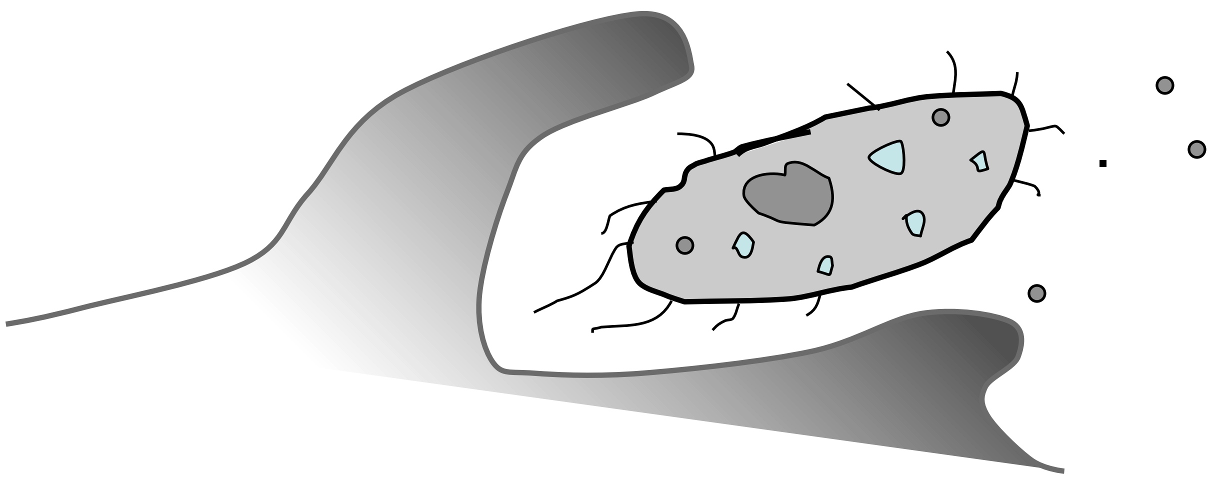
How one cell eats another

experiments and modelling elucidate mechanism of uptake



CISBIC Open Day *July 6, 2010*

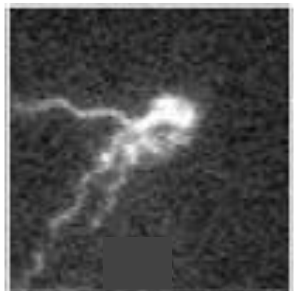
Robert Endres
Imperial College London

http://www3.imperial.ac.uk/biologicalphysics
r.endres@imperial.ac.uk

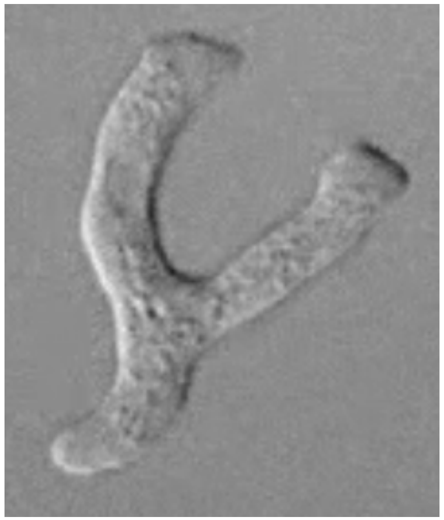
Where physics meets biology….

Chemosensing and motility

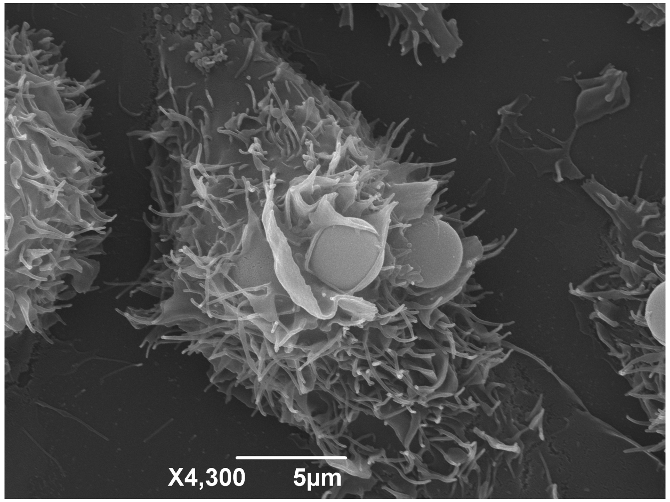
bacteria



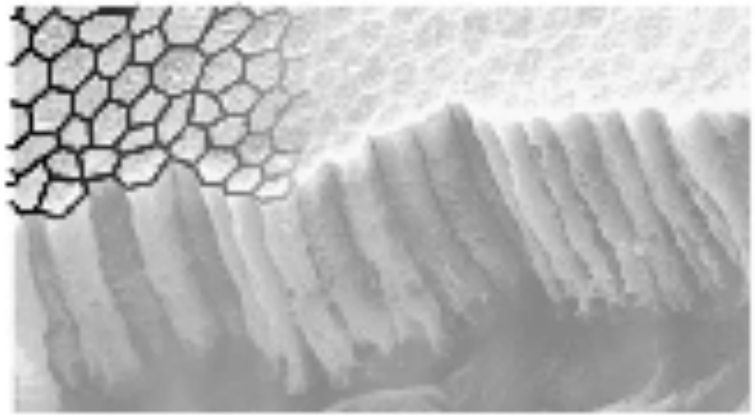
amoeba



Eating, uptake, and destruction



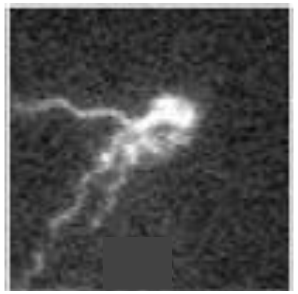
Cell packing



Where physics meets biology….

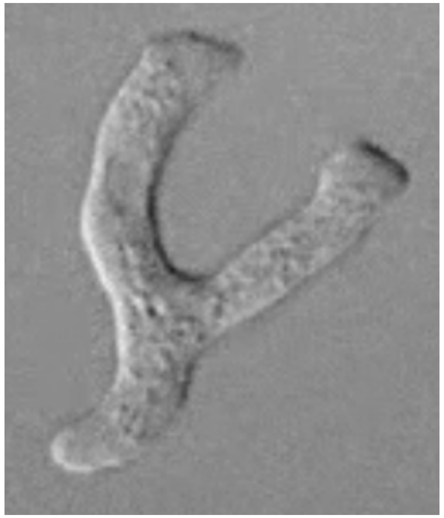
Chemosensing and motility

bacteria

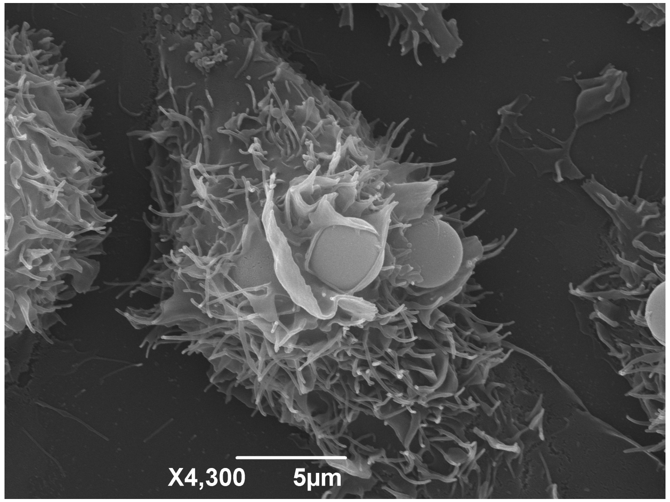


- Ligand diffusion
- Fluid mechanics
- Torgue generation
- Biased random walk

amoeba



Eating, uptake, and destruction



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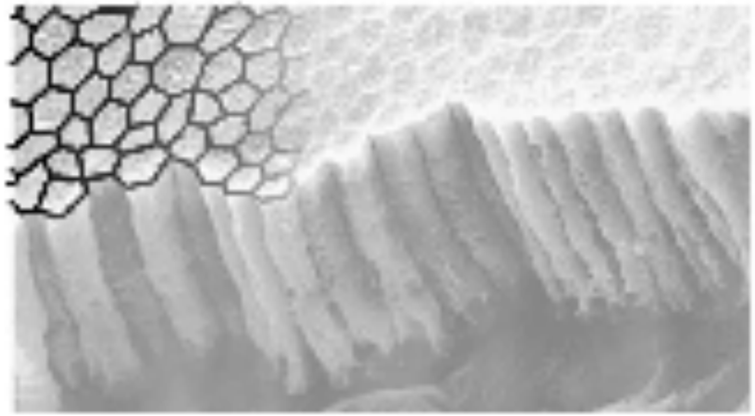
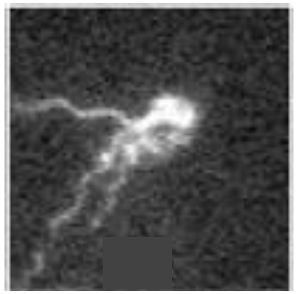


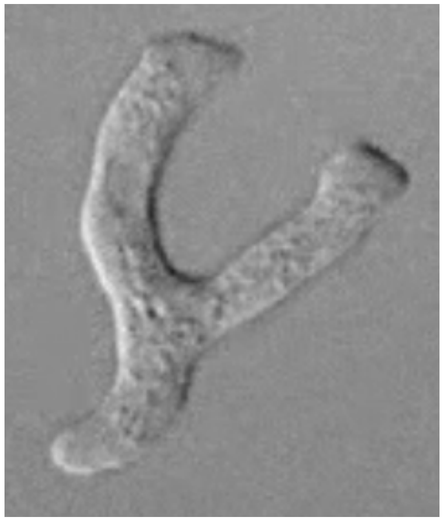
Where physics meets biology….

Chemosensing and motility

bacteria

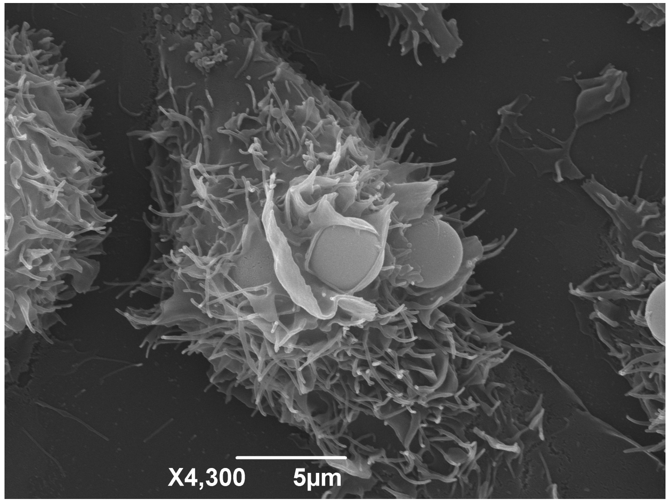


amoeba

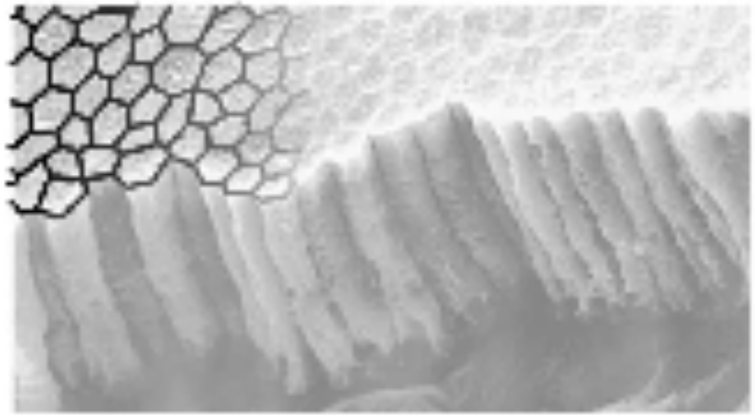


- Ligand diffusion
- Cell-shape changes
- Remodelling of cytoskeleton
- Adhesion
- Migration

Eating, uptake, and destruction



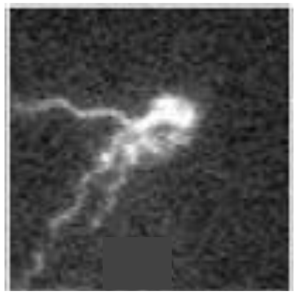
Cell packing



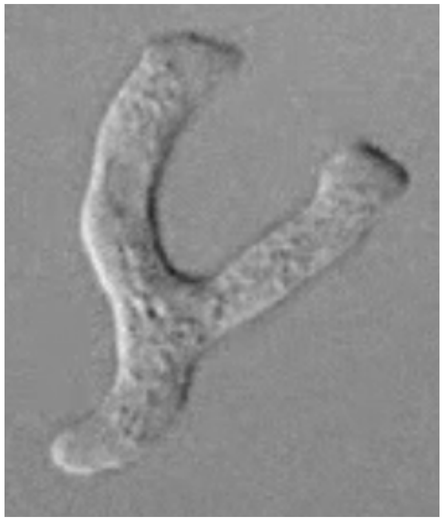
Where physics meets biology….

Chemosensing and motility

bacteria

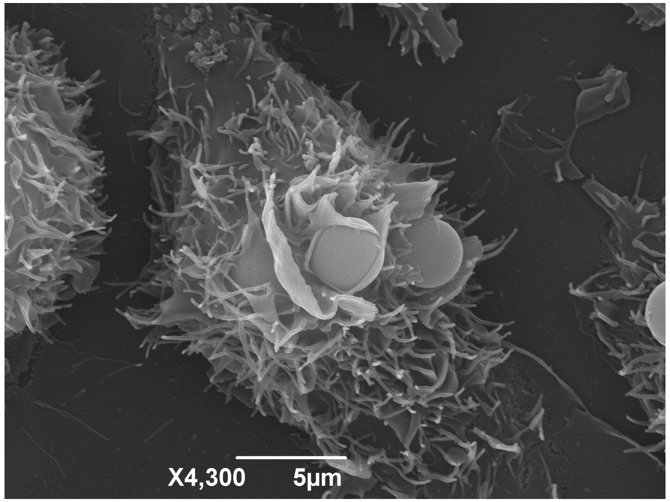


amoeba

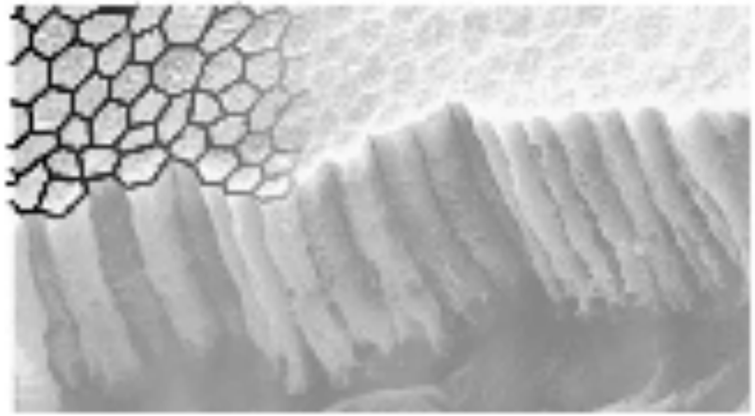


- Mechanosensing of particles
- Cell-shape changes
- Contraction by motor proteins

Eating, uptake, and destruction



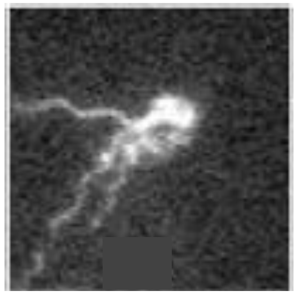
Cell packing



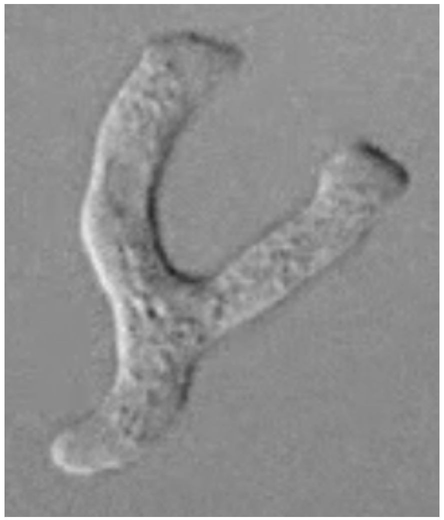
Where physics meets biology….

Chemosensing and motility

bacteria

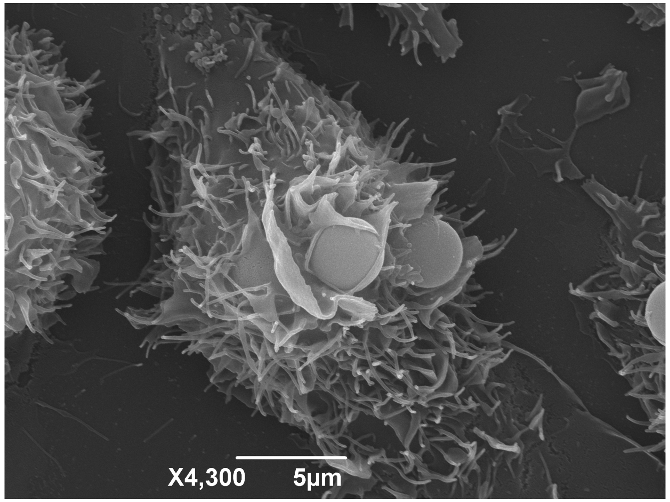


amoeba

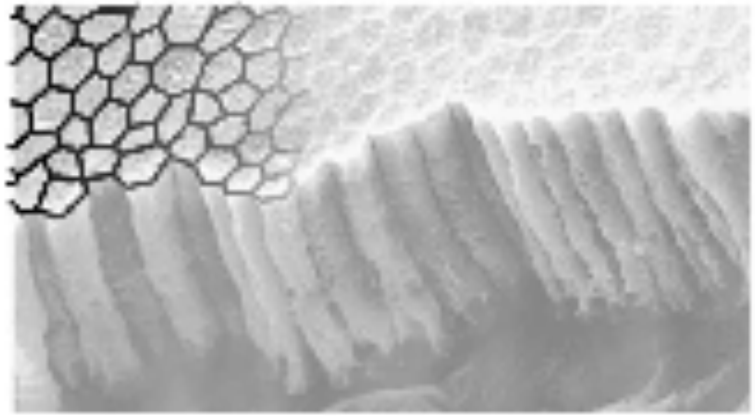


Surface tension
Contraction by
motor proteins
Cell-cell adhesion
Mechano- and
shear sensing

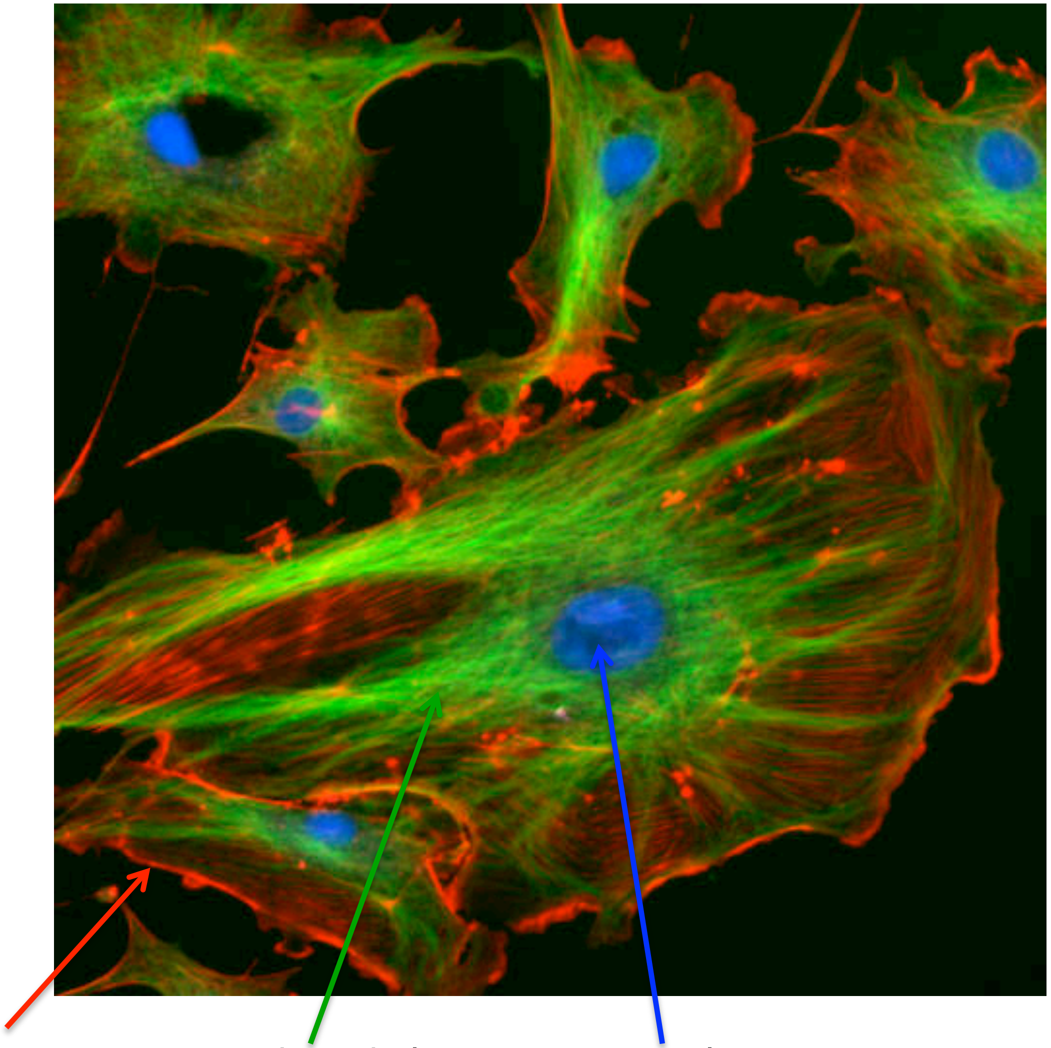
Eating, uptake, and destruction



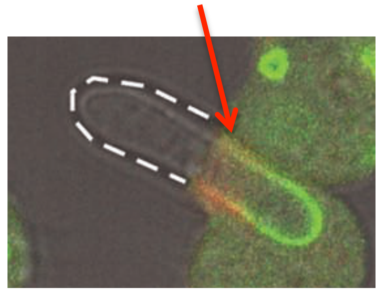
Cell packing

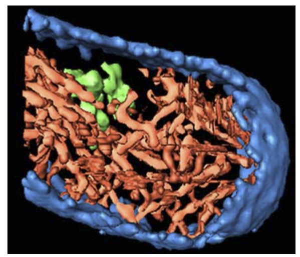


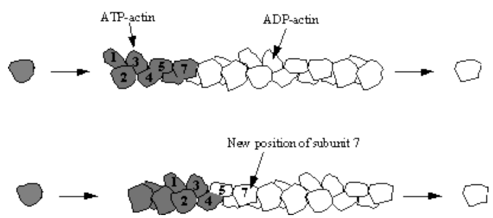
Cell cytoskeleton – very dynamic and versitile for cell-shape changes in phagocytosis, migration, adhesion, and packing



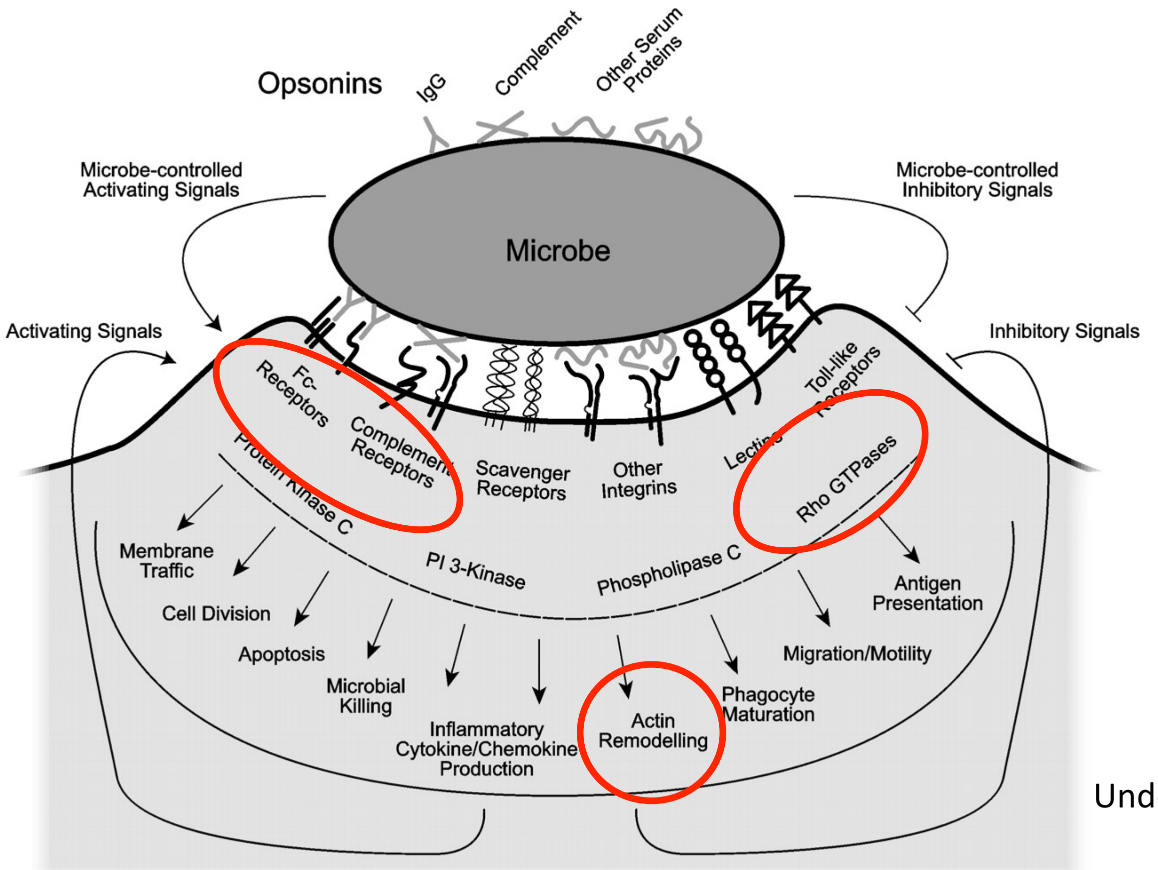
Actin:





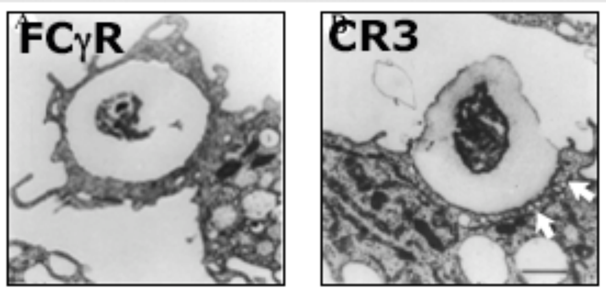


Daunting signalling complexity in phagocytosis



Underhill & Ozinsky (2002)

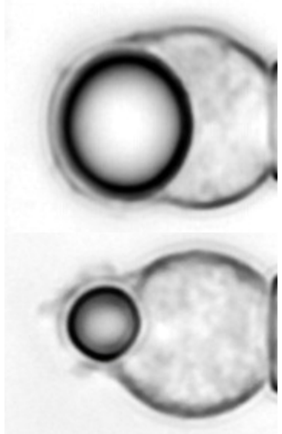
about 140 different molecular species are involved



Allen & Aderem (1996)

Geometric and biophysical aspects of phagocytosis

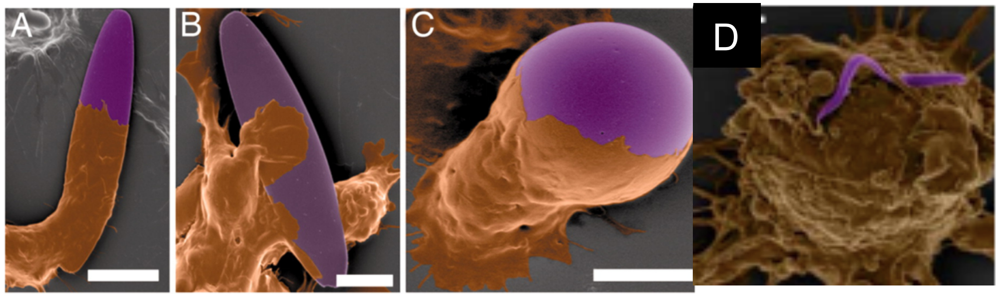
1. Particle-size independence



Herant et al. (2006)

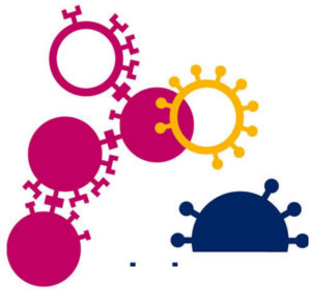
2. Shape dependence

3. Elastic properties



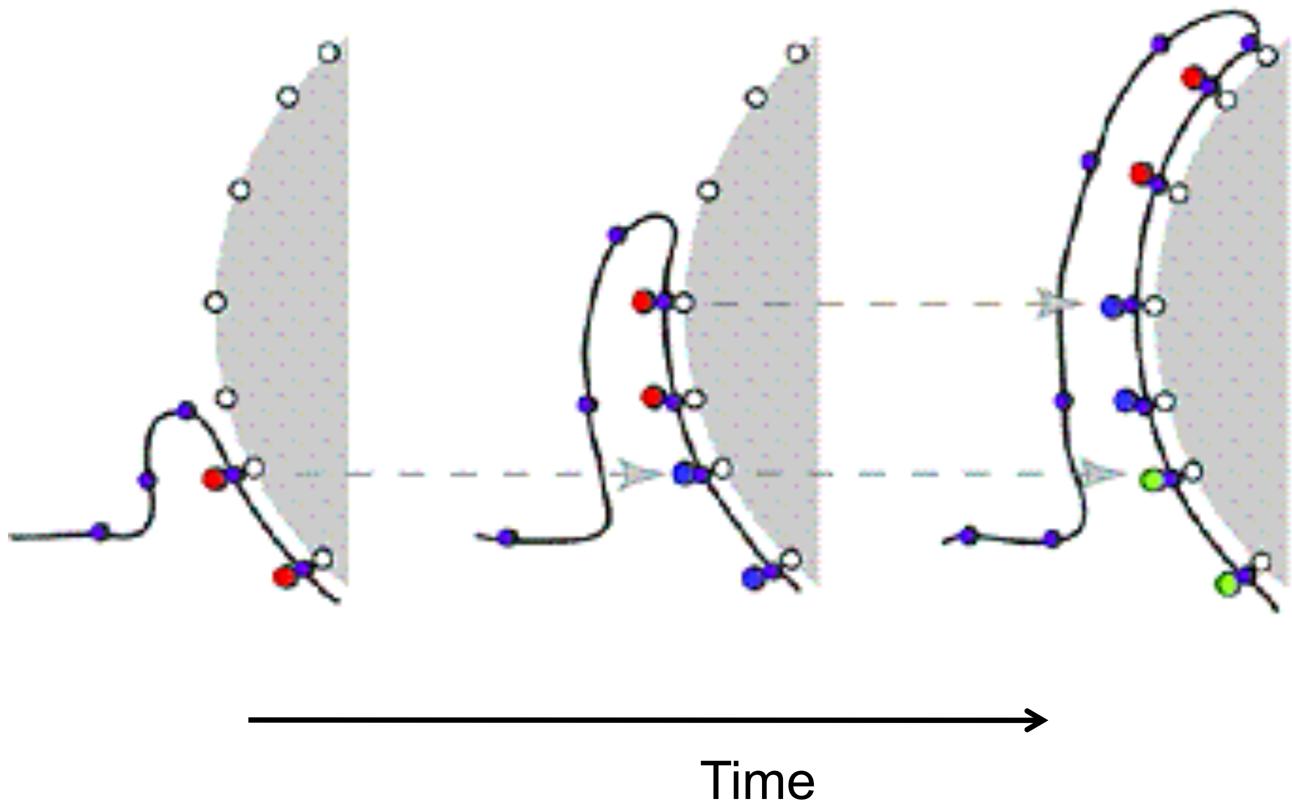
Champion et al. (2006,2009)

4. Ligand density



Conceptual Zipper mechanism

for explaining dependence on ligand density

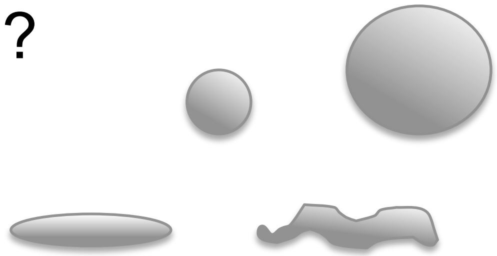


Griffin et al. (1975), Swanson (2008)

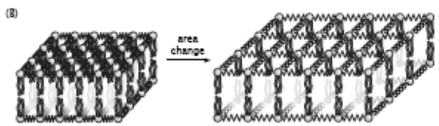
Outline of (remainder of) talk

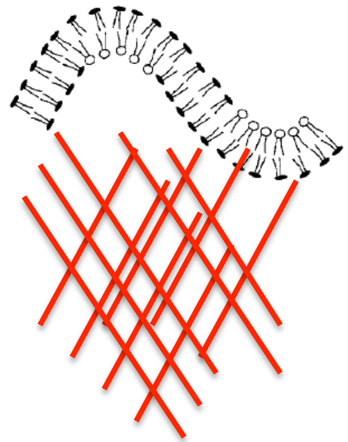
Can the Zipper mechanism explain the

- Geometric requirements of particle?
  - Size independence
  - Shape dependence



- Energetic requirements of cell?
  - Ligand-receptor binding
  - Surface tension
  - Membrane bending
  - Remodelling of cytoskeleton



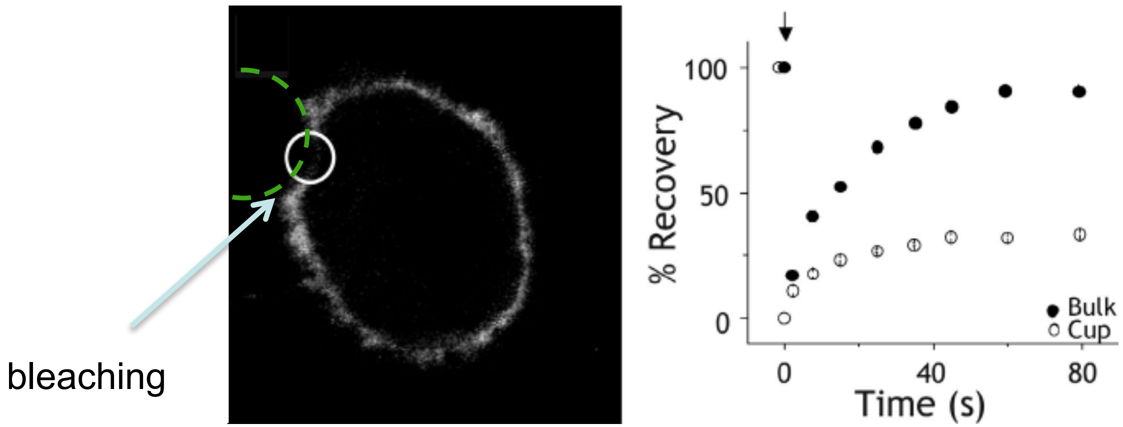


Mechanistic idea for modelling Zipper mechanism

Ratchet

1. **Unidirectional** engulfment, even **without pauses**

2. **Immobilization** of proteins and lipids in cups from FRAP



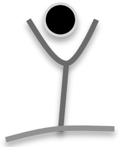
Corbett-Nelson et al. (2006)

3. Ratchets used before in modelling actin-driven motility

Useful modelling techniques

Ordinary differential equations – biochemical reactions:

$$\frac{dn}{dt} = k_+(1-n)c - k\_n$$

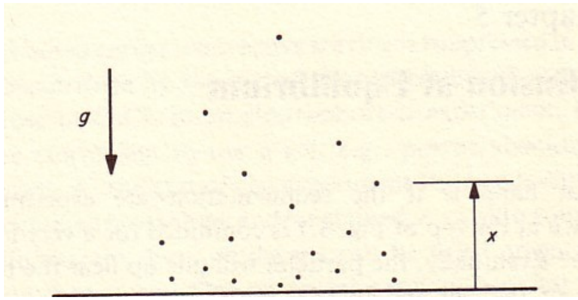


Partial differential differential equations – diffusion:

$$\frac{\partial c}{\partial t} = D\frac{\partial^2 c}{\partial x^2} + ...$$

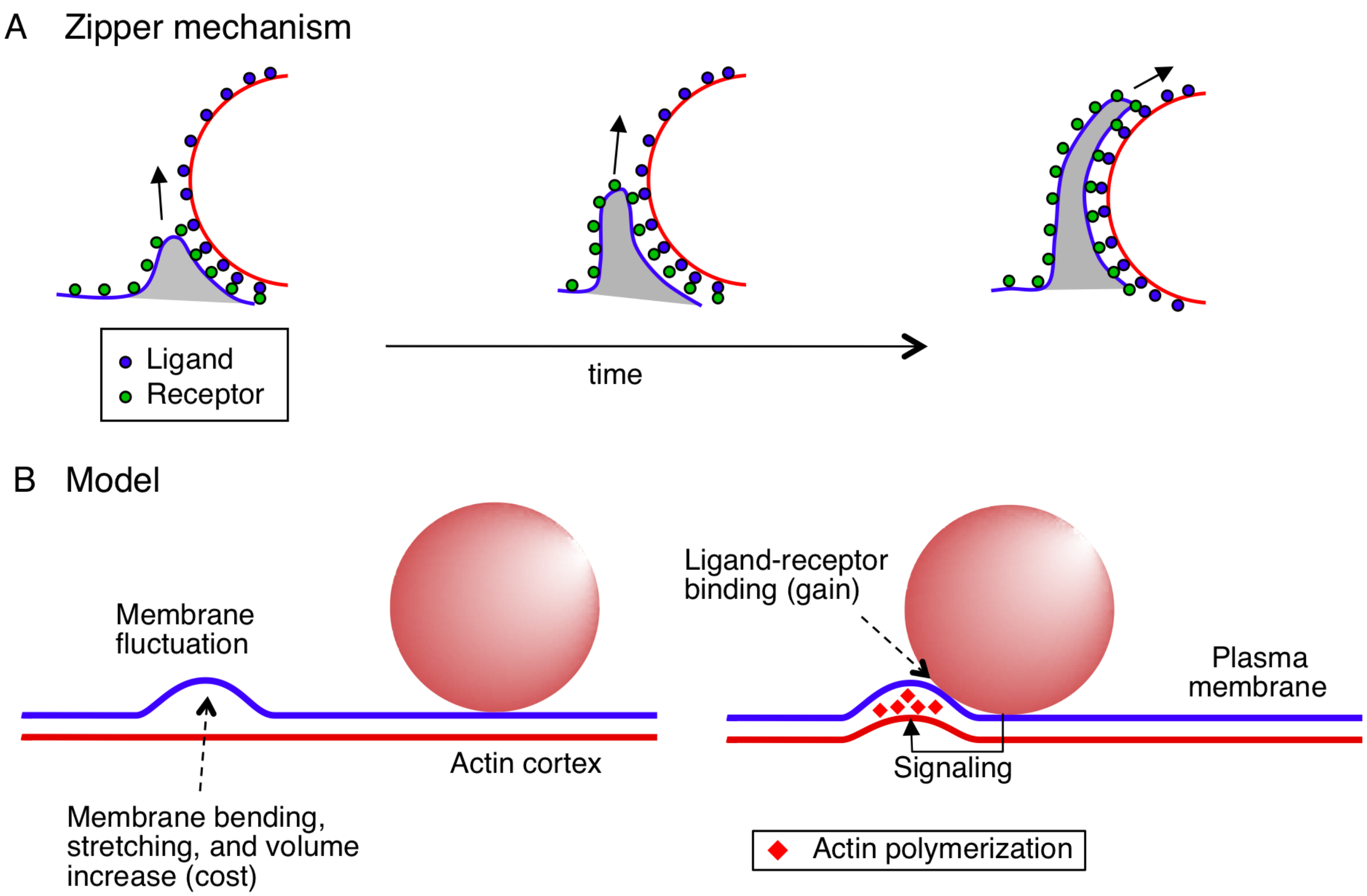
Monte Carlo simulations:

Accept or reject trial moves based on energies for equilibrium properties



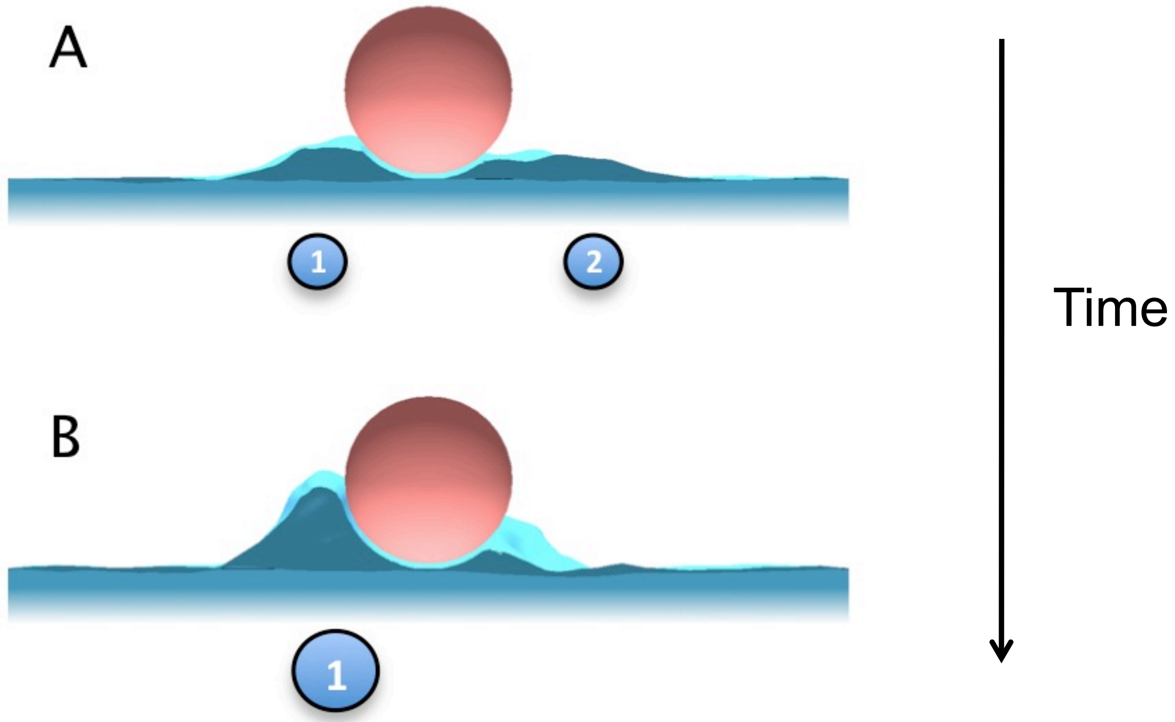
Discretization

Implementation of zipper mechanism



Ligand-receptor binding induces actin polymerization, making membrane deformation effectively irreversible → **ratchet**.

Ratchet model in action



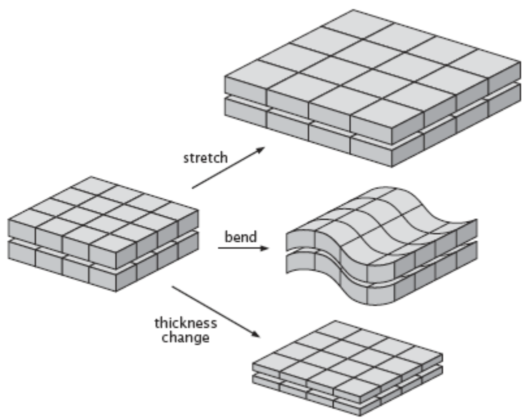
① Membrane deformation near particle is stabilized.
② Membrane deformation far from particle is retracted later.

Model for cell membrane

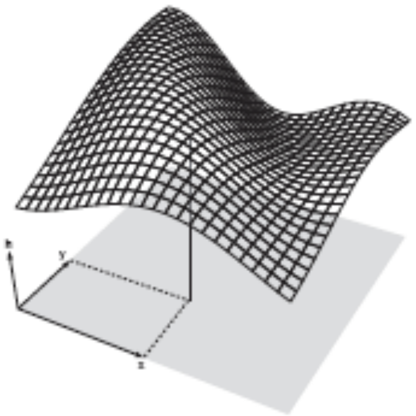
Cell-membrane energy:

$$E = \overbrace{\frac{\kappa_B}{2}\oint (\underbrace{C_1 + C_2}_{=2\overline{C}} - C_0)^2 \, dA}^{\text{bending}} + \overbrace{\Sigma A}^{\text{membrane stretching}} + \overbrace{PV}^{\text{vesicle swelling}} + \overbrace{E_{LR}}^{\text{Ligand-receptor binding}}$$

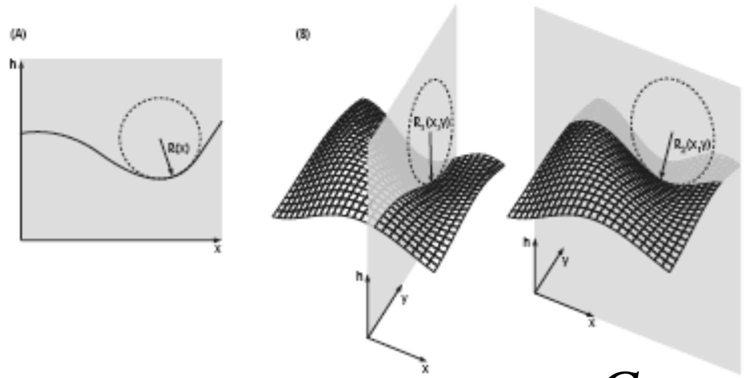
membrane deformations



height profile *h*(*x*,*y*)



membrane curvature



C_1 C_2

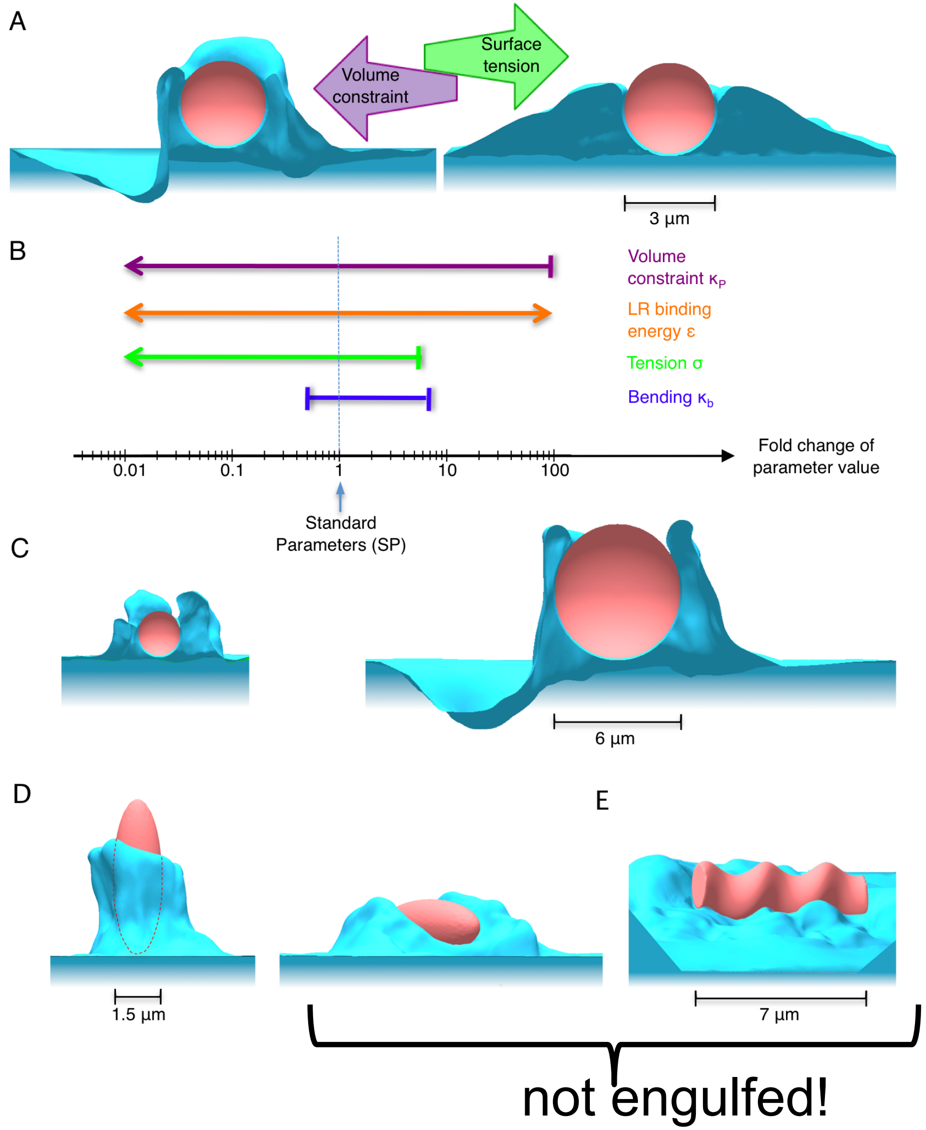
Successful engulfment for wide range of parameters

Cup shape

Parameter range

Particle size

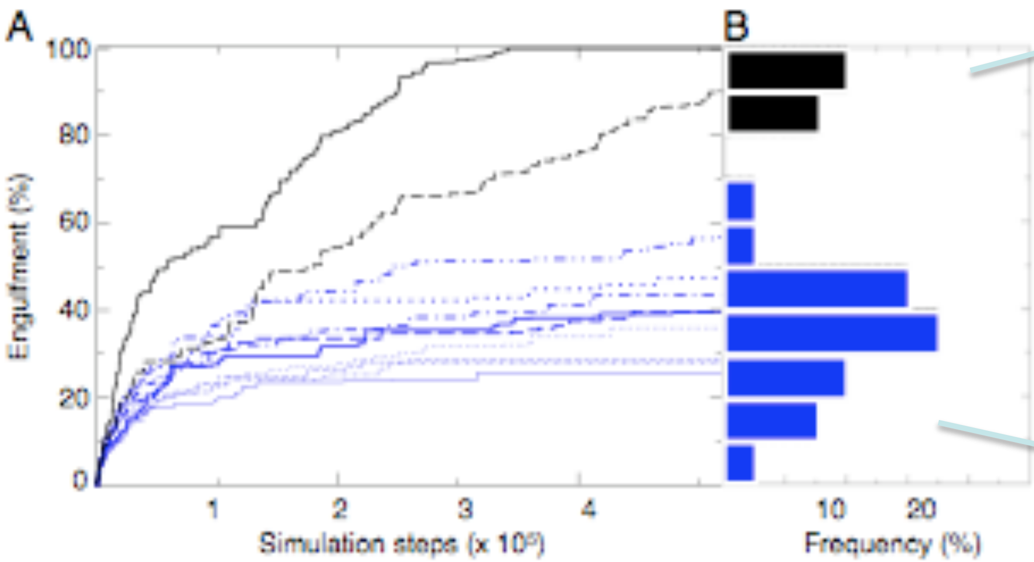
Particle shape and orientation

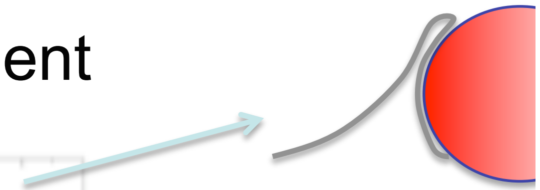


Model confirms mechanical bottleneck

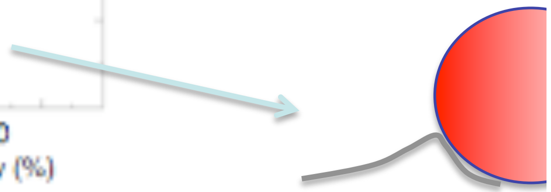
Simulation

Experiment





Mechanical bottleneck at half-engulfment



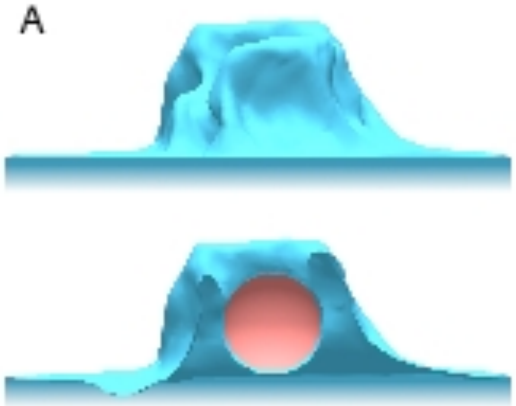
Trajectories for different values of membrane-surface tension

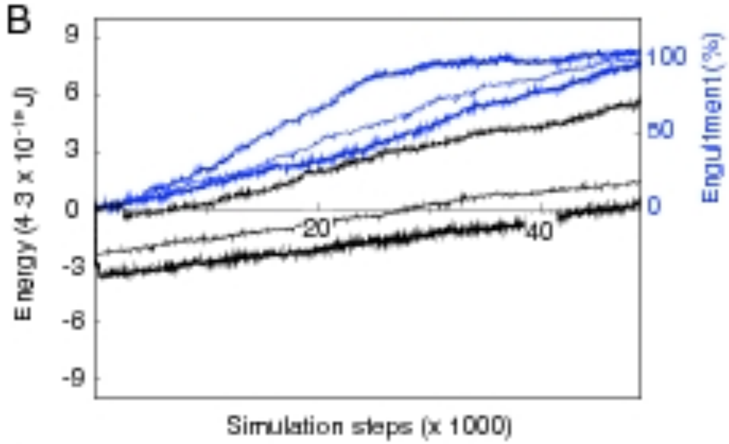
Bimodal distribution from experiments with WT-FcR after 10 min.

Van Zon *et el*. (2009)

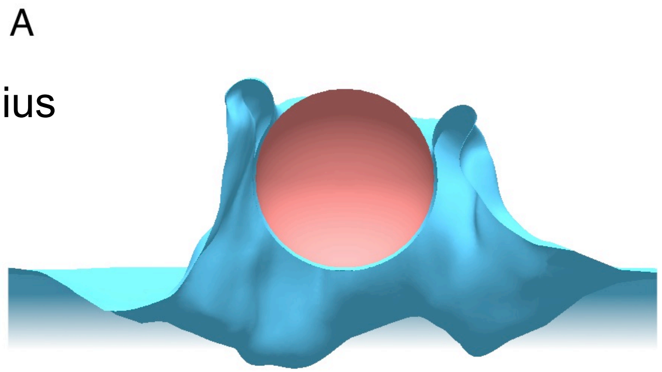
Energetic requirements:
engulfment by active zipper

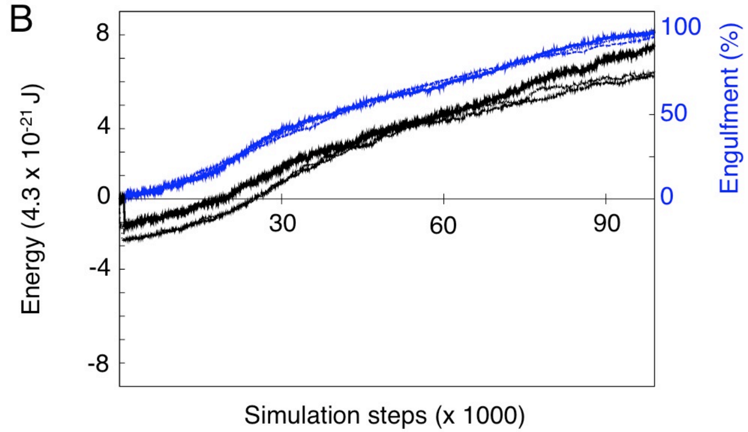
Small,
1.5 µm-radius
particle





Large,
3 µm-radius
particle

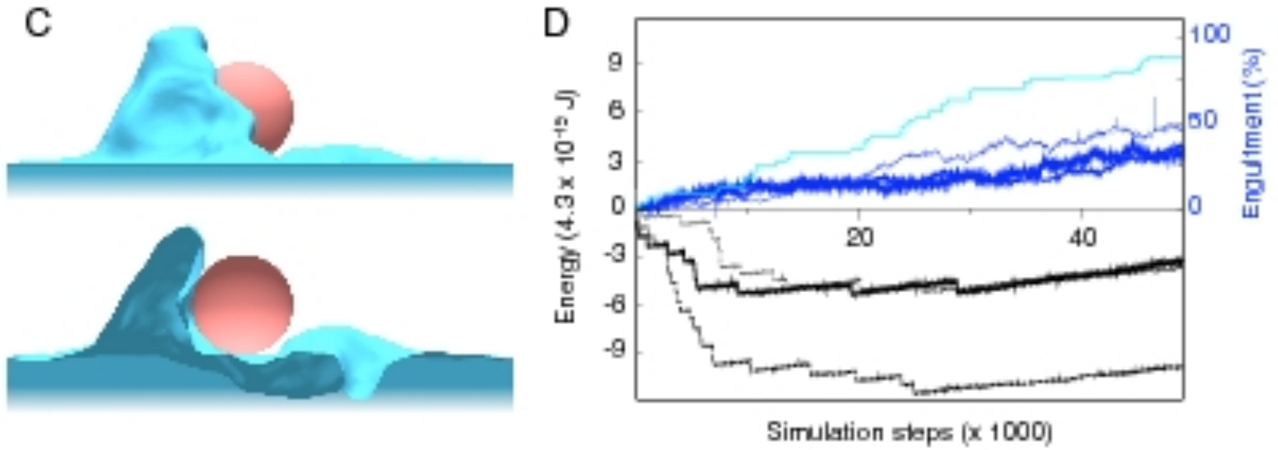




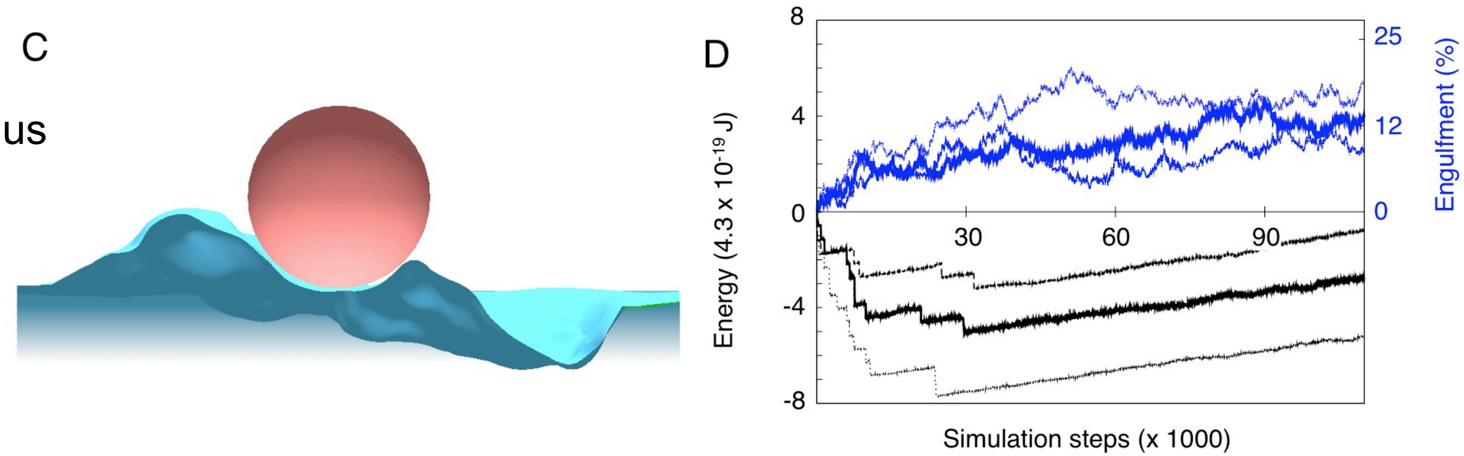
→ Active zipper easily engulfs small and large particles

Engulfment by passive zipper

Small,
1.5 μm-radius
particle



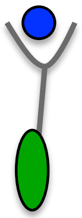
Large,
3 μm-radius
particle



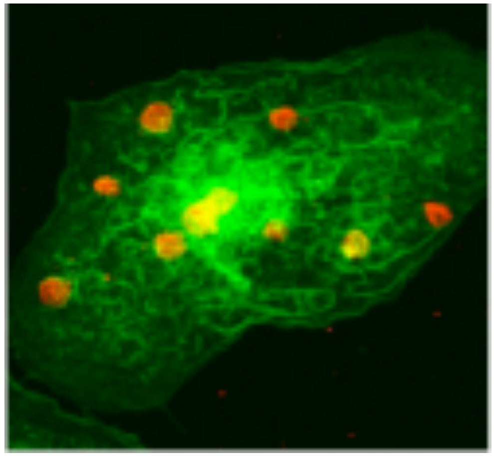
Passive zipper ONLY engulfs small particles - slowly with highly variable cups

3d imaging with confocal microscopy

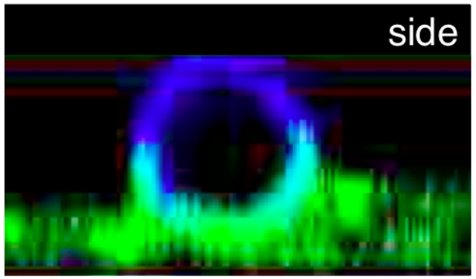
Fcγ receptor



Green Fluorescent Protein



COS-7 cell



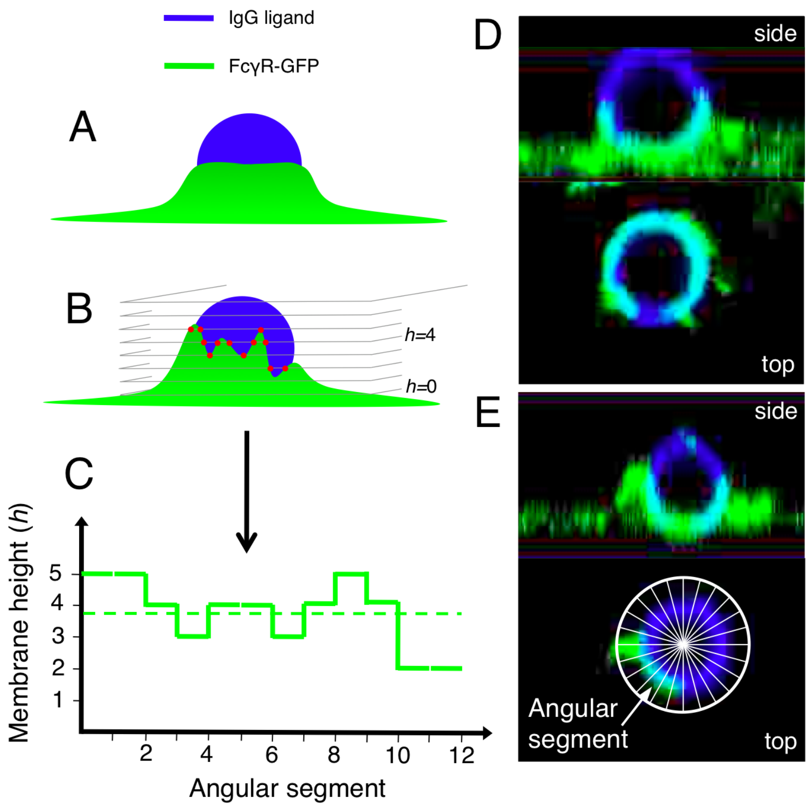
Phagocytic cup

Image analysis

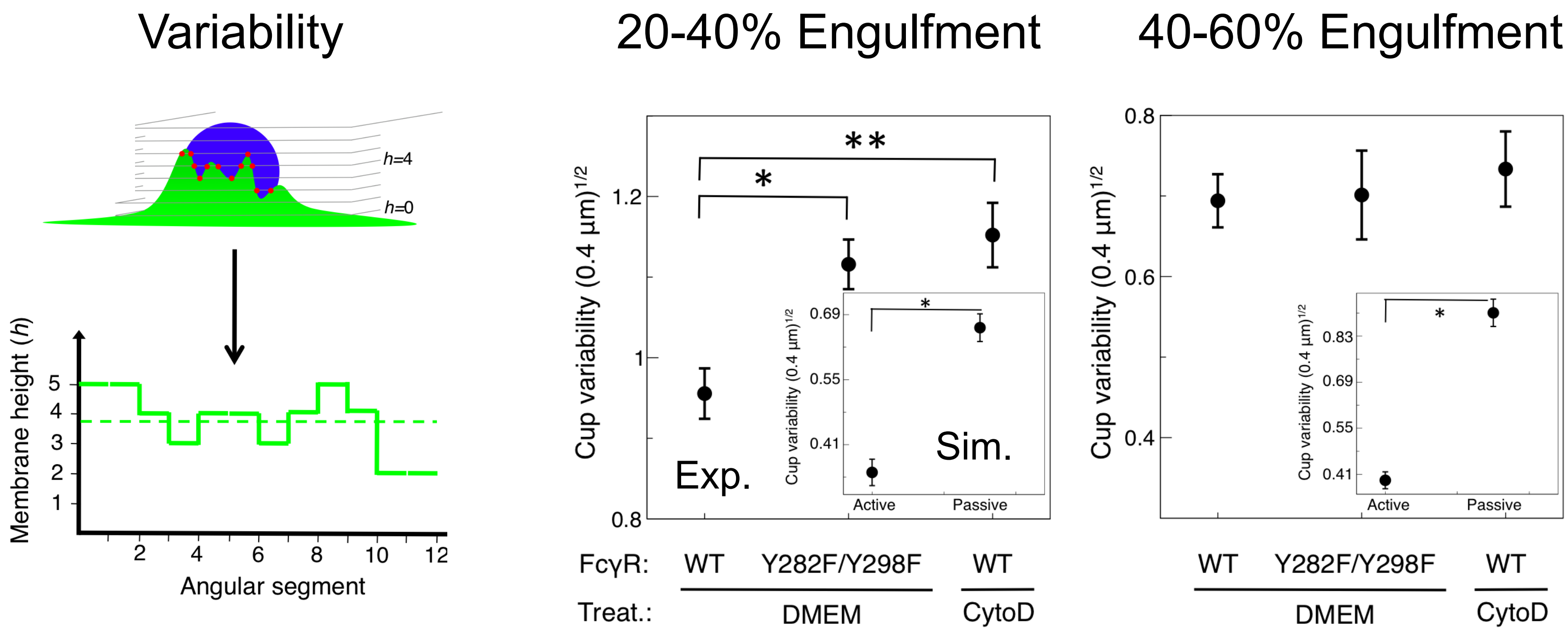
(a) Cells expressing wild-type Fcγ receptor = **active zipper**

(b) Cells expressing signalling-dead mutant receptor
(c) Cells transfected with cytochalasin D
} **passive zippers**

Cup height and variability



Variability of cup shape – exp vs. theory

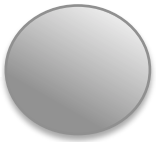


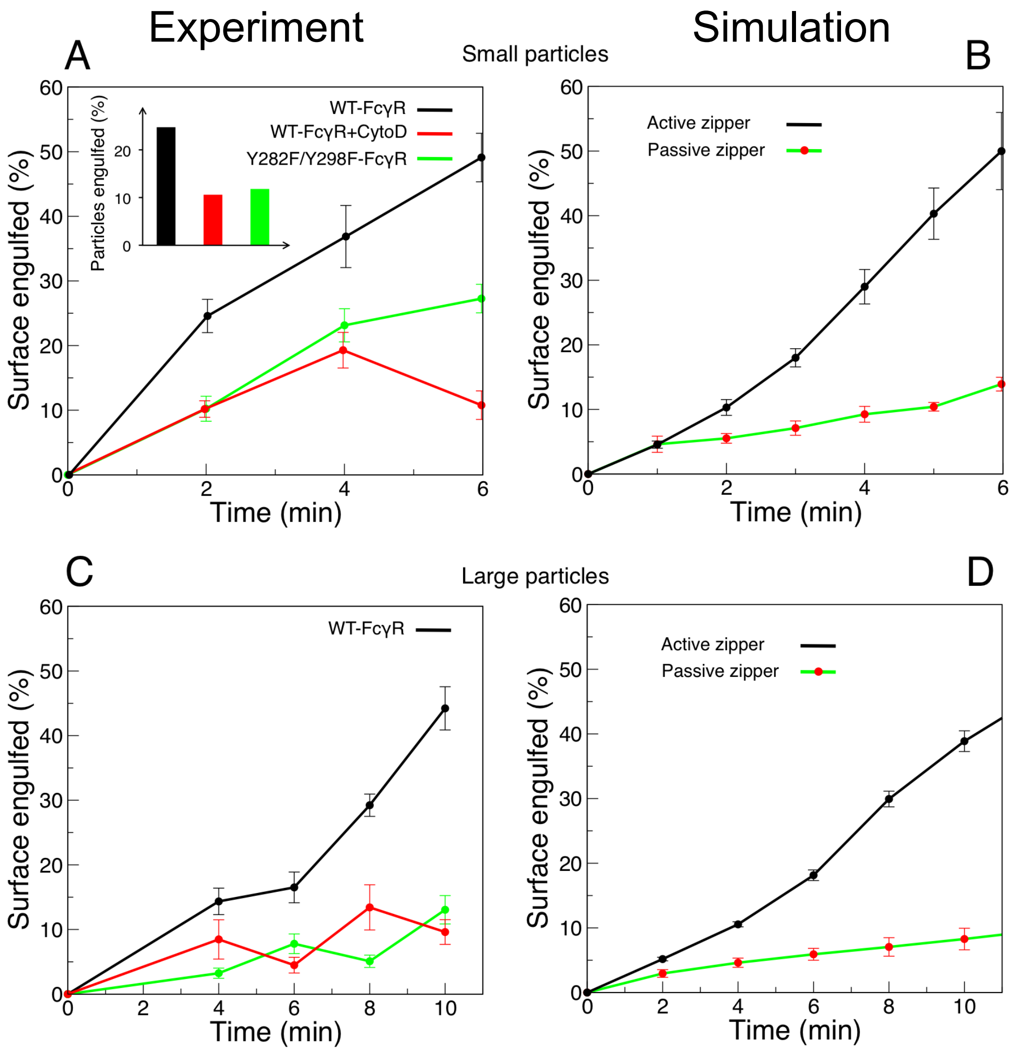
Passive zipper produces more variable cup shapes than active zipper.

Engulfment time – exp vs. theory

Small particle

Large particle

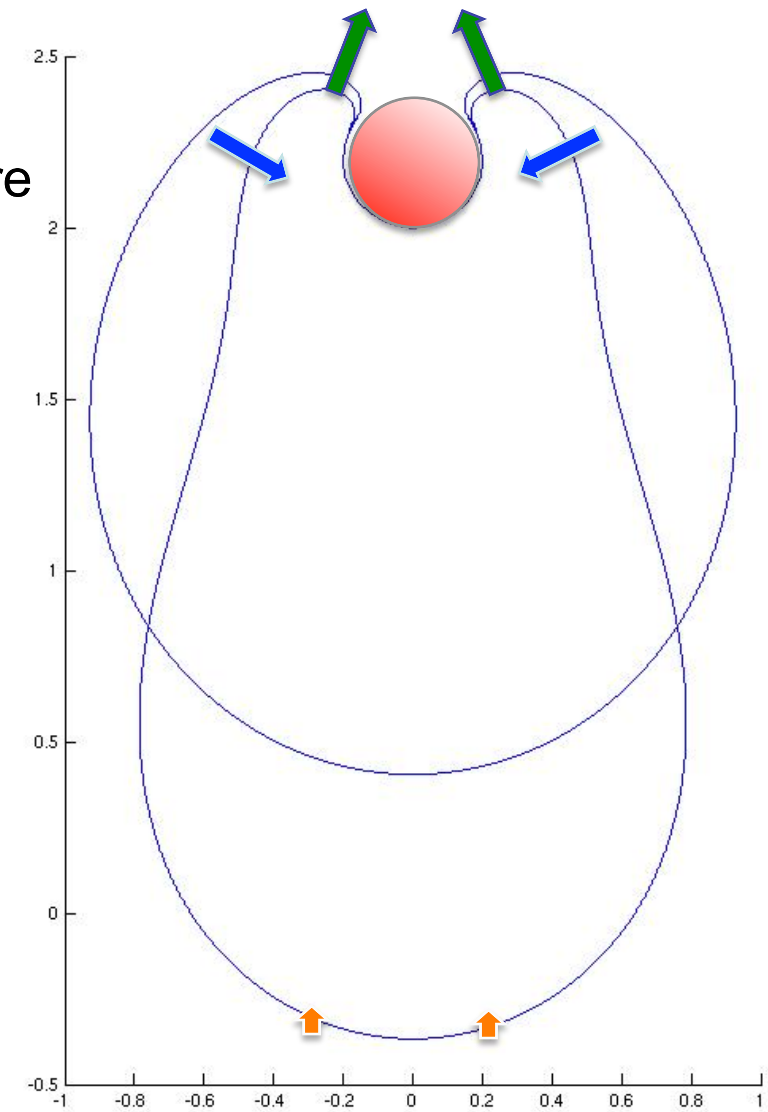




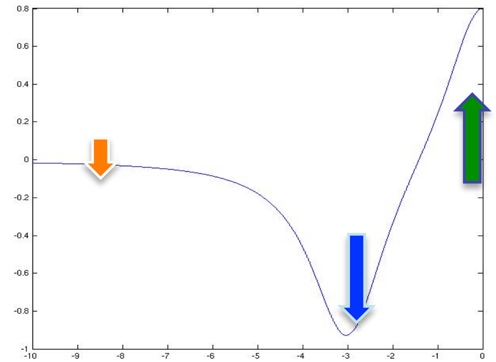
Active zipper engulfs significantly faster than passive zipper

Outlook – model of signalling and cytoskeleton

Cell shape for given cell-pressure profile



Applied pressure profile:

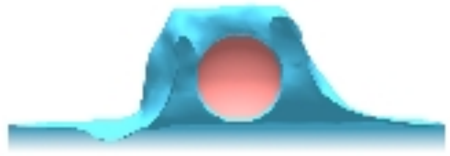


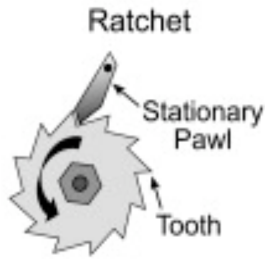
Contour length

Need to calculate pressure from model for acto-myosin cytoskeleton and signalling

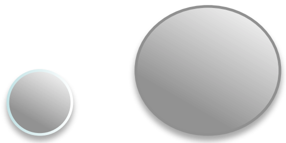
Summary

- Implemented a 3D ratchet model for Zipper mechanism.

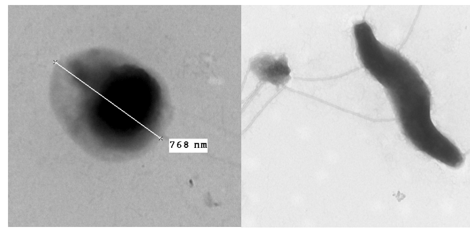




- Particle size does not matter for active zipper, but for passive zipper.

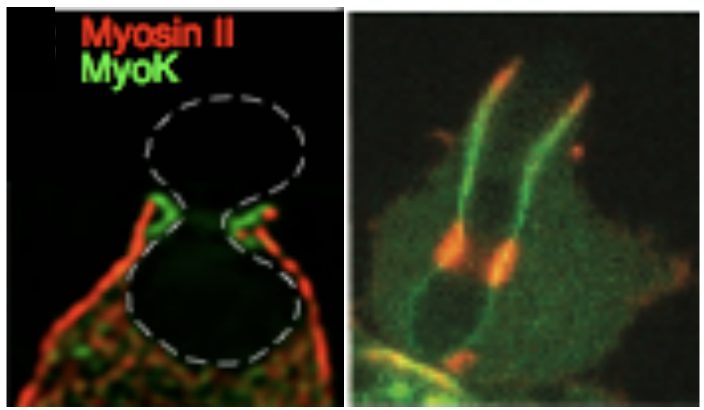


- Biochemical signalling pathways added by evolution for additional robustness?



Coccoid and helical shapes

- Shape and orientation matter for active zipper: Potentially important for host-pathogen interactions and efficient drug design.



- Current model does not close cups well, contraction by motor proteins necessary? Wave-like nature of actin polymerization?

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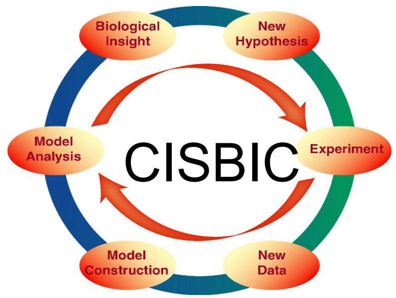
BBSRC bioscience for the future



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