Multiscale Remodeling of Biomembranes

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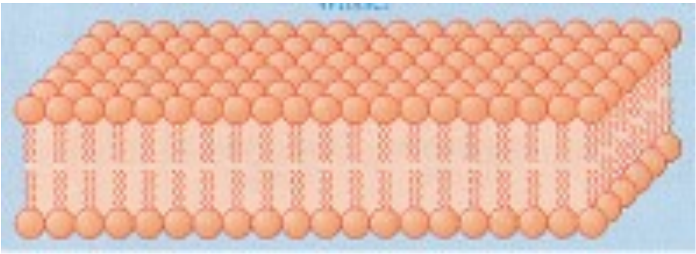
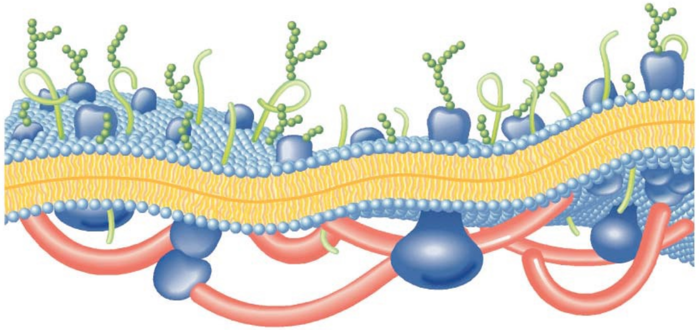
- Reminder about Biomembranes
- Remodeling of Nanovesicles
- Remodeling of Giant Vesicles
- Outlook on Endoplasmic Reticulum

Multiscale Biomembranes

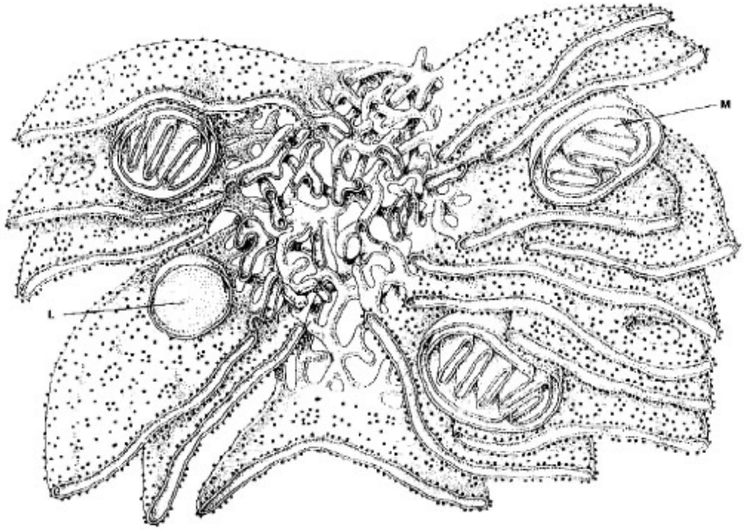
- Lipid bilayer



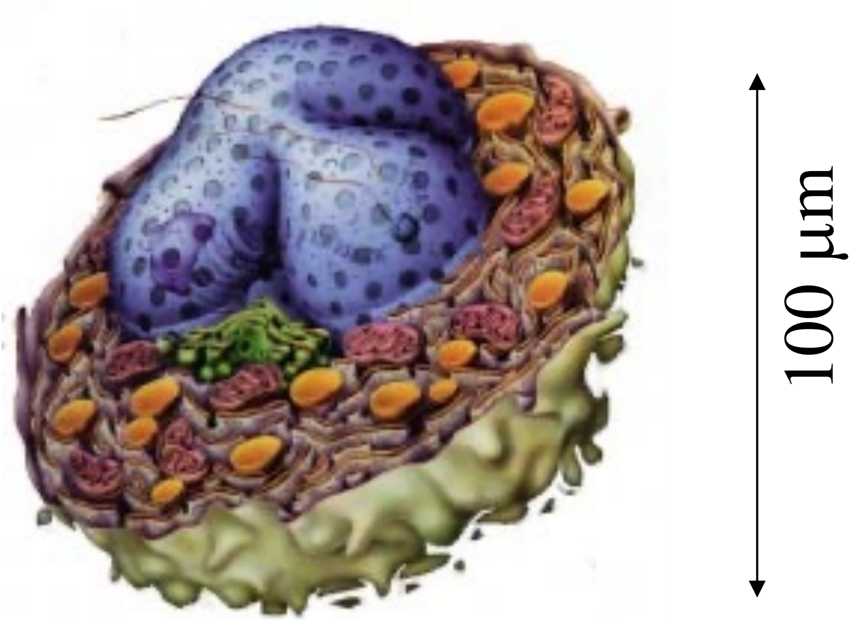
4 nm



- Biomembrane



- Endoplasmic reticulum (ER)

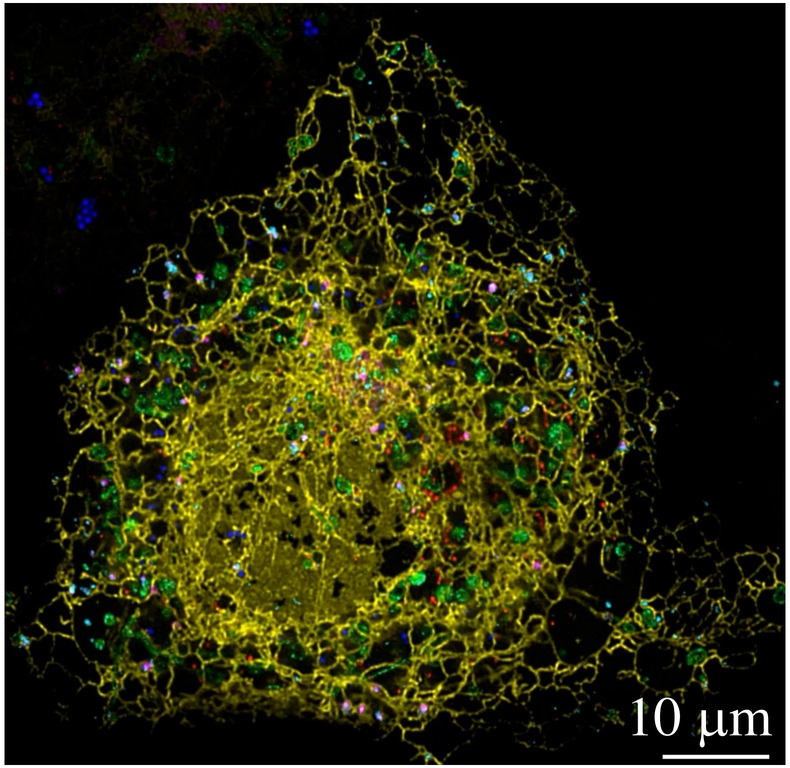


- Animal cell

Endoplasmic Reticulum (ER)

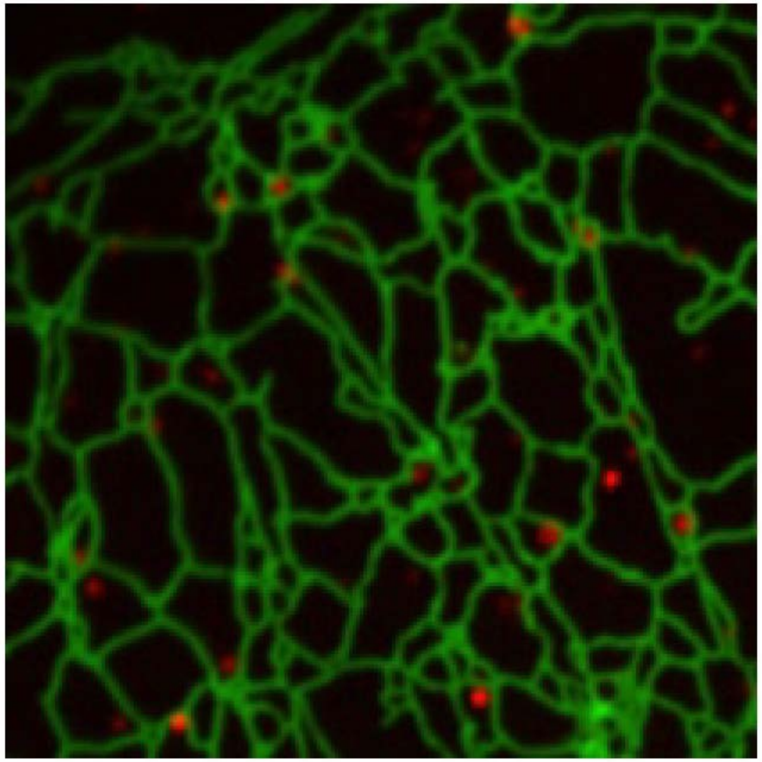
- ER = network of membrane nanotubes with three-way junctions

Tubes with yellow fluo-labels



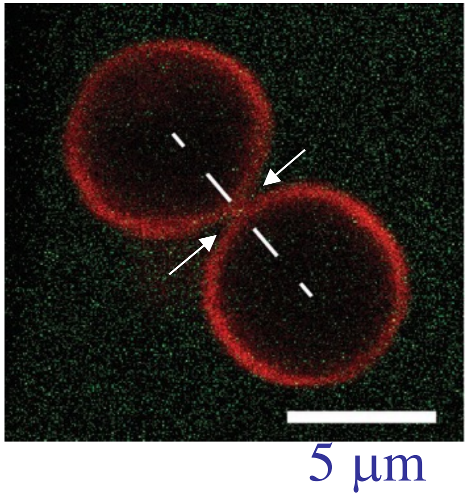
Valm et al. *Nature* (2017)

Tubes with green fluo-labels



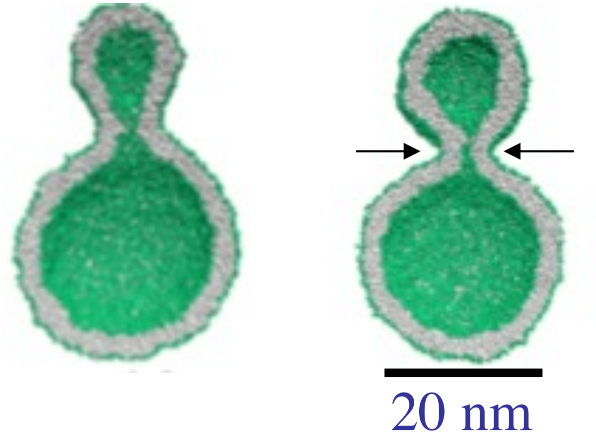
Friedmann, et al , *Mol Biol Cell* (2013)

Synthetic Membrane Compartments



- Giant unilamellar vesicles or GUVs
- Remodeling observed by optical microscopy
- Understanding in terms of curvature elasticity

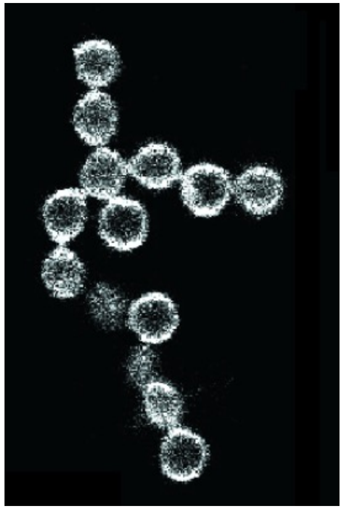
- Nanovesicles or NVs
- Electron microscopy: limited to a single snapshot for each individual nanovesicle
- Remodeling of NVs can be studied via Molecular Dynamics simulations



- In both cases: Formation of membrane necks

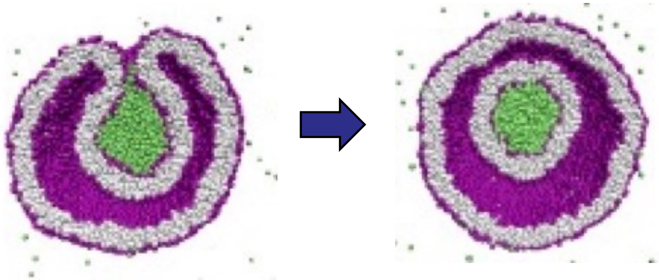
Remodeling of Shape and Topology

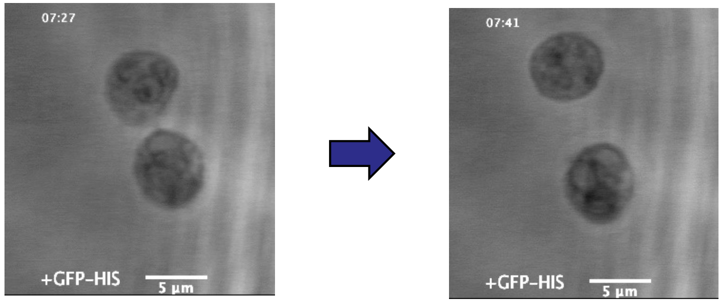
- Remodeling of membrane shape
- Polymorphism of nanovesicles and GUVs
- Multispherical shapes with many necks:



Topology of single sphere!

- Remodeling of membrane topology
- Membrane fission and fusion
- Requires formation of membrane neck:





- Introduction to Biomembranes
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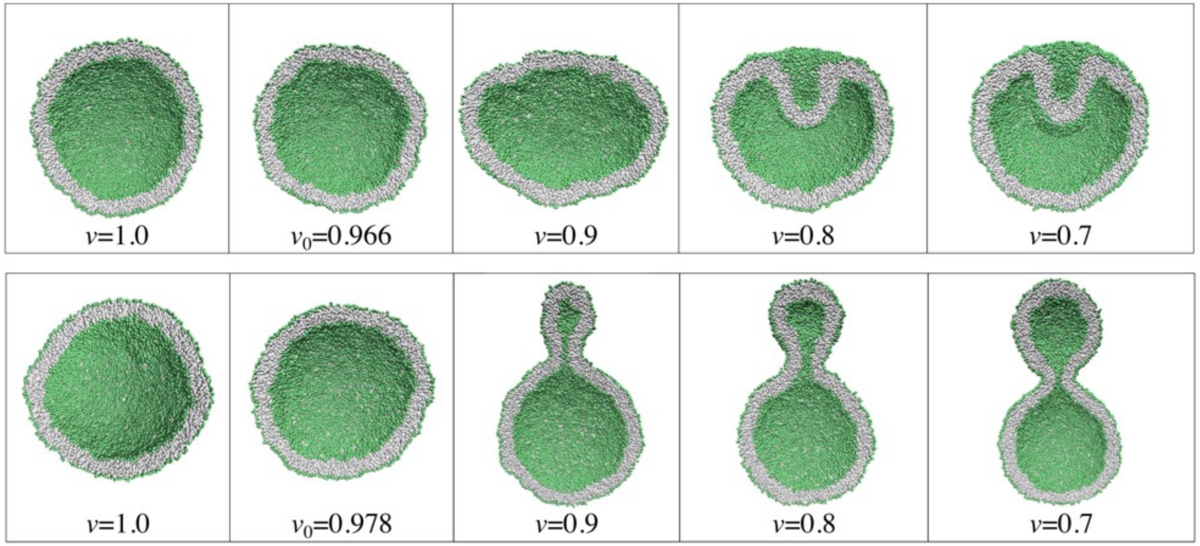
Shape Remodeling of Nanovesicles

- Spherical NVs by assembly of 10^4 lipids
- Midsurface of bilayer has diameter of 35 nm
- Shape remodeling by volume reduction:

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

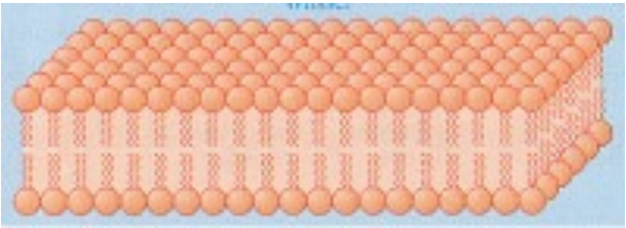


- Controlled by lipid numbers assembled in two leaflets:

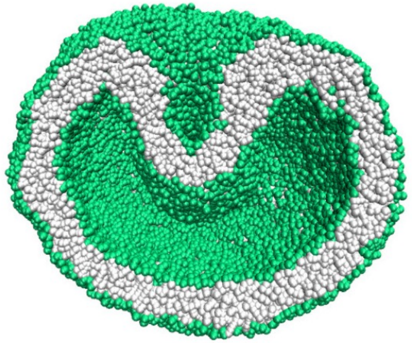
  N_{il} lipids in inner leaflet, N_{ol} lipids in outer leaflet

Bilayer and Leaflet Tensions

planar bilayer



bilayer of nanovesicle



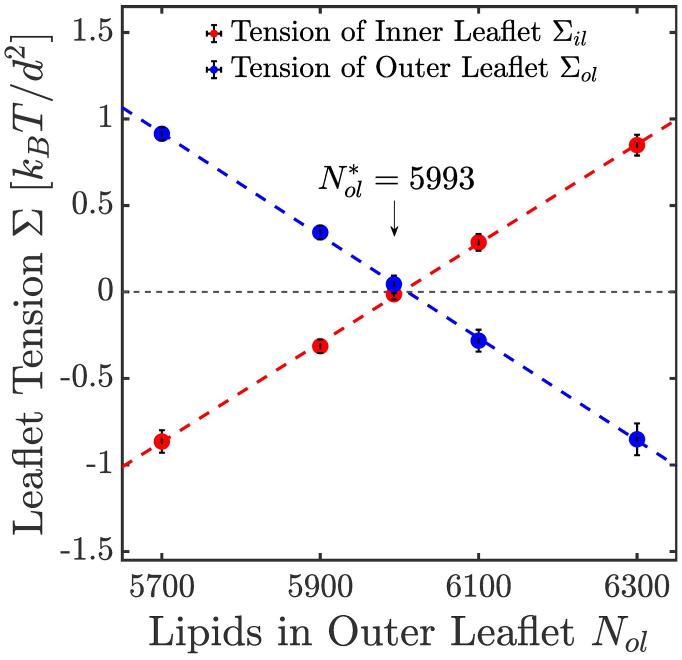
- Each bilayer consists of two leaflets, *l*1 and *l*2
- Mechanical bilayer tension $\Sigma = \Sigma_{l1} + \Sigma_{l2}$
- Leaflet tensions Σ_{l1} and Σ_{l2} of two leaflets, *l*1 and *l*2
- Low bilayer tension to avoid membrane rupture
- Tensionless bilayers: $\Sigma = 0$ implies $\Sigma_{l2} = - \Sigma_{l1}$

  => One leaflet stretched and one leaflet compressed
- Unique reference state with tensionless leaflets, $\Sigma_{l2} = \Sigma_{l1} = 0$

Nanovesicles with Tensionless Bilayers

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

- Spherical nanovesicles with fixed total number $N_{il} + N_{ol}$ of lipids
- Reshuffling lipids from inner to outer leaflet
- Impose low bilayer tension $\Sigma = \Sigma_{il} + \Sigma_{ol} \approx 0$
- Reshuffling leads to increased Σ_{il} (red) and descreased Σ_{ol} (blue) :



- Positive tensions for stretched leaflets
- Reference state with tensionless leaflets
- Negative tensions for compressed leaflets

One stretched and one compressed leaflet define stress asymmetry between leaflets

Nanovesicles with Tensionless Leaflets

- Reference state with vanishing leaflet tensions $\Sigma_{il} = \Sigma_{ol} = 0$
- But different lipid numbers N_{ol} and N_{il} in two leaflets
- Lipid number asymmetry of reference states:
  - Vesicle diameter of 35 nm : $N_{ol} = 1.5\, N_{il}$
  - Vesicle diameter of 19 nm : $N_{ol} = 2.0\, N_{il}$
  - Vesicle diameter of 13 nm : $N_{ol} = 2.9\, N_{il}$

Aparna Sreekumari
and RL, *Soft Matter* (2022)

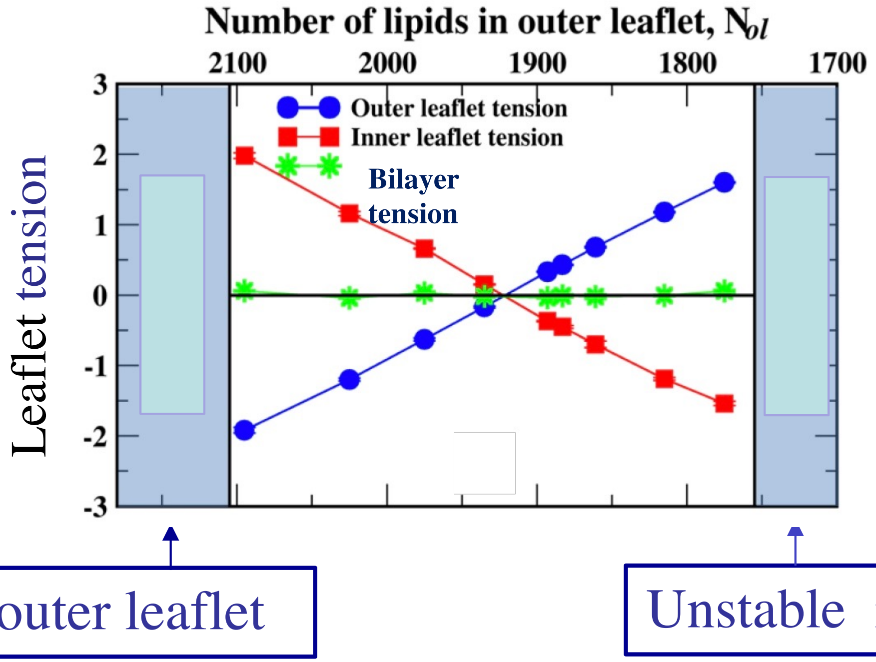
$\Rightarrow$ Lipid number asymmetry of reference state
depends on vesicle size

- In addition: different areas per lipid in outer and inner leaflet
- Outer leaflet more densely packed than inner leaflet

Stability of Assembled Bilayers

A. Sreekumari and RL, *Soft Matter* (2022)

- Reference states with tensionless leaflets = most stable states
- Stability of assembled bilayers in general?
- Stability regime bounded by two instability lines
- Nanovesicle with 19 nm diameter:



- Limited range of lipid numbers
- Limited range of leaflet tensions

Unstable outer leaflet

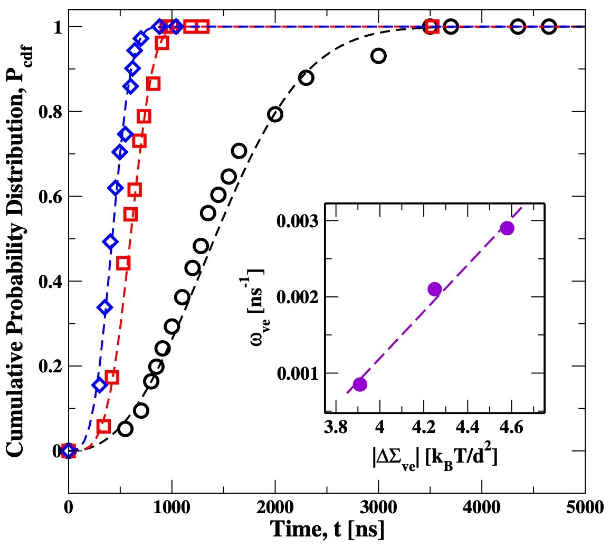
Unstable inner leaflet

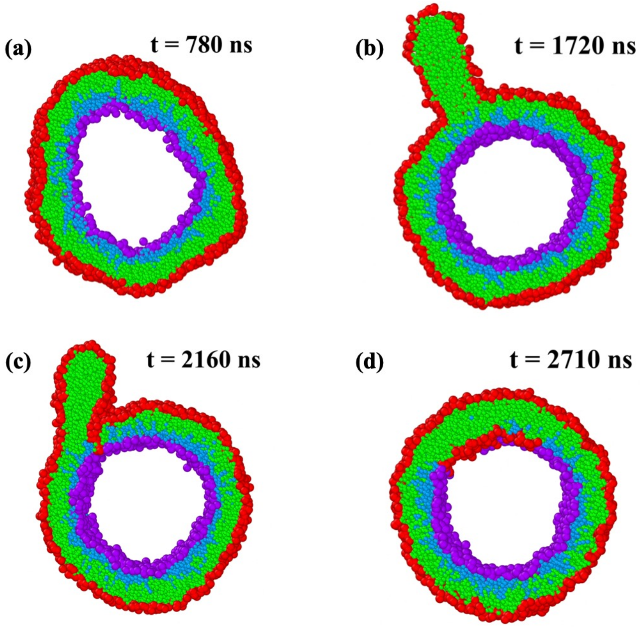
Instabilities of Assembled Bilayers

- Flip-flops of lipids between leaflets:

A. Sreekumari and RL, *Soft Matter* (2022)

- Structural instabilities:





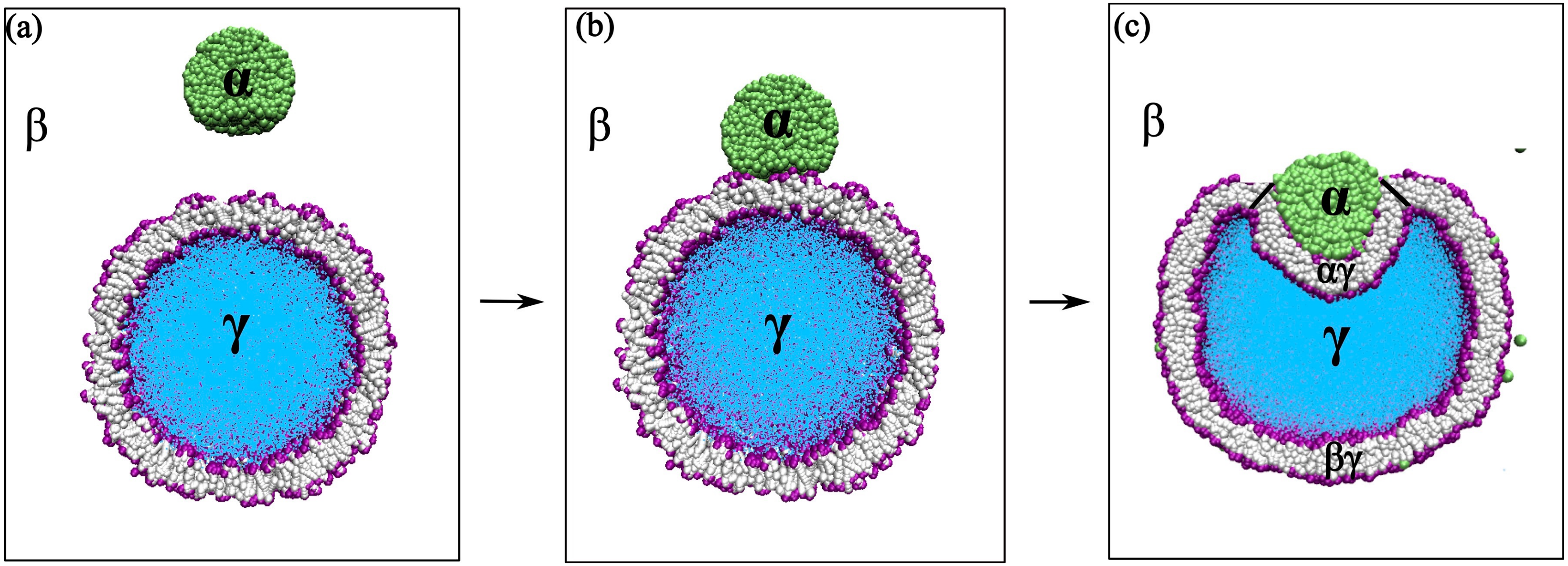
- Inset: Flip-flop rate controlled by leaflet tensions
- Sigmoidal shape implies ageing

- Self-healing process
- Restored bilayer with reduced leaflet tensions

Endocytosis of Droplets by NVs

R. Ghosh et al (*under revision*)

- Adhesion of liquid droplets to NVs:



- Liquid-liquid phase separation leads to α droplet within β phase
- Spreading -> partial engulfment -> complete engulfment -> …

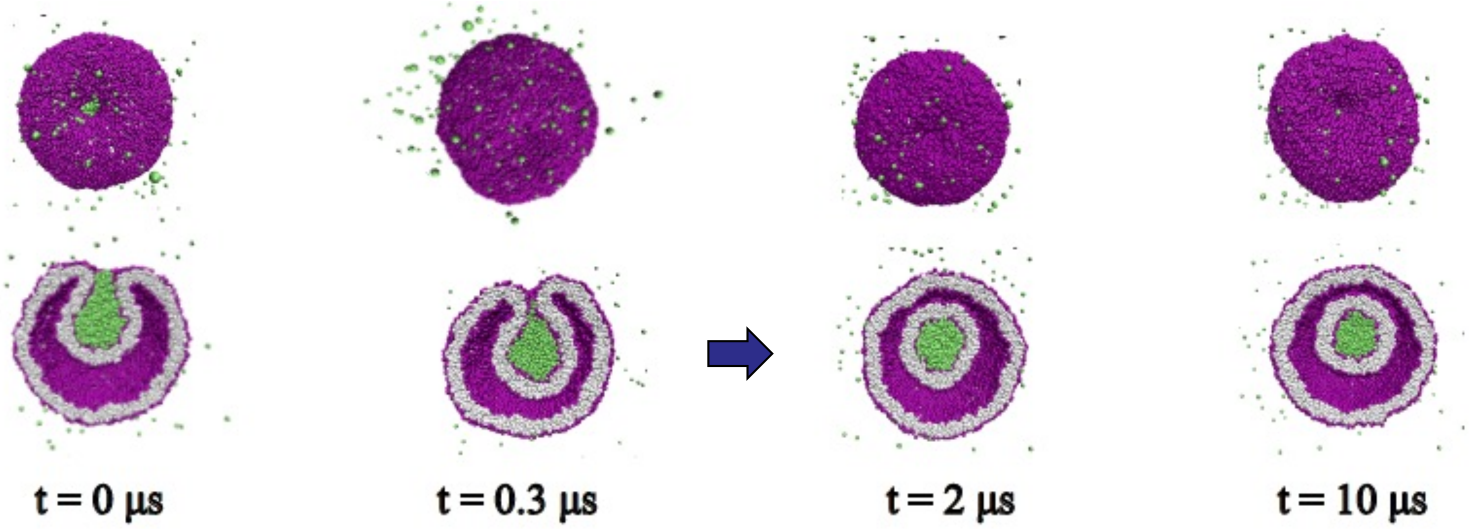
Pathway I: Complete Endocytosis

R. Ghosh et al (*under revision*)

- Complete axisymmetric engulfment of droplet followed by fission of membrane neck and division of nanovesicle into two nested daughter vesicles :

Top view:

Side view:



- Remodeling of membrane topology: One NV into two NVs

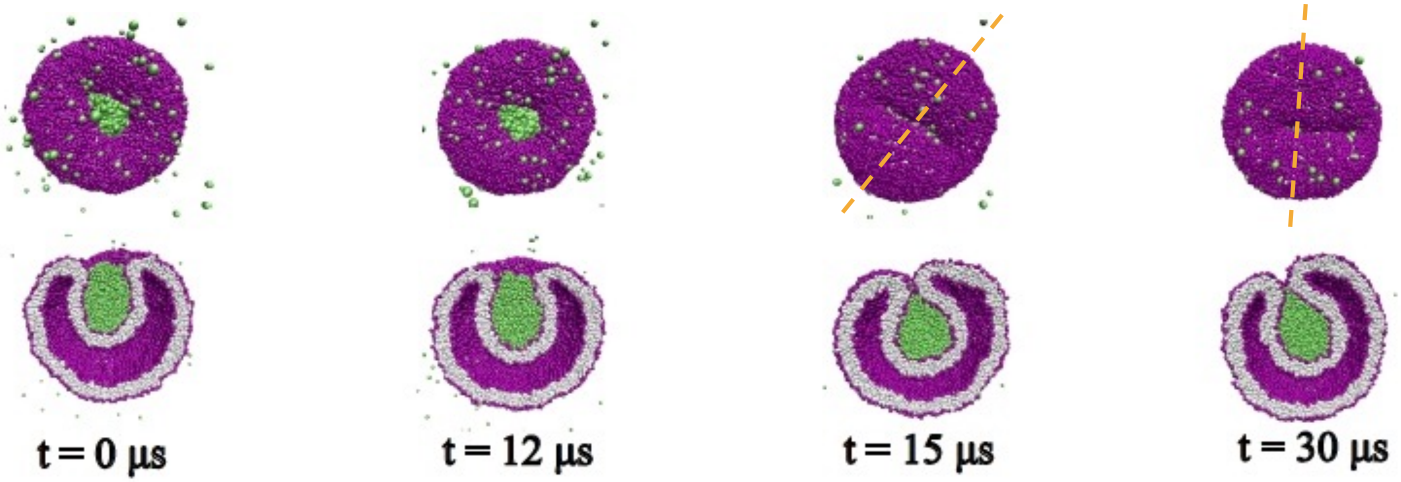
Pathway II: Incomplete Endocytosis

R. Ghosh et al (*under revision*)

- Complete non-axisymmetric engulfment of droplet leading to tight-lipped membrane neck which prevents neck fission and vesicle division:

Top view:

“Orthogonal“ side view:



- Pathway controlled by leaflet tensions plus line tension of contact line between NV membrane and droplet

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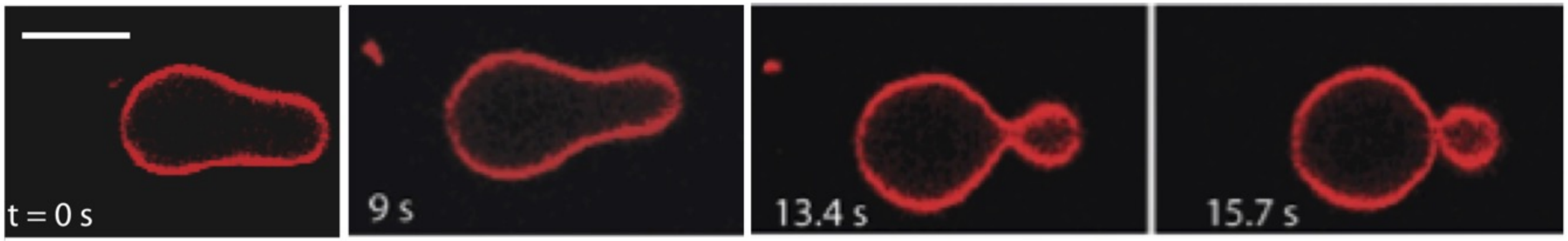
Budding of Giant Vesicles

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

T.ripta Bhatia et al, *Soft Matter* (2020)



Scale bar: 5 μm

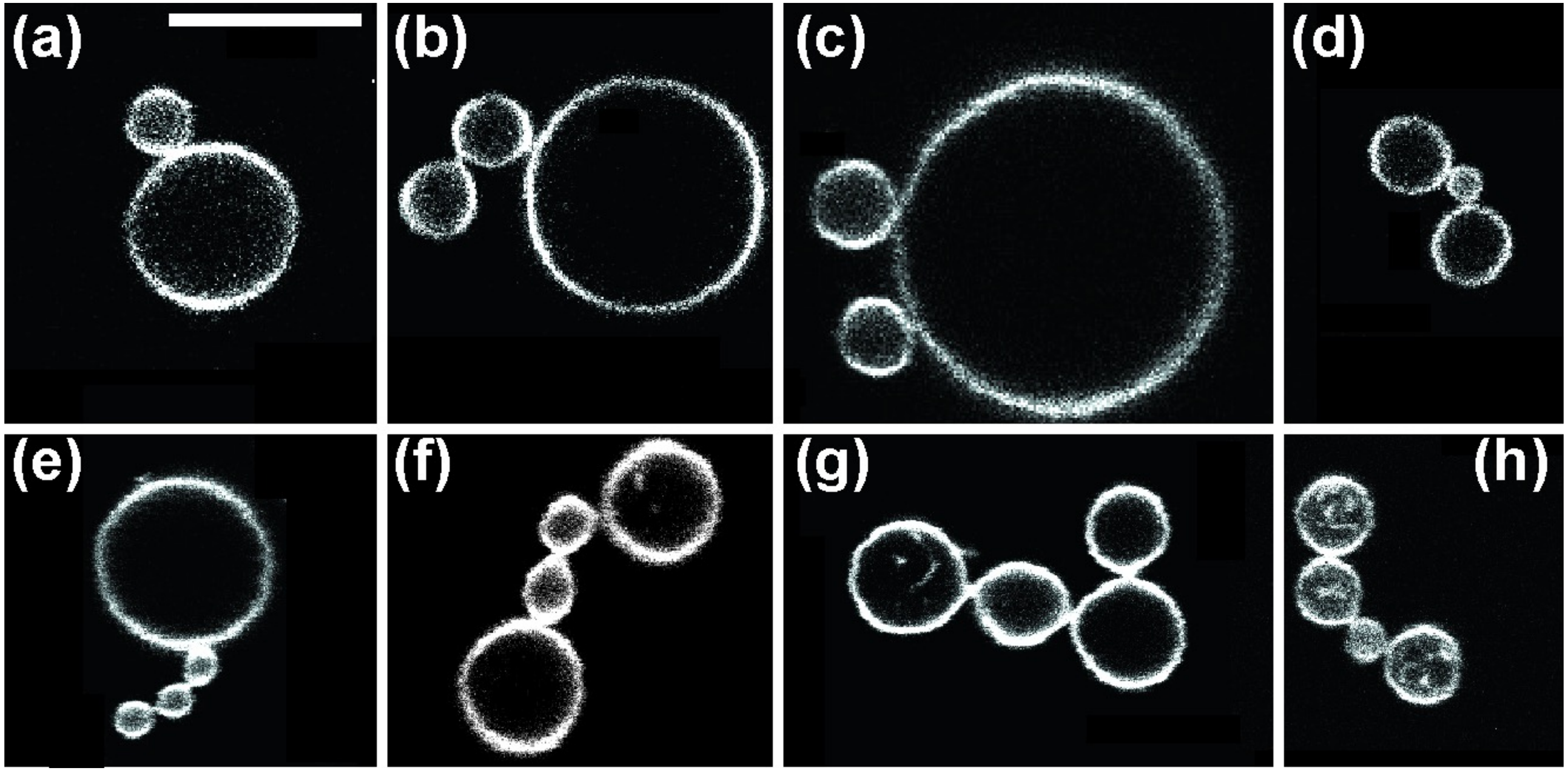
'Fluid worm hole in three dimensions'

- Membrane exposed to asymmetric sucrose/glucose solutions
- Membrane forms two spheres connected by a single neck
- Driven by transbilayer asymmetry = spontaneous curvature

Multispheres with Many Necks

Scale bar: 10 μm

T. Bhatia et al, *Soft Matter* (2020)



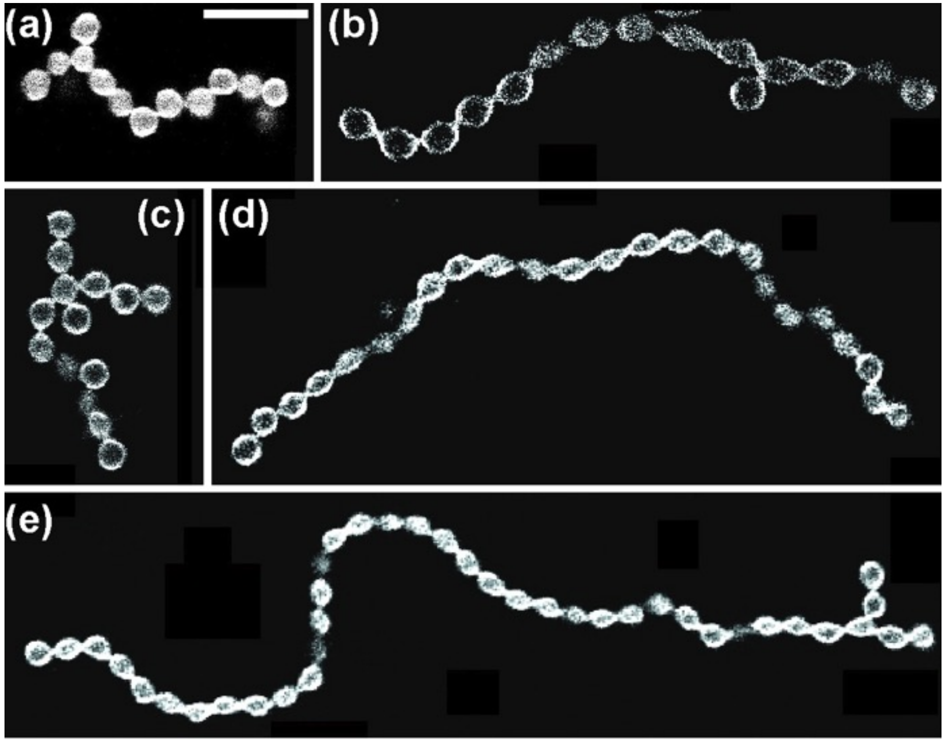
- One membrane forms several spheres connected by necks
- Each shape involves only two different sphere radii

Equally Sized Spheres

T. Bhatia et al, Soft Matter (2020)

- Multispheres consisting of N_* equally sized spheres:

$N_* = 14$
branched



$N_* = 15$
branched

$N_* = 15$
branched

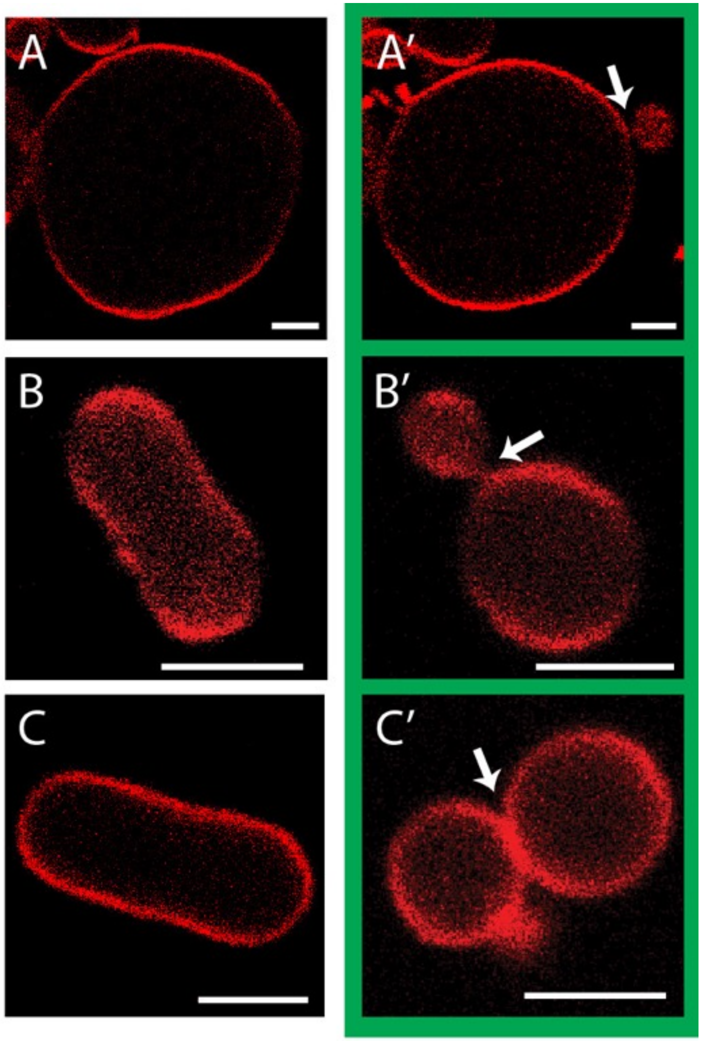
$N_* = 24$
linear

$N_* = 39$
branched

- Surprising mobility: linear ⇔ branched chains

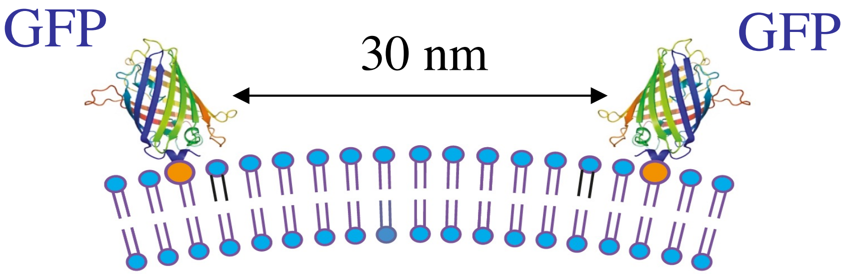
Controlled Budding of GUVs

J. Steinkühler et al, *Nature Comm* (2020)



No GFP + GFP

- Vesicles exposed to His-tagged GFP in the exterior solution
- GFP binds to anchor lipids in the vesicle membrane
- Spontaneous curvature fine-tuned by nanomolar concentration of GFP
- Low densities of membrane-bound GFP generate strongly curved membranes

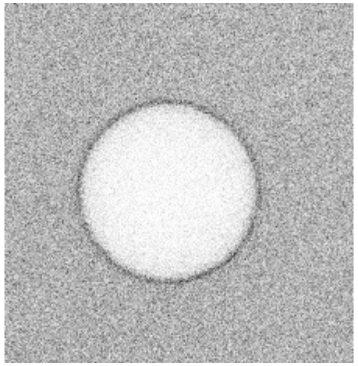


Neck Fission and Division of GUVs

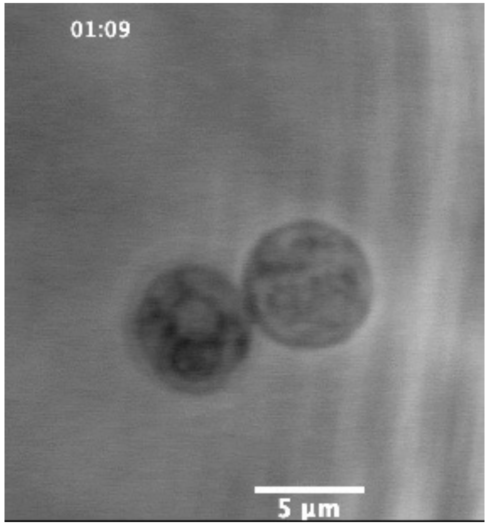
J. Steinkühler et al: *Nature Comm*. (2020)

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

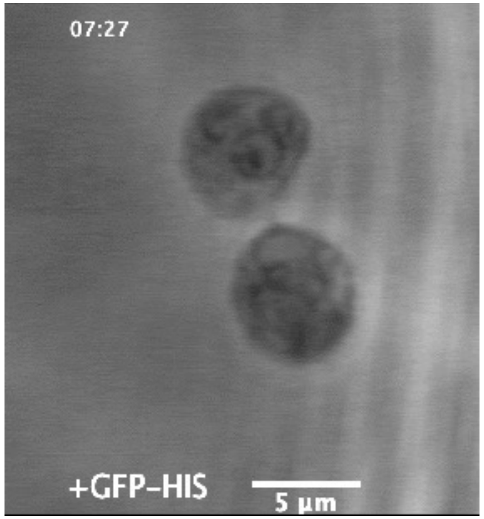
  Increase in GFP -> Neck cleavage -> Two daughter GUVs



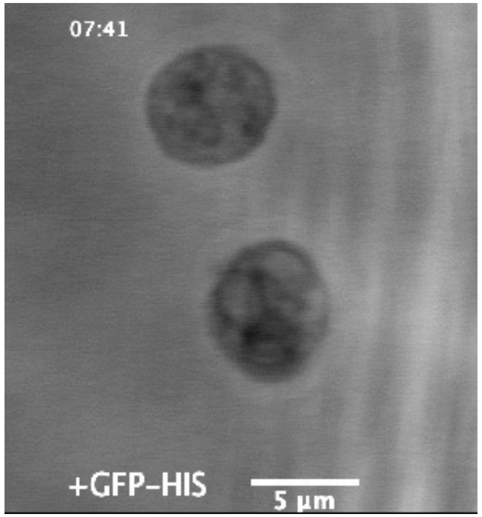
Adsorption of GFP onto GUV membrane



Deflation leads to dumbbell with membrane neck



Directly after neck cleavage

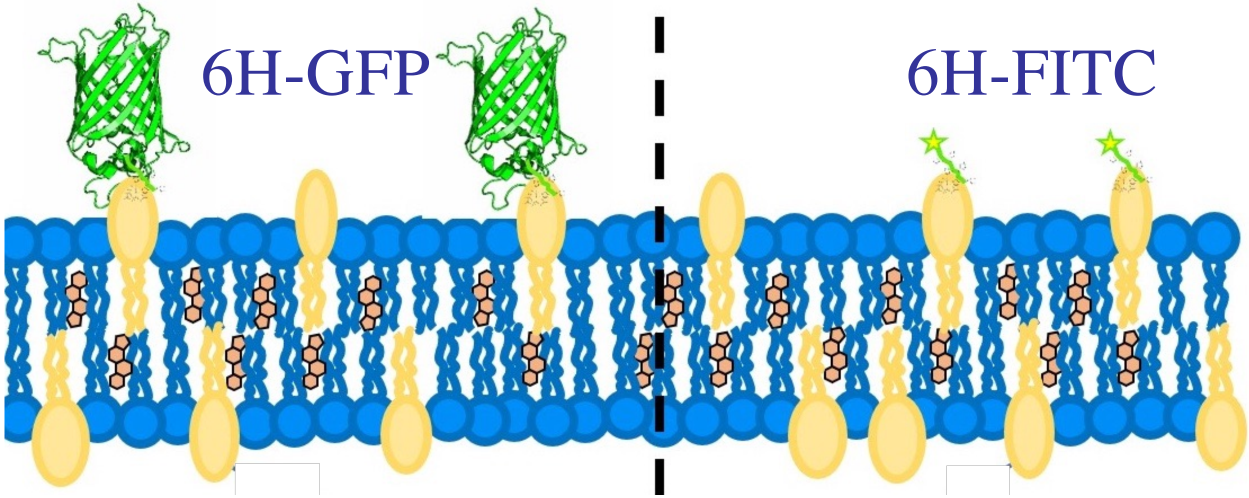


Complete division into two smaller GUVs

Binding of His-Tagged Fluorophores

Shreya Pramanik et al, *Soft Matter* (2022)

- His-tagged GFP generates large spontaneous curvature
- What about other His-tagged molecules?
- Tag of six histidines (6H) binding to NTA(Ni) anchor lipids
- Compare 6H-GFP, a large dye, with 6H-FITC, a small one



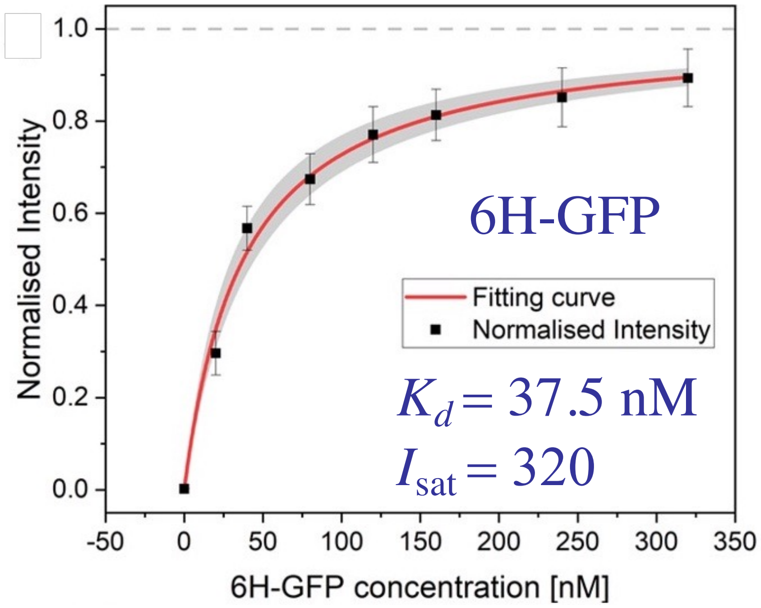
Fluorescence Intensity versus Concentration

Pramanik et al, *Soft Matter* (2022); Poster 75

- Fluorescence intensity I versus molar concentration X
- Binding of at most one 6H-dye to each anchor lipid implies

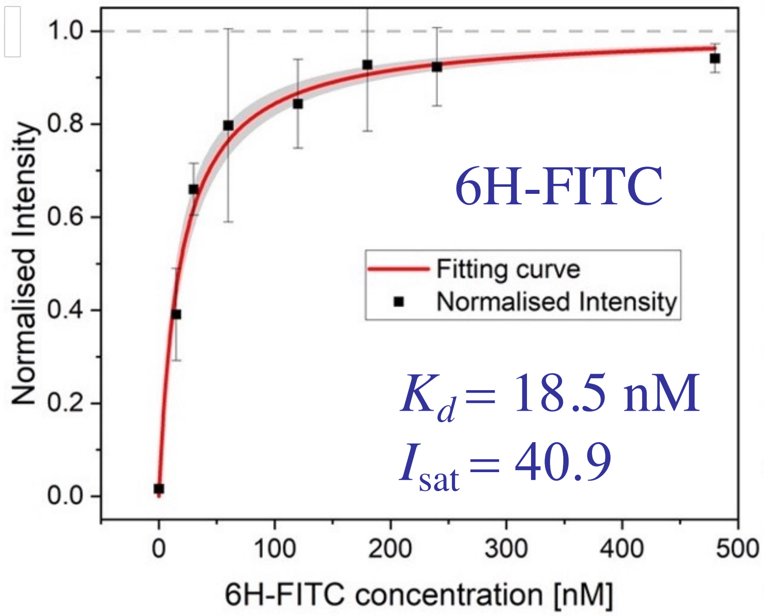
$$I = I_{\mathrm{sat}}\, X / (K_d + X)$$

Dissociation equilibrium constant K_d and saturation intensity I_{sat}


6H-GFP

$K_d = 37.5$ nM

$I_{\mathrm{sat}} = 320$


6H-FITC

$K_d = 18.5$ nM

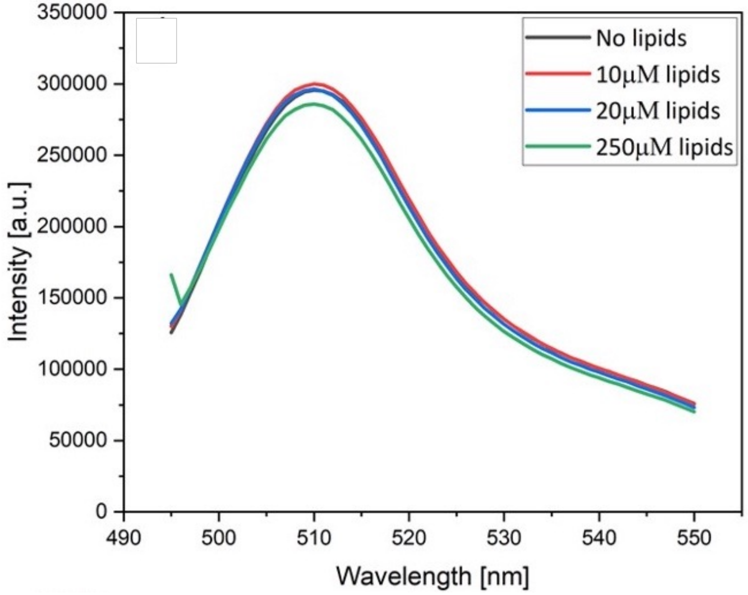
$I_{\mathrm{sat}} = 40.9$

Binding Affinity and Quenching

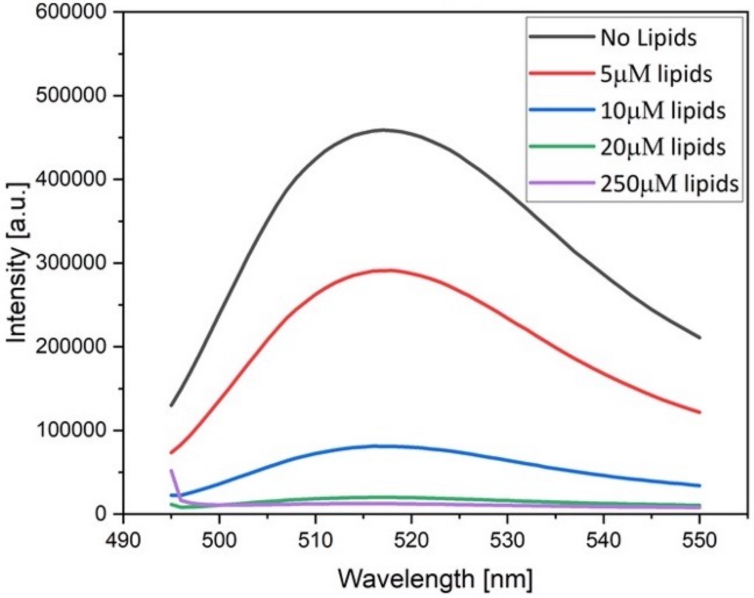
Pramanik et al, *Soft Matter* (2022); Poster 75

- Smaller K_d of 6H-FITC implies increased binding affinity
- Smaller I_{sat} implies reduced brightness of bound 6H-FITC
- Both conclusions can be reconciled by fluorescence quenching
- Confirmed by independent measurements:

6H-GFP



6H-FITC



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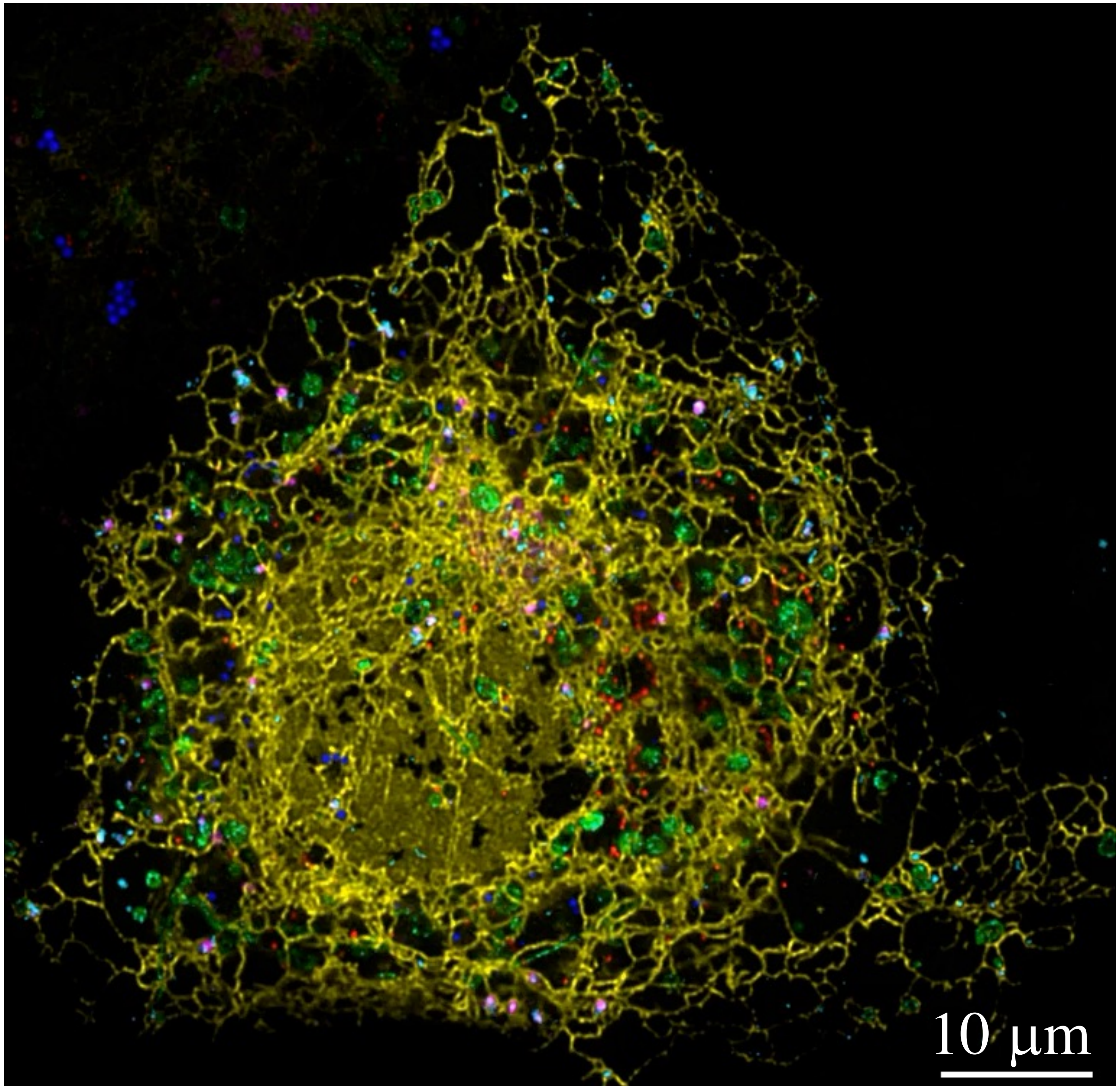
Morphological Complexity of ER

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 !

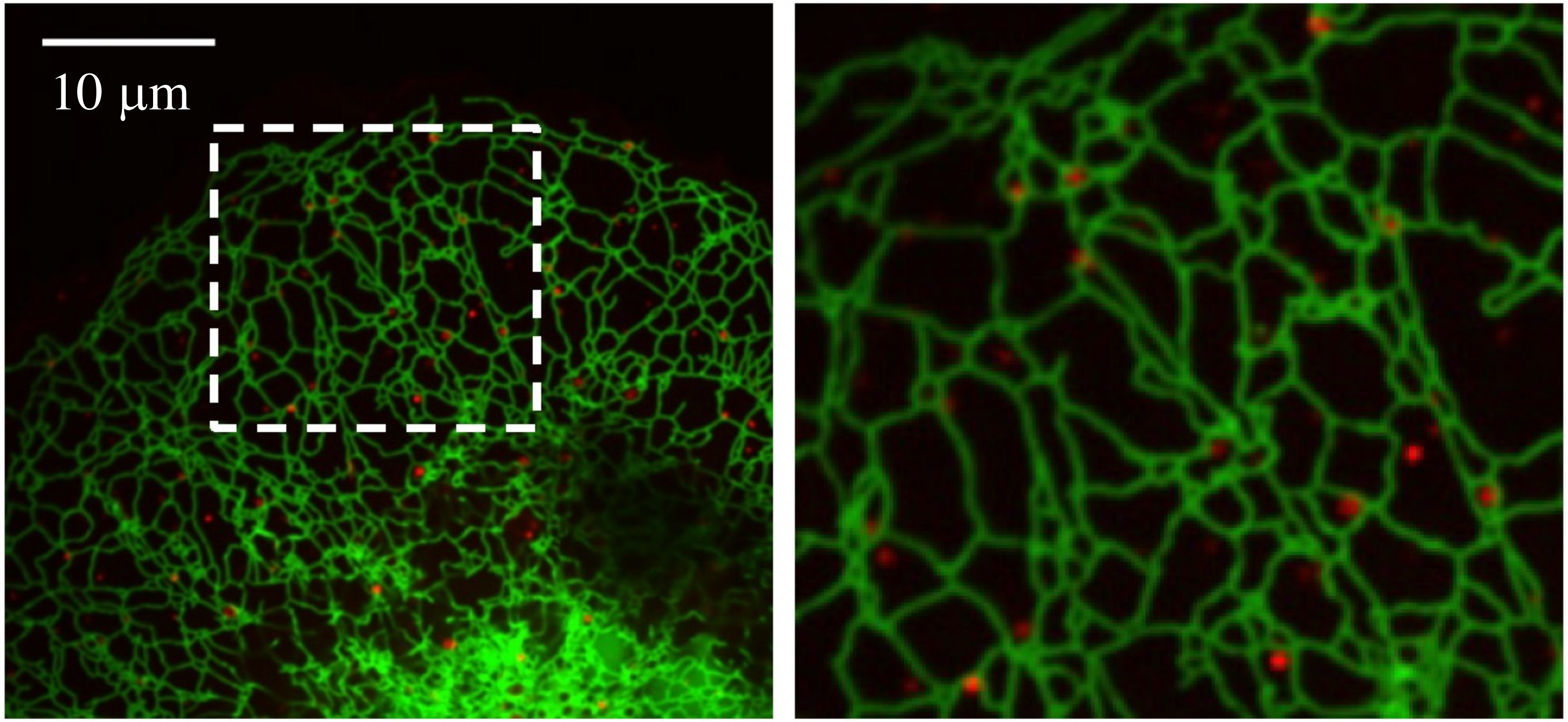
Multiscale Morphology of ER

Hierarchy of four length scales:

- Thickness of ER bilayer ~ 4 nm
- Diameter of nanotubes ~ 80 nm
- Mesh size of polygons ~ 1 μm
- Extension of reticular network ~ 80 μm

Reticular Networks, In Vivo

- Membrane nanotubes connected by junctions

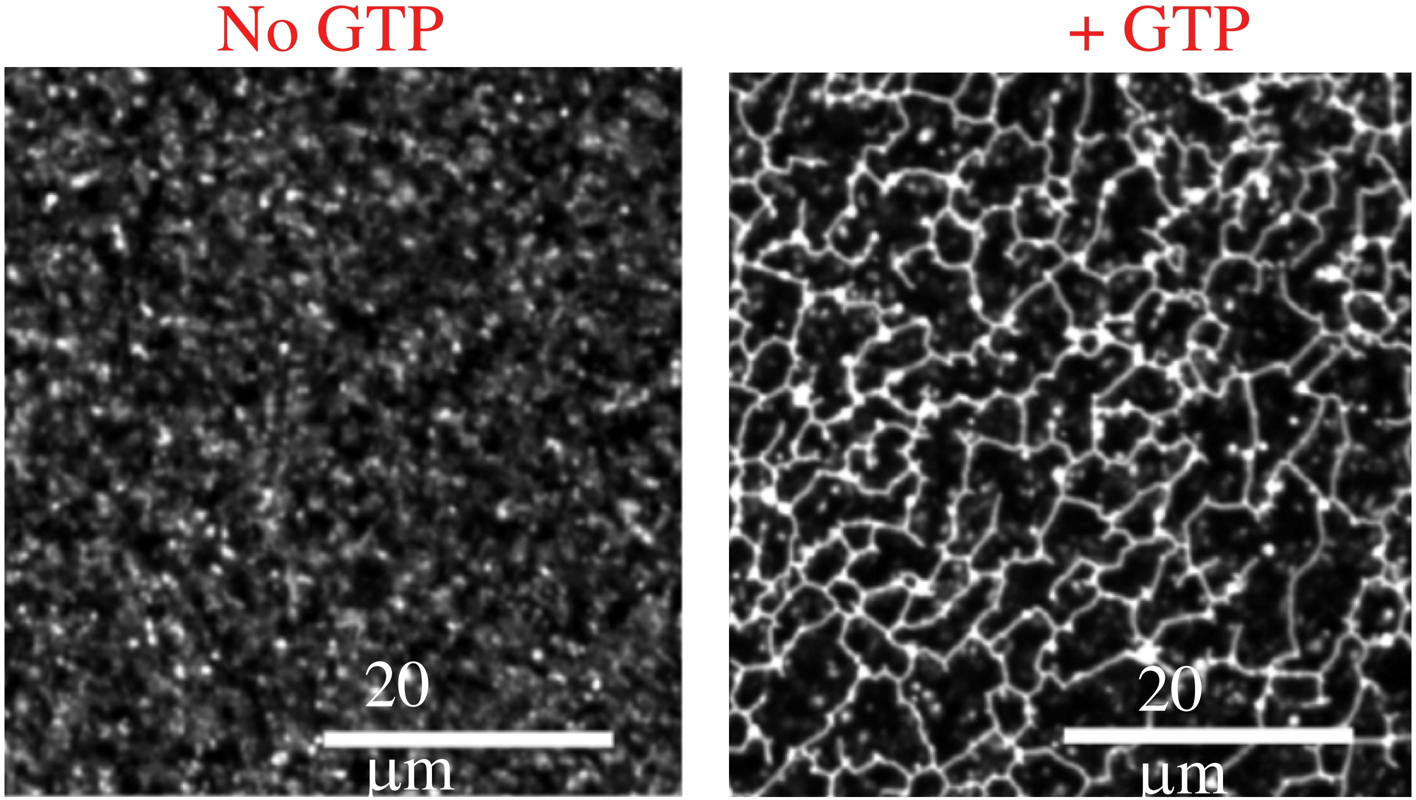


Friedmann, et al , *Mol Biol Cell* (2013)

- Primarily three-way junctions, at which three tubules meet
- Irregular polygons with angles of 120°

Reticular Networks, In Vitro

Powers et al, *Nature* (2017)



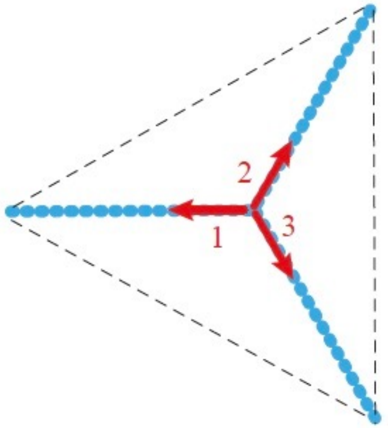
- Left: Proteoliposomes with membrane GTPase
- Right: Network formation after addition of GTP
- No cytoskeletal components, only membranes !

Junctions and Membrane Tension

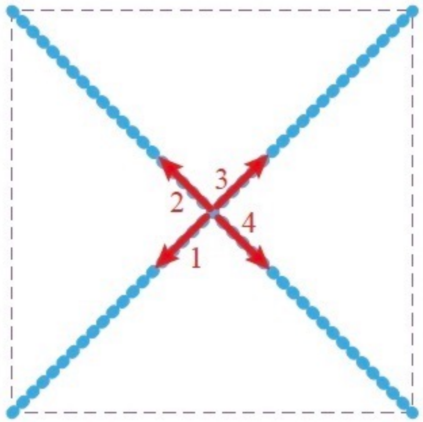
RL, *Adv. Colloid Interface Sci*. (2022)

- Prevalence of three-way junctions observed in vivo since the 1980s
- But no explanation in the available ER literature

- Each three-way junction formed by three fluid nanotubes
- Force balance at a stationary three-way junction implies that all tubes experience the same membrane tension and form contact angles of 120°:



three-way

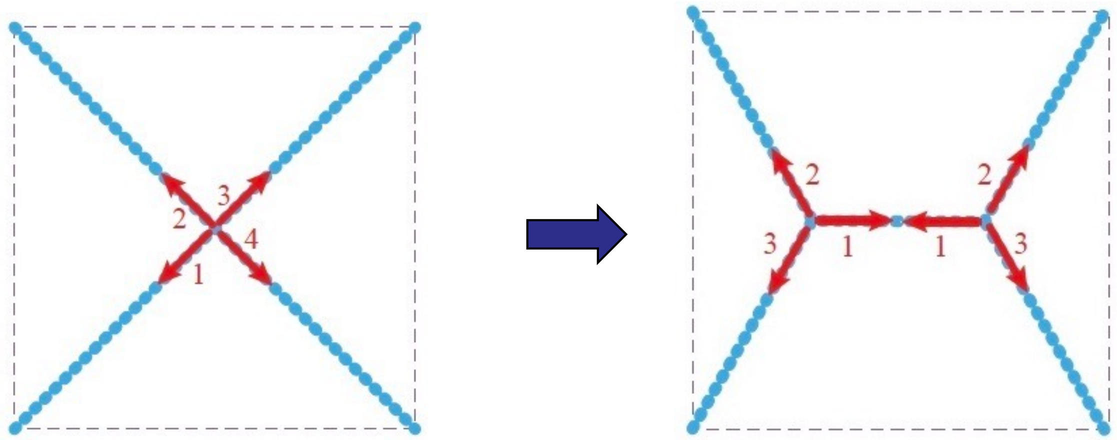


four-way

- Likewise, a stationary four-way junction between four nanotubes would lead to contact angles of 90°

Prevalence of Three-Way Junctions

- However, the total tube length can be reduced by transforming the four-way junction into two three-way junctions



This transformation is possible because the ER membrane is fluid

- Network of two three-way junctions represents a simple example of a Steiner minimal tree as studied in mathematical graph theory
- Conclusion: Significant membrane tension favors three-way junctions
- But how is this tension generated? => new project of Shreya Pramanik

Coworkers

Experiment


Rumiana
Dimova


Tripta
Bhatia


Jan
Steinkühler


Ziliang
Zhao


Shreya
Pramanik

Theory


Simon
Christ

Simulation


Andrea
Grafmüller


Markus
Miettinen


Rikhia
Ghosh


Vahid
Satarifard


Aparna
Sreekumari

Collaborations with the labs of:
Joachim Spatz, Seraphine Wegner, Petra Schwille, Anna Shnyrova