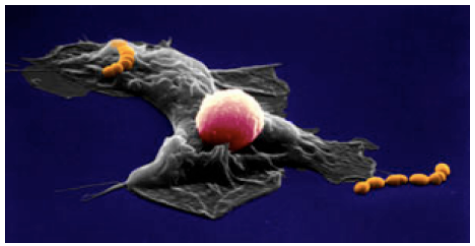
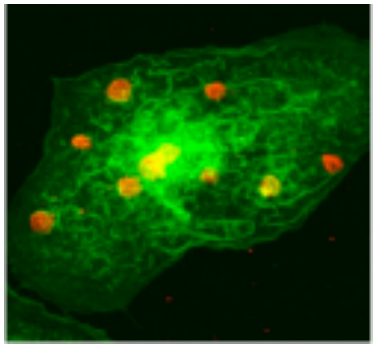
How one cell eats another –

Experiments and modelling elucidate early signalling events and biophysical requirements for uptake





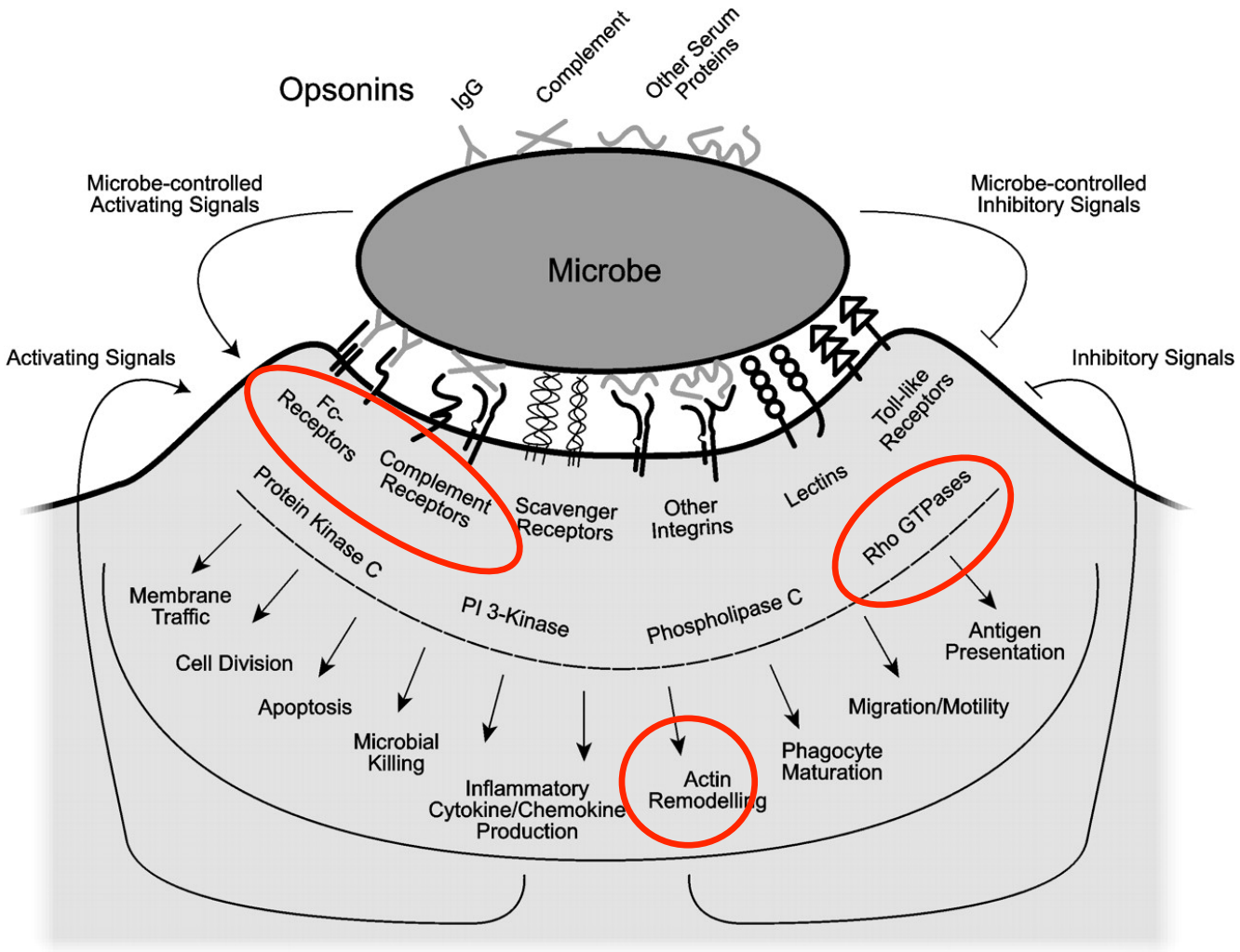
Robert Endres

Biological Physics Group at Imperial College:

http://www3.imperial.ac.uk/biologicalphysics

IOP – Complexity in Biological Systems – Bath, 20 May 2010

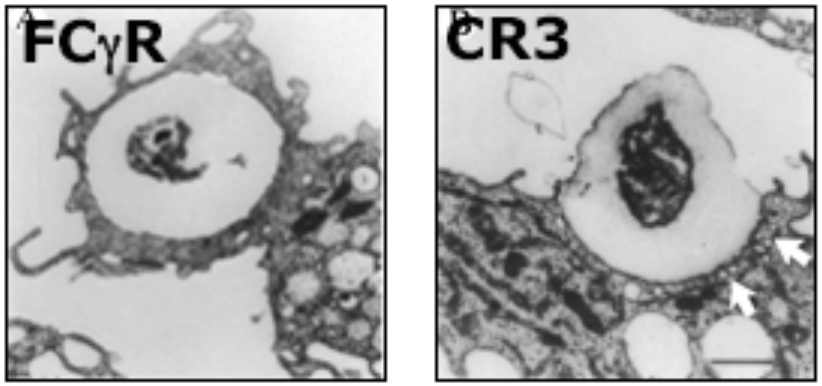
Daunting signalling complexity in phagocytosis



about 140 different molecular species are involved

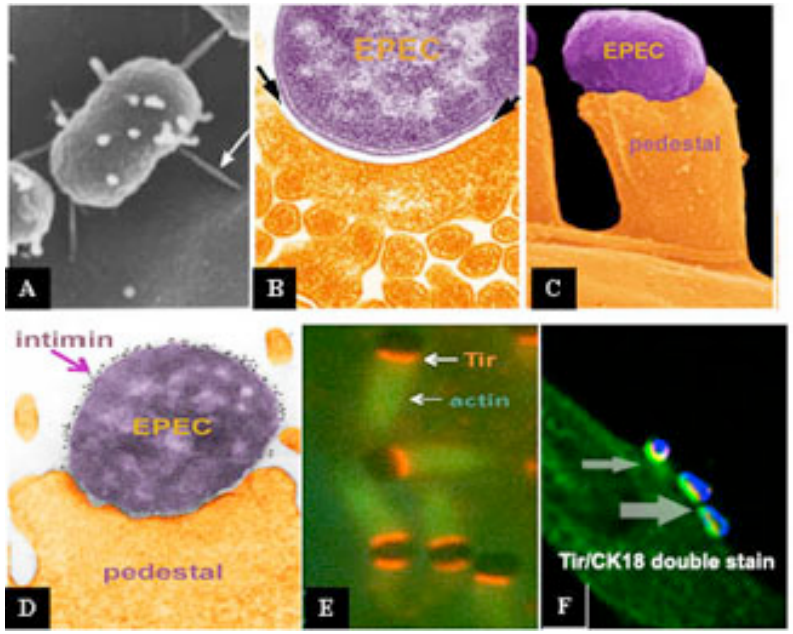
Underhill & Ozinsky (2002)

Actin-rich protrusions in phagocytosis…



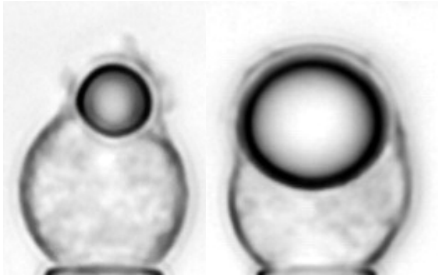
Allen & Aderem (1996)

…and anti-phagocytosis



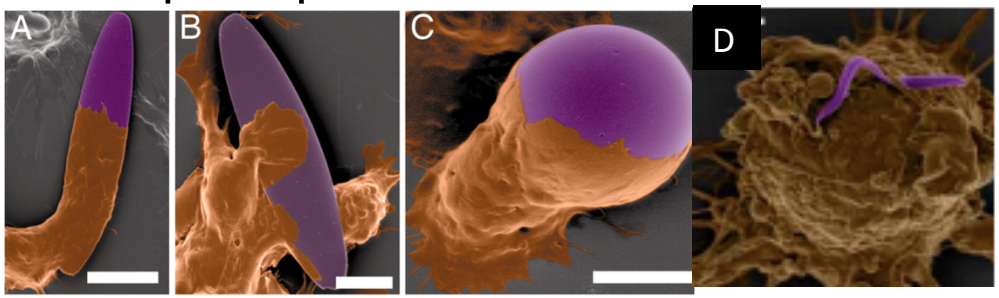
Universal biophysical aspects of phagocytosis

1. Size (in)dependence (endocytosis vs phagocytosis)



Herant et al. (2006)

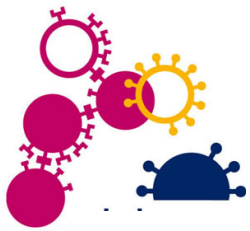
2. Shape dependence

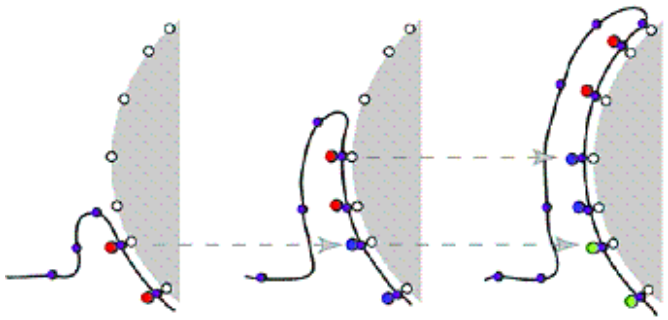


Champion et al. (2006,2009)

3. Elastic properties

4. Ligand density → Zipper mechanism





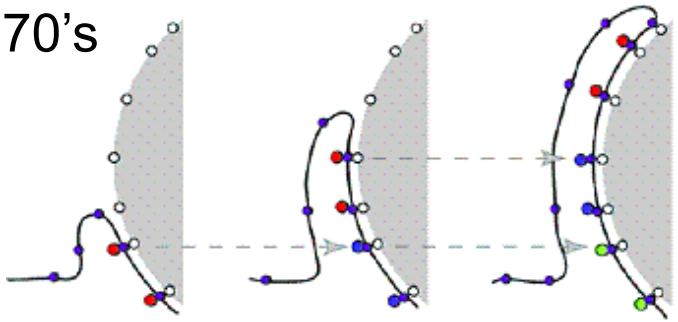
Griffin et al. (1975), Swanson (2008)

Outline

1. Minimal biophysical model of early uptake in phagocytosis
    - Cup-shape and energetic requirements
2. Experimental verification using Fcy-R and IgG-coated latex particles
3. Work in progress - extension of model and new experiments
    - Model of acto-myosin cytoskeleton
    - Experimental role of myosin isoforms
4. Summary

The Zipper mechanism: generally accepted but untested

1970's

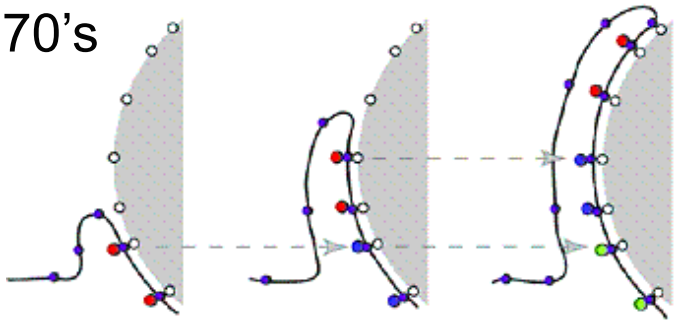


Zipper mechanism:
Unidirectional, sequential ligand -receptor interactions guide membrane around particle.

→ Can it explain biophysical aspects?

The Zipper mechanism: generally accepted but untested

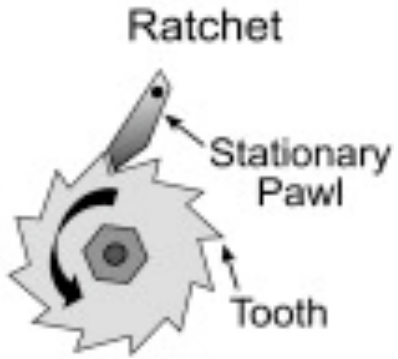
1970's



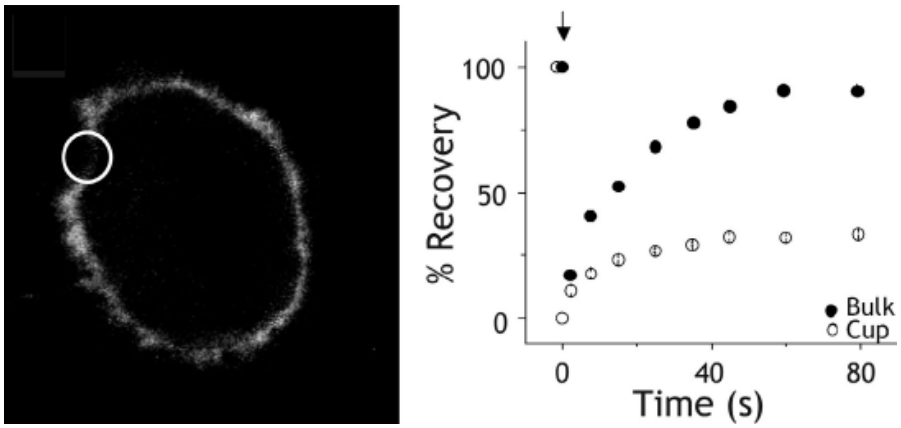
Zipper mechanism:
Unidirectional, sequential ligand -receptor interactions guide membrane around particle.

→ Can it explain biophysical aspects?

Ratchet-like mechanism ?



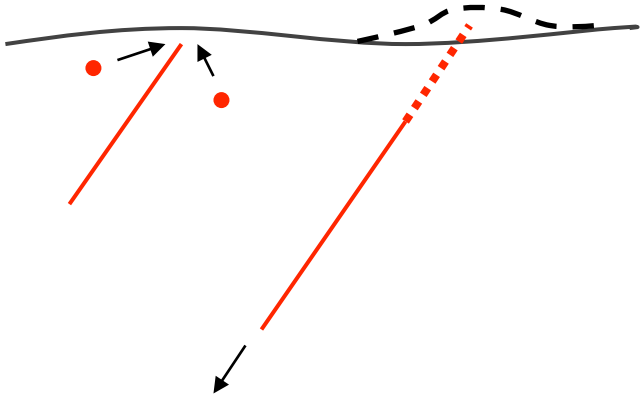
FRAP: Immobilization of proteins and lipids in cups



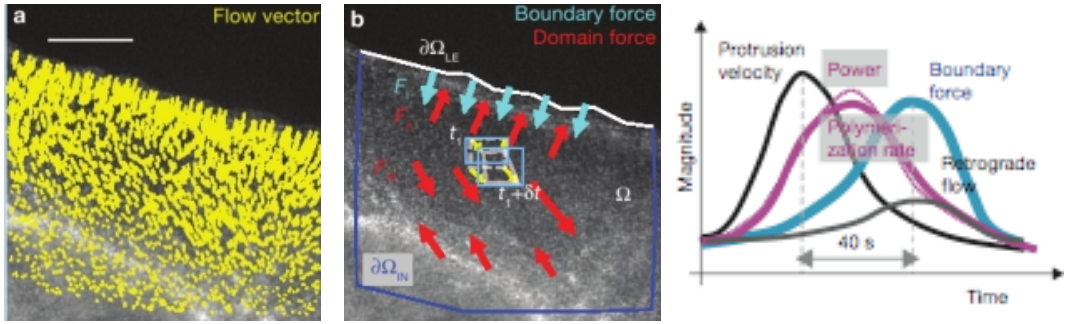
Corbett-Nelson et al. (2006)

Model ingredients

Actin polymerizes at barbed end

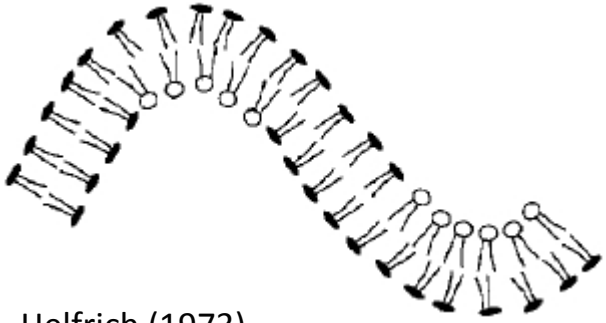


Fluorescent speckle microscopy



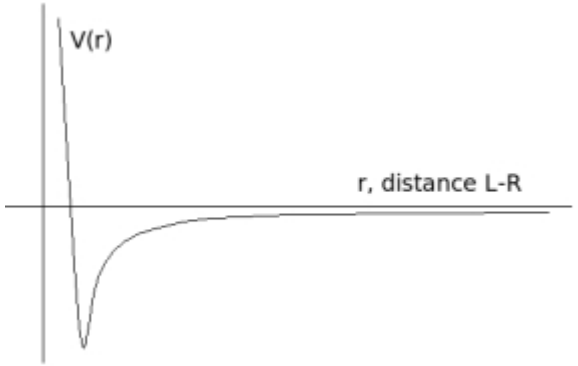
Ji et al. (2008)

Membrane energy: bending, surface tension, volume constraint

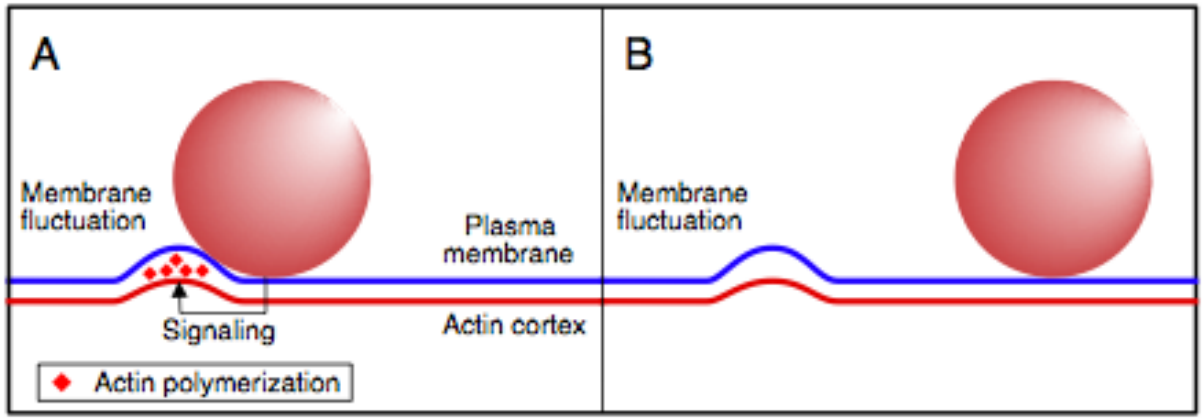


Helfrich (1973)

Ligand-receptor binding



Minimal biophysical model for zipper mechanism



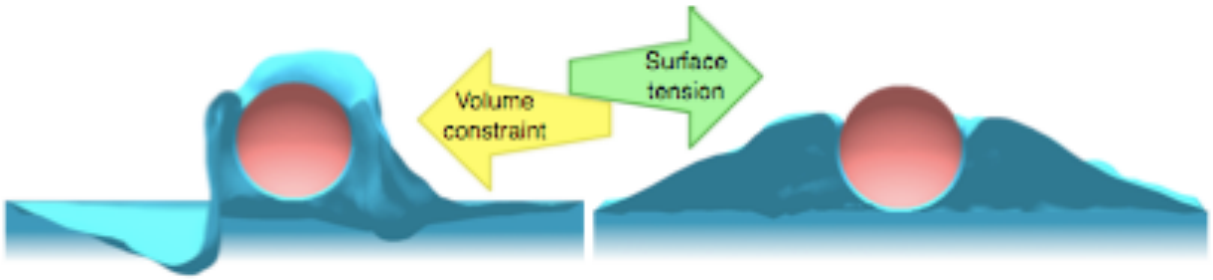
(1) Random, thermal membrane fluctuation.

(2a) If near particle, ligand-receptor binding leads to actin polymerization, stabilizing fluctuation → irreversible → ratchet.

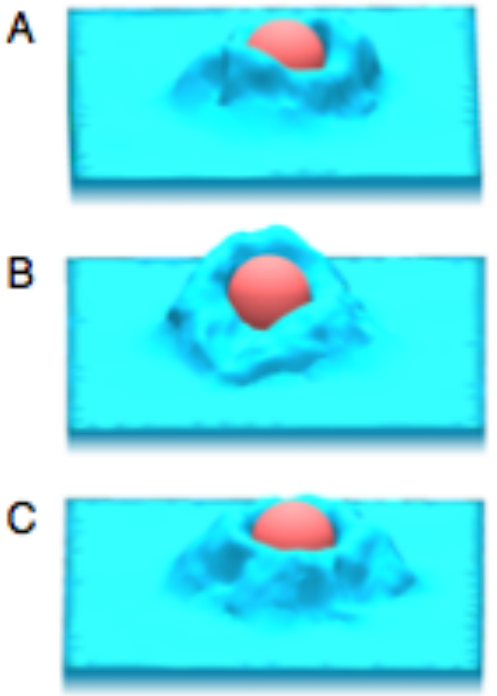
(2b) If away from particle, no stabilization and membrane fluctuation may be reversed at a later time.

(3) Model implemented with finite-element simulations and Monte Carlo algorithm.

Engulfment over wide range of parameters



different parameters
→ robustness

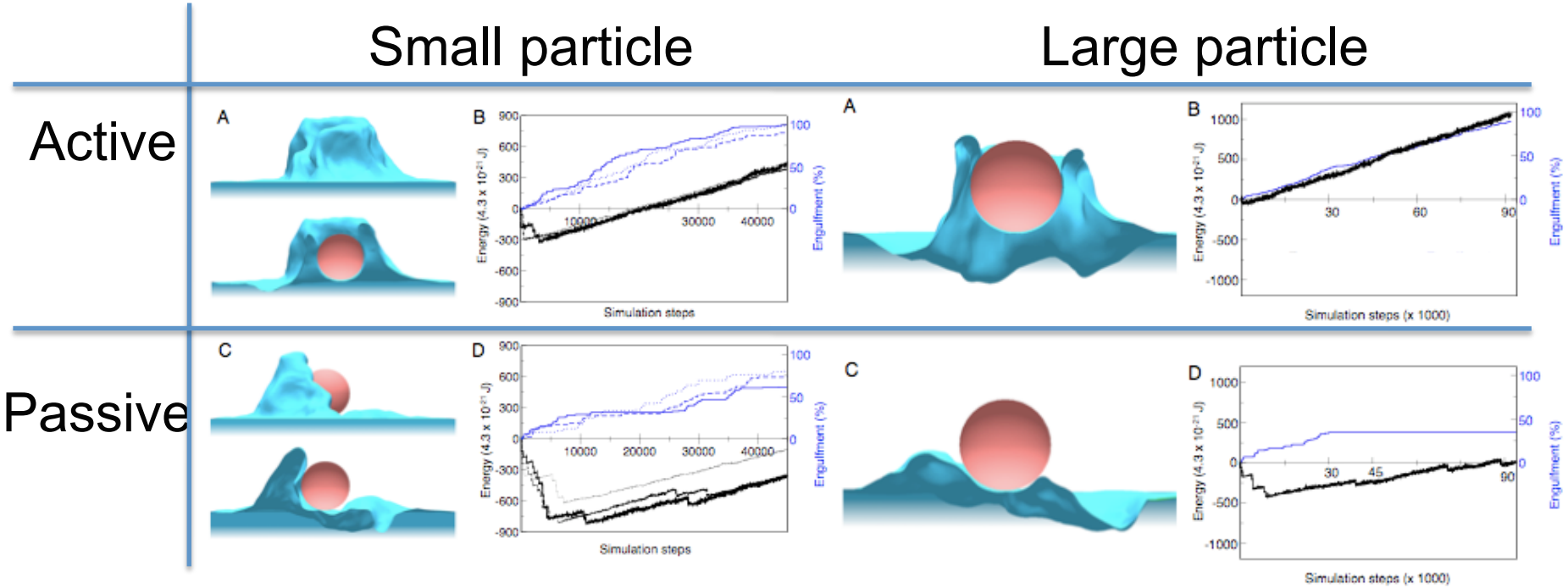


same parameters
→ variability

How important is actin-polymerization?

Would “passive” ligand-receptor binding be sufficient?

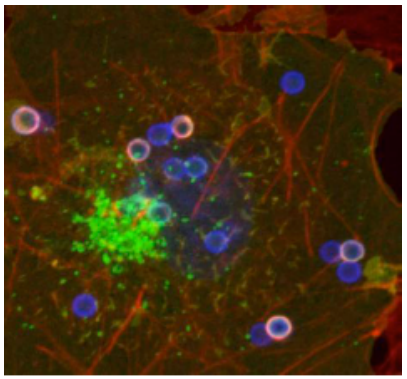
“Active” versus “passive” engulfment

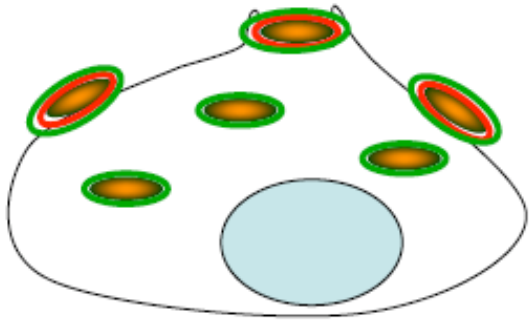


- Passive engulfment still works for small particles, but is much slower and cups are more variable.
- Can we experimentally test these two predictions?

Imaging of phagocytosis using transfected fibroblasts

Phagocytic assay





Time series data of FcR dynamics during uptake (imaging of 3µm particles)

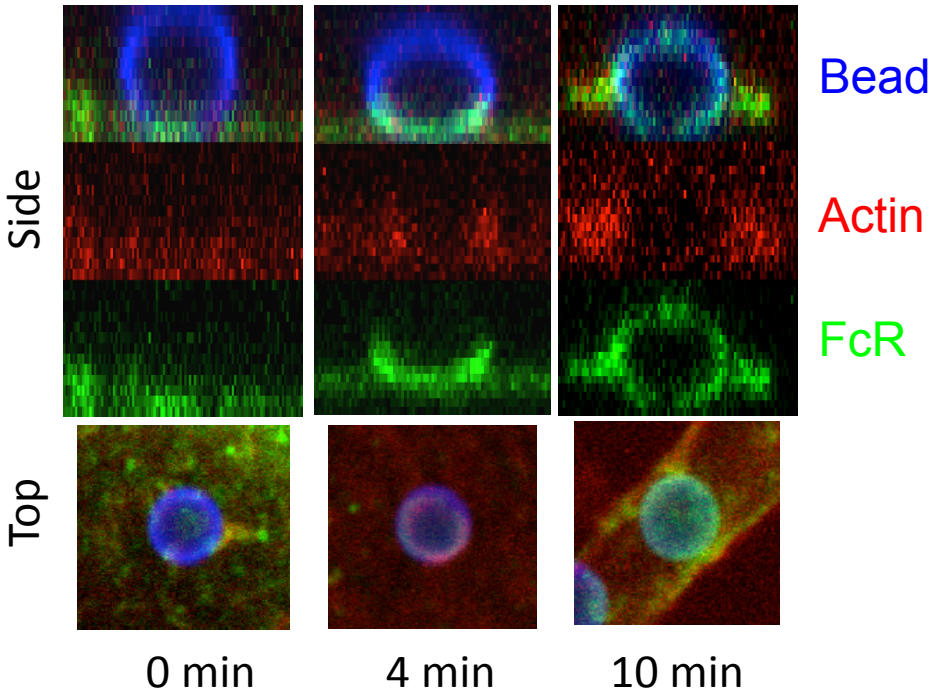
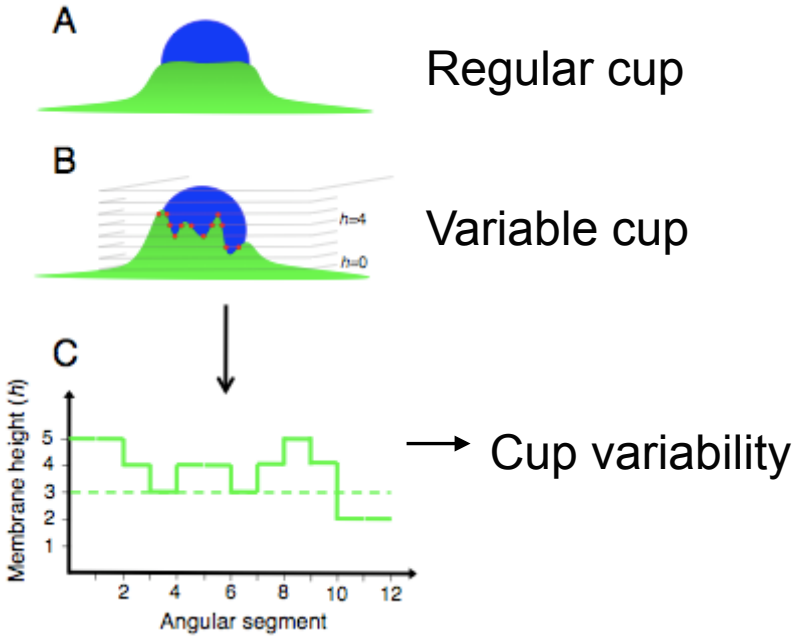


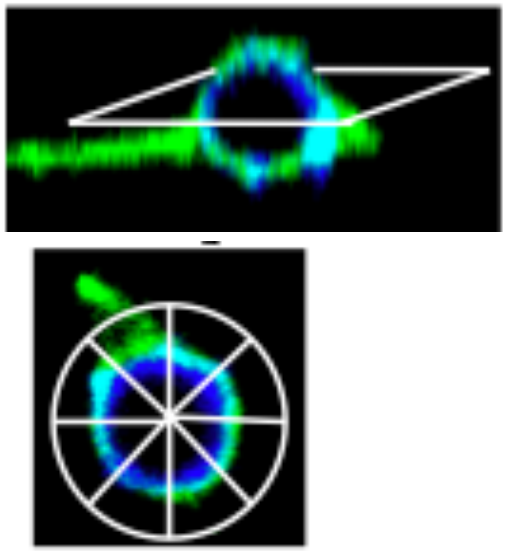
Image analysis of 3d-data from confocal microscopy

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

(b) Cells expressing signalling-dead mutant receptor
(c) Cells transfected with cytochalasin D

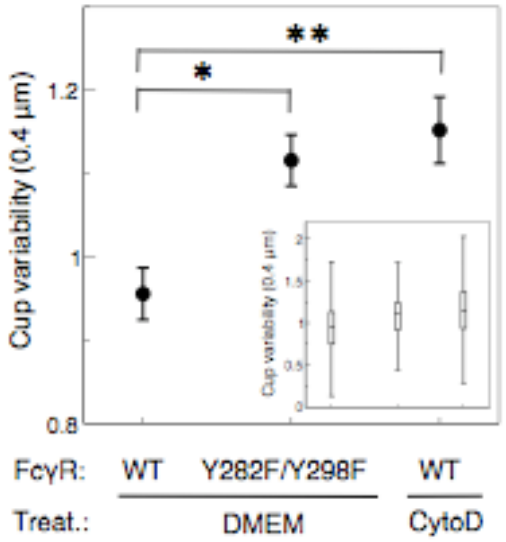
} **passive zippers**





Experiments confirm our predictions

Passive-zipper cells
have more variable cups



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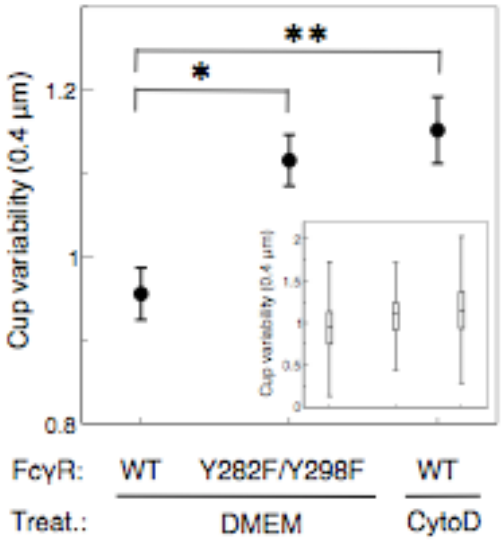
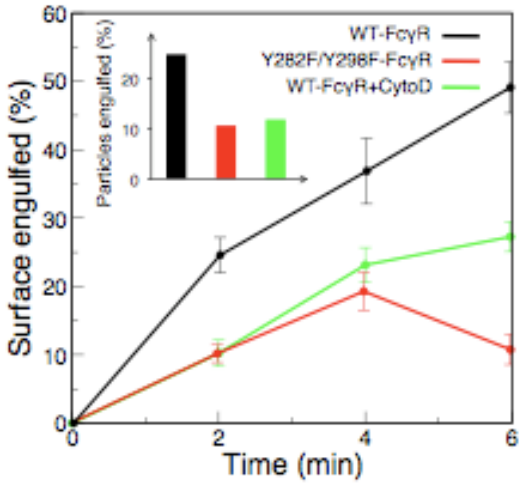


Passive-zipper cells
engulf more slowly



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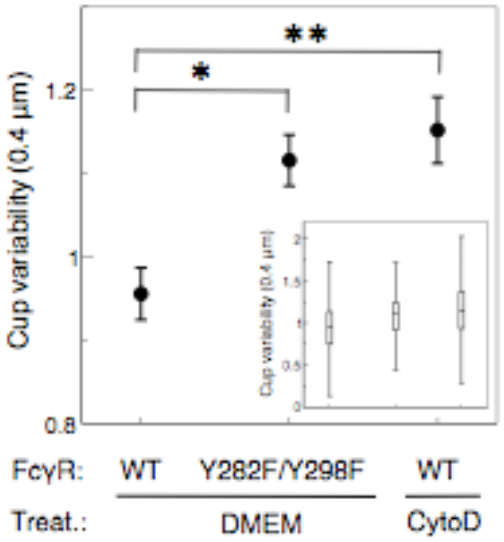

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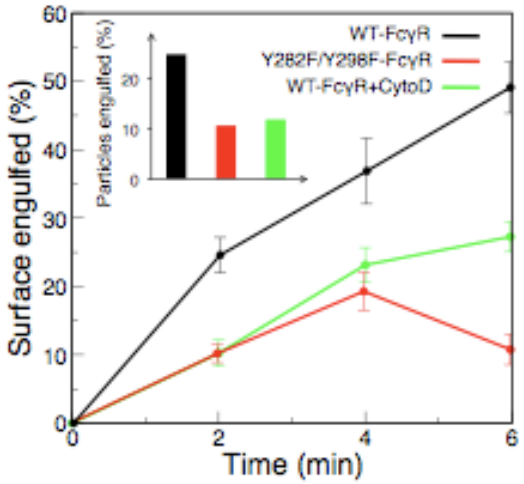
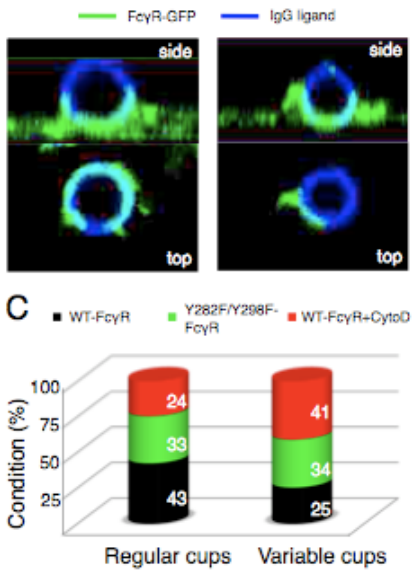
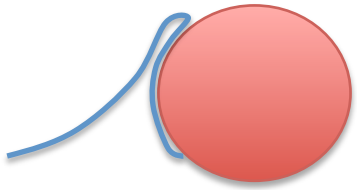




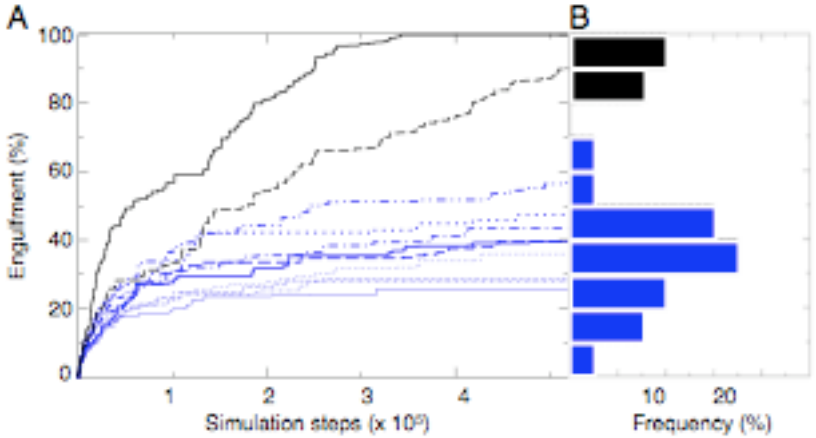
Universal
features in
cup shape

Model confirms mechanical bottleneck….

Mechanical bottleneck



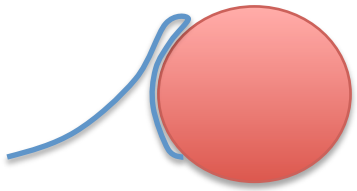
Simulations Experiment



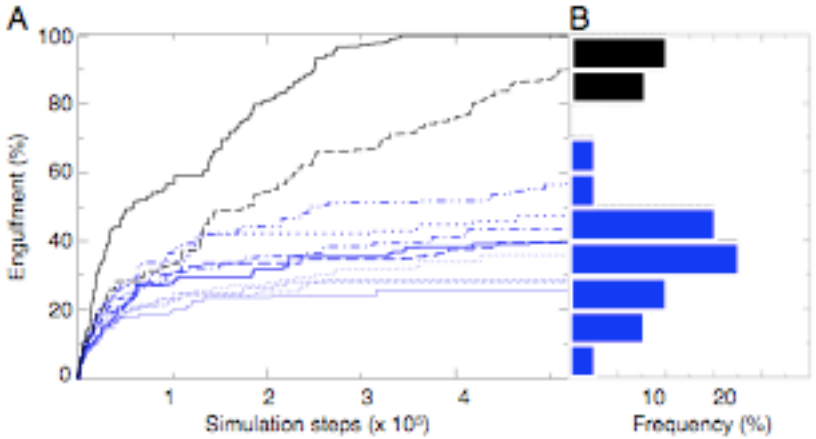
Bimodal distribution from experiments with WT-FcR after 10 min.

Model confirms mechanical bottleneck….

Mechanical bottleneck



Simulations Experiment

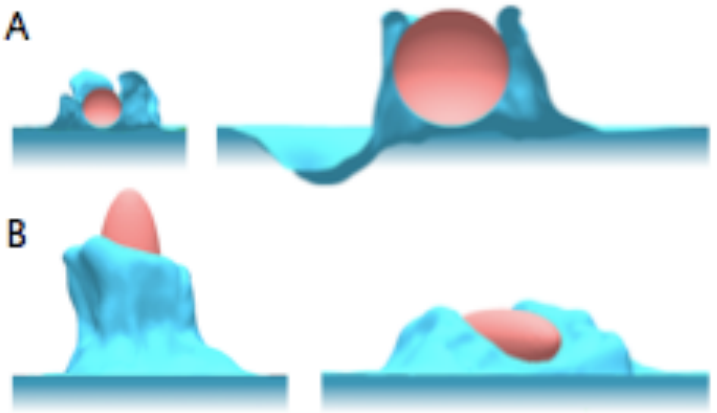


Bimodal distribution from experiments with WT-FcR after 10 min.

….and shape dependence

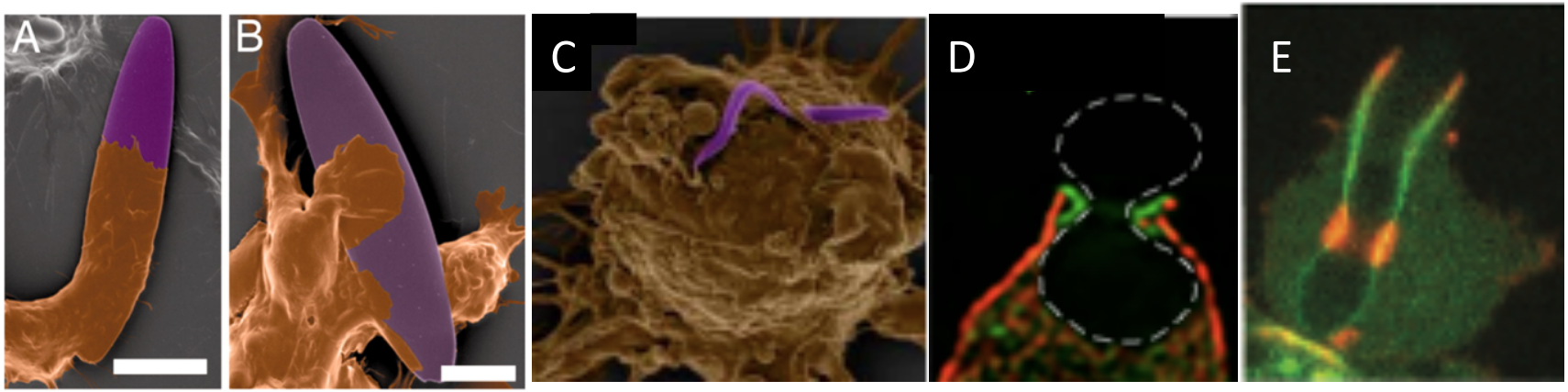
Size independence

Shape dependence



→Model difficulties with cup closure

New improved model:

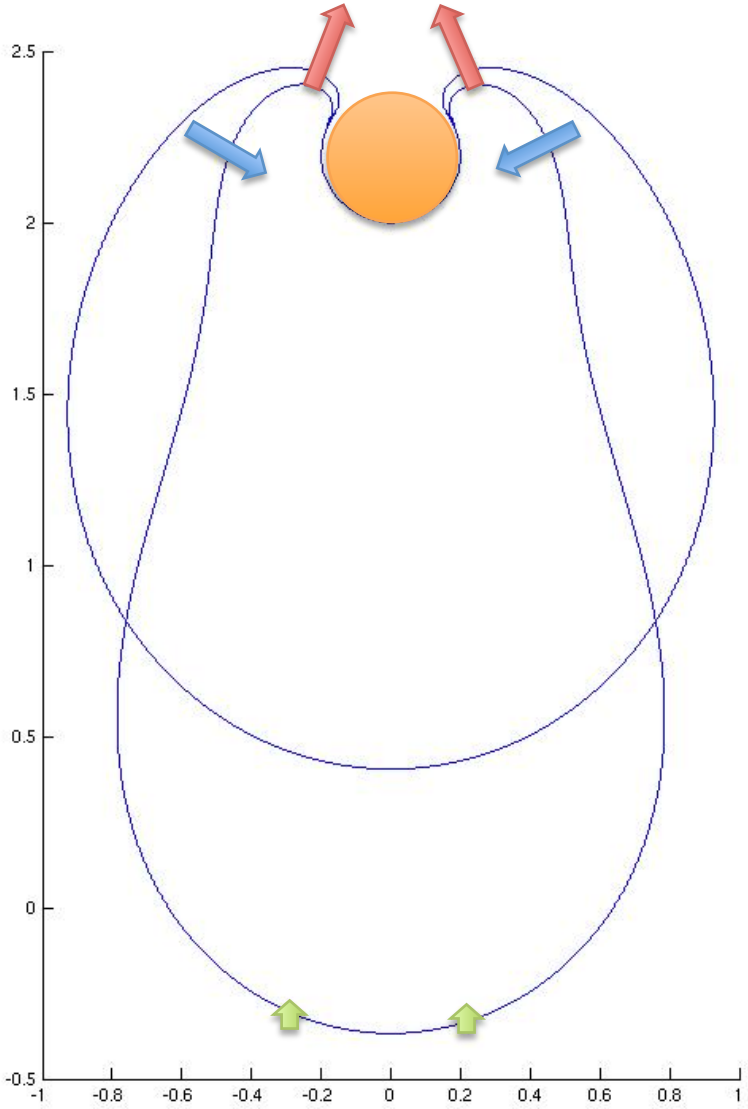


Address the following fundamental questions:

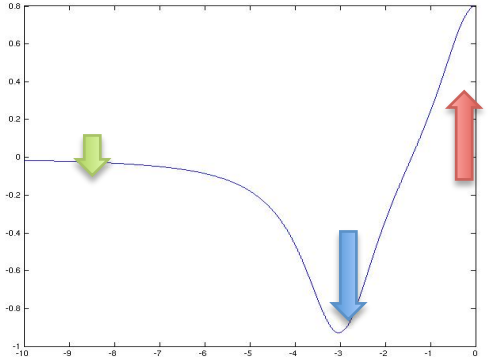
(1) Particle-shape dependence
(2) Cup closure, particle squeezing
(3) Actin waves at cup
(4) Particle stiffness dependence

→ Extend model to include acto-myosin cytoskeleton

Preliminary: cell shape for given cell-pressure profile



Applied pressure profile:



Contour length

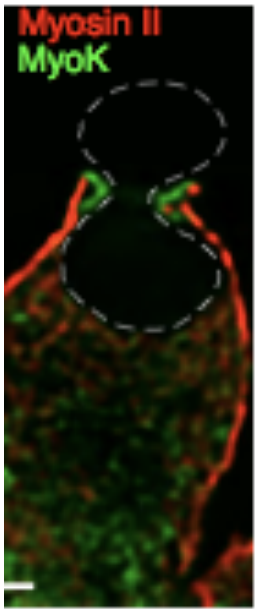
Next: calculate pressure from model for acto-myosin cytoskeleton and signalling

New experiments:

1) Role of motor proteins in cup closure and particle squeezing

Imaging by confocal microscopy of motor proteins using anti-bodies and GFP-constructs

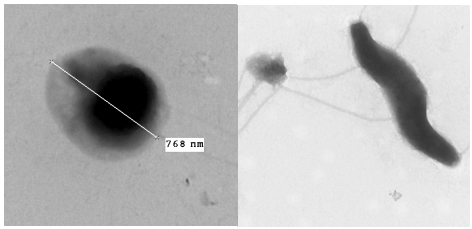
Knock-downs by RNAi and imaging by SEM



2) Shape dependence of bacterial uptake

Human pathogen *Campylobacter*

Unfortunately very small, taken up by epithelia via endocytosis, avoids lysosome



Coccoid and helical shapes

Summary

- Importance of **universal biophysical requirements** in phagocytosis:
  (a) ligand density, (b) particle shape and (c) stiffness.

- **Minimal biophysical model** uses ratchet-like mechanism for zipper.

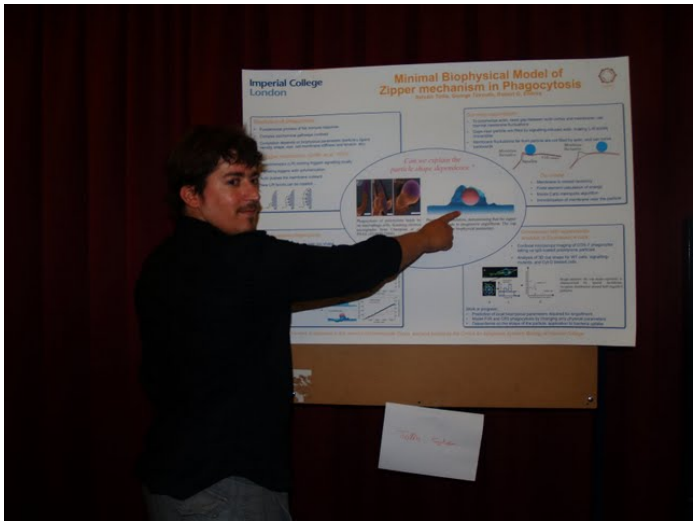
- **Model predictions** for active and passive zippers:
  Removal of actin-driven processes leads to slower engulfment and highly variable cups. This is confirmed by experiments.

- **Active zipper:** shape but not size matters.
  Passive zipper: size matters as well.

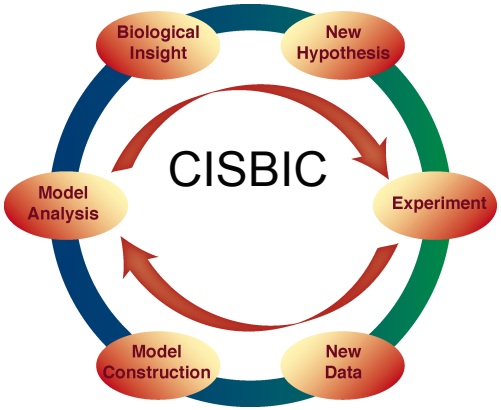
- Active processes make phagocytosis **robust**, and enable engulfment of large particles.

- **New developments:** model of acto-myosin cytoskeleton and experiments and myosins.

Acknowledgements



Sylvain Tollis in Croatia '09



- Members of the Biological Physics group:
  - Sylvain Tollis
  - Anna Dart
  - George Tzircotis
- Members of Vania Braga's lab

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