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Sub-project 2 : Progress report

Emmanuelle Caron
& Ozan Kahramanogullari

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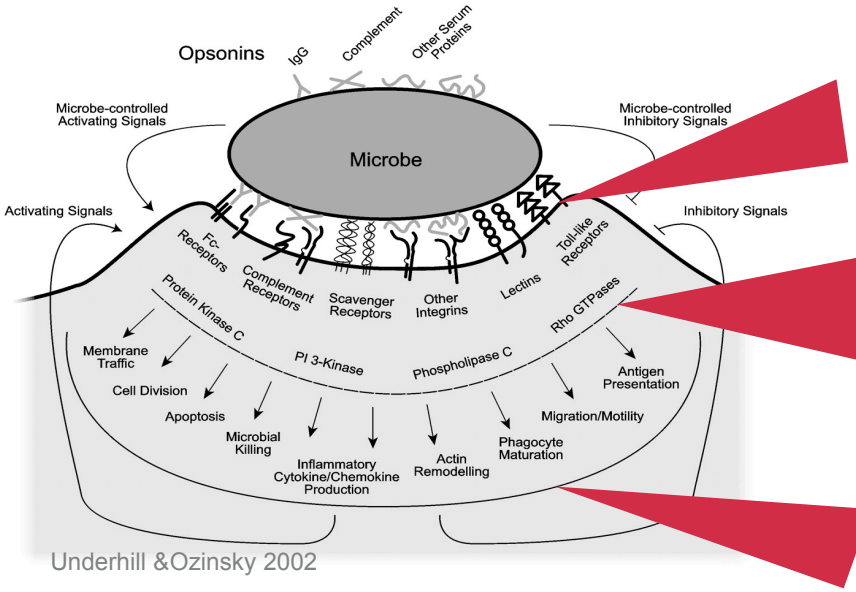
Microbial encounters with immune cells



Underhill &Ozinsky 2002

Diversity receptors vs conservation mechanism ?

How many essential regulators? Minimum phagocytic machine?

Integration of phagocytosis & cytokine production? Improved vaccine design?

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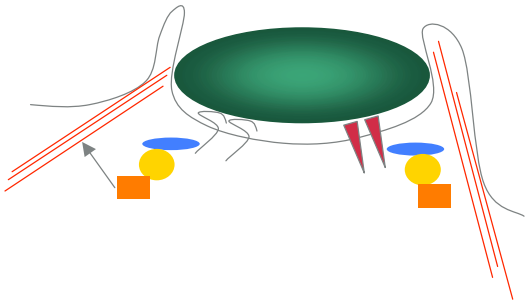
Subproject 2: People & Objectives



George Tzircotis
Emmanuelle Caron
(Marine van Berleere)
Sylvain Tollis
Robert Endres
(Jeroen van Zon)
Martin Howard
Ozan Kahramanogullari
Philippa Gardner
Luca Cardelli

Life Sciences

Maths

Computing

- *systems understanding of phagocytic uptake*
- *predictive models*
- *application to vaccine design*

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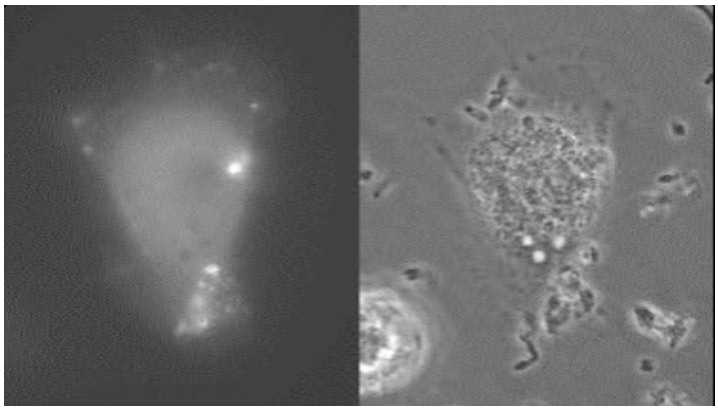
SP2 : Questions & Specific Milestones



• Local 3D remodelling of Cytoskeleton and Membrane
• Hijacked or prevented by pathogens
• Phagocytic cup as a discrete system
• Some regulators known (actin, Rho-family proteins)

➔ *Image early receptor signalling; combine with RNAi to refine understanding of early signalling networks; compare bacteria & beads*
➔ *Model 1st steps of FcγR-mediated phagocytosis*
➔ *Model Rho GTPase cycle, actin polymerization; signalling networks*
➔ *Validate and extend models and their predictions*

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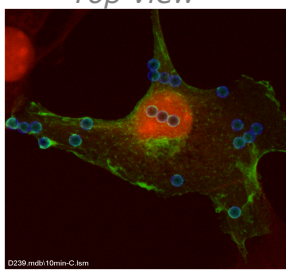
SP2: Previous progress

- Phagocytic assays, RNAi in place (cell lines)
- Mini-screen Rho GTPases (FcγR- and CR3-mediated uptake)
- Time series data on FcγR dynamics during uptake (imaging)
- Working PDE model of phagocytic cup formation, based on FcγR and actin dynamics

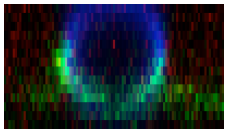
$$E = \int dA(2\kappa H^2 + \sigma)$$

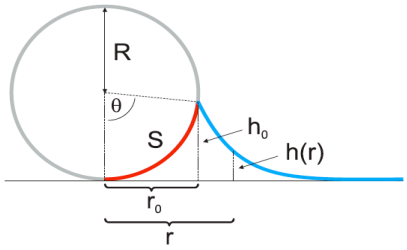
- Working process calculus models of the Rho protein cycle and Arp2/3 based actin dynamics

Top view



Side view





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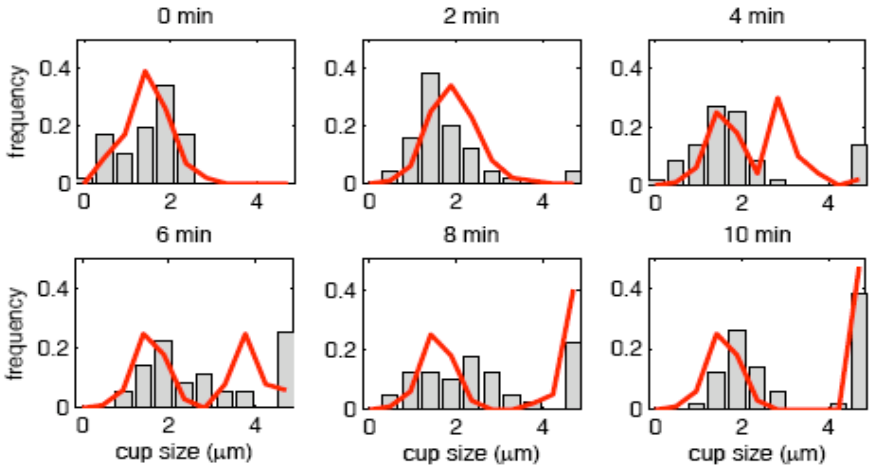
SP2: Recent progress

1. Finalisation PDE model



Reasonable fit model / measurements,
Unexpected variability in cup progression,
Reaching bimodal distribution with gaps for $\pi R/2 < S < \pi R$

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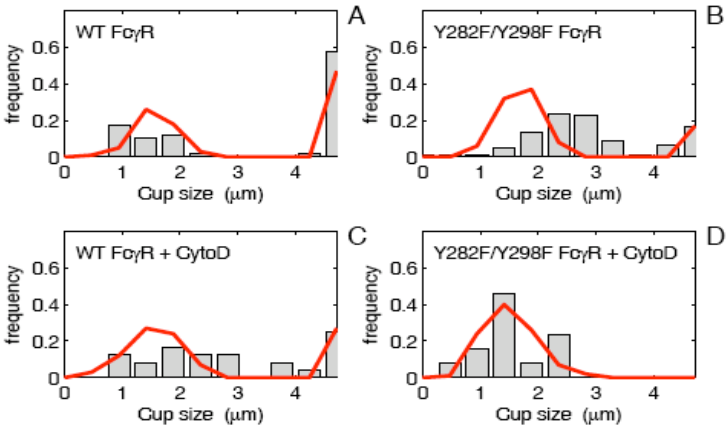
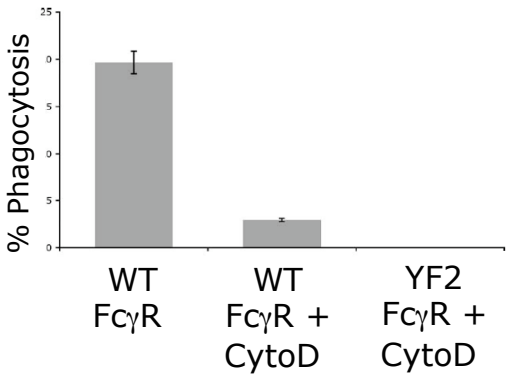
45 min measurements



Phagocytic assays



A mechanical bottleneck explains cup variability + Influence of preexisting membrane folds

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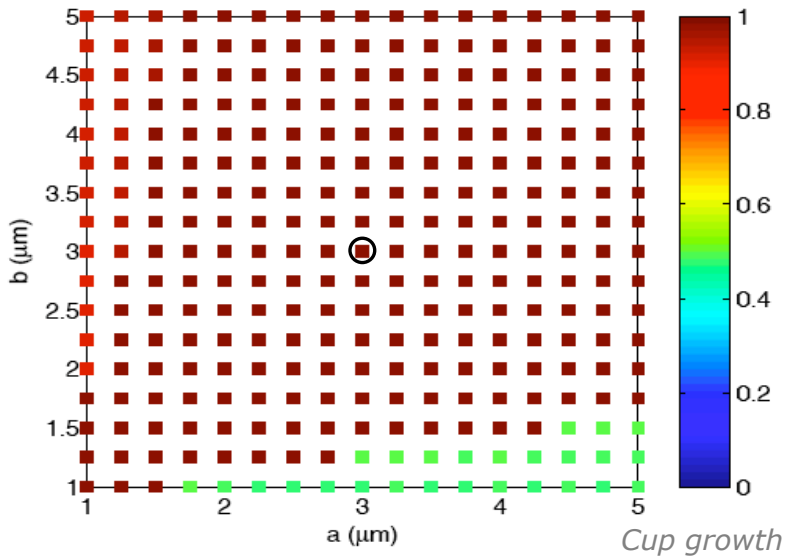
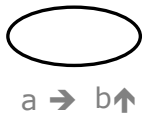
Model explains that particle shape influences uptake





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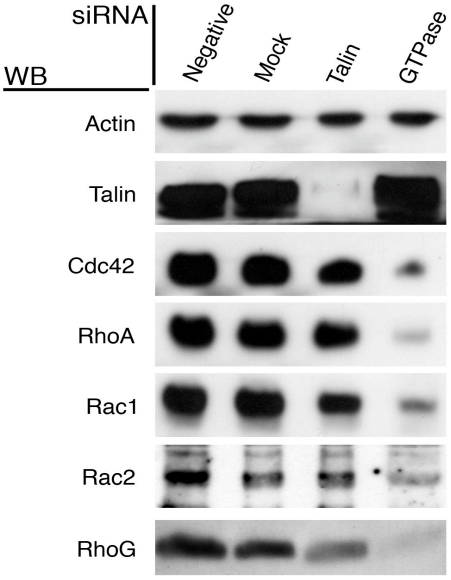
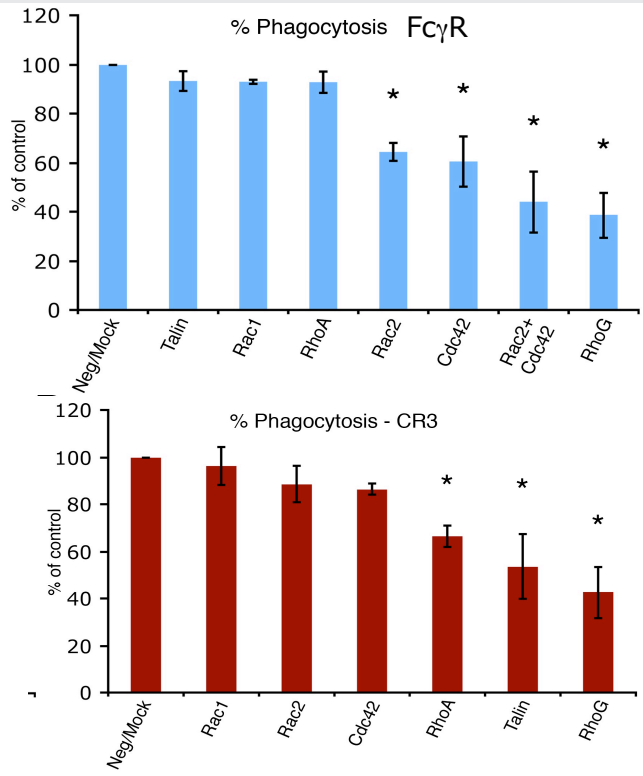
SP2: Recent progress

2. Validation RNAi miniscreen





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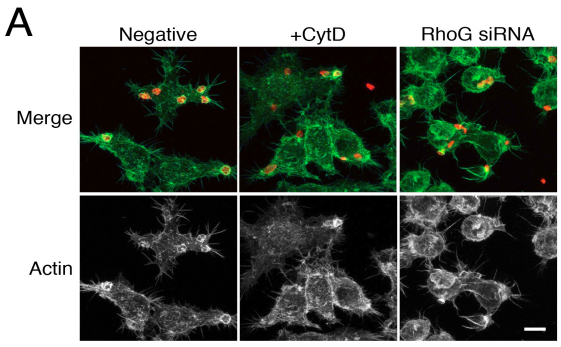
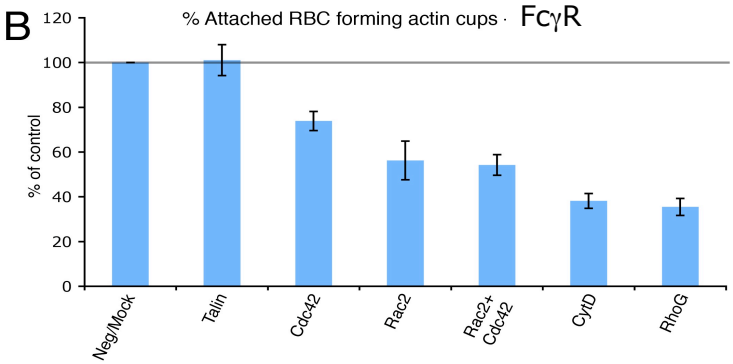
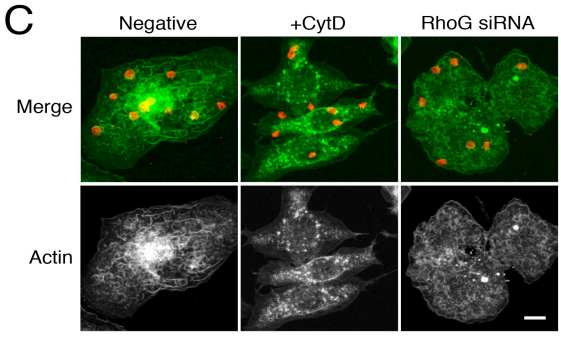
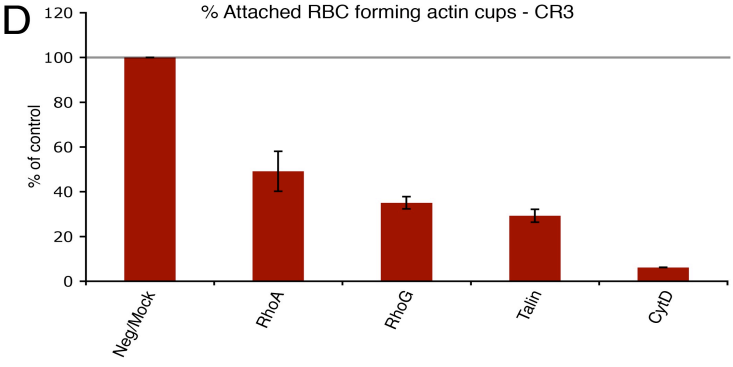
RhoG, a universal regulator of phagocytic uptake?









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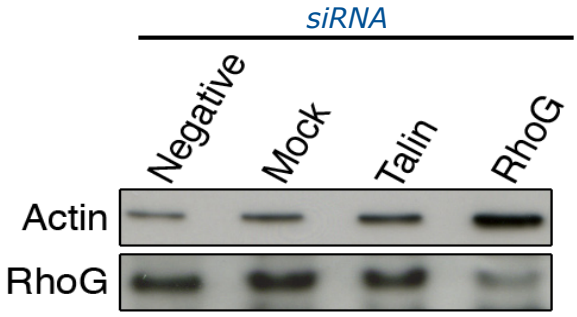
RhoG, a universal regulator of phagocytic uptake?

Bone marrow-derived, in vitro differentiated, macrophages



Also working in THP-1-derived human macrophages, tested in COS-7 cells

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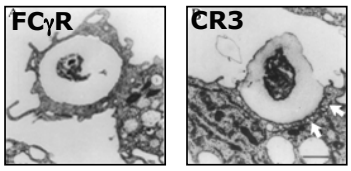
SP2 - Conclusions

- **Modelling the early phases of FcγR-mediated uptake has revealed unexpected features of phagocytosis: importance of preexisting structures, existence of a mechanical bottleneck controlling cup progression.**
- **Actin polymerization during phagocytosis of inert particles is controlled by subsets of Rho-family proteins, with a conserved role for RhoG as an early regulator of FcγR- and CR3-mediated phagocytosis.**



- **Computer languages and tools have been developed to describe signalling pathways and three-dimensional processes.**

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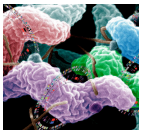
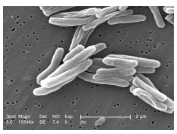
SP2: Outlook

- Spatio-temporal control of uptake of bacteria and inert particles (RhoG and other regulators)
- Larger siRNA screens for regulators of bacterial uptake
- Understanding the nature and regulation of the mechanical bottleneck - Role of biophysical constraints - Modelling CR3-dependent uptake
- Integrating SP2



  - with SP1: Impact of capsule and bacterial shape on Campylobacter binding, cup progression & uptake;
  - with SP3: Impact of Notch signalling on phagocytic signalling



  - with SP4: identifying regulators of BCG uptake (phagosys)

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