Multiresponsive Behavior of Biomembranes and Vesicles

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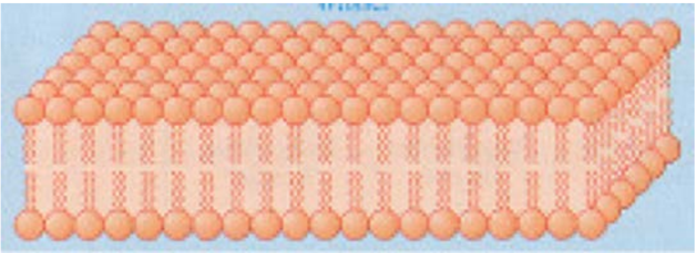
- Responses of Shape and Composition
- First 20 years, 1986 - 2006

- Aqueous Two Phase Systems
- Spontaneous Tubulation
- Engulfment of Nanoparticles

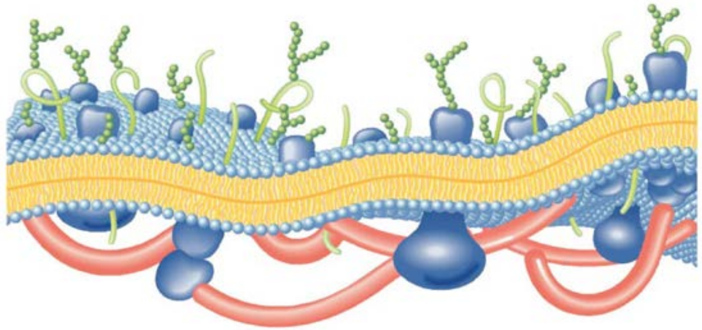
- Compositional Responses
- Ongoing Membrane Projects

Multiscale Membranes

• Lipid bilayer

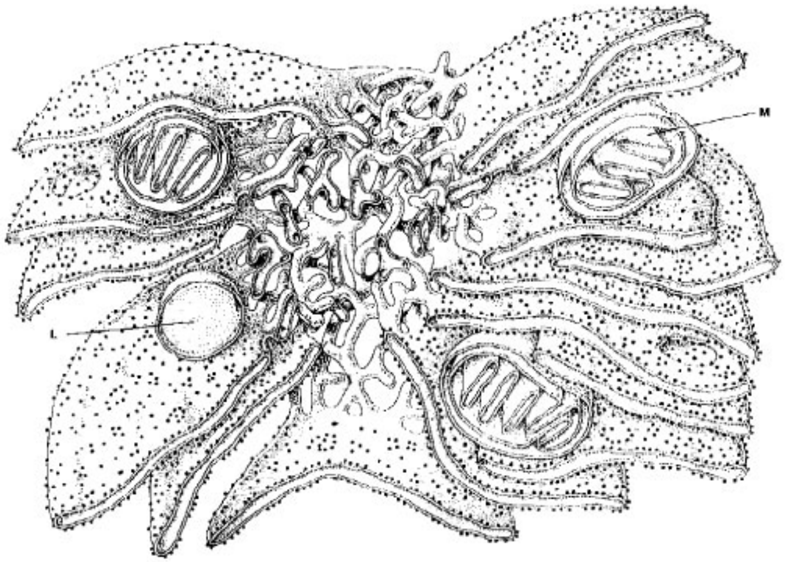


4 nm



• Biomembrane

400 nm



• Endoplasmic reticulum (ER)

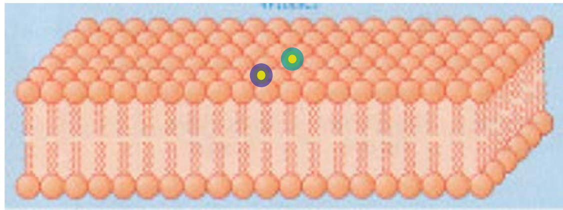
100 μm

• Animal cell

Membrane Fluidity

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

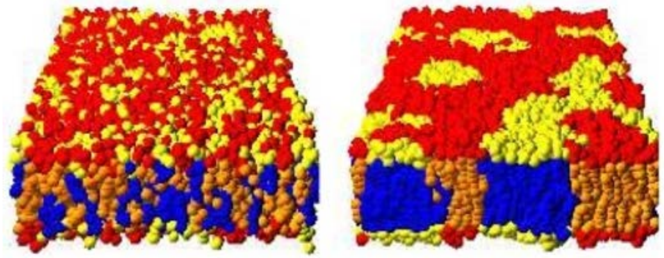
  Diffusion constant ~ μm^2/s



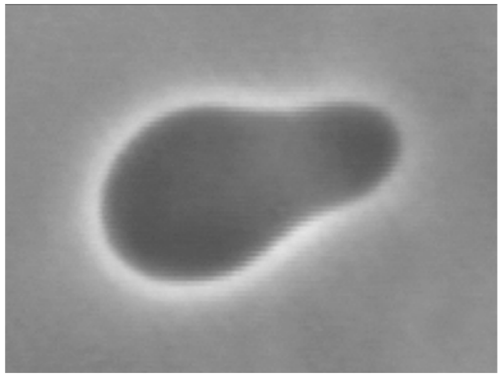
4 nm

lipid swapping ~ ns

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



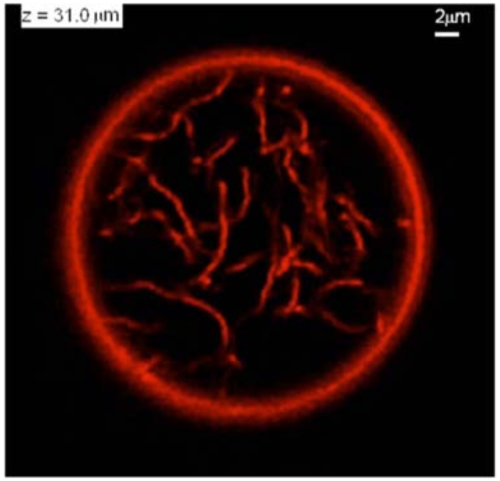
- Flexibility => Morphological responses, budding, tubulation, ... Direct evidence for fluidity



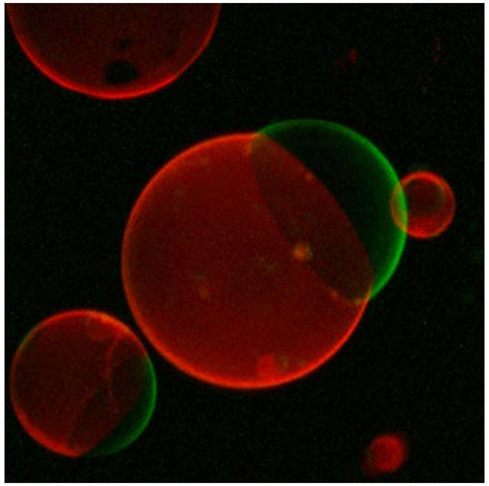
40 μm

Multiresponsive Behavior

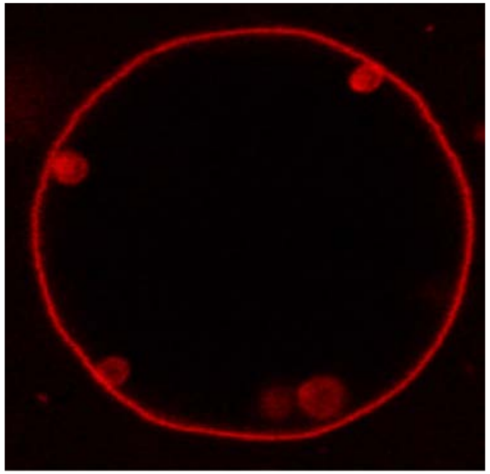
- Giant unilamellar vesicles (GUVs), tens of micrometers
- Remodelling in response to various perturbations:



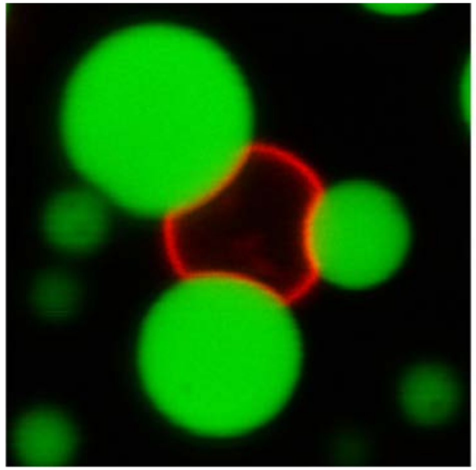
Nanotubes from polymer adsorption, tube width ~ 100 nm



Formation of intra-membrane domains, 2D phase separation



Small buds from protein adsorption, bud size ~ µm



Remodelling by adhering or partially wetting droplets

„Nothing is more practical than a good theory“

Immanuel Kant

„As simple as possible but not simpler“

Albert Einstein

Fruitful interplay between theory and experiment

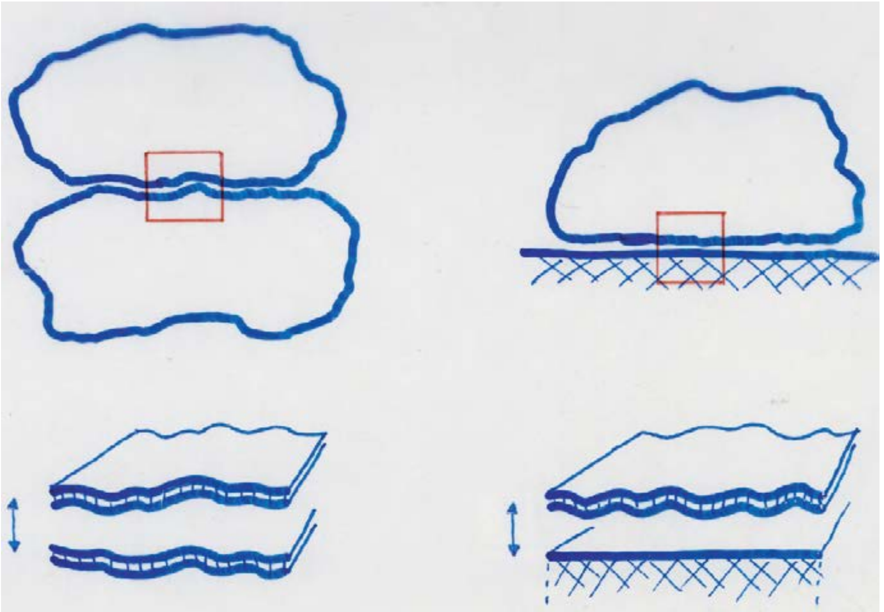
First 20 years, 1986 – 2006:

- Unbinding Transition
- Shapes of Vesicles
- Domain-induced Budding
- Molecular Bilayers

Interacting Membranes

- Unbinding transition,
  theoretical prediction

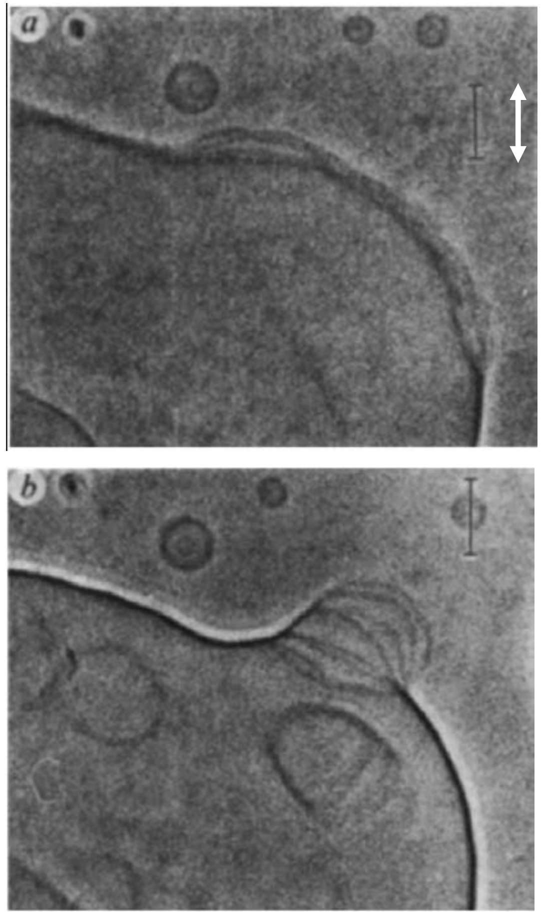
RL, Leibler: *PRL* (1986)



- Thermally excited undulations overcome van der Waals attraction
- Continuous transition

- Unbinding transition,
  experimental observation

Mutz, Helfrich: *PRL* (1989)



10 μm

22.4 ° C

22.1 ° C

Shapes of Vesicles

- Shape energy of a vesicle

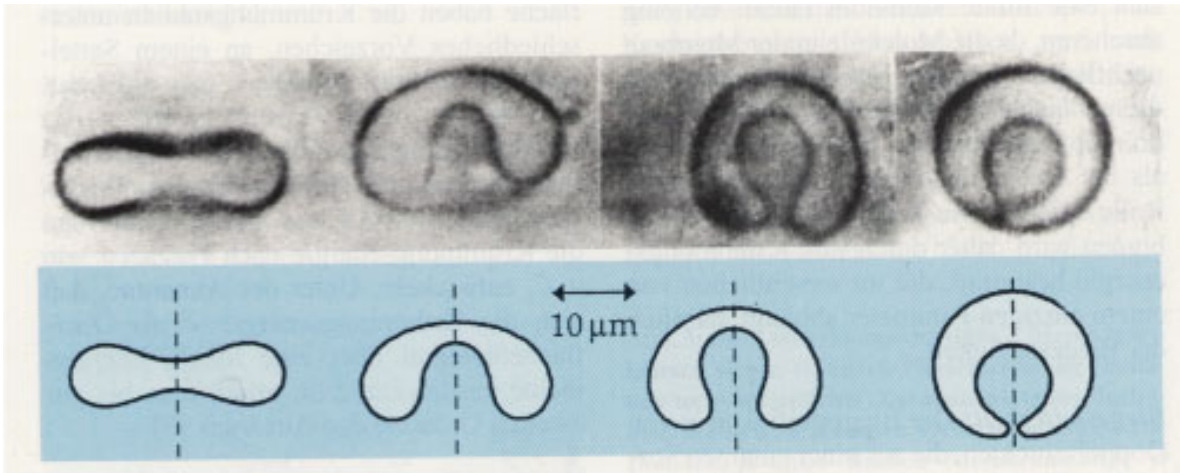
Deuling, Helfrich: *J. Physique* (1976)
Seifert et al: *Phys. Rev. A* (1991)

$$E\{\text{Shape}\} = -\Delta P\, V + \Sigma\, A + \text{curvature energy}$$

- Two geometric parameters: volume V, area A
- Two elastic parameters: bending rigidity κ, spont curv m
- Use κ and A to define energy and length scale

=> only two dimensionless parameters: $V/A^{3/2}$ and $m\,A^{1/2}$

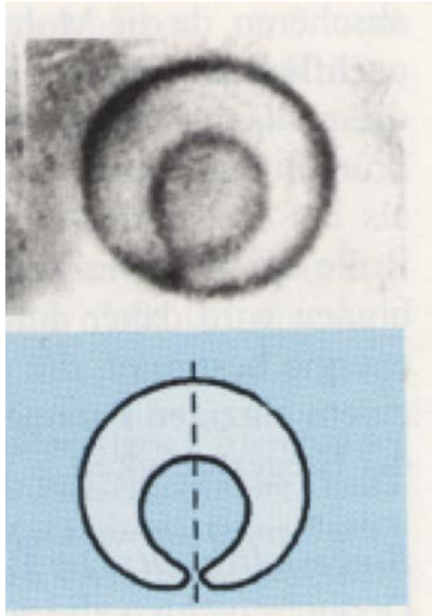
- Collaboration with Erich Sackmann
- Temperature-induced budding



Berndl et al: *Europhys. Lett.* (1990)

Budding and Membrane Necks

- Mother vesicle forms large sphere with radius R_1 and mean curvature $M_1 = 1/R_1$
- In-bud forms small sphere with radius R_2 and mean curvature $M_2 = -1/R_2$
- In-bud and mother vesicle are connected via narrow, funnel-like membrane neck



- Limit shape arising via closure of open necks
- Neck closure condition: $M_1 + M_2 = 2m$
- Relation between geometry and elastic parameter !
- Giant vesicles: both M_1 and M_2 can be measured

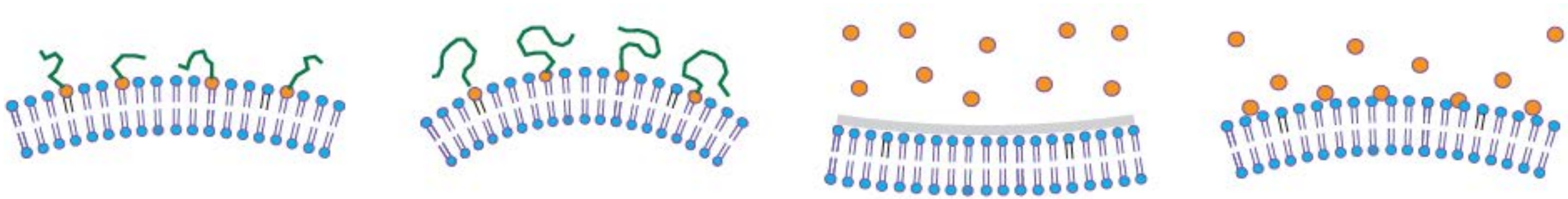
  Example: negative spont curv $m = -1/(8.6\ \mu m)$

Seifert et al: *Phys. Rev. A* (1991)

Spontaneous Curvature

- Spont curvature m describes bilayer asymmetry
- Many molecular mechanisms can generate asymmetry
- Mechanisms with universal features:

RL, Döbereiner: *Europhys. Lett.* (1998)



Anchored polymers, longer chains increase m

Depletion of solutes

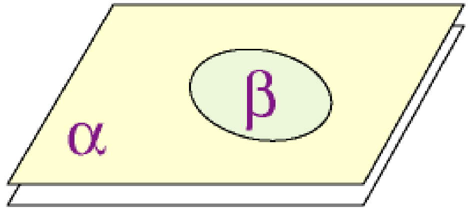
Adsorption of solutes

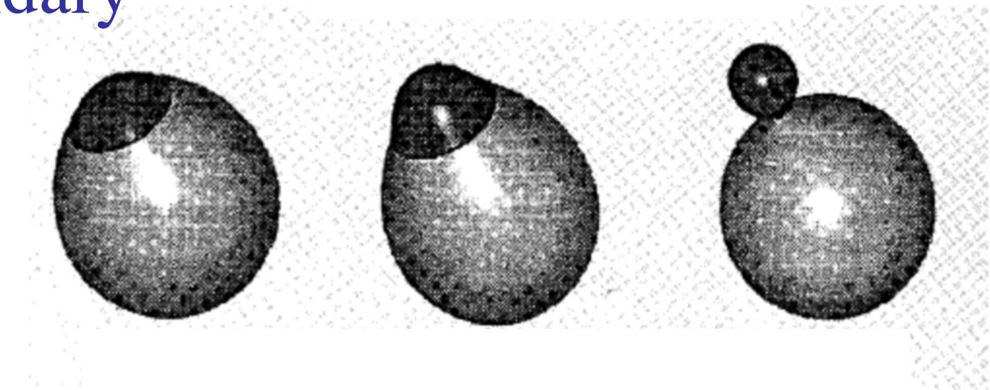
- Spontaneous curvature describes response of bilayer membrane to asymmetric molecular interactions

Domain-Induced Budding

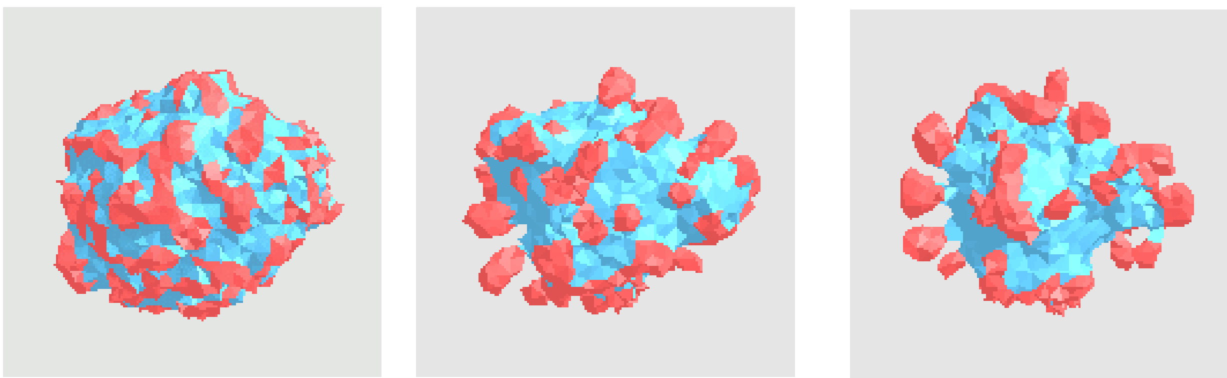
RL: *J. Physique* (1992) Jülicher, RL: *PRL* (1993)

- Intramembrane α and β domains separated by domain boundaries
- Line tension λ of domain boundary as a new membrane parameter
- Competition of line tension λ, rigidity κ, and spont curv m





- Coarsening and budding of many domains:



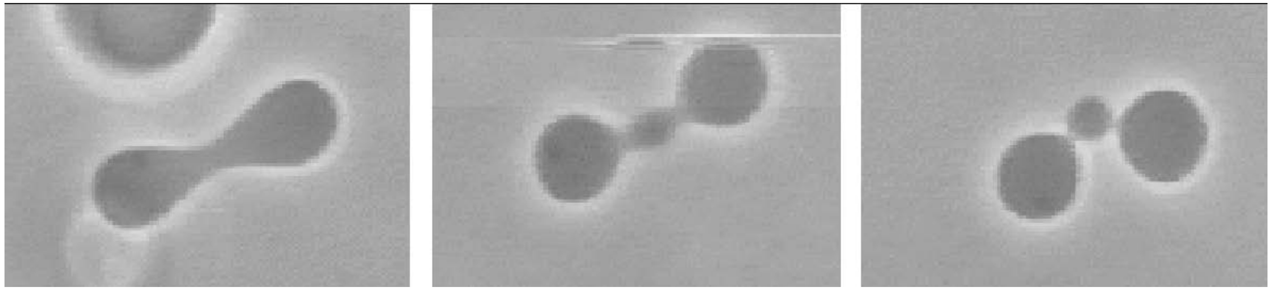
MC simulations of 2-component vesicles

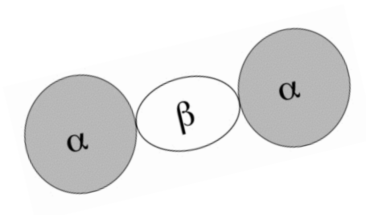
Kumar et al: *PRL* (2001)

Domain-Induced Buds: Experiment

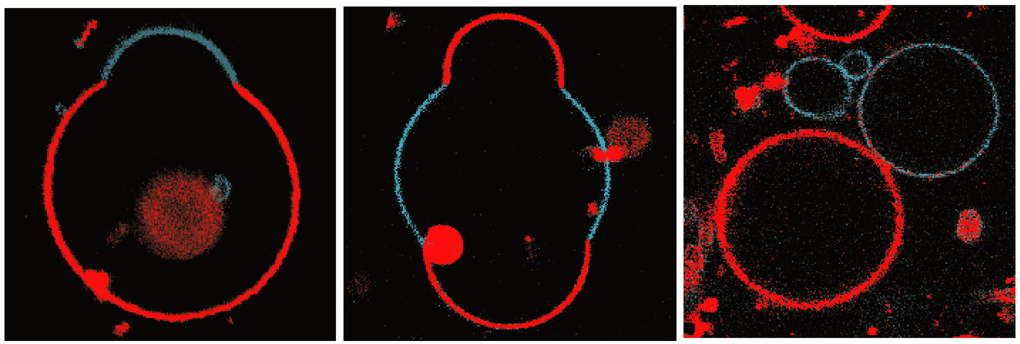
- Lipid mixture of DOPC/cholesterol/spingomyelin
- Deflation of vesicle -> budded shapes
- Vesicles with three (curvature) domains, presumably formed by two lipid phases:

RL, Dimova: *J. Phys. CM* (2003)





- Same lipid mixture + two fluo-labels:



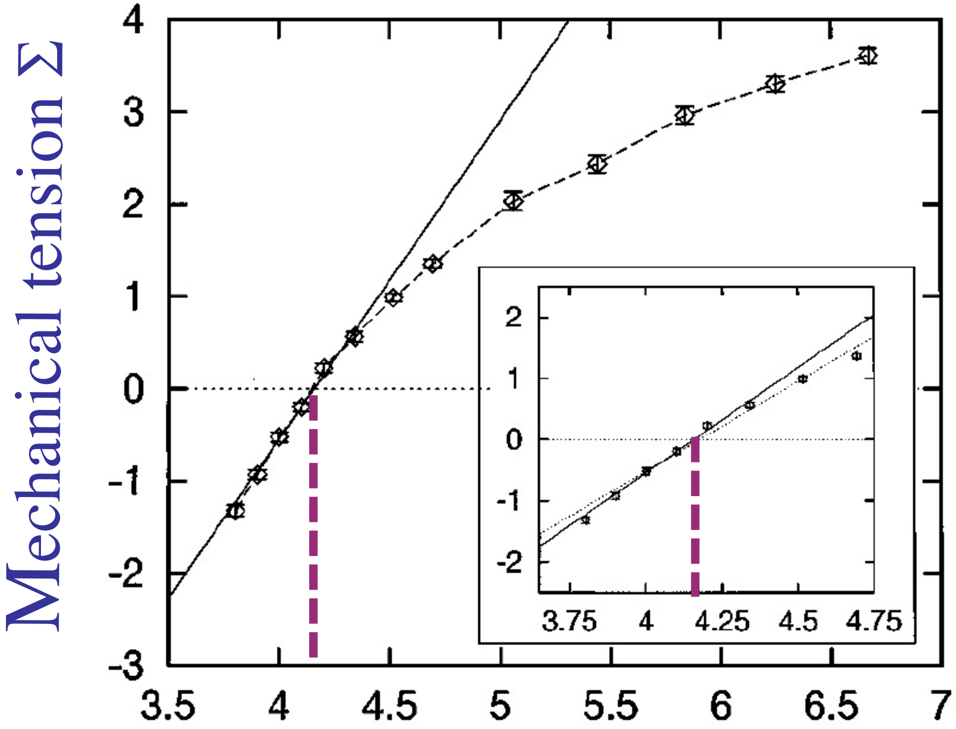
Bacia et al: *PNAS* (2005)
Semrau et al: *PRL* (2008)
and other groups

Baumgart et al: *Nature* (2003)

Molecular Bilayers: Simulations

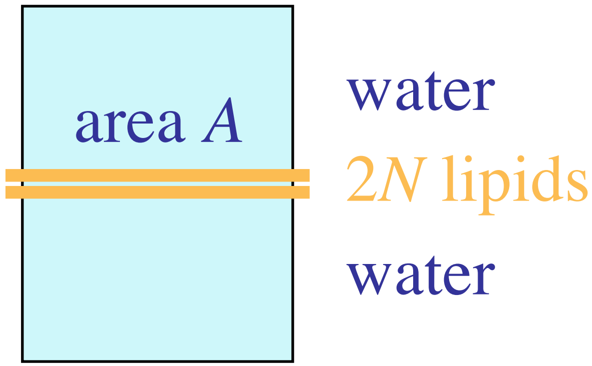
Goetz, RL: *J. Chem. Phys.* (1998)

- Mechanical tension Σ determined by molecular area A_{mo}



Molecular area A_{mo}

simulation box:



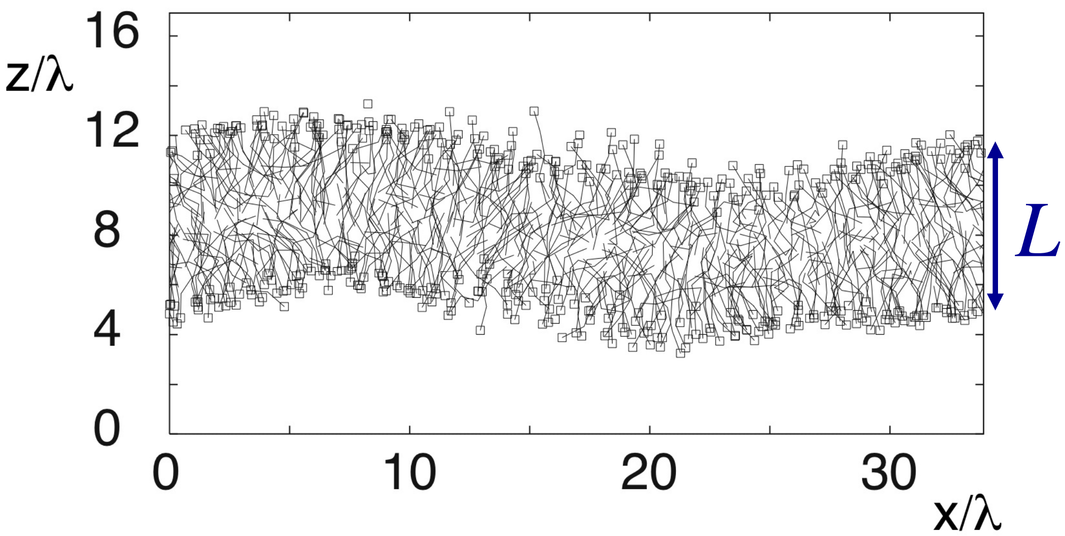
$A_{mo} = A / N$

- Tensionless state for molecular area $A_{mo} = A_0$
- Area compressibility modulus K_A from slope of $\Sigma\,(A_{mo})$ at A_0

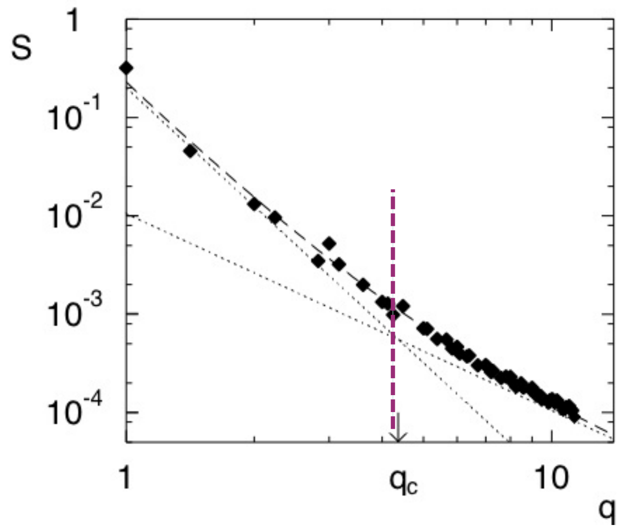
Emergence of Bending Rigidity

Goetz et al: *PRL* (1999)

- Tensionless membrane
  => bending fluctuations



- Fluctuation spectrum $S(q)$



- Bending rigidity κ emerges for wave length > 6 nm

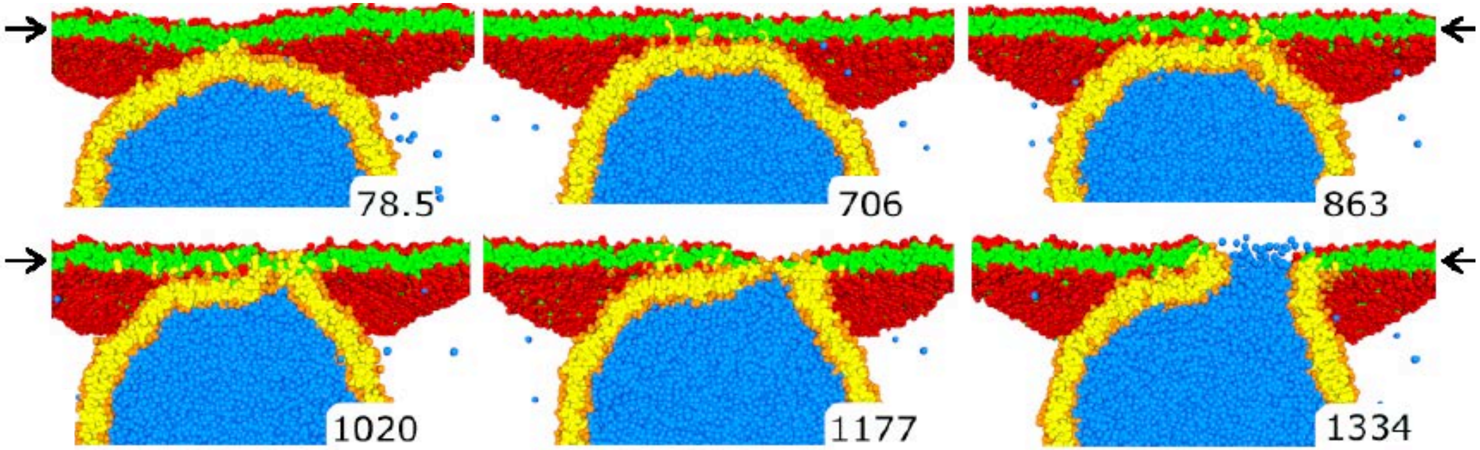
- Quantitative relation: $\kappa = K_A L^2 / 48$

K_A = area compressibility modulus

Membrane Fusion

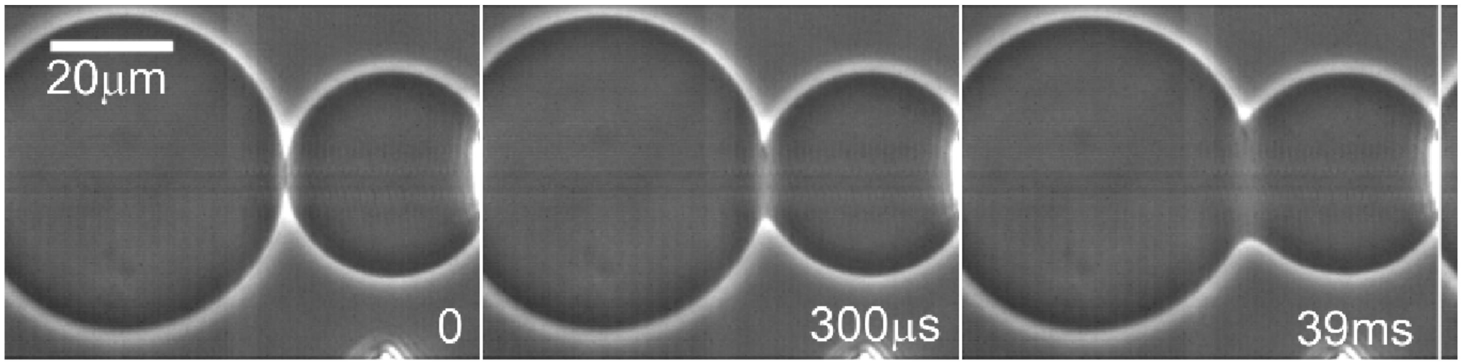
- Computer Simulations: Fusion induced by tension

Shillcock, RL: *Nature Materials* (2005) Grafmüller et al: *PRL* (2007)



- Micropipette Experiments: Fusion induced by specific adhesion

Haluska et al: *PNAS* (2006)



„Nothing is more practical than a good theory“

Immanuel Kant

„As simple as possible but not simpler“

Albert Einstein

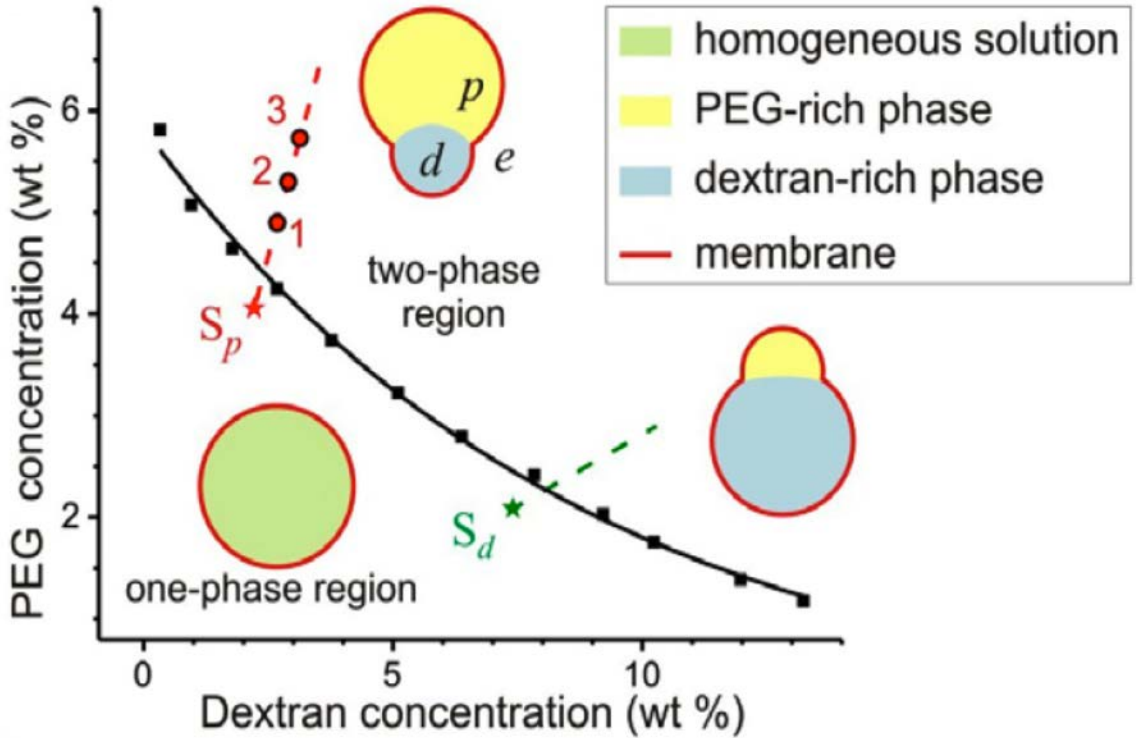
Fruitful interplay between theory and experiment II

- Aqueous Two Phase Systems
- Spontaneous Tubulation
- Engulfment of Nanoparticles
- Compositional Responses
- Ongoing Membrane Projects

GUVs + Aqueous Phase Separation

Li, RL, Dimova, *JACS* (2008); *PNAS* (2011)
Liu, Agudo. ... RL, *ACS Nano* (2015)

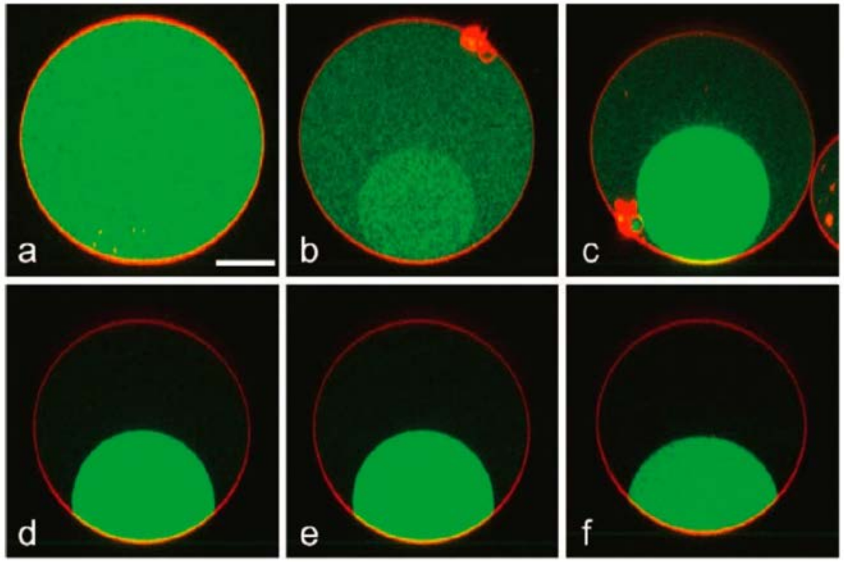
- GUVs filled with aqueous polymer solution
- Example: PEG and dextran
- Increase polymer concentration via deflation:

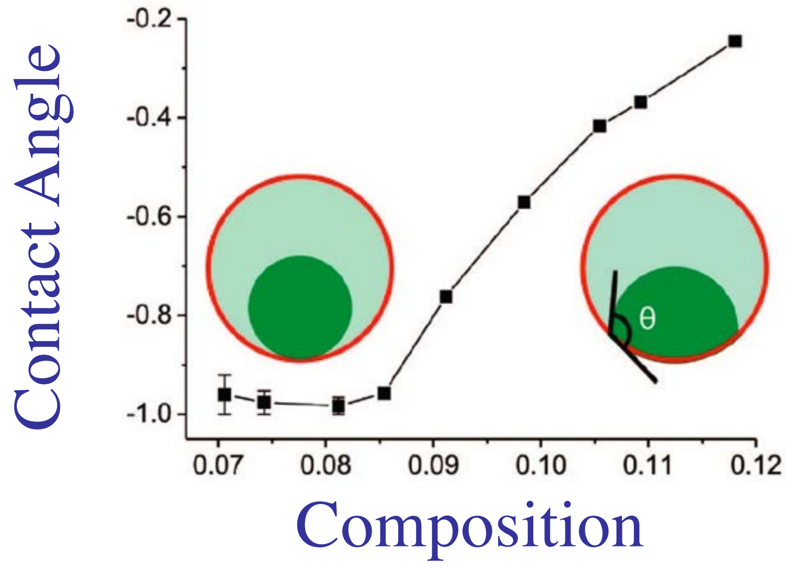


1st Surprise: Wetting Transition

Li et al, *JACS* (2008)

• Shape evolution for vesicle during deflation:





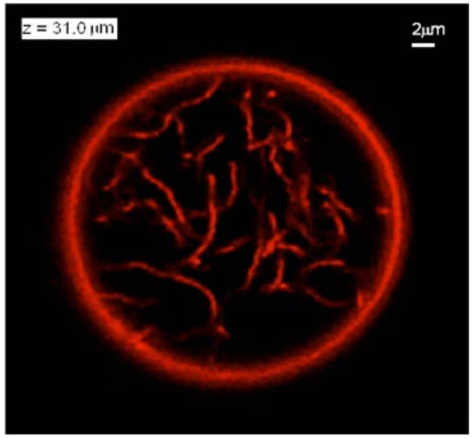
• Low deflation: zero contact angle => complete wetting

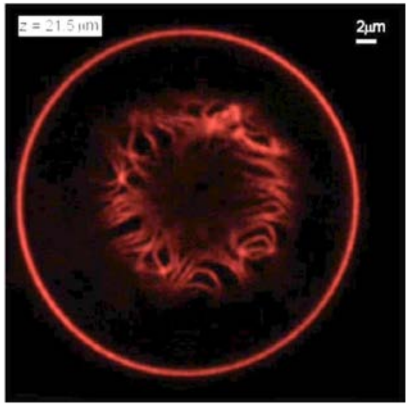
• High deflation: finite contact angle => partial wetting

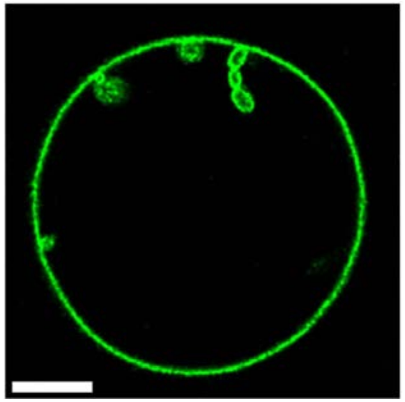
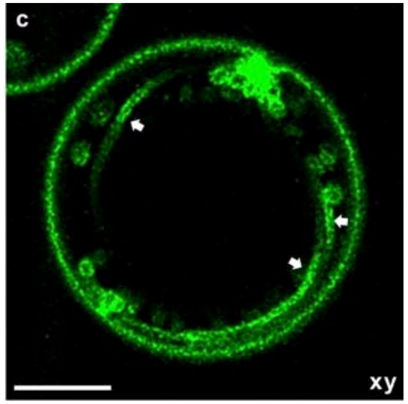
2nd Surprise: Membrane Nanotubes

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

- Membranes labeled by fluorescent dyes
- Lipid mixture of DOPC, DPPC, cholesterol
- Liquid-disordered (red) and liquid-ordered phase (green)





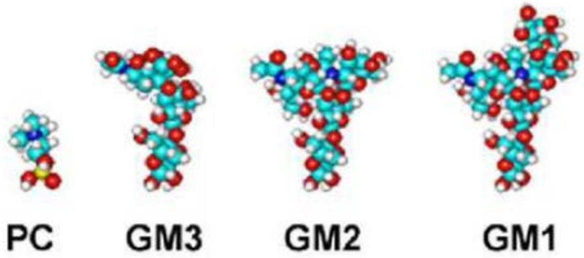



- Spontaneous tube formation without external forces
- Inward-pointing tubes reveal large negative spont curv
- Tubes can be necklace-like or cylindrical

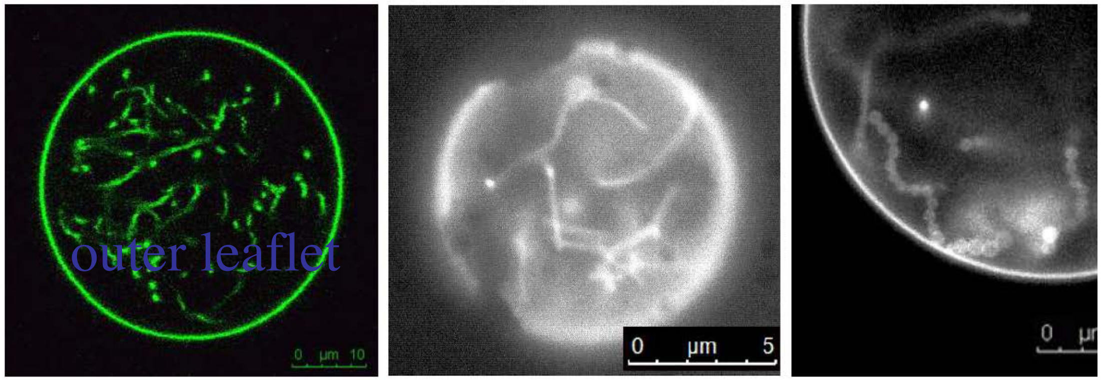
Spont Tubulation of GM1 Membranes

Rumiana Dimova

- Mixture of POPC and ganglioside (GM1):



- Spontaneous formation of in-tubes:

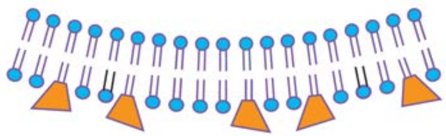


POPC + 10 % GM1

outer leaflet

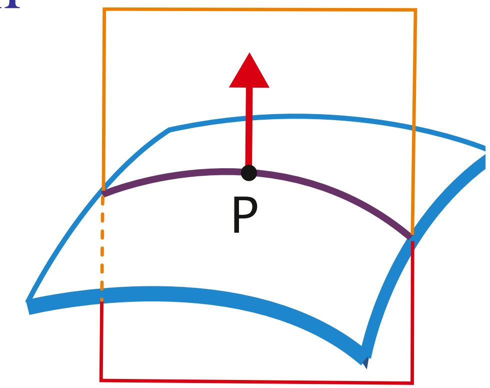
inner leaflet

- In-tubes reveal negative spont curv:



Uniform Membranes

- Basic assumption: laterally uniform composition
- Laterally uniform elastic parameters κ and *m*
- Shape described by local mean curvature *M*
- Curvature energy: $$E_{cu} = \int dA\, 2\, \kappa\, (M - m)^2$$

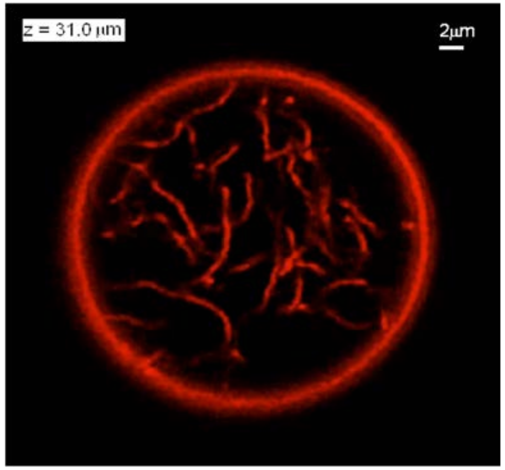


- Membrane prefers mean curvature *M* = spont curv *m*

- In contact with adhesive surface, contact area A_{bo}
- Adhesion energy: $E_{ad} = - W A_{bo}$
- Adhesive strength W > 0 is binding free energy per area
- Membrane wants to spread onto adhesive surface

Spontaneous Tubulation: Theory

RL, Faraday Discuss. (2013)



- Theoretical analysis based on separation of length scales: $R_{tu} \ll R_{ve}$
- Shape energy as shape functional: Euler-Lagrange equation + scale transform
- Decomposition of membrane tension:

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

Spontaneous tension $\boxed{\sigma = 2\,\kappa\, m^2}$

Mechanical tension $\Sigma \approx -\,\sigma\, R_{tu}/R_{ve} \ll \sigma$

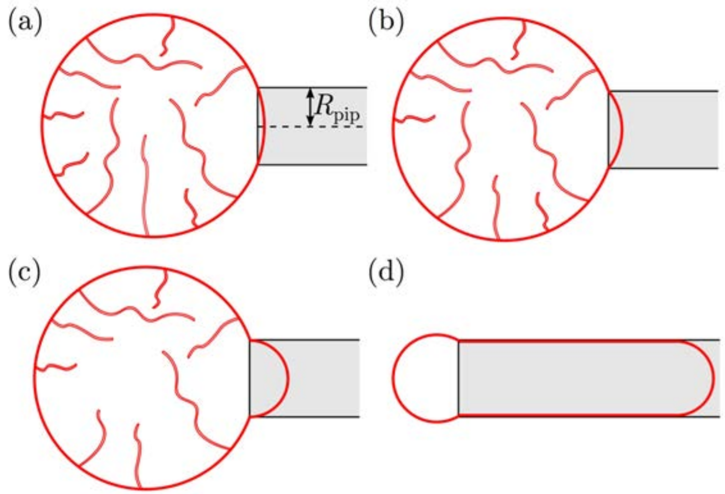
$\Rightarrow$ Membrane tension is dominated by spontaneous tension σ

$\Rightarrow$ Spont curv m from tension $\hat{\Sigma} \approx \sigma = 2\,\kappa\, m^2$

Spont Tension from Experiment

Bhatia et al (under review)

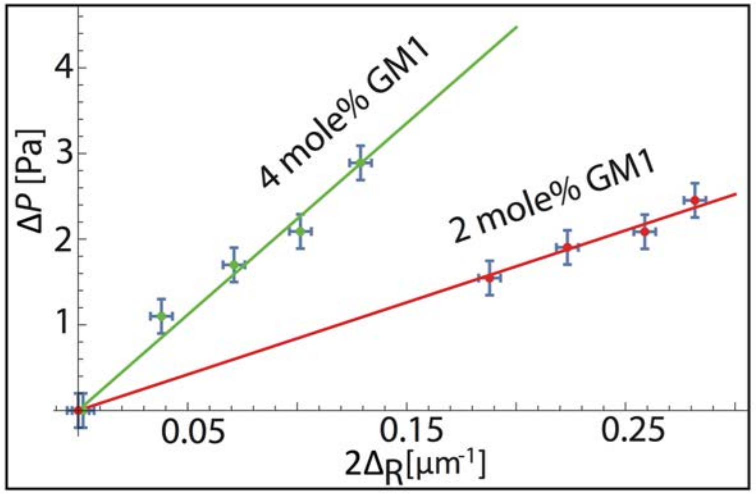
- Retraction of tubes by micropipettes:



Initial aspiration up to hemispherical tongue

then vesicle starts to flow into micropipette

Aspiration pressure



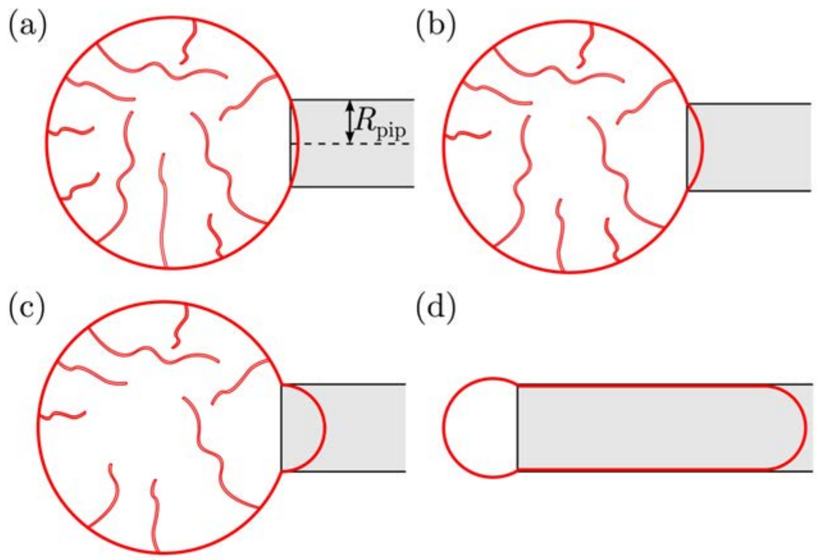
Initial aspiration:

Aspiration pressure versus geometric quantity Δ_R

Slope = spontaneous tension σ

Robustness of Tubulated Vesicles

- Mechanical tension Σ << spontaneous tension σ
- Nanotubes provide area reservoir for mother vesicle
- More robust against mechanical perturbations
- Example: Micropipette aspiration



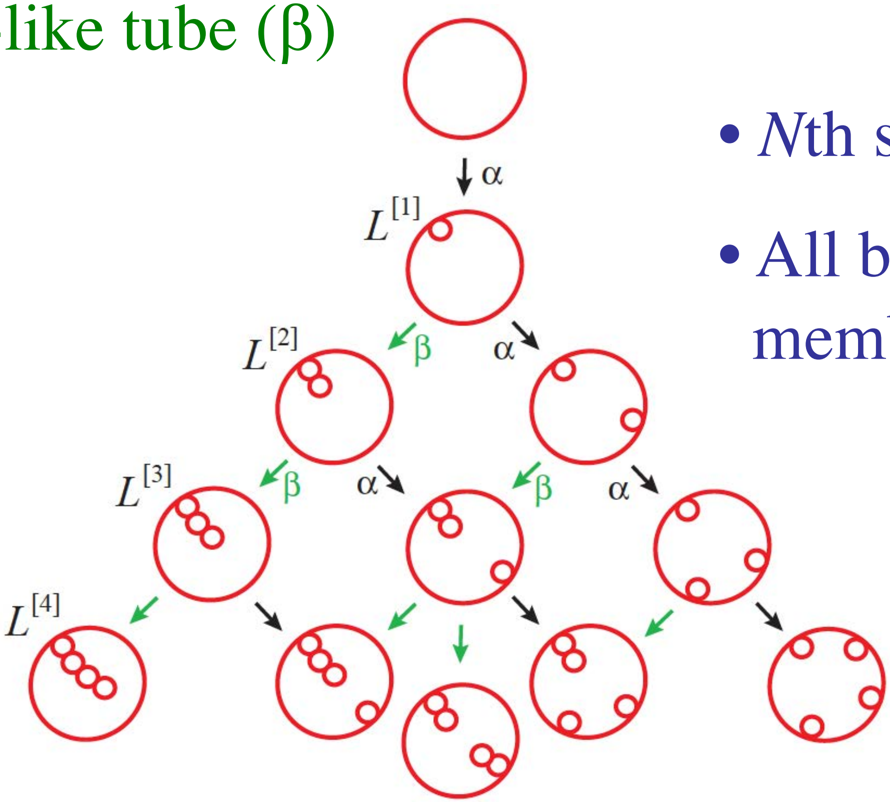
Initial aspiration up to hemispherical tongue

then vesicle starts to flow like a liquid droplet

- Mother vesicle = droplet with interfacial tension σ

Nucleation and Growth of Tubes

- Vesicle membrane with large spont curv *m*
- Osmotic deflation of GUV in discrete steps
- At each step, nucleation of new bud (α) or extension of necklace-like tube (β)

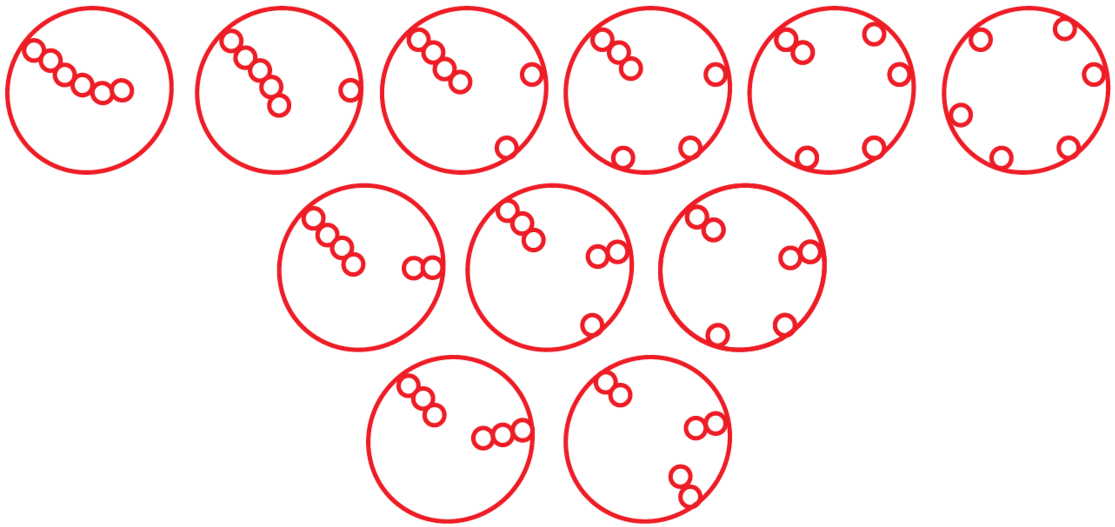


- *N*th step leads to *N* in-beads
- All beads are connected by membrane necks (not visible)

=> Buds are nuclei for necklace-like tubes

Morphological Complexity

- After 6th step, 11 morphologies with 6 beads:



- All beads are connected by membrane necks
- All morphologies have the same area, volume, and curvature energy
- Energy landscape contains 11 intersecting branches
- For large N, # of N-bead morphologies grows as exp[c √N]

Engulfment of Nanoparticles

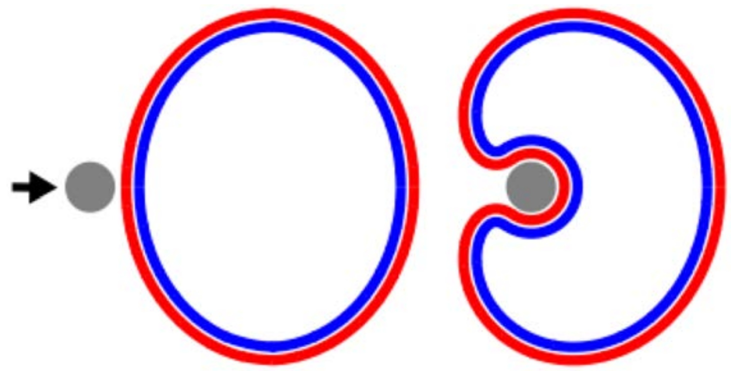
Agudo-Canalejo, RL: *ACS Nano* (2015) *Nano Letters* (2015)
Soft Matter (2016) and (2017)

- Nanoparticles interacting with membranes, vesicles and cells:

  biomedical imaging,

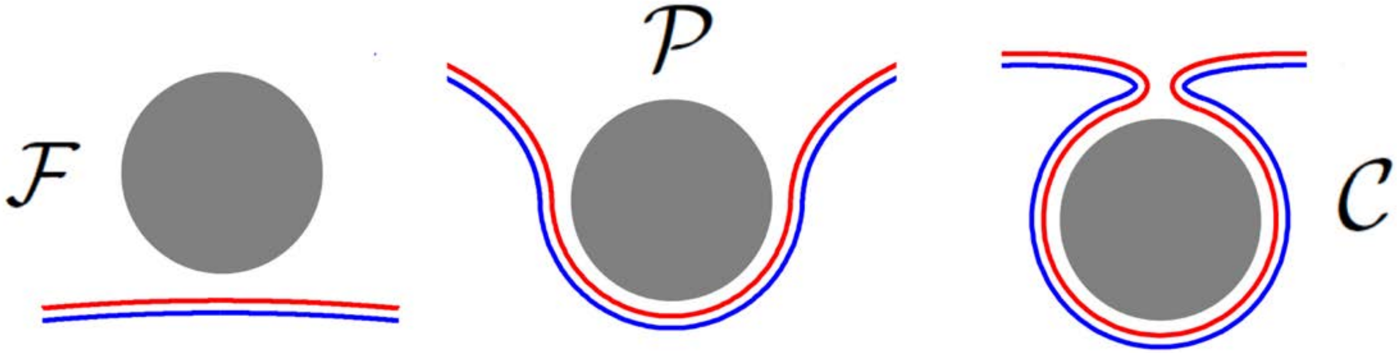
  drug delivery, nanotoxicity,

  endocytosis, virus infection ...



- Important control parameters:
  - Adhesive strength W ~ surface chemistry
  - Particle size R_{pa}
  - Spontaneous curvature m

(In)Stability of Particle States



Free

Partially engulfed

Completely engulfed

Onset of engulfment

Opening of neck

- Competition between adhesion and bending:

Adhesion length $R_W = (2\kappa/|W|)^{1/2}$

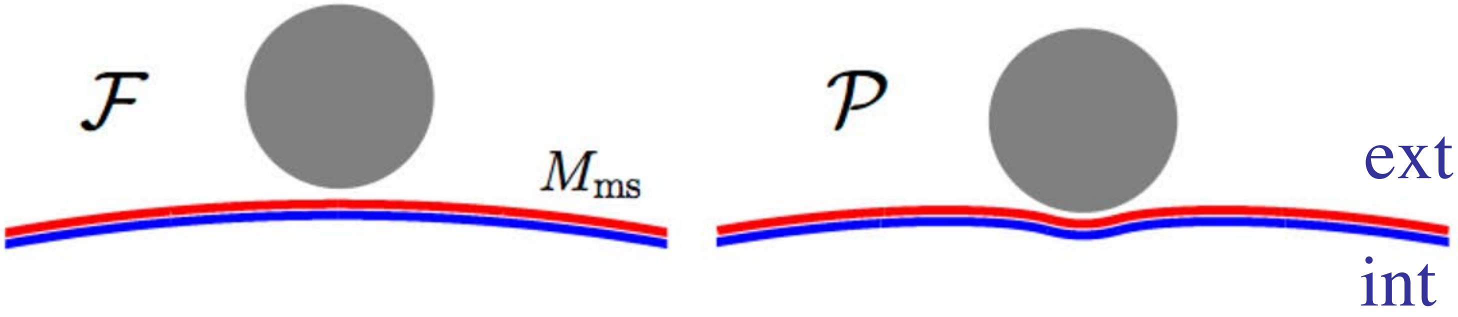
Basis Length = Adhesion Length

- Competition of adhesive strength W and bending rigidity κ
- Adhesion length $R_W = (2\kappa/|W|)^{1/2}$

adhesion regime	lipid bilayer	adhesive material	κ [10^{-19} J]	$\|W\|$ [mJ/m^2]	R_W [nm]
strong	DMPC	silica	0.8 [a]	$0.5 - 1$ [b]	13 - 18
strong	eggPC	glass	$\simeq 1$	0.15 [c]	26
weak	DOPC/DOPG	coated glass	0.4 [d]	3×10^{-4} [d]	510
ultraweak	DOPC/DOPG	glass	0.4 [d]	10^{-5} [d]	2800

Agudo-Canalejo, RL: *ACS Nano* (2015)

Onset of Adhesion



- Membrane starts to spread over particle if mean curvature M_{ms} is not too large:

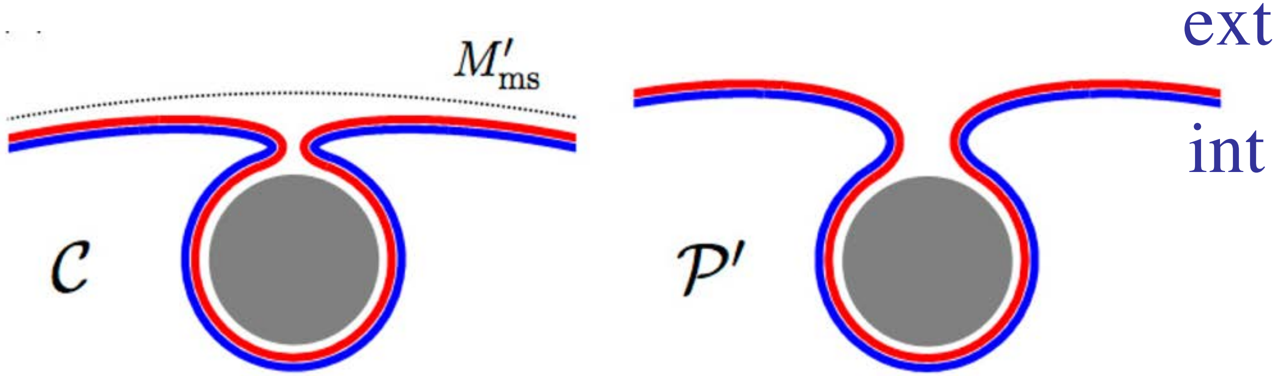
$$M_{ms} < M_{fr} = 1/R_W - 1/R_{pa}$$

- Example: threshold value $M_{fr} = 0$

no spreading

onset of adhesion

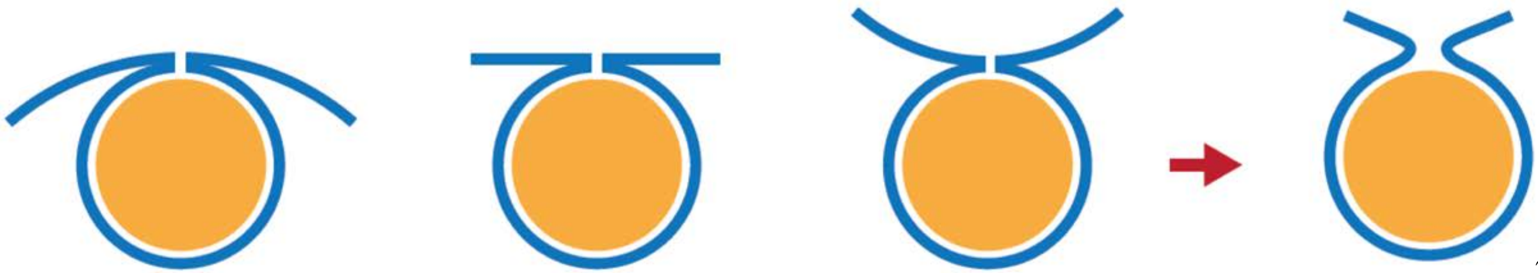
Opening of Membrane Neck



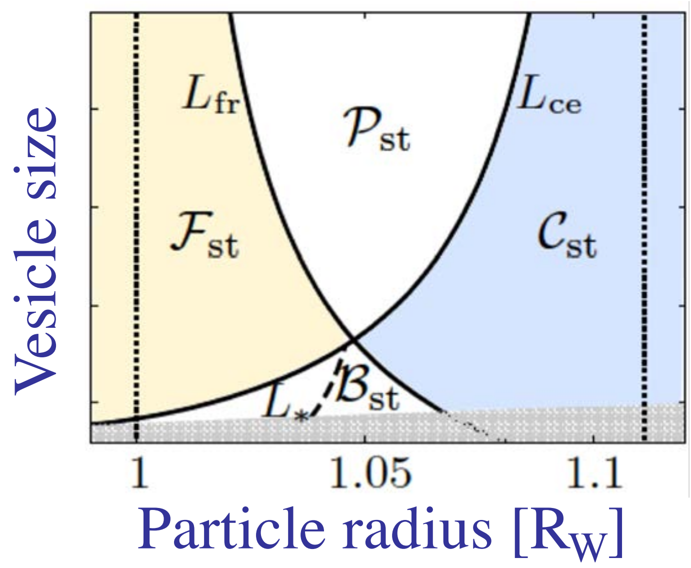
- Membrane neck starts to open if mean curvature M'_{ms} is not too large

$$M'_{ms} < M_{ce} = 2\,m - 1/R_W + 1/R_{pa}$$

- Example: threshold value $M_{ce} = 0$



Consequences of Stability Relations

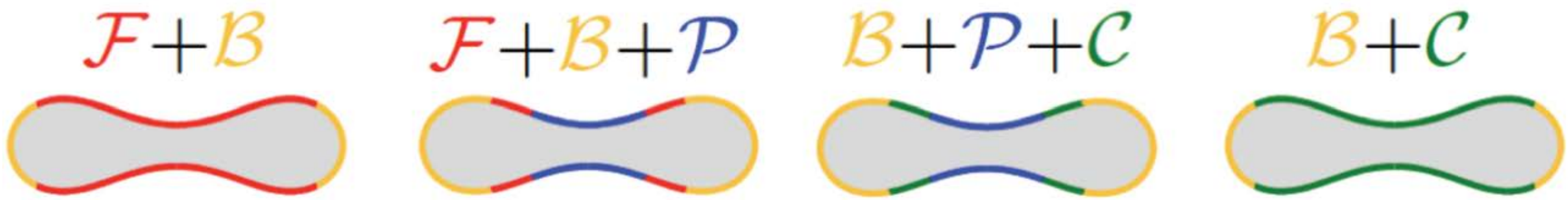


- Critical particle sizes for engulfment

  Combination of two stability relations for ***F*** and ***C*** states leads to four stability regimes $\boldsymbol{F}_{st}$, $\boldsymbol{B}_{st}$, $\boldsymbol{C}_{st}$ and $\boldsymbol{P}_{st}$

- Engulfment patterns for many particles

  Nonspherical shapes have variable segment curvature
  Different engulfment states coexist on the same vesicle



Compositional Responses

- Allow spatially dependent membrane composition
- Membrane is partitioned into different segments
- Assumption: uniform composition within each segment
- Segment i with rigidity κ_i and spont curv m_i
- Possible mechanisms for segmentation:

  adhesive surfaces, lipid phase separation,

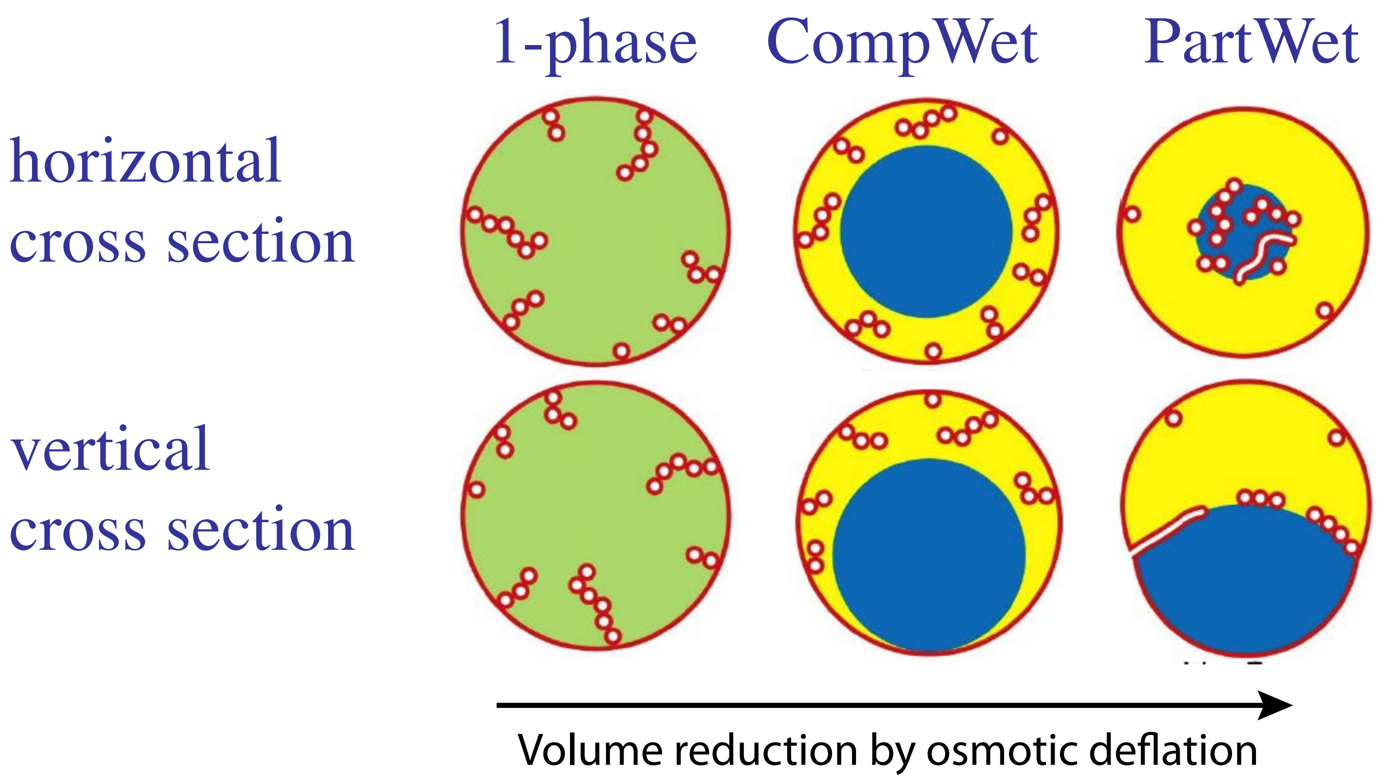
  highly curved segments, partial wetting, ...

  - Example 1: Partial wetting of membranes
  - Example 2: Receptor-mediated endocytosis
  - Example 3: ESCRT-induced budding

Partial Wetting and Tubulation

Liu et al: *ACS Nano* (2015)

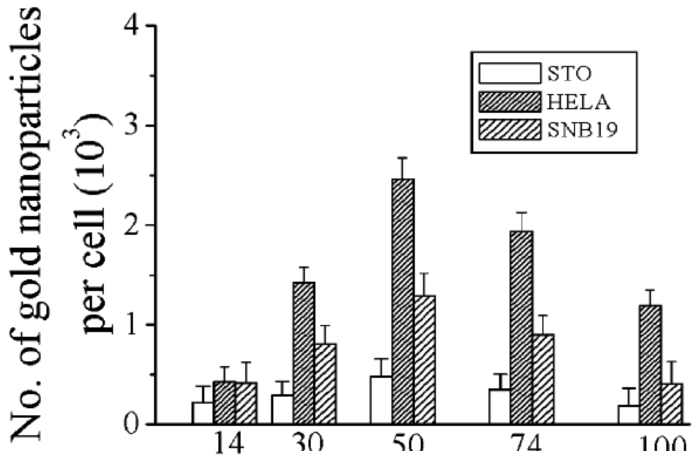
- Three morphologies with nanotubes:



- Tubes only formed by membrane segments in contact with PEG-rich phase (yellow)
- PartWet: Two segments with different spont curv

Receptor-Mediated Endocytosis

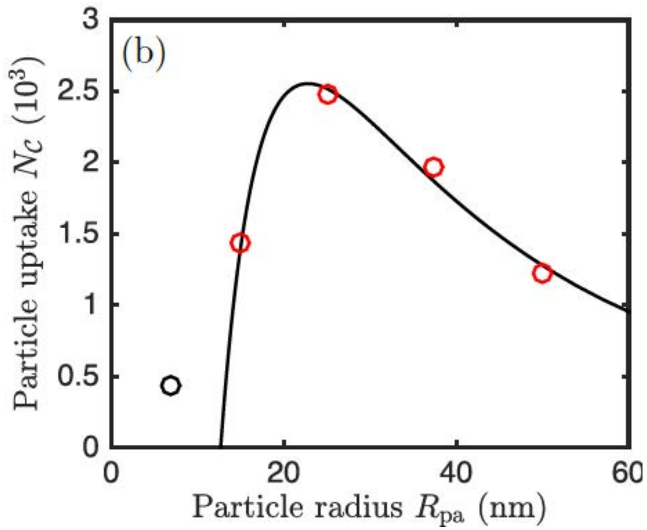
Chithrani et al, *Nano Letters* (2007)



- Uptake of gold nanoparticles by cells
- Particles bind to transferrin receptors
- Assembly of clathrin-coated vesicles

Non-monotonic size-dependence !

- Cell membrane with two types of segments, bound and unbound
- Bound segment contains protein coat with spont curv $m_{bo} = -1/(40 \text{ nm})$
- Good agreement with exp data:



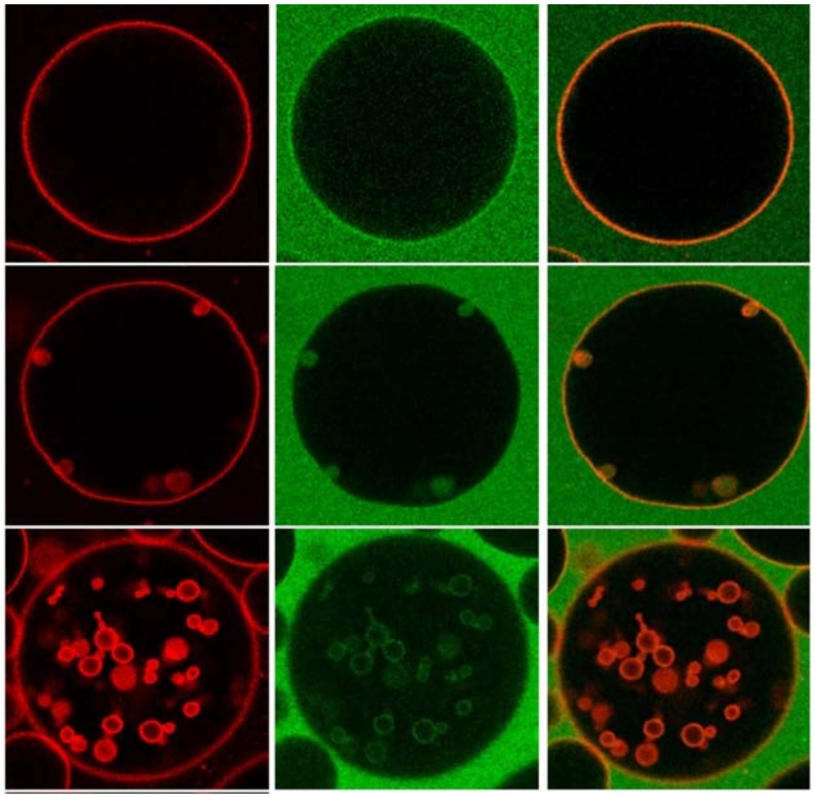
Agudo-Canalejo, RL: *ACS Nano* (2015)

ESCRT-Induced Budding

Avalos Padilla et al, unpublished

- Sequential addition of three ESCRT proteins to GUVs:

+ ESCRT 1
Vps20



adsorption of Vps20

+ ESCRT 2
Vps32

multiple buds + necks

+ ESCRT 3
Vps24

fission of necks

- Putative mechanism: buds from membrane domains with increased spont curv

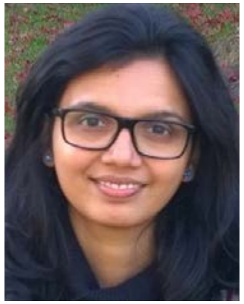
Ongoing Membrane Projects

- Budding and neck formation of nanovesicles:

  Talk by Rikhia Ghosh

- Curvature from lipids with large head groups:

  Poster by Aparna Sreekumari



- Micropipette aspiration of tubulated vesicles:

  Poster by Tripta Bhatia

- ESCRT-induced budding and fission:

  Poster by Yunuen Avalos-Padilla

Ongoing Membrane Projects

- Aqueous nanodroplets at lipid bilayers:

  Poster by Vahid Satarifard

- Giant vesicles from plasma membranes:

  Poster by Jan Steinkühler

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