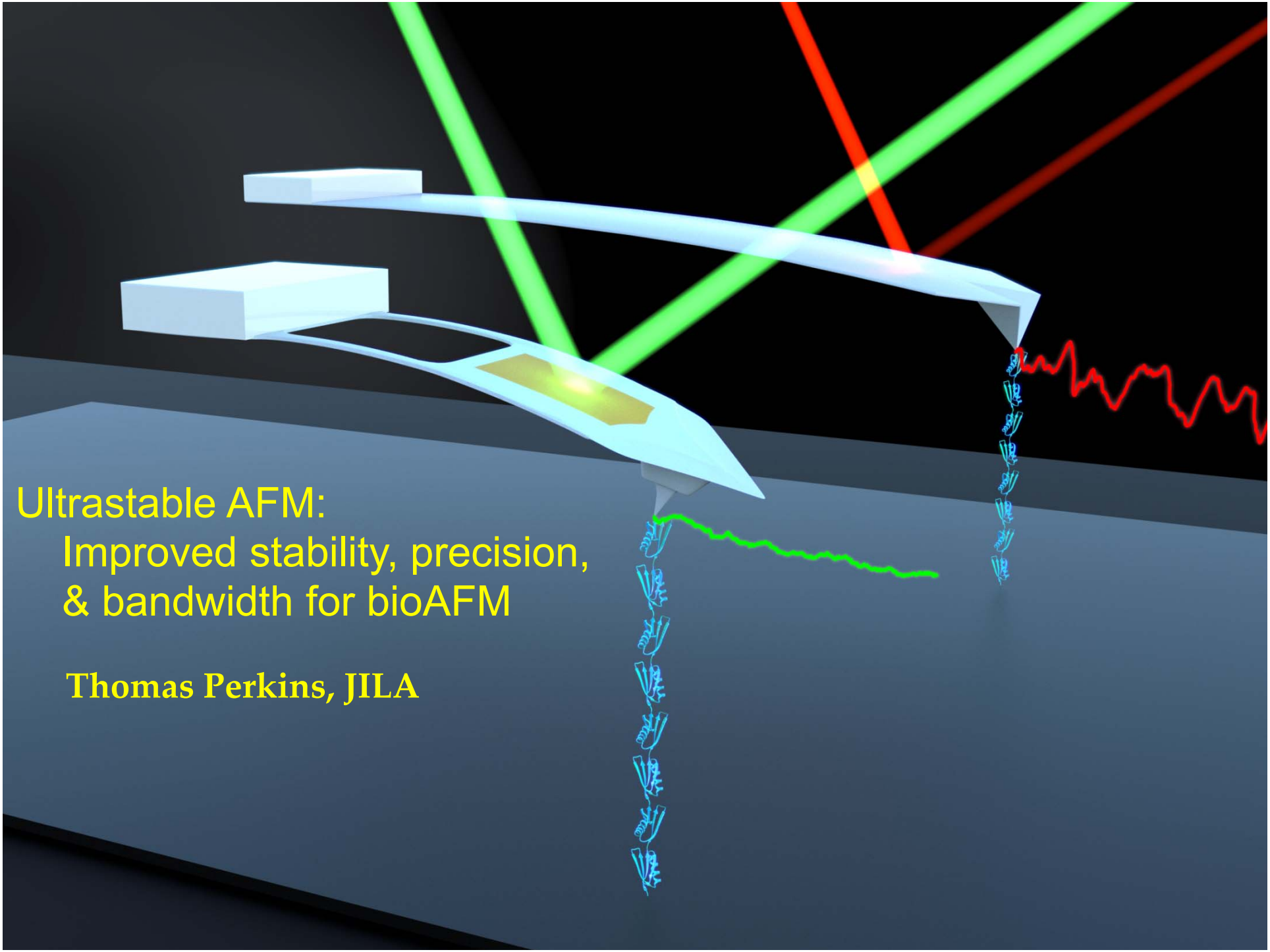
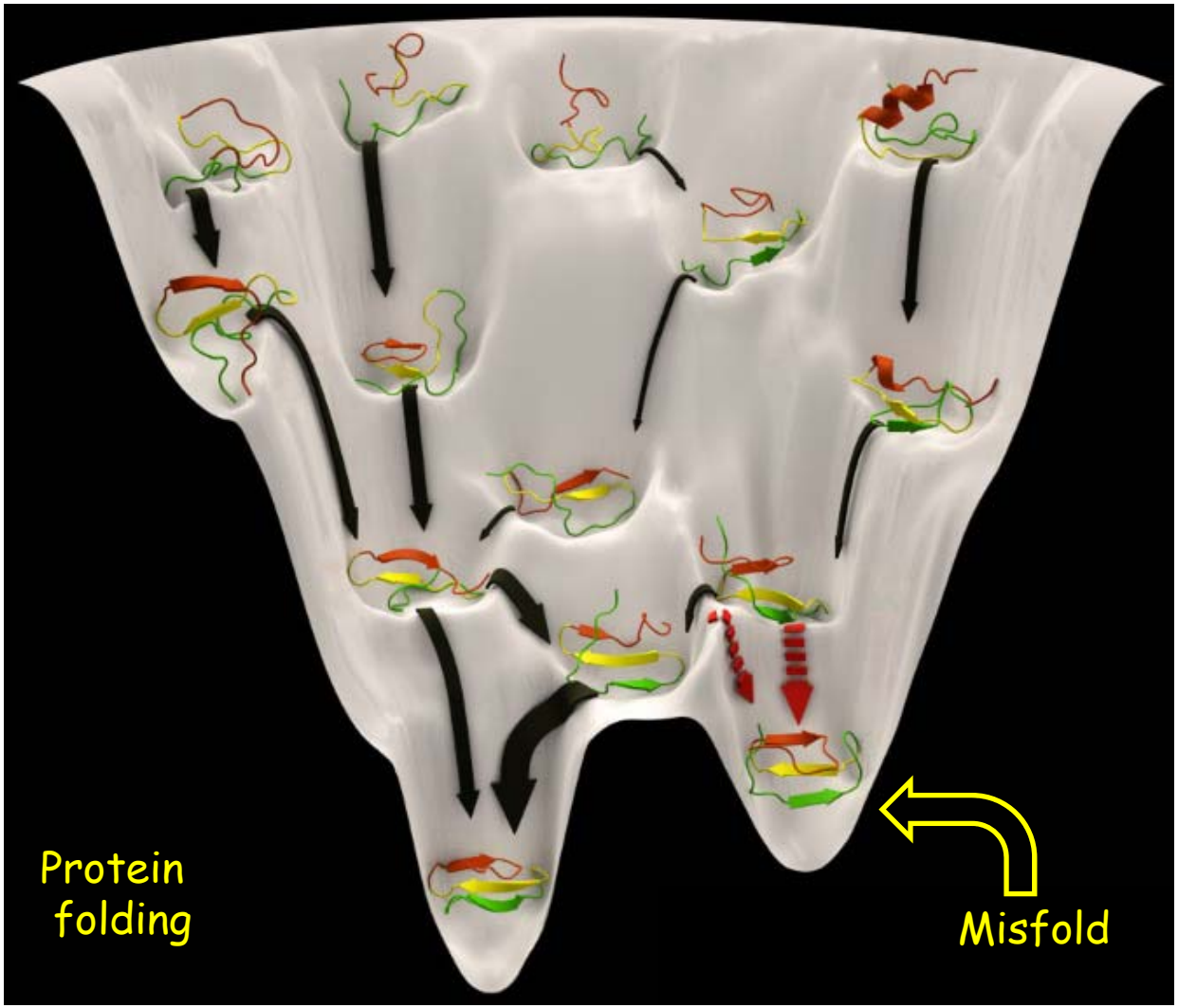

Ultrastable AFM:
Improved stability, precision,
& bandwidth for bioAFM
Thomas Perkins, JILA

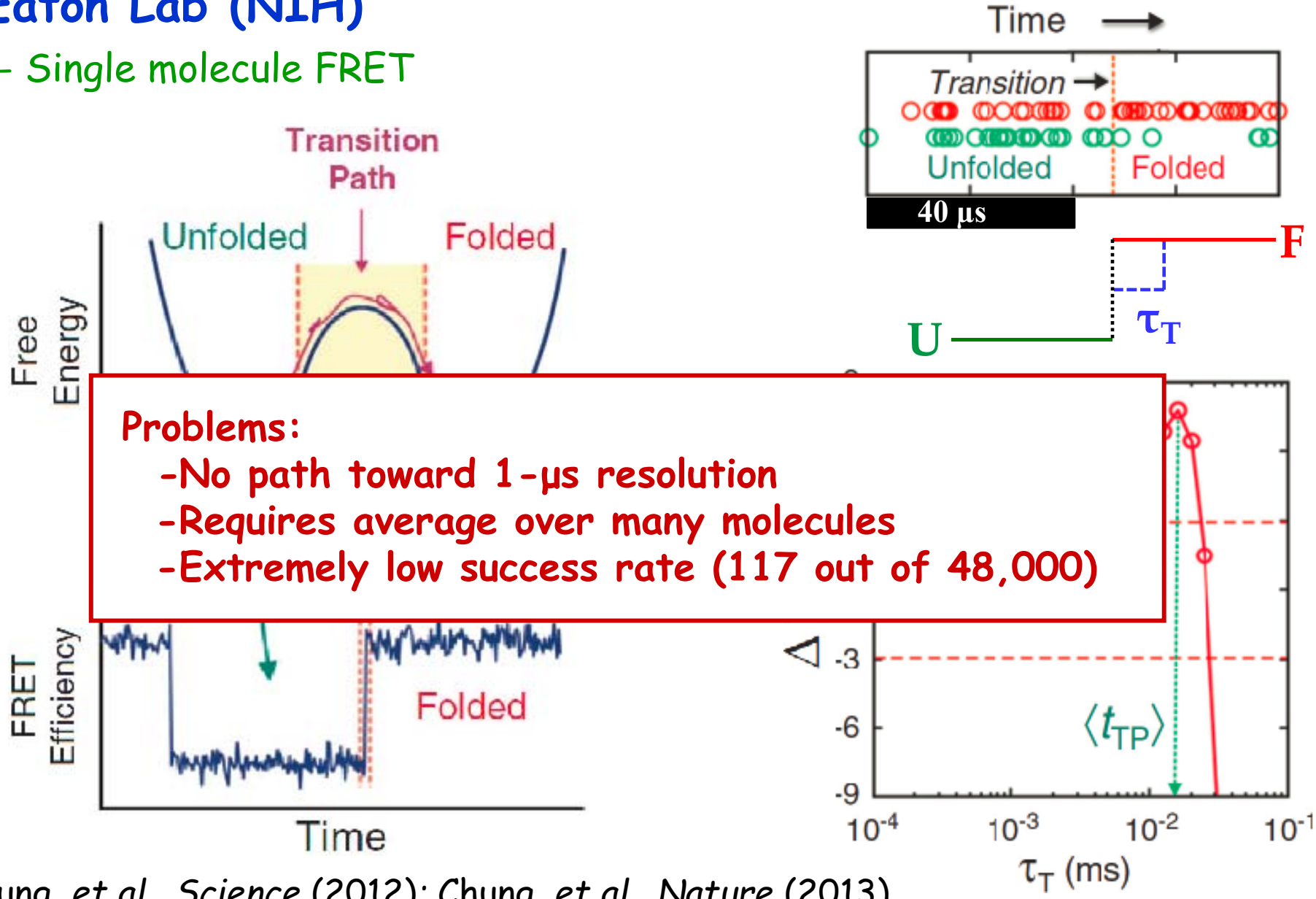
Protein folding: a 50-year old problem in biology

- Goal: Identify states, pathways, and dynamics



Current state of the art: 10-μs temporal resolution

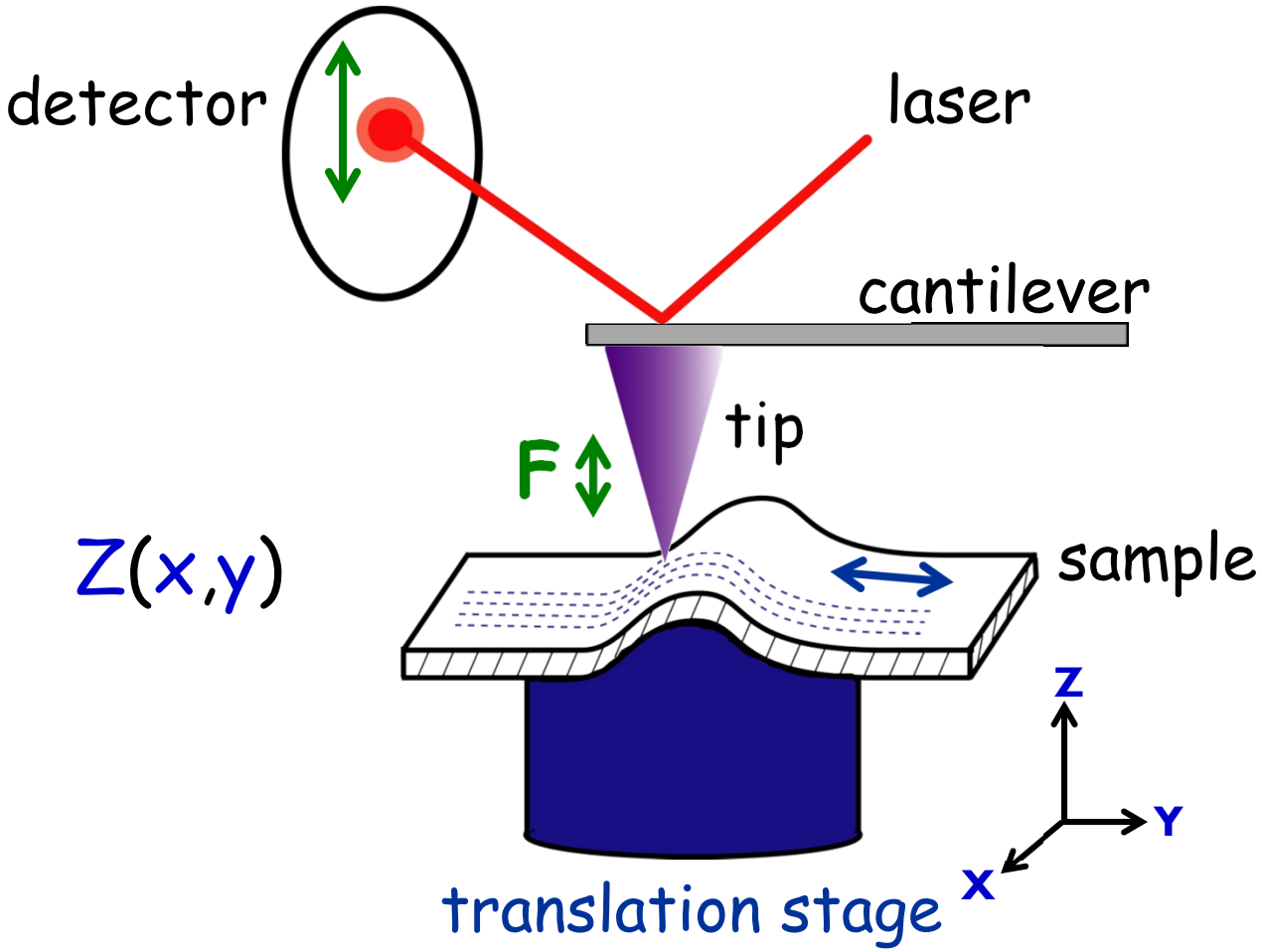
- **Eaton Lab (NIH)**
  - Single molecule FRET



Problems:
- -No path toward 1-μs resolution
- -Requires average over many molecules
- -Extremely low success rate (117 out of 48,000)

Chung, et al., Science (2012); *Chung, et al., Nature* (2013)

Basics of atomic force microscopy



Membrane proteins: a frontier in structural biology

Motivation:

Target for 50% of future drugs

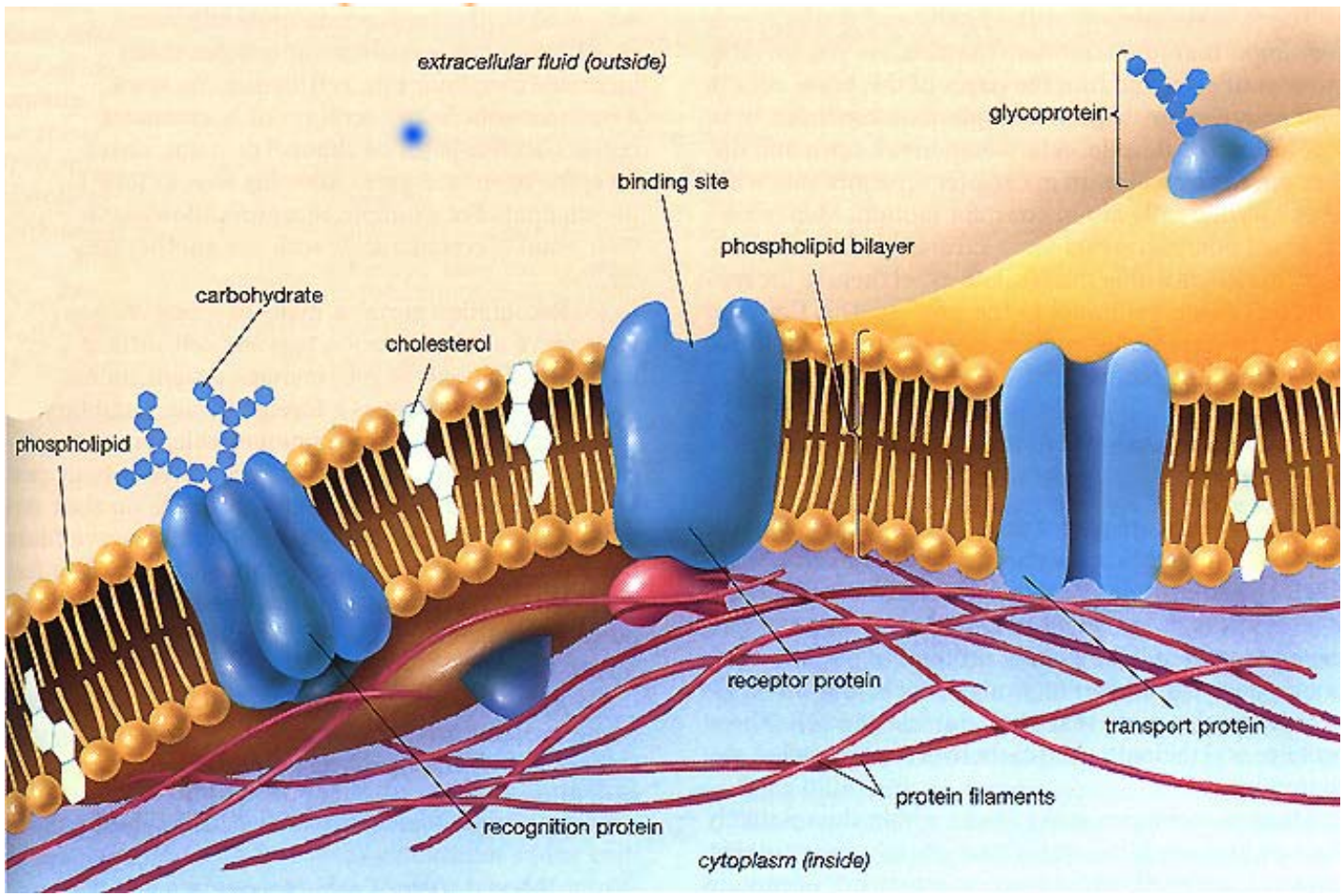
30% of genome

1% of Protein Data Bank

Problem:

Difficult to characterize by:

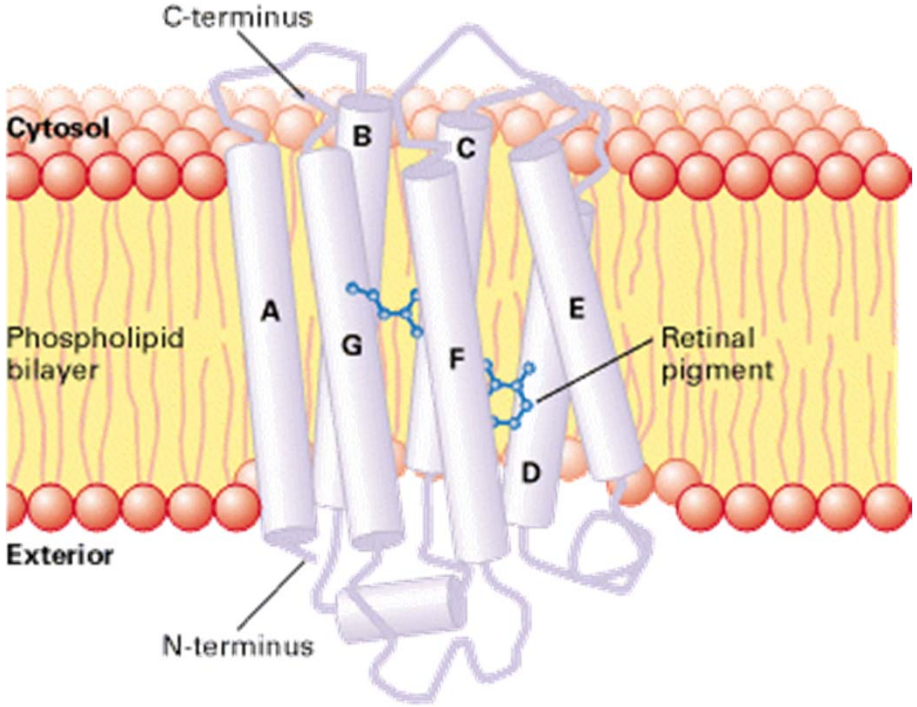
Crystallography, NMR spectroscopy, & electron microscopy

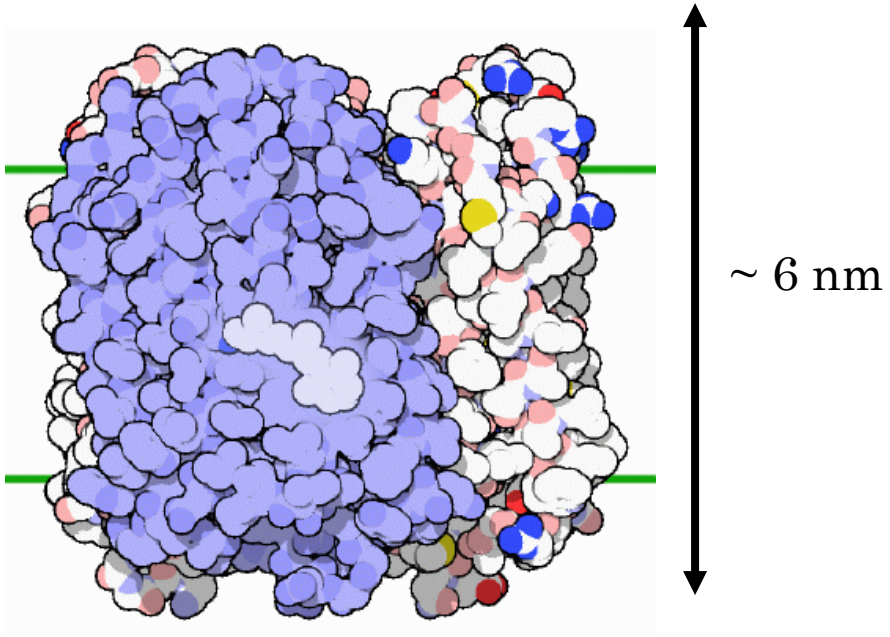


Bacteriorhodopsin is a model membrane protein

- Trimers of BR make "purple membrane"
- Extensively characterized by AFM

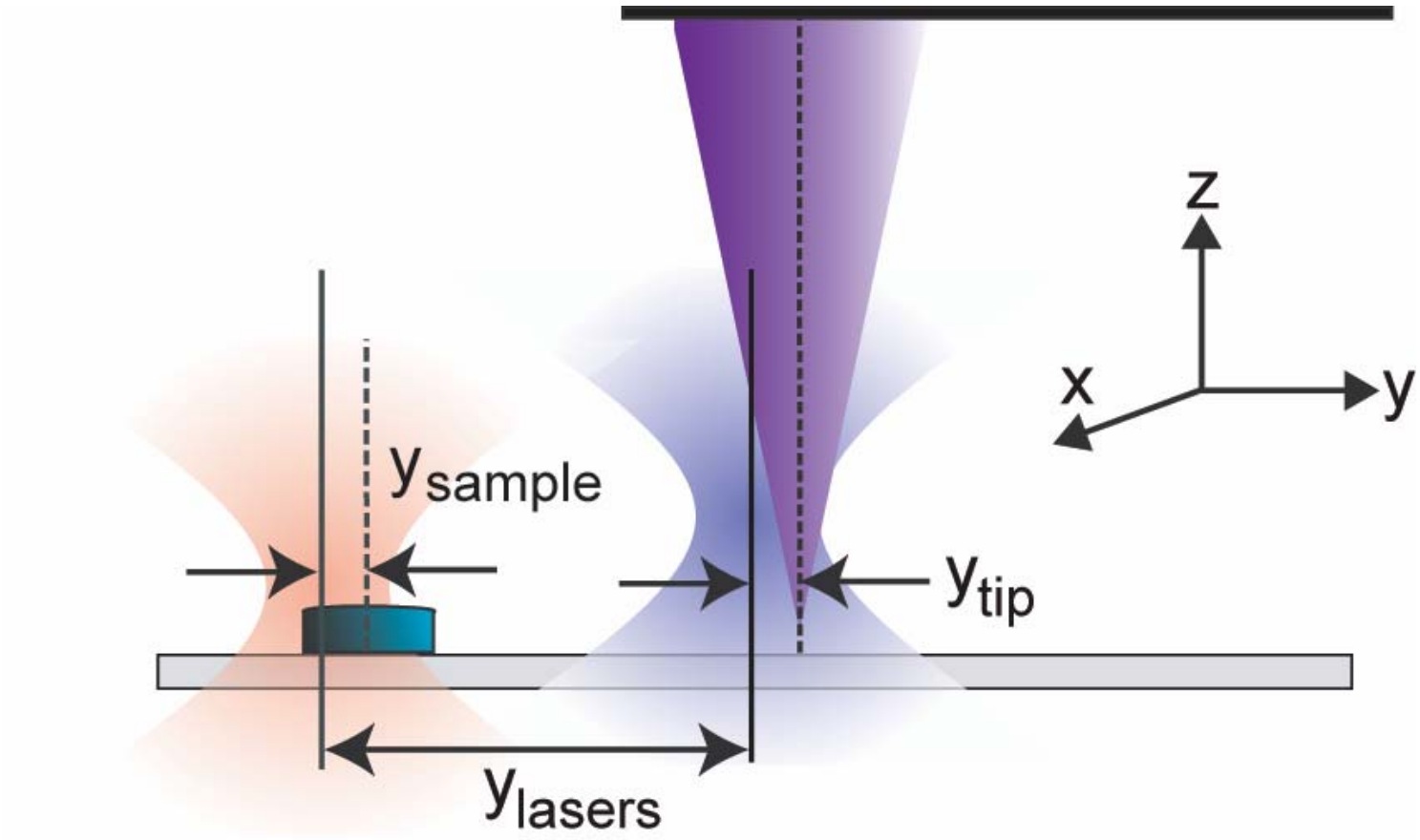
  (Hansma, Gaub, Engel, Müller, …)





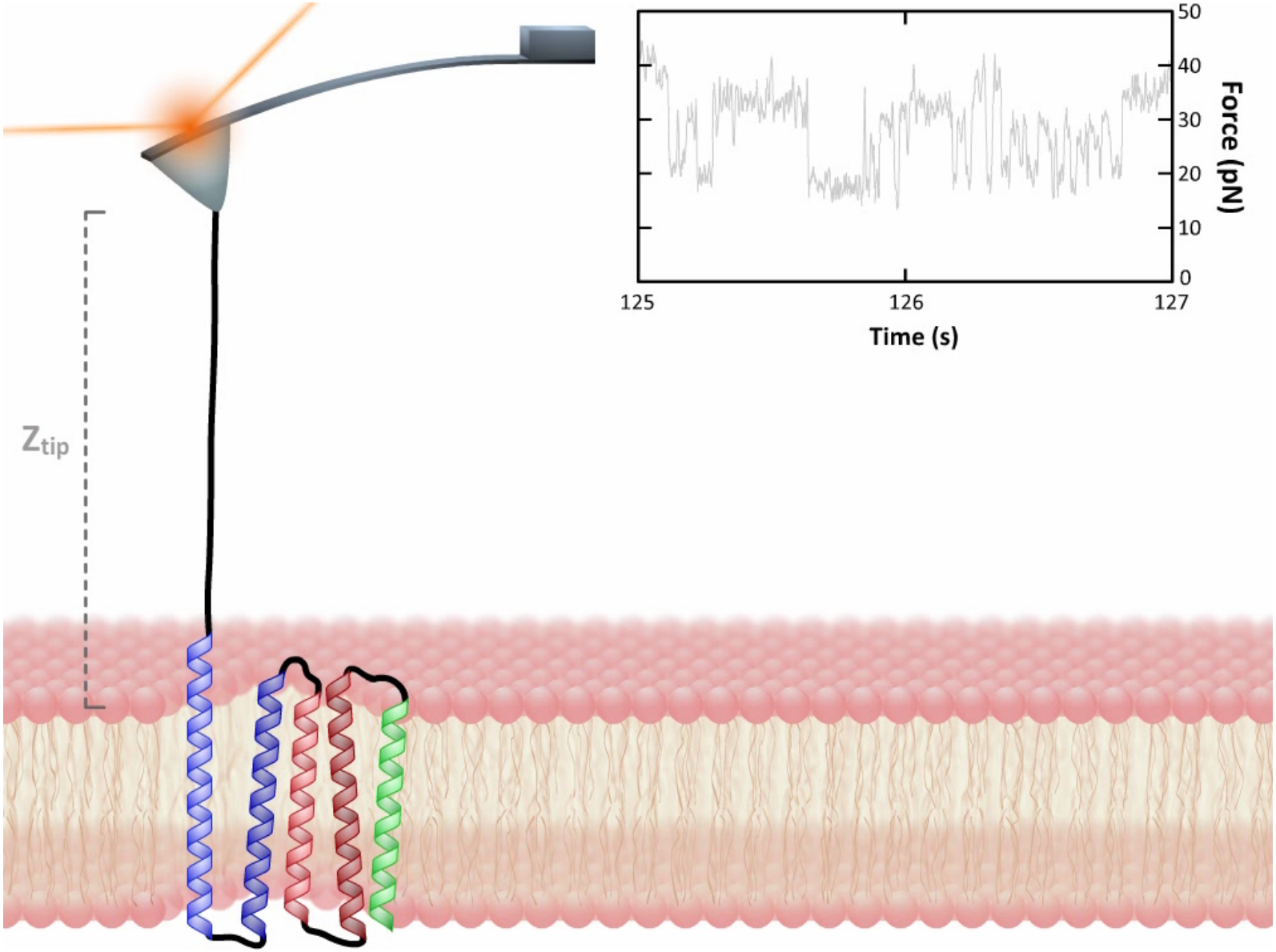
Optically stabilized AFM

- Locally measure
- Actively stabilize



King, *et al.*, *Nano Letters*, 2009

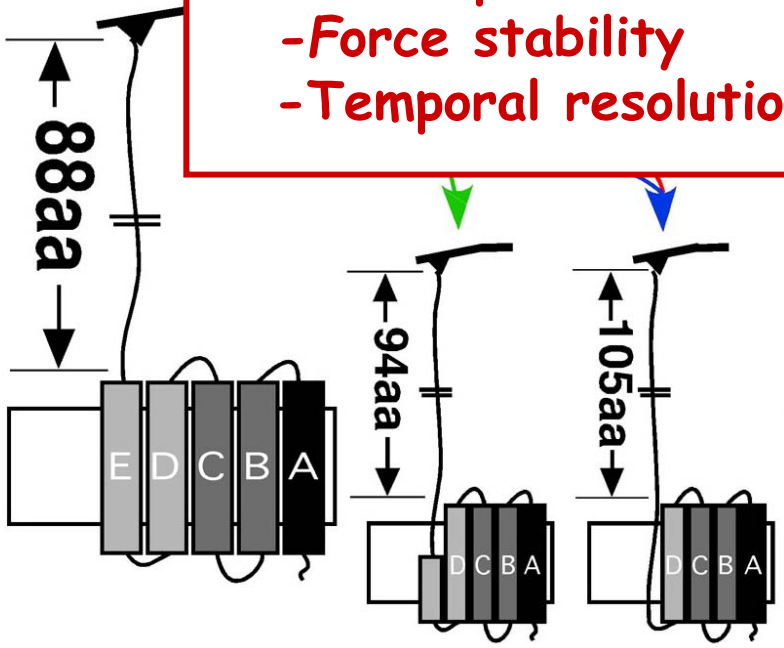
Folding and unfolding of a membrane protein



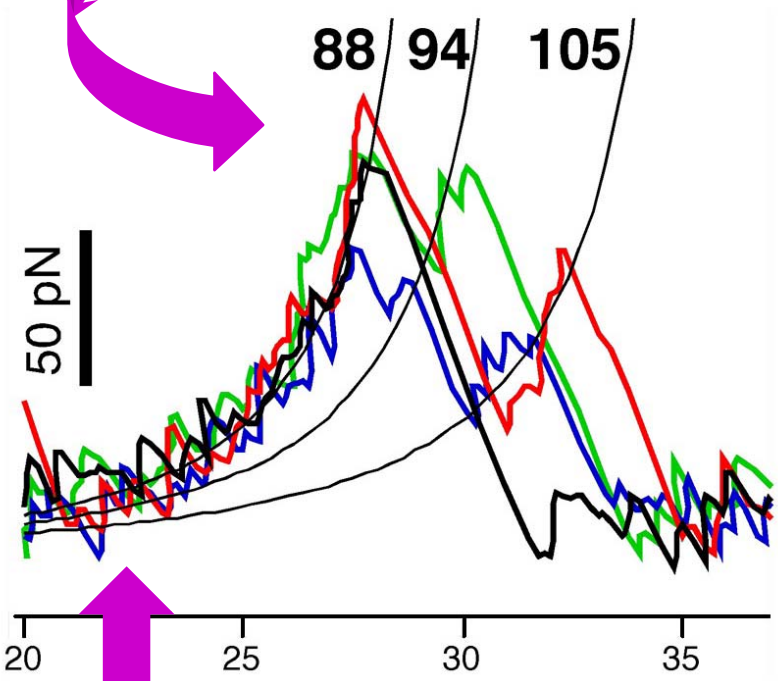
Challenges to interpretation

- **Unfolding intermediates previous described**
  - Gaub and Muller lab

Need improved:
-Force precision
-Force stability
-Temporal resolution



Fast pulling:
high forces

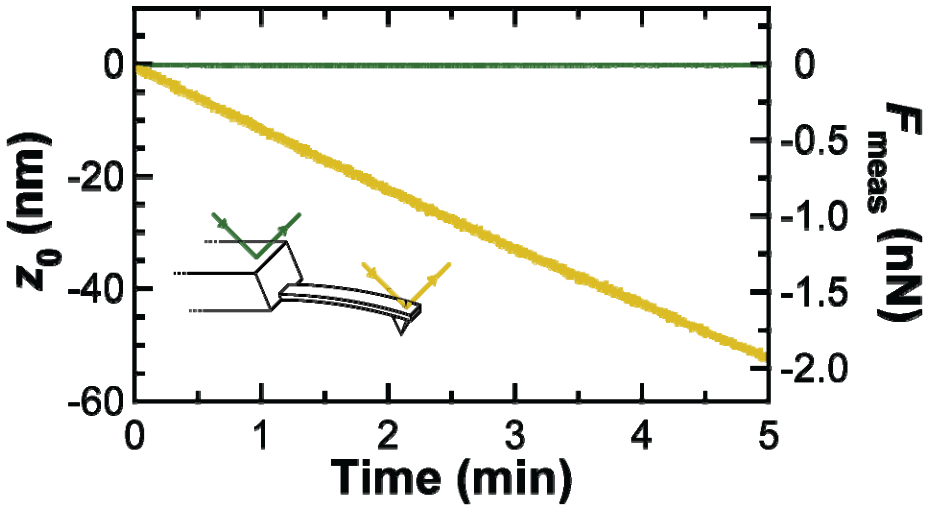


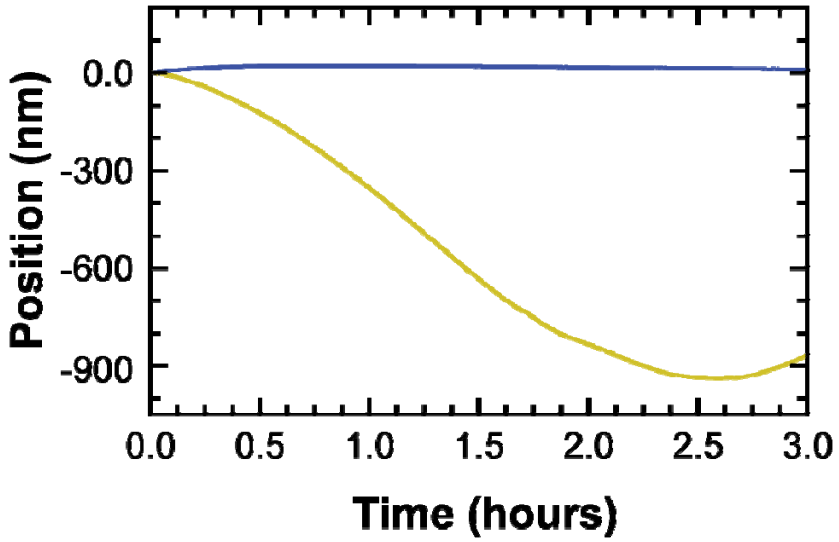
Slow pulling:
low forces

Sapra, *et al., J. Mol Bio.* (2008)

Force drift arises from gold coating

- **Cantilever position drifting**
  - Not external opto-mechanical stability
- **Gold coating causes drift**
  - Removing gold dramatically improves force stability

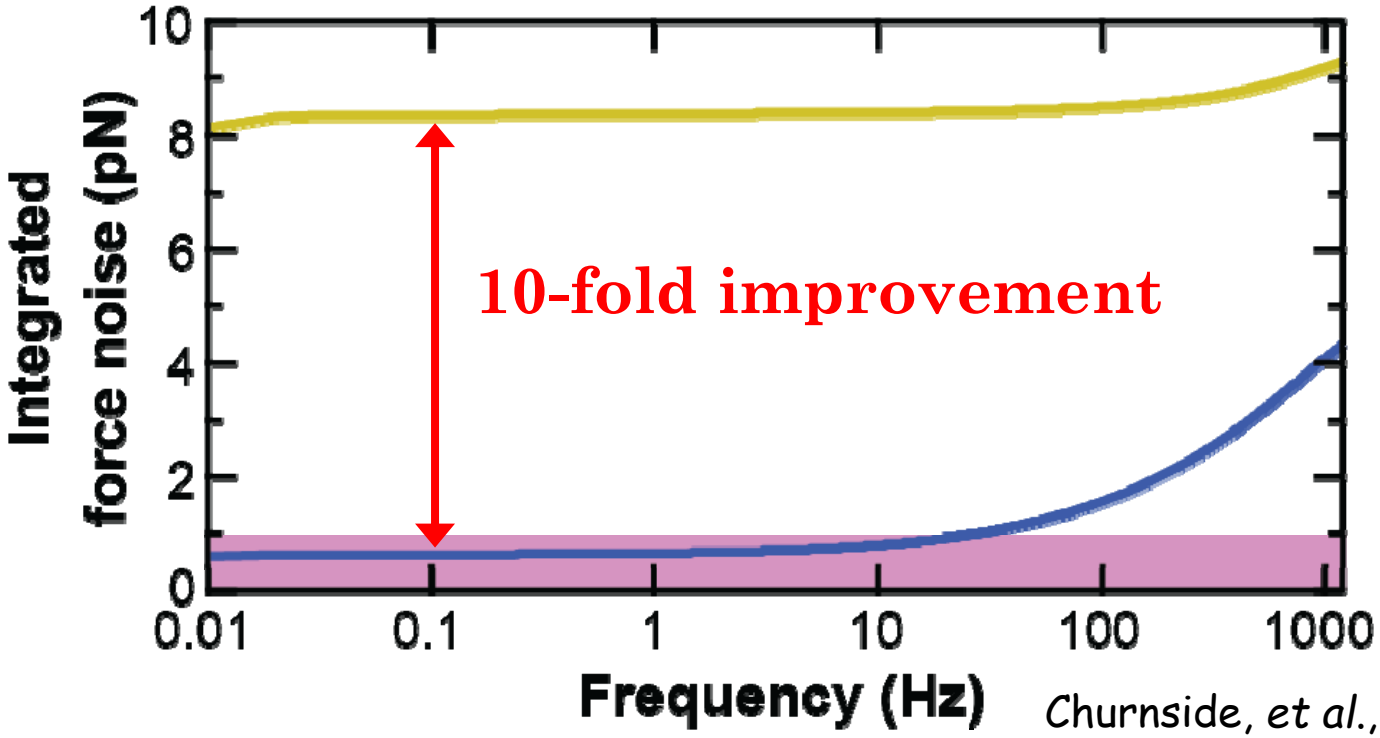




Churnside, *et al.*, *Nano Letters* (2012)

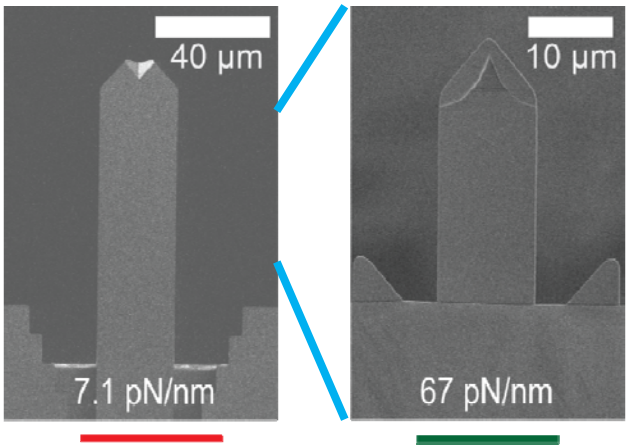
Sub-pN force precision and stability for bioAFM

- **Reflectivity not essential**
  - Commercial and US-AFM
- **Routine**
  - Achieved for 60% of cantilevers tested ($N = 14$)
- **Timely**
  - Achieved 30 min after wetting
  - No long "settling" required



Churnside, *et al.*, *Nano Letters* (2012)

Distinct temporal regimes in AFM force spectroscopy

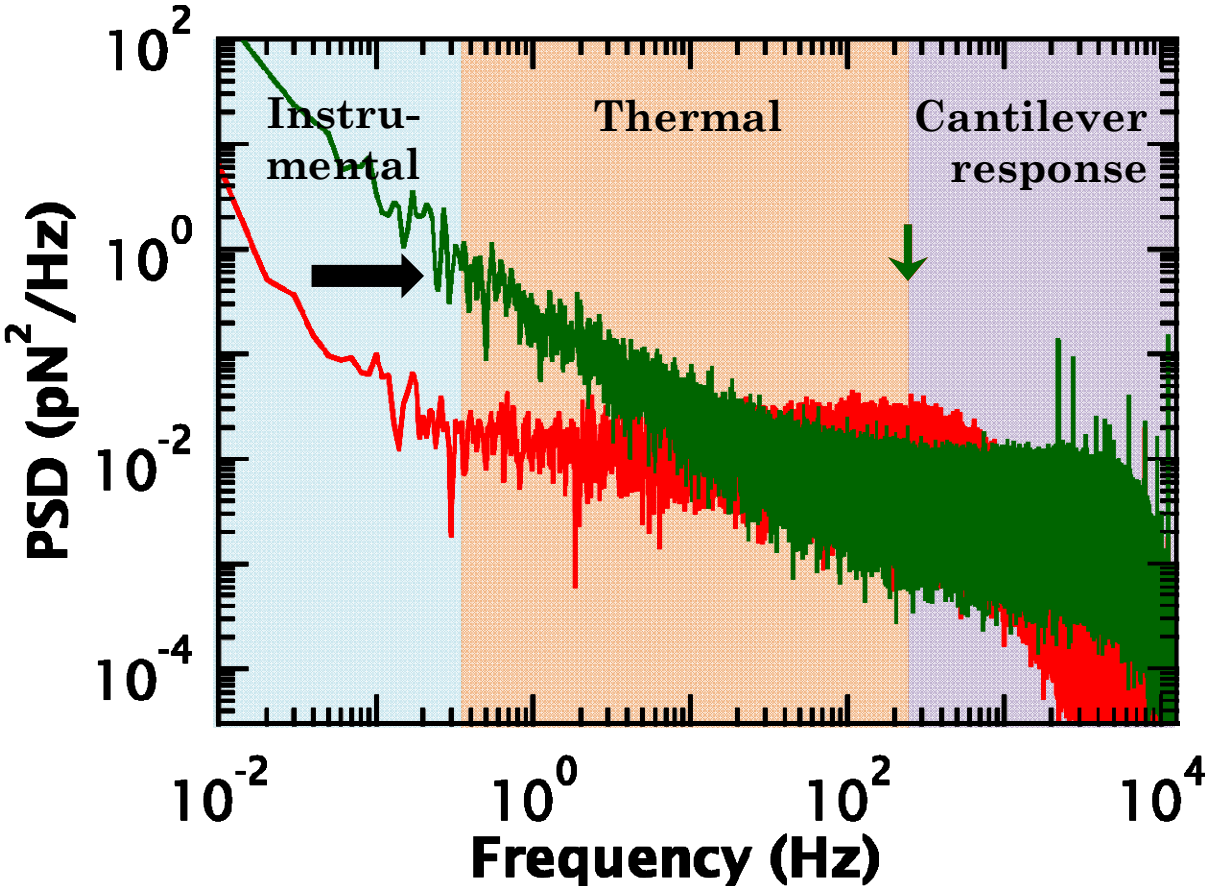


Design goals:

- Soft and short

Challenge:

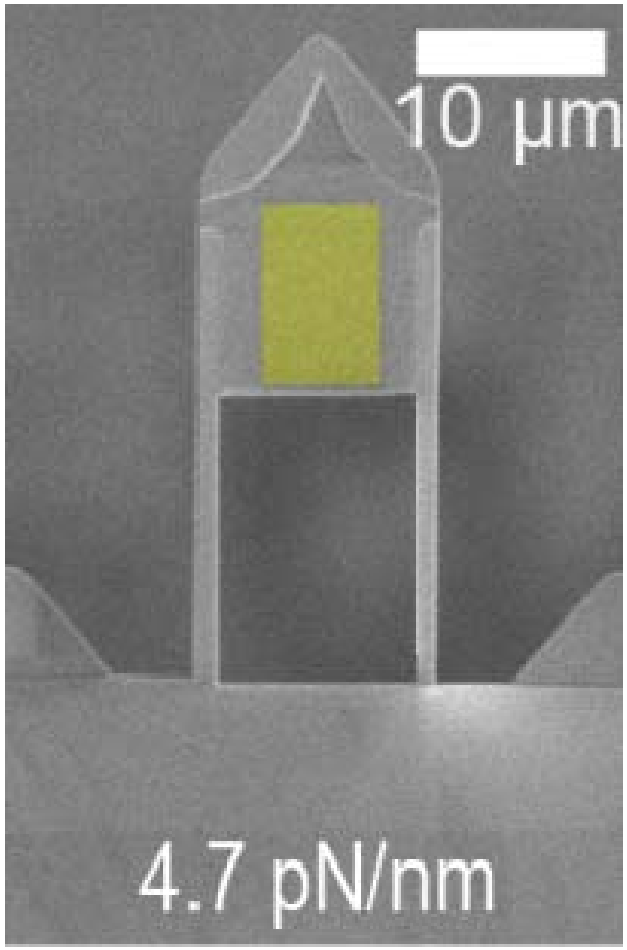
$$k \propto \frac{wt^3}{l^3}$$

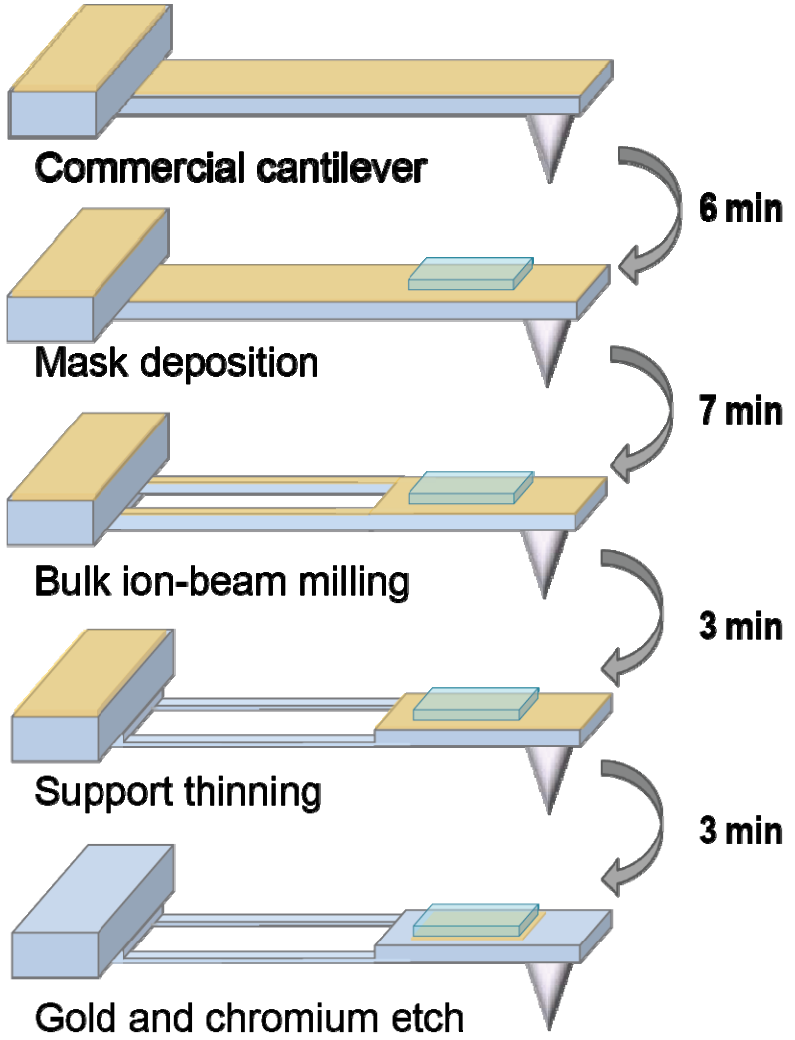


Efficient production of FIB-modified cantilevers

- **Ten-fold decrease in hydrodynamic drag and stiffness**
  - FIB-modified

see: Hodges, *Rev. Sci Instrum.* 2001

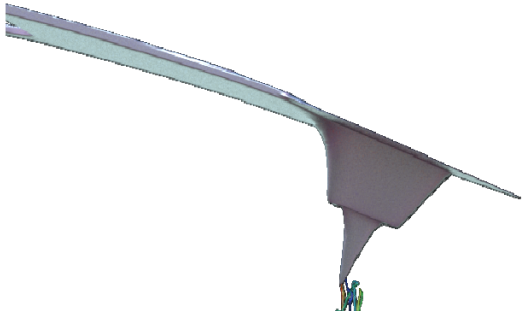


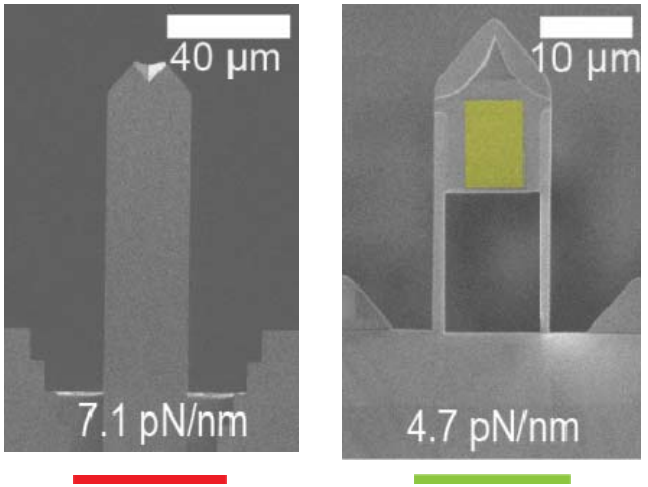


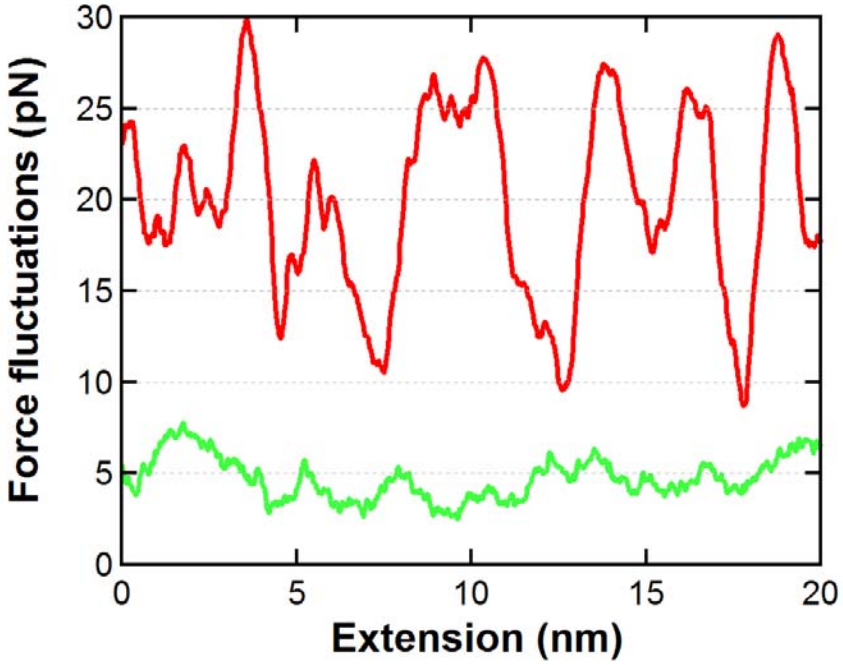
Bull, *et al., ACS Nano* (2014)

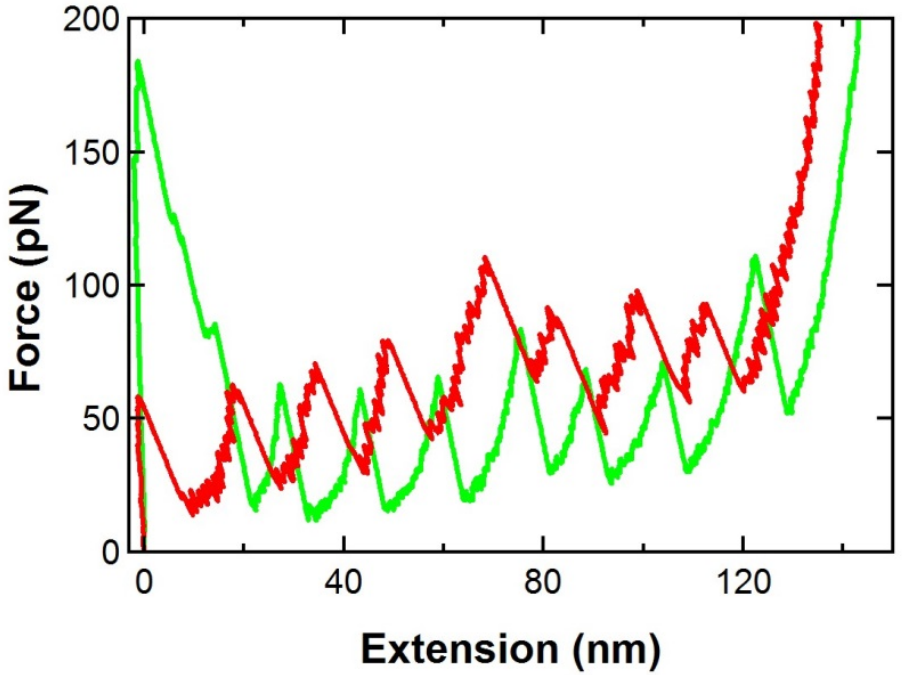
Modified cantilevers improve biophysical data

- **Unfolding a polyprotein**
  - Widely used single-molecule assay





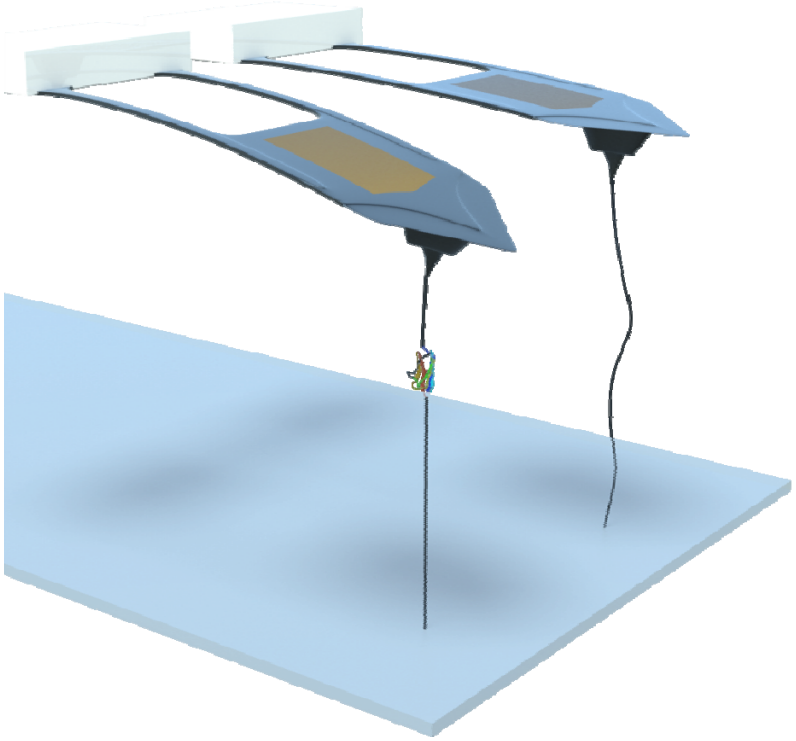


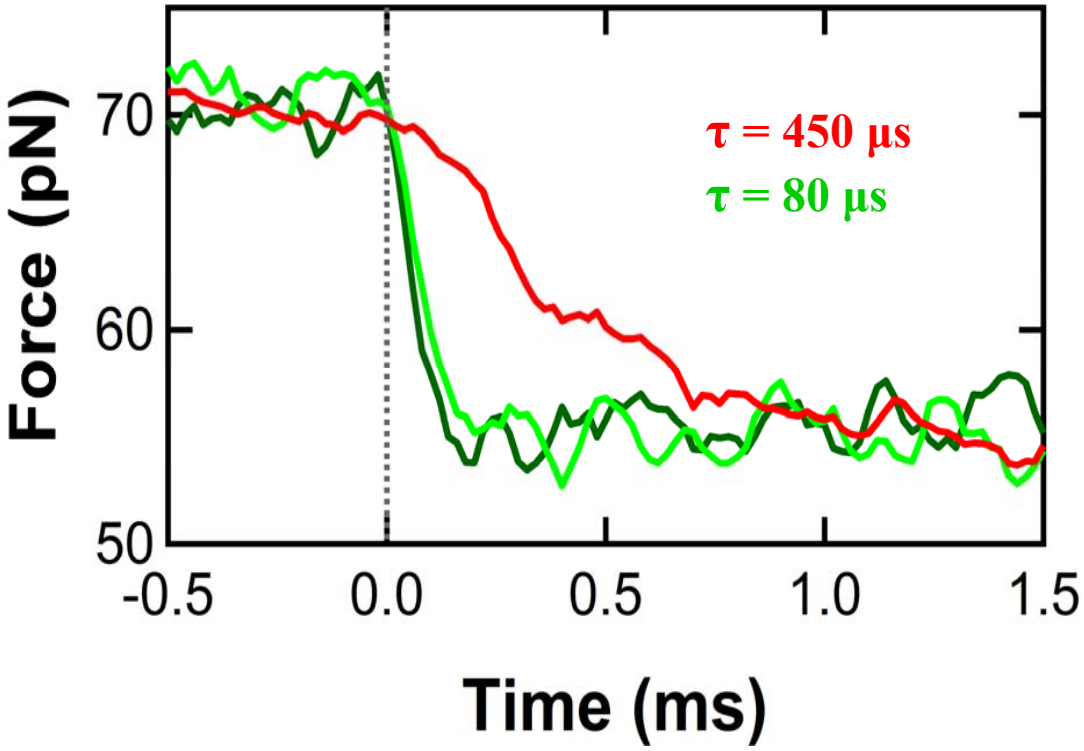


Sensitive but responsive cantilevers

- **Measuring the response function**
  - Fast protein dynamics masked by cantilever response

$$\tau_{\text{lever}} = \frac{\beta_{\text{lever}}}{k_{\text{lever}}}$$

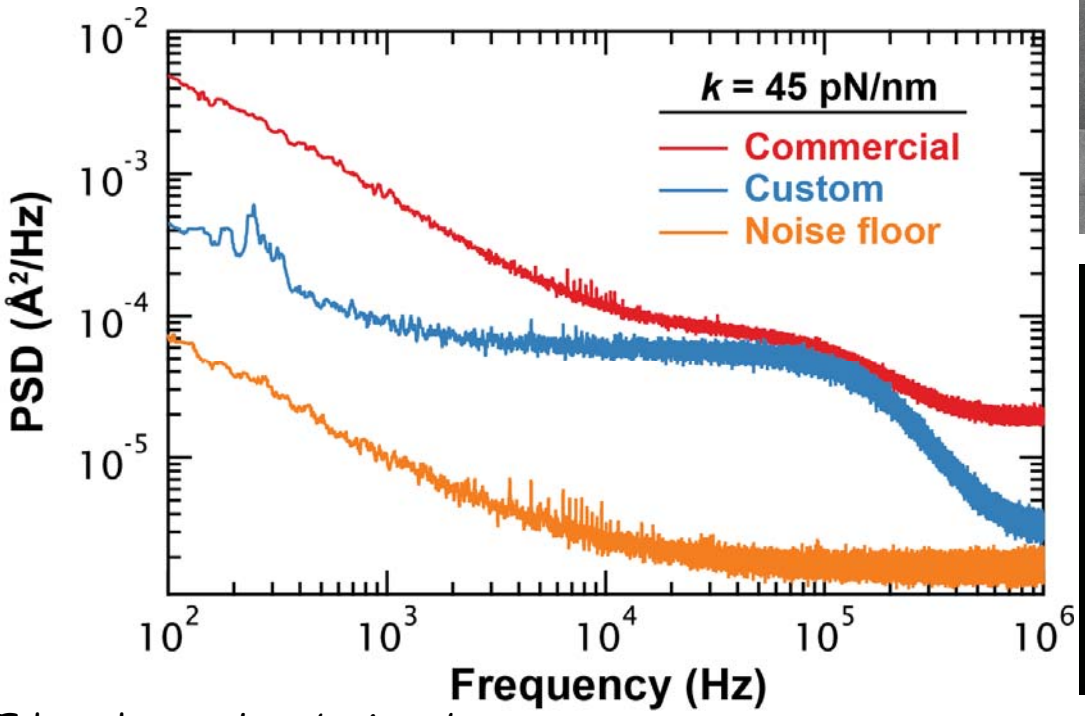


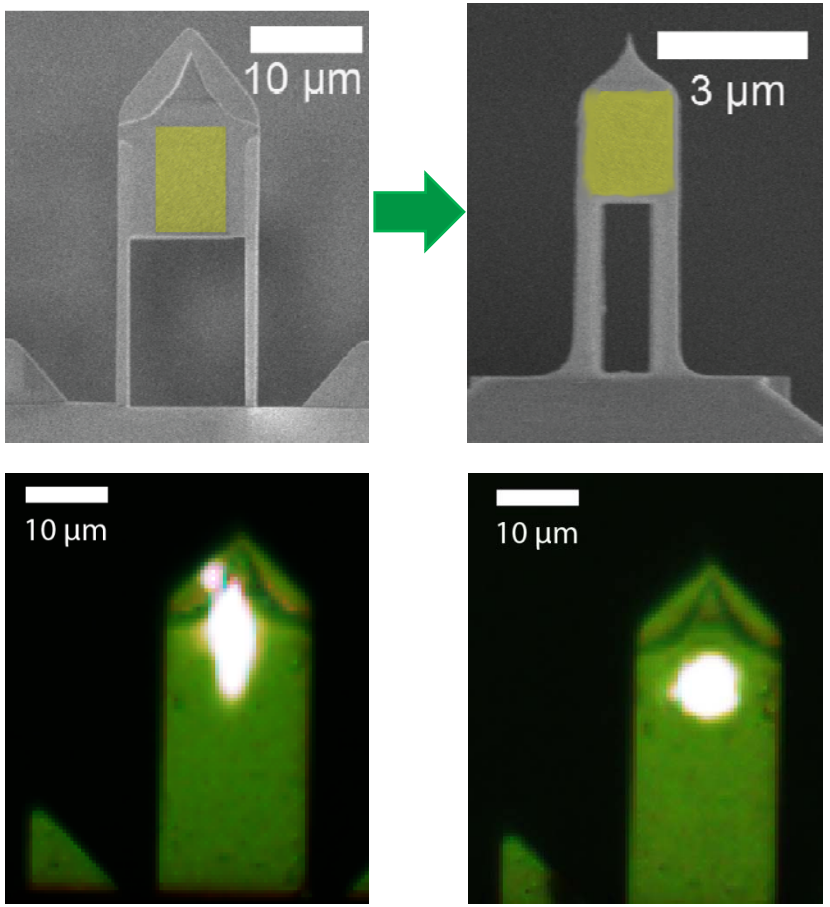


Bull, *et al.*, *ACS Nano* (2014)

Next step: modifying and detecting ultrashort cantilevers

- **Established an efficient fabrication process**
  - Compensated for significant bending
- **Develop new detection laser**
  - Very small spot size (3 μm)
    - Retrofitted into commercial AFM

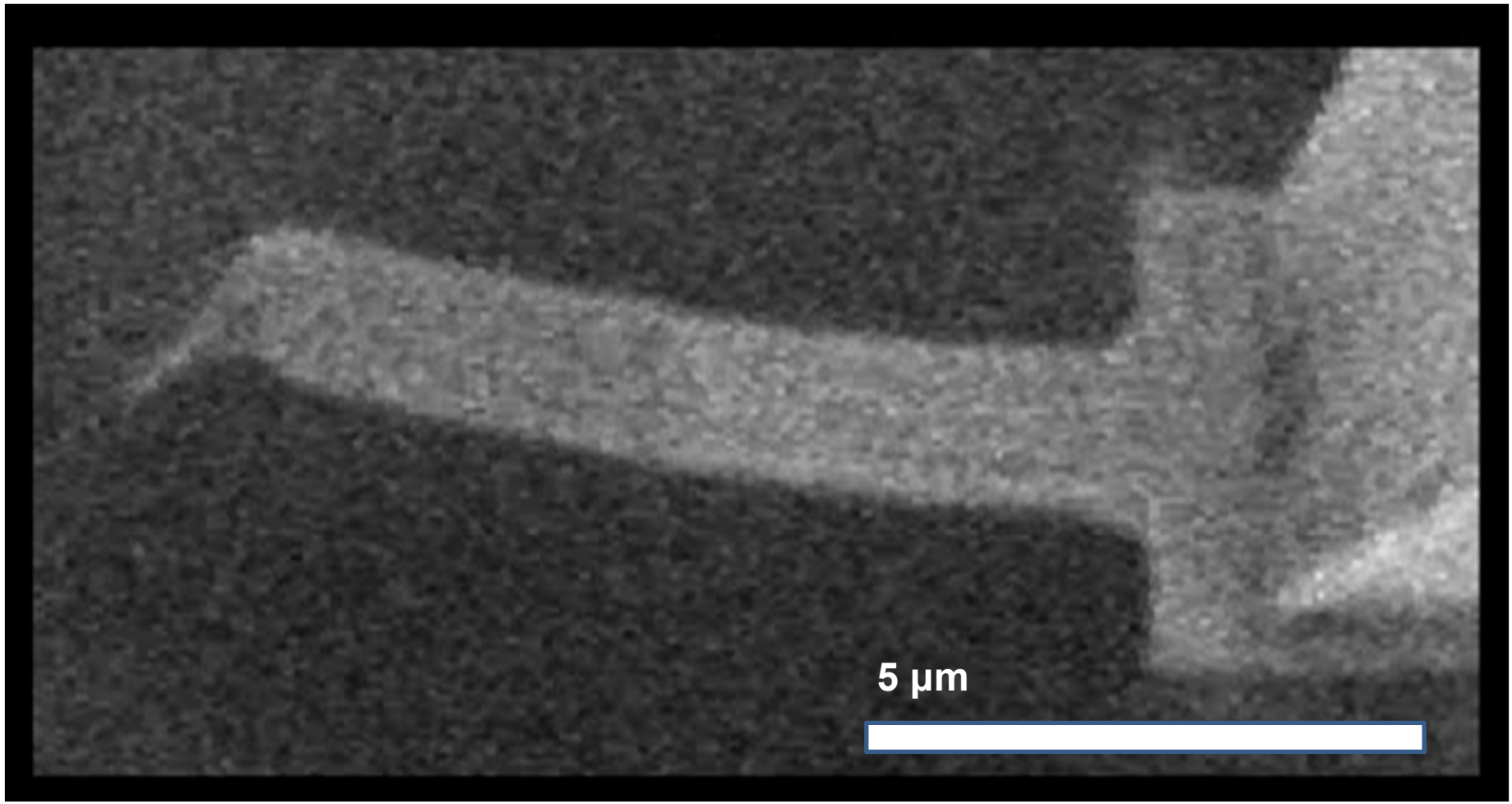




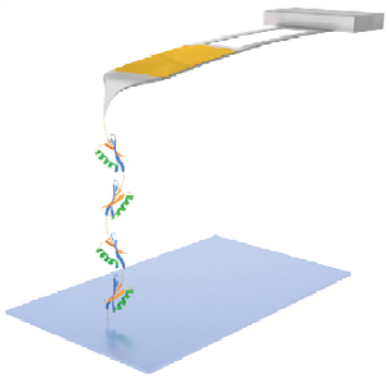
Edwards, *et al.*, *submitted*

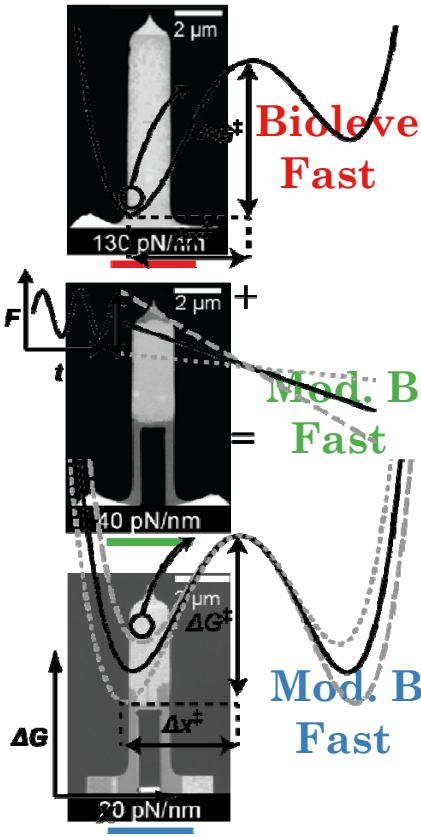
Modification routinely done by skilled undergrads

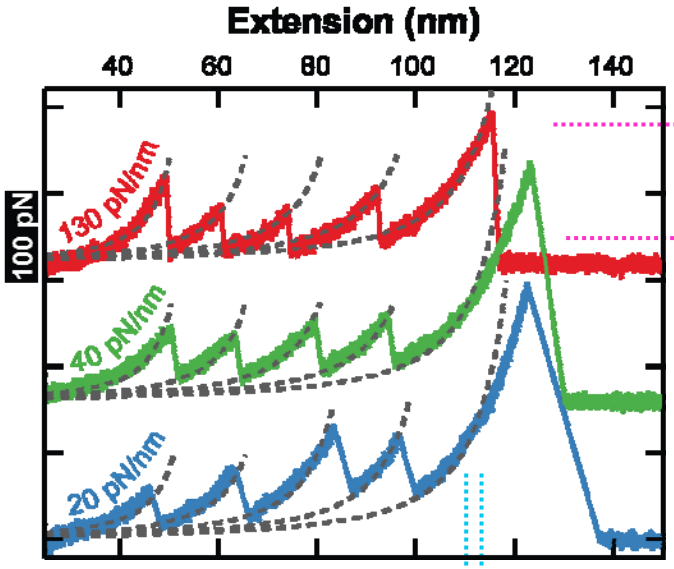
- **Real-time imaging improves yield**
  - Thinning bends cantilevers in opposite direction
- **Rate: 2-4/hr**
  - Limit: handling

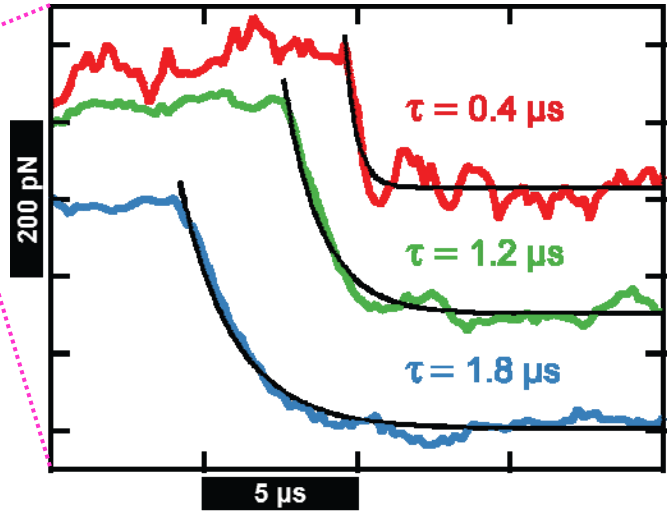


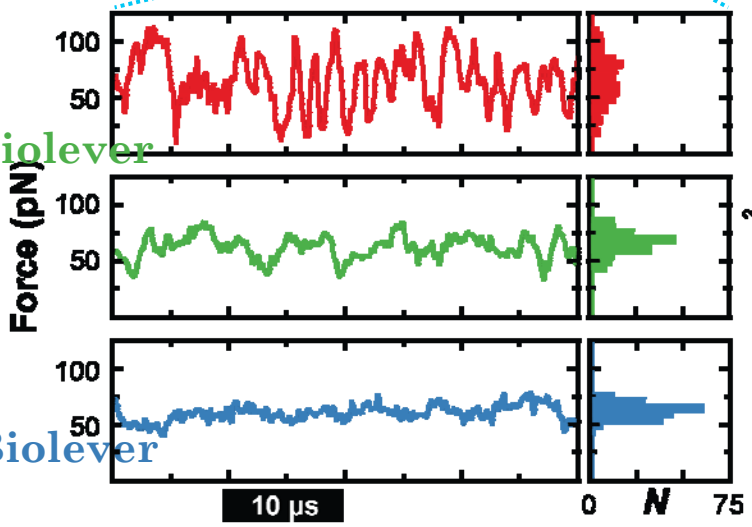
Probing protein unfolding with 1-μs resolution

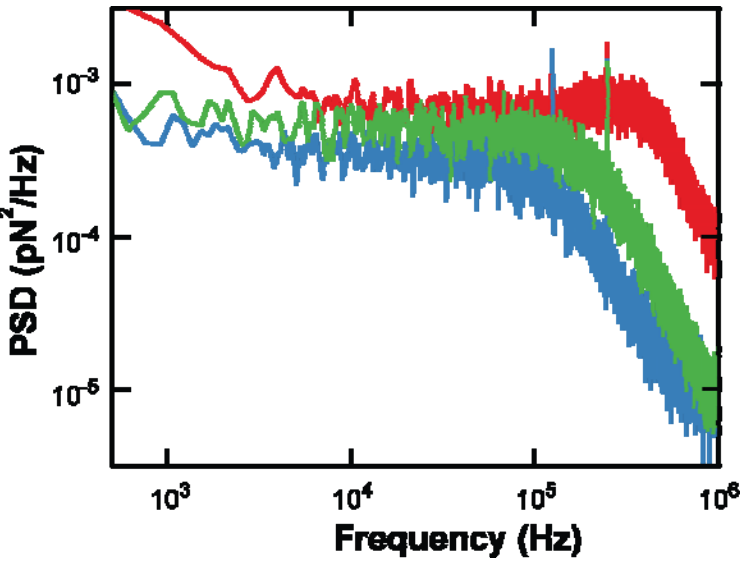




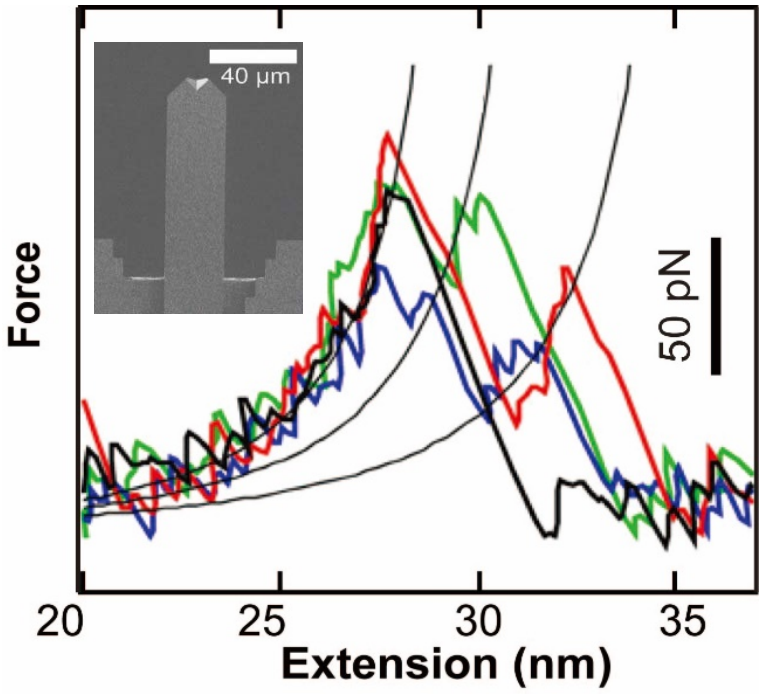




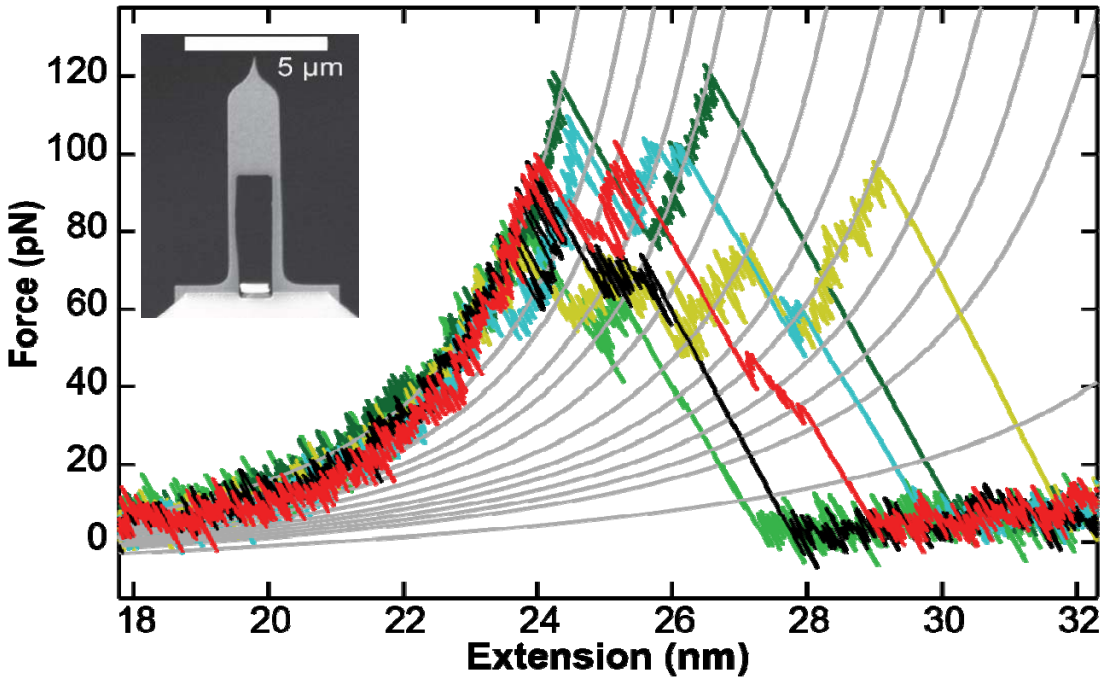




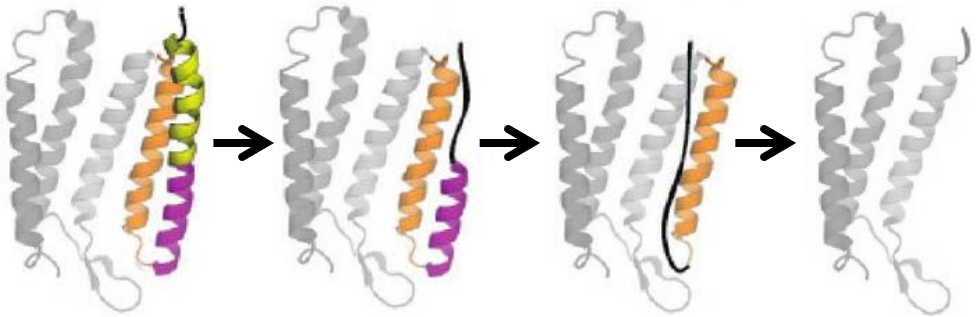
Putting it all together:
Plethora of new folding intermediates revealed in bR



(Zocher, *ACS Nano*, 2012)

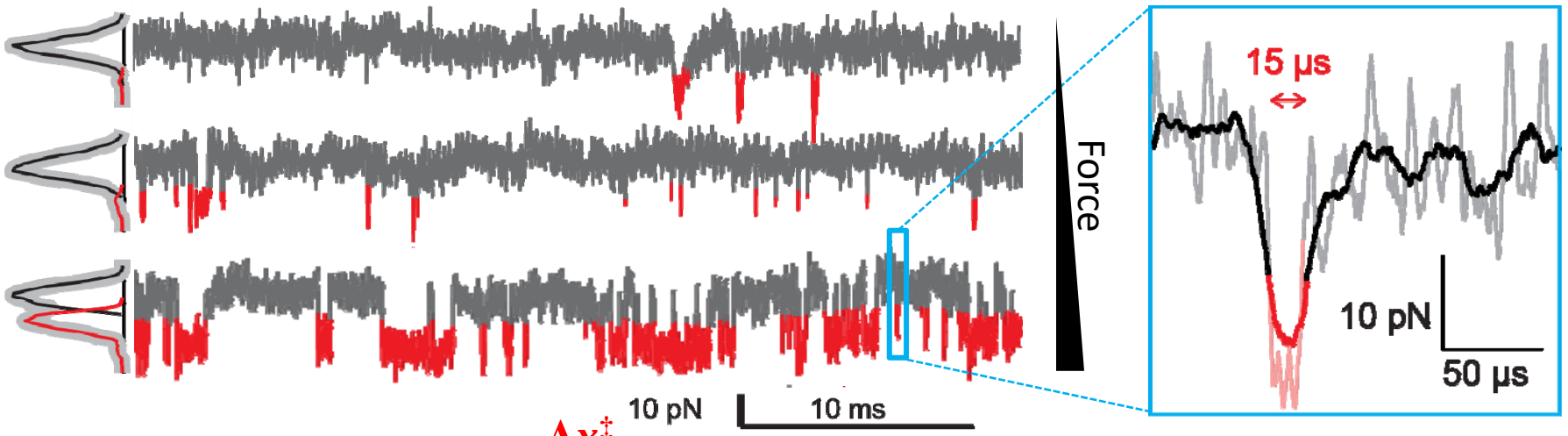


(Perkins lab, unpublished)



- Underlying instrumental advances:
  - Time resolution improved:100-fold
  - Force precision improved: 20-fold
  - Force stability improved: 10-fold

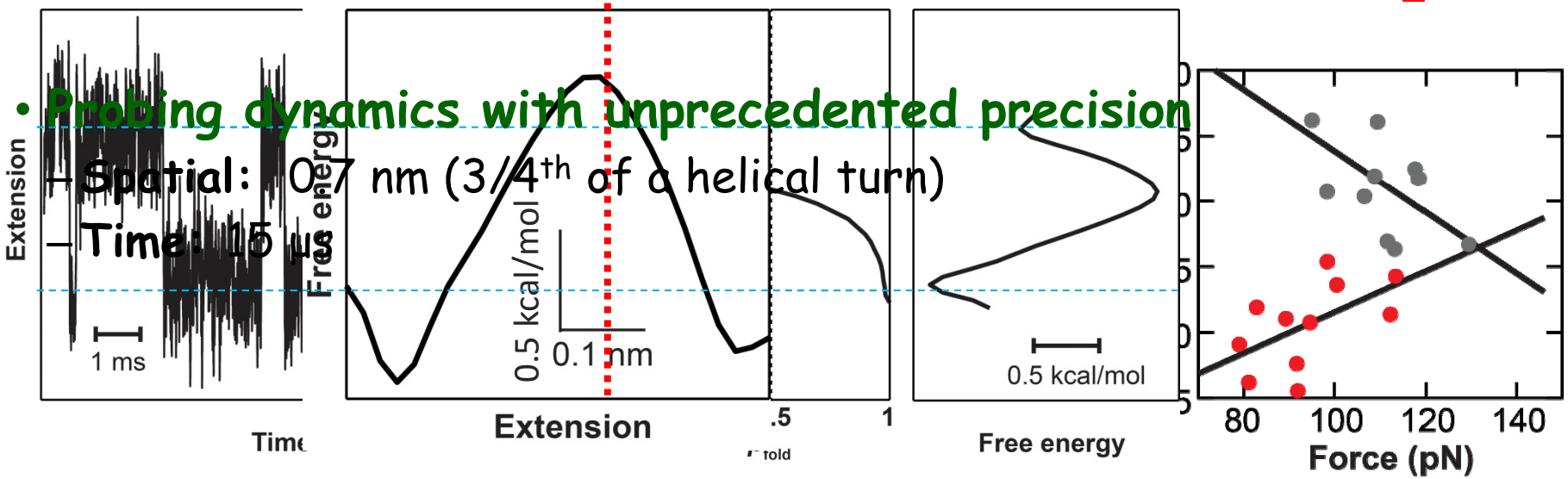
A new regime for AFM: *equilibrium folding and unfolding*



$\Delta x^{\ddagger}$

$\Delta x^{\ddagger} = 0.15 \pm 0.08$ nm

- **Probing dynamics with unprecedented precision**
  - Spatial: 07 nm (3/4th of a helical turn)
  - Time: 15 μs



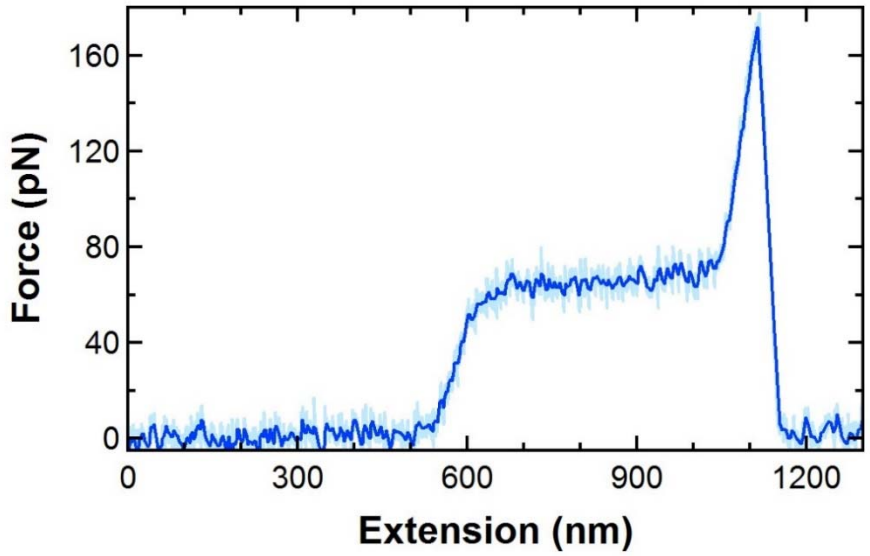
$$G(x) = \beta^{-1} ln\left(-D(x)\frac{dp_{fold}}{dx}\right)$$ Chodera & Pande, *PRL*, 2011

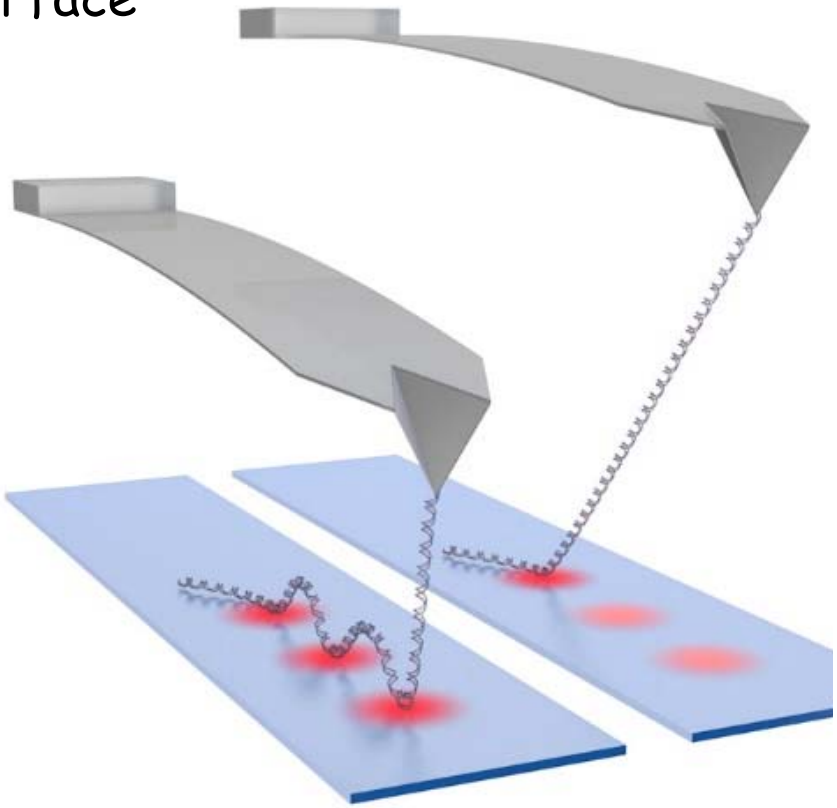
$$k \propto e^{-F\Delta x^{\ddagger}/k_B T}$$

High-quality, high-throughput force spectroscopy

- **Current limit: nonspecific attachment**
  - Adsorb biomolecules randomly onto surface
- **Solution: site-specific anchoring**
  - Pursued by many groups

Zimmermann, *et al.*, *Nature Protocols* (2010)
Taniguchi, *et al.*, *Langmuir* (2010)
Stahl, *et al.*, *PNAS* (2012)
Popa, *et al.*, *JACS* (2013)
…



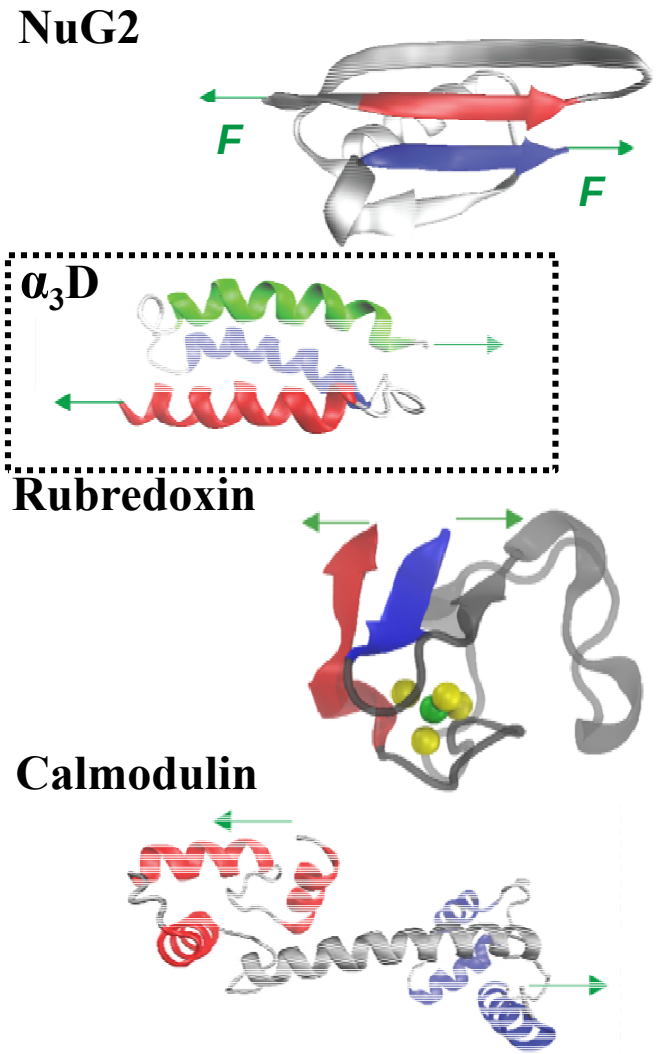


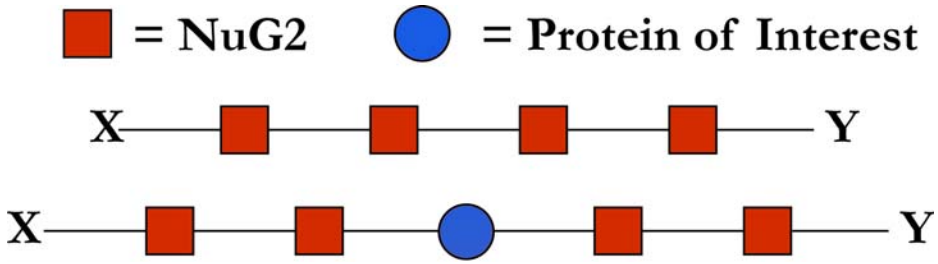
- **Dramatically increased throughput**
  - Non-specific: 5 HQ traces/month
  - Specific: 5 HQ traces/min

Walder, *et al.*, in preparation

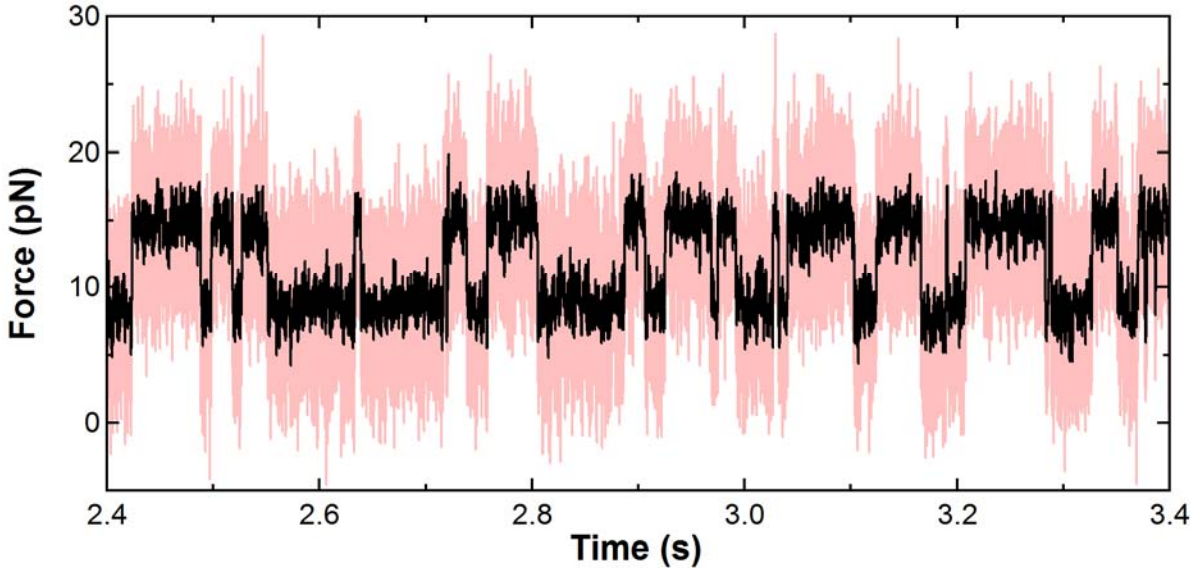
Accelerating studies of diverse proteins

- Modular construct
  - Covalent surface anchoring
  - Reversible tip attachment



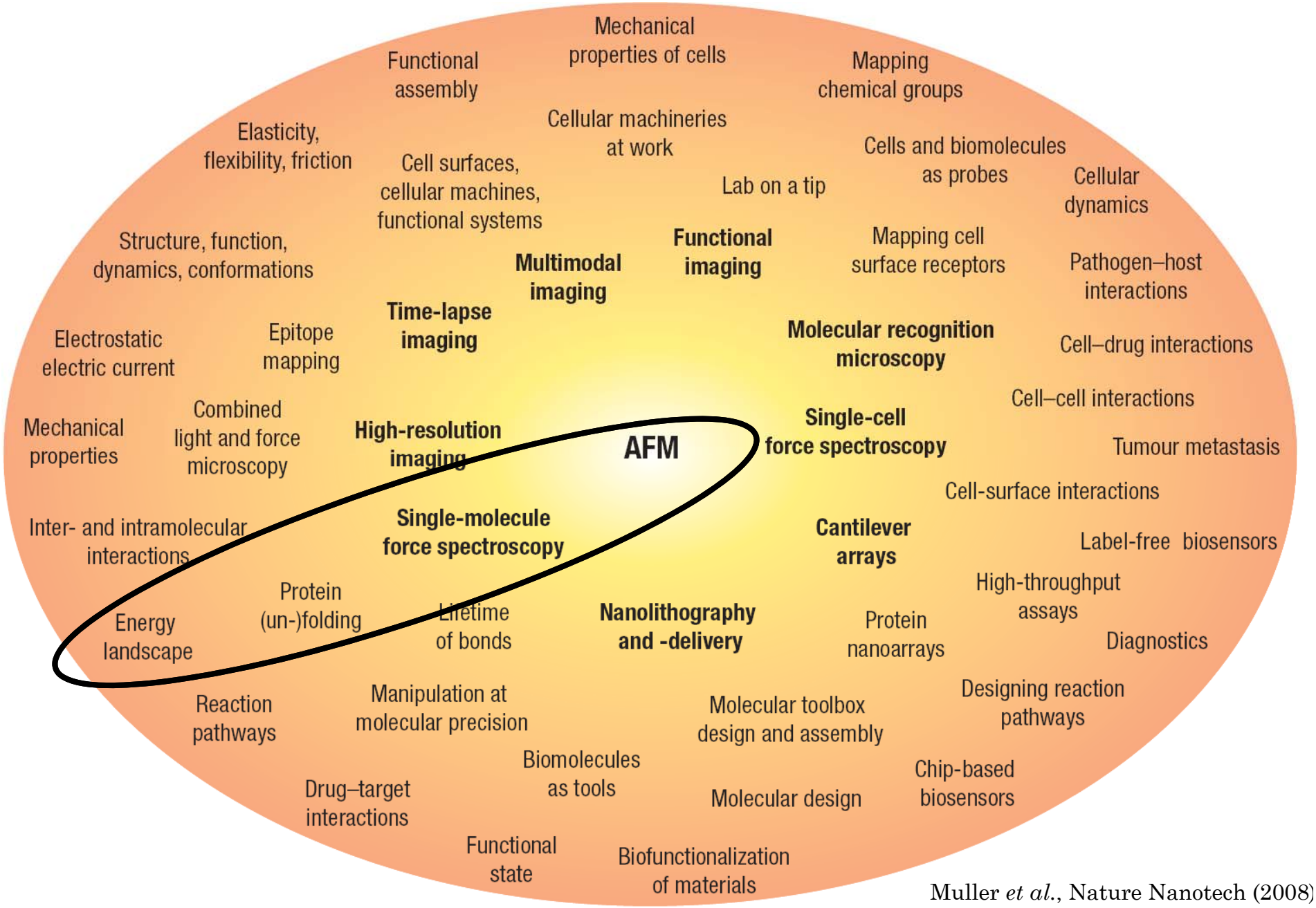


LeBlanc, *et al., in preparation*



- Unprecedented precision for AFM
- *Metrological advances still needed!*

Advances in metrology broadly enable bio-AFM



Muller *et al.*, Nature Nanotech (2008)

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Marc-Andre LeBlanc

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