Membrane Remodelling in Viral fusion and Migrasome Formation

EBSA 2023

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School of Chemistry
The Raymond and Beverly Sackler
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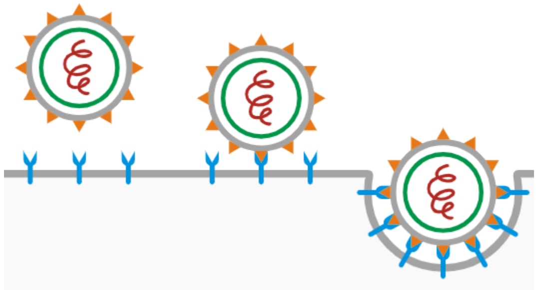
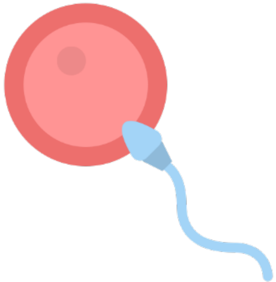
Tel Aviv University

בית הספר לכימיה
הפקולטה למדעים מדויקים
ע״ש ריימונד ובברלי סאקלר
אוניברסיטת תל אביב

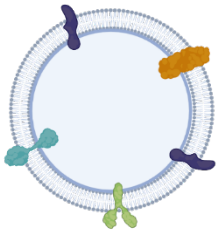
Biophysical and biochemical parameters govern membrane processes



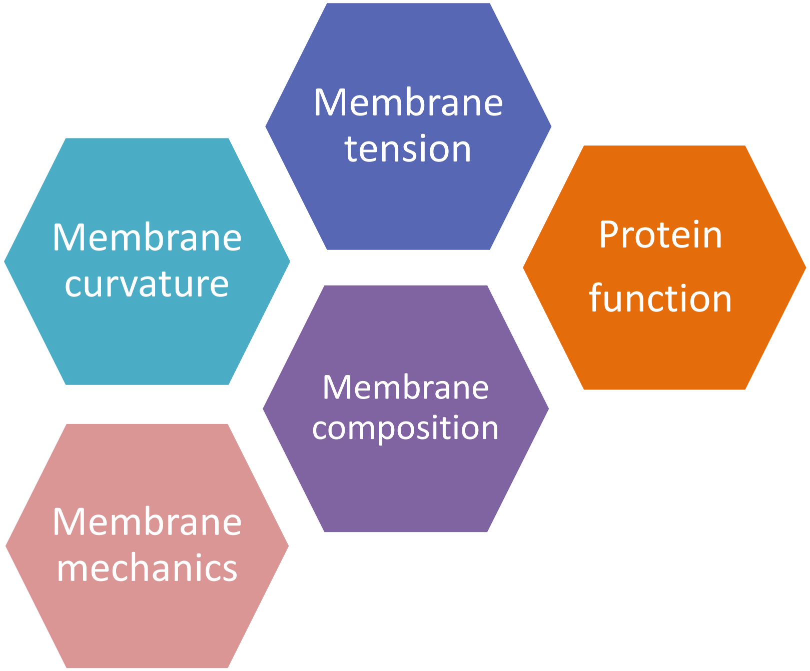
Membrane fusion in viral infection



Membrane fusion in fertilization

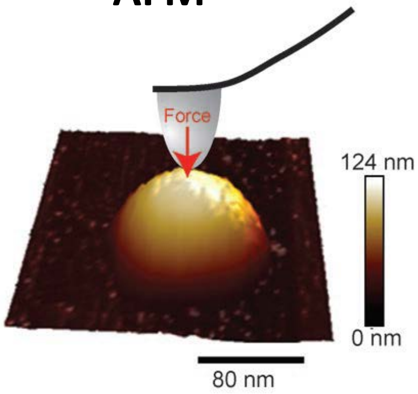


Membrane remodelling during vesicle formation



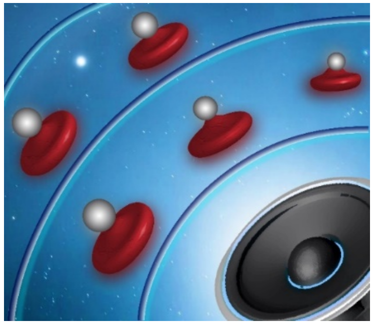
Pushing and pulling membranes:
A toolbox for membrane biophysics studies

AFM



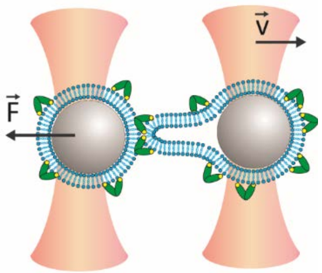
- *R. Sorkin et. al., Small, 2018*
- *D. Vorselen…R. Sorkin et. al., Nat Comm 2018*

Acoustic Force Spectroscopy



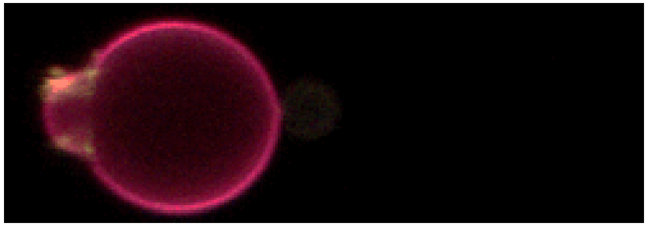
- *R. Sorkin et. al., MBoC, 2018*
- *E. Dekel, …, R. Sorkin et al, Nat Comm 2021*

Optical Tweezers Force Spectroscopy



- *R. Sorkin et. al., Biophys. J. 2020*
- *S. K. Cheppali, R. Dharan and R. Sorkin, J. Mem Bio. 2022*
- *S.K Cheppali… R. Sorkin, ACS Appl. Mater. Interfaces 2022*

Optical Tweezers+ micropipette aspiration + microscopy



- *R. Dharan…R. Sorkin, PNAS 2022*
- *R. Dharan… Y. Huang…R. Sorkin, Nat Comm, 2023*

Outline

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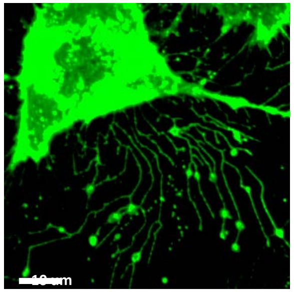
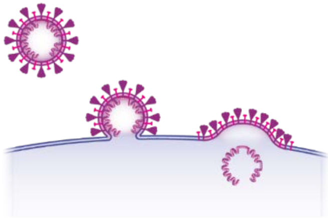
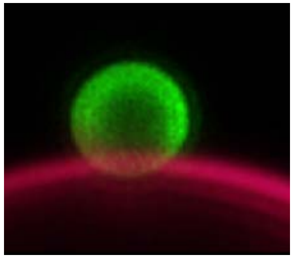


2. New assays towards better understanding of membrane fusion in viral infection



3. Effect of membrane tension on fusion



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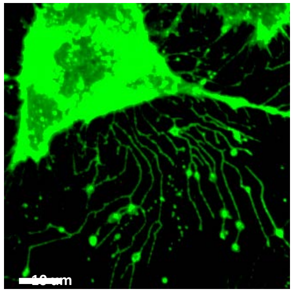

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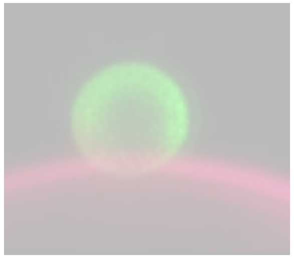


Biophysics of migrasome formation

In collaboration with:
Kozlov Group, TAU

Li Yu Lab, Tsinghua Uni

Raviv Dharan

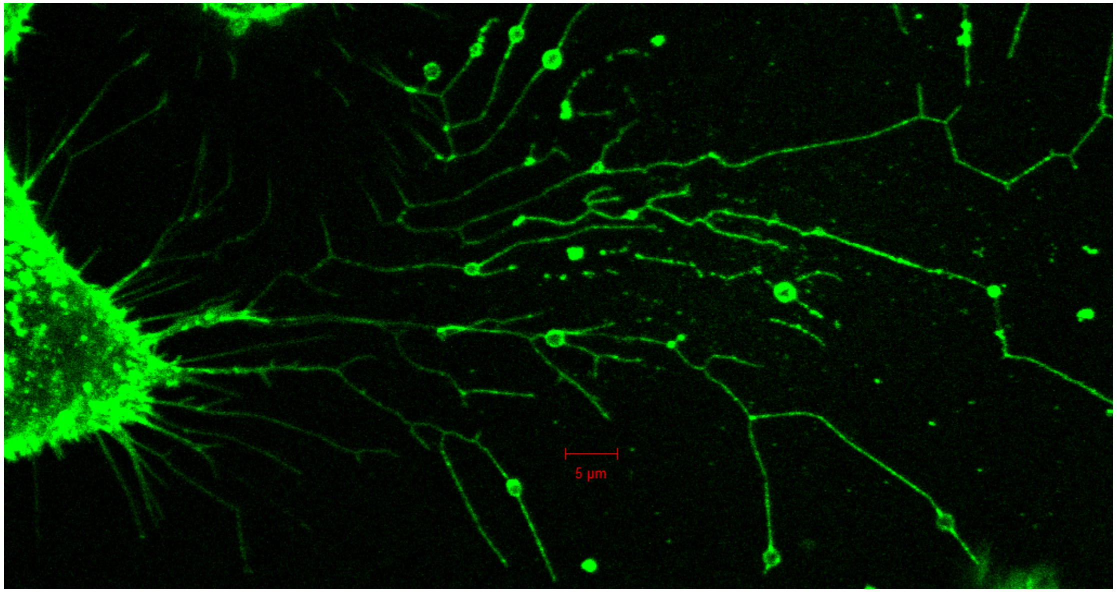
Shahar Goren

Alisa Vaknin

Misha Kozlov

Li Yu

Yuwei Huang



Migrasomes are new members of the EV family

L. Ma et al, Li Yu Lab, Cell Research 2015

- In zebrafish organ morphogenesis, release chemokine signals to defined regions of the embryo.
  Organ morphogenesis is impaired in mutants (TSPAN knockouts)

D. Jiang et al, NCB 2019

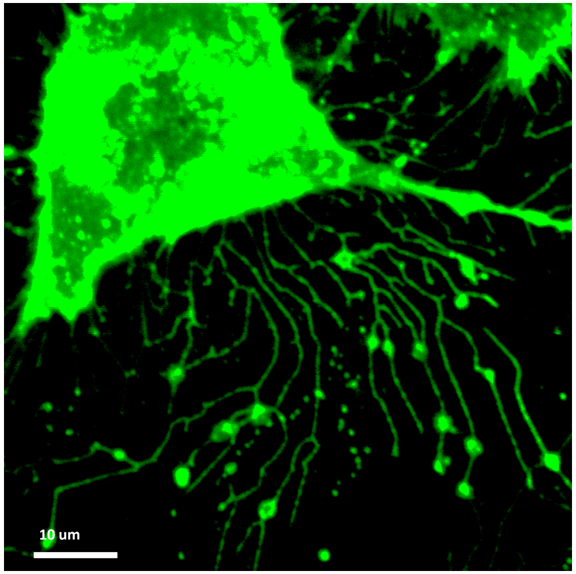
- Lateral transfer of mRNA and proteins
  by migrasomes modifies recipient cells

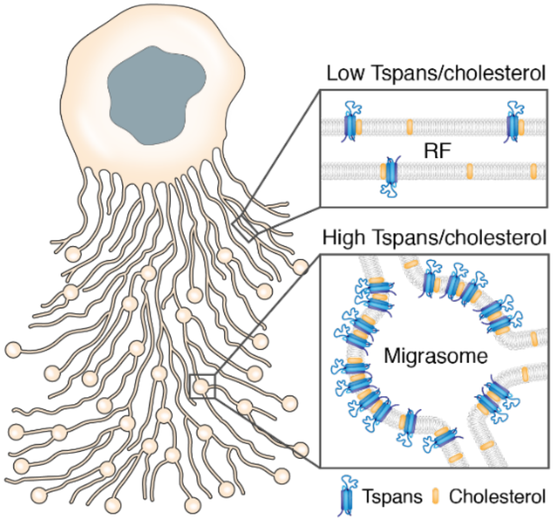
M. Zhu, Cell Research, 2020

- Mediate mitochondria quality control

H. Jiao, Cell, 2021

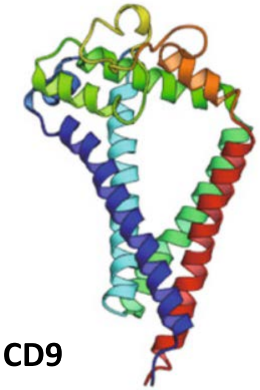
Migrasome formation necessitates Tetraspanins





Enriched with tetraspanin proteins

TSPN protein family

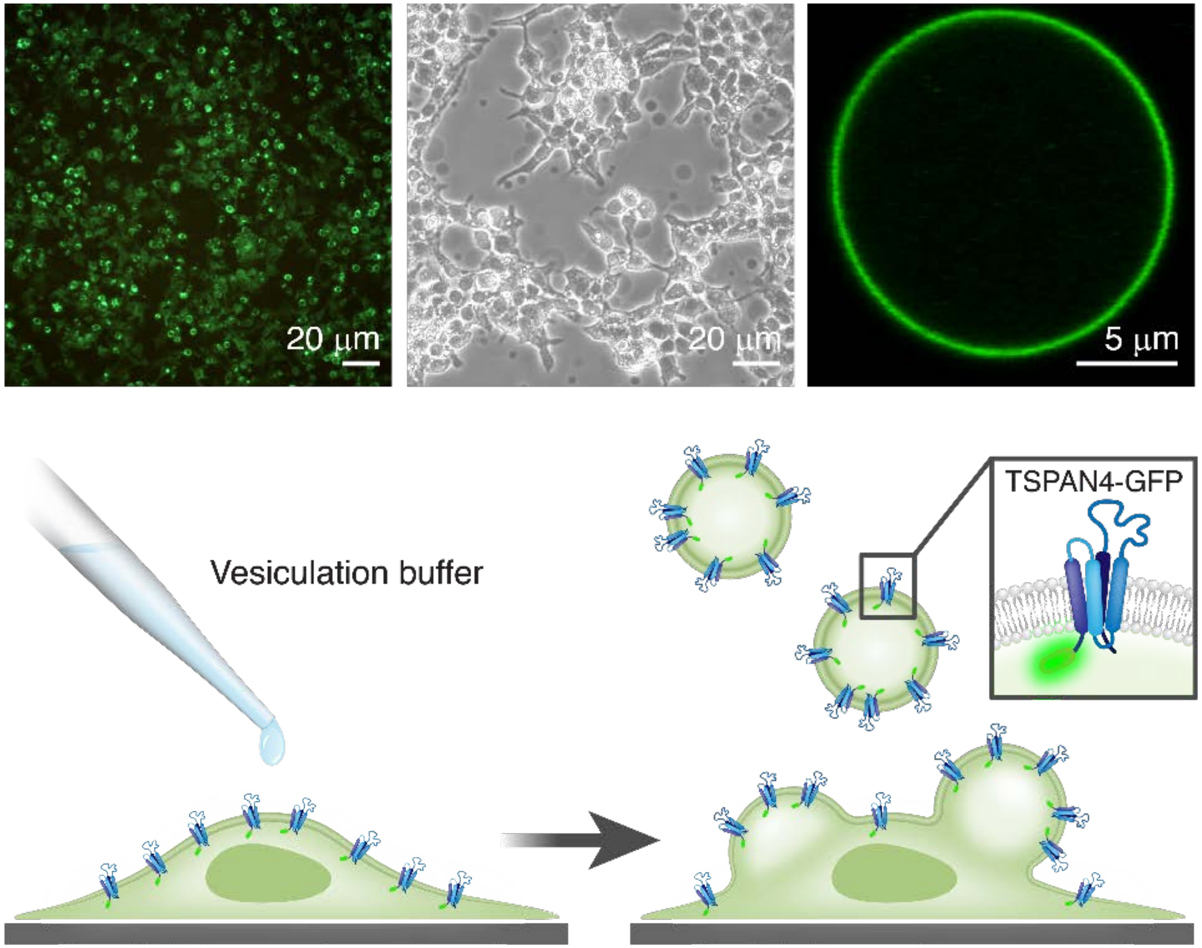


CD9

Rie Umeda et al. Nat Comm 2020

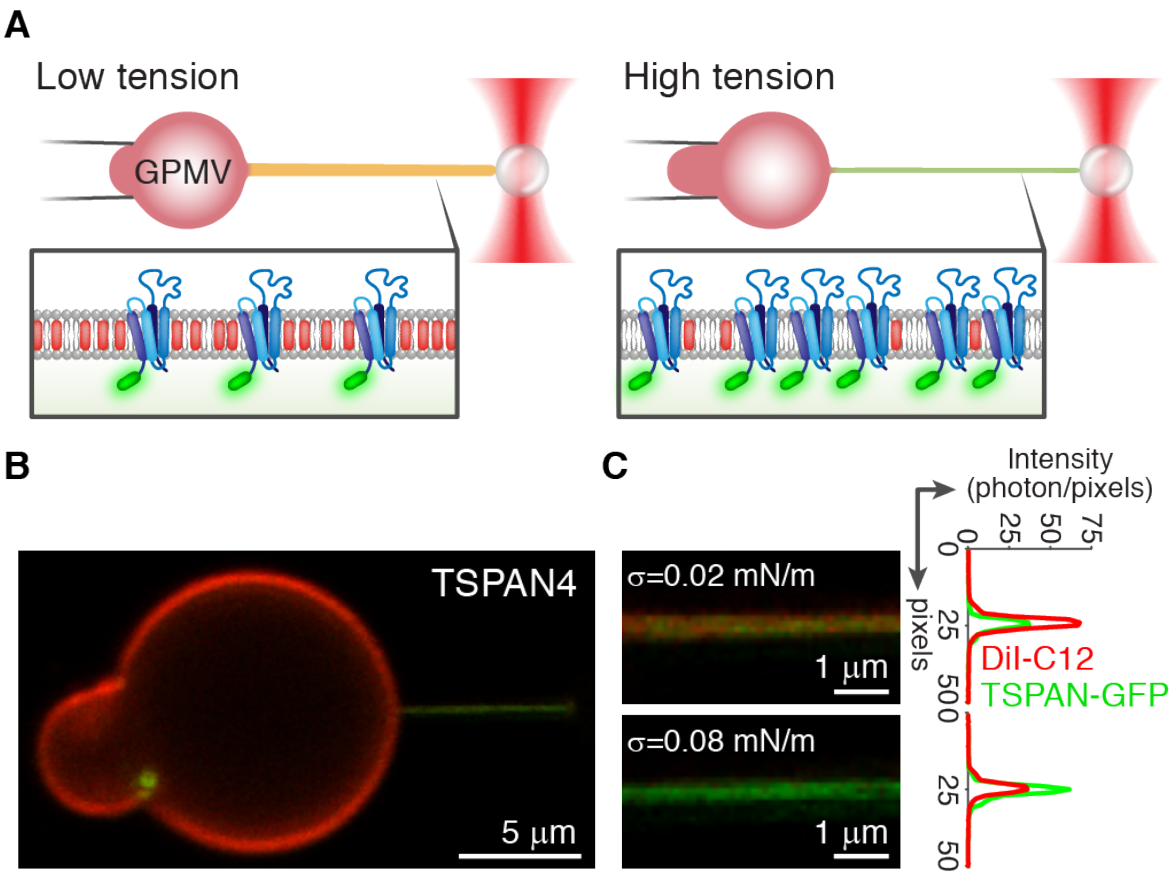
- Small proteins with four transmembrane domains
- Associated with various biological processes including cell adhesion, motility, membrane fusion and signaling in diverse organs
- The transmembrane region is highly conserved and has a **cone-like shape**

TSPAN4 GPMVs



R. Dharan...R. Sorkin, PNAS, 2022

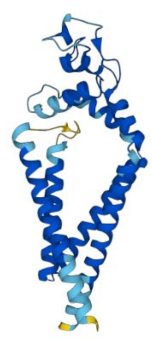
TSPN4 is curvature sensitive



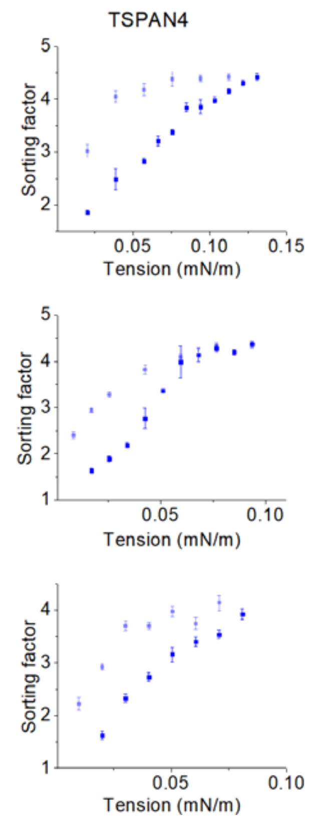
R. Dharan...R. Sorkin, PNAS, 2022

Sorting factor hysteresis

Rie Umeda et al. Nat Comm 2020

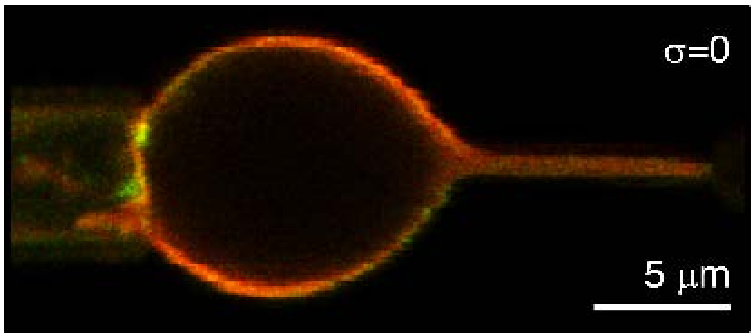


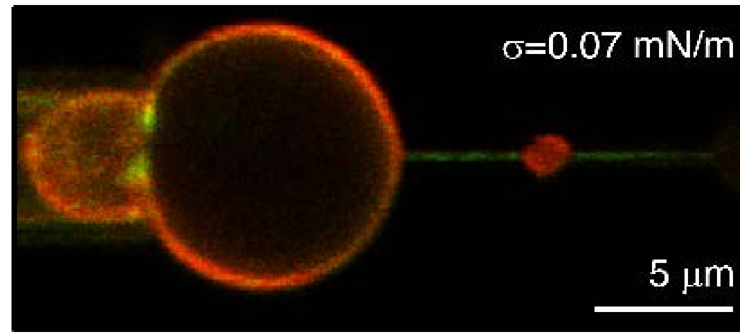
AlphaFold prediction



R. Dharan...R. Sorkin, PNAS, 2022

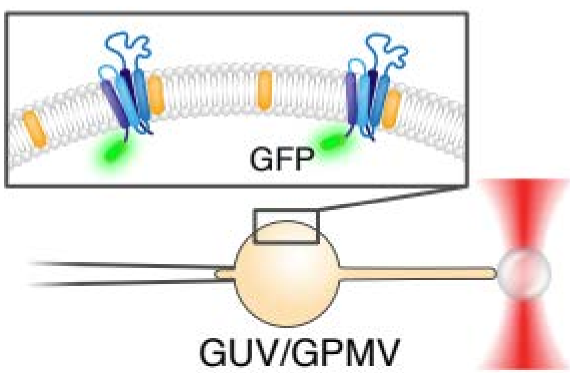
Sudden tension increase induces vesicle formation

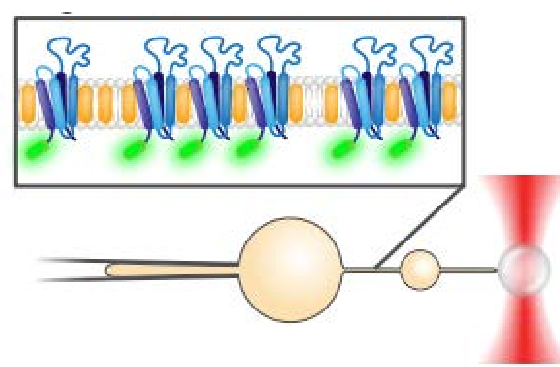




Low tension (wide tether)

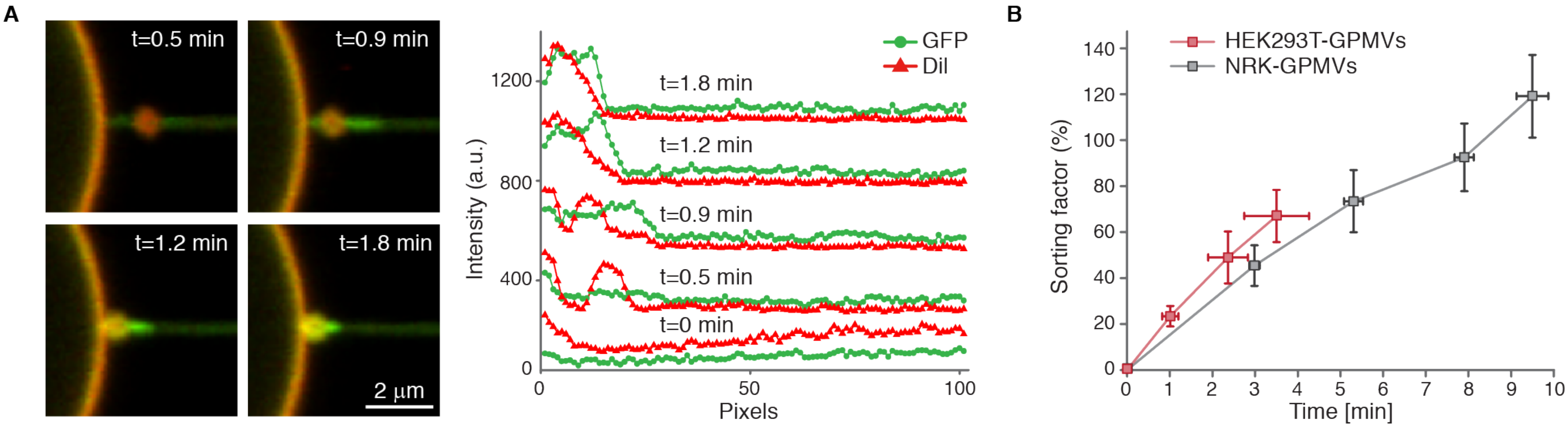
High tension (narrow tether)





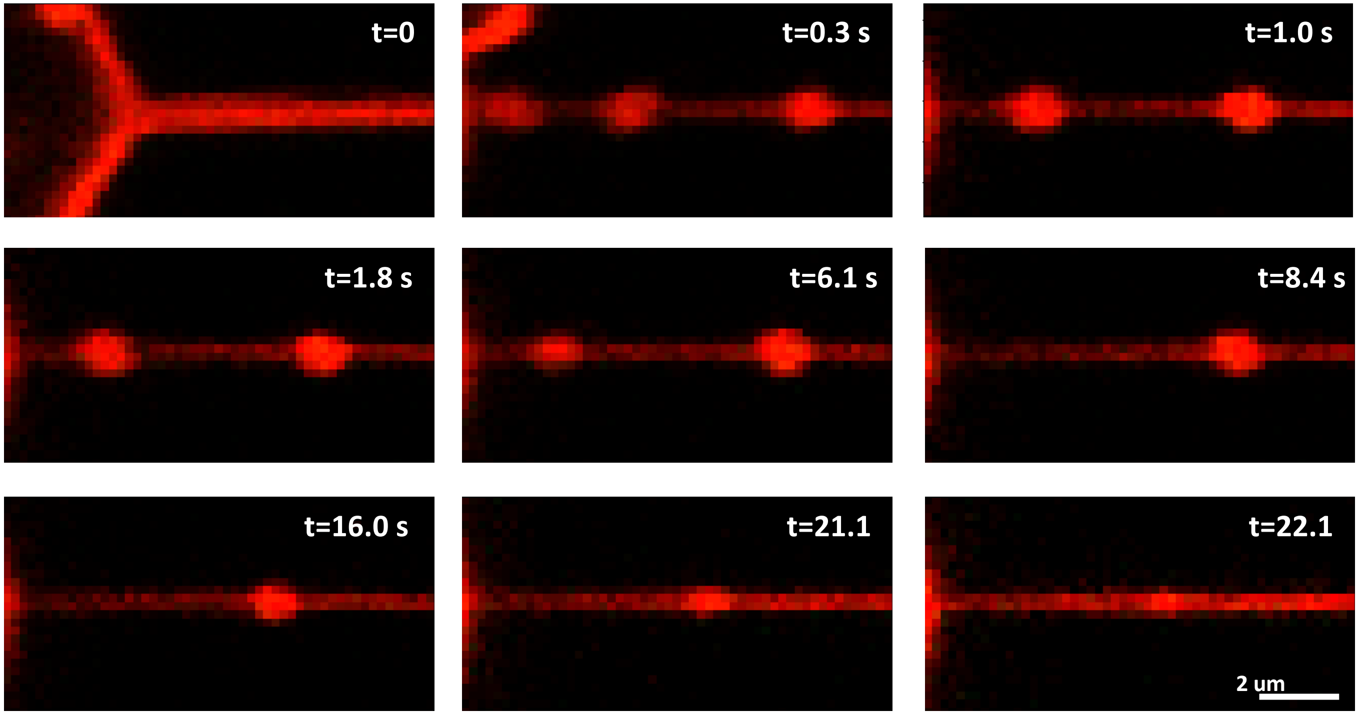
R. Dharan, Y. Huang...R. Sorkin, Nat Comm 2023

TSPAN migrates to the swelling and stabilizes it



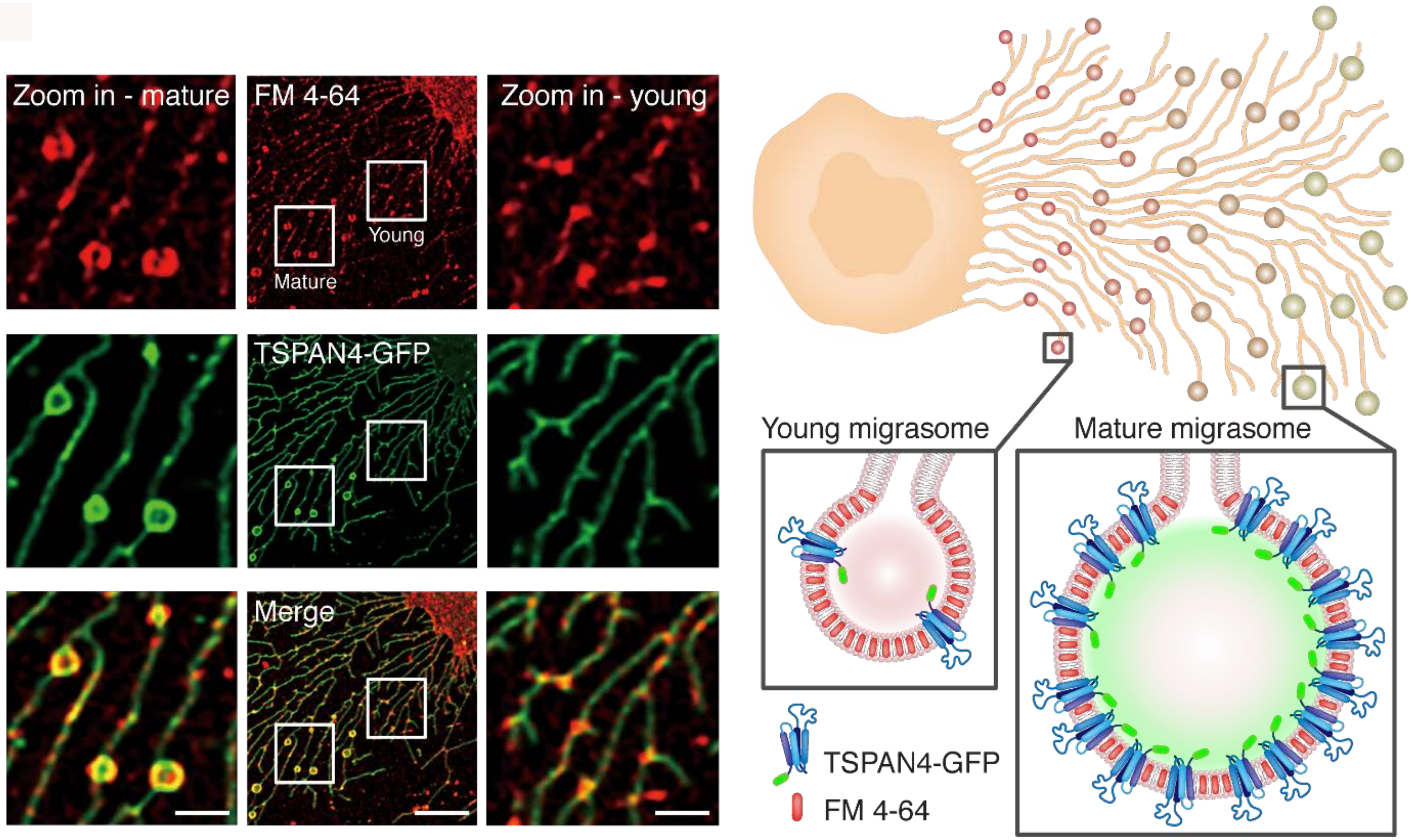
R. Dharan, Y. Huang...R. Sorkin, Nat Comm 2023

GPMVs without TSPAN-control



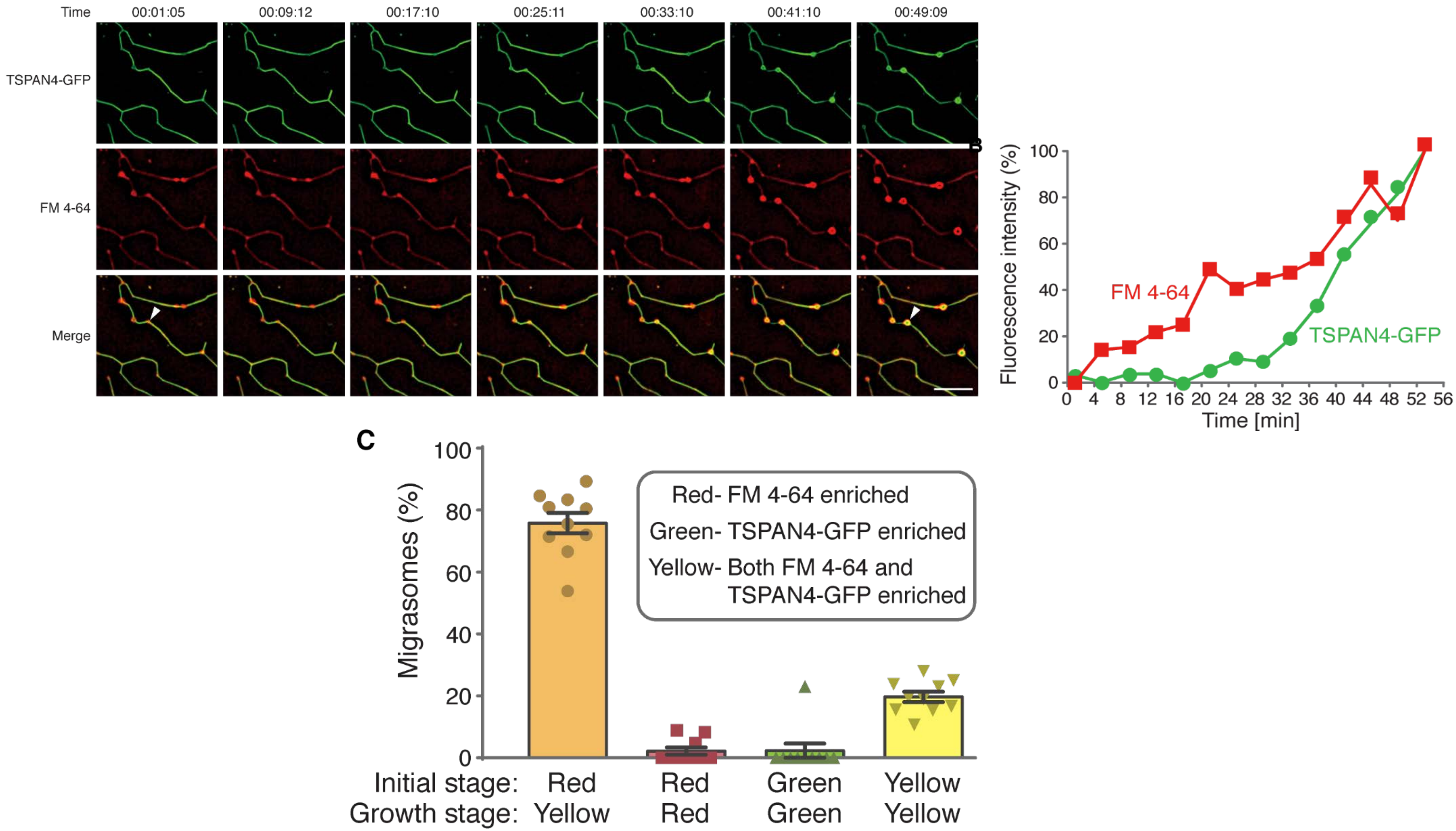
R. Dharan, Y. Huang...R. Sorkin, Nat Comm 2023

Initial stage of migrasome formation is TSPAN independent

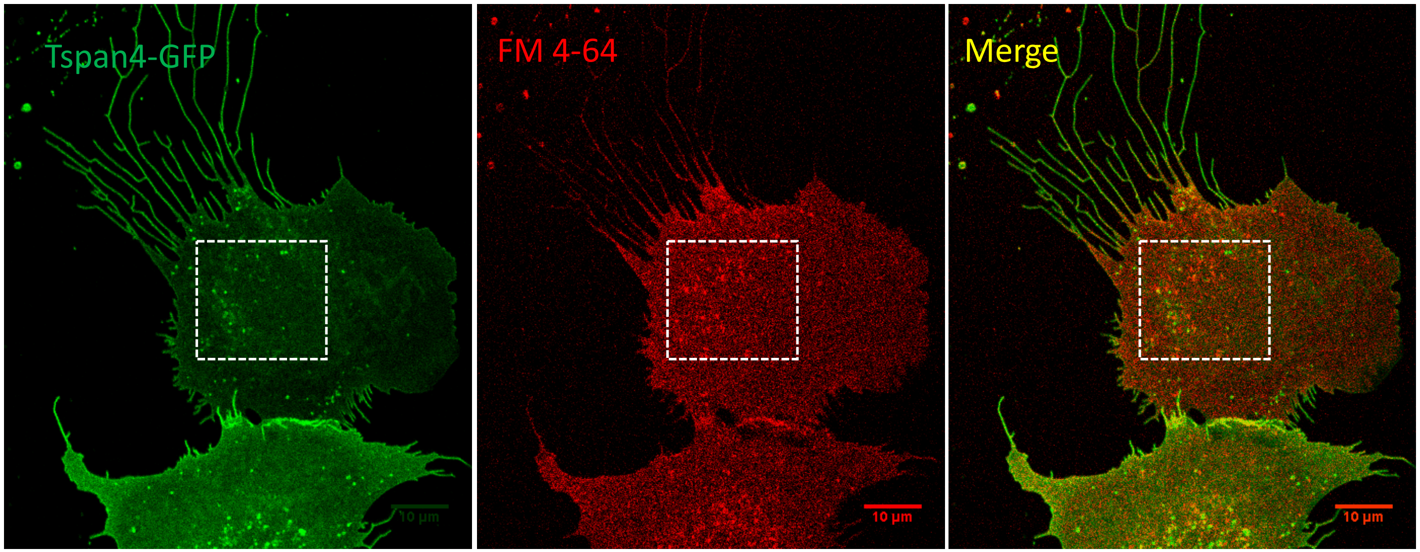


R. Dharan, Y. Huang...R. Sorkin, Nat Comm 2023

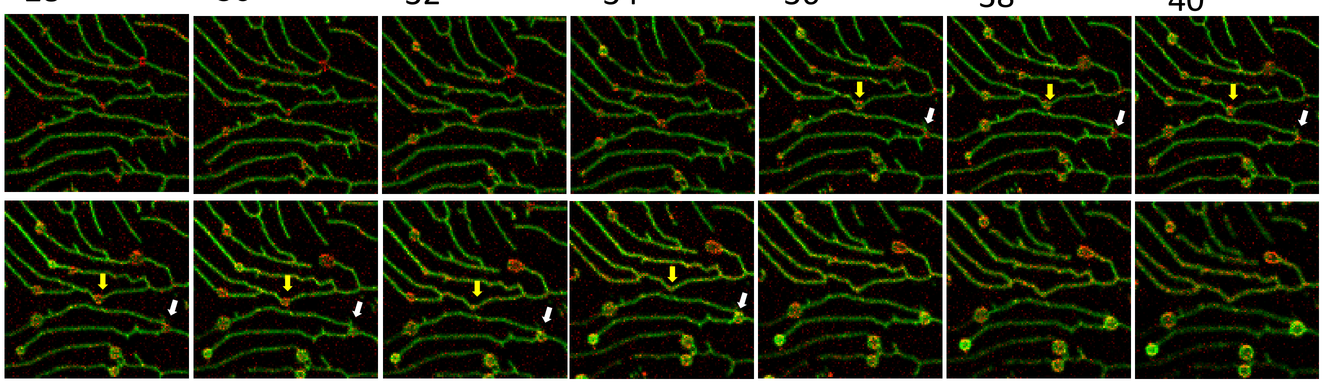
TSPAN is recruited to migrasomes



TSPAN4 stabilizes migrasomes



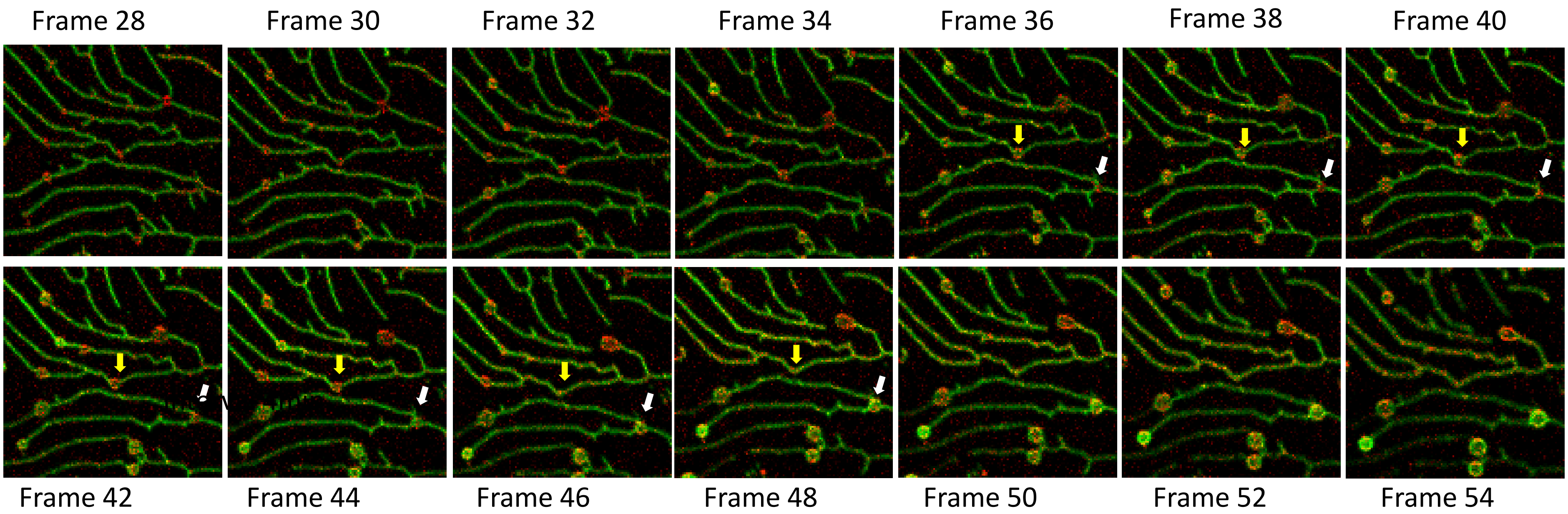
Frame 28 | Frame 30 | Frame 32 | Frame 34 | Frame 36 | Frame 38 | Frame 40



Interval 4min

Frame 42 | Frame 44 | Frame 46 | Frame 48 | Frame 50 | Frame 52 | Frame 54

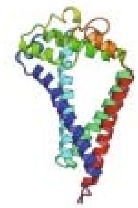
TSPN stabilizes migrasomes



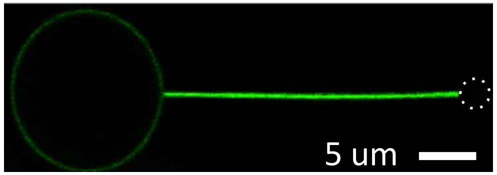
R. Dharan, Y. Huang...R. Sorkin, Nat Comm 2023

Suggested migrasome formation mechanism

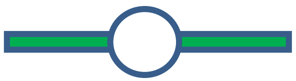
1. Curvature driven sorting due to TSPAN4 shape

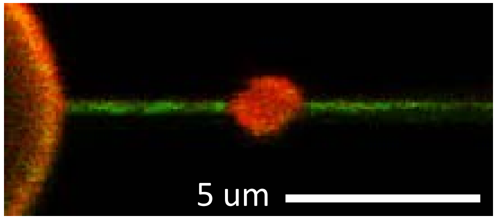


Umeda et. al., 2020

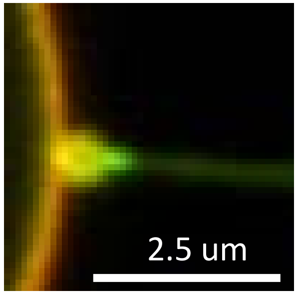


2. Swelling of migrasome like structures due to tension increase





3. Formation of macro-domains that have lower curvature → migration to lower curvature regions, migrasome stabilization



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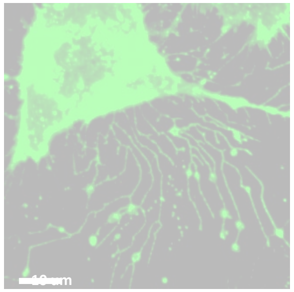

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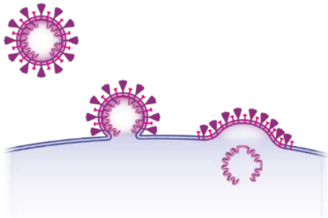

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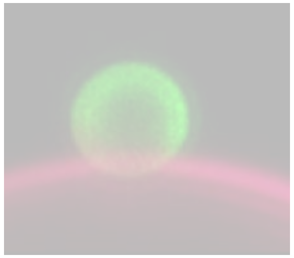
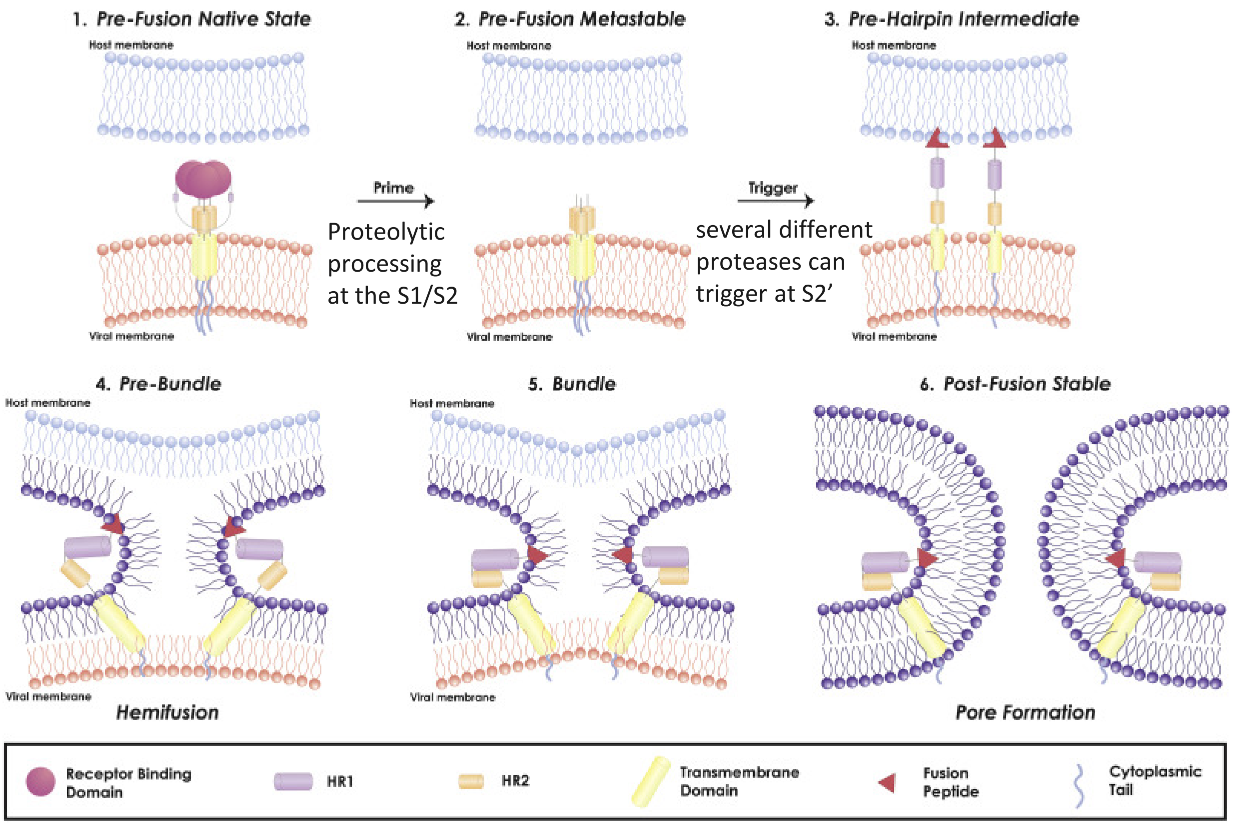


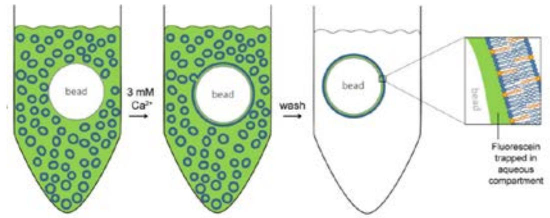
Membrane Fusion of SARS-CoV-2



Tang, T., Bidon, M., Jaimes, J.A., Whittaker, G.R., Daniel, S., Coronavirus membrane fusion mechanism offers as a potential target for antiviral development, Antiviral Research, 2020

Unravelling coronavirus membrane fusion: supported natural membranes

Coating colloids with synthetic membranes



Brouwer I. et al Nat Comm 2015   Sorkin R. et al, Biophys. J, 2020

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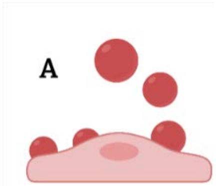
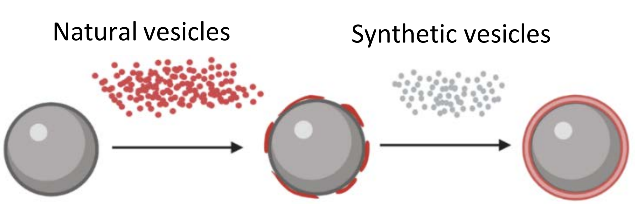
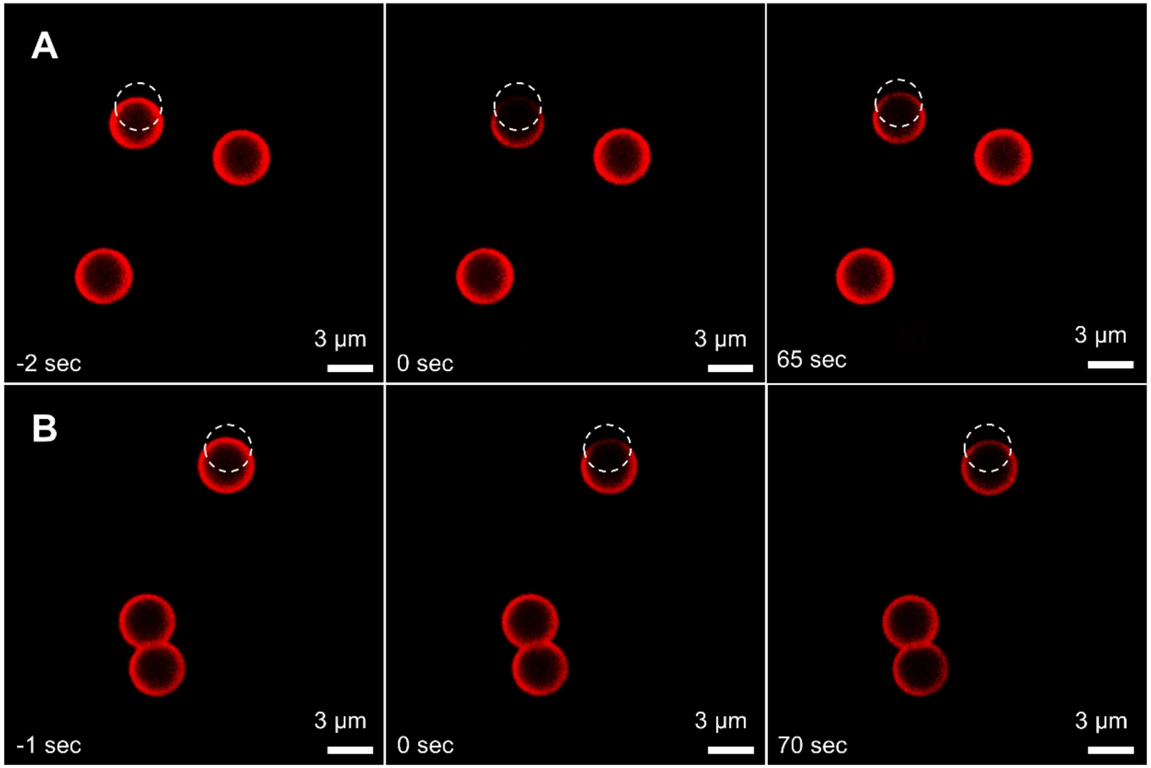




Mixing natural and synthetic vesicles – control protein concentration

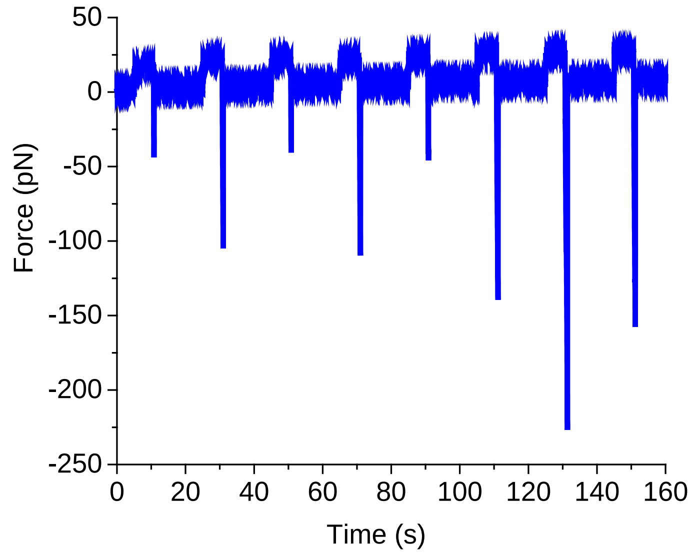
Bead-supported natural membranes are mobile



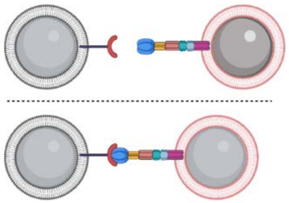
D ~ 0.3 μm^2/sec M ~ 70%

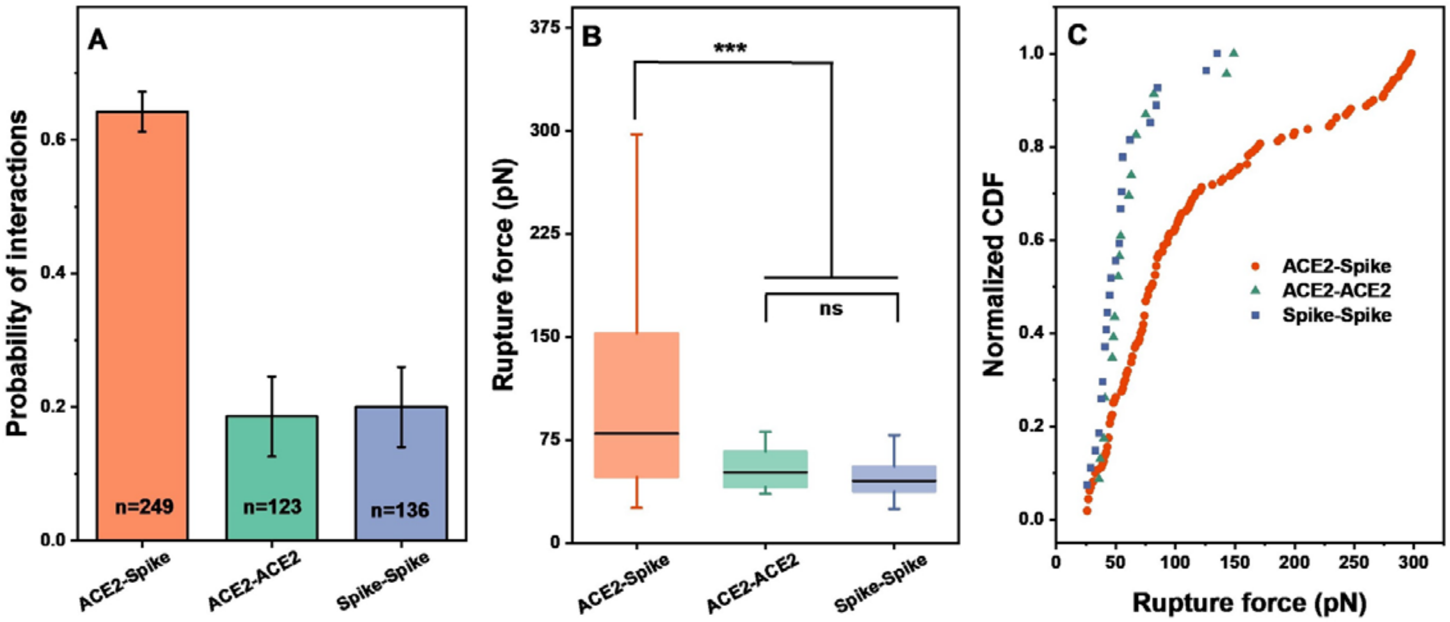
S.K Cheppali… R. Sorkin, ACS Appl. Mater. Interfaces 2022

Optical tweezers unbinding force measurement

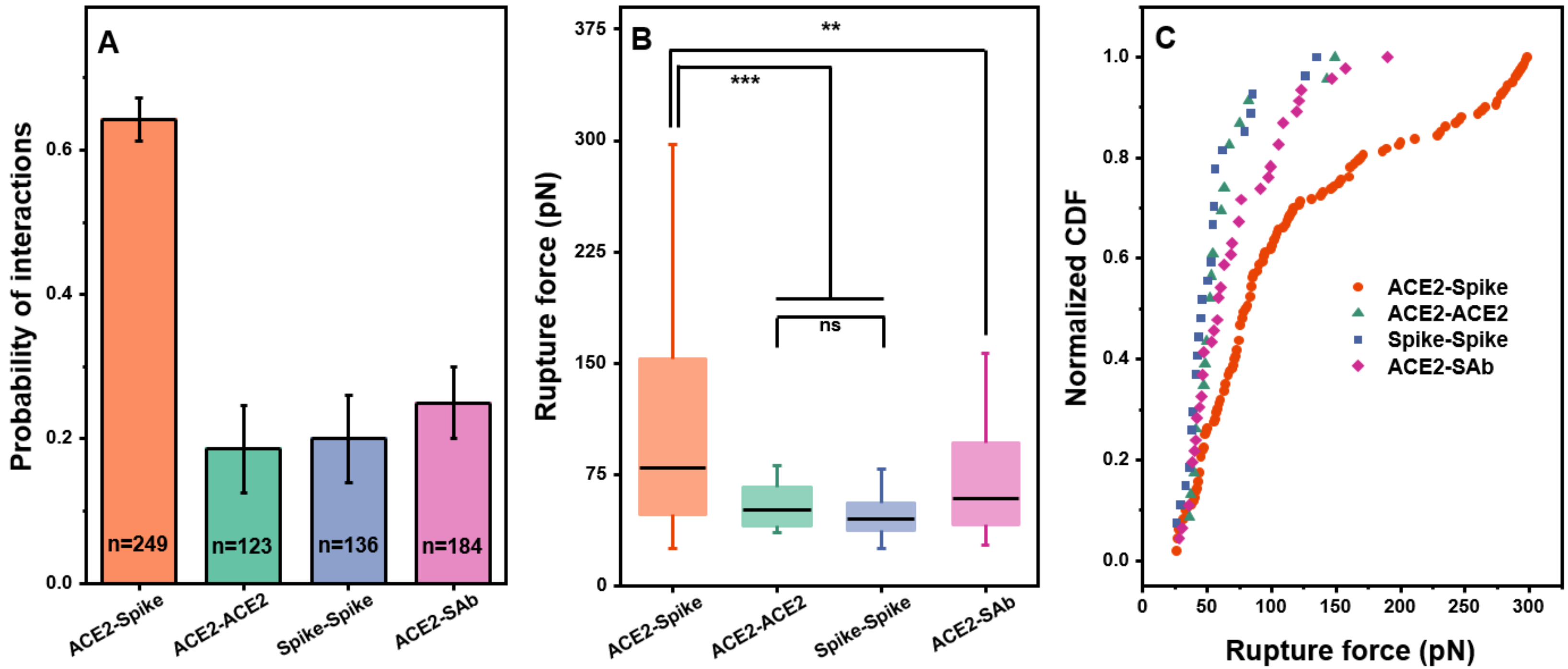


Validating protein presence and activity: ACE2- S interactions





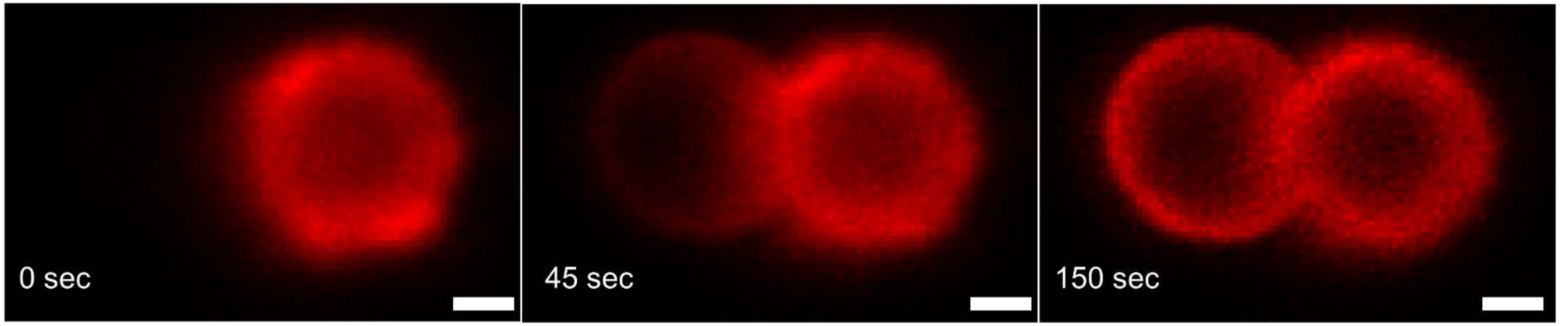
S-Ab successfully blocks S-ACE2 interactions



S.K Cheppali… R. Sorkin, ACS Appl. Mater. Interfaces 2022

Next steps

Hemifusion can be observed in OT experiments



Here hemifusion is induced by adding Ca^{2+} (liposomes have PS lipids),

next step: can exposed FP lead to hemi(fusion)?

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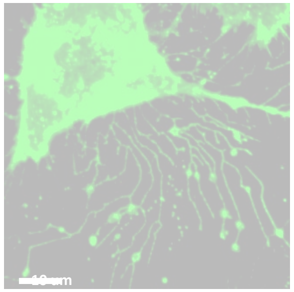

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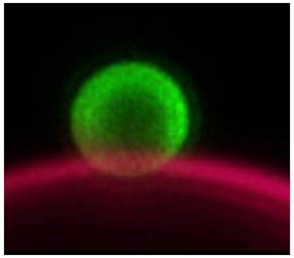
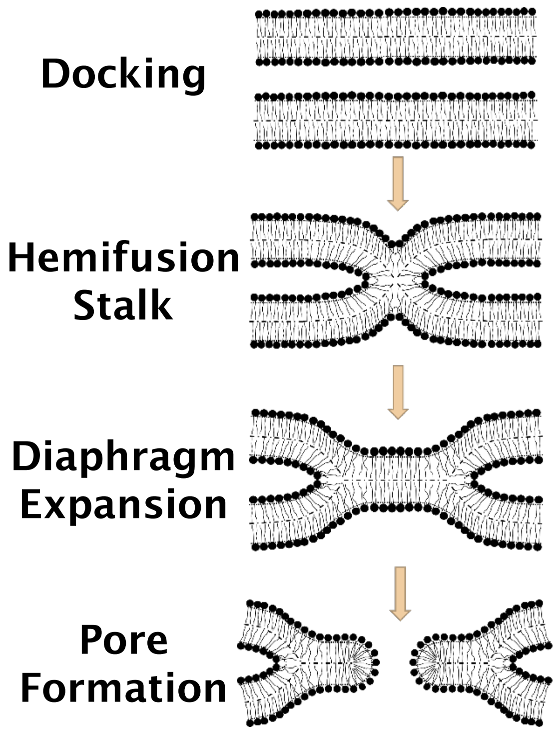
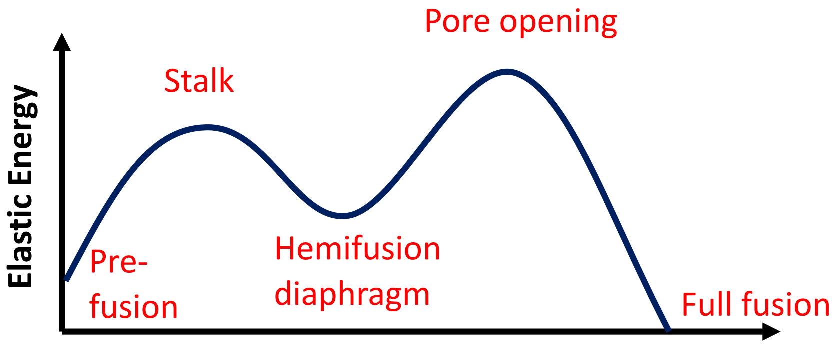


Fusion Energy Barriers

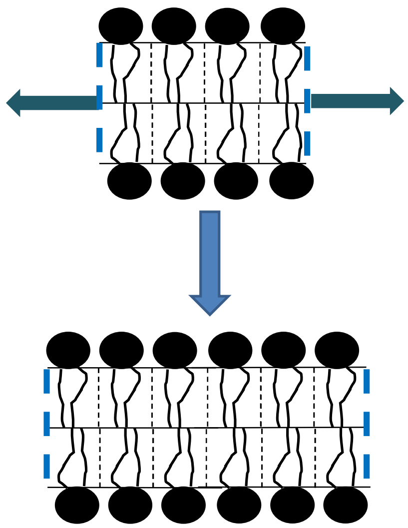


The classic model of the fusion process (Kozlov&Markin)



Membrane Tension

- Membrane tension is as free energy per unit area needed to stretch a flat membrane element.

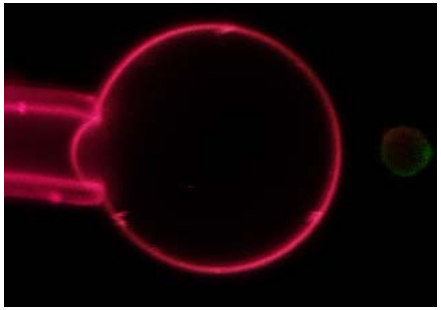


$$\gamma = \left.\frac{\partial F}{\partial A}\right|_{J,K,J_0=const}$$

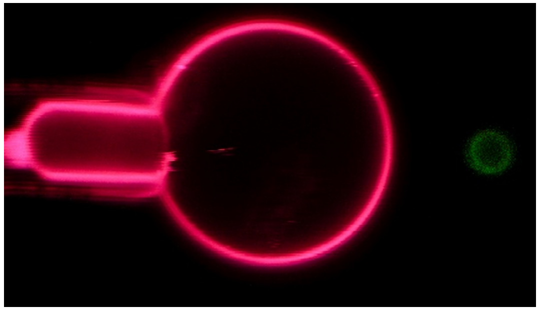
- Membrane tension usually varies between 0.005 - 0.1$[\frac{mN}{m}]$ and can reach 0.5 $[\frac{mN}{m}]$ in migrating cells.

Membrane Tension Manipulation

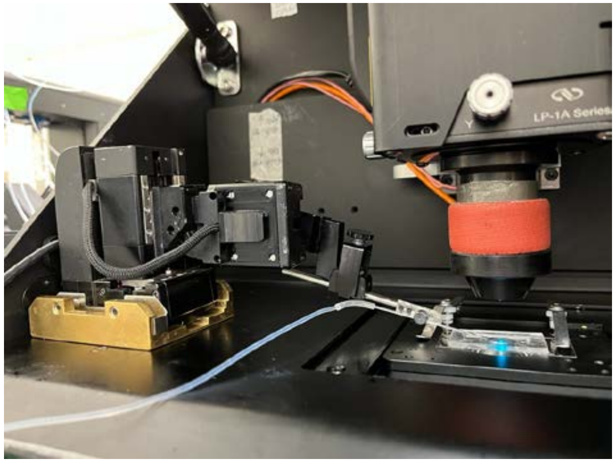
Low Aspiration



High Aspiration



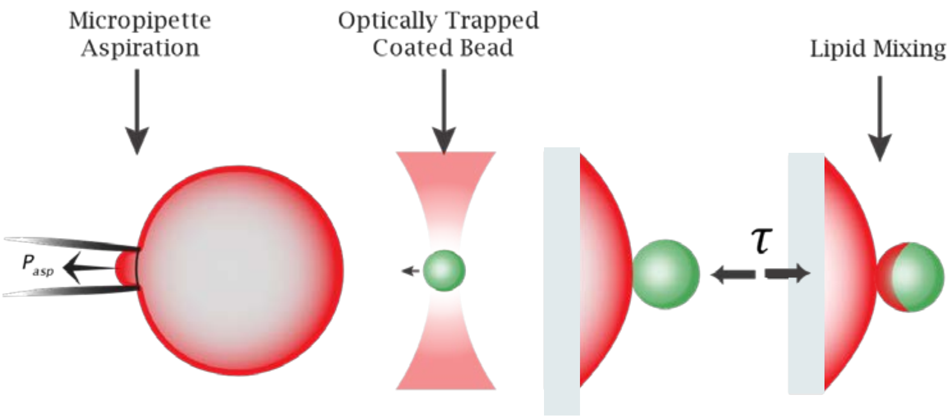
Micropipette Setup in OT

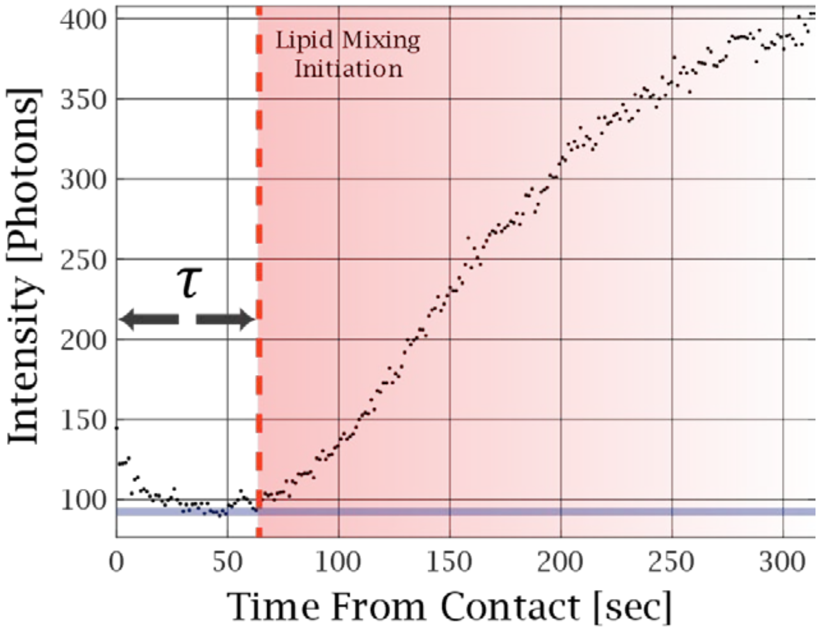


Tension: $$\gamma_{asp}[\frac{J}{m^2}] = \frac{\Delta P \cdot R_{pip}}{2(1-\frac{R_{pip}}{R_{ve}})}$$

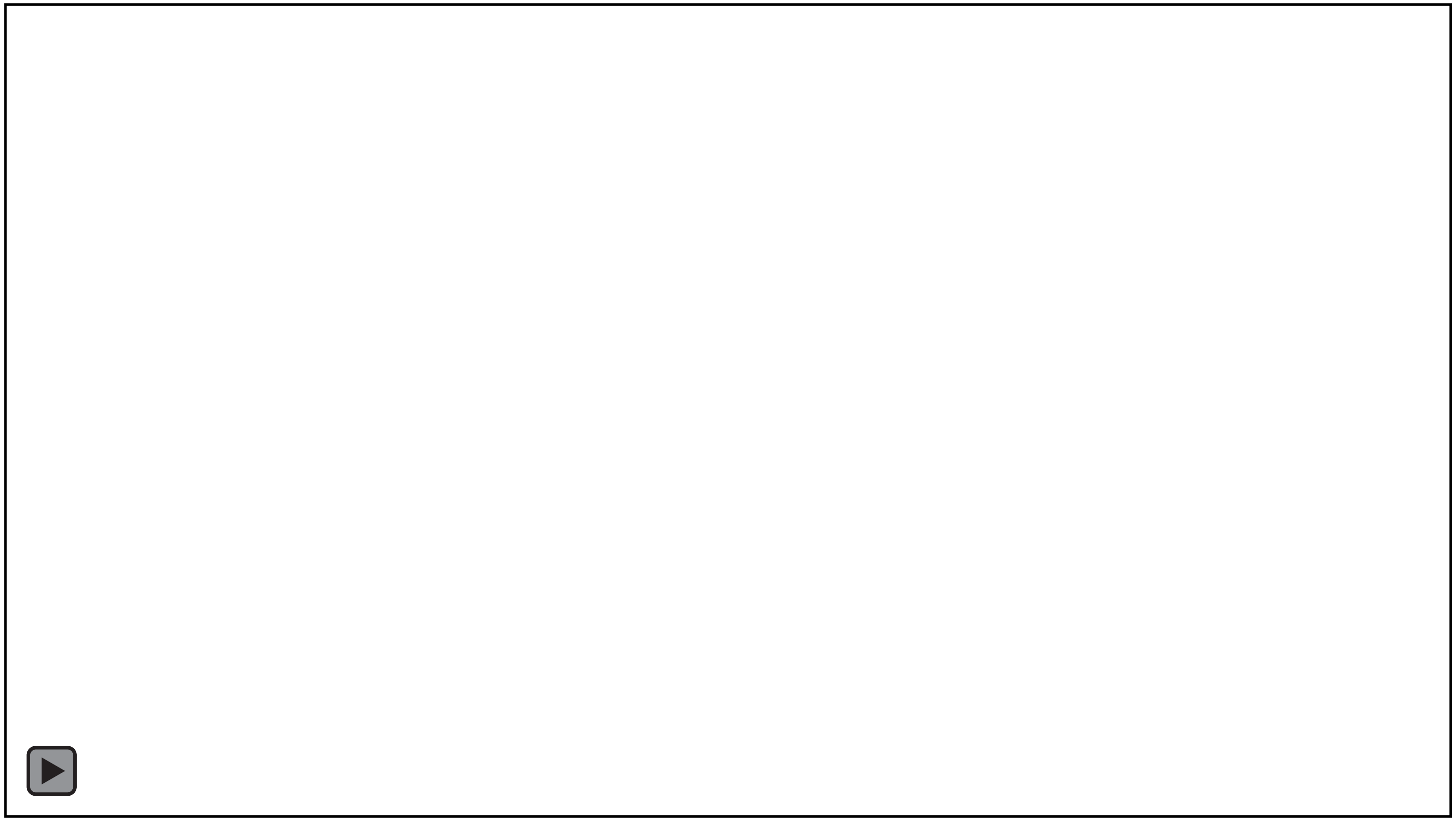
Project in collaboration with Gonen Golani and Ulrich Schwarz

Hemifusion Detection



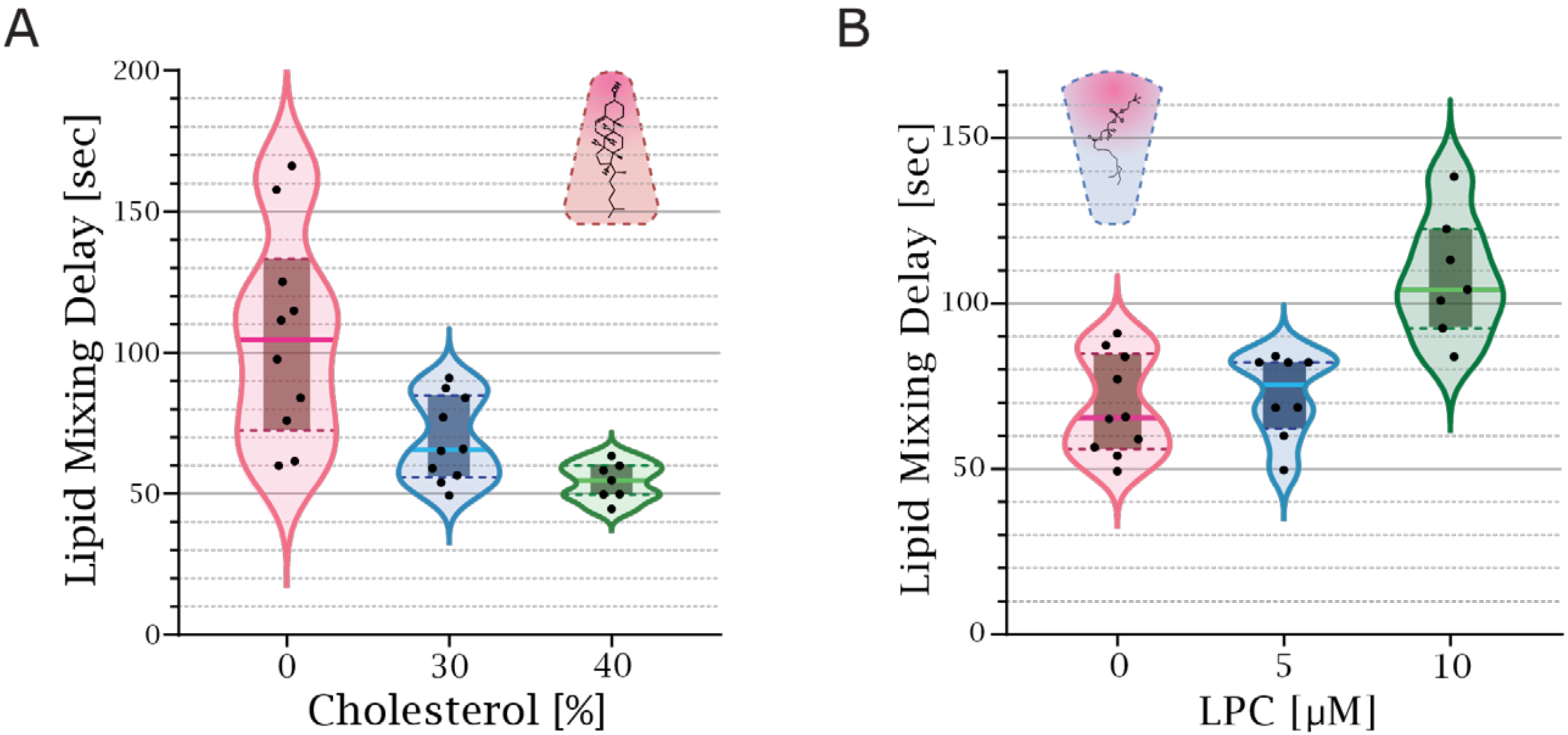


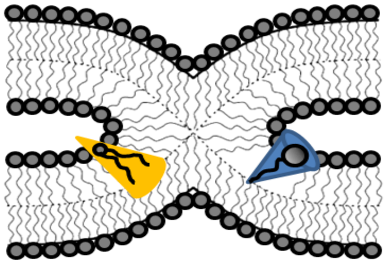
Hemifusion Detection



Protein free, Ca^{2+} mediated hemifusion of 20% PS containing membranes

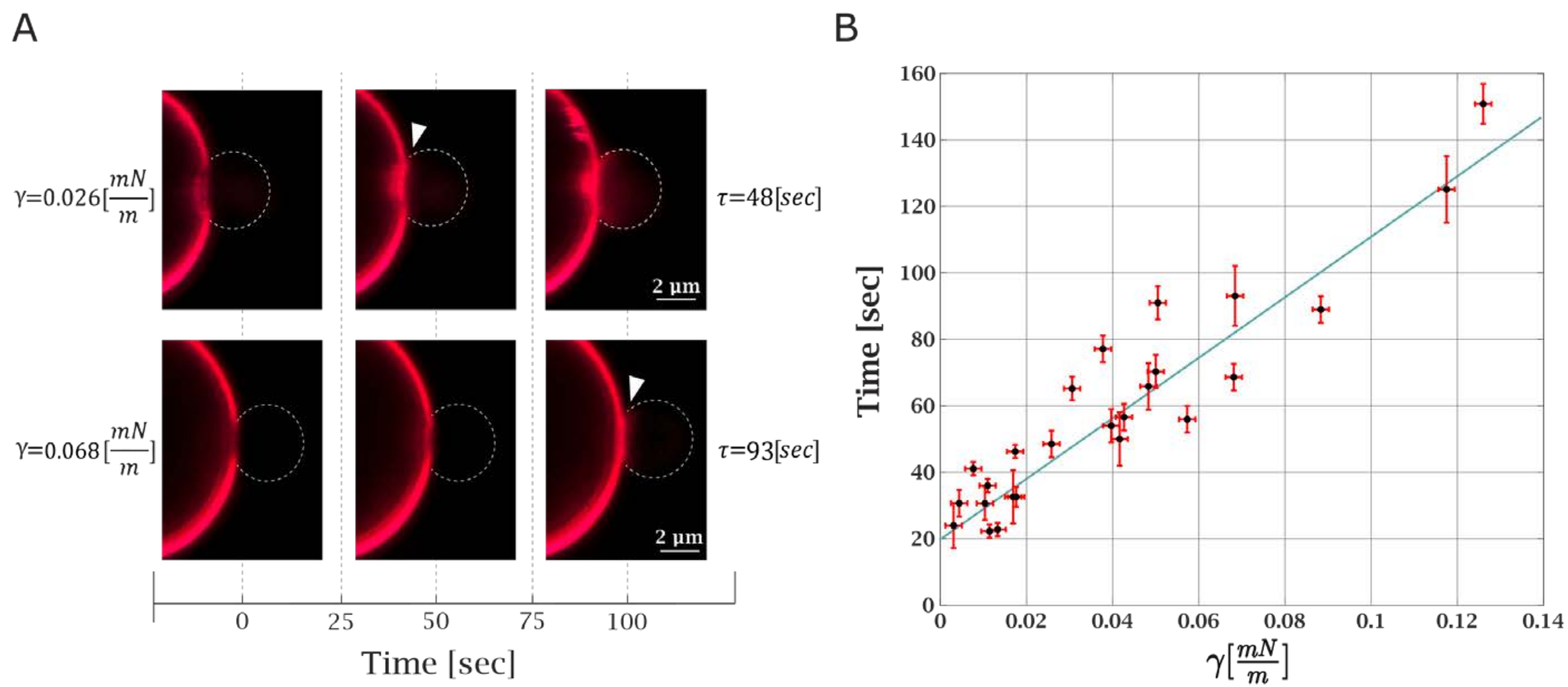
Method Validation





M. Kozlov, Cell, 2005

Tension Inhibits Lipid Mixing



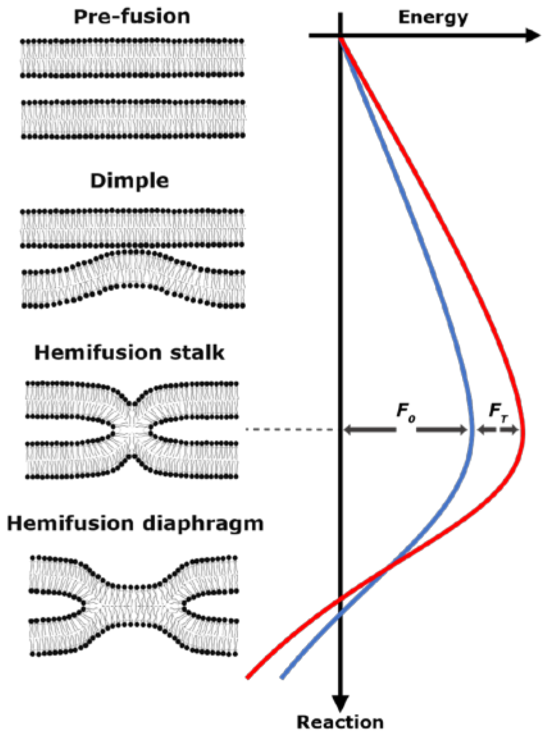
Lipid Mixing Energy Barrier

Lipid mixing energy barrier:
F= tension independent (elastic energy, dehydration repulsive forces) + tension dependent.

$$F = F_0 + \frac{1}{2}\Delta A * \gamma$$

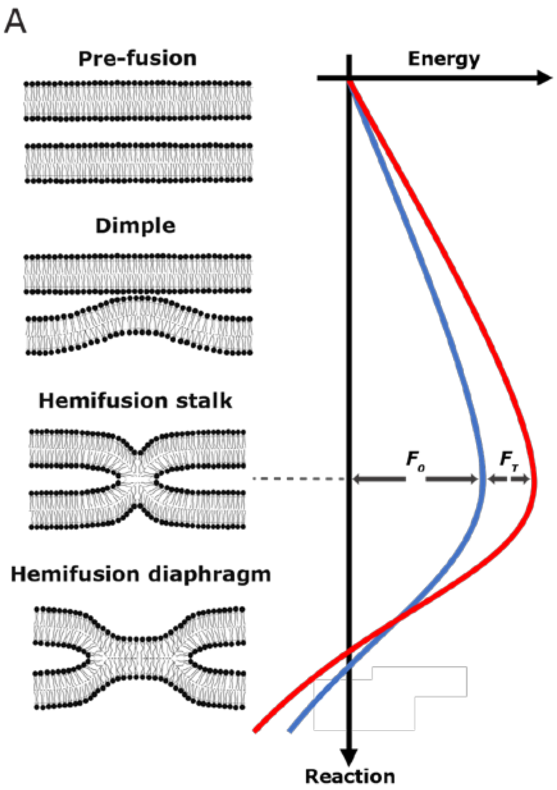
Lipid mixing time delay ∝ first passage time over the energy barrier -in our model - the stalk energy.

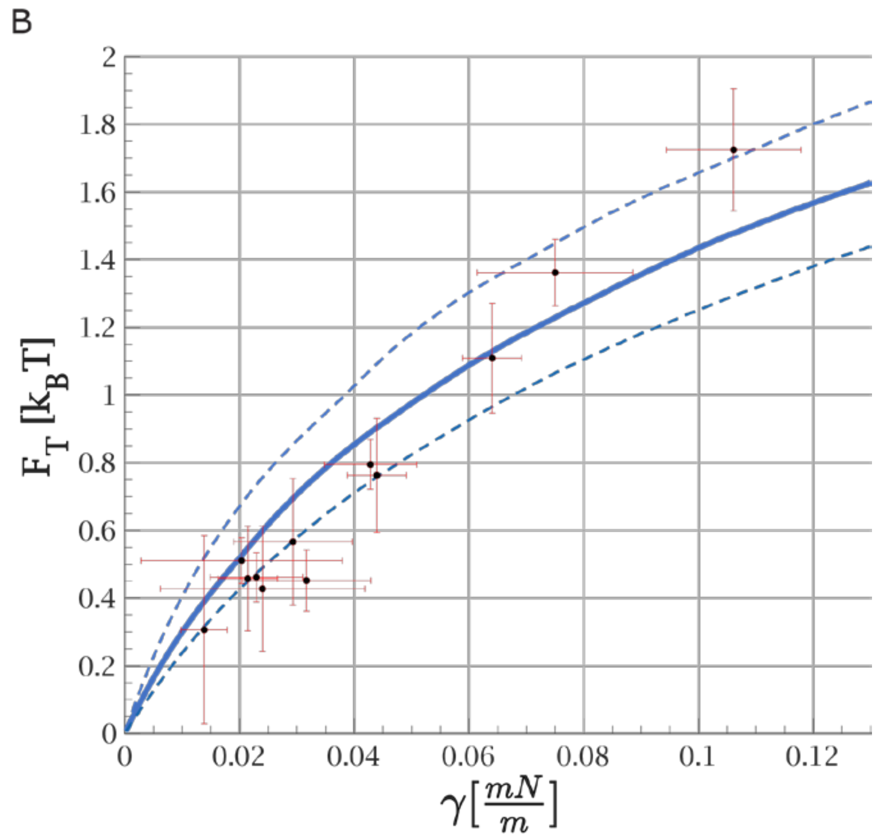
$$\tau_{[sec]} = \tilde{\tau} * e^{\frac{F}{k_B T}}$$



in collaboration with Gonen Golani and Ulrich Schwarz

Membrane Tension Inhibits Lipid Mixing by Increasing the Hemifusion Stalk Energy





$$F_T = k_B T \ln\left(\frac{\tau}{\tau_0}\right) = \frac{1}{2}\Delta A(\gamma) * \Delta\gamma$$

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