Multispherical Shapes and More

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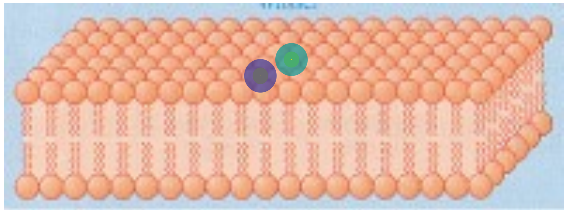
- Reminder about Biomembranes
- Elasticity of Fluid Membranes
- Multispherical Shapes of Vesicles
- Constant-Mean-Curvature Surfaces
- Remodeling of Membrane Topology
- Outlook on Endoplasmic Reticulum

Biomembranes are Fluid Surfaces

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

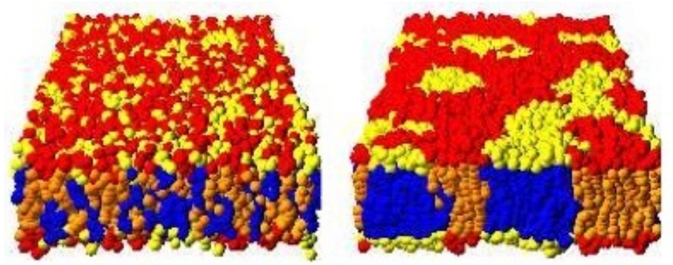
  Diffusion constant ~ $\mu m^2/s$



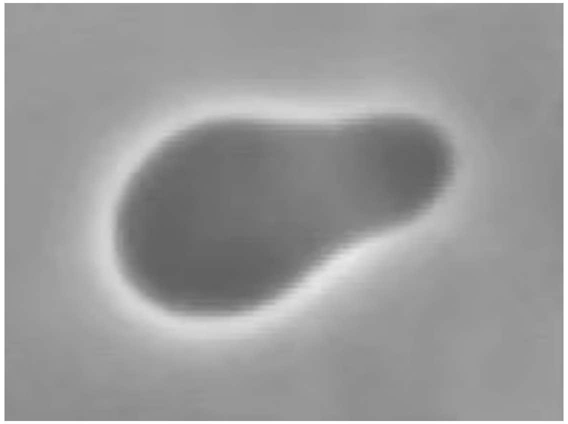
4 nm

lipid swapping ~ ns

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



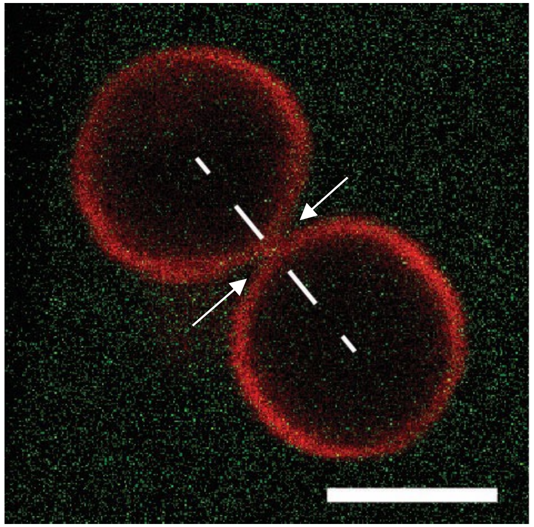
- Flexibility => Morphological responses, budding, tubulation, ... Direct evidence for fluidity



20 µm

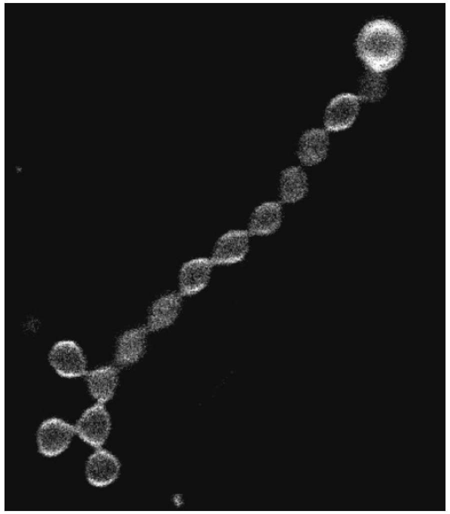
Morphological Complexity of GUVs

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



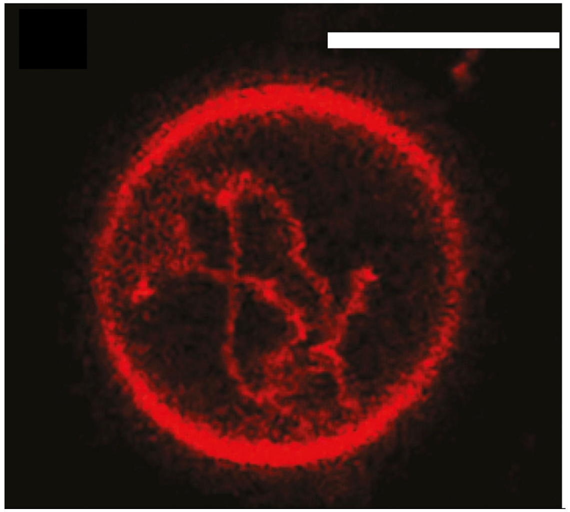
Dumbbell, (1+1)-sphere, one membrane neck

Steinkühler et al, *Nature Comm* (2020)



(1+14)-sphere, 14 necks

Bhatia et al, *Soft Matter* (2020)

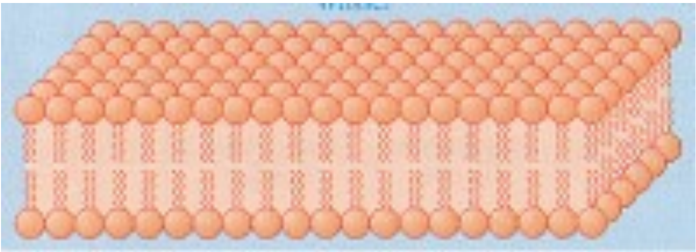


Long nanotubes, width of 100 nm

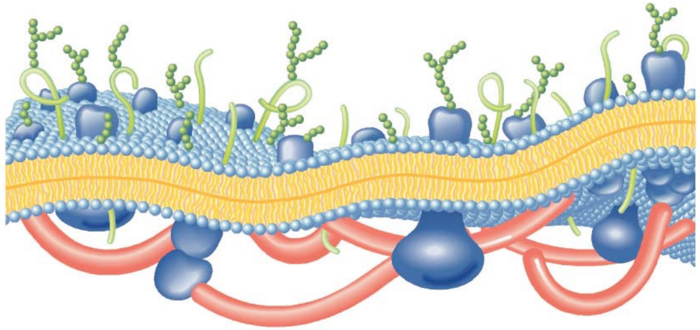
Bhatia et al, *ACS Nano* (2018)

Fluid Architecture of Biomembranes

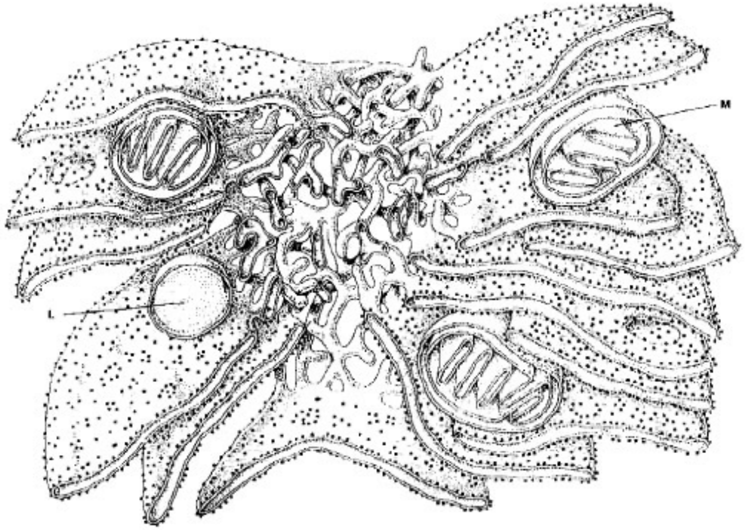
• Lipid bilayer



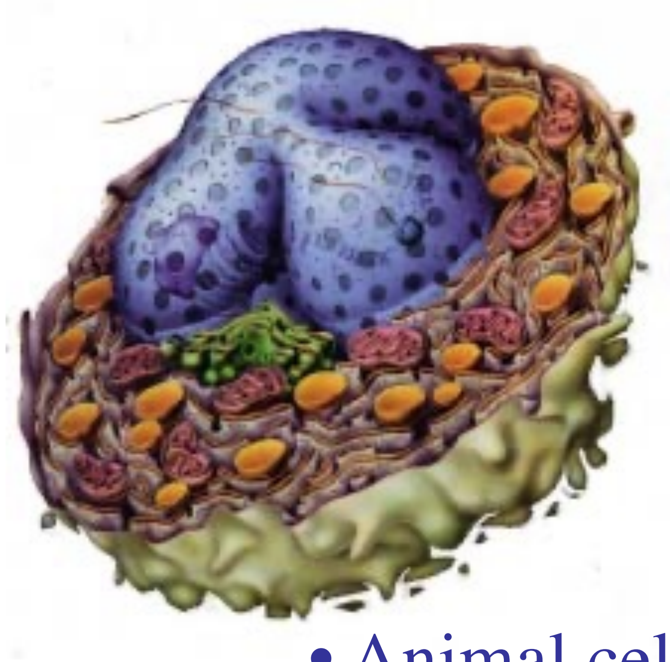
4 nm



• Biomembrane



• Endoplasmic reticulum (ER)



100 µm

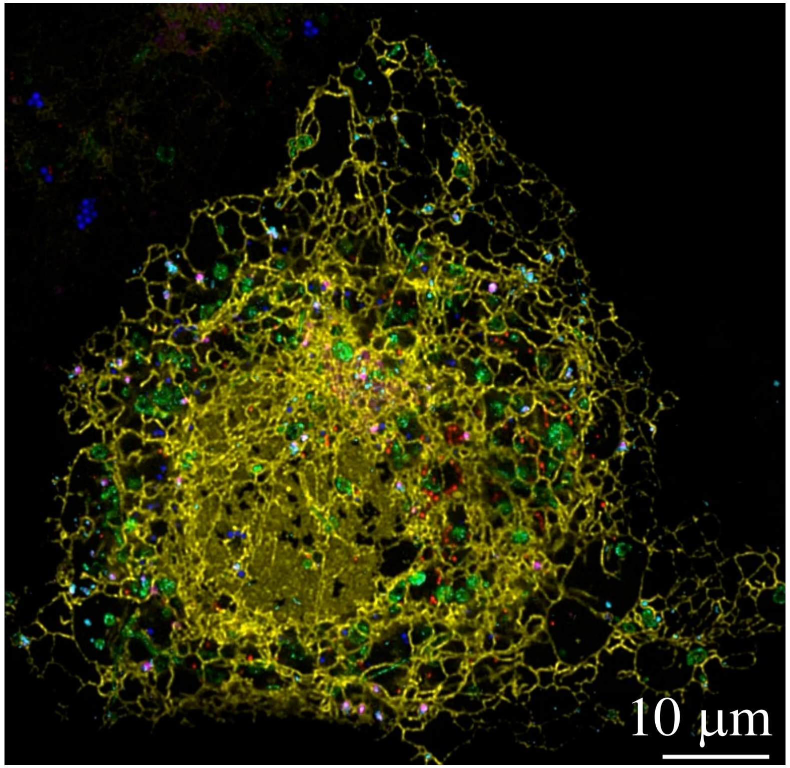
• Animal cell

Endoplasmic Reticulum (ER)

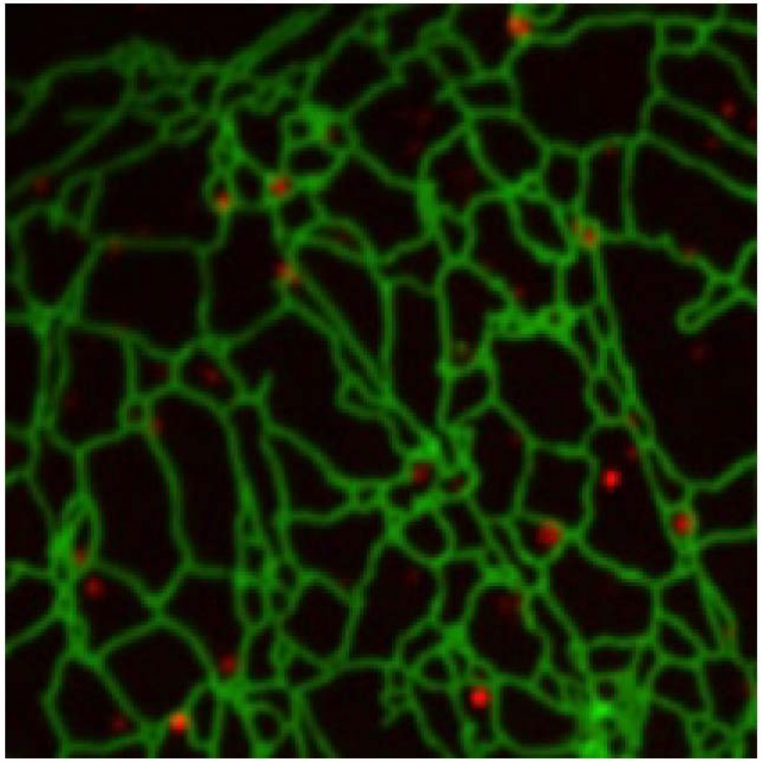
- ER = network of membrane nanotubes with three-way junctions

Tubes with yellow fluo-labels

Valm et al. *Nature* (2017)



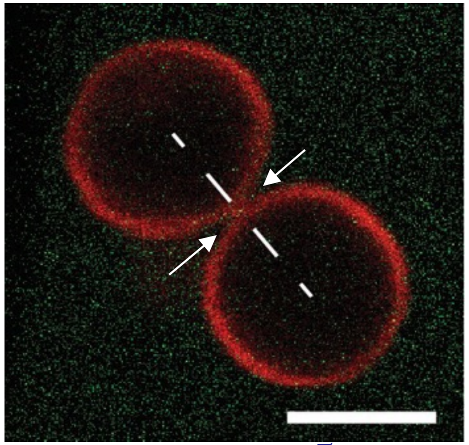
Tubes with green fluo-labels



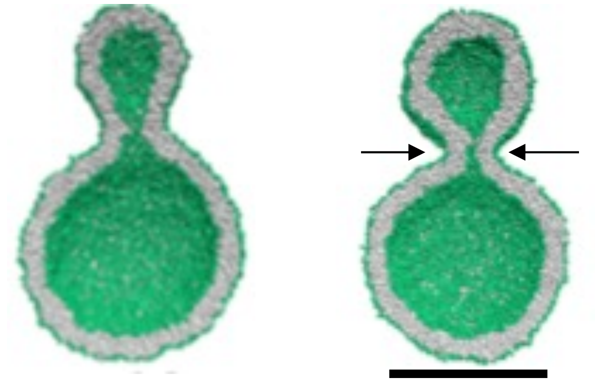
Friedmann, et al , *Mol Biol Cell* (2013)

Synthetic Membrane Compartments

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



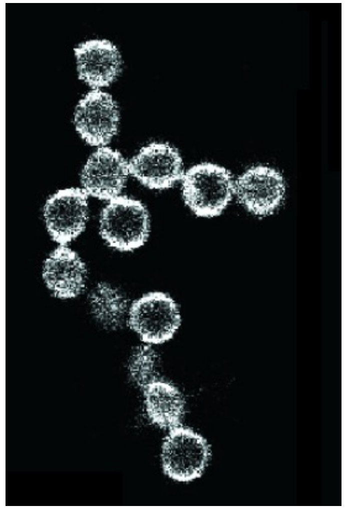
- Nanovesicles or NVs
- Electron microscopy: limited to a single snapshot for each individual nanovesicle
- Remodeling of NVs can be studied via Molecular Dynamics simulations



- In both cases: Formation of membrane necks

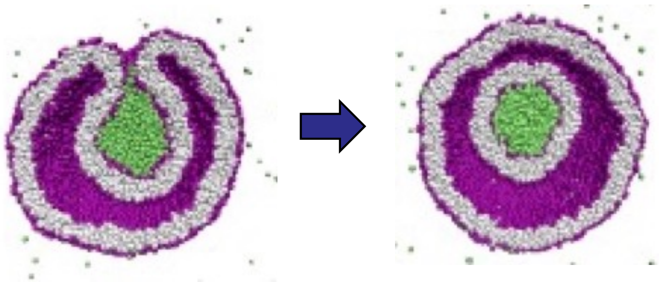
Remodeling of Shape and Topology

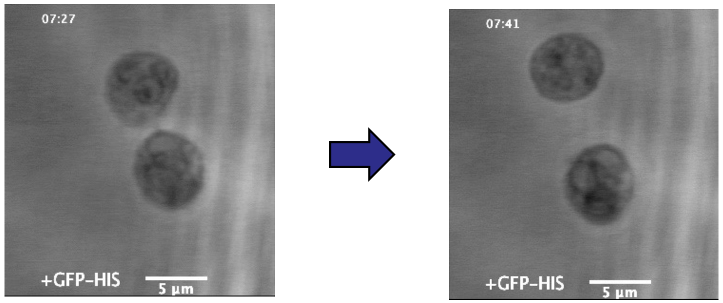
- Remodeling of membrane shape
- Polymorphism of nanovesicles and GUVs
- Multispherical shapes with many necks:



Topology of single sphere!

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





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Elasticity of Fluid Membranes

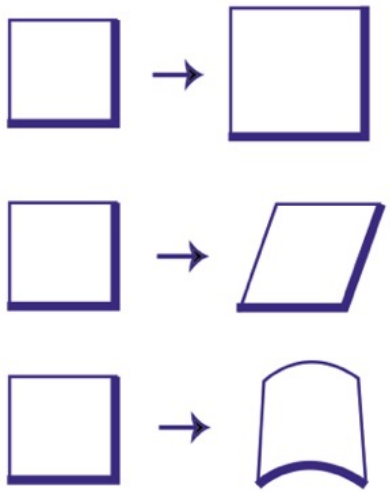
- Biomembrane as thin elastic sheet

- Elastic Deformations

  Stretching

  Shearing

  Bending



- Fluid Membranes

  Membrane tension

  Shear -> Flow

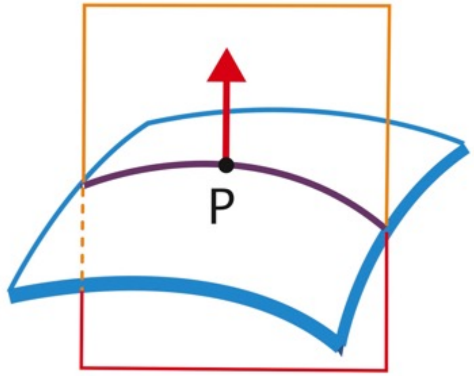
  Curvature elasticity

Elastic Energy

- Elastic stretching: Area A and stretching tension Σ_{st}

Hooke's law $\Sigma_{st} = K_A\,(A - A_0)/A_0$

area compressibility modulus K_A, optimal area A_0



- Stretching energy $E_{st} = \frac{1}{2} K_A\,(A - A_0)^2/A_0$

- Elastic bending: Mean curvature M

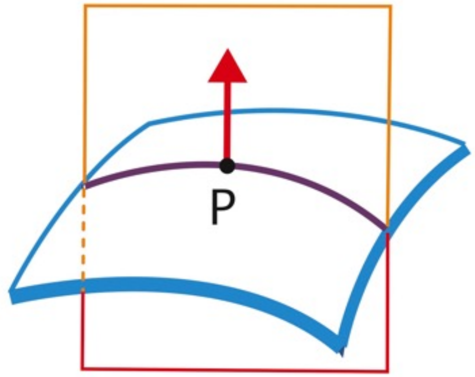
Bending energy $E_{be} = \int dA\; 2\,\kappa\,(M - m)^2$

bending rigidity κ, spontaneous curvature m

- Elastic energy = Stretch energy + bending energy

$$E_{el} = E_{st} + E_{be} = \frac{1}{2} K_A\,(A - A_0)^2/A_0 + E_{be}$$

Curvatures from Diff Geometry

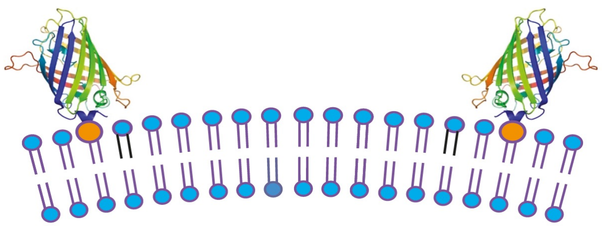


- Consider any smoothly curved surface
- Normal section through surface creates smooth curve on surface
- Smooth curve has 1-dim curvature C_{ns}
- Rotation of normal section changes curvature C_{ns} within the range $C_{min} \leq C_{ns} \leq C_{max}$
- Principal curvatures $C_1 = C_{min}$ and $C_2 = C_{max}$
- Mean curvature $M = (C_1 + C_2)/2$
- Gaussian curvature $G = C_1 C_2$

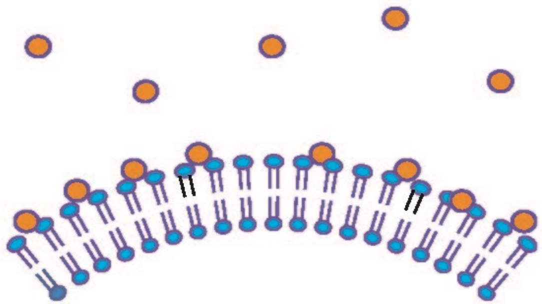
Spontaneous Curvature

- Lipid bilayer consists of two monolayers or leaflets
- Spontaneous or preferred curvature *m* describes transbilayer asymmetry = asymmetry between two leaflets

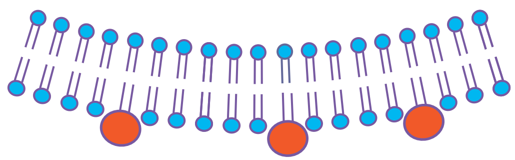
- Different molecular mechanisms for spont curvature:



Binding of GFP to outer leaflet



Adsorption layer of glucose



Adsorption of glycolipid GM1

Minimization of Elastic Energy

- Minimization with prescribed vesicle volume V
- Shape functional: $F = -\,\delta P\, V + E_{\mathrm{st}} + E_{\mathrm{be}}$

  with $E_{\mathrm{st}} = \frac{1}{2} K_A\, (A - A_0)^2/A_0$ and $\Sigma_{\mathrm{st}} = K_A\, (A - A_0)/A_0$
- Pressure difference $\delta P = P_{in} - P_{ex}$ is Lagrange multiplier
- Alternative procedure:
  Minimization with prescribed V and prescribed area A
- Shape functional: $F = -\,\delta P\, V + \Sigma\, A + E_{\mathrm{be}}$
- Two minimization procedures are equivalent:

  Lagrange multiplier $\Sigma =$ stretching tension Σ_{st}

Shape Equation

- Shape functional: $F = -\delta P\, V + \Sigma\, A + E_{\text{be}}$
- Minimization with respect to normal displacements => Euler Lagrange equation or shape equation:

$$\delta P = 2\,\Sigma\, M - 2\kappa\, \Delta_{LB}\, M - 4\kappa\,[M - m]\,[M\,(M + m) - G]$$

- Mean curv M, Gaussian curv G, Laplace-Beltrami Δ_{LB}
- Spontaneous curvature m, bending rigidity κ
- Spherical membrane with radius R:

$M = 1/R$, $M^2 = 1/R^2 = G$, simplified shape equation:

$$\delta P = 2\,(\Sigma + 2\kappa m^2)\, M - 4\kappa\, m\, M^2$$

Ou-Yang, Helfrich, *Phys. Rev. A* (1989)

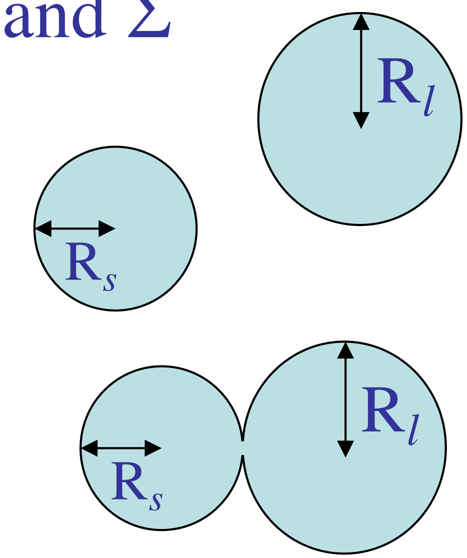
Multispherical Shapes

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

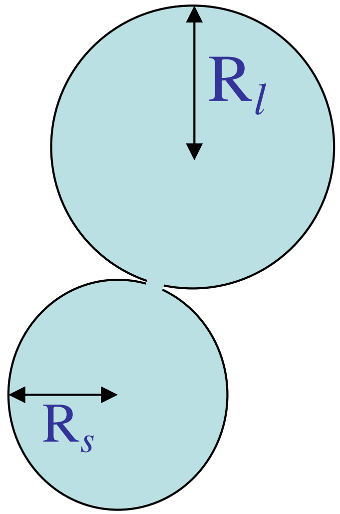
- Shape equation for spheres;

$$\delta P = 2\,(\Sigma + 2\kappa m^2)\,M - 4\kappa\, m\, M^2$$

- Quadratic in mean curvature M
- Two solutions M_l and M_s for fixed δP and Σ
- Large sphere with radius $R_l = 1/M_l$
- Small sphere with radius $R_s = 1/M_s$
- Puncture spheres, connect punctures of two spheres by membrane neck



Stability of Closed Necks



- Positive spont curvature $m > 0$
- Dumbbell with closed membrane neck corresponds to (1+1)-sphere
- Large and small sphere with radius R_l and R_s
- Neck curvature $M_{ne} = (1/2)\,(1/R_l + 1/R_s)$
- Closed neck is stable if $0 < M_{ne} \leq m$
- Stable necks for sufficiently large spont curvature m
- Local relation between geometry and material parameter

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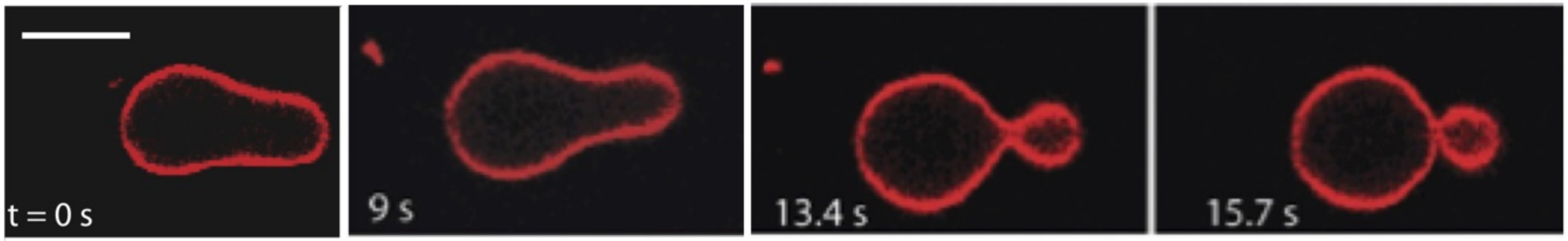
Budding of Giant Vesicles

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

Bhatia et al, *Soft Matter* (2020)



Scale bar: 5 μm

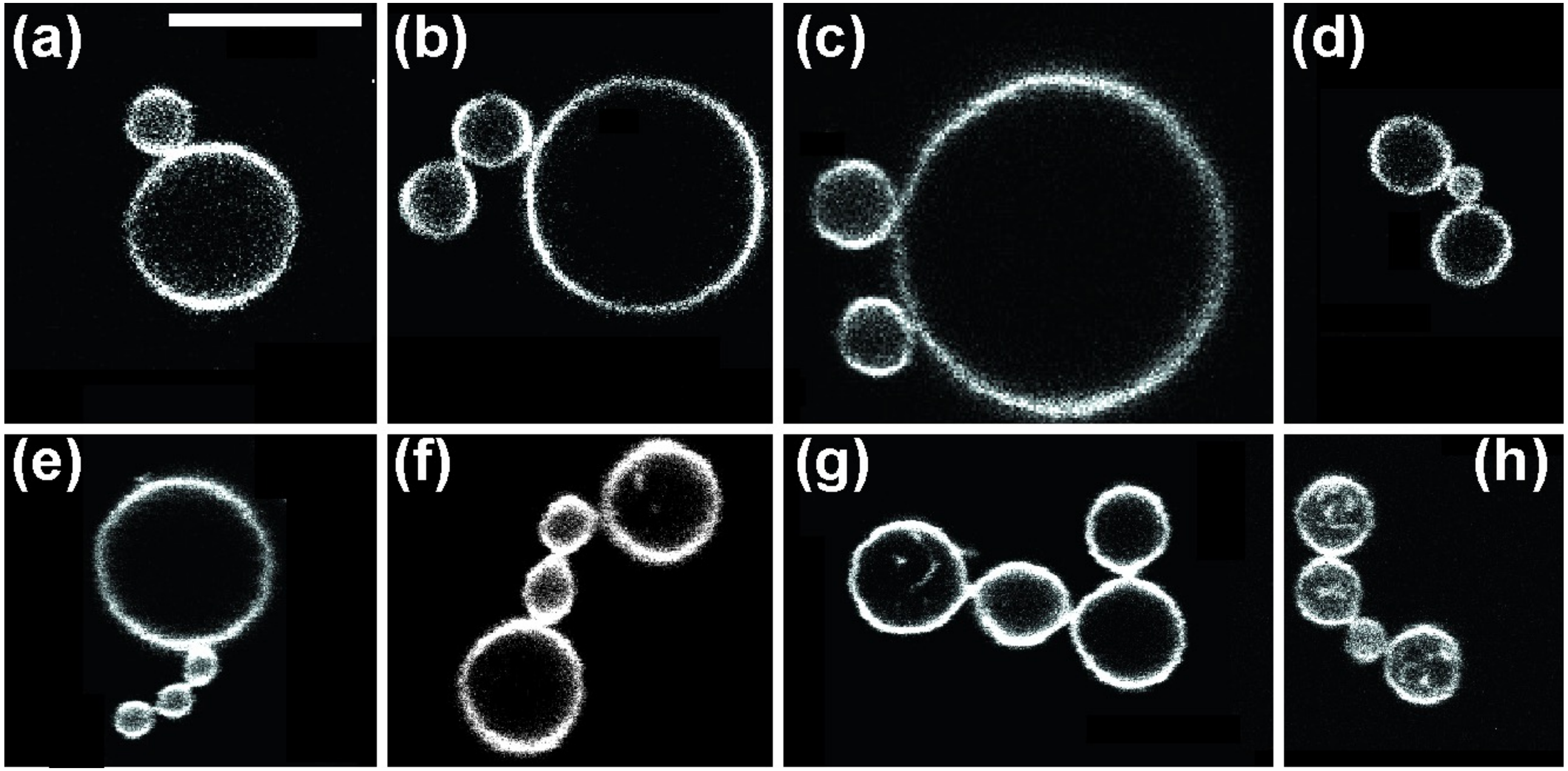
'Fluid worm hole in three dimensions'

- Membrane exposed to asymmetric sucrose/glucose solutions
- Membrane forms two spheres connected by a single neck
- Same membrane system leads to proliferation of necks !

Multispheres with Several Necks

Scale bar: 10 μm

Bhatia et al, *Soft Matter* (2020)



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

$(1+N_s)$-Multispherical Vesicles

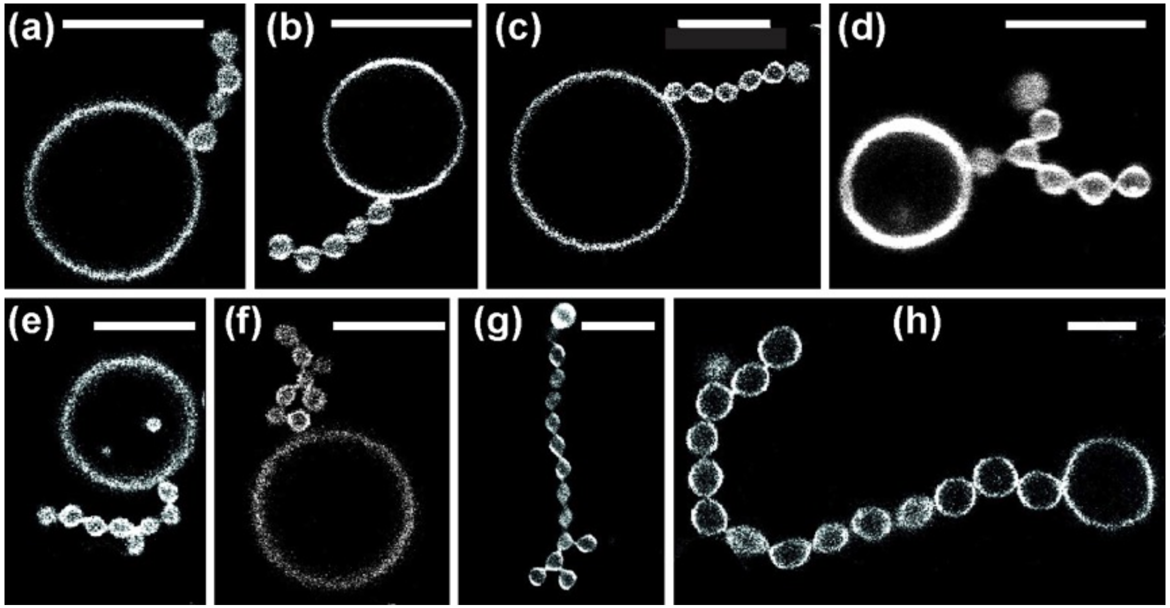
Bhatia et al, Soft Matter (2020)

- $(1+N_s)$-spheres with one large sphere and a chain of N_s small spheres:

(1+4) (1+5) (1+6) (1+7)

linear chains

branched chain



branched chains

(1+8) (1+8) (1+14) (1+15)

Multispheres: Geometry

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

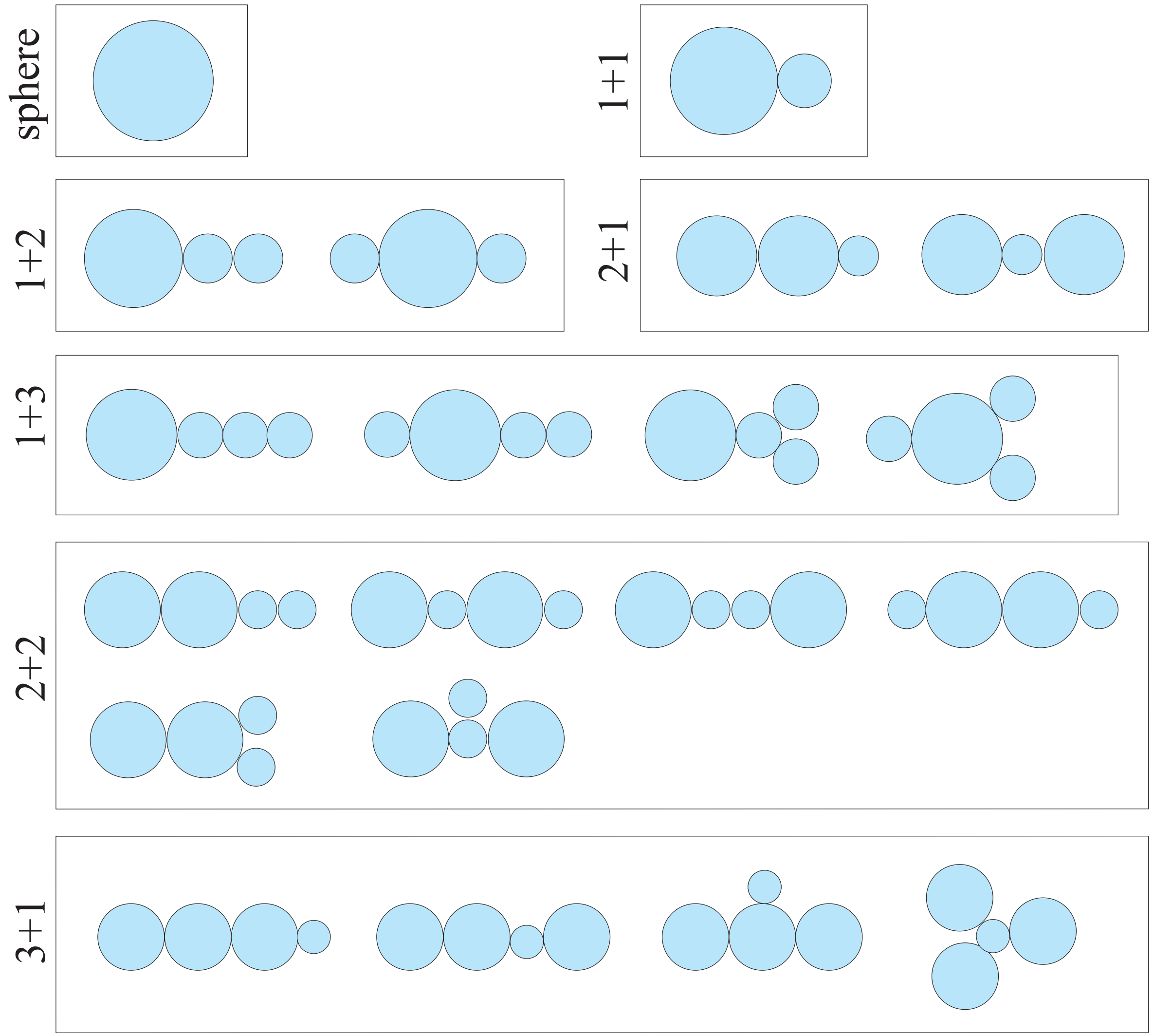
- Multispheres with large and small spheres
- Rescaled large sphere radius r_l and small sphere radius r_s
- Multispheres consisting of N_l large and N_s small spheres
- $(N_l + N_s)$-geometry determined by two simple equations:

$$N_l \, r_l^{\,2} + N_s \, r_s^{\,2} = 1$$

$$N_l \, r_l^{\,3} + N_s \, r_s^{\,3} = v$$

- Two nonlinear equations for two unknowns r_l and r_s
- Depend on single parameter, volume-to-area ratio v
- Two simple equations generate morphological complexity

Multispheres up to $N_l + N_s \leq 4$



Different Types of Membrane Necks

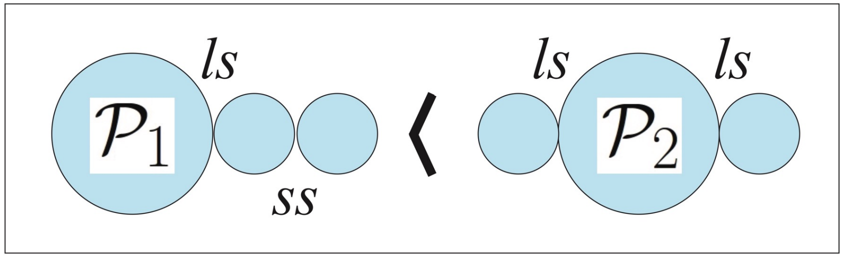
- Three types of closed membrane necks:

  ss-necks between two small spheres with radius R_s

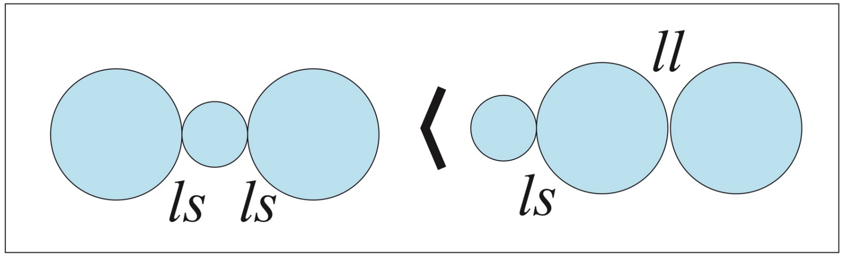
  ls-necks between large and small sphere, radius R_l and R_s

  ll-necks between two large spheres with radius R_l

(1+2)

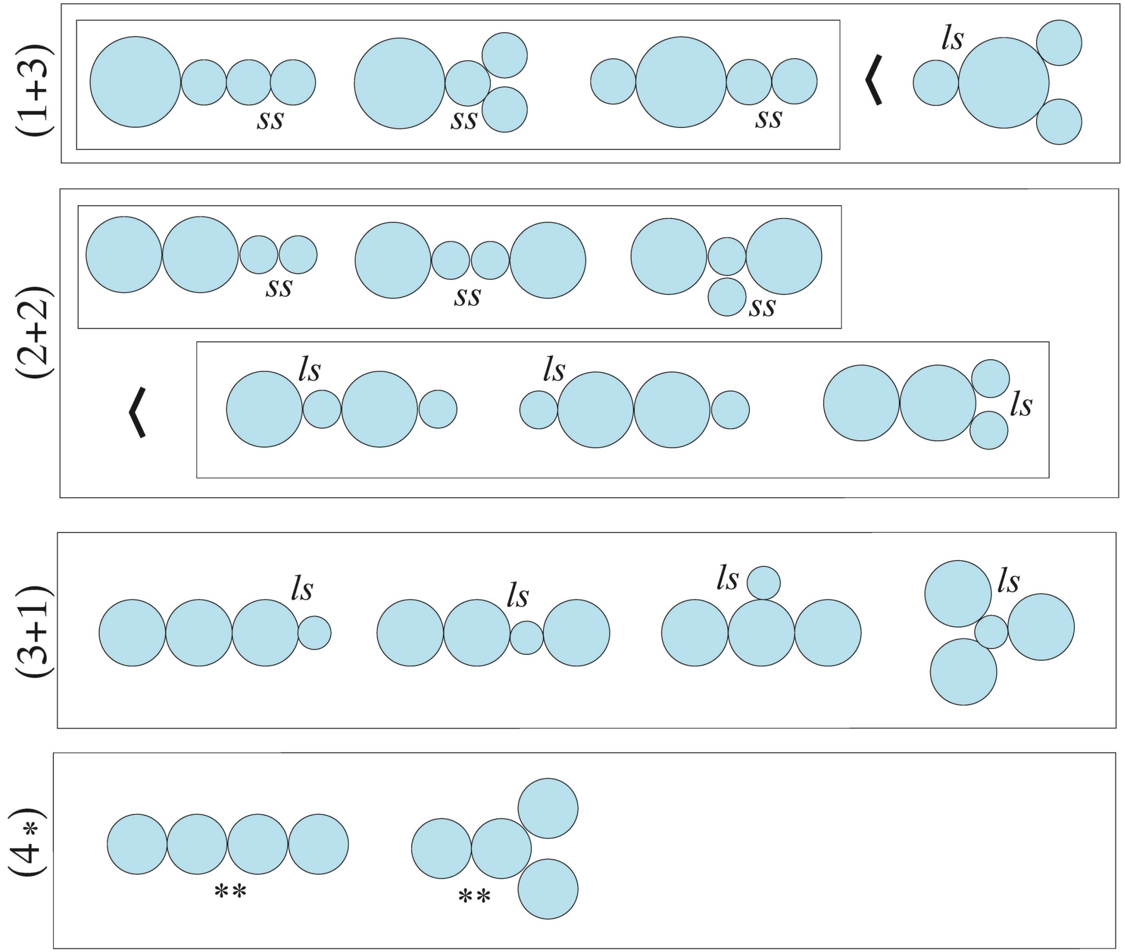


(2+1)



- Stability conditions: $0 < M_{ne} = (1/2)\ (1/R_l + 1/R_s) \leq m$
- Strongest condition and smallest stability regime for *ss*-neck
- Weakest condition and largest stability regime for *ll*-neck
- Stability regime of multisphere from least stable neck

Multispheres with $N_l+N_s = 4$

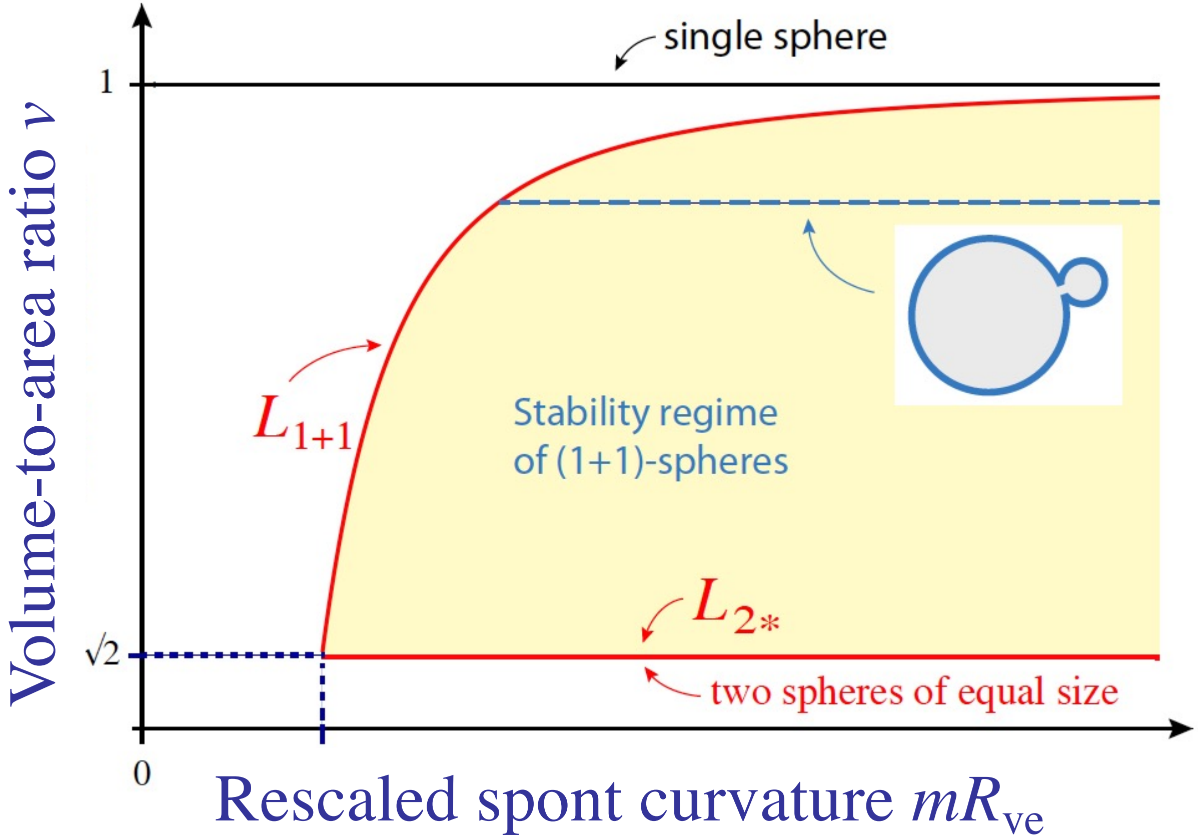


Each shape displays least stable neck

Four equally sized spheres with radius R*

Stability Regime for (1+1)-Spheres

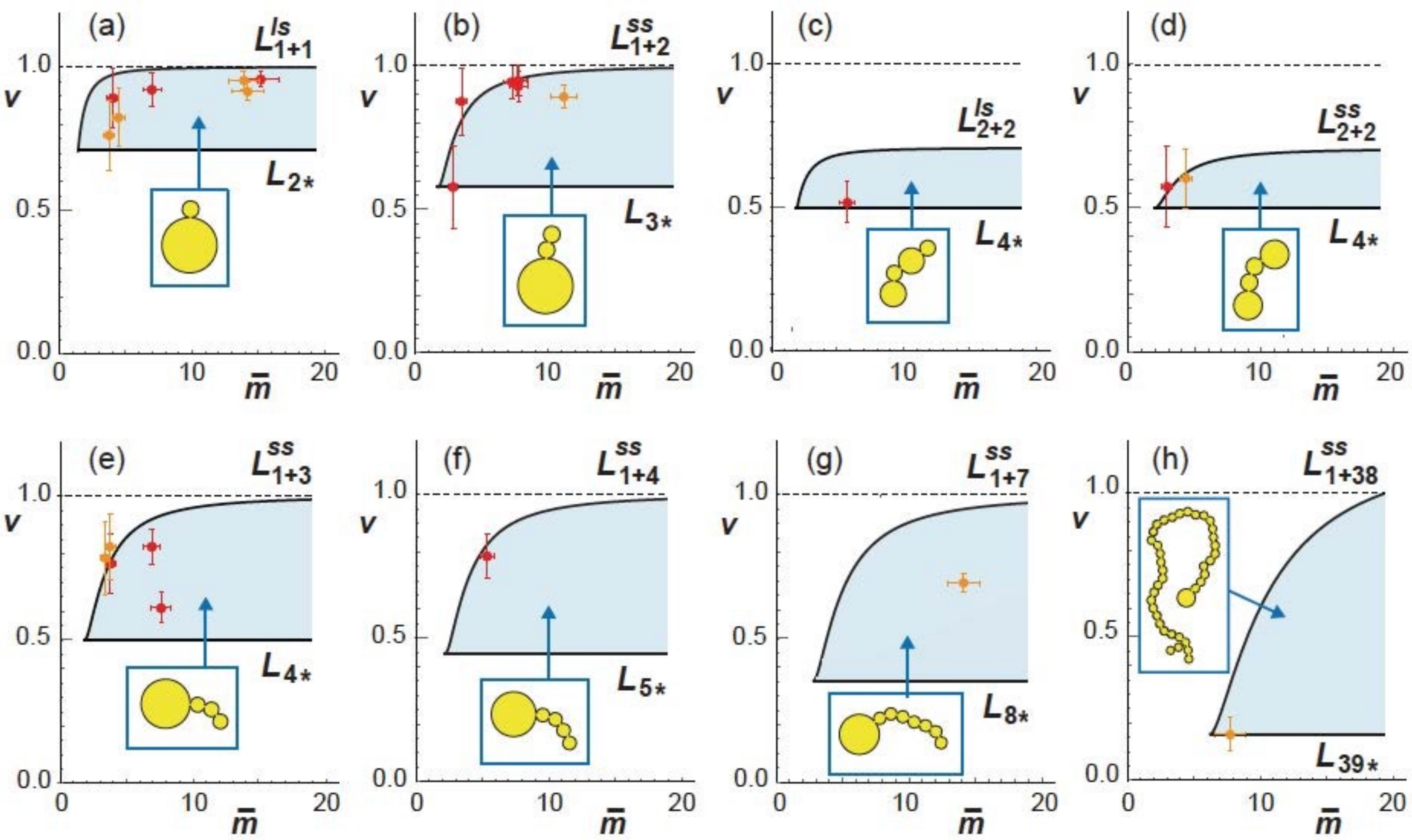
- Vesicle size R_{ve} as basic length scale
- Two dimensionless shape parameters: volume-to-area ratio v and rescaled spont curvature mR_{ve}



within yellow stability regime: shape of (1+1)-sphere depends only on v but not on mR_{ve}

Stability Regimes for (N_l+N_s)-Spheres

Bhatia et al, *Soft Matter* (2020)



N_* Equally Sized Spheres

Bhatia et al, Soft Matter (2020)

- Multispheres consisting of N_* equally sized spheres:

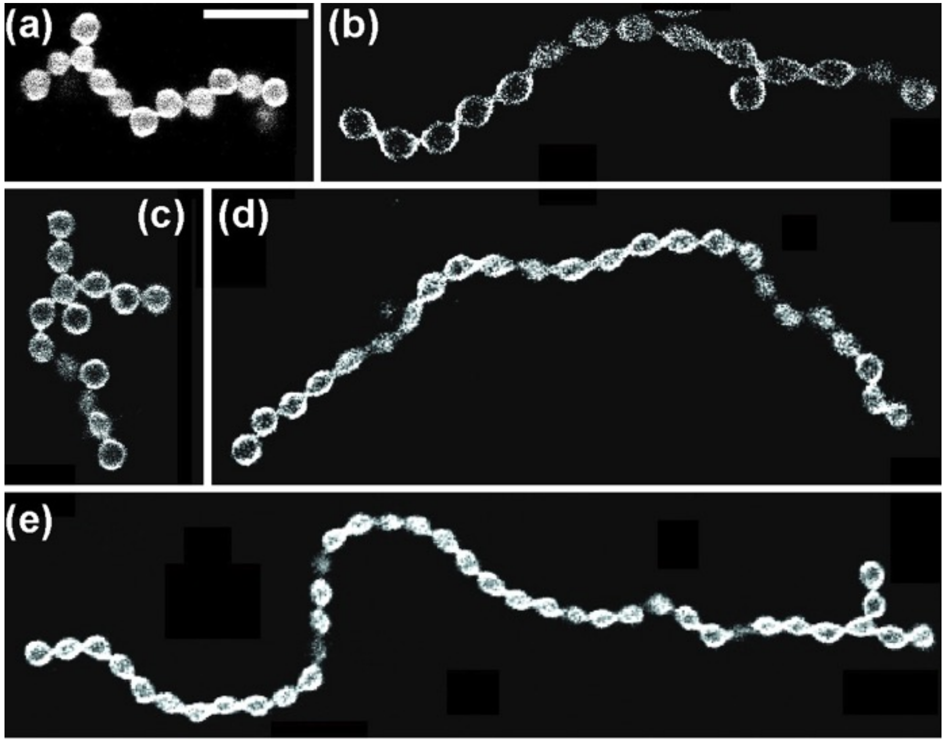
$N_* = 14$ branched

$N_* = 15$ branched

$N_* = 15$ branched

$N_* = 24$ linear

$N_* = 39$ branched



- Each (N_*)-multisphere has constant mean curvature $M = 1/R_*$
- New examples for constant-mean-curvature (CMC) surfaces

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CMC Surfaces and Curvature Elasticity

- Euler Lagrange equation or shape equation:

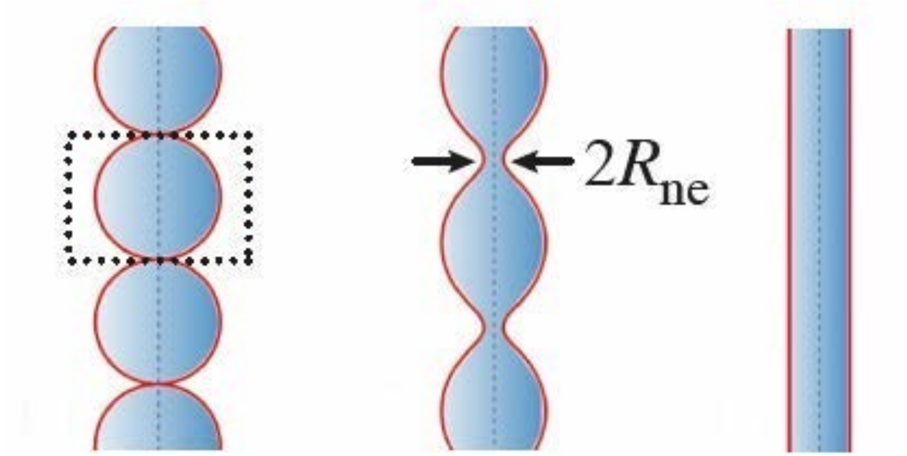
$$\delta P = 2\,\Sigma\, M - 2\kappa\,\Delta_{LB}\, M - 4\kappa\,[M - m]\,[M\,(M + m) - G]$$

- Constant mean curvature $M = \mathrm{m}$
- Simplified shape equation: $\delta P = 2\,\Sigma\, M$
- Each CMC surface solves shape equation for $M = m$
- Bending energy of such a CMC surface vanishes !

CMC Surfaces: Unduloids

Delaunay 1841

- Unduloids: tubular CMC shapes with a periodic sequence of necks and bellies



Multisphere Unduloid Cylinder

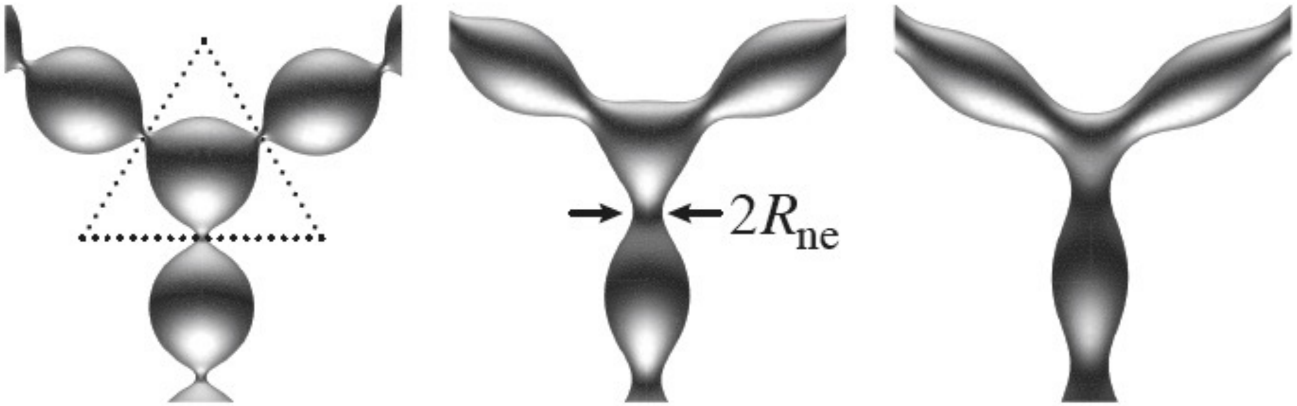
- Neck radius R_{ne}
- Belly radius R_{bel}
- $M = 1/(R_{ne}+R_{bel})$

- Multisphere for $R_{ne} = 0$, cylinder for $R_{ne} = R_{bel}$
- One-parameter family of CMC shapes with the same M
- Neck radius varies from $R_{ne} = 0$ to $R_{ne} = 1/(2\ M)$

CMC Surfaces: Triunduloids

- Triunduloids:
  three unduloidal arms connected by three-way junction

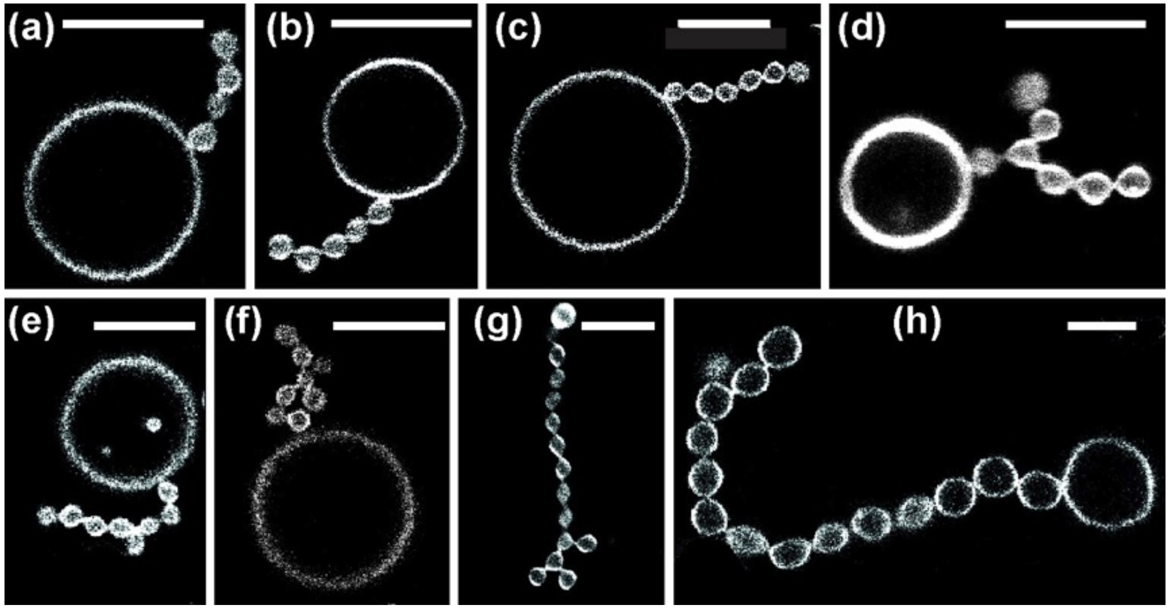
Grosse-Brauckmann and Polthier. 1997



- Multispherical shape for $R_{ne} = 0$, but no cylindrical arms
- One-parameter family of CMC shapes with the same M
- Neck radius varies from $R_{ne} = 0$ to $R_{ne} = 1/(3\ M)$

Generalized CMC Surfaces

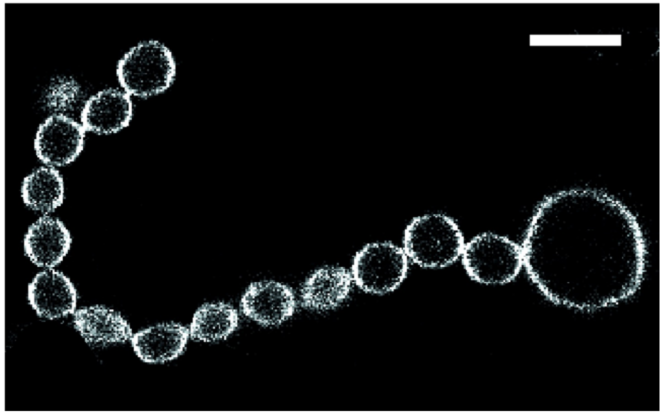
- Multispheres consisting of large and small spheres are generalized CMC Surfaces with two values of M



- Mean curvature of large spheres $M_l = 1/R_l$
- Mean curvature of small spheres $M_s = 1/R_s$

Size of Individual Spheres

- So far: Individual spheres have radii of a few microns



Example: (1+15)-sphere

Large sphere radius $R_l = 6\ \mu m$

Small sphere radius $R_s = 2\ \mu m$

Imaging by light microscopy

- Size of small spheres ~ inverse spont curvature
- Larger spont curvature leads to smaller radius R_s
- Several experimental systems with $R_s \sim 100$ nm
- Membrane nanotubes

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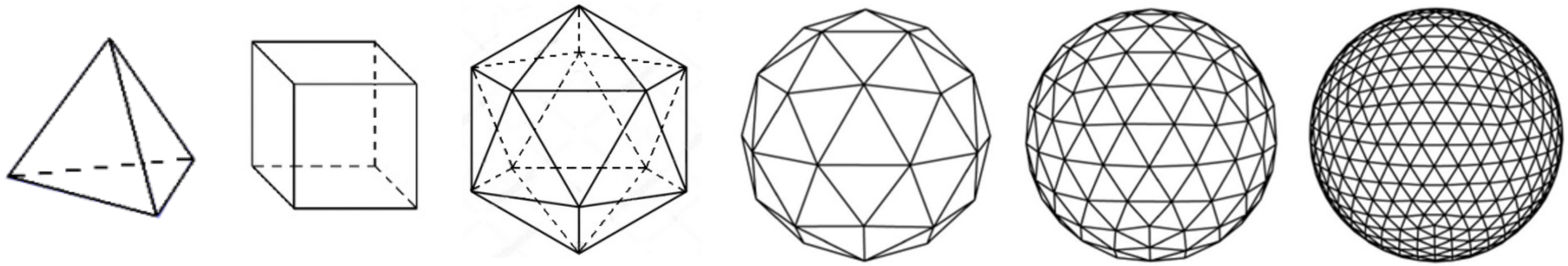
Topology of Surfaces

- Closed surface with F faces, E edges, and V vertices

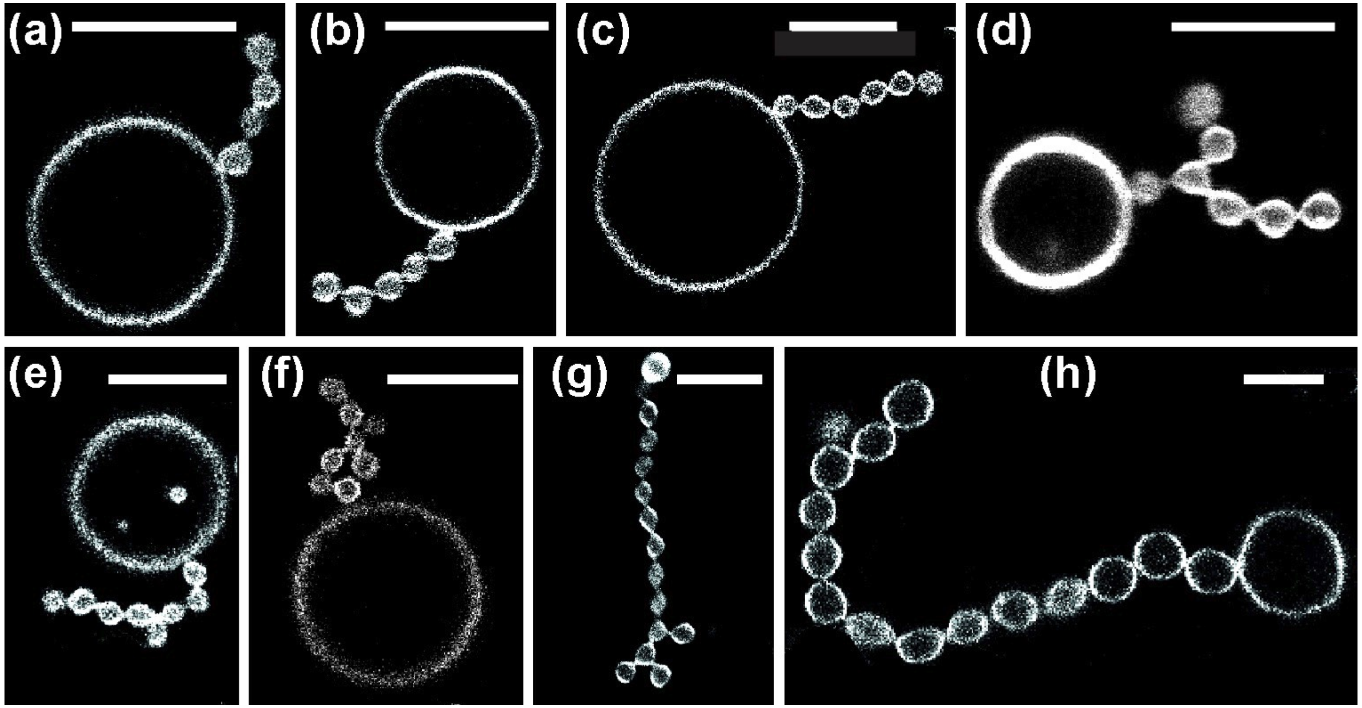


tetrahedron cube icosahedron sphere

- Euler characteristic $\chi = F - E + V$
- For tetrahedron, cube, ..., and sphere: $\chi = 2$
- Euler characteristic is topological invariant
- Euler characteristic is additive: $\chi \ = 2 + 2 = 4$ for two spheres

Topology of Multispheres

• All multispheres have the same topology as a single sphere !

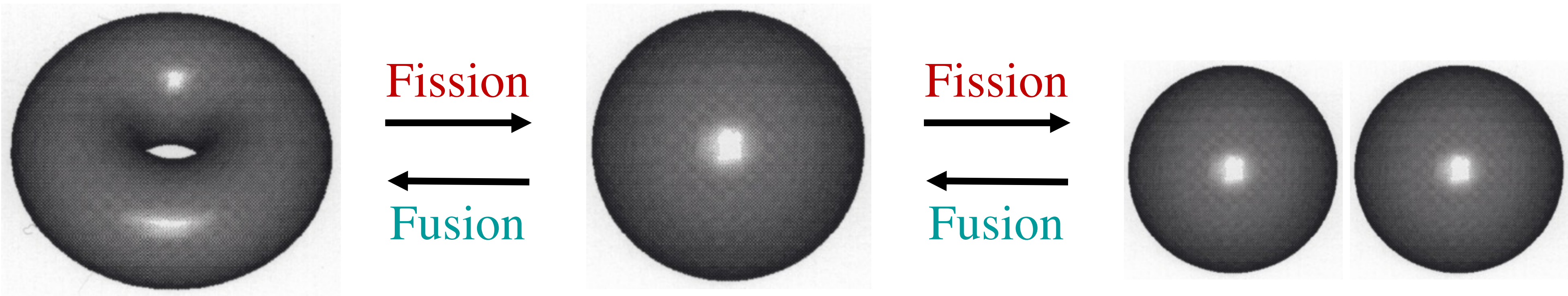


All multispheres have the same Euler characteristic

$$\chi = 2$$

Remodeling of Membrane Topology

- Two surfaces have the same topology iff they can be smoothly transformed into each other without rupture
- Topological classification via Euler characteristic χ :

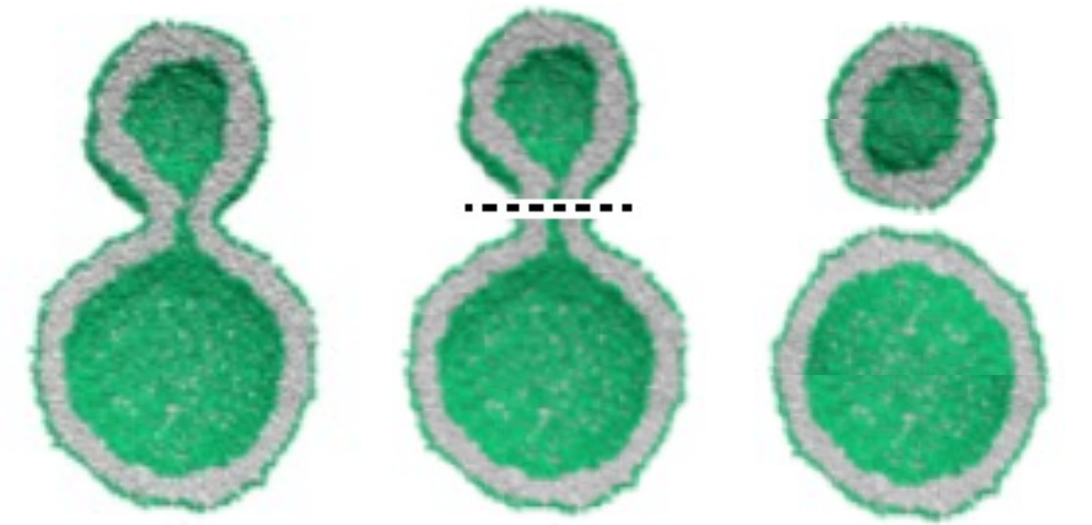


$\chi = 0$ $\chi = 2$ $\chi = 4$

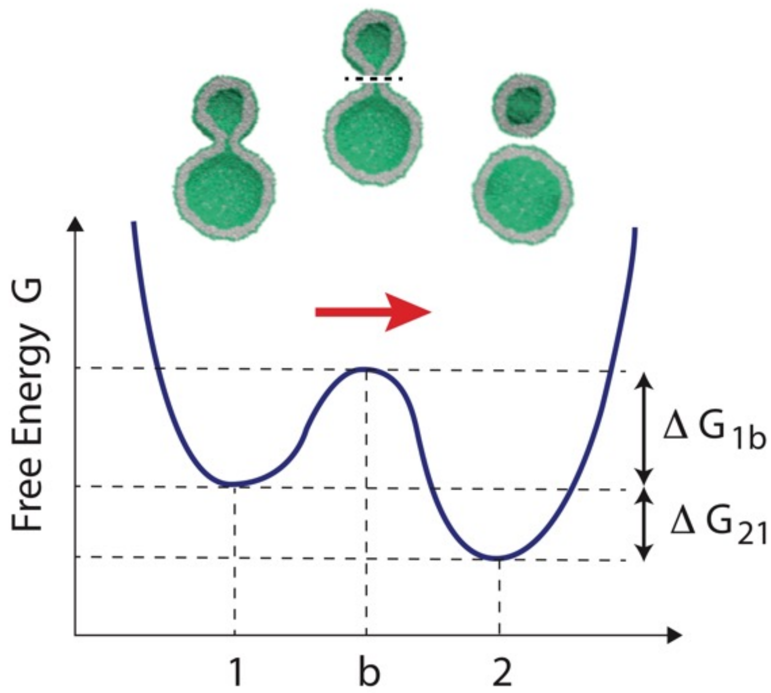
- Topological transformation $\Leftrightarrow$ change $\Delta\chi = \chi_{fin} - \chi_{ini}$
- Fission: Euler characteristic $\Delta\chi > 0$
- Fusion: Euler characteristic $\Delta\chi < 0$

Fission of Membrane Necks

- Membrane fission implies disrupture/cleavage of membrane
- Work of cleavage proportional to length of cut
- Shortest possible cut for dumbbell across membrane neck:

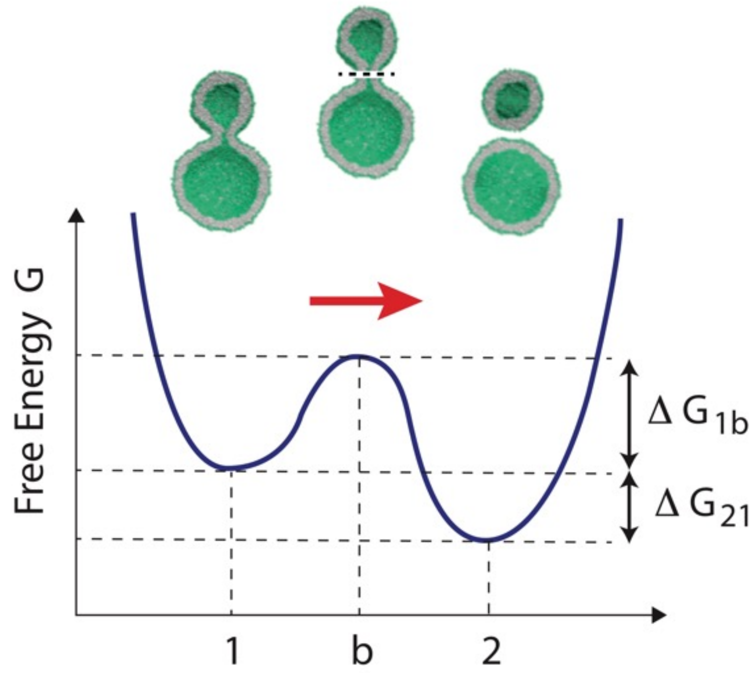


Dumbbell Broken neck Two spheres



Free Energy Landscape

- Free energy difference ΔG_{21}
- Free energy barrier ΔG_{21}
- Fission process is ‚downhill‘ or exergonic for negative ΔG_{21}
- Free energy barrier determines fission rate

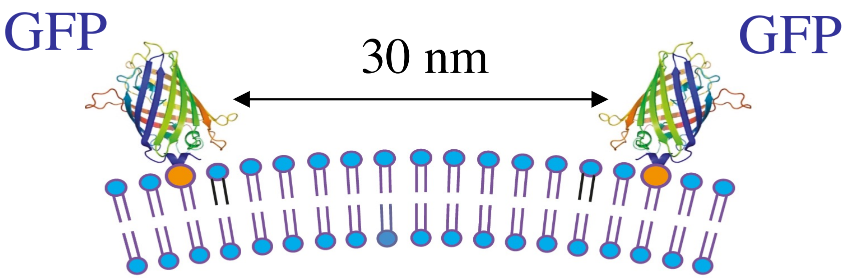


- ΔG_{21} dominated by Gaussian curvature energy E_G
- Change in Gaussian curvature energy $\Delta E_G = 2\,\pi\,\Delta\chi\,\kappa_G$ proportional to Gaussian curvature modulus κ_G
- Fission is ‚downhill‘ for negative κ_G

Fine Tuning of GUV Morphologies

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

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

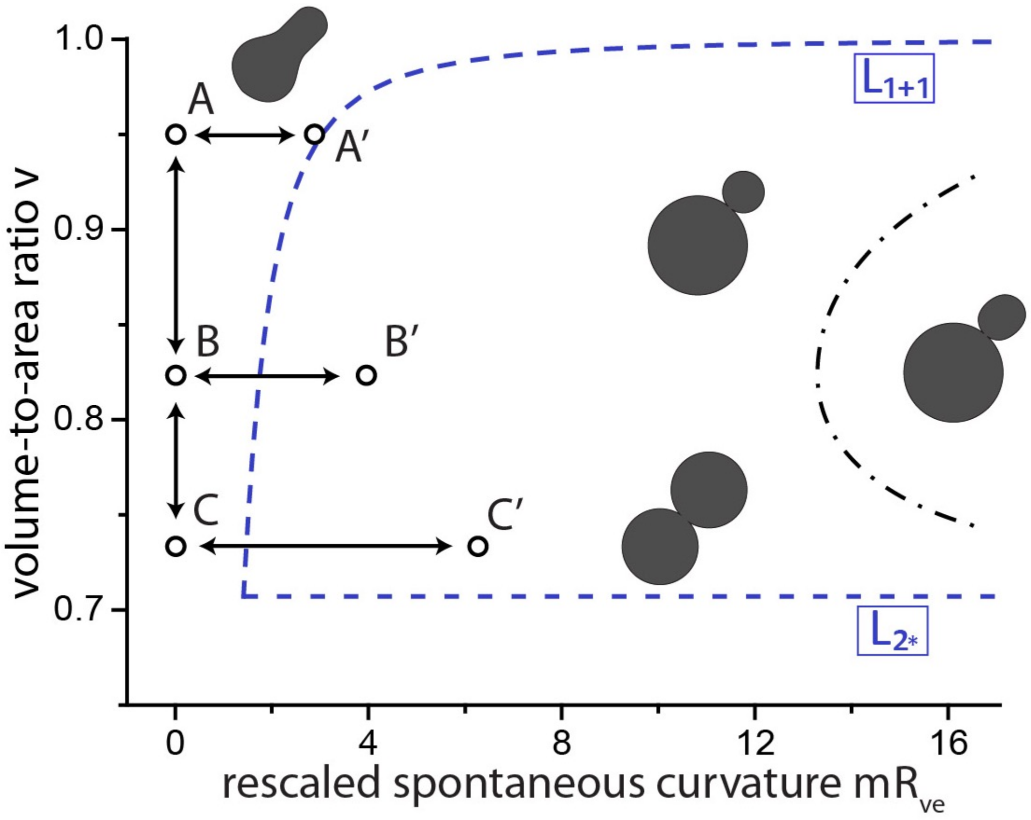


His-tagged GFP
NTA-lipids

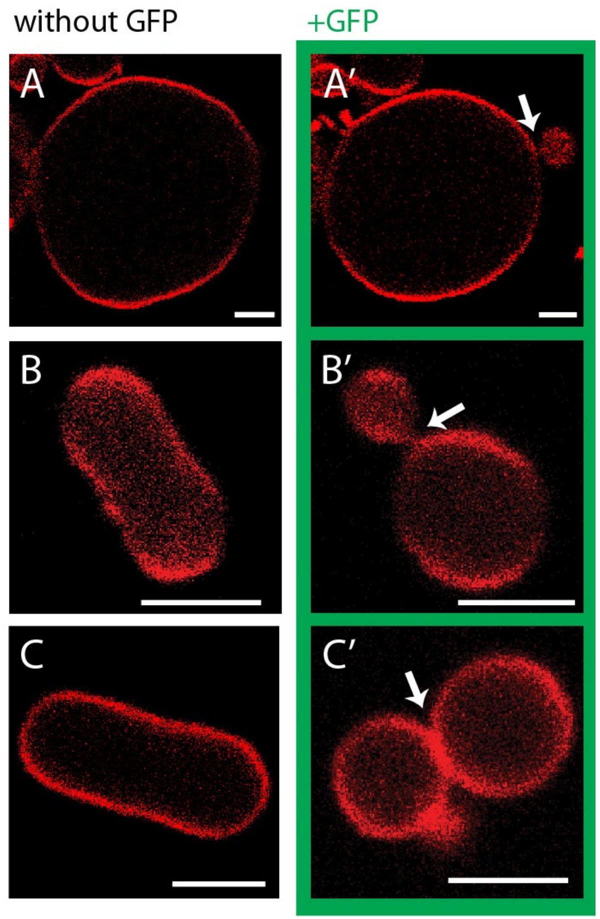
- Dilute regime, no crowding !
- Nanomolar GFP concentration X as control parameter
- Density Γ of bound GFP increases linearly with X
- Spont curvature m increases linearly with $\Gamma \sim X$

Controlled Budding of GUVs

- Morphology determined by volume and spont curvature (rescaled):



- Volume via osmotic conditions
  Sp-curvature via GFP concentration



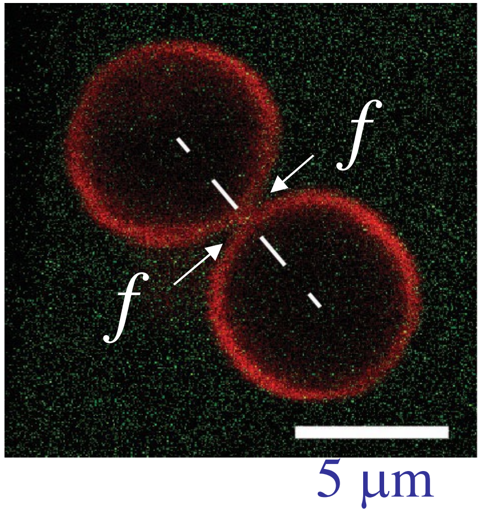
Steinkühler et al, *Nature Comm.* (2020)

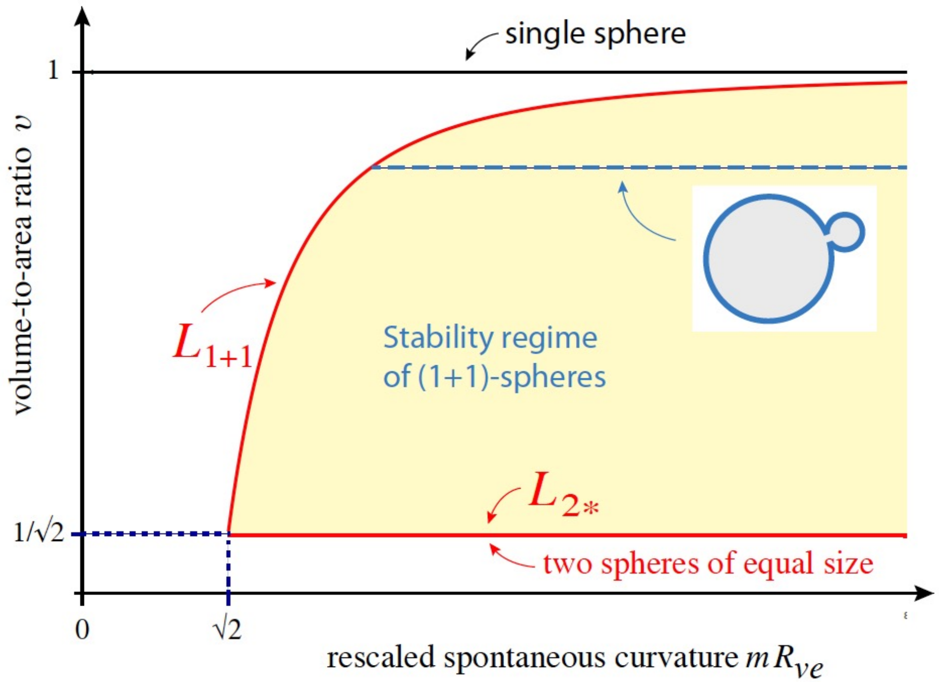
Constriction Force from Spont Curvature

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

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

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





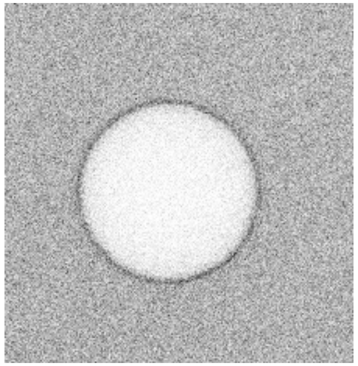
- Increase of m for fixed v
- Fixed shape of (1+1)-sphere
- Constriction force f increases

Neck Fission and Division of GUVs

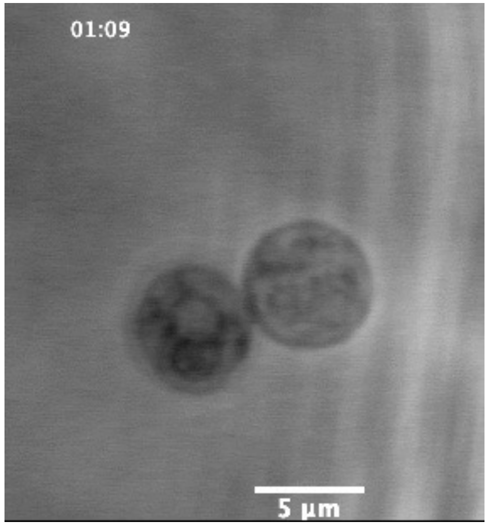
Steinkühler et al: *Nature Comm.* (2020)

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

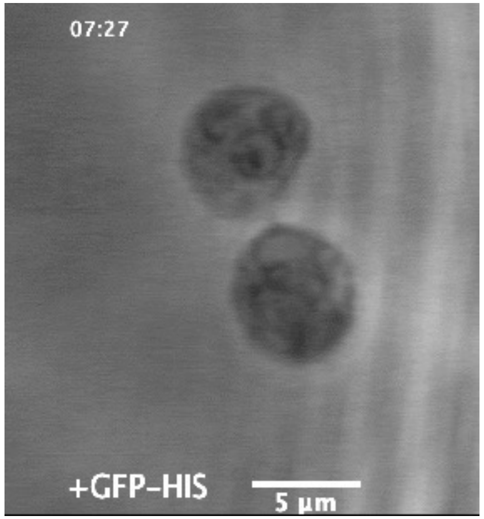
  Increase in GFP -> Neck cleavage -> Two daughter GUVs



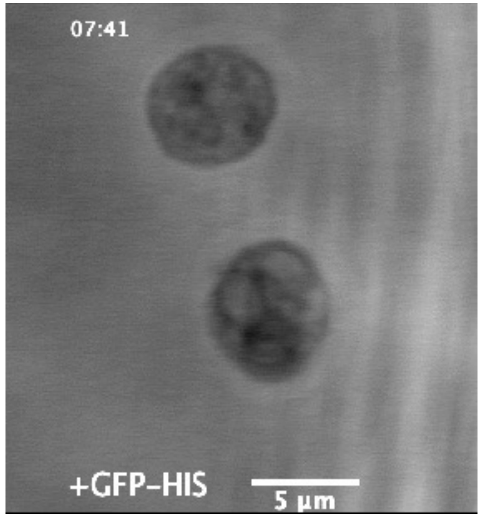
Adsorption of GFP onto GUV membrane



Deflation leads to dumbbell with membrane neck



Directly after neck cleavage

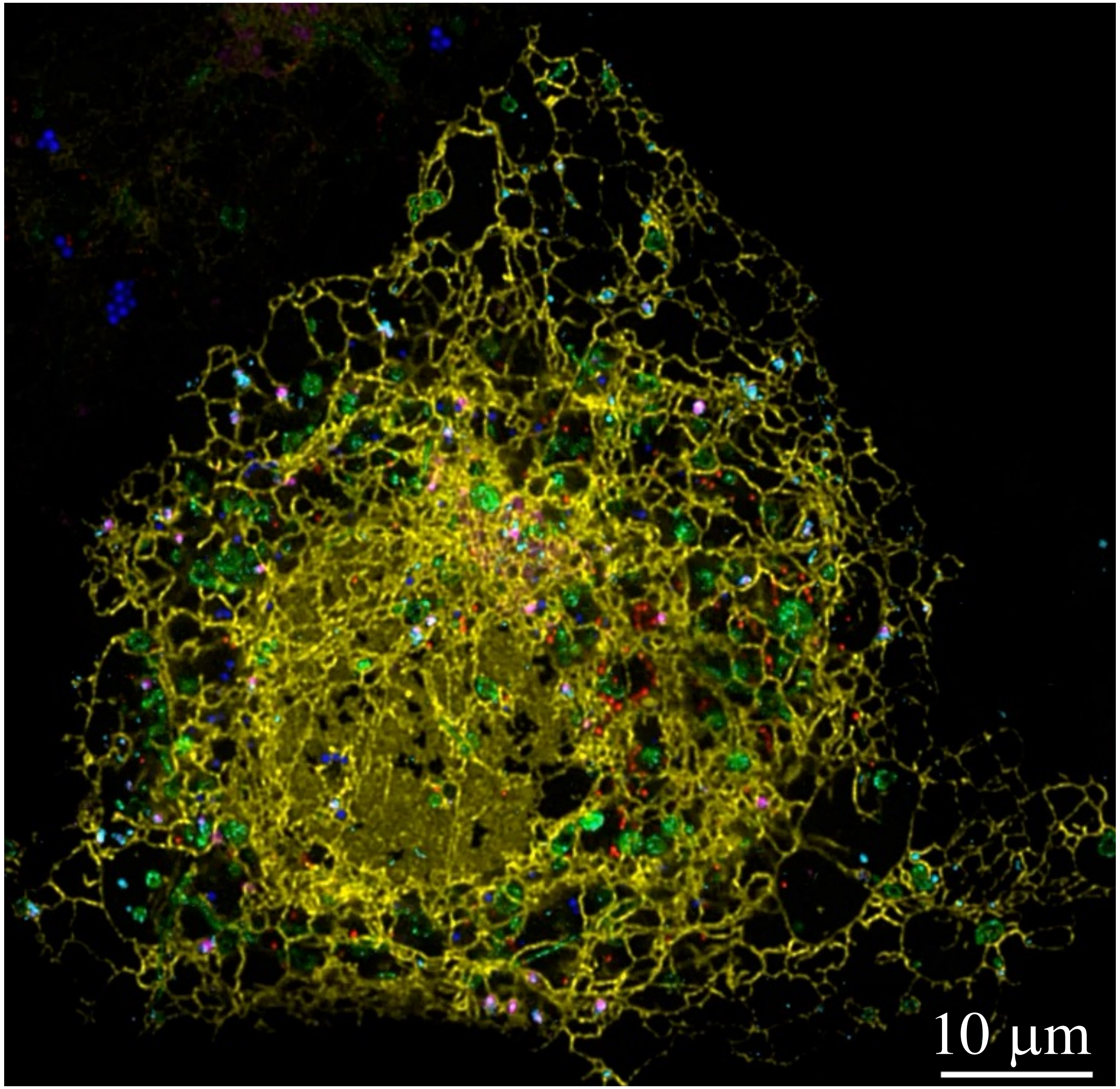


Complete division into two smaller GUVs

- Elasticity of Fluid Membranes
- Multispherical Shapes of Vesicles
- Constant-Mean-Curvature Surfaces
- Remodeling of Membrane Topology
- Outlook on Endoplasmic Reticulum

Morphological Complexity of ER

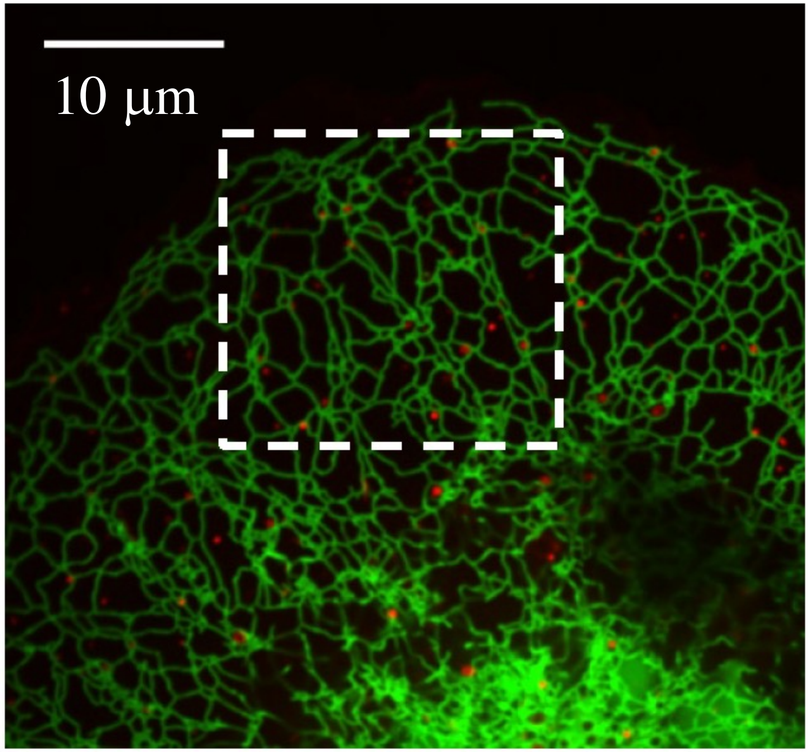
Valm et al. *Nature* (2017)

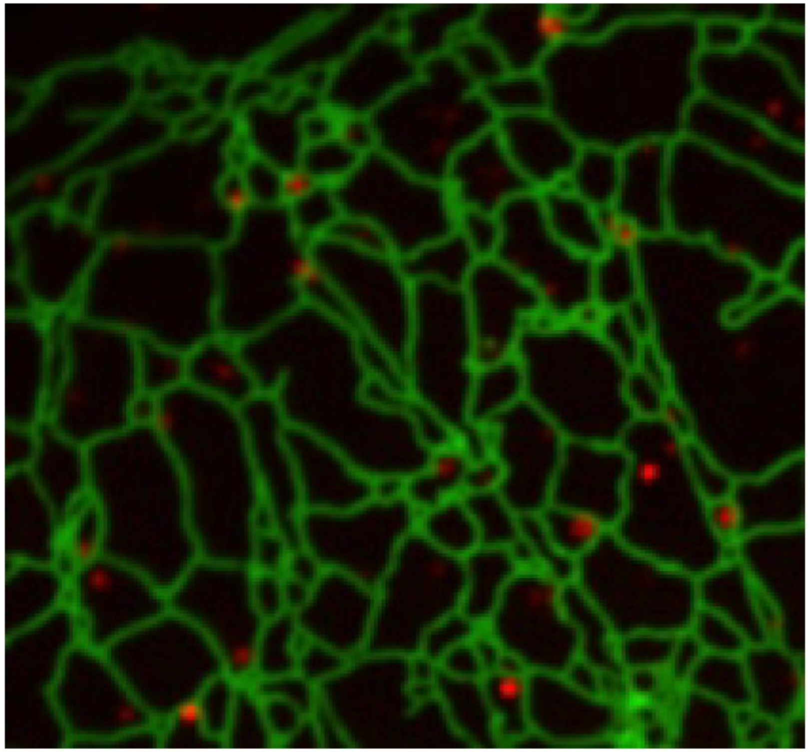


- Membrane-enclosed organelle
- Each eukaryotic cell contains only one ER
- Network of membrane nanotubes (yellow)
- Tubes have a width of ~ 80 nm
- Reticular network ~ cell size ~ 80 μm
- Meshsize of irregular polygons ~ 1 μm
- Network formed by a single membrane !

Reticular Networks, In Vivo

- Membrane nanotubes connected by junctions





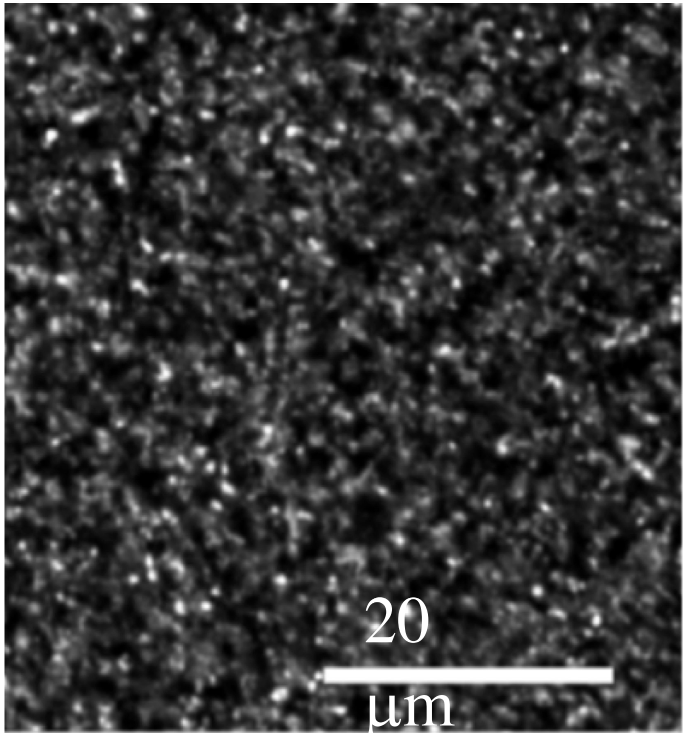
Friedmann, et al , *Mol Biol Cell* (2013)

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

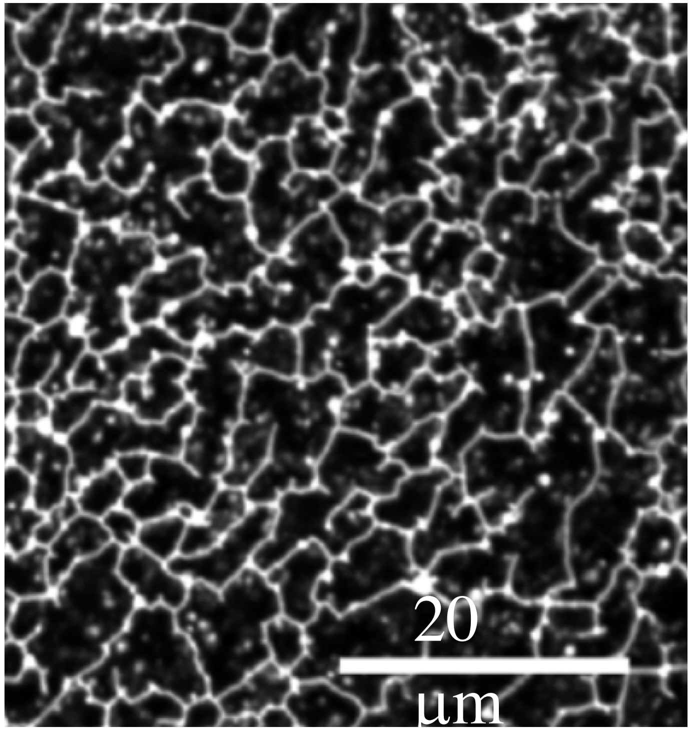
Reticular Networks, In Vitro

Powers et al, *Nature* (2017)

No GTP



+ GTP

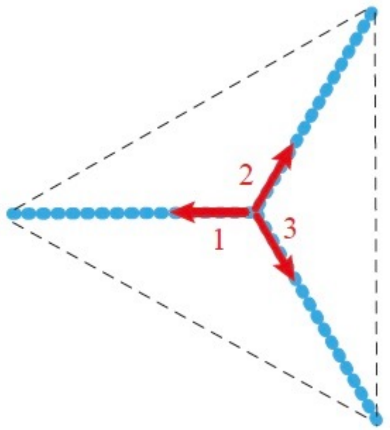


- Left: Proteoliposomes with membrane GTPase
- Right: Network formation after addition of GTP
- No cytoskeletal components, only membranes !

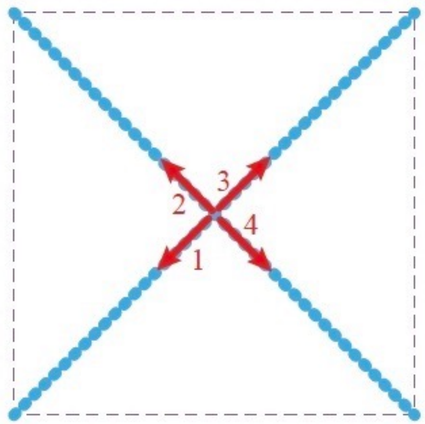
Junctions and Membrane Tension

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^{o} :



three-way

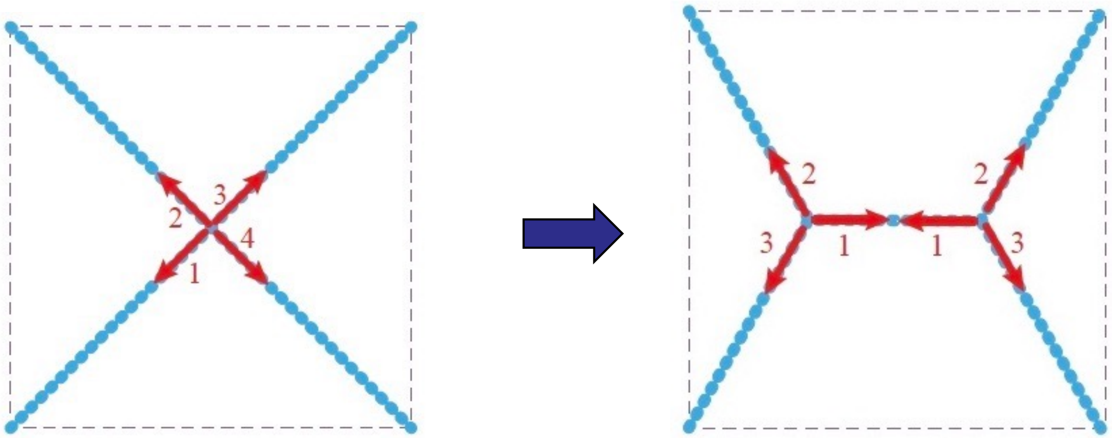


four-way

- Likewise, a stationary four-way junction between four nanotubes would lead to contact angles of 90^{o}

Prevalence of Three-Way Junctions

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



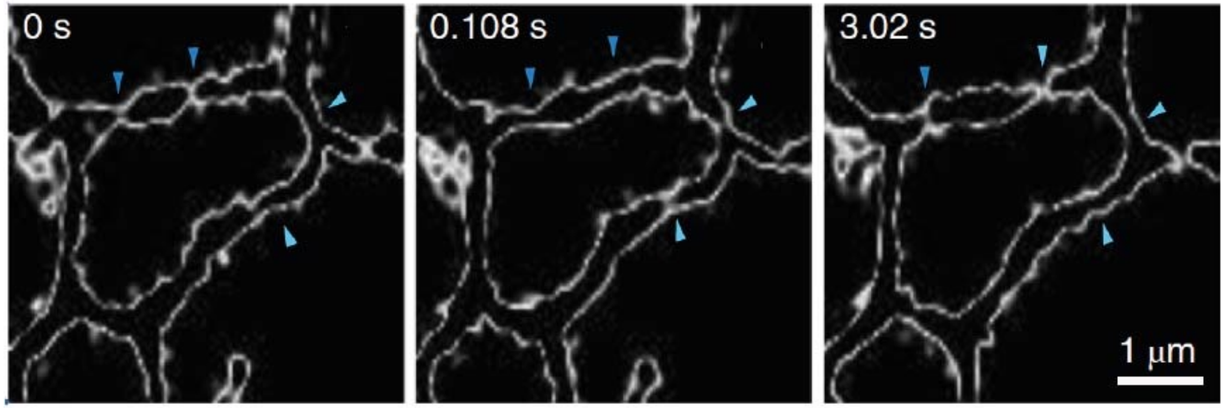
This transformation is possible because the ER membrane is fluid

- 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?

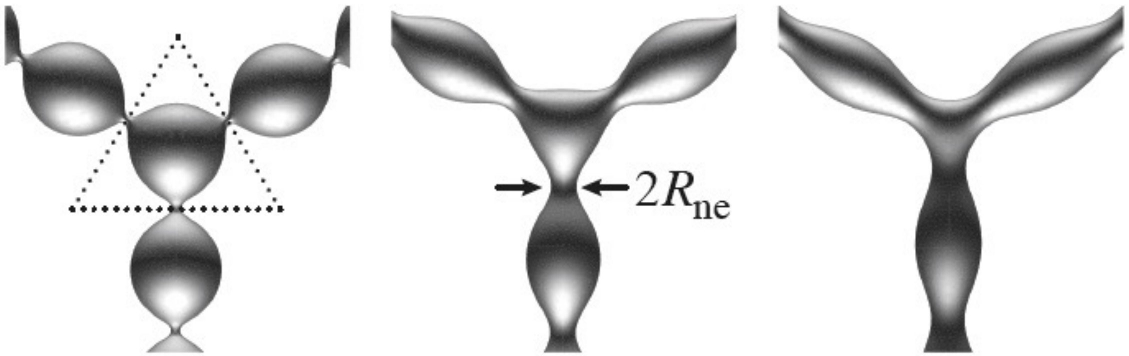
Images of Three-Way Junctions

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

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



- Single junction ~ fluctuating triunduloid !



Coworkers

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