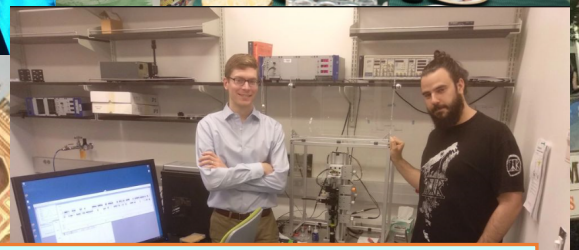
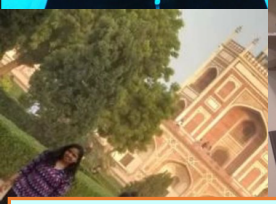
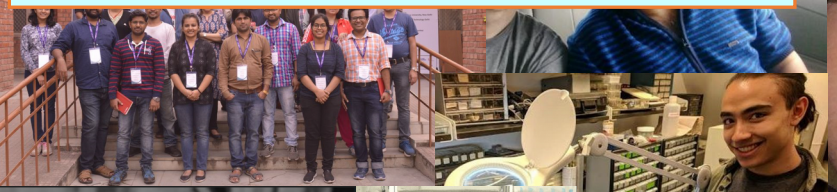
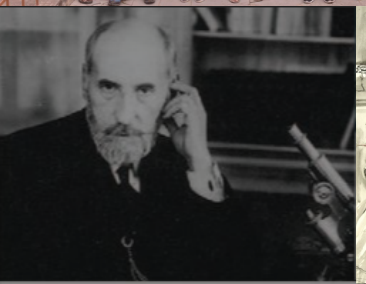
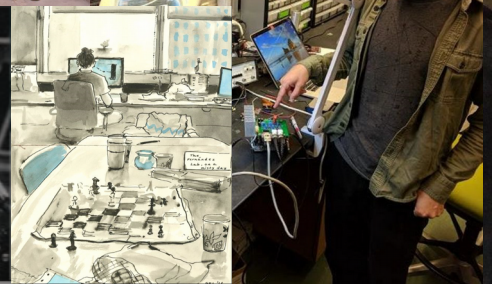



Look us up in zeptowatt.com

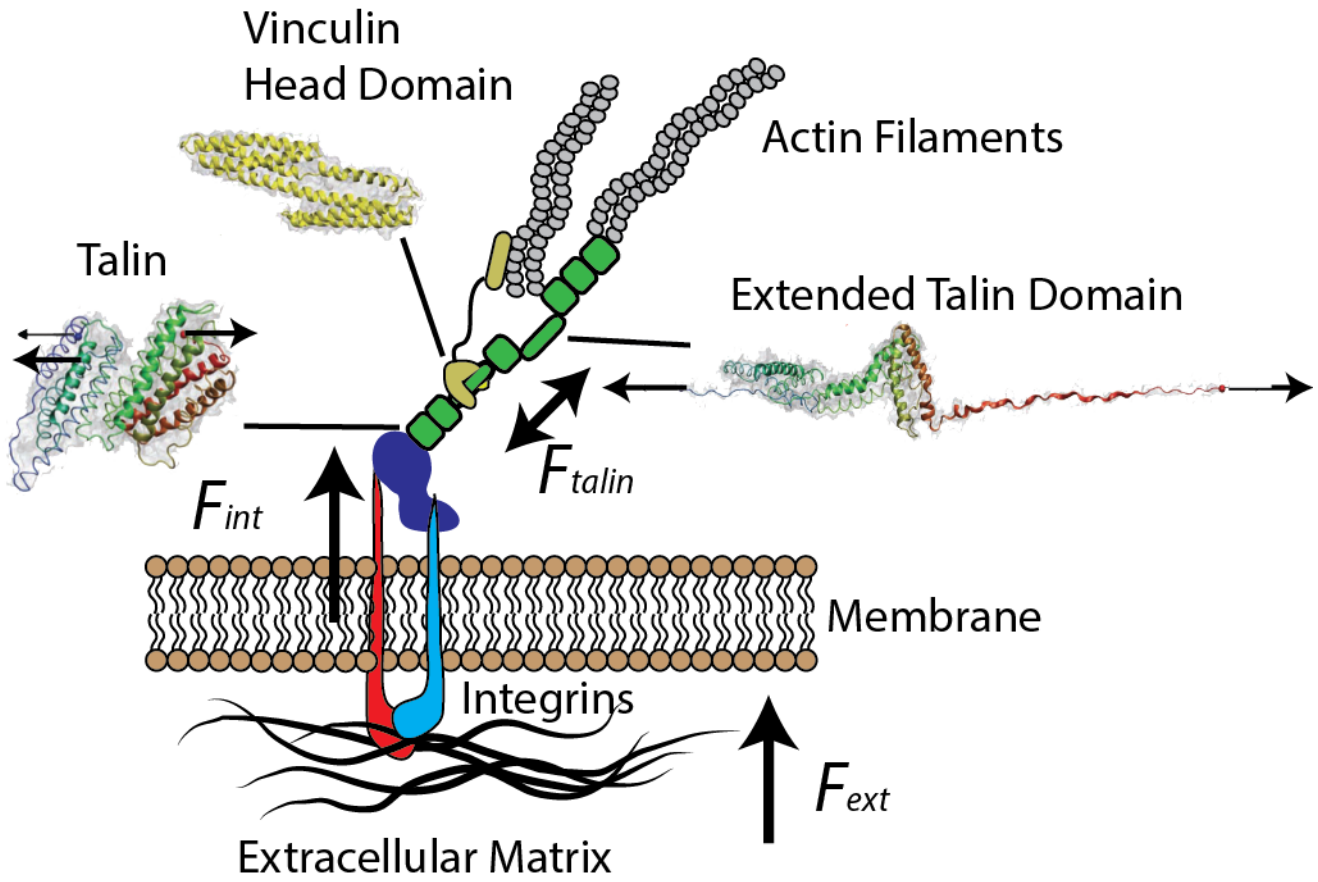




EVERYTHING IN BIOLOGY IS MECHANICAL

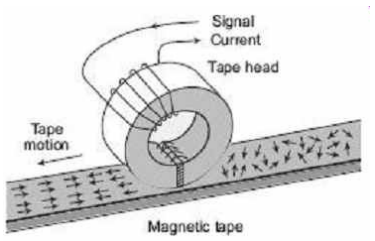
FERNANDEZ LAB

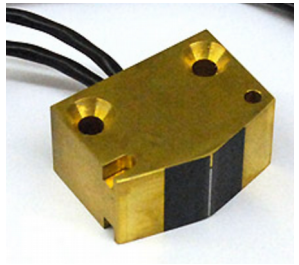
– HOME PUBLICATIONS SCIENTISTS PHOTOS DOWNLOADS CONTACT

Signal transduction by the mechanical force sensor talin



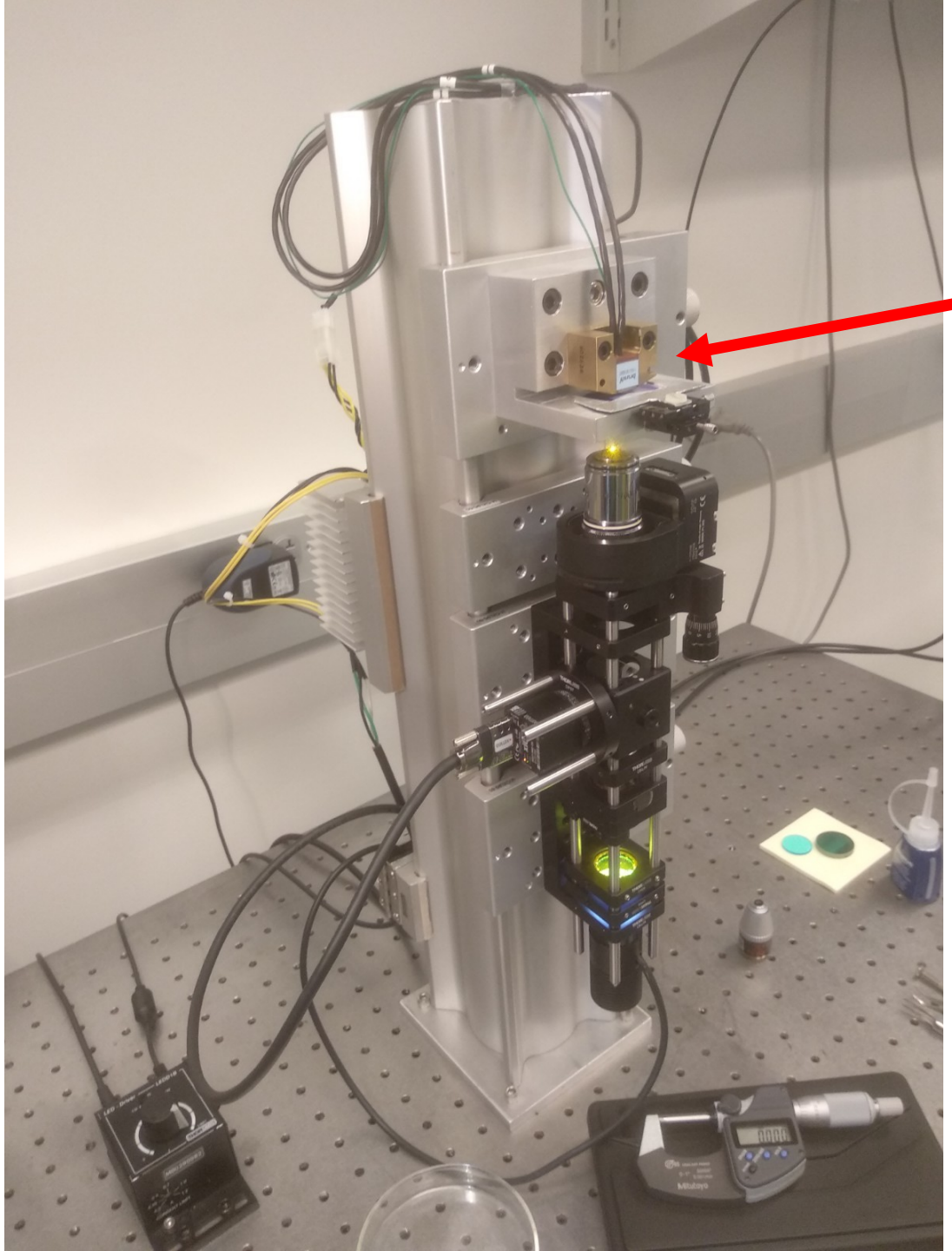
Listening to heavy talin : Dr. Rafael Tapia Rojo

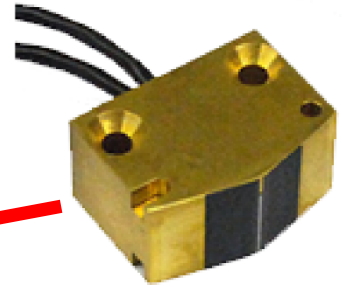




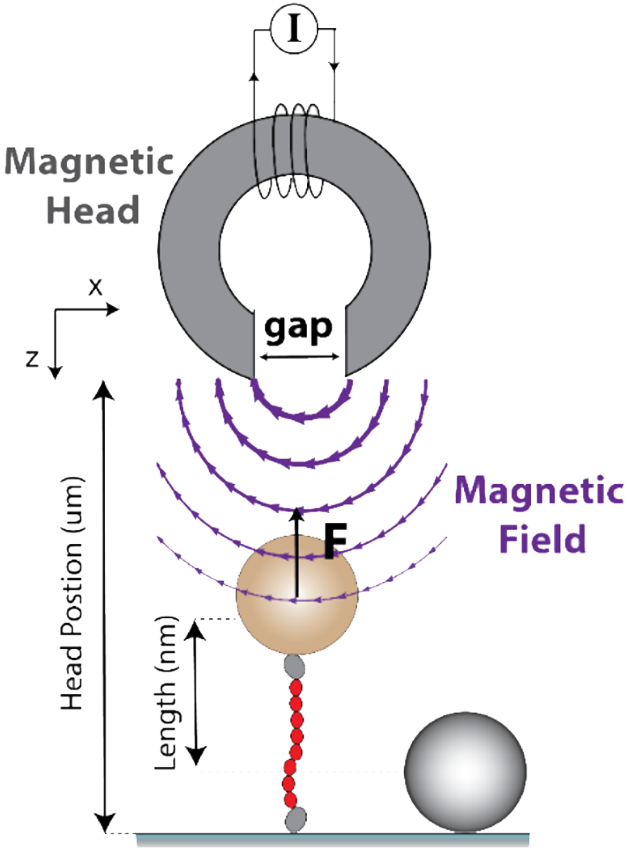
Tapia-Rojo et al., 2019 **PNAS,** 116 (16) 7873-7878

MT_3

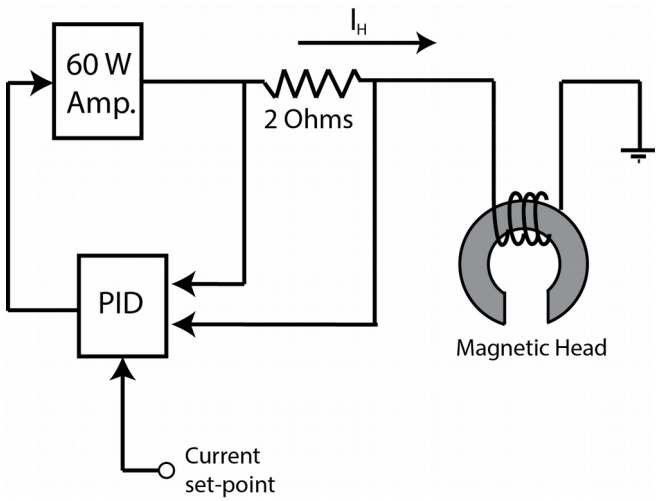


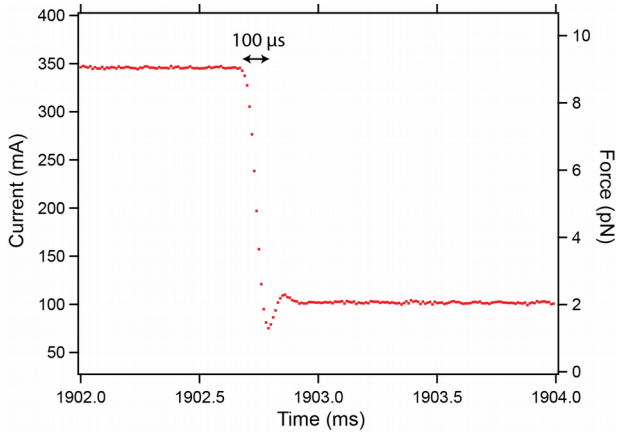


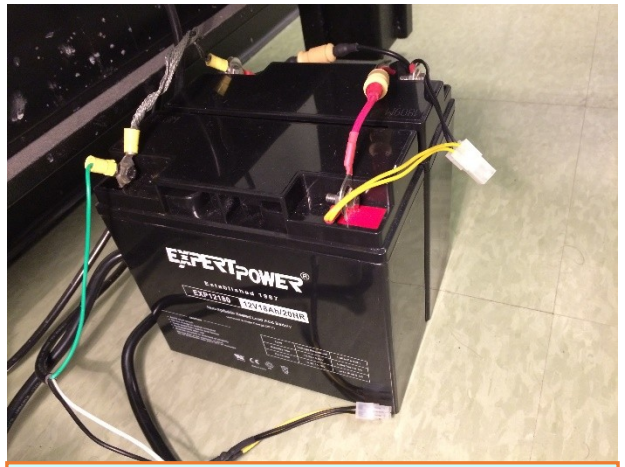
tape head



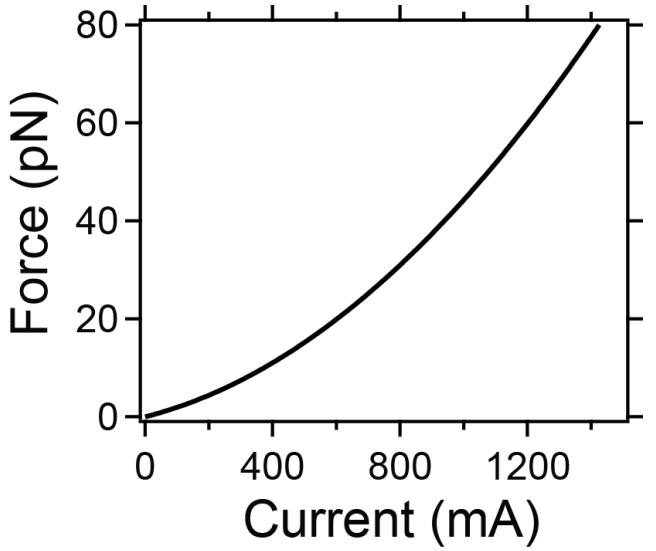
> 10 kHz bandwith with sub-pN resolution





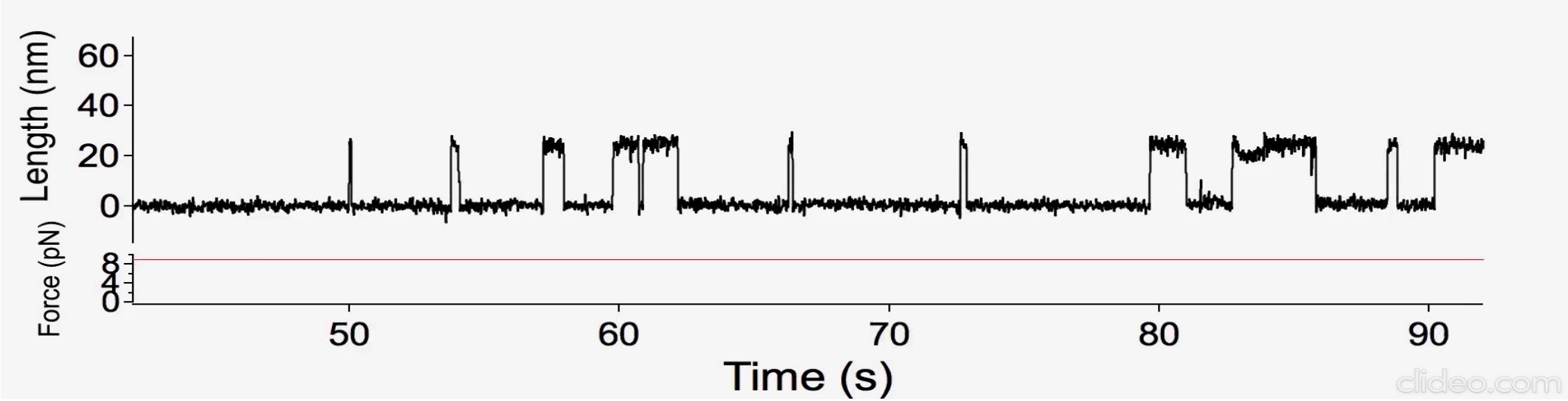


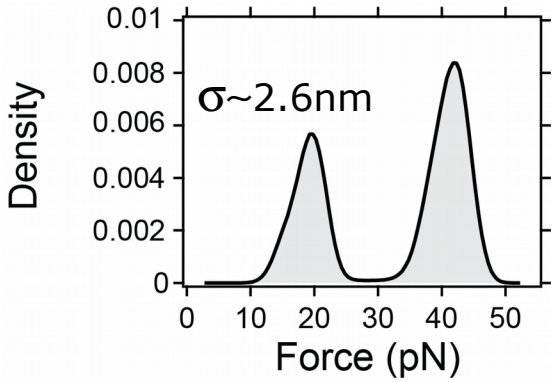
Battery powered

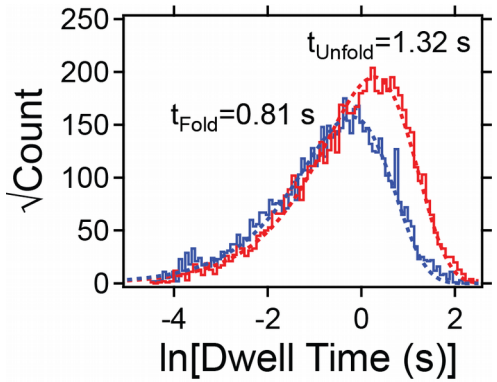


$$F(I) = 2.78610^{-5} \cdot I^2 + 0.016 \cdot I$$

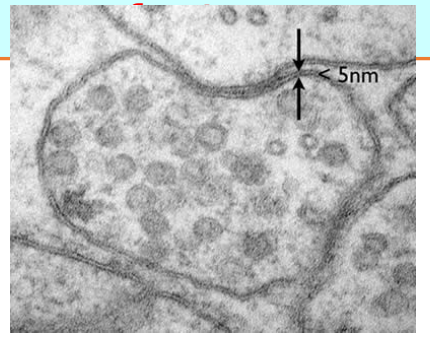
5 hour long recording of talin at 1400 fps with a total drift of 7 nm



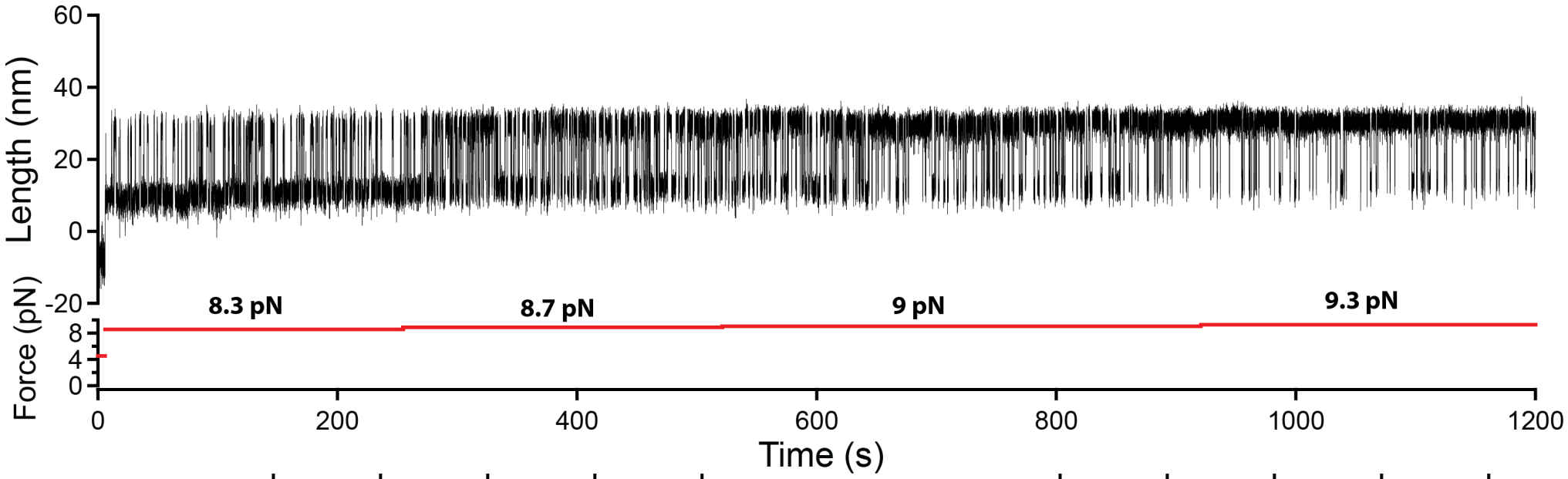




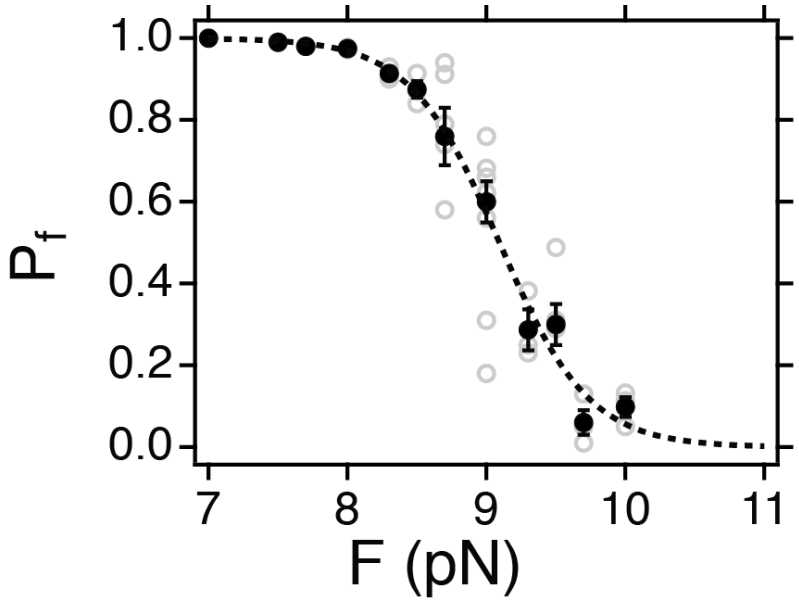
MT_3 has the resolution of an electron microscope at >1400

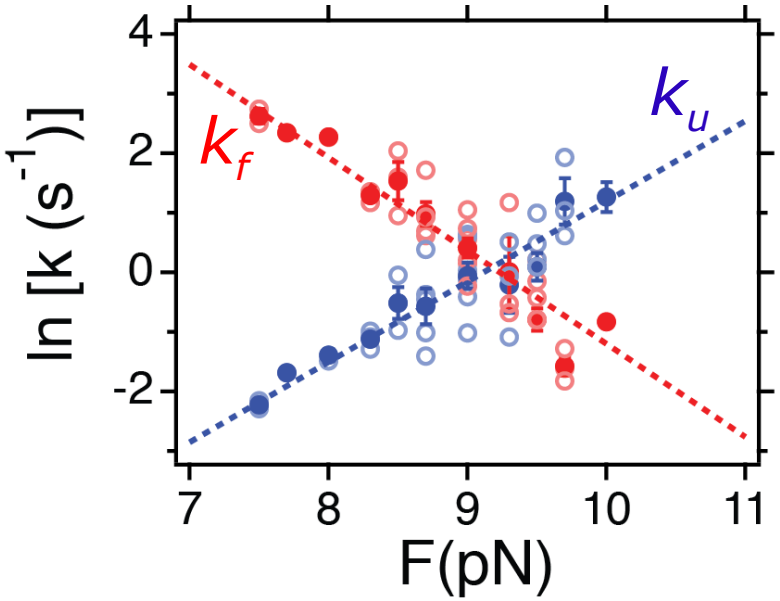


Measuring Talin R3 (IVVI) dynamics with MT3



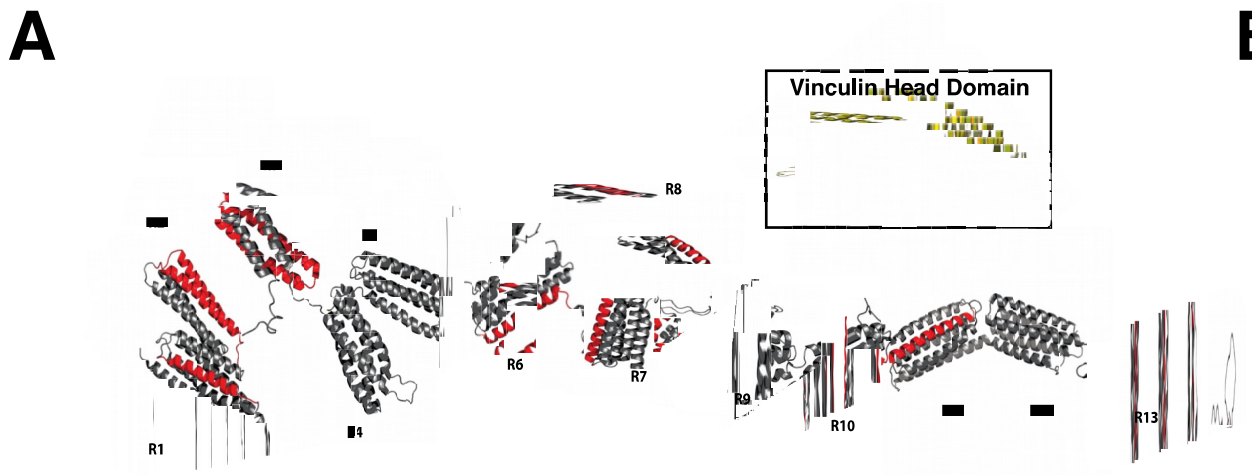
Rafael Tapia-Rojo

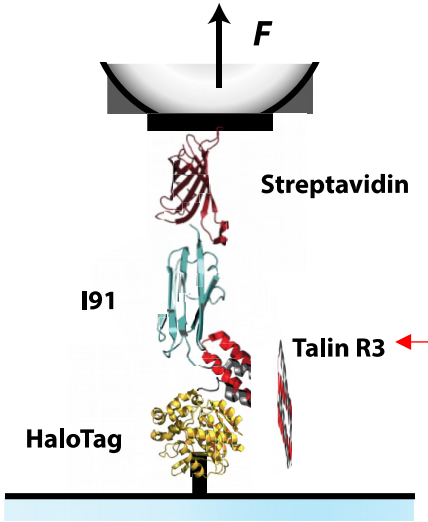




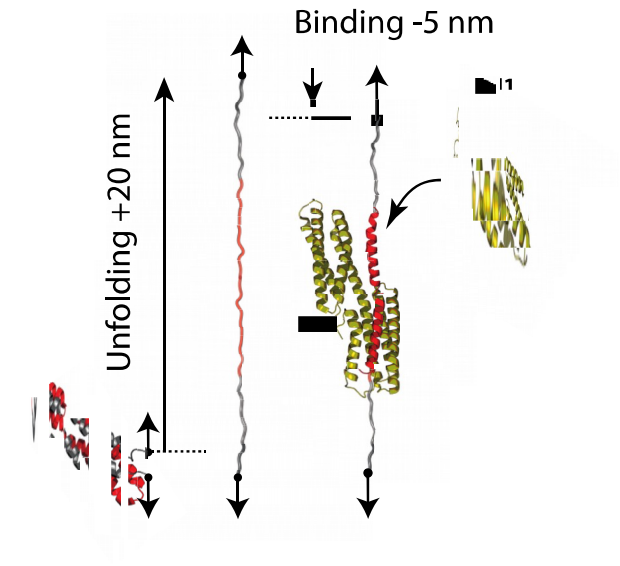
Alvaro Alonso-Caballero

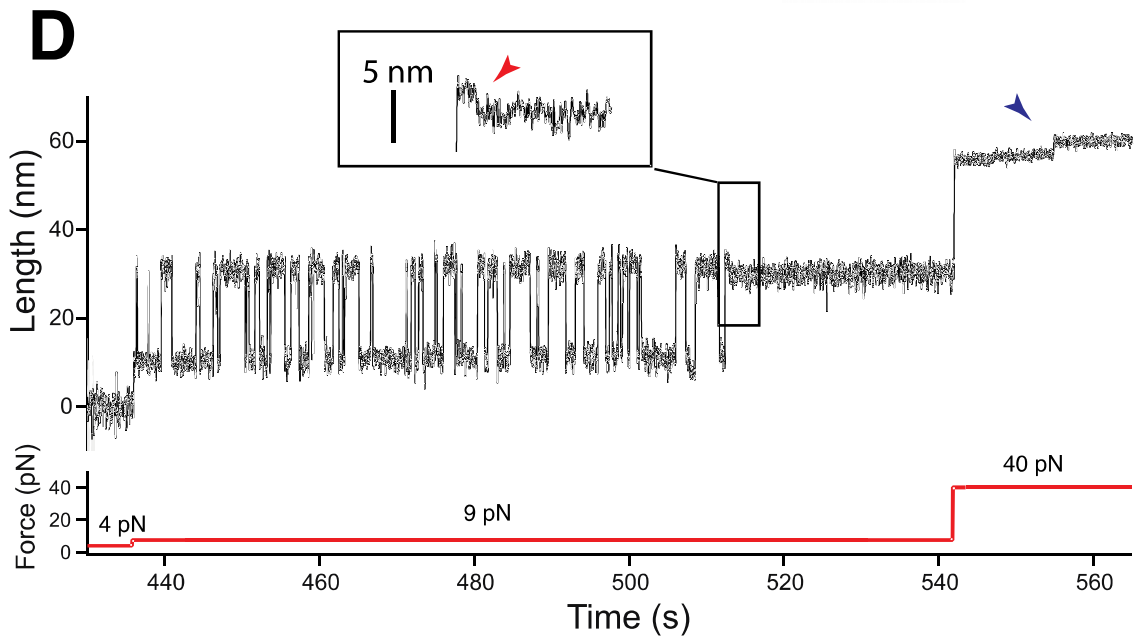
Vinculin binding to talin does mechanical work



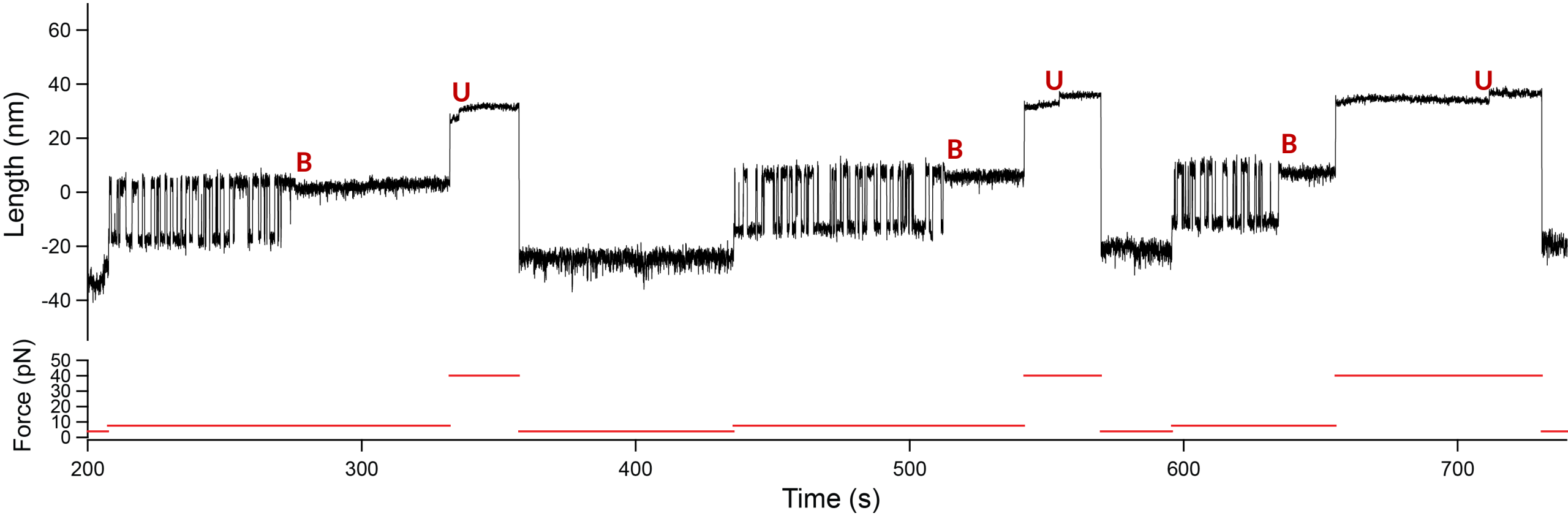


Barsukov, et al., 2013, **JBC,** 288: 8238 - 8249

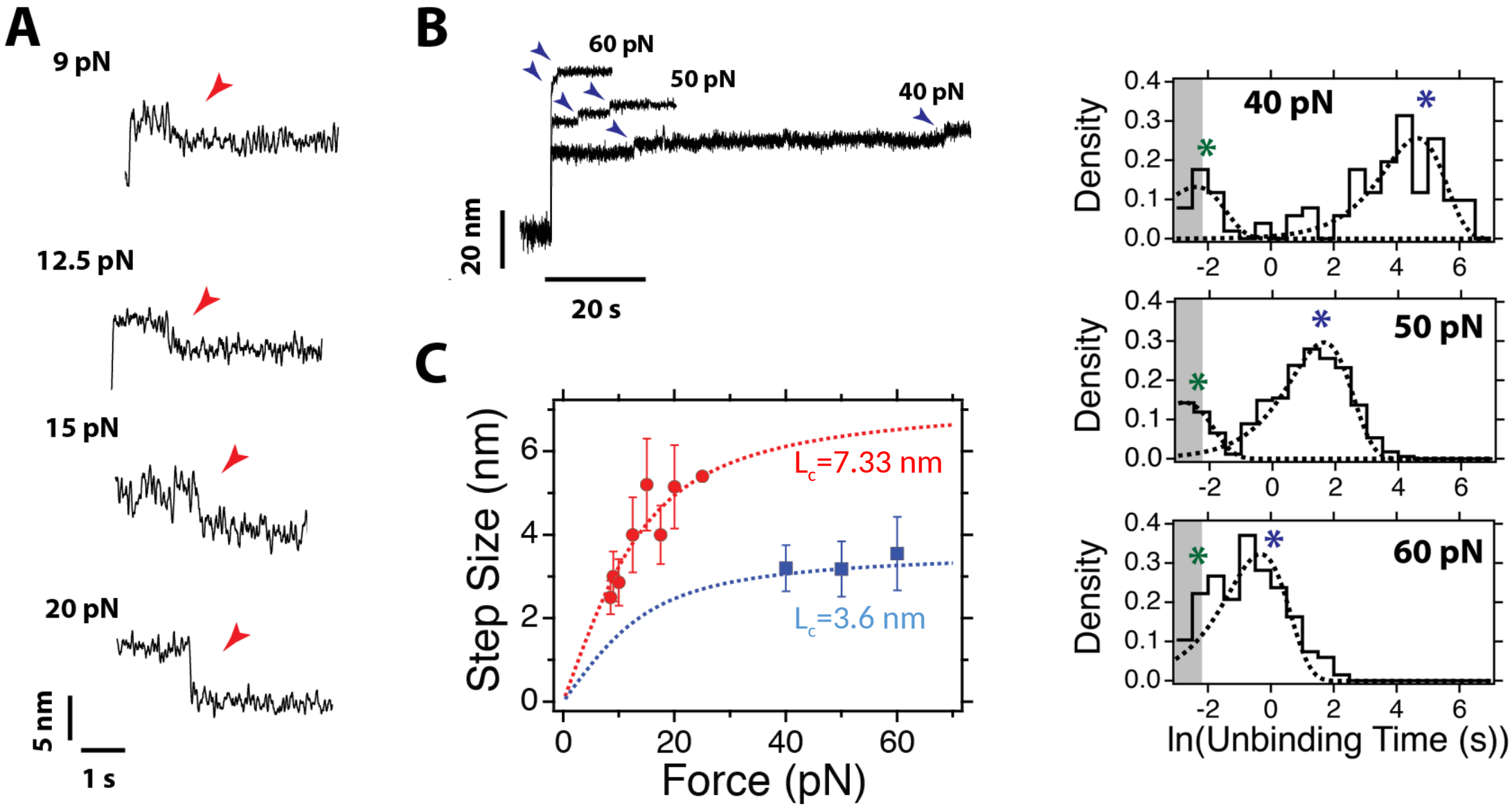




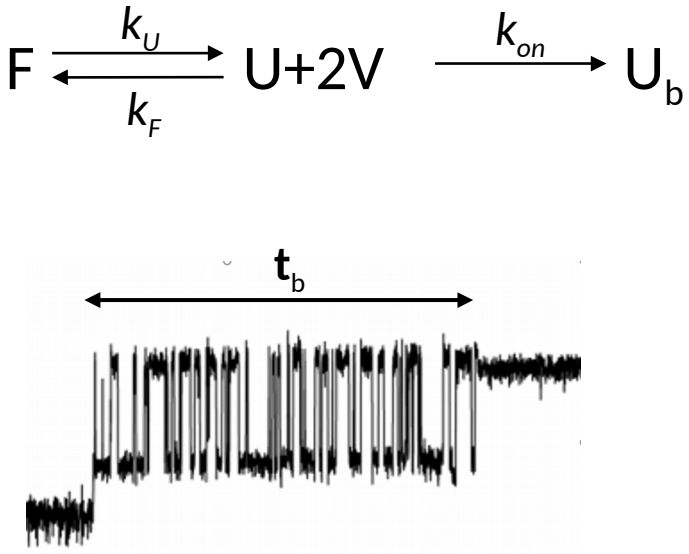
Vinculin binding/unbinding can be cycled repeatedly

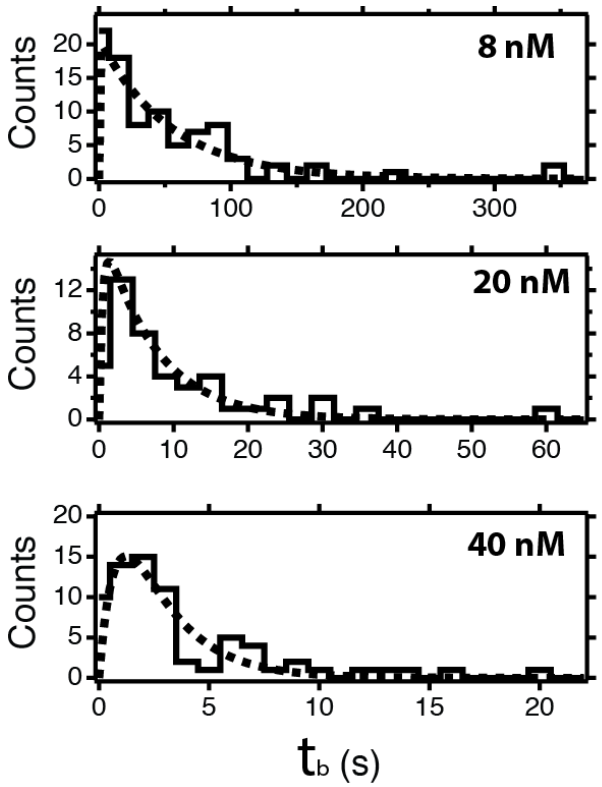


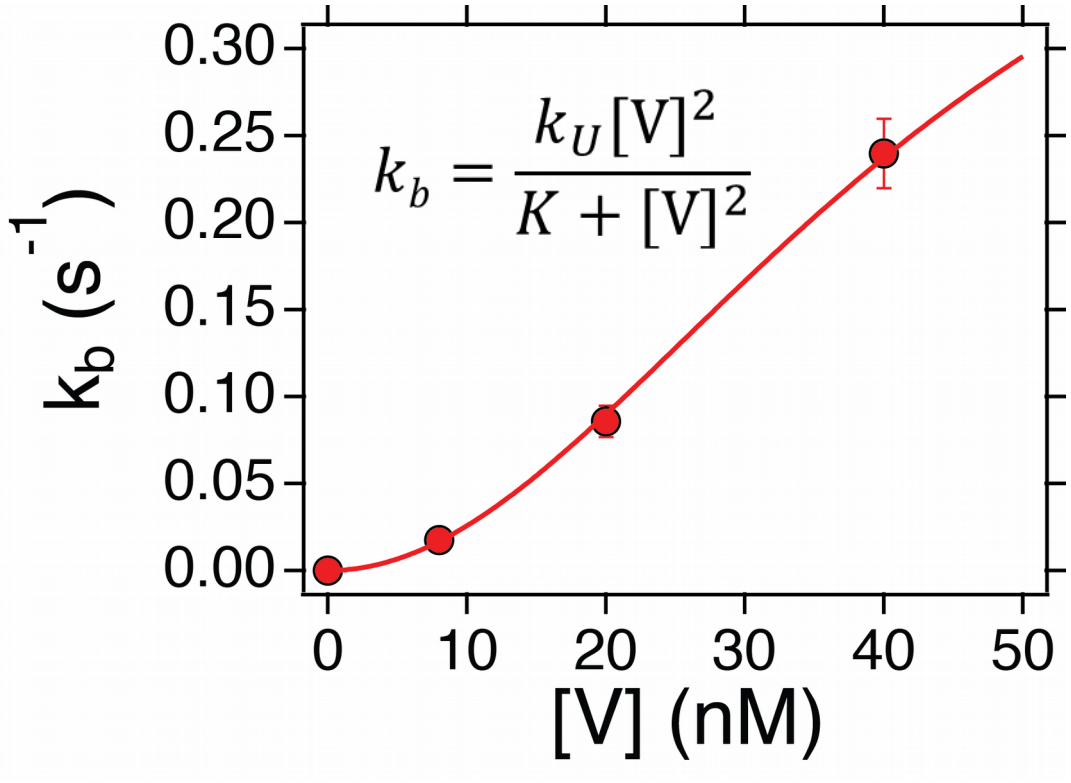
Two vinculin molecules bind simultaneously, they unbind separately, one very fast, the second more slowly.



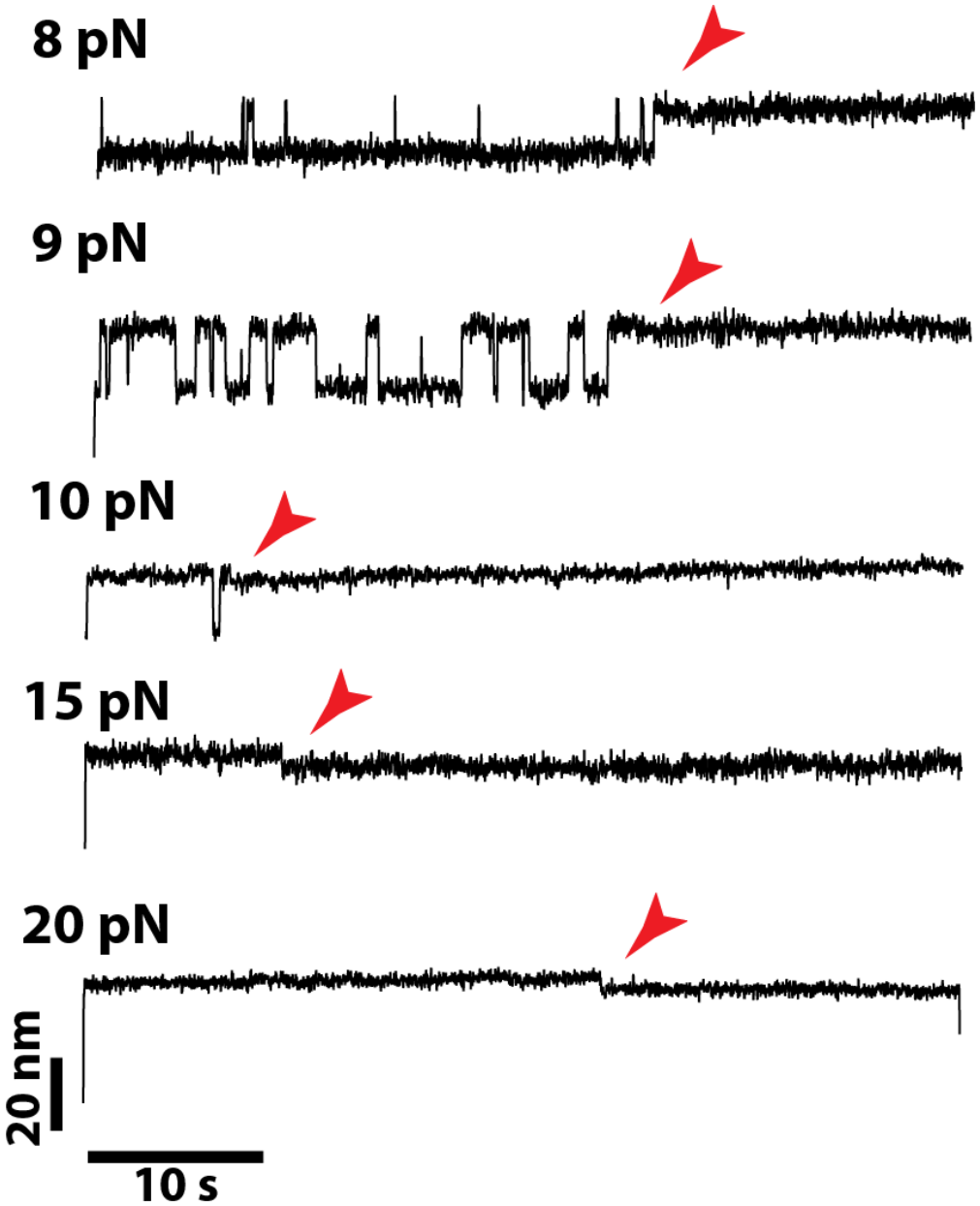
Measuring the rate of reaching the bound-state, k_b, at 9 pN





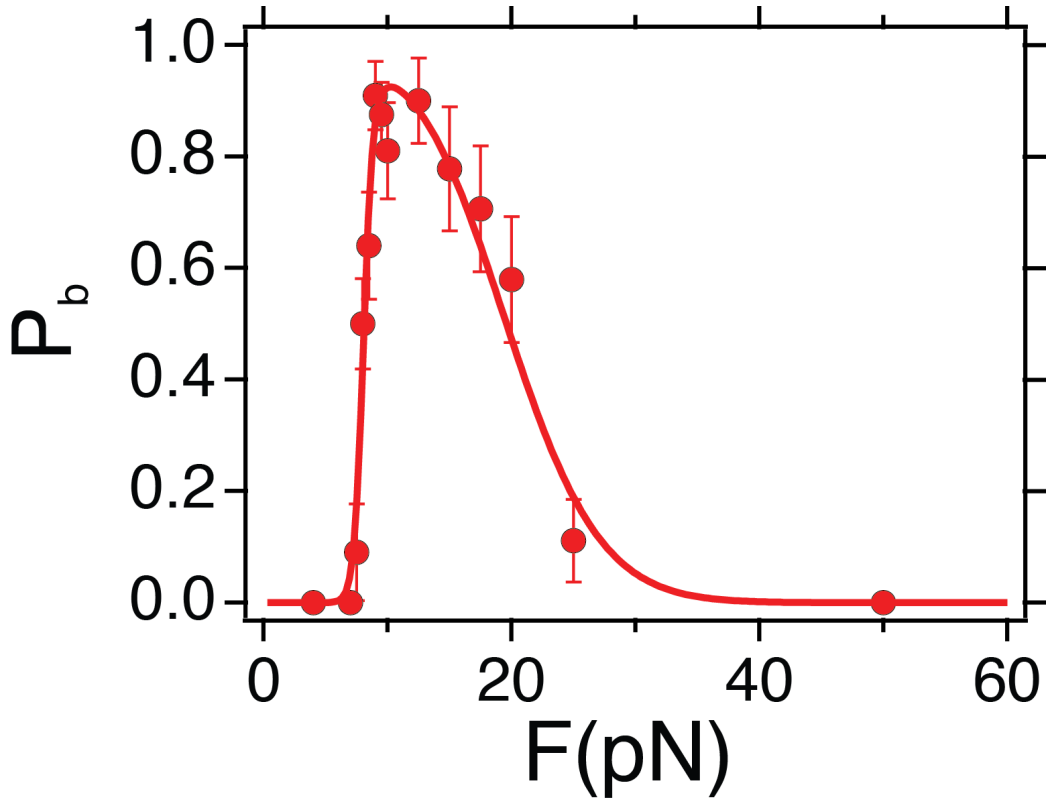


Measure the bound-state probability, P_b, as a function of force

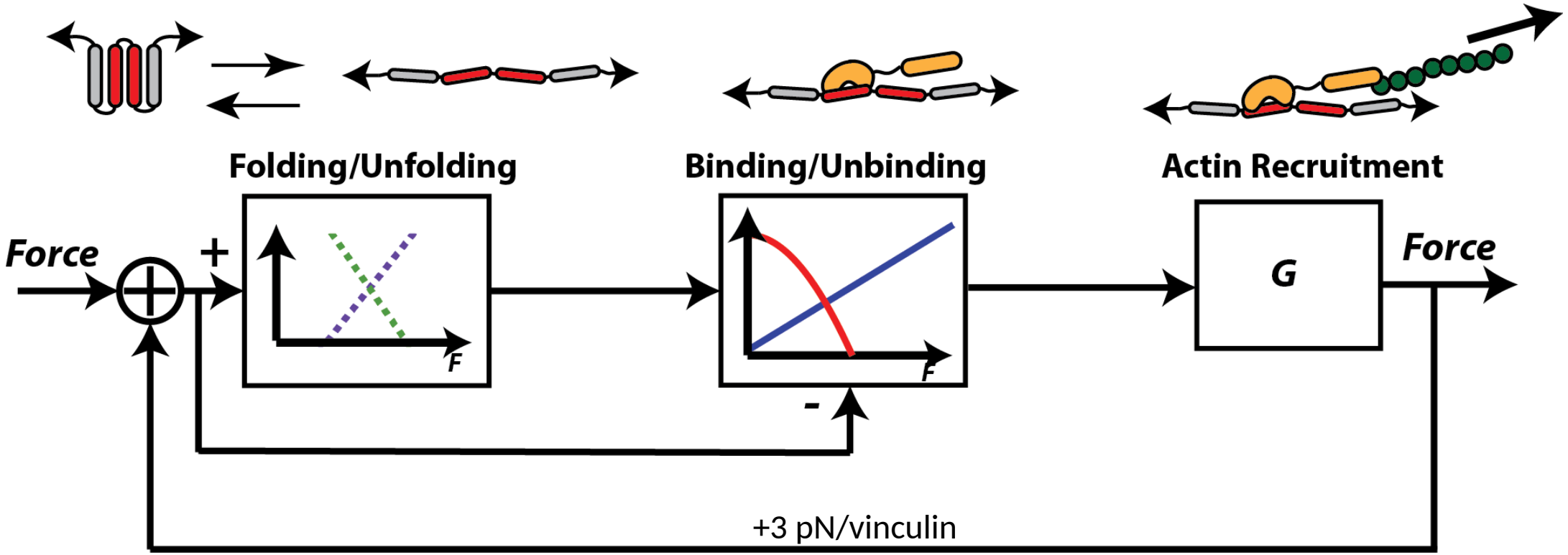


$$P_b = 1 - exp\left[-\frac{k_u k_{on} t}{k_u + k_F + k_{on}}\right]$$

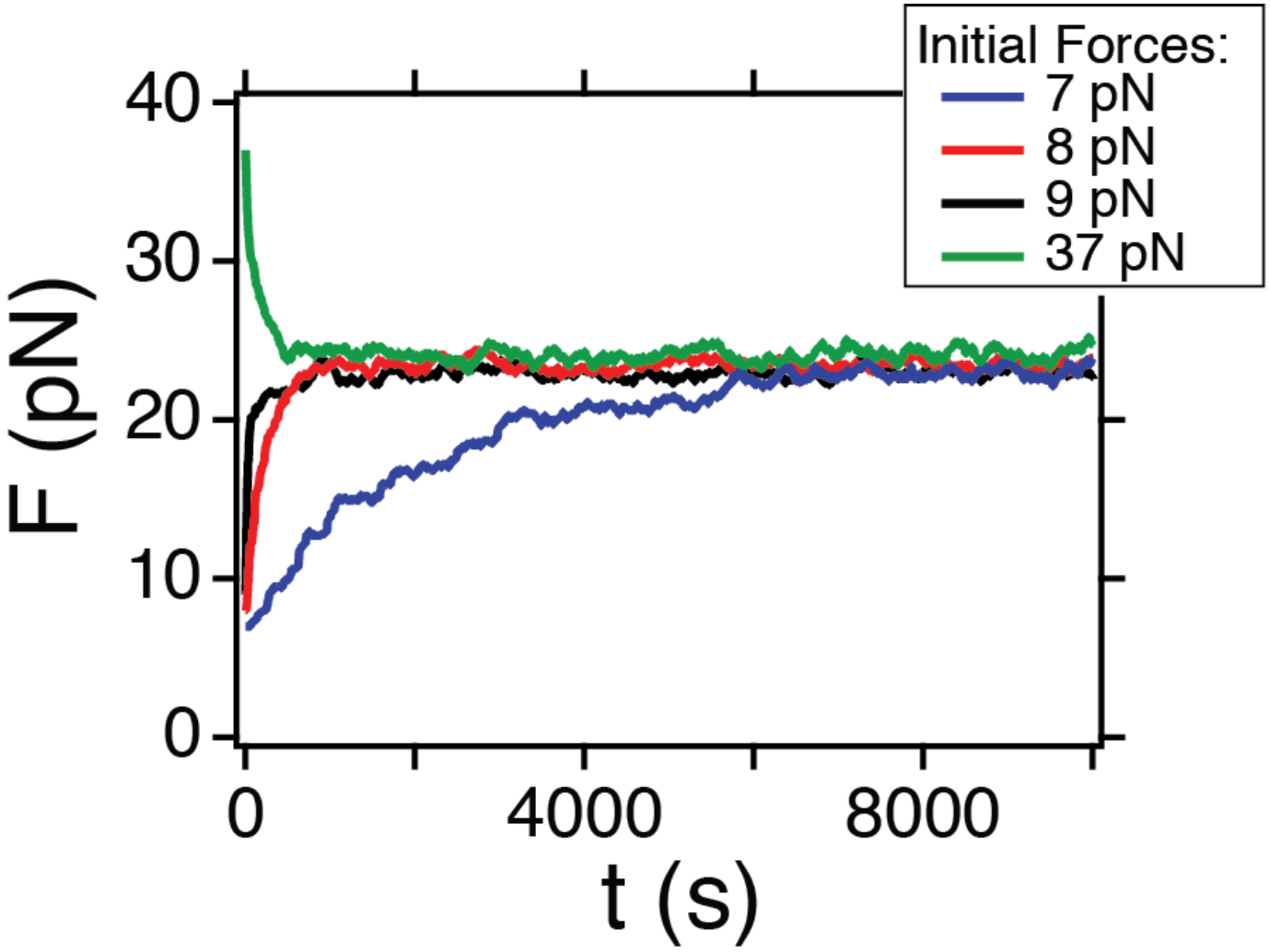
$$k_{on} = [\mathrm{V}]^2 A e^{-\Delta W_b / kT} \quad ; \quad \Delta W_b = F \Delta \mathrm{L}$$



Talin-vinculin mechanical control system

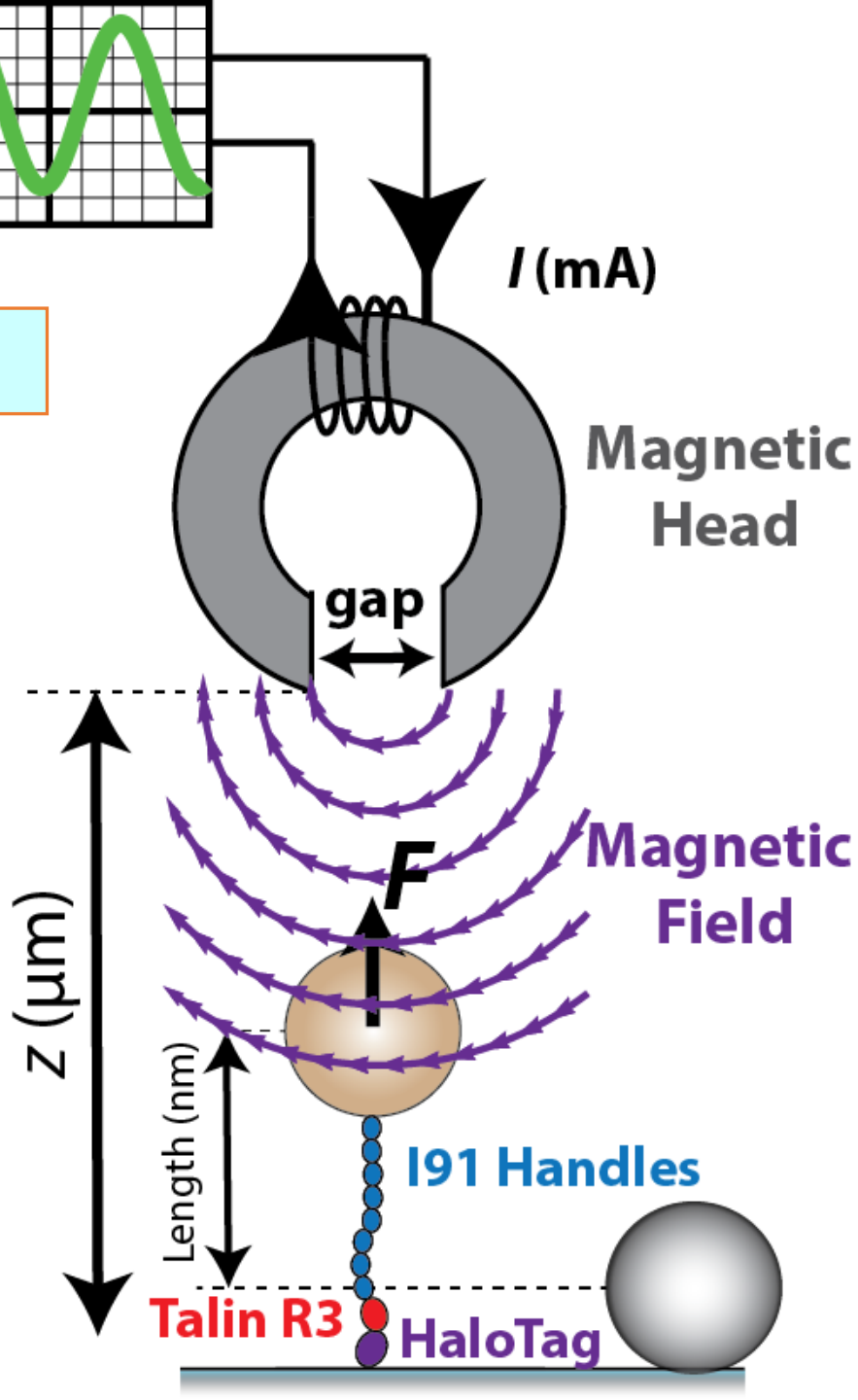


Monte-Carlo simulation of the talin-vinculin control system predicts a negative-feedback equilibrium at 23 pN

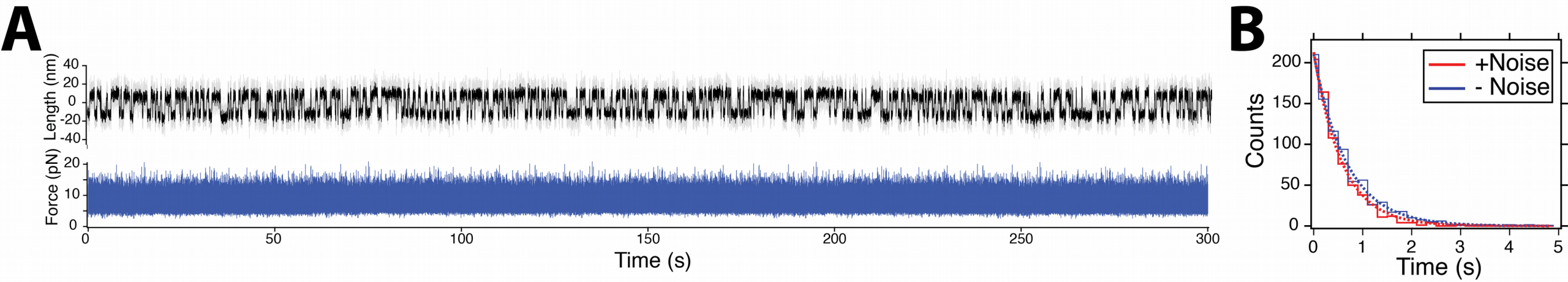


MT_3: the next level.

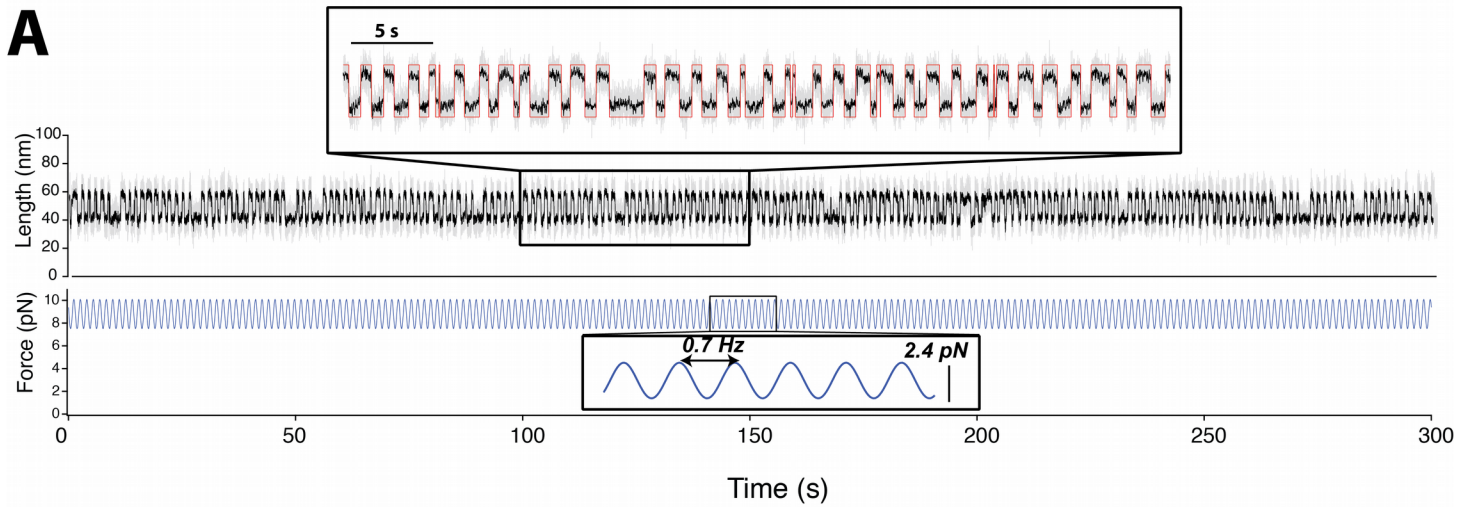
Mechanical signals in biology are noisy and contain periodic signals

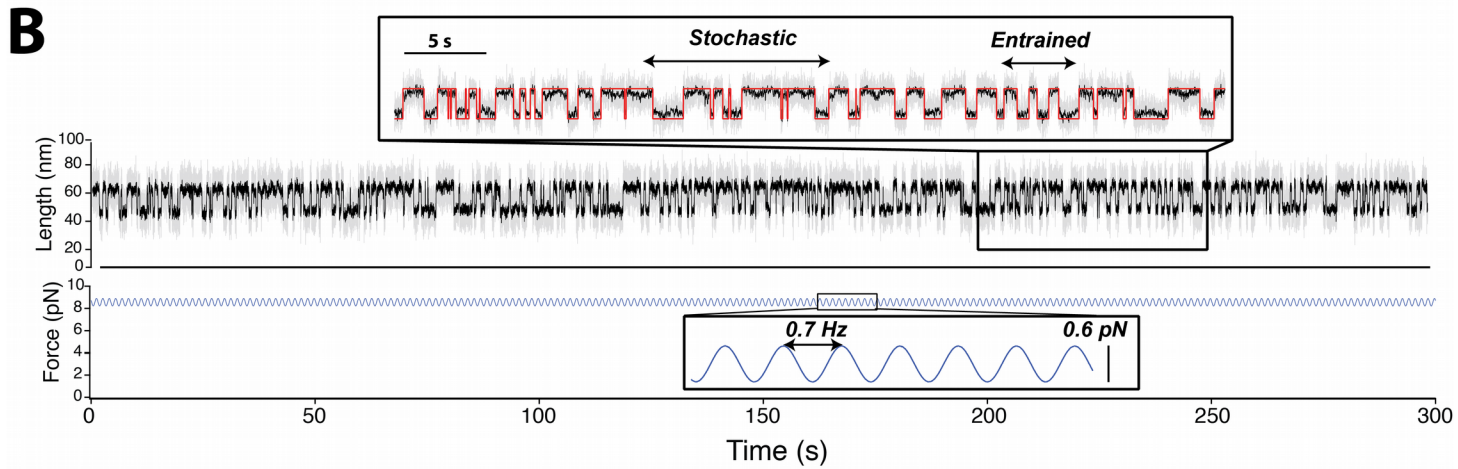


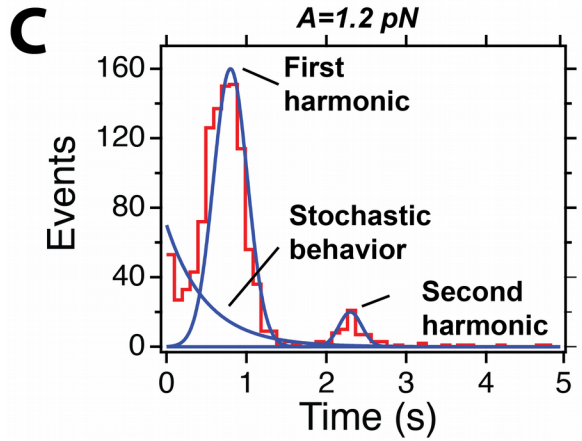
Talin rejects mechanical noise

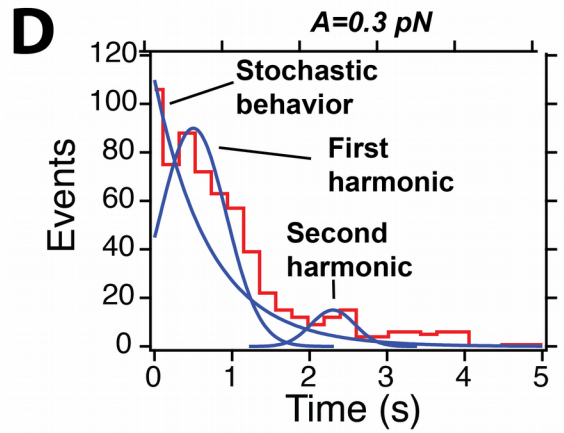


Talin entrains with periodic signals

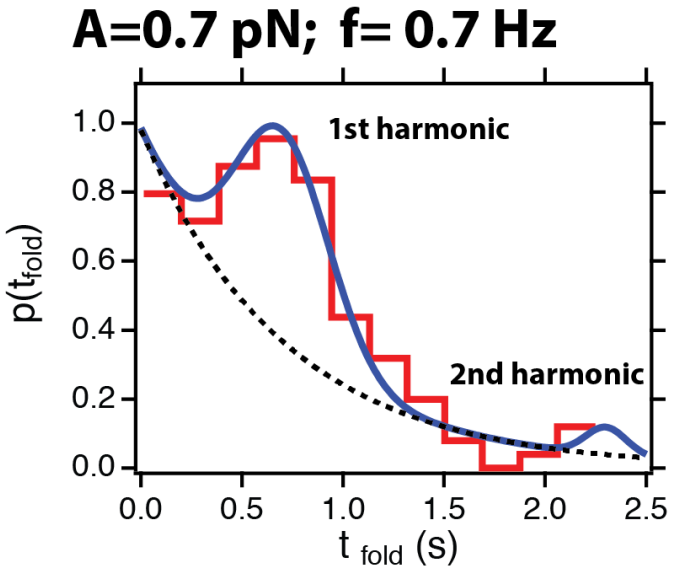


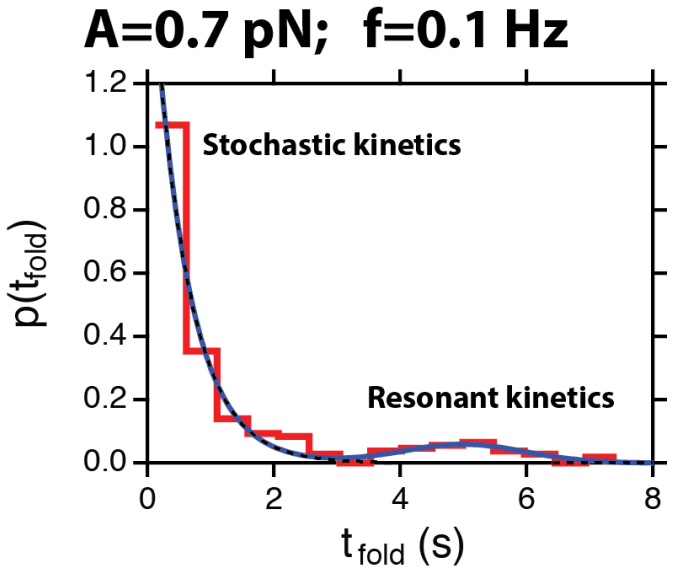


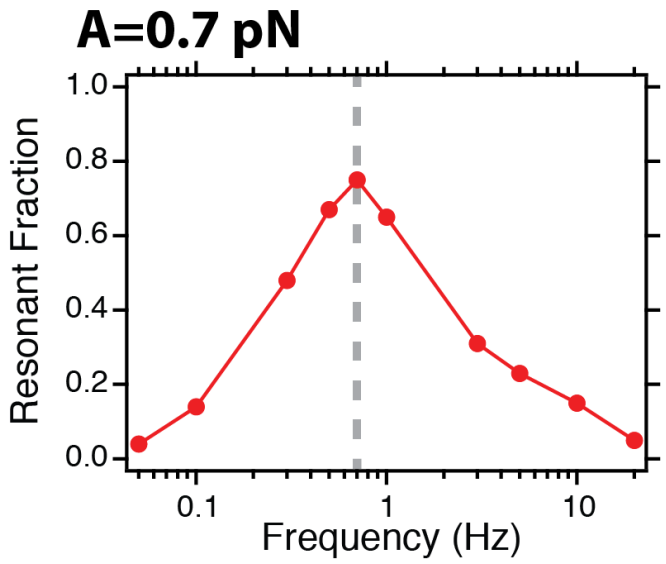


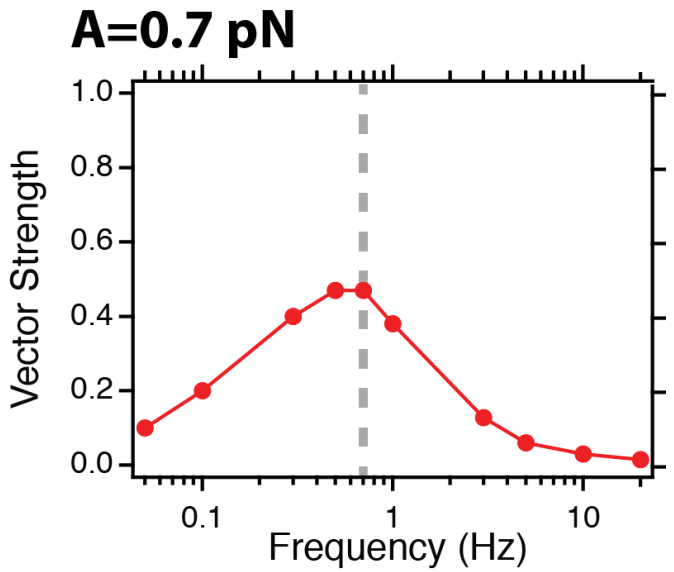


Entrainment is frequency dependent

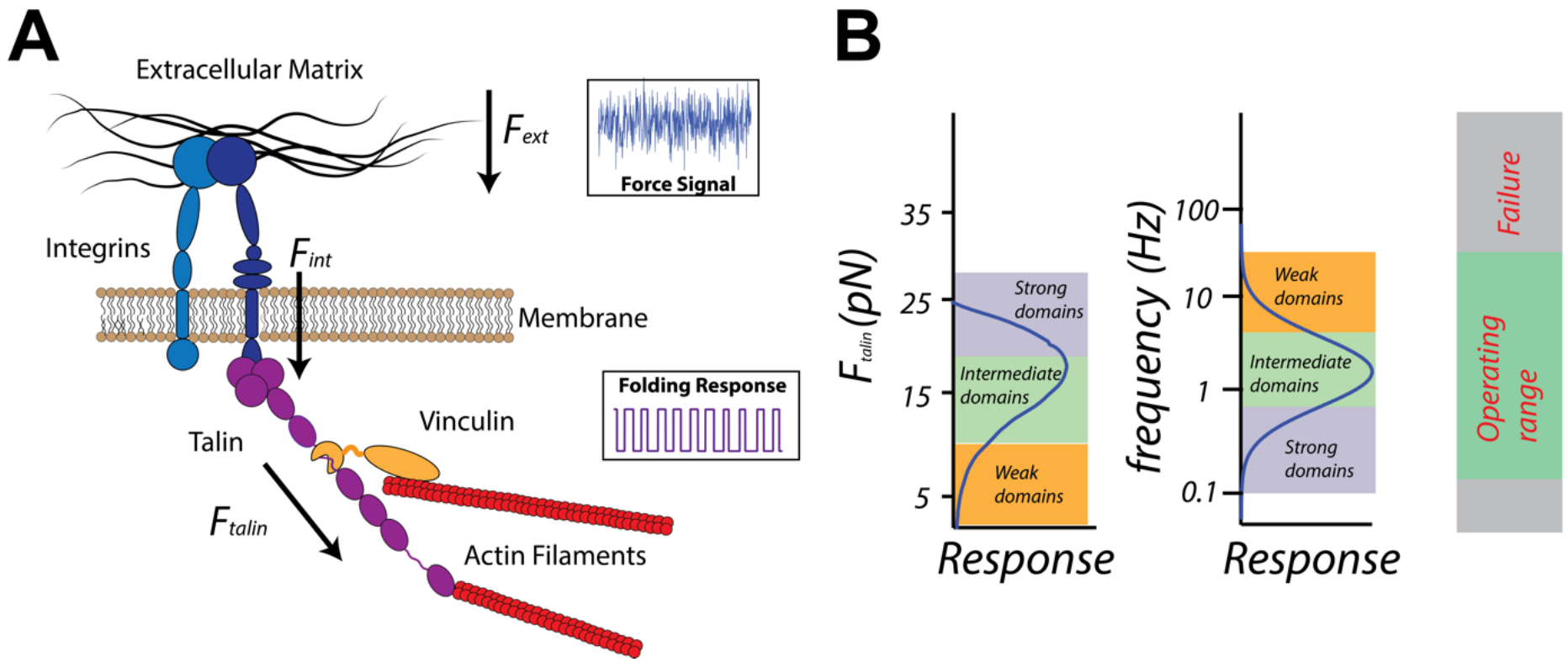






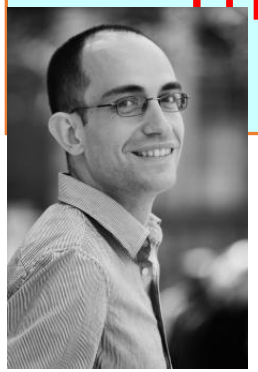


Stochastic resonance identifies periodic signals in noisy mechanical environments

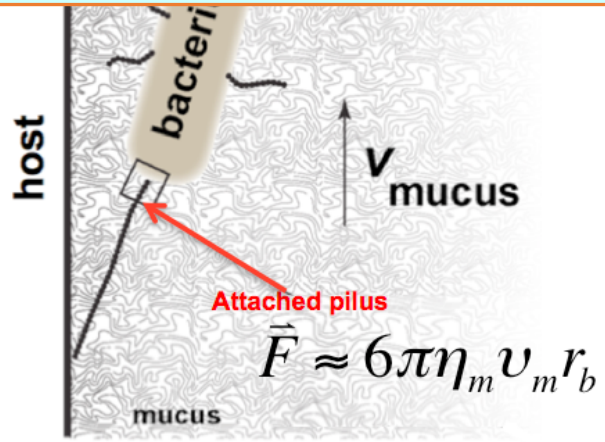


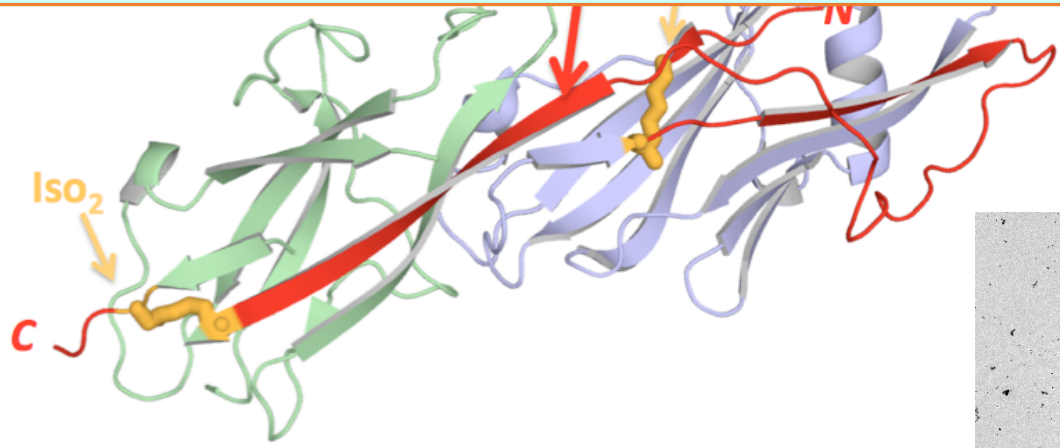
heart beat?, respiration?, rigidity sensing, cancer?

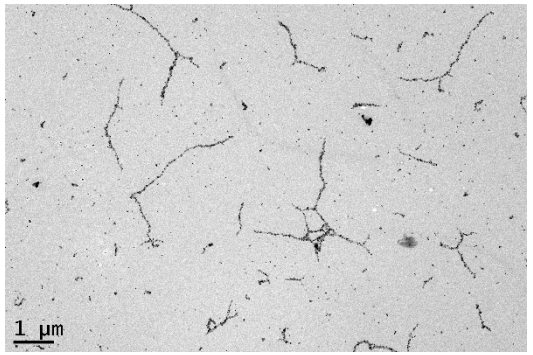
Gram-positive pili are the largest single polypeptide proteins known.
They have specialized features to resist large mechanical shocks!

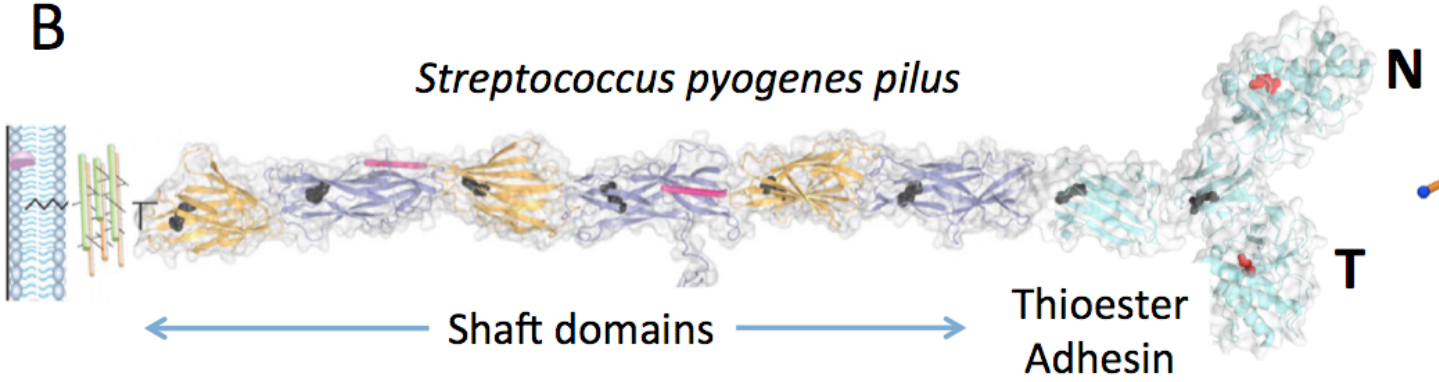


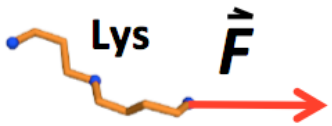
Jorge Alegre-Cebollada

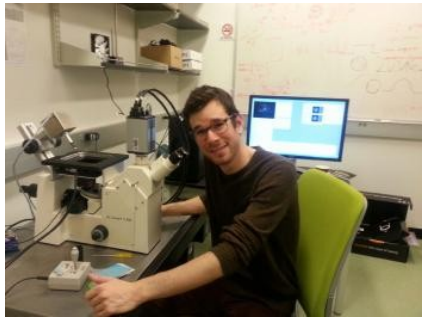












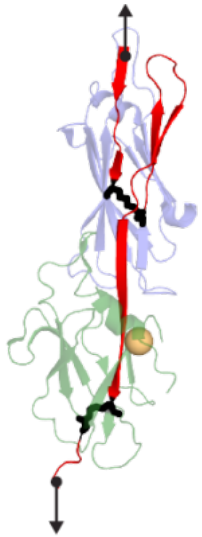
Daniel Eschelman MD/PhD (2018)

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

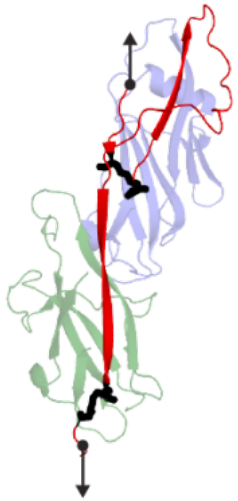
Echelman et al., 2016, **PNAS,** 113:2490-2495

IDL's are common

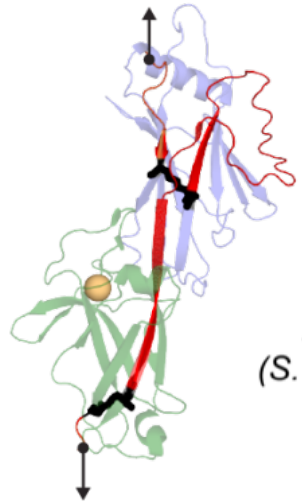
SpaA
(C. diphtheriae)



SpaD
(C. diphtheriae)

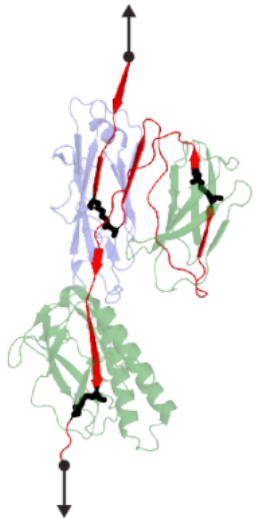


FimA
(A. oris)

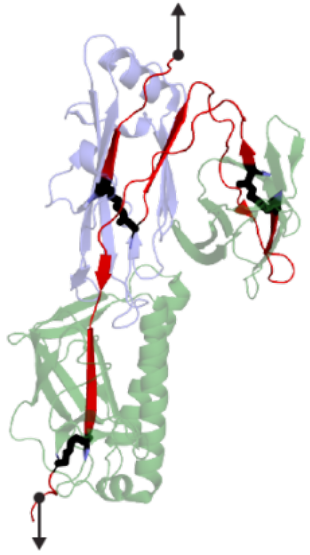


FimP
(A. oris)

AgI/II
(S. mutans)

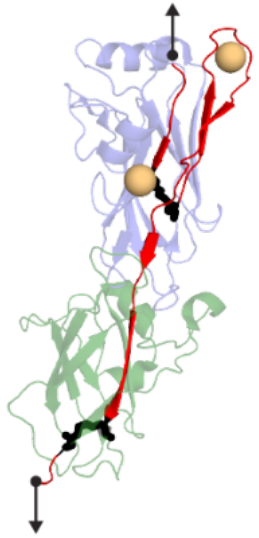


RrgB
(S. pneumoniae)

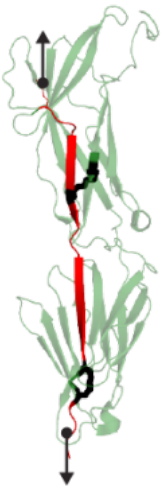


BP-2a
(S. agalactiae)

BP-2b
(S. agalactiae)

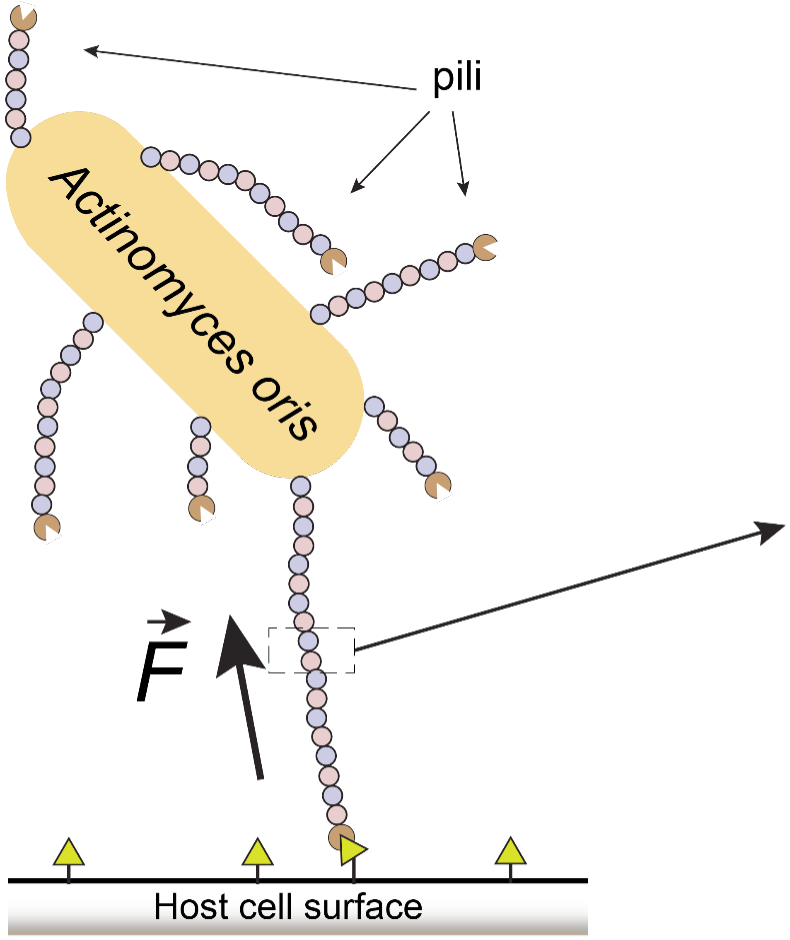


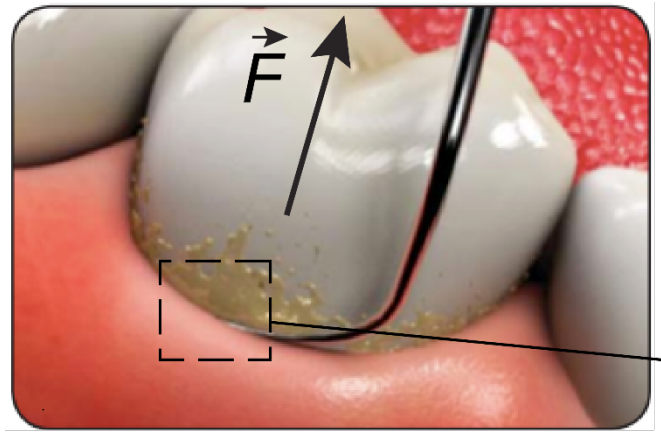
Gbs80
(S. agalactiae)

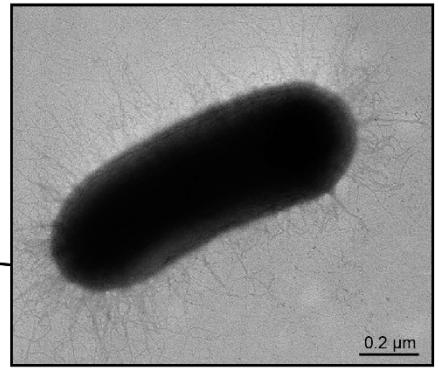


Spy0128
(S. pyogenes)

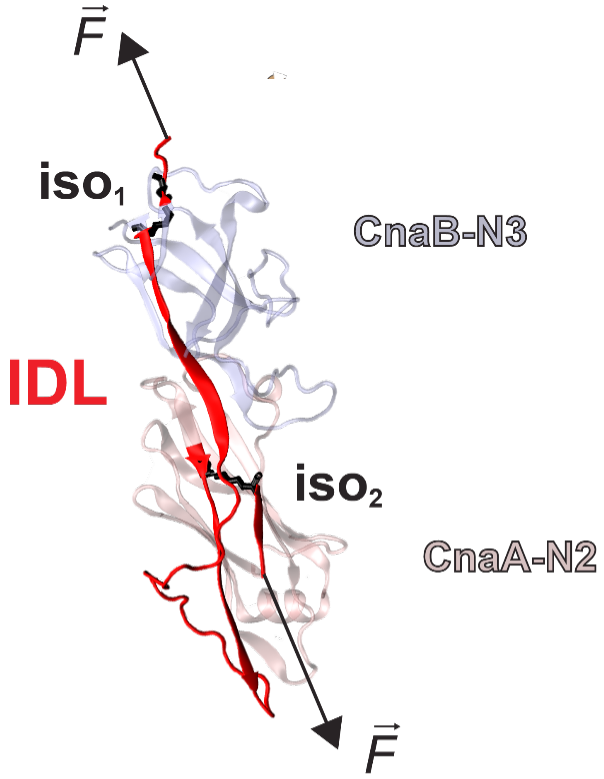
FimA







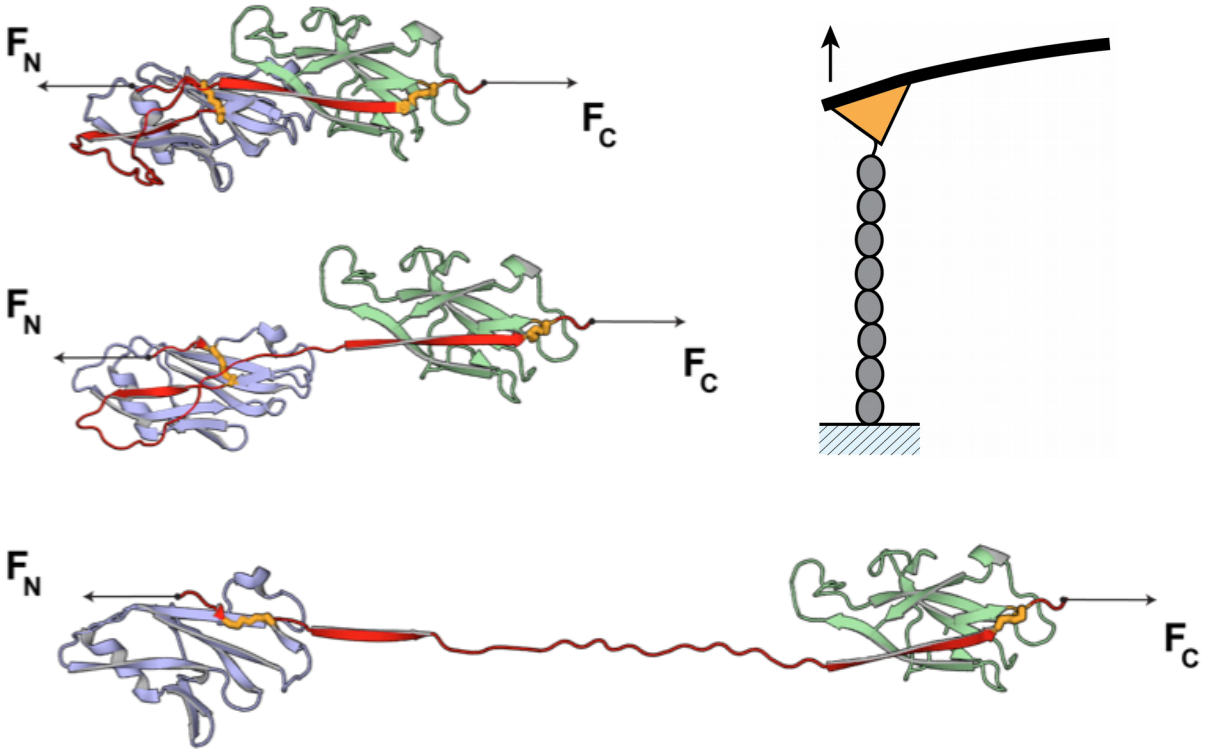
Mishra, A. *et al.* DOI:10.1128/JB.01952-06.

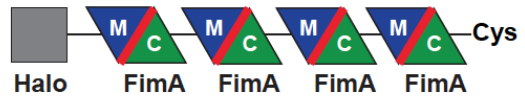


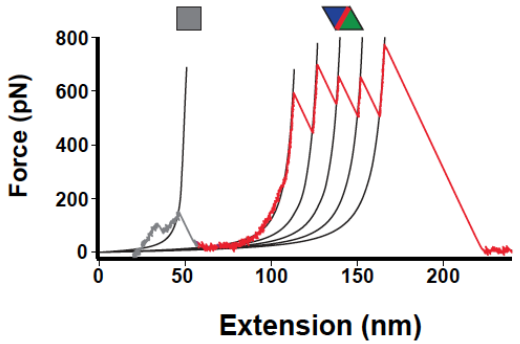
FimA

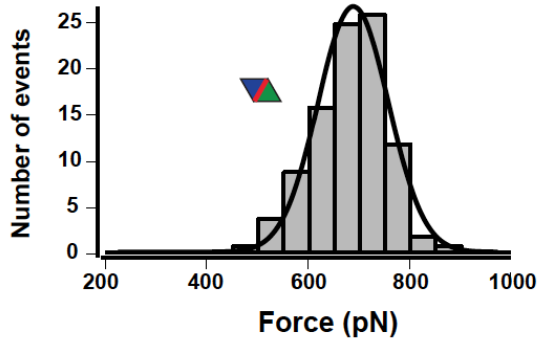
Dr. Alvaro Alonso Caballero

The FimA IDL's require a large force to unfold (~700 pN)

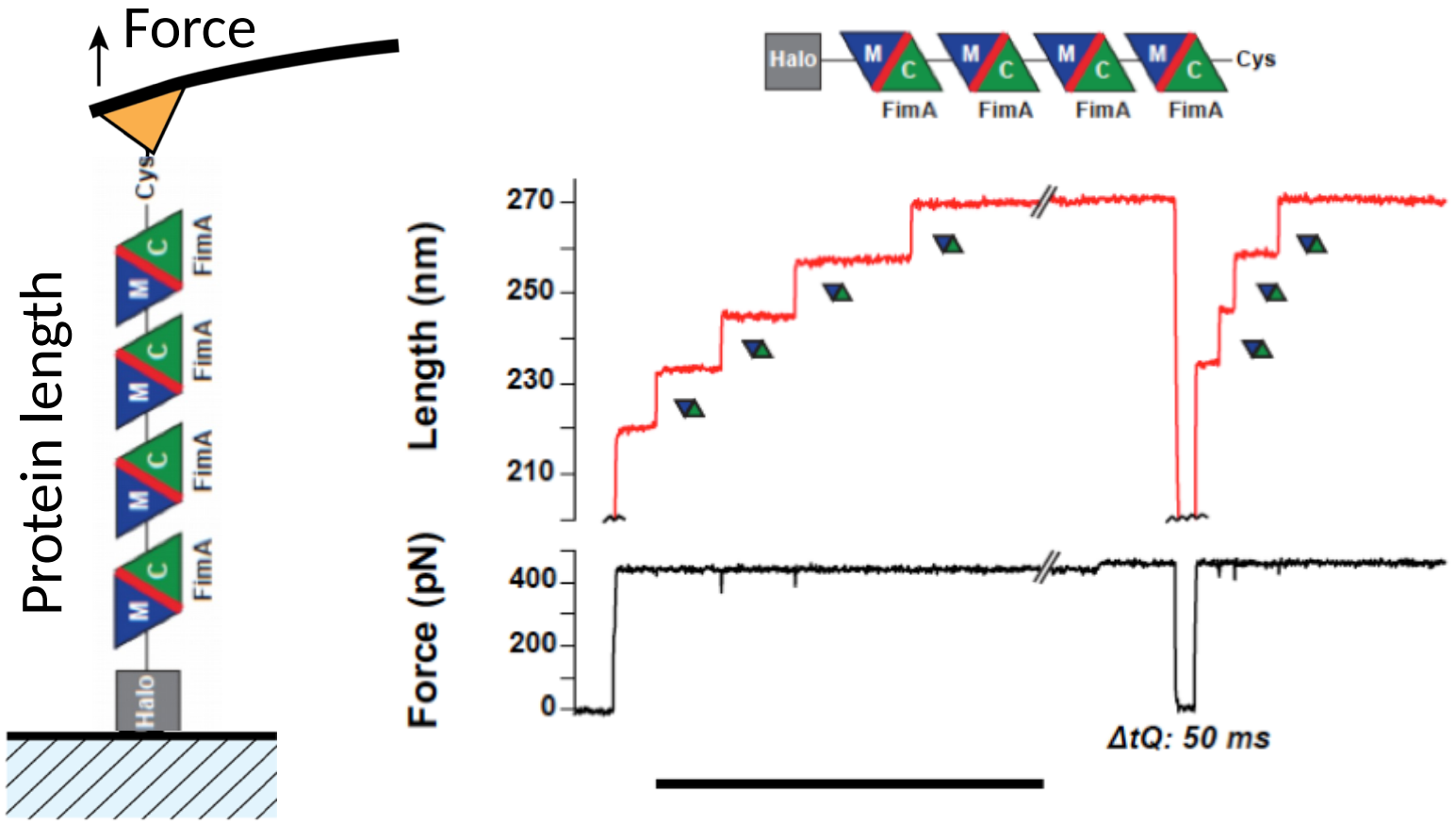




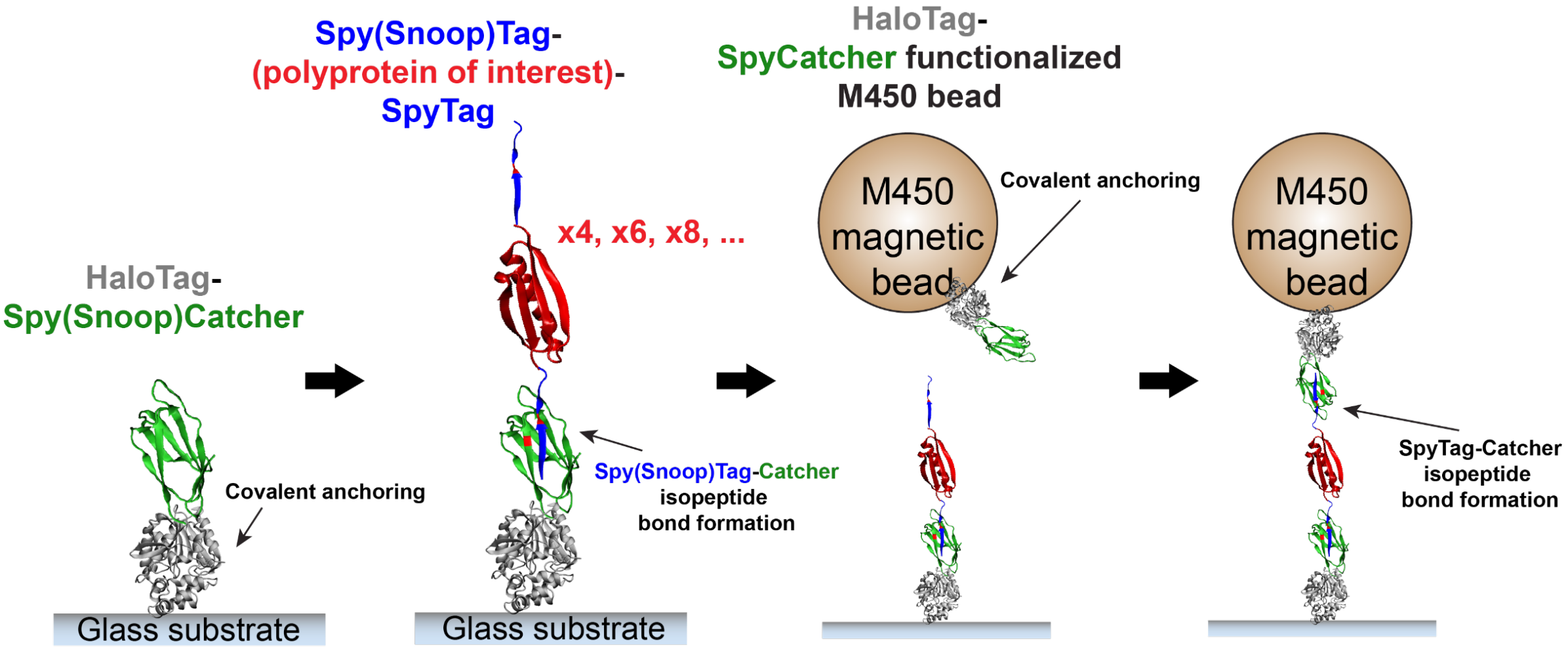




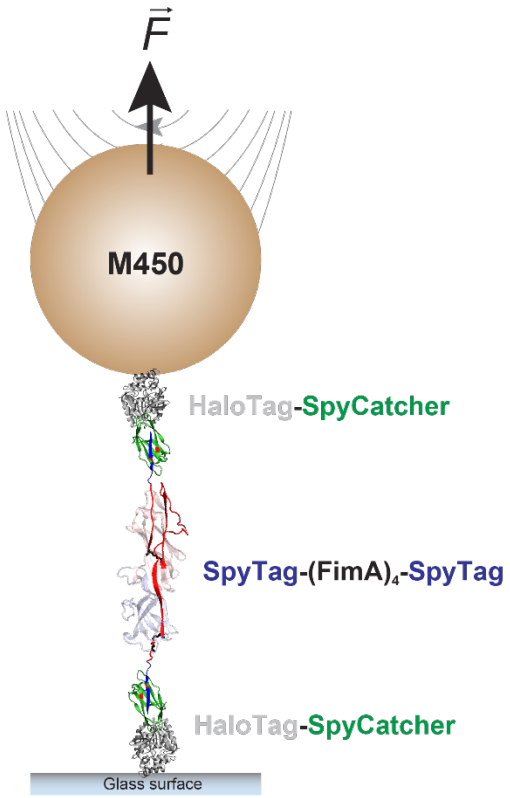
FimA refolding requires that the force drops <10 pN

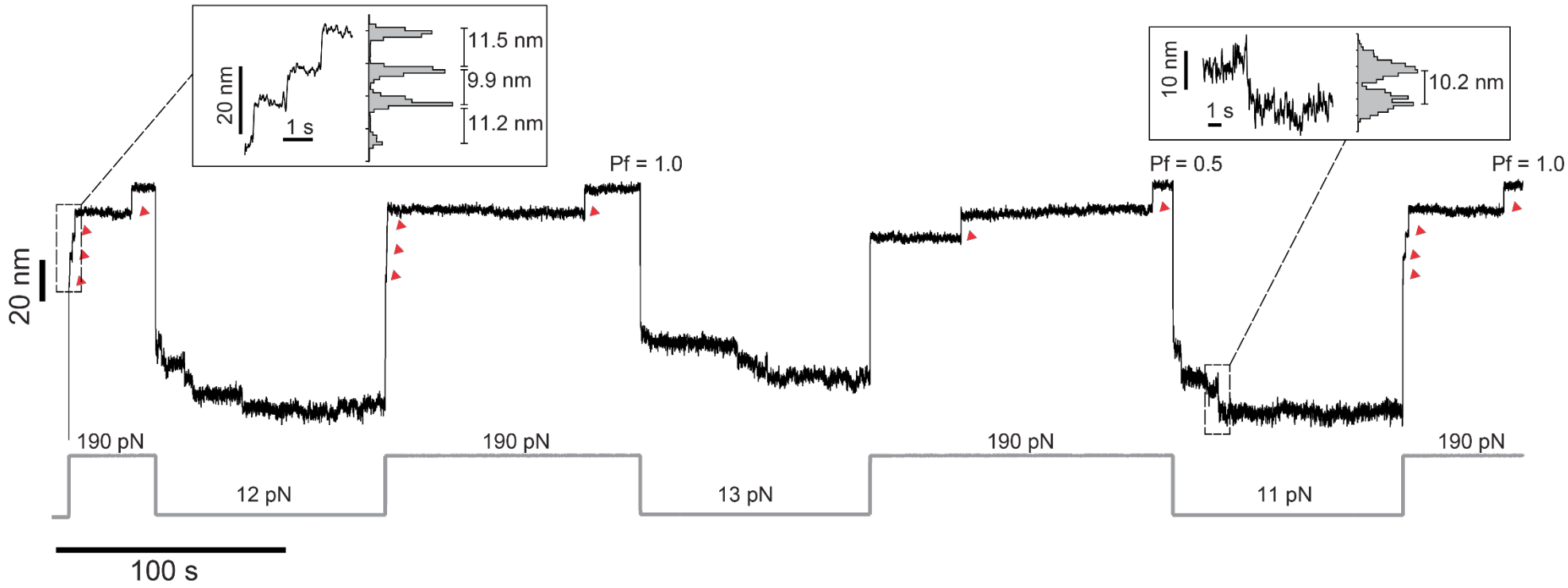


Double-covalent and split-protein technique



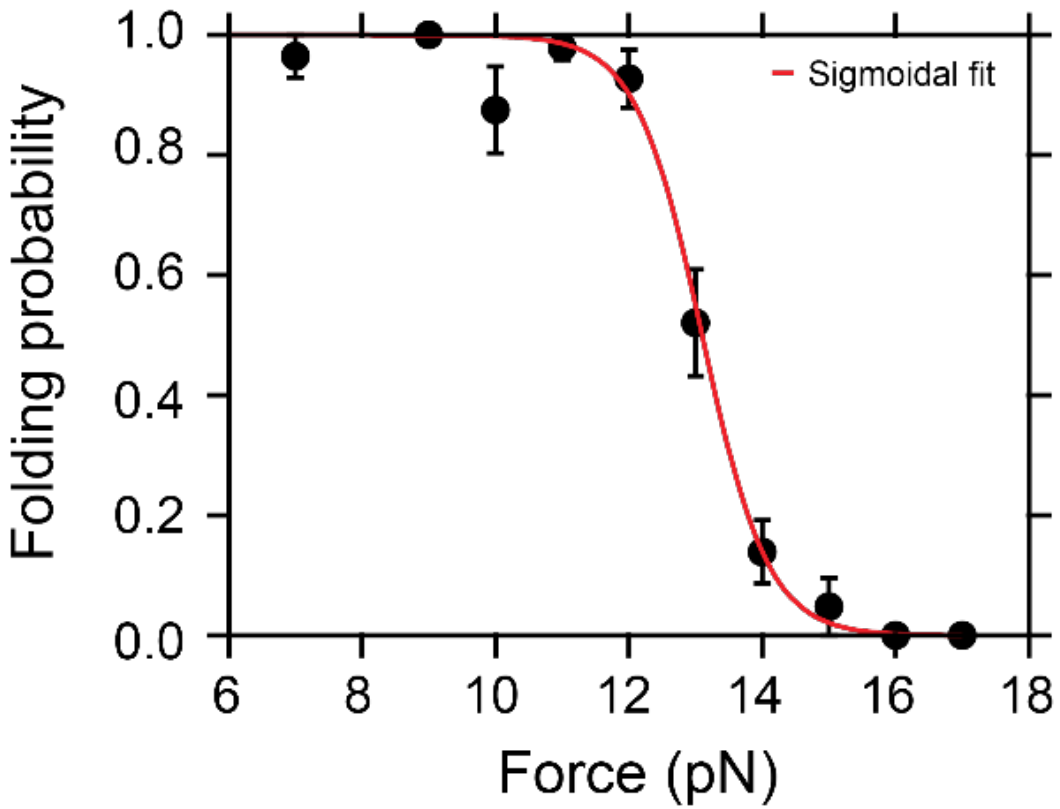
FimA IDL's, Not-folding, Not-unfolding (P425)



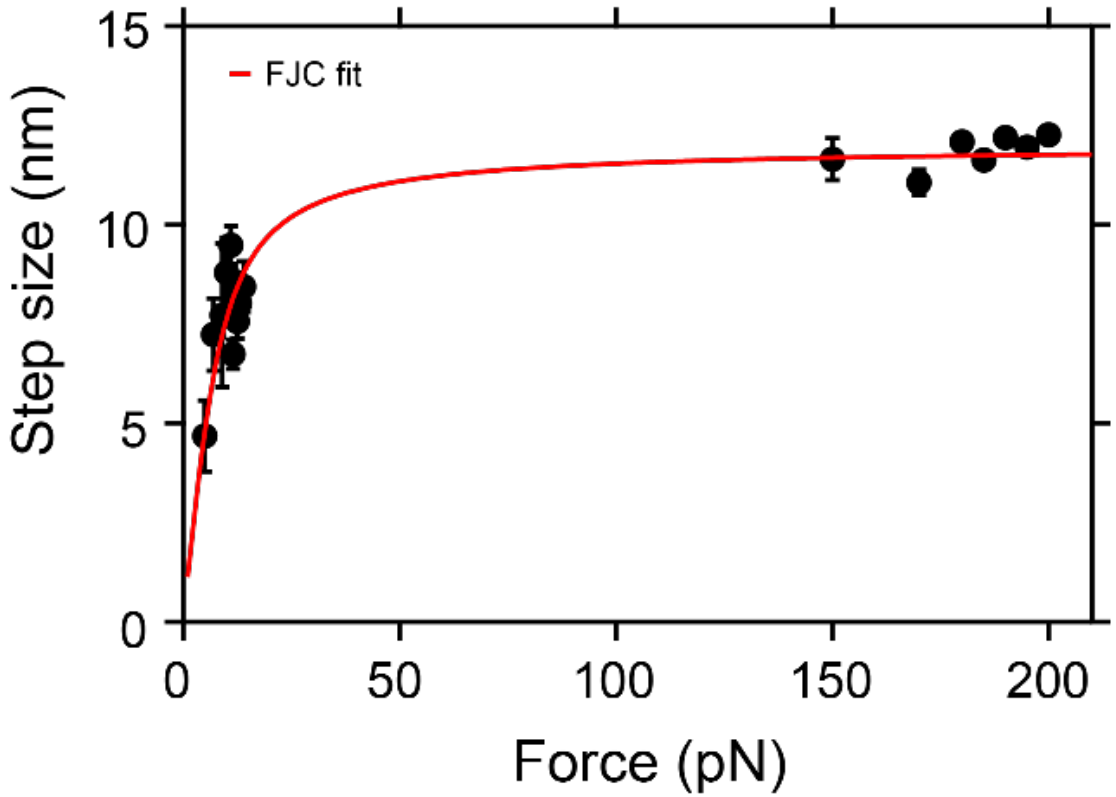


FimA

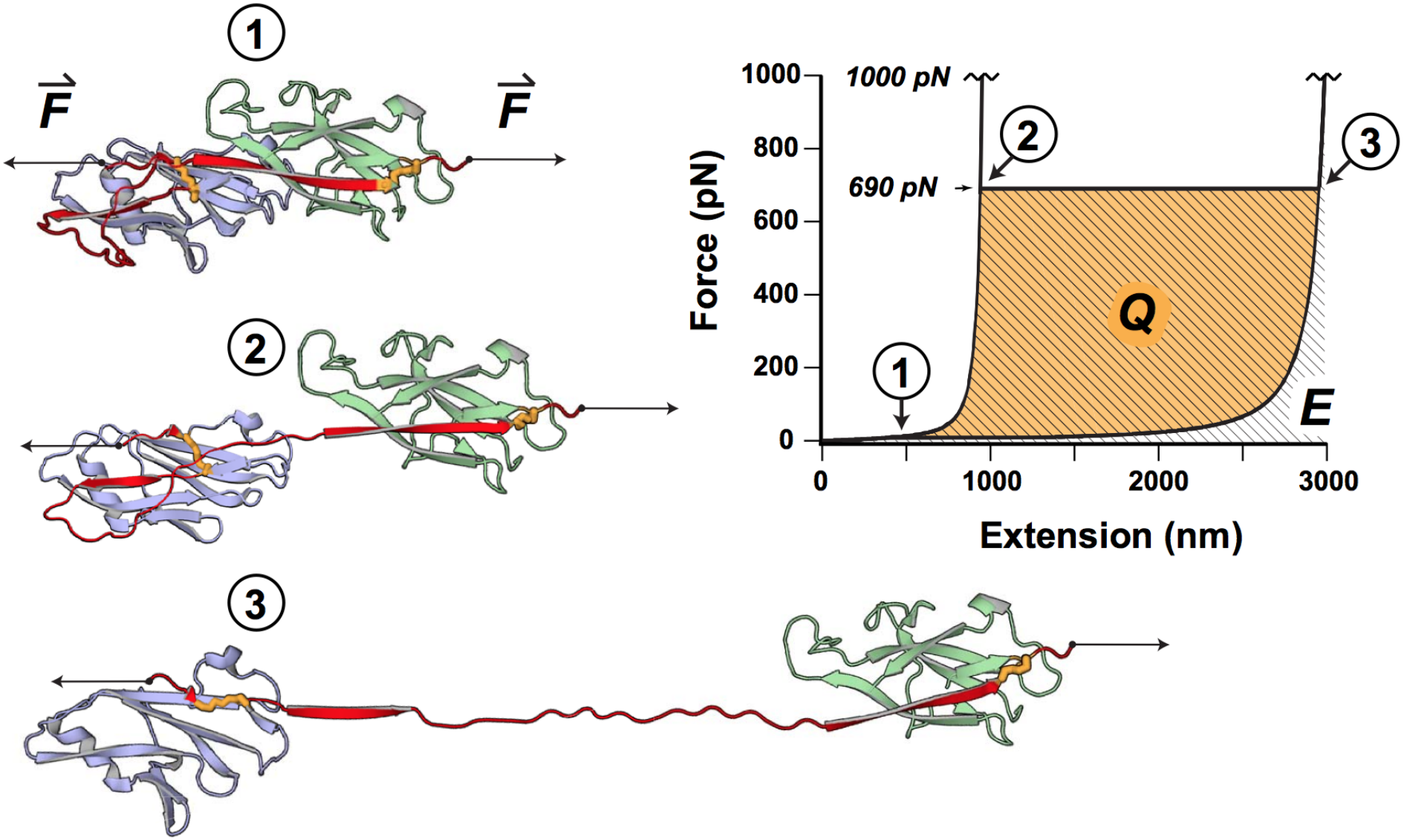
IDL Folding contraction probability



IDL extension as a function of force

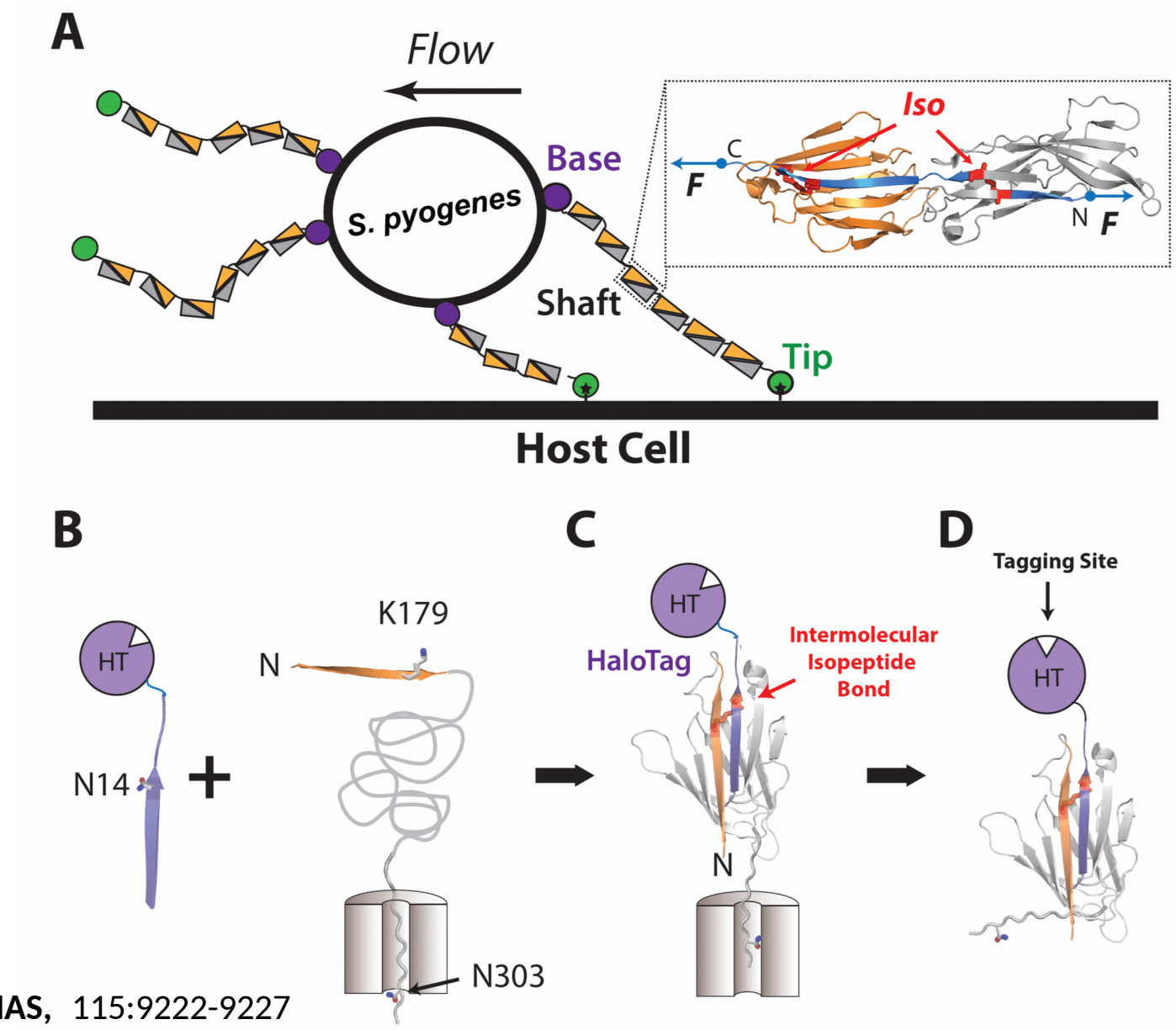


IDL's are very effective shock dissipaters



IDL folding = inter-domain polymer collapse and

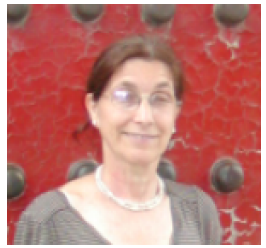
Blocking isopeptide bond formation in pili



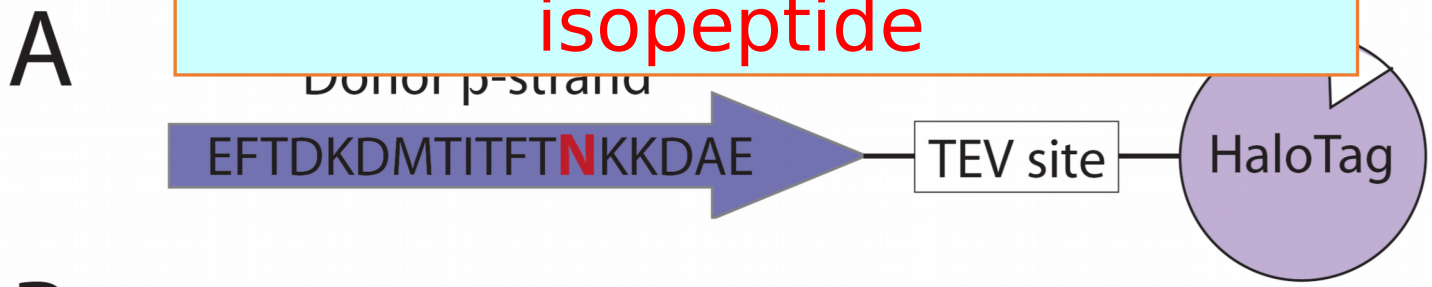
Rivas-Pardo et al., 2018, **PNAS,** 115:9222-9227

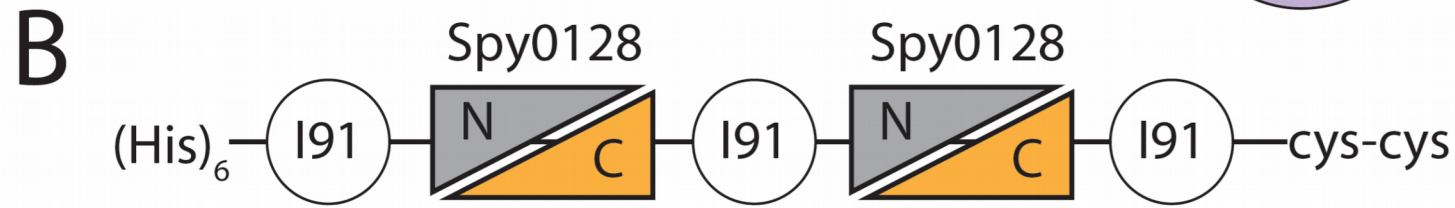
design of a blocking isopeptide

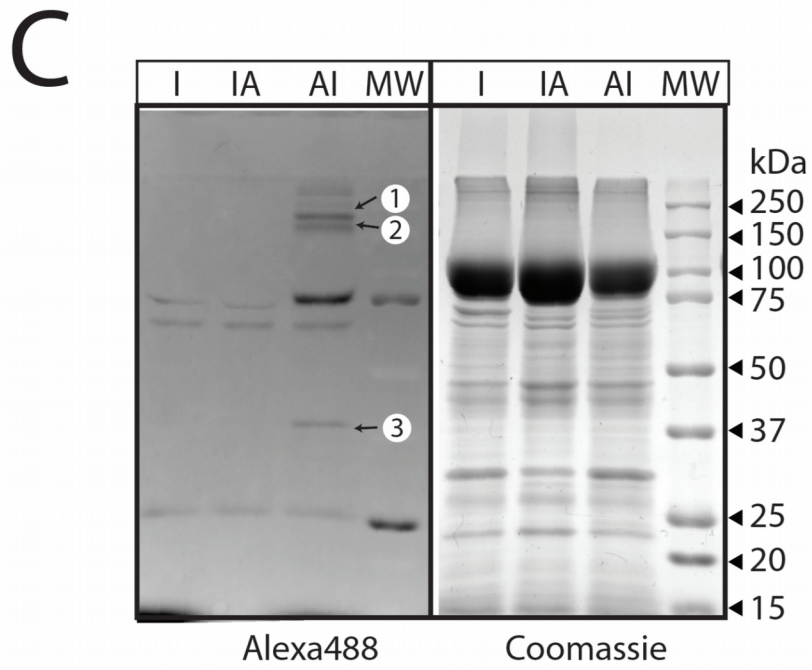
Andrés Rivas

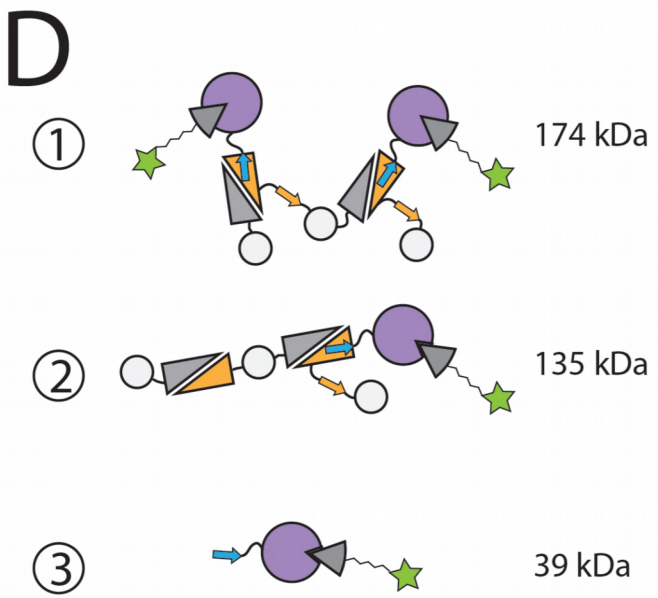


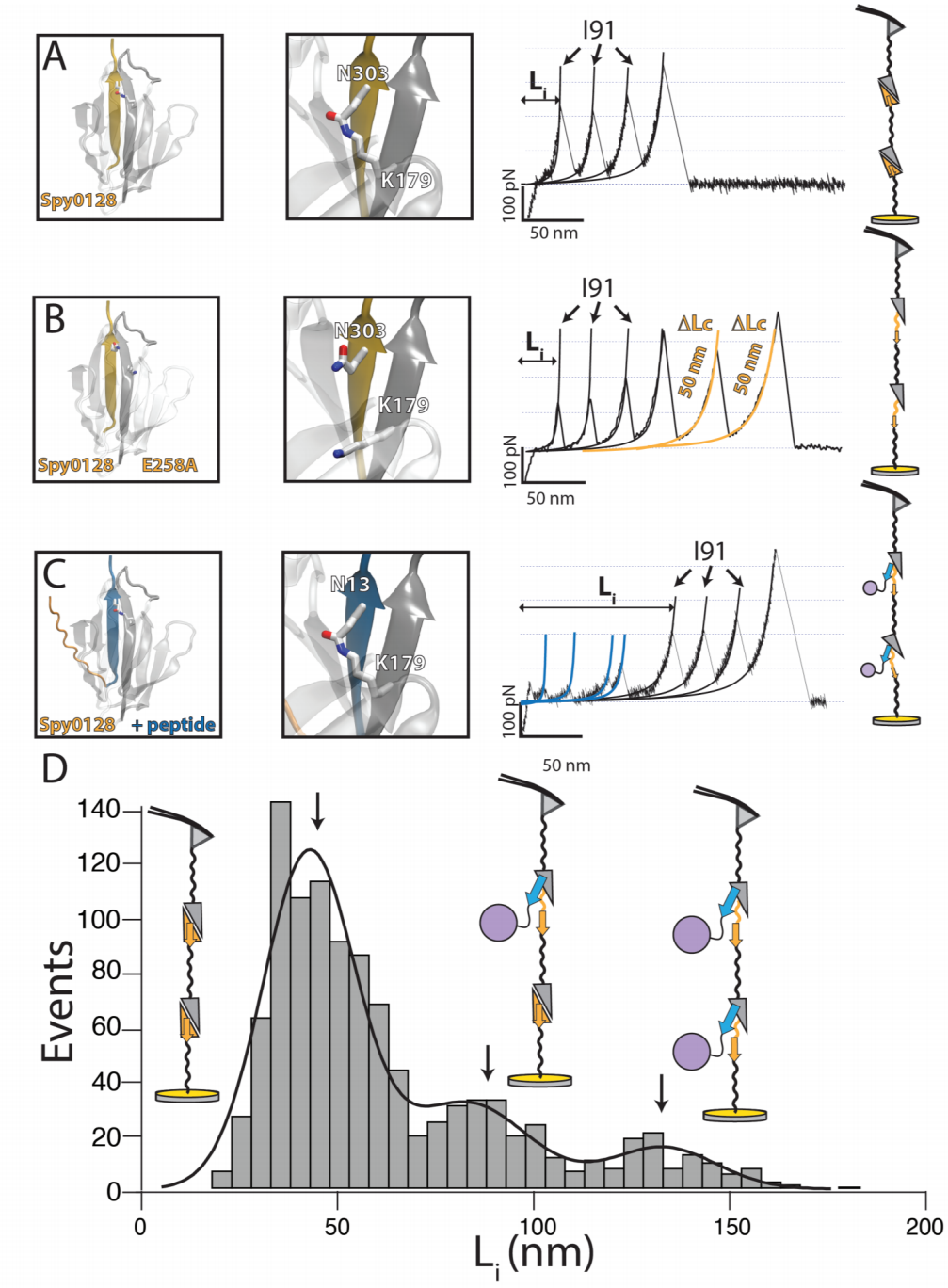
Carmelu Badilla



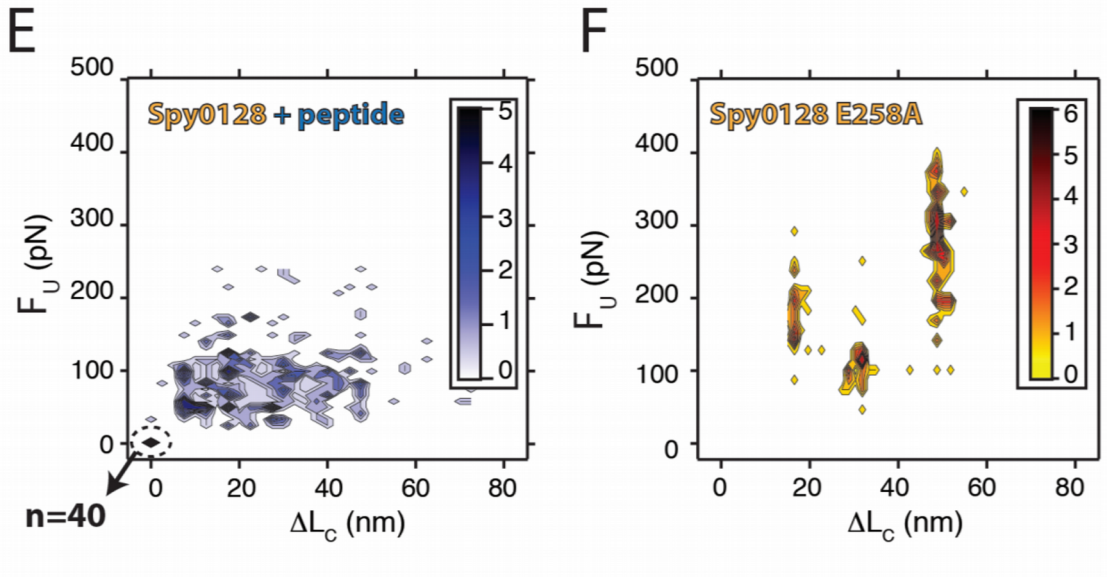




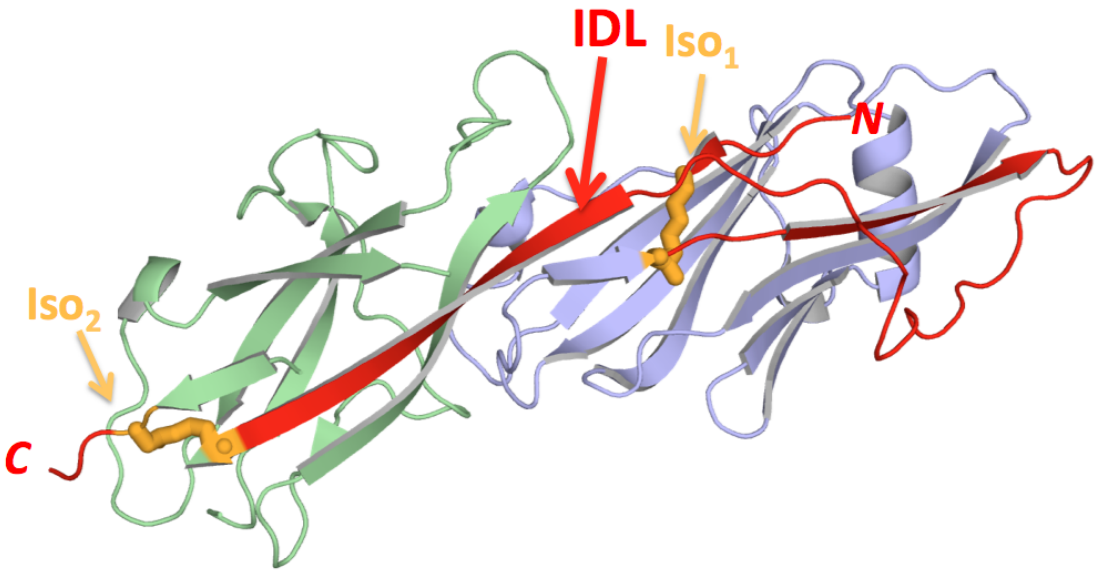




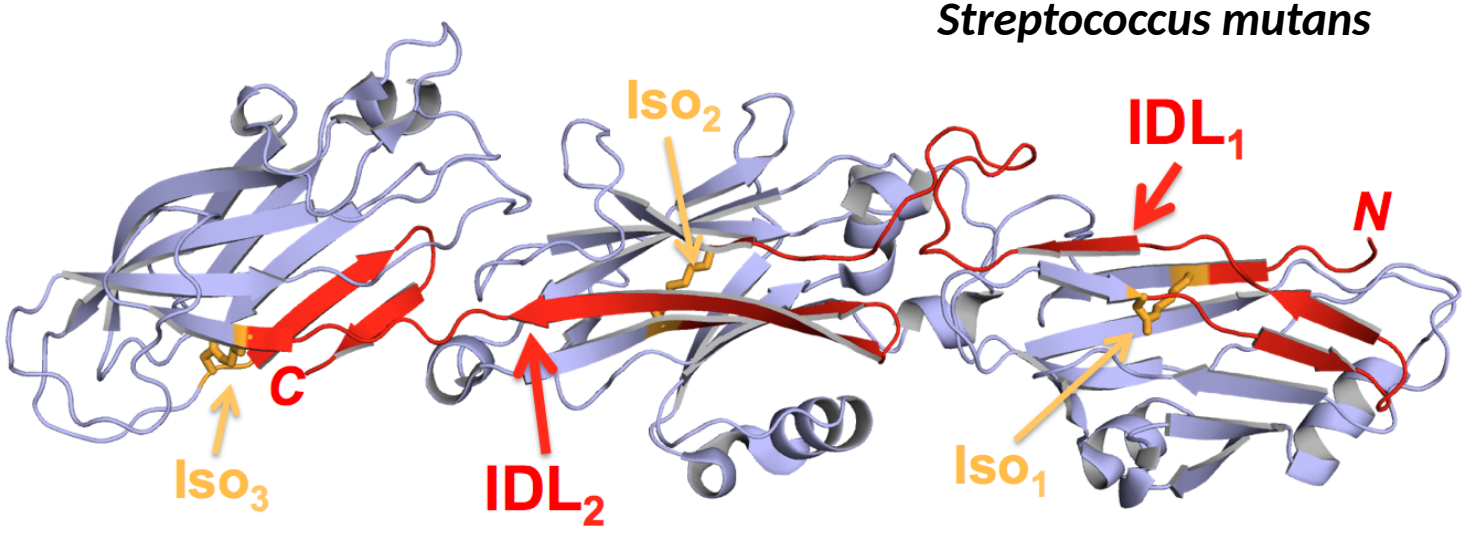
A blocking isopeptide is far more effective in knocking out the mechanical stability of pili than an isopeptide mutation



FimA *Actinomyces oris*

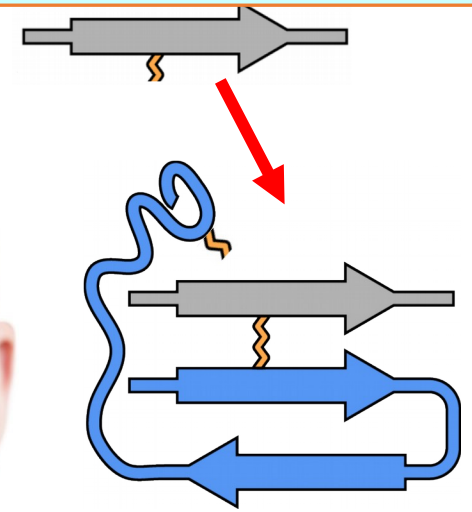


Streptococcus mutans



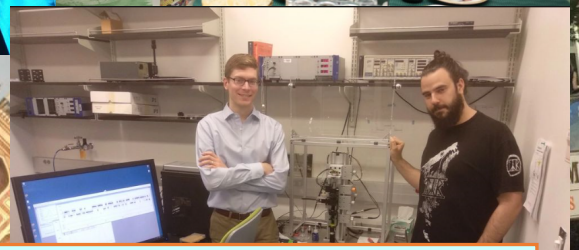
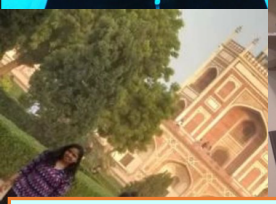
Rational design of antiadhesive peptide antibiotics

A new type of toothpaste

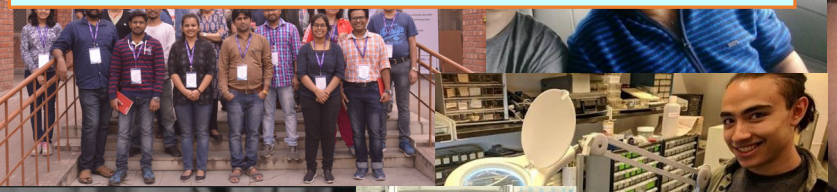
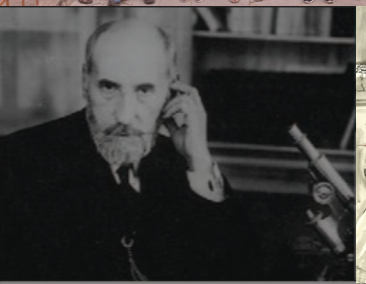
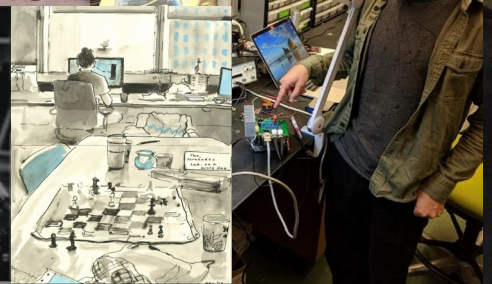


A new type of peptide

A vaccine against dental caries?

Look us up in zeptowatt.com

EVERYTHING IN BIOLOGY IS MECHANICAL

FERNANDEZ LAB

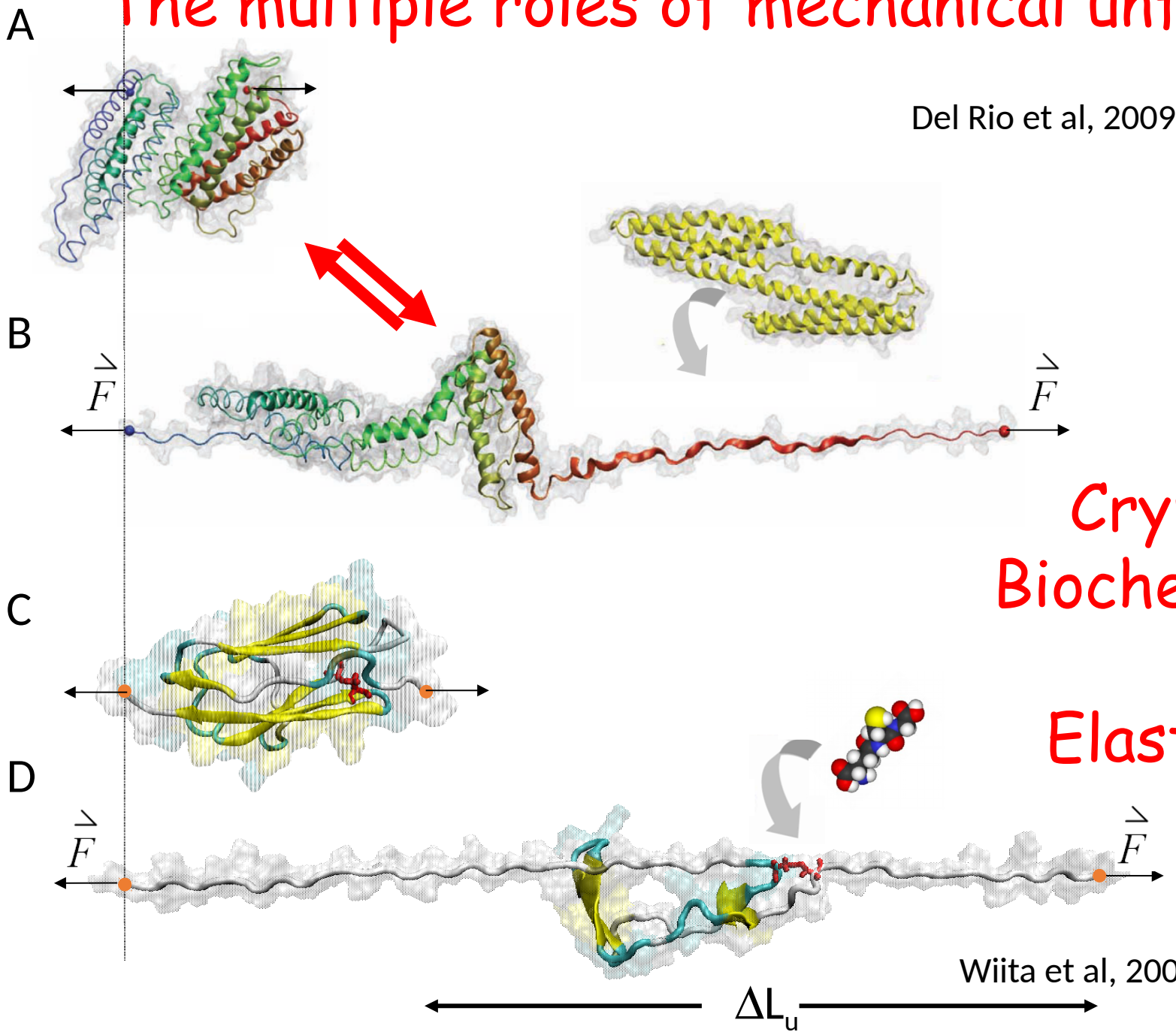
– HOME PUBLICATIONS SCIENTISTS PHOTOS DOWNLOADS CONTACT

Instrumentation

1.- AFM

2.- Magnetic Tweezers

The multiple roles of mechanical unfolding



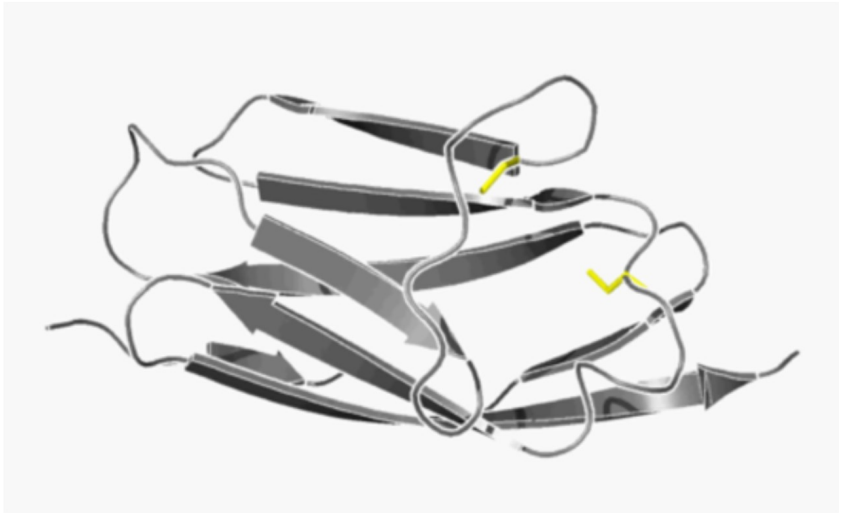
Del Rio et al, 2009, **Science**, 323: 638-641

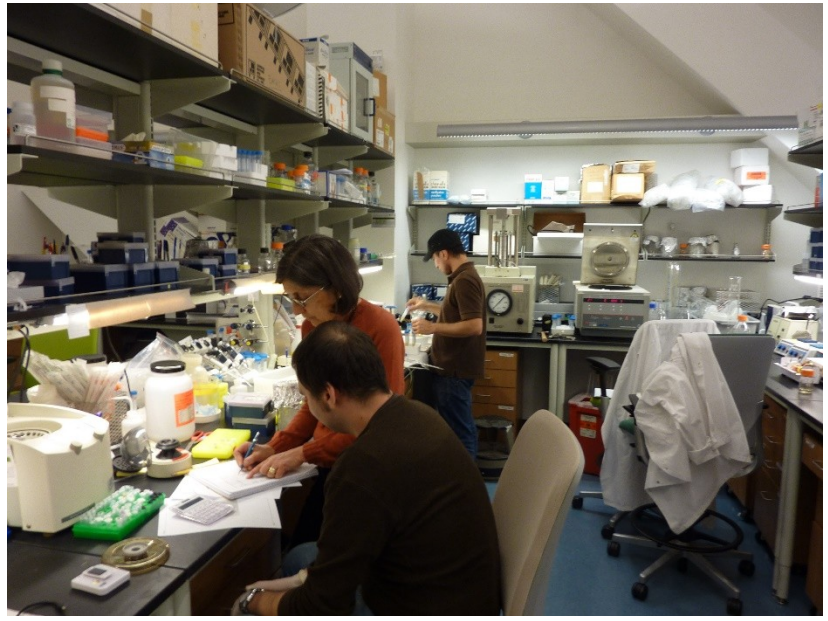
Cryptic Biochemistry

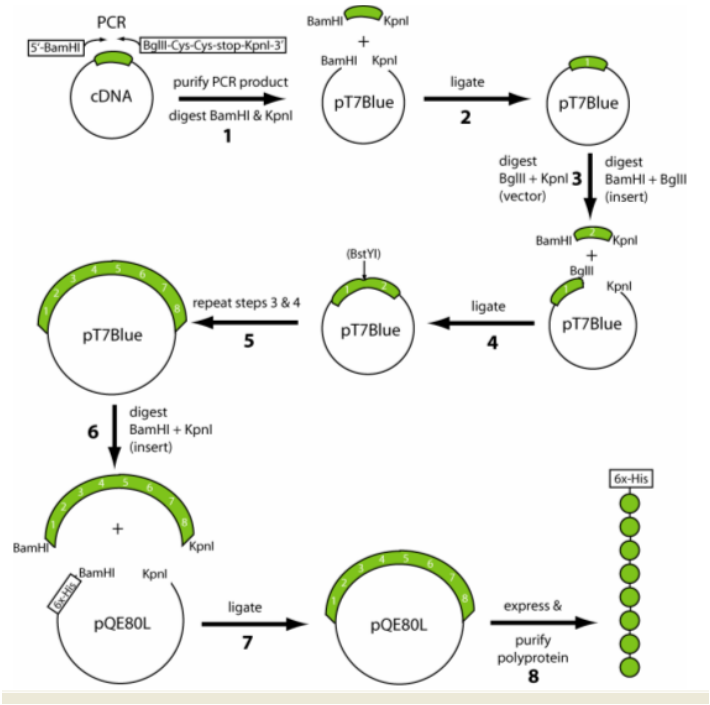
Elasticity

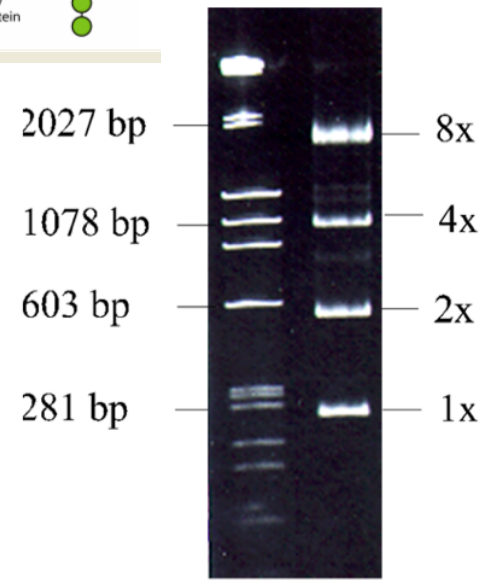
Wiita et al, 2007, **Nature**, **450**: 124-127

Polyprotein engineering for force spectroscopy
I: DNA engineering

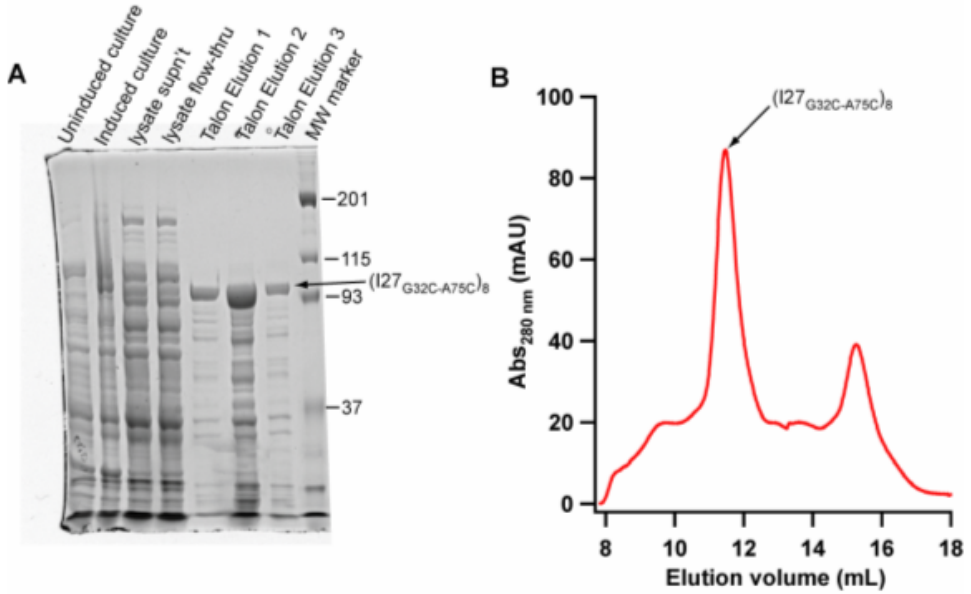


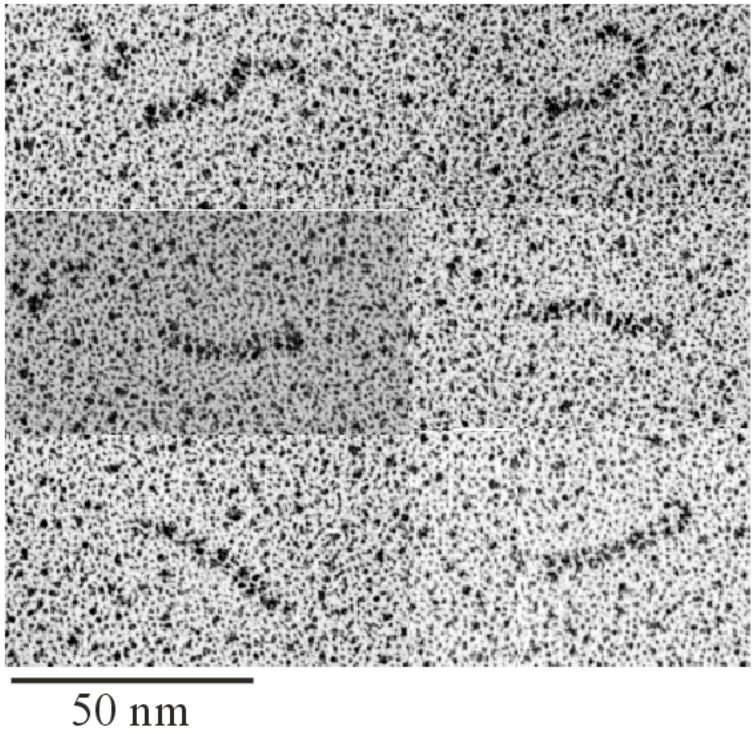


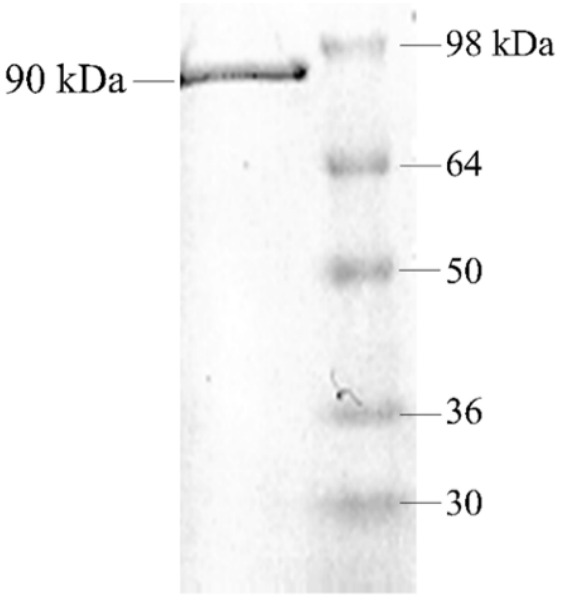




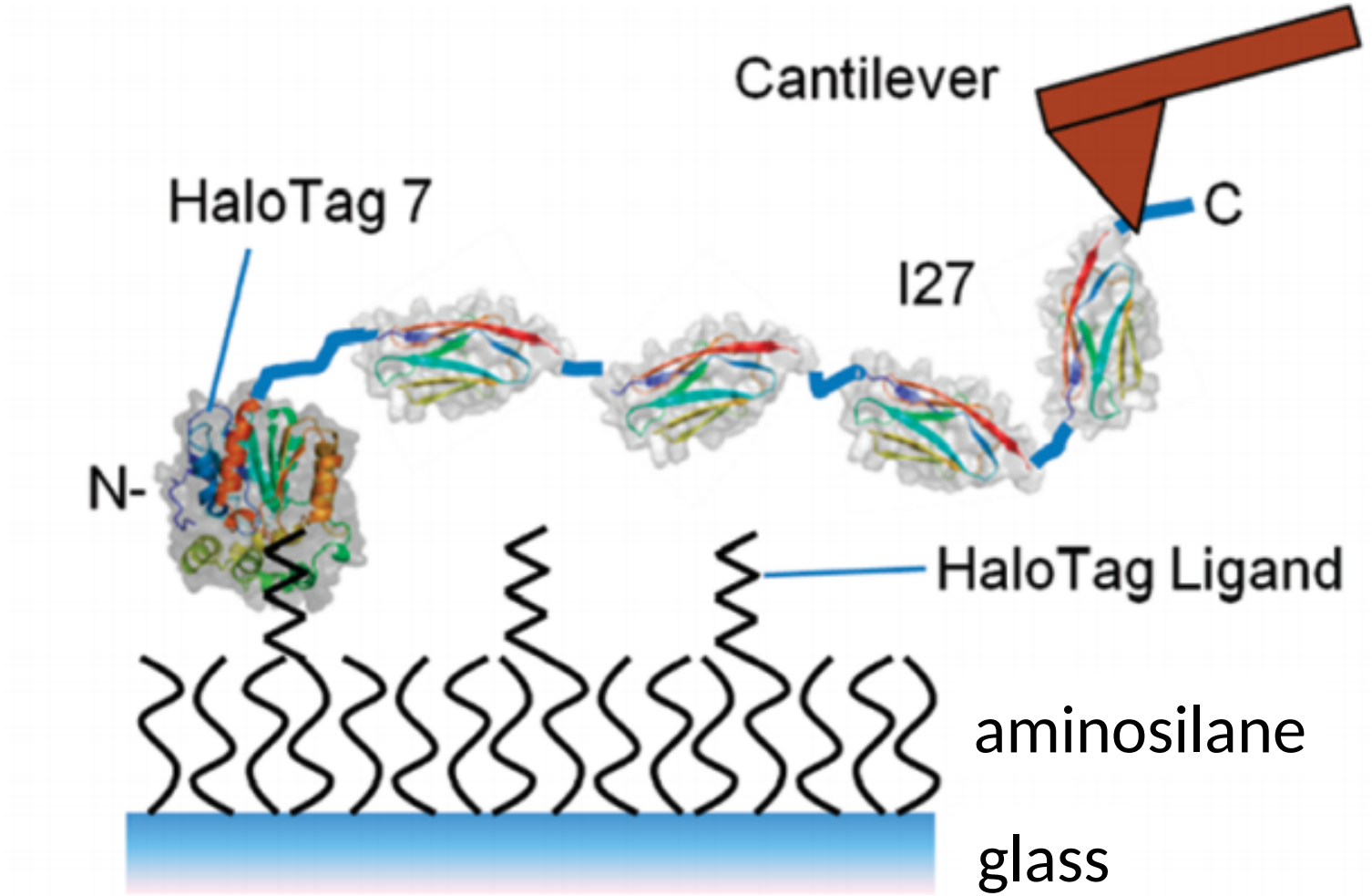
Protein engineering II; expression and purification





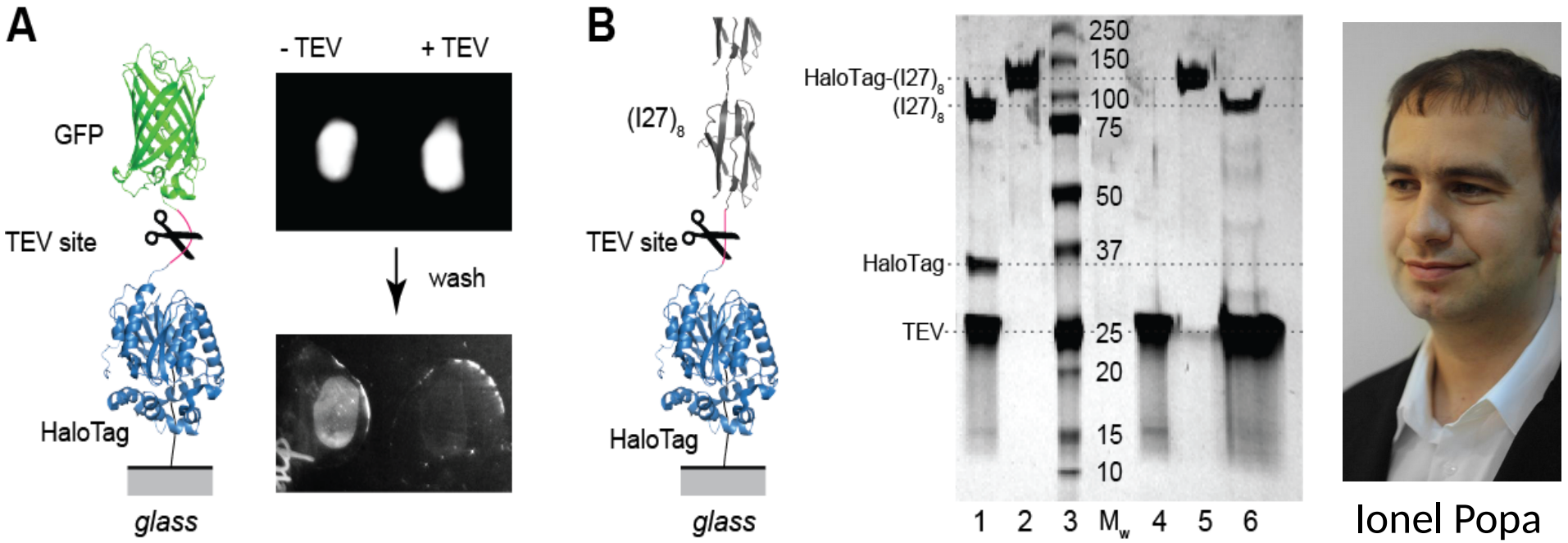


Polyprotein engineering III; anchoring



Taniguchi and Kawakami *Langmuir* **2010,** *26(13)*, 10433–10436

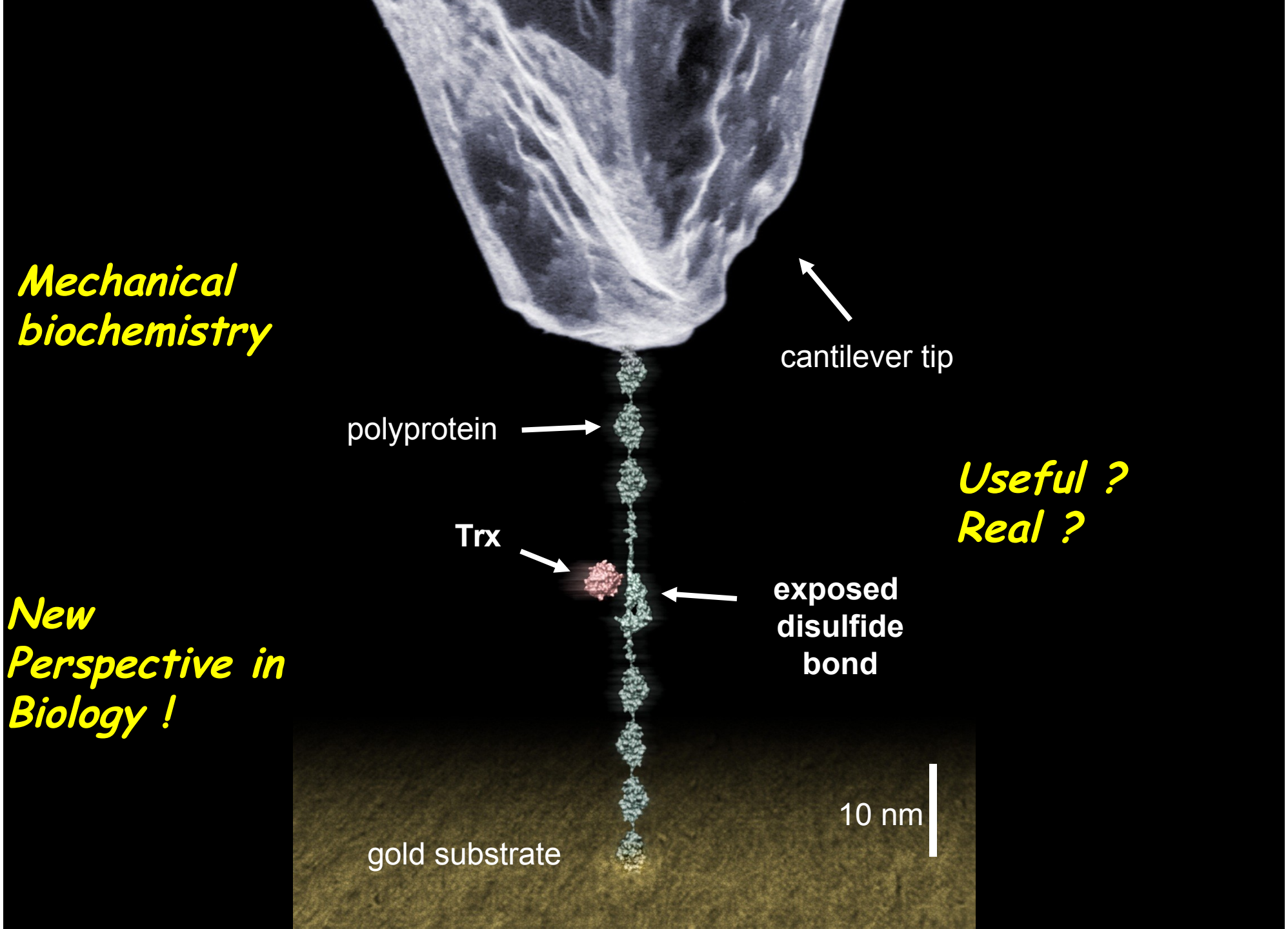
HaloTag and chloroalkane chemistry for the covalent anchoring of polyproteins



Ionel Popa

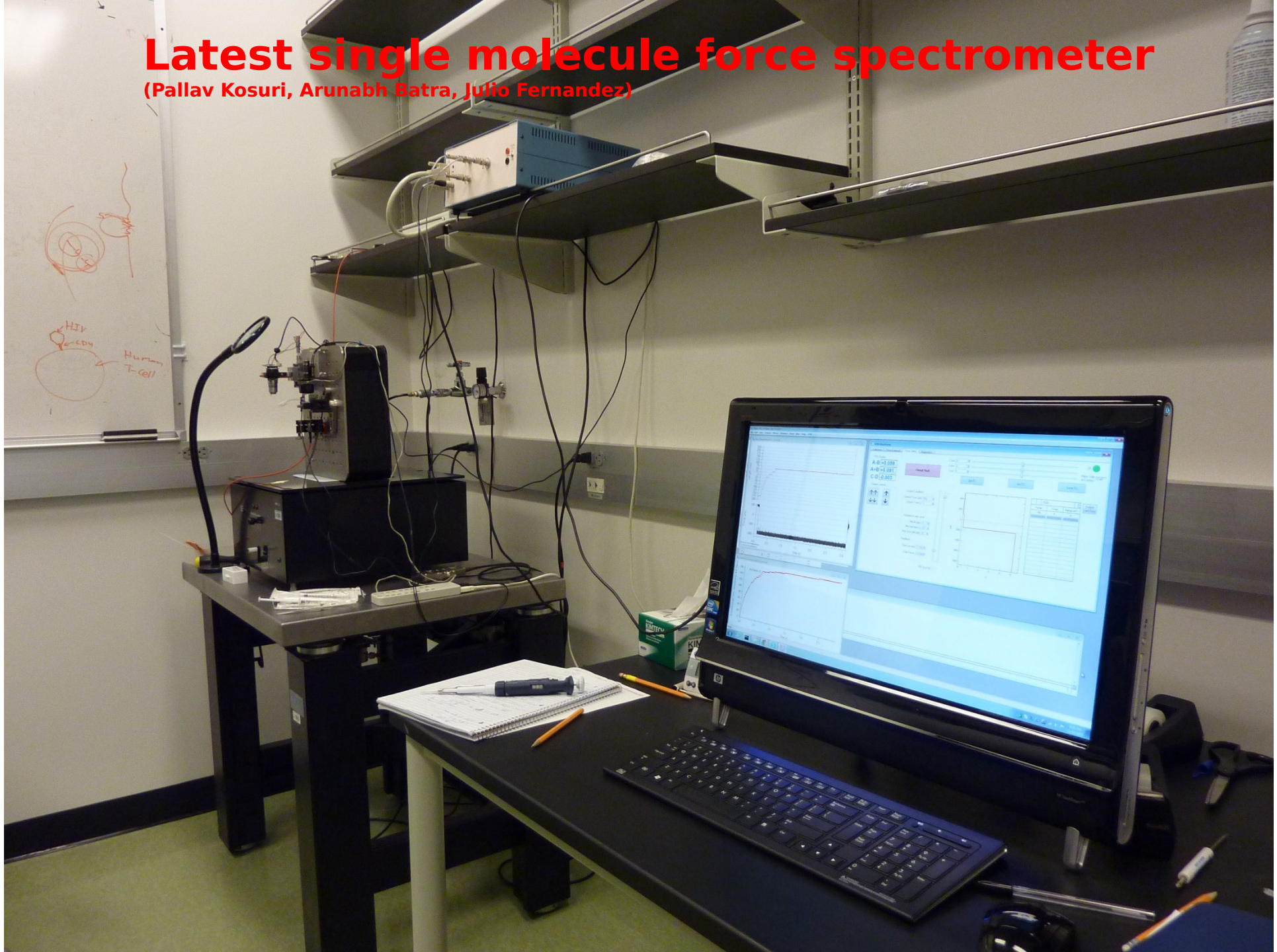
Nanomechanics of HaloTag Tethers **JACS 2013**

Ionel Popa,*,† Ronen Berkovich,† Jorge Alegre-Cebollada,† Carmen L. Badilla,† Jaime Andrés Rivas-Pardo,† Yukinori Taniguchi,‡ Masaru Kawakami,‡ and Julio M. Fernandez*,†

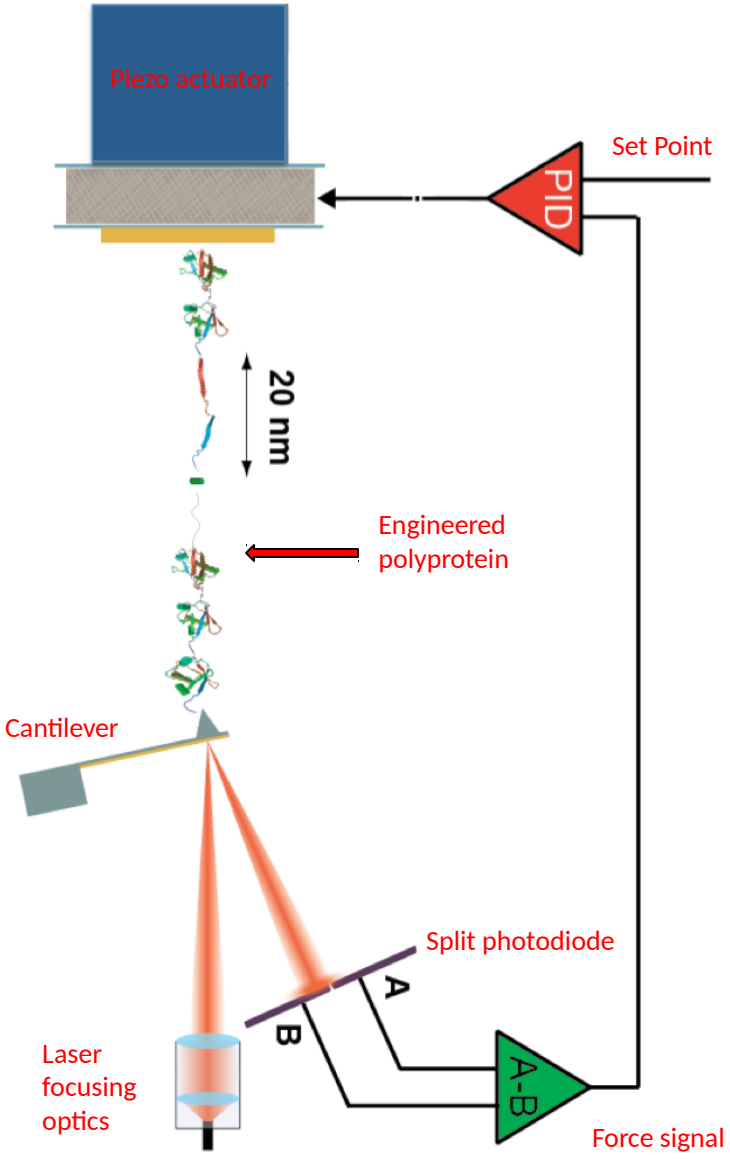

Mechanical biochemistry
cantilever tip
polyprotein
Useful ? Real ?
Trx
exposed disulfide bond
New Perspective in Biology !
10 nm
gold substrate

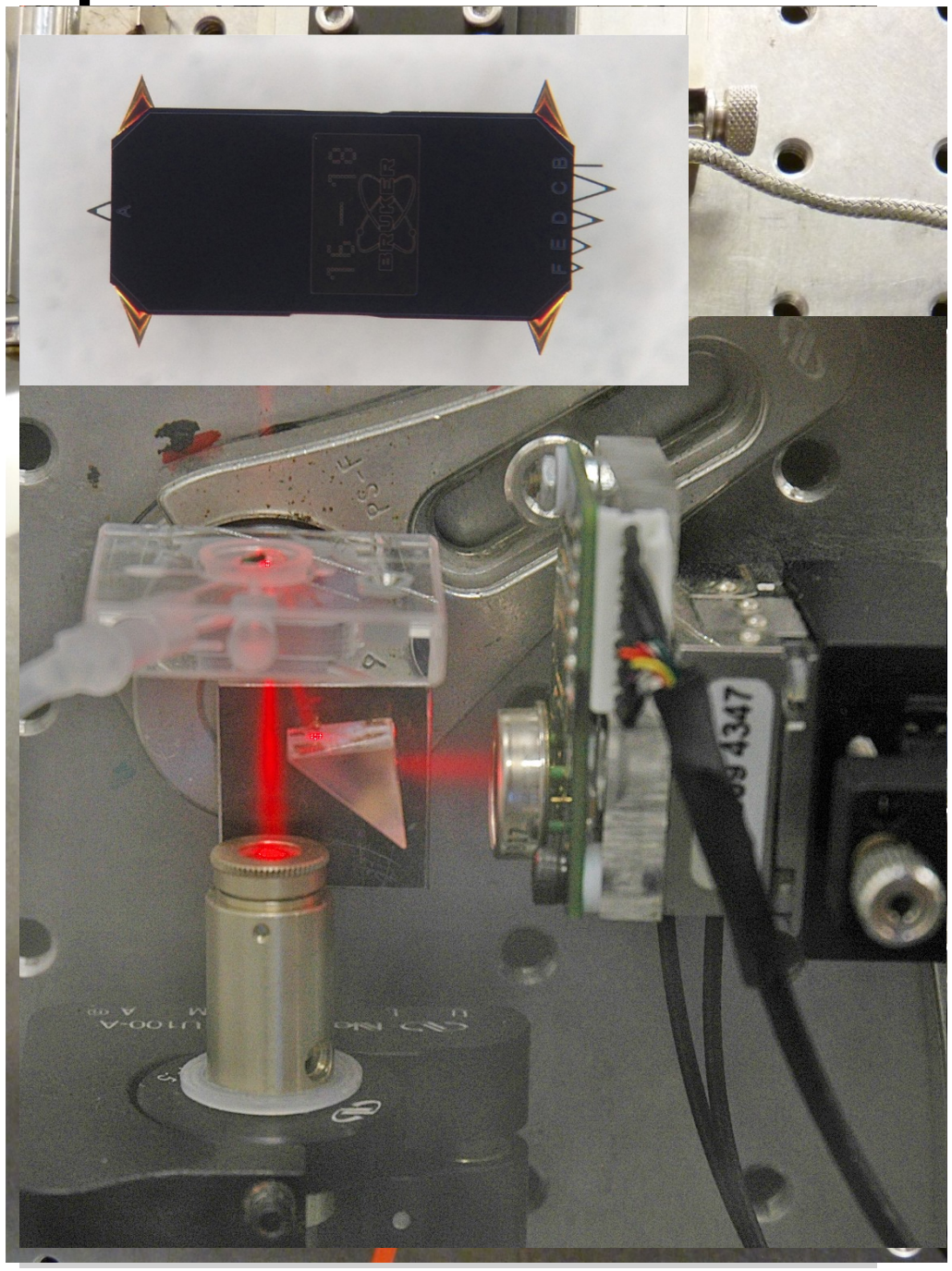
Latest single molecule force spectrometer

(Pallav Kosuri, Arunabh Batra, Julio Fernandez)

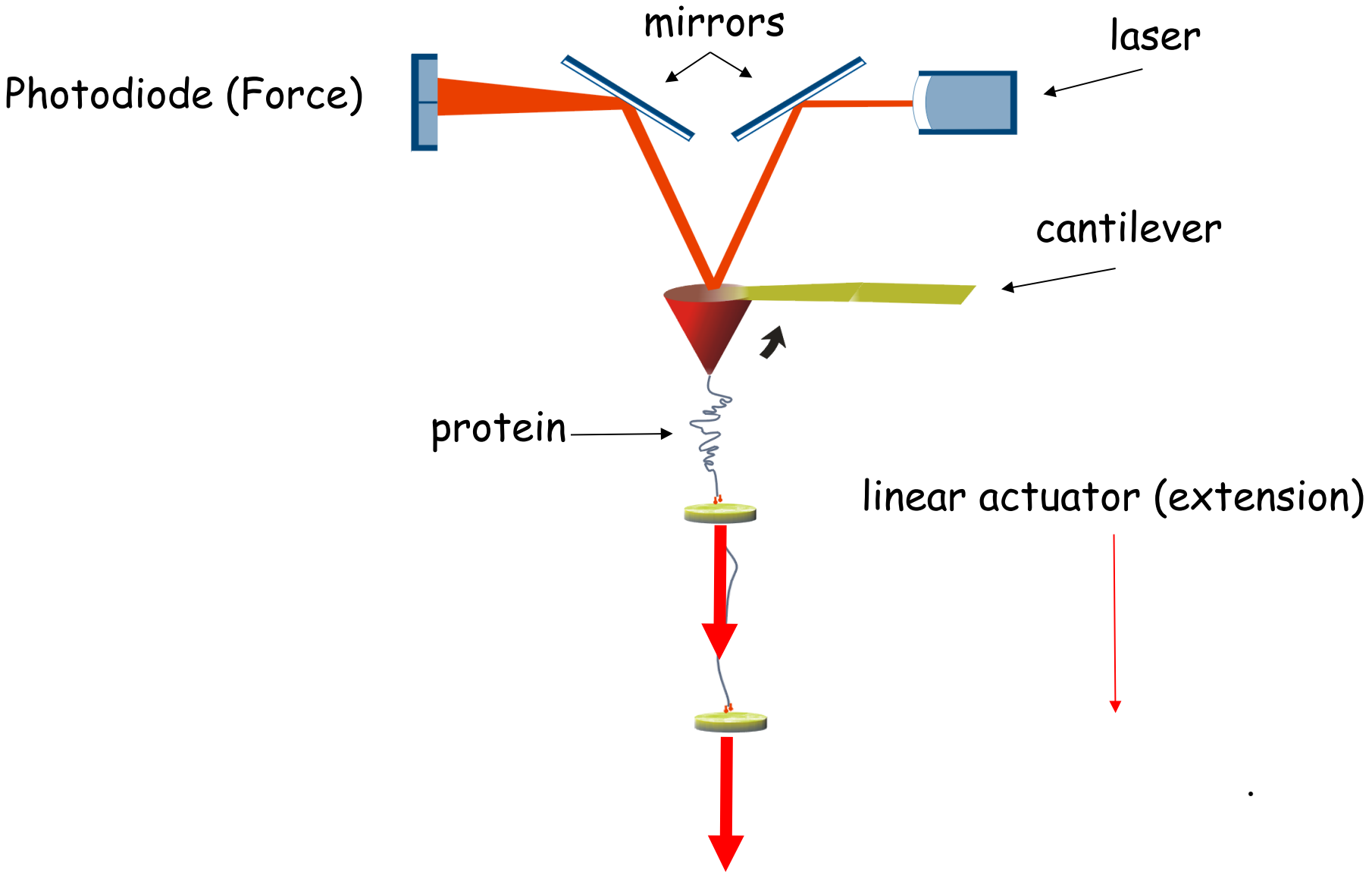


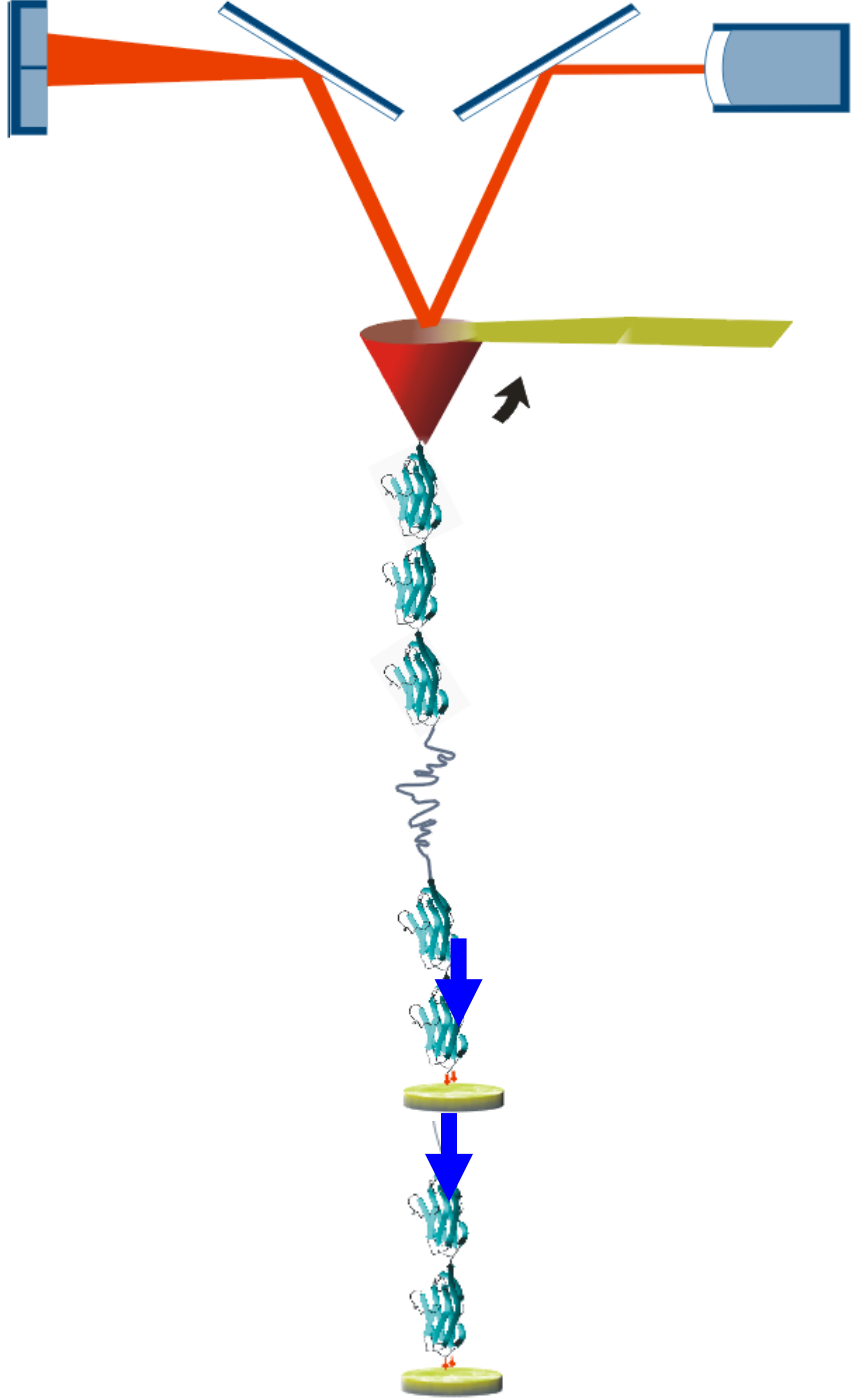
Force sensor and piezoelectric actuator

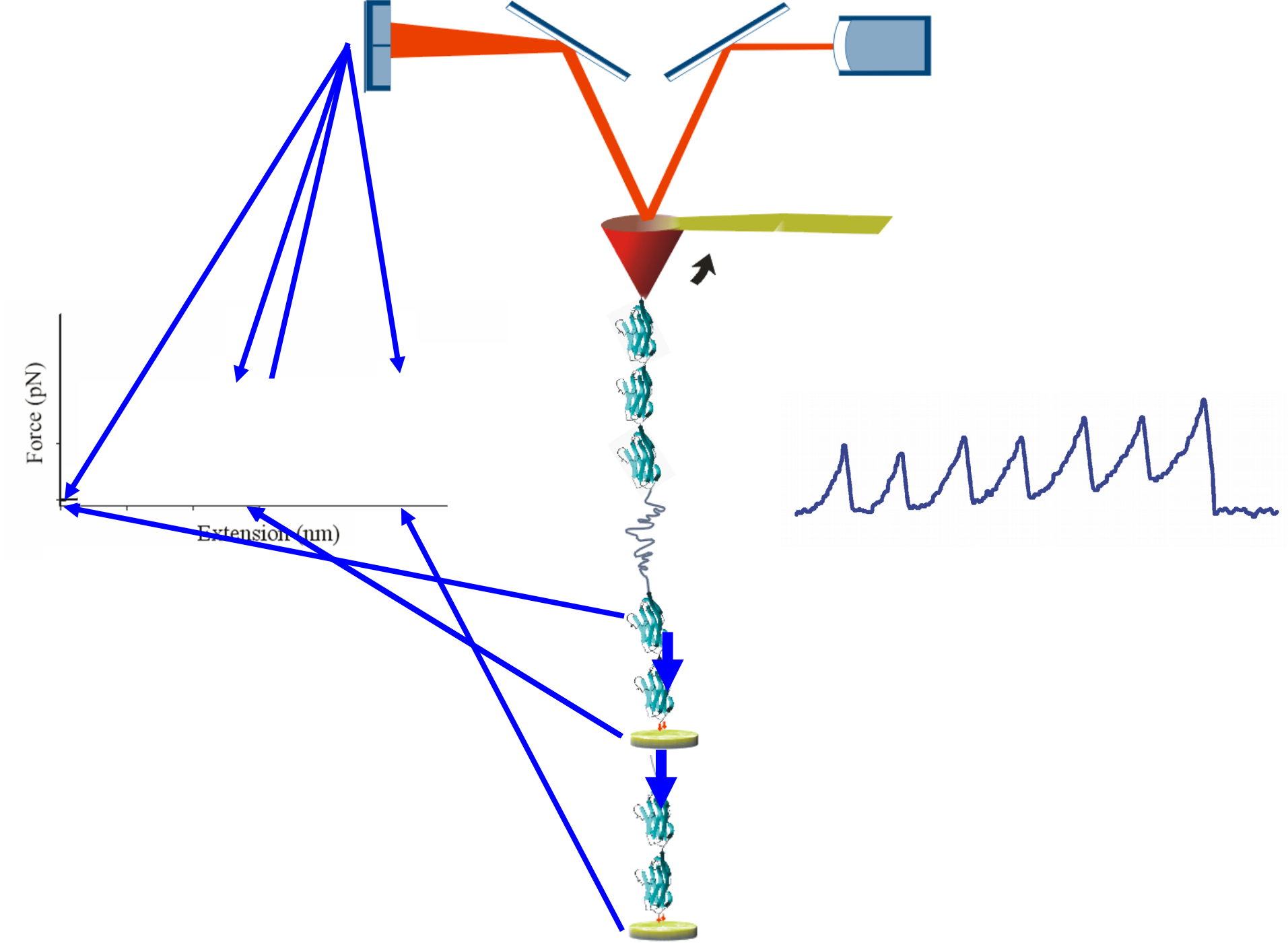


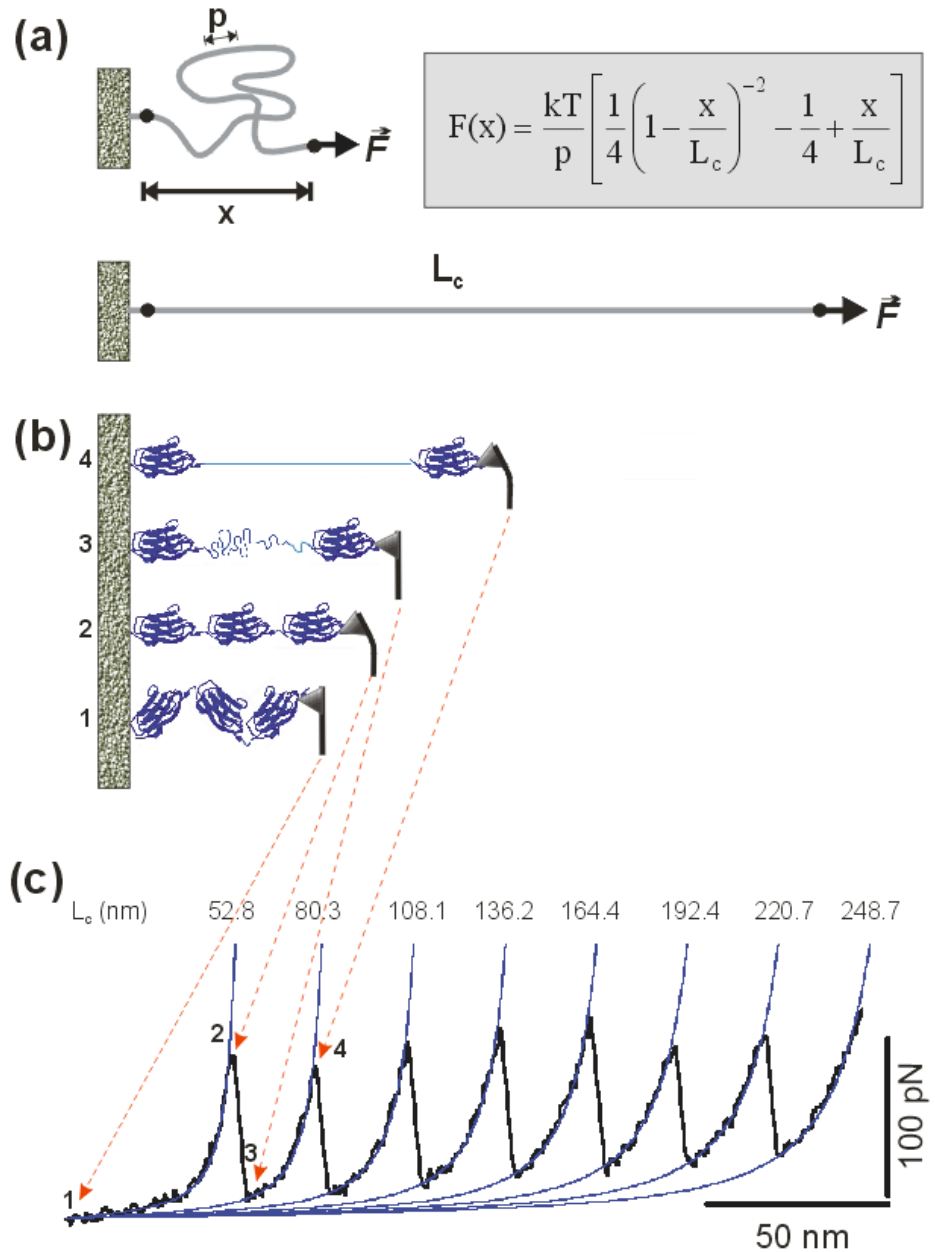


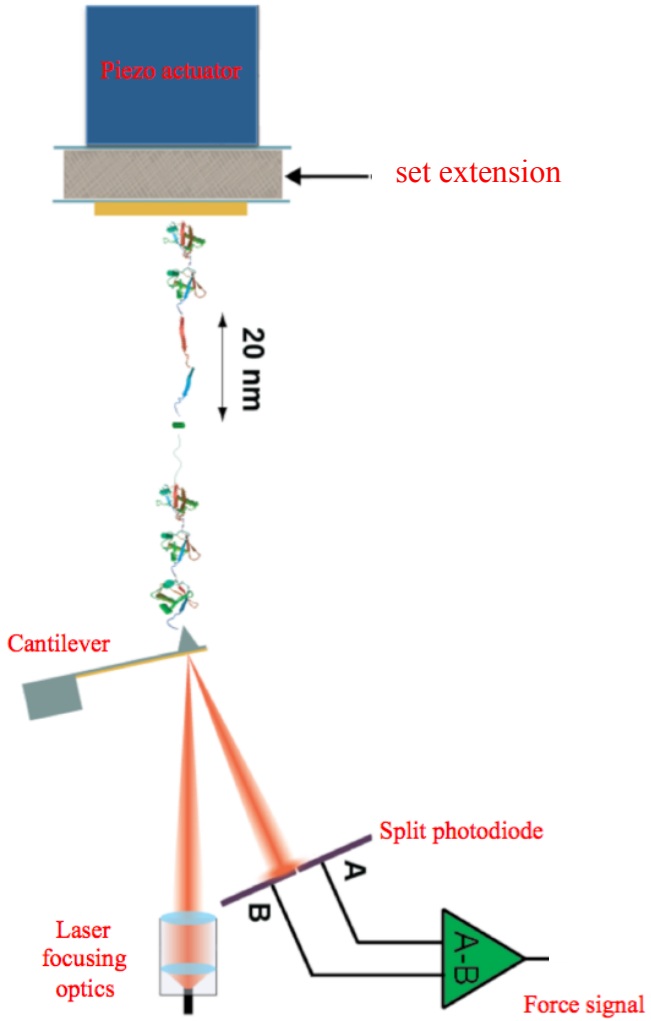
We can stretch a single protein and measure how does the restoring force changes with the extension.



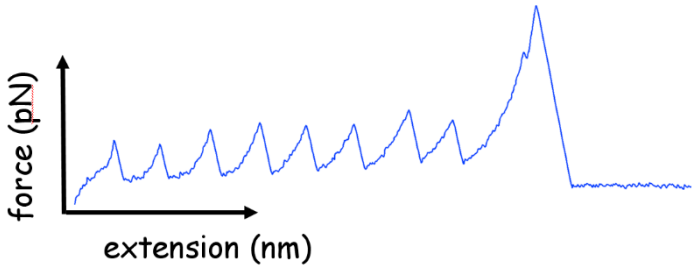



Force (pN)
Extension (nm)

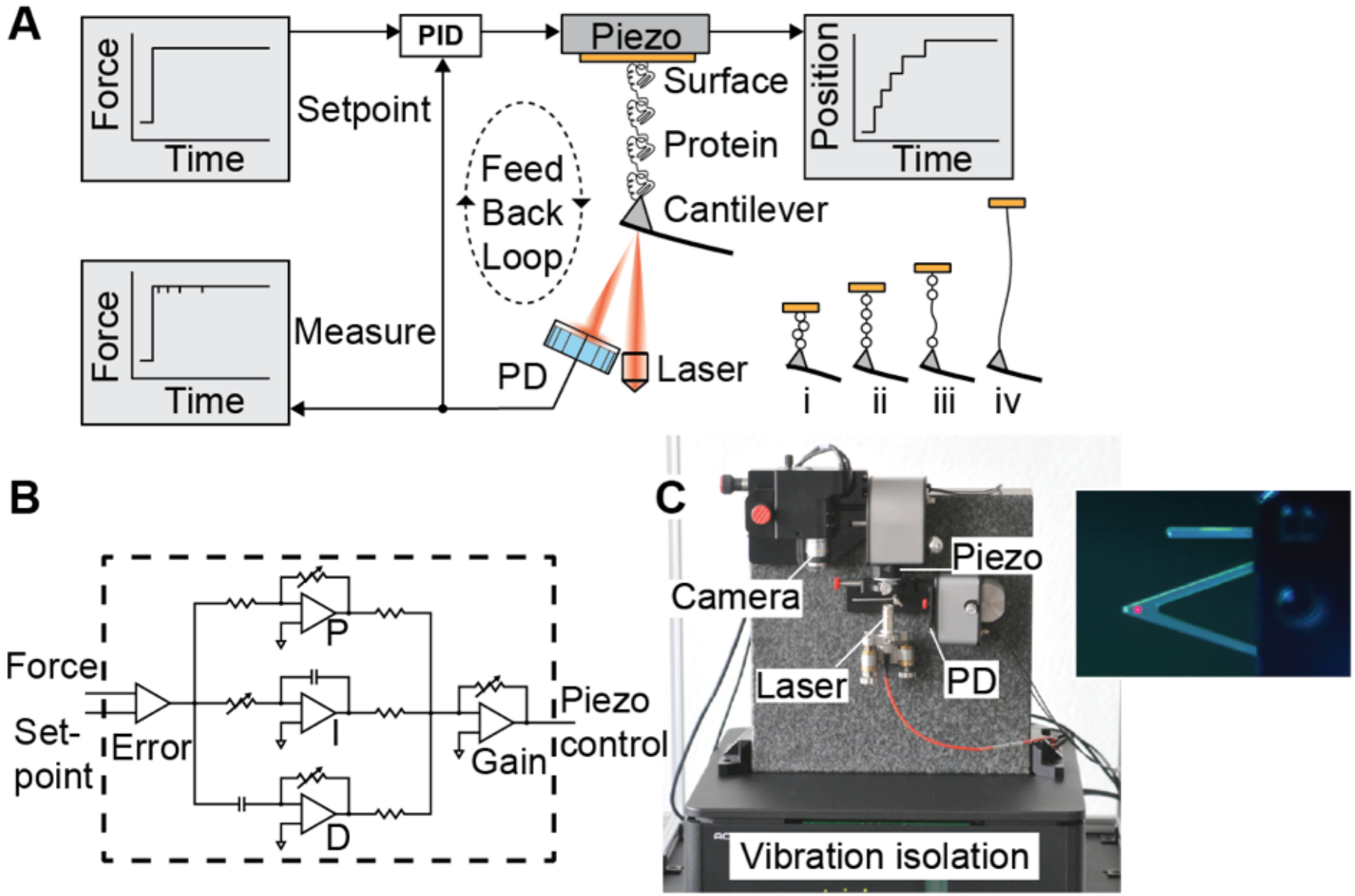

(a)
p
F
x
$F(x) = \frac{kT}{p}\left[\frac{1}{4}\left(1-\frac{x}{L_c}\right)^{-2} - \frac{1}{4} + \frac{x}{L_c}\right]$
L_c
F
(b)
4
3
2
1
(c)
L_c (nm)
52.8
80.3
108.1
136.2
164.4
192.4
220.7
248.7
1
2
3
4
100 pN
50 nm



Constant velocity mode



Force-clamp spectroscopy apparatus

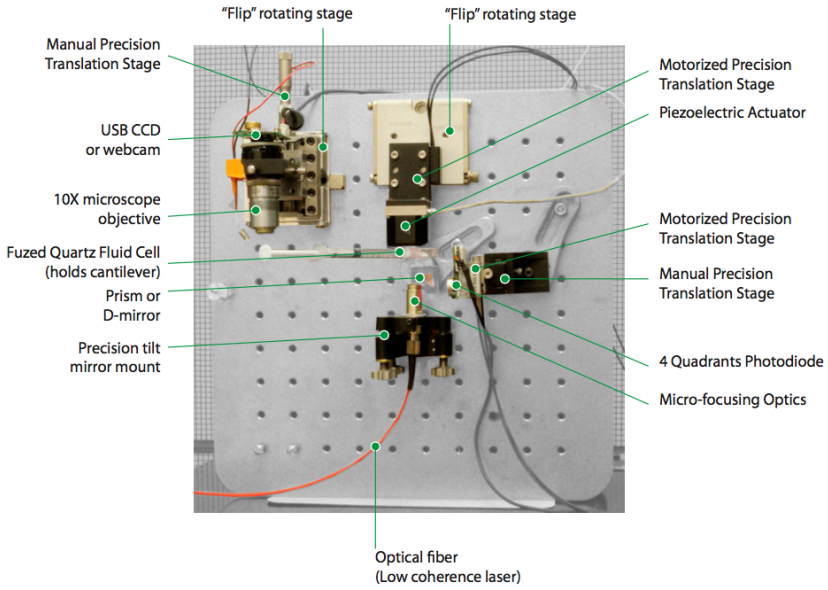


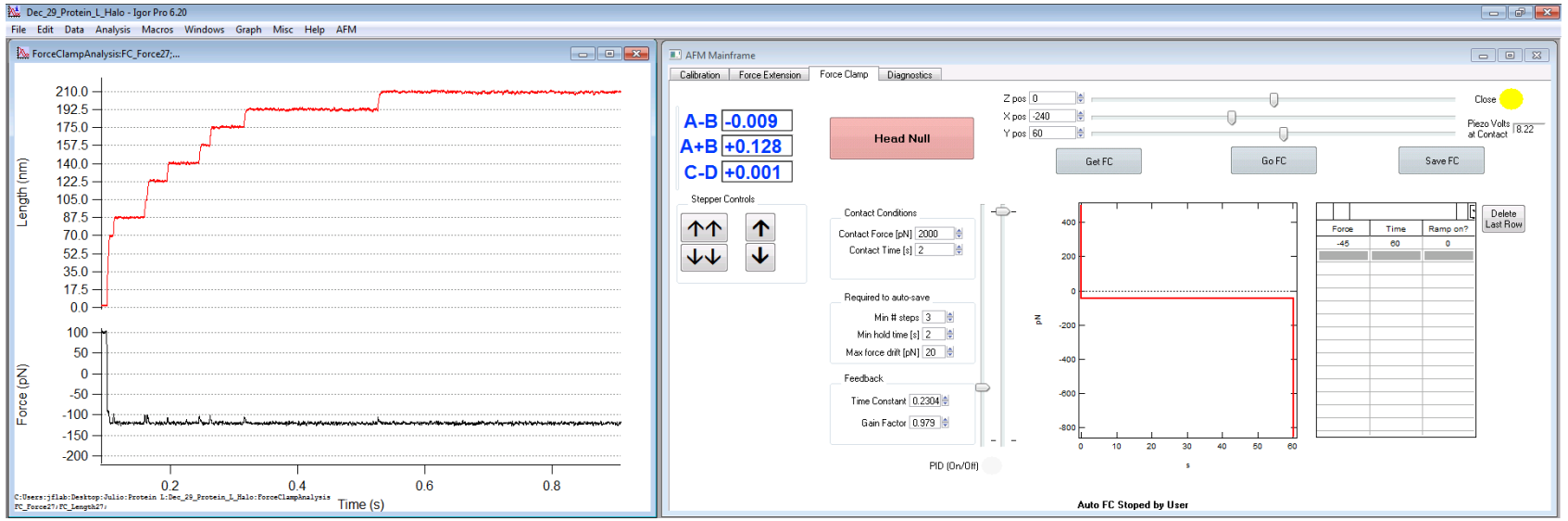
Luigs & Neumann Feinmechanik und Elektrotechnik GmbH

Introducing the AFS

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

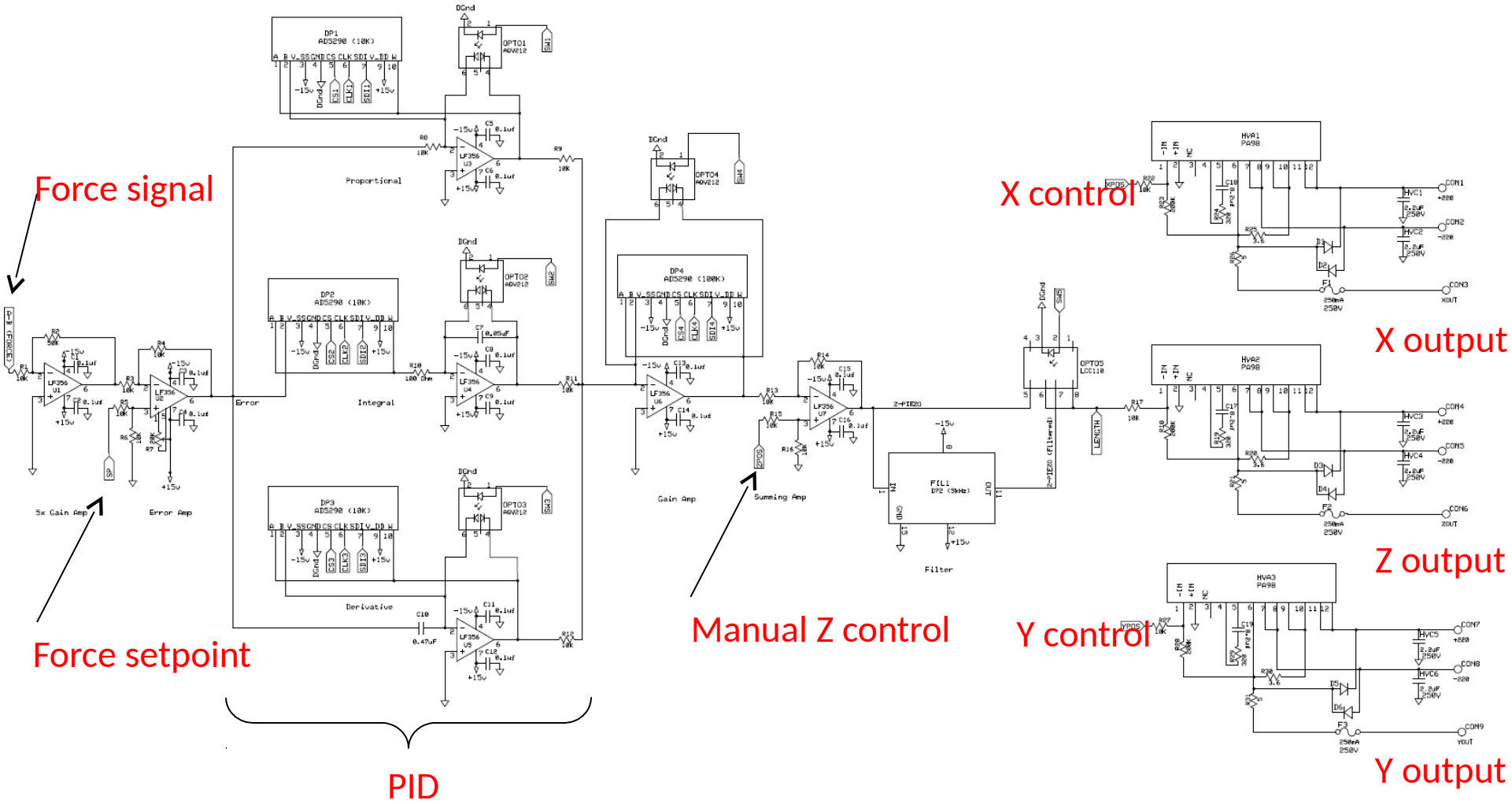
$$u(t) = MV(t) = K_p e(t) + K_i \int_0^t e(\tau)\,d\tau + K_d \frac{d}{dt} e(t)$$

Force measurement

Force setpoint

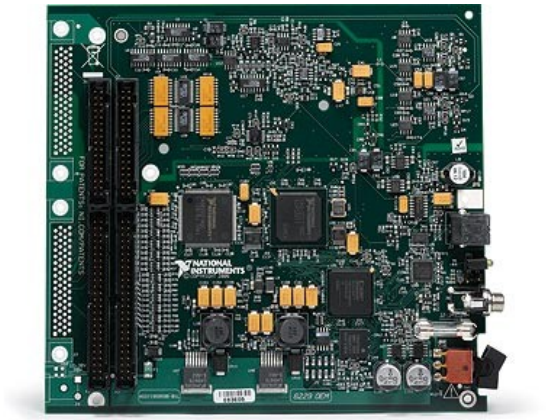
PID

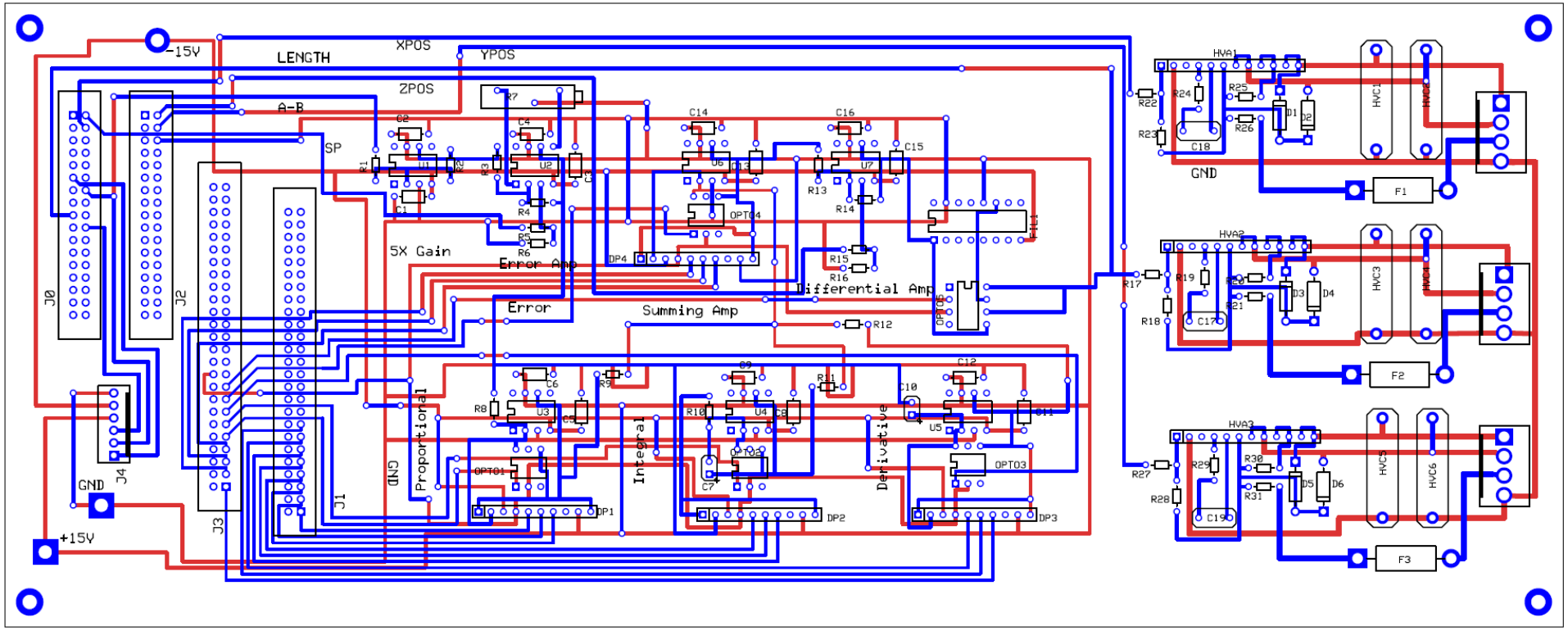
Z position



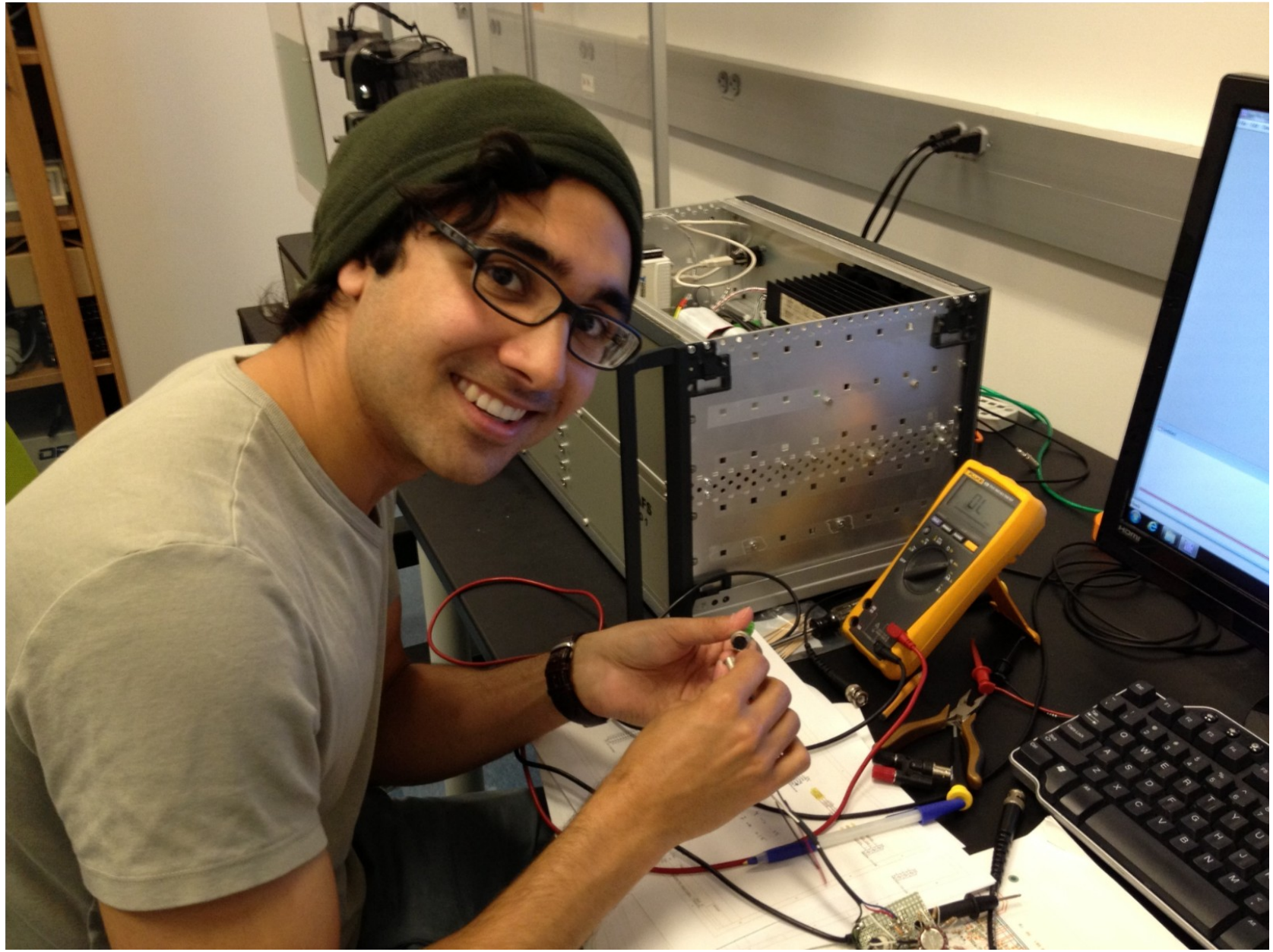
AFS: Circuit boards

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



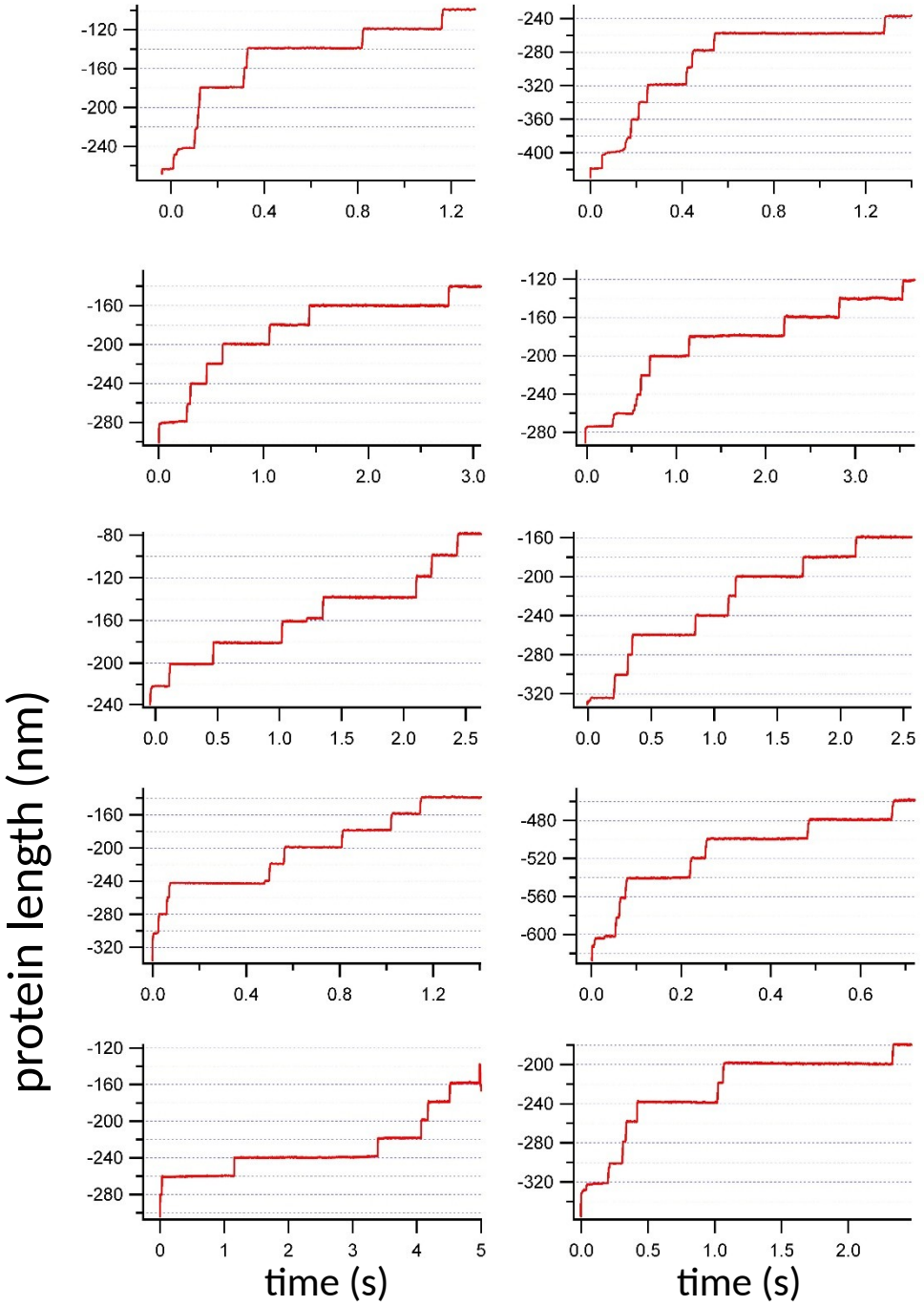


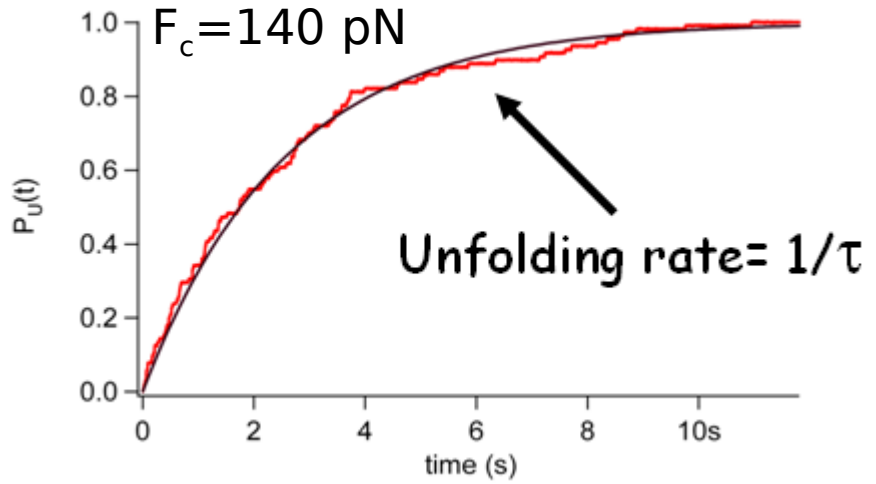
Pallav Kosuri (PhD;2012)
applying the final touches to the L&N prototype

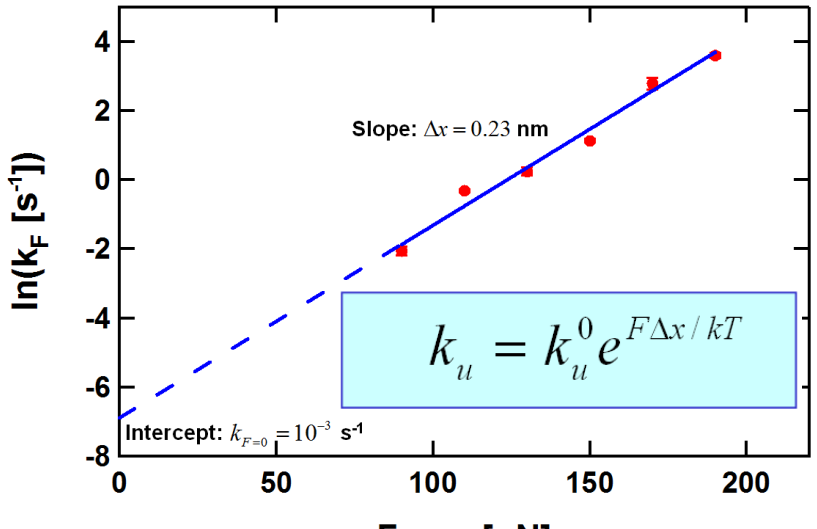


Unfolding polyproteins at constant force

(Hongbin Li)

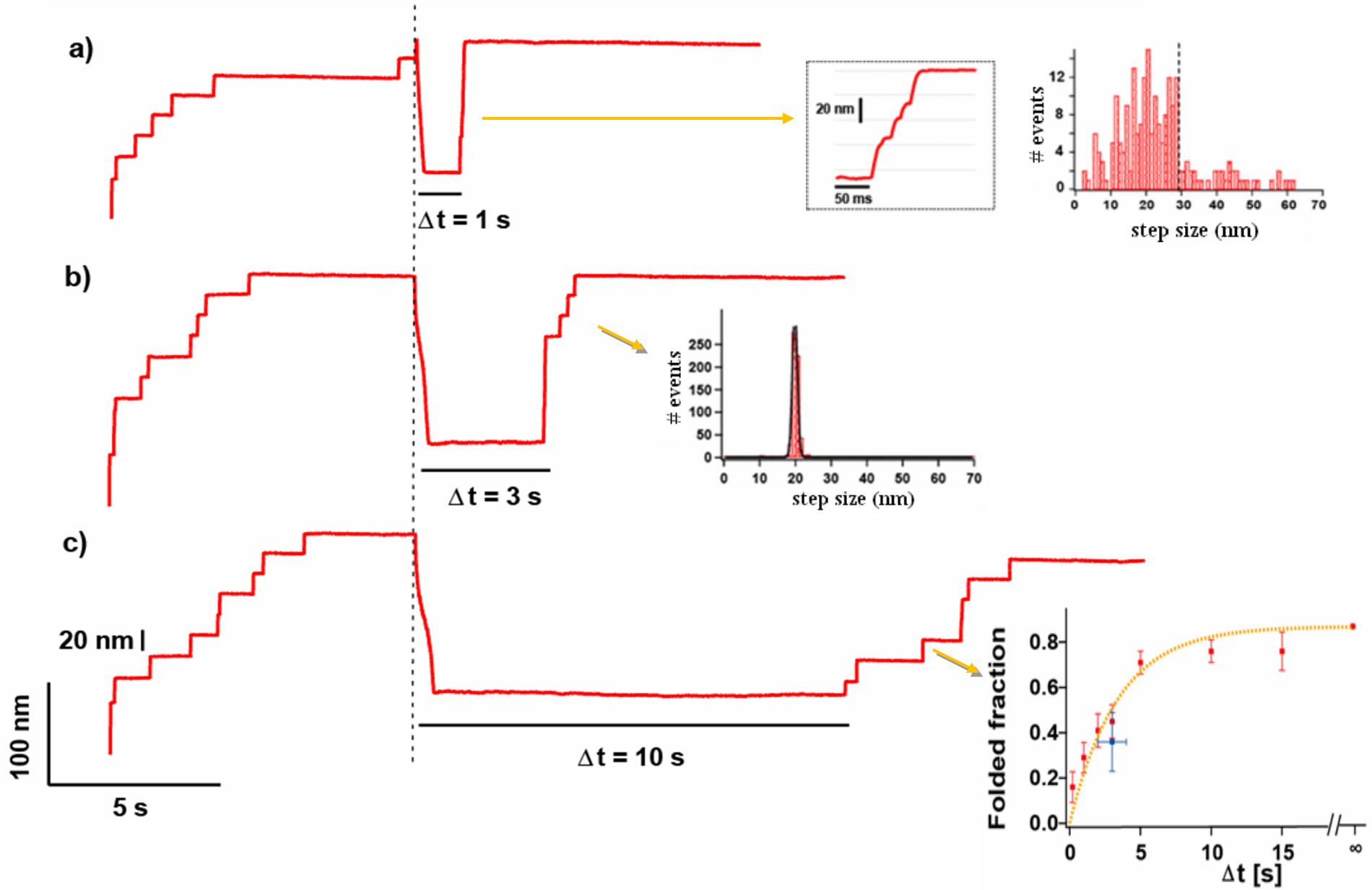




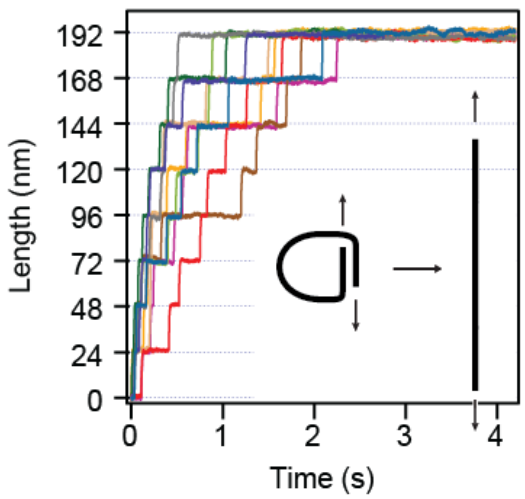


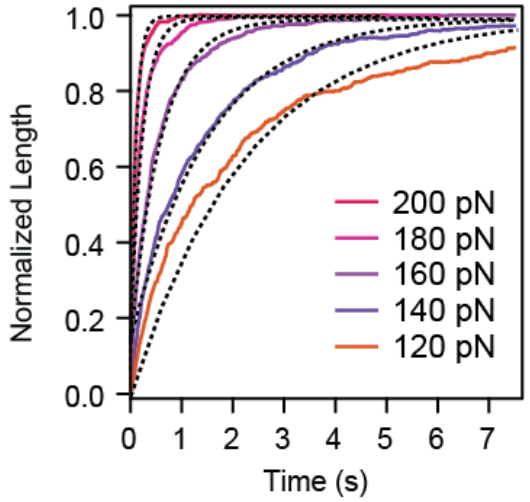
Force-quench; molten globules and folding

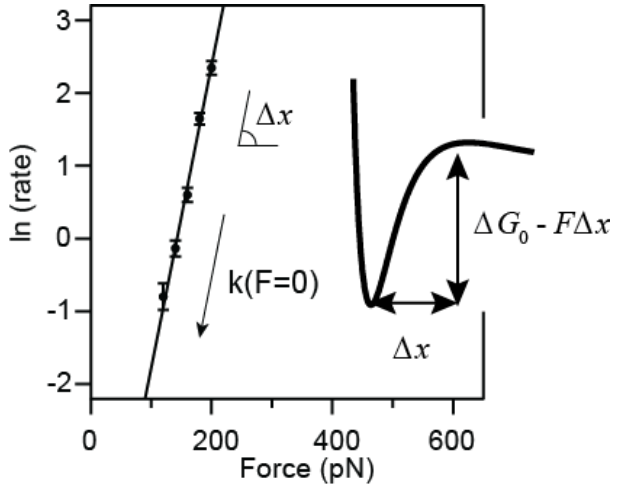
(Sergi Garcia-Manyes)

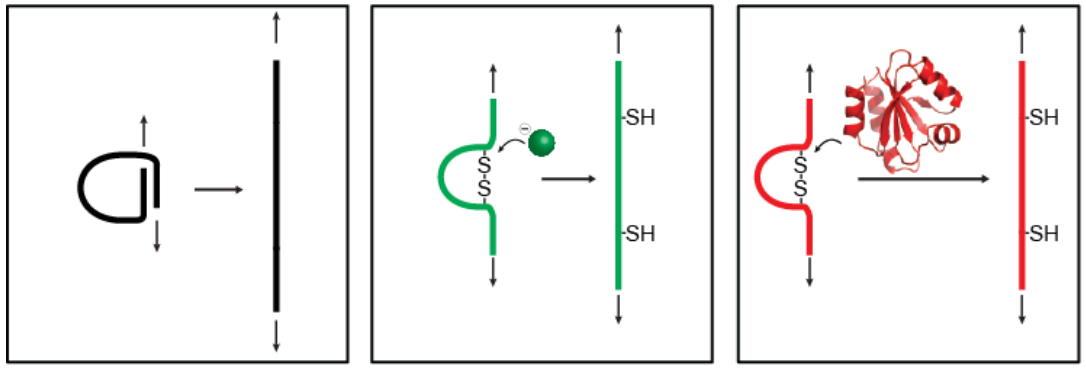


Force dependent reactions

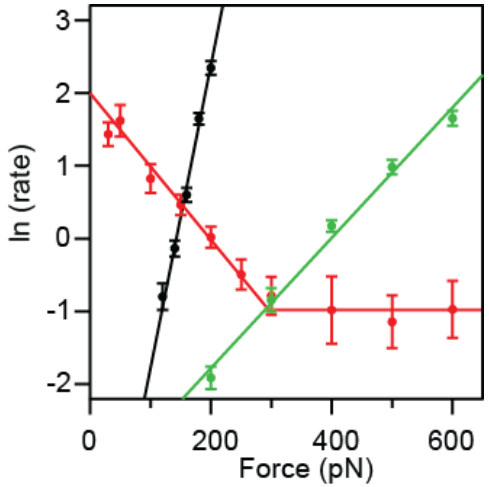






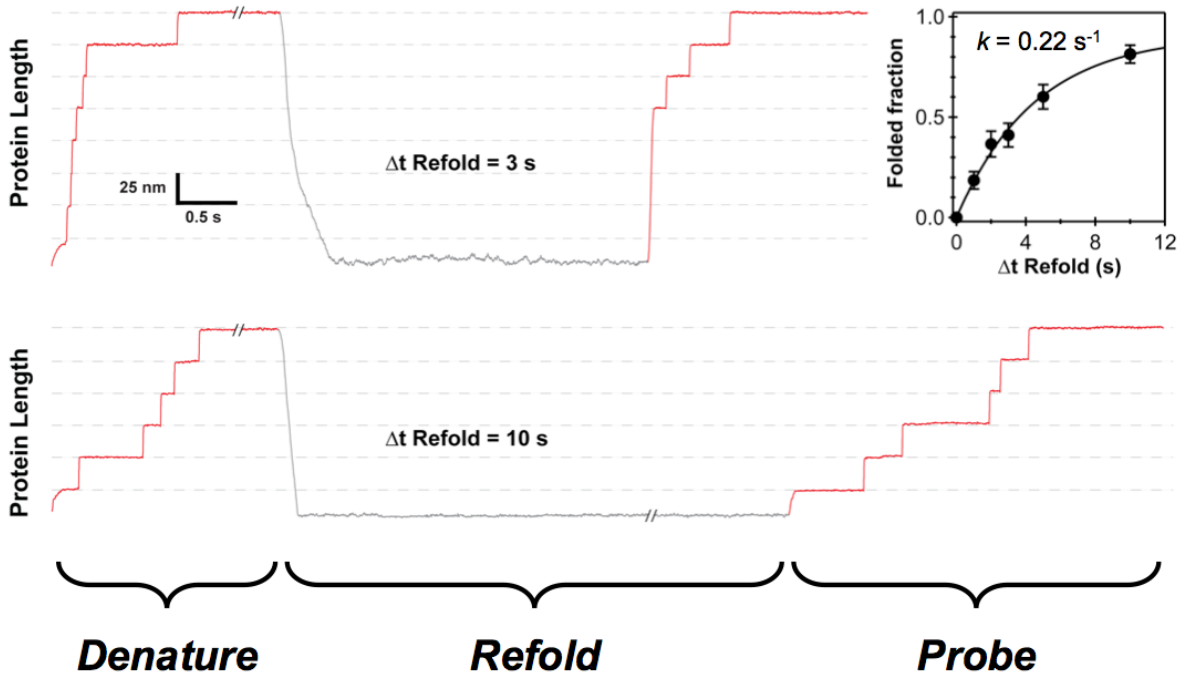


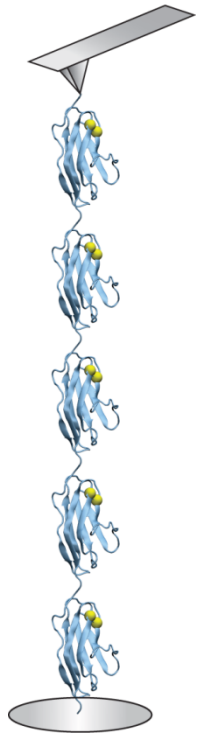
Unfolding | Chemical Reactions | Enzymatic Reactions

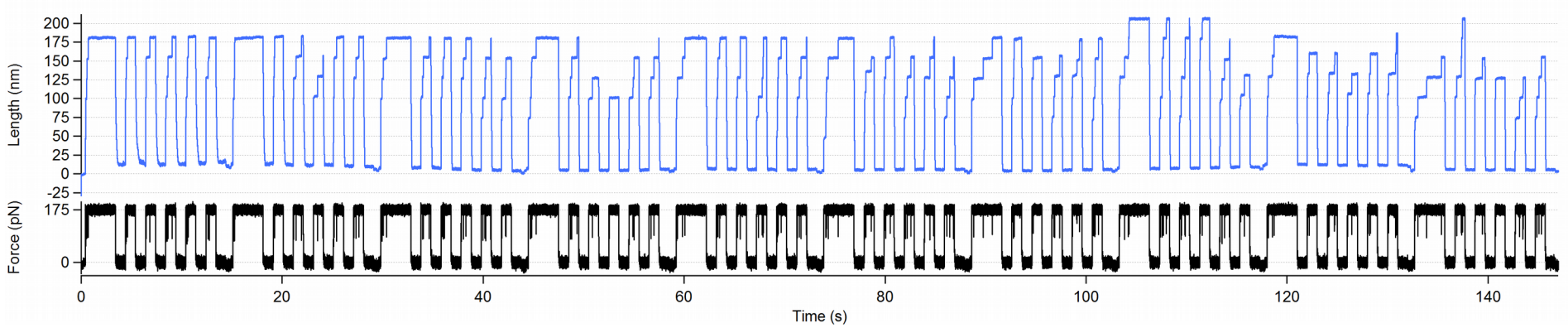


Popa I., et al, *Nat. Prot.* (2013)

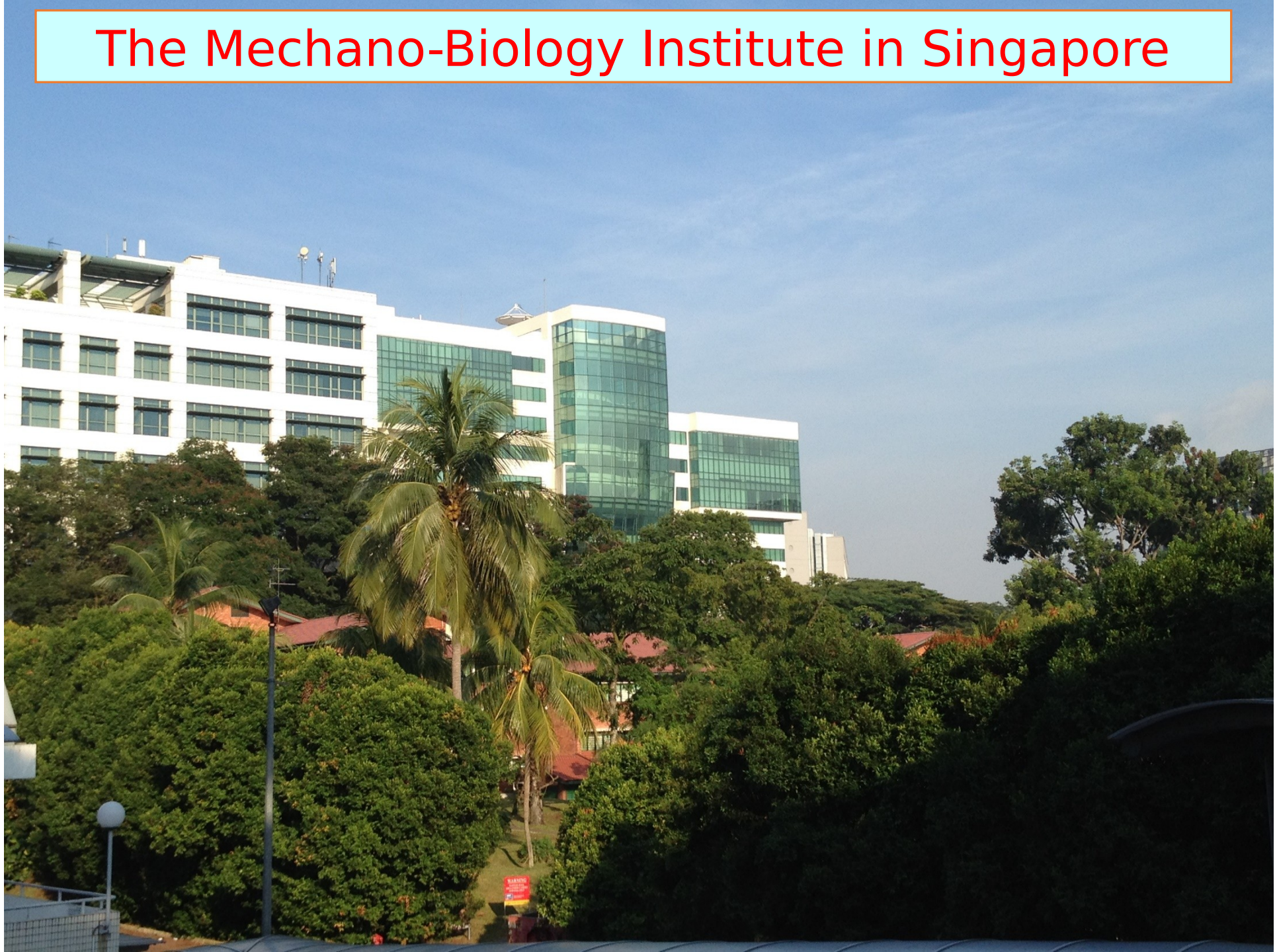
Unfolding and refolding dynamics (Titin I27)







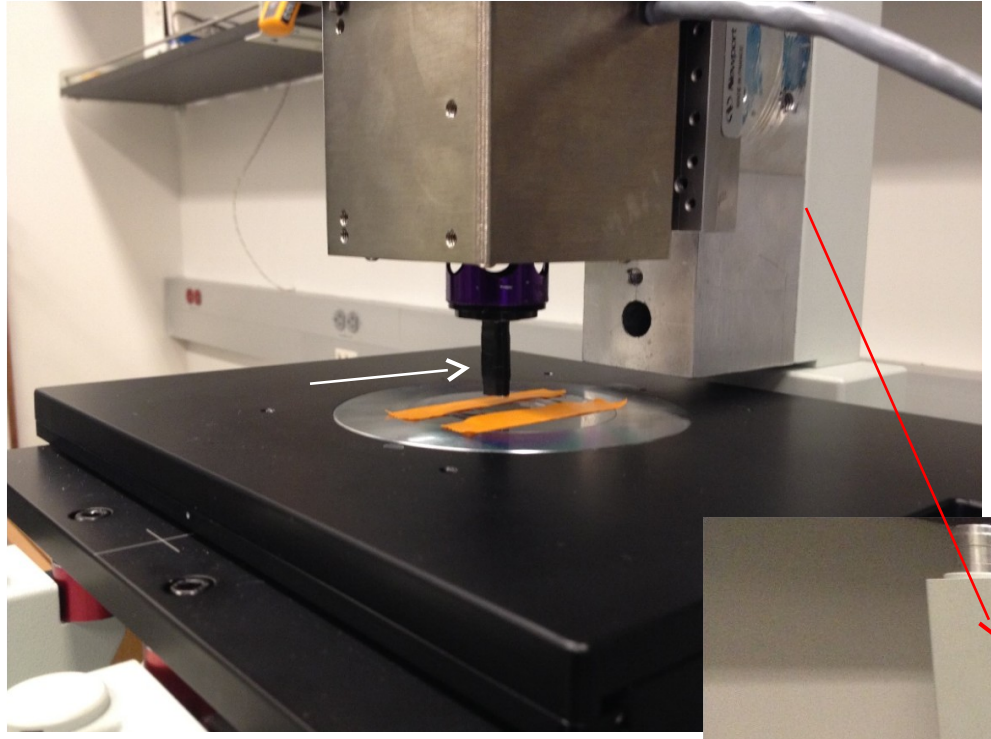
The Mechano-Biology Institute in Singapore



Refolding of titin polyproteins using Magnetic Tweezers at the MBI in Singapore

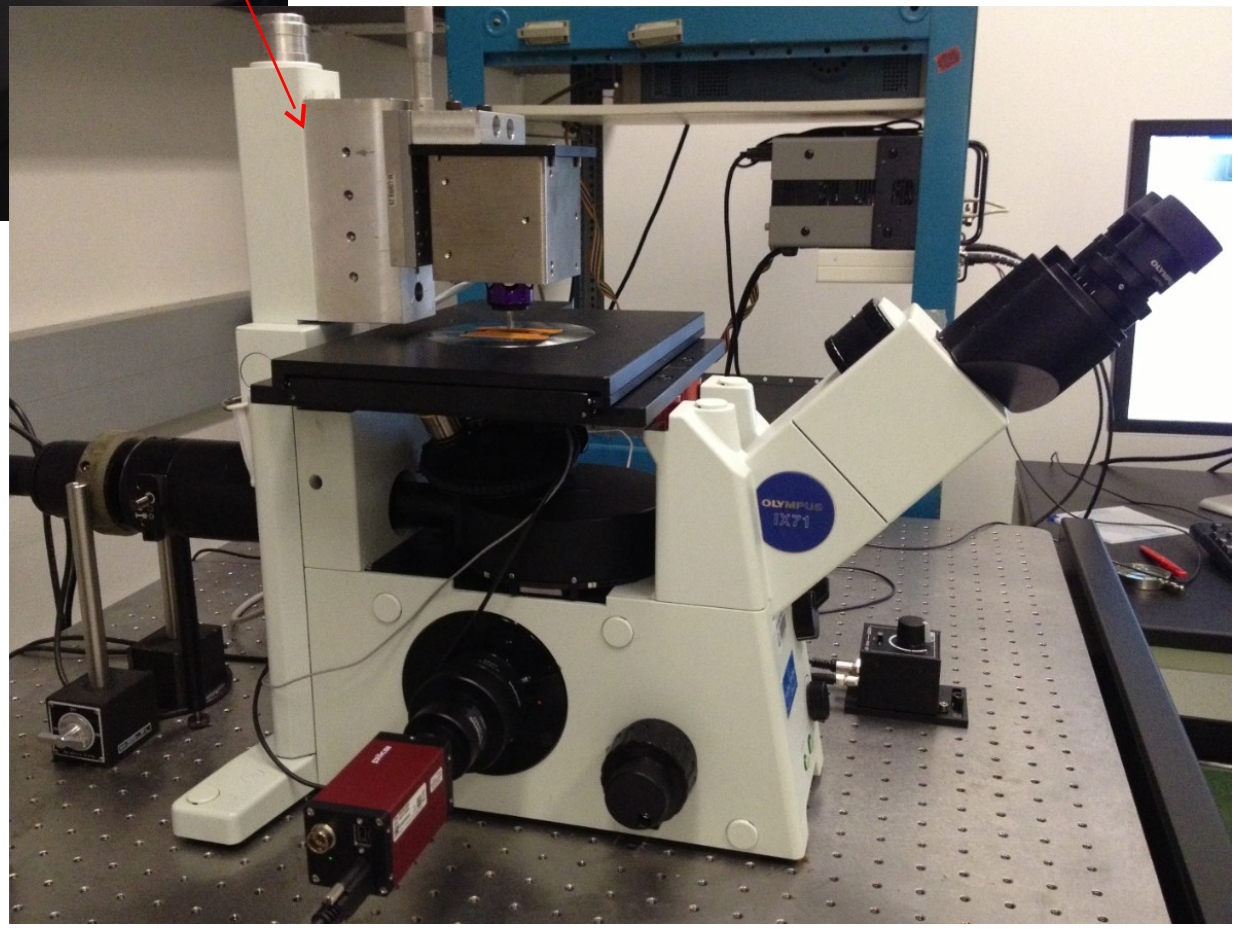
Mike Sheetz

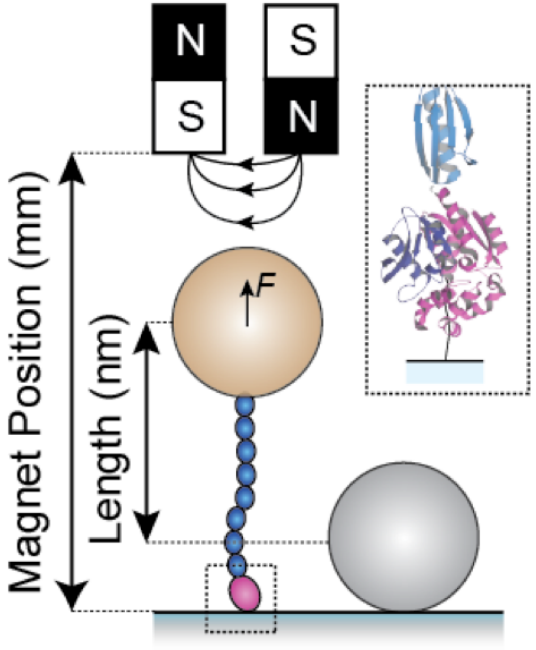
MBI
MECHANOBIOLOGY INSTITUTE
Singapore



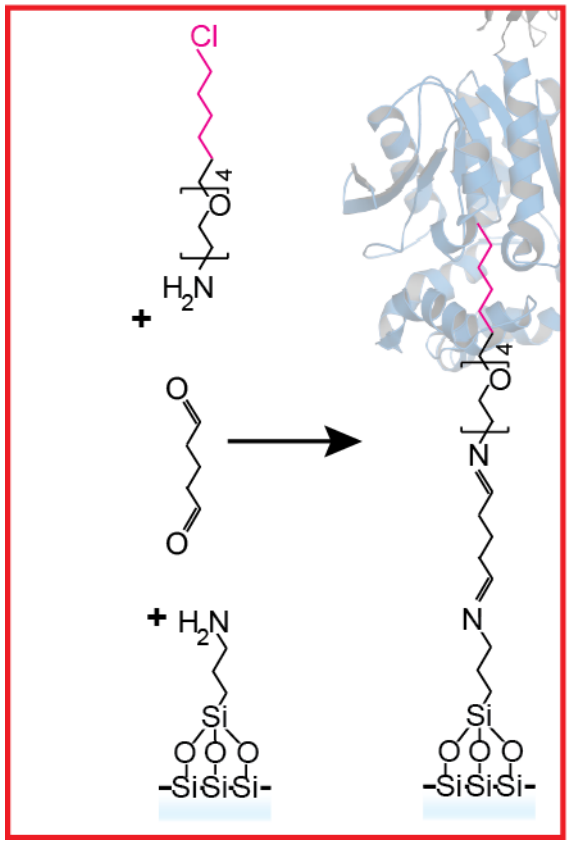
HaloTag and magnetic tweezers

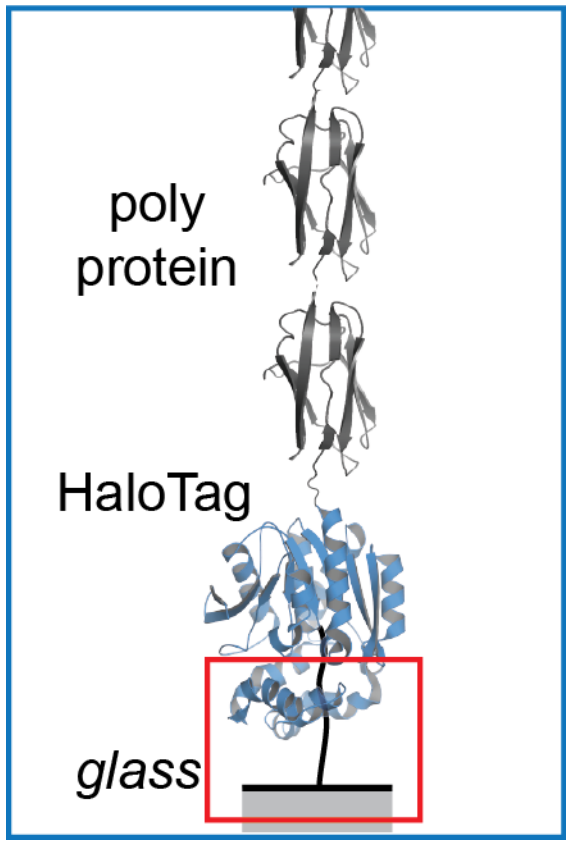
Ionel Popa

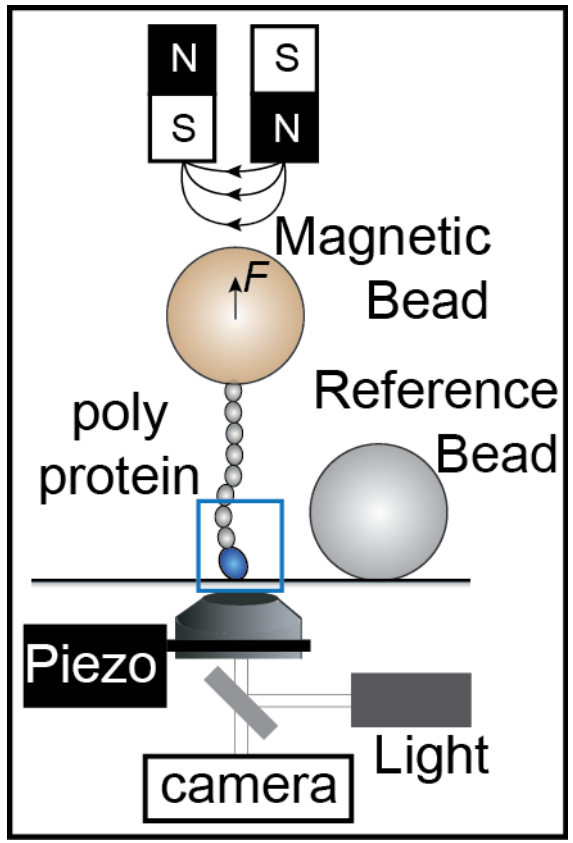




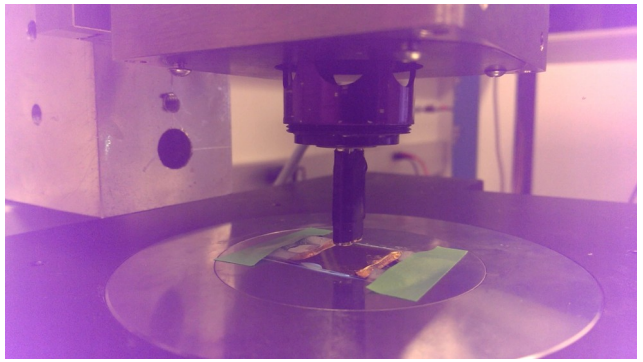
HaloTag anchored polyproteins

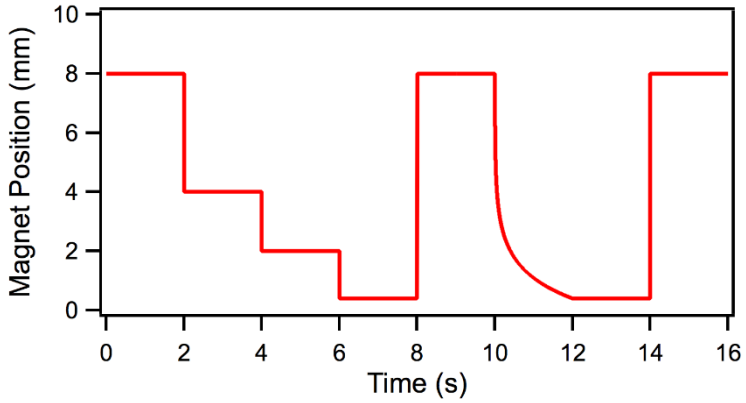


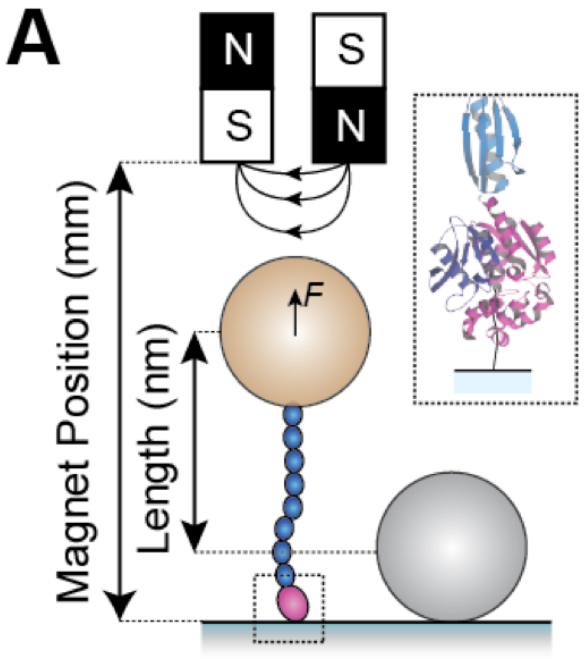


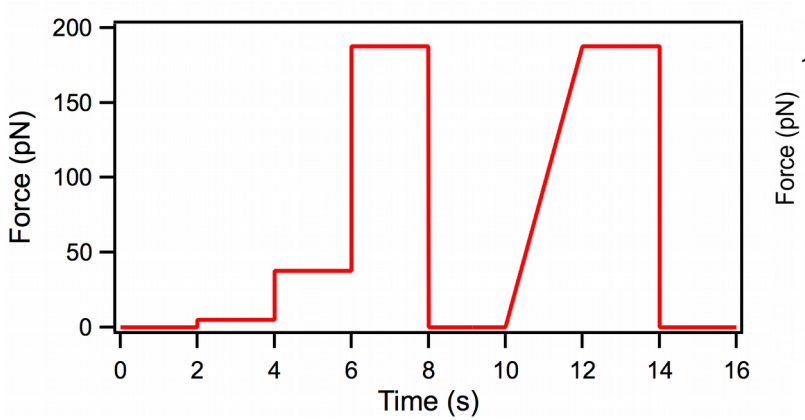


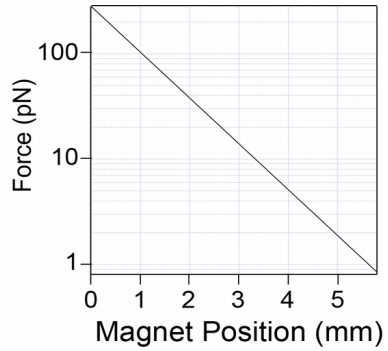
Moving coil and control of magnet position/force

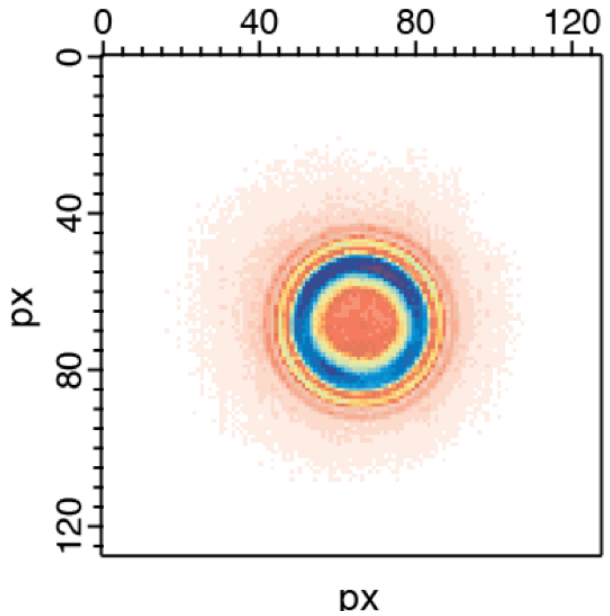


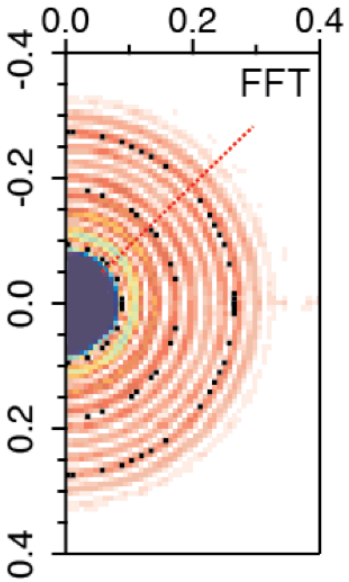


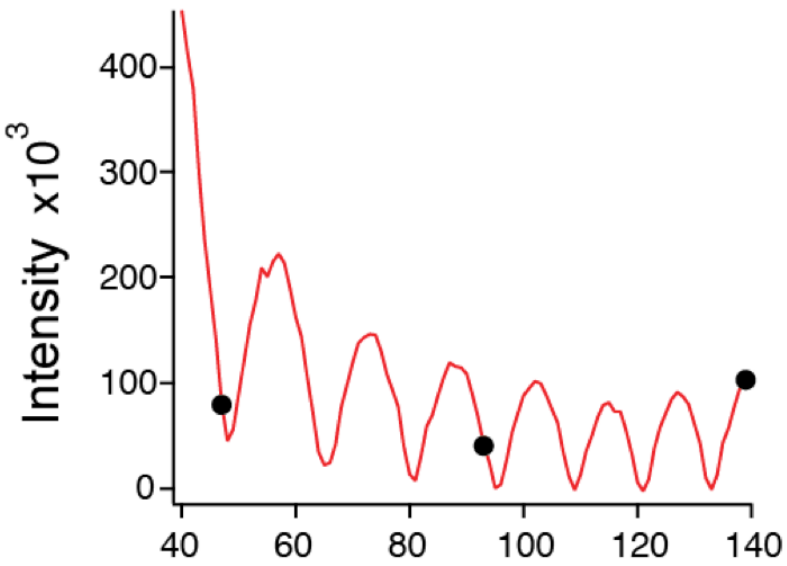


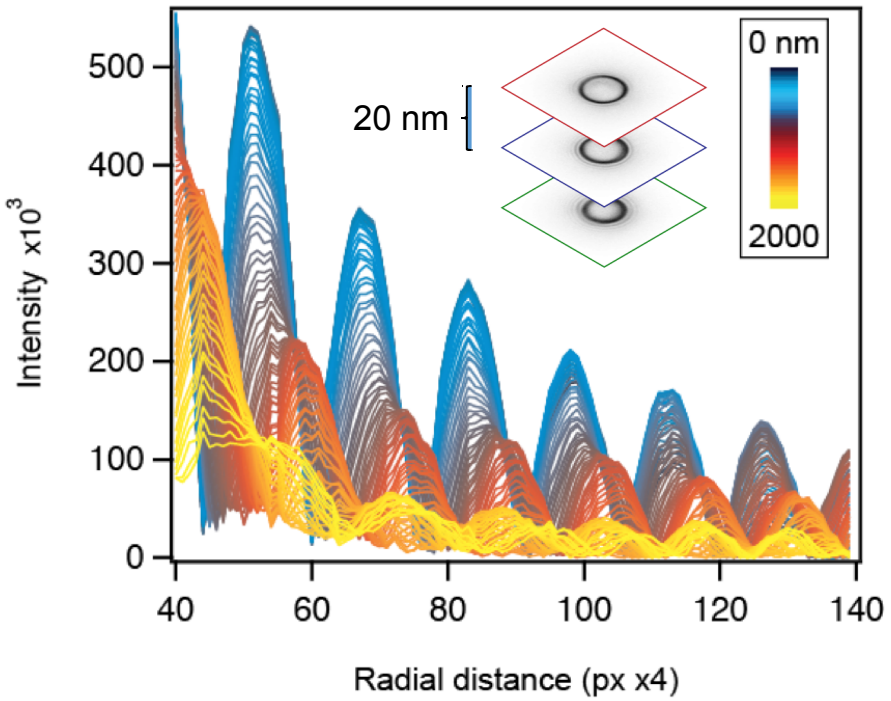


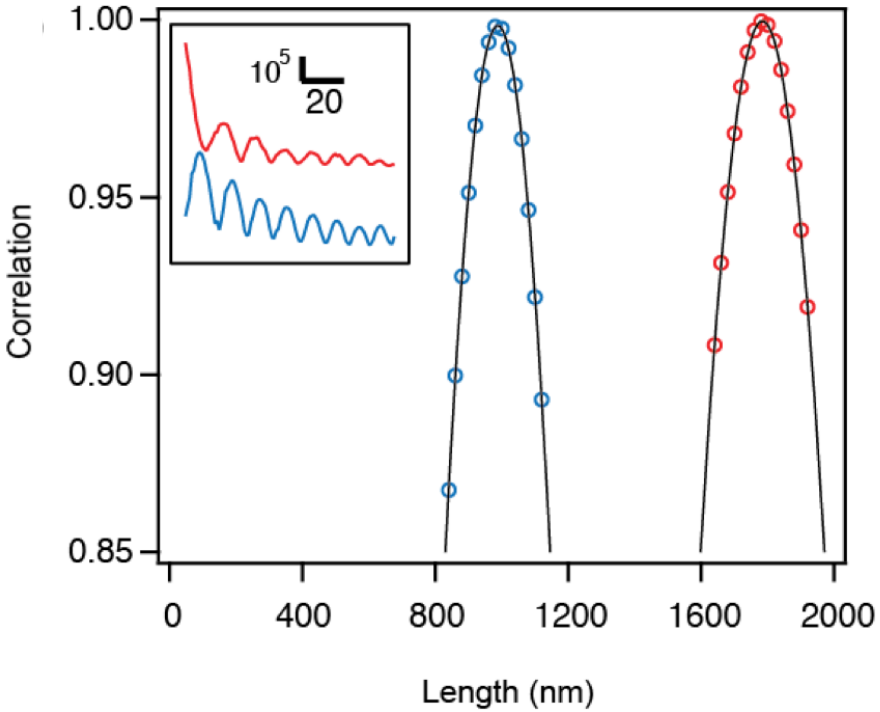



0
40
80
120
px
px

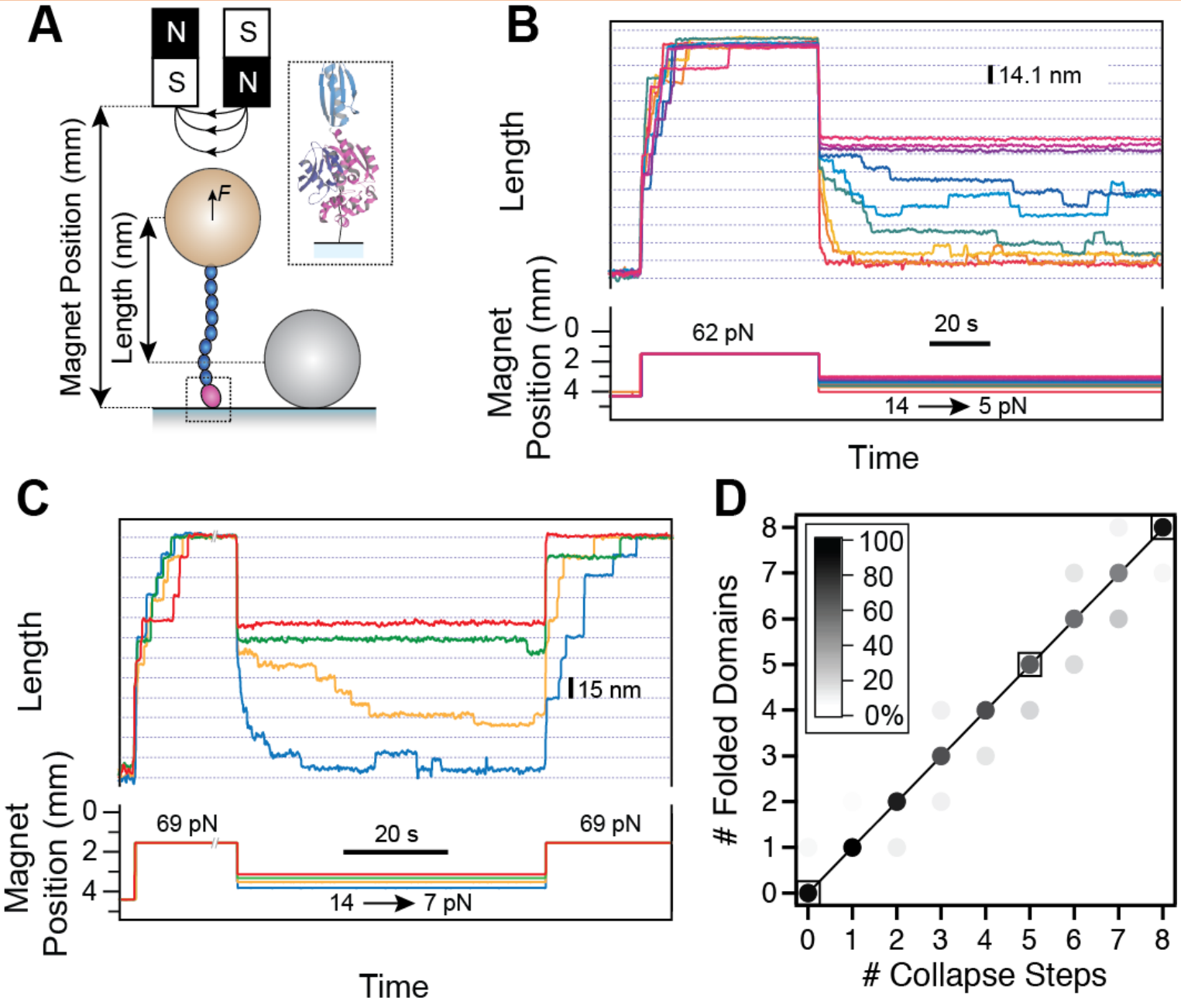

0.0
0.2
0.4
-0.4
-0.2
0.0
0.2
0.4
FFT


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

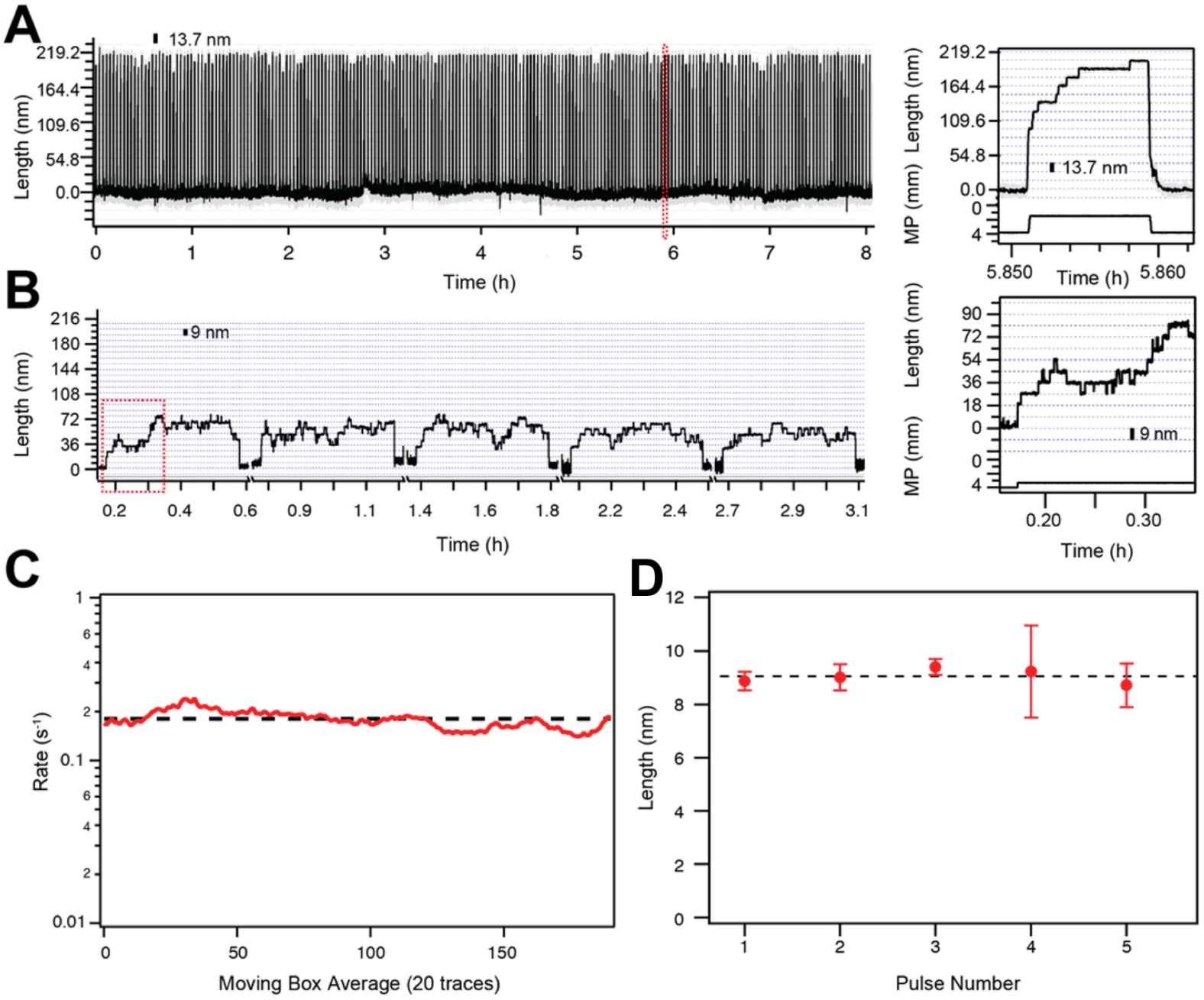

Intensity x10^3
500
400
300
200
100
0
20 nm
0 nm
2000
40
60
80
100
120
140
Radial distance (px x4)


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

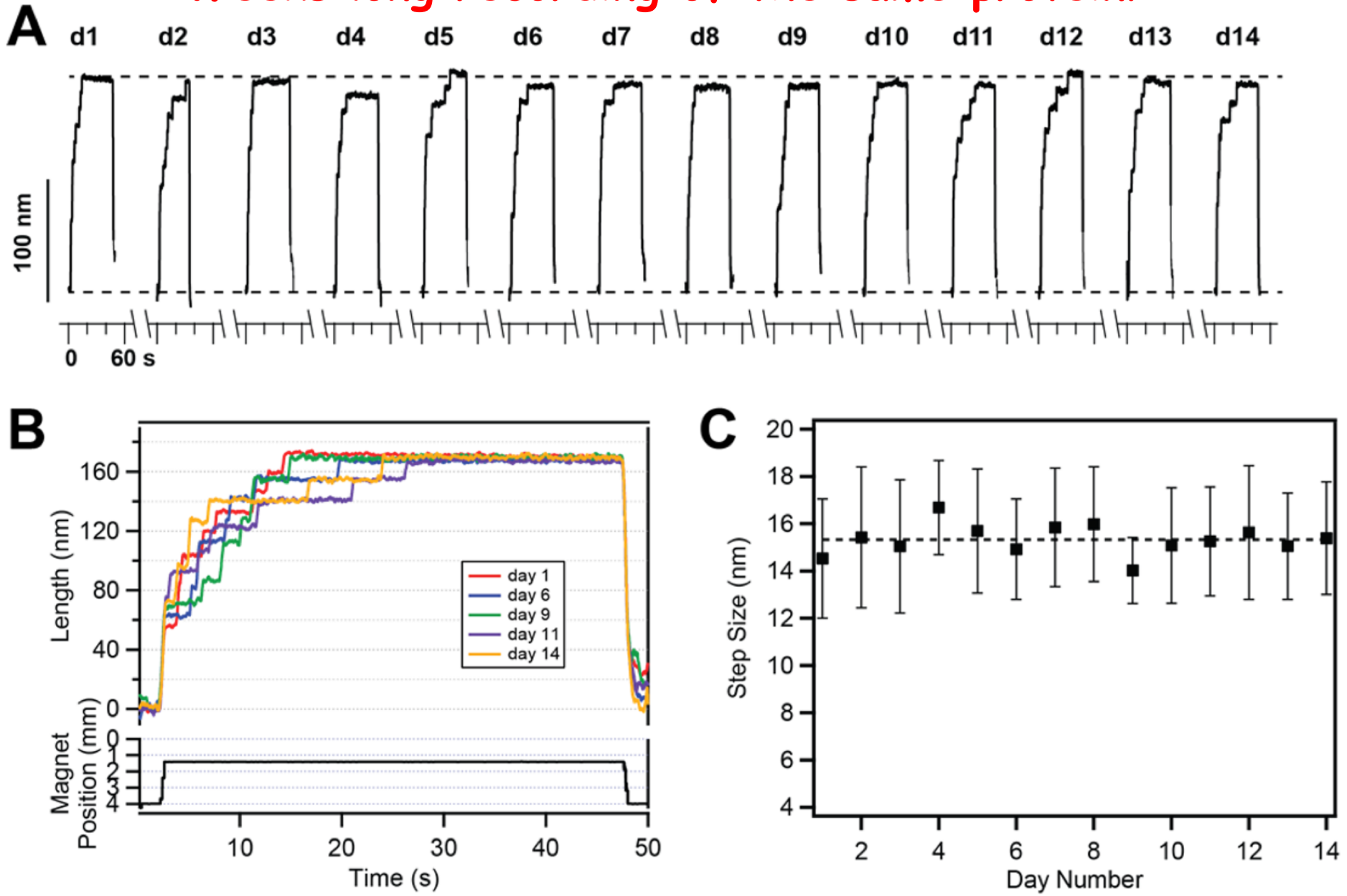
Collapse and folding dynamics of protein L_8



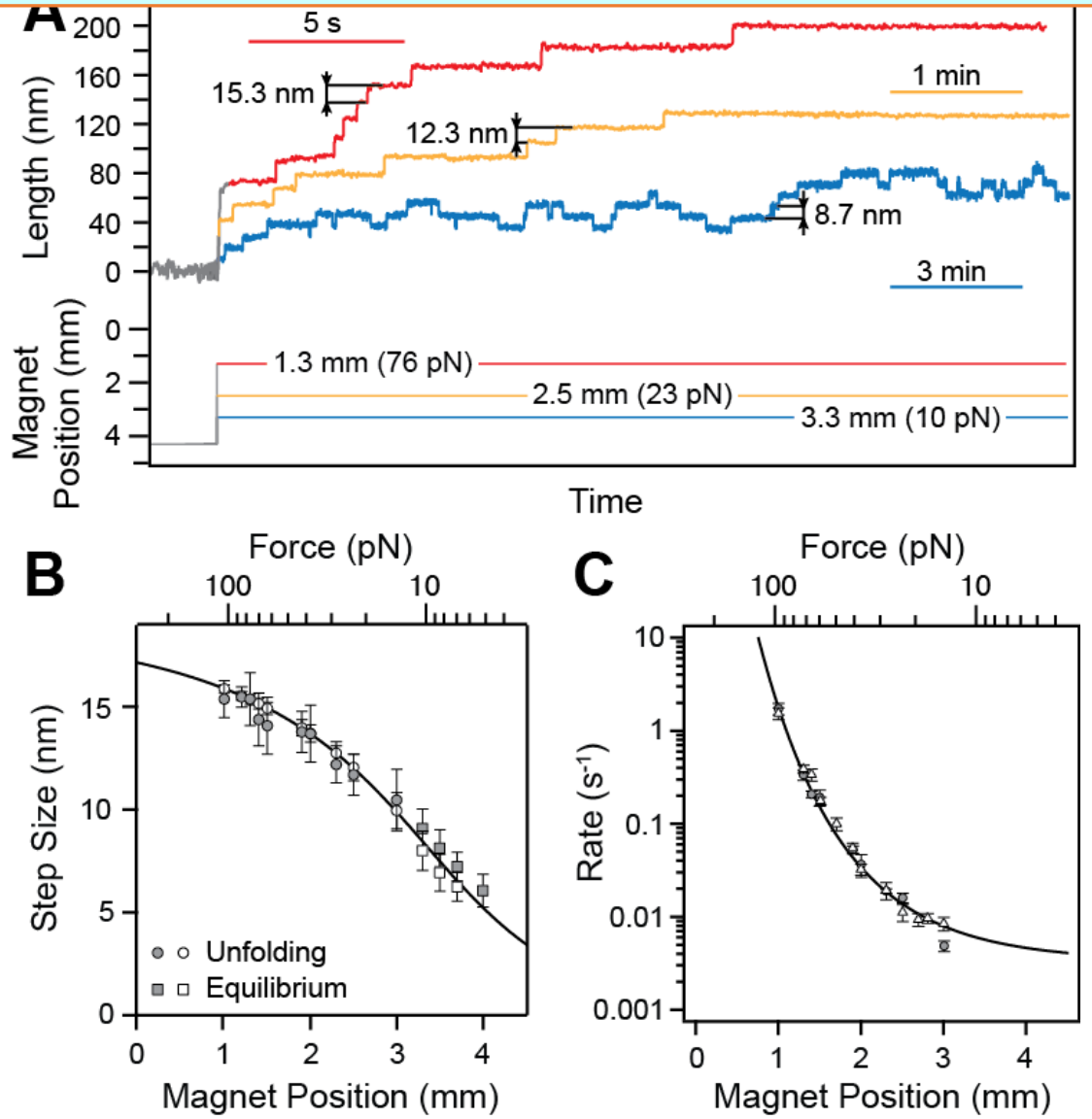
Stable recordings of a single protein



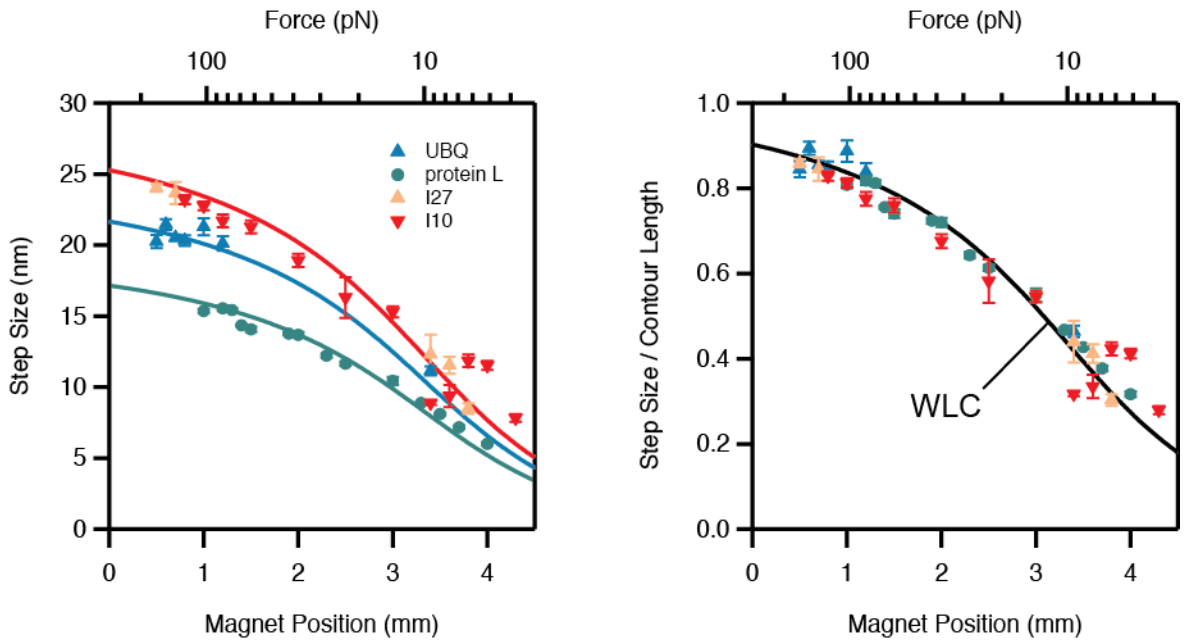
Weeks long recording of the same protein!



Both, the step sizes and rates are force dependent.

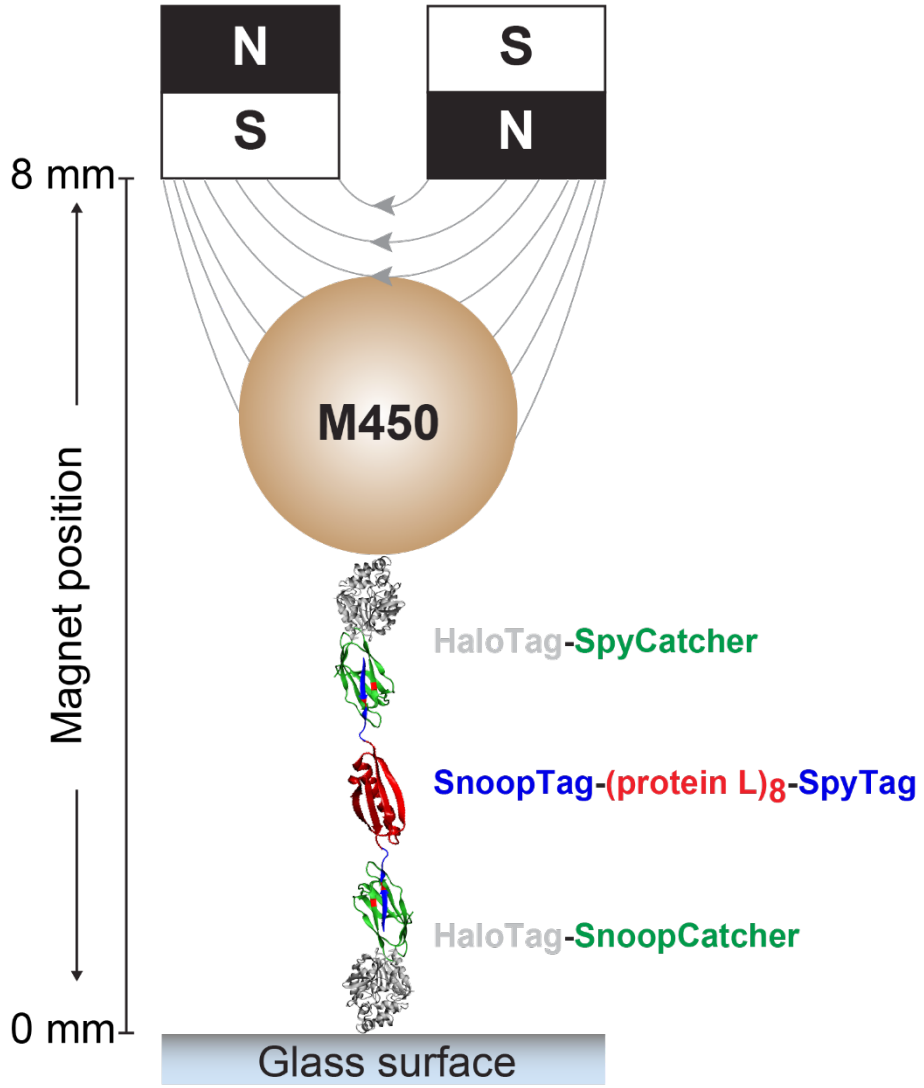


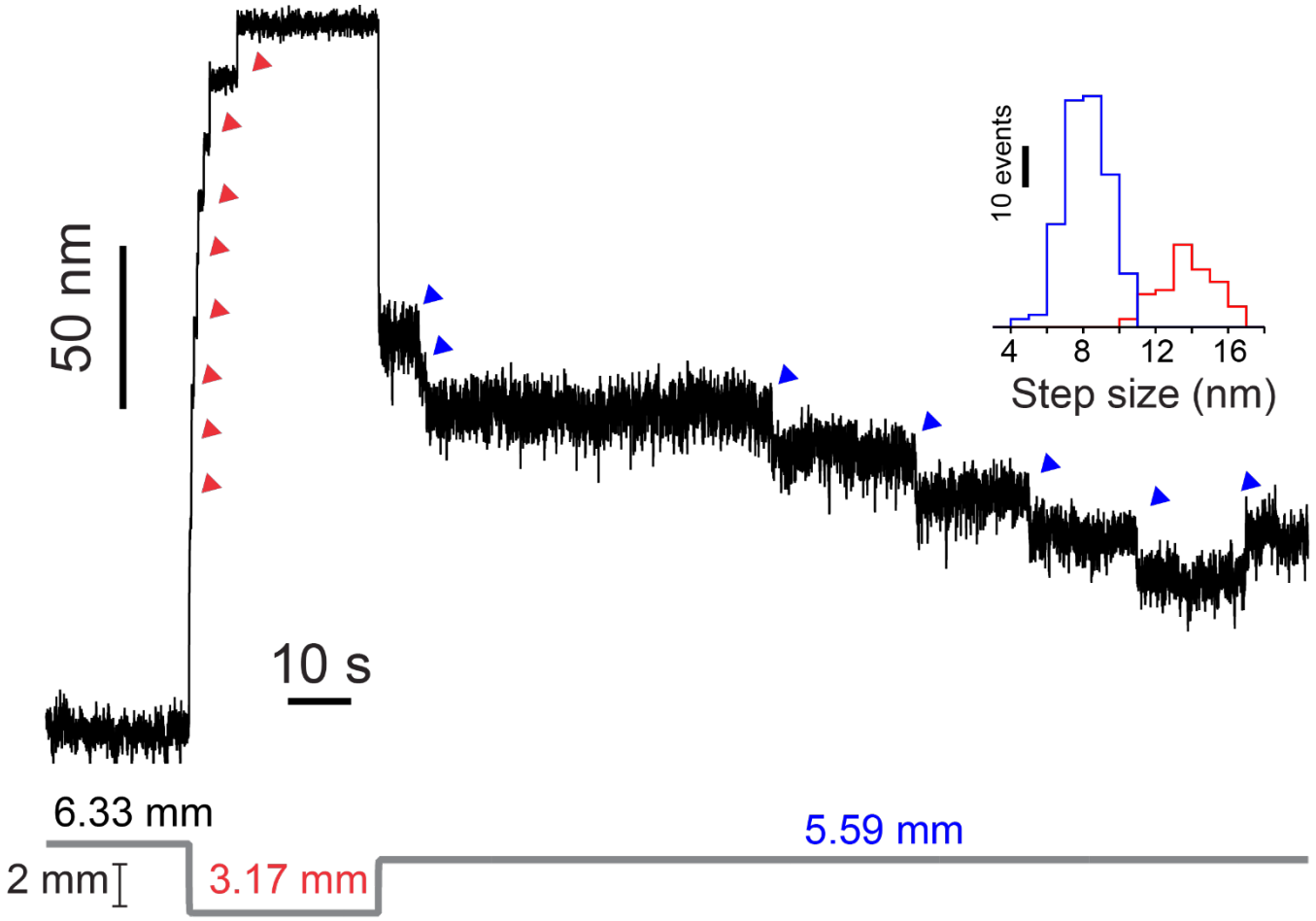
Force-dependent step sizes: a universal property of proteins.



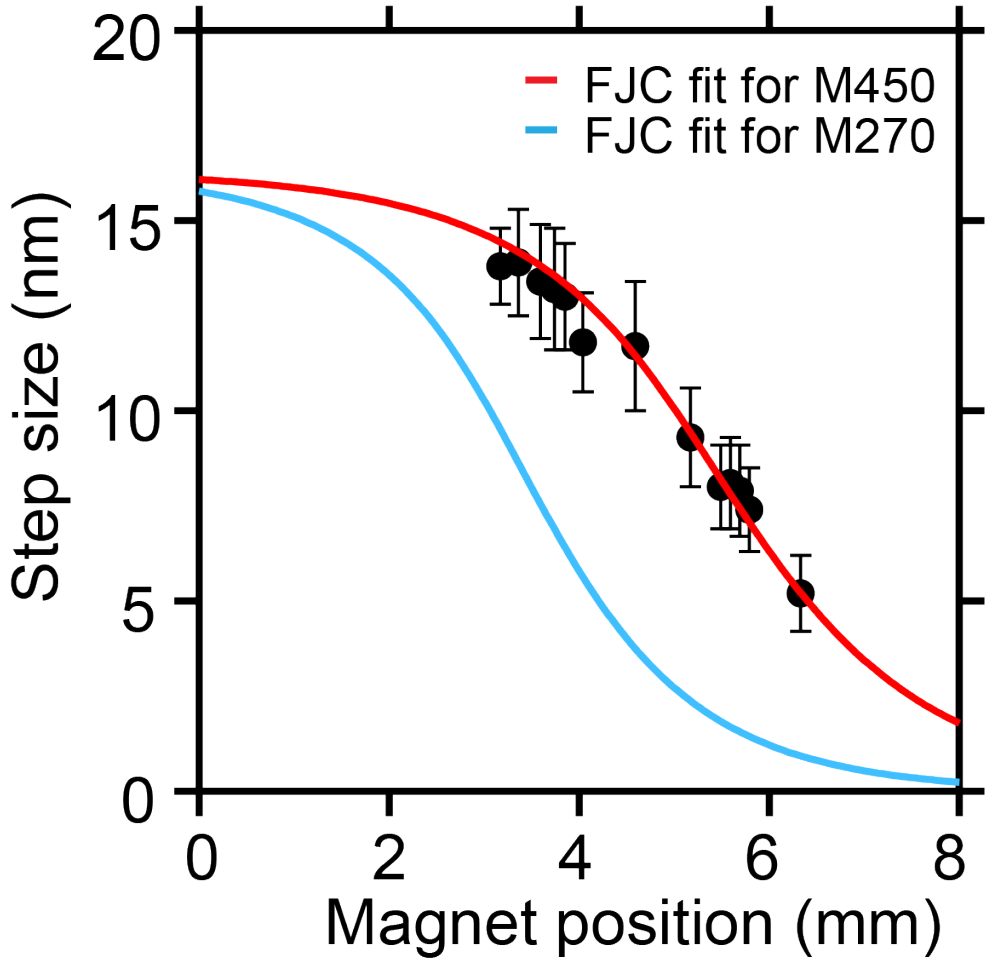
Folding under force is dominated by polymer elasticity!

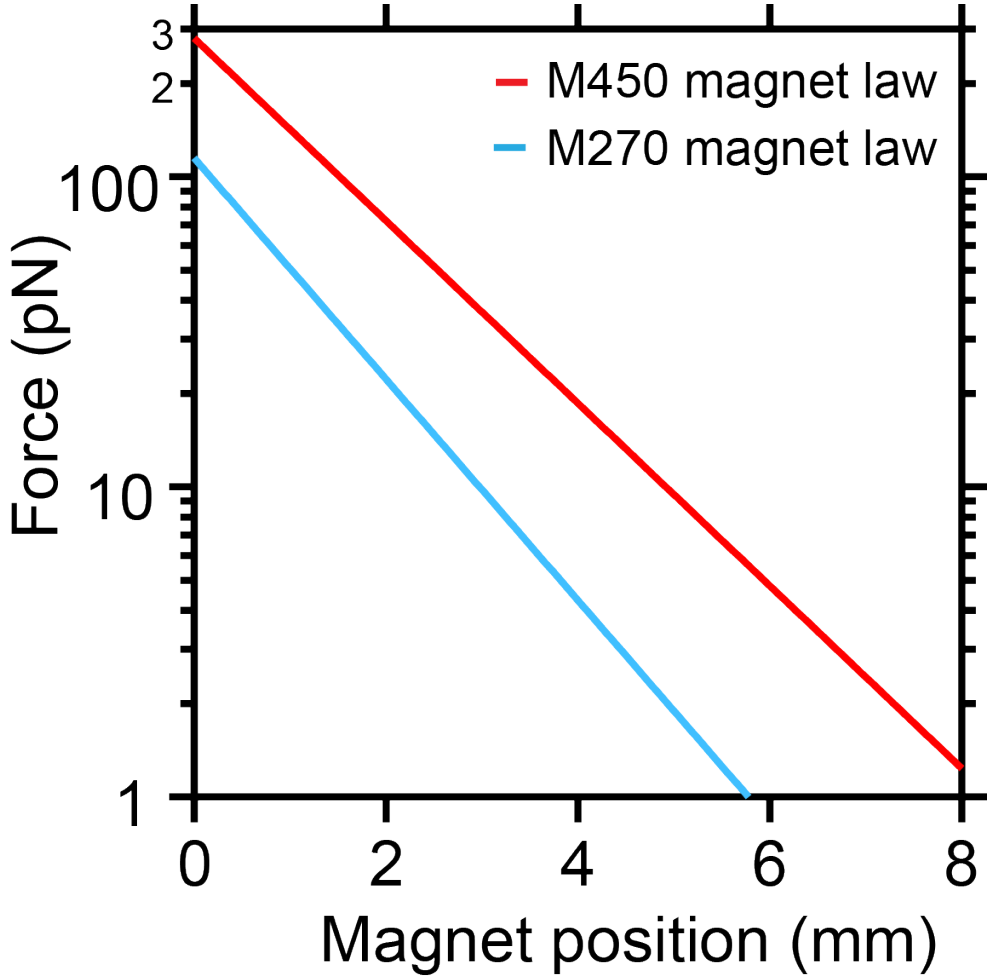
Calibration of M450





Calibration

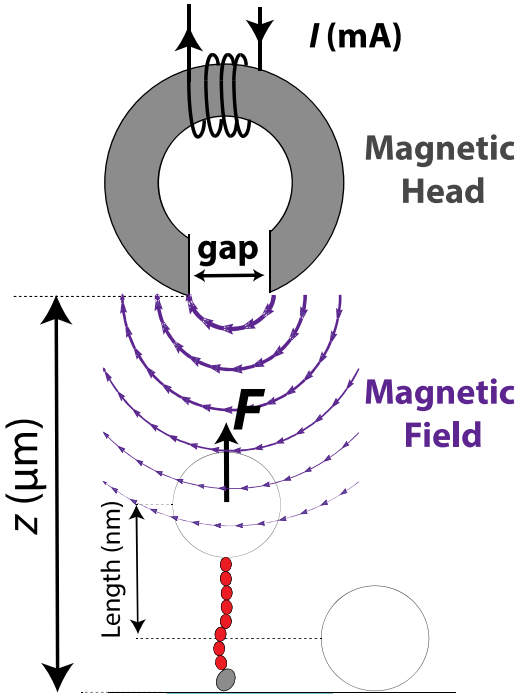




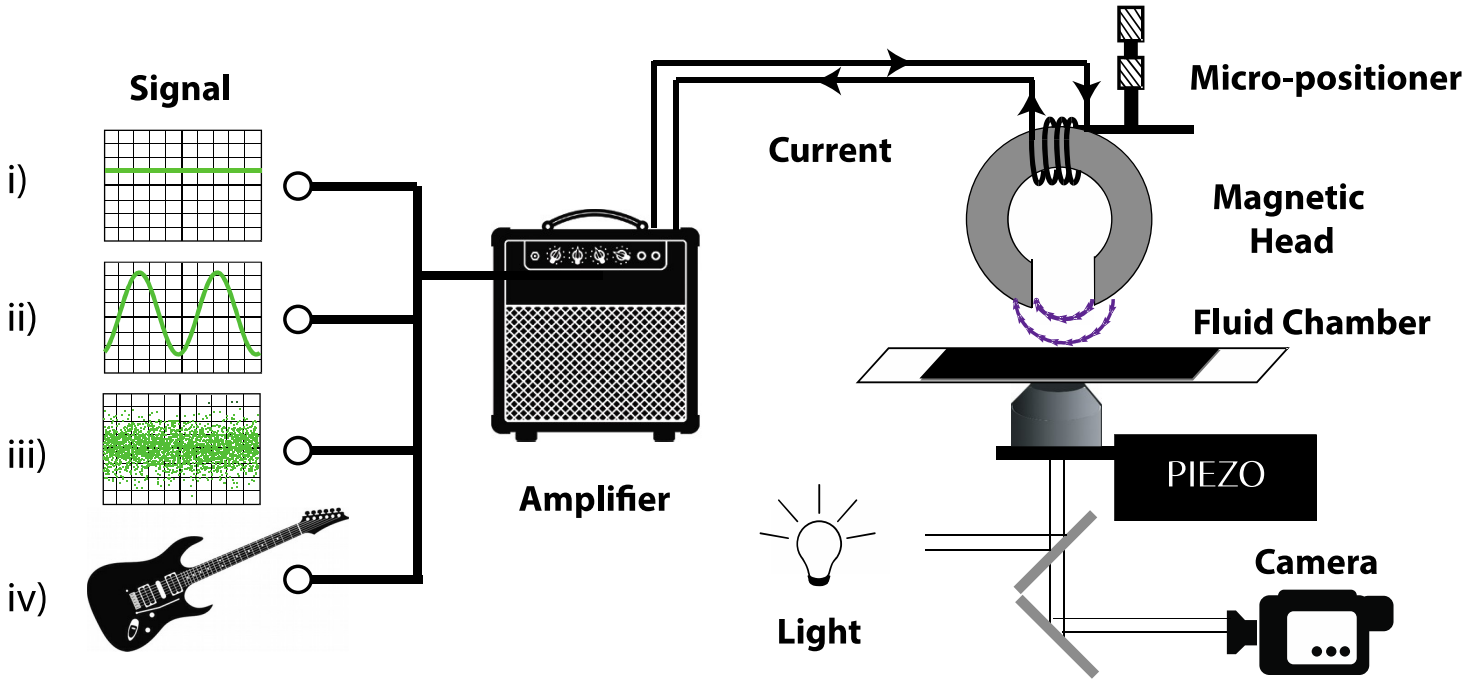
MT_2_EM
H

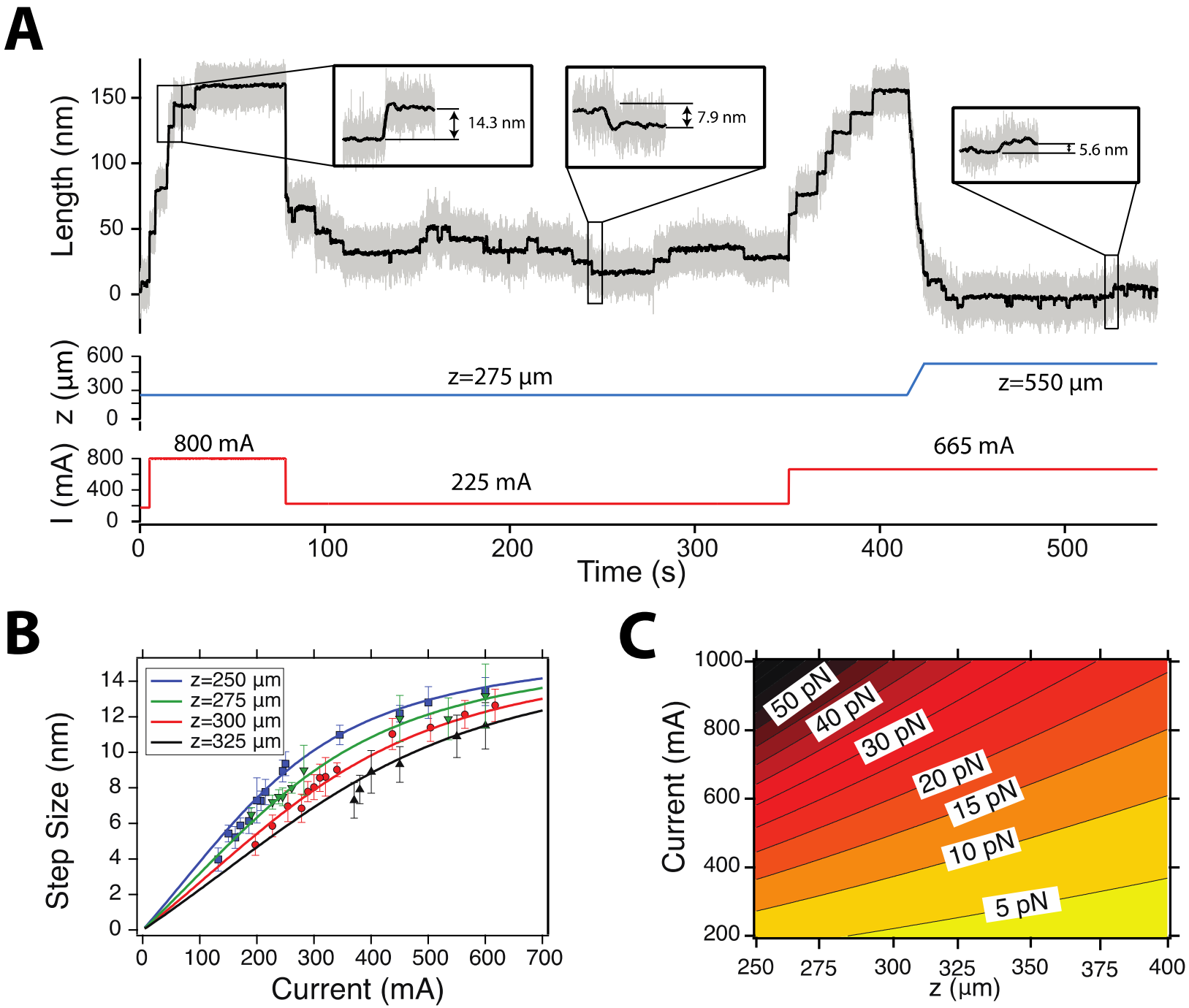
A

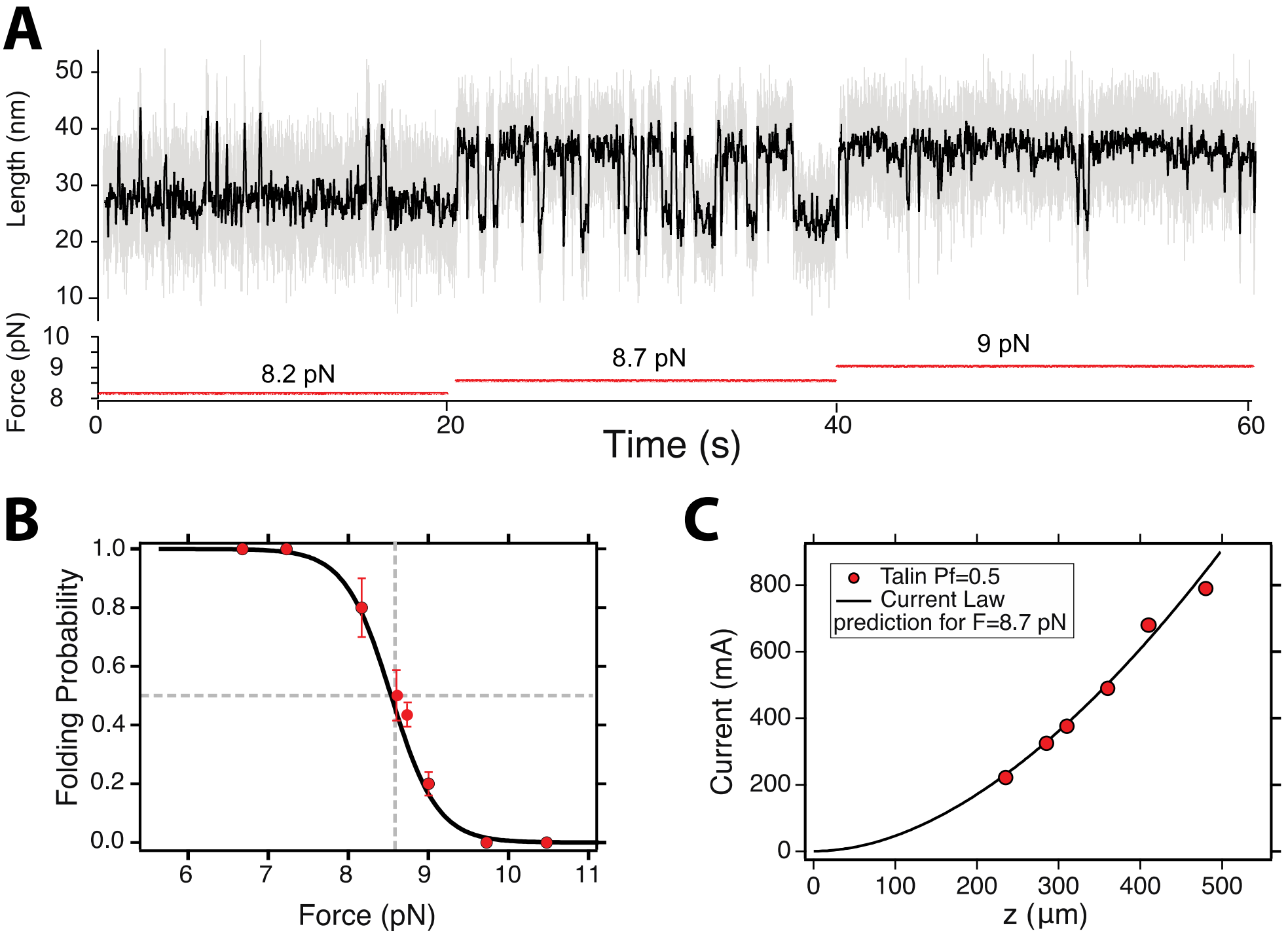
B

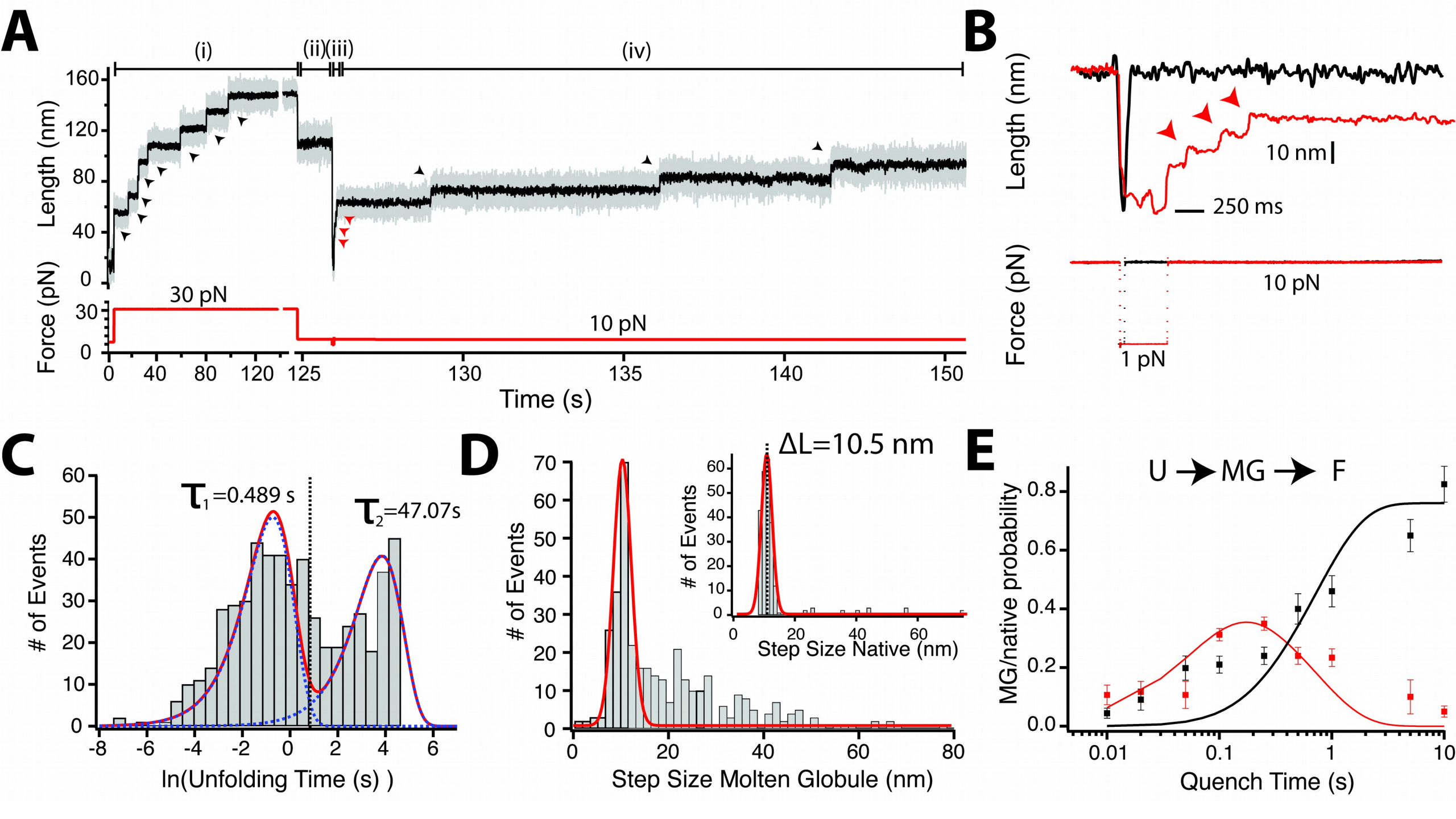

I (mA)
Magnetic Head
gap
Magnetic Field
F
z (µm)
Length (nm)

C

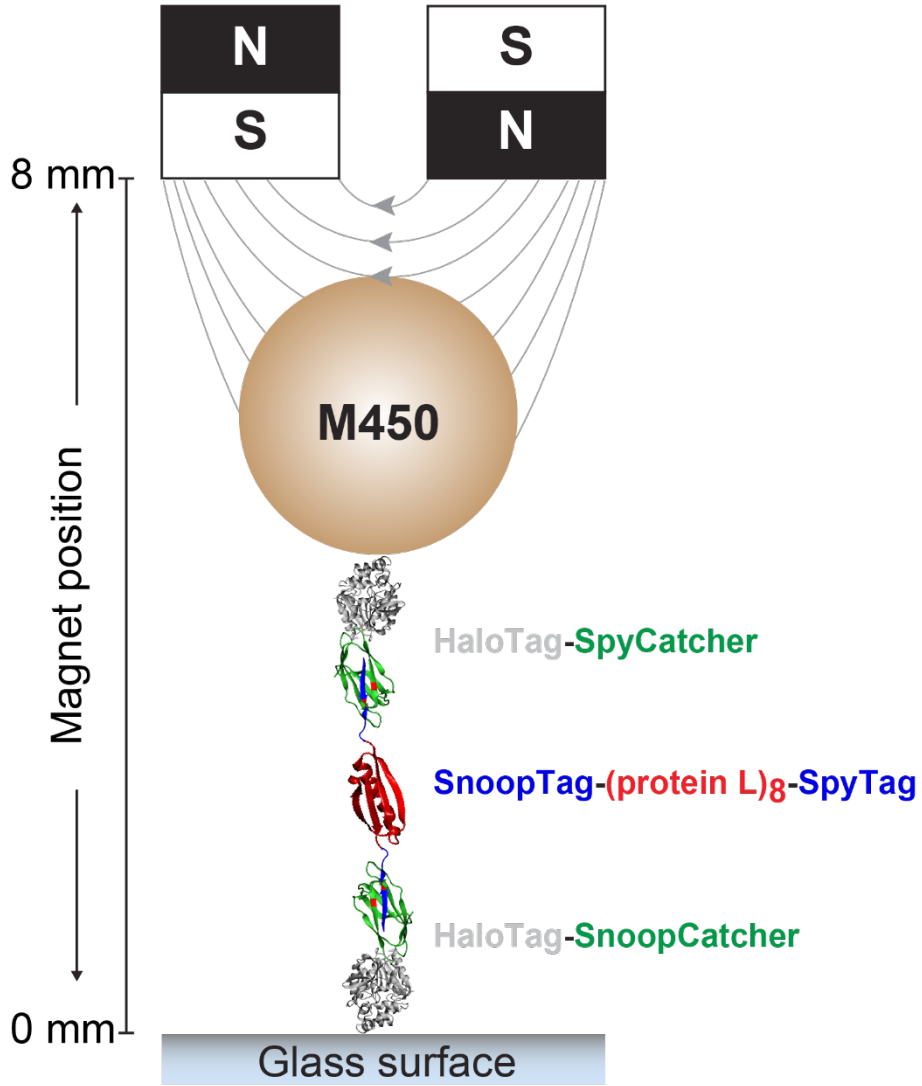

Signal
i)
ii)
iii)
iv)
Amplifier
Current
Micro-positioner
Magnetic Head
Fluid Chamber
PIEZO
Camera
Light

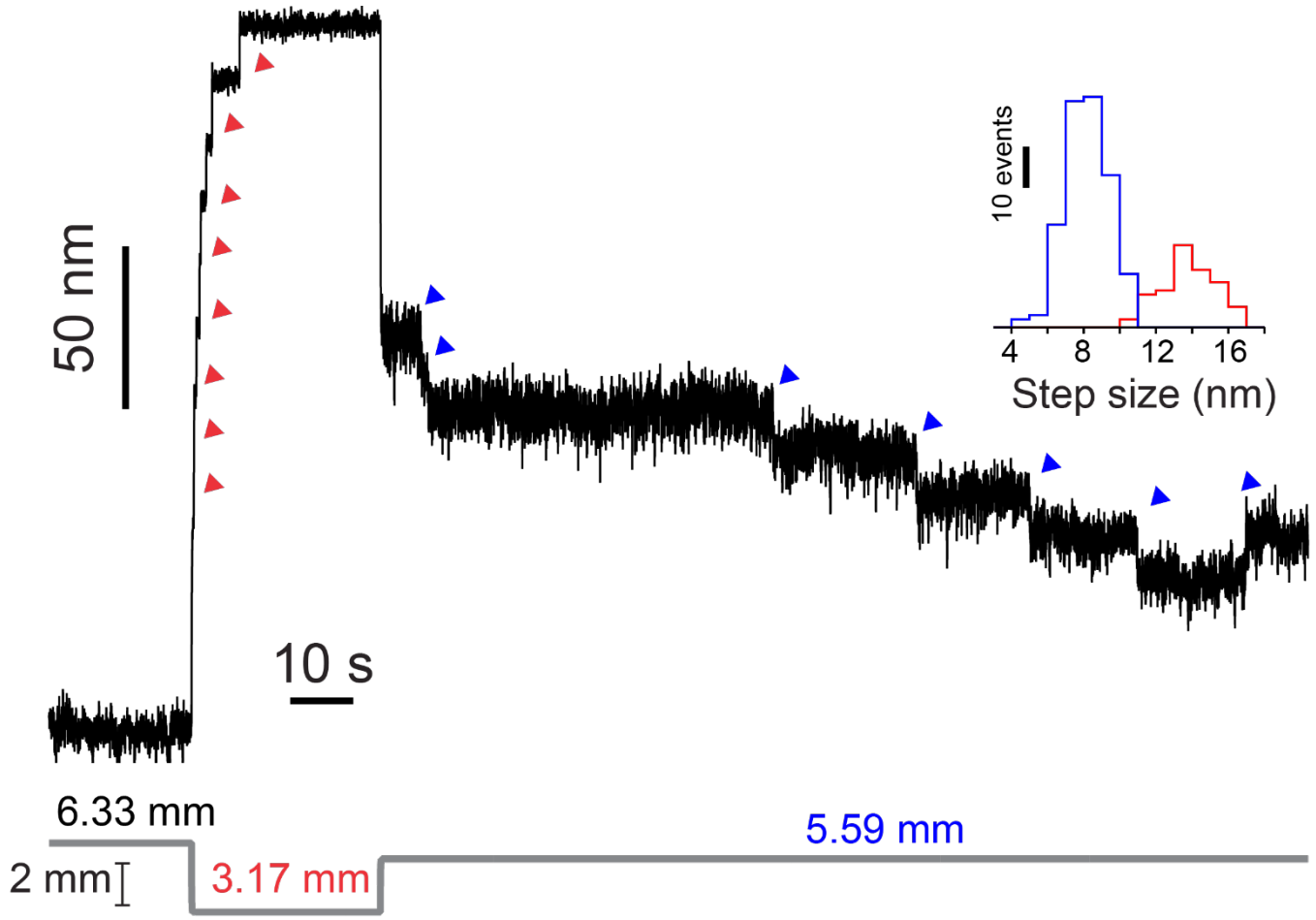

A
Length (nm)
150
100
50
0
14.3 nm
7.9 nm
5.6 nm
z (µm)
600
300
0
z=275 µm
z=550 µm
I (mA)
800
400
0
800 mA
225 mA
665 mA
0
100
200
300
400
500
Time (s)
B
Step Size (nm)
14
12
10
8
6
4
2
0
z=250 µm
z=275 µm
z=300 µm
z=325 µm
0
100
200
300
400
500
600
700
Current (mA)
C
Current (mA)
1000
800
600
400
200
50 pN
40 pN
30 pN
20 pN
15 pN
10 pN
5 pN
250
275
300
325
350
375
400
z (µm)


A
Length (nm)
50
40
30
20
10
Force (pN)
10
9
8
8.2 pN
8.7 pN
9 pN
0
20
40
60
Time (s)
B
Folding Probability
1.0
0.8
0.6
0.4
0.2
0.0
6
7
8
9
10
11
Force (pN)
C
Talin Pf=0.5
Current Law
prediction for F=8.7 pN
Current (mA)
800
600
400
200
0
0
100
200
300
400
500
z (µm)

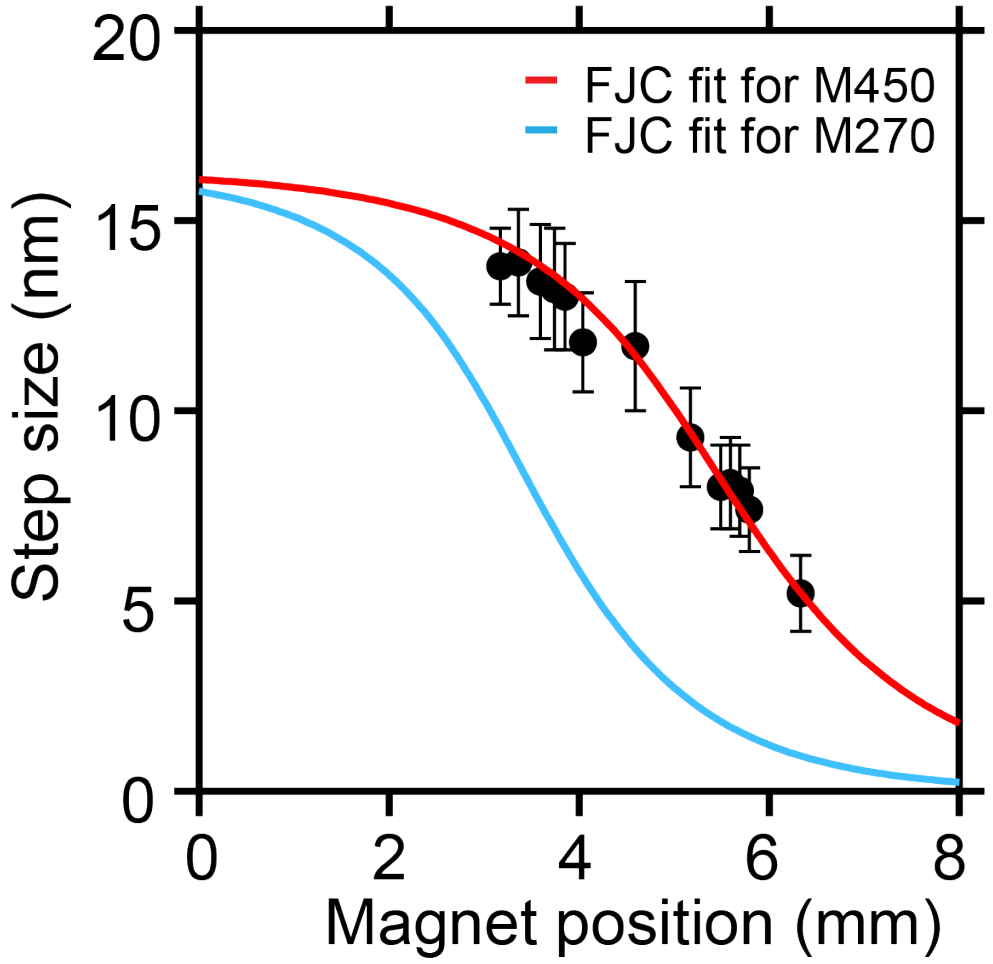

A
(i)
(ii)(iii)
(iv)
Length (nm)
Force (pN)
30 pN
10 pN
Time (s)
B
Length (nm)
10 nm
250 ms
Force (pN)
10 pN
1 pN
C
τ1=0.489 s
τ2=47.07s
# of Events
ln(Unfolding Time (s))
D
ΔL=10.5 nm
# of Events
Step Size Native (nm)
Step Size Molten Globule (nm)
E
U → MG → F
MG/native probability
Quench Time (s)

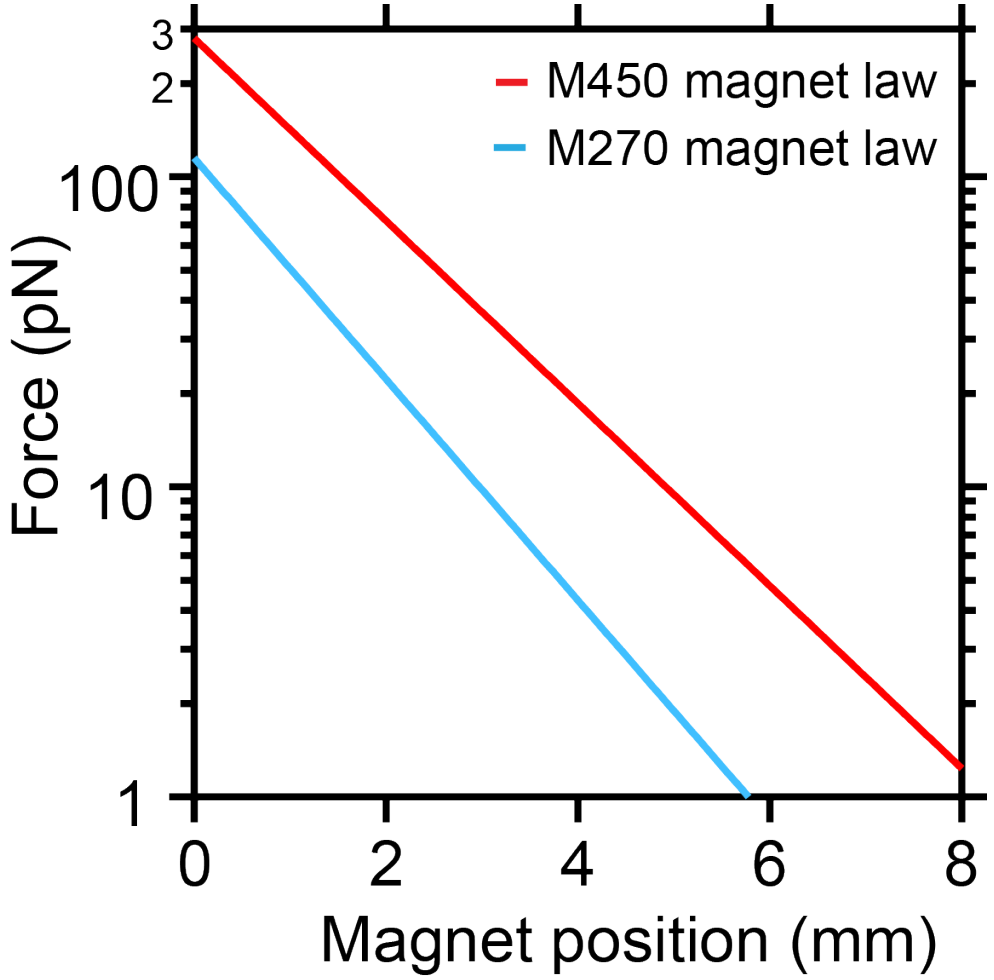
Calibration





Calibration





MT_3: the next level.

Mechanical signals in biology are noisy and contain periodic signals