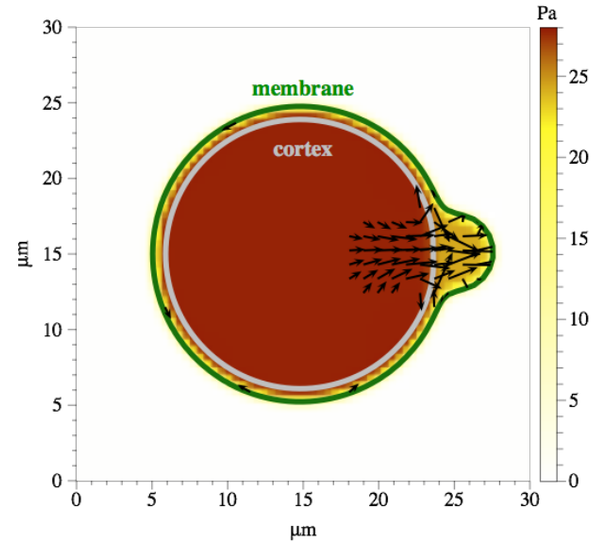
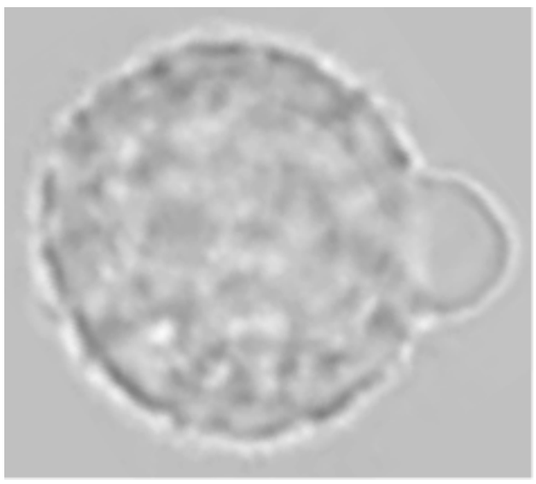
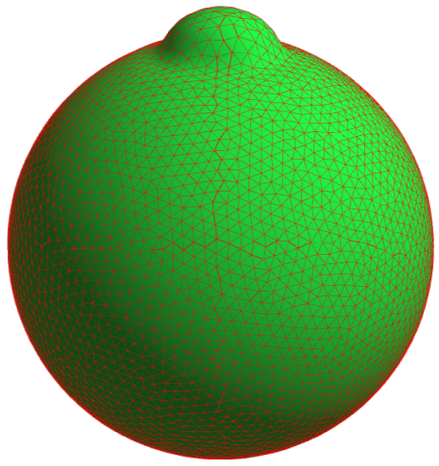


3D Computational Modeling of Bleb Initiation Dynamics

Wanda Strychalski

Department of Mathematics, Applied Mathematics, and Statistics
Case Western Reserve University
Cleveland, OH

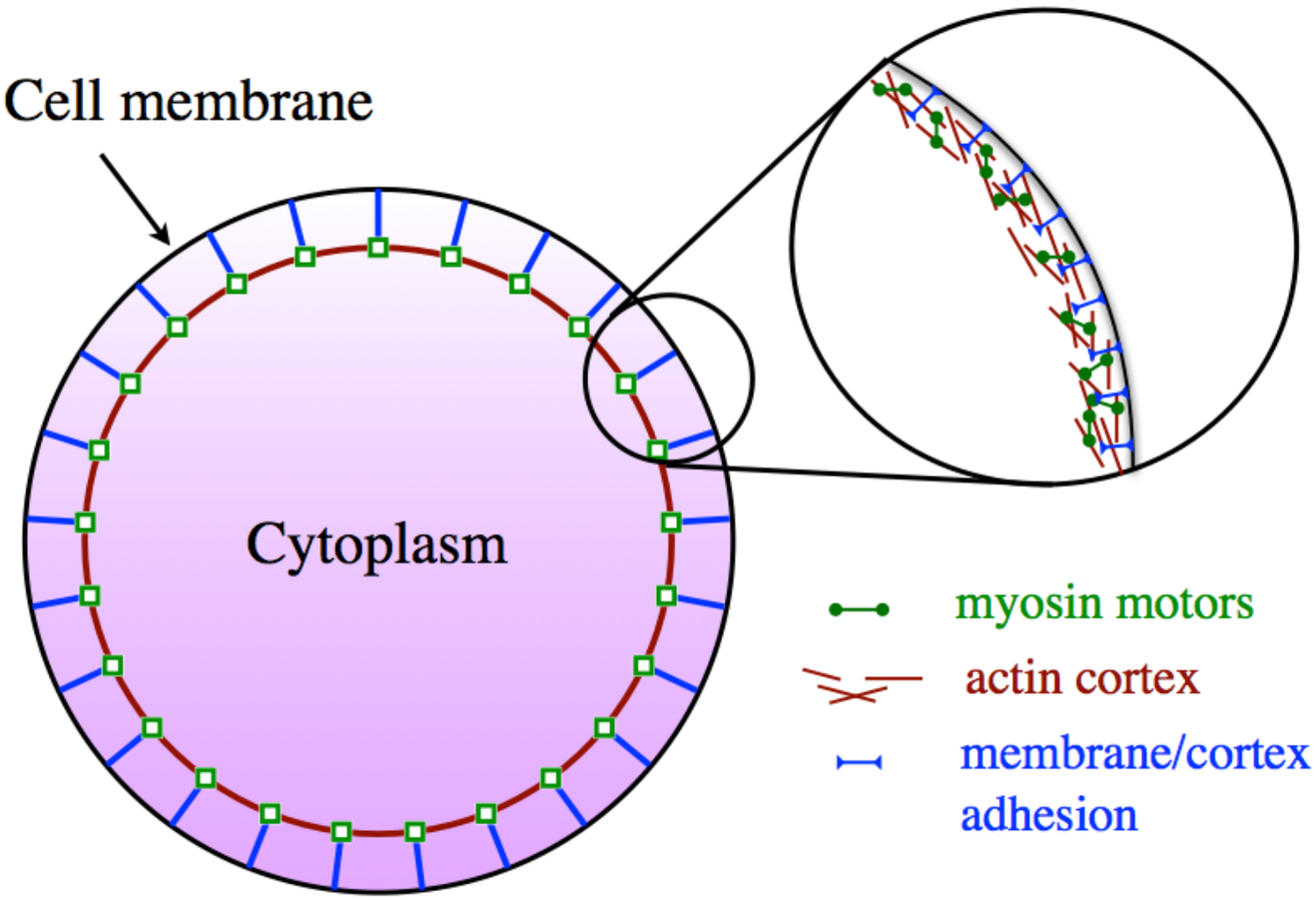




Components of the Cell

Actin Cytoskeleton

- Gives the cell mechanical support
- Scaffold for molecular motors
- Actin fiber is composed of polymers
- Cortex (specialized layer of cytoskeleton)



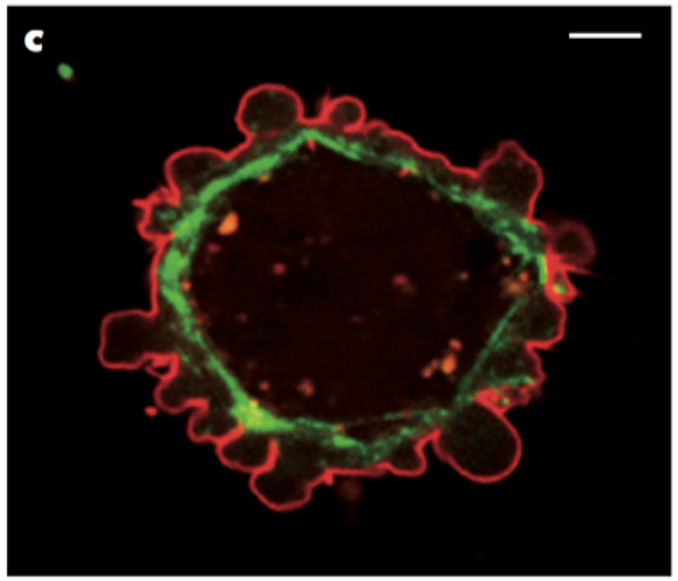
Myosin Molecular Motors

- Slide actin filaments with respect to each other
- Source of tension

Cytoplasm

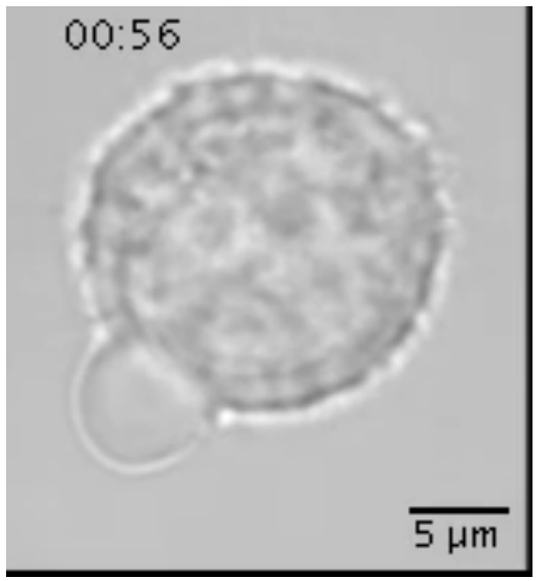
- Mixture of water, proteins, organelles, internal cytoskeleton
- Liquid part called cytosol

Blebs



Charras, G., and Paluch, E., *Blebs lead the way: how to migrate without lamellipodia*, Nat. Rev. Mol. Cell Biol., 9 (2008), 730-6.

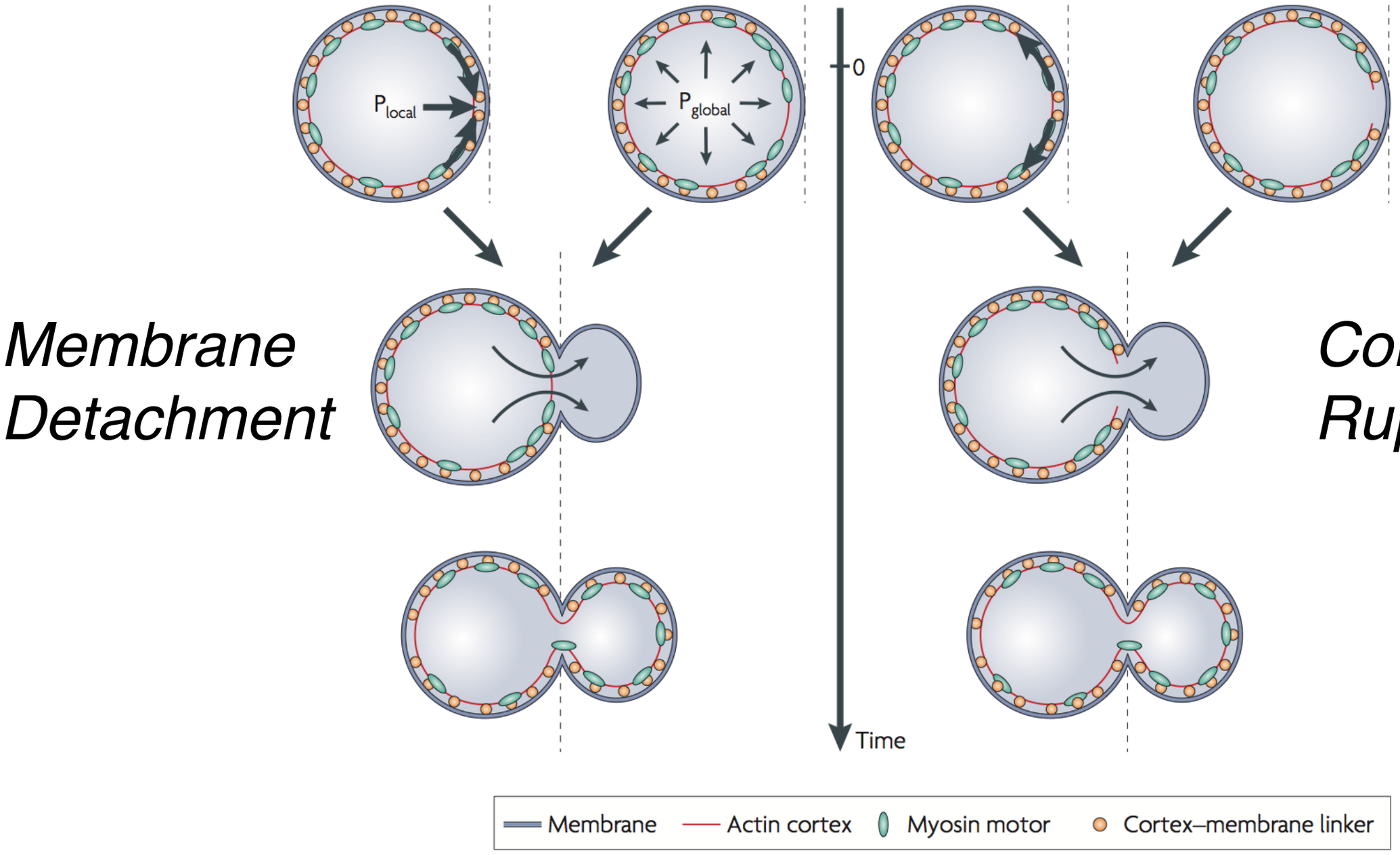
- Spherical membrane protrusions
- Membrane and cortex separate
  - disruption in actin cortex or membrane/cortex adhesion proteins



Tinevez, J. Y. et. al., *Role of cortical tension in bleb growth*, P. Natl. Acad. Sci. USA, 106 (2009), 18581-6.

- Cytosolic flow drives membrane expansion (~30 s)
  - cortex reforms and bleb contracts (1-2 minutes)

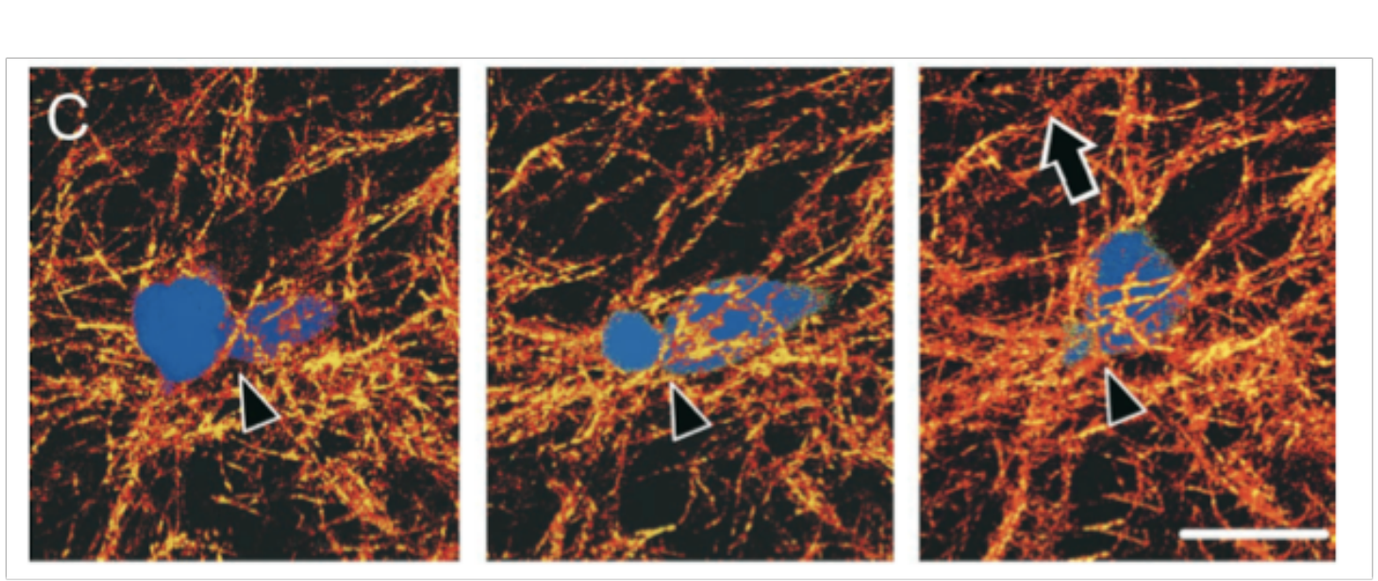
Bleb Initiation



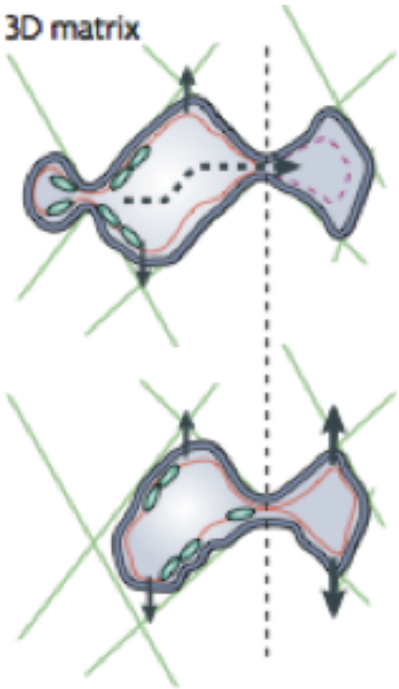
Charras, G., and Paluch, E., ***Blebs lead the way: how to migrate without lamellipodia***, Nat. Rev. Mol. Cell Bio., 9 (2008), 730-6.

Motivation

- Blebs observed during cytokinesis, and apoptosis, cell spreading, and cell migration
  - Used as leading edge protrusion during motility

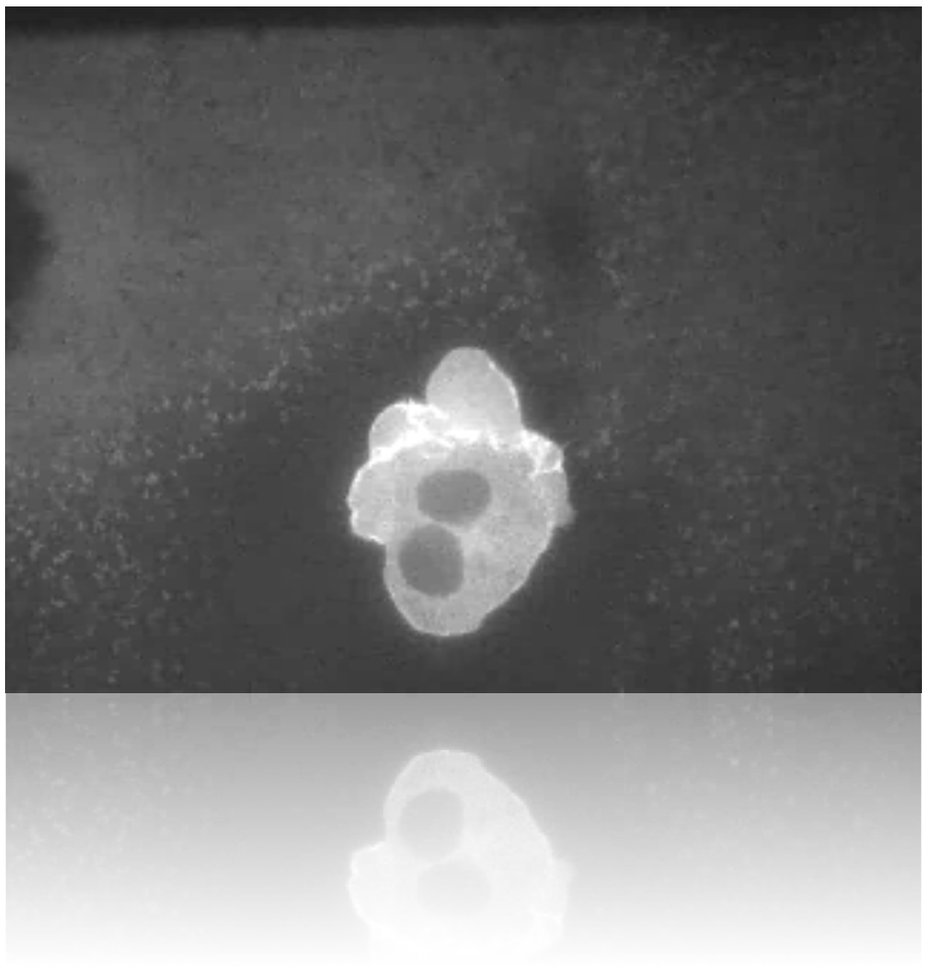


Wolf, K. et. al. *Compensation mechanism in tumor cell migration: mesenchymal-amoeboid transition after blocking of pericellular proteolysis*. J. Cell Biol. (2003) 160, 267-277.



Charras, G., and Paluch, E., *Blebs lead the way: how to migrate without lamellipodia*, Nat. Rev. Mol. Cell Biol., 9 (2008), 730-6.

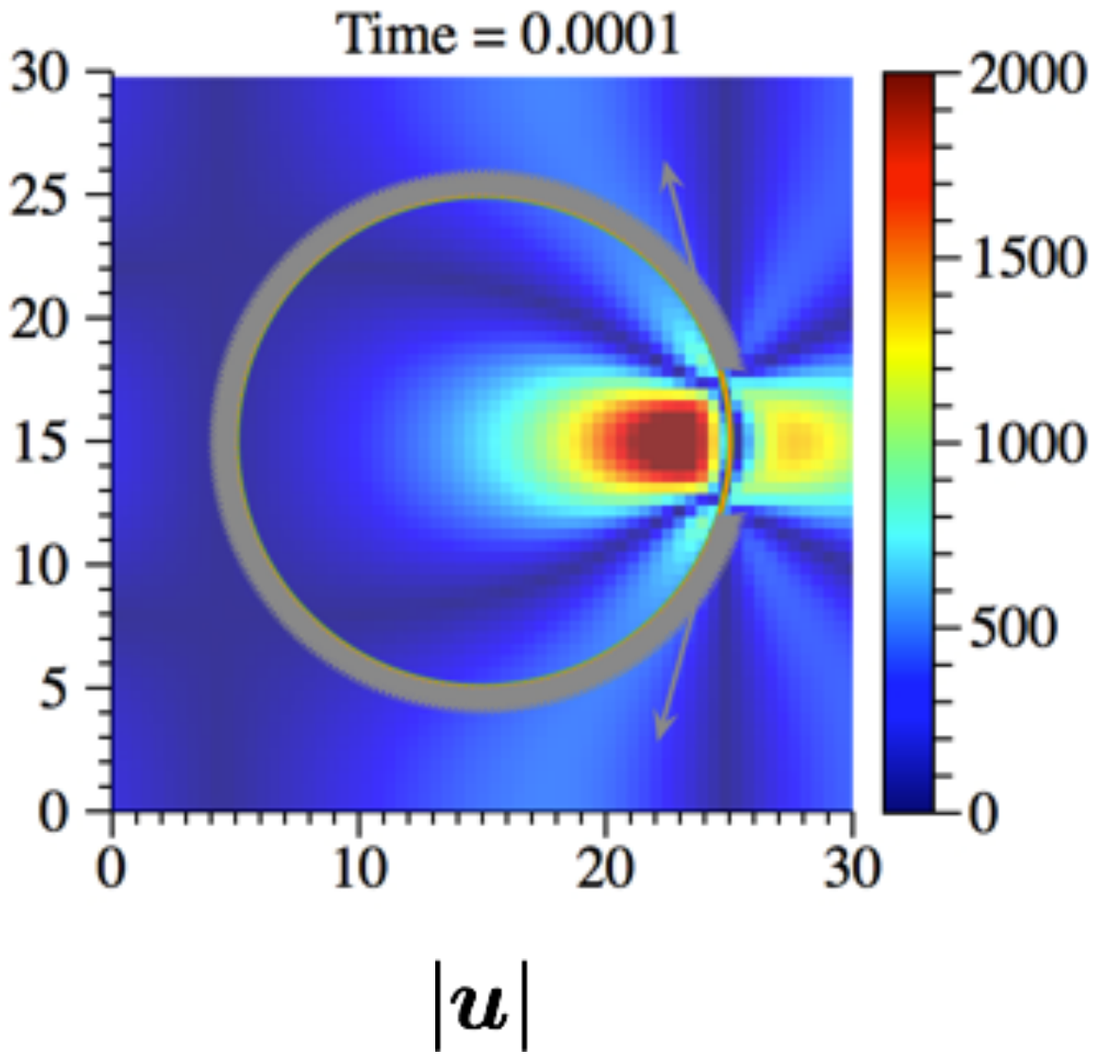
Blebbing Dynamics



- Goal is to investigate blebbing-based cell motility
- First, we want to develop a deep understanding of blebbing dynamics
  - Focus on cytoplasmic rheology and bleb initiation

Fink, R.D. Fundulus deep cells in yolk sac of killifish embryo, http://www.mtholyoke.edu/courses/rfink/Researchvideopages/rvideo3.htm

Cortical Rupture

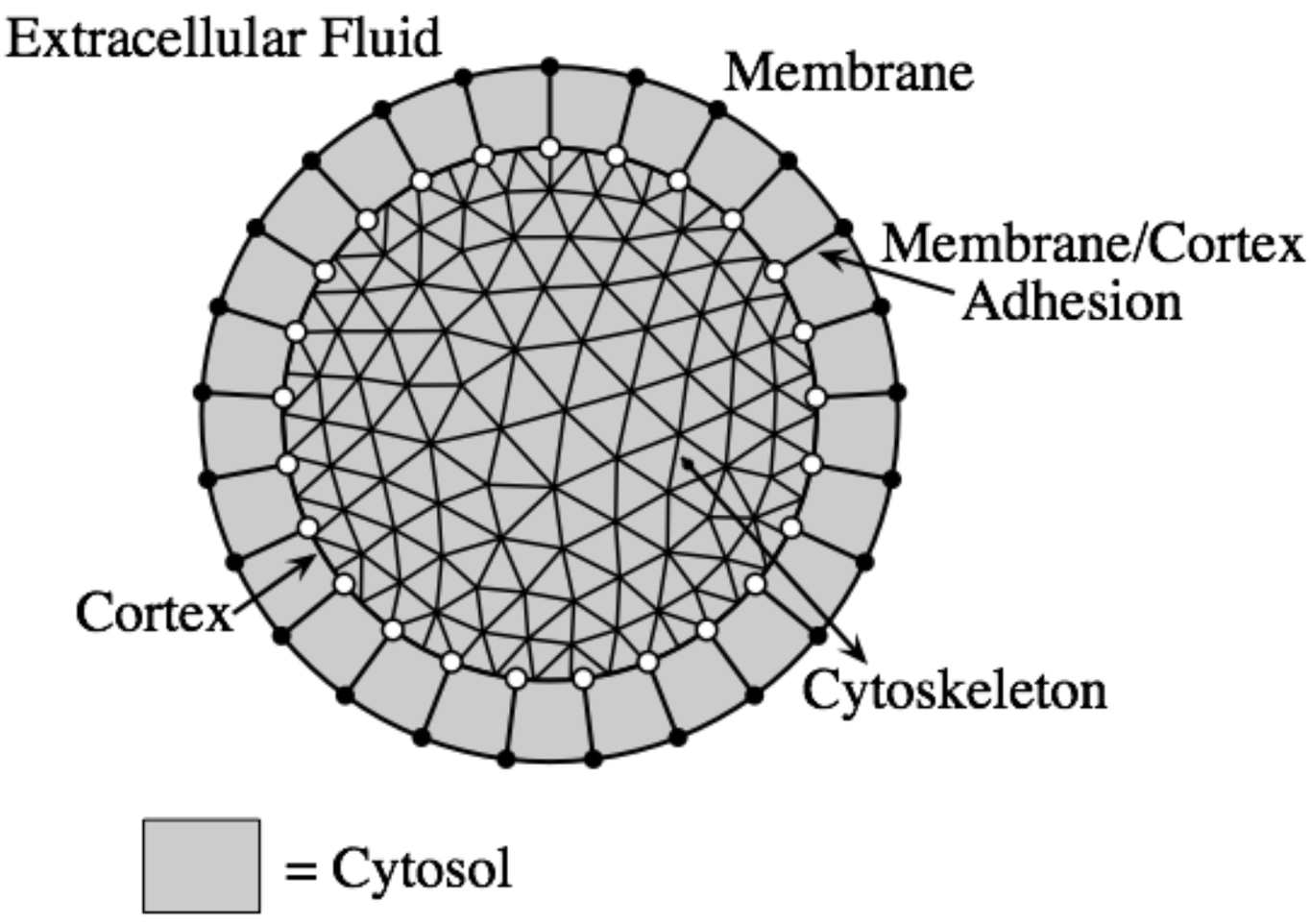


- Can’t simulate cortical ablation in 2D model
  - viscous fluid cytoplasm
- Large tangential forces on the cortex
- Really need a 3D model to take into account the line tension around the bleb neck

Questions

- How does the mechanism of bleb initiation affect cell shape and expansion dynamics?
- Do results from 2D model hold in 3D?
  - How does intracellular pressure vary over space and time?
  - How do the material properties of the cytoplasm affect blebbing dynamics?
    - Viscosity, permeability, elasticity

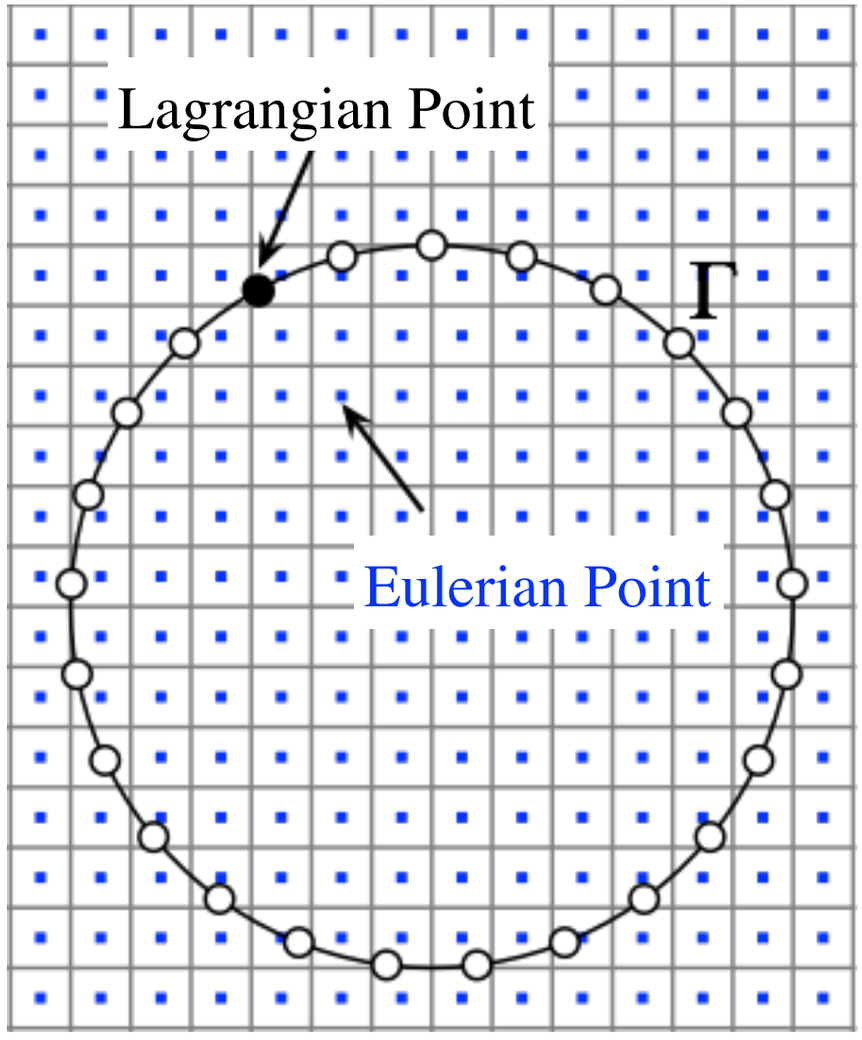
Model Schematic



- Membrane, cortex, and cytoskeleton are elastic structures immersed in fluid
- Fluid obeys Stokes' equations
- Cortex and cytoskeleton are elastic, permeable materials
- Membrane-cortex adhesion modeled by discrete elastic springs

Immersed Boundary Method

- Model put into IB framework
- Method for simulating coupled fluid/structure interaction



<u>Lagrangian variables</u>
Membrane, cortex, cytoskeleton

<u>Eulerian variables</u>
Cytosolic velocity, pressure

Peskin, C.S. ***Numerical analysis of blood flow in the heart,*** J. Comput. Phys. 25 (3) (1977) 220–252.

Immersed Boundary Method

1. Compute immersed boundary forces at t^{n+1}

   using $\Gamma^n = \boldsymbol{X}^n(s,t)$.

2. Spread the forces onto nearby Eulerian points:

$$\boldsymbol{f}^{n+1}(\boldsymbol{x},t) = \int_\Gamma \boldsymbol{F}^{n+1}\left(\boldsymbol{X}(s,t),t\right)\delta\left(\boldsymbol{x}-\boldsymbol{X}(s,t)\right)ds$$

3. Solve Stokes equations with $\boldsymbol{f}^{n+1}$ to obtain

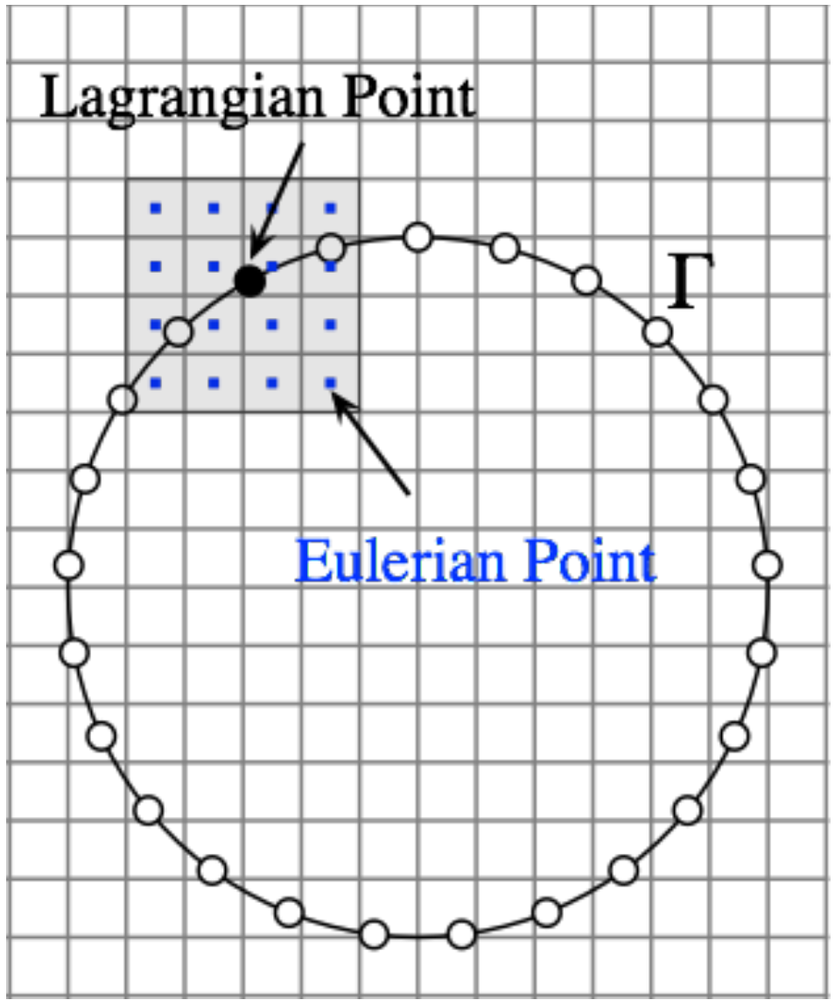
   $\boldsymbol{u}^{n+1}, p^{n+1}$.

4. Interpolate the velocity to boundary points:

$$\boldsymbol{U}\left(\boldsymbol{X}(s,t),t\right) = \int_\Gamma \boldsymbol{u}^{n+1}(\boldsymbol{x},t)\delta\left(\boldsymbol{x}-\boldsymbol{X}(s,t)\right)d\boldsymbol{x}$$

5. Update the boundary points:

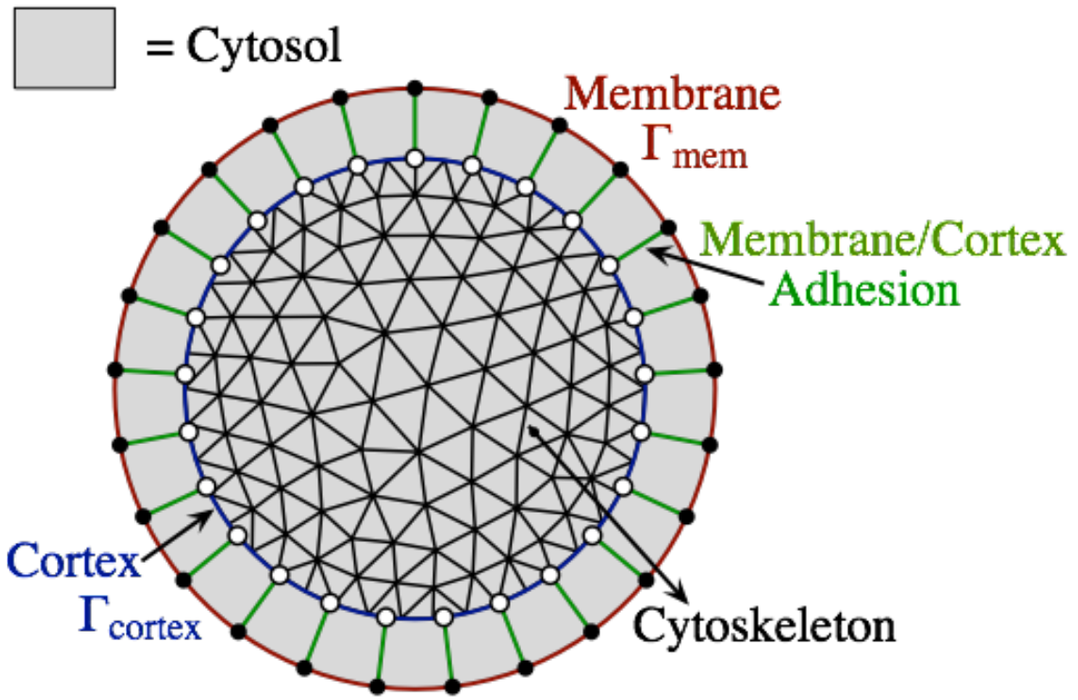
$$\boldsymbol{X}(s,t) = \boldsymbol{X}^n(s,t) + \Delta t\,\boldsymbol{U}^{n+1}\left(\boldsymbol{X}(s,t),t\right)$$



Fluid Equations

$$\mu \Delta \boldsymbol{u} - \nabla p + \boldsymbol{f}^{\text{mem}}_{\text{elastic}} + \boldsymbol{f}^{\text{mem/cortex}}_{\text{adhesion}} + \boldsymbol{f}^{\text{cortex}}_{\text{drag}} + \boldsymbol{f}^{\text{cyto}}_{\text{drag}} = \mathbf{0}$$

$$\nabla \cdot \boldsymbol{u} = 0$$



Drag Forces:

- due to relative motion of the cortex or cytoskeleton

$$\boldsymbol{F}^{\text{cortex}}_{\text{drag}} = \xi \left(\mathcal{S}^* \boldsymbol{u} - \boldsymbol{U}_{\text{cortex}} \right)$$

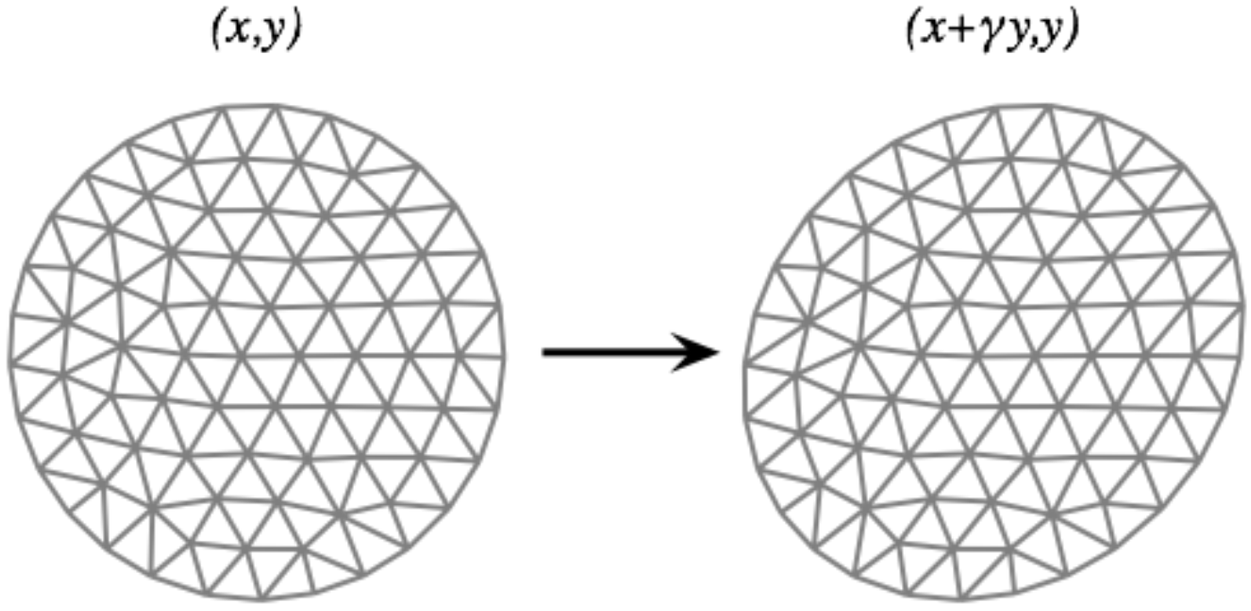
$$\boldsymbol{F}^{\text{cyto}}_{\text{drag}} = \frac{\mu}{\kappa} \left(\mathcal{S}^* \boldsymbol{u} - \boldsymbol{U}_{\text{cyto}} \right)$$

Force balances on cortex and cytoskeleton:

$$\boldsymbol{F}^{\text{cortex}}_{\text{drag}} + \boldsymbol{F}^{\text{cortex}}_{\text{elastic}} + \boldsymbol{F}^{\text{cortex/mem}}_{\text{adhesion}} + \boldsymbol{F}^{\text{cortex/cyto}}_{\text{adhesion}} = \mathbf{0}$$

$$\boldsymbol{F}^{\text{cyto}}_{\text{drag}} + \boldsymbol{F}^{\text{cyto}}_{\text{elastic}} + \boldsymbol{F}^{\text{cyto/cortex}}_{\text{adhesion}} = \mathbf{0}$$

Computing Elasticity



- Shell elasticity for membrane and cortex
- Lattice spring model for cortex

$$\boldsymbol{F}_i\, dA_i = \sum_j k_{ij}^{\text{cortex}} \left(\frac{\left\| \boldsymbol{X}_i^{\text{cortex}} - \boldsymbol{X}_j^{\text{cortex}} \right\|}{d\ell_{ij}} \right) \frac{\boldsymbol{X}_i^{\text{cortex}} - \boldsymbol{X}_j^{\text{cortex}}}{\left\| \boldsymbol{X}_i^{\text{cortex}} - \boldsymbol{X}_j^{\text{cortex}} \right\|}$$

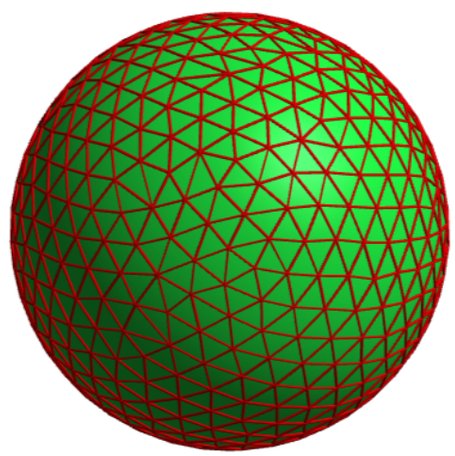
- Springs connecting membrane and cortex

$$\boldsymbol{F}_{\text{adh}}^{\text{mem/cortex}} = k_{\text{adh}}^{\text{mem/cortex}} \left(\left\| \boldsymbol{X}_{\text{mem}} - \boldsymbol{X}_{\text{cortex}} \right\| \right) \frac{\boldsymbol{X}_{\text{mem}} - \boldsymbol{X}_{\text{cortex}}}{\left\| \boldsymbol{X}_{\text{mem}} - \boldsymbol{X}_{\text{cortex}} \right\|}$$

Computing Elasticity

- Follow variational approach with assumption that deformation map is linear
  - For infinitely thin 2D membrane

    T. G. Fai et al. *Immersed boundary method for variable viscosity and variable density problems using fast constant-coefficient linear solvers I: Numerical method and results*, SIAM J. Sci. Comput. 35 (5) (2013) B1132–B1161.



  - For 3D cytoskeleton
    - Elasticity

      D. Devendran and C. S. Peskin, *An immersed boundary energy-based method for incompressible viscoelasticity*, J. Comput. Phys. 231 (2012) 4613 - 4642.

    - Permeability

      W. Strychalski et al., *A poroelastic immersed boundary method with applications to cell biology*, J. Comput. Phys. 282 (2015) 77-97.
  - Assume membrane and cytoskeleton are neo-Hookean elastic materials

Numerical Methods

- Observe spurious velocities when simulating in biologically relevant parameter regime

- Use spectral approximation to delta function using the nonuniform fast Fourier transform (NUFFT) in spreading and interpolation operators

  R. D. Guy et al. ***Spectral approximation to the discrete delta function in the immersed boundary method***, in preparation (2018)

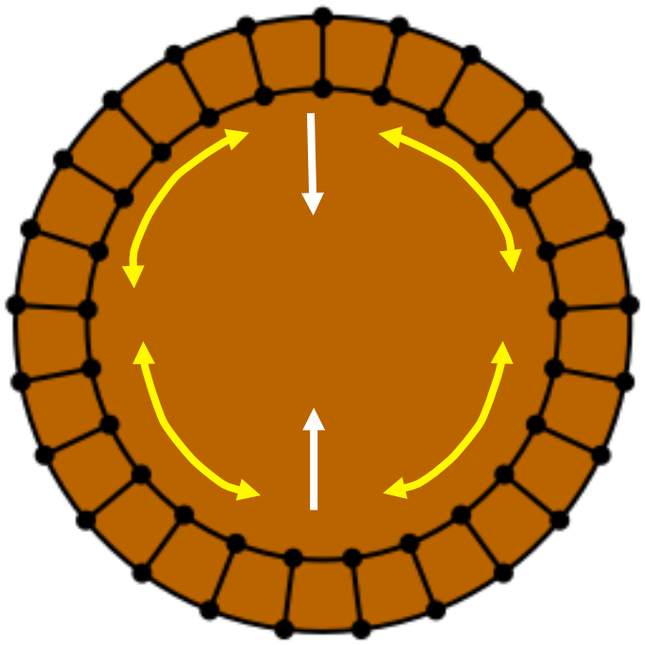
- Developed more accurate method to compute Lagrangian forces densities on the cell membrane

  - Use spherical harmonic basis functions to represent membrane

    O. Maxian et al. ***A continuous energy-based immersed boundary method for elastic shells***, J. Comput. Phys. 371 (2018) 333-362.

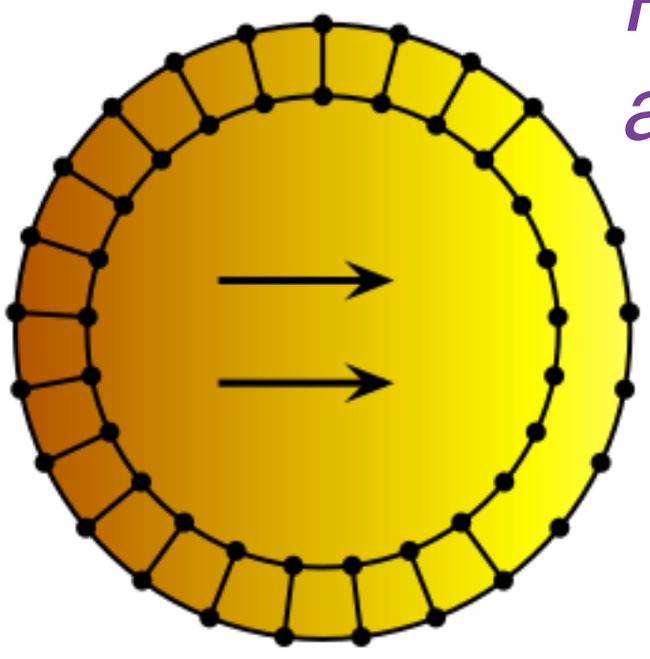
Bleb Initiation

Pressure

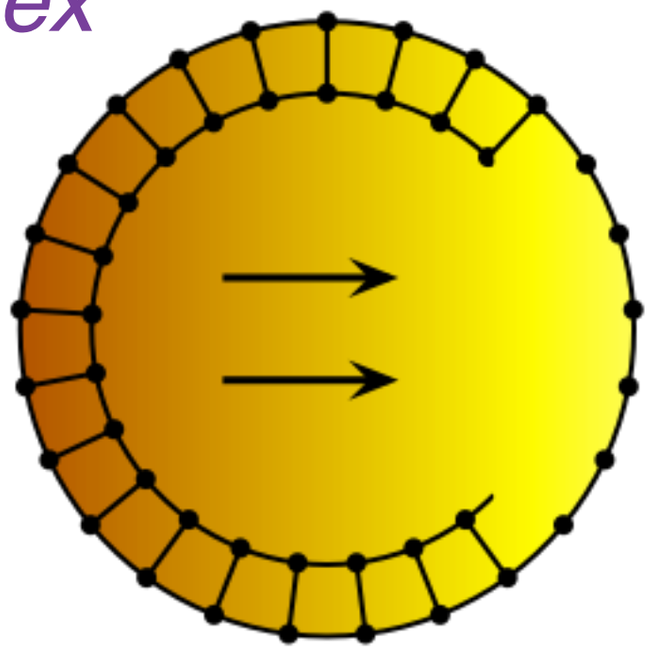


- Actomyosin contractility on cortex generates tension (yellow)
- Tension leads to normal force
- Transmitted to membrane through adhesion, resulting in intracellular pressure

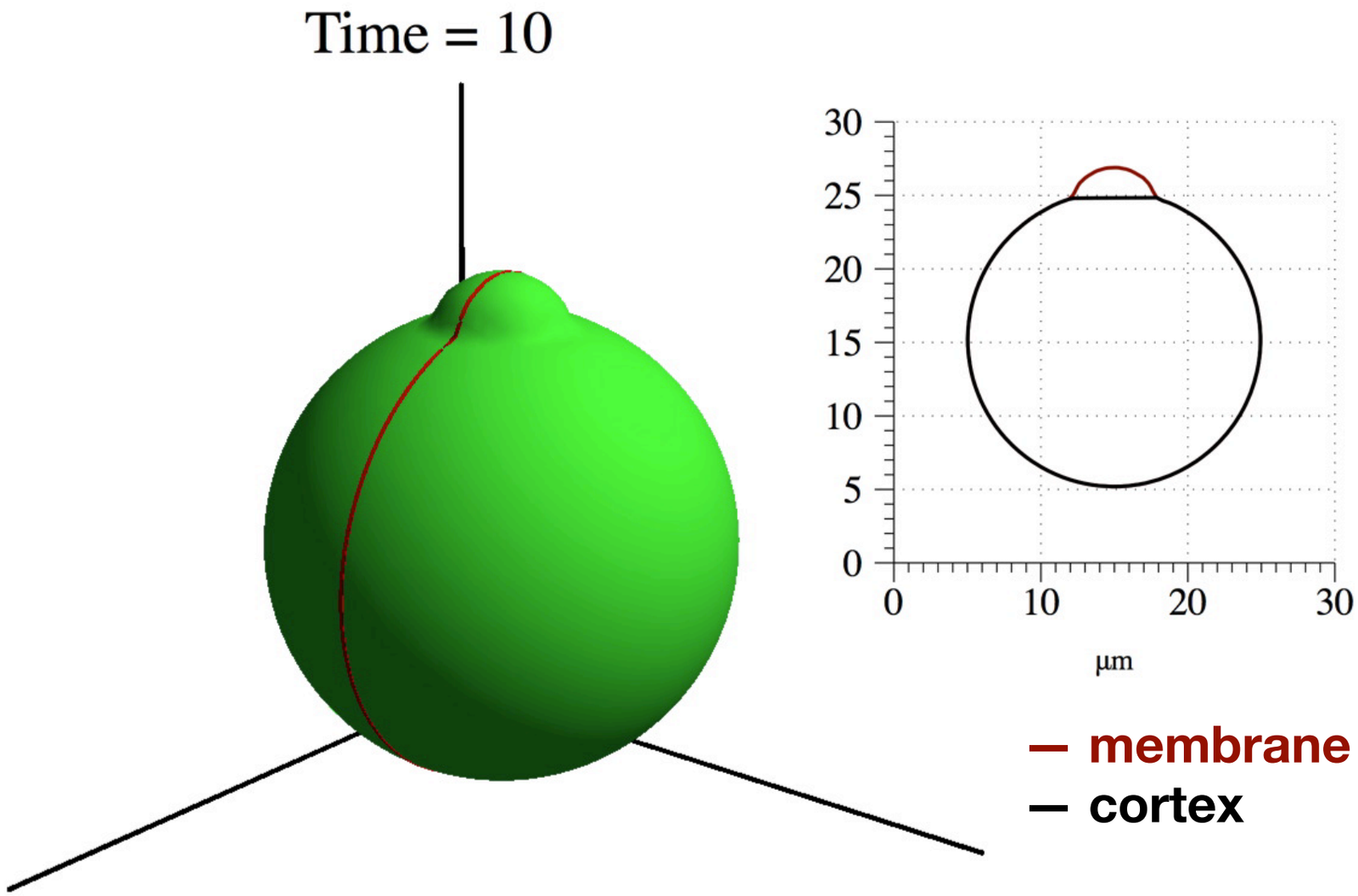
Remove membrane cortex adhesion



Remove cortex



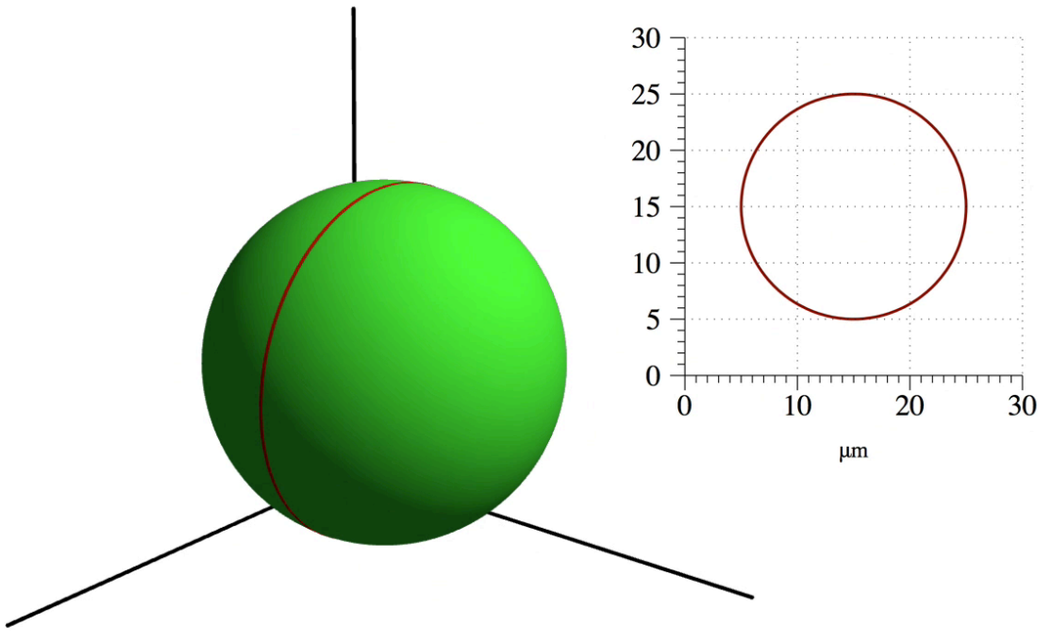
Bleb Expansion



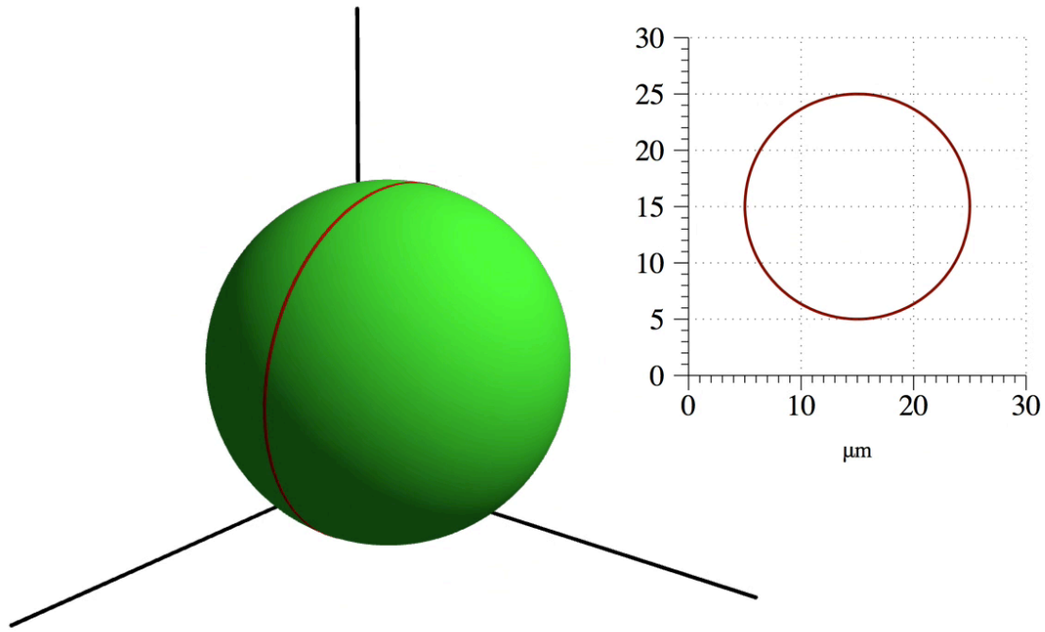
- Viscous fluid cytoplasm

Cortical Ablation

Default initial hole size



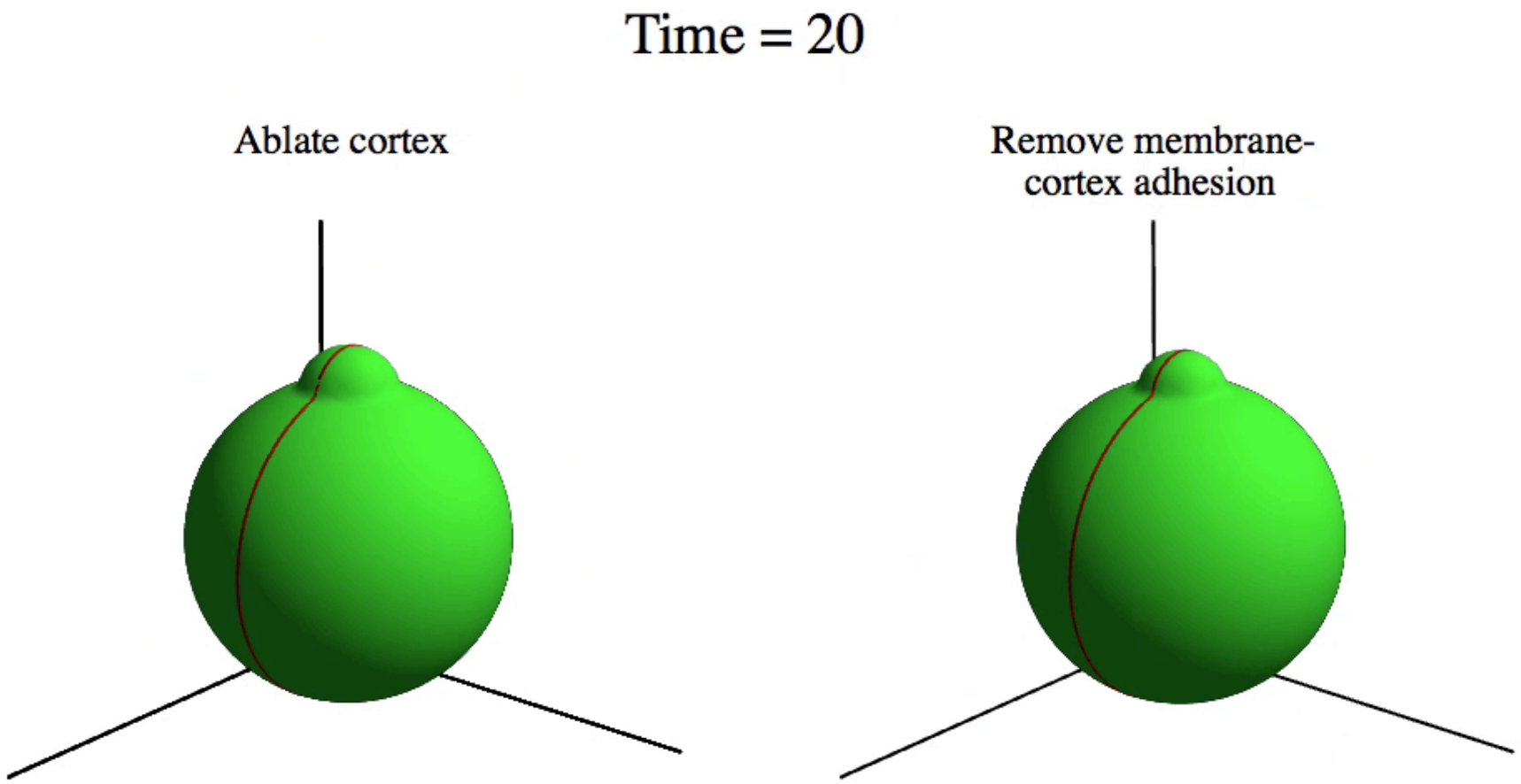
Smaller initial hole size



- Bleb morphology sensitive to initial hole size
- Fast initiation dynamics

Cortical Ablation

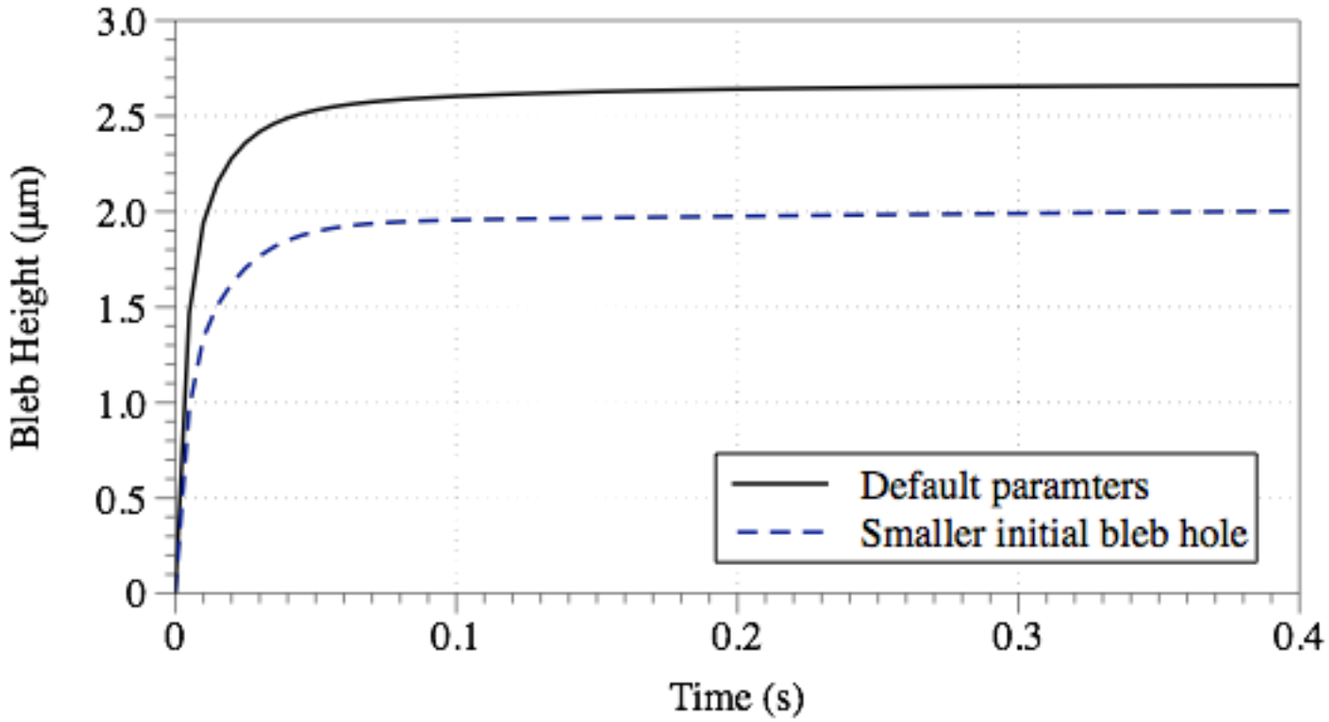
Poroelastic cytoplasm



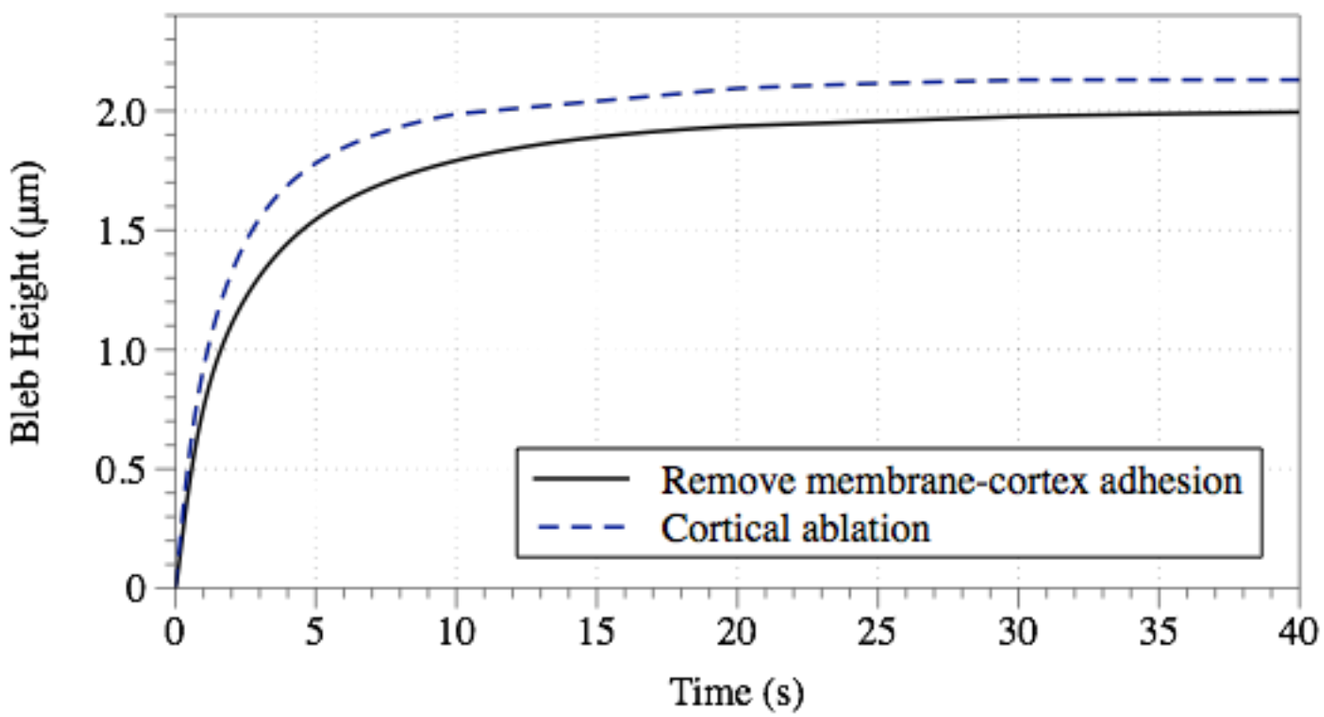
- Similar bleb shape and size regardless of initiation mechanism
- Bleb height is about 12% larger when cortex is ablated

Bleb Expansion

Viscous fluid cytoplasm
cortical ablation



Poroelastic cytoplasm



Summary of Previous Results

Strychalski W. and Guy R. D. *A Computational Model of Bleb Formation.* Math. Med. Biol. 30 (2013) 115-130.

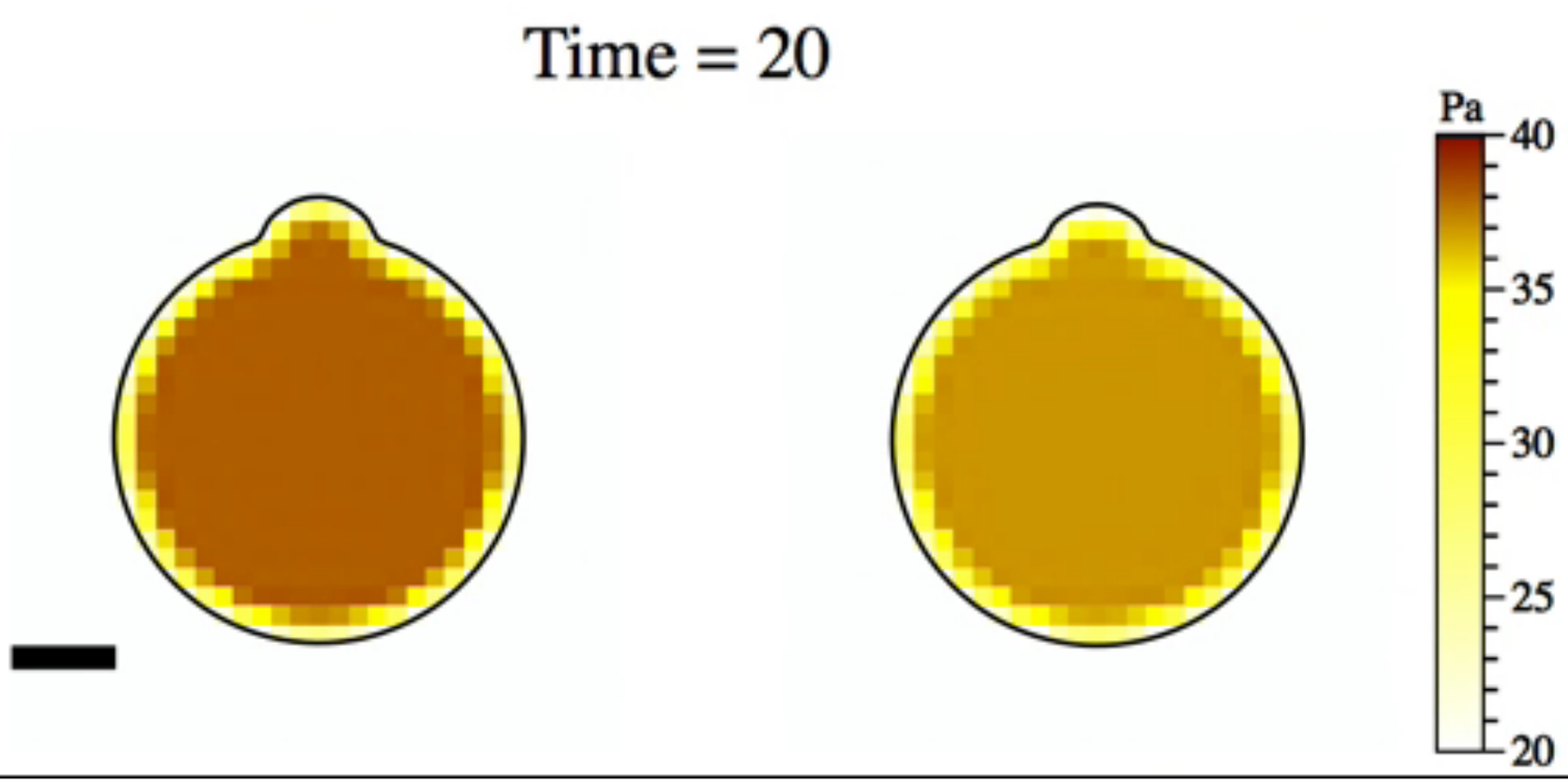
- Intracellular drag determines timescale of bleb expansion
- Fluid cytoplasm model insufficient to explain experimental data
  - Model parameters underestimate mesh (gap) size of cortex

Strychalski, W. and Guy, R.D. *Intracellular Pressure Dynamics in Blebbing Cells*, Biophys. J. (2016).

- Cytoplasmic elasticity is essential for relieving pressure in cell on timescale < 30 s
- Pressure not equilibrated in cells with multiple blebs
  - Multiple timescales in pressure dynamics
    - Short timescale for pressure propagation across the cell
    - Longer timescale from pressure equilibration

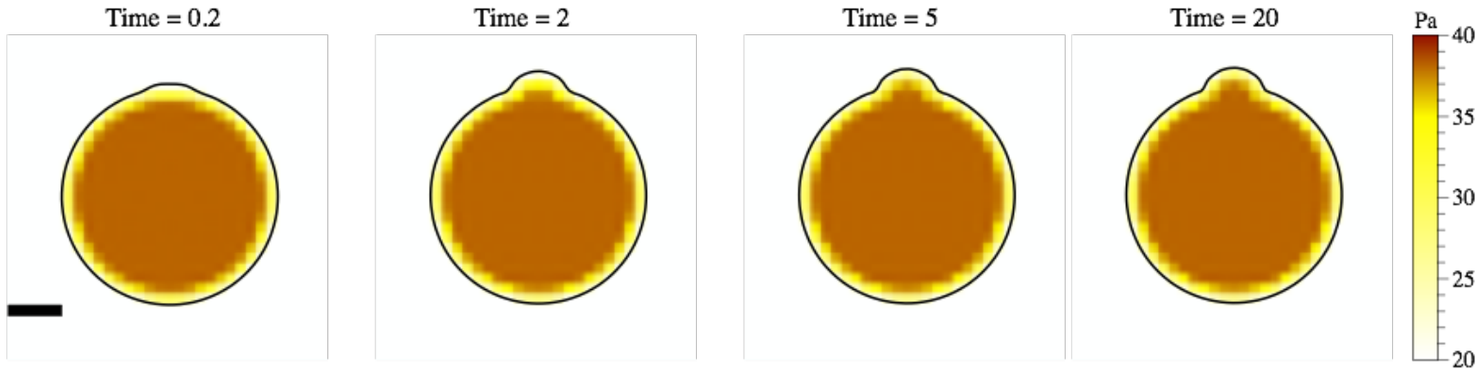
Pressure Dynamics

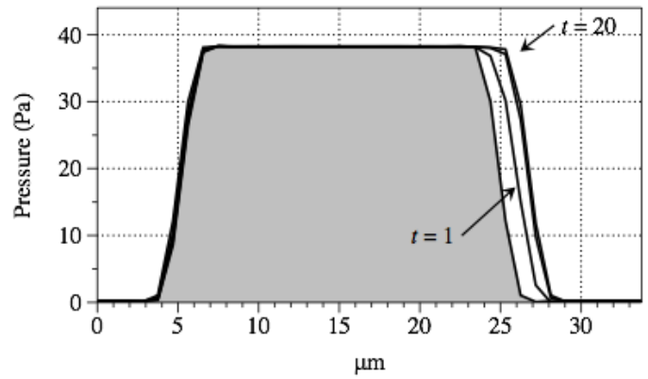
Viscous fluid cytoplasm Poroelastic cytoplasm



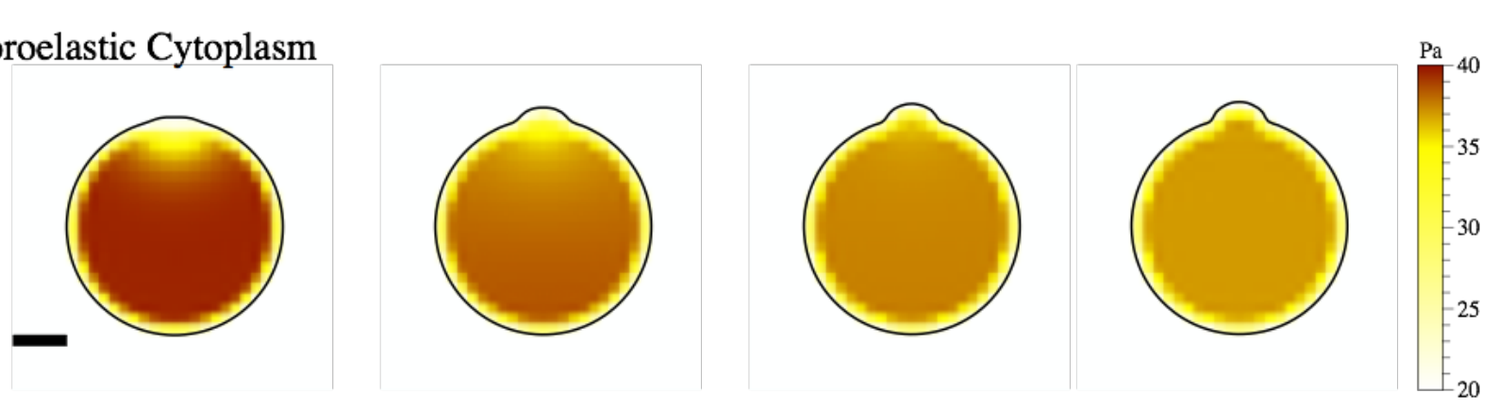
Pressure Dynamics

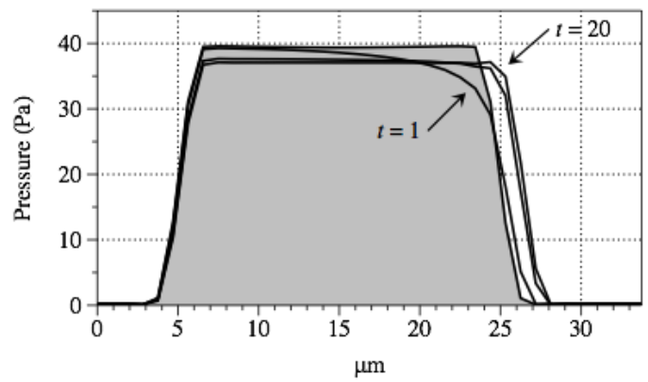
Fluid Cytoplasm





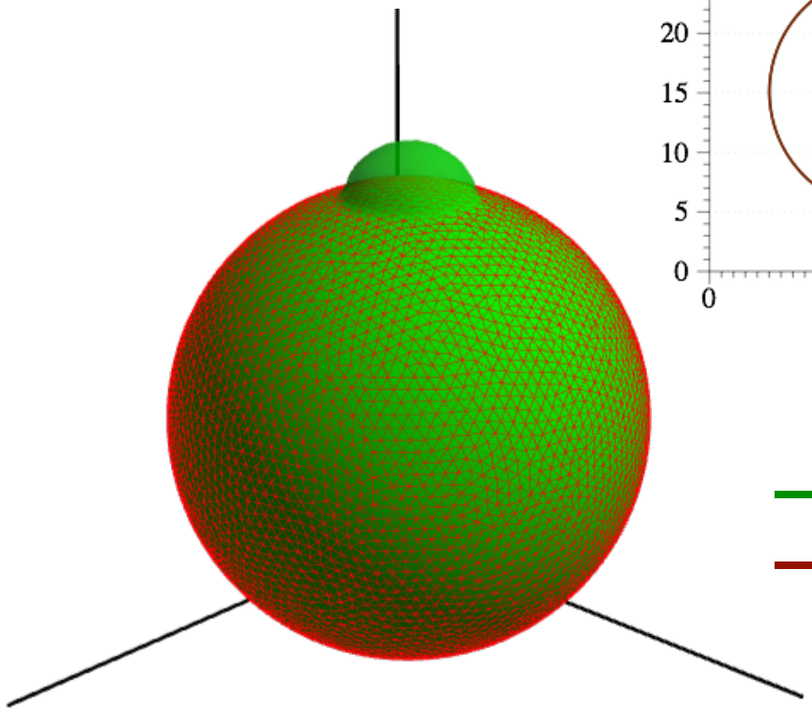
Poroelastic Cytoplasm

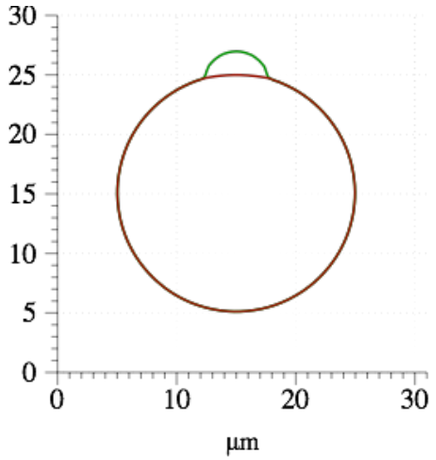




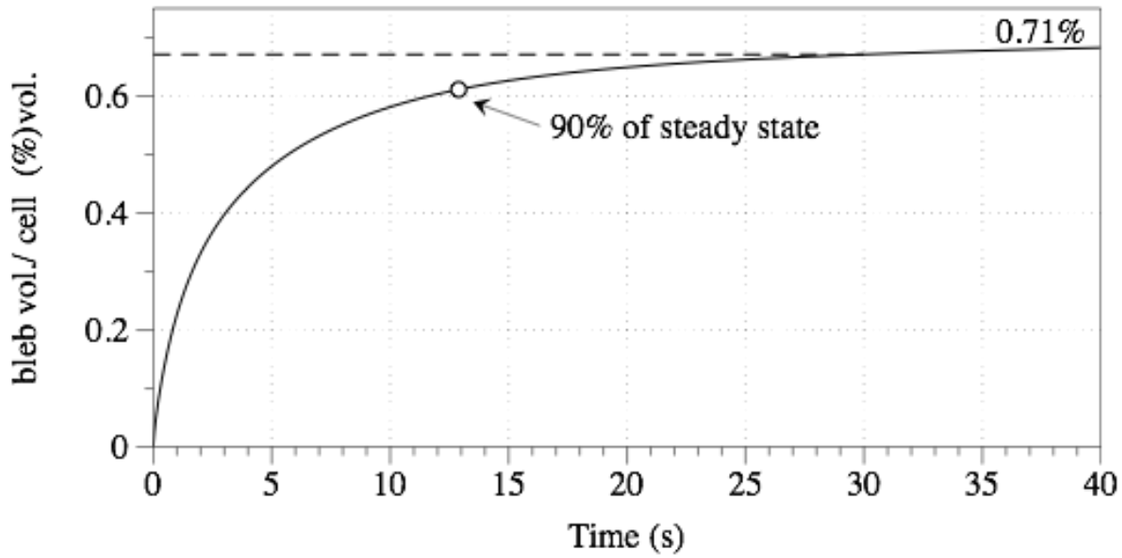
- Qualitatively similar results to 2D model
  - pressure propagates across the cells on a short time scale before equilibrating
- Observe far less pressure relief in 3D model for both cytoplasmic models
  - 10% in 3D compared to 36% in 2D simulation

Bleb Expansion Time



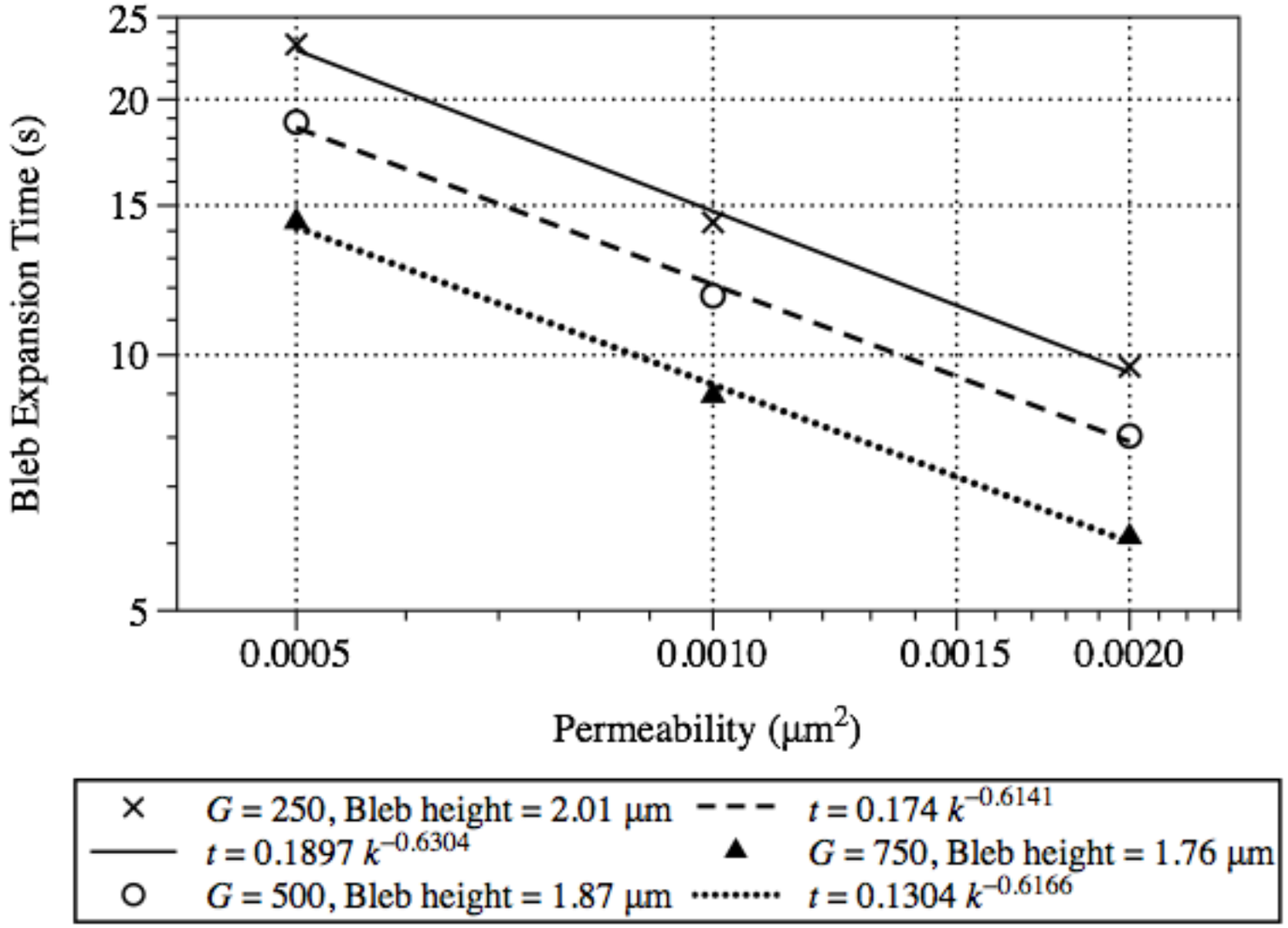


— membrane
— cortex



- Bleb volume is computed by subtracting cortex volume from membrane volume

Scaling of Bleb Time



Power law fit of 3D data is -0.6 **Power law fit of 2D data is** -0.9

- Effective diffusion coefficient for poroelastic material

$$D = G\kappa/\mu$$

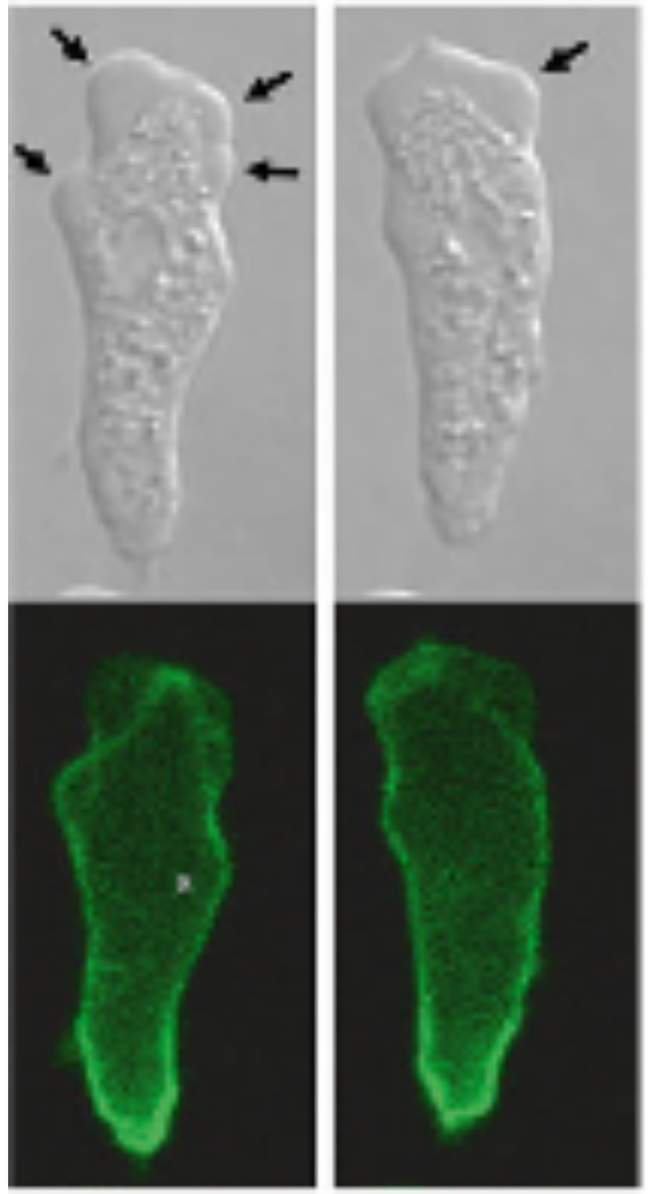
G. T. Charras et al. *Non-equilibration of hydrostatic pressure in blebbing cells*, Nature 435 (7040) (2005) 365–9.

Conclusions

- When the cytoplasm is modeled as a viscous fluid, blebs are much broader when initiated by cortical ablation than when initiated by a loss in membrane-cortex adhesion.
    - The timescale of bleb expansion is an order of magnitude faster when initiated by cortical ablation.
- When the cytoplasm is modeled as a poroelastic material, bleb shape, size, and dynamics are insensitive to the mechanism of bleb initiation.
- Poroelastic model results in bleb morphologies similar to experimental data
- Pressure propagation is qualitatively similar to the 2D model, but much less pressure is relieved in the 3D model for both fluid and poroelastic models of the cytoplasm using comparable parameter values.
- Bleb expansion dynamics from a 3D model do not obey the scaling law $D \sim G\kappa$

Future/Ongoing Work

- Blebbing in irregularly shaped cells such as Dictyostelium discoideum (Brazill Lab, CUNY Hunter)
  - Areas of negative curvature induce blebbing
  - Membrane energy model for predicting bleb nucleation sites (Emmanuel Asante-Asamani, poster session, Tuesday PP1 7:00 PM)
- Interactions with environment
- Improved algorithms methods for 3D simulations

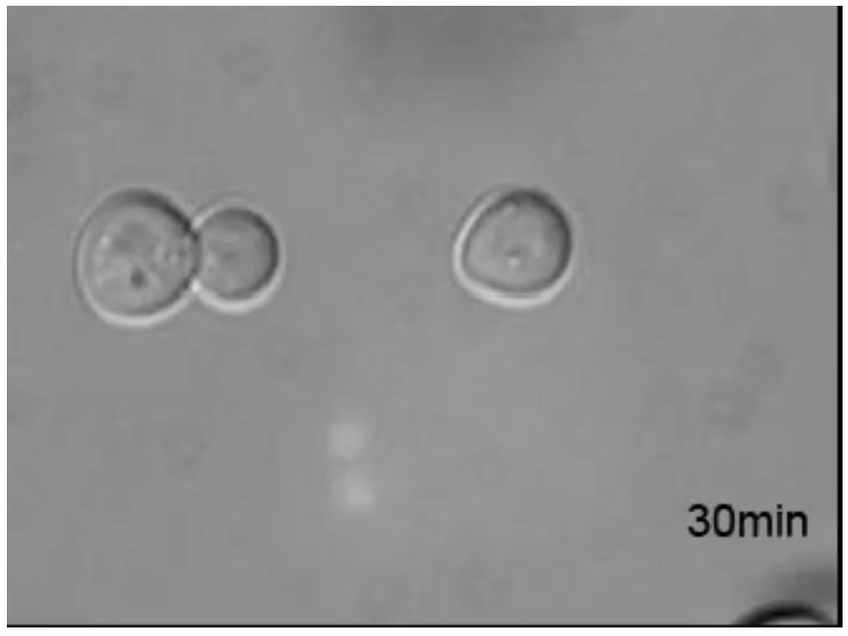


Zatulovskiy E. and Kay R.R. *Chemotatic Blebbing in Dictyostelium Cells.* Methods Mol. Biol 1407:(2016) 97-105.

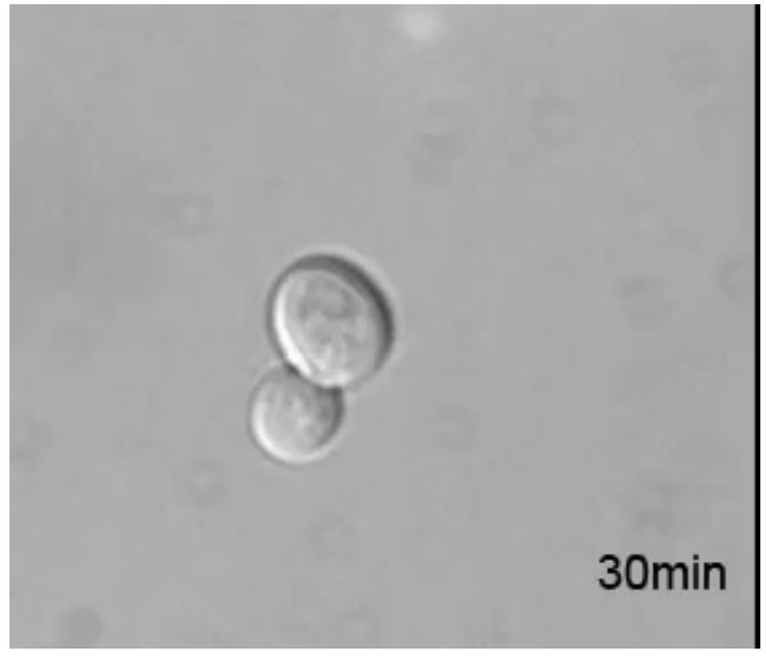
Related Systems

- Yeast mating

Initial shmoo formation

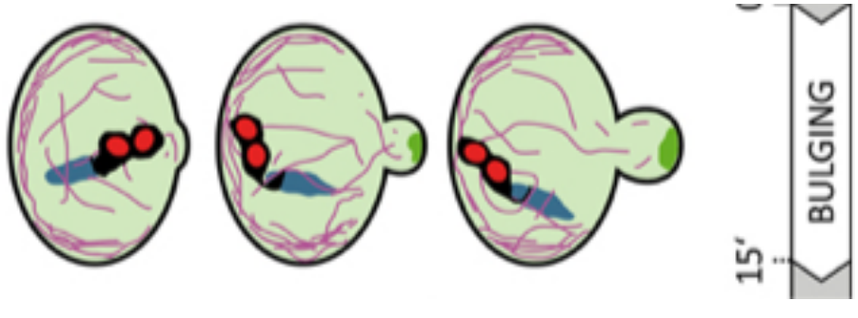


Bipolar budding



Malleshaiah et al. *The scaffold protein Ste5 directly controls a switch-like mating decision in yeast*, Nature (2010) 465 101-105

- Initial pollen tube formation after hydration



F. Volger et al. *Knockin' on pollen's door: live cell imaging of early polarization events in germinating Arabidopsis pollen*, Front. Plant Sci. (2015) 6:246.

Acknowledgements

- Bob Guy (UC Davis)
- David Hartenstine (Western Washington University)
- Alex Mogilner (New York University)
- Ewa Paluch Lab
- University College London (UCL)
- Funding agencies
  - NSF DMS-1226386 (2013 - 2016)
  - Simons Foundation #429808