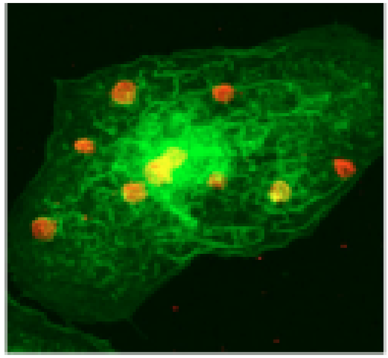
Subproject 2: Spatio-temporal control of phagocytosis

Progress report



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G23 Flowers Building, Imperial College London   September 3, 2009

Biological Physics Group:

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

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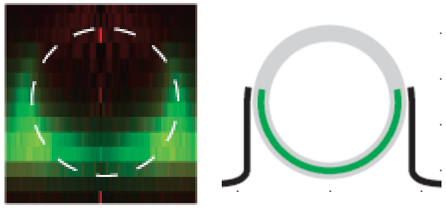
Recent achievements

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



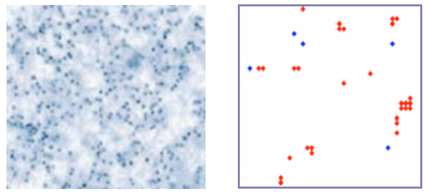
Jeroen S van Zon[1,4], George Tzircotis[1,2,4], Emmanuelle Caron[1,2] and Martin Howard[3,*]

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*]



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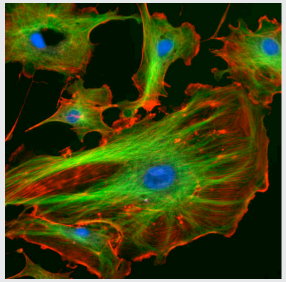
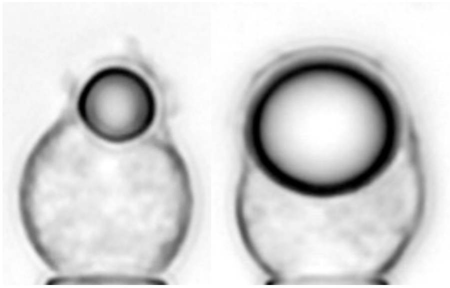
Biophysical aspects of phagocytosis

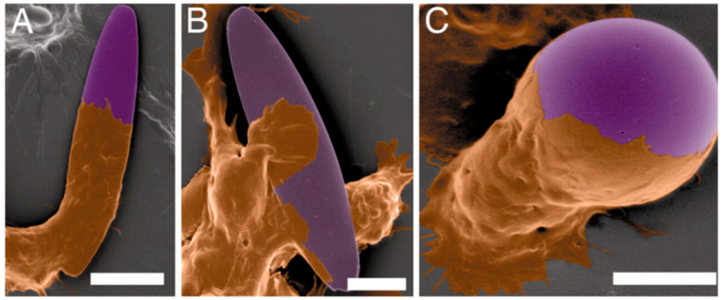


1. Size dependence (endocytosis vs phagocytosis)



Herant *et al*. (2006)

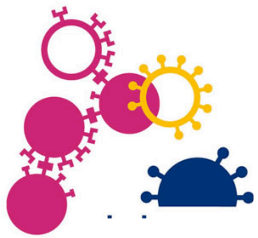
2. Shape dependence

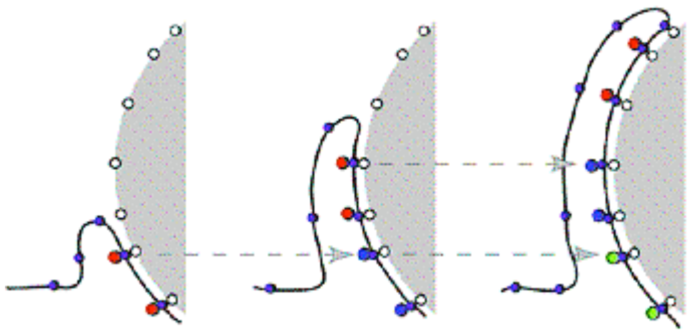


Champion *et al*. (2006)

3. Elastic properties

4. Ligand density → Zipper mechanism





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

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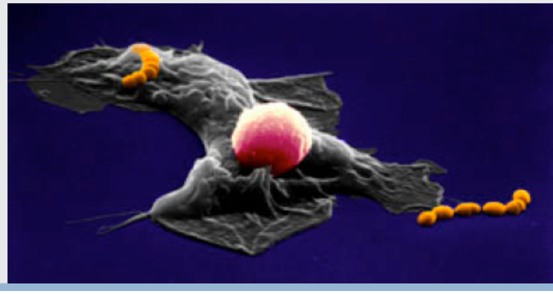
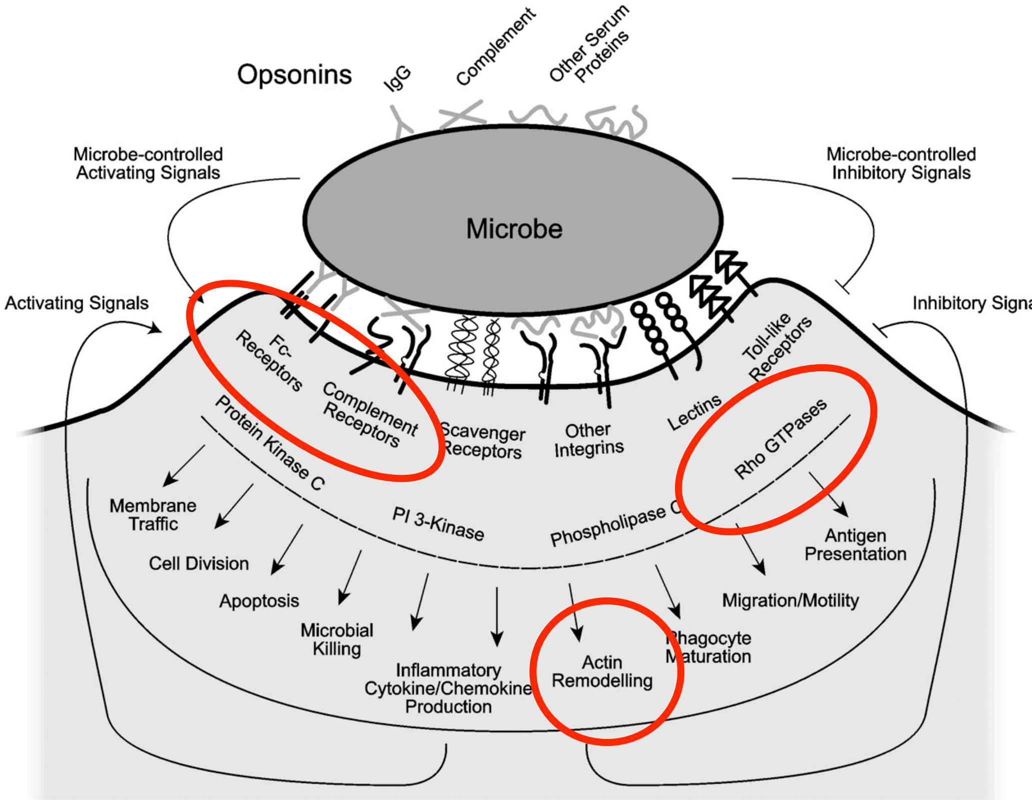
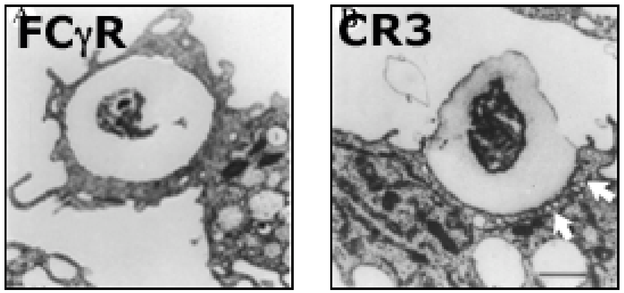
Signalling in phagocytosis





Underhill & Ozinsky (2002)



Allen & Aderem (1996)

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Objectives and Outline

1. Model of early signalling events in Fc-R phagocytosis

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

2. Process algebra model of small GTPases and actin polymerization

   (Ozan Kahramanogullari, Luca Cardelli , Philippa Gardner)

3. Early signalling events using RNA interference (RNAi) and imaging

   (George Tzirkotis, Emmanuelle Caron)

4. Extension of model and new experiments (shape dependence)

   (Sylvain Tollis, George Tzircotis, Robert Endres)

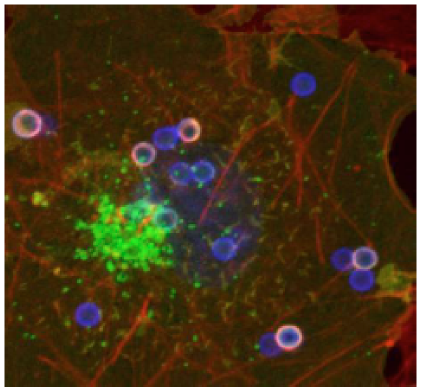
5. Collaboration with other subprojects

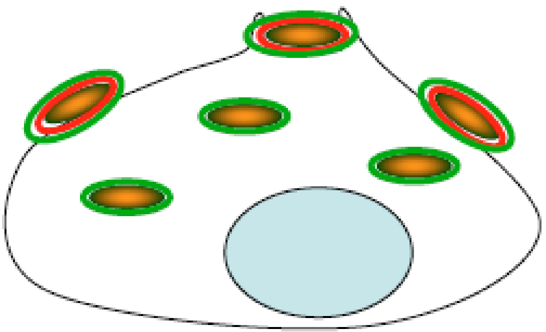
   (George Tzircotis)

6. Future directions

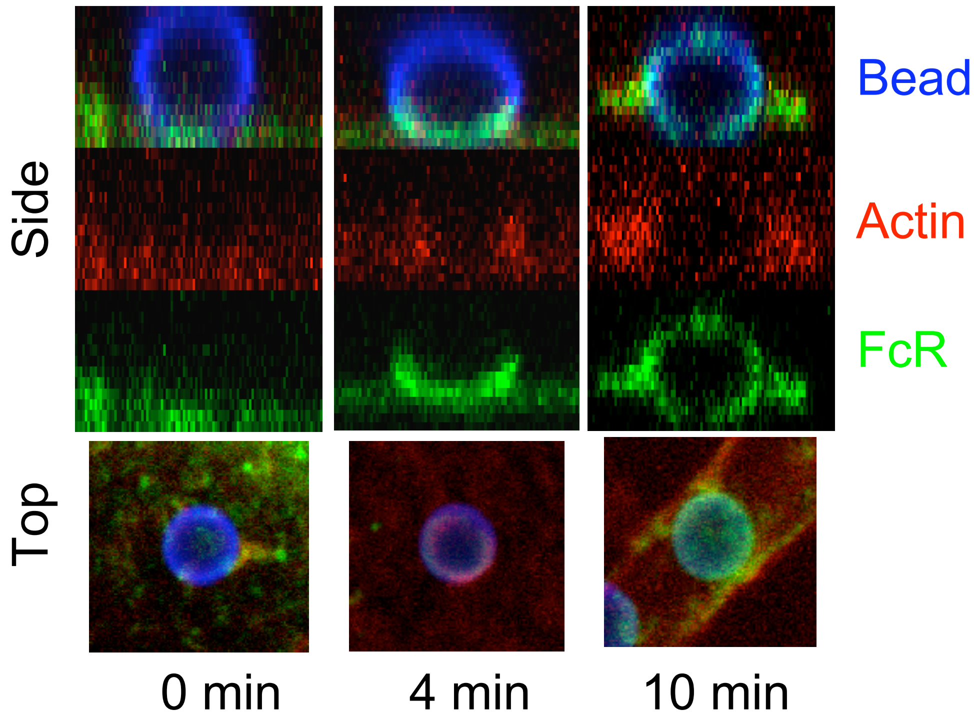
Imaging of phagocytosis

Phagocytic assay





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



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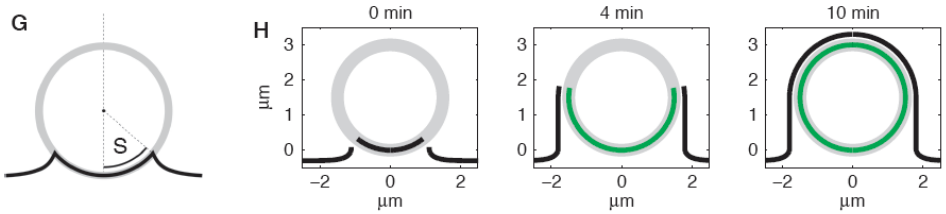
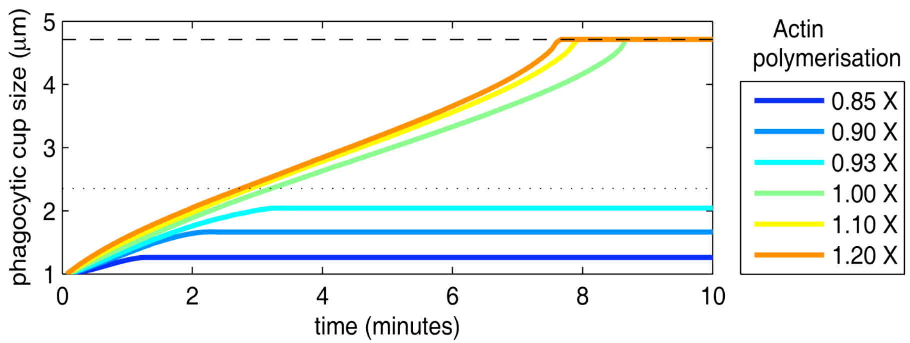
Model of early events in Fc-R phagocytosis



PDE model of phagocytic cup formation couples membrane/ cytoskeletal dynamics to receptor diffusion and signalling

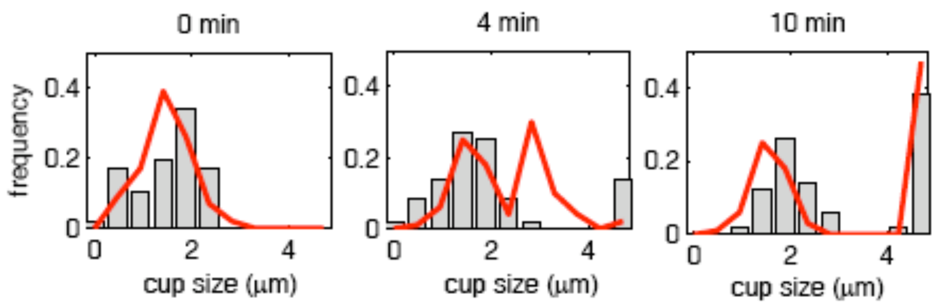
Completely determined by bending modulus and cortical tension

Predicts mechanical bottle neck



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

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



→ Bimodal distribution

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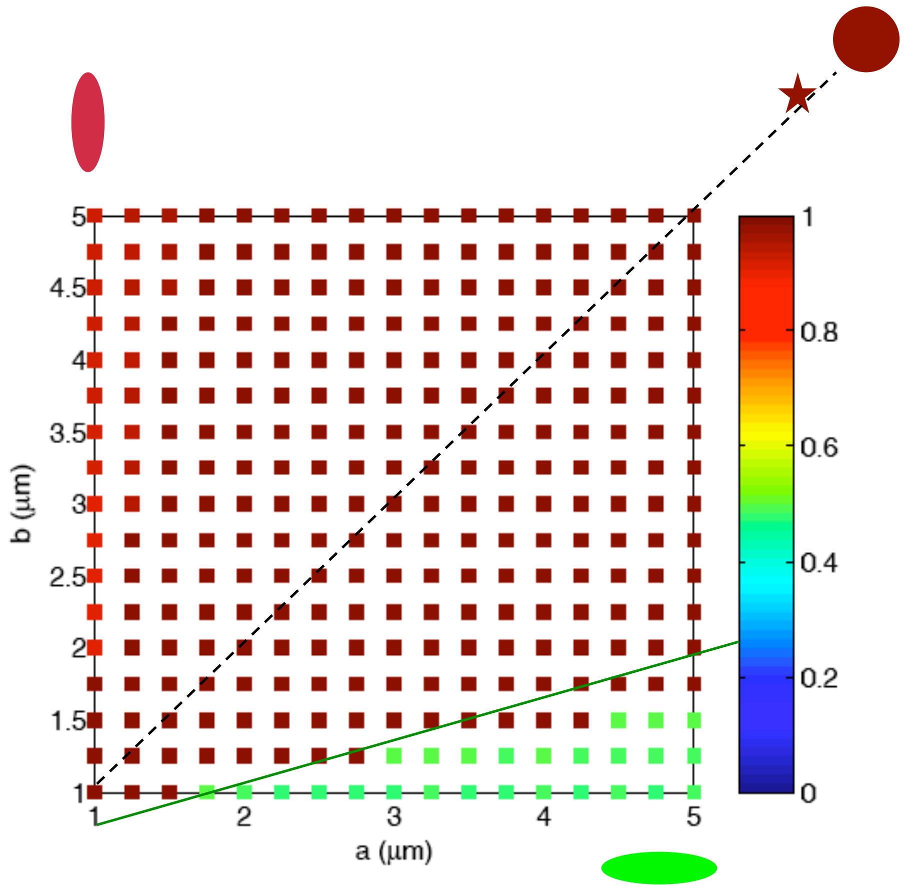
Further predictions

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



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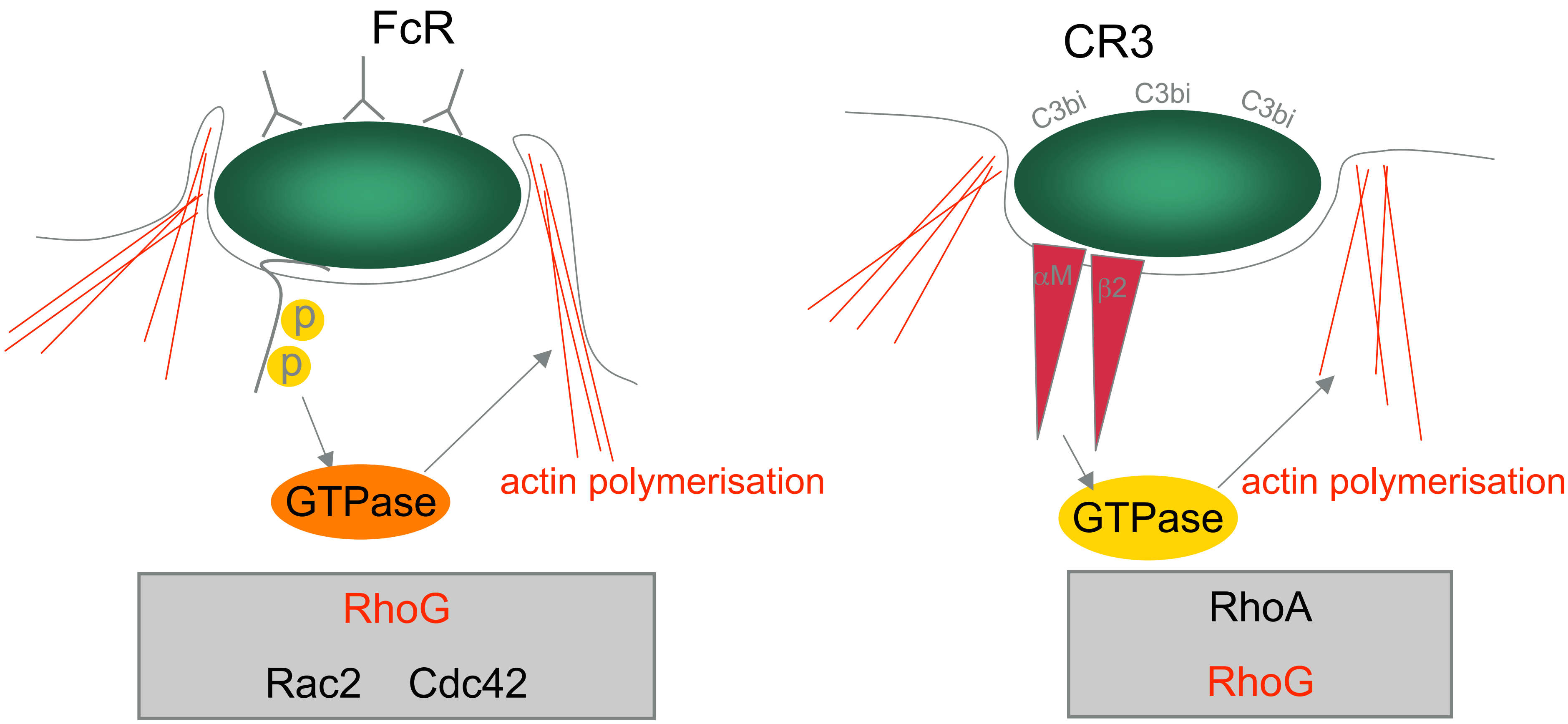
Early signalling events using RNA interference

siRNA screen of Rho GTPases in macrophage cell line



RhoG is a possible universal regulator of phagocytosis – role in FcR and CR3

Phagocytosis confirmed in bone marrow derived macrophages

RhoG is required for actin polymerisation and localises to cups

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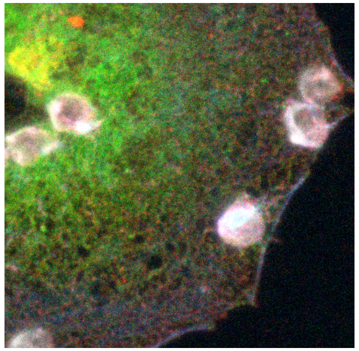
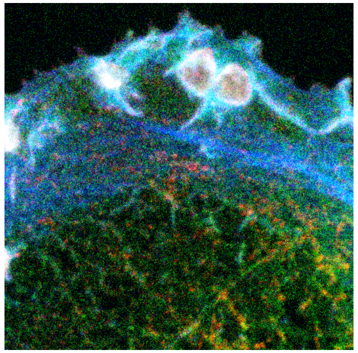
Hierarchy of small GTPases

Probe for active RhoG localises to both FcR and CR3 phagocytic cups (except for signalling-dead mutant receptors)

FcR



CR3



GTPase hierarchy: preliminary data using siRNA suggests RhoG is upstream of Cdc42 and Rac2 in FcR, but downstream of RhoA in CR3

Future work:

Use of probes for detection active Rac/Cdc42 and RhoA localisation to further resolve hierarchy of GTPases

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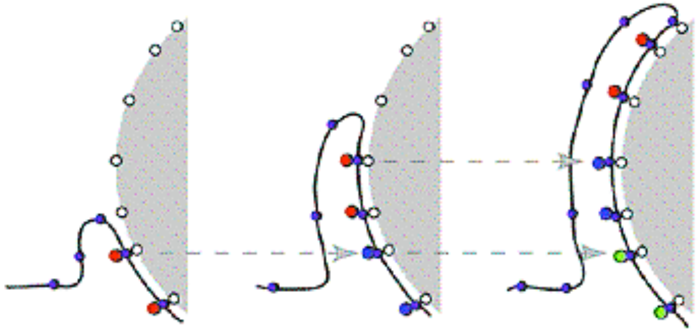
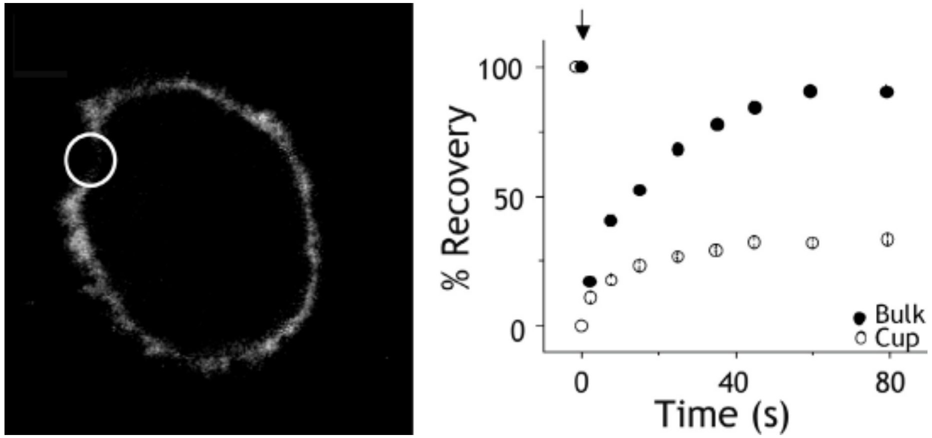
The Zipper mechanism

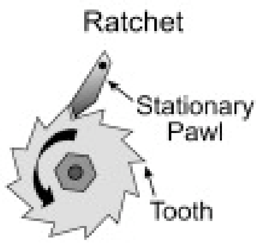


Zipper mechanism:
Unidirectional, sequential ligand-receptor interactions guide membrane around particle



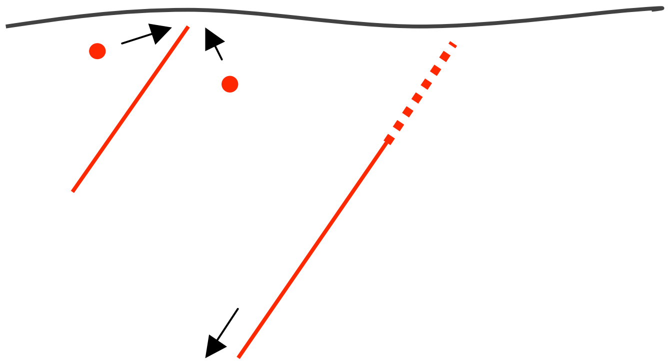
Corbett-Nelson *et al.* (2006)

FRAP points towards ratchet-like mechanism

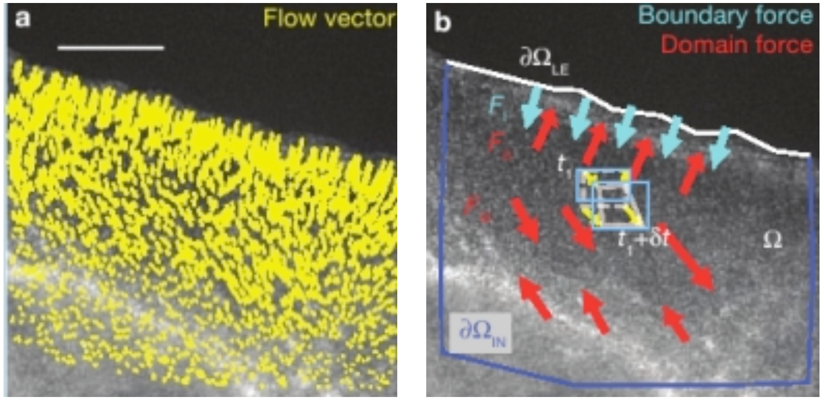


Model ingredients

Actin polymerizes at barbed end

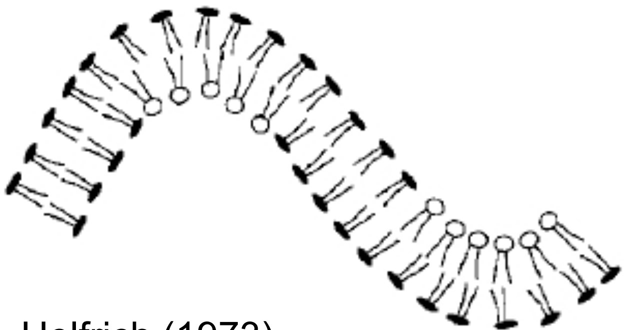


Fluorescent speckle microscopy



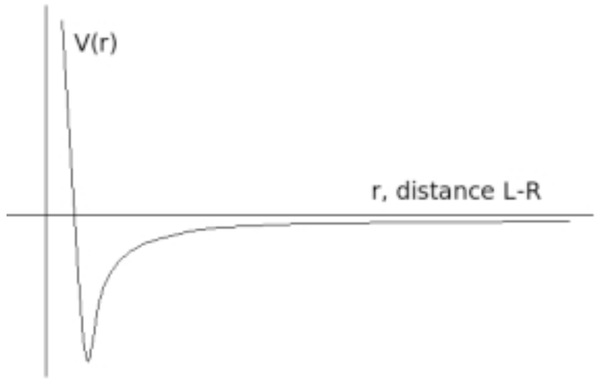
Ji *et al.* (2008)

Membrane energy: bending,
surface tension, volume constraint

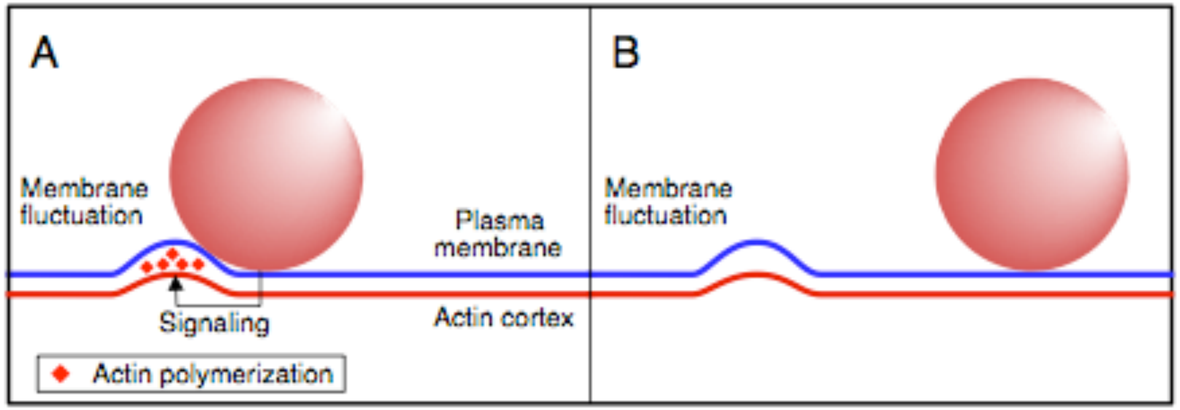


Helfrich (1973)

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.

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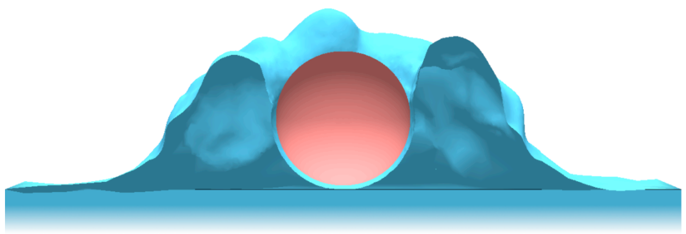
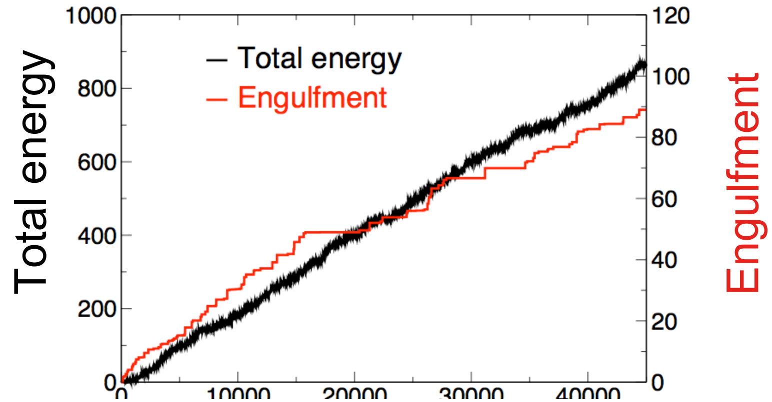
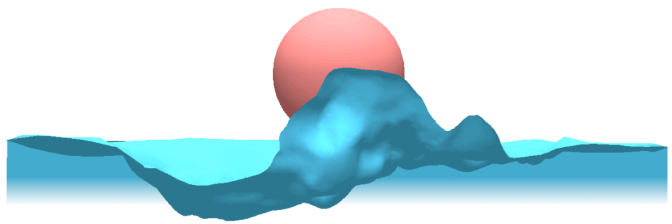
Active versus passive phagocytosis

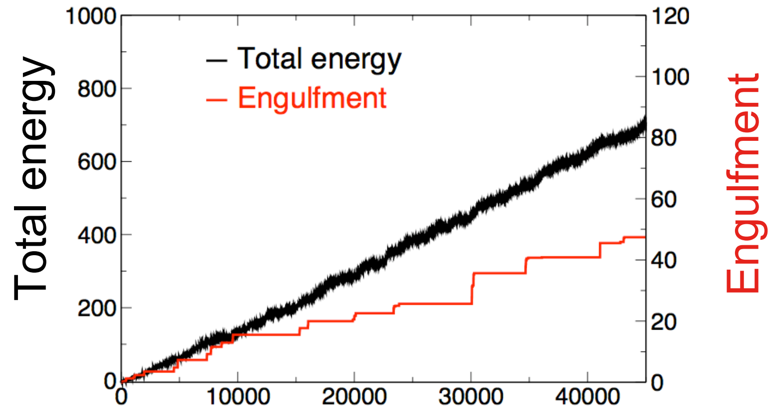
Active





Passive





→ Active engulfment completes much faster and cups are more regular

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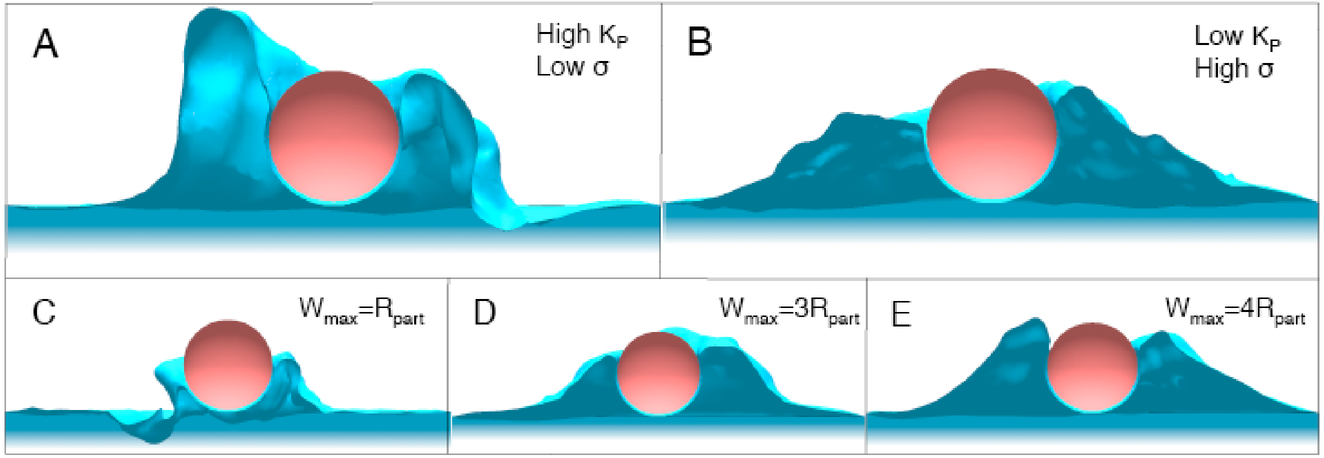
Cup shape and variability

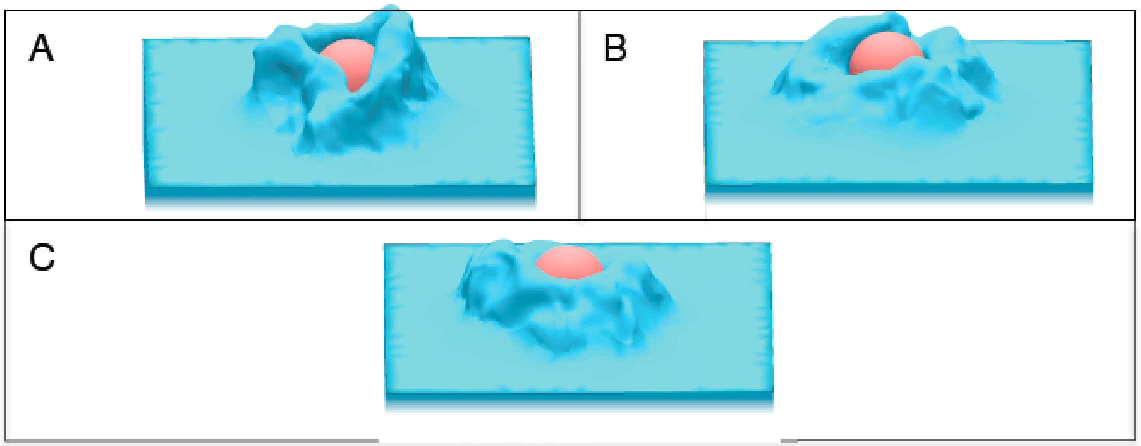
Shape



Physical parameters

Simulation parameters

Large variability



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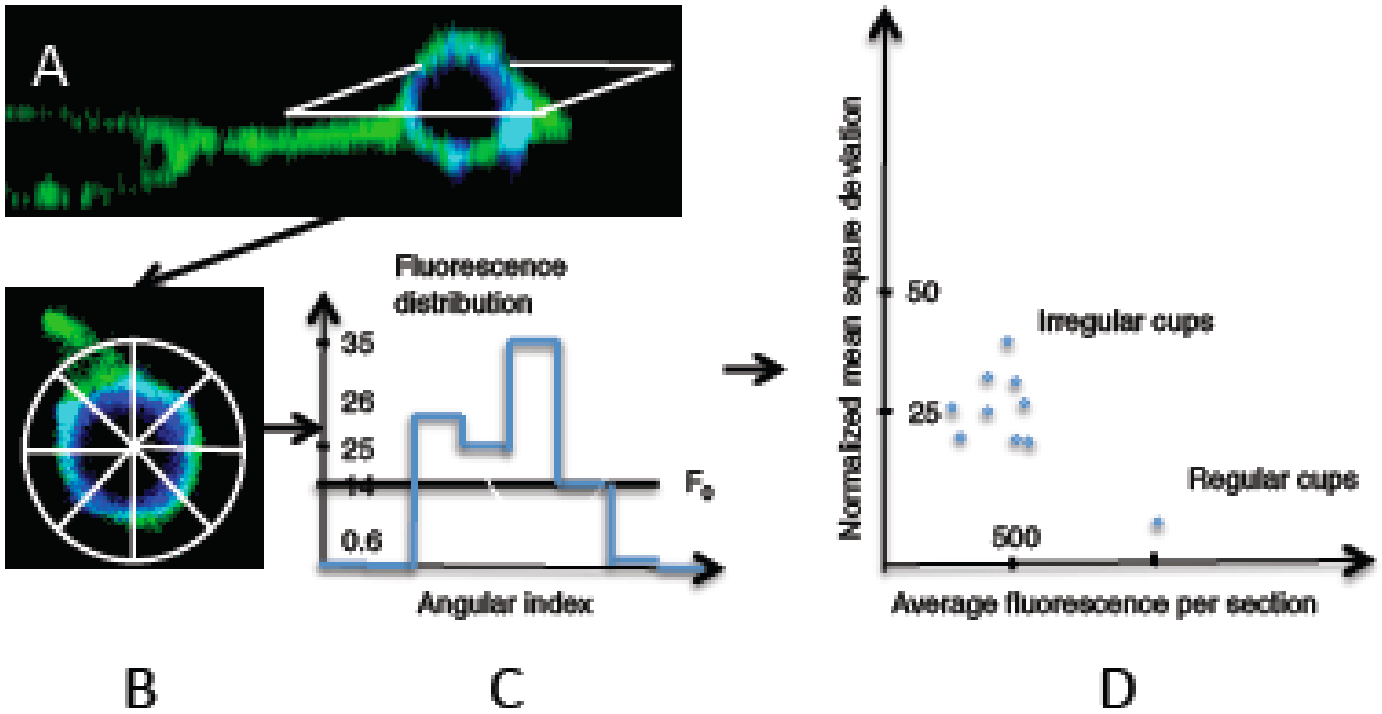
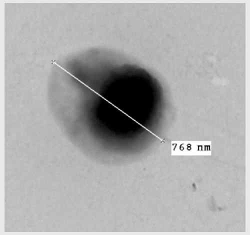
Experimental verification of predictions

Measure cup shape and variability from confocal images of wild-type (active), signalling mutant (active/passive), and cytochalasin-D treated cells (passive).

Preliminary data analysis of wild-type receptor:





Campylobacter

Collaboration with other 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. Shape dependence
2. Mechano-sensitivity during phagocytosis (squeezing of particle, soft vs stiff)
3. Later stages of phagocytosis (actin belt, motor proteins)
4. Zipper-like engulfment in other areas of biology

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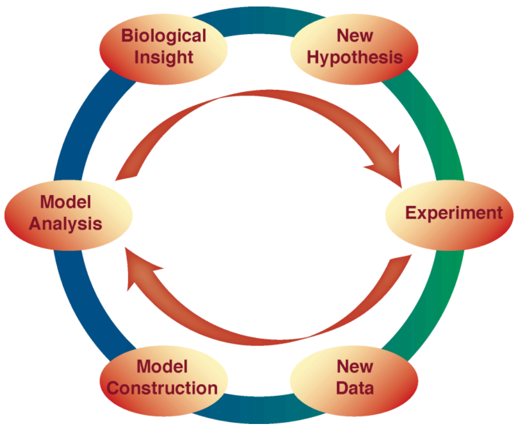
Acknowledgments

Emmanuelle Caron

- Ozan Kahramanogullari
  (Microsoft Research - University of Trento)
- Luca Cardelli
- Philippa Gardner

- Mirella Koleva
- Members of Vania Braga's lab and my group



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