

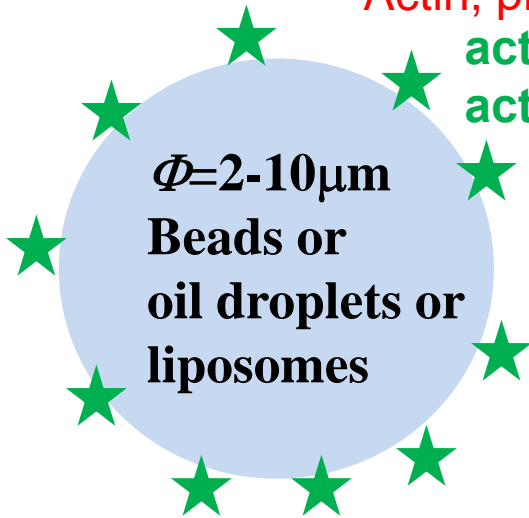


institutCurie

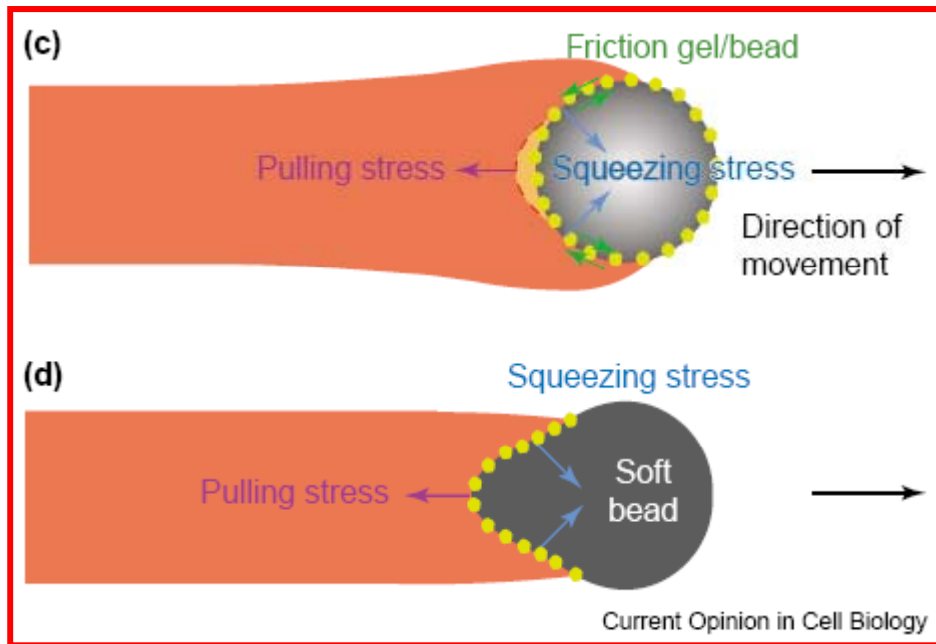
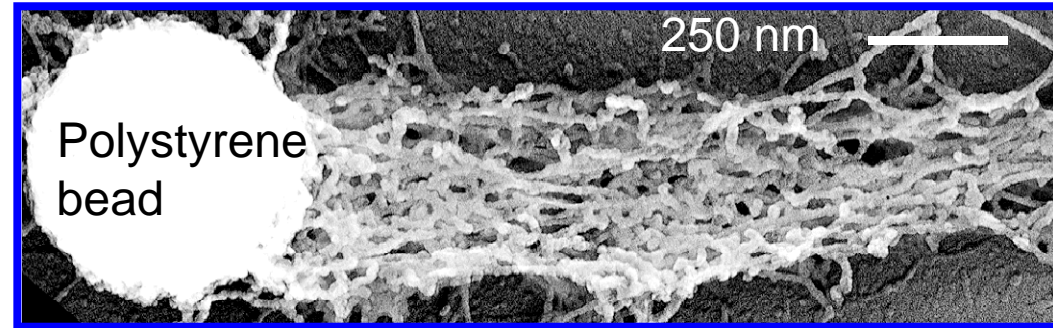
# Biomimicking systems of cell shape changes

Cécile Sykes, Laboratoire Physicochimie Curie, UMR 168  
Institut Curie/CNRS/Université Paris 6, FRANCE

Actin, proteins (or cell extracts)  
activator of the Arp2/3 complex,  
actin polymerisation



Lamellipodia like comets

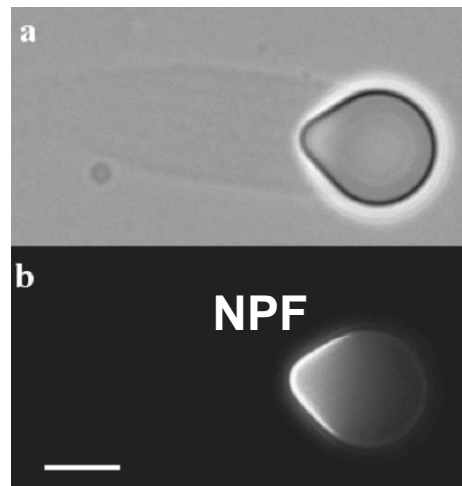
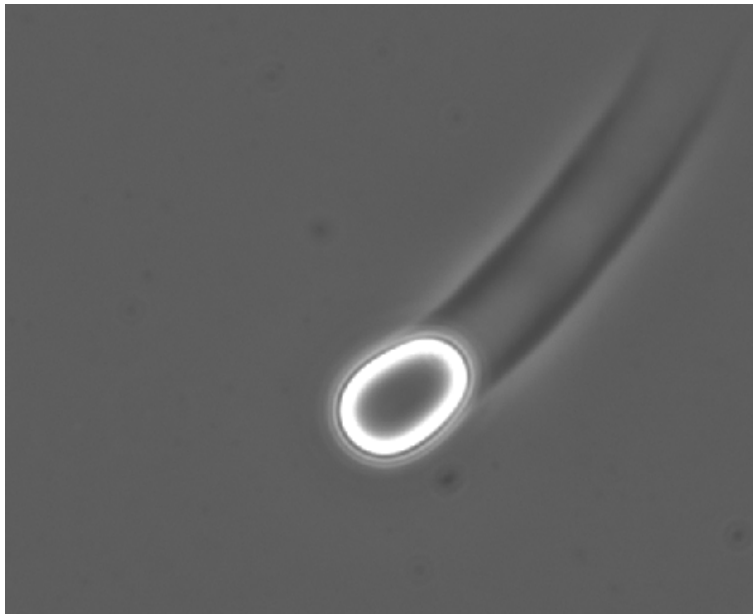


[Mastino, S. Olivier, C. Sykes, *Current Biology* 2004]



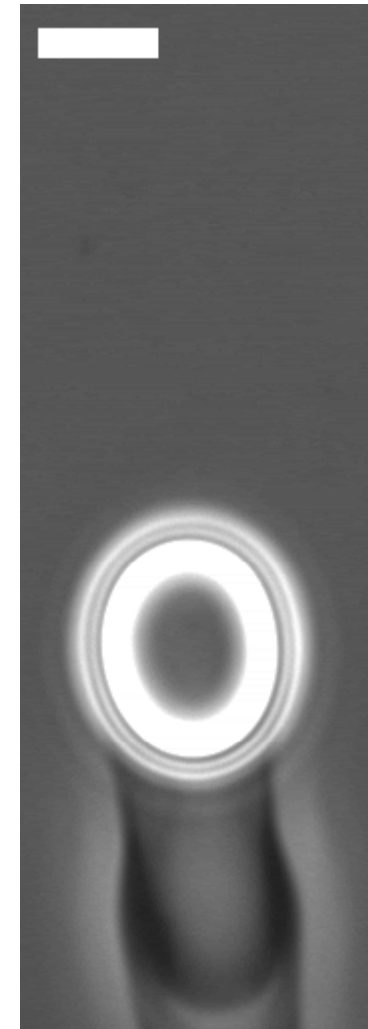
[E. Paluch, J. van der Gucht, *BJ* 2006]

$\Phi=7\mu\text{m}$



[H. Boukellal, 2004]

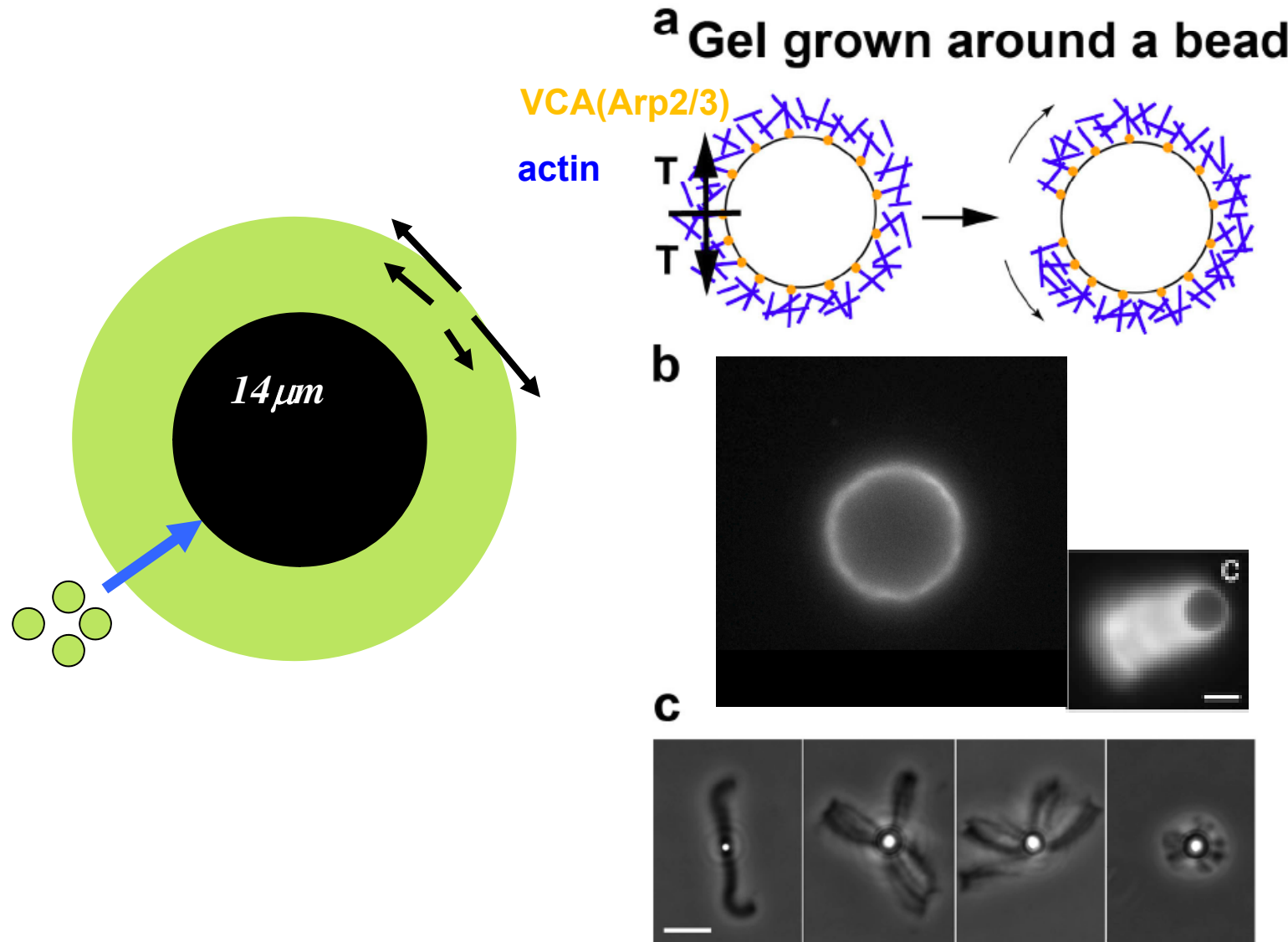
$\Phi=4\mu\text{m}$



[L. Trichet, *BJ* 2007]

**+VASP that detaches the NPF from actin**

# Gel growth-symmetry breaking-movement



*low gelsolin concentration*

[J. Plastino, Curr Op in Cell Biol 2005 ]

[J. van der Gucht PNAS 2005, E. Paluch, JCB 2006]

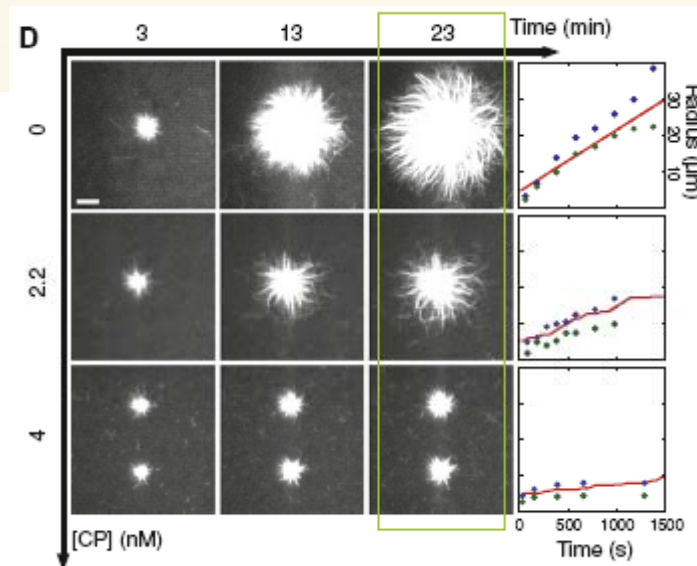
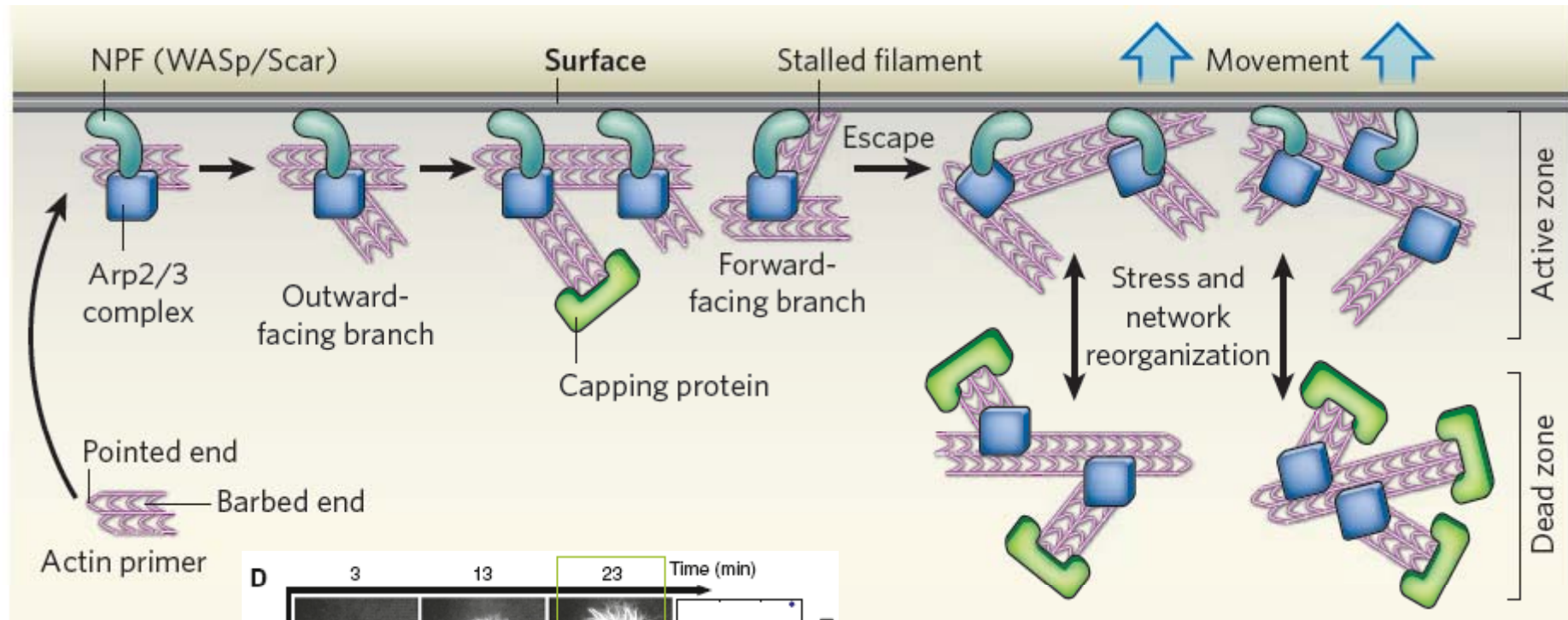
- **Formation of actin shells around beads for stress buildup**
- **Artificial corteces in liposomes (inside-out geometry of actin shells)**
- **Mechanical characterisation of artificial corteces (tube pulling)**

➤ **Formation of actin shells around beads for stress buildup**

➤ Artificial corteces in liposomes (inside-out geometry of actin shells)

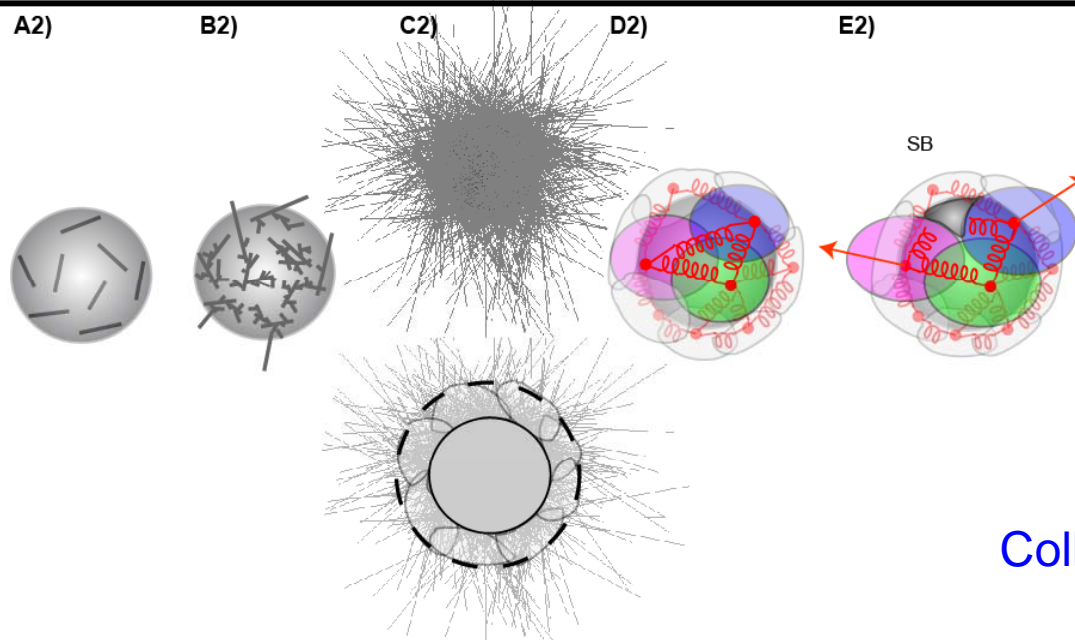
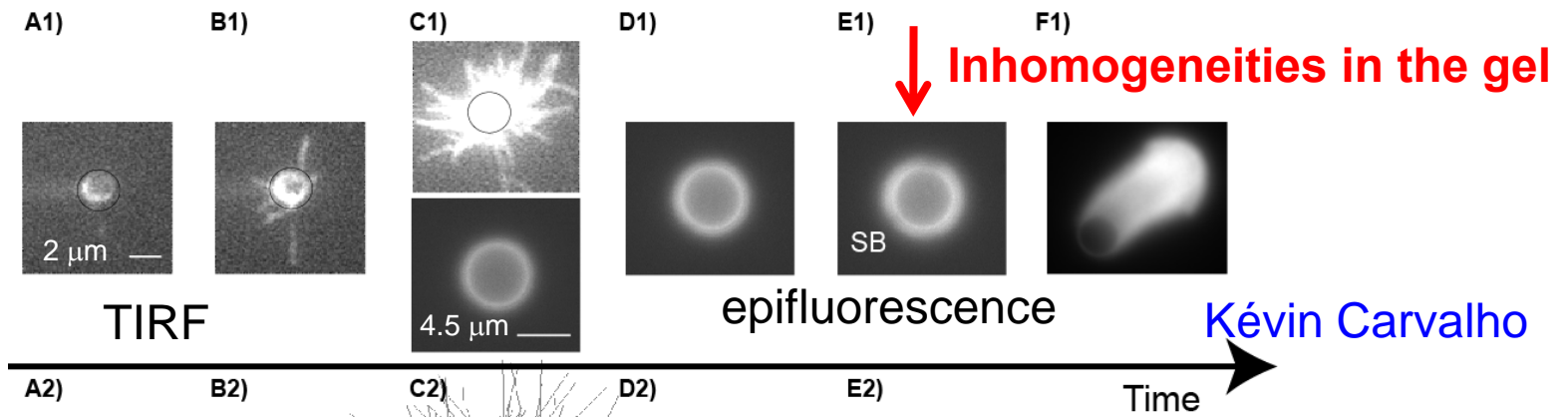
➤ Mechanical characterisation of artificial corteces (tube pulling)

# Actin gel growth through branching and capping



[Achard Current Biol 2010, Blanchoin lab]

# Actin gel growth and stress build up in the presence of Arp2/3 and CP



Agnieszka Kawka

Coll

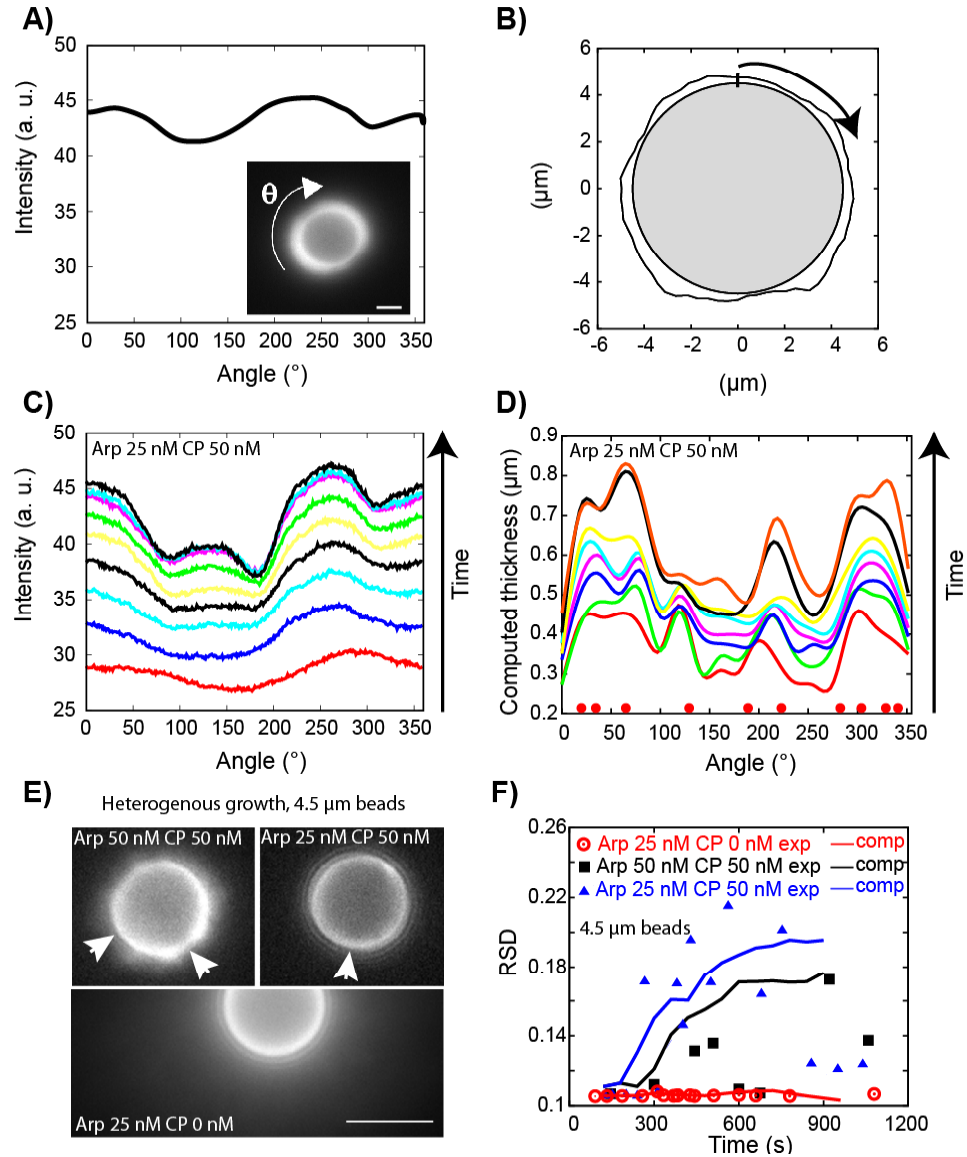
Laurent Blanchoin  
Jean-Louis Martiel

Symmetry breaking does not happen in all Arp2/3 and CP concentration conditions

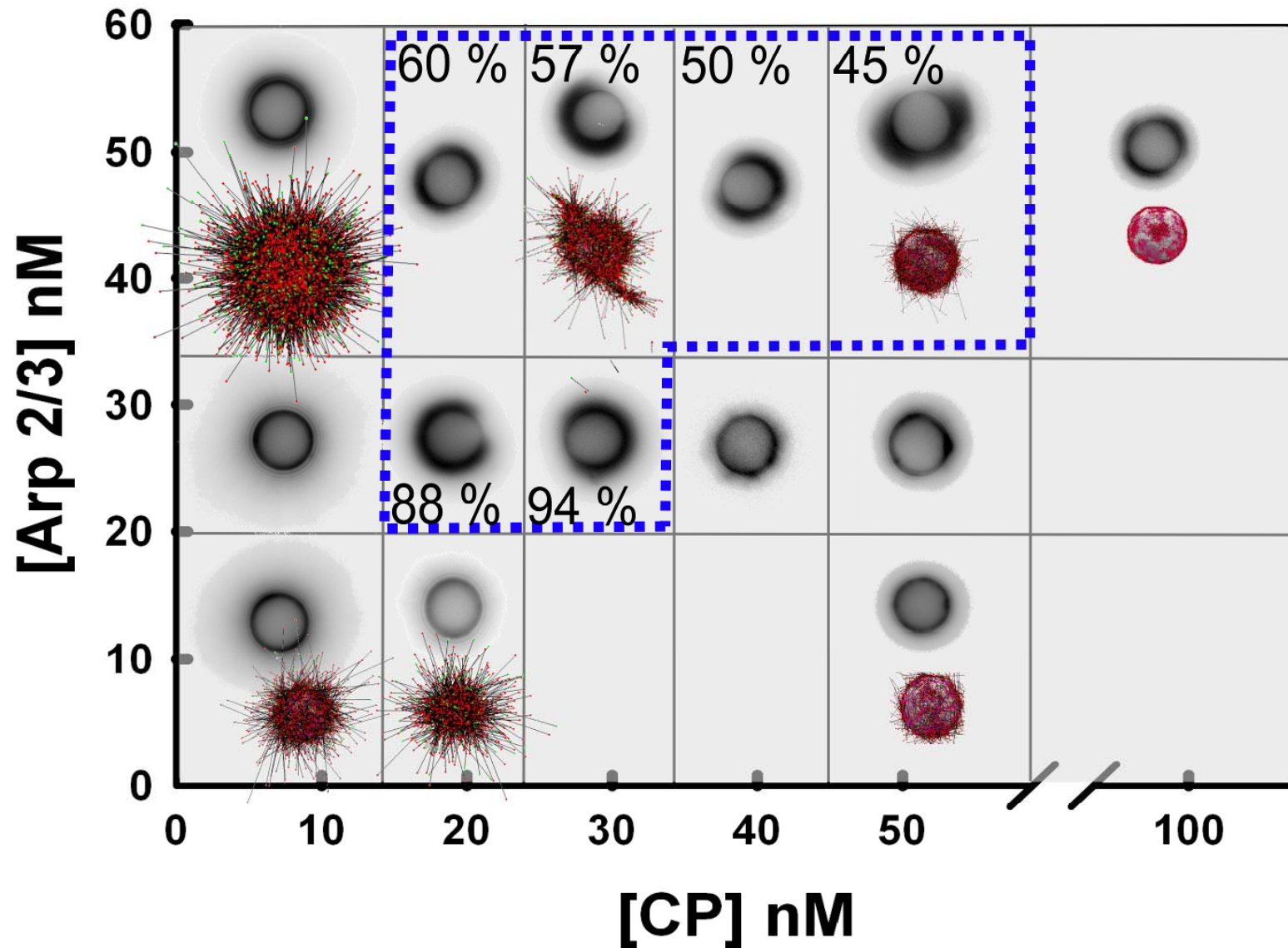


# Actin heterogeneities

## No symmetry breaking



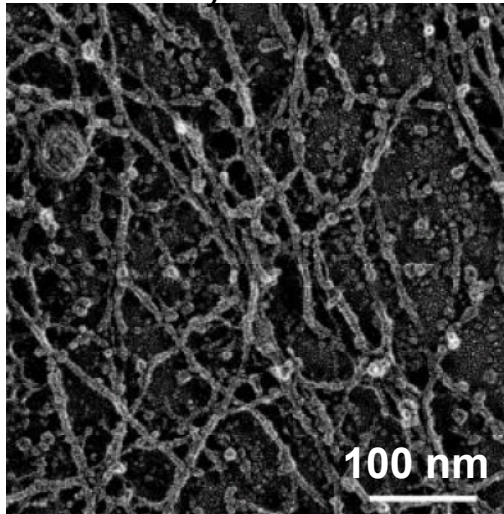
# Predictive morphology diagram



- Formation of actin shells around beads for stress buildup
- **Artificial corteces in liposomes (inside-out geometry of actin shells)**
- Mechanical characterisation of artificial corteces (tube pulling)

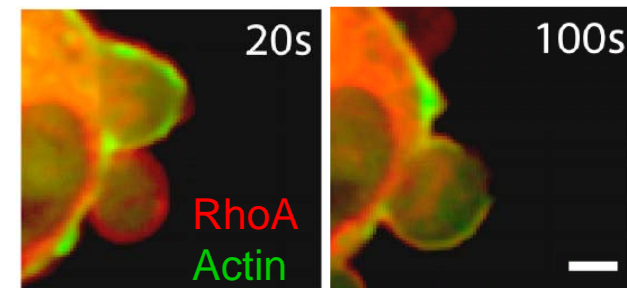
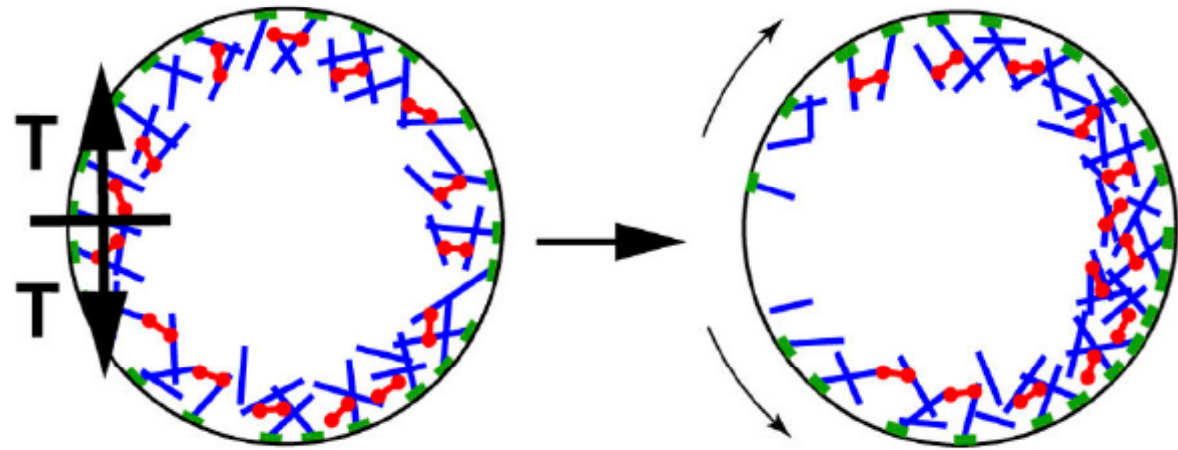
# Mimicking the **actomyosin** cortex in cells

Rat kidney fibroblast



[Morone et al., 2006]

- Filaments next to the membrane
- No specific orientation
- Meshsize ~100nm
- $50\text{nm} < \text{thickness} < 2\mu\text{m}$



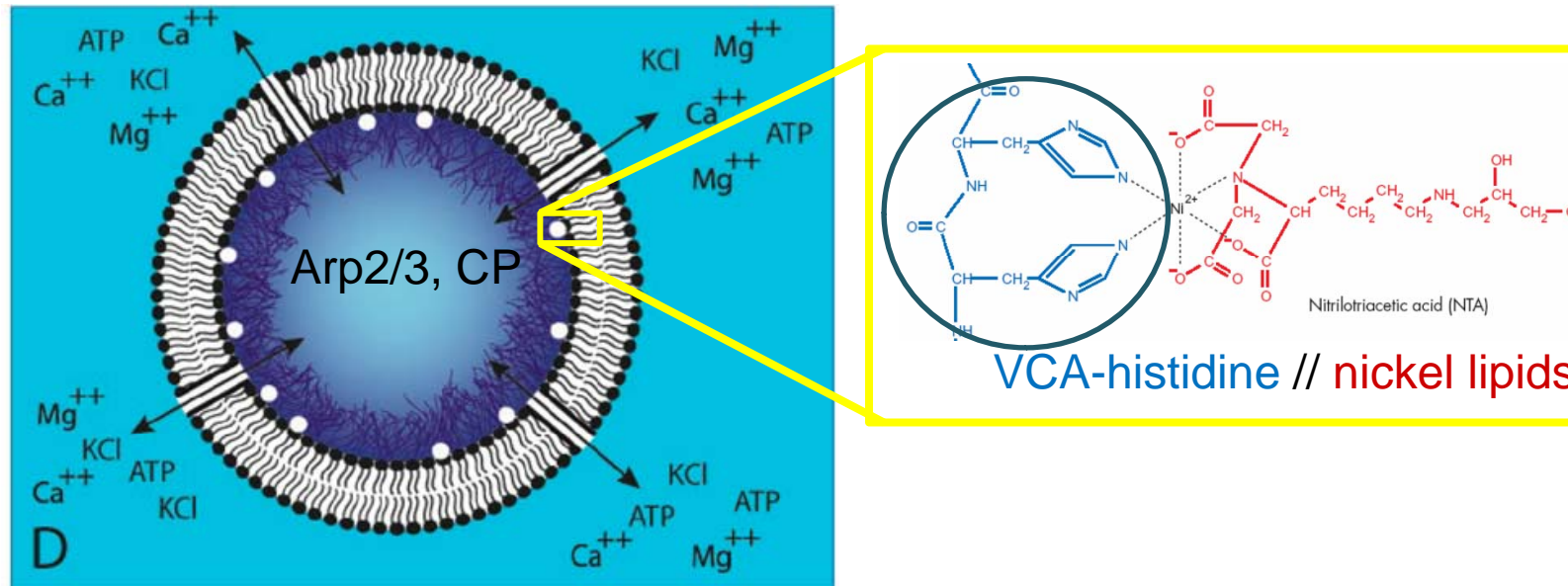
[Charras et al., JCB 2006]

- **Nucleation: unclear**
- Tethering: ERM proteins  
(Ezrin, Radixin, Moesin)
- **Myosin II**

# Actin polymerisation at the liposome inner membrane

$\alpha$ -hemolysin ~1nm diameter  
Cut-off = 3kDa

[Noireaux et al. 2004]

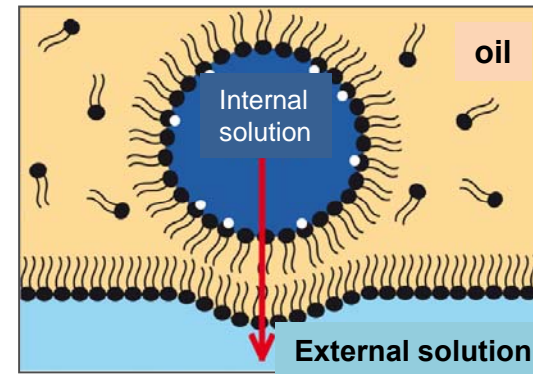
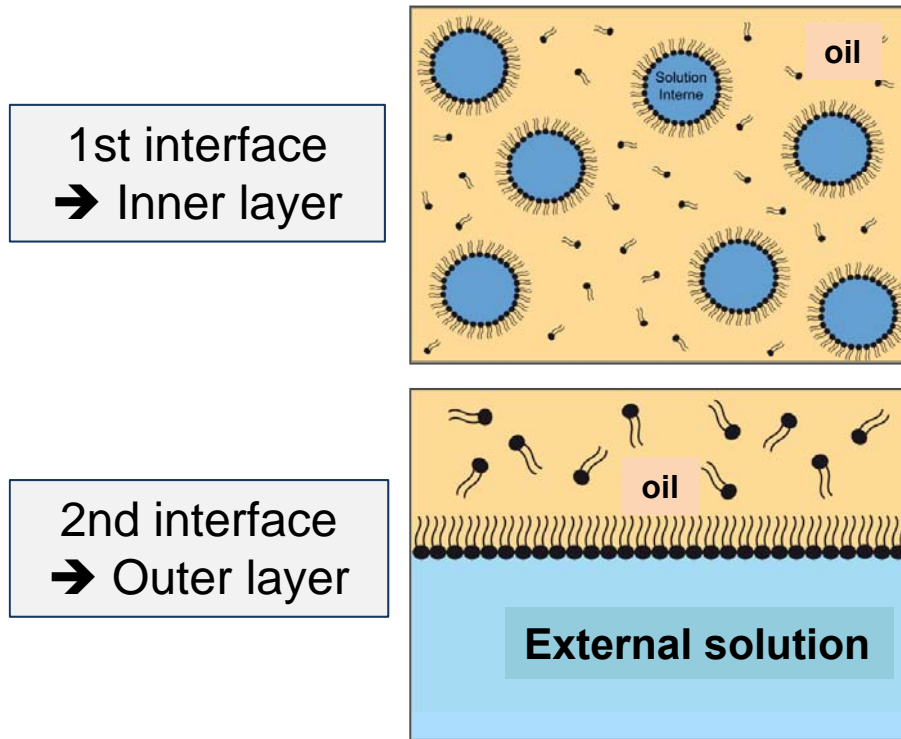


[L.-L. Pontani et al., BJ, 2009]

Polymerisation after pore incorporation or upon T° increase

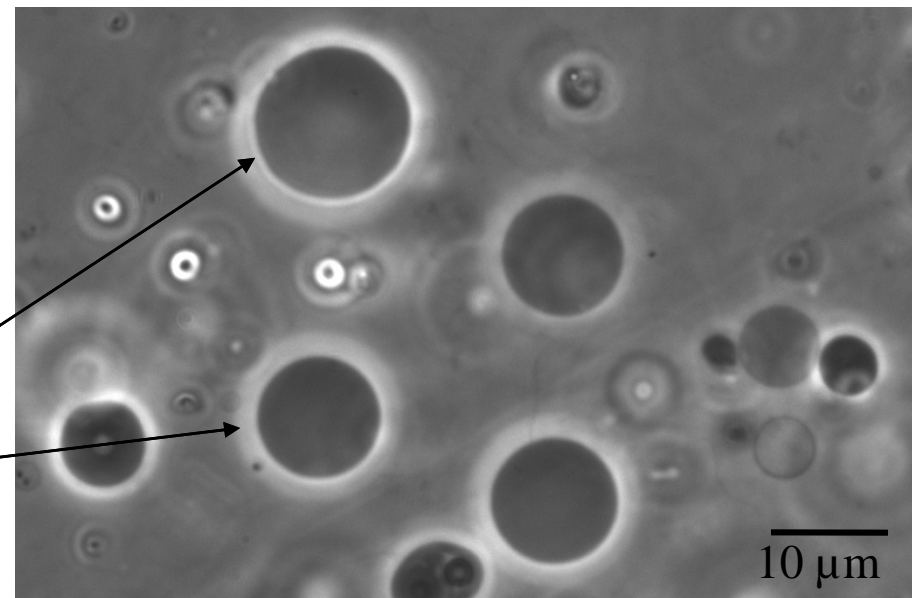
# Inverted emulsion technique

[Pautot et al. 2003]



Sucrose inside/glucose outside

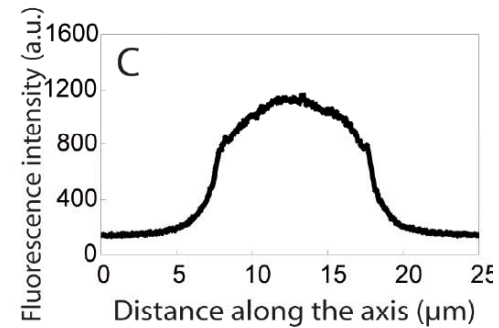
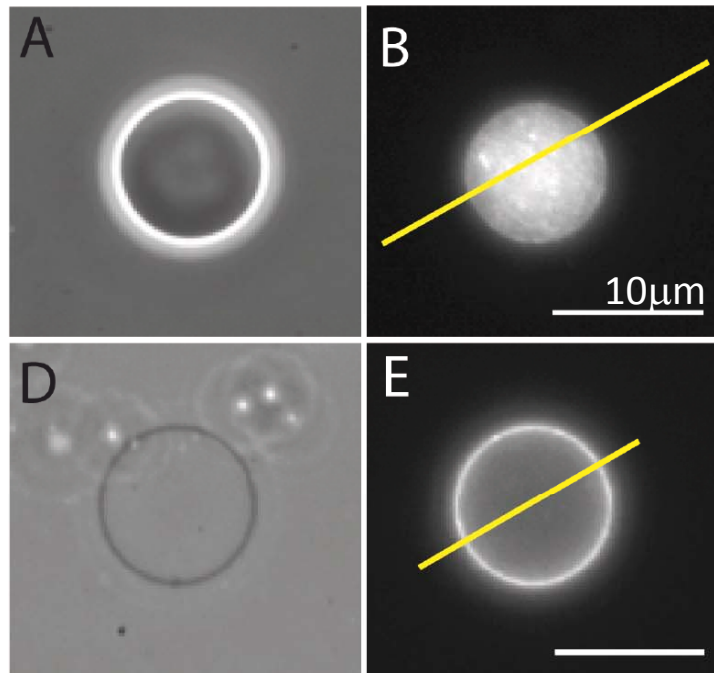
→ High contrast  
= effective encapsulation



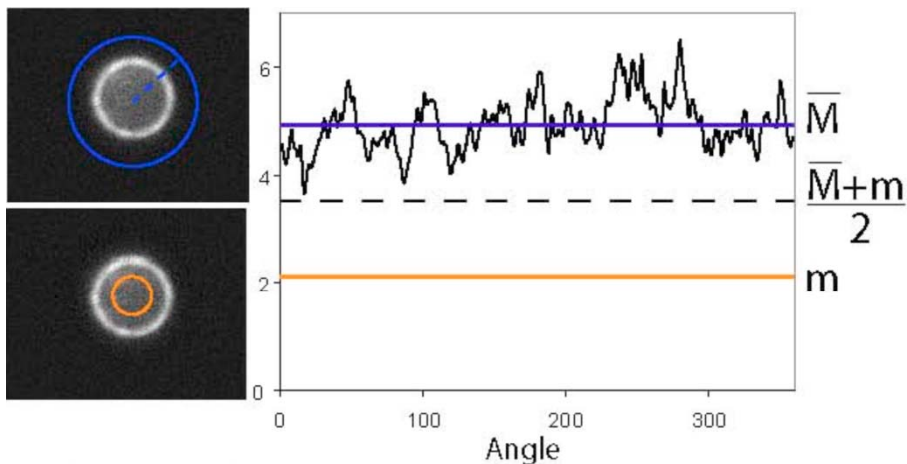
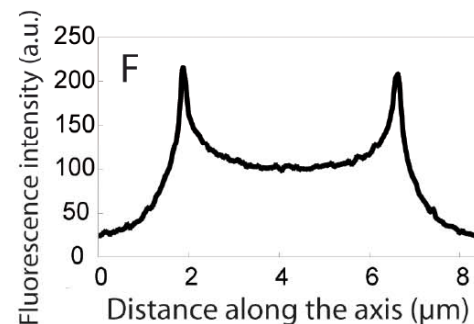
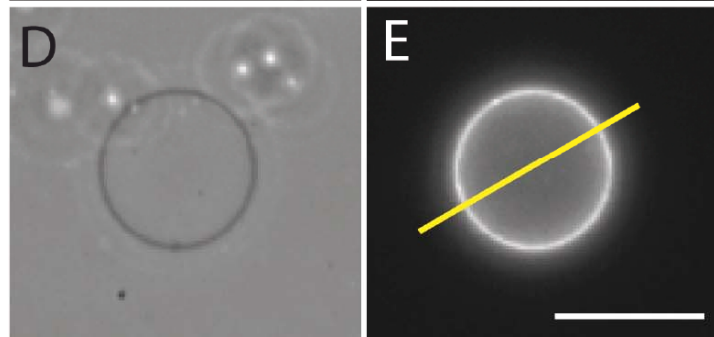
[L.-L. Pontani, BJ 2009]

# Triggering the polymerisation

Without the pores



With the pores



$$C = \frac{M - m}{M + m}$$

Shell fluorescence if  $C > 0.01$

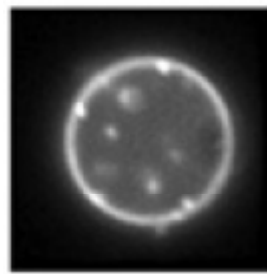
[L.-L. Pontani, BJ 2009]  
Without pores, Karine Guevorkian

# Artificial corteces

- produce dynamic actin polymerisation  
vanish in LatA treatment
- are specific of the Arp2/3 machinery

[L.-L. Pontani et al., BJ, 2009]

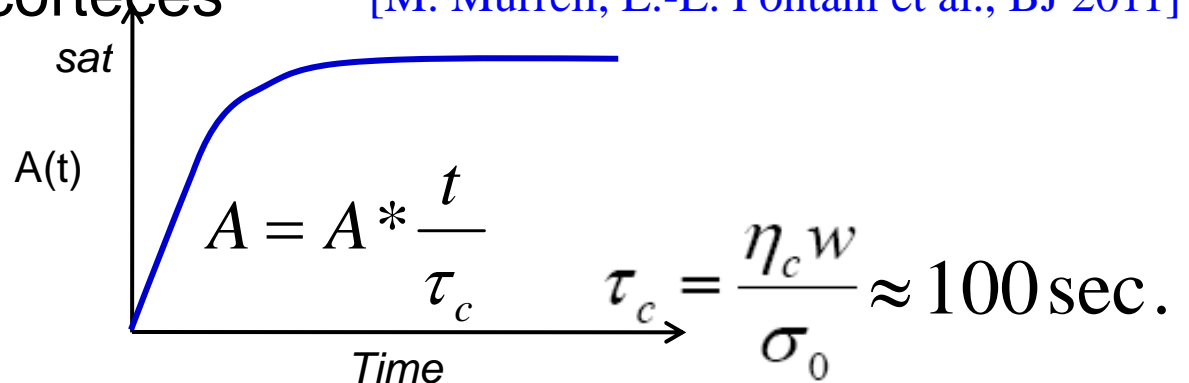
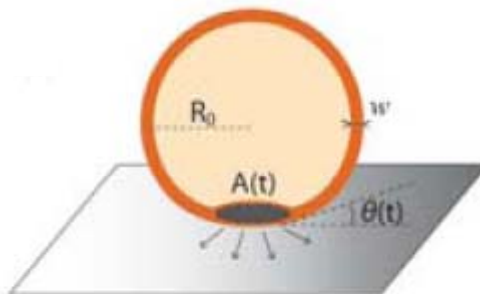
- are able to reproduce the endocytosis of the Shiga toxin



[W. Römer, L.-L. Pontani et al., Cell, 2010]

- spread like cellular corteces

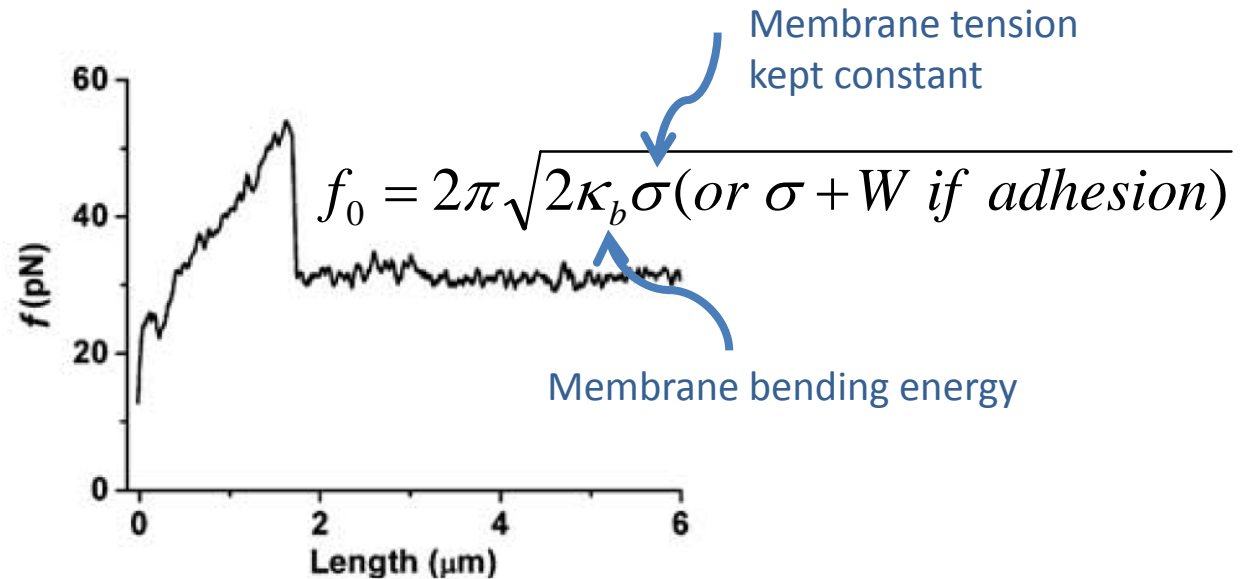
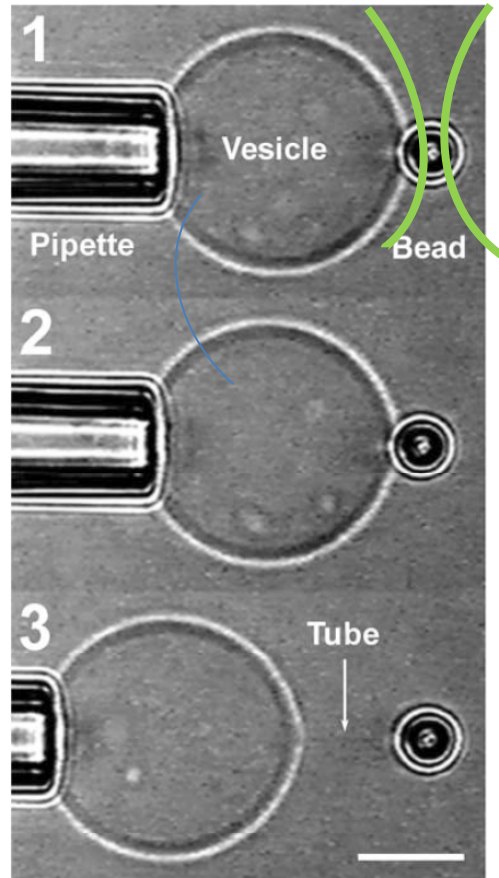
[M. Murrell, L.-L. Pontani et al., BJ 2011]





- Formation of actin shells around beads for stress buildup
- Artificial corteces in liposomes (inside-out geometry of actin shells)
- **Mechanical characterisation of artificial corteces (tube pulling)**

# Tube pulling experiments for membranes prepared by electroformation



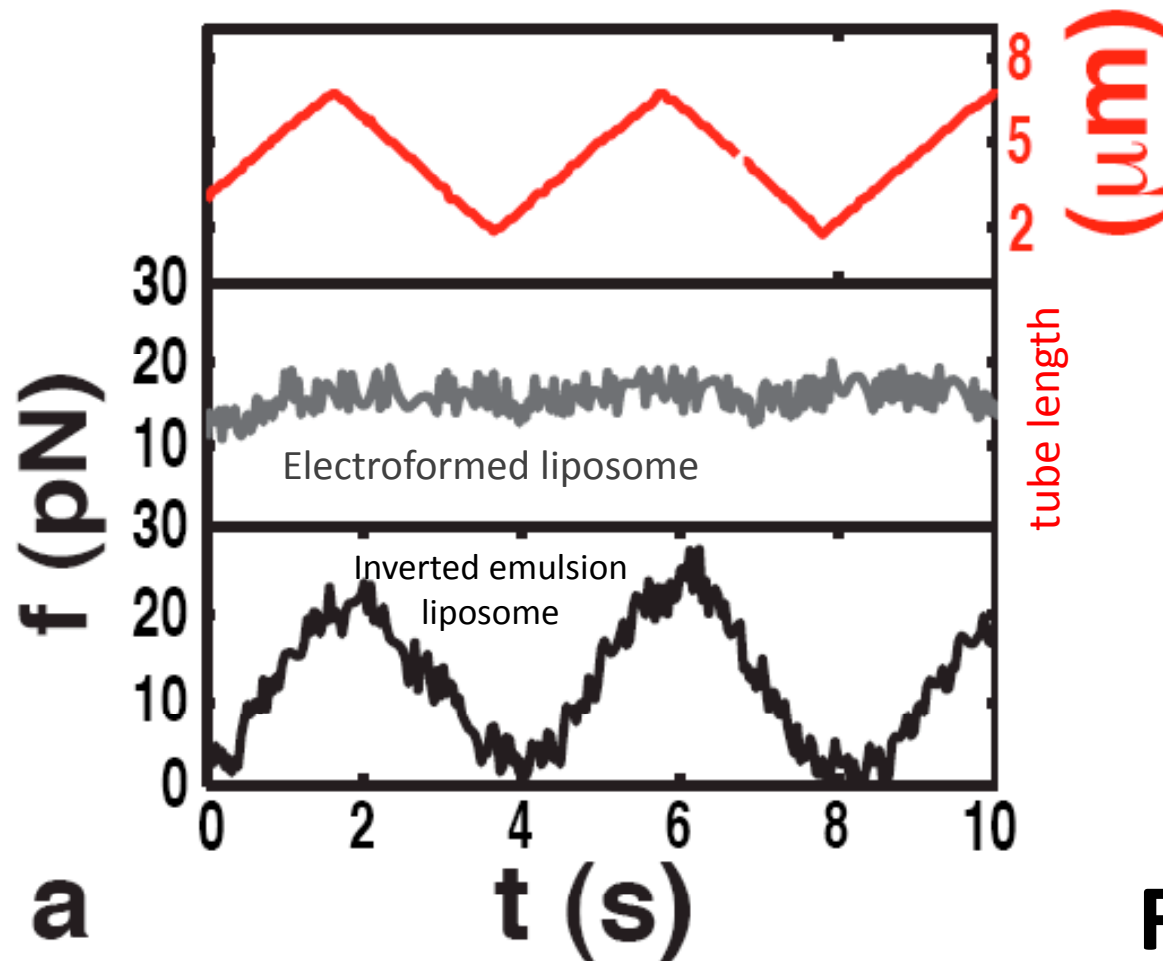
The force for pulling a tube is independent of tube length for membranes prepared by electroformation, at constant membrane tension

[A. Roux, EMBO 2005]

For membranes prepared by the inverse emulsion technique???

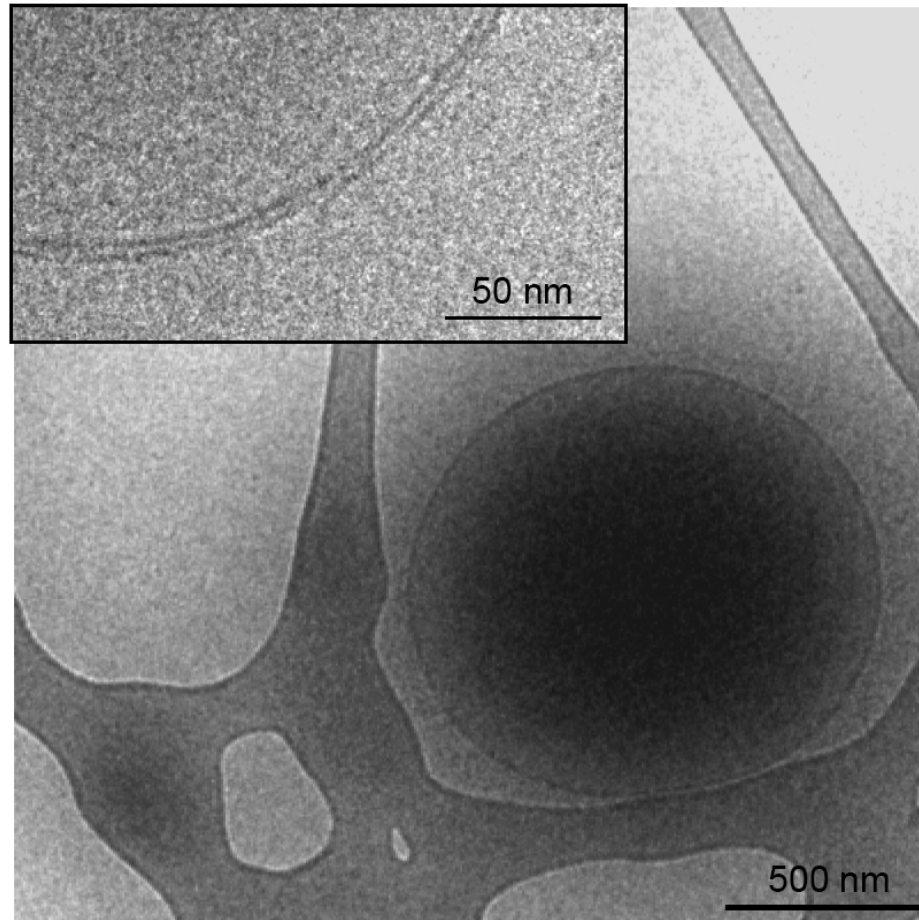
C. Campillo

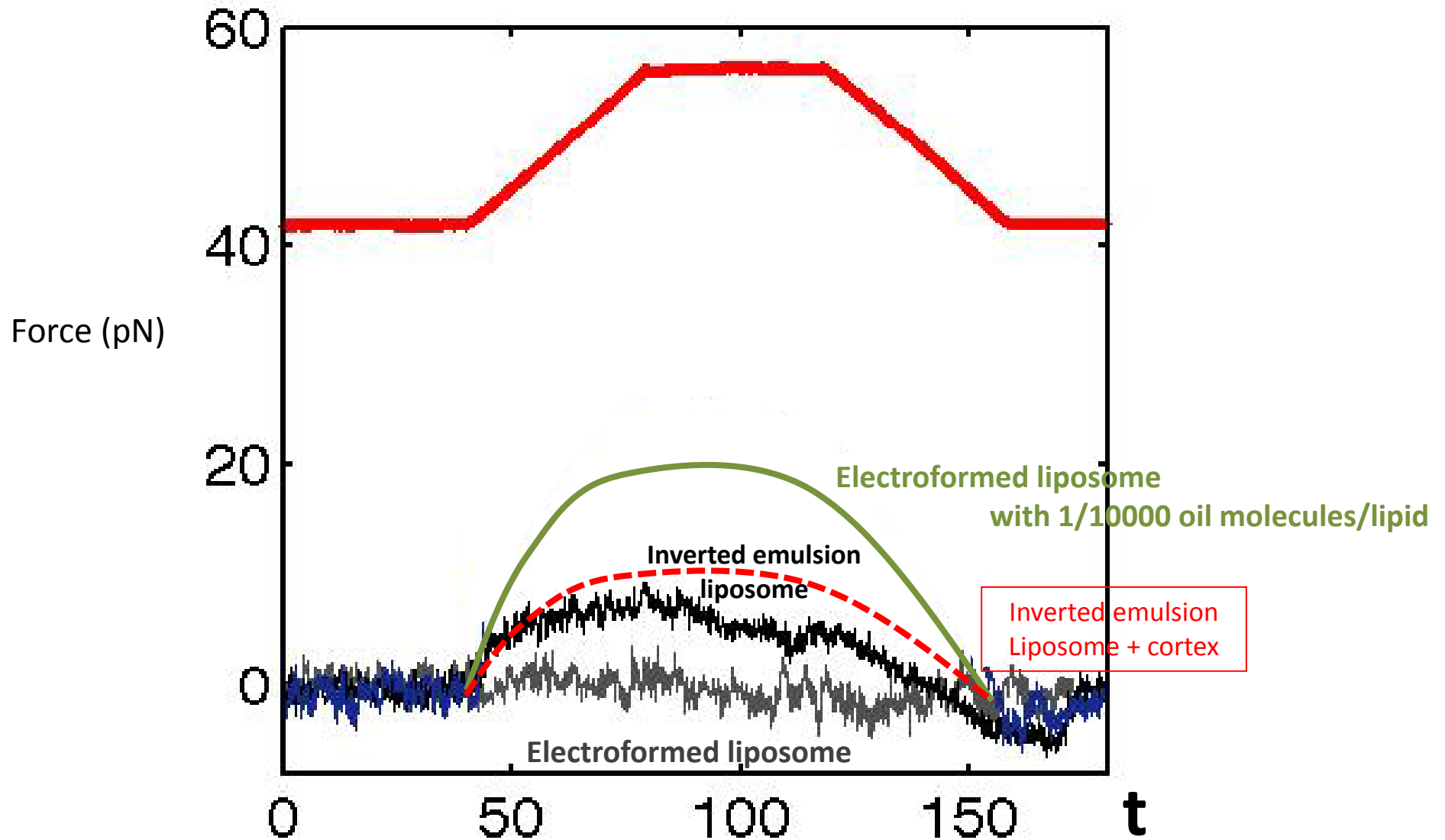
For membranes prepared by the inverted emulsion technique,  
force depends on tube length



**Friction!!**

Thickness:  $4.56 \pm 0.22$  nm (n=54), same as a “pure” lipid bilayer





**Take home message:**  
the difference between electroformed liposomes and cells should not be attributed solely to the cytoskeleton, but mainly to membrane proteins

# Conclusion

- ✓ **Actin shell breakage: stress builds up under specific protein conditions**
- ✓ **Actin shells can be produced in liposomes**
- ✓ **The composition of the bilayer membrane controls dynamic mechanical properties (probed by tube pulling)**



## **Biomimetism of cell movement**

Julie Plastino (assistant professor)

Florian Rückerl

Timo Betz

Clément Campillo

Kévin Carvalho

ANR Agence Nationale de de la Recherche

*Léa-Laetitia Pontani*

*Mike Murrell*

## **Collaborators**

Laurent Blanchoin

Ludger Johannes

Pierre Nassoy

Jean-François Joanny

Margaret Gardel (Chicago)

Patricia Bassereau

