A scanning electron micrograph (SEM) showing a cell with a prominent phagocytic cup. The cup is a deep, bowl-like structure formed by the cell membrane, containing several small, rounded particles. The cell surface is covered in fine, hair-like projections (microvilli). The background is a textured, granular surface.

# [ Investigating Forces for Uptake and Cup Closure – The Role of Myosins in Phagocytosis ]

Anna Dart

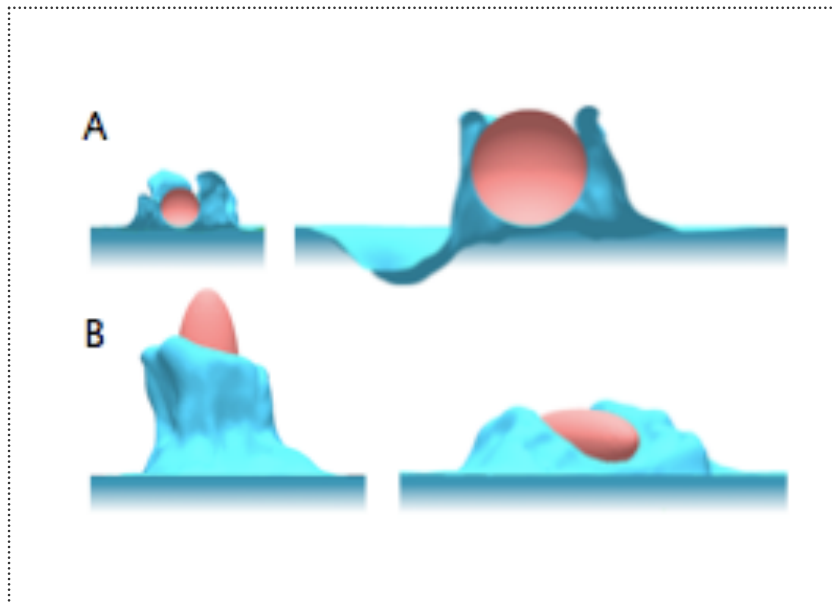
# Fundamental questions about the biophysical and signalling properties of phagocytosis



- Particle-size dependence
- Particle-shape dependence
- Cup closure, particle squeezing
- Actin waves at cup

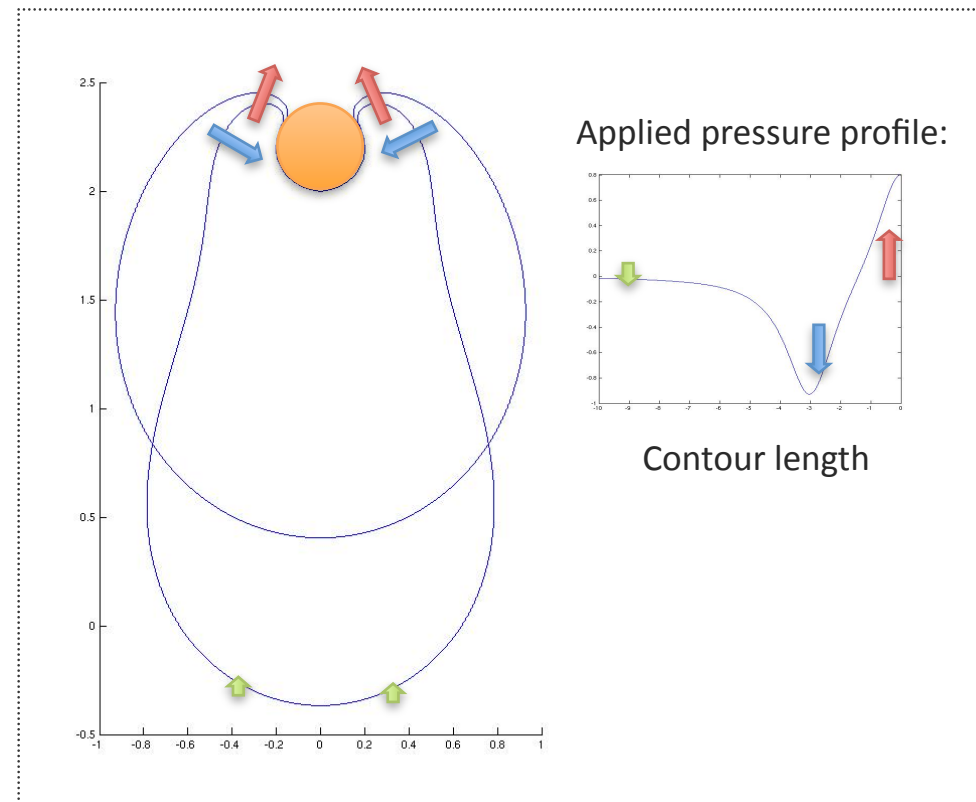
# [ Integration with modelling ]

Previous Model



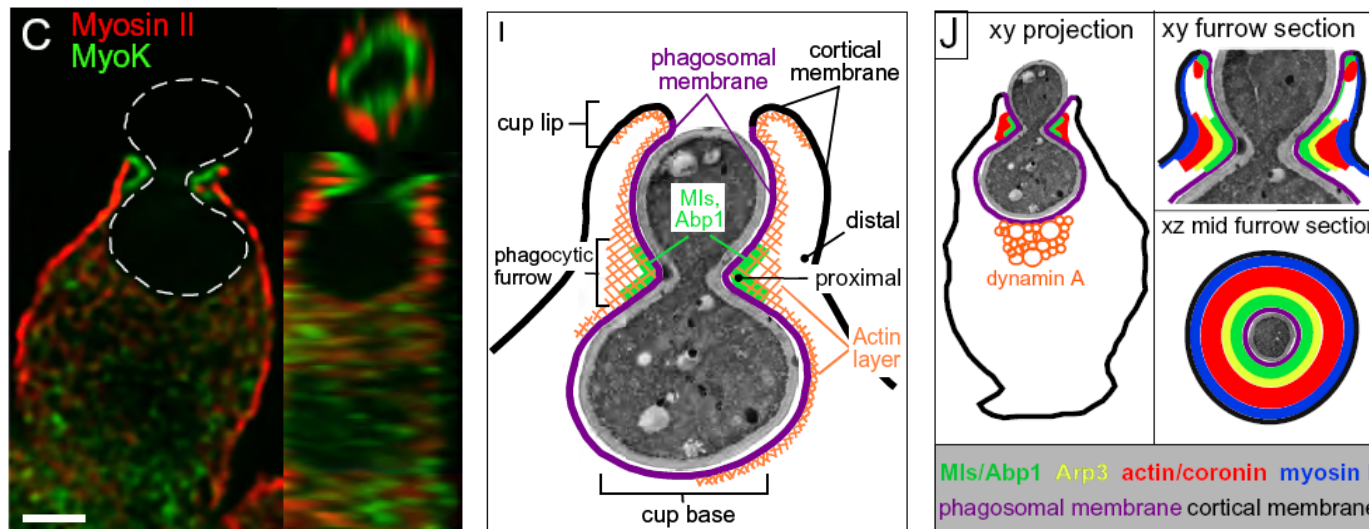
Work by Sylvain Tollis

Future Model



# Specific aims

- Model has difficulties with cup closure
- Address the question of cup closure and particle squeezing:
  - By extending the model to include the acto-myosin cytoskeleton
  - And experimentally by investigating the role of myosins



Dieckmann  
et al., 2010

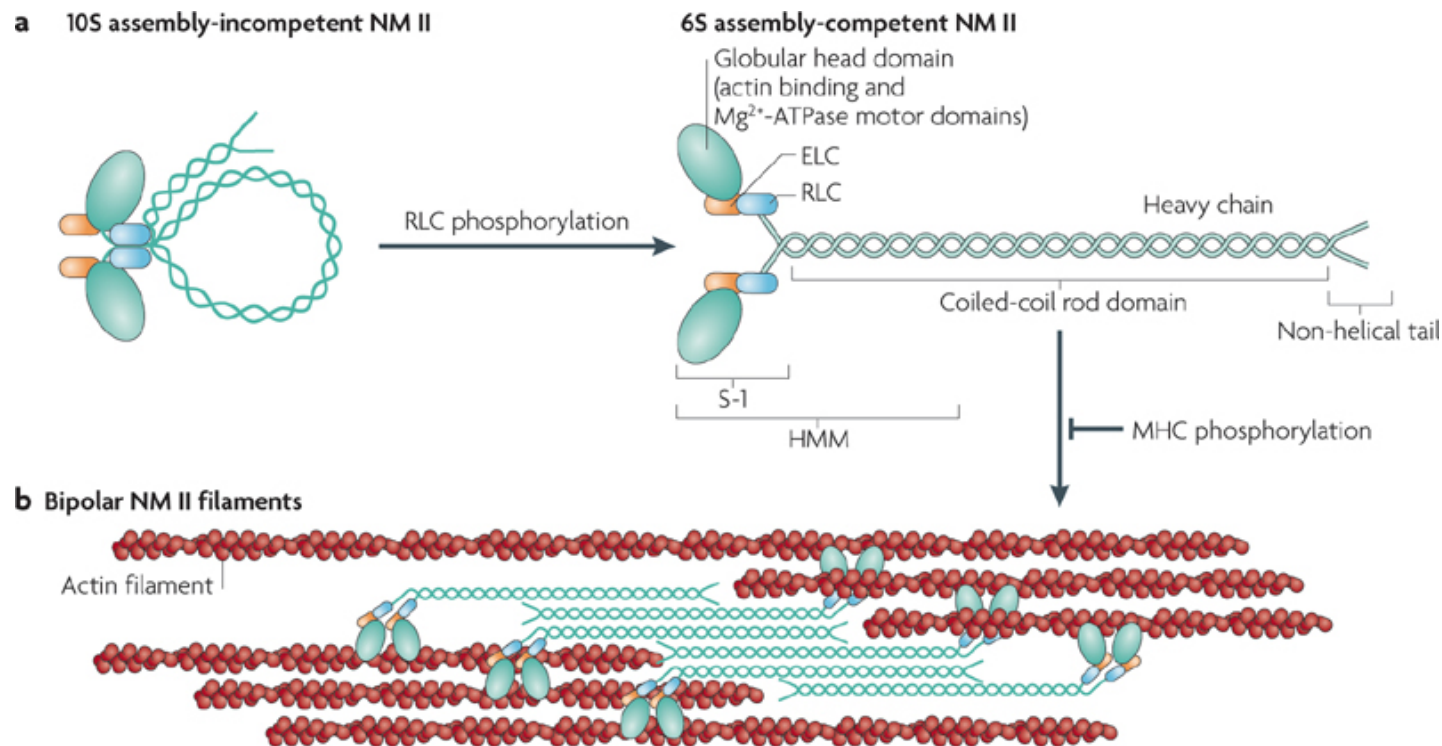


# [ What are myosins? ]

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- Myosins are actin-based motor proteins with known or predicted roles in many types of eukaryotic motility
- Myosins interact with actin filaments and couple hydrolysis of ATP to conformational changes that result in the movement of myosin and an actin filament relative to each other
- Known functions include cell adhesion, cell migration, cell division (cytokinesis), growth cone extension, maintenance of cell shape and phagocytosis
- Also involved in signal transduction pathways, such as myosin II is required for F-actin polymerisation during CR3-mediated phagocytosis

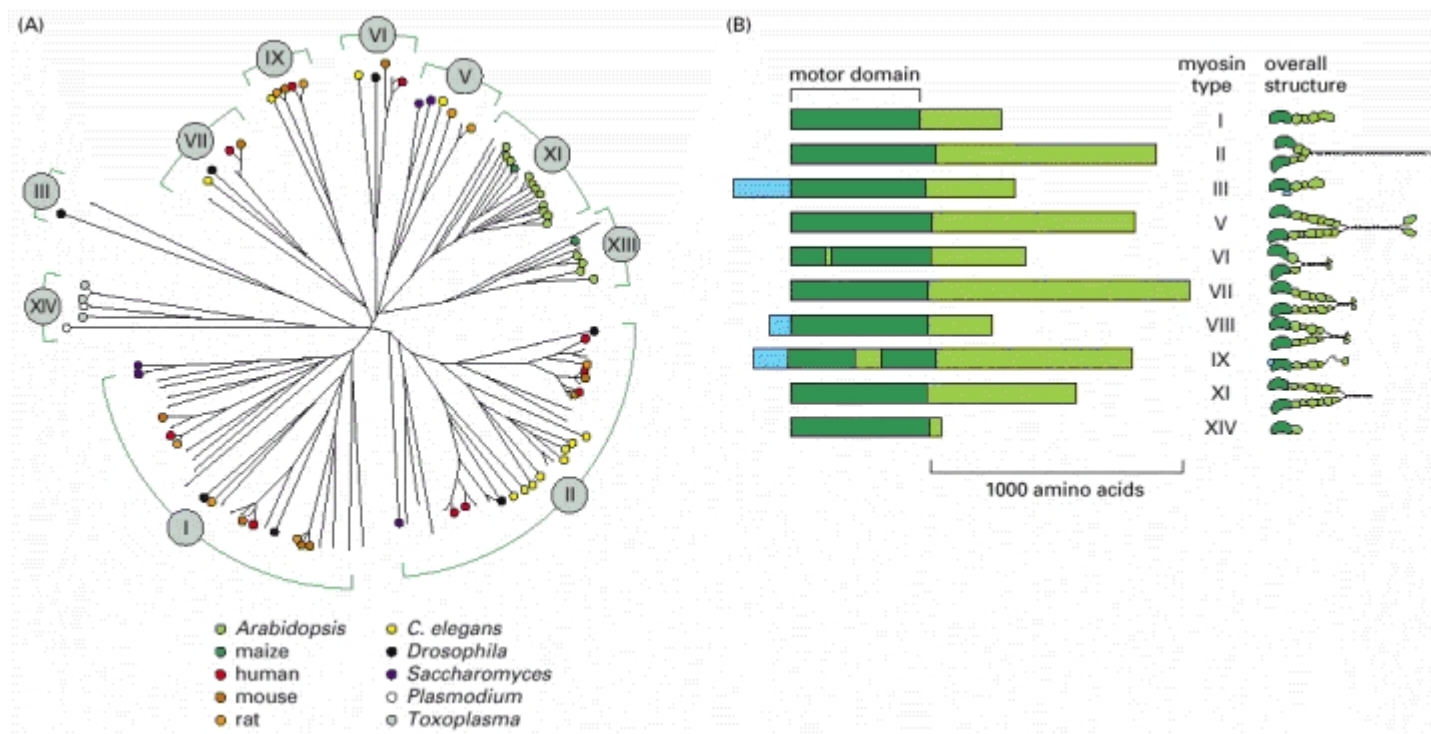
# Domain structure of prototypical myosin II, a conventional myosin



Nature Reviews | Molecular Cell Biology

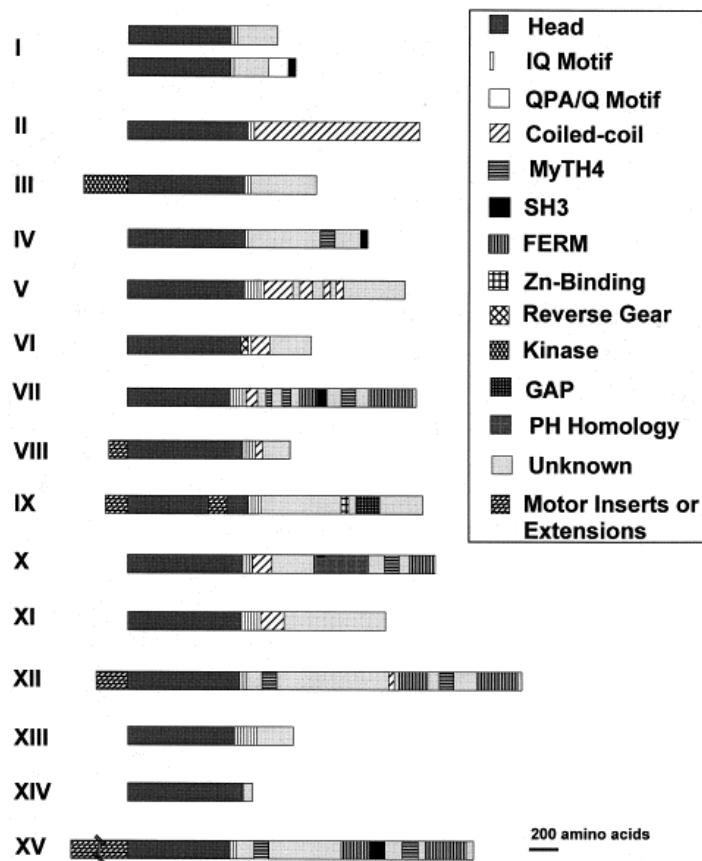
Actin cross-linking and contractile functions

# Domain structure of myosins



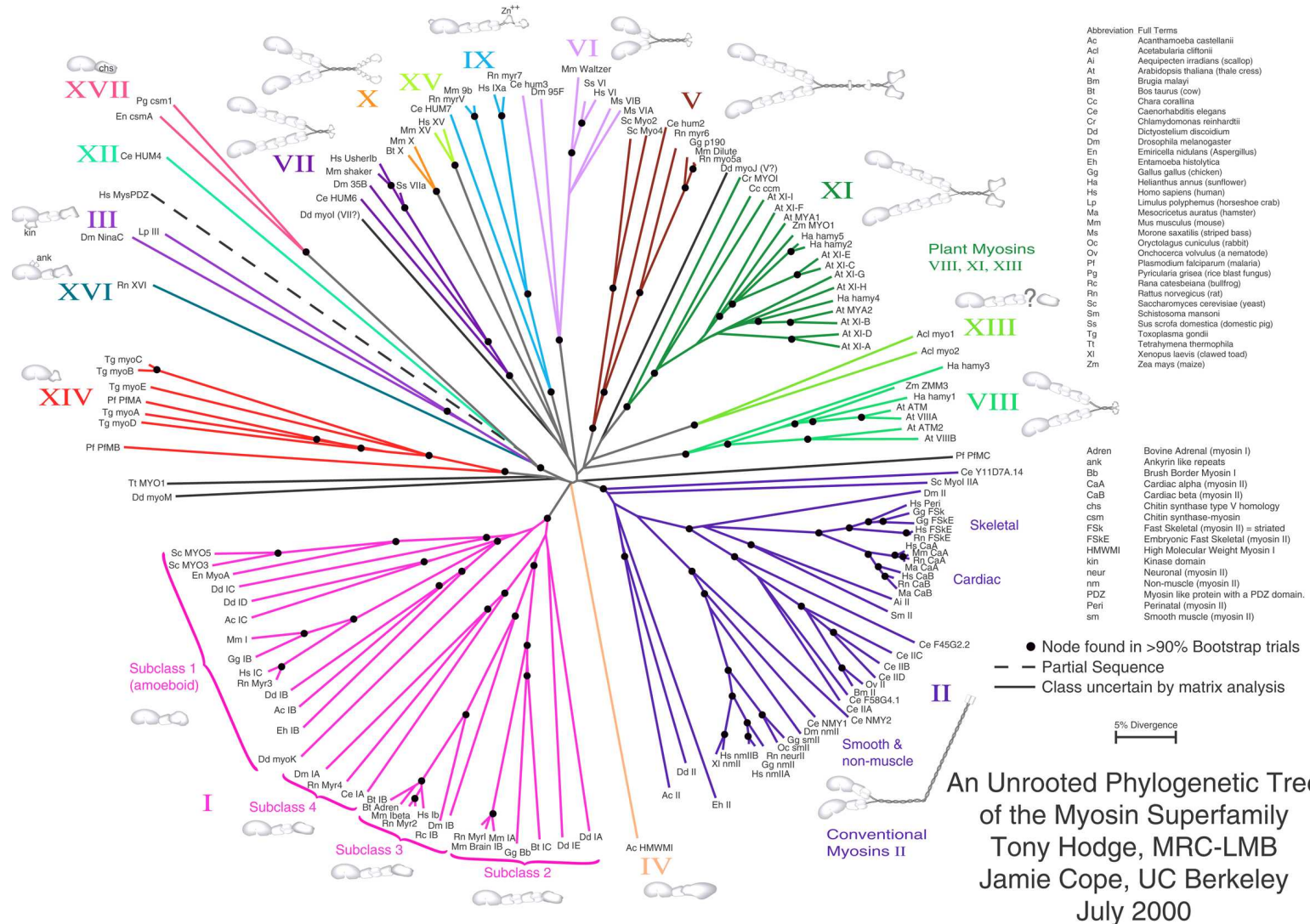
All myosins share similar motor domains (shown in *dark green*), but their C-terminal tails (*light green*) and N-terminal extensions (*light blue*) are very diverse. Many myosins form dimers, with two motor domains per molecule, but a few (such as I, IX, and XIV) seem to function as monomers, with just one motor domain.

# Domain structure of myosins

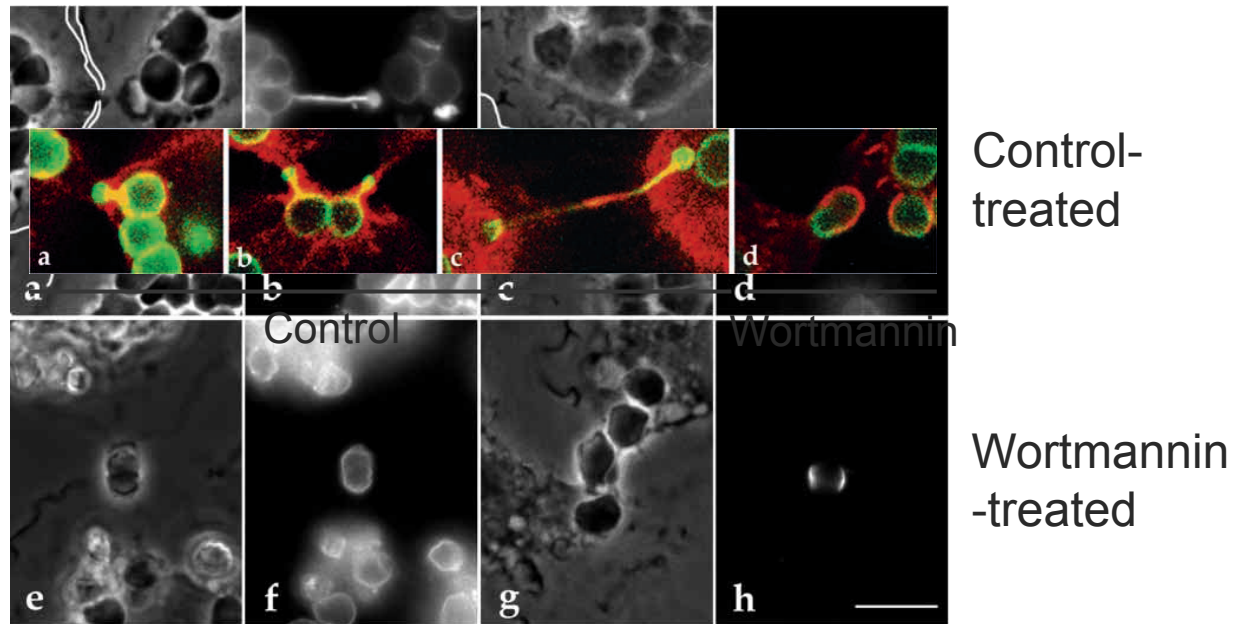


- 1) a motor region (head). A core motor (catalytic) domain which interacts with actin and binds ATP.
- 2) a neck region (or 'lever arm') composed of IQ motifs (from none to six) which have the consensus sequence (IQxxxRGxxxR) and bind either light chains or calmodulin.
- 3) a tail region which is extremely variable in sequence length, domain composition and organisation.

# The Myosins



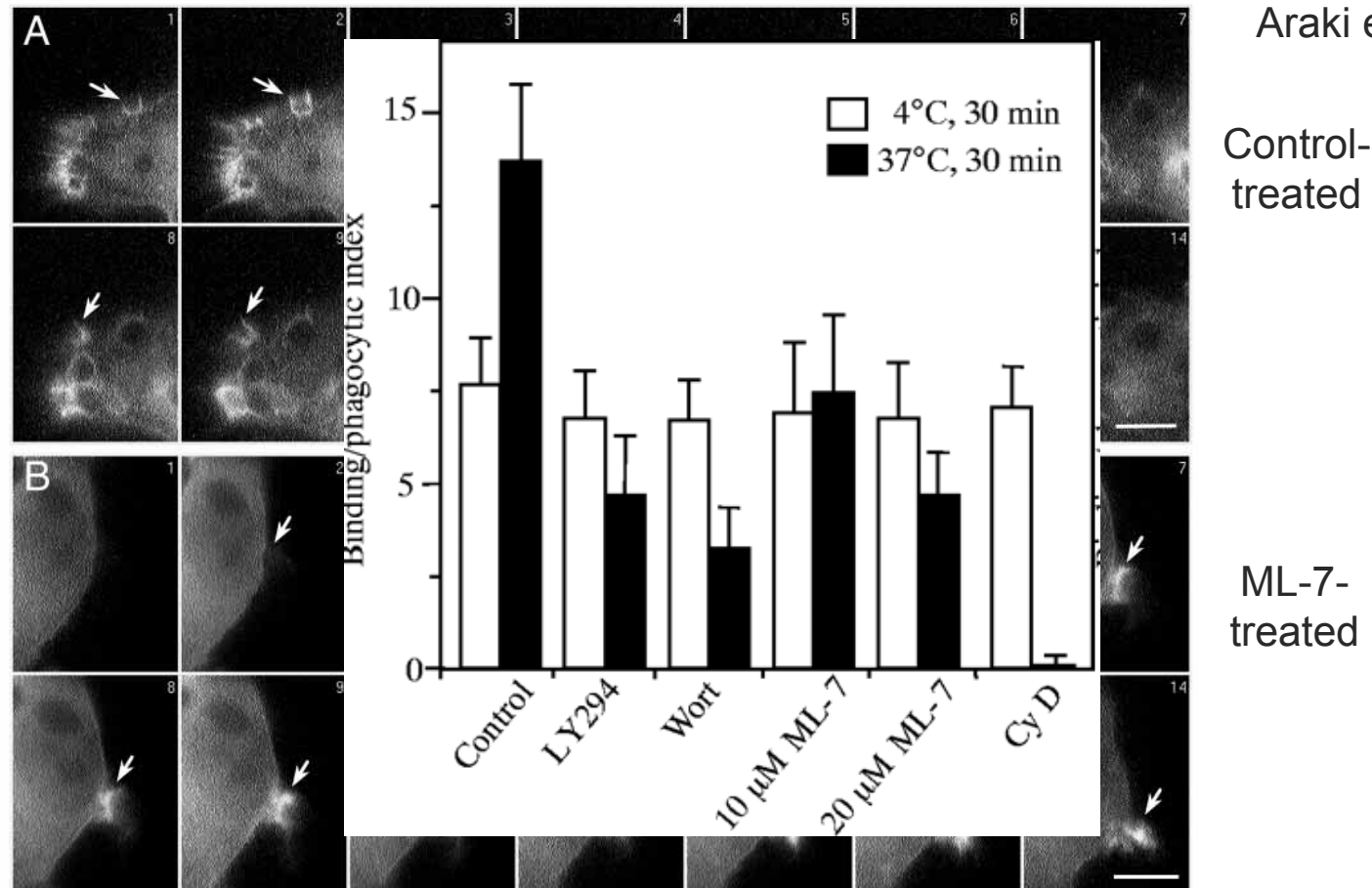
# Why look at myosin involvement in phagocytosis?





# Myosin-based contractility in phagocytosis

Araki et al., 2003

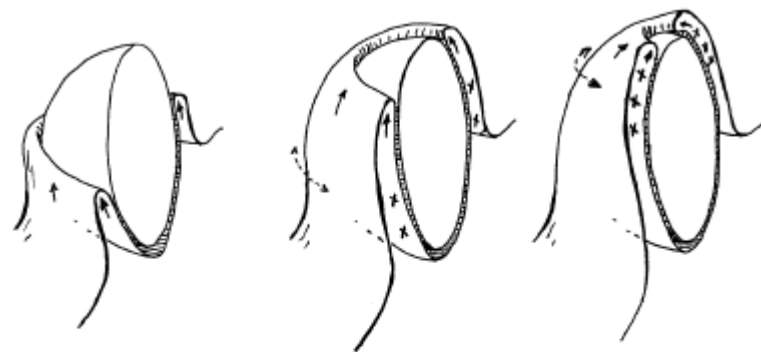


Time-lapse sequences of live macrophages expressing EGFP-actin, showing actin dynamics during Fc $\gamma$ R-mediated phagocytosis of IgG-red blood cells.

# Myosin-based contractility in phagocytosis

Two possible explanations for ML-7 inhibition are:

1. myosin contraction between the FcγR and ligand, leading to closure of the phagosome
2. the tight-fitting particle fluidizes the membrane, decreasing intraphagosomal pH and activating hydrolases



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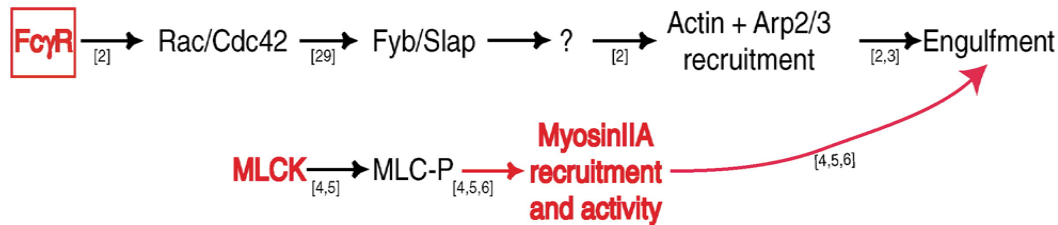
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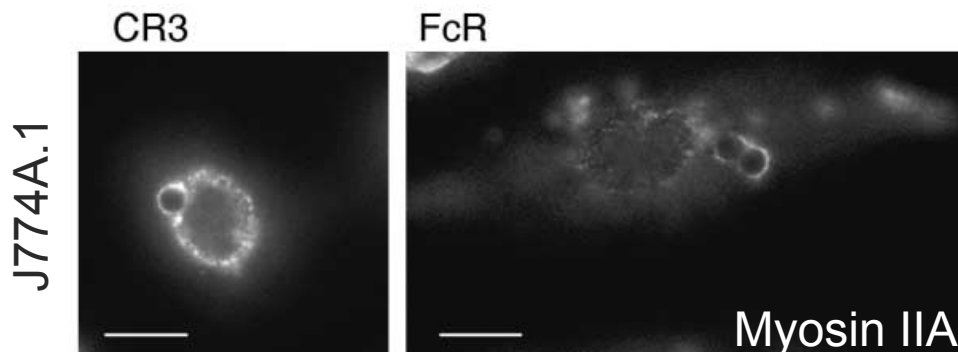


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# Myosin II in phagocytosis

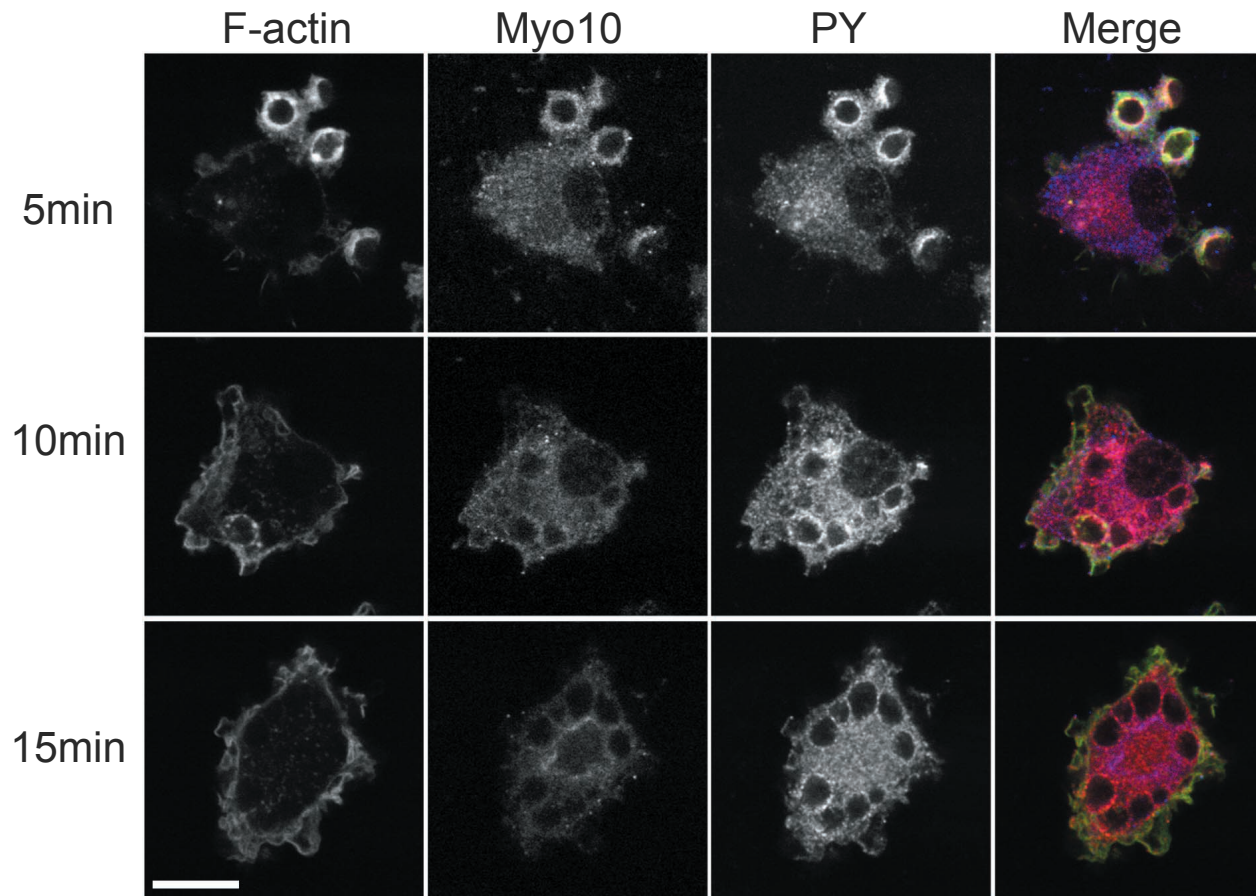


Myosin II plays a role in particle internalisation during both FcγR- and CR3-mediated phagocytosis but is only required for actin cup assembly downstream of the CR3.



Olazabal et al., 2002

# Myosin X in FcγR-mediated phagocytosis



Myosin X is recruited to phagocytic cups and expression of a truncated myosin X tail inhibits the phagocytosis of large particles (6 $\mu$ m) but not small ones (2 $\mu$ m).

**Predicted Mechanism:** Myosin X binds to PIP3 in membrane through its PH domain, the motor head domain engages actin filaments and moves towards the barbed ends

# [Experiments]

## Investigate cup closure

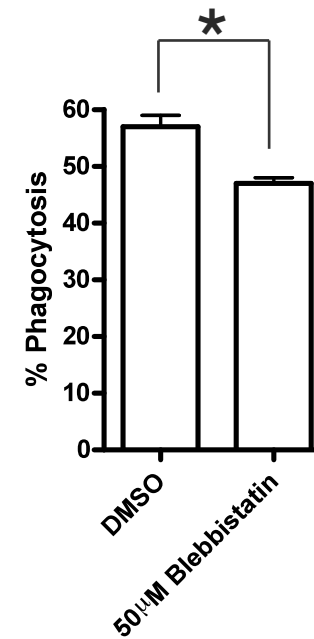
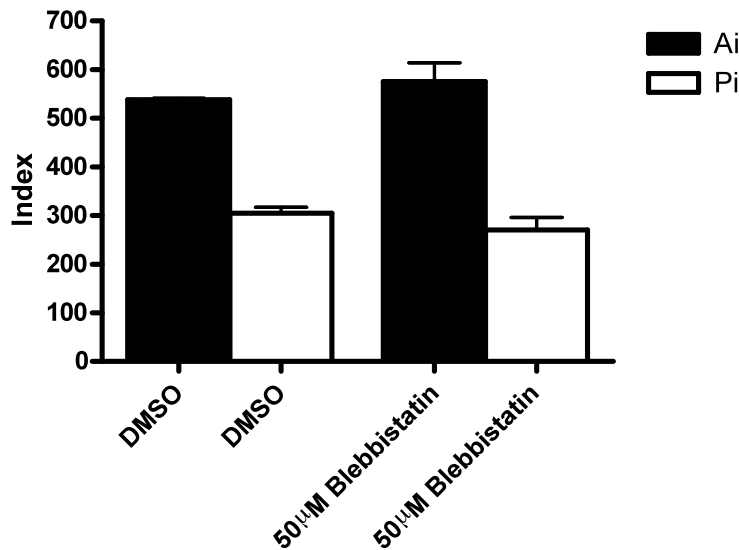
Role of motor proteins in cup shape and closure:

- J774 macrophages treated with myosin inhibitors – blebbistatin, ML-7 or BDM. Observe cup shape by confocal and SEM.
- J774 macrophages labelled with myosin-specific antibodies. Use confocal microscopy to look at localisation.
- COS-7 cells transfected with FcγR and GFP-tagged myosins during phagocytic challenge with IgG-opsonised beads. Observe cup shape by confocal microscopy.
- J774 macrophages transfected with siRNA to knockdown specific myosins. Observe cup shape by confocal and SEM.



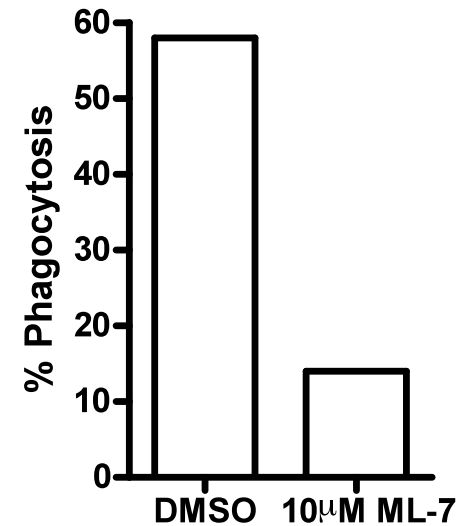
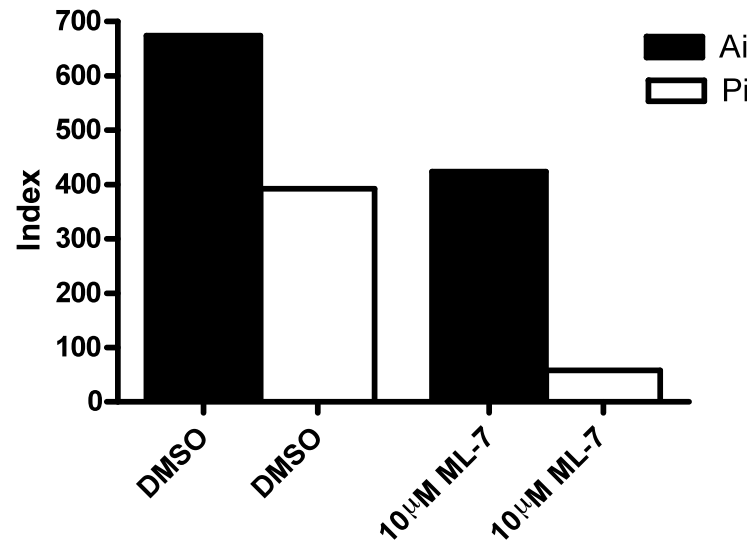
# Blebbistatin-treatment of J774A.1 cells undergoing FcγR-mediated phagocytosis

- Blebbistatin is a small molecule inhibitor showing high affinity and selectivity toward non-muscle myosin II. Importantly, it does inhibit myosin from classes I, V and X.

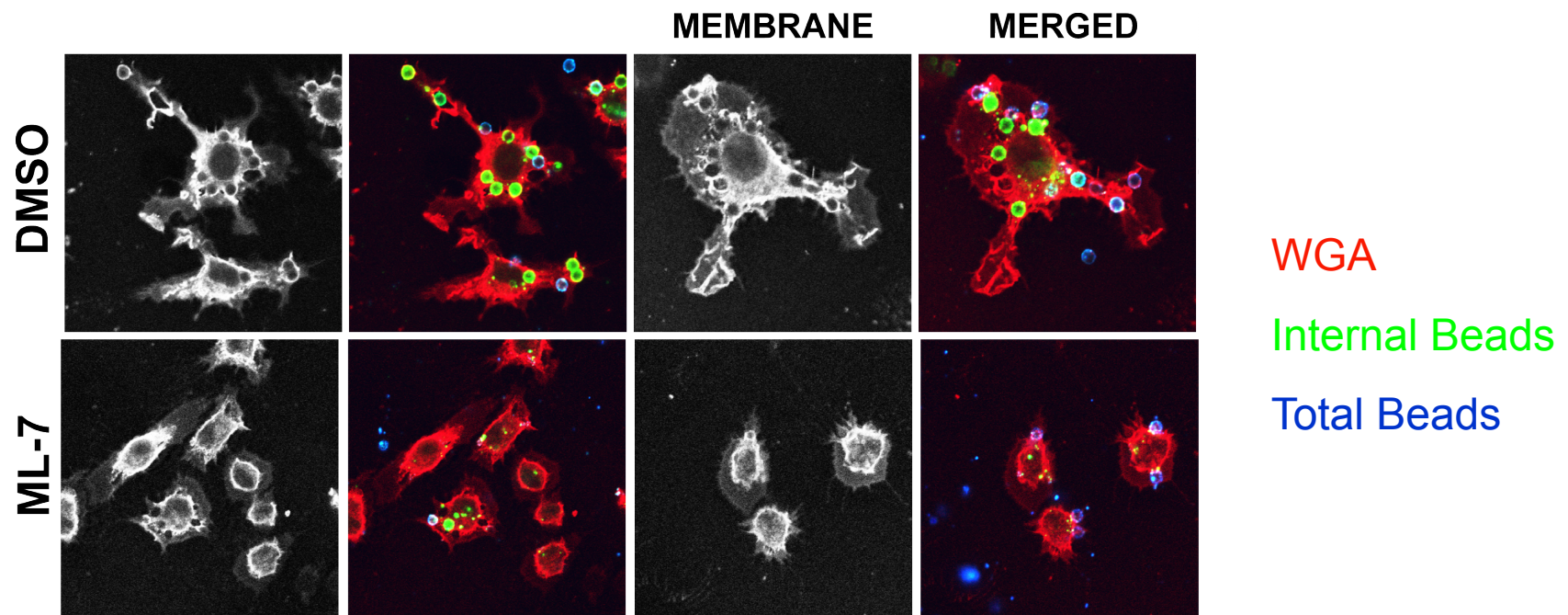


# ML-7-treatment of J774A.1 cells undergoing FcγR-mediated phagocytosis

- ML-7 is an inhibitor of myosin light chain kinase (MLCK) and inhibition of MLCK results in selective perturbation of myosin II function.

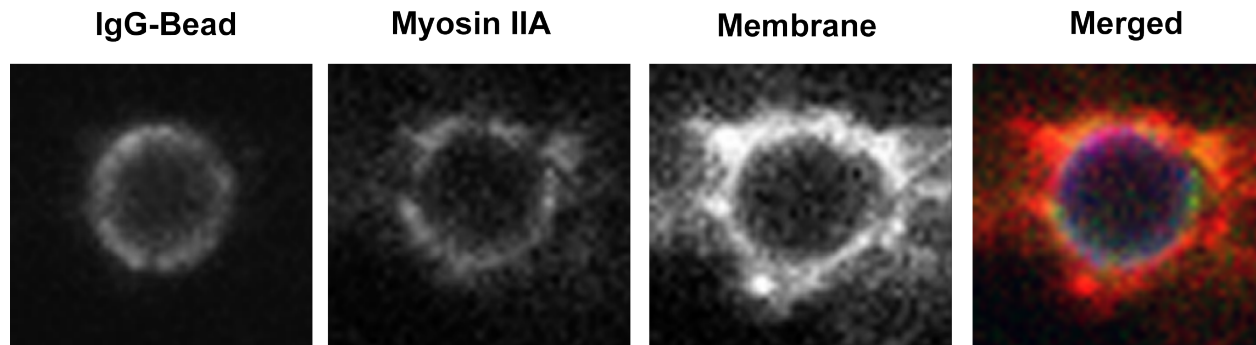
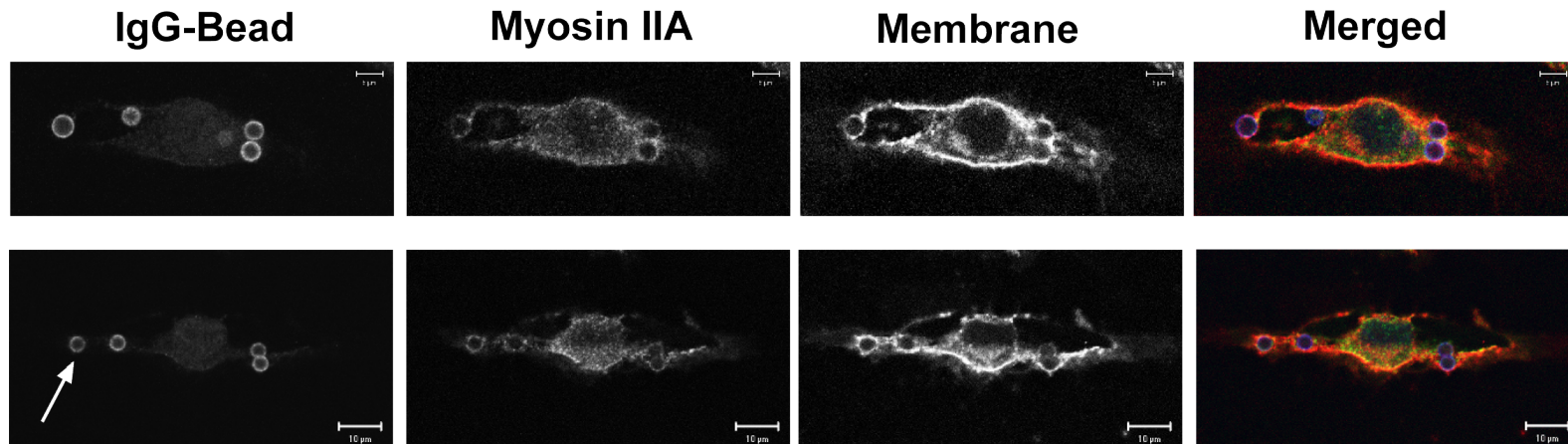


# ML-7-treatment of J774A.1 cells undergoing FcγR-mediated phagocytosis

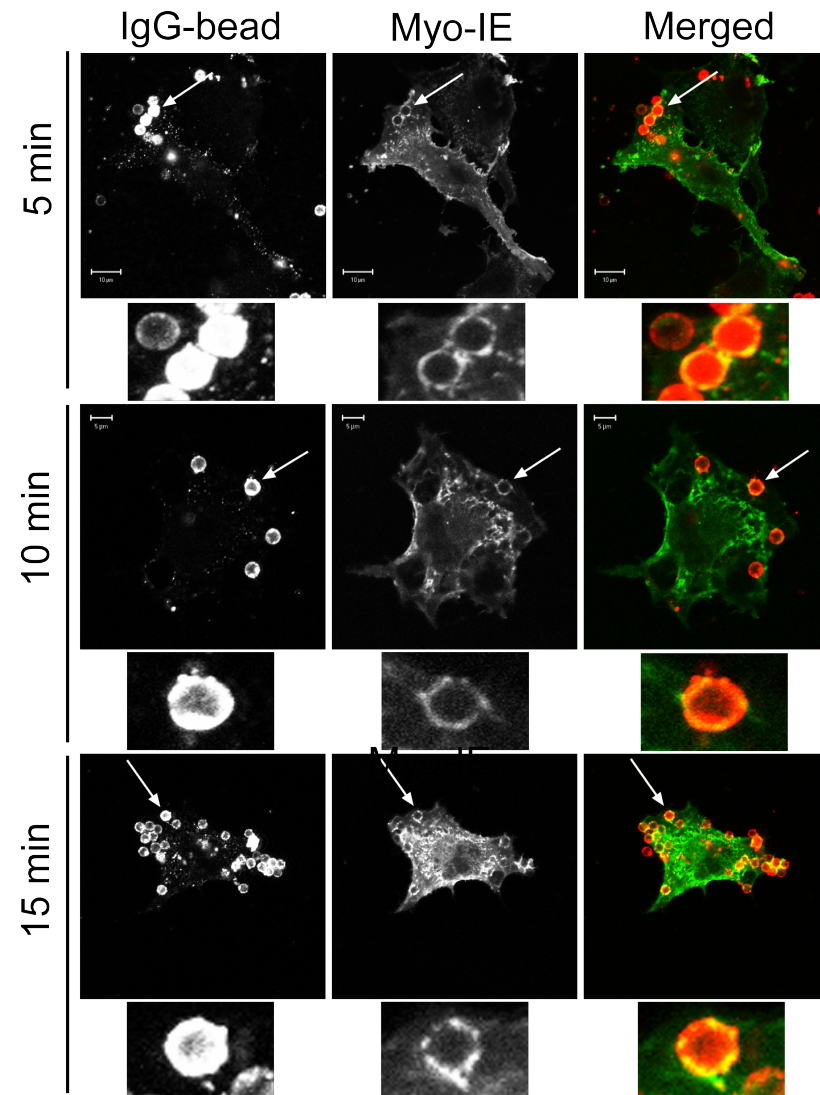
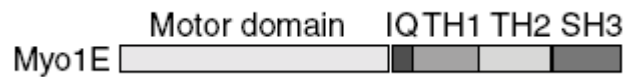


# Myosin IIA recruitment to FcγR phagocytic cups in J774A.1 macrophages

J774A.1

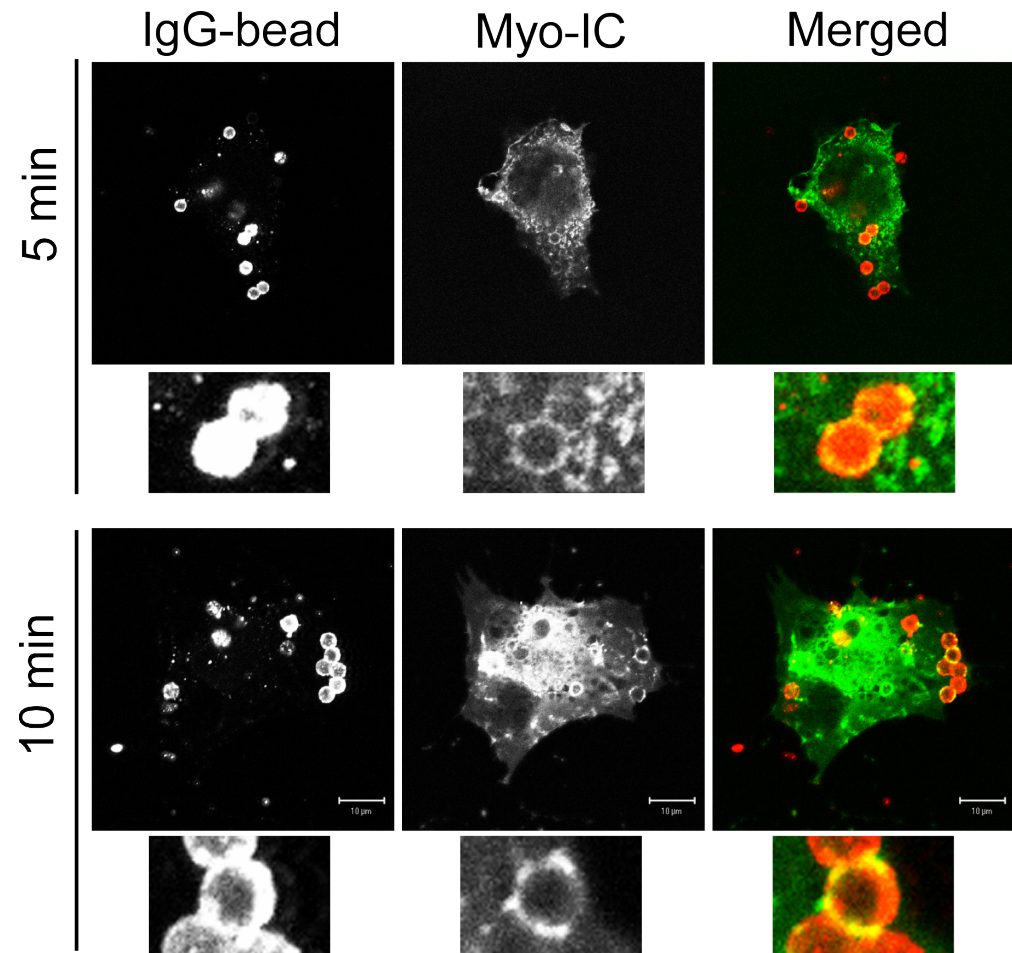
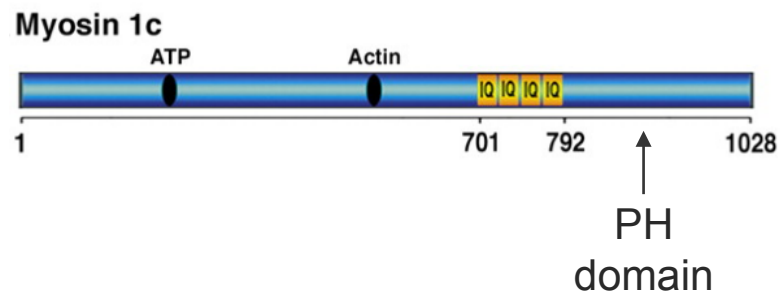


# Myosin IE recruitment to FcγR phagocytic cups in receptor-transfected COS-7 cells



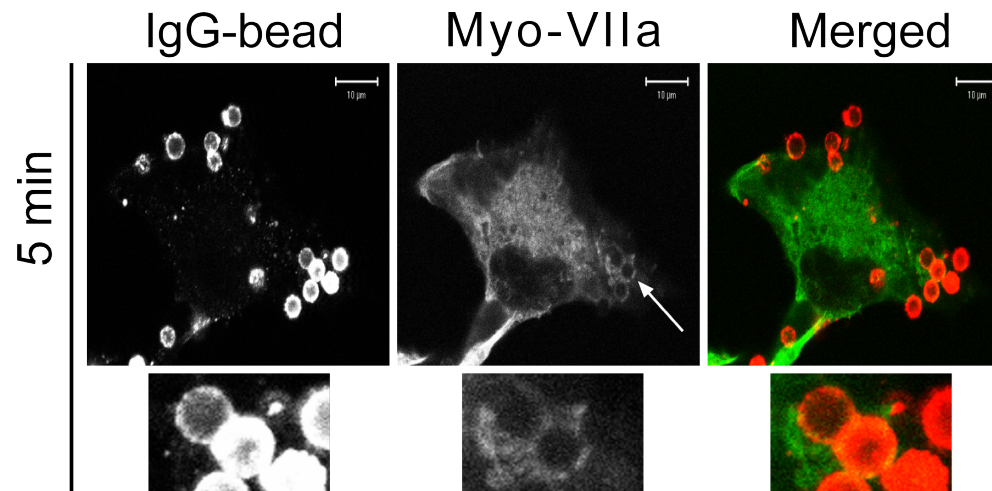


# Myosin IC recruitment to FcγR phagocytic cups in receptor-transfected COS-7 cells





# Myosin VIIa recruitment to FcγR phagocytic cups in receptor-transfected COS-7 cells



## [ Future work ]

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- Identification of the key myosins involved in FcγR-mediated phagocytosis
- Confocal imaging of key myosins during the time course of phagocytosis, in particular MyoIE and Myo1G.
- Confocal imaging of phagocytic cups with key myosins knocked down.
- SEM

# Future work

- 3D-image analysis/reconstruction of phagocytic cup shapes (by Sylvain)

