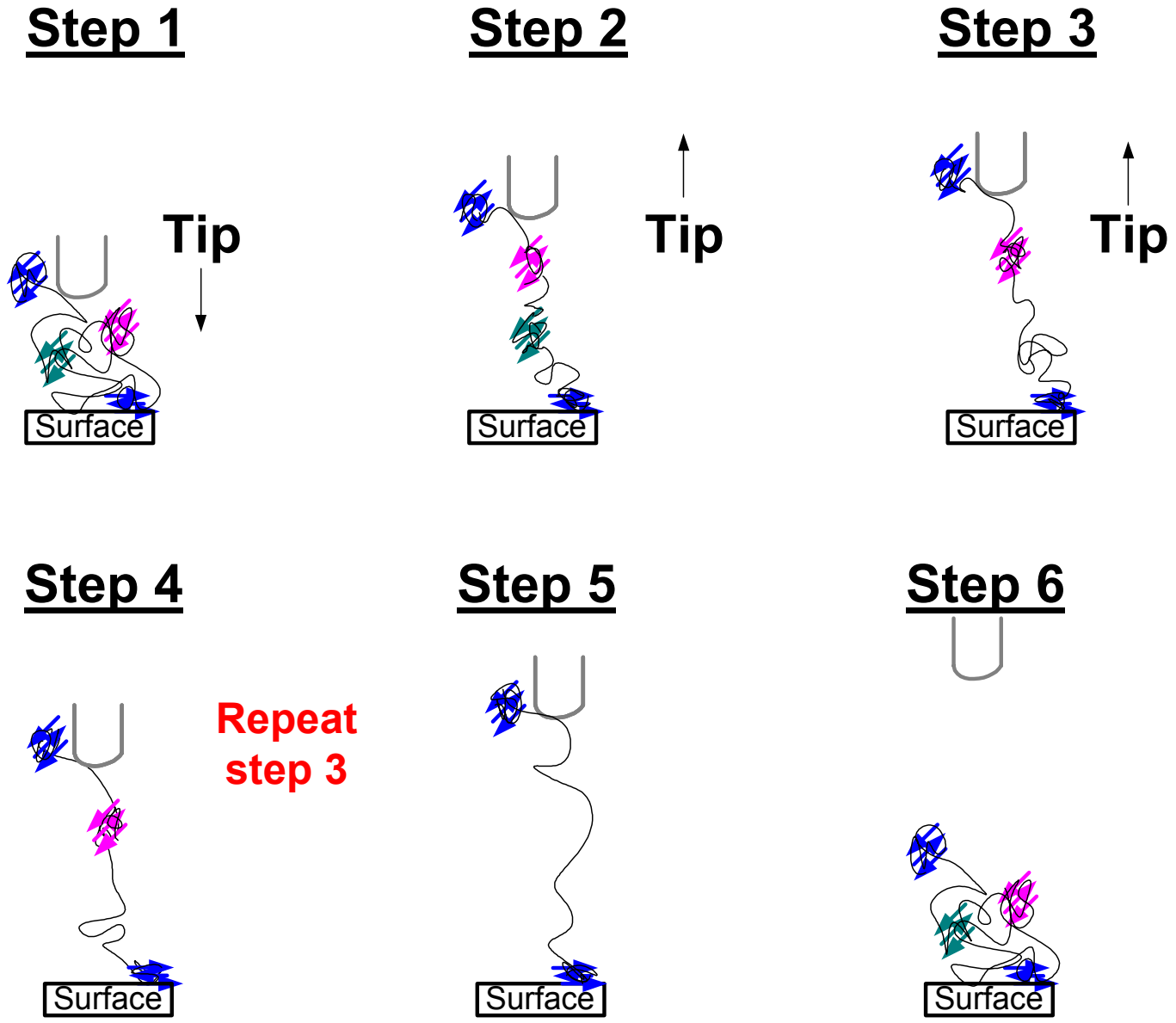
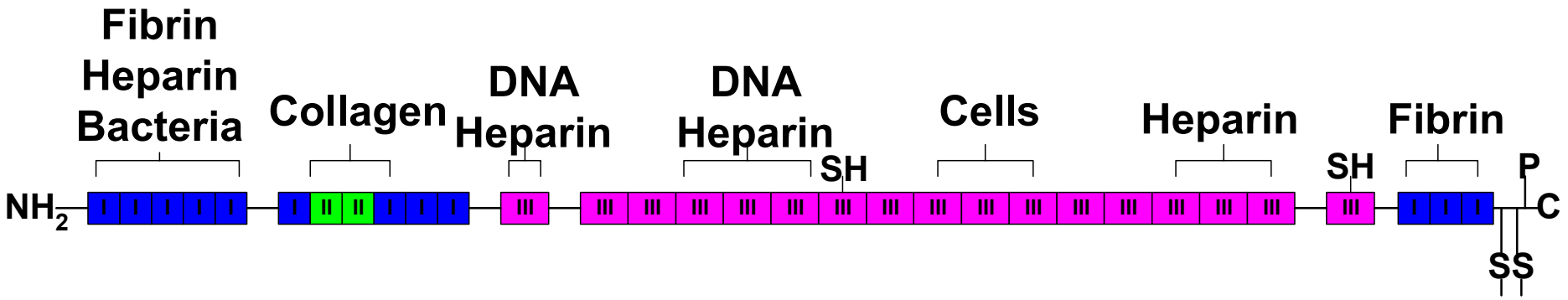
Unfolding Fibronectin Domains at Surfaces using Single Molecule Force Spectroscopy

Pamela Meadows
Jason Bemis
Gilbert Walker

Protein Stretching and Unfolding via AFM
Unfolding of each pearl in the string of pearls



Characteristics of Fibronectin

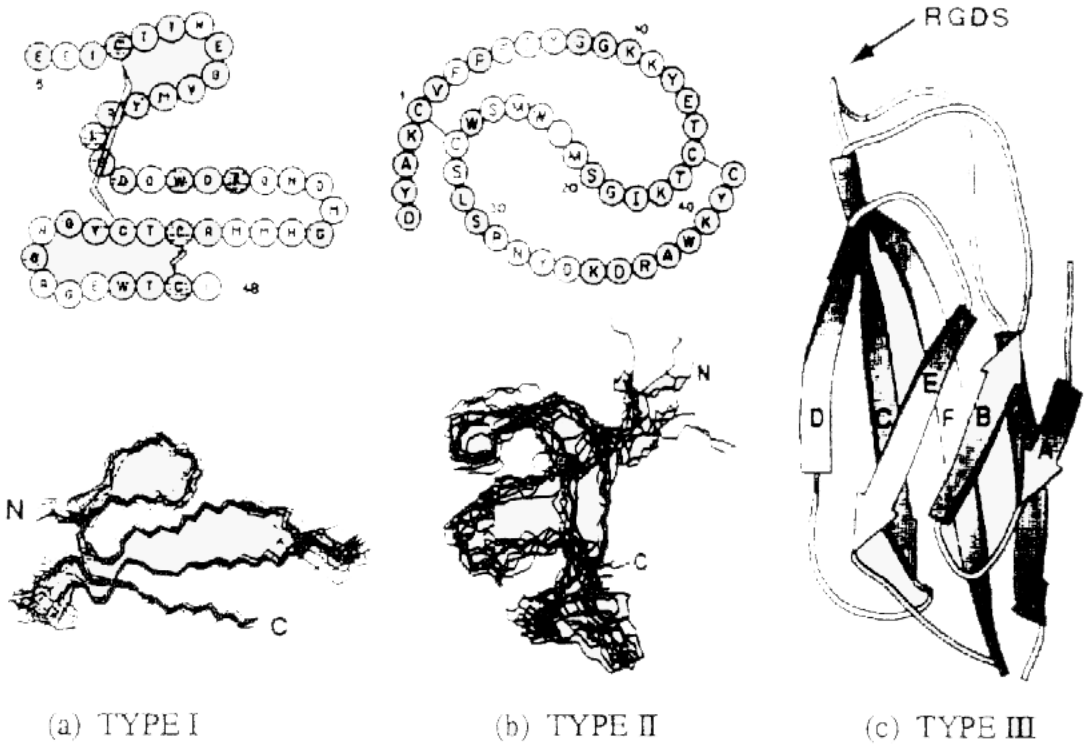


- Adhesion promoting protein
- 140 nm in length and 2nm in diameter
- Dimer linked via disulfide bond (monomer shown above)

Domains	*Type I*	*Type II*	*Type III*
# in FN	12	2	17
Avg # of res.	45	60	90
Max. length	16nm	22nm	33nm

What unfolding events do we see with AFM?

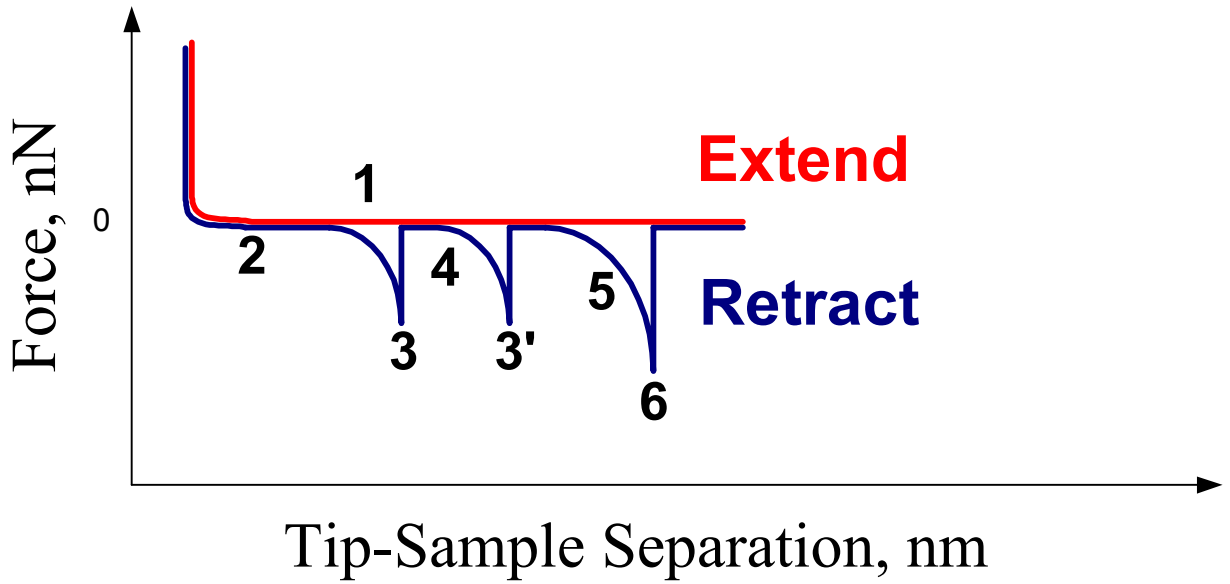
The homologous type I, II and III modules are symbolized by, ▒, ▒, ■, respectively.



Fibronectin, Ed, D.F. Mosher (San Diego, Academic Press 1989)

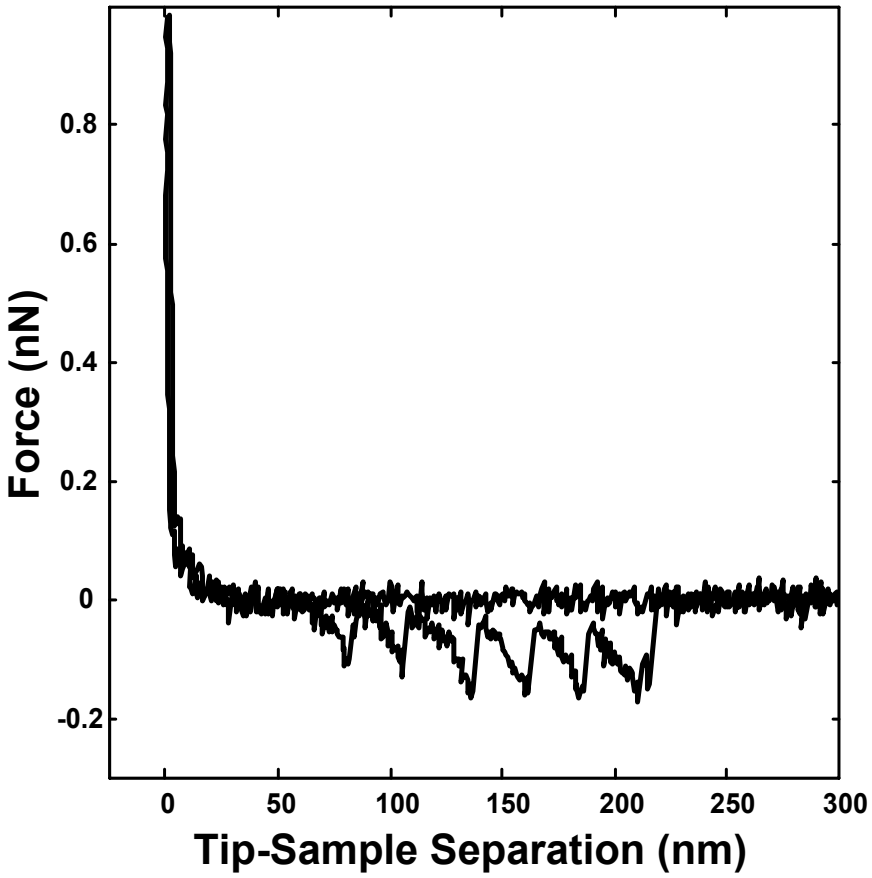
Type III domains lack disulfide bonds and therefore unfold with our loading rates.

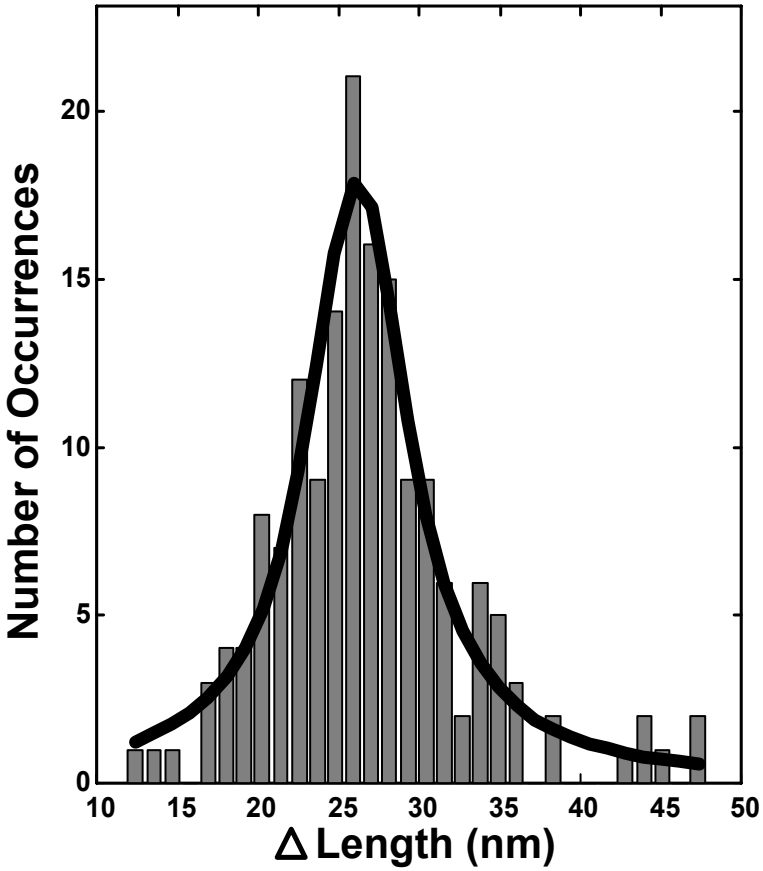
Force Plot of a Multidomain Protein Unfolding



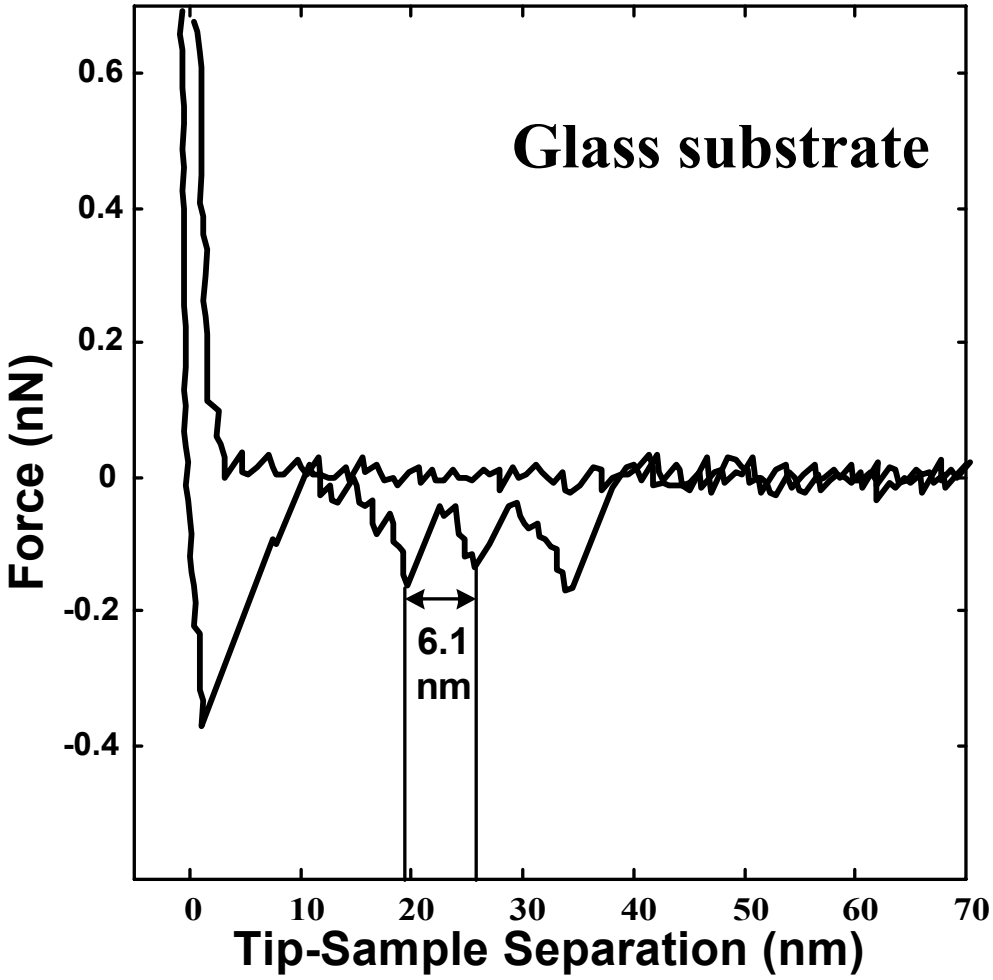
3 and 3' represent domain unfolding while 6 represents protein-tip rupture

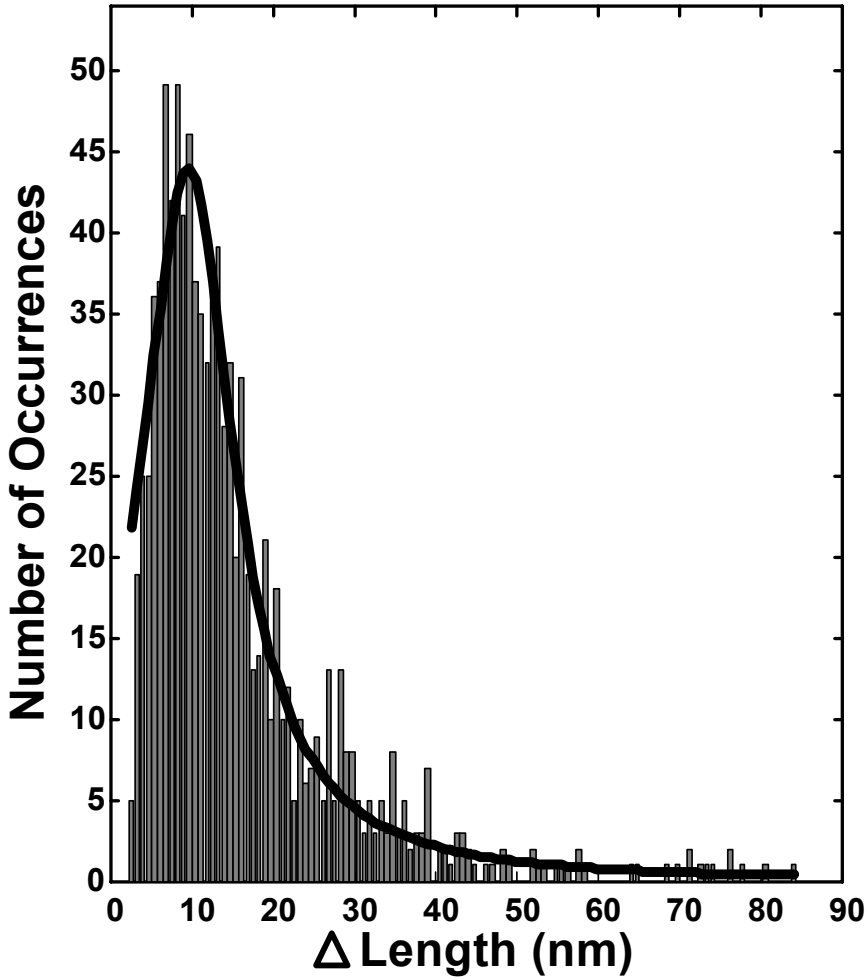
Aggregated proteins unfold "properly"



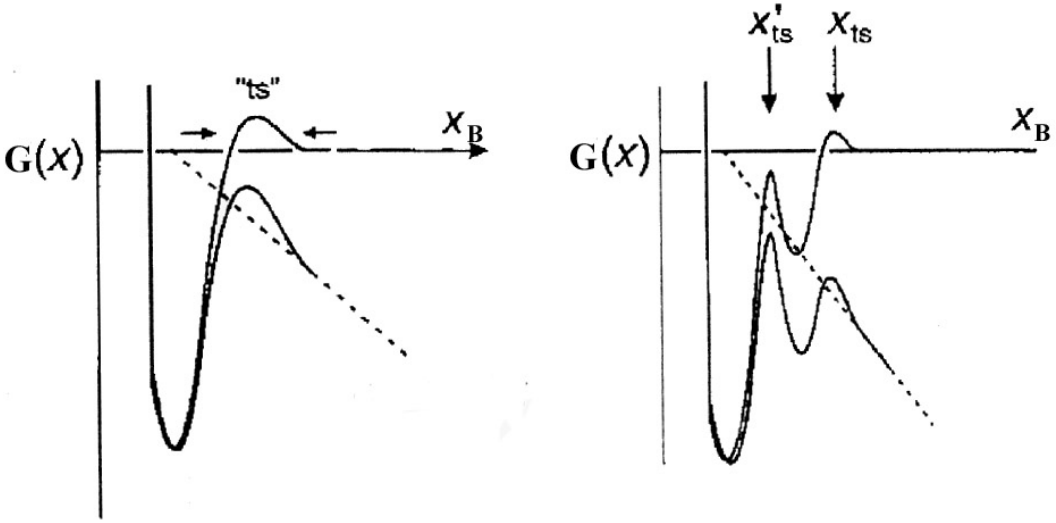


Isolated Proteins can be denatured on surfaces





Force Spectroscopy Reveals Barriers to Unfolding



After Evans, E. *Faraday Disc*. **1998,** 111, 1-26.

$$\Delta G^{\ddagger}(F) = \Delta G^{\ddagger} - Fx_{ts}$$

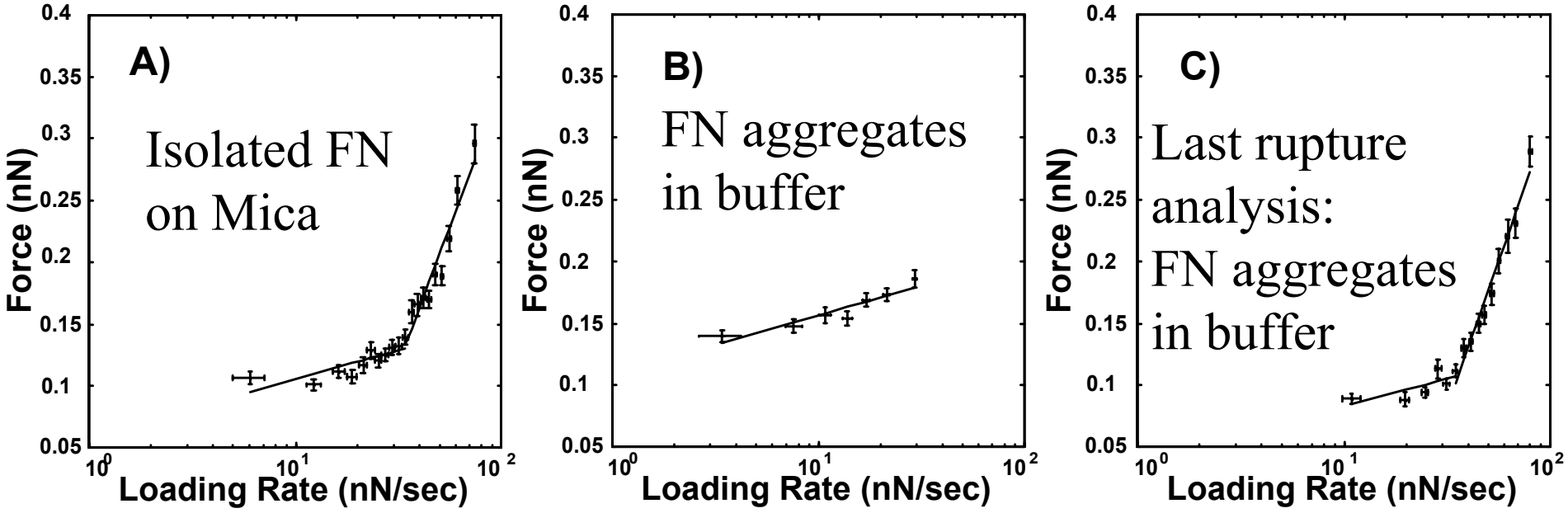
$$\Delta G^{\ddagger} = -k_B T \ln\left(\frac{k_{off} h}{k_B T}\right)$$

$$\text{Slope} = \frac{k_B T}{x_{TS}}$$

- Unfolding is an activated process.
- With forced unfolding, inner barriers can be observed.

From the slope and y-intercept of a loading rate vs. rupture force plot, x_{ts} and k_{off} can be determined.

Rupture force vs. loading rate



Two sloped regions implies two barriers; first, unfolding, second, pulling away a domain partially denatured at the surface

These data also give us thermodynamic parameters for unfolding (~20kcal/mol), and describe the volume over which the stress is distributed during rupture of the protein from the surface