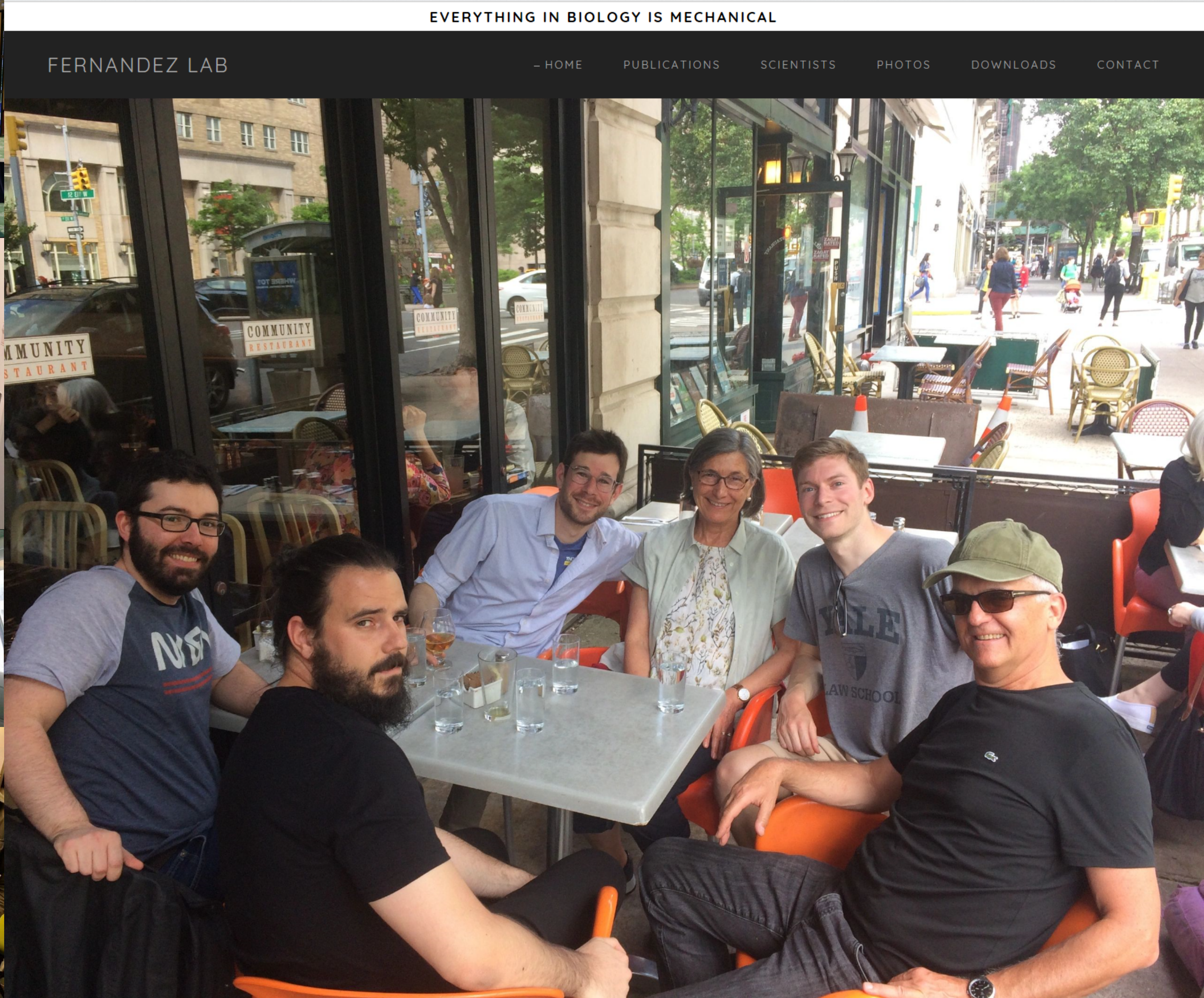
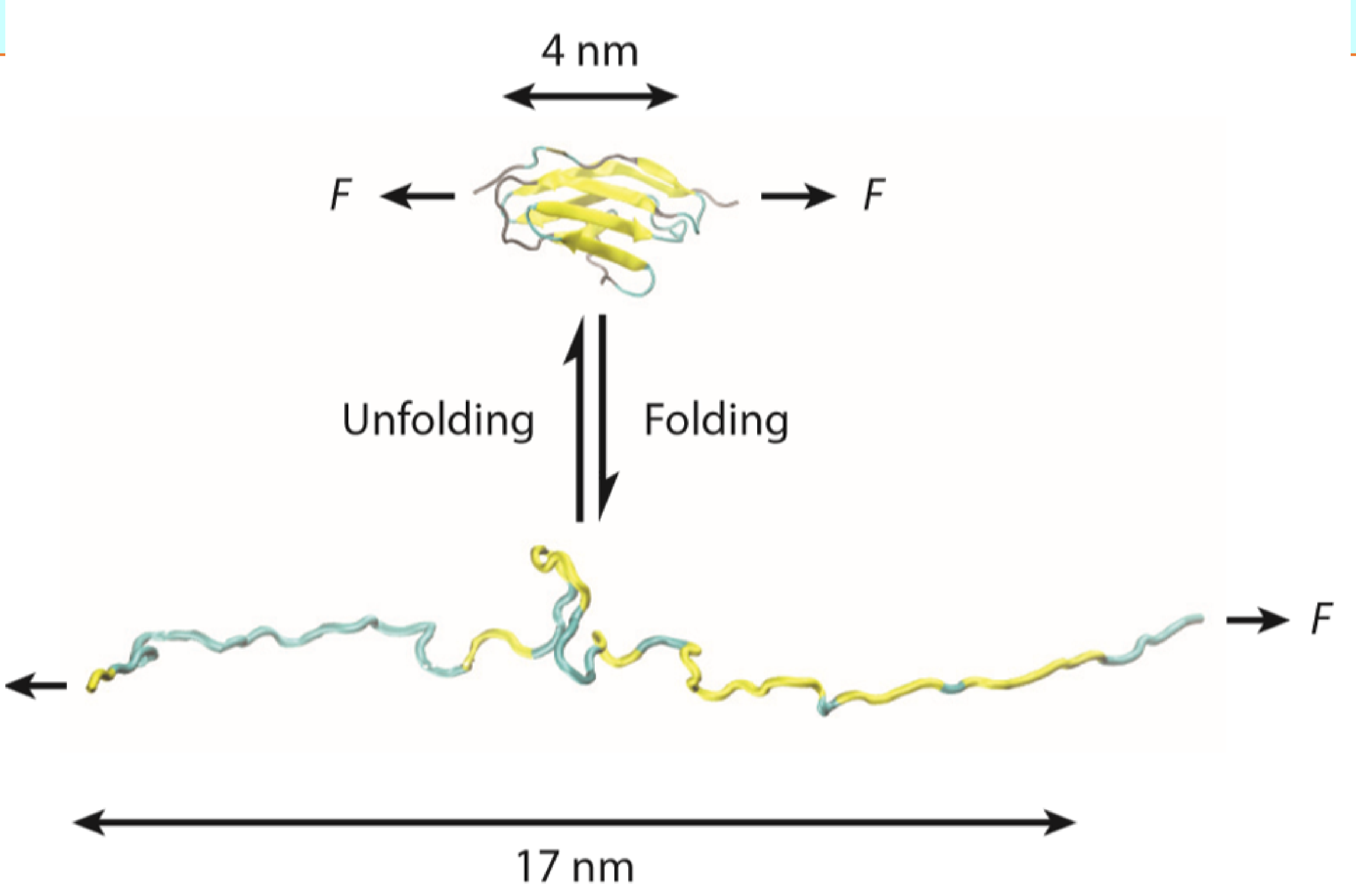
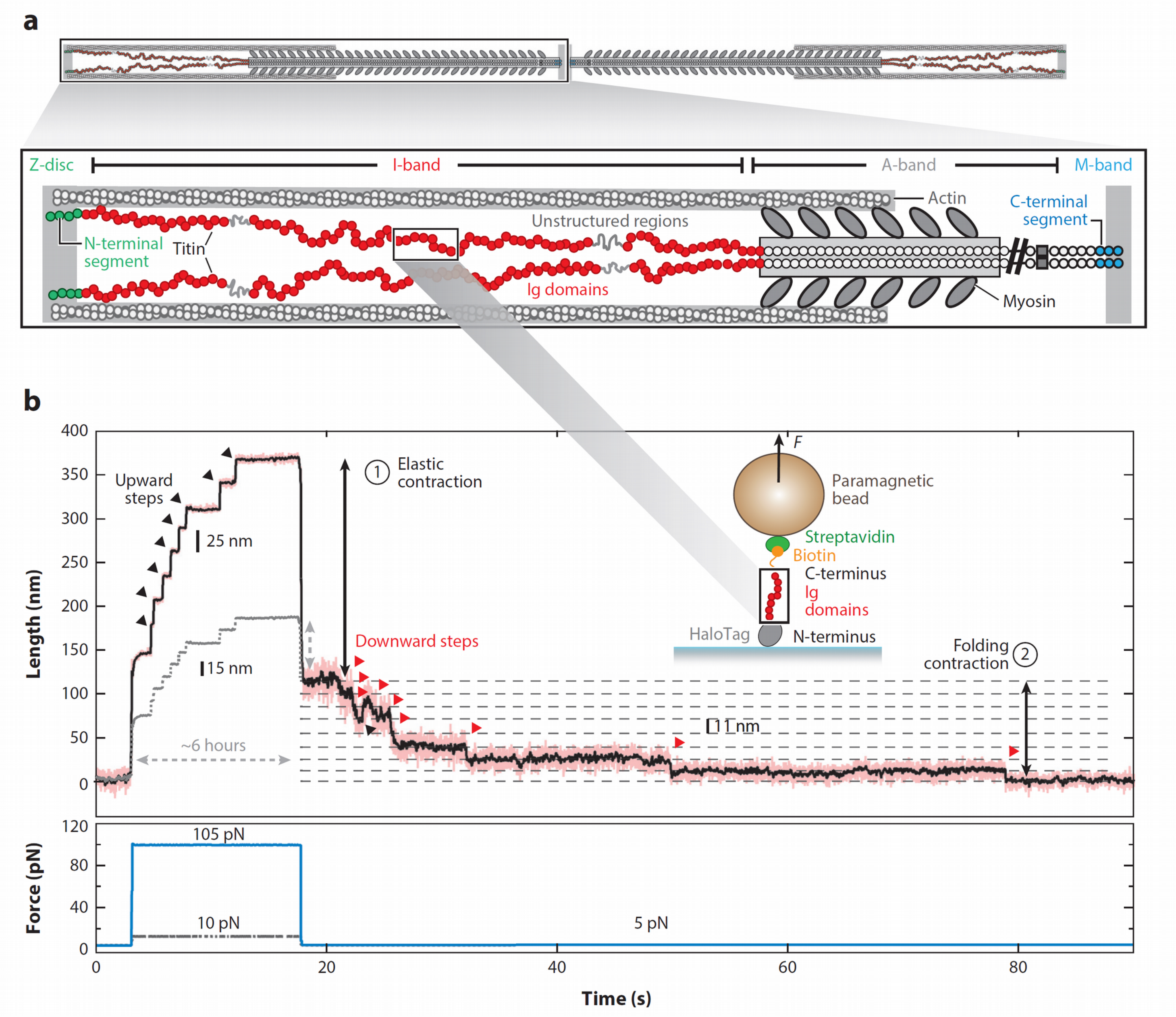
Look us up in zeptowatt.com



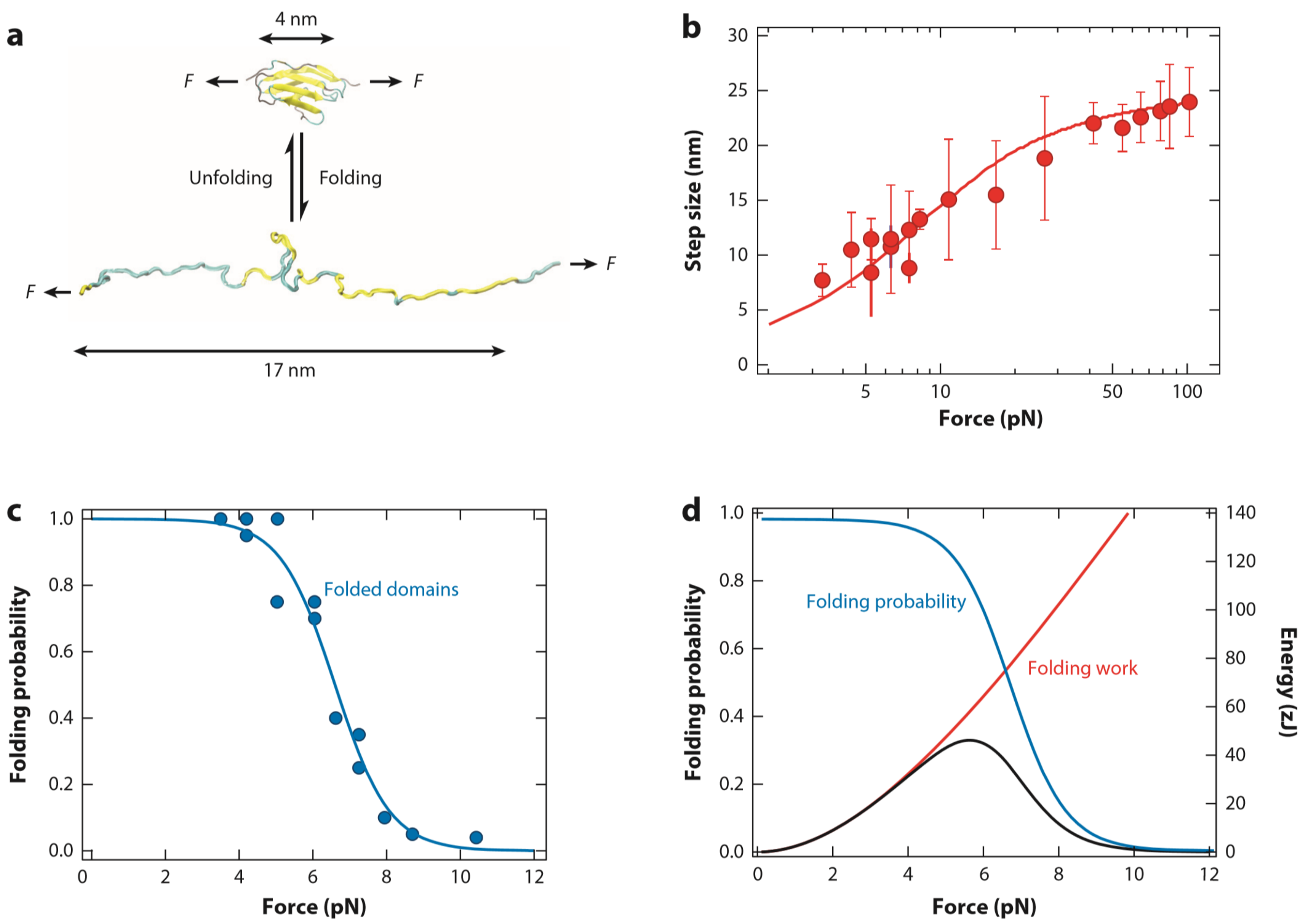
The amazing mechanical power of titin folding



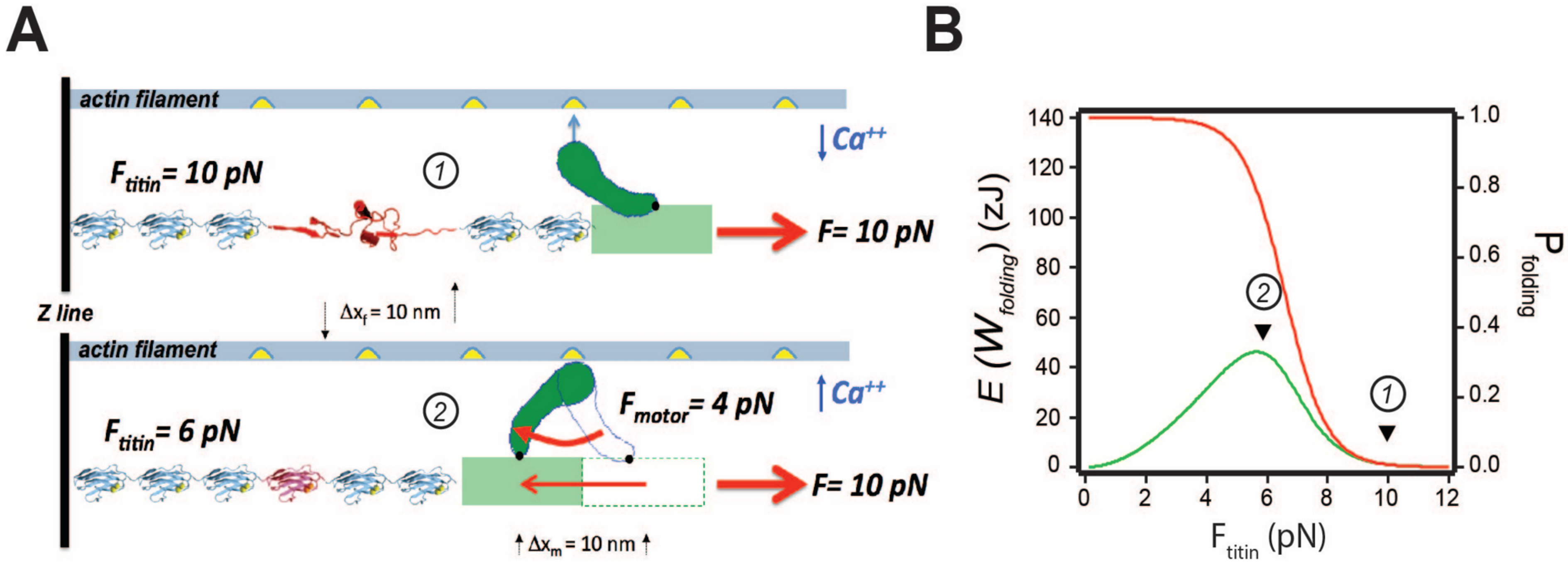
Titin stores and releases elastic energy by folding/unfolding



Titin Ig folding work is similar to ATP-driven myosin motors



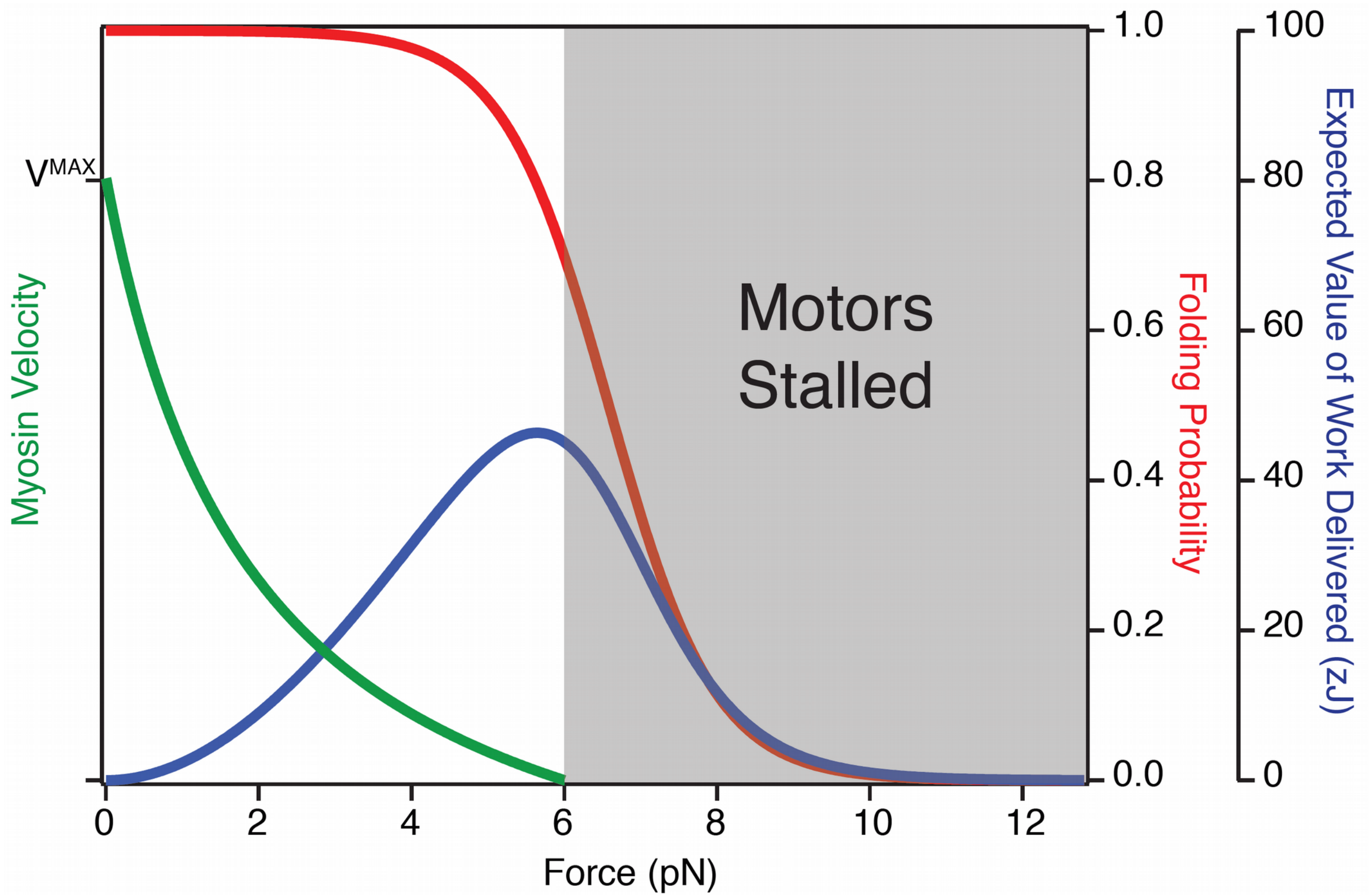
Is titin placed to deliver mechanical power during muscle contraction?

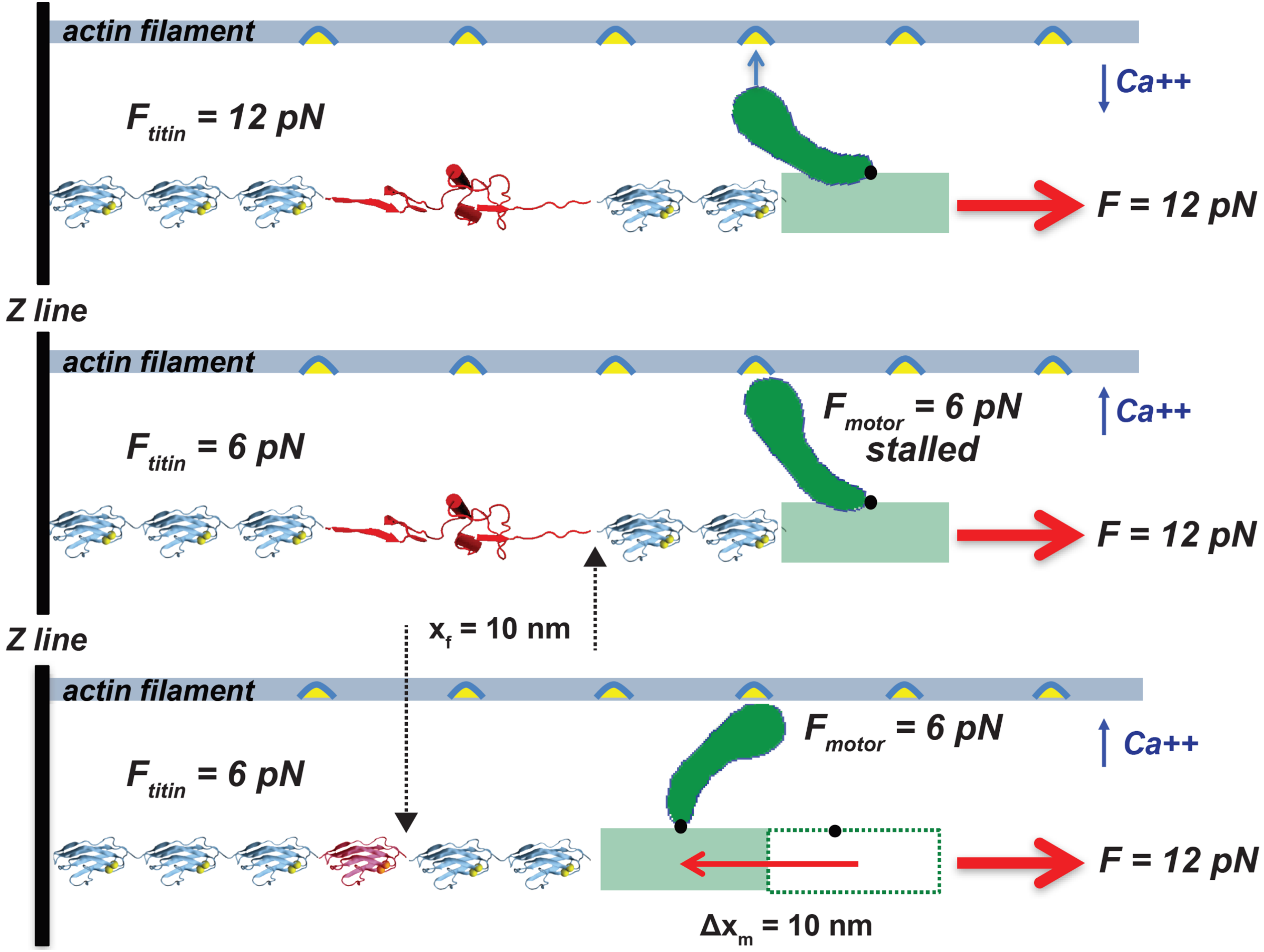


Rivas-Pardo et al., 2016, **Cell Reports,** 14:1339-1347

Eckels et al., 2018, **Annual Reviews of Physiology,** 80:327-351

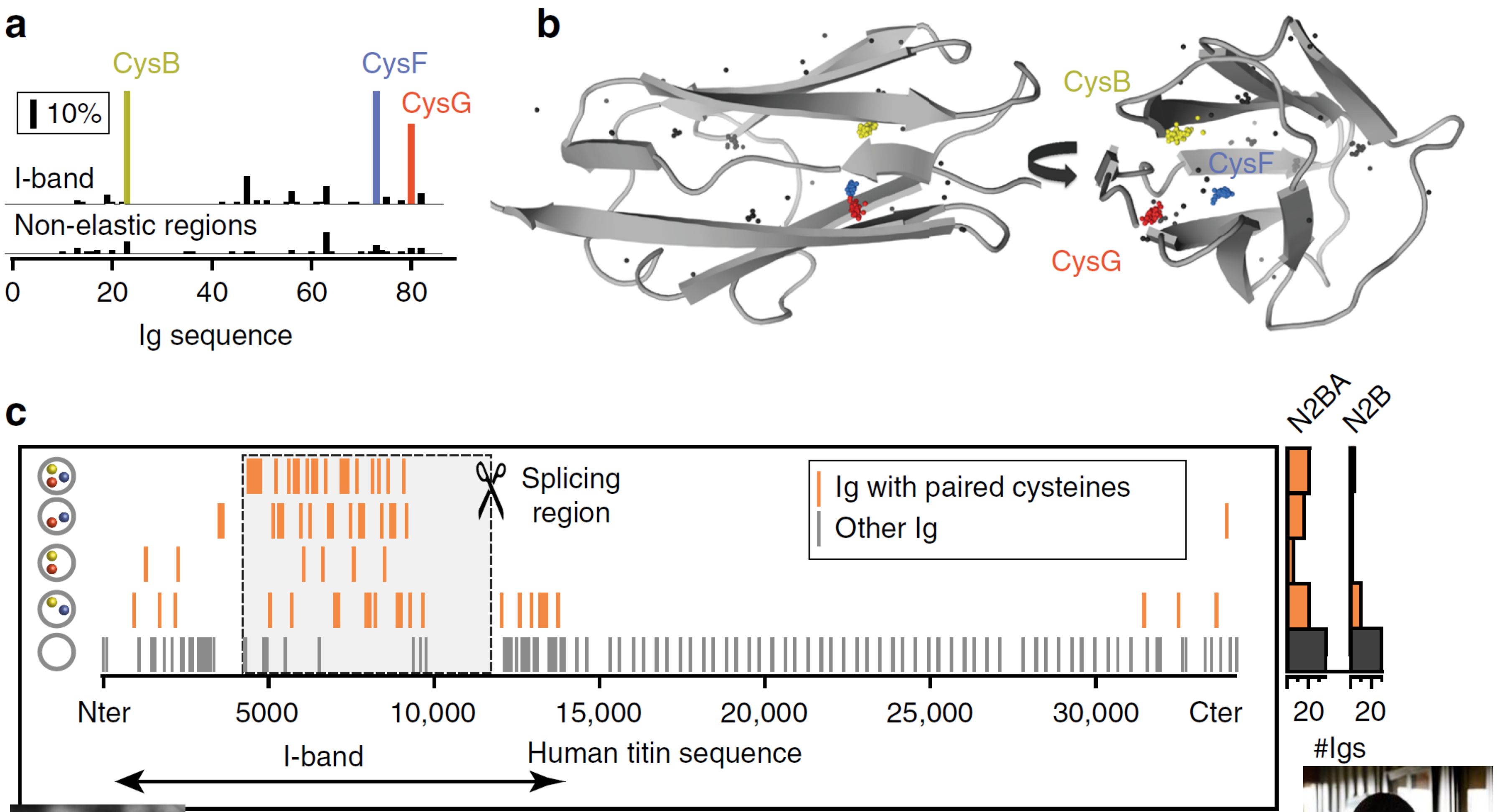
Titin folding does mechanical work at forces where myosin motors are stalled





actin filament
Ca++
F titin = 12 pN
F = 12 pN
Z line
actin filament
F motor = 6 pN
stalled
Ca++
F titin = 6 pN
F = 12 pN
Z line
x f = 10 nm
actin filament
F motor = 6 pN
Ca++
F titin = 6 pN
F = 12 pN
Δx m = 10 nm

Cryptic cysteines regulate titin folding



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

Kosuri, et al., 2012, **Cell,** 151: 794 - 806

Alegre-Cebollada, et al., 2014, **Cell,** 156: 1235 - 1246

Giganti, et al., 2018, **Nature Comm,** 9: 185

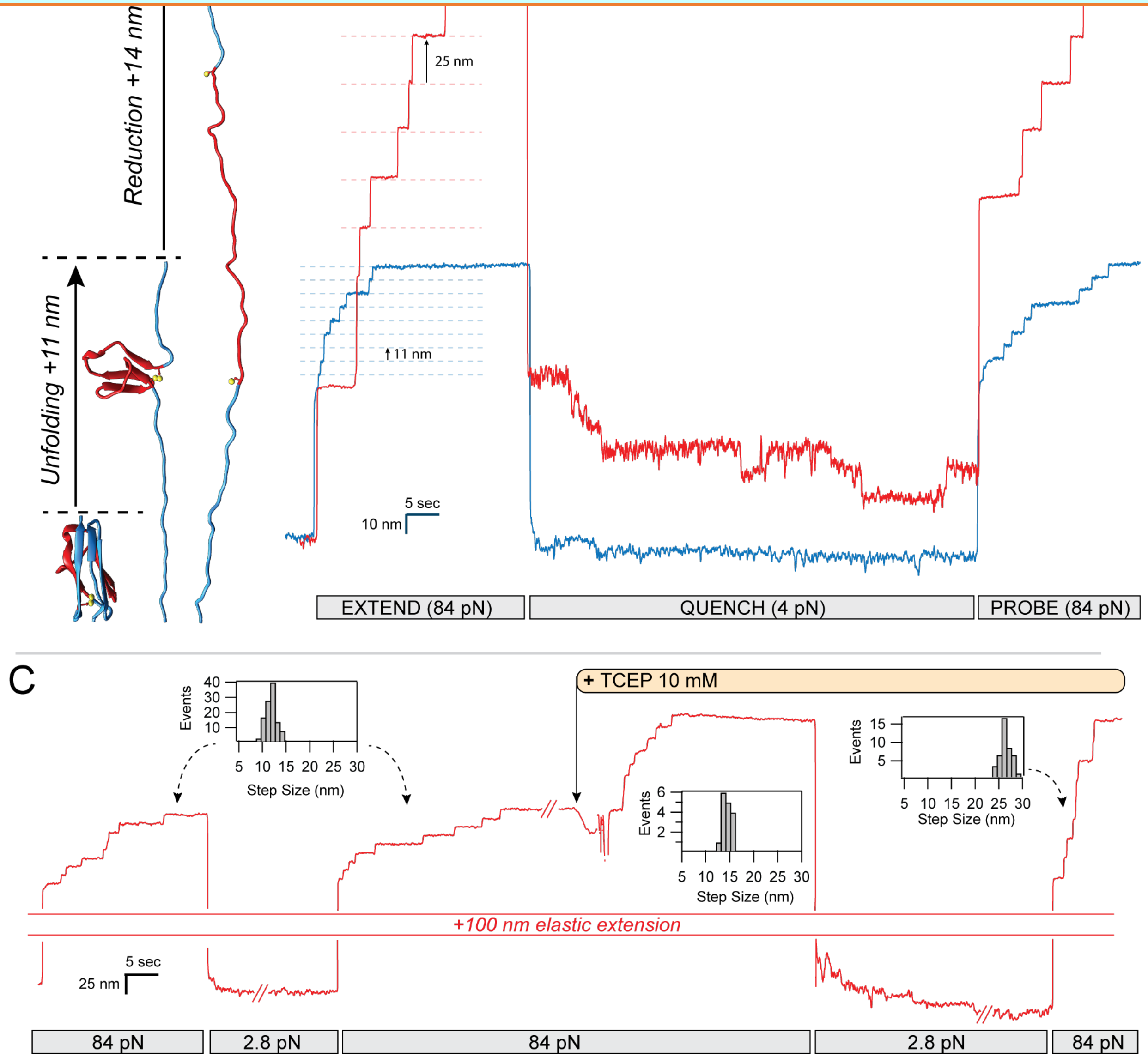
Jorge Alegre-Cebollada

Pallav Kosuri

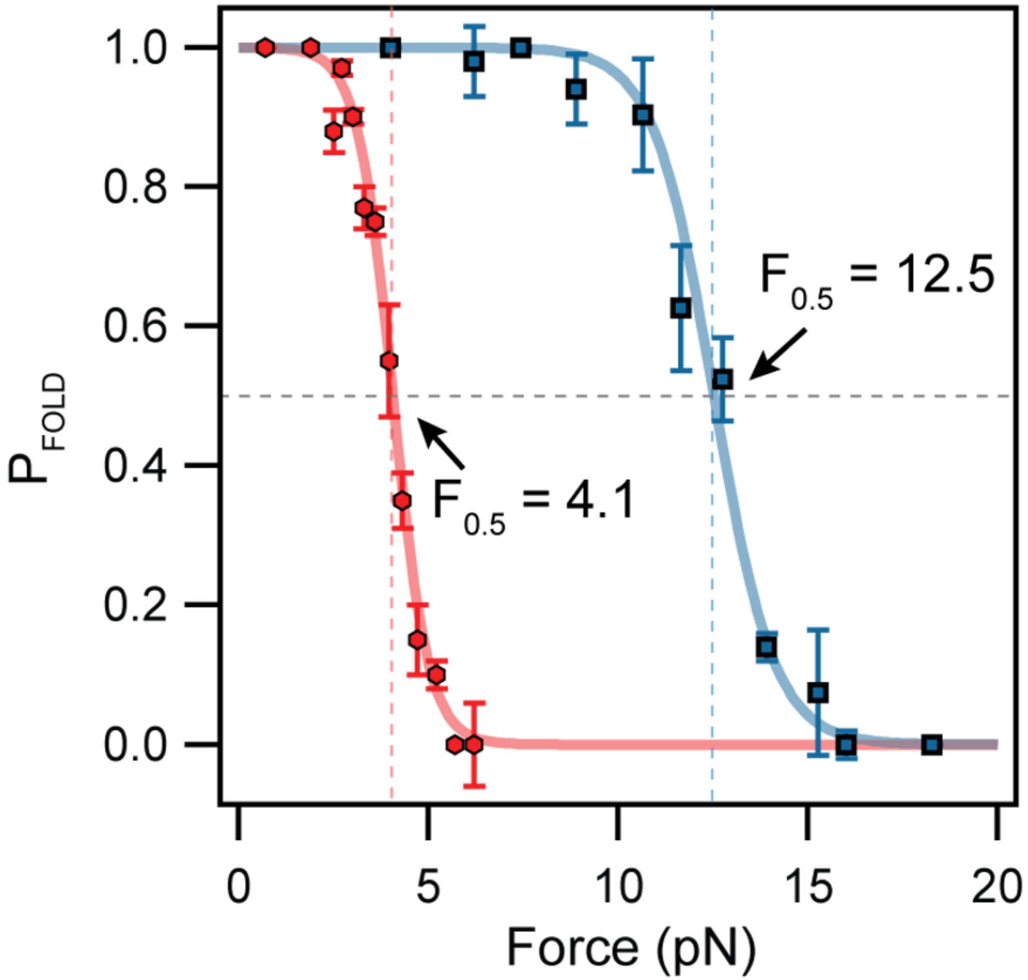
Redox state of a titin domain can be easily controlled

Edward Eckels

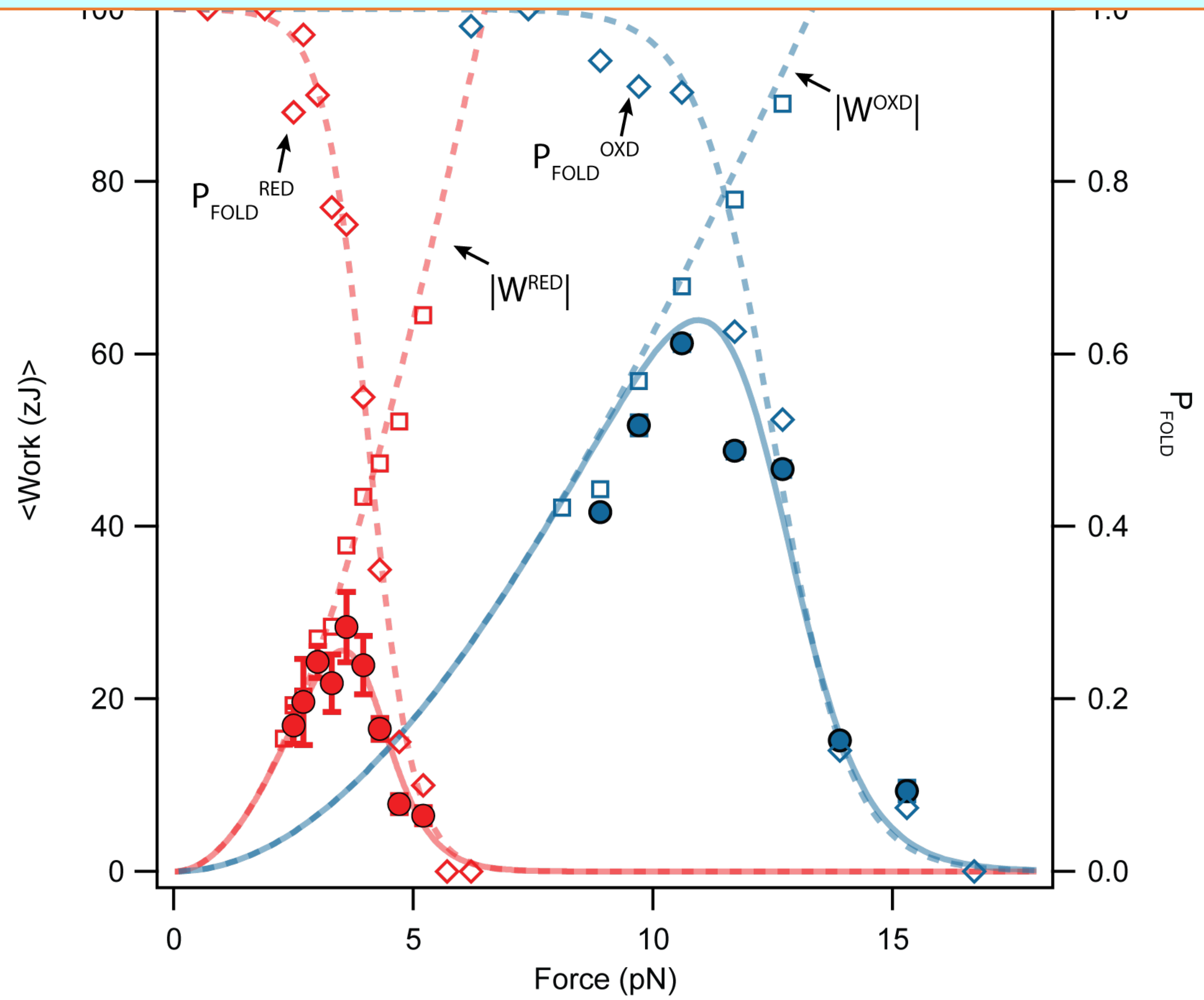
Shubhasis Haldar



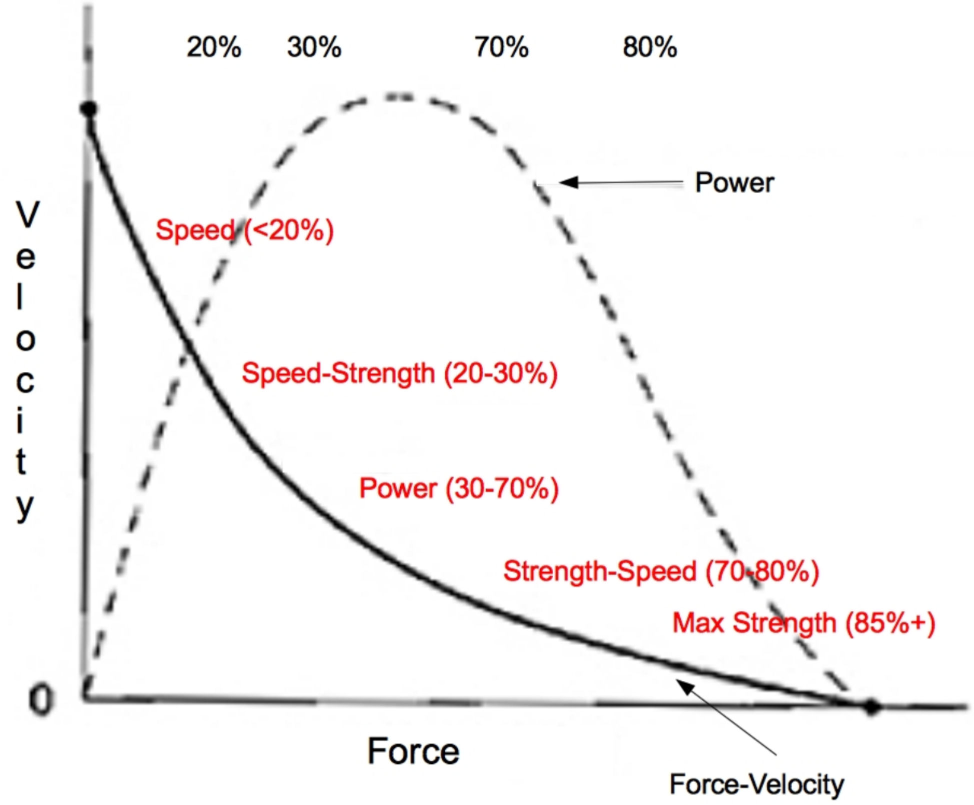
Disulfides shift titin domain folding to higher forces



S-S bonded domains do twice the work at three times the load

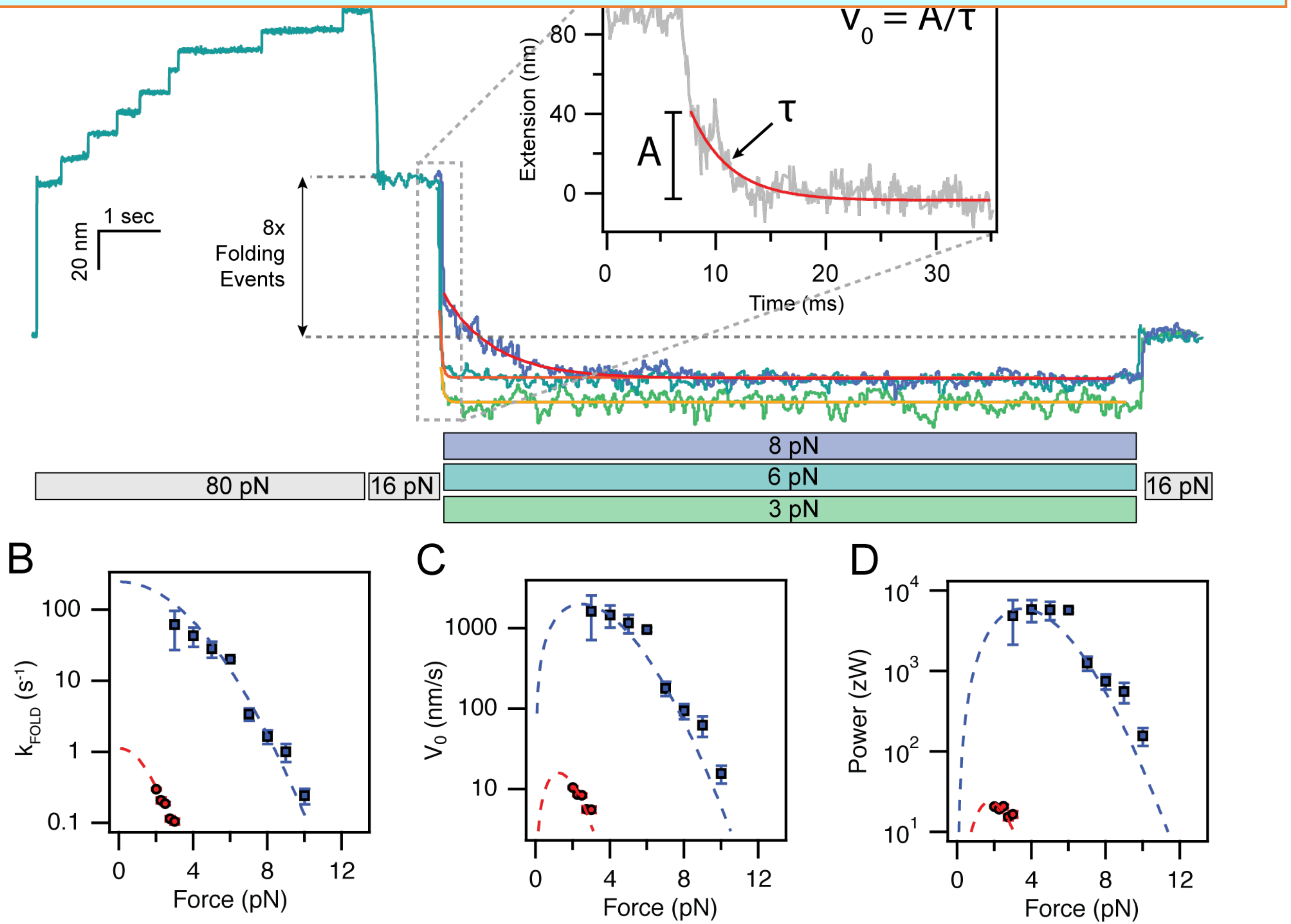


Velocity vs Force, and Power

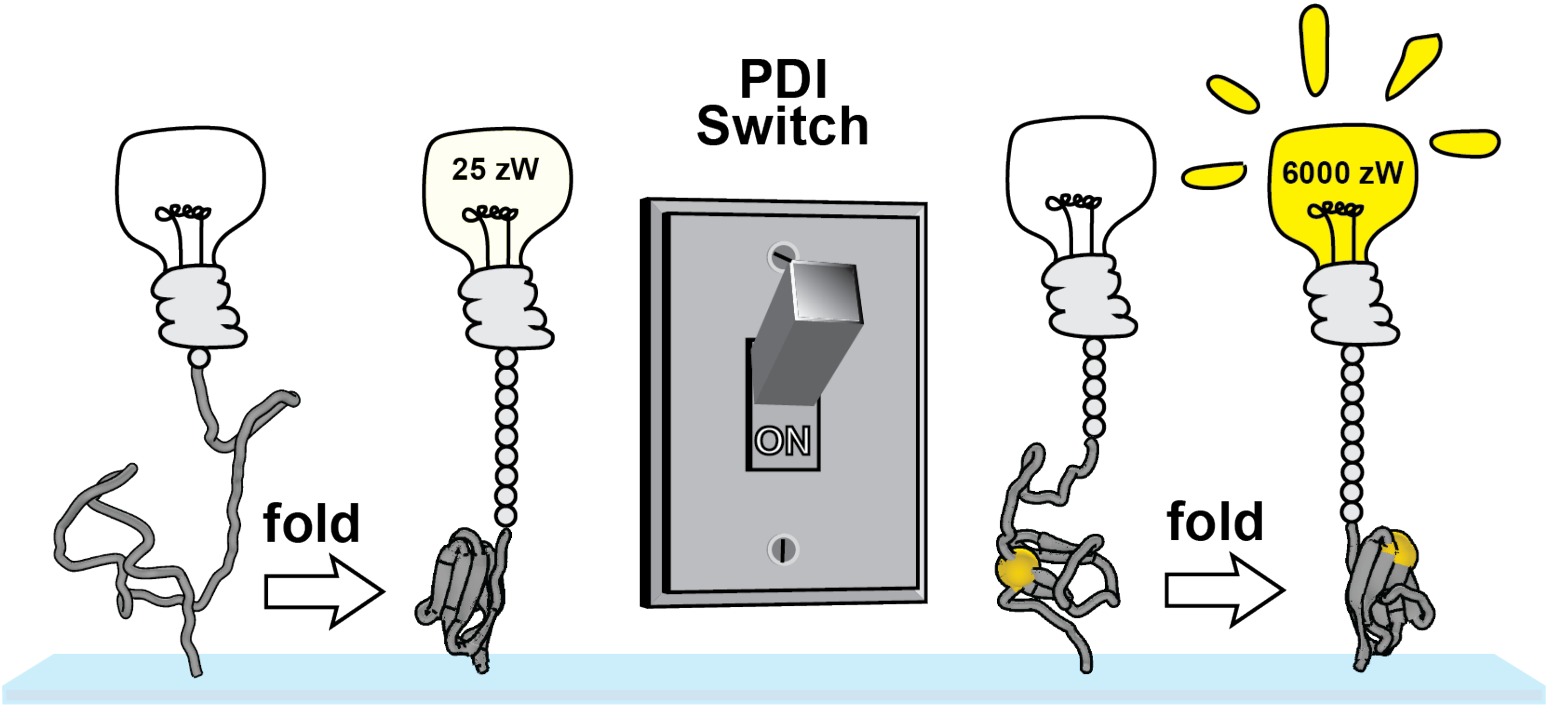


bodyrecomposition.com

Disulfide bonds greatly increase the power output of folding

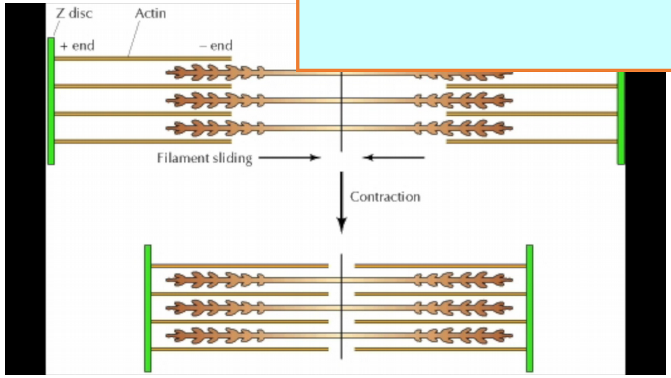


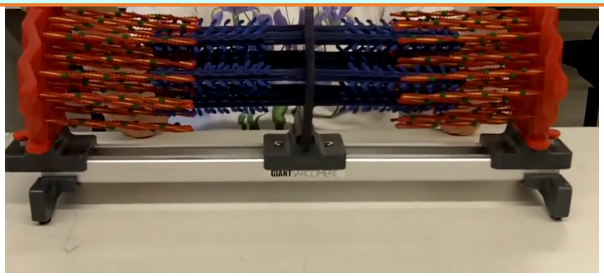
Disulfide bonds are the power switches of titin

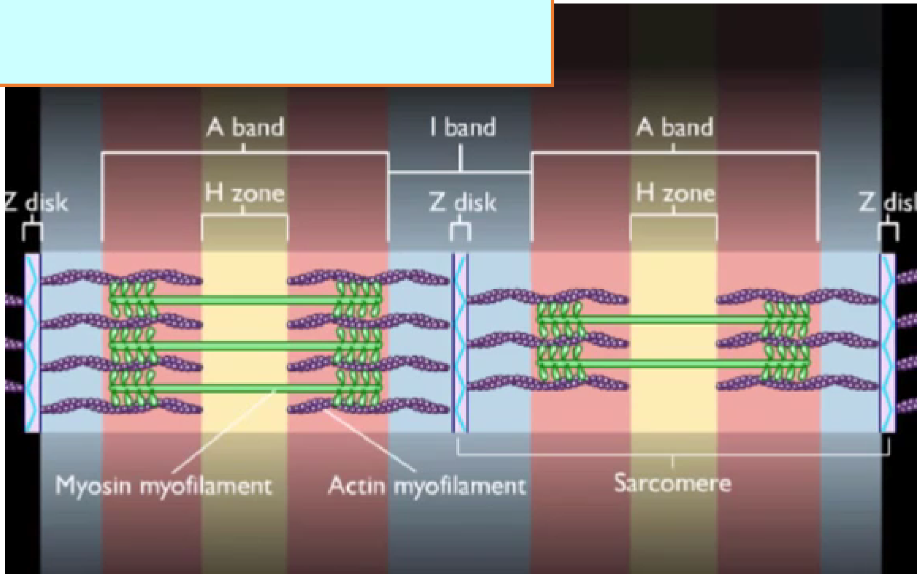


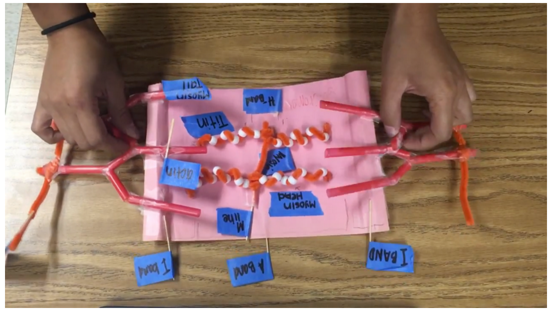
Eckels et al., 2019, **Cell Reports,** 27:1836-1847

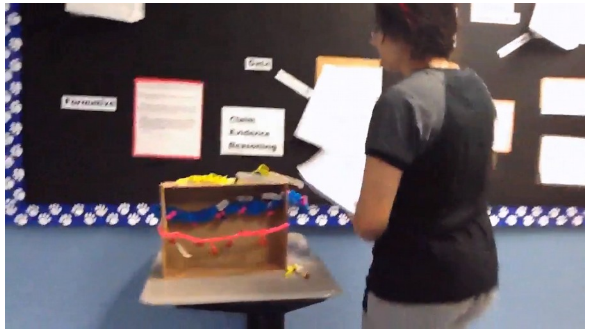
We have assumed that the power output from a contracting muscle is due solely to actin-myosin

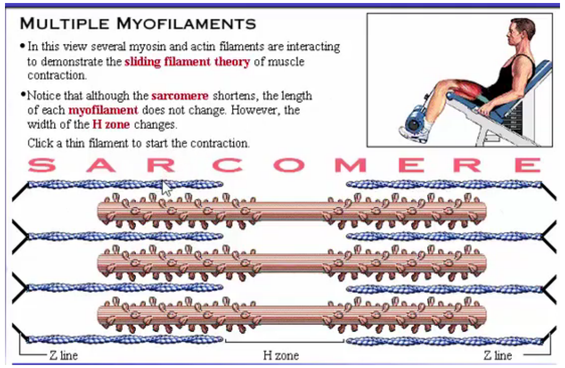


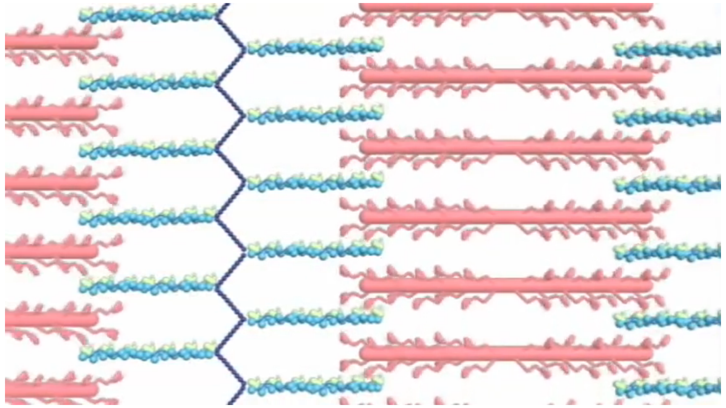


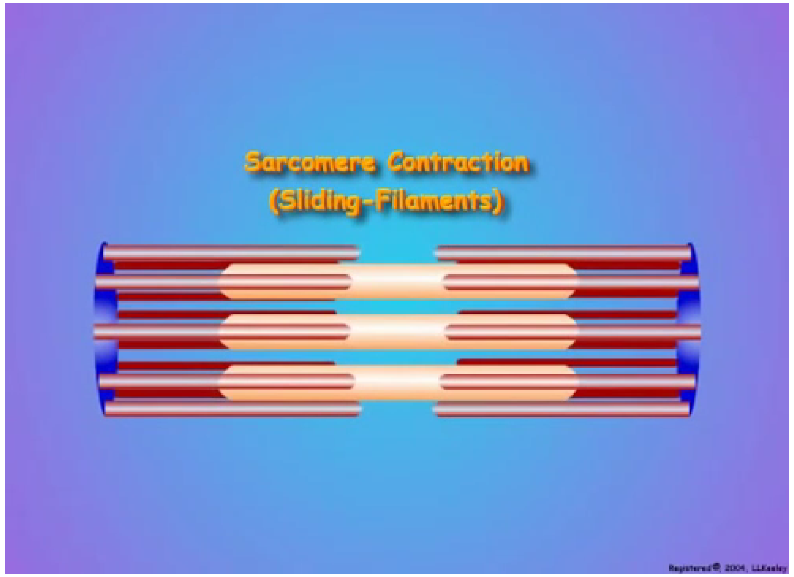




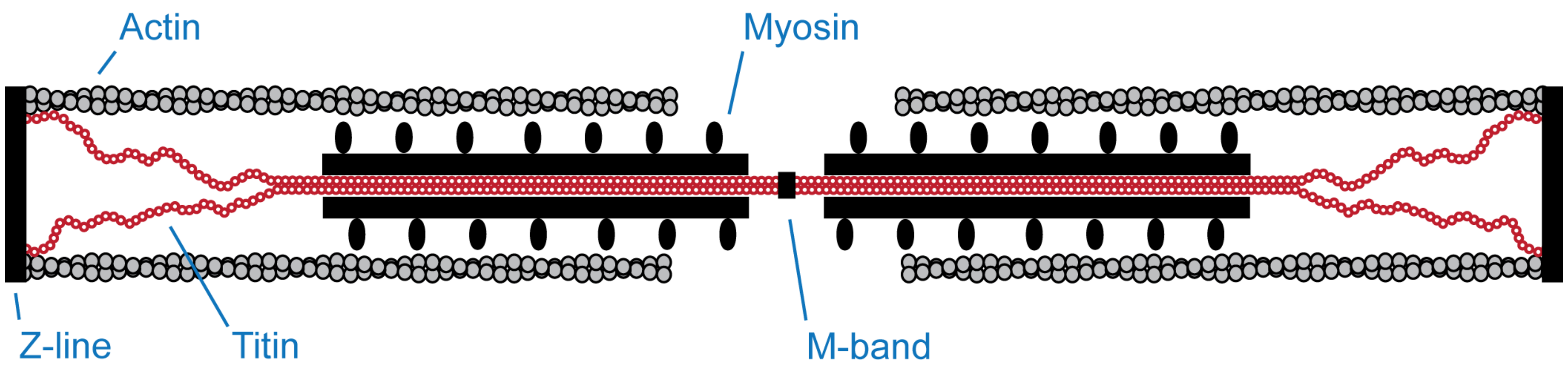


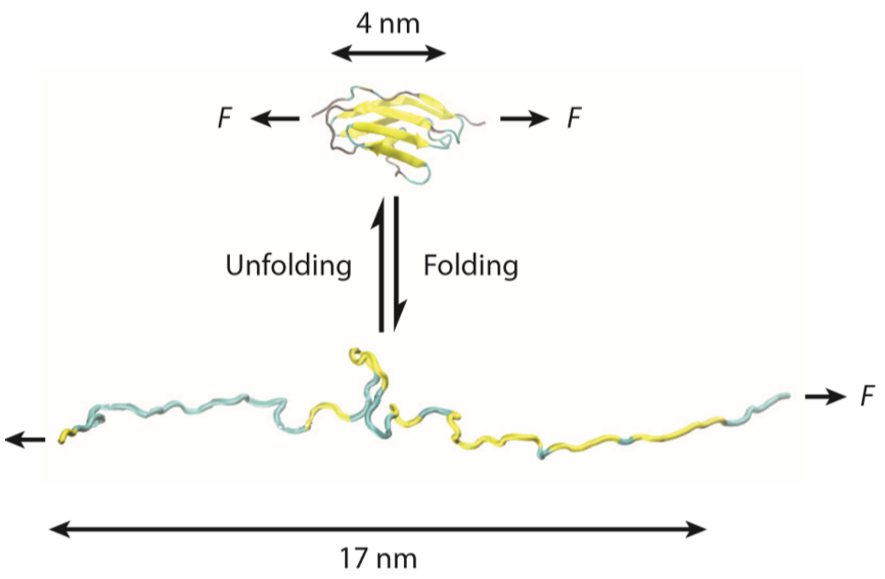






This view needs urgent revision

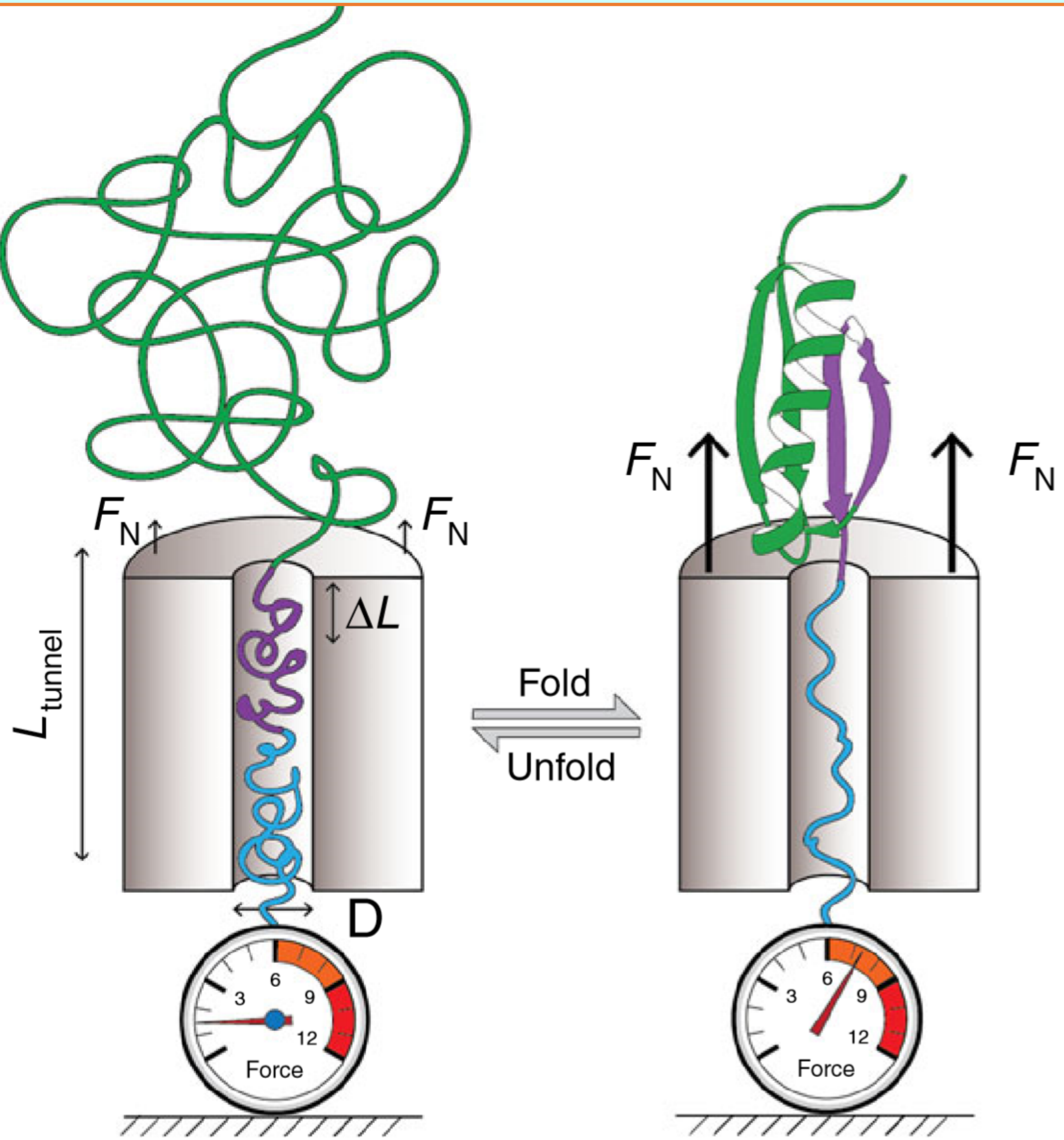




We must include titin folding as the crucial missing component

Eckels et al., 2018, **Annual Reviews of Physiology,** 80:327-351

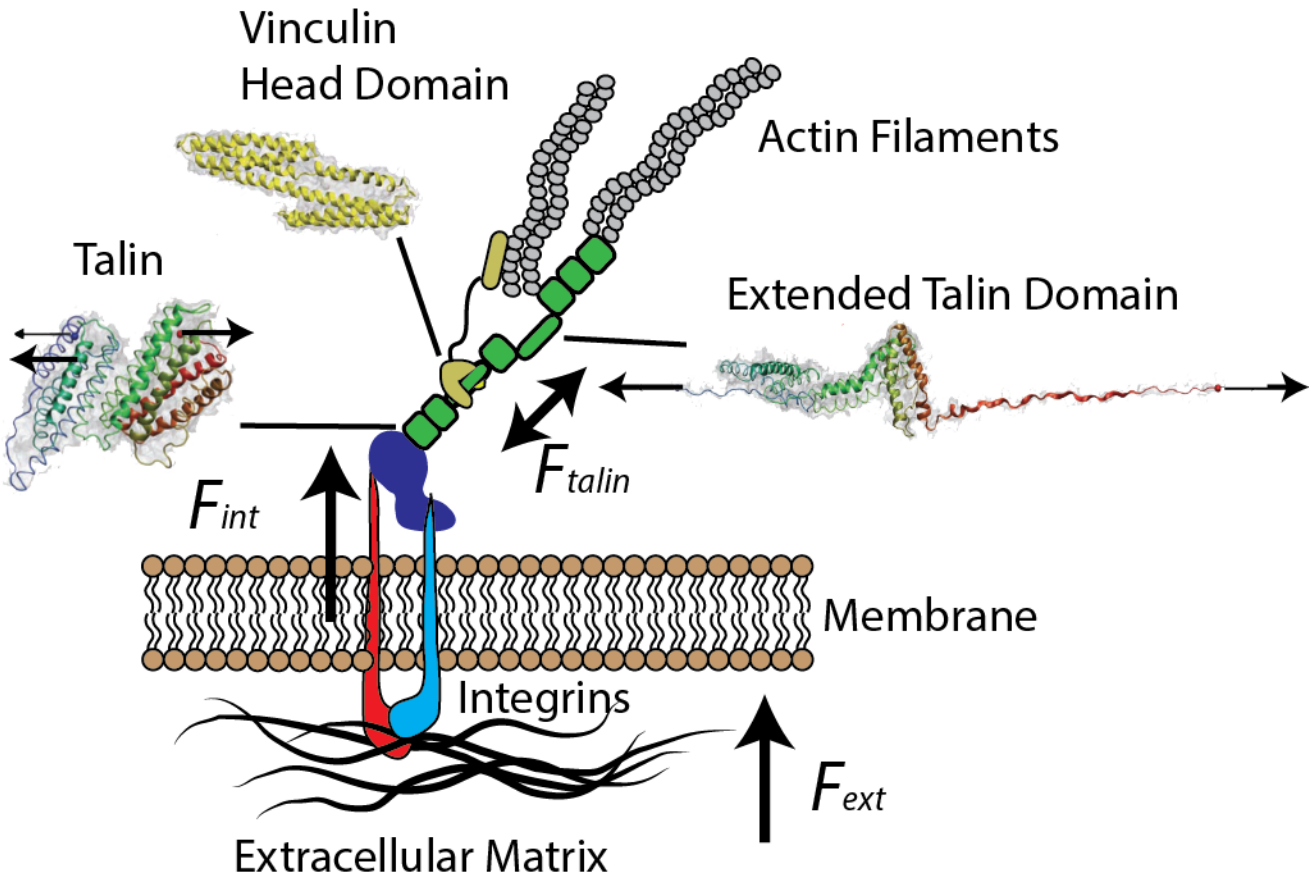
Protein folding powers translocation of proteins across pores?



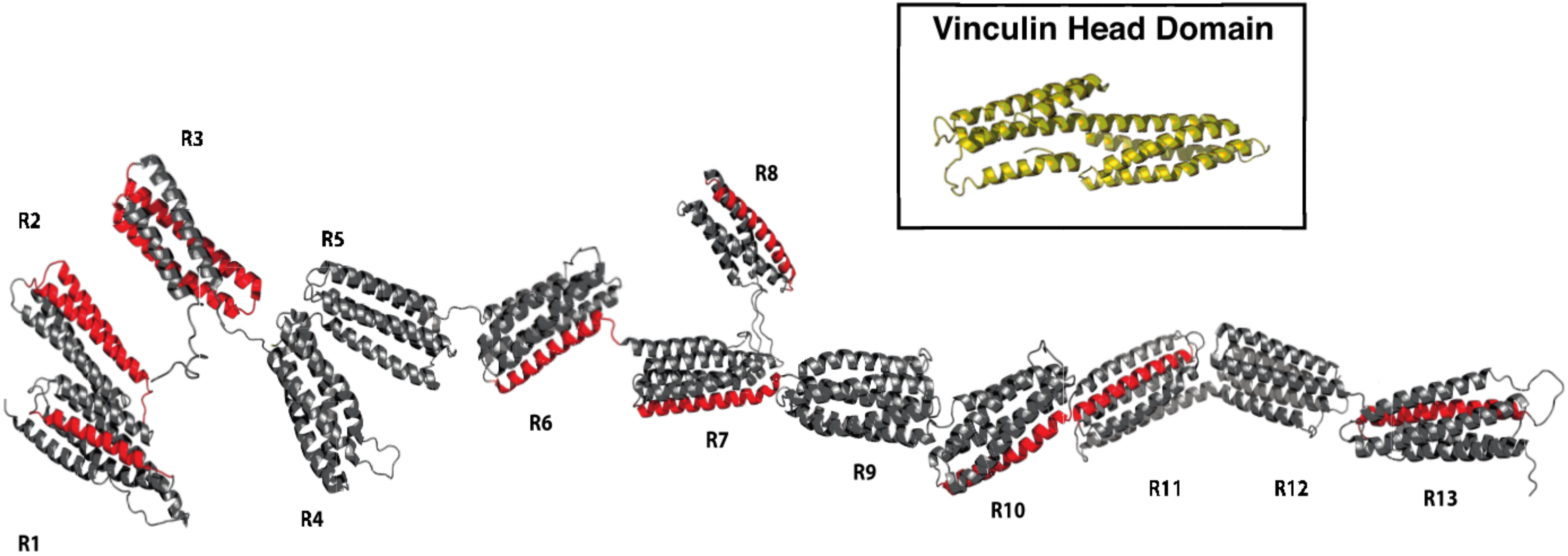
Shubhasis Haldar

Haldar, et al., 2017, **Nature Communications,** 8: 668

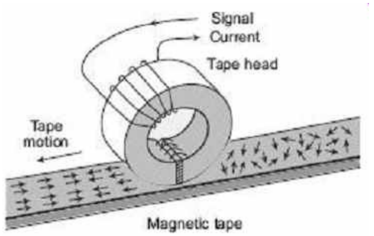
Signal transduction by the mechanical force sensor talin

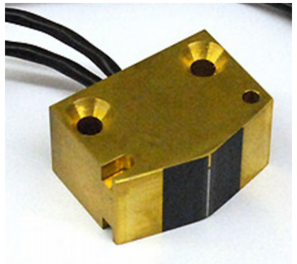


13 domains of Talin



Listening to heavy talin : Dr. Rafael Tapia Rojo

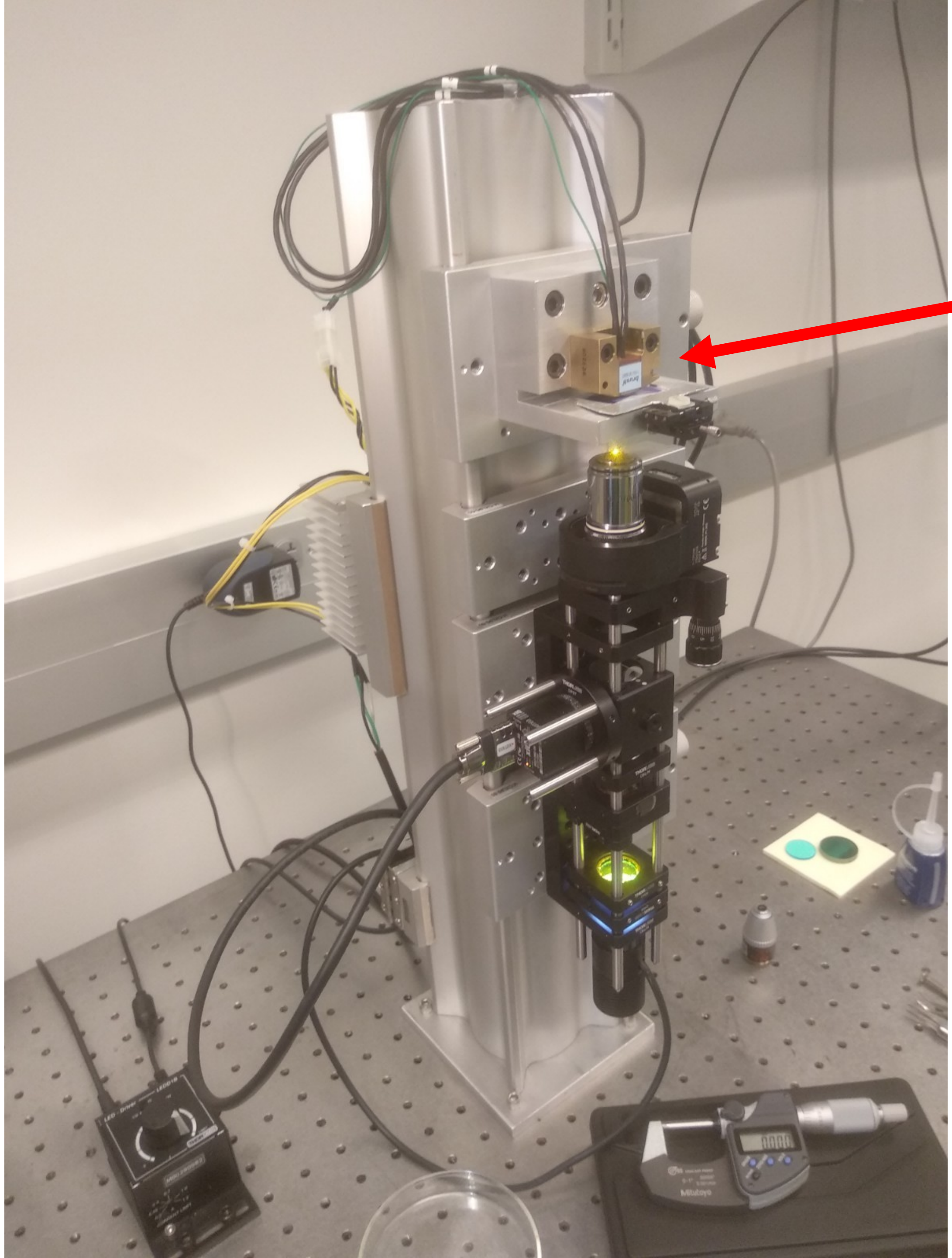


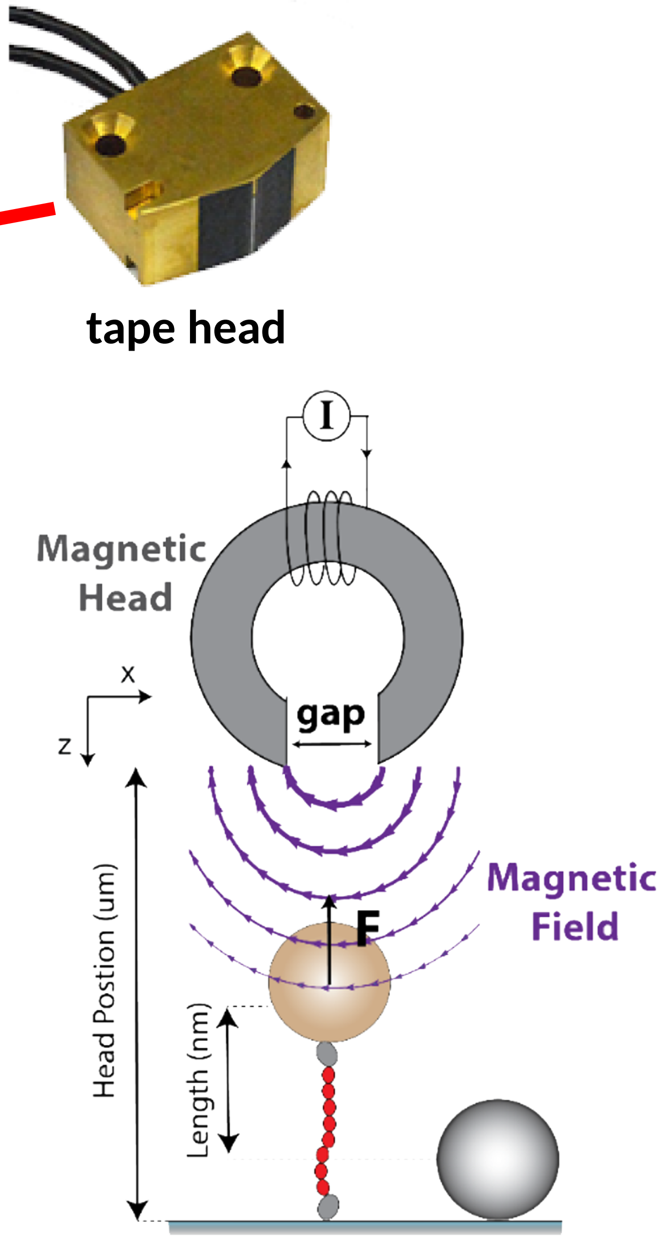


Tapia-Rojo et al., 2019 **PNAS,** 116 (16) 7873-7878

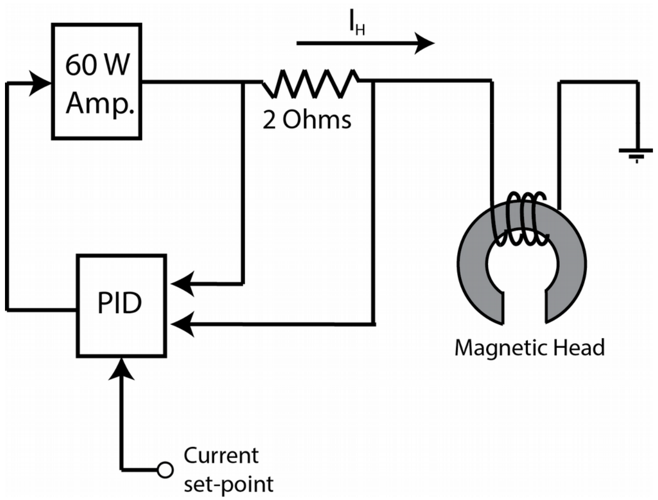
MT_3

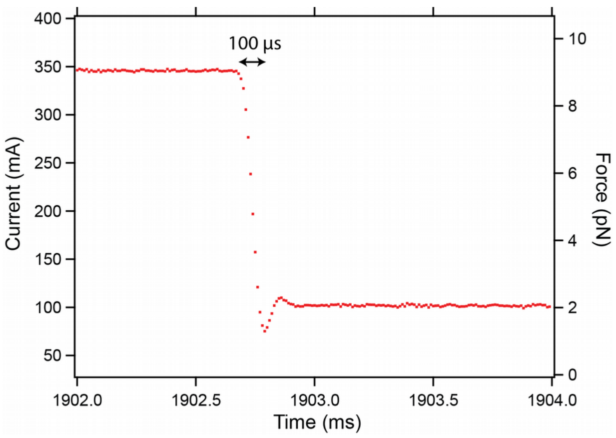

AKAI
AKAI

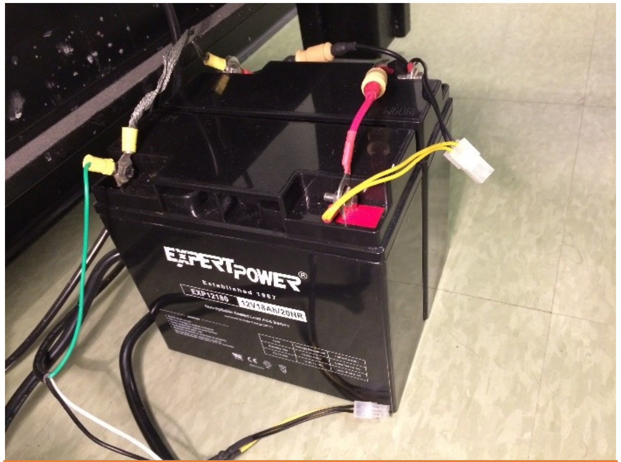


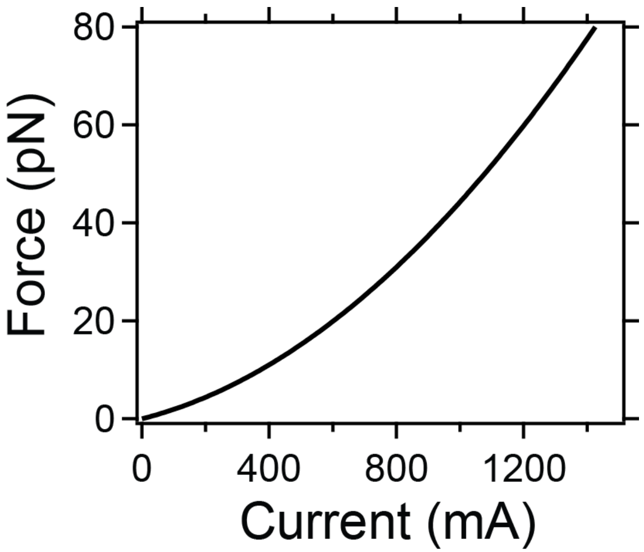

tape head
I
Magnetic
Head
gap
x
z
Magnetic
Field
F
Head Postion (um)
Length (nm)

> 10 kHz bandwith with sub-pN resolution





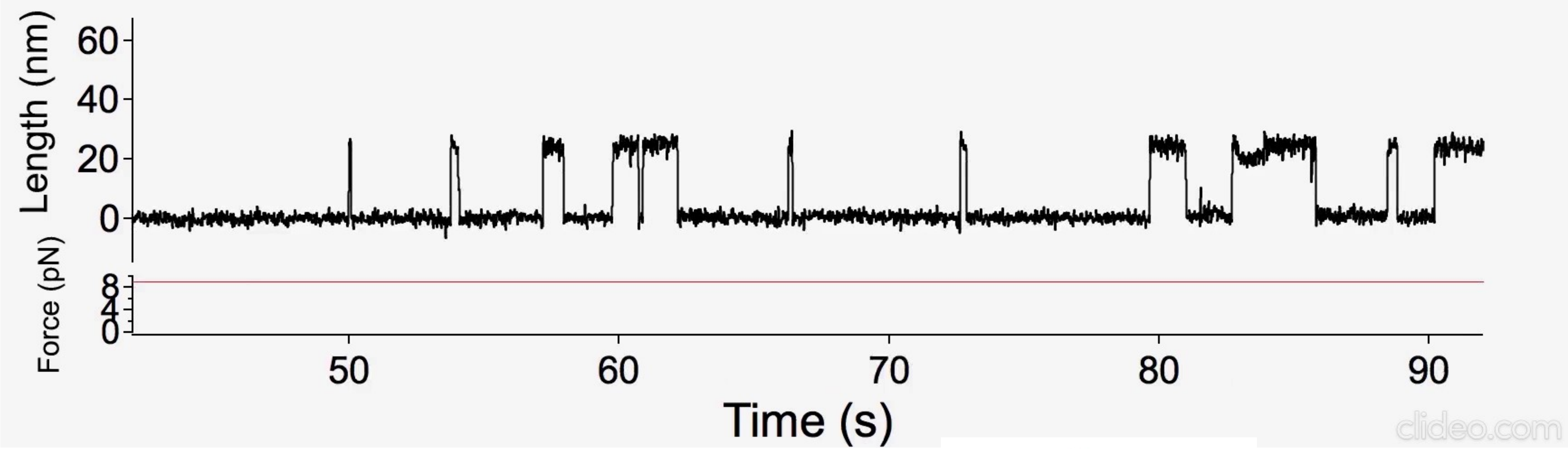


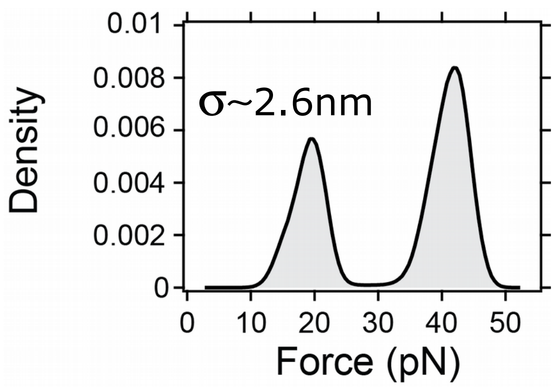


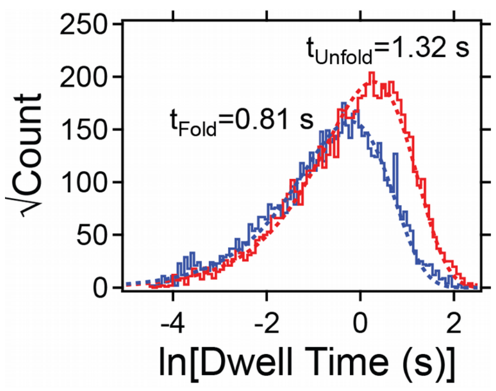
Battery powered

$$F(I) = 2.786 10^{-5} \cdot I^2 + 0.016 \cdot I$$

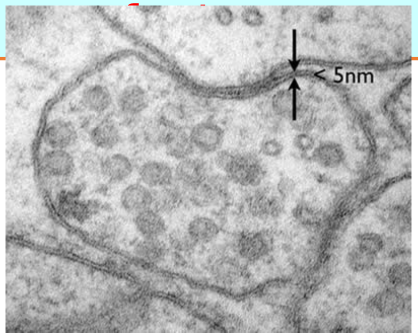
5 hour long recording of talin at 1400 fps with a total drift of 7 nm



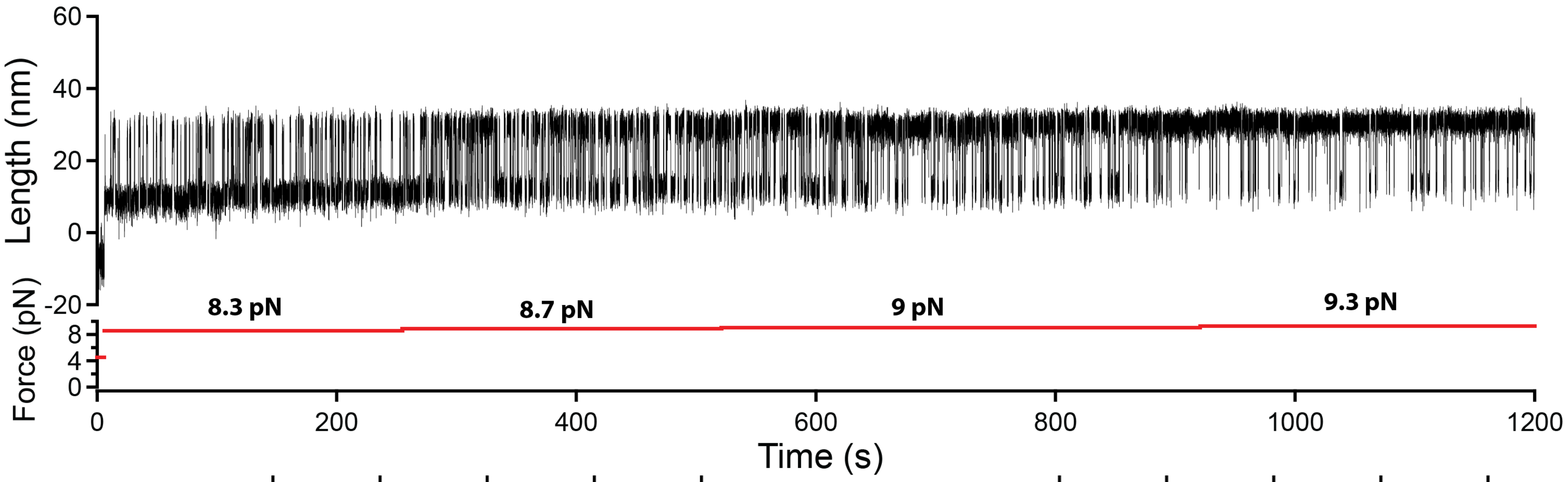




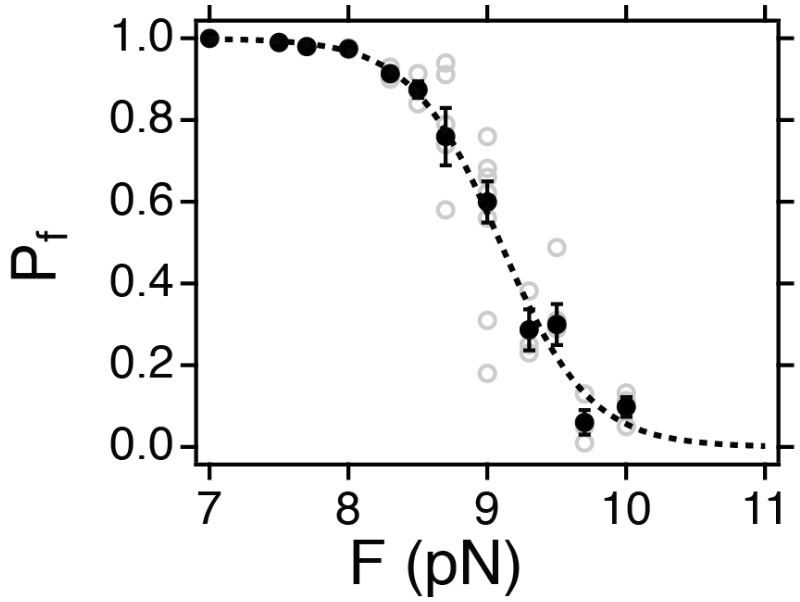
MT_3 has the resolution of an electron microscope at >1400

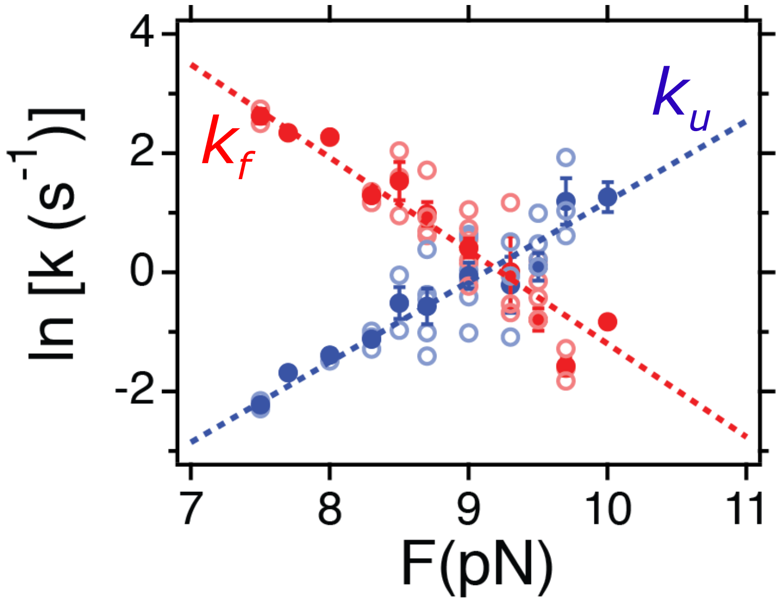


Measuring Talin R3 (IVVI) dynamics with MT3



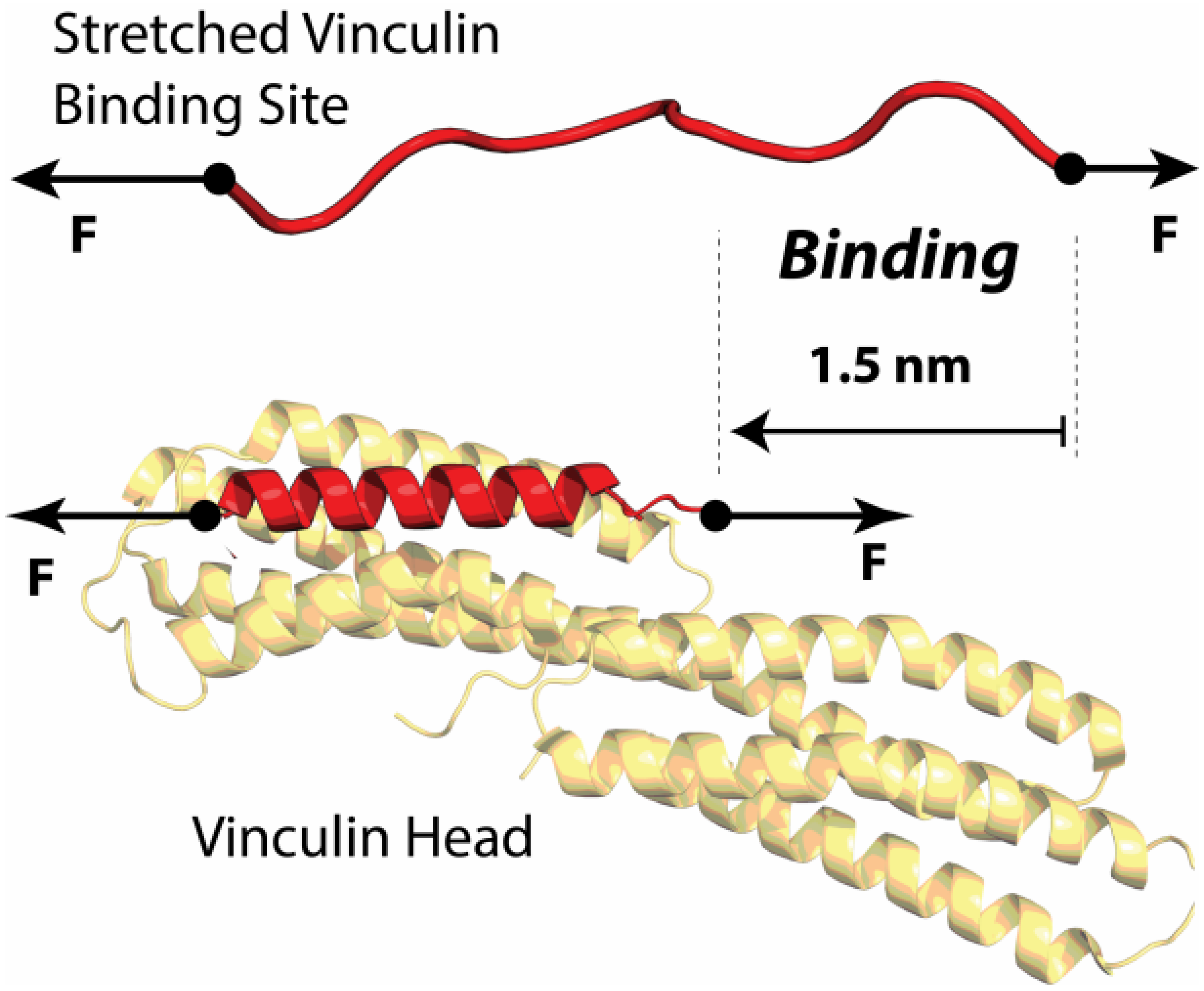
Rafael Tapia-Rojo



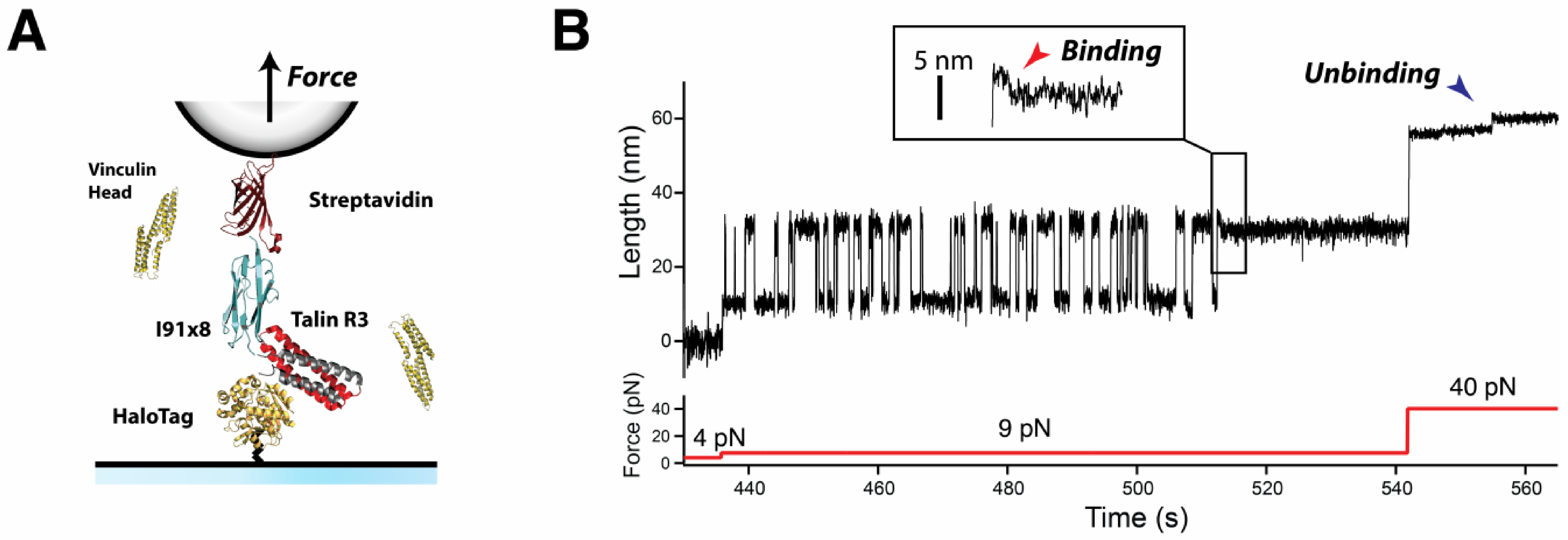


Alvaro Alonso-Caballero

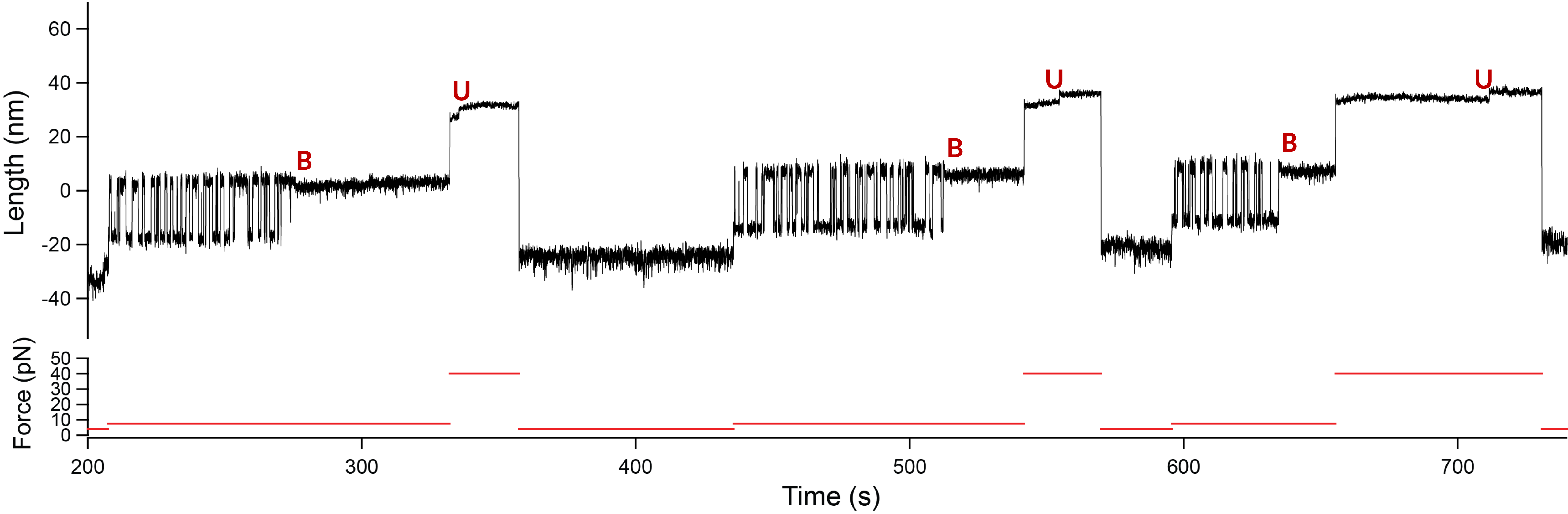
Vinculin binding to talin does mechanical work



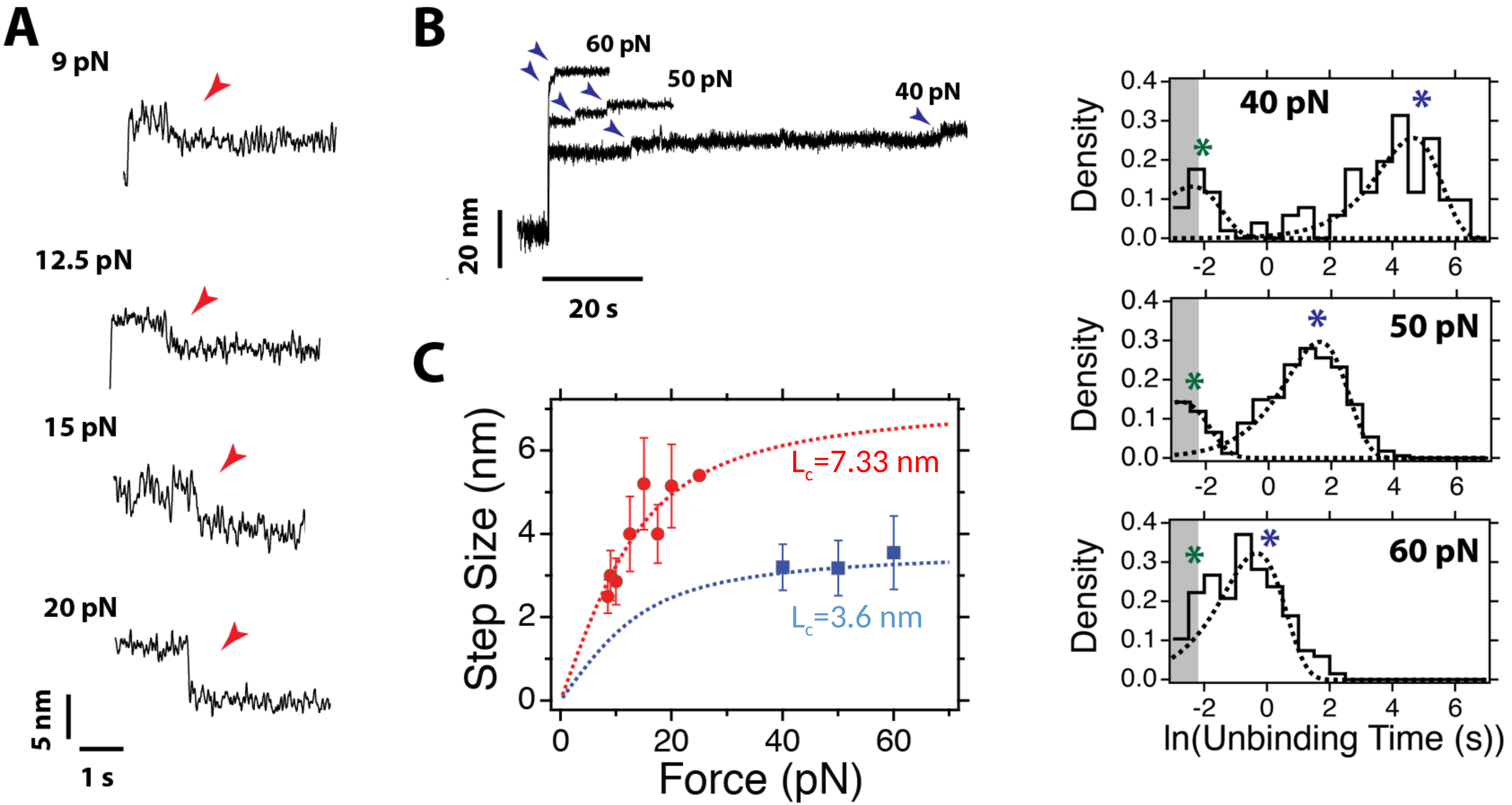
Vinculin binding to talin does mechanical work



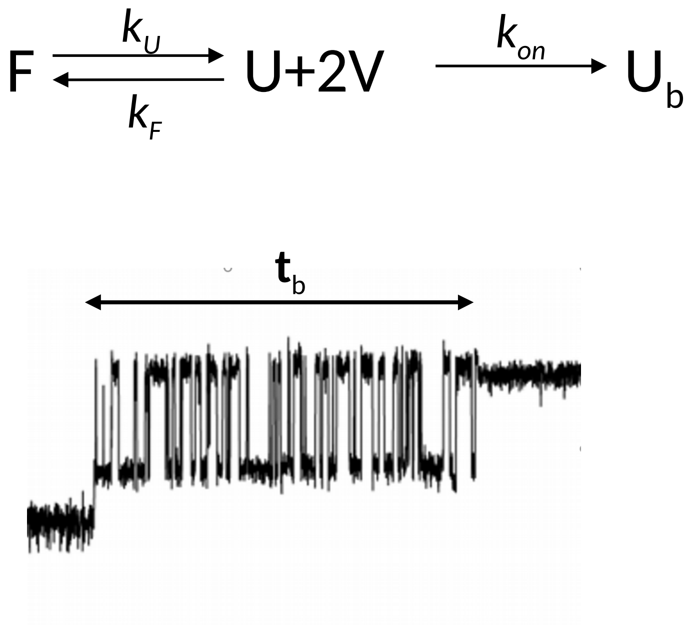
Vinculin binding/unbinding can be cycled repeatedly

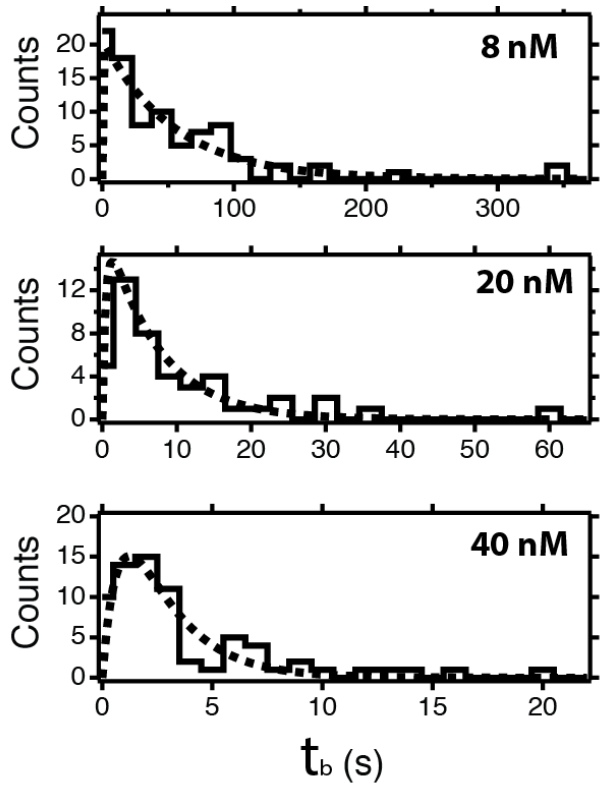


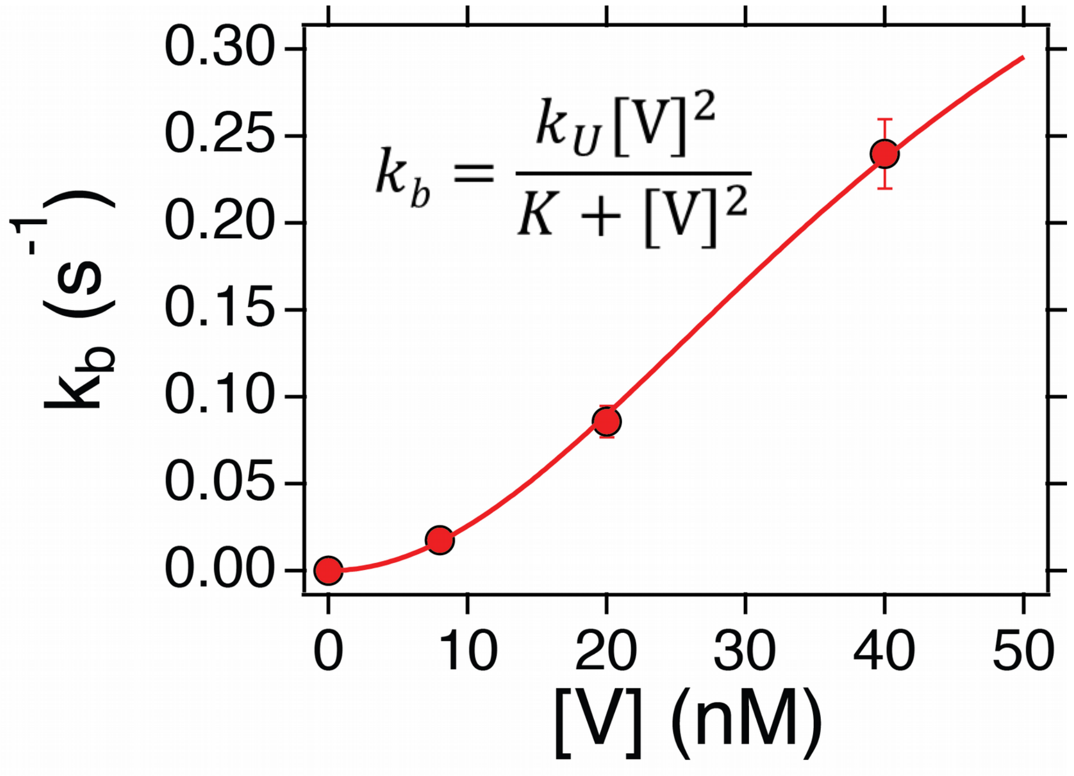
Two vinculin molecules bind simultaneously,
they unbind separately, one very fast, the second more slowly.



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

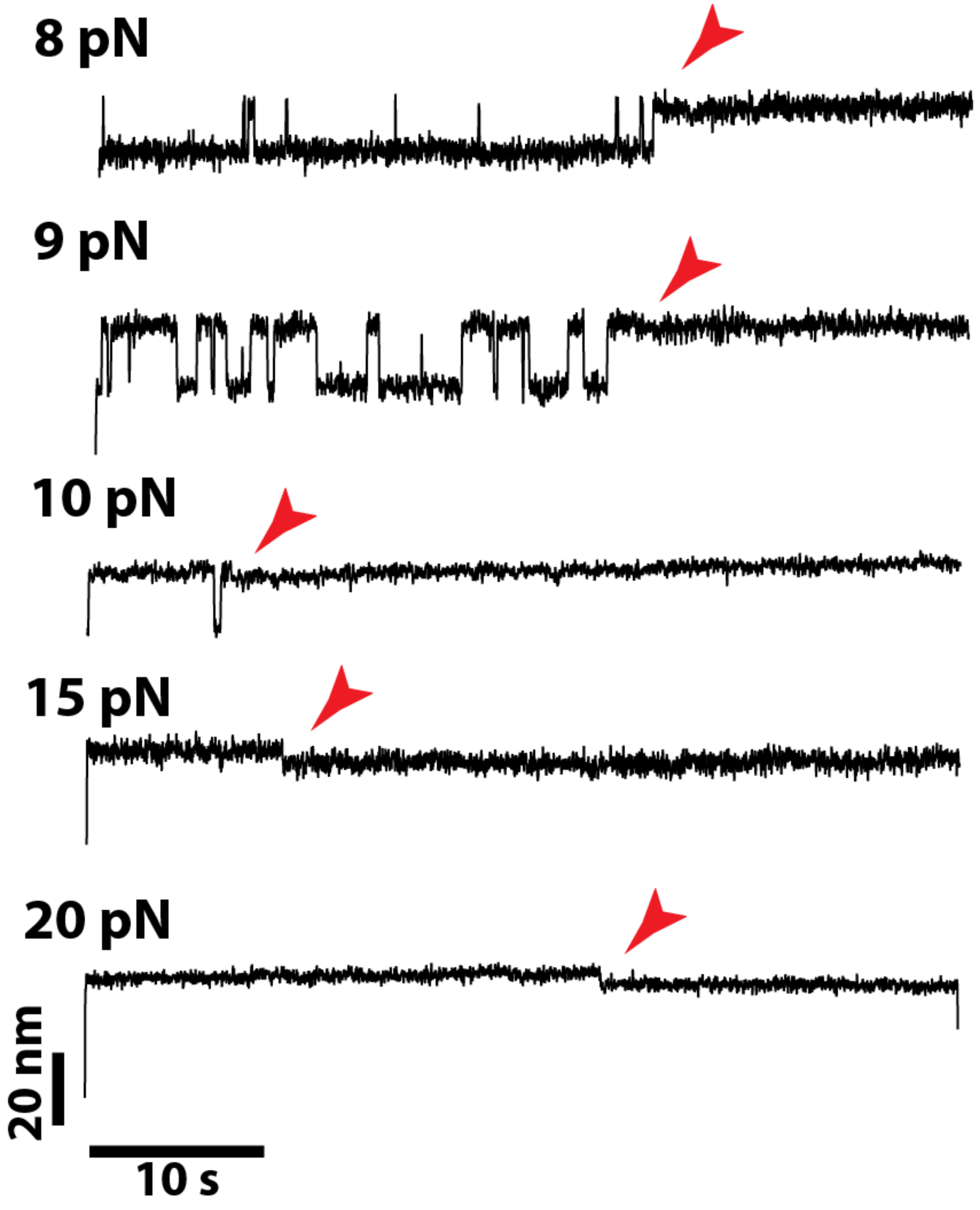






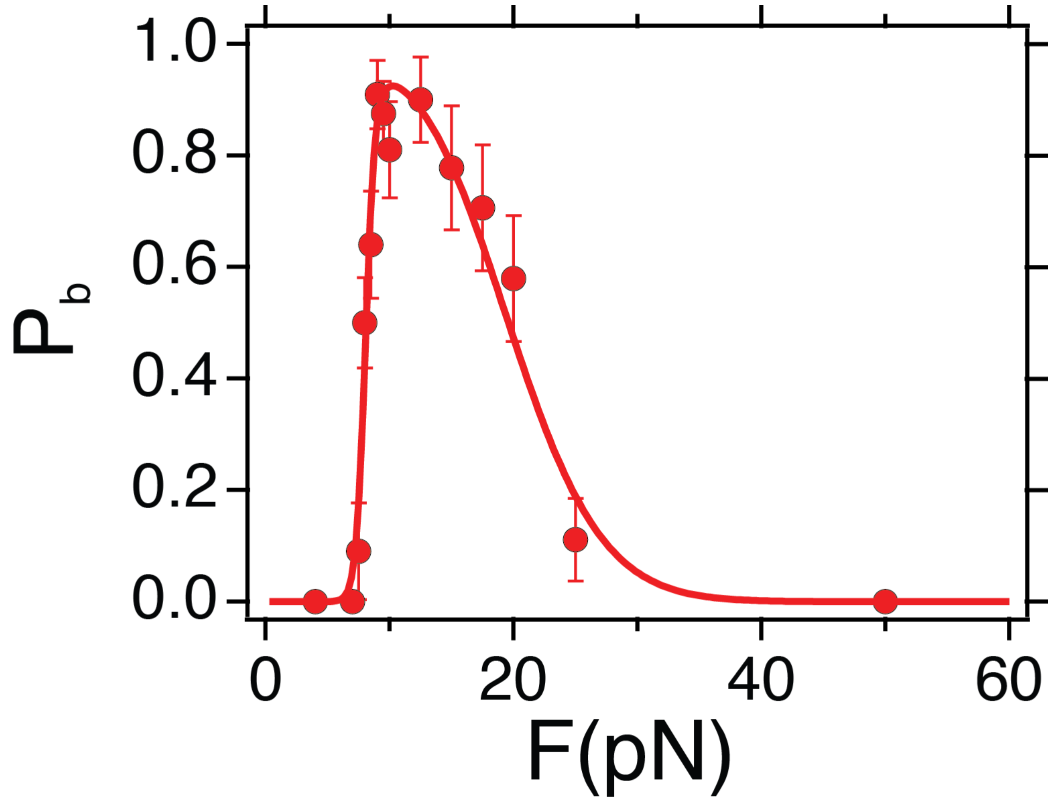
$$k_b = \frac{k_U [V]^2}{K + [V]^2}$$

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

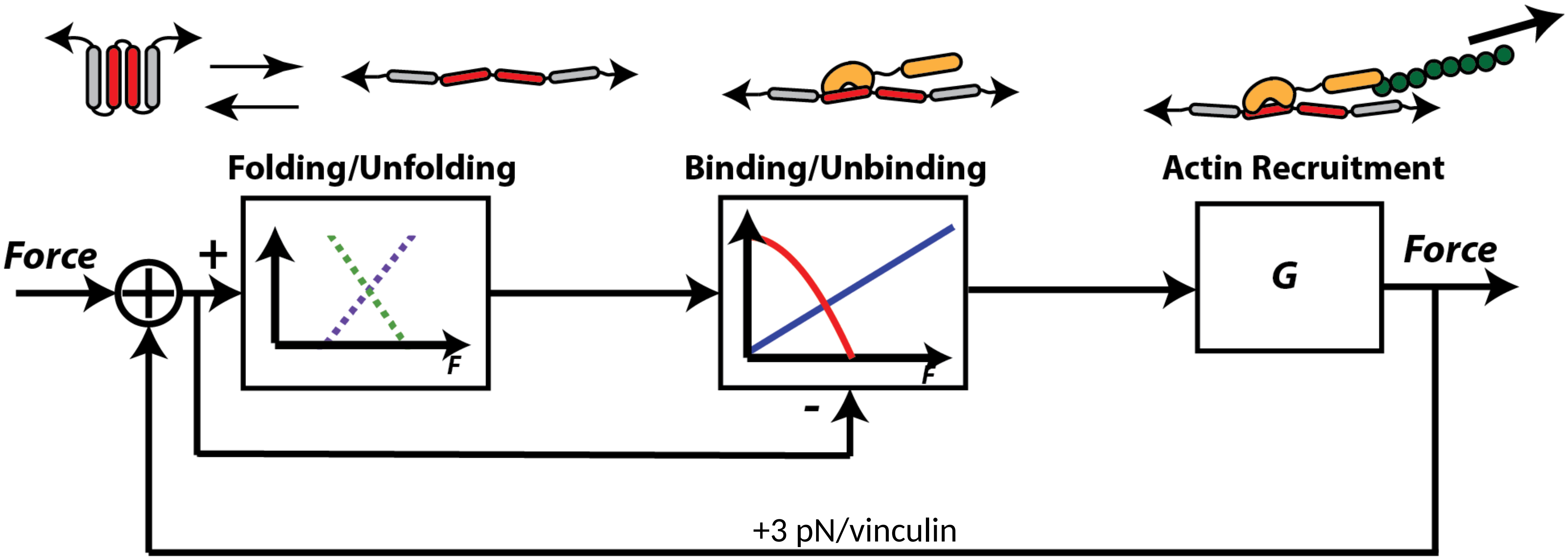


$$P_b = 1 - exp\left[-\frac{k_u k_{on} t}{k_u + k_F + k_{on}}\right]$$

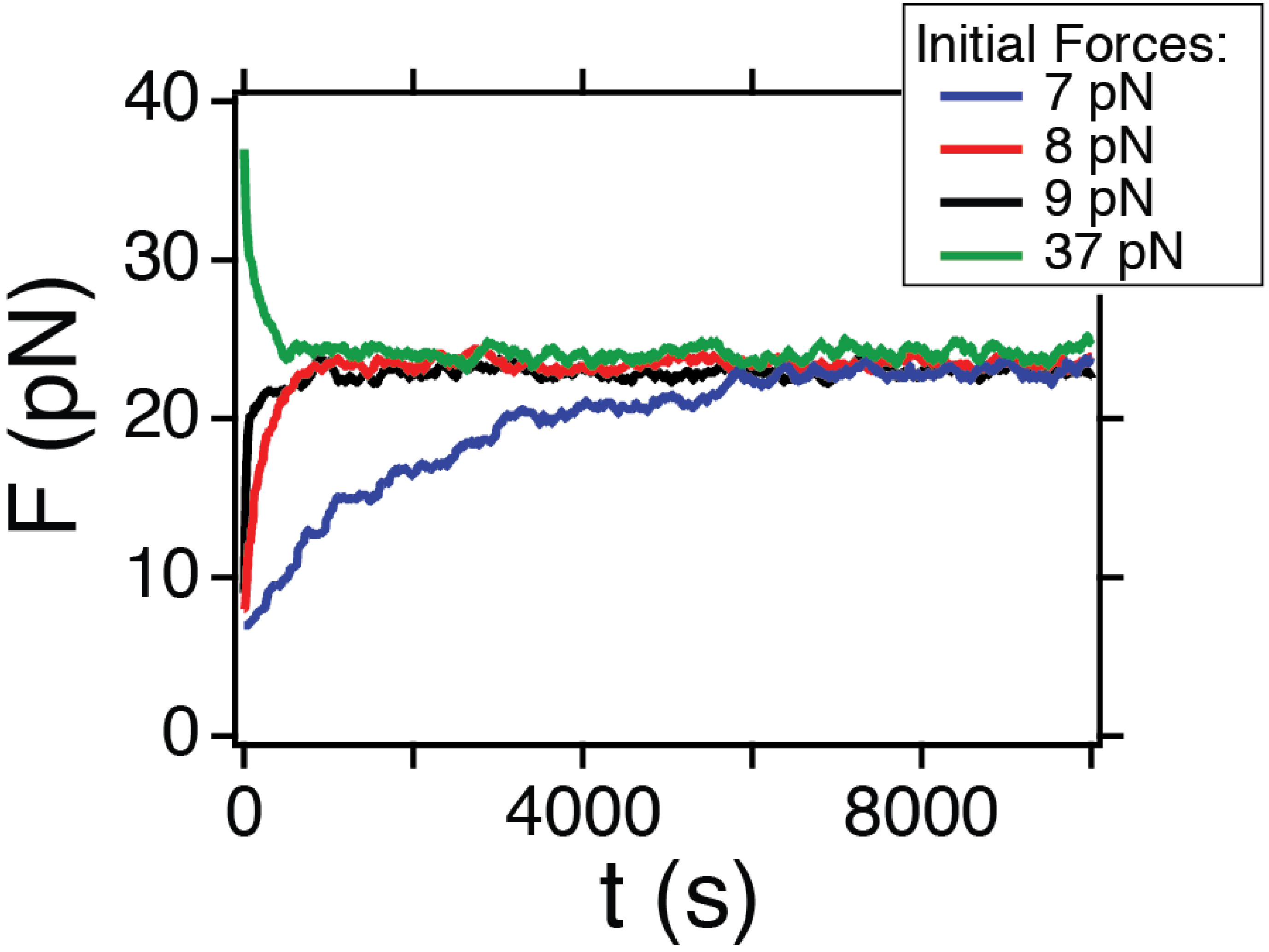
$$k_{on} = [V]^2 A e^{-\Delta W_b/kT} ;\ \Delta W_b = F\Delta L$$



Talin-vinculin mechanical control system

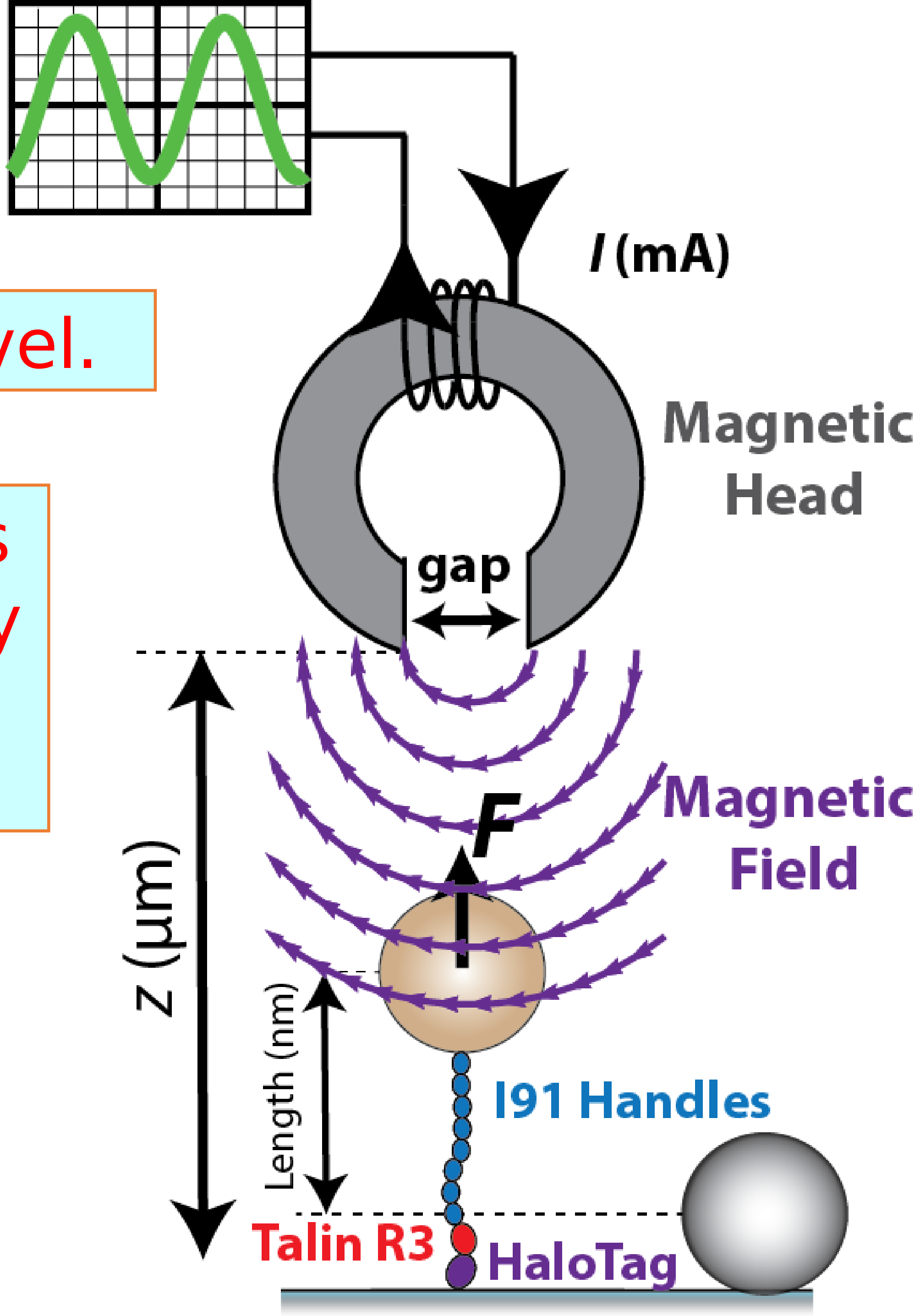


Monte-Carlo simulation of the talin-vinculin control system predicts a negative-feedback equilibrium at 23 pN

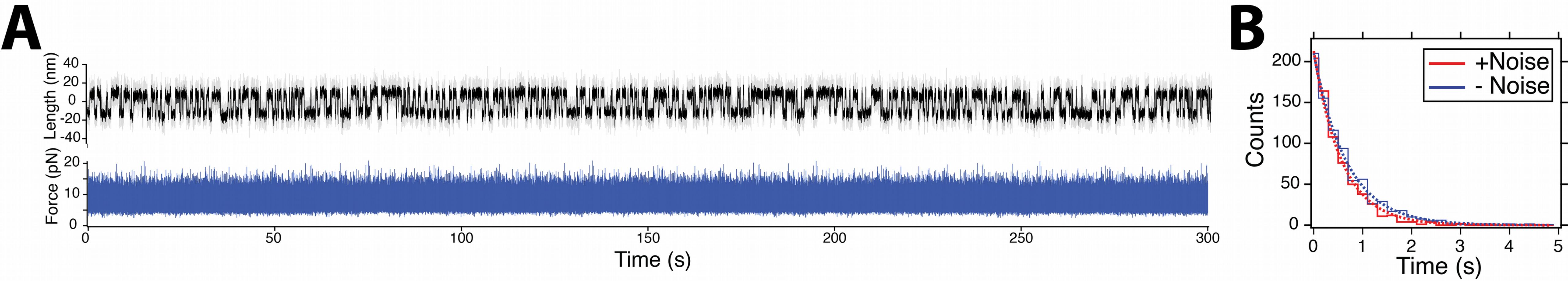


MT_3: the next level.

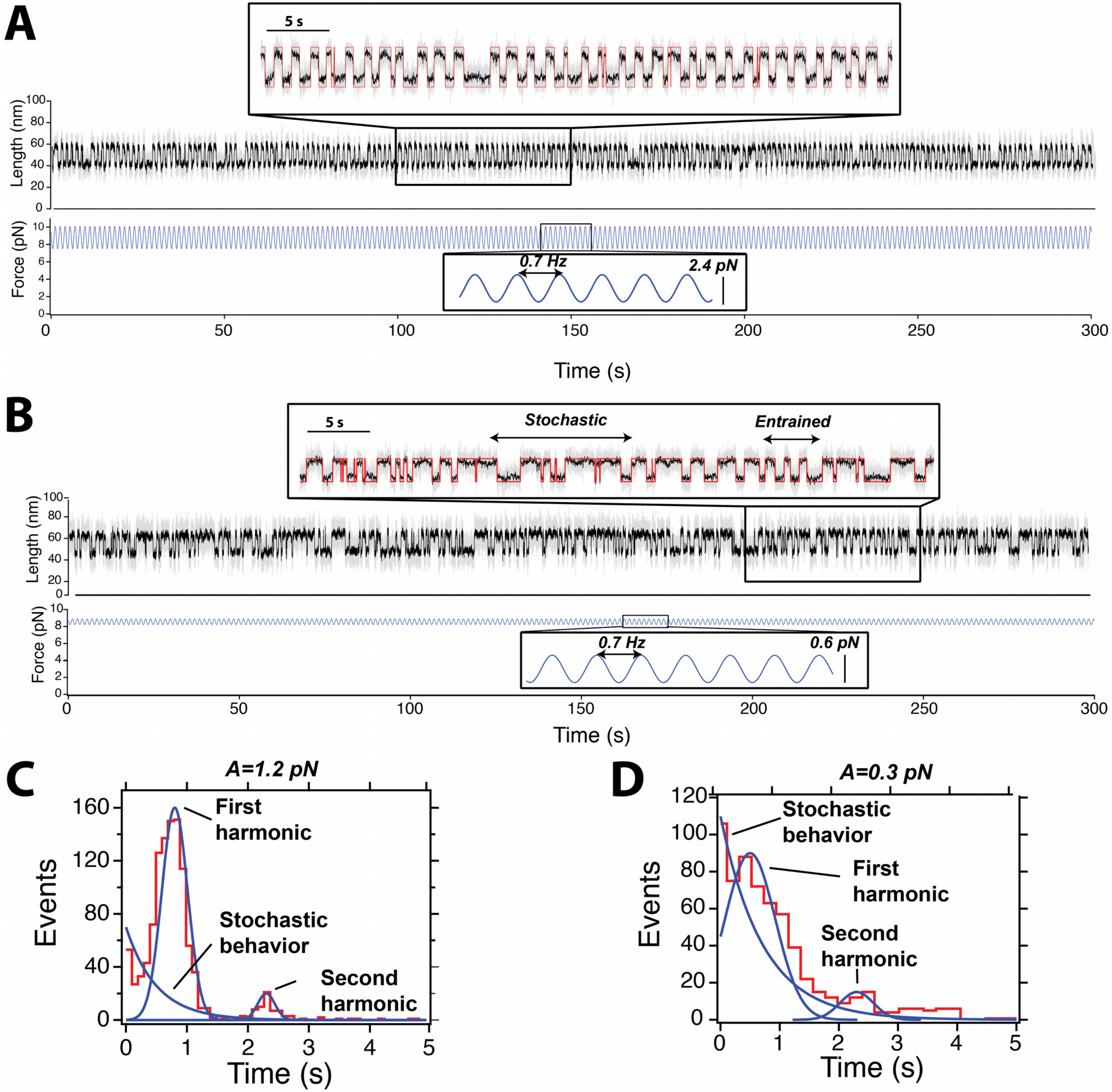
Mechanical signals in biology are noisy and contain periodic signals



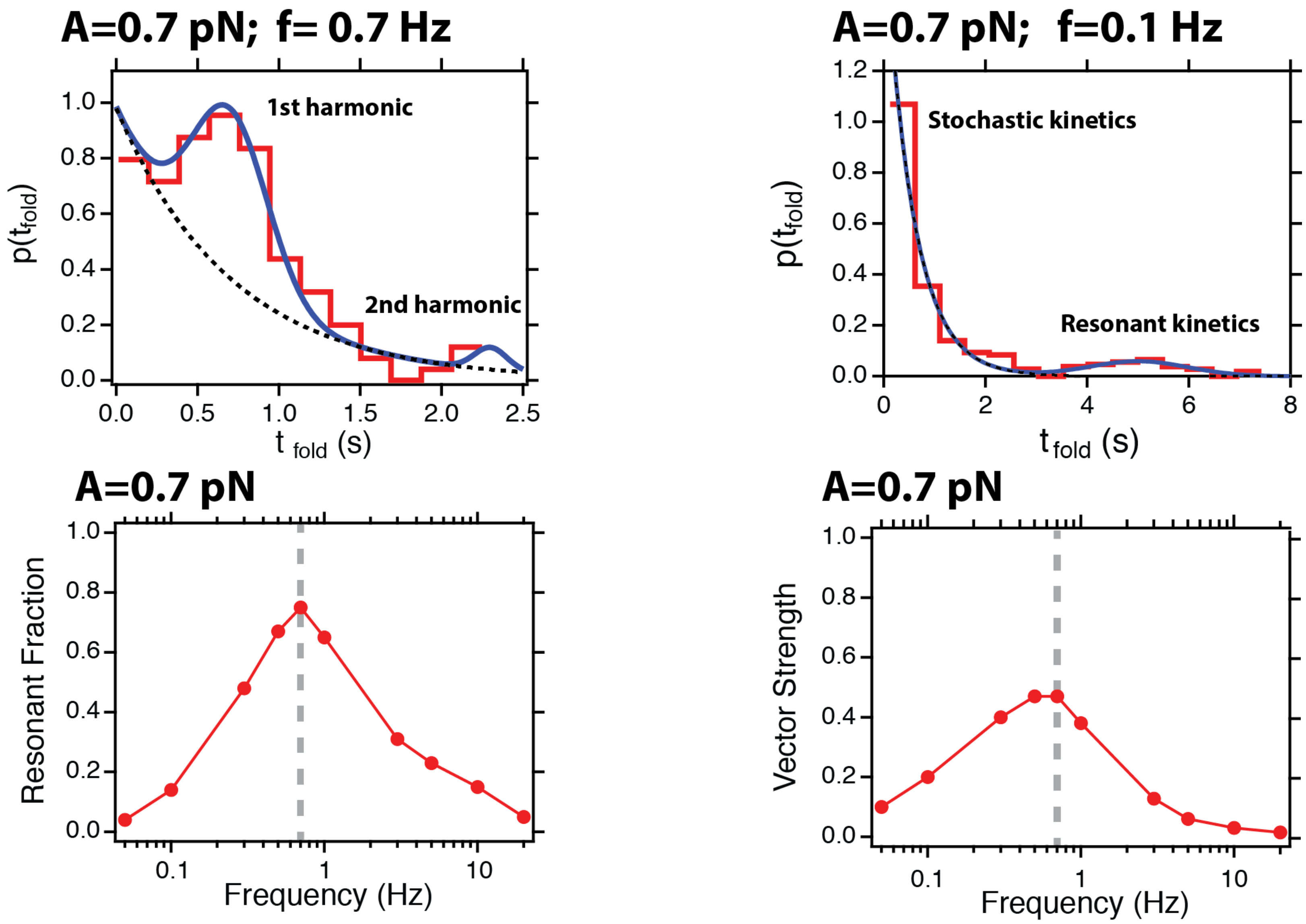
Talin rejects mechanical noise



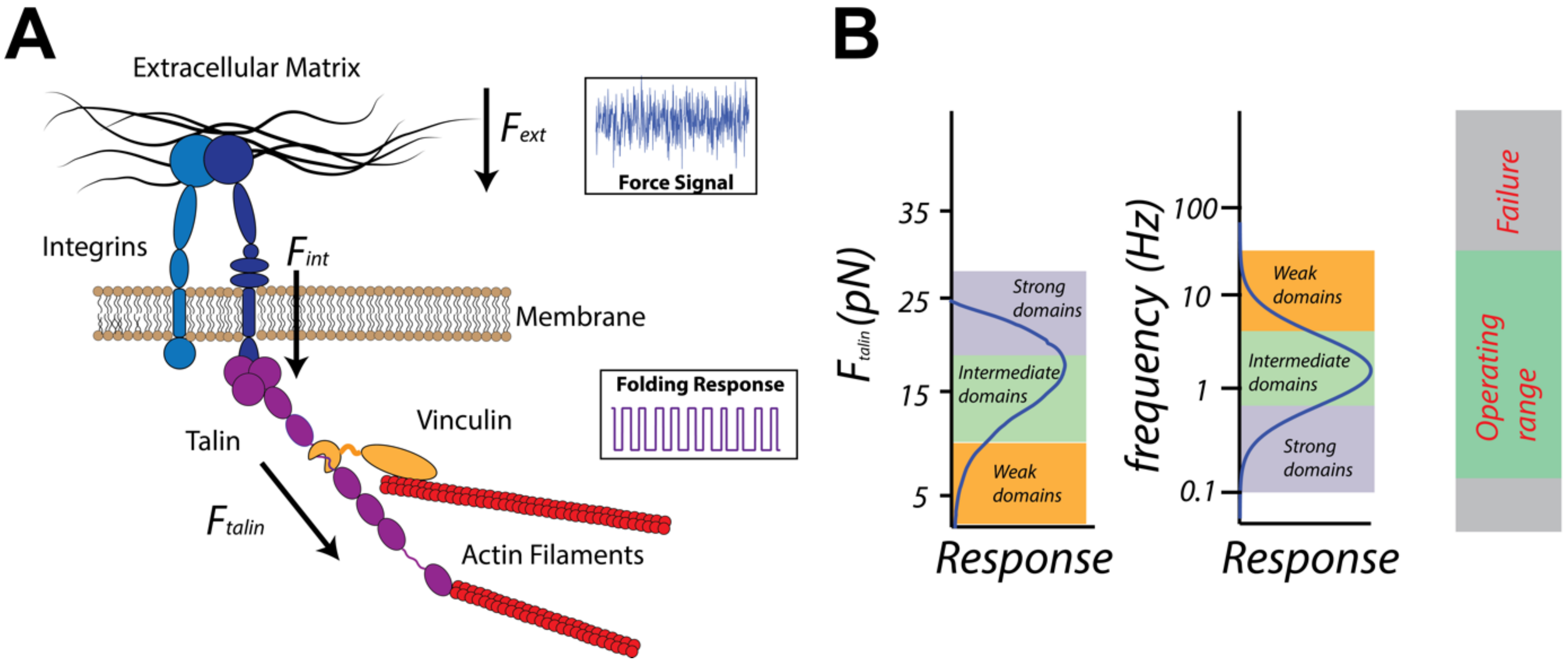
Talin entrains with periodic signals



Entrainment is frequency dependent



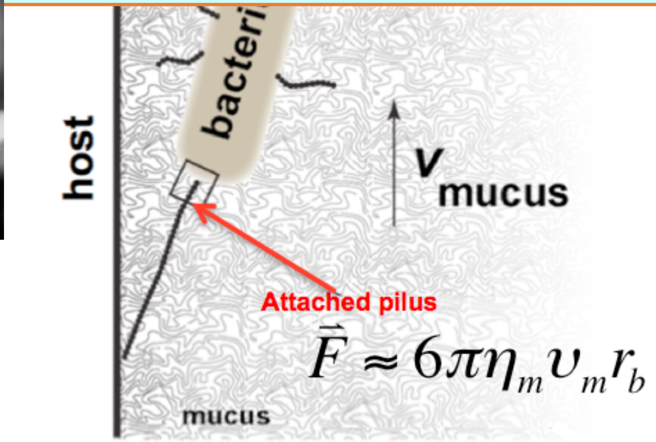
Stochastic resonance identifies periodic signals in noisy mechanical environments

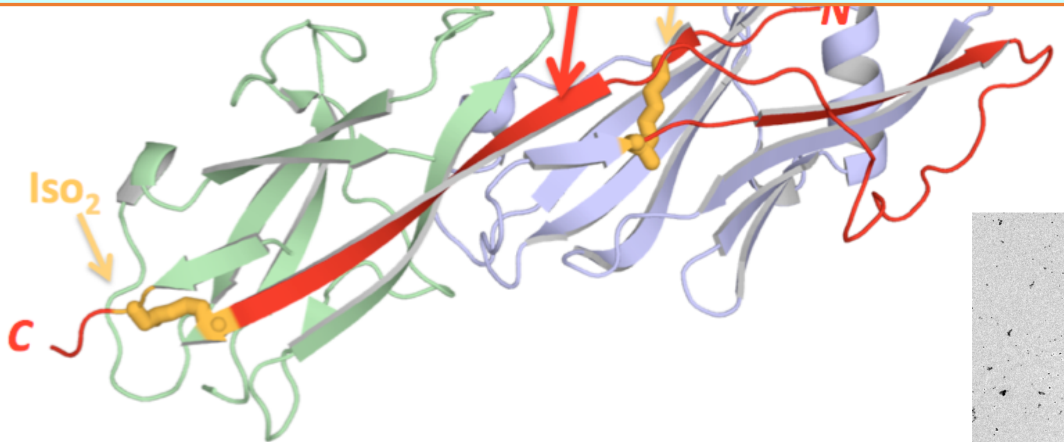


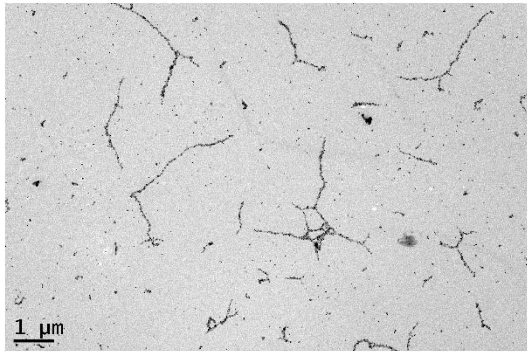
heart beat?, respiration?, rigidity sensing, cancer?

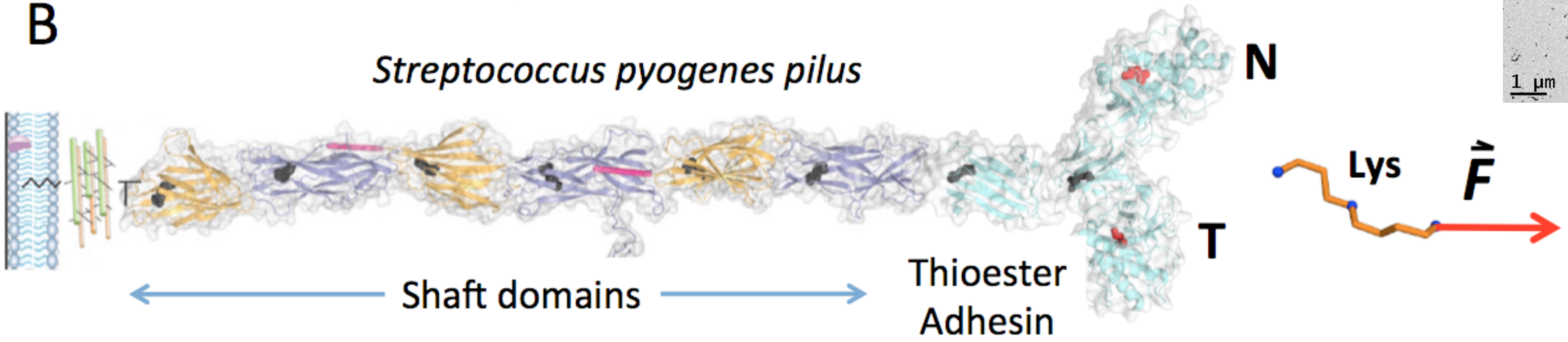
Gram-positive pili are the largest single polypeptide proteins known.
They have specialized features to resist large mechanical shocks!

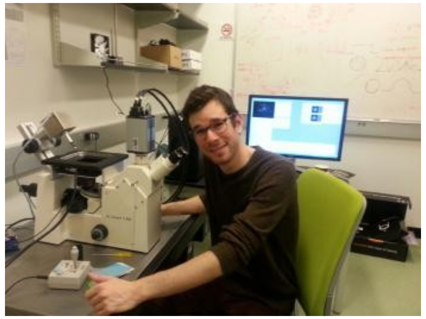
Jorge Alegre-Cebollada









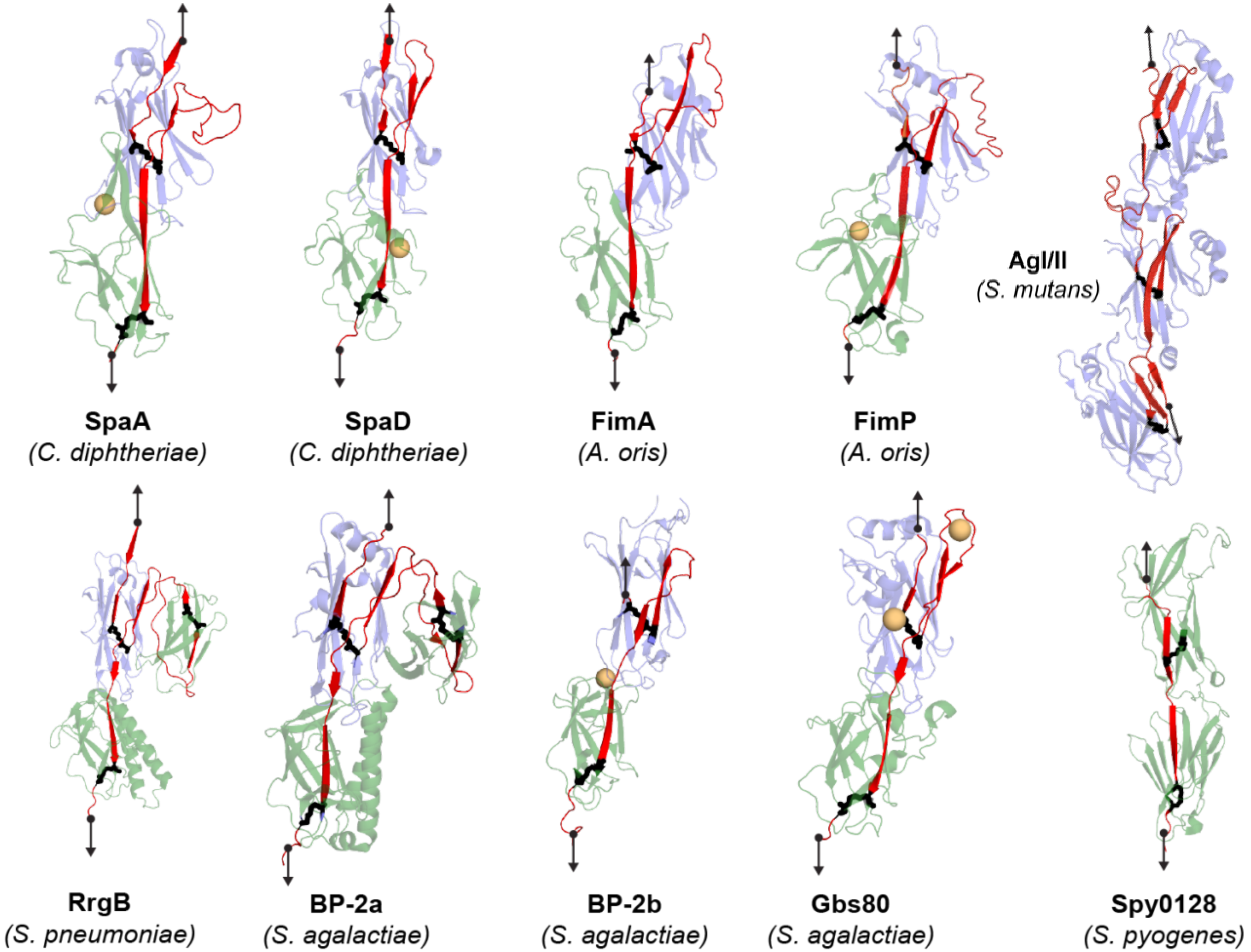


Daniel Eschelman
MD/PhD (2018)

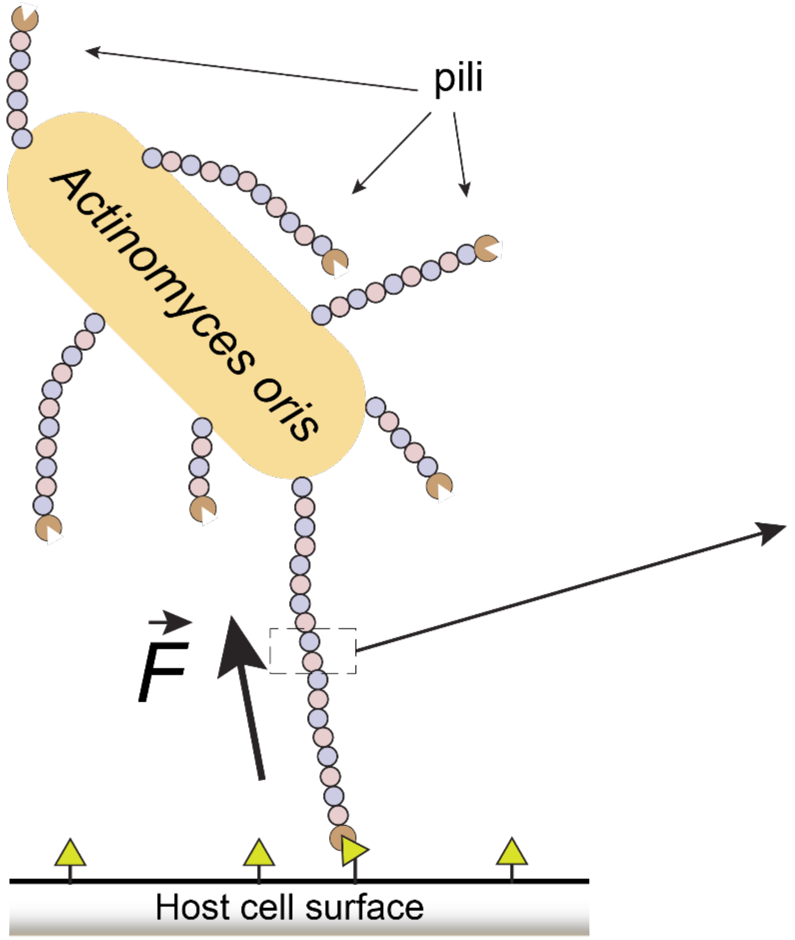
Alegre-Cebollada et al., 2010, **JBC,** 285:11235-11242

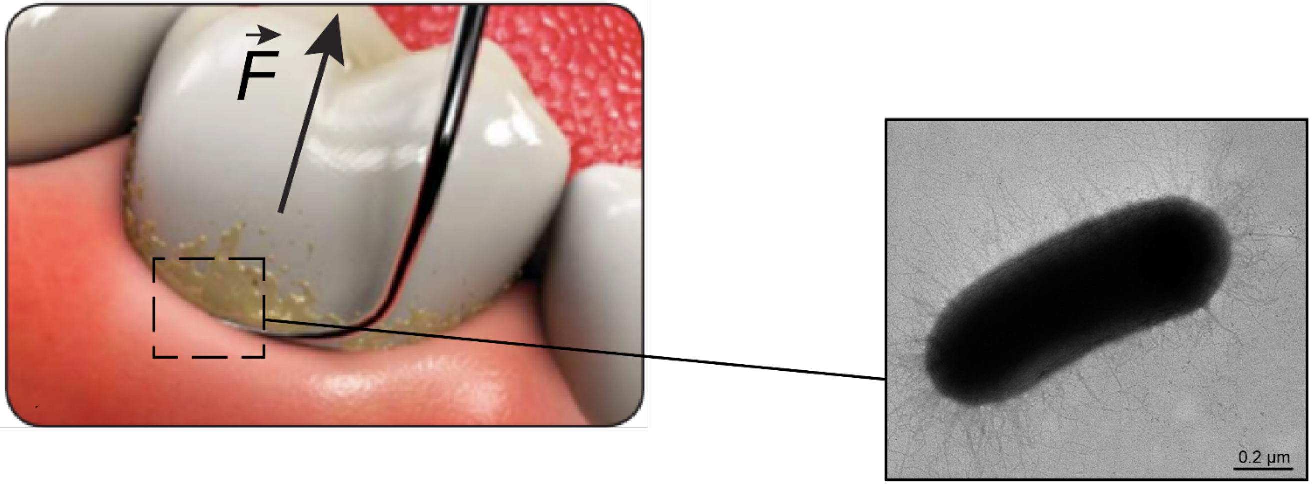
Echelman et al., 2016, **PNAS,** 113:2490-2495

IDL's are common

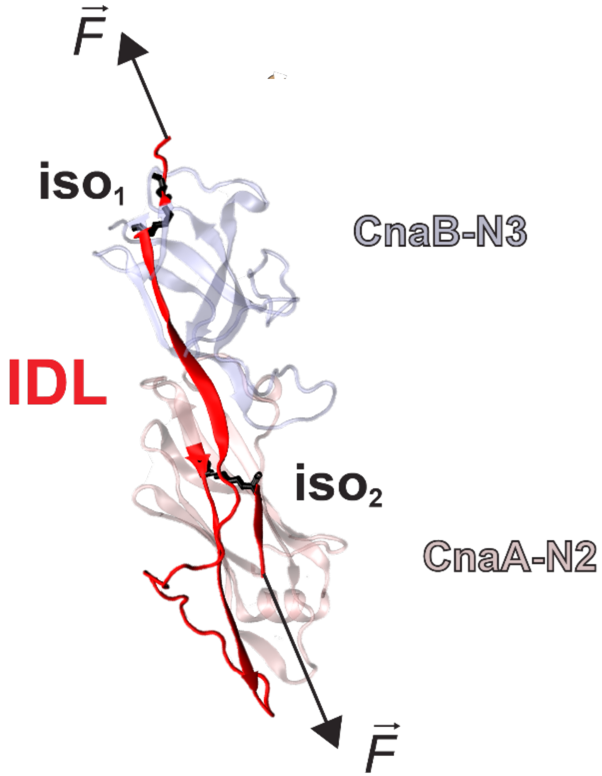


FimA





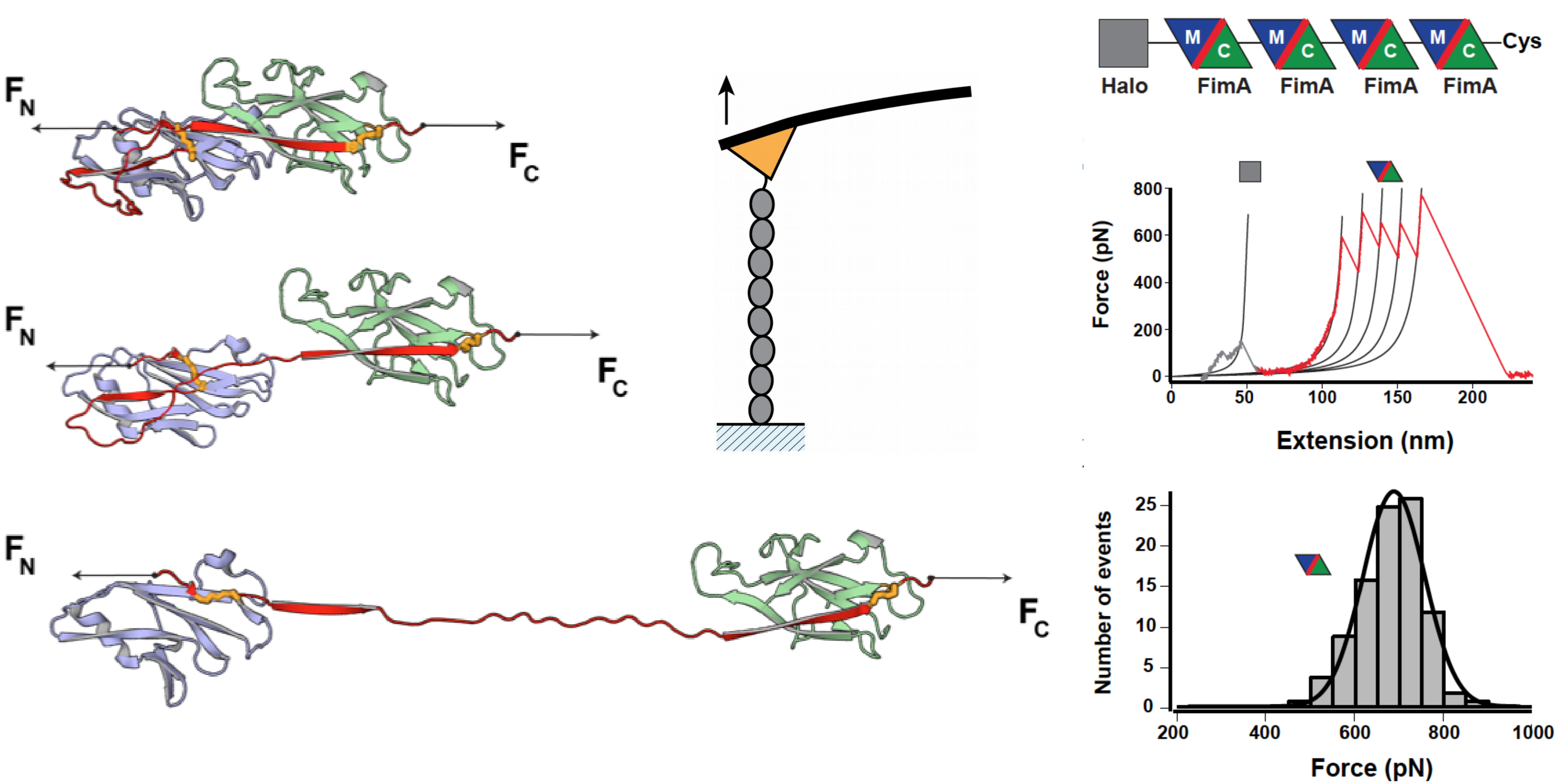
Mishra, A. *et al.* DOI:10.1128/JB.01952-06.



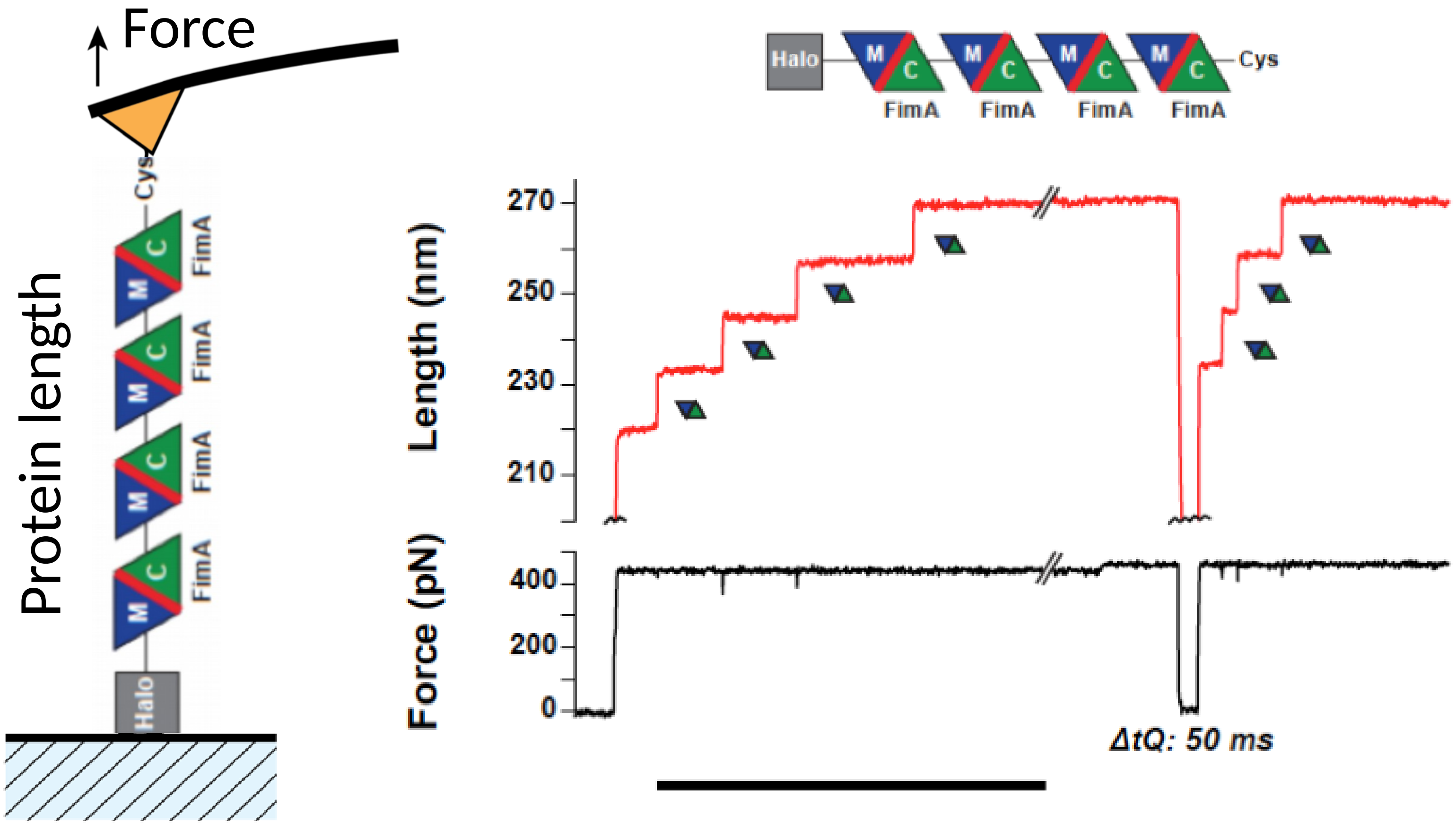
FimA

Dr. Alvaro Alonso

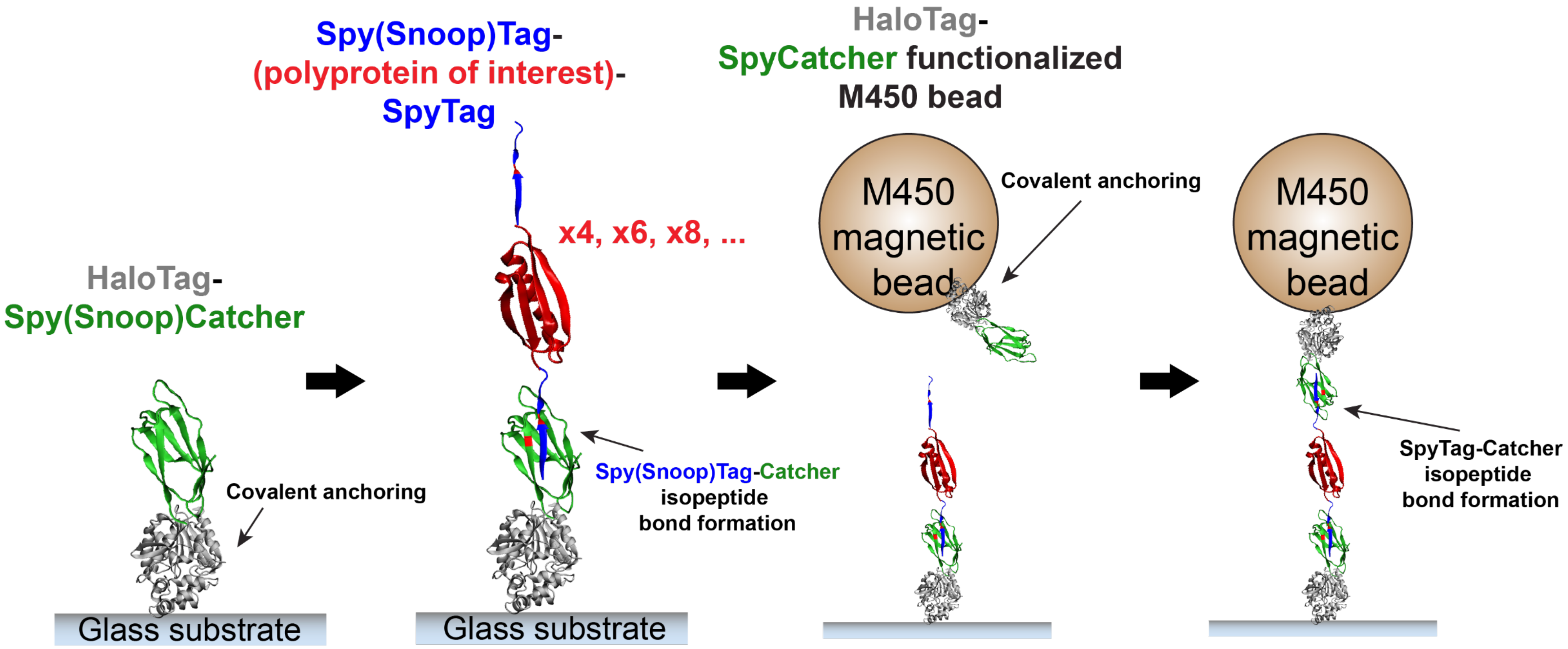
The FimA IDL's require a large force to unfold (~700 pN)



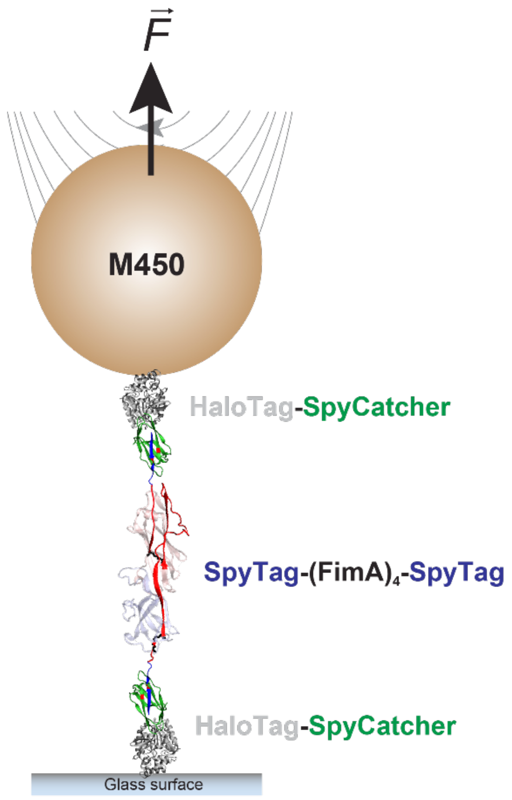
FimA refolding requires that the force drops <10 pN

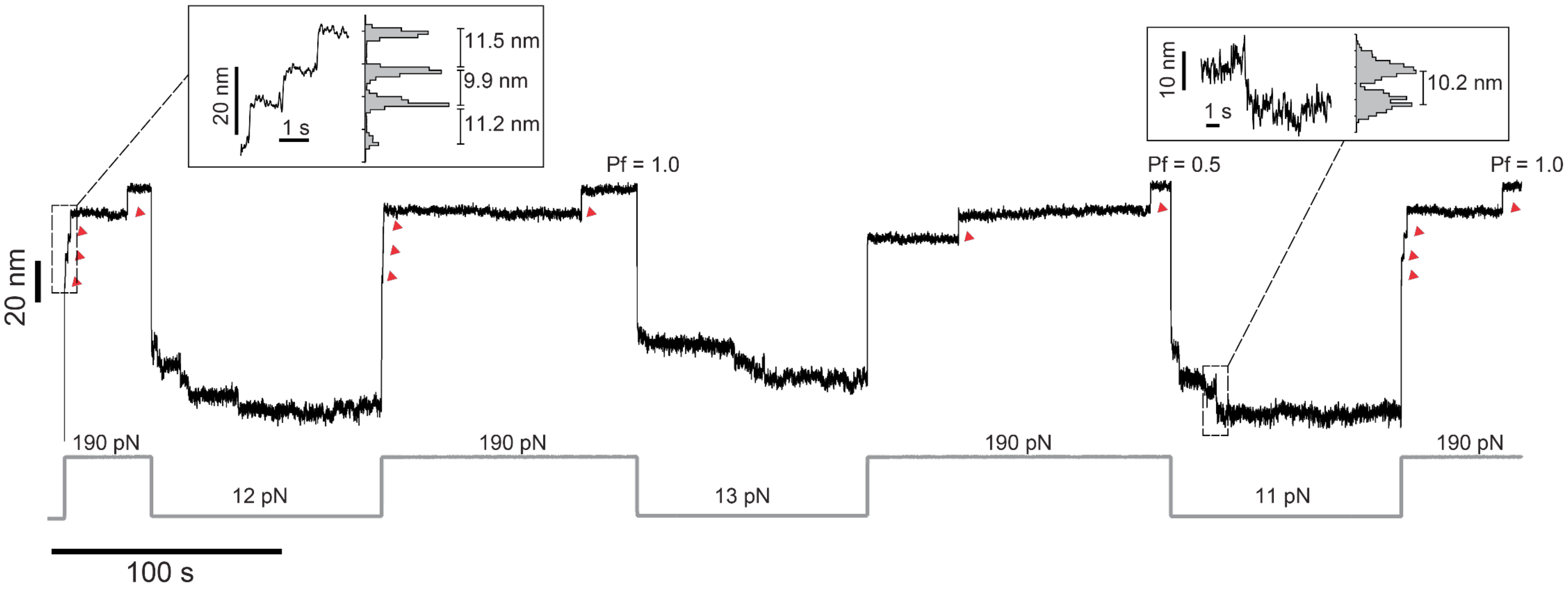


Double-covalent and split-protein technique



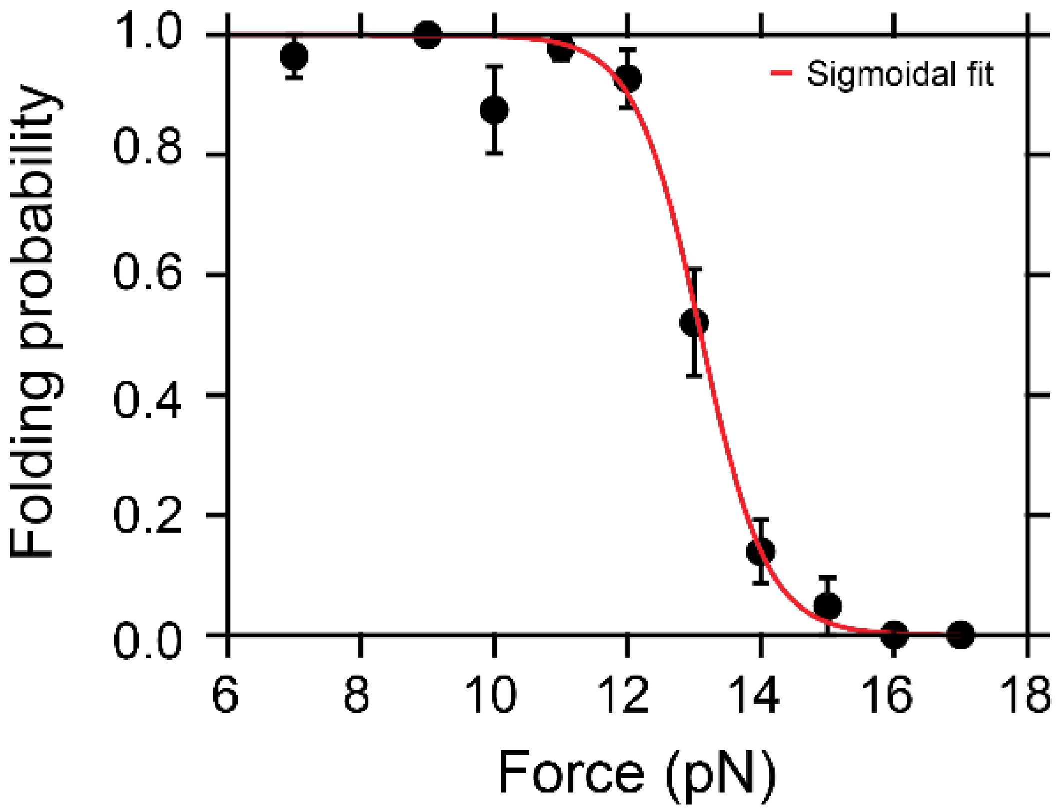
FimA IDL's, Not-folding, Not-unfolding (P425)



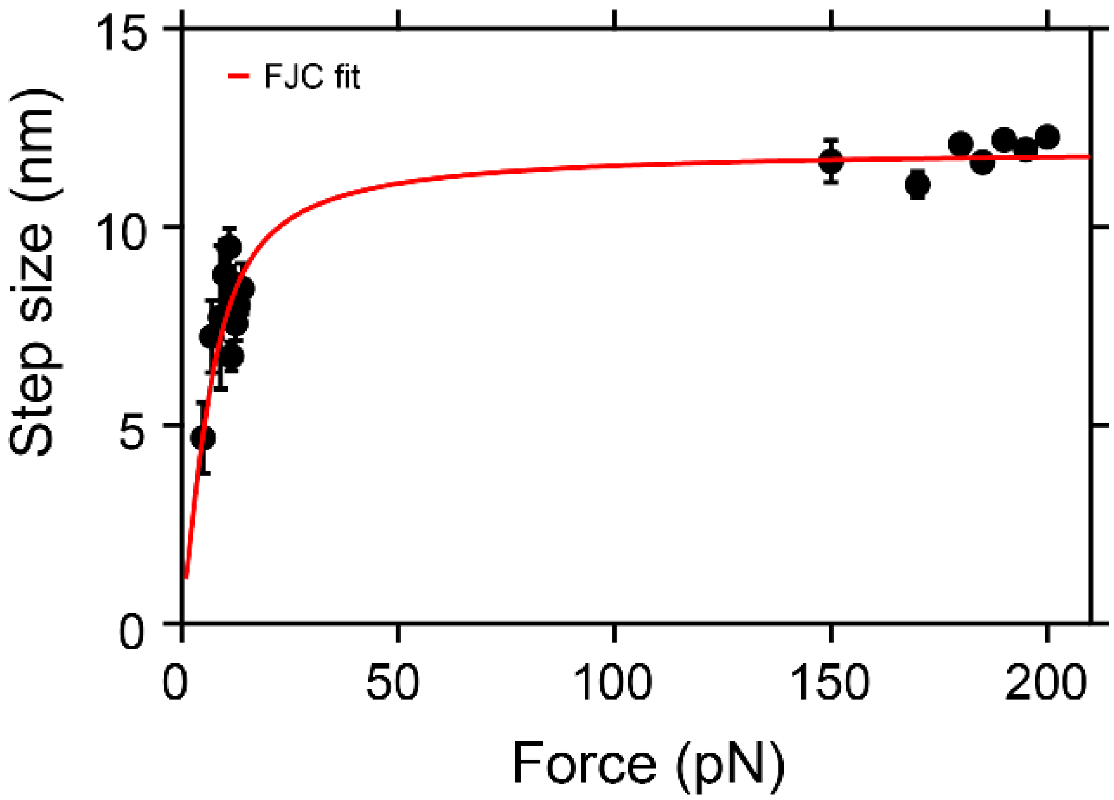


FimA

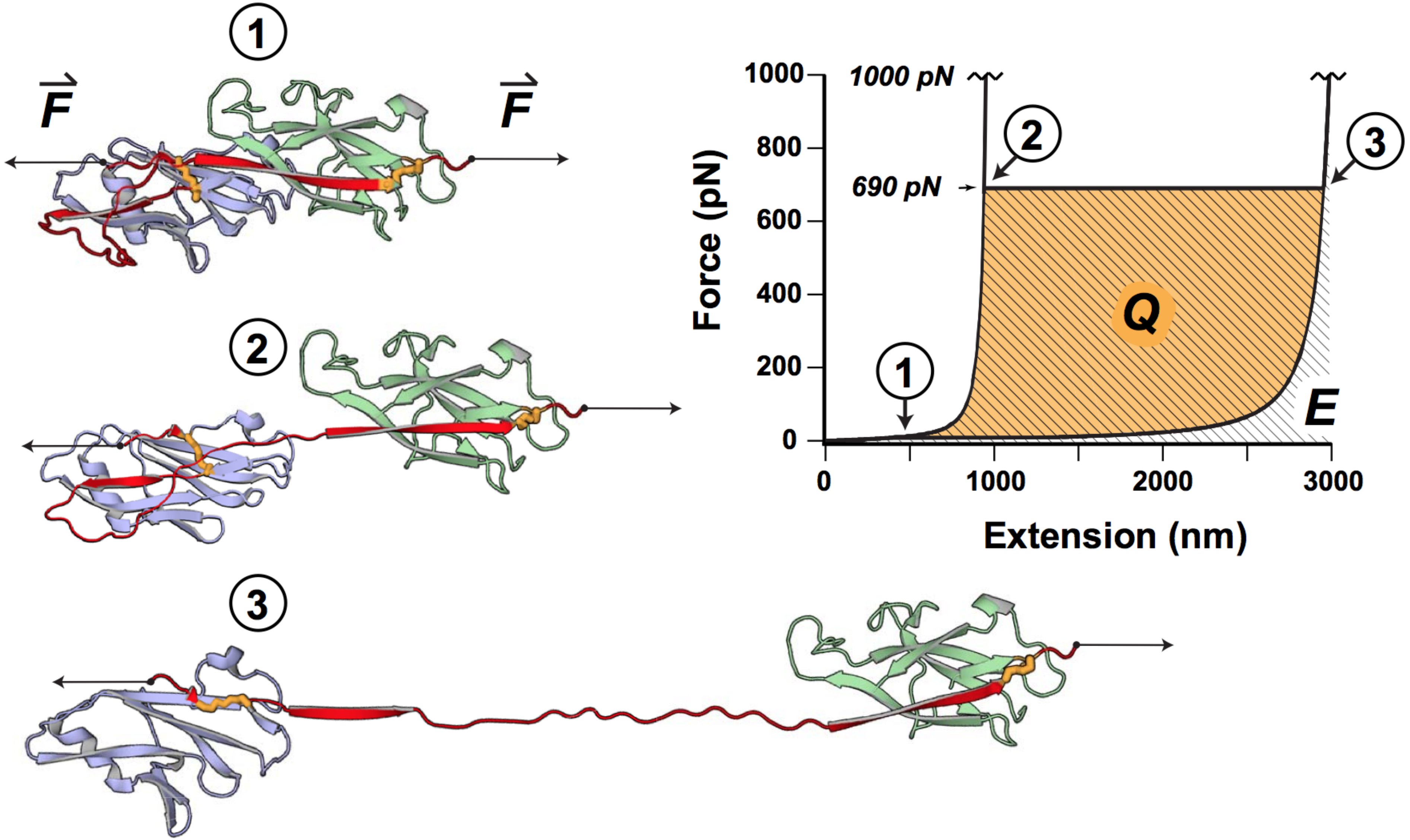
IDL Folding contraction probability



IDL extension as a function of force

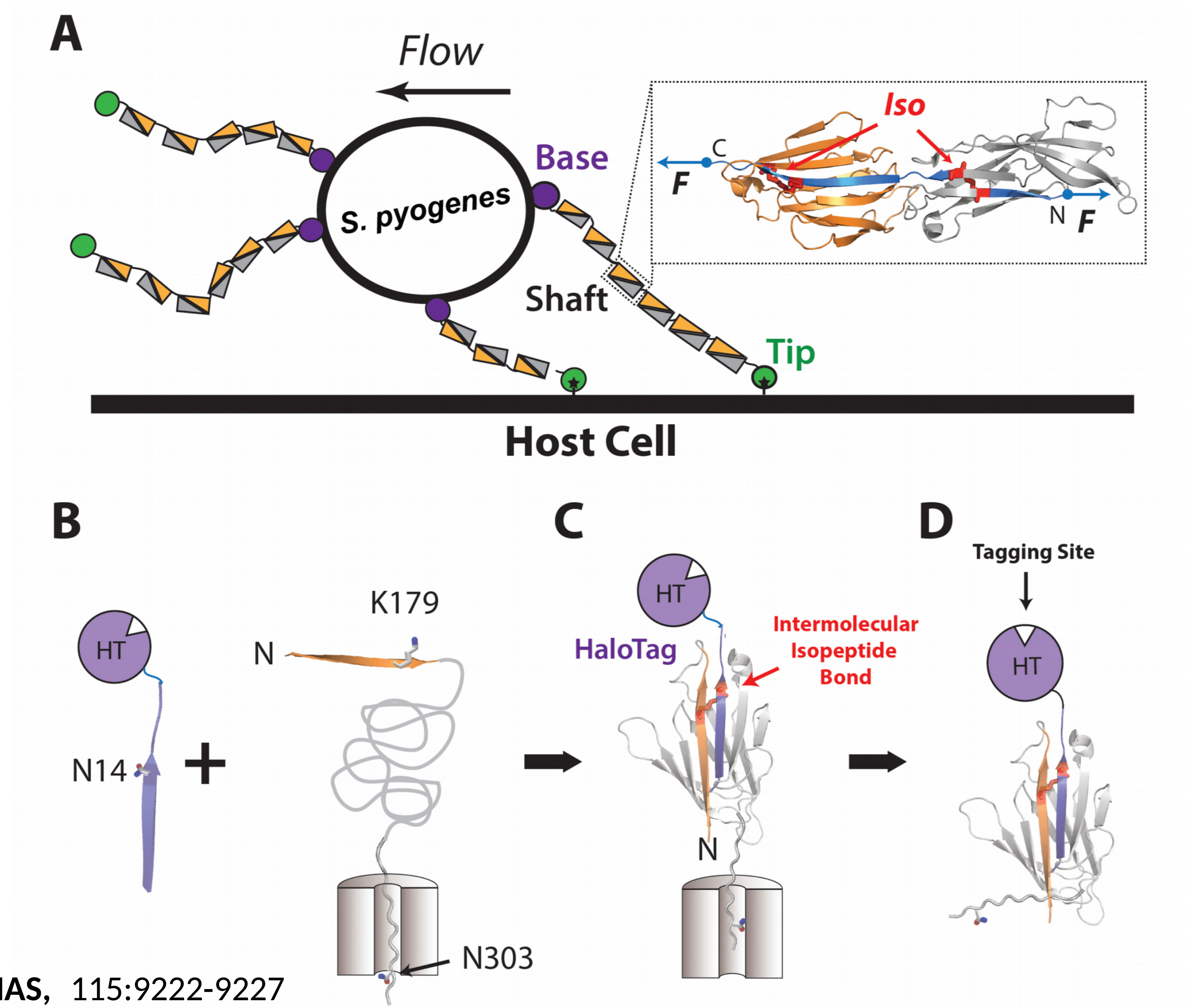


IDL's are very effective shock dissipaters



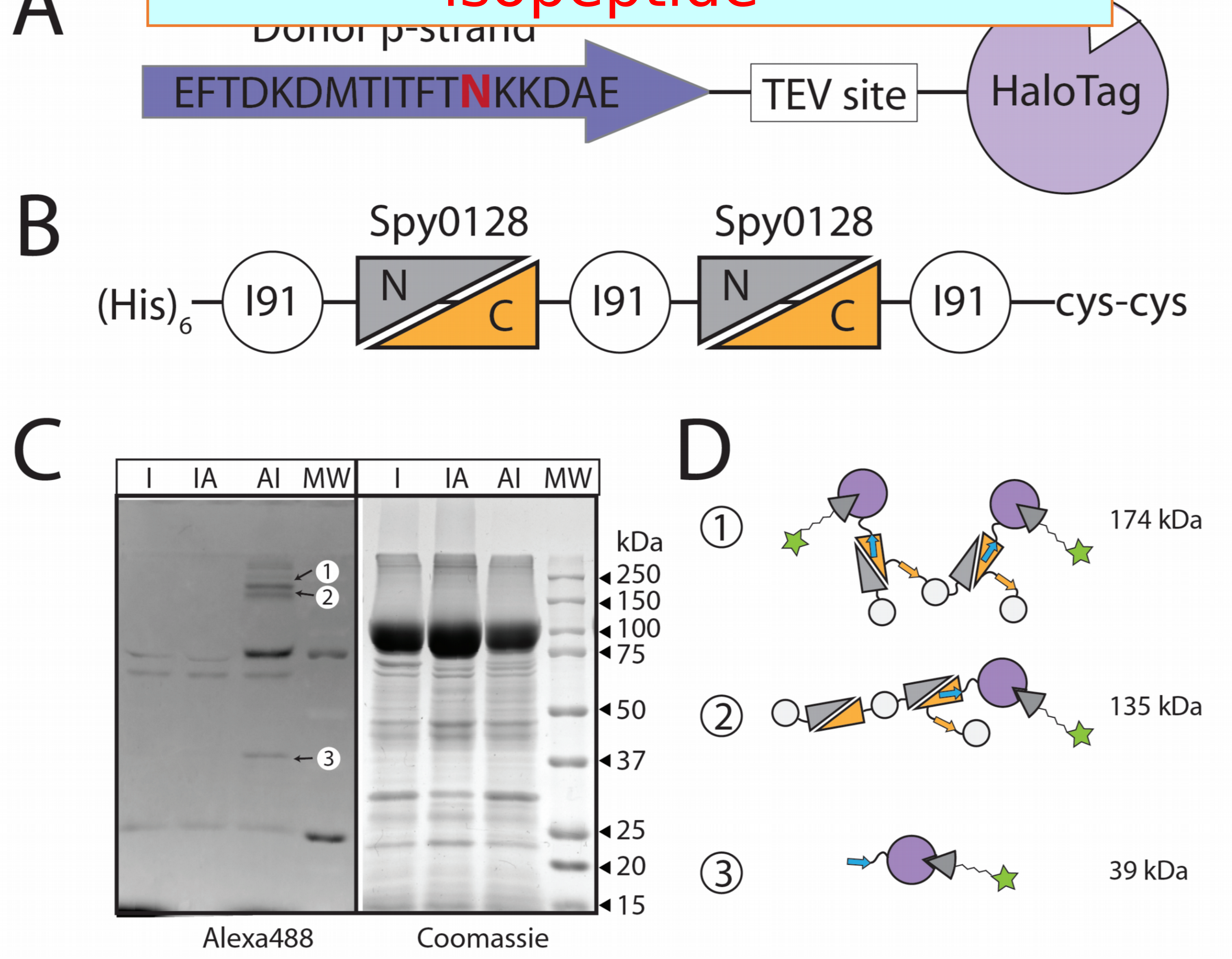
IDL folding = inter-domain polymer collapse and binding

Blocking isopeptide bond formation in pili

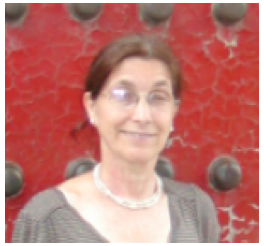


Rivas-Pardo et al., 2018, **PNAS,** 115:9222-9227

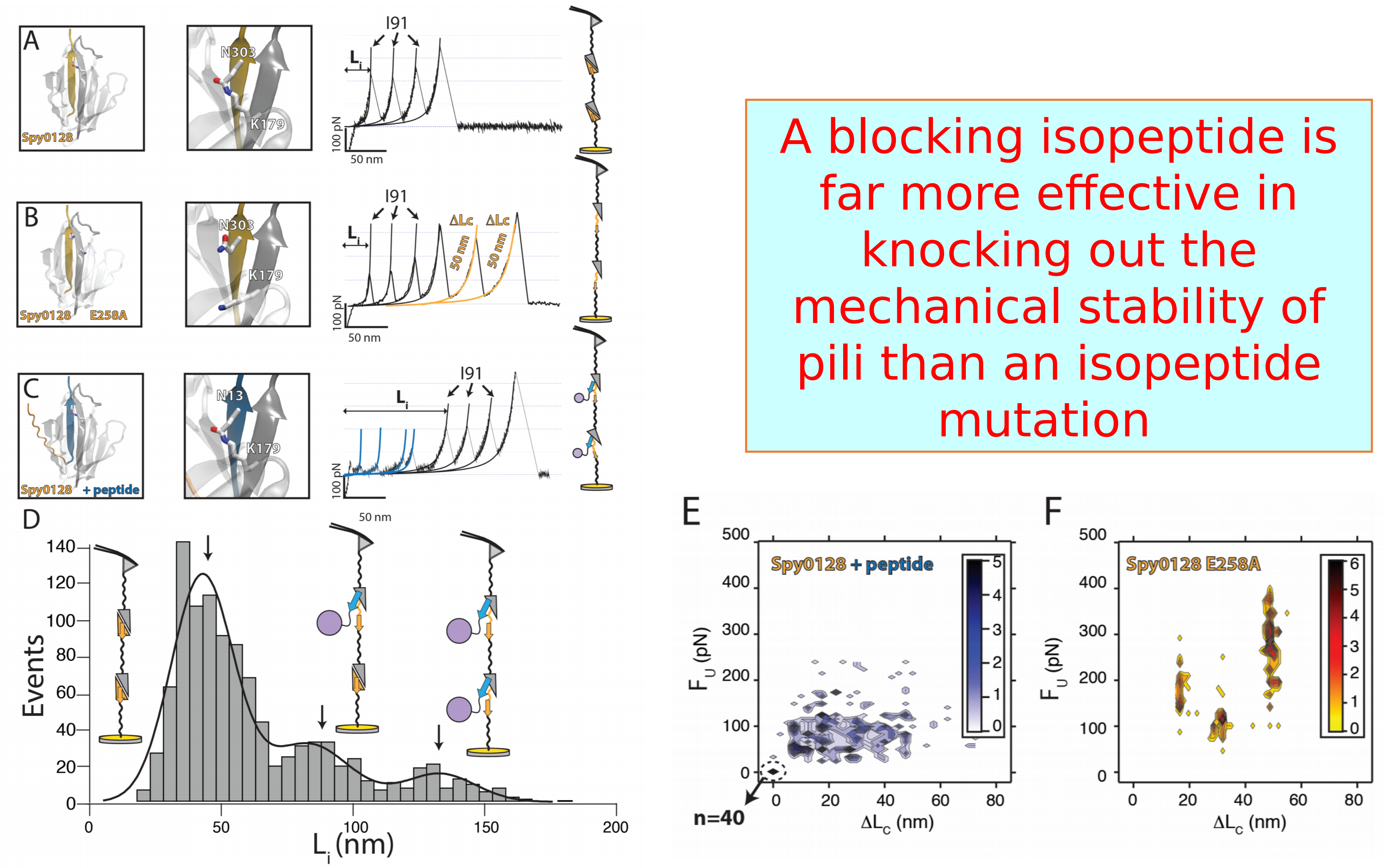
design of a blocking isopeptide



Andrés Rivas

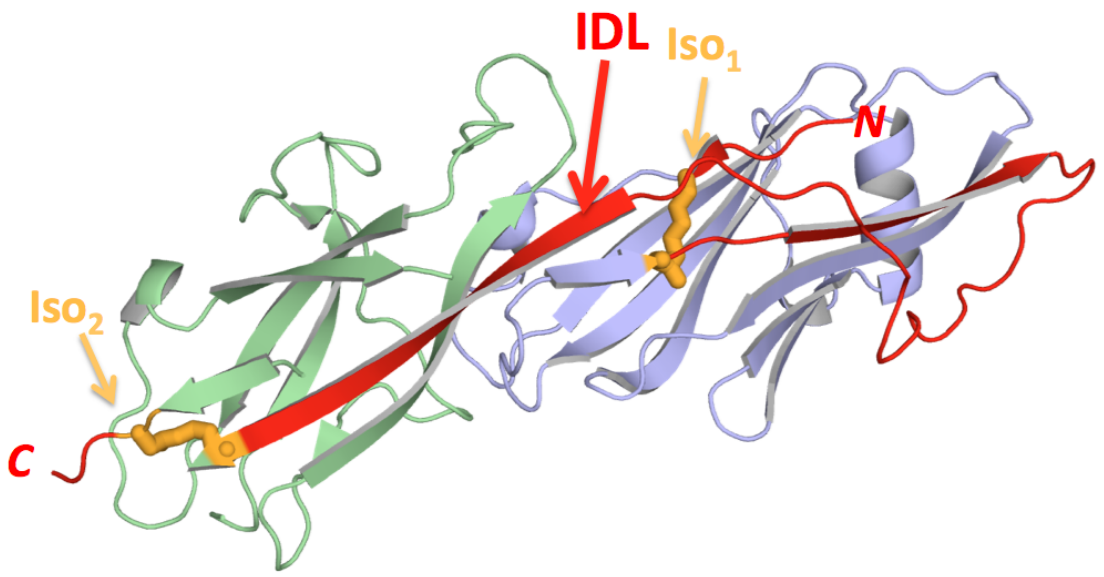


Carmelu Badilla

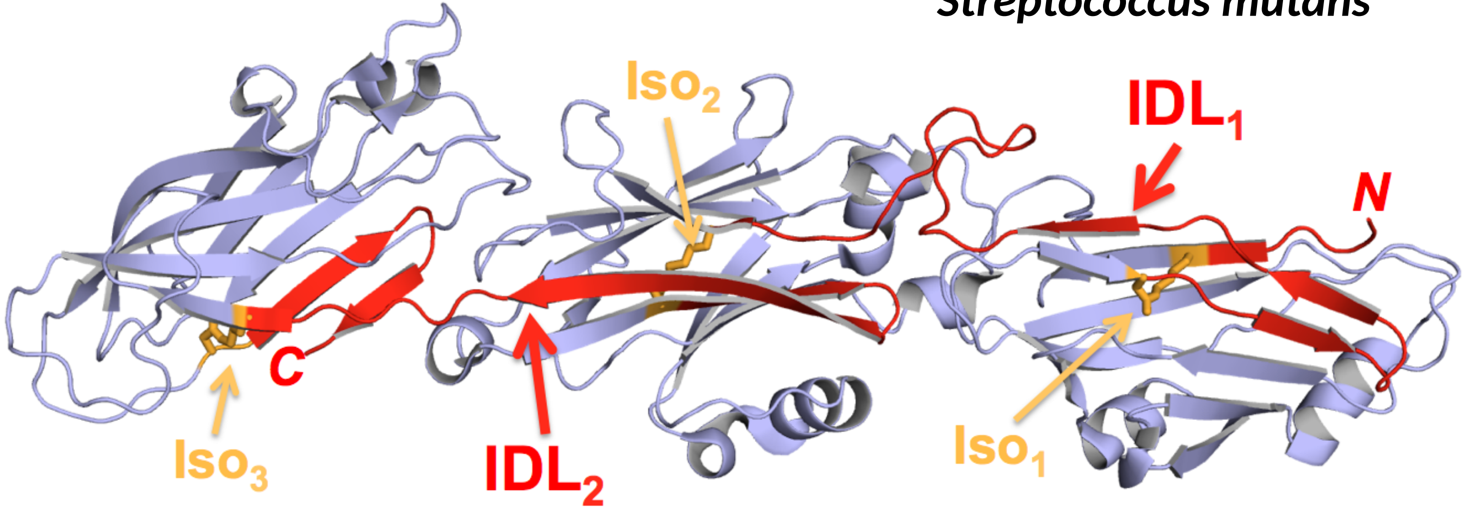


A blocking isopeptide is far more effective in knocking out the mechanical stability of pili than an isopeptide mutation

FimA *Actinomyces oris*

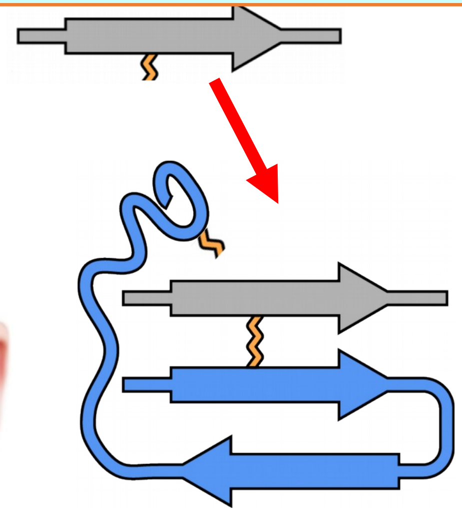


Streptococcus mutans



Rational design of antiadhesive peptide antibiotics

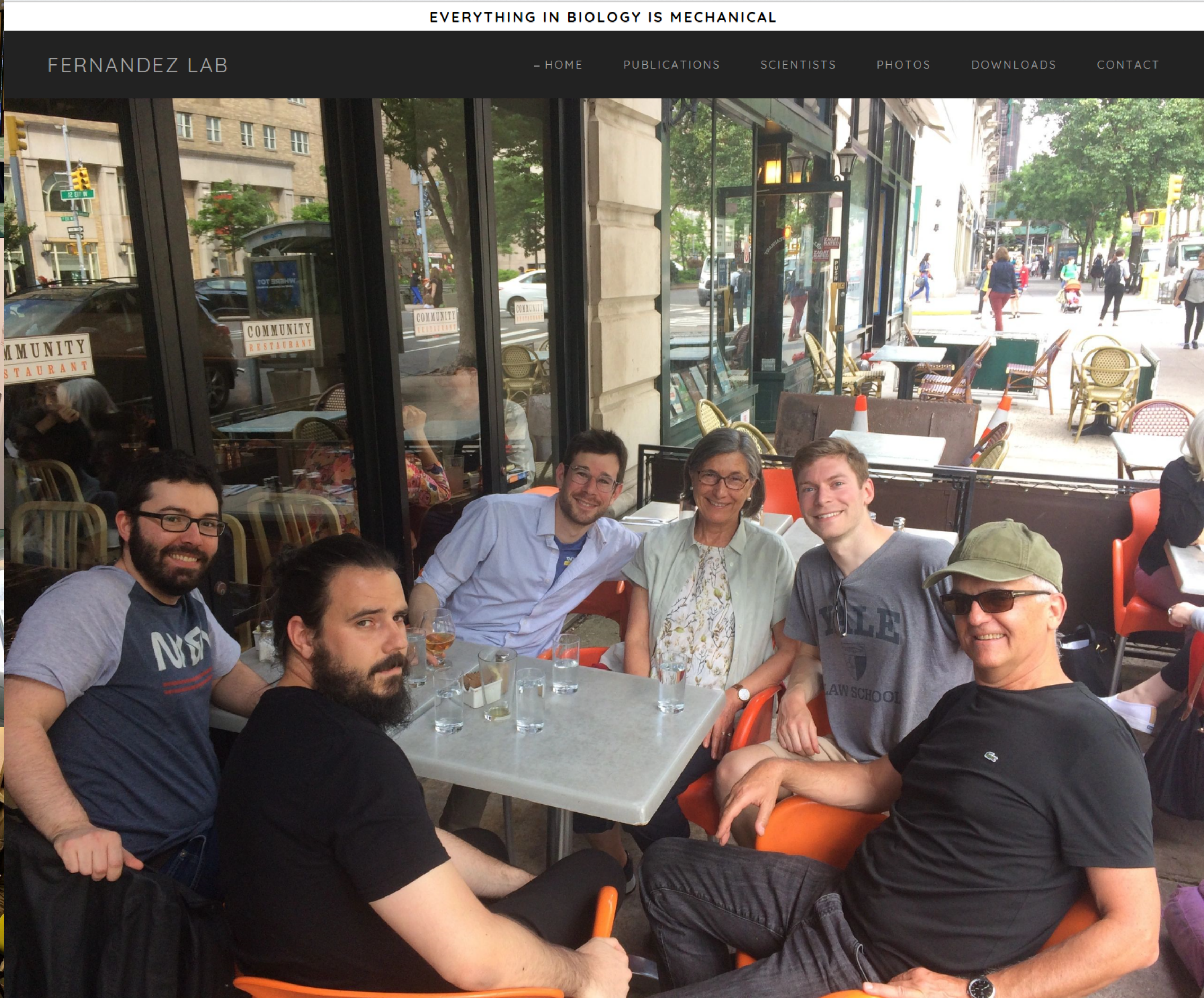
A new type of toothpaste



A new type of peptide

A vaccine against dental caries?

Look us up in
zeptowatt.co
m



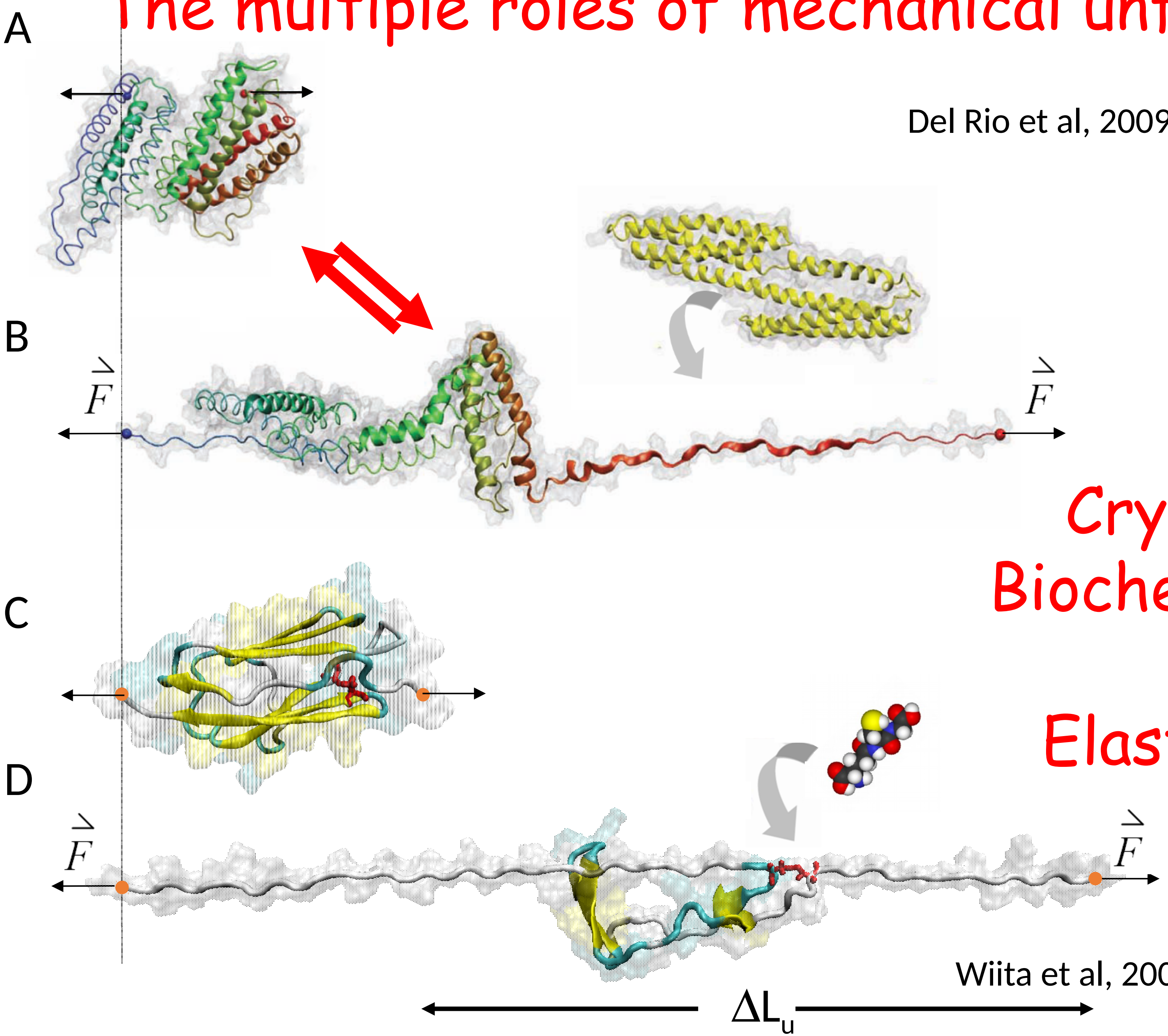
Instrumentation

1.- AFM

2.- Magnetic Tweezers

The multiple roles of mechanical unfolding

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

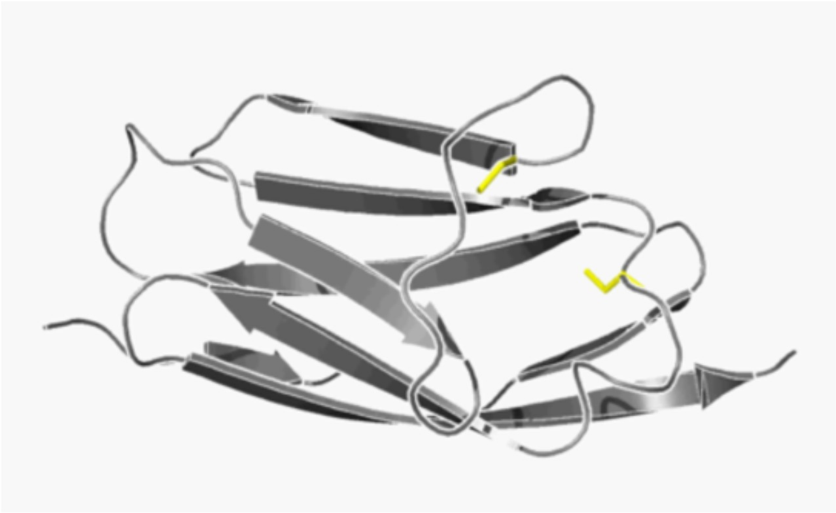


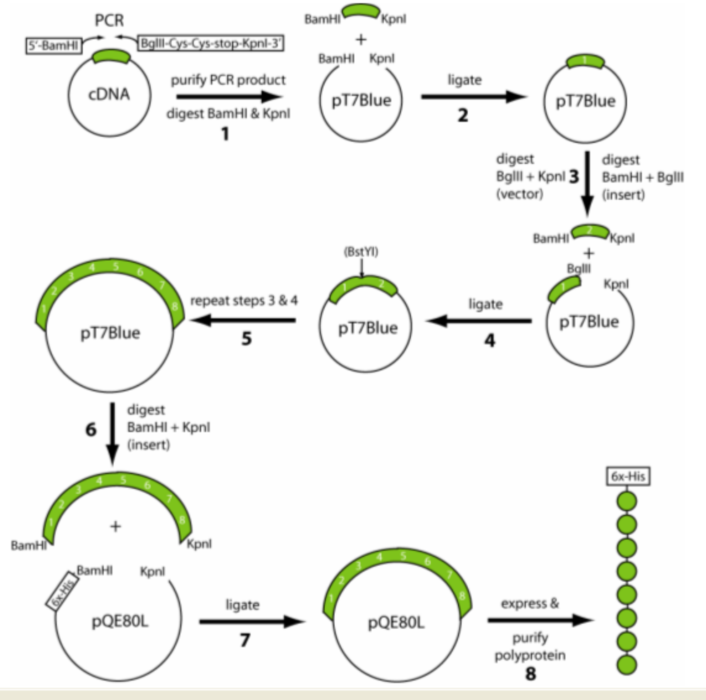
Cryptic Biochemistry

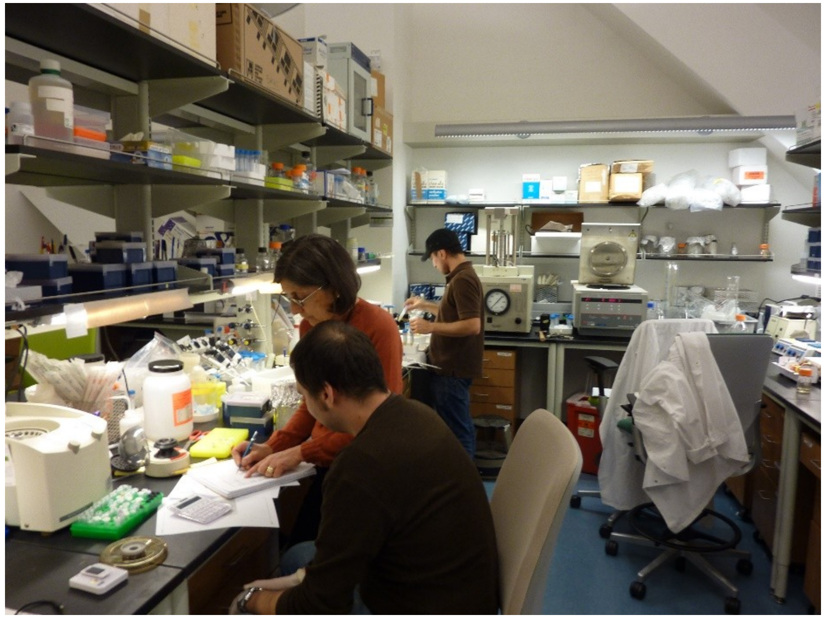
Elasticity

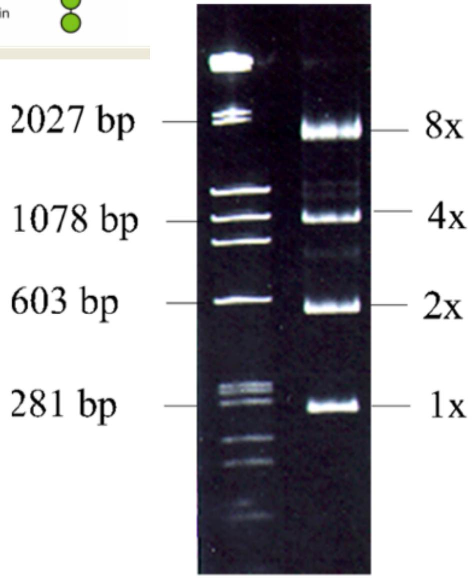
Wiita et al, 2007, **Nature**, **450**: 124-127

Polyprotein engineering for force spectroscopy I: DNA engineering

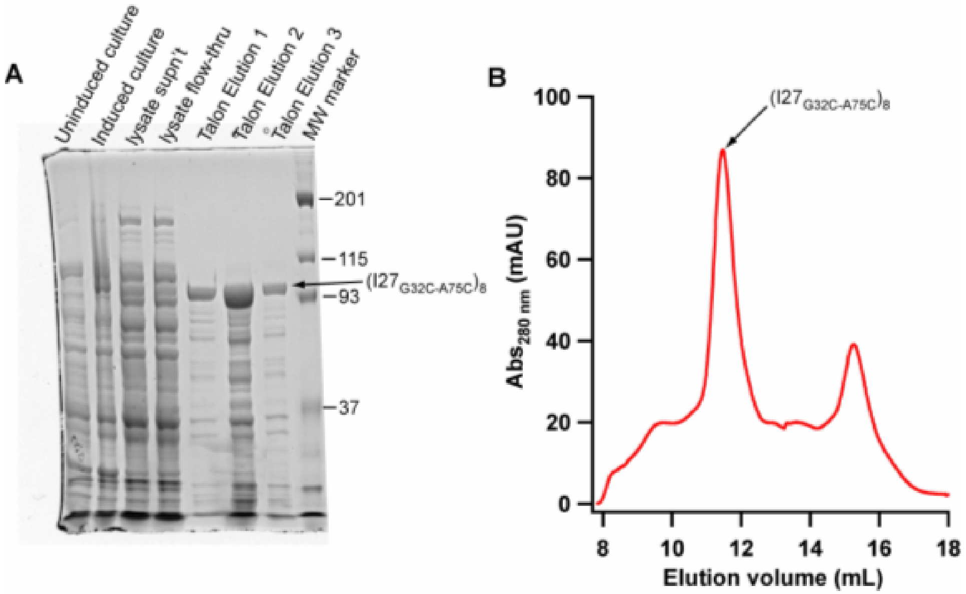


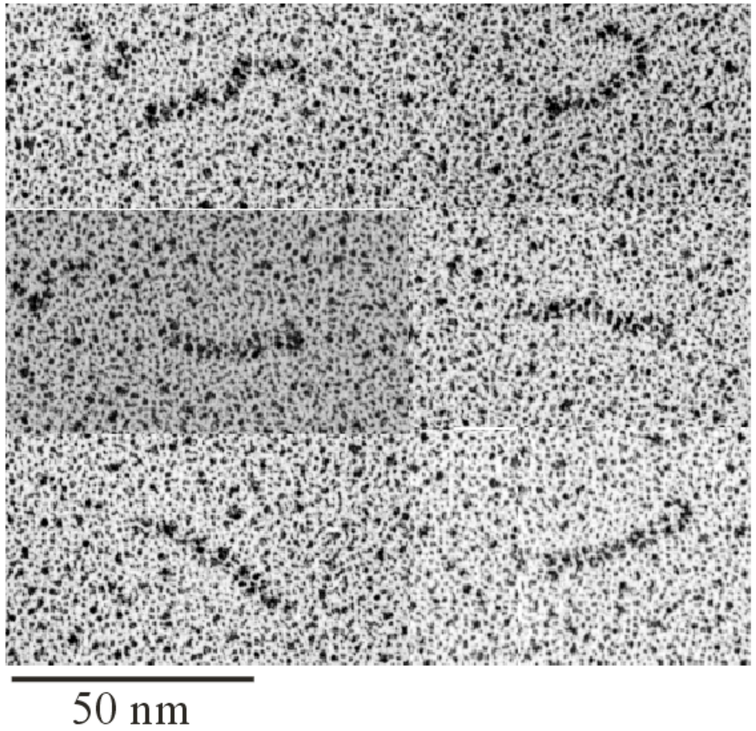


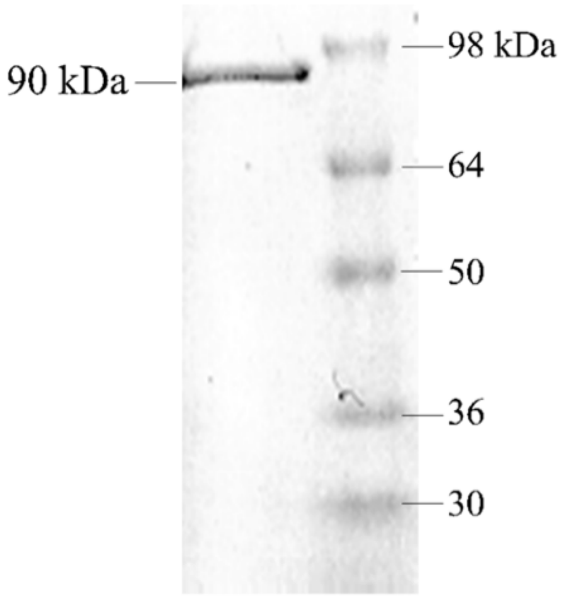




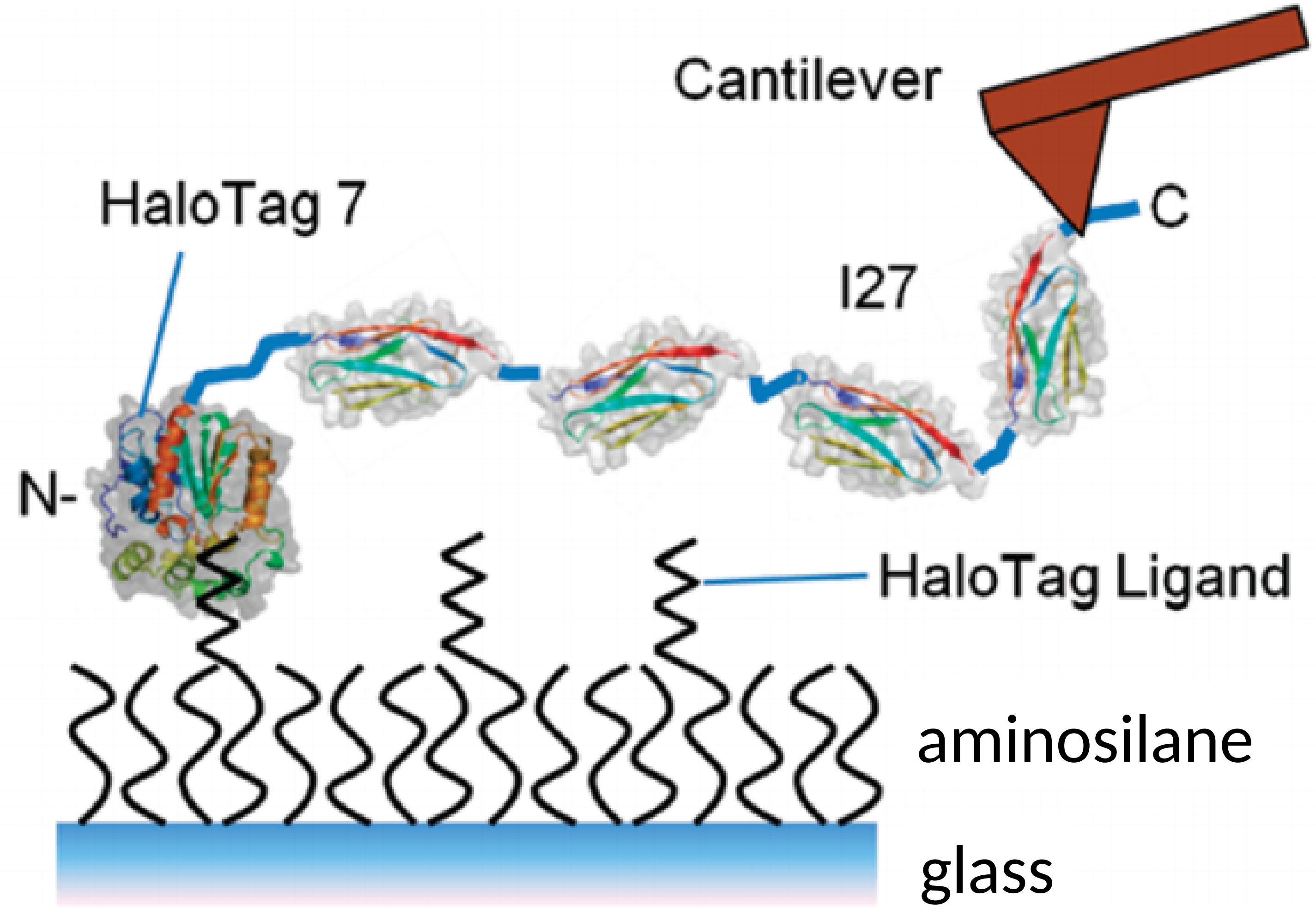
Protein engineering II; expression and purification





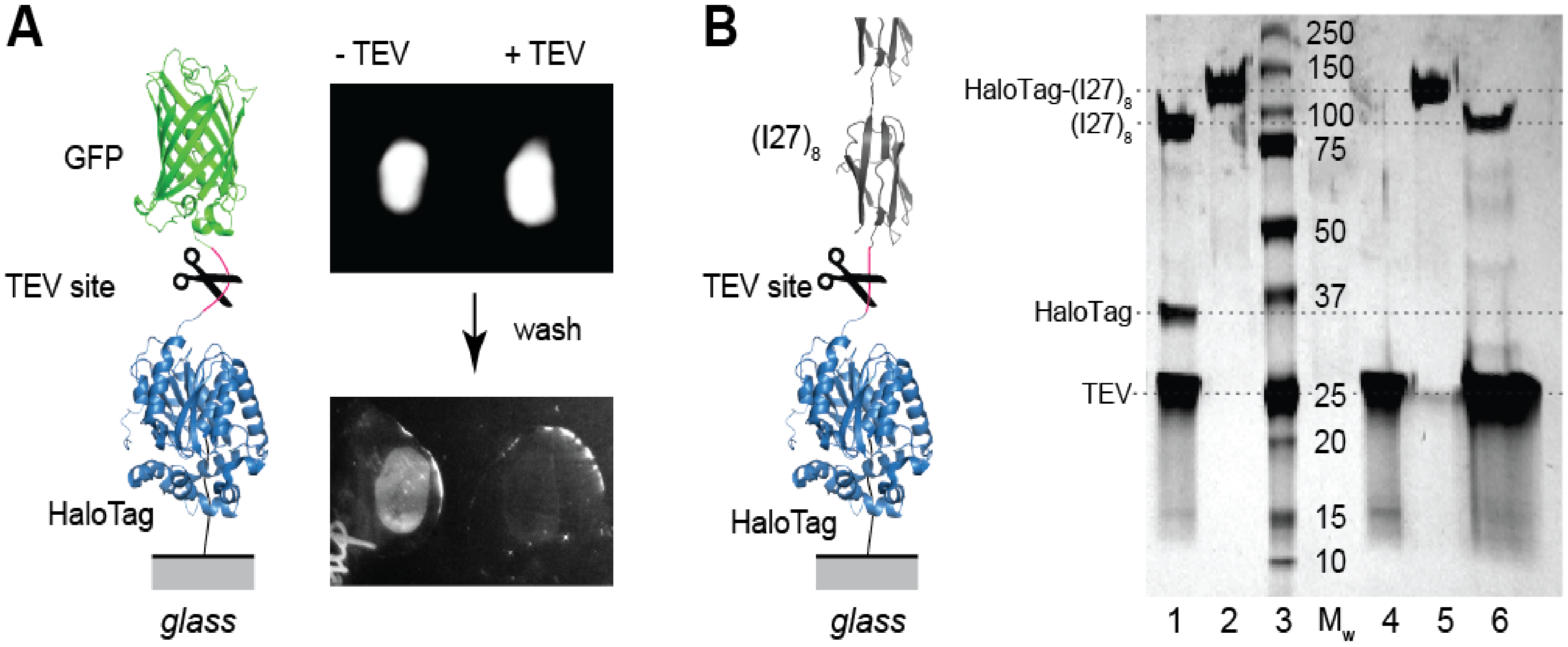


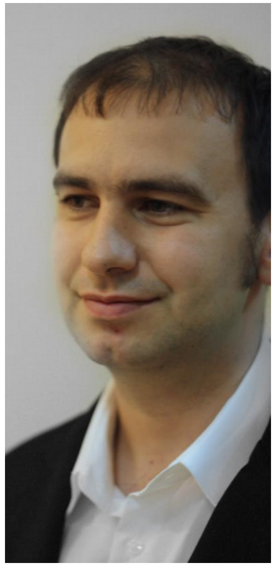
Polyprotein engineering III; anchoring



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

HaloTag and chloroalkane chemistry for the covalent anchoring of polyproteins



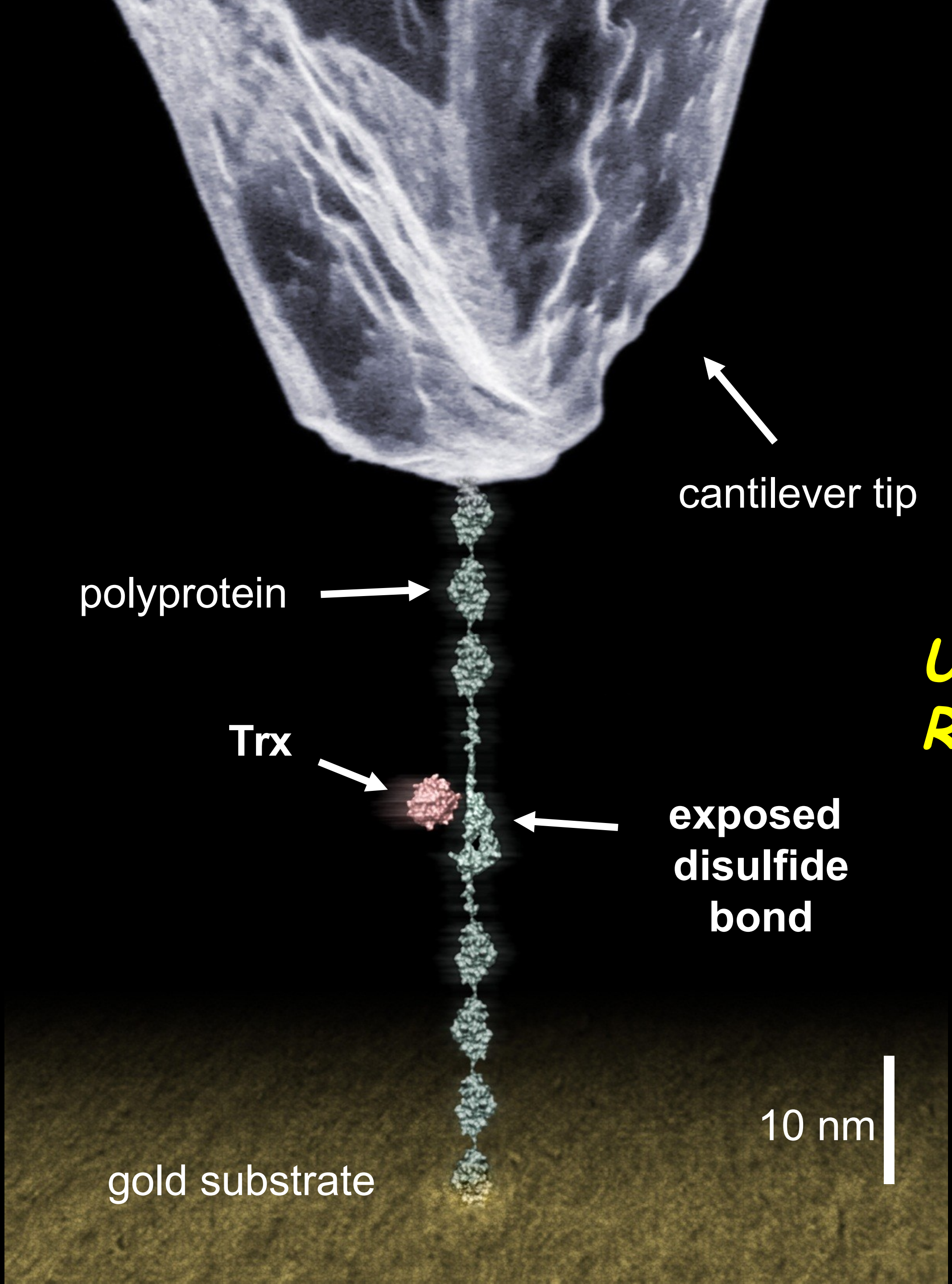


Ionel Popa

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

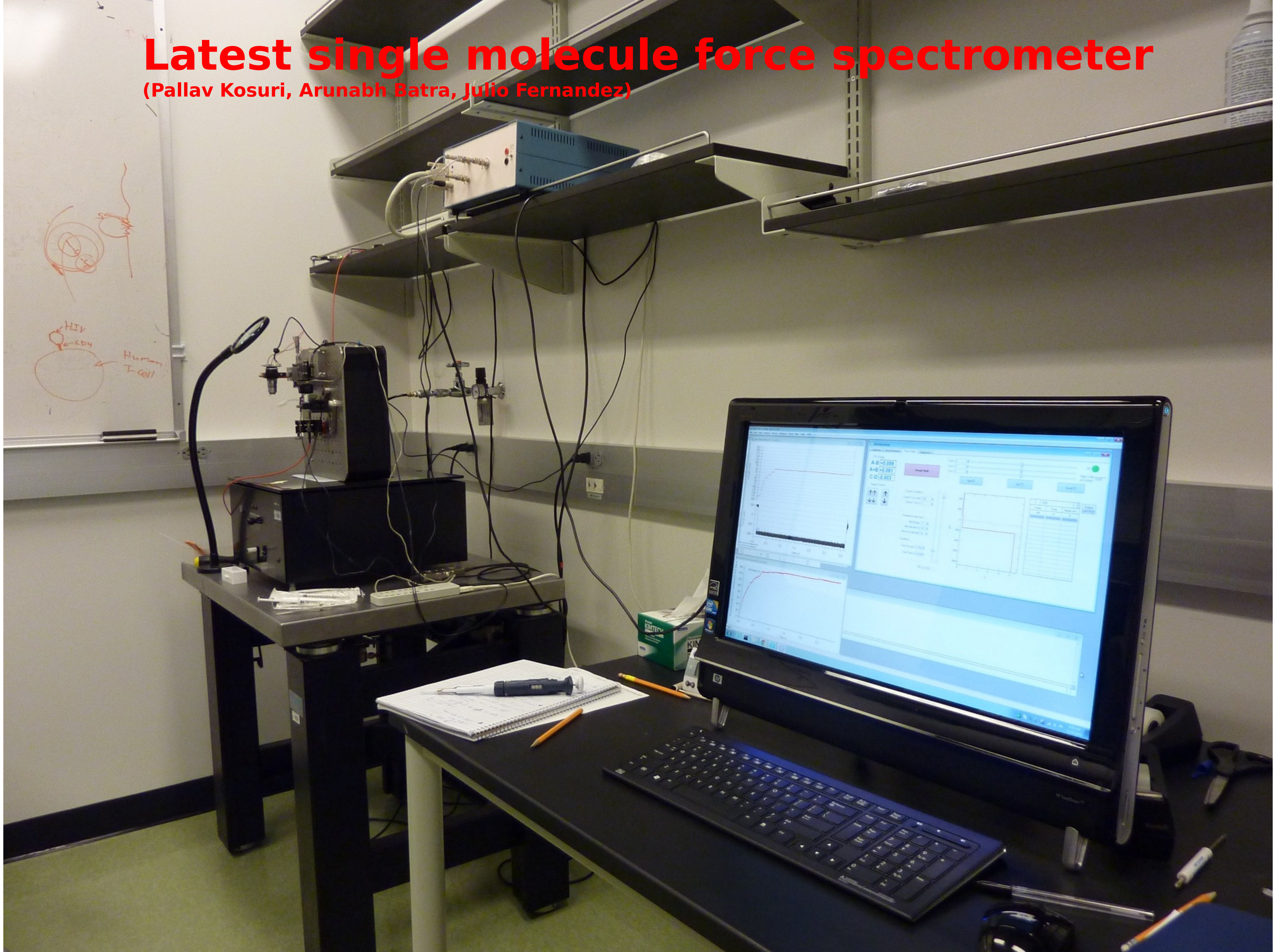


Useful ?
Real ?

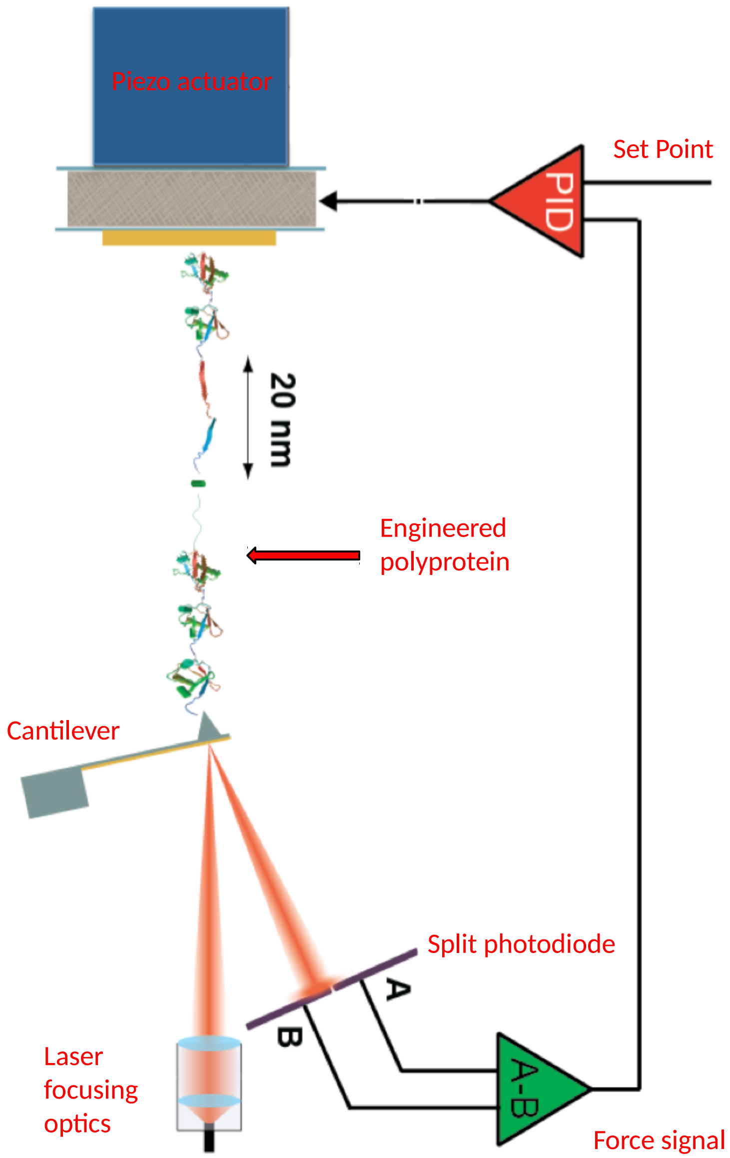
New Perspective in Biology !

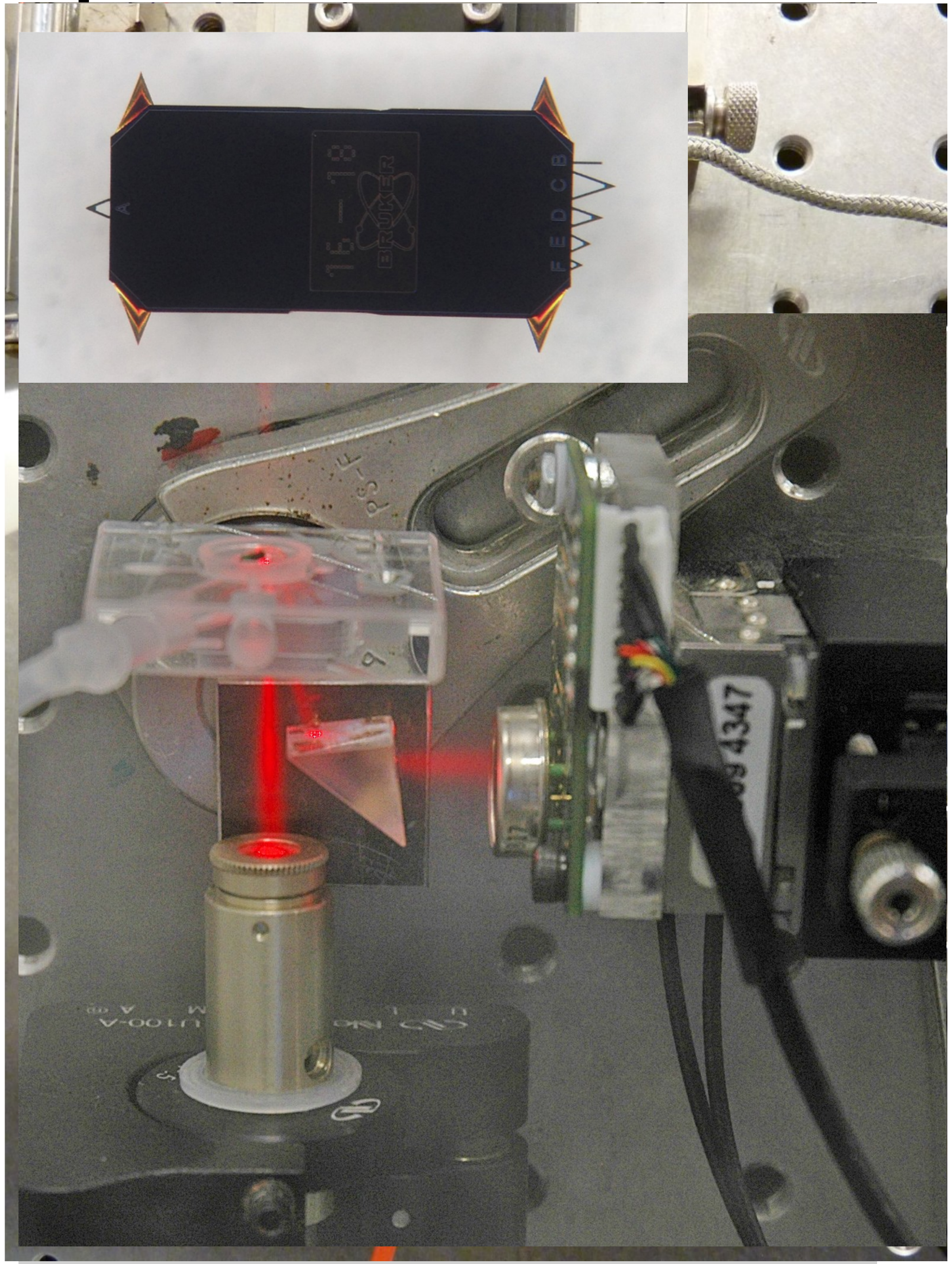
Latest single molecule force spectrometer

(Pallav Kosuri, Arunabh Batra, Julio Fernandez)

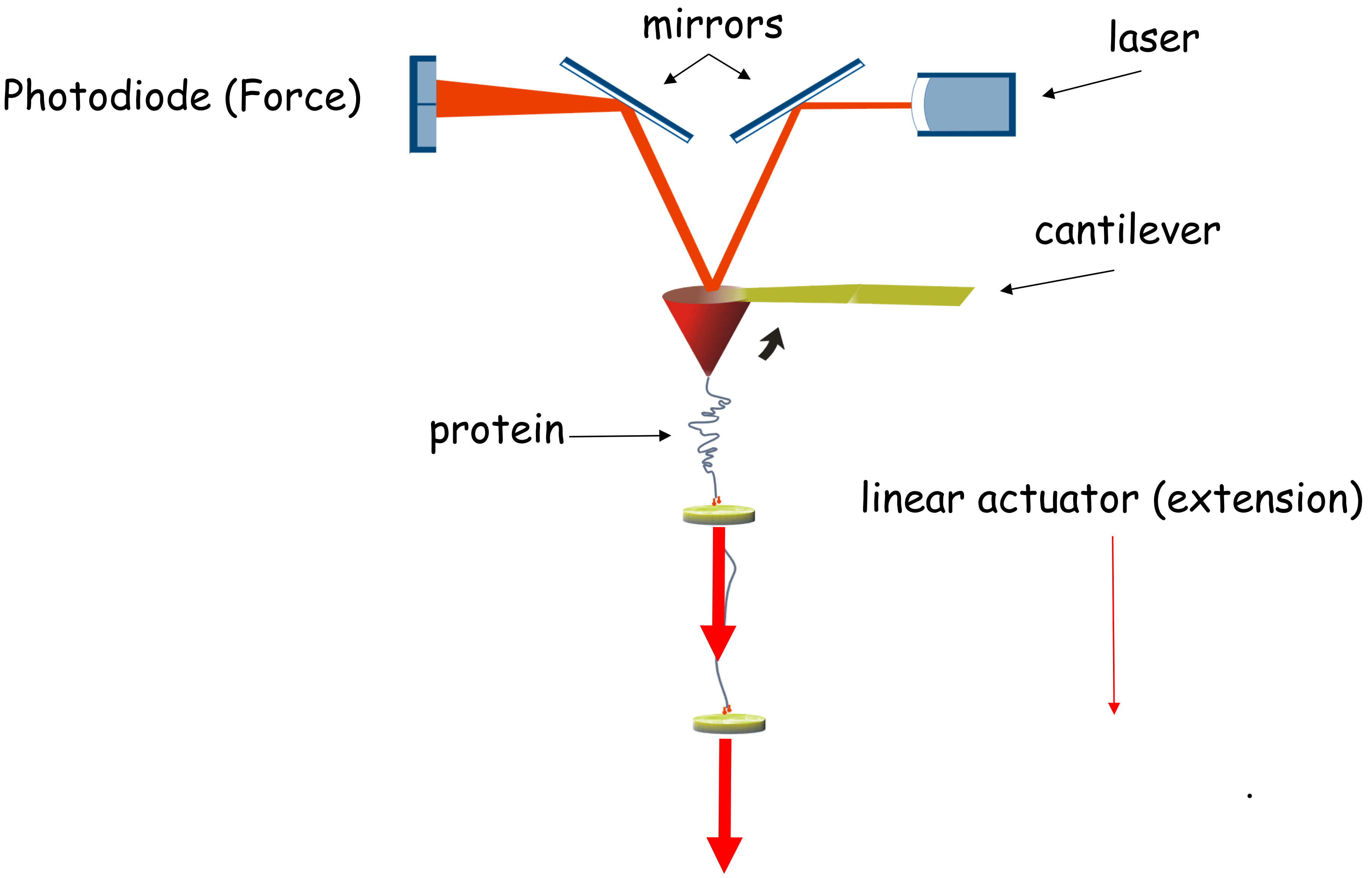


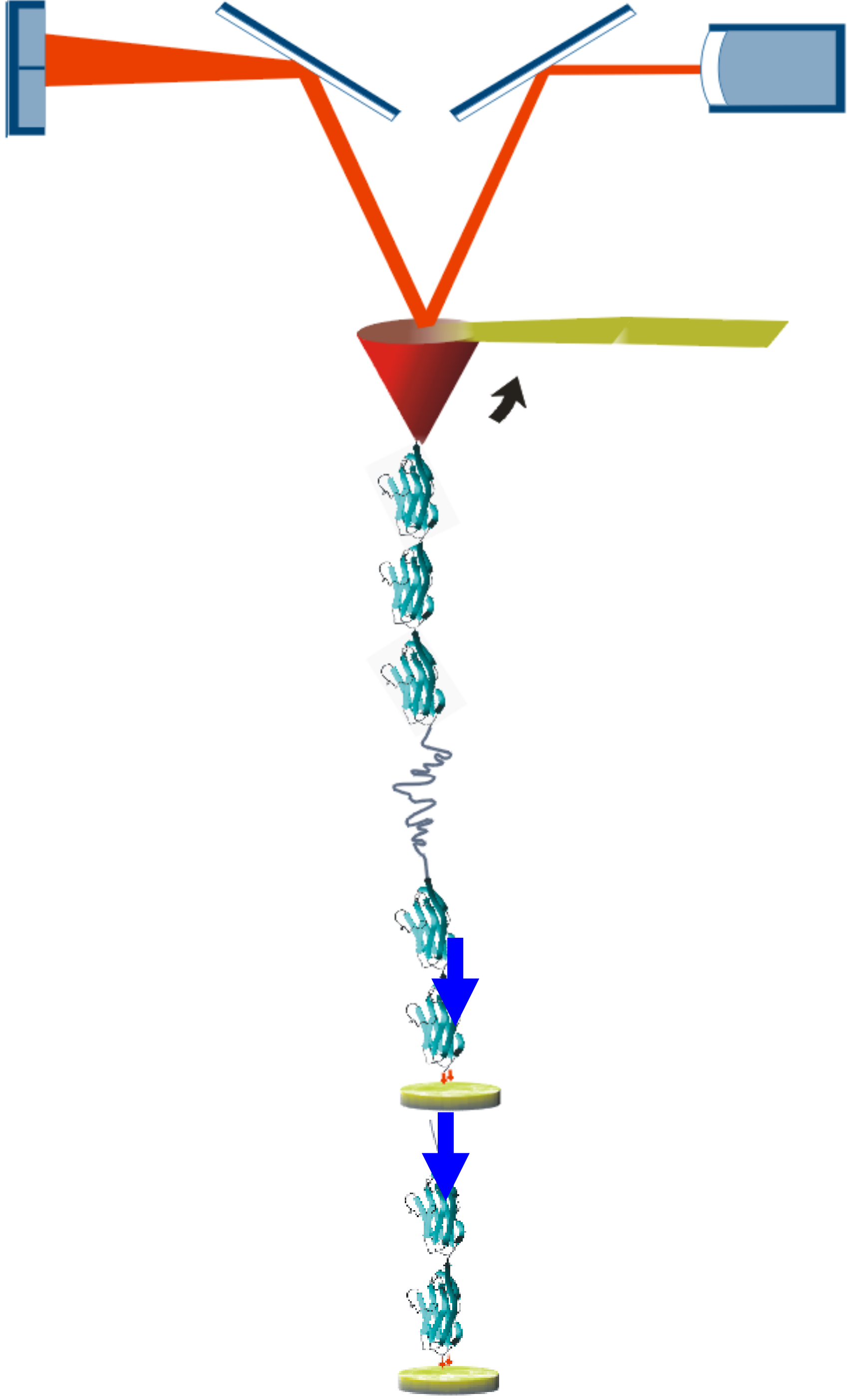
Force sensor and piezoelectric actuator

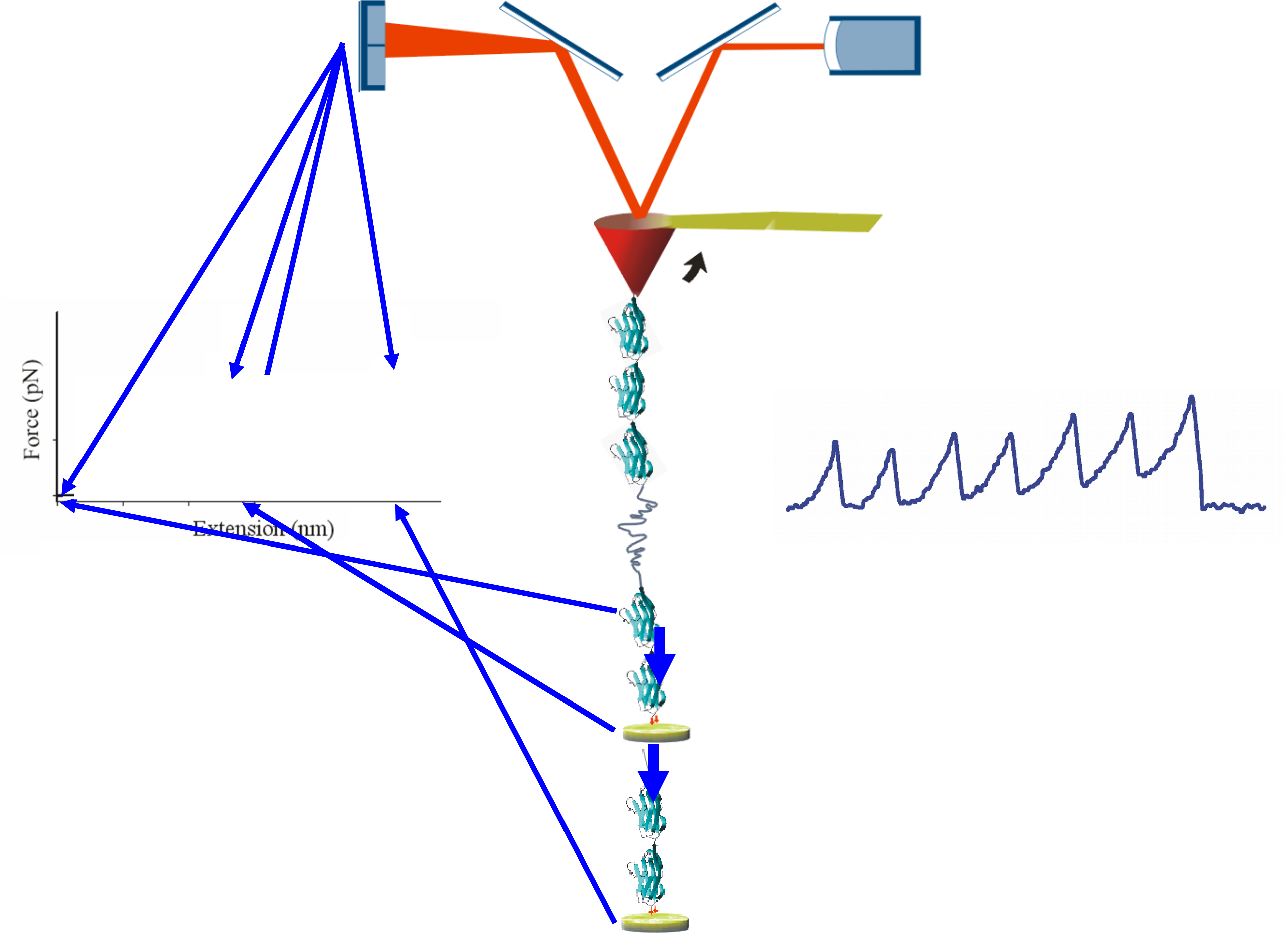


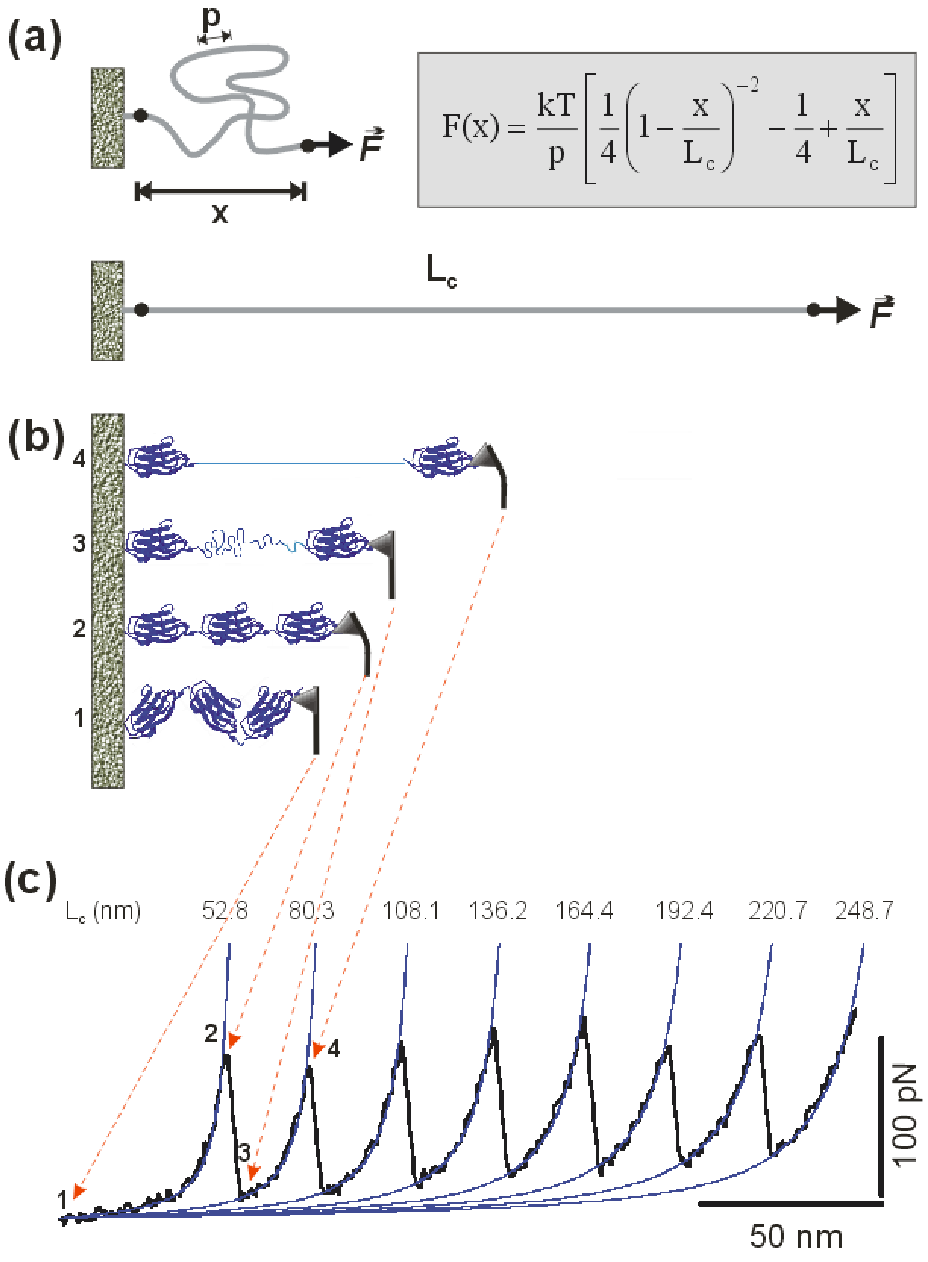


We can stretch a single protein and measure how does the restoring force changes with the extension.

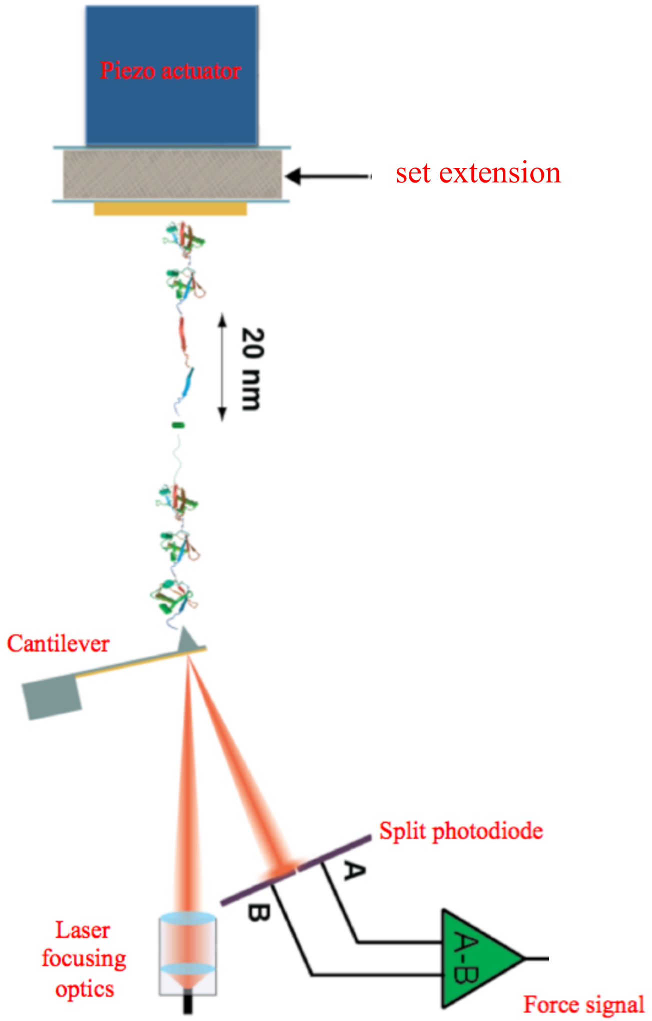




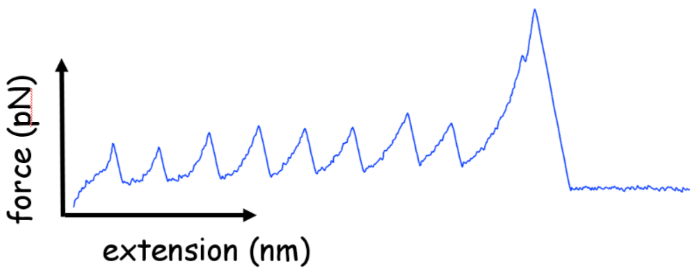

Force (pN)
Extension (nm)



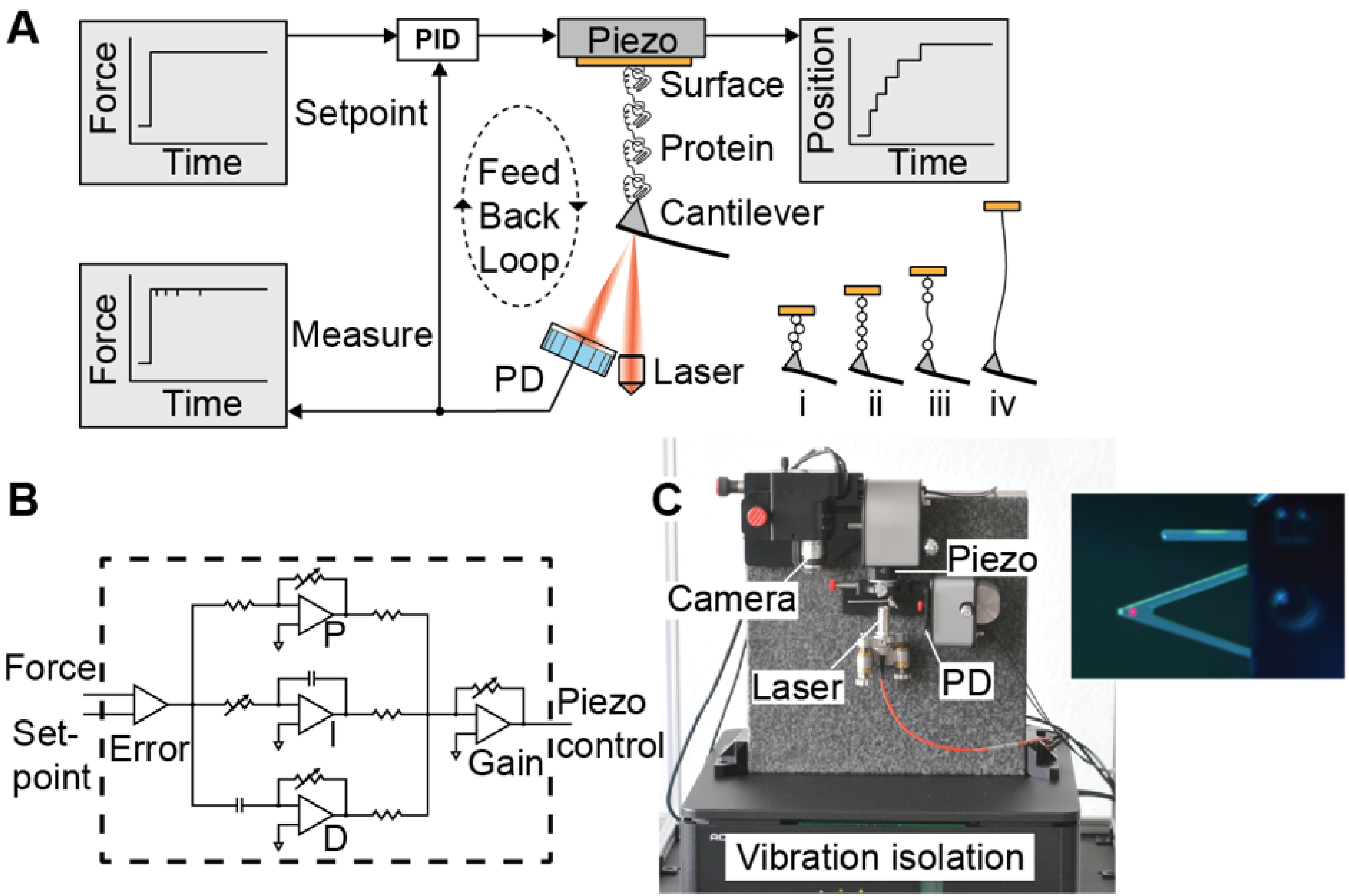
(a)
p
x
$F(x) = \frac{kT}{p}\left[\frac{1}{4}\left(1-\frac{x}{L_c}\right)^{-2} - \frac{1}{4} + \frac{x}{L_c}\right]$
L_c
(b)
4
3
2
1
(c)
L_c (nm)
52.8
80.3
108.1
136.2
164.4
192.4
220.7
248.7
100 pN
50 nm



Constant velocity mode



Force-clamp spectroscopy apparatus

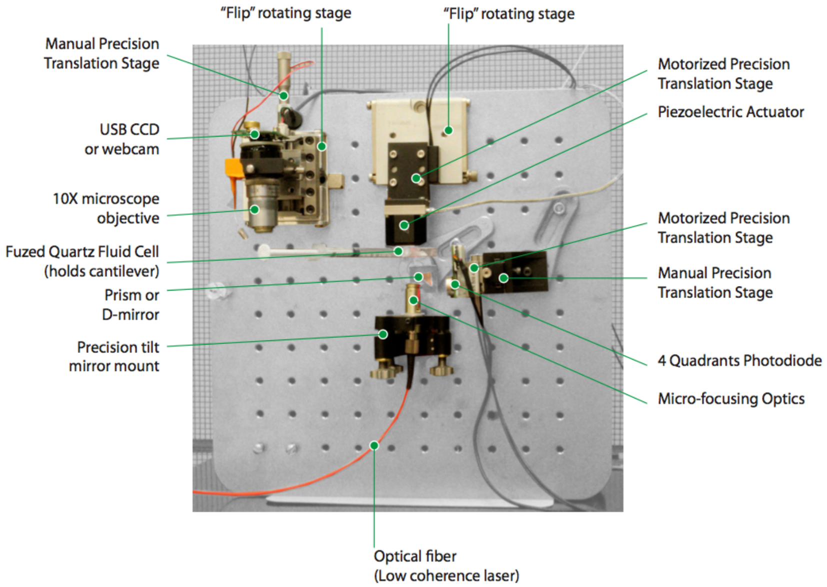


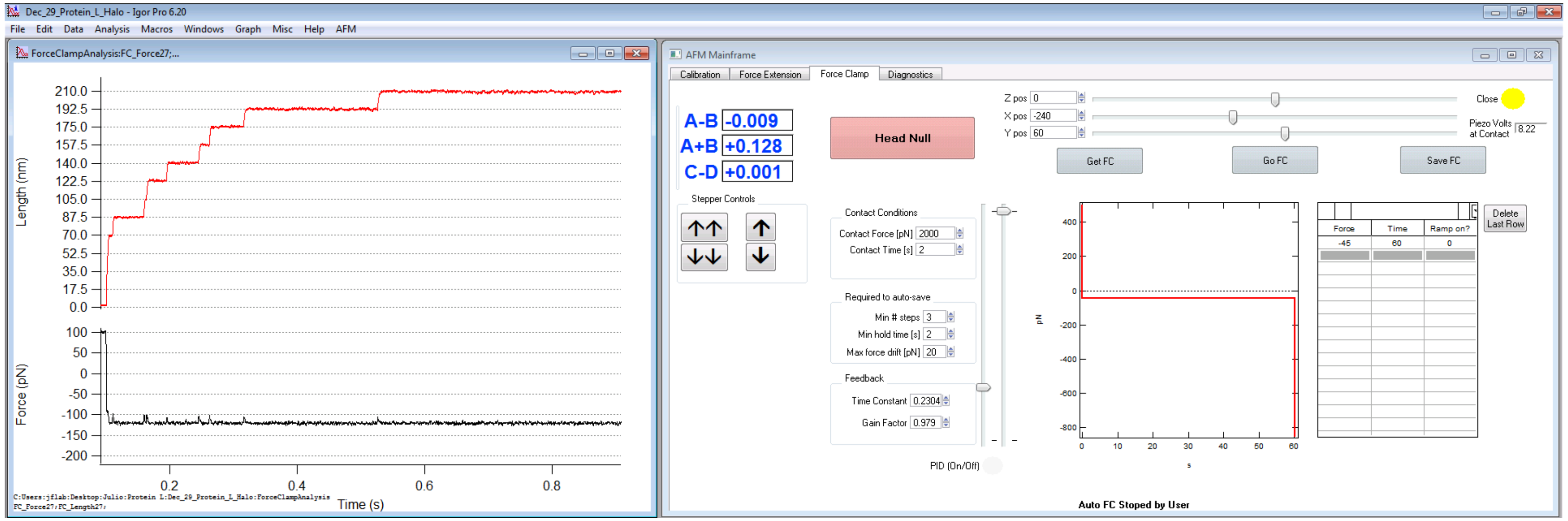
Luigs & Neumann Feinmechanik und Elektrotechnik GmbH

Introducing the AFS

Single Molecule Atomic Force Spectrometer

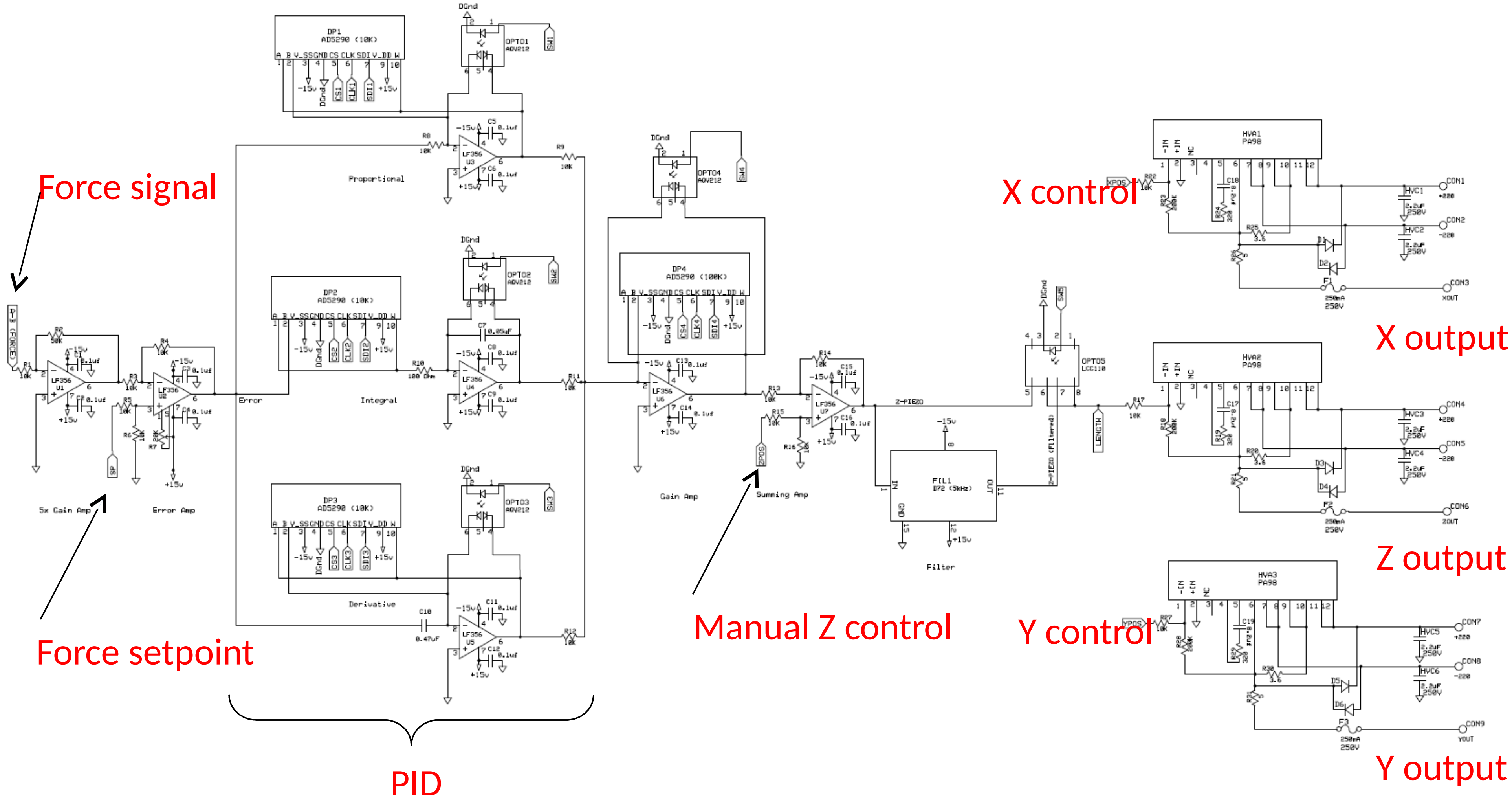
- Force-clamp and force-extension
- Sub-nanometer resolution
- Sub-millisecond time resolution
- Protein folding and unfolding
- Bond cleavage and formation
- Fully automated operation
- Powerful analysis software
- Simple user interface





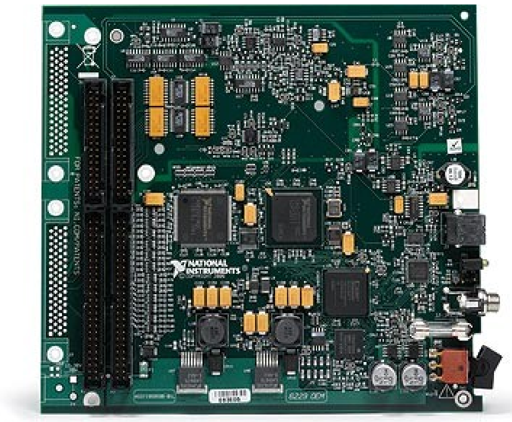
AFS: Feedback electronics

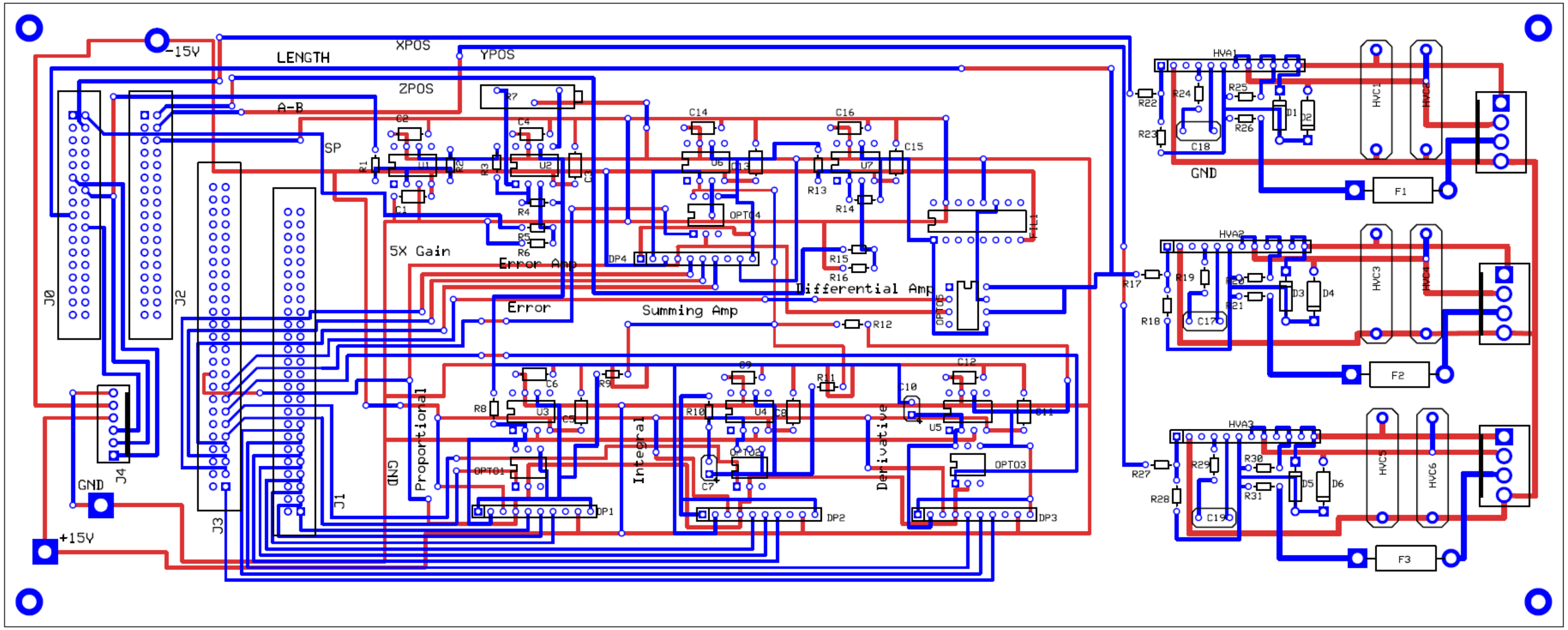
$$u(t) = \mathrm{MV}(t) = K_p e(t) + K_i \int_0^t e(\tau)\,d\tau + K_d \frac{d}{dt} e(t)$$



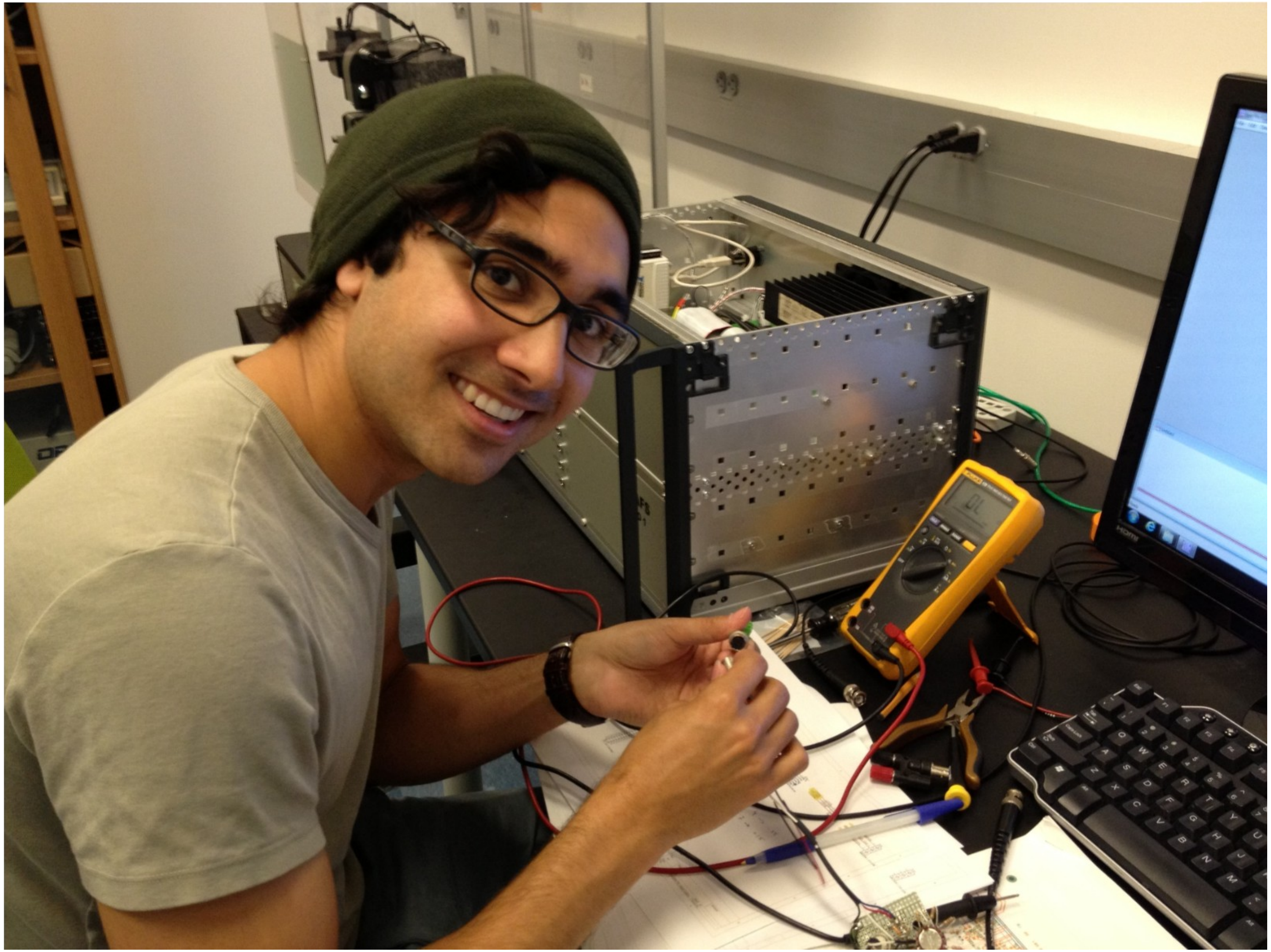
AFS: Circuit boards

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



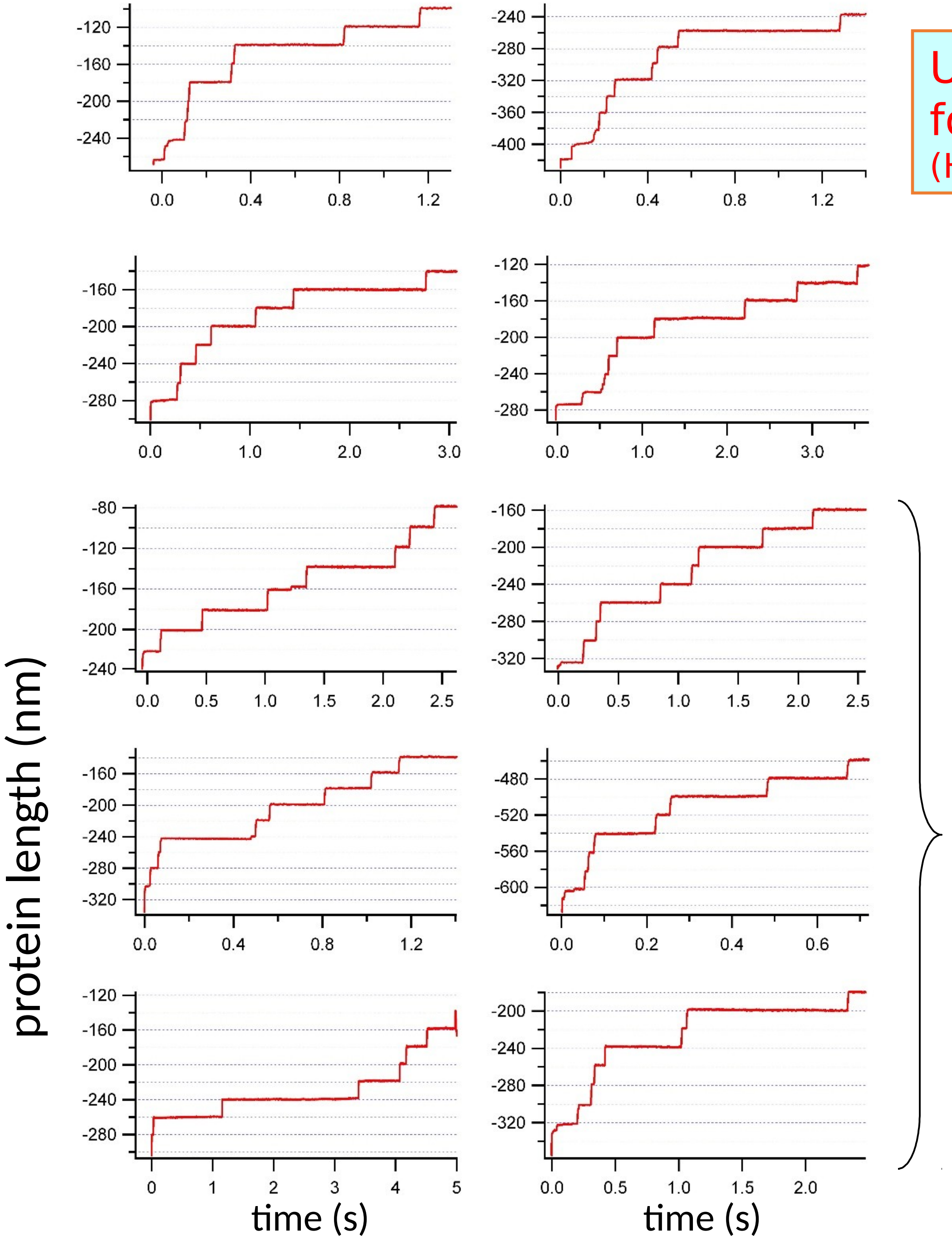


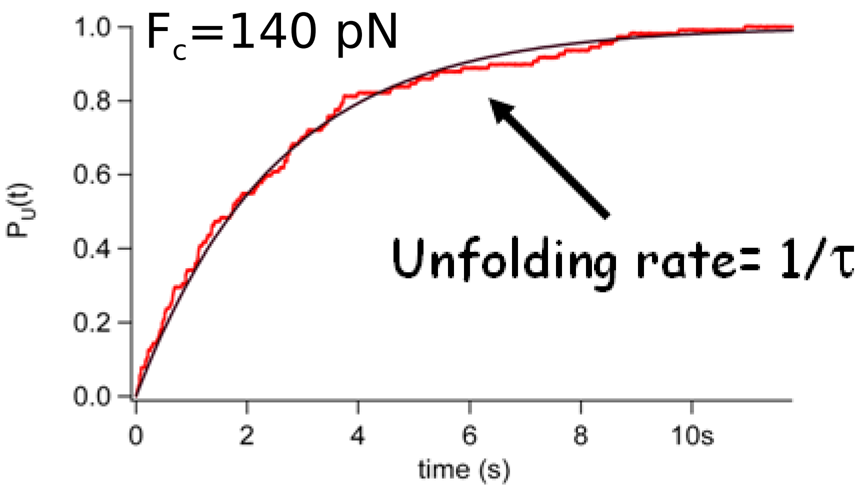
Pallav Kosuri (PhD;2012)
applying the final touches to the L&N prototype

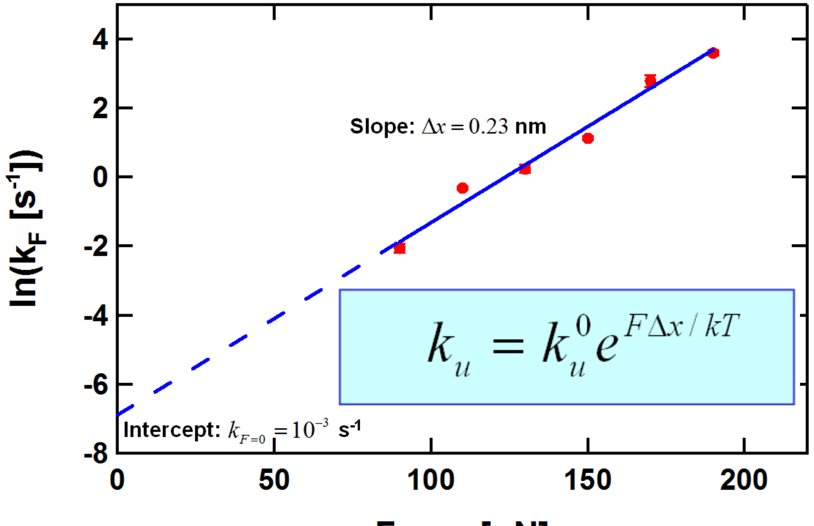


Unfolding polyproteins at constant force

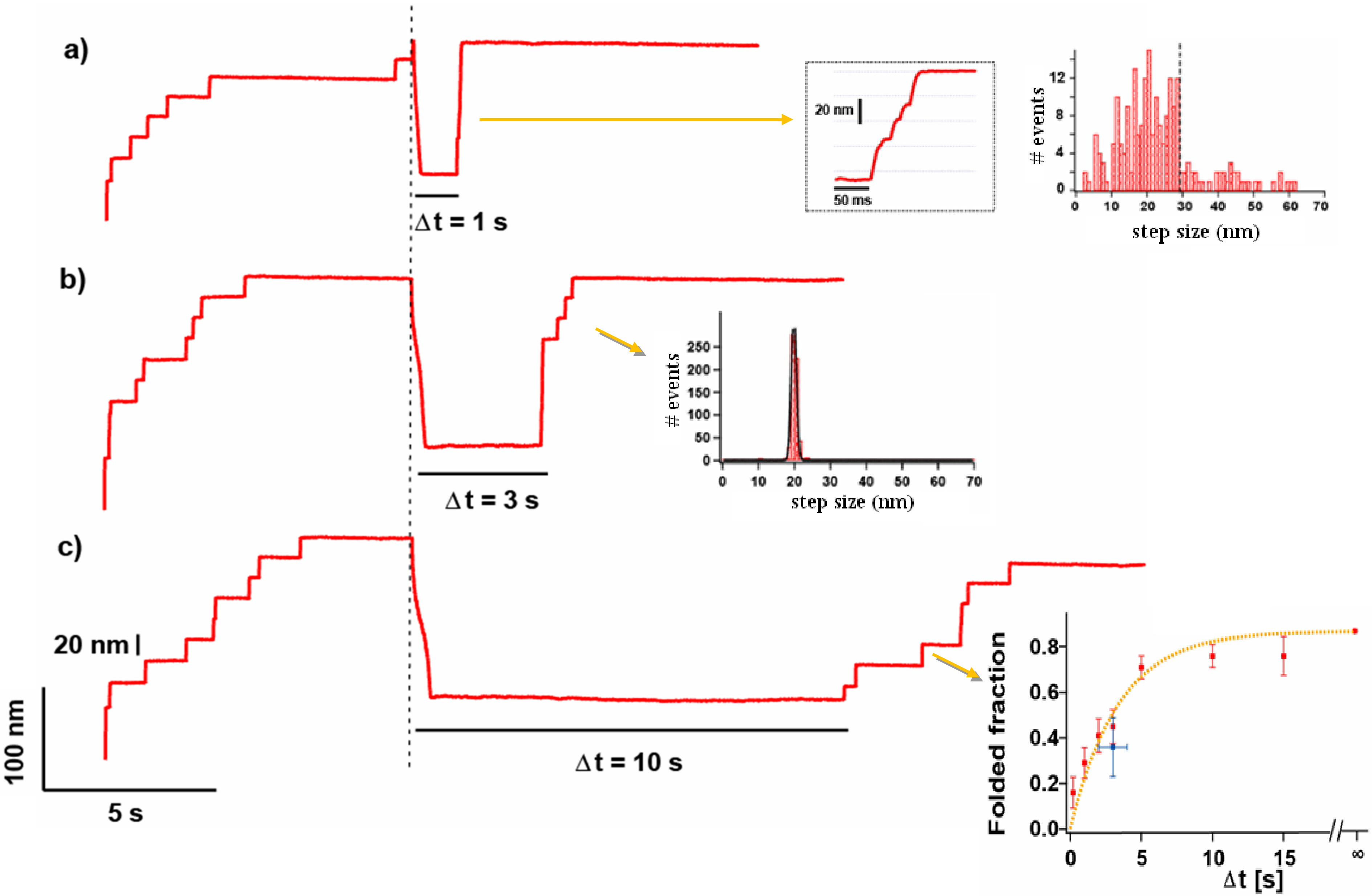
(Hongbin Li)



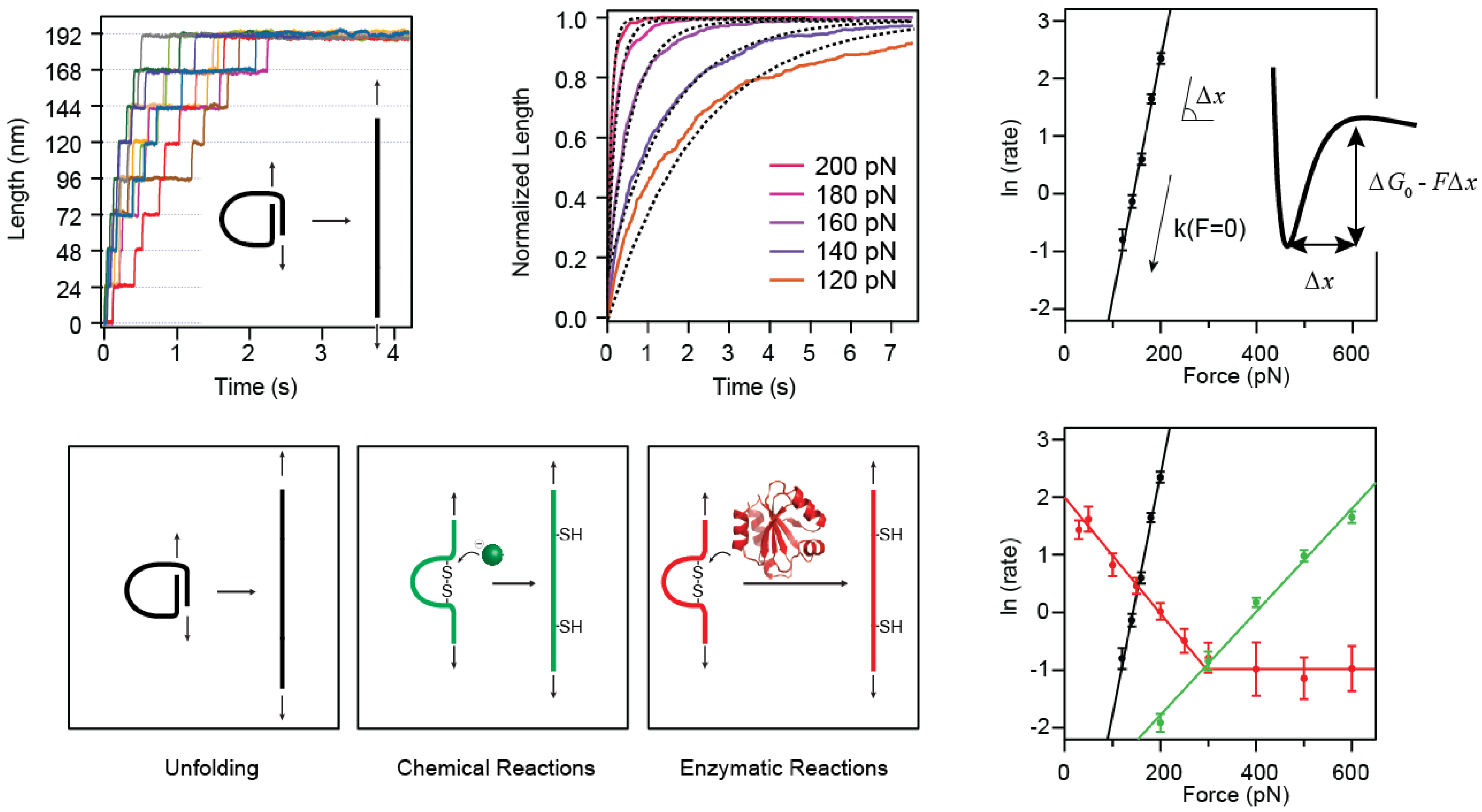




Force-quench; molten globules and folding
(Sergi Garcia-Manyes)

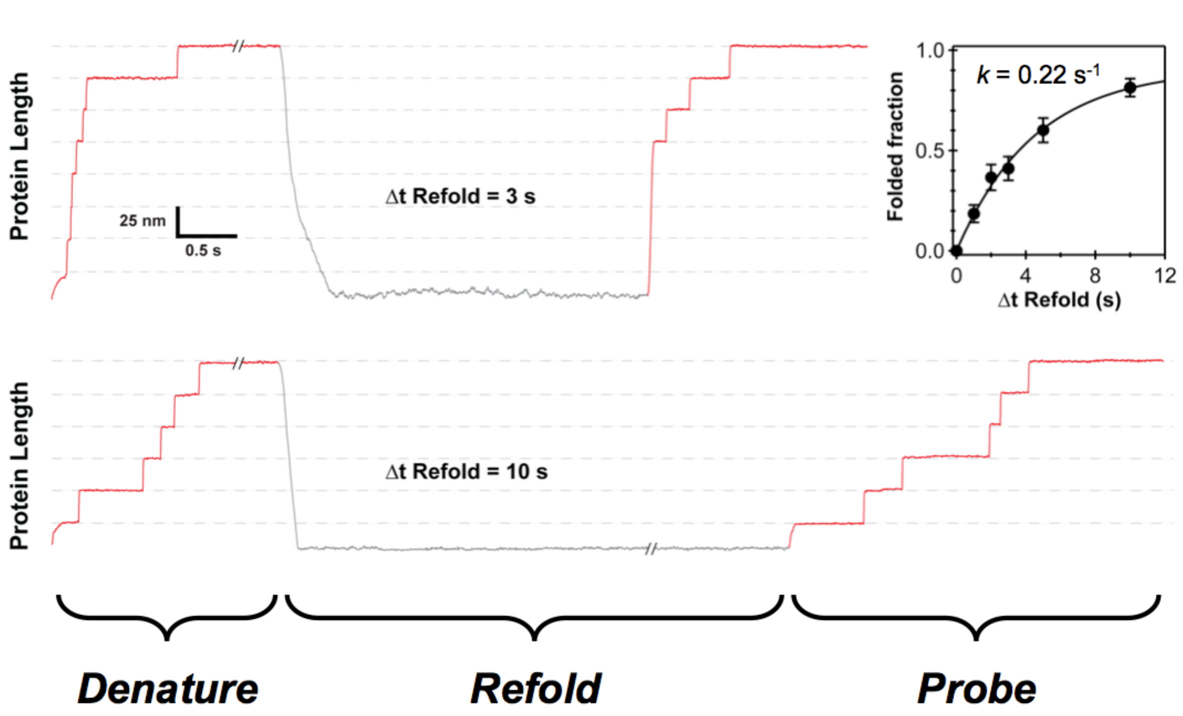


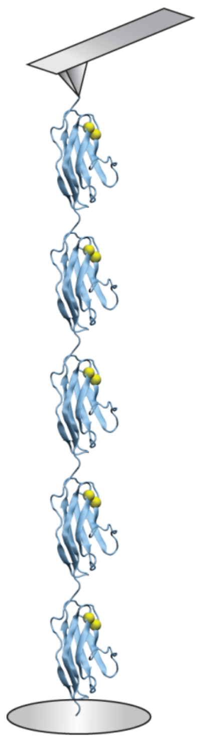
Force dependent reactions

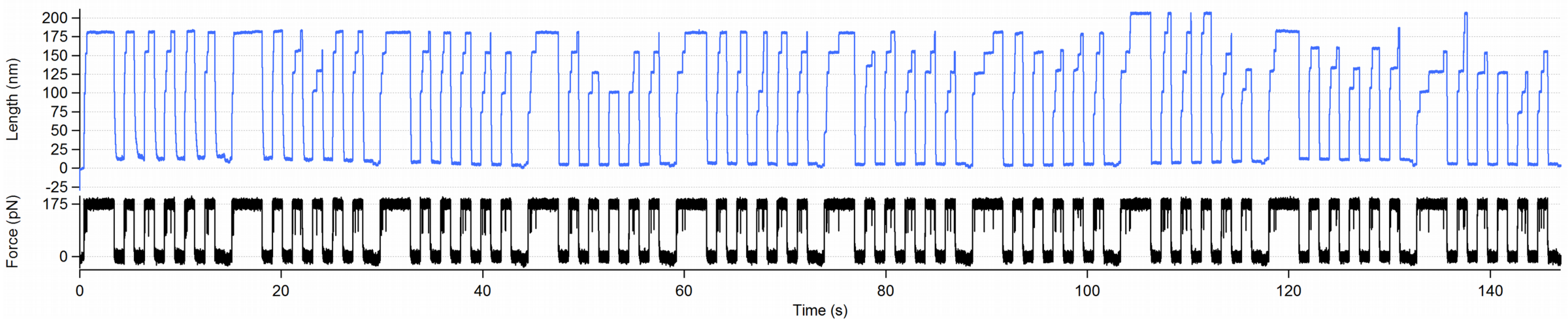


Popa I., et al, *Nat. Prot.* (2013)

Unfolding and refolding dynamics (Titin I27)



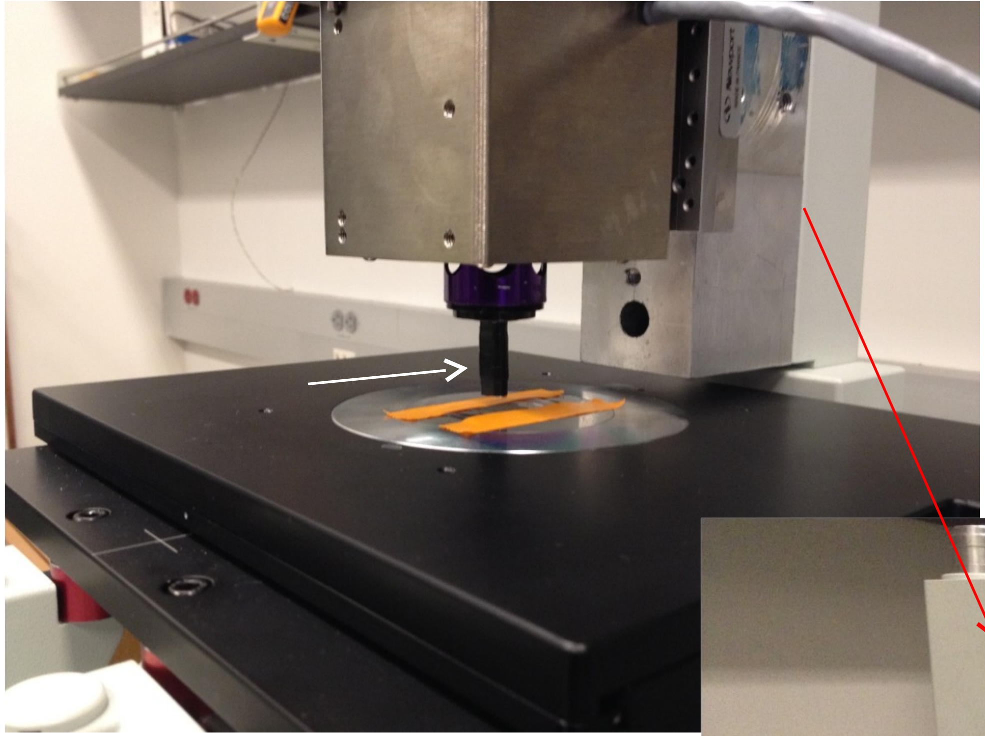




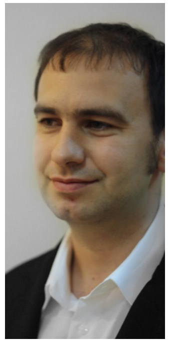
The Mechano-Biology Institute in Singapore

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

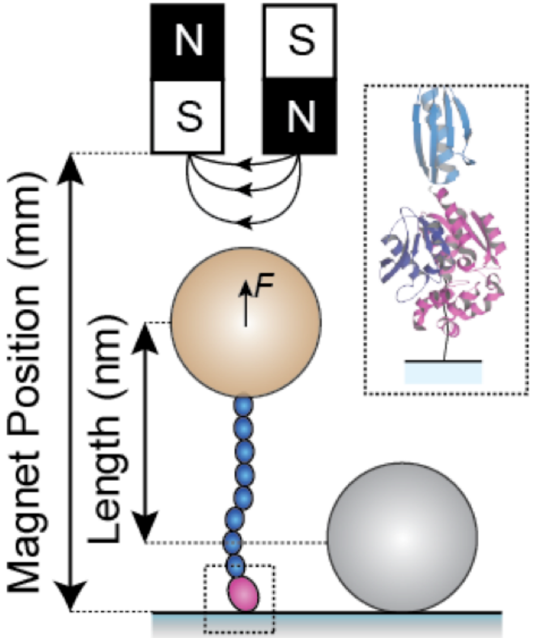
MBI
MECHANOBIOLOGY INSTITUTE
Singapore

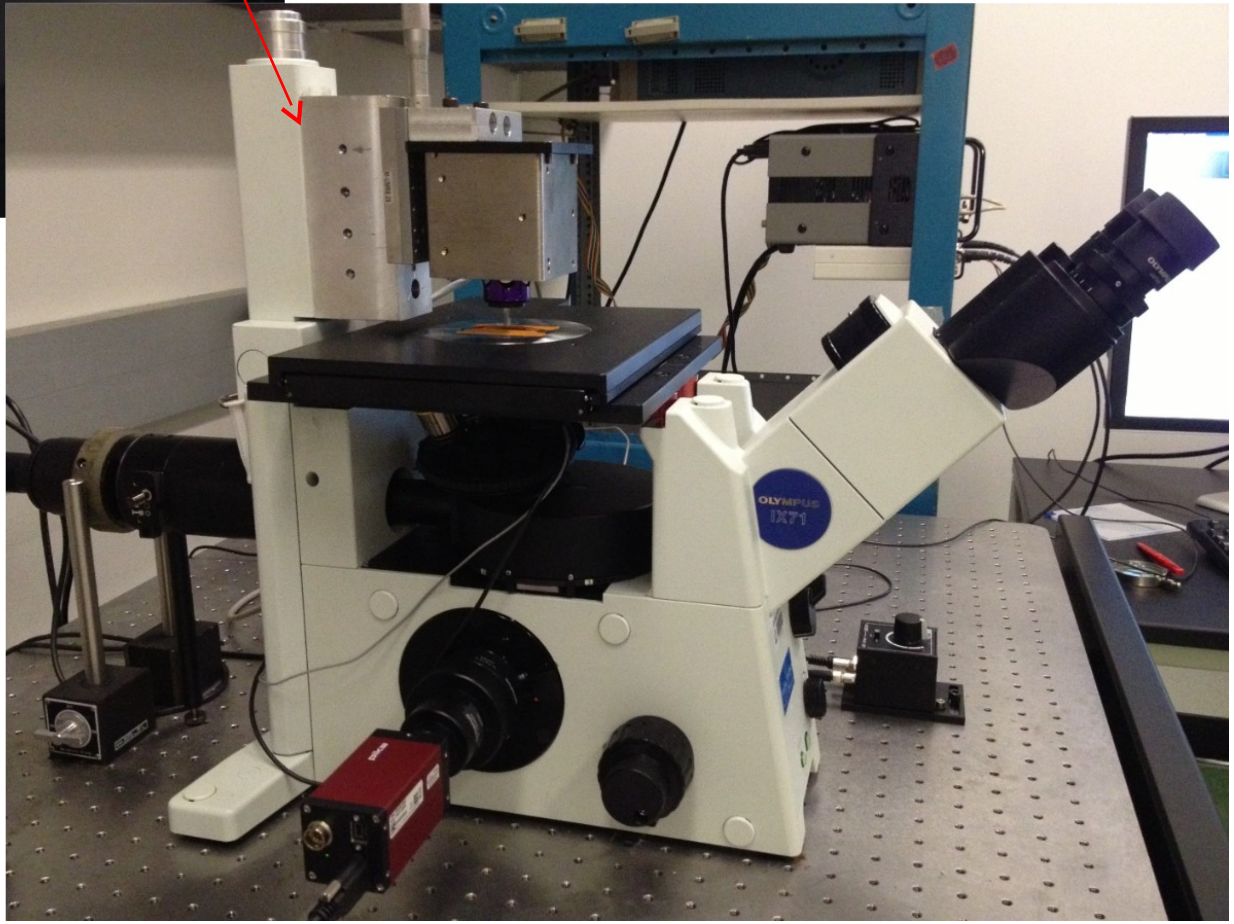


HaloTag and magnetic tweezers

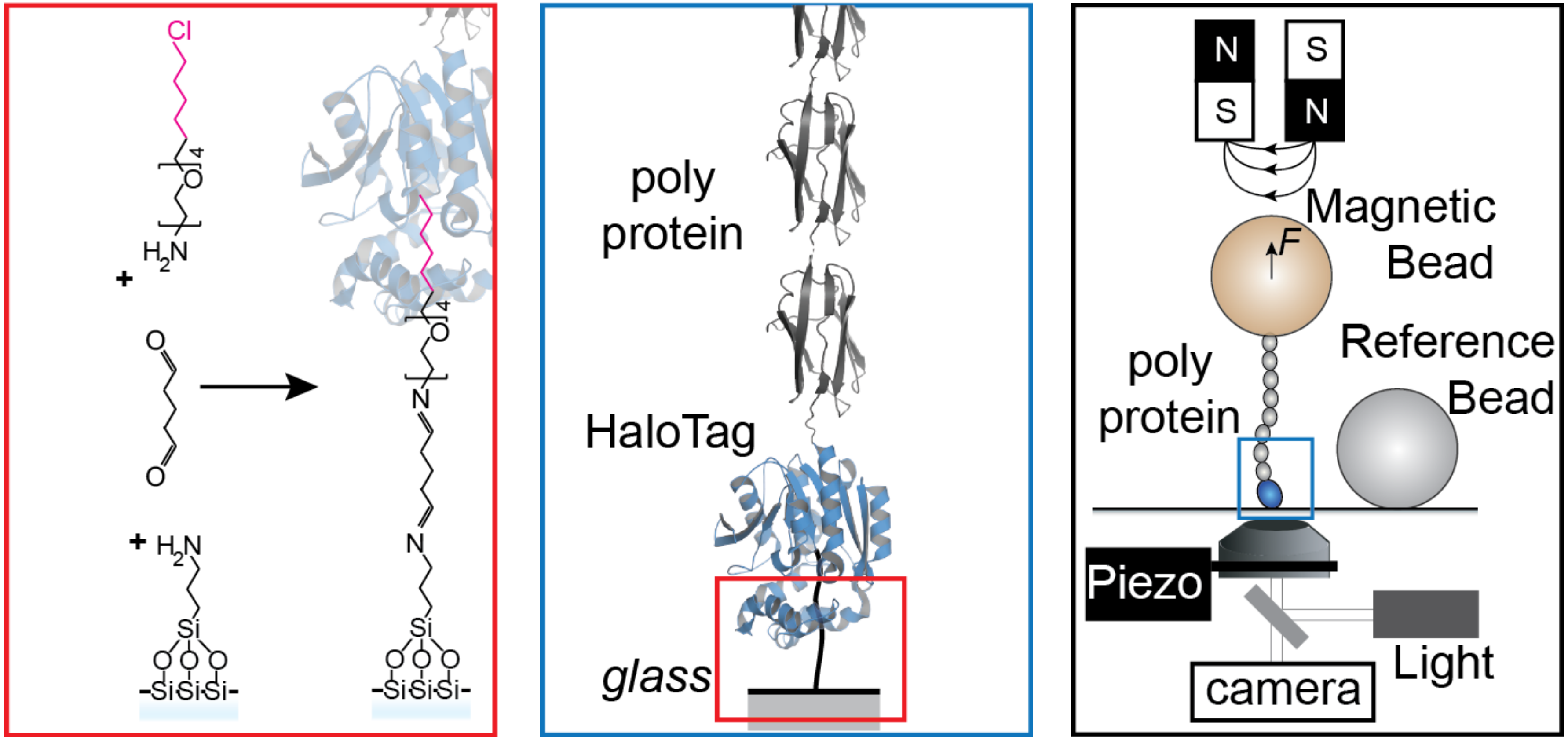


Ionel Popa

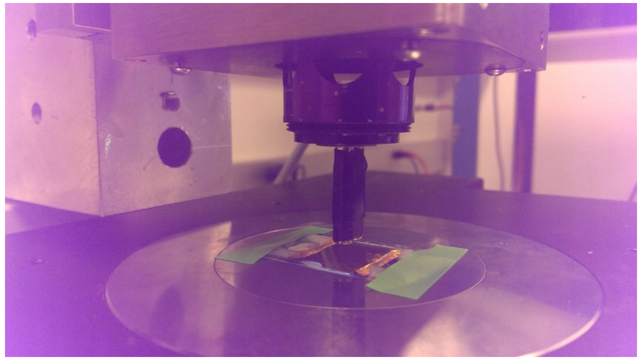


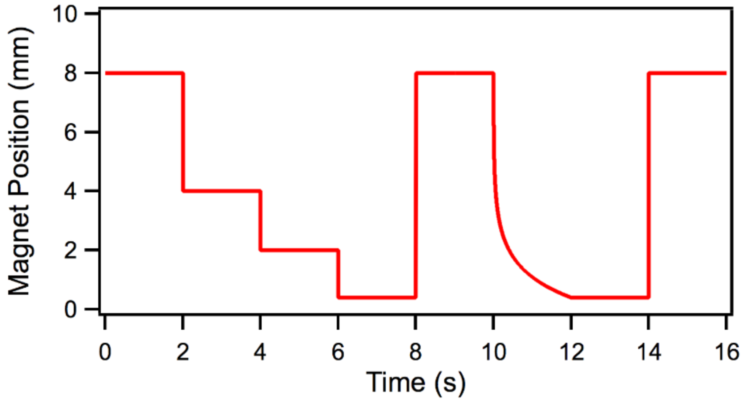


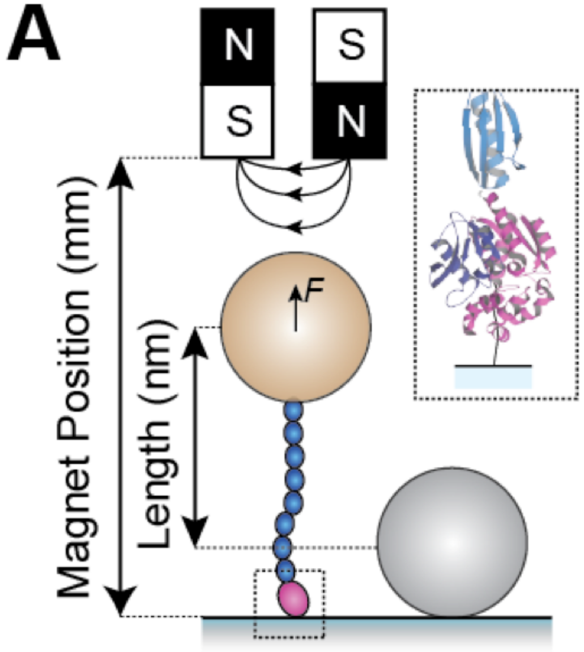
HaloTag anchored polyproteins

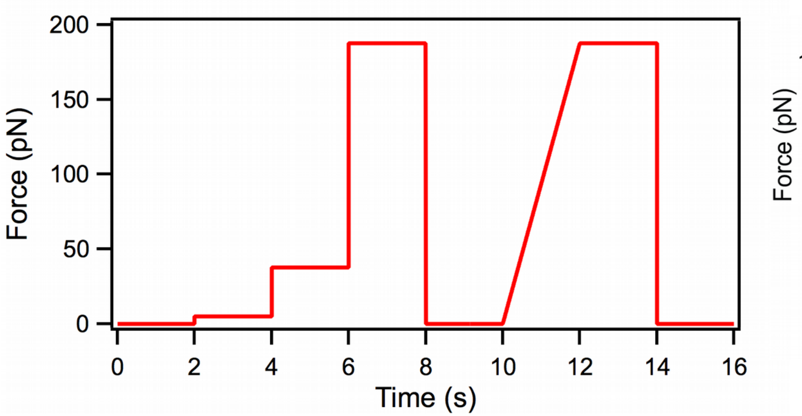


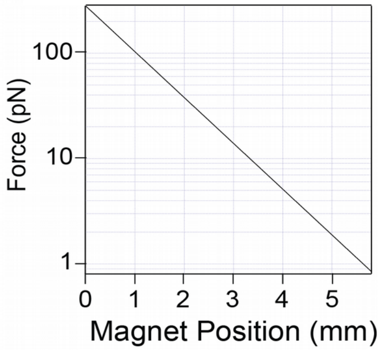
Moving coil and control of magnet position/force

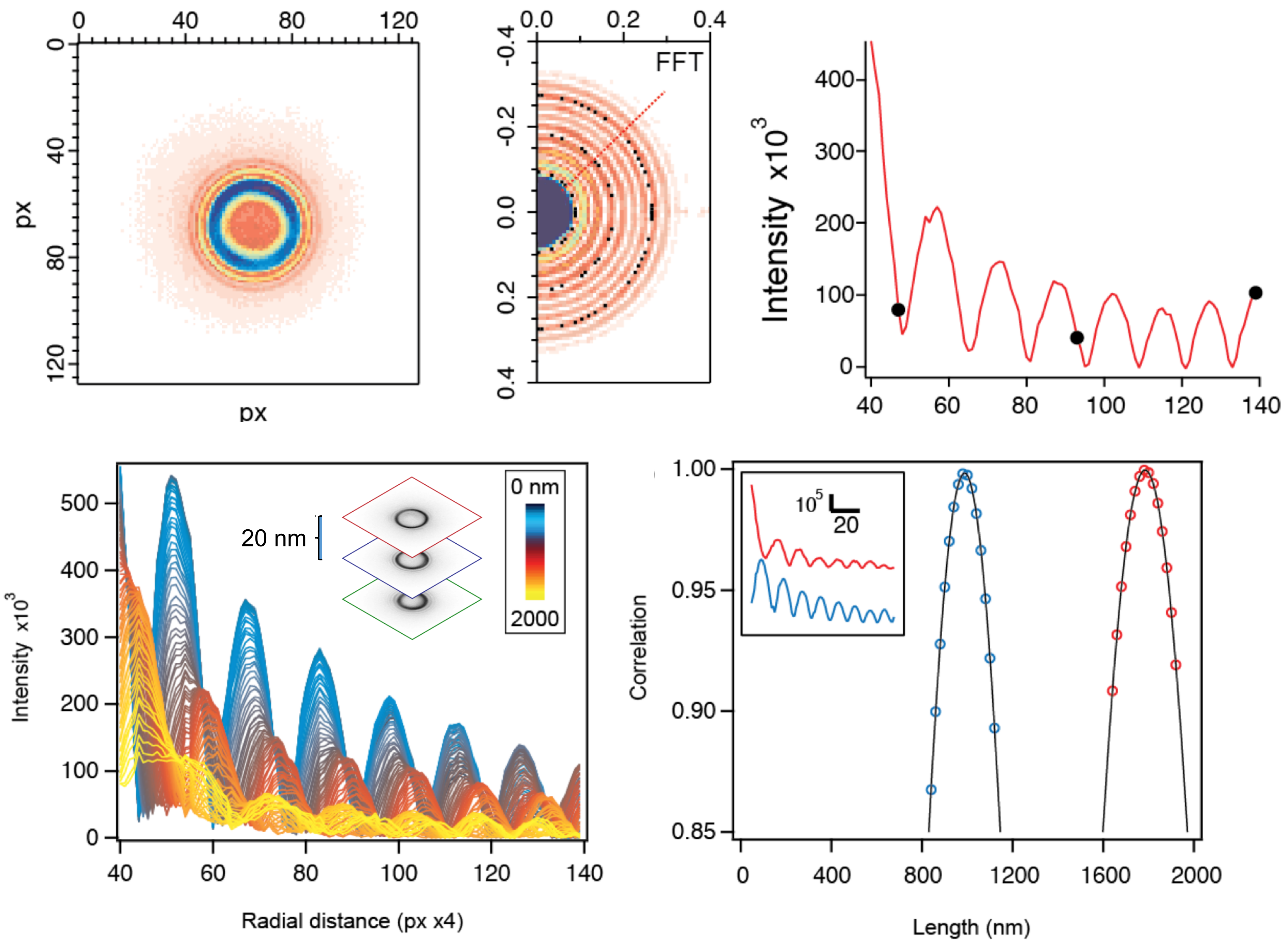






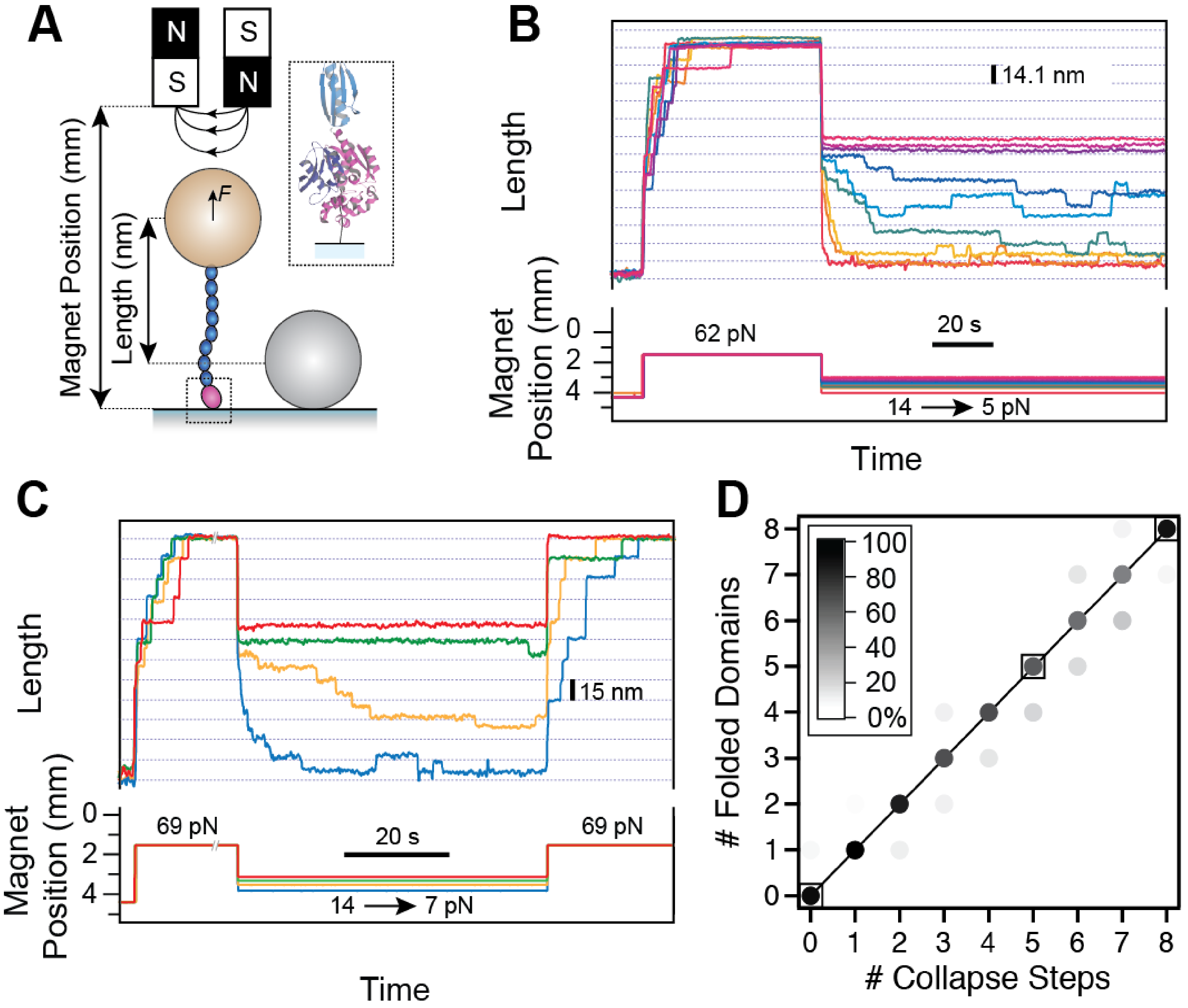




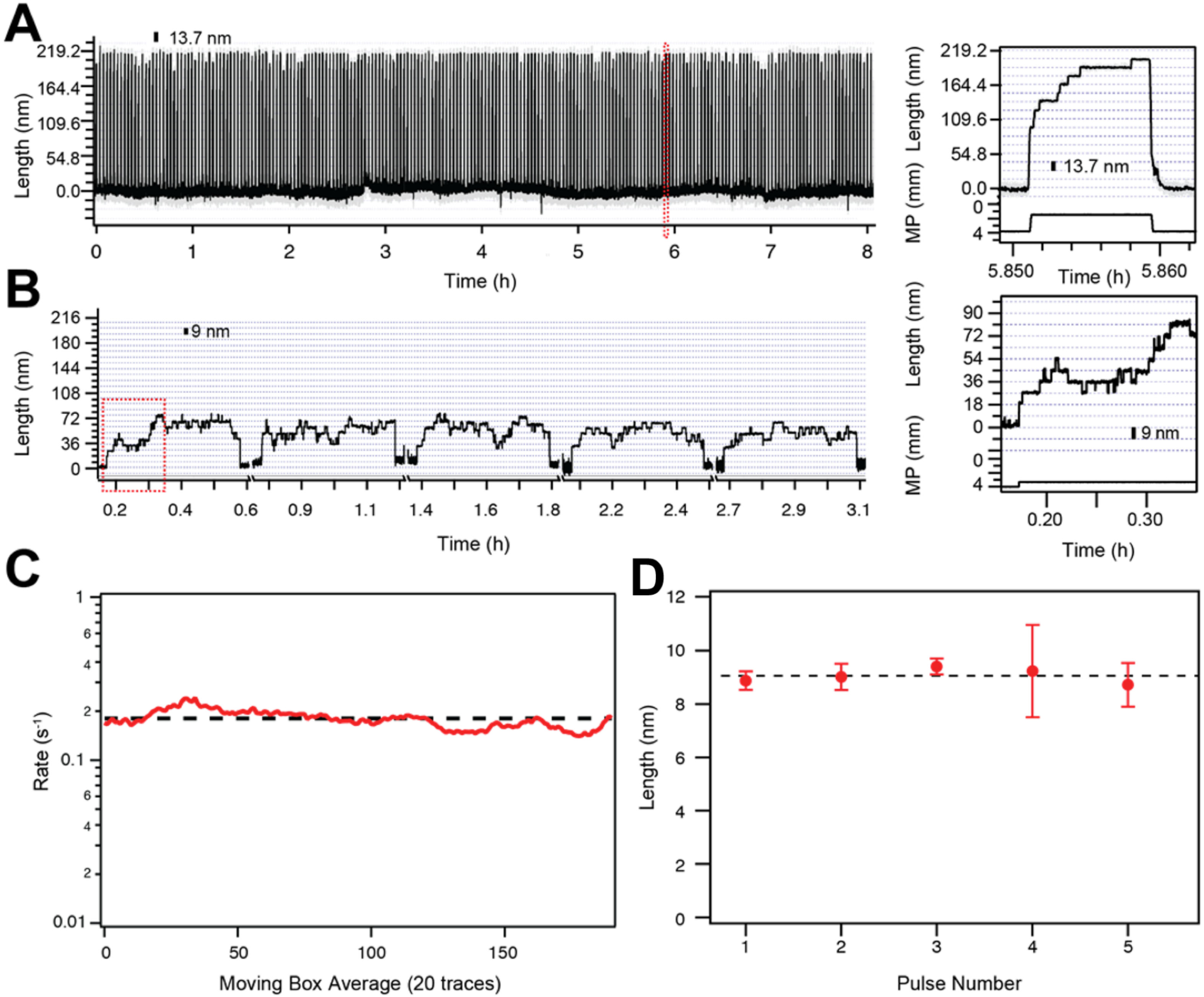


px
FFT
Intensity x10^3
20 nm
0 nm
2000
Radial distance (px x4)
Correlation
10^5
20
Length (nm)

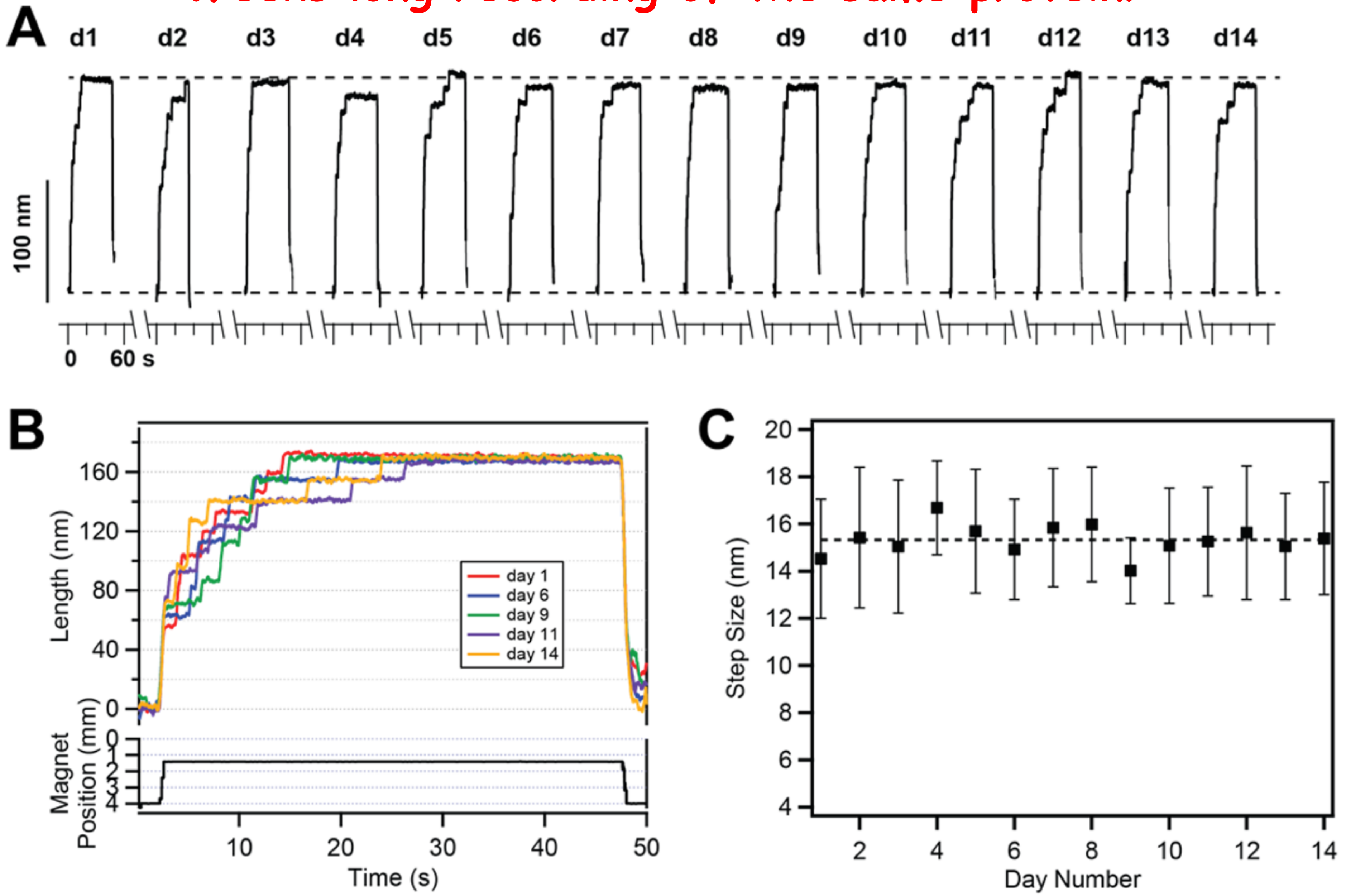
Collapse and folding dynamics of protein L_8



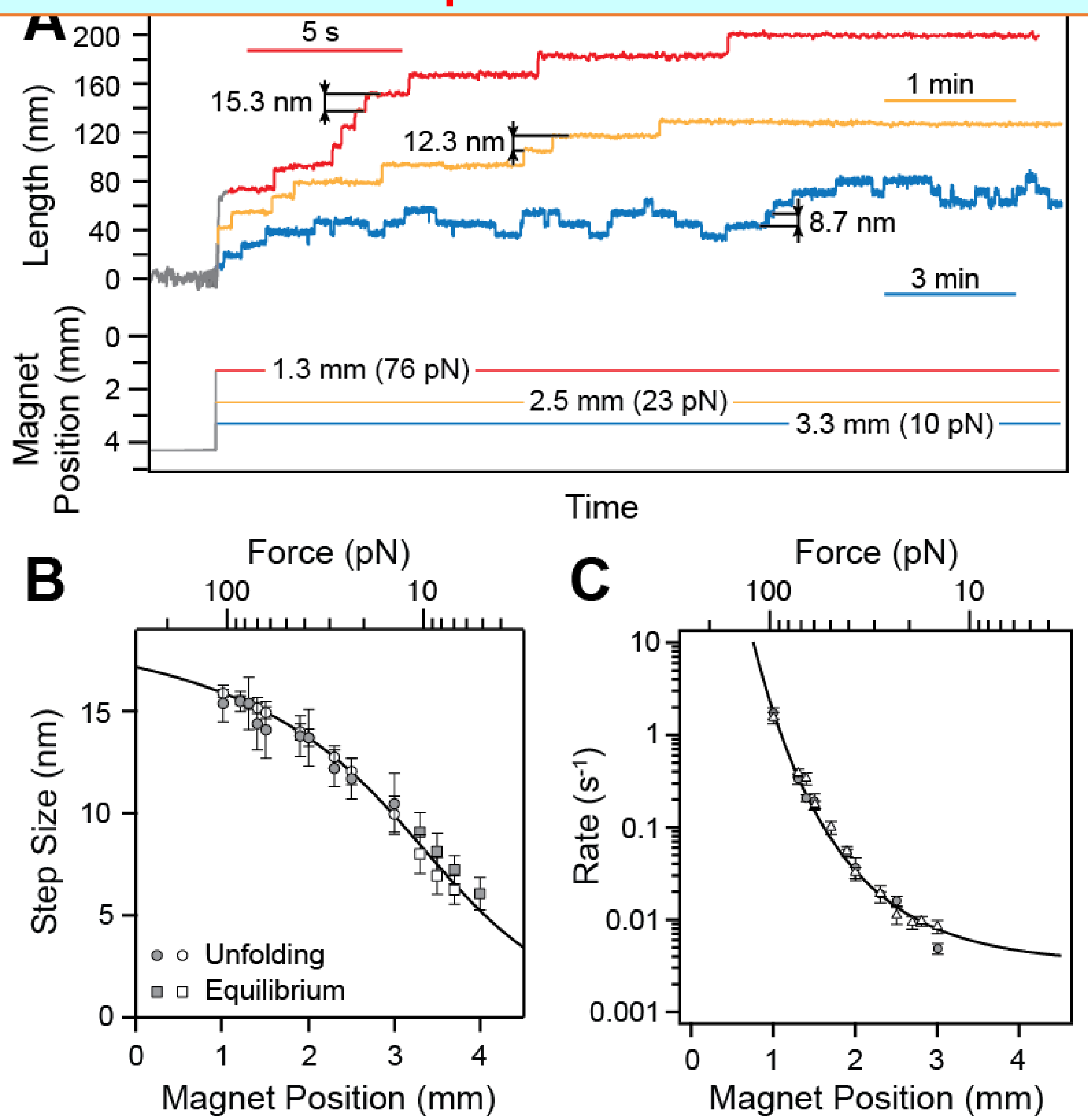
Stable recordings of a single protein



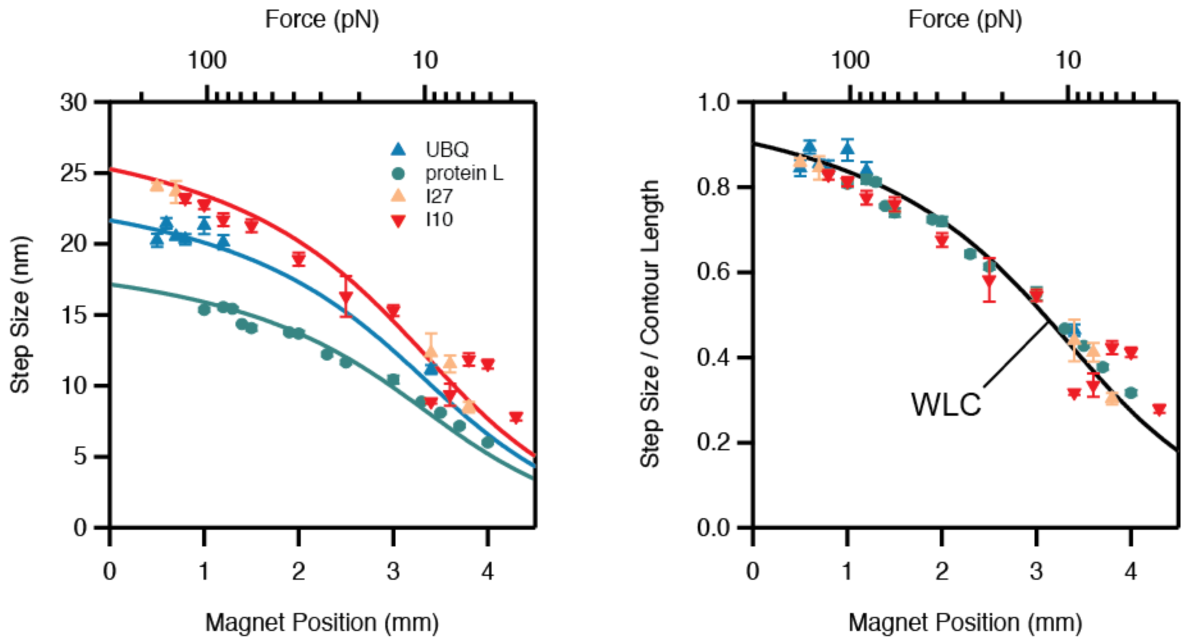
Weeks long recording of the same protein!



Both, the step sizes and rates are force dependent.

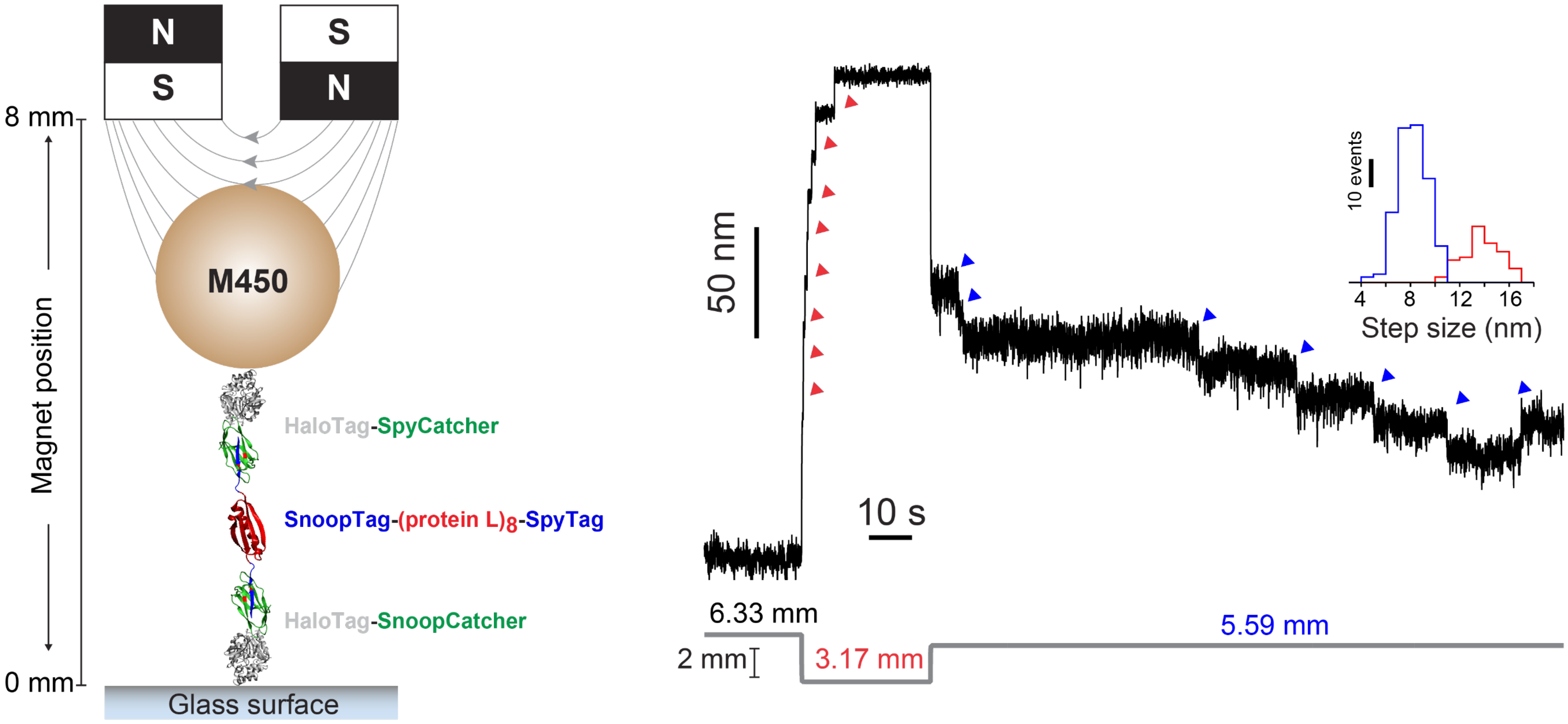


Force-dependent step sizes: a universal property of proteins.

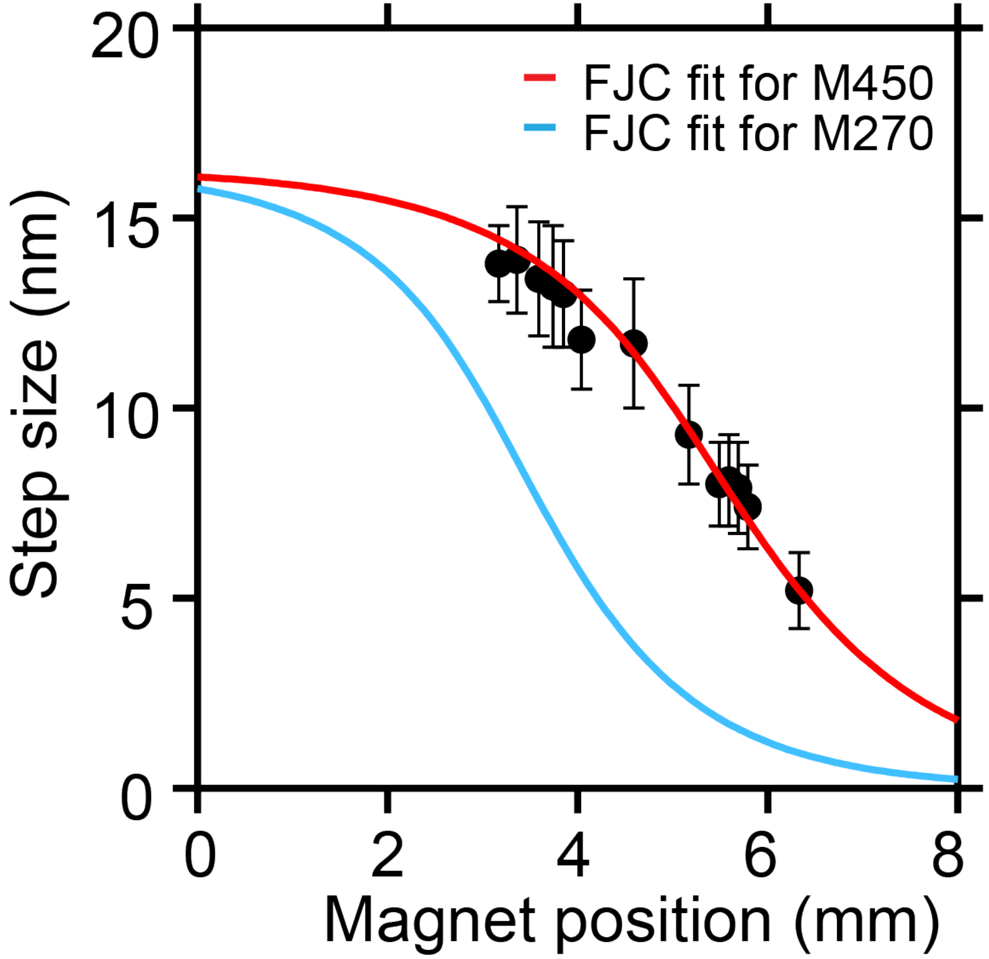


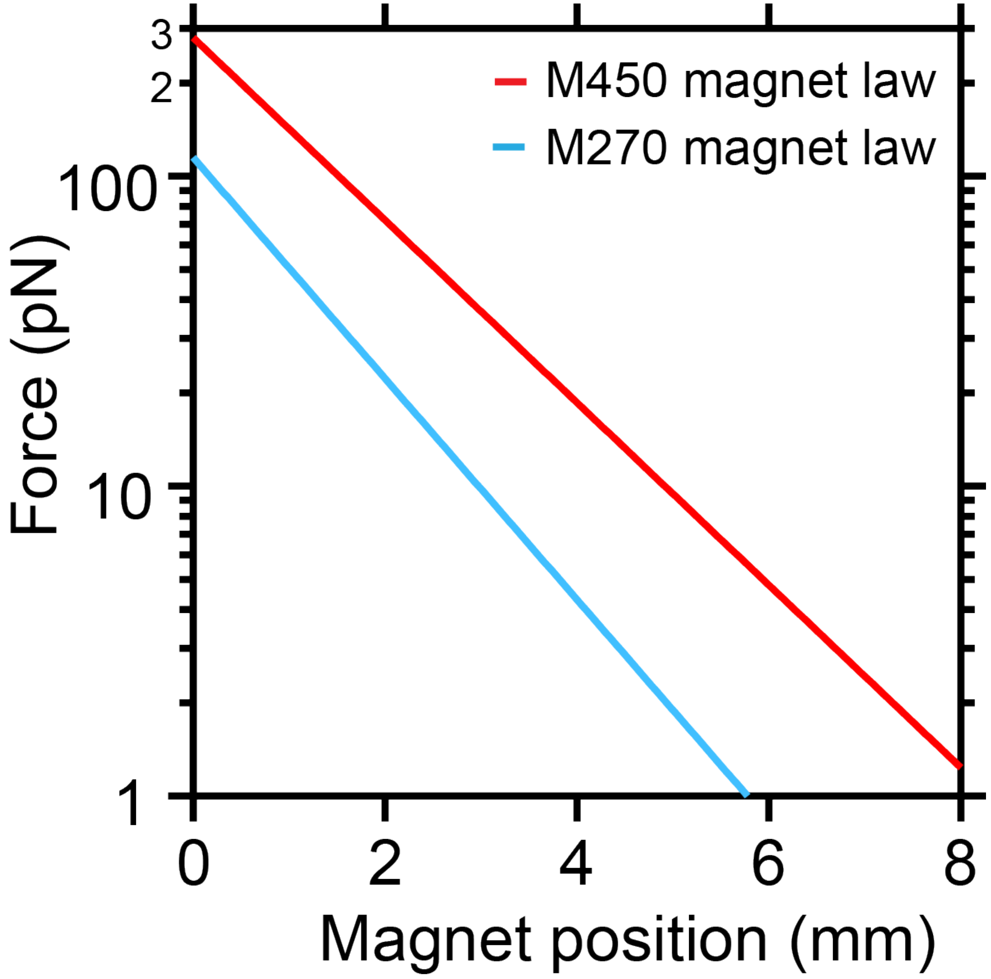
Folding under force is dominated by polymer elasticity!

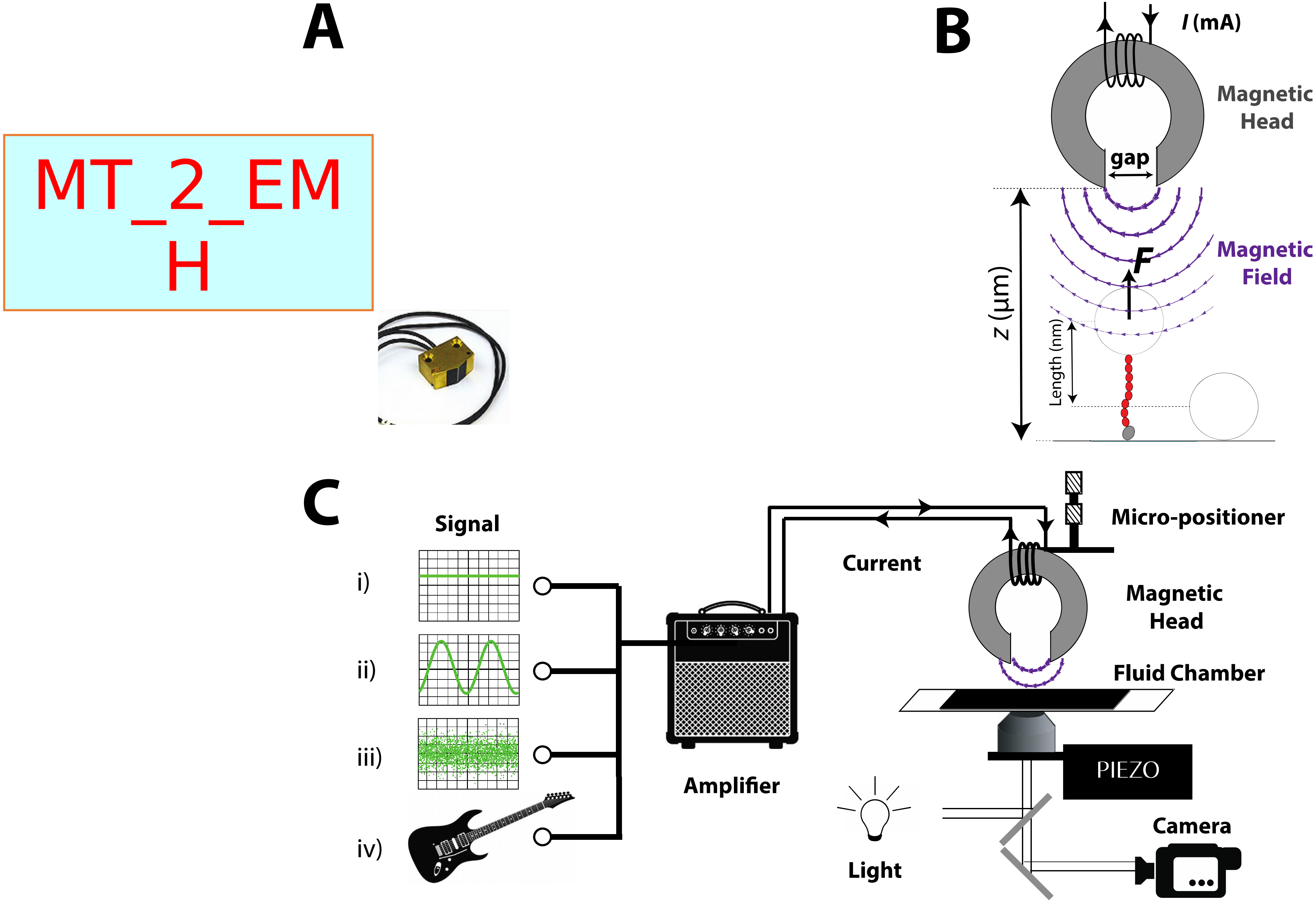
Calibration of M450

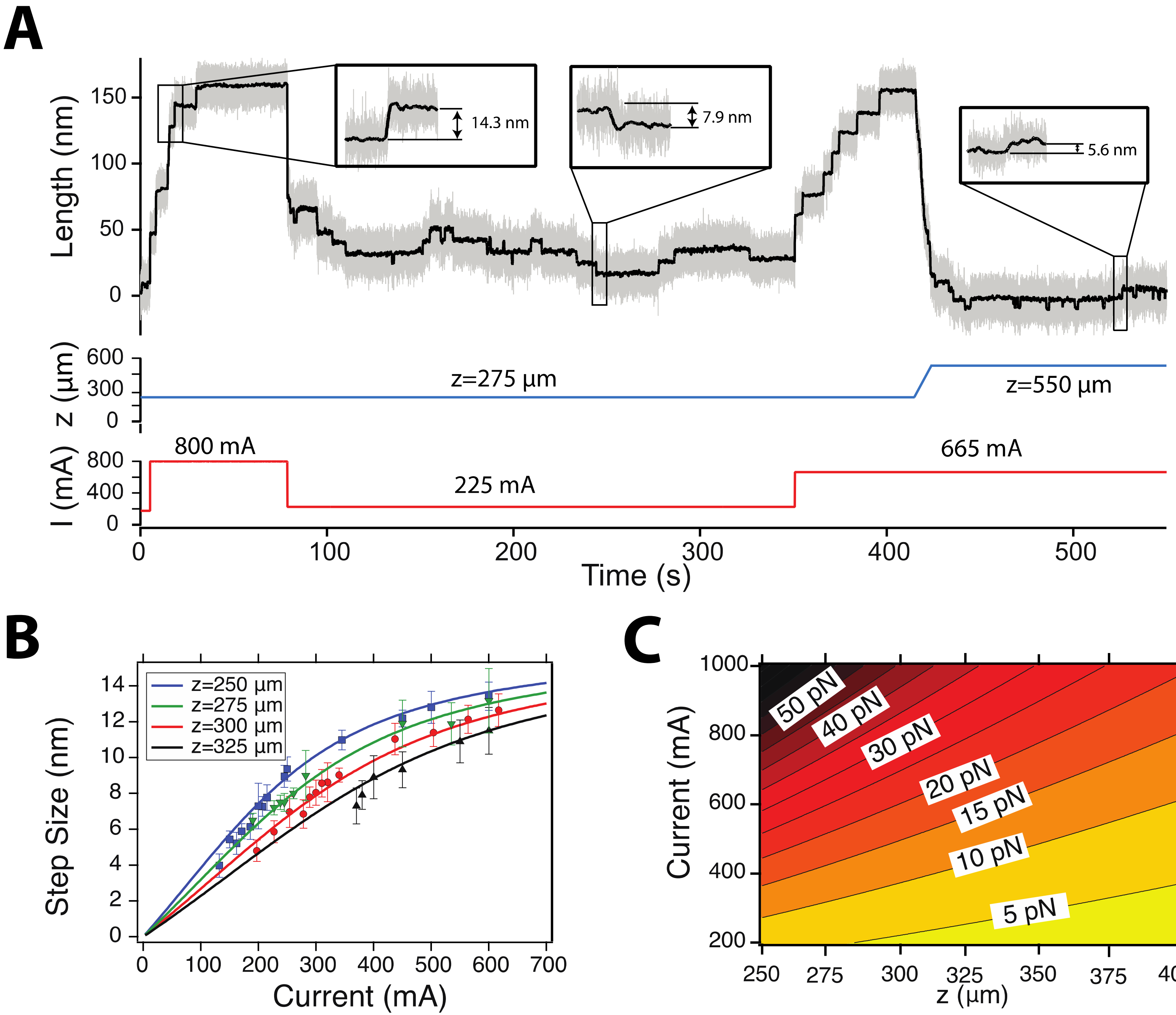


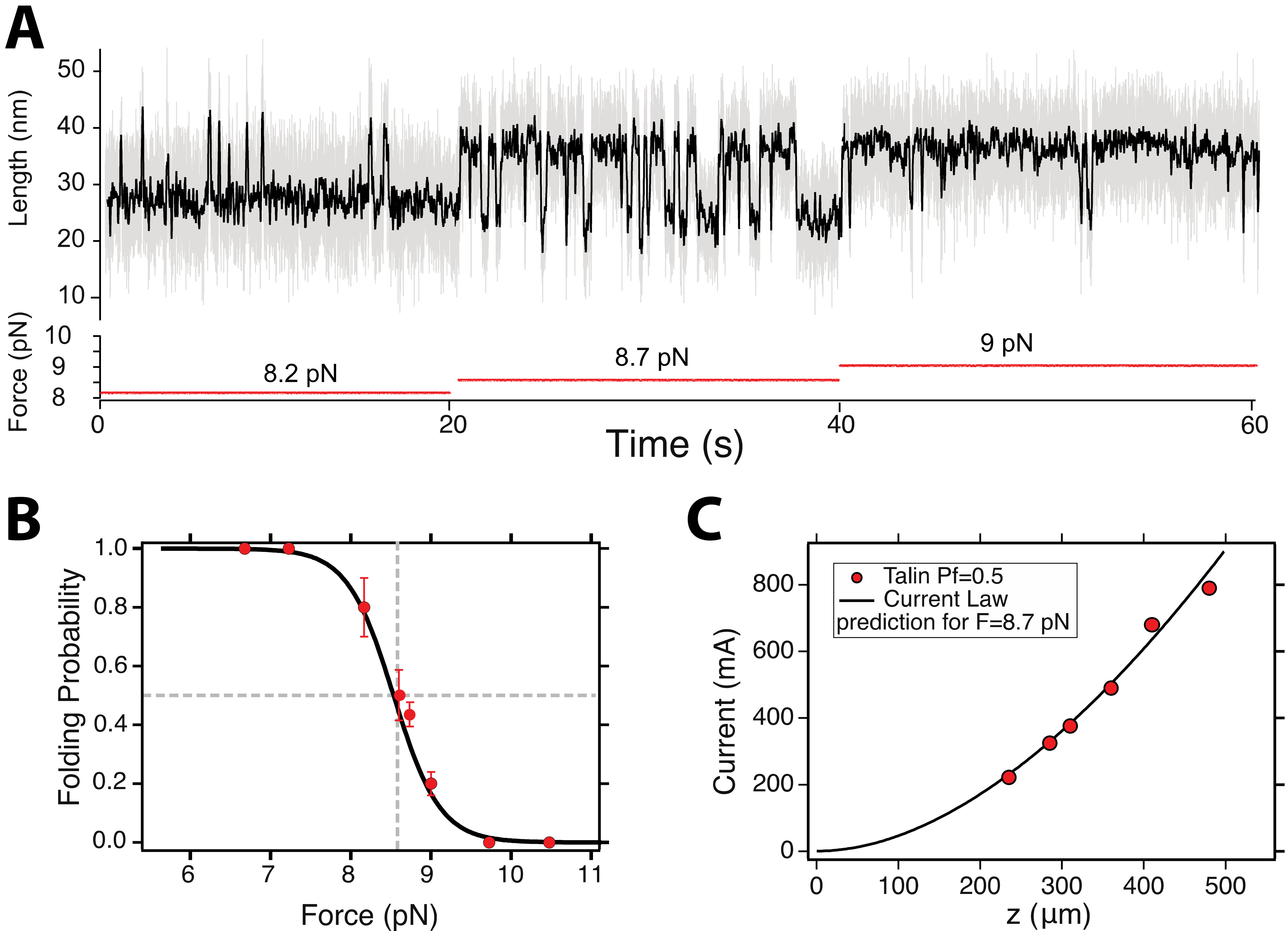
Calibration

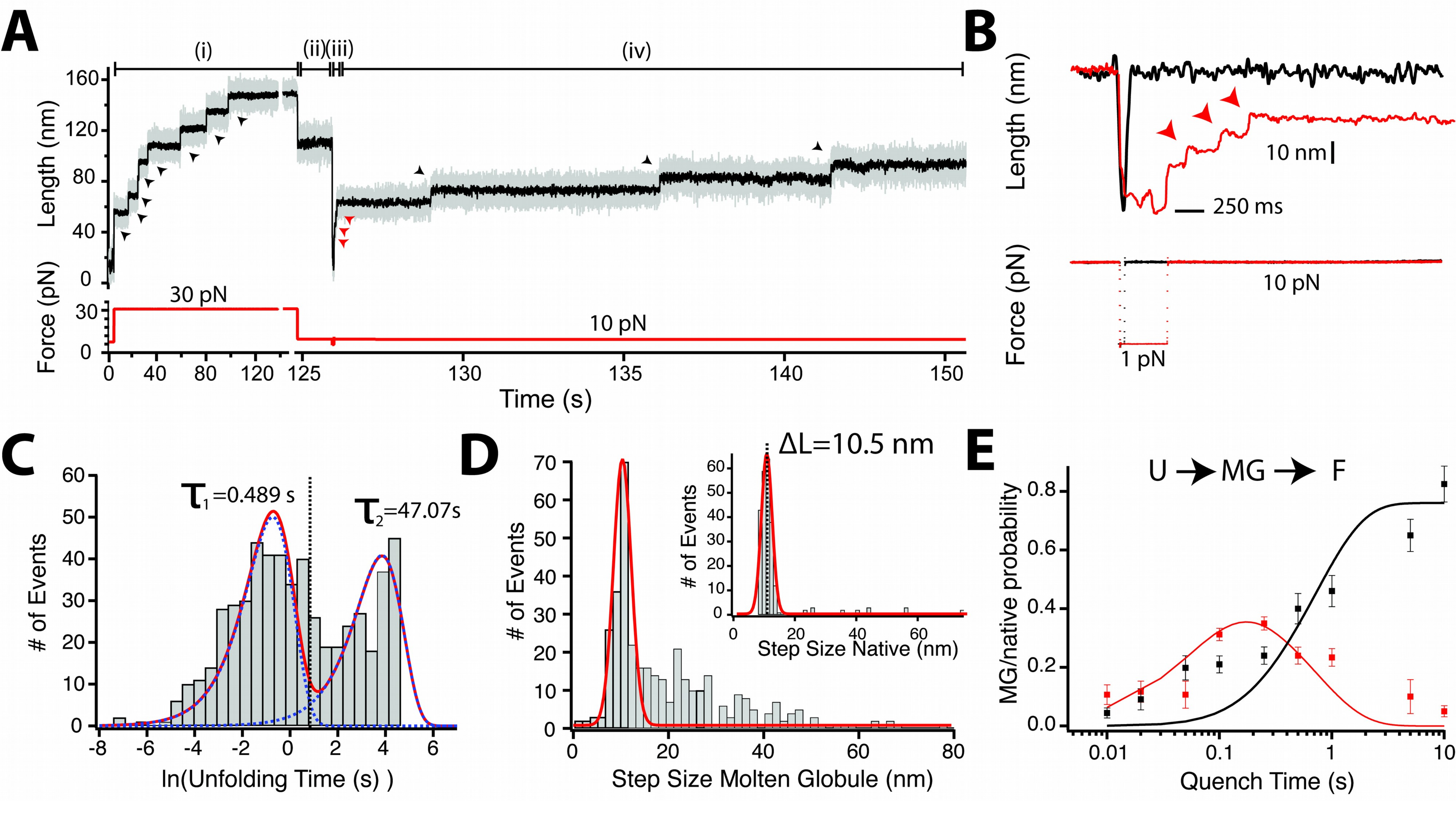





A
MT_2_EM
H
B
I (mA)
Magnetic
Head
gap
Magnetic
Field
F
z (µm)
Length (nm)
C
Signal
i)
ii)
iii)
iv)
Amplifier
Current
Micro-positioner
Magnetic
Head
Fluid Chamber
PIEZO
Light
Camera

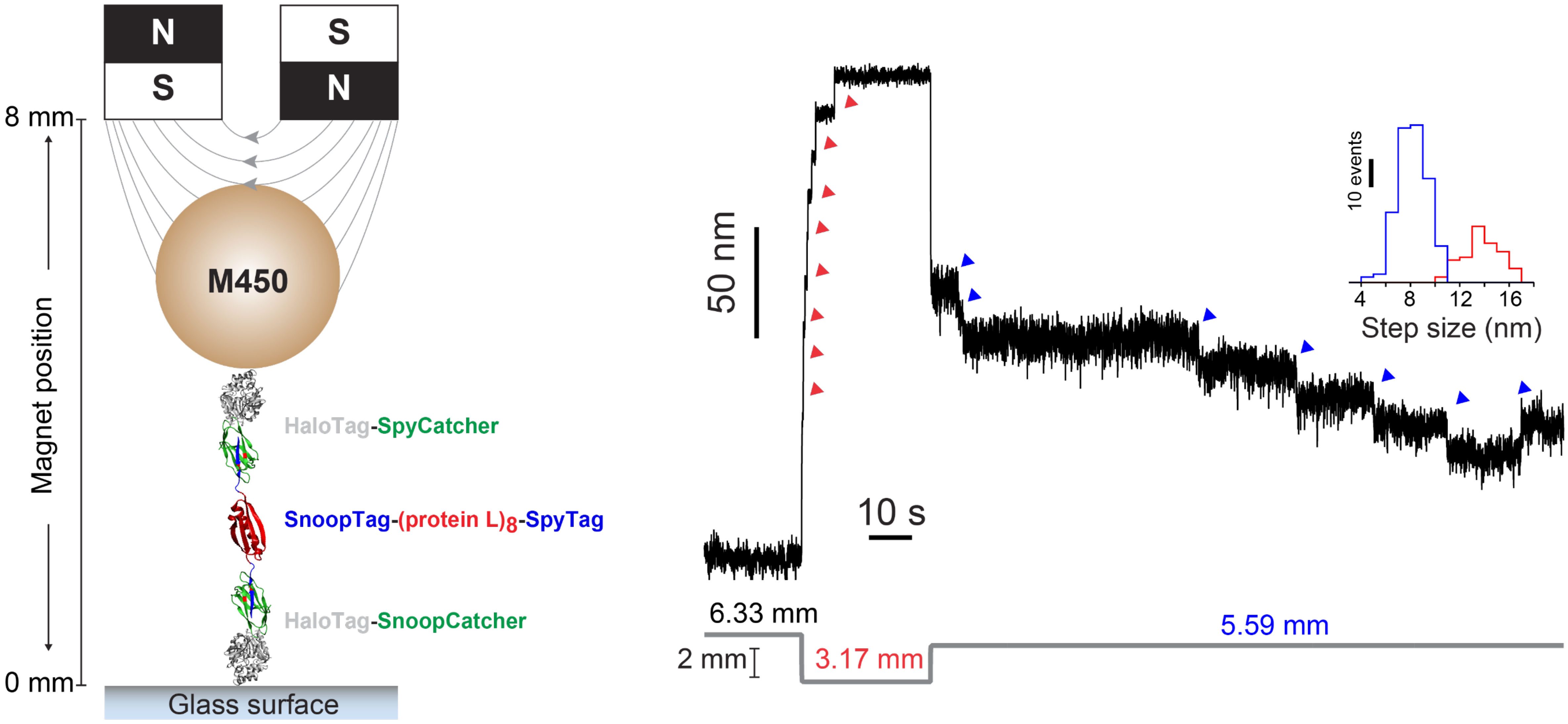

A
Length (nm)
150
100
50
0
14.3 nm
7.9 nm
5.6 nm
z (µm)
600
300
0
z=275 µm
z=550 µm
I (mA)
800
400
0
800 mA
225 mA
665 mA
0
100
200
300
400
500
Time (s)
B
Step Size (nm)
z=250 µm
z=275 µm
z=300 µm
z=325 µm
Current (mA)
C
Current (mA)
z (µm)
50 pN
40 pN
30 pN
20 pN
15 pN
10 pN
5 pN


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)

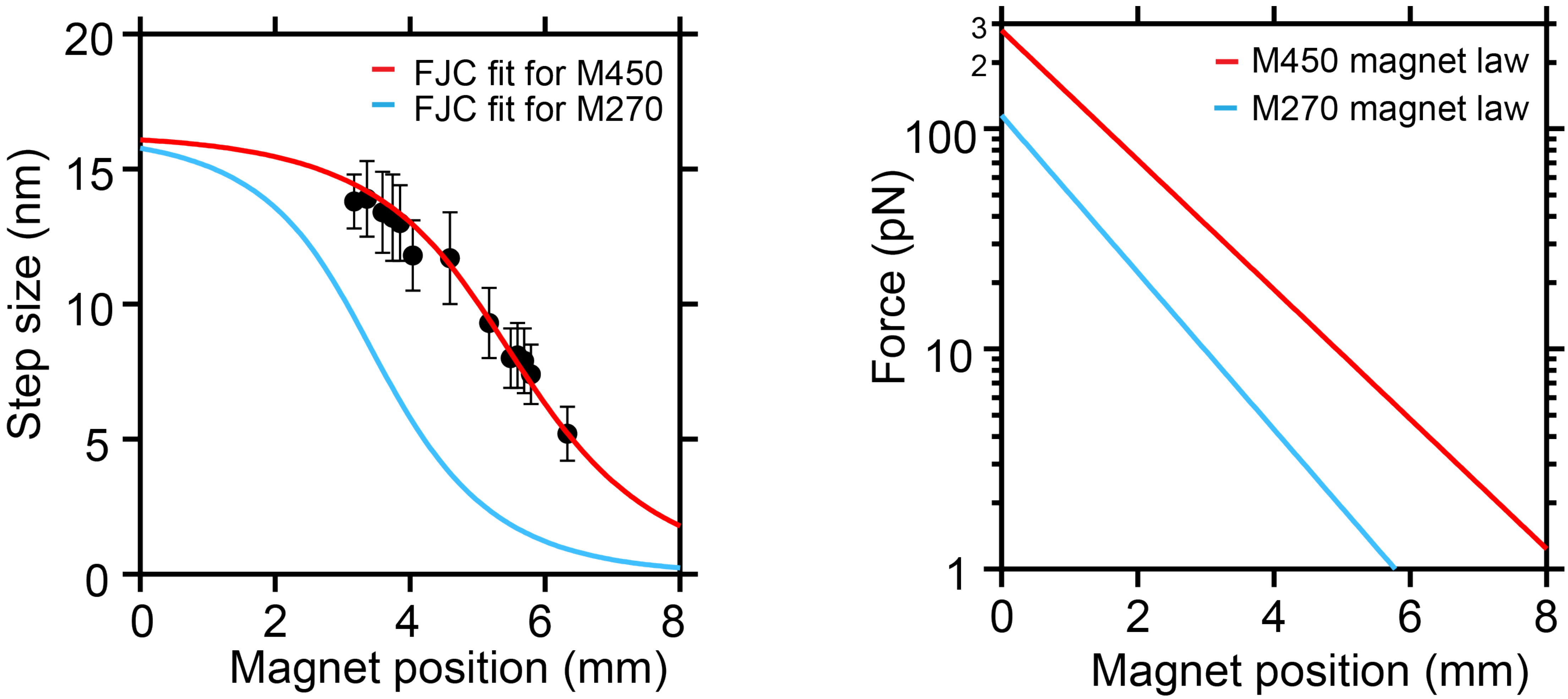


A
(i)
(ii)
(iii)
(iv)
Length (nm)
160
120
80
40
0
30 pN
10 pN
Force (pN)
30
0
0
40
80
120
125
130
135
140
150
Time (s)
B
Length (nm)
10 nm
250 ms
Force (pN)
10 pN
1 pN
C
τ1=0.489 s
τ2=47.07s
# of Events
ln(Unfolding Time (s))
D
ΔL=10.5 nm
# of Events
Step Size Native (nm)
Step Size Molten Globule (nm)
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