On the role of passive and active cytoskeleton forces in shape determination, function and dynamics of cells

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Christian Hernandez-Padilla, Virginia Tech, USA
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Harshad D Vishwasrao, NIH, Maryland, USA
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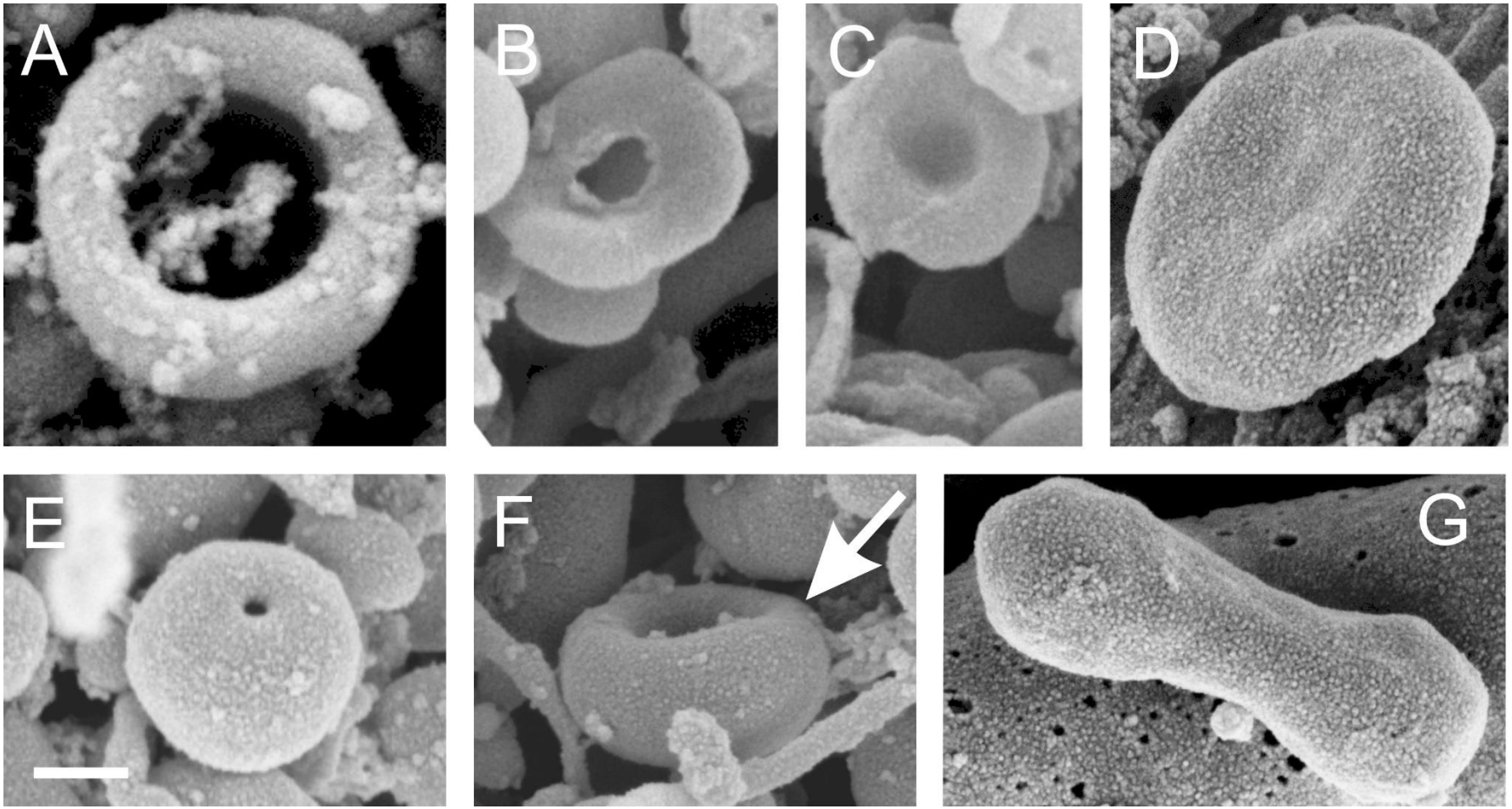
Konstantinos Konstantopoulos, Johns Hopkins University, USA

EU Horizon MSC project FarmEVs

Napoli, IBBR-CNR, March 28th , 2024

Different shapes of extracellular vesicles



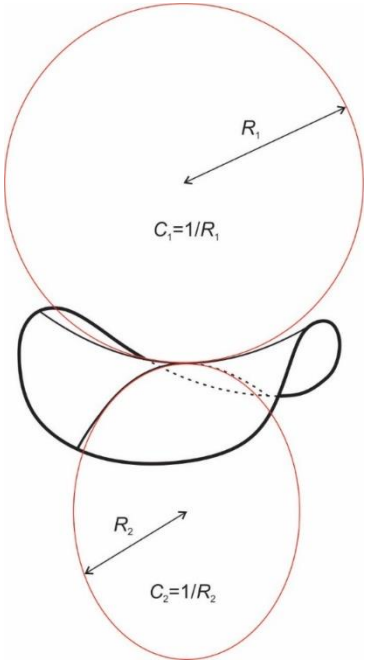
V. Kralj-Iglič, G. Pocsfalvi, L. Mesarec, V. Šuštar, H. Hagerstrand, A. Iglič: Minimizing isotropic and deviatoric membrane energy – an unifying formation mechanism of different cellular membrane nanovesicle types, PLOS ONE: 15(12): e0244796, 2021. DOI: 10.1371/journal.pone.0244796.

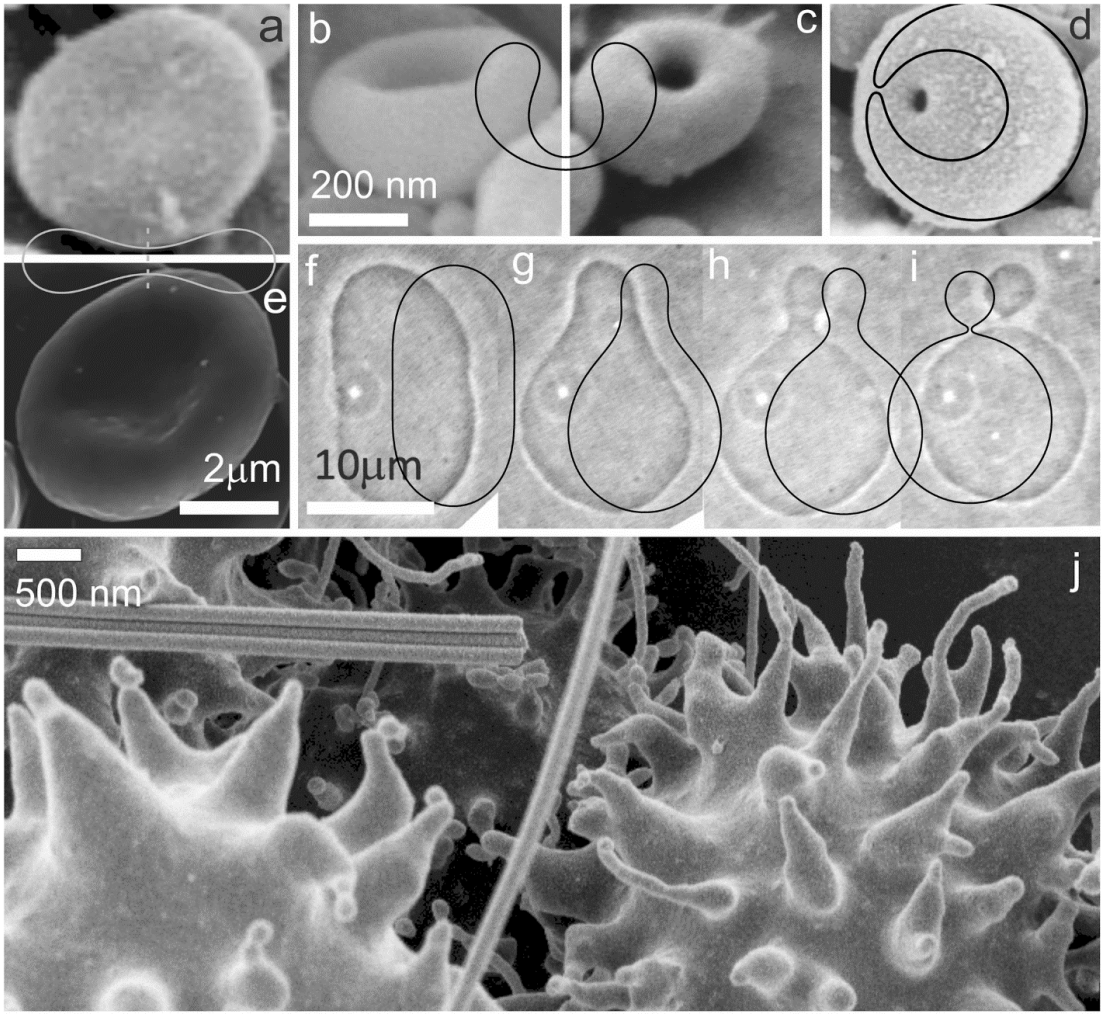
$$W_{\text{bend}} = \frac{k_c}{2}\int (2H)^2\,\mathrm{d}A \;+\; k_G\int K\,\mathrm{d}A \;+\; k_n A(\langle H\rangle - H_0)^2$$

minimisation of Helfrich -Evans-Skalak isotropic bending energy W_{bend}

$H = (C_2 + C_1)/2$

$K = C_1\, C_2$





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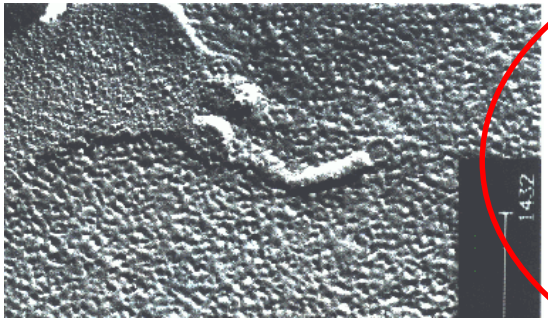
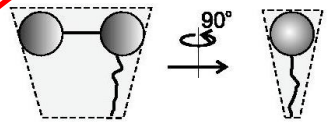
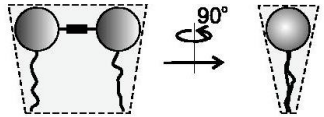
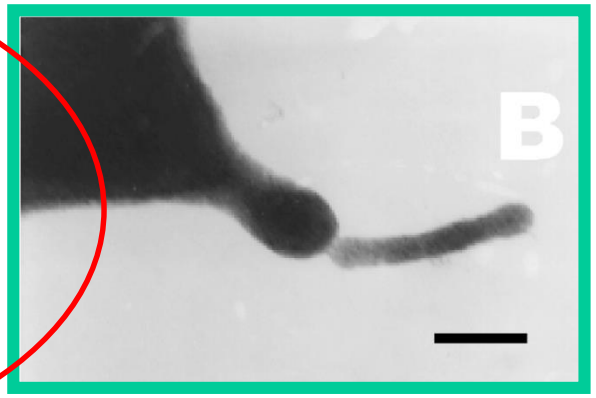
Anisotropic Detergents



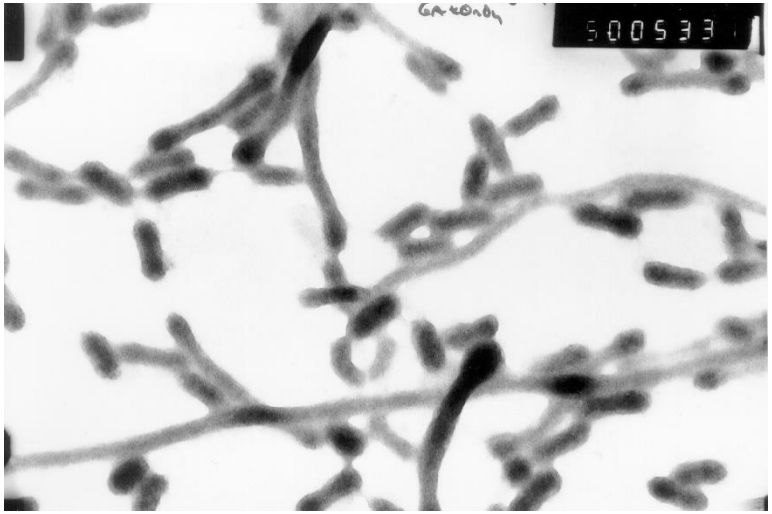


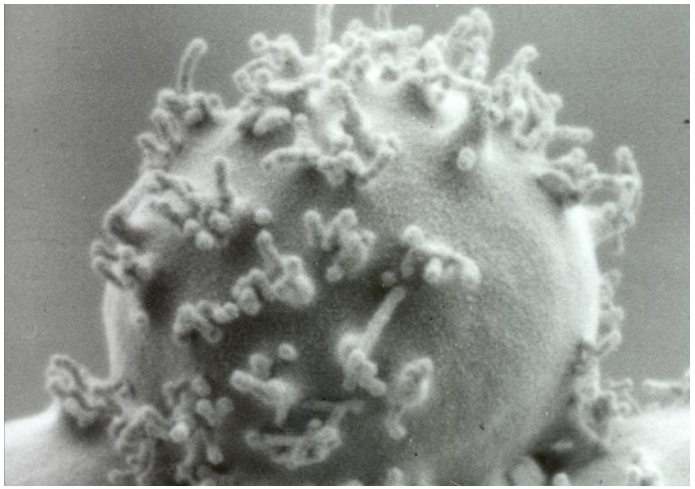




Erythrocytes+dodecylmaltoside

Erythrocytes + dioctyldiQAS





Erythrocytes+dodecylmaltoside

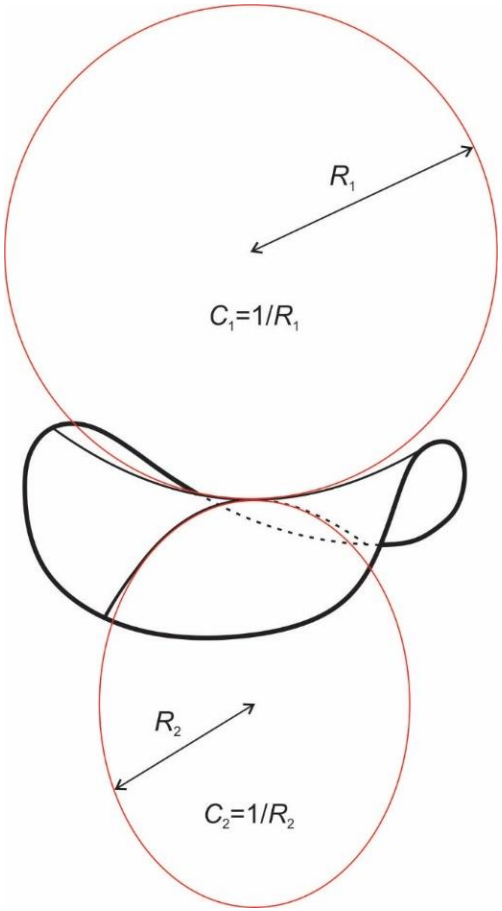
Kralj-Igiič et al., Eur Biophys J, 34 (2005) 1066; Kralj-Iglič et al., Phys.Rev. E, 61 (2000) 4230

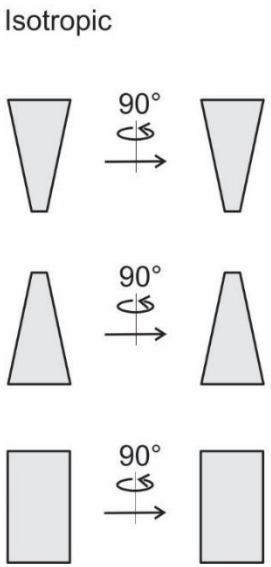
$$E_i = (2K_1 + K_2)(H - H_m)^2 - K_2(D^2 - 2DD_m\cos(2\omega) + D_m^2)$$

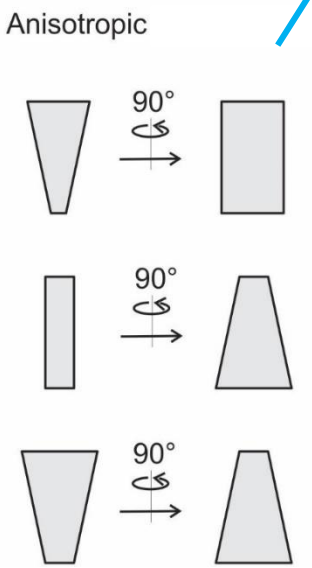
$$H = (C_2 + C_1)/2 \qquad D = (C_2 - C_1)/2 \qquad D_m = (C_{2m} - C_{1m})/2$$

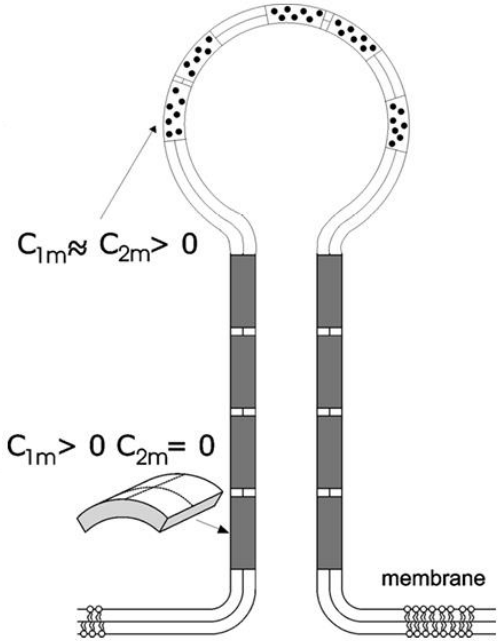
$$H_m = (C_{2m} + C_{1m})/2$$

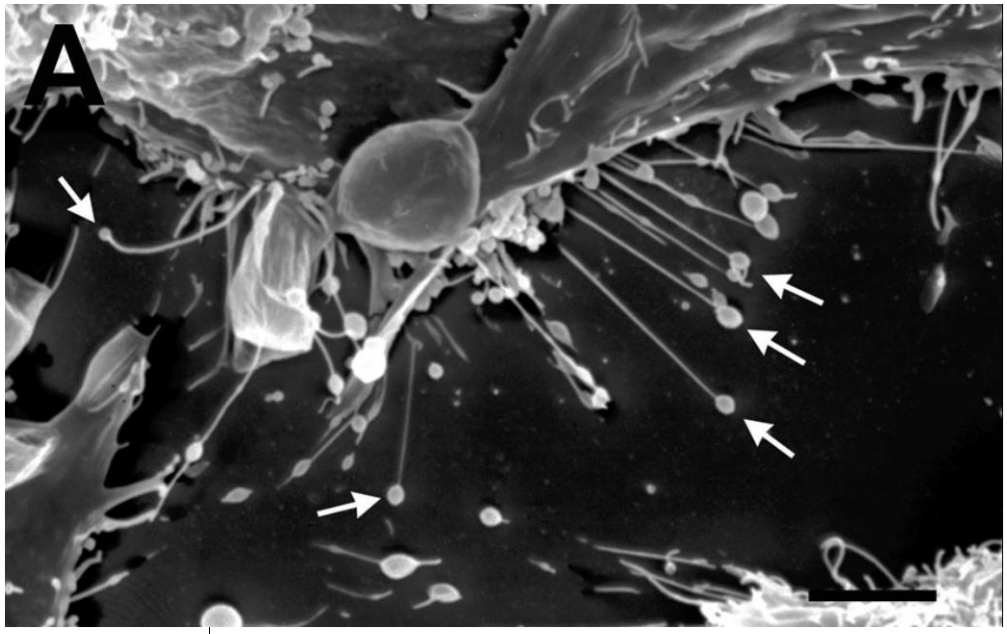
Kralj-Iglič et al., Eur. Phys. J. B; 1999; Kralj-Iglič et al., Phys. Lett. A, 2002
Fošnarič et al., J. Chem. Info. Model., 2005; Fošnarič et al., Phvs. Rev. E. 2006





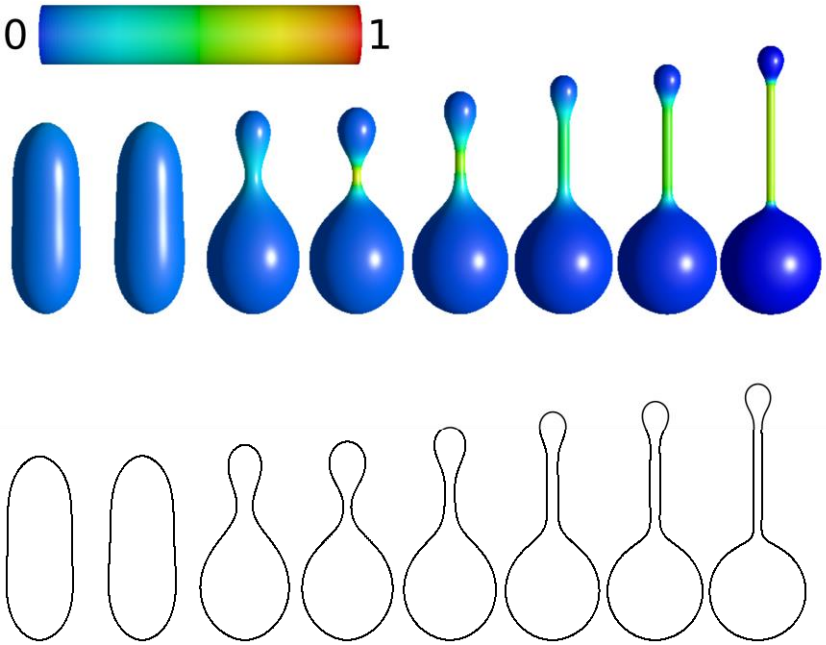






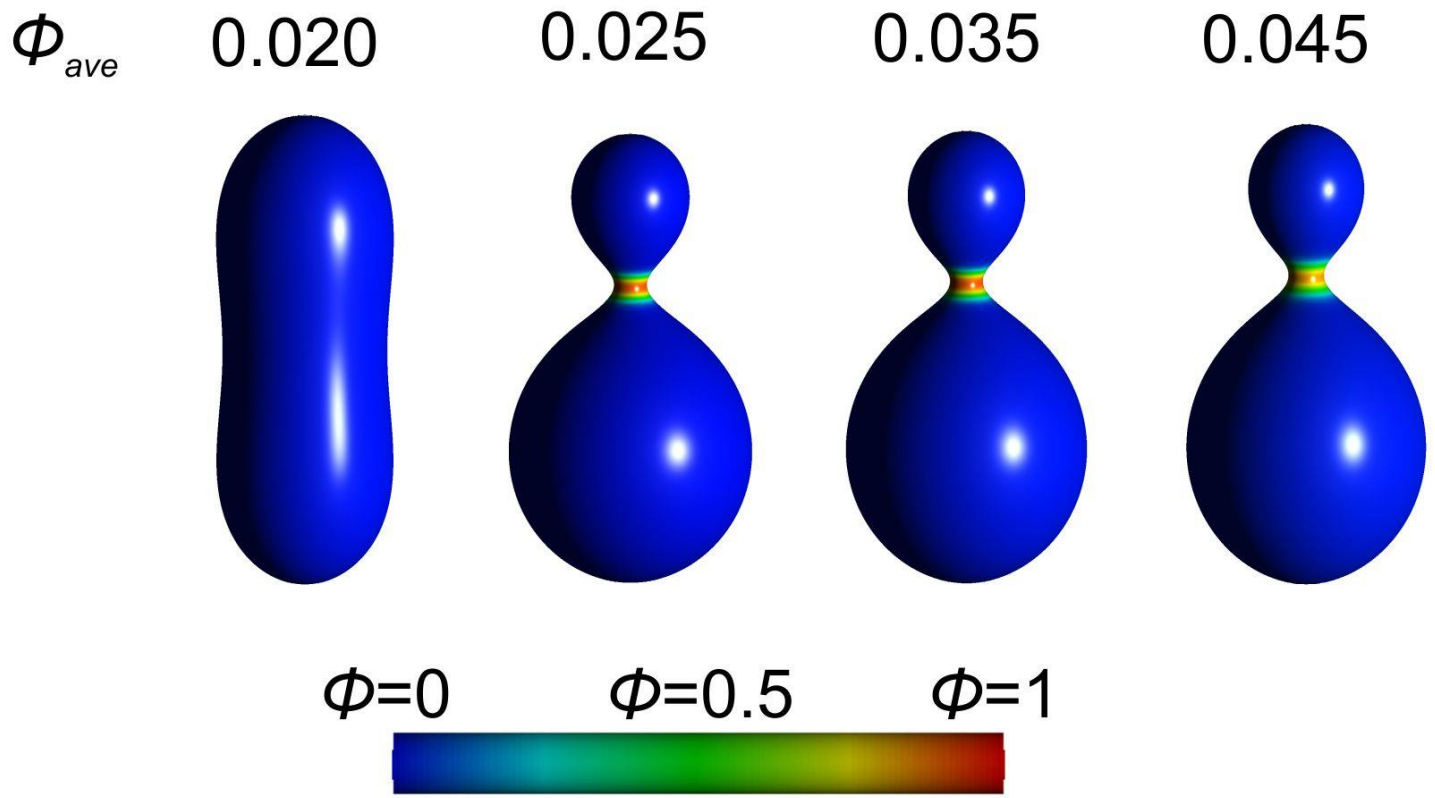
Schara K., Janša V., Šuštar V., Dolinar D., Pavlič J.I., Lokar M., Kralj-Iglič V., Veranič. P., Iglič A.: Mechanisms for the formation of membranous nanostructures in cell-to-cell communication., Cell. Mol. Biol. Lett., 14, 636-656, 2009.

Veranič P., Lokar M., Schuetz G.J., Weghuber J., Wieser S., Hagerstrand H., Kralj-Iglič V., Iglič A.: Different types of cell-to-cell connections mediated by nanotubular structures, Biophys. J., 95: 4416-4425, 2008.



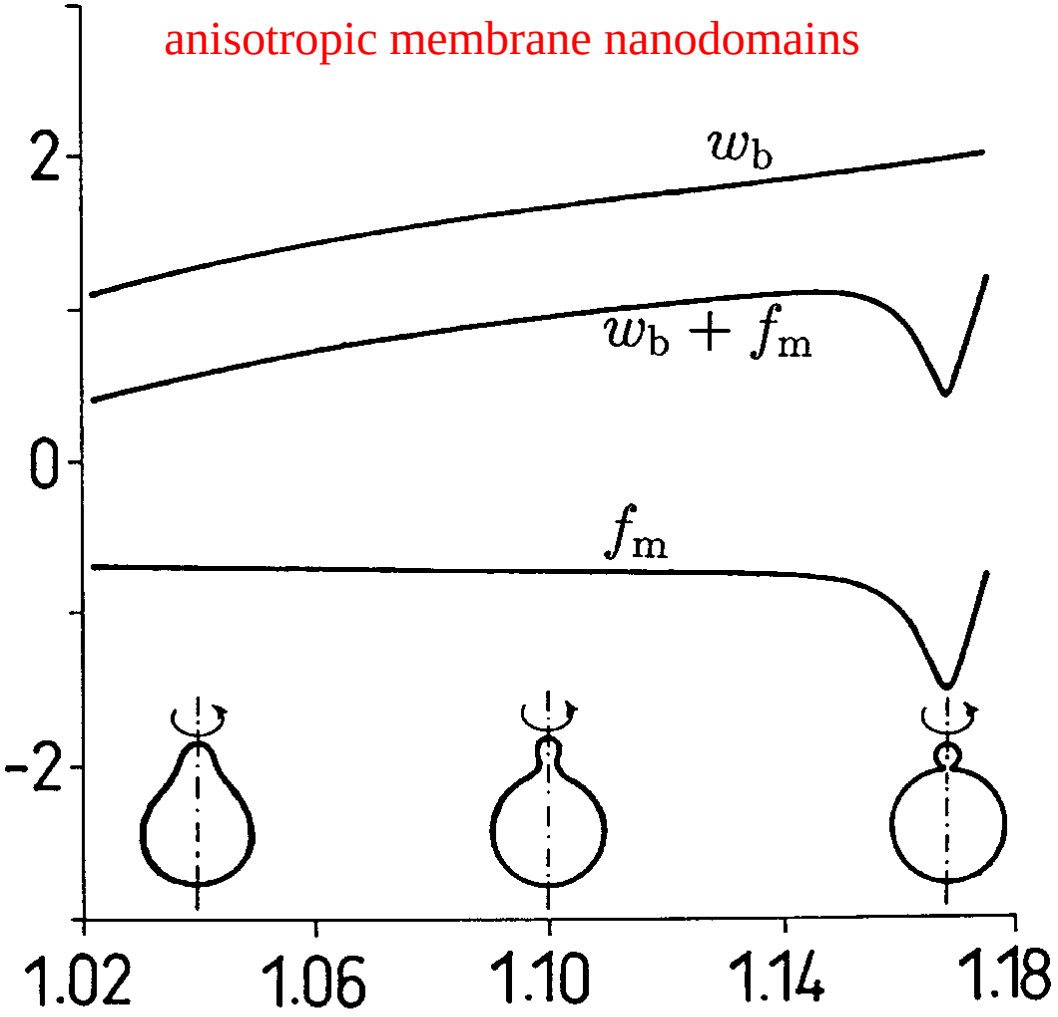
N. Bobrovska, W. Gozdz, V. Kralj-Iglič, A. Iglič: On the role of anisotropy of membrane components in formation and stabilization of tubular structures in multicomponent membranes, PLOS ONE, 8 e73941, 2013.

2-COMPONENT MEMBRANE
anisotropic saddle-like nanodomains



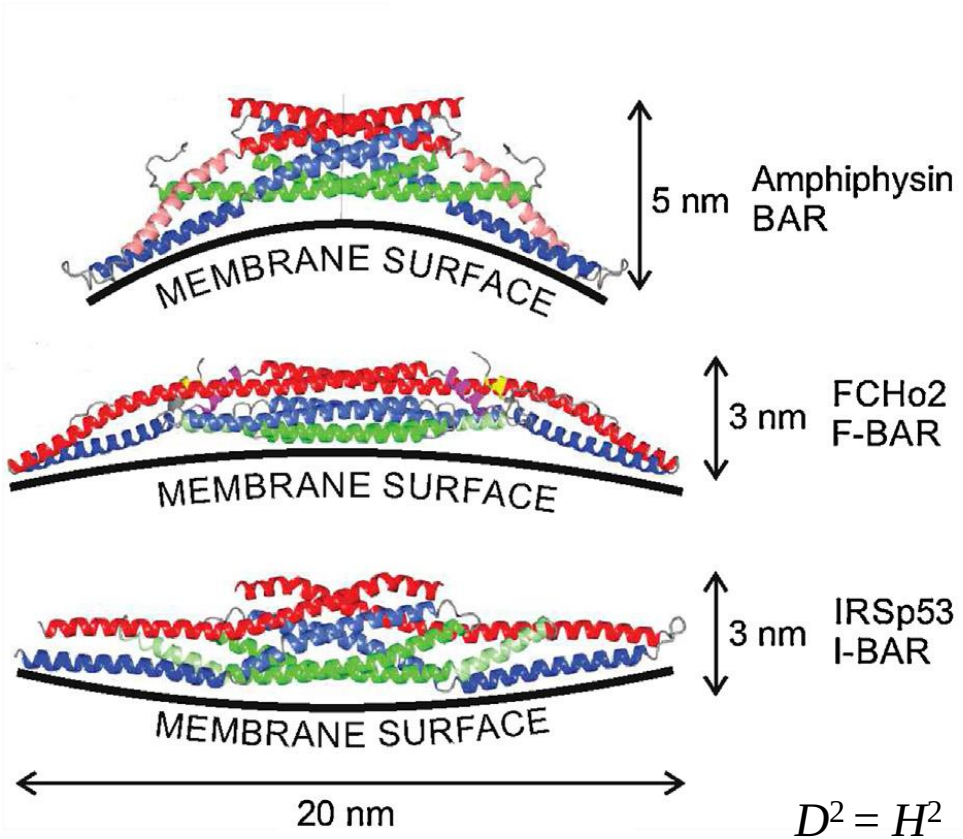
L. Mesarec, M. Drab, S. Penič, V. Kralj-Iglič, A. Iglič: On the role of curved membrane nanodomains and passive and active skeleton forces in the determination of cell shape and membrane budding, Int. J. Mol. Sci. 22(5): 2348, 2021

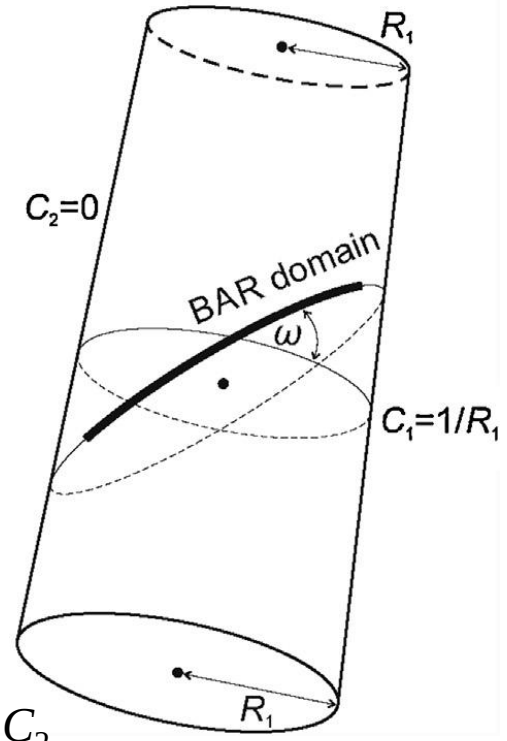
anisotropic membrane nanodomains



Kralj-Iglič V., Heinrich V., Svetina S., Žekš B.:
Free energy of closed membrane with anisotropic inclusions, Eur. Phys. J. B.,10, 5-8, 1999.

SIMPLE EXAMPLE: BAR domains attached to the membrane surface





$$D^2 = H^2 - C_1 C_2$$

$$f_{bar} = \frac{K_p L_0}{2}(C - C_p)^2$$

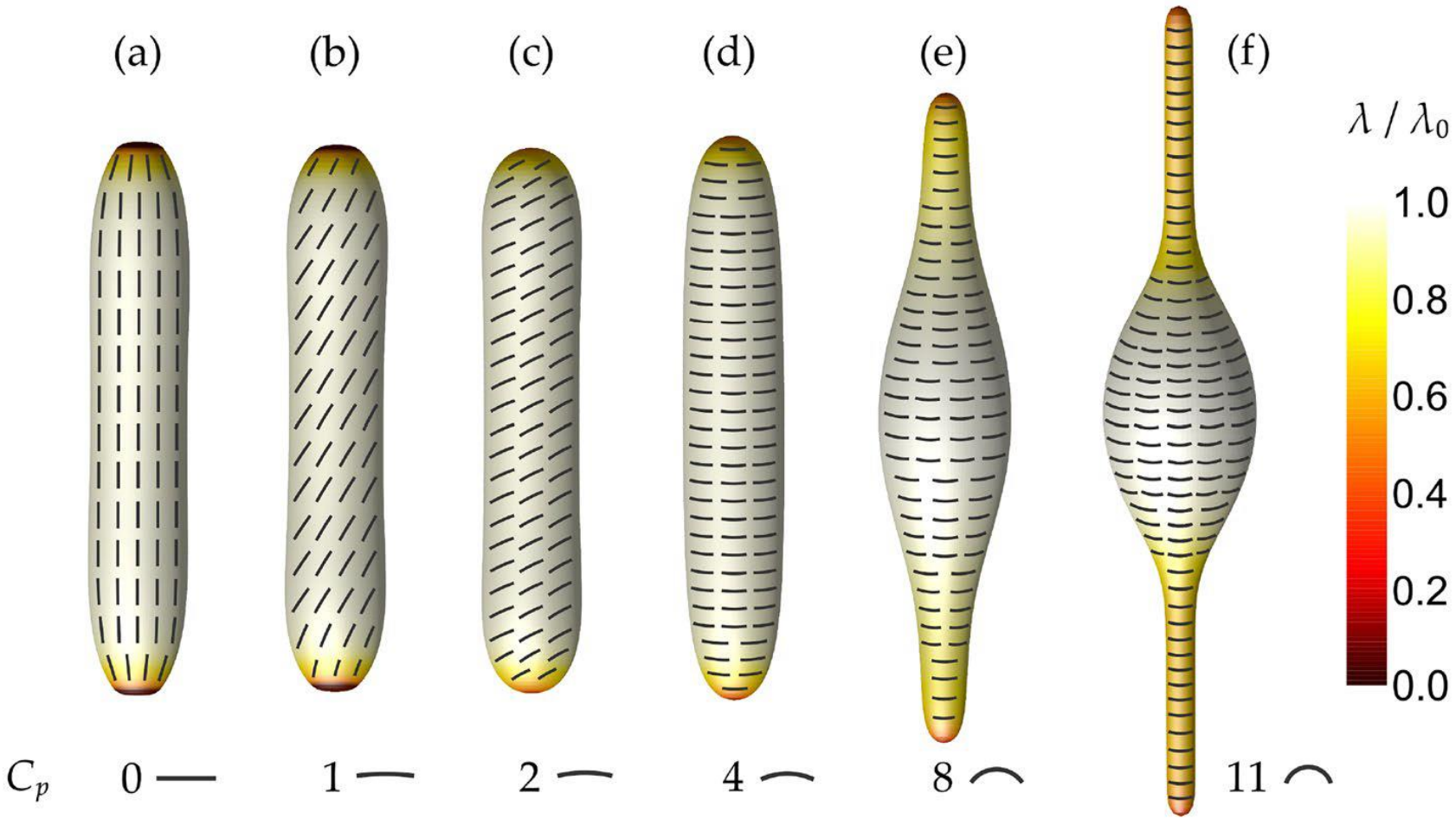
$$H = (C_1 + C_2)/2 \qquad D = (C_1 - C_2)/2$$

$$C = H + D\cos(2\omega)$$

L. Mesarec, W. Góźdź, V. Kralj-Iglič, S. Kralj, A. Iglič: Coupling of nematic in-plane orientational ordering and equilibrium shapes of closed flexible nematic shells. Scientific Reports 13:10663, 2023.

Perutkova et al., J. Biomech. 43: 1612-1617, 2010
Kabaso et al., Mini Rev. Med. Chem. 11: 272-282, 2011

Iglič, Slivnik, Kralj-Iglič: Elastic properties of biological membranes influenced by attached proteins, J Biomech., 40, 2492-2500, 2007



$$f_{bar} = \frac{K_p L_0}{2}(C - C_p)^2$$

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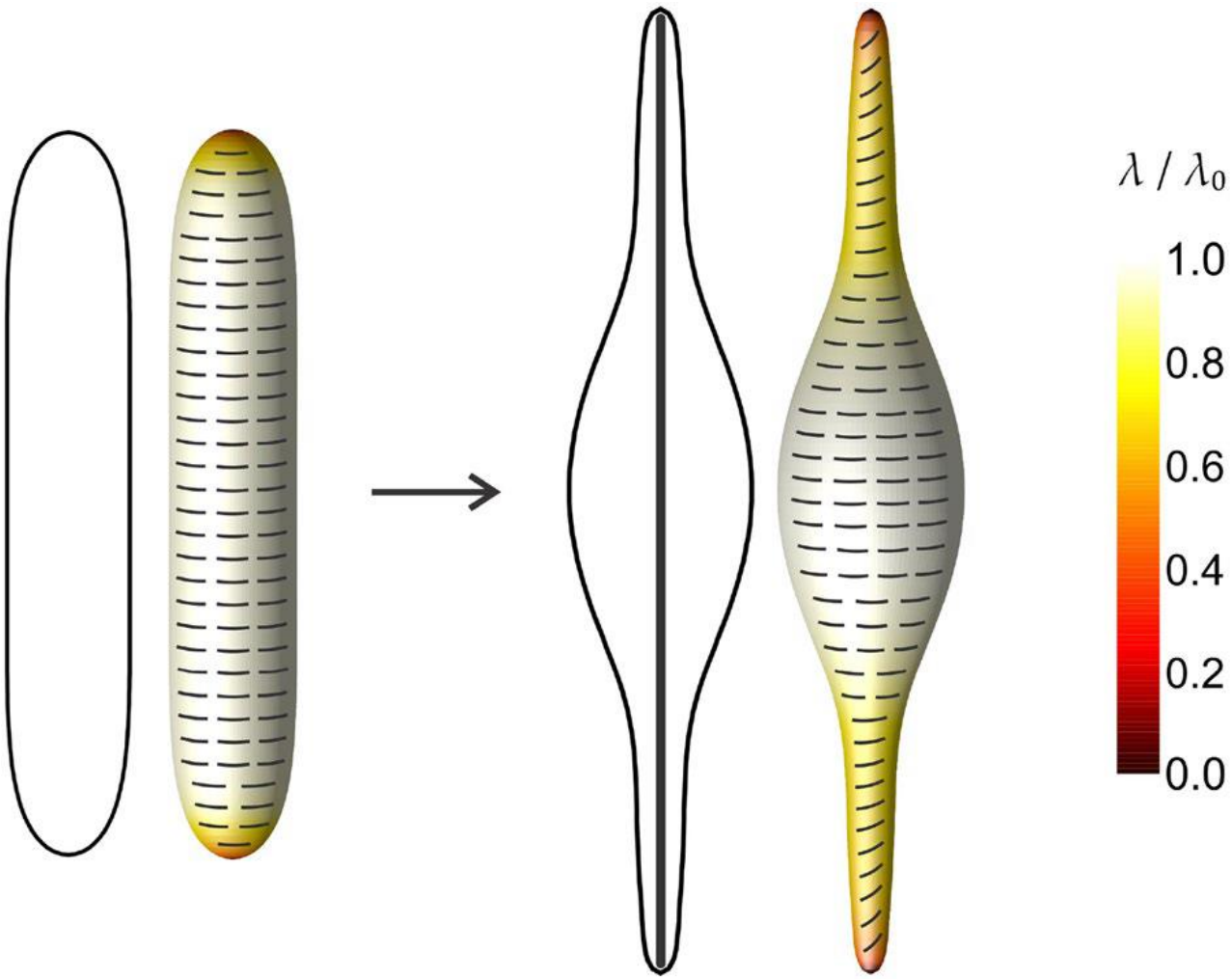
Influence of passive force



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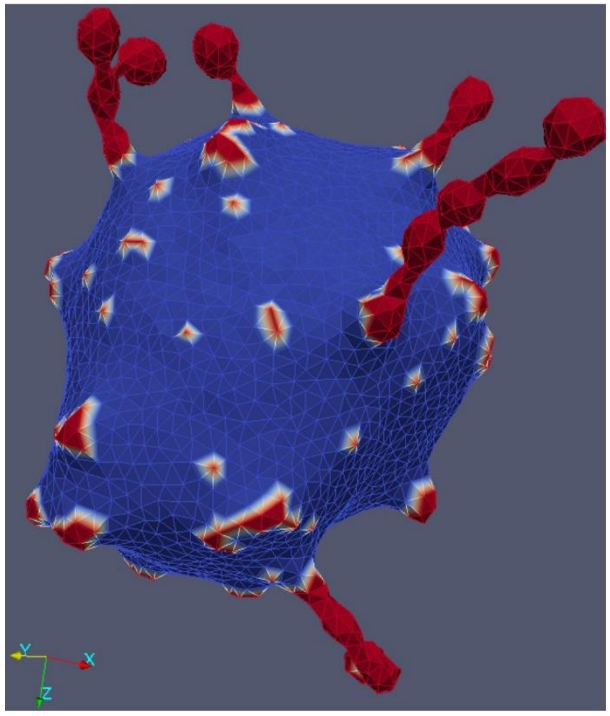
Influence of passive force

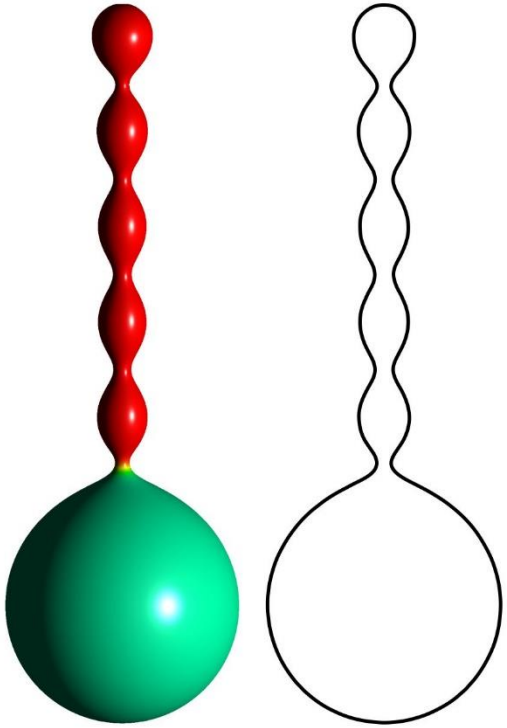
isotropic membrane inclusions: only **undulated** protrusions

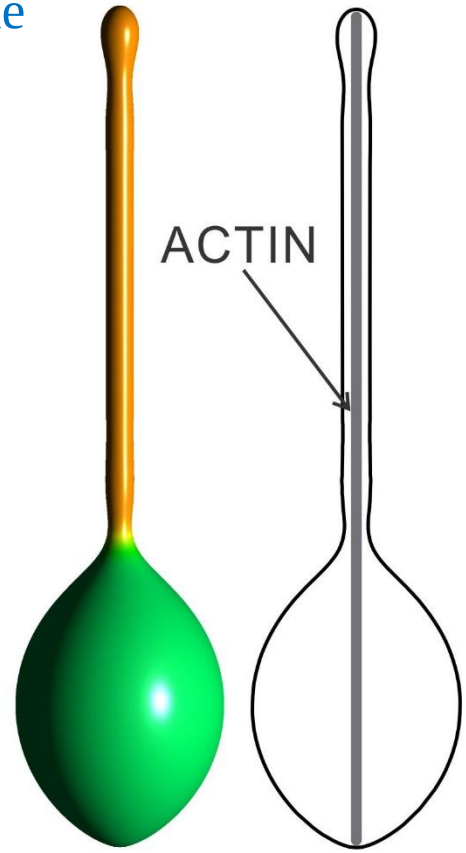
L. Mesarec, W. Góźdź, S. Kralj, M. Fošnarič, S. Penič, V. Kralj Iglič, A. Iglič, European Biophysics Journal, 46: 705-718, 2017.

2 component membrane

force







MC simulations
(fluctuations included)

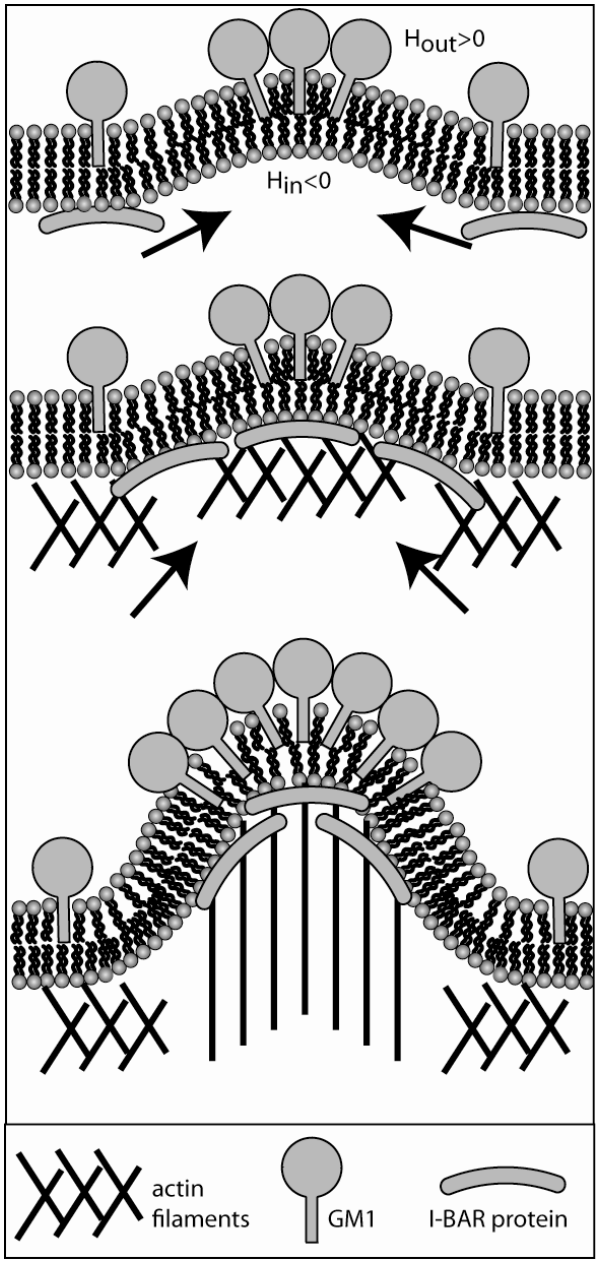
minimisation of the membrane free energy

Membrane dynamics and cell migration

· theoretical model of efficient phagocytosis driven by curved membrane proteins and active cytoskeleton forces

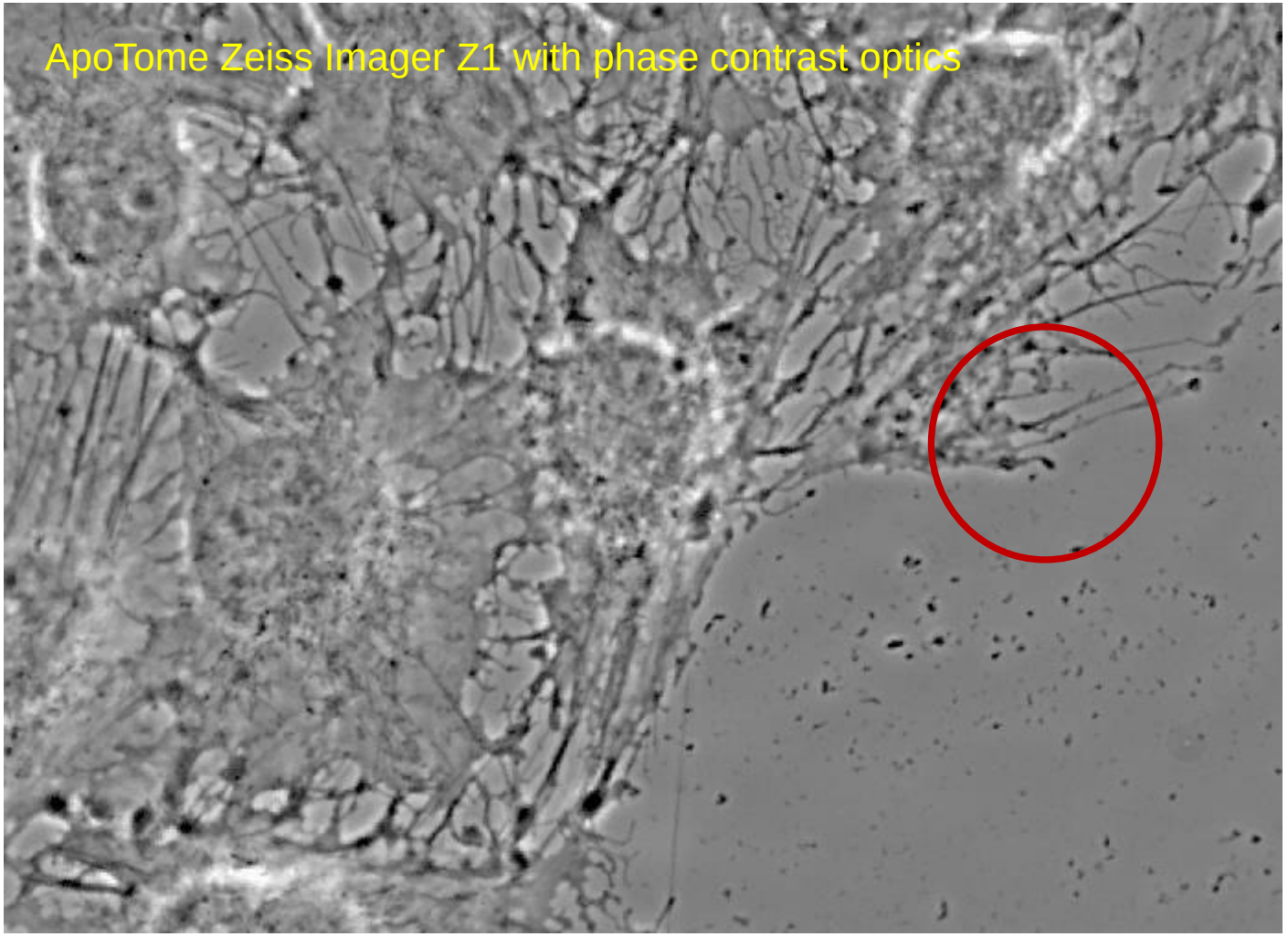
· a minimal physical model for curvotaxis driven by curved protein complexes at the cell's leading edge, that exert protrusive forces on the membrane (representing the pressure due to actin polymerization)

· theoretical model for the origin of coiling of cellular protrusions around fibers

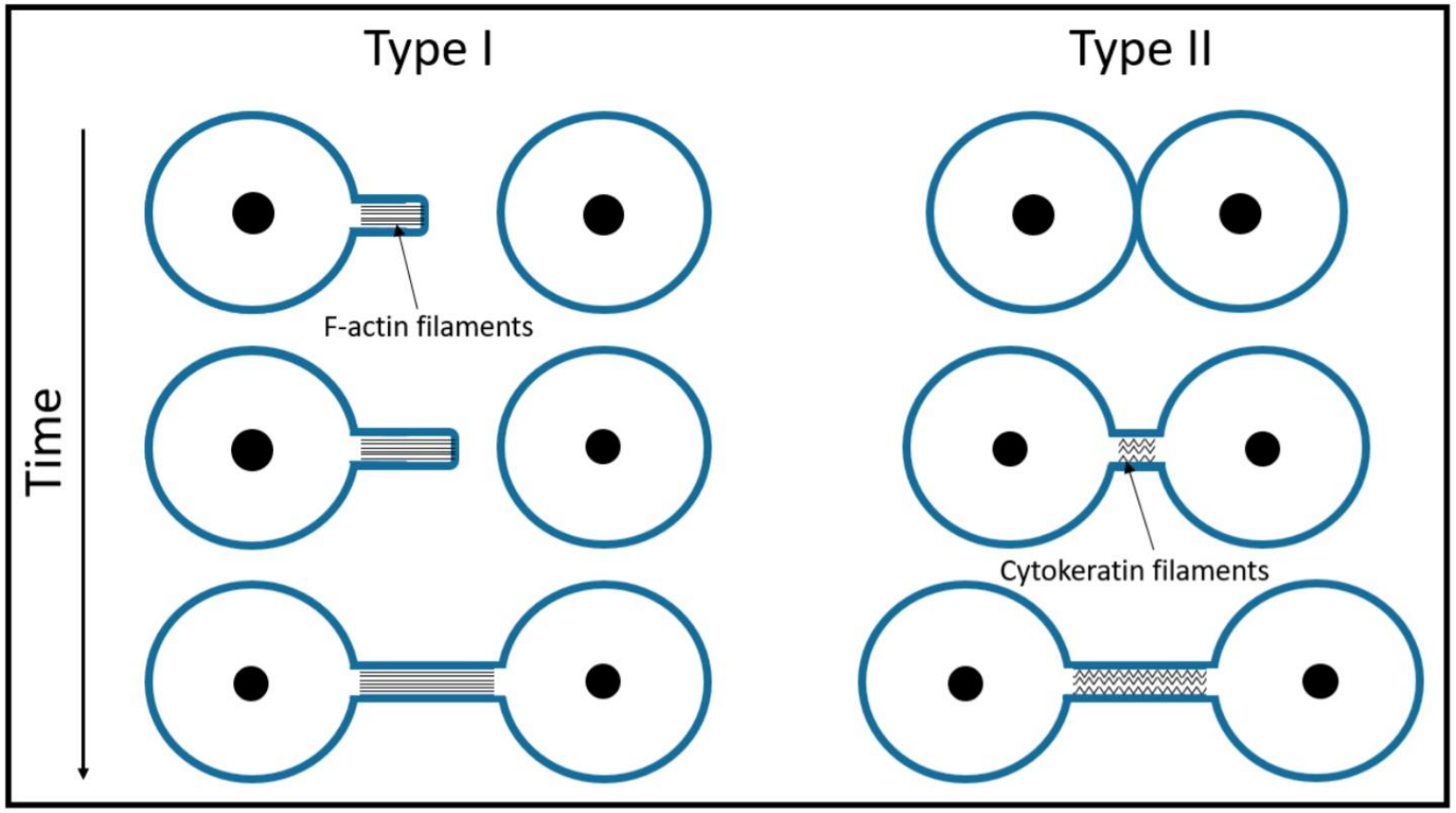


Kabaso, Lokar, Kralj-Iglič, Veranič, Iglič, Int. J. Nanomed., 6: 495–509, 2011

Human urothelial line RT4 live (nanotubes of type I – with actin filaments)



Veranič P., Lokar M., Schuetz G.J., Weghuber J., Wieser S., Hagerstrand H., Kralj-Iglič V., Iglič A.: Different types of cell-to-cell connections mediated by nanotubular structures, Biophys J., 95: 4416-4425, 2008.



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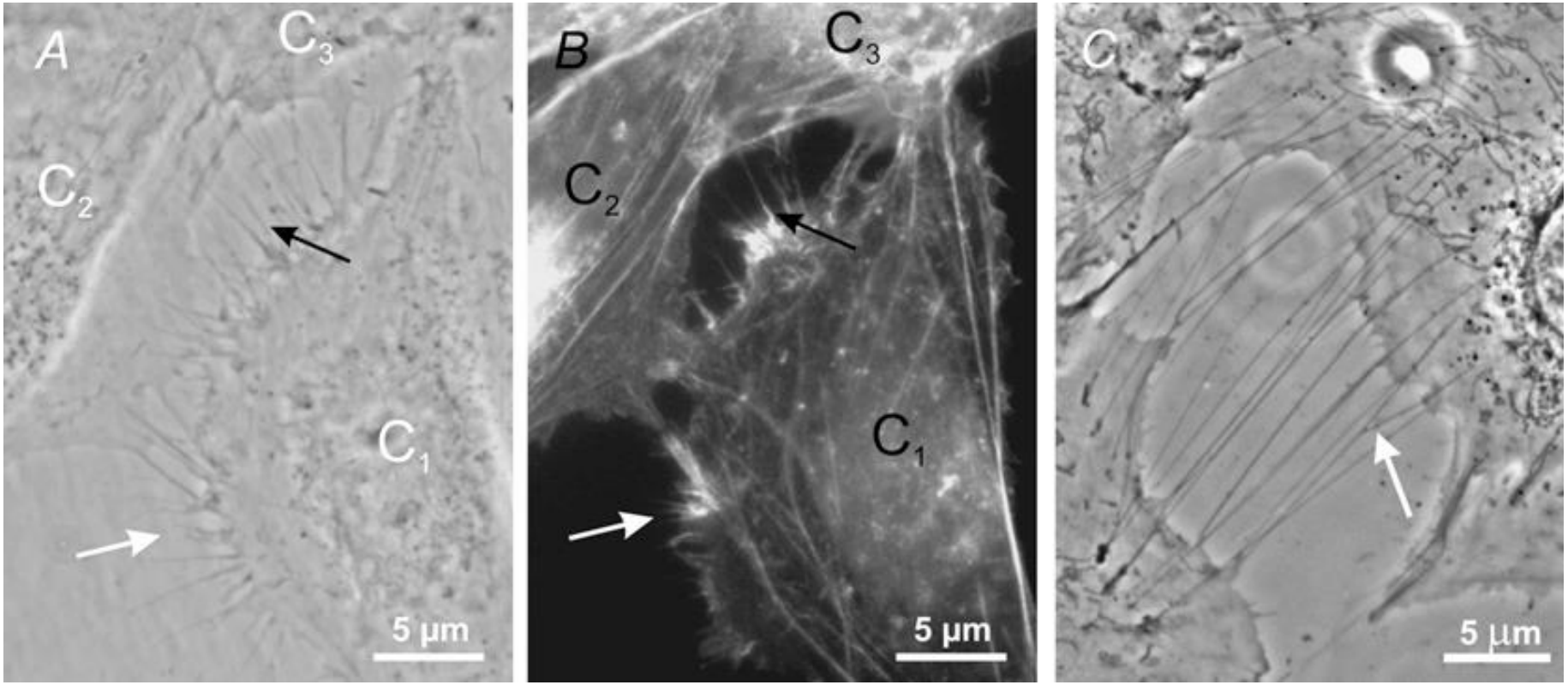
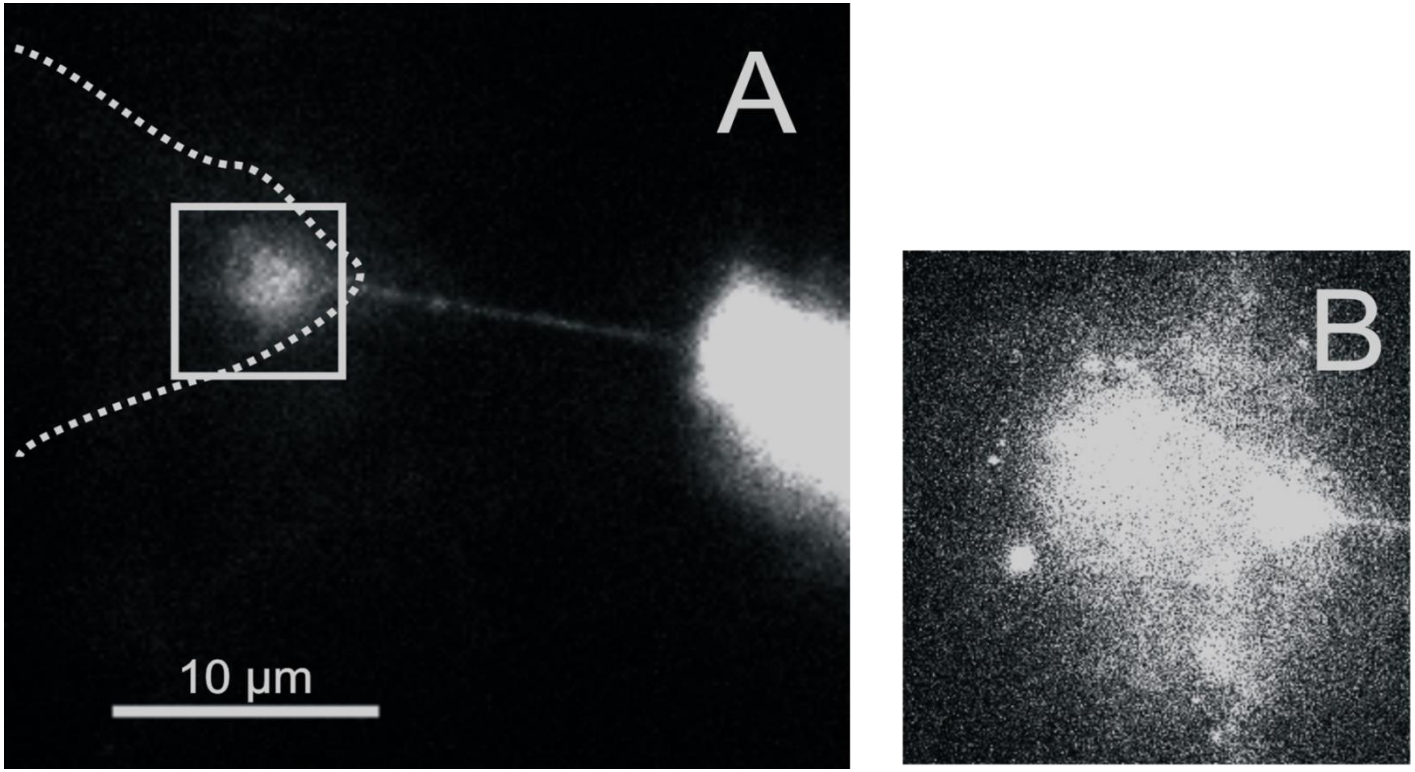
M. Drab, D. Stopar, V. Kralj-Iglič, A. Iglič: Inception mechanisms of tunneling nanotubes, Cells, 8(6): 626, 2019.



Type I nanotubes. A is a phase contrast image of live T24 cells, whereas B is a fluorescence micrograph showing **actin labeling of the same cells as in A** after 15 min of paraformaldehyde fixation. Cell C1 is approaching the cells C2 and C3 (see Movie S1). The white arrows in A and B indicate short and dynamic membrane protrusion with which the approaching cell explores its surroundings. The black arrow in A points at protrusions that have already connected to the target cell. In all these multiple tubular connections, actin filaments are present (arrows in B). Bridging nanotubes of type I can be more than 20 mm in length and occasionally bifurcations are seen (arrow in C).

Veranič P., Lokar M., Schuetz G.J., Weghuber J., Wieser S., Hagerstrand H., Kralj-Iglič V., Iglič A.: Different types of cell-to-cell connections mediated by nanotubular structures, Biophys J., 95: 4416-4425, 2008.

Transport of material through nanotubes



Exchange of actin-GFP via a bridging nanotubule between two T24 cells. Stable actin-GFP transfected T24 cells were frequently found to be interconnected by TNTs. Occasionally, connections were observed between a high expressing cell (right) and a cell devoid of actin-GFP (left; cell border is indicated by dashed line). The spreading of actin GFP into the second cell is clearly visible as a cone of fluorescence growing into a GFP-actin negative cell. Panel *B* is a magnified region of the framed area in *A*.

Veranič P., Lokar M., Schuetz G.J., Weghuber J., Wieser S., Hagerstrand H.,
Kralj-Iglič V., Iglič A.: Different types of cell-to-cell connections mediated by nanotubular structures,
Biophys J., 95: 4416-4425, 2008.

Theoretical model of efficient phagocytosis driven by curved membrane proteins and active cytoskeleton forces

Soft Matter

PAPER

Cite this: *Soft Matter*, 2023, 19, 31

A theoretical model of efficient phagocytosis driven by curved membrane proteins and active cytoskeleton forces†

Raj Kumar Sadhu, *[a] Sarah R. Barger,[b] Samo Penič,[c] Aleš Iglič,[c] Mira Krendel,[d] Nils C. Gauthier[e] and Nir S. Gov *[a]

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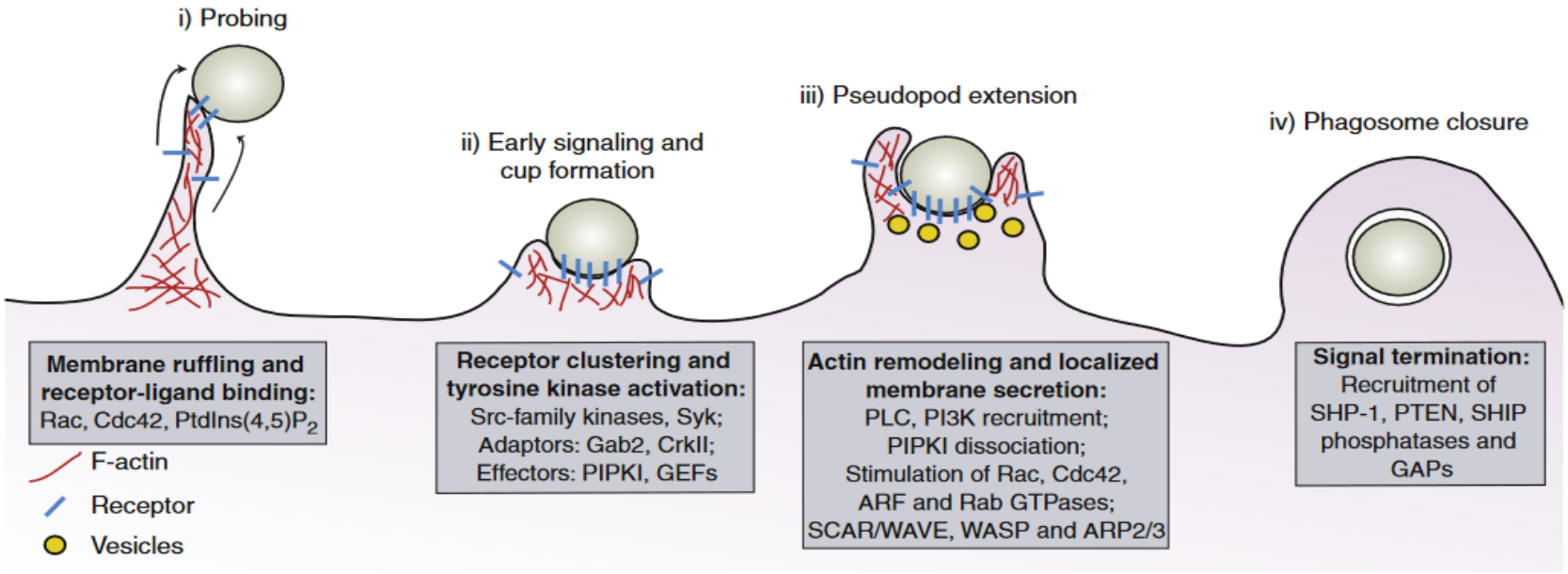
REVIEW

SUBJECT COLLECTION: CELL MIGRATION

A minimal cell model for lamellipodia-based cellular dynamics and migration

Raj Kumar Sadhu[1,*], Aleš Iglič[2] and Nir S. Gov[3,*]

Current Biology Vol 21 No 14
R534



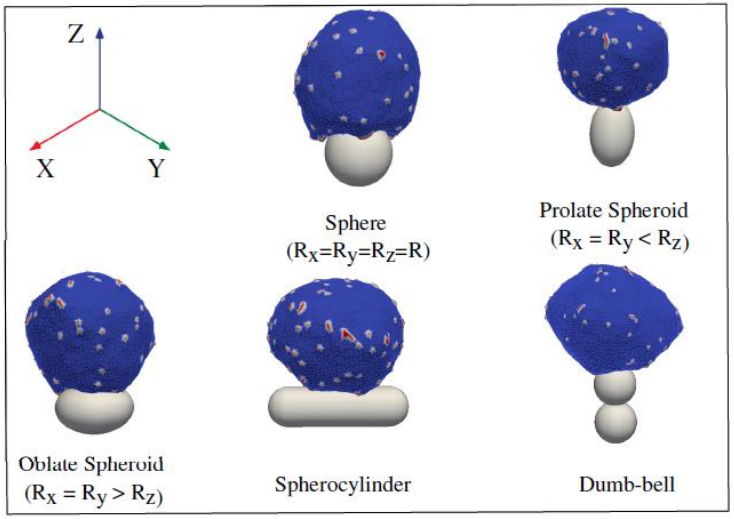
- Phagocytosis is the process of engulfment or internalization of comparatively larger particles (0.5 to 20 microns) by the cell.
- Phagocytosis plays important role for single-cell organisms, as a way of nutrient uptake and also in the immune system, to destroy foreign elements and dead cells.
- Phagocytosis is a complex process that involves different stages, such as, probing, early signaling and cup formation, pseudopod extension, phagosome closure etc.
- Phagocytosis is mainly performed by macrophages, neutrophils, dendritic cells etc.

Open Question

- how the actin cytoskeleton is coordinated spatio-temporally during the phagocytosis process?

Our aim

- to explains the dynamics and self-organization of the membrane and the actin cytoskeleton during the engulfment process, based on curved membrane activators of actin polymerization.
- phagocytosis for different shapes of engulfed particles

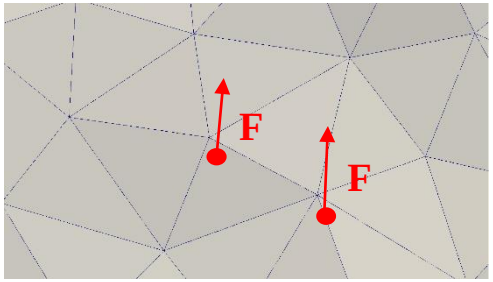


Theoretical backgrounds (Monte Carlo simulations)

The vesicle is constructed by N vertices, connected with bonds, forming a 3D triangulated surface.

The total free energy of the vesicle have four contributions:

- Bending energy, $W_b = \frac{k}{2}\int (C_1 + C_2 - C_0)^2\, dA$ $(k = bending\ rigidity)$
- Protein-protein interaction energy, $W_a = -\sum_{i<j} \mathcal{H}(r_0 - r_{ij})$ $(w = protein - protein\ interaction\ strength)$
- Energy due to active forces, $W_F = -\mathrm{F}\sum_i \hat{n}_i.\vec{x_i}$ $(\mathrm{F} = active\ cytoskeleton\ force)$
- Adhesion energy, $W_A = -E_{ad}\int_A dA$ $(E_{ad} = adhesion\ strength)$



The dynamics of the vesicle consists of (1) vertex move and (2) bond flip.

Vertex move allows vesicle shape to fluctuate; bond flip allows diffusion of proteins on the vesicle surface.

In a given movement, associated with a change in free energy ΔE, the movement occurs with rate is $e^{-\Delta E/k_B T}$ if ΔE > 0, or unity otherwise.

The parameter value used here are: Total number of vertices, N = 3127; k = 20 $k_B T$; w = 1 $k_B T$; c_0 = 1 l_{min}^{-1} (for nonzero curvature) or zero.

Experimental evidence of curved proteins and that they recruit actin filaments

© 2014. Published by The Company of Biologists Ltd | Journal of Cell Science (2014) 127, 1279–1292 doi:10.1242/jcs.140756

RESEARCH ARTICLE

The inverse BAR domain protein IBARa drives membrane remodeling to control osmoregulation, phagocytosis and cytokinesis

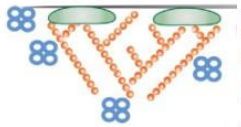
Joern Linkner[1,*], Gregor Witte[2,*,‡], Hongxia Zhao[3], Alexander Junemann[1], Benjamin Nordholz[1], Petra Runge-Wollmann[2], Pekka Lappalainen[3] and Jan Faix[1,*,‡]

ABSTRACT

Here, we analyzed the single inverse Bin/Amphiphysin/Rvs (I-BAR) family member IBARa from *Dictyostelium discoideum*. The X-ray structure of the N-terminal I-BAR domain solved at 2.2 Å resolution revealed an all-α-helical structure that self-associates into a 165-Å zeppelin-shaped antiparallel dimer. The structural data are consistent with its shape in solution obtained by small-angle X-ray scattering. Cosedimentation, fluorescence anisotropy, and fluorescence and electron microscopy revealed that the I-BAR domain bound preferentially to phosphoinositide-containing vesicles and drove the formation of negatively curved tubules.

BAR domains are grouped into three subfamilies: BAR and F-BAR domain proteins, in combination with actin polymerization, have been shown to be key players in initiating and stabilizing endosomal vesicles by generating positive membrane curvature and bending the plasma membrane inwards (Peter et al., 2004; Frost et al., 2008), whereas the subfamily of the inverse BAR domain (I-BAR) proteins induce negative membrane curvature to promote cell protrusions (Millard et al., 2005; Suetsugu et al., 2006a; Mattila et al., 2007; Saarikangas et al., 2009).

The mammalian I-BAR domain protein family comprises the five members IRSp53 (also



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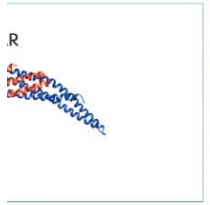

Fig. 1. The domain structures of BAR family members (left side) and the structures of BAR domain dimers (right side).

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ure
; CDC42; filopodia;

F-actin

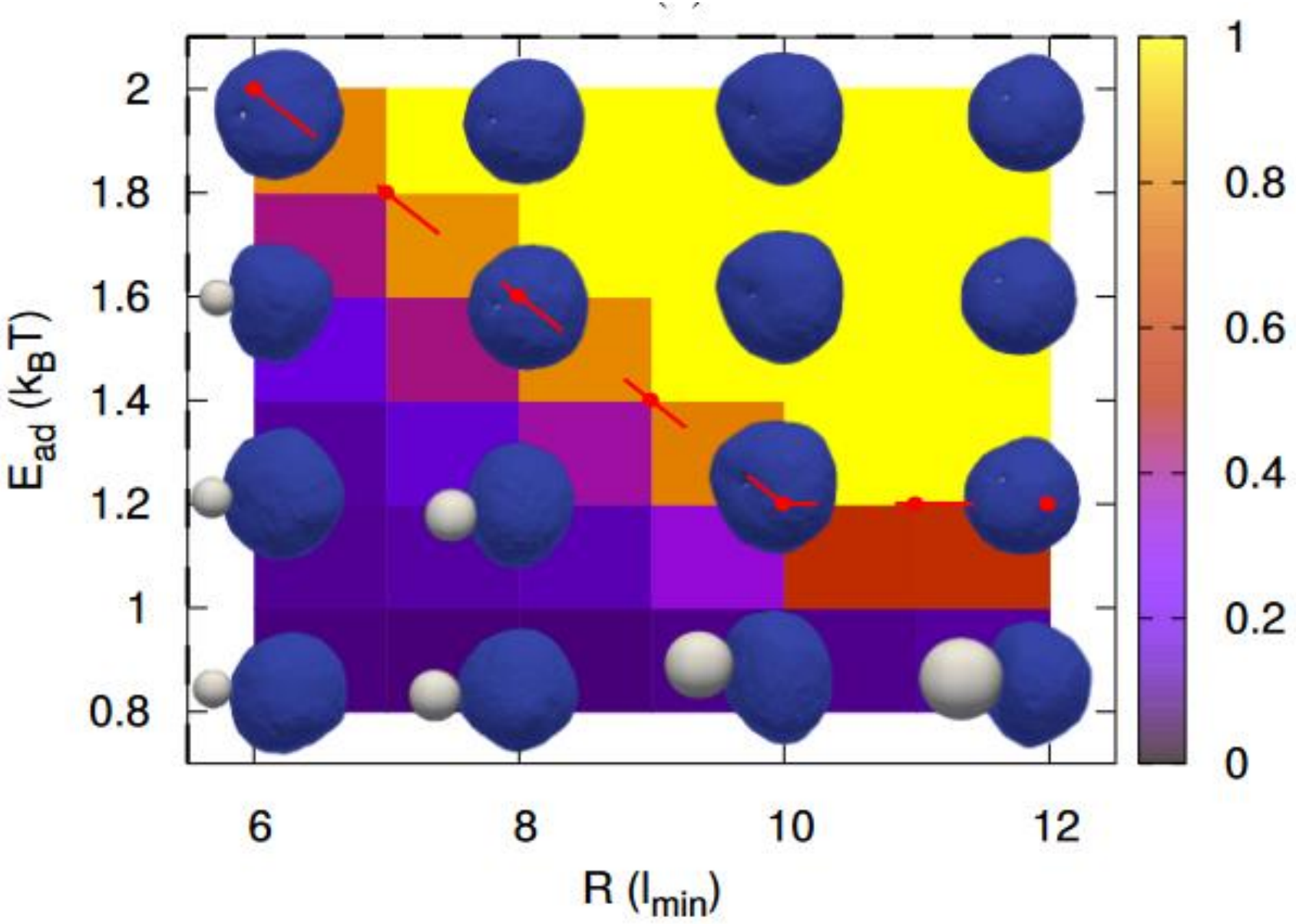
particles (NTY). We observed an impaired uptake of both types of particles by the null mutant (Fig. 5D) and, as expected, the ingestion of NTY was less efficient than that of the spherical FLY particles. In line with these findings, IBARa was detected at the rim of constricting phagocytic cups (Fig. 5E; supplementary material Movie 5). Thus, in endocytosis, IBARa contributes predominantly to phagocytosis.

Engulfment of spheres by protein-free vesicle: too small or too large particles are not engulfed

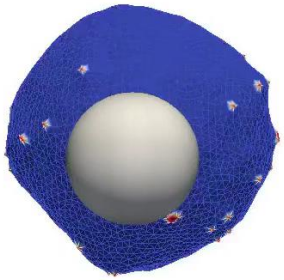
R=8; E_{ad} = 1.30

R=10; E_{ad} = 1.30

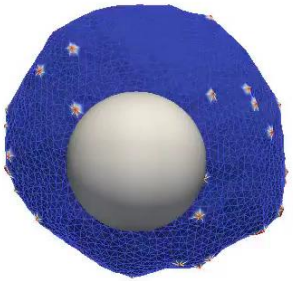
- For small E_{ad}, the particle is not engulfed.
- For medium E_{ad} particle is engulfed for an optimal radius.
- For large E_{ad} the particle is engulfed even for a small radius.
- We note that there is an optimal radius; in our next results, we fix R= 10.
- Engulfment process can be explained as a competition between adhesion energy gain and bending energy cost.


2
1.8
1.6
1.4
1.2
1
0.8
E_{ad} (k_BT)
6
8
10
12
R (l_{min})
1
0.8
0.6
0.4
0.2
0

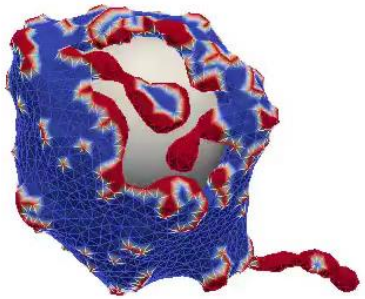
Curved (passive) proteins aggregate at the highly curved leading edge of the engulfing membrane and reduce the bending energy there



E_{ad} = 1.0, ρ = 2.4 %

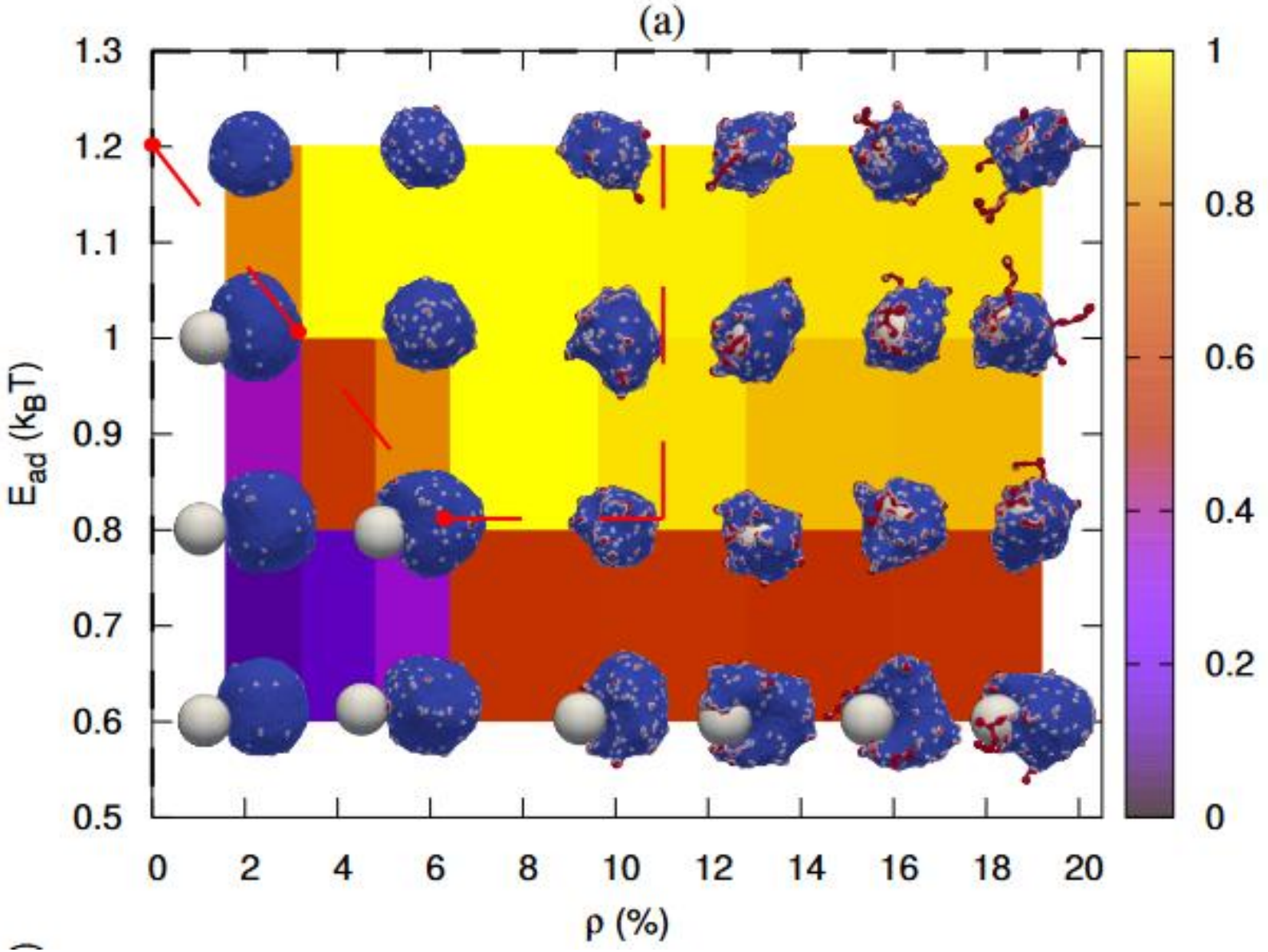


E_{ad} = 1.0, ρ = 4.8 %



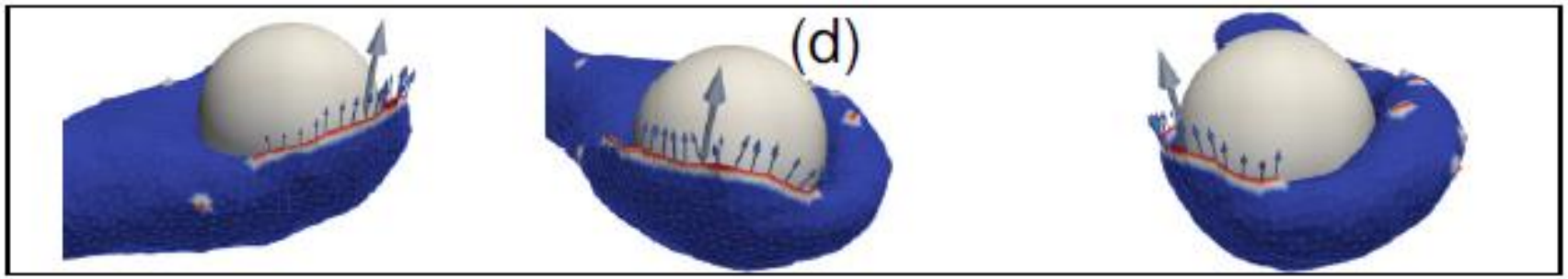
E_{ad} = 1.0, ρ = 19.2 %

- For the next results, we use the optimal radius R=10 l_{min}
- For small E_{ad} the engulfment is partial.
- For intermediate E_{ad}, the engulfment is complete as we increase ρ.
- For large E_{ad}, the process is mainly driven by adhesion energy, and the particle is engulfed without any proteins.
- For large ρ, the engulfment again becomes partial.
- Comparison of bending and adhesion energy explains the process.


(a)
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
E_{ad} (k_BT)
0
2
4
6
8
10
12
14
16
18
20
ρ (%)
1
0.8
0.6
0.4
0.2
0

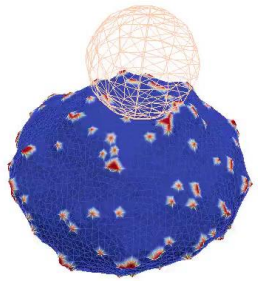
Active forces allow the engulfment at smaller protein density, by the formation of only an arc-like protrusion

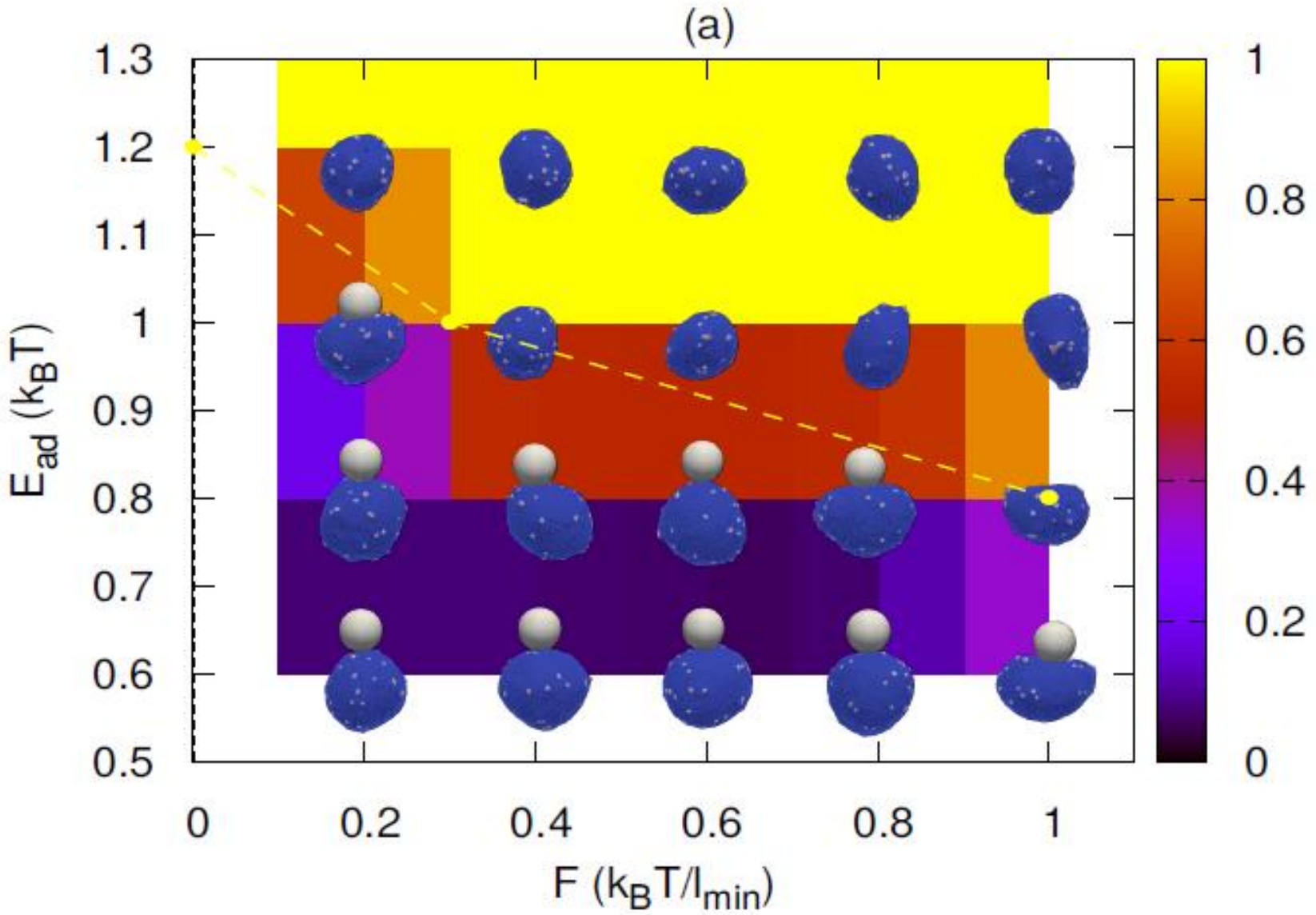
- For small E_{ad} there is no engulfment.
- For intermediate E_{ad} increasing F results in complete engulfment.
- For large E_{ad} engulfment happens without any force.



The presence of curved (convex) proteins reduces the bending energy cost by self-organizing with a higher density at the highly curved leading edge of the engulfing membrane, which forms the circular rim of the phagocytic cup that wraps around the particle. This allows the engulfment to occur at much smaller adhesion strength.

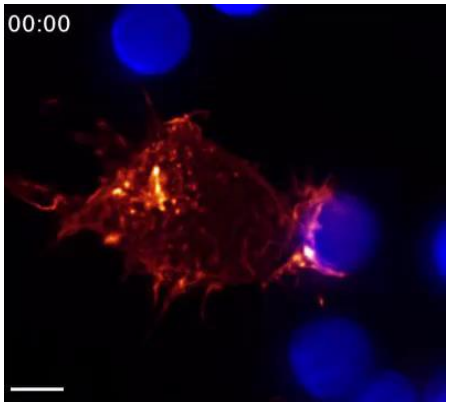
When the curved membrane-bound protein complexes locally recruit actin polymerization machinery, which leads to outward forces being exerted on the membrane, we found that engulfment is achieved more quickly and at a lower protein density.




(a)
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
E_{ad} (k_BT)
0
0.2
0.4
0.6
0.8
1
F (k_BT/l_{min})
1
0.8
0.6
0.4
0.2
0

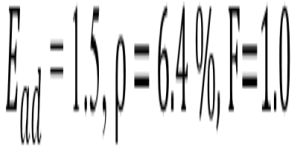
For large protein density, the proteins form rim of fragmented cluster at much smaller adhered fraction

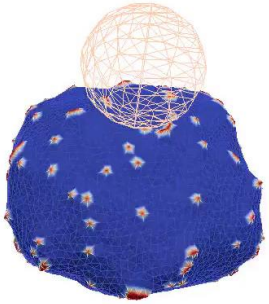
- For large ρ, the rim of the protein cluster is formed much before the full engulfment.
- The protein clusters are highly fragmented at early stage.
- These observations match well with recent high resolution experimental images.

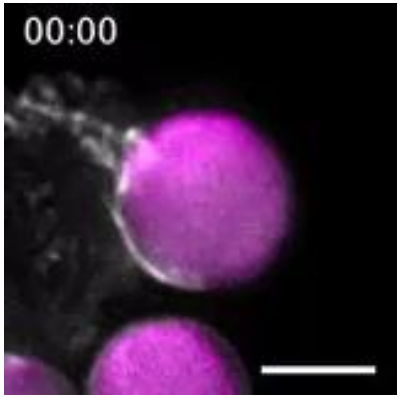


E_{ad} = 1.5, ρ = 6.4 %, F=1.0

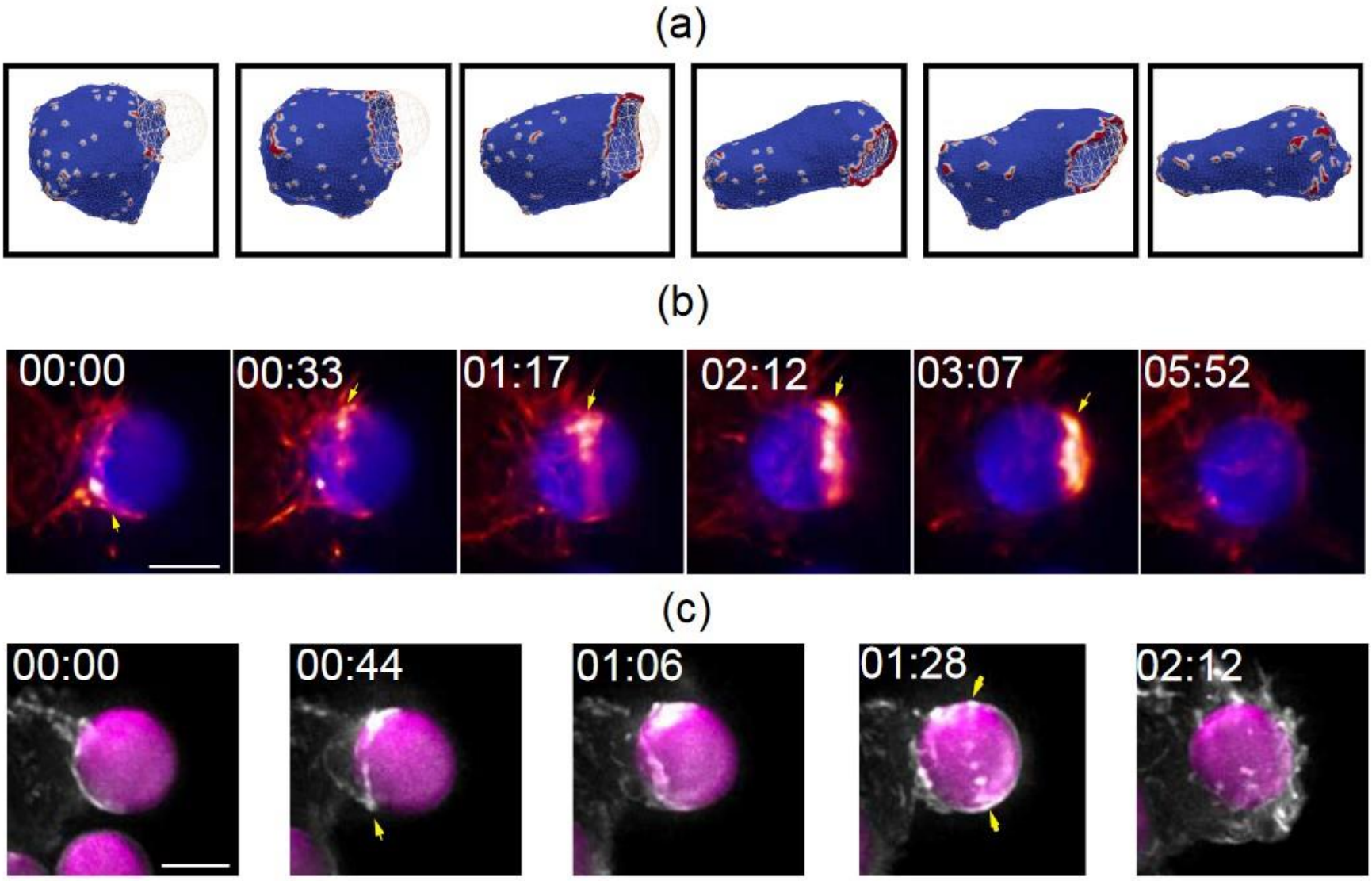
E_{ad} = 1.5, ρ = 4.8 %, F=2.0





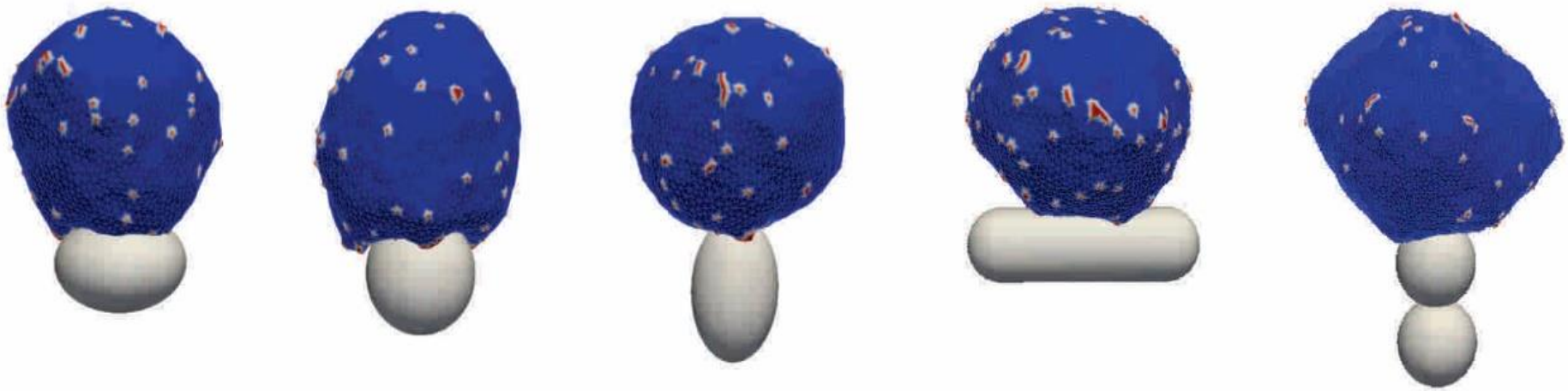


R. Kumar Sadhu, **S. R. Barger**, S. Penič, A. Iglič, **M. Krendel**, **N.C. Gauthiere**, N.S. Gov: A theoretical model of efficient phagocytosis driven by curved membrane proteins and active cytoskeleton forces. Soft Matter 19, 31-43, 2023.

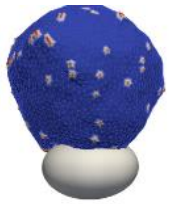


R. Kumar Sadhu, **S. R. Barger**, S. Penič, A. Iglič, **M. Krendel**, **N.C. Gauthiere**, N.S. Gov: A theoretical model of efficient phagocytosis driven by curved membrane proteins and active cytoskeleton forces. Soft Matter 19, 31-43, 2023.

Influence of the shape of the particle



Non-spherical particles are more difficult to engulf than the spherical particles of same surface area

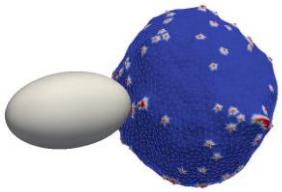


Oblate Spheroid
($R_X = R_Y > R_Z$)

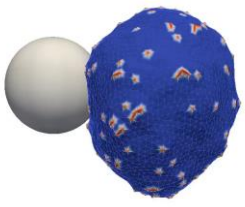
Prolate Spheroid
($R_X = R_Y < R_Z$)

- For passive, we use E_{ad}=1.0, ρ=4.8%; For active, E_{ad}=1.0, ρ=1.6%; F=1; area is constant (sphere with R=10).
- Starting from side is beneficial.
- Active case engulfs faster and spans larger region in both the oblate and prolate side.

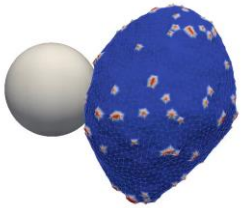
PASSIVE



Rx=Ry=8.4, Rz=14.16

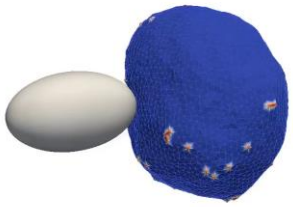


Rx=Ry=11.4, Rz=7.96

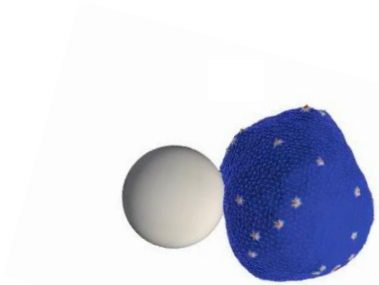


Rx=Ry=11.4, Rz=7.96

ACTIVE



Rx=Ry=8.4, Rz=14.16



Rx=Ry=12, Rz=6.77

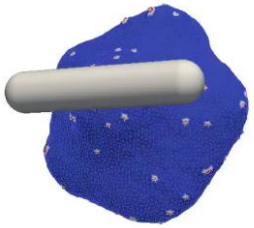
Engulfment of non-spherical particles: sphero-cylindrical viruses and dumb-bell shapes

Parameters: N=6127, E_{ad} = 1.5, ρ = 1.6 %, F=1.5

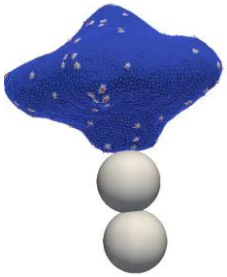
from top (passive + active)

- initializing from side is beneficial.
- initializing from top ends up with partial engulfment.
- when we make all the proteins at the back passive, it completes the engulfment.
- for dumb-bell shape, passive vesicle performs well.

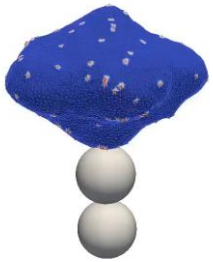
from side (passive)

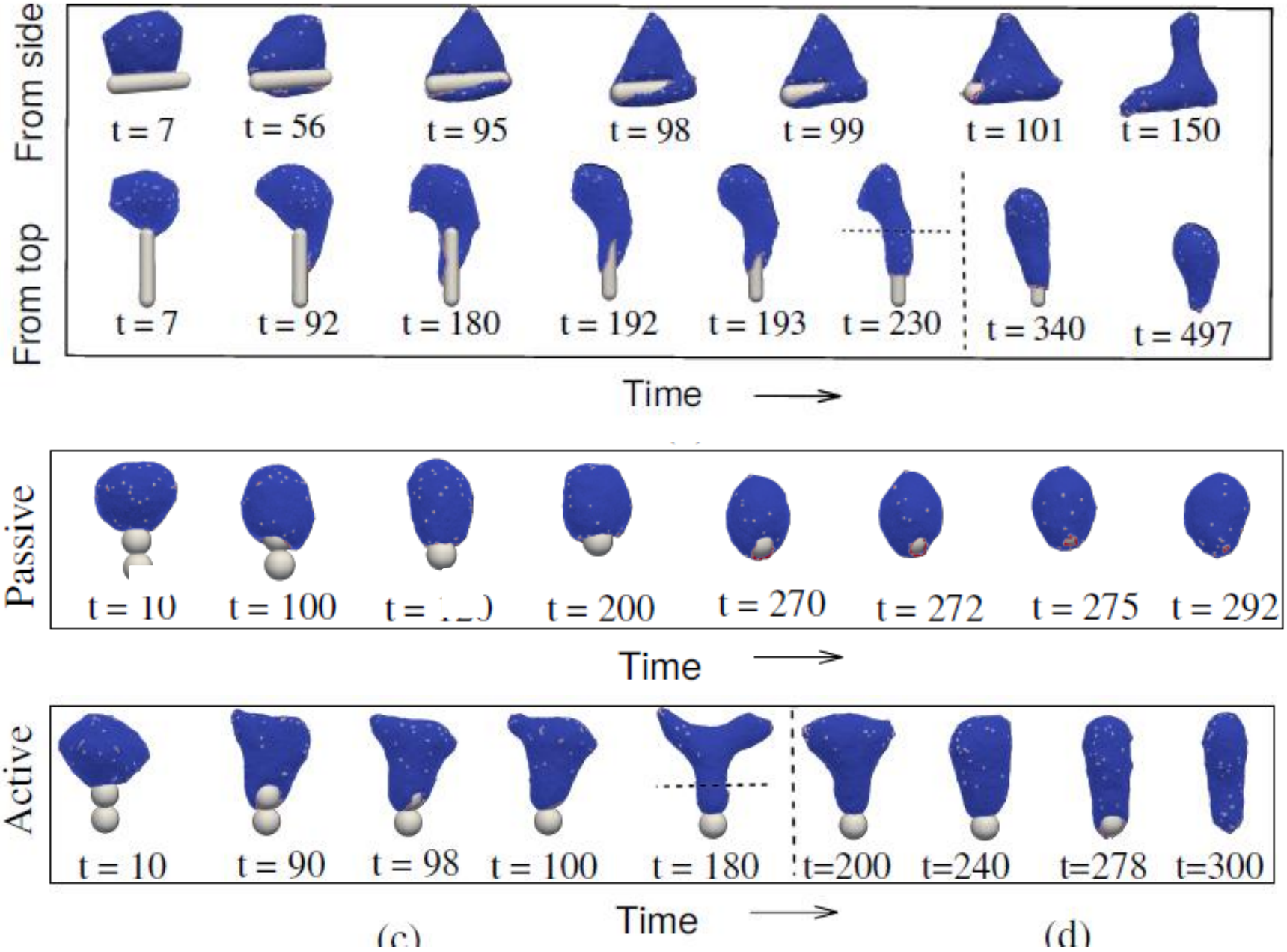


passive



passive + active




From side
From top
t = 7
t = 56
t = 95
t = 98
t = 99
t = 101
t = 150
t = 7
t = 92
t = 180
t = 192
t = 193
t = 230
t = 340
t = 497
Time
Passive
t = 10
t = 100
t = 200
t = 270
t = 272
t = 275
t = 292
Time
Active
t = 10
t = 90
t = 98
t = 100
t = 180
t=200
t=240
t=278
t=300
Time
(c)
(d)

Summary

We study the engulfment of spherical and non-spherical particles by a 3D vesicle.

We explain self-organization of actin cytoskeleton on the phagocytic cup based on curved actin nucleators.

The addition of curved proteins and active cytoskeleton force enhances the engulfment process.

The process of engulfment could be explained as a competition between adhesion energy gain and bending energy cost.

Non-spherical particles are more difficult to engulf than spherical ones with same area.

For non-spherical particles, the engulfment time crucially depends upon the initial orientation of vesicle with the particle.

Our results qualitatively agrees well with the experiments.

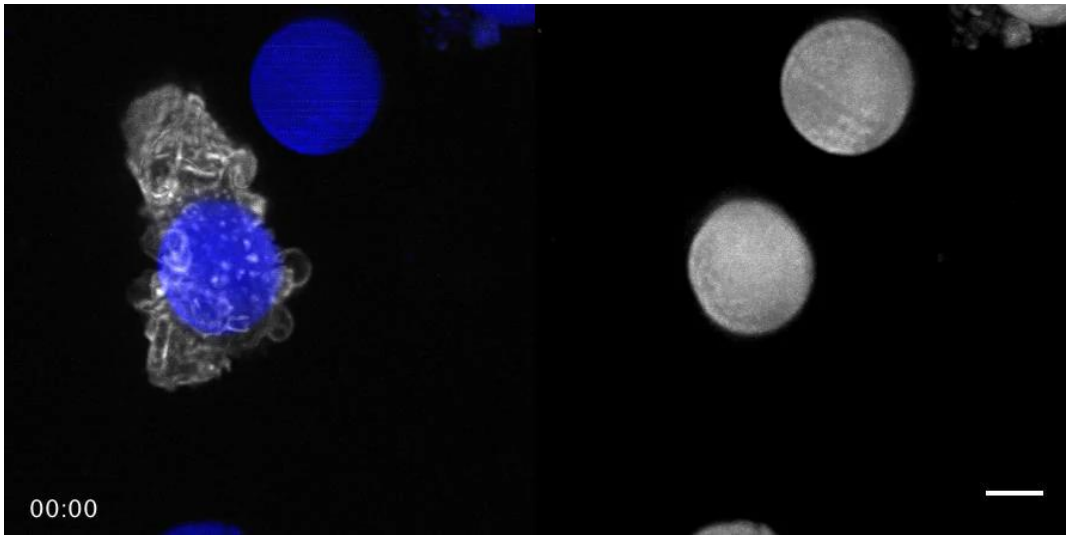
Future directions

- **Engulfment of soft objects in presence of contractile forces**

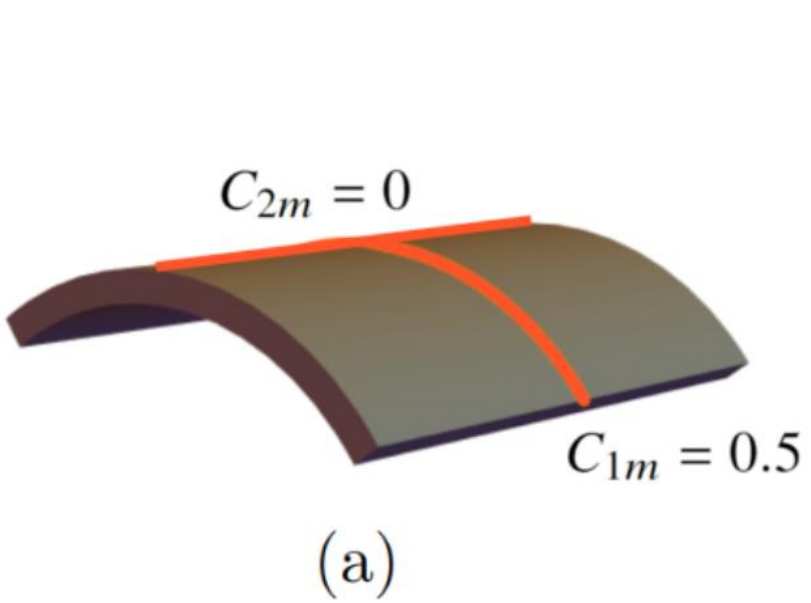
In our model particles are rigid. If the particles are soft, contractile forces due to Myosin II activity will play a major role, that can constrict the objects.

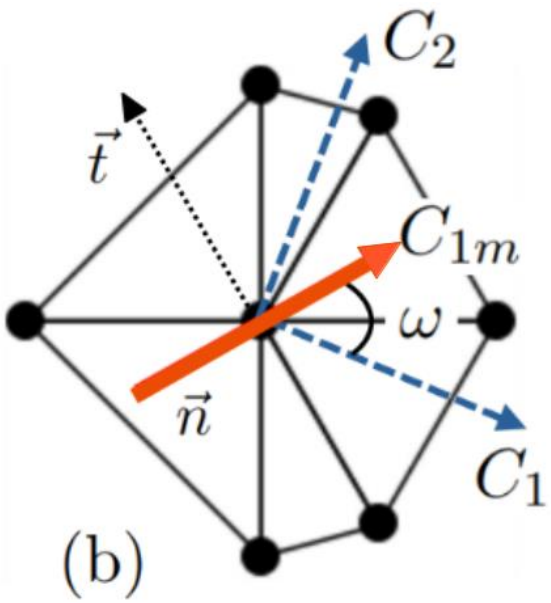
- **Engulfment of dynamic objects**

Different viruses and bacteria's have developed strategies to avoid engulfment. It will be interesting to model a dynamic target trying to avoid engulfment.



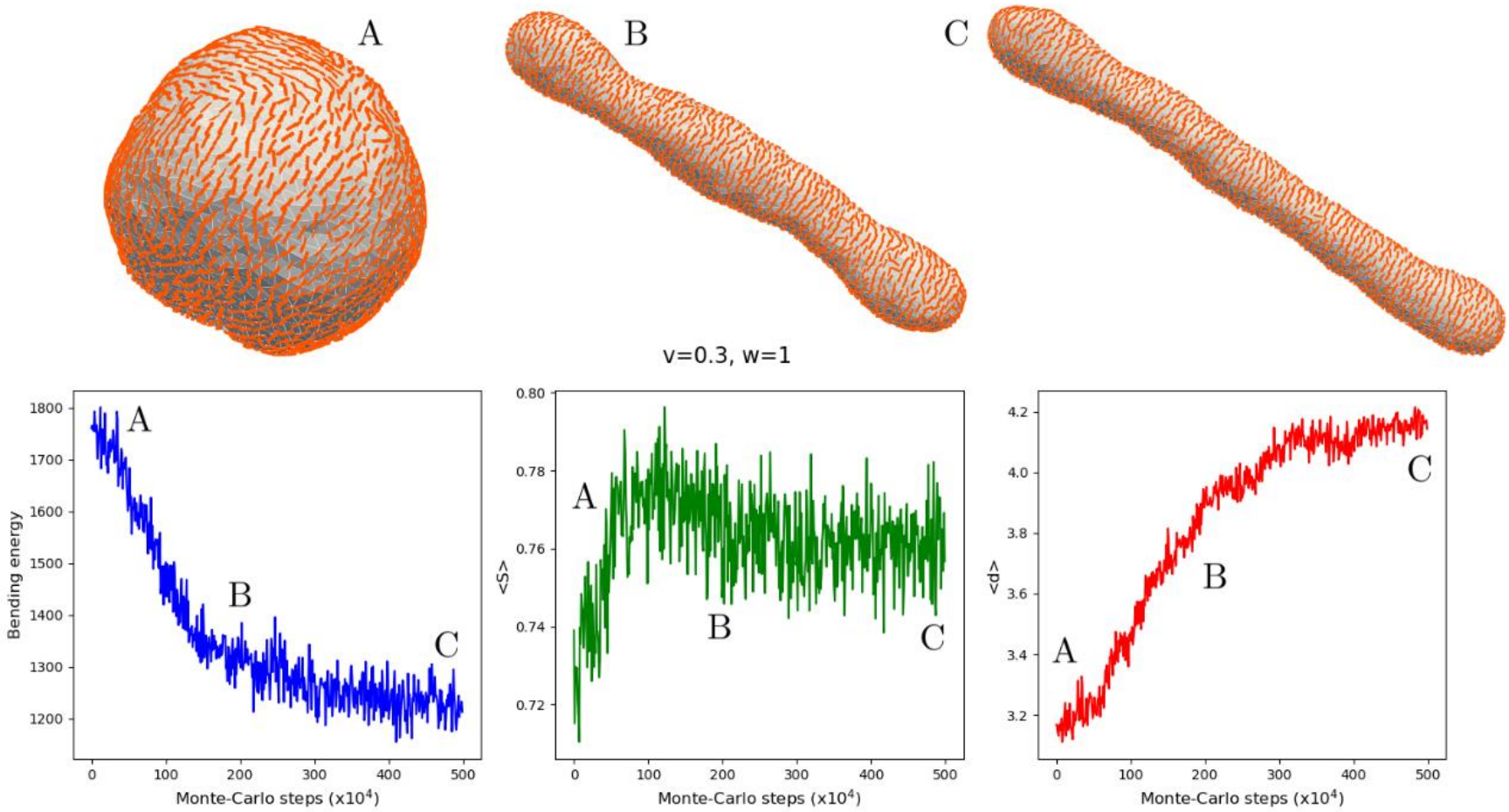
- **considering the ANISOTROPIC shape of the membrane attached banana-like BAR proteins and nematic direct nearest-neighbour direct interactions between BAR proteins**

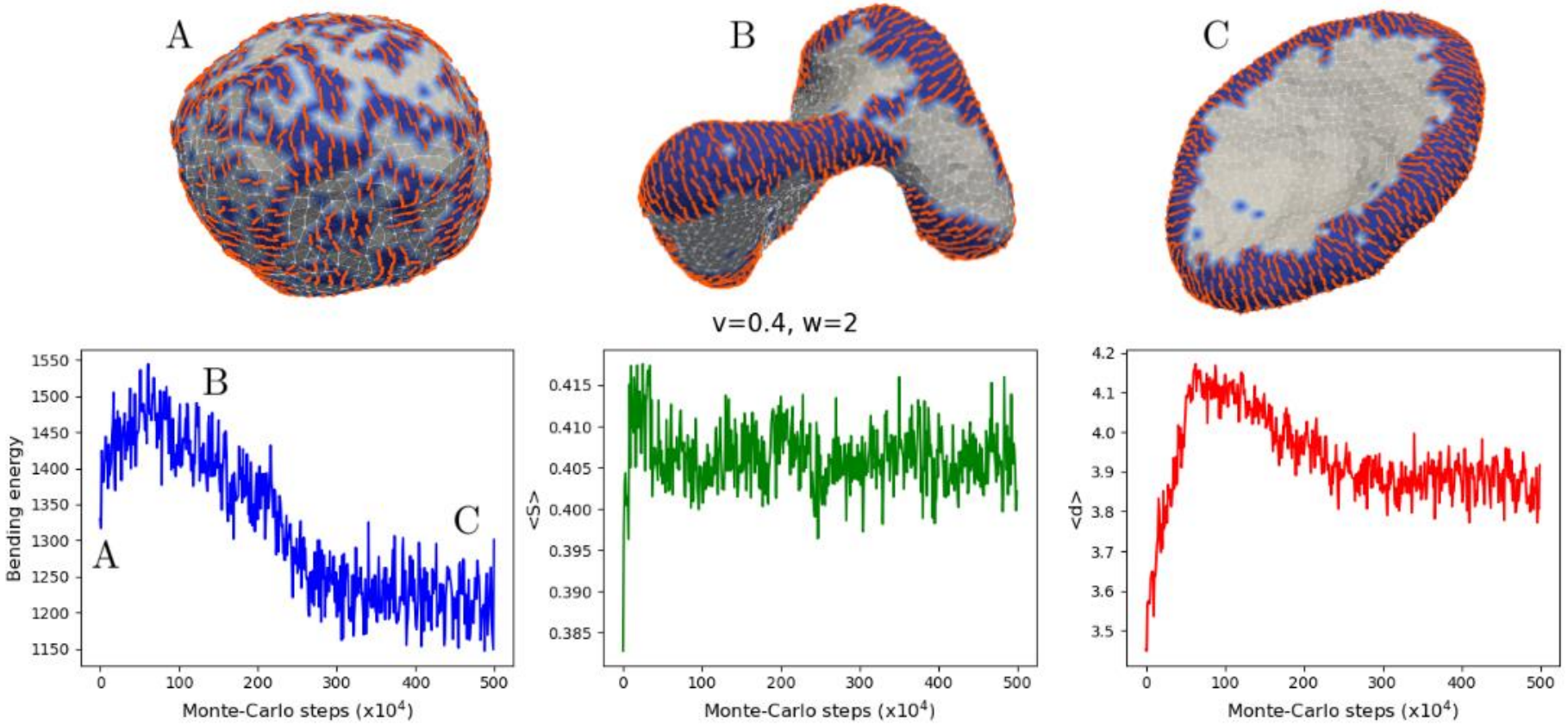




Y. Ravid, S. Penič, N. Gov, V. Kralj-Iglič, A. Iglič: NUMERICAL STUDIES OF TRIANGULATED VESICLES WITH ANISOTROPIC MEMBRANE INCLUSIONS, Advances in Biomembranes and Lipid Self-Assembly, Elsevier. 2024 (accepted for publication)

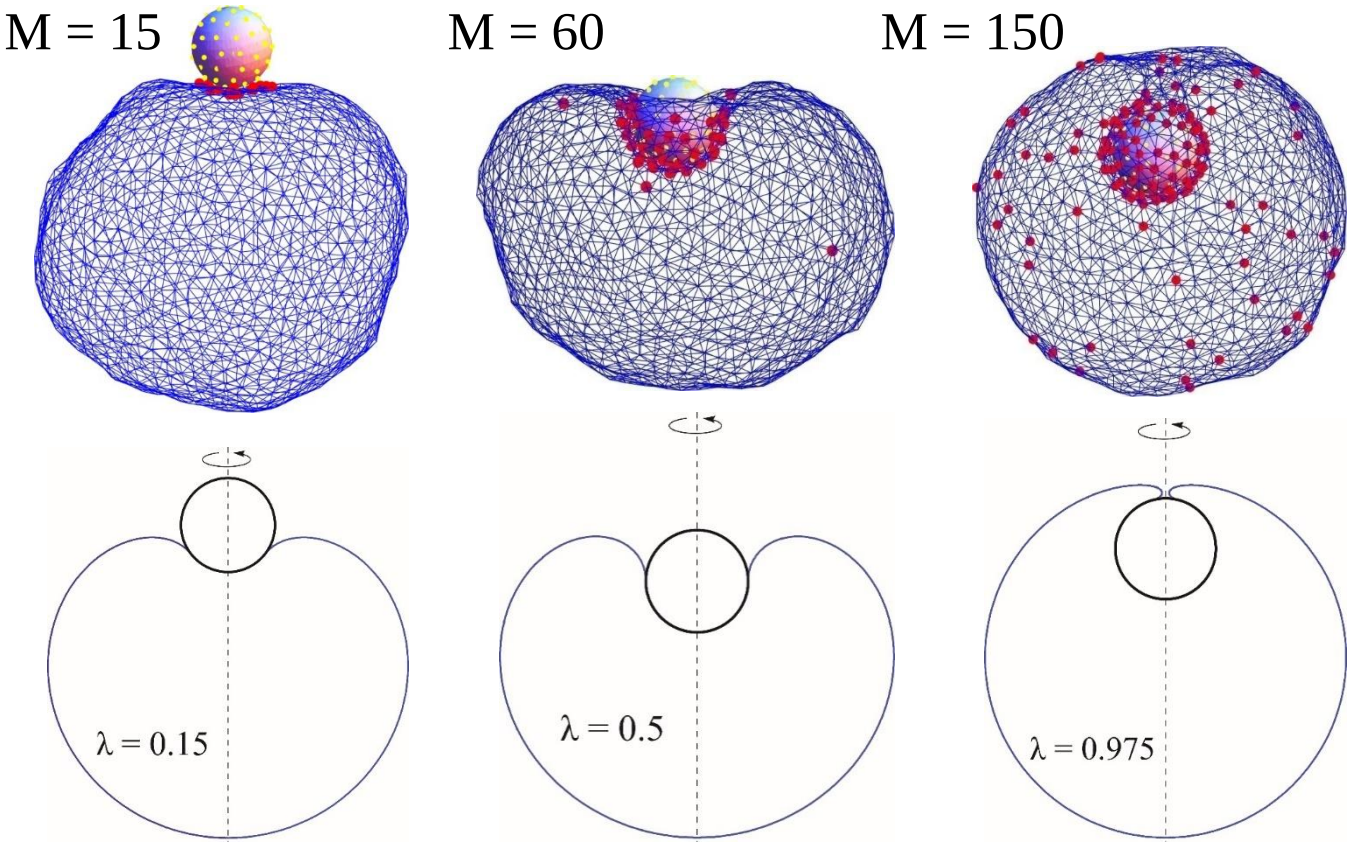
- **considering nematic direct nearest-neighbour direct interactions between BAR proteins** (M. Drab et al., in preparation)




A
B
C
v=0.4, w=2
Bending energy
<S>
<d>
Monte-Carlo steps (x10^4)
Monte-Carlo steps (x10^4)
Monte-Carlo steps (x10^4)

· considering also the membrane electrostatics

MC simulations + mathematical modelling: minimisation of bending and electrostatic



M = number of mobile charged molecules in the membrane

Fošnarič, May, Kroll & Iglič, J. Chem. Phys., 2009

A minimal physical model for curvotaxis driven by curved protein complexes at the cell's leading edge, that exert protrusive forces on the membrane (representing the pressure due to actin polymerization)

PNAS RESEARCH ARTICLE | BIOPHYSICS AND COMPUTATIONAL BIOLOGY

A minimal physical model for curvotaxis driven by curved protein complexes at the cell's leading edge

Raj Kumar Sadhu[a,1,2], Marine Luciano[b,c], Wang Xi[d], Cristina Martinez-Torres[e], Marcel Schröder[f], Christoph Blum[f], Marco Tarantola[f], Stefano Villa[f], Samo Penič[g], Aleš Iglič[g], Carsten Beta[e,h], Oliver Steinbock[i], Eberhard Bodenschatz[f], Benoît Ladoux[d], Sylvain Gabriele[c], and Nir S. Gov[a,2]

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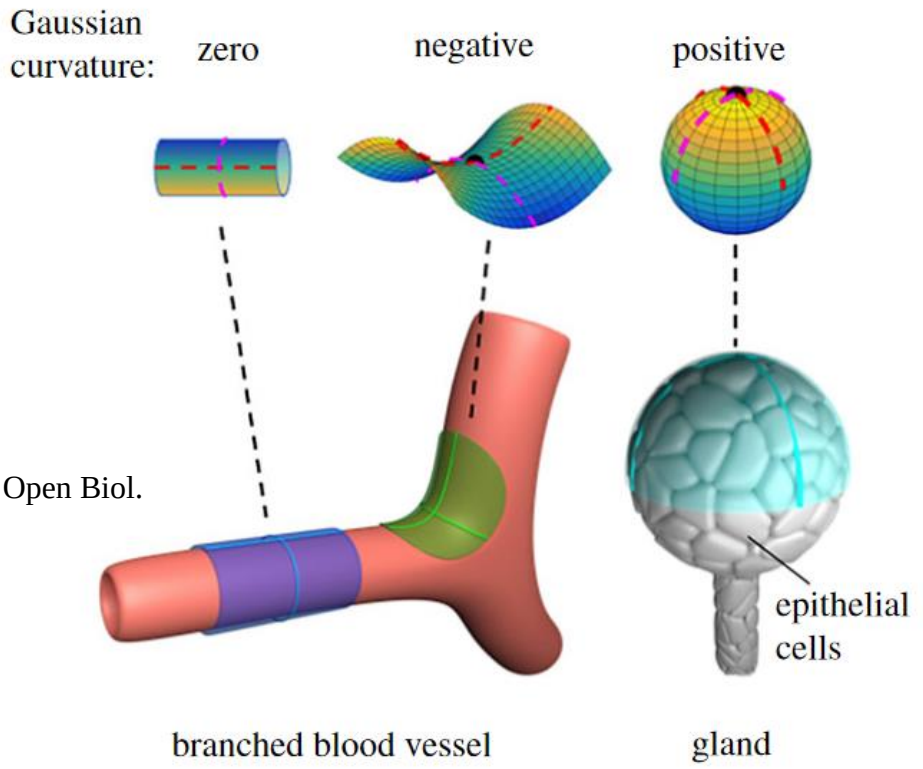
REVIEW

SUBJECT COLLECTION: CELL MIGRATION

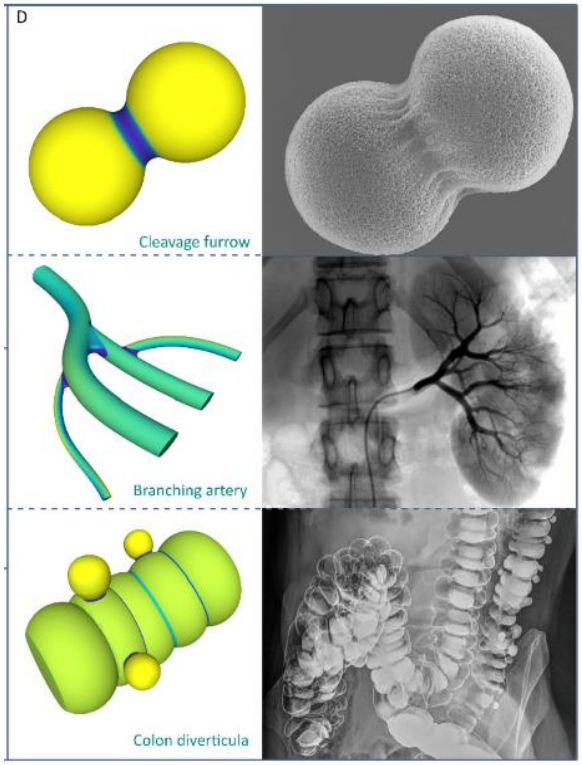
A minimal cell model for lamellipodia-based cellular dynamics and migration

Raj Kumar Sadhu[1,*], Aleš Iglič[2] and Nir S. Gov[3,*]

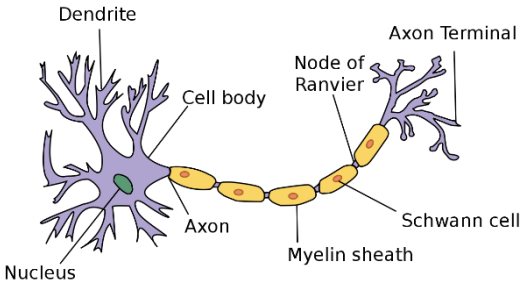
Motivation



Richard K. Assoian, Open Biol. 9: 190155 (2019)



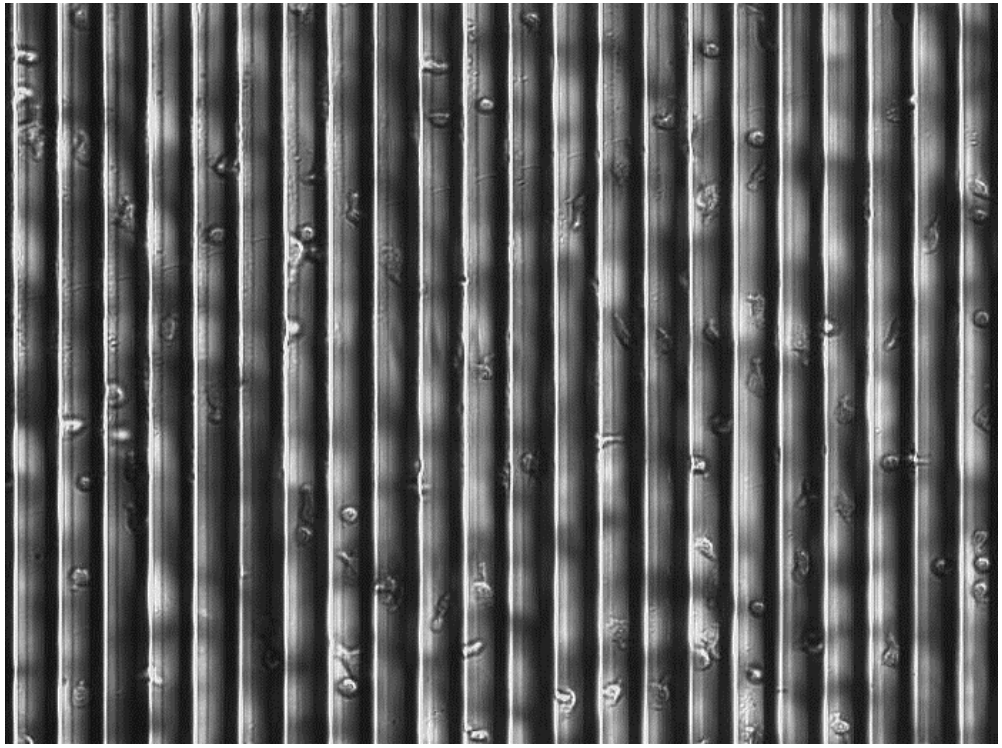
Sebastien J.P. Callens, Biomaterials 232, 119739 (2020)

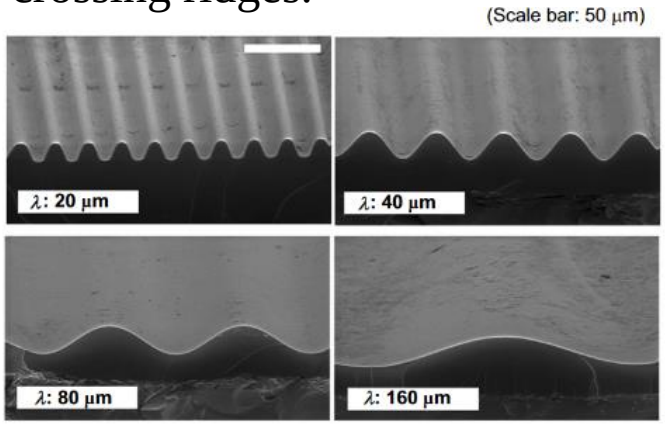


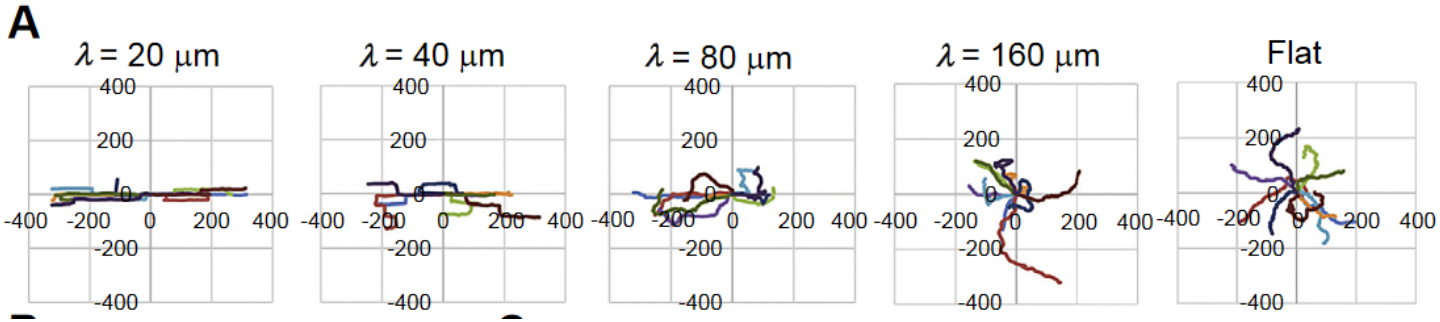
- Cells often adhere and spread on curved surfaces, such as the fibers of the extra-cellular matrix (ECM), as well as cylindrical protrusions of other cells, such as glia cells.
- Most of the cell biological studies examine function and molecular mechanisms using cells on flat surfaces.
- It is important to understand the spreading and movement of cells on curved surfaces, that will have important application in biomaterial design or tissue engineering.

Experiments: cells spreading and migrating on curved surfaces

- T cells prefer concave surfaces (or grooves) and tend to migrate along the groove [K H Song, Biomaterials, 51, 151 (2015)].
- Myosin II-inhibited T cells migrate with straight trajectories with minimally crossing ridges.







PNAS RESEARCH ARTICLE | BIOPHYSICS AND COMPUTATIONAL BIOLOGY

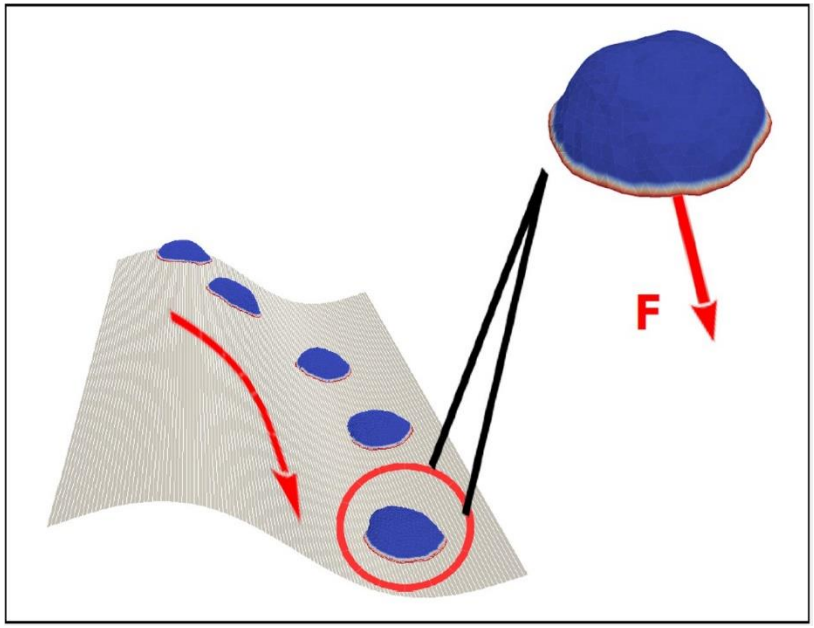
A minimal physical model for curvotaxis driven by curved protein complexes at the cell's leading edge

Raj Kumar Sadhu[a,1,2], Marine Luciano[b,c], Wang Xi[d], Cristina Martinez-Torres[e], Marcel Schröder[f], Christoph Blum[f], Marco Tarantola[f], Stefano Villa[f], Samo Penič[g], Aleš Iglič[g], Carsten Beta[e,h], Oliver Steinbock[i], Eberhard Bodenschatz[f], Benoît Ladoux[d], Sylvain Gabriele[c], and Nir S. Gov[a,2]

Edited by Pascal Silberzan, Institut Curie, Paris, France; received April 27, 2023; accepted January 29, 2024 by Editorial Board Member Herbert Levine

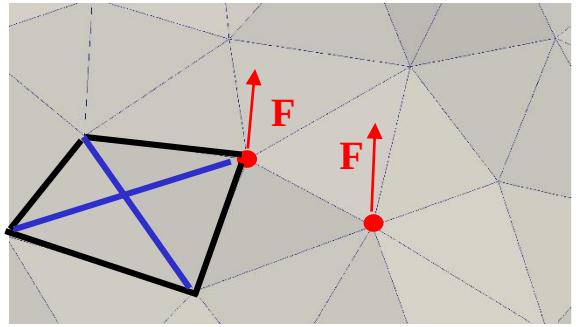
Significance

How cells migrate when exposed to curvature cues (curvotaxis), is important for understanding cell migration inside complex tissues, however, the underlying mechanisms are not well understood. Here, we use a theoretical "minimal cell" model, which is formed using a closed vesicle containing curved membrane proteins coupled with active (cytoskeleton) forces, that self-organizes to form a motile phenotype. Using this "minimal



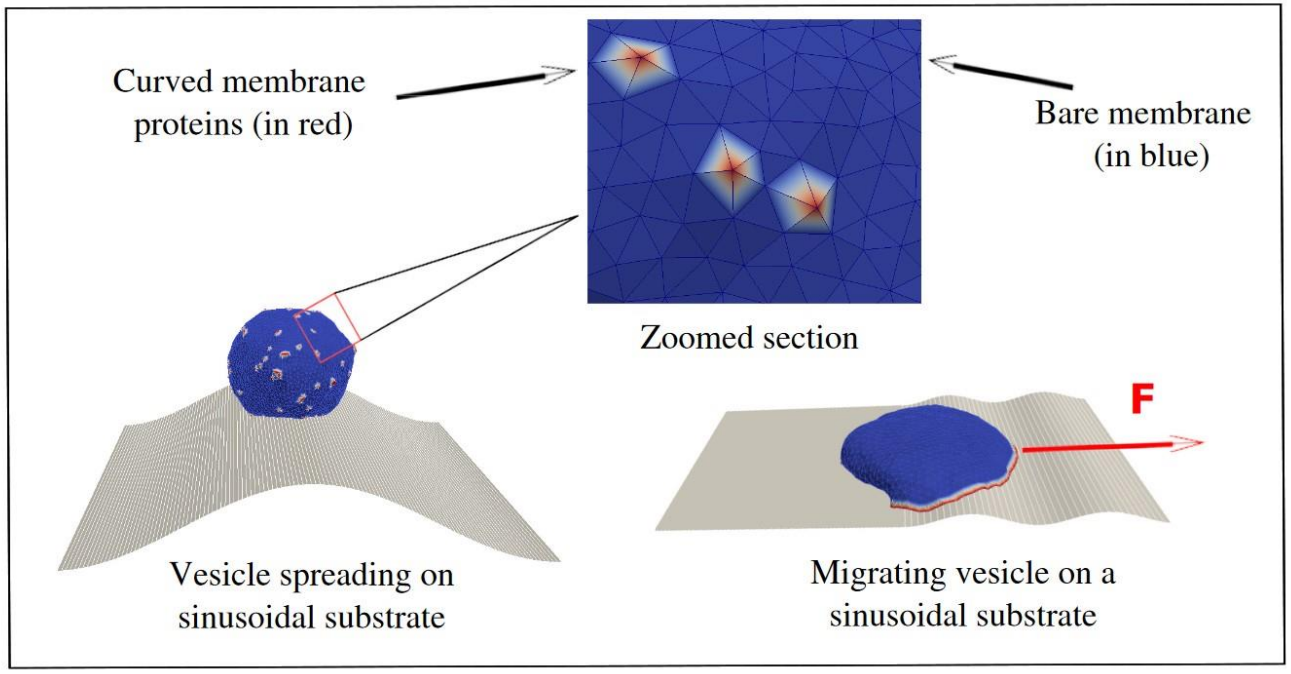
(zoomed version, showing triangulated mesh)

Red dots: proteins with spontaneous curvature



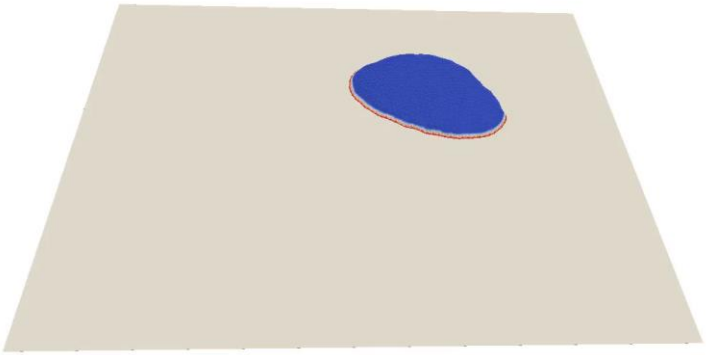
Theoretical/Monte Carlo model

(as described before)



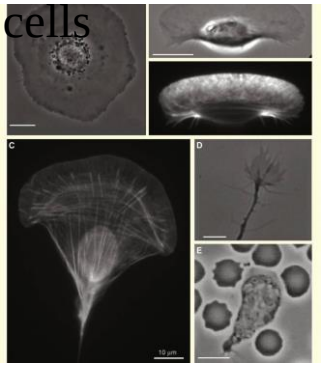
‘Minimal Cell’ spreading and migrating on flat substrate

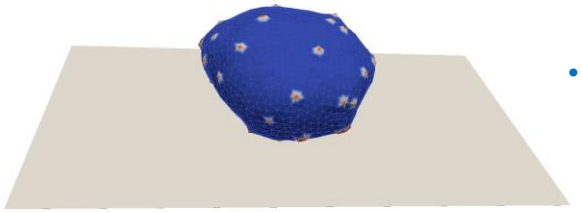
Migrating phenotype on flat substrate



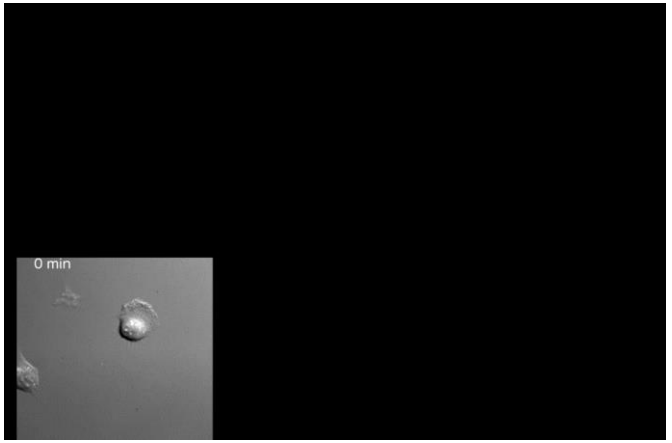
Two-arc shapes formed on flat substrate

Shape of motile cells





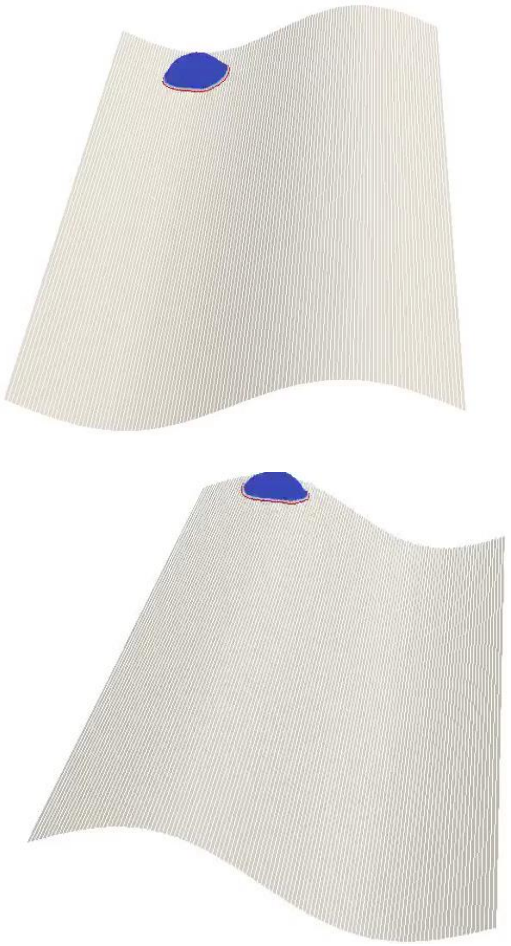
- The shape of the motile vesicle is very close to the shape observed in experiments.
- Real cells have the mechanism to repolarize itself that is absent in our model

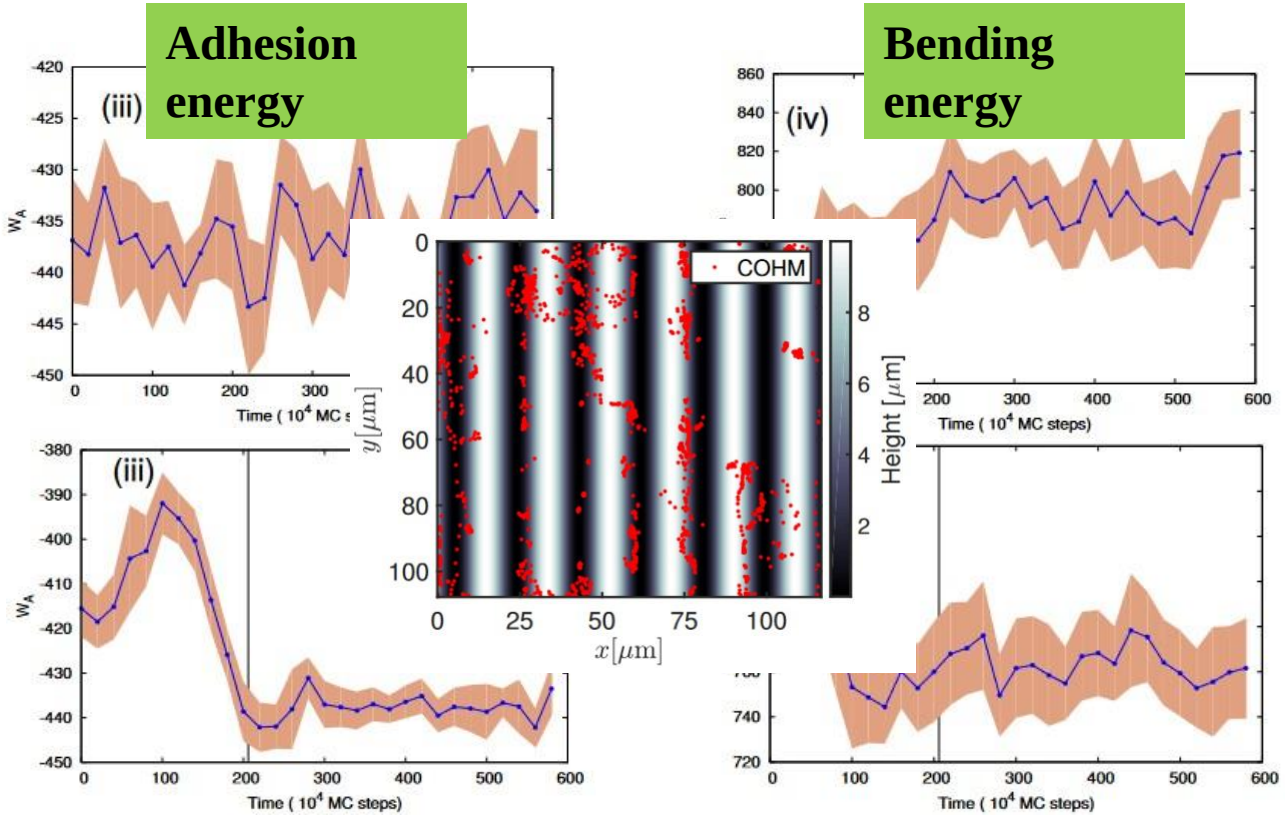


R. K. Sadhu, S. Penič, A. Iglič, N. S. Gov: Modelling cellular spreading and emergence of motility in the presence of curved membrane proteins and active cytoskeleton forces.
Eur. Phys. J. Plus 136:495, 2021.

Vesicle prefers to stay on the minimum and migrates along the axis

R. K. Sadhu, M. Luciano, W. Xi, C. Martinez-Torres, M. Schröder, C. Blum, M. Tarantola, S. Villa, S. Penič, A. Iglič, C. Beta, O. Steinbock, E. Bodenschatz, B. Ladoux, S. Gabriele, Nir S. Gov: A minimal physical model for curvotaxis driven by curved protein complexes at the cell's leading edge. Proceedings of the National Academy of Sciences USA (PNAS), 121 (12): e2306818121, 2024.

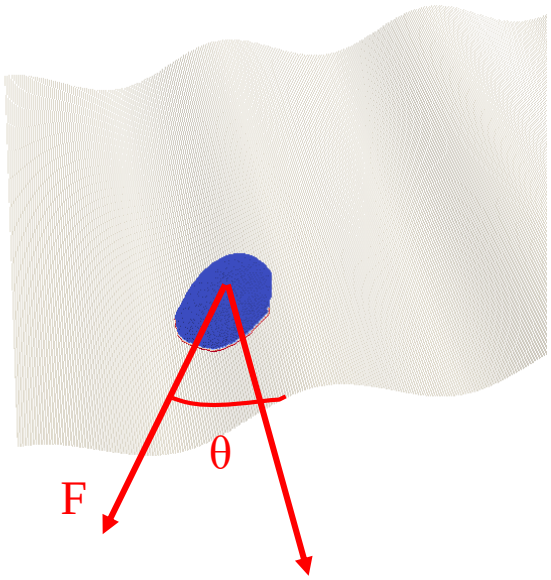




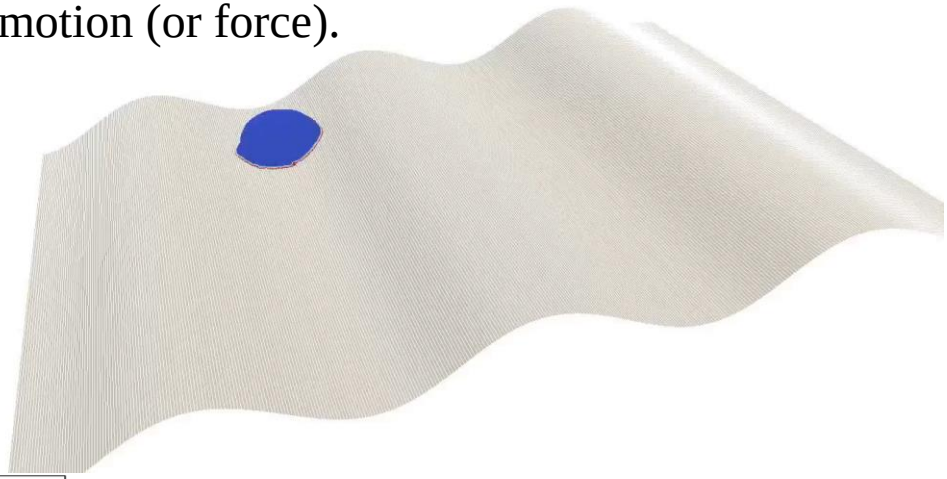
- Vesicles prefer to move along the axis in the minimum of the substrate.
- The minimum is energetically favorable compared to the maximum.
- Dictyostelium discoideum (D.d.) Cells prefer to stay on the grooves.

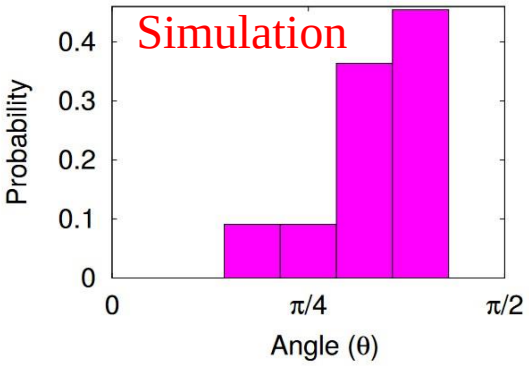
Comparison with experiments: Cells cross the ridges at higher angles

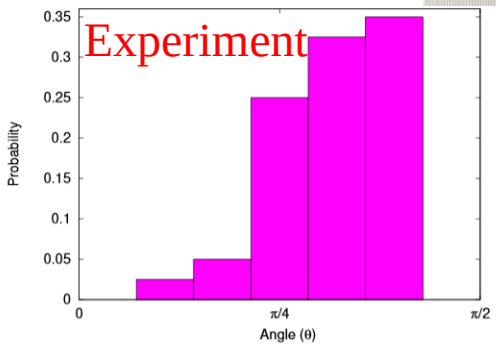
R. K. Sadhu, M. Luciano, W. Xi, C. Martinez-Torres, M. Schröder, C. Blum, M. Tarantola, S. Villa, S. Penič, A. Iglič, C. Beta, O. Steinbock, E. Bodenschatz, B. Ladoux, S. Gabriele, Nir S. Gov: A minimal physical model for curvotaxis driven by curved protein complexes at the cell's leading edge. Proceedings of the National Academy of Sciences USA (PNAS), 121 (12): e2306818121, 2024.



- We define the angle of crossing the ridges (maxima) as the angle between the axial direction and the direction of motion (or force).







- We note that the vesicle cross at higher angles (more than $\pi/4$).
- The experimental data agrees well with the simulation.

Cells having larger size than sinusoidal wavelength: oscillations in cell speed

R. K. Sadhu, **M. Luciano**, W. Xi, C. Martinez-Torres, M. Schröder, C. Blum, M. Tarantola, S. Villa, S. Penič, A. Iglič, C. Beta, O. Steinbock, E. Bodenschatz, B. Ladoux, **S. Gabriele**, Nir S. Gov:
A minimal physical model for curvotaxis driven by curved protein complexes at the cell's leading edge.
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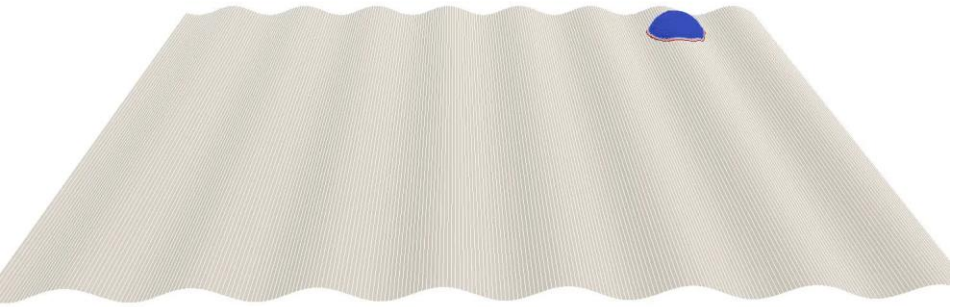
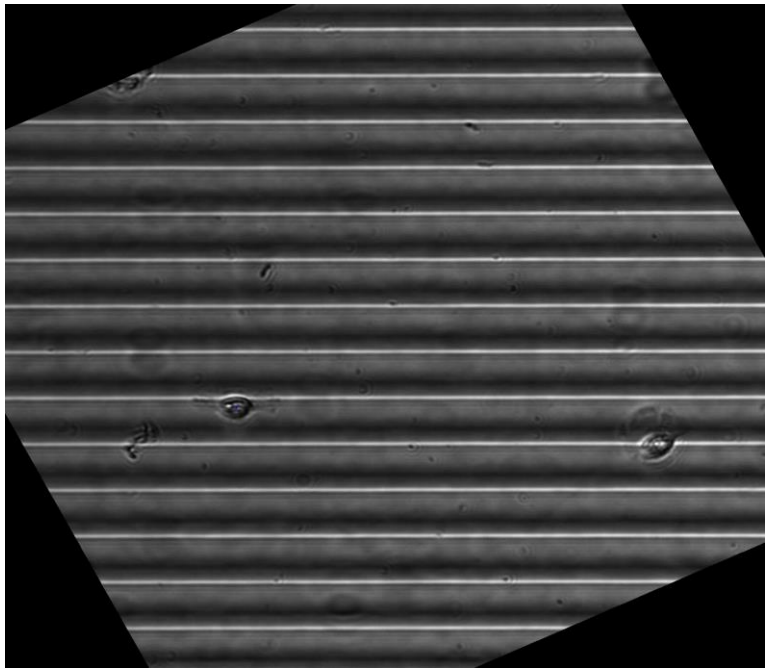
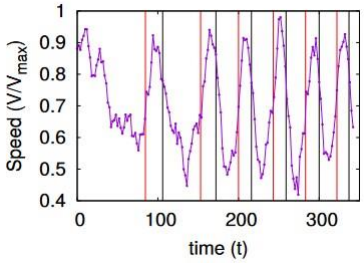
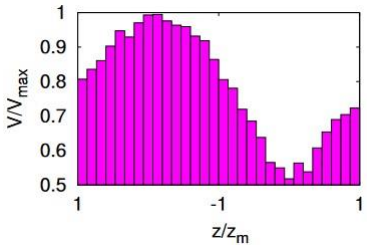
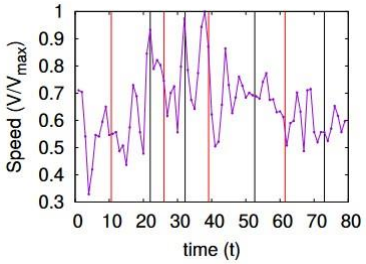
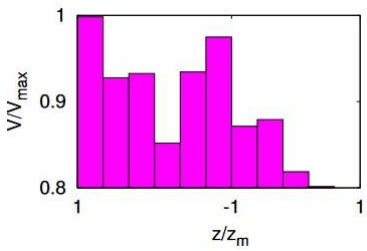








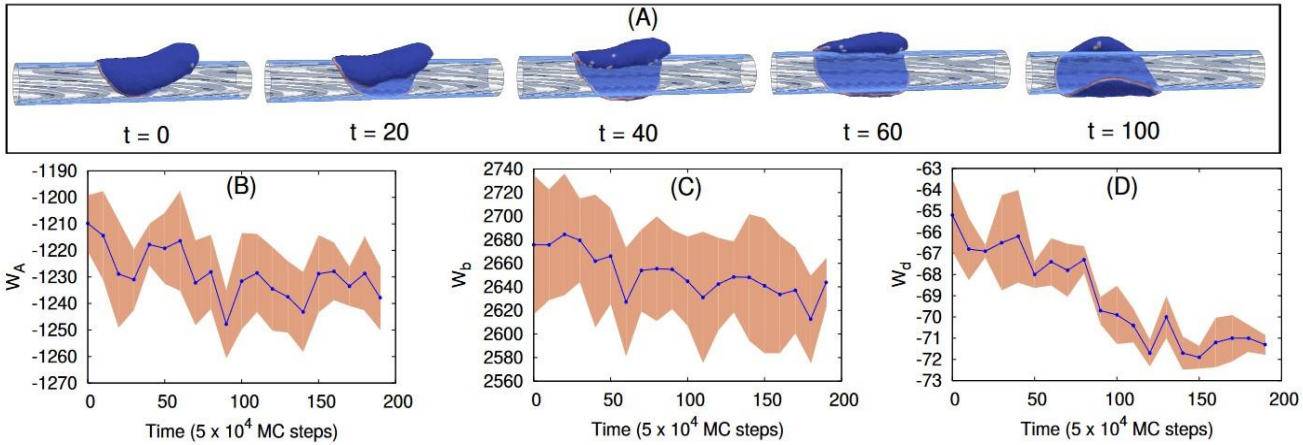


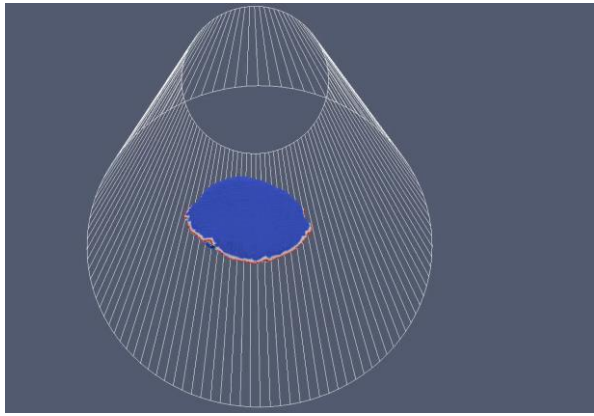


- Migrating karatocytes on sinusoidal substrate.
- Migration speed shows oscillatory behavior.
- The oscillation is periodic for simulation.

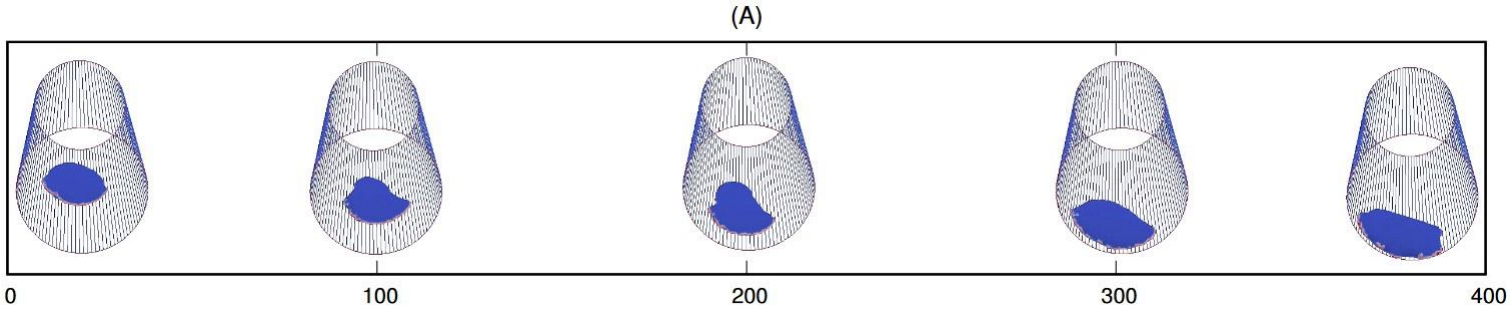
Vesicles migrating outside of cylindrical fibers and inside tube

R. K. Sadhu, M. Luciano, W. Xi, C. Martinez-Torres, M. Schröder, C. Blum, M. Tarantola, S. Villa, S. Penič, A. Iglič, C. Beta, O. Steinbock, E. Bodenschatz, B. Ladoux, S. Gabriele, Nir S. Gov: A minimal physical model for curvotaxis driven by curved protein complexes at the cell's leading edge. Proceedings of the National Academy of Sciences USA (PNAS), 121 (12): e2306818121, 2024.





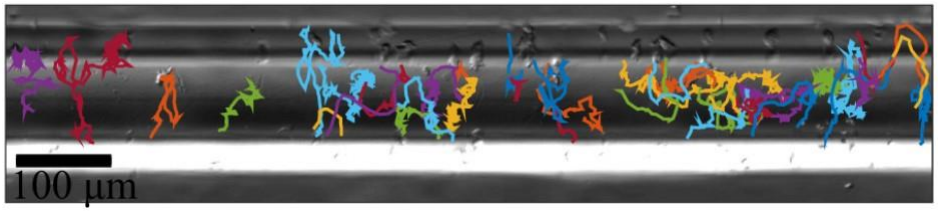
- Vesicle prefers to migrate circumferentially when outside of cylindrical substrate
- Vesicle inside cylinder moves axially.
- These behaviors can be explained by energy minimization.

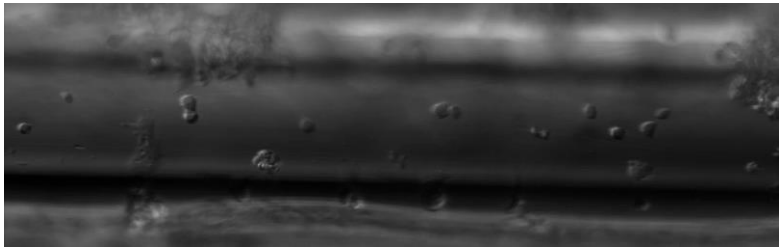


Experiments on fiber and tube qualitatively agree with our simulation

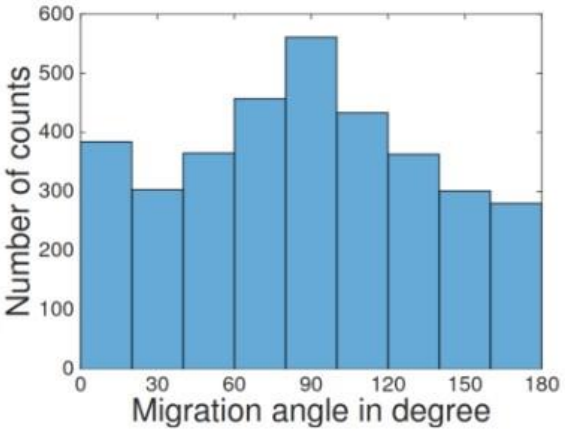
R. K. Sadhu, M. Luciano, **W. Xi**, C. Martinez-Torres, **M. Schröder**, **C. Blum**, M. Tarantola, S. Villa, S. Penič, A. Iglič, C. Beta, O. Steinbock, **E. Bodenschatz**, **B. Ladoux**, S. Gabriele, Nir S. Gov: A minimal physical model for curvotaxis driven by curved protein complexes at the cell's leading edge. Proceedings of the National Academy of Sciences USA (PNAS), 121 (12): e2306818121, 2024.

Dictyostelium discoideum (D.d.) migrating **outside** of an optical fiber



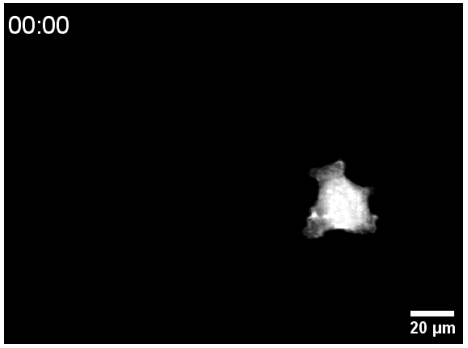


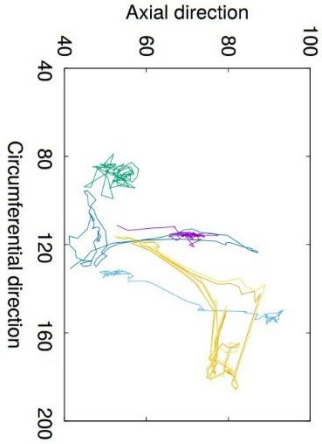
- **outside** of a fiber, Dictyostelium discoideum (D.d.) cells are preferable migrating circumferentially



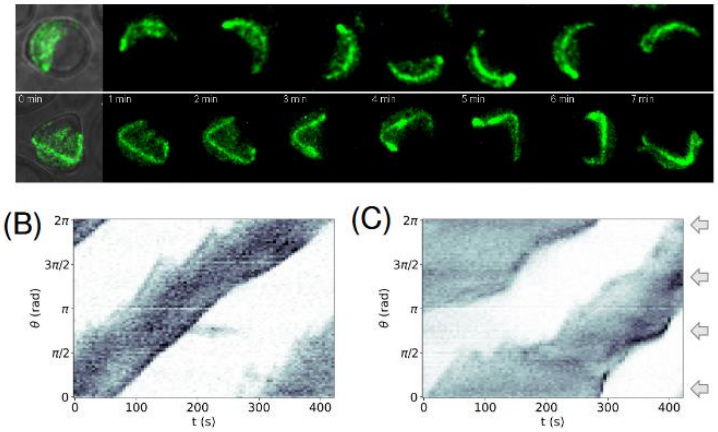
- **inside** the tube, MDCK cells prefer to migrate along the axis

MDCK cells migrating inside tube

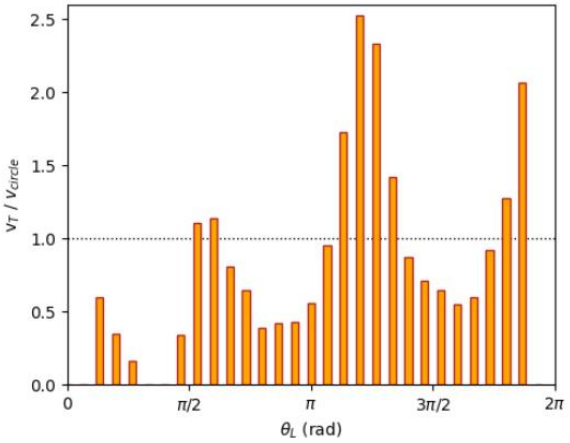


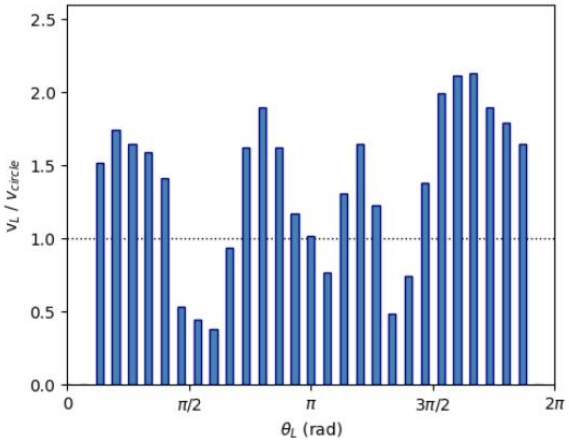


Cells migrating on micropillars of circular and triangular cross-section

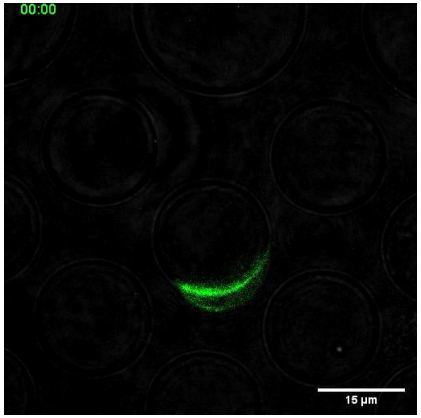


triangular cross-section



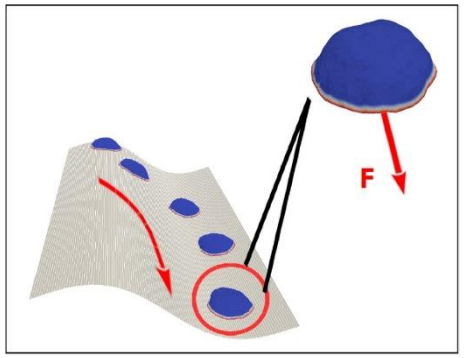


- Dictyostelium discoideum cells are shown to **rotate persistently on pillars with circular cross-section**.
- On pillars with **triangular cross-section, the cells slow down periodically whenever they cross the higher curvature corners**.
- In our **simulation**, we se fibers with elliptical cross-sections and observe **similar behavior.**



R. K. Sadhu, M. Luciano, W. Xi, **C. Martinez-Torres**, M. Schröder, C. Blum, M. Tarantola, S. Villa, S. Penič, A. Iglič, **C. Beta**, O. Steinbock, E. Bodenschatz, B. Ladoux, S. Gabriele, Nir S. Gov: A minimal physical model for curvotaxis driven by curved protein complexes at the cell's leading edge. Proceedings of the National Academy of Sciences USA (PNAS), 121 (12): e2306818121, 2024.

Summary



- We study the spreading and migration of cells on cylindrical and sinusoidal surfaces using a “minimal cell” model.
- For sinusoidal surfaces, the minima (grooves) are more favorable energetically than the maxima (ridges).
- On the minima of sinusoidal surface, the vesicle moves along the axis, while, when they transit from one to another minima, they cross the ridges at higher angles.
- For cylindrical substrate, the vesicle align or migrates circumferentially outside of a cylinder, while axially inside.
- Most of our results agree well with the experiments, and our model predictions are successfully verified with new experiments.
- Some aspects of “curvotaxis” can be explained using physical principles.

Theoretical model for the origin of coiling of cellular protrusions around fibers

nature communications

Article

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Experimental and theoretical model for the origin of coiling of cellular protrusions around fibers

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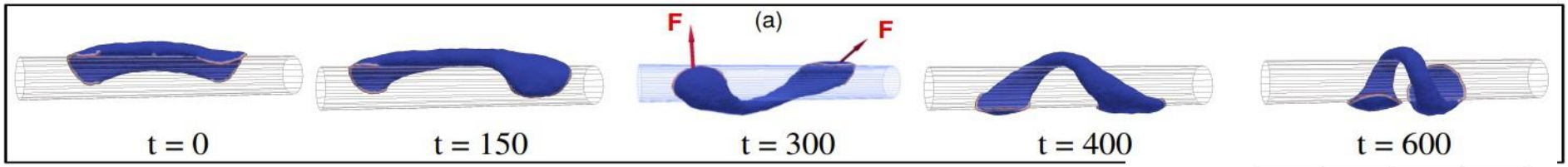
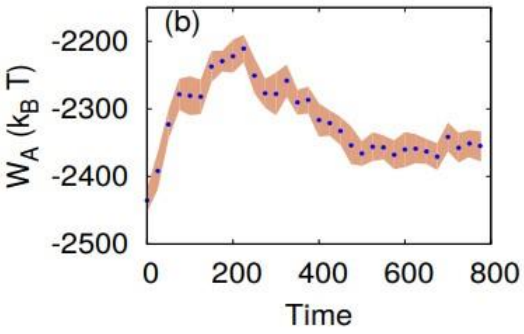
Raj Kumar Sadhu[1,8], Christian Hernandez-Padilla[2], Yael Eshed Eisenbach[3], Samo Penič[4], Lixia Zhang[5], Harshad D. Vishwasrao[5], Bahareh Behkam[2], Konstantinos Konstantopoulos[6], Hari Shroff[5,7], Aleš Iglič[4], Elior Peles[3], Amrinder S. Nain[2] & Nir S. Gov[1]

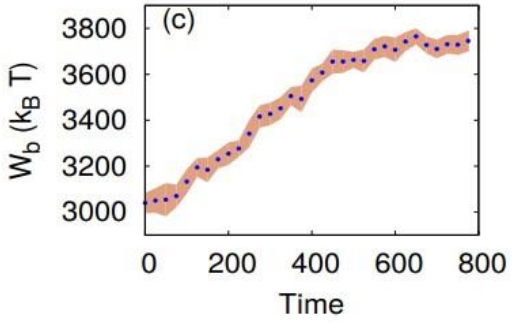
Protrusions at the leading-edge of a cell play an important role in sensing the extracellular cues during cellular spreading and motility. Recent studies provided indications that these protrusions wrap (coil) around the extracellular fibers. However, the physics of this coiling process, and the mechanisms that drive it, are not well understood. We present a combined theoretical and experimental study of the coiling of cellular protrusions on fibers of different geometry. Our theoretical model describes membrane protrusions that are produced by curved membrane proteins that recruit the protrusive forces of actin polymerization, and identifies the role of bending and adhesion energies in orienting the leading-edges of the protrusions along the azimuthal (coiling) direction. Our model predicts that the cell's leading-edge coils on fibers with circular cross-section (above some critical radius), but the coiling ceases for flattened fibers of highly elliptical cross-section. These predictions are verified by 3D visualization and quantitation of coiling on suspended fibers using Dual-View light-sheet microscopy (diSPIM). Overall, we provide a theoretical framework, supported by experiments, which explains the physical origin of the coiling phenomenon.

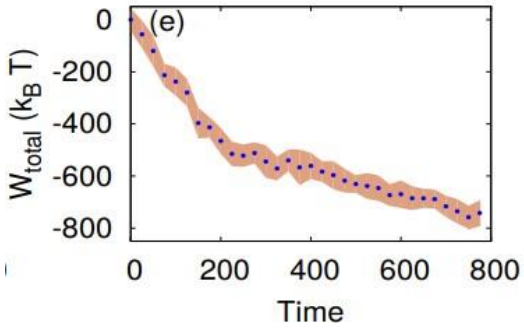
Circumferential orientation is energetically favourable

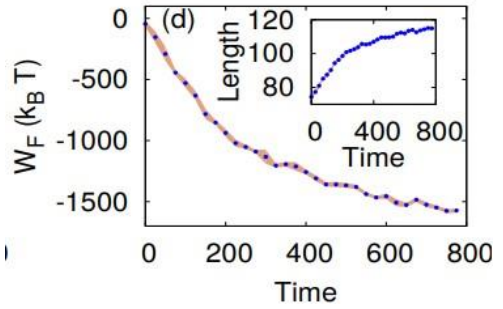
R. K. Kumar Sadhu, C. Hernandez-Padilla, Y.E. Eisenbach, S. Penič, L. Zhang, H.D. Vishwasrao, B. Behkam, K. Konstantopoulos, H. Shroff, A. Iglič, E. Peles, A. S. Nain, N. S. Gov: Experimental and theoretical model for the origin of coiling of cellular protrusions around fibers. Nature Communications 14: 5612, 2023.







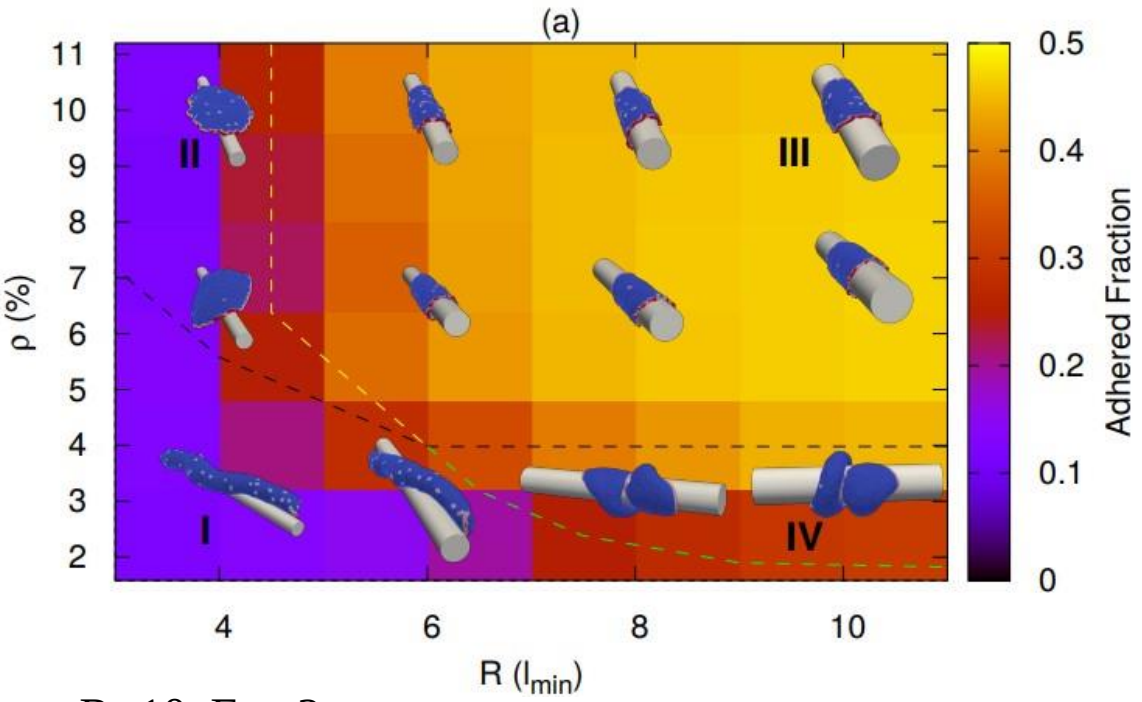




While the coiling is driven by active work done, initial reorientation is driven by adhesion and bending energies

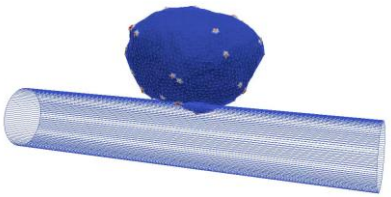
- Two-arc shape placed axially reorients along circumferential direction.
- In the process of coiling, the bending energy increases, while the adhesion energy shows non-monotonic variation.
- The active energy decreases throughout.

Vesicle spreading on fiber: phase diagram in the ‘protein-density’-’fiber radius’ plane



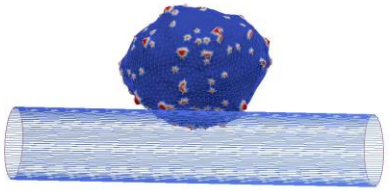
- For small R, the vesicle does not spread or coil.
- For large R, the vesicle coils for small ρ.
- The vesicle can also form axially rotating crescent

R=10, E_{ad}=2, ρ=2.4%, F=2



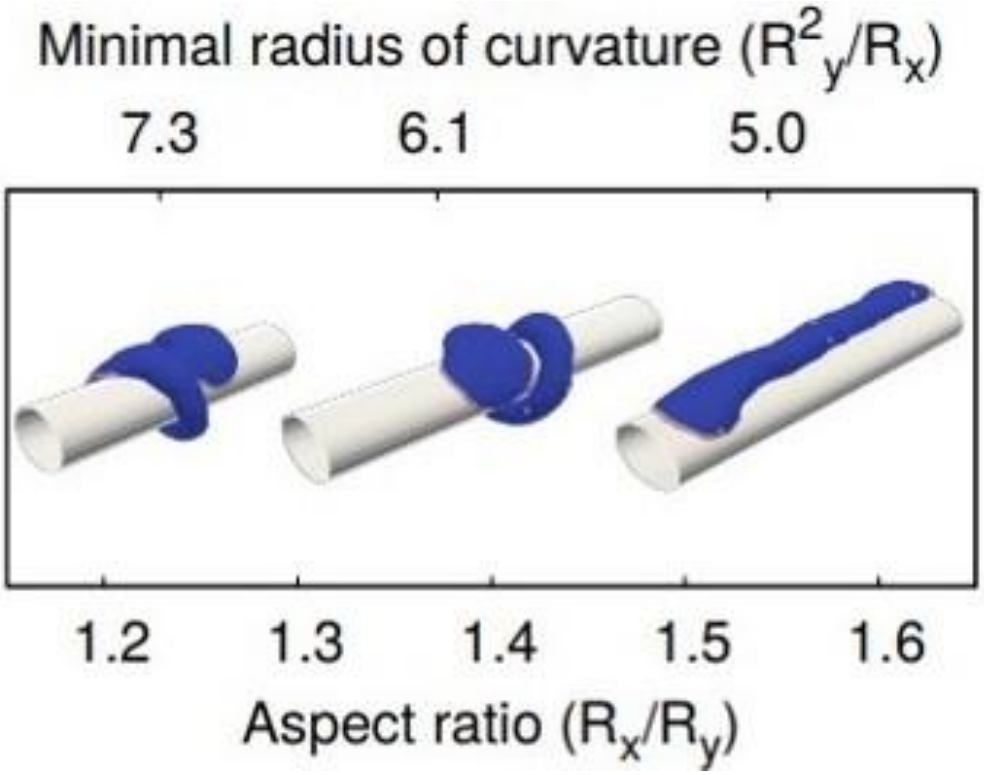
R=10, E_{ad}=1, ρ=2.4%, F=2

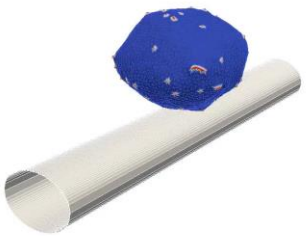
R=10, E_{ad}=1, ρ=3.2%, F=2



R. K. Kumar Sadhu, C. Hernandez-Padilla, Y.E. Eisenbach, S. Penič, L. Zhang, H.D. Vishwasrao, B. Behkam, K. Konstantopoulos, H. Shroff, A. Iglič, E. Peles, A. S. Nain, N. S. Gov: Experimental and theoretical model for the origin of coiling of cellular protrusions around fibers. Nature Communications 14: 5612, 2023.

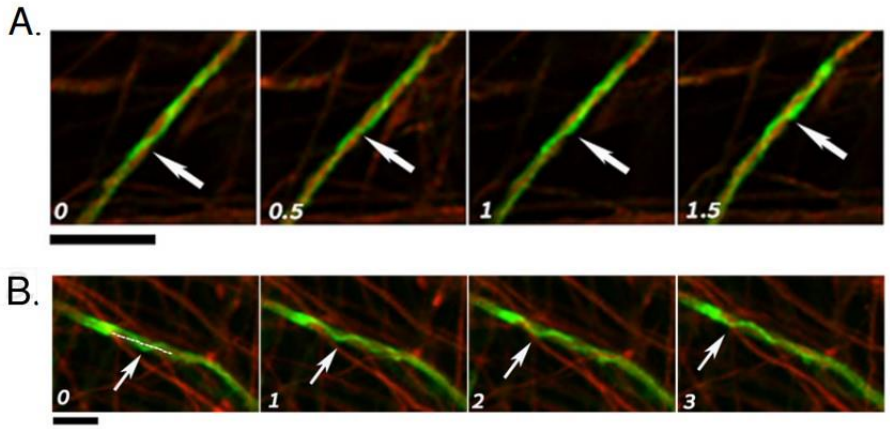
Coiling is not possible for a fiber with flat (elliptical) cross-section with large aspect ratio (Sadhu et al., Nature Communications 14: 5612, 2023)

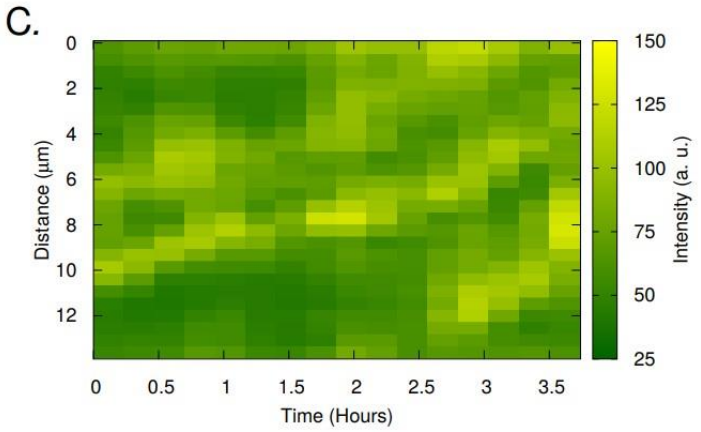




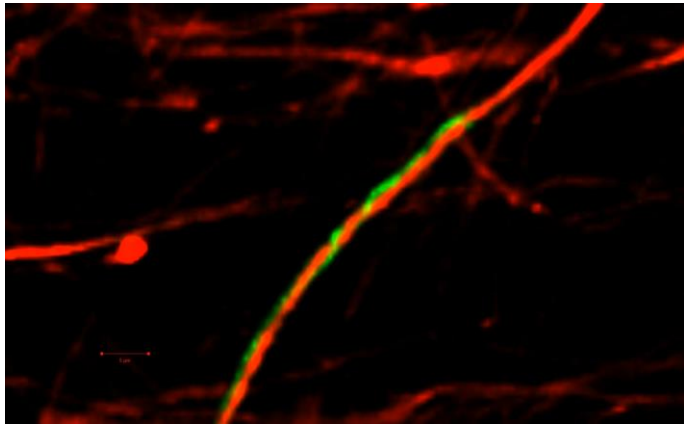
Imaging of early-stage myelination process

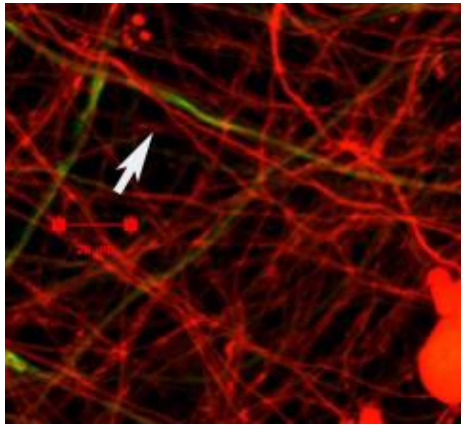
R. K. Kumar Sadhu, C. Hernandez-Padilla, **Y.E. Eisenbach**, S. Penič, L. Zhang, H.D. Vishwasrao, B. Behkam, K. Konstantopoulos, H. Shroff, A. Iglič, **E. Peles**, A. S. Nain, N. S. Gov: Experimental and theoretical model for the origin of coiling of cellular protrusions around fibers. Nature Comm. 14: 5612, 2023.





For the generation of Schwann cell-dorsal root ganglia (DRG) neuron myelinating cultures we used mice expressing S-MAG-GF.

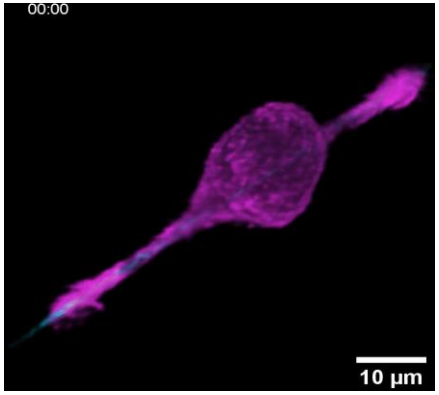


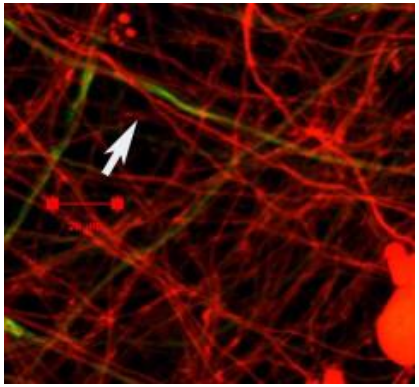


- Early stage myelination shows similar coiling around axons.
- Myelination is known to occur on axons for which diameter exceeds a critical threshold.

Summary

Sadhu et al., Nature Communications 14: 5612, 2023





- We study the coiling of cellular protrusions around cylindrical fibers using experimental and theoretical technique.
- The leading protrusions found to wrap around the fiber, and also on axons.
- Coiling of protrusions seems to cease when the fiber becomes flat.
- Our model explains the coiling of cellular protrusions around fibers and axons, and also explains the disappearance of coiling on flat fibers.
- Our work shows that many biological processes could be explained using simple physical principals.

Thank you !