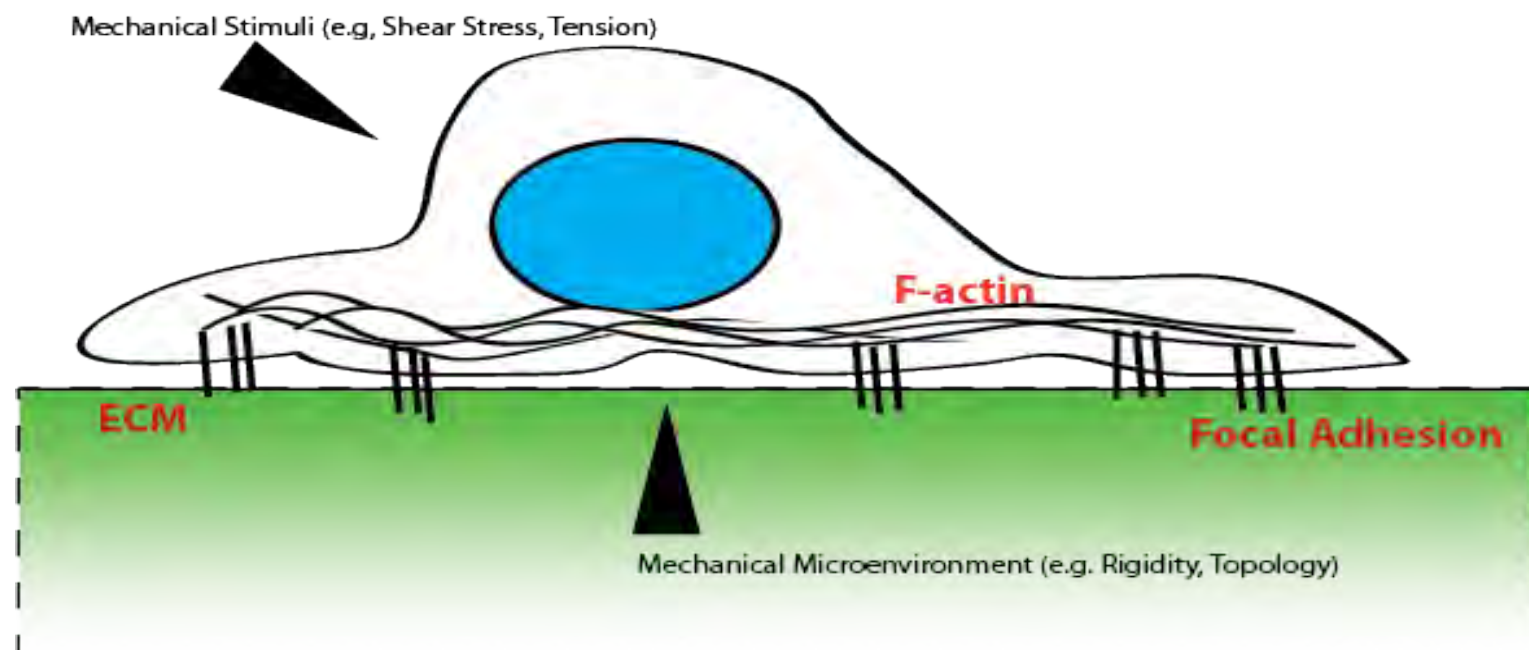
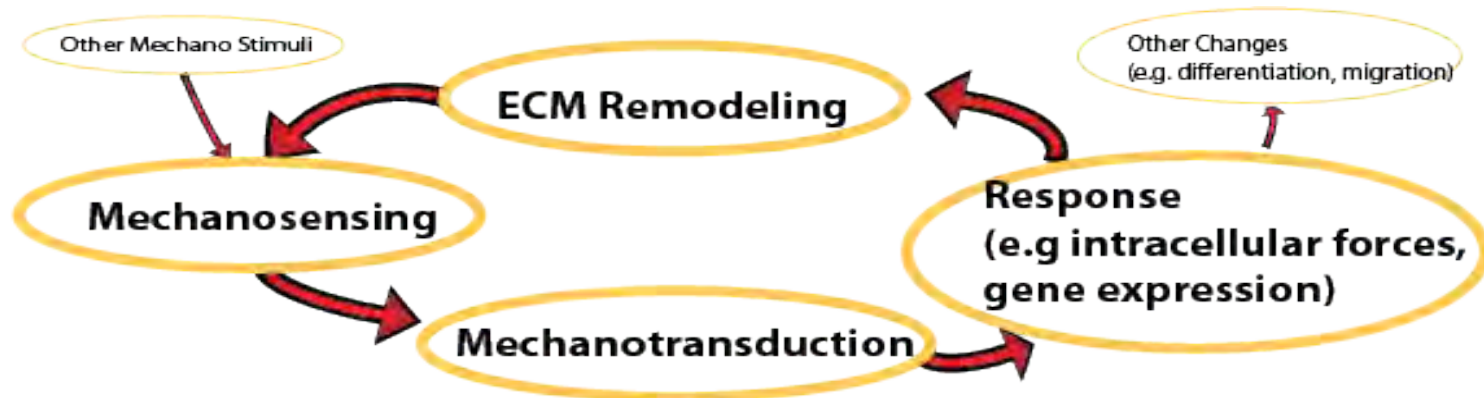


# Biological Applications of MechanoBiology

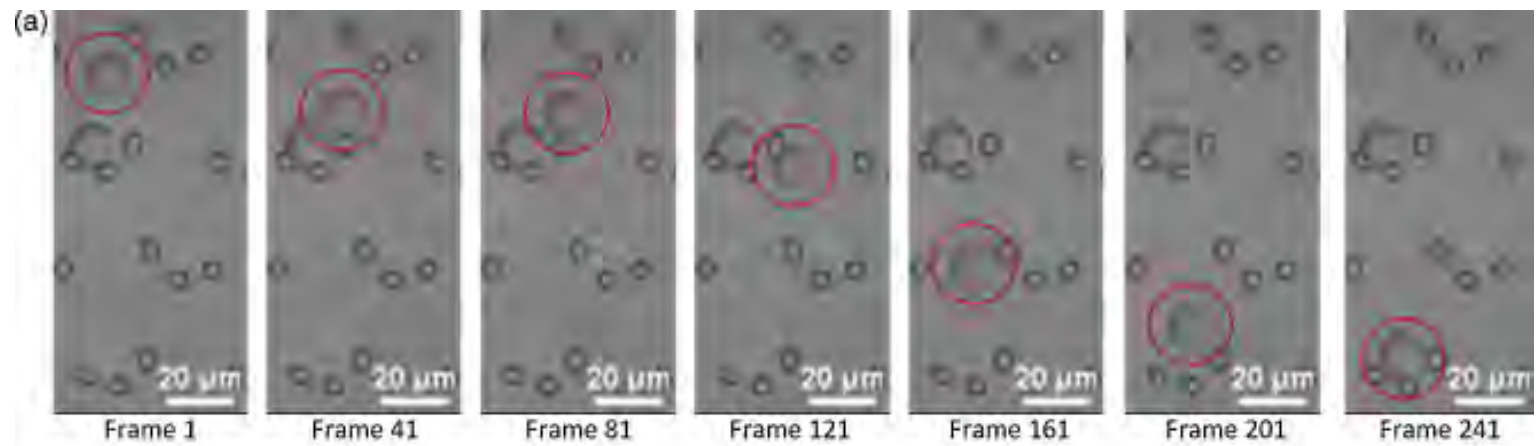
FYS4715 Høst 2020

Xian Hu(Edna)



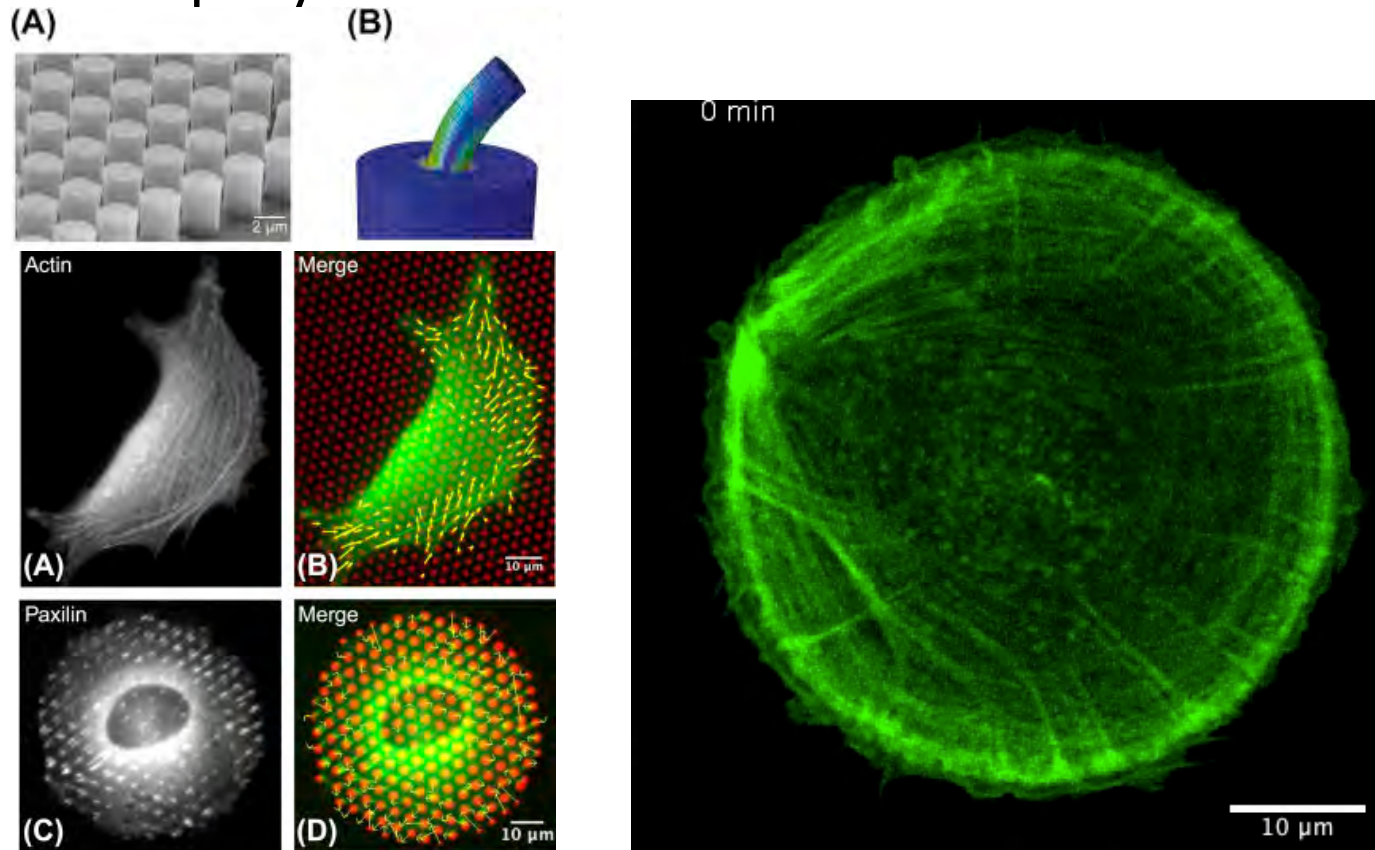
# Cellular Rigidity

Microfluidics device that capture CTC by rigidity



Tan et al, 2010 Biosensors and Bioelectronics

# Cell exert physical forces on substrate

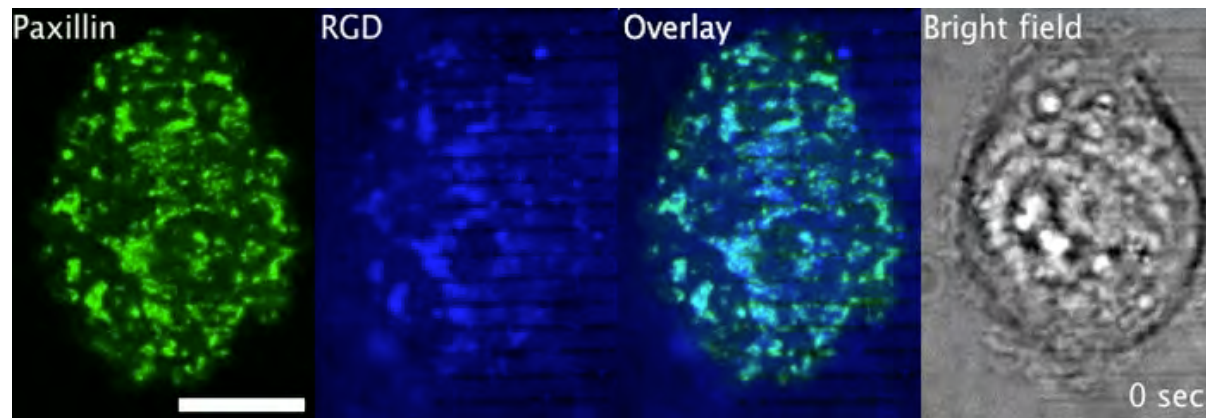
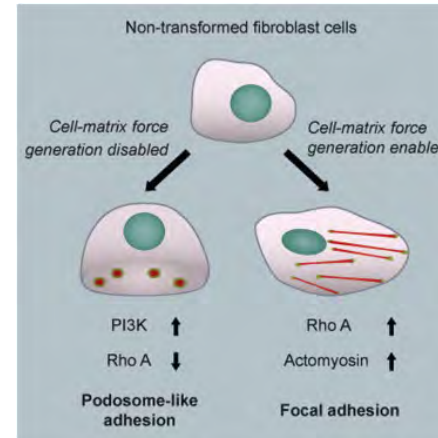


Gupta et al, 2015 Methods in Cell Biology

Tee et al, 2015 NCB

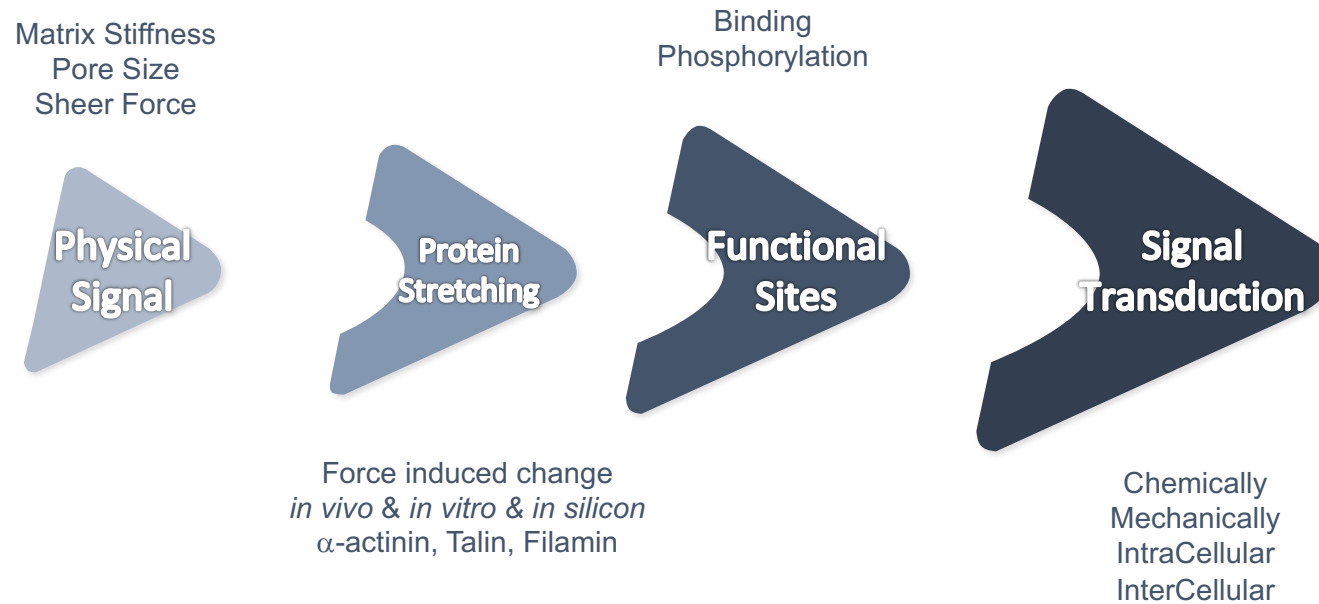
# Cell Matrix Attachment

- Fibroblast: Focal Adhesion
- Immune Cells: Podosome
- Convertible



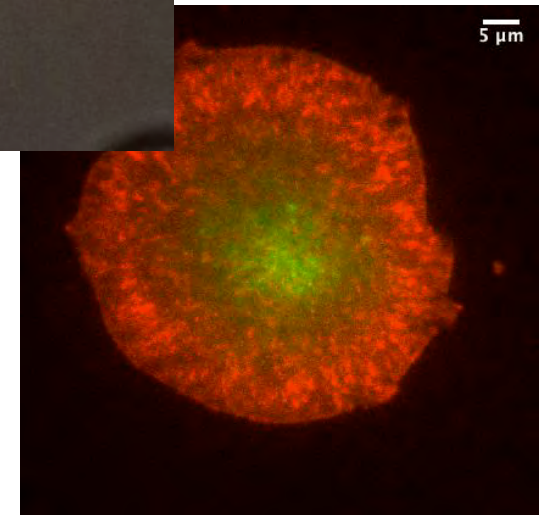
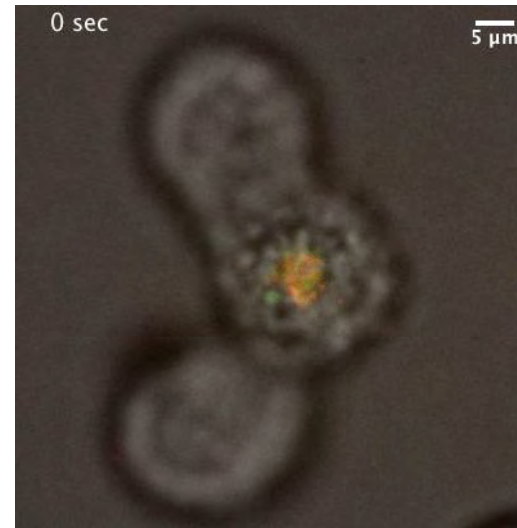
Yu et al, Cell Reports 2013

# Rigidity Sensing and Molecular Mechanism

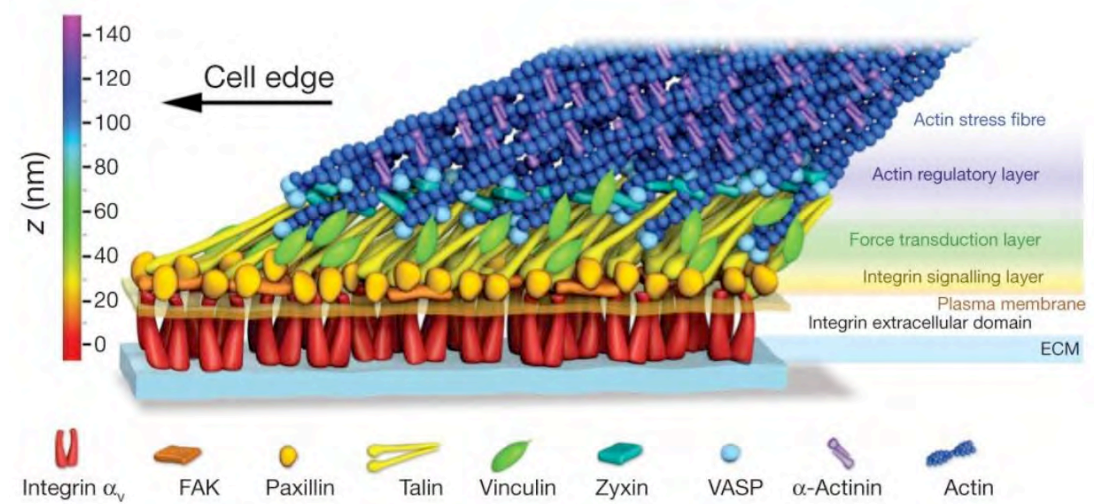
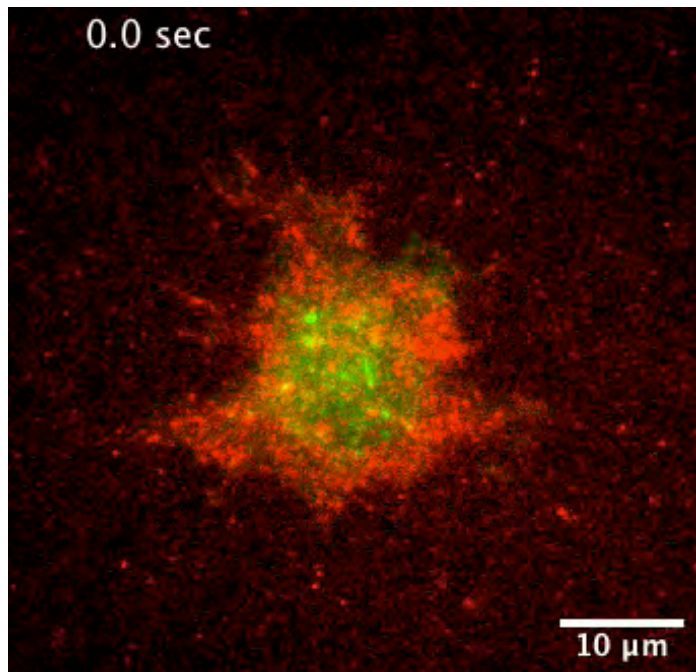


# Understand Complex Function in Cells

- Cells has many complex functions, integrated by different cellular states—high noise
- Need to standardize experimental procedure, isolate the events and reapeat
- Rigidity Sensing- Cell Sensing Matrix-Cell Binding to Matrix- Cell Spreading Assay
- Have several different experiments to test the same thing

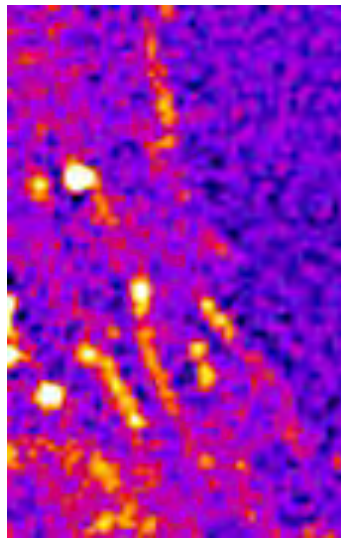


# Focal Adhesion

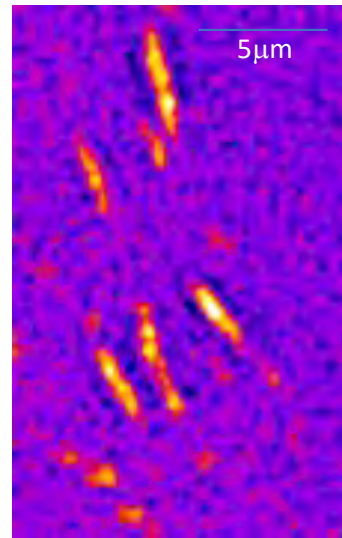




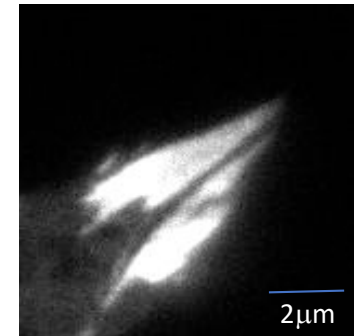
# Dynamics of Focal Adhesion Proteins



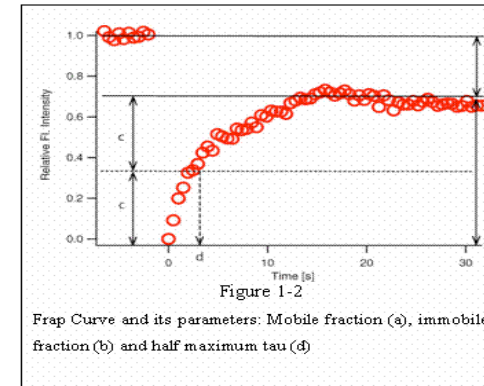
Talin-GFP



Vinculin-mCherry



Vinculin-GFP

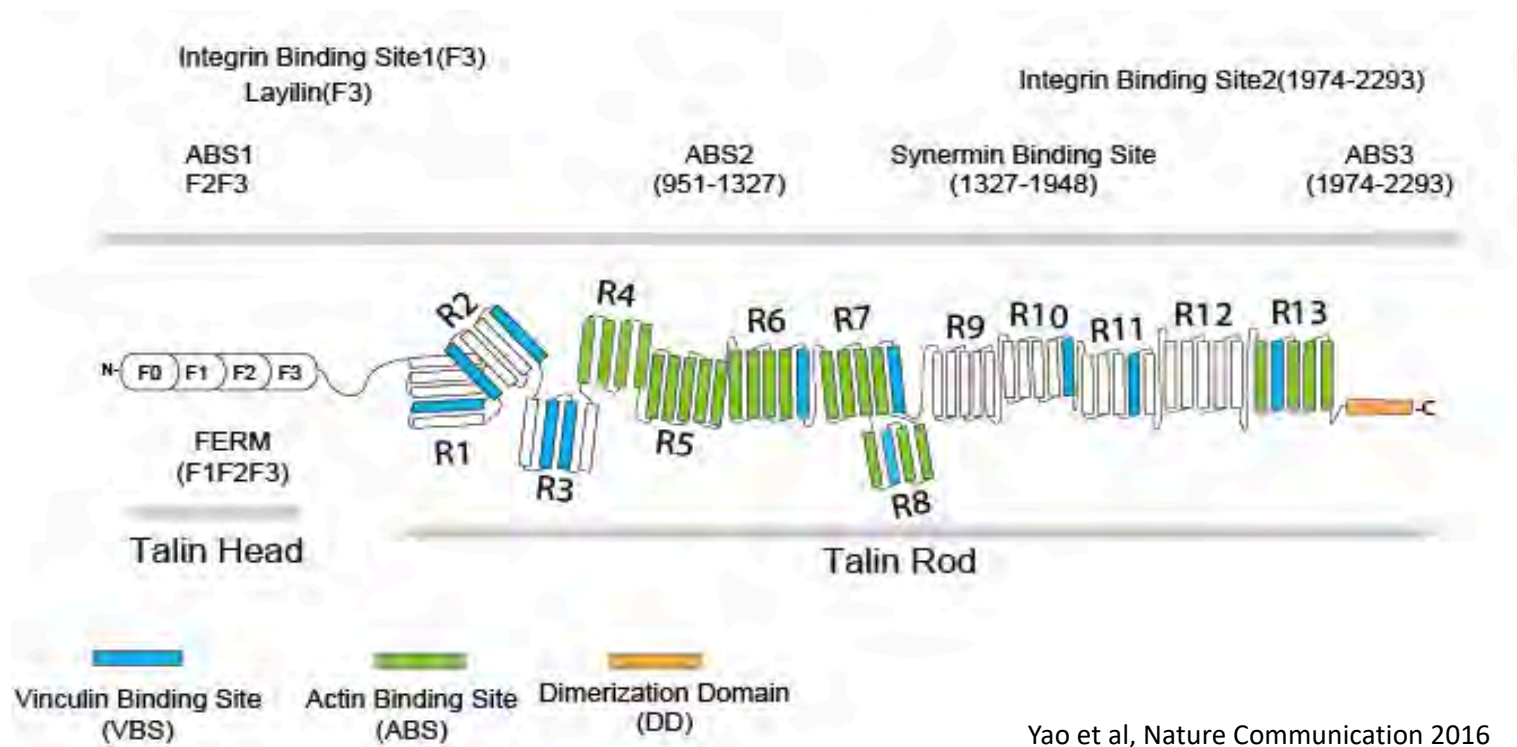


Glass/Fibronectin      MEF(Fibroblast)  
100ms/Frame  
Stream Mode

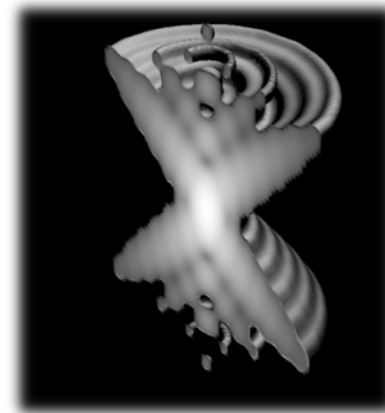
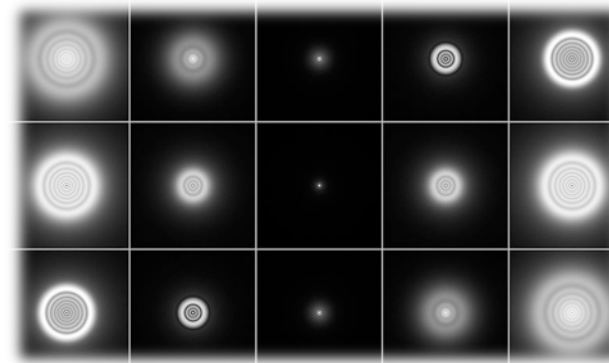
# Dynamics of FA Proteins

Protein Name	FRAP t1/2(s)	Cell Type
Vinculin	1.9/14/50/11/21	HelaJW/MEF/ REF52
Paxillin	1.5/25/17.7/53.2/20	HelaJW/MEF/ Bovine capillary endothelial cells/MEF, TIRF FRAP/ REF52
FAK	2.1/7/6.7/14	HelaJW/MEF/ Bovine capillary endothelial cells/ REF52
Zyxin	0.5/12/7/10	HelaJW/MEF/ REF52
Talin	2.2/23/77/18	HelaJW/MEF/ Bovine capillary endothelial cells/ REF52
ILK	9.2/12	HelaJW/ REF52
□-actinin	36/14.1/27	MEF/ Bovine capillary endothelial cells/ REF52
beta3-Integrin	38.5	Bovine capillary endothelial cells
p130Cas	5.8/14	MEF / REF52
Tensin	55	REF52
Src/CSK	9	REF52
Pinch	14	REF52
VASP	0.7/15	HelaJW/ REF52

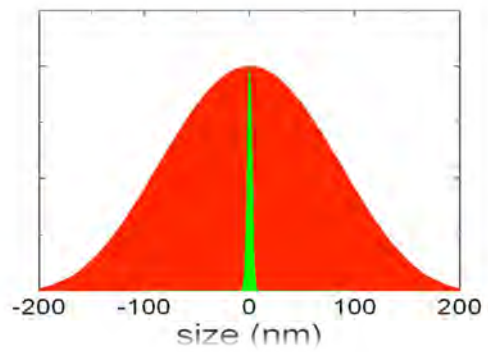
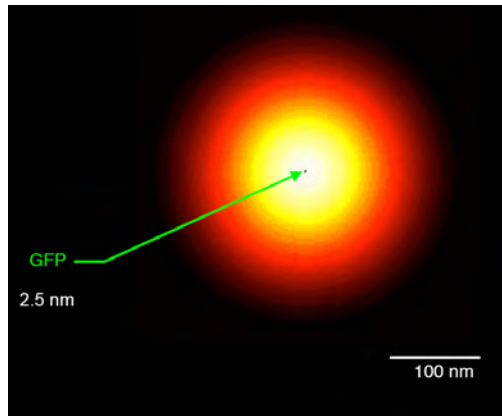
# Talin



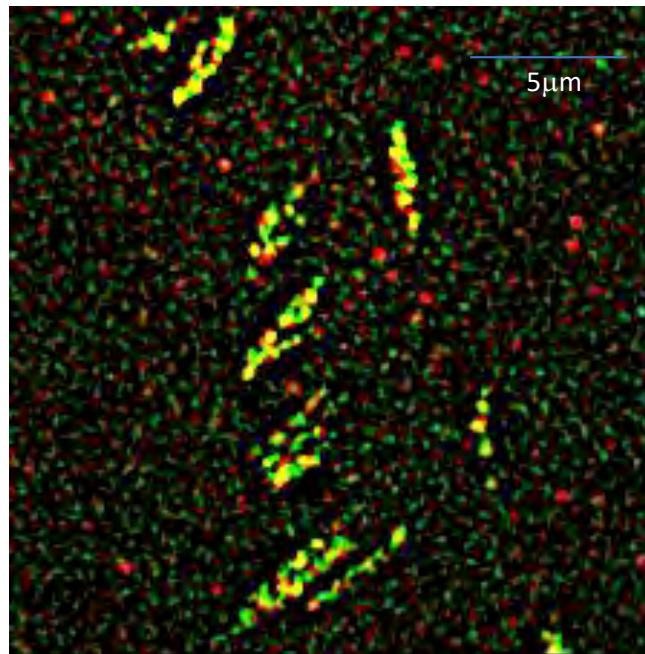
# Resolution Limit/PSF



# Single Molecule Localization



# Talin Stretching *in vivo*

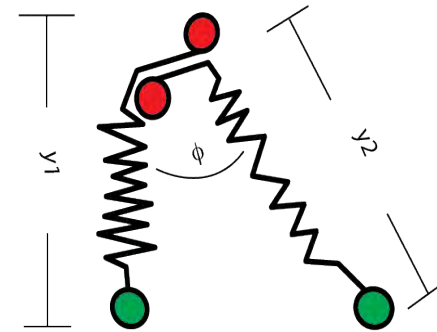


GFP-Talin-mCherry

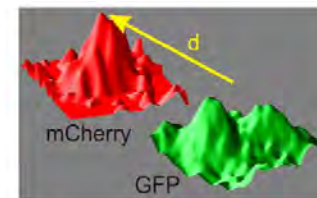
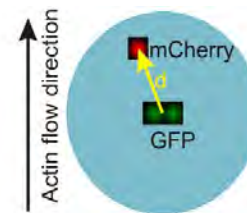
Paired Single Molecule  
Live Cell Imaging  
site

GFP: integrin binding site  
mCherry: actin binding site

100ms/frame, stream mode, total duration 10s

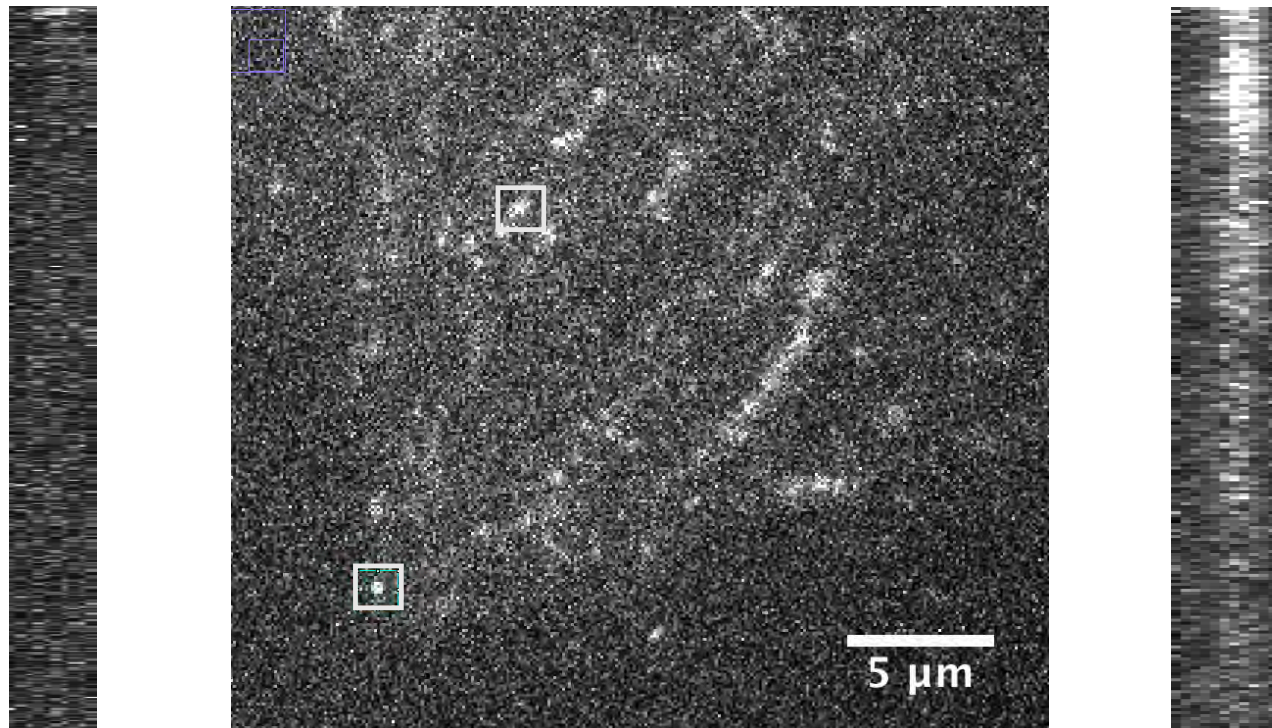


Labeling of Talin N&C

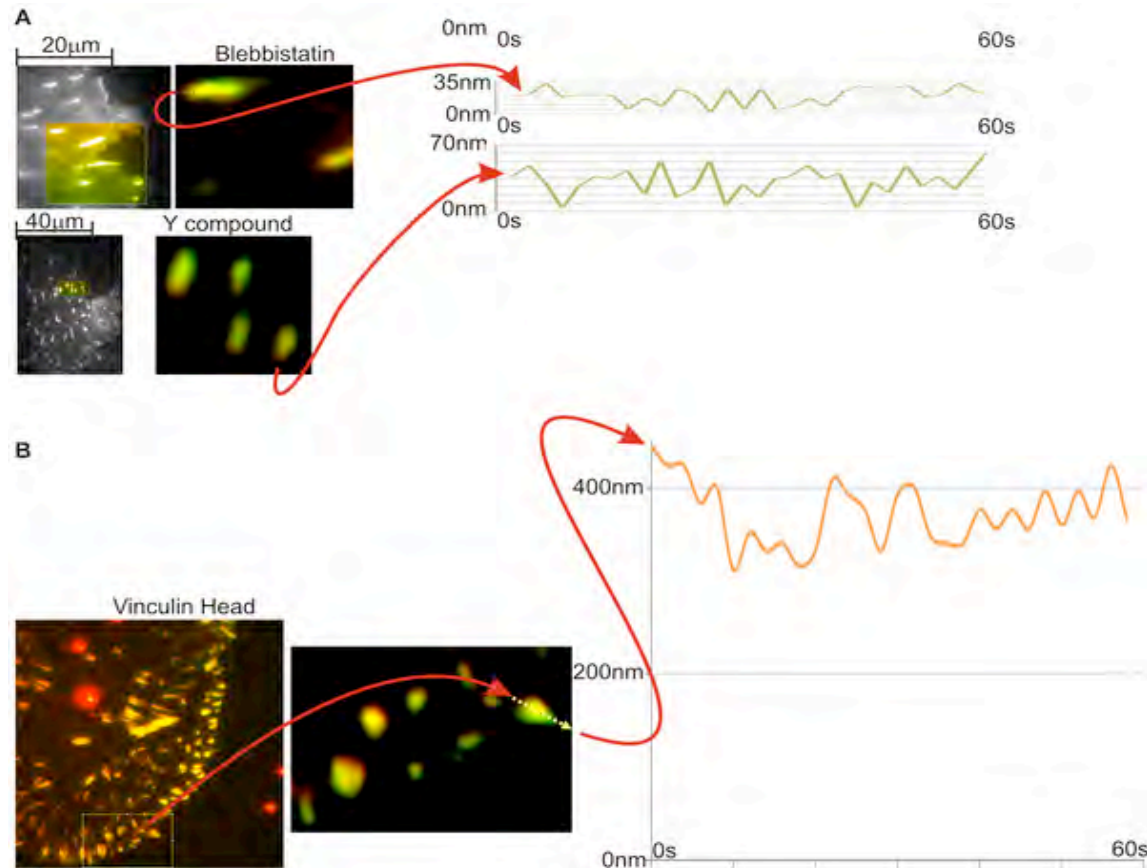


Dimer Tolerant Detector: Search for Signal Pairs

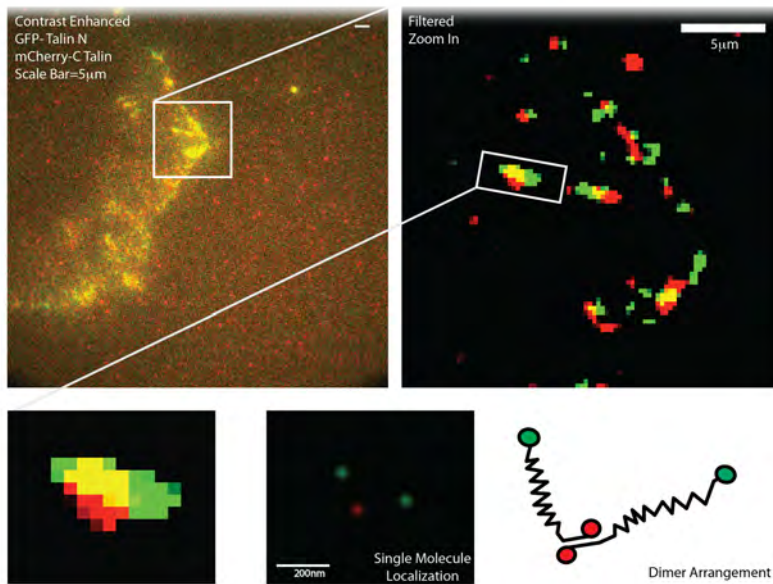
# Single Molecule Imaging



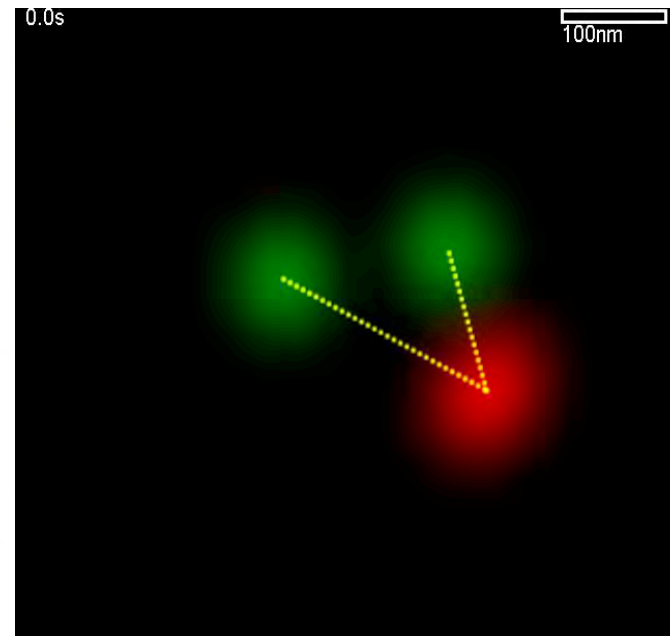
# Talin Stretching *in vivo*





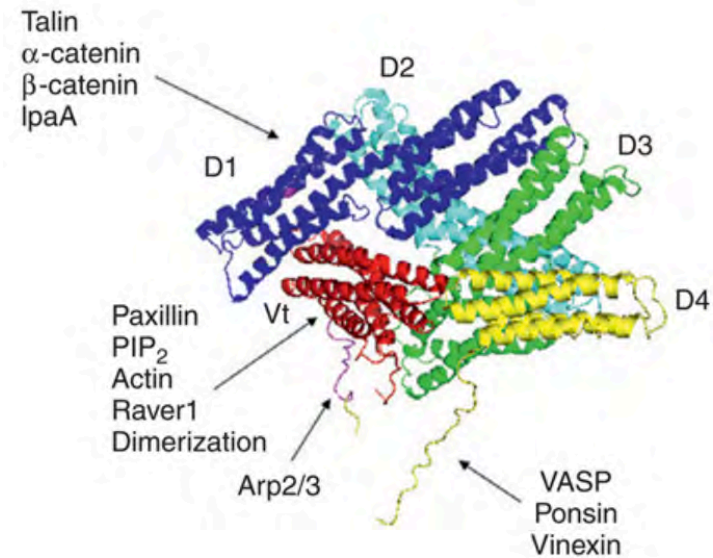


GFP-Talin-mCherry  
Paired Single Molecule Dynamics



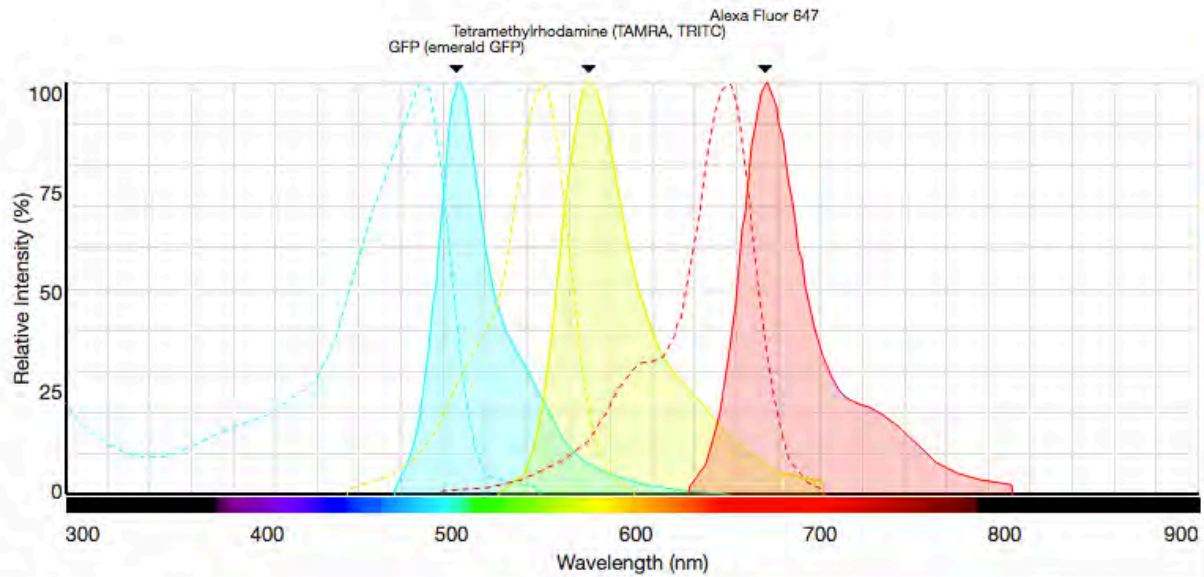
# Vinculin

- Function:
  - adaptor
  - non enzymatic activity
  - regulating FA assembly
  - force transmission
- Work with talin:
  - one talin binding site
  - activated by talin



# Vinculin and Talin

- **Question:** vinculin recruitment event *in situ*
- **Challenge:** Three color live cell imaging with single molecule localization accuracy.
  - Wavelength: ~~Blue, Green, Red~~, Far-red
  - Short term imaging
  - Superresolution localization precision
  - Organic dyes: delivering system: eDHFR-TMP



**Legend**

- GFP (emerald GFP)
- Tetramethylrhodamine (TAMRA, TRITC)
- Alexa Fluor 647

Pointer Location: 635nm, 7%  
 Fluorophores: Tetramethylrhodamine (TAMRA, TRITC), Alexa Fluor 647

**Key**  
 Excitation: - - - Emission: - - - -

**Fluorophores** | [Light Sources](#) | [Excitation Filters](#) | [Emission Filters](#) | [Reset](#)

Category (Optional)	Fluorophore	Excitation	Emission	Action
1. <a href="#">Click to Select</a>	GFP (emerald GFP)	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<a href="#">Remove</a>
2. <a href="#">- All Categories -</a>	Tetramethylrhodamine (TAMRA, TRITC)	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<a href="#">Remove</a>
3. <a href="#">Click to Select</a>	Alexa Fluor 647	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<a href="#">Remove</a>

[+ Add](#)

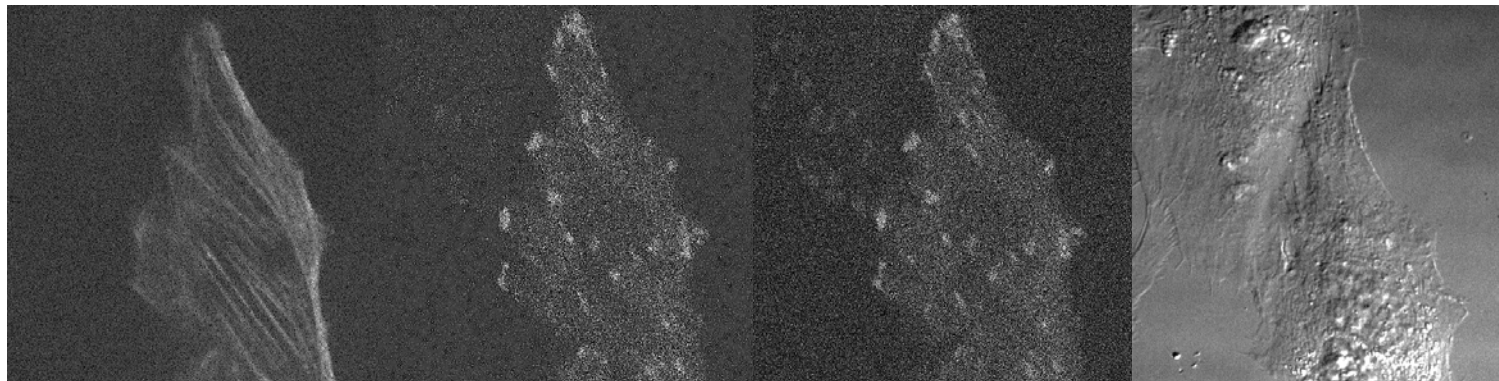
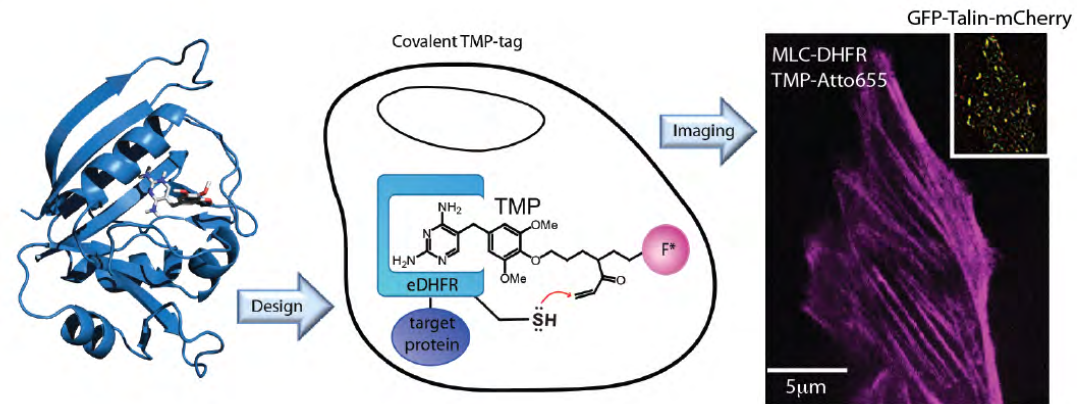
**Display Options**

- Grid
- Labels
- Excitation Plots
- Emission Plots
- Light Sources
- Filters
- Show Emission Plot Normalization

## Choosing a Fluorophore

Excitation and emission	Together with filter set/laser line
Brightness	quantum yield, extinction coefficient
Photo stability	quenching and photo-bleaching rate
Size	GFP 27kDa
Blinking or photometry	Localization or quantitation
Maturation speed	mCherry ~ 15min
Labeling Ratio	monomer/oligomer
Delivering method	SNAP, CLIP, eDHFR-TMP
Biocompatibility	Membrane Permeability, Cytotoxicity, PH stability(pKa)

# Labeling Vinculin



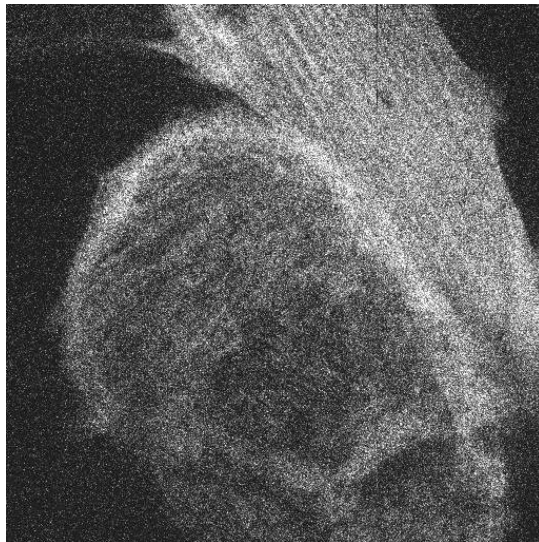
MLC-DHFR, TMP Atto655

GFP-Talin

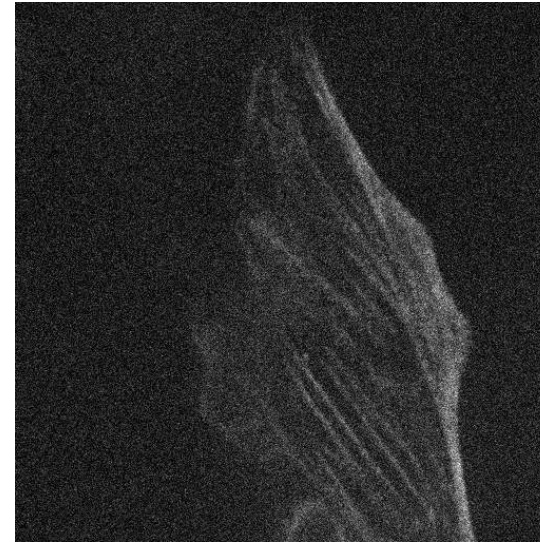
Talin-mCherry

DIC

# BioCompability

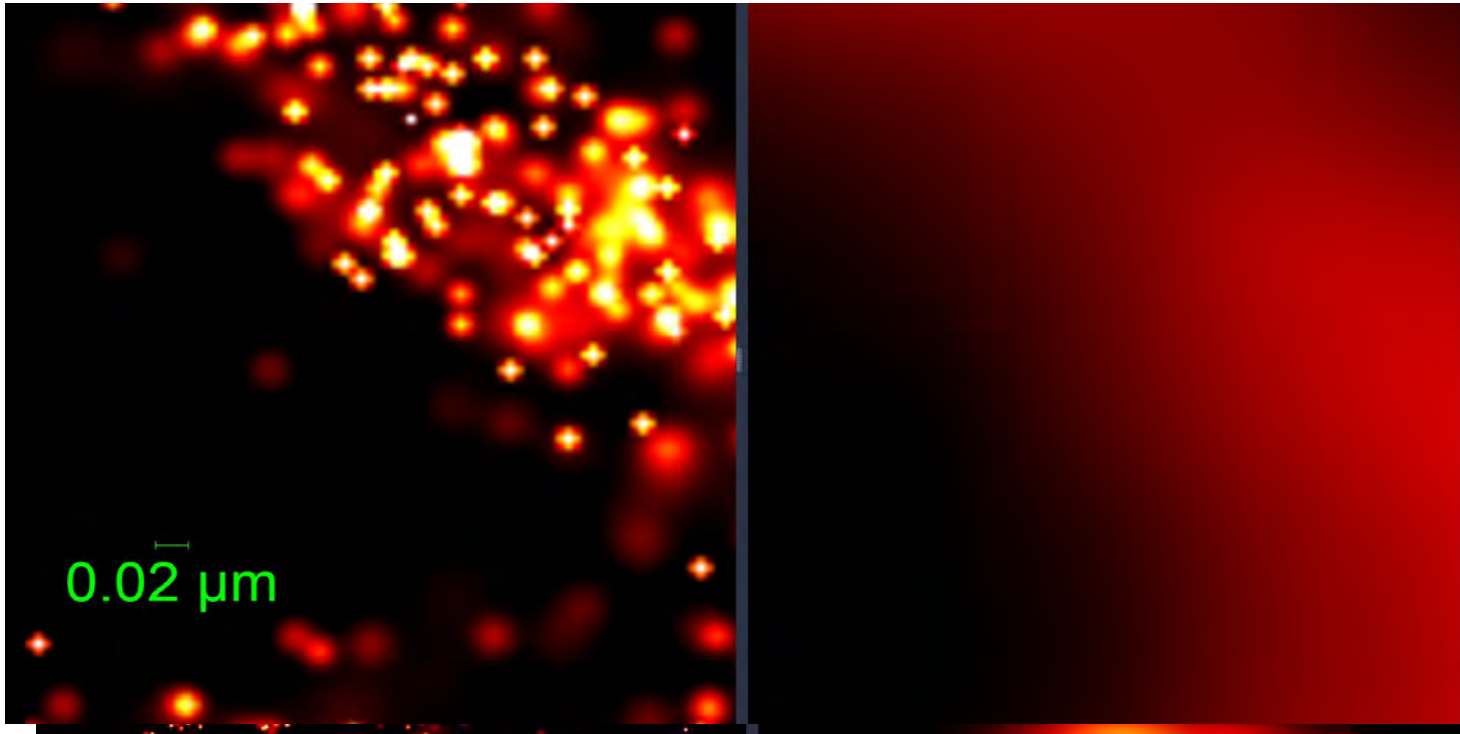


20 uM TMP-Fluorescein,  $\tau=55$  sec N=5



20 uM TMP-Atto655,  $\tau=64$  sec N=5

# Single Molecule Localization

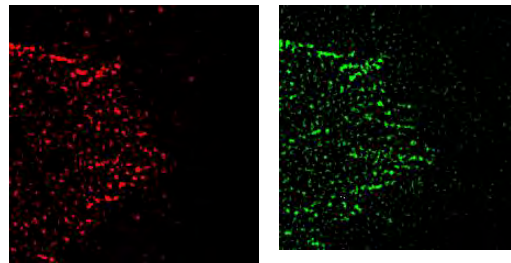


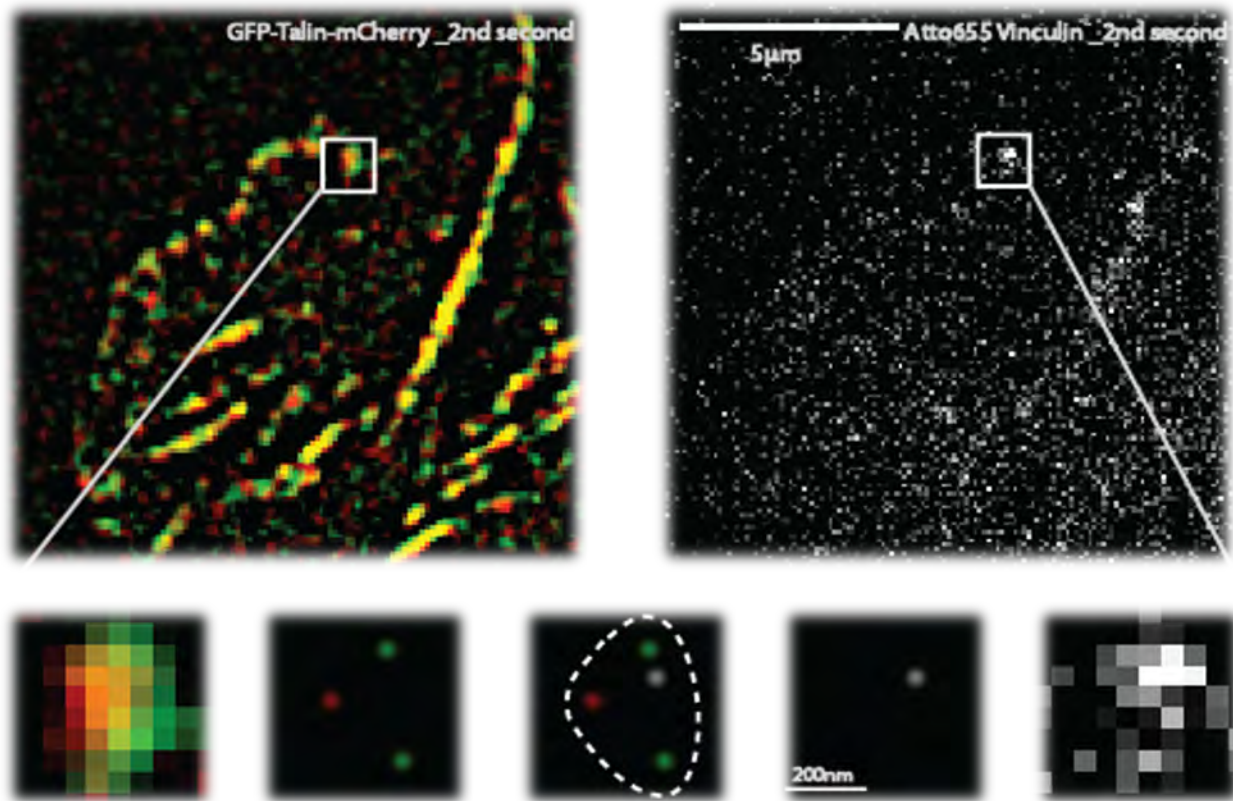


5 $\mu$ m



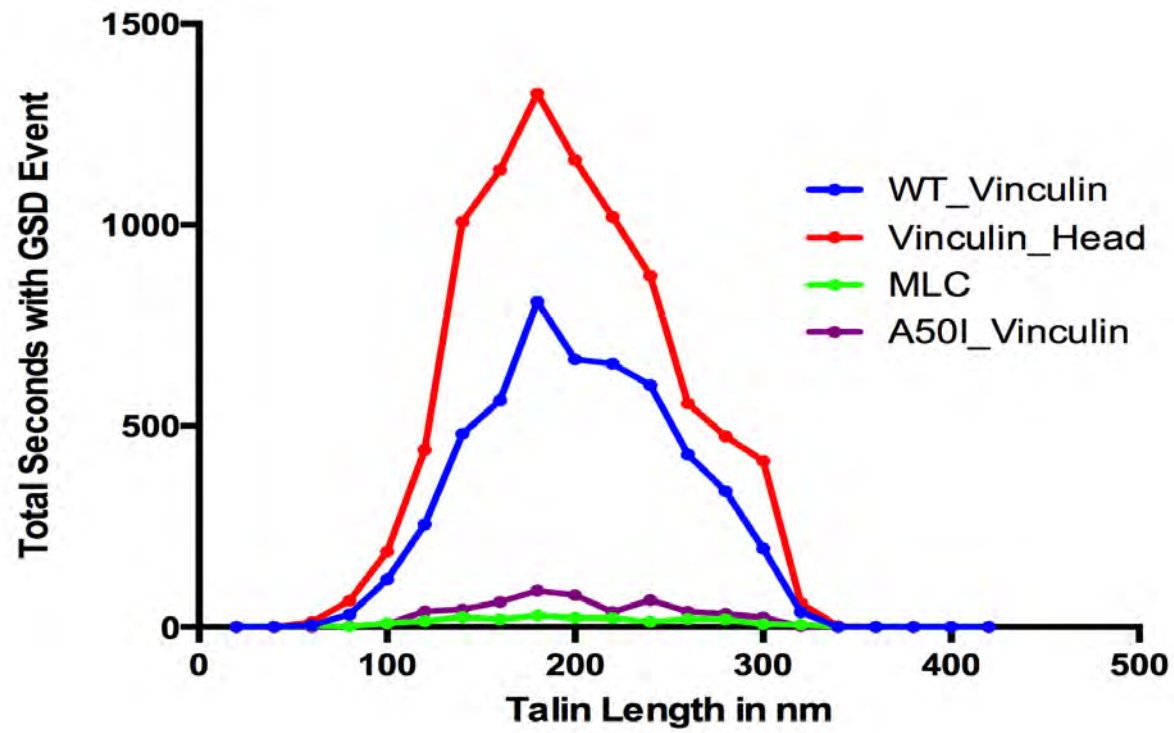
GFP-Talin-mCherry  
Paired Single Molecule

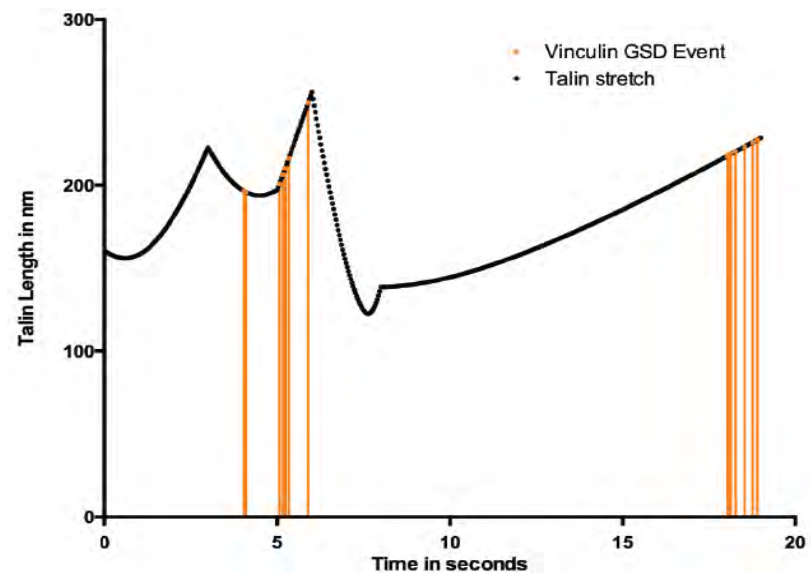
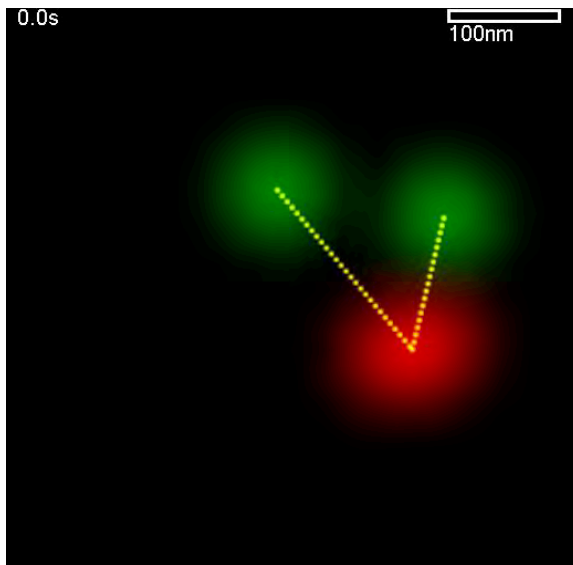




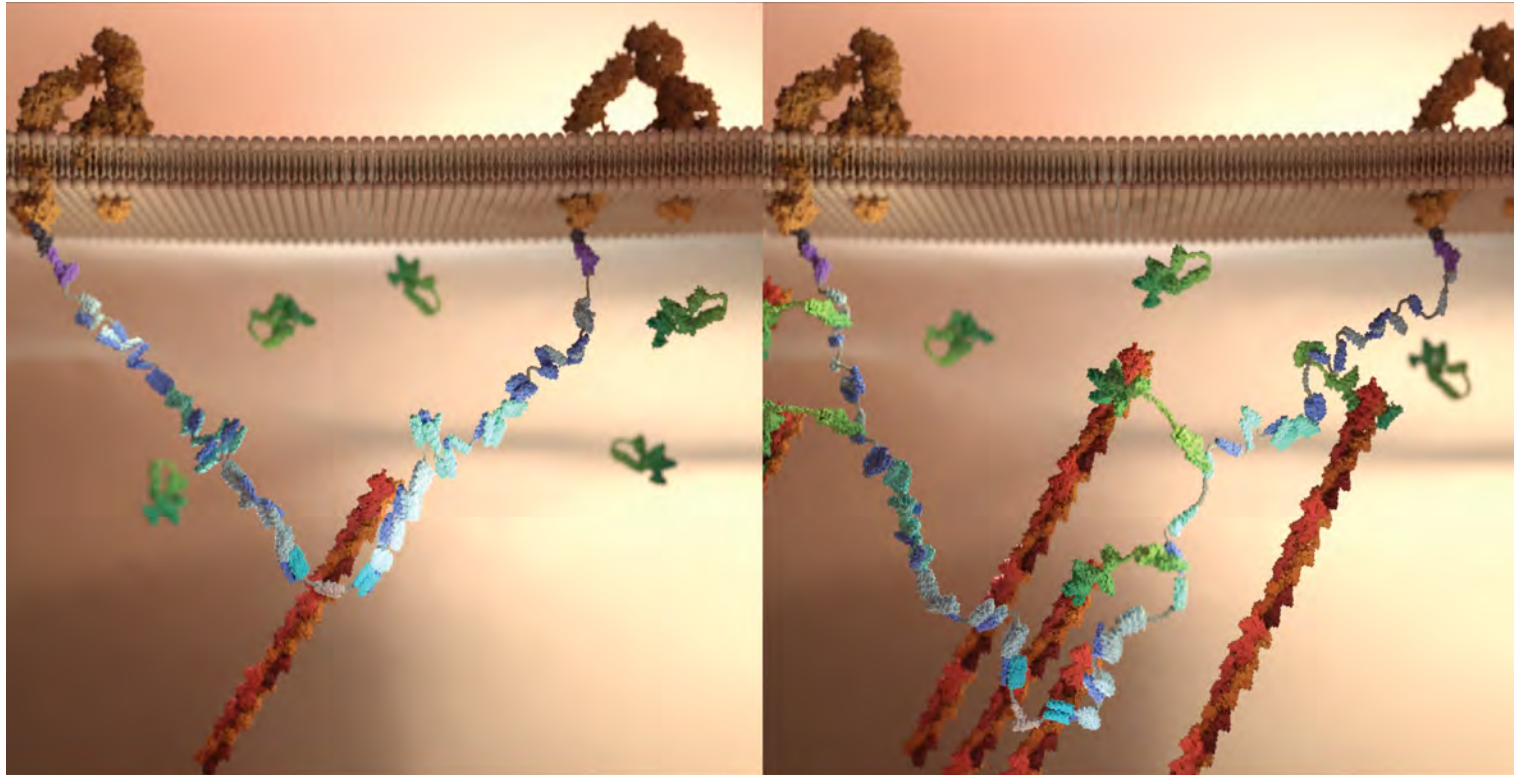
- Simultaneous Imaging of
  - GFP: N terminal, Integrin Binding
  - mCherry: C terminal, Actin Binding, Dimerization
  - Atto655: Vinculin

Vinculin binds to talin most when talin is 180nm





- GFP: N terminal, Integrin Binding
- mCherry: C terminal, Actin Binding
- Atto655: Vinculin



Integrin Complex

F-actin

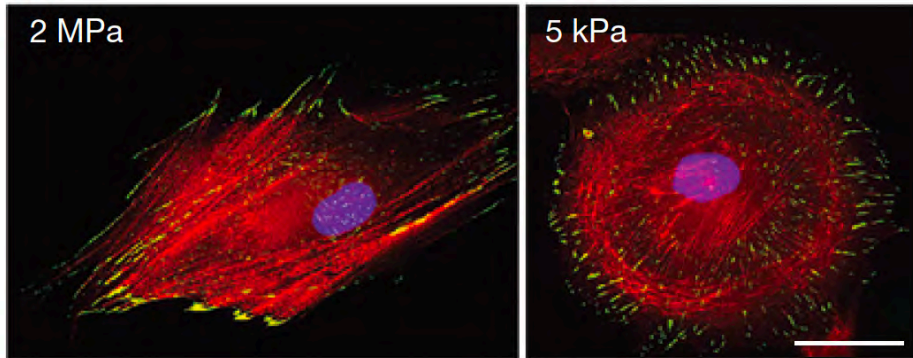
Talin (Unstretched)  
Talin (Stretched)

Vinculin(Auto-inhibition)  
Vinculin(Activated)

Hu et al, Pro Sci. 2017

# Rigidity sensing is impaired in transformed cell

6 h

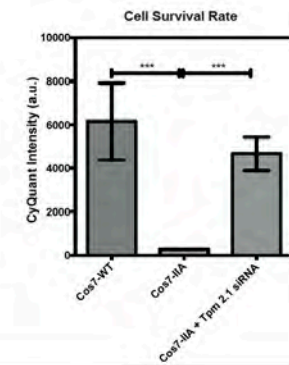
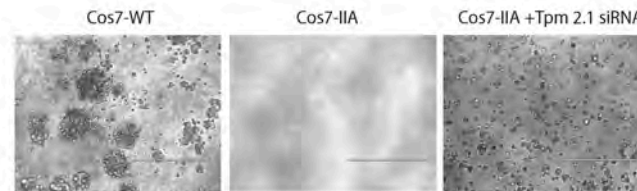
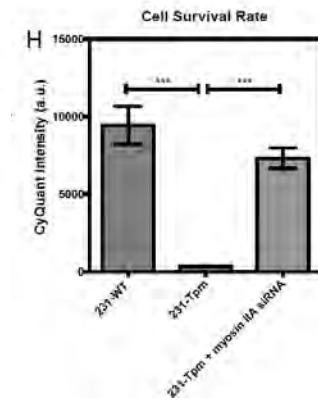
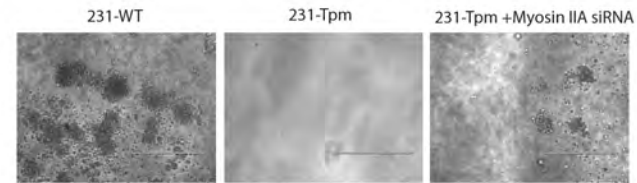
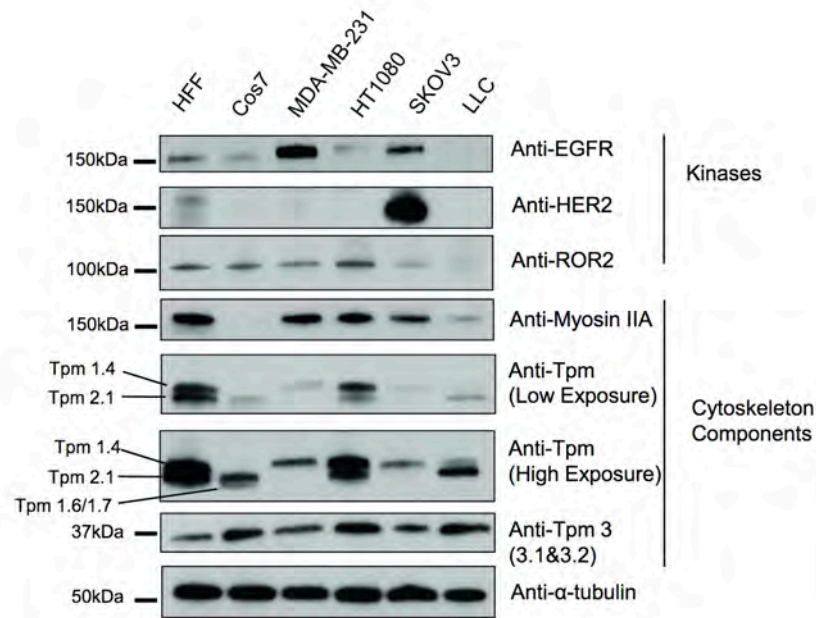


Normal Cell behave differently on soft substract

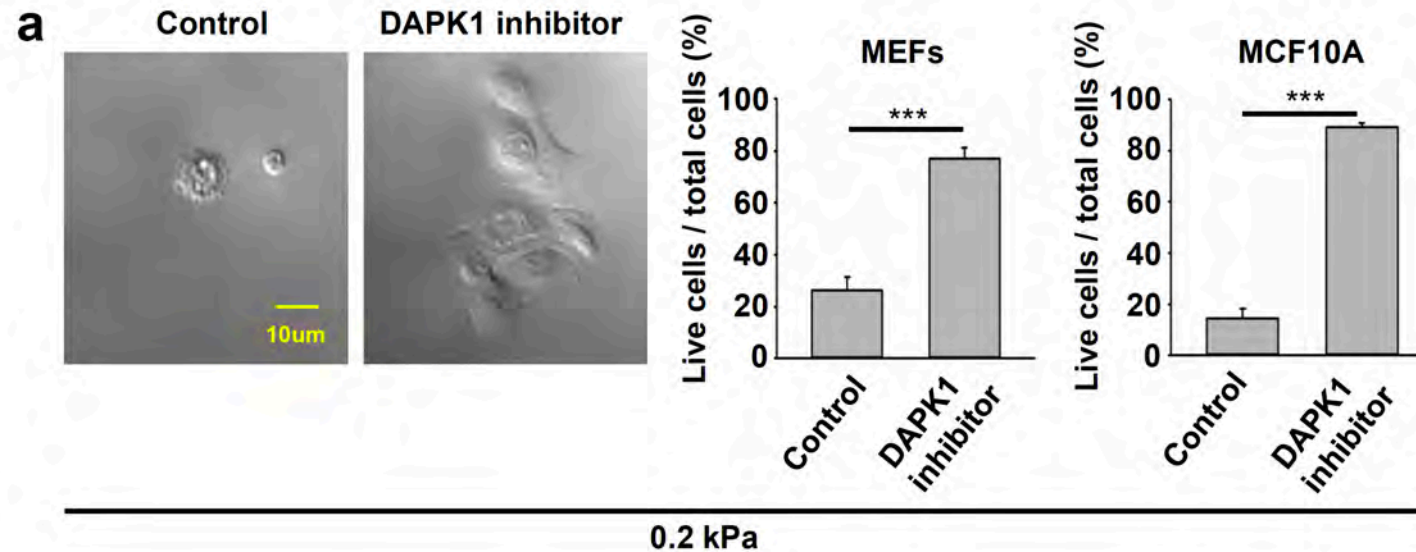
Prager-Khoutorsky, M. *et al*; Nat Cell Bio 2011

Yang, B. , Wolfenson,H., Nakazawa, N. *et al*, Nat Mater 2019

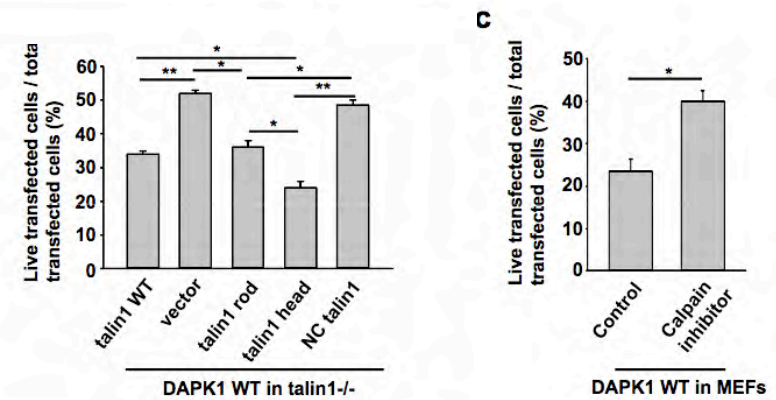
# The Missing Mechanosensing Component



# Missing Link between Sensing and Apoptosis



- DAPKI activity linked with Talin cleavage





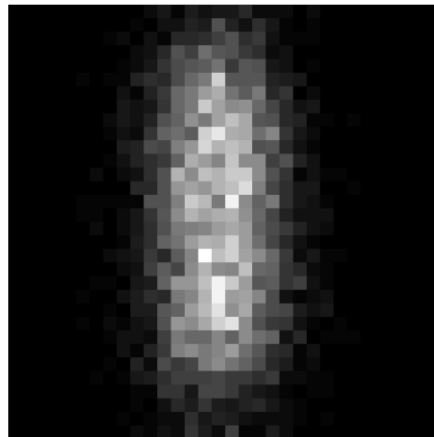
# Bio Imaging For Quantitative Measurement

- Qualitative and Quantitative
- What Makes Quantitative Measurement Possible:
  - Scientific grade detectors are linear
  - Fluorescent proteins are linear

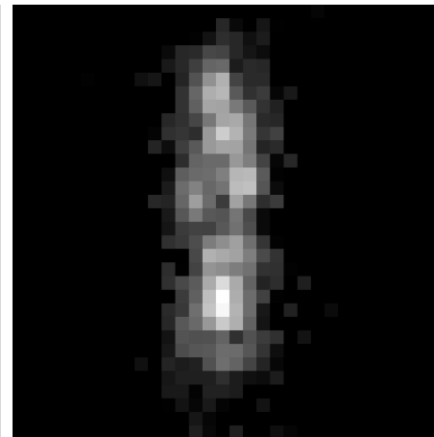
- **Live Cell Imaging: Photon budget**
- Artifacts in Quantitative Imaging
  - Protein expression level?
  - Bleaching
  - Laser power
  - Light efficiency of microscope
  - Undersampling



line object

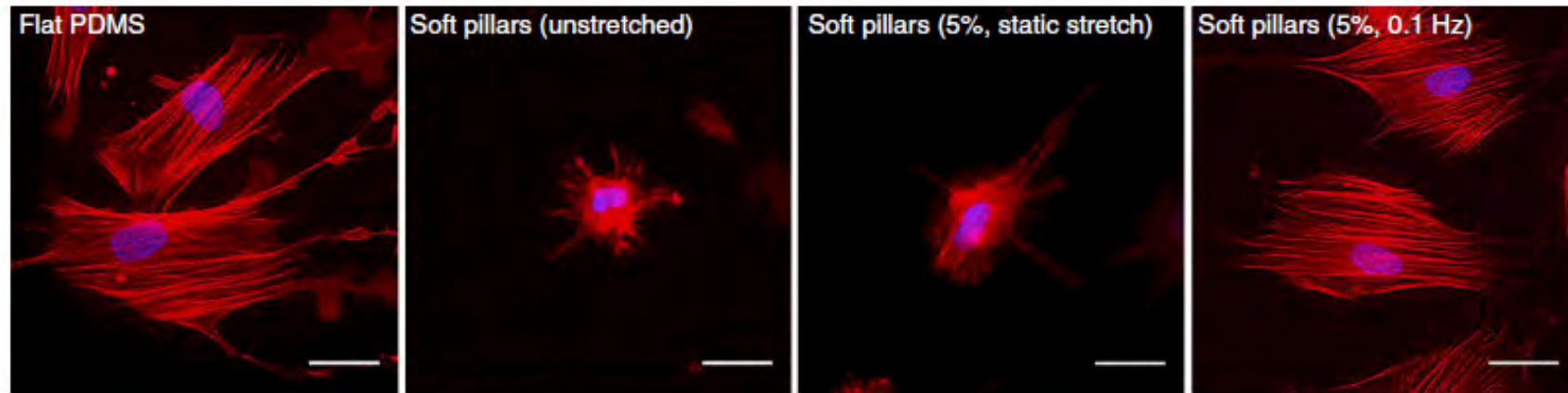
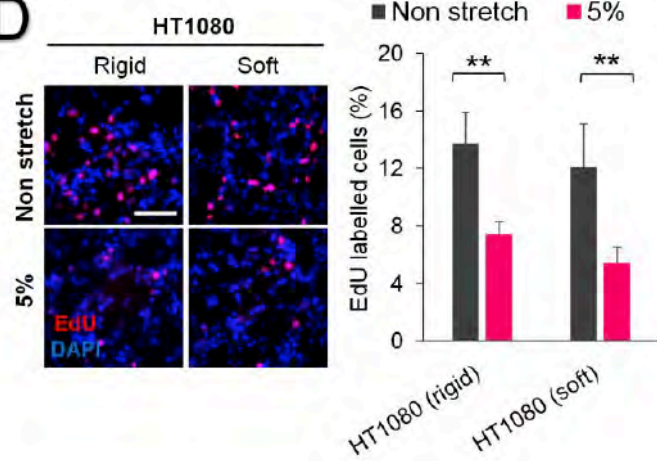
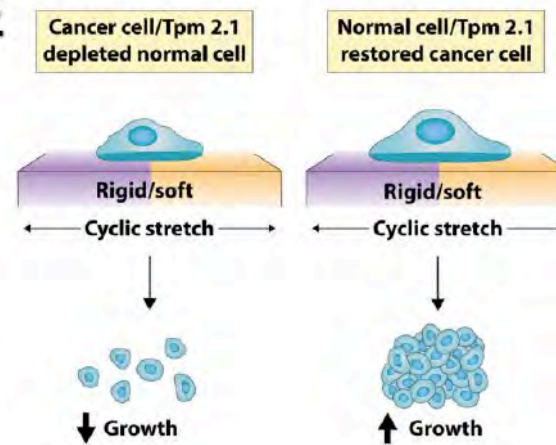


convolved with PSF and Poisson noise



Undersampling simulation

Simulation by Felix Margadant

**a****D****E**

Cui et al, Nat Commun, 2015

Tijore et al, BioArchive, 2018

Thank you



UiO : **University of Oslo**



Prof. Michael Sheetz  
Prof. Alexander Bersdhasky  
Prof. Harry Yu  
Prof. Oddmund Bakke

*Talin Project:* Xian Hu, Salma Jalali, MingXi Yao, Felix Margadant, Michael Sheetz

*Endosome Project:* Xian Hu, Duarte Mateus, Vinodha Manovasegaran,  
Felix Margadant & Oddmund Bakke