Interactions of Liquid Droplets with Biomembranes

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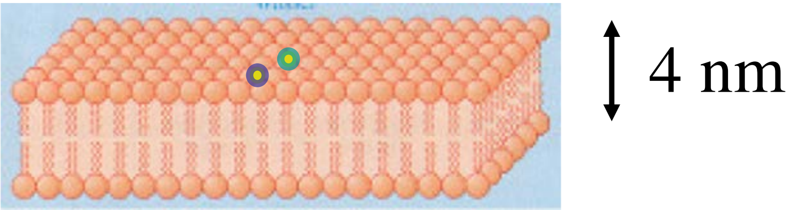
- Intro: Membranes and GUVs
- GUVs + Aqueous Two-Phase Systems
- Theory of Fluid-Elastic Scaffolding
- GUVs + Membraneless Organelles
- Nanodroplets at Membranes
- Outlook: More on GUVs

Biomembranes are Fluid Bilayers

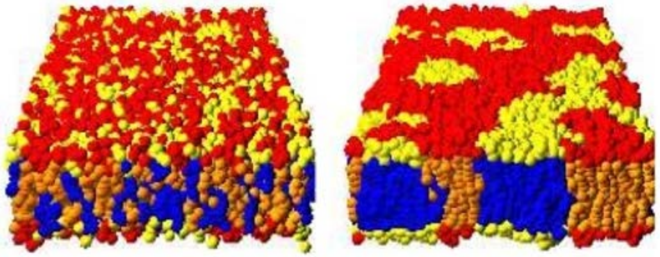
- Fluid membranes, i.e., fast lateral diffusion:

  Diffusion constant ~ µm^2/s

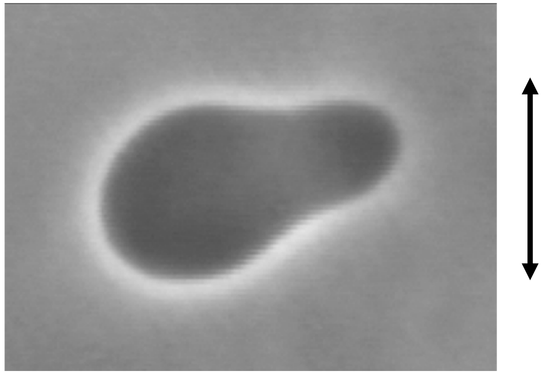


lipid swapping ~ ns

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

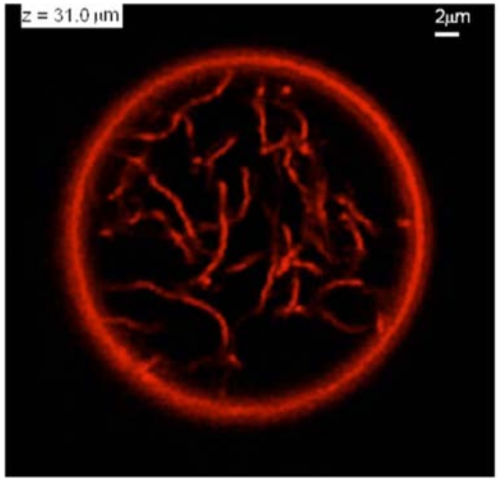


- Flexibility => Morphological responses, budding, tubulation, ... Direct evidence for fluidity

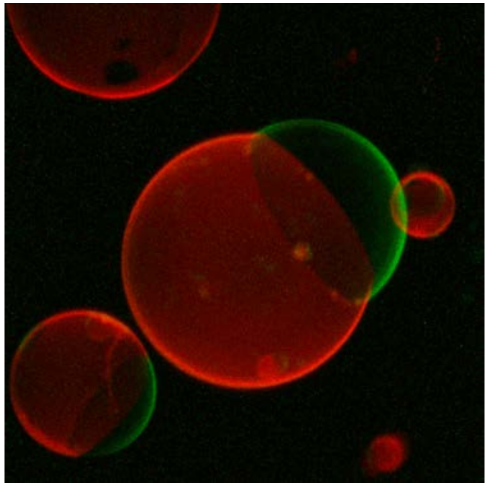


Multiresponsive Behavior

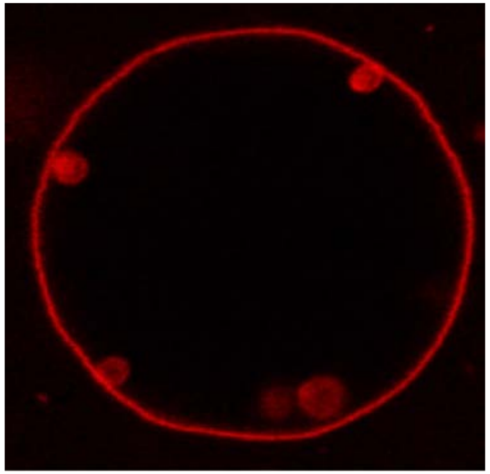
- Giant unilamellar vesicles (GUVs), tens of micrometers
- Remodelling in response to various perturbations:



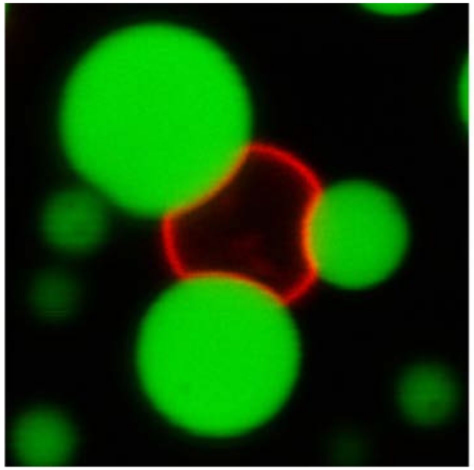
Nanotubes from polymer adsorption, tube width ~ 100 nm



Formation of intra-membrane domains, 2D phase separation



Small buds from adsorption of two ESCRT proteins



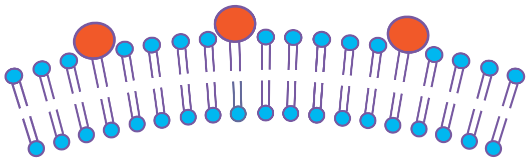
Shaping GUVs by membrane-less organelles, FUSb

- What are the forces that drive remodelling processes?

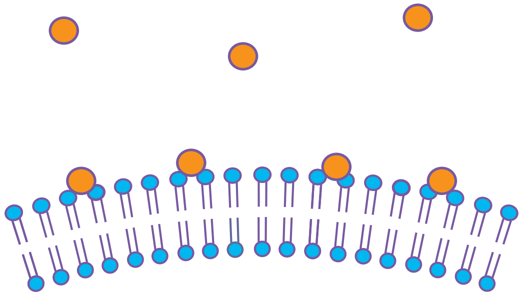
Spontaneous = Preferred Curvature

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

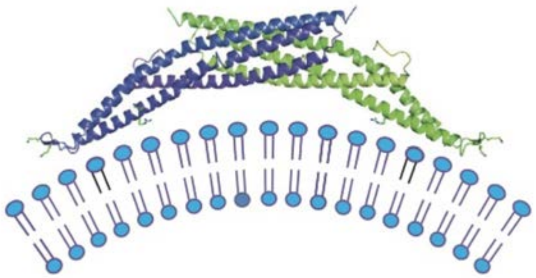
- Different molecular mechanisms for bilayer asymmetry:



Asymmetric
composition,
e.g., ganglioside



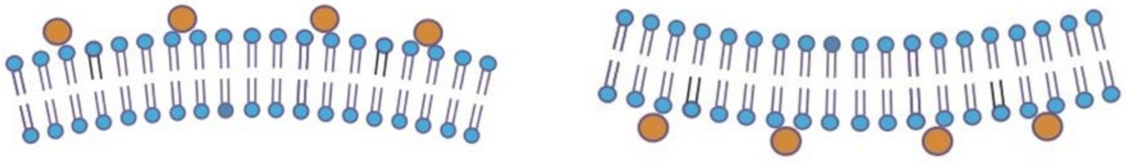
Asymmetric
adsorption of
small molecules



Asymmetric
protein coats,
e.g. BAR-domain

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

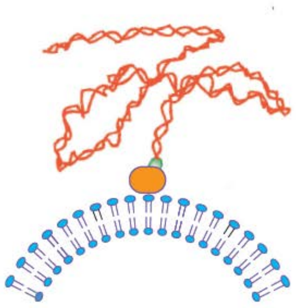
Mean curvature M is positive (negative) if membrane bulges towards exterior (interior) compartment

Local Curvature Generation

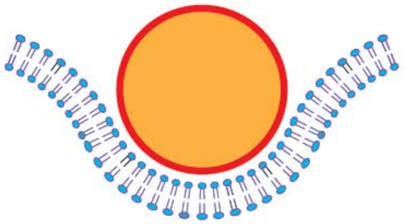
RL, *Faraday Disc*. (2013); *Biol. Chem*. (2014)

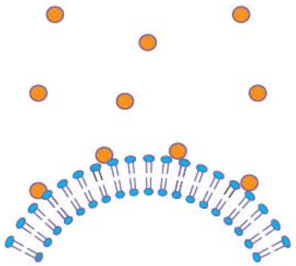
- Local curvature generated on nanoscopic scales:

Anchored polymer

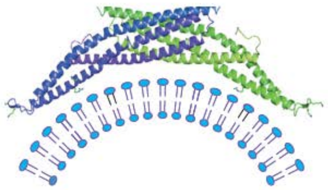


‘Large’ particle

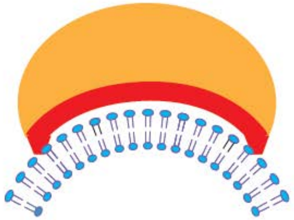


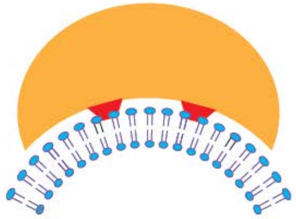


‘Small’ adsorbate particles



BAR-domain protein





Nonspherical Janus particles

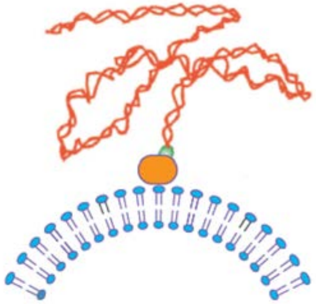
Induced Fit

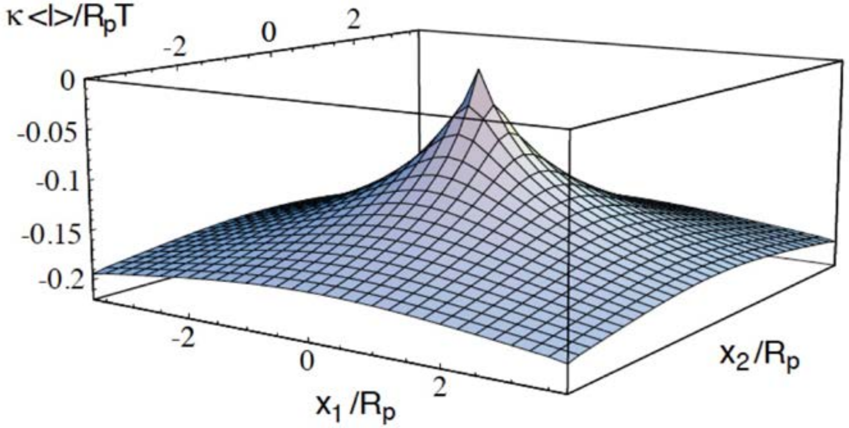
Conformational Selection

Intimate Relation to Spont Curvature

- Curvature profile $M(x)$ for one bound ‘particle’
- Example: Anchored polymer

Breidenich, Netz, RL
Europhys. Lett. (2000)





- ‘Particle’ density Γ_{ex} and Γ_{in} on outer and inner leaflet
- Spontaneous curvature m for dilute regime:

$$m = \int \mathrm{d}A \;\; M(x) \;\; [\, \Gamma_{\text{ex}} - \Gamma_{\text{in}} \,]$$

Spont Curv from Mol Simulations

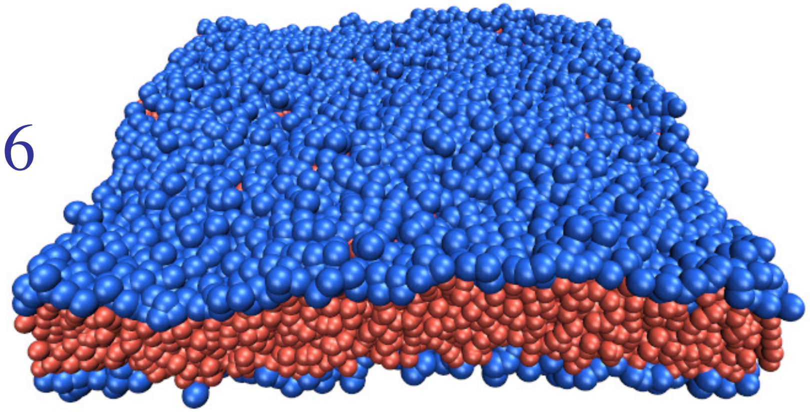
Rozycki, RL, *J. Chem. Phys.* (2015); *J. Chem. Phys.* (2016)

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

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

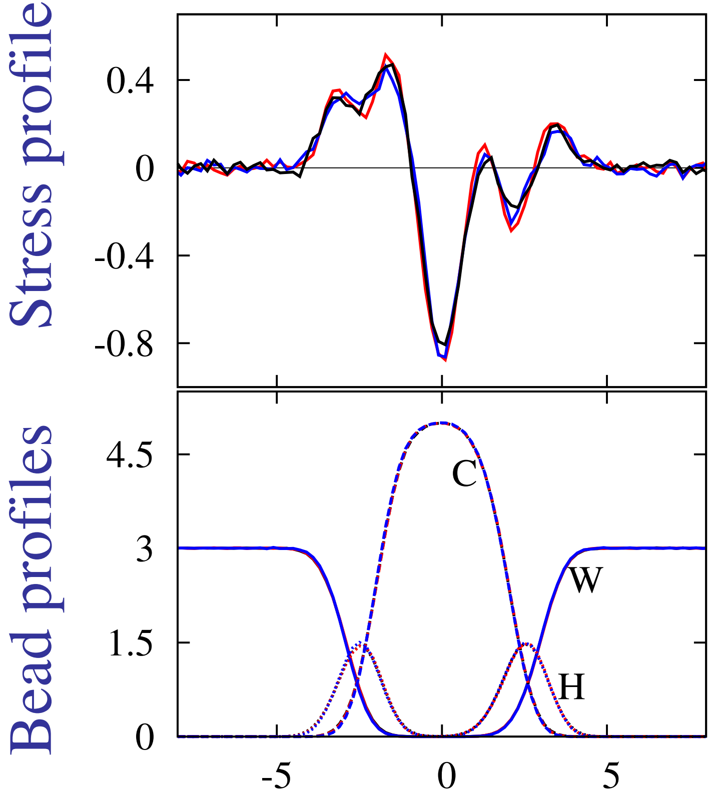
outer leaflet with N_{ex} lipids

$\phi = 0.56$

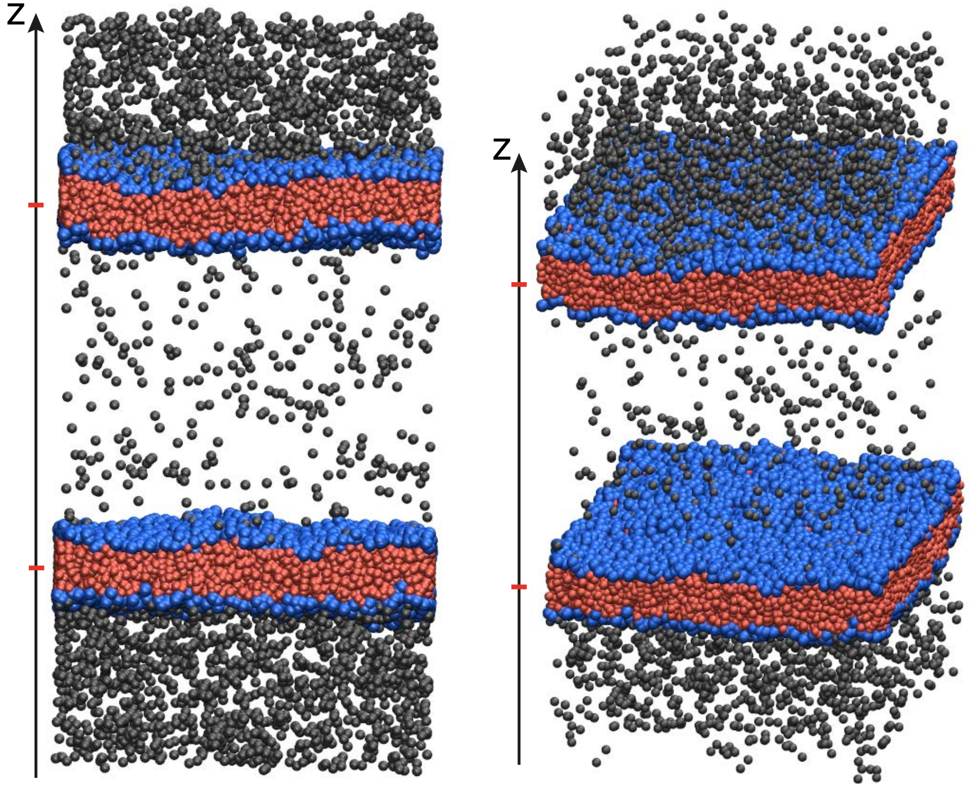


inner leaflet with N_{in} lipids

$\phi = 0.51$



Asymmetric Adsorption and Depletion



Particle concentration X_{ex}

Bilayer 1

Particle concentration X_{in}

Bilayer 2

Particle concentration X_{ex}

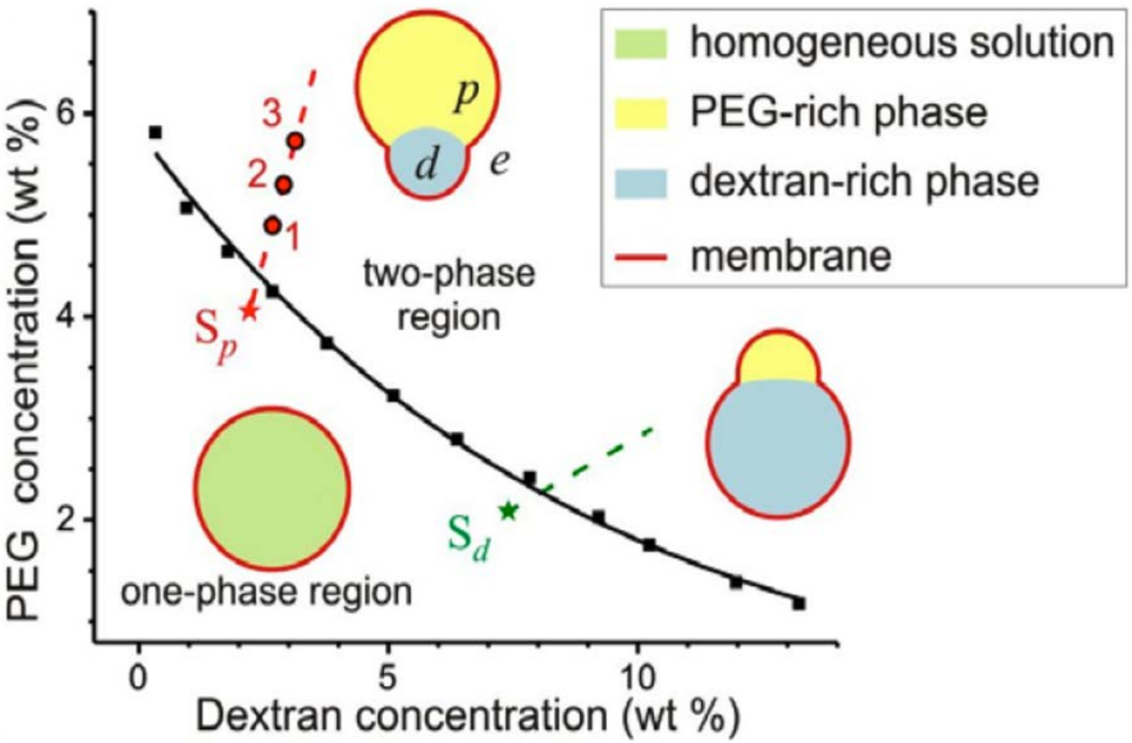
- Spont curv proportional to $\pm\,(X_{ex} - X_{in}) = \pm\,\Delta X$
- Example: 1 nm particles, $\Delta X = 100$ mM

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)

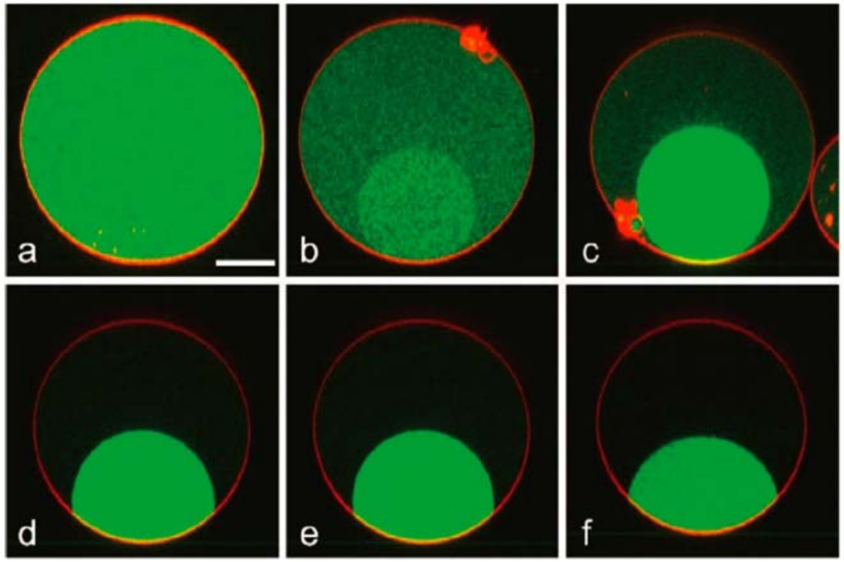
- GUVs filled with aqueous polymer solution
- Example: PEG and dextran
- Increase polymer concentration via deflation:

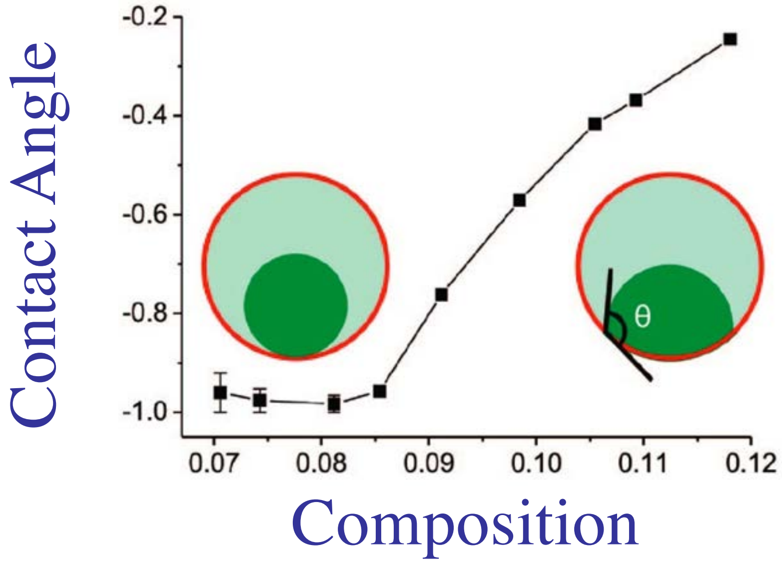


1st Surprise: Wetting Transition

Li et al, *JACS* (2008)

- Shape evolution for vesicle during deflation:



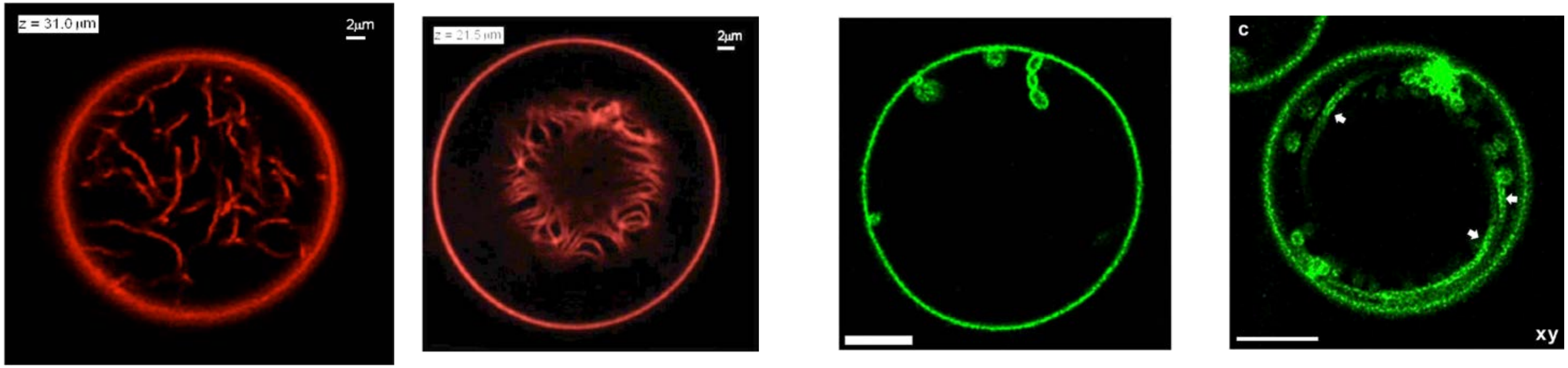


- Low deflation: zero contact angle => complete wetting
- High deflation: finite contact angle => partial wetting

2^{nd} 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)

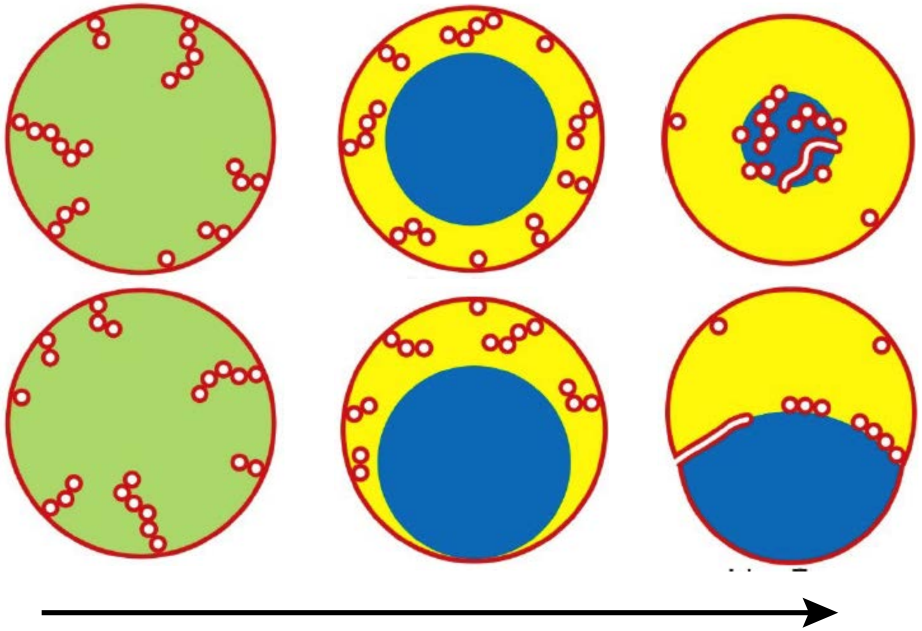


- Spontaneous tube formation without external forces
- Inward-pointing tubes reveal large negative spont curv
- Tubes can be necklace-like or cylindrical

Nanotubes and Wetting

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

- Tubulation followed by phase separation:



1-phase CW PW

horizontal cross section

vertical cross section

Volume reduction by osmotic deflation

- CW: Nanotubes stay away from αβ interface
- PW: Nanotubes adhere to αβ interface

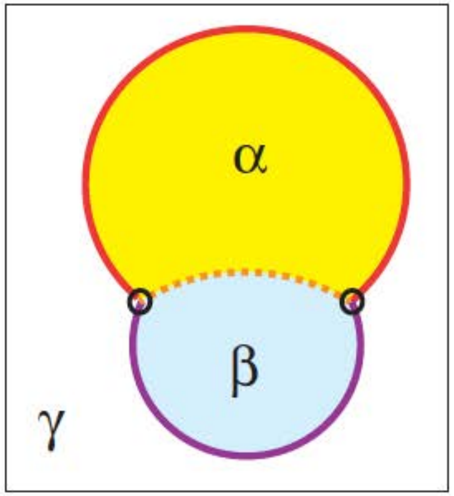
Membranes and Droplets: Theory

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

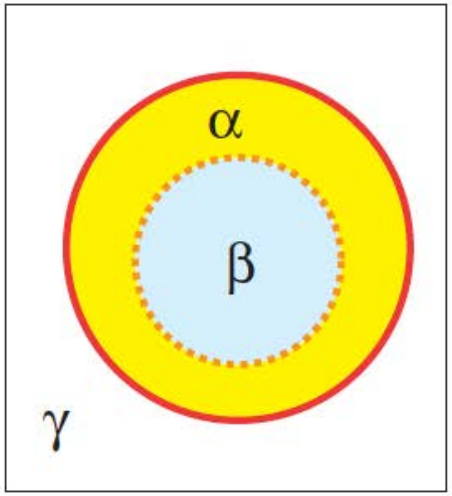
- In-wetting: Droplets at inner leaflet
- Nanoscopic description: smooth bends
- Mescoscopic description: three spherical caps
- Droplets without membranes
- Phase diagram and wetting transitions
- Out-wetting: Droplets at outer leaflet
- Mechanisms of fluid-elastic scaffolding

In-Wetting Morphologies

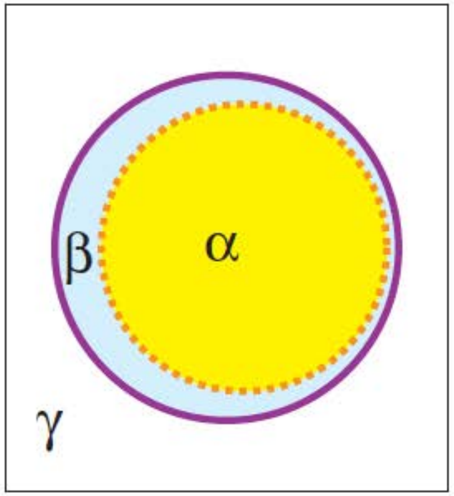
- Three aqueous phases α, β, γ
- Phase coexistence of α and β, γ is ext spectator phase
- GUV membrane encloses α and β



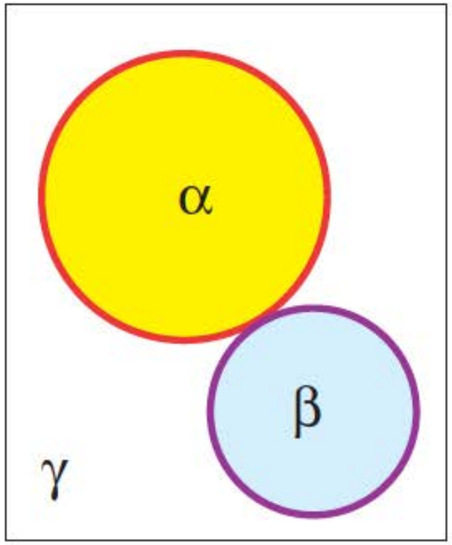
Partial wetting by α and β



Complete wetting by α



Complete wetting by β

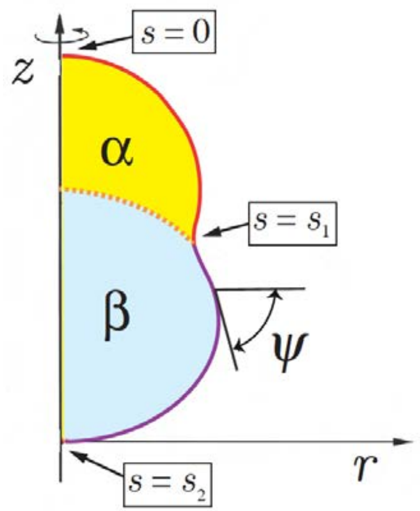


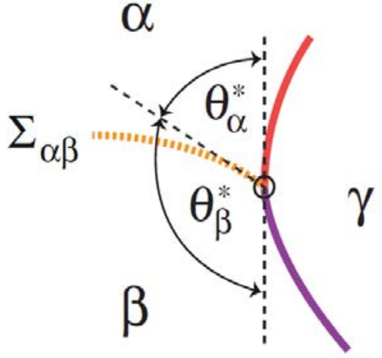
Complete wetting by γ, Membrane neck

Nanoscopic Scales

- 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

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

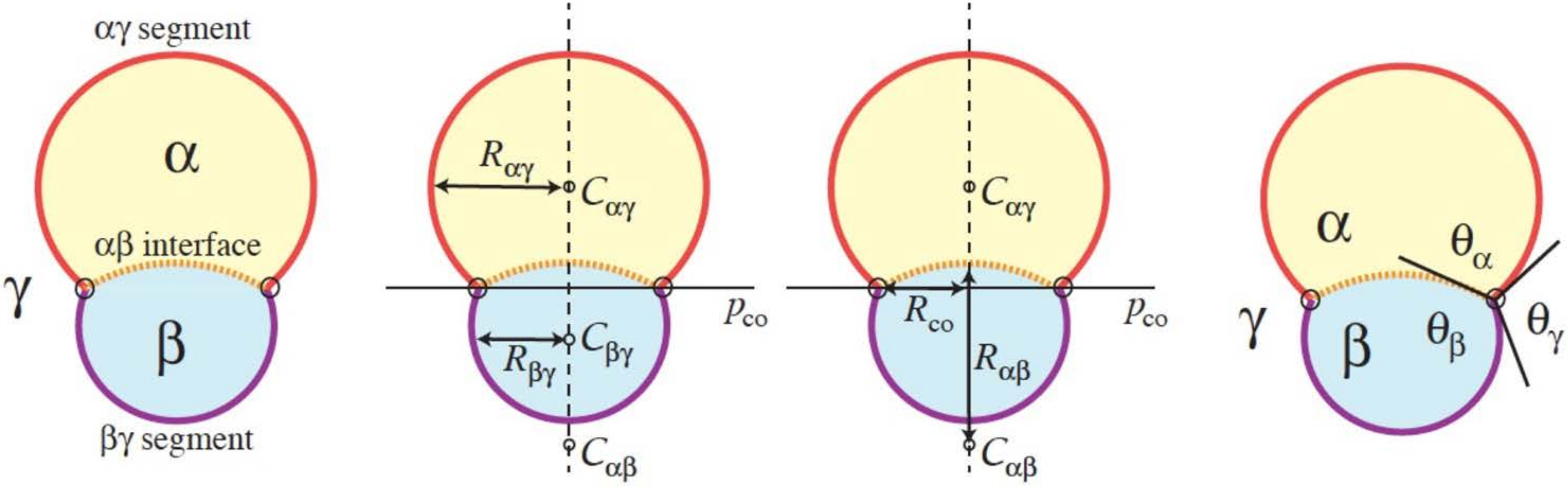
- Complex matching conditions along contact line
- Simplification if $\alpha\gamma$ and $\beta\gamma$ segments have identical curvature elastic properties
- Special case: zero spontaneous curvatures

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 three sphere centers $C_{\alpha\gamma}$, $C_{\beta\gamma}$, and $C_{\alpha\beta}$

Shape Equations

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

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

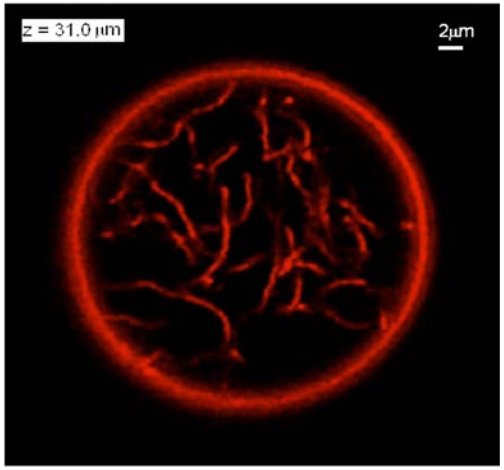
- Effective membrane tension:

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

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

Spontaneous Tension: Theory

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 $\sigma = 2\,\kappa\, m^2$ curves the membrane

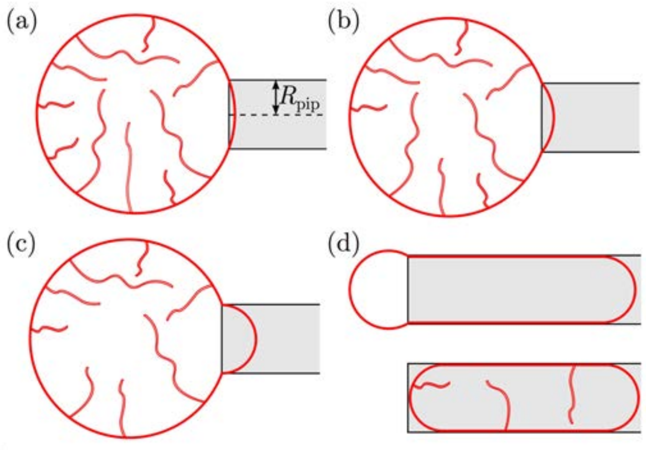
- Presence of nanotubes implies dominance of spontaneous tension, mechanical tension can be ignored
- Example: Spont curvature $\approx -1/(100\text{ nm})$ implies

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

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

Spontaneous Tension: Experiment

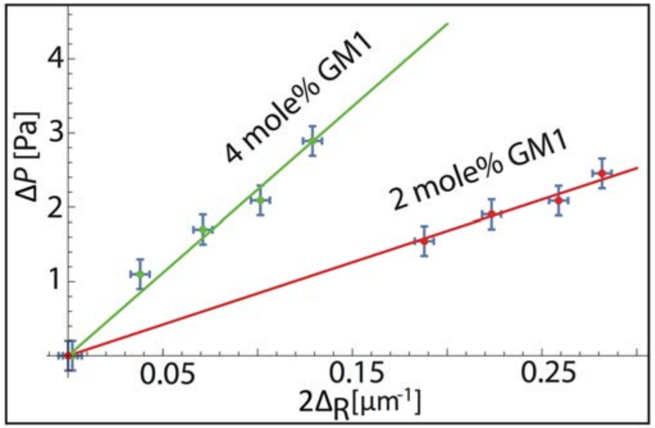
- Retraction of tubes by micropipettes: Bhatia et al, *ACS Nano* (2018)



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

Tensions and Angles

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

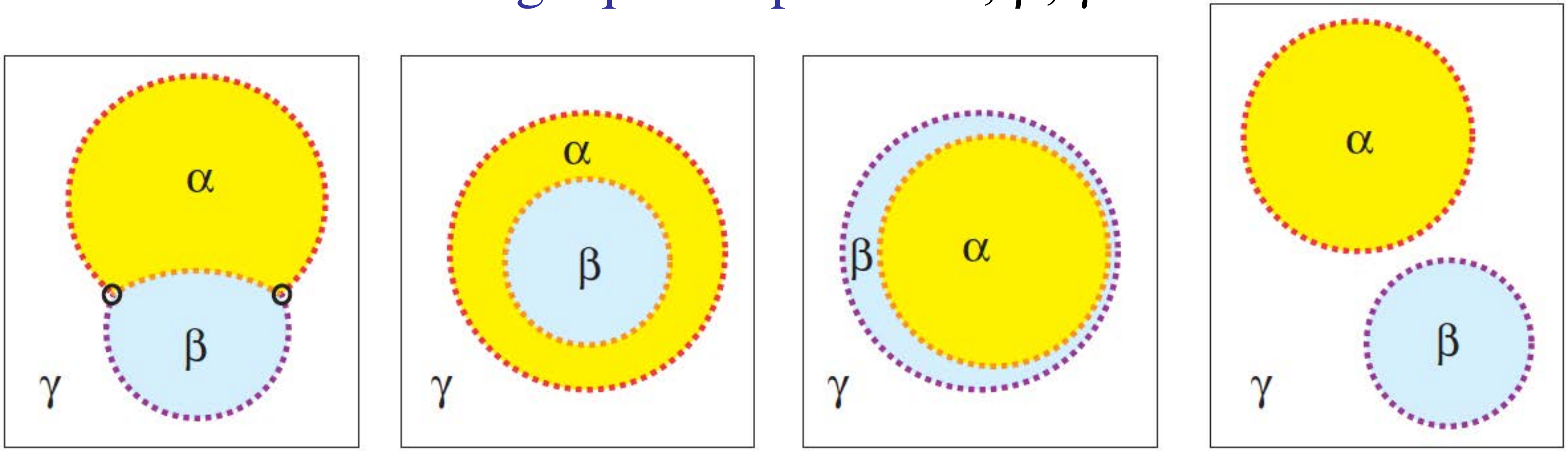
- Combine three shape equations with geometric relation
- Eliminate mean curvature $M_{\alpha\beta}$
- Relation between effective tensions and contact angles:

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

- 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



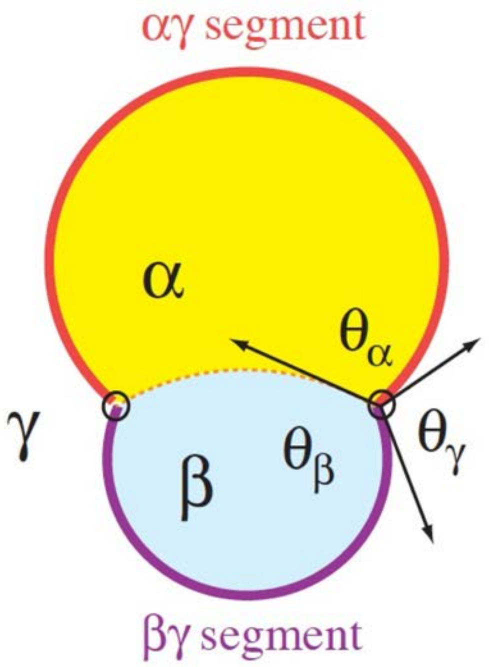
- Similar relation:

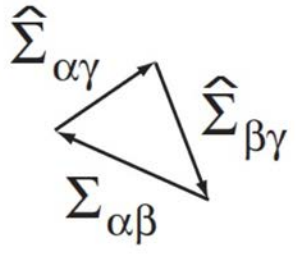
$$M_{\alpha\gamma}\left(\frac{\Sigma_{\alpha\gamma}}{\Sigma_{\alpha\beta}} - \frac{\sin\theta_\beta}{\sin\theta_\gamma}\right) = M_{\beta\gamma}\left(\frac{\Sigma_{\beta\gamma}}{\Sigma_{\alpha\beta}} - \frac{\sin\theta_\alpha}{\sin\theta_\gamma}\right)$$

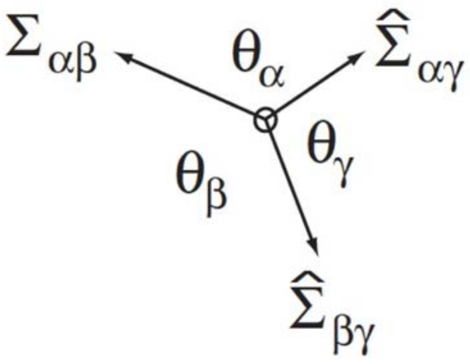
- But interfacial tensions Σ_{ij} are material parameters
- Expressions in two parentheses vanish separately

Special Parameter Regimes

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







Total segment tension

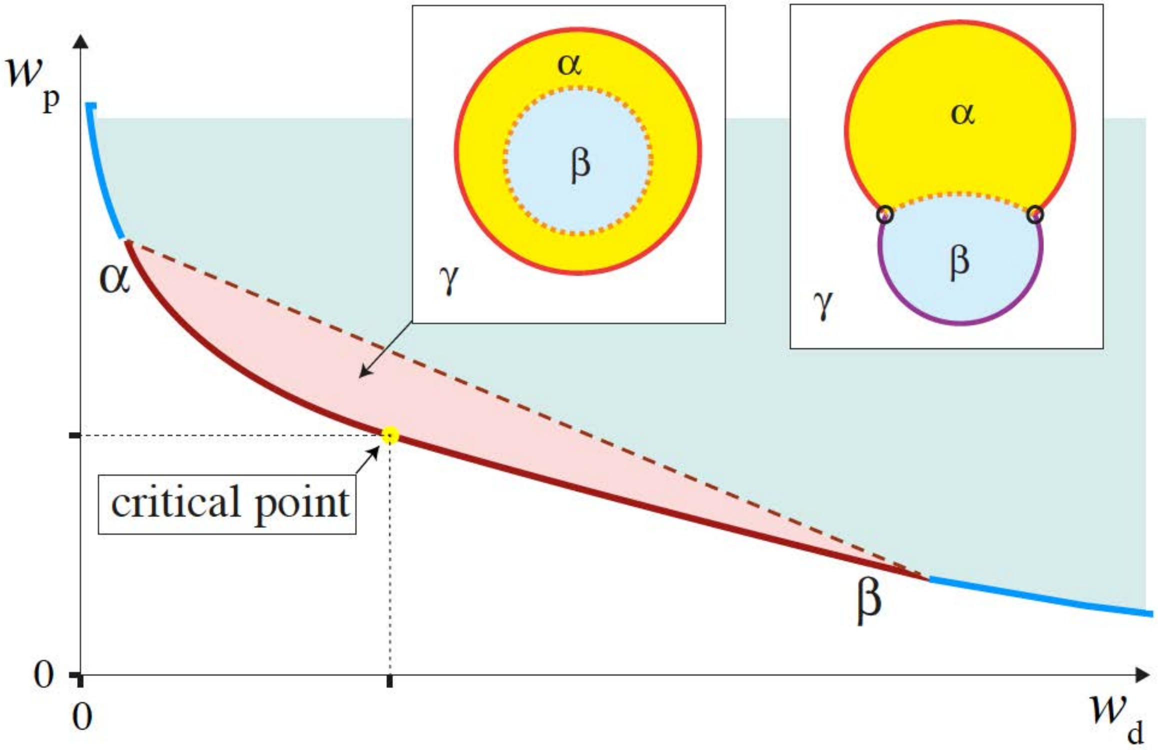
$$\hat{\Sigma}_{j\gamma} = \Sigma_{j\gamma} + \sigma_{j\gamma}$$

Sum of mechanical and spontaneous tension

- Force balance: $\dfrac{\hat{\Sigma}_{\alpha\gamma}}{\Sigma_{\alpha\beta}} = \dfrac{\sin\theta_\beta}{\sin\theta_\gamma}$ and $\dfrac{\hat{\Sigma}_{\beta\gamma}}{\Sigma_{\alpha\beta}} = \dfrac{\sin\theta_\alpha}{\sin\theta_\gamma}$

Phase Diagram

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



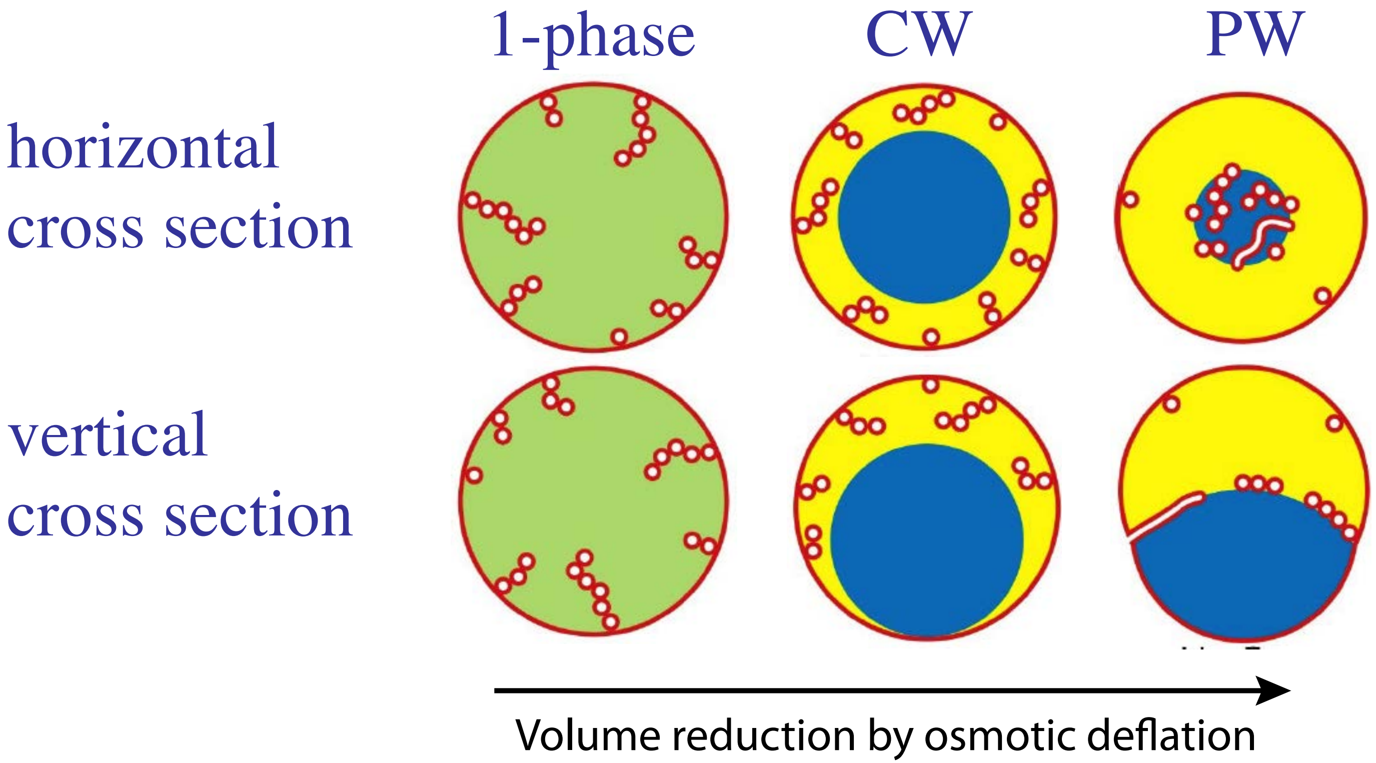
coexistence region
= two subregions:
pink: complete wetting
blue: partial wetting

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

Nanotubes and Wetting

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

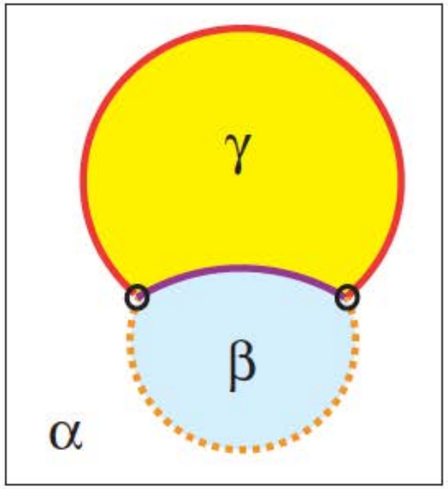
- Tubulation followed by phase separation:



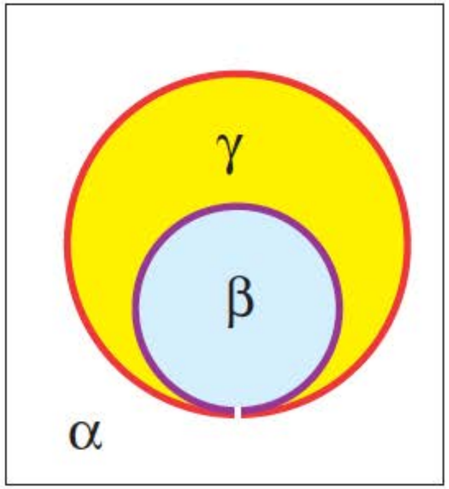
- CW: Nanotubes stay away from $\alpha\beta$ interface
- PW: Nanotubes adhere to $\alpha\beta$ interface

Out-Wetting Morphologies

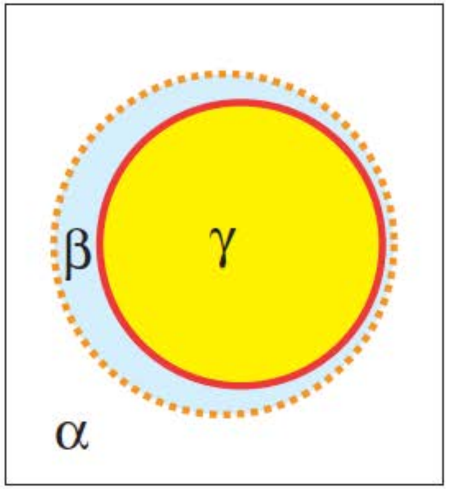
- Three aqueous phases α, β, γ
- Phase coexistence of α and β in exterior solution
- GUV membrane encloses spectator phase γ



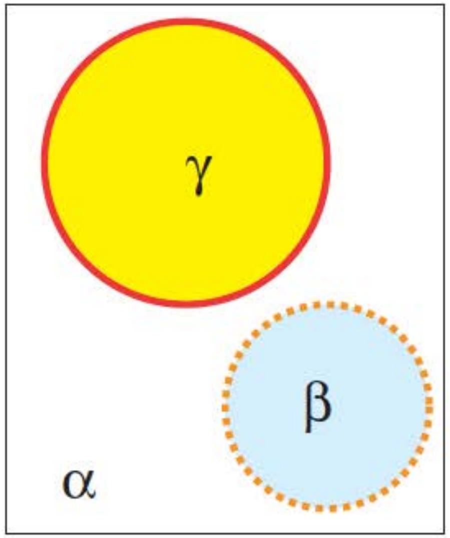
Partial wetting by α and β



Complete wetting by γ, Membrane neck



Complete wetting by β

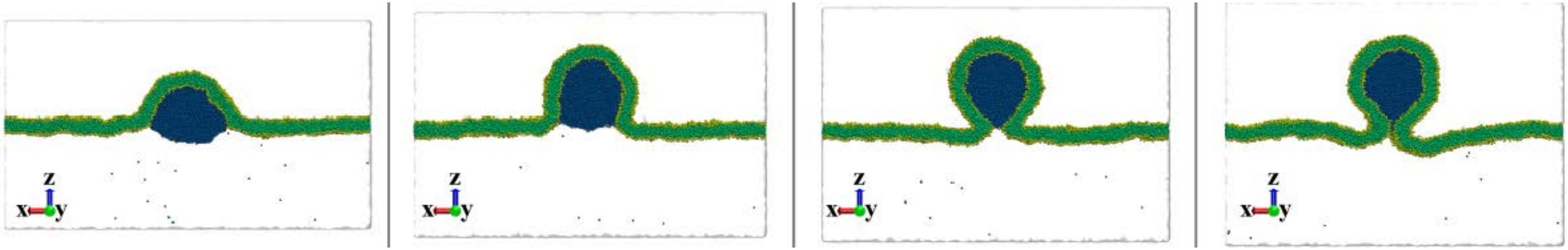


Complete wetting by α

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
- Membrane deformation can reduce interfacial area
- Droplet generates bilayer asymmetry and spont curv
- Example: Engulfment of droplet by membrane

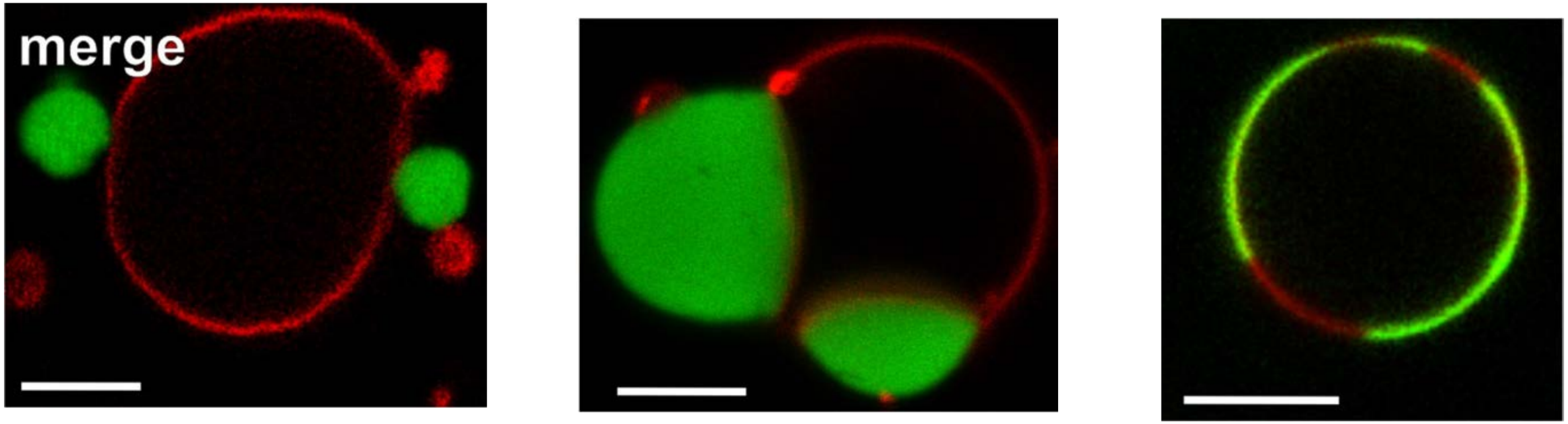


Vahid Satarifard (unpublished)

Membraneless Organelles

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

- Membrane-less organelles 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:



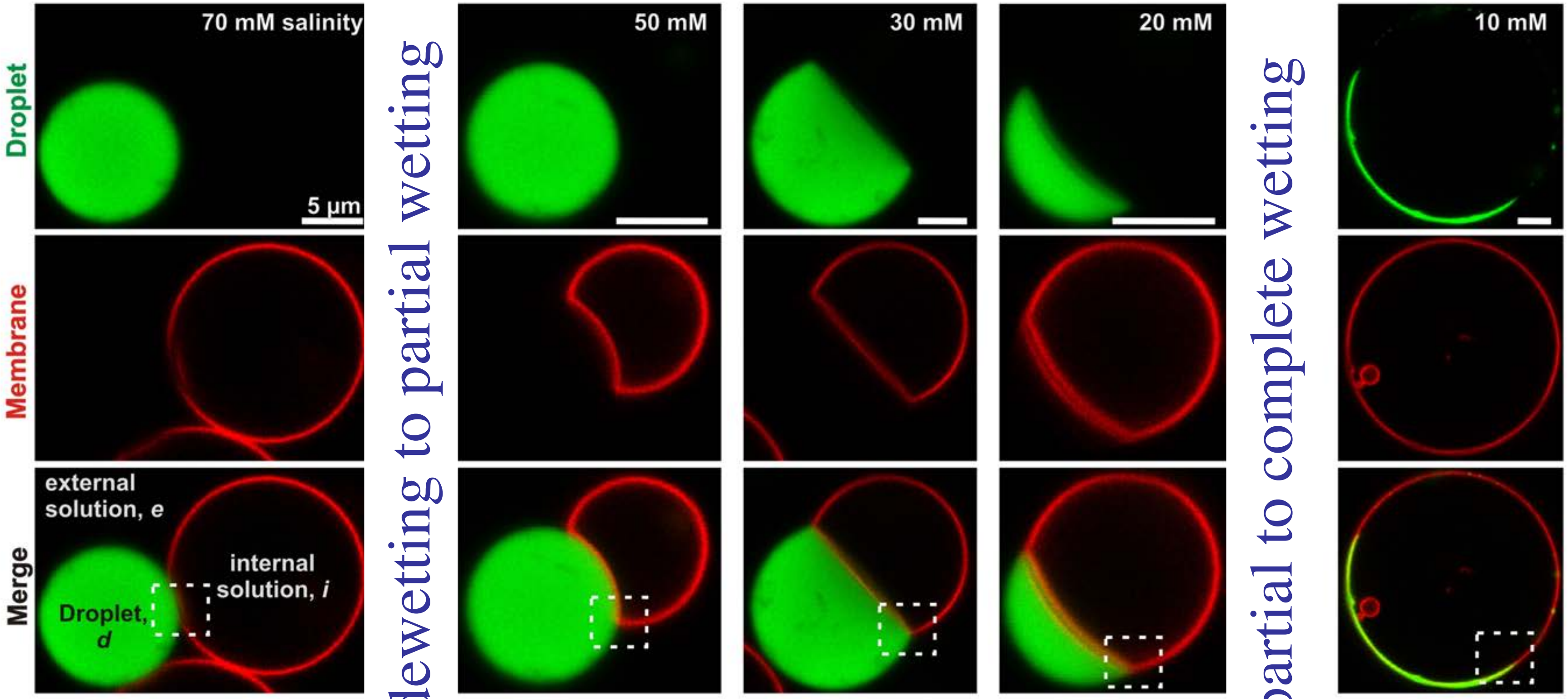
dewetting for high salt

partial wetting for intermediate salt

complete wetting for low salt

Two Wetting Transitions

- GUV + FUS-rich organelle + salt



dewetting to partial wetting

partial to complete wetting

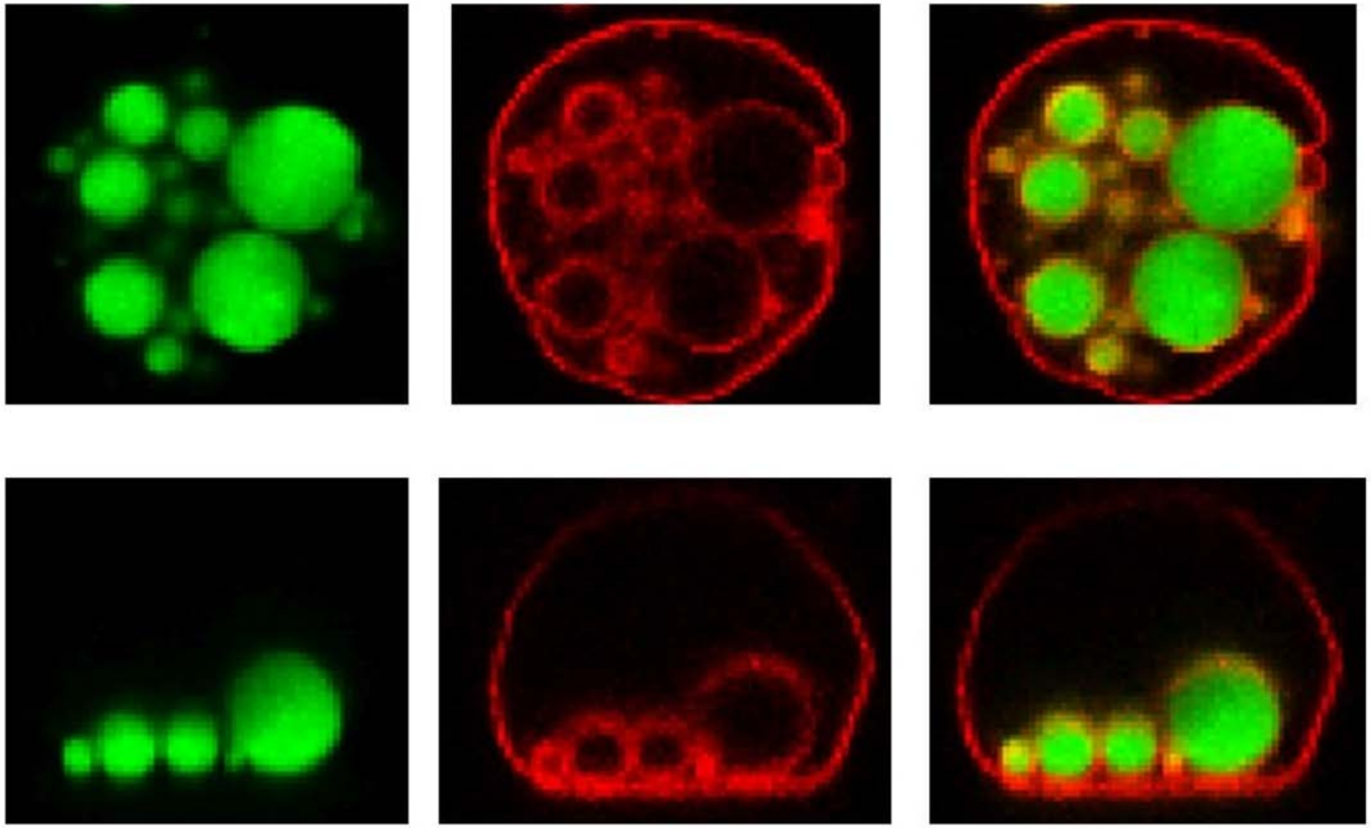
dewetting
for high salt

partial wetting for
intermediate salt

complete wetting
for low salt

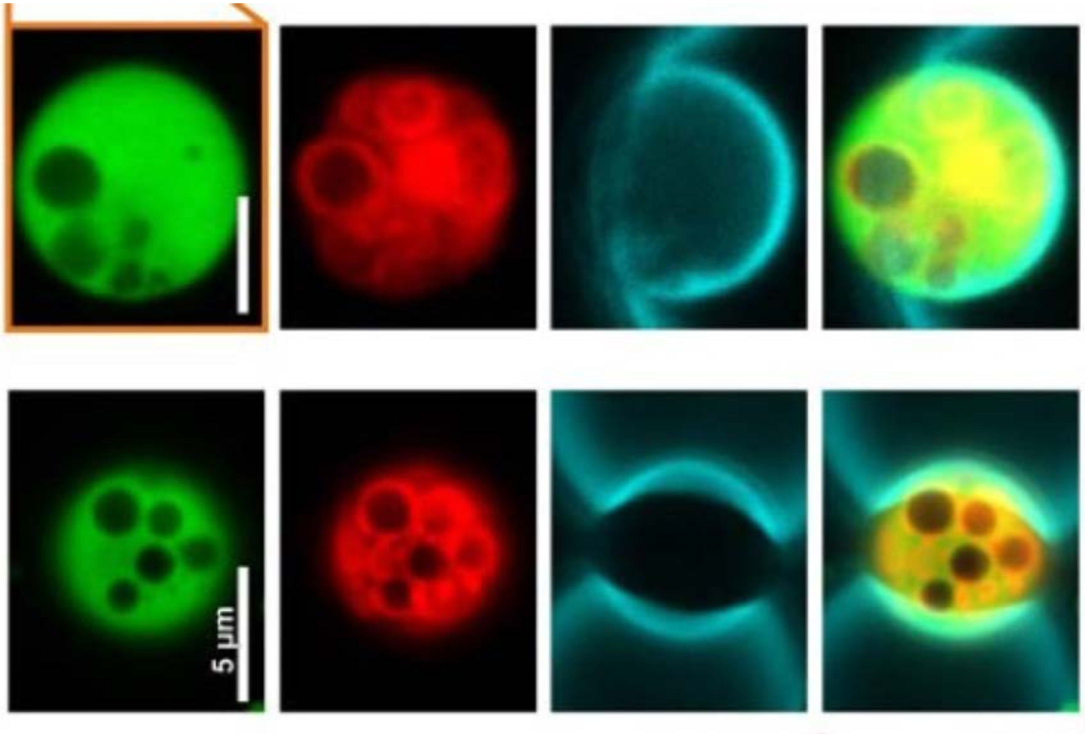
Engulfment of Organelles

- Green FUS-rich organelles engulfed by red GUV:



Two Types of Membranes

- Two types of GUVs with different lipid compositions
- FUS is green, GUV-1 is red, GUV-2 is blue



single blue
GUV-2

two blue
GUV-2s

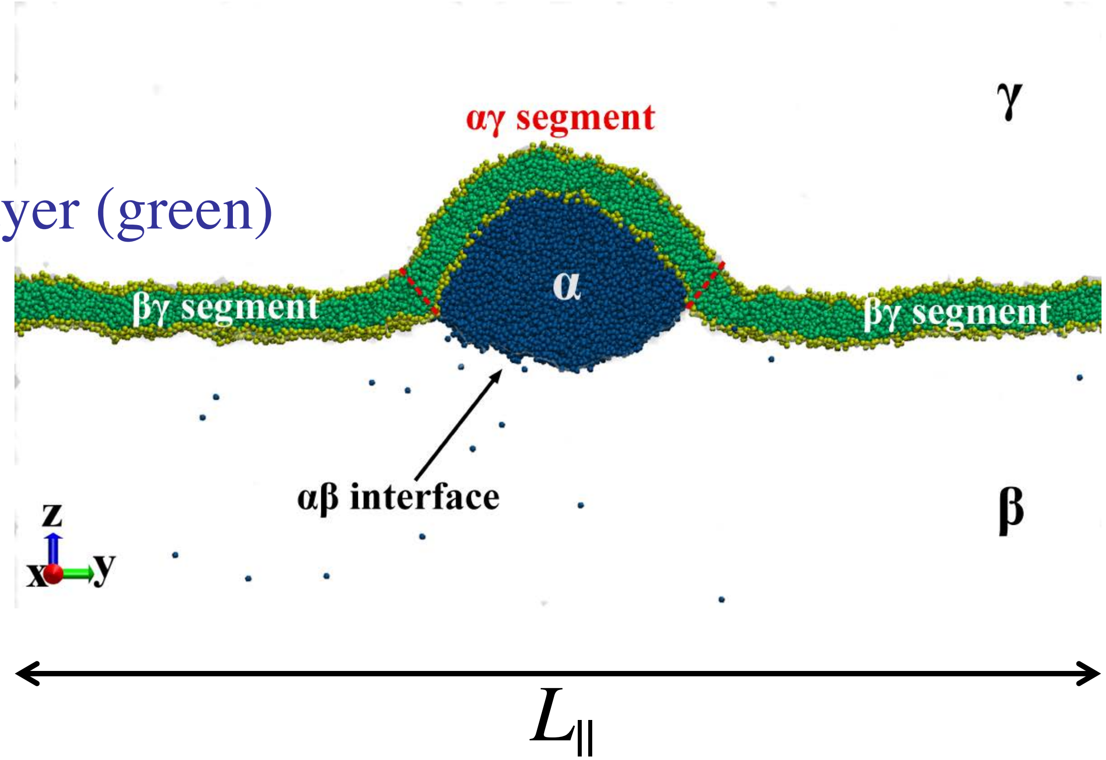
- red GUV-1s enclosed by green FUS-rich organelle
- green organelle partially engulfed by blue GUV-2s

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)

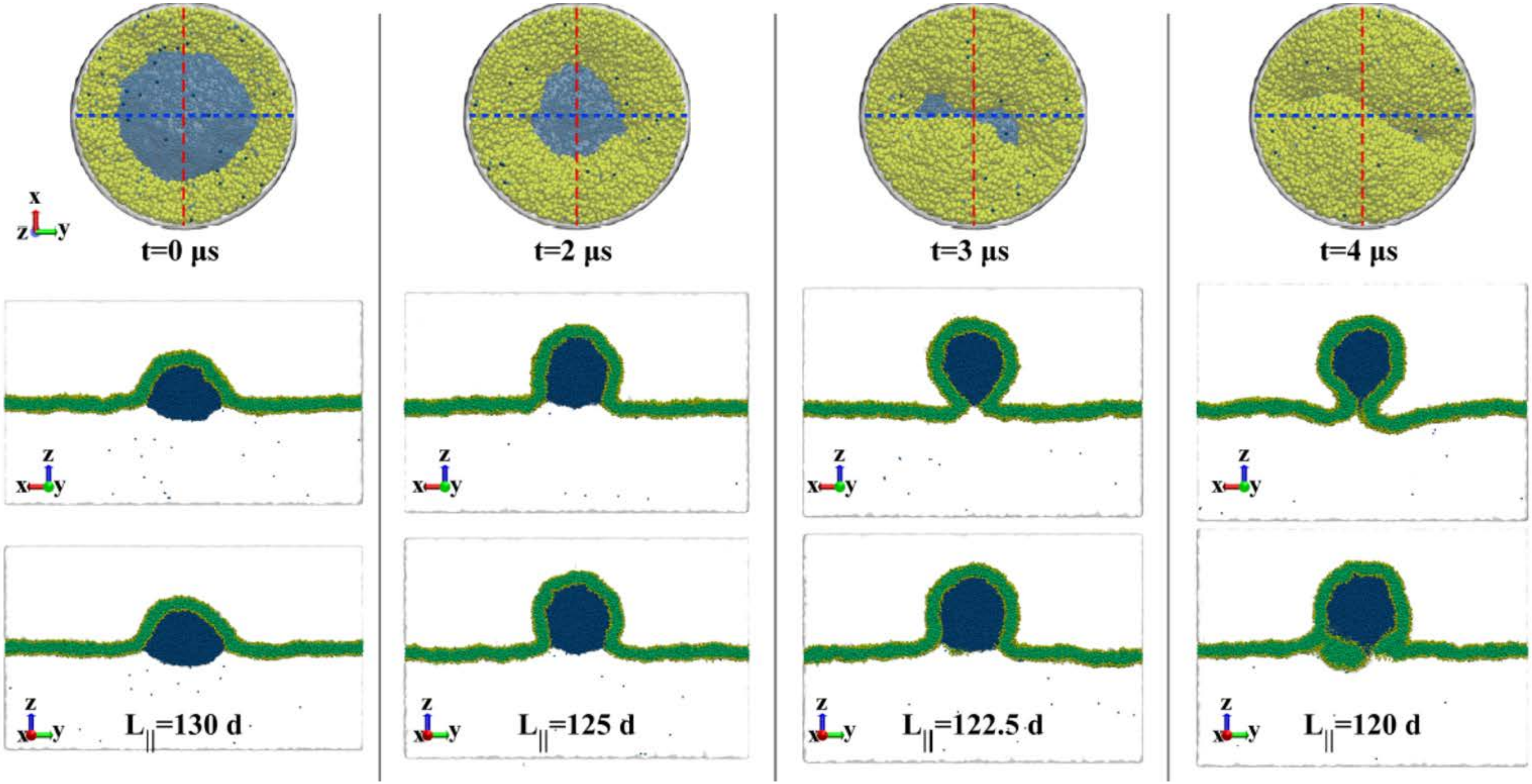


three aqueous phases α, β, γ

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

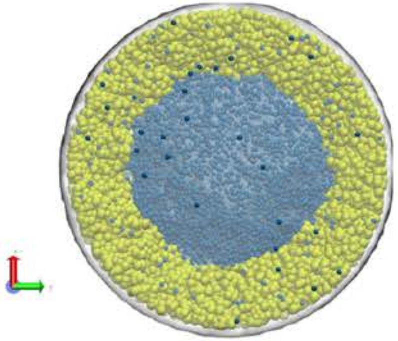
three surface segments αβ, αγ, βγ

Engulfment from Different Angles

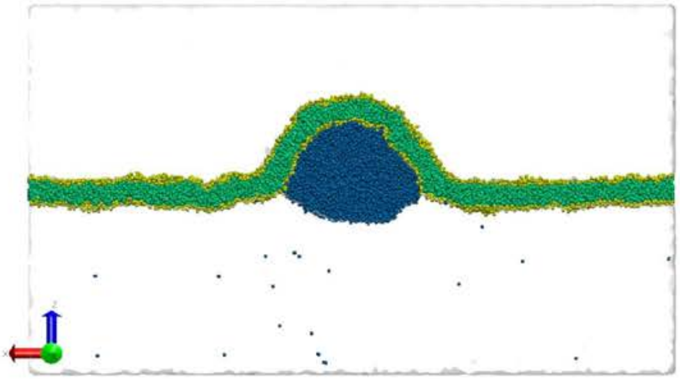


Engulfment Movie

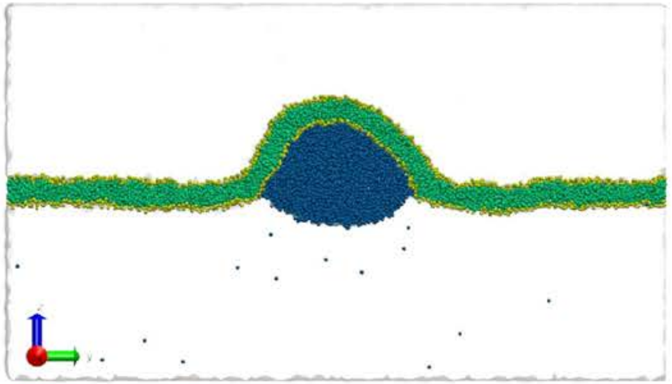
Bottom View



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

Coworkers

Rumiana
Dimova

Vahid
Satarifard

Andrea
Grafmüller

Potsdam: No mountains ☹
but much waters ☺

Tony Hyman, Titus Franzmann
Dresden