Rates and transition paths from force spectroscopy experiments

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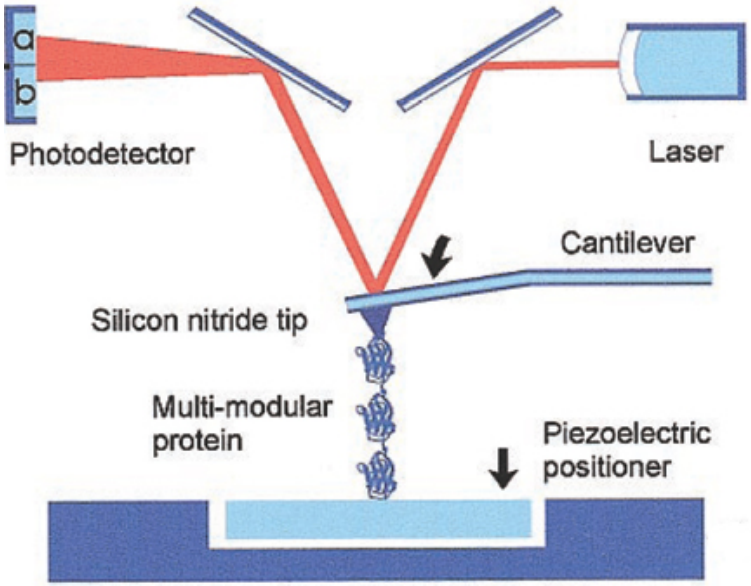
Outline

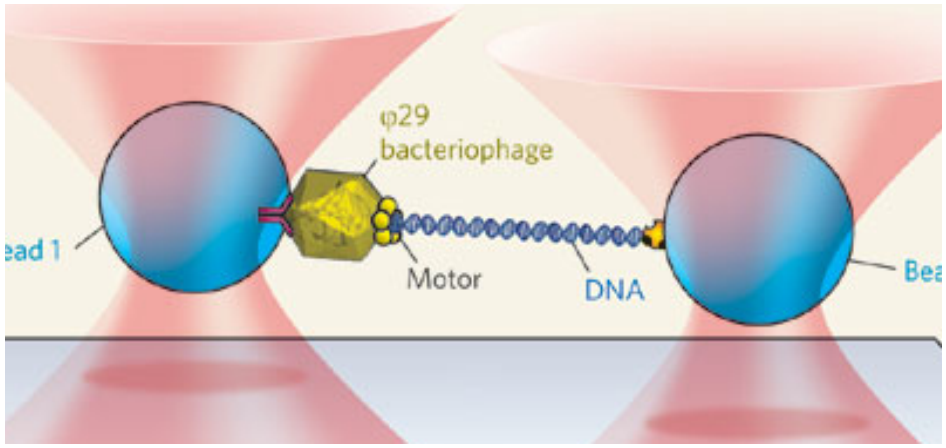
* Atomic force spectroscopy
* Extracting information from the experiments
* Artifacts of the apparatus
* Conclusions

Motivation

Single-molecule force spectroscopy



Atomic Force Microscopy (AFM)



Optical Tweezers

Single-molecule

Dr. Gerhard Hummer

Dr. Attila Szabo

mpibp
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für biophysik

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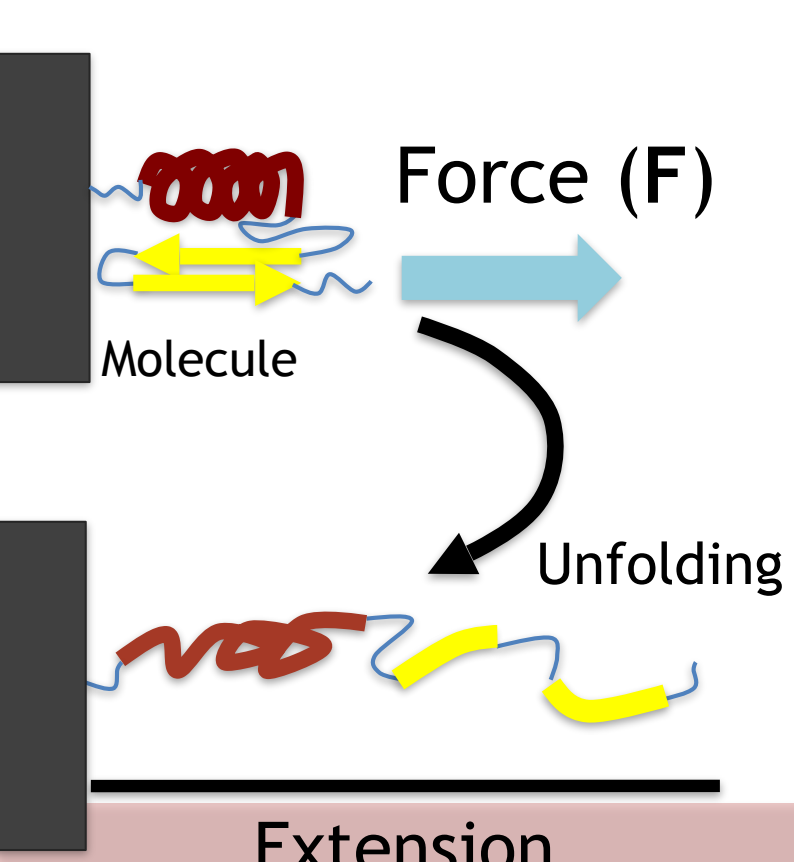
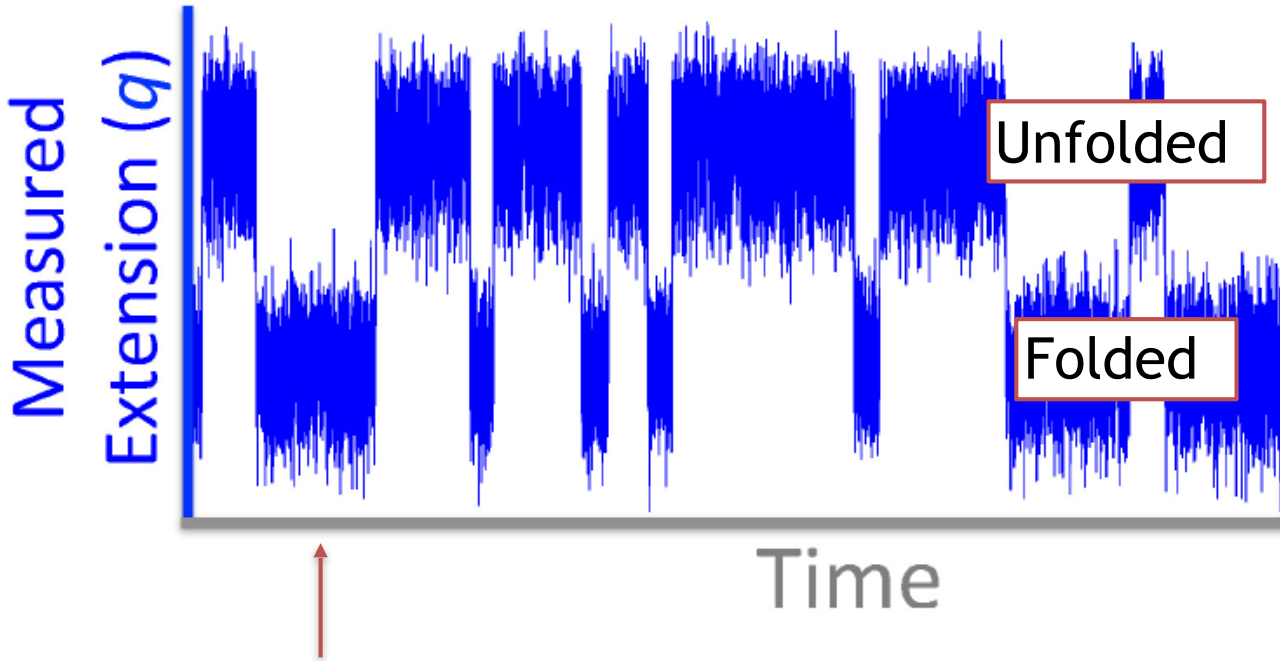
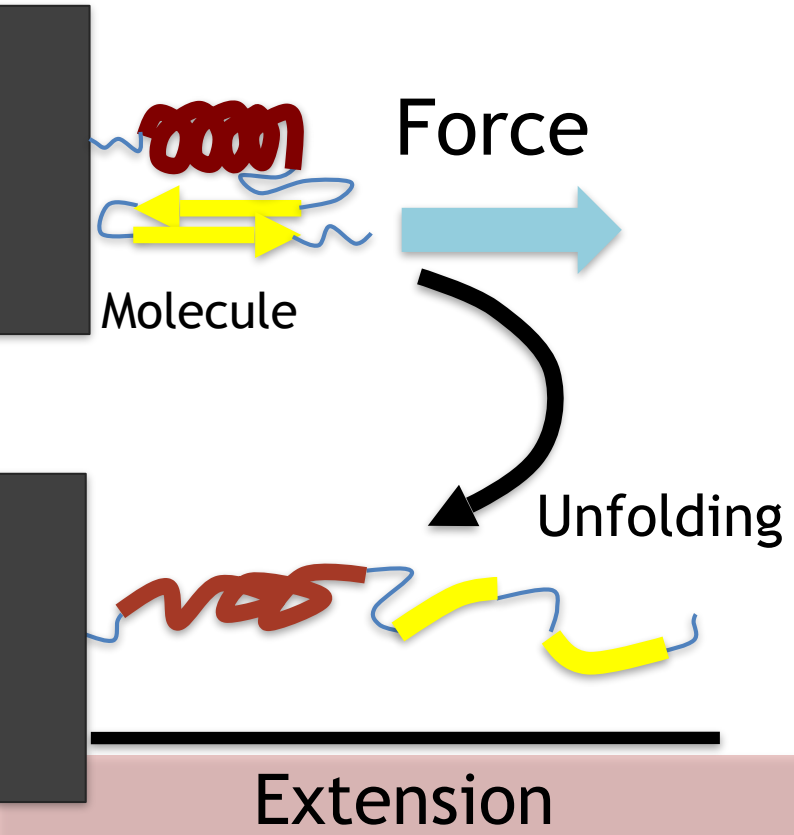




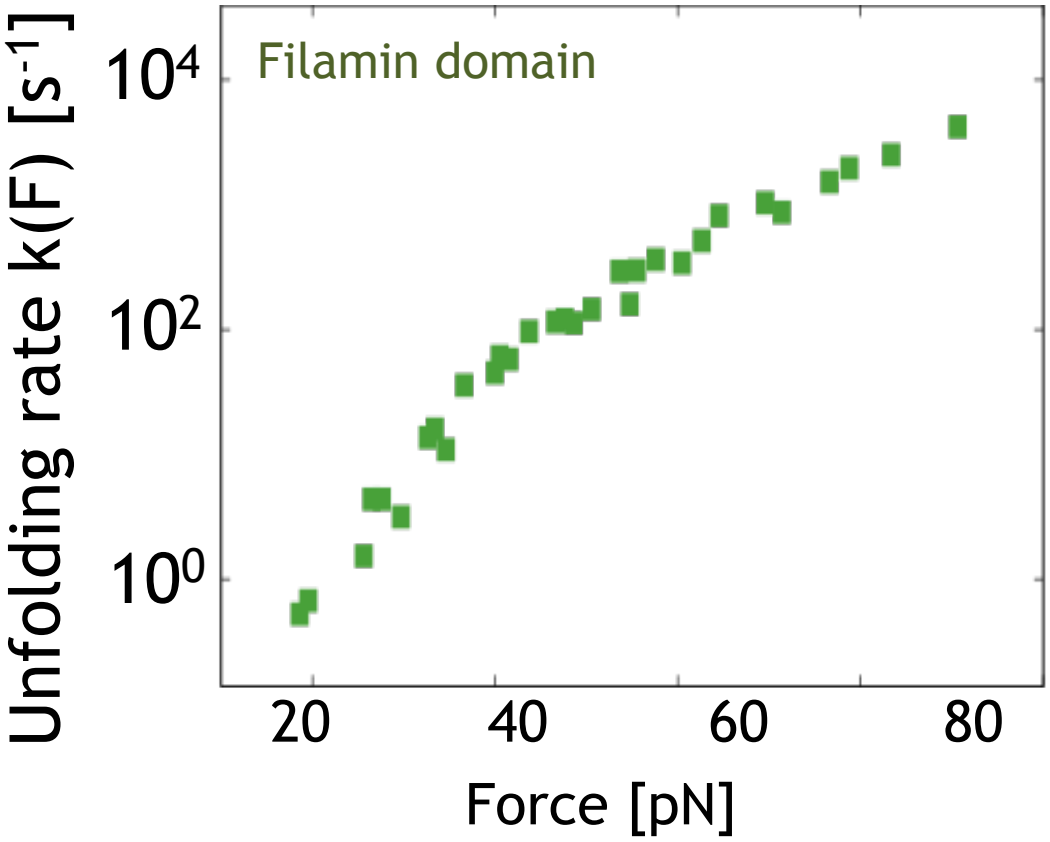
Inverse of mean residence time
= **Unfolding rate**

$k(F)$

Single-molecule



Force-dependent unfolding rate k(F) as a function of force:

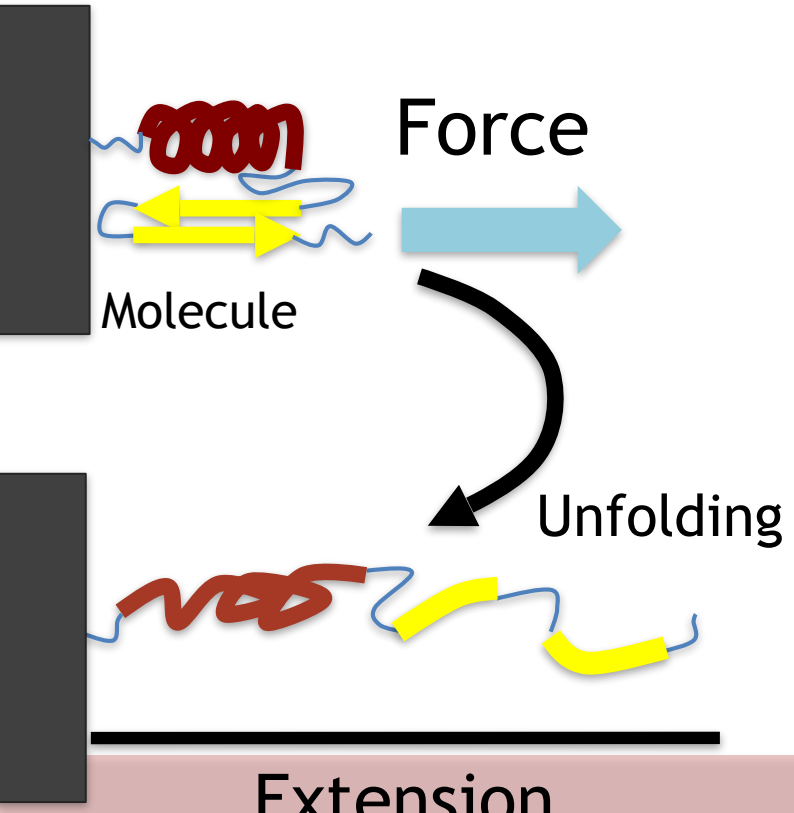


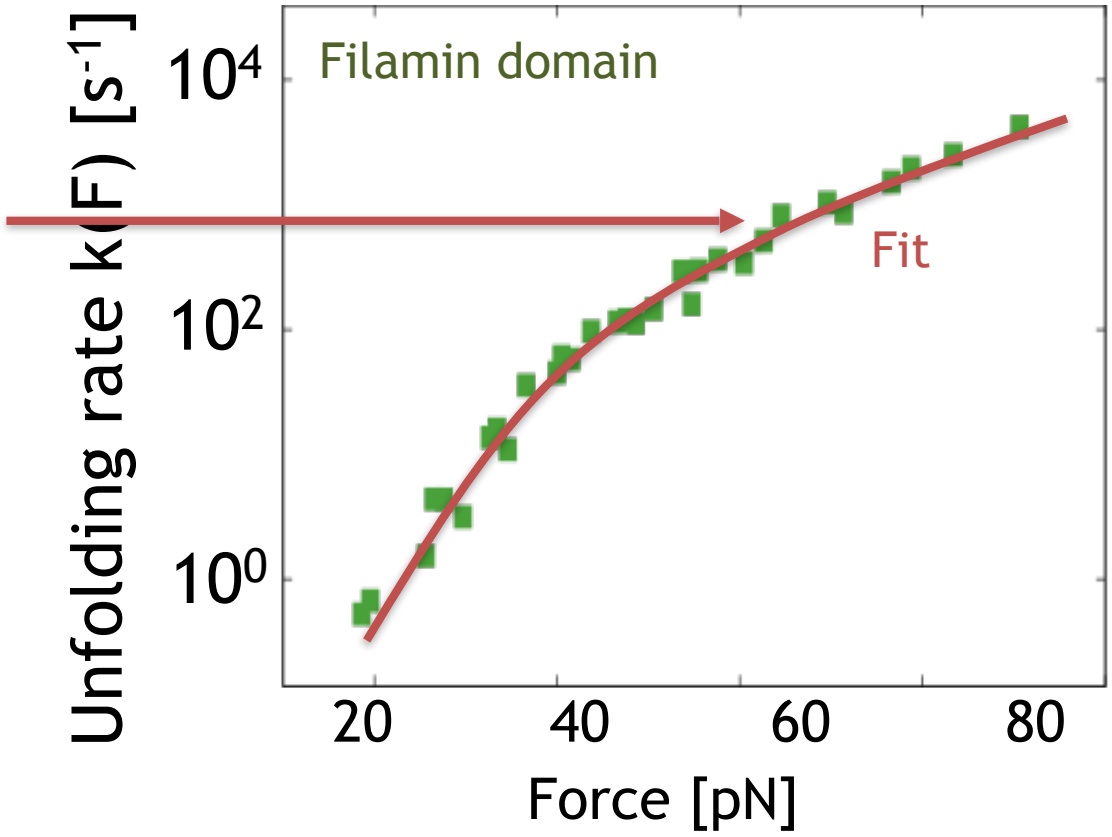
What can we learn from the data? **Information at F=0?**

Extracting information from the experimental traces

Single-molecule

Force-dependent unfolding rate k(F) as a function of force:

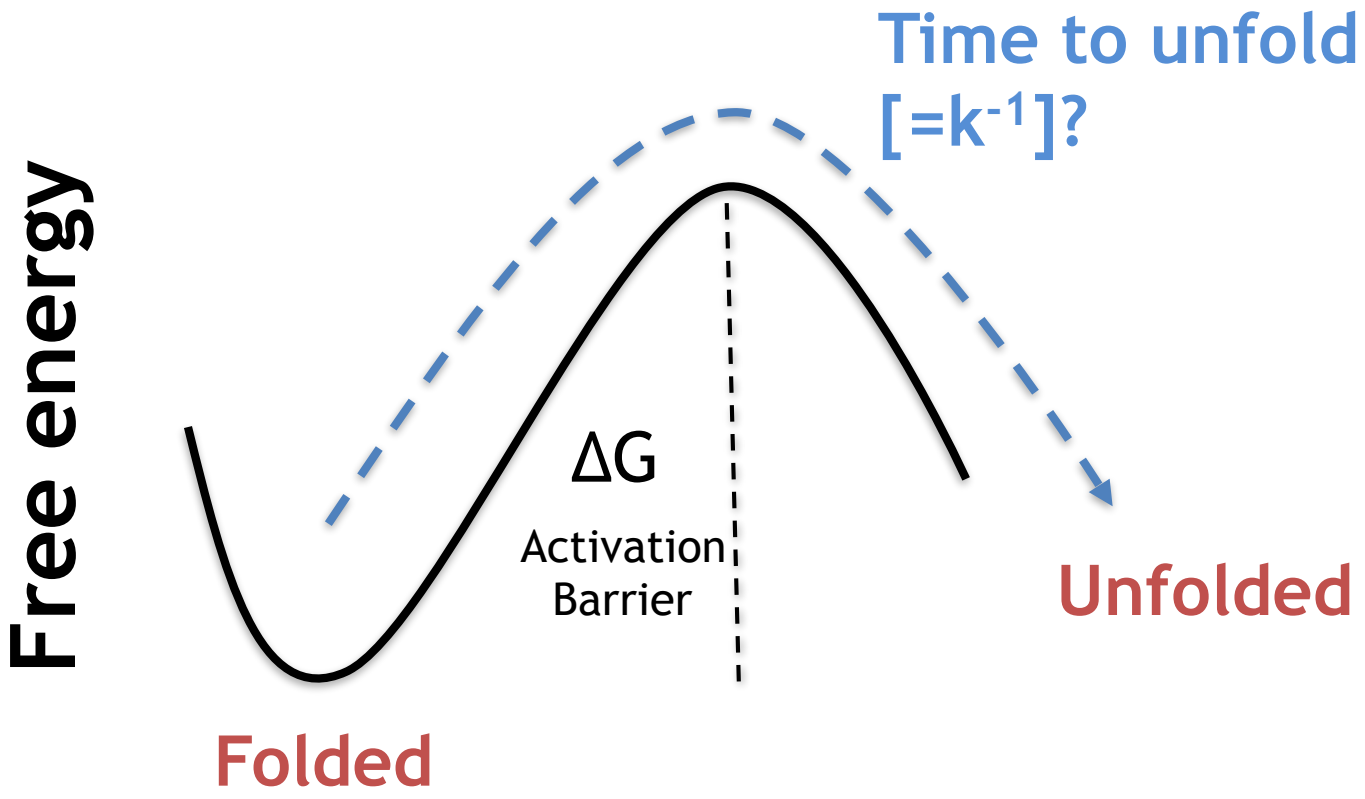




What can we learn from the data? **Information at F=0?**

The unfolding process is modelled in 1D using **Kramers Theory:** Single-well potential

Single-molecule

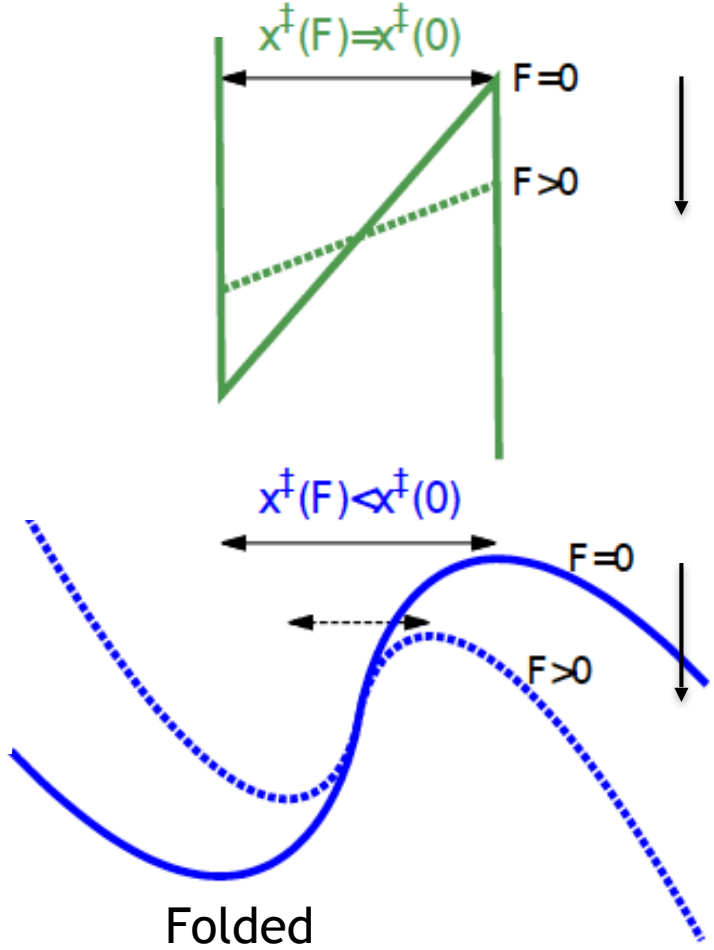


$$k_0^{-1} = \frac{1}{D}\int_{\ddagger} e^{\beta G(x)}\, dx \int_{\text{well}} e^{-\beta G(x)}\, dx$$

Effect of force on a general class of 1D free energy surfaces:

Single-molecule

$$G(x)=G_0(x)-Fx$$



Force lowers the activation barrier

Energy surfaces that allows us to calculate k(F), and the probability distribution of rupture forces p(F) analytically

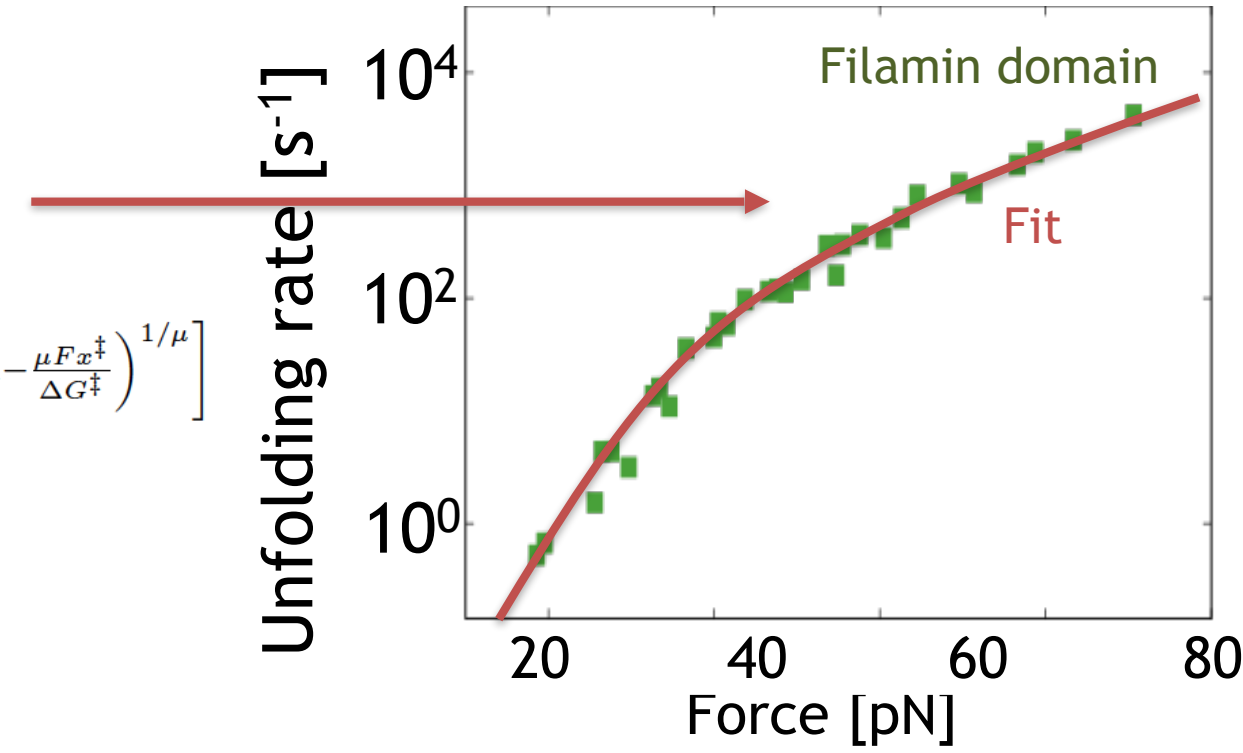
Cossio, Hummer, Szabo. ***Biophysical Journal*** *111, 832-840* (2016).

Single-molecule

Kramers theory over free Energy surfaces:

Analytical expression of **unfolding rate**

$$k(F) = k_0 \left(1 - \frac{\mu F x^{\ddagger}}{\Delta G^{\ddagger}}\right)^{2-\frac{1}{\mu}} e^{\beta \Delta G^{\ddagger}\left[1-\left(1-\frac{\mu F x^{\ddagger}}{\Delta G^{\ddagger}}\right)^{1/\mu}\right]}$$



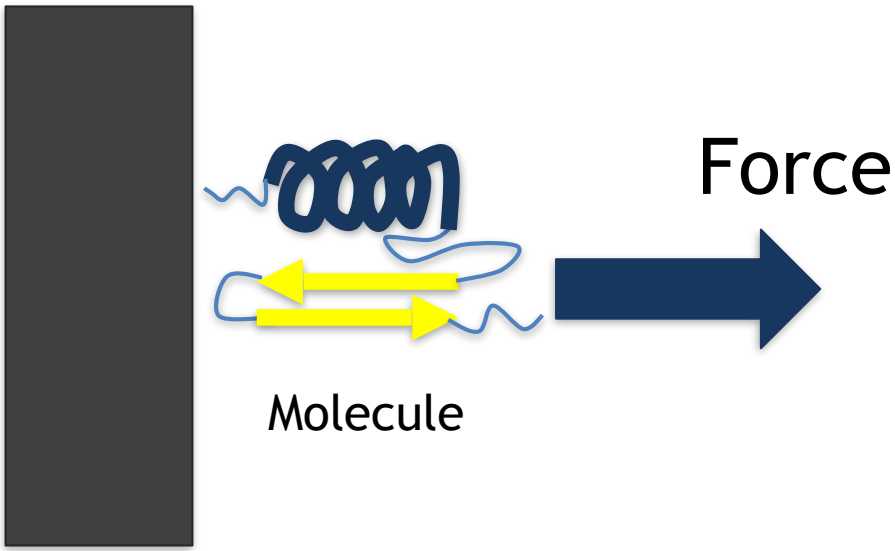
Extract information of the intrinsic (F=0) free energy and dynamics

Artifacts of the apparatus

However, there are debates in the field of atomic force spectroscopy…

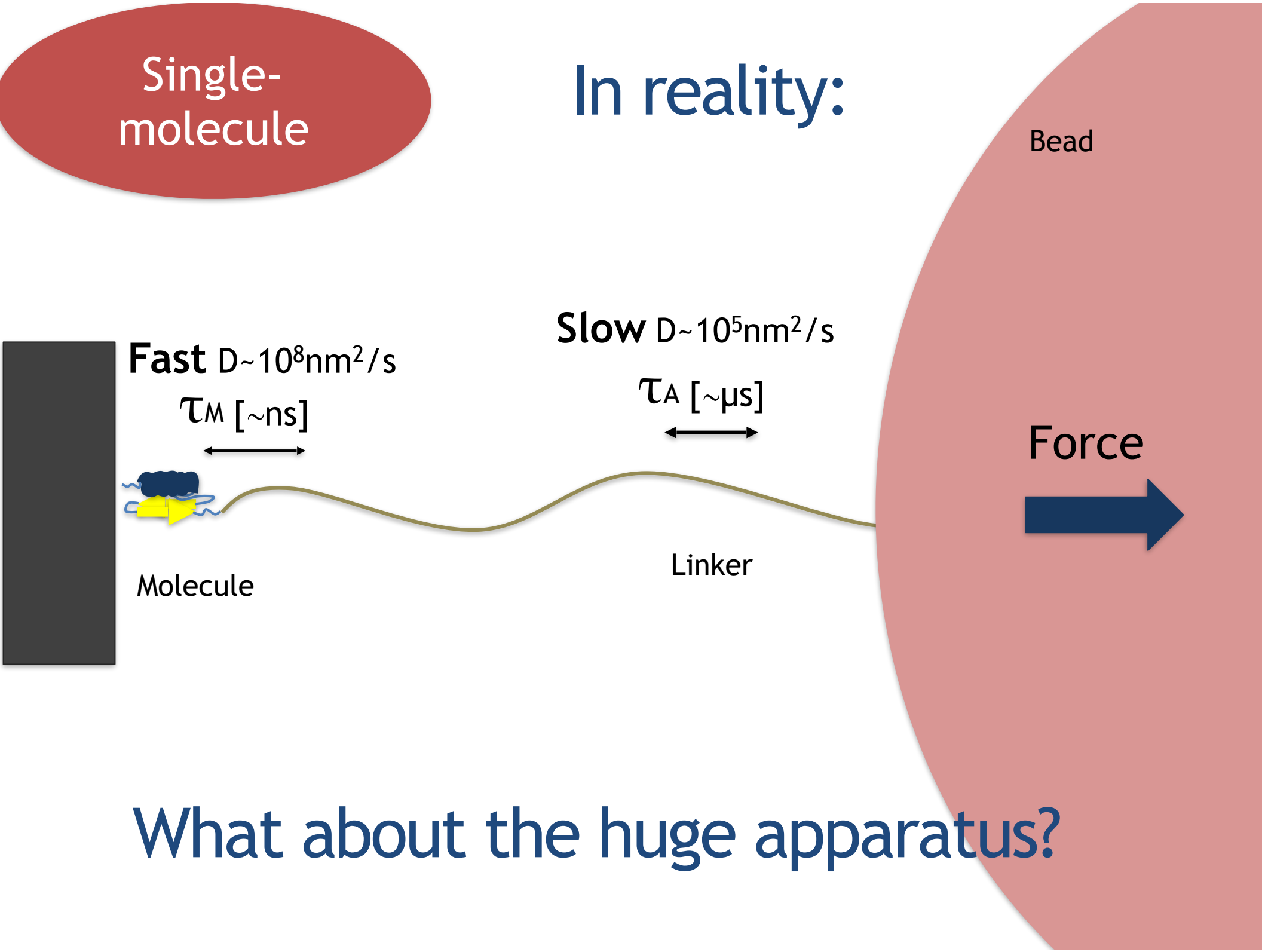
Single-molecule

Ideal case:



Single-molecule

In reality:



What about the huge apparatus?

Single-molecule

What are the artifacts of the apparatus on the measured rate of unfolding?

Effects of apparatus:

Hyeon C, Thirumalai D (2008) Multiple probes are required to explore and control the rugged energy landscape of RNA hairpins. J. Am. Chem. Soc. 130(5):1538–1539.

Hyeon C, Morrison G, Thirumalai D (2008) Force-dependent hopping rates of RNA hairpins can be estimated from accurate measurement of the folding landscapes. Proc. Natl. Acad. Sci. U.S.A. 105(28):9604–9609.

Hyeon C, Morrison G, Pincus DL, Thirumalai D (2009) Refolding dynamics of stretched biopolymers upon force quench. Proc. Natl. Acad. Sci. U.S.A. 106(48):20288–20293.

West DK, Paci E, Olmsted PD (2006) Internal protein dynamics shifts the distance to the mechanical transition state. Phys. Rev. E 74(6):061912.

Dudko OK, Hummer G, Szabo A (2008) Theory analysis and interpretation of single-molecule force spectroscopy experiments. Proc. Natl. Acad. Sci. U.S.A. 105(41):15755–15760.

Hummer G, Szabo A (2010) Free energy profiles from single-molecule pulling experiments. Proc. Natl. Acad. Sci. U.S.A. 107(50):21441–21446.

Maitra A, Arya G (2010) Model Accounting for the Effects of Pulling-Device Stiffness in the Analyses of Single-Molecule Force Measurements. Phys. Rev. Lett. 104(10):108301.

Maitra A, Arya G (2011) Influence of pulling handles and device stiffness in single-molecule force spectroscopy. Phys. Chem. Chem. Phys. 13(5):1836–1842.

Berkovich R, Garcia-Manyes S, Urbakh M, Klafter J, Fernandez JM (2010) Collapse Dynamics of Single Proteins Extended by Force. Biophys. J. 98(11):2692–2701.

Berkovich R, Garcia-Manyes S, Klafter J, Urbakh M, Fernandez JM (2010) Hopping around an entropic barrier created by force. Biochem. Biophys. Res. Commun. 403(1):133–137.

Hinczewski M, von Hansen Y, Netz RR (2010) Deconvolution of dynamic mechanical networks. Proc. Natl. Acad. Sci. U.S.A. 107(50):21493–21498.

Dudko OK, Graham TGW, Best RB (2011) Locating the Barrier for Folding of Single Molecules under an External Force. Phys. Rev. Lett. 107(20):208301.

Friddle RW, Noy A, De Yoreo JJ (2012) Interpreting the widespread nonlinear force spectra of intermolecular bonds. Proc. Natl. Acad. Sci. U.S.A. 109(34):13573–13578.

Berkovich R et al. (2012) Rate limit of protein elastic response is tether dependent. Proc. Natl. Acad. Sci. U.S.A. 109(36):14416–14421.

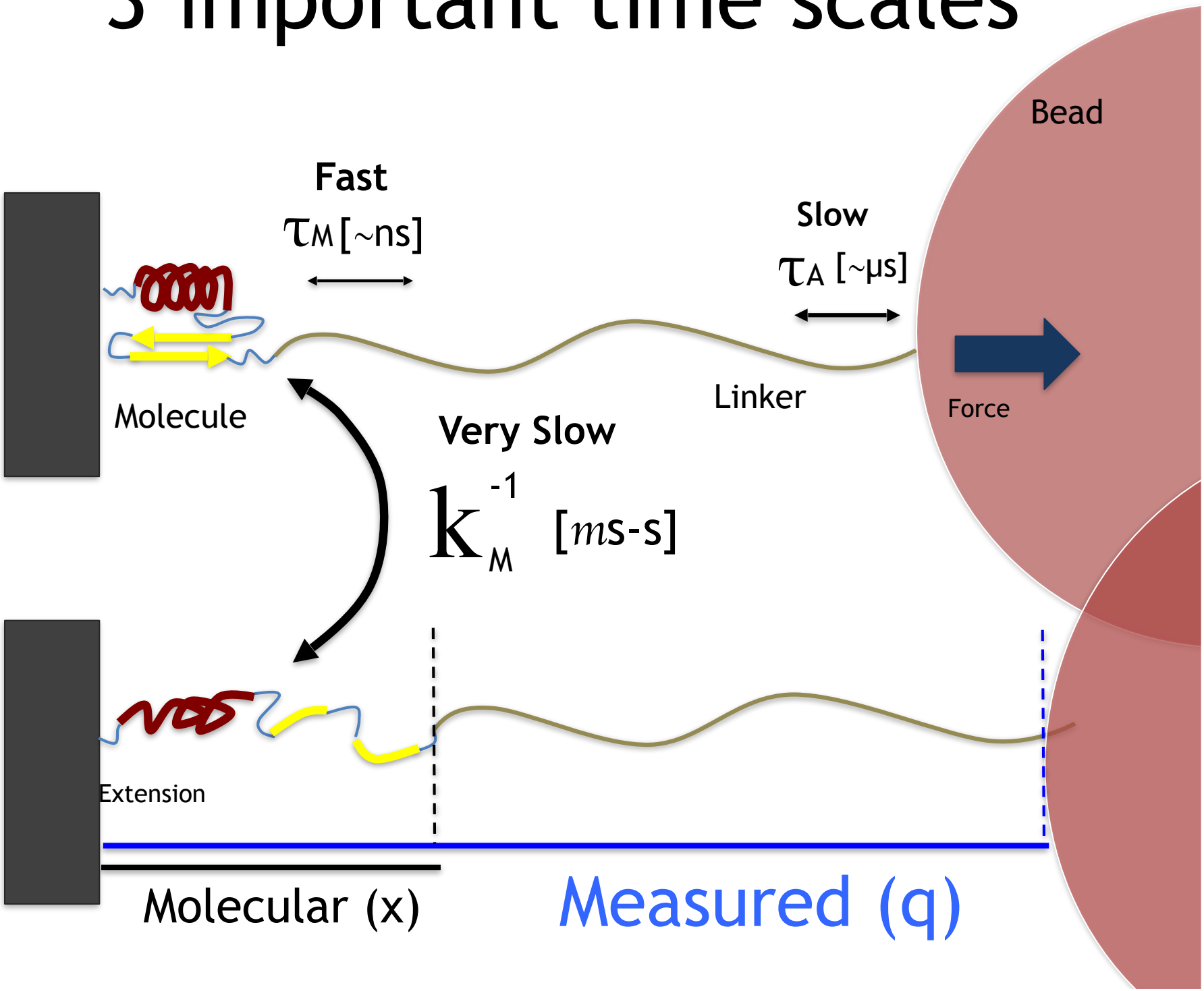
(and many more…)

The conclusions vary from one extreme where the **effect is negligible**, to the other, where the observed **rates have little to do with the dynamics** of the molecule of interest.

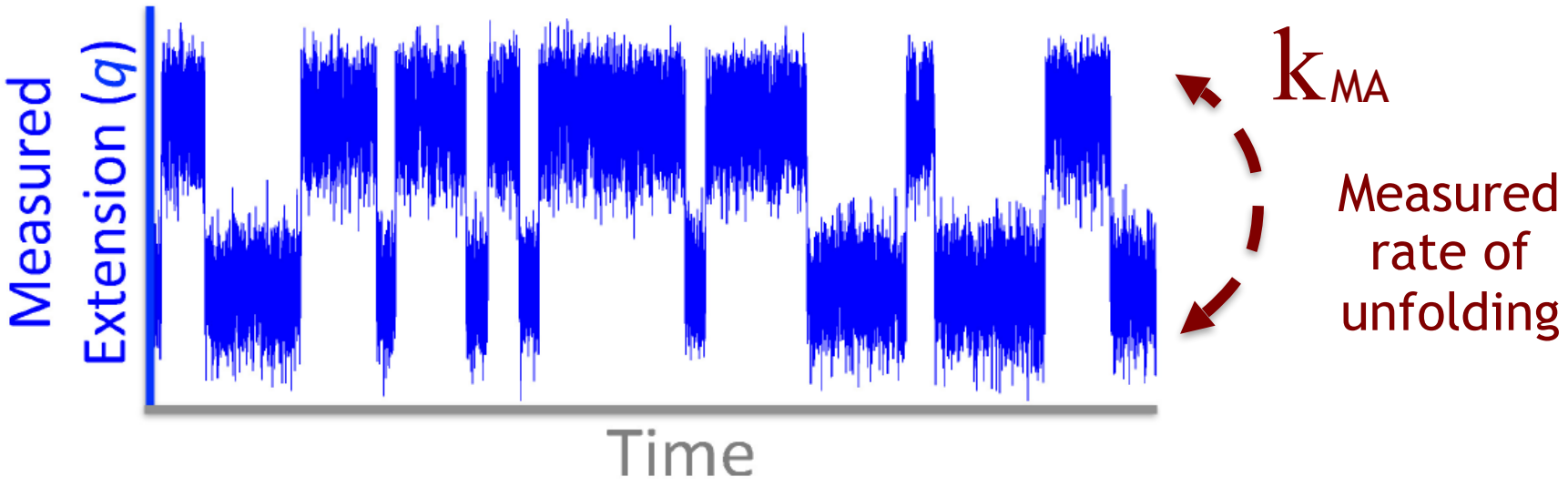
Single-molecule

How does the measured rate compare to the molecular rate (in the ideal case that force could be applied directly to the molecule)?

3 important time scales

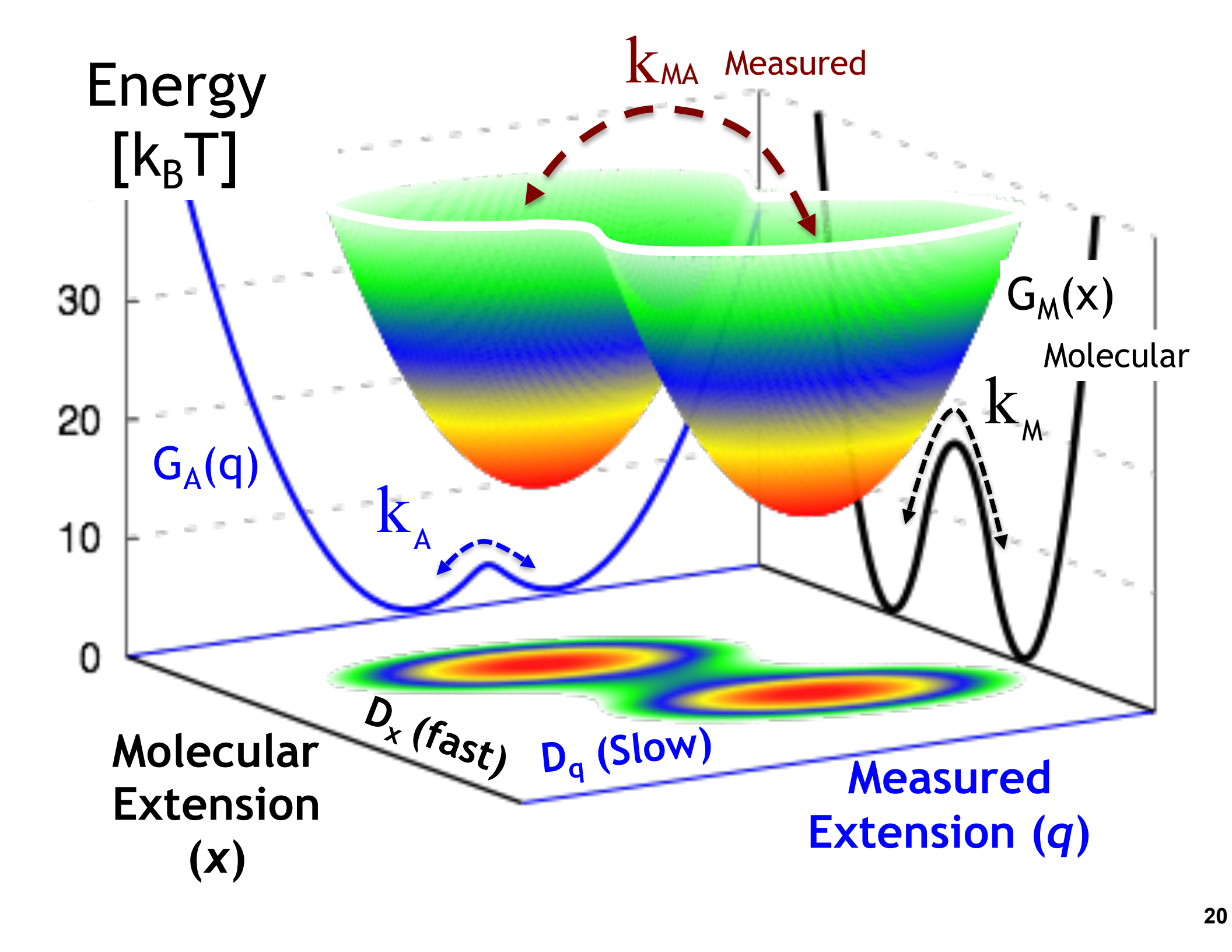


Output from the experiment:



The molecular rate k_M is hidden.

The simplest picture that captures the essential physics: **anisotropic 2D diffusion** over the measured extension and the hidden molecular extension.


Energy
[k_BT]
k_{MA} Measured
30
20
10
0
$G_A(q)$
k_A
$G_M(x)$
Molecular
k_M
D_x (fast)
D_q (Slow)
Molecular
Extension
(x)
Measured
Extension (q)

Single-molecule

- Using **Langer's Theory** and following ref [1], in the limit of high anisotropic diffusion (very slow apparatus)[2]:

$$\frac{1}{k_{MA}} \approx \frac{1}{k_M(1 - V''(q_\cup - x_\cup)/|G_o''(x_\cap)|)} + \frac{1}{k_A}$$

Measured

Molecular

Linker stiffness

Molecular stiffness

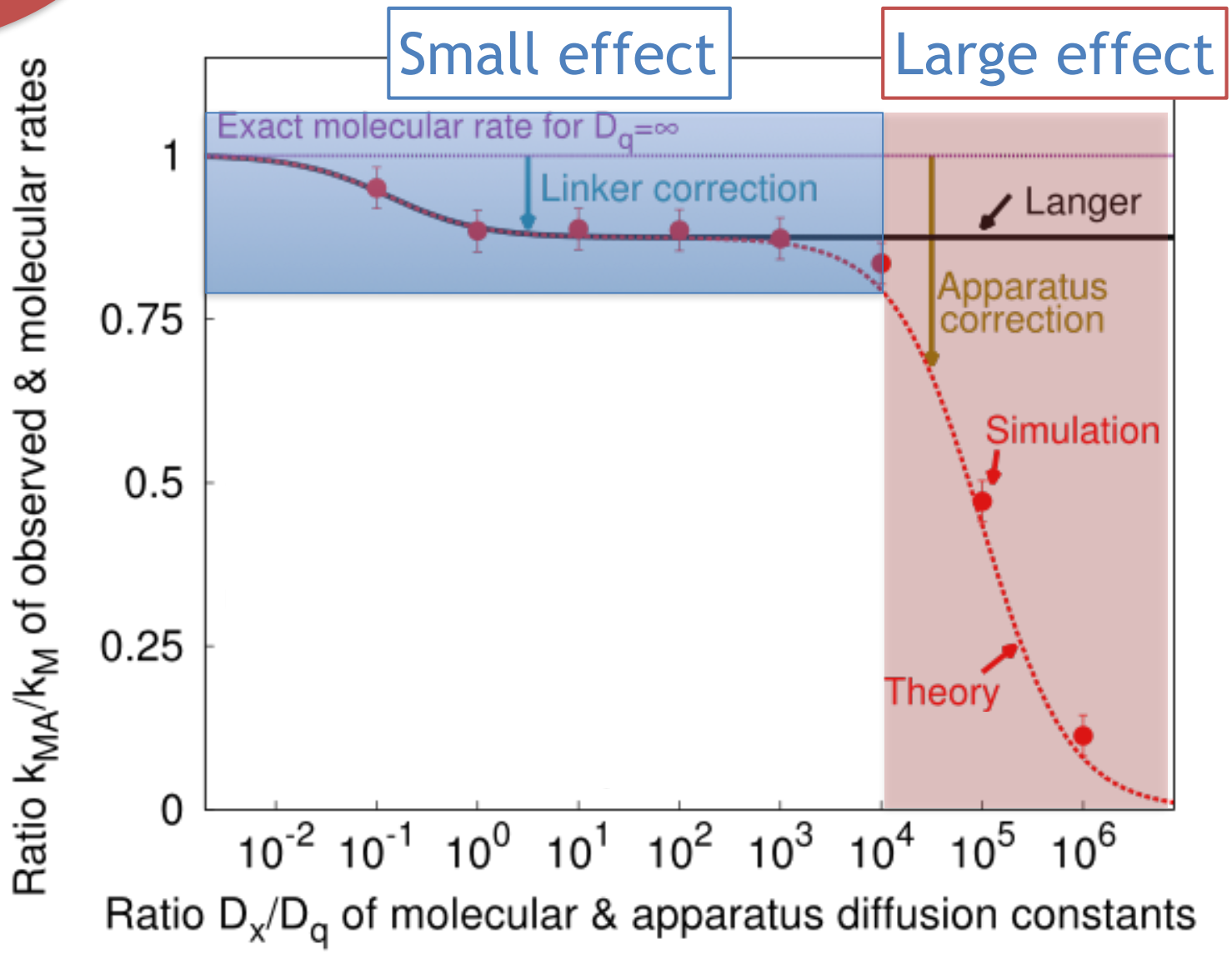
Apparatus artifact

[1] Berezhkovskii AM, Szabo A, Greives N, Zhou HX. **J Chem Phys** (2014).
[2] Cossio, Hummer, Szabo. ***Proc Natl Acad Sci U S A*** (2015).

Single-molecule

Validation with 2D Brownian dynamics:

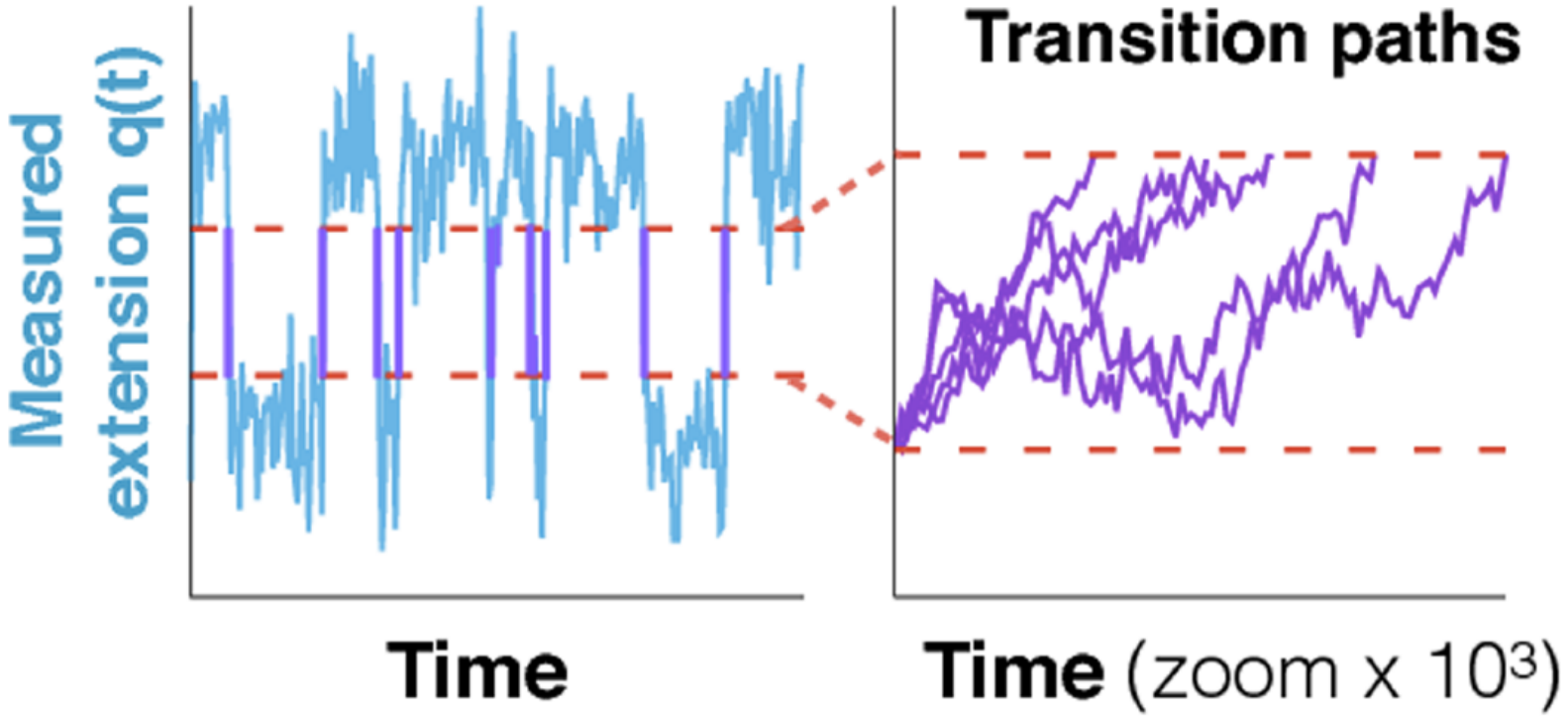
Use the analytical expressions to know in which regime the system is.



Cossio, Hummer, Szabo. ***Proc Natl Acad Sci U S A*** (2015).

Single-molecule

What about the transition paths?



Single-molecule

Recently it was possible to directly measure transition paths with force spectroscopy

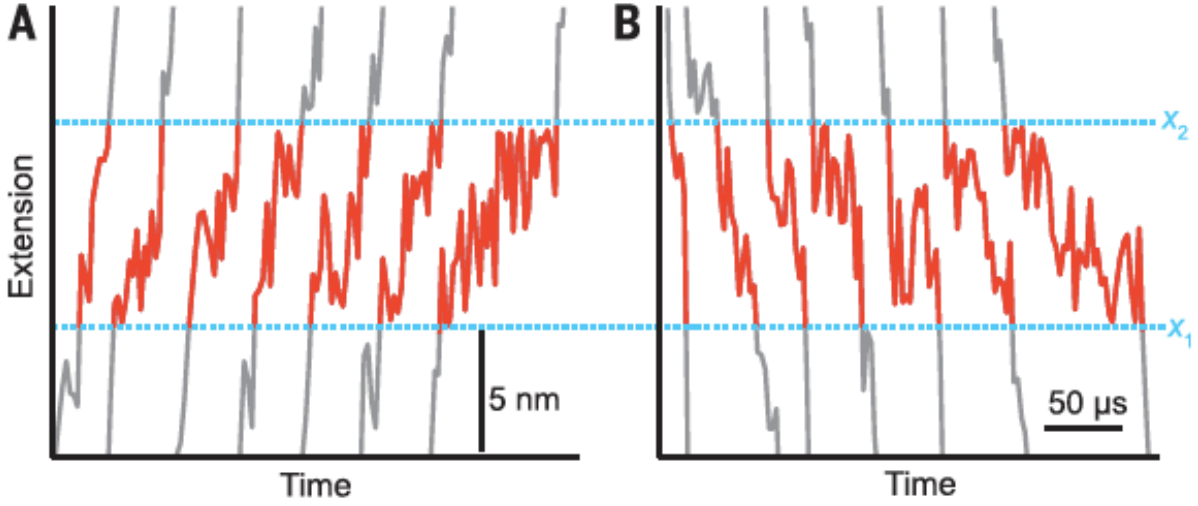
REPORT

Direct observation of transition paths during the folding of proteins and nucleic acids

Krishna Neupane[1], Daniel A. N. Foster[1], Derek R. Dee[1], Hao Yu[1], Feng Wang[2], Michael T. Woodside[1,2,*]
+ See all authors and affiliations

Science 08 Apr 2016:
Vol. 352, Issue 6282, pp. 239-242
DOI: 10.1126/science.aad0637

Fig. 2. Transition paths for a DNA hairpin. Selection of transition paths for (**A**) unfolding and (**B**) refolding. Boundaries x_1 and x_2 (cyan) demark the barrier region. Transition paths display a wide variety of shapes and transit times.



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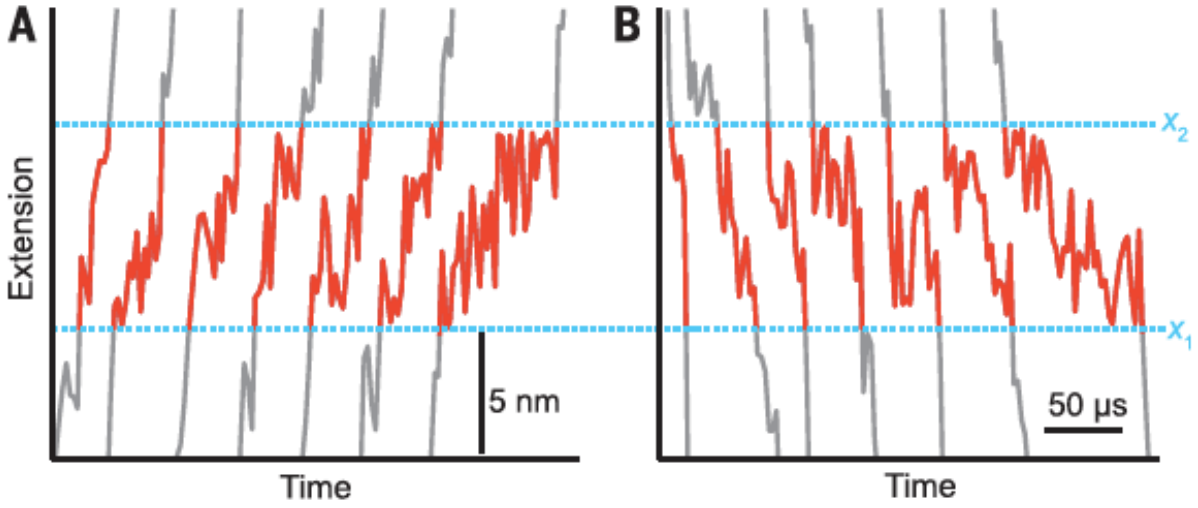
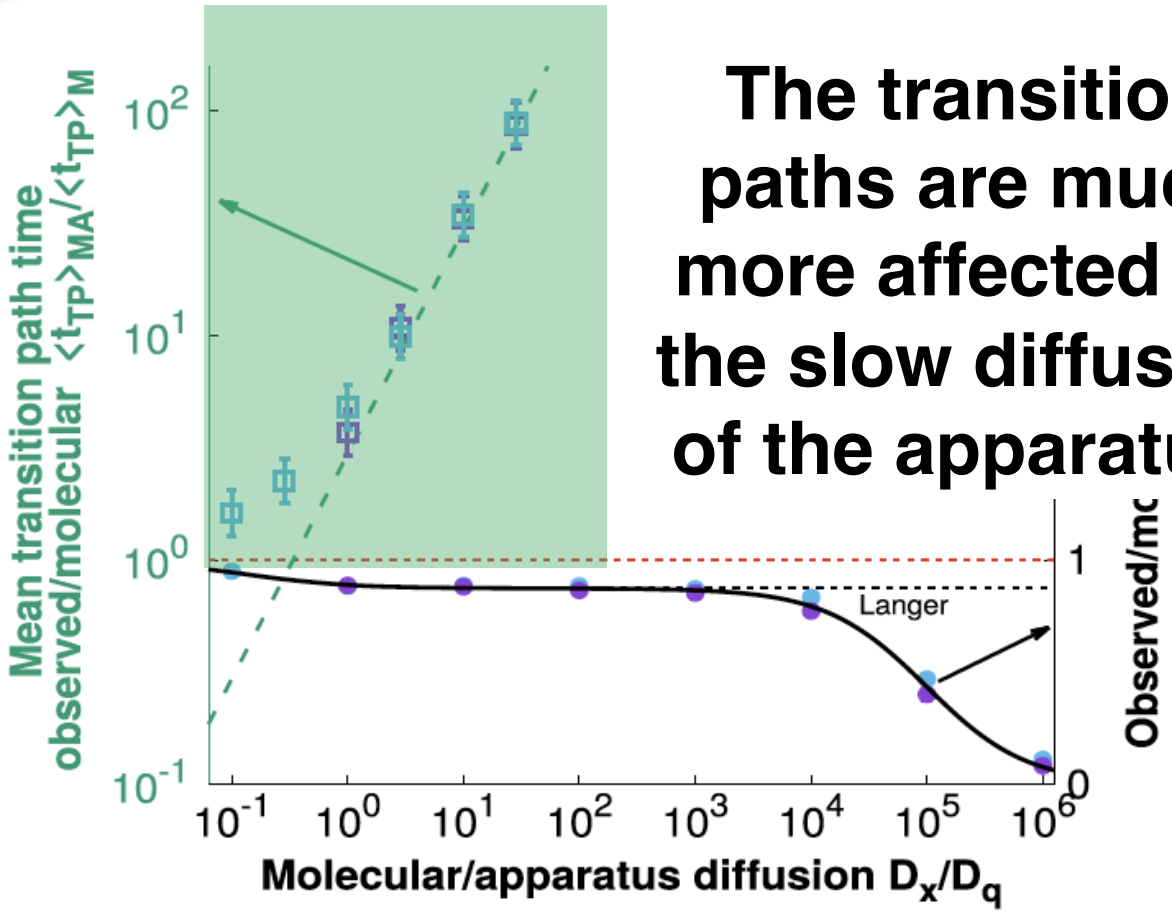


The authors claimed that the transition path time depends only on the **molecule**.

Single-molecule

Artifacts of the apparatus:



The transition paths are much more affected by the slow diffusion of the apparatus!

Cossio, Hummer, Szabo. ***J Chem Phys*** (2018).

Single-molecule

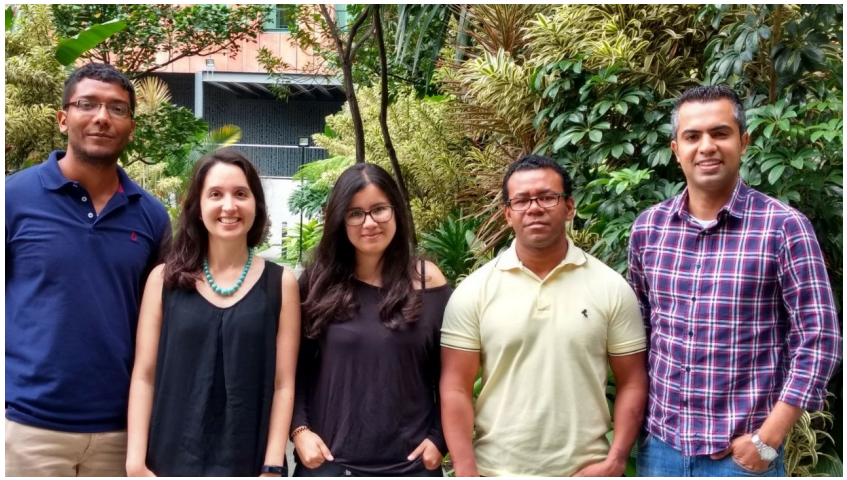
Conclusions

Our theory provides accurate analytic expressions to:

- extract information of the intrinsic free energy and dynamics of the molecule
- assess the effects of the pulling device on the unfolding transition rate and paths.

Acknowledgements

My group:



Prof. Hummer

Dr. Szabo

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