# Understanding
# Membranes and Vesicles

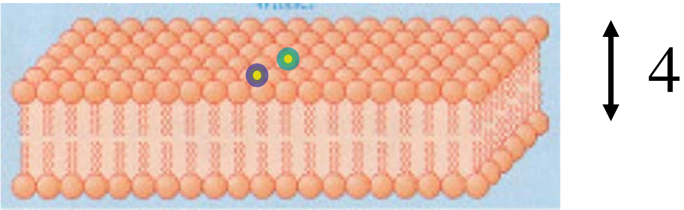
Reinhard Lipowsky
*MPI of Colloids and Interfaces, Potsdam*

- Short Introduction to Biomembranes
- Importance of Spontaneous Curvature
- Morphological Complexity
- Controlled Division of GUVs
- Endocytosis of Nanoparticles
- Membranes and Biomolecular Condensates

# Biomembranes are Fluid Bilayers

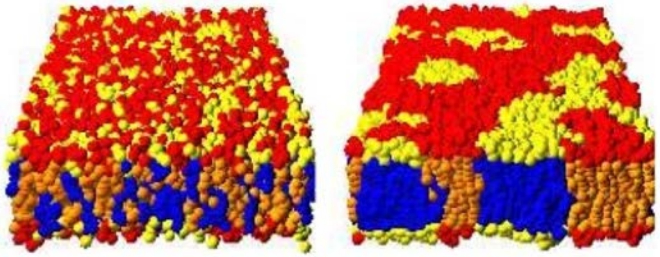
- Fluid membranes, i.e.,
  fast lateral diffusion:

  Diffusion constant ~ µm$^2$/s

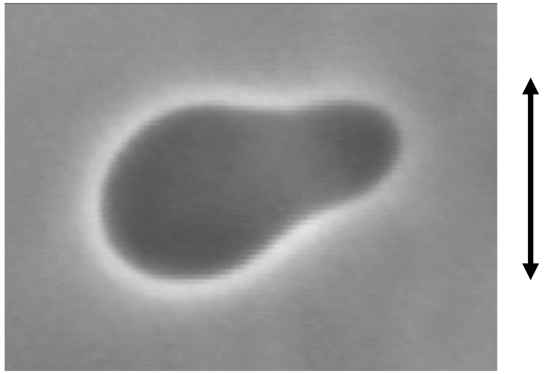


lipid swapping ~ ns

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

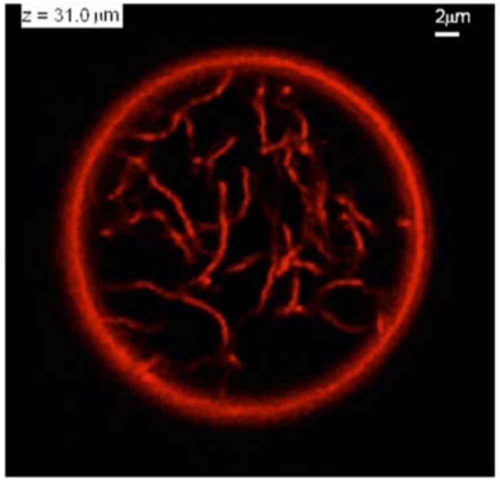


- Flexibility =>
  Morphological responses,
  budding, tubulation, …
  Direct evidence for fluidity

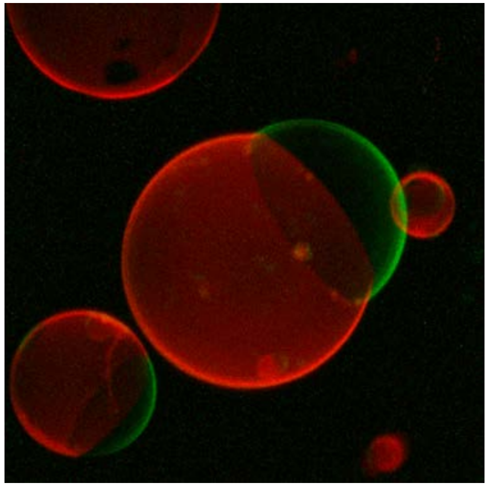


# Multiresponsive Behavior of GUVs

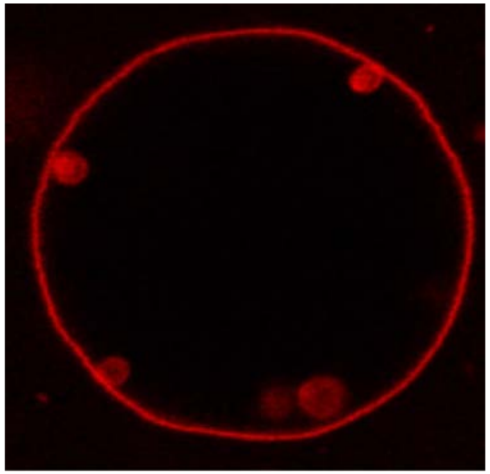
- GUVs = giant unilamellar vesicles
- GUVs exposed to different environments
- GUVs respond by membrane remodeling



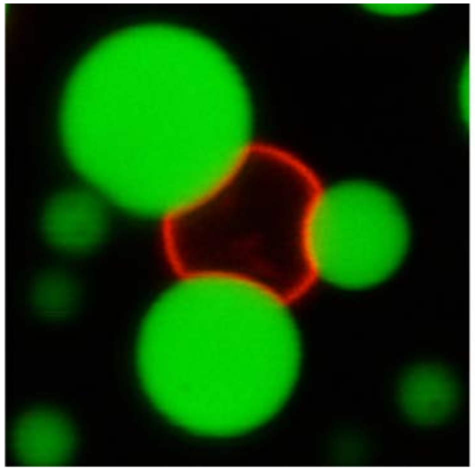
Nanotubes from polymer adsorption, tube width ~ 100 nm



Formation of intra-membrane domains, 2D phase separation



Small buds from adsorption of two ESCRT proteins

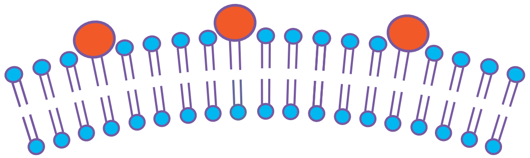


Shaping GUVs by biomolecular condensates

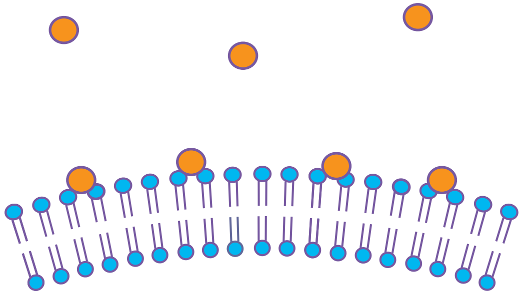
# Key Parameter: Spontaneous Curvature

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

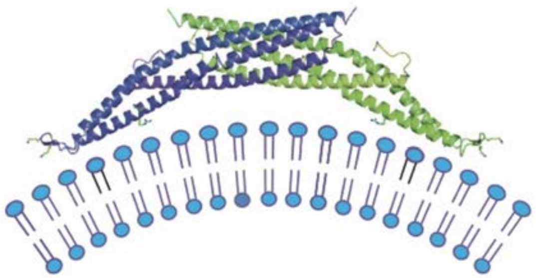
- Different molecular mechanisms for bilayer asymmetry:



Asymmetric composition, e.g., ganglioside



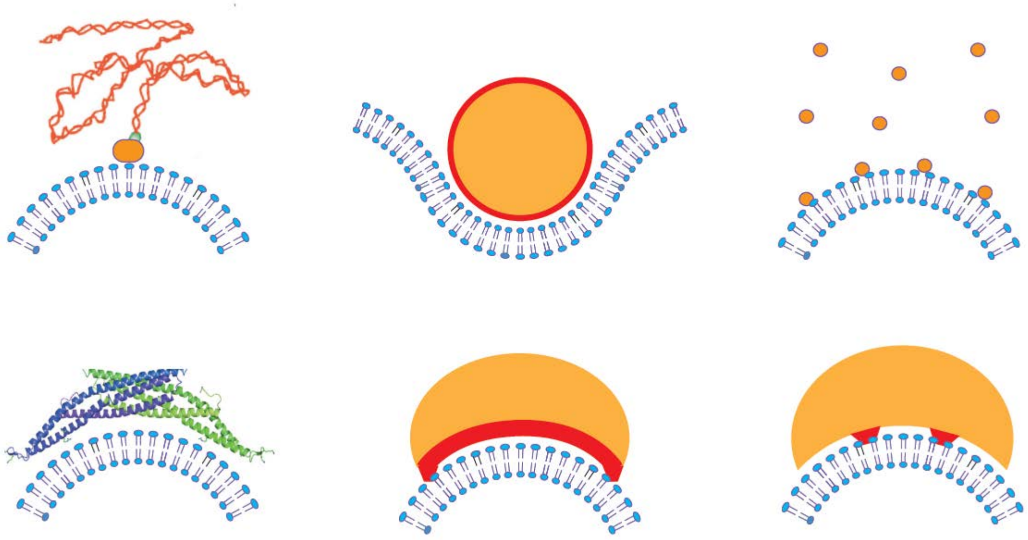
Asymmetric adsorption of small molecules



Asymmetric protein coats, e.g. BAR-domain

# Importance of Sp-Curvature I

- Bridges the gap between molecular scales and micron scales
- Spatially uniform membranes: Micrometer-sized shape of GUVs depends only on volume, area, and spont curvature
- Spont curvature determined by local bilayer asymmetry
- Emergent property on length scales that exceed about 6 nm
- Asymmetry may include lipids, proteins, block-copolymers, nanoparticles, ...



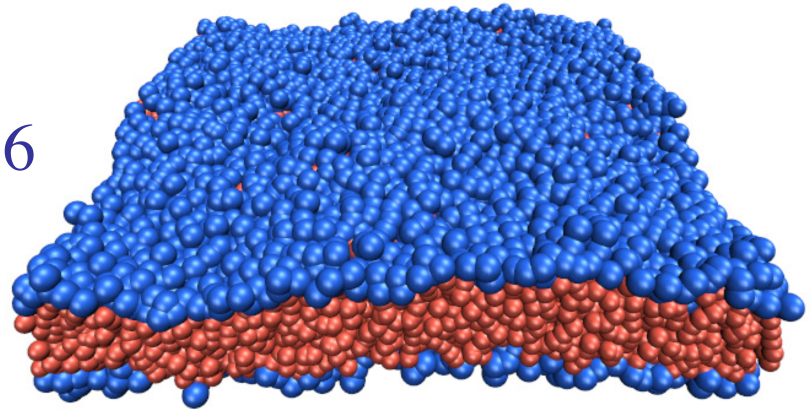
# Sp-Curvature from MD Simulations

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

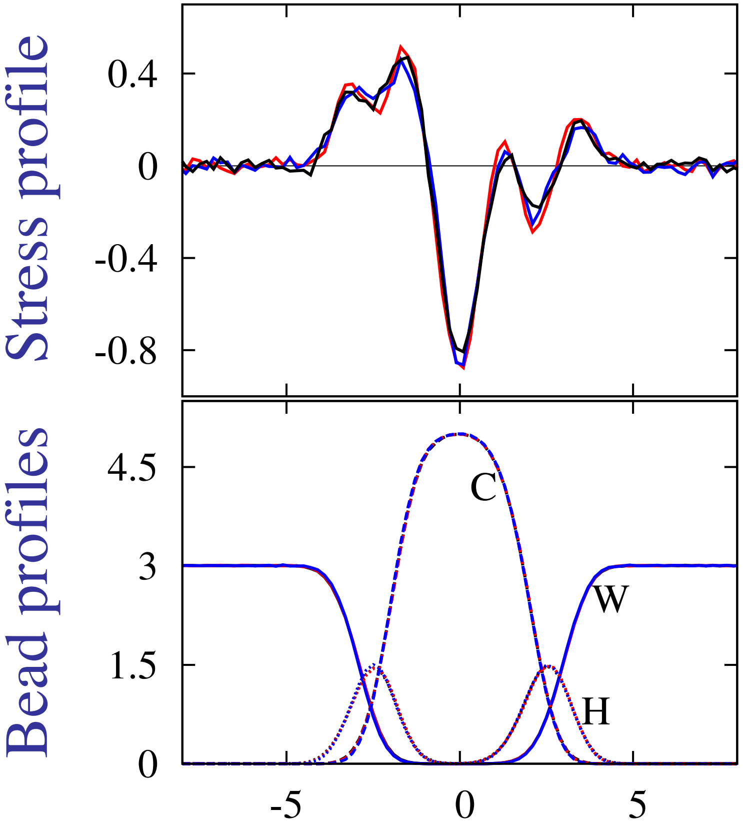
outer leaflet with $N_{ex}$ lipids



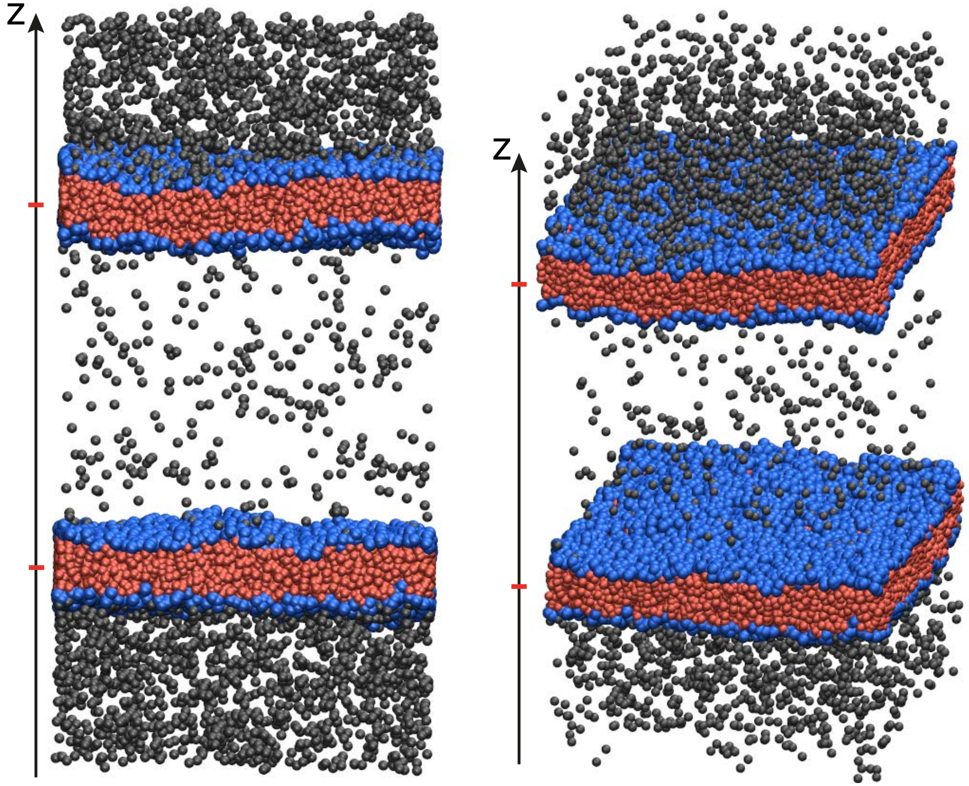
$\phi = 0.56$

inner leaflet with $N_{in}$ lipids

$\phi = 0.51$



# Asymmetric Adsorption and Depletion



Particle concentration $X_{\text{ex}}$

Bilayer 1

Particle concentration $X_{\text{in}}$

Bilayer 2

Particle concentration $X_{\text{ex}}$

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

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

# Importance of Sp-Curvature II

Sp-curvature crucial for:

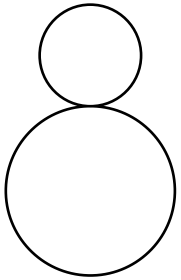
- Size of membrane buds, geometry of membrane necks
- Domain-induced budding of phase separated membranes
- Spontaneous formation of membrane nanotubes
- Constriction forces around membrane necks
- Endocytosis of nanoparticles
- ...

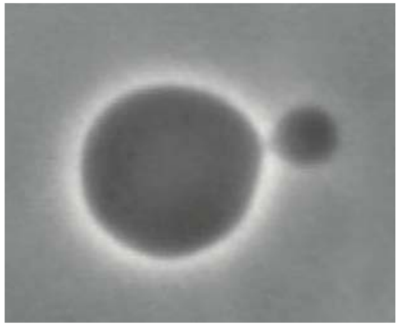
Two challenges:

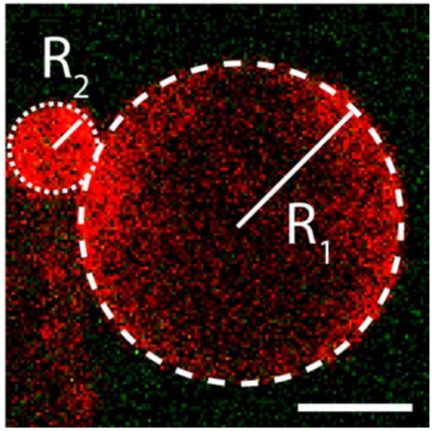
- How to measure or deduce the sp-curvature $m$?
- How to specify and control the sp-curvature $m$?

# Stable Buds and Membrane Necks

- Positive sp-curvature $m > 0$ leads to spherical membrane segments connected by closed necks = dumbbells:



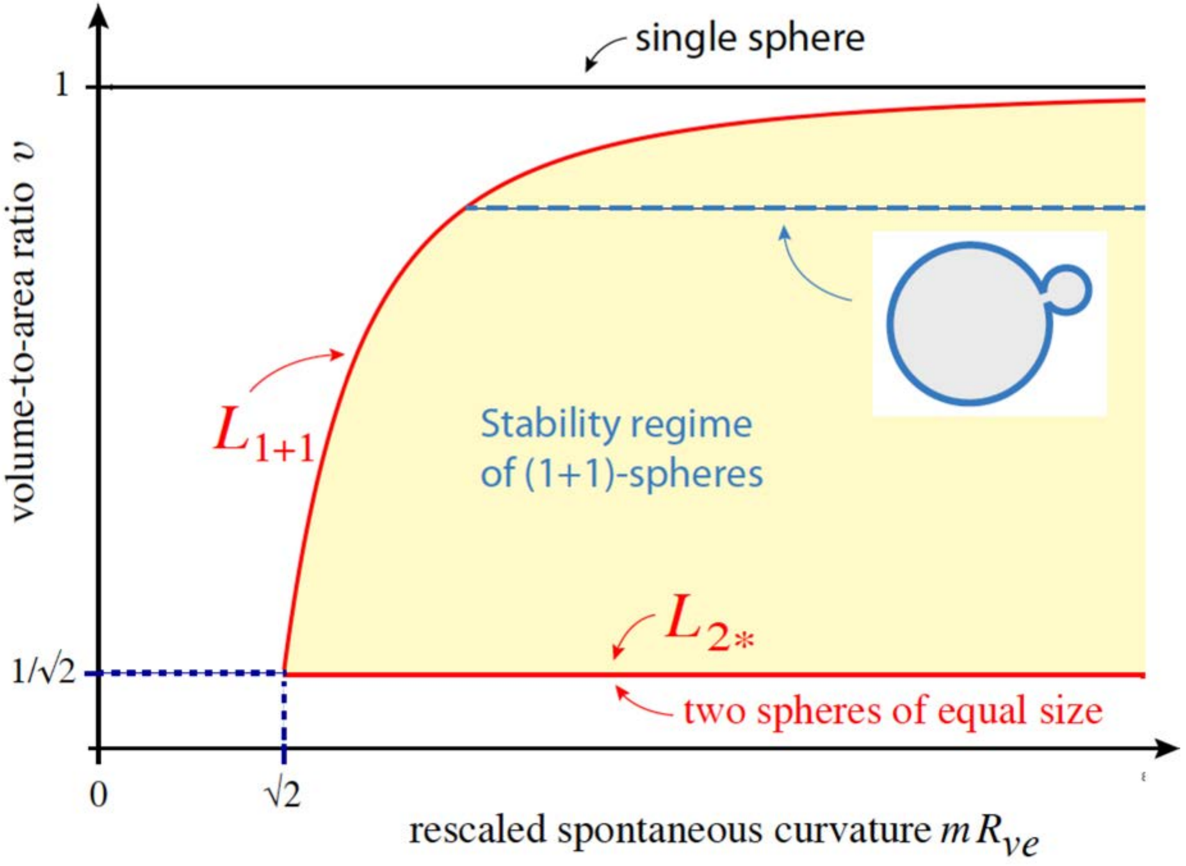




- Sphere radii $R_1$ and $R_2$
- Mean curvatures $M_1 = 1/R_1$ and $M_2 = 1/R_2$
- Neck curvature $M_{ne} = (1/2)\,(M_1 + M_2)$
- Closed neck is stable if $0 < M_{ne} \leq m$
- Local relation between geometry and material parameter

# Stability Regime of Dumbbells

- Vesicle area $A$, vesicle size $R_{\rm ve} = [A/(4\pi)]^{1/2}$
- Dimensionless volume $v \sim V/R_{\rm ve}^3$ with $0 < v \leq 1$
- Dimensionless sp-curvature $mR_{\rm ve} > 0$

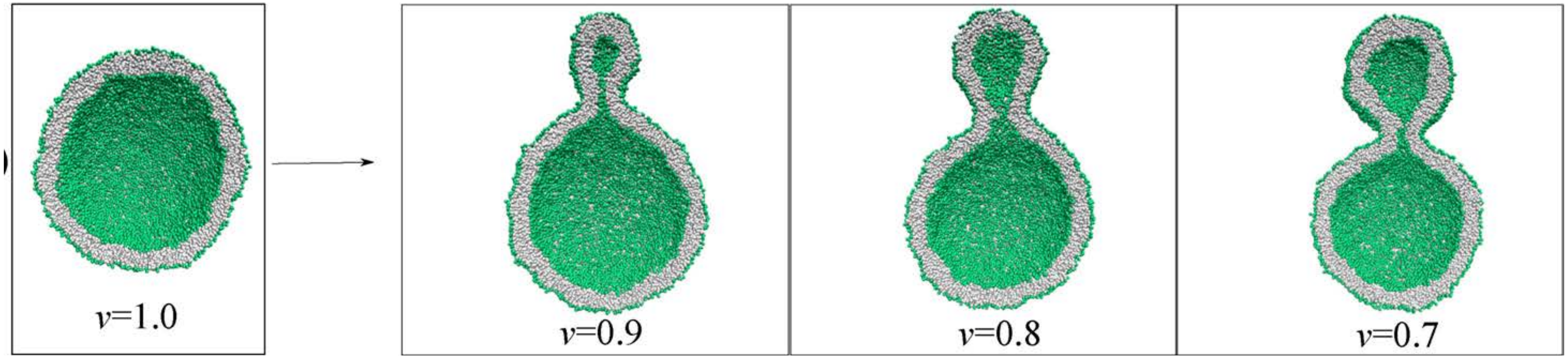


within yellow
stability regime:
dumbbell shape
depends only
on $v$ and not on $mR_{\rm ve}$

# Budding of Nanovesicles: MD

Rikhia Ghosh, Vahid Satarifard, A. Grafmüller, RL (in preparation)

- Spherical nanovesicle with diameter of 40 nm
- Decreasing vesicle volume *v* , corresponding to deflation
- Formation of dumbbell with closed neck dumbbell:

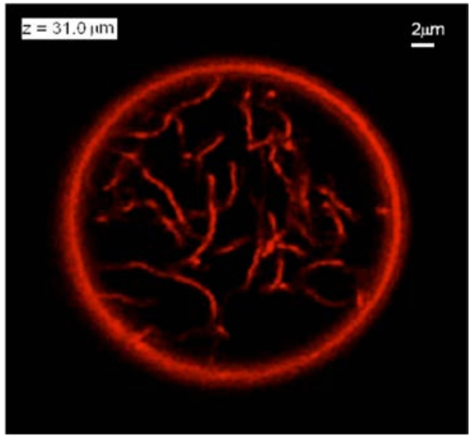


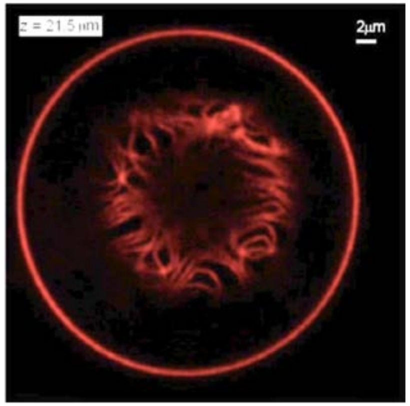
- Spontaneous curvature (sp-curvature)
- Spontaneous tubulation
- Morphological complexity
- Controlled division of GUVs
- Membrane tension(s)
- Endocytosis of nanoparticles
- Biomolecular condensates at membranes

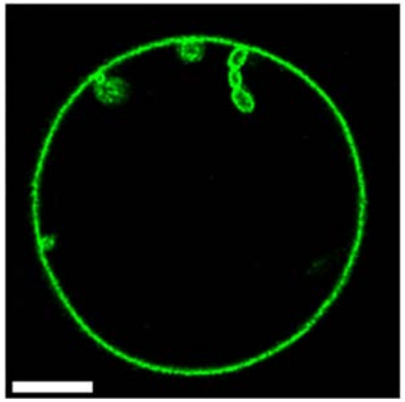
# Large Sp-Curvature: Nanotubes

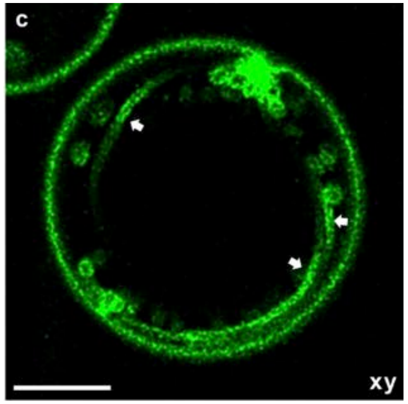
Li ... Dimova, *PNAS* (2011) Liu ... RL, *ACS Nano* (2016)

- Lipid mixture of DOPC, DPPC, cholesterol
- Small amounts of fluorescently labeled lipids
- Liquid-disordered (red) and liquid-ordered phase (green)





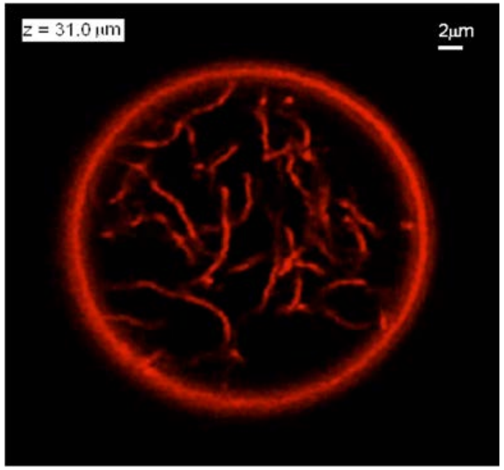




- Spontaneous tube formation without external forces
- Complex patterns of nanotubes

# Sp-Tubulation and Sp-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 $\Sigma$ 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 $\approx -1/(100\ \text{nm})$

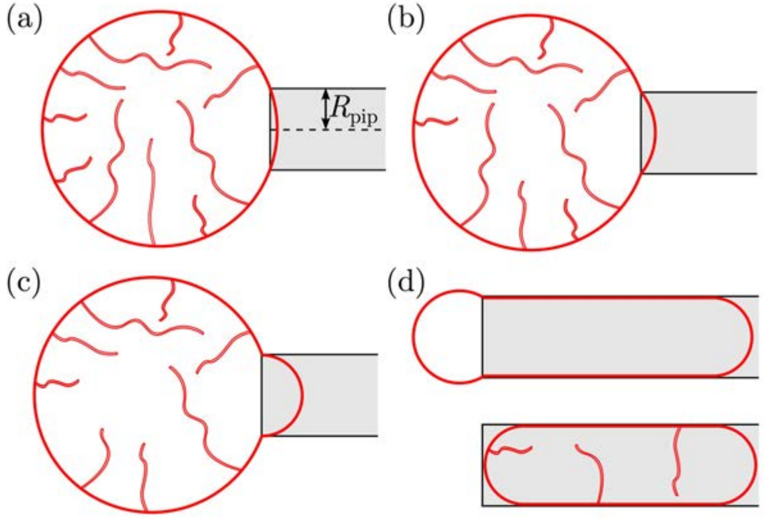
Spontaneous tension $\sigma \approx 10^{-2}$ mN/m

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

# Robustness of tubulated GUVs

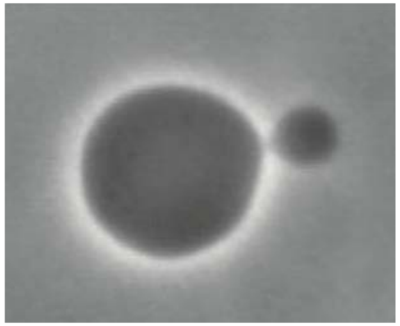
Bhatia ... RL: *ACS Nano* (2018)

- Conventional GUVs: Membrane rupture under osmotic inflation, strong adhesion, micropipette aspiration, ...
- Membrane nanotubes provide area reservoir
- Tubulated GUVs have very low mechanical tension and do not rupture under strong mechanical perturbations
- Robustness demonstrated for inflation and aspiration
- Membrane tension dominated by sp-tension $\sigma = 2\,\kappa\, m^2$
- Mother vesicle behaves like liquid droplet with interfacial tension = sp-tension of membrane



# How Do Nanotubes Form?

Liu et al, *ACS Nano* (2016)

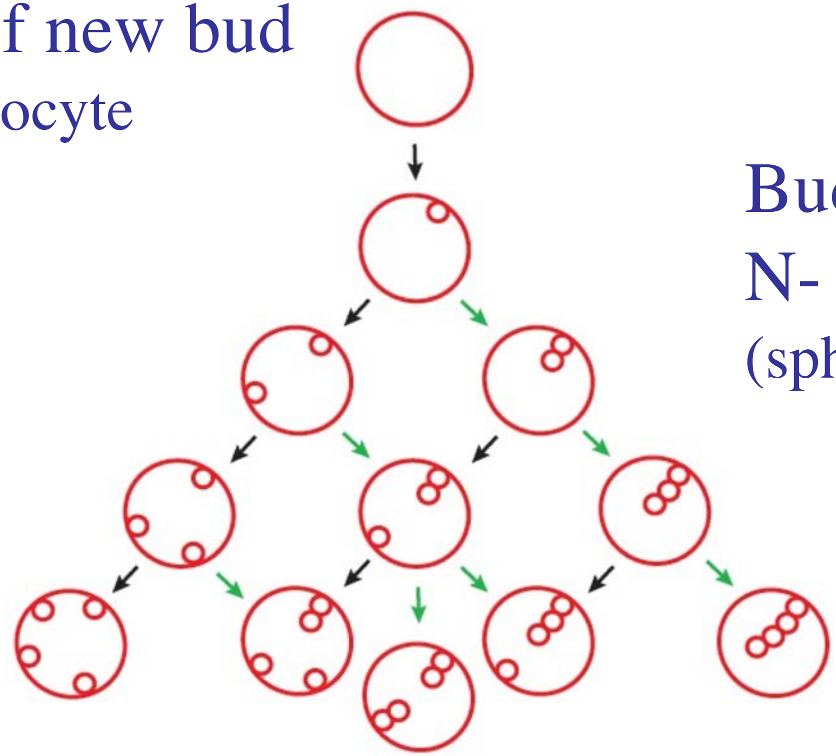


- Tubulation intimately related to budding
- Osmotic deflation of spherical GUV
- Small deflation step leads to single bud
- Bud and mother vesicle connected by membrane neck
- Bud acts as nucleation site for necklace-like tube
- Several pathways for subsequent deflation steps:
    - Formation of new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

# Nucleation and Growth of Tubes

Liu et al, *ACS Nano* (2016)
RL, *J. Phys. D* (2018)

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

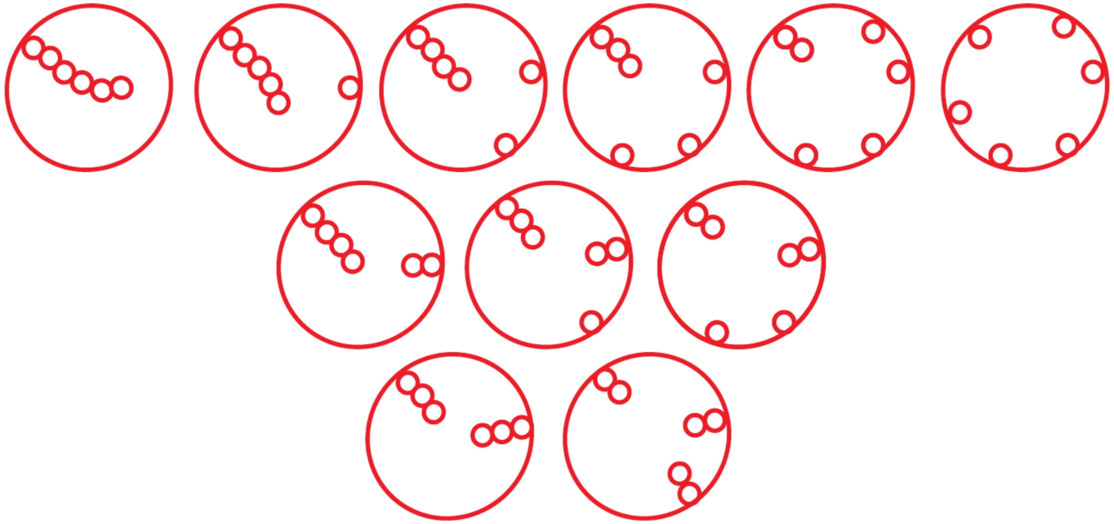


Formation of new bud
(oblate-stomatocyte
bifurcation)

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

# Morphological Complexity

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



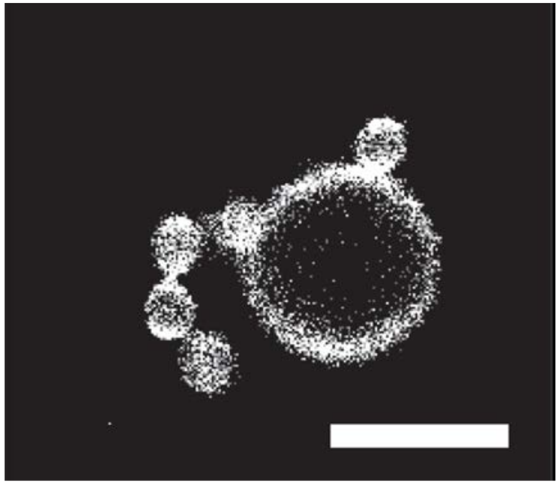
- All beads are connected by membrane necks
- All morphologies have the same area, volume, and curvature energy
- Rugged energy landscape contains 11 intersecting branches
- For large N, number of N-spherule morph grows as $\exp[c \sqrt{N}]$

# GUVs Exposed to Two Simple Sugars

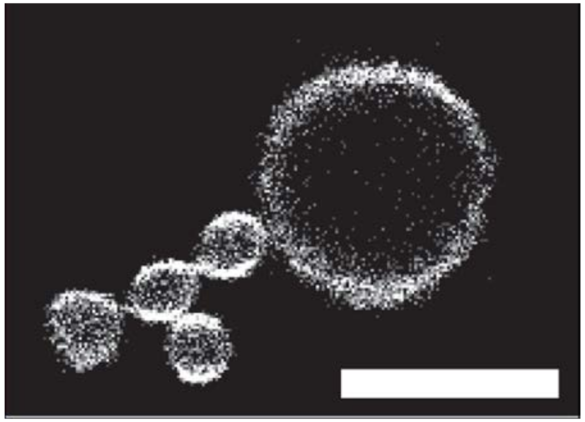
Tripta Bhatia, J. Steinkühler, R. Dimova, RL (in preparation)

- Out-Necklaces

Linear

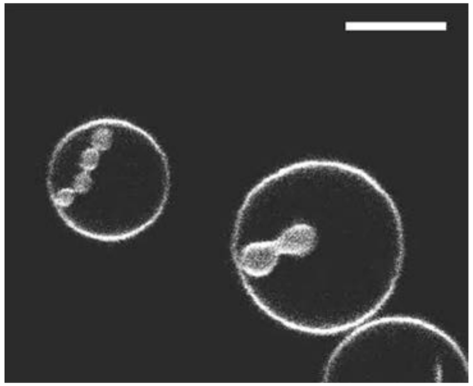


Branched



- In-Necklaces

Linear



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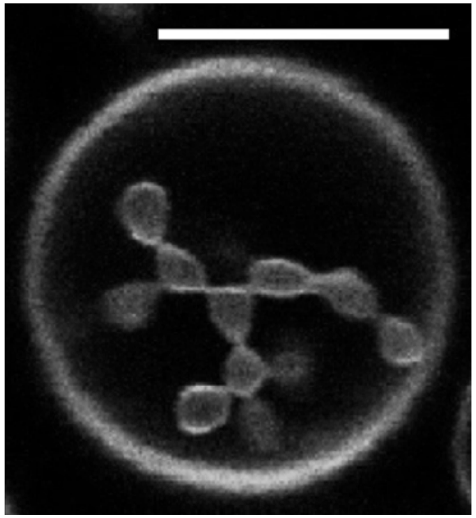
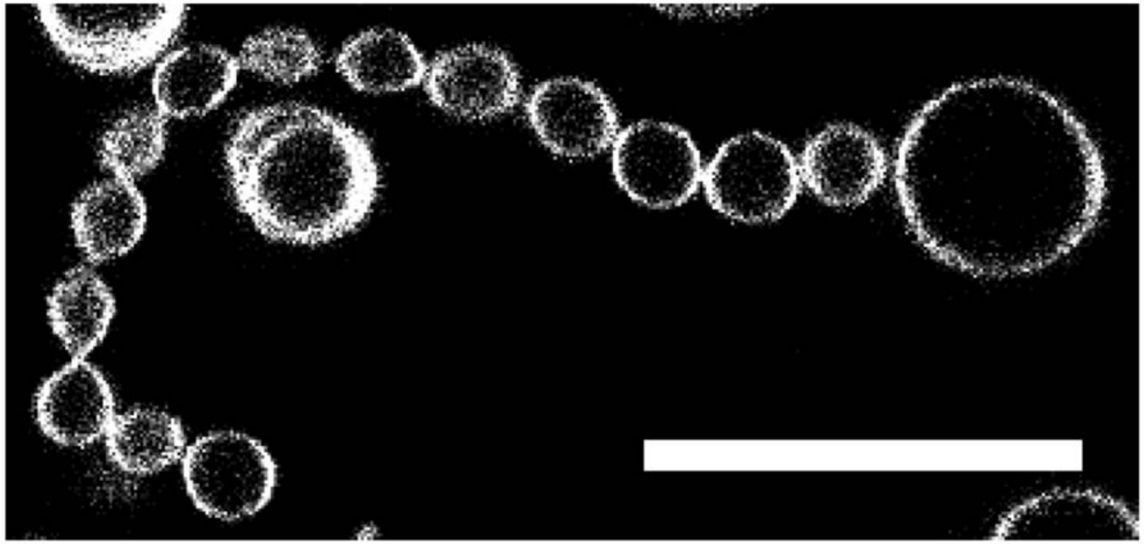


- Each budded shape formed by a single membrane!

# Morph Complexity: Experiment

- Out-Necklace with 14 beads:

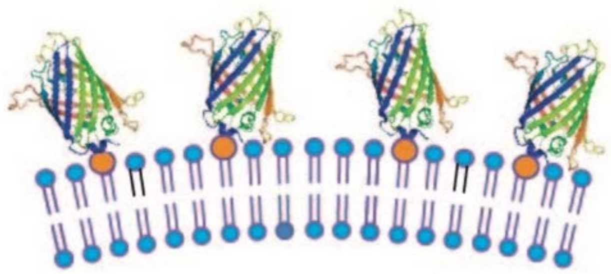


- Intermediate complexity between single buds and patterns of nanotubes
- SponCurv varied by changing sugar composition

# Controlled Variation of Sp-Curvature

Jan Steinkühler, ... , S. Wegner, R. Dimova, RL (in preparation)

- Binding of GFP to certain anchor lipids:



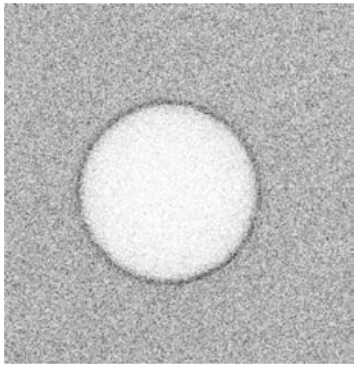
His-tagged GFP
NTA-lipids

- GFP solution concentration $X$ as control parameter
- Density $\Gamma$ of bound GFP increases linearly with $X$
- Sp-curvature $m$ increases linearly with $\Gamma$
- Dilute regime: separation of bound GFPs much larger than lateral size of GFP

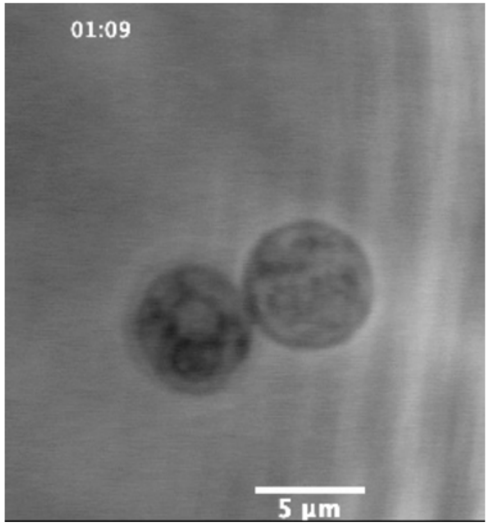
# Controlled Division of GUVs

- Osmotic deflation + GFP binding
- Osmotic deflation: Spherical GUV -> dumbbell GUV

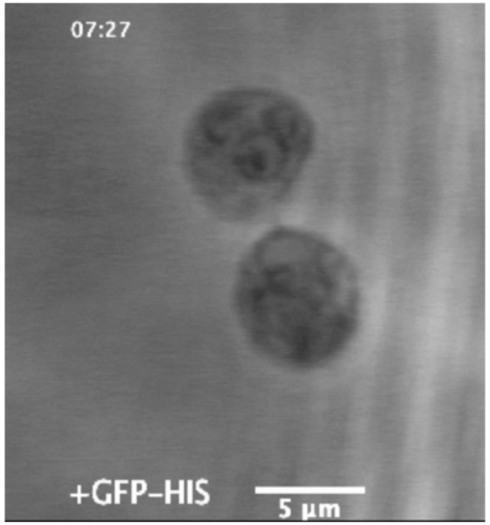
  Increase in GFP -> Neck cleavage -> Two daughter GUVs



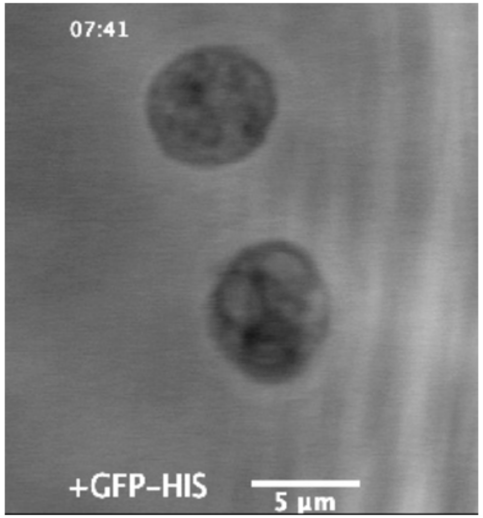
Adsorption of GFP onto GUV membrane



Deflation leads to dumbbell with membrane neck



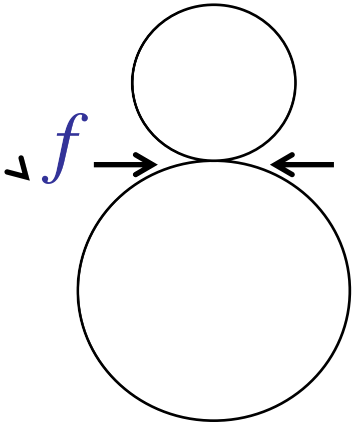
Directly after neck cleavage



Complete division into two smaller GUVs

# Constriction Force from Sp-Curvature

RL, *Advances in Biomembranes and Lipid Selfassembly* Vol. 30 (2019) Ch. 3

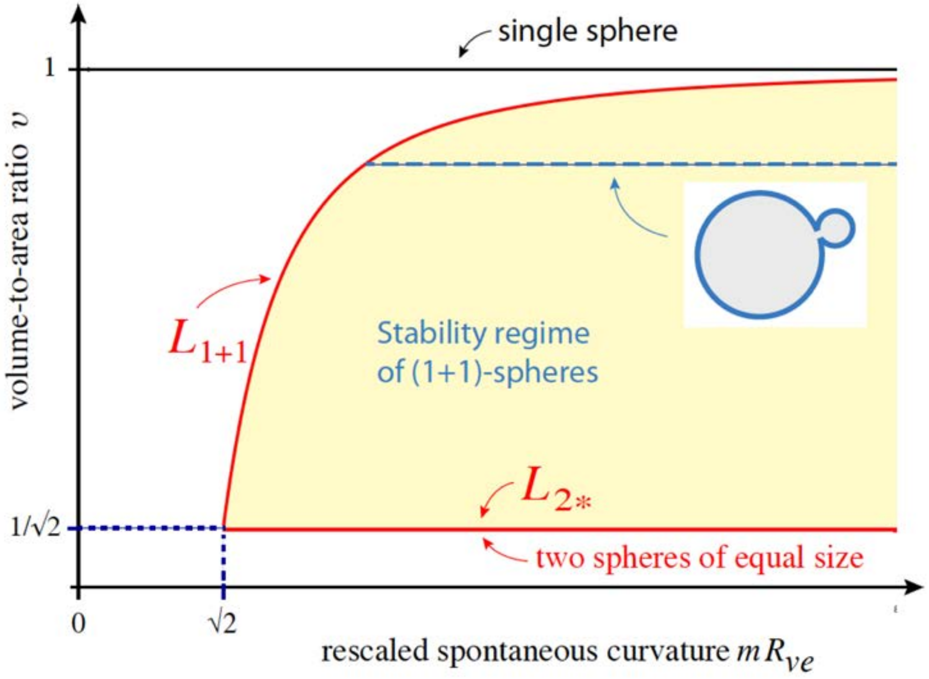


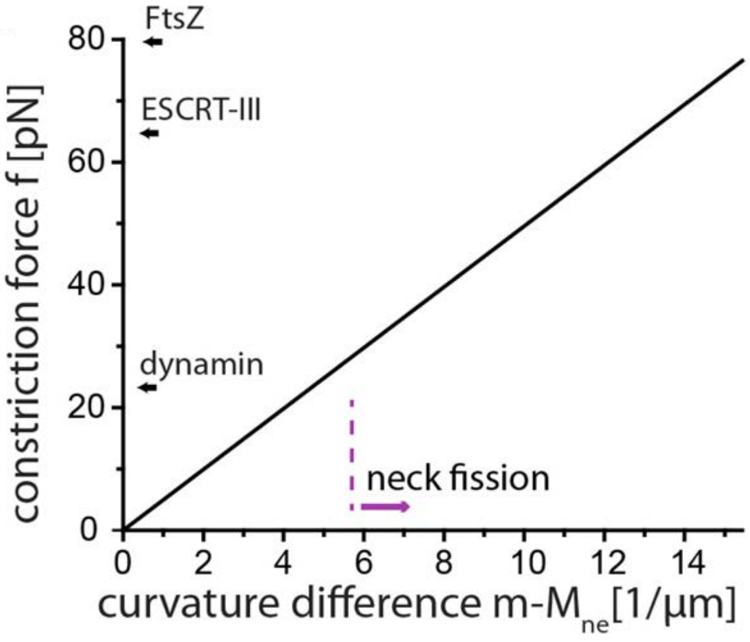
- Sp-curvature generates constriction force

$$f = 8\pi\,\kappa\,(\,m - M_{\mathrm{ne}}\,)$$

acting radially on closed membrane neck:

- Force increases with increasing sp-curvature:





- Spontaneous curvature (sp-curvature)
- Spontaneous tubulation
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# Membrane Tension

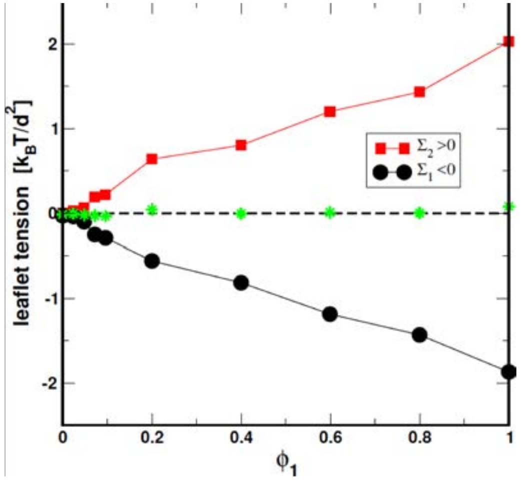
- Uniform membranes: Laterally uniform composition and spatially uniform environment
- Uniform membranes experience mechanical tension $\Sigma$ and spontaneous tension $\sigma = 2\,\kappa\, m^2$
- These two tensions act on the whole membrane
- Optimal packing of lipids ⇔ low mech tension $\Sigma$
  ⇔ tensionless bilayer

- But lipid membranes are molecular bilayers
- What about tensions within individual leaflets?

# Bilayer Membranes and Leaflet Tensions

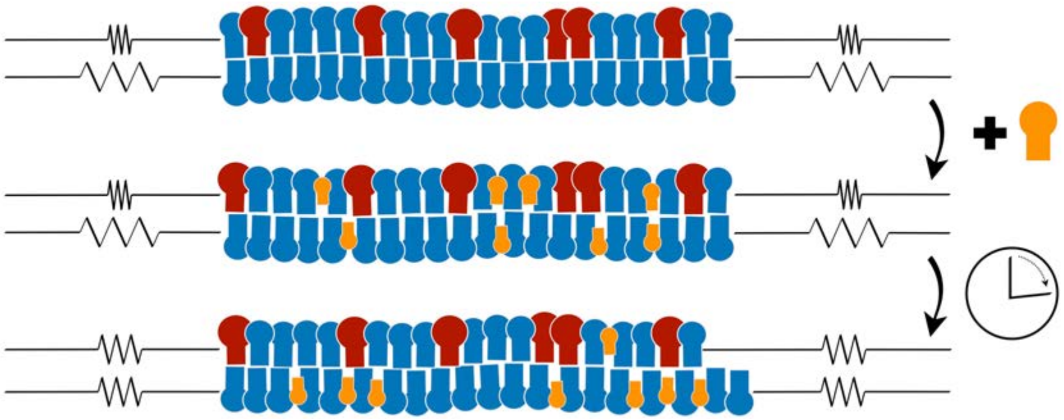
- Bilayer with two leaflets:
  - Two leaflet tensions $\Sigma_1$ and $\Sigma_2$ with $\Sigma_1 + \Sigma_2 = \Sigma$
  - Tensionless bilayer: $\Sigma = 0$
  - Leaflet tensions for binary mixture

Sreekumari, RL , J. Chem. Phys. (2018)



- Leaflet tensions and flip-flops:

Miettinen, RL , Nanoletters (2019)

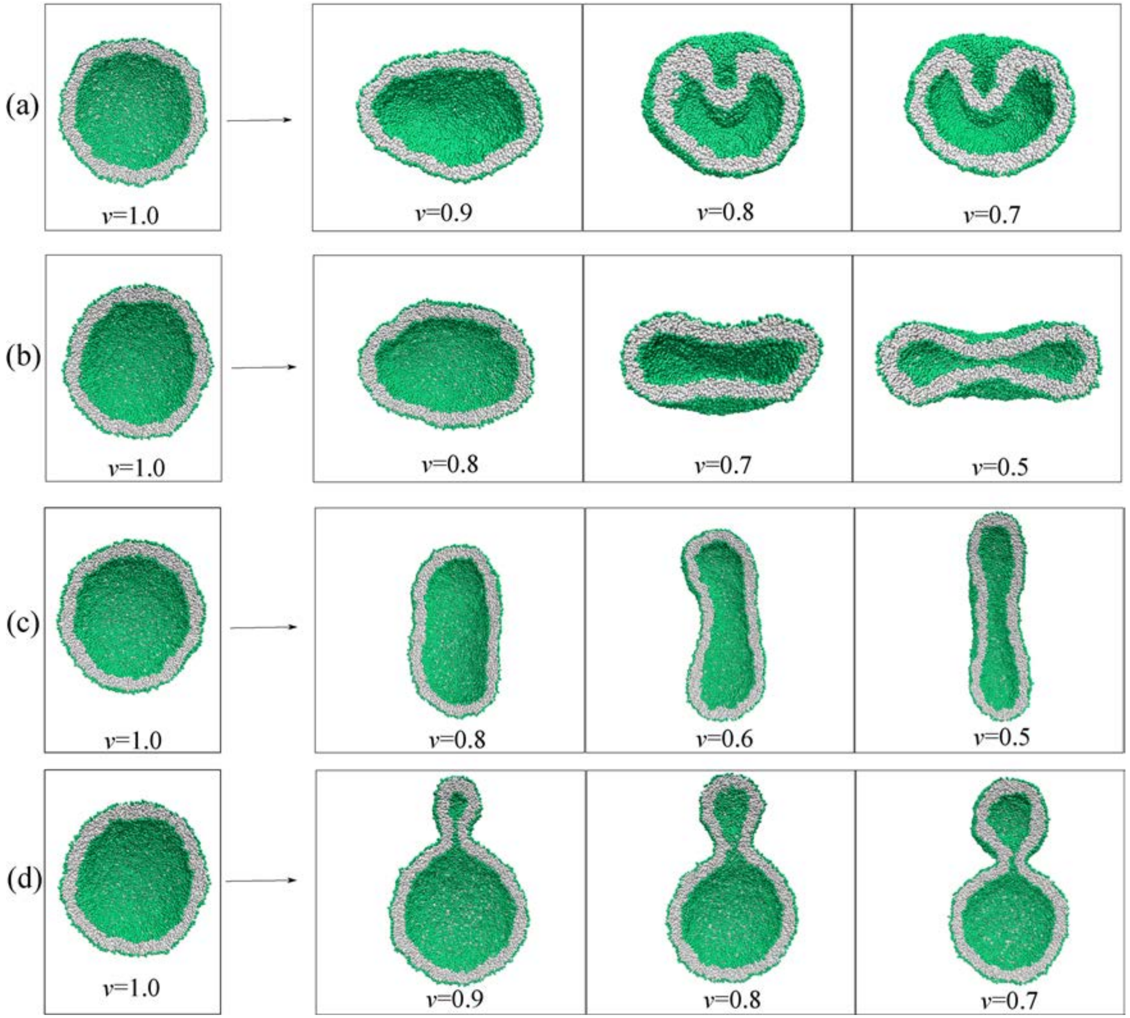


- Add cholesterol
- Leaflet tensions relax towards $\Sigma_1 = \Sigma_2 = 0$

# Leaflet Tensions and Nanovesicles

Rikhia Ghosh, Vahid Satarifard, A. Grafmüller, RL (in preparation)

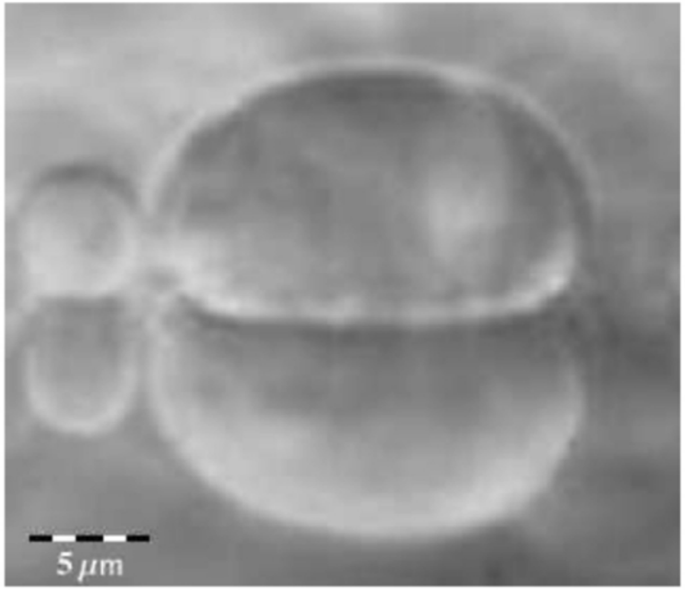
- Polymorphism of spherical nanovesicle:

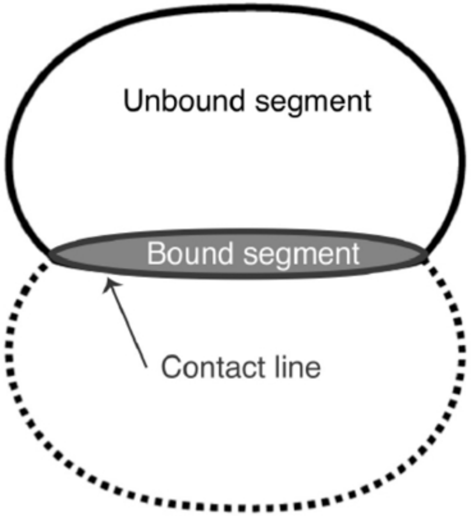


- Four spherical vesicles
- Same volume
- Same total # of lipids
- Reduction of volume: very different shapes
- Shape transformations determined by leaflet tensions $\Sigma_1$ and $\Sigma_2$

# Tension of Nonuniform Membranes

- Nonuniform membrane composition or nonuniform environment leads to segmentation of membranes
- Example: Adhesion of GUV





# Tensions of Membrane Segments

- Two environments, α and β, partition membrane into two membrane segments, *a* and *b*
- Total membrane area $A = A_a + A_b$
- Mechanical tensions $\Sigma_a$ and $\Sigma_b$
- Free energy terms:

$$\Sigma_a \ A_a + \Sigma_b \ A_b = \Sigma_a \ A + ( \Sigma_b - \Sigma_a ) \ A_b$$

- Adhesion free energy per unit area

$$\mathrm{W}_{ba} = \Sigma_b - \Sigma_a$$

- Membrane prefers $\beta$-environment: $\mathrm{W}_{ba} < 0$

- Spontaneous curvature (sp-curvature)
- Spontaneous tubulation
- Morphological complexity
- Controlled division of GUVs
- Membrane tension(s)
- Endocytosis of nanoparticles
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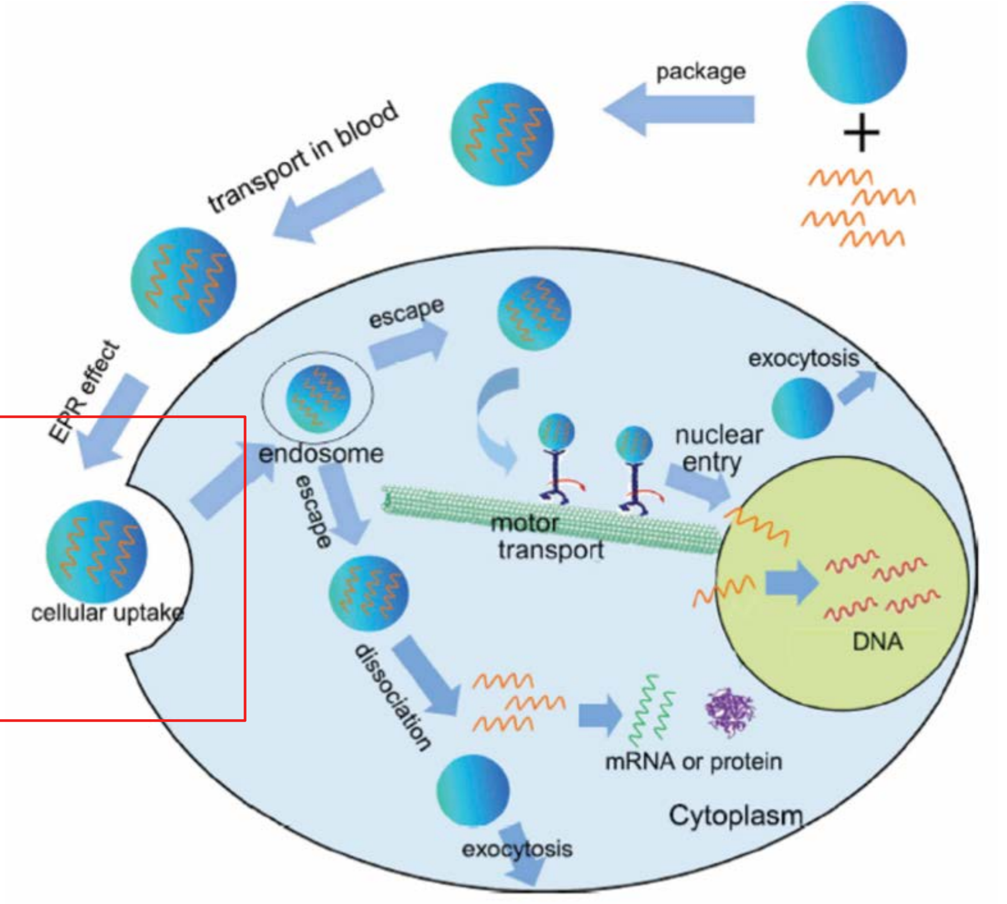
# Targeting Nanoparticles to Cells

- Nanoparticles (NPs) as drug delivery systems:

Transport of NPs
towards cells

Transport across
cell membrane
by endocytosis

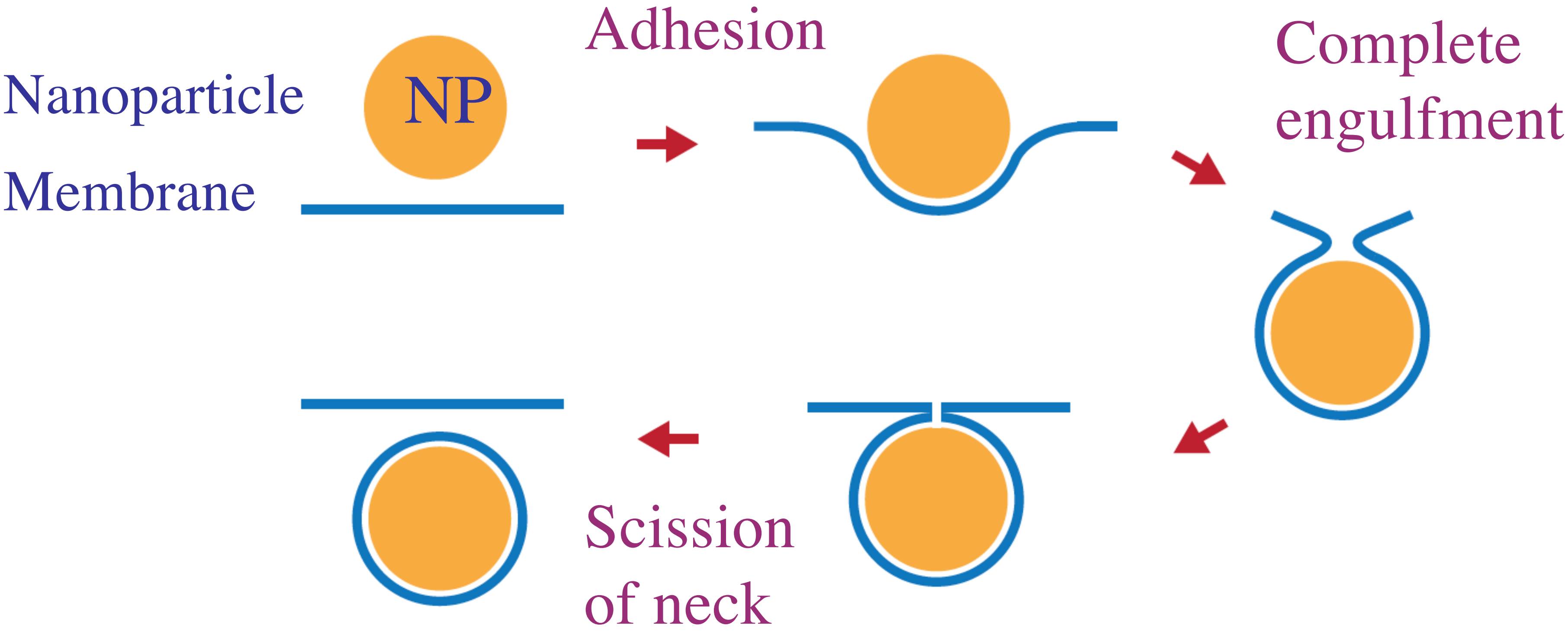


H.-M. Ding and Y.-Q. Ma, *Small* (2015)

- Endocytic pathway also used by virusses,

  airborne ultrafine particles, ...

# Endocytosis of Nanoparticles

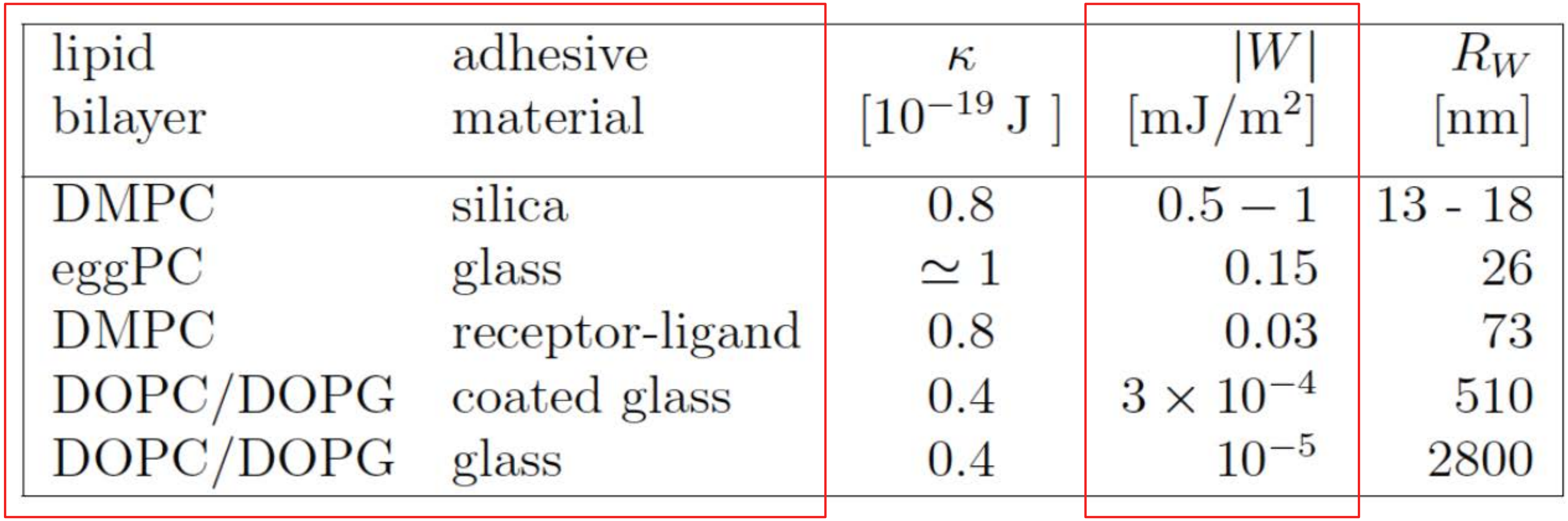


- Three steps: Adhesion, Complete Engulfment, Scission
- All steps governed by local stability relations

Agudo-Canalejo, RL: *ACS Nano* (2015); Soft Matter (2016)

# Adhesive Strength

- Adhesion free energy proportional to contact area
- Adhesive strength |W| = adhesion free energy per area
- Adhesive strength $|W|$ reflects NP surface chemistry and membrane composition



| lipid bilayer | adhesive material | $\kappa$ $[10^{-19}$ J $]$ | $\|W\|$ $[\mathrm{mJ/m^2}]$ | $R_W$ [nm] |
|---|---|---|---|---|
| DMPC | silica | 0.8 | $0.5 - 1$ | 13 - 18 |
| eggPC | glass | $\simeq 1$ | 0.15 | 26 |
| DMPC | receptor-ligand | 0.8 | 0.03 | 73 |
| DOPC/DOPG | coated glass | 0.4 | $3 \times 10^{-4}$ | 510 |
| DOPC/DOPG | glass | 0.4 | $10^{-5}$ | 2800 |

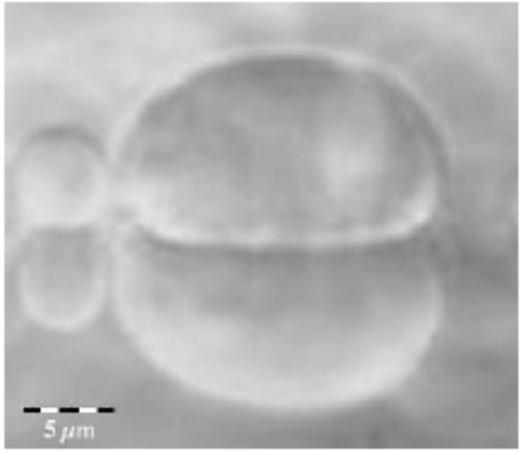
- Adh strength |W| varies over orders of magnitude

# Adhesion Length

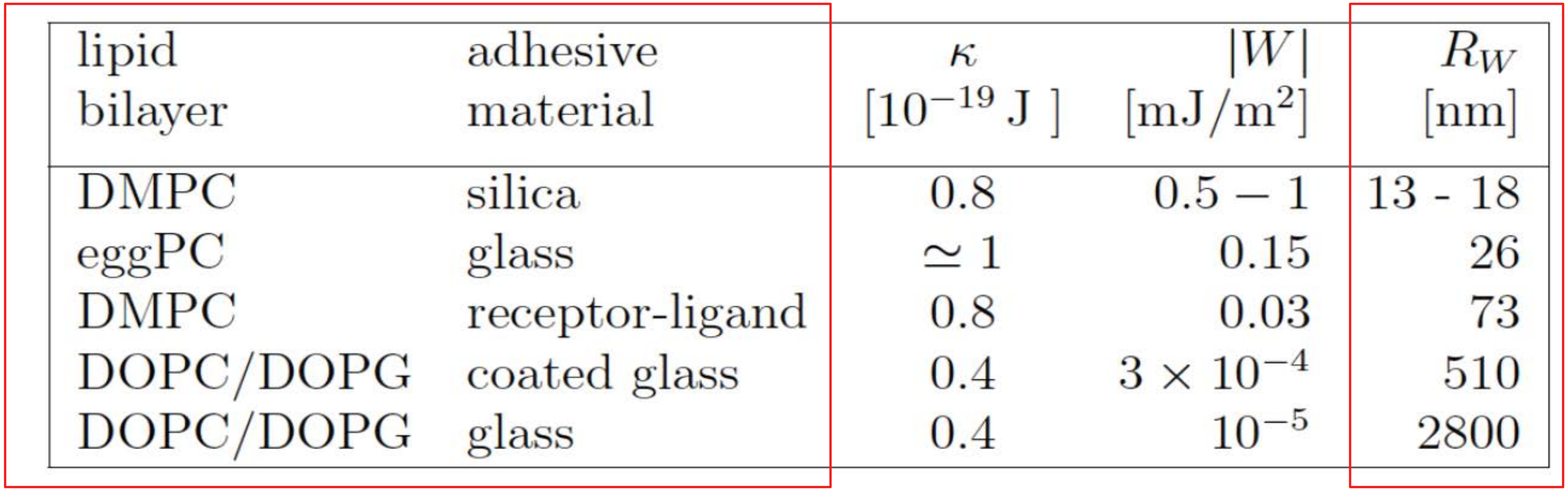
- Competition between $\kappa$ and $|W|$ :

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

- Experimentally accessible via membrane curvature along contact line



Gruhn et al, *Langmuir* (2007)



| lipid bilayer | adhesive material | $\kappa$ [$10^{-19}$ J ] | $\lvert W \rvert$ [mJ/m$^2$] | $R_W$ [nm] |
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- Strong/weak adhesion = small/large adhesive length $R_W$

# Onset of Adhesion: Local Criterion

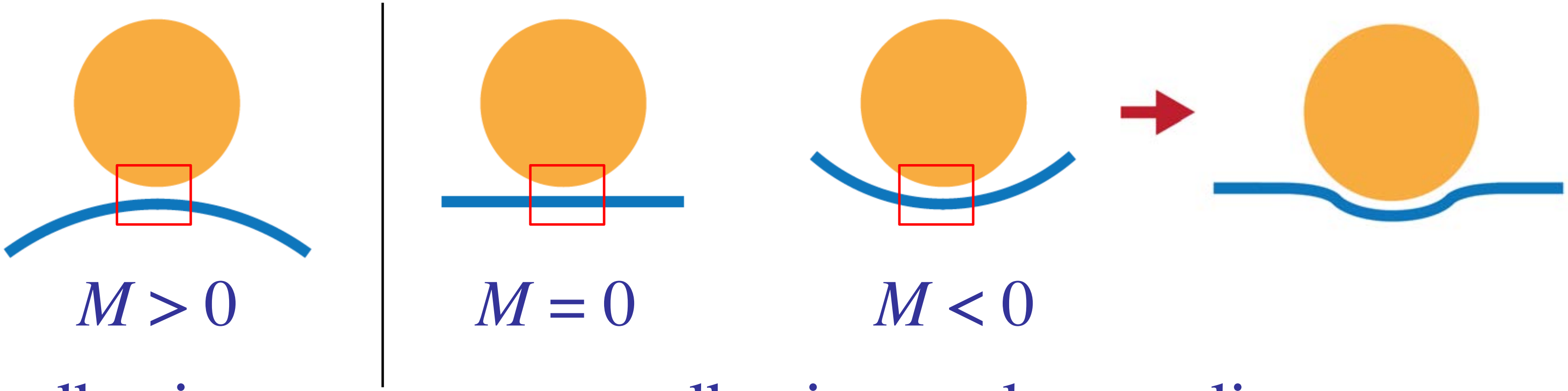
Agudo-Canalejo and RL, *ACS Nano* + *Nano Letters* (2015)

- Membrane starts to spread over particle if

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

contact curvature
$M_{\text{co}}$ is threshold
value for $M$

- Example: $M_{\text{co}} = 0$



$M > 0$ | $M = 0$ | $M < 0$

no adhesion | adhesion and spreading

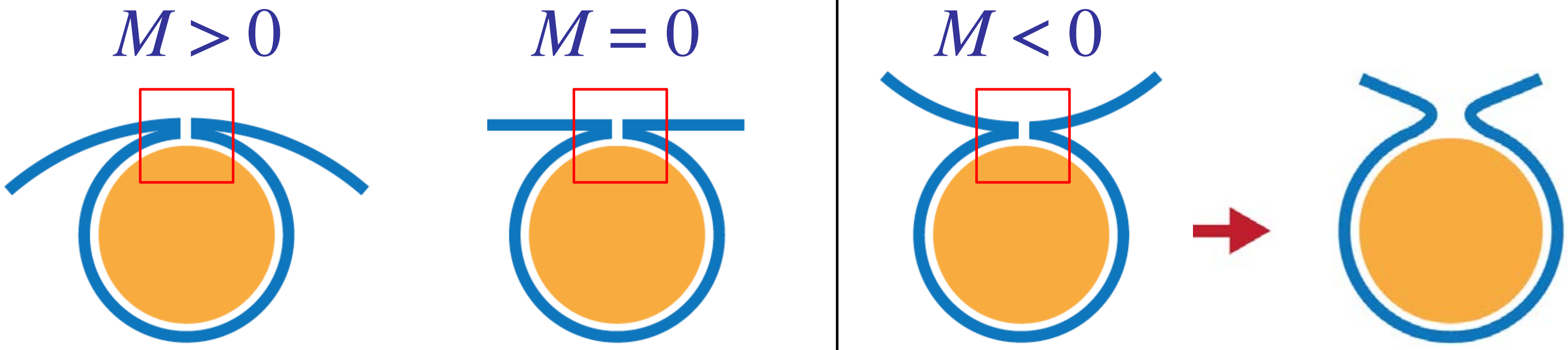
# Complete Engulfment: Local Criterion

- Closed membrane neck is stable if membrane curvature

$$\boxed{M \geq 2m - M_{co}}$$

2nd threshold value for $M$
depends on spont curvature $m$

- Example: $2m - M_{co} = 0$

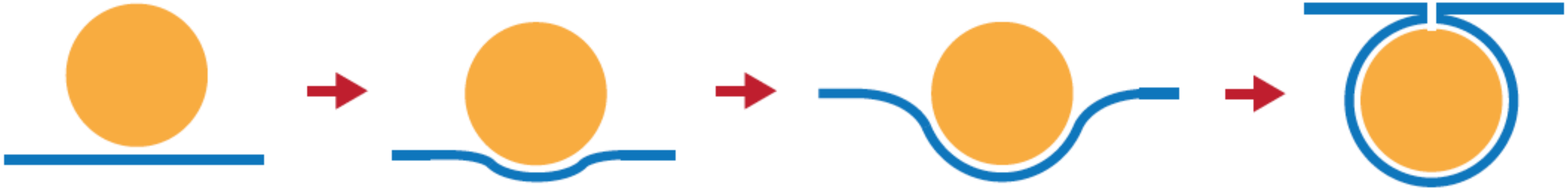


closed neck is stable

closed neck is unstable
and opens up

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

# From Adhesion to Engulfment



Adhesion

Complete engulfment

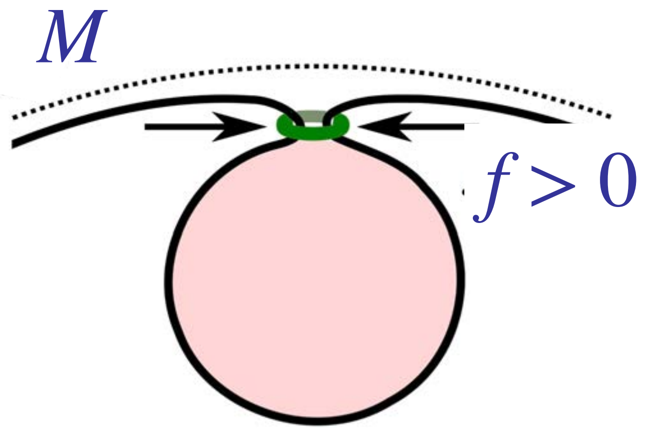
- Two local conditions for onset of adhesion and stability of closed neck
- Combination of both local conditions:

$$\boxed{2m - M_{\text{co}} \leq M \leq M_{\text{co}}}$$

- Technical detail: Limit of small particle size $R_{\text{pa}}$

Agudo-Canalejo and RL, *Soft Matter* (2017)

# Constriction Force from Adhesion



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

- Generalized neck curvature

$$M_{\mathrm{ne}} = (1/2)\,(M + M_{\mathrm{co}})$$

- Contact mean curvature depends on adhesion length $R_W = (2\kappa/|W|)^{1/2}$

- Constriction force

$$f = 8\pi\,\kappa\,(\,M_{\mathrm{ne}} - m\,)$$

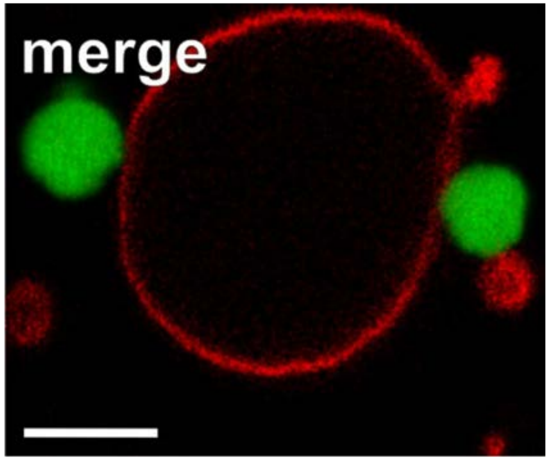
- Negative sp-curvature $m < 0$ increases force

- Positive sp-curvature $m > 0$ decreases force

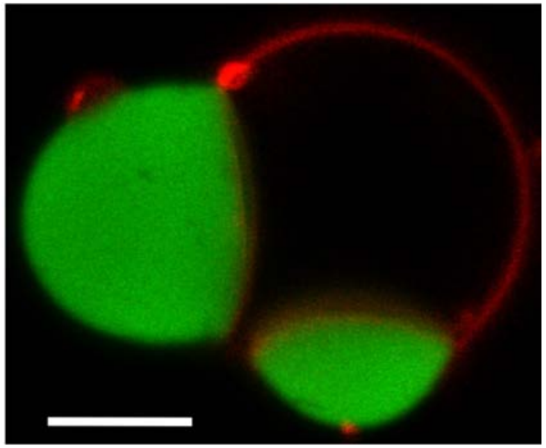
# Biomolecular Condensates

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

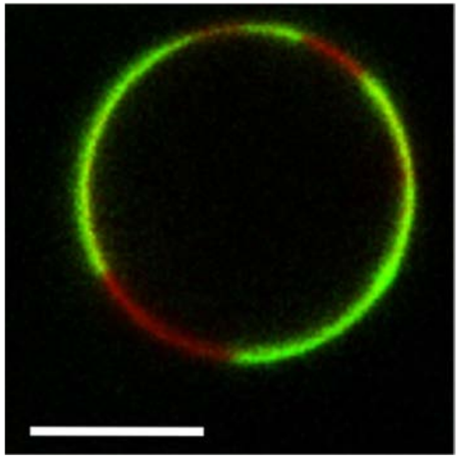
- Biomolecular condensates that behave like liquid droplets
- Enriched in intrinsically disordered proteins (IDPs)
- Example for IDP: RNA-binding protein FUS
- Interaction of FUS-droplets with GUVs, two subsequent wetting transitions:



dewetting for
high salt



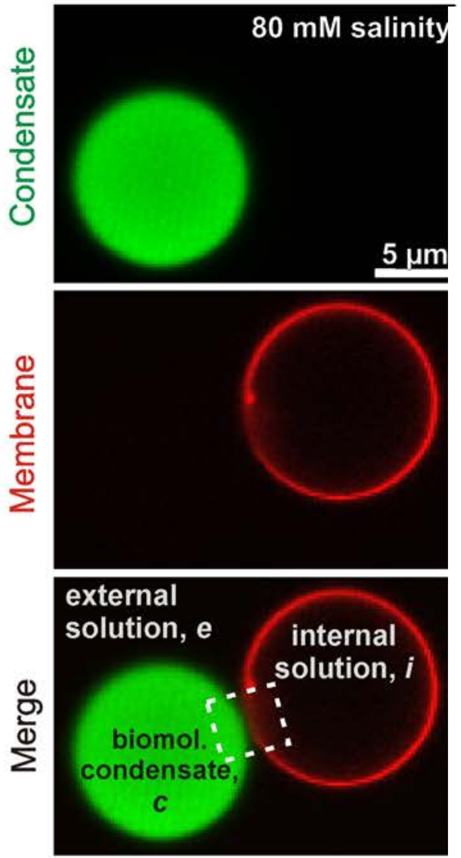
partial wetting for
intermediate salt



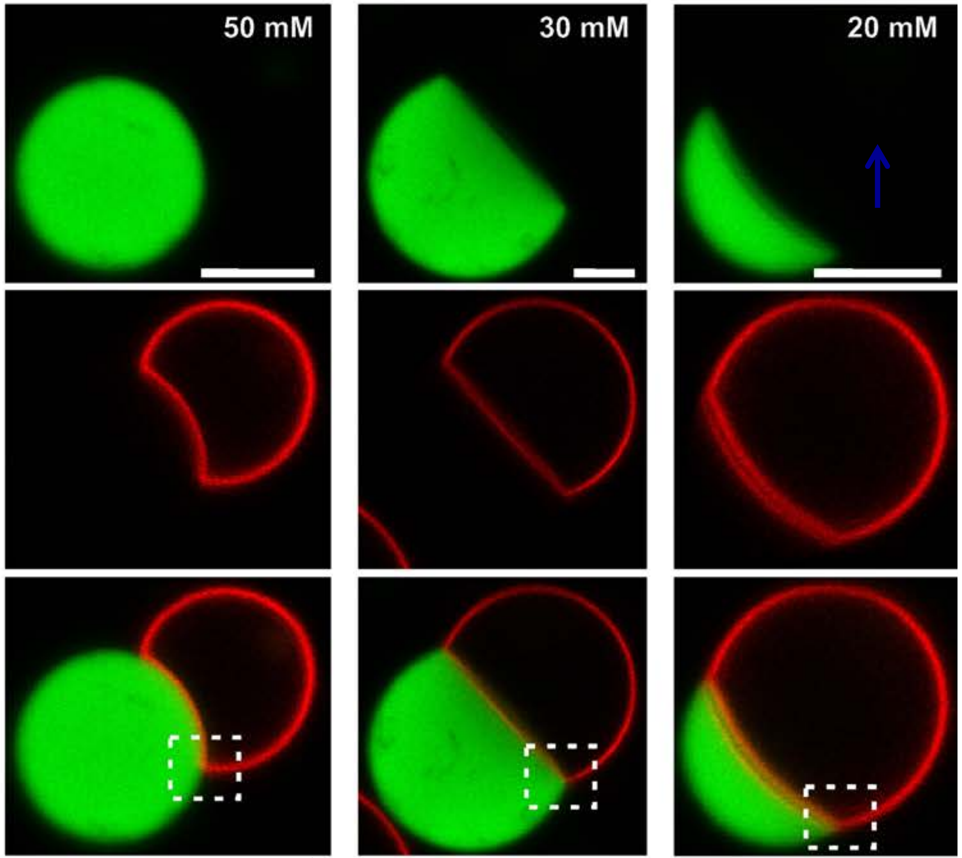
complete wetting
for low salt

# Two Wetting Transitions

- GUV + FUS-rich organelle + salt



dewetting to partial wetting



partial to complete wetting

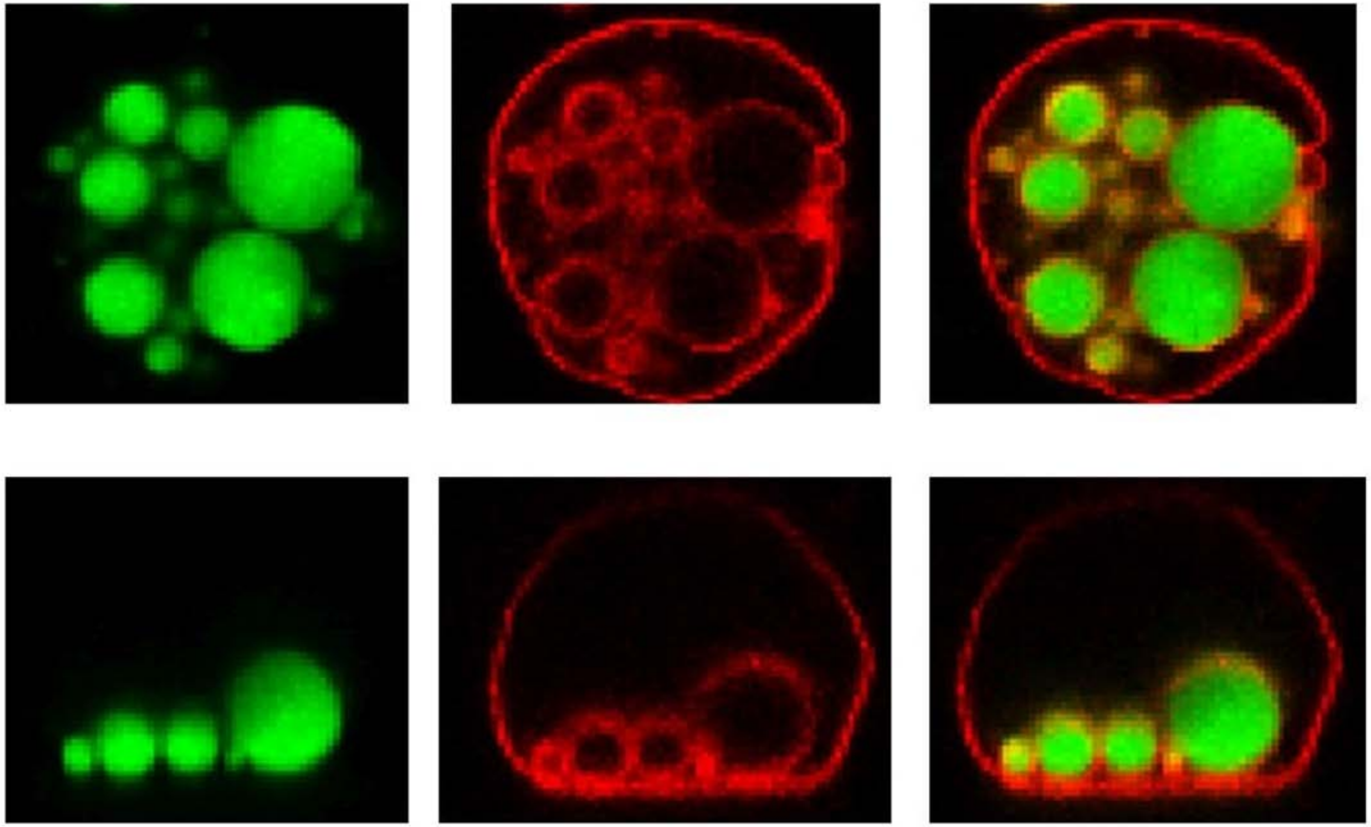
dewetting
for high salt

partial wetting for
intermediate salt

complete wetting
for low salt

# Engulfment of Condensates

- Green FUS-rich condensates engulfed by red GUV:

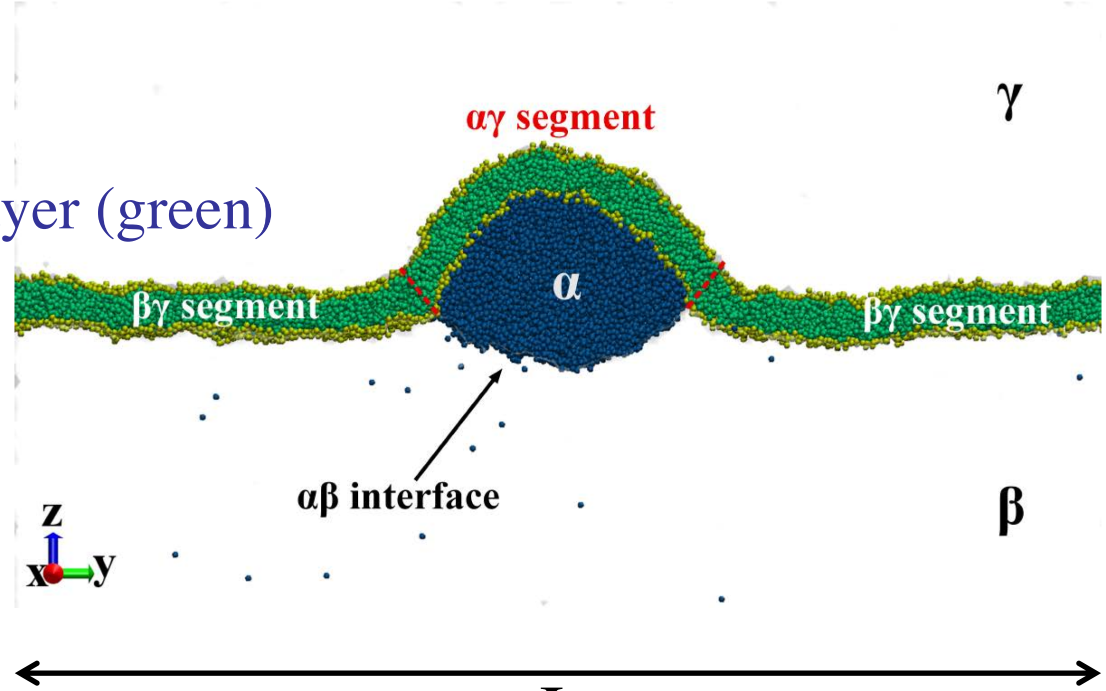


# Lipid Bilayer + Nanodroplet

Satarifard, Grafmüller, RL: *ACS Nano* (in press)

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

bilayer (green)

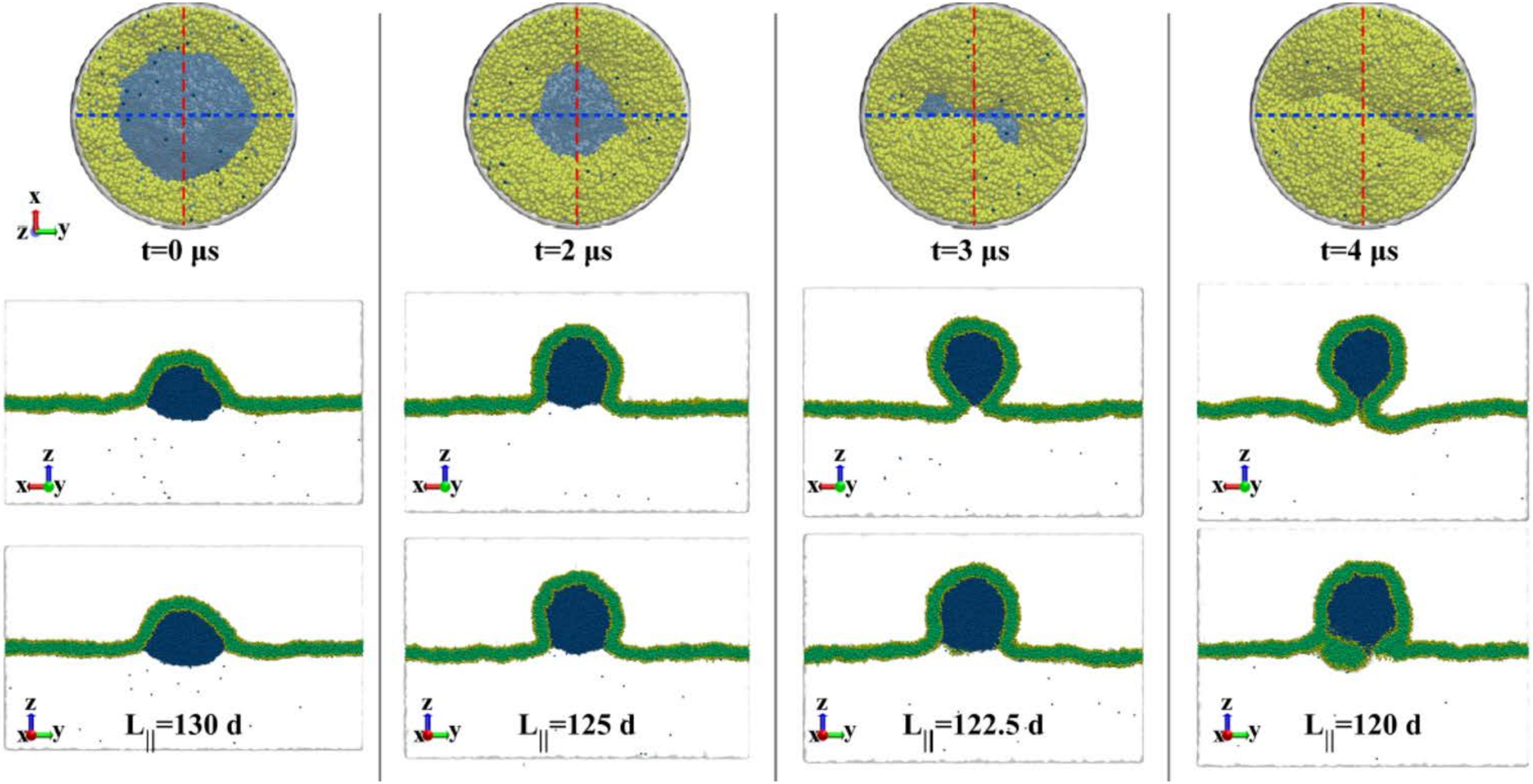


three aqueous phases $\alpha$, $\beta$, $\gamma$

$\alpha$ droplet (blue) coexists with $\beta$ phase (white)

three surface segments $\alpha\beta$, $\alpha\gamma$, $\beta\gamma$

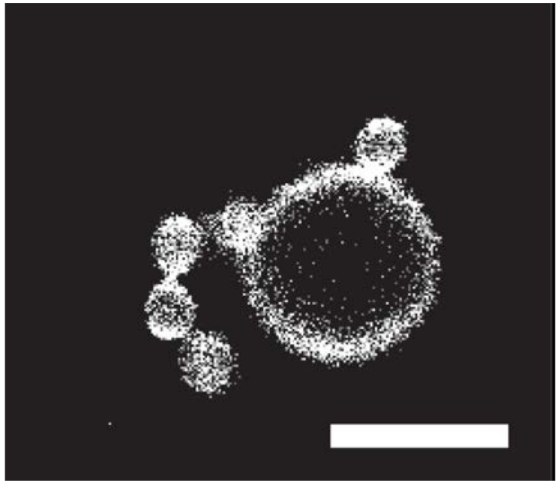
# Engulfment of Nanodroplets



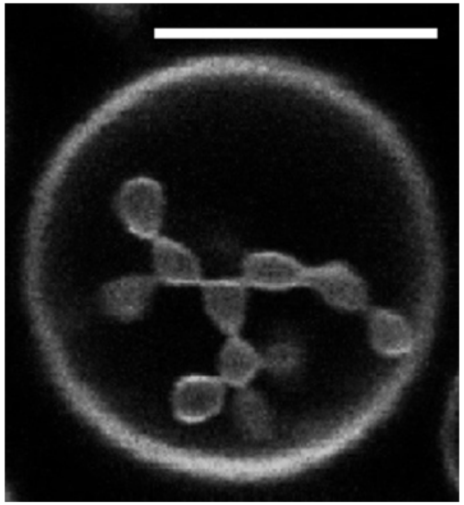
- Tight-lipped membrane neck from negative line tension

# Outlook: Smart Compartments

- Positive SponCurv: out-buds or out-beads
- Buds/beads filled with drugs or agents
- Divison into many small compartments
- Multiplication of delivery systems



- Negative SponCurv: in-buds or in-beads
- Storage and delivery of nanoparticles (NPs)
- Uptake of NPs by in-tubes
- Storage of NPs by closed necks
- Release of NPs by neck opening

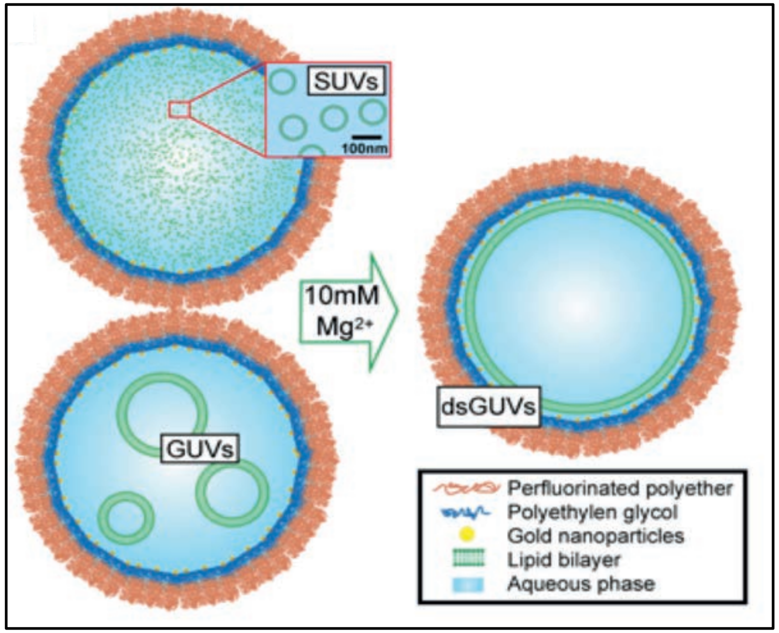


# Outlook: GUVs à la Carte

- Combine control of spontaneous curvature with microfluidic methods to produce monodisperse GUVs
- Very promising method: Droplet-supported GUVs

Weiss ... Spatz, *Nature Materials* (2018)

- Water-in-Oil emulsion droplets
- Formation of droplet-supported GUVs by pico-injection
- Additional components by additional pico-injections
- Control of SpoCurv



# Summary and Outlook

- Importance of spontaneous curvature
- Morphological complexity of GUVs
  uniform membranes => multi-domain membranes
- Controlled Division of GUVs
  binding of GFP => photoresponsive proteins
- GUVs a la carte:
  dsGUVs with controlled spont curvature
- Controlling morphological complexity
- Smart storage and delivery systems

- Membranes, Theo

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  Andrea Grafmüller
  Markus Miettinen
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  Titus Franzmann
  Seraphine Wegner
  Solveig Bartelt