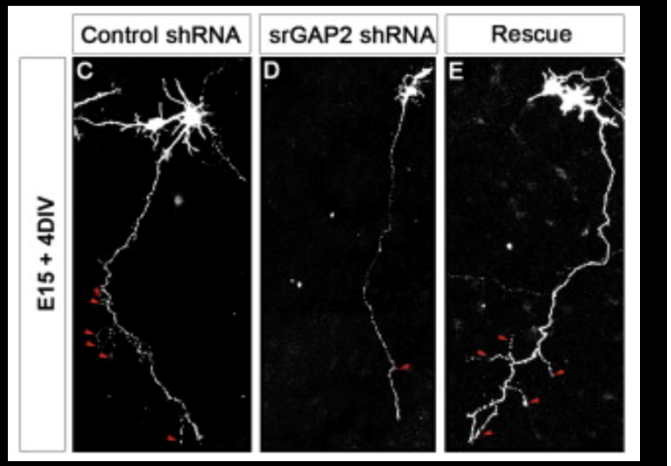
srGAP2 – neuronal migration (epilepsy/schizophrenia)

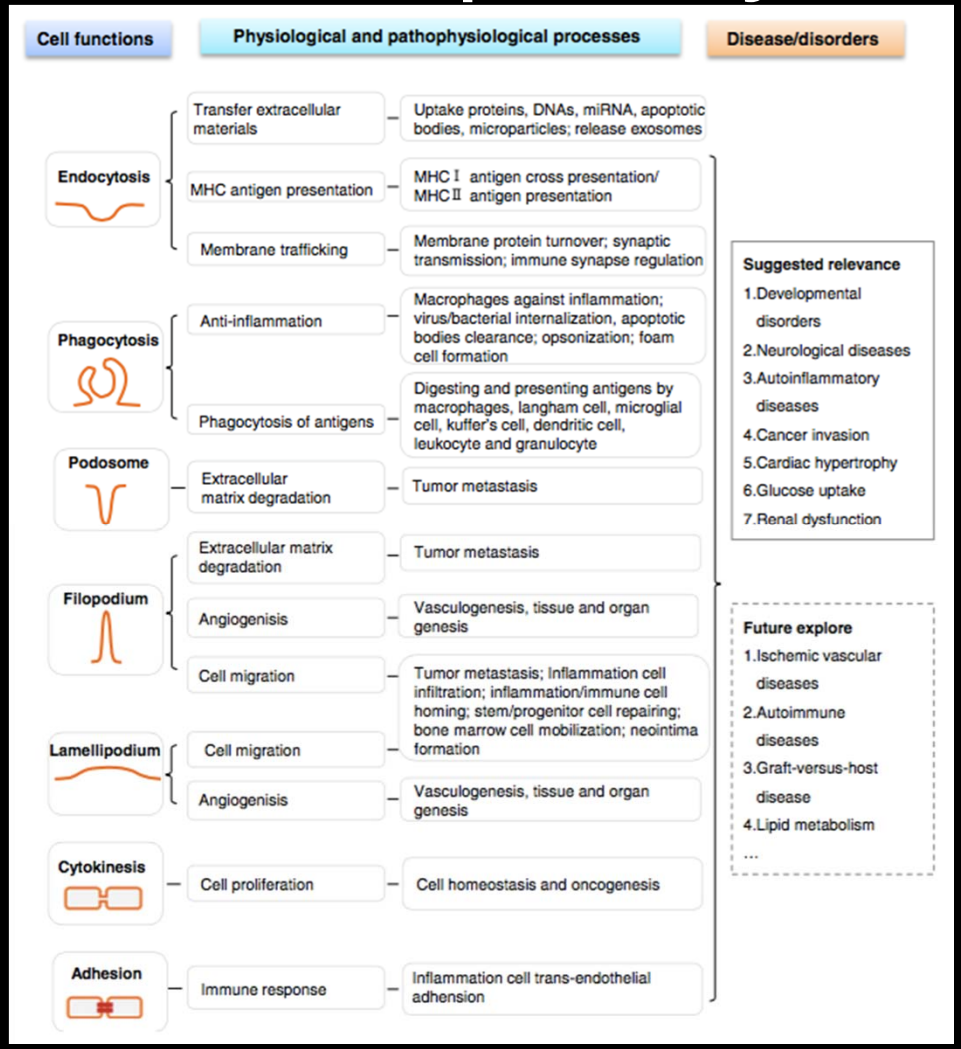
Supplementary Movie 5
Control EGFP-expressing neurons
Imaged at E15 + 3DIV
1 frame every 16 minutes
Total duration: 8 hours 48 minutes

Supplementary Movie 6
srGAP2-EGFP- expressing neurons
Imaged at E15 + 3DIV
1 frame every 16 minutes
Total duration: 10 hours 24 minutes



Guerrier *Cell* 2009

Tightly regulated spatially and temporally



Liu 2015

Limitations of *in vivo* techniques

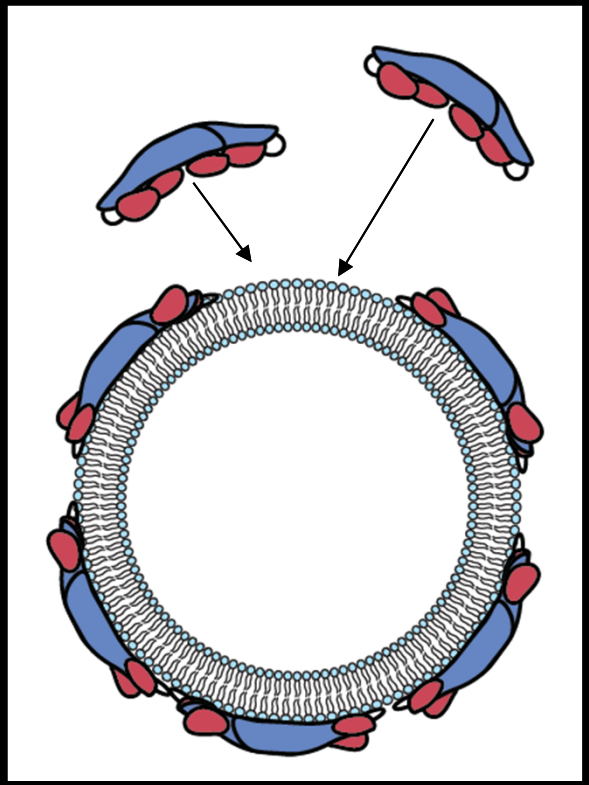
- Unable observe nanoscale membrane dynamics
- Cannot directly observe assembly/activity of membrane-bound remodeling proteins
- No current tools to measure lipid dynamics or the physical characteristics of membrane at sites of deformation

How to directly observe membrane remodeling?

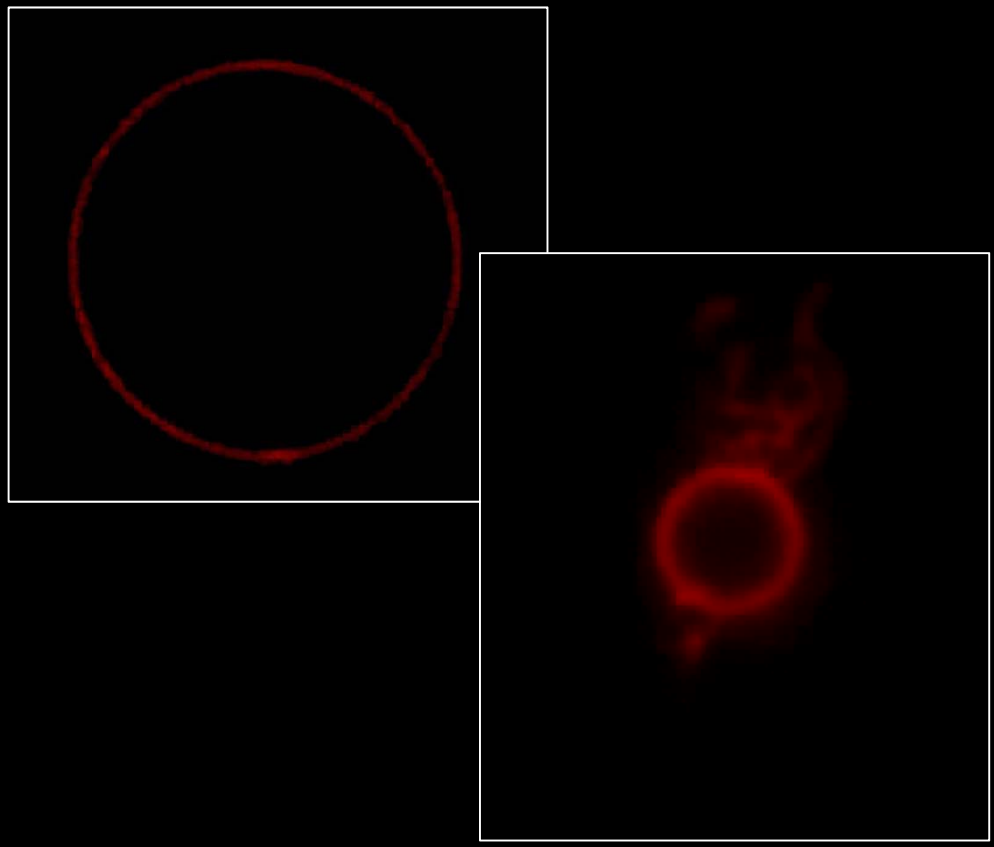
- *In vitro* – simplified system, purified components
  - Manipulate lipid composition
  - Protein density
  - Directly visualize membrane remodeling
  - Evaluate/measure the important physical parameters affecting membrane curvature

Directly observing membrane binding and deformation

Giant Unilamellar Vesicle

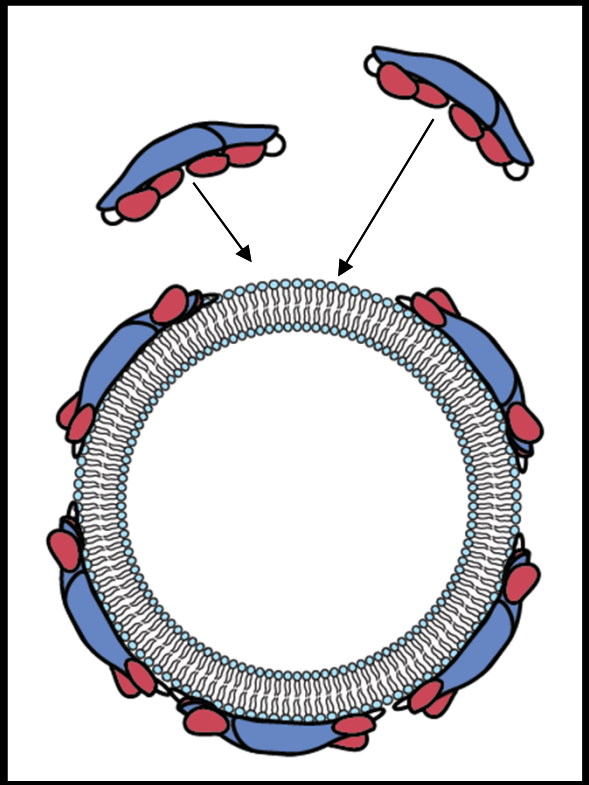


F-BAR – SNAP549



Directly observing membrane binding and deformation

Giant Unilamellar Vesicle

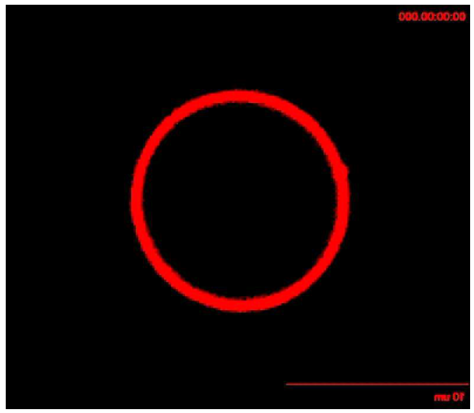


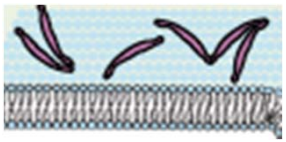
F-BAR – SNAP549

Protein mobility – detecting stable scaffolds

Nwk$^{F\text{-}BAR}$-coated vesicles

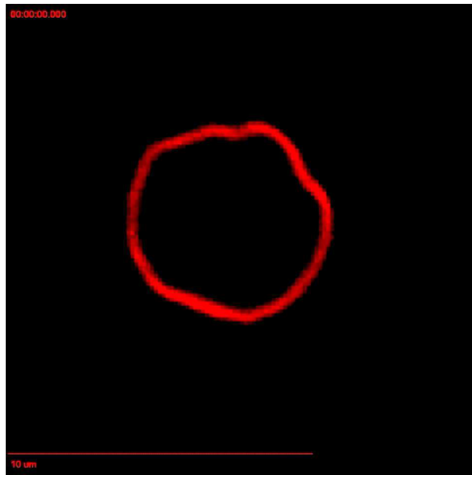
Undeformed

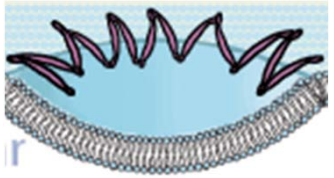




Individually bound –
Fast recovery

Deformed

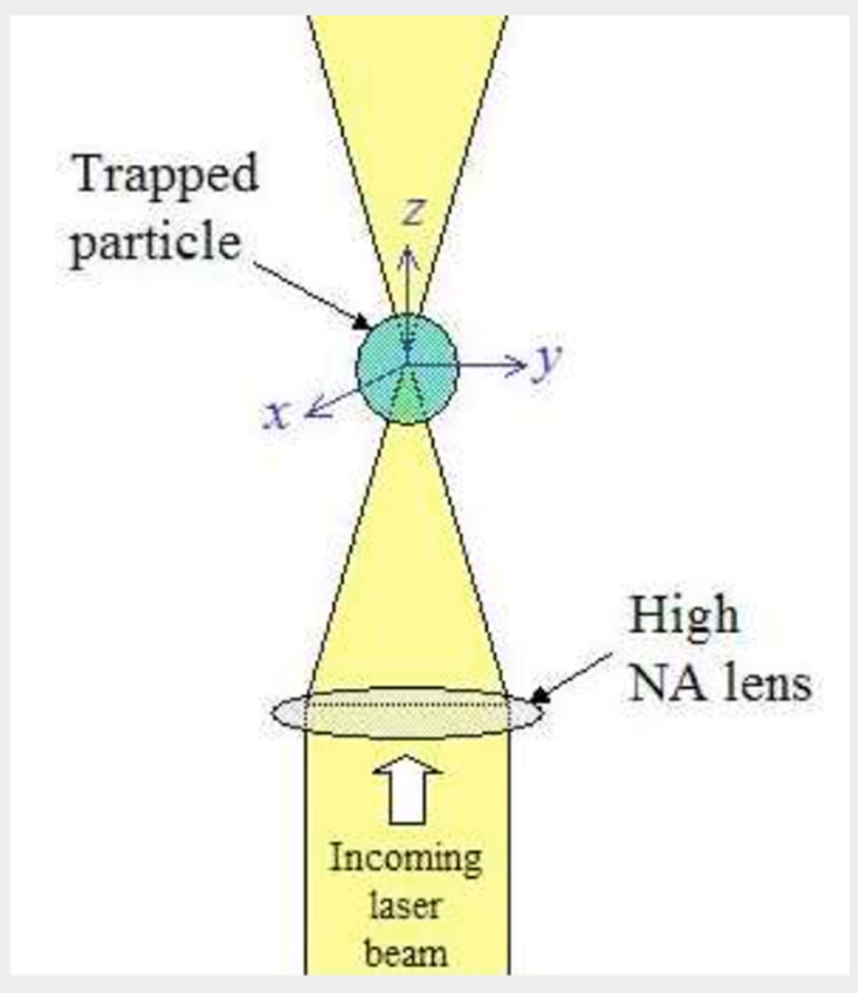




Assembled –
Slow recovery

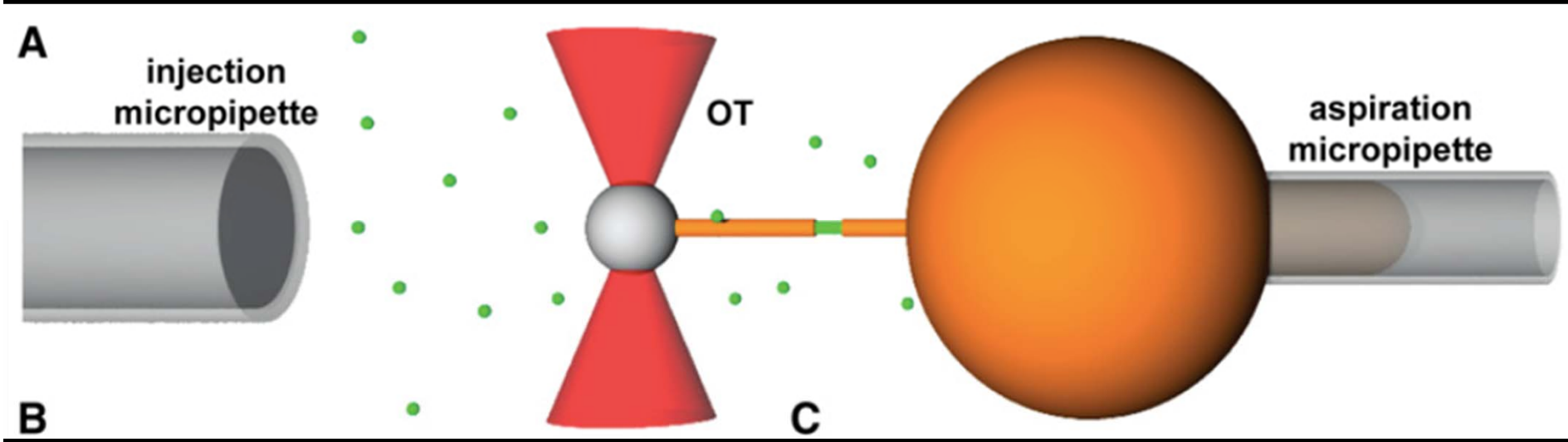
Kelley 2015

Optical Trap



- Highly focused laser beam
- Exerts small (pN) forces
- “Beam Waist” has a strong electric gradient
- Objects get stuck in center, where electric force is greatest

Optical Trap + Giant Vesicles



Injector delivers labeled protein locally

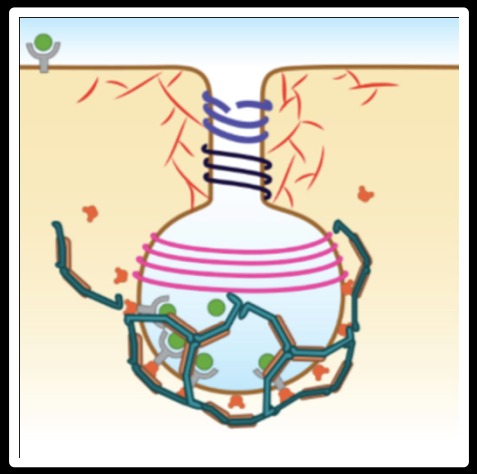
Trapped bead sticks to membrane, forms membrane tubule

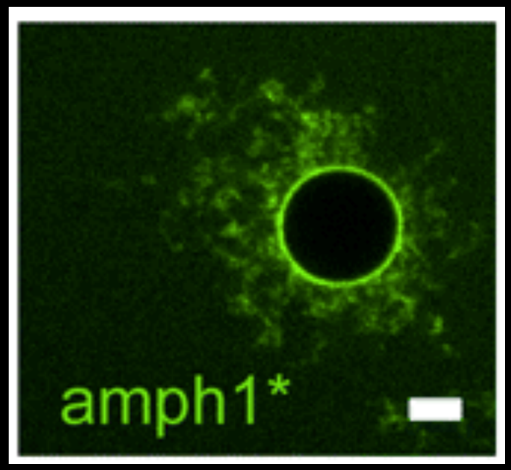
Aspirator sets membrane tension and holds vesicle in place

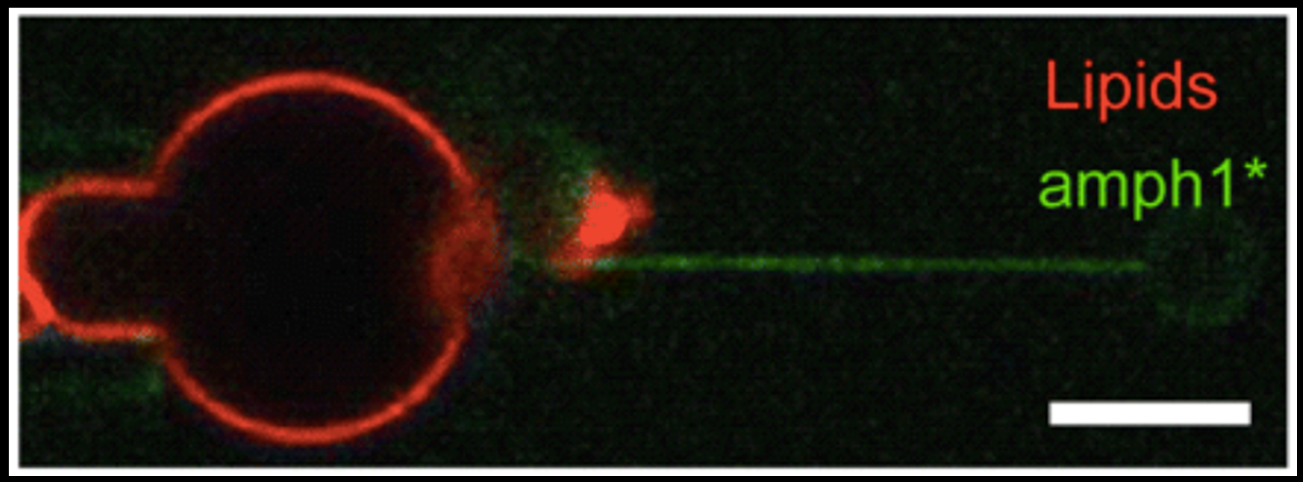
Morlot 2012

Protein activity is dependent on protein density/ membrane shape

- Mechanical action of BAR domains as function of:
- Amphyphysin
  - Tubulates membranes
  - Important for endocytosis

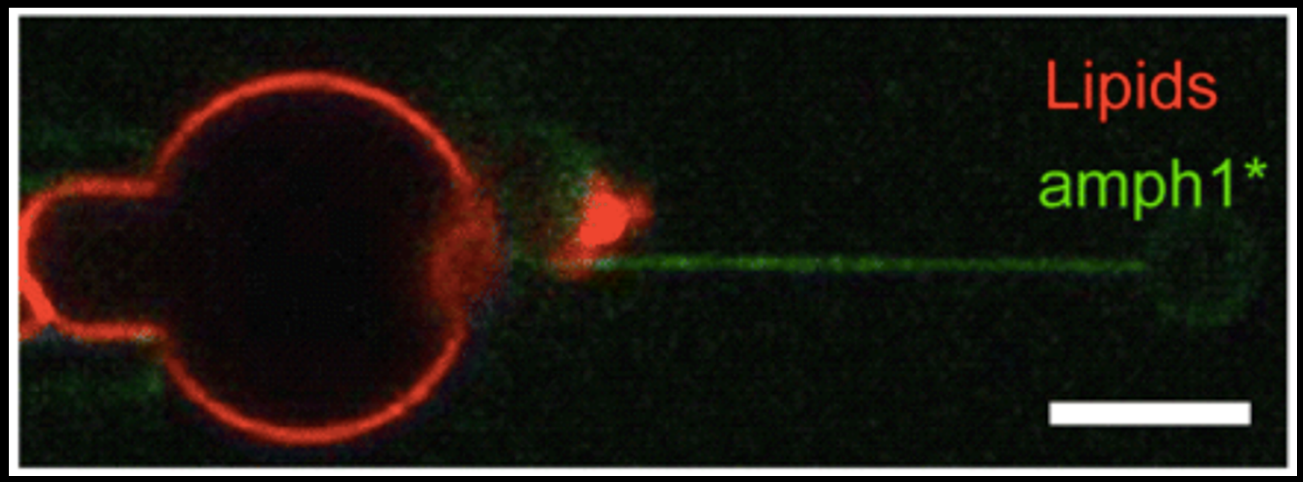




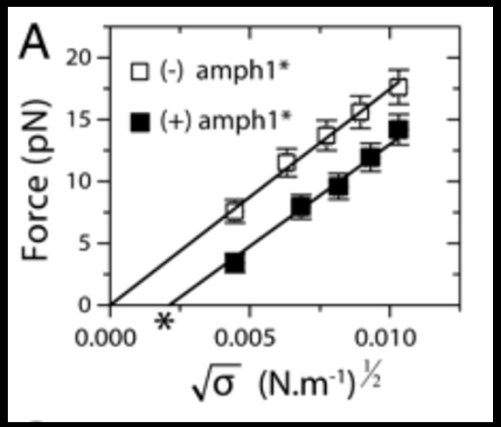


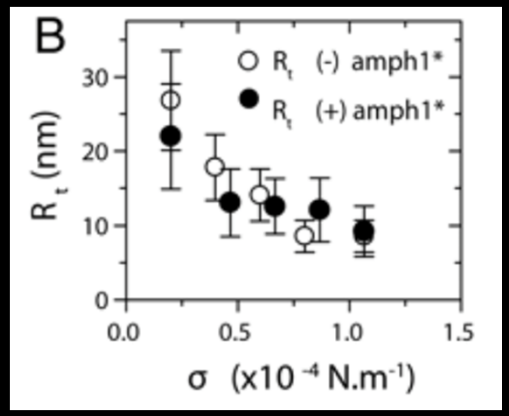
Sorre 2011

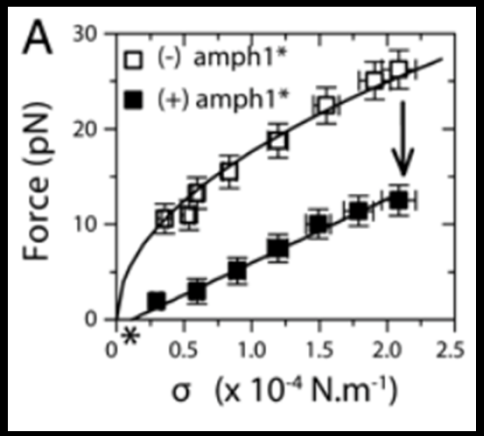
Curvature preference independent of protein concentration

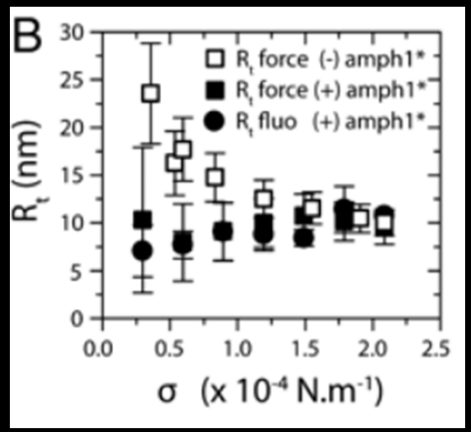


Mechanical impact on membrane depends on protein density

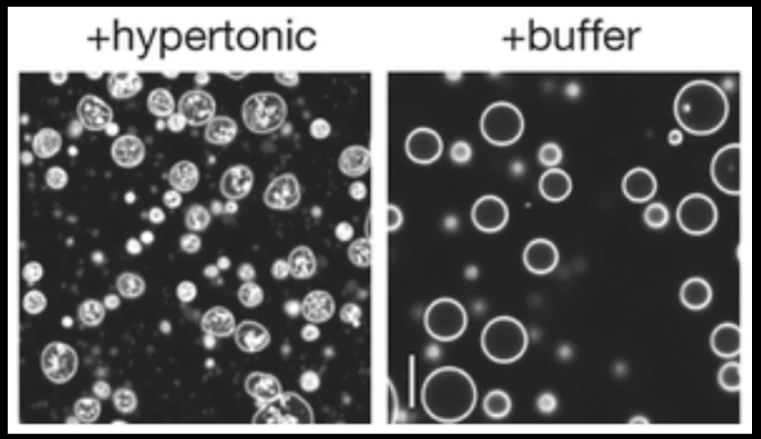


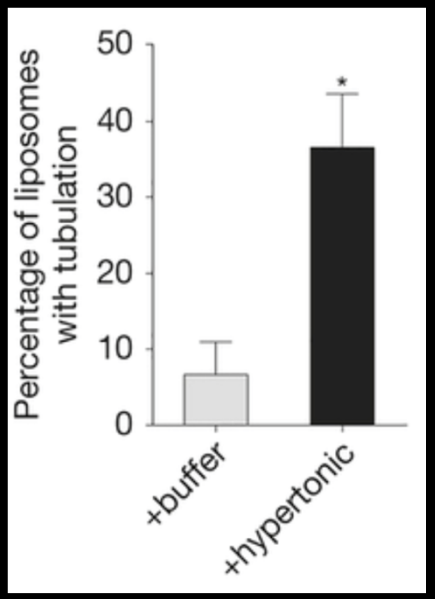


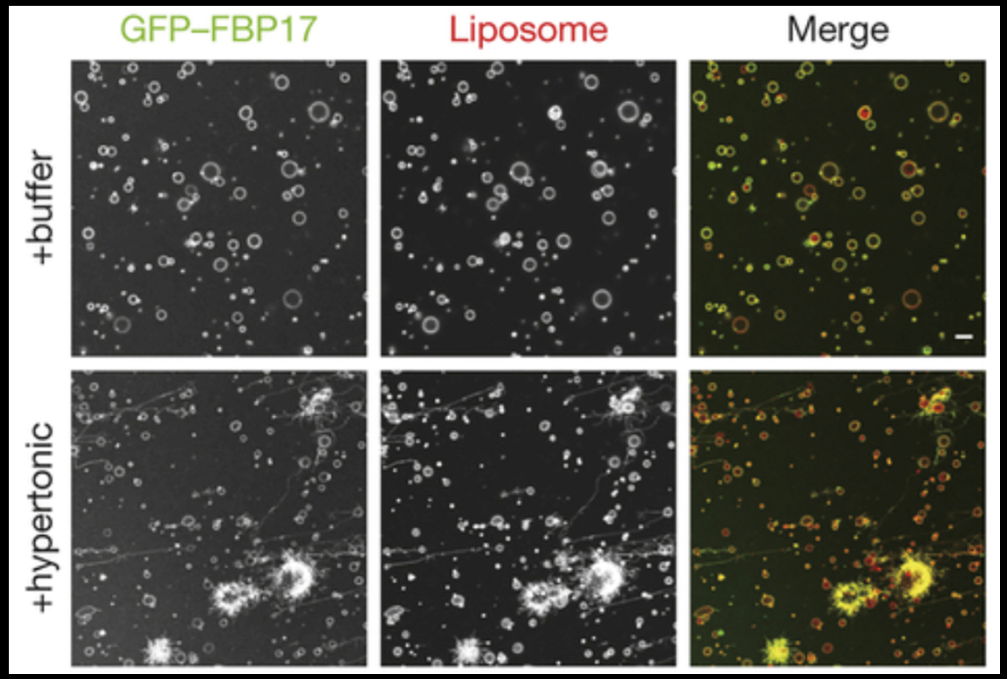




Membrane tension





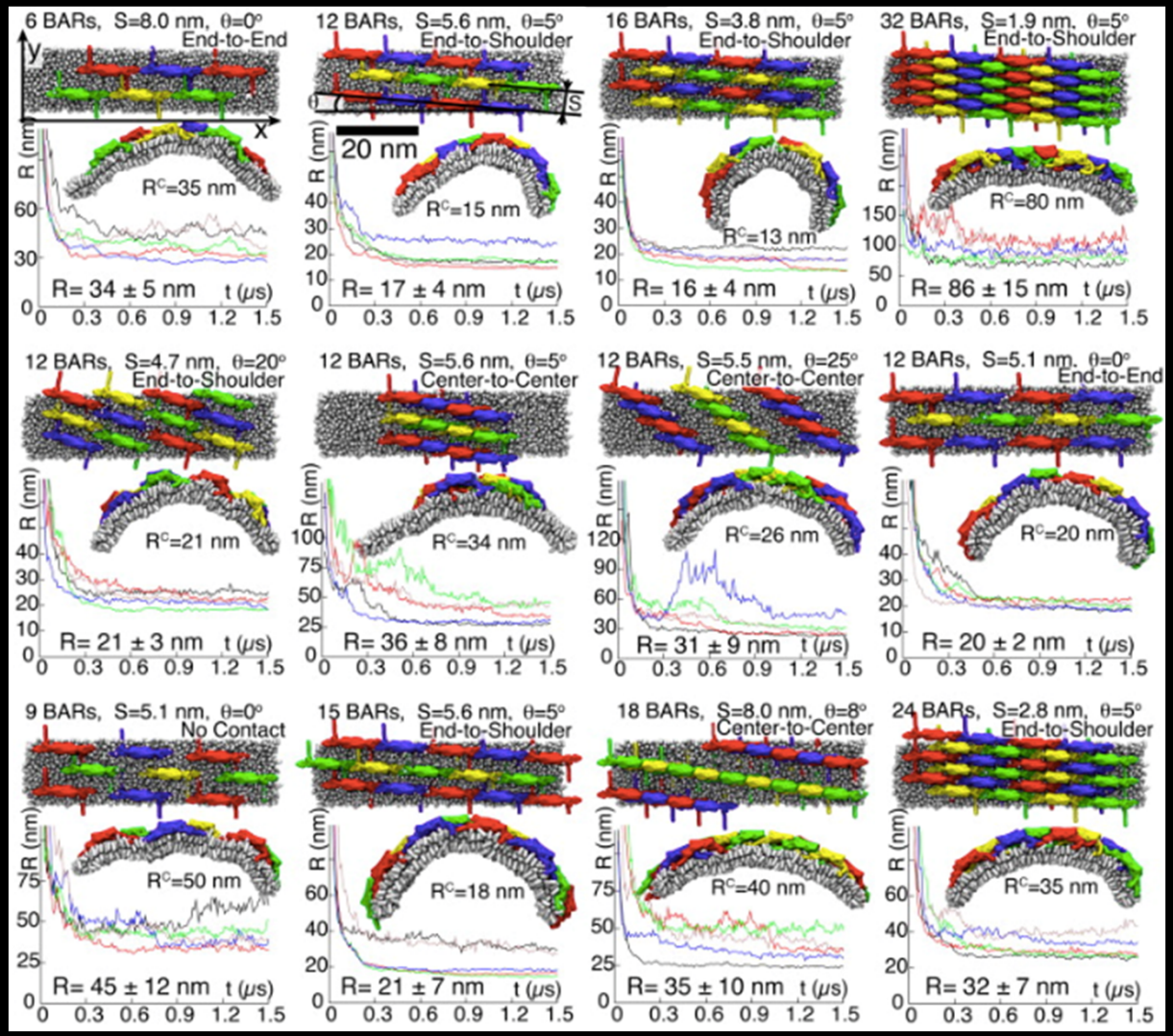


Tsujita 2015

Overall: physical parameters regulate membrane remodeling

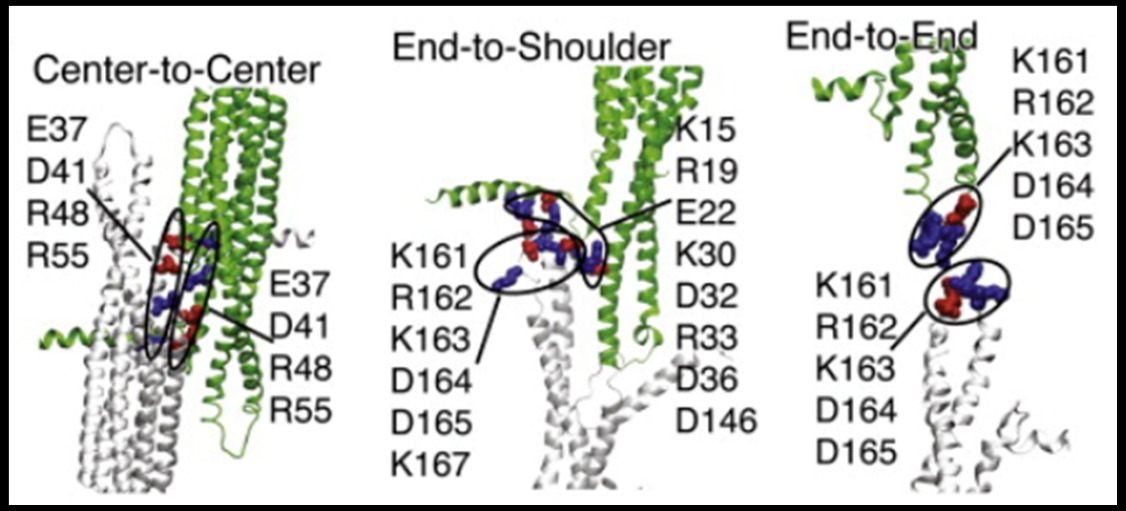
- Protein density
- Membrane tension
- Membrane shape
  - composition

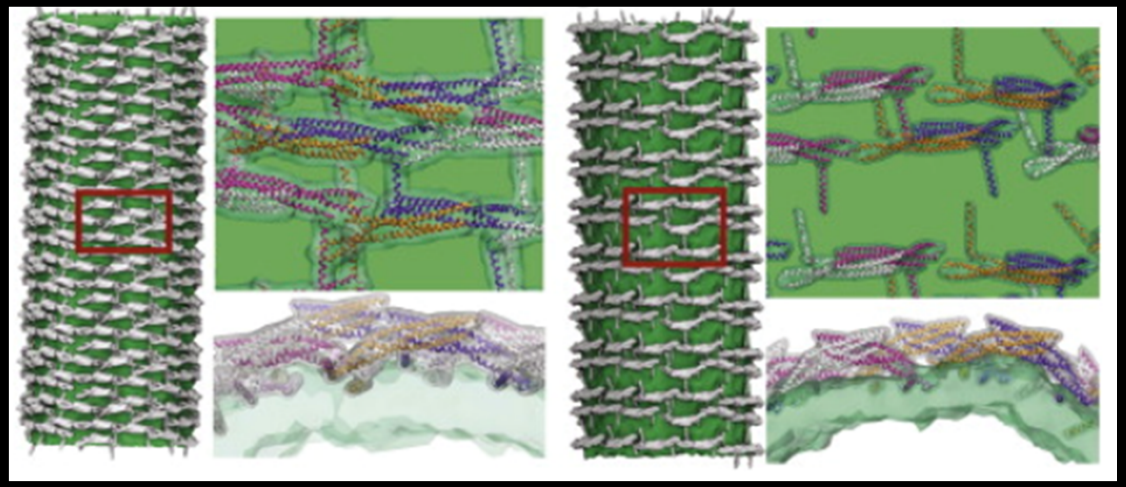
Molecular dynamic simulations



Yin 2009

BAR domains can assemble differently dependent on intermolecular interactions





Yin 2009

Questions that cannot be answered experimentally (yet)

- What roles do lipid dynamics and composition play?
- How do these physical parameters affect stability and/or assembly of individual BAR domains on the membrane?
- How do all these factors come together to induce curvature *in vivo*?