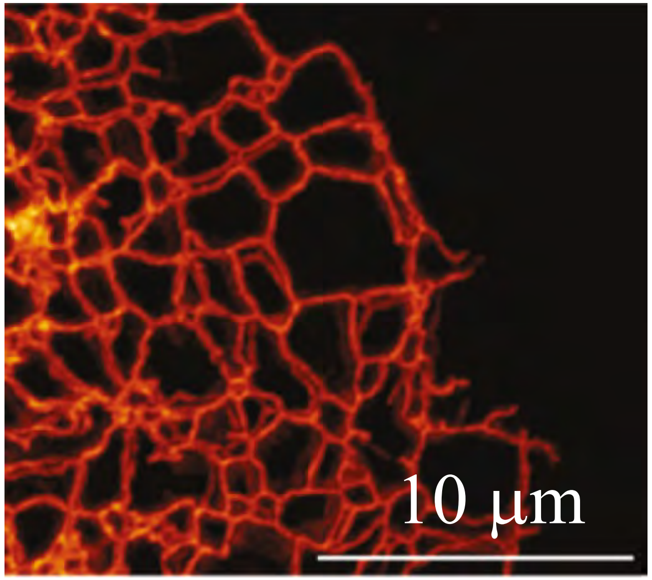
Fascinating Membrane Architecture of the Endoplasmic Reticulum (ER)

Reinhard Lipowsky
MPI of Colloids and Interfaces, Potsdam, Germany

- Introduction to ER
- Molecules of ER Membrane
- Membrane Nanotubes
- Three-Way Junctions
- Coupling to GTP-Hydrolysis
- Perspectives

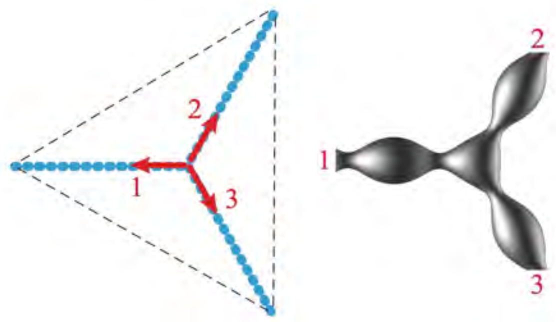


Coworkers

Shreya Pramanik
Rumiana Dimova

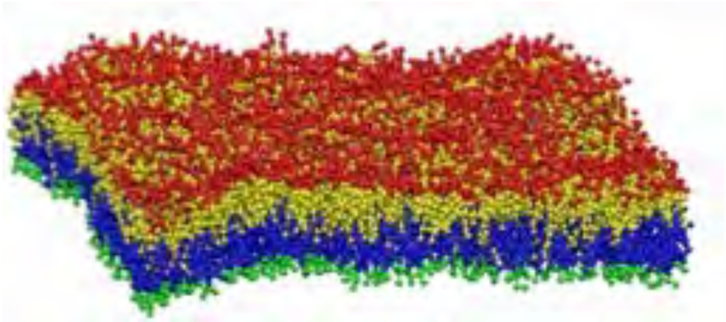
Amelie Benk
Miroslaw Tarnawski
Joachim Spatz

RL, S. Pramanik, A. S. Benk, M. Tarnawski, J. P. Spatz, R. Dimova:
Elucidating the Morphology of the ER: Puzzles and Perspectives.
ACS Nano 17 (June 2023)

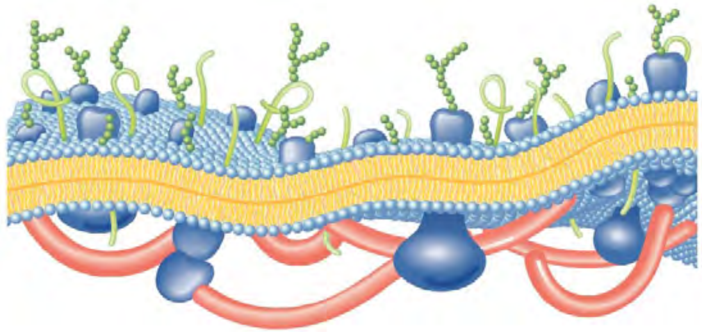


Fluid Architecture of Biomembranes

• Lipid bilayer, two leaflets

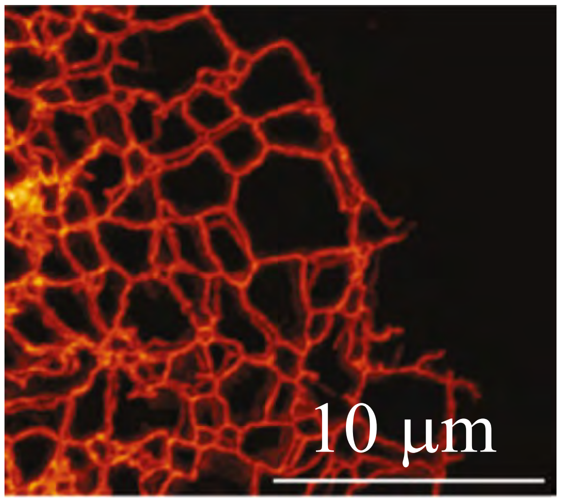


4 nm

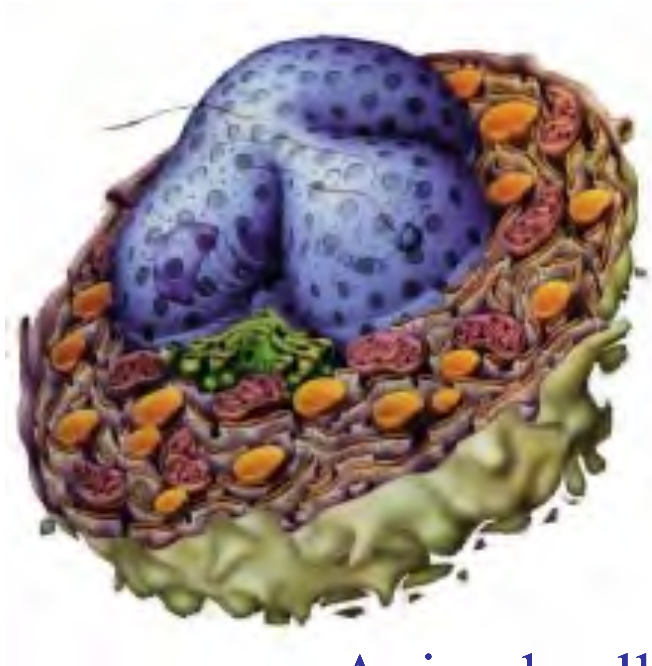


• Biological membrane

Lippincott-Schwartz (2022)



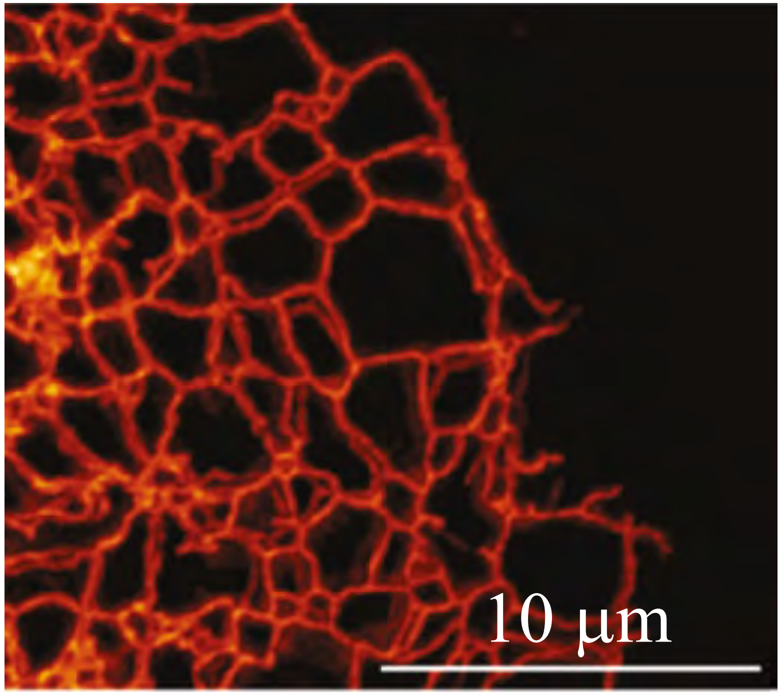
• Endoplasmic reticulum



100 μm

• Animal cell

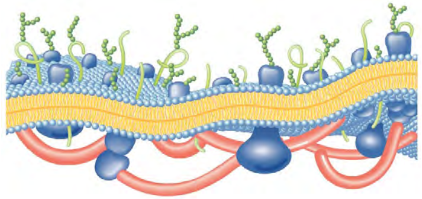
Basic Aspects of ER



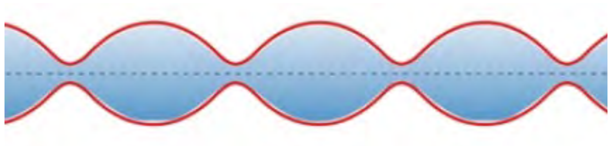
- Membrane-enclosed organelle
- Each eukaryotic cell contains only one ER
- Network of membrane nanotubes
- Tubes have a width of 50 to 100 nm
- Tubes connected by junctions
- Tubes form irregular polygons
- Average size of polygons ~ 1 μm
- Cell-sized network, extension of ~ 80 μm
- Network formed by a single membrane !
- Network is truly three-dimensional

Multiscale Architecture of ER Membrane

- Structure of ER, bottom-up:

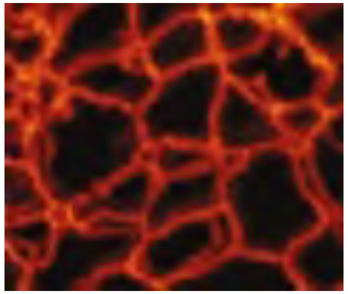


1 Thickness of ER lipid bilayer: 4 - 5 nm



2 Width of nanotubes: 50 – 100 nm

3 Mesh size of network: 0.5 - 2 μm

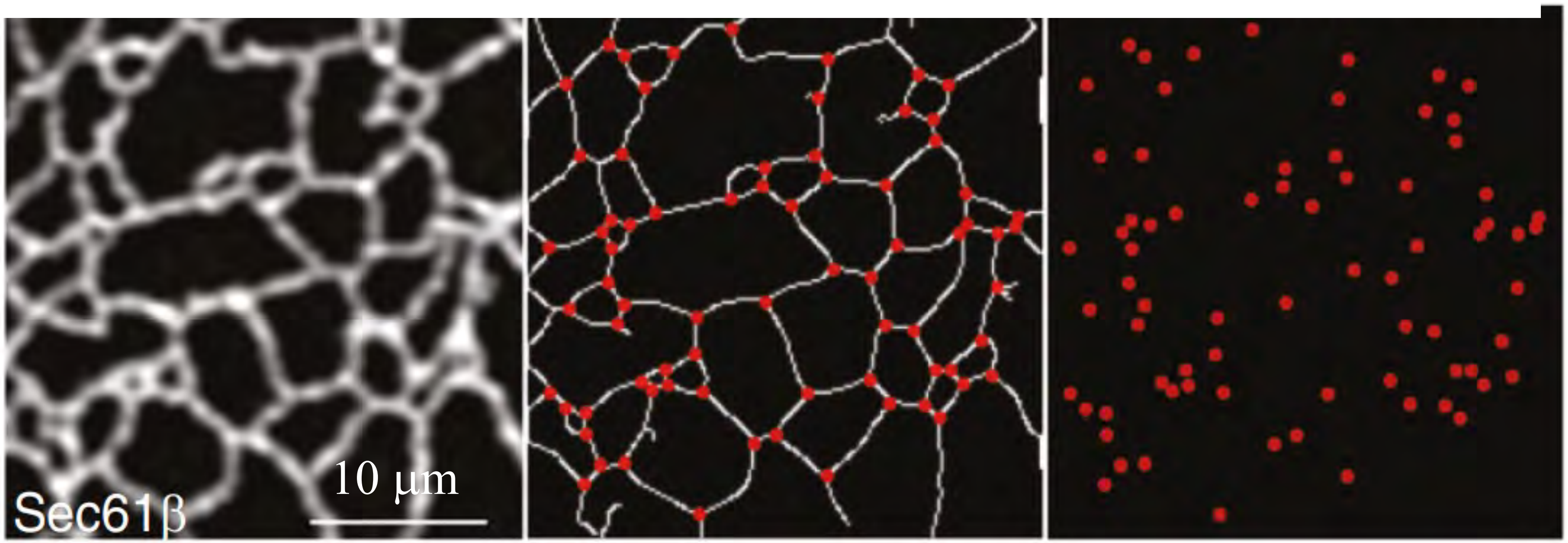


4 Extension of tubular network: 50 – 100 μm

- ER involves hierarchy of four distinct length scales that vary over 4 – 5 orders of magnitude !

Three-Way Junctions, In Vivo

Obara et al , *Cold Spring* (2023)

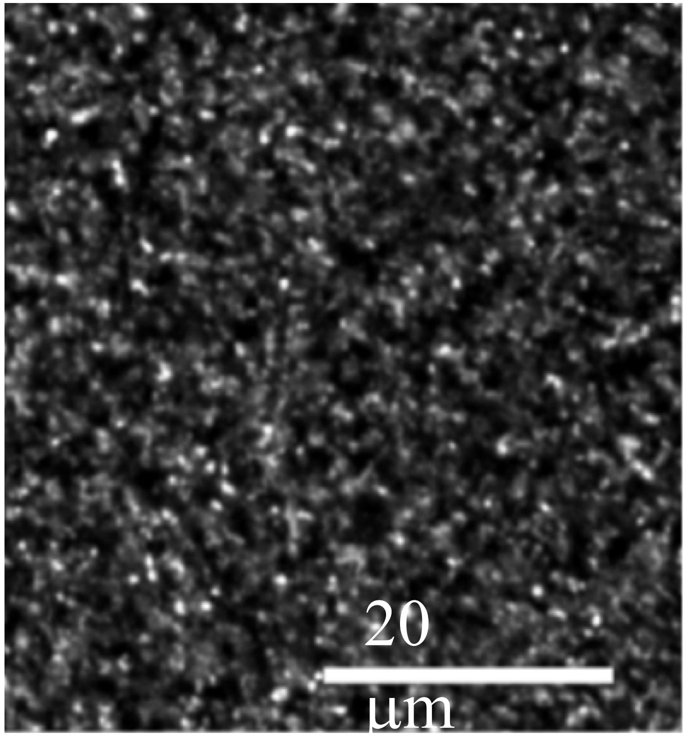


- Three-way junctions, at which three nanotubes meet
- Tubules form irregular polygons with angles of ~ 120^{o}

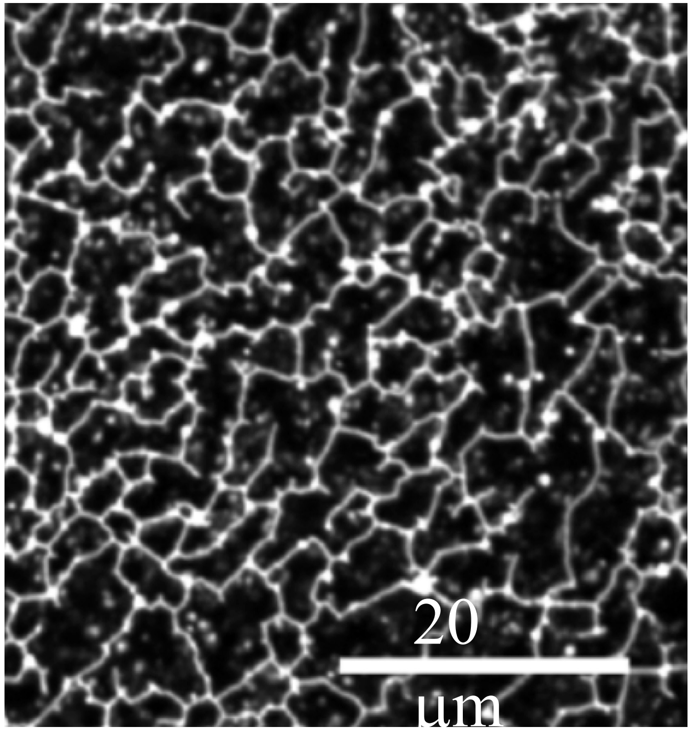
Three-Way Junction, In Vitro

Powers, … , Rapoport, *Nature* (2017)

No GTP



+ GTP



- Left: Proteoliposomes with membrane GTPase
- Right: Network formation after addition of GTP
- Three-way junctions and polygons with angles of ~ 120^o

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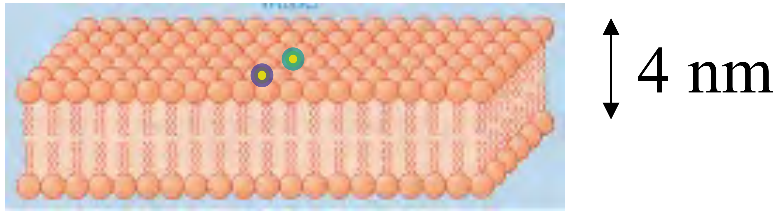
Lipid Bilayer + Membrane Proteins



• Lipid bilayers in their fluid state

• Membrane proteins for curvature generation

• Membrane proteins for GTP hydrolysis (Membrane GTPase)

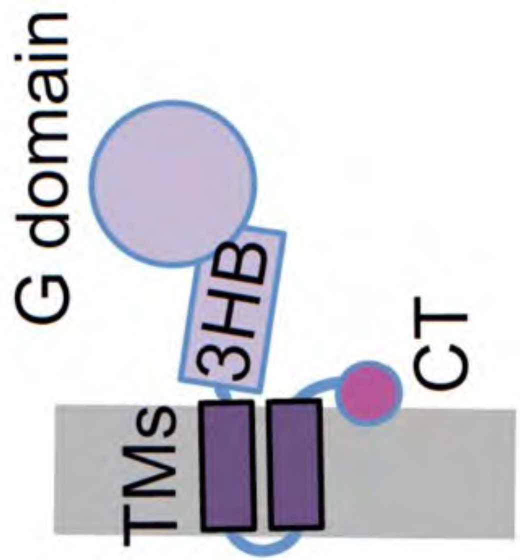
• In-vitro reconstitution often uses pairs of membrane proteins

• Special membrane protein atlastin:

  - G domain for GTP hydrolysis

  - Bilayer asymmetry from oriented proteins

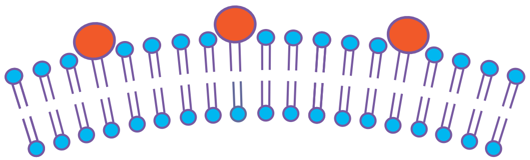
• In-vitro reconstitution of tubular networks using lipid bilayers with atlastin only



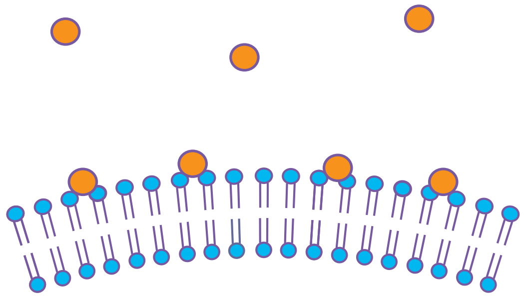
Bilayer Asymmetry and Spont Curvature

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

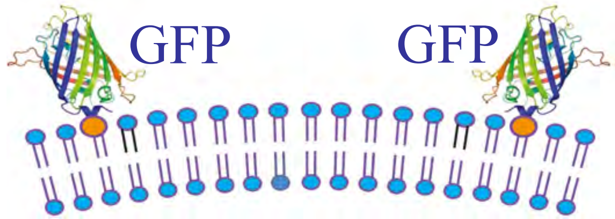
- Different molecular mechanisms for bilayer asymmetry:



Asymmetric
composition,
e.g., ganglioside



Asymmetric
adsorption of
small molecules

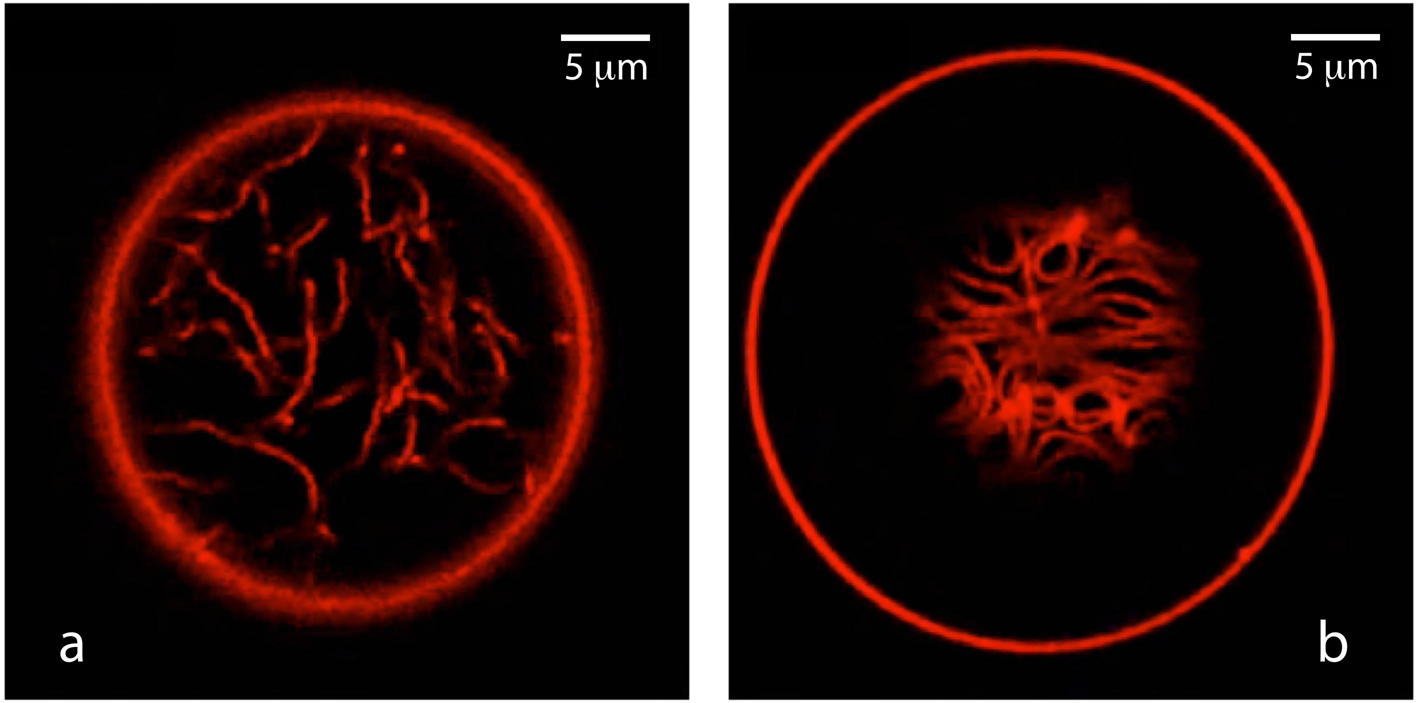


Asymmetric binding
of proteins,
dilute coverage

Jan Steinkühler et al,
Nature Comm (2020)

Membrane Nanotubes

- GUVs engulfing two condensate droplets
- Membrane segment exposed to PEG-rich condensate droplet forms many nanotubes with a diameter of about 100 nm
- Different patterns of nanotubes:

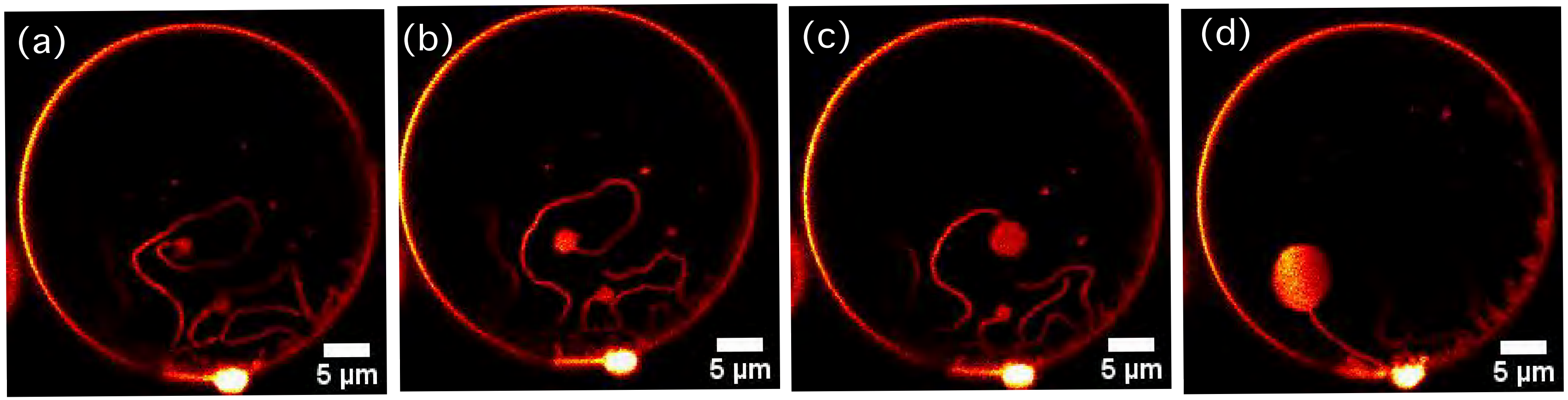


Yonggang Liu et al, *ACS Nano 10* (2016)

From Tubes to Sheets and Back

Ziliang Zhao, Vahid Satarifard, RL, Rumiana Dimova (unpublished)

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



Tube transformed into sheet, starting from inner tube end;
Inverse transformation from sheet to tube also observed

Multispherical Tubes

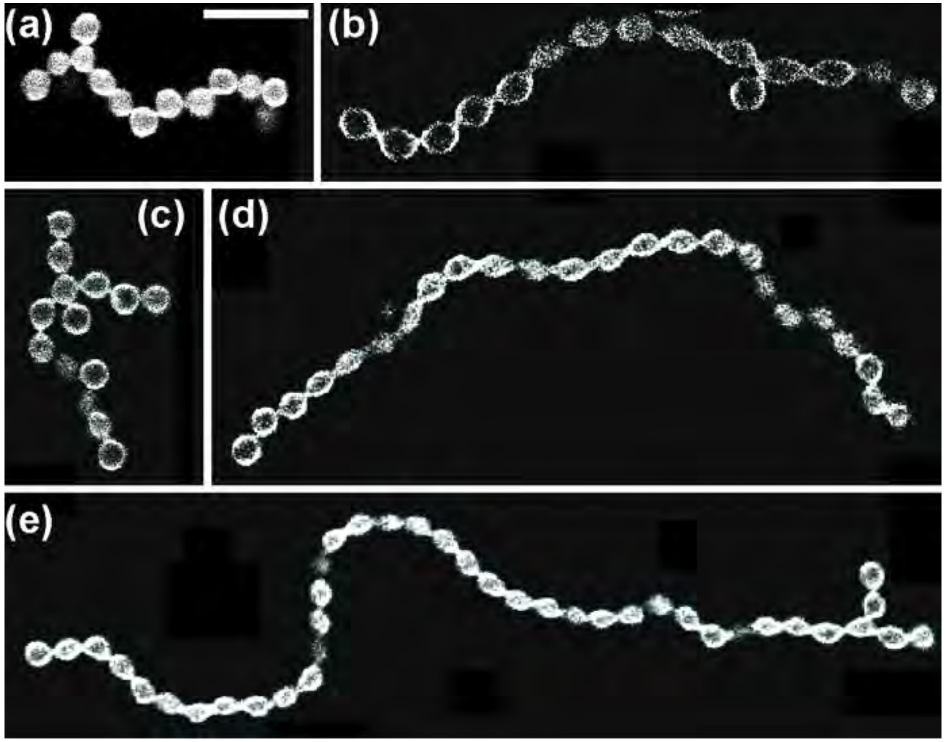
Tripta Bhatia et al, *Soft Matter* (2020)

- Tubes consisting of N_* equally sized spheres:

$N_* = 14$
branched

$N_* = 15$
branched

$N_* = 39$
branched



$N_* = 15$
branched

$N_* = 24$
linear

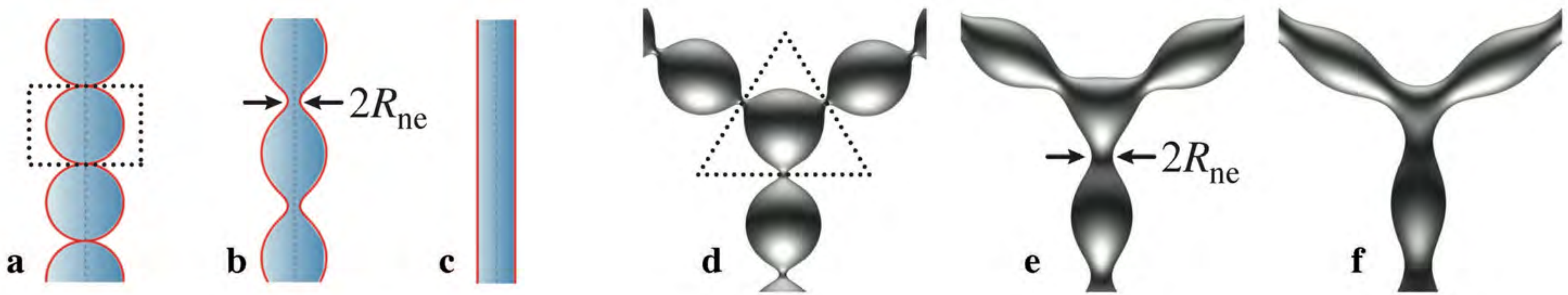
- Branched chains ⇔ Multispherical junctions

Unduloids and Triunduloids

RL et al, *ACS Nano 17* (June 2023)

- Differential geometry: Shapes with constant mean curvature M
- Shapes have minimal bending energy for M = spont curvature m

Unduloids: Triunduloids:



Große-Brauckmann et al, *Visualization and Mathematics* (1997)

- Open and closed membrane necks
- Multispherical shapes in (a) and (d) with closed necks
- Triunduloids provide models for three-way junctions

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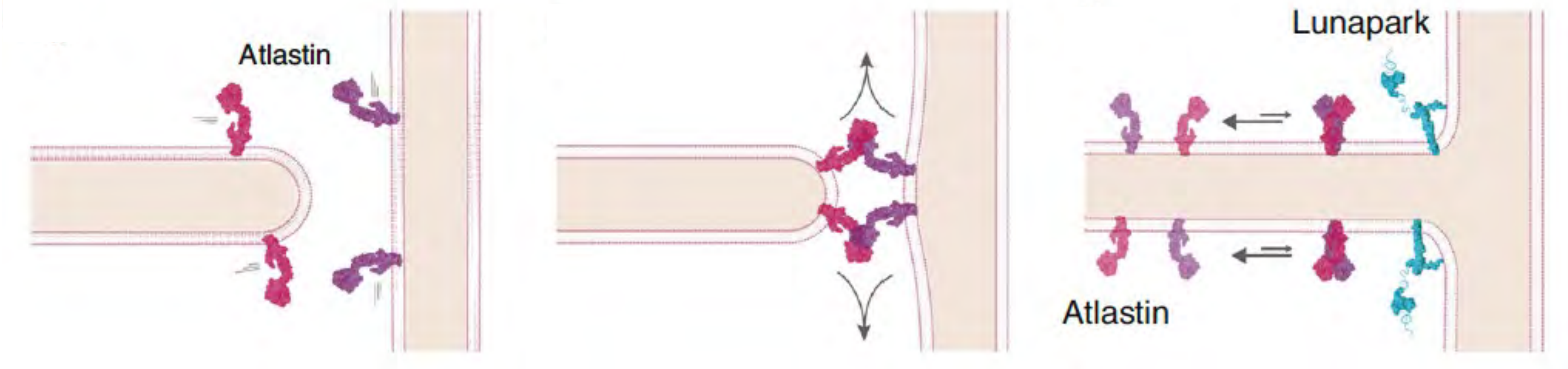
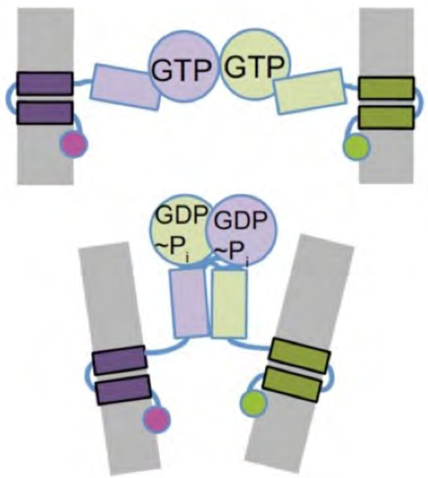
Formation of Junctions

• Trans-dimerization of atlastin anchored to different membranes:



Obara et al , *Cold Spring* (2023)

• Blow-up:
Dimerization coupled
to GTP-hydrolysis



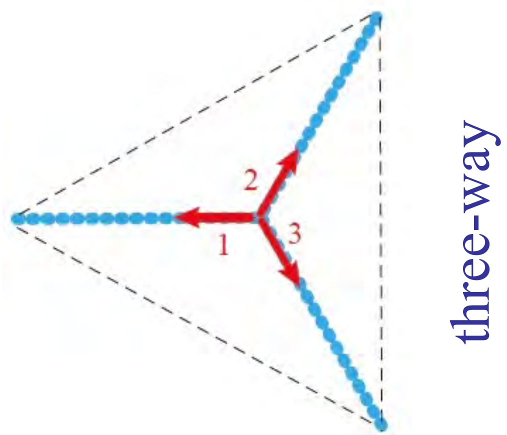
Liu et al, *PNAS* (2015)

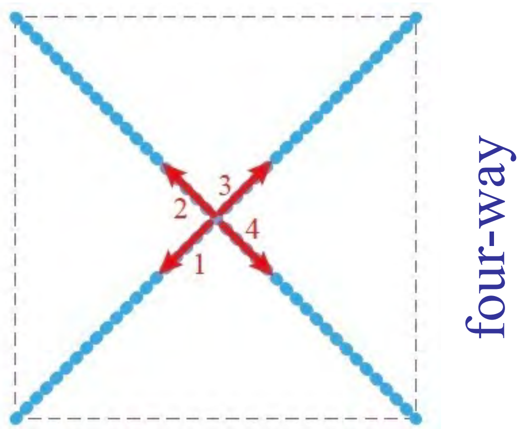
• Membrane fusion

Three-Way Junctions, Force Balance

RL et al, *ACS Nano 17* (June 2023)

- Prevalence of three-way junctions observed in vivo since the 1980s
- But no explanation provided in the 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° :

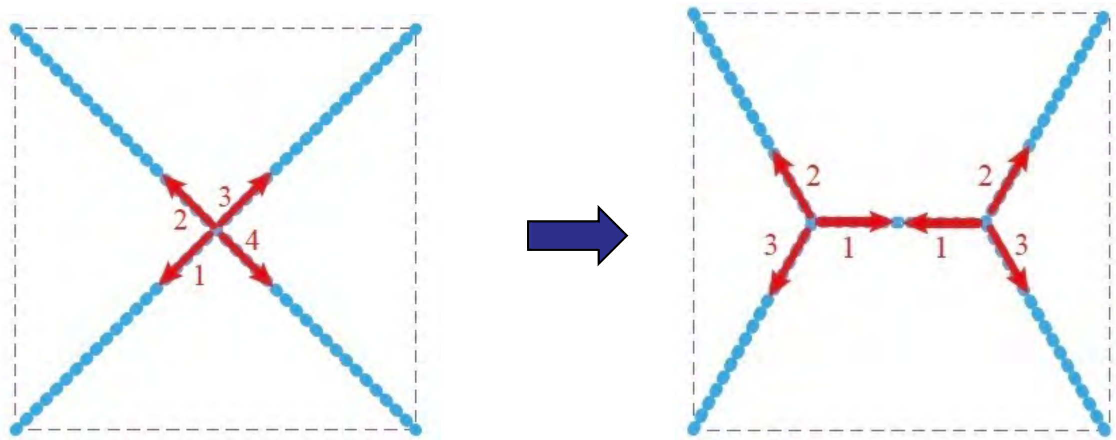




- Likewise, a stationary four-way junction between four nanotubes would lead to contact angles of 90°

Three-Way Junctions and Tension

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



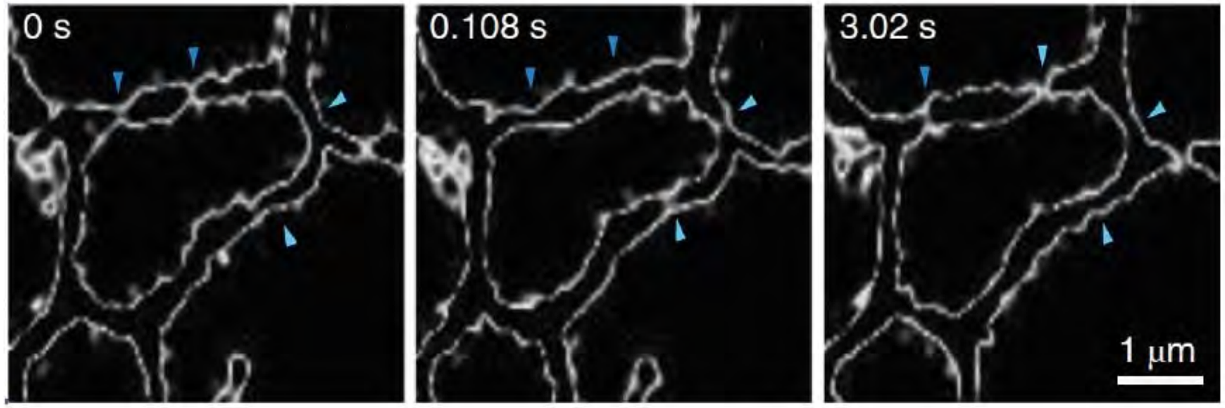
This transformation is possible because the ER membrane is fluid

- 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? And how can it be measured?

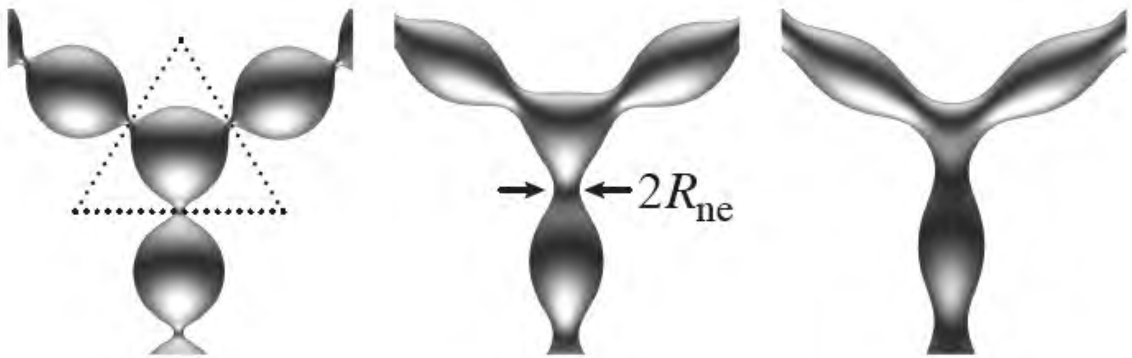
Shape of Three-Way Junctions

Holcman et al, *Nature Cell Biology* (2018)

- Time lapse movie:
- Closure and opening of membrane necks, blue arrow-heads



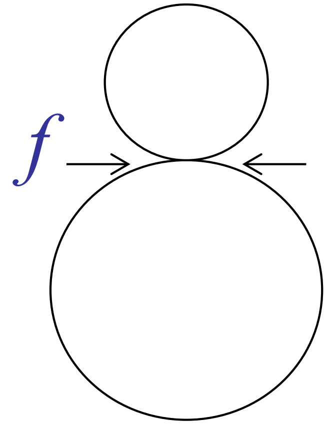
- Single junction ~ fluctuating triunduloid !



- Closed membrane necks are prone to undergo membrane fission!

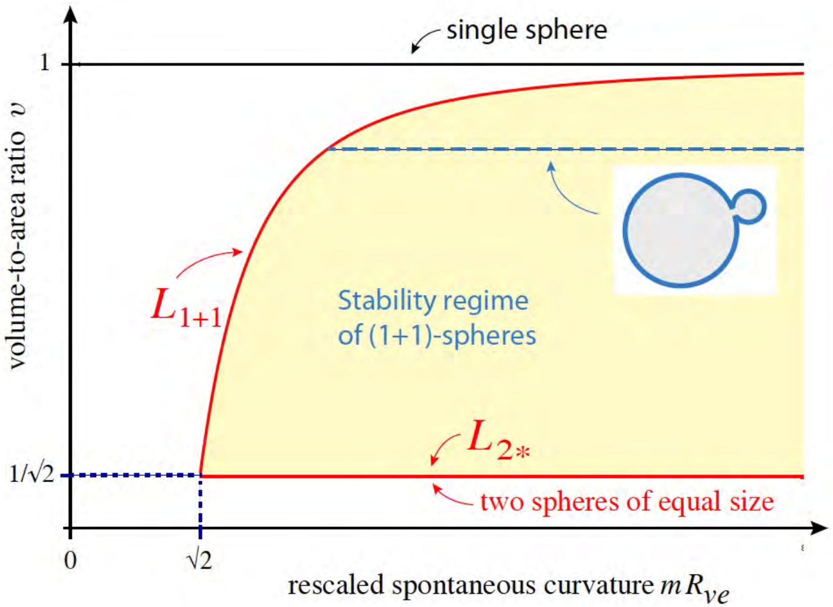
Constriction Force at Membrane Neck

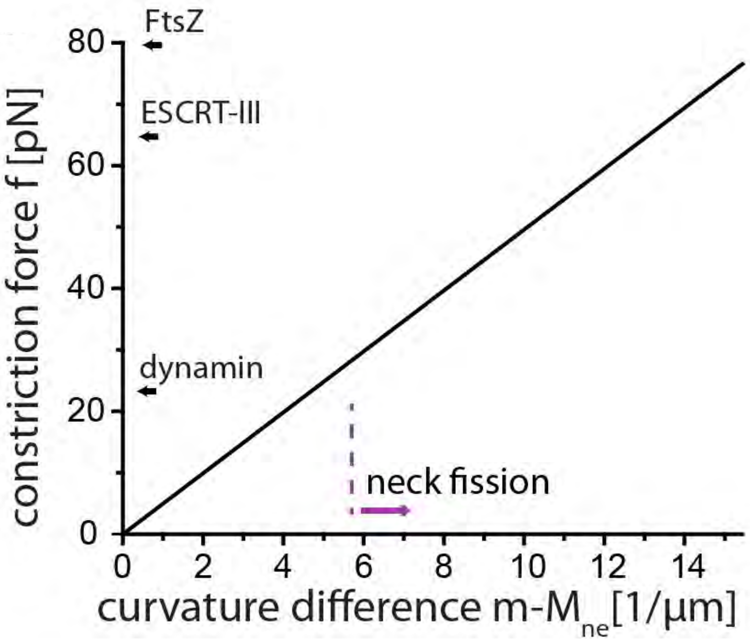
RL, *Advances in Biomembranes and Lipid Selfassembly* (2019)



- Spontaneous curvature m generates constriction force $f = 8\pi\,\kappa\,(\,m - M_{ne}\,)$ when m exceeds the neck curvature M_{ne}

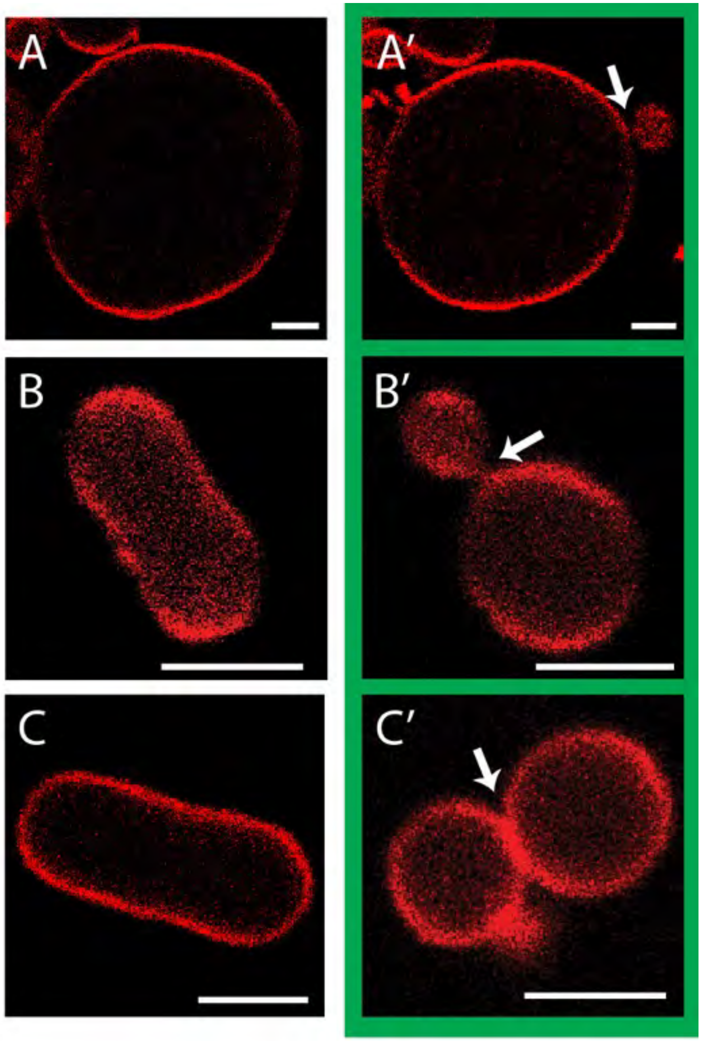
- Force increases with increasing spont curvature:





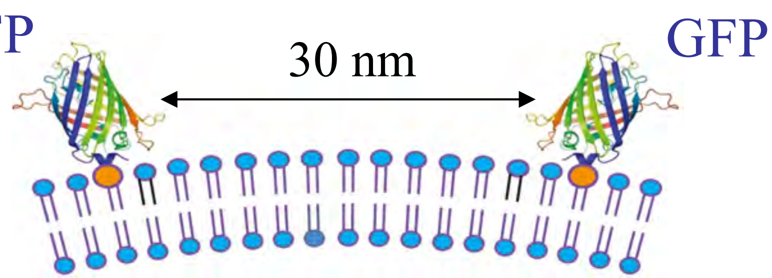
Controlled Formation of Necks

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

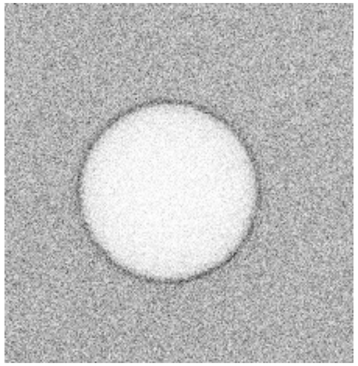


Neck Fission and GUV Division

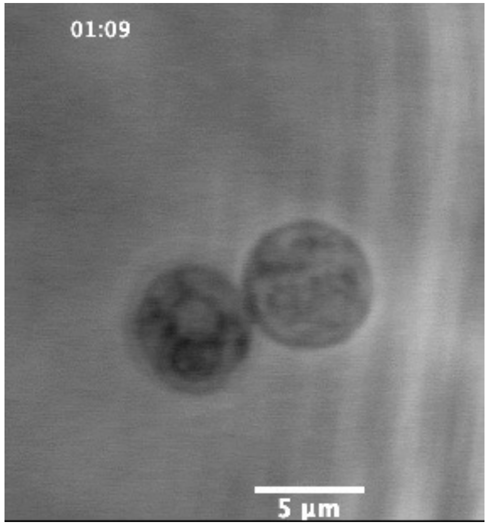
J. Steinkühler et al: *Nature Comm*. (2020)

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

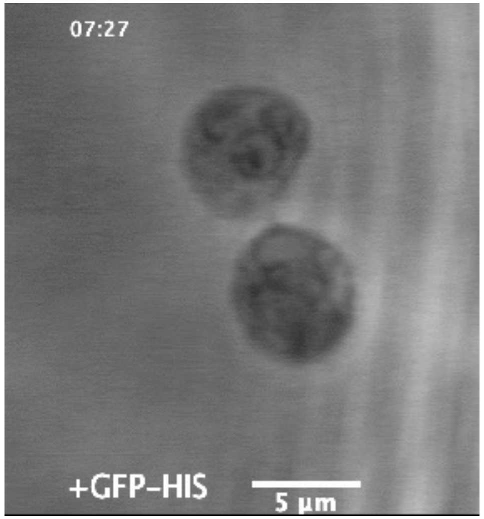
  Increase in GFP -> Neck cleavage -> Two daughter GUVs



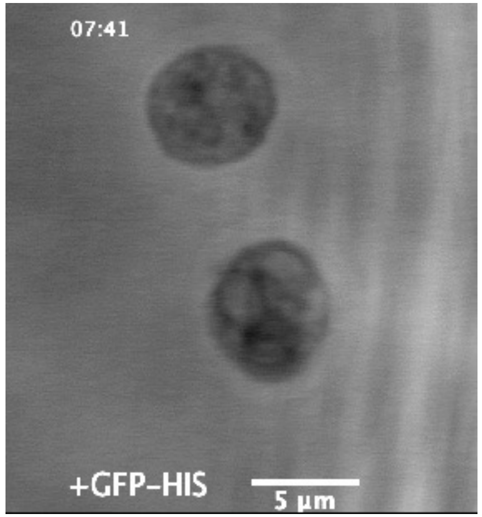
Adsorption of GFP onto GUV membrane



Deflation leads to dumbbell with membrane neck



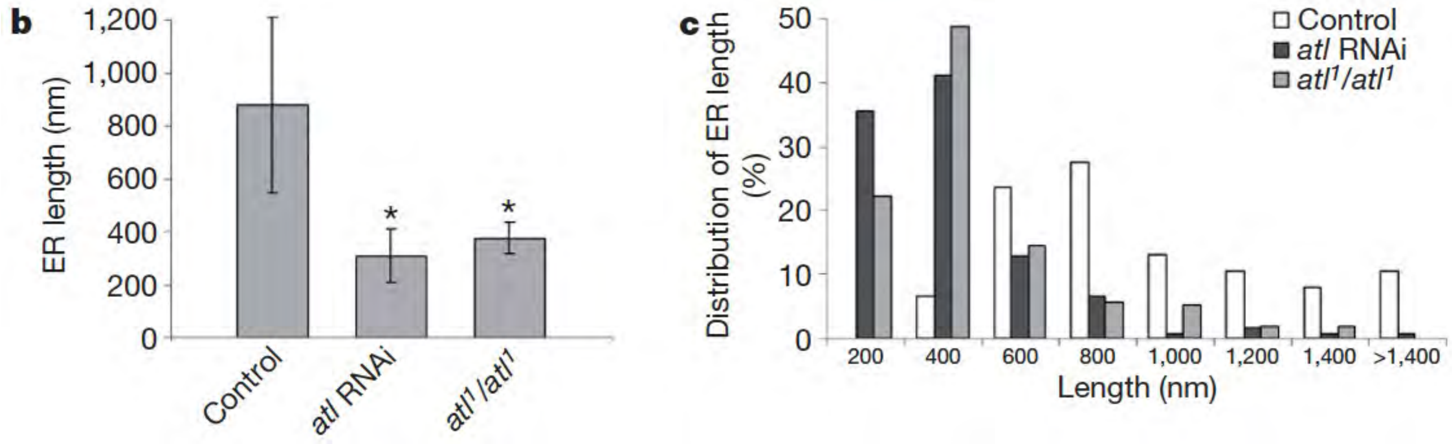
Directly after neck cleavage



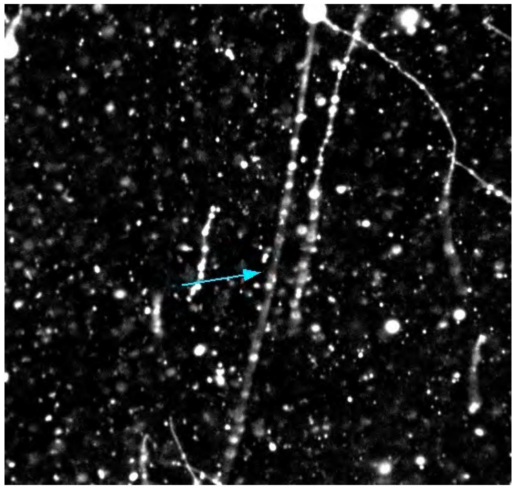
Complete division into two smaller GUVs

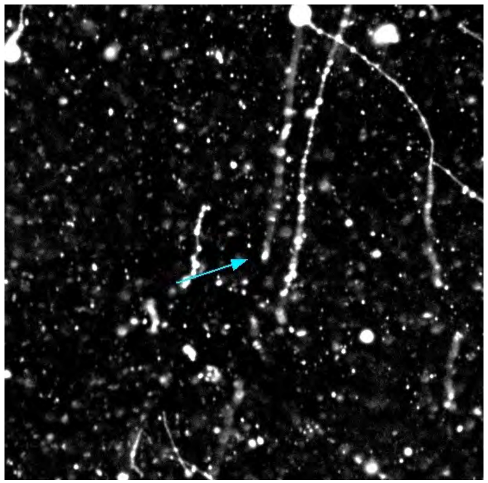
Observed Fragmentation of ER Nanotubes

- In vivo after knockdown of atlastin: Orso et al, *Nature 460* (2009)



- In vitro after interrupting supply of GTP: Powers et al, *Nature 543* (2017)

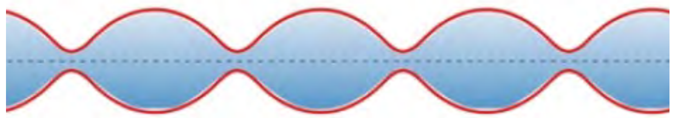




Tension and Fragmentation

RL et al, *ACS Nano 17* (June 2023)

- Cylindrical membrane tube of length L and radius r_0
- Membrane experiences effective tension Σ
- Persistaltic modes of tube, ℓ_n
- Most unstable mode ℓ_* with period $2\pi r_0$
- Mean-squared amplitude of ℓ_* :



$$\langle |\ell_*|^2 \rangle = \frac{k_B T}{2\pi (L/r_0)\Sigma}$$

small for large tension Σ

large for small tension Σ

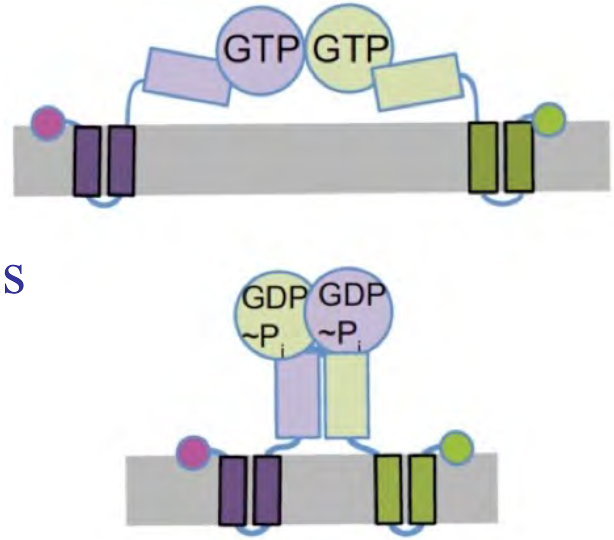
- Simple criterion for fragmentation:

$$\langle |\ell_*|^2 \rangle \geq r_0^2 \quad \text{or} \quad \Sigma \leq \Sigma_1 \equiv \frac{k_B T}{2\pi r_0 L}$$

← tension threshold

- No fragmentation for $\Sigma >> \Sigma_1$

Tension via Cis-Dimerization



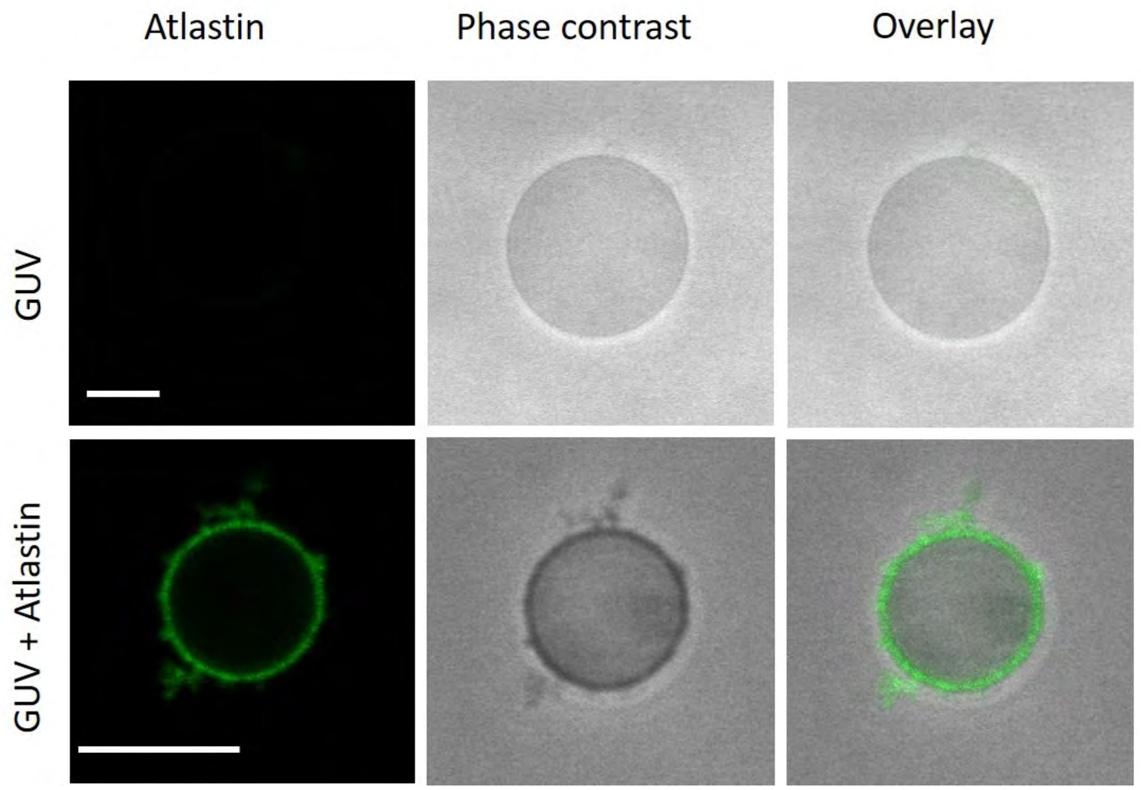
Liu et al, *PNAS* (2015)

- Proposal: Membrane tension from cis-dimerization of atlastin
- Cis-dimerization coupled to GTP-hydrolysis
- Observed for solid-supported bilayers
- So far, the functional significance for this dimerization process remained elusive
- Thus, cis-dimerization seemed to waste a lot of GTP
- Now, cis-dimers viewed as active „hot spots“ that change excess membrane area for undulations and generate membrane tension
- On the nano scale, tension prevents tube fragmentation
- On the micron scale, tension explains network geometry

Proteo-GUVs with Atlastin, PoC

RL et al, *ACS Nano* (June 2023)

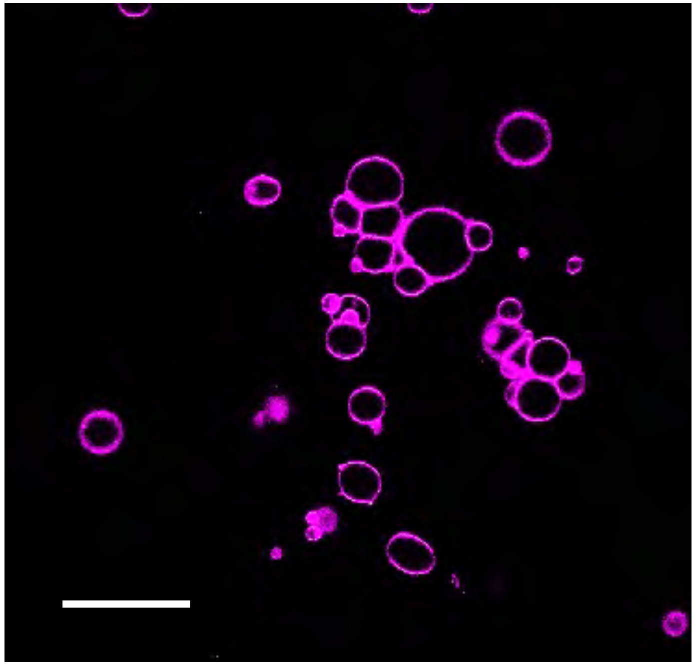
- Atlastin fluorescently labeled by Oregon Green
- GUVs exposed to protein buffer with detergent
- Removal of detergent by BioBeads



Shreya Pramanik

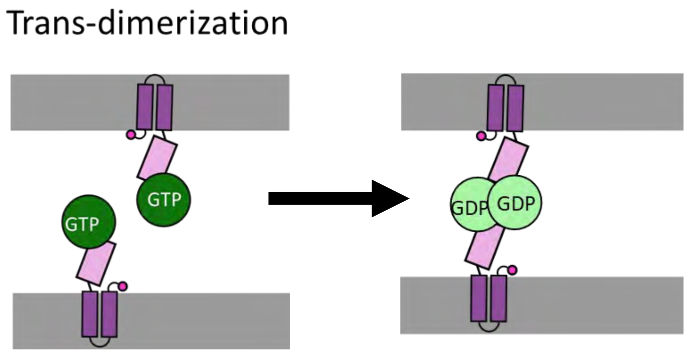
GTP-induced Adhesion of Proteo-GUVs

- Proteo-GUVs with atlastin
- Addition of GTP leads to vesicle adhesion:



50µm

The proteo-GUVs stick to each other on the addition of GTP.



Proteo-GUVs + GTP

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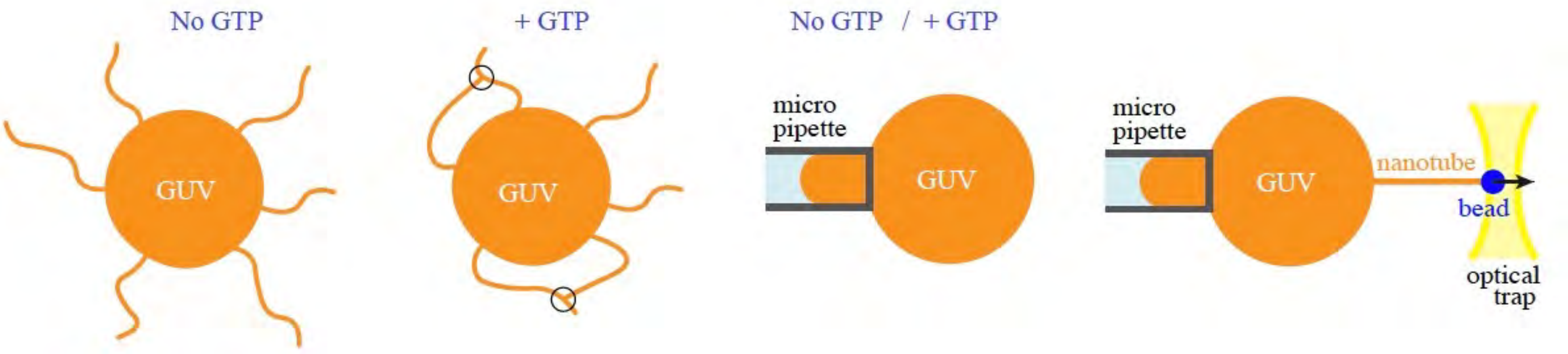
Perspectives for Proteo-GUVs

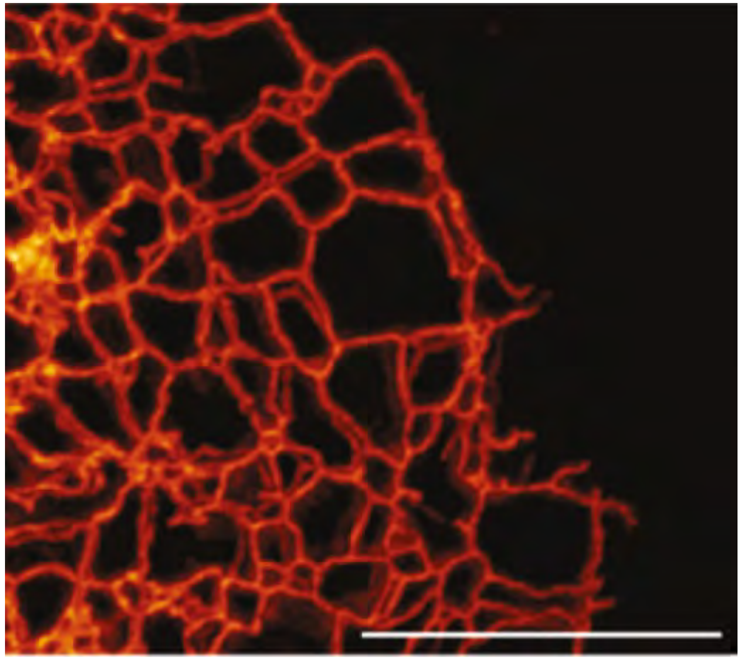
- GTP as a new control parameter for GUV morphology
- Formation of nanotubes without GTP
- Formation of tubular junctions by addition of GTP
- Change of undulation spectrum by addition of GTP
- Direct measurement of tension via micropipette aspiration
- Tension measurement via tube pulling ...



Topological Complexity of ER

- ER membrane creates a bicontinuous structure by separating the ER lumen from the surrounding cytosol
- Topology of any closed surface characterized by its genus
- Genus is an integer that counts the number of “handles“

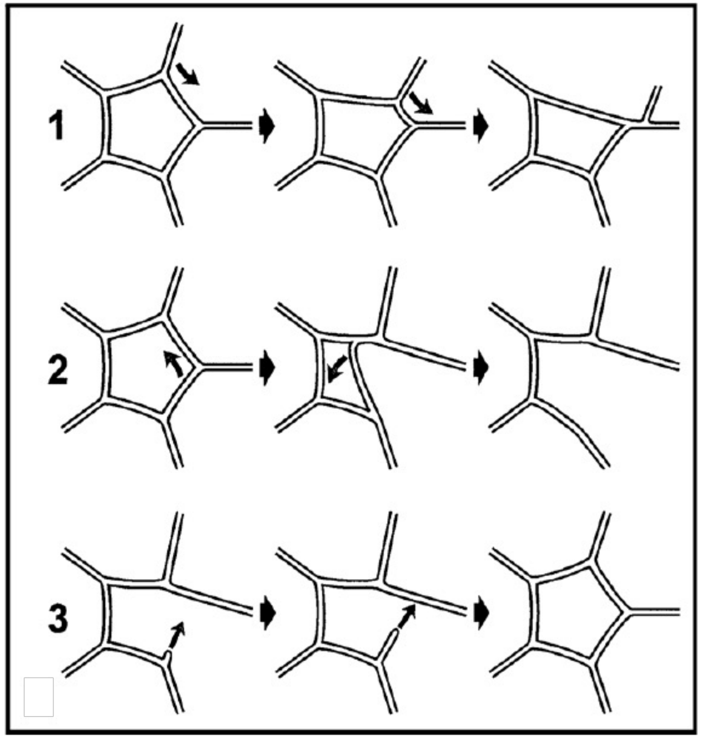
- Genus of ER membrane is equal to the number of irregular polygons formed by the nanotubes
- Small section of ER on the right contains more than 40 polygons
- Whole ER contains many thousands of polygons



Movements of Junctions

- Local changes in the network connectivity
- Three basic dynamic processes observed in vivo:

Lee and Chen, *Cell* (1988)
Baumann and Walz, Int. Rev. Cyt (2001)



1 Sliding:

No change in number of polygons

2 Ring closure:

Removal of one polygon by fission

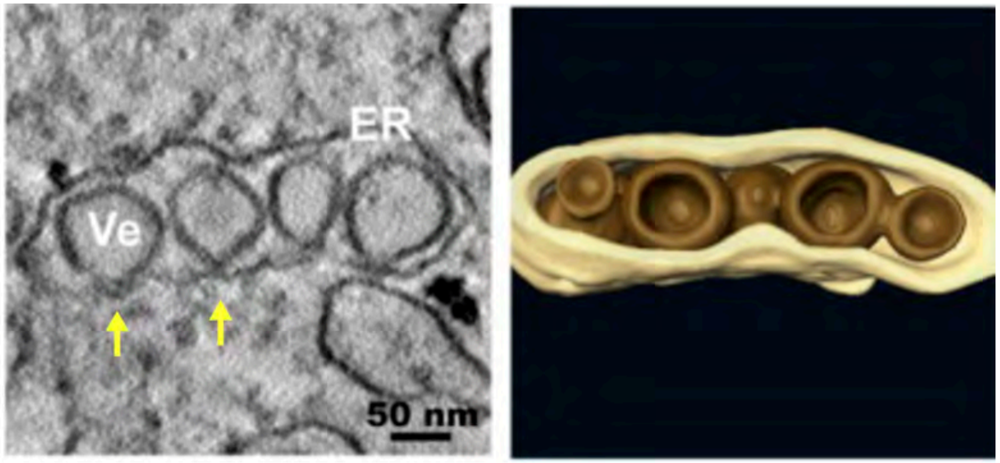
3 Branching

Creation of one polygon by fusion

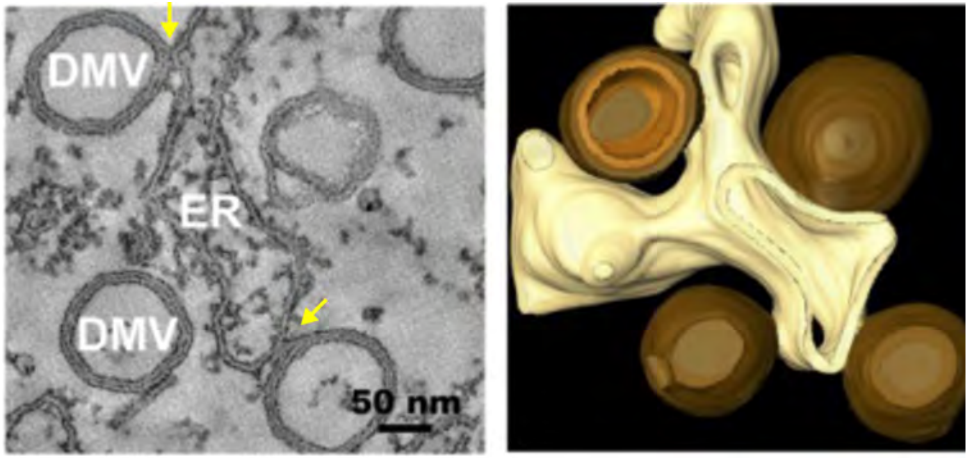
ER Remodeling by Viruses

Romero-Brey and Bartenschlager, *Viruses* (2014)

- Viral replication at organelle membranes
- ER membrane is “the favorite niche for viral replication“
- Viruses create replication factories at ER membrane
- Electron tomography of replication factories:



Dengue virus creates in-buds



Hepatitis C virus creates out-buds

Yellow arrows = ER membrane necks