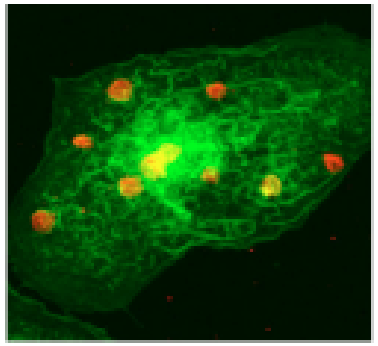
Imperial College London

CISBIC Subproject 2: How one cell eats another - experiments and modelling elucidate early signalling events and biophysical requirements for uptake.

Robert Endres
George Tzircotis
Sylvain Tollis

BBSRC Grant Holders' Workshop
7. January 2010



Biological Physics Group at Imperial College:
http://www3.imperial.ac.uk/biologicalphysics

bbsrc
biotechnology and biological sciences research council

Imperial College London

Outline

1. Model of early signalling events in Fc-R phagocytosis: mechanical bottle neck

   (Jeroen van Zon, George Tzircotis, Emmanuelle Caron, Martin Howard)

2. Extension of model and new experiments: variability and energetic requirements

   (Sylvain Tollis, George Tzircotis, Robert Endres)

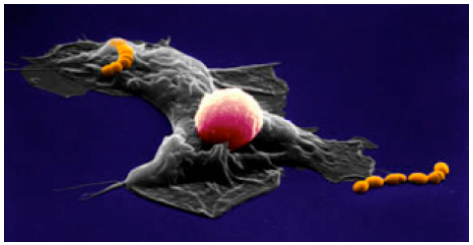
3. Summary

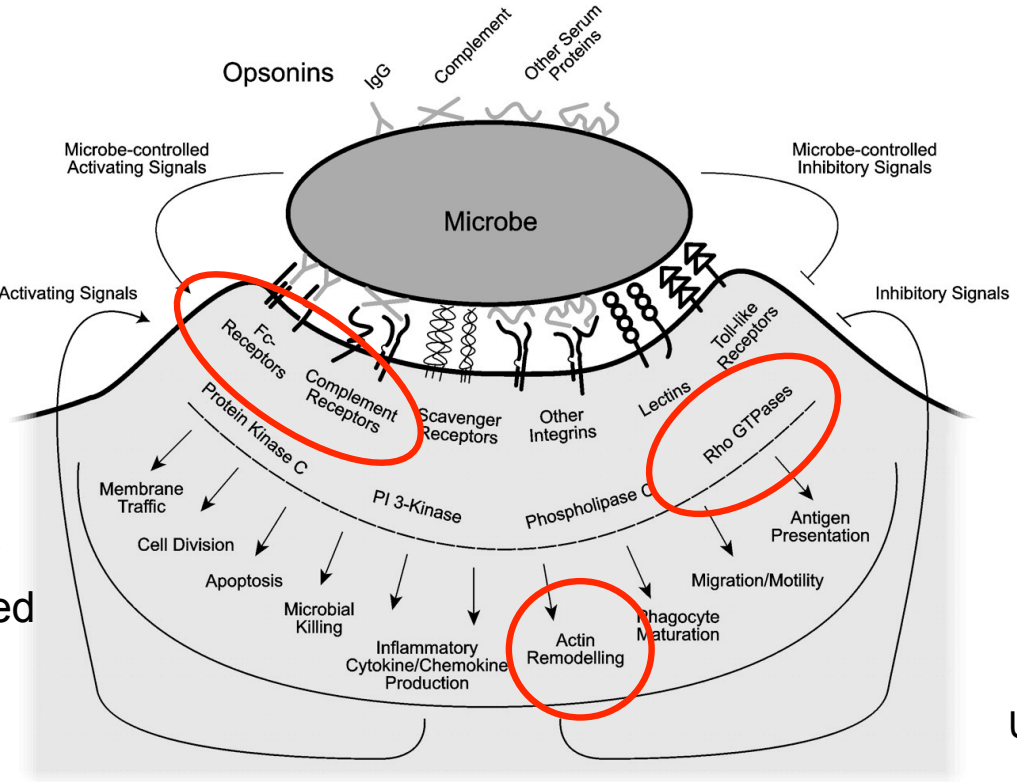
4. Future directions

CISBIC

Imperial College London

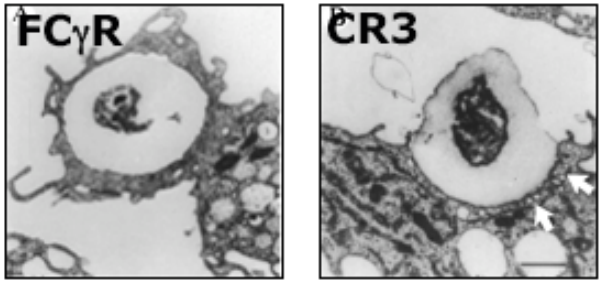
Daunting signalling complexity in phagocytosis





about 140 different molecular species are involved

Underhill & Ozinsky (2002)

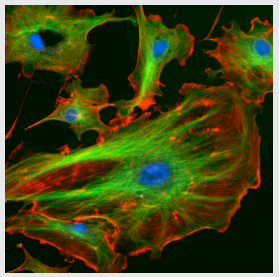


Allen & Aderem (1996)

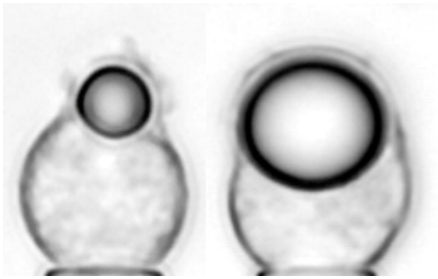
CISBIC

Imperial College London

Universal biophysical aspects of phagocytosis

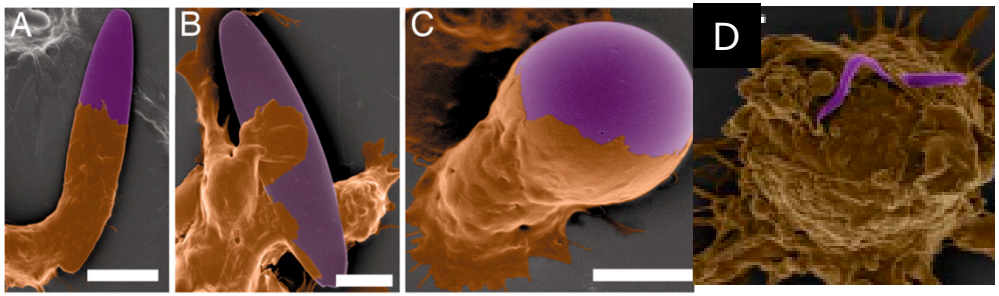


1. Size (in)dependence (endocytosis vs phagocytosis)



Herant *et al*. (2006)

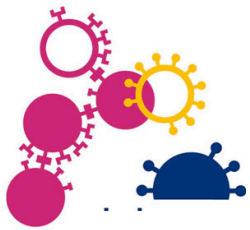
2. Shape dependence

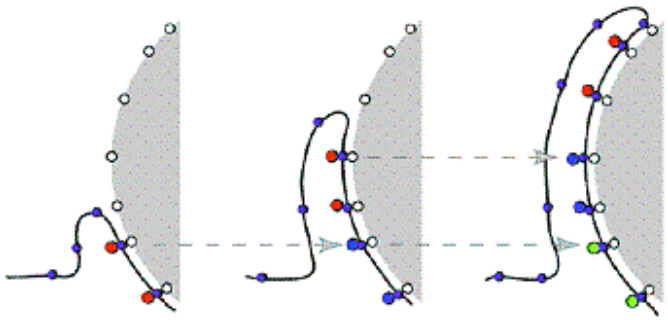


Champion *et al*. (2006,2009)

3. Elastic properties

4. Ligand density → Zipper mechanism



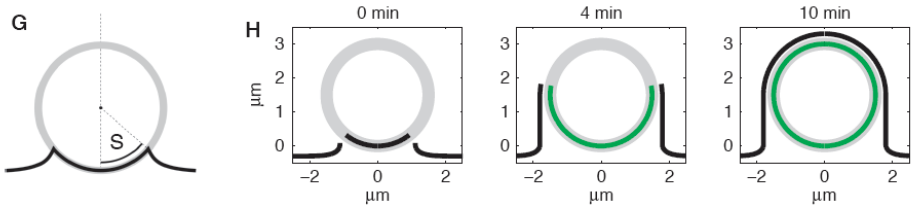


Griffin *et al*. (1975), Swanson (2008)

CISBIC

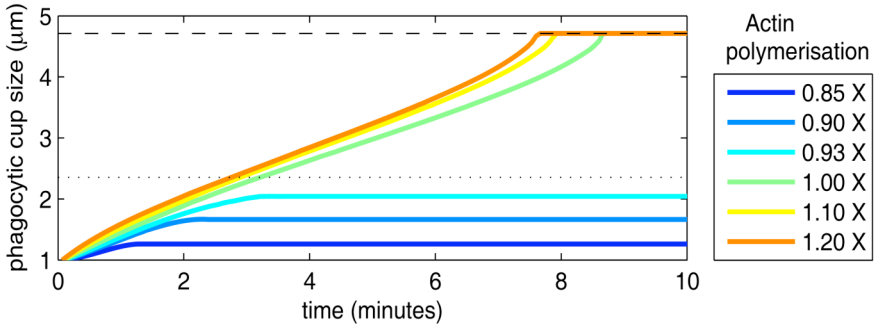
Imperial College London

Model of early events in Fc-R phagocytosis

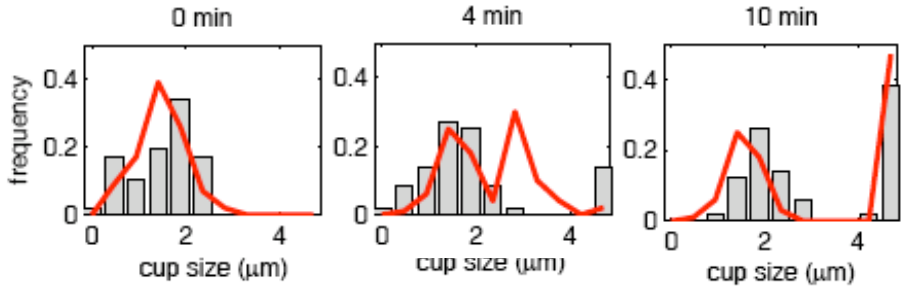


Model for phagocytic cup formation:
Membrane dynamics, receptor diffusion and actin force.

Completely determined by bending modulus and cortical tension.



Predict mechanical bottleneck:
Half cup is point of max resistance due to cortical tension.
If force from actin can overcome this point, then cup completes.



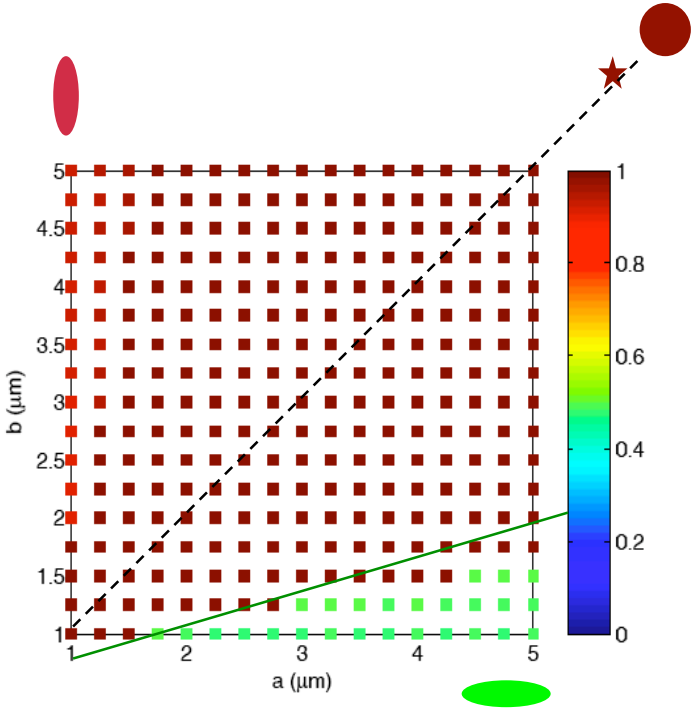
Experimental verification: Cup progression: actual data (grey bars) vs. model (red line).

→ Bimodal distribution

CISBIC

Imperial College London

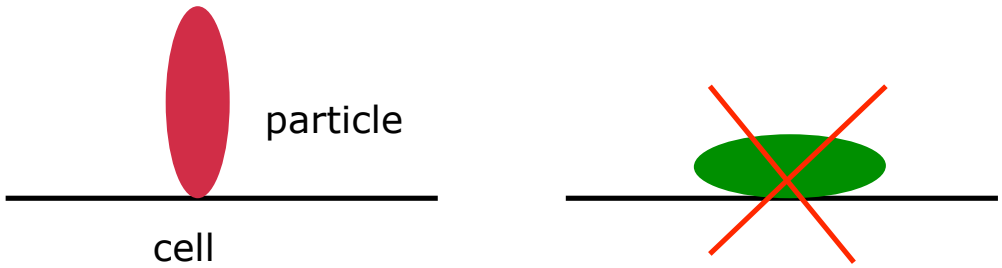
Model of early events in Fc-R phagocytosis



Influence of particle shape on uptake:

Tall/thin particles are phagocytosed, short/flat particles are not phagocytosed.

Further experiments using 6μm beads show similar phagocytosis dynamics and excellent agreement with model.



CISBIC

Imperial College London

Remaining/new open questions:

- In experimental data, large variability in cup shape and large particle-to-particle variation → stochastic model
- Even with signalling-dead receptor mutant or cytochalasin-D treatment lead to engulfment for some particles.
- How important are active (actin-driven) processes?
- Influence of particle shape without assuming rotational symmetry.

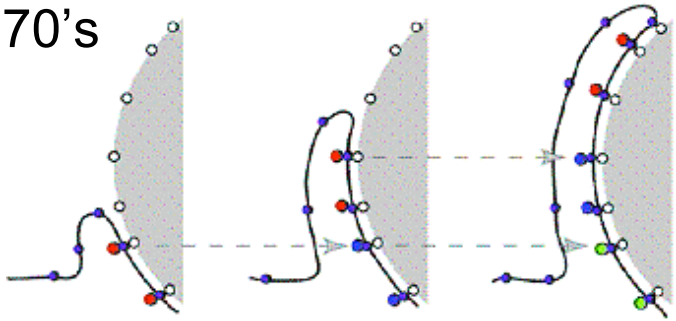
→ New, extended model

CISBIC

Imperial College London

The Zipper mechanism - generally accepted but untested

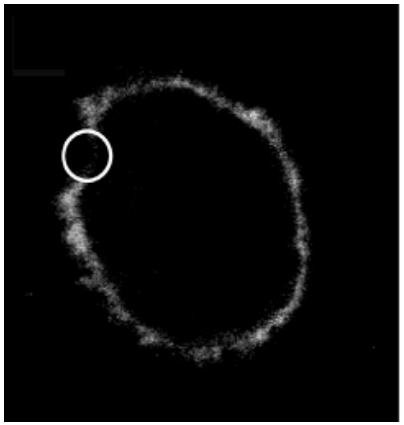
1970's

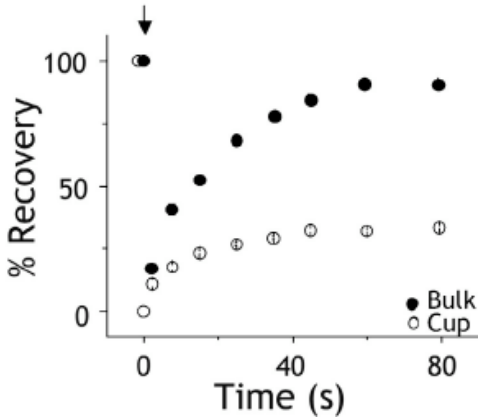


Zipper mechanism:
Unidirectional, sequential ligand-receptor interactions guide membrane around particle.

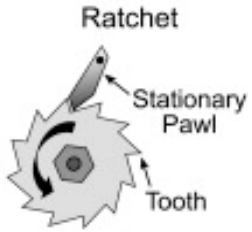
→ Can it explain biophysical aspects?

FRAP: Immobilization of proteins and lipids in cups





ratchet-like mechanism ?



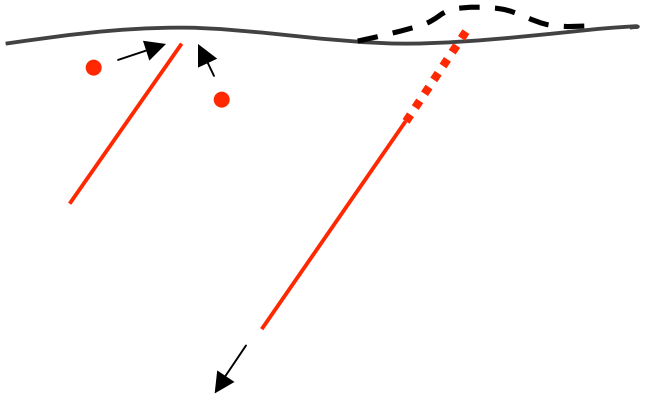
Corbett-Nelson *et al*. (2006)

CISBIC

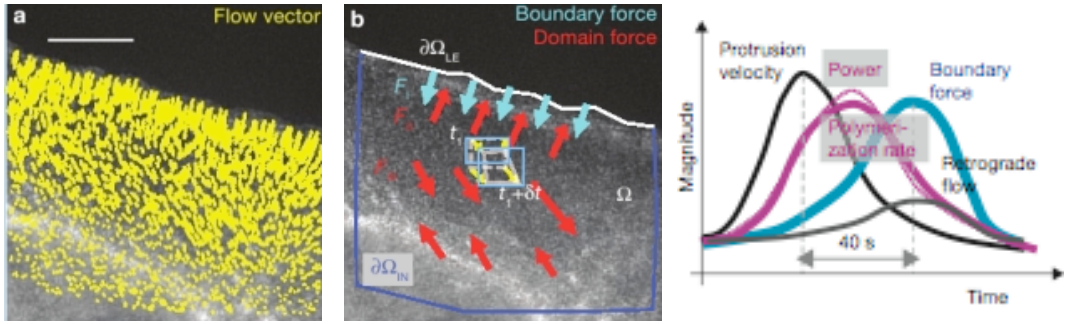
Imperial College London

Model ingredients

Actin polymerizes at barbed end

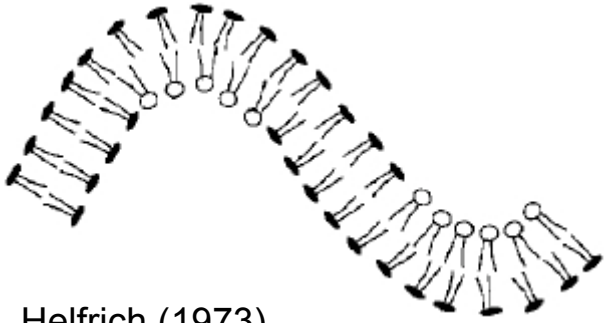


Fluorescent speckle microscopy



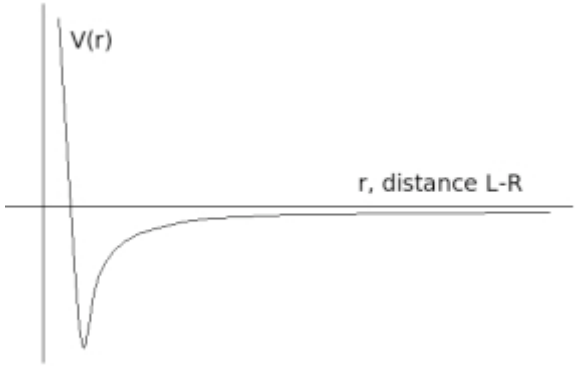
Ji *et al*. (2008)

Membrane energy: bending, surface tension, volume constraint



Helfrich (1973)

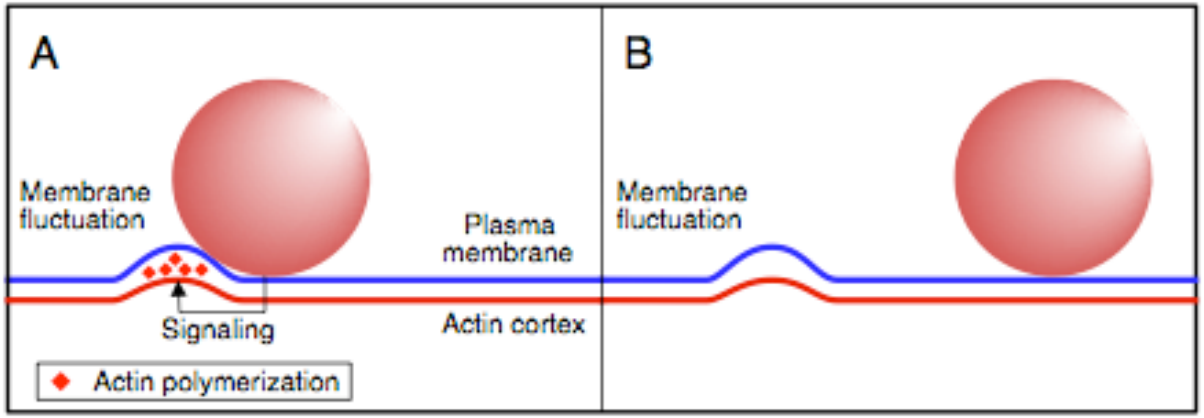
Ligand-receptor binding



CISBIC

Imperial College
London

Minimal biophysical model for zipper mechanism



(1) Random, thermal membrane fluctuation.

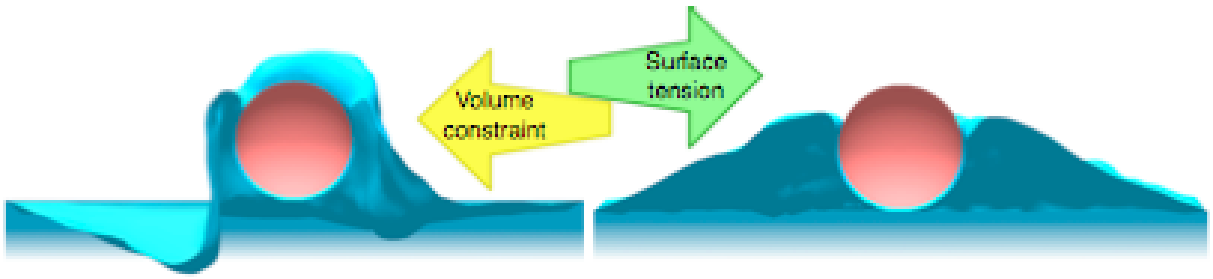
(2a) If near particle, ligand-receptor binding leads to actin polymerization, stabilizing fluctuation → irreversible → ratchet.

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

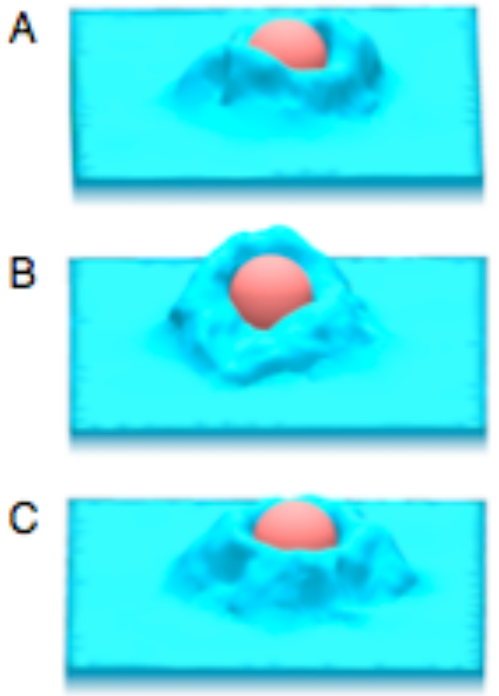
(3) Model implemented with finite-element simulations and Monte Carlo algorithm.

Imperial College London

Engulfment over wide range of parameters



different parameters
→ robustness



same parameters
→ variability

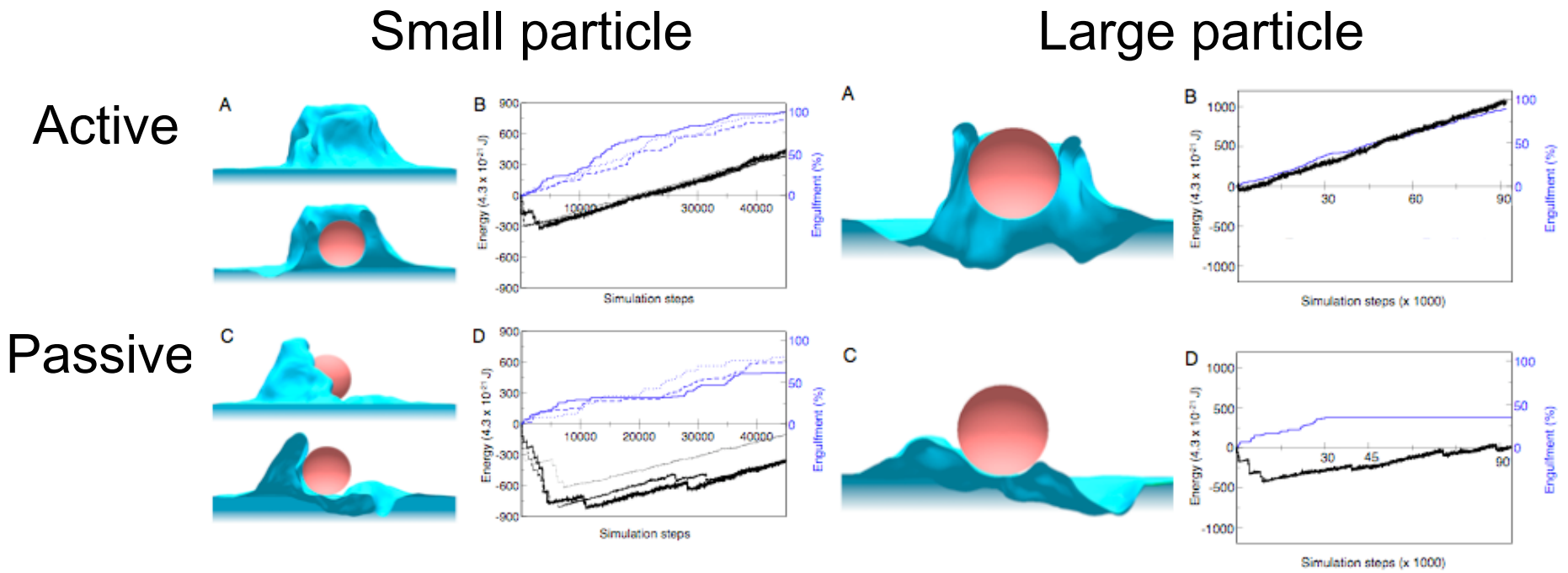
How important is actin-polymerization?

Would “passive” ligand-receptor binding be sufficient?

CISBIC

Imperial College London

“Active” versus “passive” engulfment



Passive engulfment surprisingly still works, but is much slower and cups are more variable.

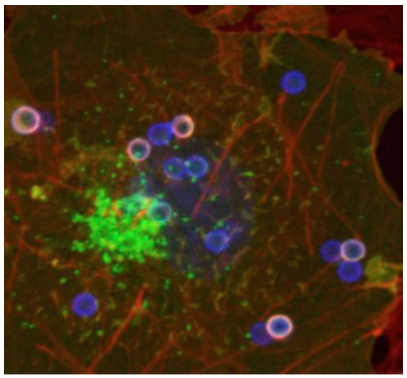
Can we experimentally test these two predictions?

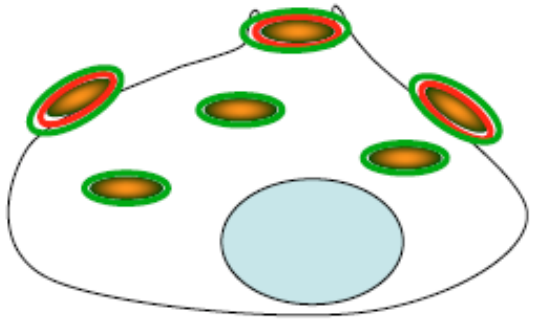
CISBIC

Imperial College London

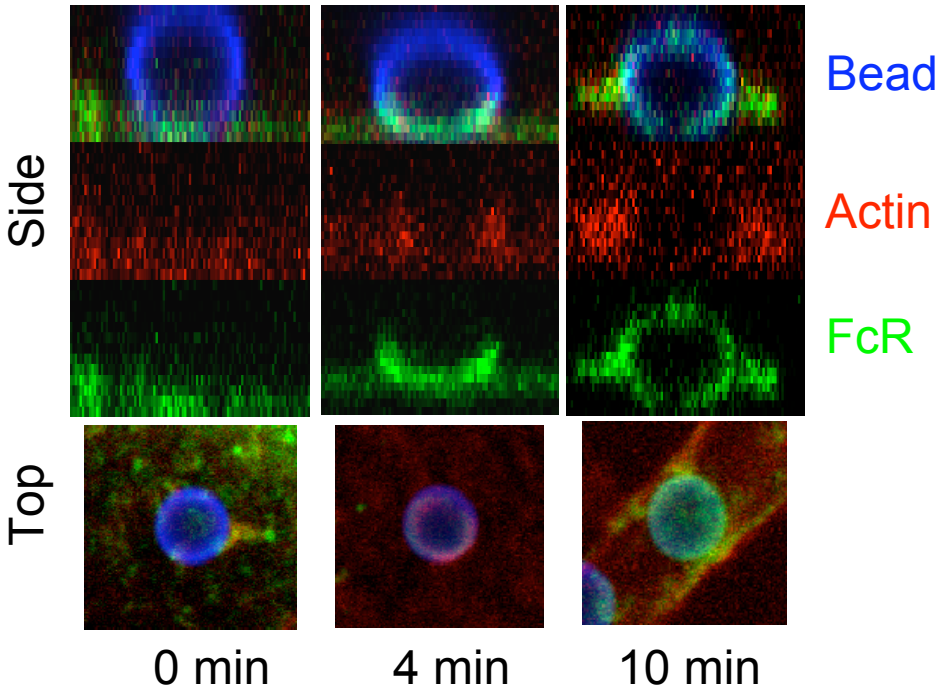
Imaging of phagocytosis using transfected fibroblasts

Phagocytic assay





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



CISBIC

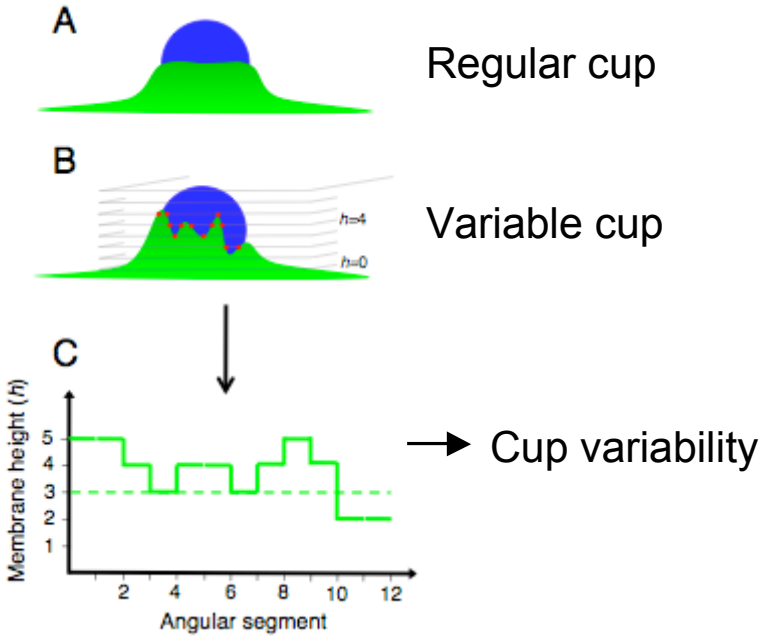
Imperial College London

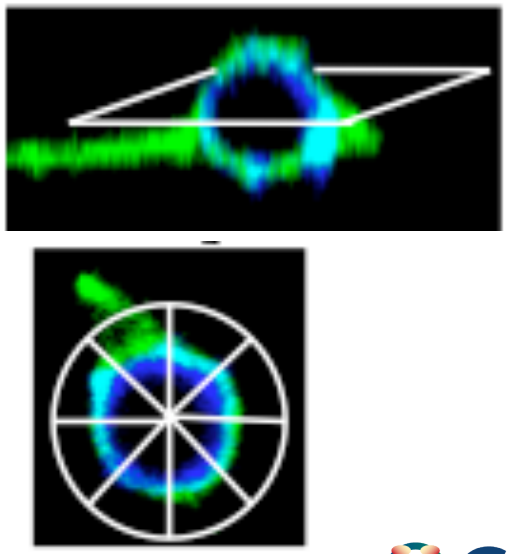
Image analysis of 3d-data from confocal microscopy

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

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

} **passive zippers**



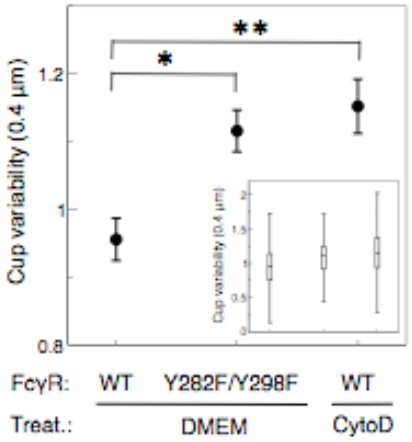


CISBIC

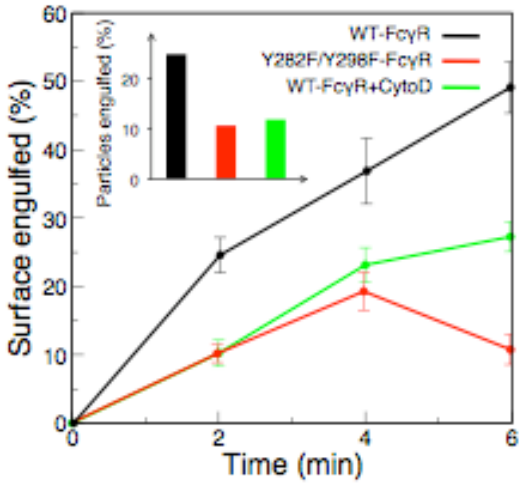
Imperial College London

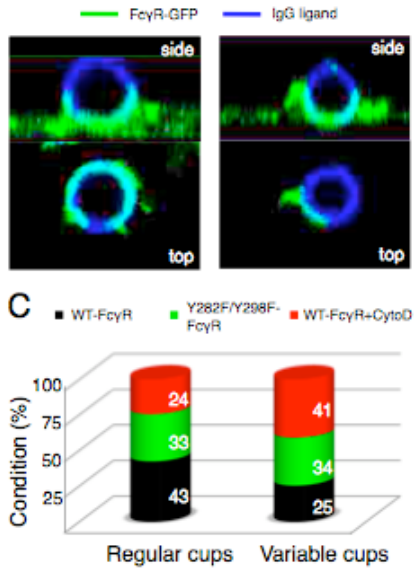
Experiments confirm our predictions

Passive-zipper cells have more variable cups



Passive-zipper cells engulf more slowly





Universal features in cup shape

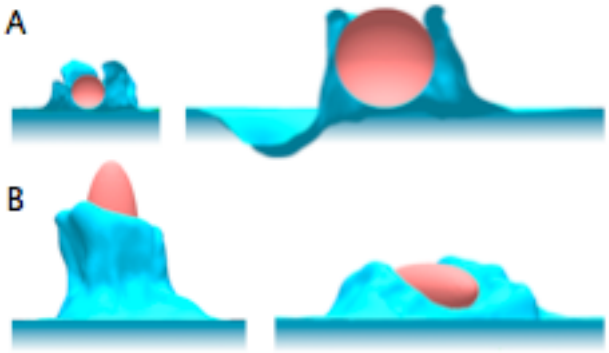
CISBIC

Imperial College London

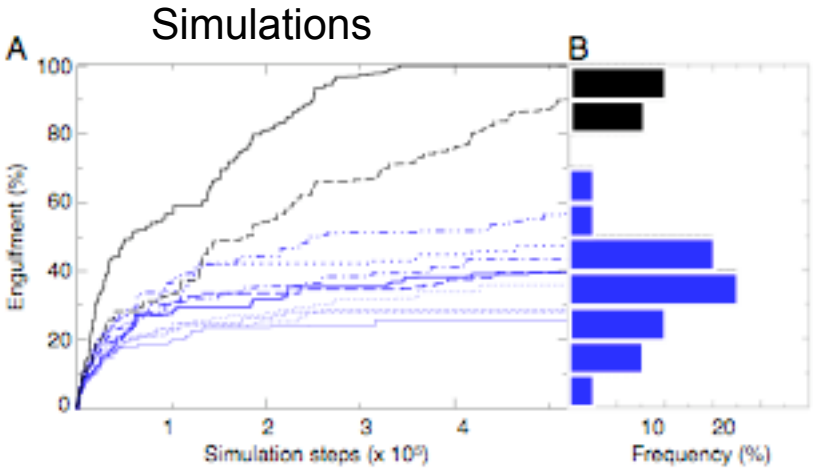
Model also confirms shape dependence and bottleneck

Size independence

Shape dependence



Mechanical bottle neck



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

CISBIC

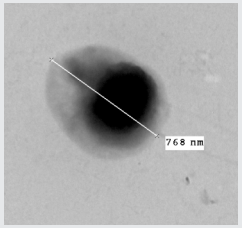
Imperial College London

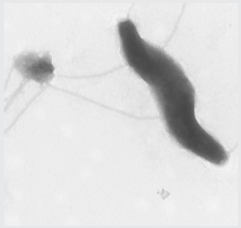
Summary

- Importance of universal biophysical requirements in phagocytosis: (a) ligand density, (b) shape, (c) stiffness.
- Minimal biophysical model proposes ratchet-like mechanism for zipper.
- Model predicts that a removal of actin-driven processes leads to slower engulfment and highly variable cups. This is confirmed by experiments.
- Active processes make phagocytosis robust, and enable engulfment of large particles.

CISBIC

Imperial College London





Campylobacter

Collaborations with other CISBIC subprojects

Sub-project 1 – Phagocytosis of *Campylobacter* – effect of glycosylation mutants (Emily Kay)

Sub-project 3 – Notch signalling and phagocytosis. Effect of Jgd stimulation on uptake (Anna Rose)

Future directions

1. Inclusion of signalling pathways (Rho GTPases) in model
2. Shape dependence (tubercular mycobacteria, helical *Campylobacter*)
3. Mechano-sensitivity during phagocytosis (squeezing of particle, soft vs stiff particles)
4. Later stages of phagocytosis (actin belt, motor proteins, actin waves)
5. Zipper-like engulfment in other areas of biology (bacteria)

CISBIC

Imperial College
London

Recent achievements

S. Tollis, G. Tzircotis, R.G. Endres, **The zipper mechanism in phagocytosis: Energetic requirements and variability in phagocytic cup shape**, submitted.

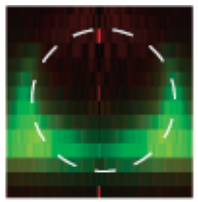
Molecular Systems Biology 5; Article number 298; doi:10.1038/msb.2009.59
Citation: *Molecular Systems Biology* 5:298

www.molecularsystemsbiology.com

molecular systems biology

A mechanical bottleneck explains the variation in cup growth during FcγR phagocytosis

Jeroen S van Zon[1,4], George Tzircotis[1,2,4], Emmanuelle Caron[1,2] and Martin Howard[3,*]

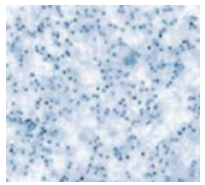


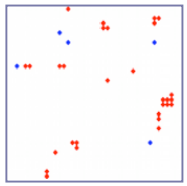
OPEN ACCESS Freely available online

PLoS one

Biophysical Mechanism for Ras-Nanocluster Formation and Signaling in Plasma Membrane

Thomas Gurry[1,2], Ozan Kahramanoğulları[1,3], Robert G. Endres[1,4]*





CISBIC

Imperial College London

Acknowledgments

- Members of Vania Braga's lab
- Members of the Biological Physics group
- Jeroen van Zon, Martin Howard, and Emmanuelle Caron (†)

Funding by BBSRC

bbsrc
biotechnology and biological sciences research council

….and BBSRC grant BB/G000131/1 on "Engineering Principles in Chemotaxis Signalling Pathways"

CISBIC