The Fluid Architecture of Biomembranes and Vesicles

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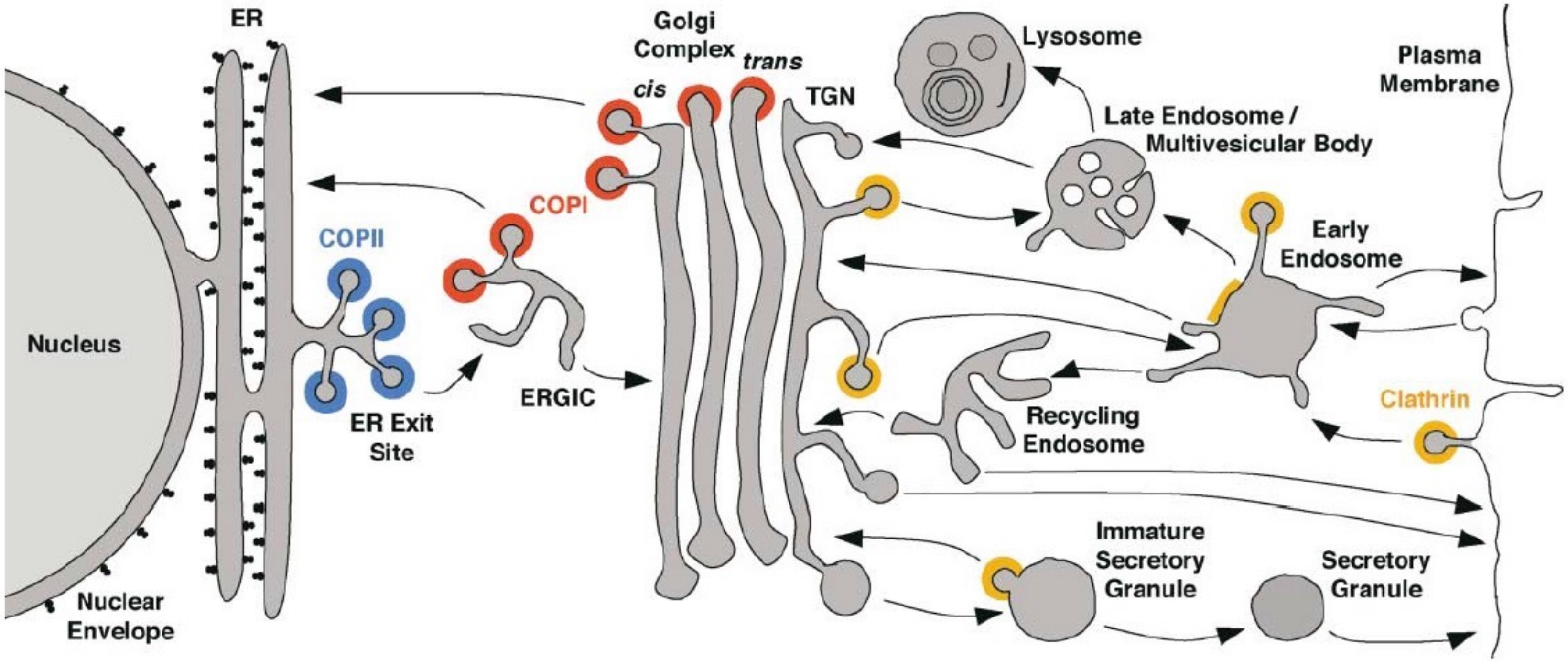
- Challenge of (Intra)Cellular Membranes
- Basic Aspects of Biomembranes
- Membrane Remodeling at the Nanoscale
- Membrane Remodeling at the Micron-Scale
- Giant Vesicles and Condensate Droplets
- Short Outlook on Endoplasmic Reticulum

Intracellular Membranes and Vesicles

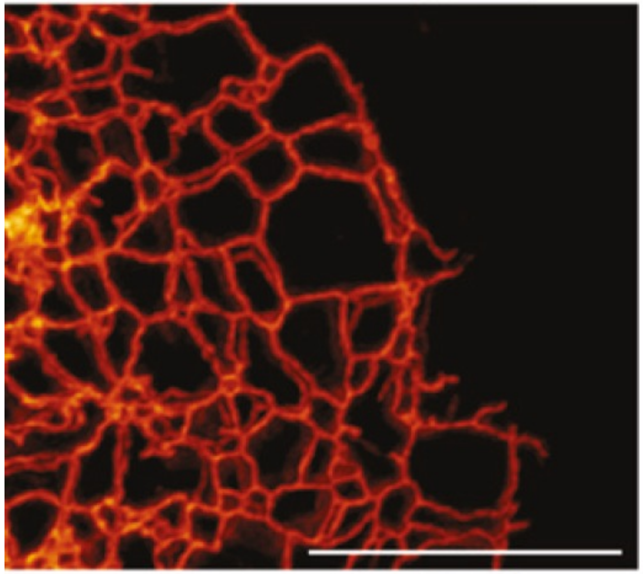


Bonifacio,Glick, *Cell* (2004)

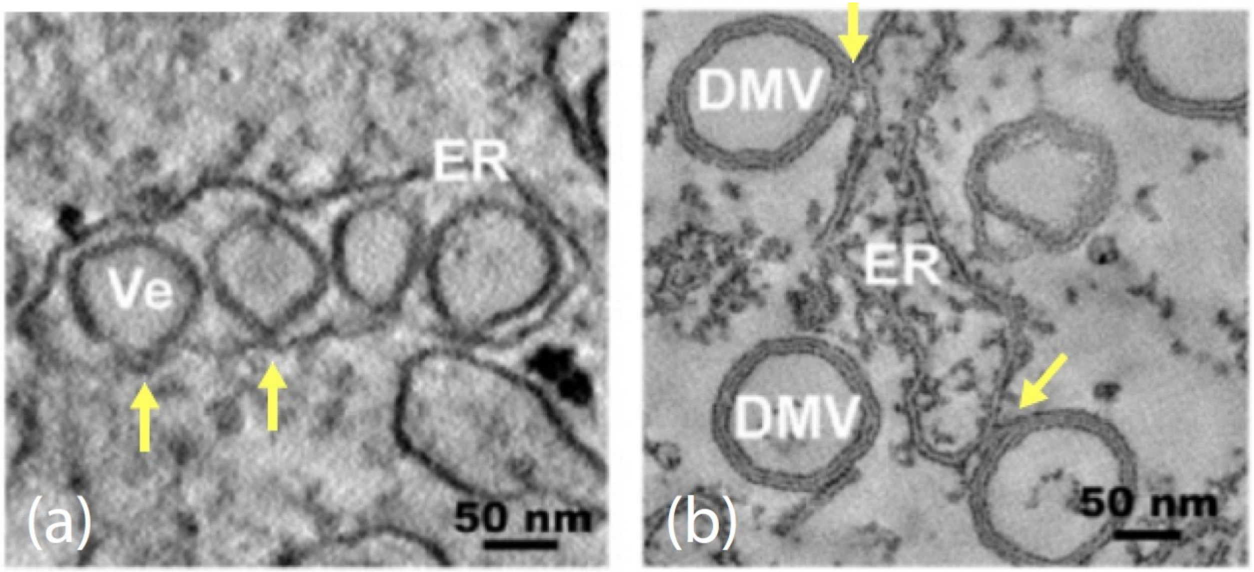
- Each cell of our body contains many membrane compartments
- Membrane fusion and fission change number of compartments
- Challenge for synthetic biosystems !

Membrane Morphology of Endoplasmic Reticulum (ER)

Obara et al , *Cold Spring* (2023)



Romero-Brey et al, *Viruses* (2014)



ER in healthy cells:

Network of membrane nanotubes and three-way junctions

ER membrane in sick cells, remodeling by (a) Dengue virus; (b) Hepatitis C virus:

(a) In-budded vesicles (Ve) and (b) out-budded double-membrane vesicles (DMV)

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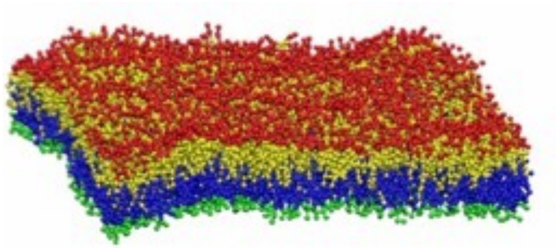
Basic Aspects I: Lipid Bilayers

• Universal building block of all biomembranes

• Each bilayer consists of two leaflets:



• Symmetric bilayers with two identical leaflets

• Asymmetric bilayers with two different leaflets

• Asymmetries can arise from different lipid composition or different lipid densities or different aqueous solutions adjacent to the two leaflets …

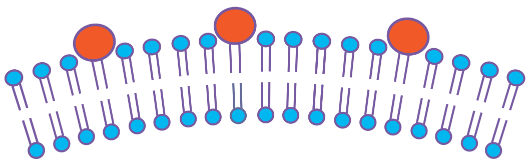
• Asymmetric densities imply distinct leaflet tensions

• On the nanoscale, bilayer asymmetry implies spontaneous curvature

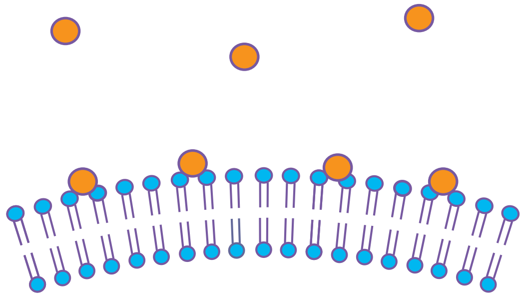
Bilayer Asymmetry and Spont Curvature

- Spontaneous or preferred curvature *m* describes (trans)bilayer asymmetry = asymmetry between two leaflets

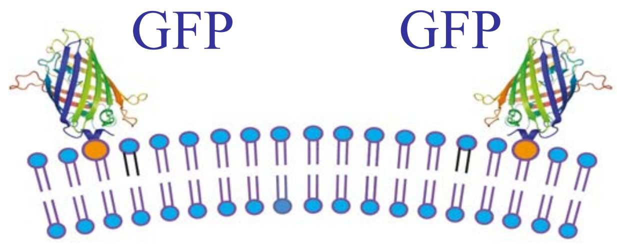
- Different molecular mechanisms for bilayer asymmetry:



Asymmetric composition, e.g., ganglioside



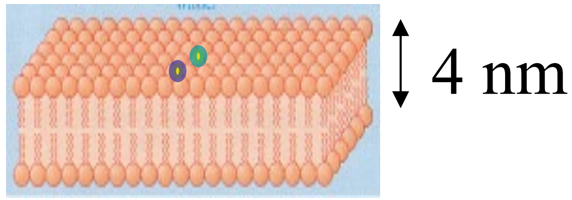
Asymmetric adsorption of small molecules



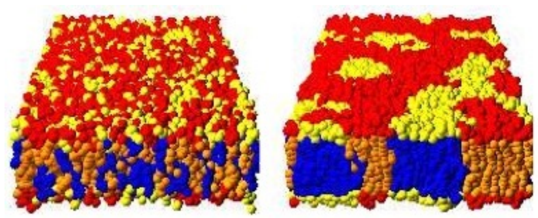
Asymmetric binding of proteins, dilute coverage

Basic Aspects II: Fluidity

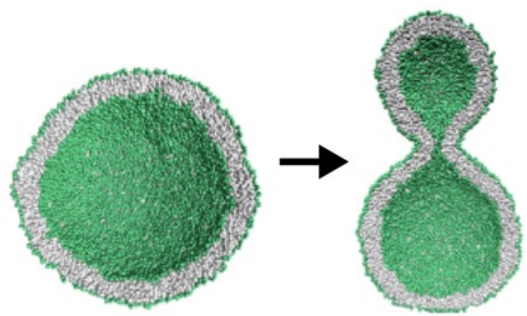
- Molecular scale: Fast lateral diffusion of all molecular components



- Fluidity leads to pattern formation in/by membranes
- Pattern formation traditionally called “remodeling“
- Remodeling of lipid composition: demixing and membrane domains

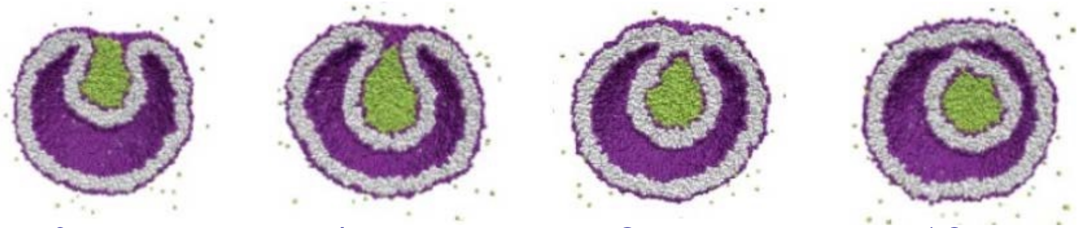


- Remodeling of membrane shape: sphere into dumbbell, two spheres connected by membrane neck



Remodeling of Membrane Topology

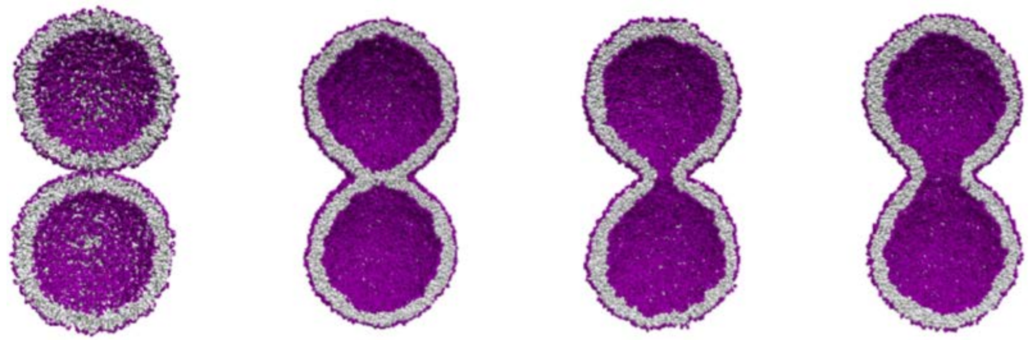
- Remodeling of membrane topology via fission and fusion
- Fission transforms one membrane compartment into two:



0 µs   4 µs   8 µs   12 µs

Ghosh et al , *Nature Commun* (2023)

- Fusion transforms two membrane compartments into one:

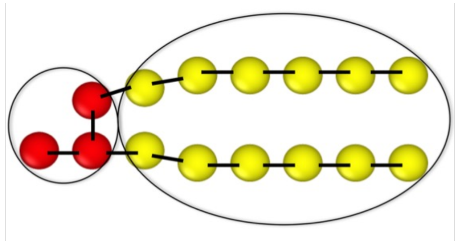


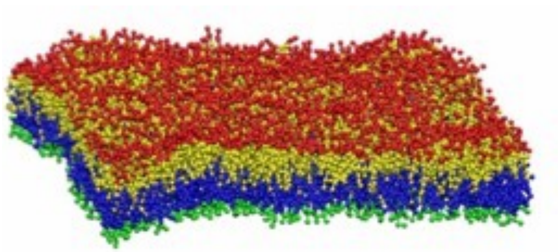
0 µs   0.2 µs   0.3 µs   0.5 µs

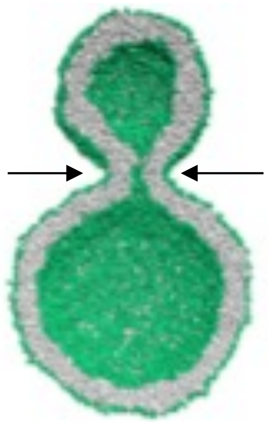
Lipowsky et al , *Biomolecules* (2023)

Assembly of Bilayers and Nanovesicles

- Assembly of lipids into two leaflets
- Coarse-grained lipids, in-silico assembly
- Leaflet 1 assembled from N_1 lipids
- Leaflet 2 assembled from N_2 lipids







- Lipid numbers N_1 and N_2 are simple assembly parameters, easy to control in the simulations

From Lipid Numbers to Leaflet Tensions

Bartosz Rozycki and Lipowsky, *J. Chem. Phus.* (2015)
Lipowsky, Rikhia Ghosh et al, *Biomolecules* (2023)

- Different lipid numbers N_1 and N_2

  generate different leaflet tensions Σ_1 and Σ_2
- Bilayer tension $\Sigma = \Sigma_1 + \Sigma_2$
- Each leaflet tension can be positive or negative, corresponding to a stretched or compressed leaflet

- Stress asymmetry between leaflets, $\Delta\Sigma = \Sigma_2 - \Sigma_1$
- Stress asymmetry $\Delta\Sigma$ provides important control parameter
- All remodeling processes at the nanoscale controlled by $\Delta\Sigma$

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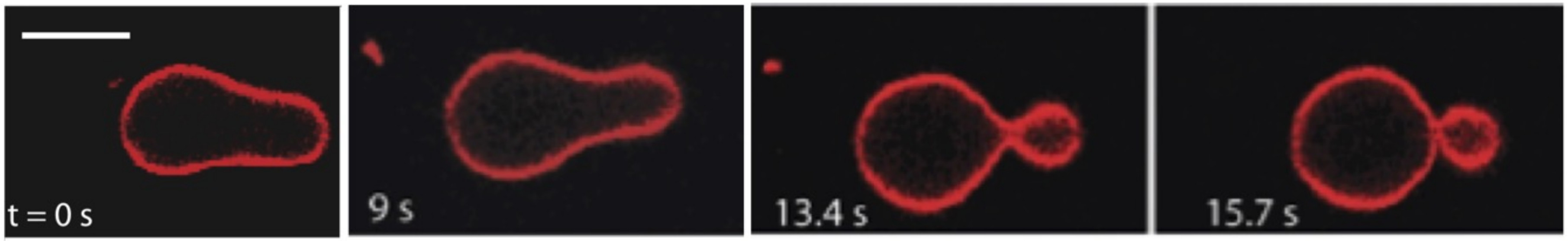
Out-Budding of Giant Vesicles

Tripta Bhatia et al, *Soft Matter* (2020)

- Pear-like vesicle transformed into two-sphere vesicle
- Snapshots from time lapse over 16 s:



Scale bar: 5 μm

- Membrane exposed to asymmetric sucrose/glucose solutions
- Membrane forms two spheres connected by a single neck
- (1+1)-sphere consisting of one large and one small sphere

Stability of Two-Sphere Shapes

Lipowsky, *Giant Vesicle Book* (2019)

- Morphology diagram defined by two parameters: volume-to-area ratio v and spontaneous curvature m

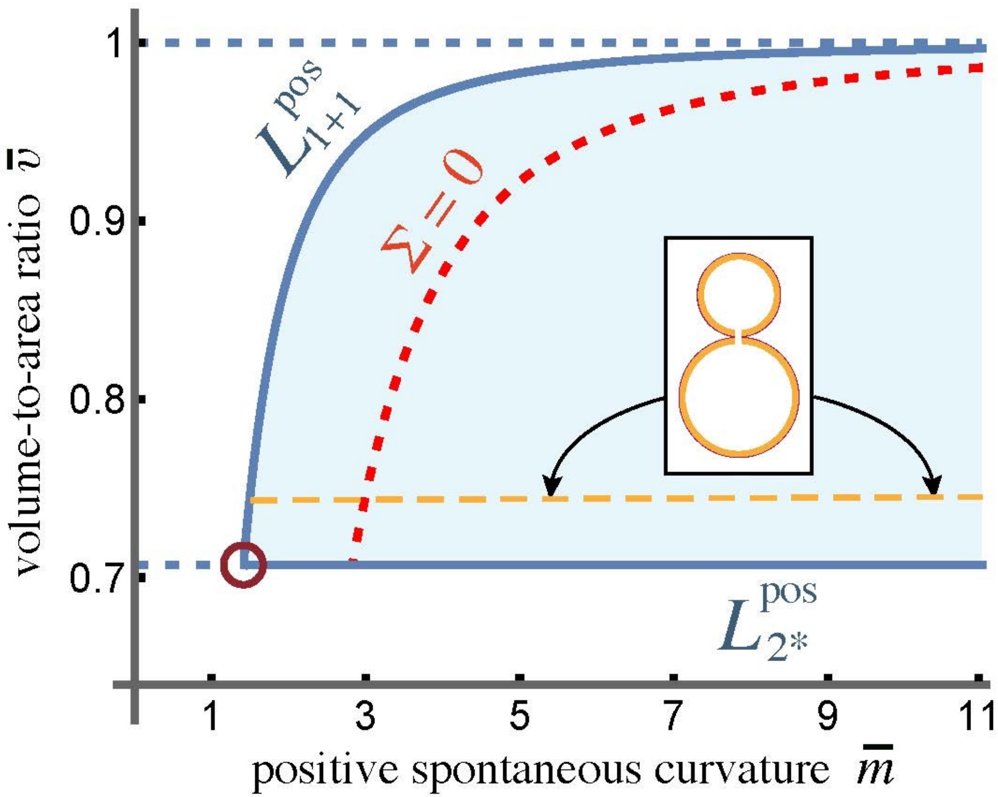
- Stability regime is subregion of morphology diagram

- Bounded by two lines of limit shapes L_{1+1} and L_{2*}

- Two-sphere shapes independent of spontaneous curvature m

- But, for larger m, membrane neck compressed by larger constriction force

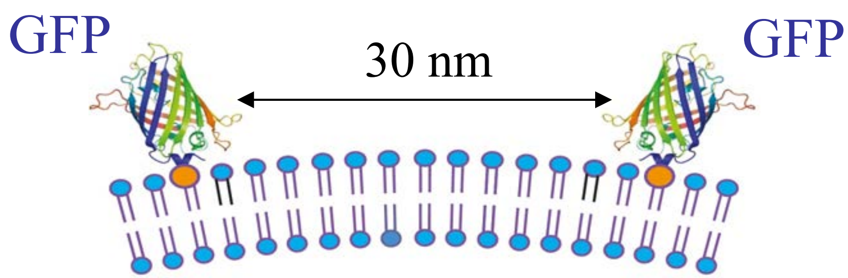
$$f = 8\pi\,\kappa\,(\,m - M_{ne}\,)$$



Fine-Tuning of Spontaneous Curvature

Jan Steinkühler et al, *Nature Commun* (2020)

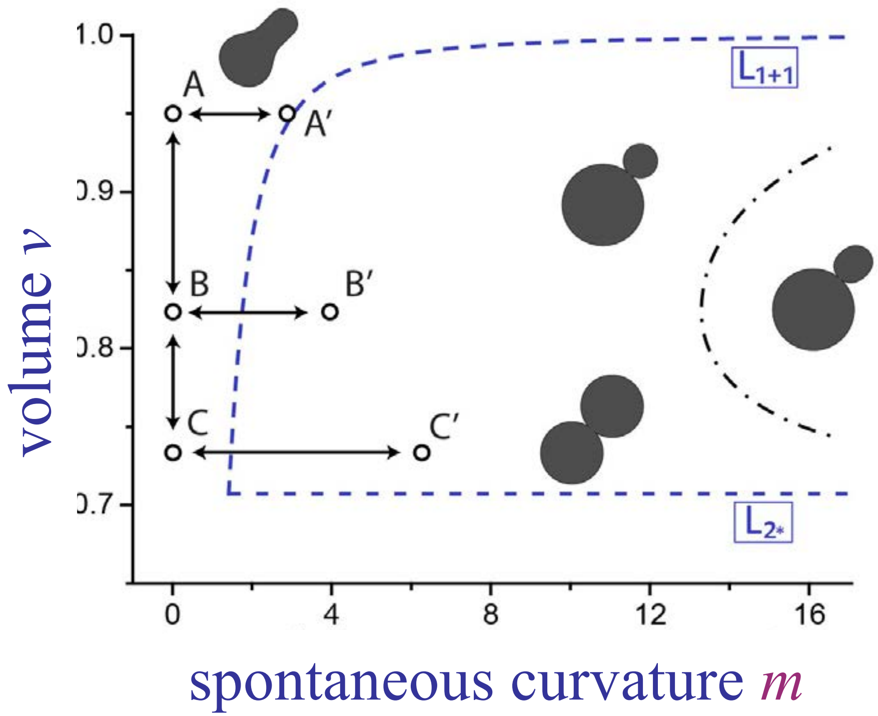
- Vesicles exposed to His-tagged GFP in exterior solution
- GFP binds to anchor lipids in the vesicle membrane
- Fine-tuning of spontaneous curvature by membrane-bound GFP
- High curvature from low densities of membrane-bound GFP:

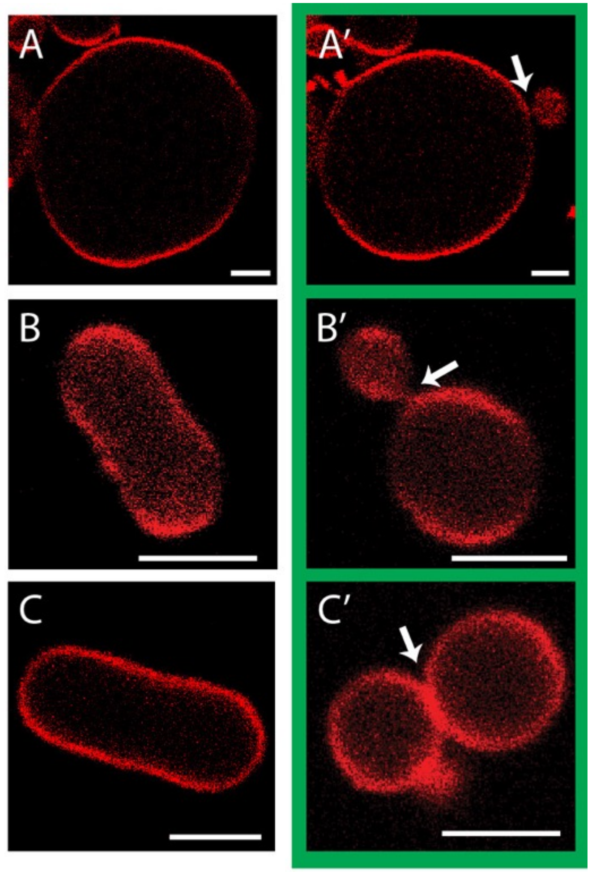


Morphology Diagram, Experiment

Jan Steinkühler et al, *Nature Commun* (2020)

- Vesicle volume *v* changed by osmotic inflation/deflation
- Spontaneous curvature *m* controlled by GFP concentration
- Morphology diagram:



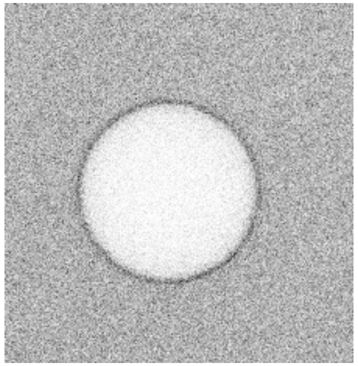


Fission and Division of GUVs

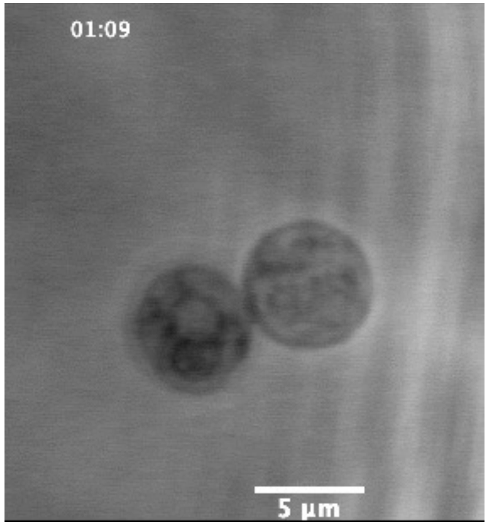
Jan Steinkühler et al: *Nature Commun*. (2020)

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

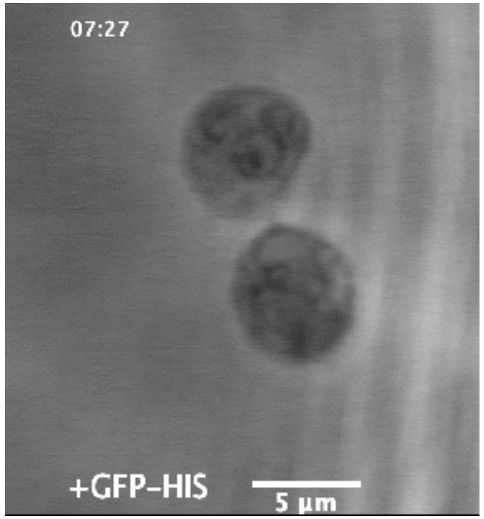
Increase in GFP -> Neck cleavage -> Two daughter GUVs



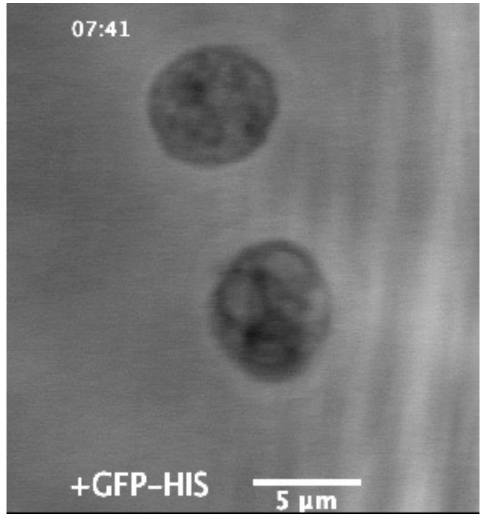
Adsorption of GFP onto GUV membrane



Deflation leads to dumbbell with membrane neck



Directly after neck cleavage

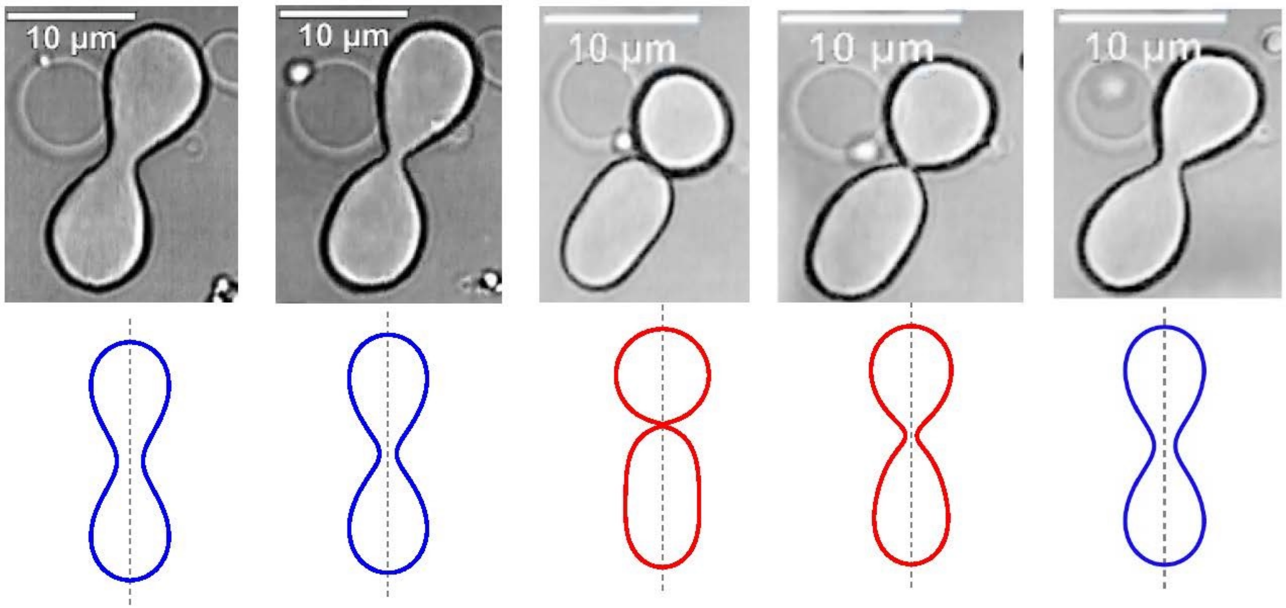


Complete division into two smaller GUVs

Active Shape Oscillations of Giant Vesicles

Simon Christ et al, *Soft Matter* (2021)

- Min proteins D and E in interior solution
- MinD/E binds to membrane and unbinds via ATP hydrolysis
- Cyclic closure and opening of membrane neck:



Symmetric (blue) Asymmetric (red)

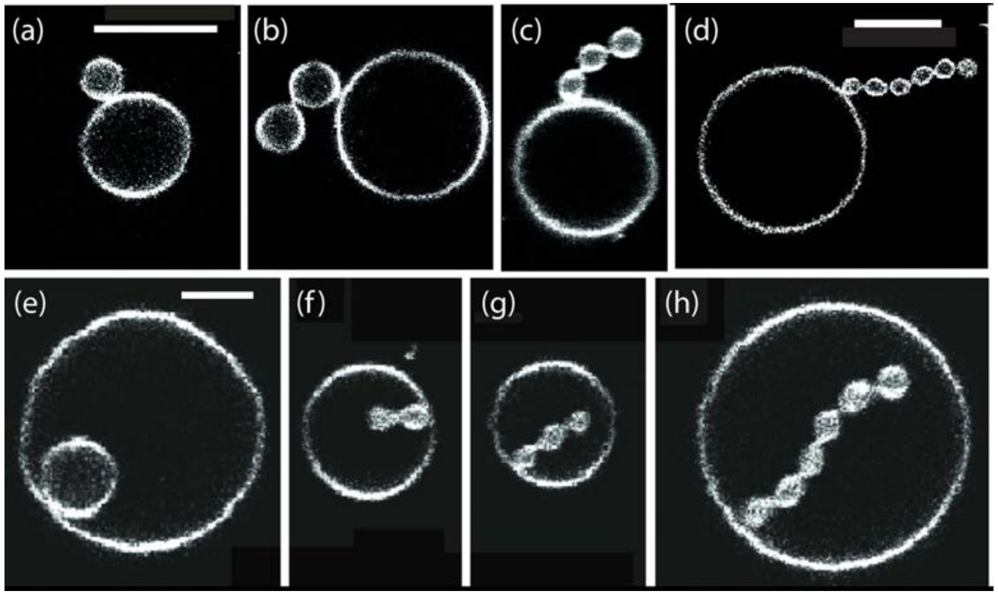
Multispherical Shapes of Giant Vesicles

Tripta Bhatia et al, *Soft Matter* (2020)

- (1+1)-spheres are the simplest multispheres
- Multispheres consisting of small and large spheres:

- Multispheres with out-buds

- Multispheres with in-buds



Scale bars: 10 μm

Scale bar: 5 μm

- All spheres connected by membrane necks
- All small spheres have the same radius
- Likewise: all large spheres have the same radius

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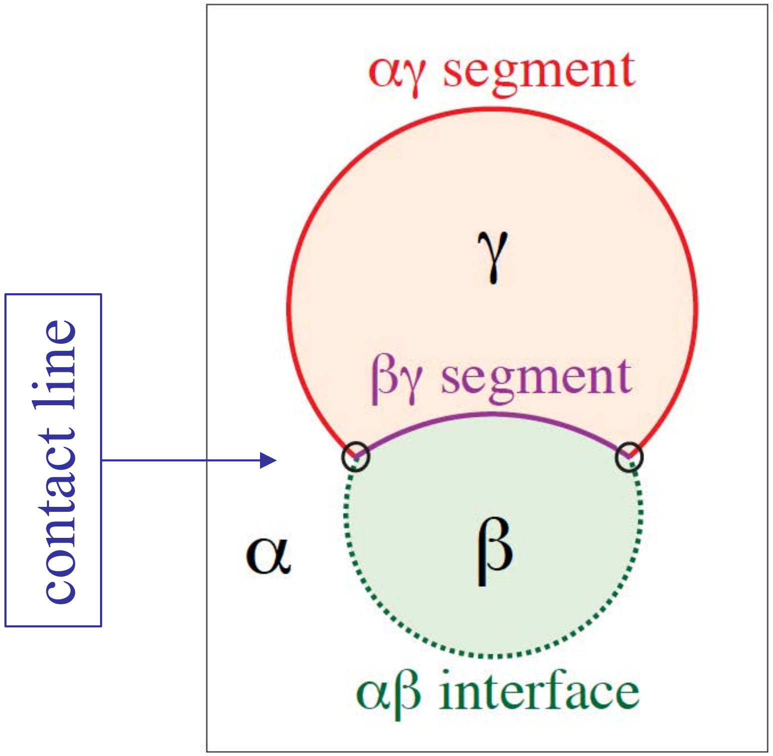
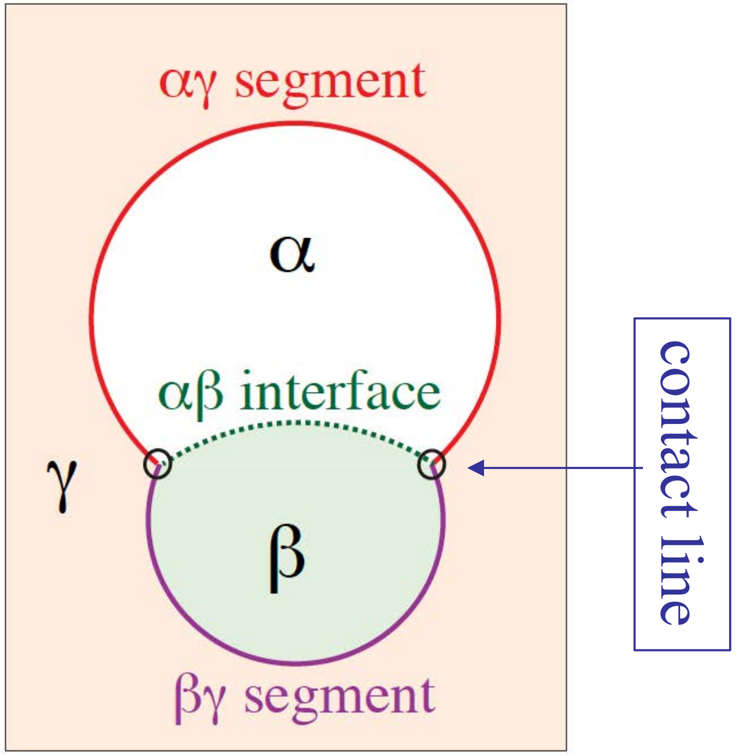
Giant Vesicles and Condensate Droplets

- Liquid-liquid phase separation in exterior solution:



- Liquid-liquid phase separation in interior solution:



Lipowsky, *Membranes* (2023)

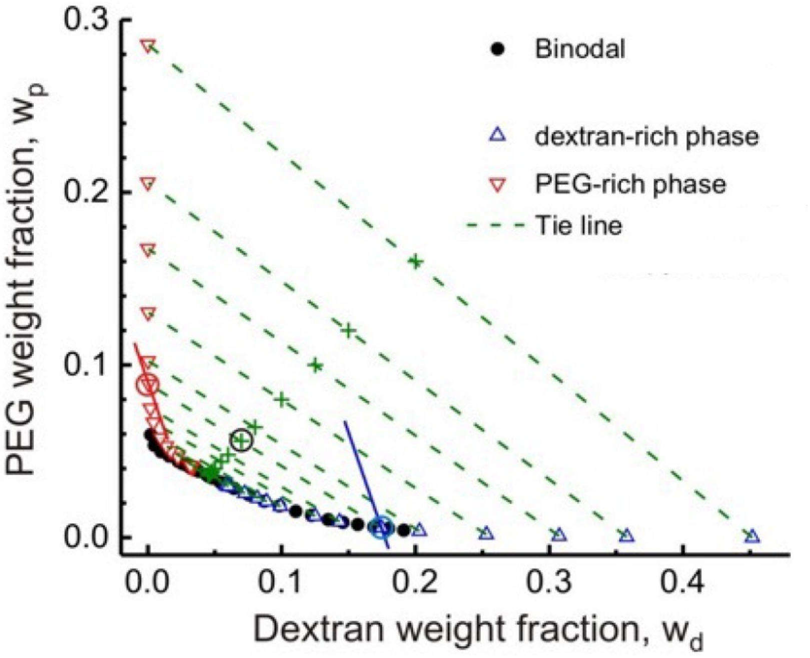
Liquid-liquid (αβ) interface pulls at the membrane by capillary forces, thereby generating membrane "kinks“

Condensates and Interfacial Tension

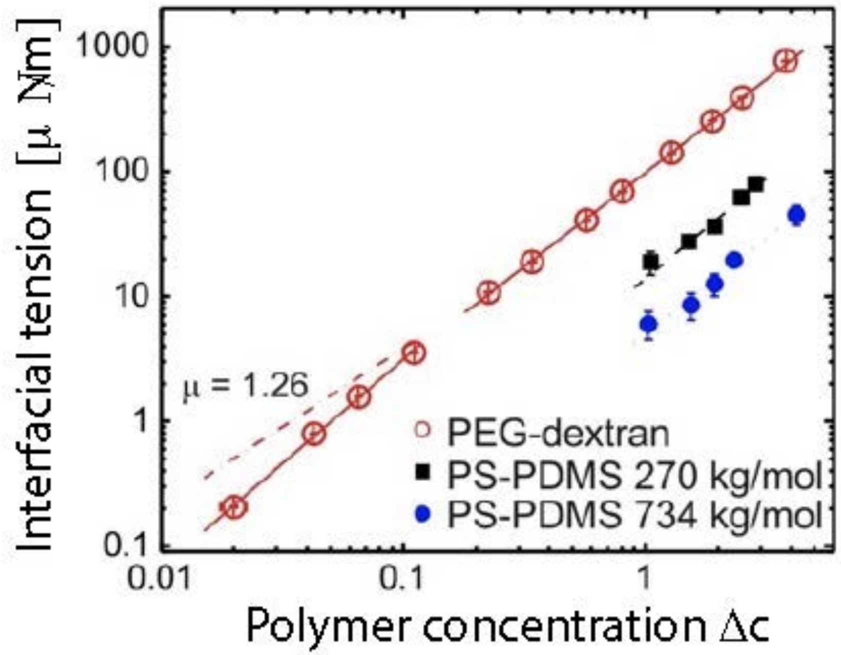
Yonggang Liu et al, *Langmuir (*2012)

- Condensates in aqueous solution of PEG and dextran
- Segregative phase separation into PEG-rich and dextran-rich phase

- Phase diagram:



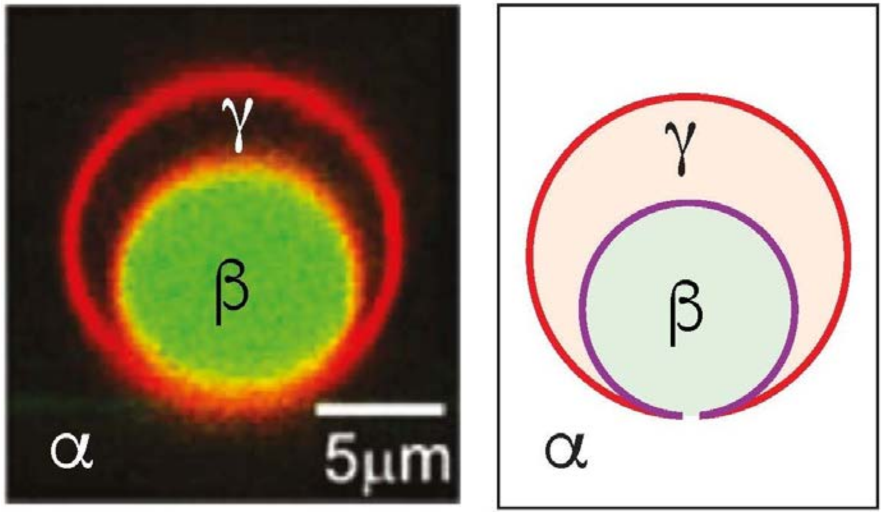
- Interfacial tension:



Droplet Engulfment versus Tubulation

- Two competing remodeling processes:

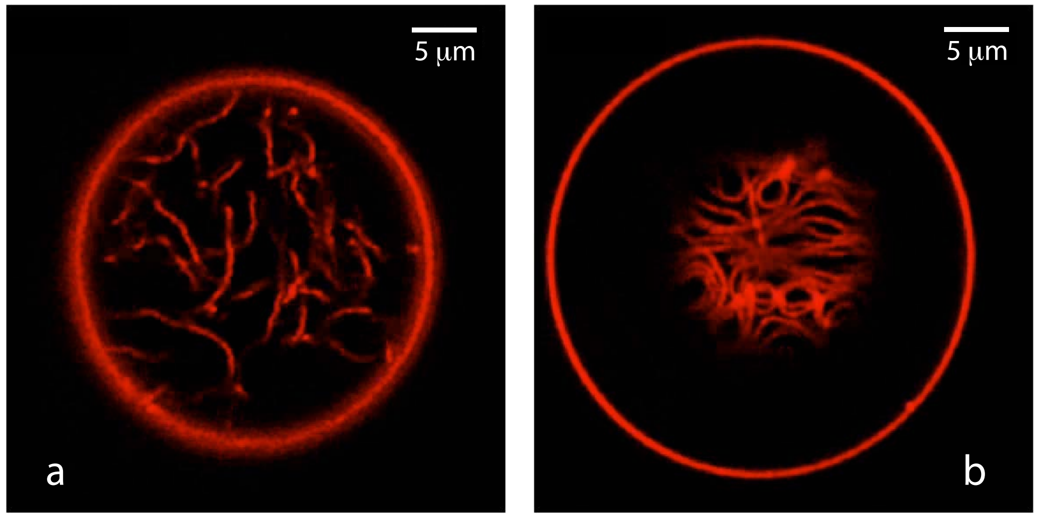
- Complete engulfment of condensate droplet:



Yanhong Li et al, *JPC B (*2012)

Interfacial tension large compared to curvature-elastic tension, $2\kappa m^2$

- Tubulation of membrane segment in contact with PEG-rich phase:



Yonggang Liu et al, *ACS Nano (*2016)

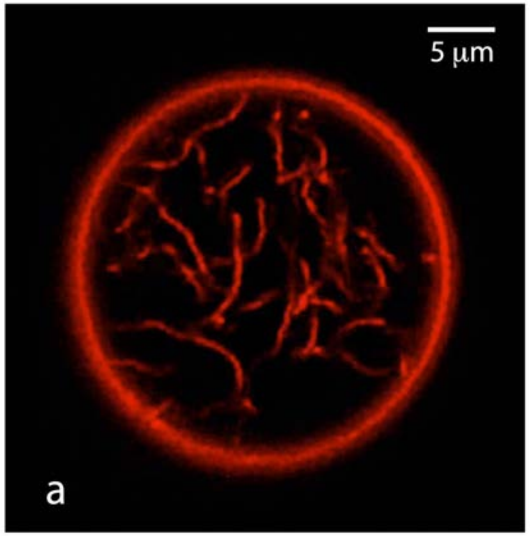
Curvature-elastic tension large compared to interfacial tension

Lipowsky, *Giant Vesicle Book* (2019)

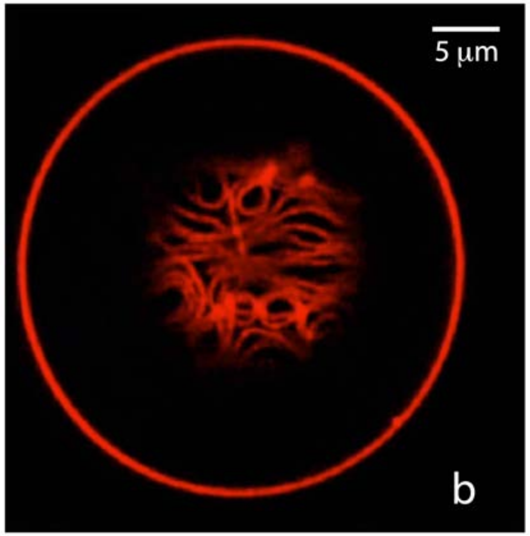
Tubular Patterns and Wetting

Yonggang Liu et al, *ACS Nano 10* (2016)

- Membrane segment exposed to PEG-rich phase forms many nanotubes with a diameter of about 100 nm
- Different patterns of nanotubes:



Tubes immersed in PEG-rich phase if membrane is completely wetted (CWet)

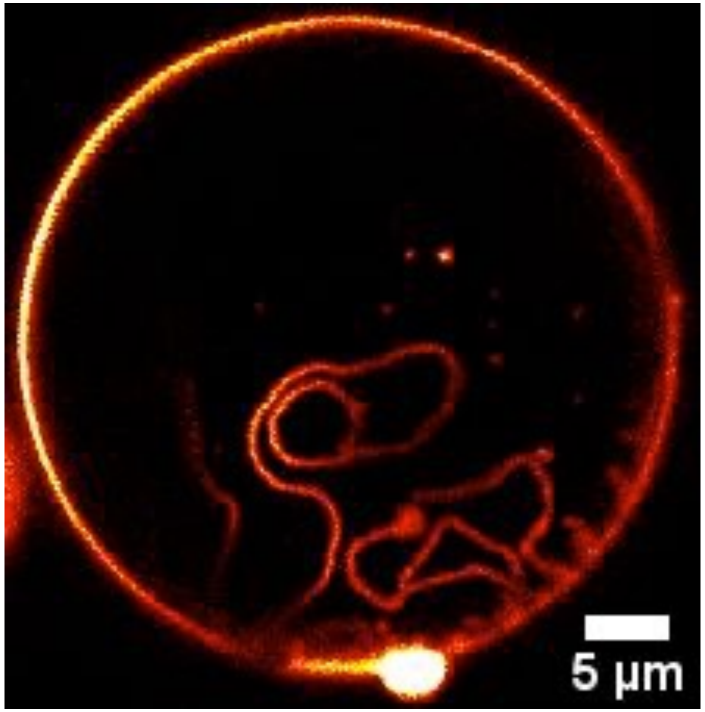


Tubes adhere to liquid-liquid interface if membrane is partially wetted (Pwet)

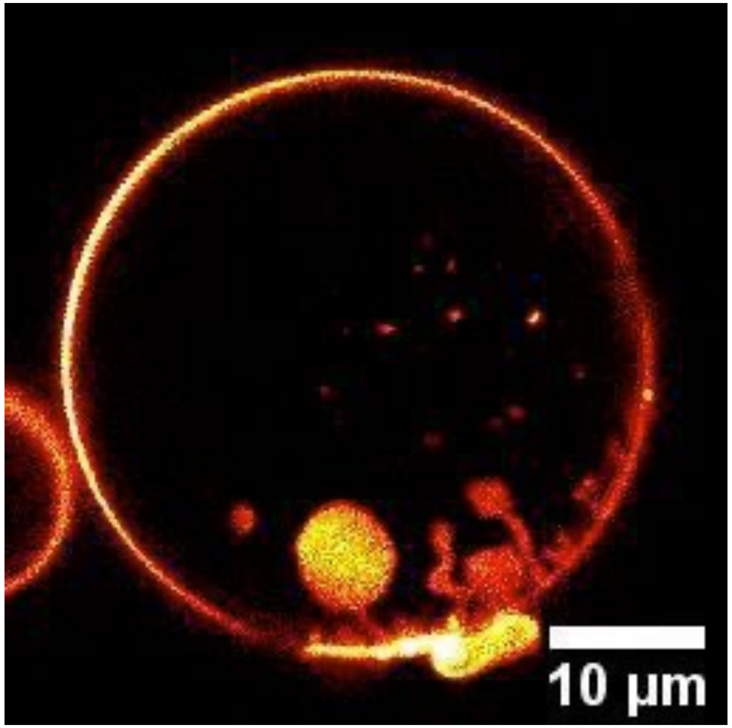
From Tubes to Sheets and Back

Ziliang Zhao et al, *PNAS* (2024)

- PWet: many nanotubes adhering to PEG-dextran interface
- Transformation between tubes and sheets:



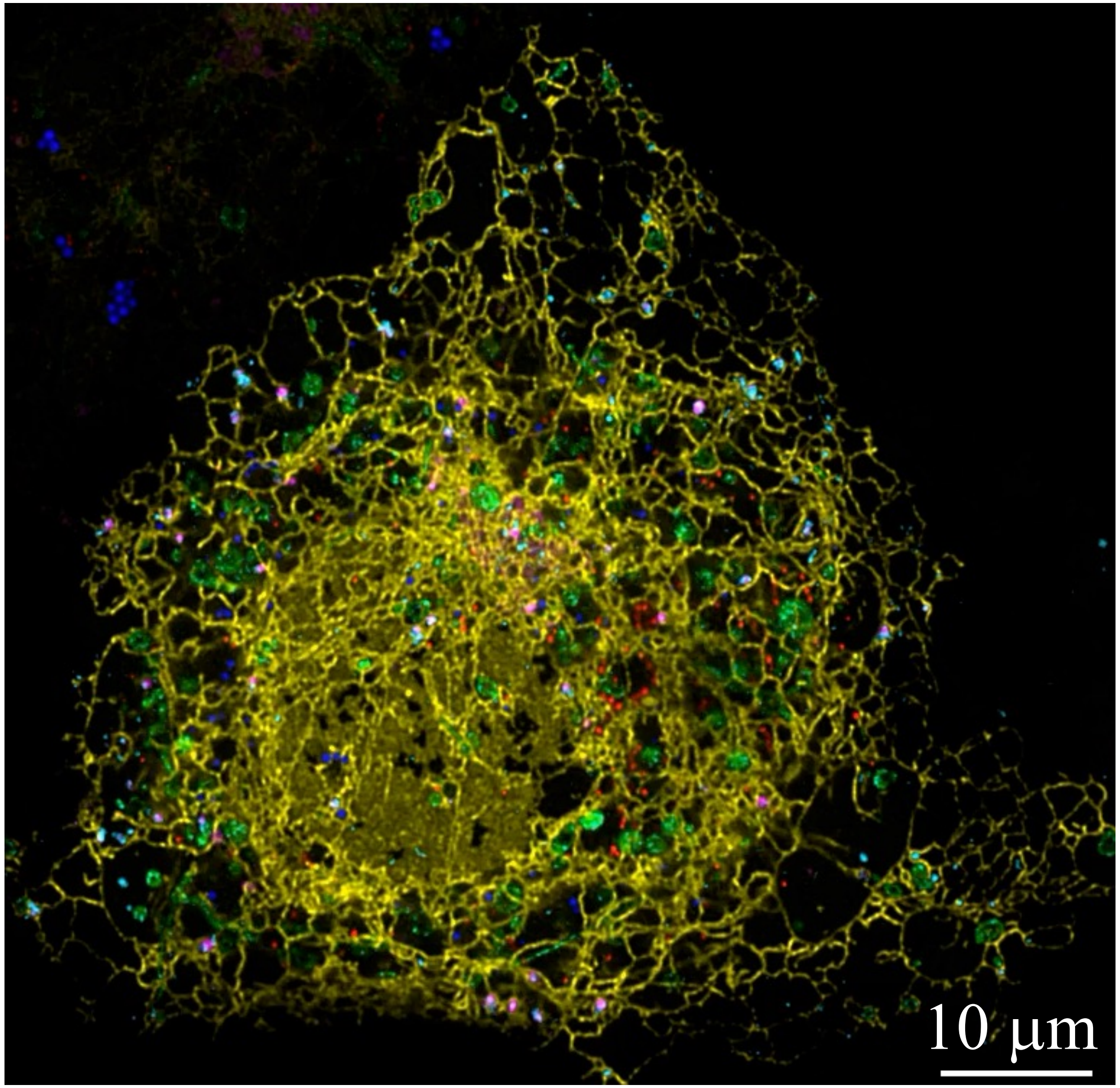
Tube transformed into sheet



Sheet transformed into tube

Morphological Complexity of ER

Valm et al. *Nature* (2017)



- Membrane-enclosed organelle
- Each eukaryotic cell contains only one ER
- Network of membrane nanotubes (yellow)
- Tubes have a width of ~ 80 nm
- Reticular network ~ cell size ~ 80 μm
- Meshsize of irregular polygons ~ 1 μm
- Network formed by a single membrane !

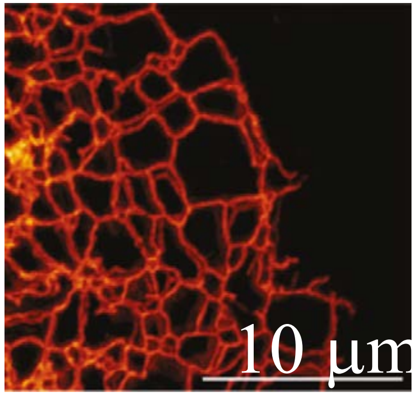
Networks with Three-Way Junctions

Lipowsky, Shreya Pramanik et al, *ACS Nano* (2023)

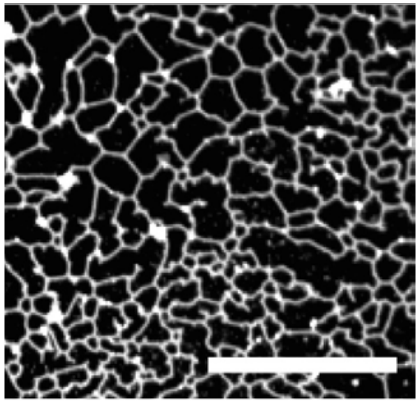
- Three-way junctions provide link between three nanotubes
- Observed for a long time
  - in vivo example: Lippincott-Schwartz lab
  - in vitro example: Rapoport lab
- But unknown mechanism

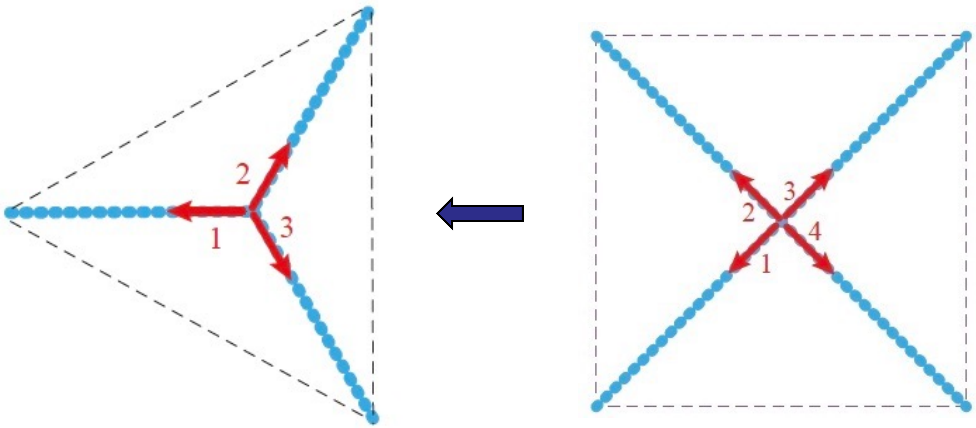
- Force balance at junction
- Proposed mechanism based on membrane tension
- Favors Transformation of four-way into three-way junction

in vivo



in vitro



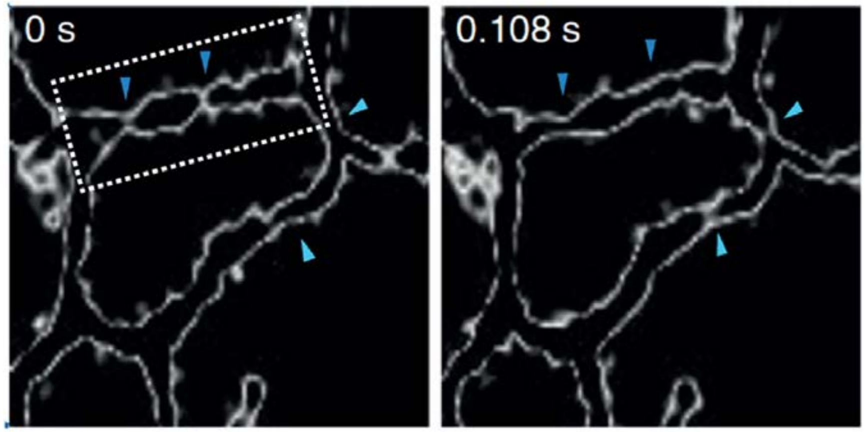


three-way four-way

Shape of Three-Way Junctions

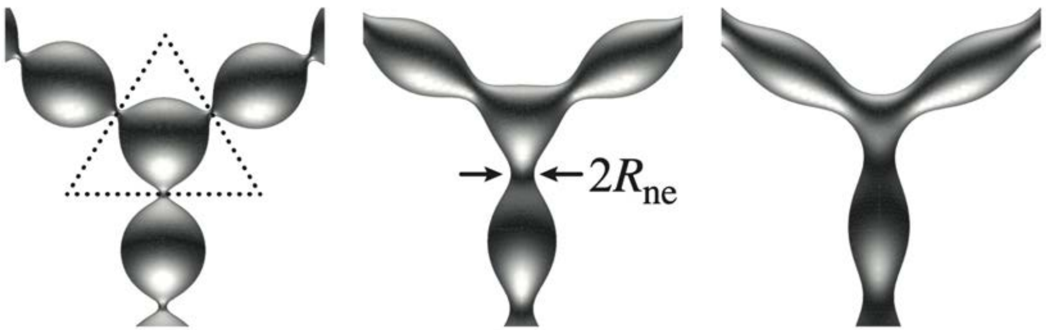
Lipowsky, Shreya Pramanik et al, *ACS Nano* (2023)

- Time-lapse of mesh with 5 junctions:
- Nanotubes undergo peristaltic modes
- Membrane necks close and reopen



Holcman et al, *Nat. Cell Biol.* (2018)

- Junctions resemble triunduloids:
- Closed neck => neck fission
- Membrane tension prevents necks from closing and nanotubes from fragmentation



Große-Braukmann et al, *Visualization* (1997)

Coworkers

Experiments, Micron-Scale


Rumiana Dimova


Tripta Bhatia


Jan Steinkühler


Ziliang Zhao


Agustin Mangiarotti


Shreya Pramanik

Theory


Simon Christ

Simulations, Nanoscale


Andrea Grafmüller


Bartosz Rozycki


Markus Miettinen


Rikhia Ghosh


Vahid Satarifard


Aparna Sreekumari


Miftakh Zamaletdinov

Fruitful collaborations with
Seraphine Wegner, Petra Schwille, and Joachim Spatz

****

Polymorphism of Nanovesicles

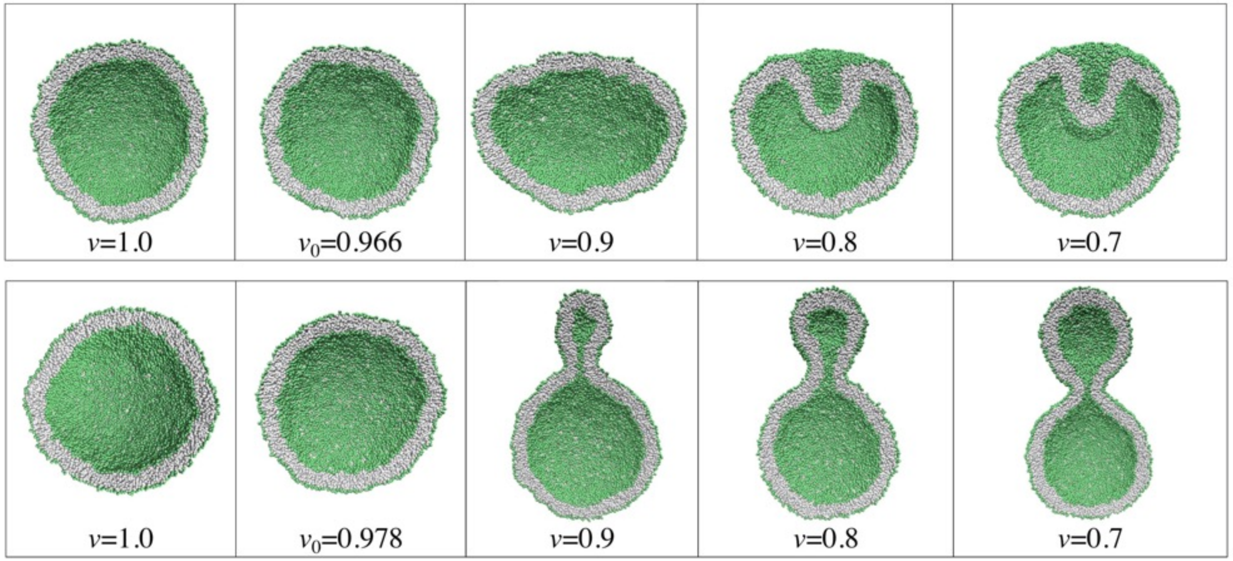
Rikhia Ghosh et al, *Nano Letters* (2019)

- Changes of vesicle volume by osmotic deflation and inflation
- Spherical vesicles with different stress assymmetry $\Delta\Sigma = \Sigma_{ol} - \Sigma_{il}$
- Reduction of volume v leads to distinct nonspherical shapes:

In-Budding

Positive $\Delta\Sigma$: Outer leaflet stretched, Inner leaflet compressed => In-Bud

Negative $\Delta\Sigma$: Outer leaflet compressed, Inner leaflet stretched => Out-Bud



Out-Budding

Multipheres of Equally Sized Spheres

Tripta Bhatia et al, Soft Matter (2020)

- Multispheres consisting of N_* equally sized spheres:

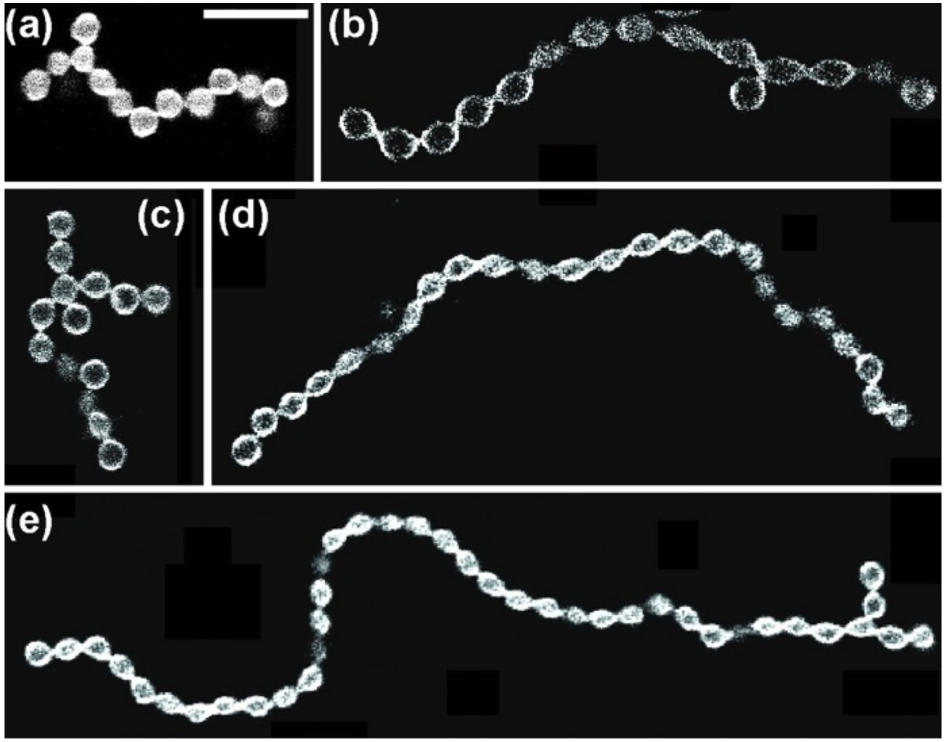
$N_* = 14$ branched

$N_* = 15$ branched

$N_* = 15$ branched

$N_* = 24$ linear

$N_* = 39$ branched



- Surprising mobility: linear ⇔ branched chains