Imperial College London

International Symposium on
Membrane-Less Organelles in Cell Life and Disease
Seville, 8 March 2023

Conversion-limited phase separation in biomolecular condensation

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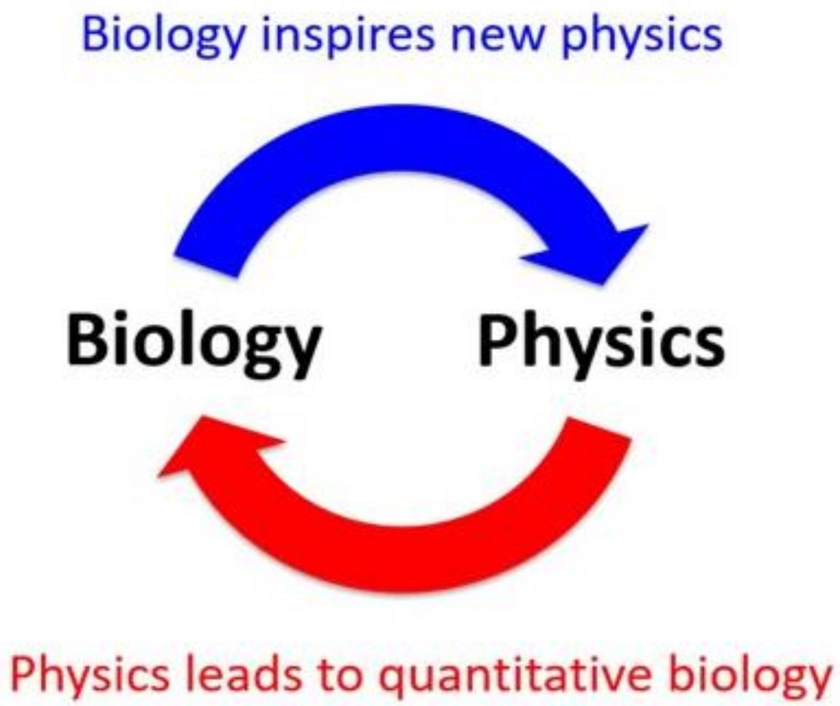
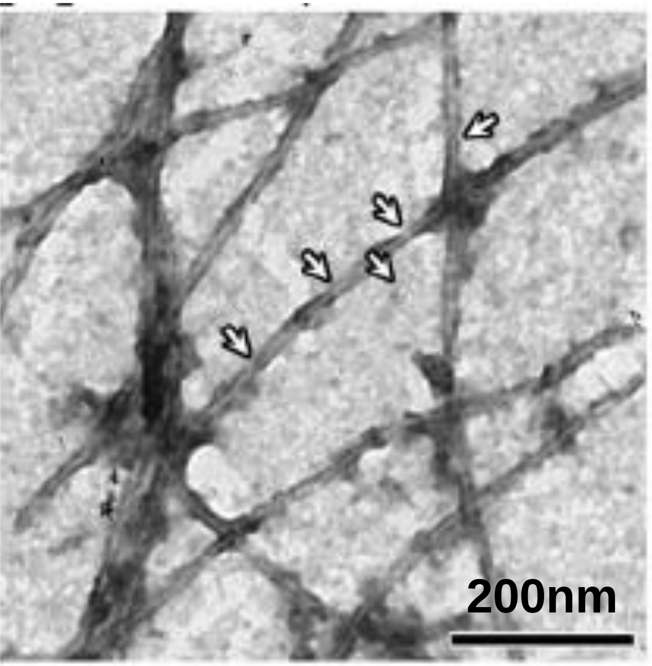
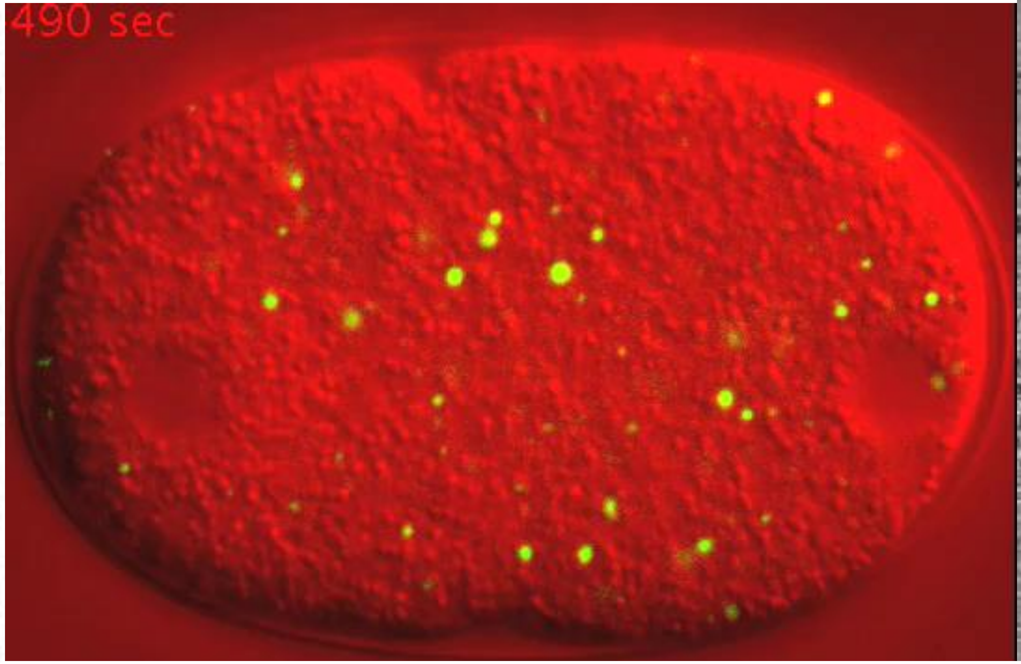
Universality in Biology

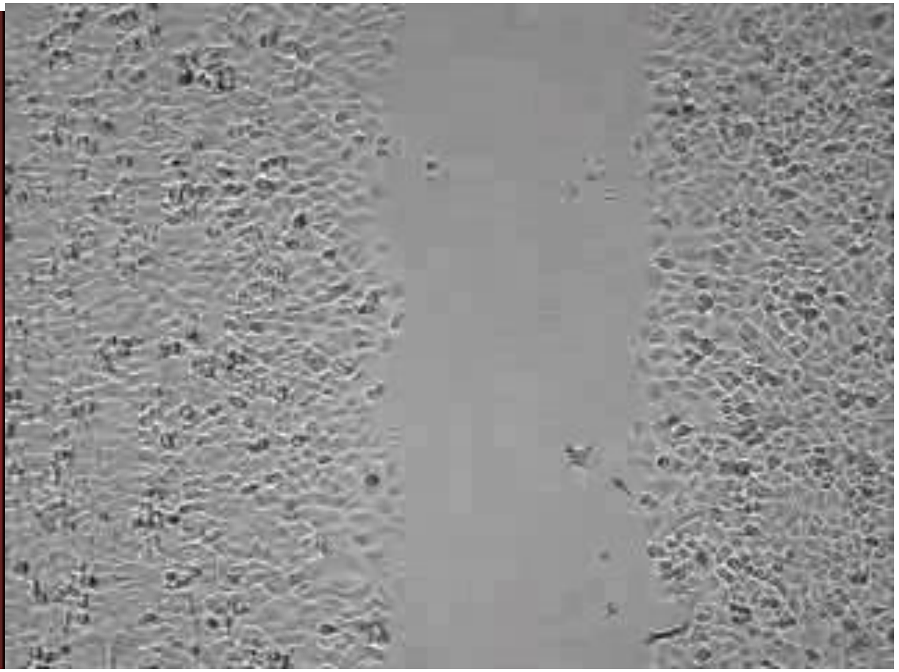

Biology inspires new physics
Biology
Physics
Physics leads to quantitative biology

Novel Biology ↔ Novel Physics





C Brangwynne et al. (2009) Science



https://www.youtube.com/watch?v=EimBzUSmak8

Amyloid fibrils

B: Relevant to many neurogenerative diseases

P: Dynamics of polymeric assembly

Biomolecular condensates

B: Key organisational principles inside cells

P: Novel modes of phase separation

Tissue dynamics

B: Tissue morphogenesis, wound healing, collective motions in organisms

P: Novel **universality classes** in nonequilibrium physics

Acknowledgement

Vaux group (Oxford)
Seydoux group (Johns Hopkins)

Imperial College London

EPSRC
Engineering and Physical Sciences Research Council

BBSRC
bioscience for the future

The Leverhulme Trust

CANCER RESEARCH UK | IMPERIAL CENTRE

NIHR | Imperial Biomedical Research Centre

Plan

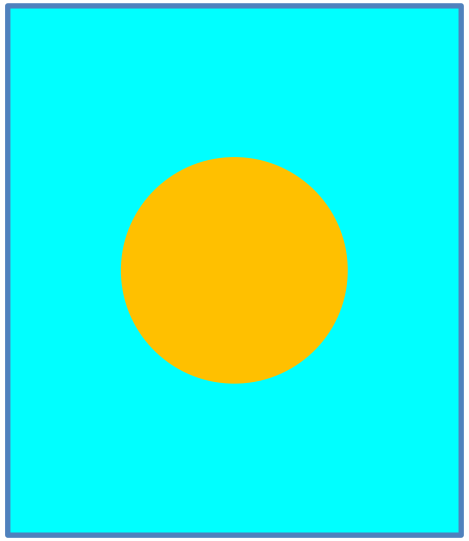
1. Coarsening in phase separation
2. Coarsening is very slow in biomolecular condensation, why?
3. Resolution – rugged energy landscape in protein conversion
4. Conversion-limited phase separation and its emergent physics
5. Summary & outlook

1. Coarsening in phase separation

Phase separation vs. emulsion

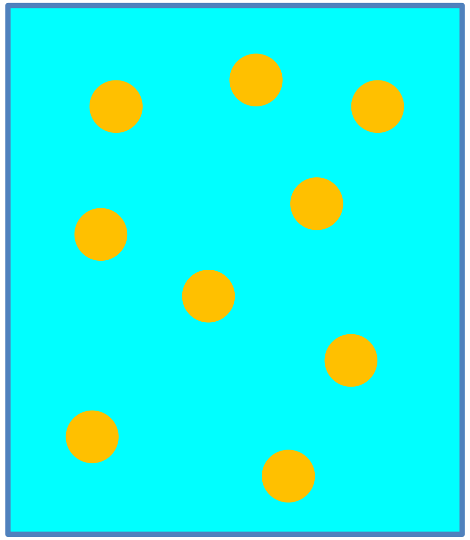
Phase separation

Free energy minimisation →

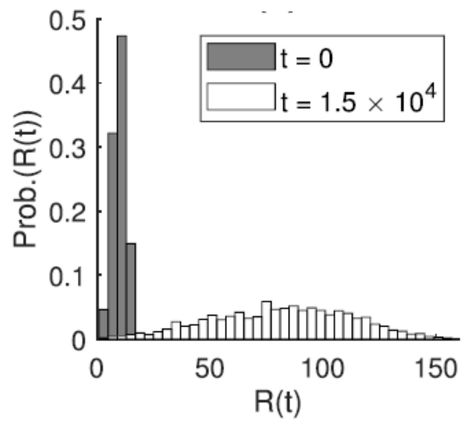


A stable emulsion

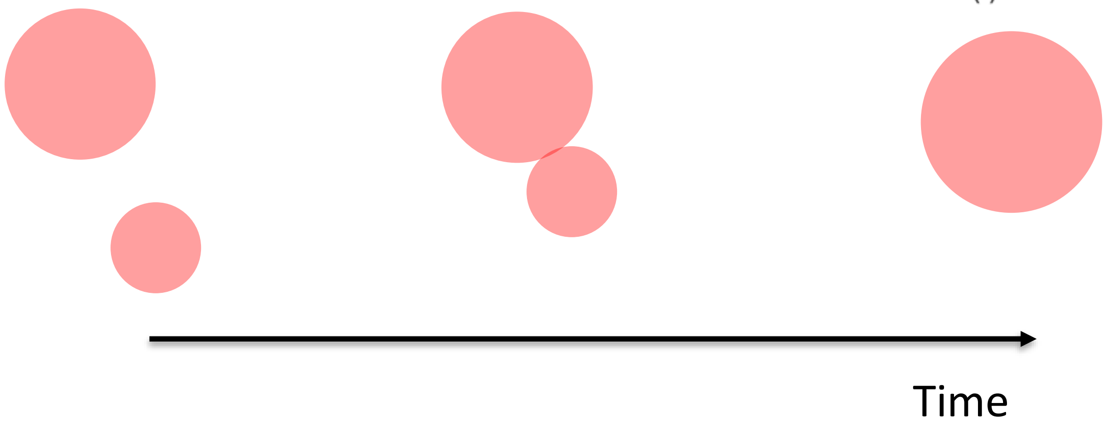
But this is what we see in cells →



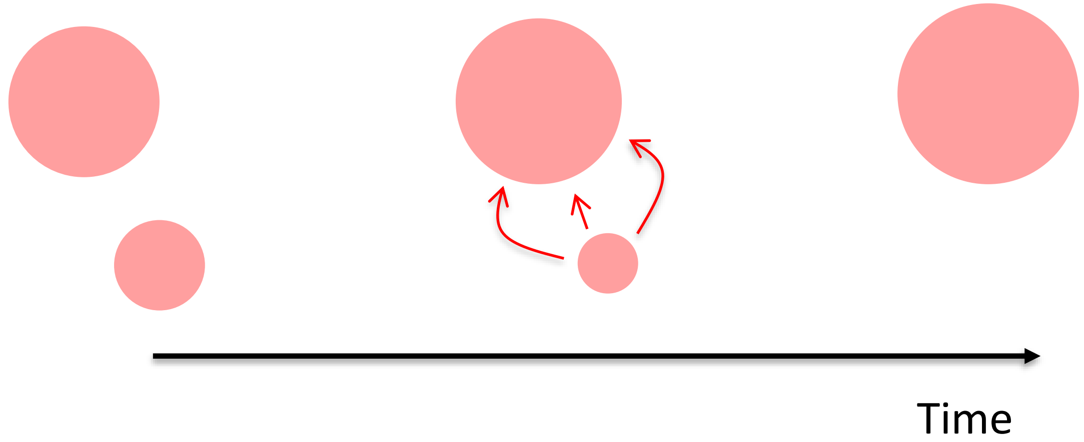
How systems coarsen



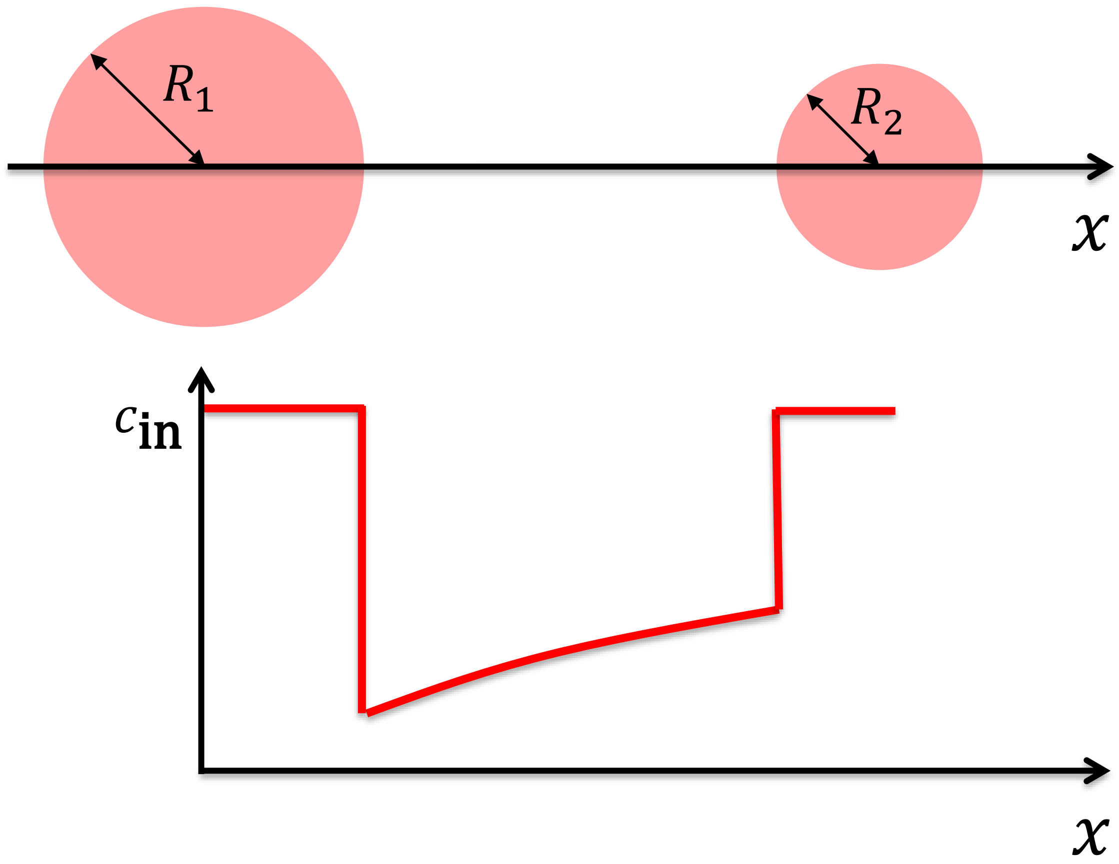
Coalescence



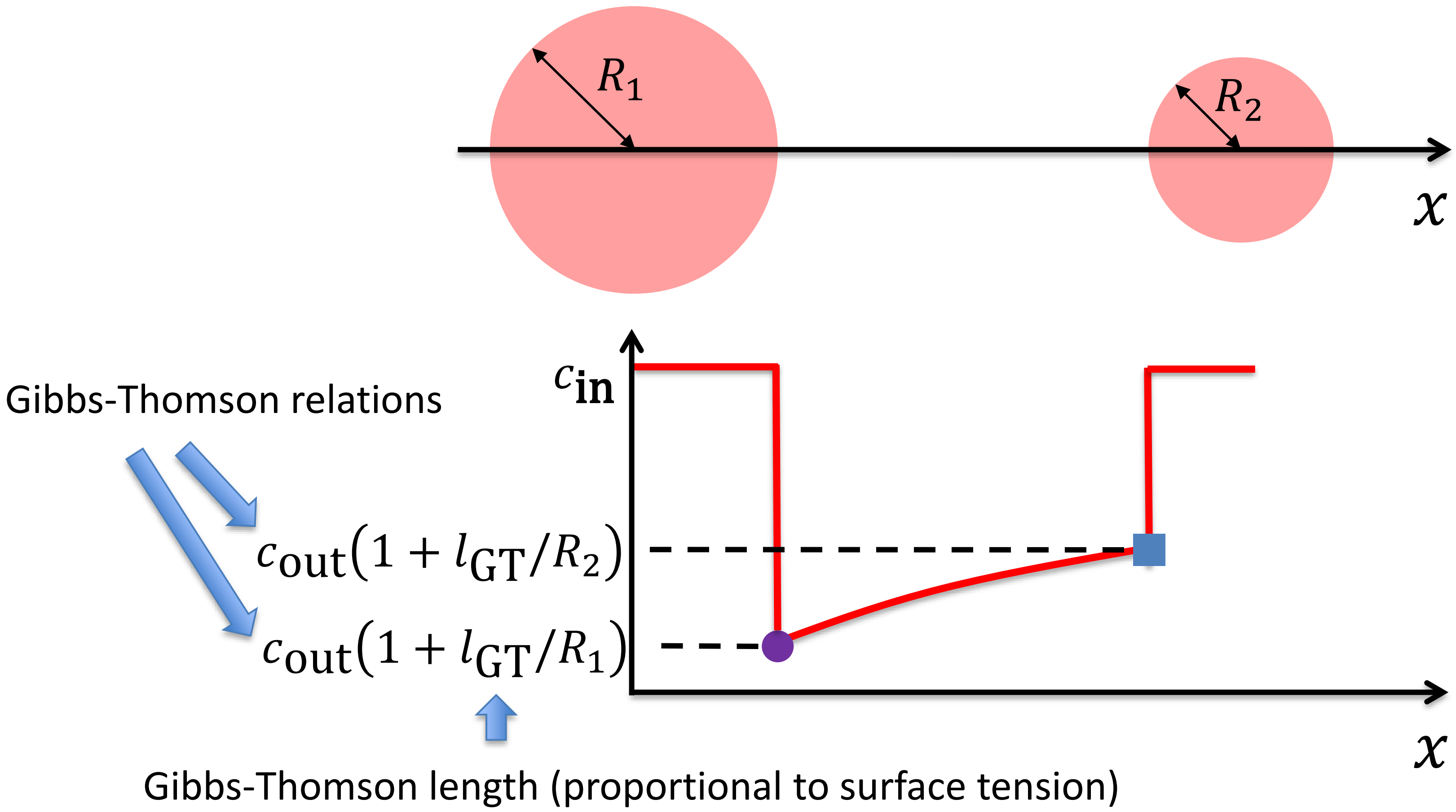
Ostwald ripening



Ostwald ripening



Ostwald ripening

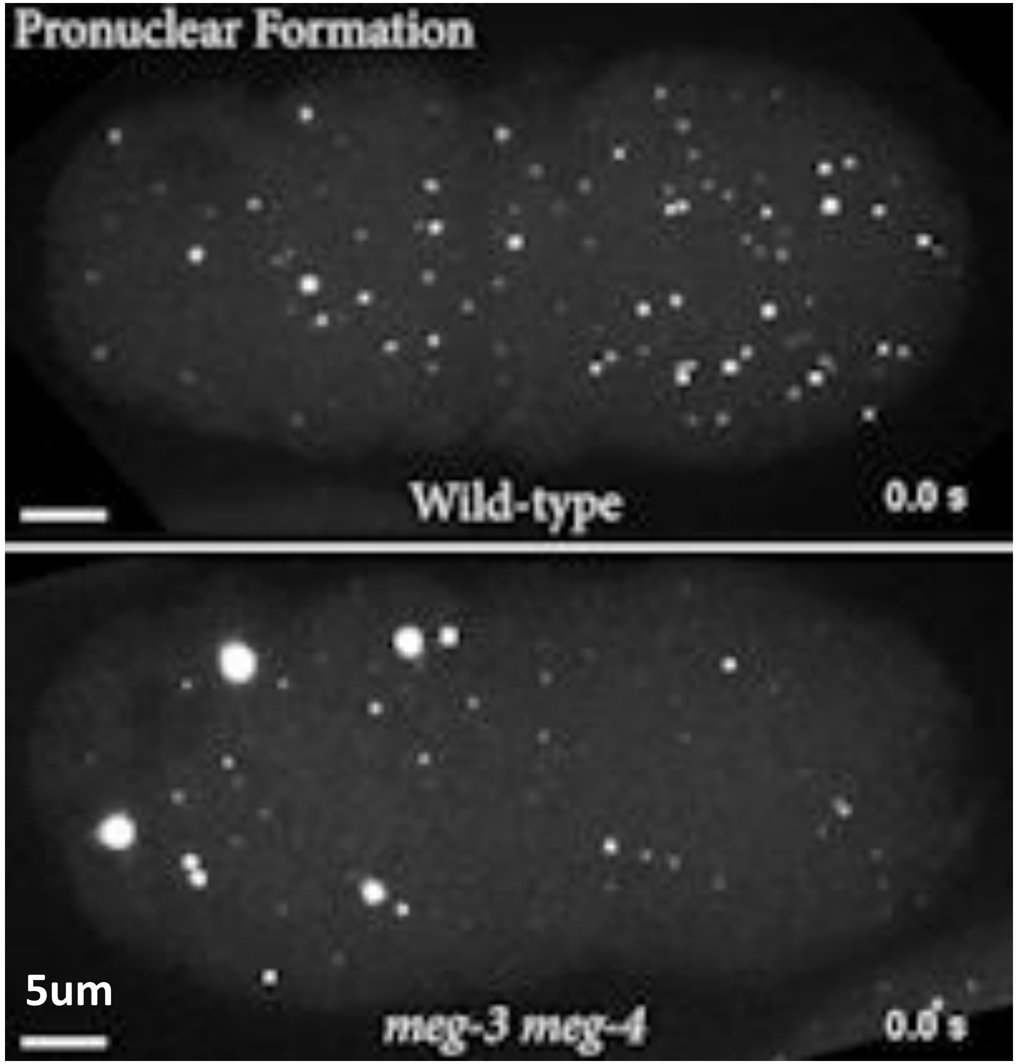


First attempts at explaining away coarsening

- 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



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

Problem: Not so simple

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

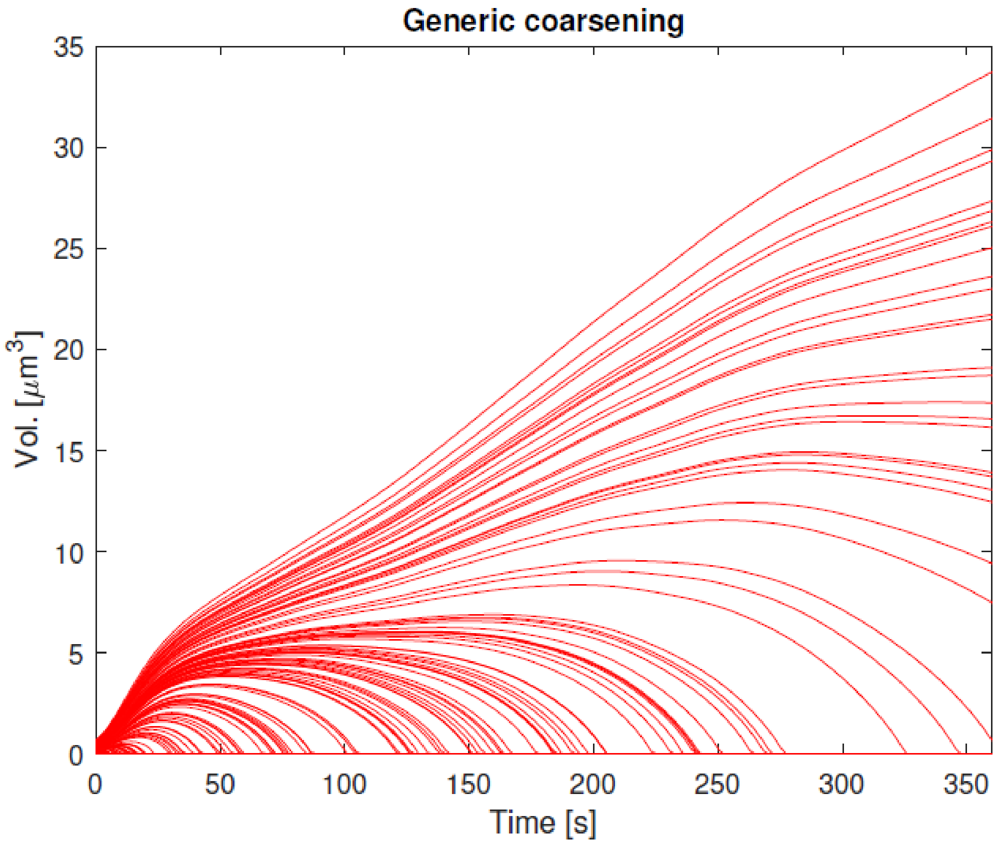


TABLE I: Parameters are estimates taken from [2]

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, $c_{\rm in}/c_{\rm out}$	20
Embryo volume	30 pL

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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 (2014) PNAS; D Zwicker et al (2015) PRE; JD Wurtz & CFL (2018) PRL]

- 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
- Mechanical suppression of drop growth via cytoskeletal networks

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

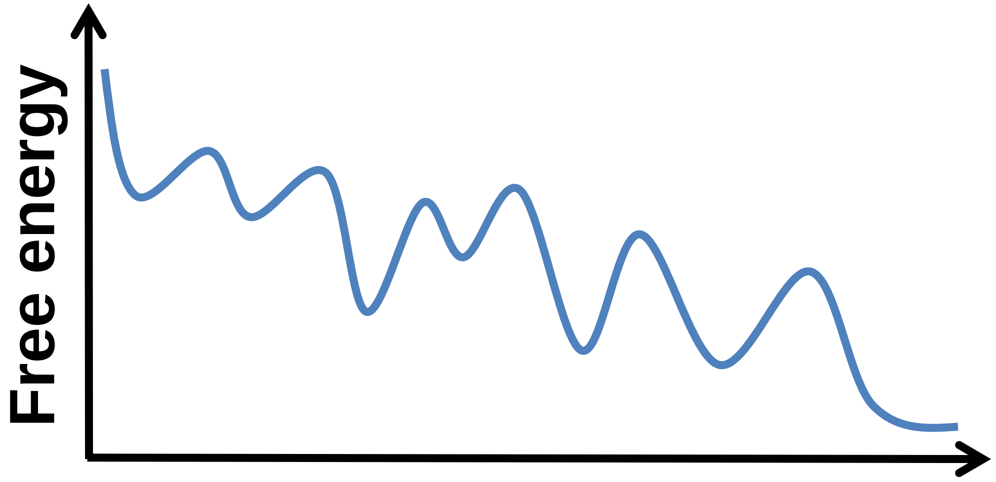
  -> Stop coalescence due to a 'rigid' network
- Pickering effects (coating of drop surface to reduce surface tension)

  -> 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*".

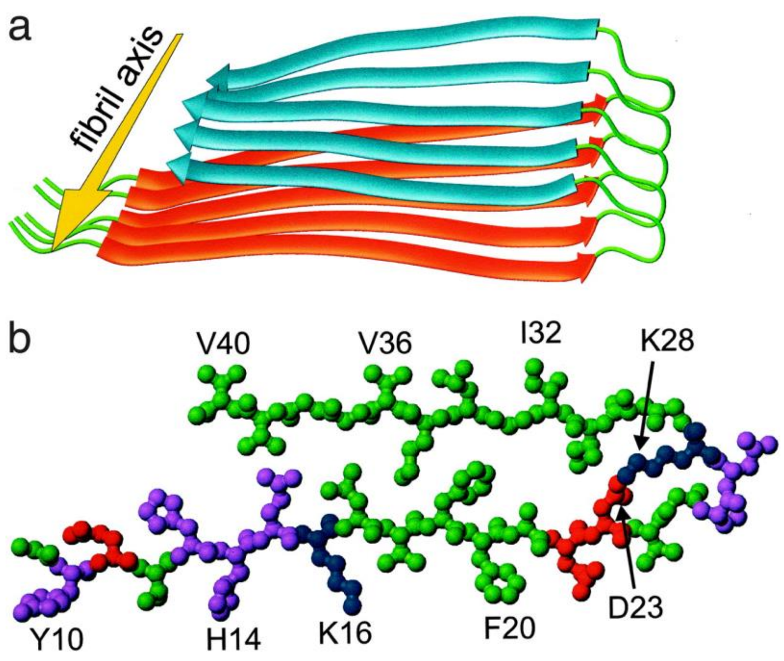
- Indeed, slow kinetics has been observed in a different kind of biomolecular system



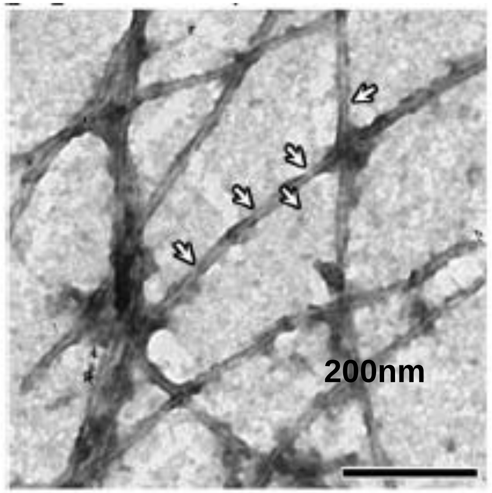
3. Resolution – rugged energy landscape in protein conversion

Drawing inspiration from another system: amyloid fibrils

Abeta amyloid fibrils



AT Petkova et al (2002) PNAS

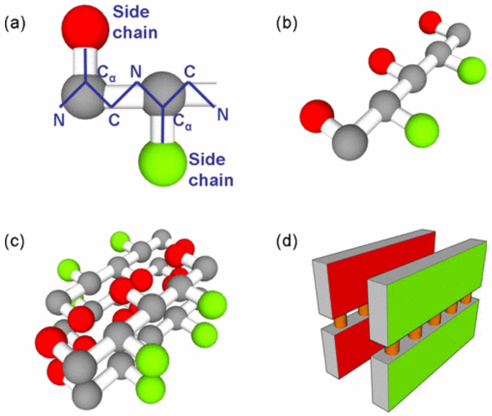


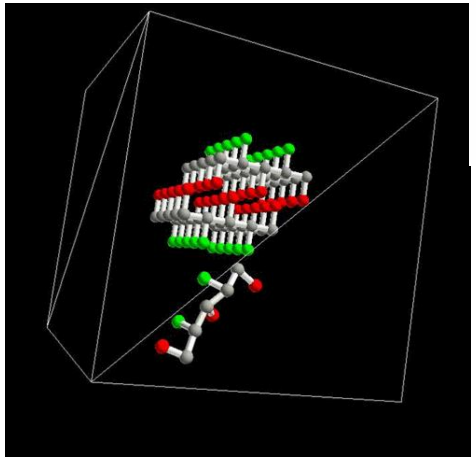
- Amyloid fibrils consist of cross beta sheets
- Fibrils elongate by incorporating free peptide monomer in solution

Slow elongation dynamics

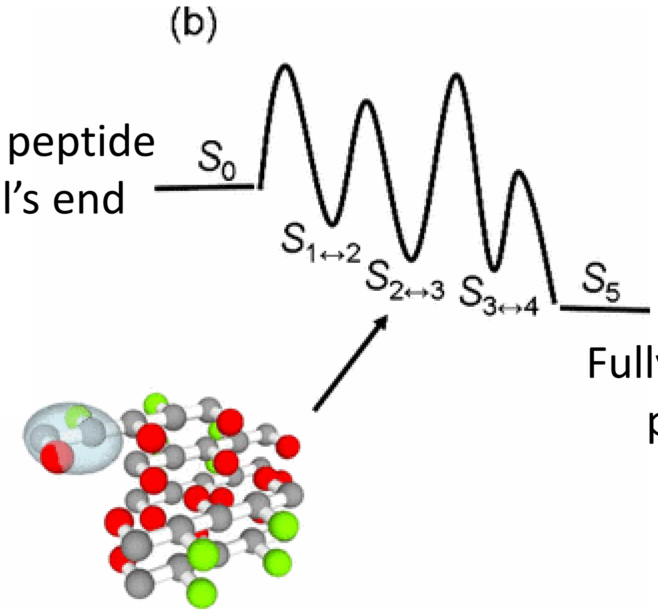
- Elongation dynamics was found to be slow (e.g., ~1 peptide per second under in vitro conditions), even though
  - No driven chemical reactions
  - No rigid networks limiting fibril growth
  - No subdiffusive behaviour

Resolution – a rugged energy landscape picture





Monomeric peptide at the fibril's end



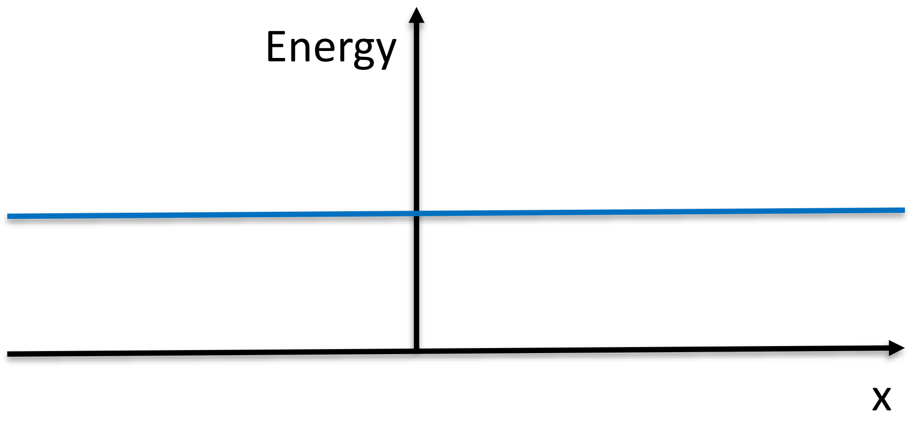
Fully fibrilised peptide

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

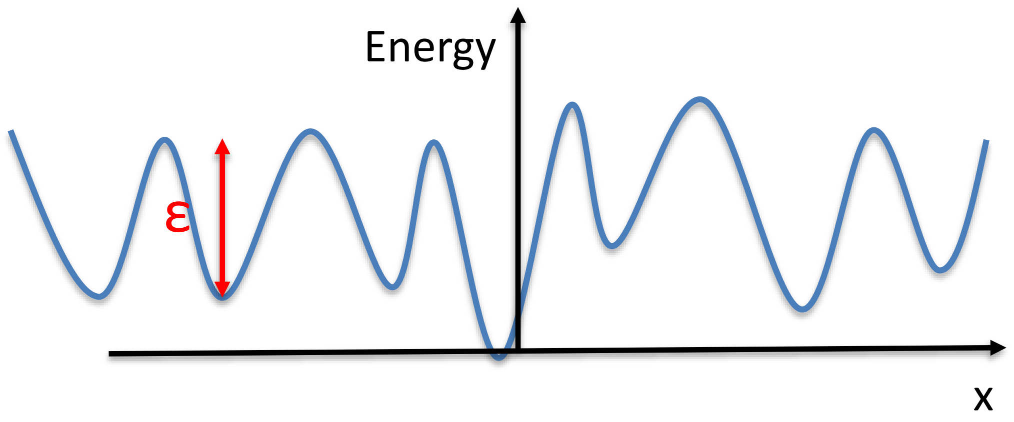
A cartoon explanation in 1D

Normal diffusion



Diffusion coefficient: D

Diffusion over a rugged energy landscape



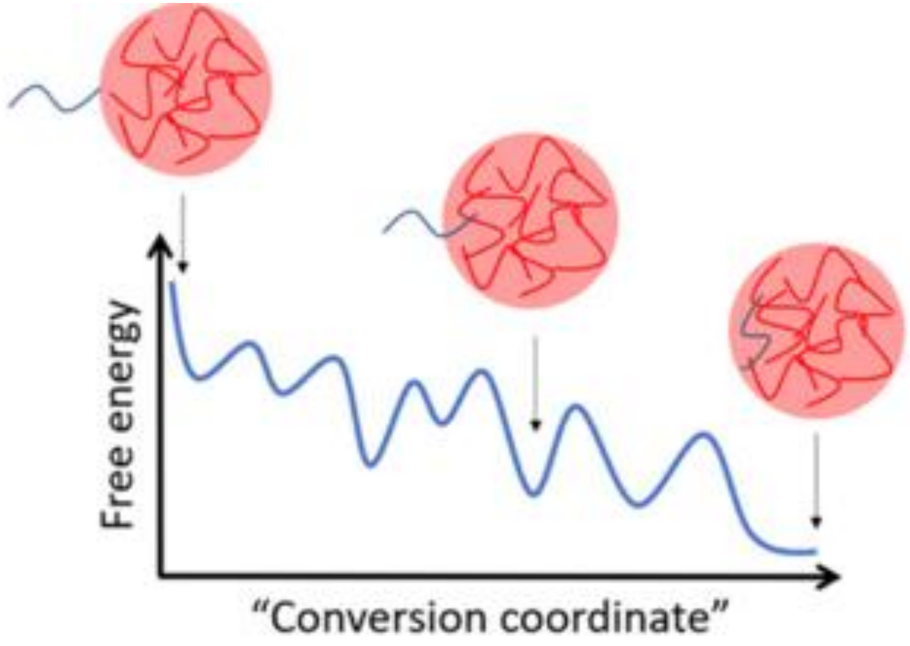
Analytical results: $D_{eff} = D \exp\left[-\left(\frac{\varepsilon}{k_B T}\right)^2\right]$

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!

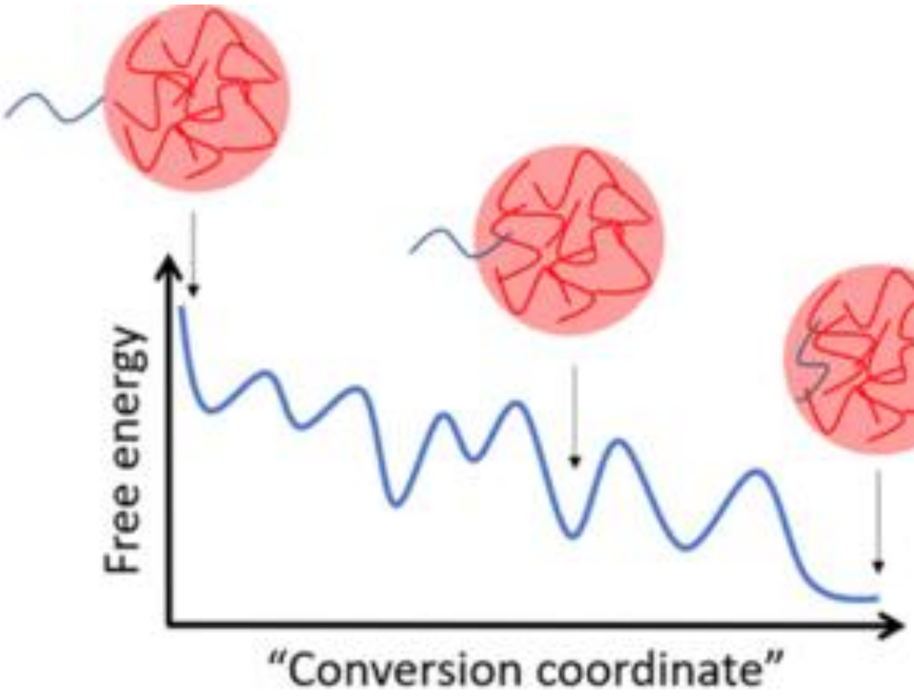
R Zwanzig (1998) *Diffusion in a rough potential.* PNAS

Back to biomolecular condensates



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

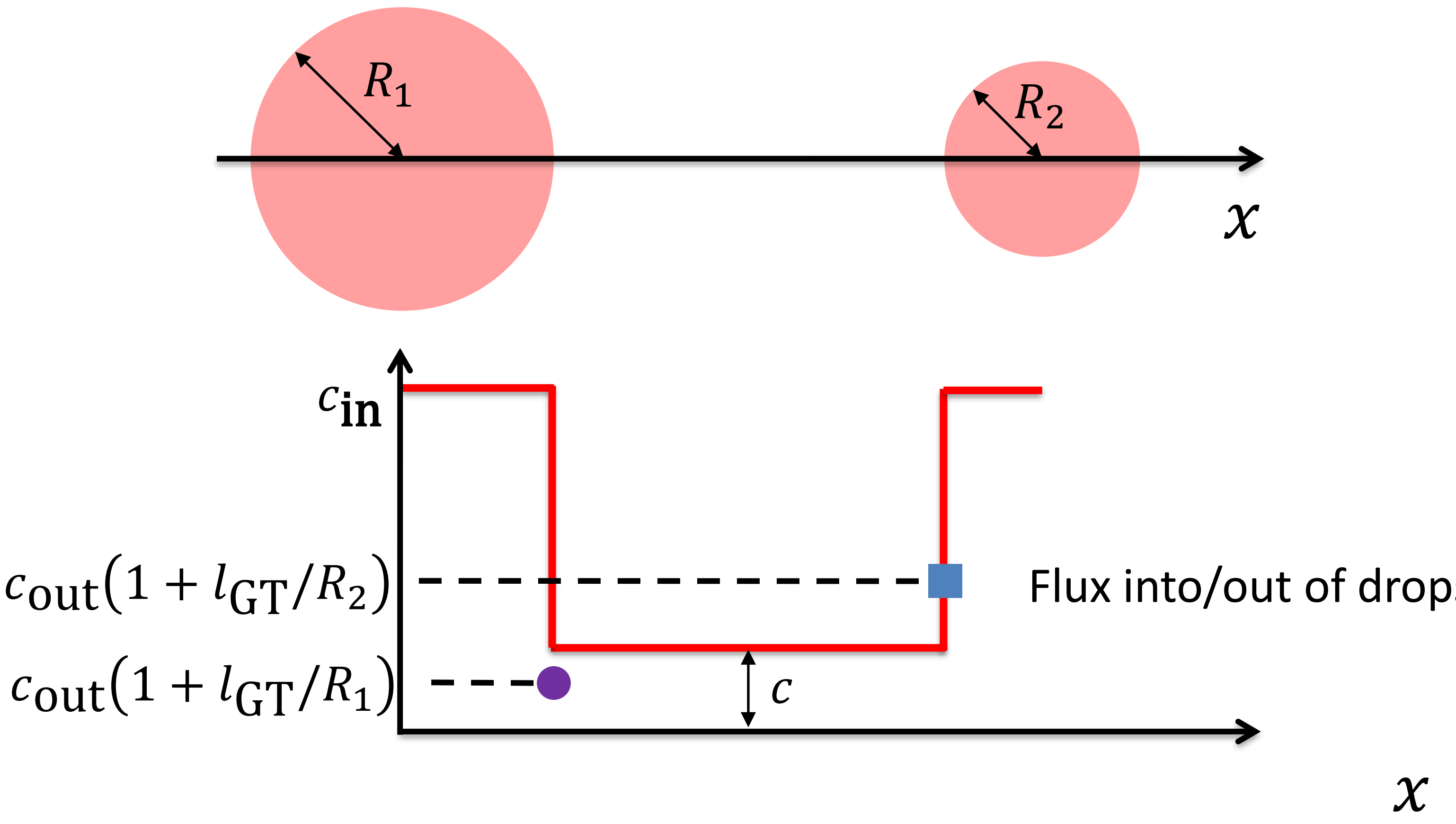
AW Folkmann, A Putnam, CFL, & G Seydoux (2021) Science
CFL (2021) Physical Review Research



4. Conversion-limited phase separation and its emergent physics

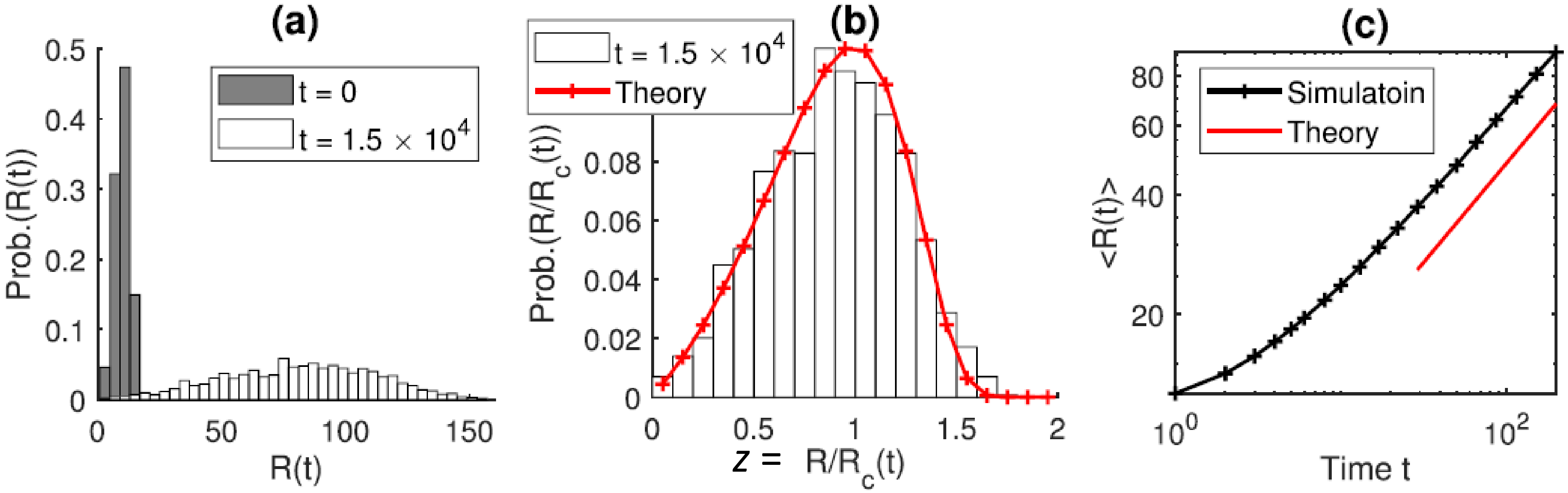
Conversion-limited phase separation

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



Flux into/out of drops proportional to $(c - c_{out})$

Late-stage universal coarsening behaviour



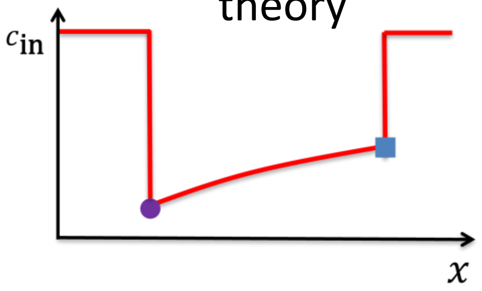
$$g(z) \propto \frac{z \exp\left(-\frac{6}{2-z}\right)}{(2-z)^5},$$

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

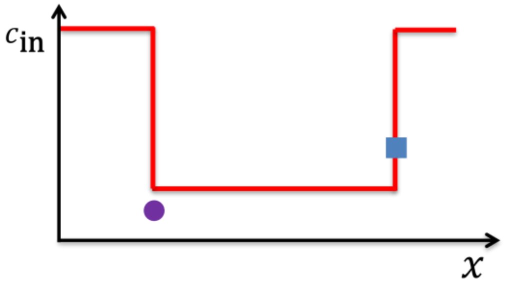
CFL (2021) *Scaling law and universal drop size distribution of coarsening in conversion-limited phase separation.* Phys Rev Res

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

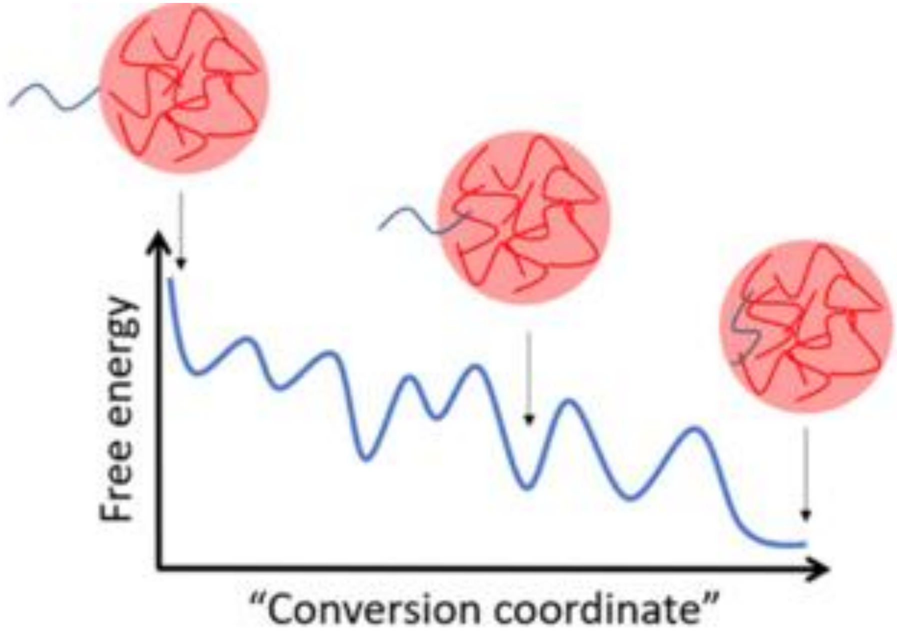
Universality class
of conversion-limited phase separation

CFL (2021) Phys Rev Res

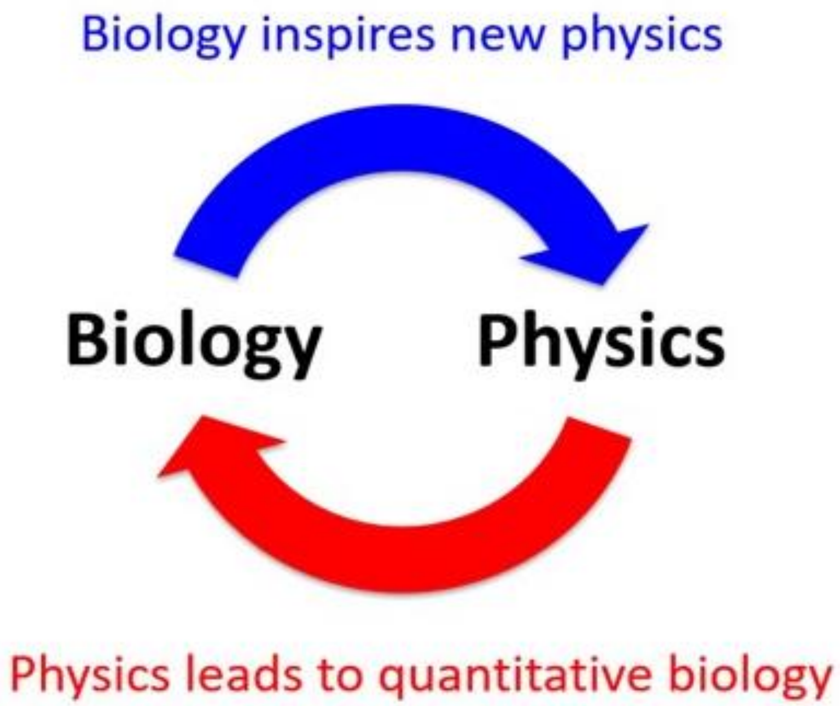
Summary

Slow coarsening kinetics in biomolecular condensation observed can be explained by conversion-limited phase separation

→ novel universal physics of phase separation



Outlook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