Biological condensates: cellular mechanisms governed by phase transitions
Isaac Newton Institute, Cambridge, 12 October 2023

Conversion-limited phase separation in biomolecular condensation

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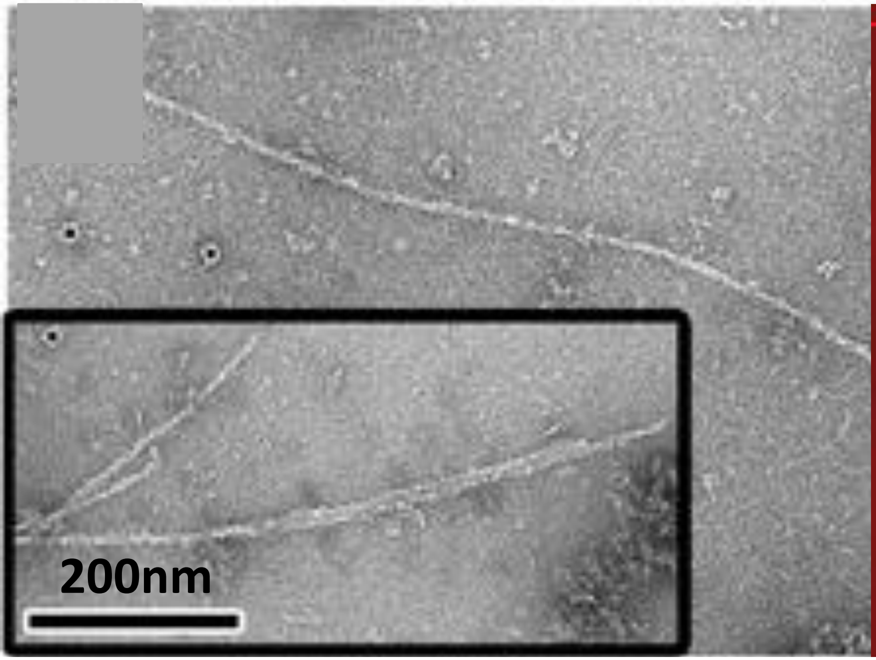
Imperial College London

Universality in Biology

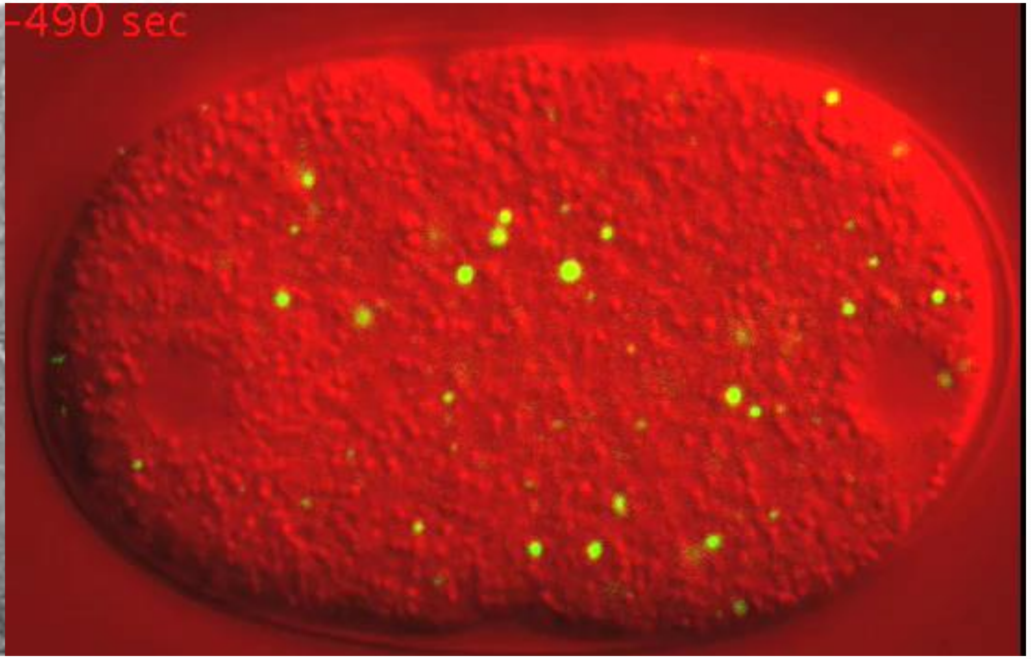
Universal physics in biology

Amyloid formation



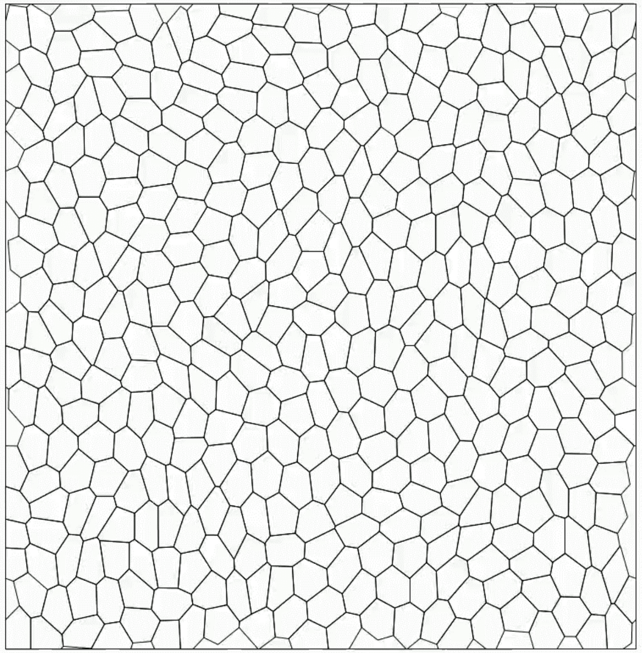
Pytowski, Lee, Foley, Vaux, Jean (2020)
Liquid–liquid phase separation of type II diabetes associated IAPP initiates hydrogelation and aggregation
PNAS

Biomolecular condensates



Brangwynne, Eckmann, Courson, Rybarska, Hoege, Gharakhani, Jülicher, Hyman (2009) Science

Active matter



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Polar Fluctuations Lead to Extensile Nematic Behavior in Confluent Tissues
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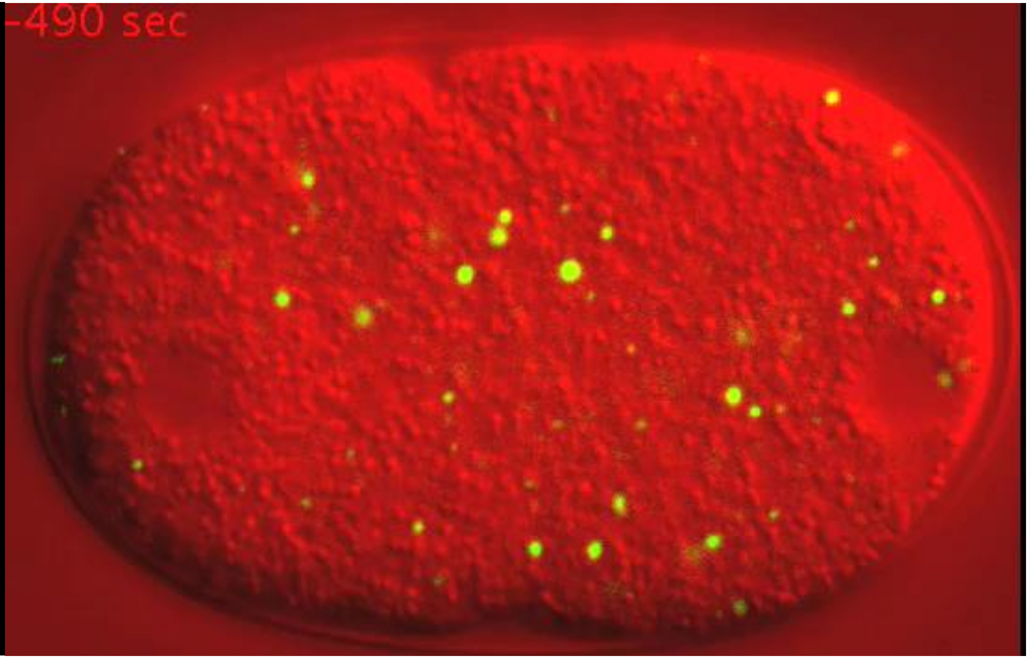

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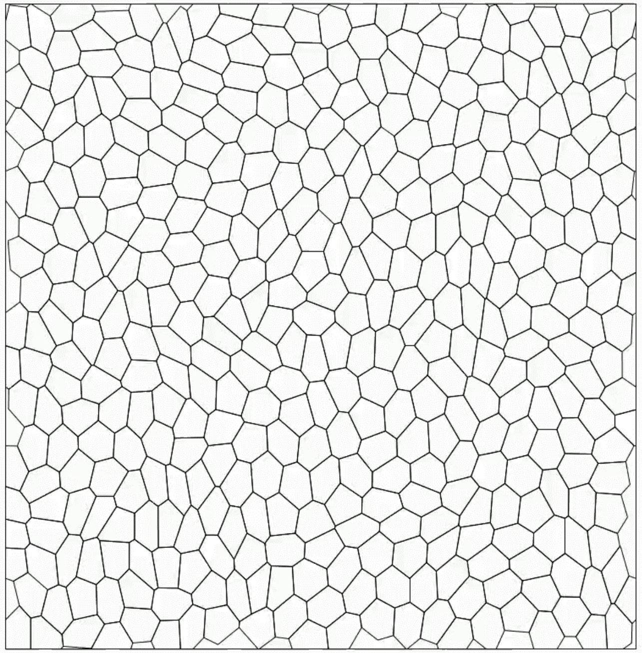

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BBSRC
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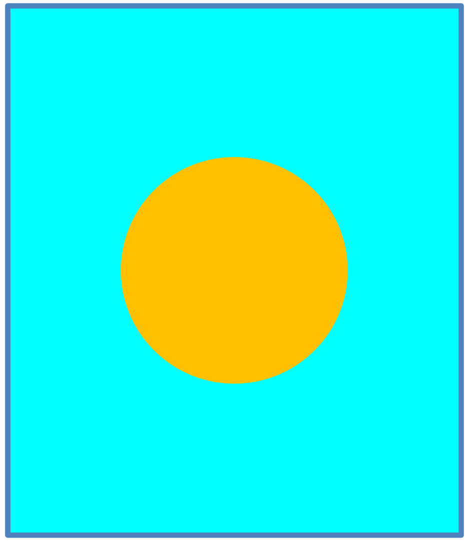
Plan

1. Coarsening in phase separation
2. Coarsening is very slow in biomolecular condensation, why?
3. Resolution – rugged energy landscape in protein conversion
4. Conversion-limited phase separation
5. Summary & outlook

1. Coarsening in phase separation

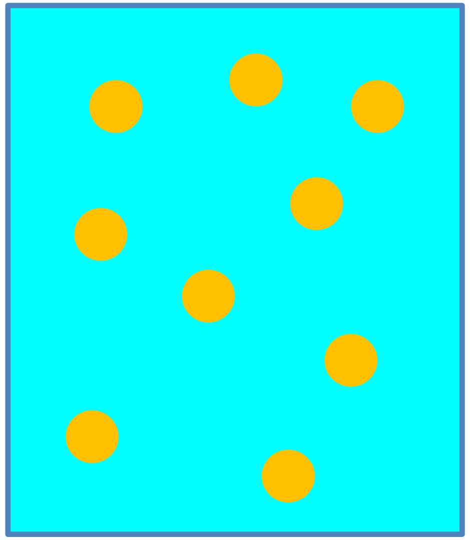
Phase separation vs. emulsion

Phase separation



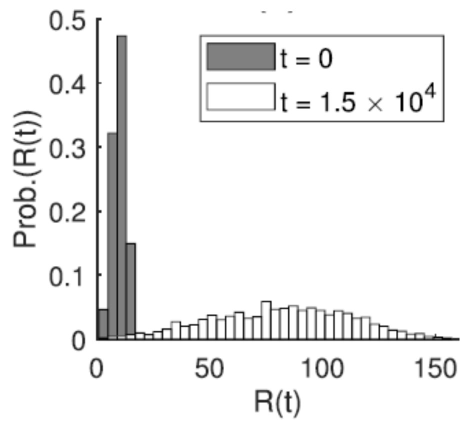
Free energy minimisation →

A stable emulsion

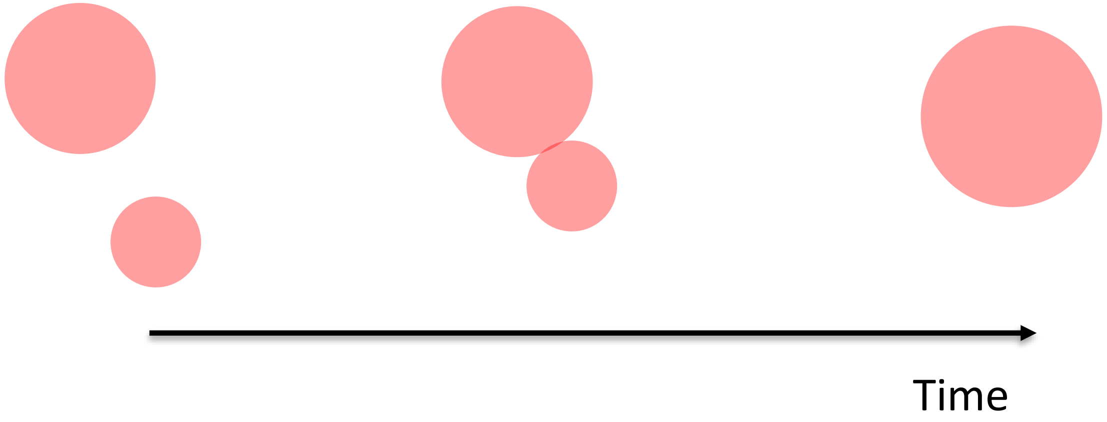


But this is what we see in cells →

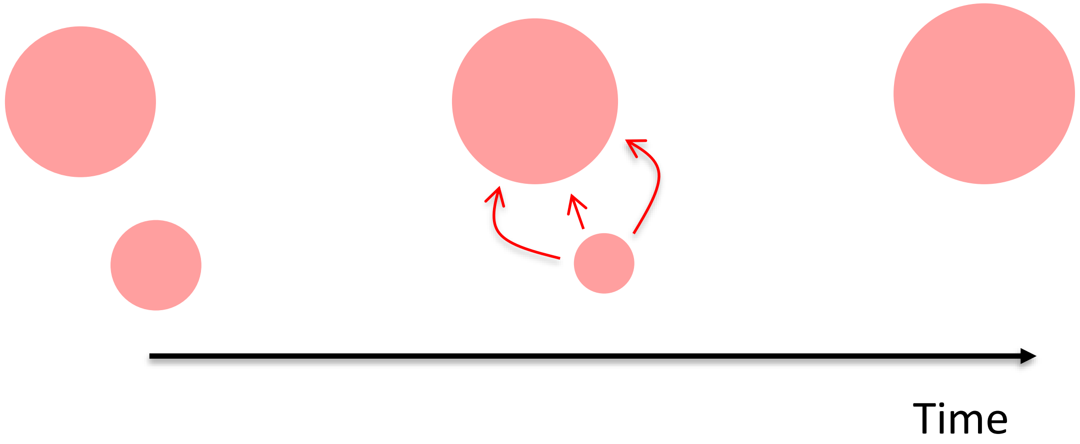
How systems coarsen



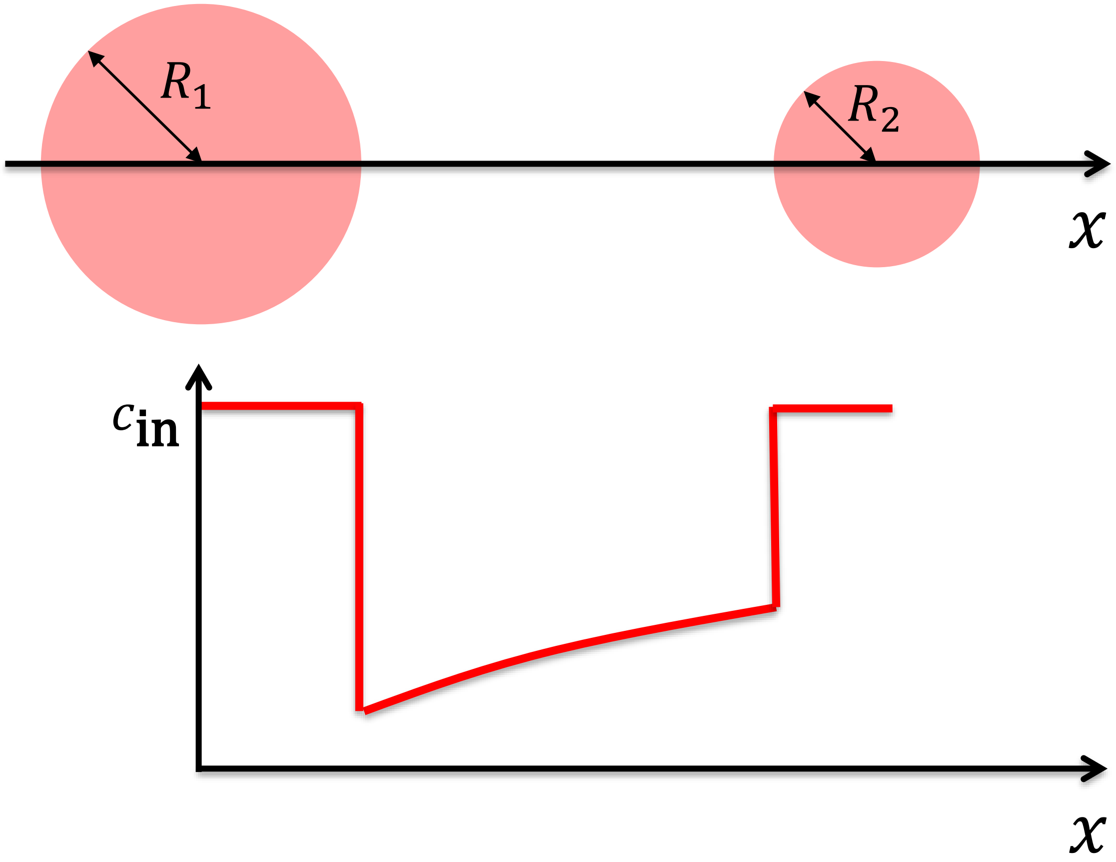
Coalescence



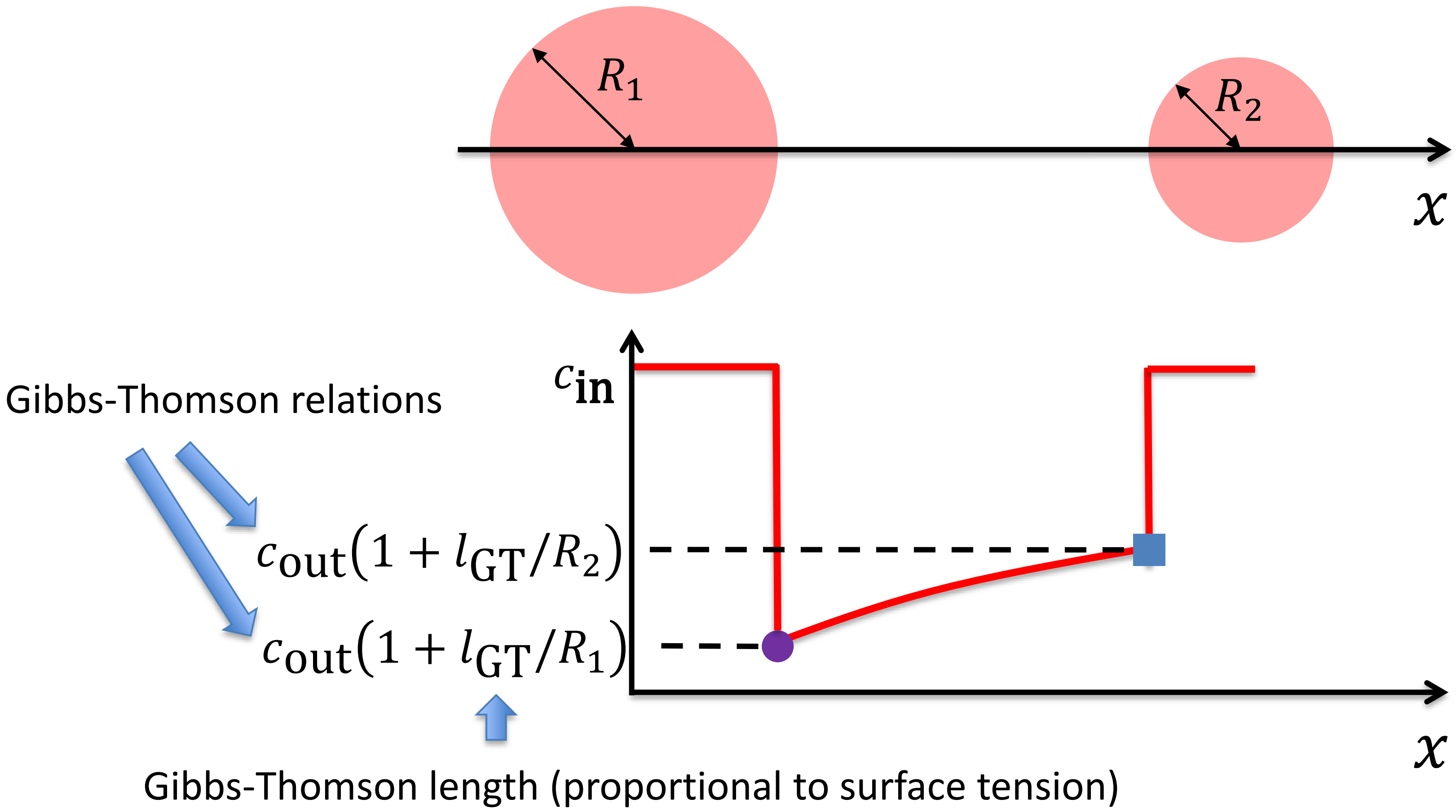
Ostwald ripening



Ostwald ripening



Ostwald ripening



First attempts at explaining away coarsening

- For coalescence
  - Almost absent because of corralling due to cytoskeleton and molecular crowding

- For Ostwald ripening
  - Almost absent because of small surface tension

Problem: Not so simple

Using typical parameters, 85% of PGL granules would have disappeared in 6min under Ostwald ripening

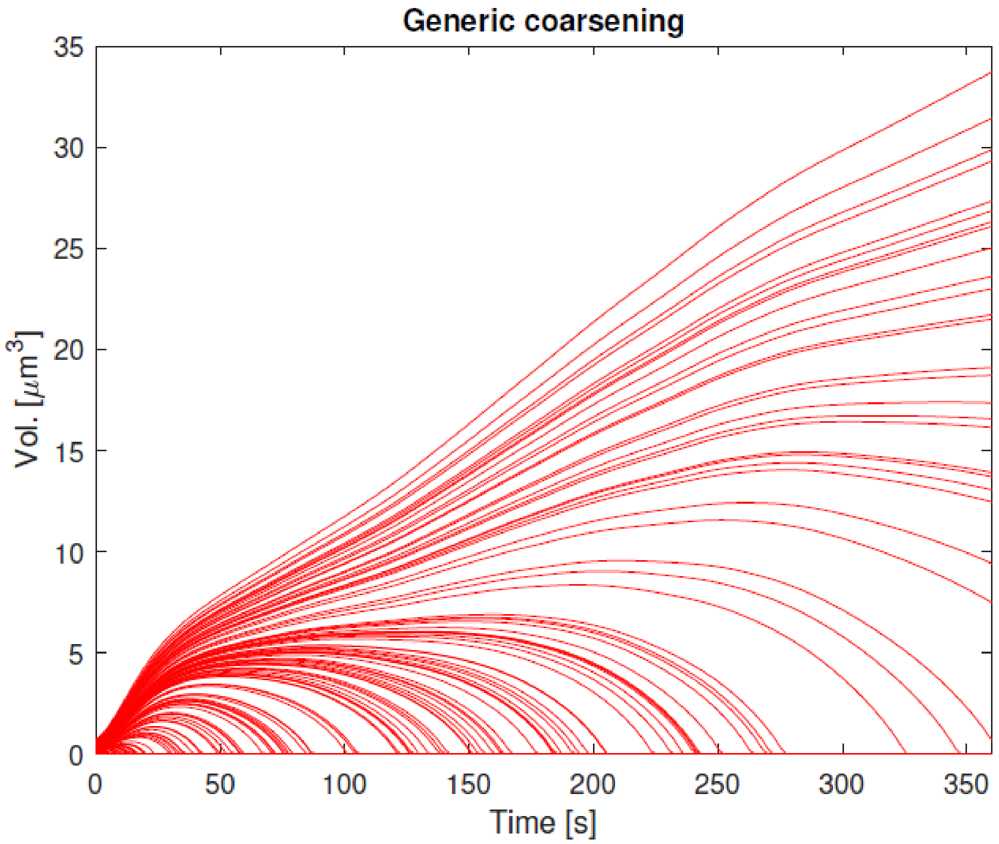


TABLE I: Parameters are estimates taken from [2]

Parameters	Value
Typical monomer volume, ν	1 nm^3
Capillary length, l_c	15 nm
Typical monomer diffusion coefficient, D	30 μm^2/s
Partition coefficient, $c_{\text{in}}/c_{\text{out}}$	20
Embryo volume	30 pL

A.W. Folkmann, A.A. Putnam, CFL, and G. Seydoux (2021)
Regulation of biomolecular condensates by interfacial protein clusters
Science 373 1218

2. Coarsening is very slow in biomolecular condensation, why?

More sophisticated resolutions needed !?

- Driven chemical reactions converting proteins between soluble and phase separating states

  [D Zwicker et al (2015) PRE; JD Wurtz & CFL (2018) PRL; Weber, Zwicker, Lee & Jülicher (2019) Rep Prog Phys]

- Mechanical suppression of drop growth via cytoskeletal networks

  [M Feric et al (2013) Nat Cell Biol; RW Style et al (2018) PRX; KA Rosowski et al (2020) Nat Phys]

- Subdiffusion of drops

  [DSW Lee et al (2021) Nat Phys]

- Pickering effects (coating of drop surface to reduce surface tension)

  [AW Folkmann et al (2021) Science]

More sophisticated resolutions needed !?

- Driven chemical reactions converting proteins between soluble and phase separating states

  -> Stop Ostwald ripening & coalescence through driven chemical reactions (2019) Rep Prog Phys]

- Mechanical suppression of drop growth via cytoskeletal networks

  -> Stop Ostwald ripening & coalescence due to a 'rigid' network l (2020) Nat Phys]

- Subdiffusion of drops

  -> Stop coalescence due to a 'rigid' network

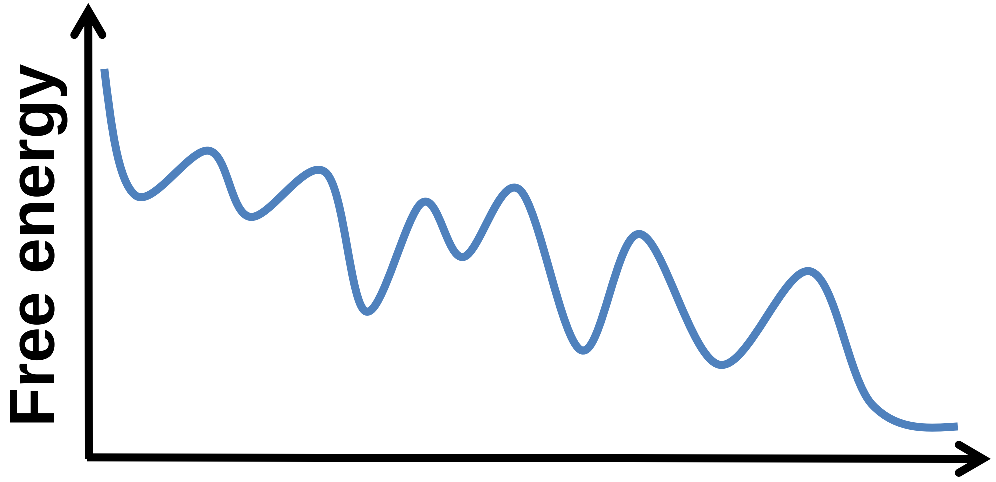
- Pickering effects (coating of drop surface to reduce surface tension)

  -> Stop Ostwald ripening & coalescence through surface tension-reducing coating

Is there a more fundamental reason?

- Occam's razor: "*Plurality must never be posited without necessity.*"

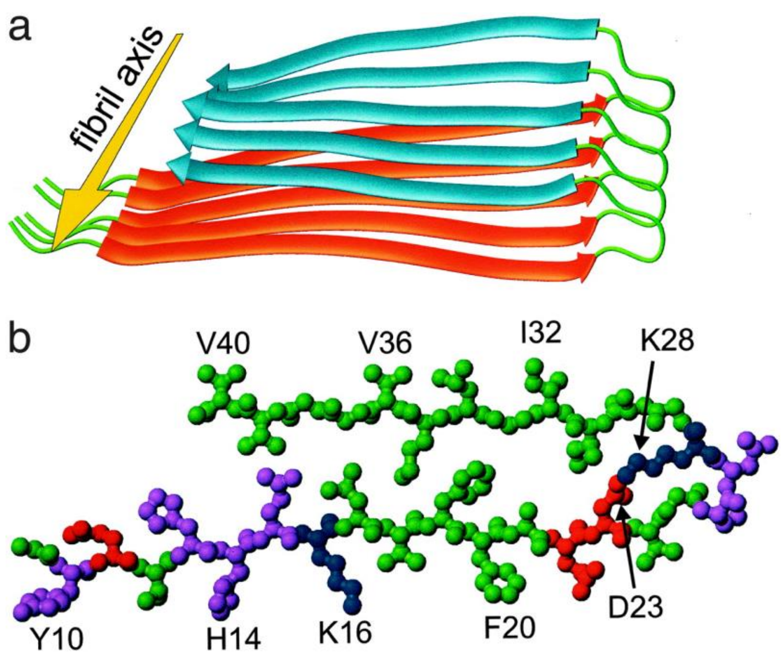
- Indeed, slow kinetics has been observed in a different kind of biomolecular system



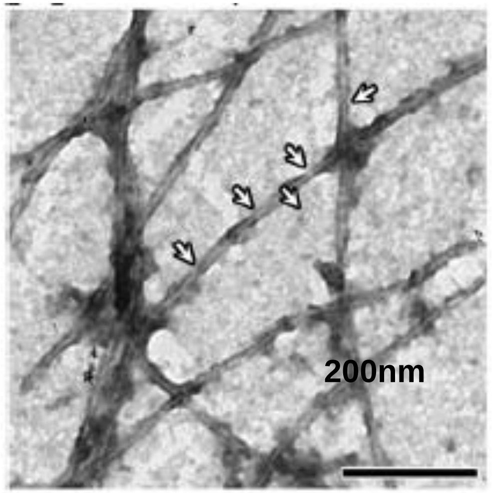
3. Resolution – rugged energy landscape in protein conversion

Drawing inspiration from another system: amyloid fibrils

Abeta amyloid fibrils



AT Petkova et al (2002) PNAS

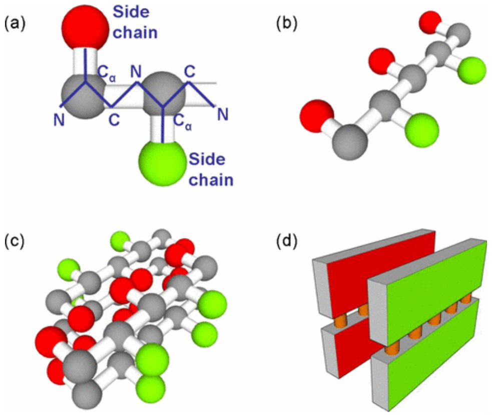


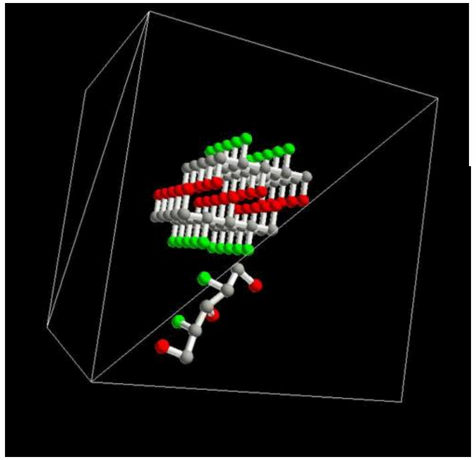
- Amyloid fibrils consist of cross beta sheets
- Fibrils elongate by incorporating free peptide monomer in solution

Slow elongation dynamics

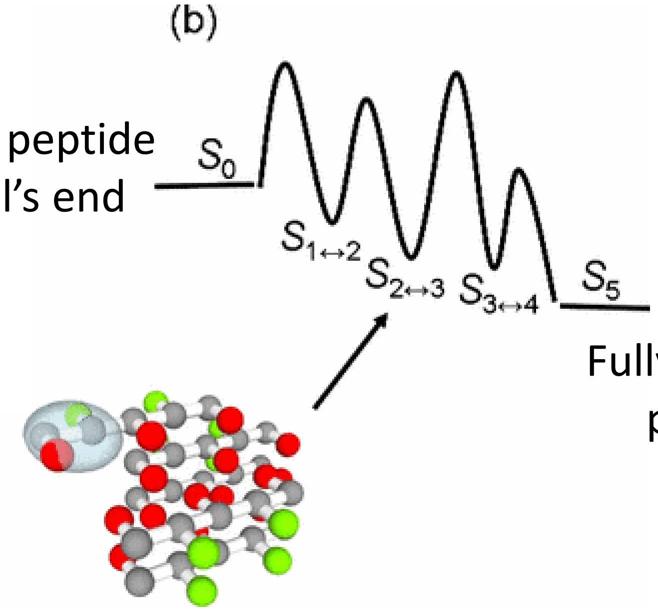
- Elongation dynamics was found to be slow (e.g., ~1 peptide per second under in vitro conditions [e.g., Ban et al (2004) JMB]), even though
  - No driven chemical reactions
  - No rigid networks limiting fibril growth
  - No subdiffusive behaviour

Resolution – a rugged energy landscape picture





Monomeric peptide at the fibril's end



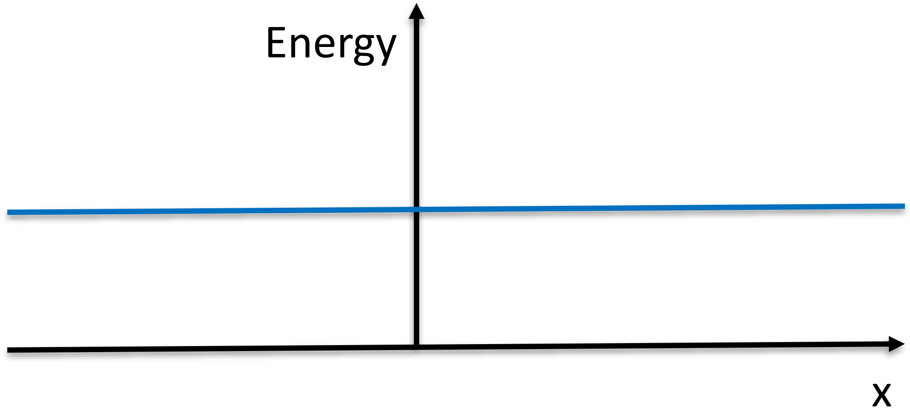
Fully fibrilised peptide

Diffusion over rugged energy landscape can be dramatically slowed down

CFL, J Loken, L Jean and DJ Vaux (2009) *Elongation dynamics of amyloid fibrils: A rugged energy landscape picture.* Phys Rev E

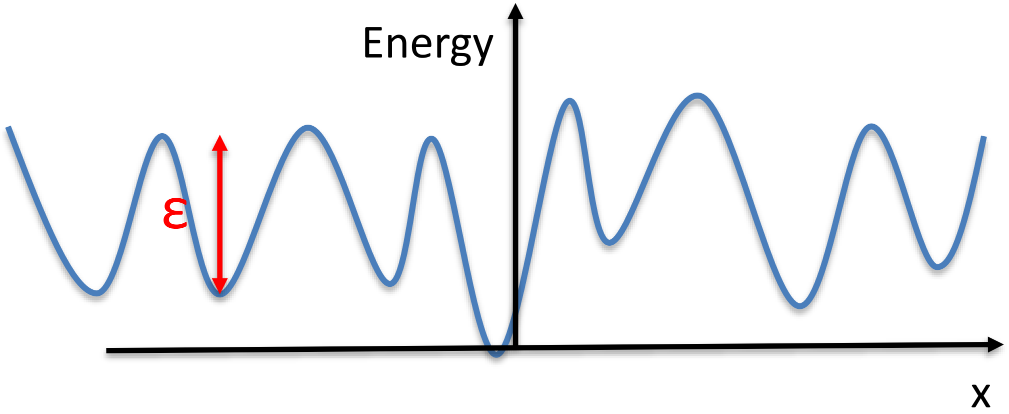
A cartoon explanation in 1D

Normal diffusion



Diffusion coefficient: D

Diffusion over a rugged energy landscape



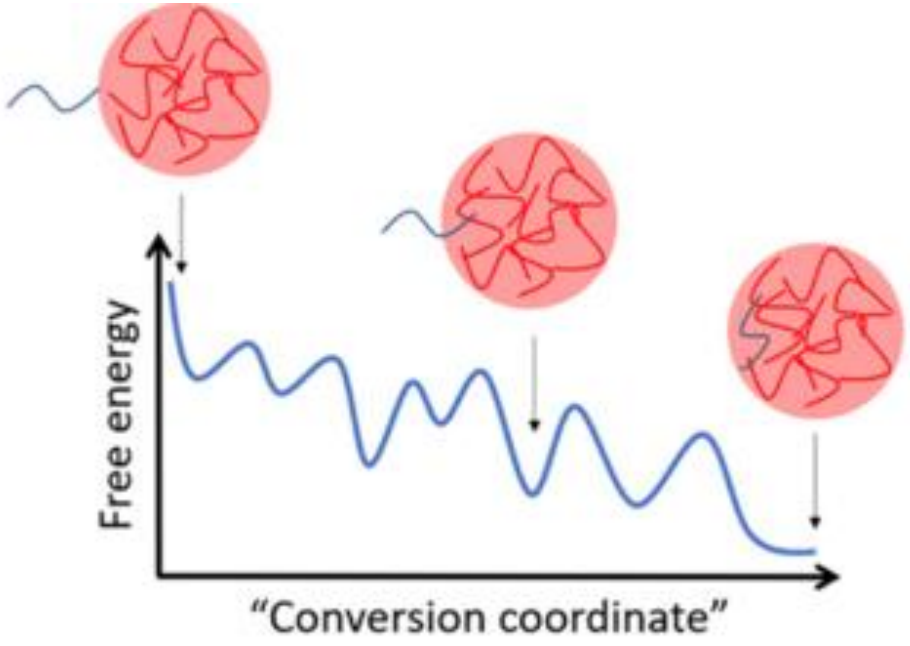
Analytical results: $D_{eff} = D \exp\left[-\left(\frac{\varepsilon}{k_B T}\right)^2\right]$

E.g.: if $\varepsilon = 2k_B T, D_{eff} \approx 0.01 \times D$

Conversion over a rugged energy landscape can be dramatically slowed down!

R Zwanzig (1998) *Diffusion in a rough potential.* PNAS

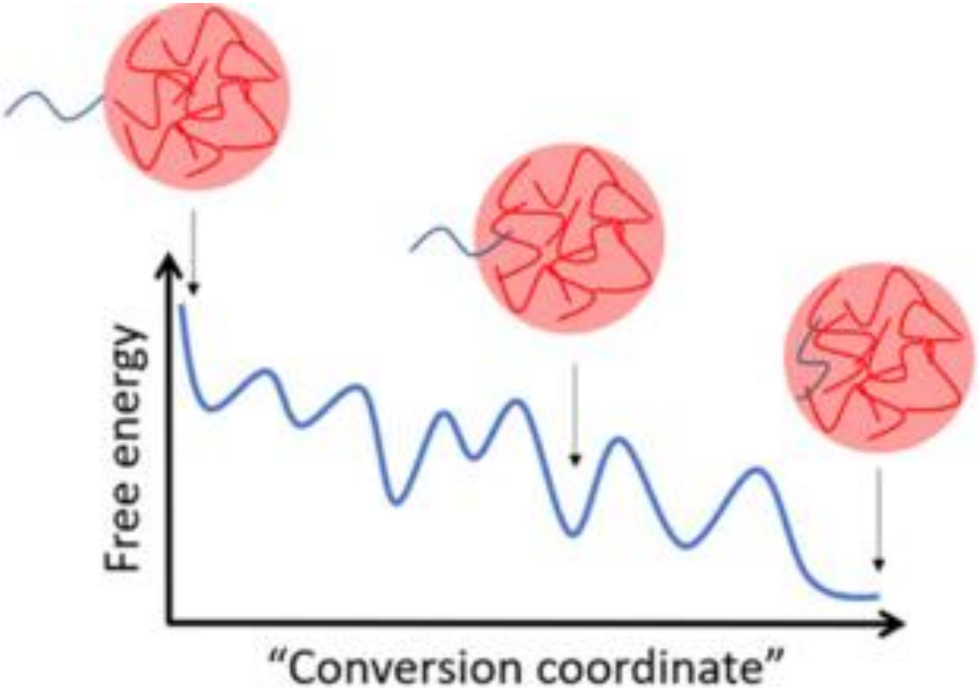
Back to biomolecular condensates



Picture from the BBC

Conversion over a rugged energy landscape can be dramatically slowed down!

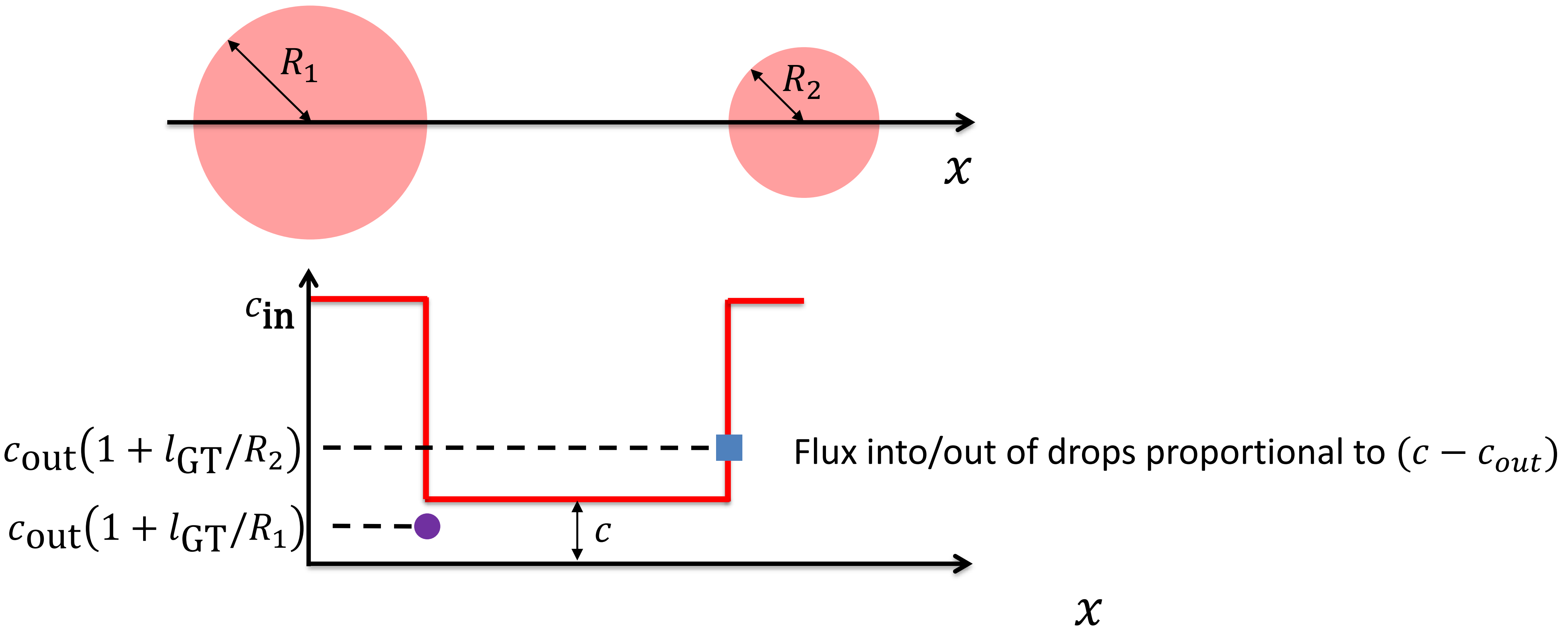
AW Folkmann, A Putnam, CFL, & G Seydoux (2021) Science
CFL (2021) Physical Review Research
See also: Choi, Holehouse, & Pappu (2020) Ann Rev Biophys
Ranganathan & Shakhnovich (2020) eLife
Takaki, Jawerth, Popović & Jülicher (2023) PRX



5. Conversion-limited phase separation

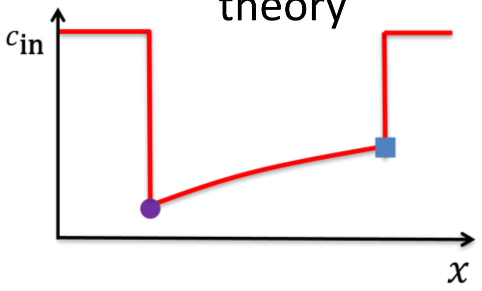
Conversion-limited phase separation

Coarsening rate limited by peptide conversion rates (free ↔ condensates), not diffusion of monomeric peptides

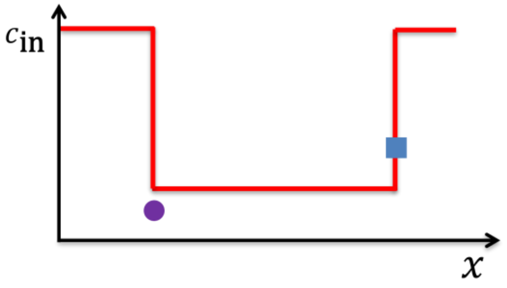


Diffusion-limited vs. conversion-limited

Standard Lifshitz-Slyozov-Wagner theory



Conversion-limited coarsening



Universal drop size distribution:

$$g_{\mathrm{LSW}}(z) \propto \frac{z^2 \exp\left(1 - \frac{3}{3-2z}\right)}{\left(1+\frac{z}{3}\right)^{7/3}\left(1-\frac{2z}{3}\right)^{11/3}},$$

$$g(z) \propto \frac{z \exp\left(-\frac{6}{2-z}\right)}{(2-z)^5},$$

Kinetic scaling:

$$\langle R(t)\rangle \sim t^{1/3}$$

$$\langle R(t)\rangle \sim t^{1/2}$$

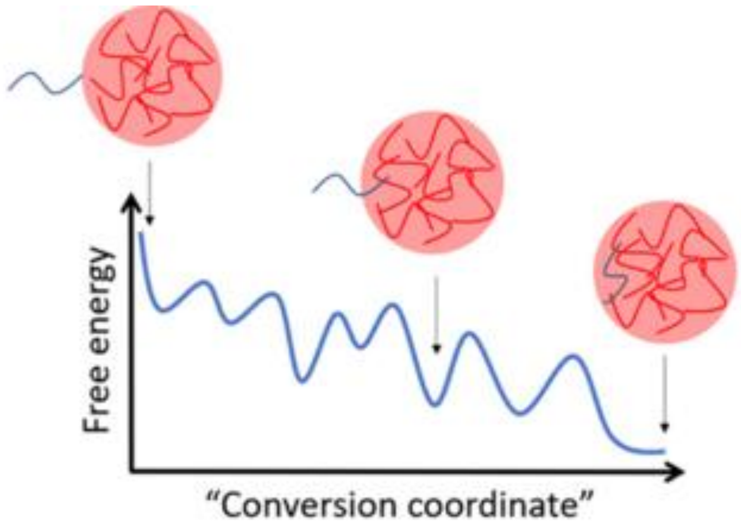
"Universality class"
of diffusion-limited phase separation

"Universality class"
of conversion-limited phase separation

Wagner (1961) Zeitschrift für Elektrochemie
CFL (2021) Phys Rev Res

Summary

Slow coarsening kinetics in biomolecular condensation observed can be explained by the slow conversion dynamics between the monomeric and phase separated states



Outlook

- Implications on the evolving viscoelastic/glassy behaviour at longer times
- How universal is conversion-limited phase separation in biomolecular condensation?
- Investigation of other universal properties