Remodeling of Biomembranes by Adhesion of Condensate Droplets

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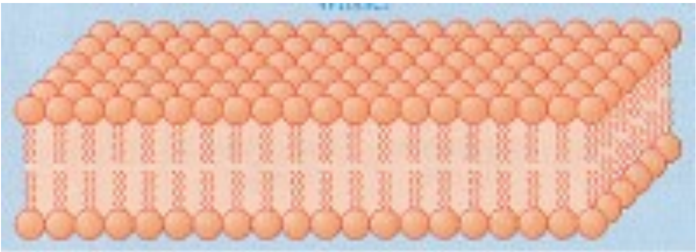
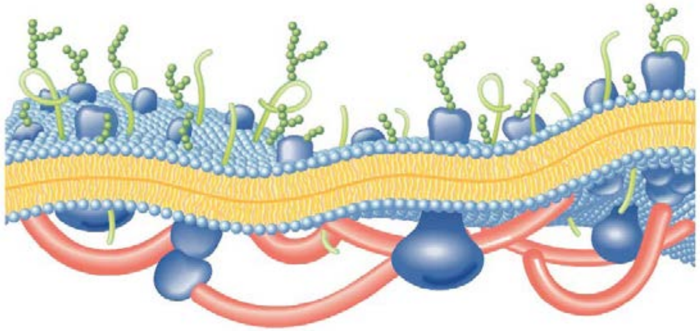
- Introduction to Membranes + Droplets
- Adhesion Morphologies
- Formation of Membrane Nanotubes
- Endocytosis by Nanovesicles

Biomembranes at the Nano- and Microscale

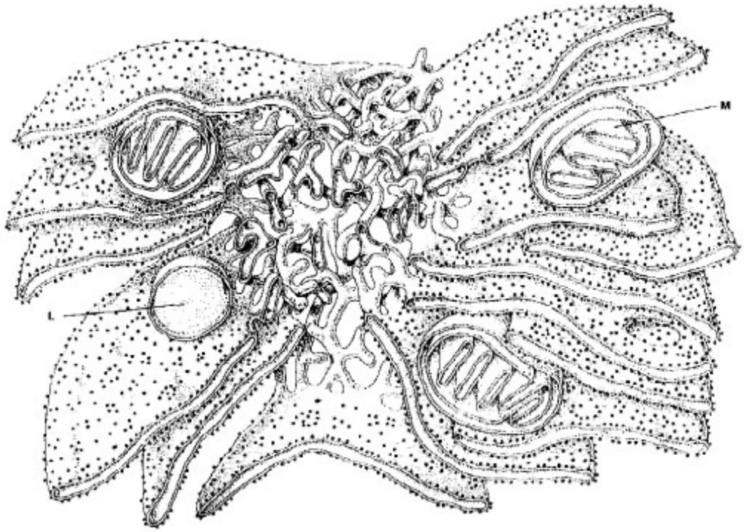
- Lipid bilayer, two leaflets



4 nm



- Biological membrane

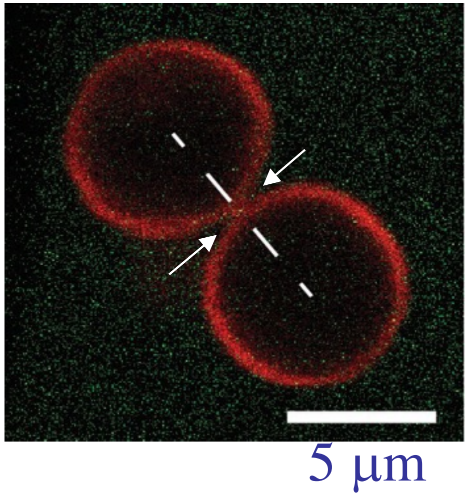


- Endoplasmic reticulum (ER)

100 μm

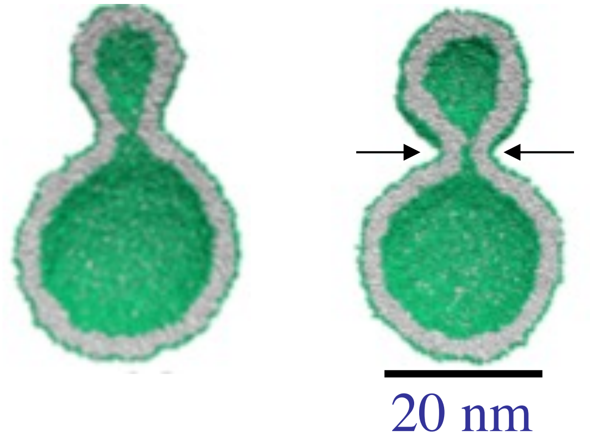
- Animal cell

Synthetic Membrane Compartments



- Giant unilamellar vesicles (GUVs)
- Morphology observed by optical microscopy
- Theory of curvature elasticity

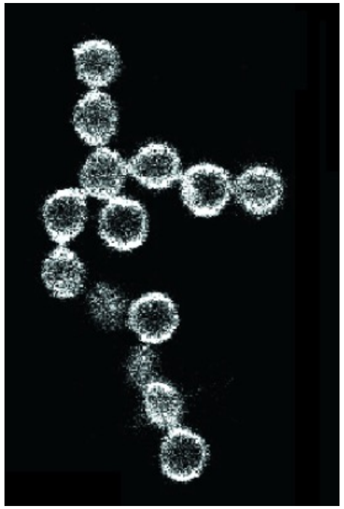
- Nanovesicles (NVs)
- Electron microscopy: limited to a single snapshot for each individual nanovesicle
- Spatio-temporal remodeling studied via Molecular Dynamics simulations



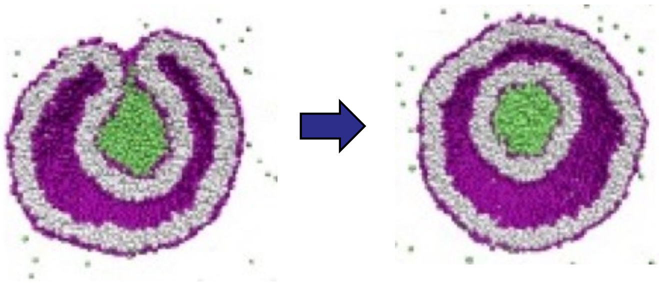
- Arrows indicate position of membrane necks with a diameter of about 10 nm

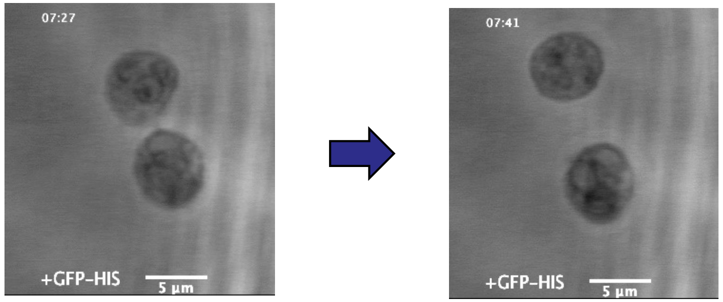
Remodeling of Shape and Topology

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



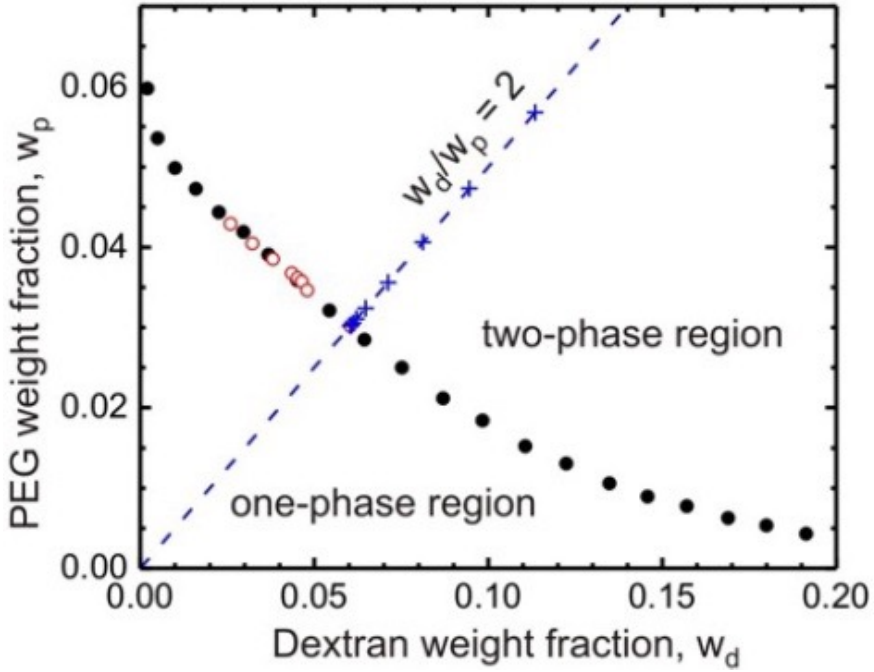
- Remodeling of membrane topology
- Via membrane fission and fusion
- Requires formation of membrane neck:

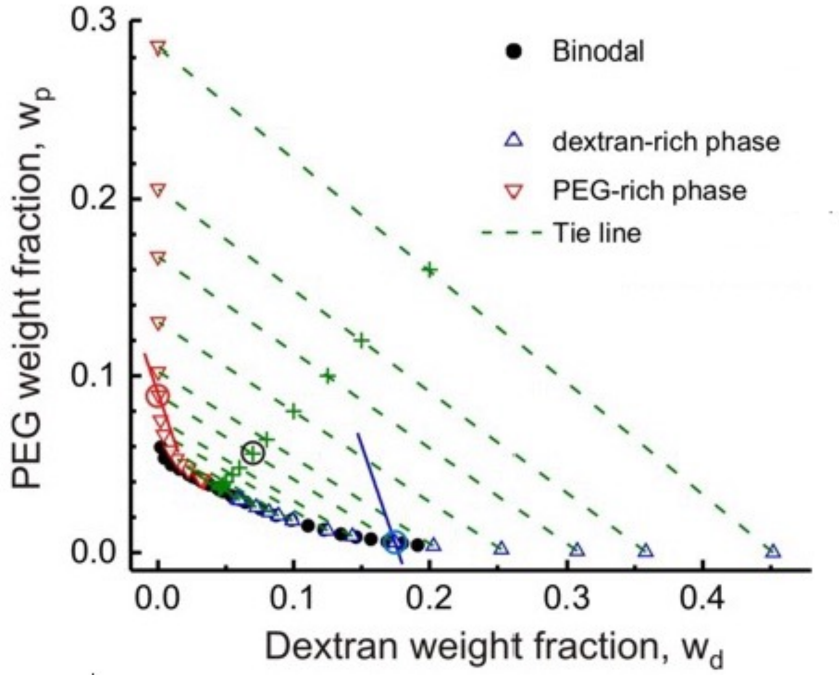




Liquid-Liquid Phase Separation (LLPS)

- Aqueous two-phase systems: PEG plus dextran



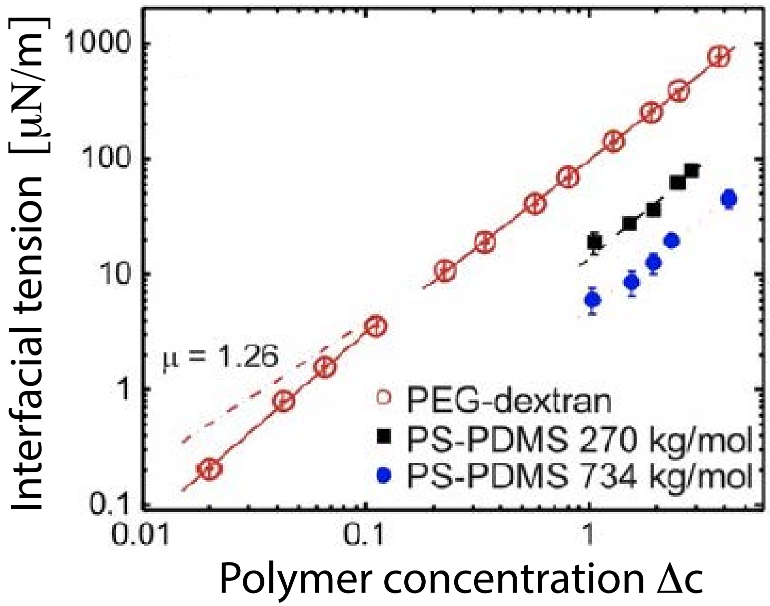


Liu et al, *Langmuir* 28, (2012)

- *Segregative* phase separation: Polymer A repels polymer B
- *Associative* phase separation: Polymer A attracts polymer B

Condensate Droplets

- Droplets formed via LLPS
- Two coexisting liquid phases α and β
- Droplets formed by minority phase β
- Droplets enclosed by αβ interface with tension $\Sigma_{\alpha\beta}$



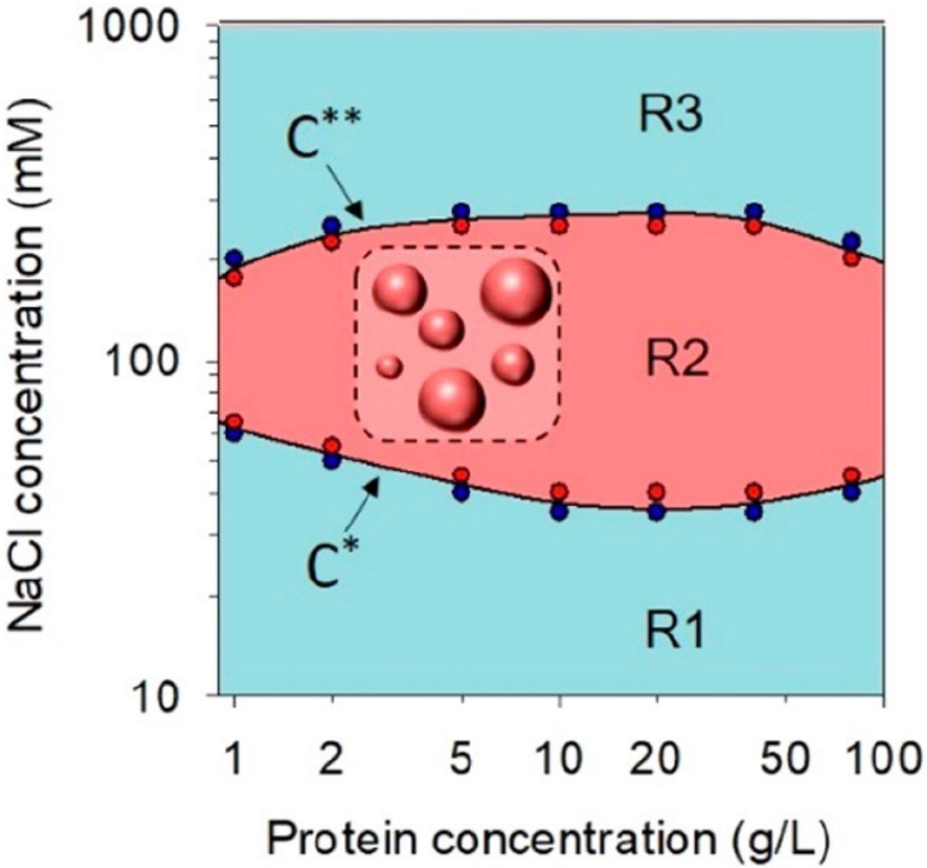
- PEG-dextran system
- Ultralow interfacial tensions $\Sigma_{\alpha\beta}$
- Varies from 10^{-3} N/m to 10^{-7} N/m
- Vicinity of critical demixing point

Liu et al, *Langmuir 28, (*2012)

LLPS in Glycinin Solutions

- Glycinin is a soybean protein
- Phase diagram for glycinin plus salt:

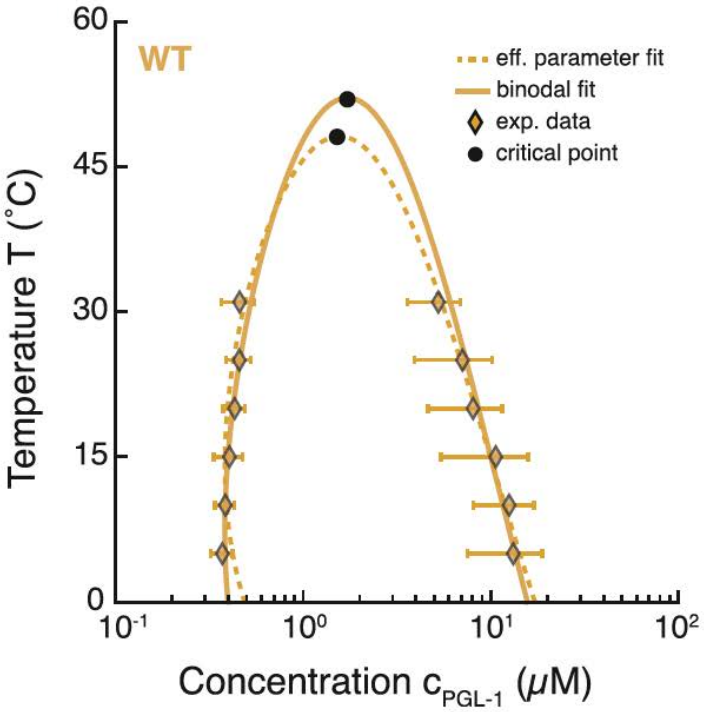
Chen et al,
ACS Macro Lett 9 (2020)



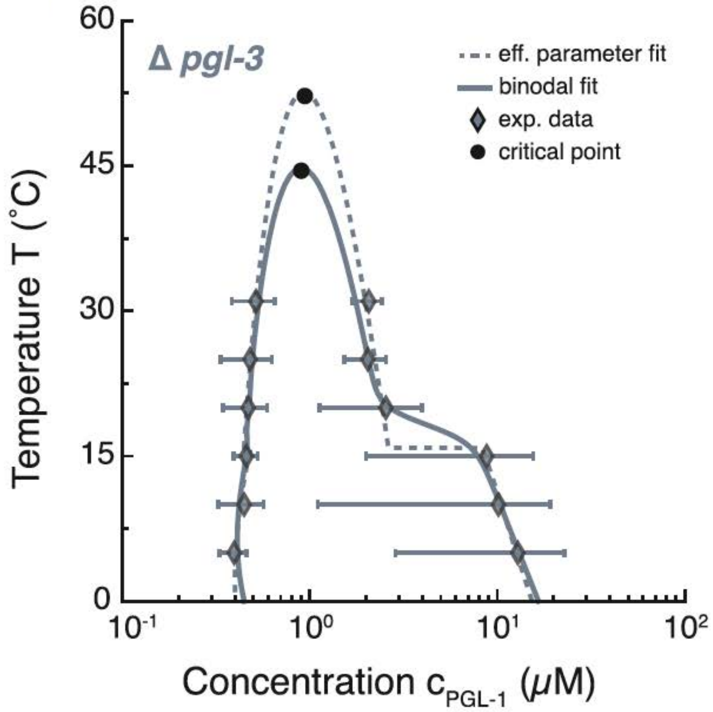
- One-phase regions R3
- Two-phase region R2
- One-phase region R1

LLPS in P Granules

- Two coexisting liquid phases α and β
- Enriched in different proteins such as PGL-1:



Phase diagram for wild-type



Phase diagram after PLG-3 deletion

Fritsch et al, *PNAS 118*, (2021)

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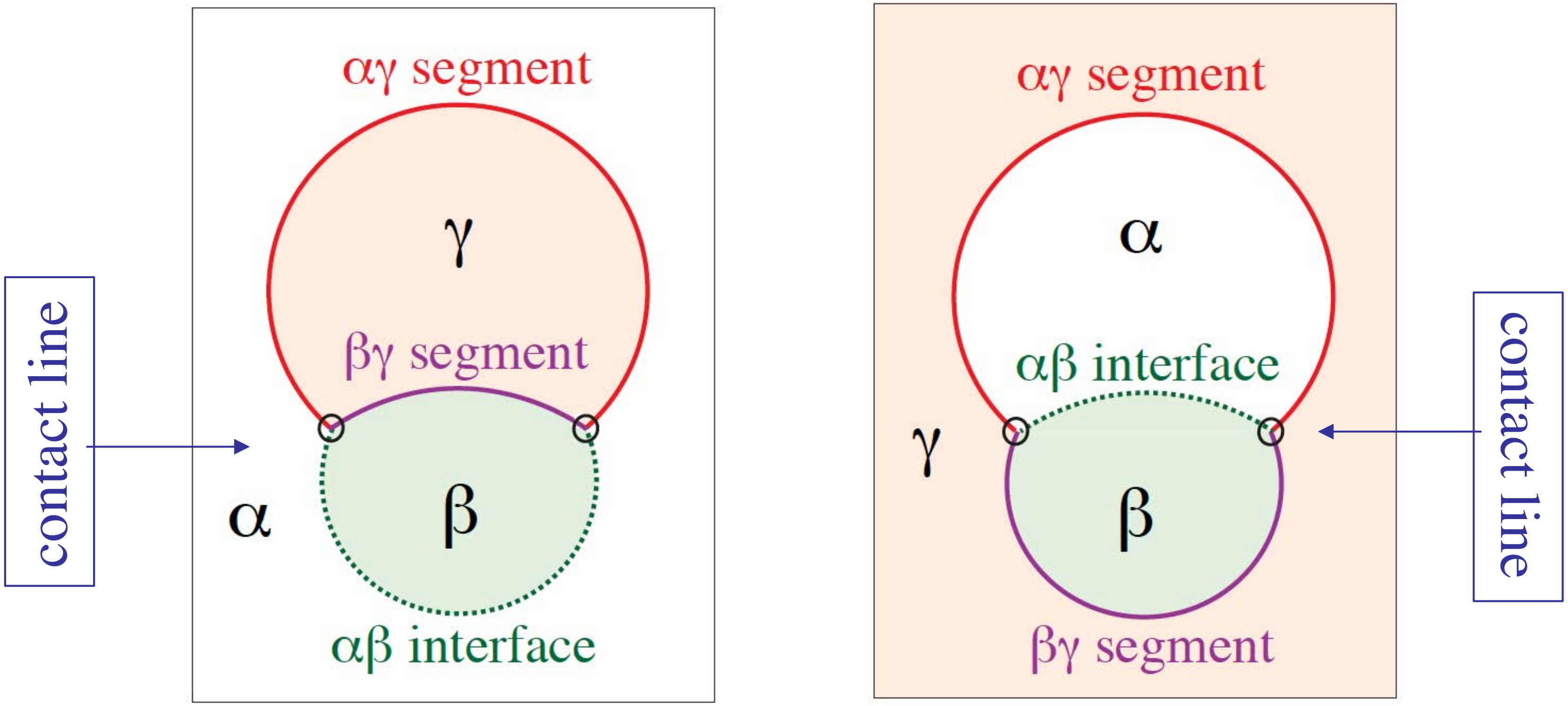
Geometry of Vesicle-Droplet Systems

- LLPS in exterior or interior aqueous solution:



Lipowsky, *Membranes 13* (2023)

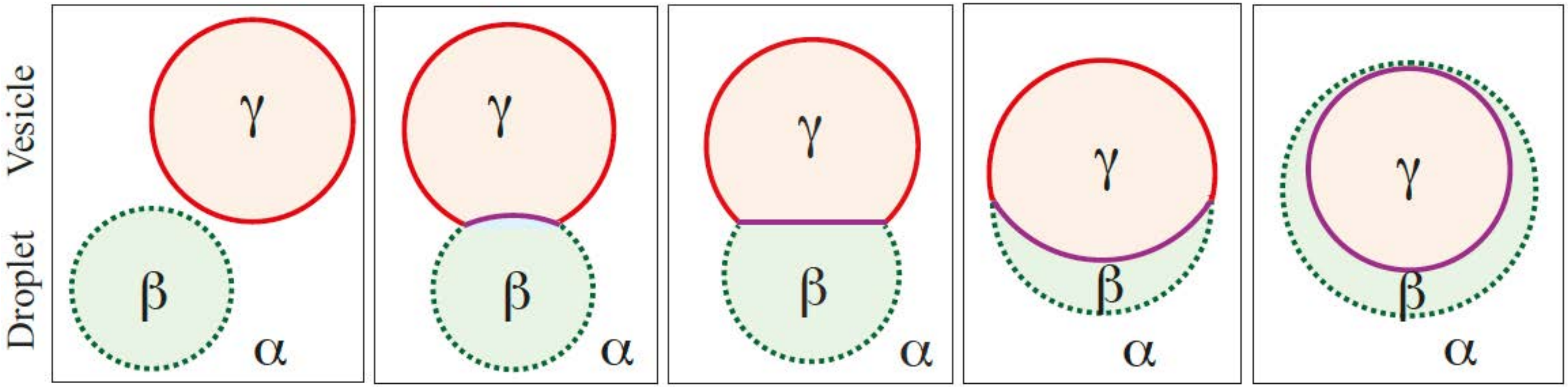
- αβ interface forms contact line with membrane and divides this membrane up into αγ segment and βγ segment

Adhesion Morphologies, Theory

• LLPS in exterior solution, adhesion of β droplet to vesicle

• No adhesion, complete dewetting:

• Strong adhesion, complete wetting



• Apparent kink of vesicle membrane (red/purple)

Adhesion Morphologies, Experiment

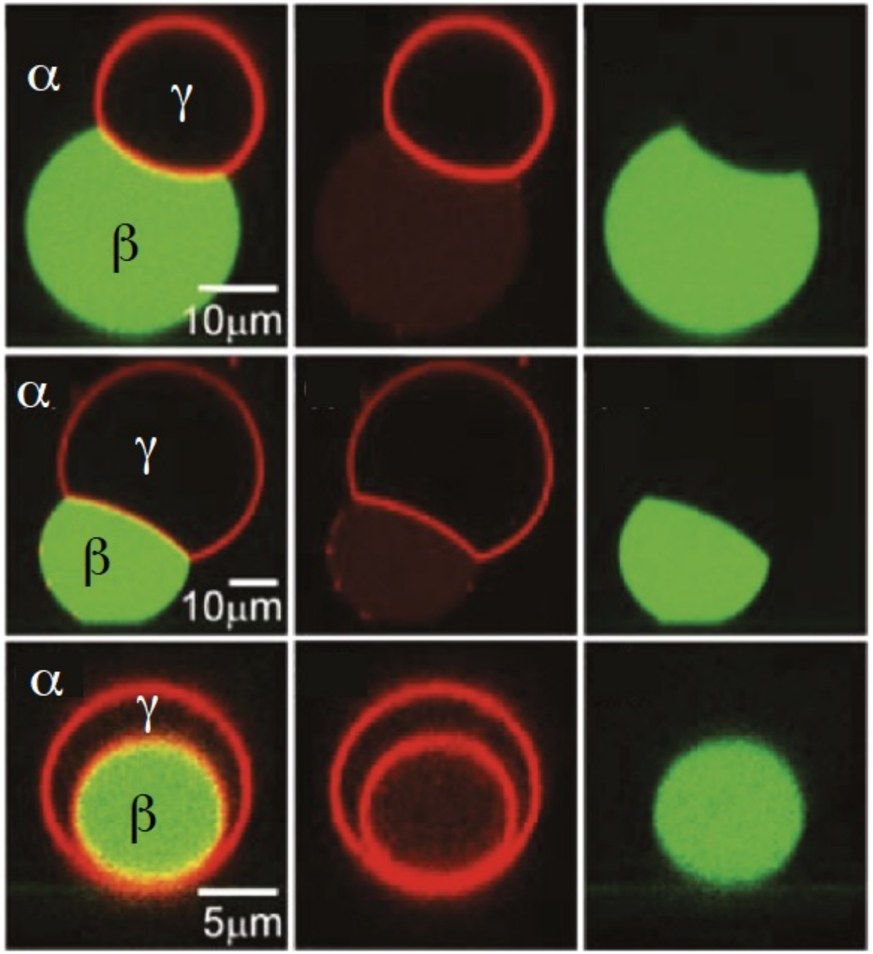
- LLPS in exterior solution, adhesion of β droplet to vesicle

- Red vesicle membrane
- Green condensate droplet dextran-rich phase β
- Weak remodeling in (a,b), vesile membrane has kink along contact line
- Strong remodeling in (c), corresponding to complete engulfment of droplet

(a)

(b)

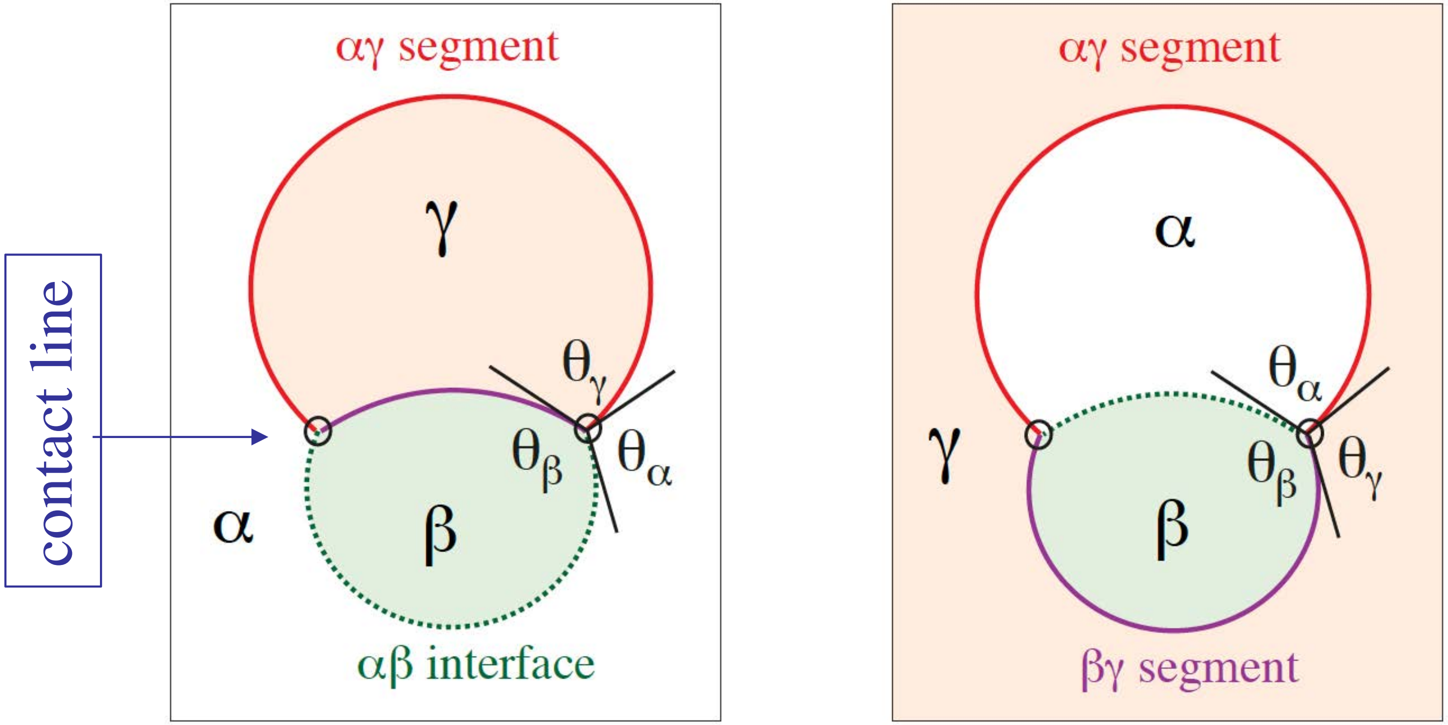
(c)



Li et al, *J. Phys. Chem. B 116*, (2012)

Contact Angles

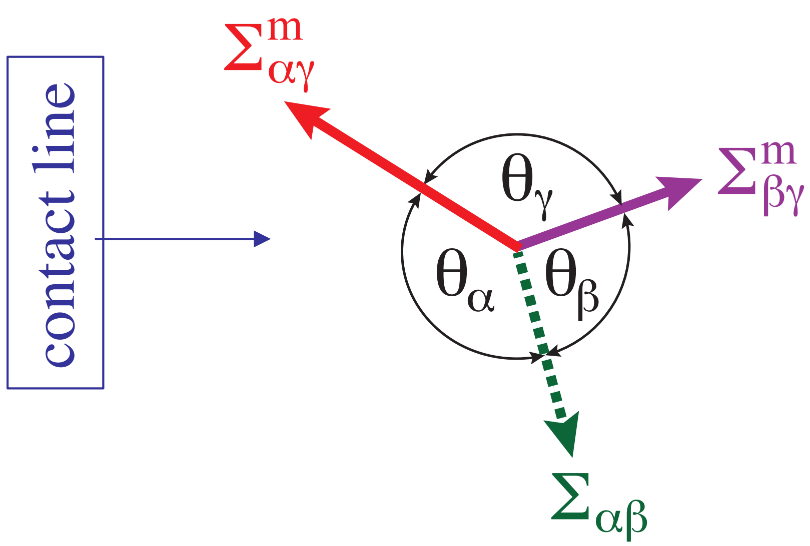
• Three contact angles θ_α , θ_β , and θ_γ with $\theta_\alpha + \theta_\beta + \theta_\gamma = 2\pi$

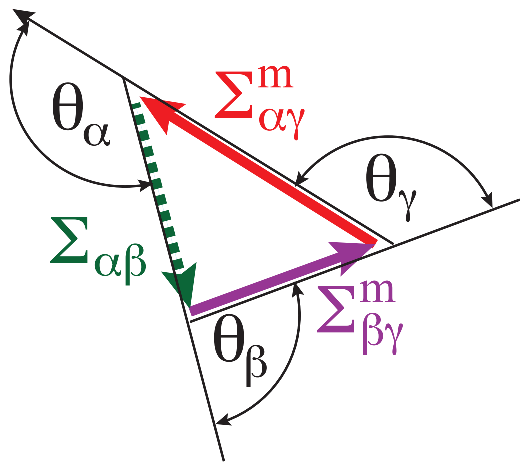


• αβ interface forms contact line with membrane and divides this membrane up into αγ segment and βγ segment

Balance of Surface Tensions

- Interfacial tension $\Sigma_{\alpha\beta}$ of $\alpha\beta$ interface is balanced by two membrane tensions $\Sigma^{m}_{\alpha\gamma}$ and $\Sigma^{m}_{\beta\gamma}$ of $\alpha\gamma$ segment and $\beta\gamma$ segment





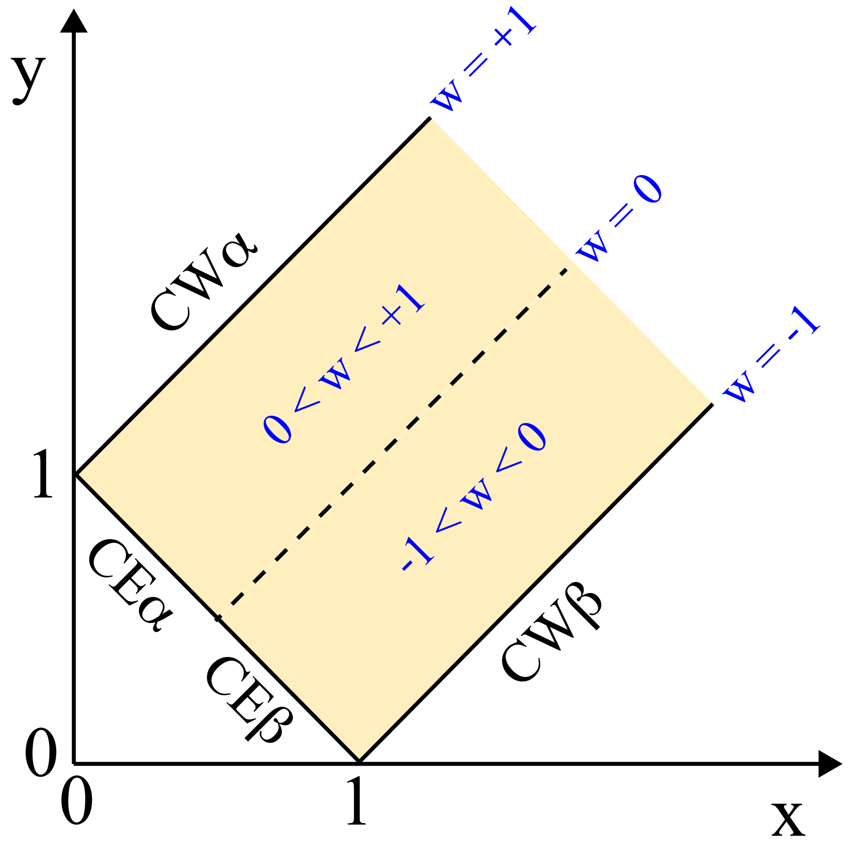
- Three surface tensions form triangle with the external angles being equal to the contact angles

Affinity Contrast

- Affinity contrast $W = \Sigma_{\beta\gamma} - \Sigma_{\alpha\gamma}$
- Rescaled affinity contrast

  $w = W / \Sigma_{\alpha\beta} = (\Sigma_{\beta\gamma} - \Sigma_{\alpha\gamma}) / \Sigma_{\alpha\beta}$
- Two tension ratios

  $x = \Sigma_{\beta\gamma} / \Sigma_{\alpha\beta}$ and $y = \Sigma_{\alpha\gamma} / \Sigma_{\alpha\beta}$
- Yellow parameter regime = force balance regime
- Complete wetting by β: w = - 1
- Complete wetting by α: w = +1



Affinity Contrast and Contact Angles

- Affinity contrast $W = \Sigma_{\beta\gamma} - \Sigma_{\alpha\gamma}$ is mechanical parameter
- Contact angles are geometric quantities
- Rescaled affinity contrast directly related to contact angles:

$$w = \frac{\Sigma^{\mathrm{m}}_{\beta\gamma} - \Sigma^{\mathrm{m}}_{\alpha\gamma}}{\Sigma_{\alpha\beta}} = \frac{\sin\theta_\alpha - \sin\theta_\beta}{\sin\theta_\gamma}$$

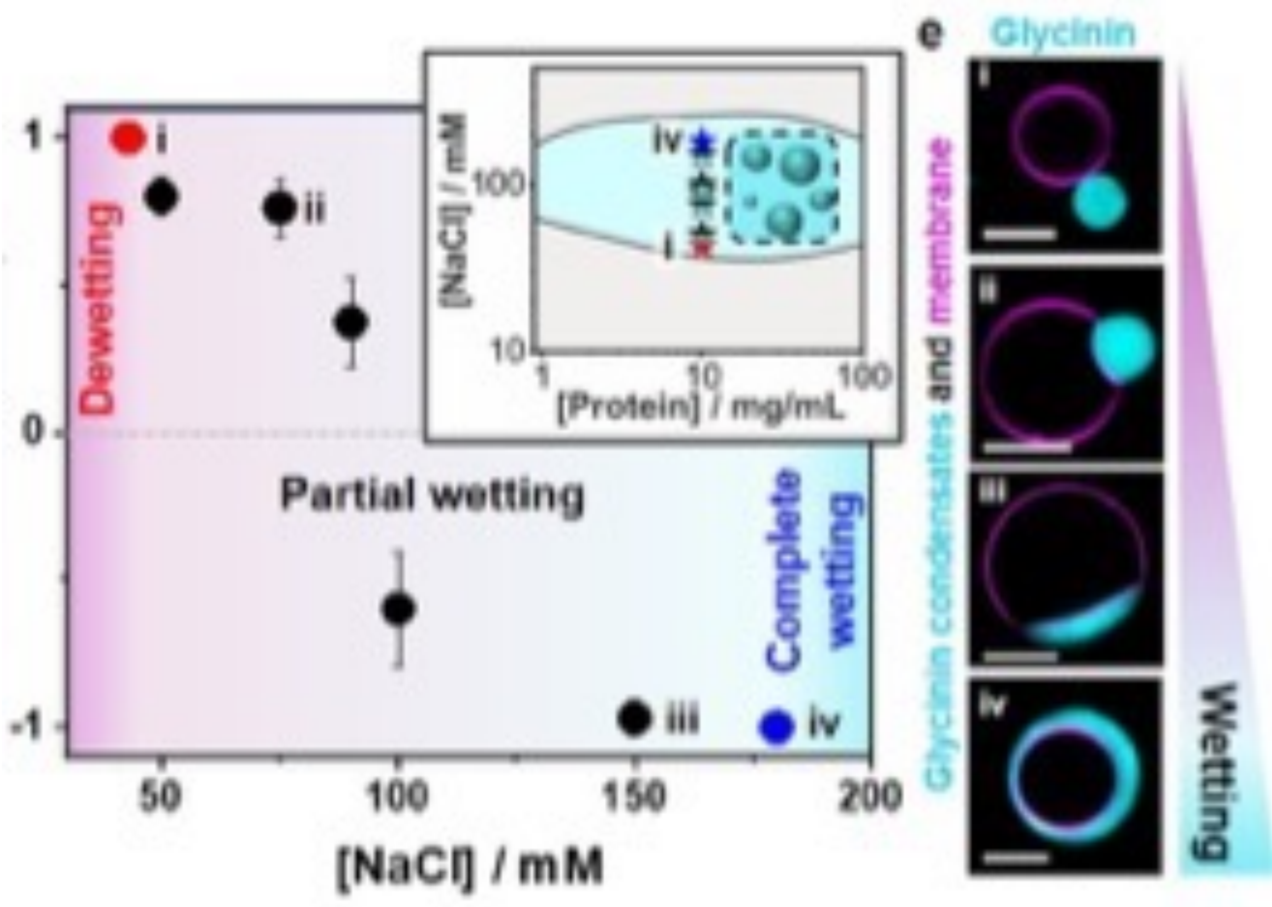
- Rescaled affinity contrast *w* can be determined experimentally by measuring the contact angles

Lipowsky, *Membranes 13* (2023)

Glycinin Condensates

- Rescaled affinity contrast *w* for droplets of glycinin condensates:

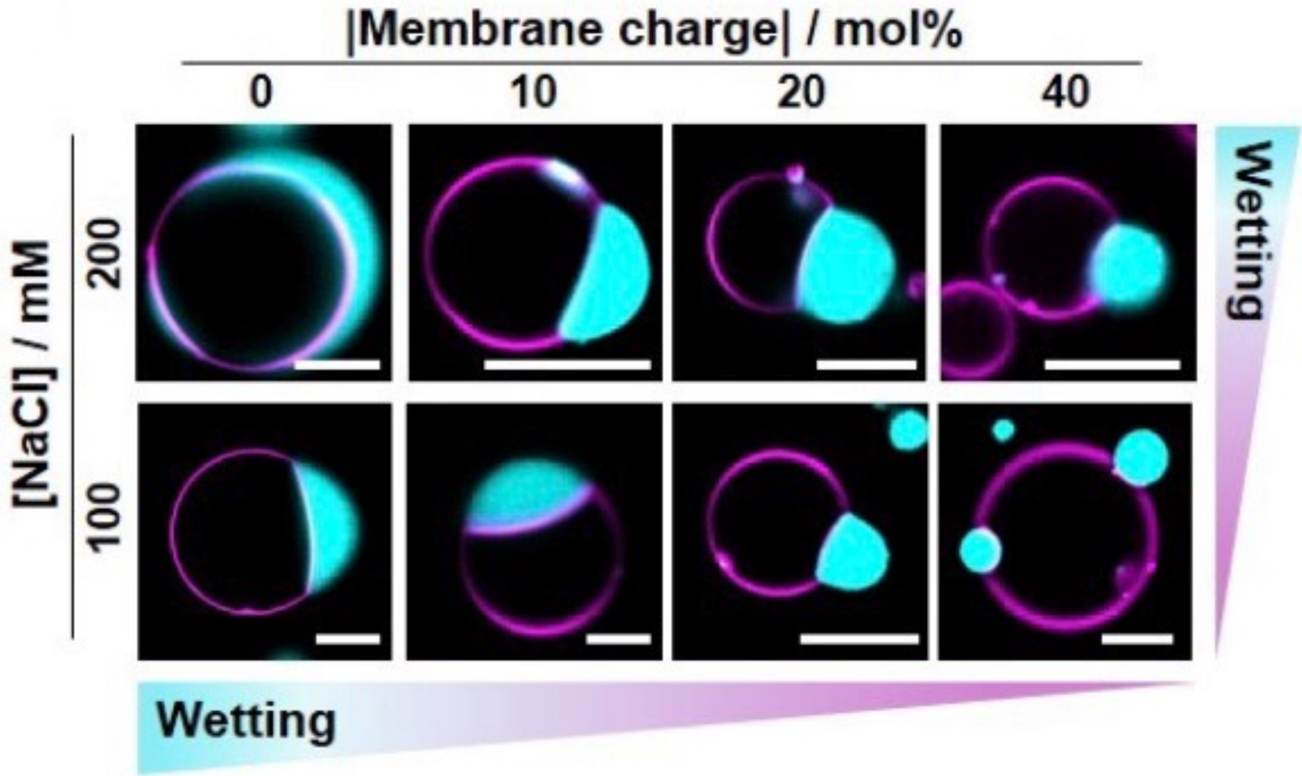
Rescaled affinity contrast *w*



Mangiarotti et al, *Nature Comm 14* (2023)

GUVs Exposed to Glycinin Condensates

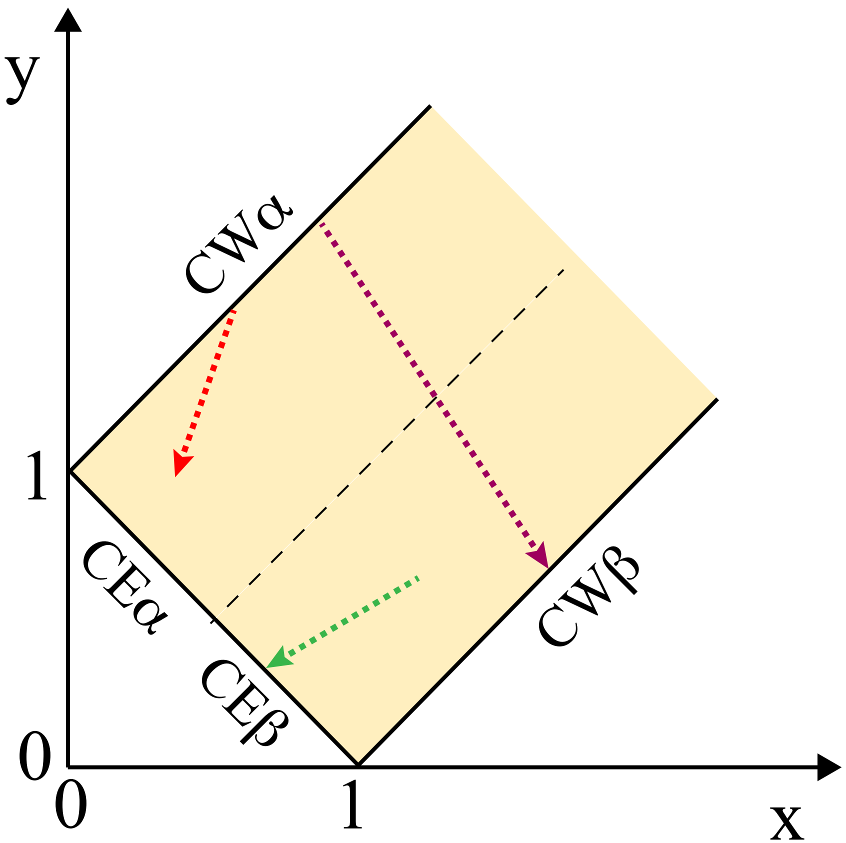
- Dewetting for low and complete wetting for high salinity
- Similar behavior as a function of membrane charge:



Mangiarotti et al, *Nature Comm 14* (2023)

Two Wetting Transitions

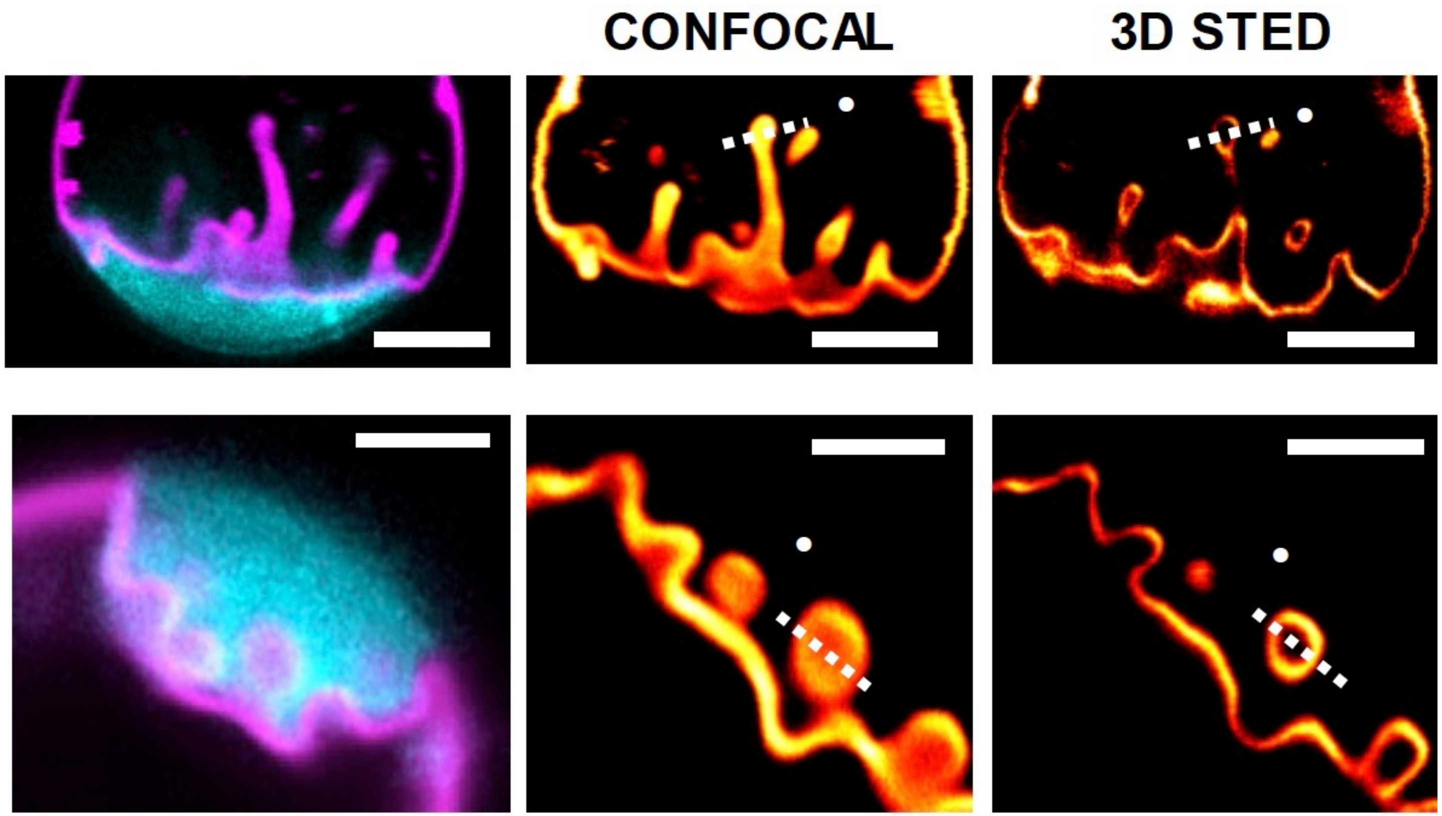
- Different control parameters that drive the vesicle-droplet system from complete wetting by α to complete wetting by β



- Corner point with x = 1 and small $y \sim \Sigma_{\beta\gamma}$
- Corner point with y = 1 and small $x \sim \Sigma_{\alpha\gamma}$

Fingering of βγ Membrane Segment

- Corner with x = 0 and small y implies tensionless βγ segment
- No constraint on membrane area of βγ segment
- Fingering of βγ segment observed for glycinin droplets:



Mangiarotti et al, *Nature Comm 14* (2023)

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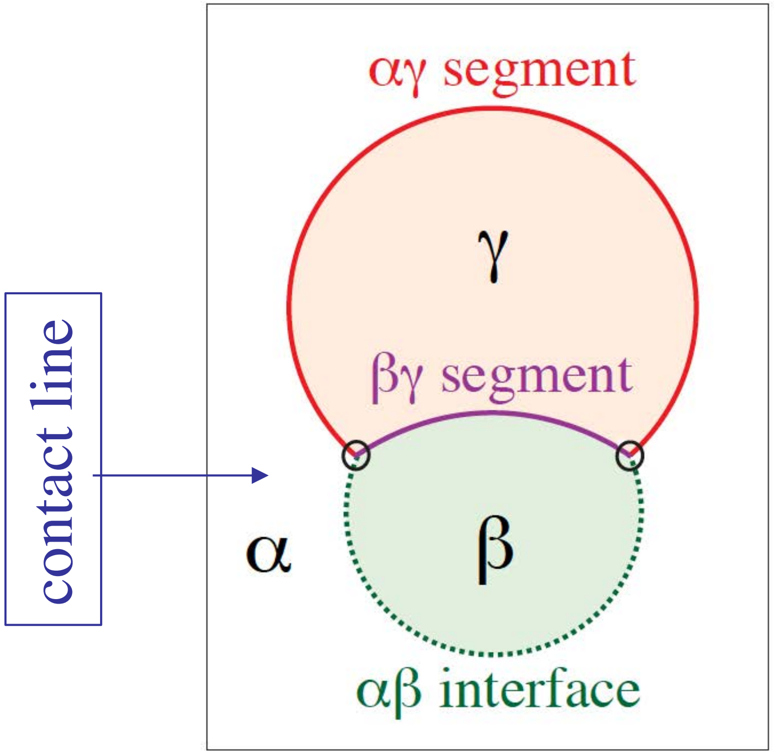
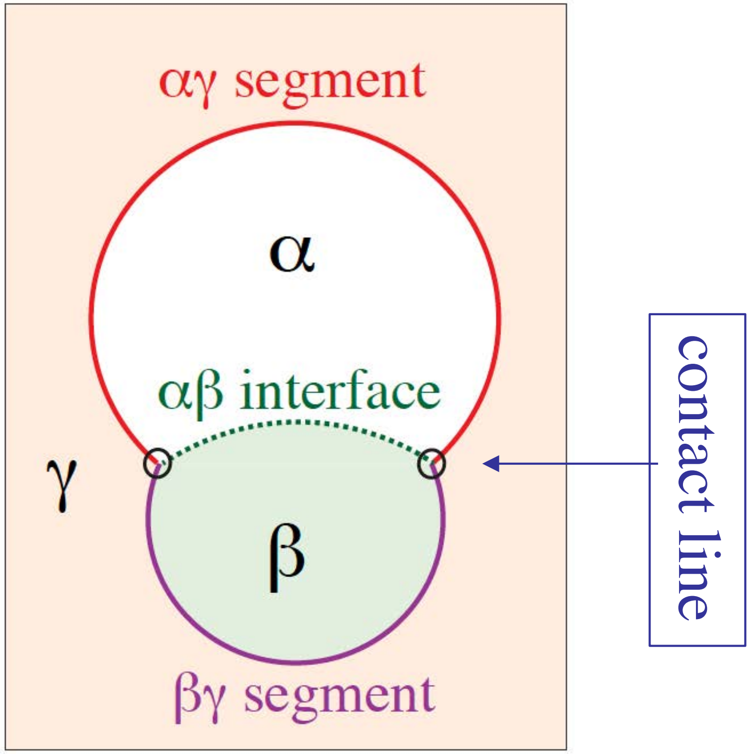
Geometry of Vesicle-Droplet Systems

- LLPS in exterior solution:



- LLPS in interior solution:

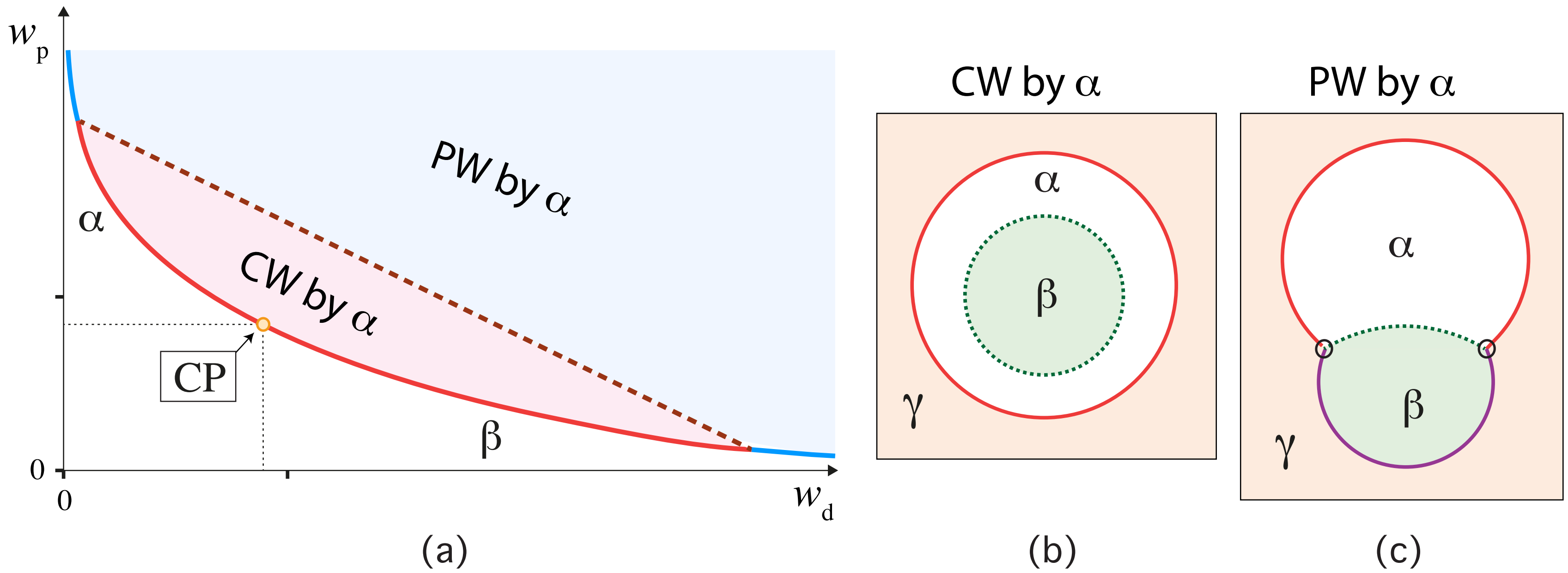


Lipowsky, *Membranes* 13 (2023)

- So far: Examples for LLPS in exterior solution
- Now: Example for LLPS in interior solution

PEG-Dextran within GUVs

- PEG-rich phase α and dextran-rich phase β



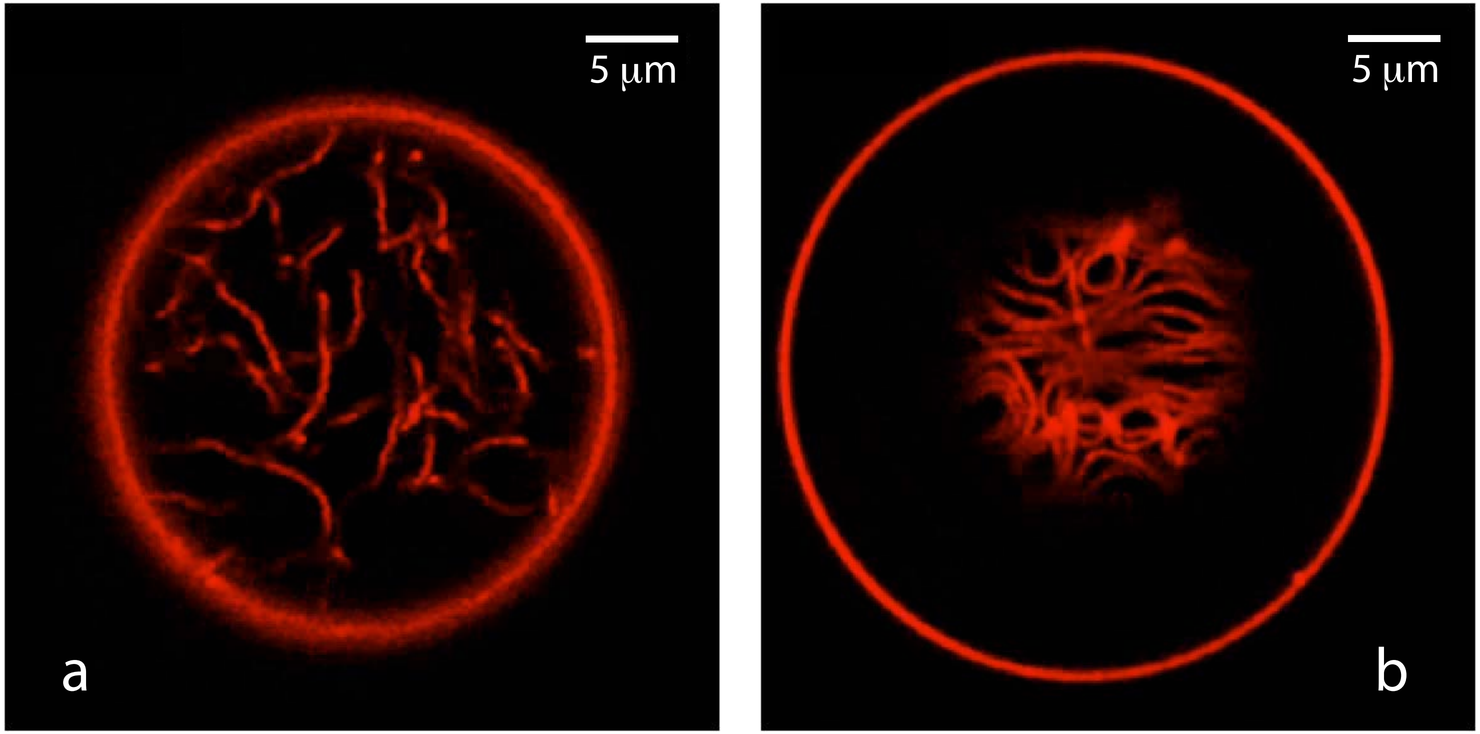
- Complete wetting (CW) by α close to critical point, partial wetting (PW) by α further away from critical point

- PW with contact line, CW without contact line.

Lipowsky, *Membranes 13* (2023)

Formation of Membrane Nanotubes

- Membranes labeled by fluorescent dyes
- αγ membrane segment forms many nanotubes

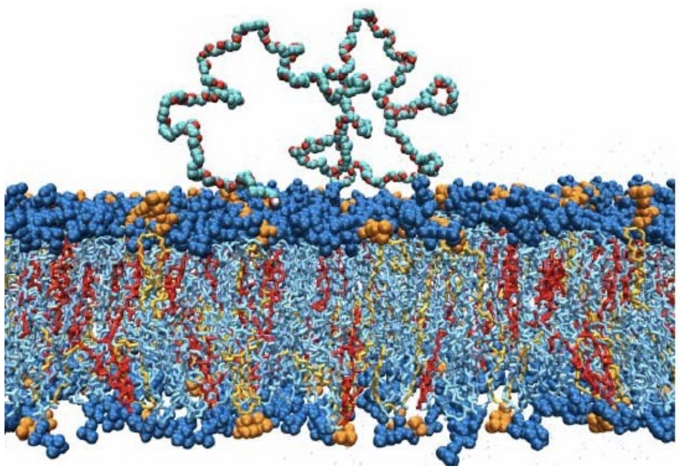


Complete wetting   Partial wetting

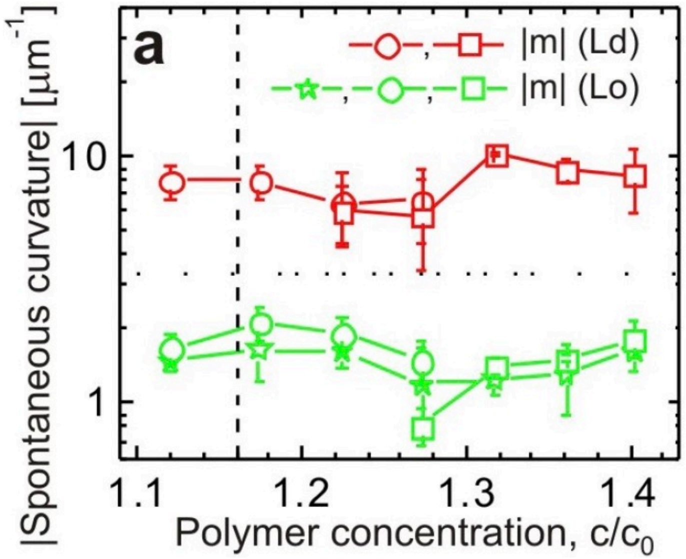
Liu et al, *ACS Nano 10* (2016)

Asymmetric PEG Adsorption

- Tubulation of αγ membrane segment exposed to PEG-rich phase
- PEG adsorption generates large membrane curvature:



PEG molecule adsorbed onto inner leaflet

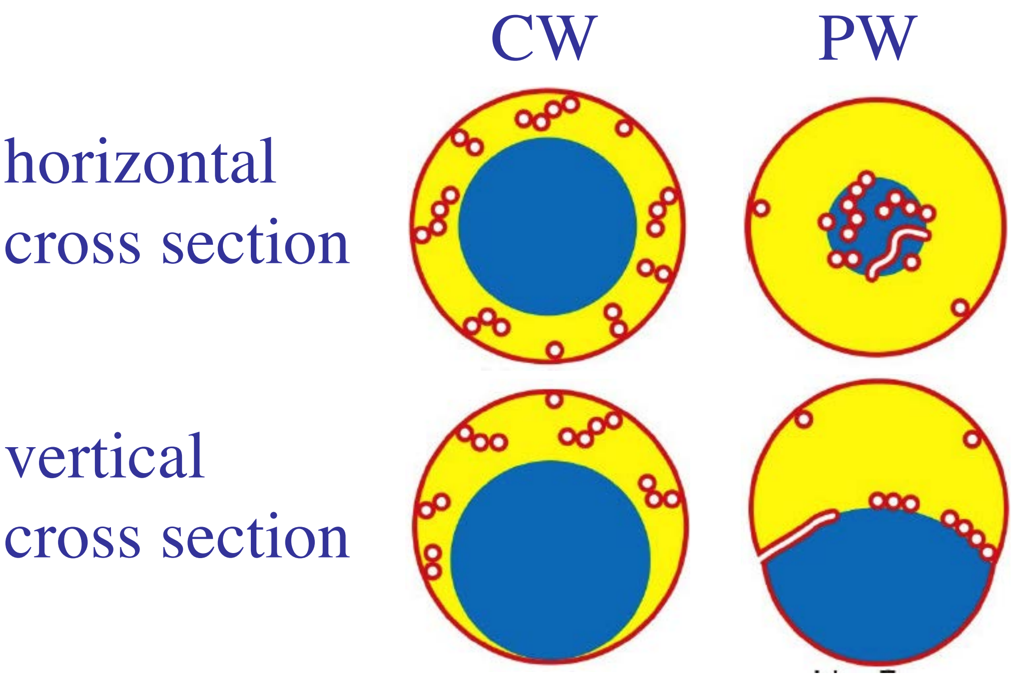


Spontaneous curvature of about 1/(125 nm)

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

Different Tube Patterns

- CW and PW lead to different tube patterns:



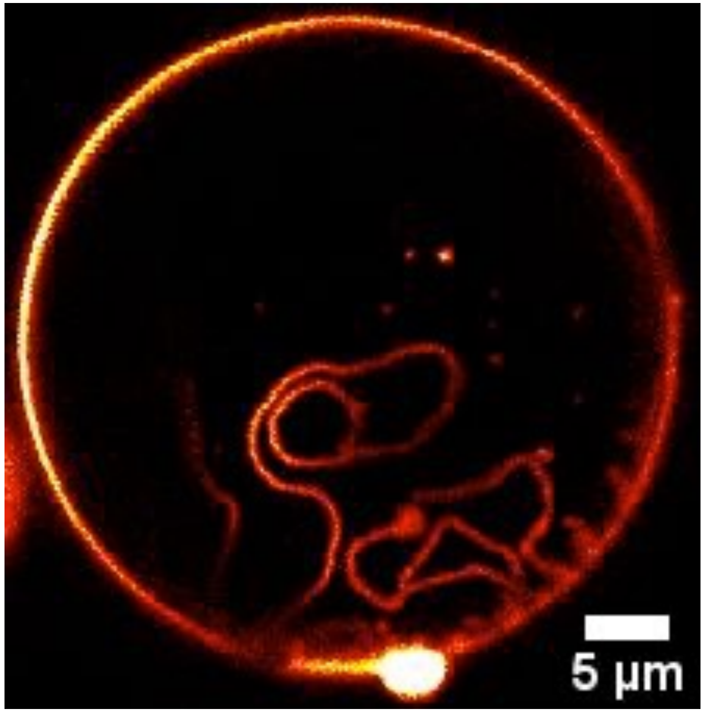
- CW: Nanotubes stay away from αβ interface
- PW: Nanotubes adhere to αβ interface

- Can distinguish CW from PW by visual inspection !

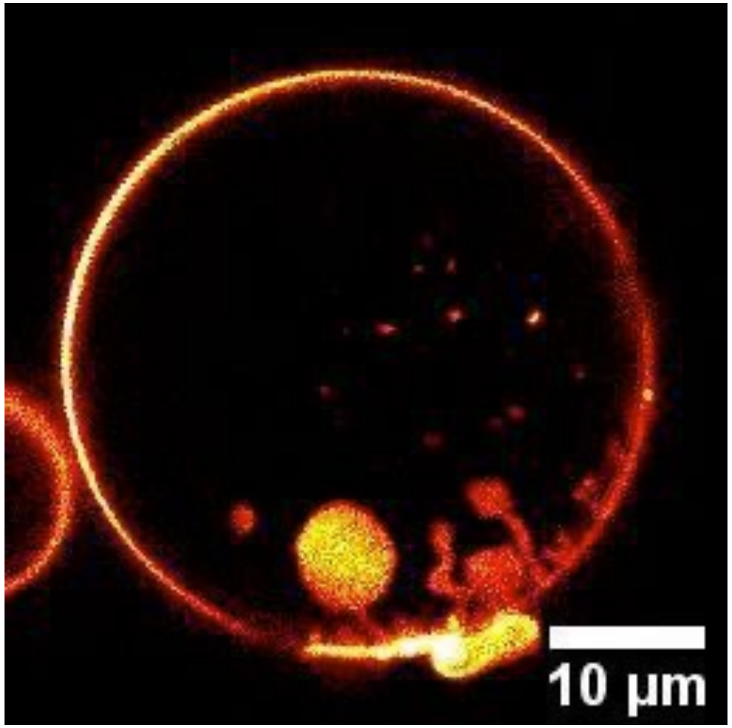
From Tubes to Sheets and Back

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

- System with many nanotubes adhering to αβ interface
- Transformation betweem tubes and sheets:



Tube transformed into sheet



Sheet transformed into tube

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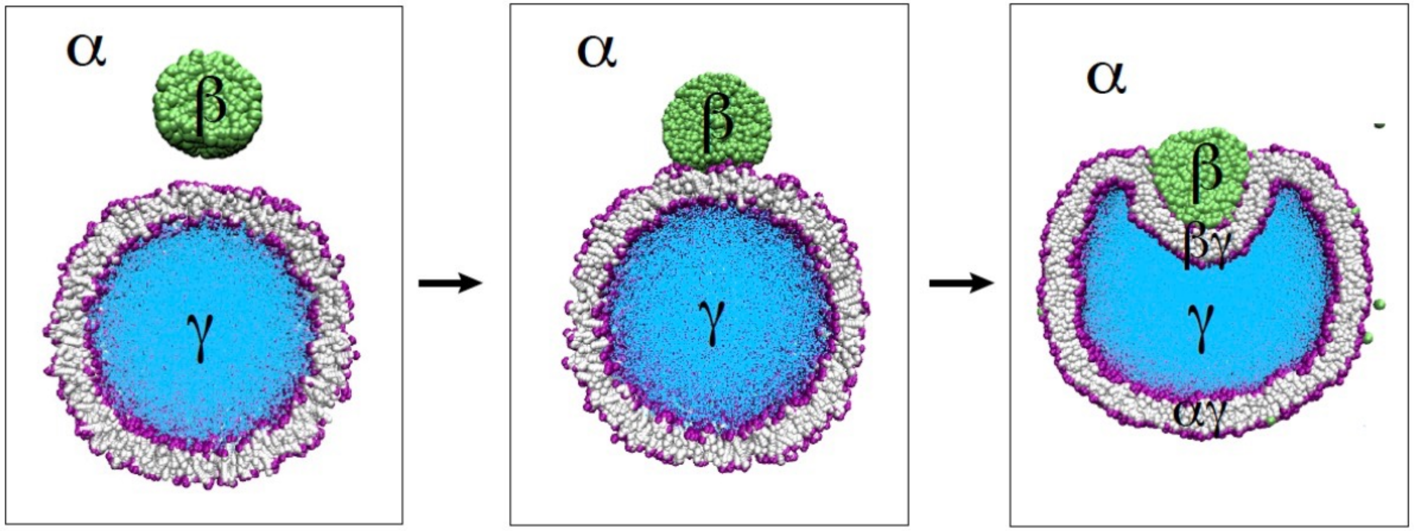
Droplet Endocytosis by Nanovesicles

- Nanovesicle assembled from about 10^4 lipids
- Nanovesicle exposed to condensate droplet:

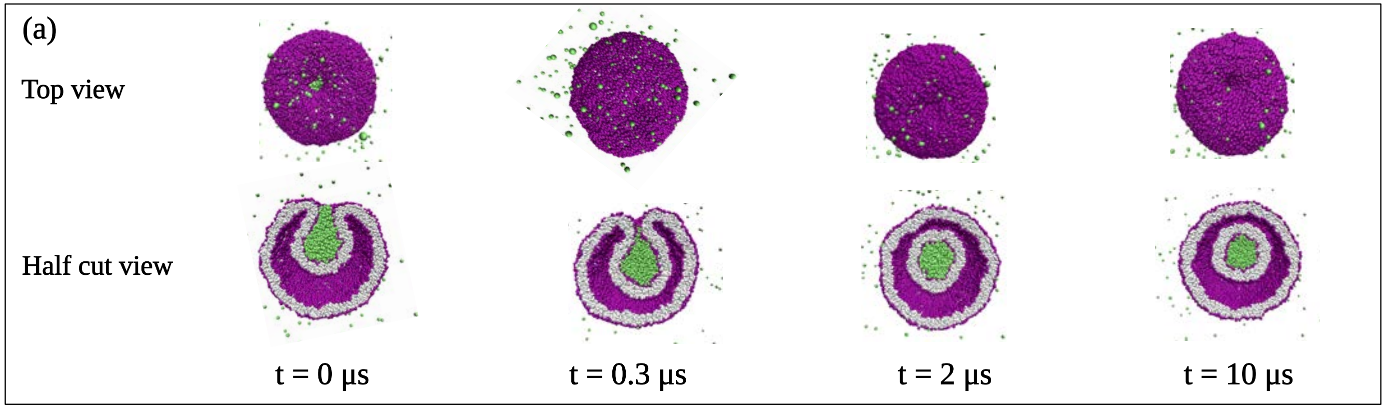


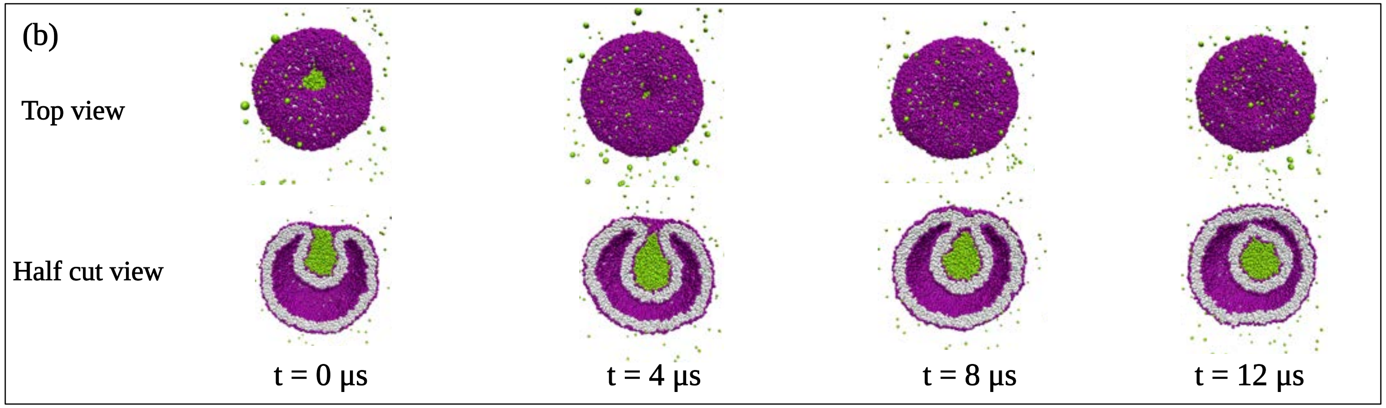
No adhesion -> Adhesion -> Engulfment

Ghosh et al, *Nature Comm 14* (2023)

Axisymmetric Engulfment + Division

• Time-lapse snapshots of vesicle-droplet system:



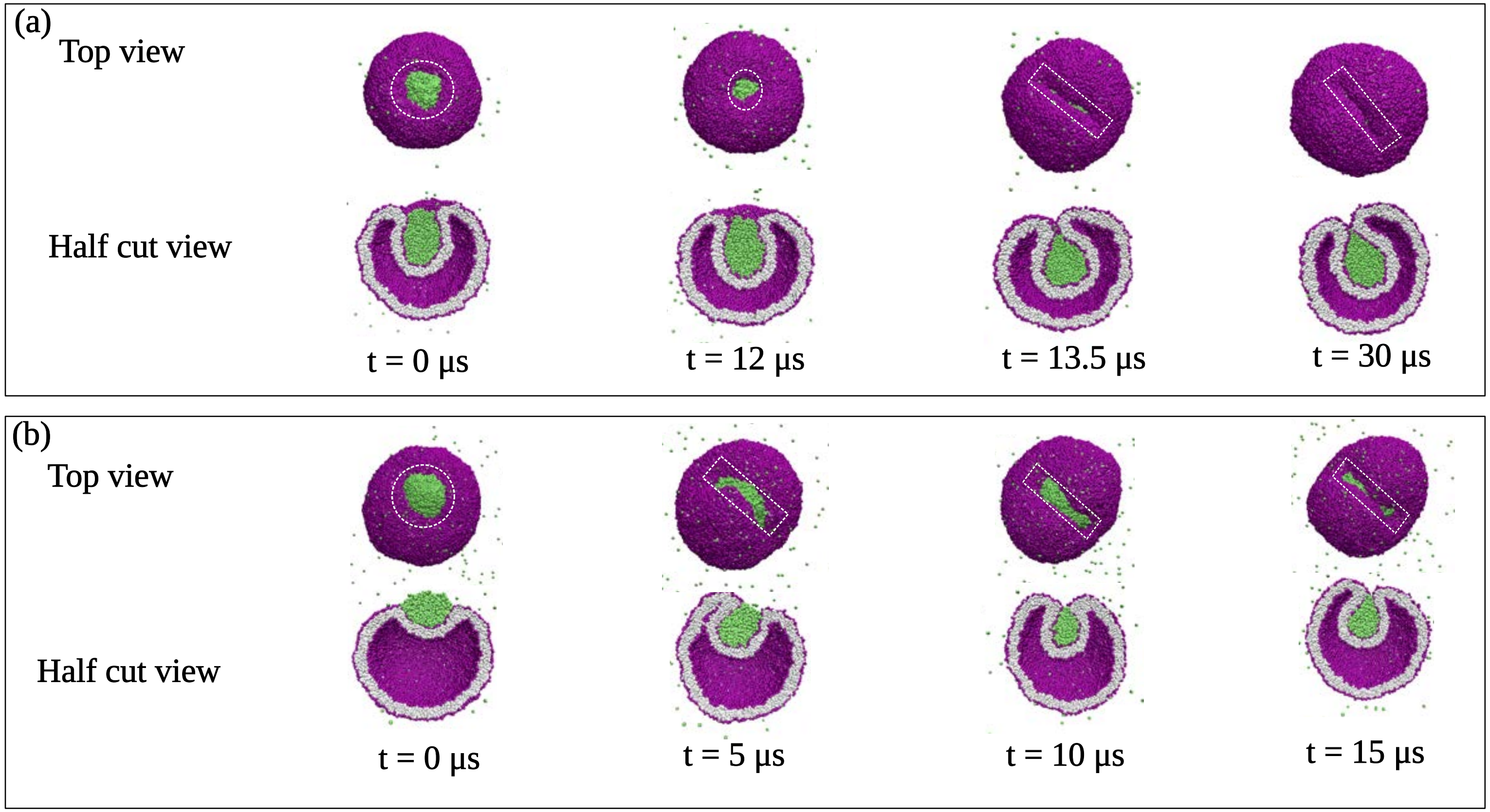


• Axisymmetric neck leads to division of nanovesicle

Ghosh et al, *Nature Comm 14* (2023)

Engulfment with Tight-Lipped Neck

- Time-lapse snapshots of vesicle-droplet system:

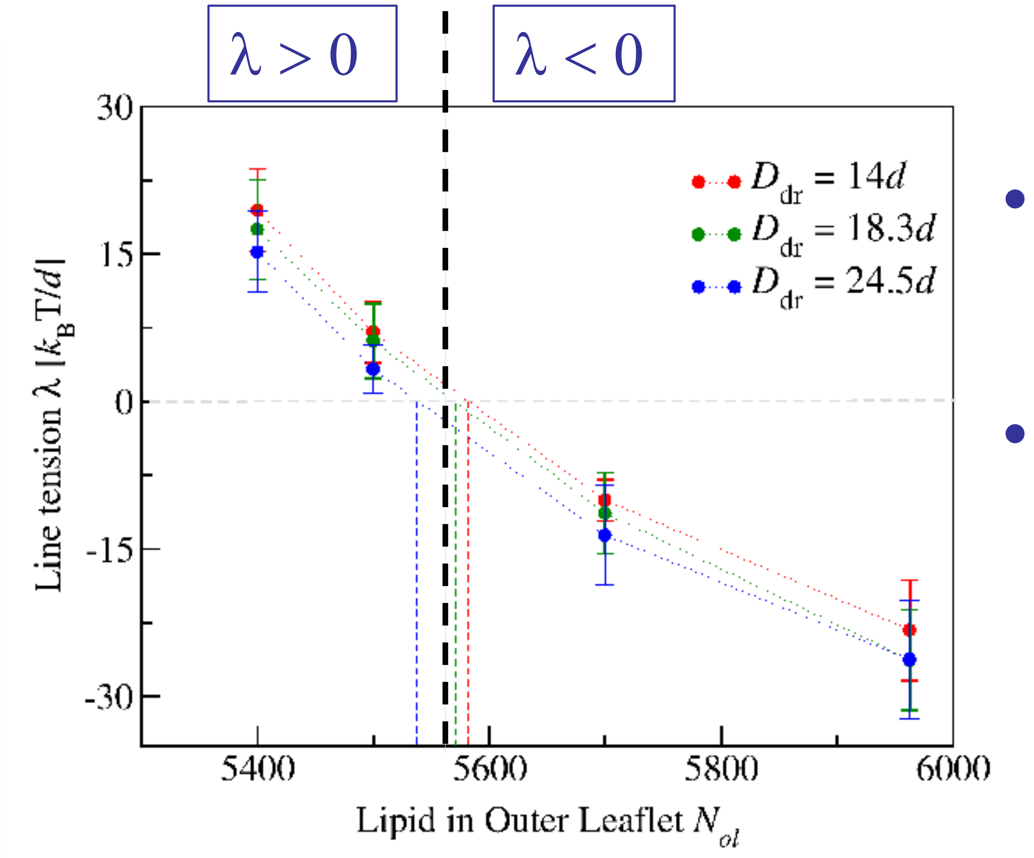


- Tight-lipped neck prevents division of nanovesicle

Ghosh et al, *Nature Comm 14* (2023)

Positive and Negative Line Tensions

- Contact line between droplet and vesicle membrane
- Line tension λ of contact line can be positive or negative:



- Positive line tension λ > 0 leads to axisymmetric engulfment
- Negative line tension λ < 0 leads to tight-lipped membrane neck

Ghosh et al, *Nature Comm 14* (2023)

Coworkers

Experiment

Rumiana Dimova

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Jan Steinkühler

Ziliang Zhao

Agustin Mamgiarotti

Shreya Pramanik

Theory

Simon Christ

Simulation

Andrea Grafmüller

Markus Miettinen

Rikhia Ghosh

Vahid Satarifard

Aparna Sreekumari

Miftakh Zamaletdinov

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