

Measuring the roughness of protein molecules using the single molecule unfolding method

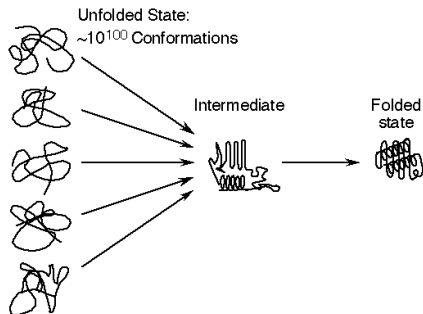
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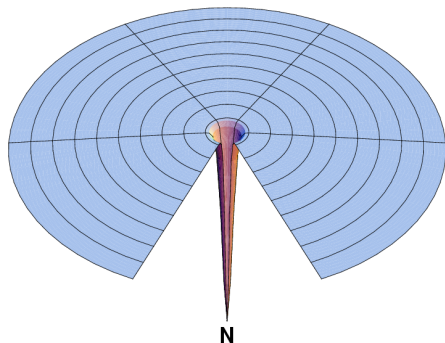
June 6, 2007

A needle in a haystack

How do we find the global minimum?

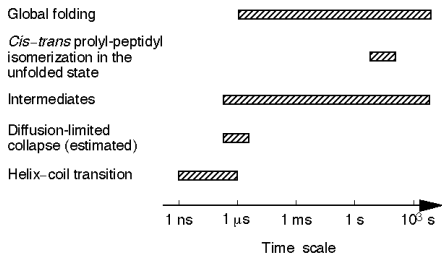


Levinthal paradox [Nolting].

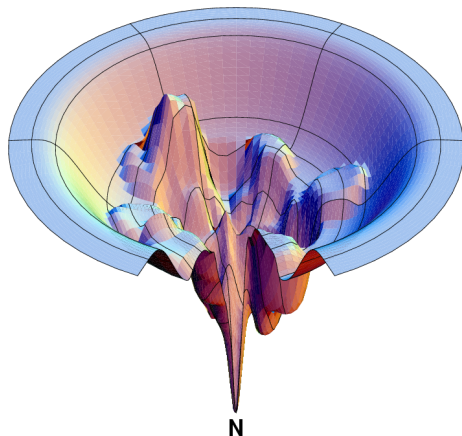


A hypothetical energy landscape [Dill].

But natural proteins *do* fold

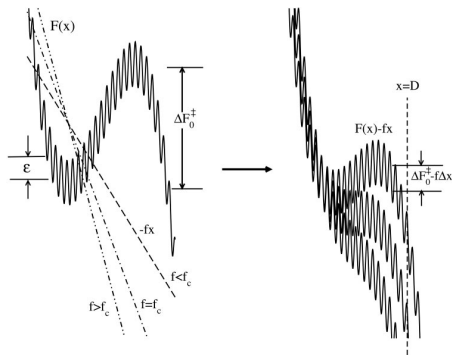
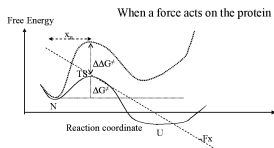
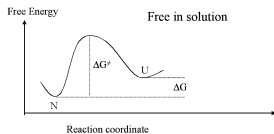


Protein folding timescales at 25°C and pH 7 [Nolting].



How do we measure this energy landscape?

Measuring the energy landscape roughness ε



Proposed energy roughness mode [Hyeon].

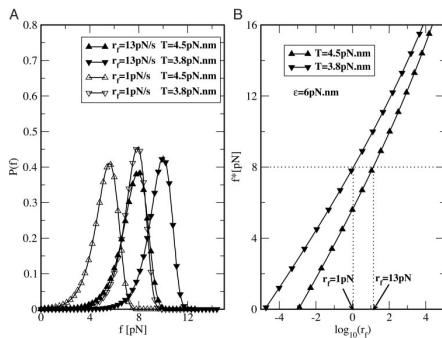
$$F(x) = F_0(x) + F_1(x).$$

Kramers' problem and diffusion in rough potentials

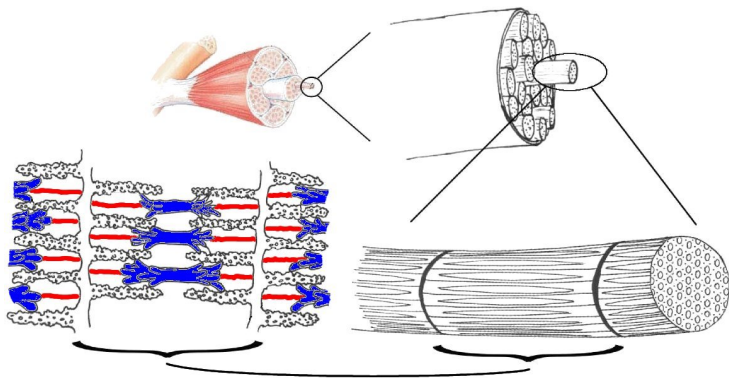
$$\varepsilon^2 \approx \frac{k_B^2 T_1 T_2}{T_2 - T_1} \cdot \left[T_1 \log \left(\frac{r_f(T_1) \Delta x(f^*)}{k_0(f^*) k_B T_1} \right) - T_2 \log \left(\frac{r_f(T_2) \Delta x(f^*)}{k_0(f^*) k_B T_2} \right) \right],$$

where

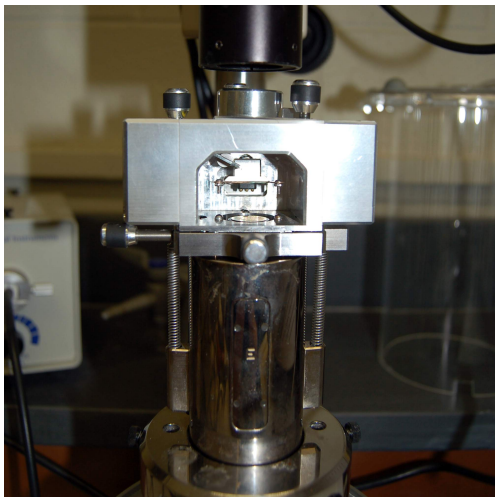
- $\Delta x(f^*)$ is the unfolding distance from the bound state to the transition state for the smooth potential $F_0(x) - f^*(x)$.
- $k_0(f^*)$ is the escape rate from the smooth potential.



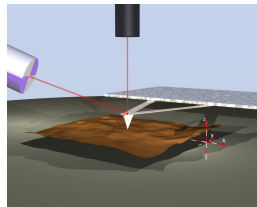
Titin and temperature dependence in muscle



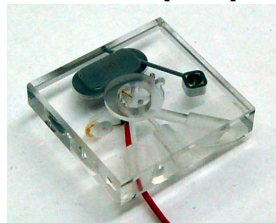
Atomic force microscopy



MultiMode head.

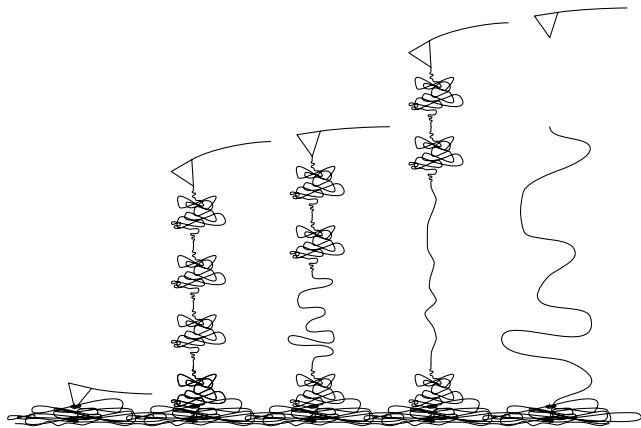


Operation[Warr].

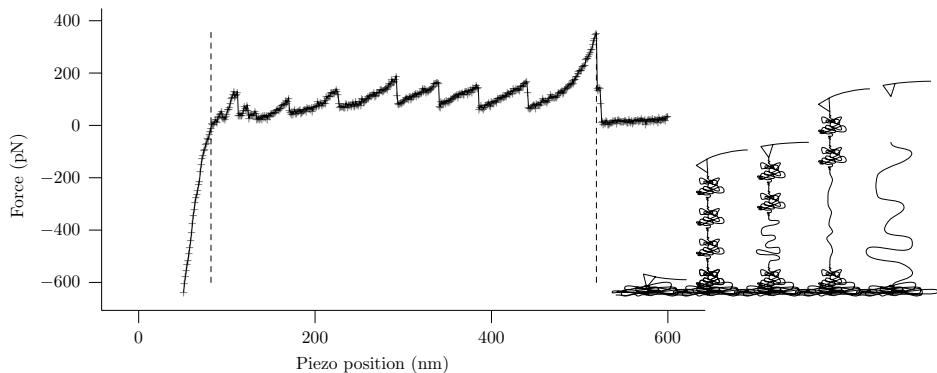


Fluid cell.

Protein unfolding, understanding the mechanics

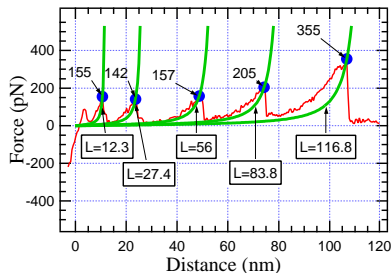


Protein unfolding, understanding the data



Protein unfolding, understanding the data

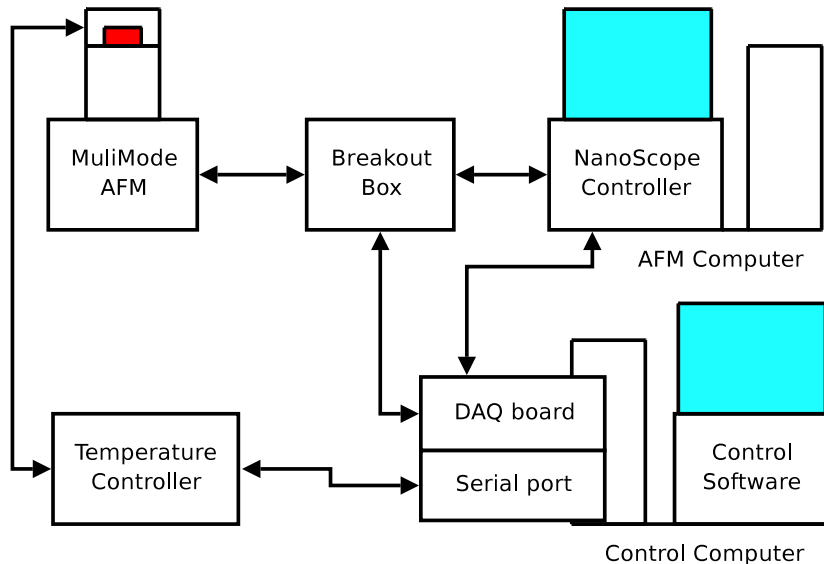
Worm like chains



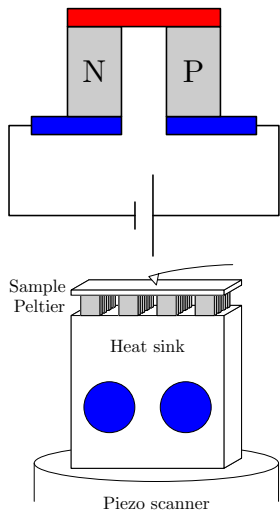
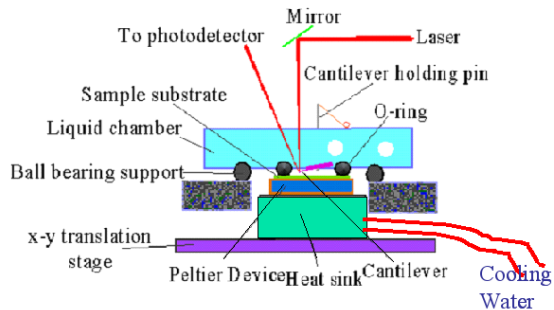
$$F(x) = \frac{k_B T}{p} \left[\frac{1}{4} \left(\frac{1}{(1 - x/L)^2} - 1 \right) + \frac{x}{L} \right],$$

where p is the persistence length and L is the contour length.

Equipment layout

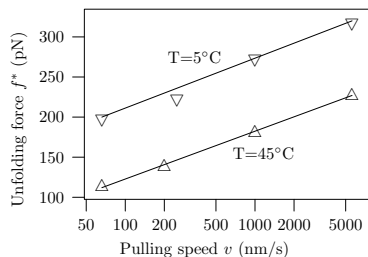


Temperature control



Temperature control module and the Peltier heater/cooler.

Temperature dependent unfolding in Ubiquitin



So the unfolding energy according to Hyeon and Thirumalai's equation is

$$\epsilon \approx 1.98 \cdot 10^{-20} \text{ J} \approx 4.8 k_B T.$$

Goals



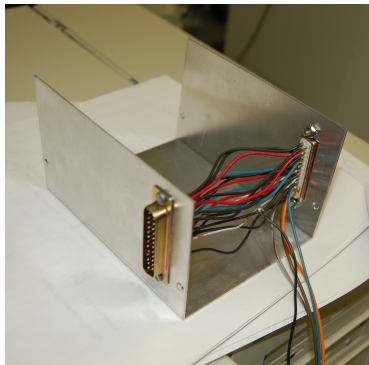
Difficulties with temperature dependent measurement

- Thermal drift and limited piezo range.
- Procedure not very reliable, needs hand tweaking.
- Many parameters to optimize once it *is* working.
- Lots of data to search through to find our signature sawtooth.

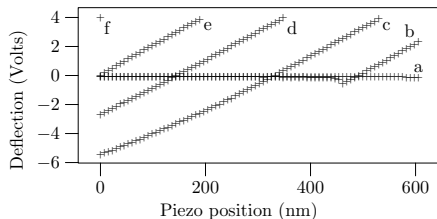
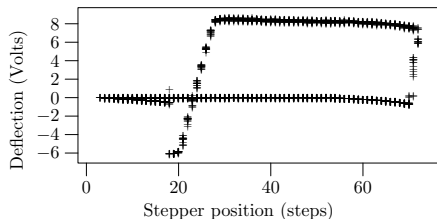
Goals

- Automate stepping motor control.
- Re-architect experiment software.
- Automate force-curve sorting.

Breaking into AFM communication



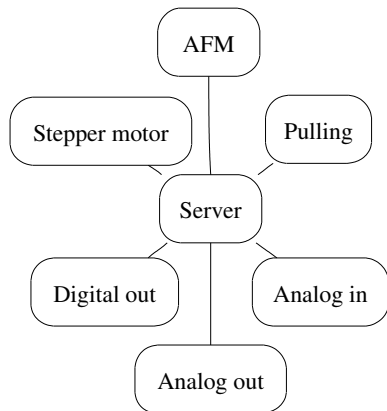
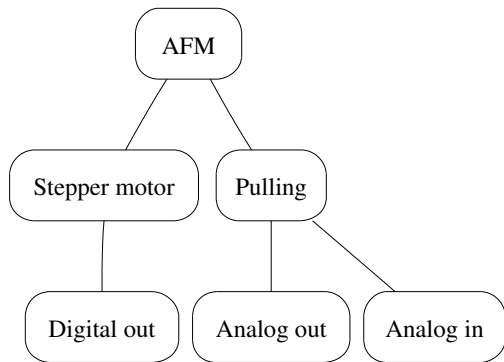
Testing the control of the stepper motor via the breakout box



Measuring backlash (~ 46 steps) and stepsize ($\sim 170\text{nm}$).

Architecture comparison

Choose for flexibility with minimal programmer time.



Tree vs. network.

The goal: Flexible control scripts in action

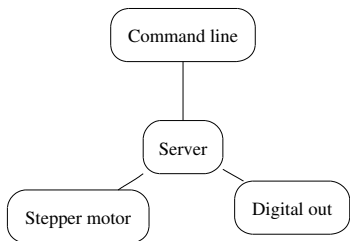
```
> approach_surface
Lost signal!
> approach_surface
> piezo_measure_distance
372 nm
> pull_protein --save 'D:\data\' &
saving data to 'D:\data\070510\'
> monitor_unfolding
> sort_data --dir 'D:\data\070510\'
...
> ls
pulls_good
pulls_bad_structure
...
```

Example script: approach_surface

```
#!/bin/sh
TARG_DEF=1 # Volts
MIN_TOT=3 # Volts
get_feedback() {
    DEF='cmmddline -c ':AFM|Get:Deflection''
    TOT='cmmddline -c ':AFM|Get:TotalPhotodiode''
}
step_closer() {
    ANS='cmmddline -c 'Stepper|Set:RelPos 1''
}
get_feedback
while [ $DEF -lt $TARG_DEF ] && [ $TOT -gt $MIN_TOT ]; do
    step_closer
    get_feedback
done
```

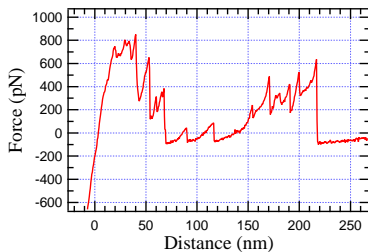
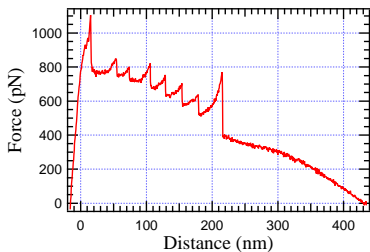
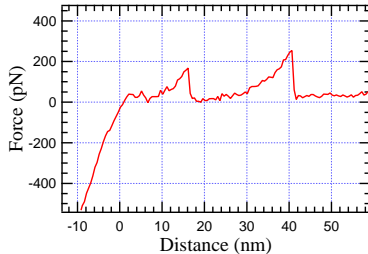
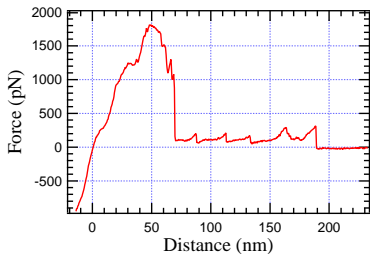
Minimize programmer time

Tracing commands

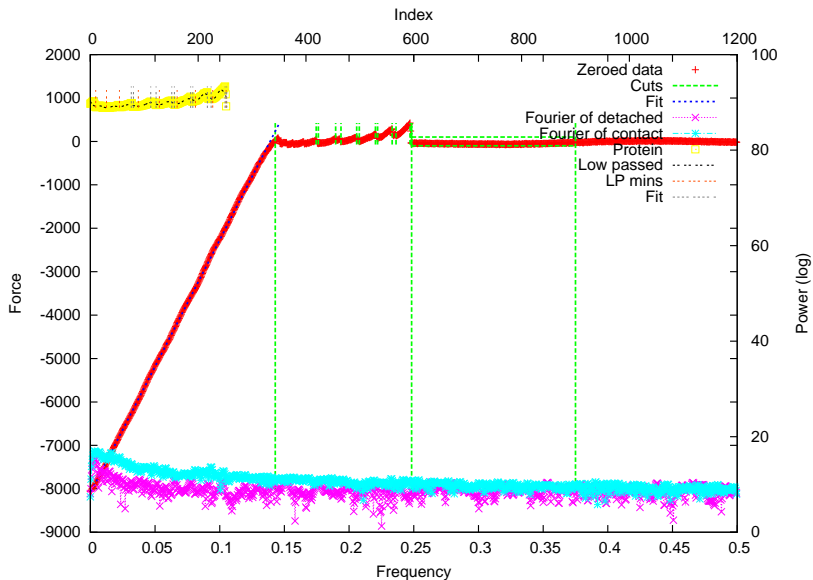


From	To	Message
Cmmdline	Stepper	Set:AbsPos 3
Stepper	Dig. Out	Set:Output 1
Dig. Out	Stepper	DONE
Stepper	Dig. Out	Set:Output 2
Dig. Out	Stepper	DONE
Stepper	Dig. Out	Set:Output 3
Dig. Out	Stepper	DONE
Stepper	Cmmdline	DONE

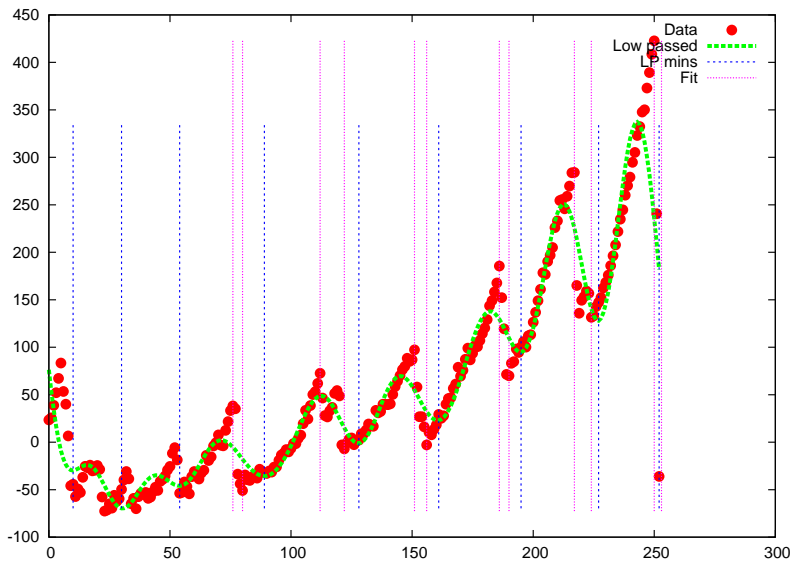
Automatically sorted pulling curves



Sorting example



Sorting example



Preliminary results

%	Good	Bad	%	Good	Bad
+	1.9	23	+	2.2	3.9
-	0.3	75	-	2.2	91.5

The sorting algorithm on training and test datasets.

Why are they so different?
What do we do about it?

Conclusions

We have

- Automated control of the stepping motor, which will allow longer data runs without interruption.
- Developed a flexible software architecture, which will ease the implementation of future refinements and reduce troubleshooting time during setup.
- Beginning development of an automatic force curve sorter, which will allow automatic experiment parameter optimization and ease the sorting of large datasets.

The next step will be putting these pieces together in order to measure Titin's temperature dependent unfolding properties.