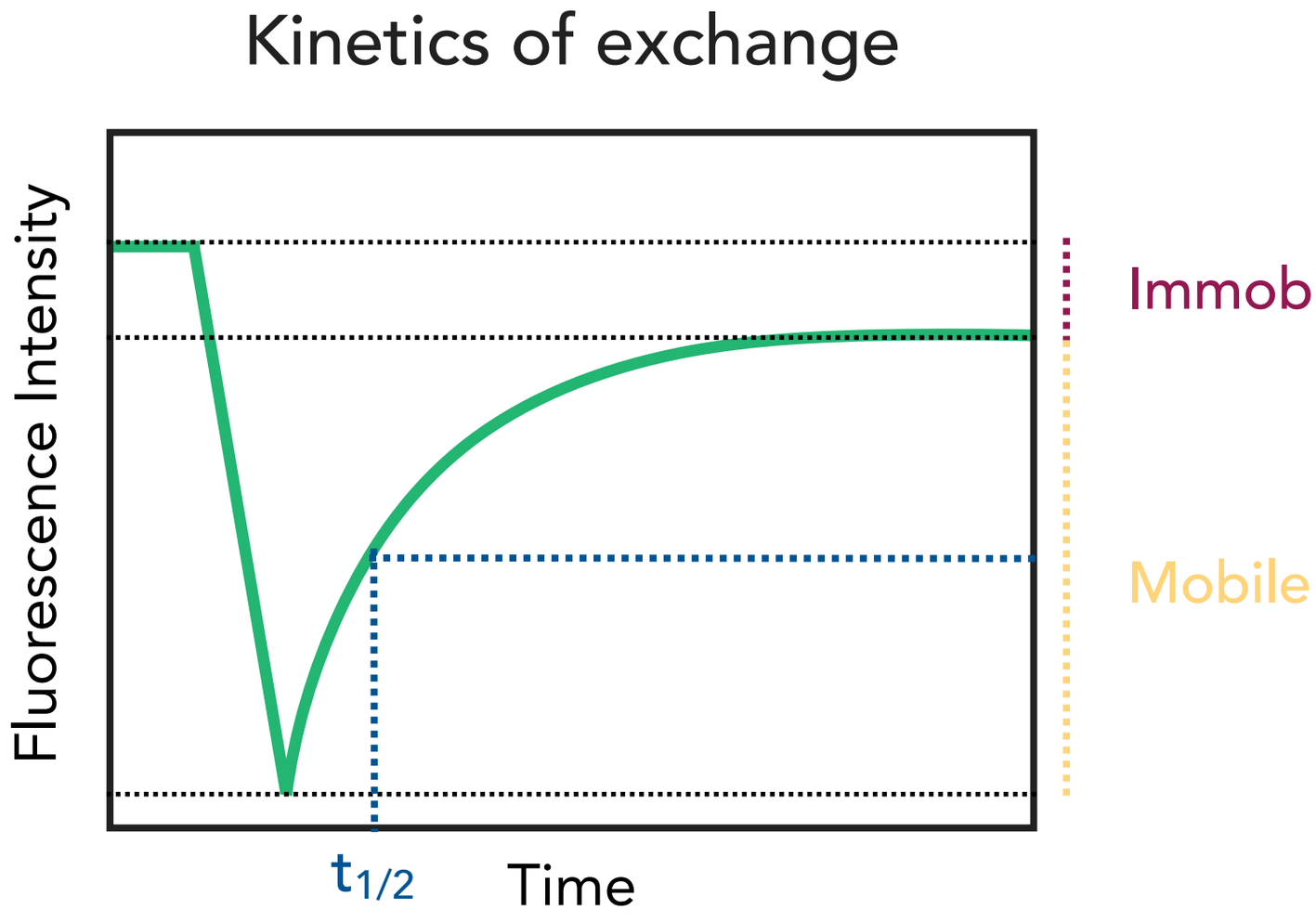
Riback et al. and Brangwynne, 2020, PMID: 32405004

Banani et al. and Rosen, 2016, PMID: 27374333

Arter, Qi et al. and Knowles, 2022, PMID: 36543777

Fluorescence Recovery After Photobleaching (FRAP)

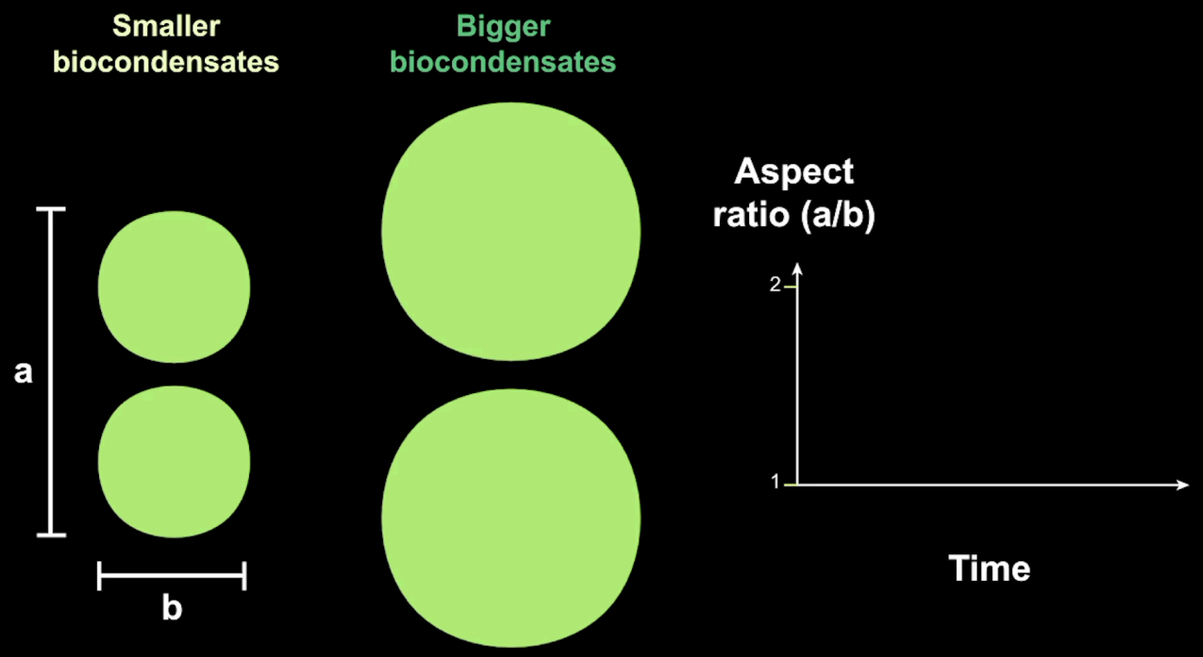




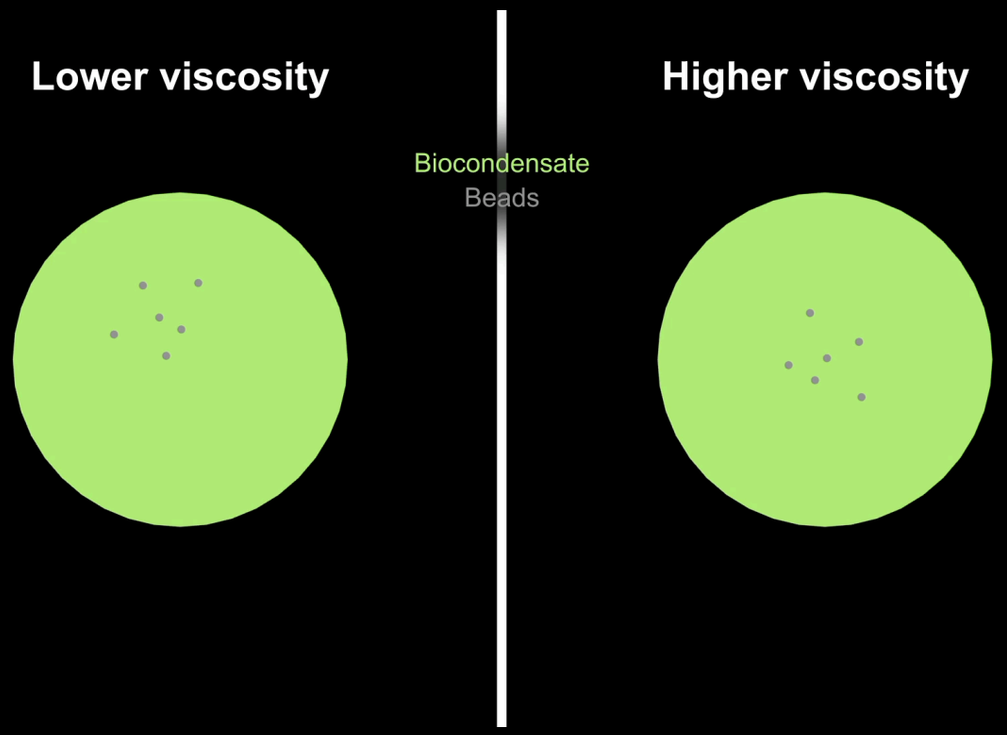
Measures: Molecular dynamics

Fusion, viscosity

Fusion



Microrheology



Measures:

Viscosity, surface tension

Viscosity

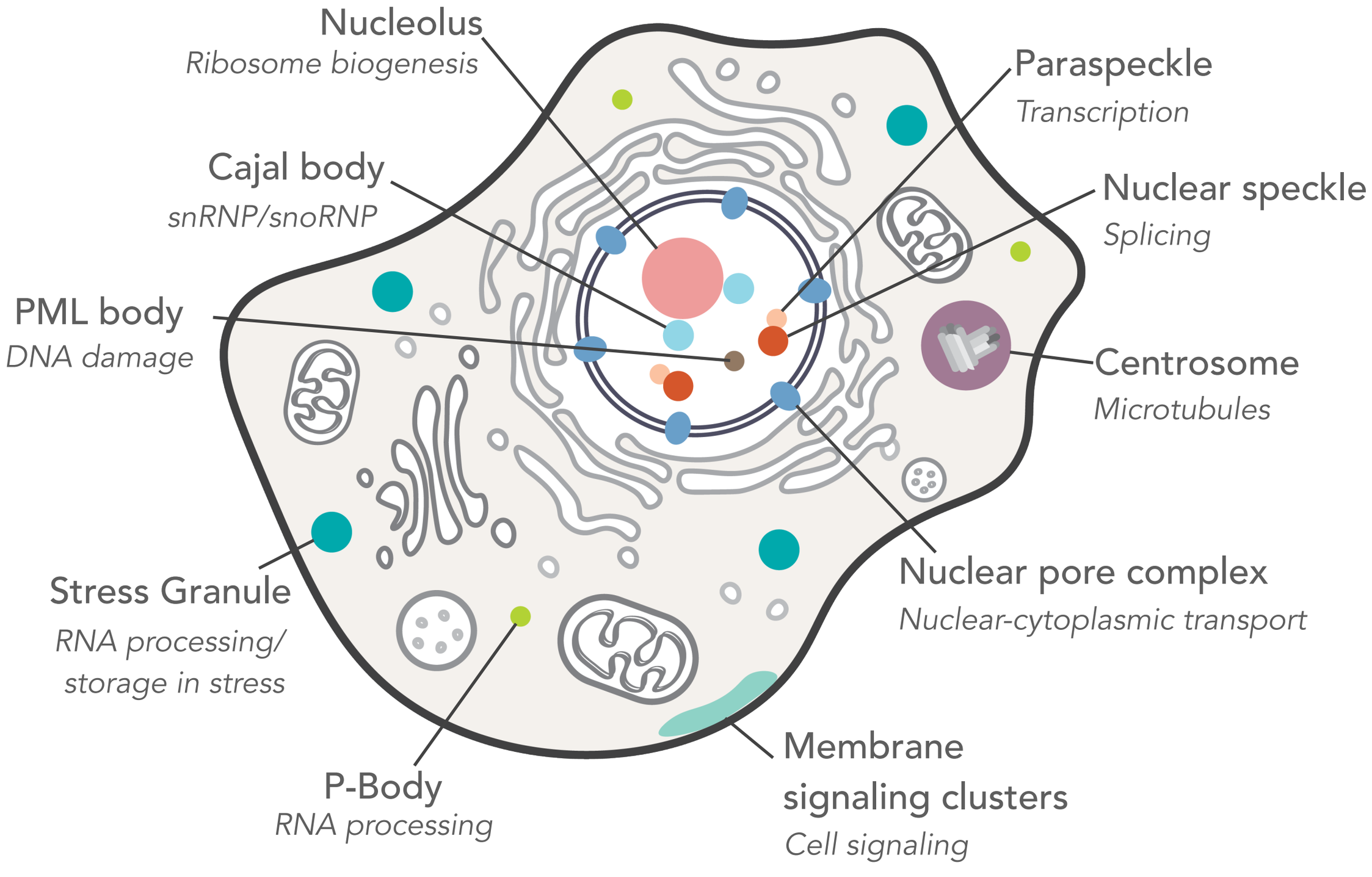
Recap: emergent features of LLPS and phase separation

- Interfacial tension, difference in diffusion of factors inside vs. out
- Shows liquid-like dynamics (fusion, high viscosity, dynamic exchange)
- Other phase transitions can lead to diverse material properties
- Various biophysical techniques in vitro and in cells can measure these features:
  - Phase diagrams: thermodynamics
  - FRAP: molecular dynamics
  - Rheology, fusion: viscosity, surface tension

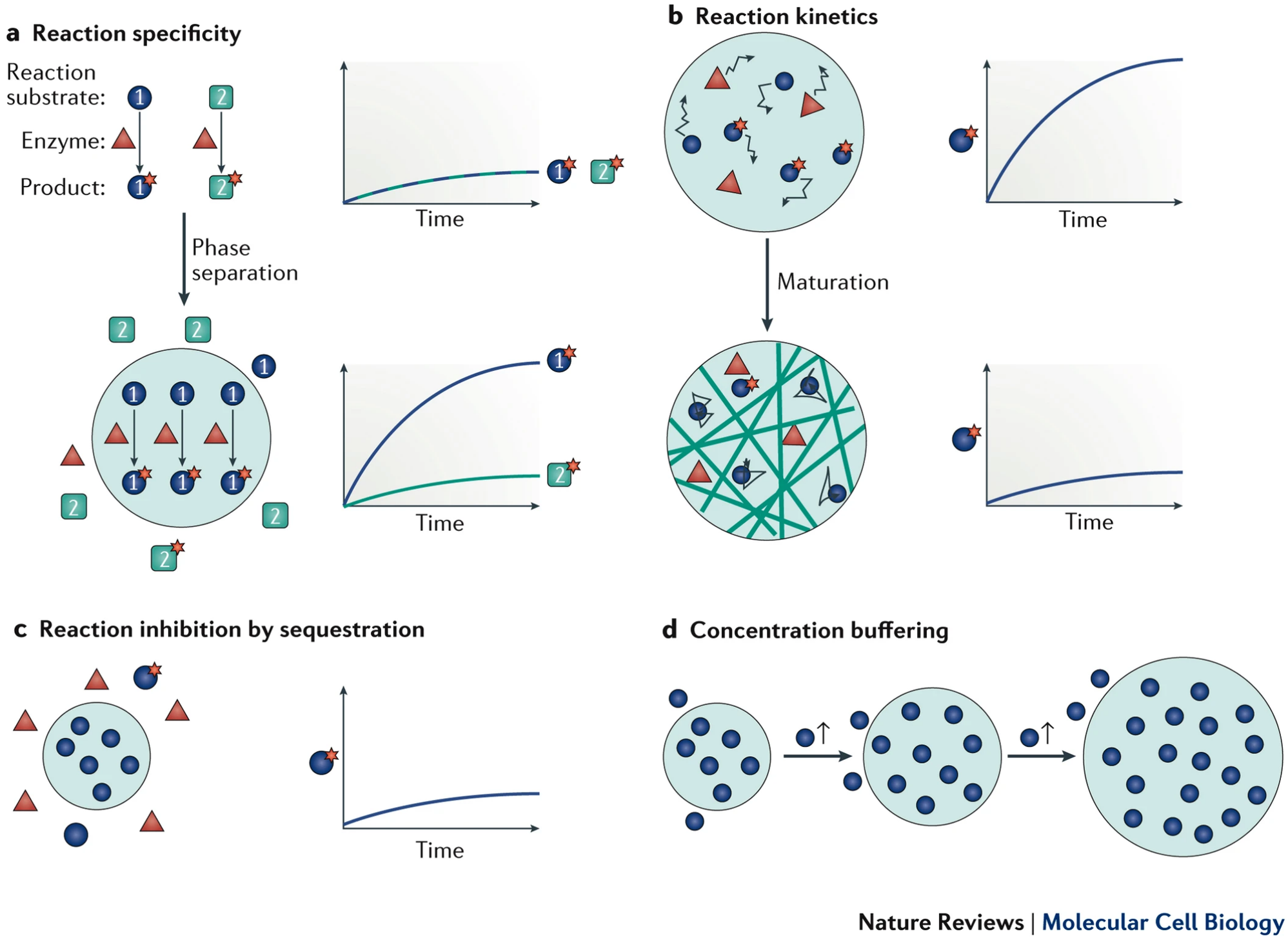
V. Questions? And break!

VI. Biological condensates and their (proposed) functions

Biomolecular condensates perform diverse functions



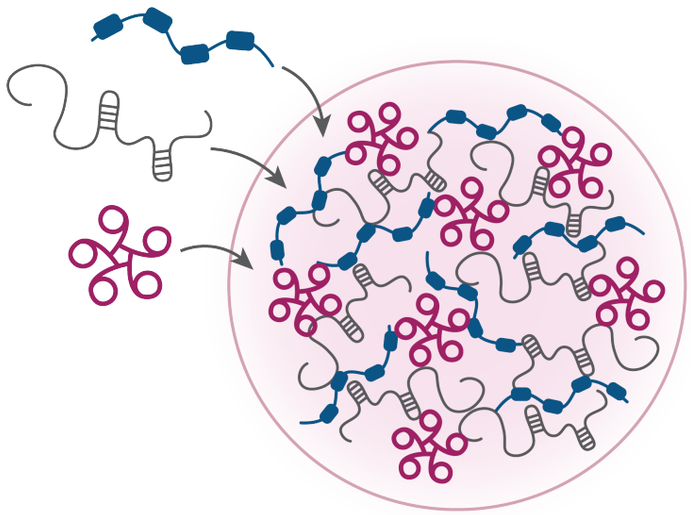
How might phase separation impact function?



Banani et al. and Rosen, 2017, PMID: 28225081

Condensates can influence biochemical reactions

Concentration and Compartmentalization



Reaction kinetics
Reaction specificity
Sequestration and storage
Buffering

Transcription

Hirose et al. 2014, PMID 24173718
Powers et al. 2019, PMID 31421981

Carbon fixation

Wang et al. 2019, PMID: 30675061
Wunder et al. 2018, PMID: 30498338

RNA degradation

Nadra et al. 2019, PMID 30197298

Immune signaling

Su et al. 2016, PMID 27056844
Du et al. 2018, PMID 29976794

Pre-mRNA processing

Nadra et al. 2019, PMID 30197298

Autophagy

Fujioka et al. 2020, PMID 32025038

RNA splicing

Ying et al. 2017, PMID 28708999

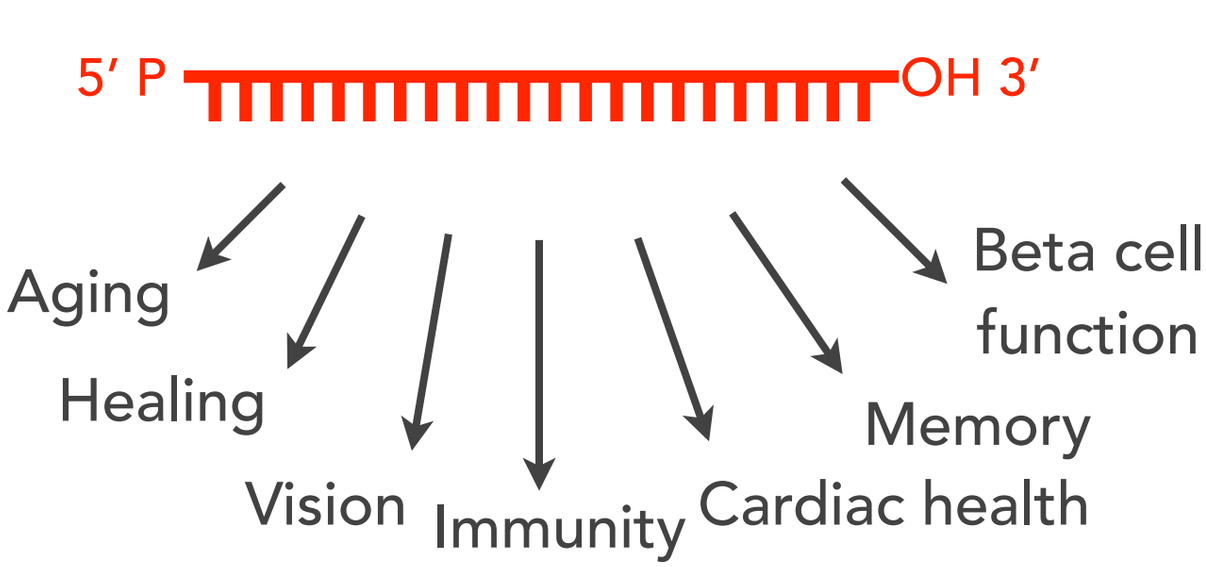
Synaptic transmission

Zeng et al. 2018, PMID 30078712

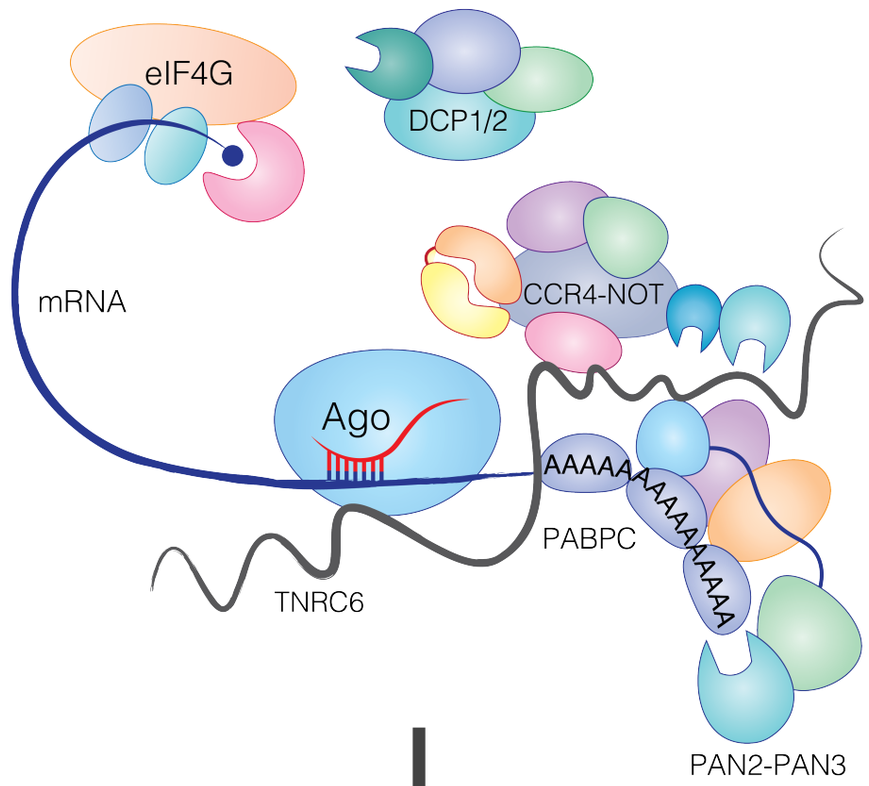
Biophysical assembly of the microRNA-induced silencing complex (miRISC)

microRNAs

- 100's of unique species
- Post-transcriptionally regulate ~50% of all mRNAs

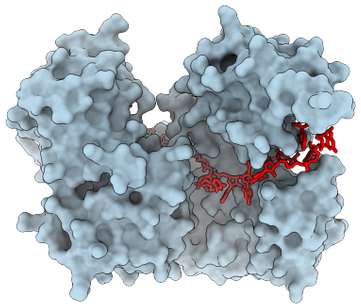


miRISC



Deadenylation of Targeted mRNAs

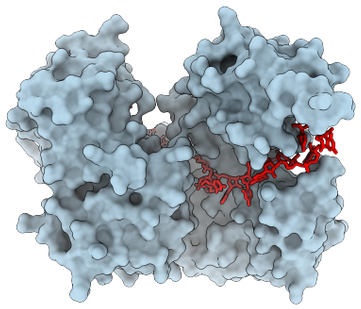
Reconstituted miRISC can phase separate

Argonaute 

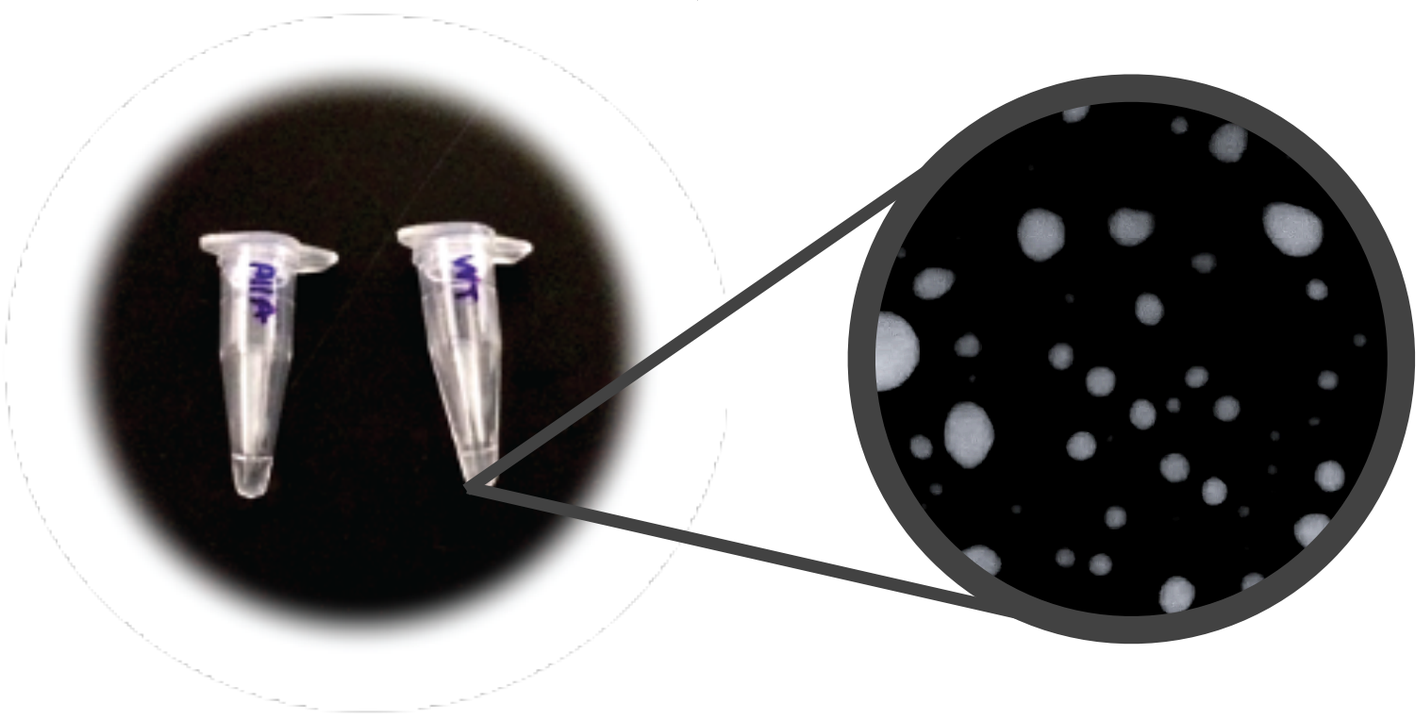
TNRC6

Sheu-Gruttadauria and MacRae 2018, PMID: 29576456

Reconstituted miRISC can phase separate

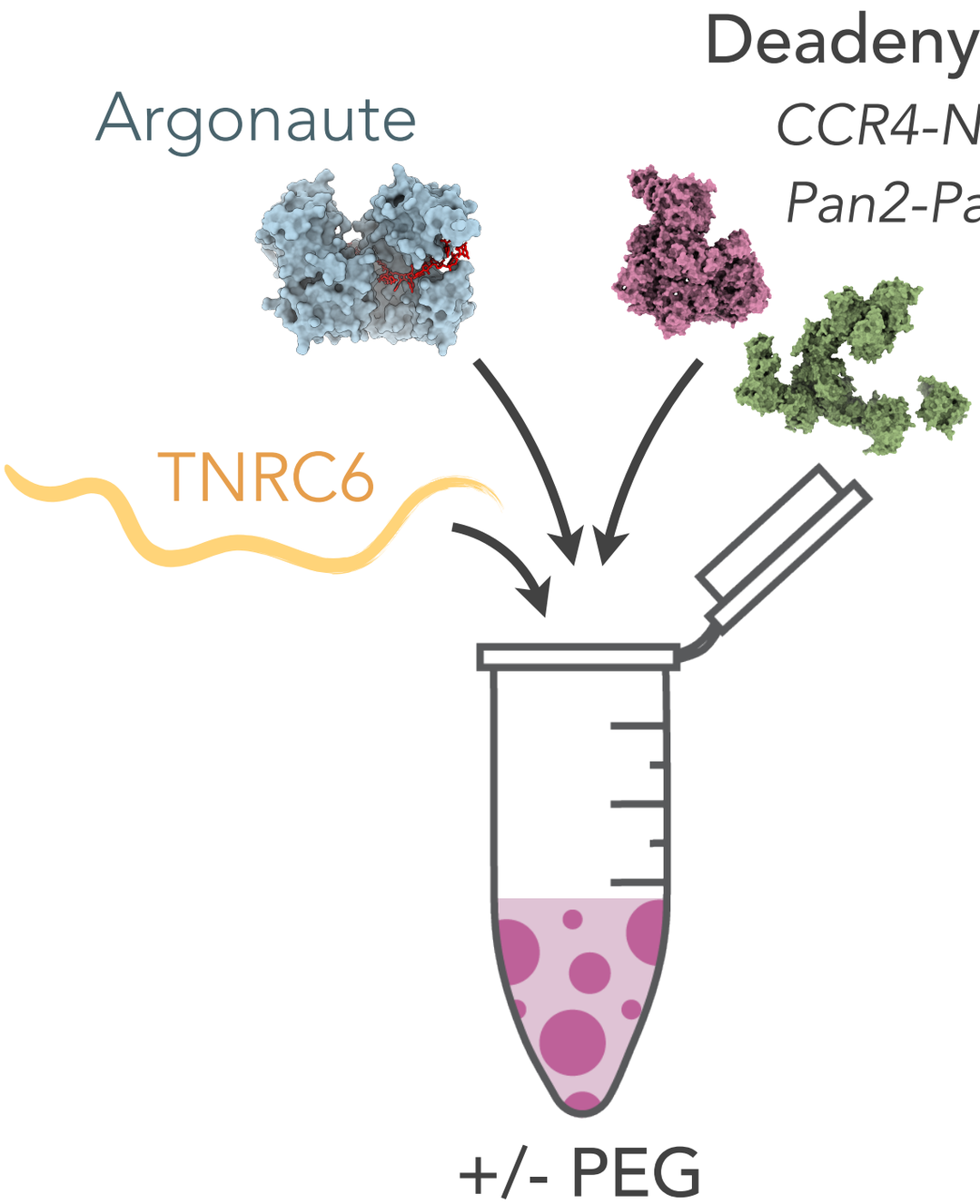
Argonaute 

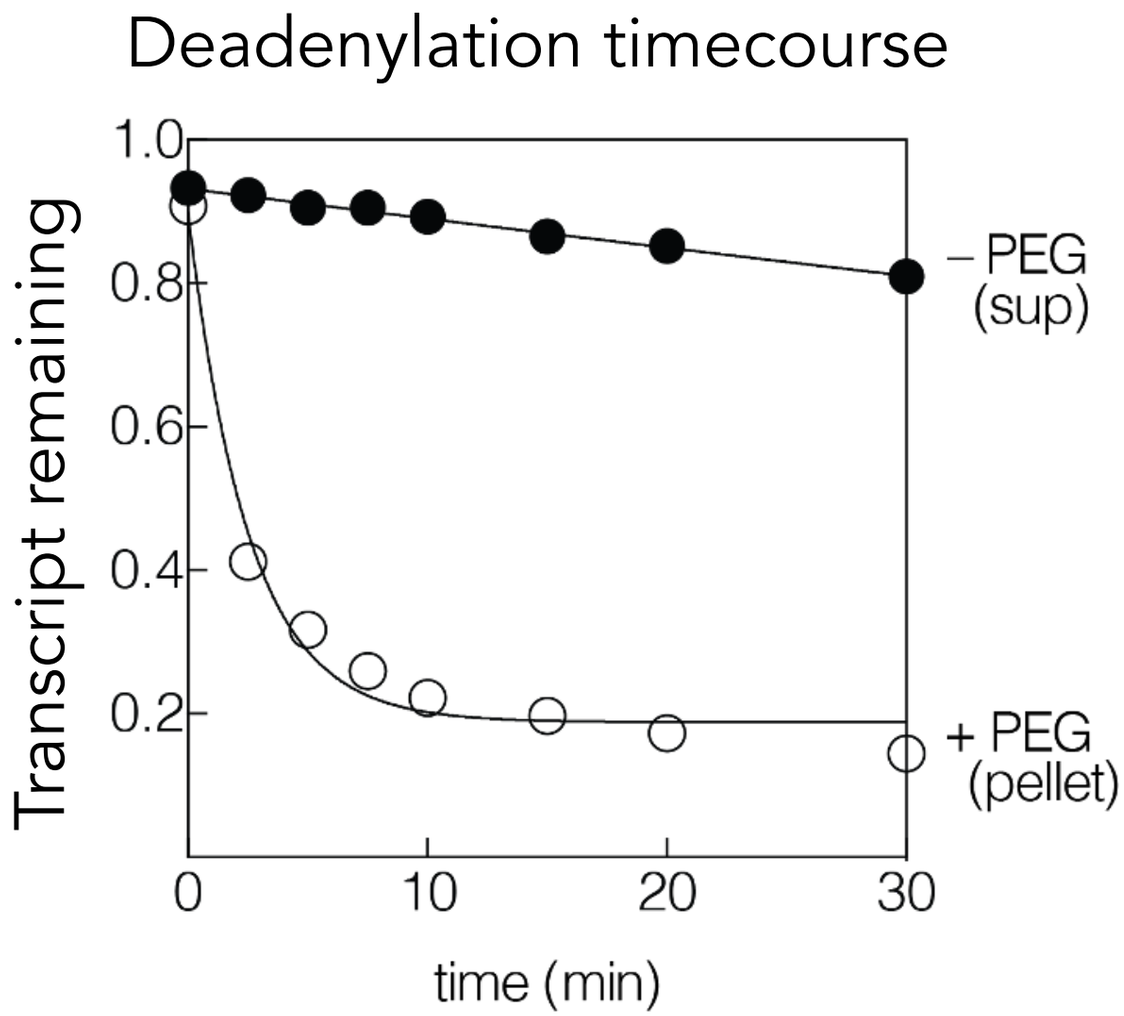
TNRC6



Sheu-Gruttadauria and MacRae 2018, PMID: 29576456

Phase separation facilitates enzymatic activity



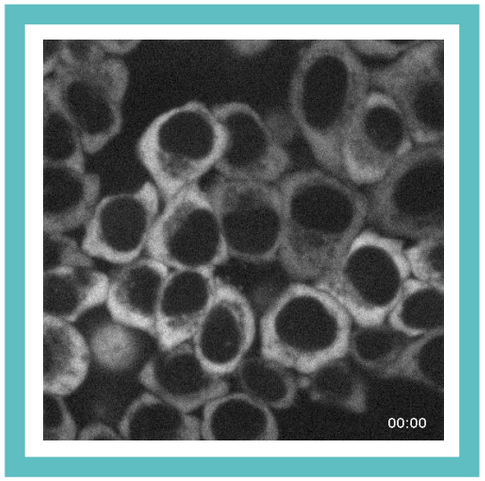


>100x faster!

Sheu-Gruttadauria and MacRae 2018, PMID: 29576456

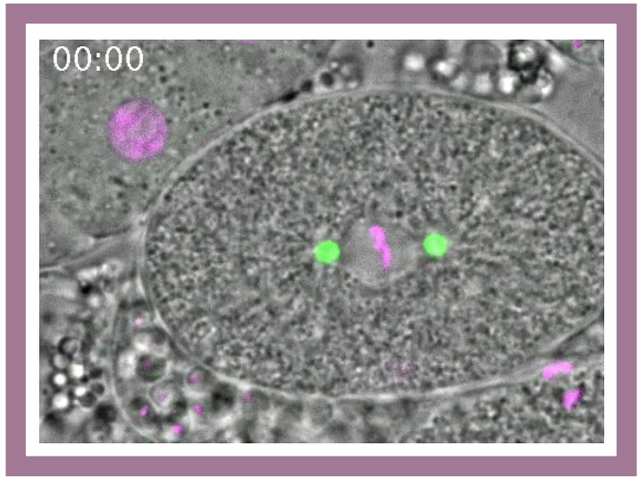
Material properties may influence function

Liquid



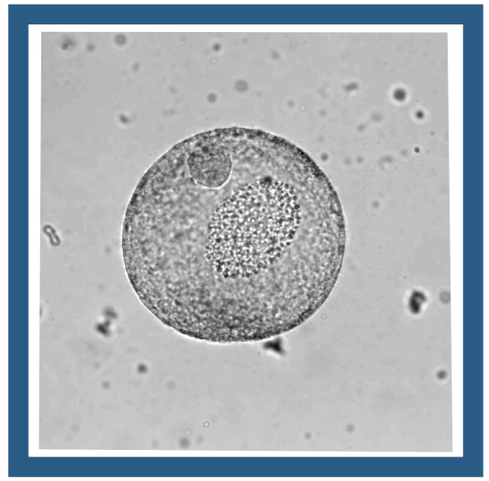
Stress Granule

Gel



Centrosome

Solid

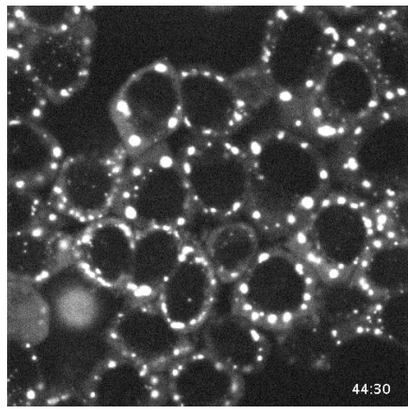


Balbiani Body

Mittasch et al. And Woodruff, 2020, PMID: 32050025; Boke et al. And Mitchison, 2016, PMID: 27471966

Material properties may influence function

Liquid

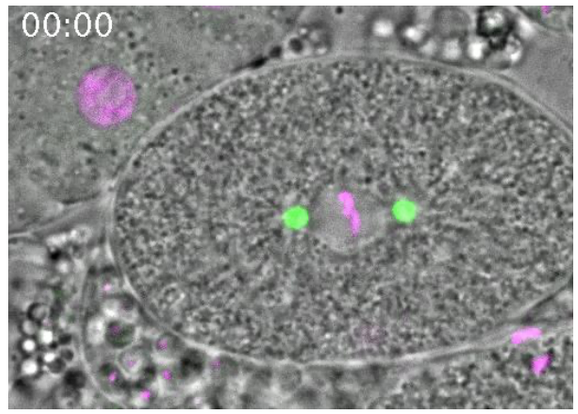


Stress Granule

↓

Responsive assembly
Dynamic processes
Reaction flux

Gel

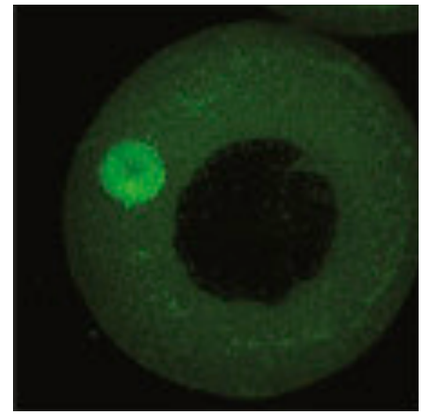


Centrosome

↓

Bear mechanical force

Solid



Balbiani Body

↓

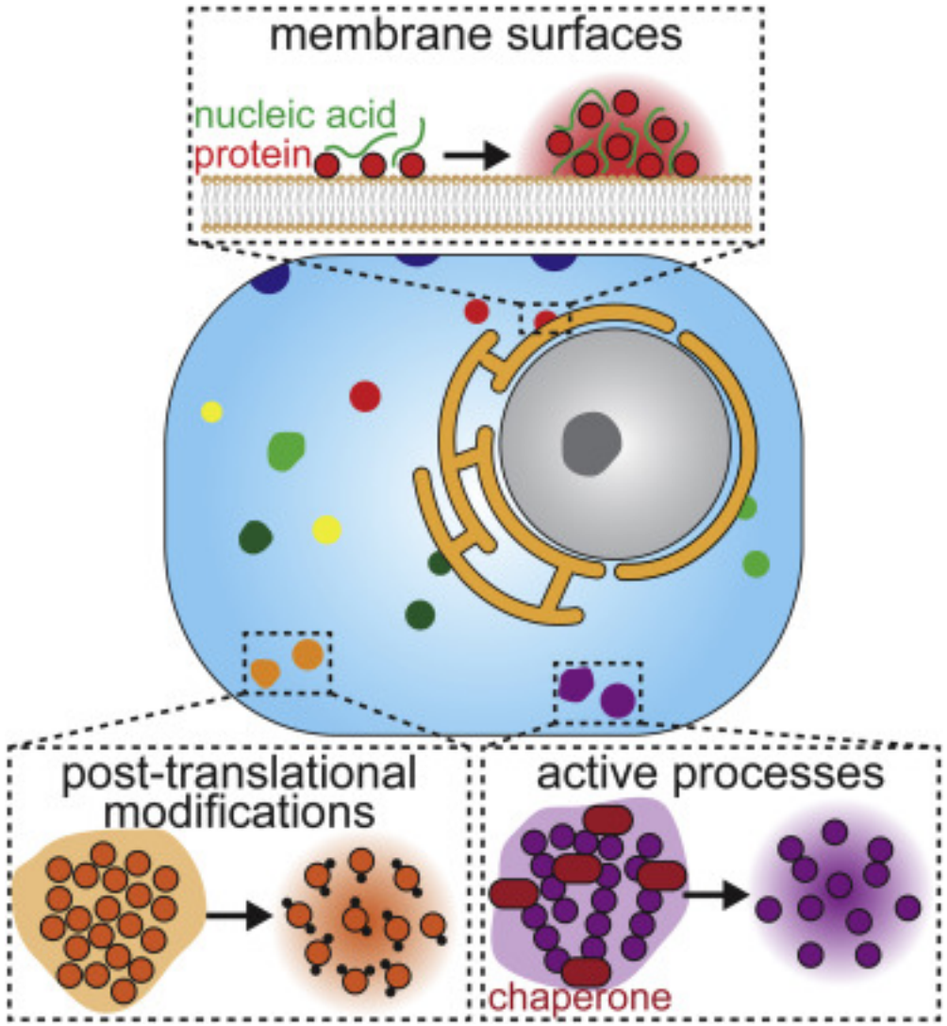
Sequestration

Mittasch et al. And Woodruff, 2020, PMID: 32050025; Boke et al. And Mitchison, 2016, PMID: 27471966

How might condensates be regulated?

Alters critical drivers/features of phase separation:

Concentration



Valency, affinity

Material state

Snead and Gladfelter 2019, PMID: 31604601

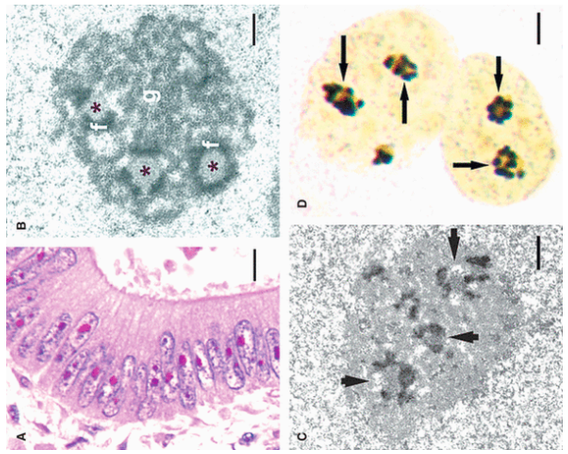
VII. Condensates in disease and therapeutics

Condensates in disease

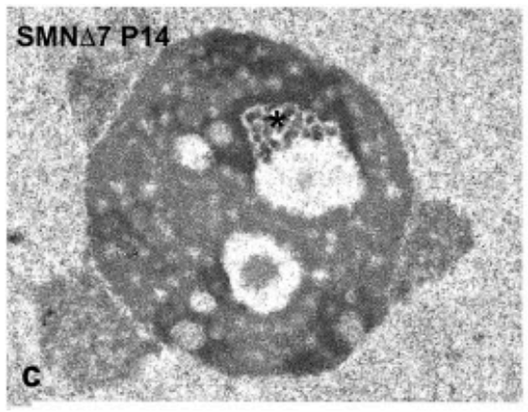
Loss of function

Disruption of normal activity

- Disrupted nucleoli in cancers
- Disrupted Cajal bodies in SMA



Many cancers

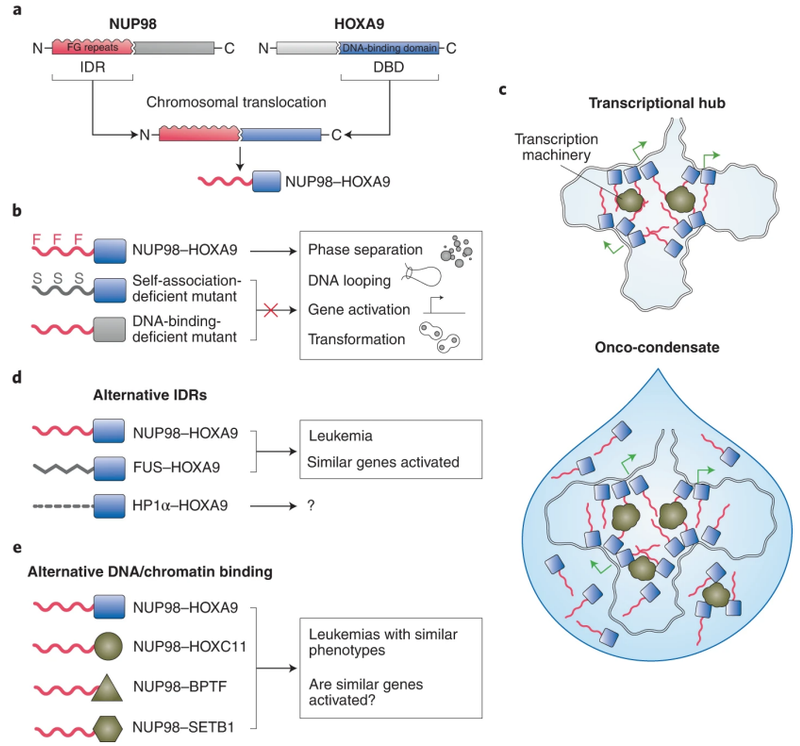


Spinal Muscular Atrophy

Gain of function

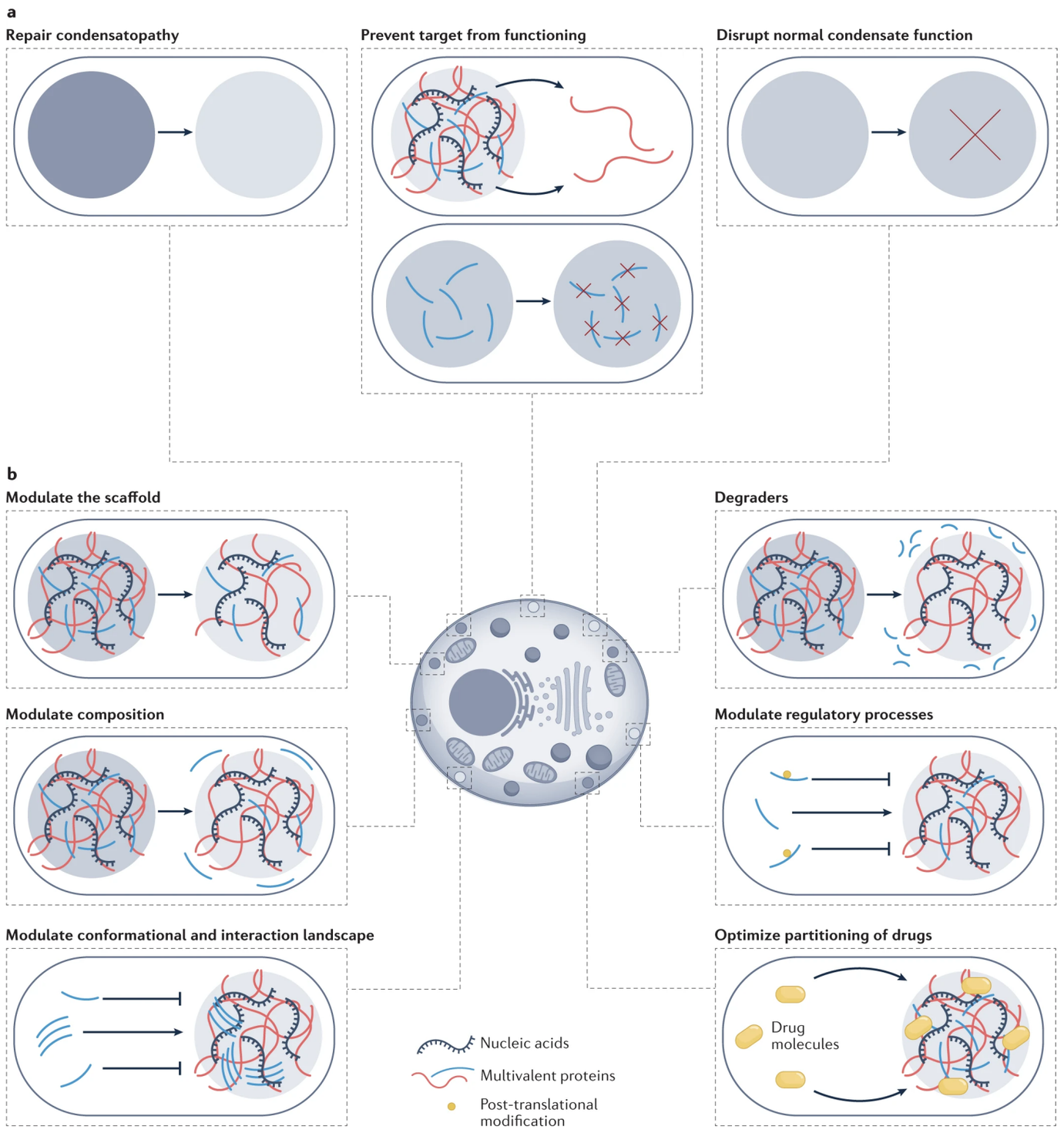
Generation of new activity

- Onco-condensates
- Plaques in neurodegeneration



Derenzini et al. 2009, PMID: 191785882009; Tapia et al. 2021, PMID: 28823932; McMillan et al. 2021, PMID: 34187600, Fasciani et al. 2020, PMID: 33169020; Hallacli et al. 2022, PMID: 35688132; Chew et al. 2019, PMID: 30767771; Prophet et al. 2022, PMID: 36302970; An et al. 2019, PMID: 30642400; Mittag and Ansari 2021, PMID: 34163089

Therapeutically targeting condensates

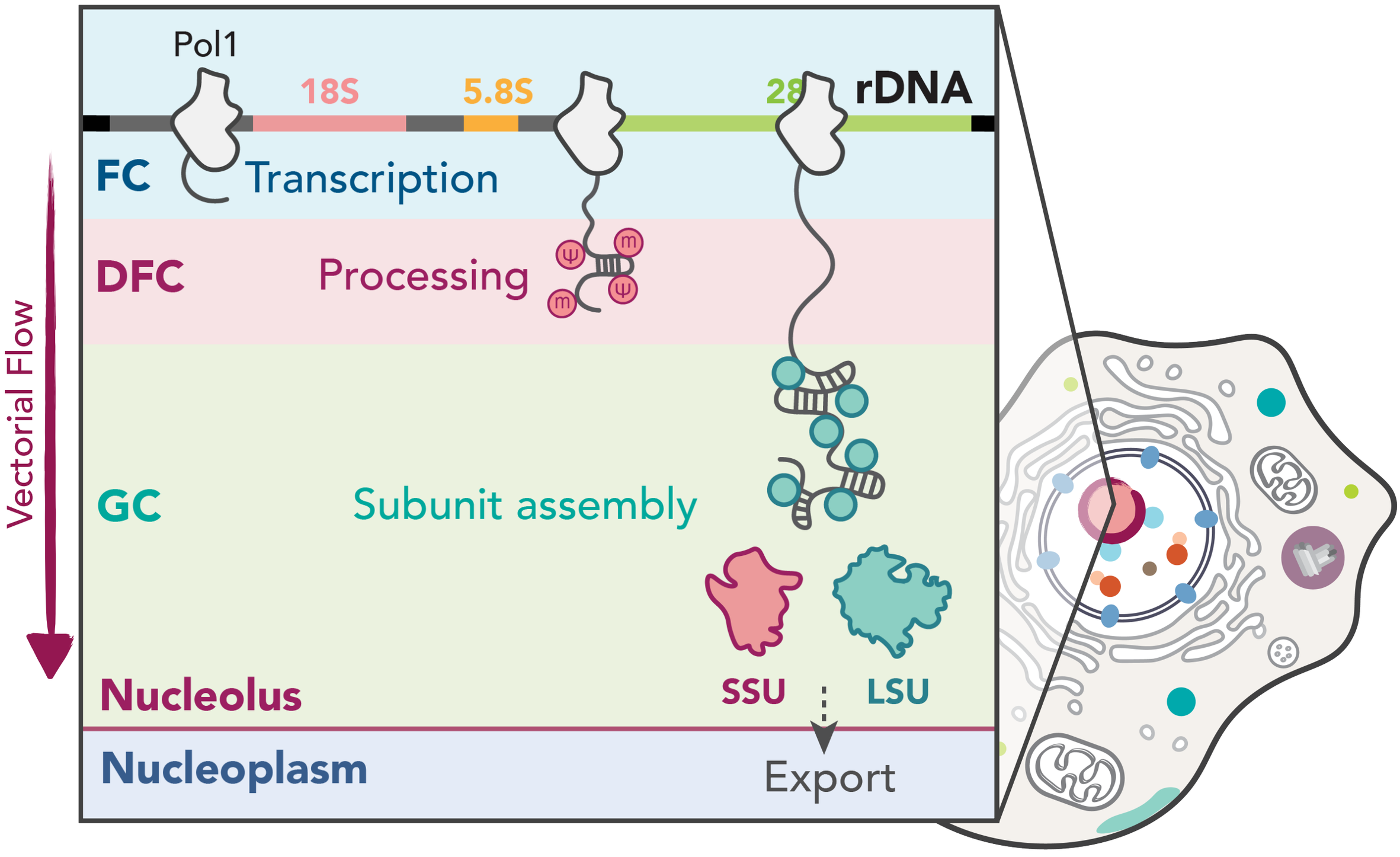


Mitrea et al. 2022, PMID: 35974095

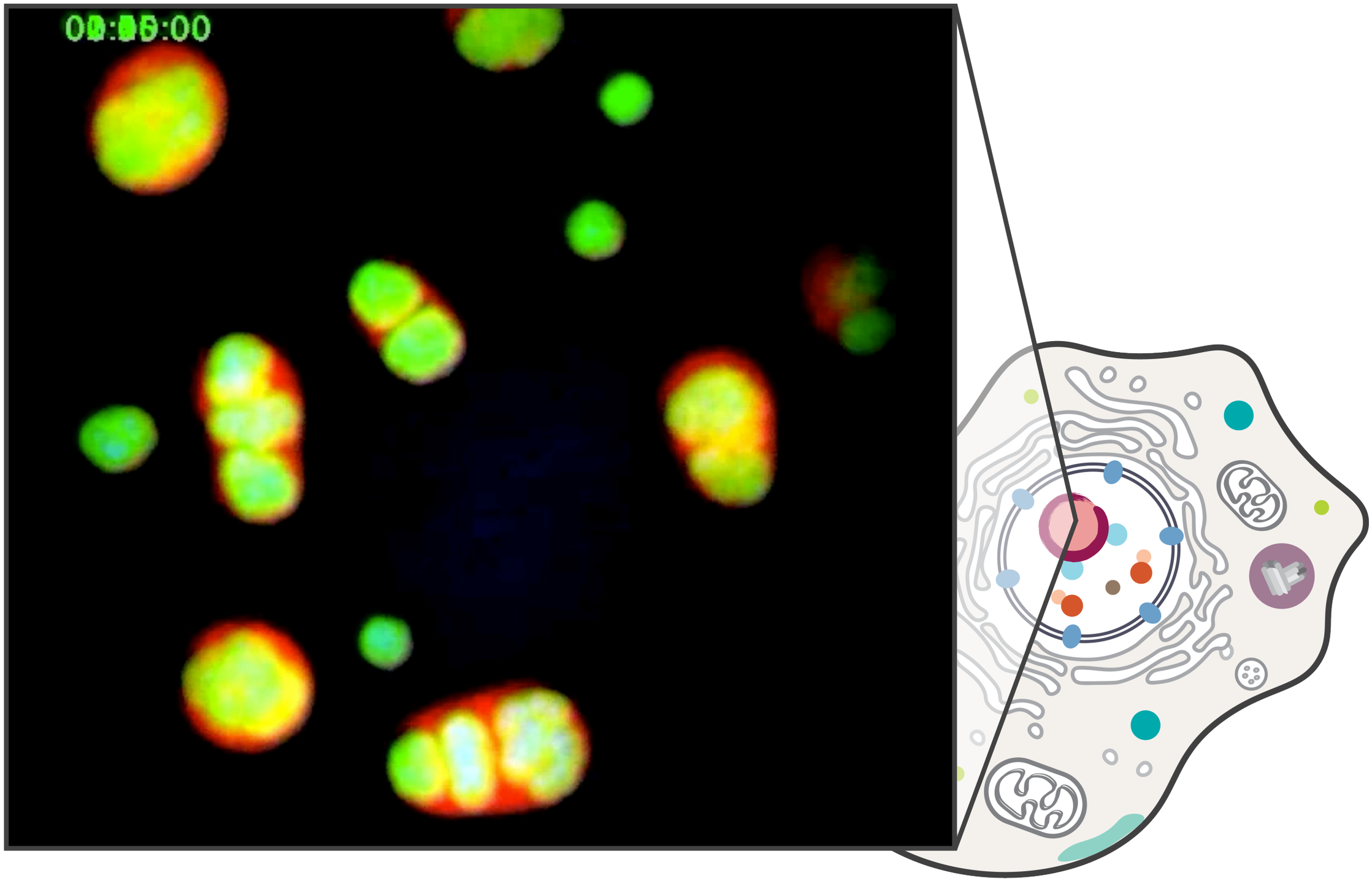
VIII. Scientific controversy

Question: what is the evidence for and against LLPS as mechanism for nucleolar assembly?

The nucleolus: a ribosome factory



The nucleolus: a multiphase condensate?



Feric et al. 2016, PMID: 27212236