Remodeling of Lipid Bilayers and Nanovesicles

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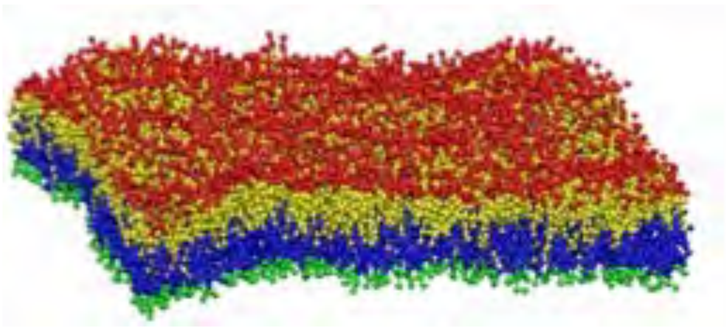
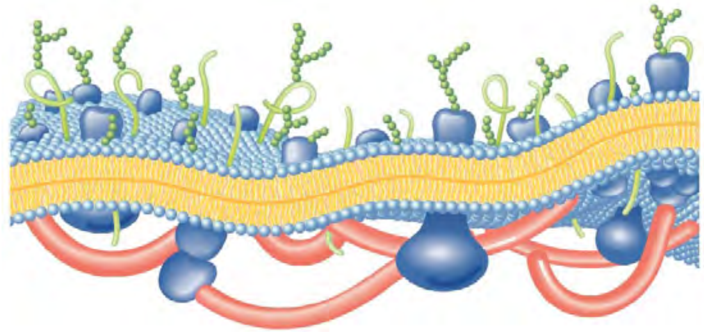
- Basic Aspects of Biomembranes
- Electron Microscopy and Molecular Dynamics
- Examples for Remodeling Processes
- Leaflet Tensions as Control Parameters
- Summary

Biomembranes at the Nano- and Microscale

- Lipid bilayer, two leaflets

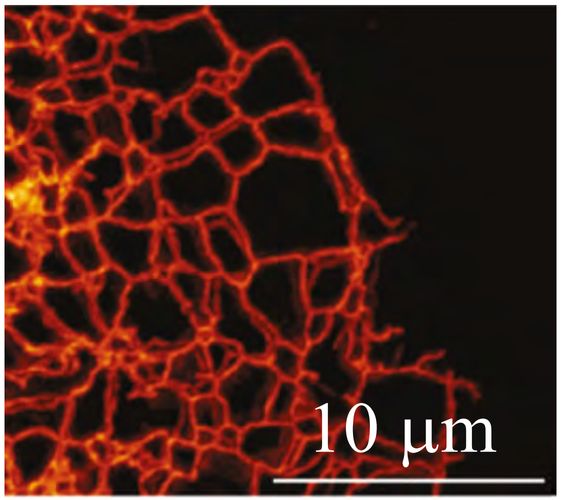


4 nm

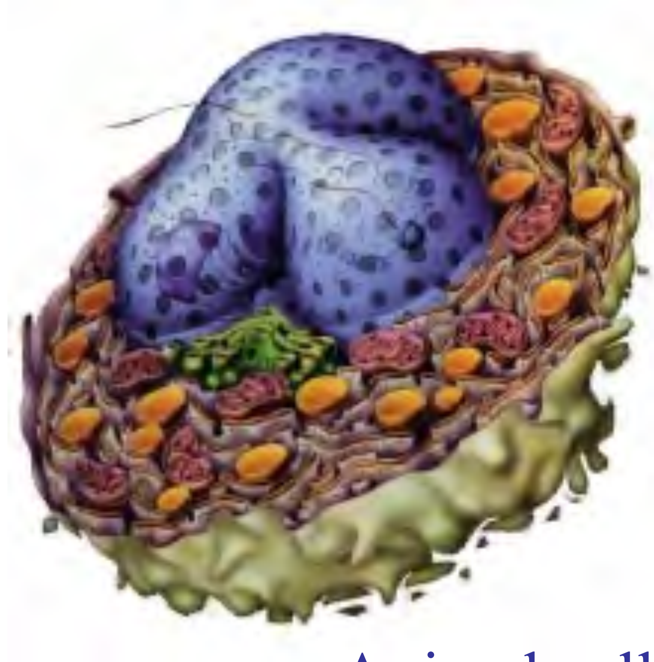


- Biological membrane

Lippincott-Schwartz (2022)



- Endoplasmic reticulum

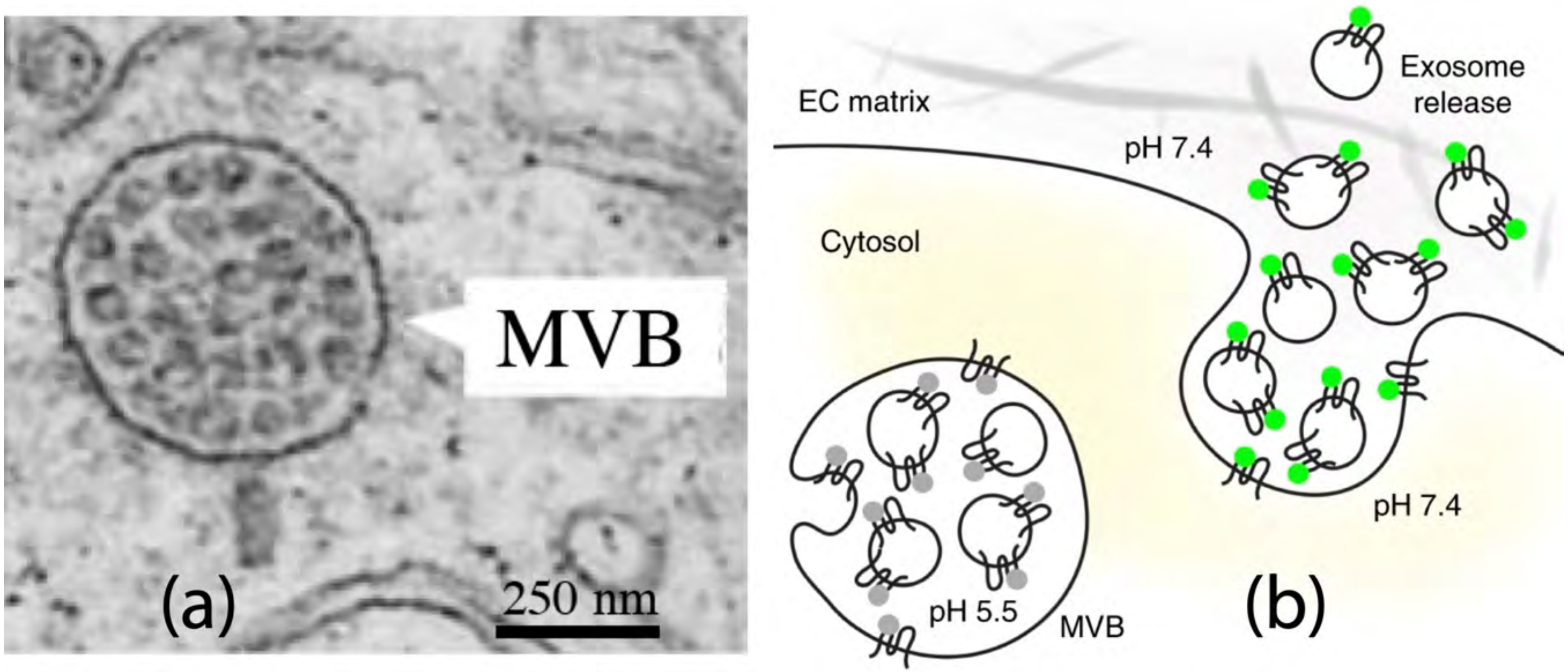


100 µm

- Animal cell

Multivesicular Bodies and Exosomes

Bartheld and Altick, *Prog. Neurobiol.* (2011)



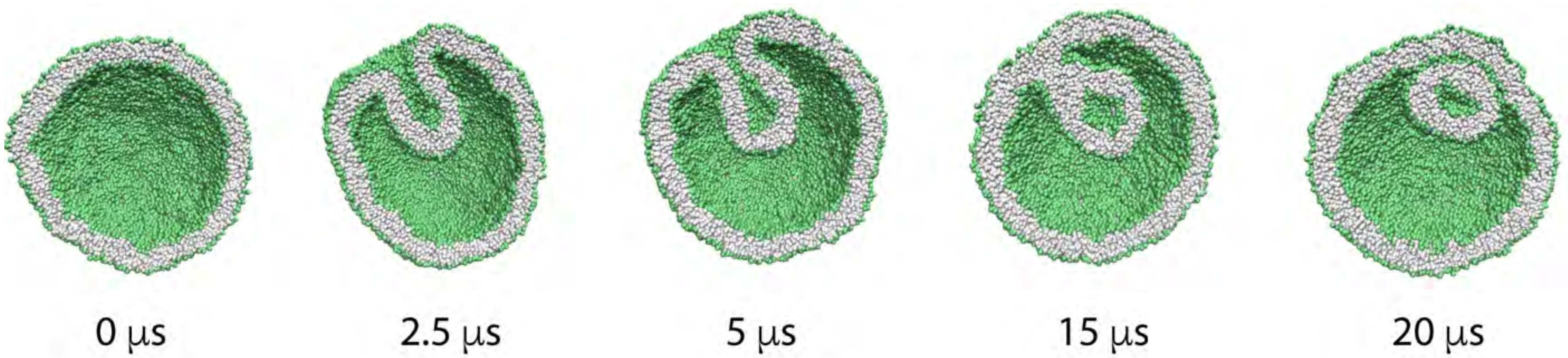
Bebelman et al, *Nature Protoc.* (2020)

- Electron microscopy produces „frozen“ snapshots
- Remodeling processes are highly dynamic!
- Molecular dynamics simulations

Molecular Dynamics Simulations: Example

Lipowsky, Ghosh et al, *Biomolecules* (2023)

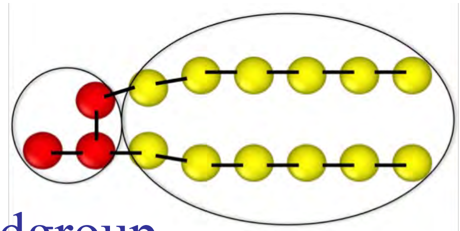
- Nanovesicle assembled from 10000 lipids
- Nanovesicle with diameter of 36 nm
- Tensionless bilayer with compressed inner leaflet
- Time evolution of nanovesicle after volume reduction:

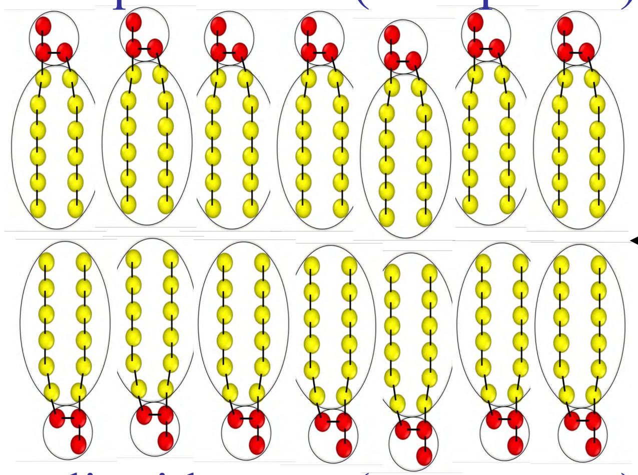


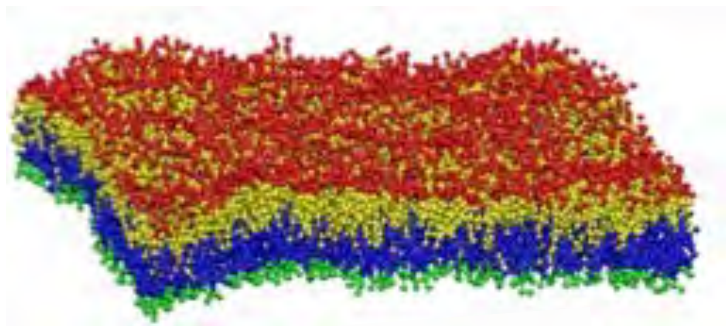
- Fission of membrane neck between outer and inner sphere

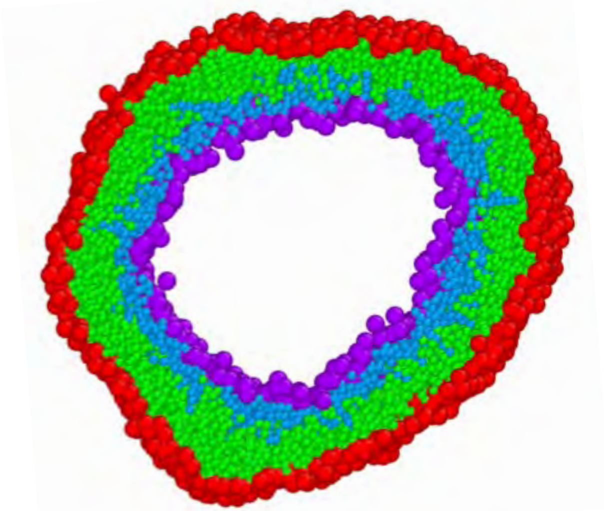
Assembly of Lipid Bilayers

- Coarse-grained lipid molecules
- Assembly of lipids into two leaflets







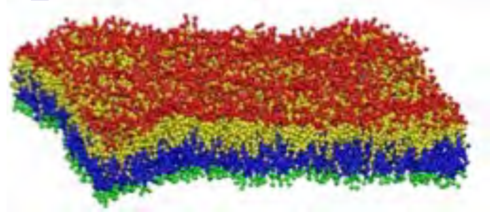


- Diff colors for lipids in diff leaflets
- Visualization of leaflet-leaflet interface
- Useful to follow lipid dynamics

Leaflet Tensions and Stress Asymmetry

Rozycki and Lipowsky, *J. Chem. Phus.* (2015); Sreekumari and Lipowsky, *Soft Matter.* (2022)

- Each leaflet experiences leaflet tension, Σ_1 and Σ_2
- Bilayer tension $\Sigma = \Sigma_1 + \Sigma_2$
- Stress asymmetry $\Sigma = \Sigma_1 - \Sigma_2$

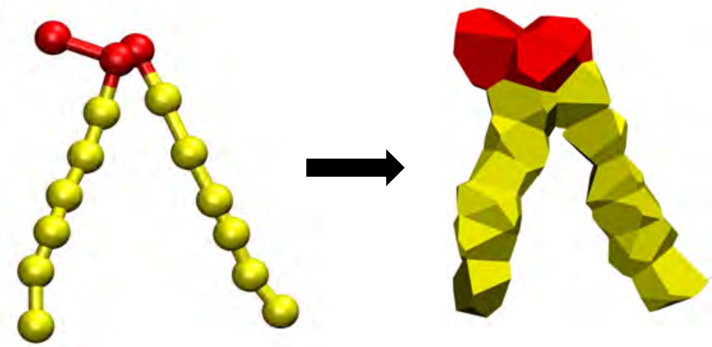


- Leaflet tensions determined by number of lipid molecules
- Different numbers of lipids => different leaflet tensions
- Each leaflet tension can be positive or negative corresponding to stretching or compression
- Tensionless bilayer $\Sigma = \Sigma_1 + \Sigma_2 = 0$ or $\Sigma_1 = -\Sigma_2$
- Tensionless bilayers are unlikely to rupture
- Tensionless bilayers can involve large leaflet tensions

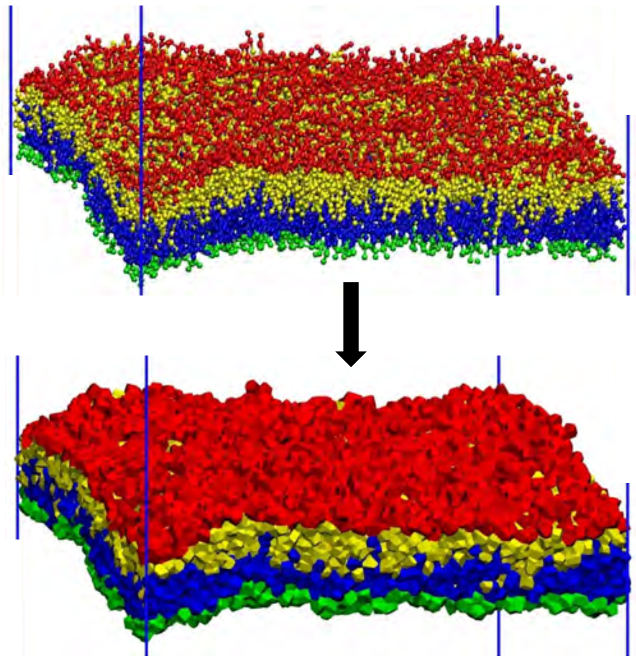
Digression: Area per Lipid / Volume per Lipid

Zamaletdinov, Miettinen, Lipowsky, *Soft Matter 19* (2023) p. 6929

- Most protocols based on area per lipid, but ambiguous
- Improved protocol based on volume per lipid



- Voronoi tesselation
- Place polyhedral cell around each molecular group
- Compute volumes of all cells
- Volume of leaflet = sum over volumes of all cells around lipids in this leaflet

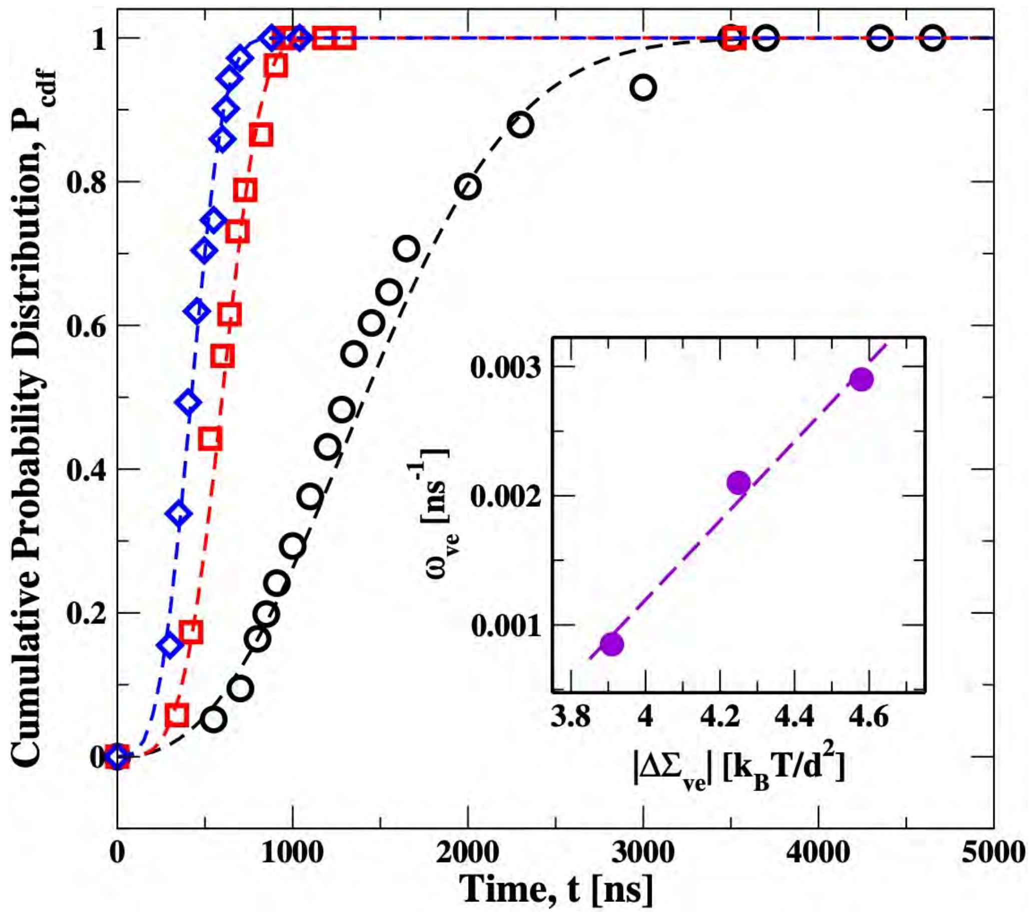


Large Stress Asymmetries: Flip-Flops

Sreekumari and Lipowsky
Soft Matter (2022)

- Cumulative distribution function for time *t* until first flip flop:

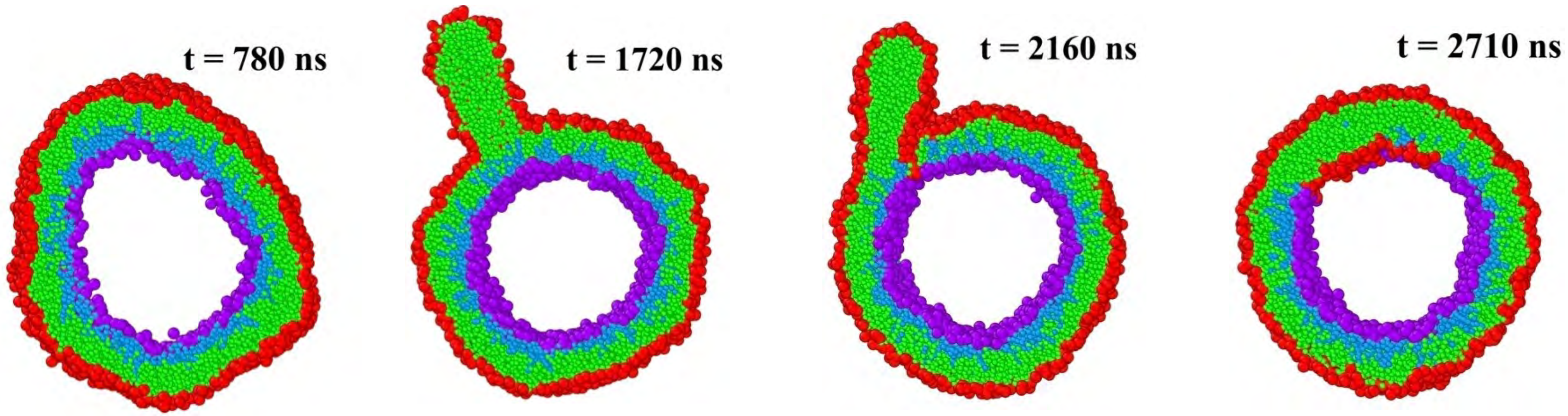
- Distribution function depends on stress asymmetry $\Delta\Sigma$
- 3 data sets for 3 different $\Delta\Sigma$:
- Good fit by Weibull distribution with sigmoidal shape
- Fit parameter = flip-flop rate ω_{pl}
- Flip-flop rate ω_{pl} increases with stress asymmetry $\Delta\Sigma_{ve}$ (inset)



Structural Instabilities and Self-Healing

- Large stress asymmetries $\Delta\Sigma$
- Structural instabilities in addition to flip-flops
- Tensionless bilayer with stretched inner leaflet and compressed outer leaflet, Time-lapse snapshots:

Sreekumari and Lipowsky
Soft Matter (2022)



Expelled micelle

Stress-incuded flips of red-green lipids

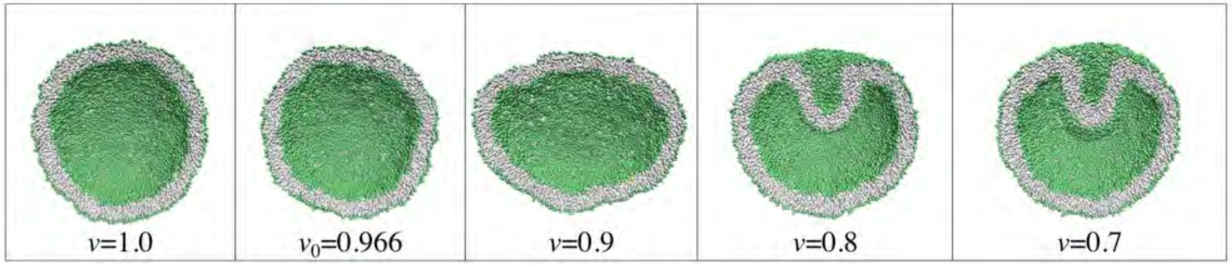
Bilayer after self-healing

Nonspherical Shapes of Nanovesicles

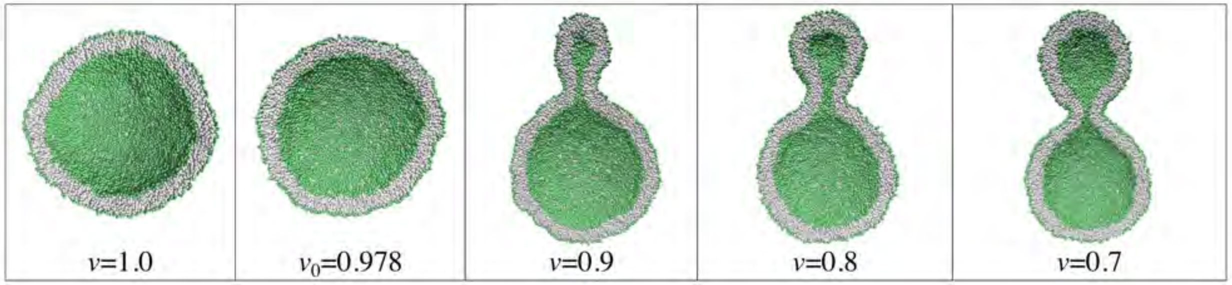
- Spherical nanovesicles with total number of 10000 lipids
- Leaflet tensions Σ_{il} and Σ_{ol} changed by reshuffling a few hundred lipids between the two leaflets

Rikhia Ghosh et al
Nano Letters (2019)

Outer leaflet stretched
Inner leaflet compressed



Outer leaflet compressed
Inner leaflet stretched

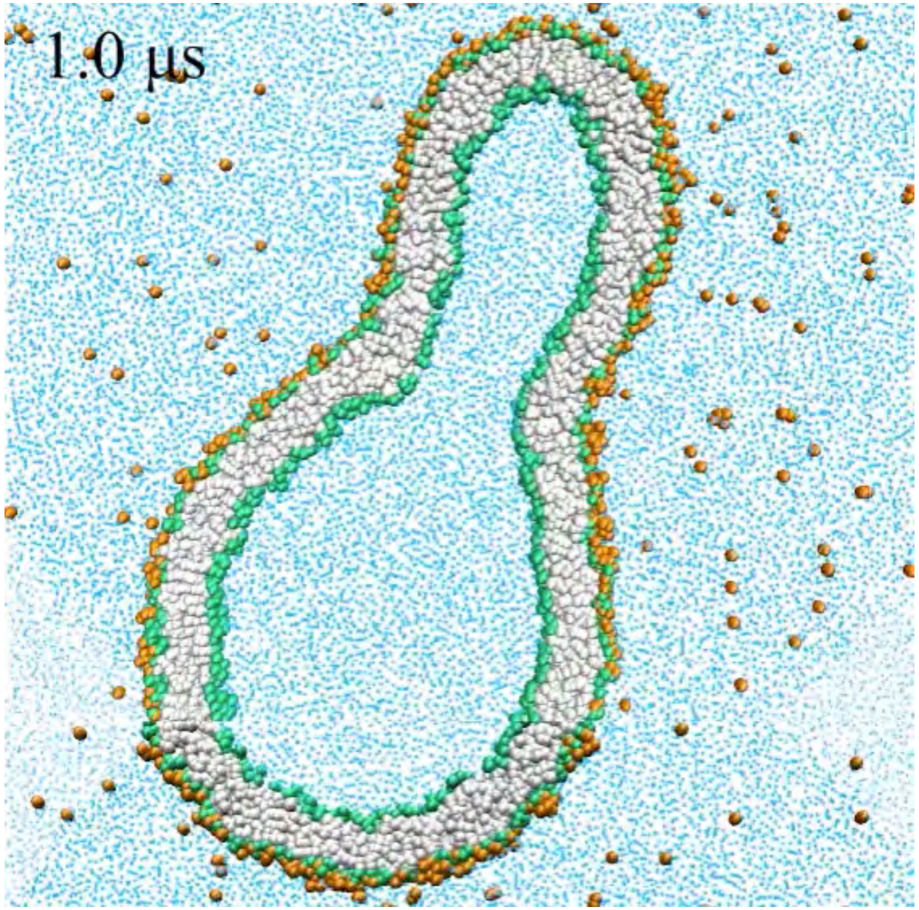


- Reduction of vesicle volume leads to distinct shape transformations

Nanovesicles Exposed to Small Solutes

Rikhia Ghosh et al,
ACS Nano 15 (2021)

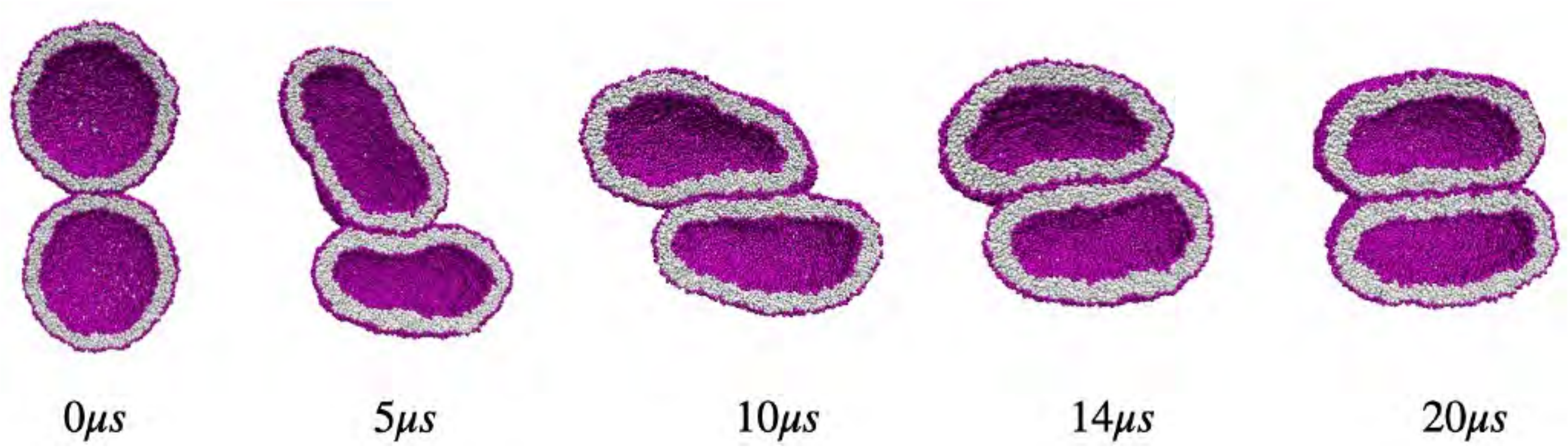
- Nanovesicle exposed to small adsorbing solutes (orange)
- Remodeling controlled by solute concentration
- Example: Dumbbell shapes with open and closed necks
- Close to the binodal line: recurrent shape changes between open and closed dumbbells



Interactions of Nanovesicles: Adhesion

Lipowsky, Ghosh et al, *Biomolecules* (2023)

- Two identical nanovesicles in close contact
- Outer leaflets stretched, inner leaflets compressed
- Each vesicle with stress asymmetry $\Delta\Sigma = 1.69\ kT/d^2$
- Increase of contact area after volume reduction
- After 20 μs, stable adhesion between two vesicles:



Interaction of Nanovesicles: Fusion

Lipowsky, Ghosh et al, *Biomolecules* (2023)

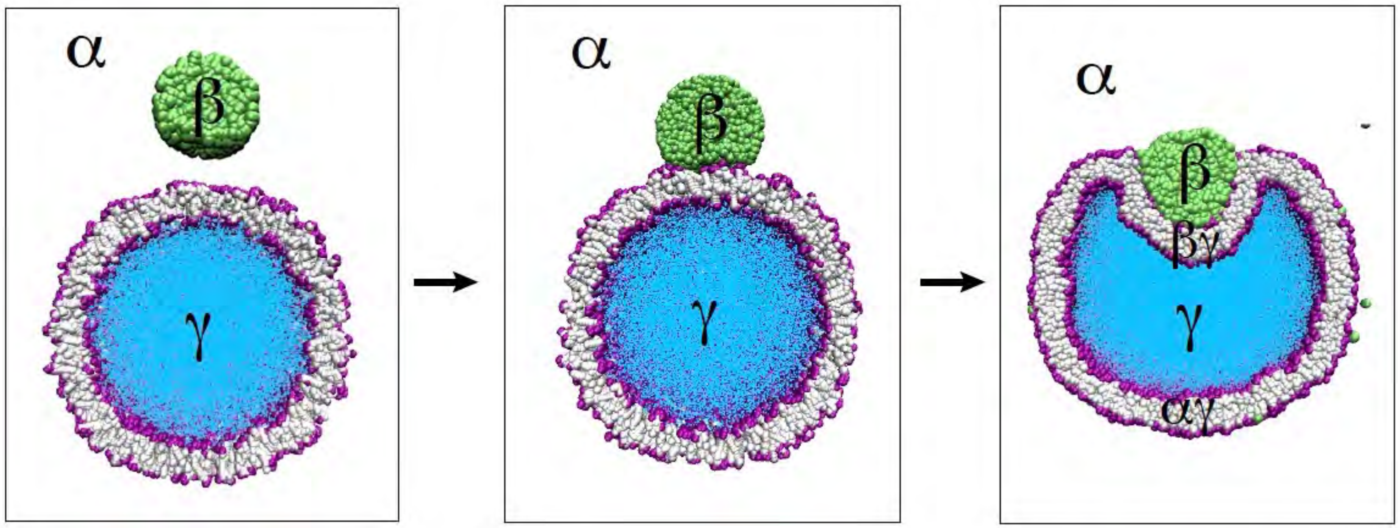
- Two identical nanovesicles in close contact
- Stress asymmetry increased to $\Delta\Sigma = 2.04\ kT/d^2$
- Vesicles undergo fast fusion:

- New fusion mechanism between bilayers with vanishing bilayer tension but stretched outer leaflets!

Endocytosis of Condensate Droplets

Rikhia Ghosh et al, *Nature Commun* (2023)

- Adhesion of condensate droplets to nanovesicles:



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

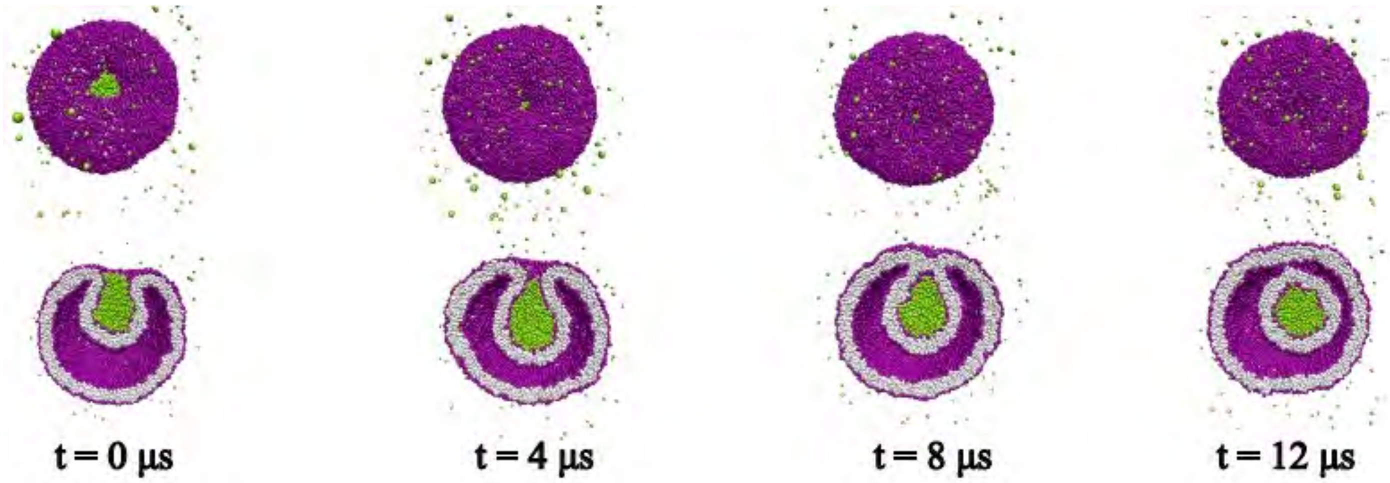
Pathway I: Endocytosis and NV Division

Rikhia Ghosh et al, *Nature Commun* (2023)

- Complete axisymmetric engulfment of droplet followed by fission of membrane neck and division of nanovesicle:

Top view:

Side view:



- Positive line tension of contact line between membrane and condensate droplet

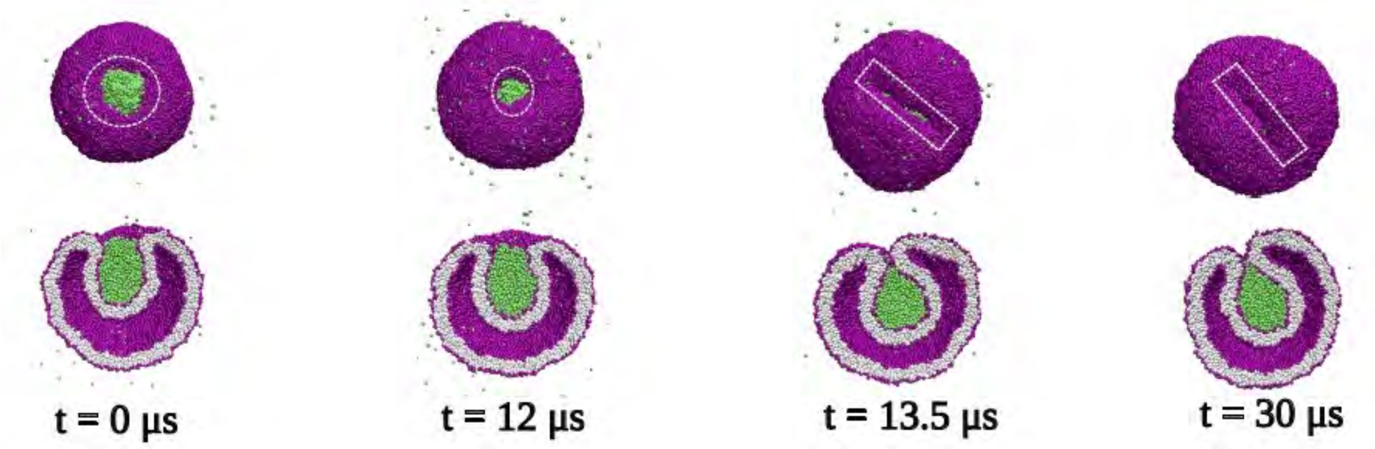
Pathway II: Incomplete Endocytosis

Rikhia Ghosh et al, *Nature Commun* (2023)

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

Top view:

"Orthogonal" side view:



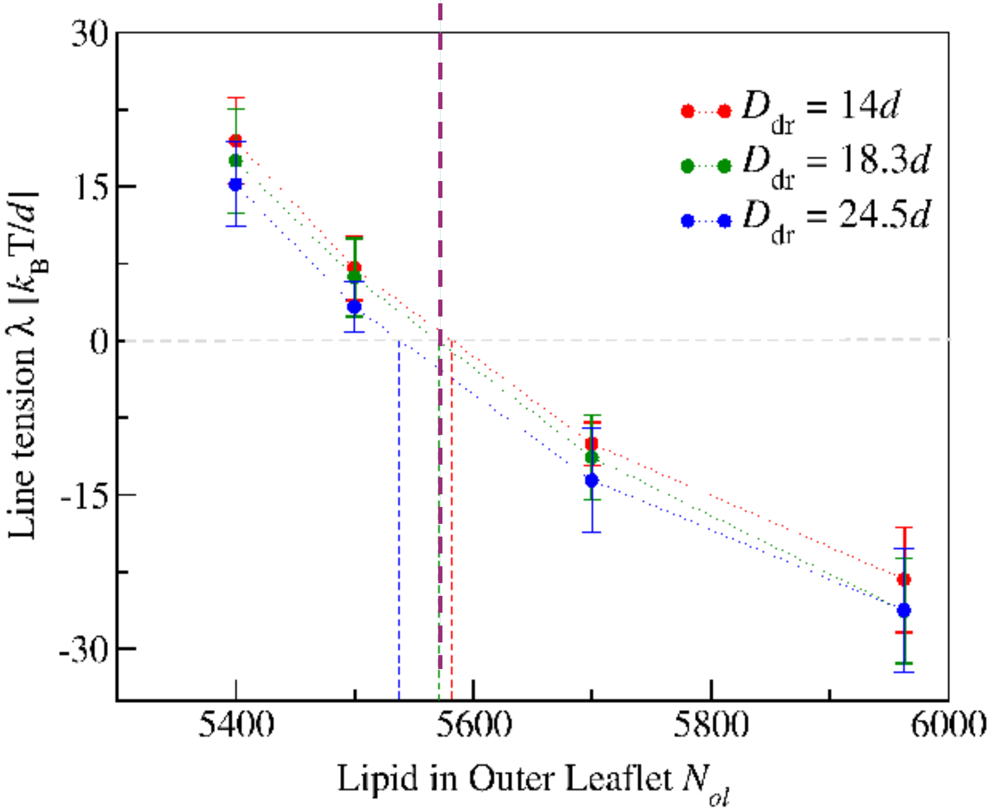
- Negative line tension of contact line between membrane and condensate droplet

Pathways controlled by Leaflet Tensions

Rikhia Ghosh et al, *Nature Commun* (2023)

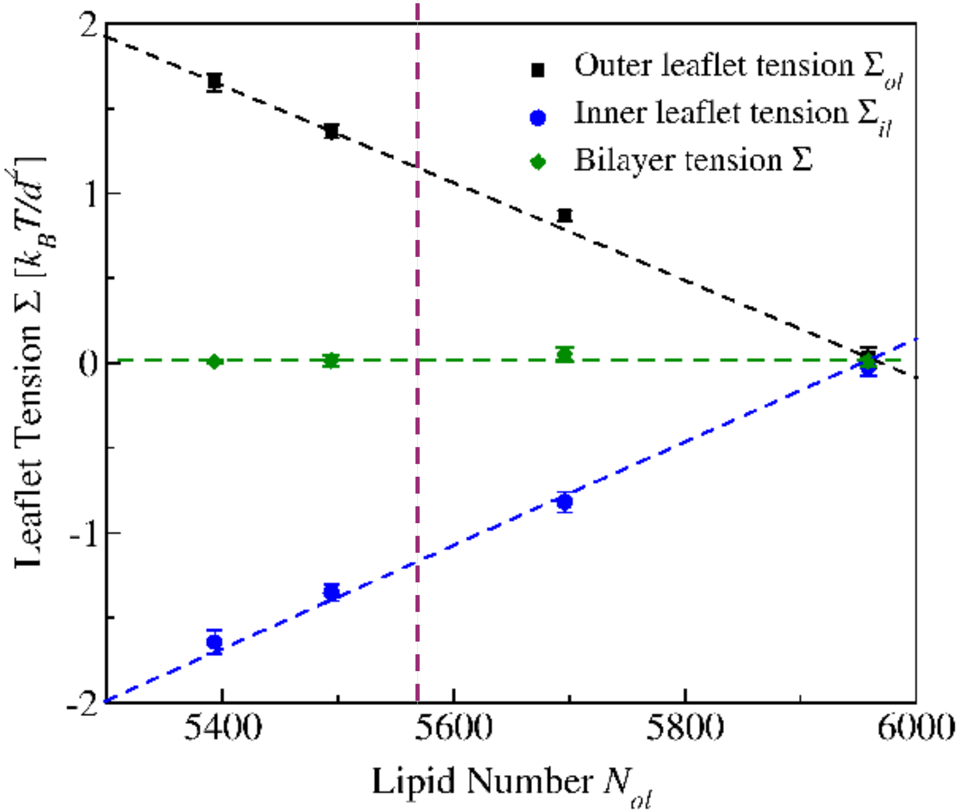
- Line tension λ versus lipid number N_{ol} in outer leaflet:

$\lambda > 0$ $\lambda < 0$



- Leaflet tensions versus lipid number N_{ol} in outer leaflet:

$\lambda > 0$ $\lambda < 0$



Summary

Leaflet tensions are key parameters to control:

- Stability and instability regimes of lipid bilayers
- Stress-induced flip-flops and structural instabilities
- Nonspherical shapes of nanovesicles
- Adhesion versus fusion for interacting vesicles

  Membrane fusion by stretched outer leaflets
- Different pathways for endocytosis of nanodroplets

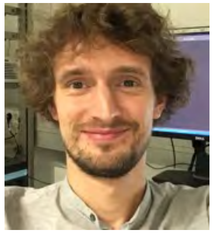
  Positive and negative line tensions of contact line
- Tight-lipped membrane necks impede endocytosis
- Different pathways for endocytosis of nanoparticles

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


Miftakh Zamaletdinov

Collaborations with: Joachim Spatz, Seraphine Wegner, Petra Schwille

Abstract - References

[1] R. Ghosh, V. Satarifard, A. Grafmüller, and R. Lipowsky: *Spherical Nanovesicles Transform into a Multitude of Nonspherical Shapes.* Nano Lett. **19**, 7703–7711 (2019).

[2] A. Sreekumari and R. Lipowsky: *Large stress asymmetries of lipid bilayers and nanovesicles generate lipid flip-flops and bilayer instabilities.* Soft Matter **18**, 6066–6078 (2022).

[3] M. Miettinen and R. Lipowsky: *Bilayer Membranes with Frequent Flip Flops have Tensionless Leaflets.* Nano Lett. **19**, 5011–5016 (2019).

[4] R. Ghosh, V. Satarifard, A. Grafmüller, and R. Lipowsky: *Budding and Fission of Nanovesicles induced by membrane adsorption of small solutes.* ACS Nano **15**, 7237–7248 (2021).

[5] R. Ghosh, V. Satarifard, and R. Lipowsky: *Different pathways for engulfment and endocytosis of droplets by nanovesicles.* Nature Commun. **14**, 615 (2023).

[6] M. Zamaletdinov, M. Miettinen, and R. Lipowsky: *Probing the elastic response of lipid bilayers and nanovesicles to leaflet tensions via volume per lipid.* Soft Matter **19**, 6929 (2023)

[7] R. Lipowsky, R. Ghosh, V. .Satarifard, A. Sreekumari, M. Zamaletdinov, B. Różycki, M. Miettinen, and A. Grafmüller: *Leaflet tensions control the spatio-temporal remodeling of lipid bilayers and nanovesicles.* Biomolecules **13**, 926 (2023).