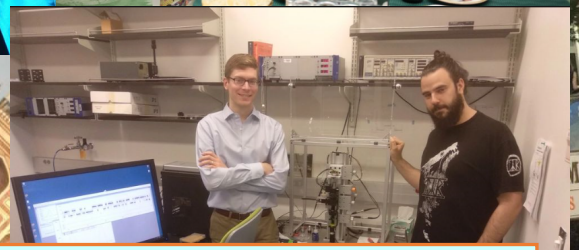
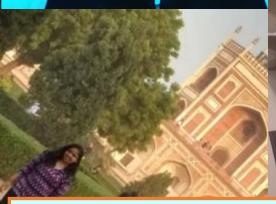
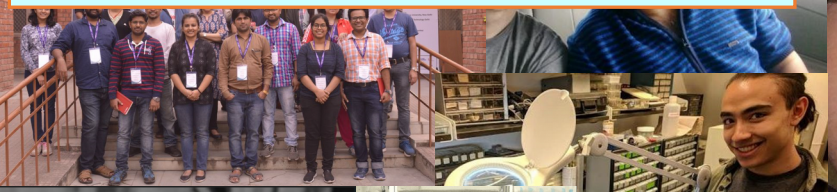
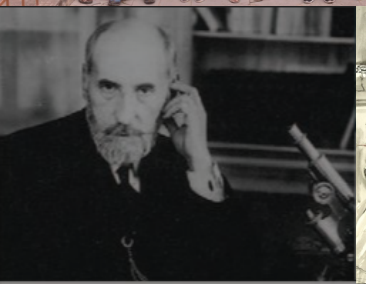
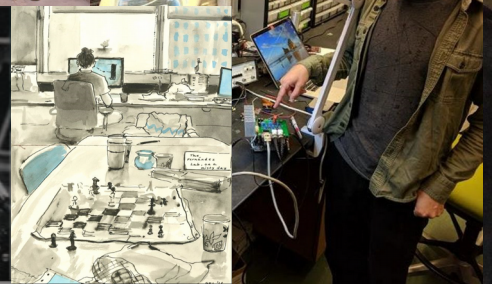



Look us up in zeptowatt.com

EVERYTHING IN BIOLOGY IS MECHANICAL

FERNANDEZ LAB

– HOME PUBLICATIONS SCIENTISTS PHOTOS DOWNLOADS CONTACT

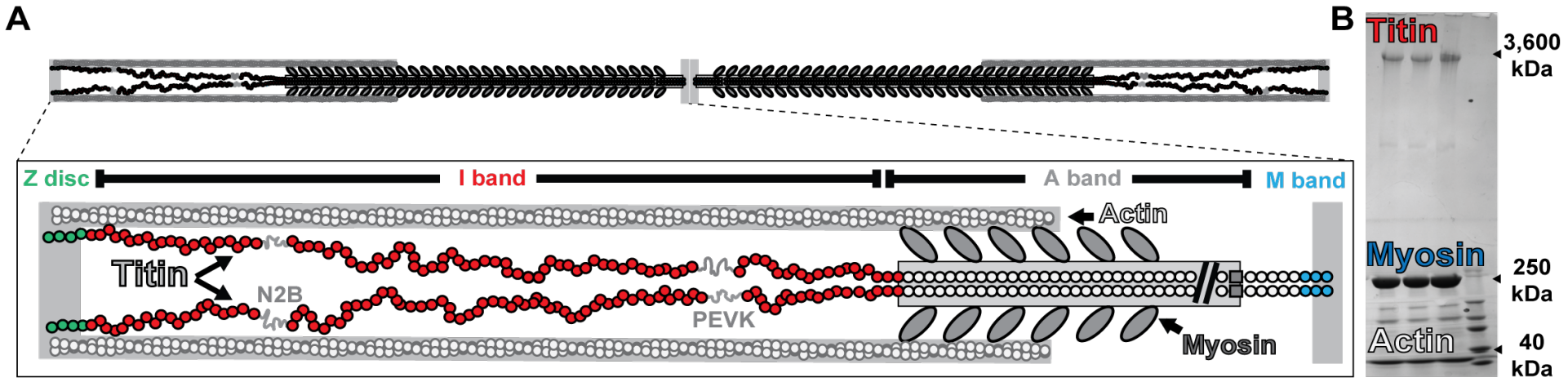
Giants under force: titin, pilin and talin

How does stretching work?

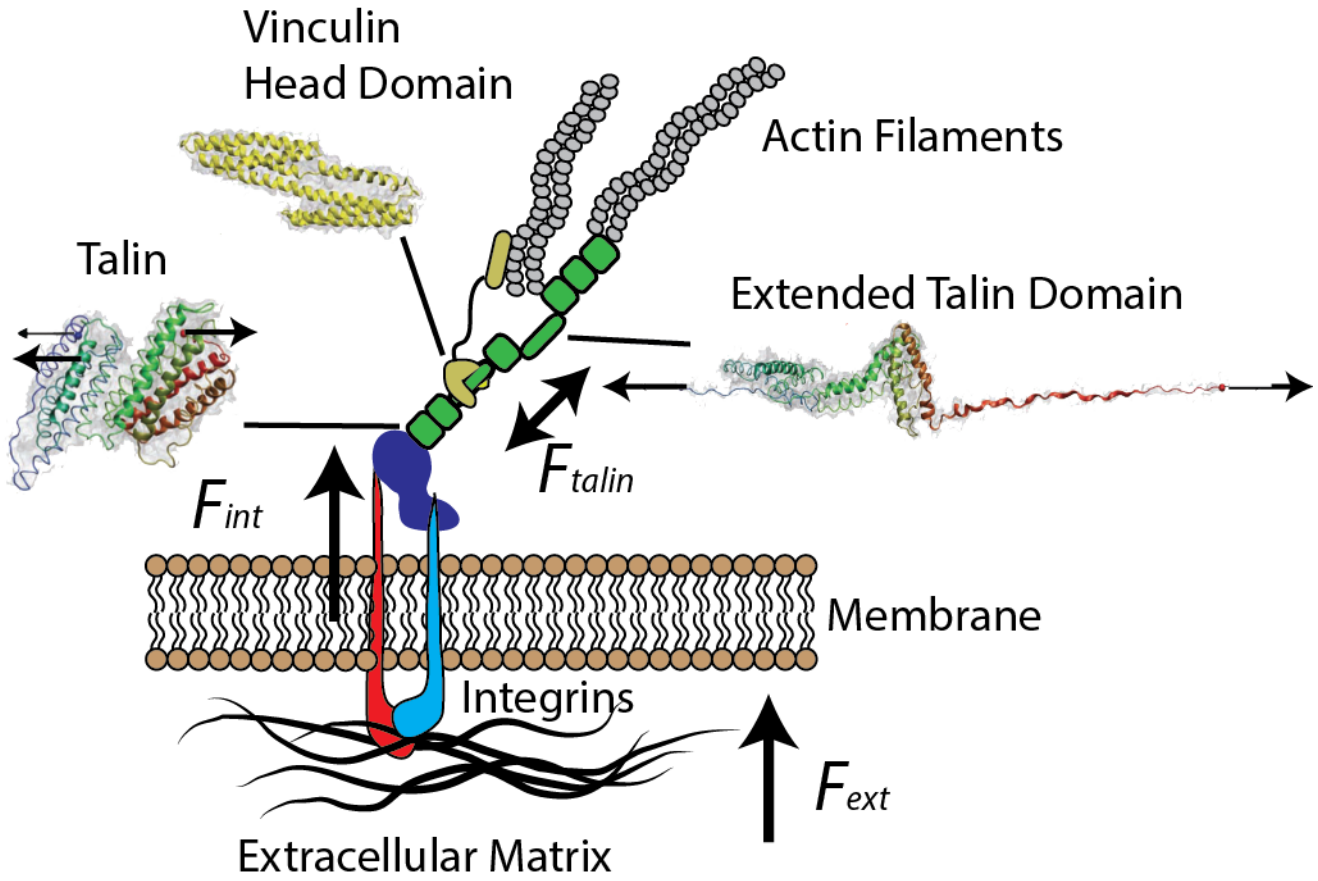
Do we understand how a man can throw a ball at >100

Does **titin** contribute to pitching a ball at >100 mph?

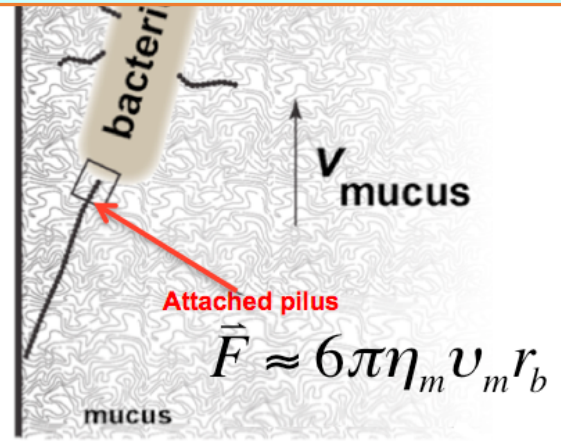
> 100 mph

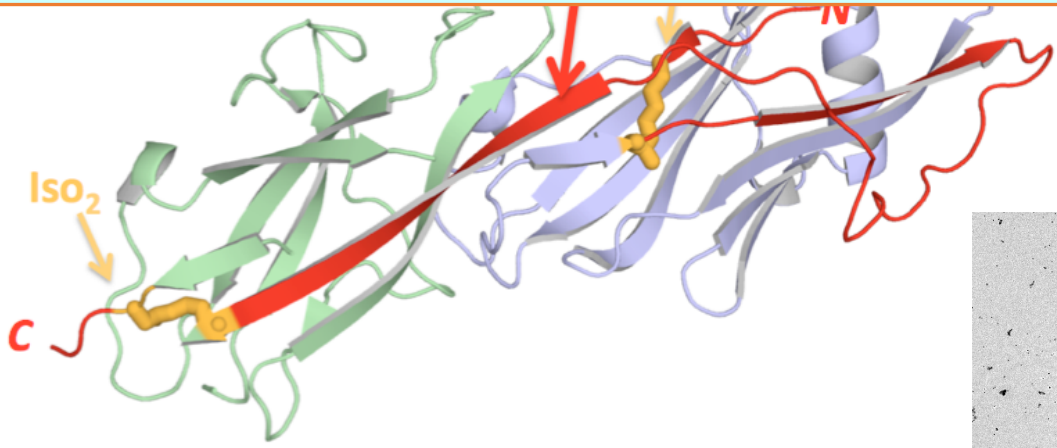


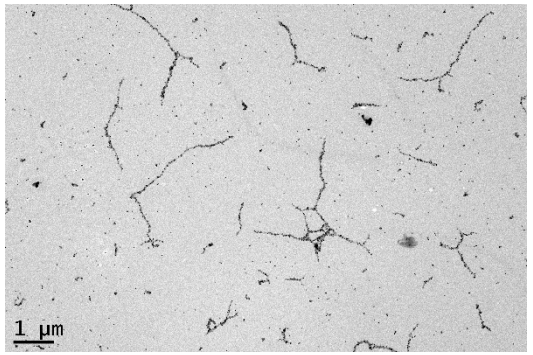
Signal transduction by the mechanical force sensor talin a crucial member of focal adhesions

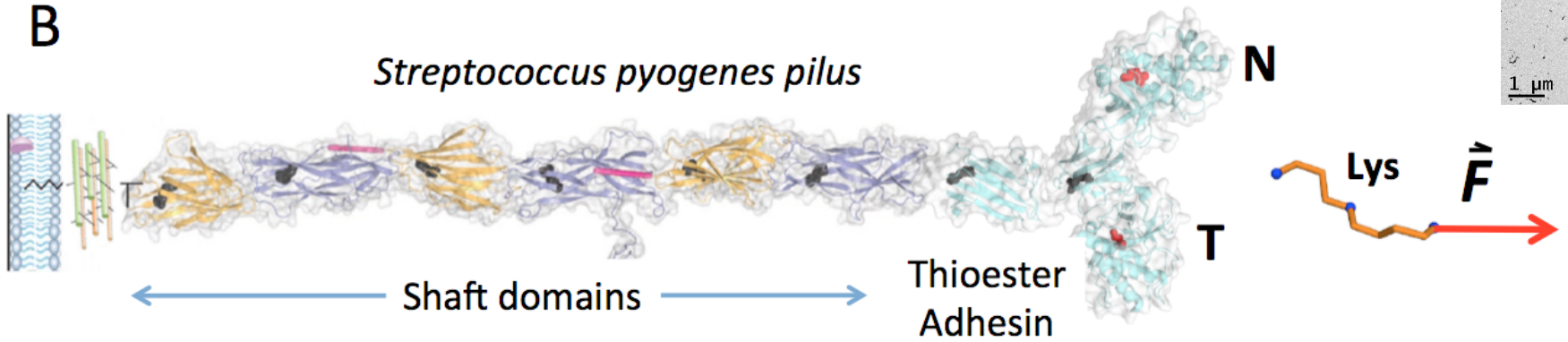


Gram-positive pili are the largest single polypeptide proteins known.
They have specialized features to resist large mechanical shocks!





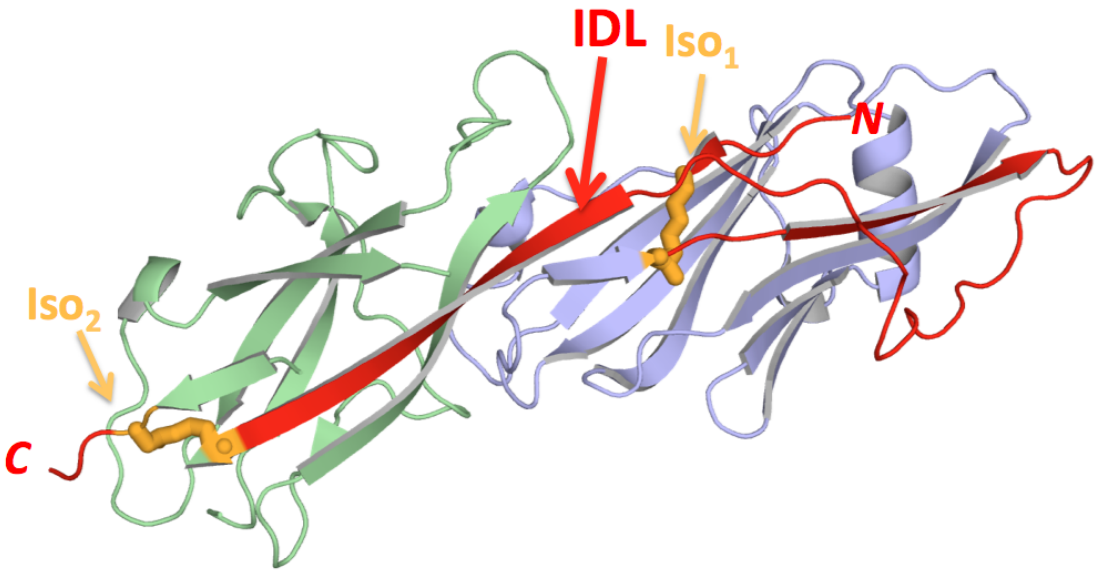




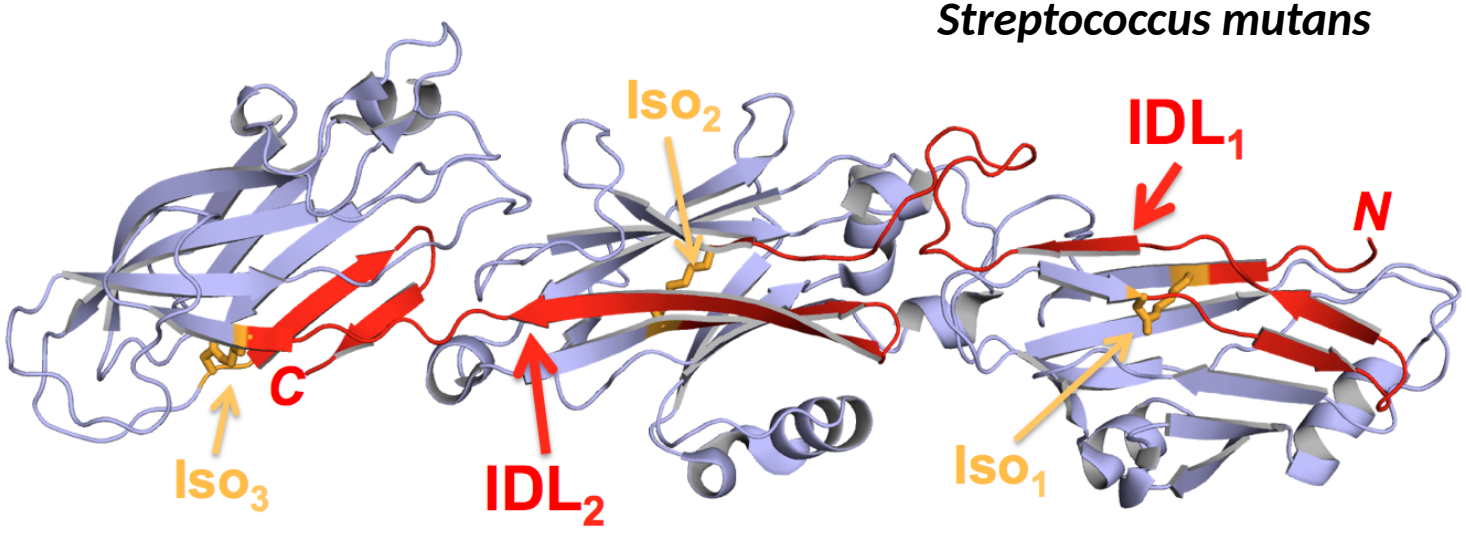
Alegre-Cebollada et al., 2010, **JBC,** 285:11235-11242

Echelman et al., 2016, **PNAS,** 113:2490-2495

FimA *Actinomyces oris*

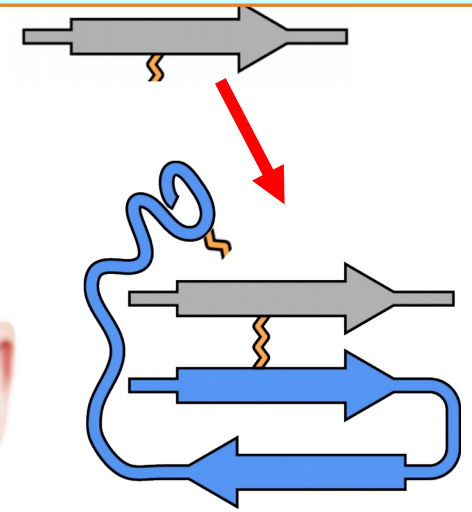


Streptococcus mutans



Rational design of antiadhesive peptide antibiotics

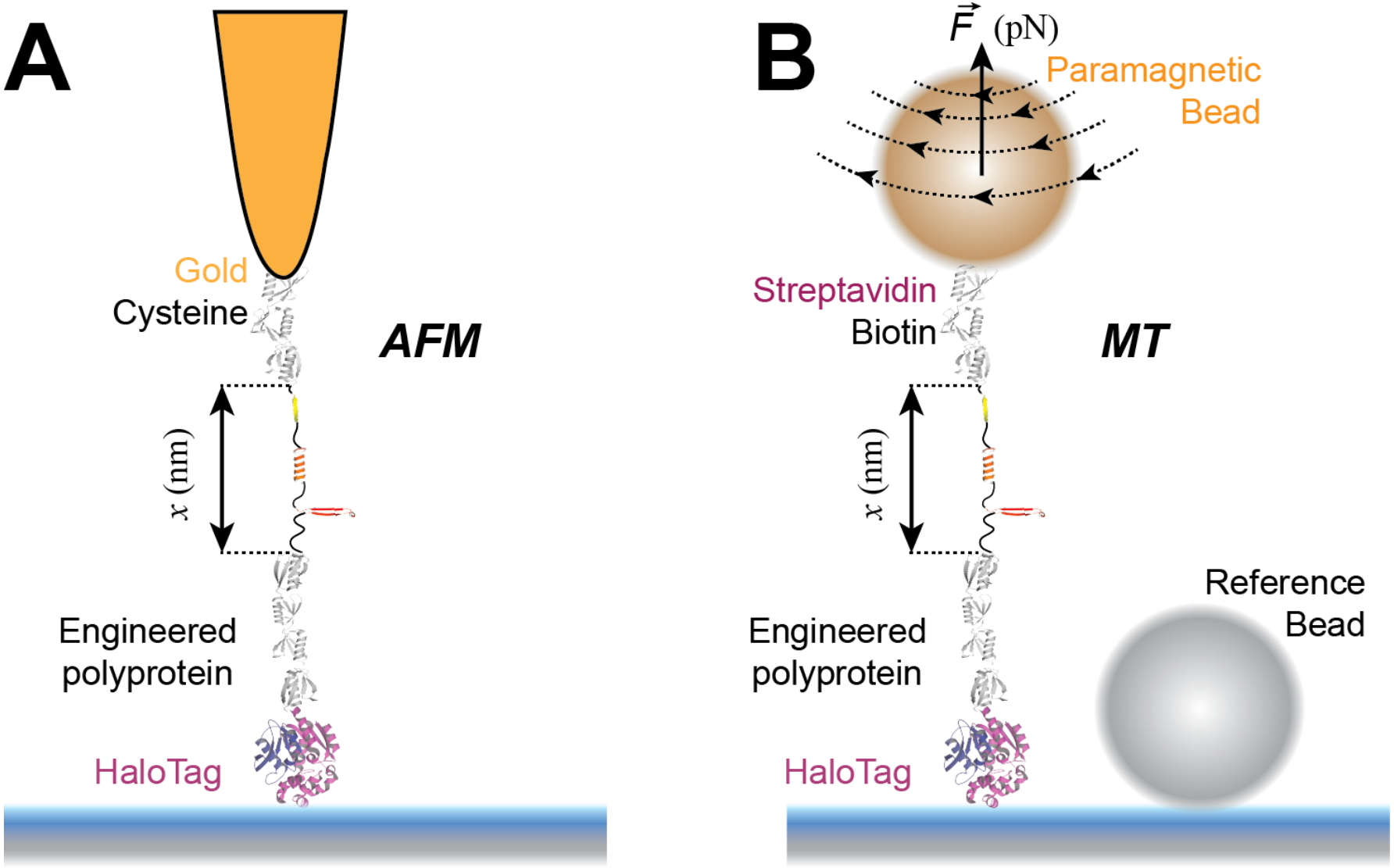
A new type of toothpaste



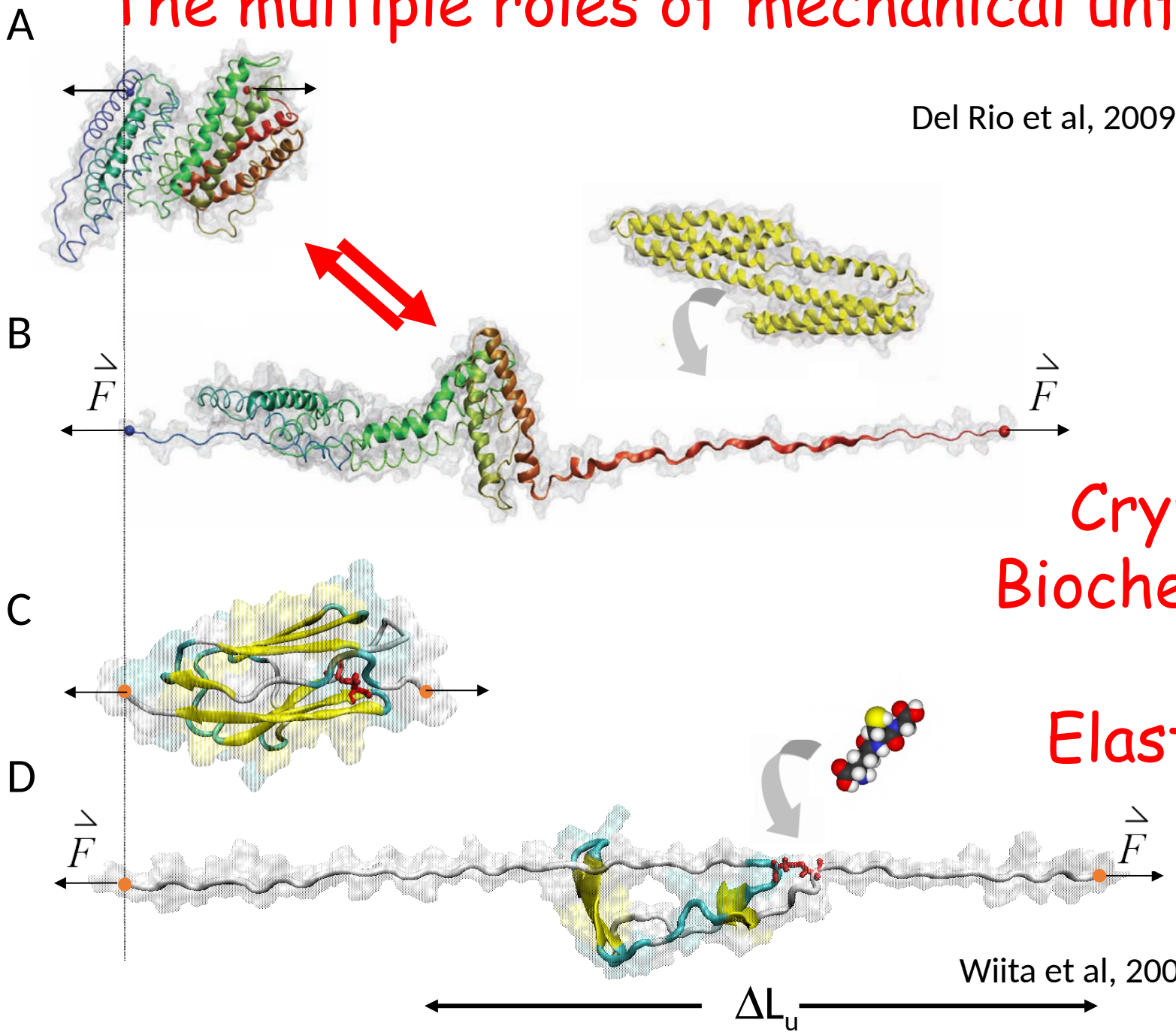
A new type of peptide

A vaccine against dental caries?
Understand to design of coughing

Force spectroscopy measures force, work and power in folding proteins



The multiple roles of mechanical unfolding



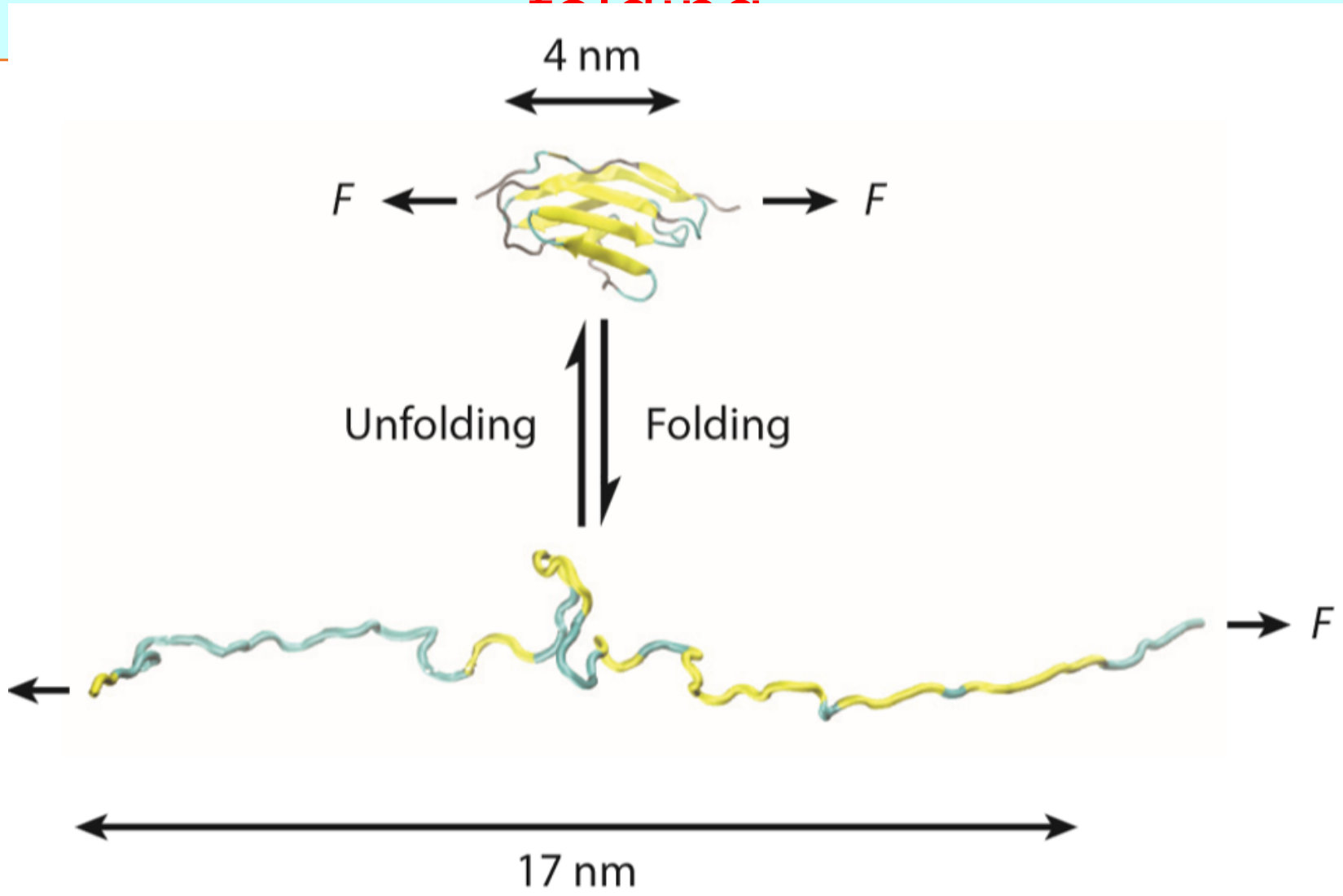
Del Rio et al, 2009, **Science**, 323: 638-641

Cryptic Biochemistry

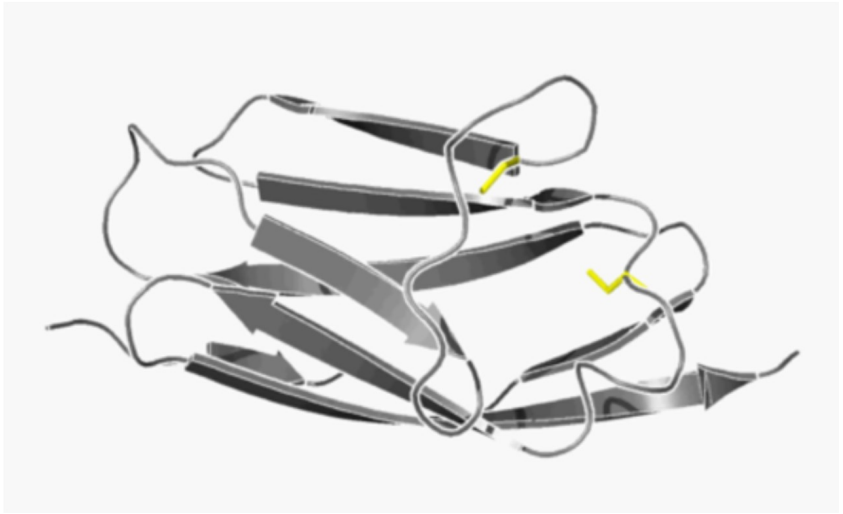
Elasticity

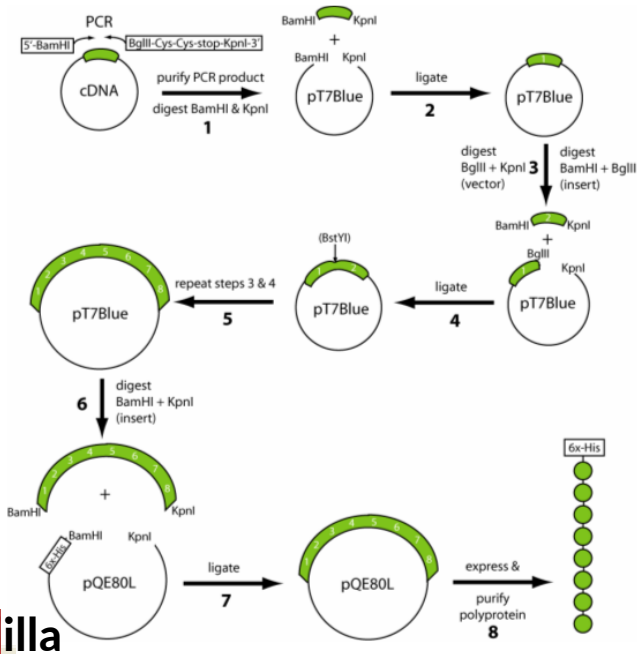
Wiita et al, 2007, **Nature**, **450**: 124-127

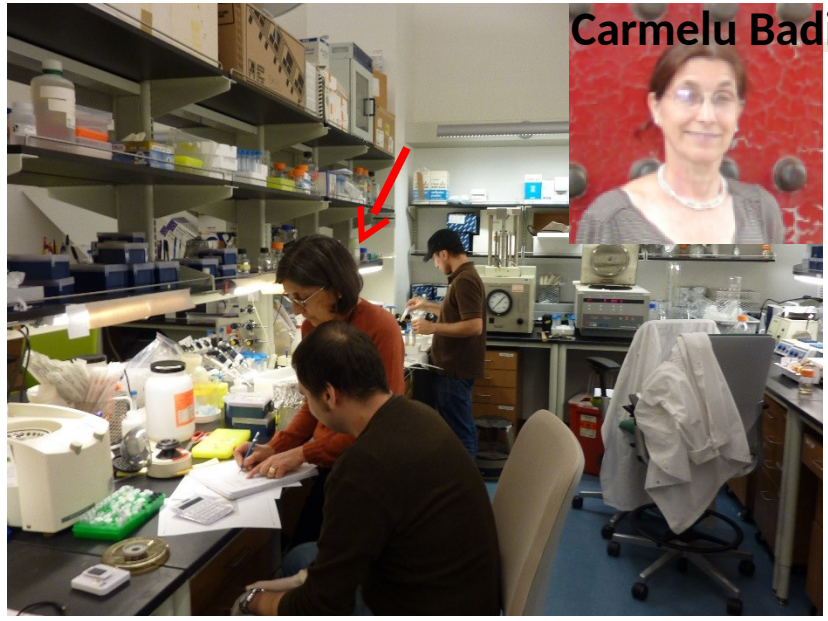
The amazing mechanical power of protein folding



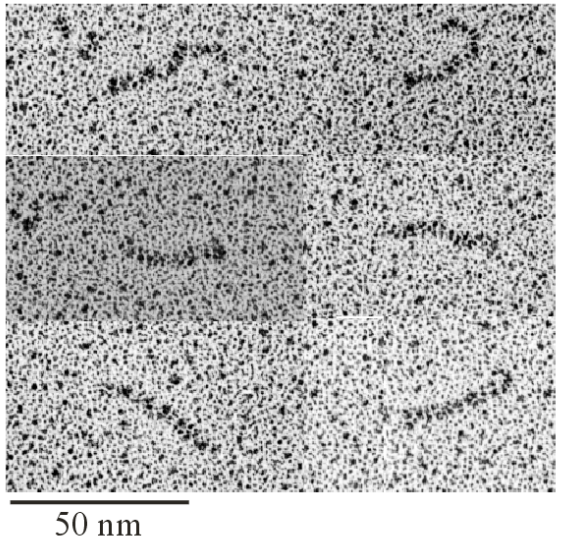
Engineering proteins for force spectroscopy

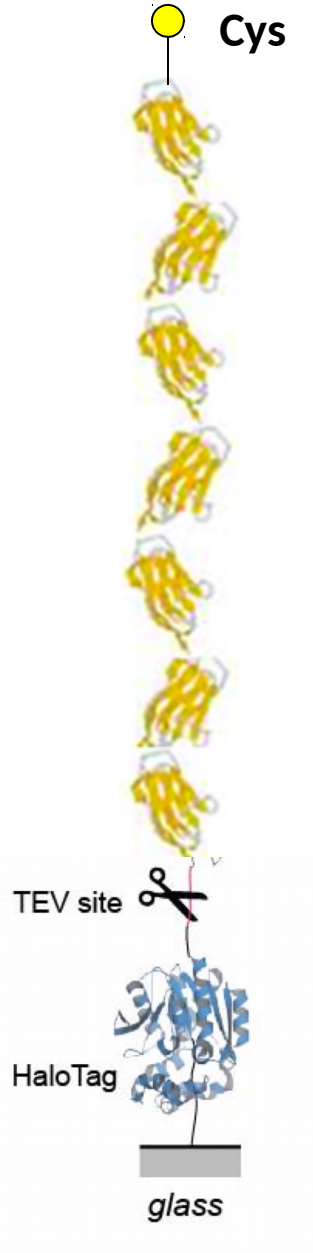


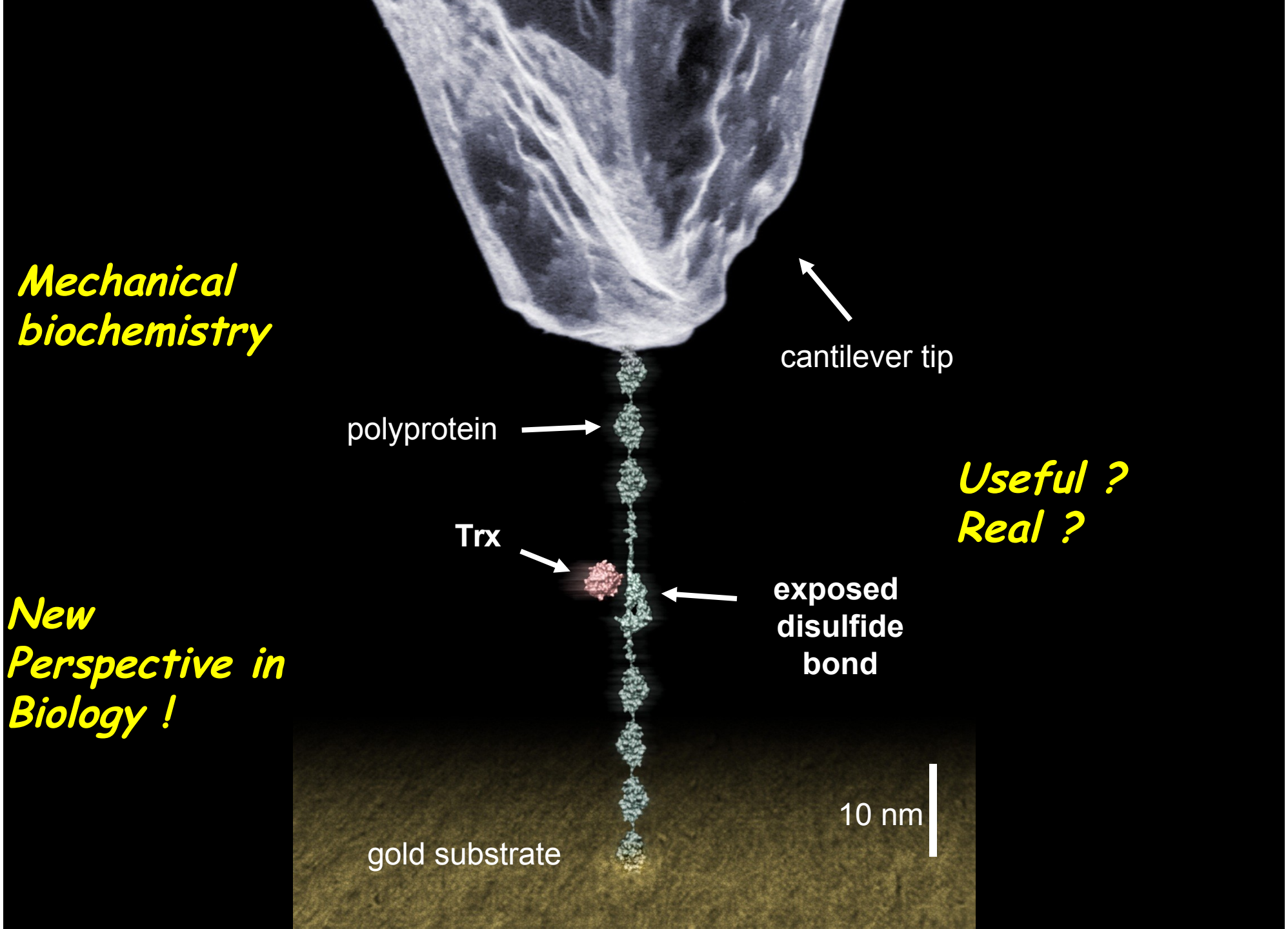




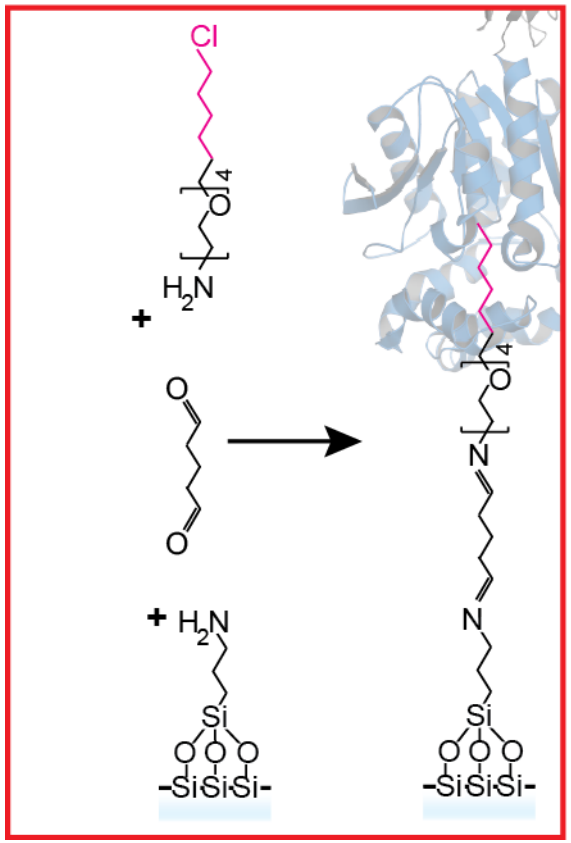
Carmelu Badilla

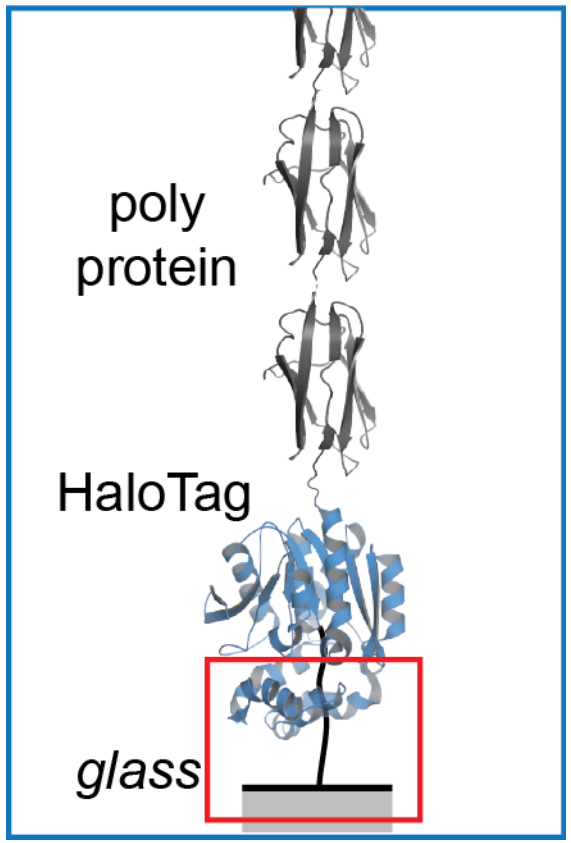


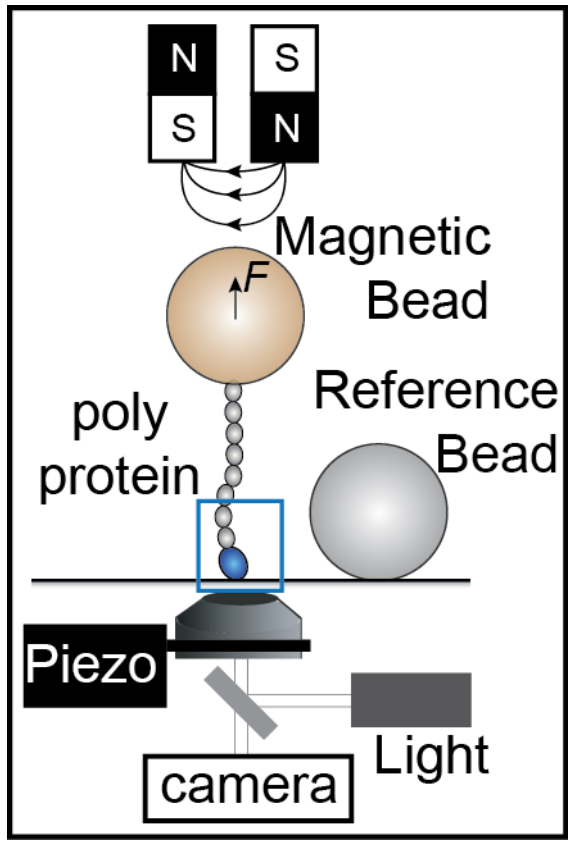



Mechanical biochemistry
cantilever tip
polyprotein
Useful ? Real ?
Trx
exposed disulfide bond
New Perspective in Biology !
10 nm
gold substrate

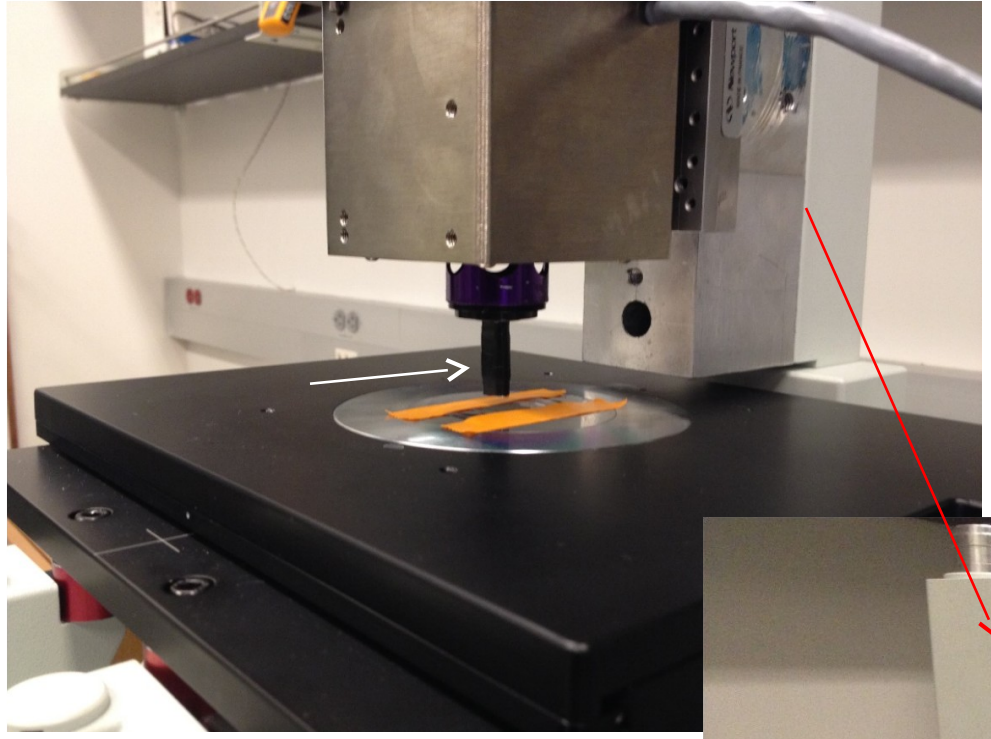
HaloTag anchored polyproteins

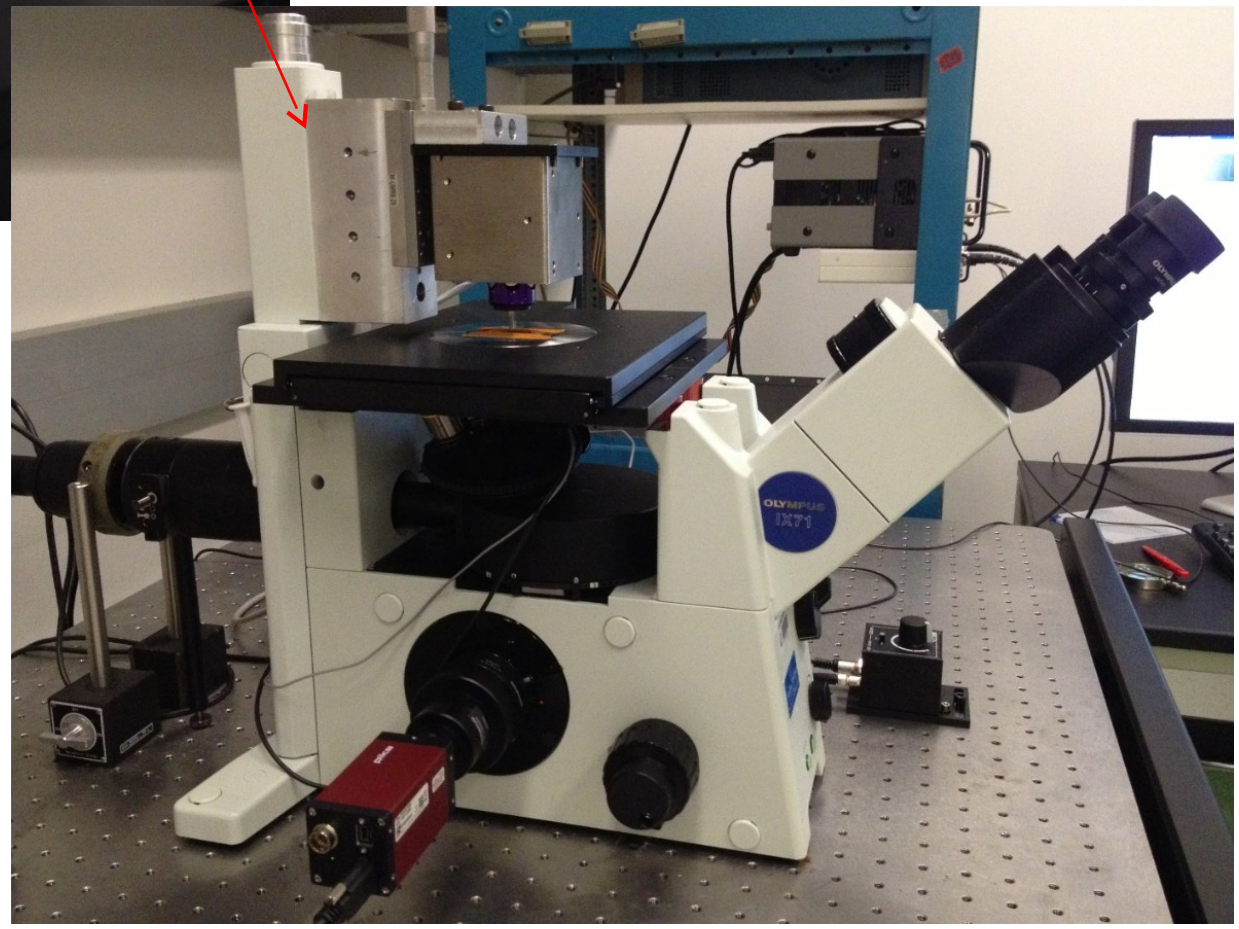


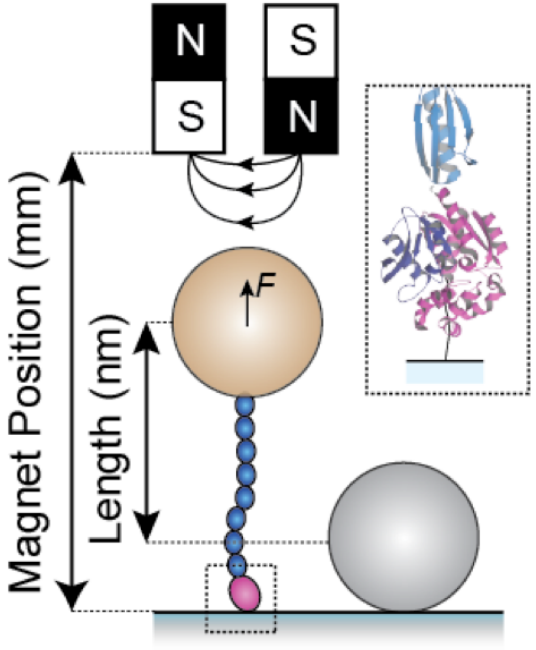




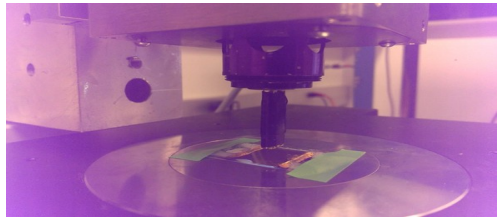
HaloTag and magnetic tweezers

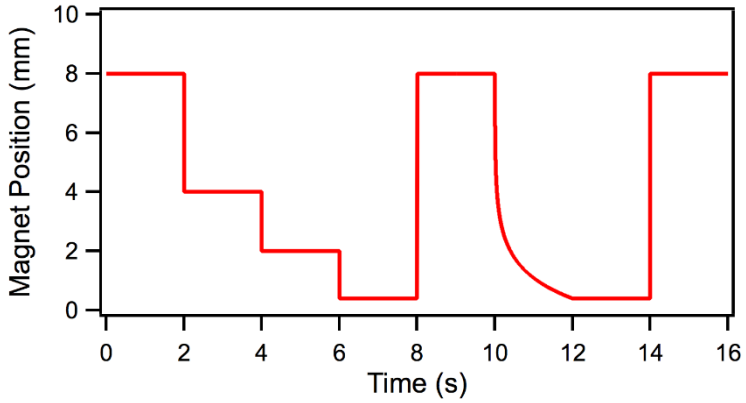


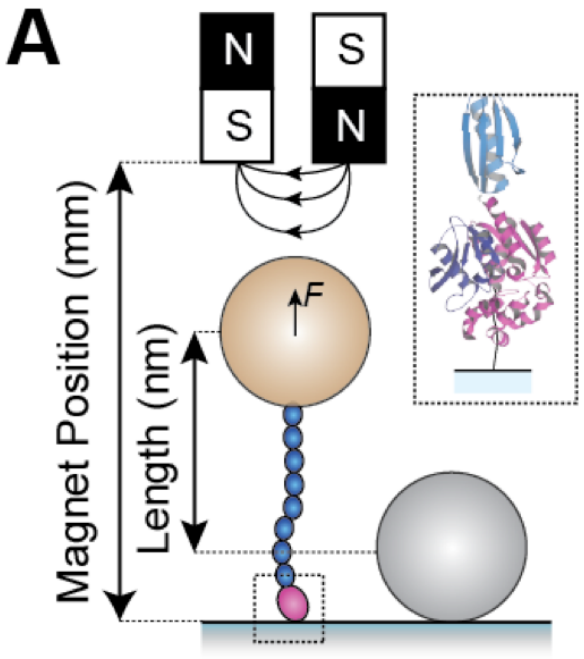


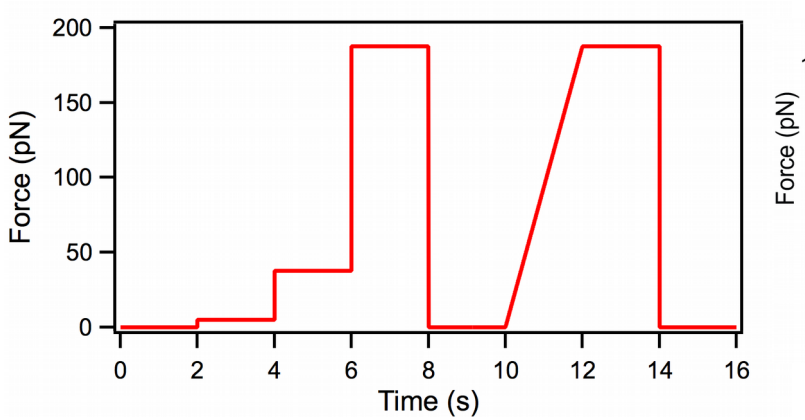


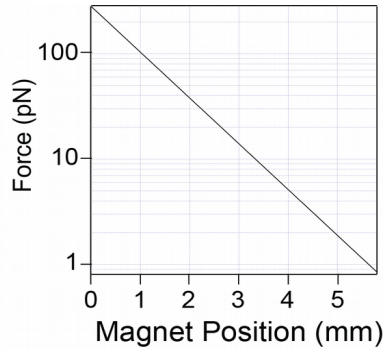
Moving coil and control of magnet position/force

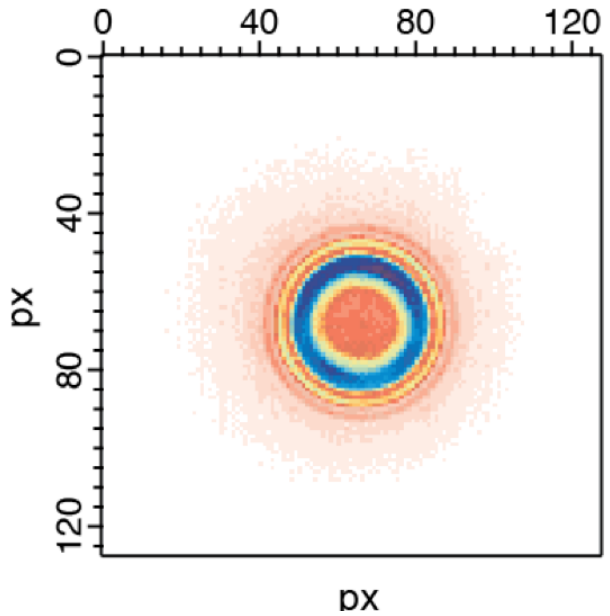


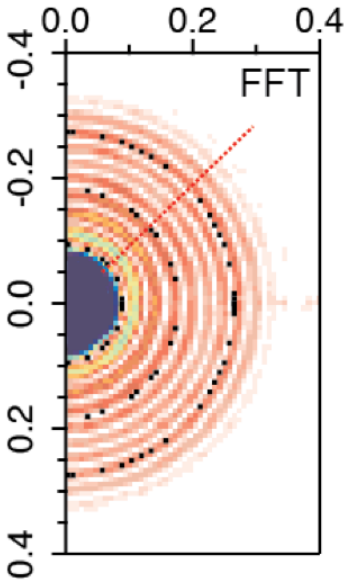


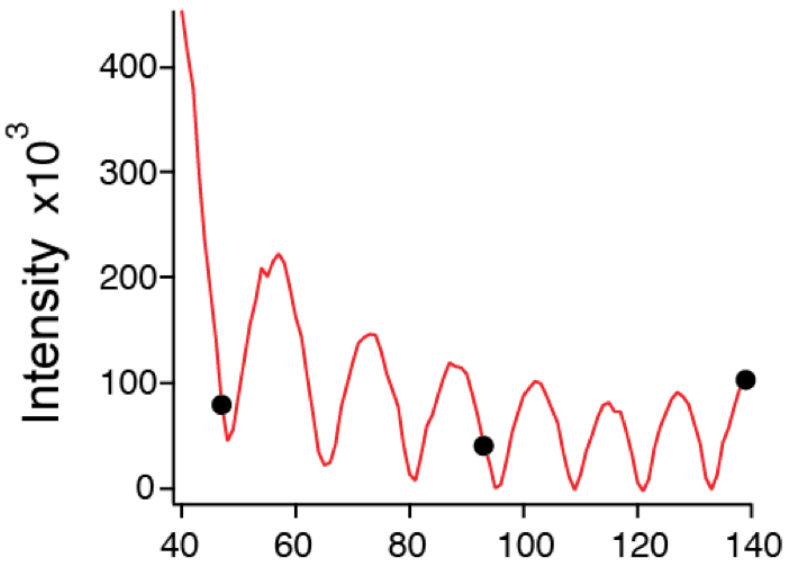


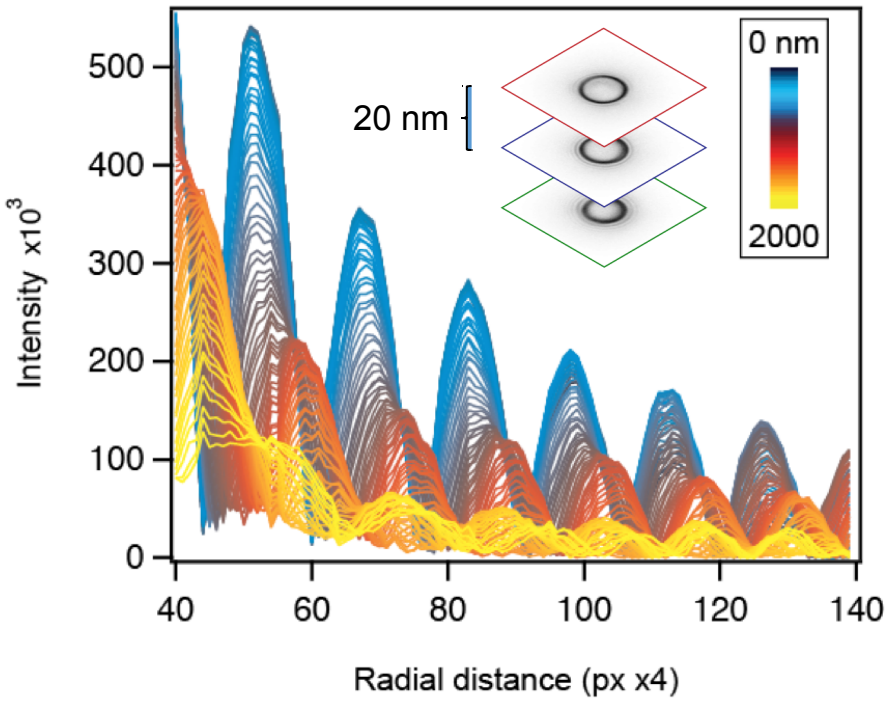


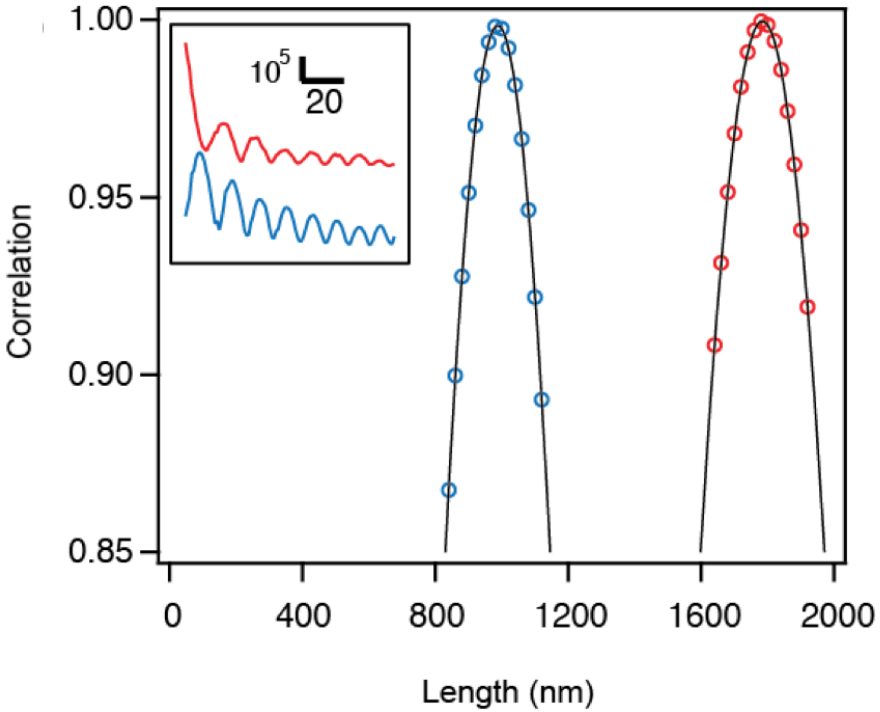



0
40
80
120
px
px

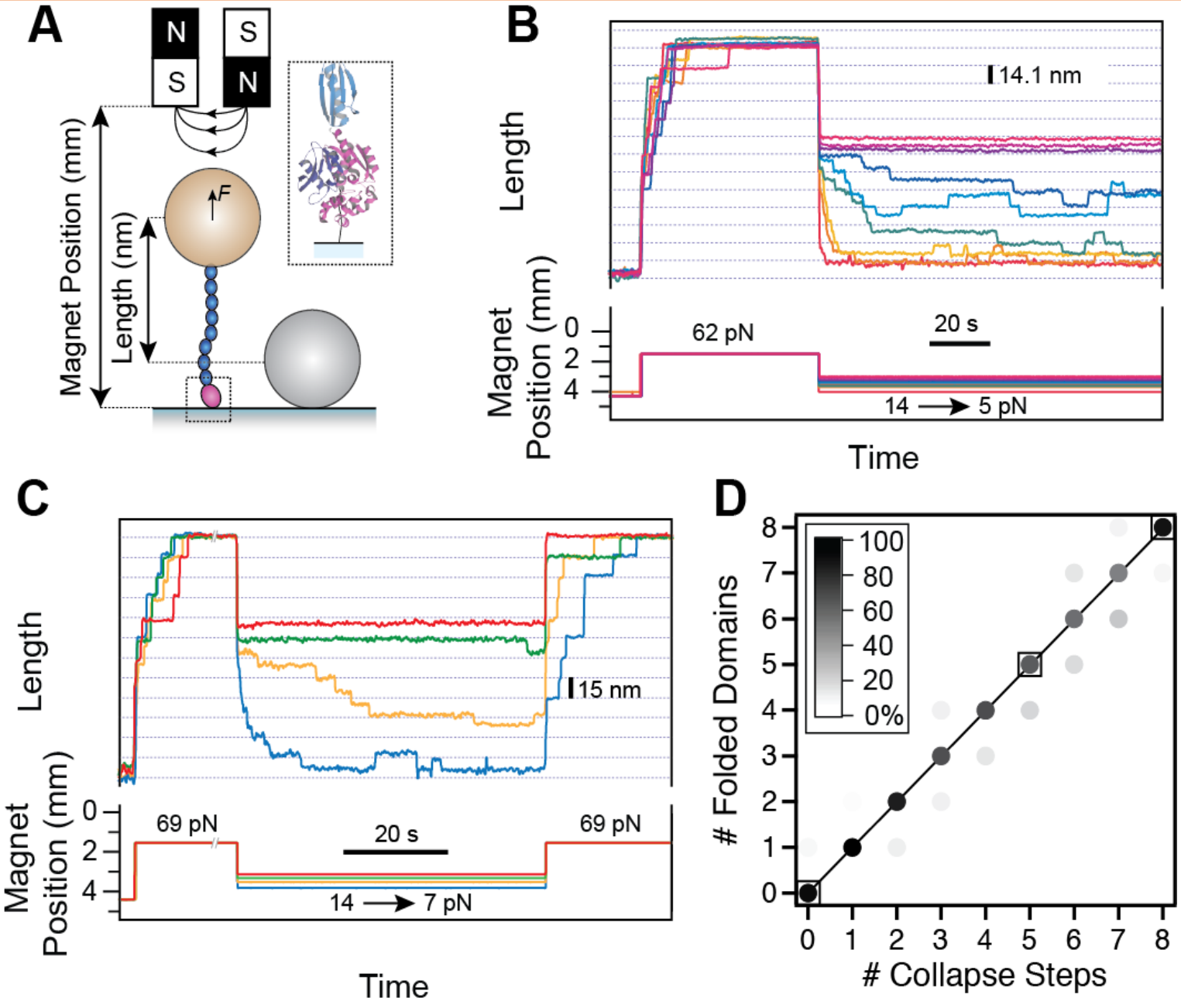

0.0
0.2
0.4
-0.4
-0.2
0.0
0.2
0.4
FFT


Intensity x10^3
400
300
200
100
0
40
60
80
100
120
140

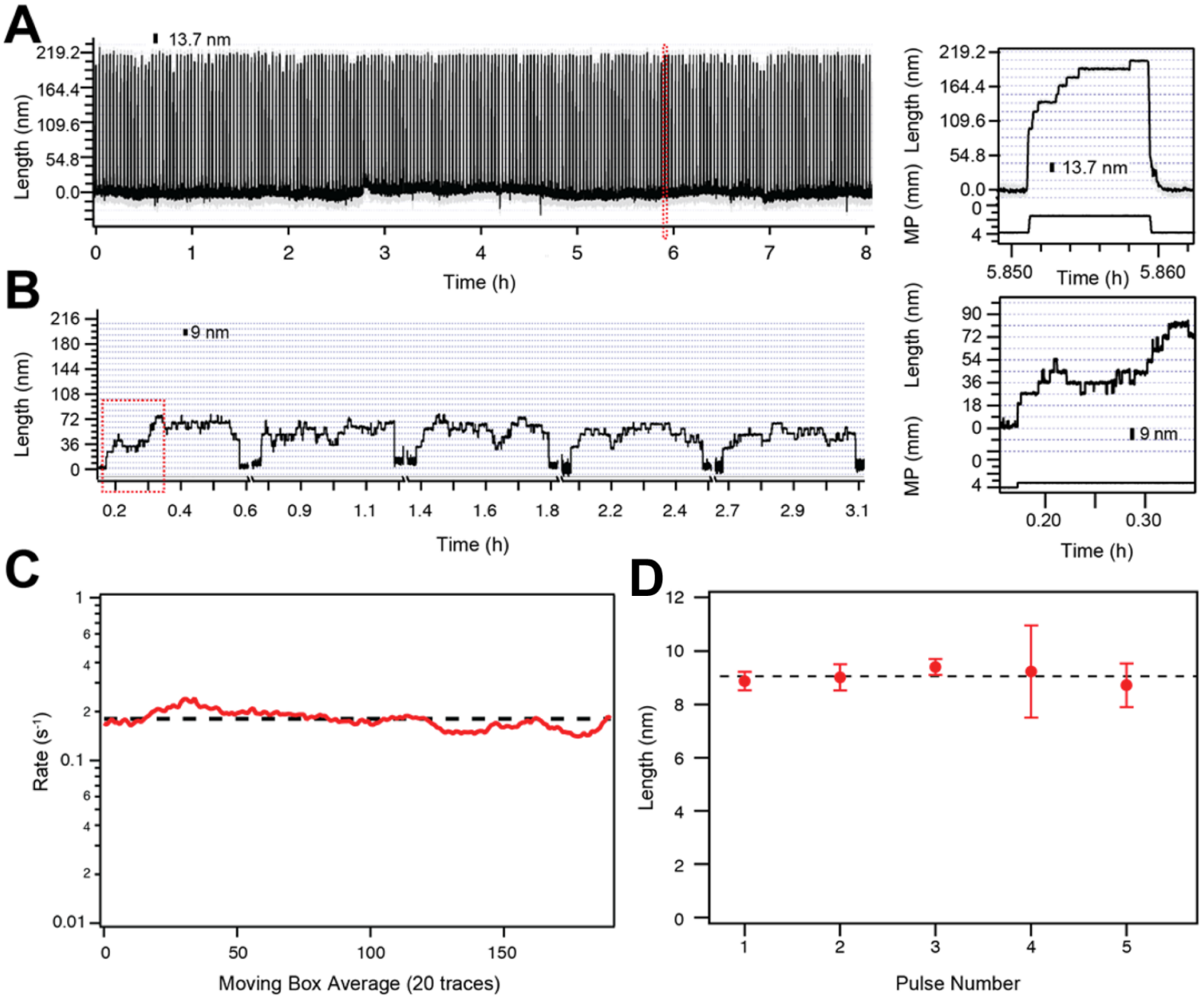

Intensity x10^3
500
400
300
200
100
0
20 nm
0 nm
2000
40
60
80
100
120
140
Radial distance (px x4)


Correlation
1.00
0.95
0.90
0.85
10^5
20
0
400
800
1200
1600
2000
Length (nm)

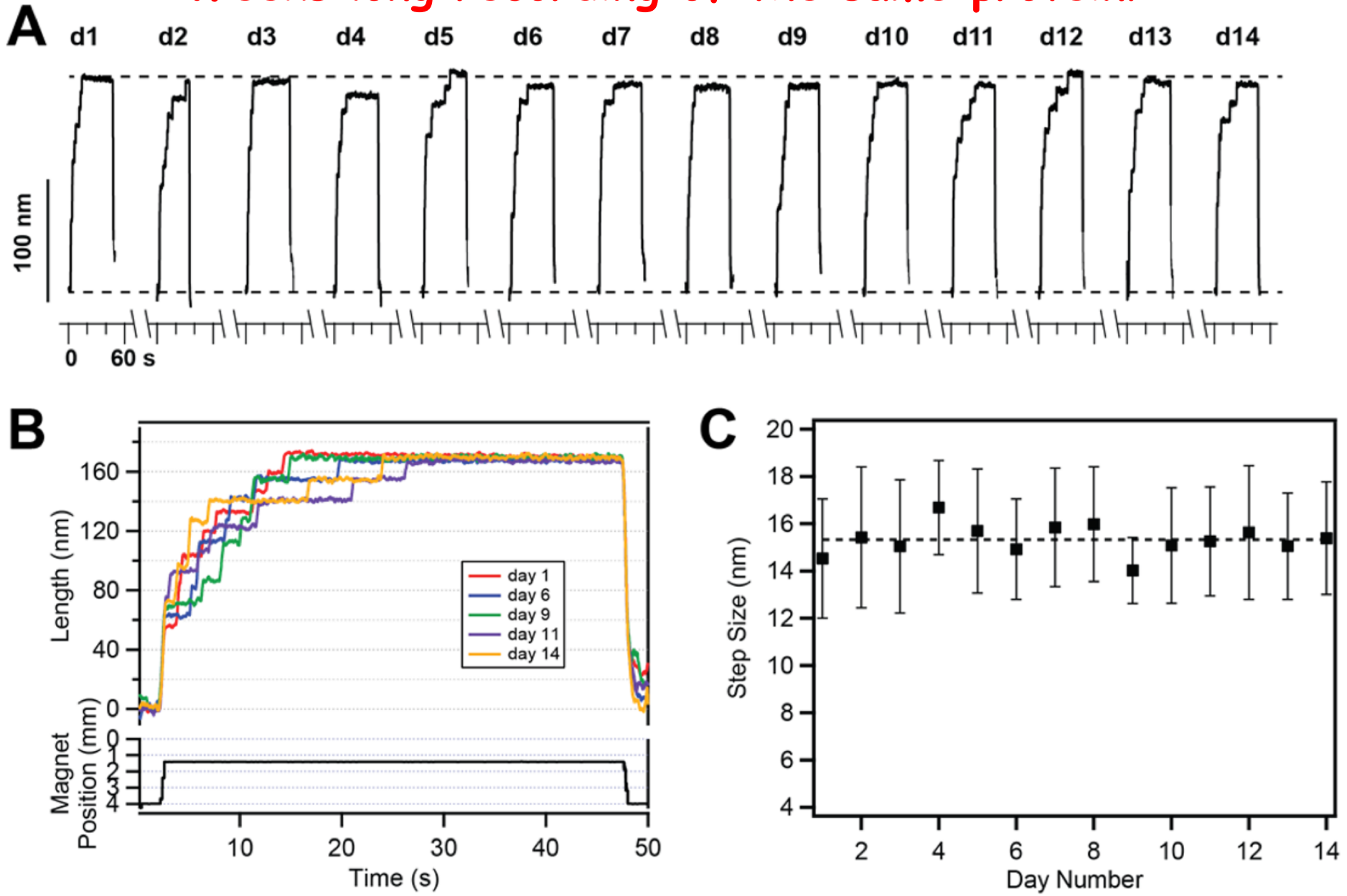
Collapse and folding dynamics of protein L_8



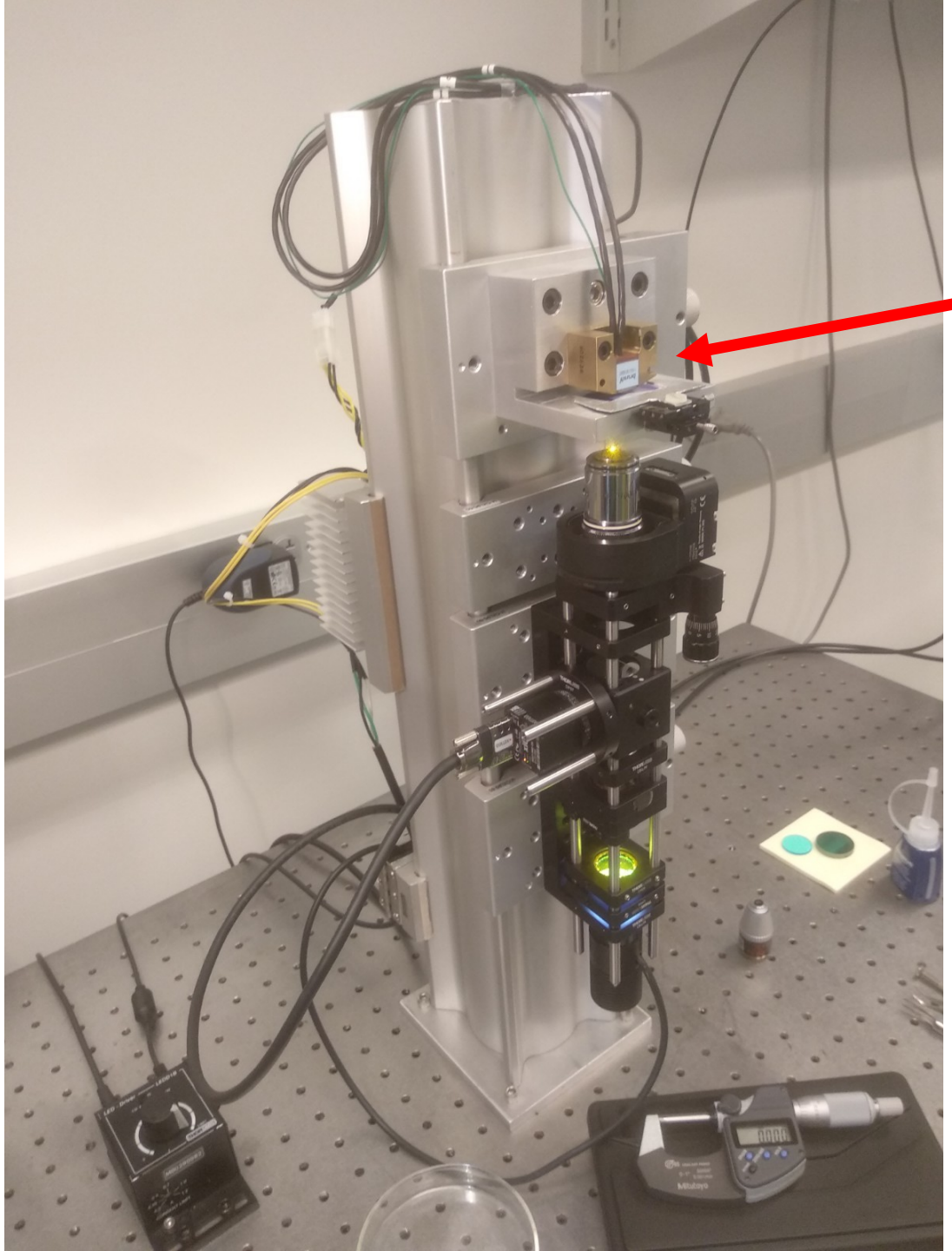
Stable recordings of a single protein

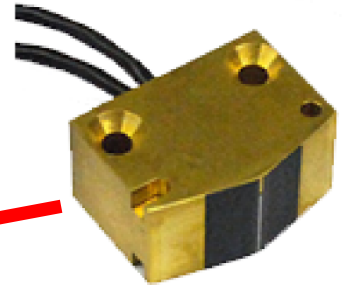


Weeks long recording of the same protein!

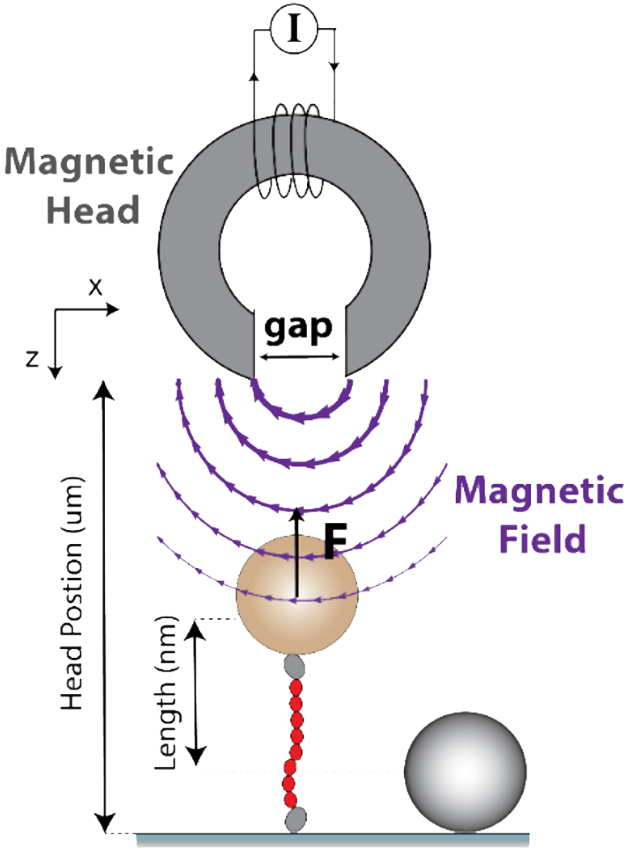


MT_3

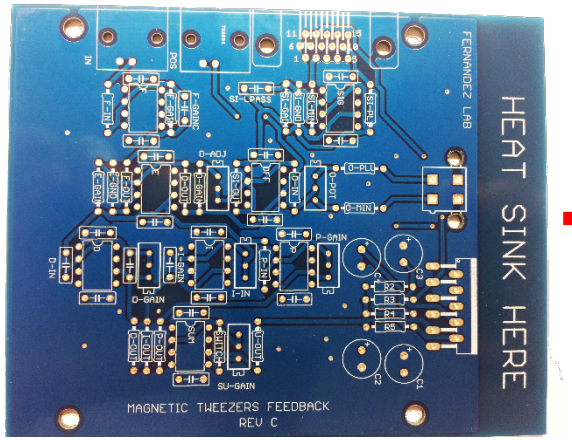




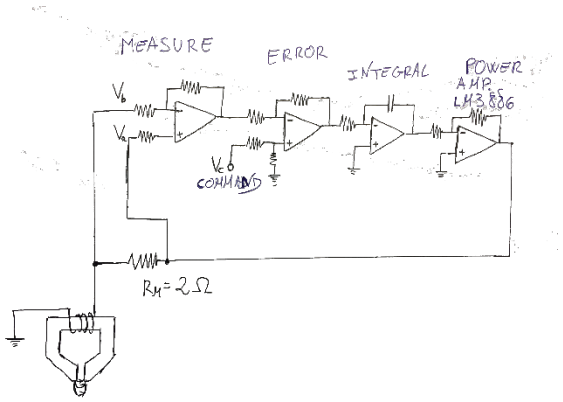
tape head

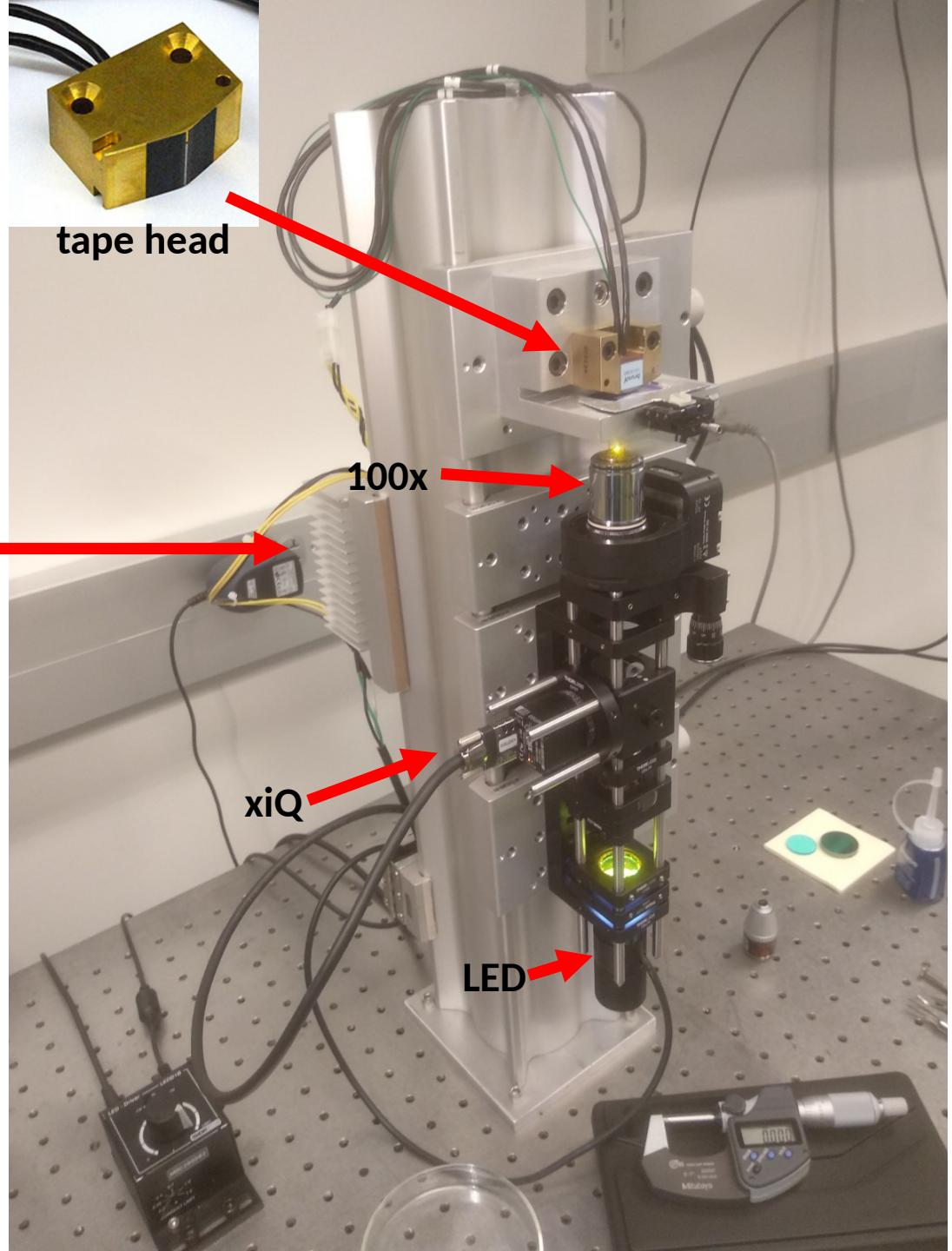


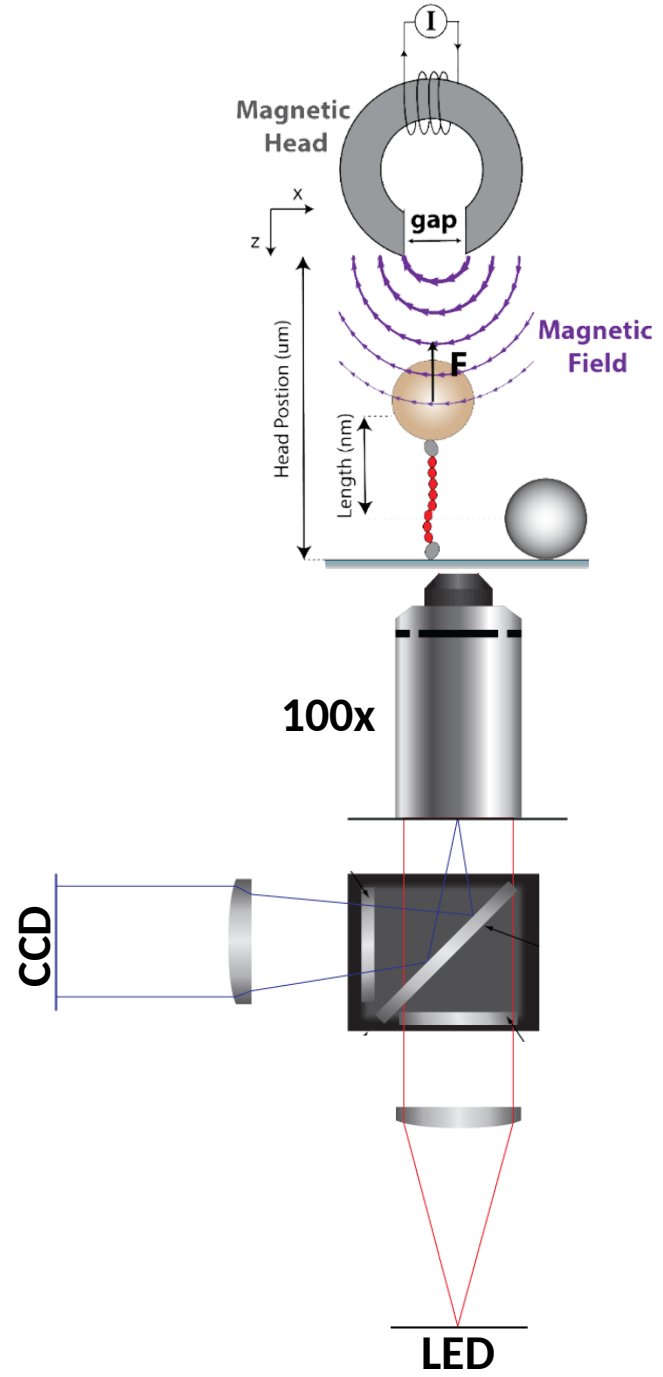
MT_3

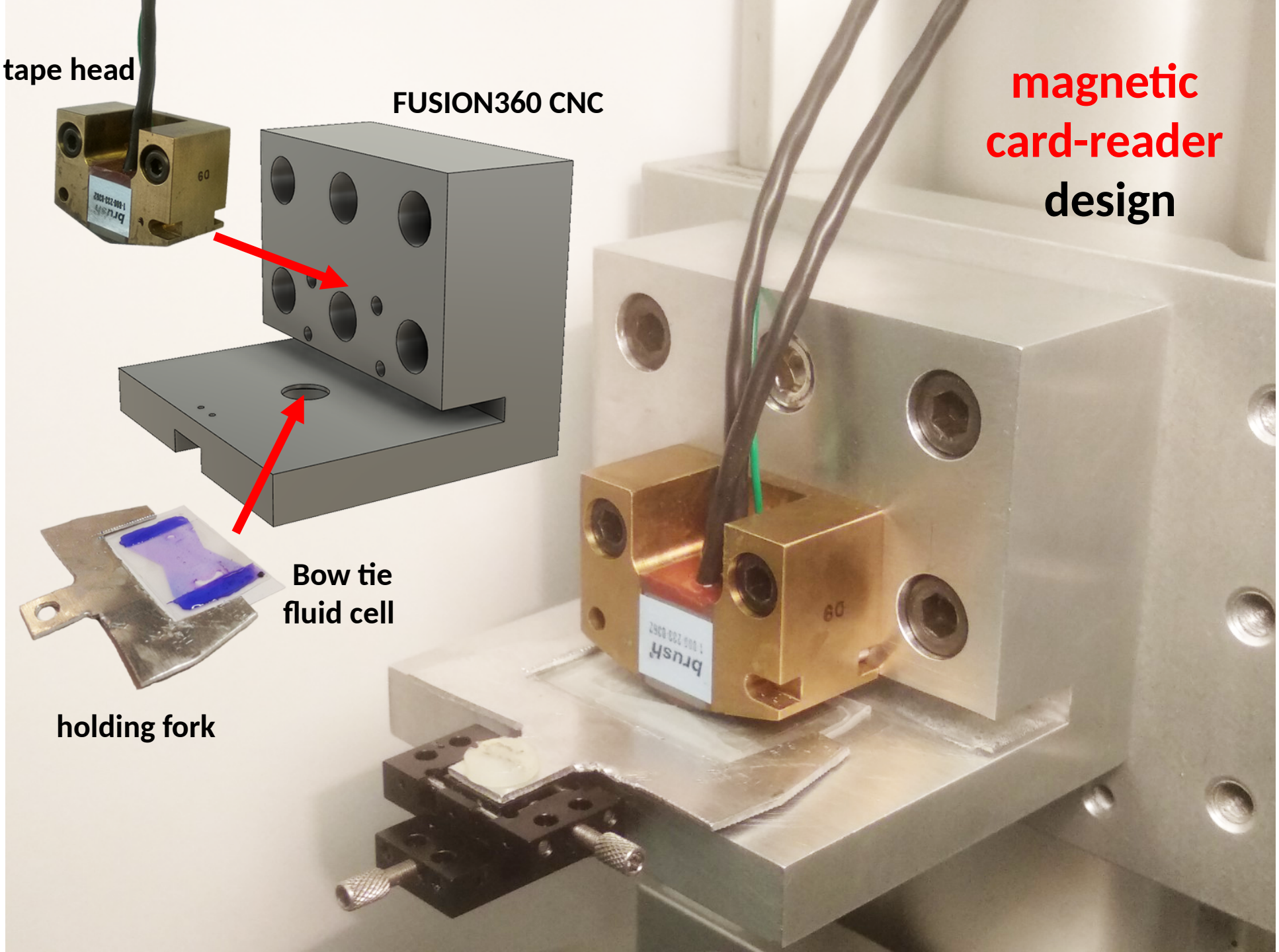


Current feedback electronics

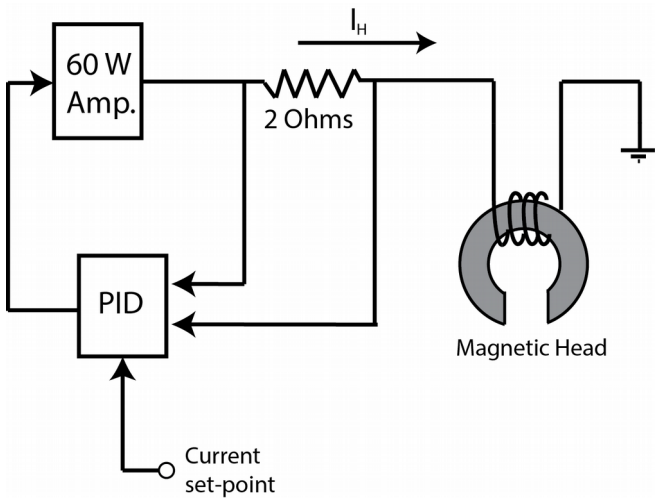


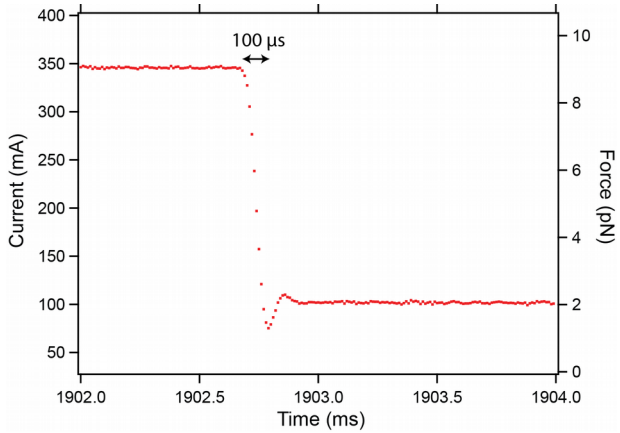


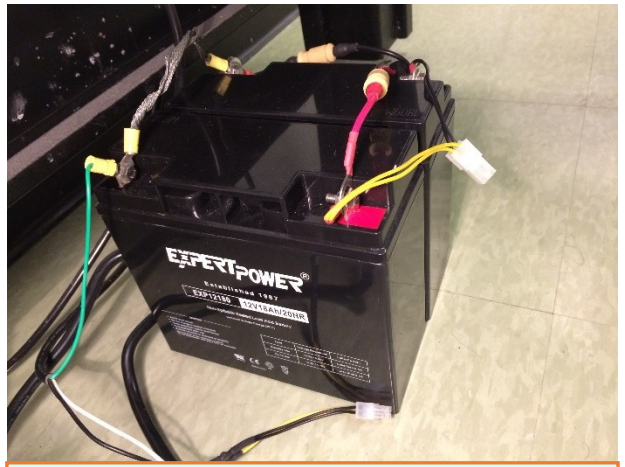



tape head
FUSION360 CNC
magnetic
card-reader
design
Bow tie
fluid cell
holding fork

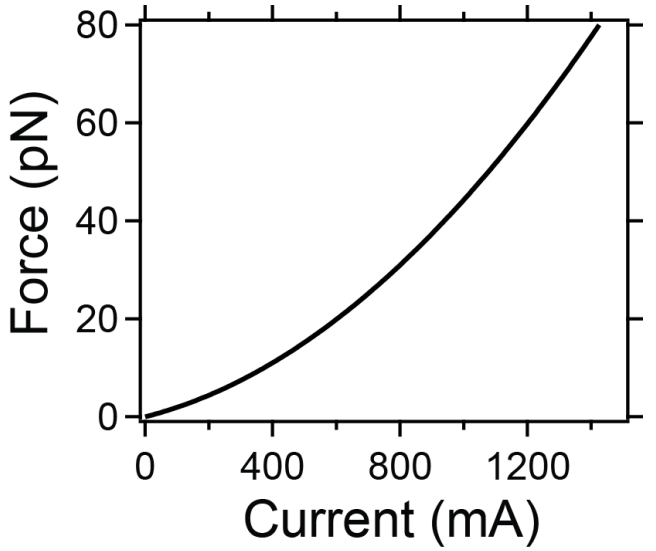
> 10 kHz bandwith with sub-pN resolution







Battery powered



$$F(I) = 2.78610^{-5} \cdot I^2 + 0.016 \cdot I$$

MT_2_EMH_Application

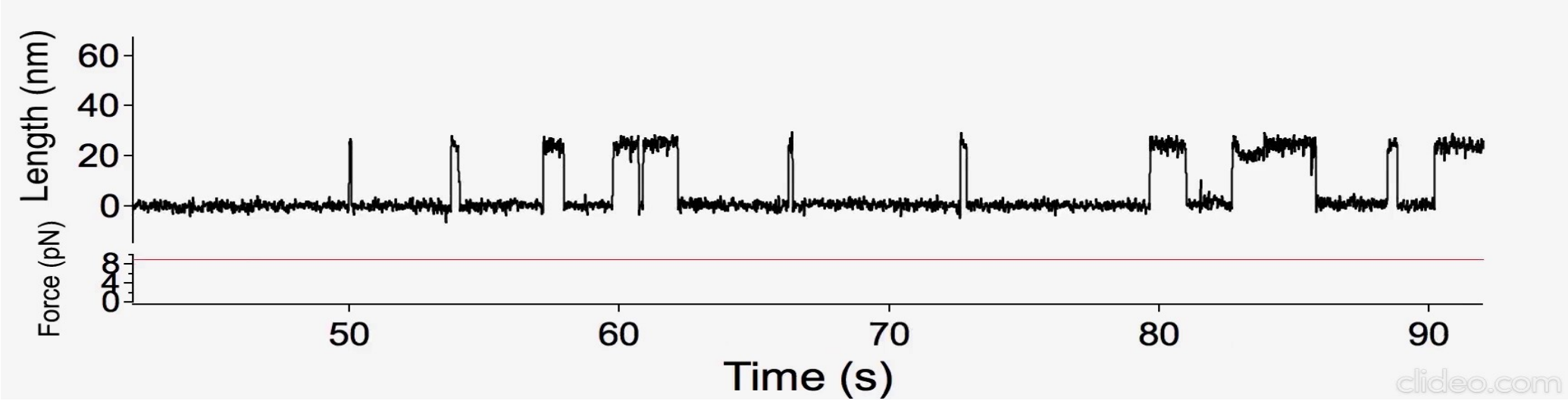
```h
#pragma once

#include <QtWidgets/QMainWindow>
#include <xiApiPlusOcv.hpp>
#include <NIDAQmx.h>
#include "ui_MT_2_EMH.h"

class MT_2_EMH : public QMainWindow
{
    Q_OBJECT
public:
    MT_2_EMH(QWidget *parent = Q_NULLPTR);
    xiAPIplusCameraOcv cam;
    TaskHandle  taskHandle_0 = 0;
    TaskHandle  taskHandle_1 = 0;
    TaskHandle  taskHandle_2 = 0;
    QGraphicsScene *scene;
    void ShowButtons(bool preview, bool init, bool R
    public slots:
    void measure();
    void show_ROI();
    void get_stack();
    void Focus_box(int value);
    void Current_box(double MP);
    void Send_pulse();
private:
    Ui::MT_2_EMHClass ui;
};
```

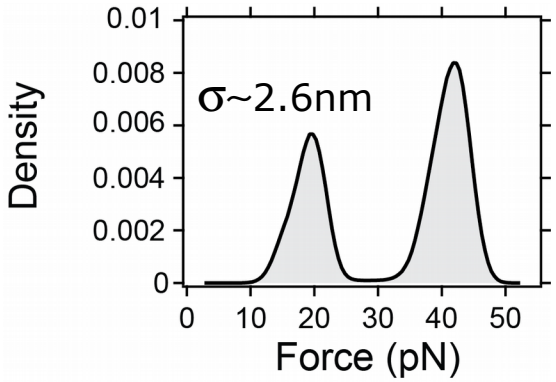
C++ code implementing “kick-ass” modules

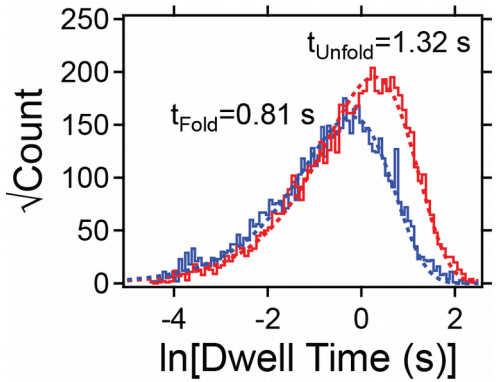
MT_2_EMH_Application | MT_2_EMH | measure()

```cpp
#include ...

using namespace ...

/////////Function Declarations/////////////////
void onmouse(int event, int x, int y, int flags, void* param);
Mat FFT_MT(Mat Img);
float RadialVector(Mat Img, float *Profile);
void SetupDisplay();
float64 AI_Buffer[500];
float AI_average;
int32 CVICALLBACK EveryNCallback(TaskHandle taskHandle, int32 everyNsamplesEventType, uInt32 nSamples, void *callbackData);

/////////////////Globals//////////////////////
Point M_1, M_2, R_1, R_2;
Point M_1S, M_2S, R_1S, R_2S;
Point H_1 = { 480,0 }; Point H_2 = { 800,1024 };//  Magnetic head only
int mouse_x, mouse_y, mouse_z;
int Save_mode_on = 0;
int mouse_c; int currentFocus; int lockFocus = 50;
float M_Stack[128][100]; float R_Stack[128][100];
__int64 now, t0;
int update_rate = 100;//samples/s
float Measuring_resistance = 2;//  in Ohm
int32 num_read;
float Karlqvist_A = 0.386;
float Karlqvist_B = 9.462;

MT_2_EMH::MT_2_EMH(QWidget *parent) { ... }

void MT_2_EMH::measure() { ... }

void MT_2_EMH::get_stack() { ... }

void SetupDisplay() { ... }

Mat FFT_MT(Mat Img) { ... }

float RadialVector(Mat Img, float *Profile) { ... }

void MT_2_EMH::show_ROI() { ... }

void onmouse(int event, int x, int y, int flags, void* param) { ... }

void MT_2_EMH::Current_box(double F) { ... }

void MT_2_EMH::Focus_box(int value) { ... }

void MT_2_EMH::Send_pulse() { ... }

void MT_2_EMH::ShowButtons(bool measure, bool savedata, bool ROI, bool stack) { ... }

int32 CVICALLBACK EveryNCallback(TaskHandle taskHandle, int32 everyNsamplesEventType, uInt32 nSamples, void *callbackData) { ... }
```

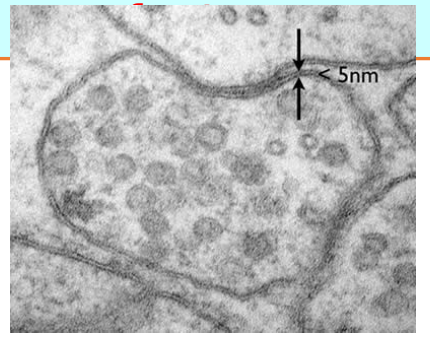
5 hour long recording of talin at 1400 fps with a total drift of 7 nm

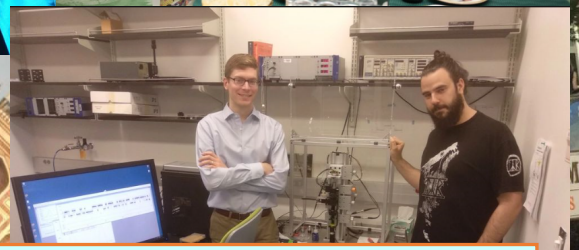
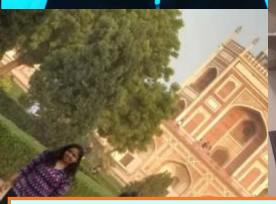




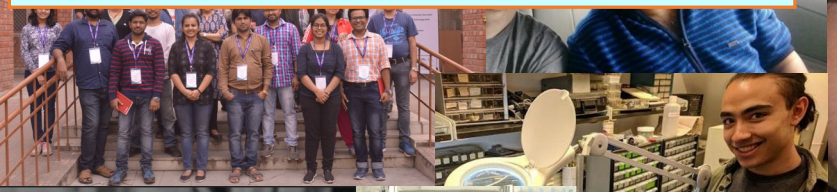
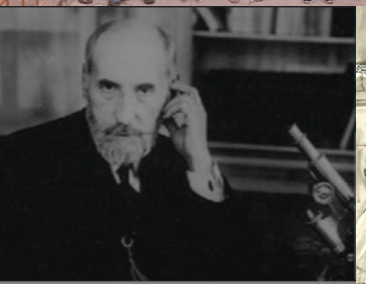
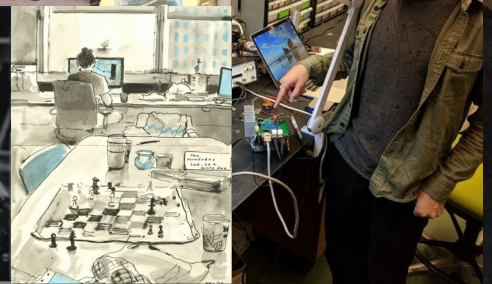


MT_3 has the resolution of an electron microscope at >1400



Look us up in zeptowatt.com

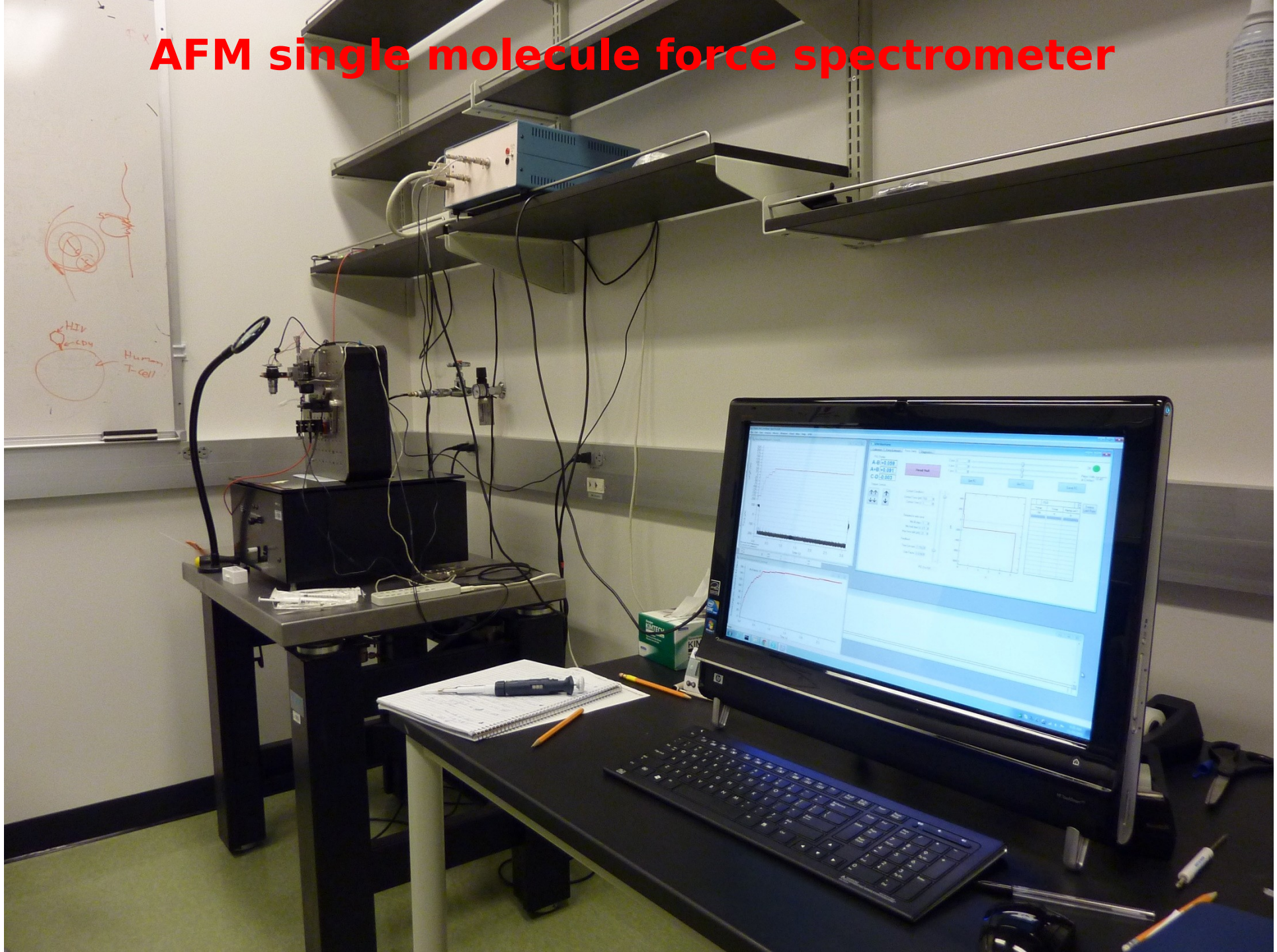




EVERYTHING IN BIOLOGY IS MECHANICAL

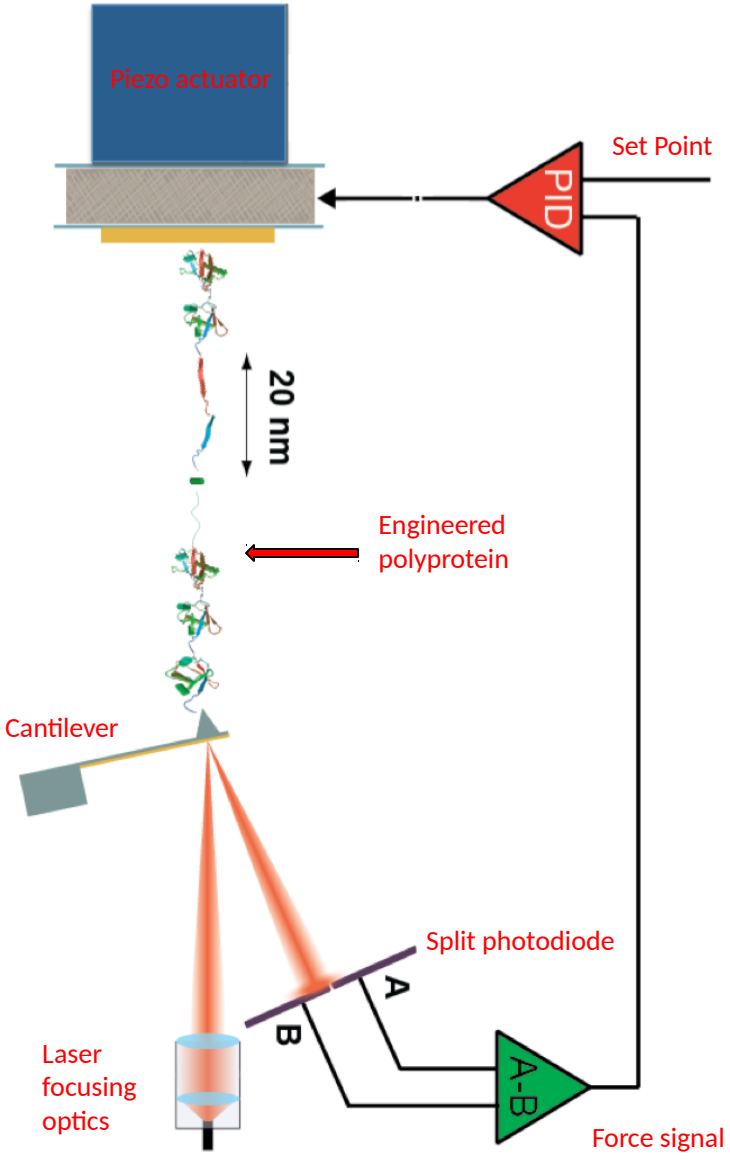
FERNANDEZ LAB

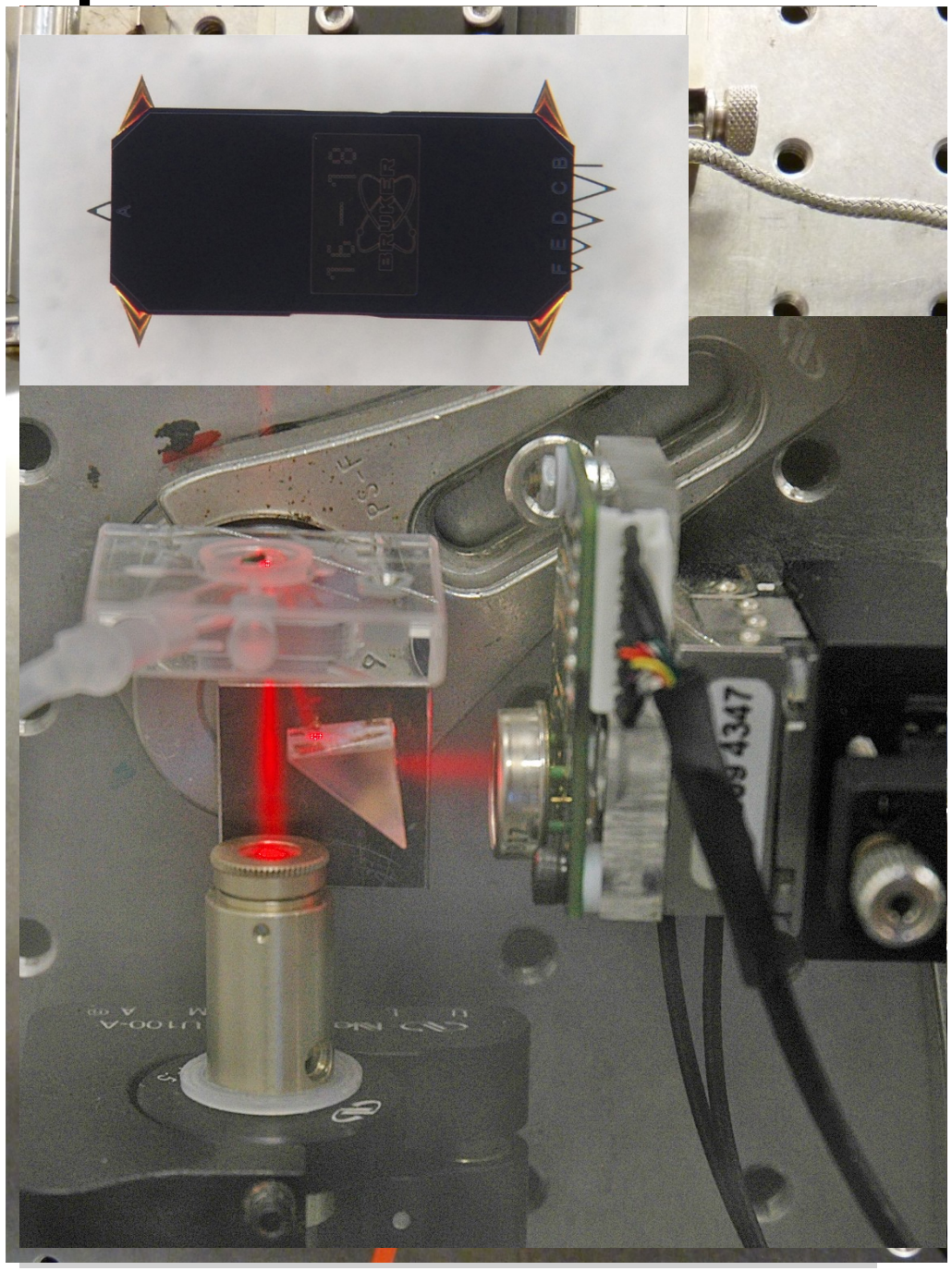
– HOME PUBLICATIONS SCIENTISTS PHOTOS DOWNLOADS CONTACT

AFM single molecule force spectrometer

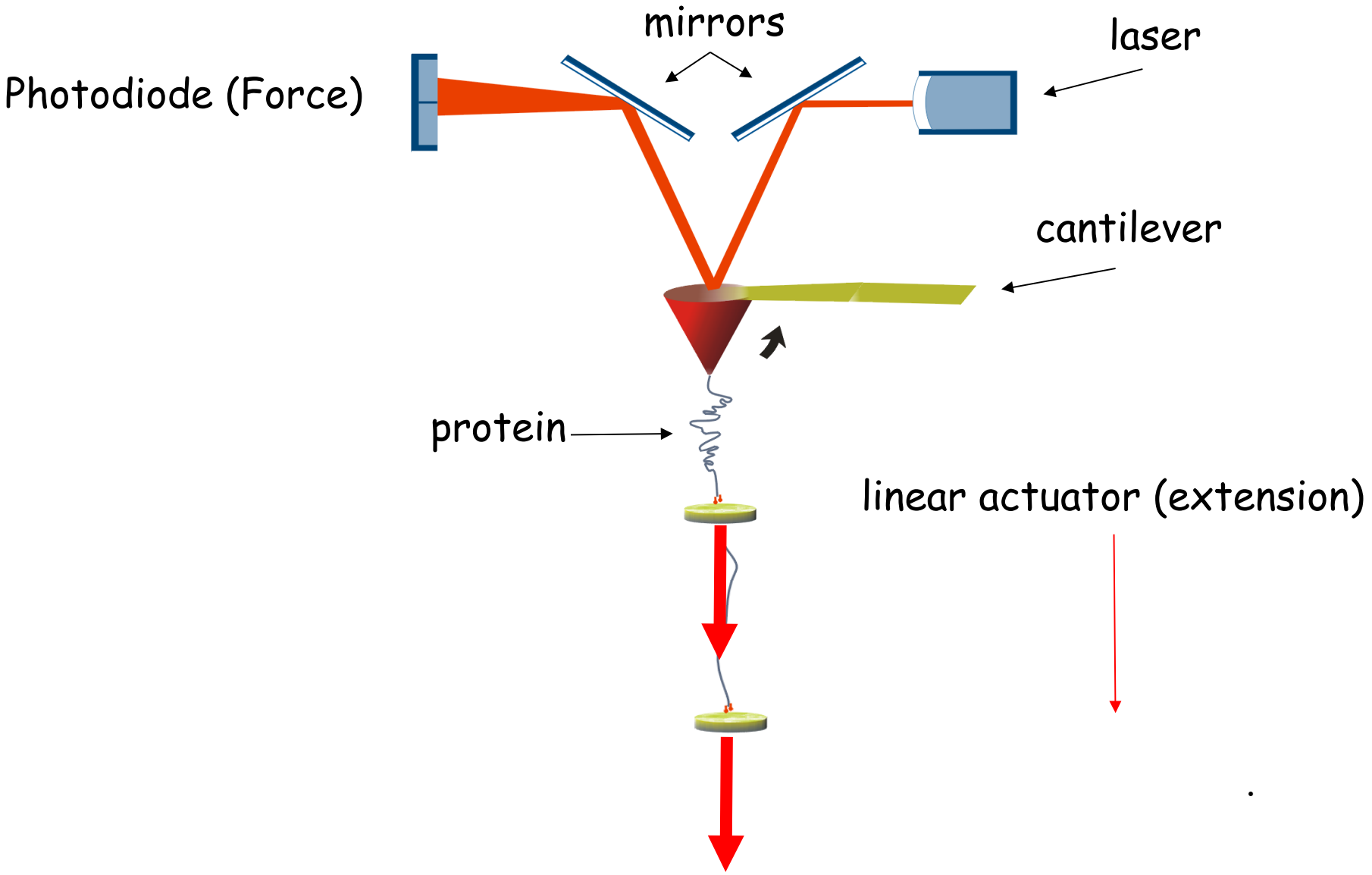


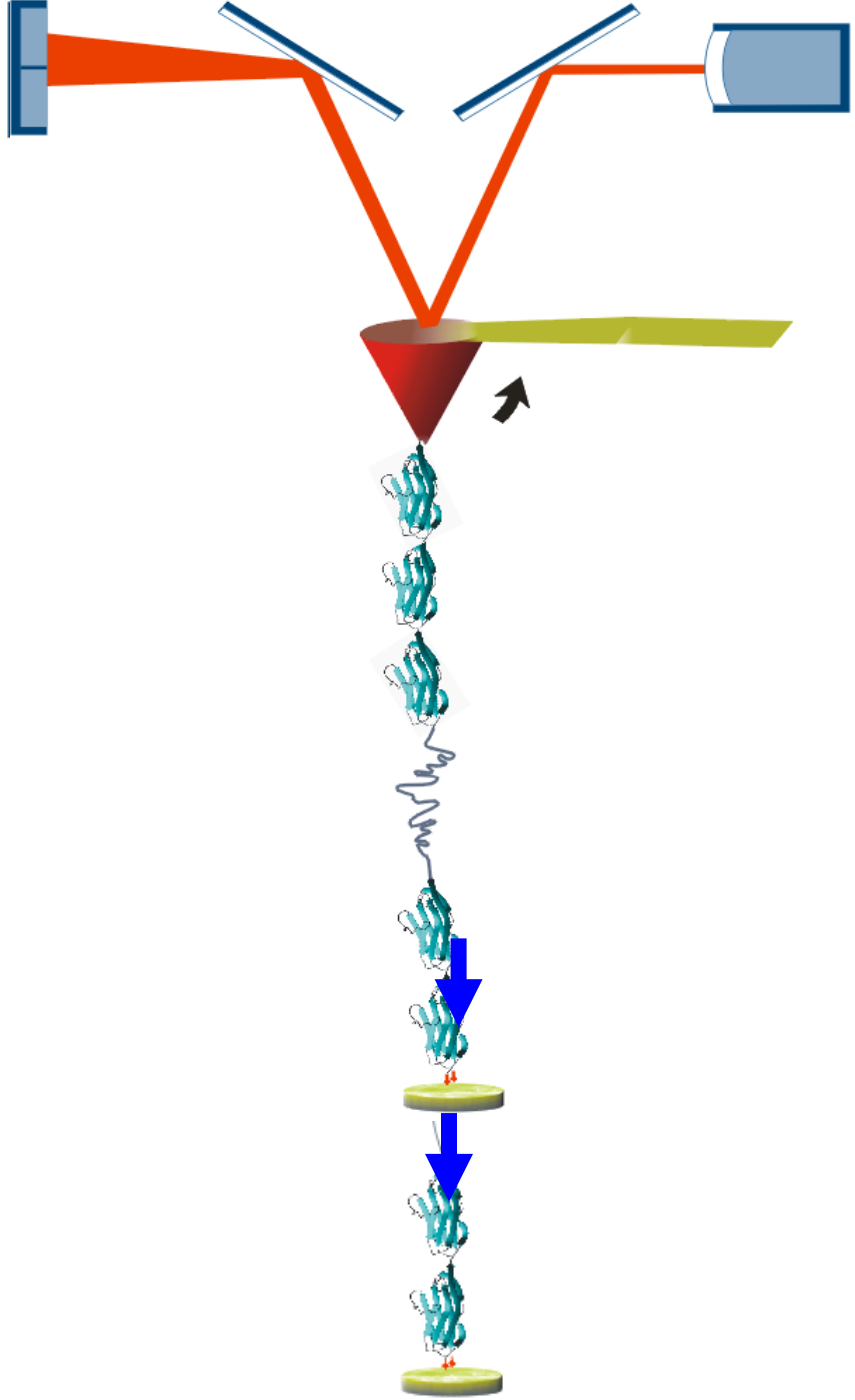
Force sensor and piezoelectric actuator

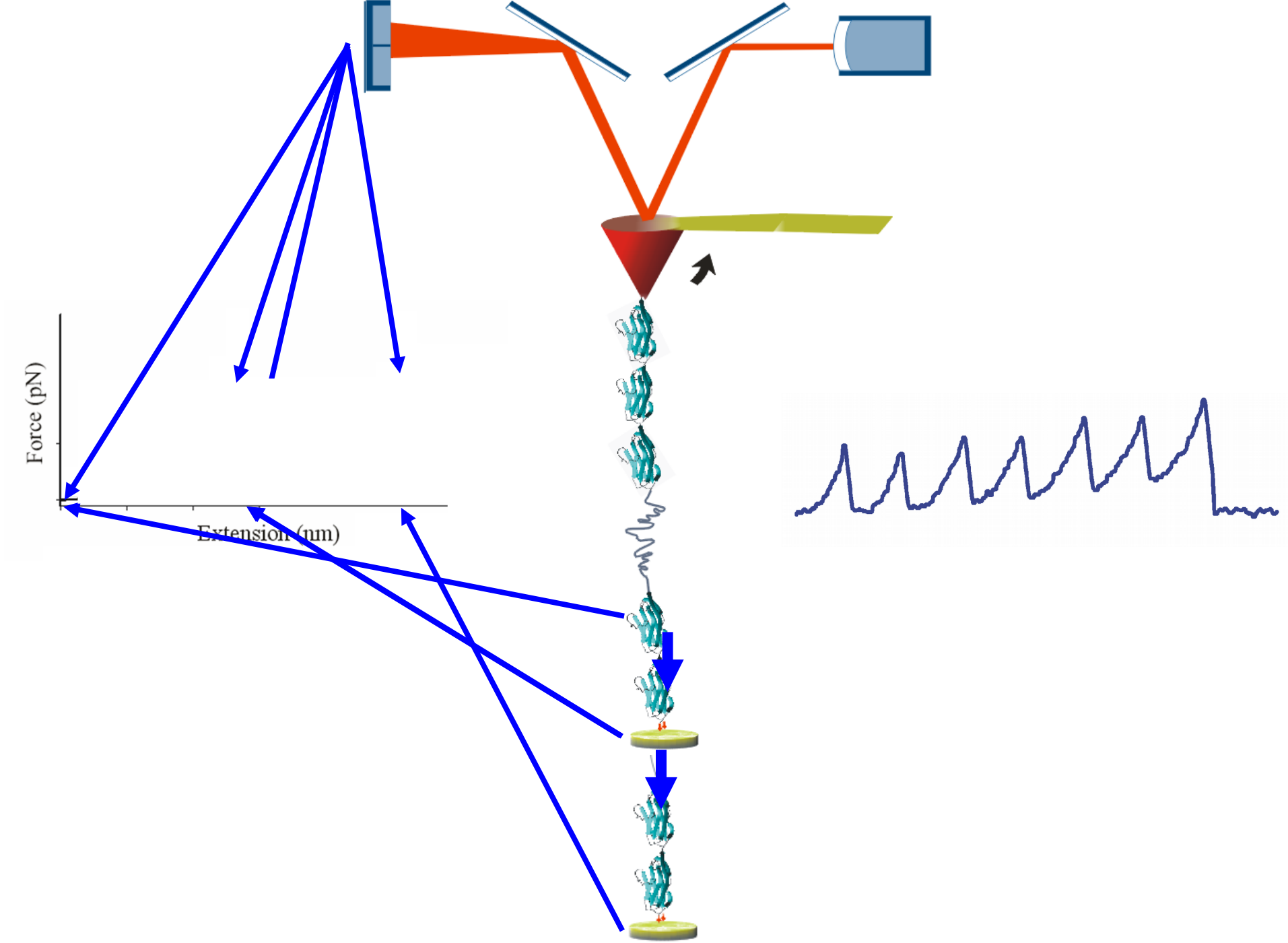


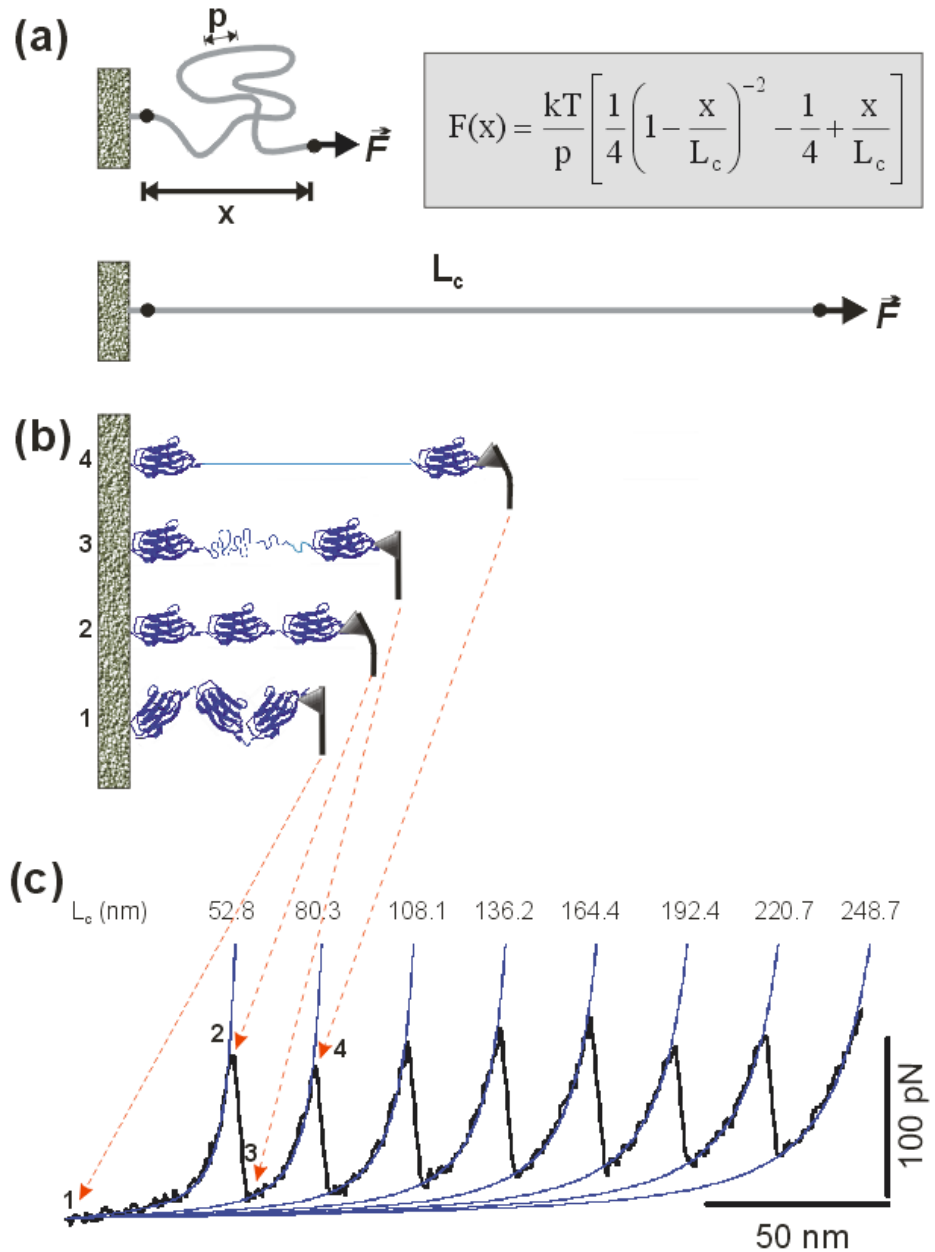


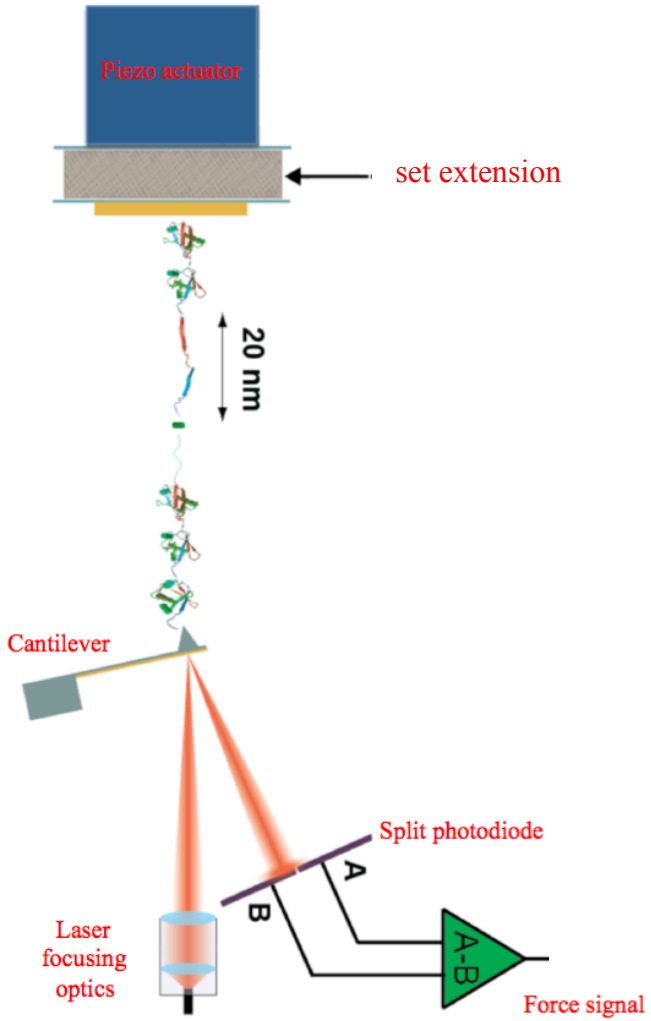
We can stretch a single protein and measure how does the restoring force changes with the extension.



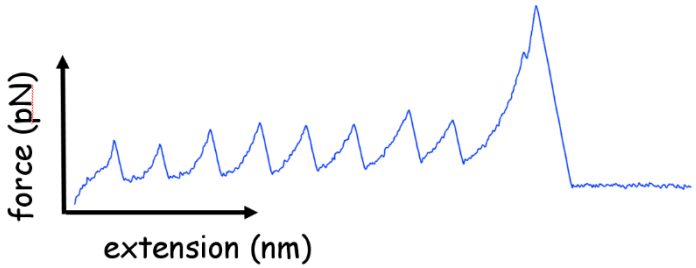



Force (pN)
Extension (nm)

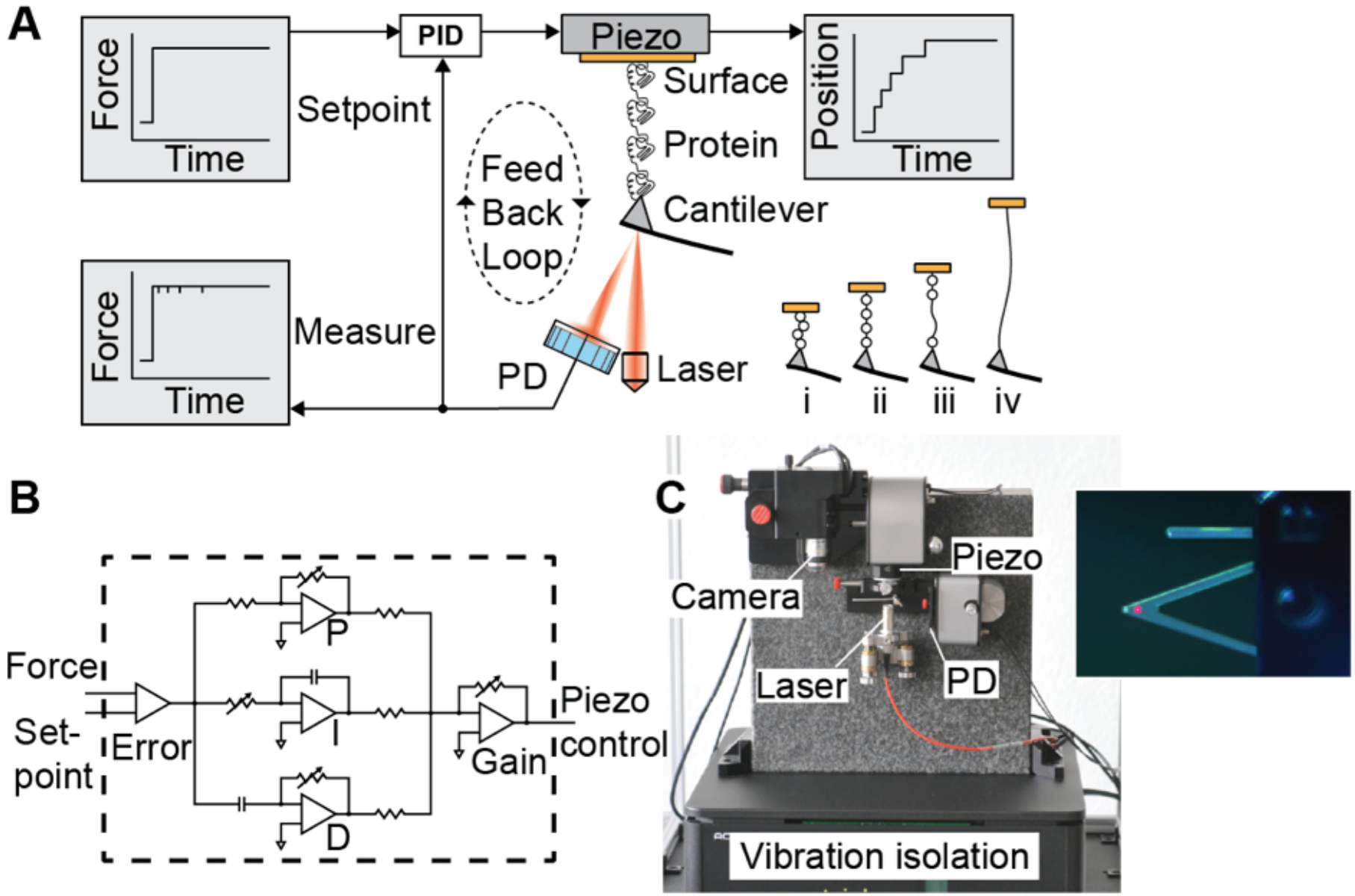

(a)
p
F
x
$F(x) = \frac{kT}{p}\left[\frac{1}{4}\left(1-\frac{x}{L_c}\right)^{-2} - \frac{1}{4} + \frac{x}{L_c}\right]$
L_c
F
(b)
4
3
2
1
(c)
L_c (nm)
52.8
80.3
108.1
136.2
164.4
192.4
220.7
248.7
1
2
3
4
100 pN
50 nm



Constant velocity mode



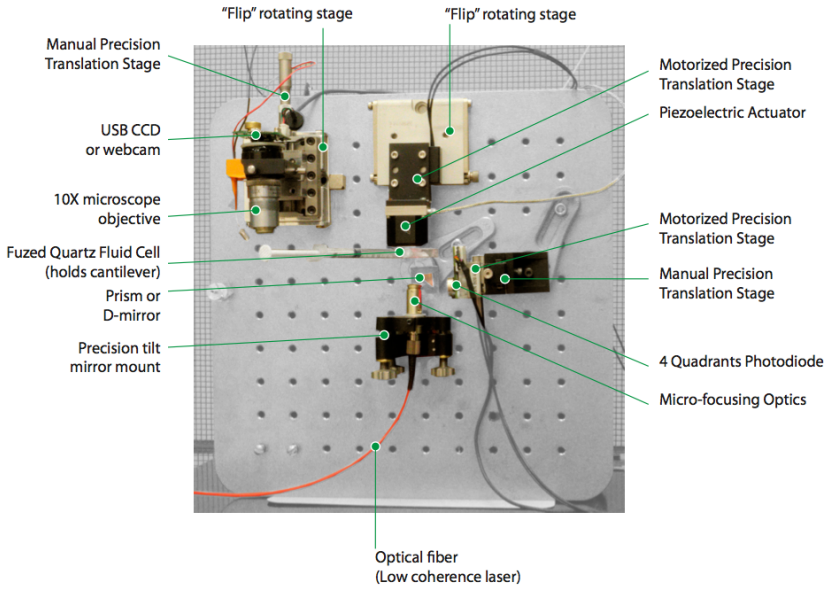
Force-clamp spectroscopy apparatus

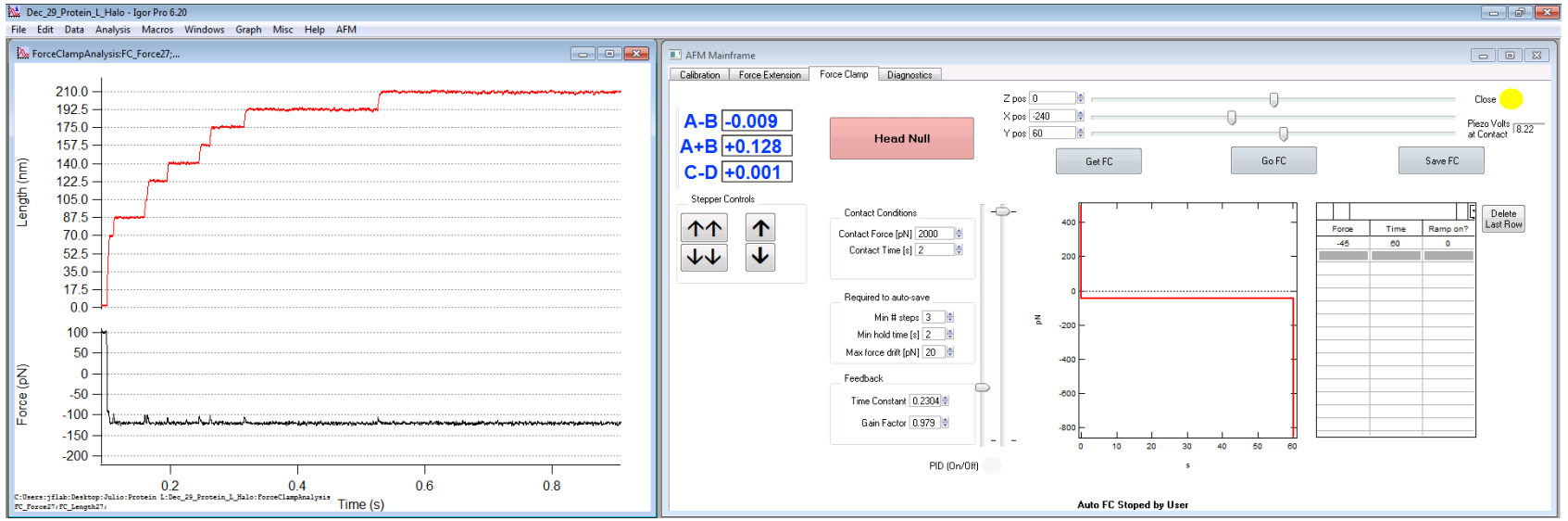


AFS Software (Igor)

Single Molecule Atomic Force Spectrometer

- Force-clamp and force-extension
- Sub-nanometer resolution
- Sub-millisecond time resolution
- Protein folding and unfolding
- Bond cleavage and formation
- Fully automated operation
- Powerful analysis software
- Simple user interface





AFS: Feedback electronics

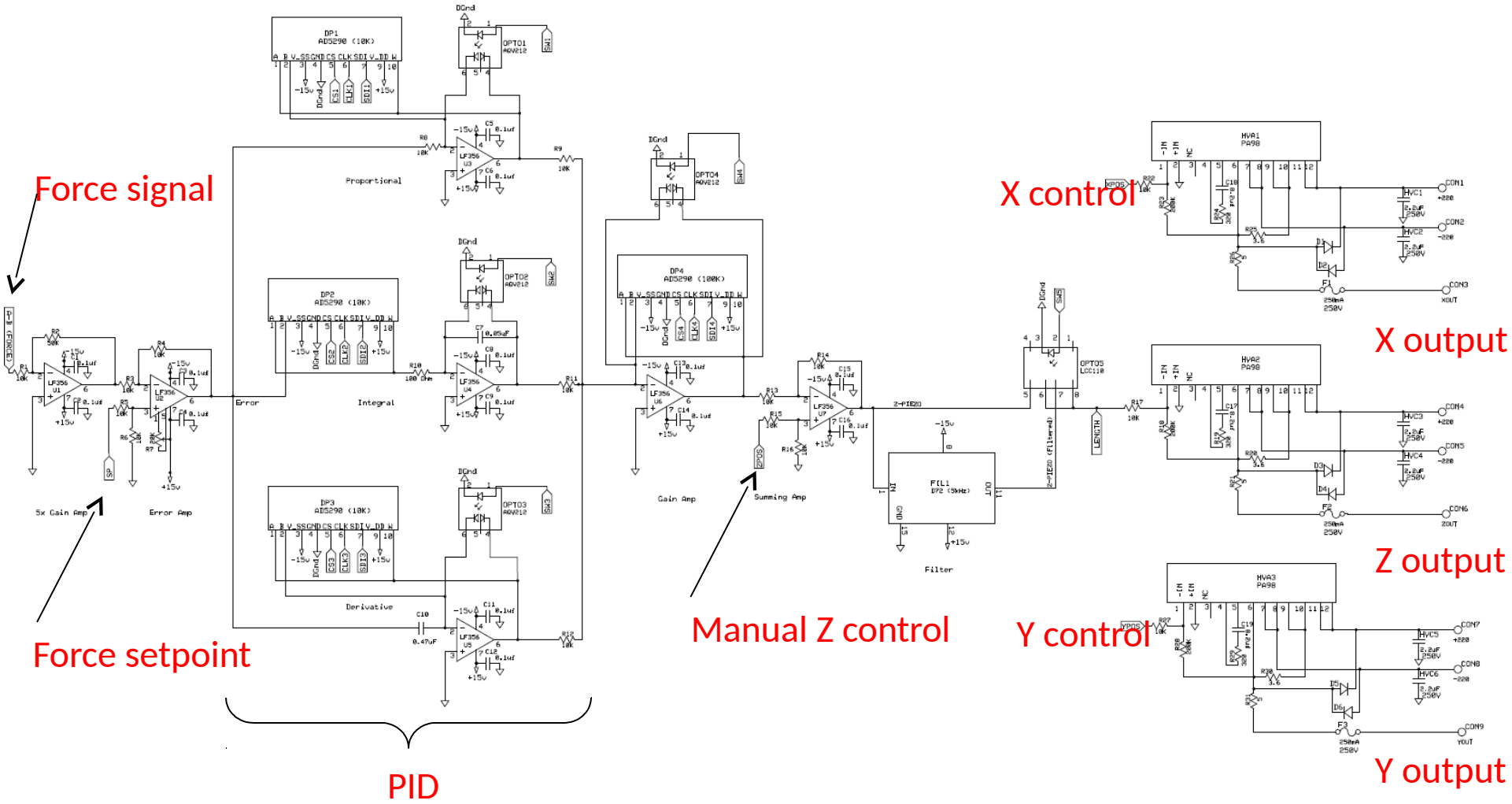
$$u(t) = MV(t) = K_p e(t) + K_i \int_0^t e(\tau)\,d\tau + K_d \frac{d}{dt} e(t)$$

Force measurement

Force setpoint

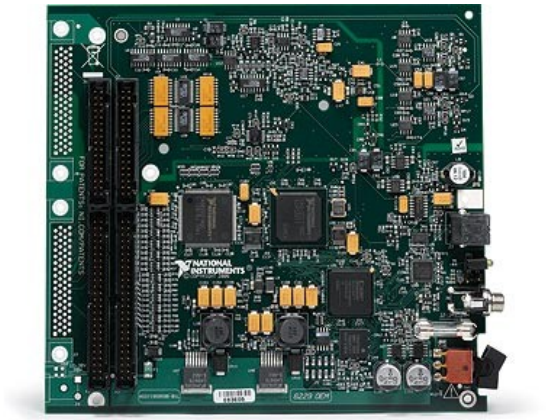
PID

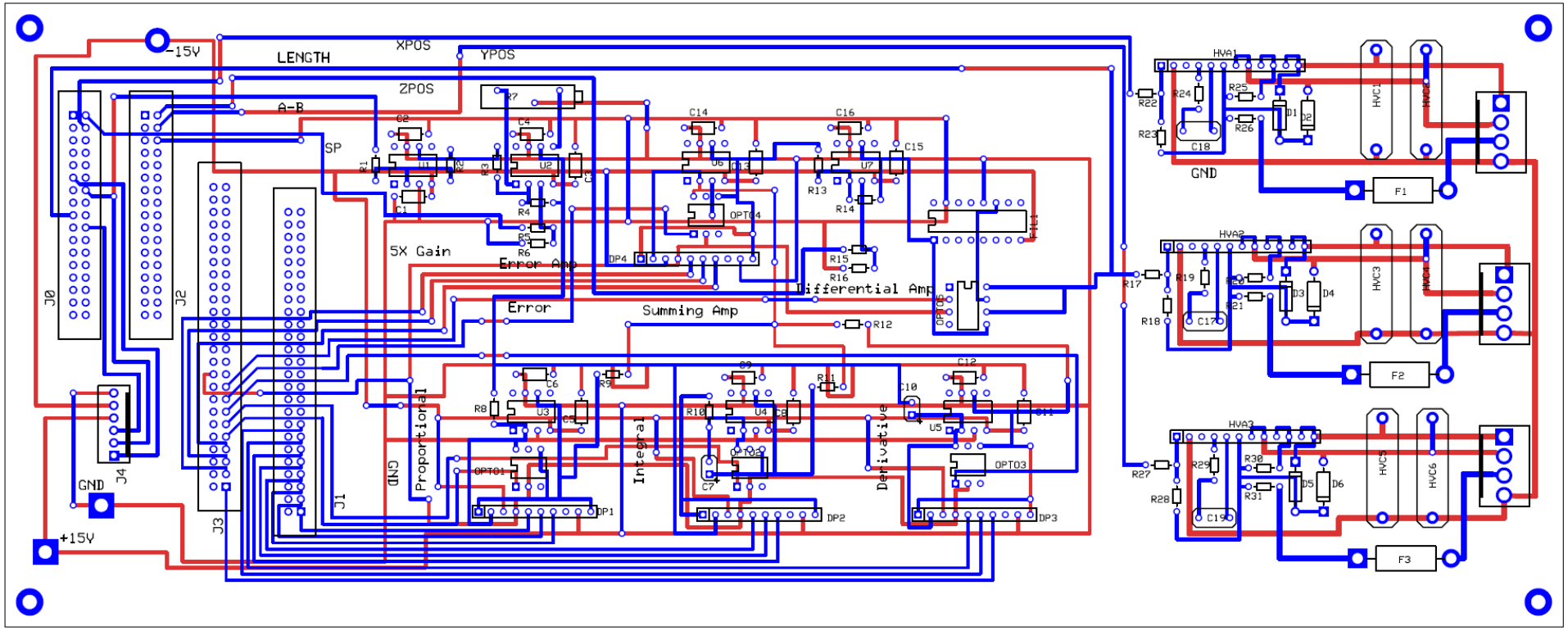
Z position



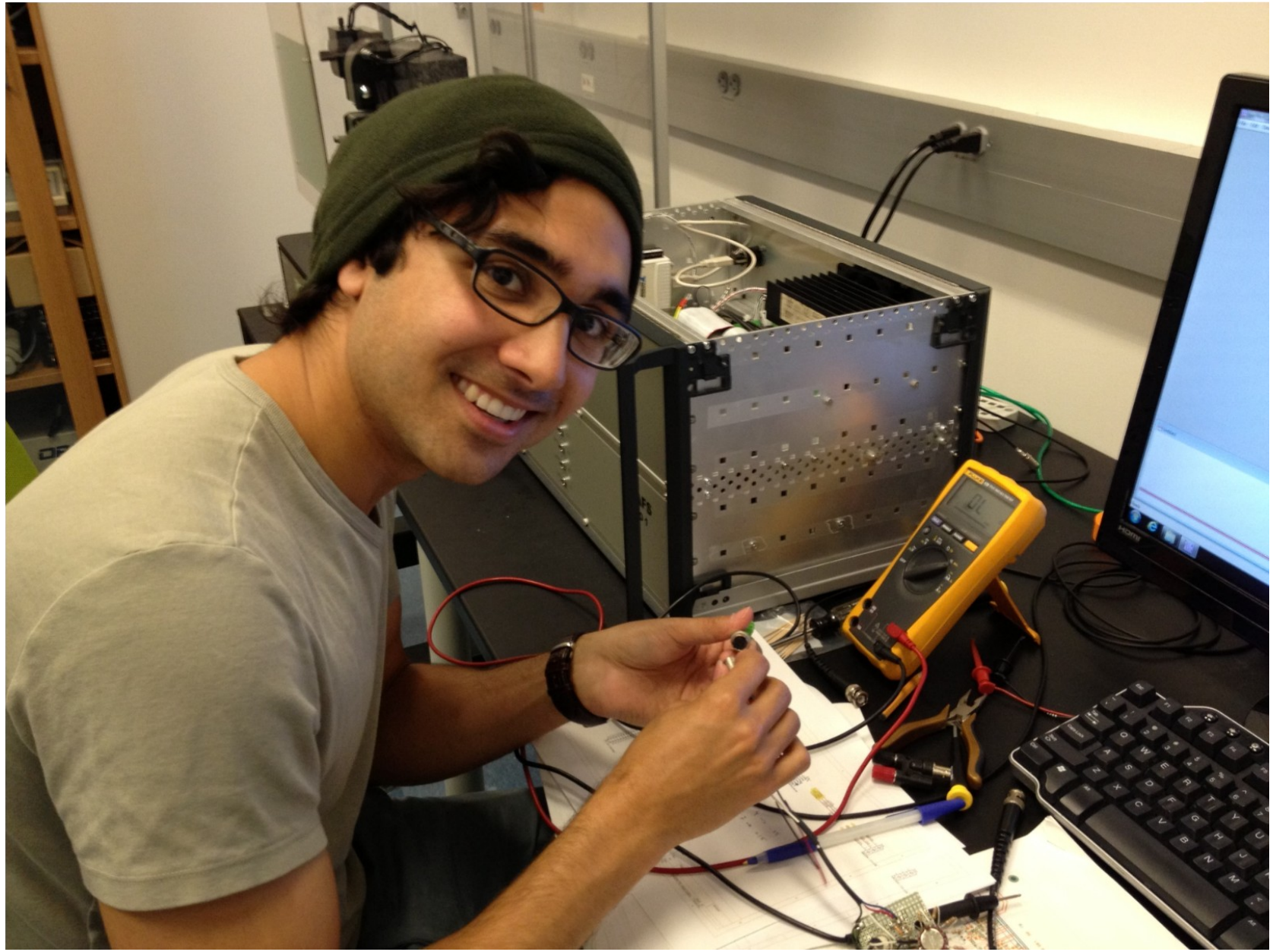
AFS: Circuit boards

- AFS controller allows complete hands-off operation
- Standard DAQ
- Connects to any computer via USB

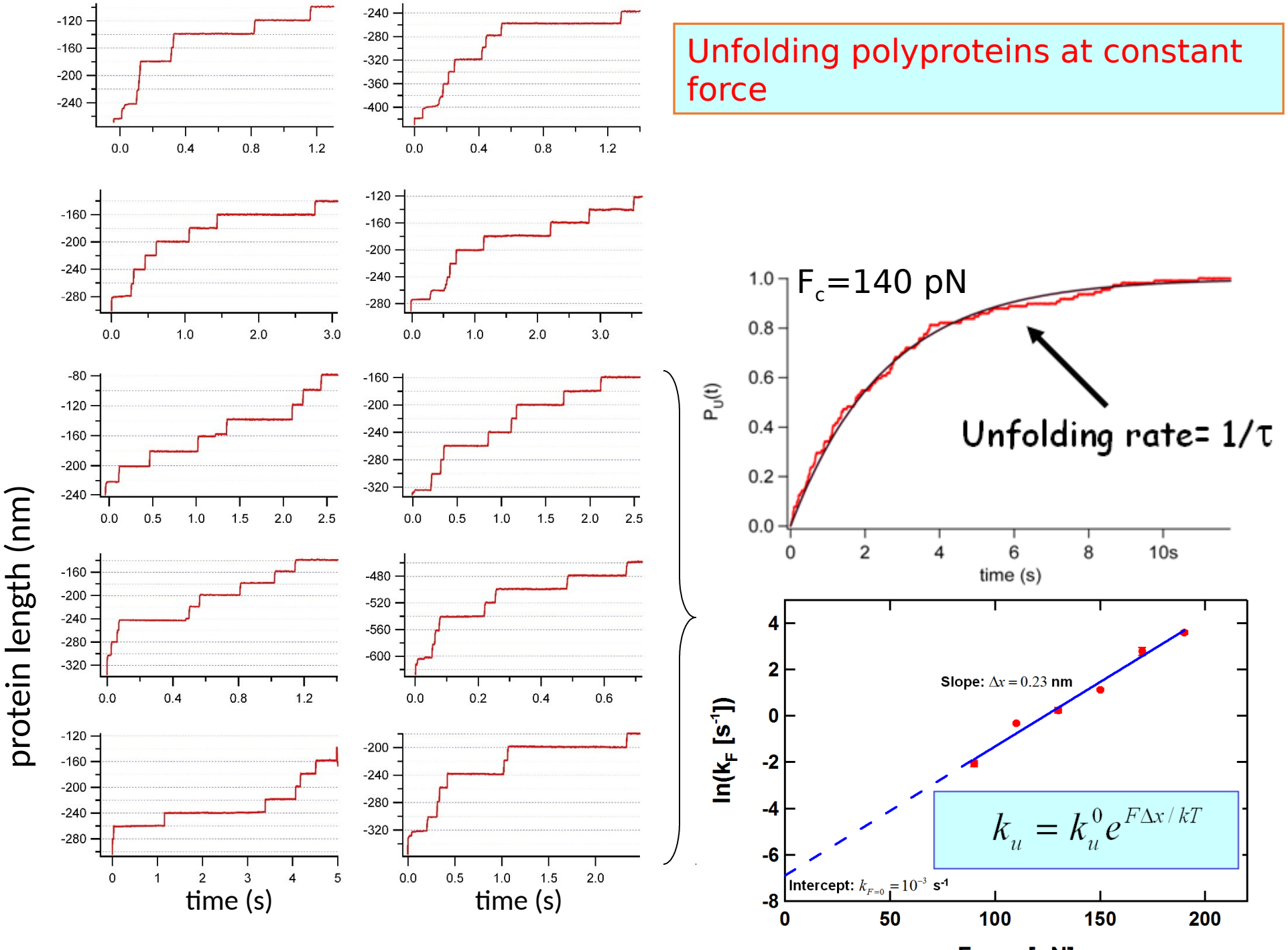




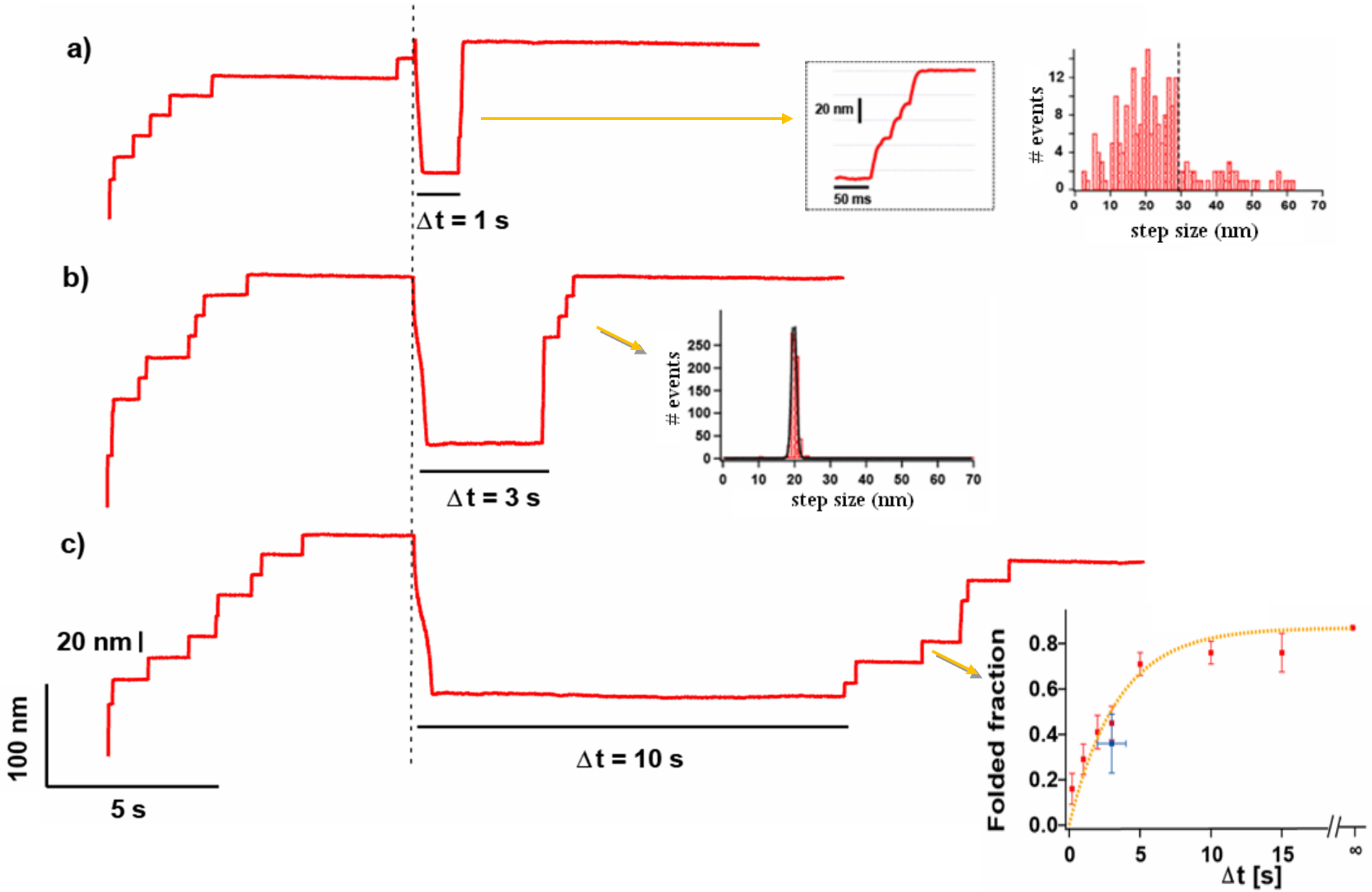
Pallav Kosuri (PhD;2012)
applying the final touches to the AFM prototype



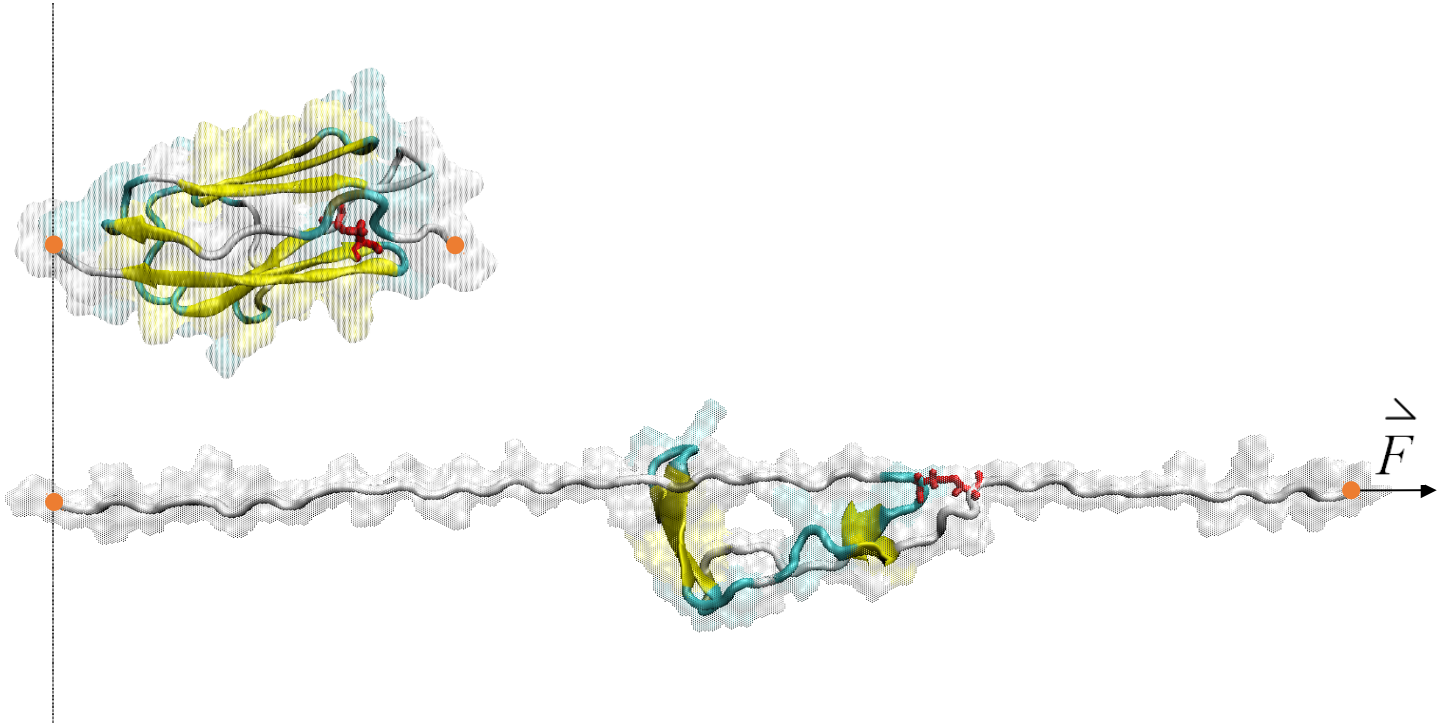
Unfolding polyproteins at constant force



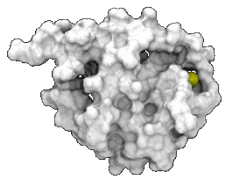
Force-quench; molten globules and folding

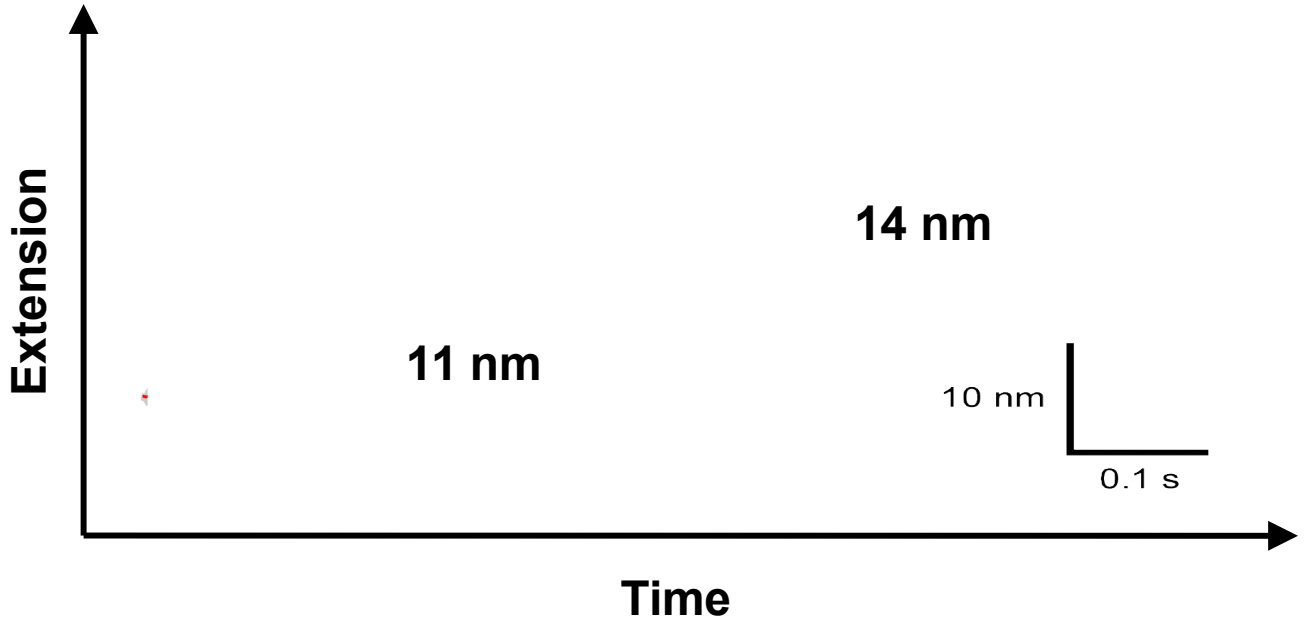


Disulfide bonds restrict the elongation of an unfolding protein, and accelerate refolding 20-50 fold making the protein stiffer

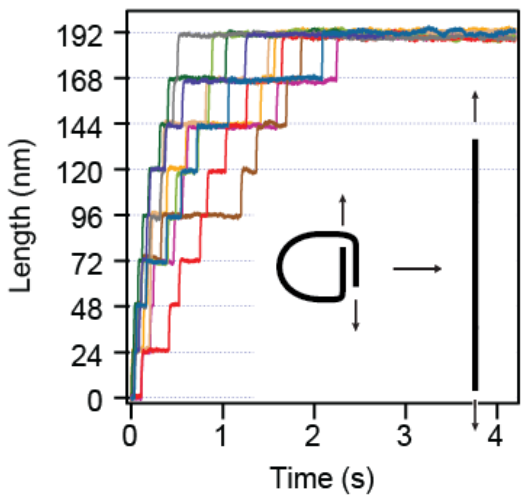


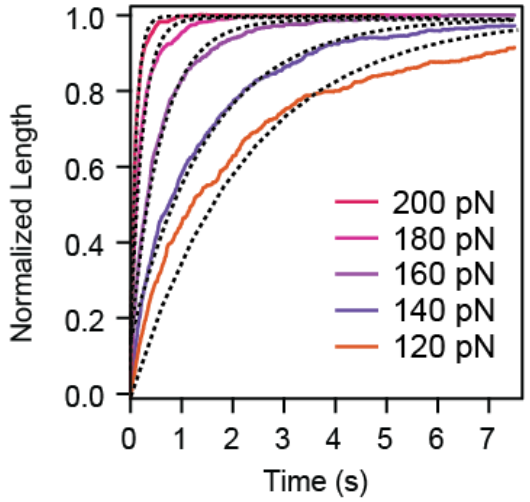
Detecting cryptic disulfide bonds in I27

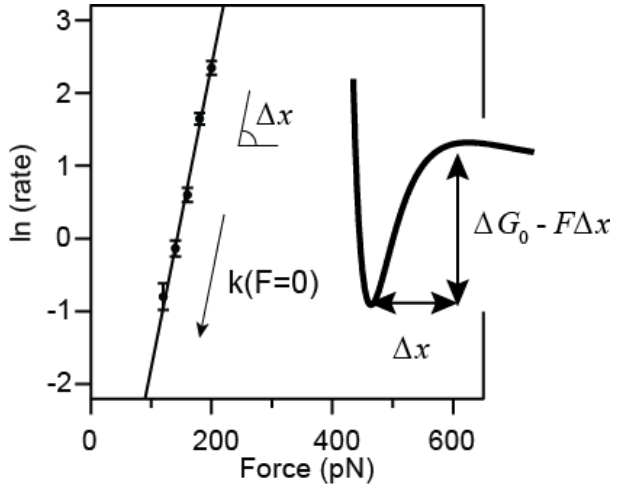


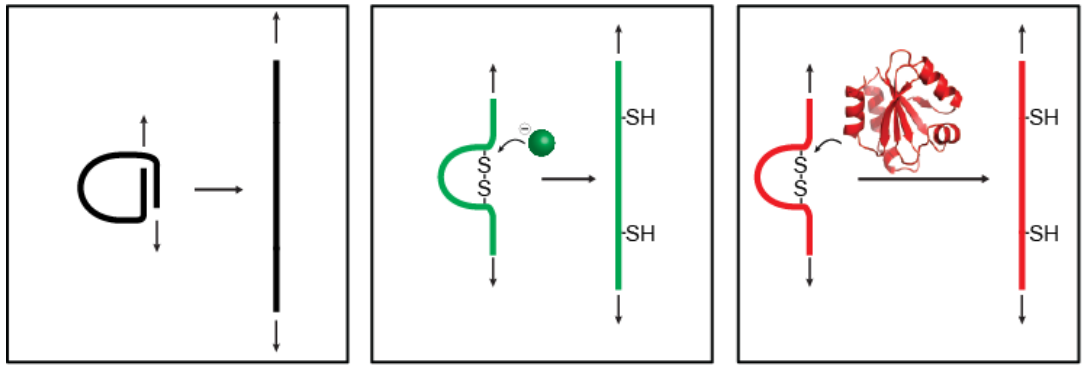


Force dependent reactions

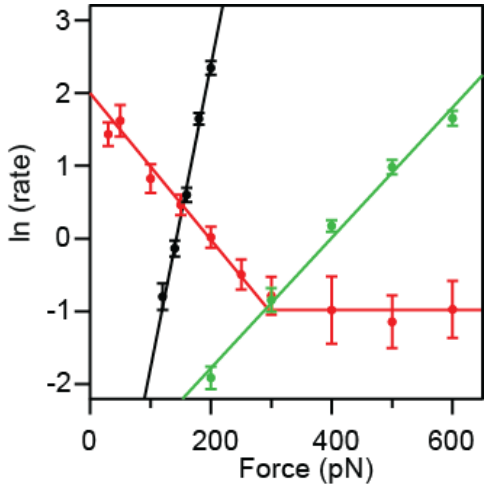








Unfolding | Chemical Reactions | Enzymatic Reactions



Popa I., et al, *Nat. Prot.* (2013)

Unfolding and refolding dynamics (Titin I27)