Morphological Complexity of Biomembranes and Synthetic Cells

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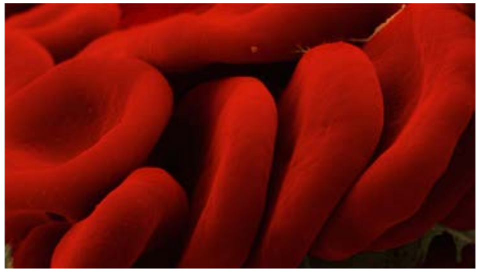
- Reminder: Shapes of Cells and Organelles
- Transbilayer Asymmetry and Sp-Curvature
- Controlled Division of Giant Vesicles
- Multispherical Shapes of Giant Vesicles
- Spontaneous Tubulation
- Concept of Membrane Tension

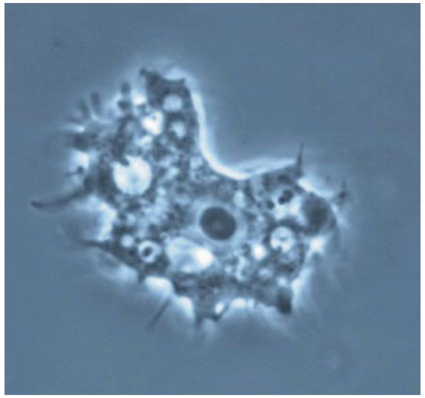
Diverse Shapes of Cells

Red blood cells



White blood cell

Single Purkinje cell



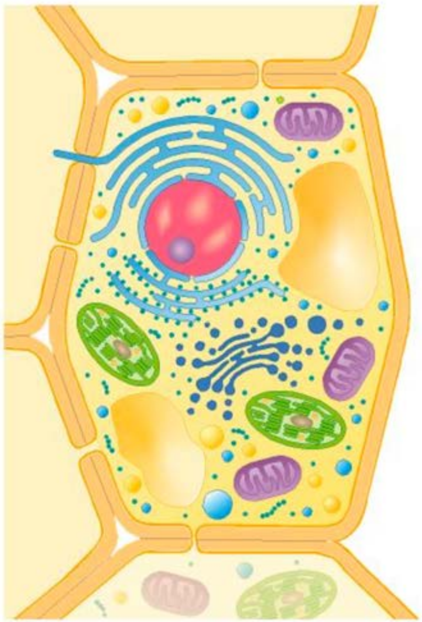
Amoeba

Cell shape = shape of plasma membranes controlled by:

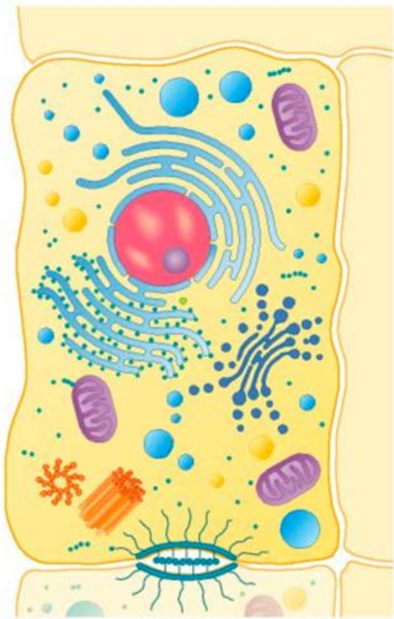
- Cell volume
- Osmotic conditions
- Membrane area and tension
- Cytoskeletal filaments
- Plants and bacteria: cell walls
- This talk: no rigid cell walls

Shapes of Membrane-bound Organelles

Organelle shape = shape of organelle membrane controlled by:



Plant

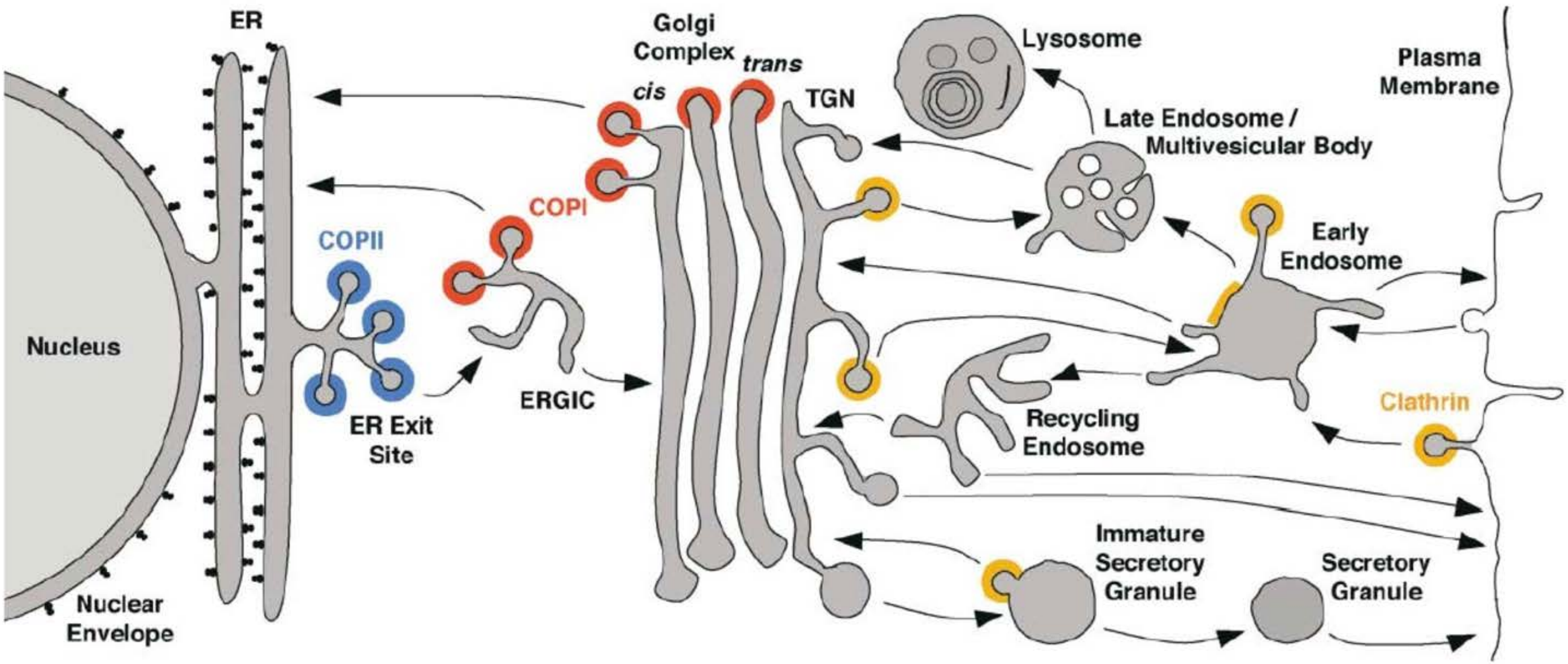


Animal

- Organelle volume
- Osmotic conditions
- Membrane area and tension
- Scaffolding proteins
- ...

Intracellular Vesicle Trafficking

Bonifacio,Glick, *Cell* (2004)

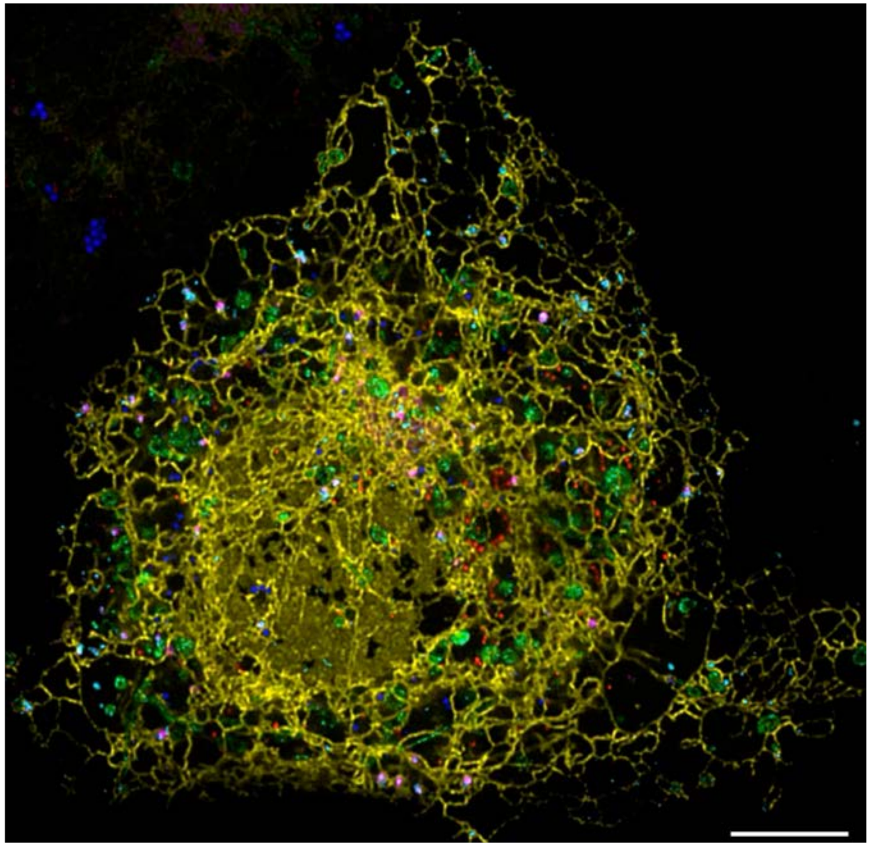


- Different colors indicate different protein scaffolds
- Budding and fission via formation of membrane necks

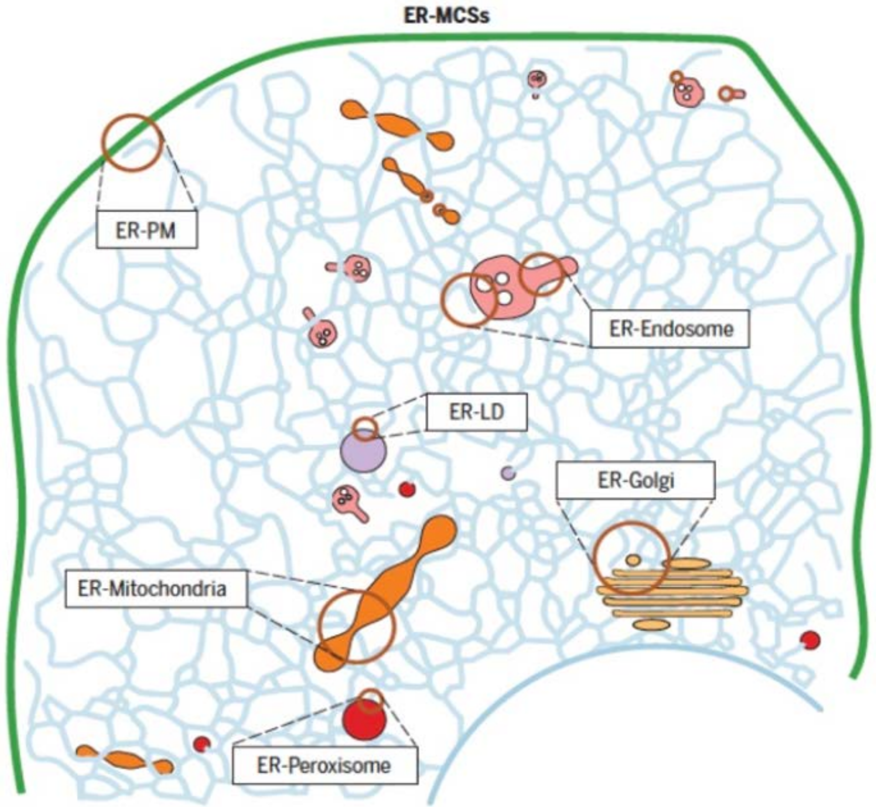
Endoplasmic Reticulum (ER)

- ER = network of membrane nanotubes with junctions

Valm et al. *Nature* (2017)



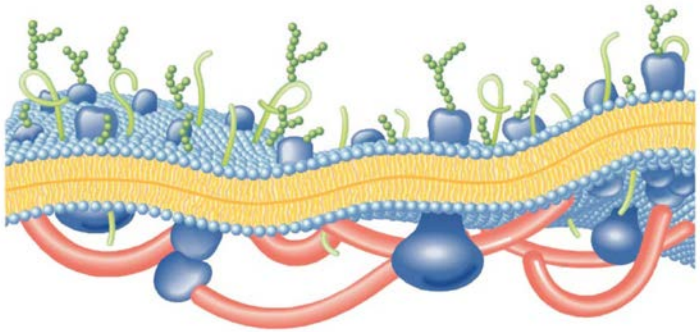
yellow reticular network



blue reticular network

Wu et al, *Science* (2018)

Transbilayer Asymmetries



- Biomembranes = molecular bilayers with two leaflets of lipids + proteins
- Different mechanisms for transbilayer asymmetry:
    - Different lipid composition in the two leaflets
    - Leaflets exposed to different aqueous solutions
    - Asymmetric binding of proteins to two leaflets
    - Unidirectional orientation of transmembrane proteins
    - ... and scaffolding proteins as another example

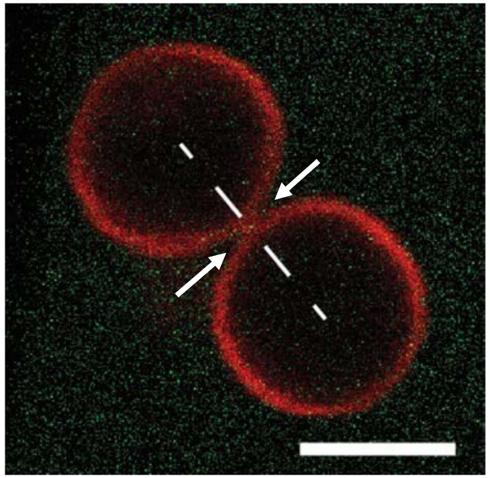
Advise to Visitors

„ If you visit a foreign island, show
some weapons the natives don‘t know “

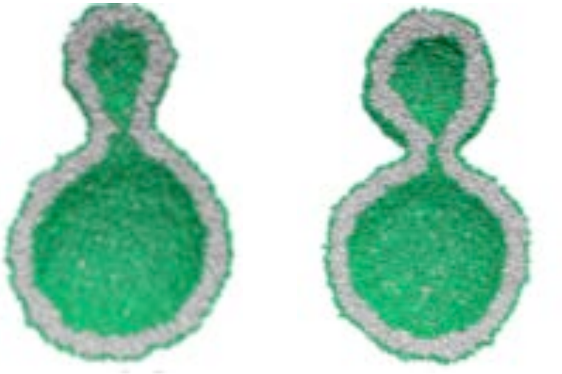
Quote from Sam Edwards

who blamed it on James Cook

Model Membranes and Synthetic Cells



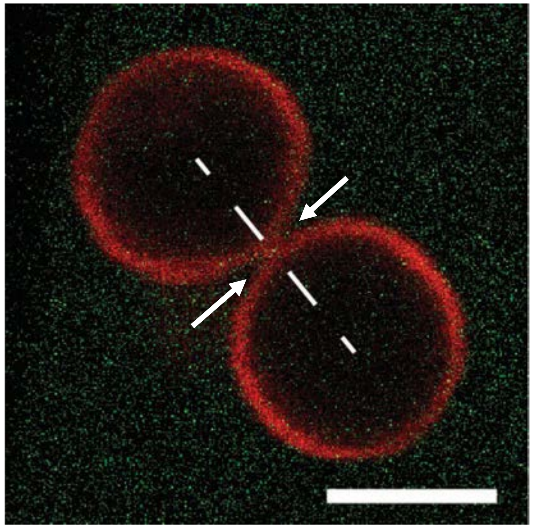
- Giant unilamellar vesicles (GUVs)
- Basic modules for synthetic cells
- Microfluidic methods to create GUVs
- Understanding based on curvature elasticity



- Nanovesicles
- Electron microscopy: limited to single a snapshot for each individual nanovesicle
- Molecular dynamics simulations: dynamics of shape transformations

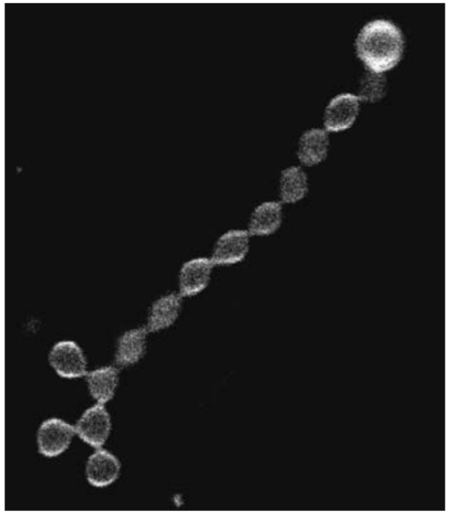
Giant Vesicles with Membrane Necks

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



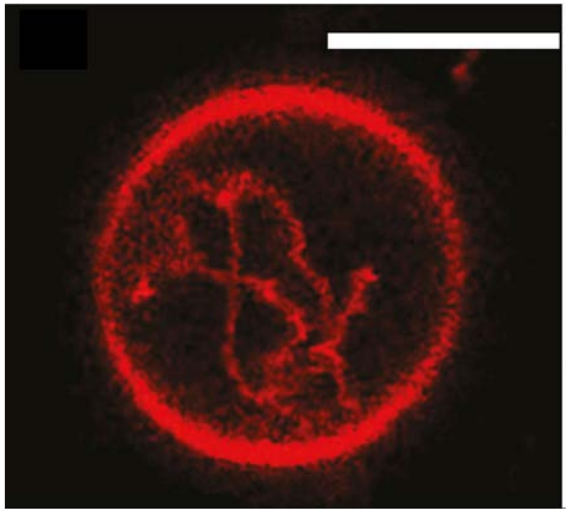
Exposed to His-tagged GFP in exterior solution

Steinkühler et al, *Nature Comm* (2020)



Sucrose inside, glucose outside

Bhatia et al, *Soft Matter* (2020)

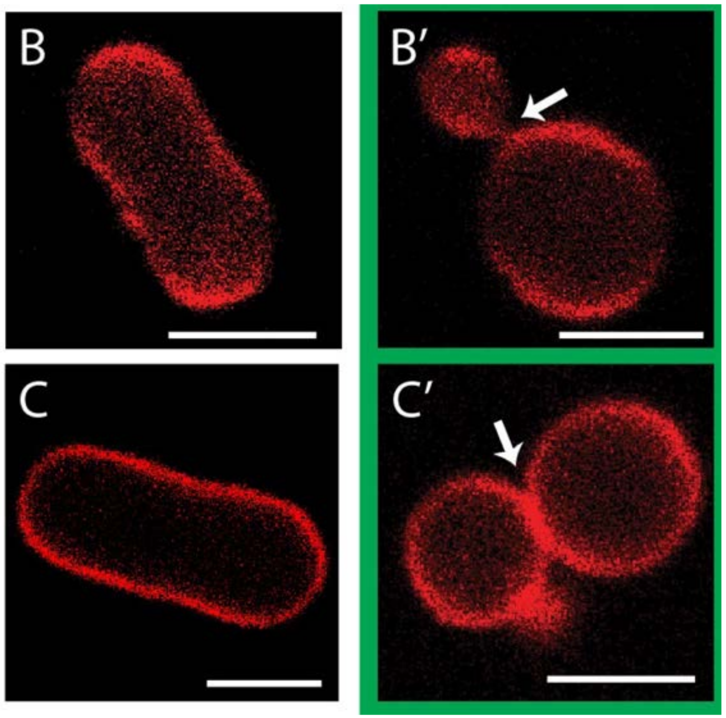


Bilayer contains GM1 with bulky head group

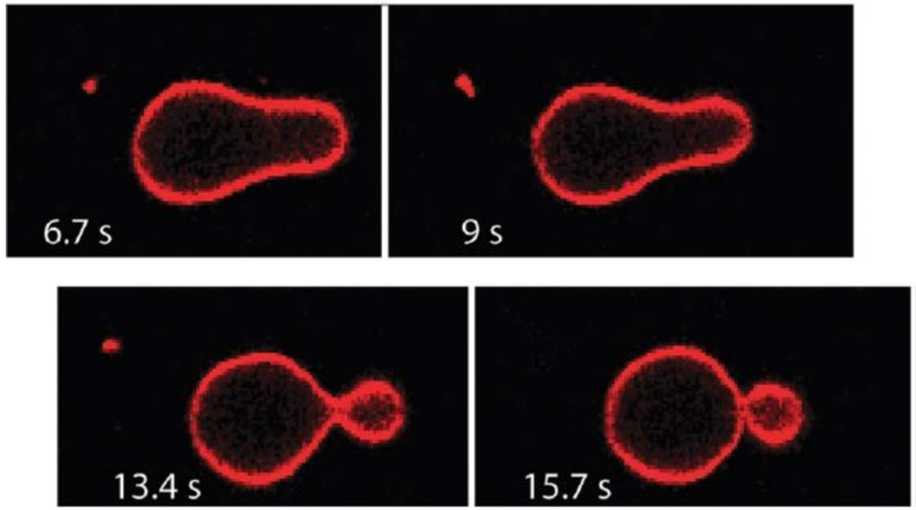
Bhatia et al, *ACS Nano* (2018)

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

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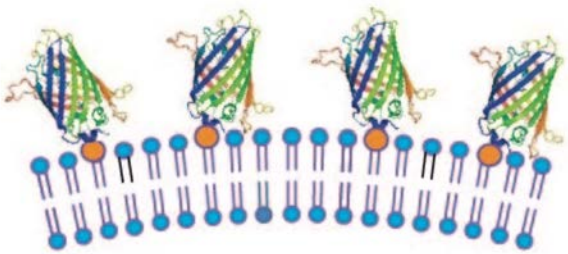
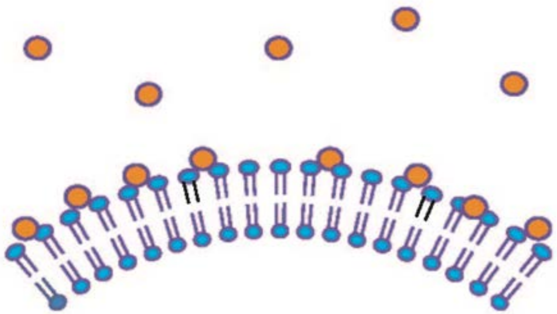
Key Parameter: Spontaneous Curvature

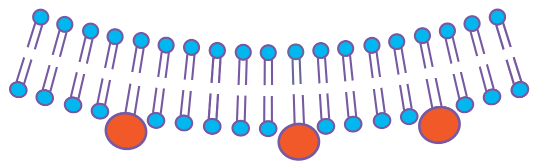
- Lipid bilayer consists of two leaflets
- Spontaneous or preferred curvature *m* describes transbilayer asymmetry = asymmetry between two leaflets
- Different molecular mechanisms for sp-curvature:



Binding of GFP to outer leaflet



Adsorption layer of glucose



Adsorption of glycolipid GM1

Importance of Sp-Curvature

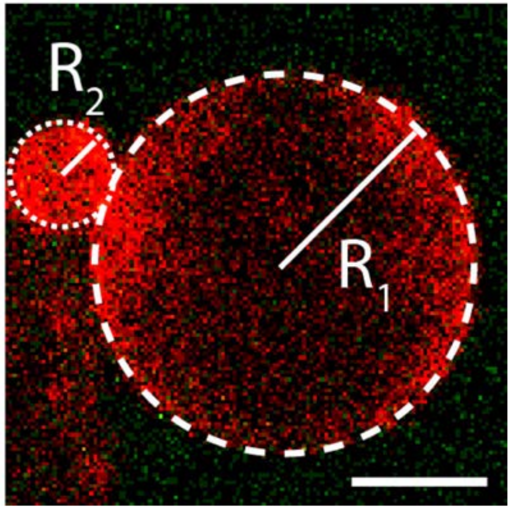
Sp-curvature crucial for:

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

Two challenges:

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

Stability of Closed Necks



- 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

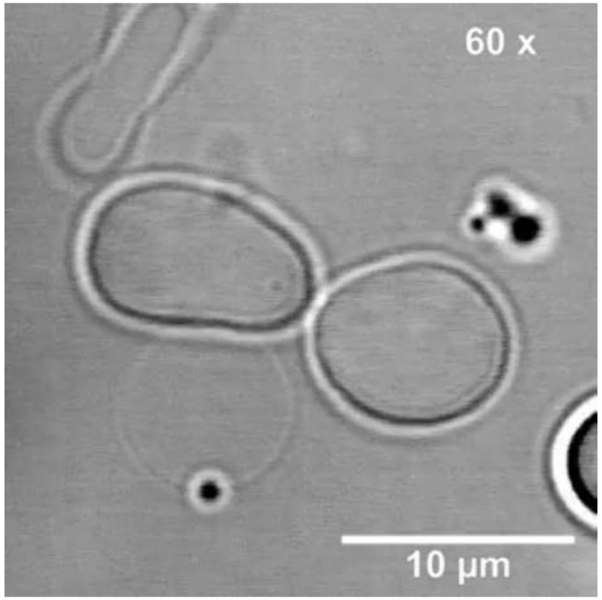
Dumbbell = (1+1)-Sphere

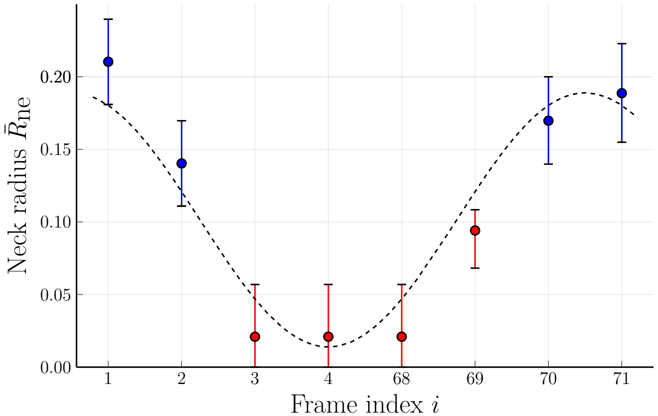
- Large and small sphere with radii R_1 and R_2
- Neck curvature $M_{ne} = (1/2)\,(1/R_1 + 1/R_2)$
- Closed neck is stable if $0 < M_{ne} \leq m$
- Local relation between geometry and material parameter

Shape Oscillations of GUVs

S. Christ, T. Litschel, P. Schwille, RL, Soft Matter (in press)

. Schwille lab



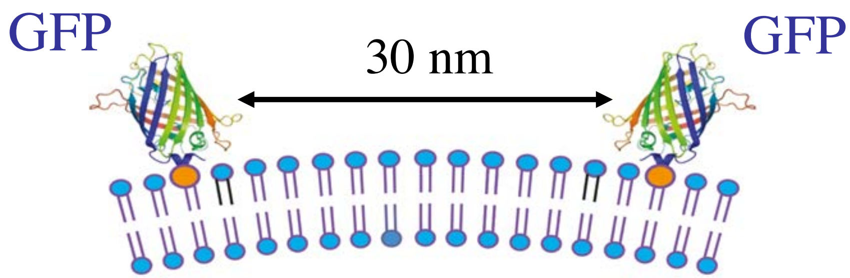


- Shape oscillations generated by Min protein system coupled to ATP
- Oscillations imaged over 25 min
- 200 frames separated by 7.6 s
- 26 complete oscillations
- Two branches of dumbbells, symmetric and asymmetric ones
- Oscillations of bound Min proteins
- Oscillations of sp-curvature
- Oscillations of neck radius

Fine Tuning of Spontaneous Curvature

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

- Binding of GFP to small mole fraction of anchor NTA-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

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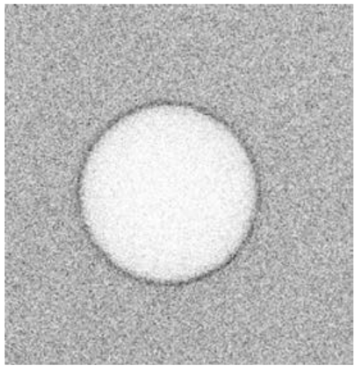
Controlled Division of GUVs

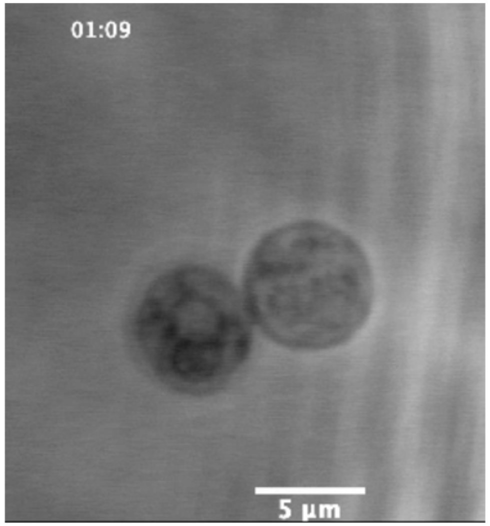
Jan Steinkühler ... RL : *Nature Comm.* (2020)

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

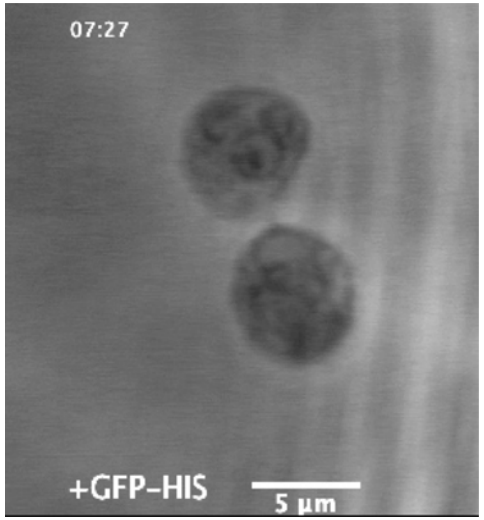
Increase in GFP -> Neck cleavage -> Two daughter GUVs



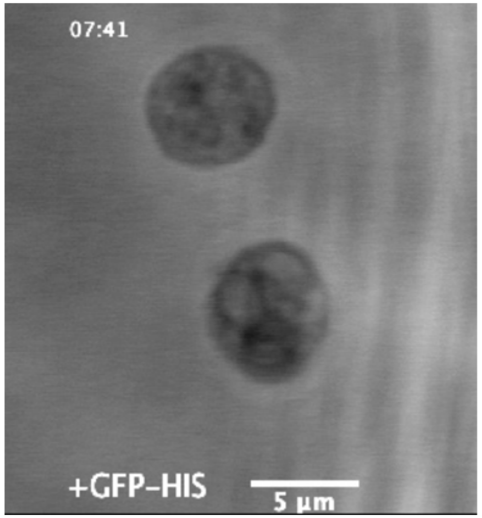
Adsorption of GFP onto GUV membrane



Deflation leads to dumbbell with membrane neck



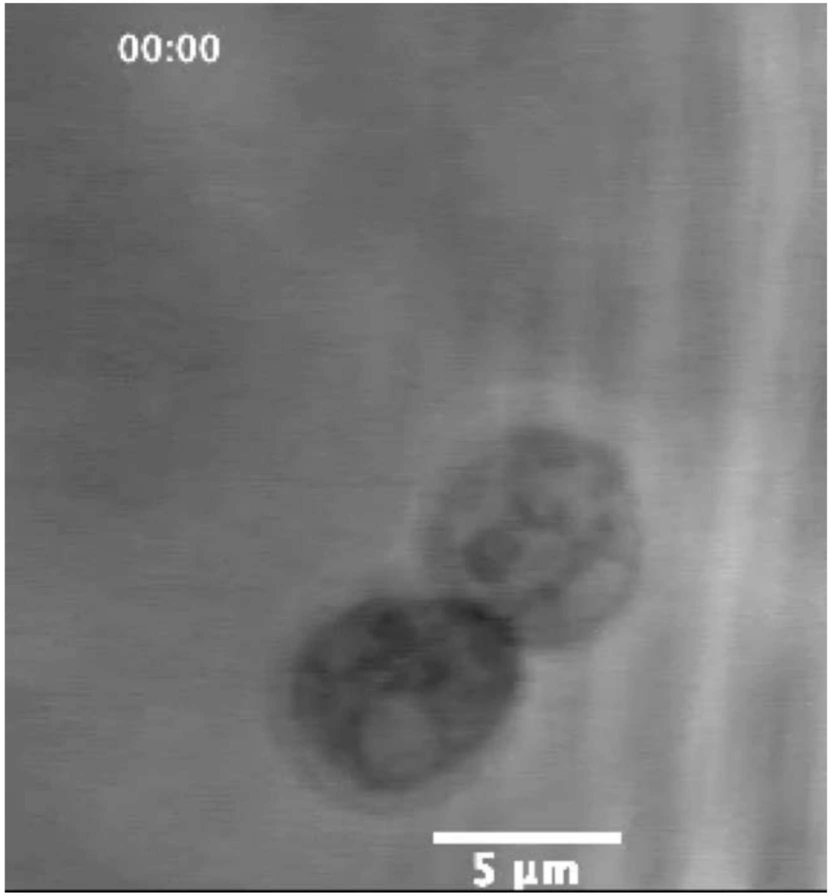
Directly after neck cleavage



Complete division into two smaller GUVs

Controlled Division of GUVs: Movie

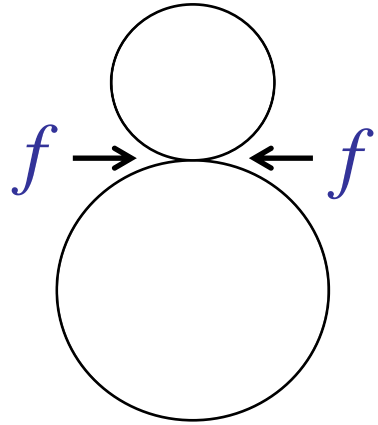
Jan Steinkühler ... RL : *Nature Comm*. (2020)



- Two-step process:
- Osmotic deflation:
  Spherical GUV -> dumbbell GUV
- Increase in GFP ->
  Neck cleavage + GUV division

Constriction Force from Sp-Curvature

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

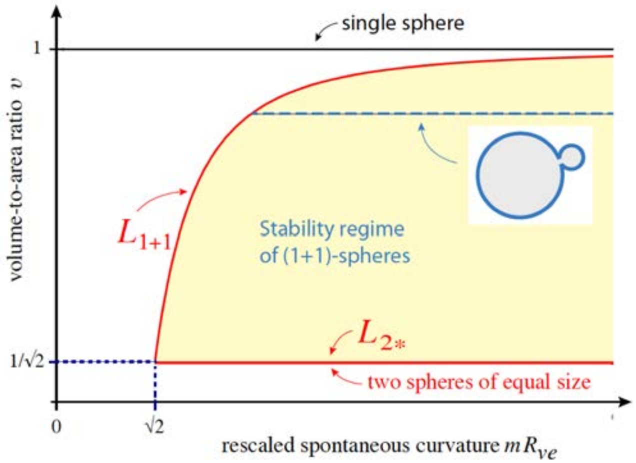


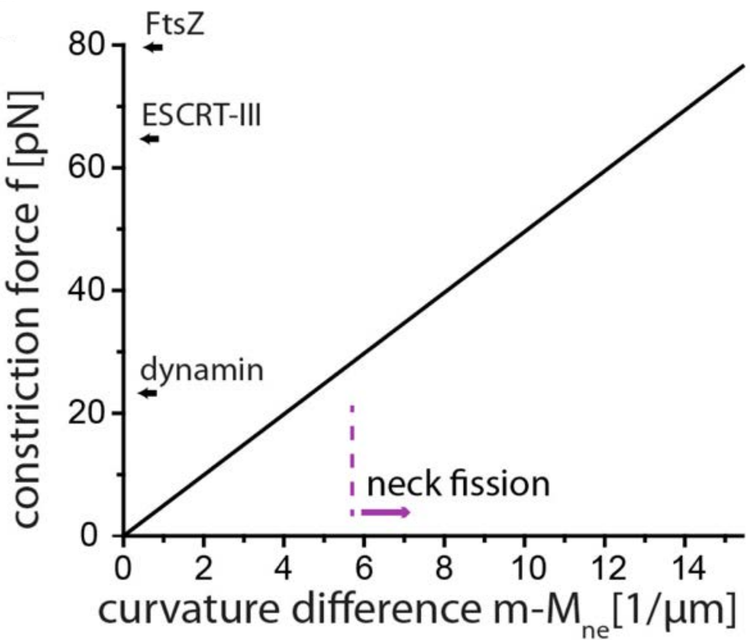
- Sp-curvature m generates constriction force f acting radially on membrane neck:

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

bending rigidity κ, neck curvature M_{ne}

- Force f increases with increasing sp-curvature m:





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Multispheres: Theory

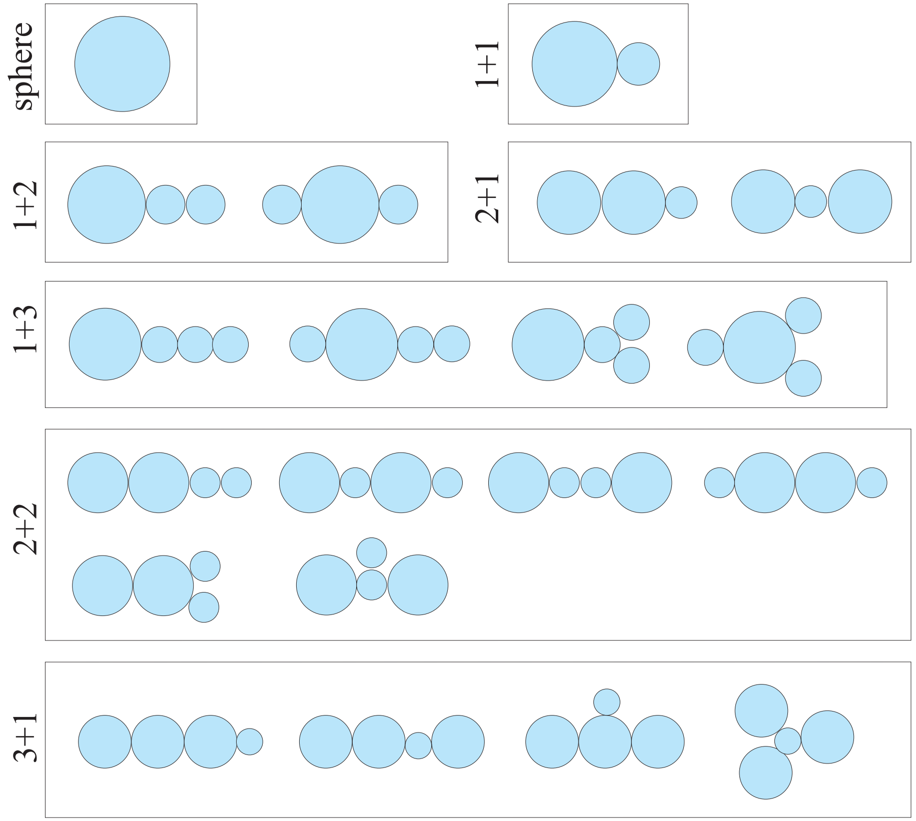
RL, *Advances in Biomembranes and Lipid Selfassembly* Vol. 30, Ch. 3 (2019)

- 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: $N_l + N_s \leq 4$

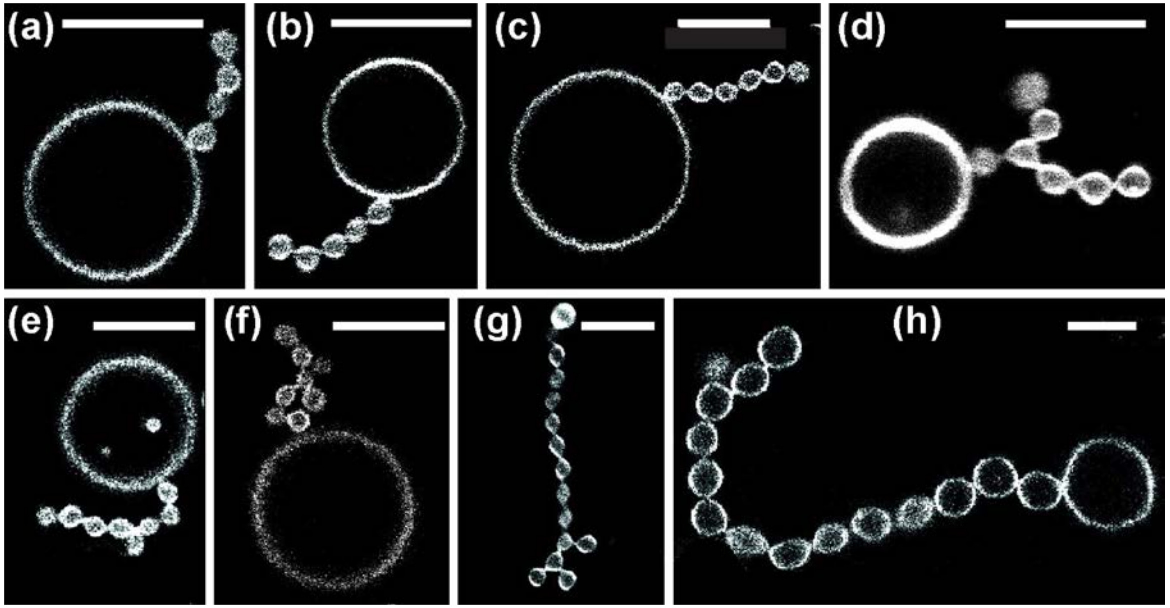
- Overlapping stability regimes



Multispheres: Experiment

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

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



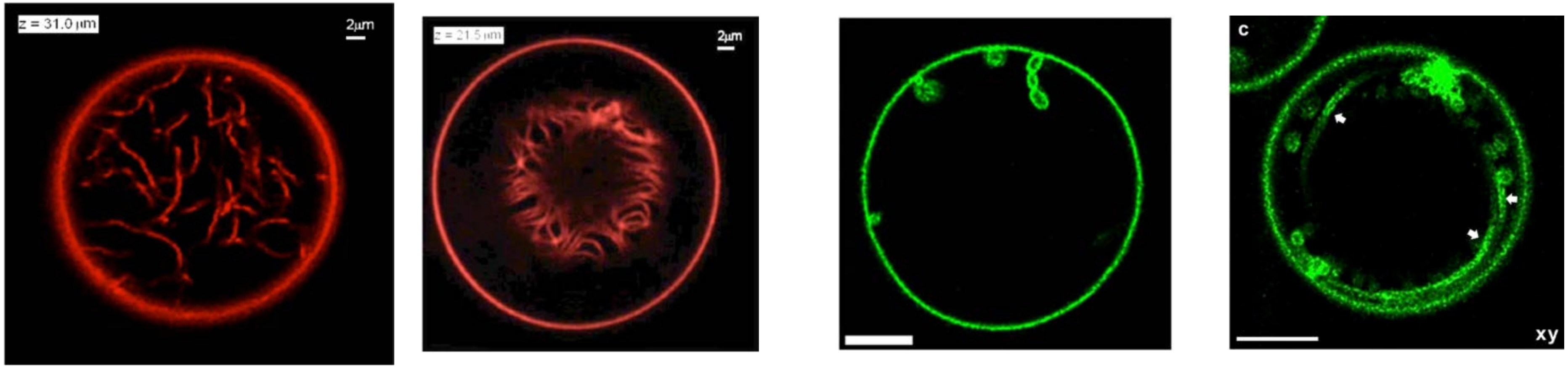
- Only two different radii, R_l and R_s
- Each shape formed by single membrane
- N_s membrane necks

- 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

Spontaneous Tubulation of GUVs

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

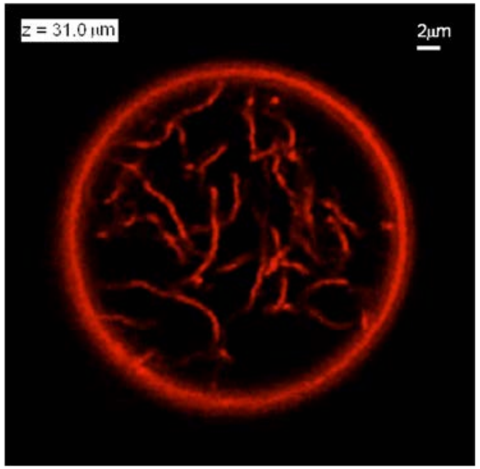
- Lipid mixture of DOPC, DPPC, cholesterol
- Small amounts of fluorescently labeled lipids
- Liquid-disordered (red) and liquid-ordered phase (green)



- 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 membrane tension has two components:

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

Elastic stress Σ stretches the membrane

Spontaneous tension $\sigma = 2\,\kappa\, m^2$

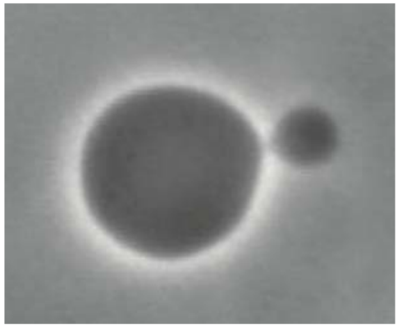
- Presence of nanotubes implies dominance of spontaneous tension, elastic stress can be ignored
- Example: Sp-curvature $\approx -1/(100\ \text{nm})$

Sp-tension $\sigma \approx 10^{-2}$ mN/m, Elastic stress $\Sigma \approx 10^{-4}$ mN/m

How Do Nanotubes Form?

Liu et al, *ACS Nano* (2016)

- Tubulation intimately related to budding



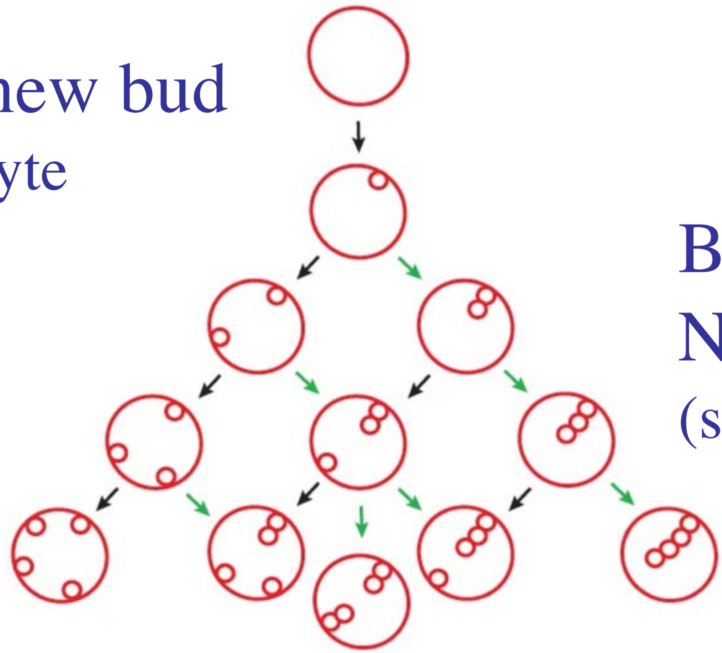
- Osmotic deflation of spherical GUV
- Small deflation step leads to single bud
- Bud and mother vesicle connected by membrane neck
- Bud acts as nucleation site for necklace-like tube
- Several pathways for subsequent deflation steps:
  - Formation of new bud
  - Bud into 2-necklace
  - N-necklace into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

Nucleation and Growth of Tubes

Liu et al, *ACS Nano* (2016)

- 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)

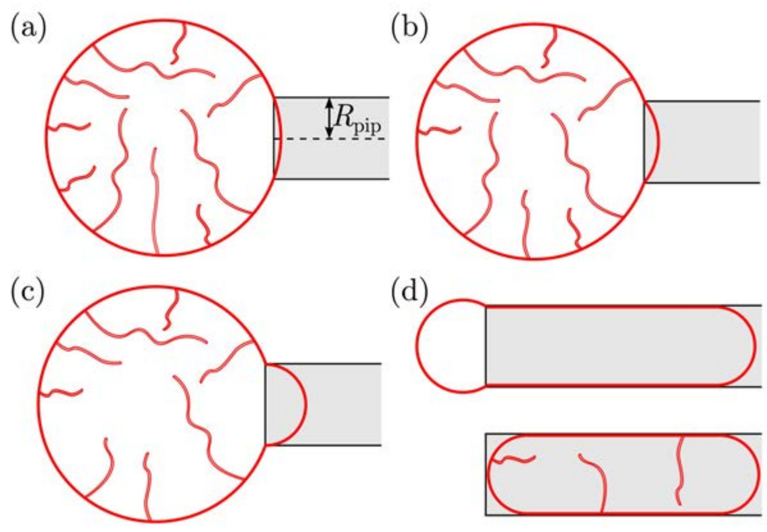


Bud into 2-necklace
N- into (N+1)-necklace
(sphere-prolate bifurcation)

Robustness of tubulated GUVs

- Conventional GUVs: Membrane rupture under osmotic inflation, strong adhesion, micropipette aspiration, ...
- Membrane nanotubes provide area reservoir
- Tubulated GUVs experience very low elastic stress and do not rupture under strong mechanical perturbations
- Robustness demonstrated for inflation and aspiration
- Mother vesicle behaves like liquid droplet with interfacial tension = sp-tension of membrane

Bhatia et al: *ACS Nano* (2018)



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- Shapes of Nanovesicles

Concept of 'Membrane Tension'

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

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

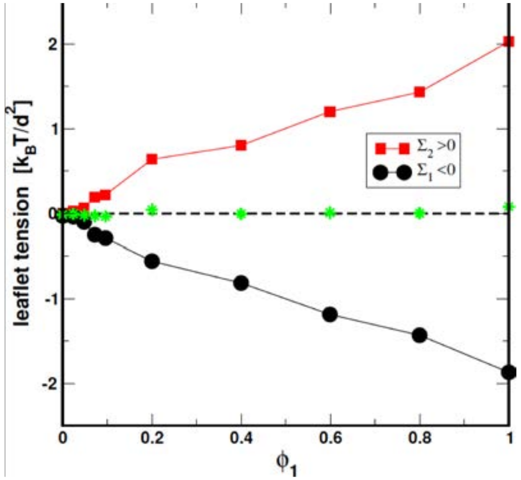
  Total tension = elastic stress Σ + sp-tension

- Elastic stress depends on GUV shape !
- Measurement of elastic stress changes this stress!
- New insights on elastic stress from molecular simulations

Bilayer versus Leaflet Stresses

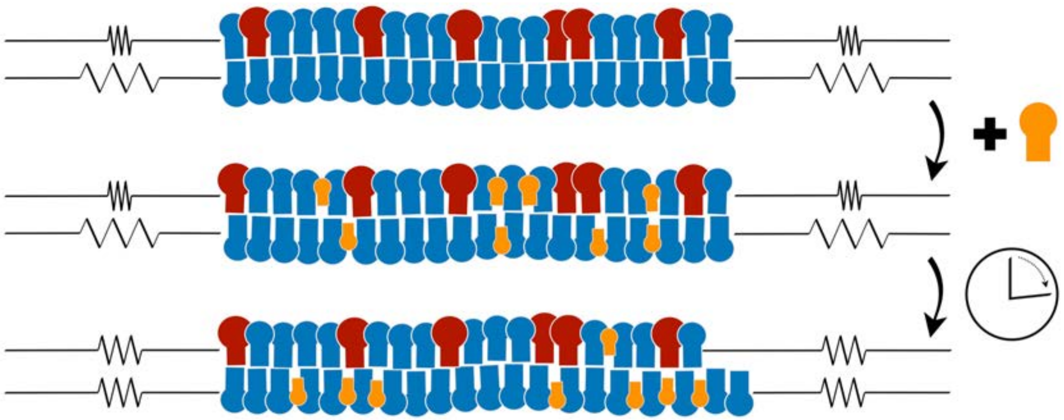
A. Sreekumari, RL , *J. Chem. Phys.* (2018)

- Bilayer with two leaflets:
  - Two leaflet stresses Σ_1 and Σ_2 with bilayer stress $\Sigma_1 + \Sigma_2 = \Sigma$
  - Tensionless bilayer: $\Sigma = 0$



- Leaflet stresses and flip-flops:

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

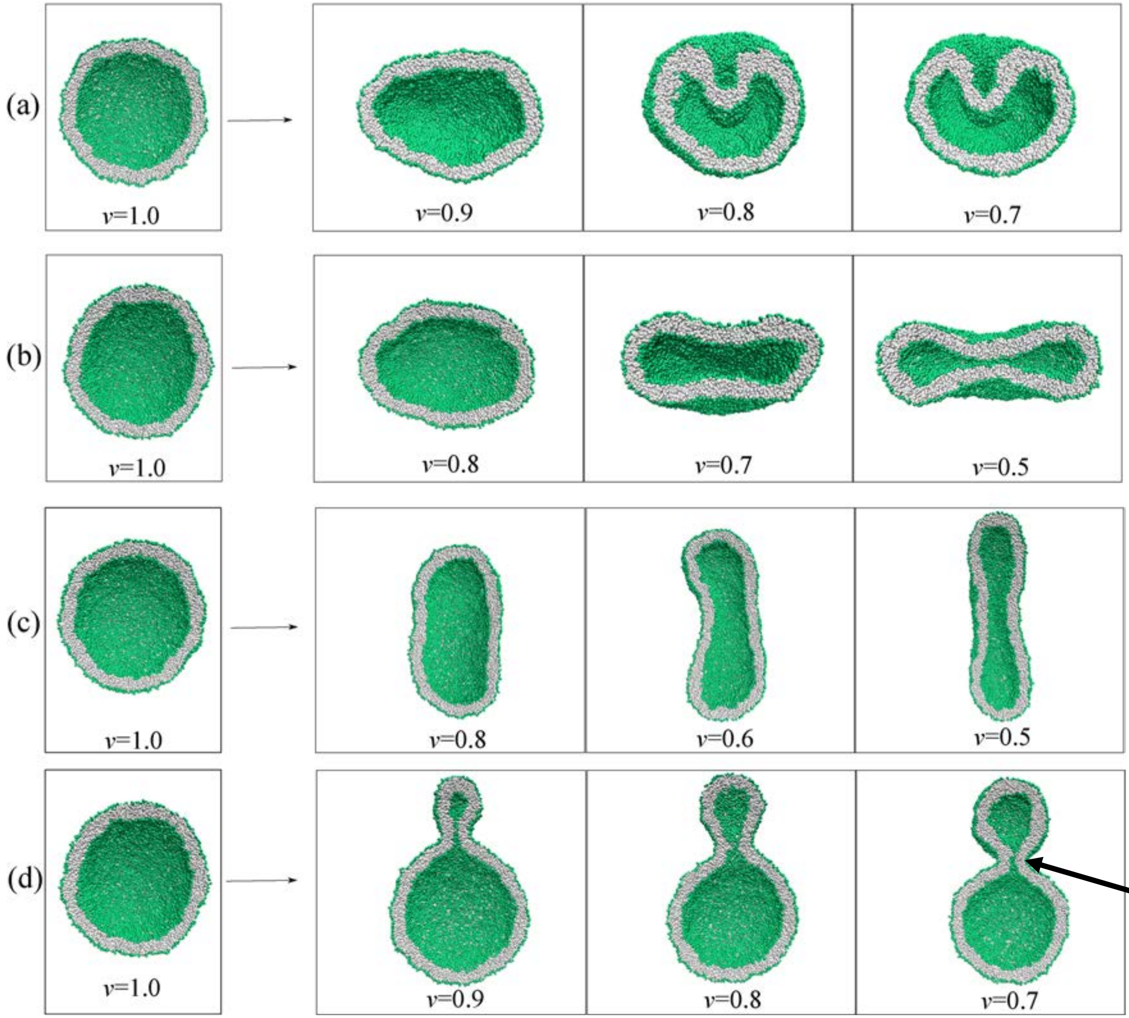


- Add cholesterol
- Flip-flops lead to tensionless leaflets with $\Sigma_1 = \Sigma_2 = 0$

Shapes of Nanovesicles

- Nanovesicle with diameter of 40 nm:

R. Ghosh, V. Satarifard et al, *Nano Letters* (2019)



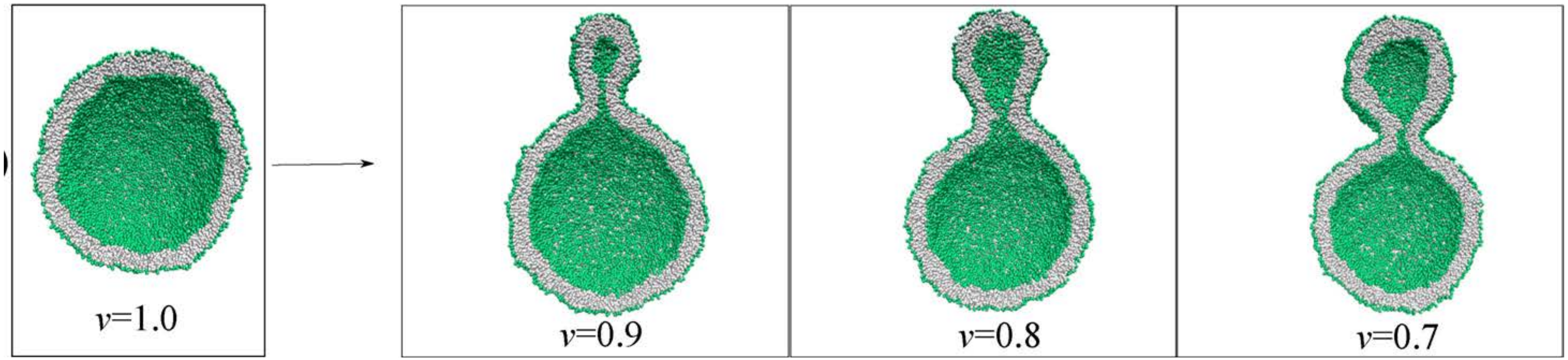
- Four spherical vesicles
- Same volume
- Same total # of lipids
- Reduction of volume: very different shapes
- Shape transformations caused by leaflet tensions

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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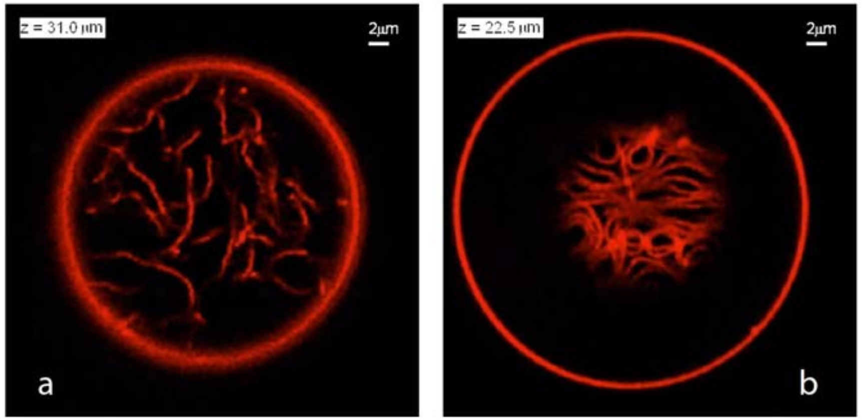
- Shapes of Nanovesicles
- Wetting of Membranes

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



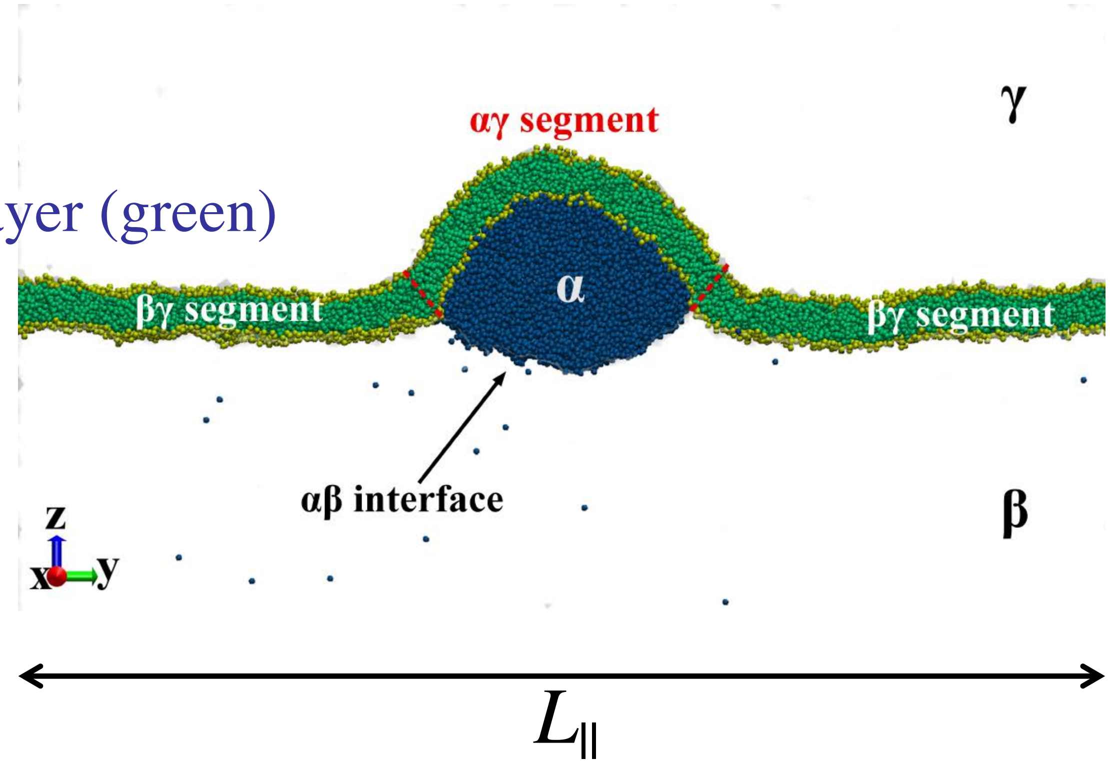
Distinct patterns of nanotubes

Lipid Bilayer + Nanodroplet

Satarifard et al, *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)

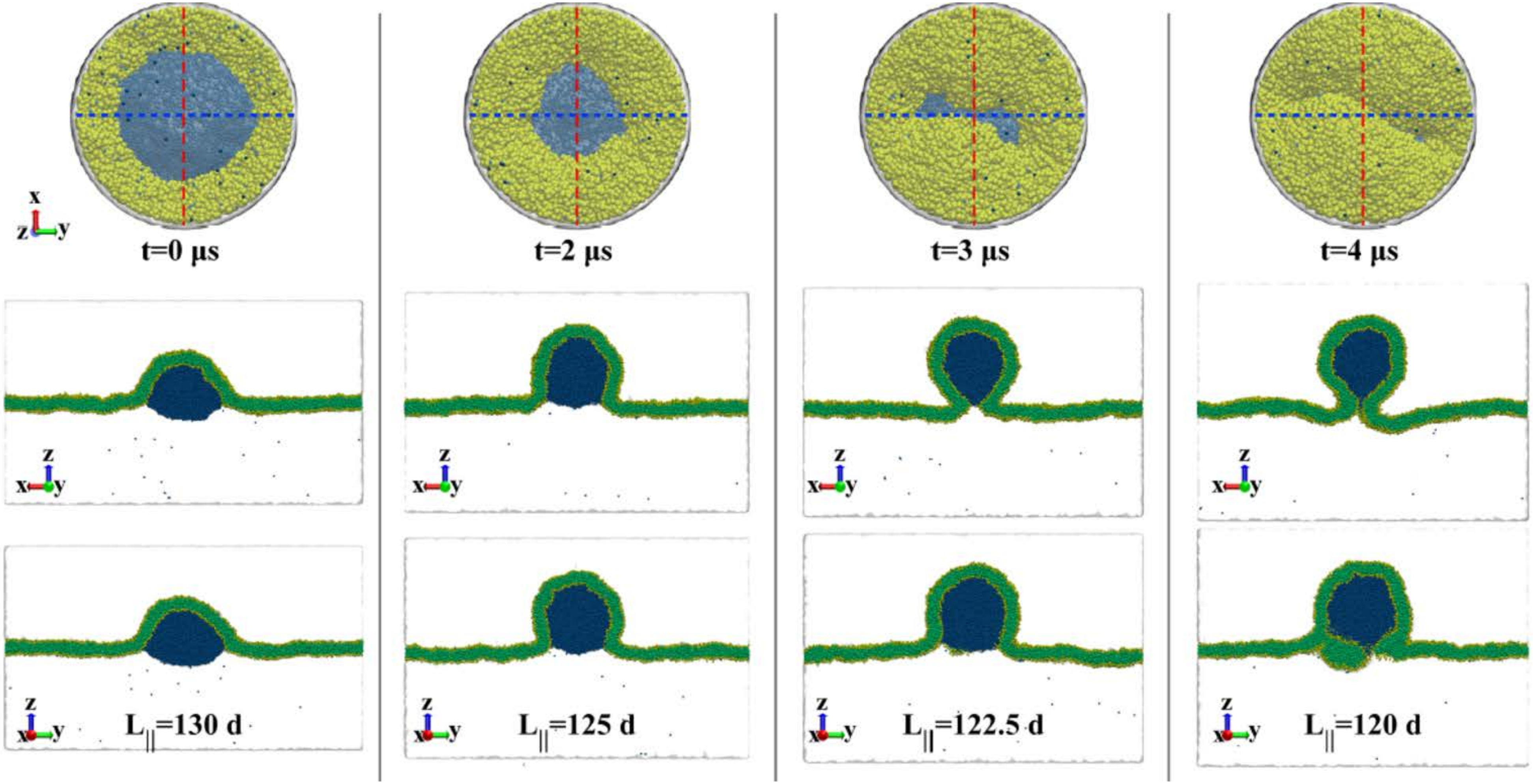


three aqueous phases α, β, γ

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

three surface segments αβ, αγ, βγ

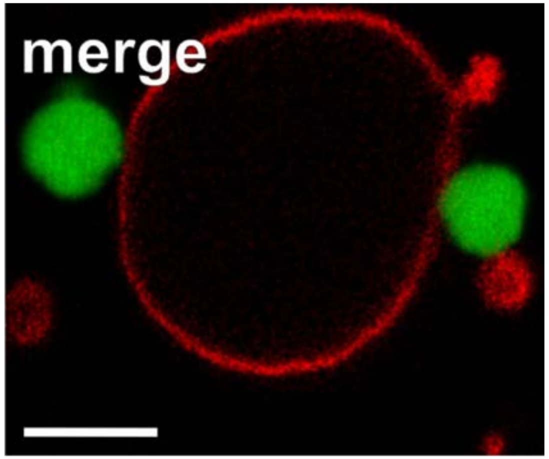
Engulfment of Nanodroplet



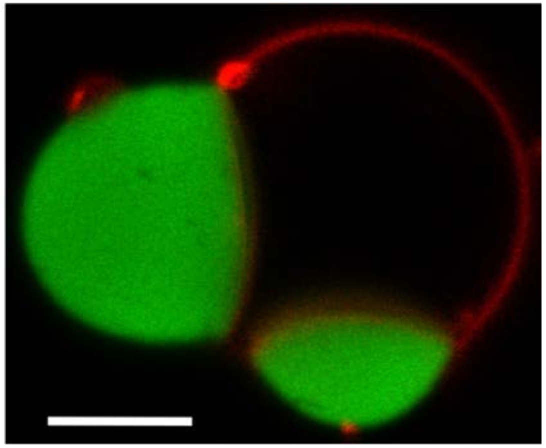
- Tight-lipped membrane neck from negative line tension

Biomolecular Condensates

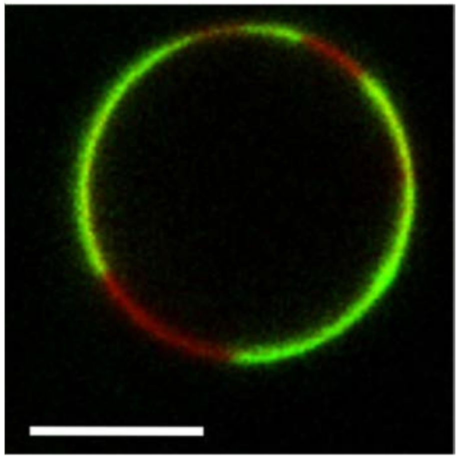
- Eukaryotic cells contain droplet-like compartments = membrane-less organelles = biomolecular condensates (BMCs)
- Wetting and molding of membranes by BMCs
  two subsequent wetting transitions



dewetting for high salt



partial wetting for intermediate salt



complete wetting for low salt

- Analogous processes in vacuoles of plant cells

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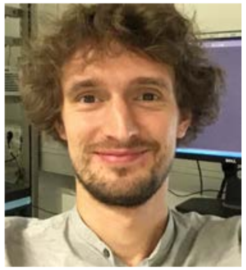

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