Morphological Complexity of Biomembranes and Vesicles

Reinhard Lipowsky

Theory & Bio-Systems, MPI-KG Potsdam

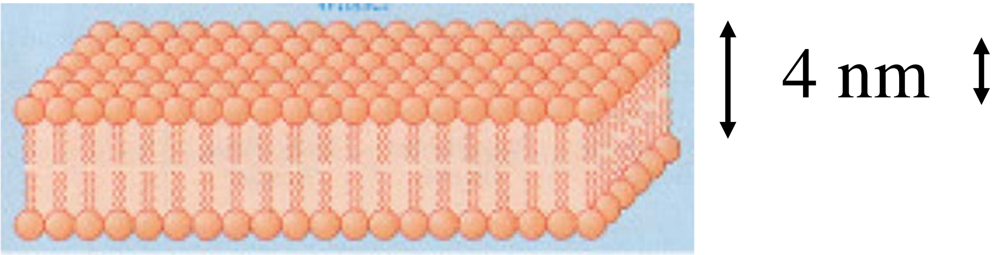
- Membranes and vesicles
- Membrane tension and curvature elasticity
- Spontaneous curvature and membrane necks
- Morphological complexity

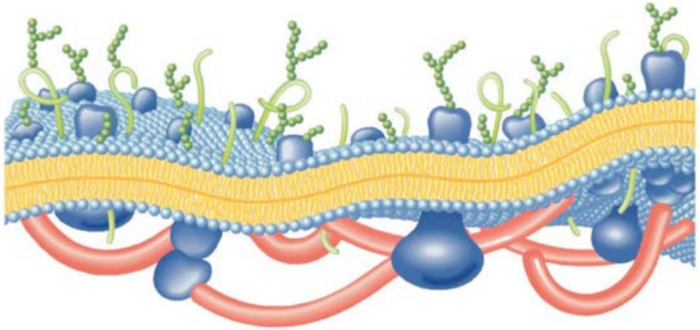
„Physical understanding from fruitful interplay between theory, simulation, and experiment"

- ☞ Vesicles and Membranes
- Membrane tension and curvature elasticity
- Spontaneous curvature and membrane necks
- Morphological complexity of vesicles

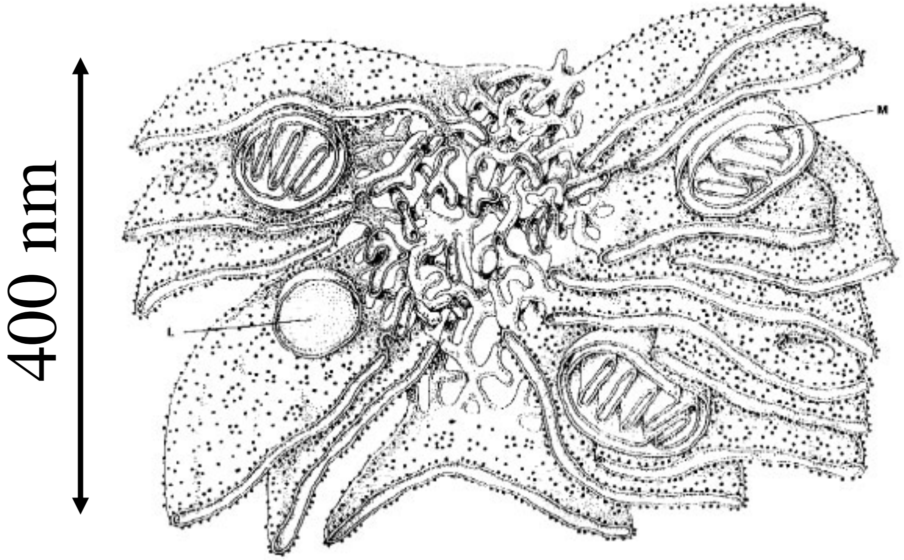
Multiscale Membranes

- Lipid bilayer

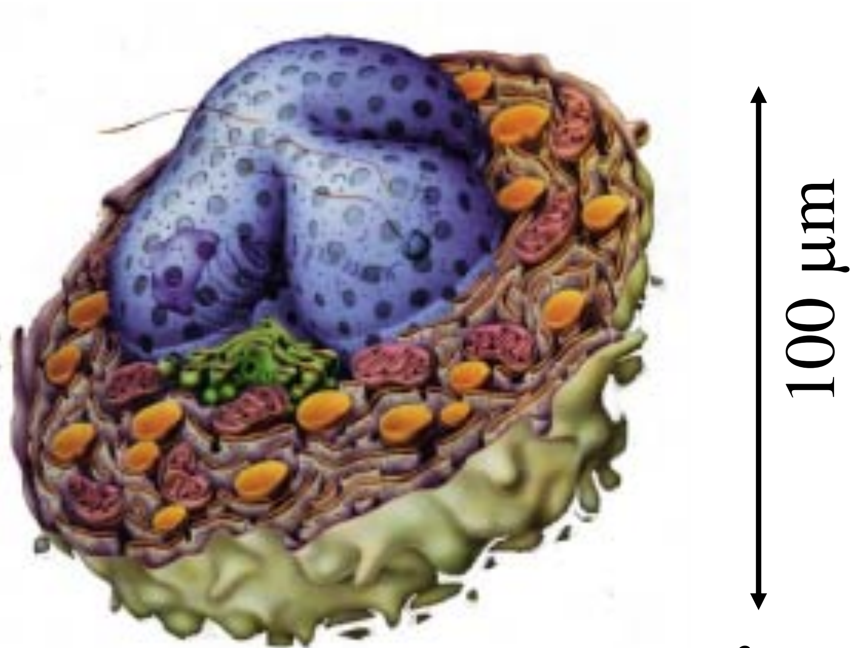




- Biomembrane



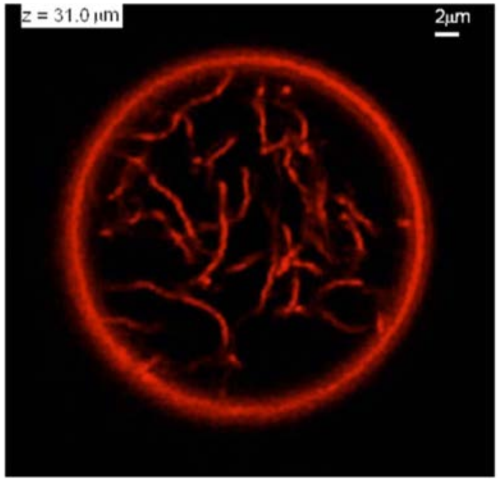
- Endoplasmic reticulum (ER)



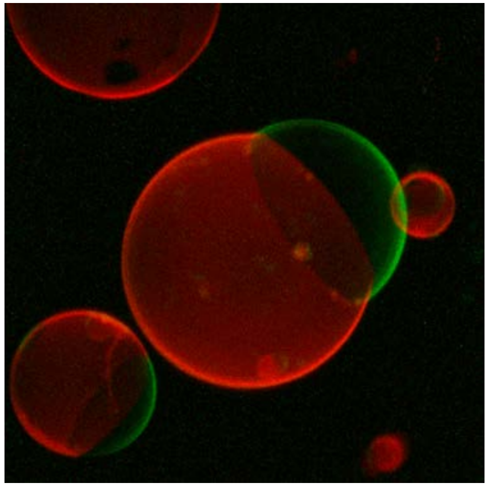
- Animal cell

Morphology of Vesicles

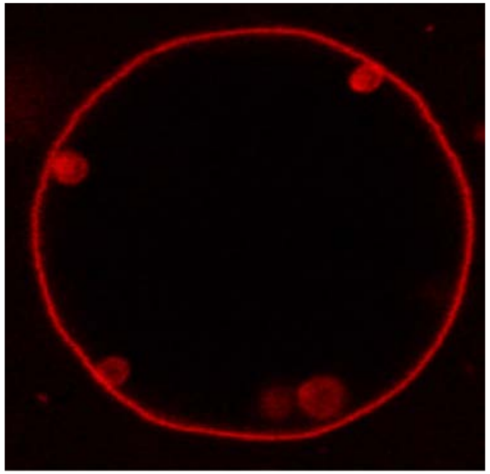
- Giant unilamellar vesicles (GUVs), tens of micrometers
- Remodelling in response to various perturbations:



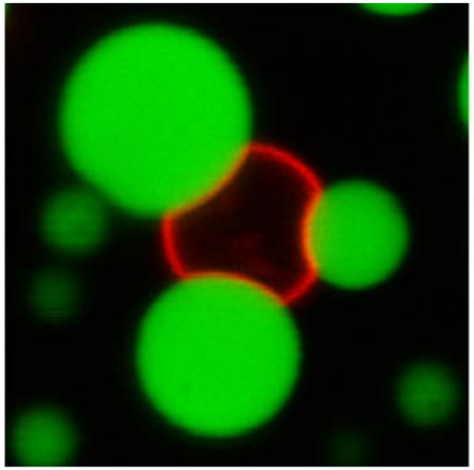
Nanotubes from polymer adsorption, tube width ~ 100 nm



Formation of intra-membrane domains, 2D phase separation



Small buds from protein adsorption, bud size ~ μm



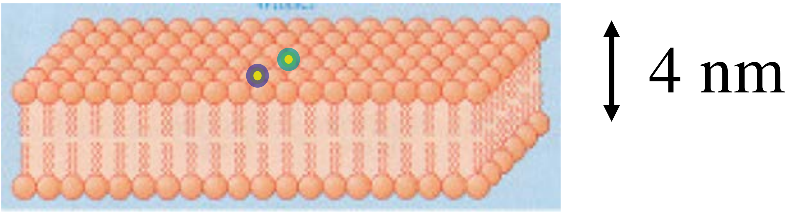
Remodelling by adhering or partially wetting droplets

- What determines these strongly nonspherical shapes of membranes and vesicles?

Membrane Fluidity

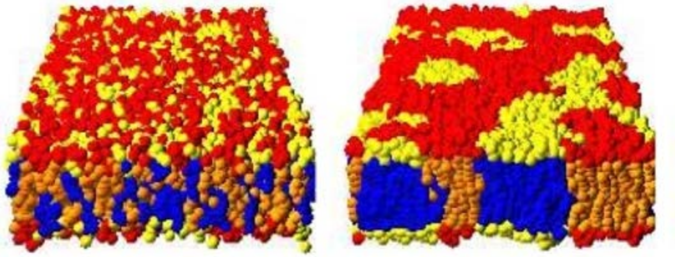
- Fluid membranes, i.e., fast lateral diffusion:

  Diffusion constant ~ μm^2/s



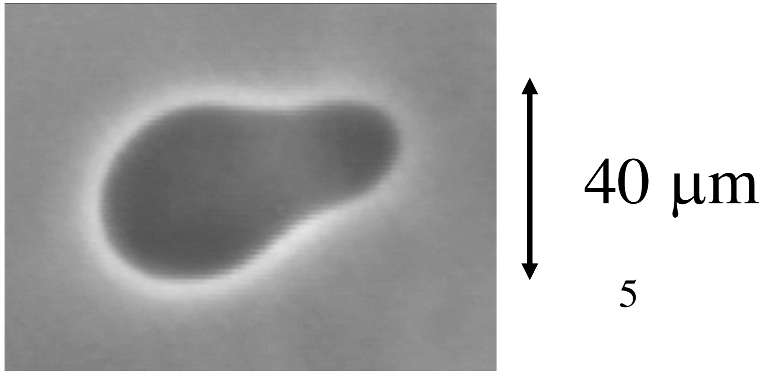
lipid swapping ~ ns

- Lateral diffusion => demixing and domain formation



- Fluidity => Flexibility

  Formation of spherical bud (with Gaussian curvature) => Direct evidence for fluidity



Membranes as Flexible Sheets

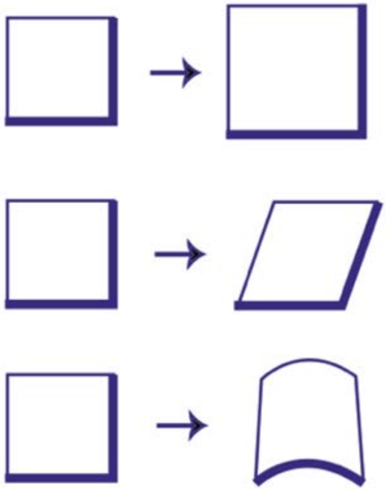
- Membrane as thin elastic sheet

- Elastic Deformations

Stretching

Shearing

Bending



- Fluid Membranes

Hardly stretchable (without rupture)

Shear -> Flow

Main contribution related to bending and curvature

Emergence of Curvature

- Fluid membranes as smooth surfaces:

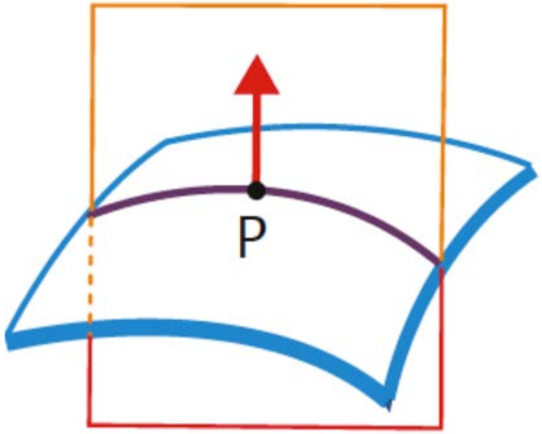
  Fluidity => no intrinsic coordinates

  Invariance under coordinate transformations

  Principal curvatures C_1 and C_2

  Mean curvature $M = (C_1 + C_2)/2$

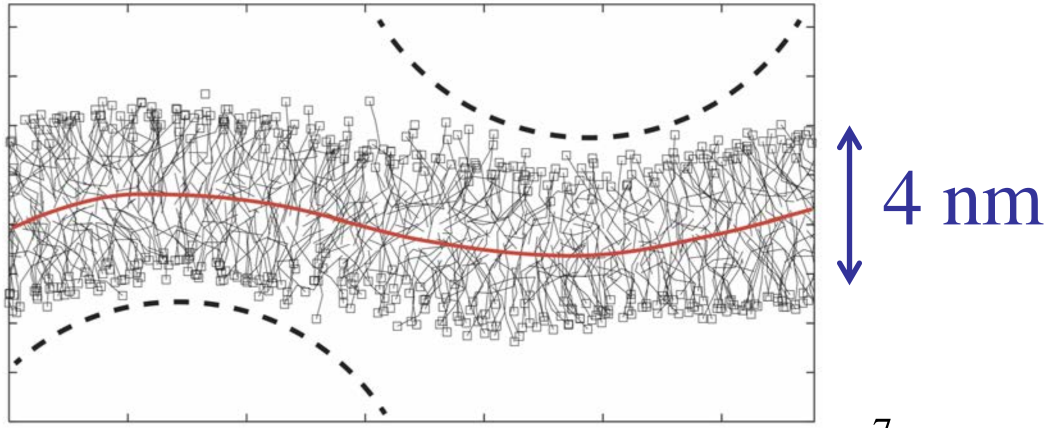
  Gaussian curvature $G = C_1 C_2$



Canham, Helfrich, E. Evans (1970s)

- Mesoscopic description, ignores molecular architecture
- Emergence of curvature on nanoscopic scales:

  Bending undulations for membrane patches > 6 nm

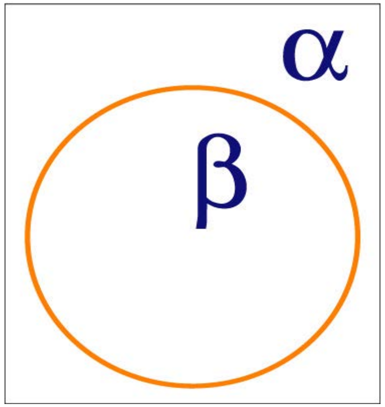


Goetz, Gompper, RL (1999)

- Vesicles and Membranes
- ☞ Membrane tension and curvature elasticity
- Spontaneous curvature and membrane necks
- Morphological complexity of vesicles

For Comparison: Liquid Droplets

- Coexistence of two liquid phases α and β
- Shape energy of a β droplet within the bulk α phase:



$$E = V_\beta (P_\alpha - P_\beta) + A_{\alpha\beta} \Sigma_{\alpha\beta}$$

Two parameters:
Pressure difference $P_\beta - P_\alpha$ and interfacial tension $\Sigma_{\alpha\beta}$

- First variation of shape energy leads to Laplace equation for mean curvature M :

$$2 M \Sigma_{\alpha\beta} = P_\beta - P_\alpha = \Delta P$$

- Interfacial tension is constant (for constant temperature)

=> Interface attains shape with constant M

Shape Energy of Vesicles

Deuling, Helfrich, *J. Physique* (1976)

- Shape energy of a vesicle

$$E = -\Delta P\, V + \Sigma\, A + \text{curvature-elastic terms}$$

- First two terms have the same form as for droplets
- $\Delta P = P_{\mathrm{in}} - P_{\mathrm{ex}}$ is again the pressure difference
- But the area term has a different meaning: in contrast to droplets, the membrane area A is essentially fixed and the tension Σ can either be viewed as a Lagrange multiplier to prescribe the area or as the elastic stress acting to stretch the weakly compressible membrane

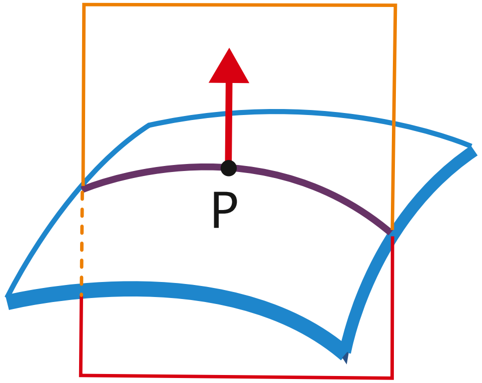
RL, *Adv. Colloid Interface Sci*. (2014)

=> While the interfacial tension $\Sigma_{\alpha\beta}$ represents a material parameter, the membrane tension Σ is shape-dependent

Curvature Elasticity

Canham (1970) Helfrich (1973) Seifert, Berndl, RL (1991)

- 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)

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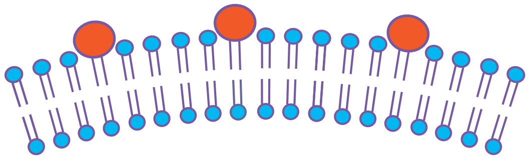
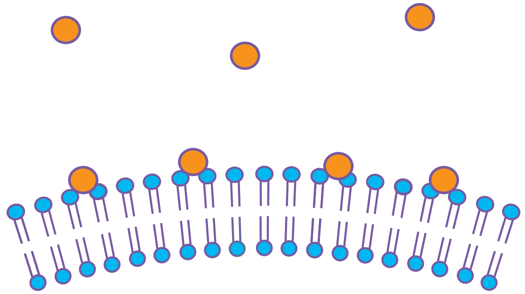
Spontaneous or Preferred Curvature

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

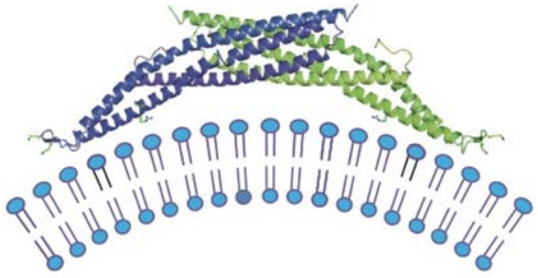
- Different molecular mechanisms for bilayer asymmetry:



Asymmetric composition, e.g., ganglioside



Asymmetric adsorption of small molecules

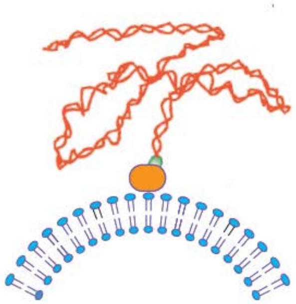


Asymmetric protein coats, e.g. BAR-domain

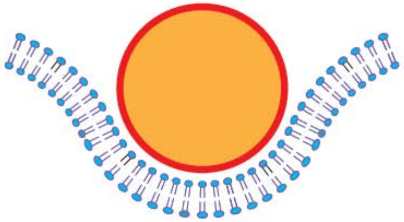
Local Curvature Generation

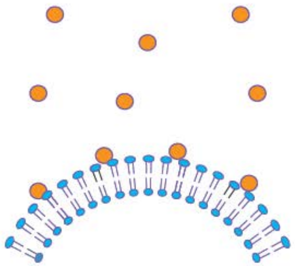
- Local curvature generated on nanoscopic scales:

Anchored polymer

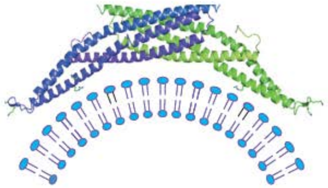


‘Large’ particle





‘Small’ adsorbate particles



BAR-domain protein

Nonspherical Janus particles

Induced Fit

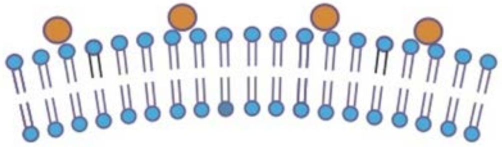
Conformational Selection

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

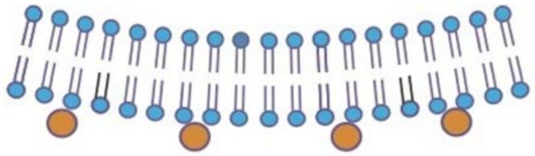
$M > 0$

exterior compartment



interior compartment

outer leaflet



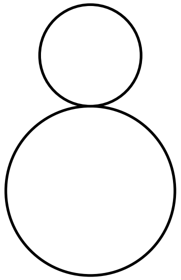
inner leaflet

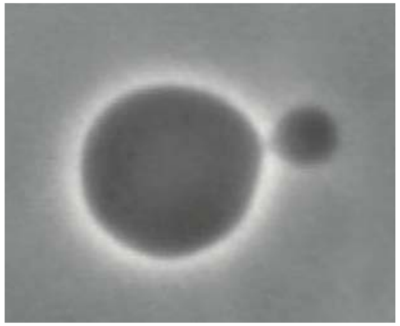
$M < 0$

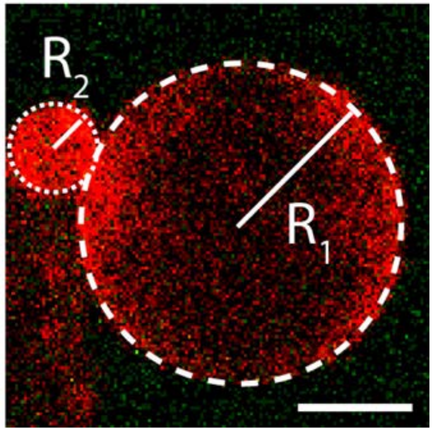
Mean curvature M is positive (negative) if membrane bulges towards exterior (interior) compartment

(1+1)-Spheres and Membrane Necks

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



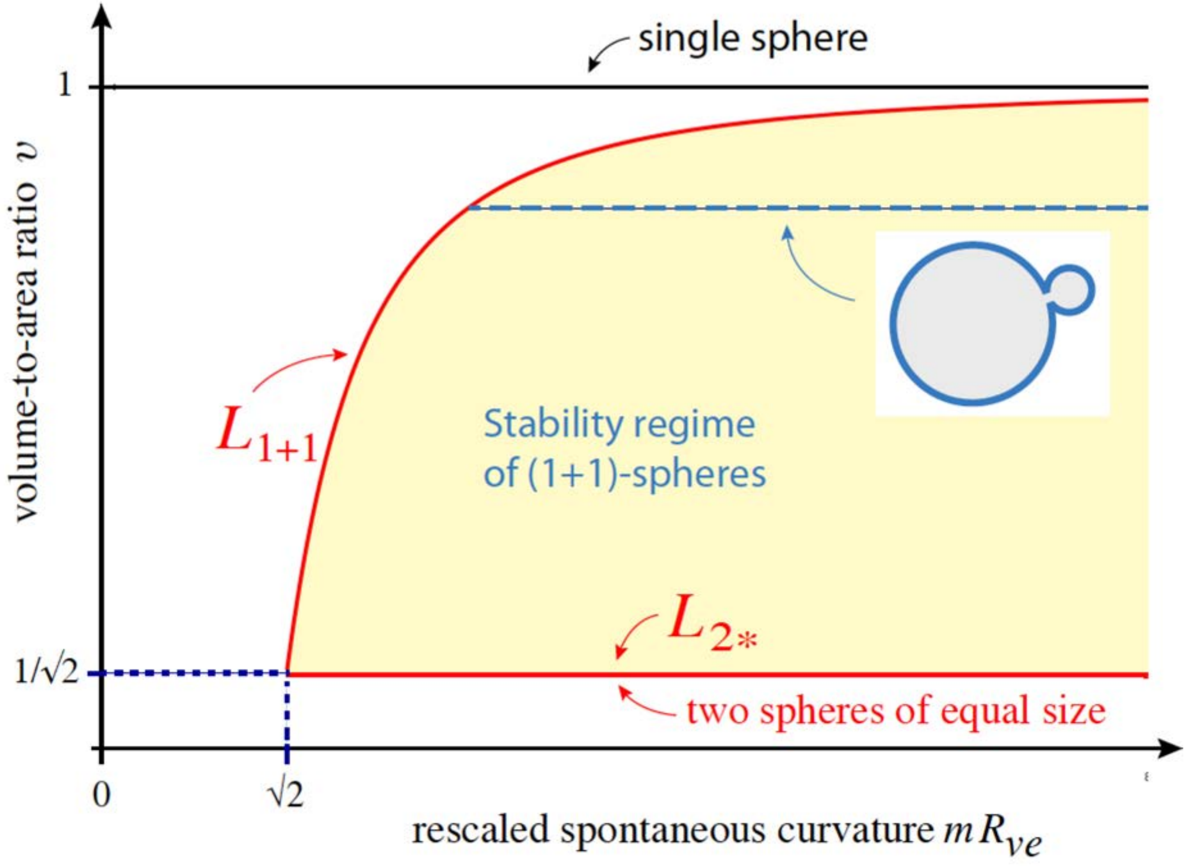




- Sphere radii R_1 and R_2
- Mean curvatures $M_1 = 1/R_1$ and $M_2 = 1/R_2$
- Neck curvature $M_{\text{ne}} = (1/2)\,(M_1 + M_2)$
- Closed neck is stable if $0 < M_{\text{ne}} \leq m$
- Local relation between geometry and material parameter

Stability Regime of (1+1)-Spheres

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



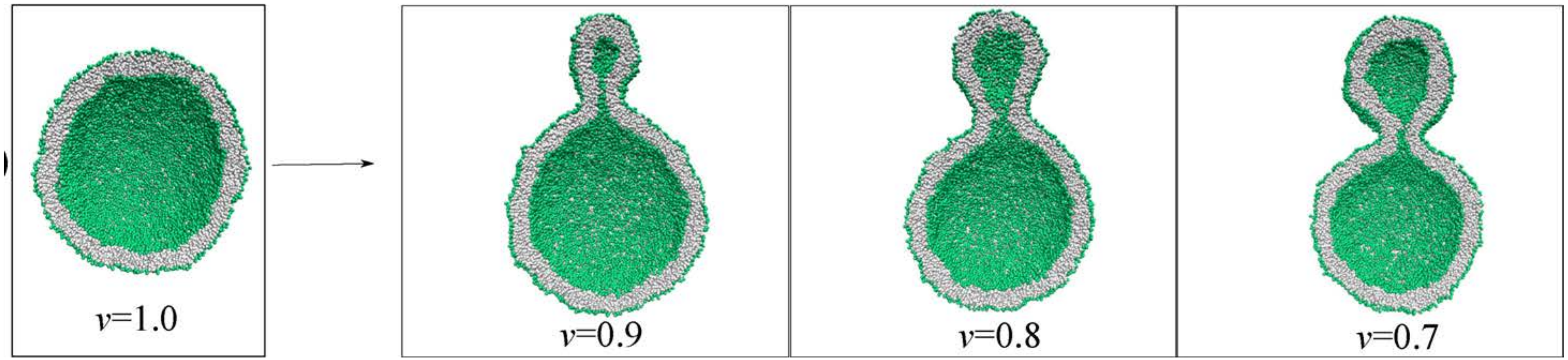
within yellow stability regime: dumbbell shape depends only on v and not on mR_{ve}

volume v:
$1 > v \geq 0.707$

Membrane Necks of Nanovesicles

Rikhia Ghosh, Vahid Satarifard, A. Grafmüller, RL. *Nano Letters* (2019)

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



- Vesicles and Membranes
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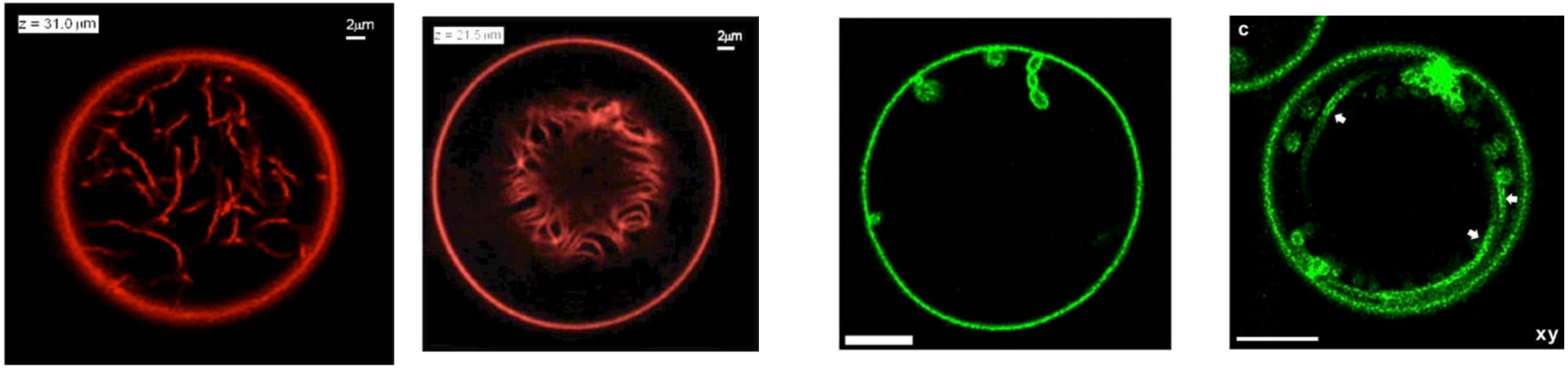
☞ Large sp-curvature: Membrane nanotubes

Controlled sp-curvature and GUV division

Large Sp-Curvature: Nanotubes

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

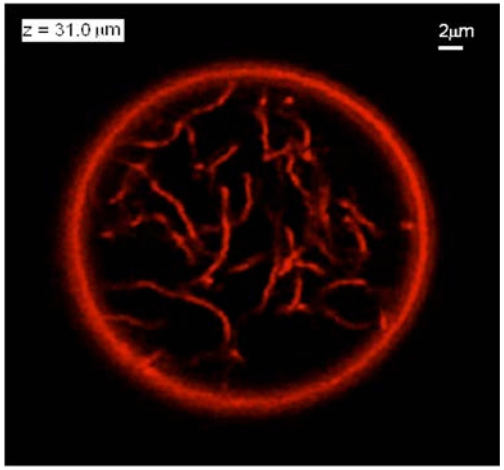
- Lipid mixture of DOPC, DPPC, cholesterol
- Small amounts of fluorescently labeled lipids
- Liquid-disordered (red) and liquid-ordered phase (green)



- 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 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

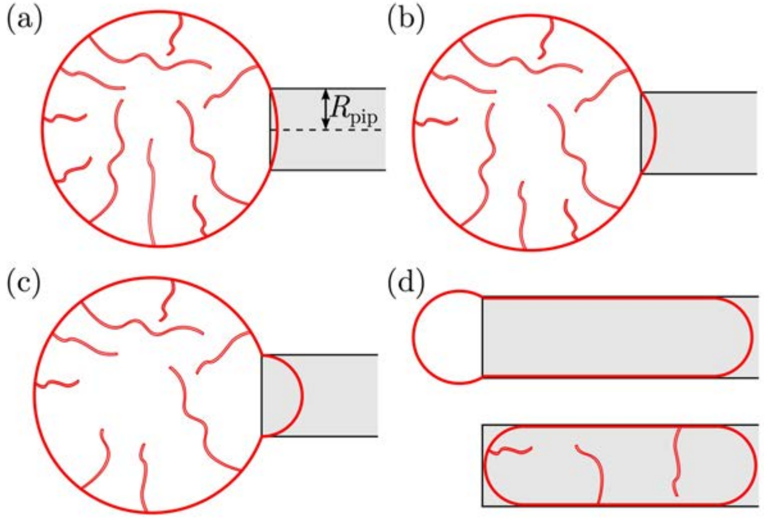
Mechanical tension $\Sigma \approx 10^{-4}$ mN/m

Robustness of tubulated GUVs

- 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

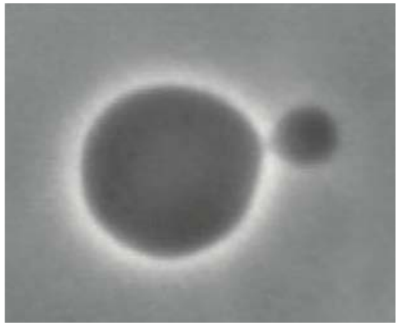
Bhatia ... RL: *ACS Nano* (2018)

- 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)



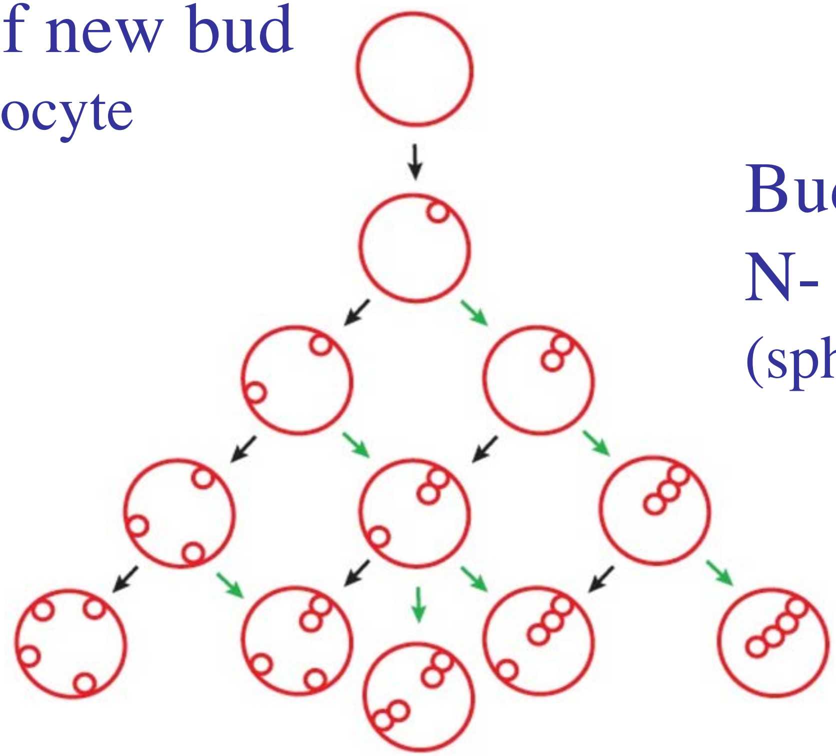
- Tubulation intimately related to budding
- Osmotic deflation of spherical GUV
- Small deflation step leads to single bud
- Bud and mother vesicle connected by membrane neck
- Bud acts as nucleation site for necklace-like tube
- Several pathways for subsequent deflation steps:
  - Formation of new bud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

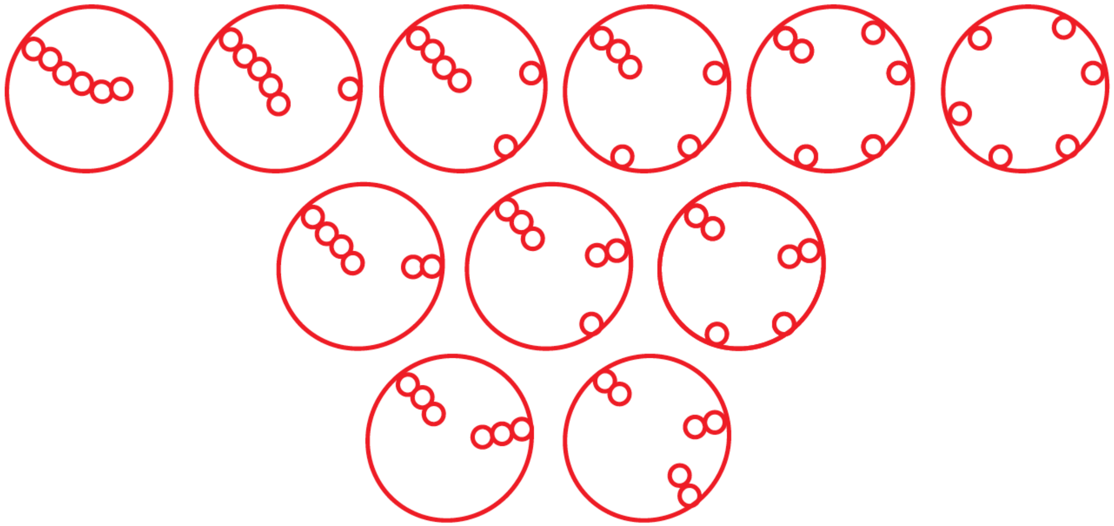
Formation of new bud
(oblate-stomatocyte bifurcation)



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

Morph Complexity: Theory

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

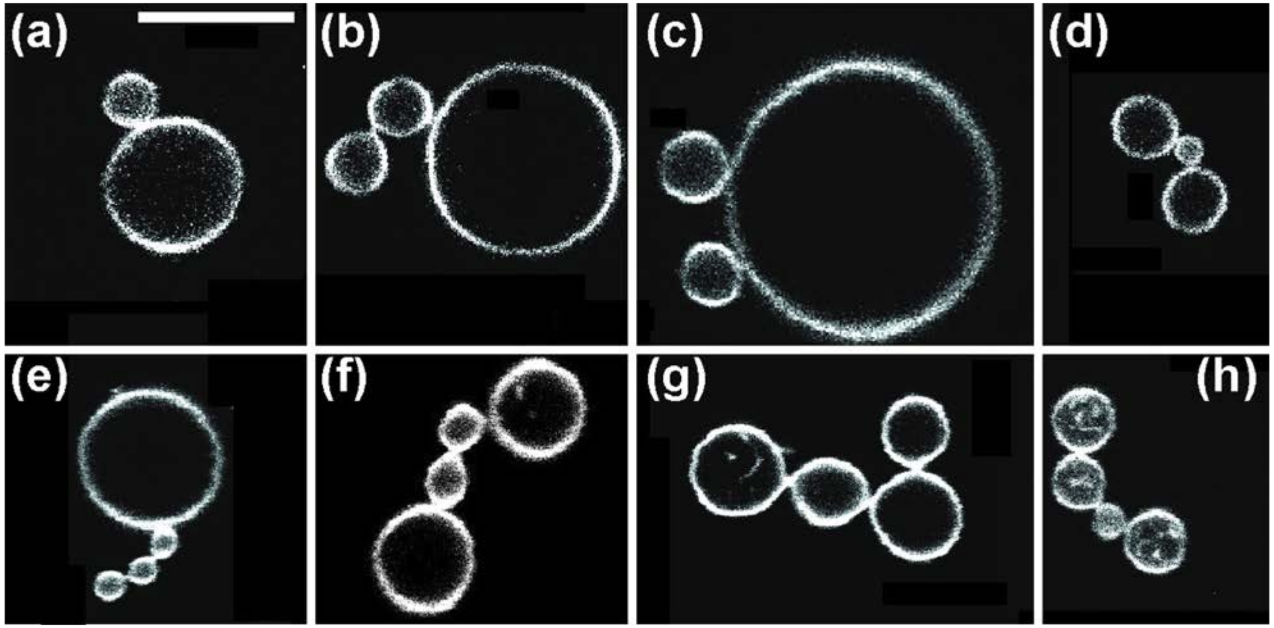


- All beads are connected by membrane necks
- All morphologies have the same area, volume, and curvature energy
- Rugged energy landscape contains 11 intersecting branches
- For large N, number of N-spherule morph grows as exp[c √N]

Morph Complexity: Experiment

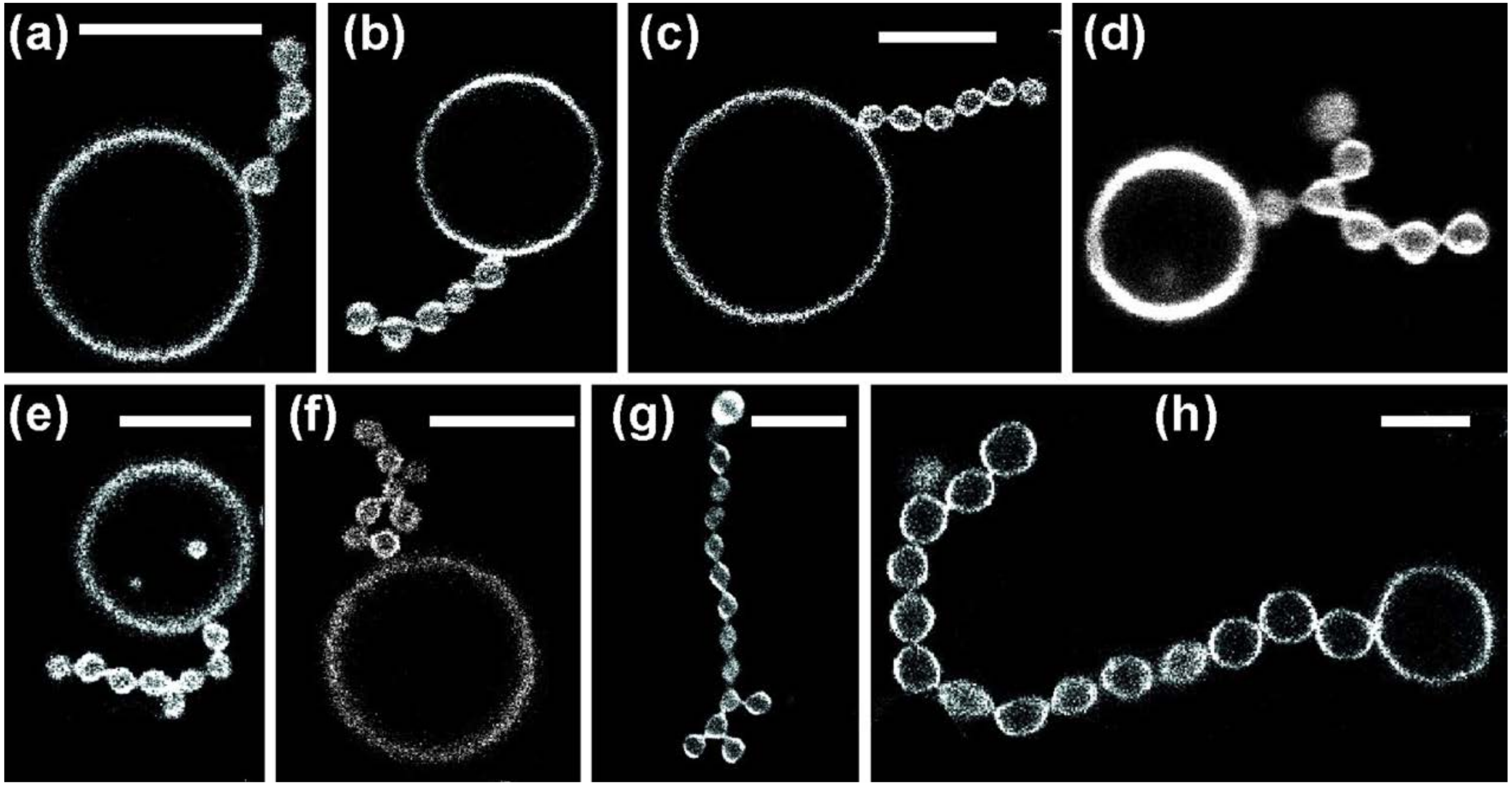
Bhatia, Christ, Steinkühler, Dimova, RL, *Soft Matter* (under revision)

- GUVs exposed to two simple sugars, sucrose and glucose
- Sugar asymmetry: sucrose inside, glucose outside
- Singe GUV membrane forms N_l large and N_s small spheres connected by closed membrane necks
- Multispheres with $N = N_l + N_s$ and $2 \leq N \leq 4$:



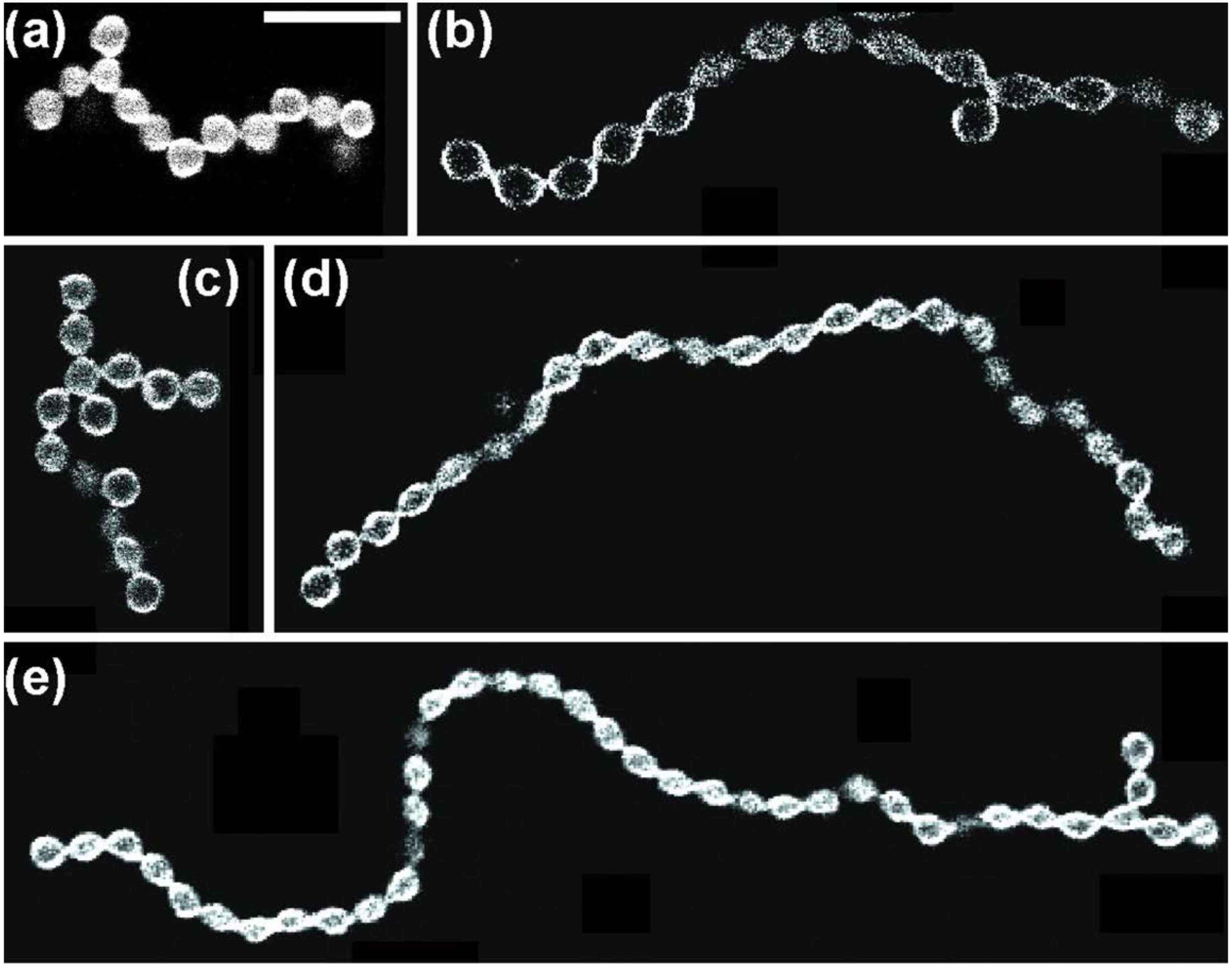
Multispheres with $N = 1 + N_s$

- One large and N_s small spheres, linear and branched chains:



- Individual small spheres are surprisingly mobile !

N_* Equally Sized Spheres



$N_* = 15$

$N_* = 24$

$N_* = 39$

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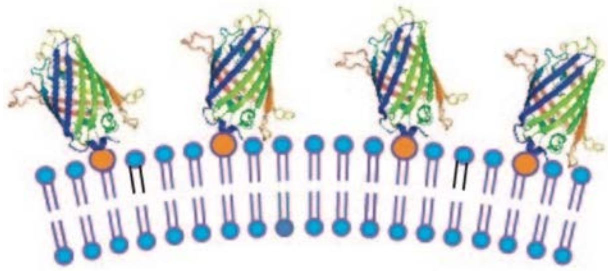
- Morphological complexity of vesicles
  - Large sp-curvature: Membrane nanotubes
  - ☞ Controlled sp-curvature and GUV division

Controlled Variation of Sp-Curvature

Steinkühler, ... , Wegner, Dimova, RL, *Nature Comm* (under revision)

- Binding of GFP to certain anchor lipids:



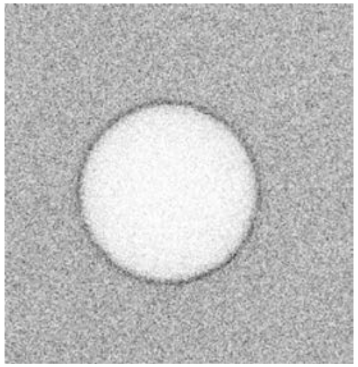
His-tagged GFP
NTA-lipids

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

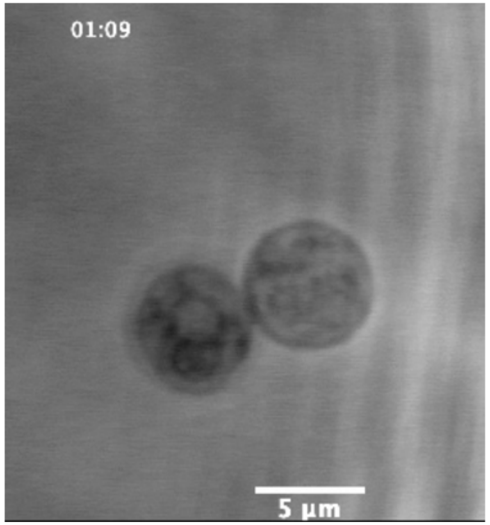
Controlled Division of GUVs

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

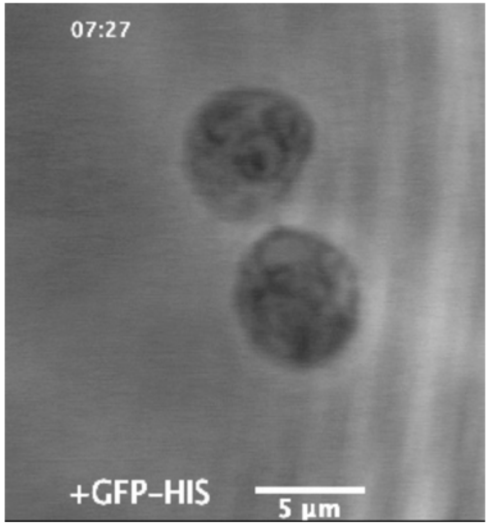
  Increase in GFP -> Neck cleavage -> Two daughter GUVs



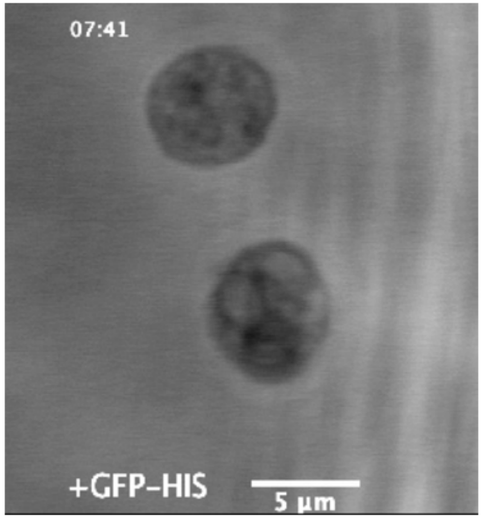
Adsorption of GFP onto GUV membrane



Deflation leads to dumbbell with membrane neck



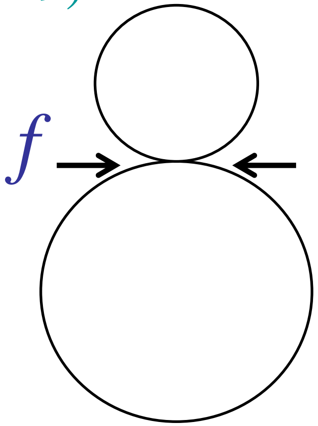
Directly after neck cleavage



Complete division into two smaller GUVs

Constriction Force from Sp-Curvature

RL, in *The Giant Vesicle Book*. ed by Dimova and Marques (2019)

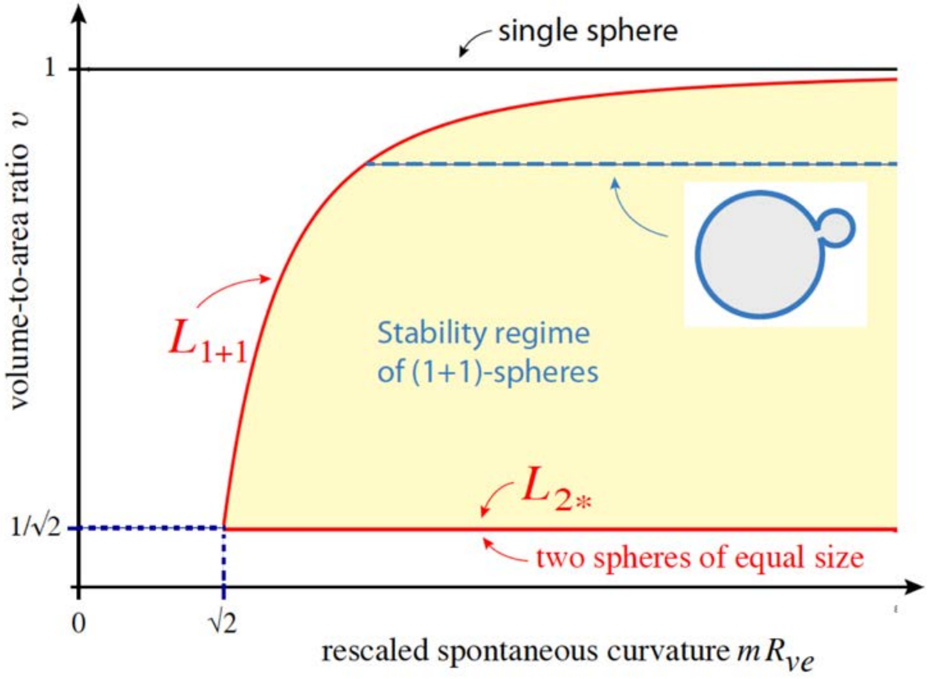


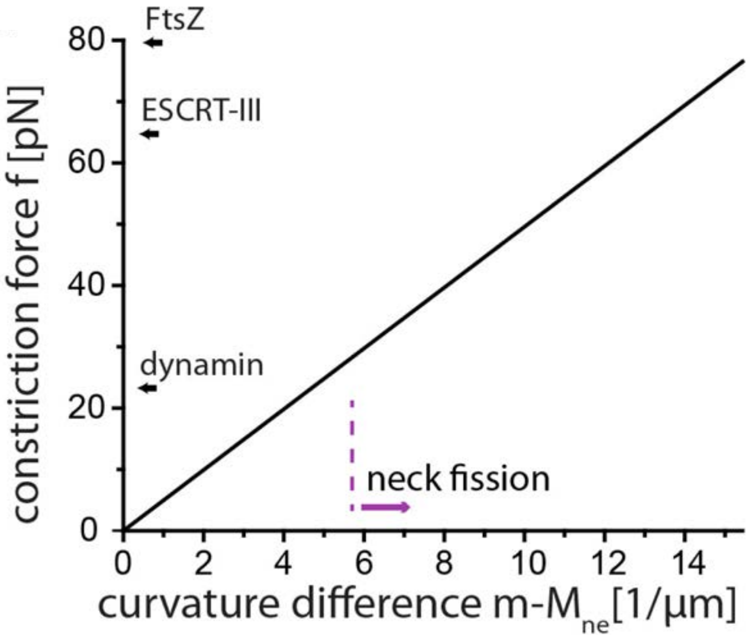
- Sp-curvature generates constriction force

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

acting radially on closed membrane neck:

- Force increases with increasing sp-curvature:





- Vesicles and Membranes
- Membrane tension and curvature elasticity
- Spontaneous curvature
- Morphological complexity of vesicles
- Outlook on related topics:

  ☞ Bilayer membranes and leaflet tensions

  Endocytosis of nanoparticles by membranes

  Wetting and engulfment of droplets

  ...

Membrane Tension

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

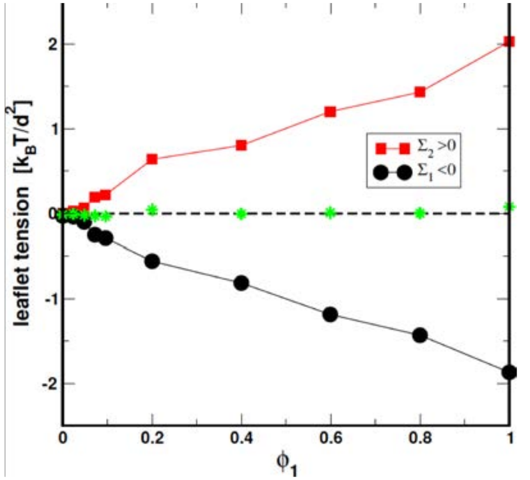
  ⇔ tensionless membrane

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

Bilayer Membranes and Leaflet Tensions

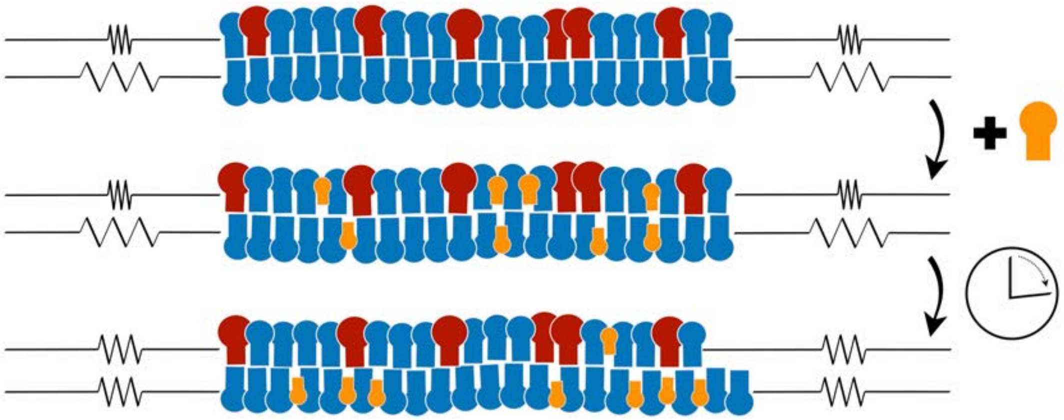
- Bilayer with two leaflets:
  - Two leaflet tensions Σ_1 and Σ_2 with $\Sigma_1 + \Sigma_2 = \Sigma$
  - Tensionless bilayer: $\Sigma = 0$
  - Leaflet tensions for binary mixture

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



- Leaflet tensions and flip-flops:

Miettinen, RL , Nanoletters (2019)

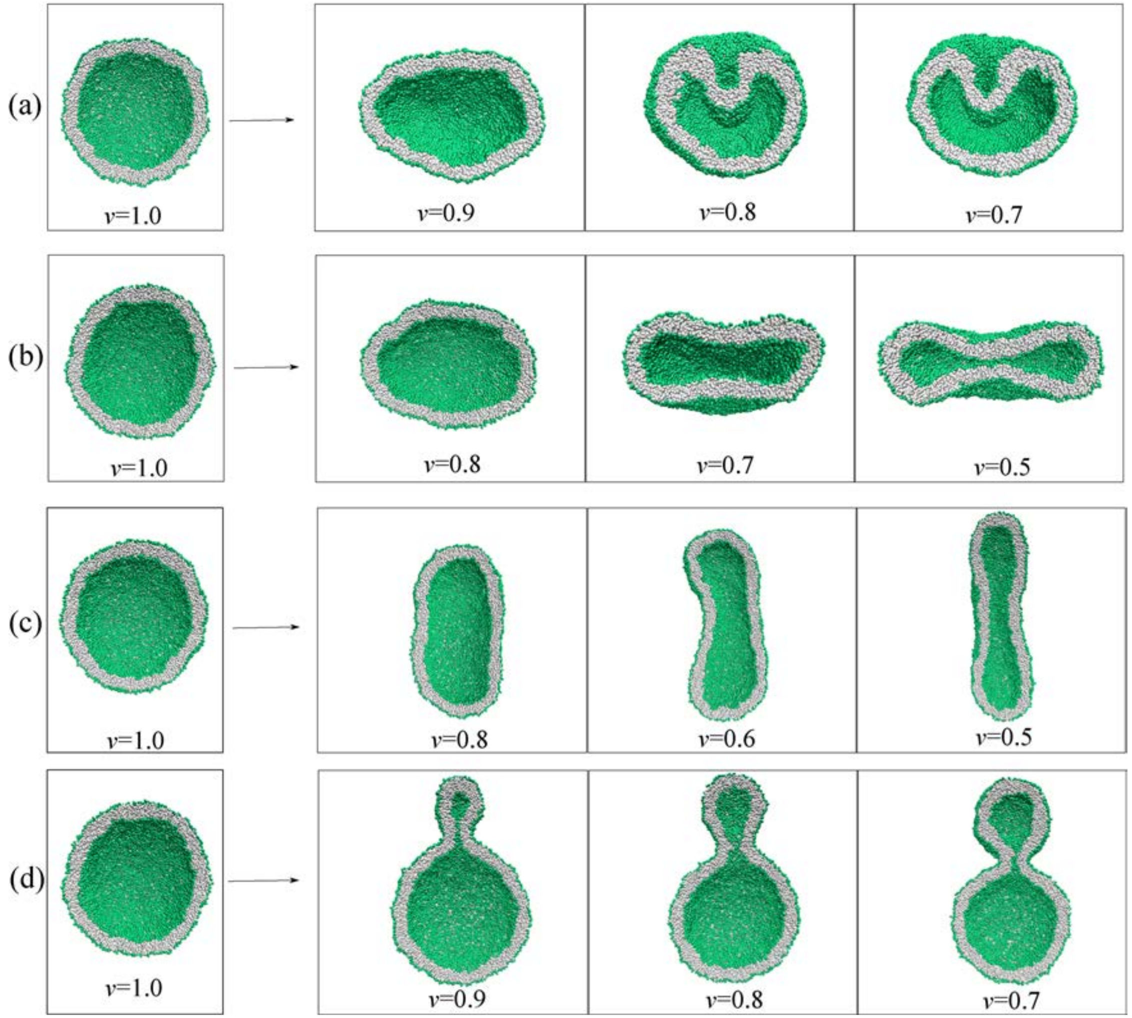


- Add cholesterol
- Leaflet tensions relax towards $\Sigma_1 = \Sigma_2 = 0$

Leaflet Tensions and Nanovesicles

Ghosh, Satarifard, Grafmüller, RL, *Nanoletters* (2019)

- Polymorphism of spherical nanovesicle:



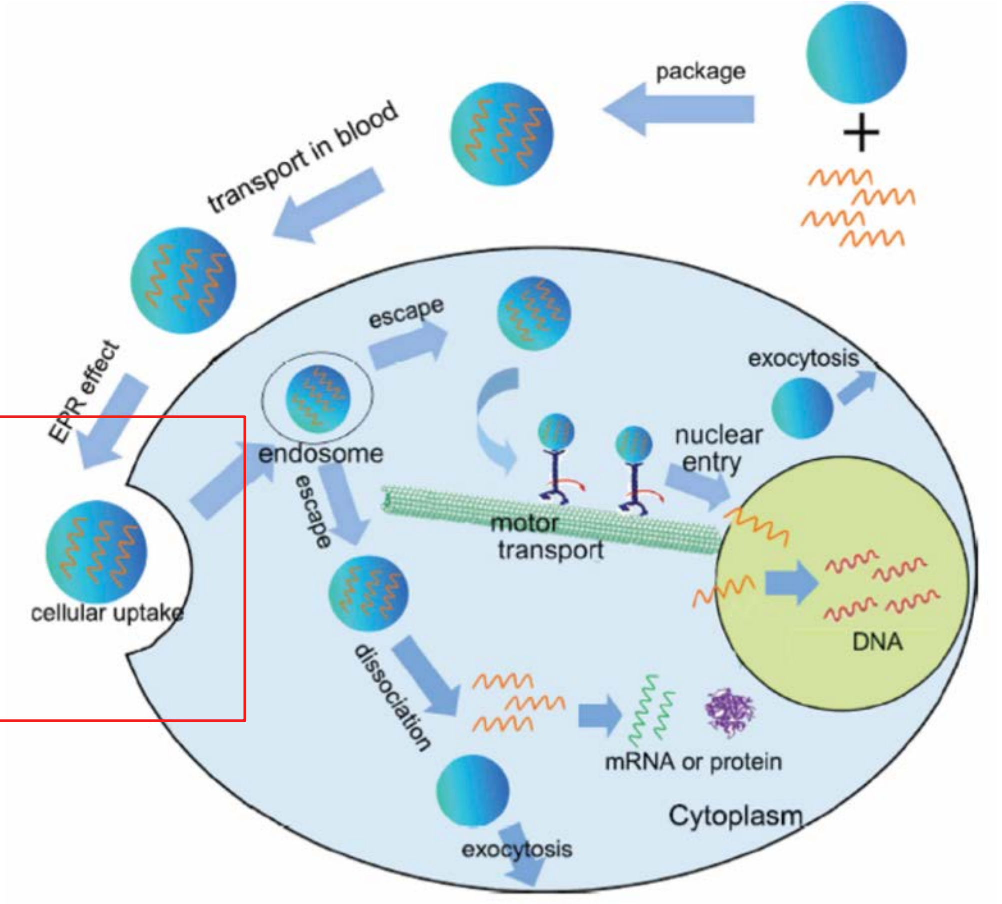
- Four spherical vesicles
- Same volume
- Same total # of lipids
- Reduction of volume: very different shapes
- Shape transformations determined by leaflet tensions Σ_1 and Σ_2

- Vesicles and Membranes
- Membrane tension and curvature elasticity
- Spontaneous curvature
- Morphological complexity of vesicles
- Outlook on related topics:
  Bilayer membranes and leaflet tensions
  ☞ Endocytosis of nanoparticles by membranes
  Wetting and engulfment of droplets
  ...

Targeting Nanoparticles to Cells

- Nanoparticles (NPs) as drug delivery systems:

Transport of NPs
towards cells

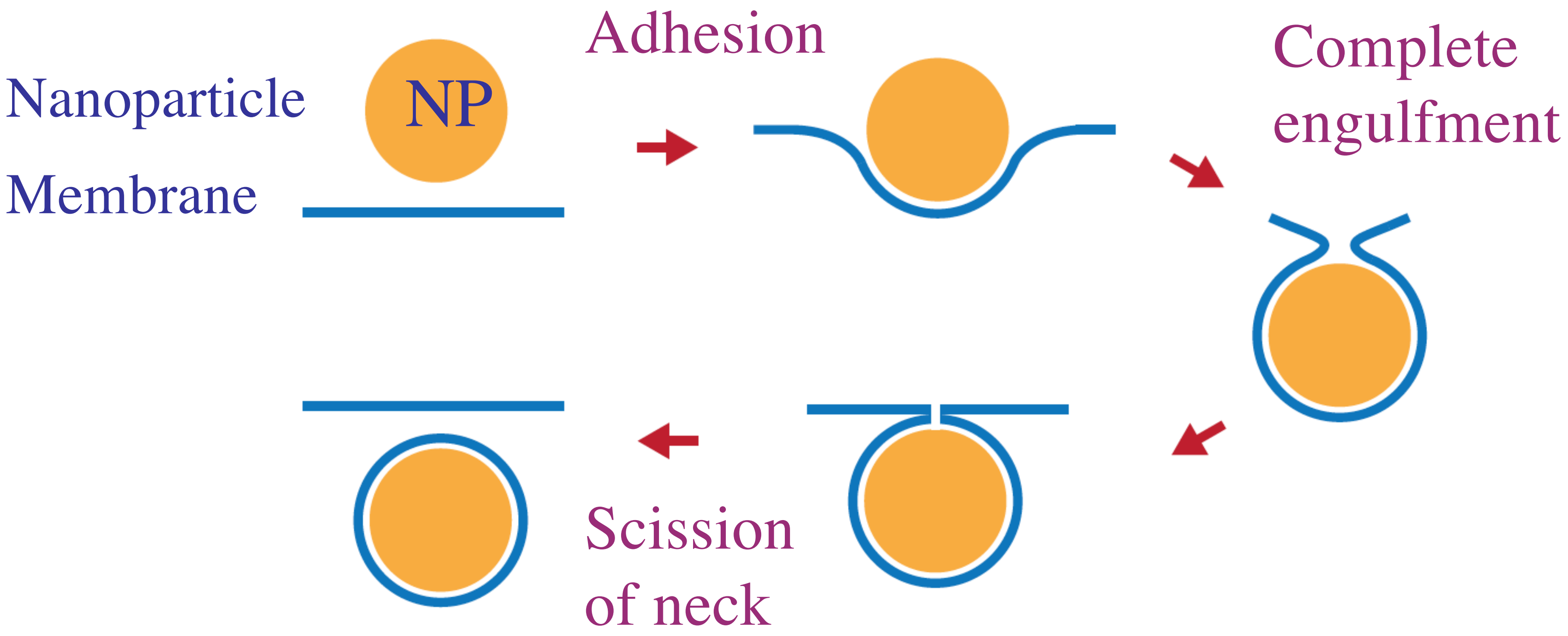


Transport across
cell membrane
by endocytosis

- Endocytic pathway also used by virusses,
airborne ultrafine particles, ...

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

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)

Adhesion: Basic Aspects

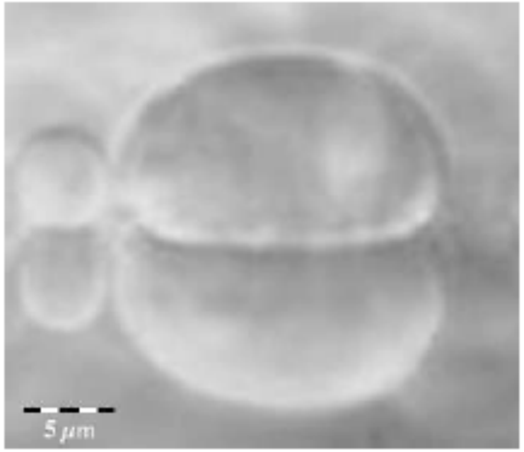
- Attractive interactions between NP and membrane
- Van der Waals, electrostatic, receptor-ligand
- Gain of adhesion free energy but increase of elastic membrane energy
- Competition between adhesion and bending
- Bending rigidity κ versus adhesive strength |*W*|

Adhesion Length

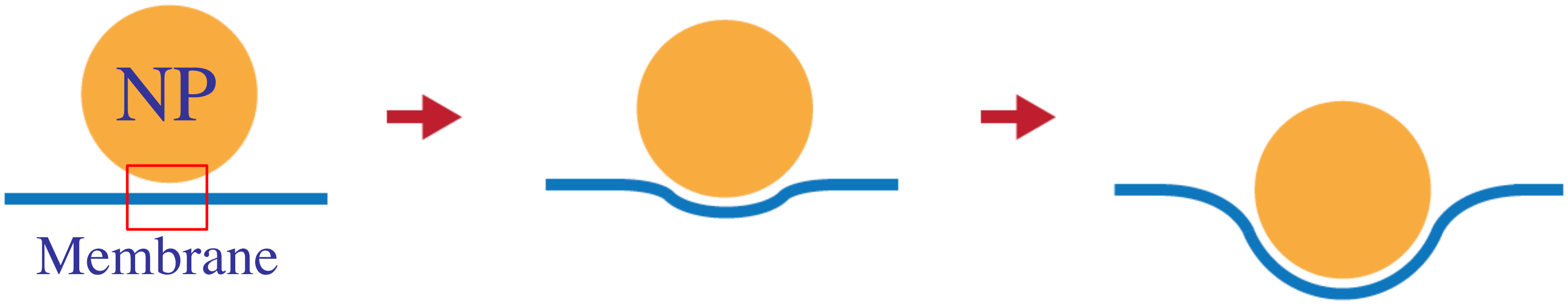
- Adhesive strength $|W|$ = adhesion free energy per area
- Bending rigidity κ and adhesive strength $|W|$ define adhesion length

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

- 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



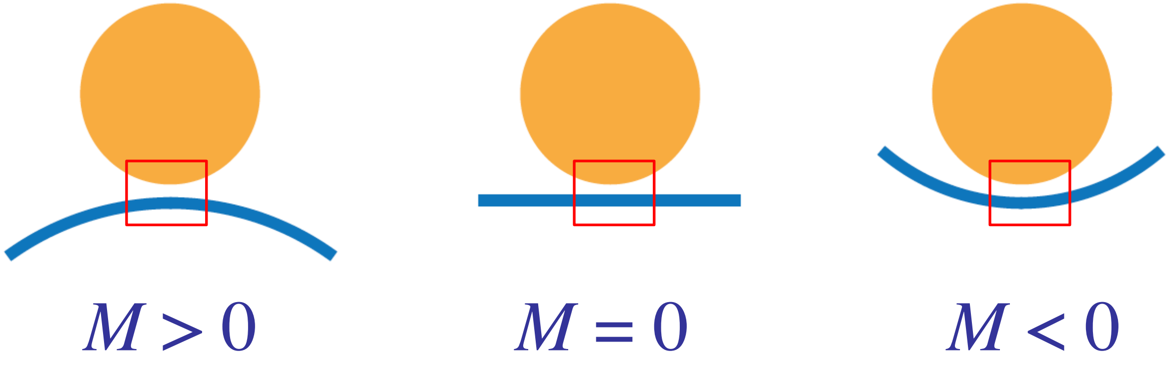
Onset of Adhesion: Key Parameters



- 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

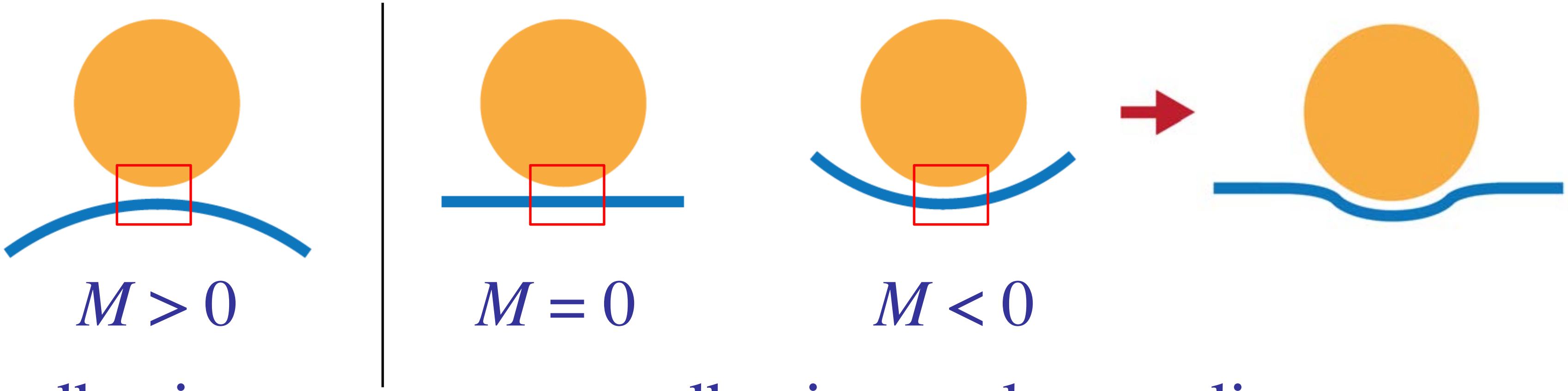
Agudo-Canalejo and RL, *ACS Nano* + *Nano Letters* (2015)

- Membrane starts to spread over particle if

$$\boxed{M \le 1/R_\mathrm{W} - 1/R_\mathrm{pa} =: M_\mathrm{co}}$$

contact curvature M_co is threshold value for M

- Example: $R_\mathrm{W} = R_\mathrm{pa}$ or $M_\mathrm{co} = 0$

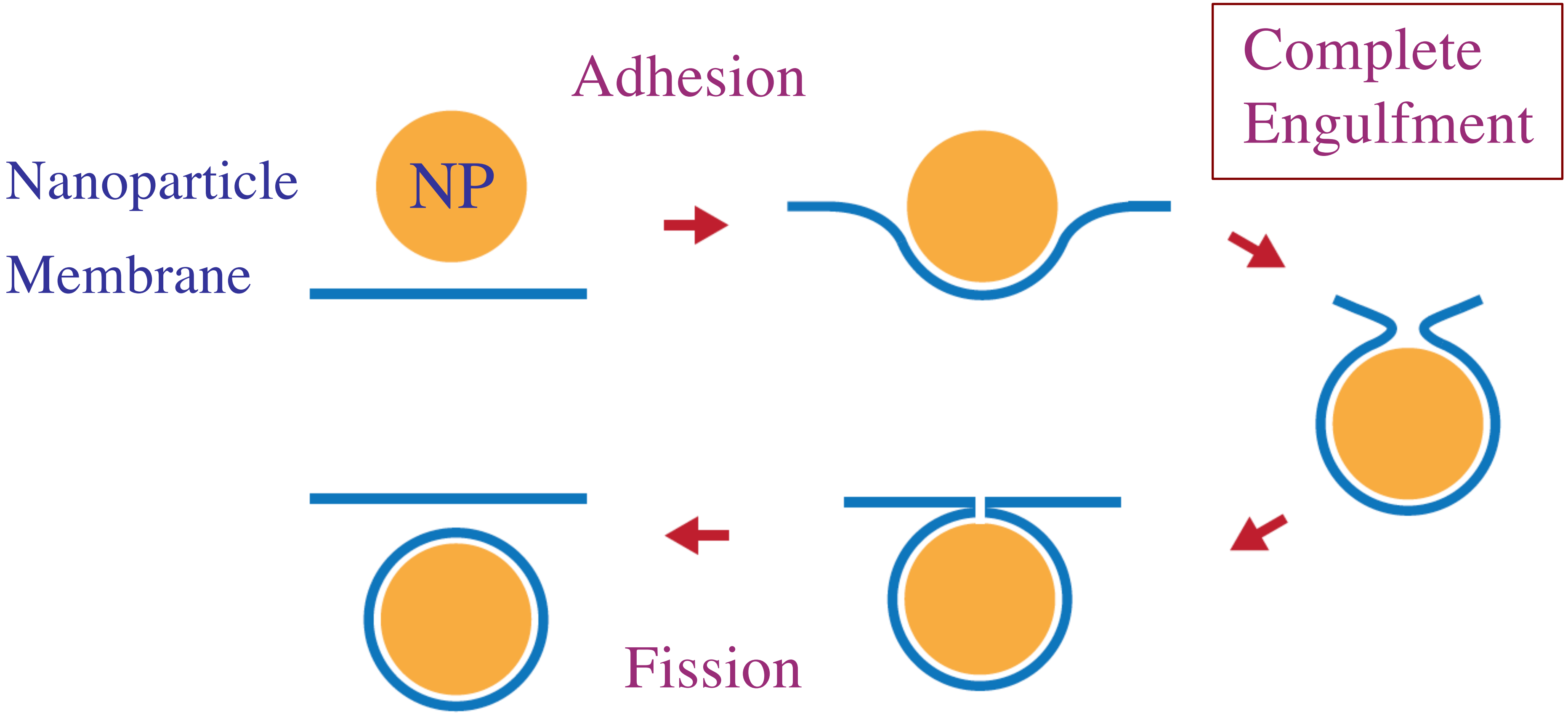


$M > 0$ $M = 0$ $M < 0$

no adhesion adhesion and spreading

- Large contact curvature M_co for small R_W or large $|W|$

Endocytosis: Complete Engulfment



Nanoparticle

Membrane

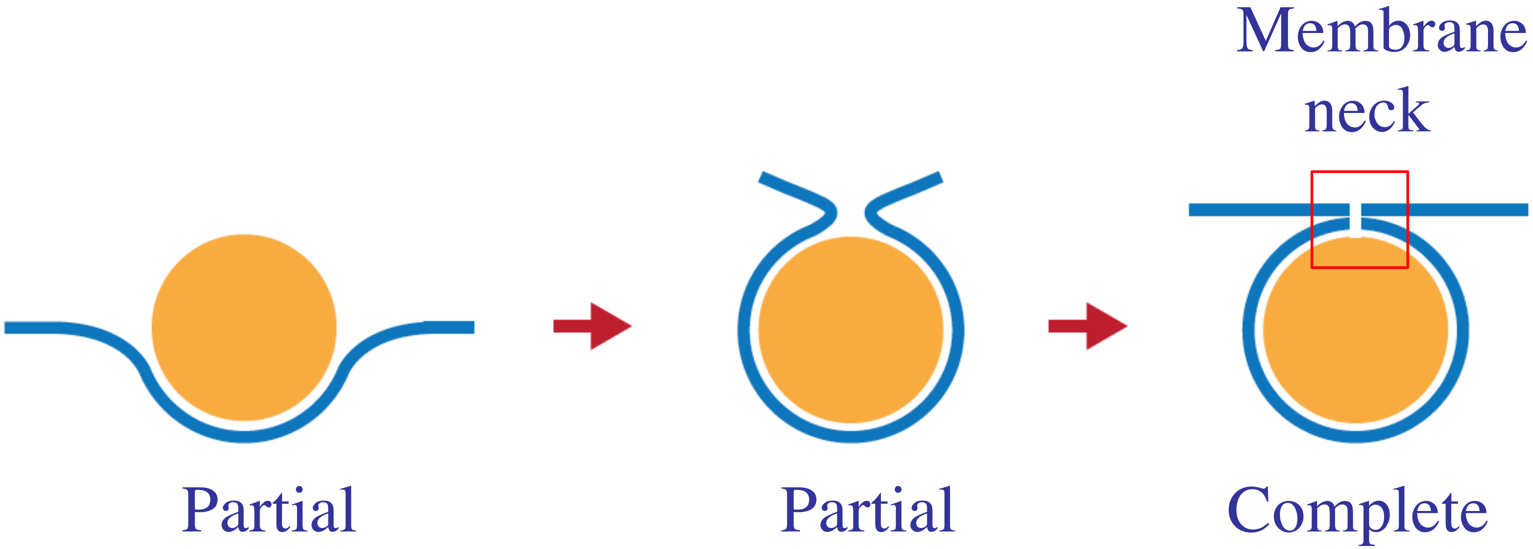
NP

Adhesion

Complete Engulfment

Fission

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

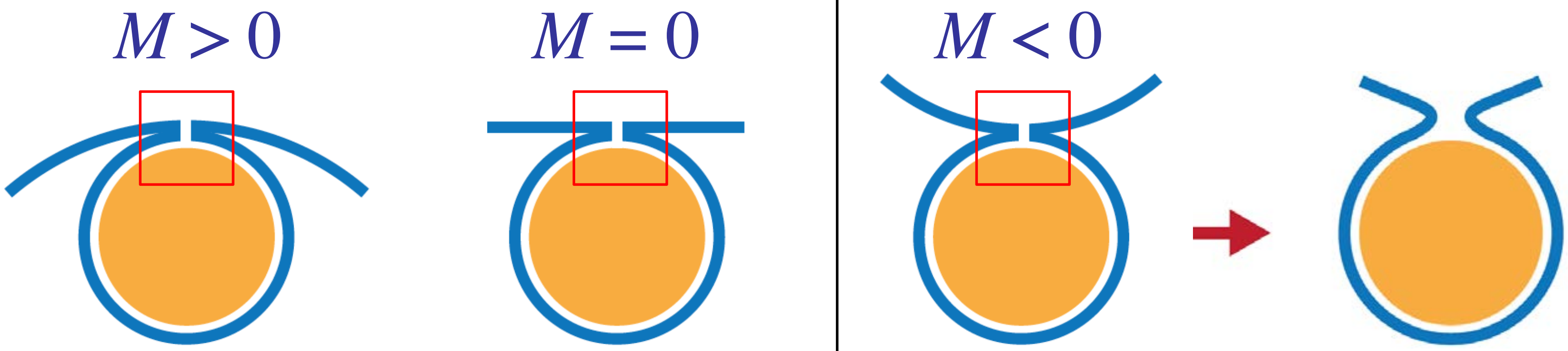
Agudo-Canalejo and RL, *ACS Nano* + *Nano Letters* (2015)

- Closed membrane neck is stable if membrane curvature

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

2nd threshold value for M

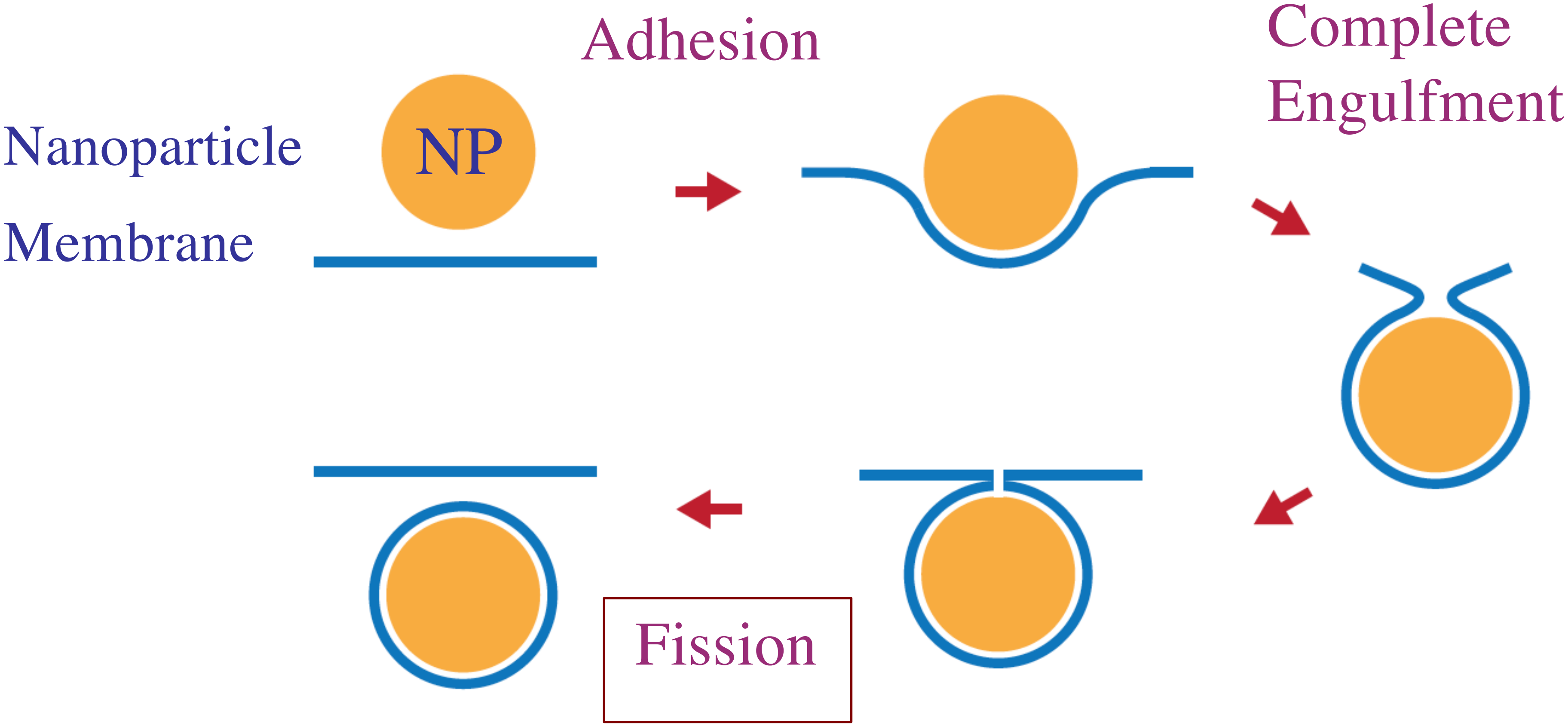
- Example: $M_{\mathrm{ne}} = 2m + 1/R_{\mathrm{pa}} - 1/R_{\mathrm{W}} = 0$



closed neck is stable

closed neck is unstable and opens up

Endocytosis: Fission



Nanoparticle

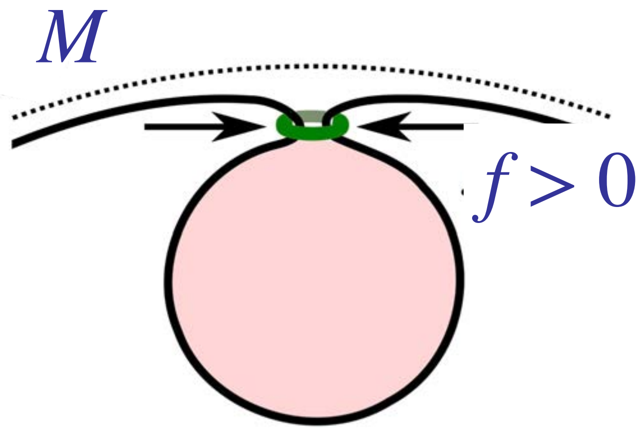
Membrane

Adhesion

Complete Engulfment

Fission

Effective Constriction Force



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

- Closed neck stability with force f :

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

- Effective constriction force

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

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

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

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

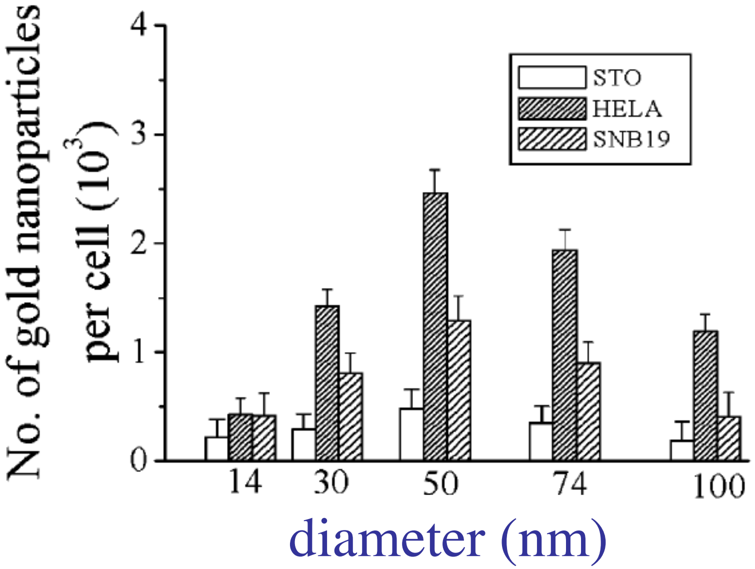
- Sufficient to create two hydrophobic bilayer edges

Receptor-Mediated Endocytosis

Chithrani et al, *Nano Letters* (2007)

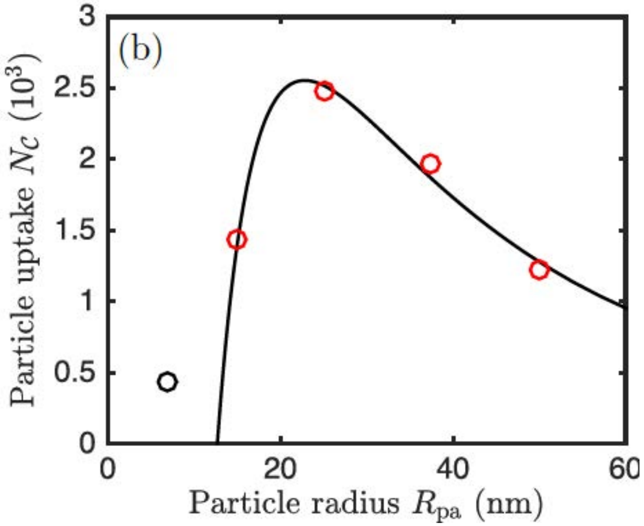
- Uptake of gold nanoparticles by cells
- Particles bind to transferrin receptors
- Assembly of clathrin-coated vesicles

Non-monotonic size-dependence !



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

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



- Vesicles and Membranes
- Membrane tension and curvature elasticity
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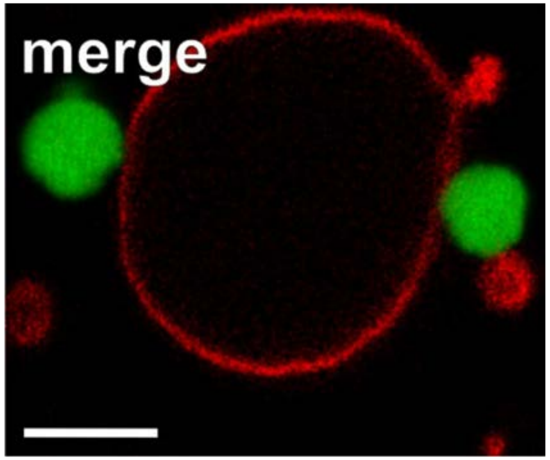
  - ☞ Wetting and engulfment of droplets
  - ...

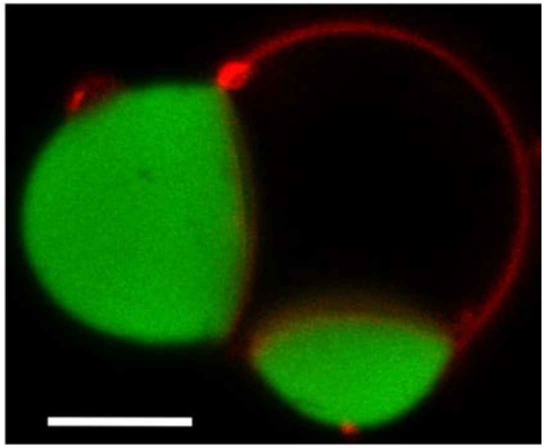
Biomolecular Condensates

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

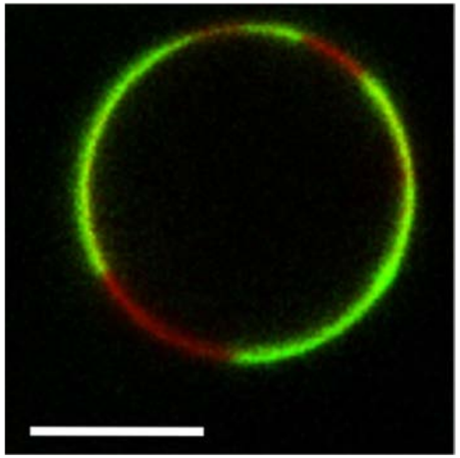
- Biomolecular condensates that behave like liquid droplets
- Enriched in intrinsically disordered proteins (IDPs)
- Example for IDP: RNA-binding protein FUS
- Interaction of FUS-droplets with GUVs,
  two subsequent wetting transitions:



dewetting for
high salt



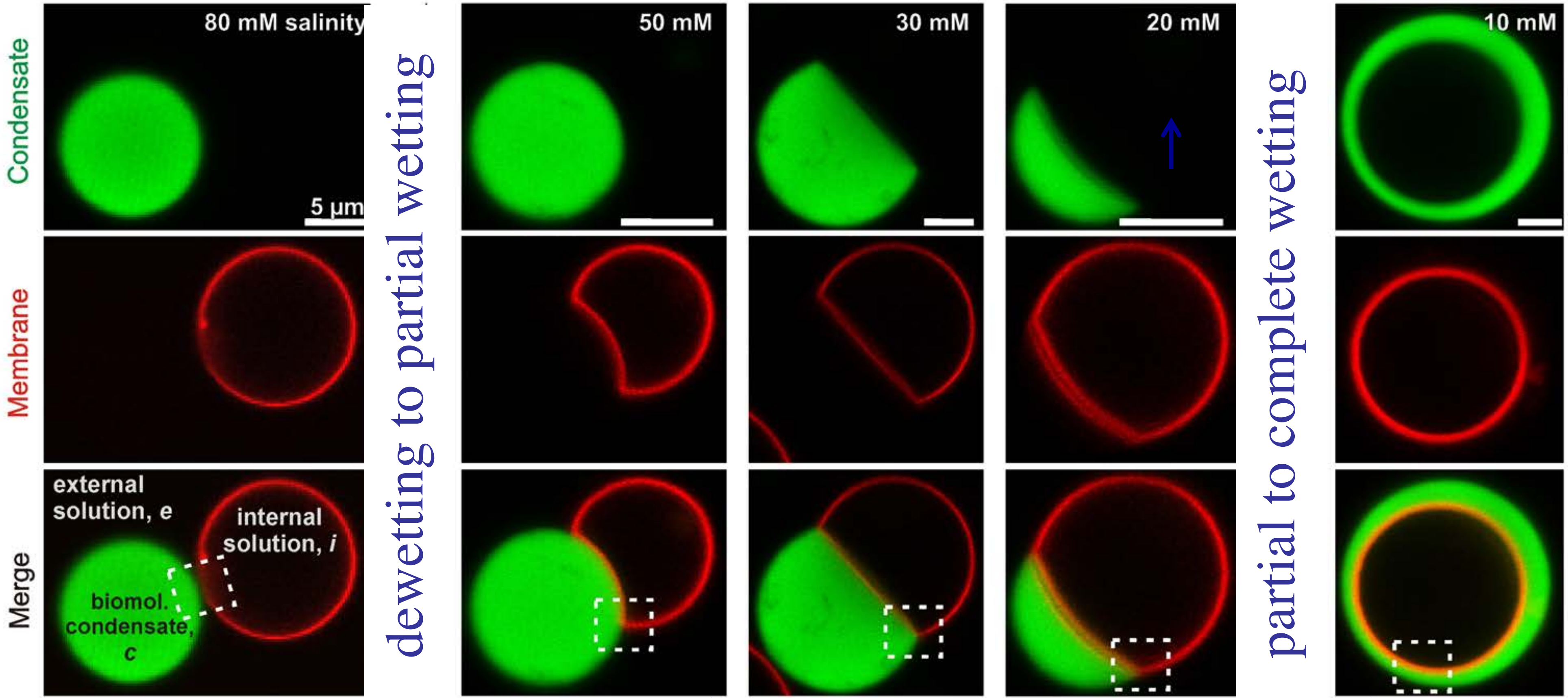
partial wetting for
intermediate salt



complete wetting
for low salt

Two Wetting Transitions

- GUV + FUS-rich organelle + salt



dewetting to partial wetting

partial to complete wetting

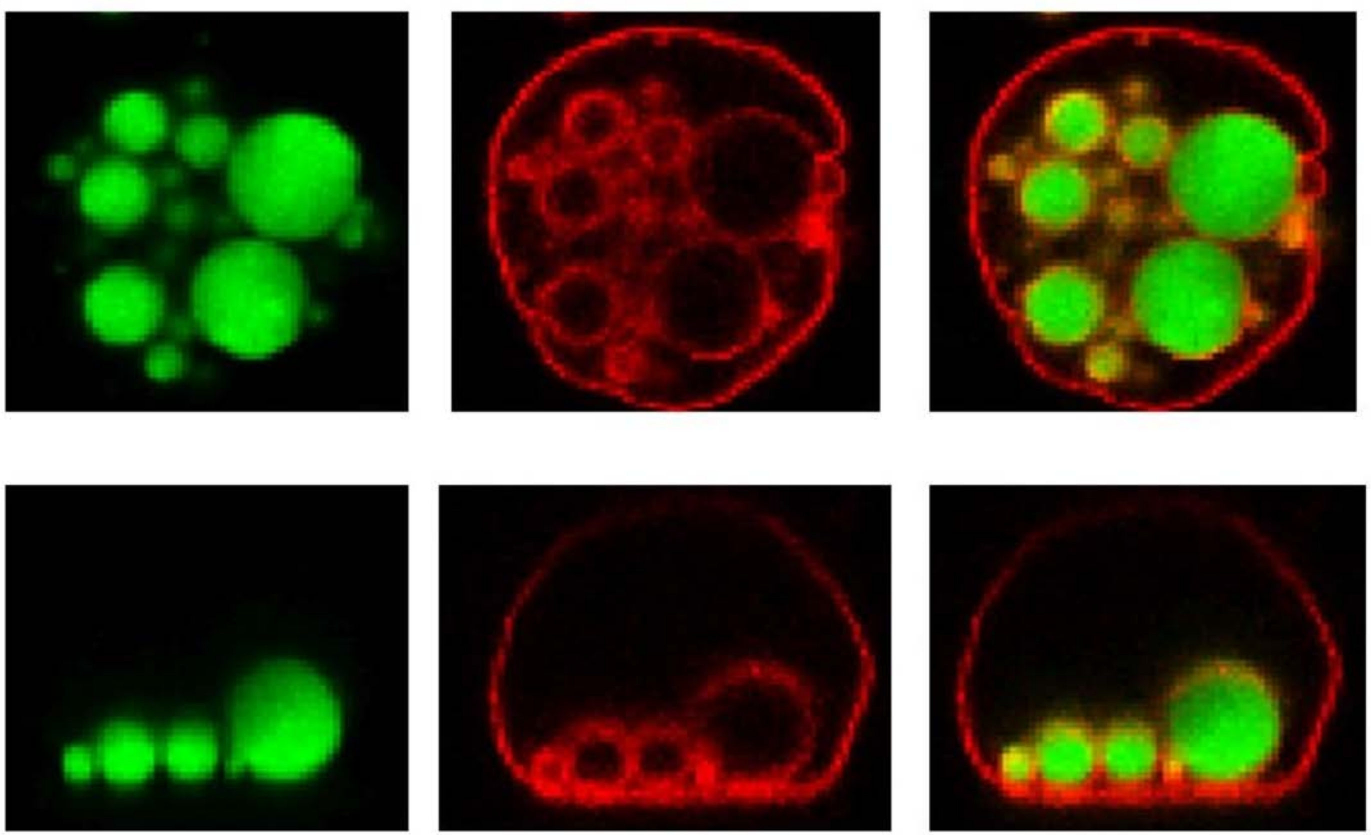
dewetting
for high salt

partial wetting for
intermediate salt

complete wetting
for low salt

Engulfment of Condensates

- Green FUS-rich condensates engulfed by red GUV:

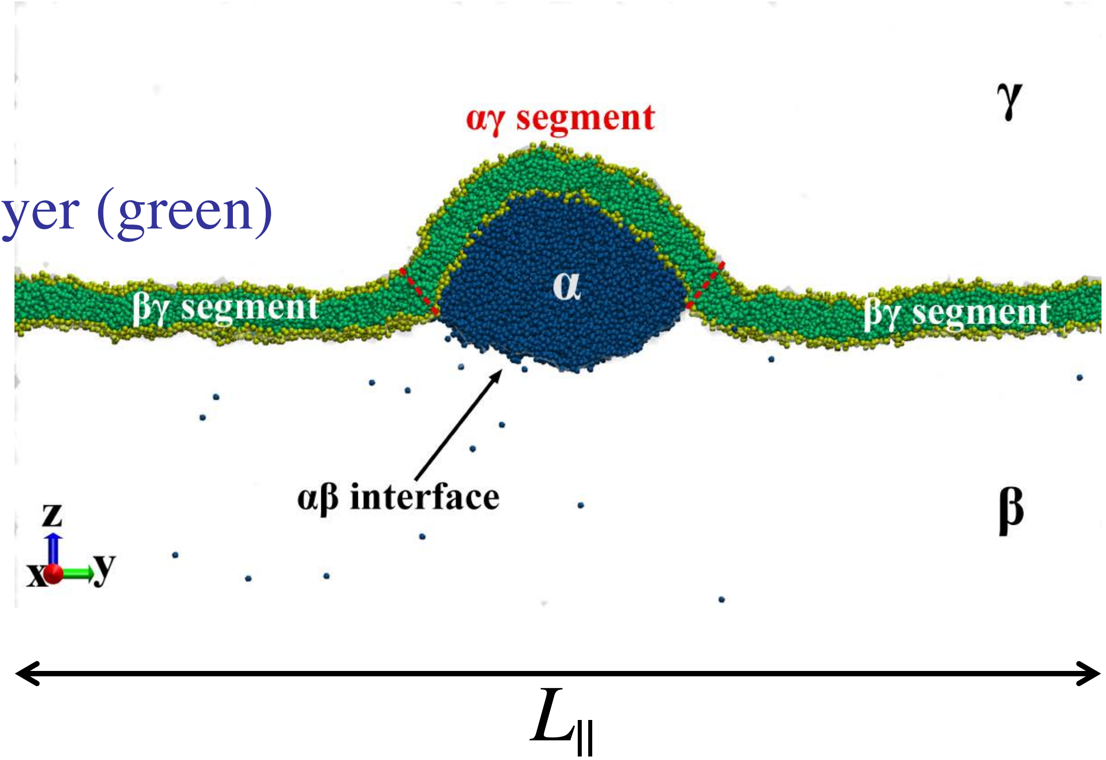


Lipid Bilayer + Nanodroplet

Satarifard, Grafmüller, RL: *ACS Nano* (2018)

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

bilayer (green)

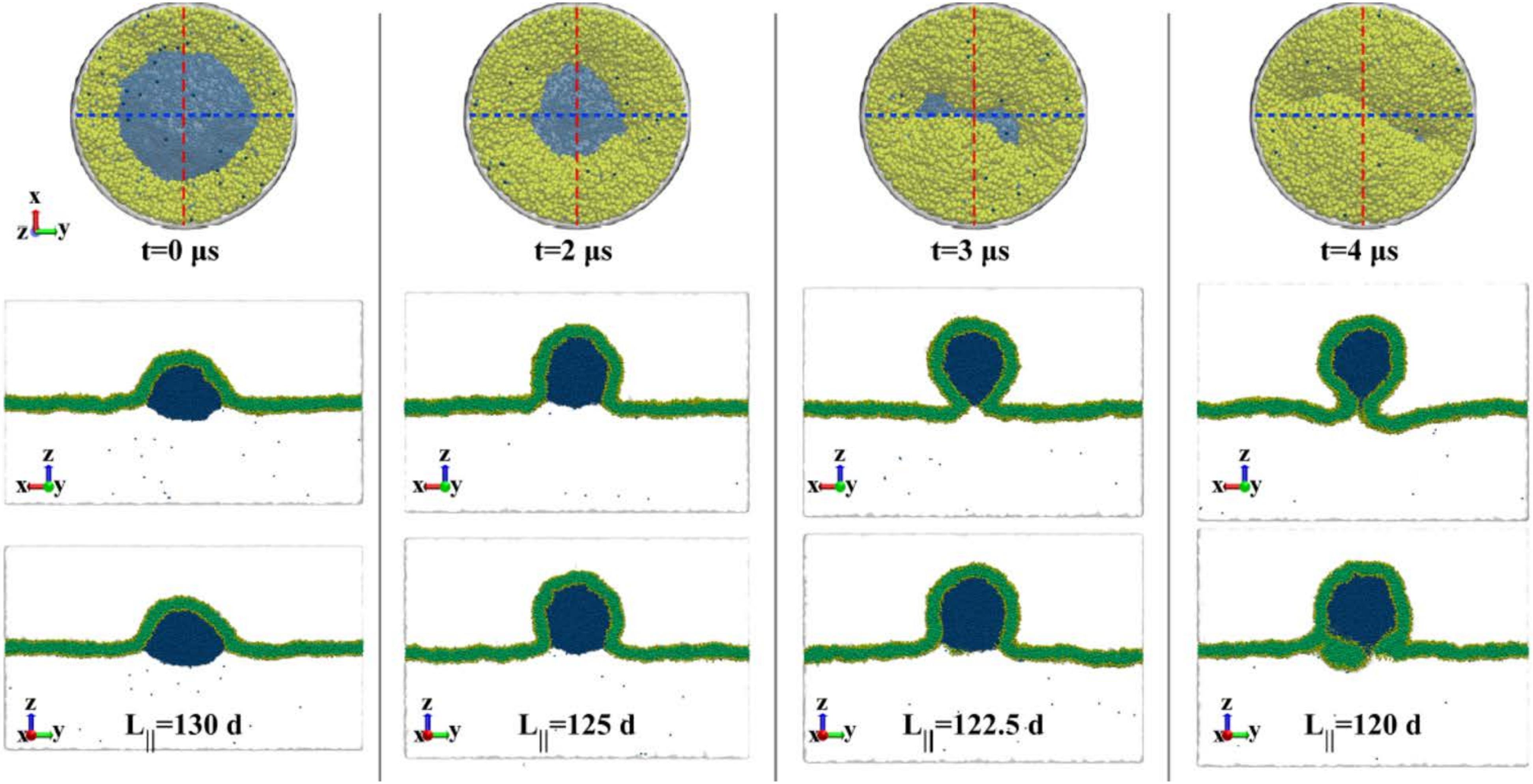


three aqueous phases α, β, γ

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

three surface segments αβ, αγ, βγ

Engulfment of Nanodroplets



- Tight-lipped membrane neck from negative line tension

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

  Jaime Agudo
  Andrea Grafmüller
  Markus Miettinen
  Aparna Sreekumari
  Rikhia Ghosh
  Vahid Satarifard
  Simon Christ

- Membranes, Exp

  Rumiana Dimova
  Tripta Bhatia
  Jan Steinkühler
  Ziliang Zhao

- Collaborations

  Joachim Spatz
  Tony Hyman
  Titus Franzmann
  Seraphine Wegner
  Solveig Bartelt