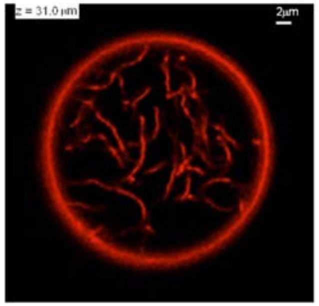
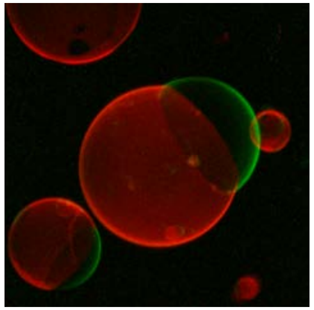
Morphological Complexity of Biomembranes

Reinhard Lipowsky
MPI of Colloids and Interfaces, Potsdam, Germany

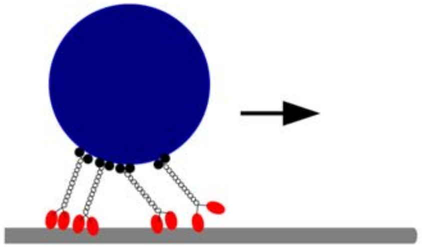
- Biomembranes and Giant Vesicles (GUVs)
- Spontaneous (Sp) Curvature
- Controlled Division of GUVs
- Constriction Forces from Sp-Curvature
- Multispherical Shapes of GUVs
- Spontaneous Tubulation
- Concept of Membrane Tension
- Outlook on Related Processes

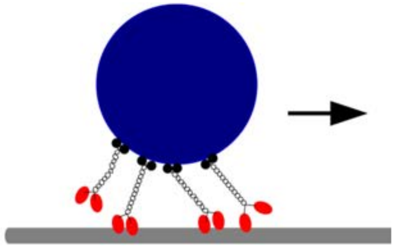
Three Basic Modules



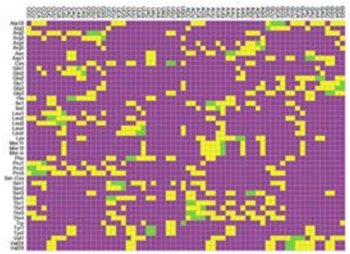


- Membrane and vesicles, fluid compartments, remodeling



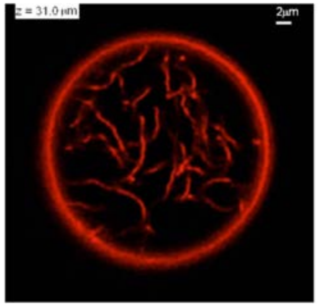


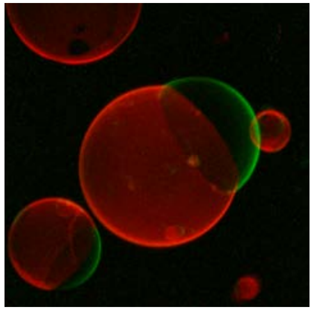
- Directed transport by molecular motors, free energy transduction



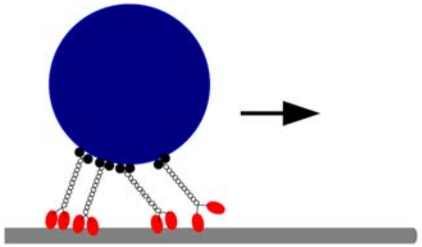
- Protein synthesis by ribosomes, information processing

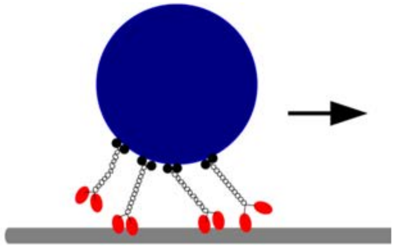
Theory <-> Experiment



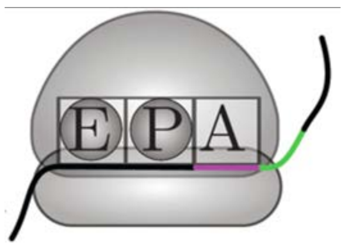


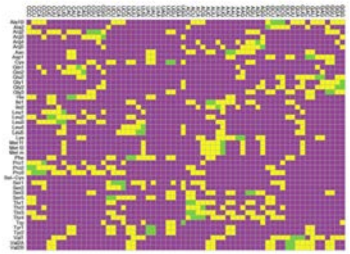
- Fruitful interplay Th <-> Exp quantitative parameter estimates, from understanding to construction





- Successful theory for transport, missing parameters: motor number, reaction free enthalpy, ...

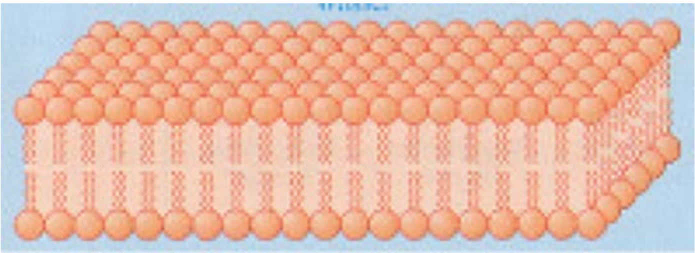




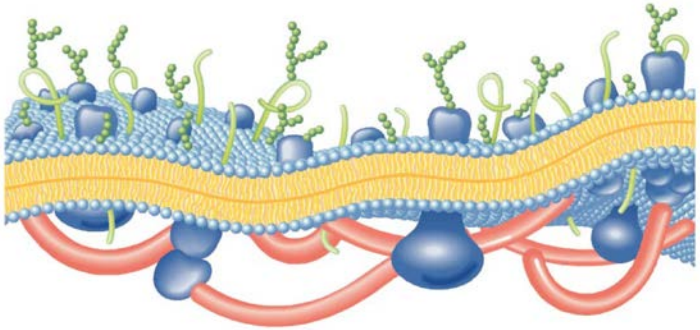
- Multistep process, codon-specfic and context-specific rates, strong coupling to other modules

Multiscale Membranes

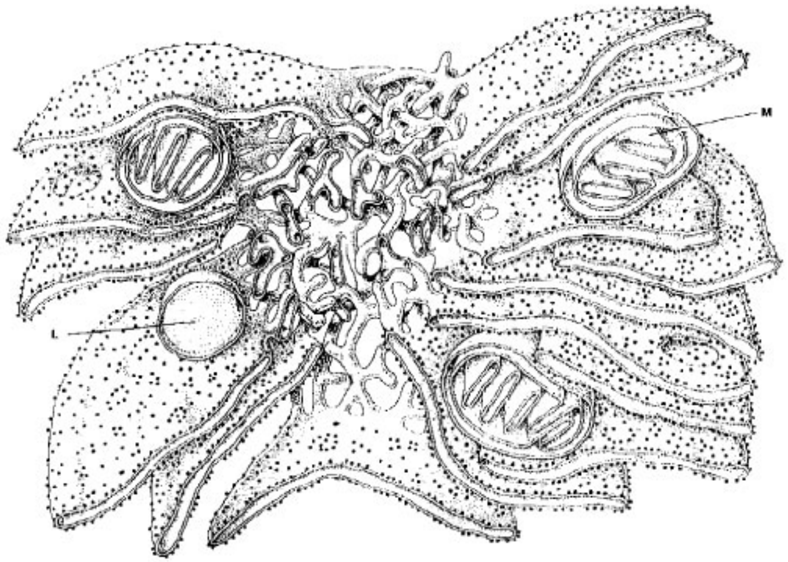
- Lipid bilayer

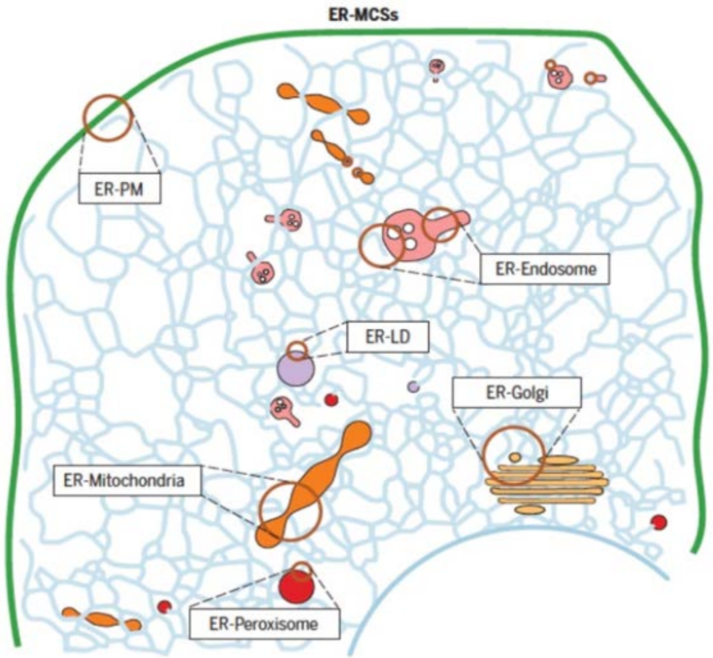


4 nm



- Biomembrane





40 μm

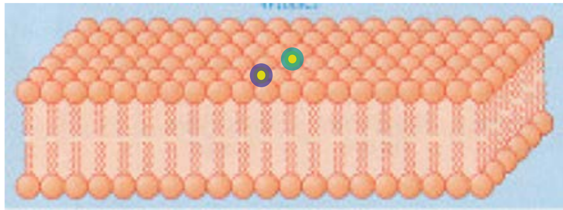
- Endoplasmic reticulum (ER)

Wu et al, *Science* (2018)

Biomembranes are Fluid Bilayers

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

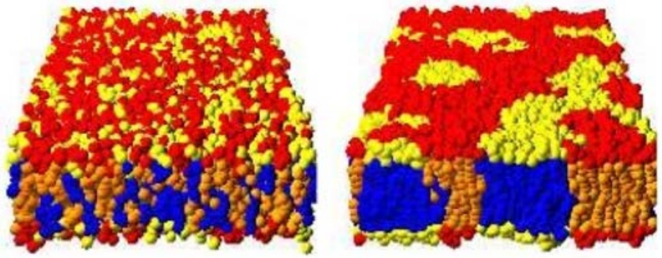
  Diffusion constant ~ $\mu m^2/s$



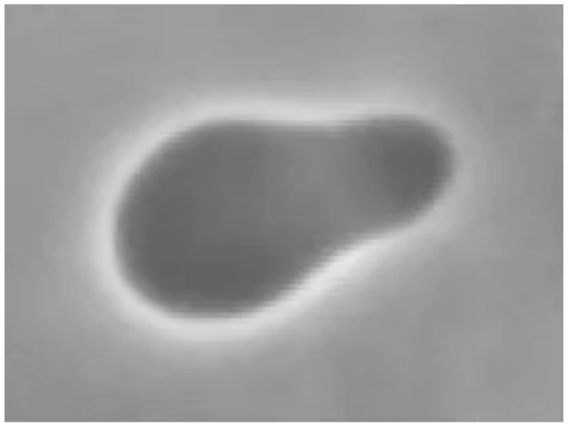
4 nm

lipid swapping ~ ns

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



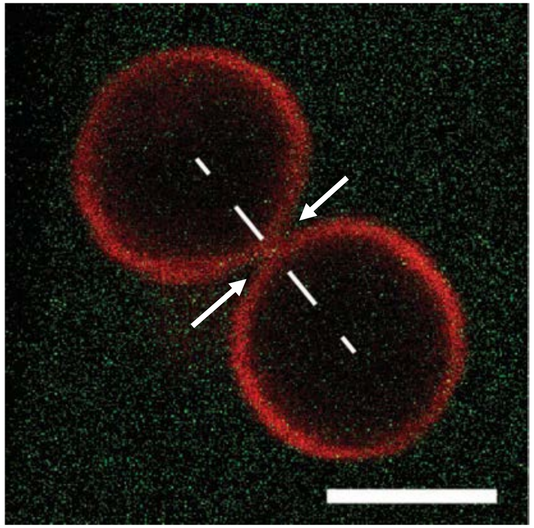
- Flexibility =>
  Morphological responses,
  budding, tubulation, ...
  Direct evidence for fluidity



20 μm

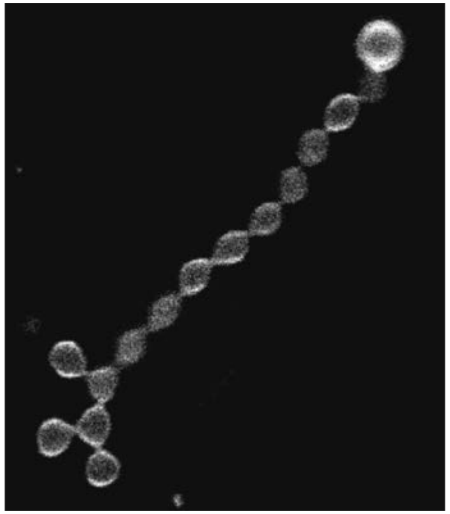
Giant Vesicles with Membrane Necks

- Giant Unilamellar Vesicles (GUVs), size of 5 – 50 μm
- Lipid bilayers, thickness of 4 -5 nm
- Many different shapes with membrane necks:



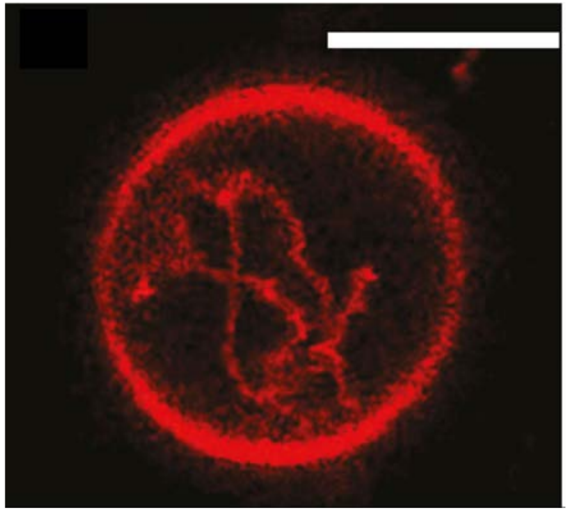
Dumbbell, (1+1)-sphere, one membrane neck

Steinkühler et al, *Nature Comm* (2020)



(1+14)-sphere, 14 necks

Bhatia et al, *Soft Matter* (2020)

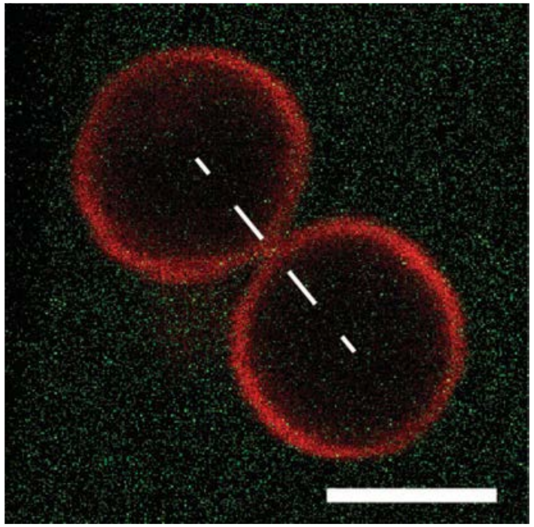


Long nanotubes, width of 100 nm

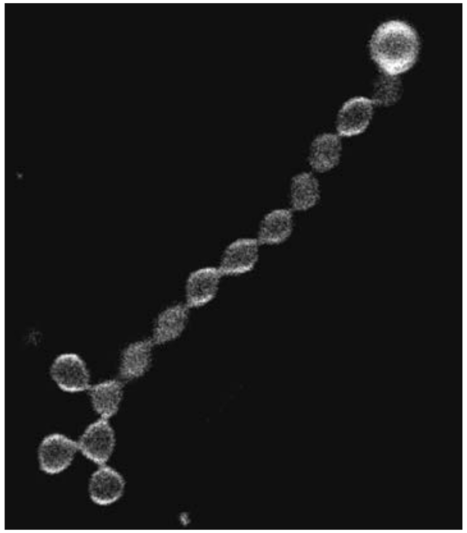
Bhatia et al, *ACS Nano* (2018)

Multiresponsive Compartments

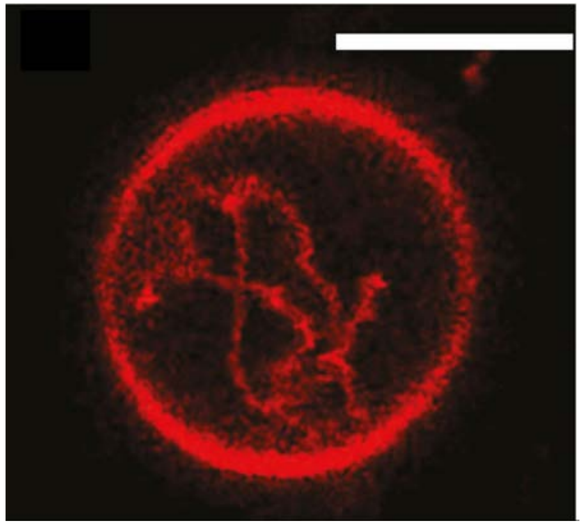
- Nanoscale: Lipid membranes provide fluid walls
- Membrane elasticity = bending and stretching
- Morphological responses via molecular interactions:



Exposed to
His-tagged GFP
in exterior solution



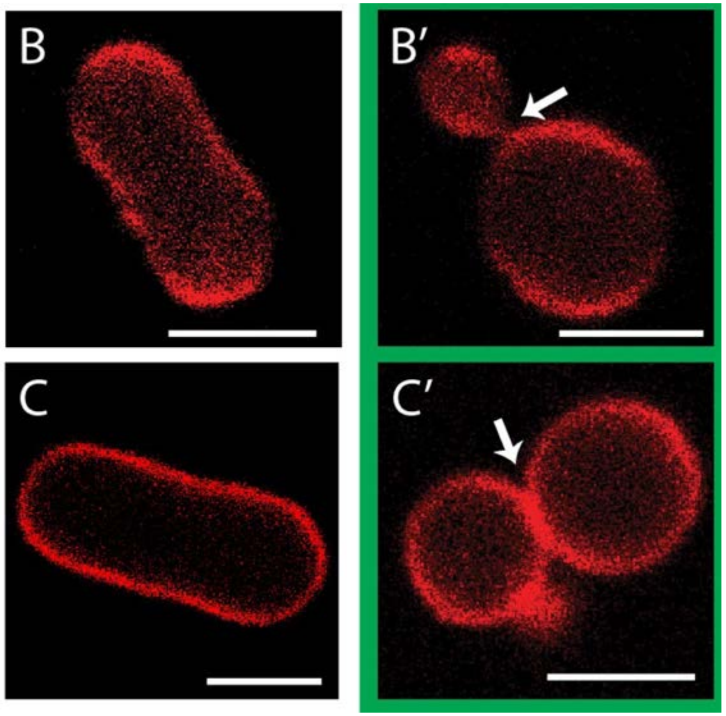
Sucrose inside,
glucose outside



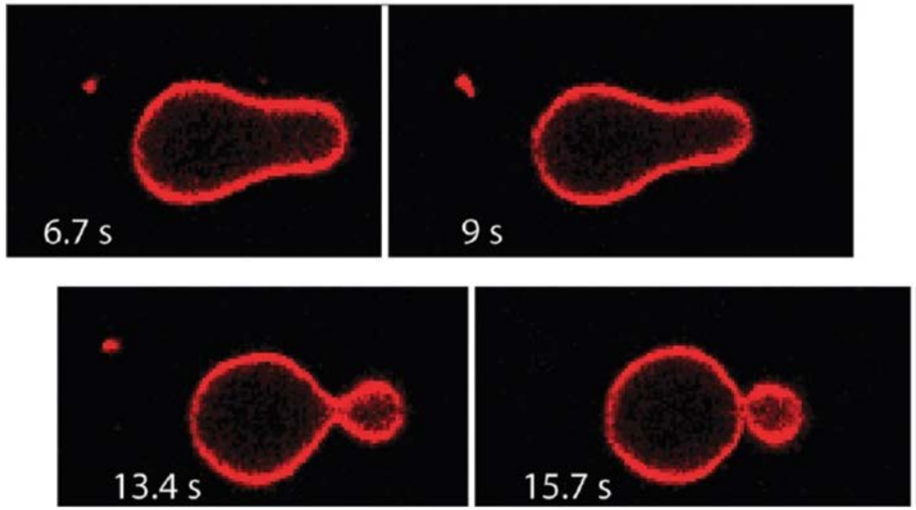
Exposed to
glycolipid GM1 with
bulky head group

Budding and Membrane Necks

Neck formation by increase of [GFP]



Neck formation by osmotic deflation:



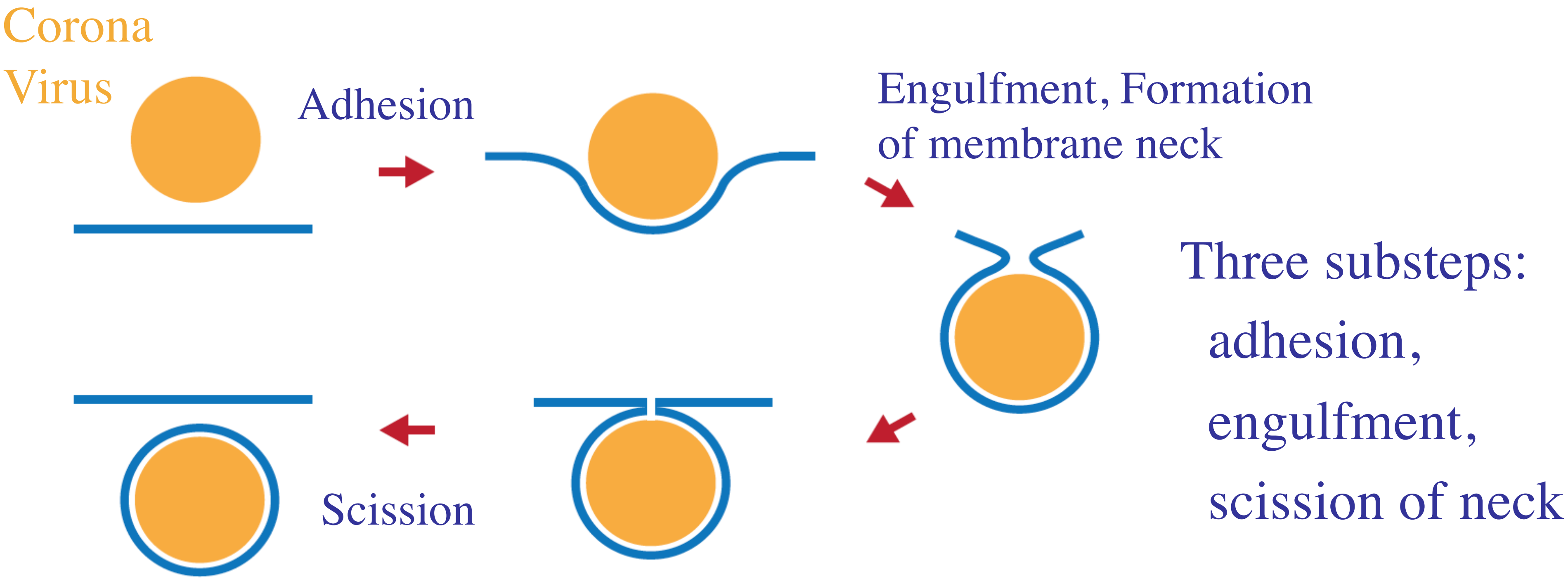
Membrane neck provides ‘wormhole in 3-dim space’

- Theory of curvature elasticity:

  Budding and neck formation ⇔ spontaneous curvature

Endocytosis of Viruses

- Crossing the cell membrane by endocytosis:



- Interplay of spontaneous curvature and adhesion length

Agudo-Canalejo, RL, *ACS Nano* (2015); *Soft Matter* (2017)

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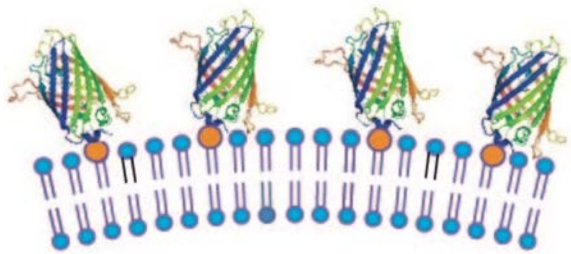
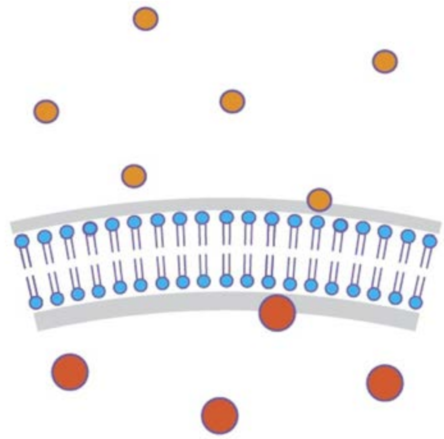
Key Parameter: Spontaneous Curvature

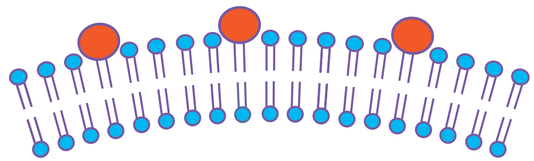
- Lipid bilayer consists of two leaflets
- Spontaneous or preferred curvature *m* describes bilayer asymmetry = asymmetry between two leaflets
- Different molecular mechanisms for sp-curvature:



Binding of GFP to outer leaflet



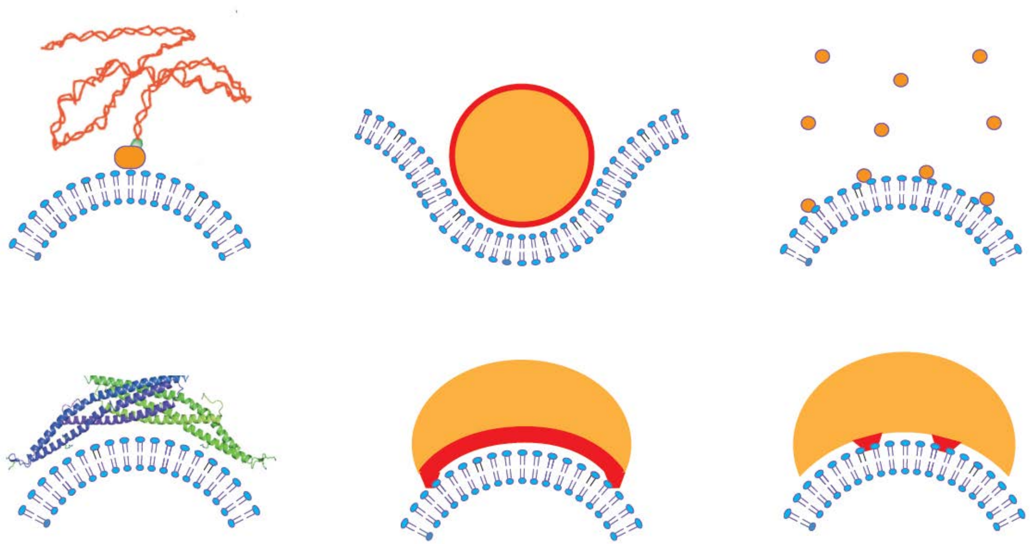
Depletion layers of glucose and sucrose



Asymmetric mole fraction of GM1 with large head group

Importance of Sp-Curvature

- Bridges the gap between molecular and mesoscopic scales
- Spatially uniform membranes: Shape of GUVs depends only on volume, area, and sp-curvature
- Emergent property on length scales that exceed about 6 nm
- Sp-curvature determined by local bilayer asymmetry
- Asymmetry from lipids (GM1), anchored polymers, bound proteins, nanoparticles, ...



Importance of Sp-Curvature

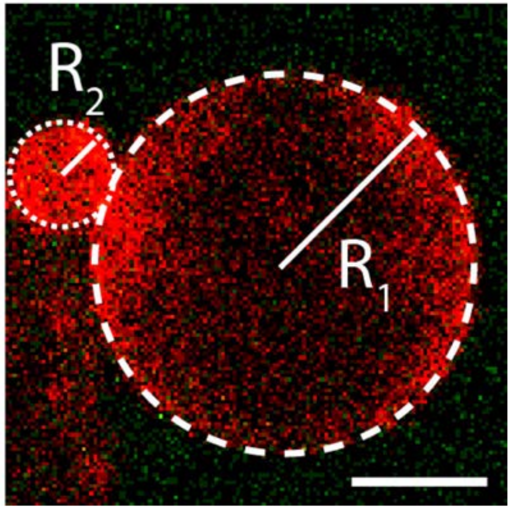
Sp-curvature crucial for:

- Size of membrane buds, stability of membrane necks
- Domain-induced budding of phase separated membranes
- Spontaneous formation of membrane nanotubes
- Endocytosis of viruses and nanoparticles
- Wetting of membranes by droplets
- Active shape oscillations of GUVs

Two challenges:

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

Stability of Closed Necks



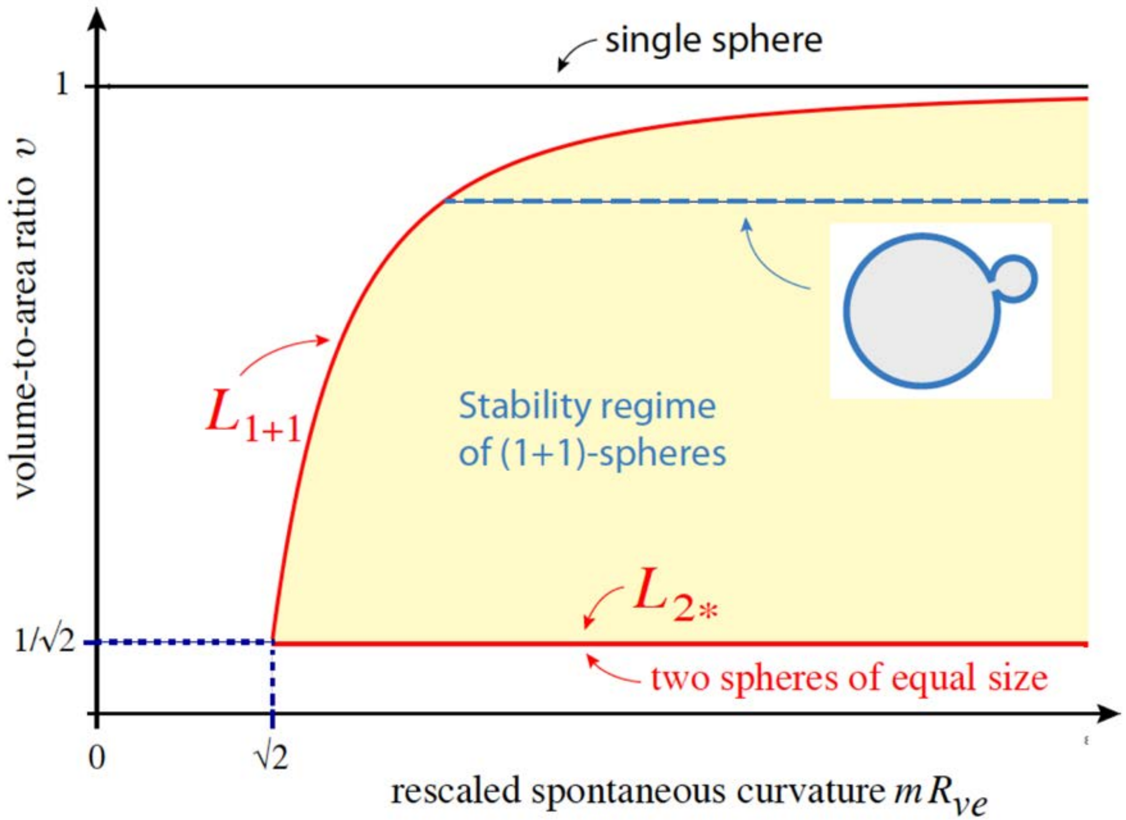
Dumbbell = (1+1)-Sphere

- Sp-curvature m can be positive or negative
- Out-buds ⇔ positive values $m > 0$
- Positive sp-curvatures m above certain threshold value ⇔ dumbbells with closed membrane necks

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

Stability Regime of Dumbbells

- Simplest case: Uniform membrane, no domains or rafts
- Vesicle size R_{ve} as basic length scale
- Two dimensionless shape parameters:
  volume-to-area ratio v and rescaled sp-curvature mR_{ve}



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

RL, *Giant Vesicle Book,* Ch. 5 (2020)

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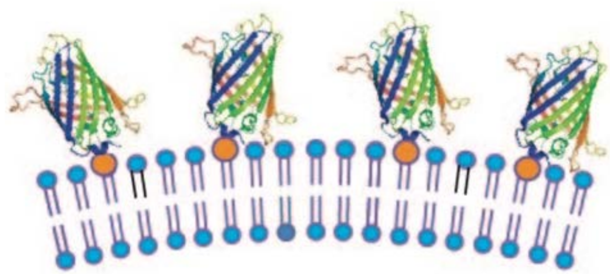
Fine Tuning of Spontaneous Curvature

Jan Steinkühler ... RL : *Nature Comm*. (2020)

• Binding of GFP to small mole fraction of anchor lipids:



His-tagged GFP
NTA-lipids

• Nanomolar GFP concentration X as control parameter

• Density Γ of bound GFP increases linearly with X

• Sp-curvature m increases linearly with $\Gamma \sim X$

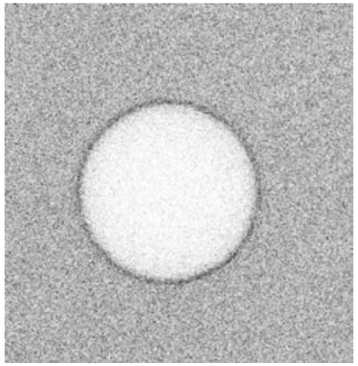
• Dilute regime: separation of bound GFPs much larger than lateral size of GFP

Controlled Division of GUVs

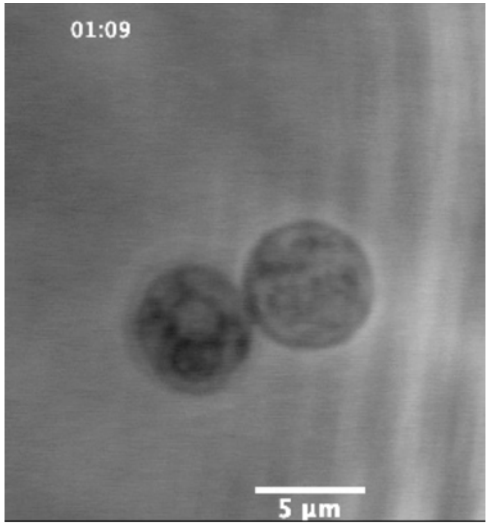
Jan Steinkühler ... RL : *Nature Comm*. (2020)

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

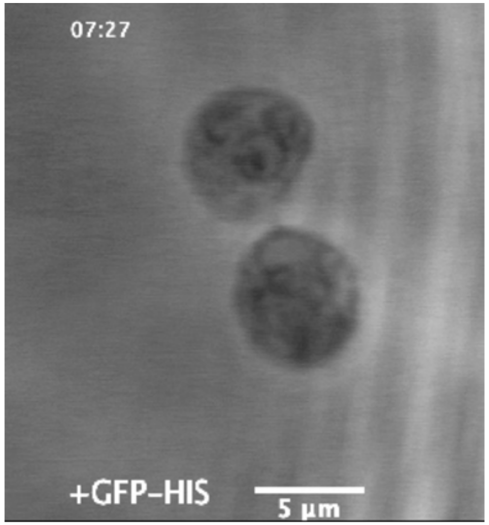
  Increase in GFP -> Neck cleavage -> Two daughter GUVs



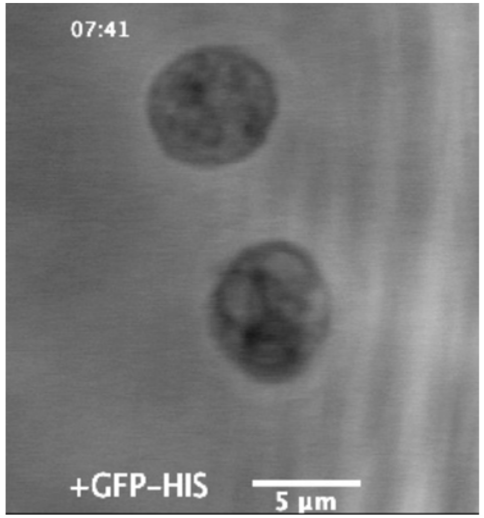
Adsorption of GFP onto GUV membrane



Deflation leads to dumbbell with membrane neck



Directly after neck cleavage



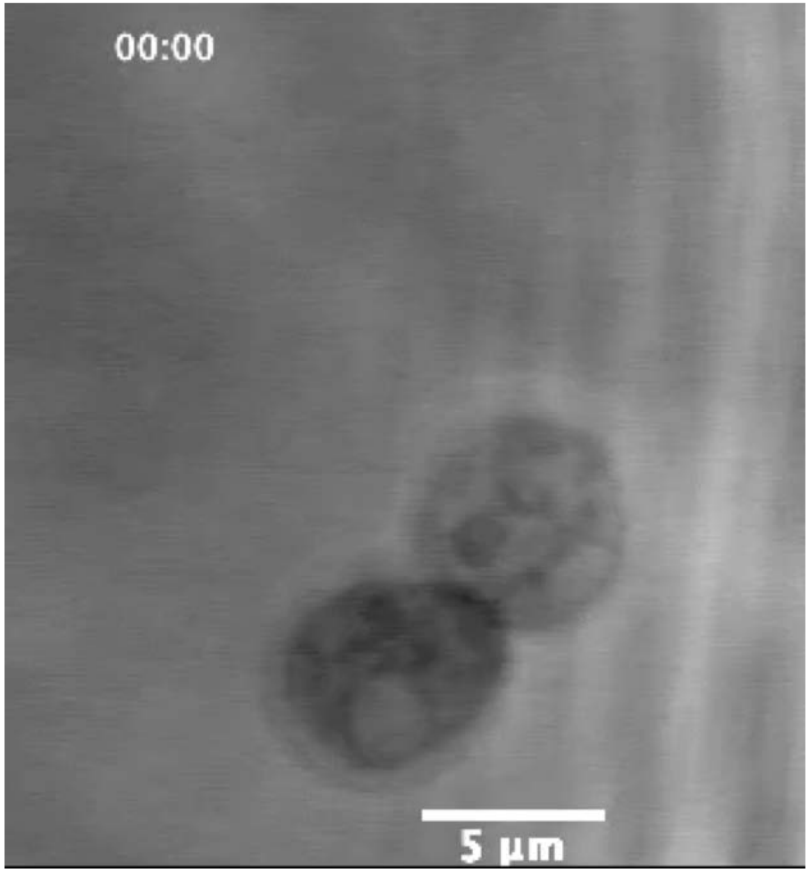
Complete division into two smaller GUVs

Controlled Division of GUVs

Jan Steinkühler ... RL : *Nature Comm*. (2020)

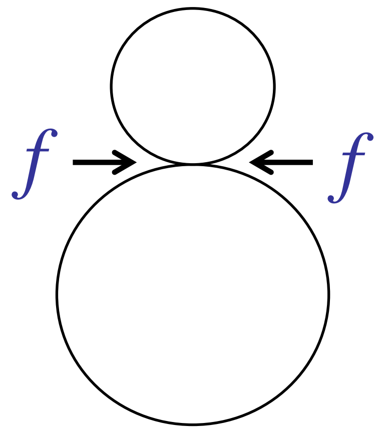
- Osmotic deflation: Spherical GUV -> dumbbell GUV

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



Constriction Force from Sp-Curvature

RL, *Giant Vesicle Book*, Ch. 5 (2020)

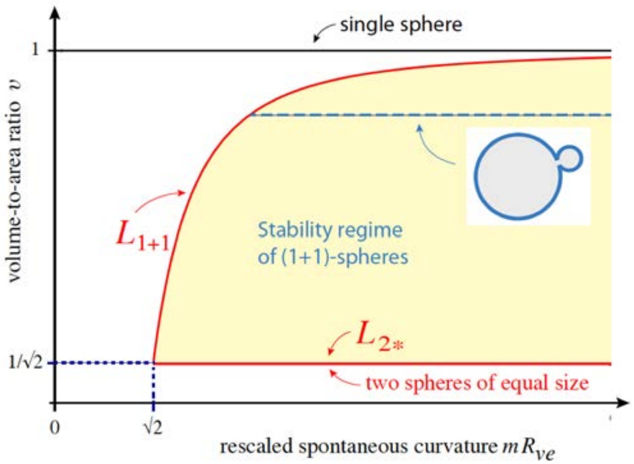


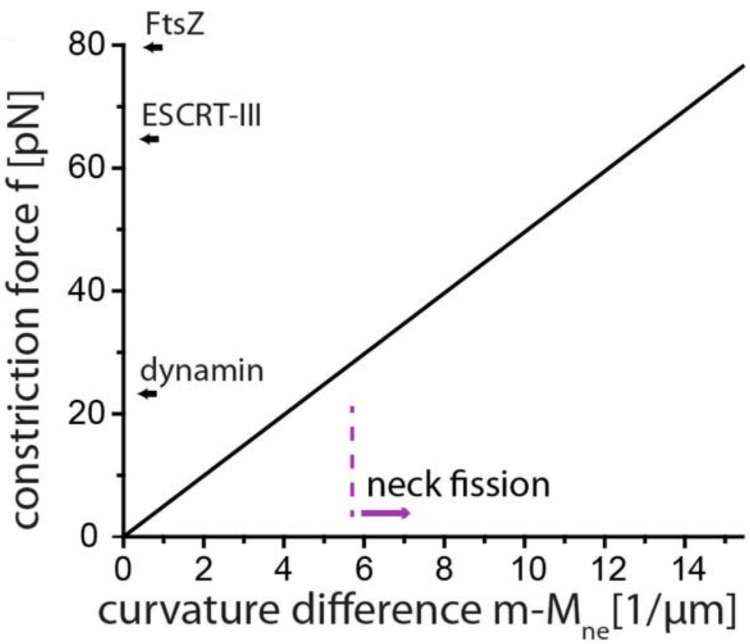
- Sp-curvature m generates constriction force f acting radially on membrane neck:

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

bending rigidity κ, neck curvature M_{ne}

- Force f increases with increasing sp-curvature m:





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Multispheres: Theory

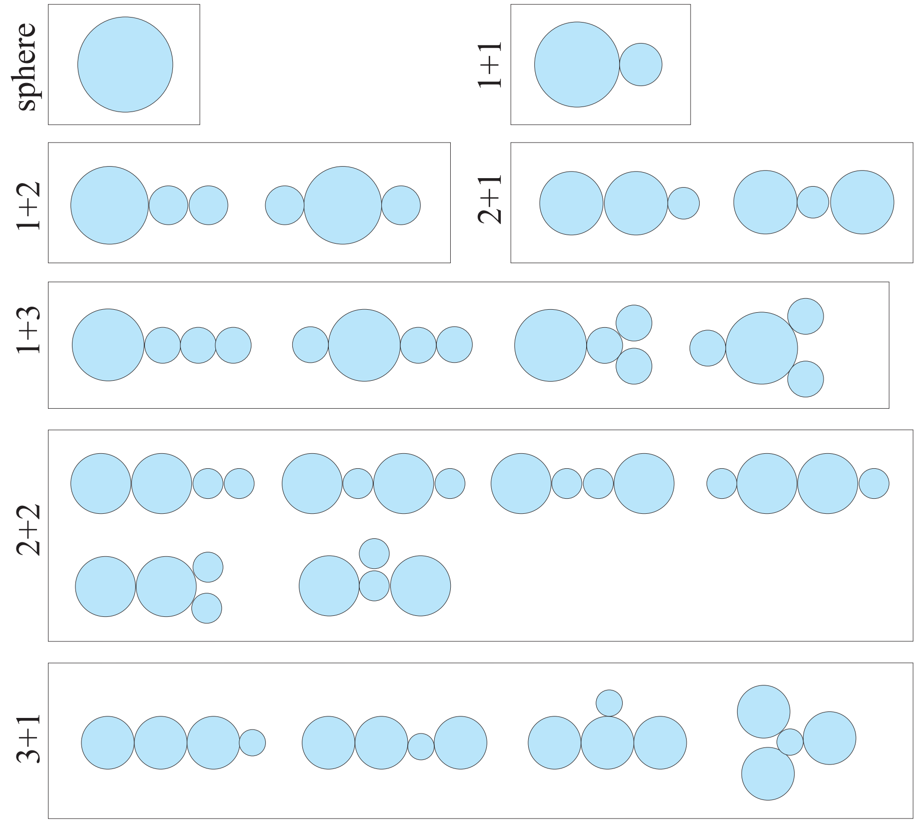
RL, *Advances in Biomembranes and Lipid Selfassembly* Vol. 30, Ch. 3 (2019)

- Single membrane forms several spheres, with pairs of neighboring spheres connected by membrane necks:

- Only two possible radii
- Large spheres with radius R_l
- Small spheres with radius R_s

- $(N_l + N_s)$-spheres
- Example: $N_l + N_s \leq 4$

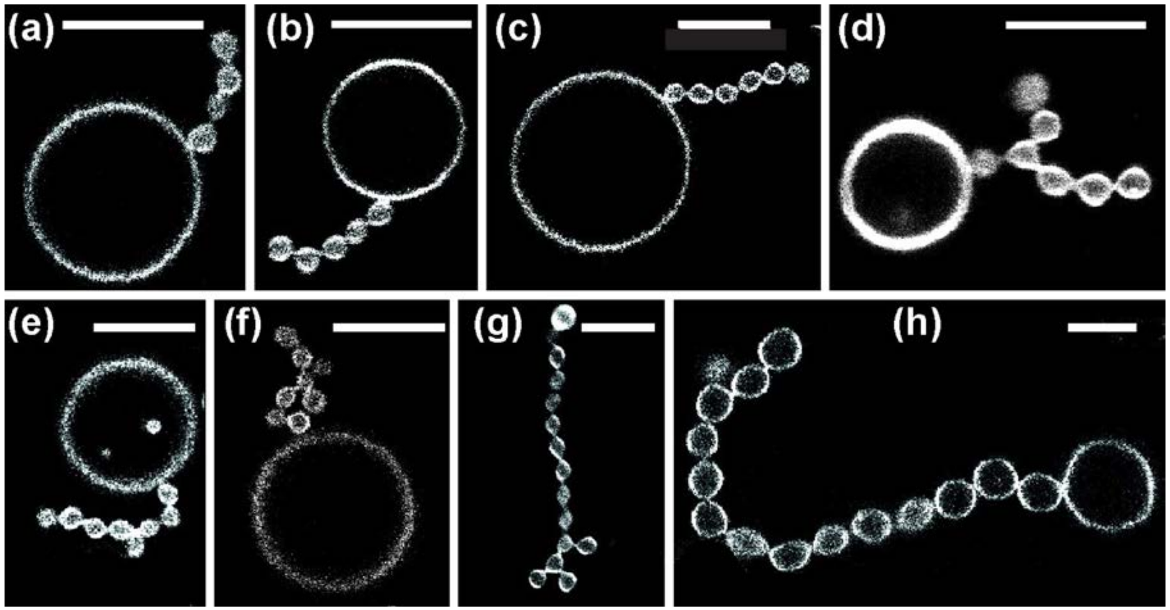
- Overlapping stability regimes



Multispheres: Experiment

- $(1+N_s)$-spheres, one large, N_s small sph:

Tripta Bhatia ... RL :
Soft Matter (2020)



- Only two different radii, R_l and R_s
- Each shape formed by single membrane
- N_s membrane necks

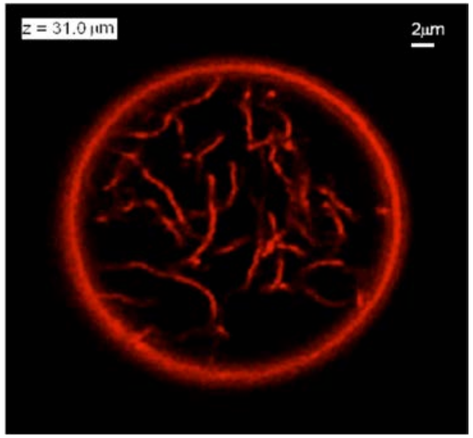
- In general: $(N_l + N_s)$-spheres with $N_l + N_s - 1$ necks
- Surprising mobility: linear ⇔ branched chains
- Degenerate case: N_* equally sized spheres

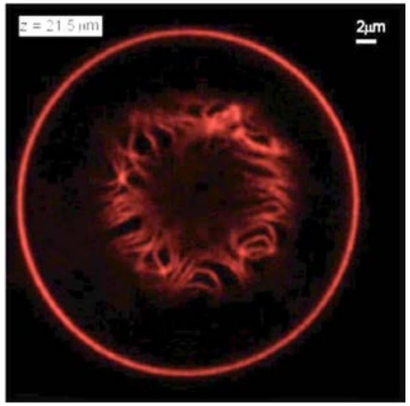
Ex: long chain with $N_* = 39$

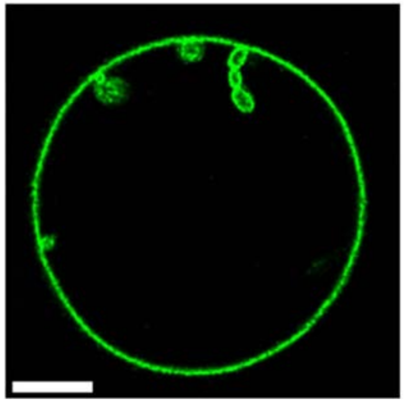
Spontaneous Tubulation of GUVs

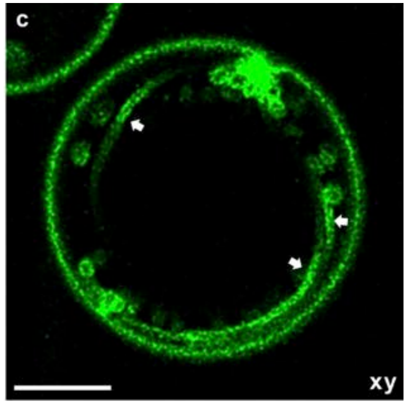
Liu … RL, *ACS Nano* (2016)

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





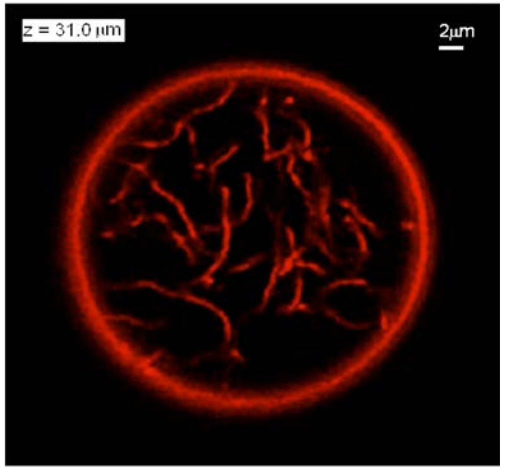




- Spontaneous tube formation without external forces
- Complex patterns of nanotubes

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 $M << m$

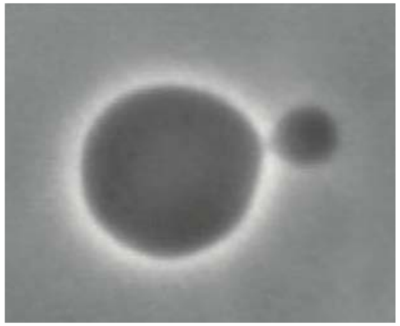
- Presence of nanotubes implies dominance of spontaneous tension, mechanical tension can be ignored
- Example: Spont curvature $\approx -1/(100\text{ nm})$

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

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

How Do Nanotubes Form?

Liu et al, *ACS Nano* (2016)



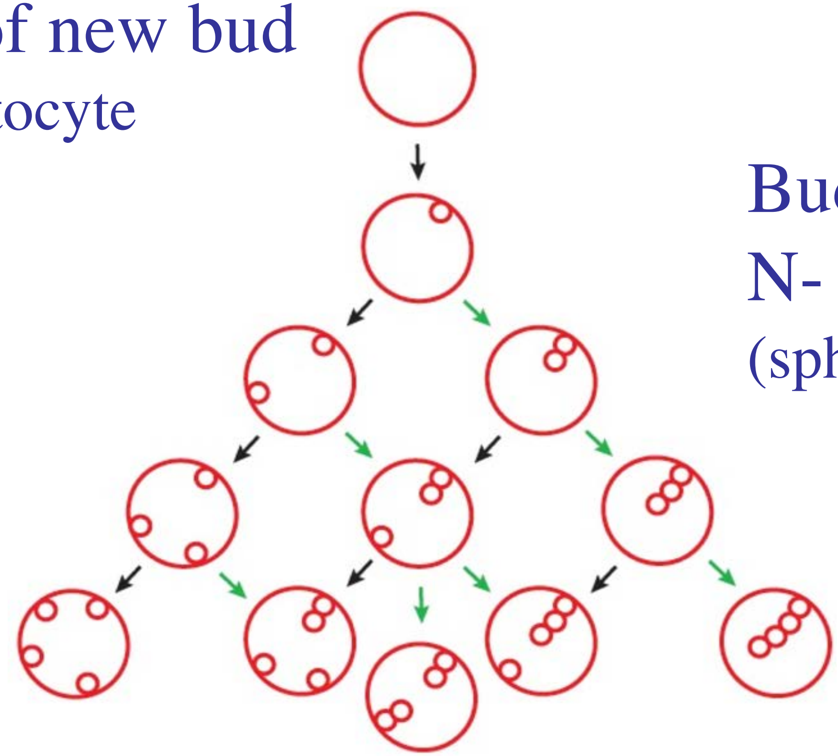
- Tubulation intimately related to budding
- Osmotic deflation of spherical GUV
- Small deflation step leads to single bud
- Bud and mother vesicle connected by membrane neck
- Bud acts as nucleation site for necklace-like tube
- Several pathways for subsequent deflation steps:
  - Formation of new bud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

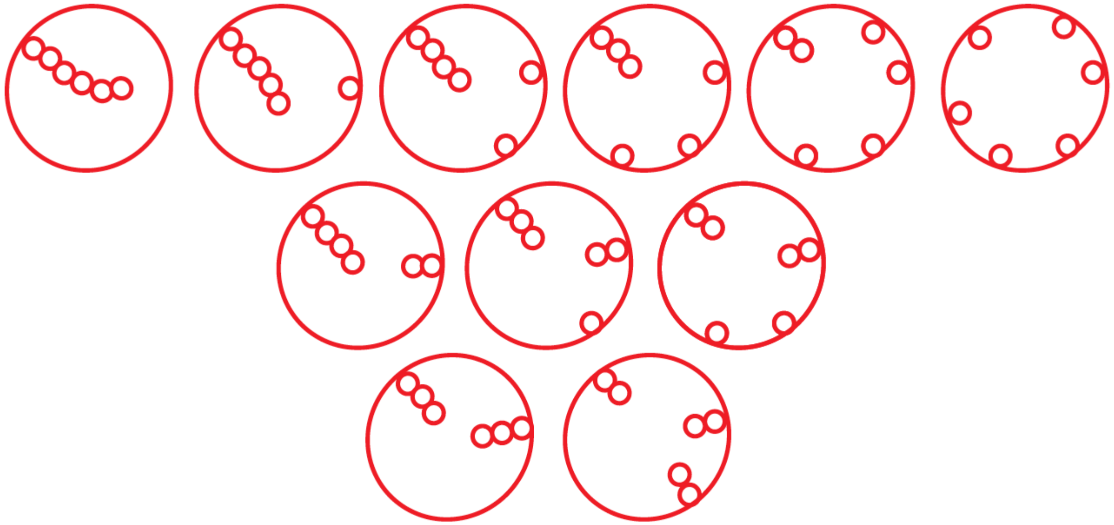
Formation of new bud
(oblate-stomatocyte bifurcation)



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

Morphological Complexity

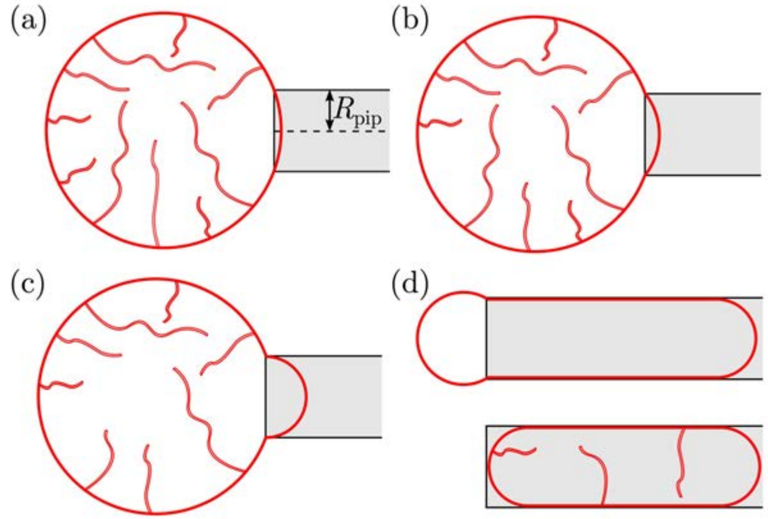
- After 6th step, 11 morphologies with 6 spherules:



- 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 √N]

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
- 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



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

- Biomembranes and Giant Vesicles (GUVs)
- Spontaneous (Sp) Curvature
- Controlled Division of GUVs
- Constriction Forces from Sp-Curvature
- Multispherical Shapes of GUVs
- Spontaneous Tubulation
- Concept of Membrane Tension
- Outlook on Related Processes

Concept of ‘Membrane Tension’

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

- Membrane tension looks simple but is complex
- Membrane tension consists of several contributions:

  Total tension = elastic stress Σ + sp-tension

- Elastic stress Σ can be viewed in two apparently distinct but nevertheless equivalent ways:

  (i) Elastic stress $\Sigma = K_A (A - A_0)/2$ to stretch membrane

  (ii) Lagrange multiplier Σ to enforce membrane area A

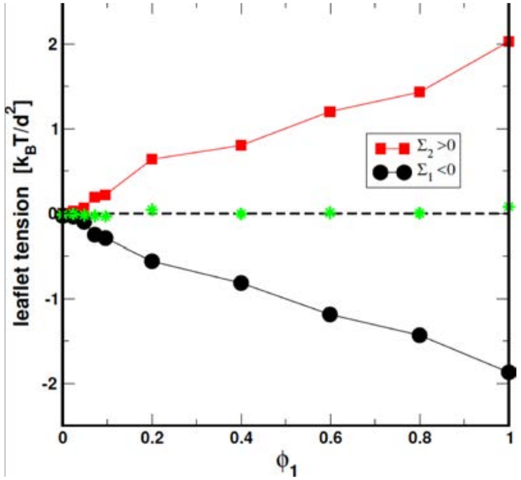
- Elastic stress depends on GUV shape !
- Measurement of Σ changes Σ (as in Qiamti,M)
- New insights on Σ from molecular simulations

Bilayer versus Leaflet Tensions

- Bilayer with two leaflets:

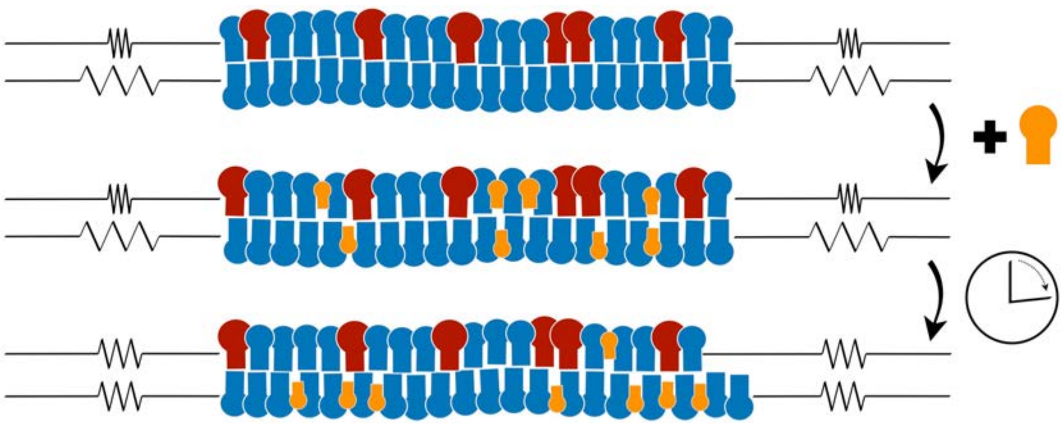
A. Sreekumari, RL , *J. Chem. Phys.* (2018)

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



- Leaflet tensions and flip-flops:

M. Miettinen, RL , *Nanoletters* (2019)

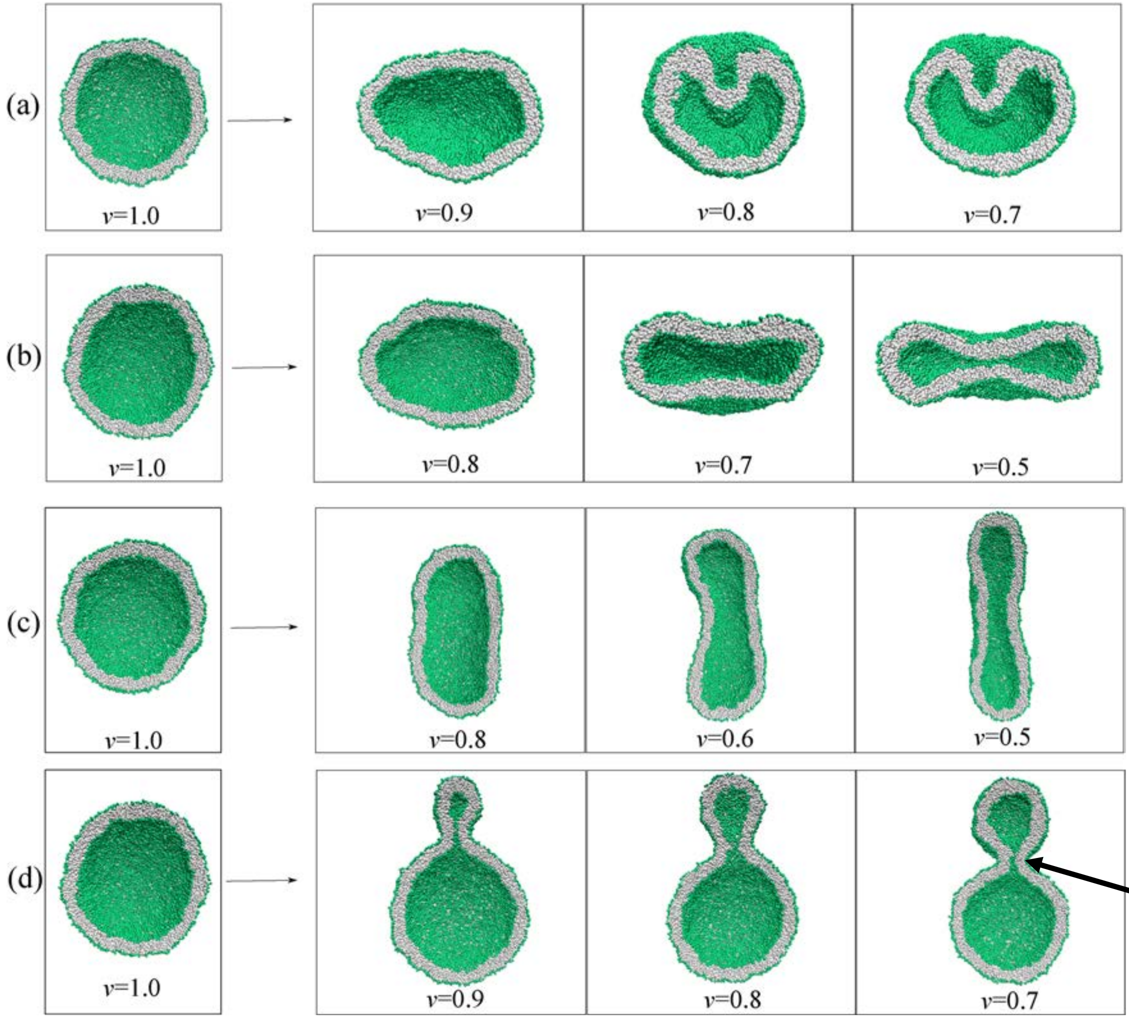


- Add cholesterol
- Flip-flops relax both leaflet tensions towards $\Sigma_1 = \Sigma_2 = 0$

Shapes of Nanovesicles

- Nanovesicle with diameter of 40 nm:

R. Ghosh ... RL,
Nano Letters (2019)



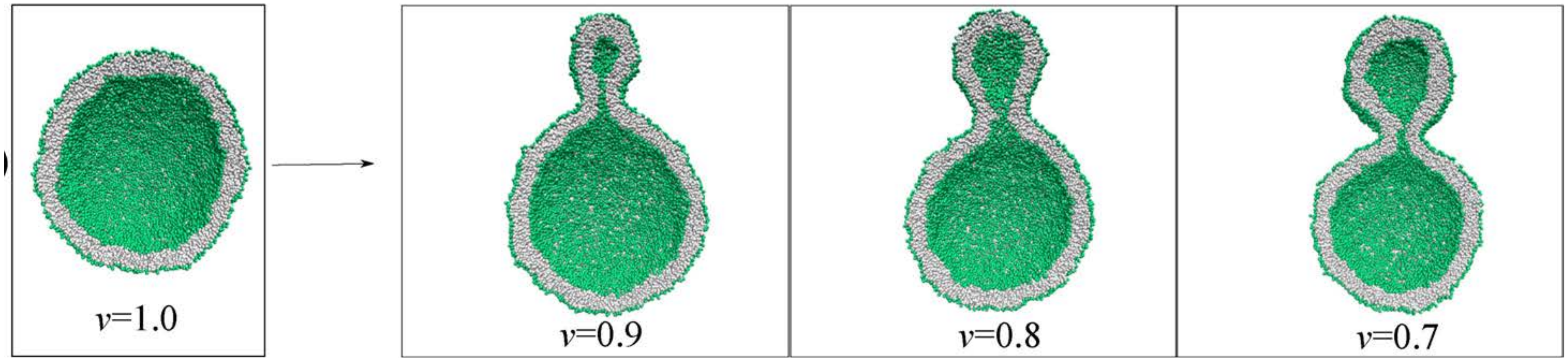
- Four spherical vesicles
- Same volume
- Same total # of lipids
- Reduction of volume: very different shapes
- Shape transformations caused by leaflet tensions

closed neck
at the nanoscale

Budding of Nanovesicles

R. Ghosh, V. Satarifard, A. Grafmüller, RL : Nano Letters (2019)

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



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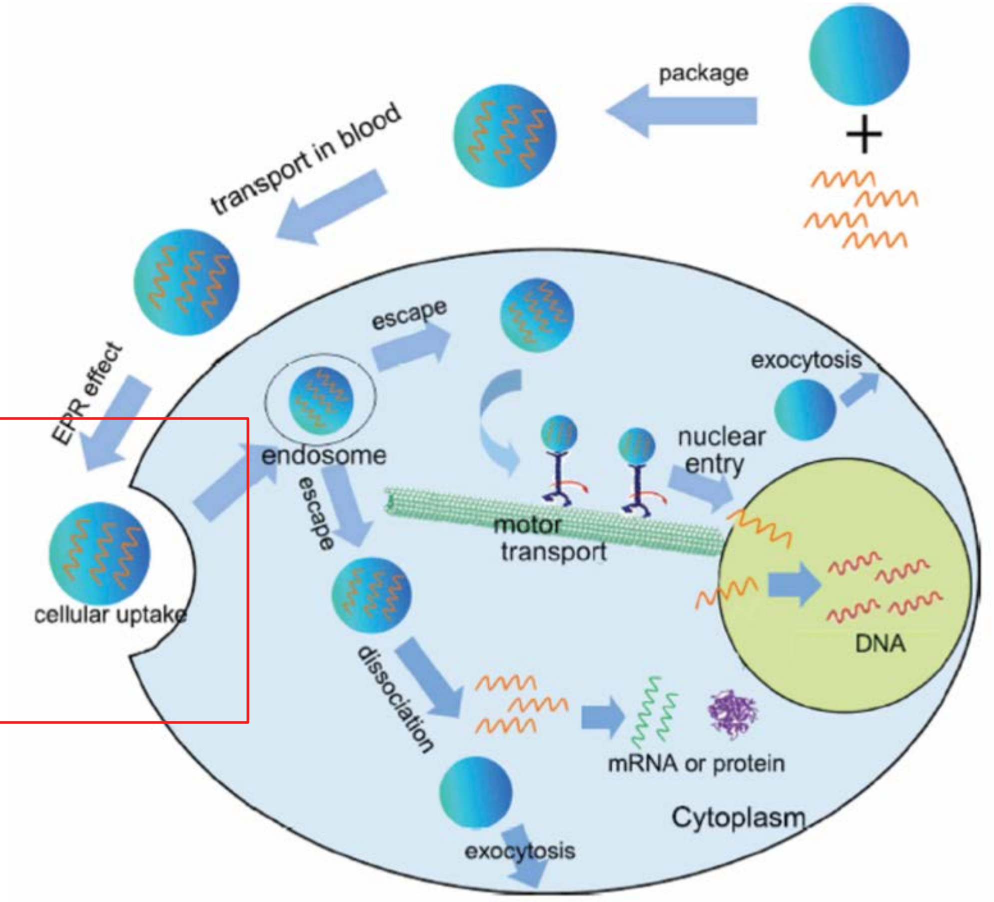
- Outlook on Related Processes
    - Endocytosis of Nanoparticles
    - Wetting of Membranes
    - Shape Oscillations

Targeting Nanoparticles to Cells

- Nanoparticles (NPs) as drug delivery systems:

Transport of NPs towards cells

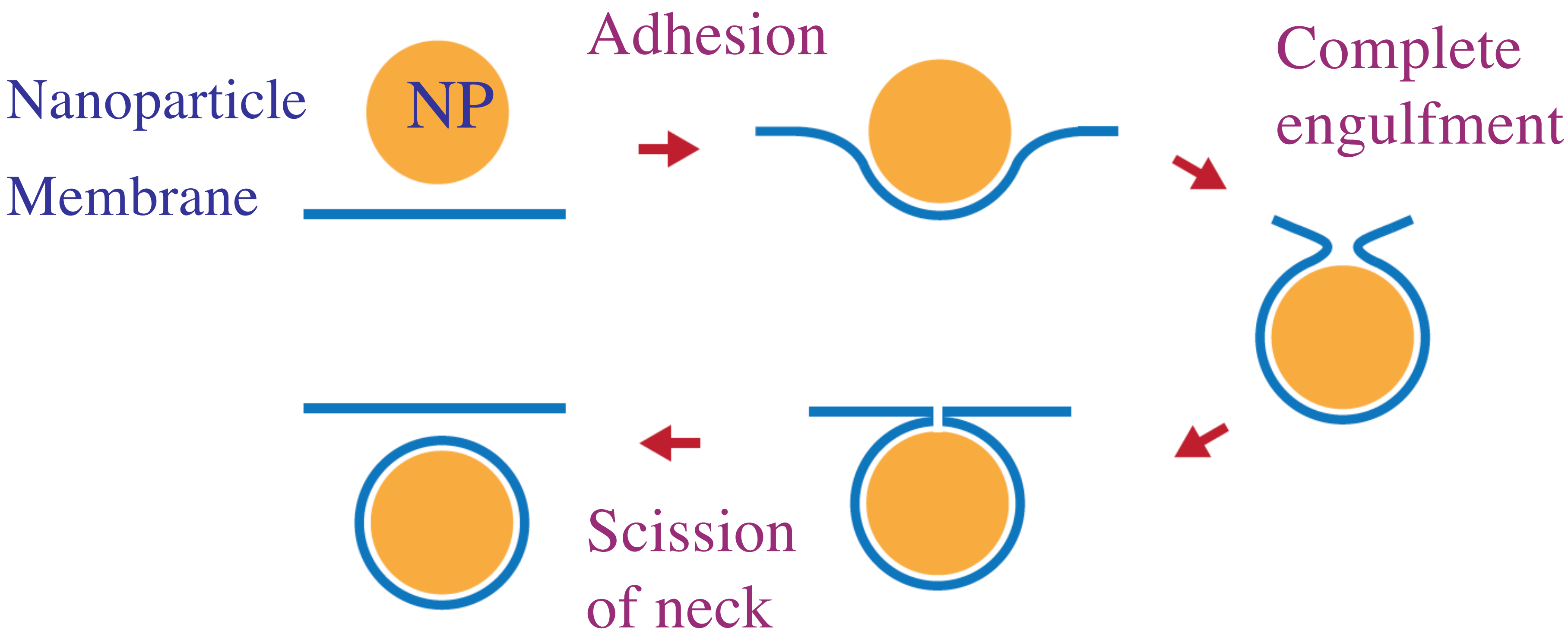
Transport across cell membrane by endocytosis



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
- Interplay between sp-curvature and adhesion length

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

Adhesive Length

- 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

- Competition between bending rigidity κ and $|W|$:

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

- Adhesion length varies from 10 nm to several μm

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

Onset of Adhesion: Local Criterion

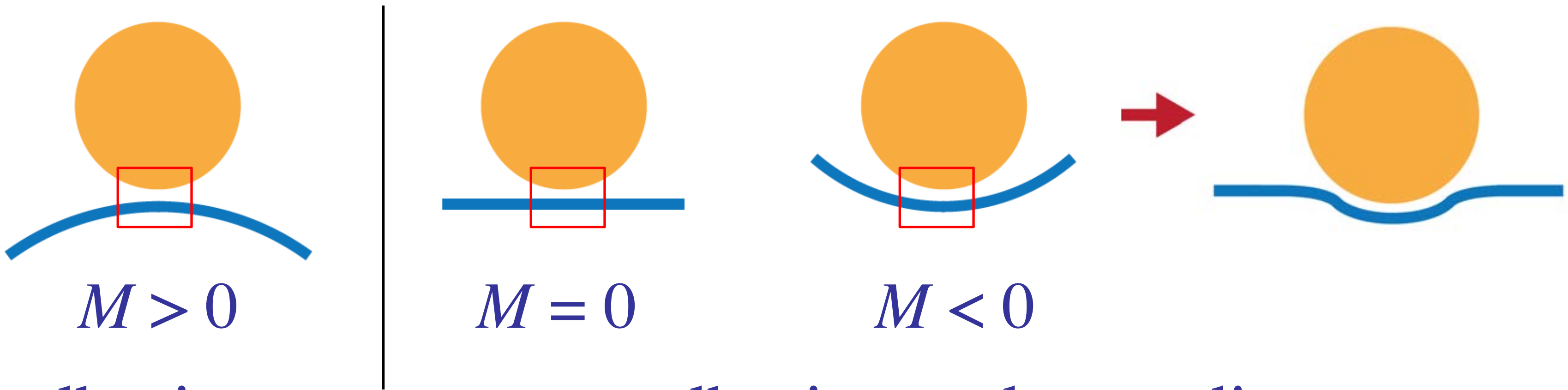
Agudo-Canalejo and RL, *ACS Nano* + *Nano Letters* (2015)

- Membrane starts to spread over particle if

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

contact curvature M_{co} is threshold value for M

- Example: $M_{co} = 0$



no adhesion

adhesion and spreading

Complete Engulfment: Local Criterion

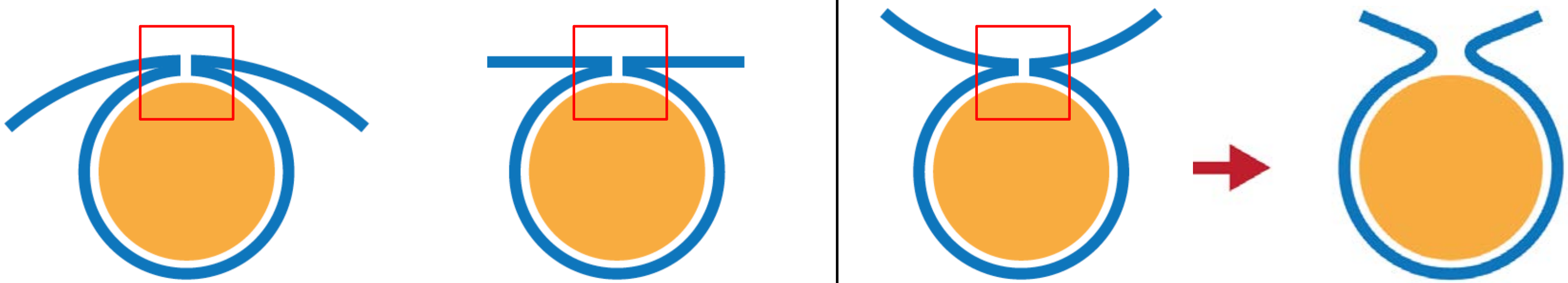
- Closed membrane neck is stable if membrane curvature

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

2nd threshold value for M depends on spont curvature m

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

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

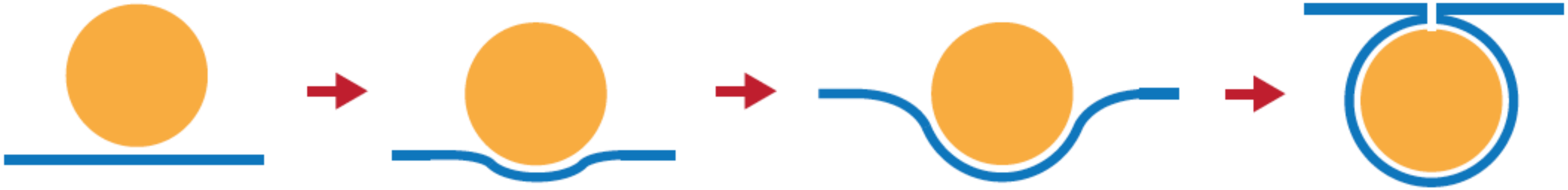


closed neck is stable

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:

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

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

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

Constriction Force from Adhesion

- Generalized neck curvature

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

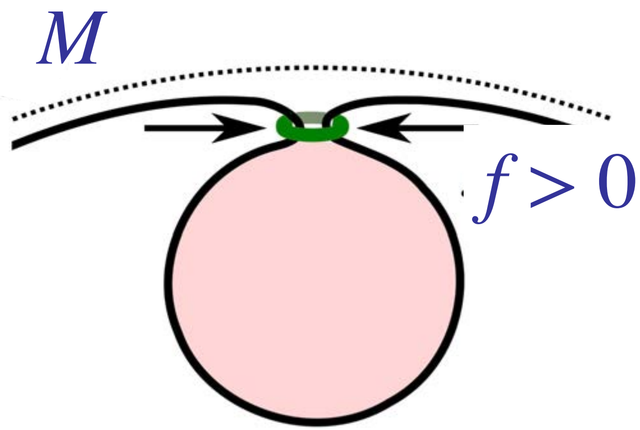
- Contact mean curvature depends on adhesion length $R_W = (2\kappa/|W|)^{1/2}$

- Constriction force

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

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

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

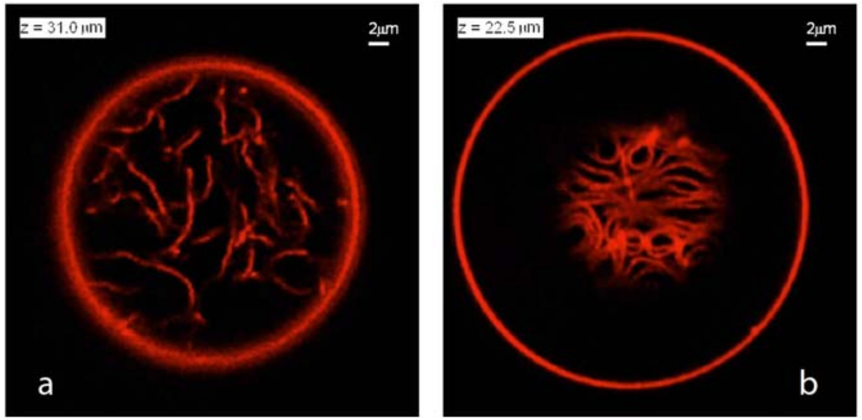


Agudo-Canalejo and RL, *Soft Matter* (2016)

Wetting of Membranes

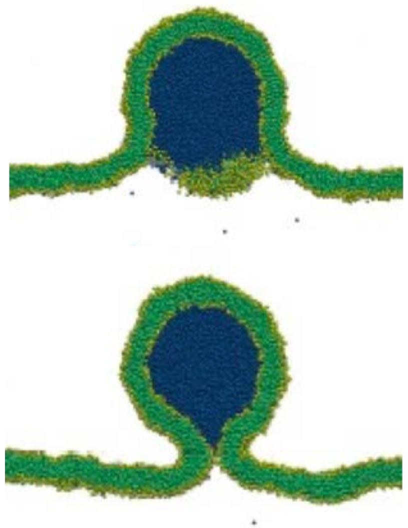
Liu et al, *ACS Nano* (2016)

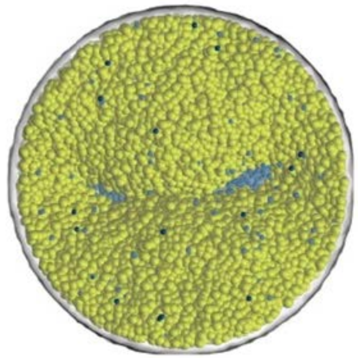
- Aqueous phase separation inside GUVs
- Polymer solutions, PEG+dextran
- Complete and partial wetting of GUV membranes



Distinct patterns of nanotubes

- Engulfment of nanodroplets
- Neck closure leads to tight-lipped neck:
- Negative line tension of contact line !

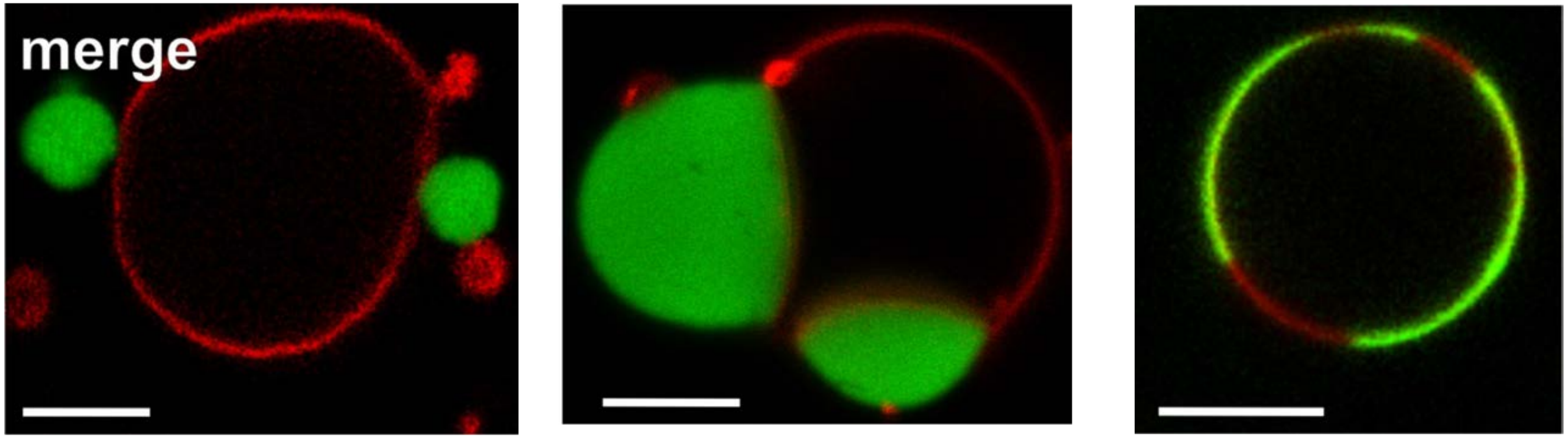




Satarifard et al, *ACS Nano* (2018)

Biomolecular Condensates

- Eukaryotic cells contain droplet-like compartments = membrane-less organelles = biomolecular condensates (BCs)
- Wetting and molding of membranes by BCs two subsequent wetting transitions:

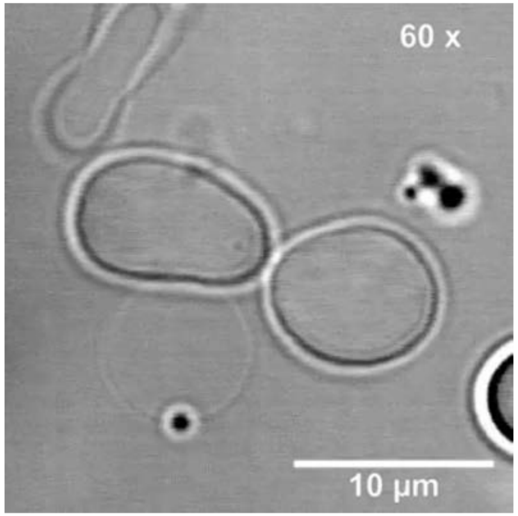


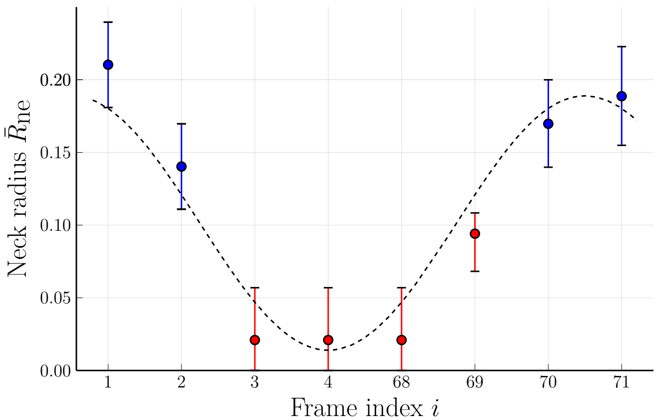
dewetting for high salt | partial wetting for intermediate salt | complete wetting for low salt

- Analogous processes in vacuoles of plant cells

Shape Oscillations of GUVs

T. Litschel ... P. Schwille: Angew. Chemie Int. Ed. (2018)
S. Christ, T. Litschel, P. Schwille, RL, submitted





- Shape oscillations generated by Min protein system coupled to ATP

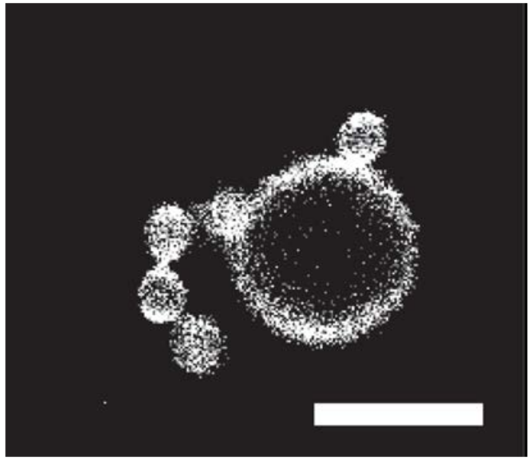
- Oscillations imaged over 25 min
- 200 frames separated by 7.6 s
- 26 complete oscillations

- Two branches of dumbbells, symmetric and asymmetric ones

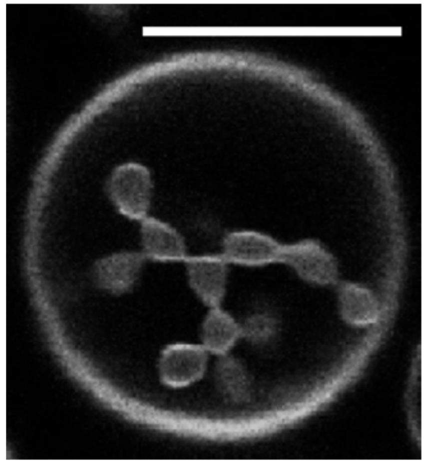
- Oscillations of bound Min proteins
- Oscillations of sp-curvature
- Oscillations of neck radius

Outlook: Smart Compartments

- Positive sp-curvature: out-buds or out-tubes
- Buds and tubes filled with drugs or agents
- Divison into many small compartments
- Multiplication of delivery systems



- Negative sp-curvature: in-buds or in-tubes
- Storage and delivery of nanoparticles (NPs)
- Uptake of NPs by in-tubes
- Storage of NPs by neck closure
- Release of NPs by neck opening



Coworkers

Rumiana Dimova

Tripta Bhatia

Simon Christ

Rikhia Ghosh

Andrea Grafmüller

Markus Miettinen

Vahid Satarifard

Jan Steinkühler

Aparna Sreekumari

Ziliang Zhao

- Collaborations:

Joachim Spatz, Seraphine Wegner, Tony Hyman, Titus Franzmann, Petra Schwille, Thomas Litschel