Multiresponsive Behavior of Membranes and Vesicles

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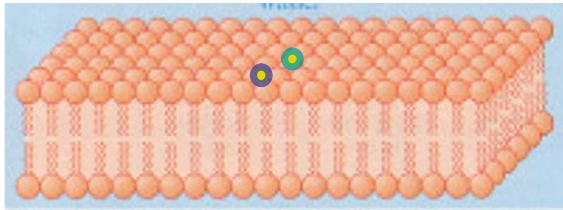
- Intro: Fluid Membranes and GUVs
- GUVs and Aqueous Two-Phase Systems
- Spontaneous Tubulation and Tension
- Morphological Complexity
- Wetting and Fluid-Elastic Scaffolding
- GUVs and Biomolecular Condensates
- Endocytosis of Nanodroplets
- Endocytosis of Nanoparticles
- Outlook: Droplet-Stabilized GUVs

Biomembranes are Fluid Bilayers

- Fluid membranes, i.e., fast lateral diffusion:

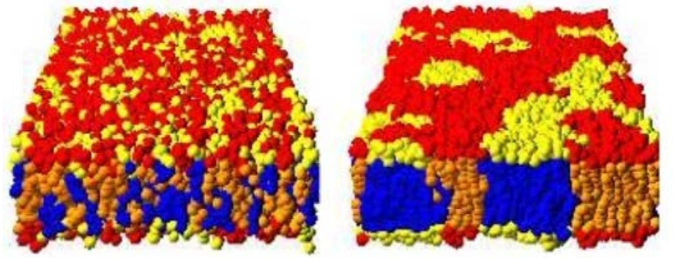
  Diffusion constant ~ $\mu m^2/s$



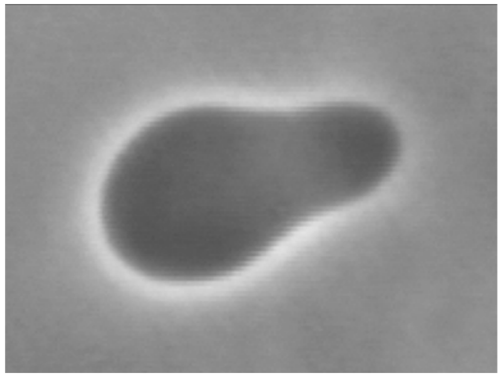
4 nm

lipid swapping ~ ns

- Lateral diffusion => Compositional responses, demixing, domain formation ...



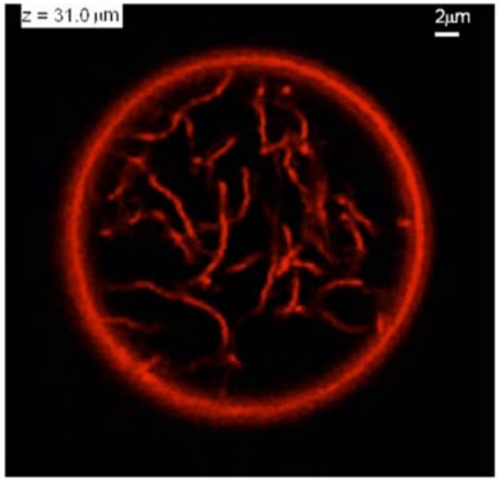
- Flexibility => Morphological responses, budding, tubulation, ... Direct evidence for fluidity



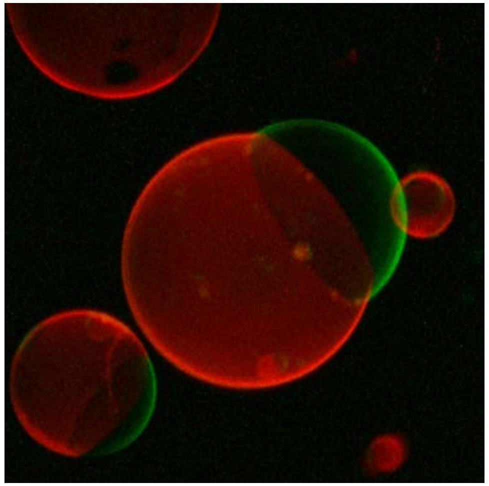
40 µm

Multiresponsive Behavior of GUVs

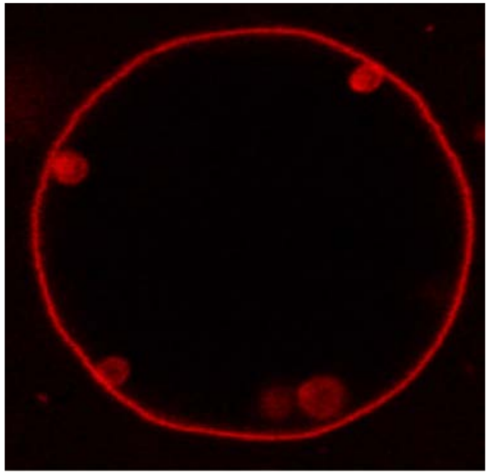
- Giant unilamellar vesicles (GUVs), tens of micrometers
- Remodelling in response to various perturbations:



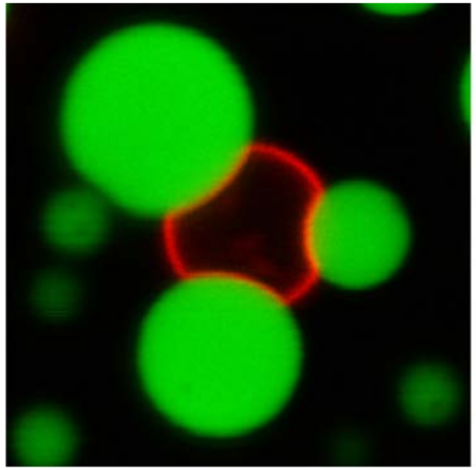
Nanotubes from polymer adsorption, tube width ~ 100 nm



Formation of intra-membrane domains, 2D phase separation



Small buds from adsorption of two ESCRT proteins



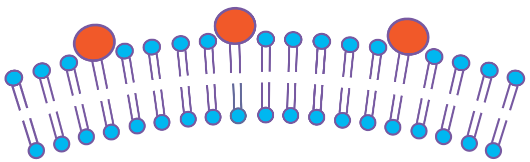
Shaping GUVs by biomolecular condensates

- What are the forces that drive remodelling processes?

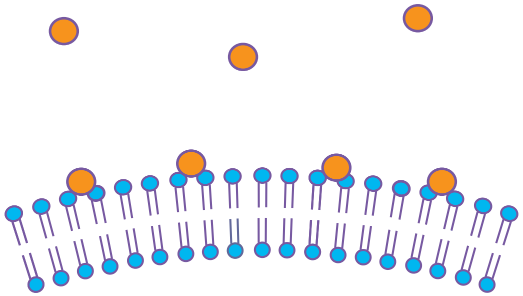
Spontaneous = Preferred Curvature

- Spontaneous or preferred curvature *m* describes
  bilayer asymmetry = asymmetry between two leaflets

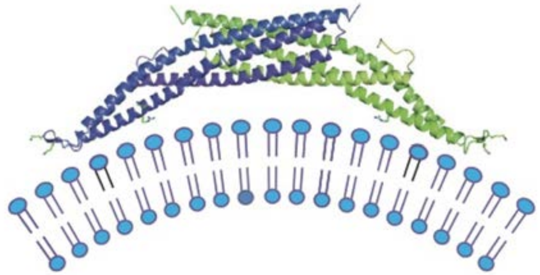
- Different molecular mechanisms for bilayer asymmetry:



Asymmetric
composition,
e.g., ganglioside



Asymmetric
adsorption of
small molecules

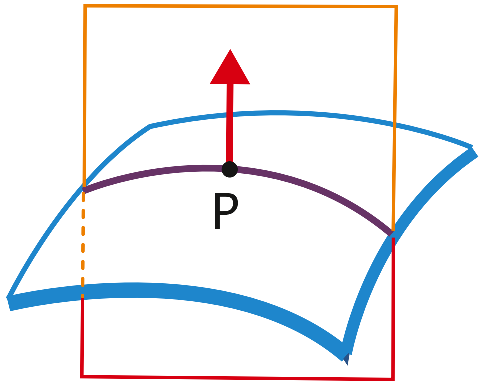


Asymmetric
protein coats,
e.g. BAR-domain

Curvature Elasticity

Helfrich, Z. Naturforschung (1973)

- Local mean curvature *M* tries to adapt to spontaneous (or preferred) curvature *m*



- Curvature or bending energy:

$$E_{cu} = \int dA \, 2 \, \kappa \, (M - m)^2$$

integral over membrane area *A*

- 2nd fluid-elastic parameter: Bending rigidity κ

  Dimensions of energy, $\kappa = 10^{-19}$ J = 20 k_B T

- Range of spontaneous curvatures *m*

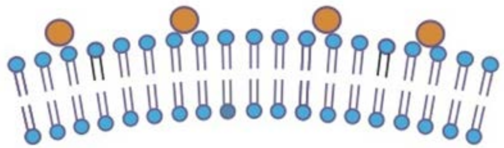
  from 1/(20 nm) to 1/(20 μm)

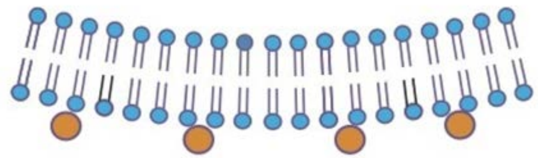
Sign of (Spontaneous) Curvature

- Mean curvature M and spontaneous curvature m can be positive or negative
- Sign defined with respect to interior/exterior compartments = with respect to inner/outer leaflet

exterior compartment — outer leaflet

$M > 0$





$M < 0$

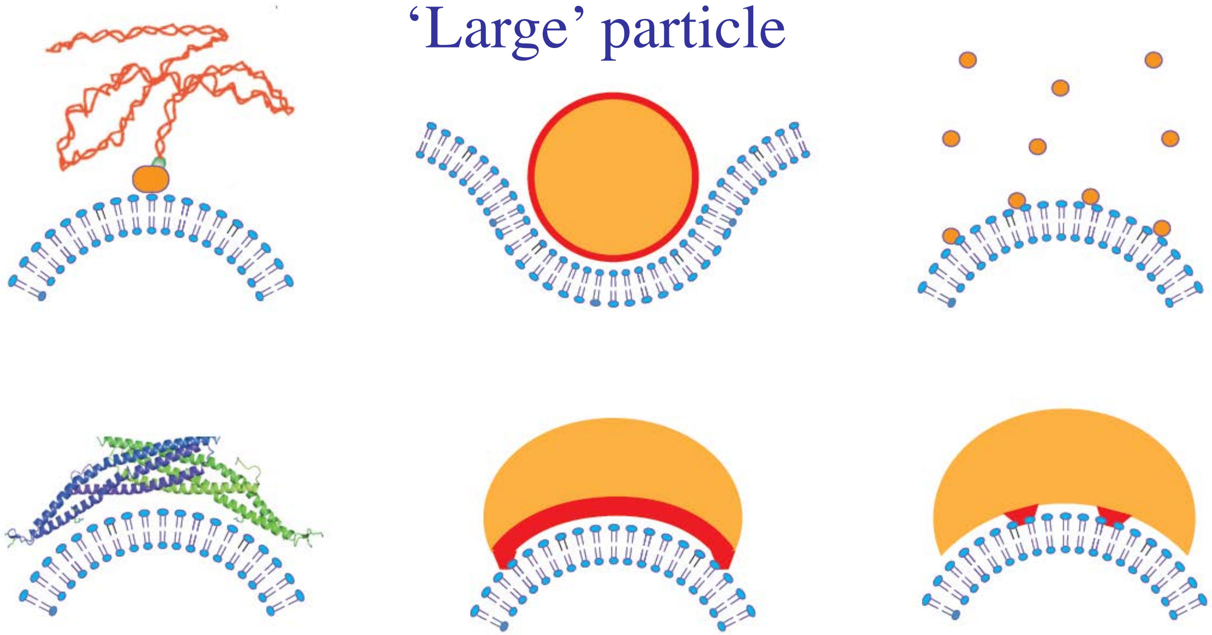
interior compartment — inner leaflet

Mean curvature M is positive (negative) if membrane bulges towards exterior (interior) compartment

Local Curvature Generation

RL, *Faraday Disc.* (2013); *Biol. Chem.* (2014)

- Local curvature generated on nanoscopic scales:



Spont Curv from Mol Simulations

Rozycki, RL, *J. Chem. Phys.* (2015); *J. Chem. Phys.* (2016)

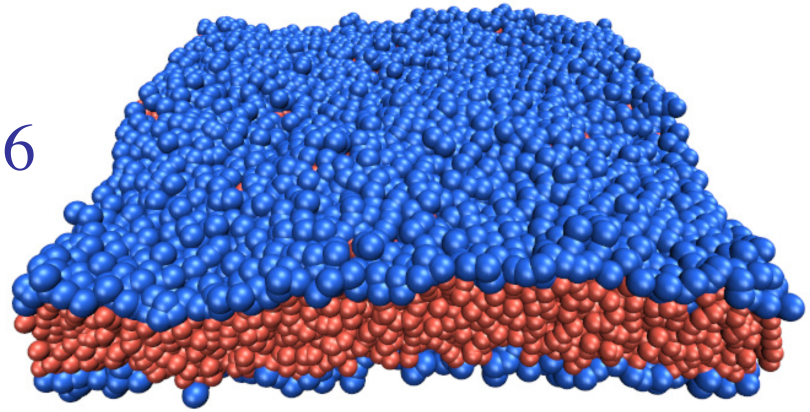
- Stress profiles and tension-free states
- Spont curv from first moment of stress profiles
- Example: Different leaflet densities

$\phi = 0.51$

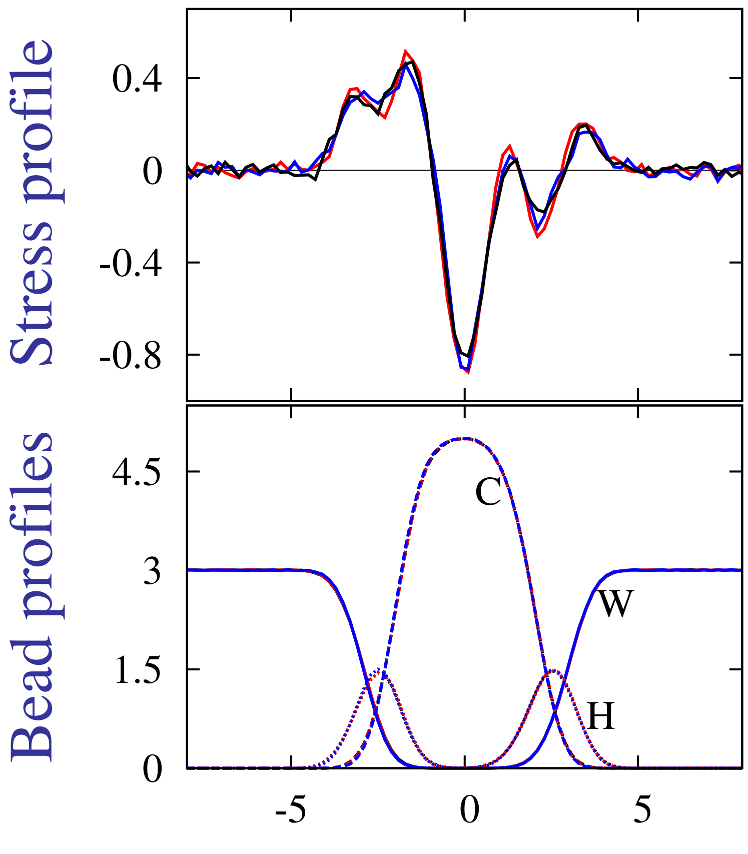
Asymmetry $\phi = N_{ex}/(N_{ex}+N_{in})$

outer leaflet with N_{ex} lipids

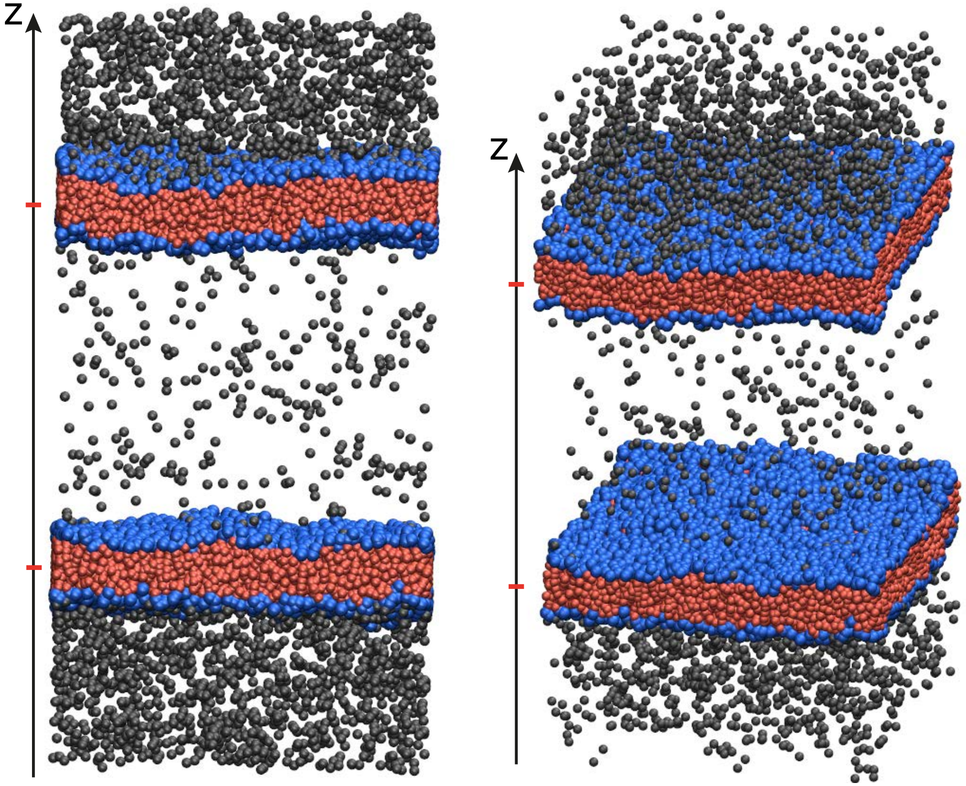
$\phi = 0.56$



inner leaflet with N_{in} lipids



Asymmetric Adsorption and Depletion



Particle concentration X_{ex}

Bilayer 1

Particle concentration X_{in}

Bilayer 2

Particle concentration X_{ex}

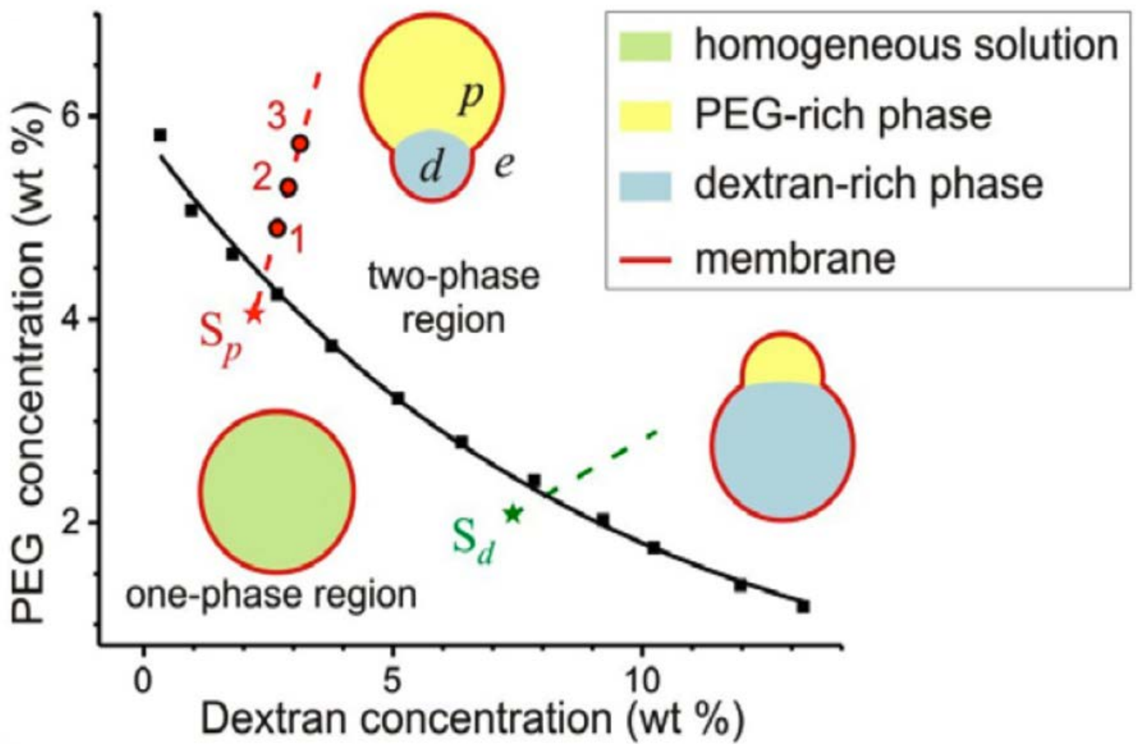
- Spont curv proportional to $\pm\ (X_{ex} - X_{in}) = \pm\ \Delta X$
- Example: 1 nm particles, $\Delta X = 100$ mM

Adsorption: $m = 1/(77\ \text{nm})$, Depletion: $m = -\ 1/(270\ \text{nm})$

GUVs + Aqueous Phase Separation

Li, RL, Dimova, *JACS* (2008); *PNAS* (2011)
Liu, Agudo. ... RL, *ACS Nano* (2015)

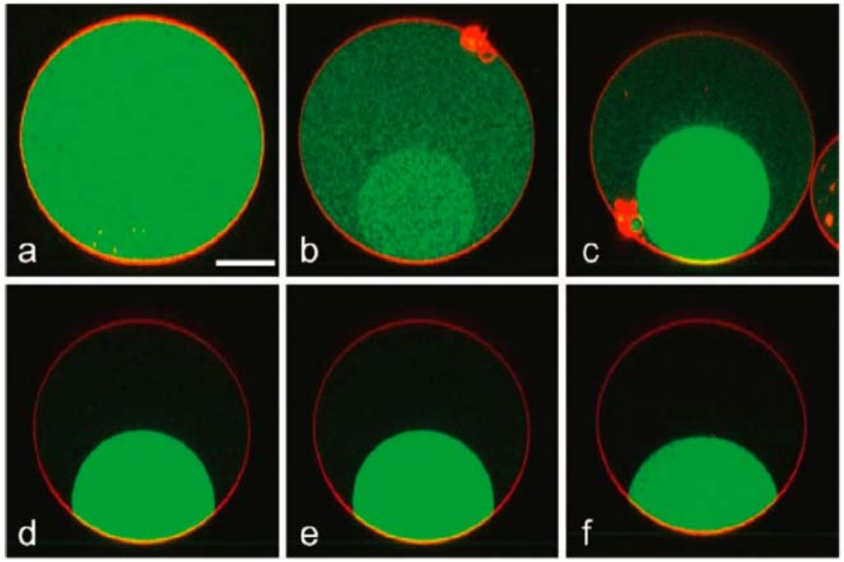
- GUVs filled with aqueous polymer solution
- Example: PEG and dextran
- Increase polymer concentration via deflation:

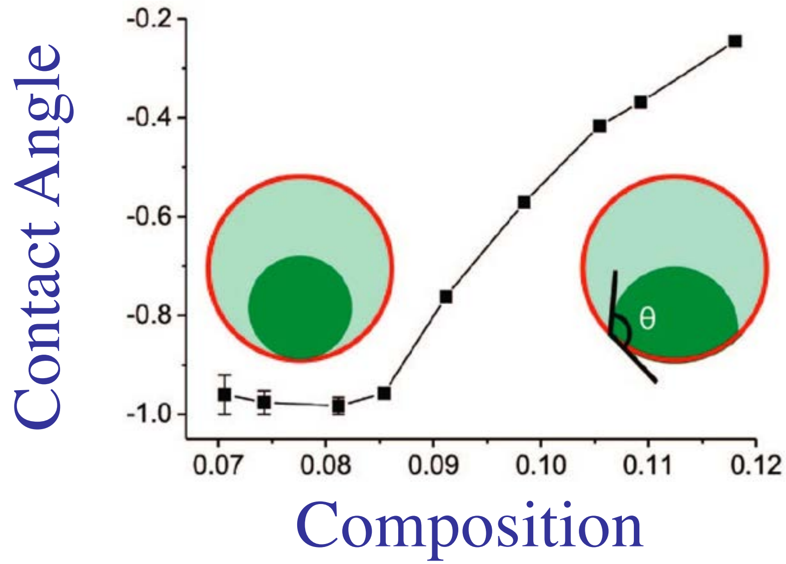


1^{st} Surprise: Wetting Transition

Li et al, *JACS* (2008)

- Shape evolution for vesicle during deflation:



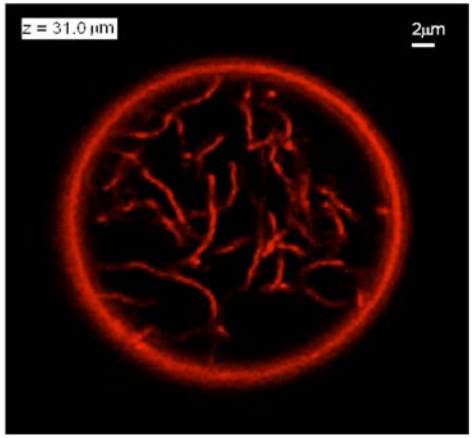


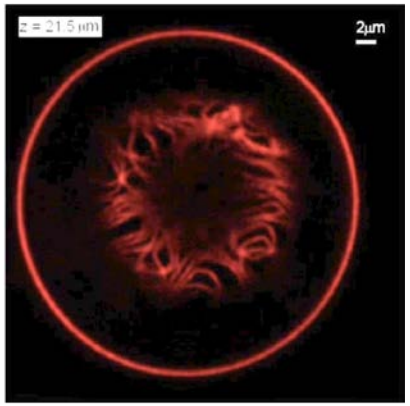
- Low deflation: zero contact angle => complete wetting
- High deflation: finite contact angle => partial wetting

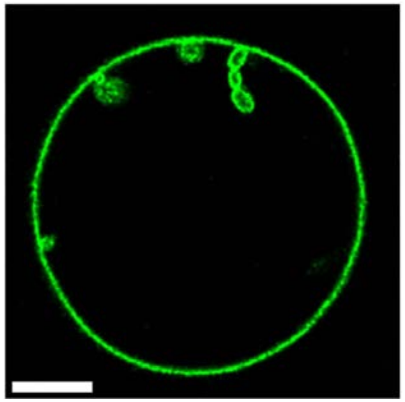
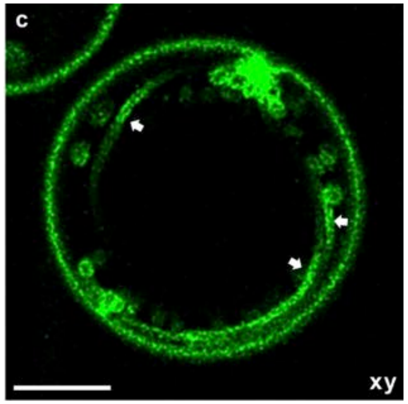
2nd Surprise: Membrane Nanotubes

Li et al, *PNAS* (2011)   Liu et al, *ACS Nano* (2016)

- Membranes labeled by fluorescent dyes
- Lipid mixture of DOPC, DPPC, cholesterol
- Liquid-disordered (red) and liquid-ordered phase (green)





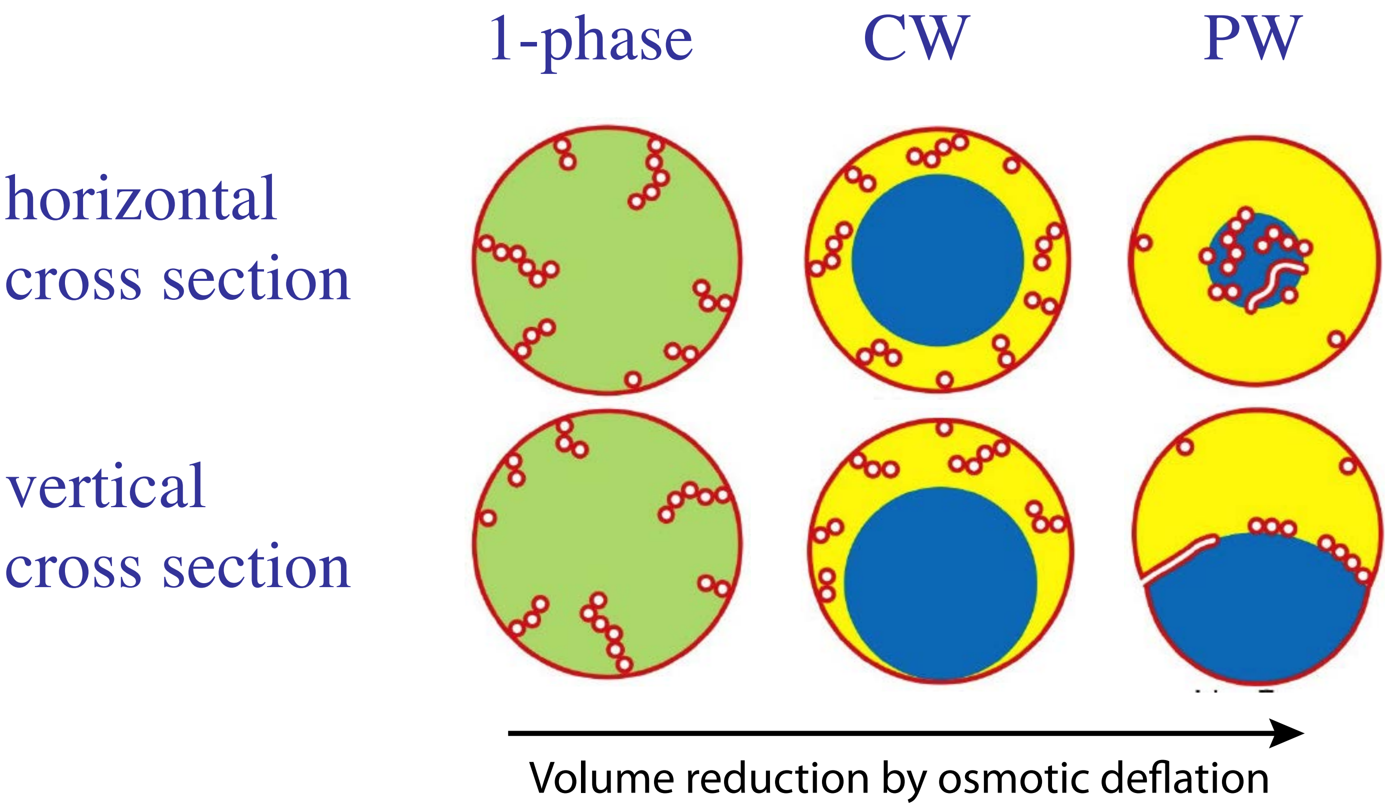



- Spontaneous tube formation without external forces
- Inward-pointing tubes reveal large negative spont curv
- Tubes can be necklace-like or cylindrical

Tubulation and Wetting

Liu, Agudo ... RL, *ACS Nano* (2015)

- Nanotubes form for different wetting morphologies:



- Tubulation does not require aqueous phase separation

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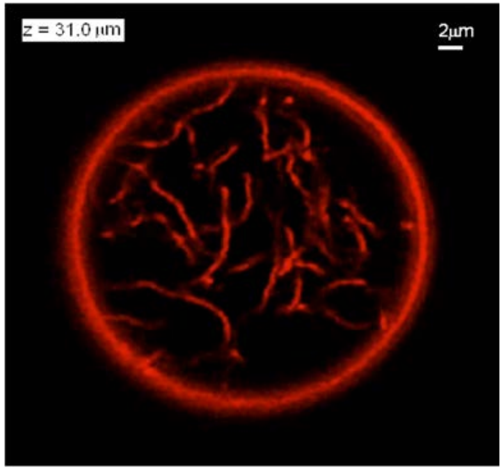
Spont Tubulation and Tension

RL, *Faraday Discuss*. (2013)



- Tubulation leads to tense mother vesicle
- Total tension in Euler-Lagrange equation has two components:

$$\hat{\Sigma} = \Sigma + \sigma$$

Mechanical tension Σ stretches the membrane

Spontaneous tension $\sigma = 2\kappa m^2$ for $M << m$

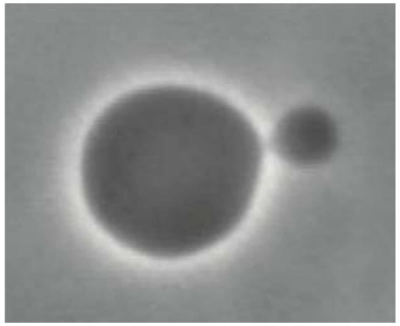
- Presence of nanotubes implies dominance of spontaneous tension, mechanical tension can be ignored
- Example: Spont curvature $\approx -1/(100 \text{ nm})$

Spontaneous tension $\sigma \approx 10^{-2}$ mN/m

Mechanical tension $\Sigma \approx 10^{-4}$ mN/m

How Do Nanotubes Form?

Liu et al, *ACS Nano* (2016)

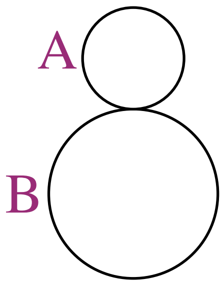


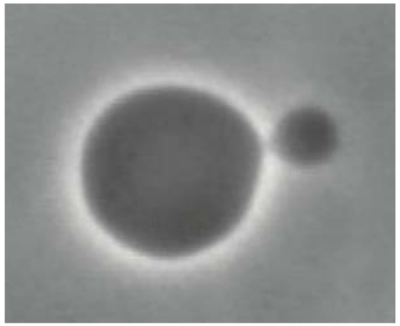
- Tubulation intimately related to budding
- Osmotic deflation of spherical GUV
- Small deflation step leads to single bud
- Bud and mother vesicle connected by membrane neck
- Bud acts as nucleation site for necklace-like tube
- Several pathways for subsequent deflation steps:
  - Formation of new bud
  - Bud into 2-necklace
  - N-necklace into (N+1)-necklace

Membrane Buds and Necks

- For $m \neq 0$, curvature elasticity leads to spherical membrane segments connected by membrane necks

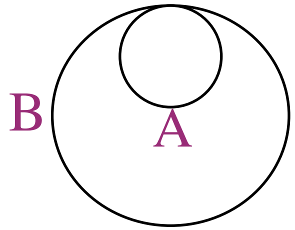
- Out-bud:

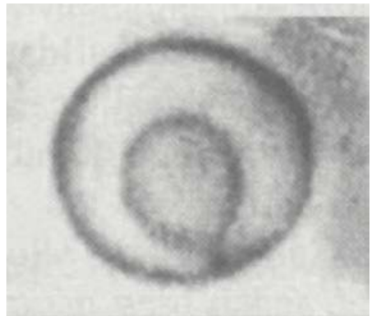




spont curv $m > \sqrt{2}/R_{ve}$

- In-bud:





spont curv $m < 0$

Seifert et al, *PRA* (1991)

- Closed neck is stable if:

$$0 < M^A + M^B \leq 2\,m$$

$$2\mathrm{m} \leq M^A + M^B < 0$$

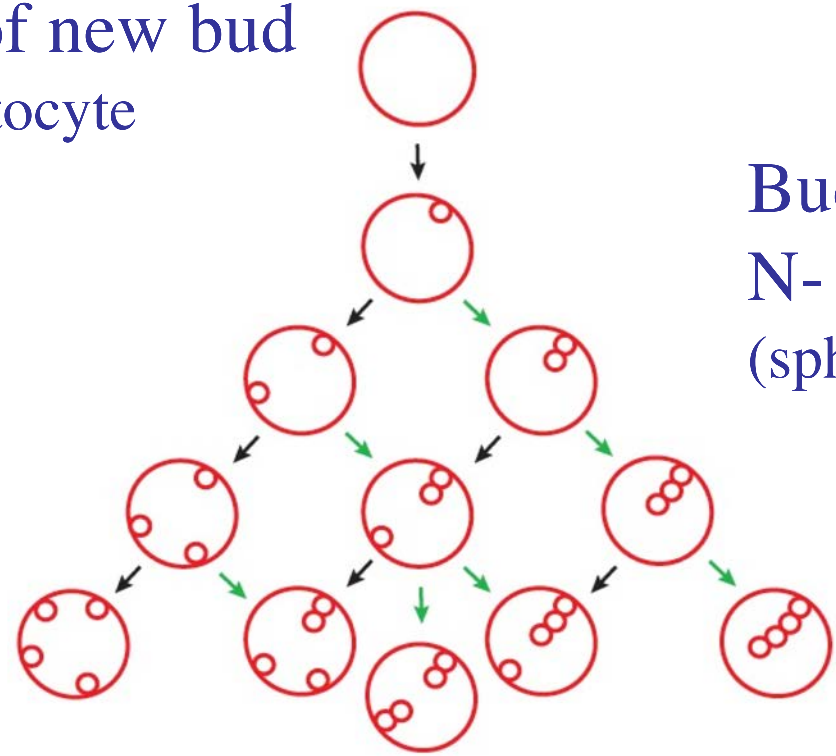
- Simple relation between geometry and material parameter

Nucleation and Growth of Tubes

Liu et al, *ACS Nano* (2016)
RL, *J. Phys. D* (in press)

- Spherical GUV, large spont curv m
- Osmotic deflation of GUV in discrete steps
- At each step, different morphological pathways:

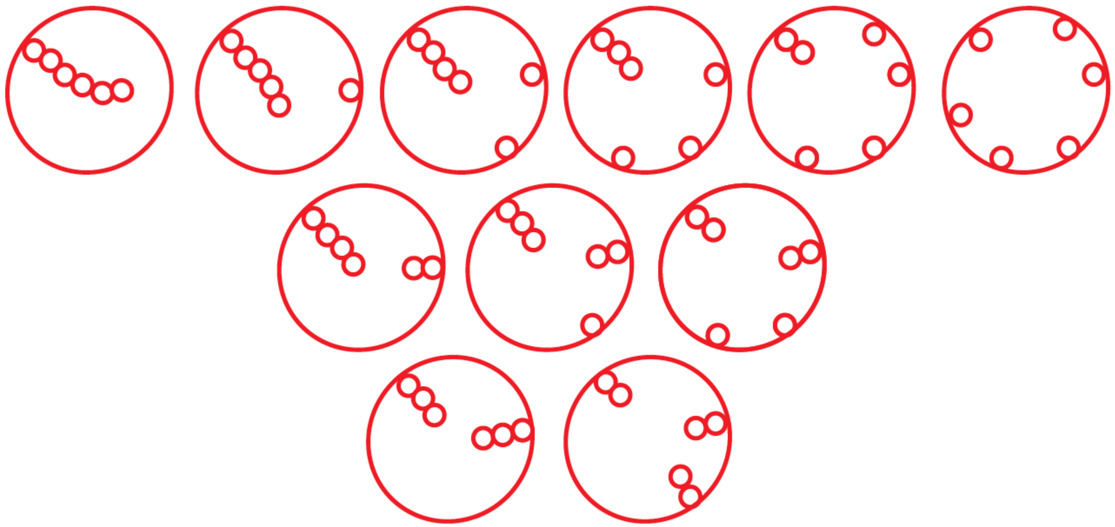
Formation of new bud
(oblate-stomatocyte bifurcation)



Bud into 2-necklace
N- into (N+1)-necklace
(sphere-prolate bifurcation)

Morphological Complexity: Theory

- After 6th step, 11 morphologies with 6 spherules:



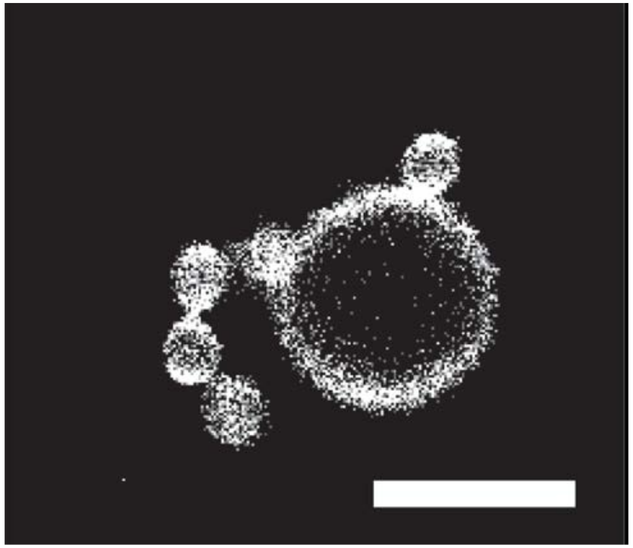
- All beads are connected by membrane necks
- All morphologies have the same area, volume, and curvature energy
- Rugged energy landscape contains 11 intersecting branches
- For large N, number of N-spherule morph grows as $\exp[c\sqrt{N}]$

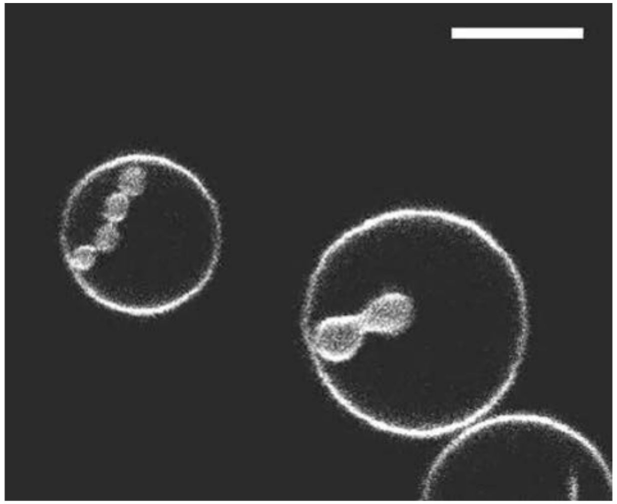
Morph Complexity: Experiment

Tripta Bhatia (unpublished)

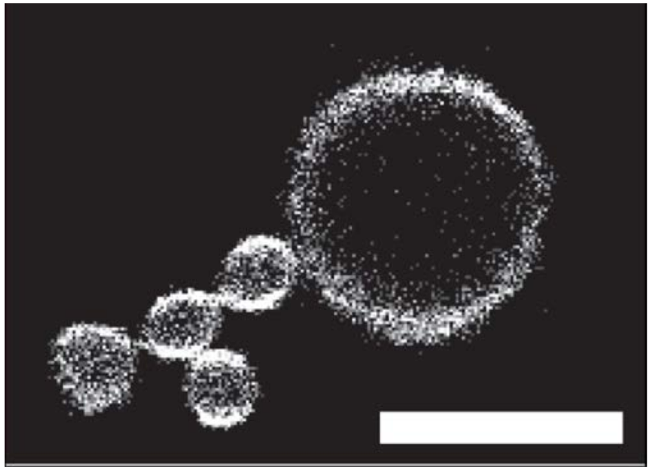
- Out-Necklaces
- In-Necklaces

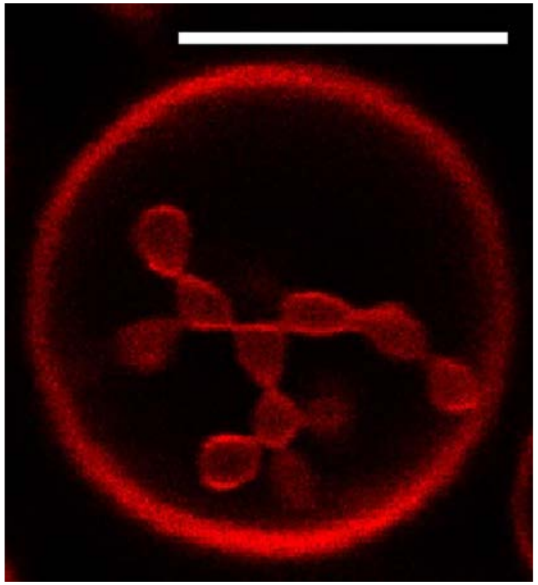
Linear





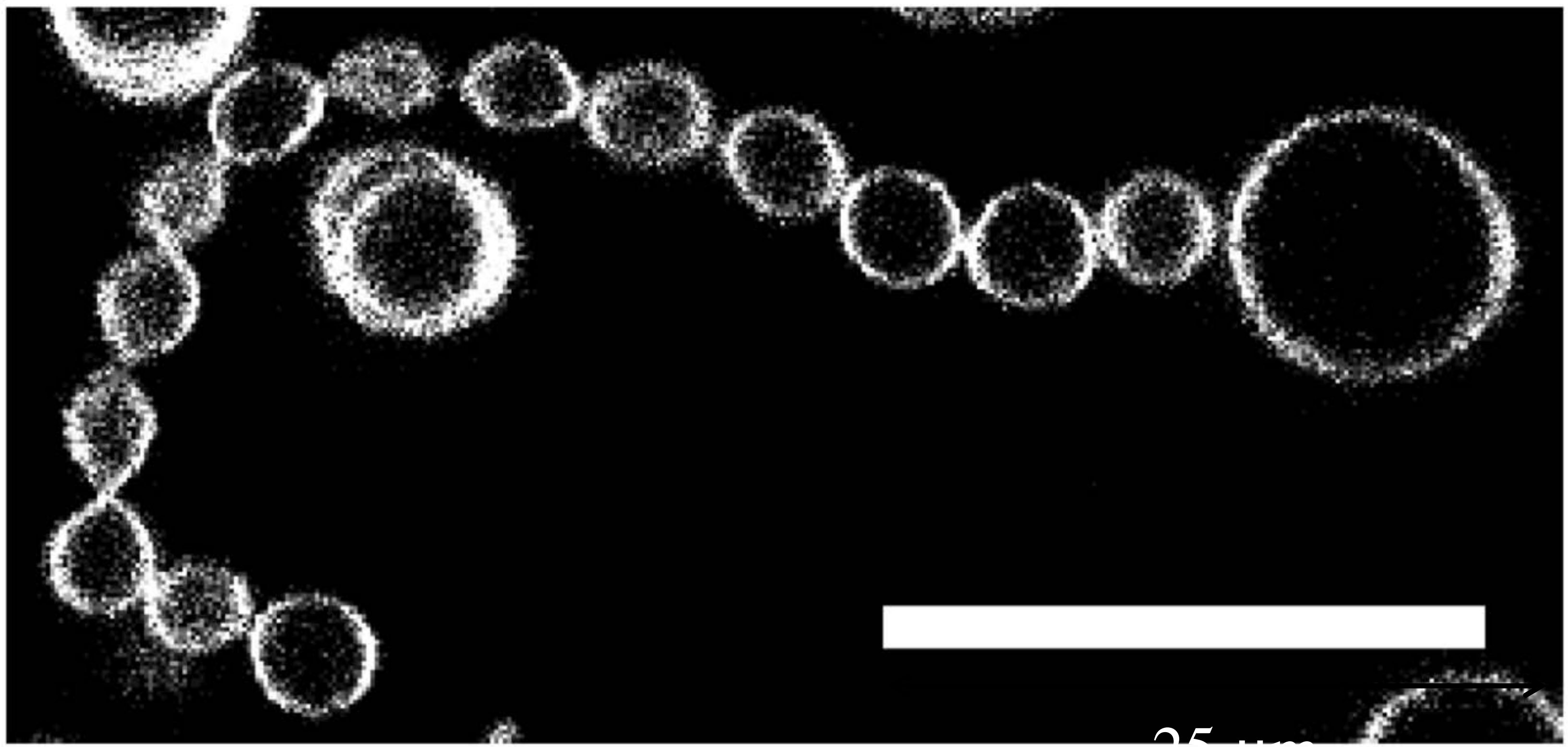
Branched





Long Necklaces

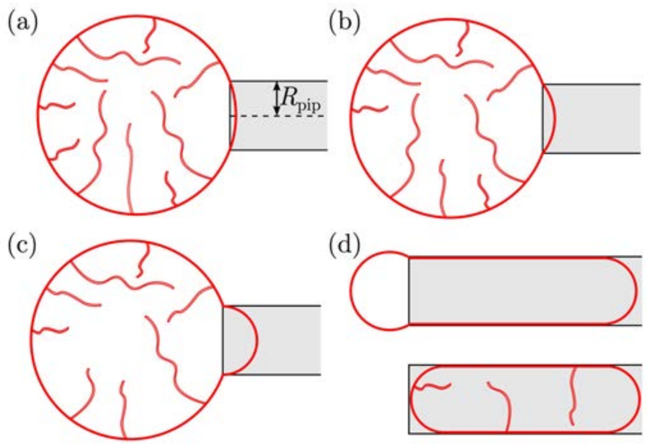
- Example: Out-Necklace with 14 beads



- Initial deflation leads to one bud
- Bud grows into long necklace

Nanotubes Increase Robustness of GUVs

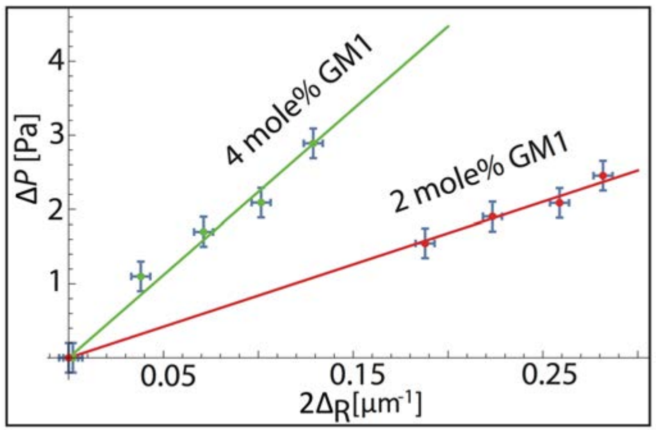
- Retraction of tubes by micropipettes: Bhatia et al, *ACS Nano* (2018)



Initial aspiration up to hemispherical tongue

then vesicle starts to flow into micropipette, increased robustness !

Suction pressure



Initial aspiration:

Aspiration pressure versus geometric quantity Δ_R

Slope = spontaneous tension σ

- Tubulated GUV behaves like a liquid droplet
- Interfacial tension = spontaneous tension

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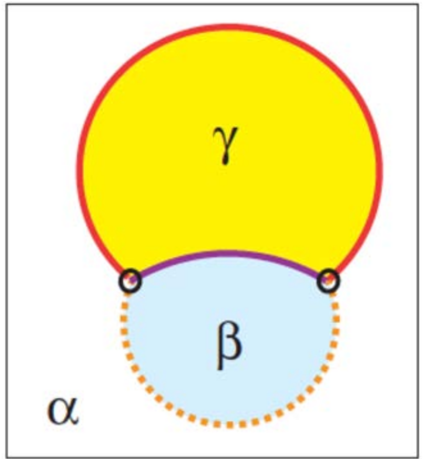
Membranes and Droplets

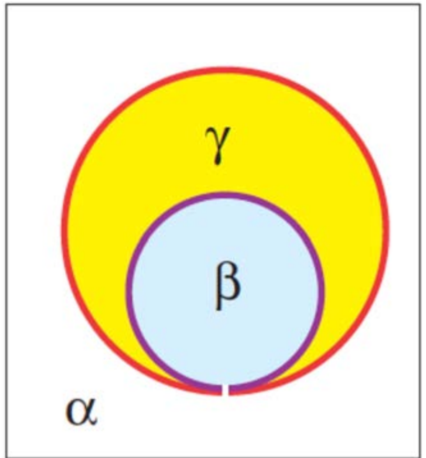
RL, *J. Chem. Phys. B* (2018)

- In-wetting: Droplets at inner leaflet
- Out-wetting: Droplets at outer leaflet
- Droplet view: Wetting morphologies
- Membrane view: Fluid-elastic scaffolding



Example 1

- Ex 1: Partial wetting, contact angles
- Ex 1: Capillary forces and deformations
- Ex 2: Complete wetting by γ phase
- Ex 2: Endocytosis of β droplet

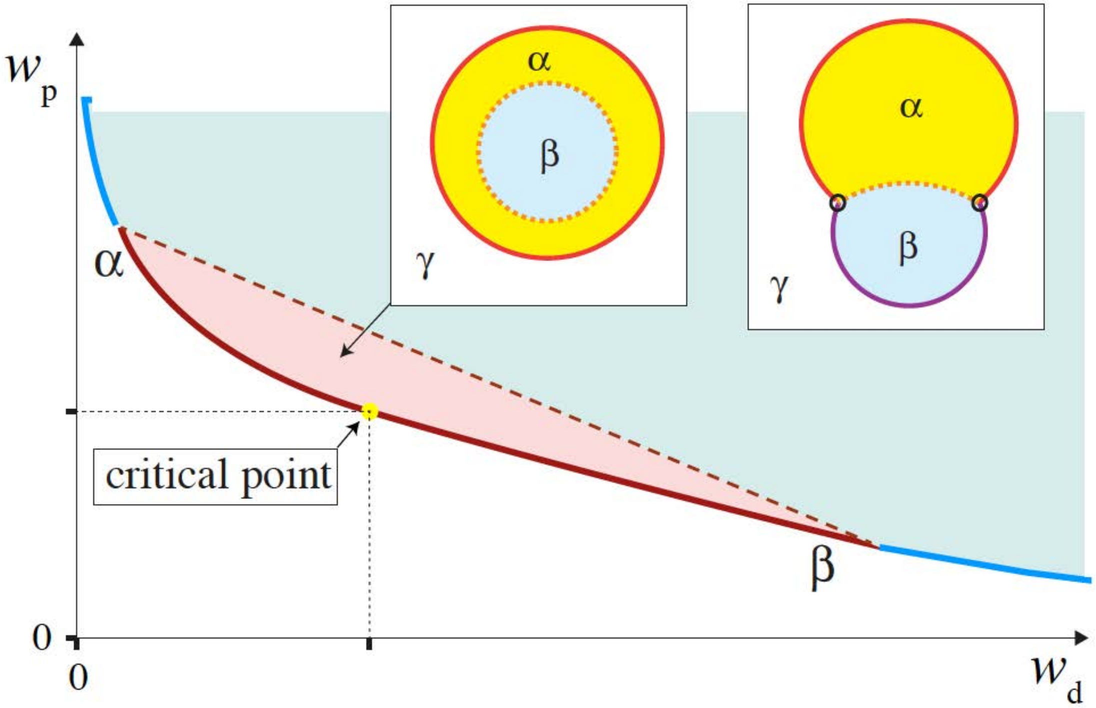


Example 2

Phase Diagram for PEG + Dextran

Liu et al, *Langmuir* (2012)

- Phase diagram of α = PEG and β = dextran:

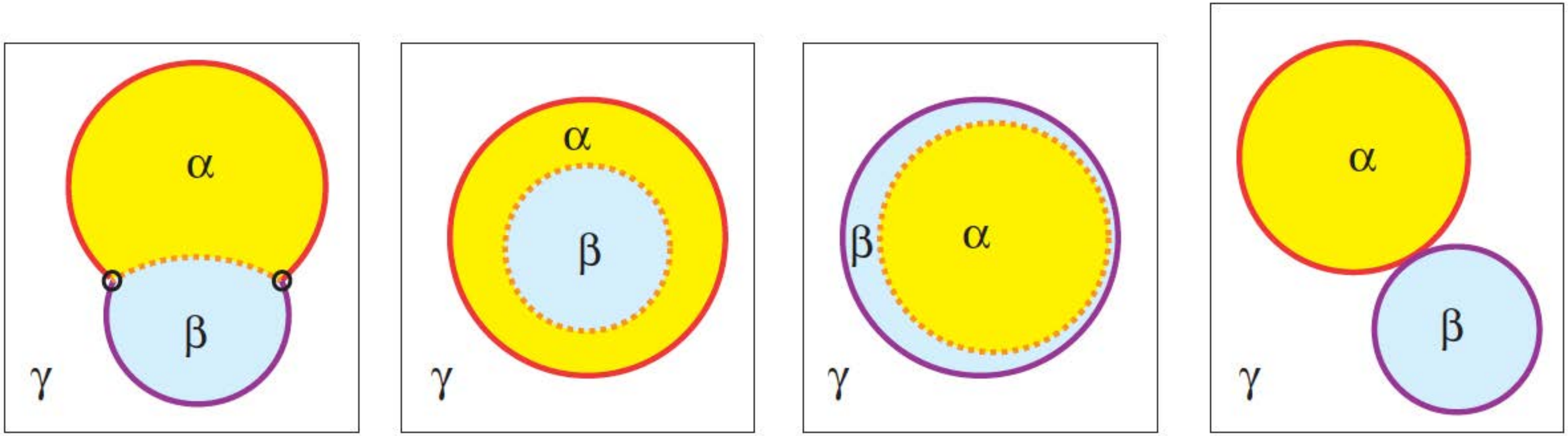


coexistence region
= two subregions:
pink: complete wetting
blue: partial wetting

- For all lipid compositions, same ordering of complete and partial wetting subregions
- Partial-to-complete wetting transition at boundary tie line

In-Wetting Morphologies

- Three aqueous phases α, β, γ
- Phase coexistence of α and β, γ is ext spectator phase
- GUV membrane encloses α and β



Partial wetting by α and β

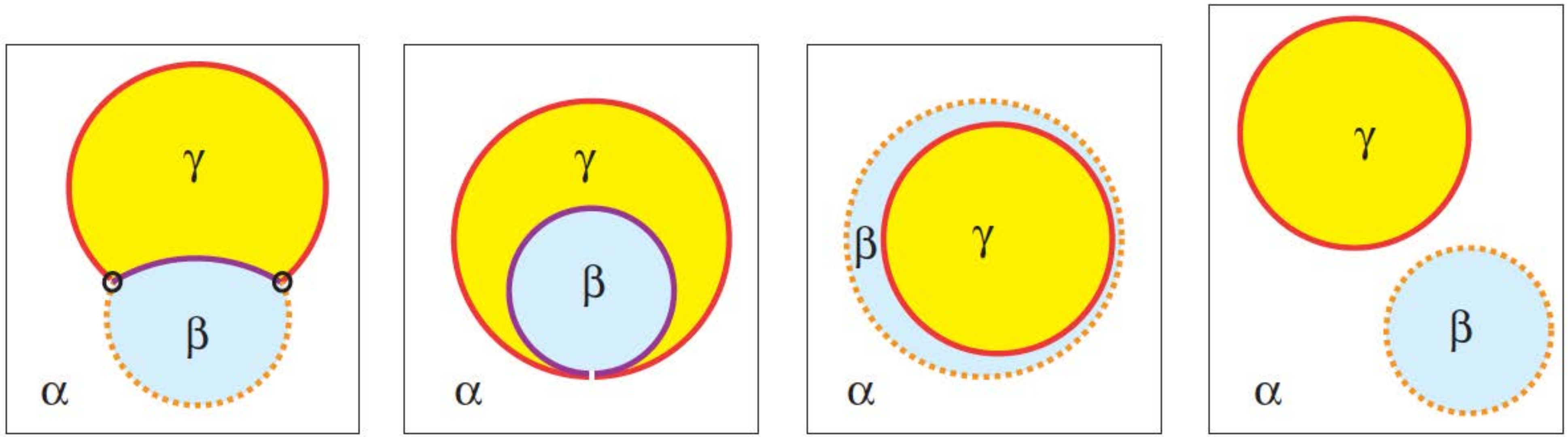
Complete wetting by α

Complete wetting by β

Complete wetting by γ, Membrane neck

Out-Wetting Morphologies

- Three aqueous phases α, β, γ
- Phase coexistence of α and β in exterior solution
- GUV membrane encloses spectator phase γ



Partial wetting by α and β

Complete wetting by γ, Membrane neck

Complete wetting by β

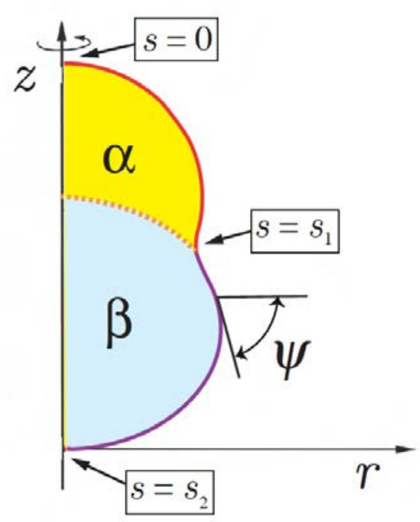
Complete wetting by α, Dewetting

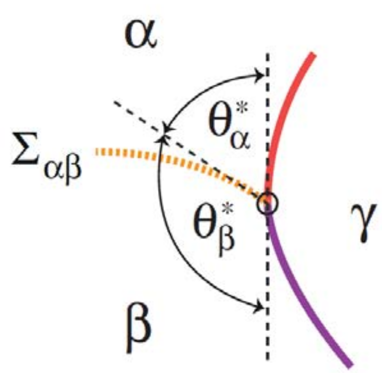
Nanoscopic Scales

RL, *J. Chem. Phys. B* (2018)

- Shapes with smooth bends and no kinks
- Axisymmetric shapes, energy minimization:

arc length s
contact line
at $s = s_1$





common tangent at contact line, two intrinsic contact angles $\theta_\alpha{}^*$ and $\theta_\beta{}^*$ with

$$\theta_\alpha{}^* + \theta_\beta{}^* = \pi$$

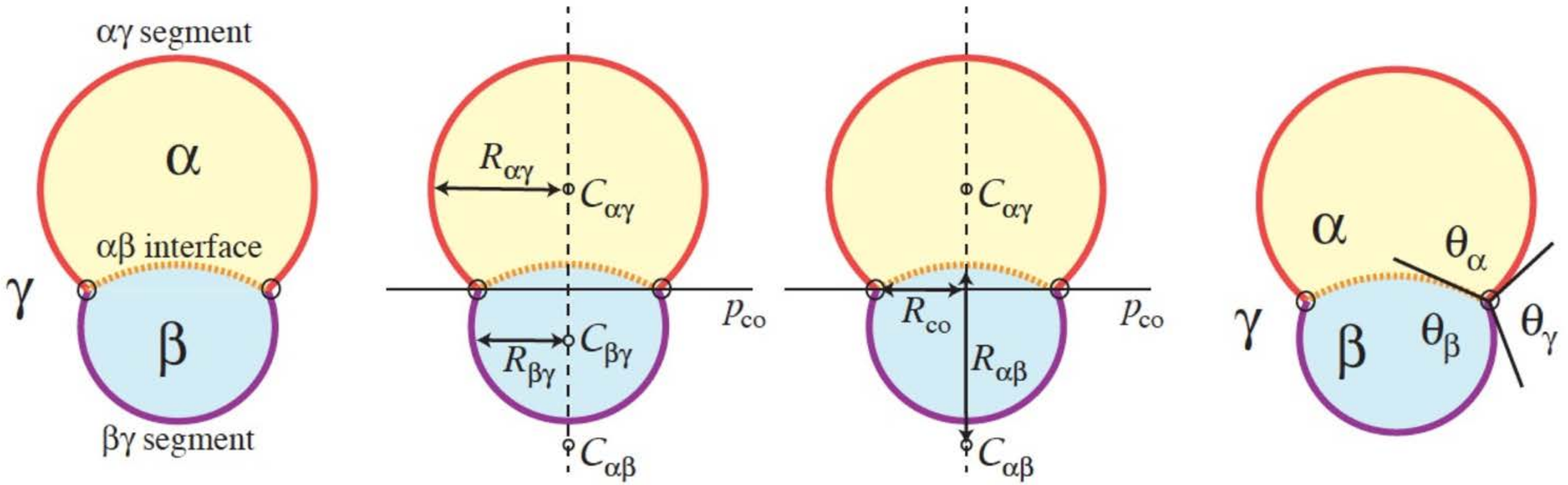
- Complex matching conditions along contact line
- Simplification if $\alpha\gamma$ and $\beta\gamma$ segments have identical curvature elastic properties

Kusumaatmaja et al, *Phys. Rev. Lett.* (2009)

Mesoscopic Scales

RL, *J. Chem. Phys. B* (2018)

- Experimental shapes consist of three spherical caps
- Apparent contact angles θ_α, θ_β, and θ_γ with $\theta_\alpha + \theta_\beta + \theta_\gamma = 2\pi$
- Geometry of three spherical caps, common contact line



- Shape determined by four radii $R_{\alpha\gamma}$, $R_{\beta\gamma}$, $R_{\alpha\beta}$, and R_{co} and by positions of three sphere centers $C_{\alpha\gamma}$, $C_{\beta\gamma}$, and $C_{\alpha\beta}$

Effective Membrane Tensions

- Shape equations for mean curvatures $M = \pm\ 1/R$
- Balance between pressures and tensions:

$$P_\alpha - P_\beta = 2\Sigma_{\alpha\beta} M_{\alpha\beta} \qquad\qquad P_j - P_\gamma = 2\,\Sigma^{\text{eff}}_{j\gamma}\, M_{j\gamma}$$

- Effective membrane tension:

$$\Sigma^{\text{eff}}_{j\gamma} \equiv \Sigma + W_{j\gamma} + \sigma_{j\gamma} - 2\kappa_{j\gamma} m_{j\gamma} M_{j\gamma}$$

- Overall lateral stress Σ from tensile forces
- Adhesive strength W from attractive molecular forces
- Spontaneous tension σ from bilayer asymmetry
- Curvature dependent term

Tensions and Angles

RL, *J. Chem. Phys. B* (2018)

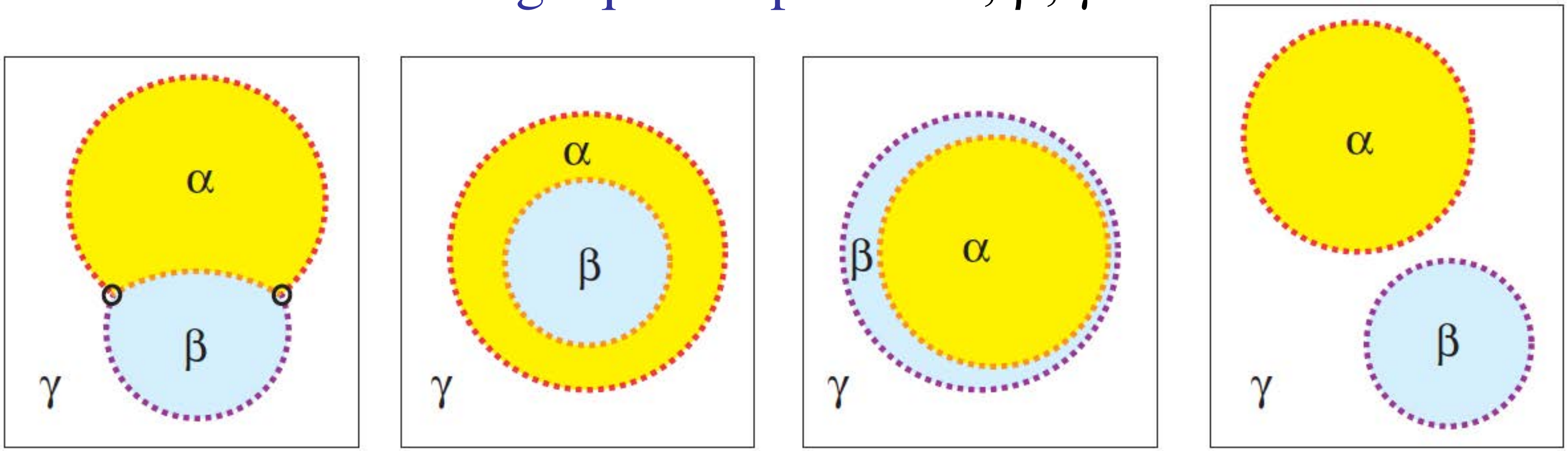
- Combine three shape equations with geometric relation
- Eliminate mean curvature $M_{\alpha\beta}$
- Relation between effective tensions and contact angles:

$$M_{\alpha\gamma}\left(\frac{\Sigma_{\alpha\gamma}^{\text{eff}}}{\Sigma_{\alpha\beta}}-\frac{\sin\theta_\beta}{\sin\theta_\gamma}\right)=M_{\beta\gamma}\left(\frac{\Sigma_{\beta\gamma}^{\text{eff}}}{\Sigma_{\alpha\beta}}-\frac{\sin\theta_\alpha}{\sin\theta_\gamma}\right)$$

- Relation depends on mean curvatures $M_{\alpha\gamma}$ and $M_{\beta\gamma}$
- Contact angles can be measured directly
- Complex parameter dependence via effective tensions

Droplets without Membrane

- Three coexisting aqueous phases α, β, γ



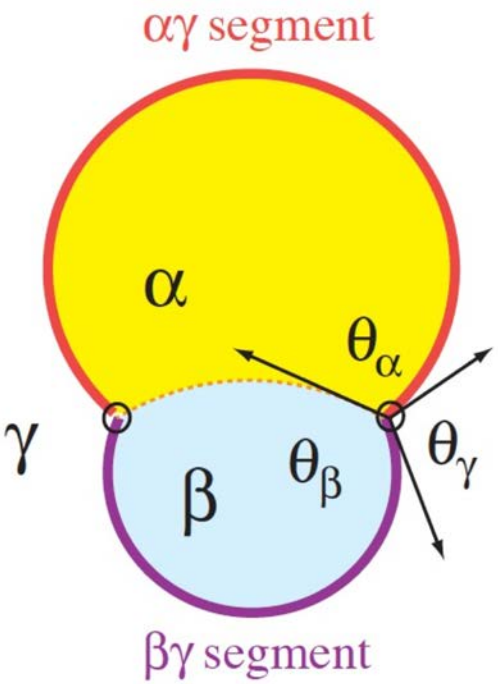
- Similar relation:

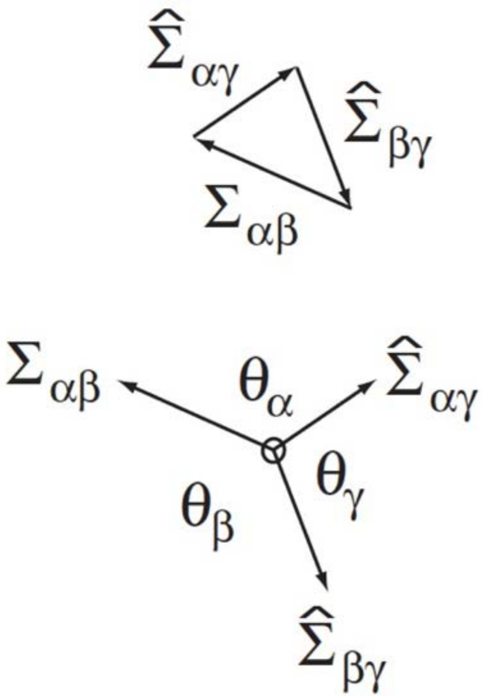
$$M_{\alpha\gamma}\left(\frac{\Sigma_{\alpha\gamma}}{\Sigma_{\alpha\beta}}-\frac{\sin\theta_\beta}{\sin\theta_\gamma}\right)=M_{\beta\gamma}\left(\frac{\Sigma_{\beta\gamma}}{\Sigma_{\alpha\beta}}-\frac{\sin\theta_\alpha}{\sin\theta_\gamma}\right)$$

- But interfacial tensions Σ_{ij} are material parameters
- Expressions in two parentheses vanish separately

Special Parameter Regimes

- Each segment has small or large spont curvatur
- Balance of tensions at apparent contact line:





Total segment tensions

$$\hat{\Sigma}_{j\gamma} = \Sigma_{j\gamma} + \sigma_{j\gamma}$$

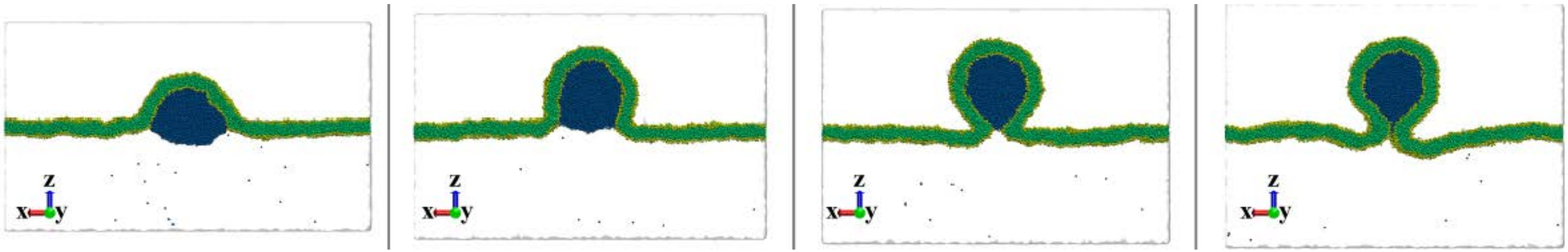
Sum of mechanical and spontaneous tensions

- Balance relations: $$\frac{\hat{\Sigma}_{\alpha\gamma}}{\Sigma_{\alpha\beta}} = \frac{\sin\theta_\beta}{\sin\theta_\gamma} \quad \text{and} \quad \frac{\hat{\Sigma}_{\beta\gamma}}{\Sigma_{\alpha\beta}} = \frac{\sin\theta_\alpha}{\sin\theta_\gamma}$$

Scaffolding Mechanisms

Scaffolding mechanisms and parameters:

- Tensile forces generate overall lateral stress Σ
- Adhesive strength $W < 0$ creates contact area
- Interfacial tension $\Sigma_{\alpha\beta}$ generates capillary forces
- Endocytosis: larger contact area + smaller interfacial area



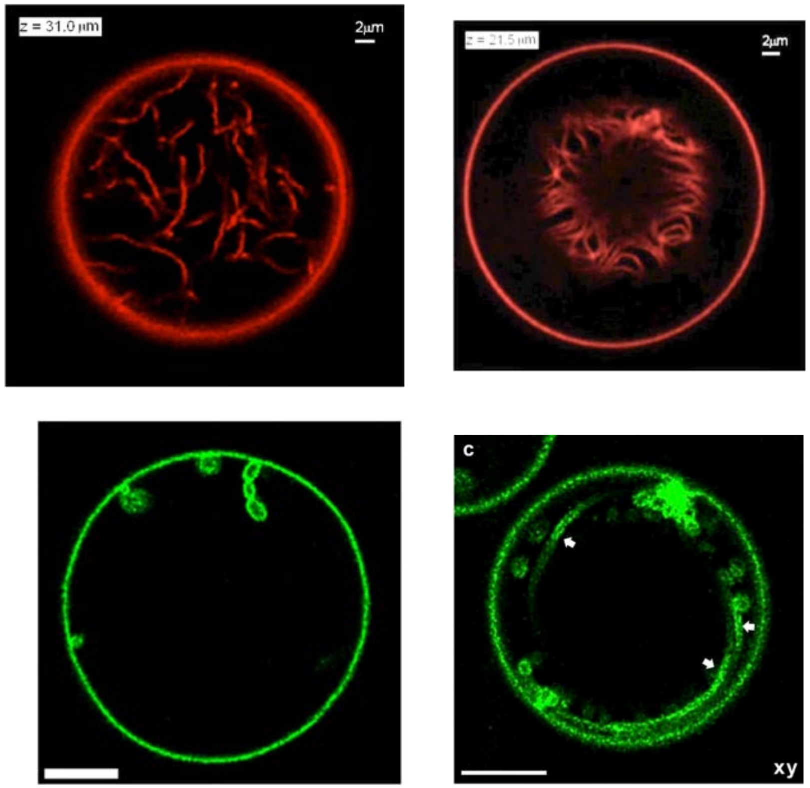
Vahid Satarifard (unpublished)

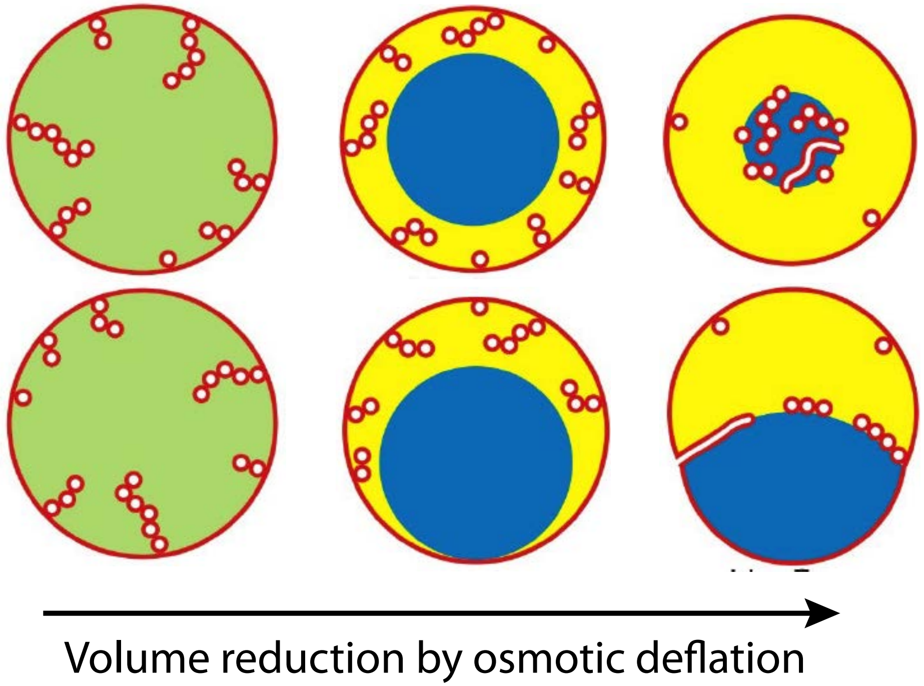
- Droplet generates bilayer asymmetry and spont curv

Droplet-Induced Curvature

Liu, Agudo ... RL, *ACS Nano* (2015)

- Tubulation of membrane segment in contact with PEG-rich phase:

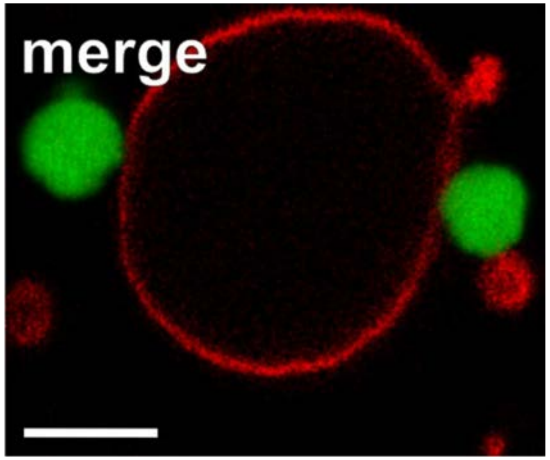




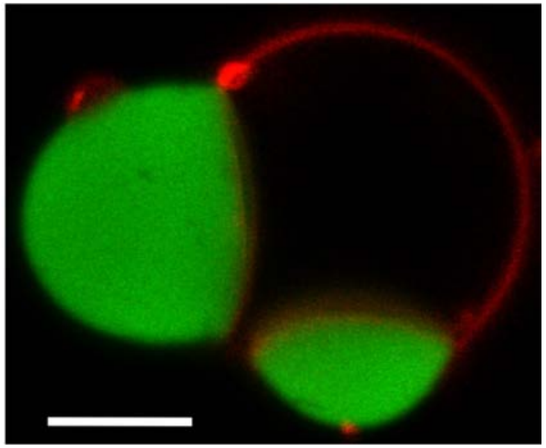
Biomolecular Condensates

Brangwynne ... Hyman, *Science* (2009)

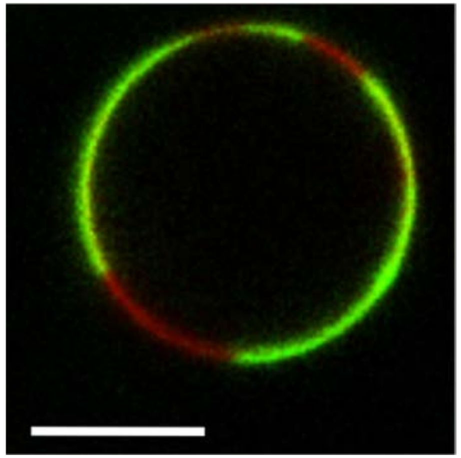
- Biomolecular condensates that behave like liquid droplets
- Enriched in intrinsically disordered proteins (IDPs)
- Example for IDP: RNA-binding protein FUS
- Interaction of FUS-droplets with GUVs, two subsequent wetting transitions:



dewetting for high salt



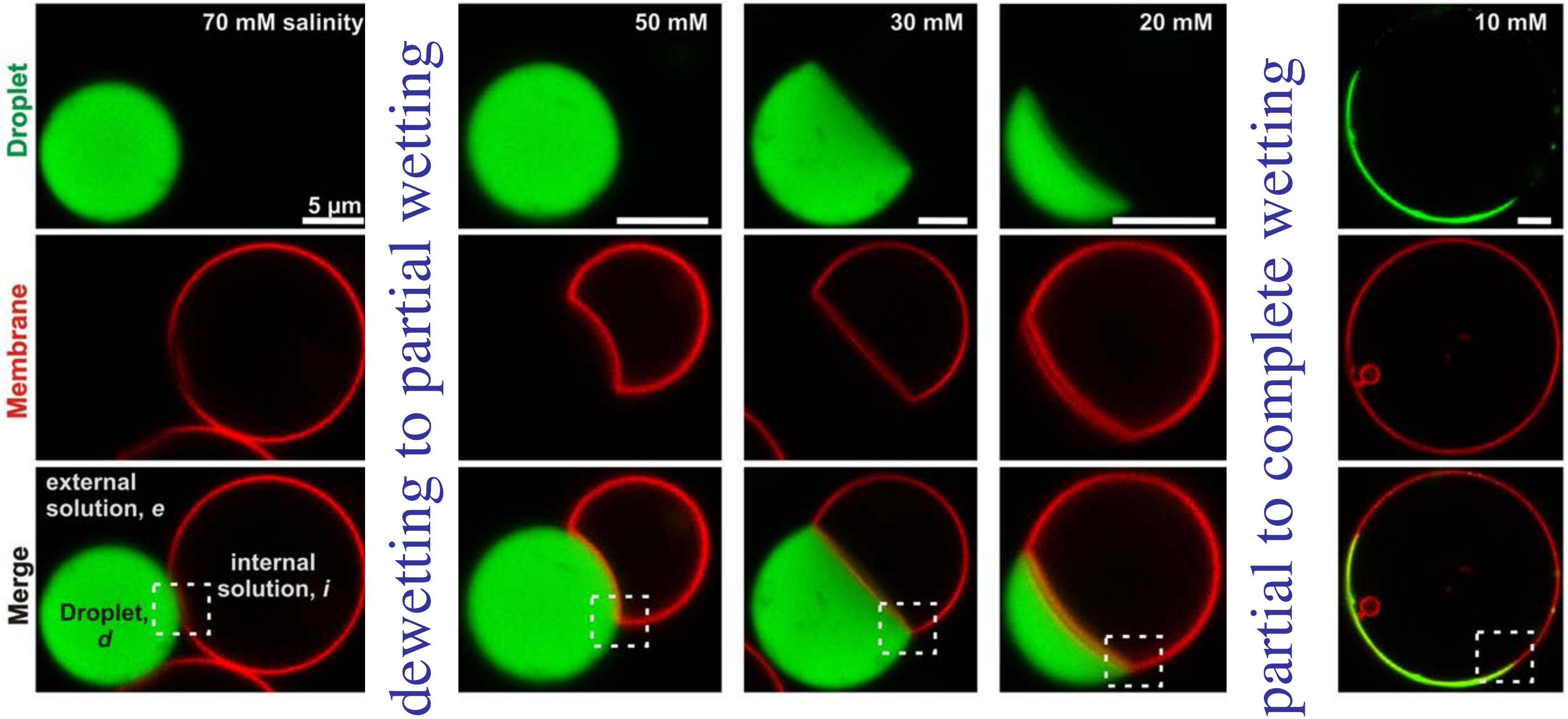
partial wetting for intermediate salt



complete wetting for low salt

Two Wetting Transitions

- GUV + FUS-rich organelle + salt



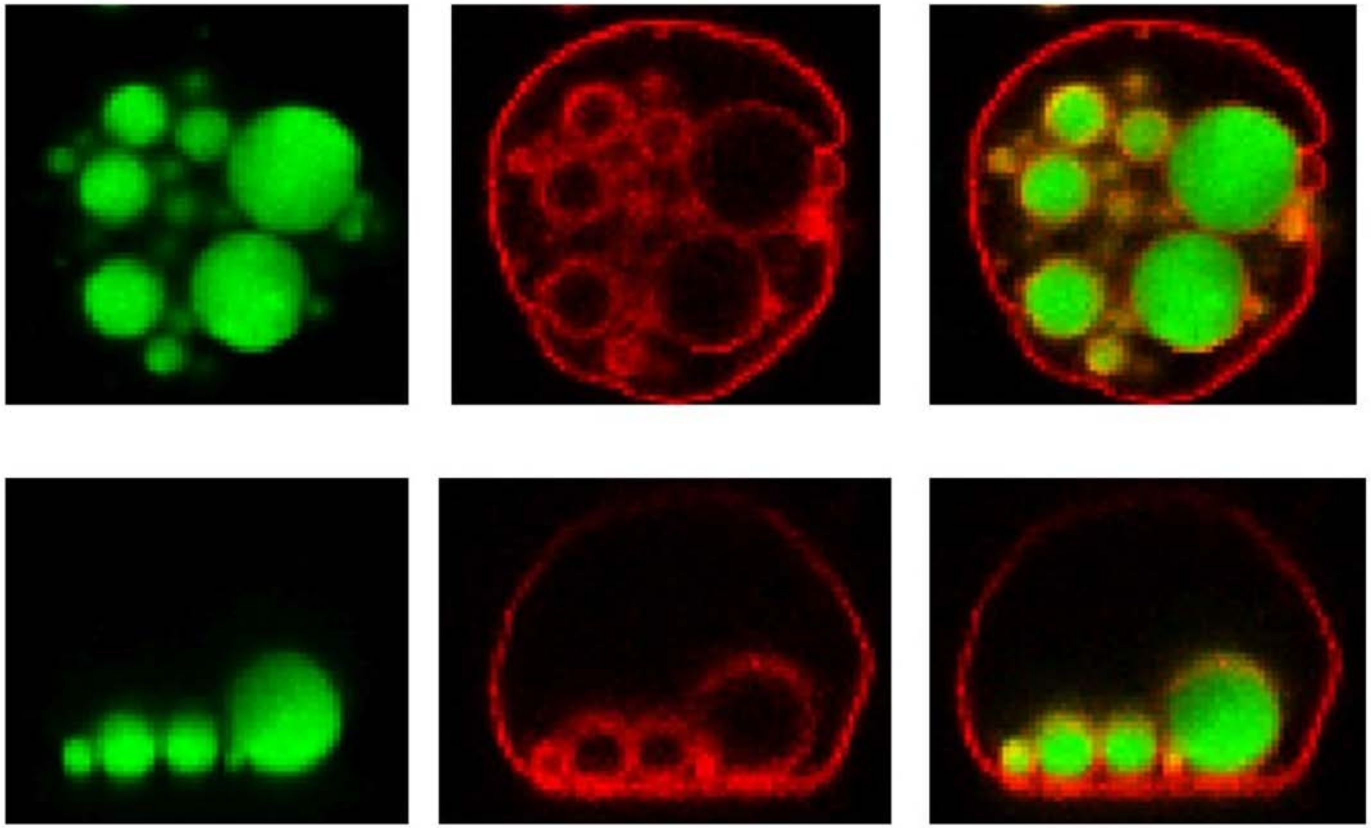
dewetting for high salt

partial wetting for intermediate salt

complete wetting for low salt

Endocytosis of Condensates

- Green FUS-rich condensates engulfed by red GUV:

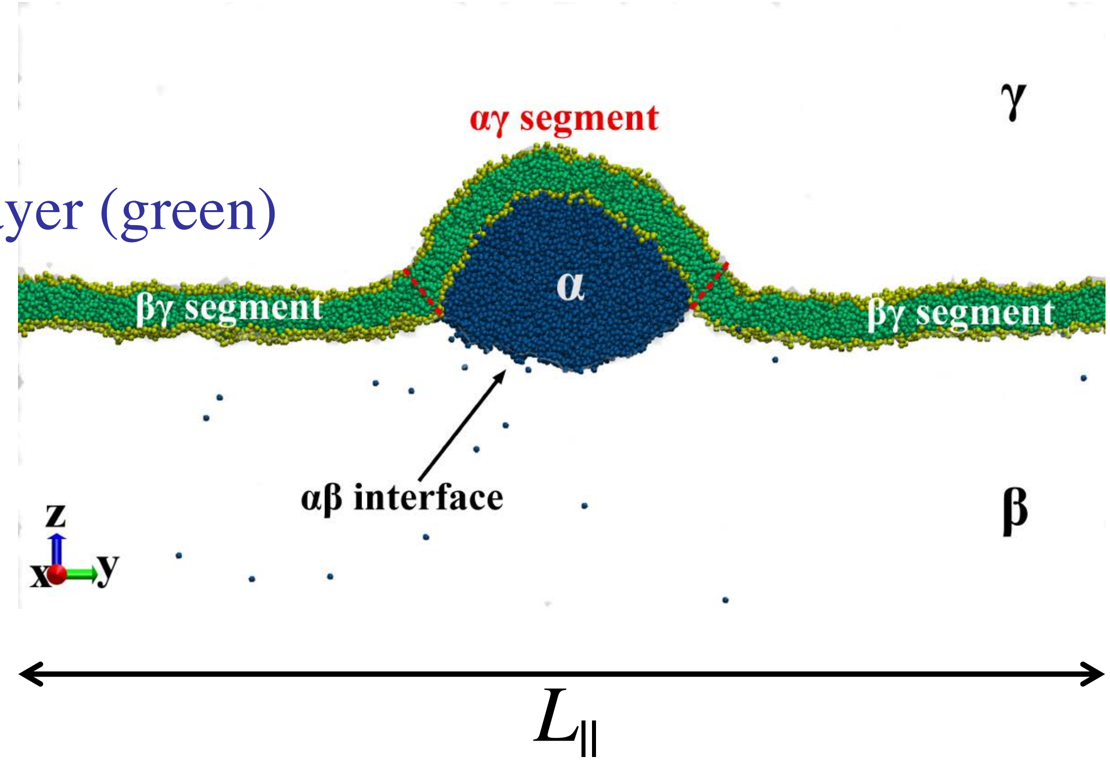


Lipid Bilayer + Nanodroplet

Satarifard, Grafmüller, RL (unpublished)

- Molecular simulations of lipid bilayer + nanodroplet
- Lateral box size $L_{||}$ determines mechanical tension
- Mechanical tension ~ size $L_{||}$ as control parameter

bilayer (green)

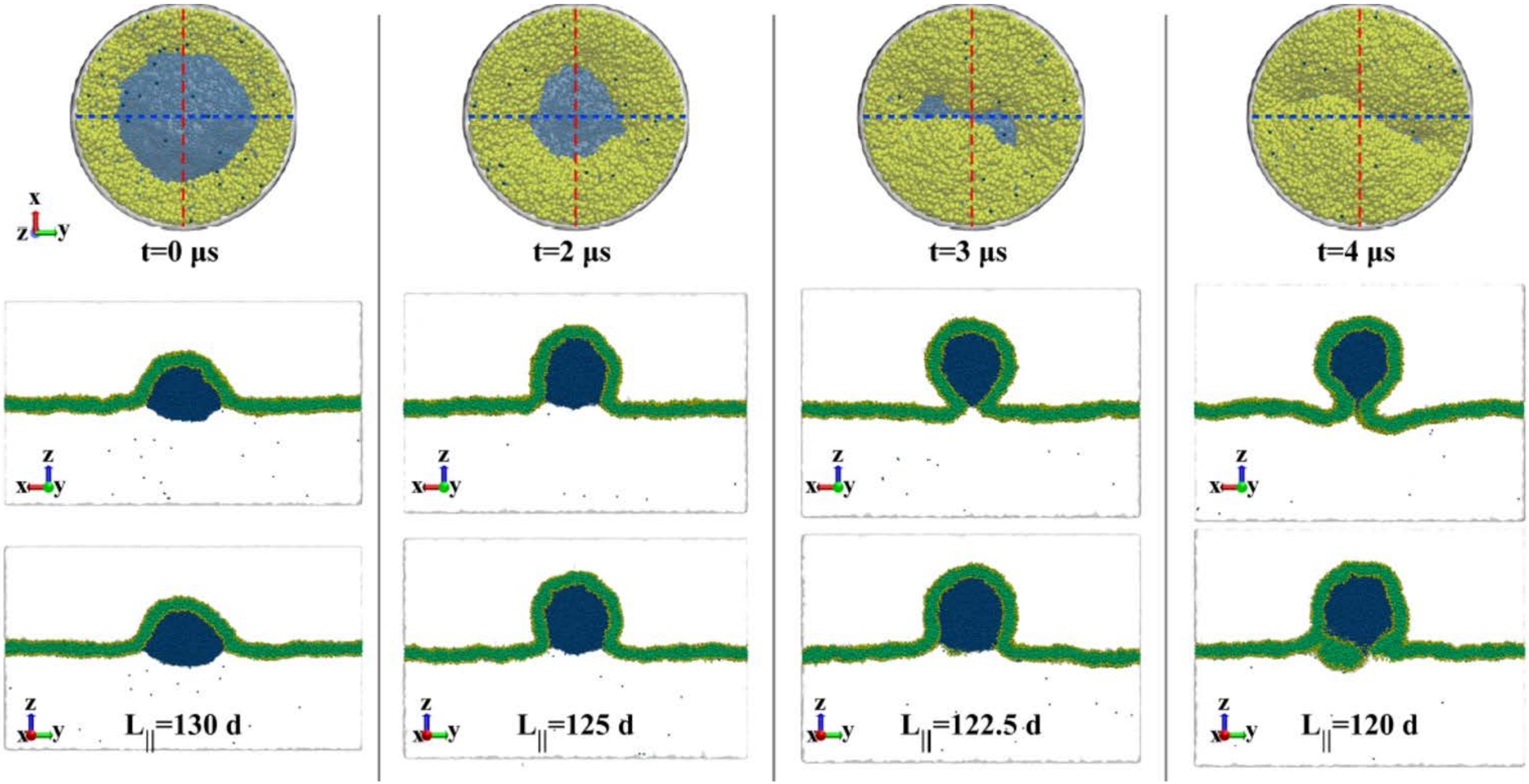


three aqueous phases α, β, γ

α droplet (blue) coexists with β phase (white)

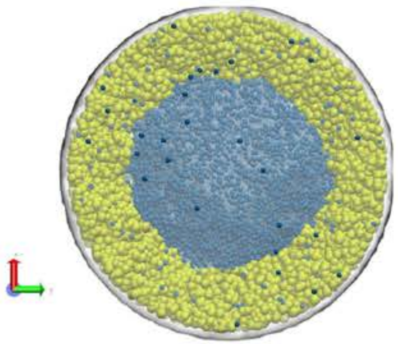
three surface segments αβ, αγ, βγ

Engulfment from Different Angles

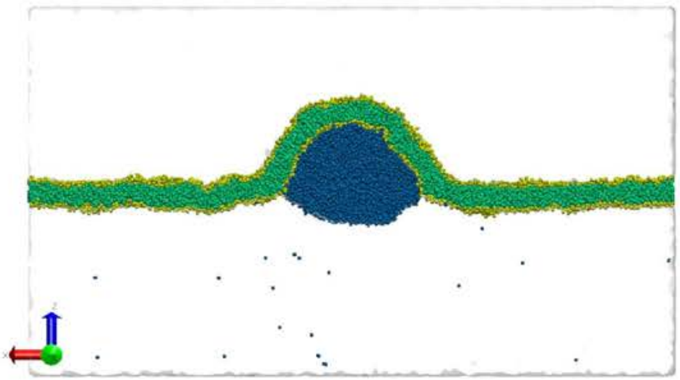


Engulfment Movie

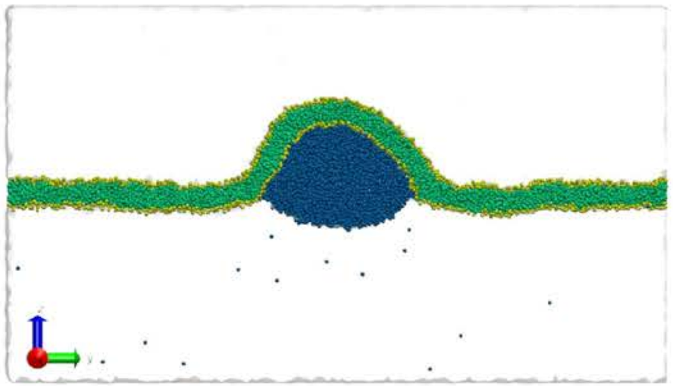
Bottom View



Cross Section 1



Cross Section 2



$L_{||}$=130 [d] **t=0 [μs]**

Vahid Satarifard
(unpublished)

- Reduction of size $L_{||}$ from 130 *d* to 120 *d*
- Axisymmetry broken for $L_{||}$ around 124 *d*
- Caused by negative line tension of contact line

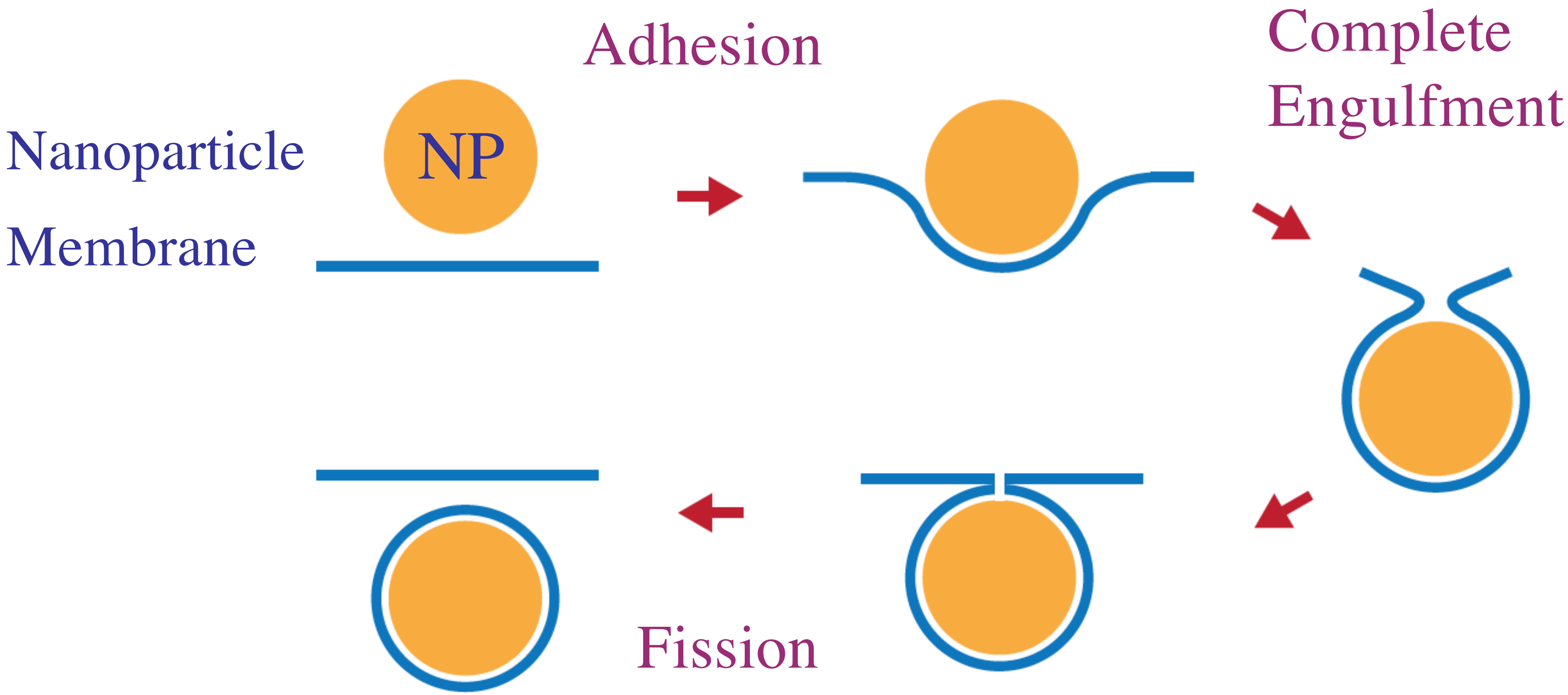
- Intro: Membranes and Giant Vesicles (GUVs)
- GUVs and Aqueous Two-Phase Systems

- Spont Tubulation and Tension
- Morphological Complexity

- Wetting and Fluid-Elastic Scaffolding
- GUVs and Biomolecular Condensates
- Endocytosis of Nanodroplets

- Endocytosis of Nanoparticles
- Outlook: Droplet-Stabilized GUVs

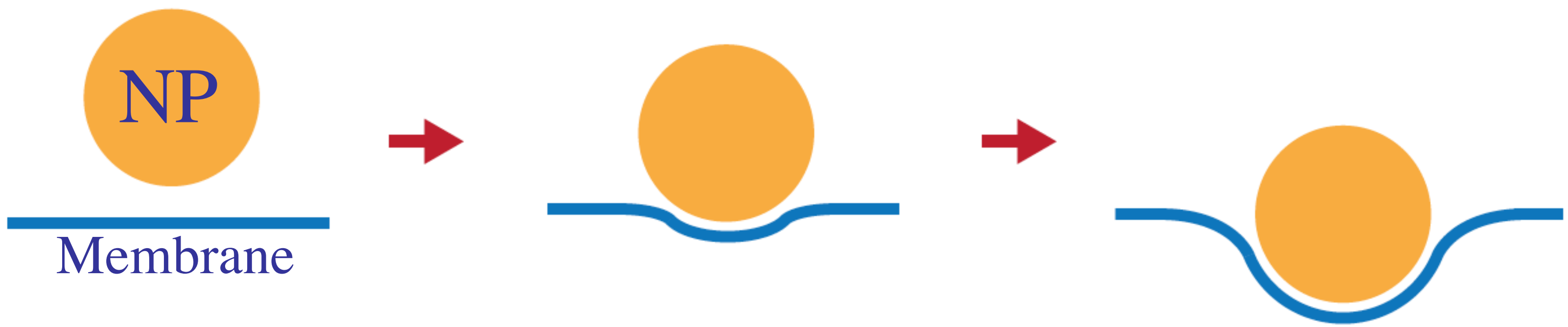
Endocytosis of Nanoparticles



• Dissecting endocytosis into three basic steps:

Onset of Adhesion, Complete Engulfment, Fission

Adhesion: Basic Aspects



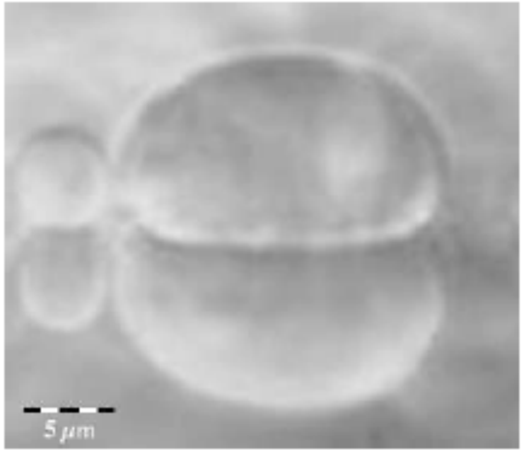
- Attractive interactions between NP and membrane
- Van der Waals, electrostatic, receptor-ligand
- Gain of adhesion free energy but increase of elastic membrane energy
- Competition between adhesion and bending
- Bending rigidity κ versus adhesive strength $|W|$

Adhesion Length

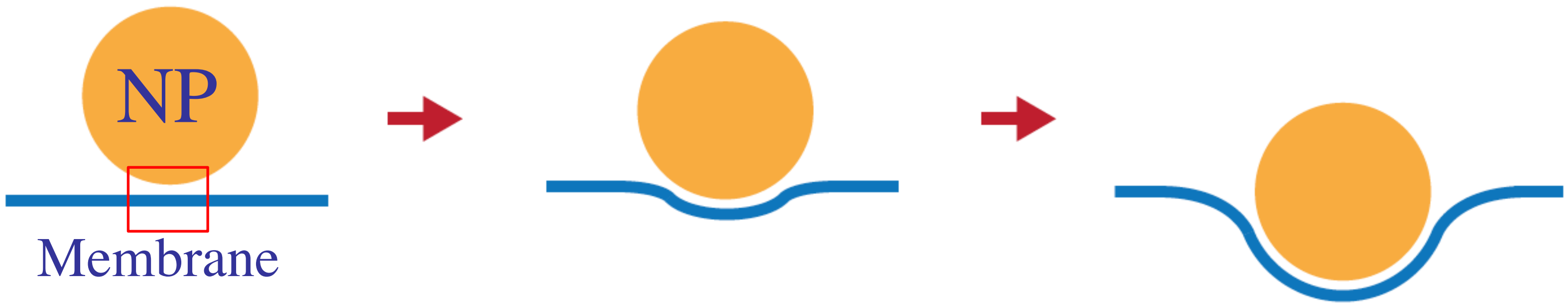
- Adhesive strength $|W|$ = adhesion free energy per area
- Bending rigidity κ and adhesive strength $|W|$ define adhesion length

$$R_W = (2\kappa/|W|)^{1/2}$$

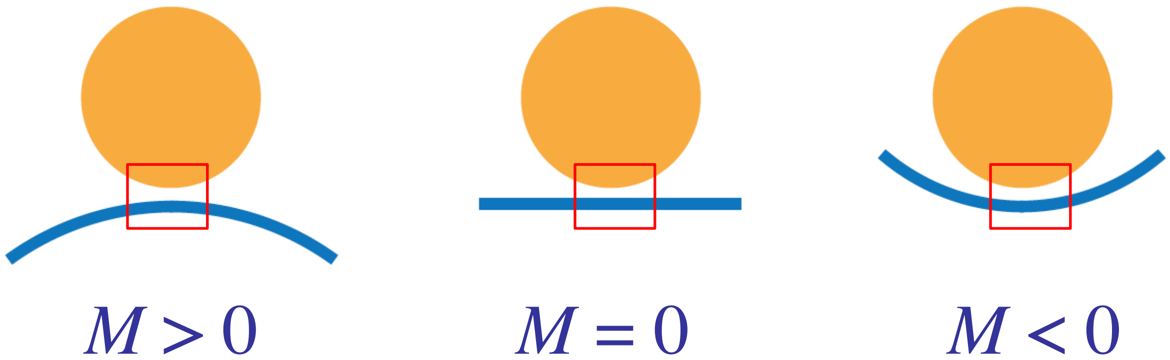
- For specific NP-membane systems, R_W varies between 10 nm and 3 μm !
- Large R_W values can be measured via membrane curvature along contact line



Onset of Adhesion: Key Parameters



- Three key parameters for onset of adhesion:
  Adhesion length R_W, Particle size R_{pa}, and
  Membrane curvature M at point of contact
- Membrane curvature M can be positive or negative:



Onset of Adhesion: Local Criterion

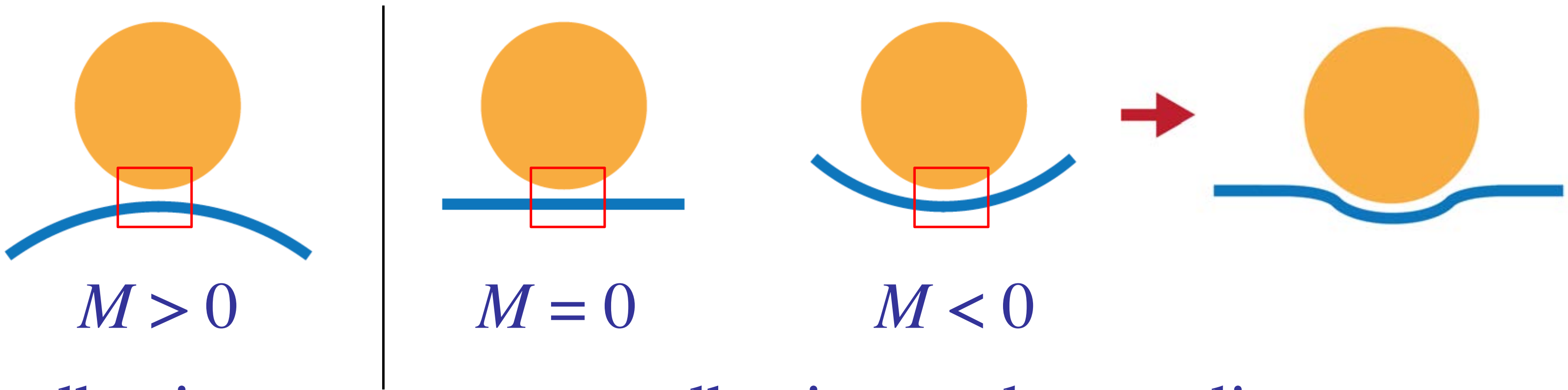
Agudo-Canalejo and RL, *ACS Nano* + *Nano Letters* (2015)

- Membrane starts to spread over particle if

$$M \leq 1/R_W - 1/R_{pa} =: M_{co}$$

contact curvature M_{co} is threshold value for M

- Example: $R_W = R_{pa}$ or $M_{co} = 0$

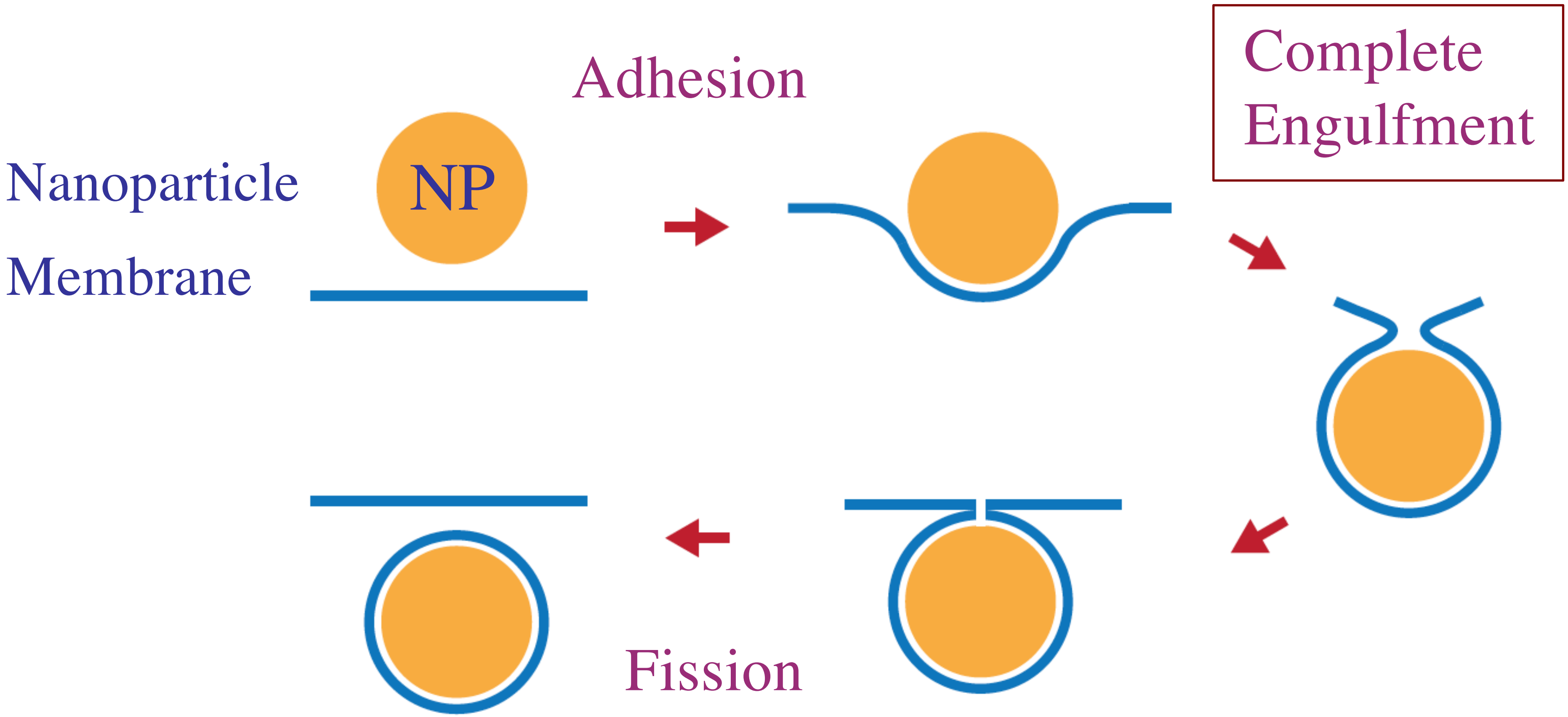


no adhesion

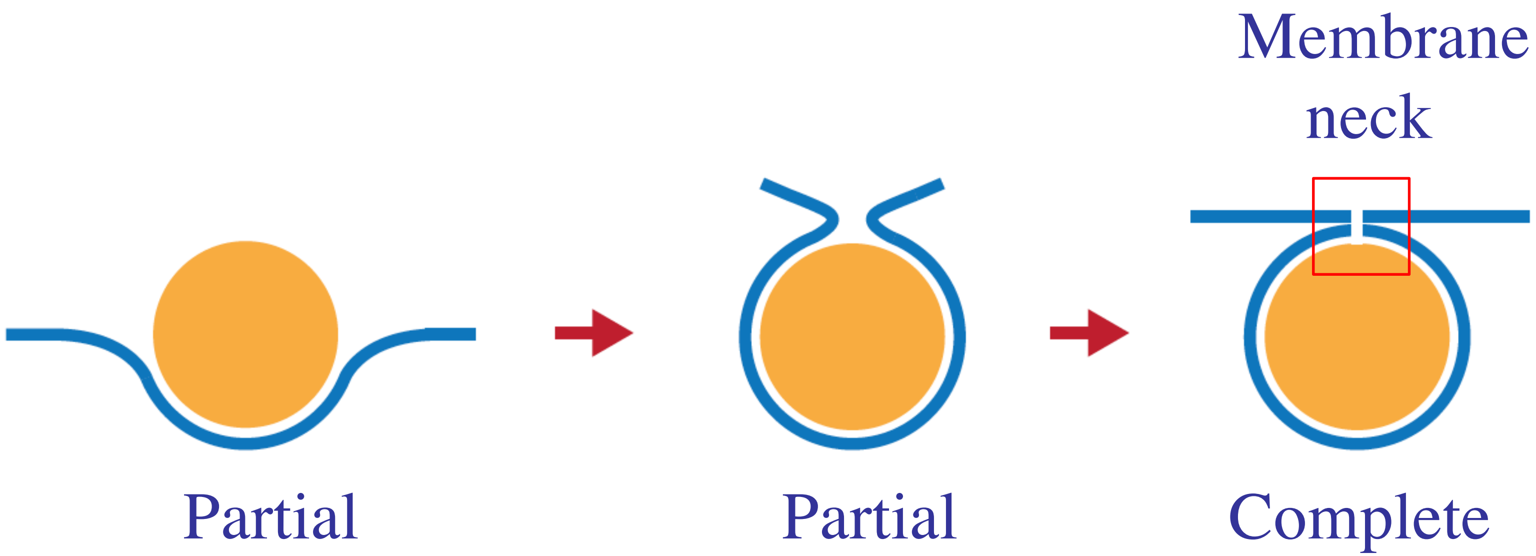
adhesion and spreading

- Large contact curvature M_{co} for small R_W or large $|W|$

Endocytosis: Complete Engulfment



Engulfment: Basic Aspects



- After onset of adhesion, membrane spreads over NP
- Membrane may engulf NP only partially or completely
- Complete engulfment involves closed membrane neck
- Necessary condition for complete engulfment:

Closed membrane neck must be stable

Neck Stability: Local Criterion

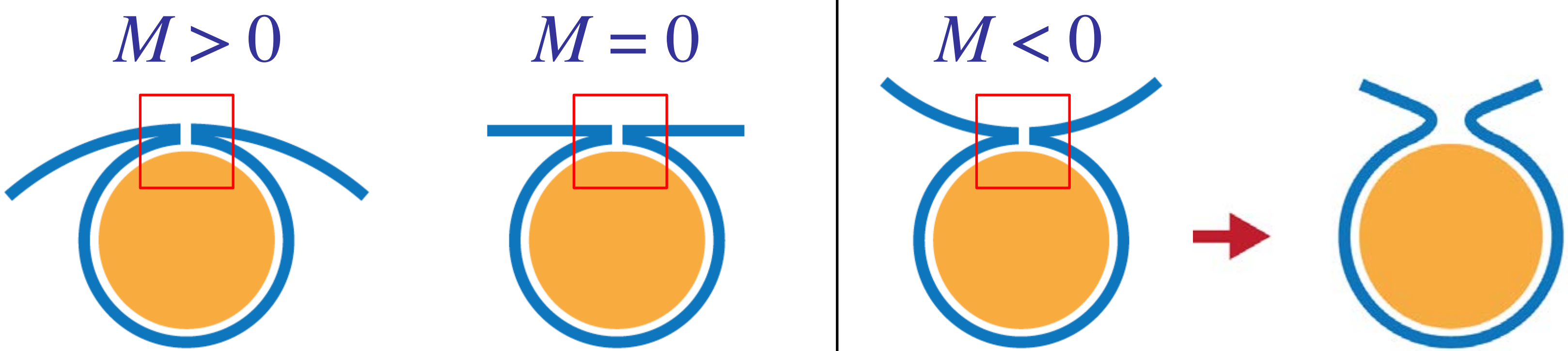
Agudo-Canalejo and RL, *ACS Nano* + *Nano Letters* (2015)

- Closed membrane neck is stable if membrane curvature

$$M \geq 2m + 1/R_{pa} - 1/R_{W} =: M_{ne}$$

2nd threshold value for M

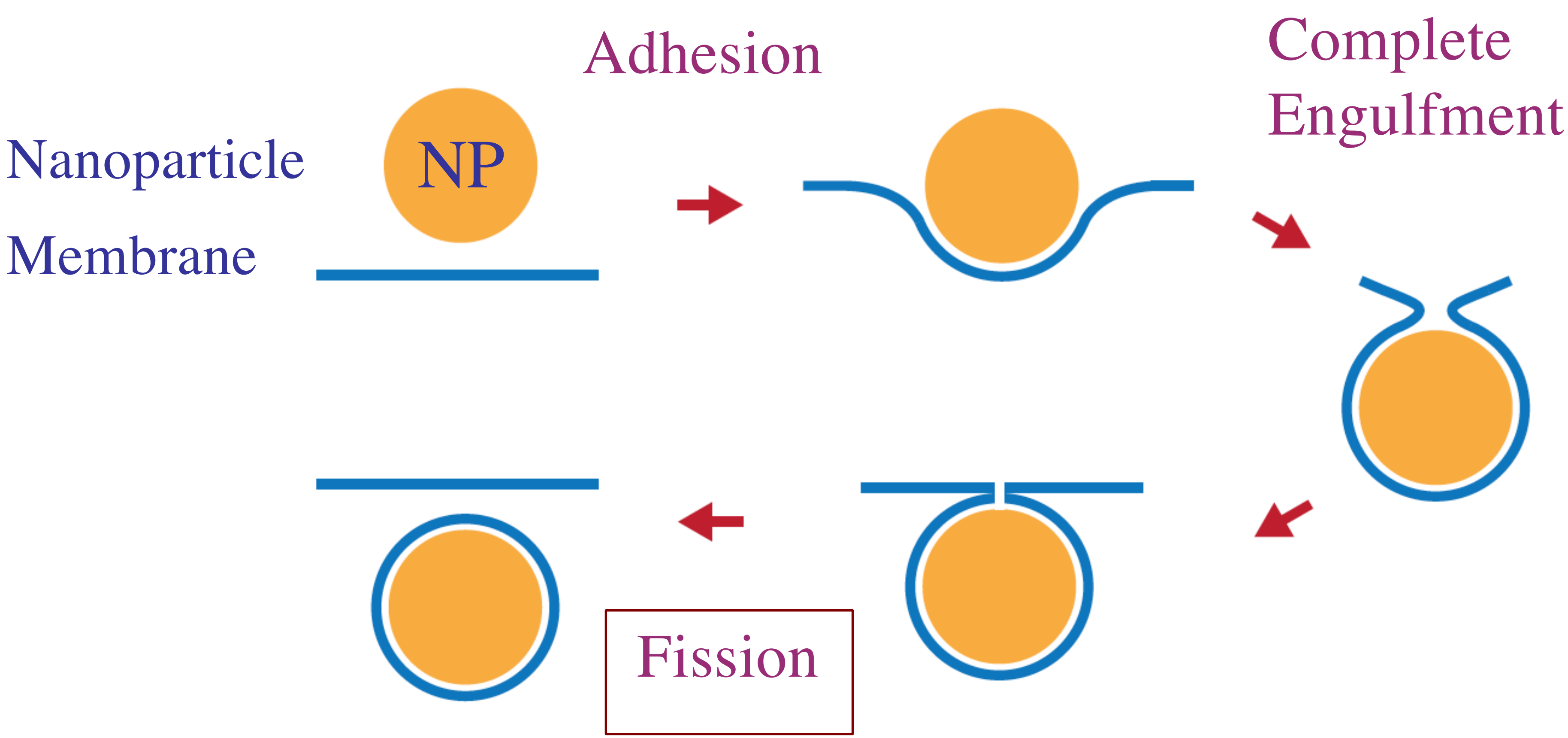
- Example: $M_{ne} = 2m + 1/R_{pa} - 1/R_{W} = 0$



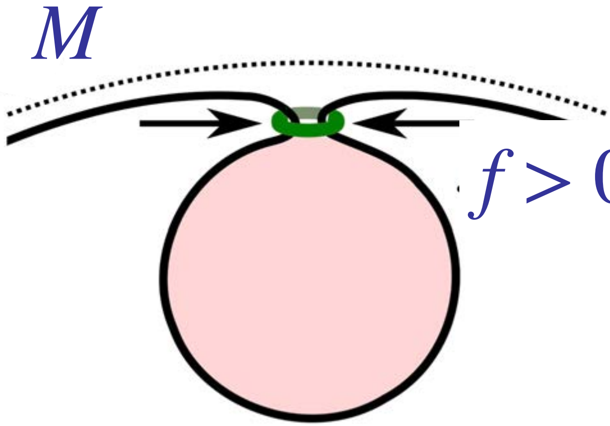
closed neck is stable

closed neck is unstable and opens up

Endocytosis: Fission



Effective Constriction Force



- Closed neck stability with force f :

$$M + M_{co} - 2m + f\,(4\pi\kappa)^{-1} \geq 0$$

- Effective constriction force

$$f_{eff} = f + 4\pi\kappa\,(M + M_{co} - 2m) \geq 0$$

- Engulfment force $f_{eng} = 4\pi\kappa\,(M + M_{co} - 2m)$

- Example: $\kappa = 4 \times 10^{-19}$ J, $M = M_{co} = 0$, $m = -1/(100\text{ nm})$

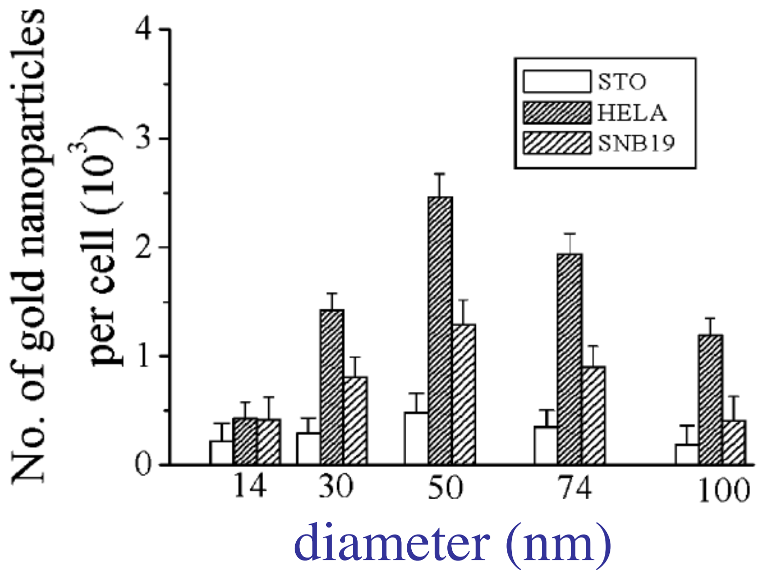
  => Engulfment force $f_{eng} = 100$ pN

- Sufficient to create two hydrophobic bilayer edges

Agudo-Canalejo and RL, *Soft Matter* (2016)

Receptor-Mediated Endocytosis

Chithrani et al, *Nano Letters* (2007)

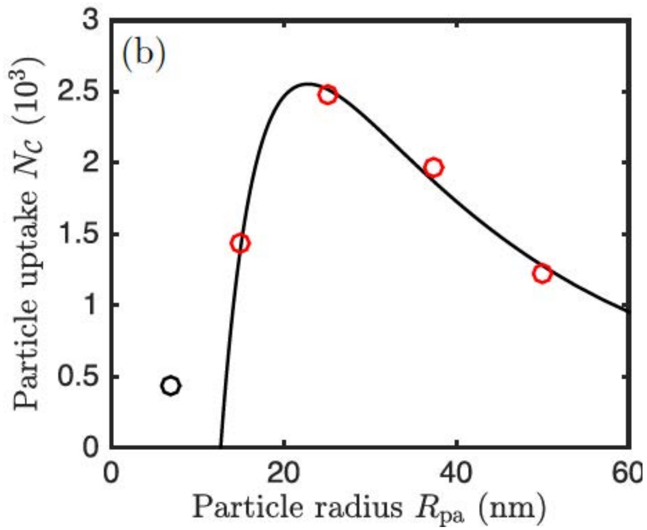


- Uptake of gold nanoparticles by cells
- Particles bind to transferrin receptors
- Assembly of clathrin-coated vesicles

Non-monotonic size-dependence !

- Cell membrane with two types of segments, bound and unbound
- Bound segment contains protein coat with spont curv m_{bo} = - 1/(40 nm)
- Good agreement with exp data:

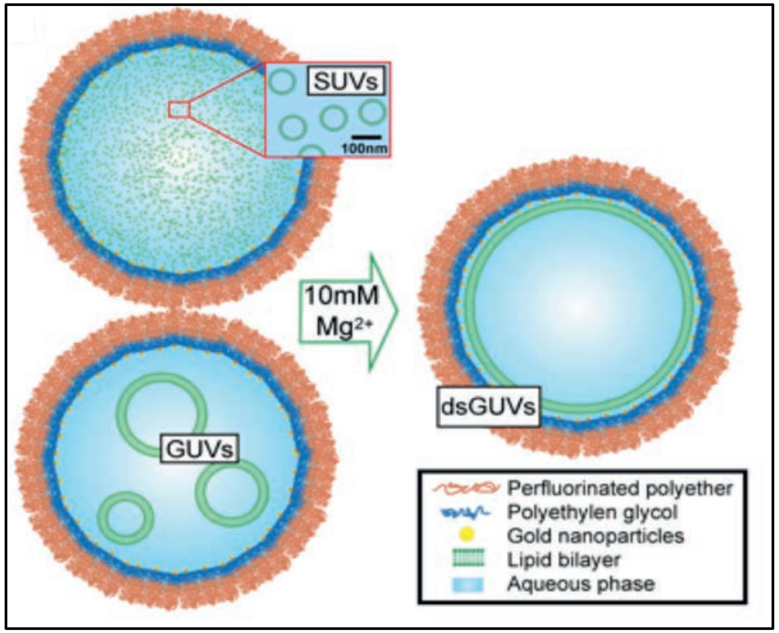
Agudo-Canalejo, RL: *ACS Nano* (2015)

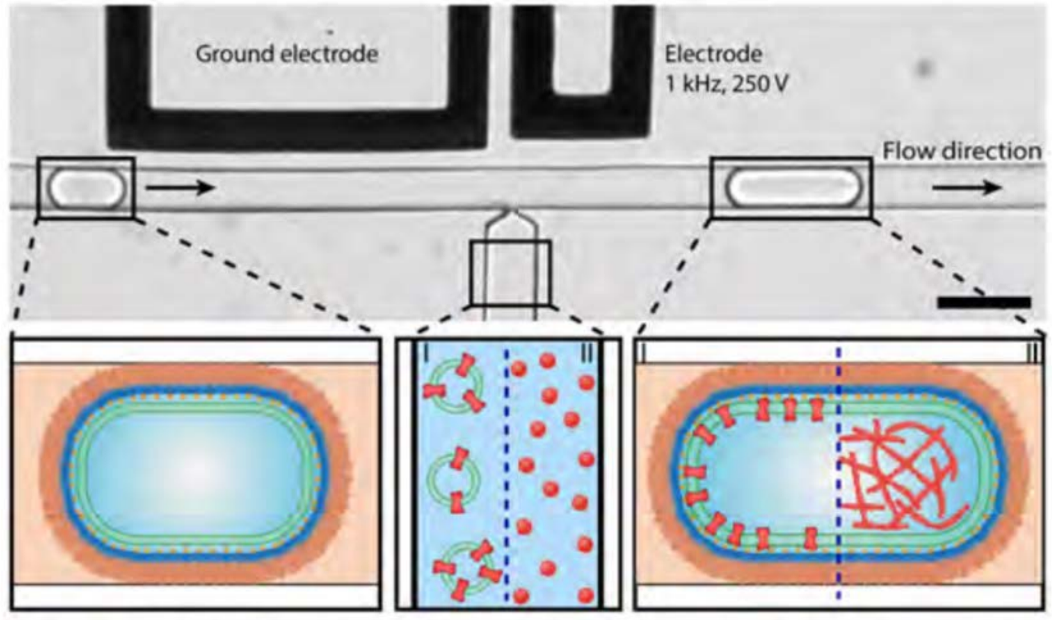


Outlook: Droplet-Stabilized GUVs

Weiss et al, *Nature Materials* (2018)

- Four MPIs within MaxSynBio, leading PI: J. Spatz

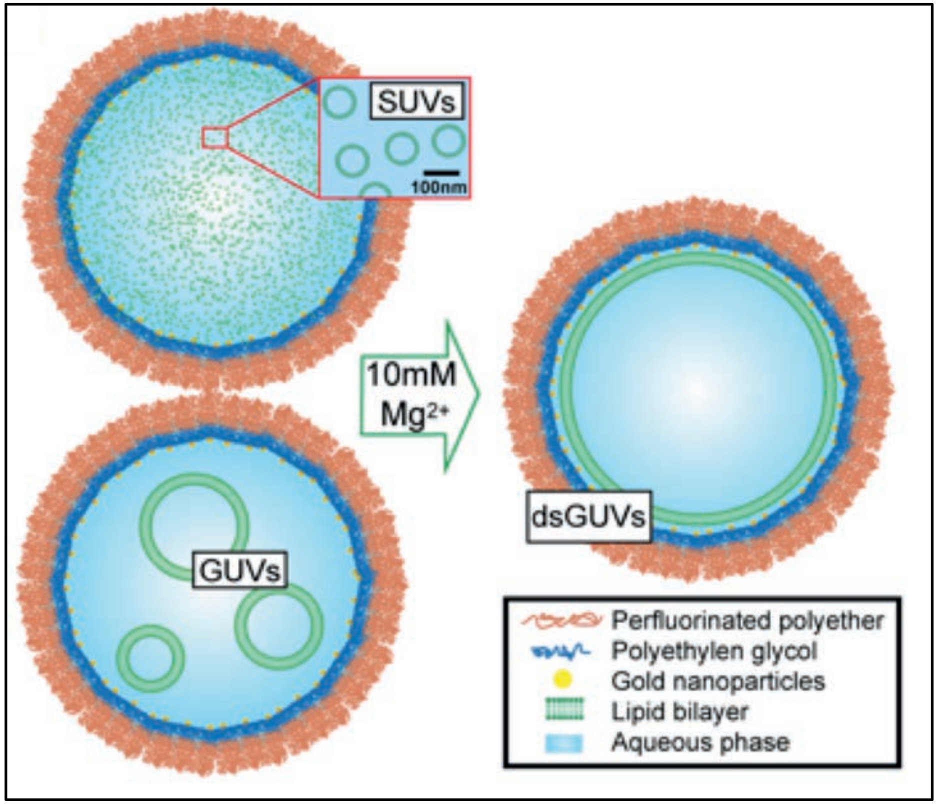




- Water-in-Oil emulsion droplets
- Formation of GUV supported by the droplet surface
- Additional components by pico-injection
- Example: ATP synthase

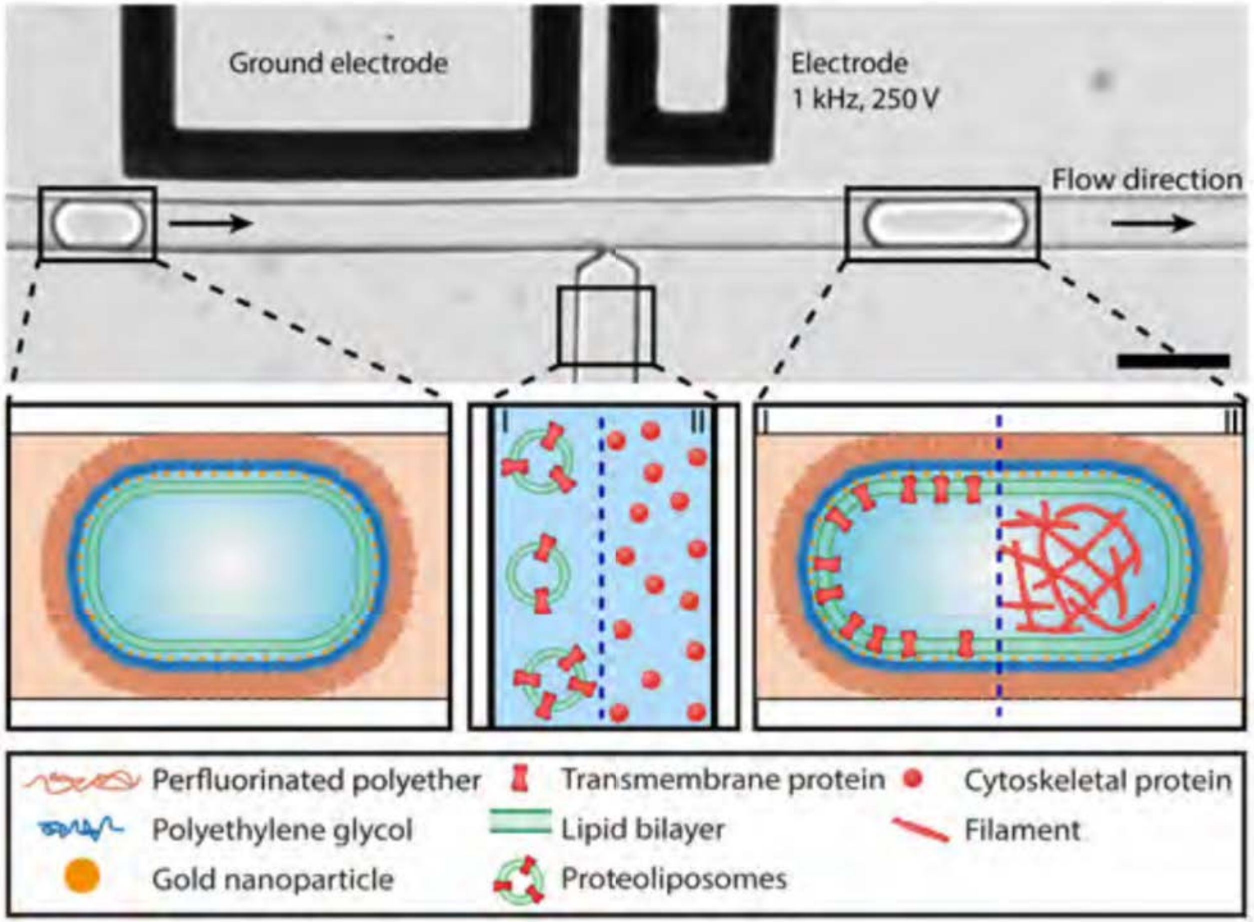
GUVs within W/O Emulsion Droplets

- Emulsion w/o droplet stabilized by surfactant
- Pico-Injection of small vesicles
- Pico-Injection of Mg^{++}
- Adhesion of vesicles to surfactant layer
- Rupture of vesicles
- Fusion of fragments
  => Formation of a GUV supported by surfactant layer



- Release of encaged GUV from droplet

Sequential Pico-Injections



- Pico-injection of membrane and cytoskeletal proteins
- Incorporation of functional ATP Synthase (FoF1-complex)

Coworkers

- Collaborations:

  Joachim Spatz, Tony Hyman, Titus Franzmann

Rumiana Dimova

Vahid Satarifard

Andrea Grafmüller

Jaime Agudo-C.