

Phase separation in living cells

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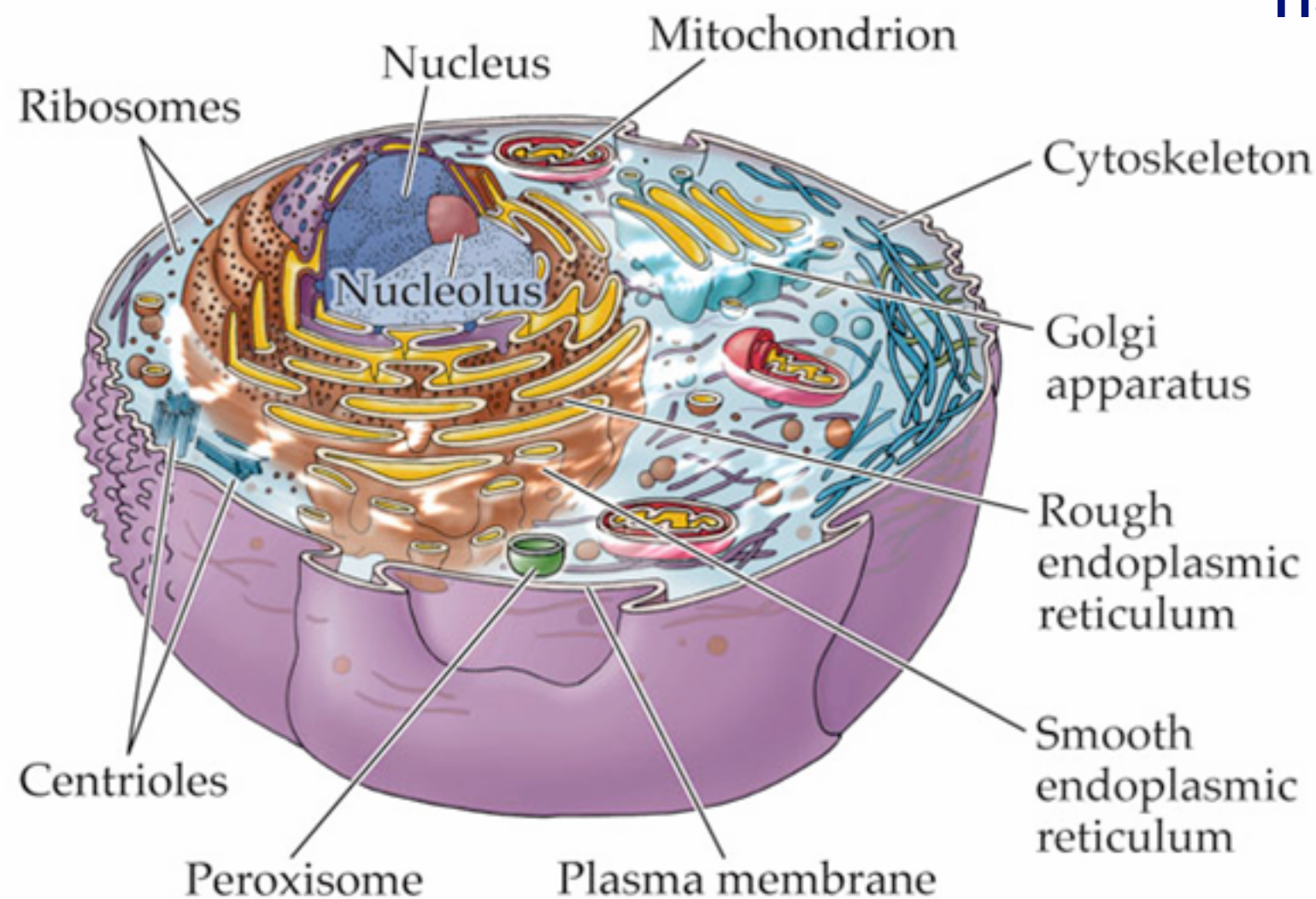
Elisabeth Friedrich-Fischer



Cellular compartments

Cell compartments separate chemically distinct environments

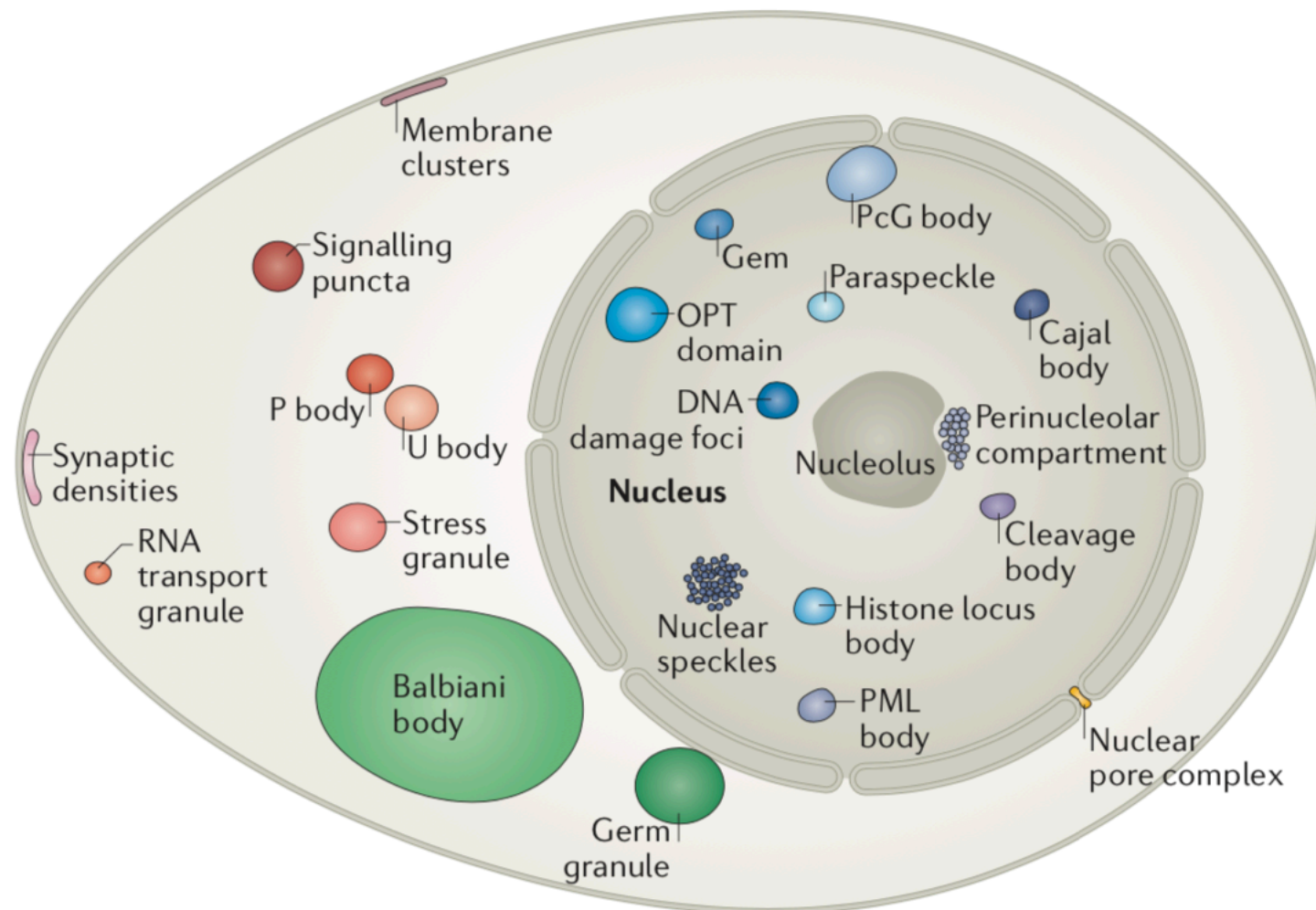
Many cell compartments have membranes



Cellular compartments

Cell compartments separate chemically distinct environments

Some compartments do not possess membranes



Outline

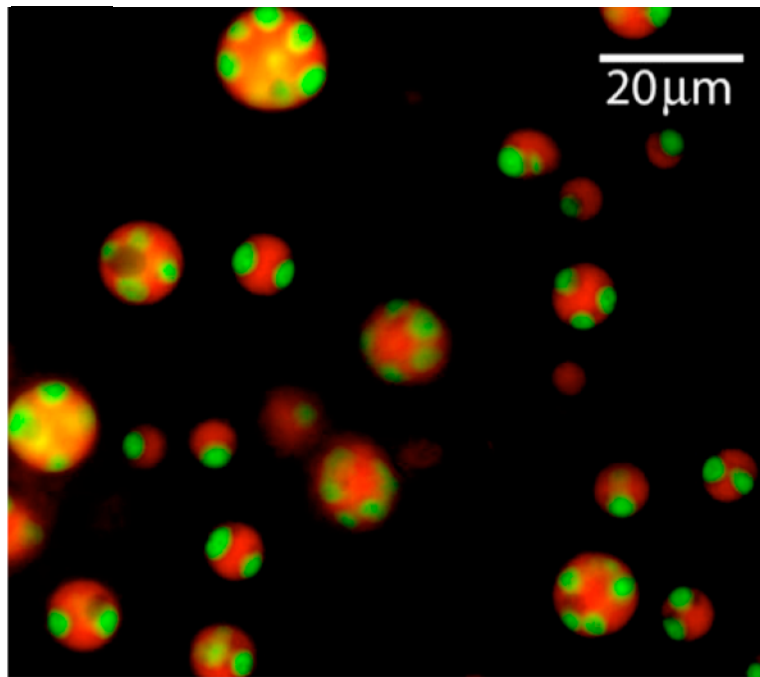
1. Introduction: protein condensates
2. Liquid-liquid phase separation in cells

Spatial segregation of droplets

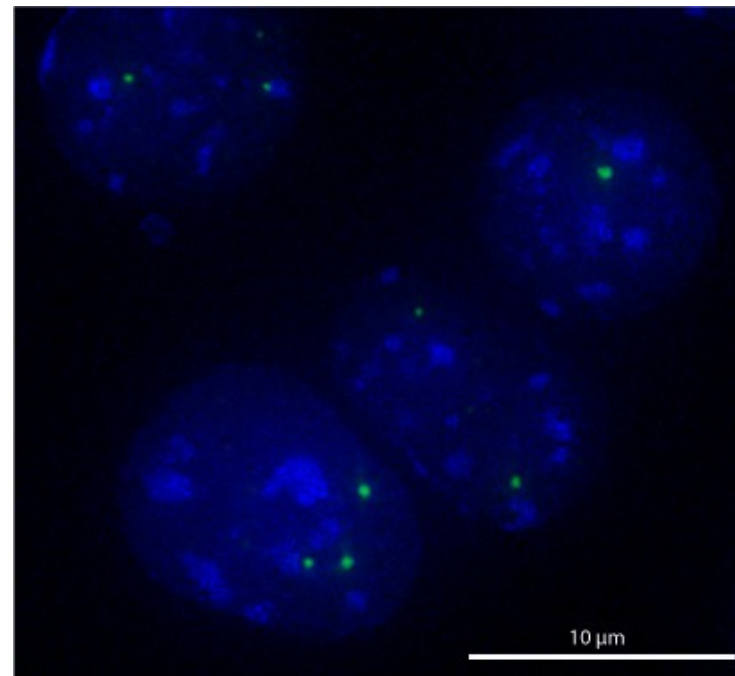
Buffering of concentration fluctuations

4. Chemically active droplets
5. Material properties of protein condensates

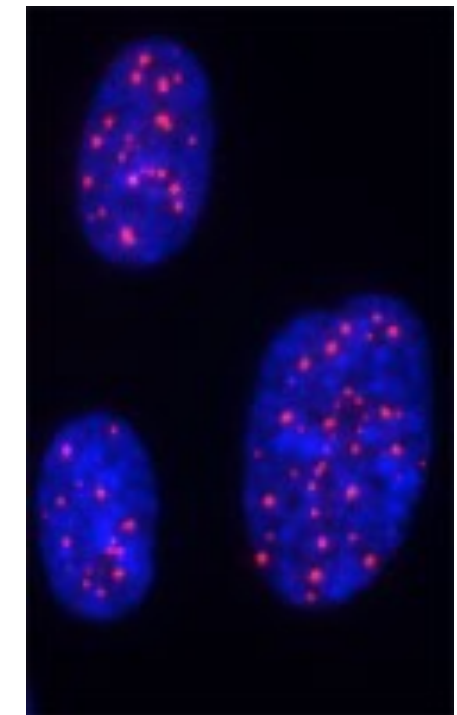
Membraneless compartments



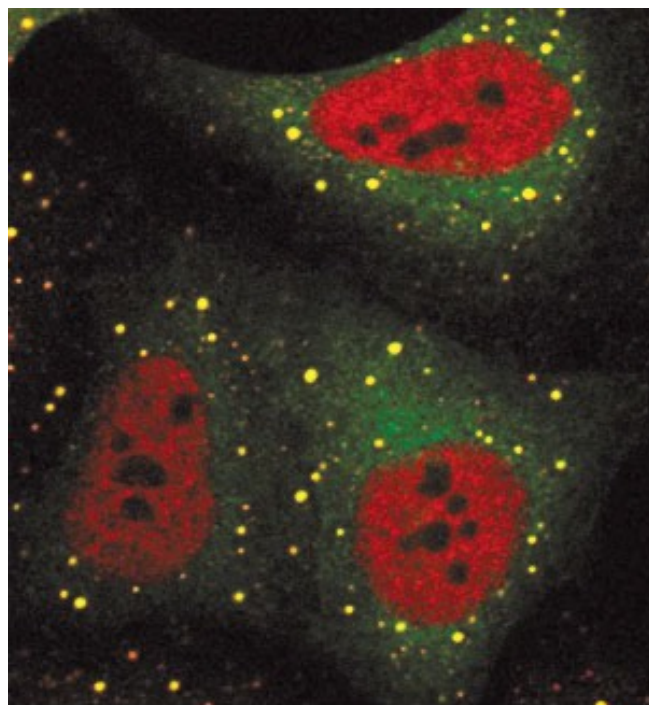
Source: C. Brangwynne
Nucleoli (green)



Source: Wikipedia
Cajal bodies (green)

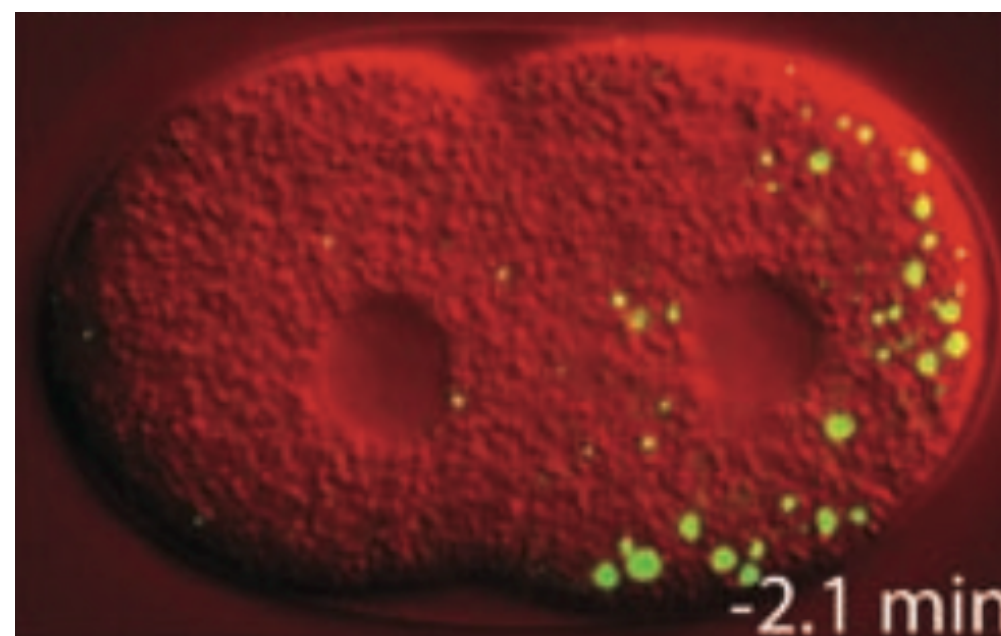


Source: Thomas G Hofmann
PML bodies (red)

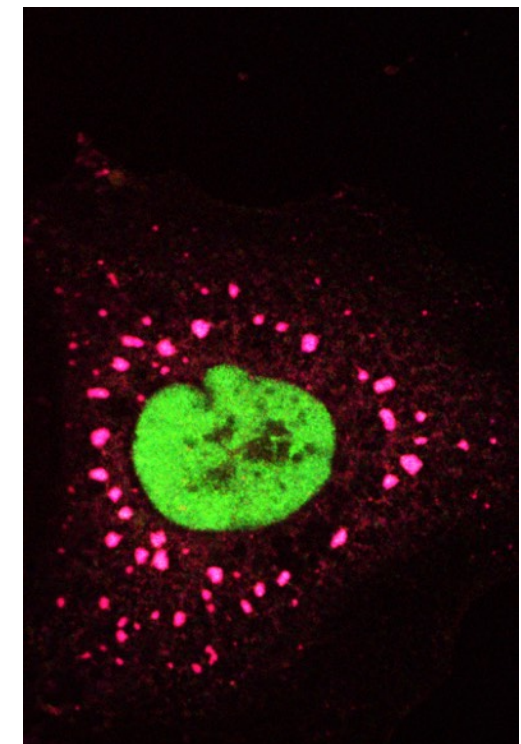


Nature Reviews | Molecular Cell Biology

P bodies (yellow)



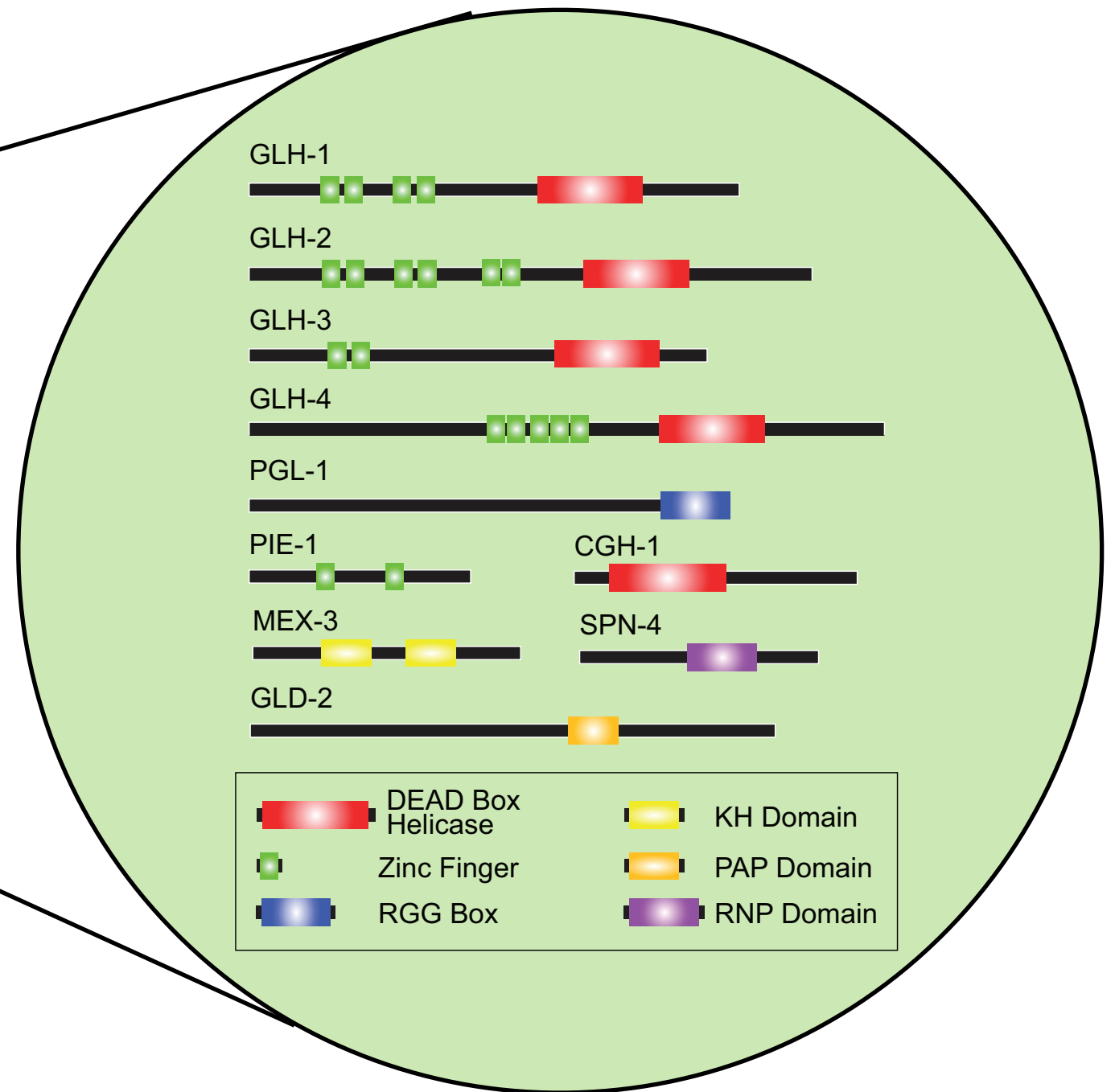
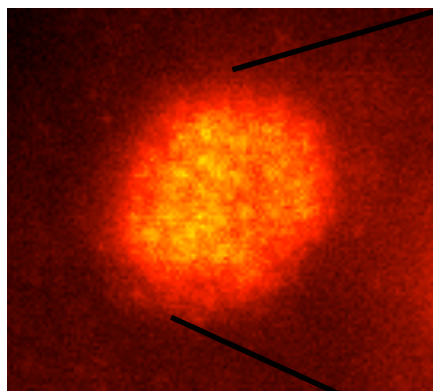
P granules (green)



Source: Christine Vande Velde
Stress granules (purple)

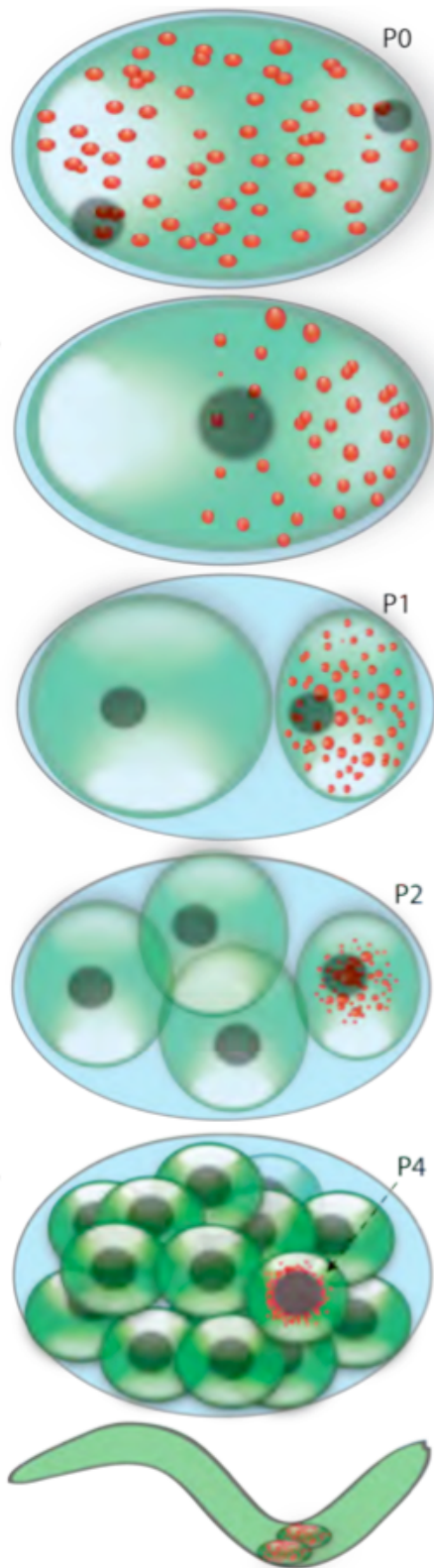
P granules

P granules (germ granules) contain many different RNA binding proteins and RNA
no membrane



implicated in specification of
germ cells

P granule segregation

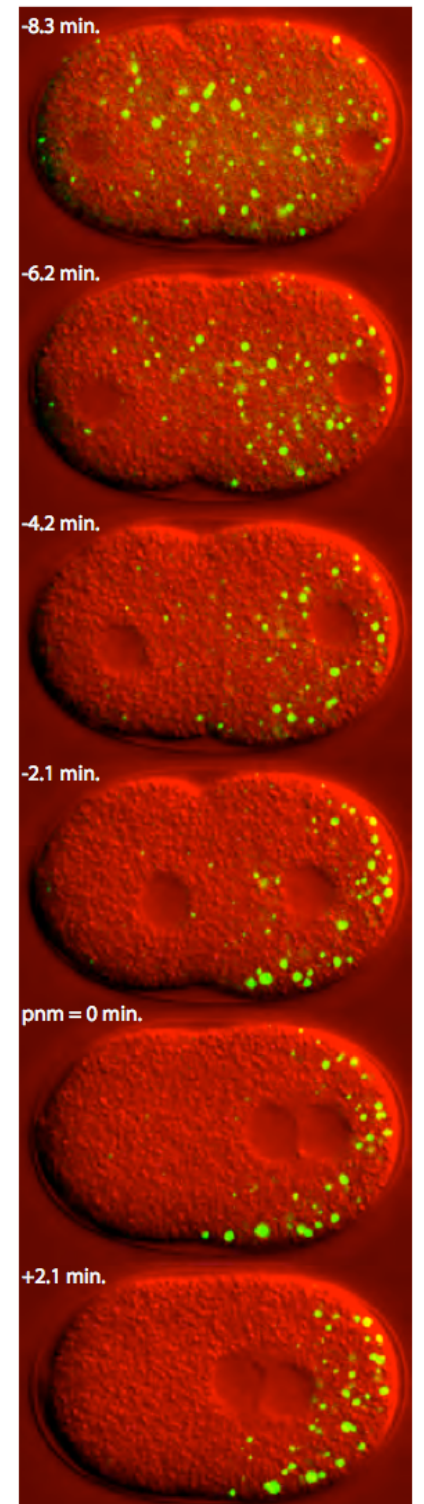


non-membrane bound assemblies
of proteins and RNA

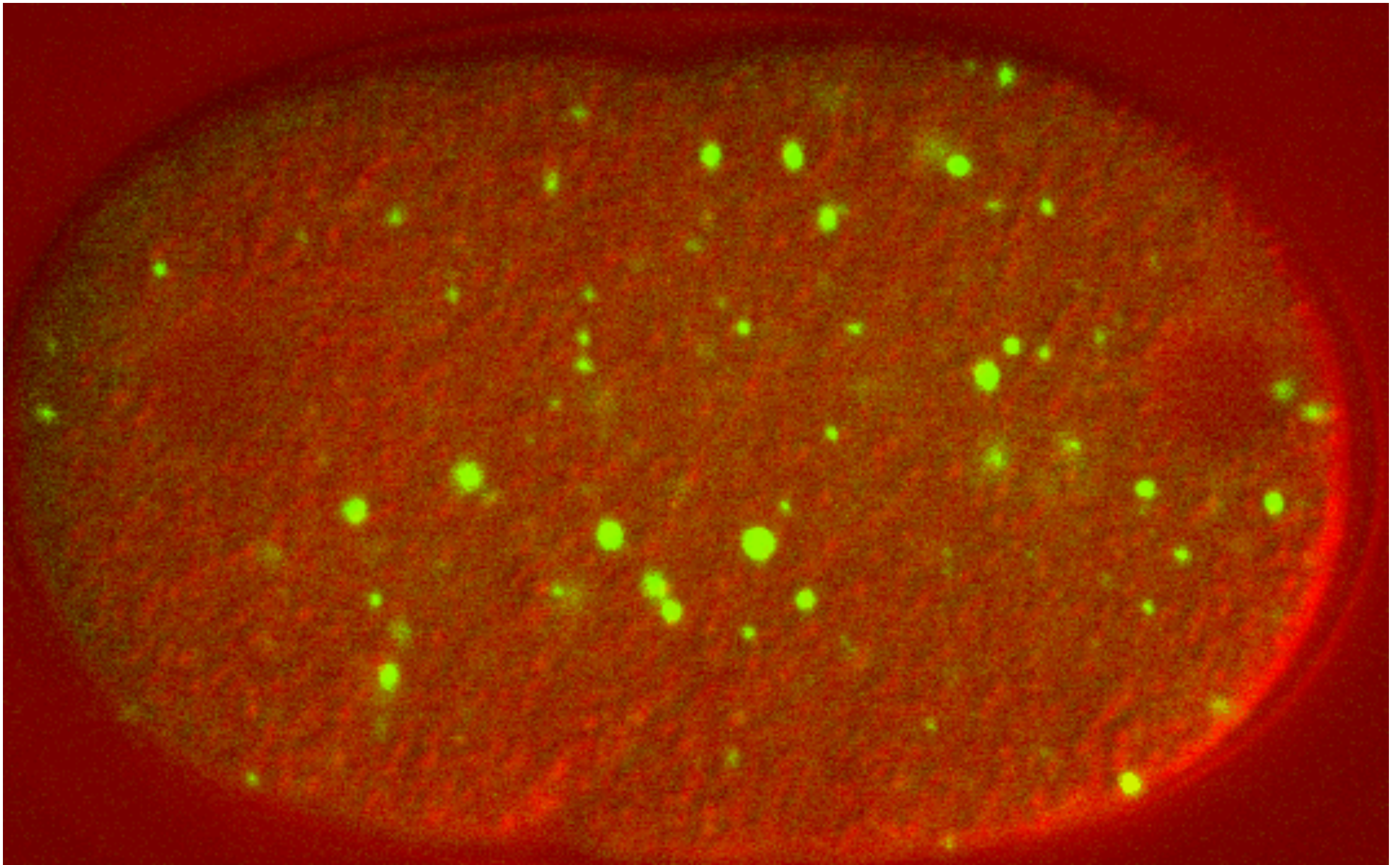
found in the cytoplasm of
germ cell precursors

implicated in specification of
germ cells

segregated during asymmetric
cell division



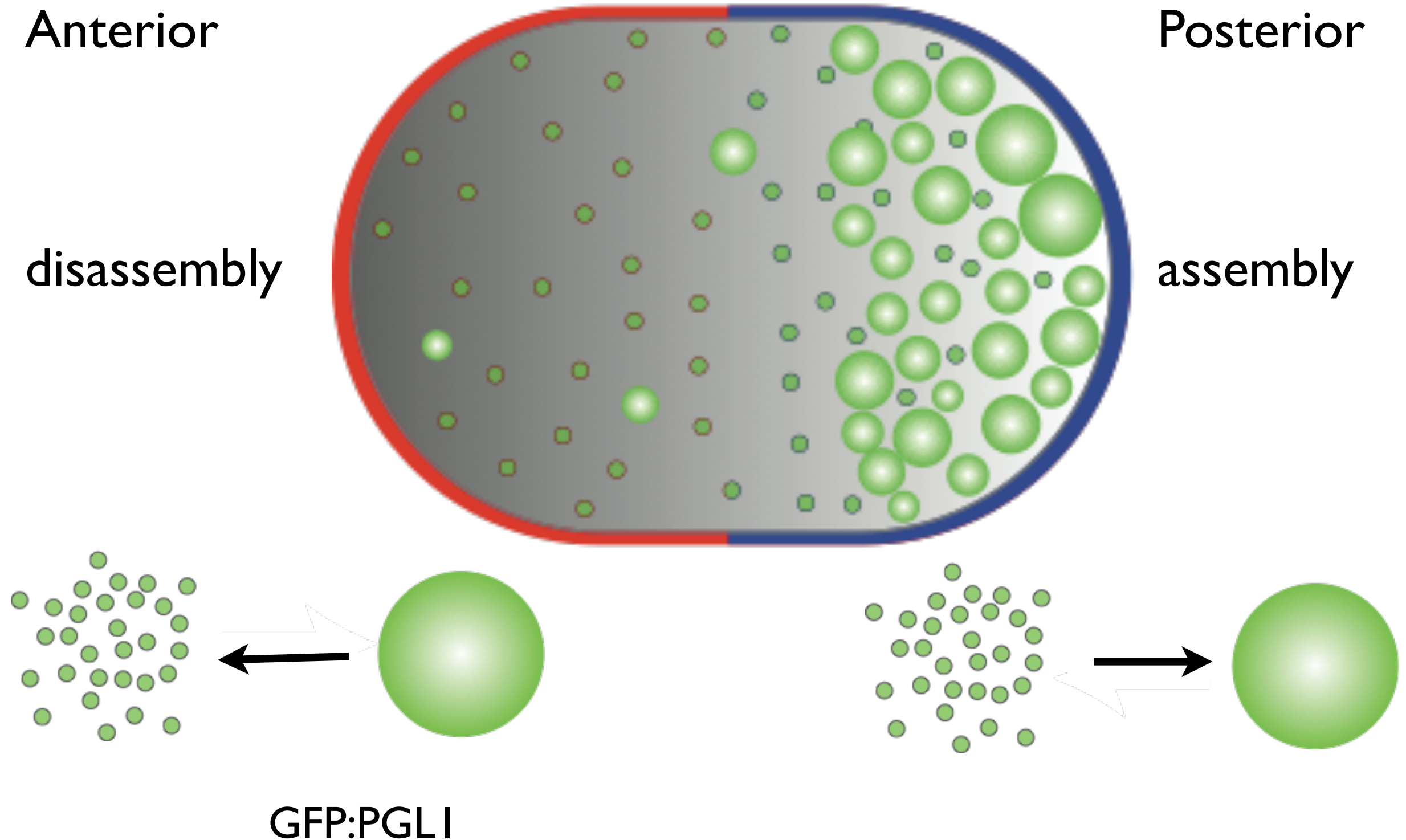
P granule segregation



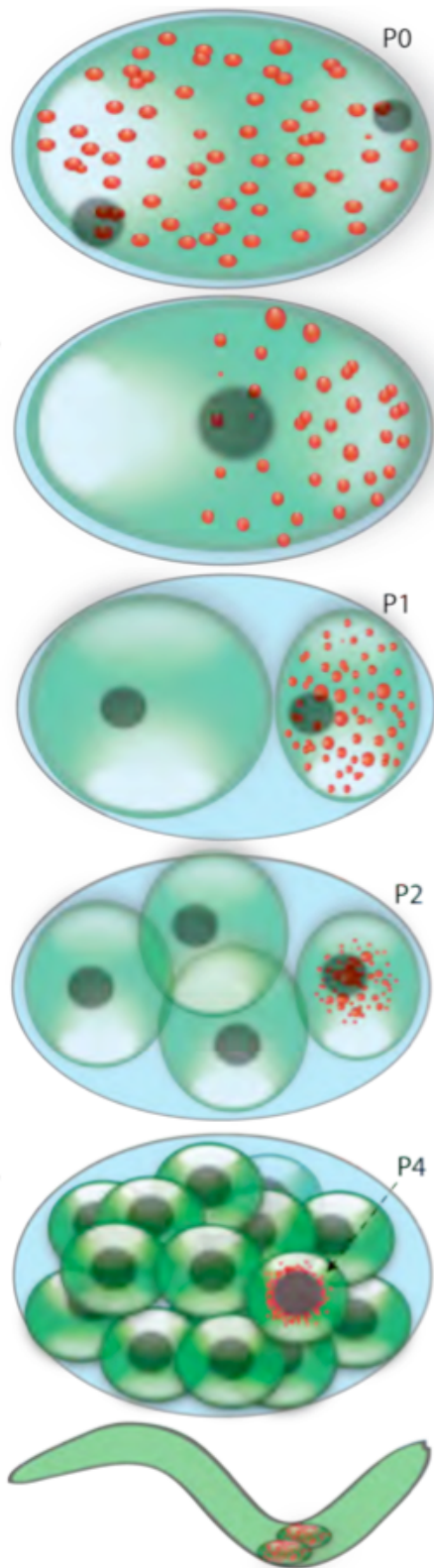
GFP:PGLI

Brangwynne, Jülicher, Hyman et. al, Science 324 (2009)

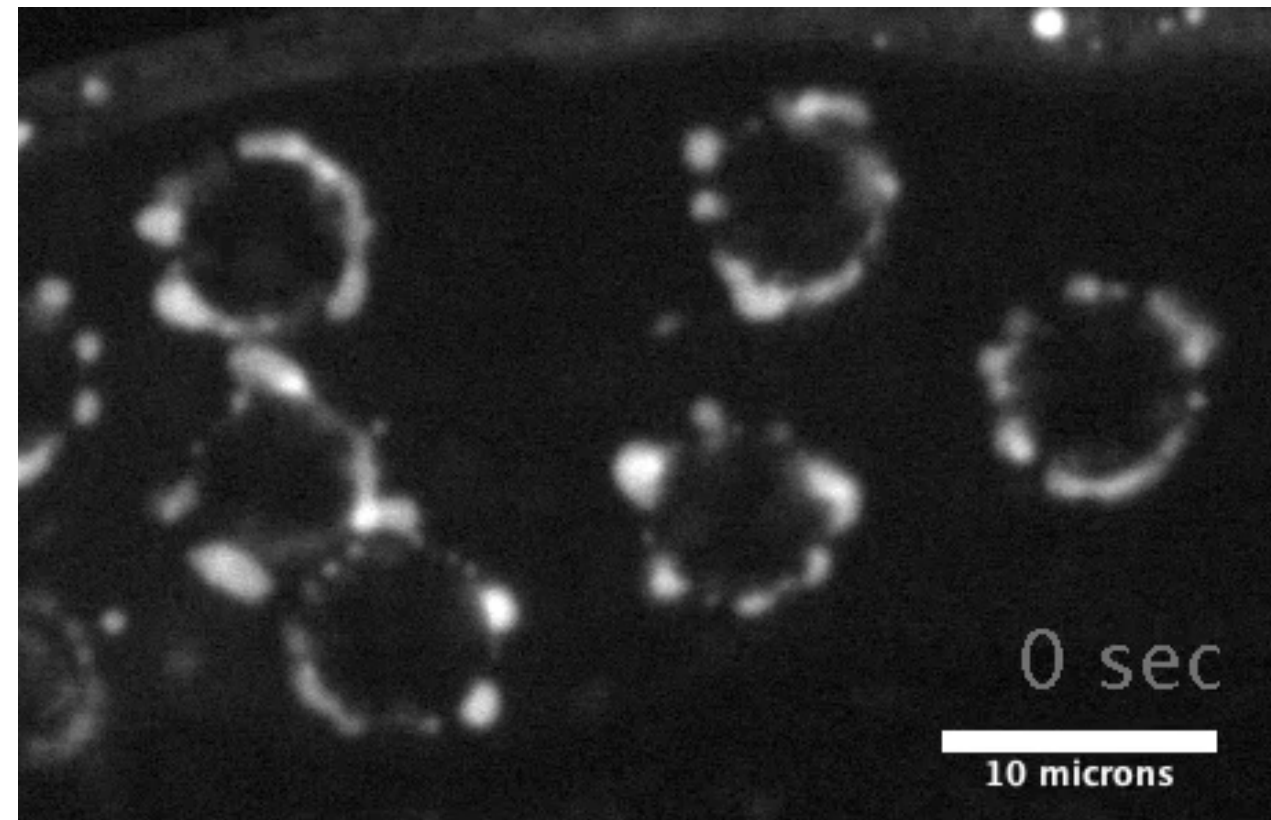
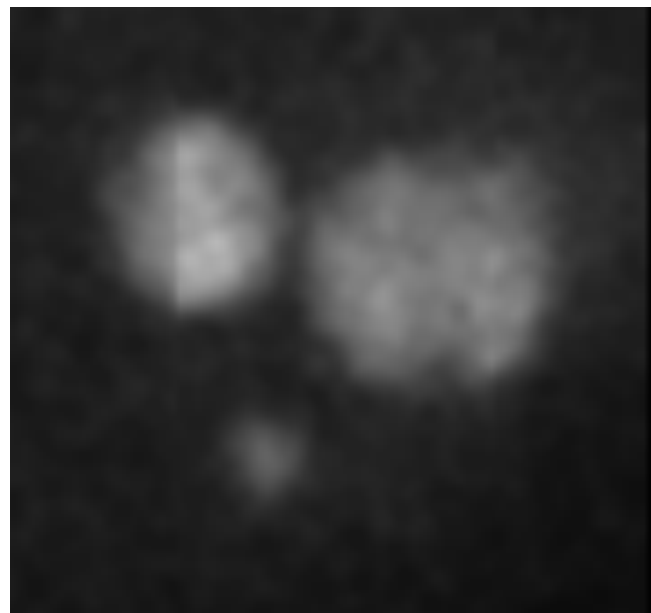
P granule assembly gradient



P granules are liquid drops

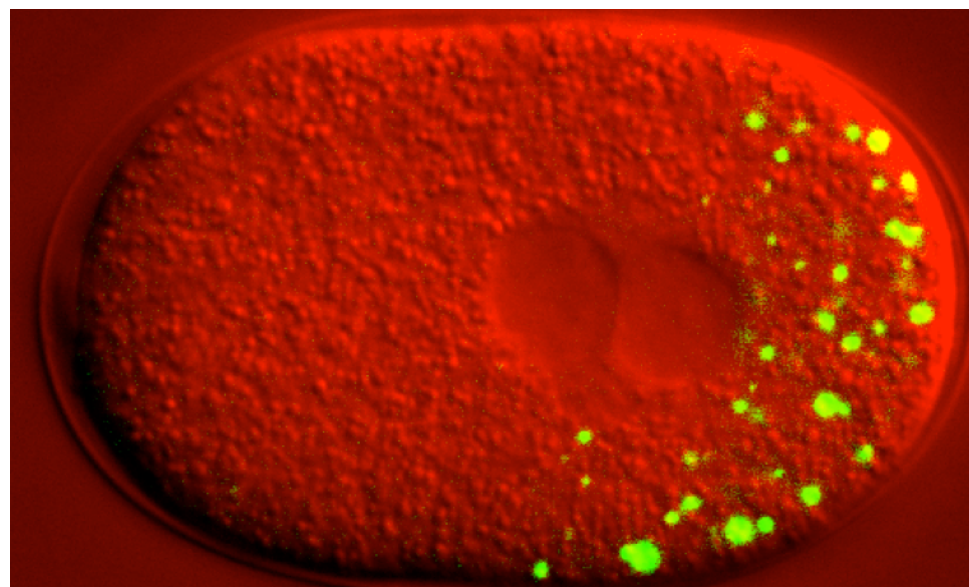
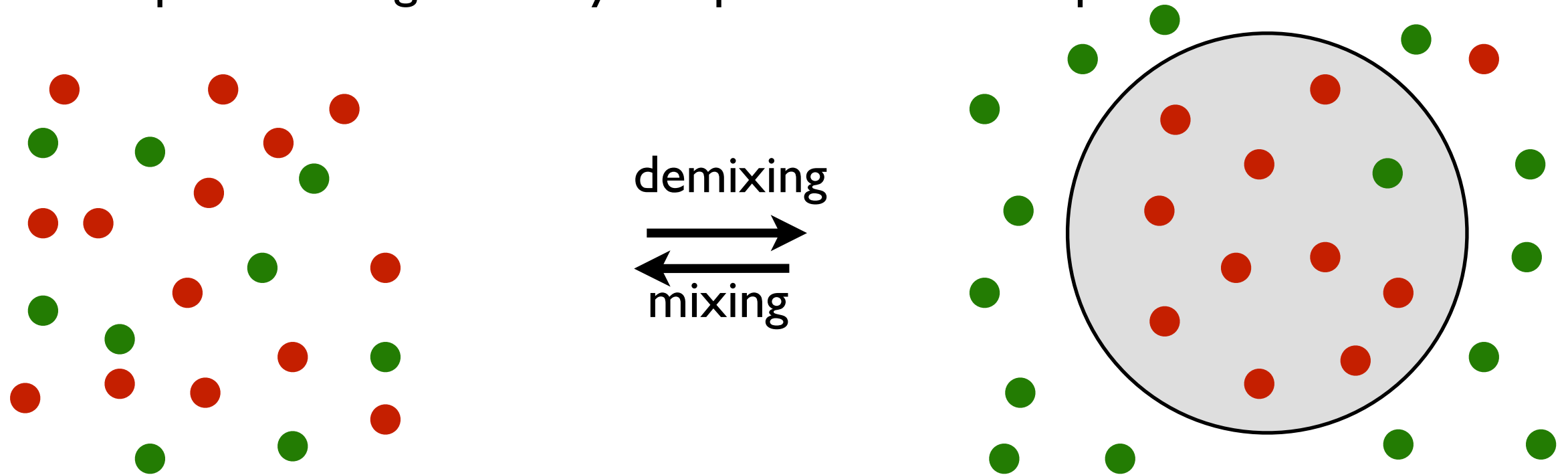


P-granule flow
and fusion



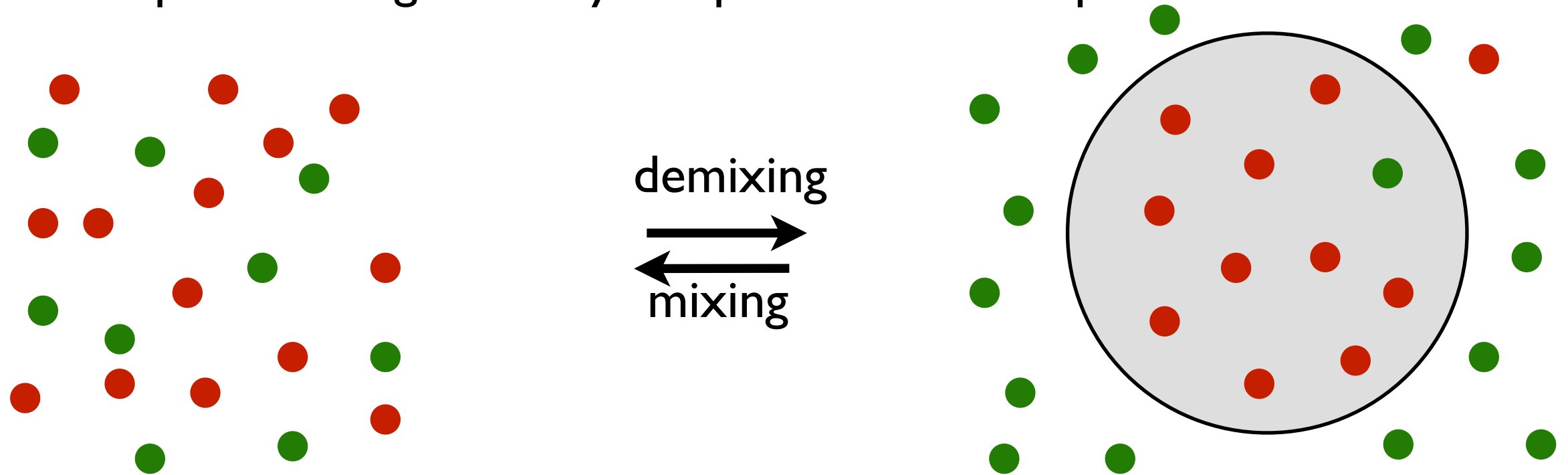
Liquid-liquid phase separation

phase separation regulated by temperature or composition

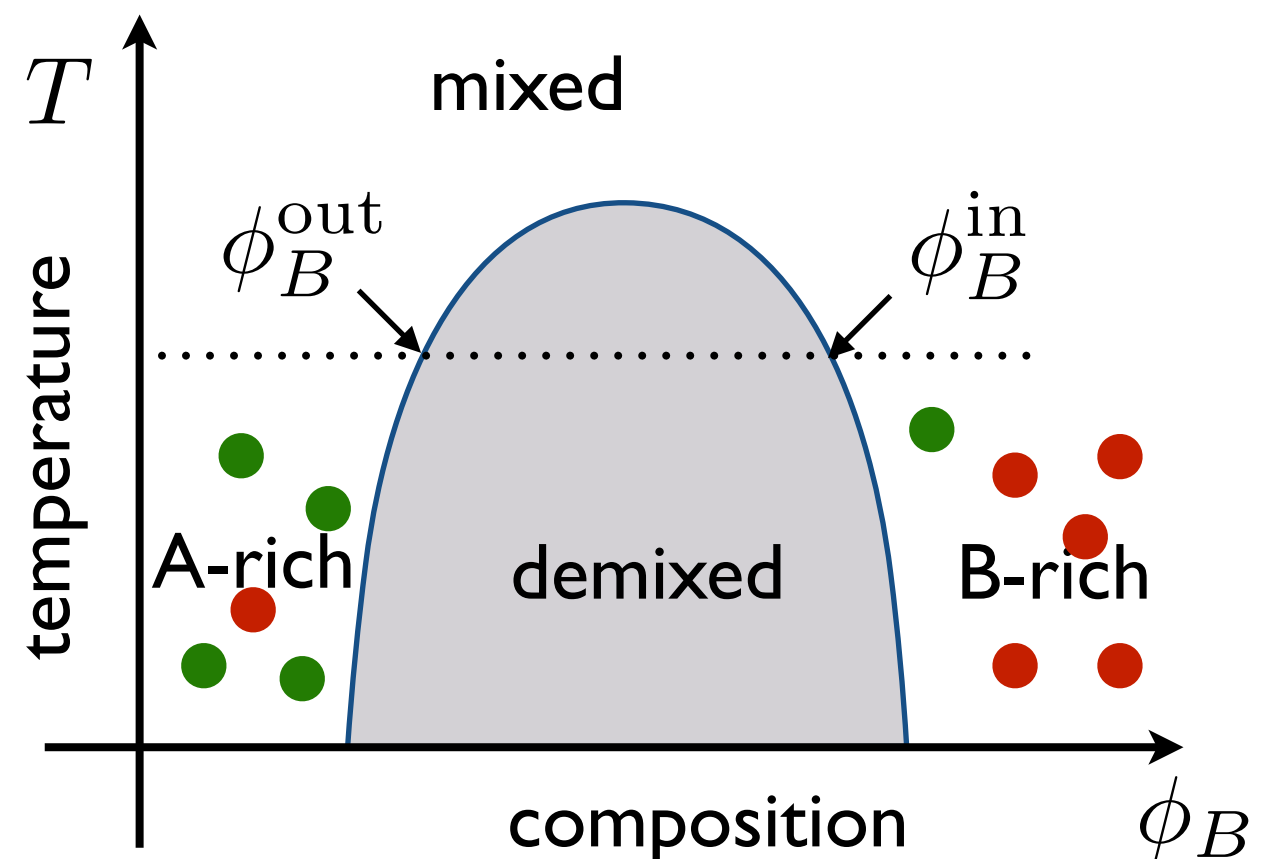


Liquid-liquid phase separation

phase separation regulated by temperature or composition



Competition between interaction energies and entropy



Phase transition in a cell

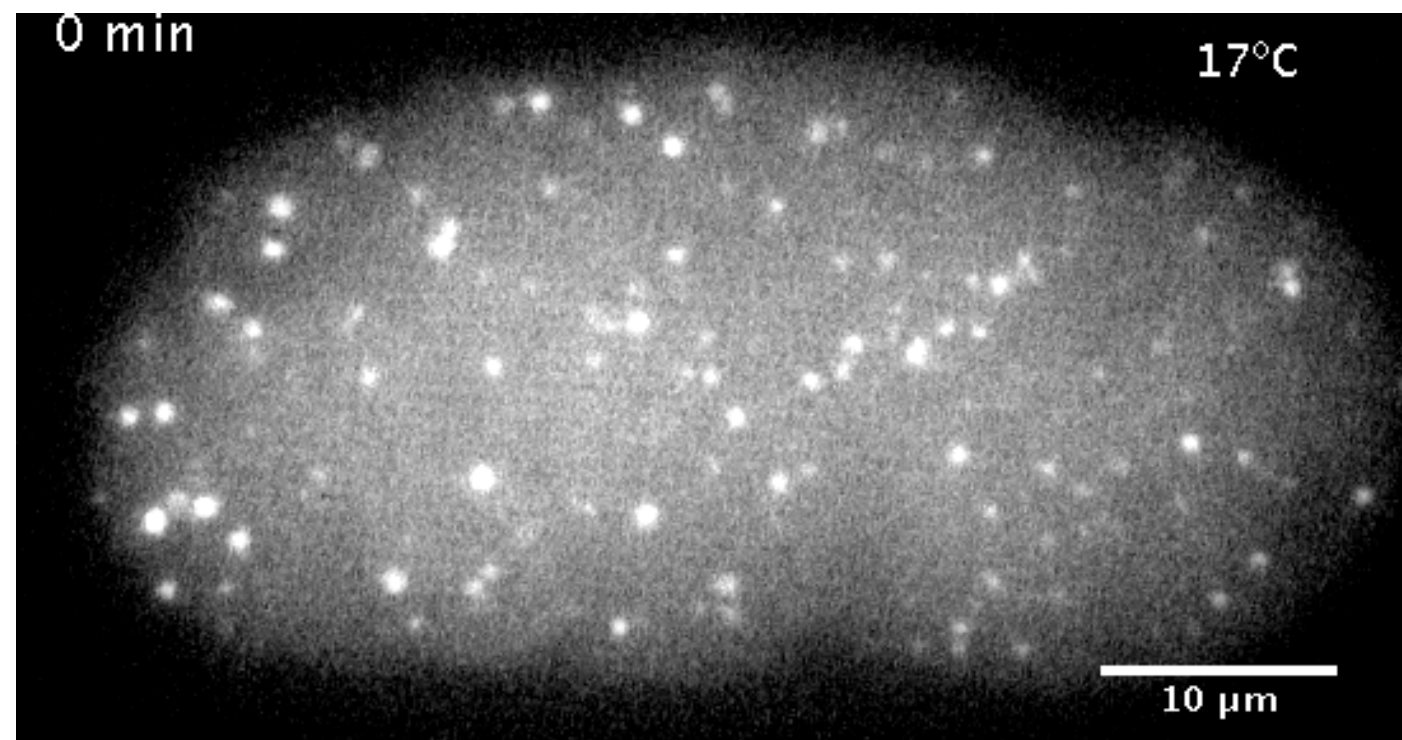
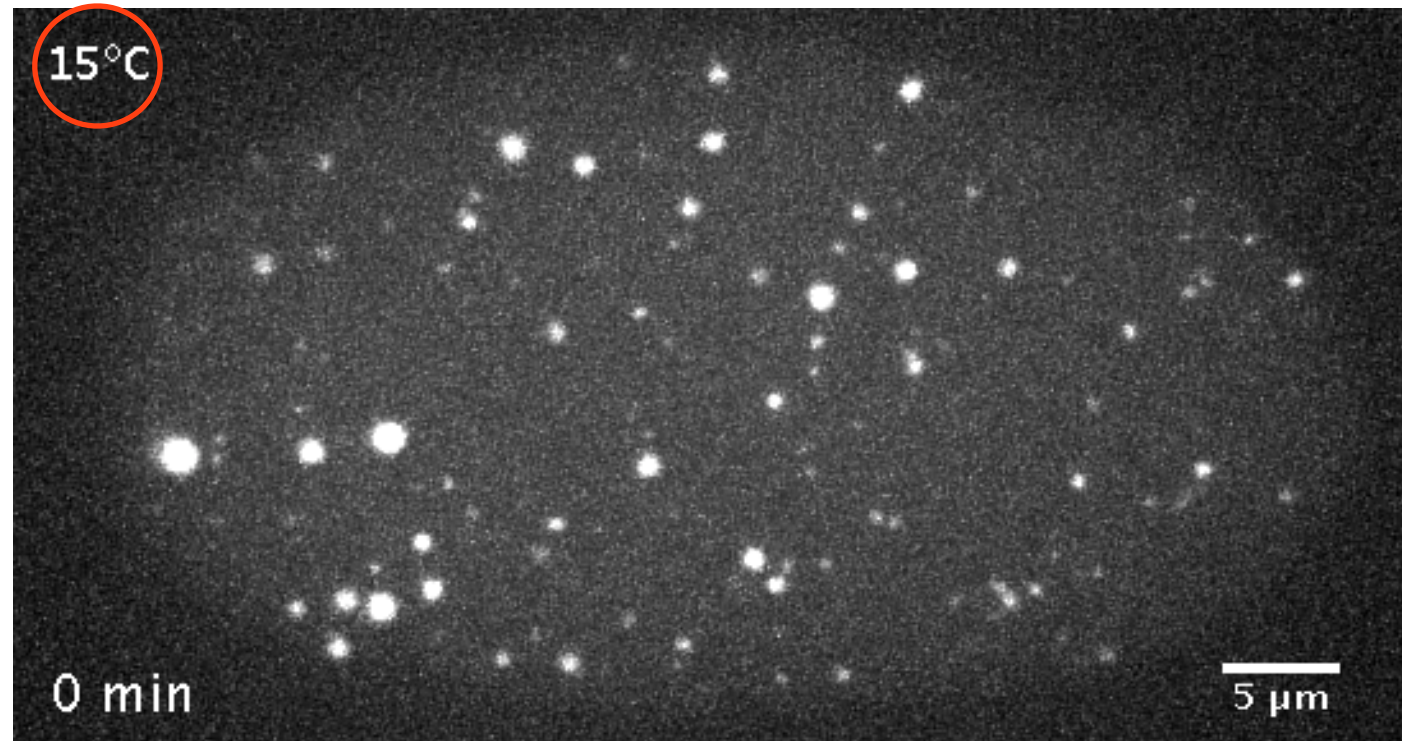
PGL-1::GFP

temperature
increase

15°C → 27°C

17°C

constant T°



Phase transition in a cell

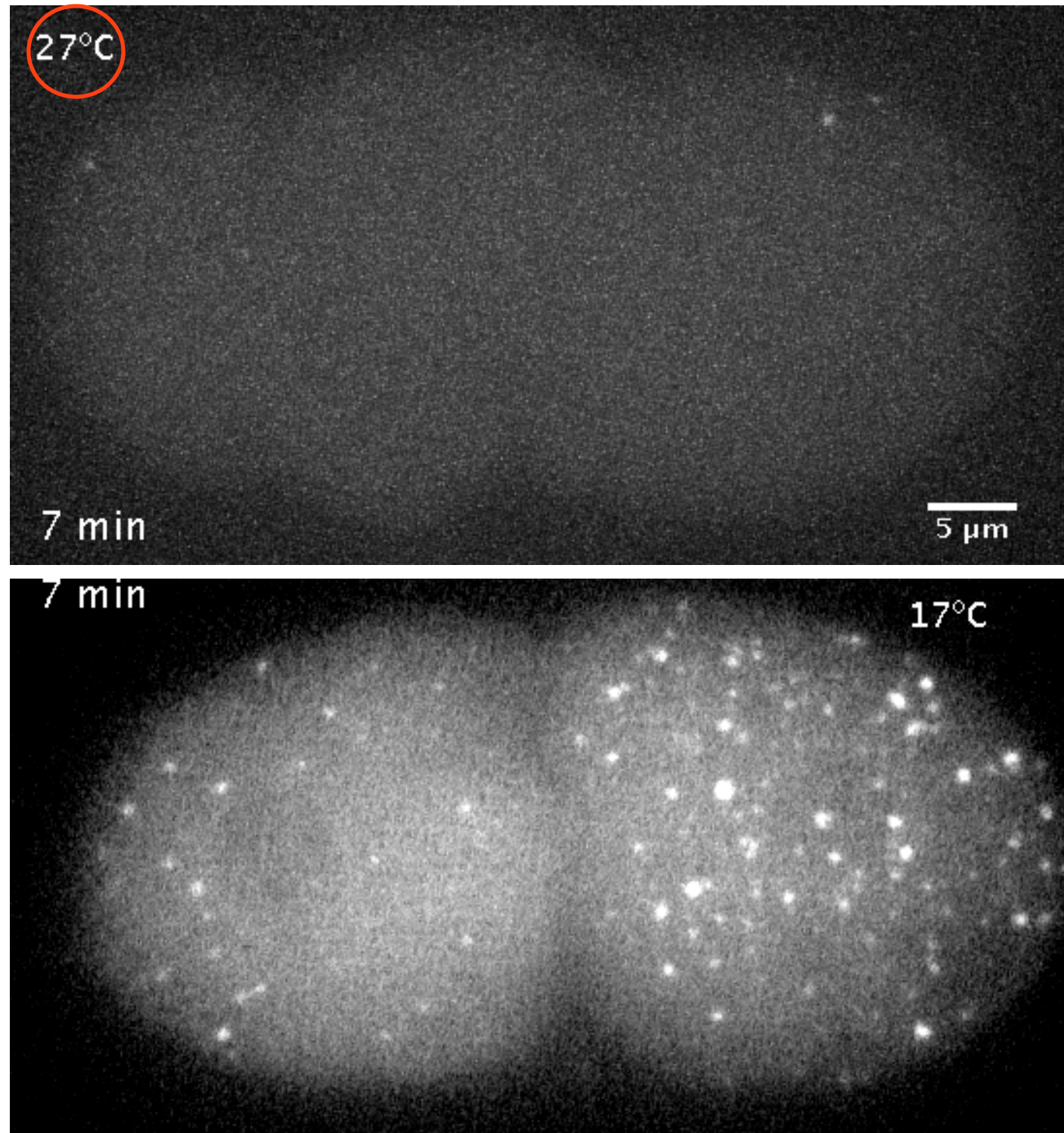
PGL-1::GFP

temperature
decrease

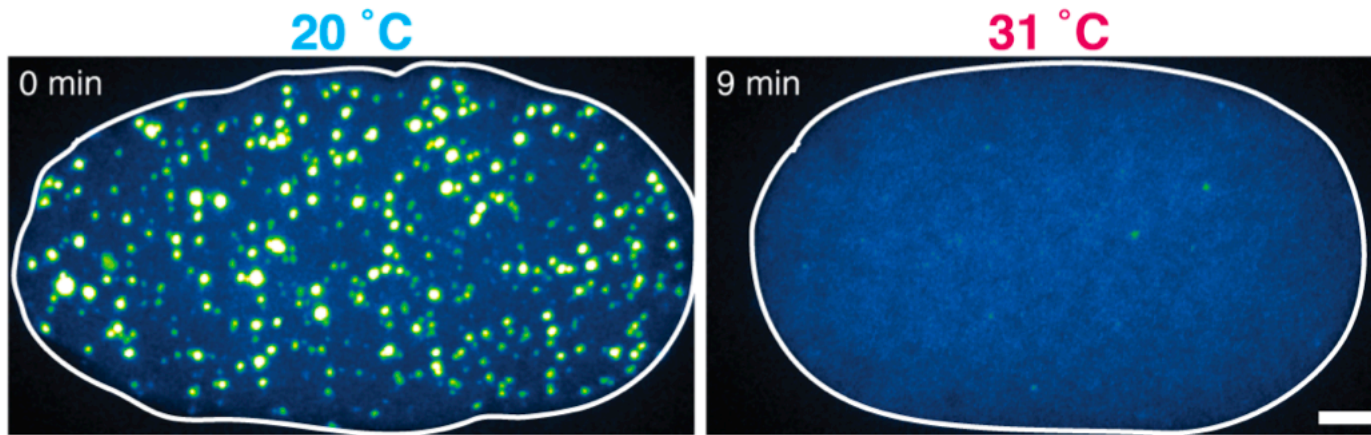
27°C → 15°C

17°C

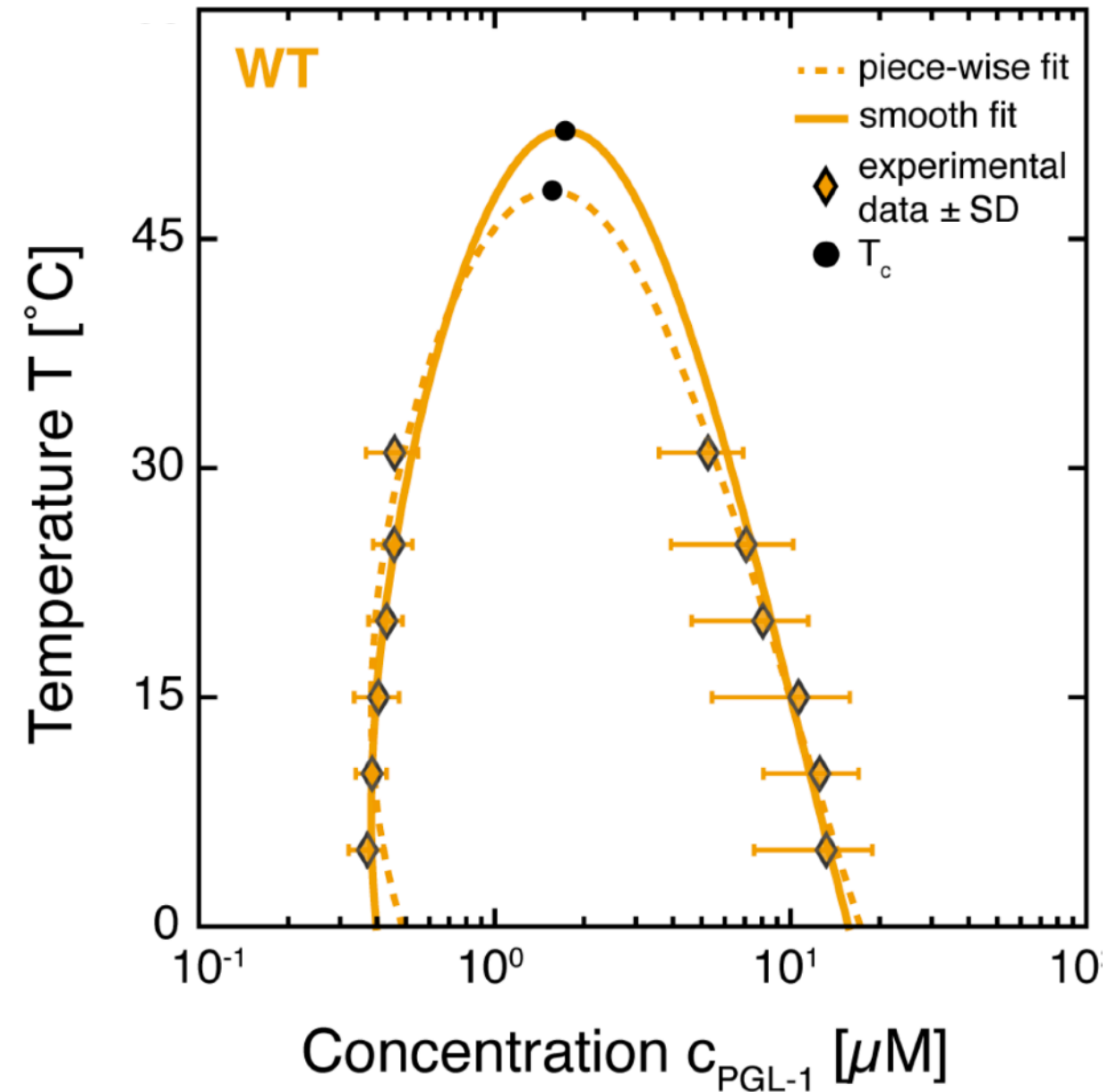
constant T°



Phase diagram



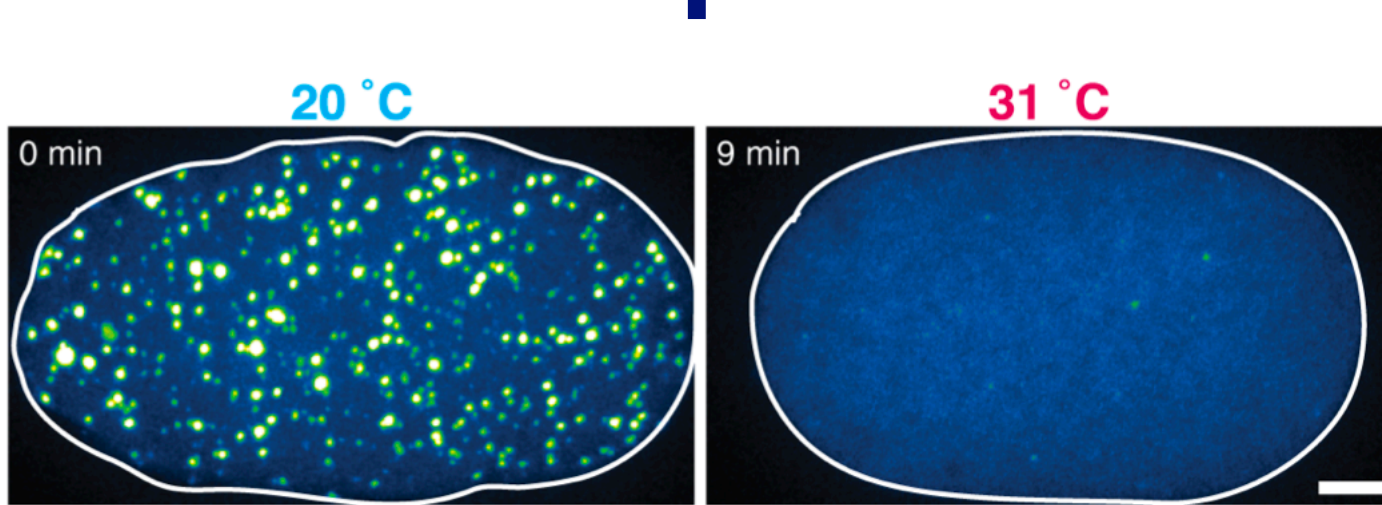
measurement of concentrations inside and outside



binary Flory Huggins Model

$$f = k_B T \left(\frac{\phi}{\nu(T)} \ln \phi + \frac{(1 - \phi)}{\nu_0} \ln (1 - \phi) + \frac{\chi(T)}{\nu_0} \phi (1 - \phi) \right)$$

Active processes: T fluctuations



heat produced by active processes

$$Q \simeq 10^3 \text{ W/m}^3$$

$$\alpha \simeq 1.4 \cdot 10^{-7} \text{ m}^2/\text{s}$$

a single ATP hydrolysis event dissipating $10k_B T$ produces a temperature perturbation

heat source

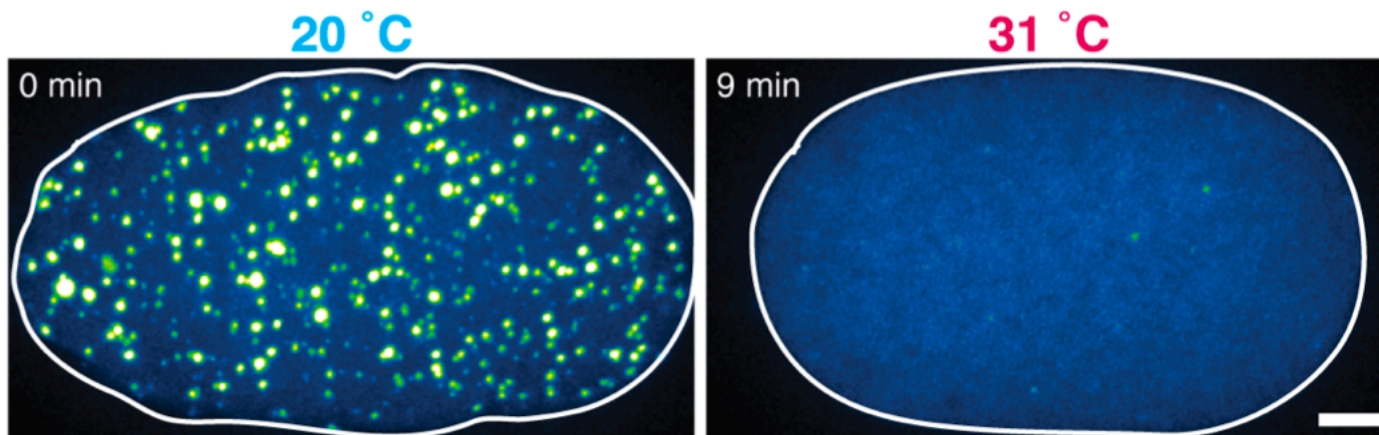
Dynamic temperature profile

$$\partial_t T = \alpha \nabla^2 T + \frac{Q}{\rho c_p}$$

thermal diffusivity

specific heat

Active processes: T fluctuations



heat produced by active processes

$$Q \simeq 10^3 \text{ W/m}^3$$

$$\alpha \simeq 1.4 \cdot 10^{-7} \text{ m}^2/\text{s}$$

heat source

Dynamic temperature profile

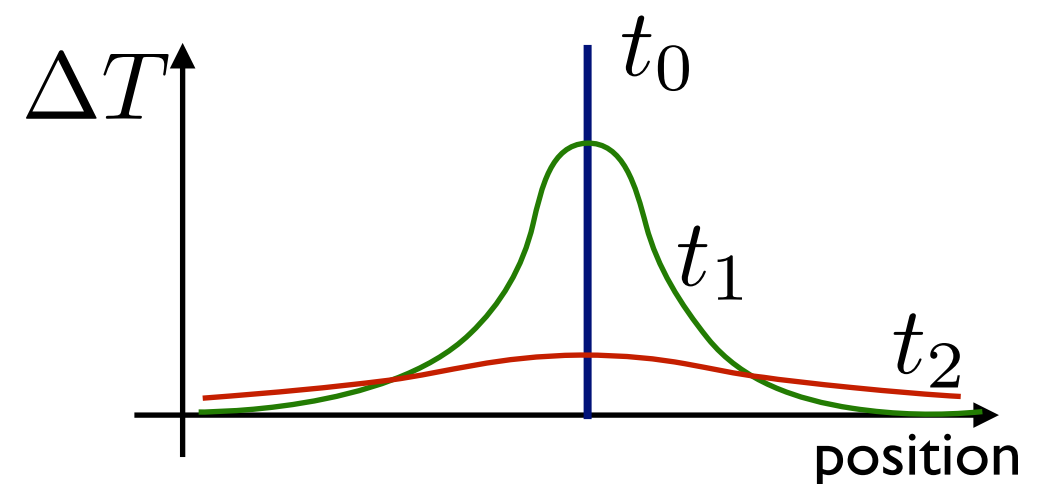
$$\partial_t T = \alpha \nabla^2 T + \frac{Q}{\rho c_p}$$

thermal diffusivity

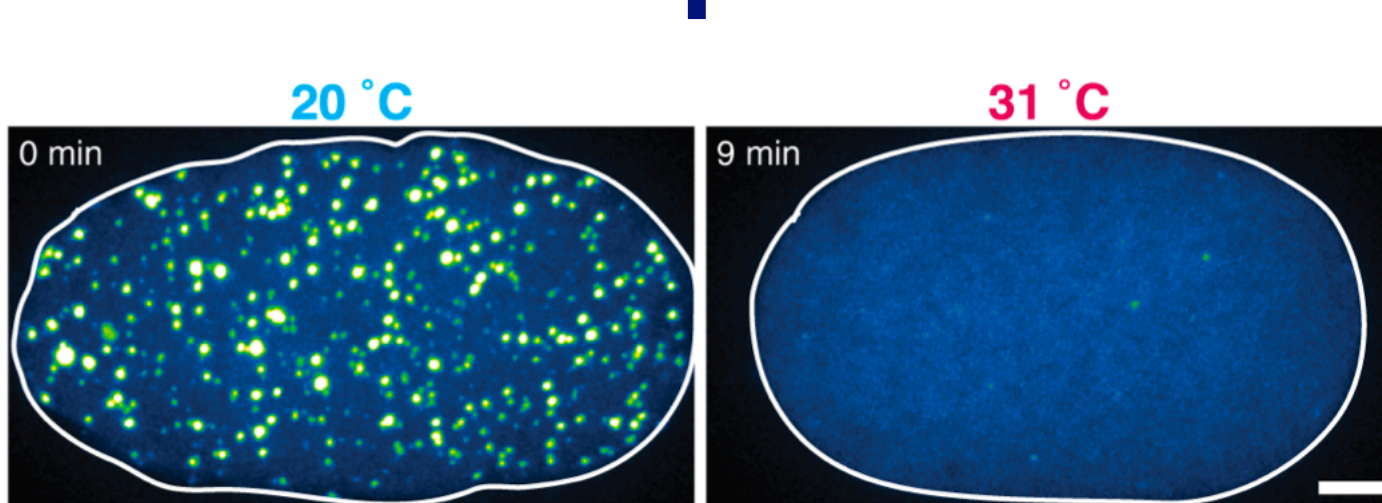
specific heat

a single ATP hydrolysis event dissipating $10k_B T$ produces a temperature perturbation

time	distance	temperature
100ps	1nm	3K
10ns	10nm	3mK
1 μ s	100nm	3 μ K



Active processes: T fluctuations



heat produced by active processes

$$Q \simeq 10^3 \text{ W/m}^3$$

heat source
Dynamic temperature profile

$$\partial_t T = \alpha \nabla^2 T + \frac{Q}{\rho c_p}$$

thermal diffusivity specific heat

$$\alpha \simeq 1.4 \cdot 10^{-7} \text{ m}^2/\text{s}$$

a single ATP hydrolysis event dissipating $10k_B T$ produces a temperature perturbation

time	distance	temperature
100ps	1nm	3K
10ns	10nm	3mK
$1\mu\text{s}$	100nm	$3\mu\text{K}$

volume elements of 100nm size
beyond $1\mu\text{s}$ timescales
are at
local thermodynamic equilibrium

Thermodynamics of phase coexistence

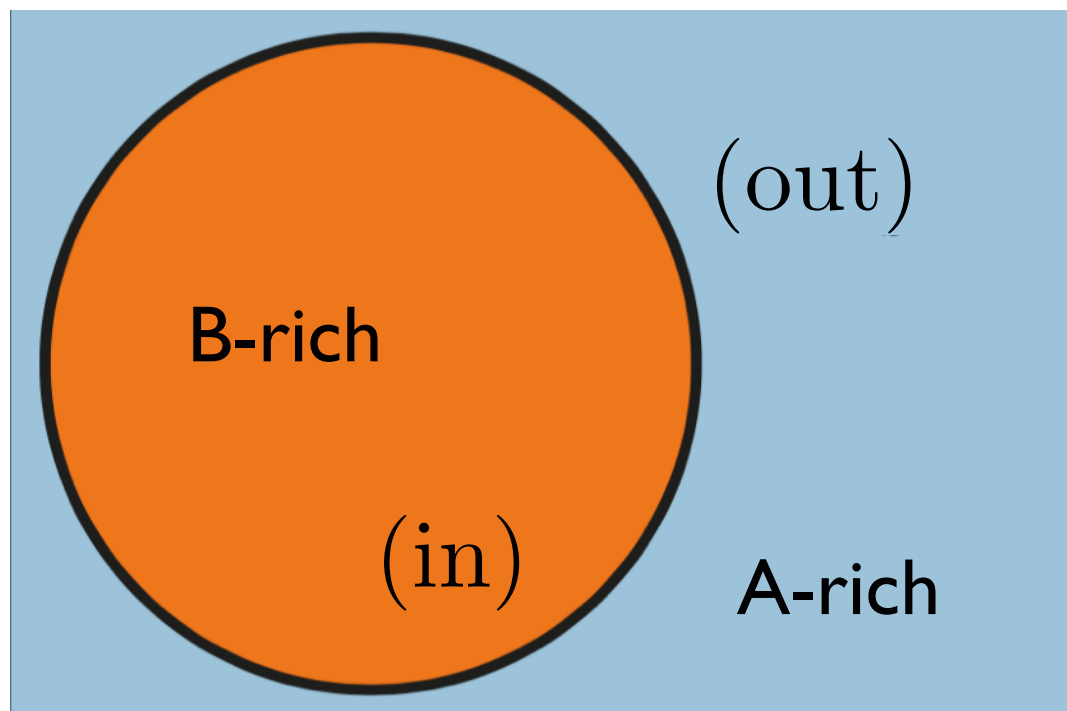
entropy $\rightarrow$ mixing
 interaction energies $\rightarrow$ demixing

$$F = U - TS$$

$$P^{\text{in}} = P^{\text{out}}$$

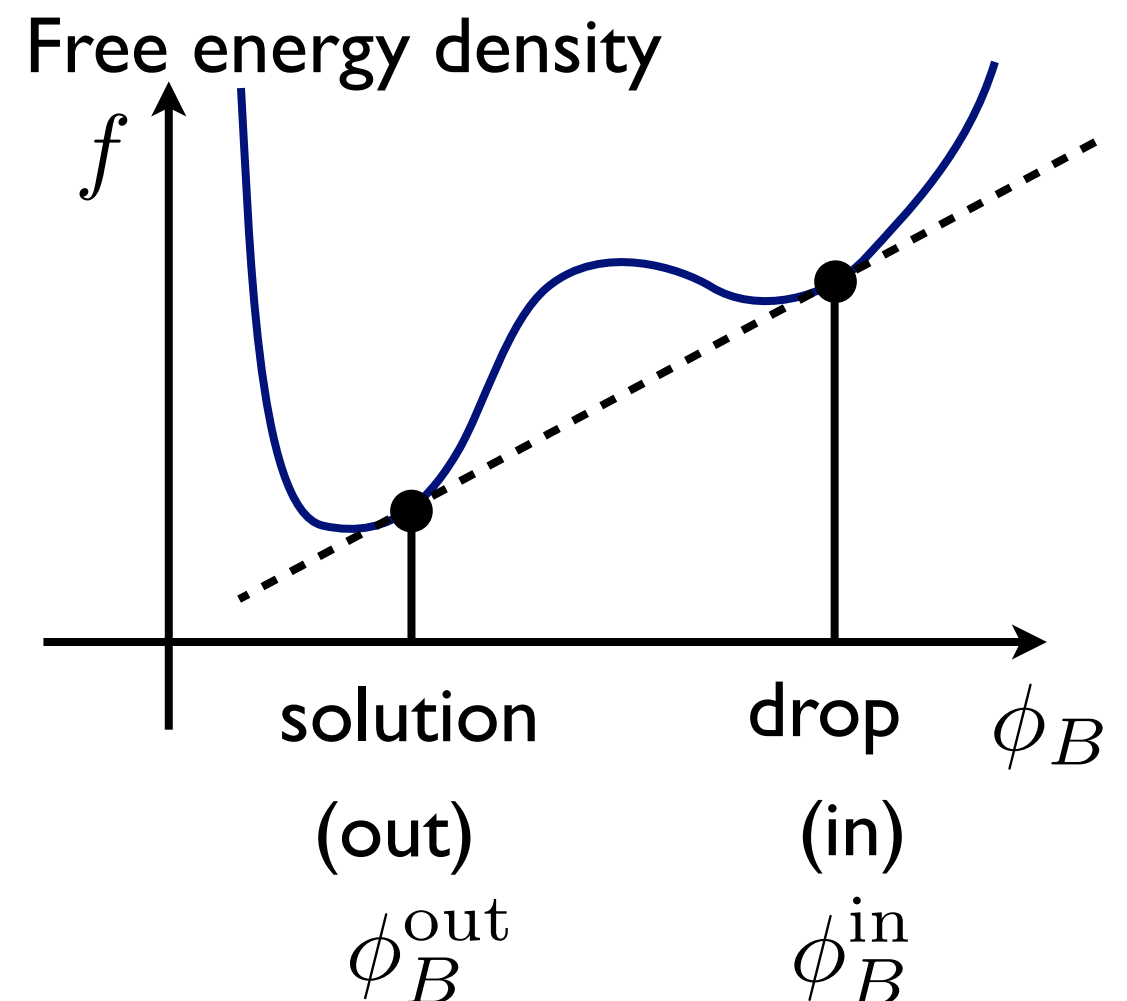
$$\mu_A^{\text{in}} = \mu_A^{\text{out}}$$

$$\mu_B^{\text{in}} = \mu_B^{\text{out}}$$



Concentration jump at droplet interface

Concentration ϕ_B



Thermodynamics of phase coexistence

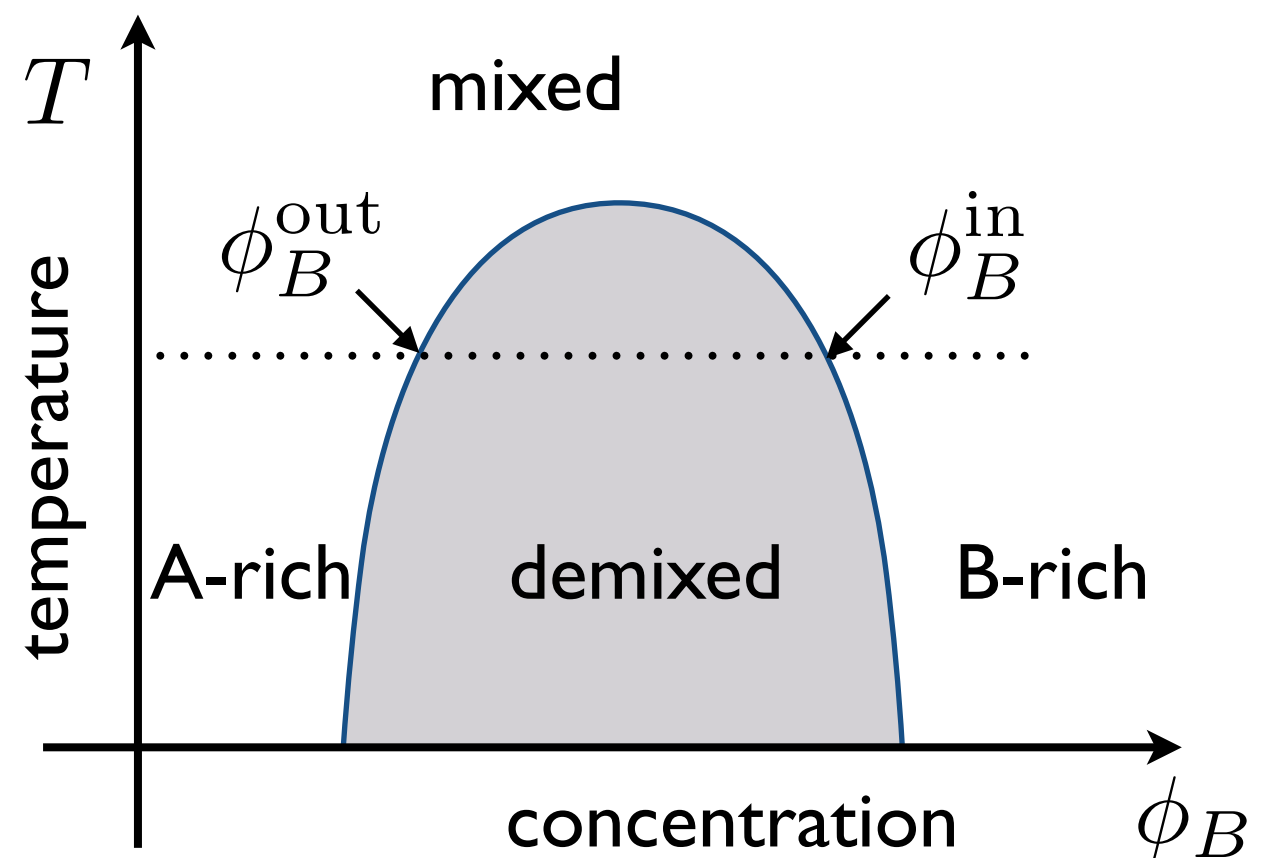
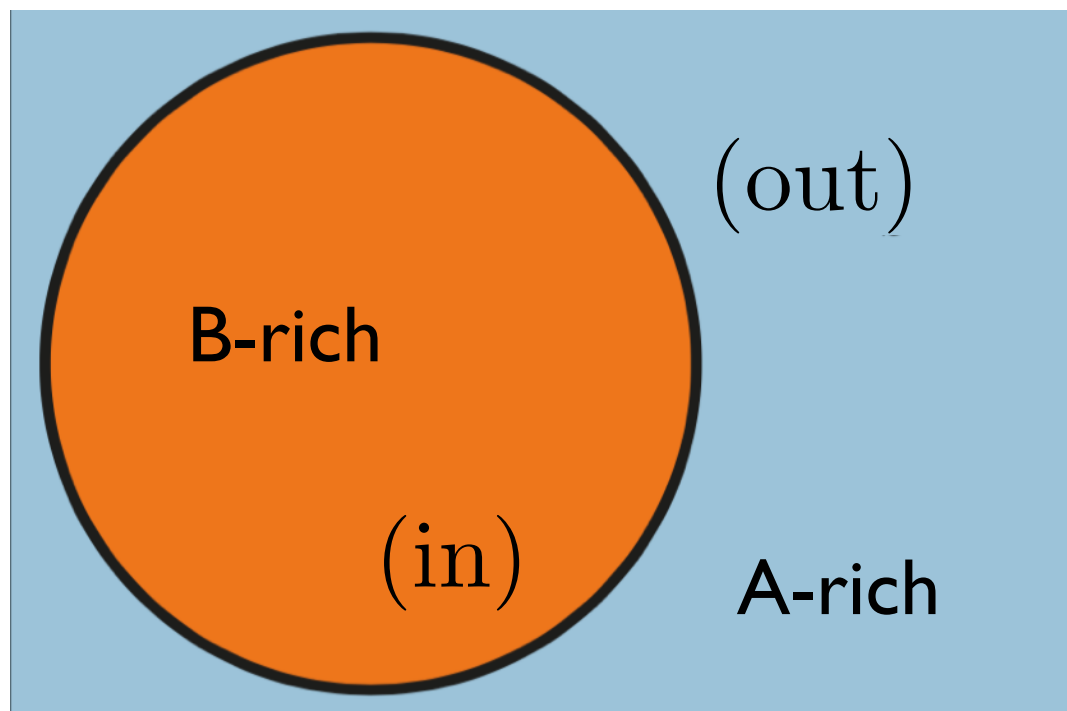
entropy

→ mixing

interaction energies → demixing

Phase diagram

Demixing of B from A



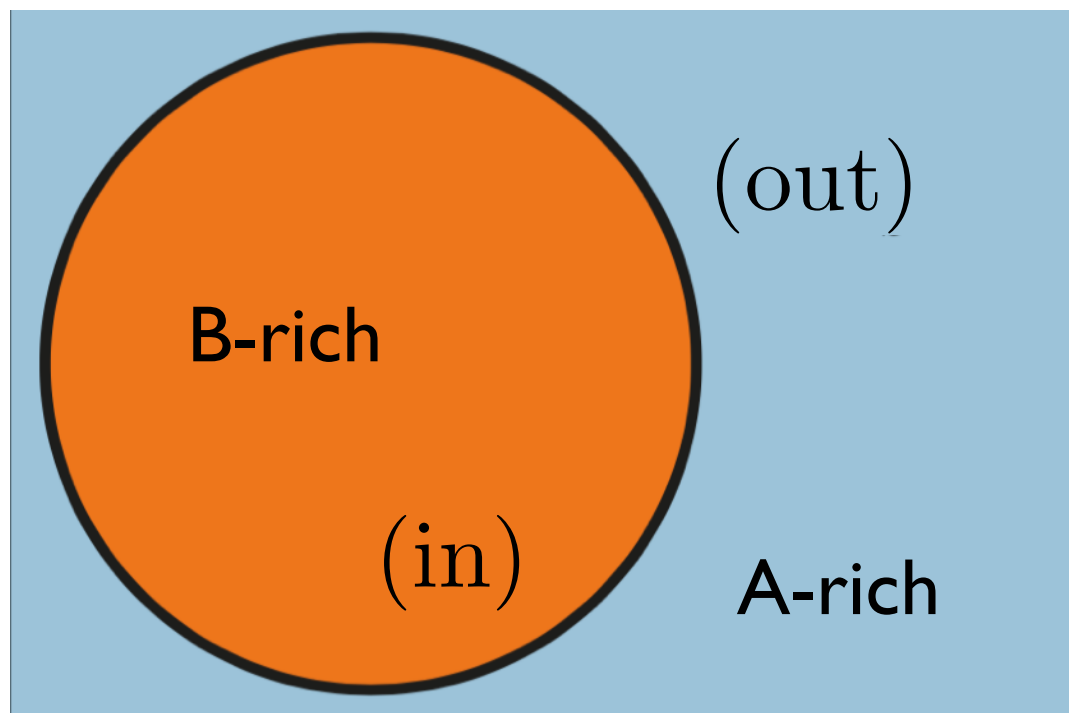
Concentration ϕ_B

Droplet coexistence

surface tension γ

radius R

Demixing of B from A



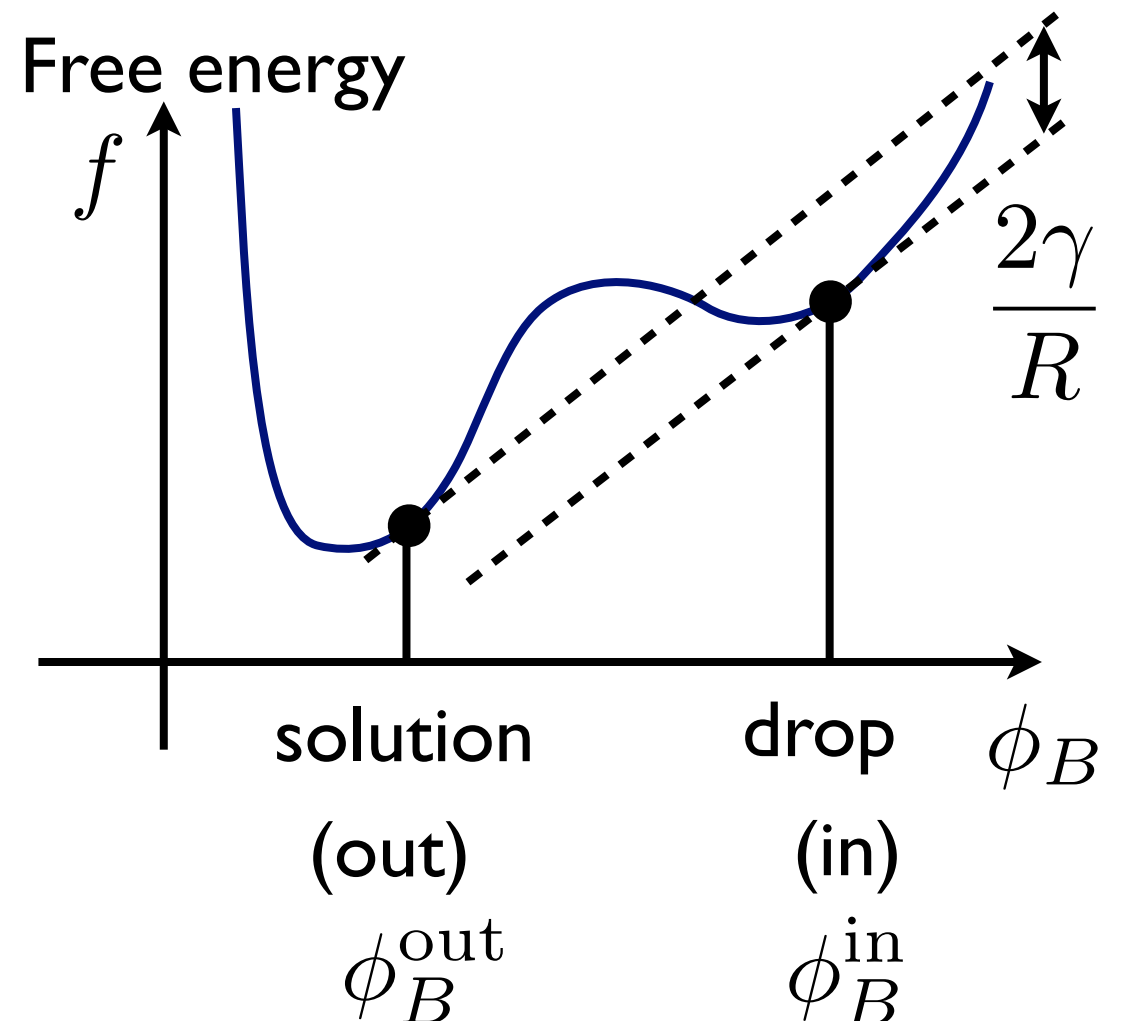
Concentration jump at droplet interface

Concentration ϕ_B

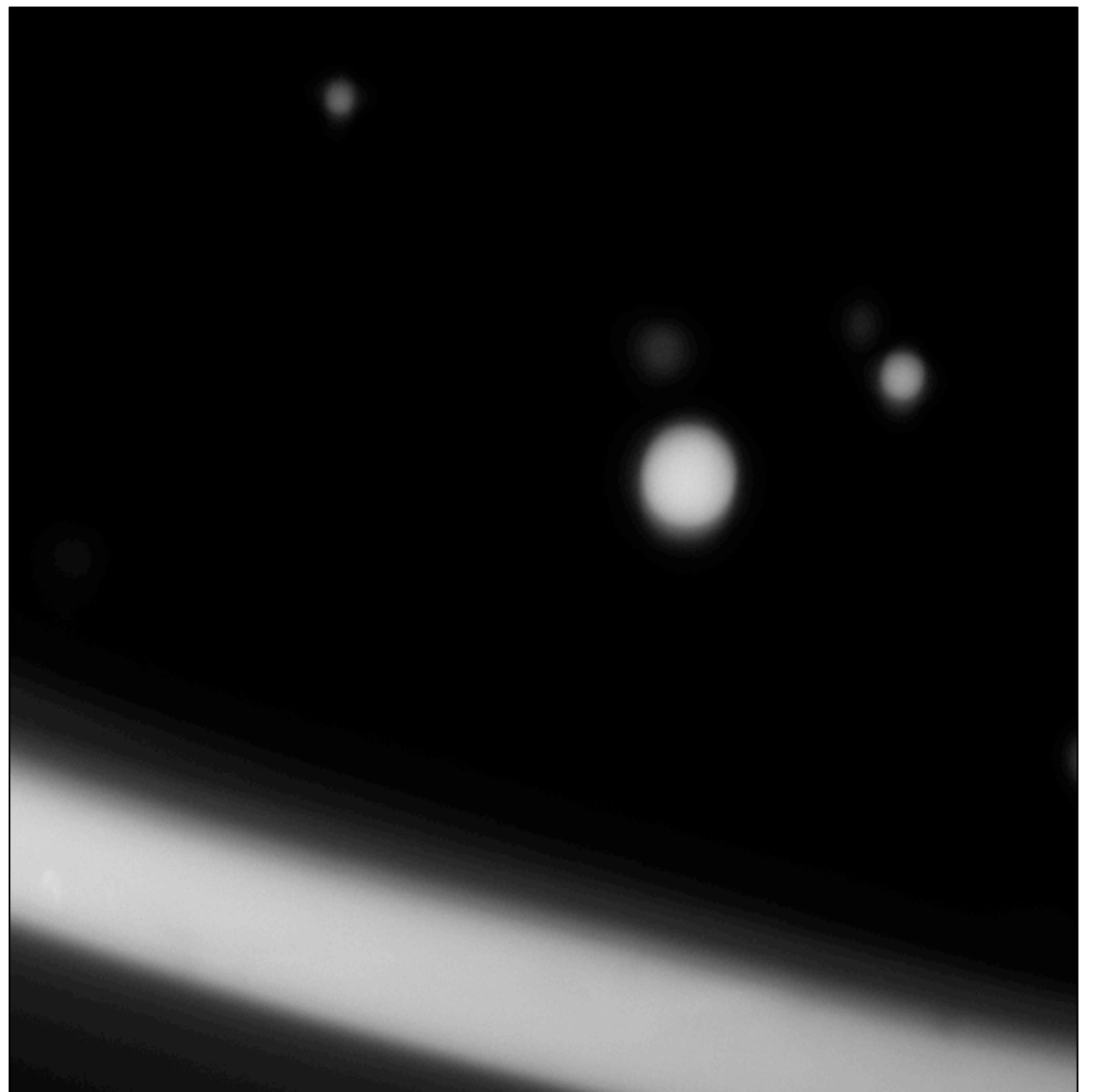
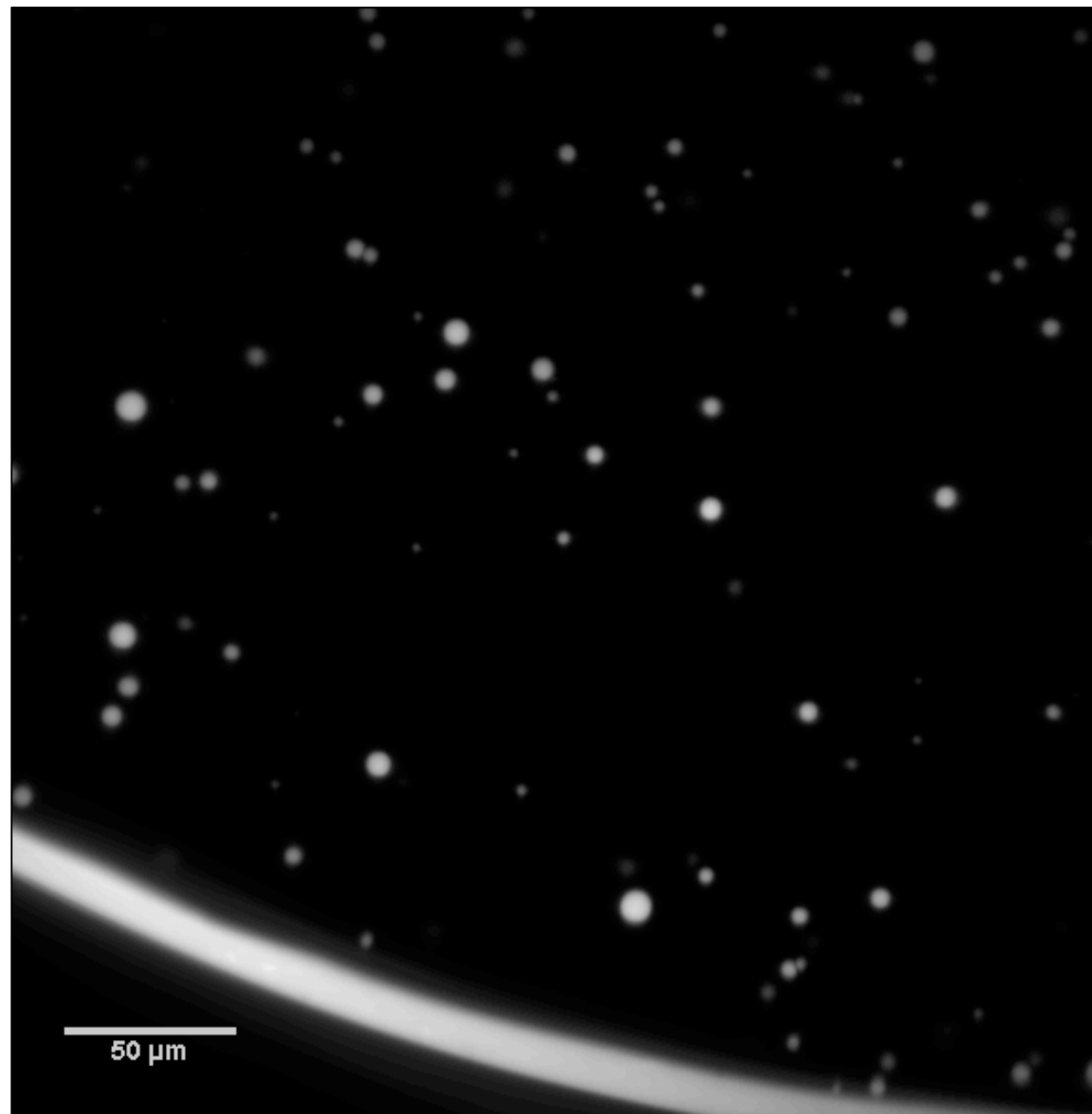
$$P^{\text{in}} = P^{\text{out}} + \frac{2\gamma}{R} \quad \text{Laplace Pressure}$$

$$\mu_A^{\text{in}} = \mu_A^{\text{out}}$$

$$\mu_B^{\text{in}} = \mu_B^{\text{out}}$$

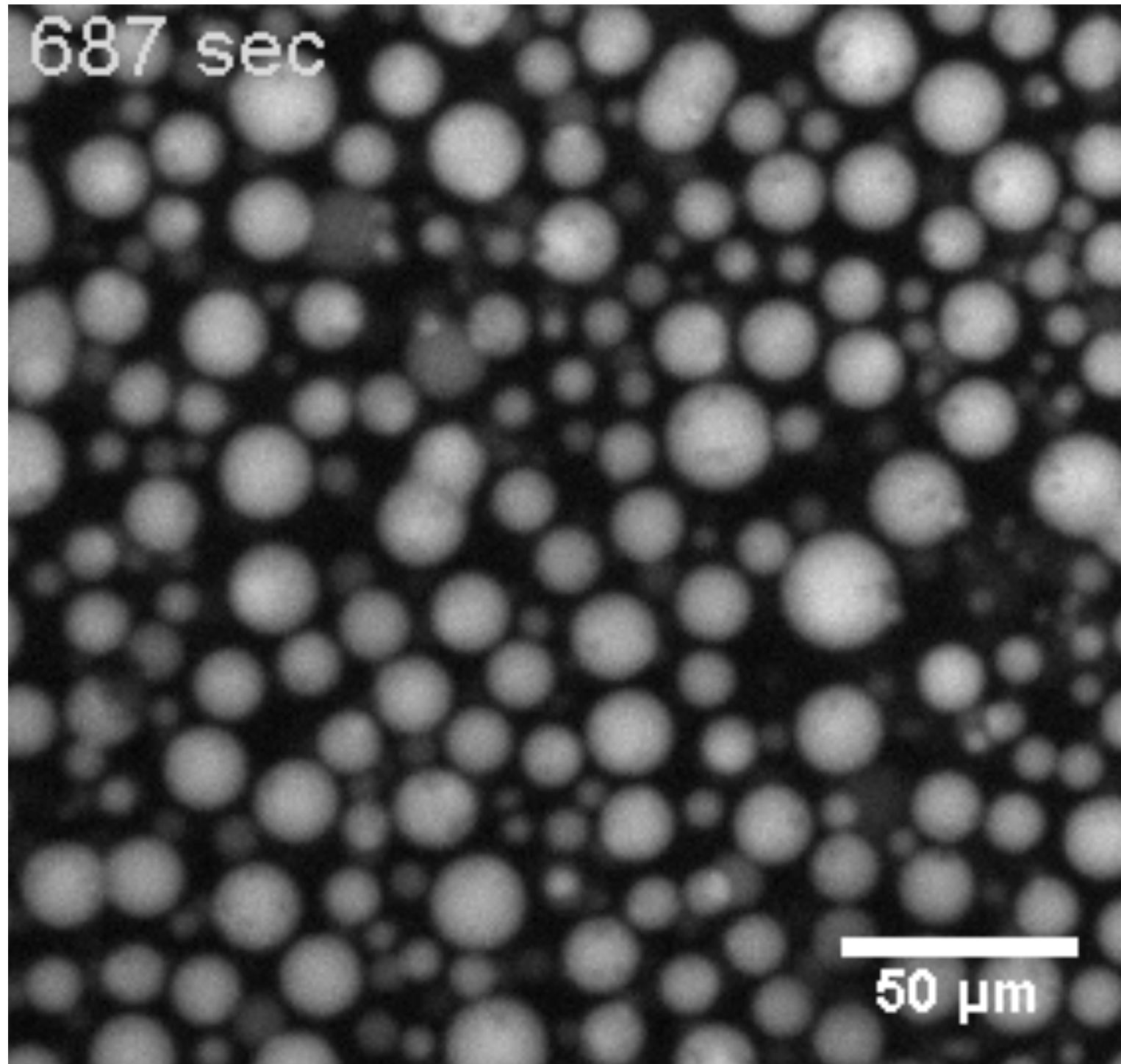


In vitro protein condensation

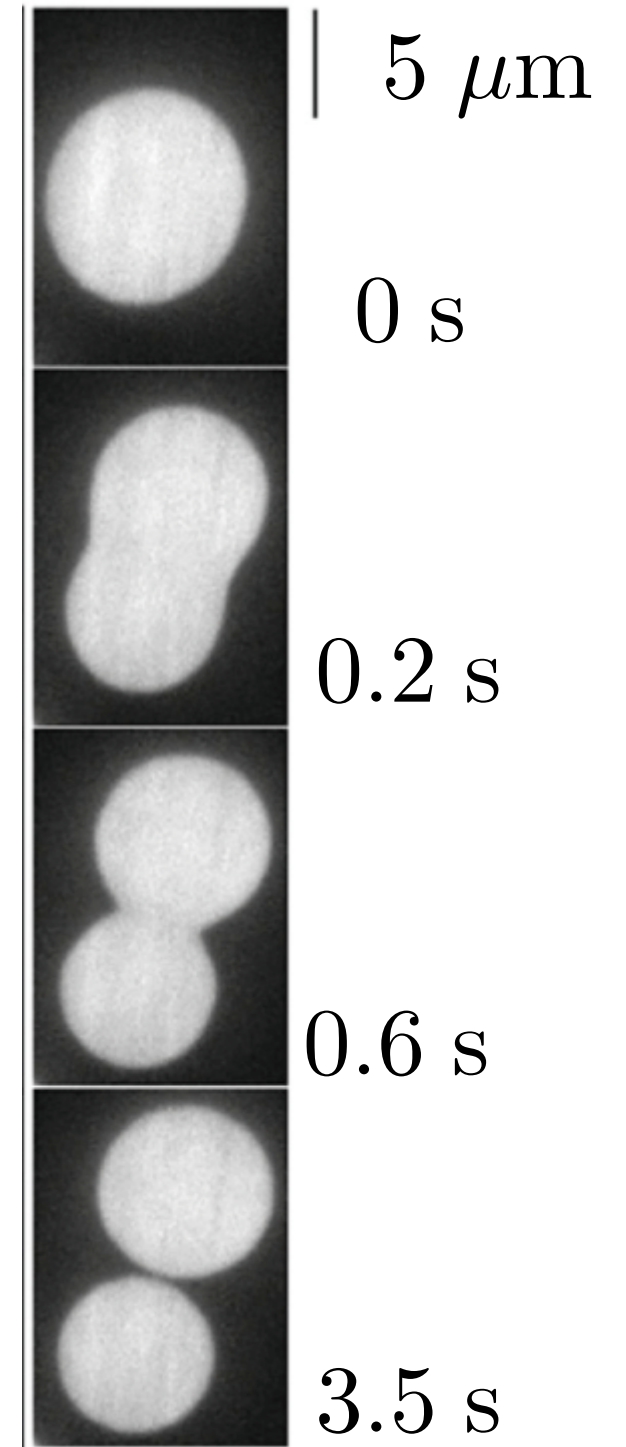


purified P granule protein PGL-3 in a physiological buffer

In vitro droplet ripening



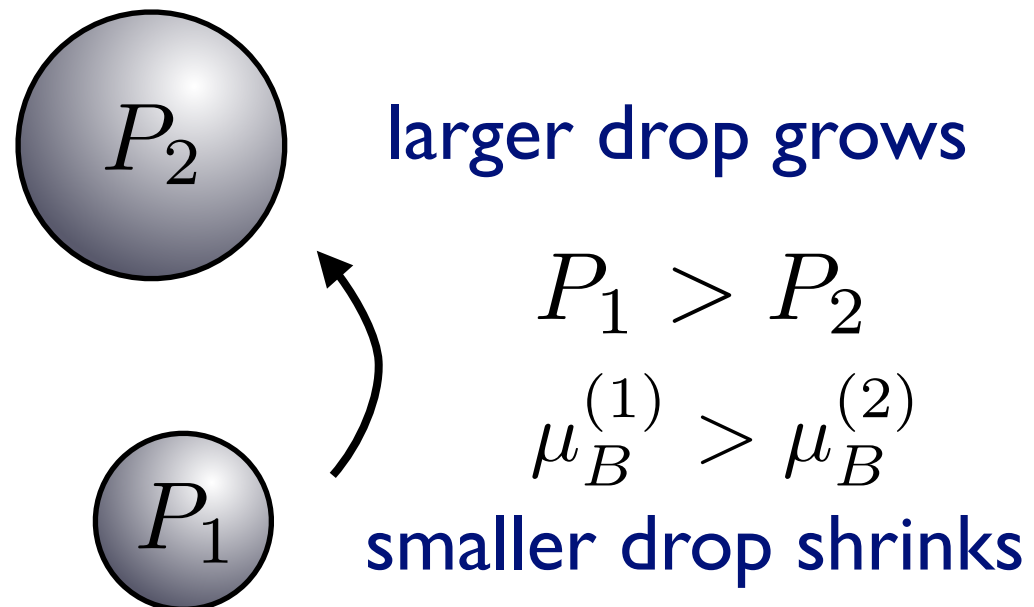
PGL-3 droplets fuse and grow



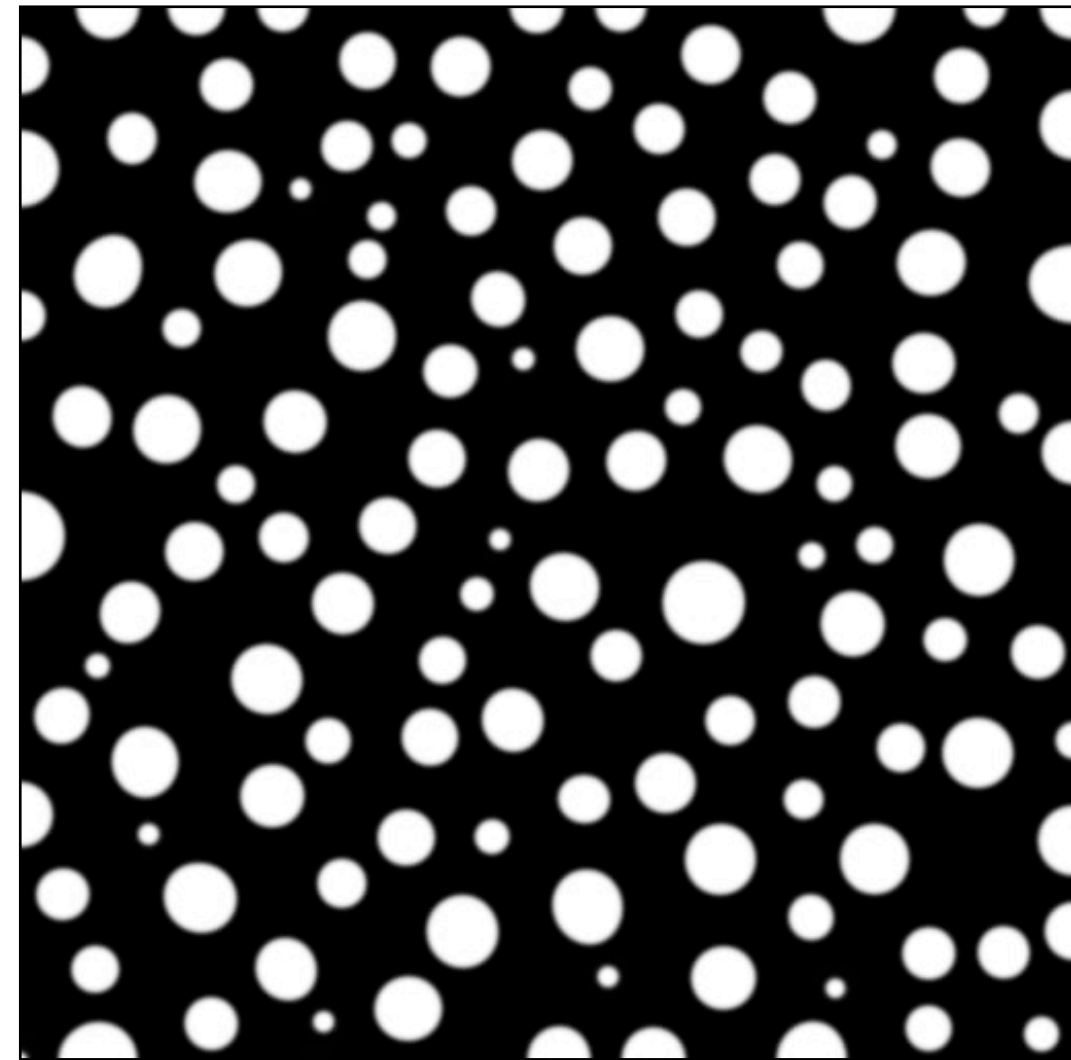
Shambaditya Saha
Louise Jawerth

Ostwald ripening

Ostwald ripening: two equal drops are unstable



Droplet ripening

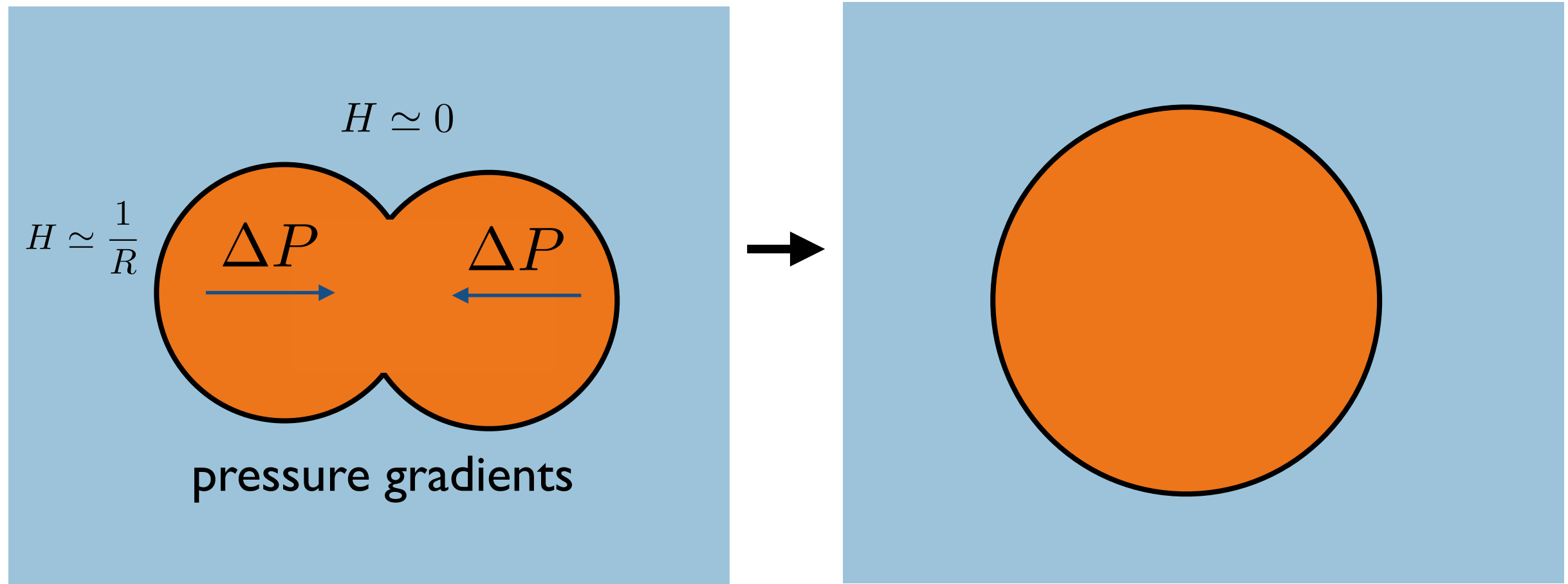


Laplace pressure

$$\Delta P = \frac{2\gamma}{R}$$

$$\partial_t \phi = \nabla \cdot (\lambda \nabla \mu)$$

Droplet fusion: hydrodynamics



Laplace pressure $P_L = 2\gamma H$

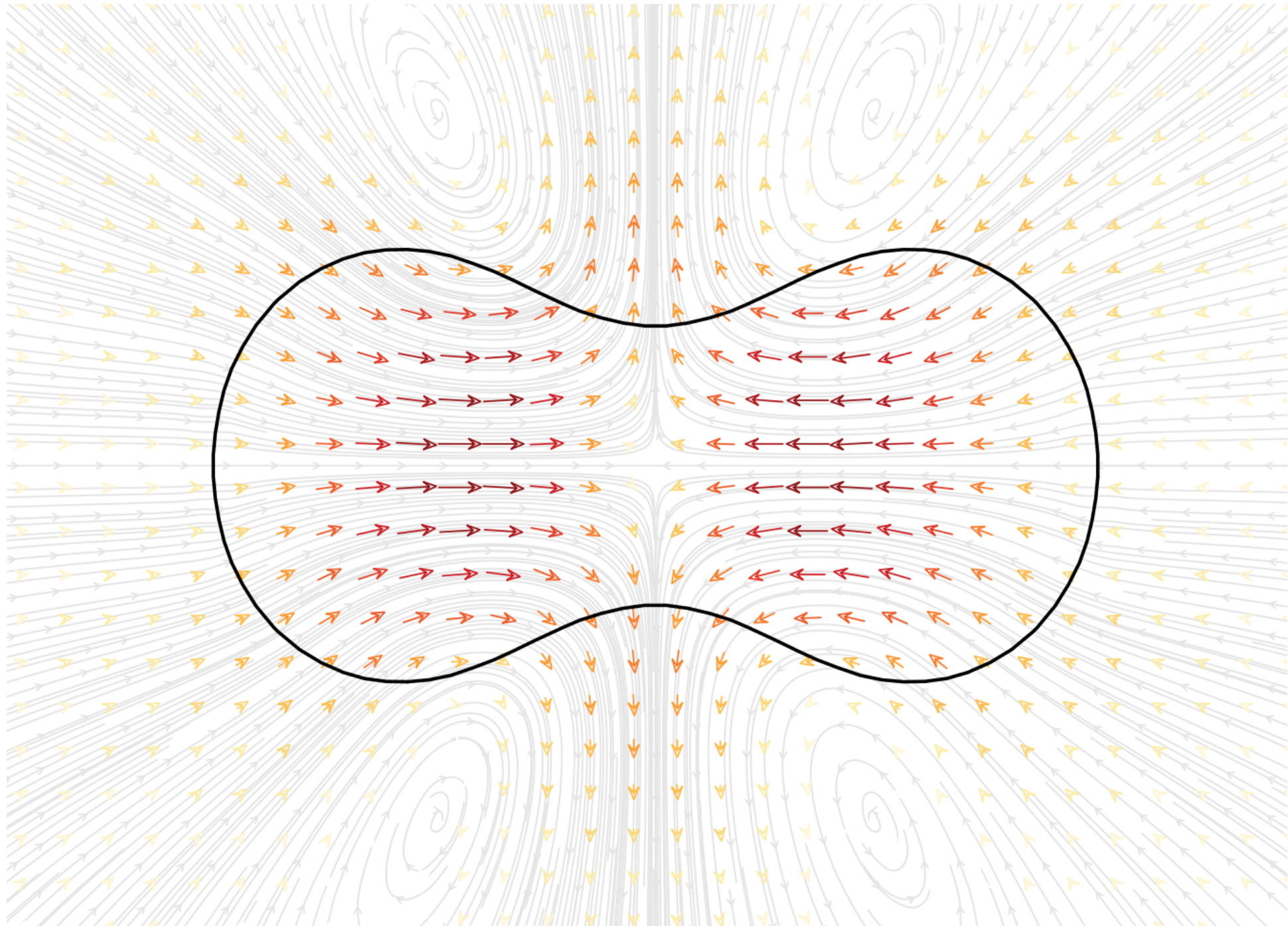
surface tension γ

mean curvature of droplet surface H

flow velocity $\eta \Delta \mathbf{v} = \nabla P$

viscosity η

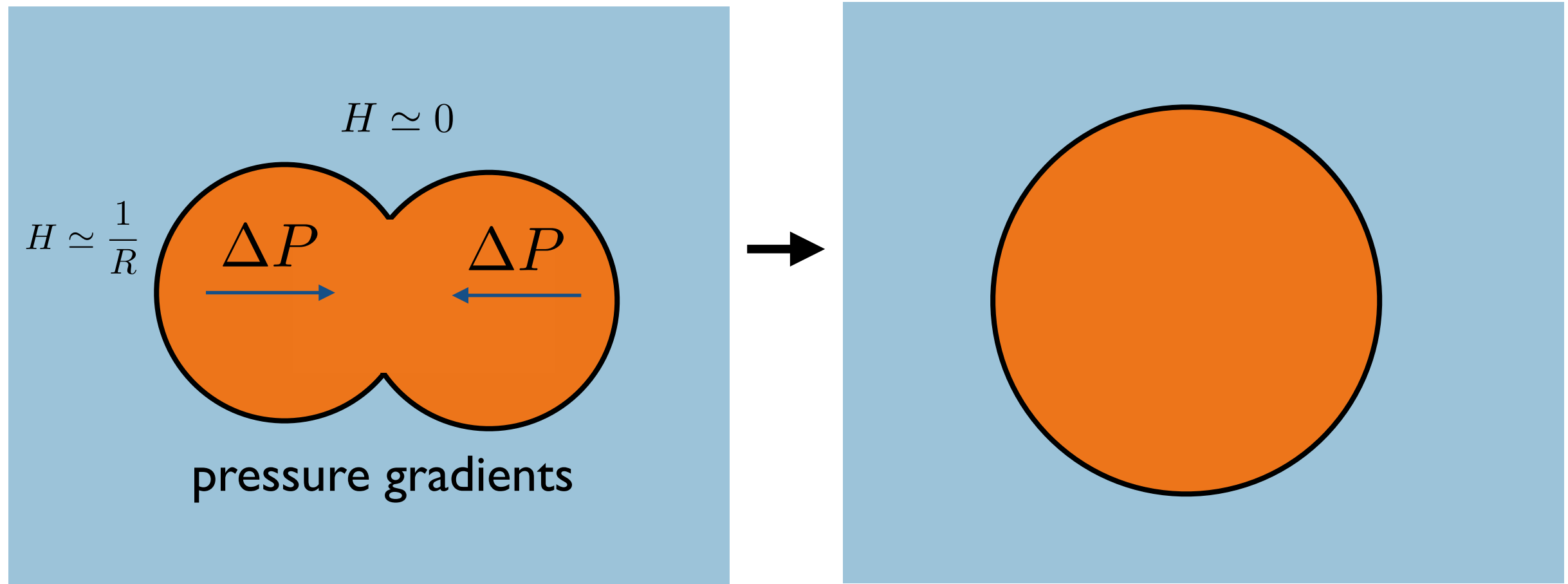
Droplet fusion: hydrodynamics



Laplace pressure $\Delta P = \frac{2\gamma}{R}$ flow velocity $\eta \Delta \mathbf{v} = \nabla P$

Rabea Seyboldt

Droplet fusion: hydrodynamics



Laplace pressure $P_L = 2\gamma H$

surface tension γ

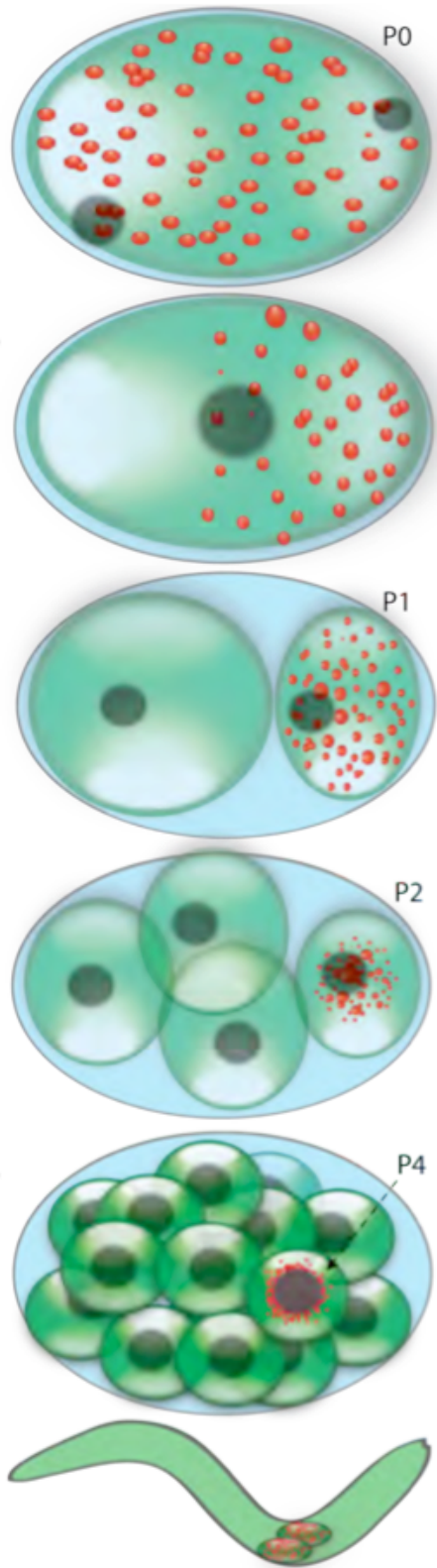
shape dynamics $\frac{dR}{dt} \sim \frac{\gamma}{\eta} \left(1 - \frac{R}{R_0}\right)$

flow velocity $\eta \Delta \mathbf{v} = \nabla P$

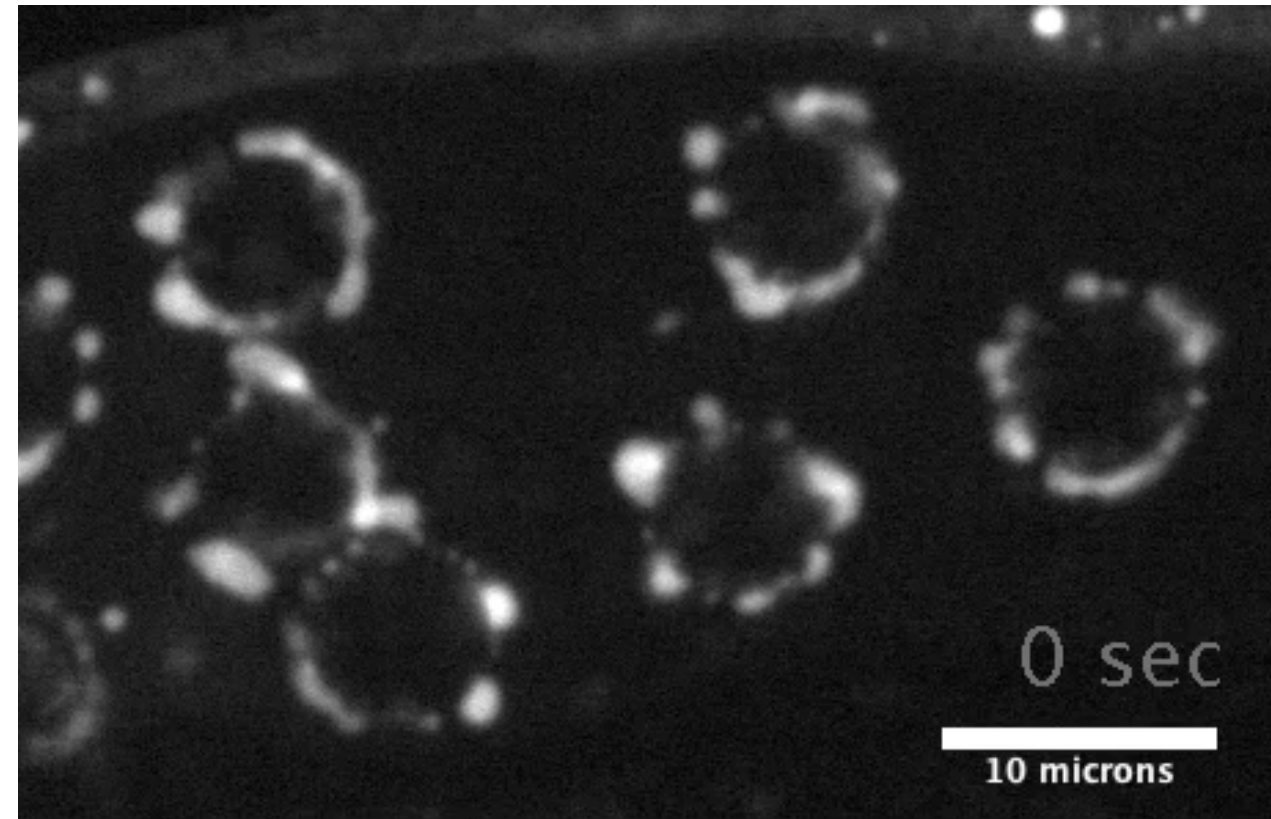
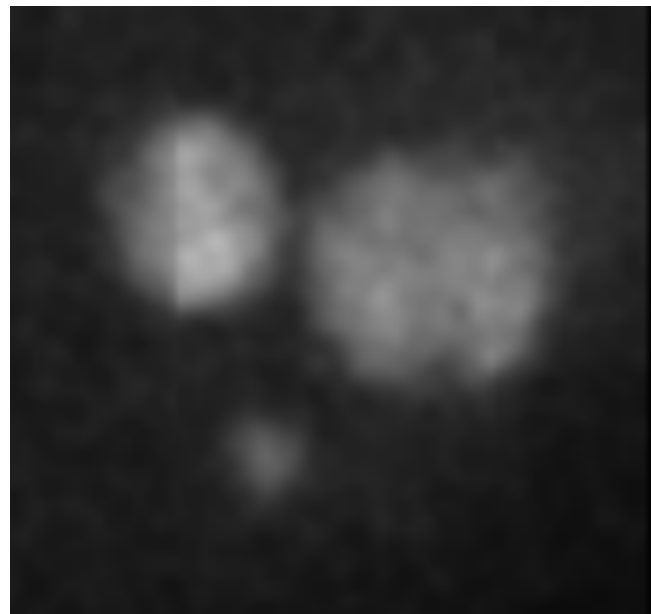
viscosity η

Time scale $\tau \simeq \frac{\eta}{\gamma} R_0$

P granules are liquid drops



P-granule flow
and fusion



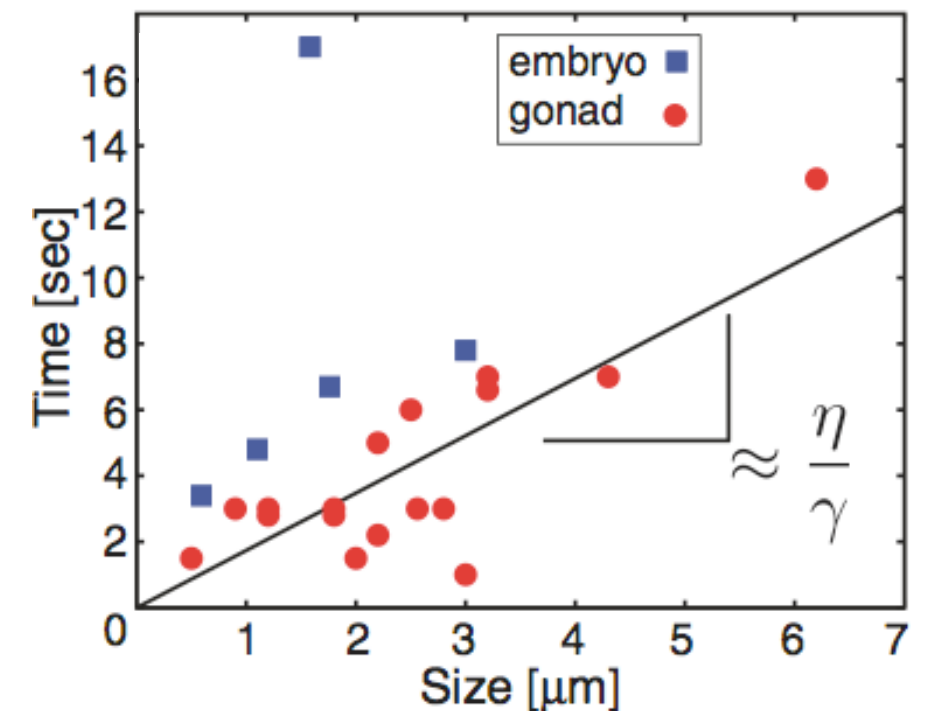
viscosity η

surface tension γ

$$\eta \simeq 1000 \eta_{H_2O}$$

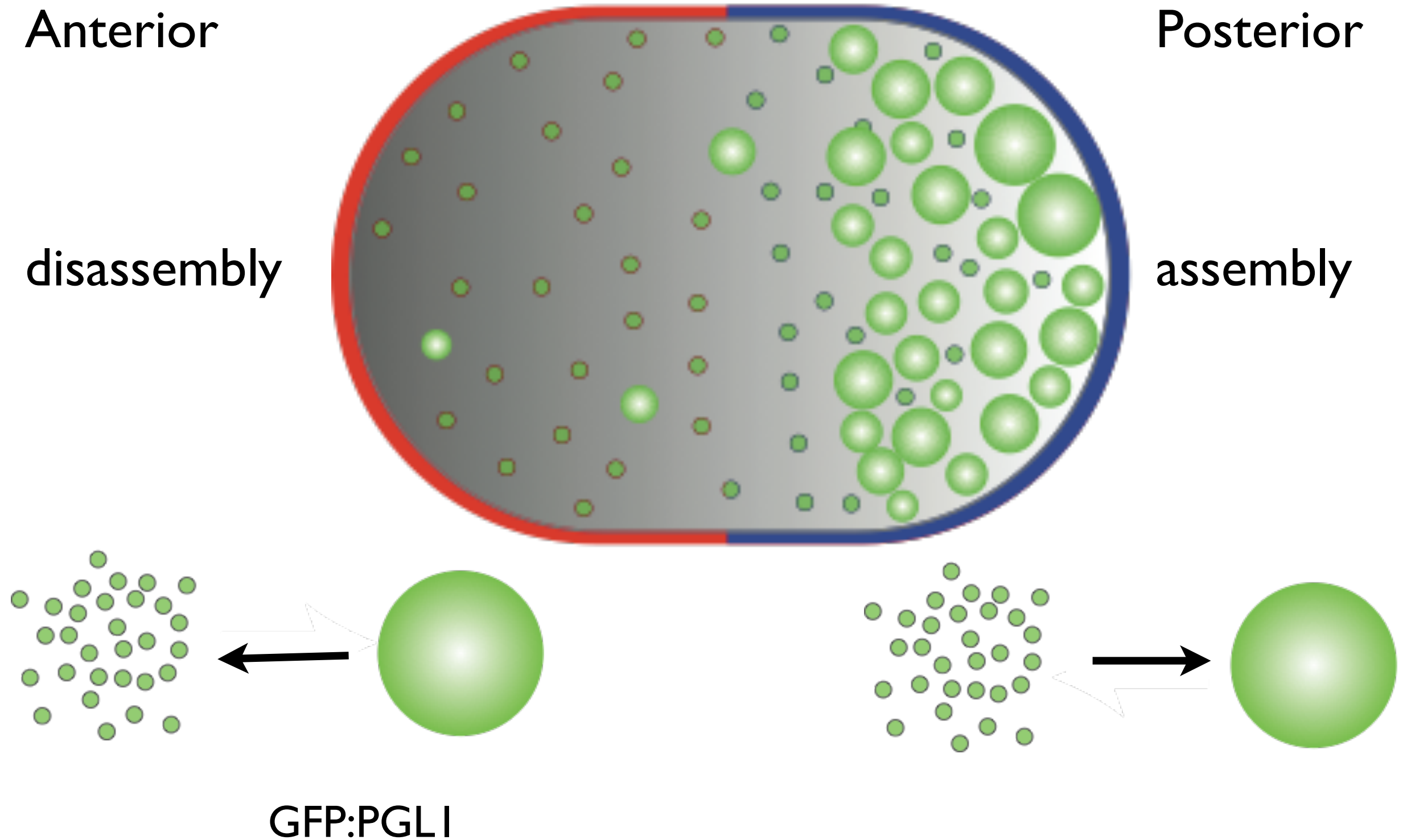
$$\eta/\gamma \simeq 2s/\mu m$$

$$\gamma \simeq 10^{-6} \text{N/m}$$



Brangwynne et. al, Science 324 (2009)

P granule assembly gradient



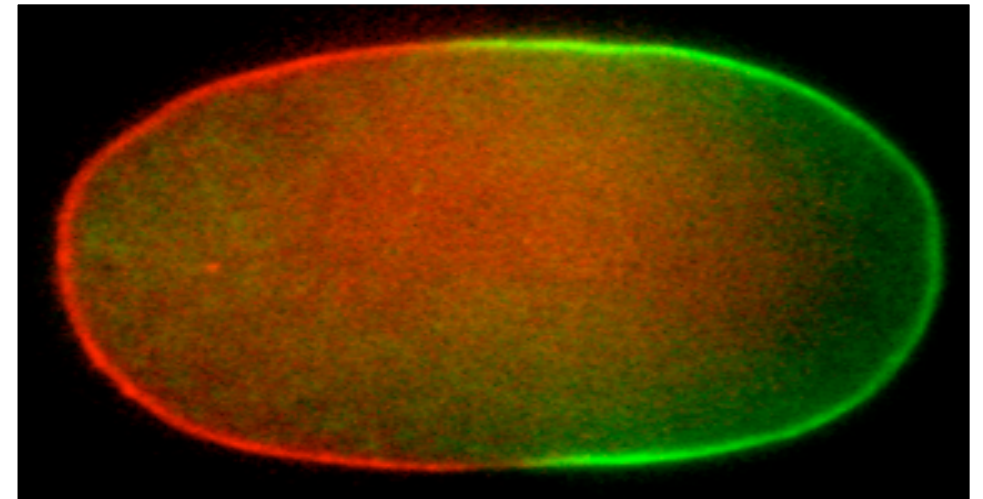
Cell polarity

Cortical asymmetry

PAR-6 / PAR-2

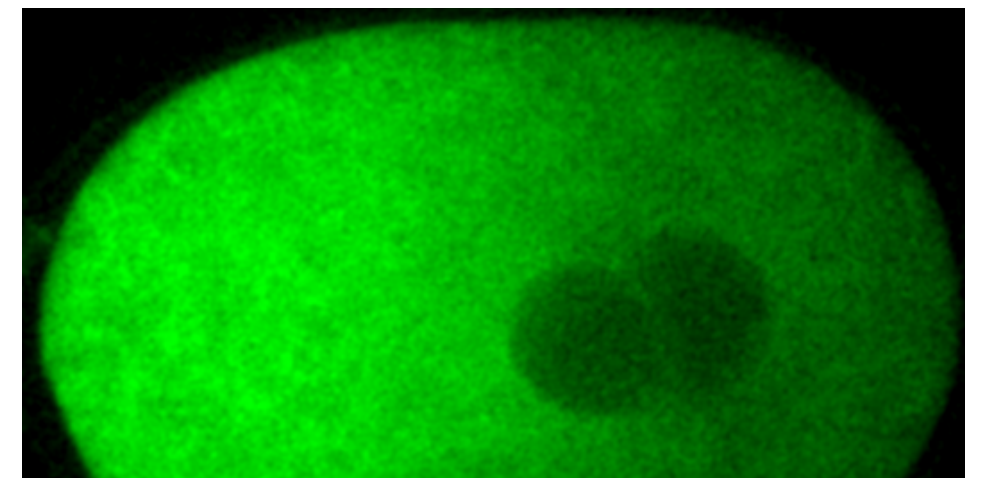
A

P



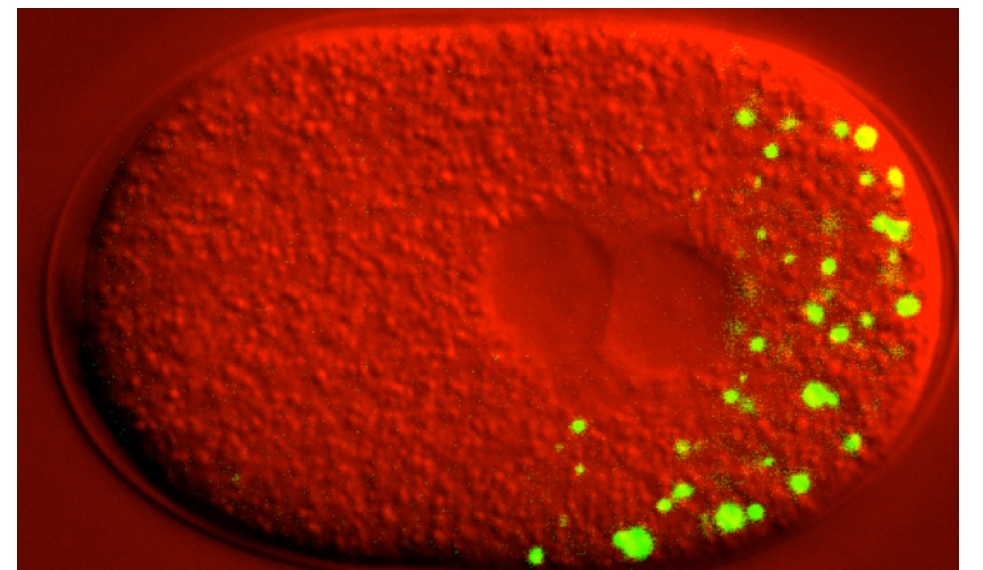
Cytoplasmic gradients

Mex5



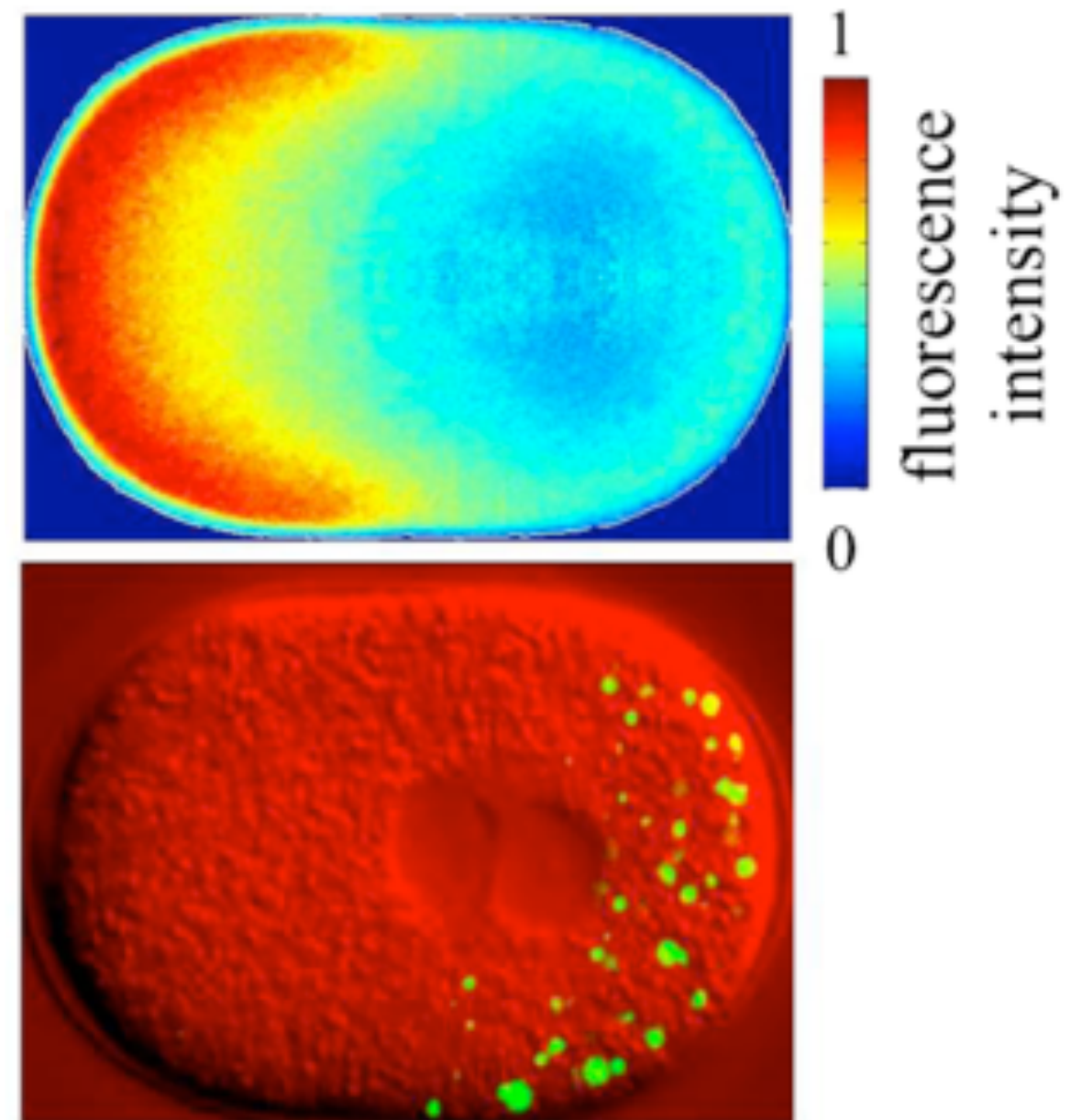
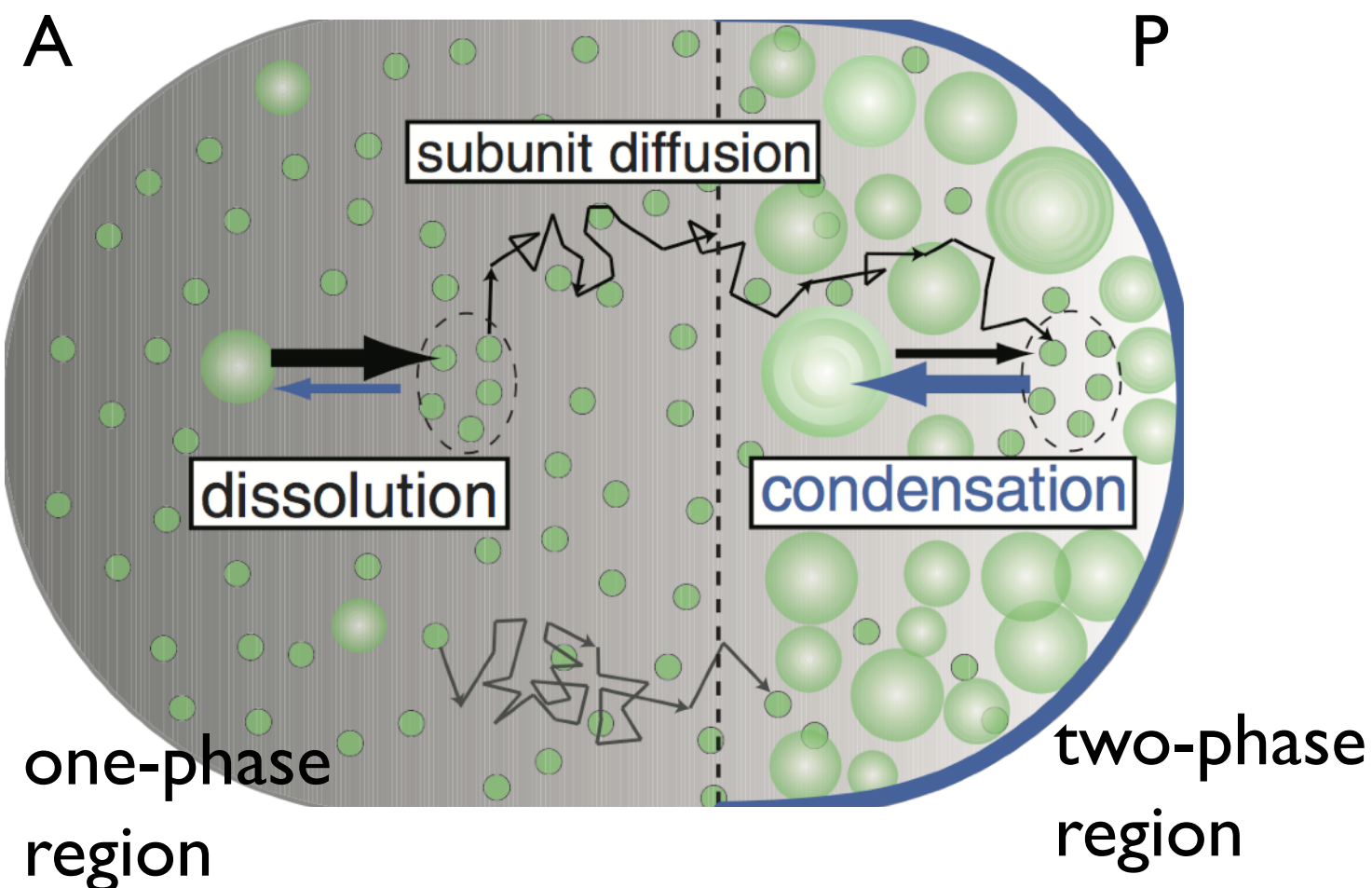
P granule segregation

PGLI



Protein gradient drives granule segregation

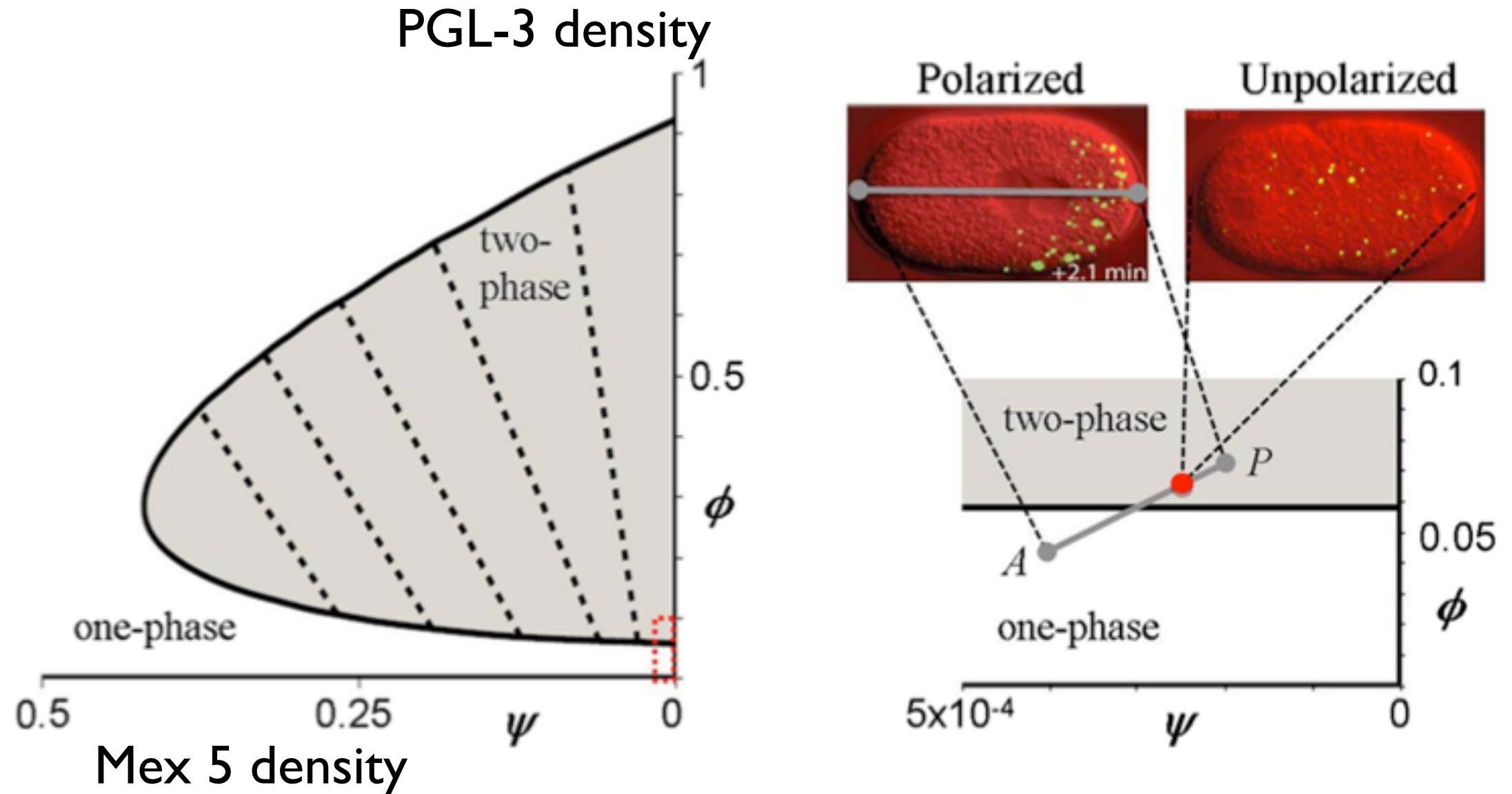
Concentration gradient
of Mex 5 protein



Chui-Fan Lee, Brangwynne, Gharakhani, Hyman, Jülicher PRL (2013)

Brangwynne et. al, Science 324 (2009) Saha et al Cell (2016)

Position dependent phase separation



PGL-3 density ϕ

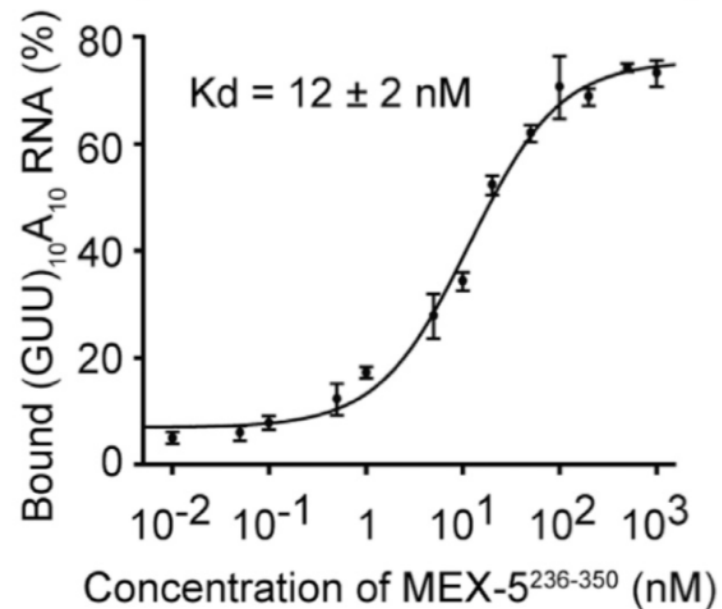
Mex 5 density ψ

solvent density θ

$$\phi + \psi + \theta = 1$$

RNA binding competition

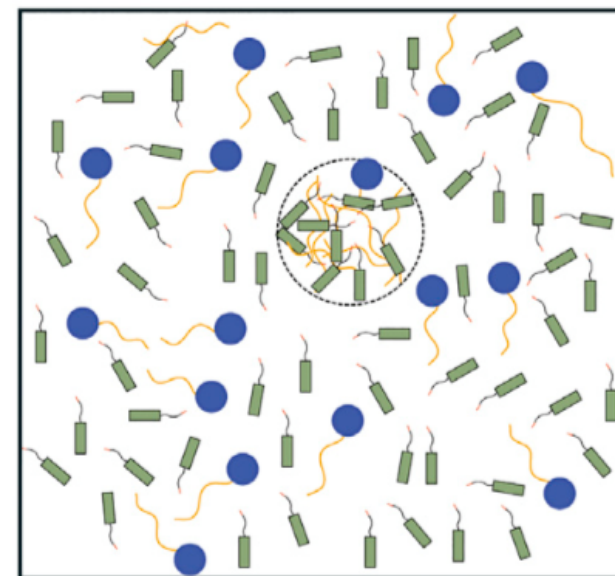
Mex-5
binds RNA



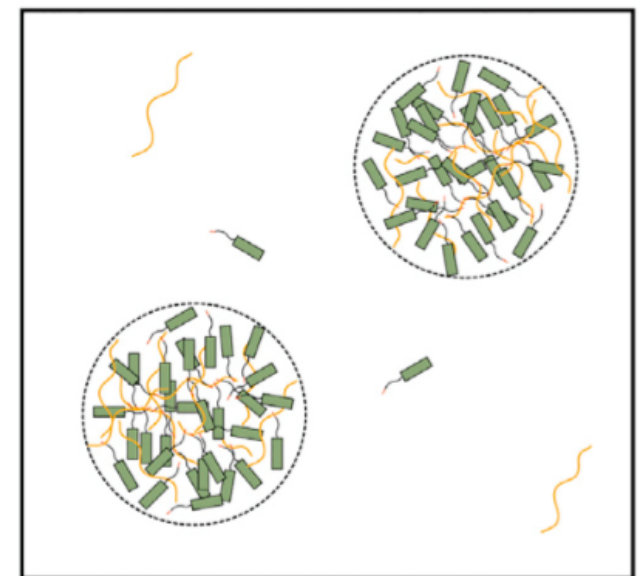
Mex-5 does not bind to PGL-3

Mex-5 binding to RNA reduces
free RNA concentration

→ PGL-3 droplet formation
inhibited

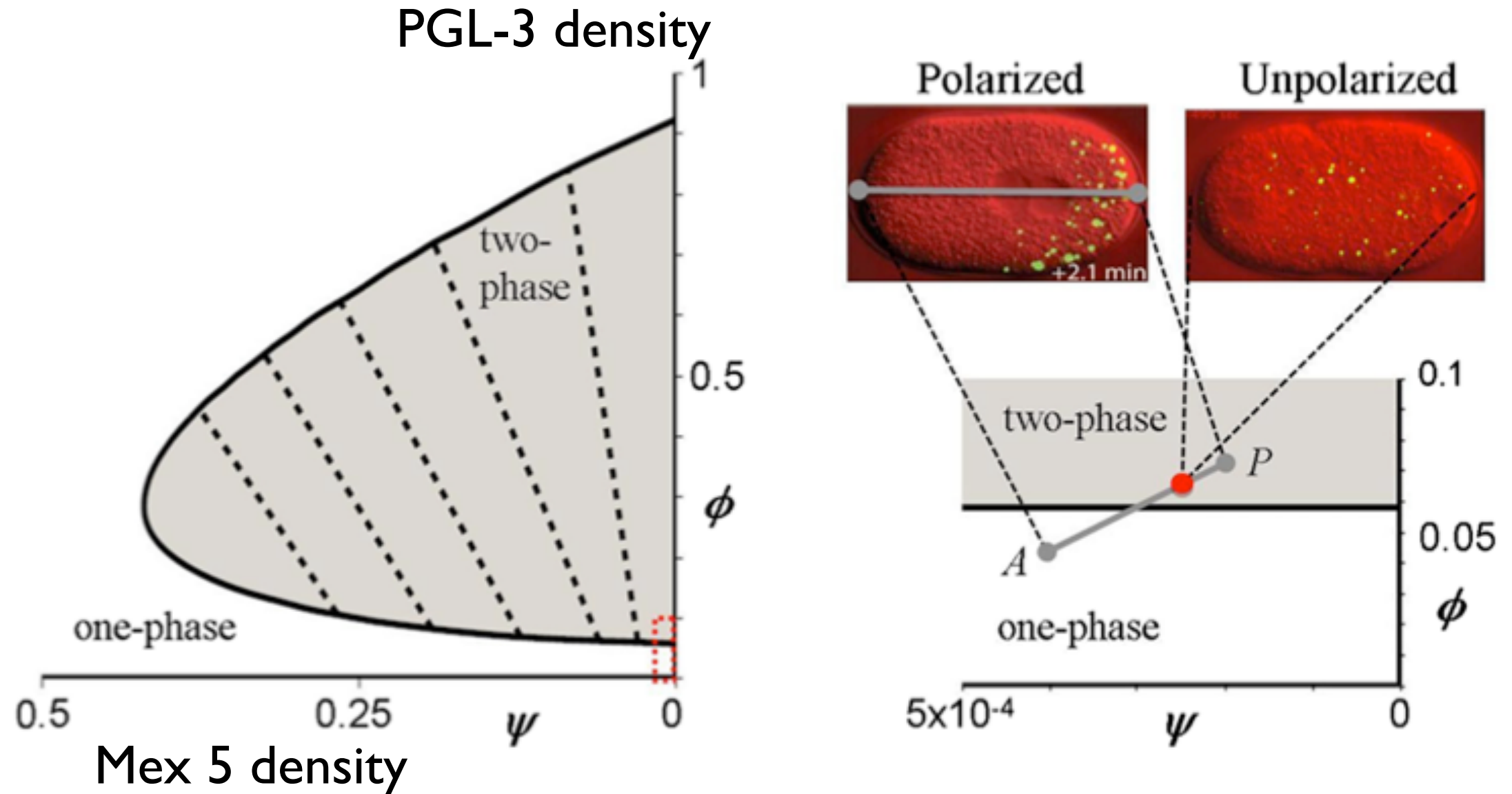


MEX-5 + mRNA + PGL-3



mRNA + PGL-3

Position dependent phase separation



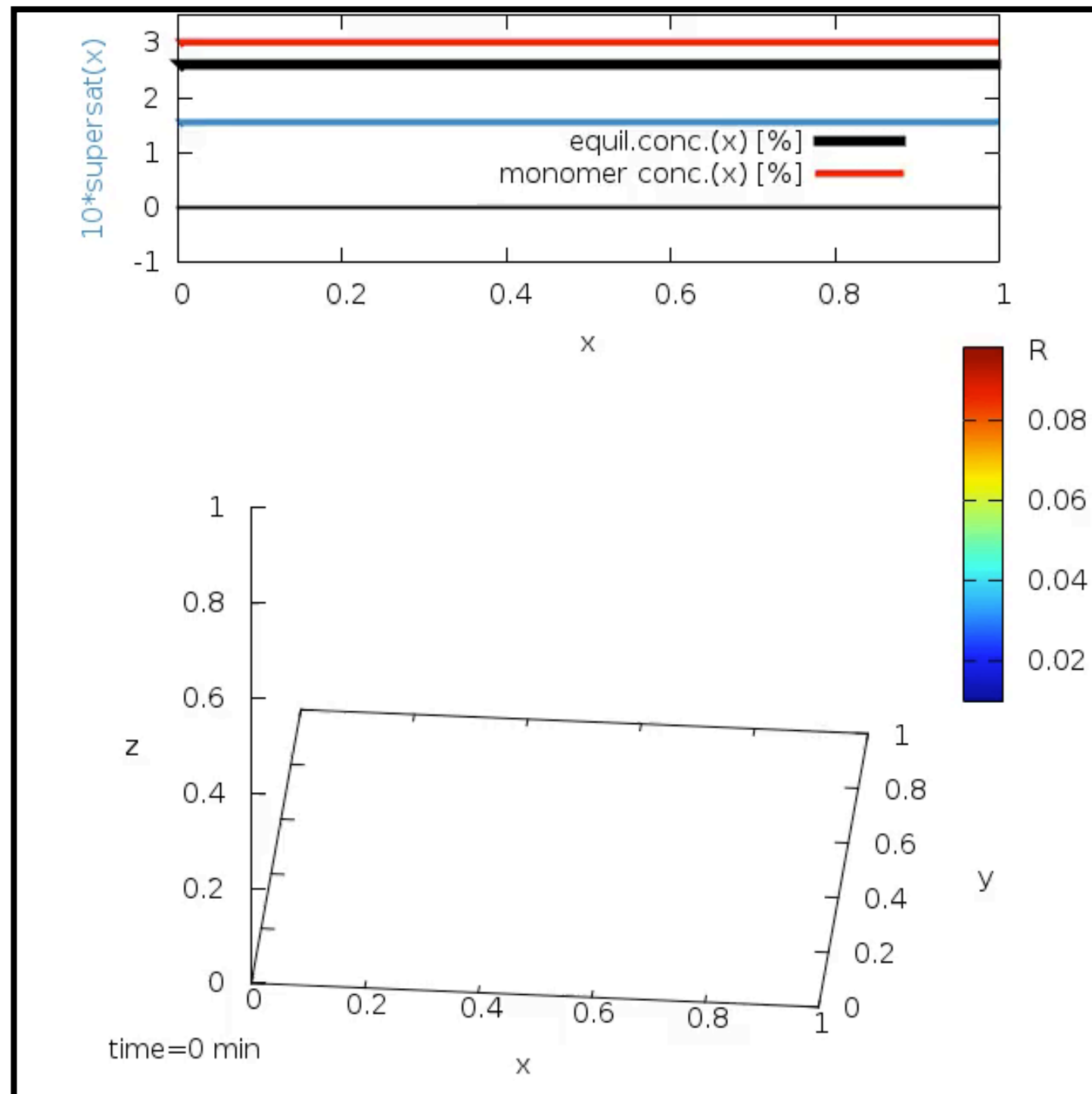
PGL-3 density ϕ

Mex 5 density ψ

solvent density θ

$$\phi + \psi + \theta = 1$$

Stochastic droplet dynamics



Effective droplet model

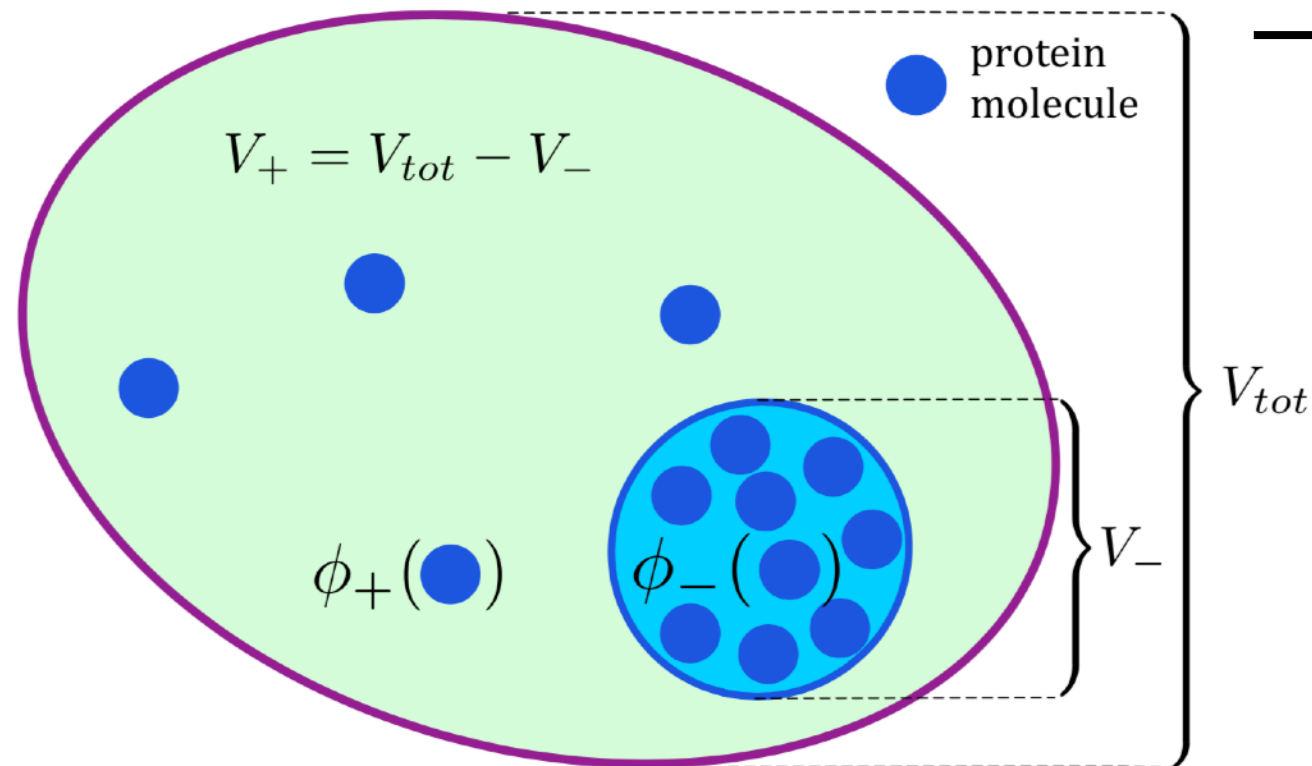
Chui-Fan Lee, Brangwynne, Gharakhani, Hyman, Jülicher PRL (2013)

Weber, Chiu-Fan Lee, Jülicher, NJP 19 (2017)

Concentration buffering

total average concentration

dilute phase concentration

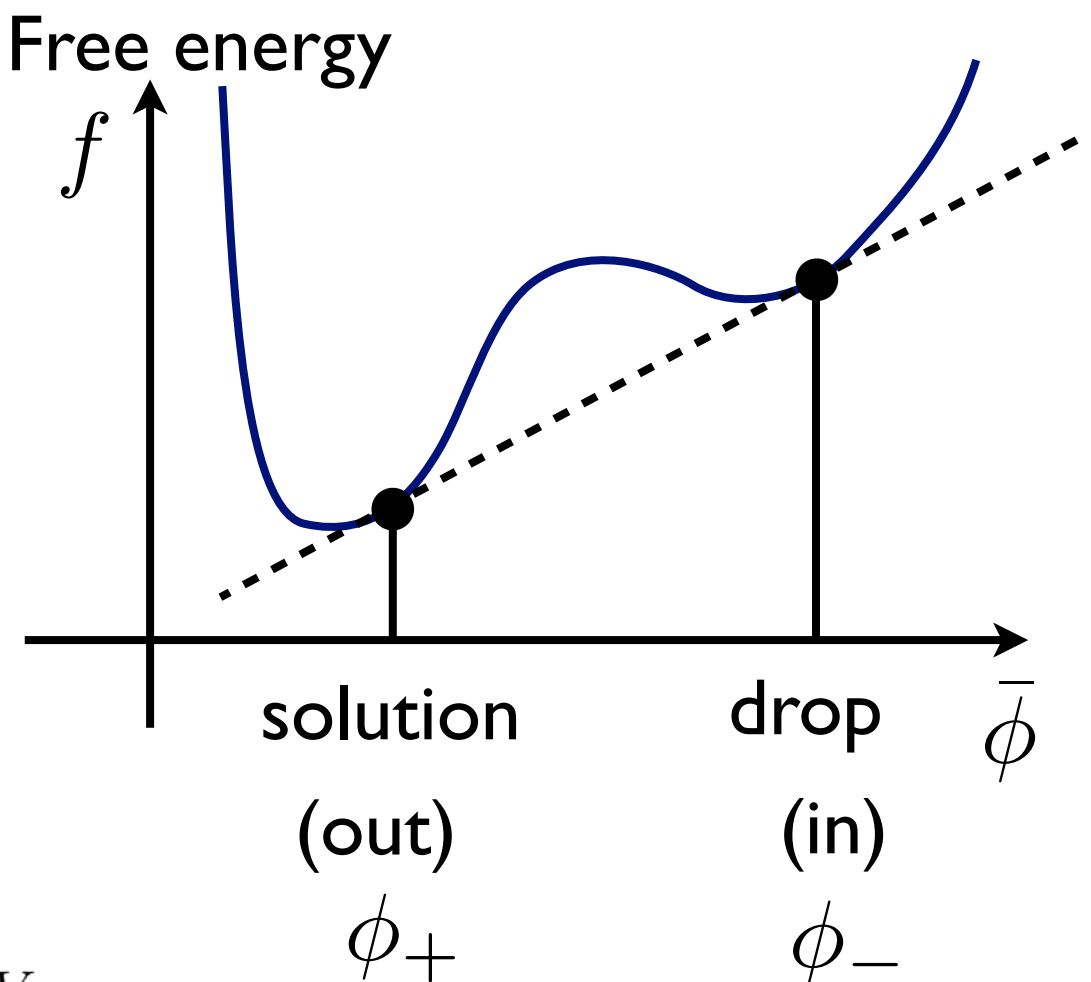


$\bar{\phi}$

ϕ_+

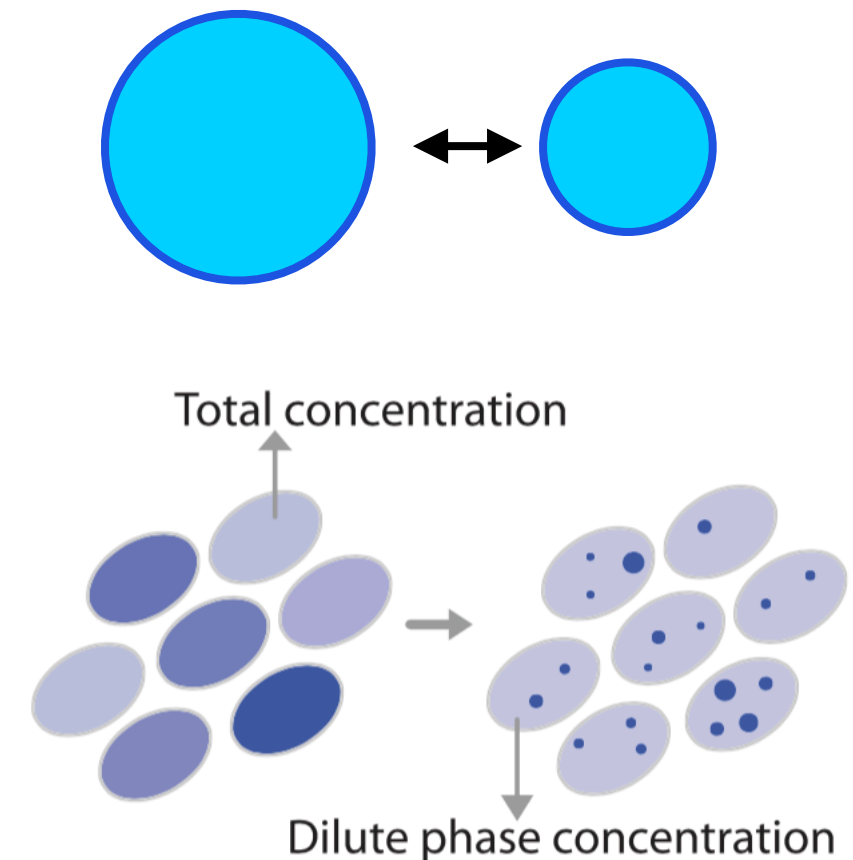
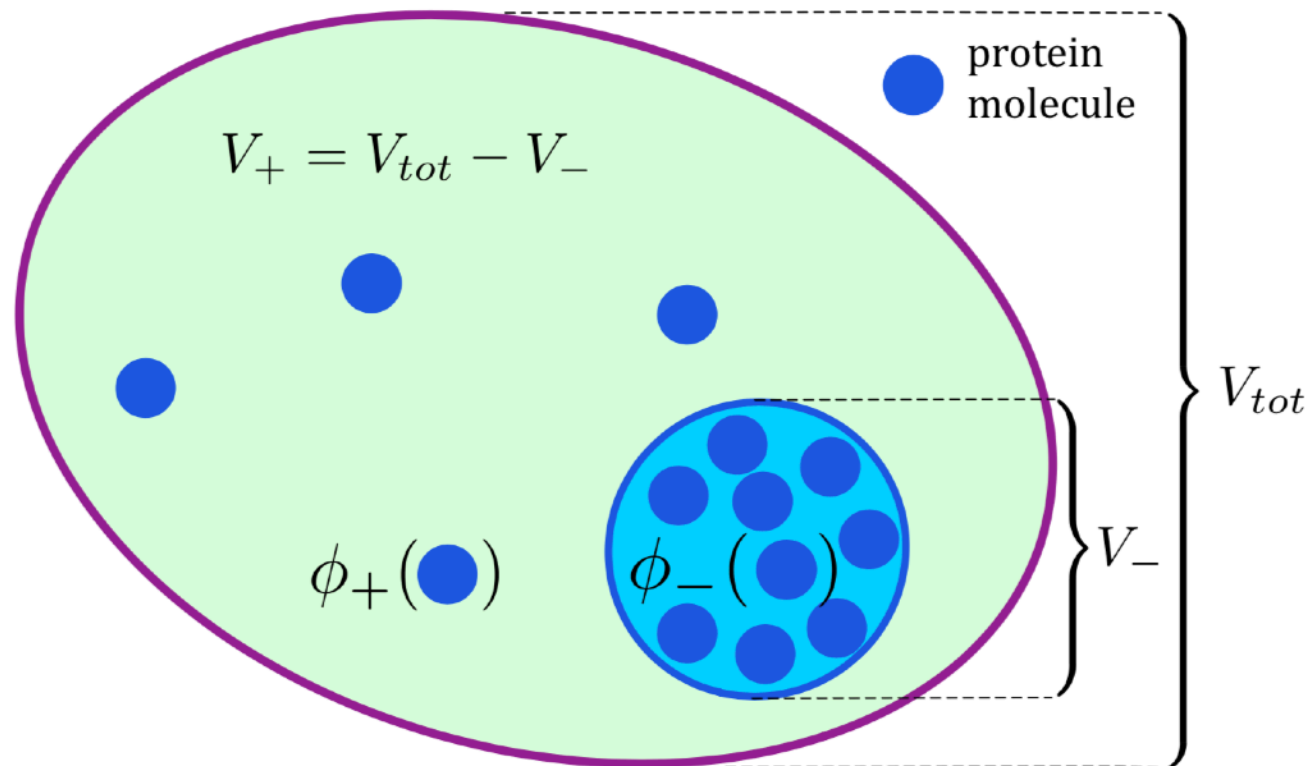
Free energy

f

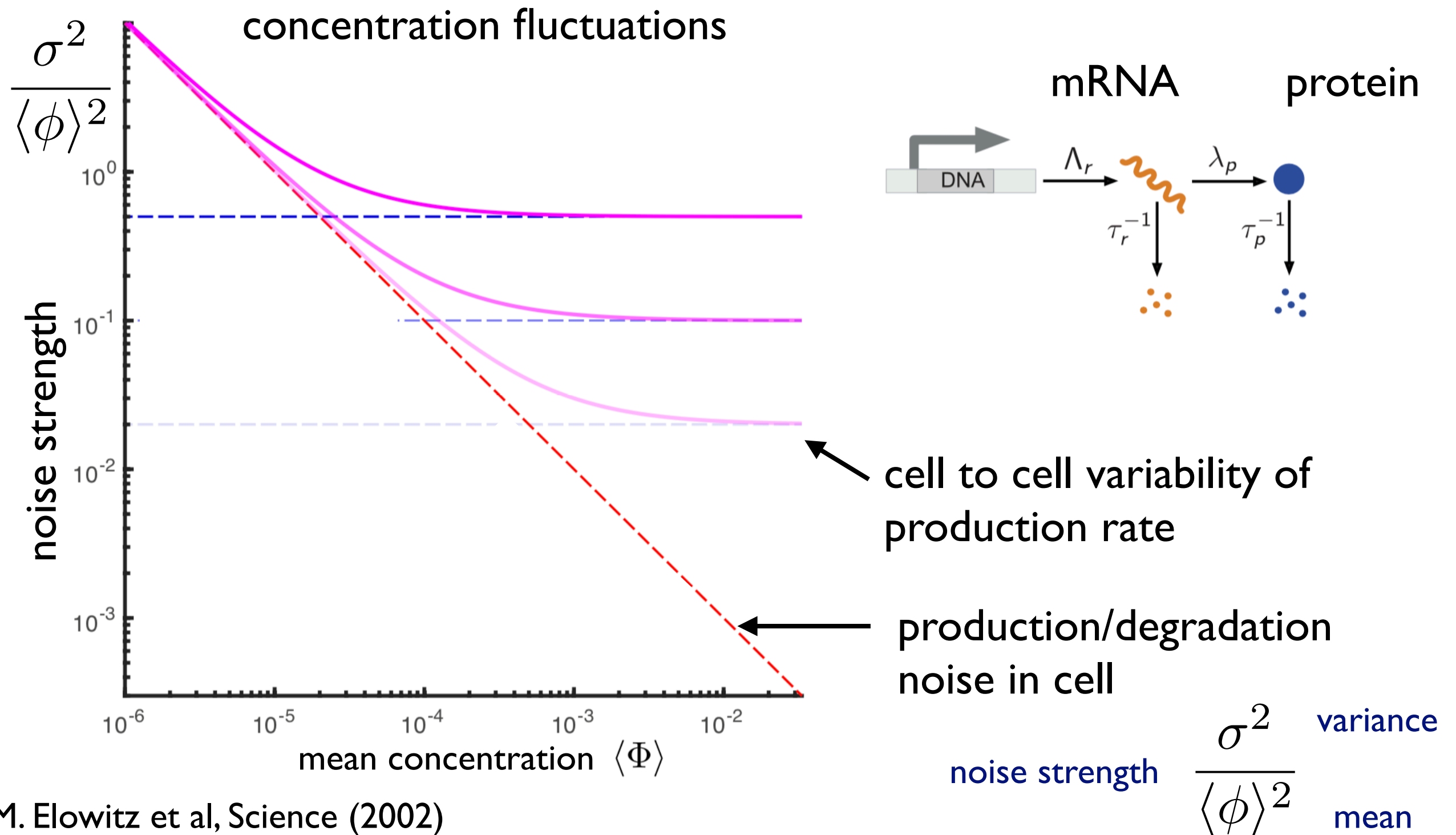


Concentration buffering

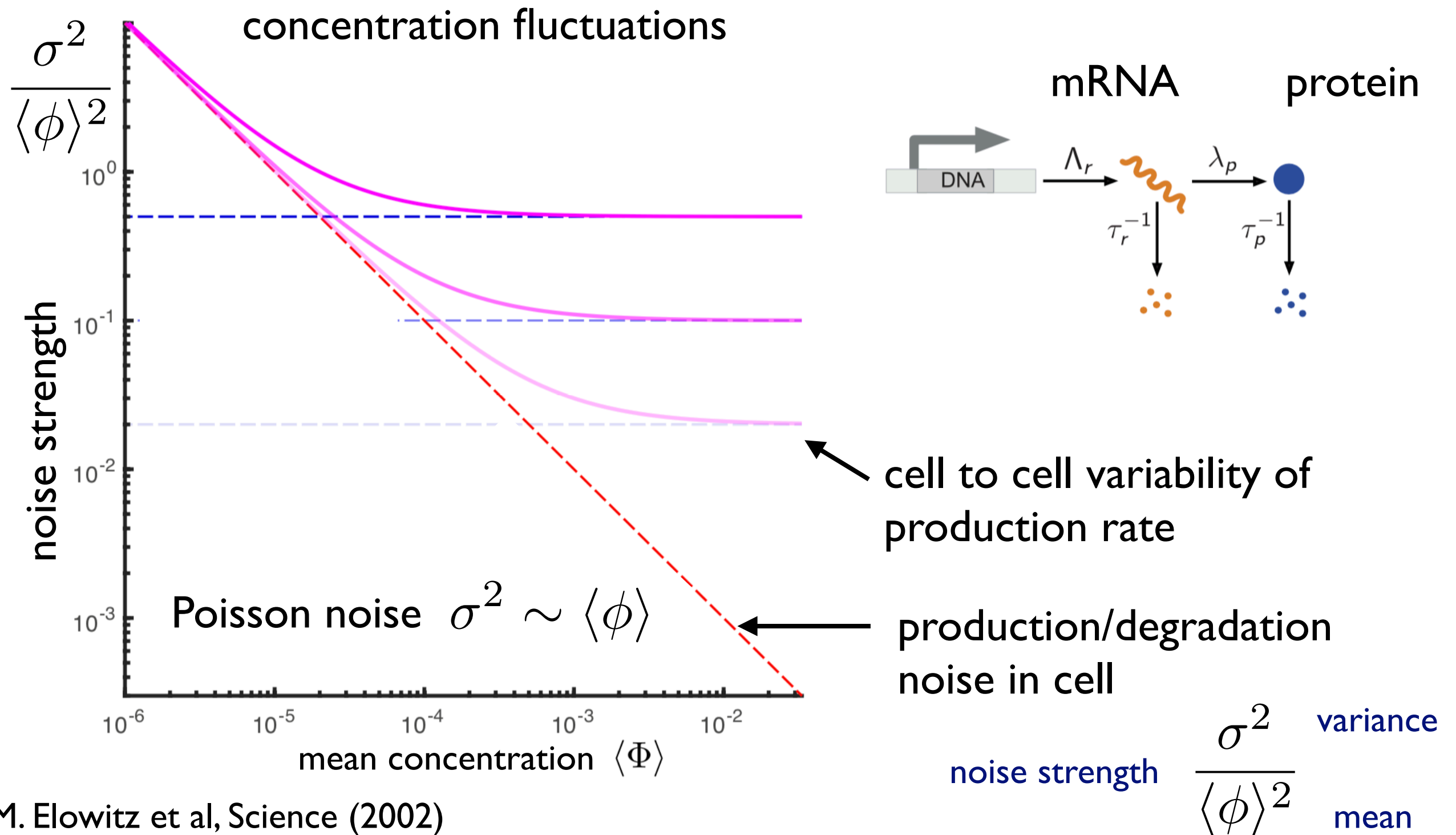
total average concentration	$\bar{\phi}$	changes in $\bar{\phi}$
dilute phase concentration	ϕ_+	unchanged ϕ_+
droplet size changes		



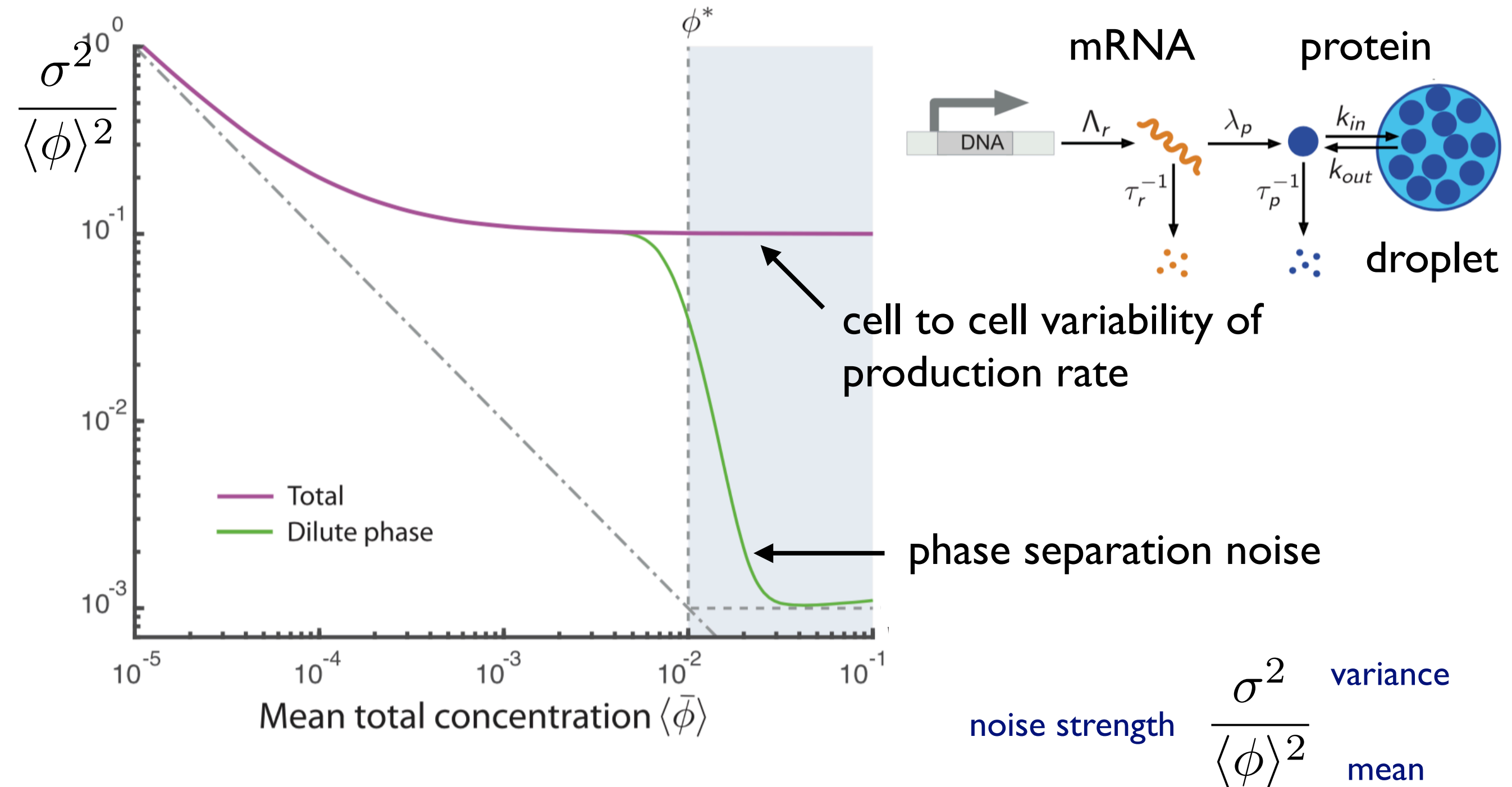
Stochastic protein production and degradation



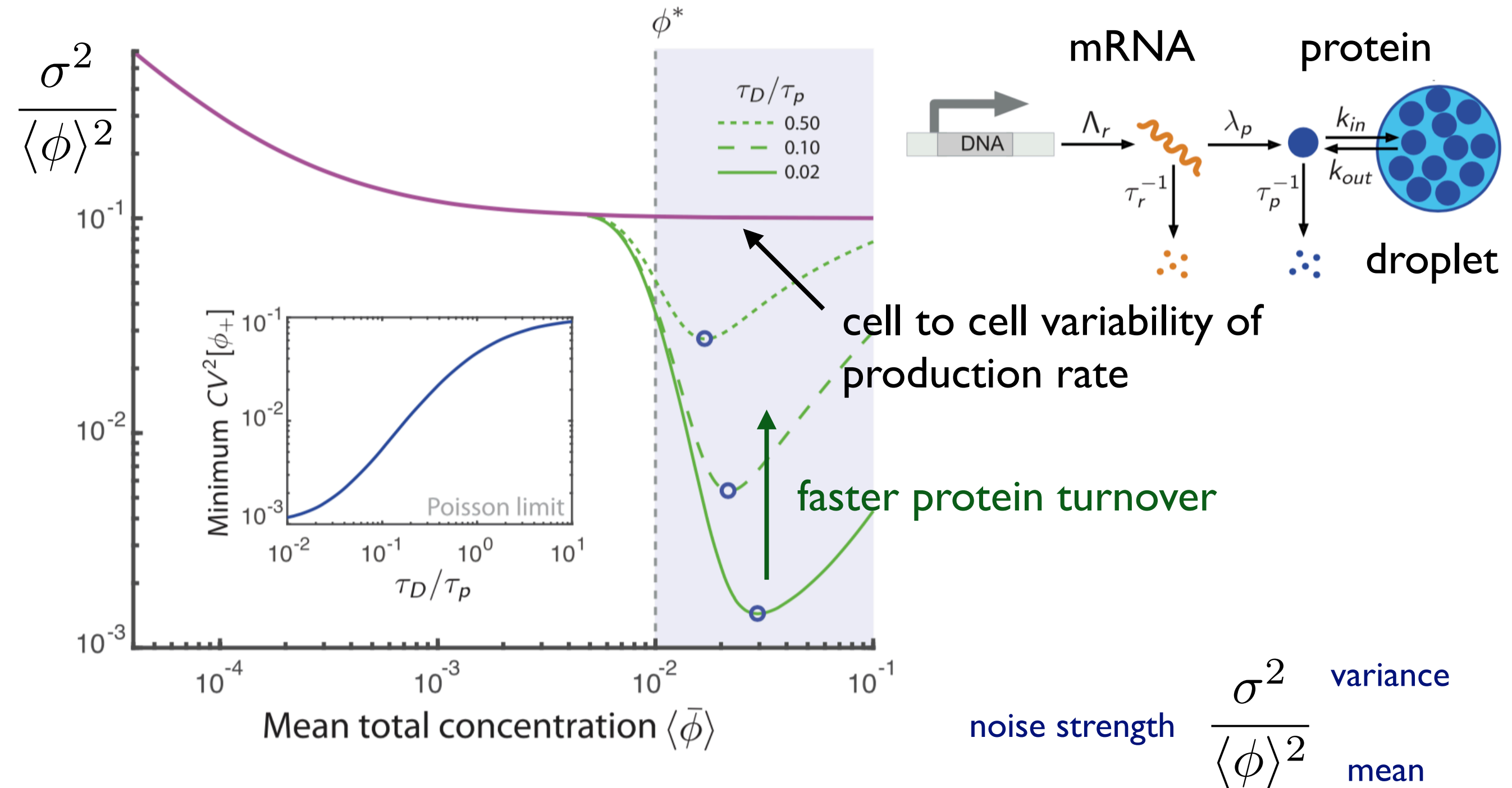
Stochastic protein production and degradation



Noise buffering by phase separation



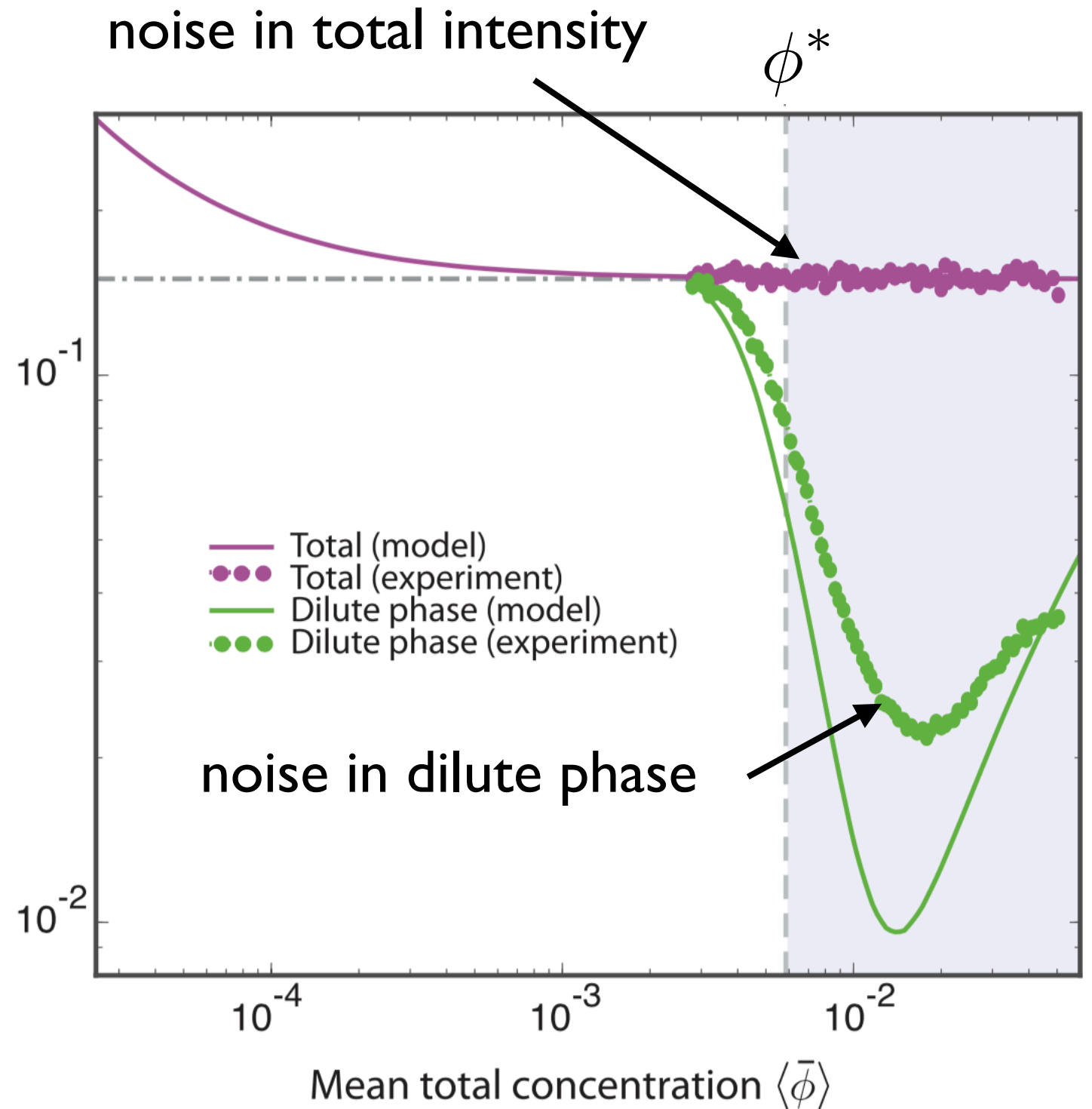
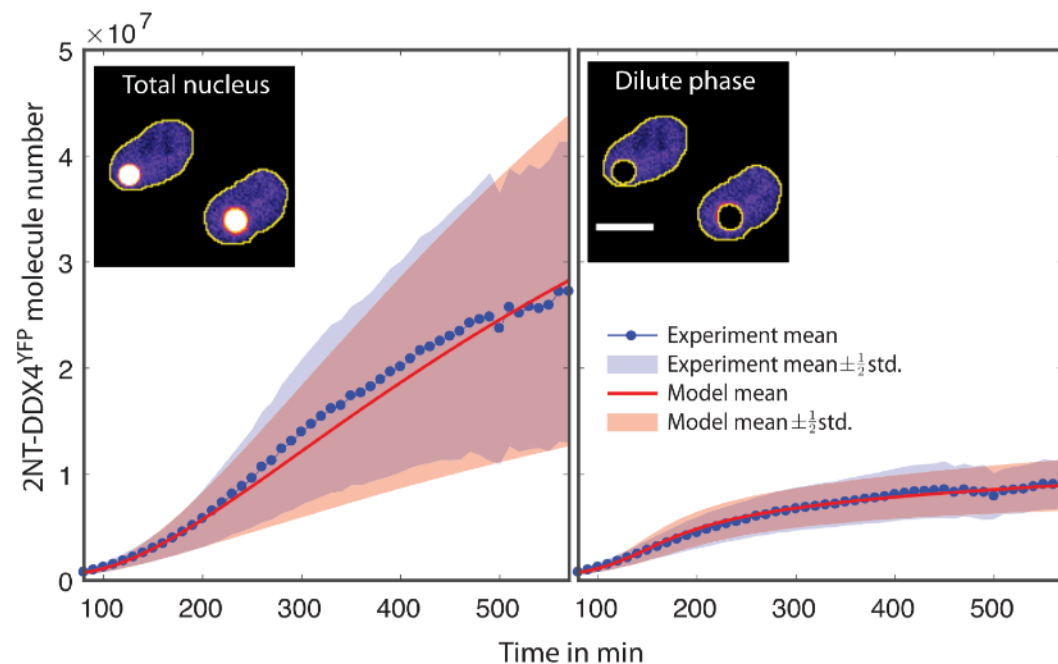
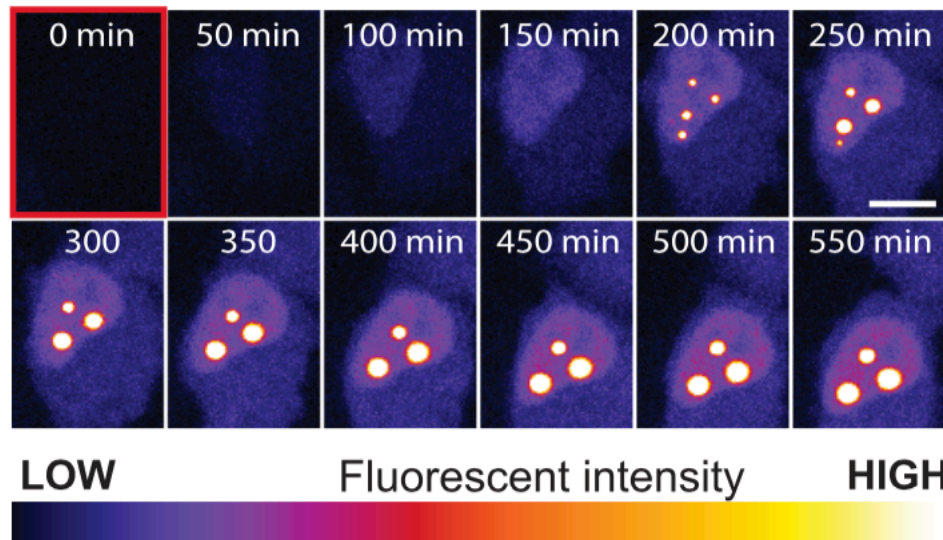
Noise buffering by phase separation



Noise buffering in Experiments

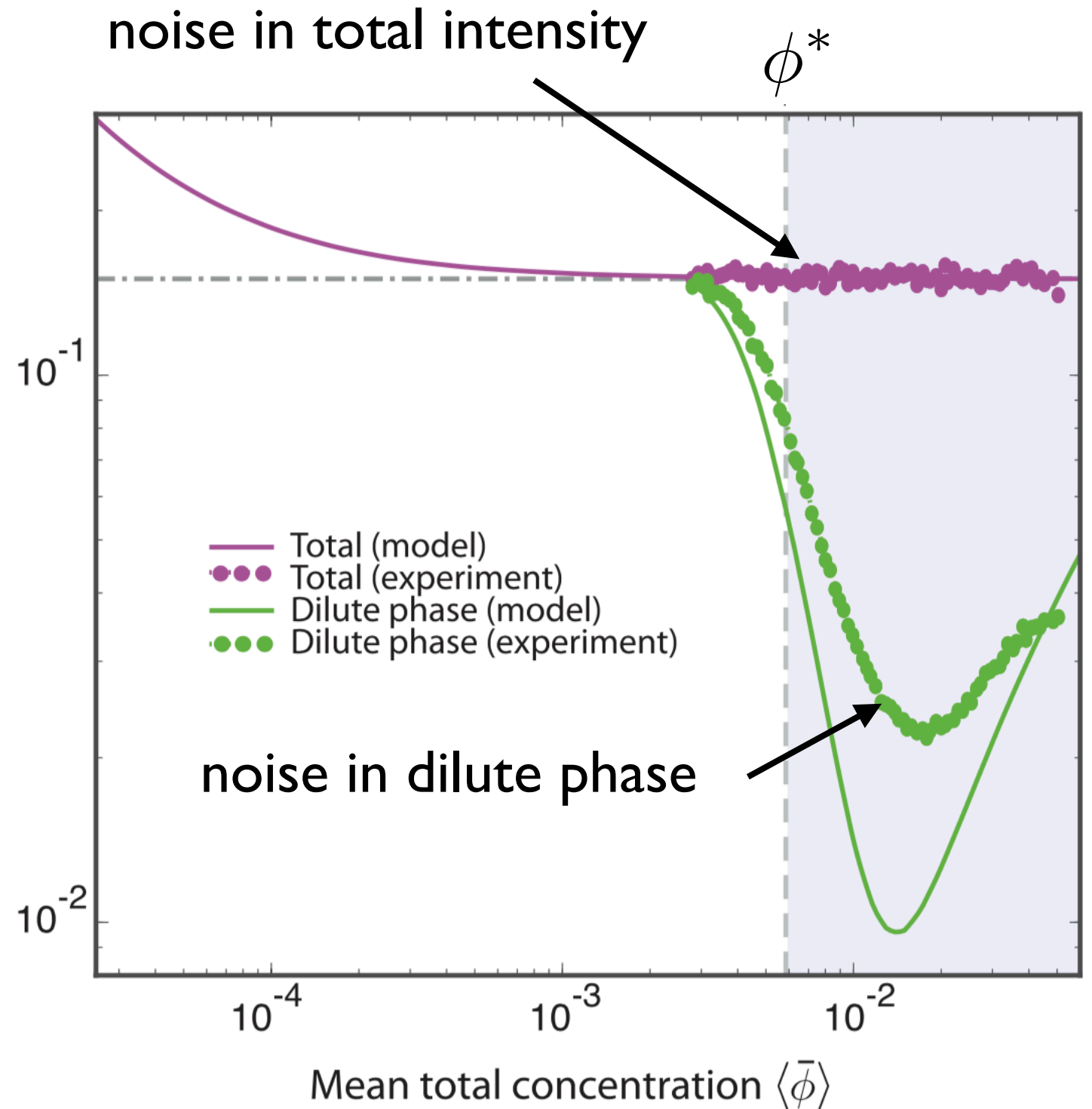
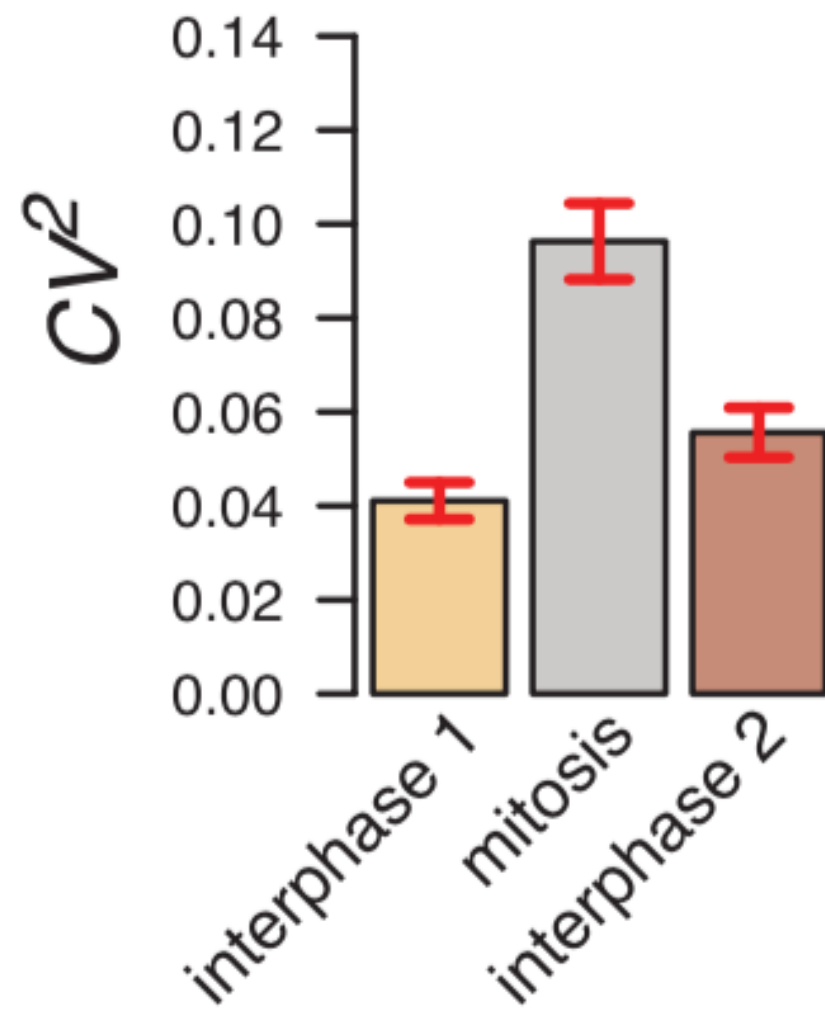


Transfection + time-lapse microscopy

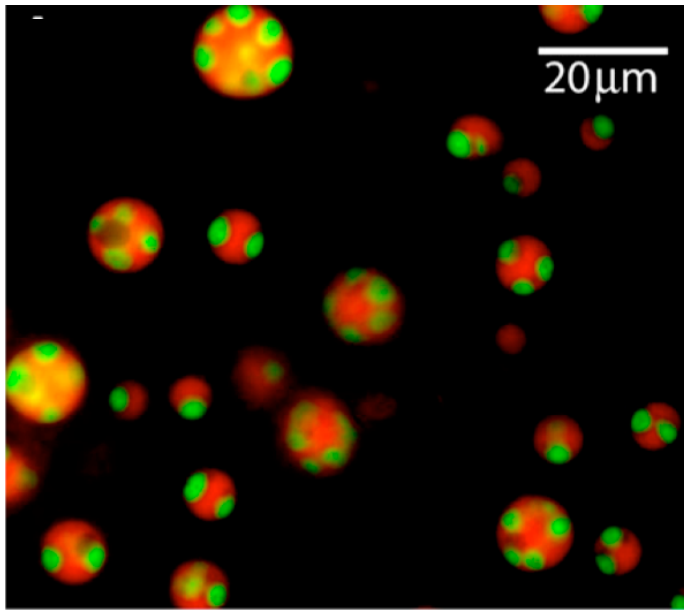


Noise buffering in Experiments

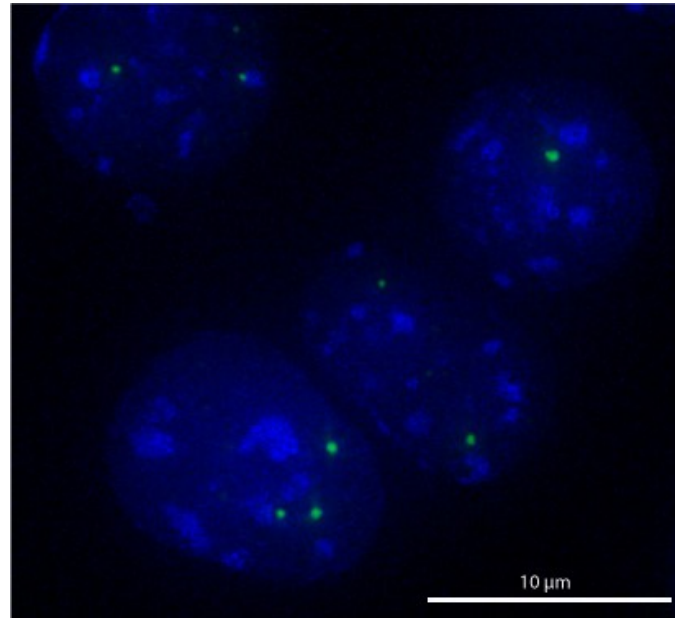
droplets dissolve during mitosis: noise increases



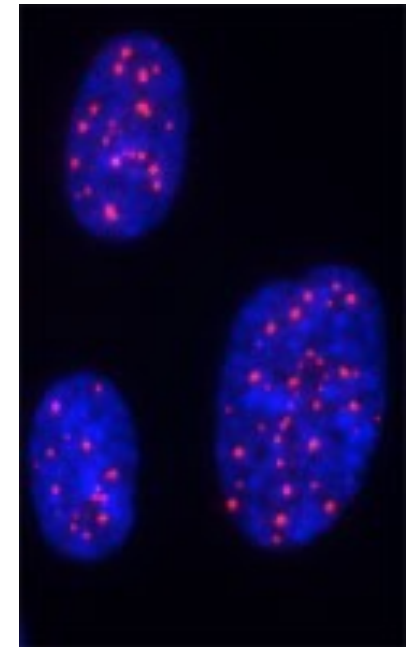
Condensates as chemical reaction centers



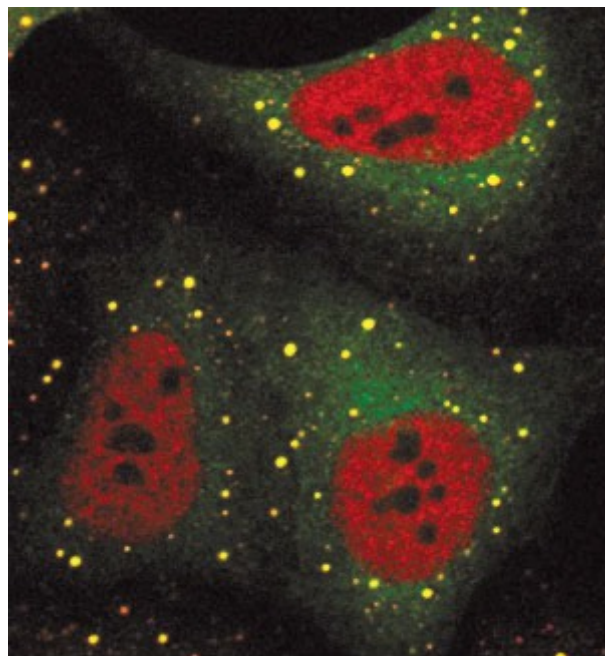
Source: C. Brangwynne
Nucleoli (green)



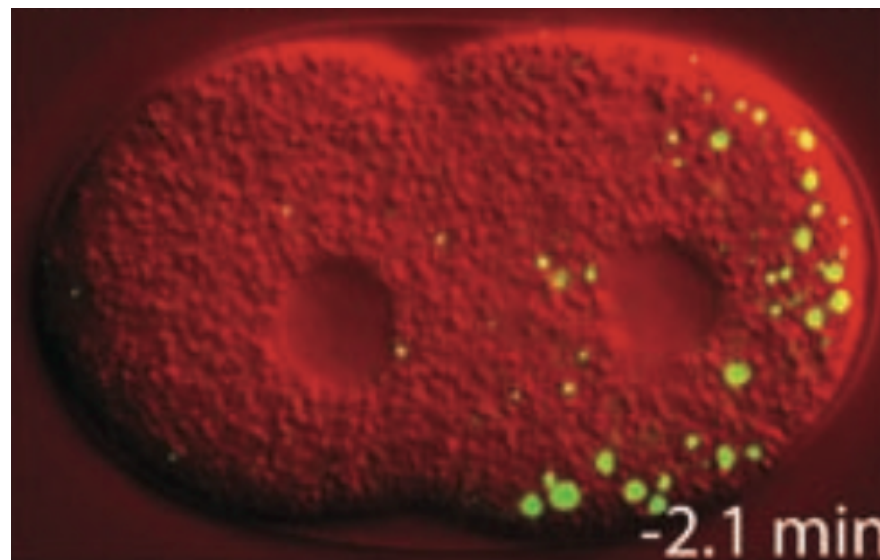
Source: Wikipedia
Cajal bodies (green)



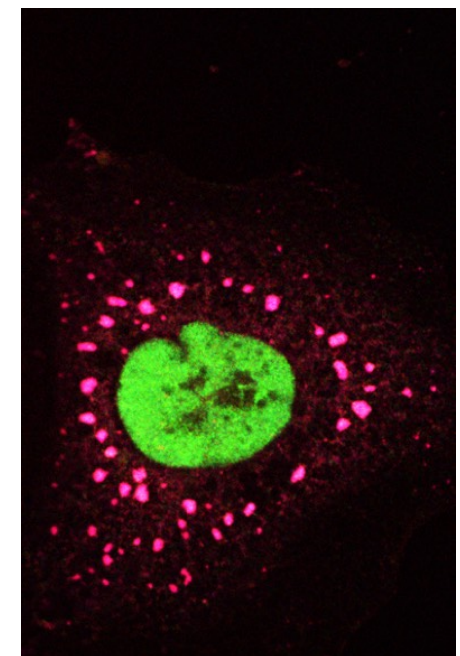
Source: Thomas G Hofmann
PML bodies (red)



Nature Reviews | Molecular Cell Biology
P bodies (yellow)



P granules (green)

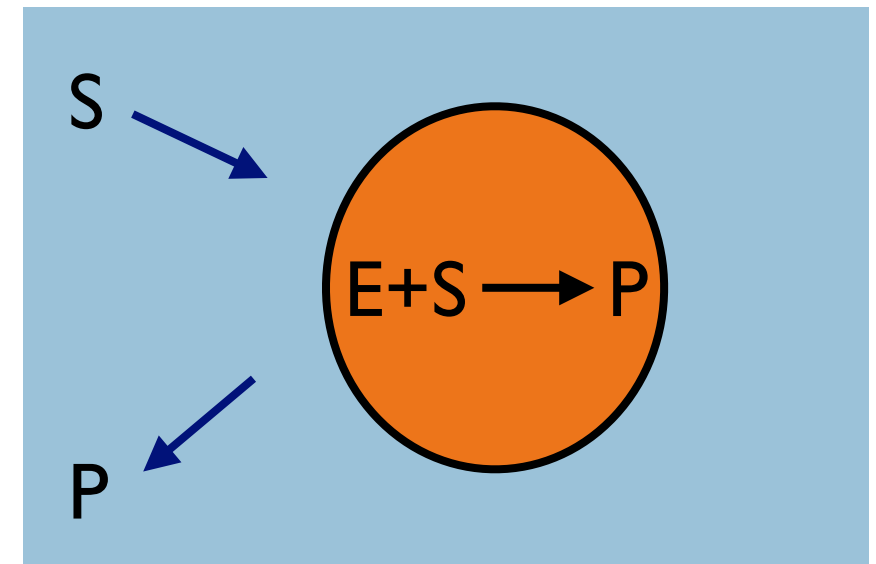


Source: Christine Vande Velde
Stress granules (purple)

Chemical reactions in droplets

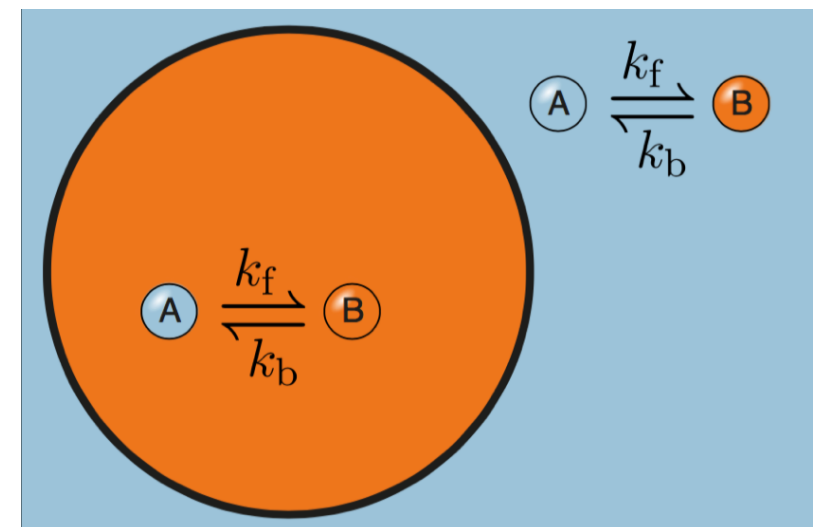
1.) droplets provide reaction centers

localise reactions
concentrate reactants
accelerate reactions

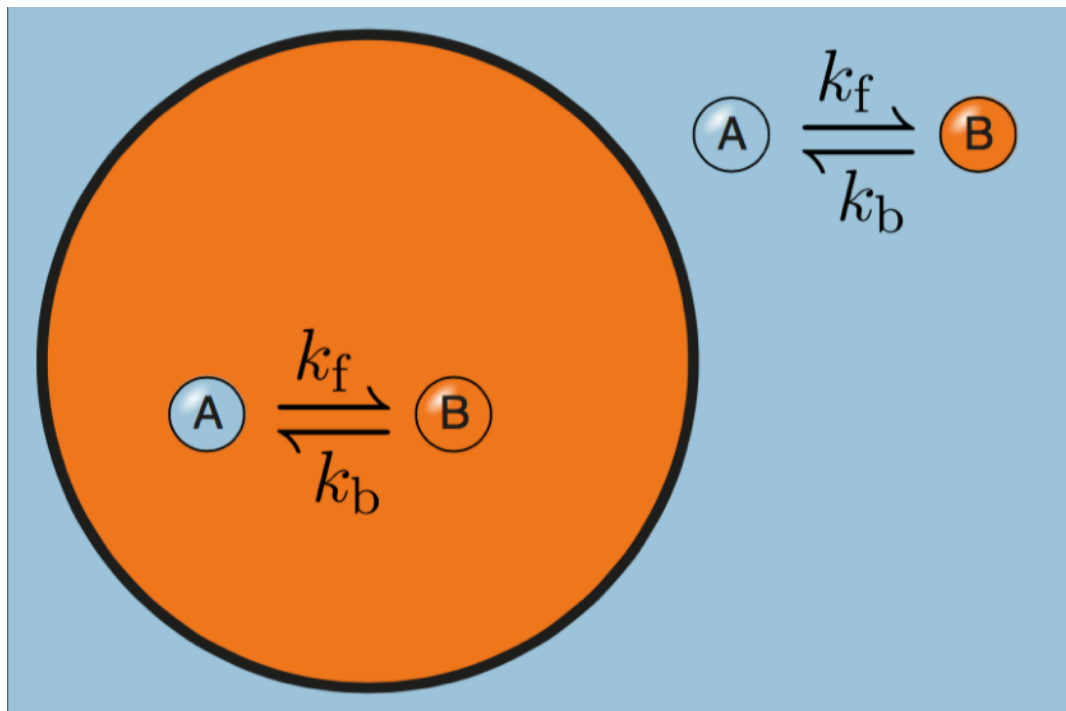


2.) droplets material is turned over by reactions

active droplets
novel droplet behaviors



Droplet turnover: detailed balance



closed system: detailed balance

$$\frac{k_f}{k_b} = \exp \left(\frac{w_A - w_B}{k_B T} \right)$$

$$\mu_n = k_B T \ln \phi_n + w_n$$

passive reactions $A \rightleftharpoons B$

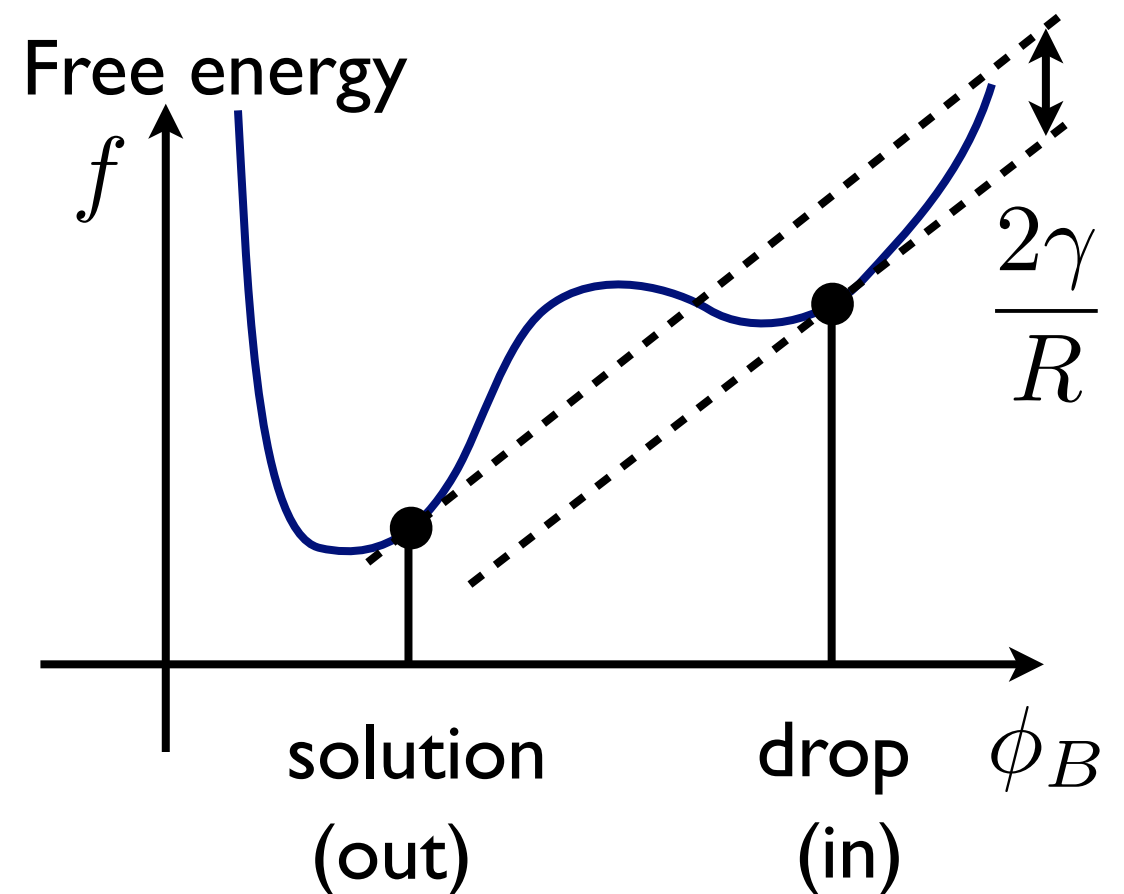
reaction-diffusion in the bulk

$$\partial_t \phi_A = D_A \nabla^2 \phi_A - r$$

$$\partial_t \phi_B = D_B \nabla^2 \phi_B + r$$

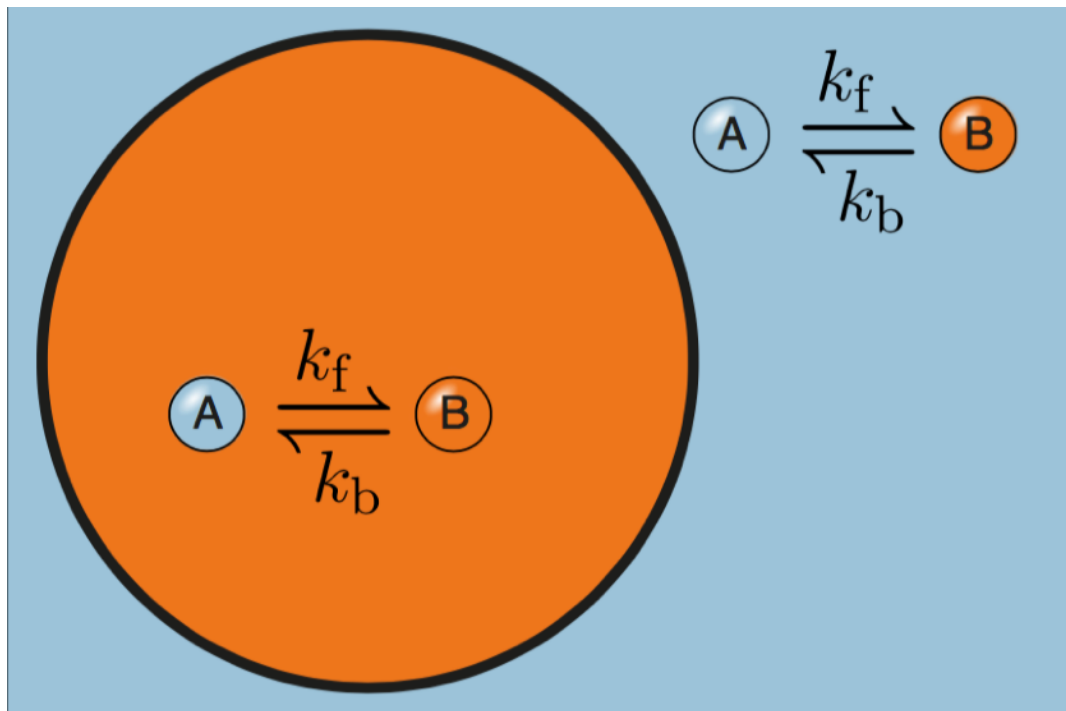
reaction-driven droplet growth

$$r = \phi_A k_f - \phi_B k_b$$



droplets dissolve!

Chemically active droplets



open system: detailed balance broken

$$\frac{k_f}{k_b} \neq \exp \left(\frac{w_A - w_B}{k_B T} \right)$$

$$\mu_n = k_B T \ln \phi_n + w_n$$

active chemical reactions

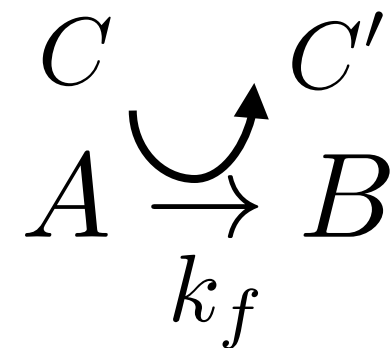
reaction-diffusion in the bulk

$$\partial_t \phi_A = D_A \nabla^2 \phi_A - r$$

$$\partial_t \phi_B = D_B \nabla^2 \phi_B + r$$

reaction-driven droplet growth

$$r = \phi_A k_f - \phi_B k_b$$



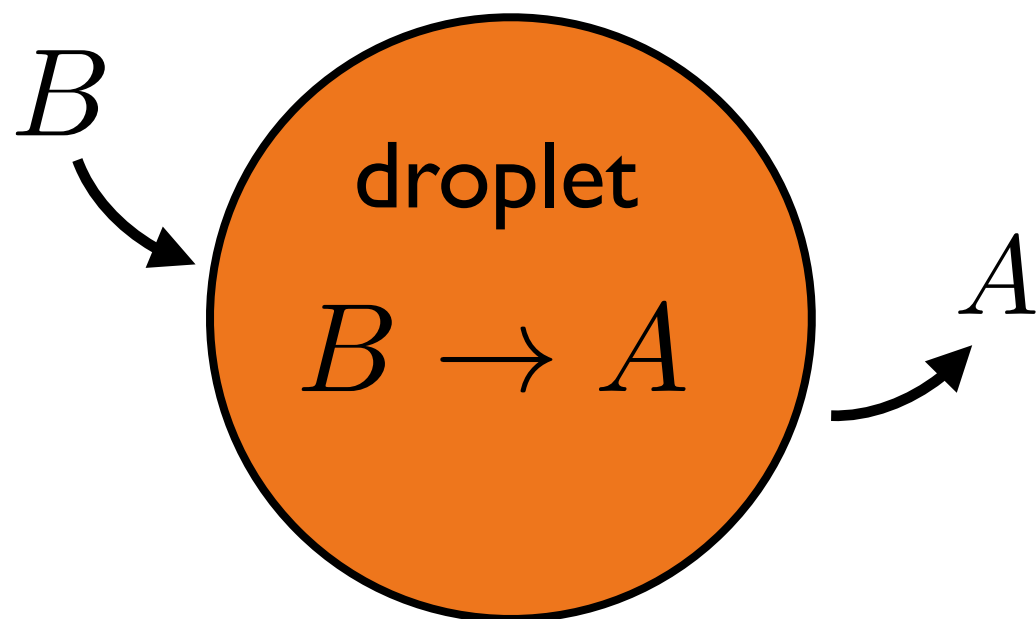
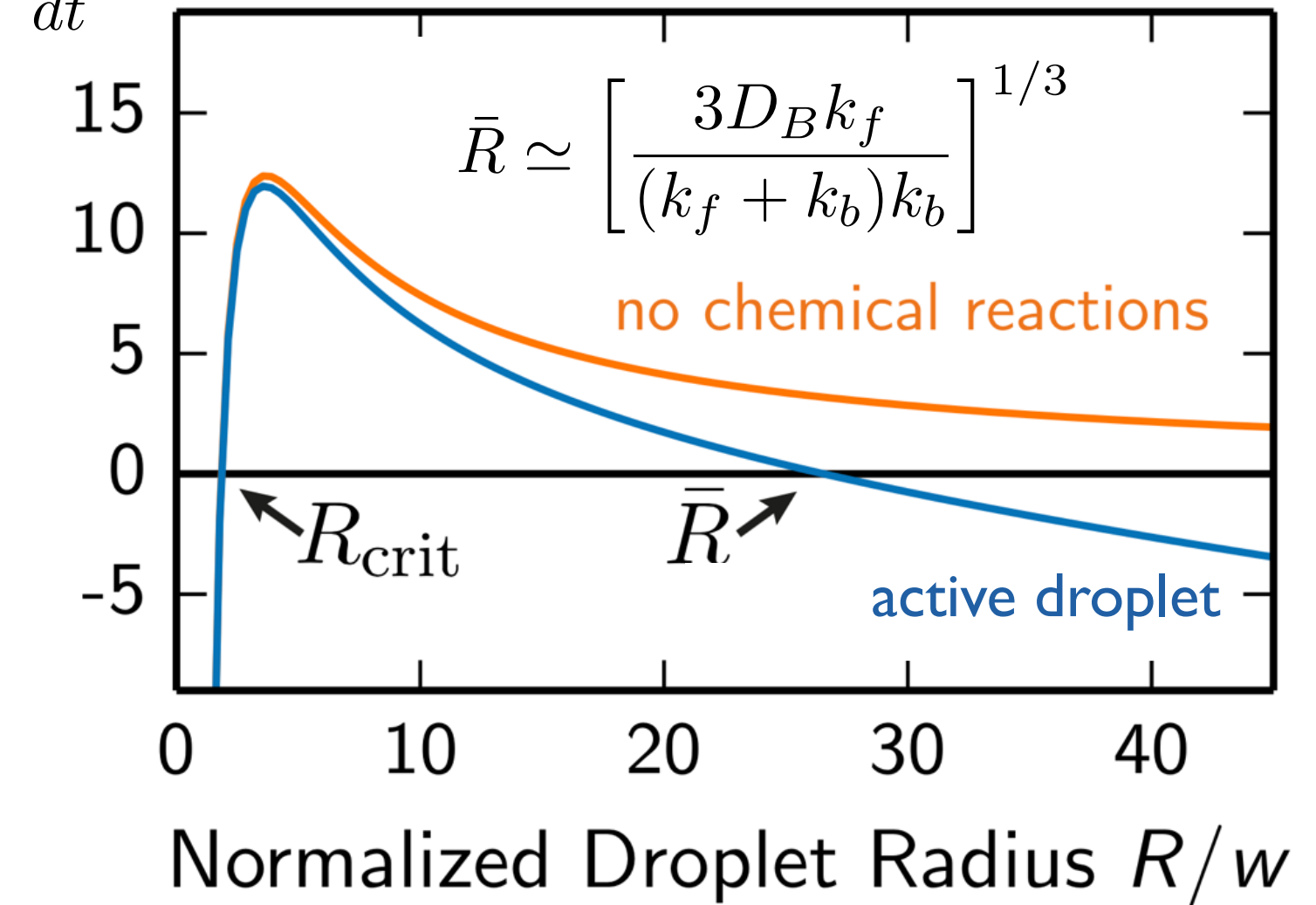
chemical energy (fuel) $\Delta\mu_C$

Steady state of active droplets

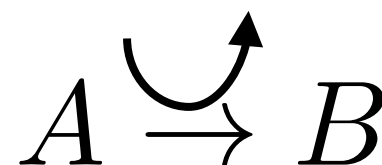
Reaction flux, no detailed balance $\frac{dR}{dt}$

$$r = \phi_A k_f - \phi_B k_b$$

suppression of Ostwald ripening: many droplets of fixed size

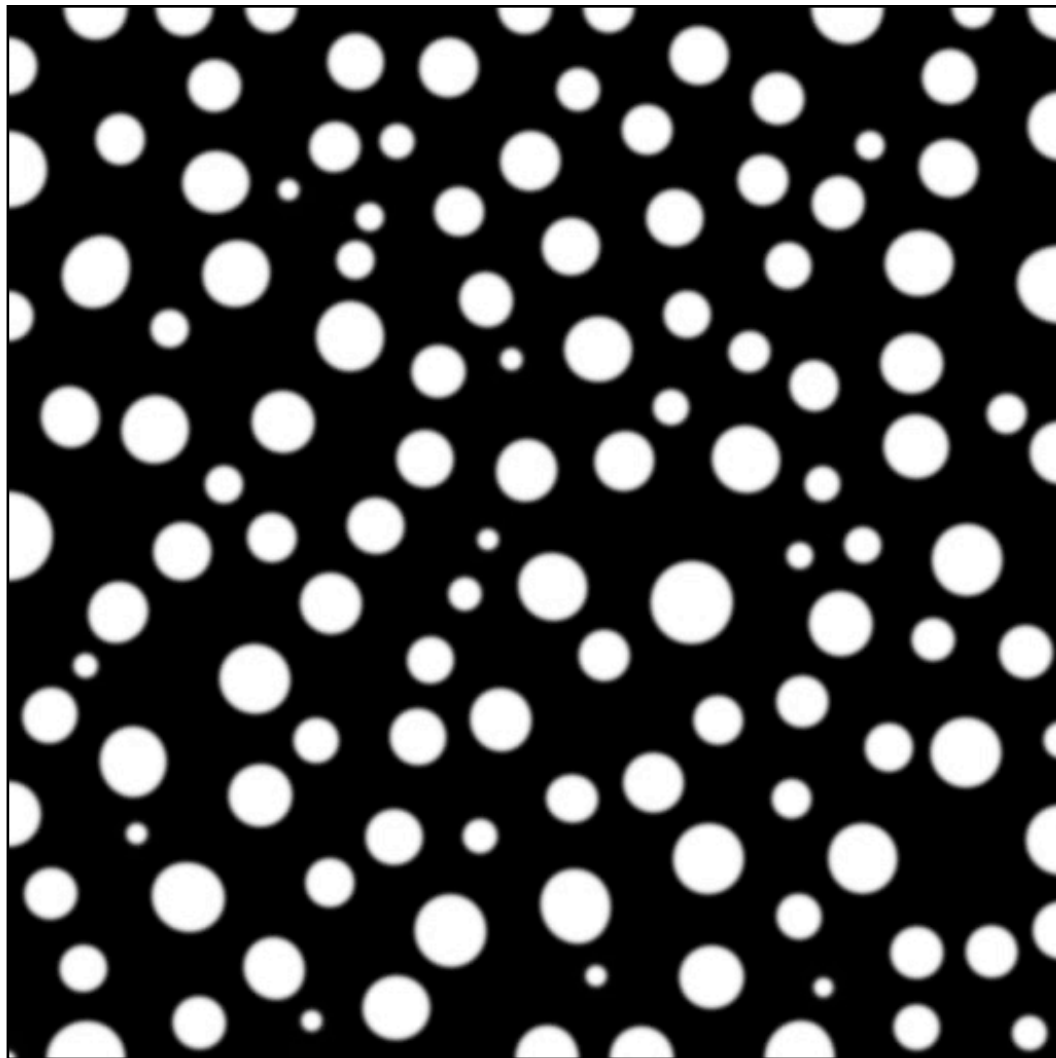


chemical energy



Dynamics of active droplets

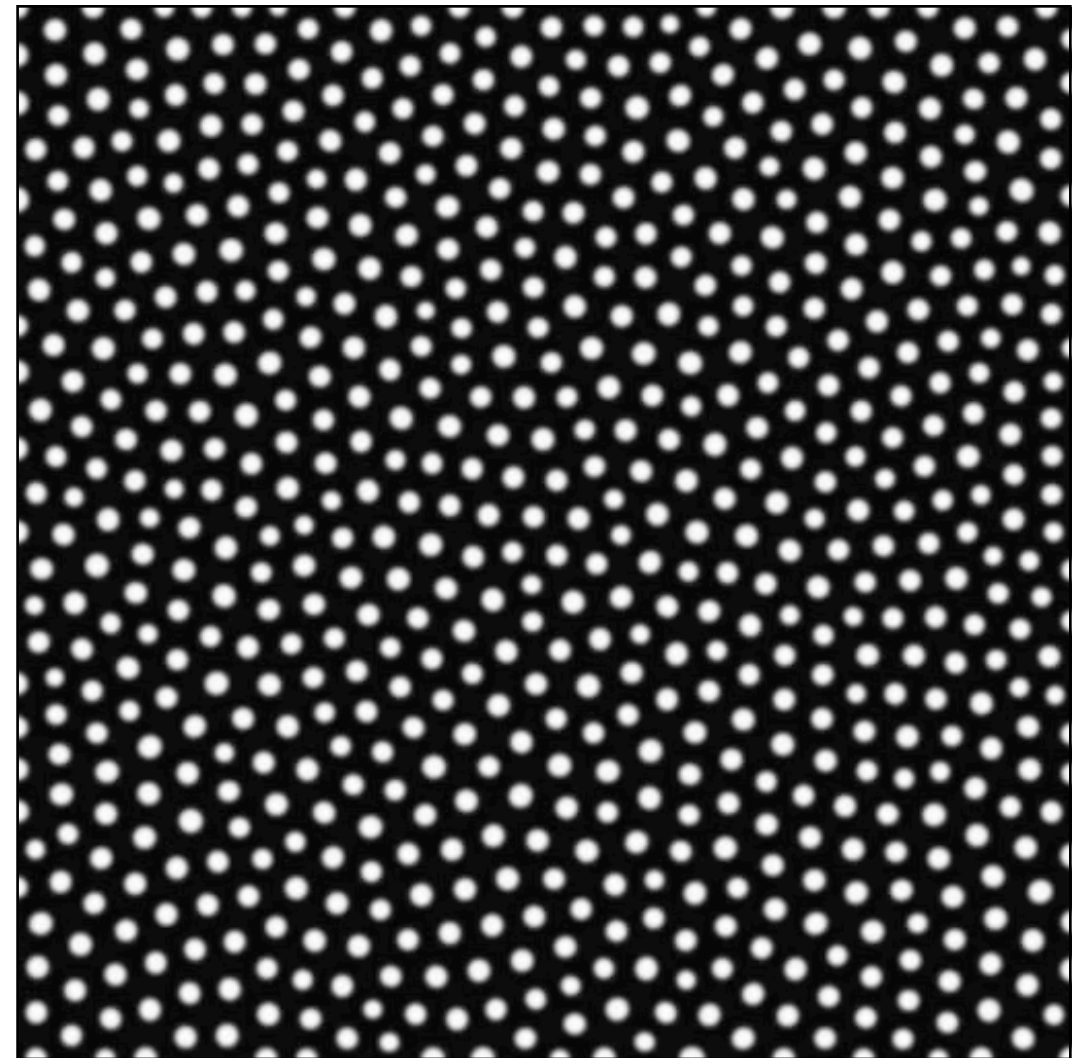
Droplet ripening
(no chemical reactions)



$$\partial_t \phi = \nabla \cdot (\lambda \nabla \mu)$$

Cahn Hilliard equation

Chemically active droplets
(supression of Ostwald ripening)

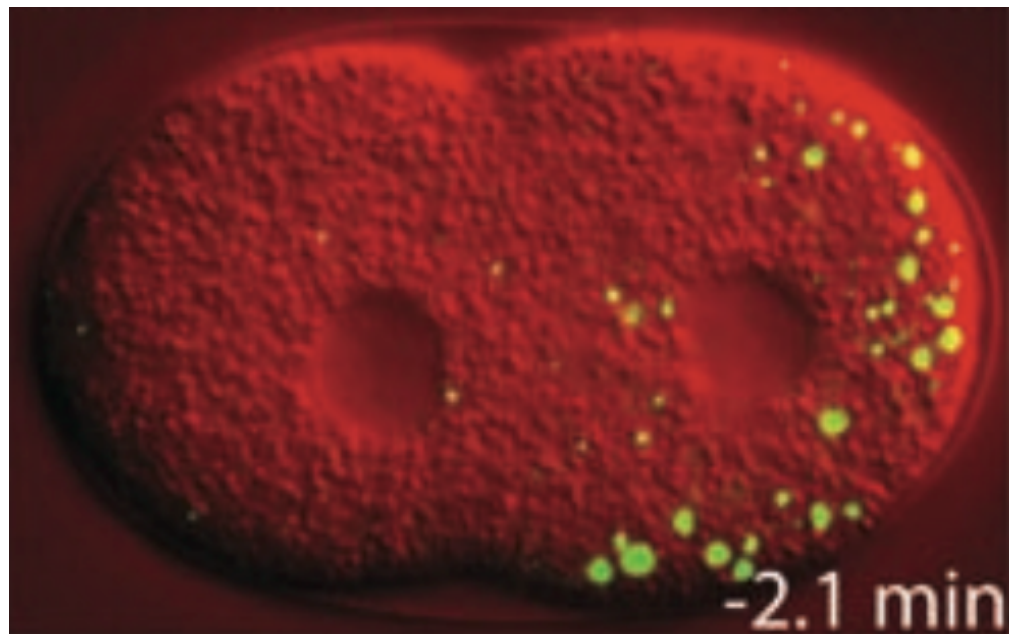


$$\partial_t \phi = \nabla \cdot (\lambda \nabla \mu) + s(\phi)$$

Zwicker, Hyman, Jülicher, Phys. Rev. E (2015)

RNA-protein assemblies organize chemistry in space

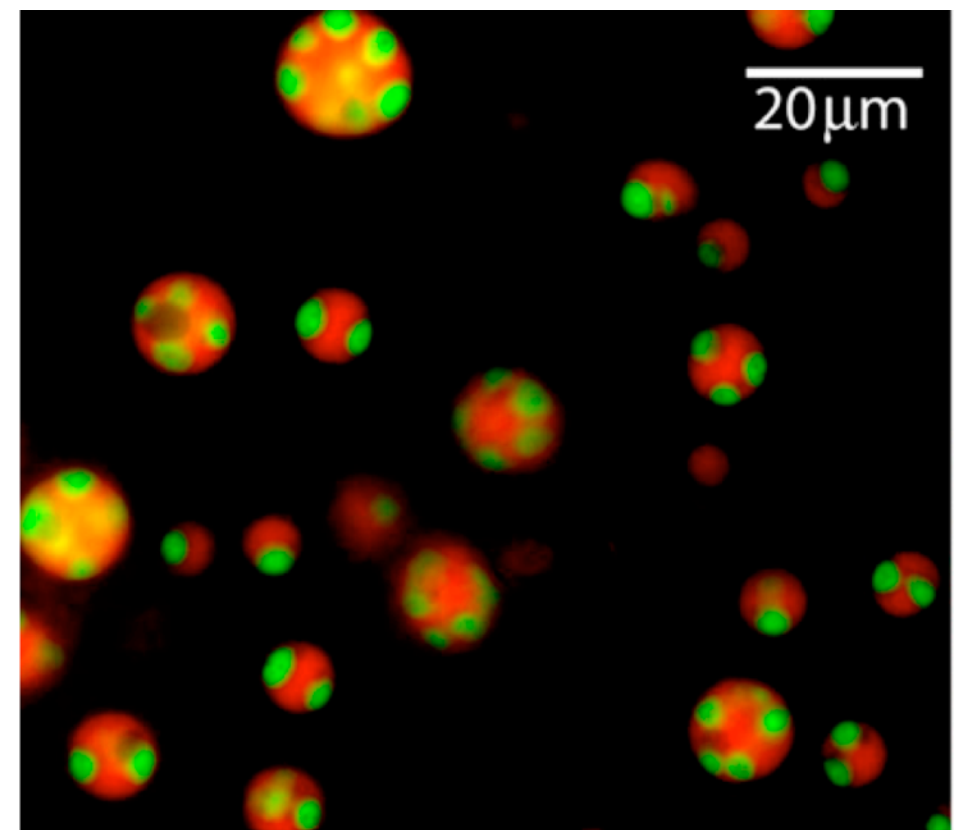
different types of RNP granules in many different cells



P-granules

stress granules
P granules
nucleoli ...

nucleolus



Brangwynne et al PNAS (2011)

Droplets in early life?



Alexander Oparin
1894-1980

coacervates:

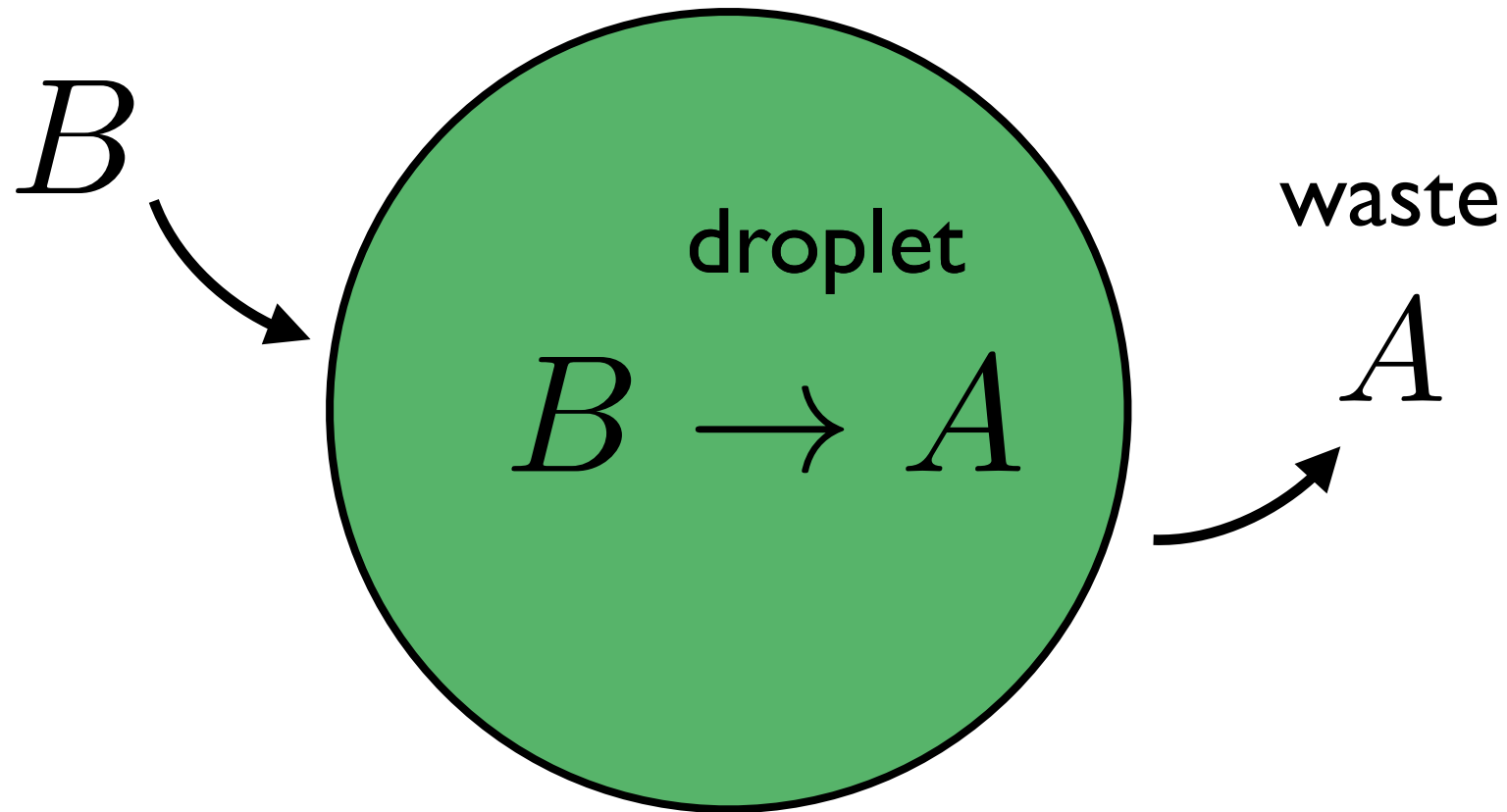
colloidal droplets formed by aggregation
of macromolecules in a liquid phase

The origin of life (1924)

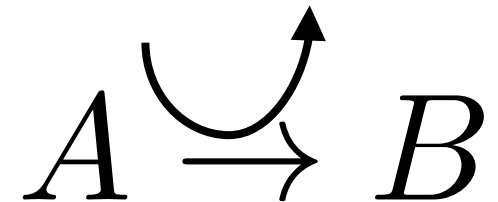
Active droplets as simple models for protocells

Confined chemical reactions

nutrient

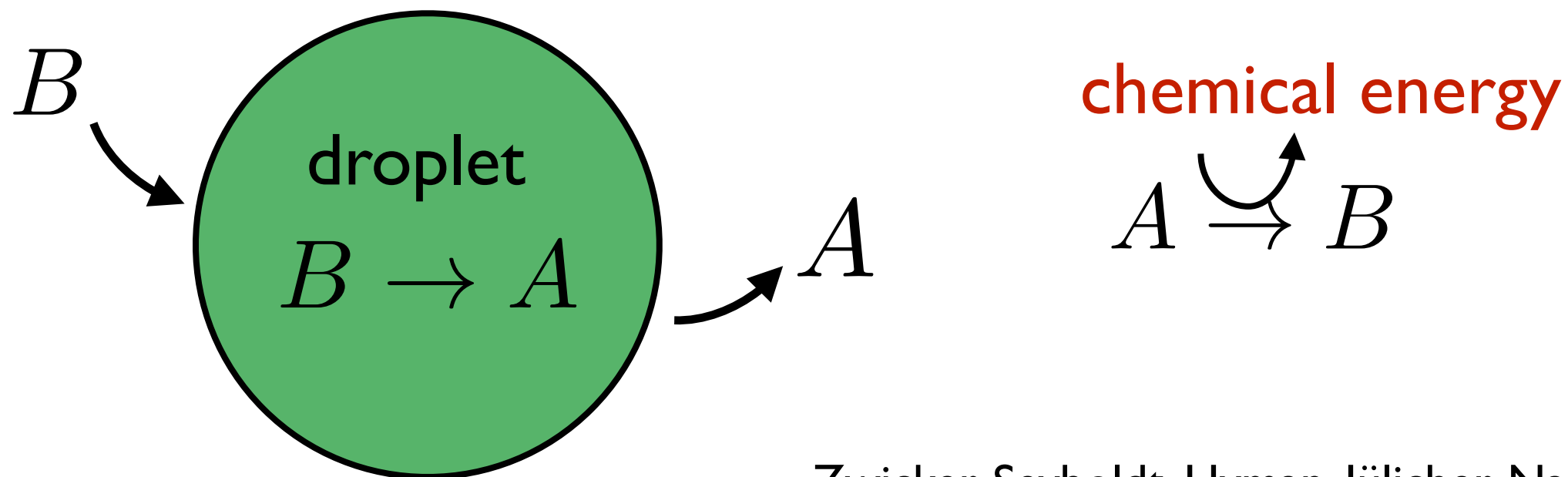
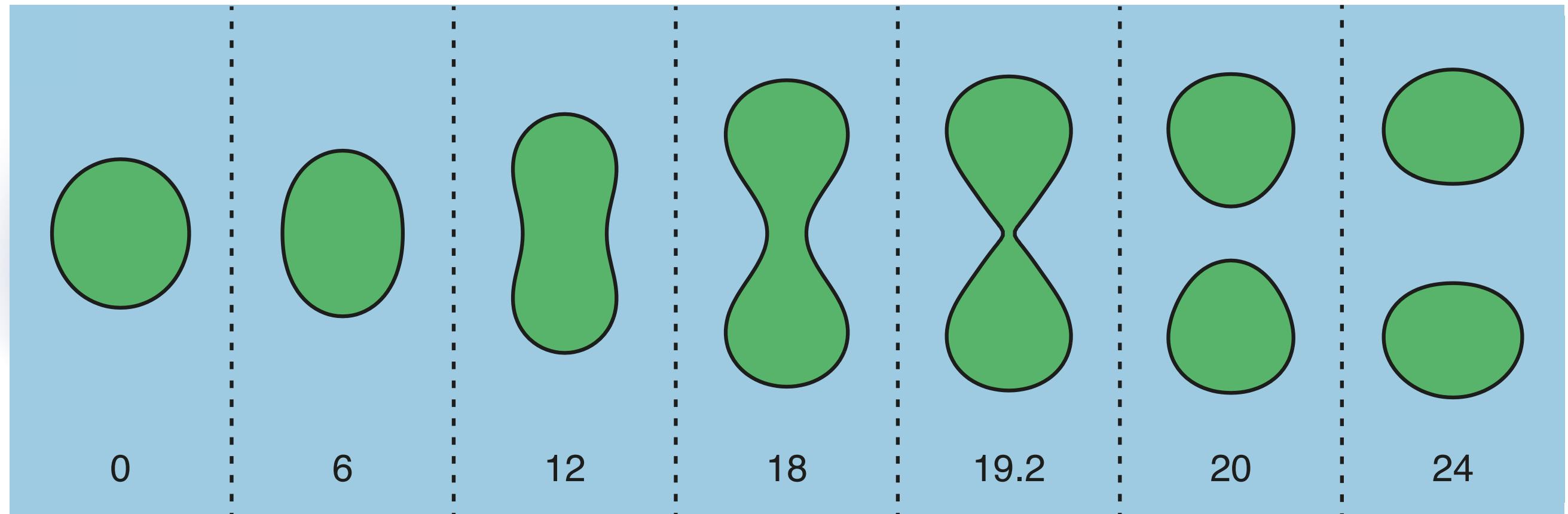


chemical fuel
or light

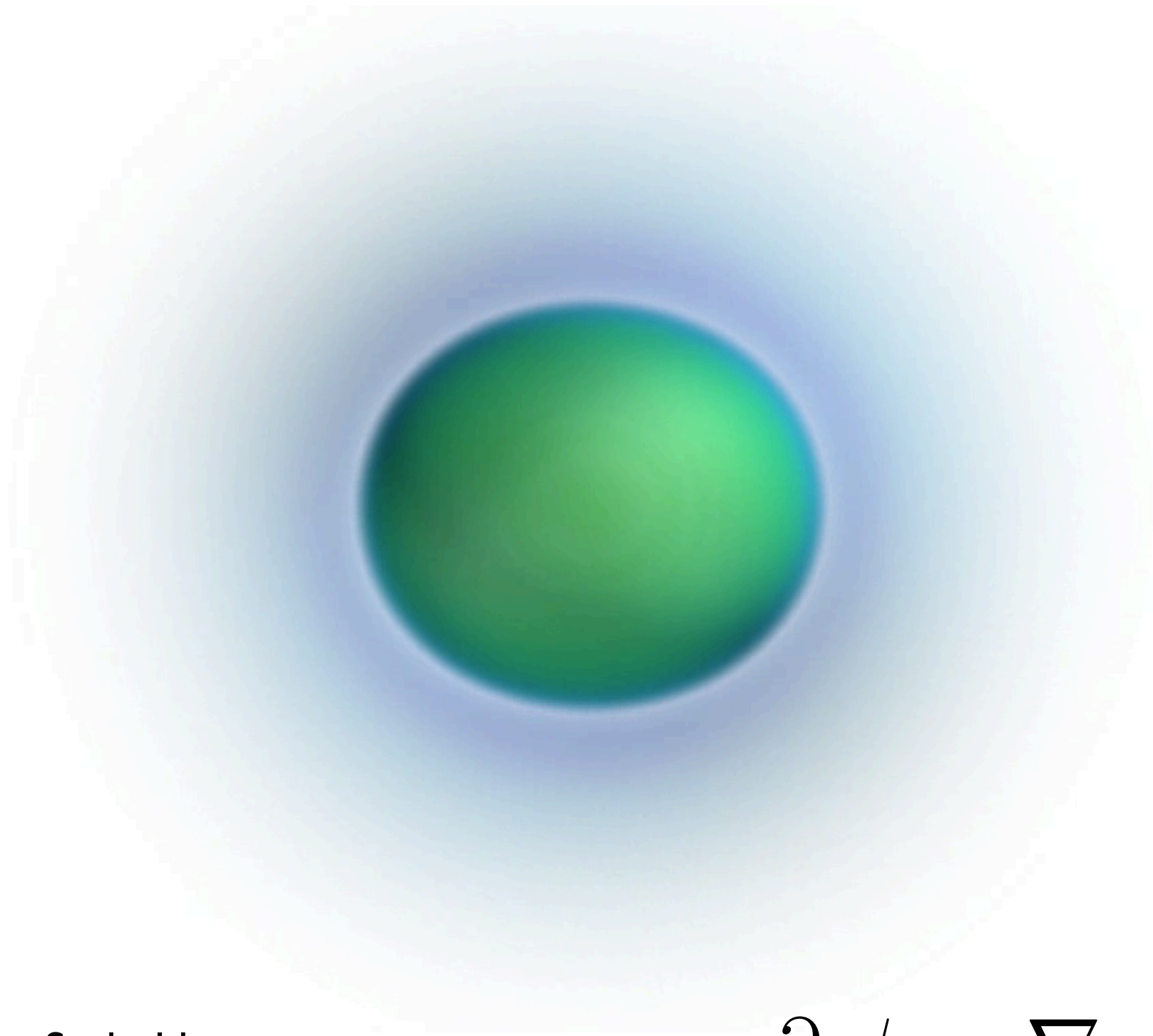


Division of active droplets

chemically driven shape instability leads to droplet division



Division of active droplets

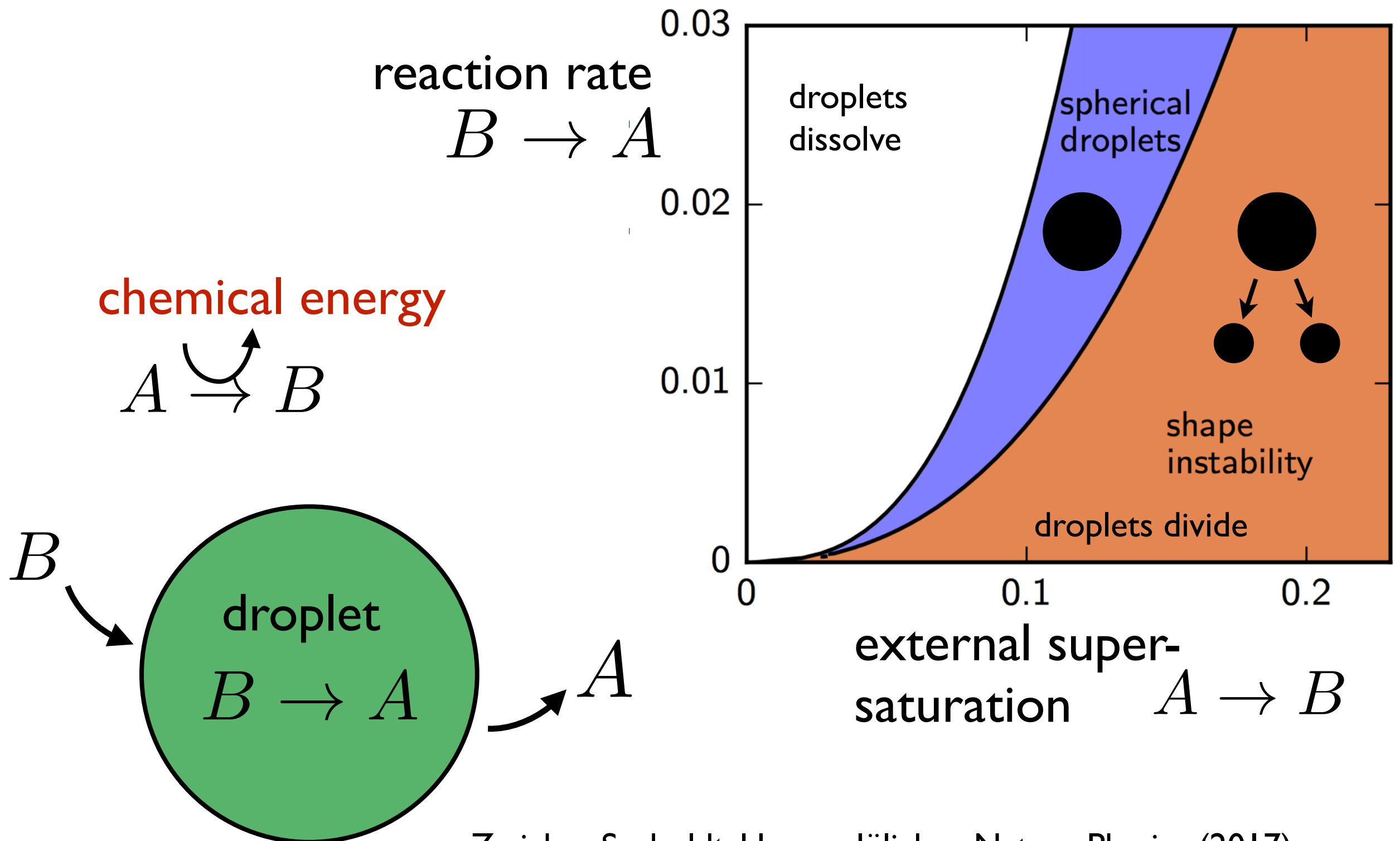


Rabea Seyboldt

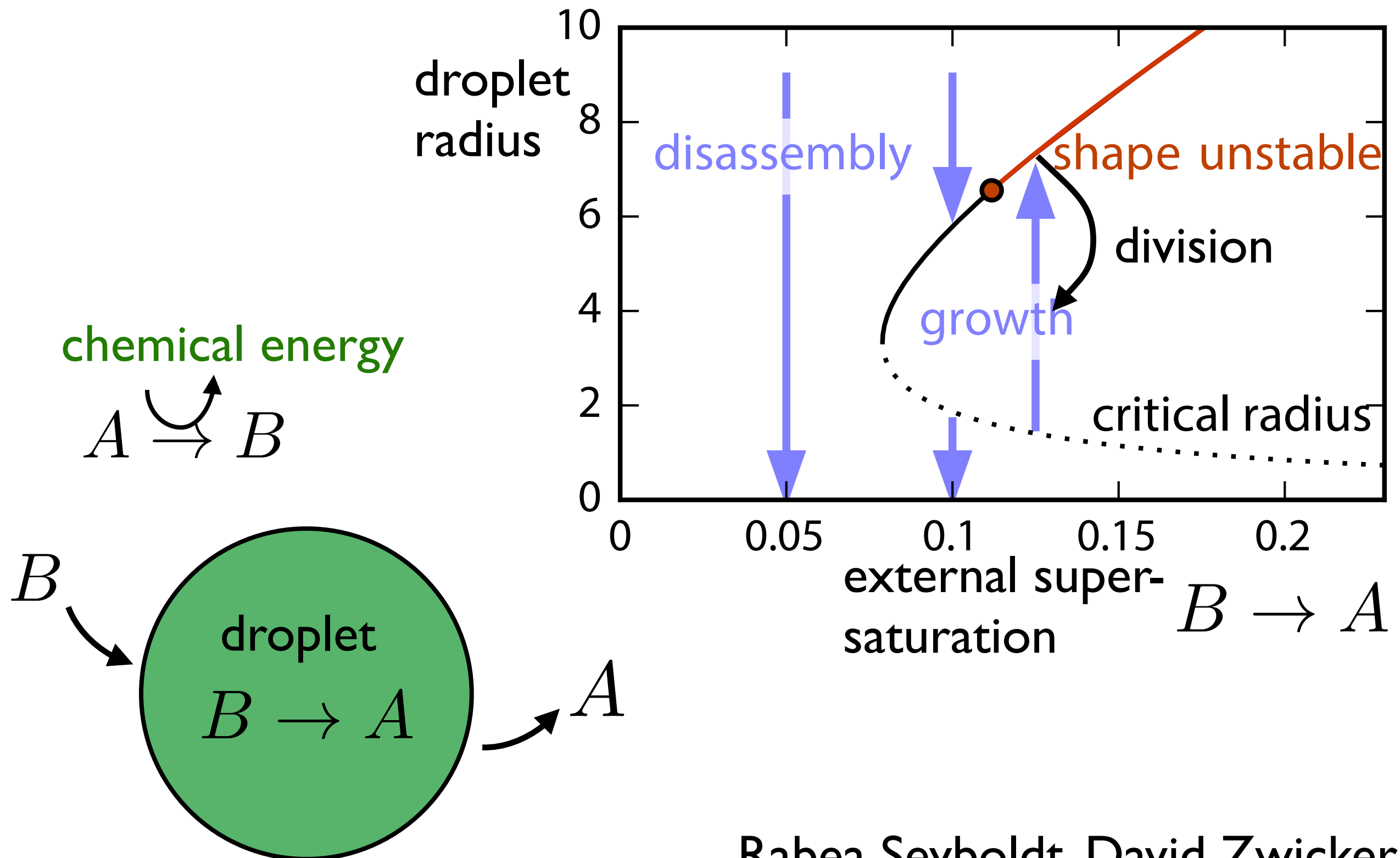
$$\partial_t \phi = \nabla \cdot (\lambda \nabla \mu) + s(\phi)$$

Cahn-Hilliard equation with chemical reactions and **energy input**

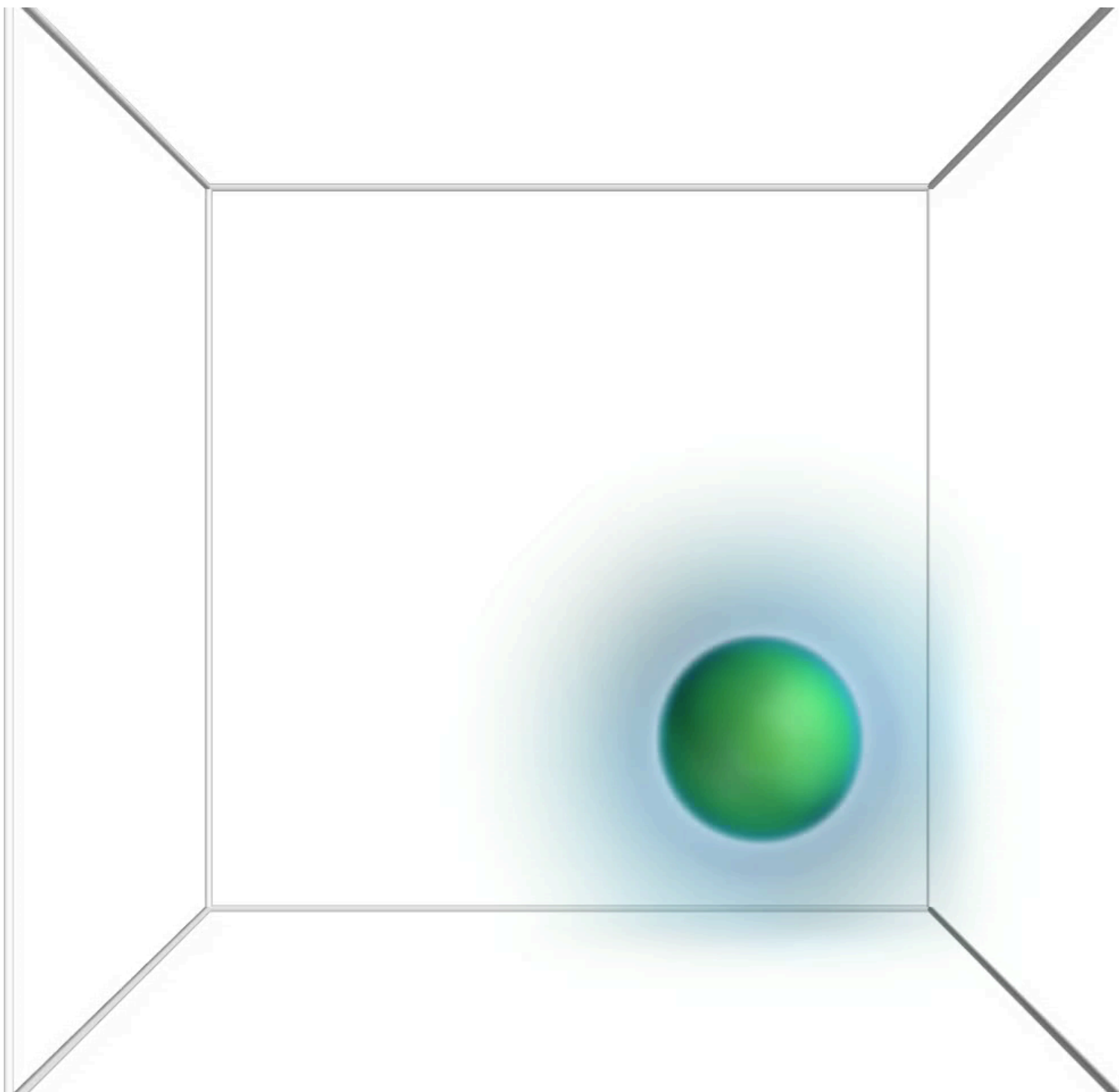
Division of active droplets



Division of active droplets



Growth-division cycles



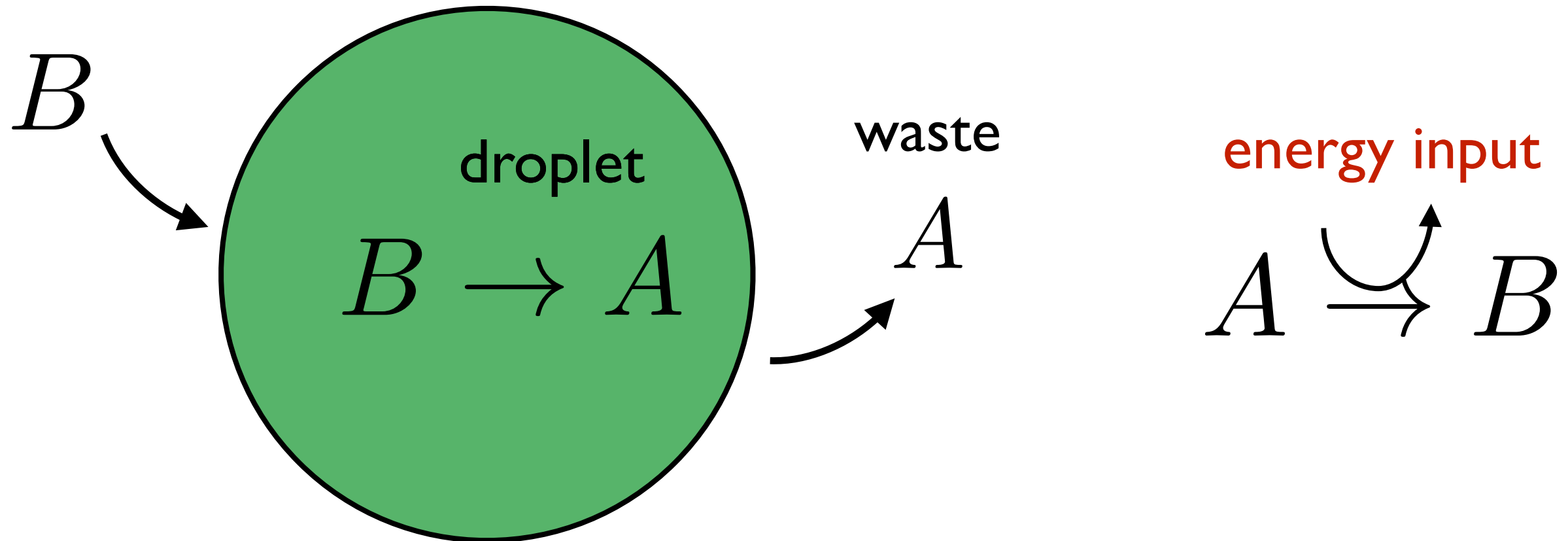
$$\partial_t \phi = \nabla \cdot (\lambda \nabla \mu) + s(\phi)$$

Simple model for protocells

Chemically active droplets:

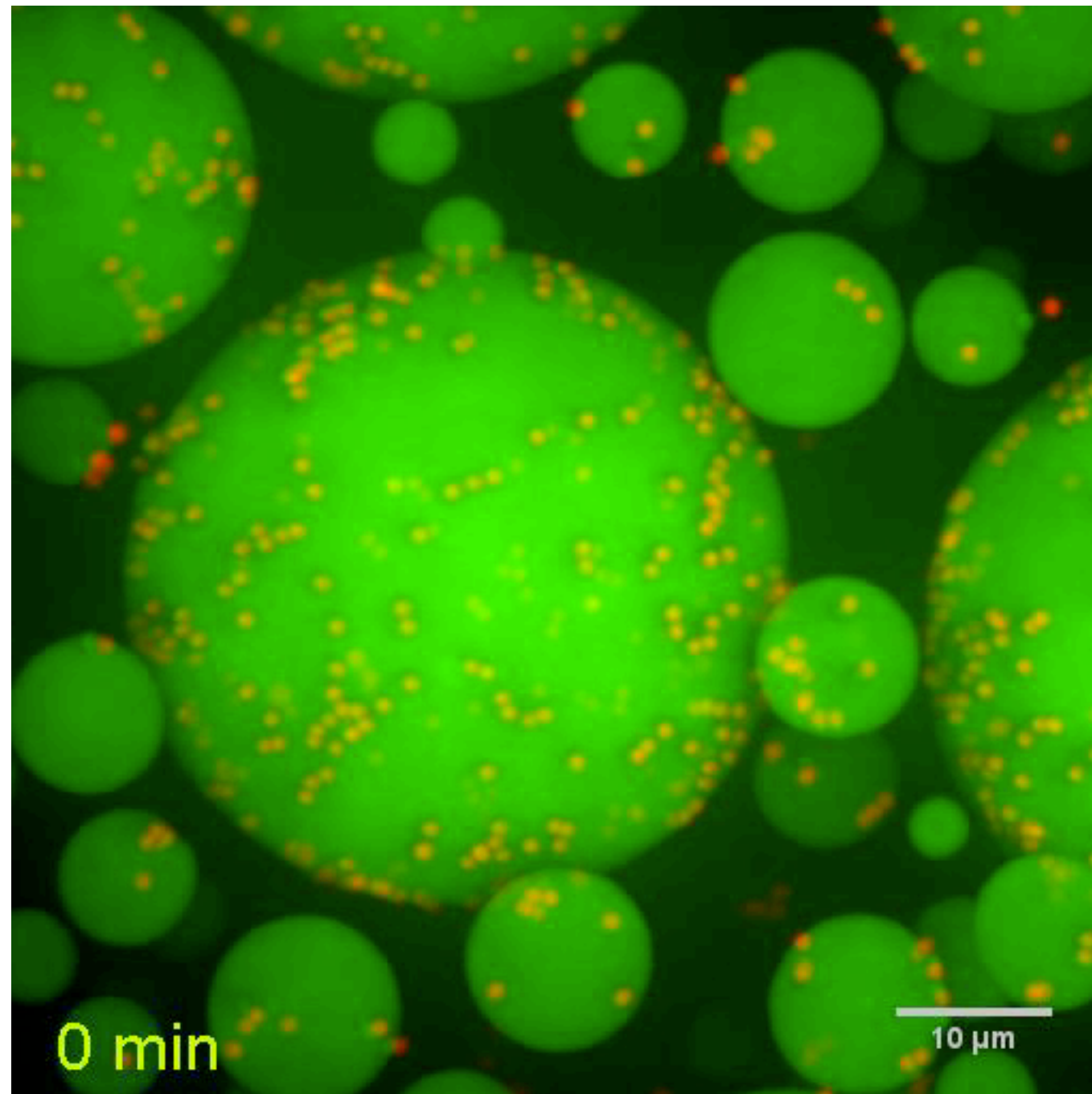
simple “metabolism”
division and growth
far from equilibrium
no membrane
no genetics

nutrient



Hardening of protein condensates

Protein condensates that are liquid-like after formation can become more solid like when older



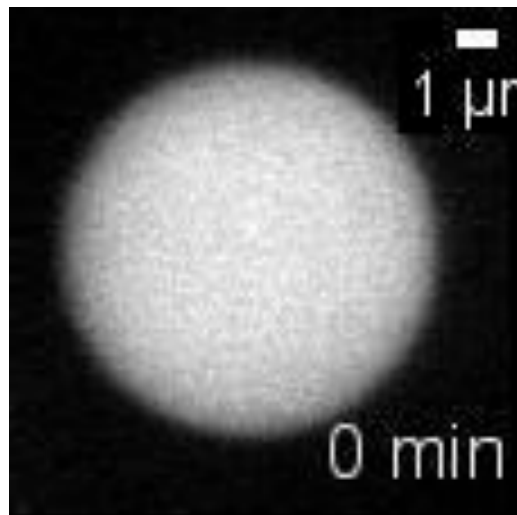
PGL3 - 40mM KCl

Louise Jawerth

Hardening of protein condensates

Protein condensates that behave liquid-like after formation can become more solid like when older

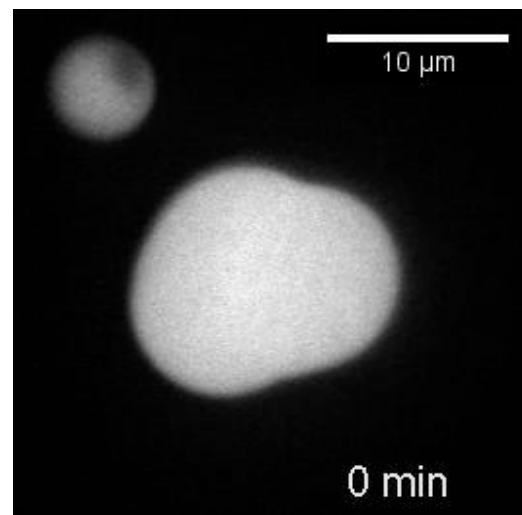
1h after preparation



PGL3

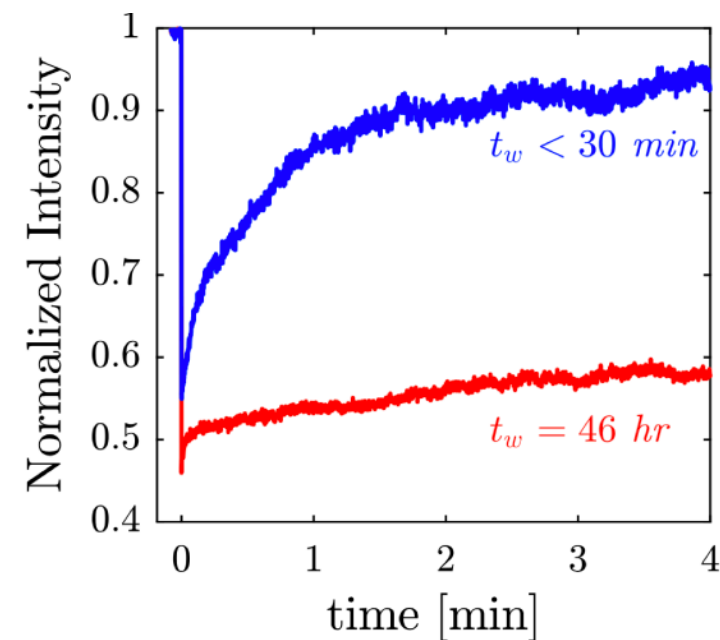
$t_w = 2 \text{ hrs}$

46h after preparation

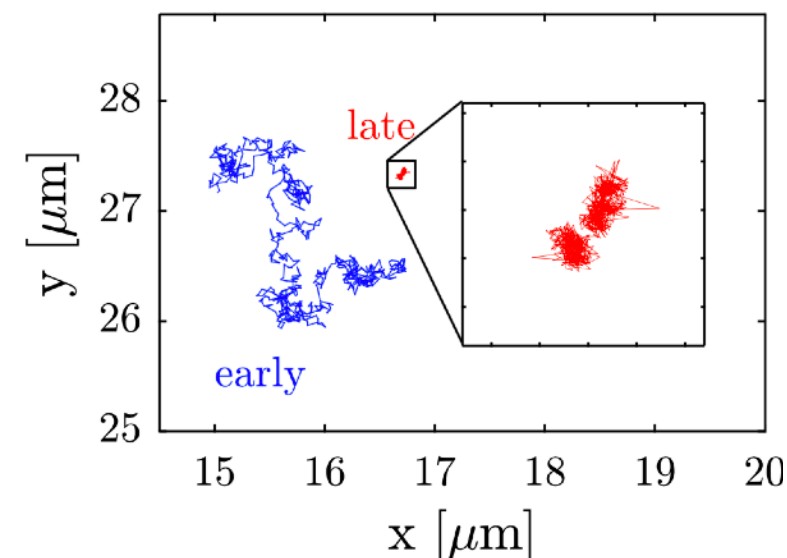


$t_w = 31 \text{ hrs}$

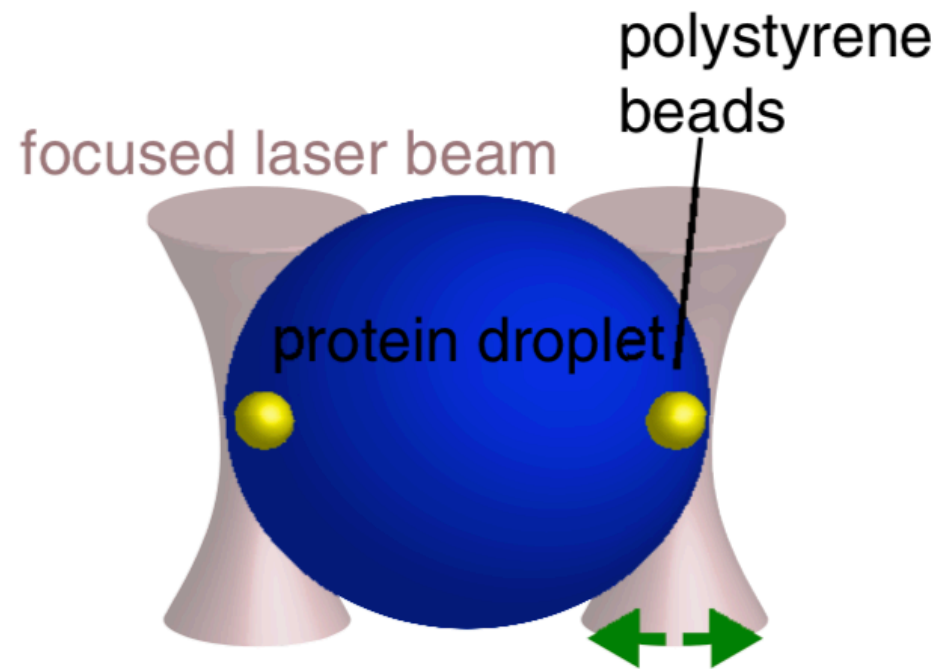
Fluorescence recovery



Diffusion of immersed beads



Pulling on condensates: material properties



measurement of force and deformation

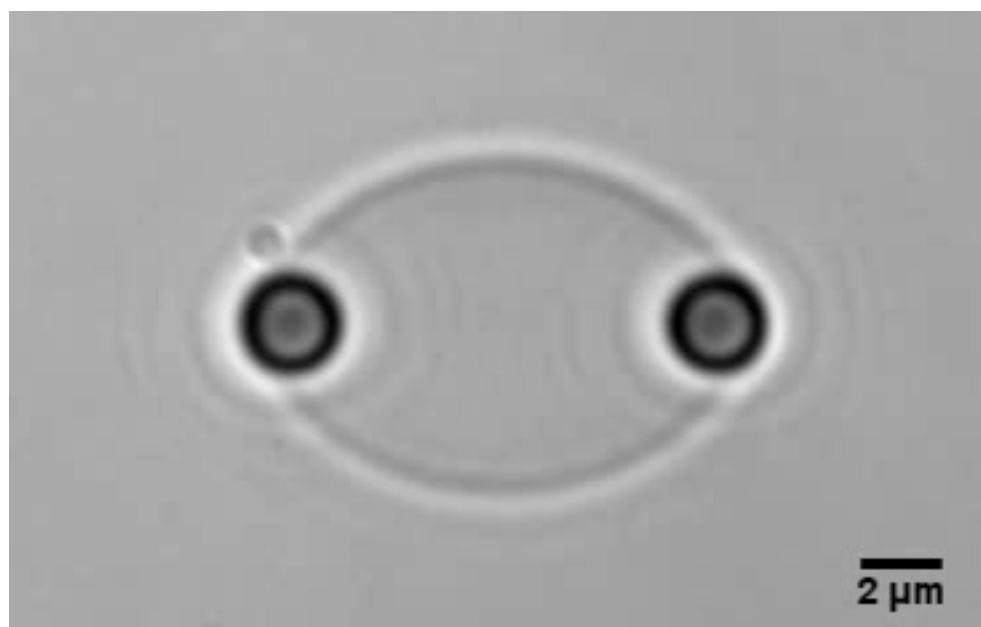
surface tension γ

viscosity η

elastic moduli E

rheology $G^* = G' + G''$

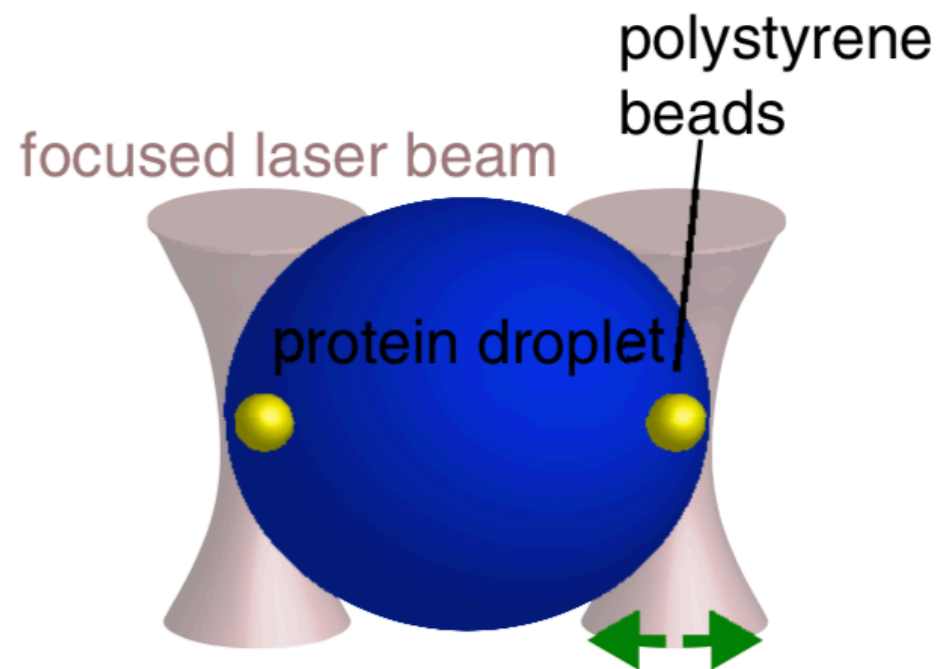
frequency dependent moduli



Jawerth et al. PRL 121 (2018)

Jawerth et al. Science (2020)

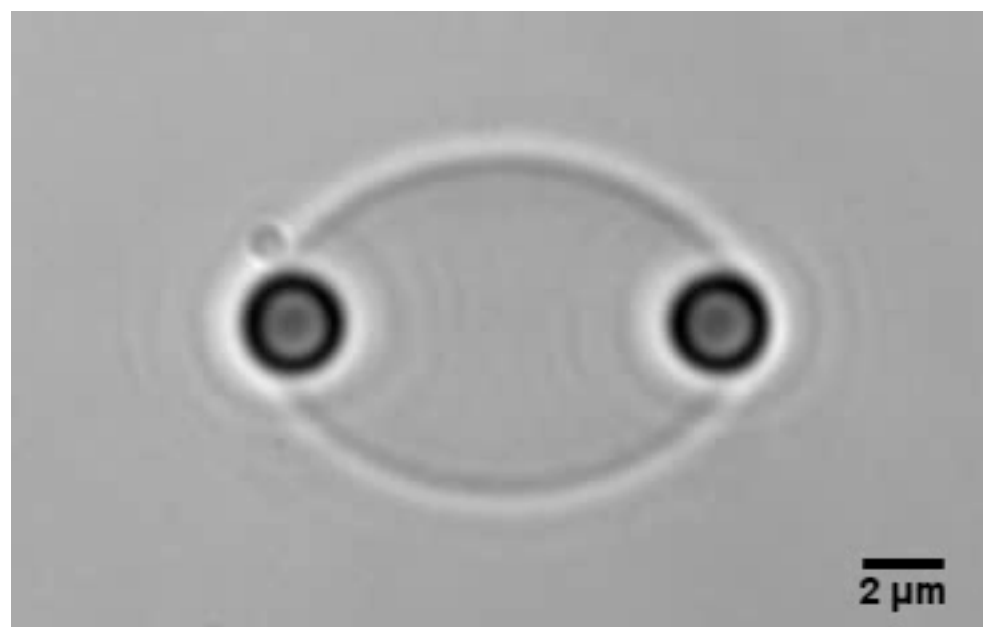
Surface tension from active micro-rheology



measurement of force and deformation

surface tension γ

$$\gamma \simeq 5 \mu\text{N/m}$$



(air-water surface tension for comparison)

$$\gamma = 70\text{mN/m}$$

Jawerth et al. PRL 121 (2018)

Jawerth et al. Science (2020)

Time periodic forcing

deformation

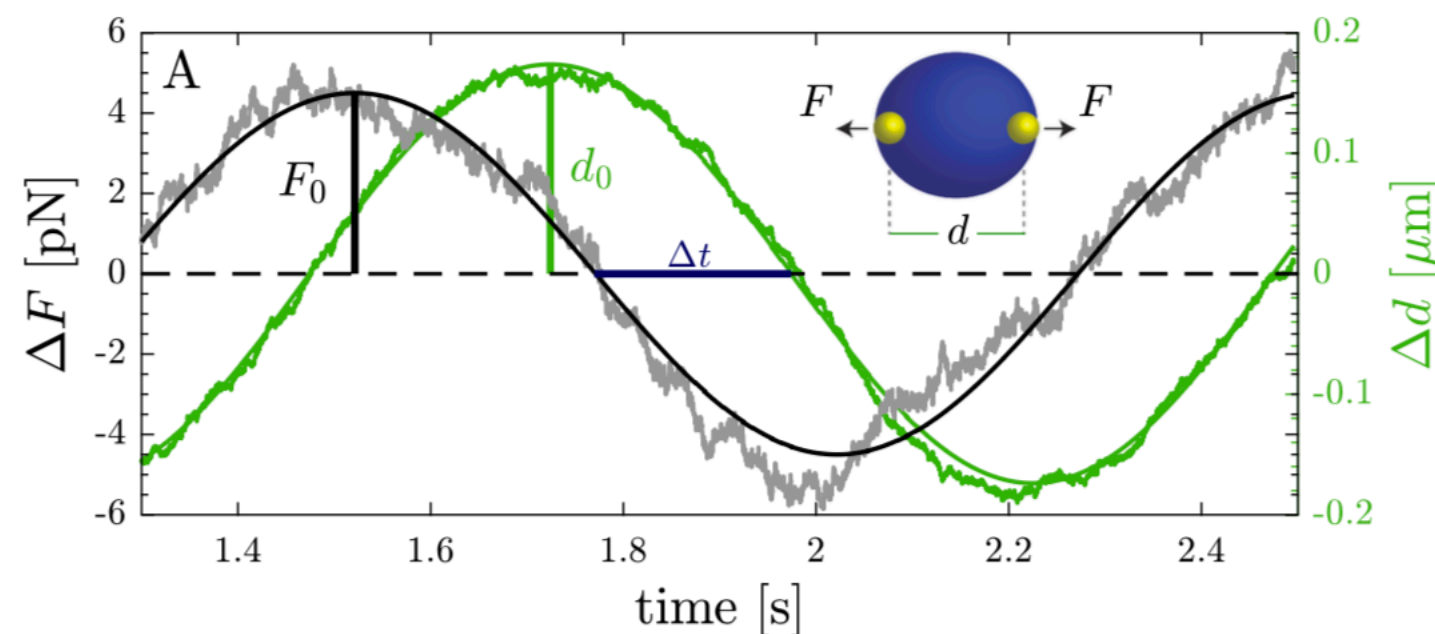
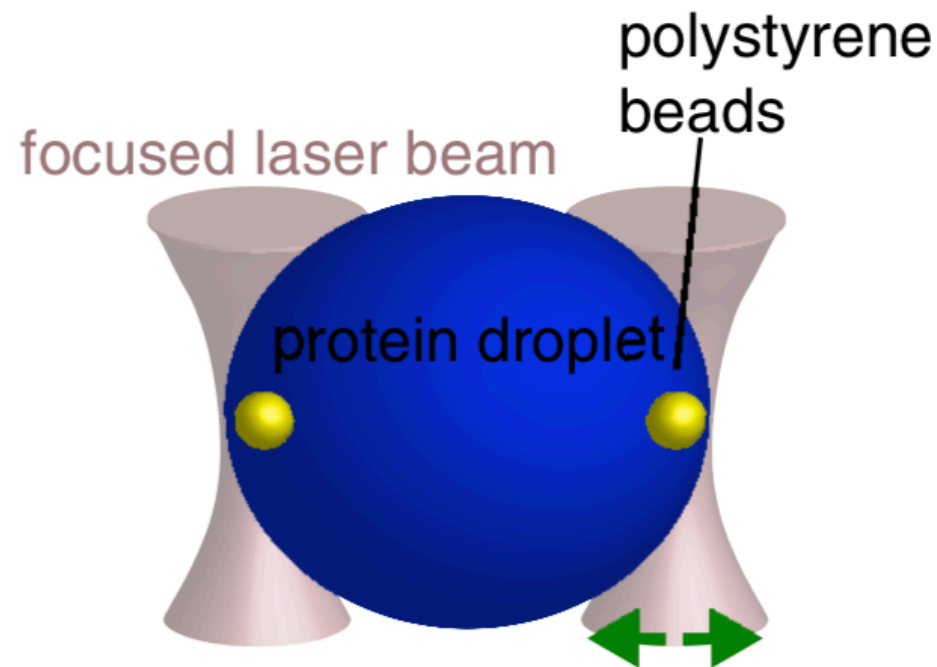
$$u(t) = \tilde{u}e^{i\omega t}$$

stress

$$\sigma(t) = \tilde{\sigma}e^{i\omega t}$$

material response

$$\tilde{\sigma} = G^*(\omega)\tilde{u}$$



storage modulus: in phase
(elastic)

loss modulus: phase shifted
(dissipation)

Jawerth et al. PRL 121 (2018)

Jawerth et al. Science (2020)

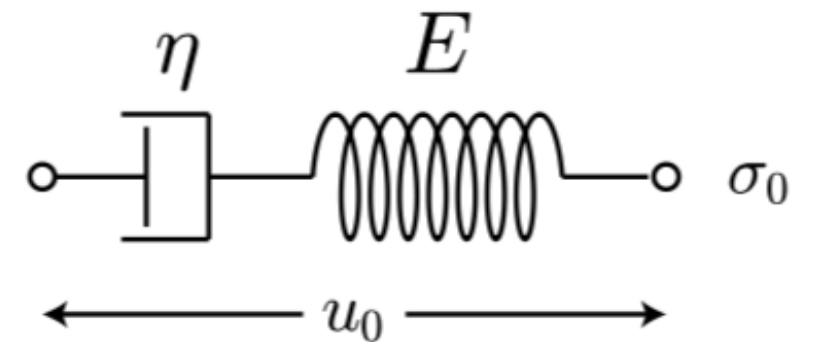
Ageing of protein condensates

$$\tilde{\sigma} = G^*(\omega) \tilde{u}$$

stress amplitude
(force per area)

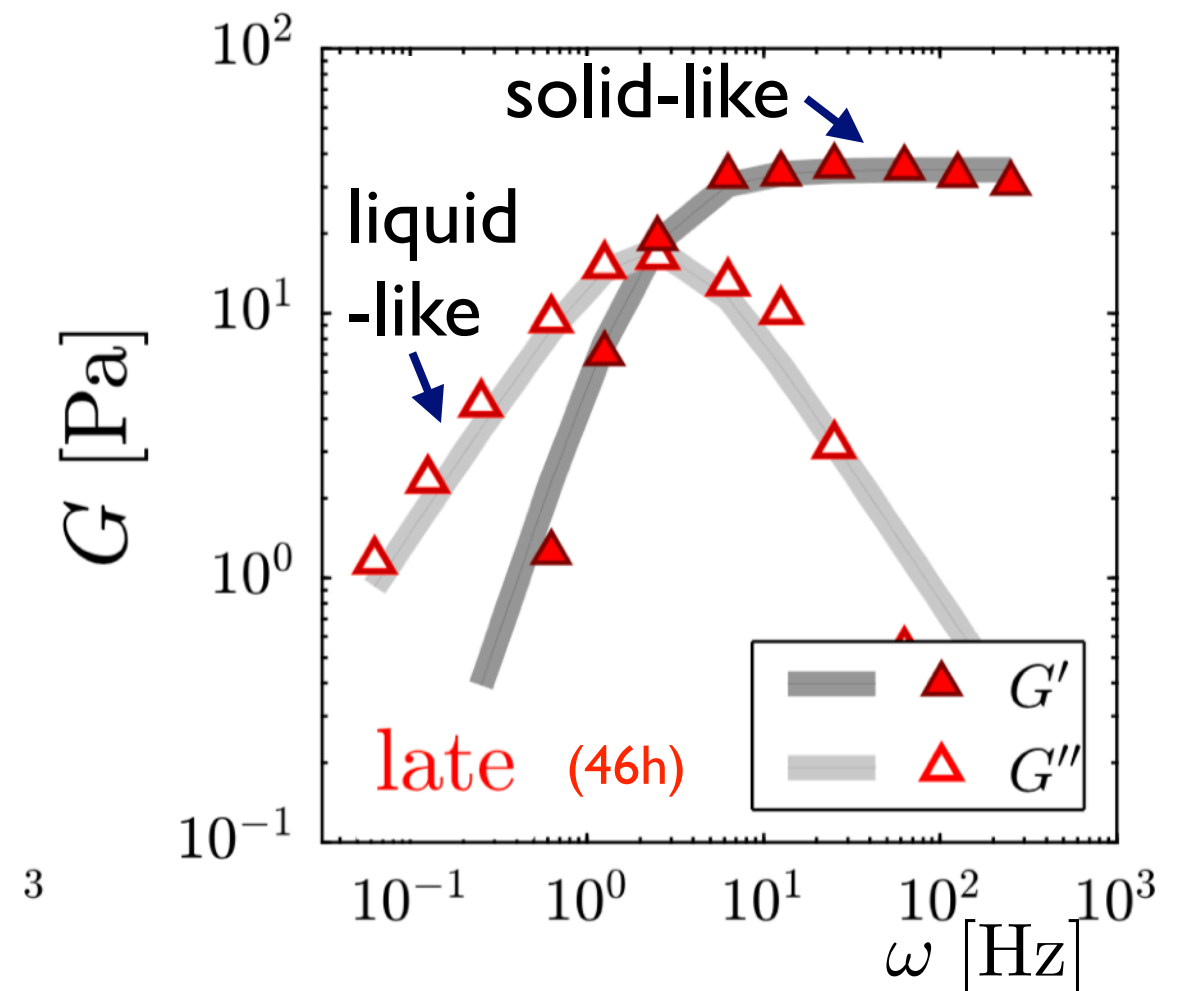
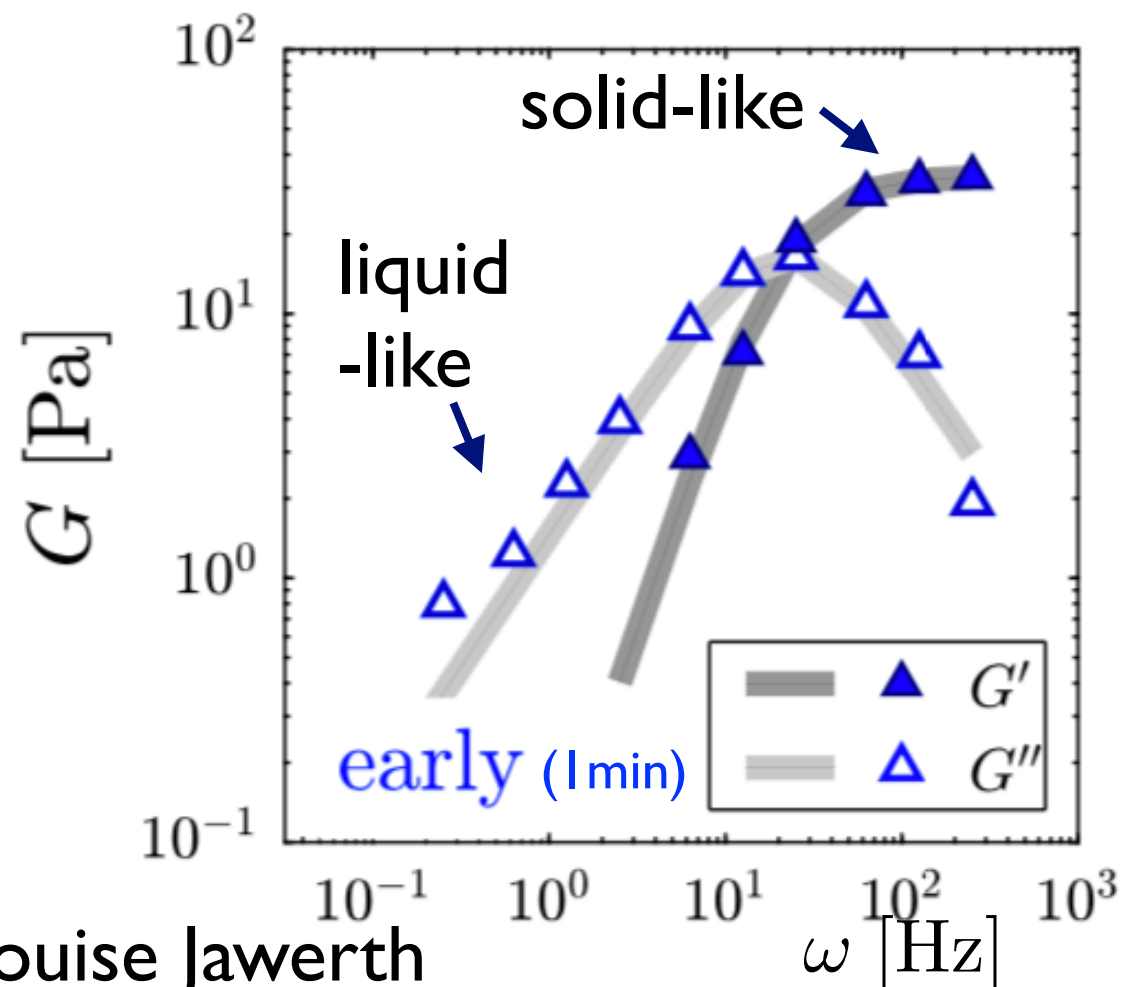
complex
modulus

deformation
amplitude



Maxwell model

$$G^* = \frac{i\omega\tau_c E}{1 + i\omega\tau_c}$$



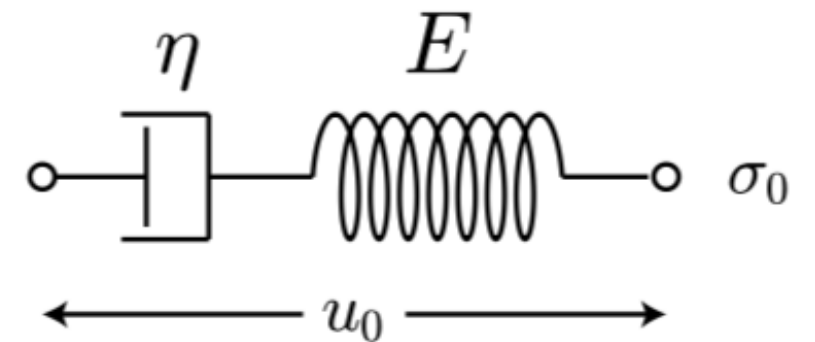
Ageing of protein condensates

$$\tilde{\sigma} = G^*(\omega) \tilde{u}$$

stress amplitude
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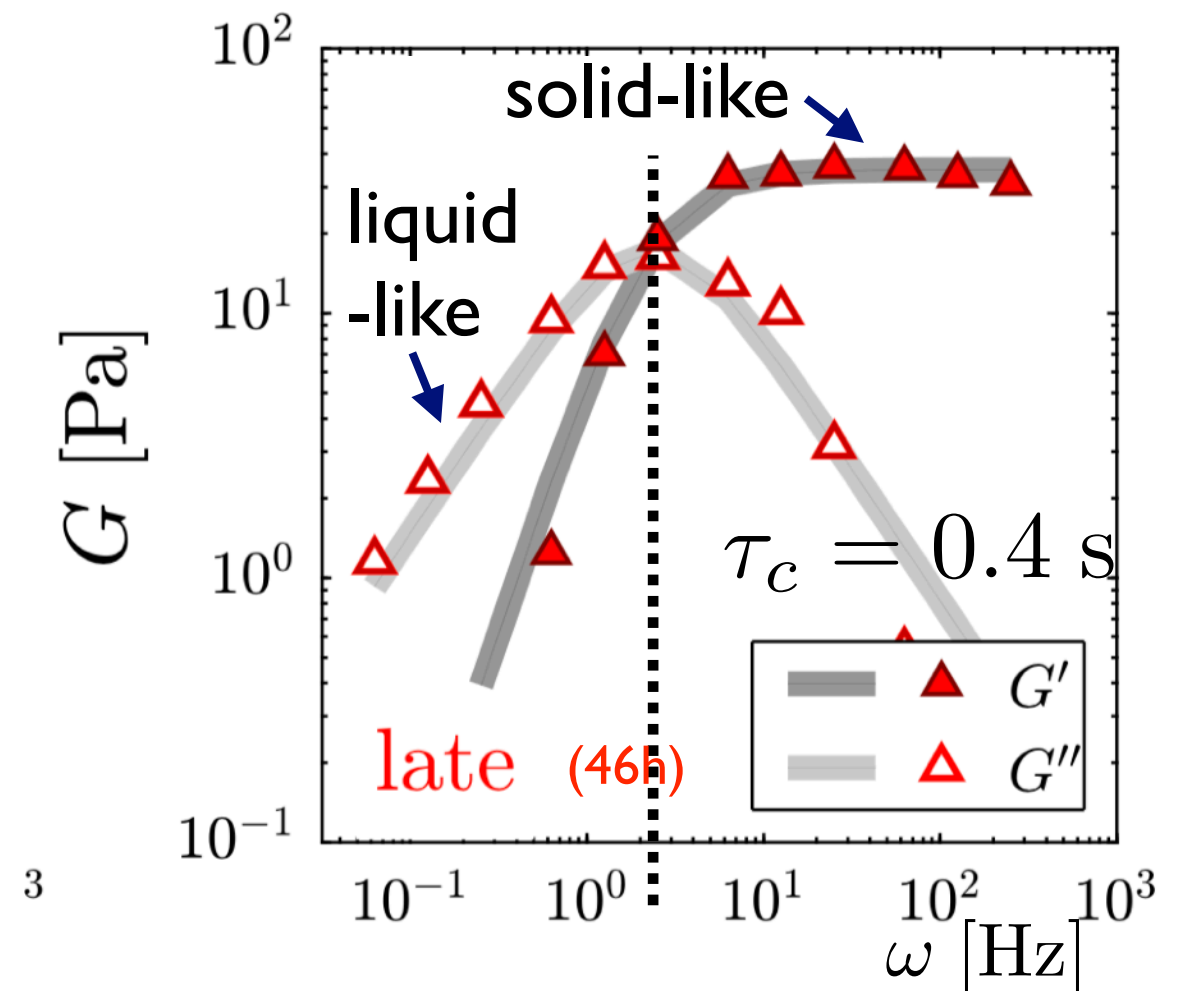
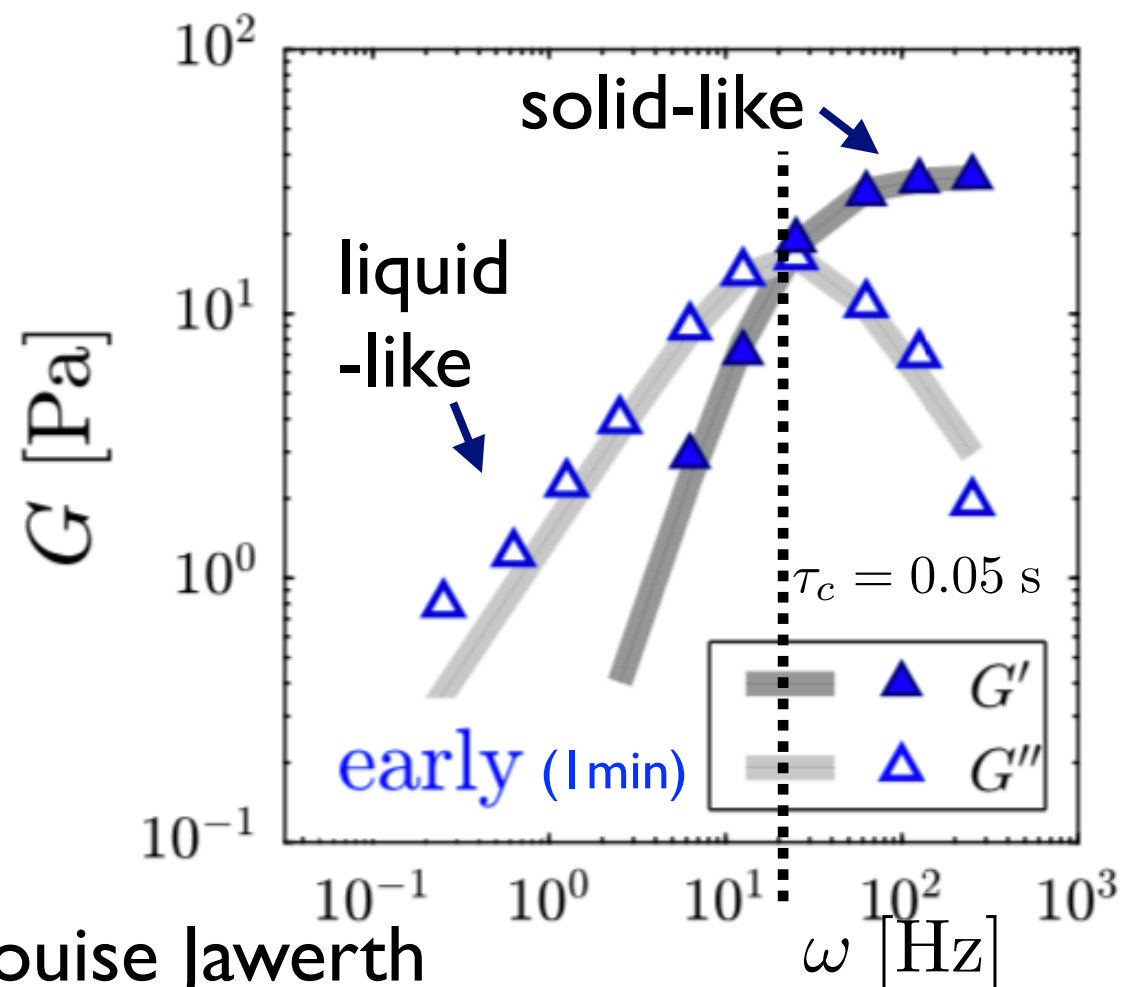
complex
modulus

deformation
amplitude



Maxwell model

$$G^* = \frac{i\omega\tau_c E}{1 + i\omega\tau_c}$$



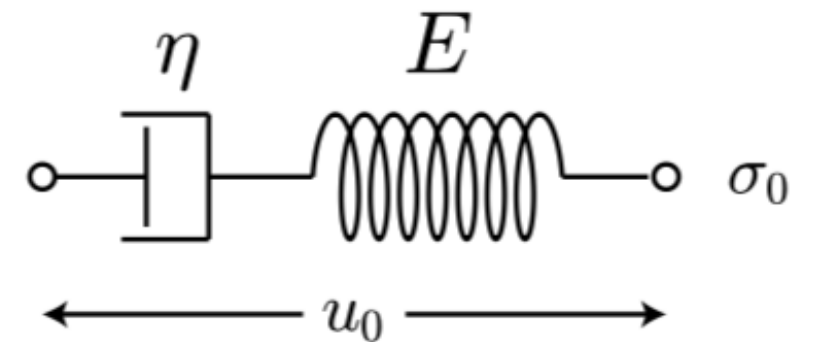
Ageing of protein condensates

$$\tilde{\sigma} = G^*(\omega) \tilde{u}$$

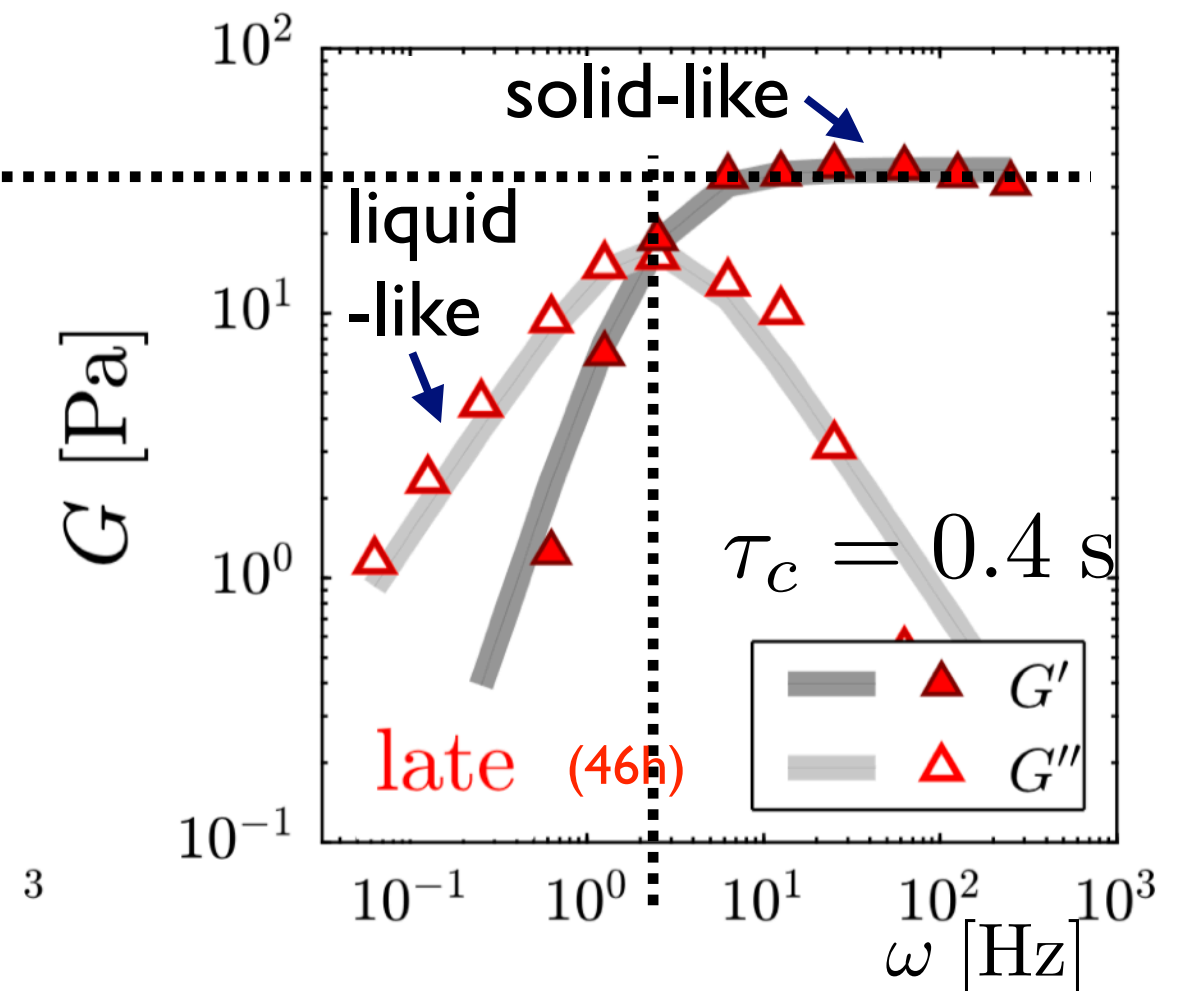
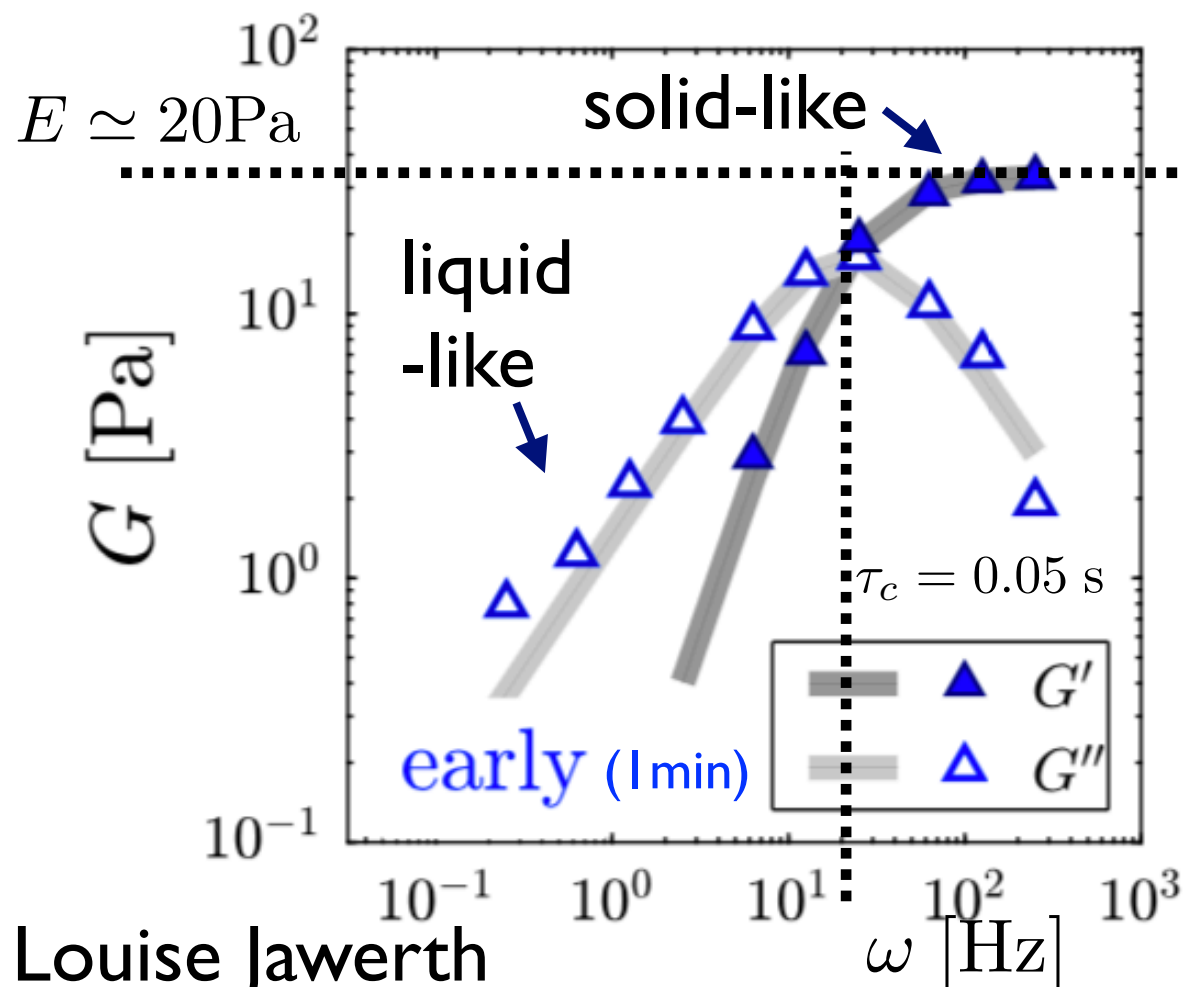
stress amplitude
(force per area)

complex
modulus

deformation
amplitude

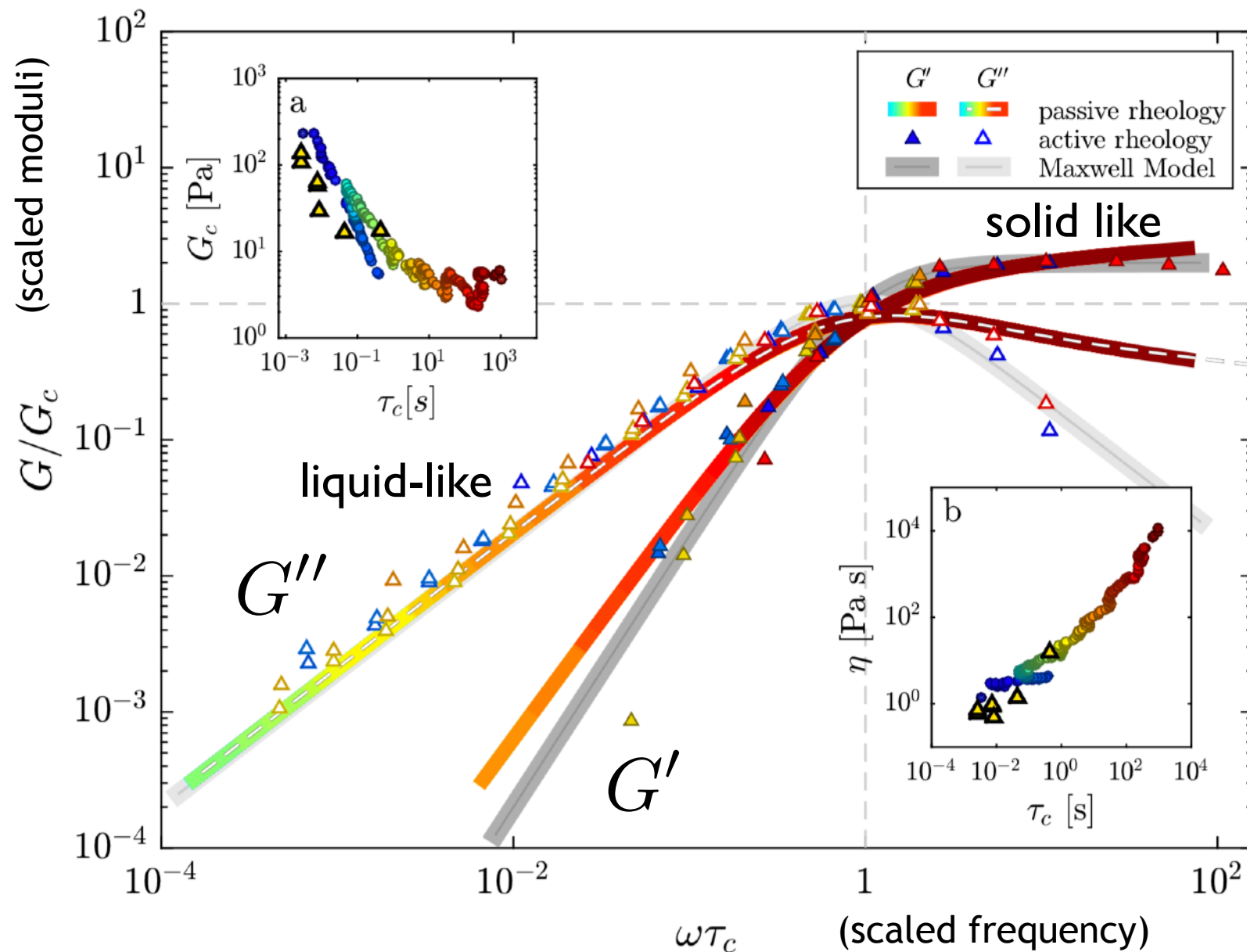


$$G^* = \frac{i\omega\tau_c E}{1 + i\omega\tau_c}$$

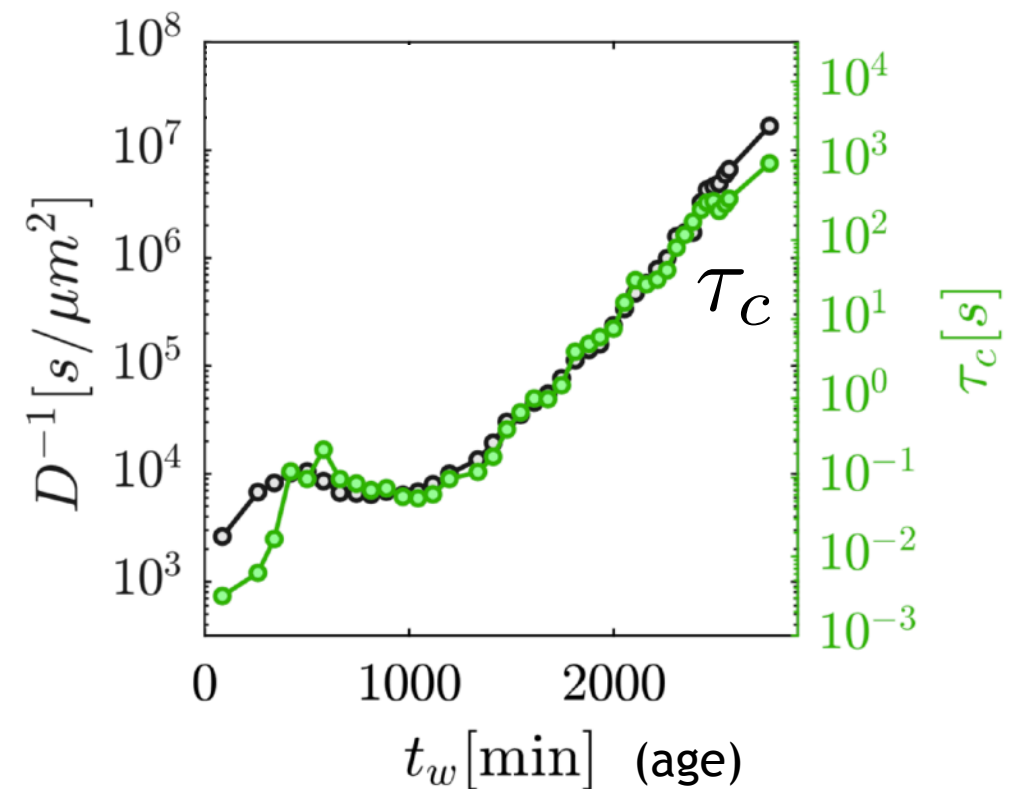


Increasing relaxation time: glassy dynamics

Rescaled frequency dependent moduli



Maxwell relaxation time for increasing age



crosslink lifetime
increases with age

crosslink density
roughly unchanged

Glassy Maxwell fluid: Maxwell glass

increasing viscosity with age

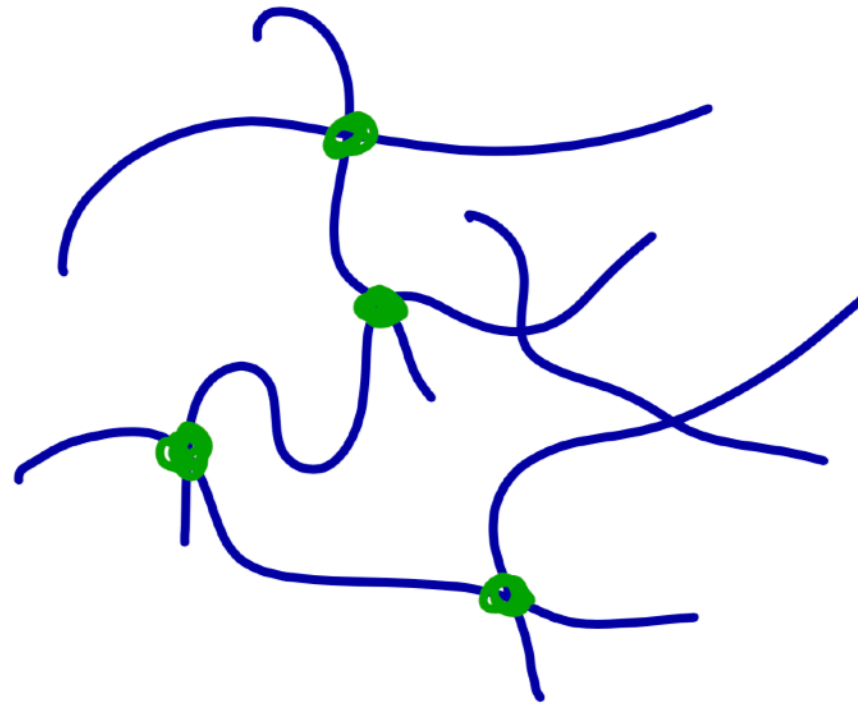
Gel formation versus ageing glass

Gelation

permanent cross-links
added

sharp transition from
fluid to solid

elastic modulus increases

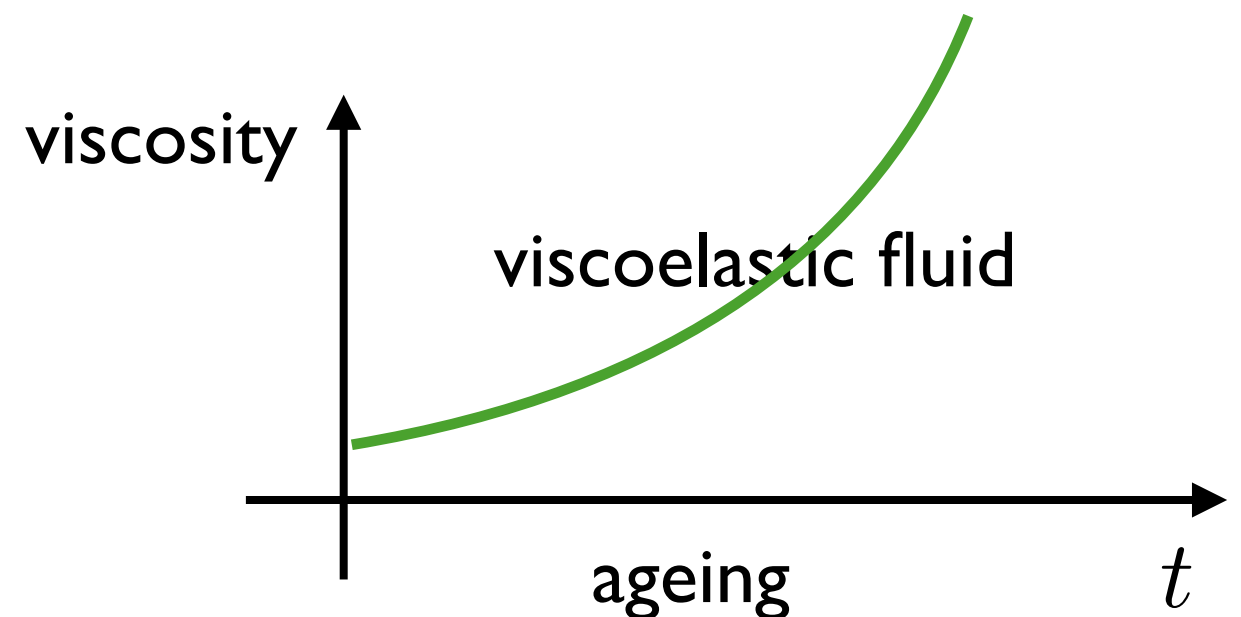
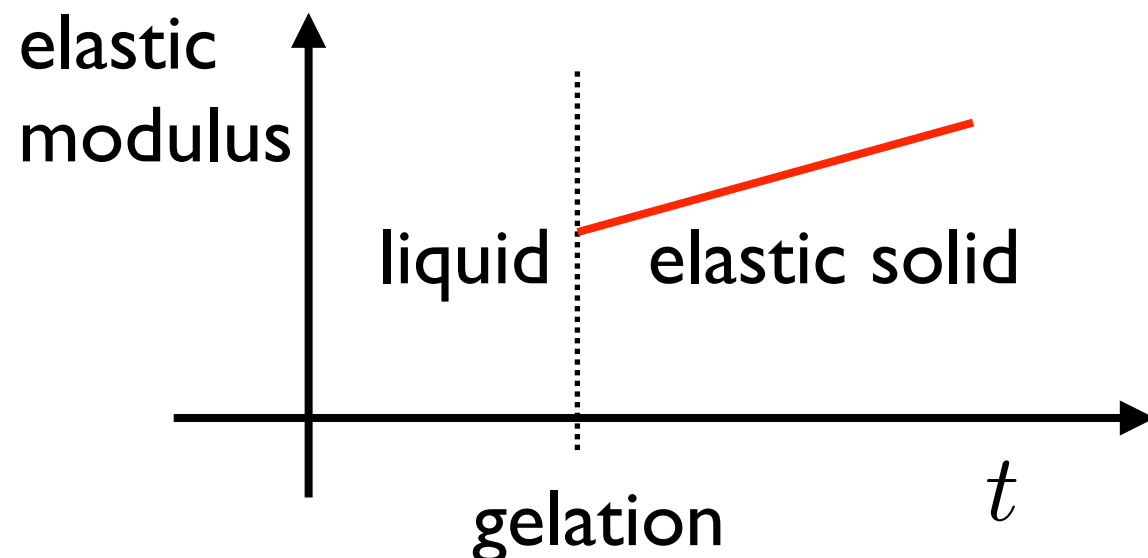


Maxwell glass

cross link life
time increases

viscosity increases
gradually

remains soft



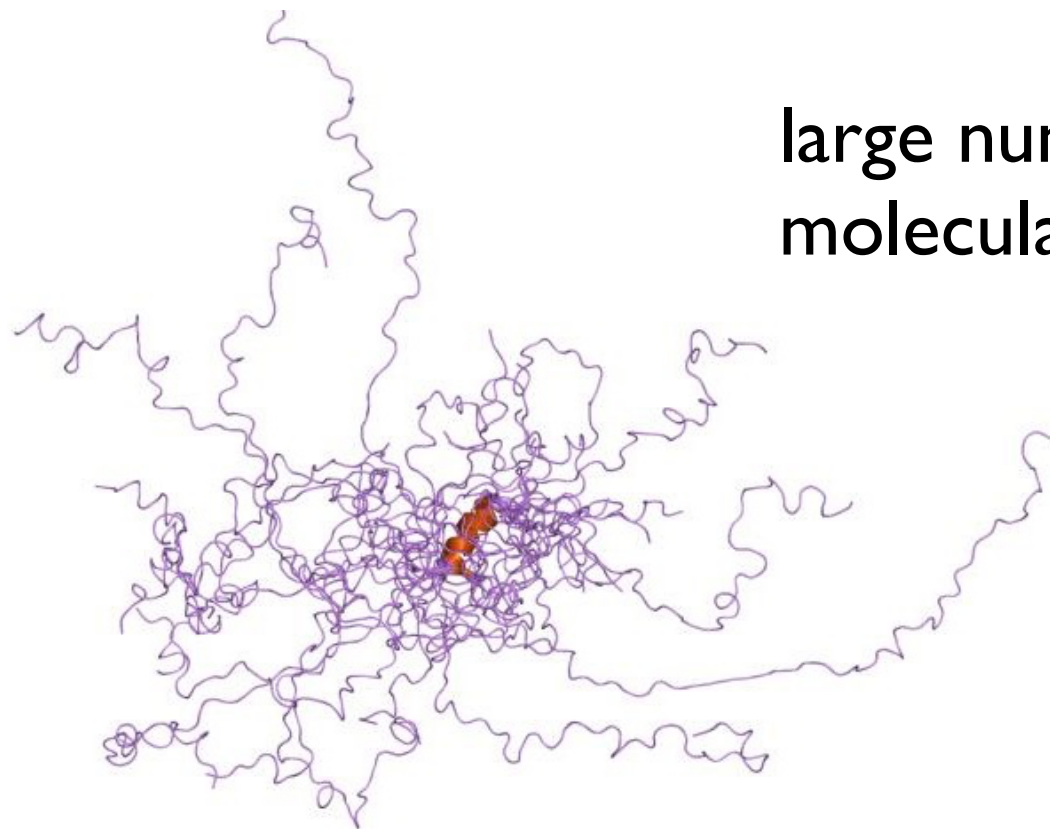
Glassy dynamics: disorder of molecular interactions

intrinsically disordered protein domains

supramolecular chemistry

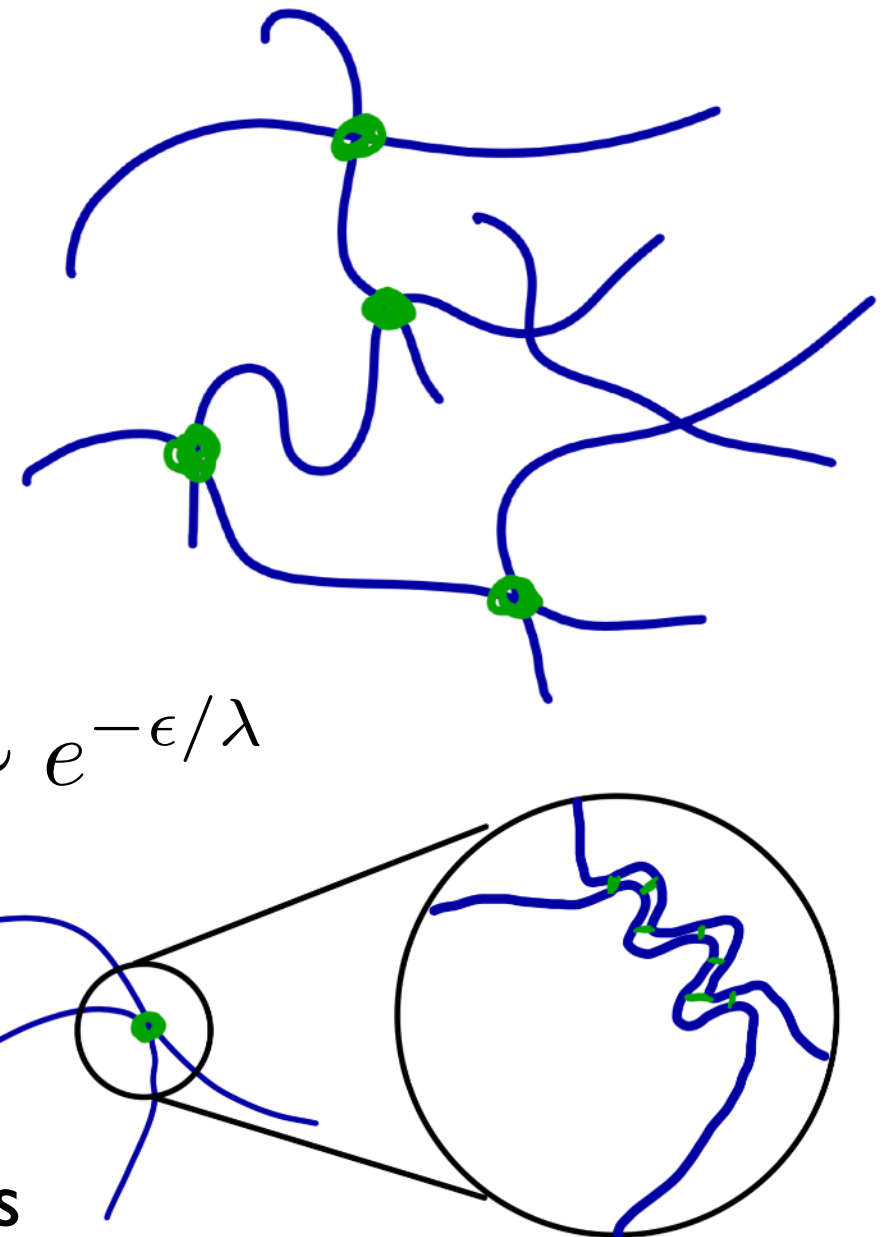
polymer physics

large number of possible
molecular configurations



$$P(\epsilon) \sim e^{-\epsilon/\lambda}$$

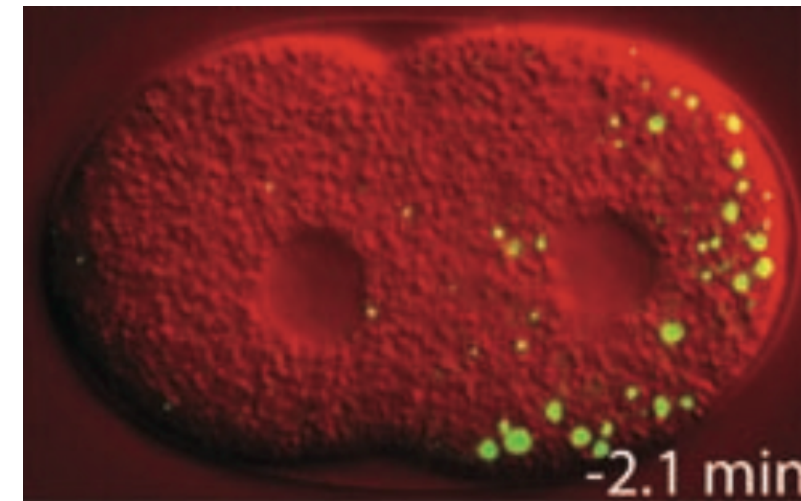
complex energy landscapes



Conclusions

Phase separation in the cell cytoplasm

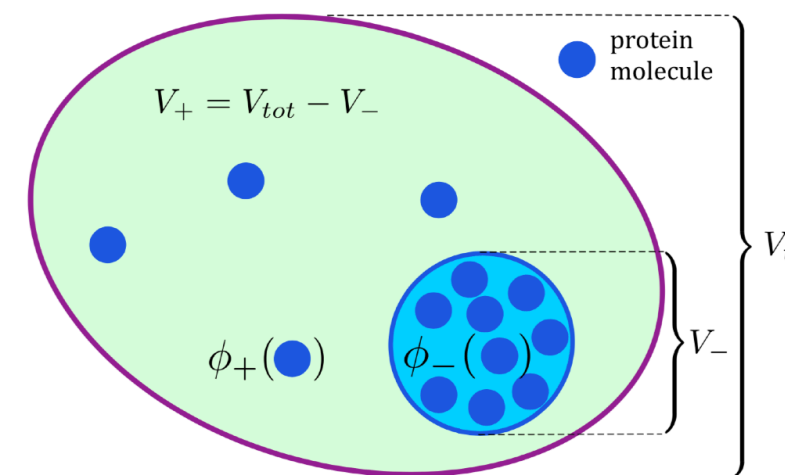
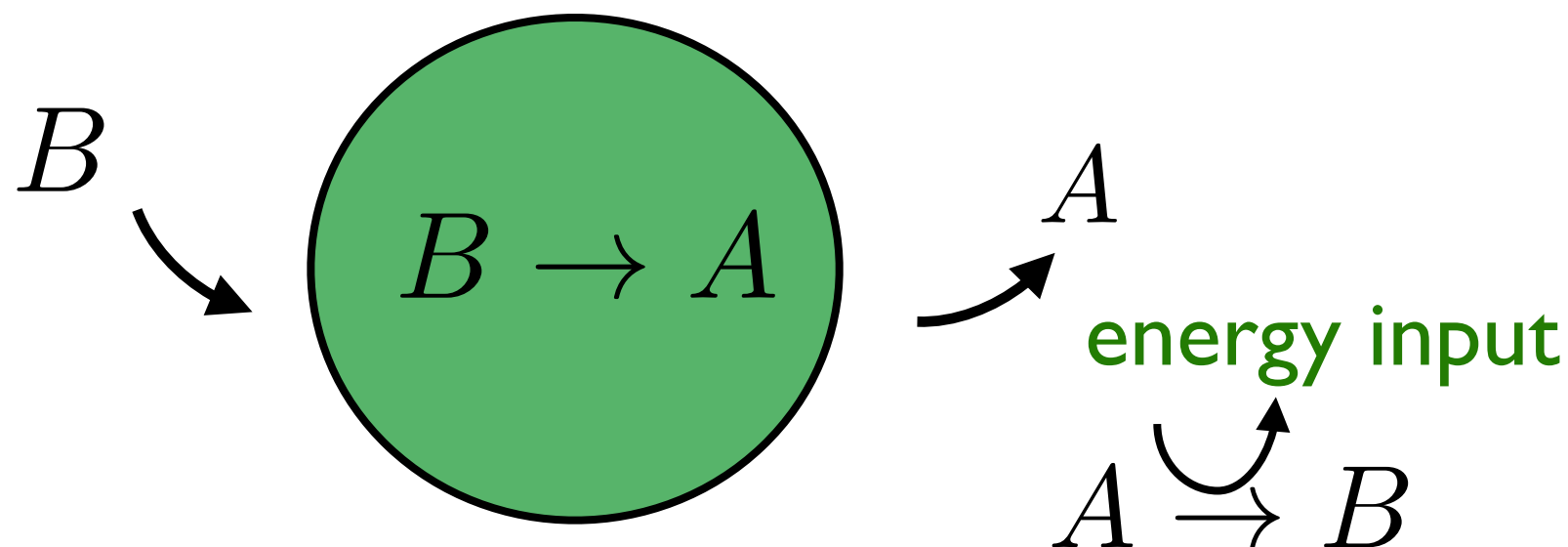
distinct chemical compartments in the cell
spatial organization of chemistry



P-granules: segregation by position
dependent supersaturation

Fluctuations: Buffering of concentration fluctuations

protocell? active droplets grow and divide



complex soft materials

Acknowledgements

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center for
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dresden

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