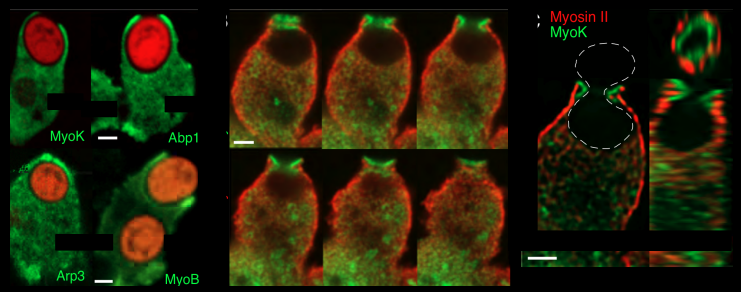
How one cell eats another: experiments and modeling elucidate biophysical requirements for uptake



S. Tollis, R. Endres, G. Tzircotis, February 15th 2010

Biological physics group, Biochemistry building 3rd floor

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

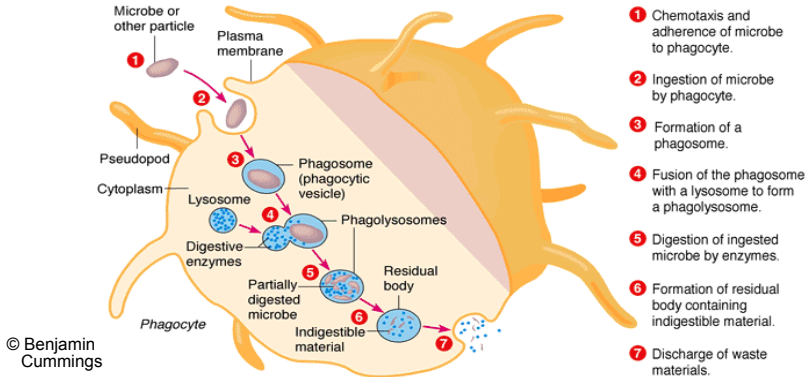
Outline

What is phagocytosis ?

Definition and functions of the phagocytic process

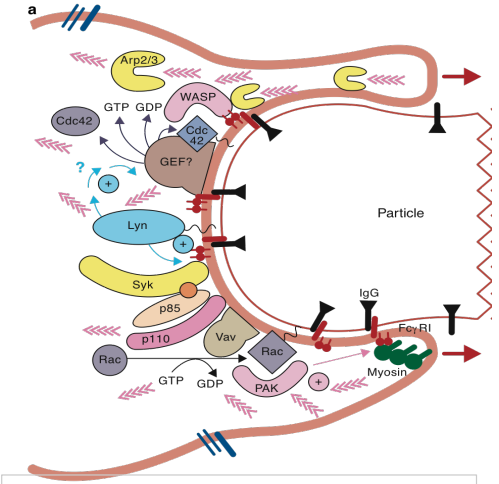
Process by which cells remove tissue debris, clear apoptotic cells, destruct invading pathogens: essential role immune response …

Stages of particle degradation: mesoscale view



What is phagocytosis: biochemical aspects

Simplified biochemical pathway (FcγR phagocytosis)



From Chimini et. al., Nature Cell Biology 2, E191-E196 (2000)

Ligand receptor binding leads to FcγR ITAM domain phosphorylation

Syk and Cdc 42 activation (via activation of a Src-family kinase)

Recruitment of WASP to complex

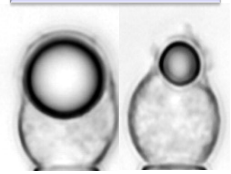
Activates Arp2/3 complex which promotes actin polymerization

What is phagocytosis: biophysical aspects

Phagocytosis requires cell-shape changes via actin network remodeling

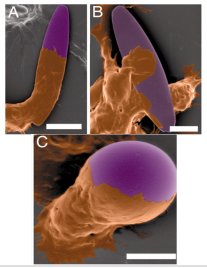
Relevance of cell/particle's physical properties + polymer physics/mechanics/fluidics...

Particle size



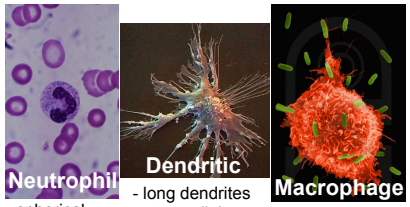
Phagocytosis of polystyrene beads by neutrophils. Optical microscope. Herant et. al., J. Cell Sci. 2006

Particle shape



SEM of polystyrene beads taken up by rat macrophage. Champion et. al., PNAS 2006

Different cells/physical properties



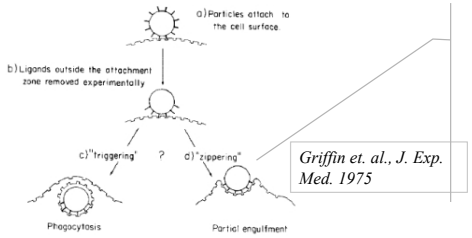
- spherical
- few memb. folds
- 10-12 µm diameter
- engulf many times

- long dendrites
- strong dialog skills
- engulf only some times when immature

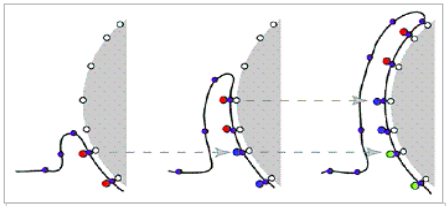
- flat far from nucleus
- lot of folds
- 20-30 µm long
- engulf many times

Ratchet model: the zipper mechanism

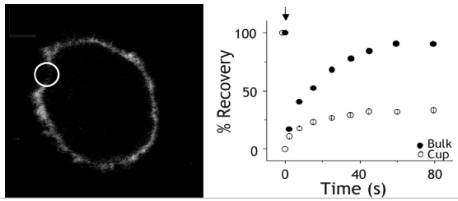
Particles partially bleached are partially engulfed



Griffin et. al., J. Exp. Med. 1975



Swanson et al., J. Leuk. Biol. 76, 1093-1103 (2004)



FRAP of RAW264.7 macrophages taking up IgG coated particles. From Corbett-Nelson et. al., J. Cell Biol. 2006

- need L/R bonds on all the particle
- L/R bonds have low mobility
- origin of specific chemistry still debated

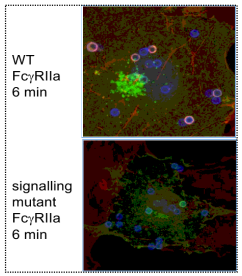
Ratchet model: actin force vs strong binding

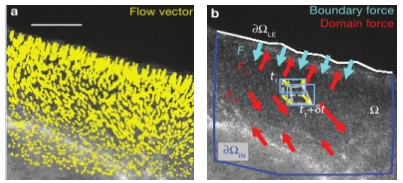
Phagocytosis is an active out of equilibrium process

Potential sources of energy to drive engulfment

Chemical (force generated by the actin network, L-R attraction)

Thermal (no direct force, disordered thermal fluctuations are rectified by the zipper)





Actin flow and intracellular forces. Polymerization drives membranes protrusions in the "good" direction. Fluorescent speckle microscopy image, from Ji et. al., Nature Cell Biology 10, 12 (2008)

Our assumption : active processes make the L-R bonds irreversible

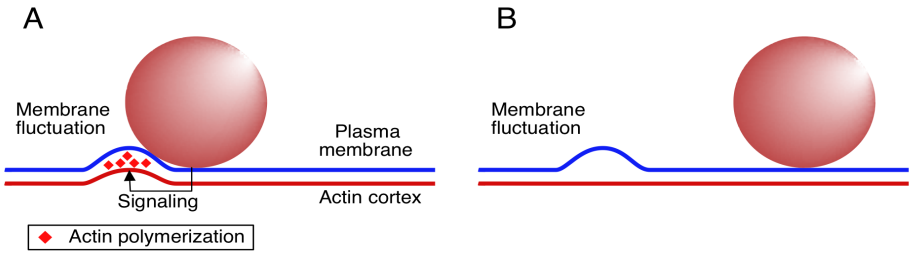
Ratchet mechanism

Ratchet model: our approach

No predictive power of the Zipper mechanism

Need for a quantitative model (kinetics of engulfment, cup shape...)

Contribution of the thermal energy: membrane fluctuations support actin polymerization



From Ref.1: Tollis et al., submitted

Ratchet model: energy & MC simulations

Membrane total energy is function of the cup shape

- Energy includes membrane resistance to stretching (tension σ), to bending (curvature stiffness κ_b), L/R attraction, local constraint on V (amplitude κ_p)
- Quantitative analysis : (some) parameters can be measured experimentally

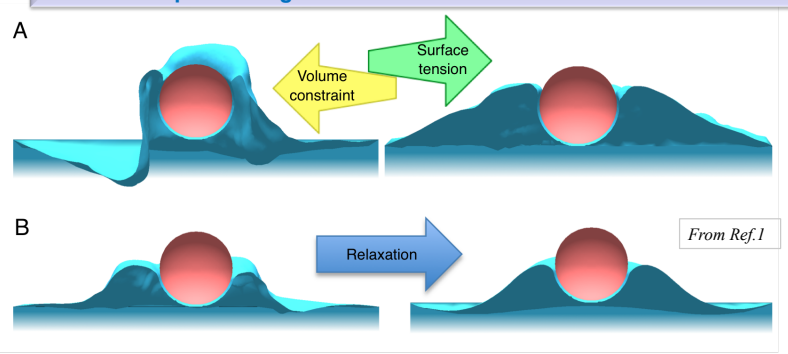
Finite elements calculations and Monte Carlo simulations

- Cell surface sampled
- MC simulations (random membrane moves)
- Move accepted according to finite temperature Metropolis criterion

How things work: movie of phagocytic engulfment

Simulation results: parameters & cup shape

Different shapes for engulfment



R_P = 1.5μm

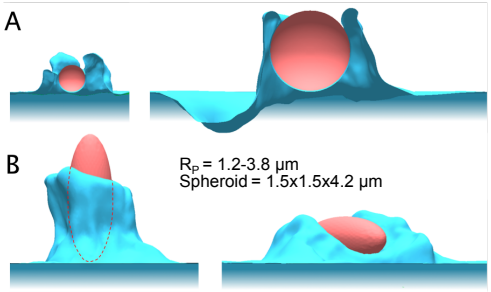
σ = 6.2-620 (x10^{-7}mNm^{-1})

K_P = 10^5-3.8 (x10^{-7}pNμm^{-5})

- FcR-mediated phagocytosis involves “thin” cups (panel A, left)
- Complement-mediated phagocytosis involves broad cups (panel A, right)
- Out of equilibrium engulfment is crucial in shaping the cup (panels C and D)

Simulation results: size & shape dependence

Particle size dependence of phagocytosis



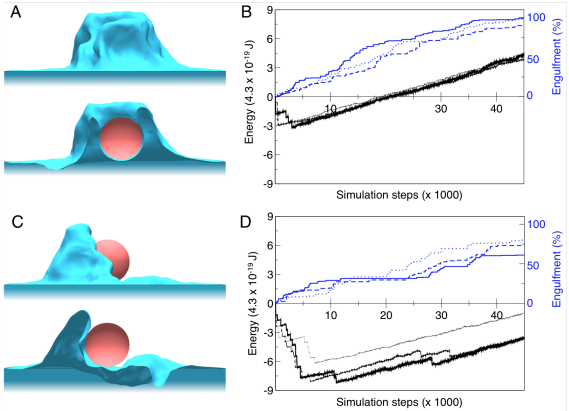
- Particles of different sizes are engulfed by cells with the same biophysical parameters
- Engulfment of big particles is more regular than for small particles
- Elongated spheroid particles are engulfed more efficiently if taken with their tip first
- All these observations are qualitatively consistent with experiments

Conclusions and general observations

- No assumed rotational symmetry: can be used to analyze complicated particle shapes
- Cup closure is slow and quite irregular
- Lateral extension of membrane trial moves may influence the cup shape: how should we estimate this parameter ?

Simulation results: active vs passive

Active versus passive engulfment



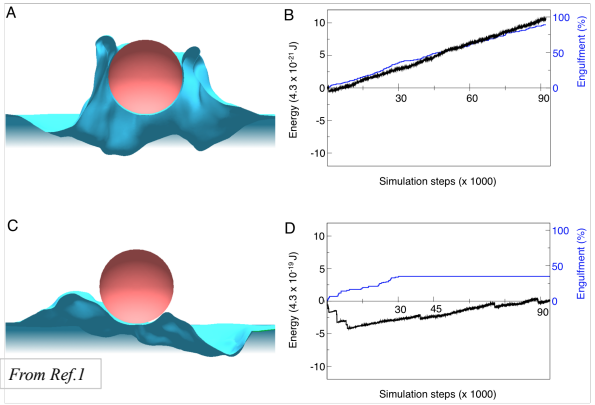
From Ref.1

- Passive phagocytosis is slower and the cup is more irregular
- We predict quantitatively the amount of extra energy needed to phagocytose

Compatible with myosin motors energy production

Simulation results: active vs passive

Active versus passive engulfment of large particles



From Ref.1

- Passive phagocytosis does not allow uptake of large particles

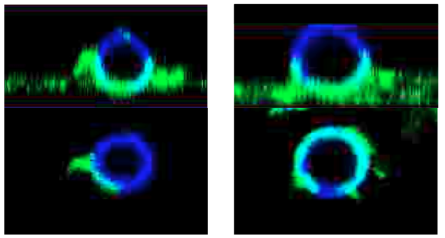
- Energy production by thermal fluctuations only is not enough

Was phagocytosis originally working only on physical grounds, and were biochemical processes added through evolution for robustness ?

Passive engulfment was unexpected: it deserved some experimental confirmation

Comparison with experiments: setup

Phagocytosis of IgG coated polystyrene beads by COS-7 phagocytes



- Experiments done here (IC-CMMI)

- IgG are intrinsically fluorescent (blue here)

- Fc Receptors genetically modified to express a GFP: label the cell membrane

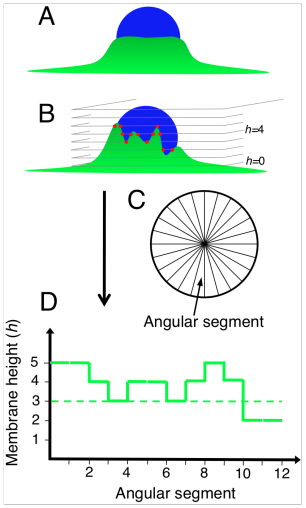
- Confocal microscopy used to reconstruct 3D phagpcytic cups

Images taken for 3 types of cells:

Wild-type	When LR bonds form, FcR signal and actin polymerizes: active engulfment
Signaling mutant	Genetically modified FcR do not signal thus actin cortex is not remodeled: passive engulfment
Cyt D treated cells	Cyt D treatment destroys the actin cortex: passive uptake

Comparison with exp. : analysis method

Image analysis: successful method



- Determine particle's engulfed area

- For particles engulfed more than 20%, determine the angular distribution of wrapping cell membrane

- Calculate average and mean square deviation of this distribution

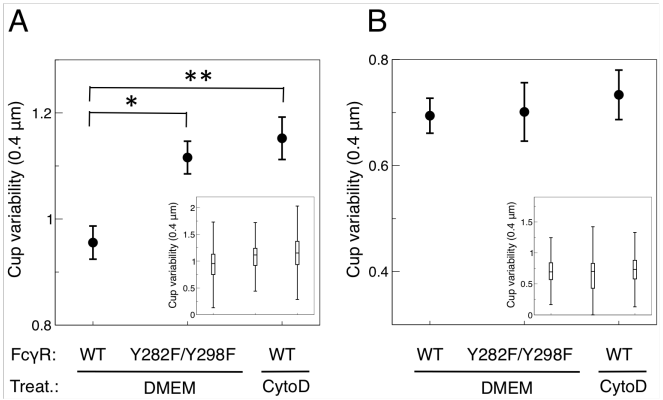
- Accuracy limited by quality of images (diffusion …)

- But a lot of data points for statistical averaging

From Ref.1

Comparison with exp.: analysis results

Significant difference in cup shape between active and passive uptake



From Ref.1

- 20-40%: cup is more regular for WT (Student's t-test p-values ≈ 10^{-4})

- 40-60%: no difference

Signature of active processes mainly in early engulfment ?

Conclusion

Results summary

- Biophysical model based on membrane fluctuations and irreversible ligand/receptor binding

- We found how biophysical parameters influence cup shape and engulfment completion

- Phagocytosis works without biochemical support but slower and in a less regular fashion: biochemical pathways may have been added through evolution for robustness

- Successful comparison with imaging experiments

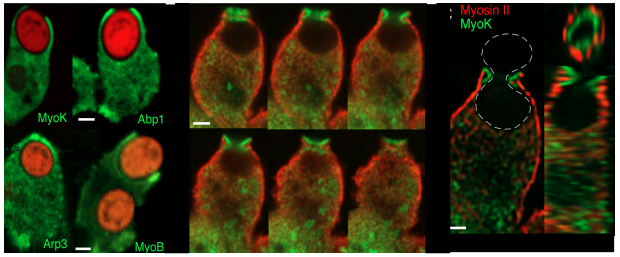
Limitations of the model and further work

- Estimated parameter one order of magnitude smaller than measured for bulk cell membrane -> Indicate that the cell may regulate these parameters locally. New experiments ?

- Problems with cup closure: need to include cytosol/cytoskeleton contractions

- Receptor microclusters and/or lipid rafts for more quantitatively accurate description

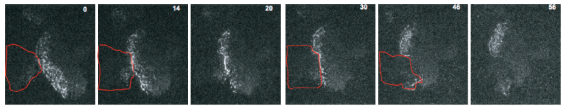
Conclusion: new project

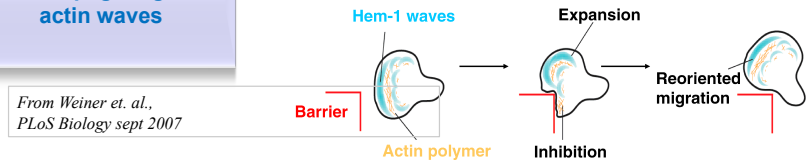


Dictyostelium eating budding yeasts: evidence for acto-myosin contractions

From Dieckmann et. al., in press

Propagating actin waves

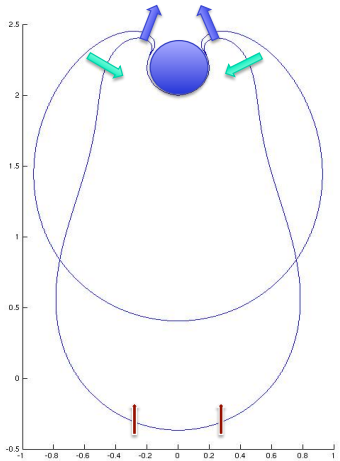




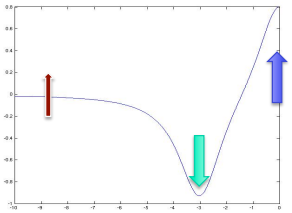
From Weiner et. al., PLoS Biology sept 2007

Conclusion: new project

Preliminary: cell shape for a given pressure/force profile



obtained using pressure profile:



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

Acknowledgement

- Members of Vania Braga's lab
- Members of the Biological Physics group

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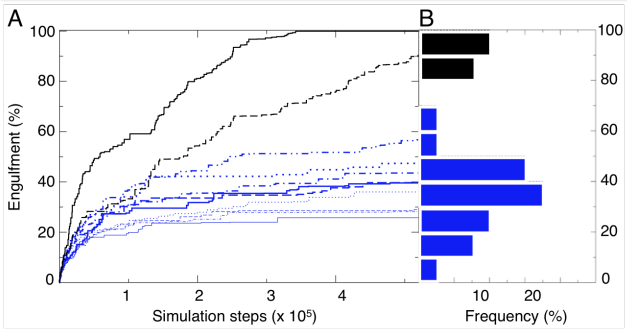
bbsrc
biotechnology and biological sciences research council

Comparison with exp.: analysis results

Engulfment stalling: how slight changes in model parameters affect completion



From Ref.1

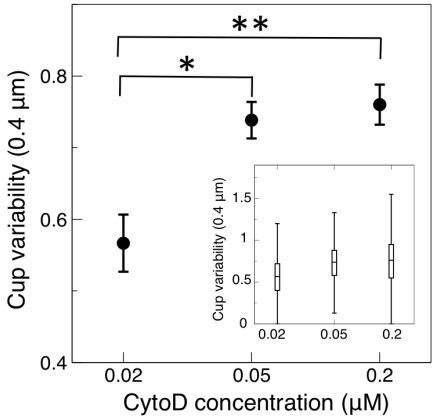
- Black: a slight increase in σ (0.8->1.1x10^{-4} mNm) does't affect a lot the cup progression

- Blue: similar steps in σ (1.4,1.6,1.9 …x10^{-4} mNm) lead to cup stalling

May explain why, for similar biophysical parameters, engulfment may succeed or not (example in B.Subtilis sporulation)

Comparison with exp.: analysis results

Efficiency of CytoD treatment



From Ref.1

- Cup variability is significantly lower for cells treated with only 0.02µM

- 0.05µM or 0.2µM lead to the same overall high cup variability

Indicates that actin dynamics survive the 0.02µM treatment

Comparison with exp. : inaccurate analysis

Image analysis: first attempt