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SMR 1564 - 22

SPRING COLLEGE ON SCIENCE AT THE NANOSCALE
(24 May - 11 June 2004)

SINGLE MOLECULE BIOPHYSICS - Part III

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Force-clamp spectroscopy of ubiquitin

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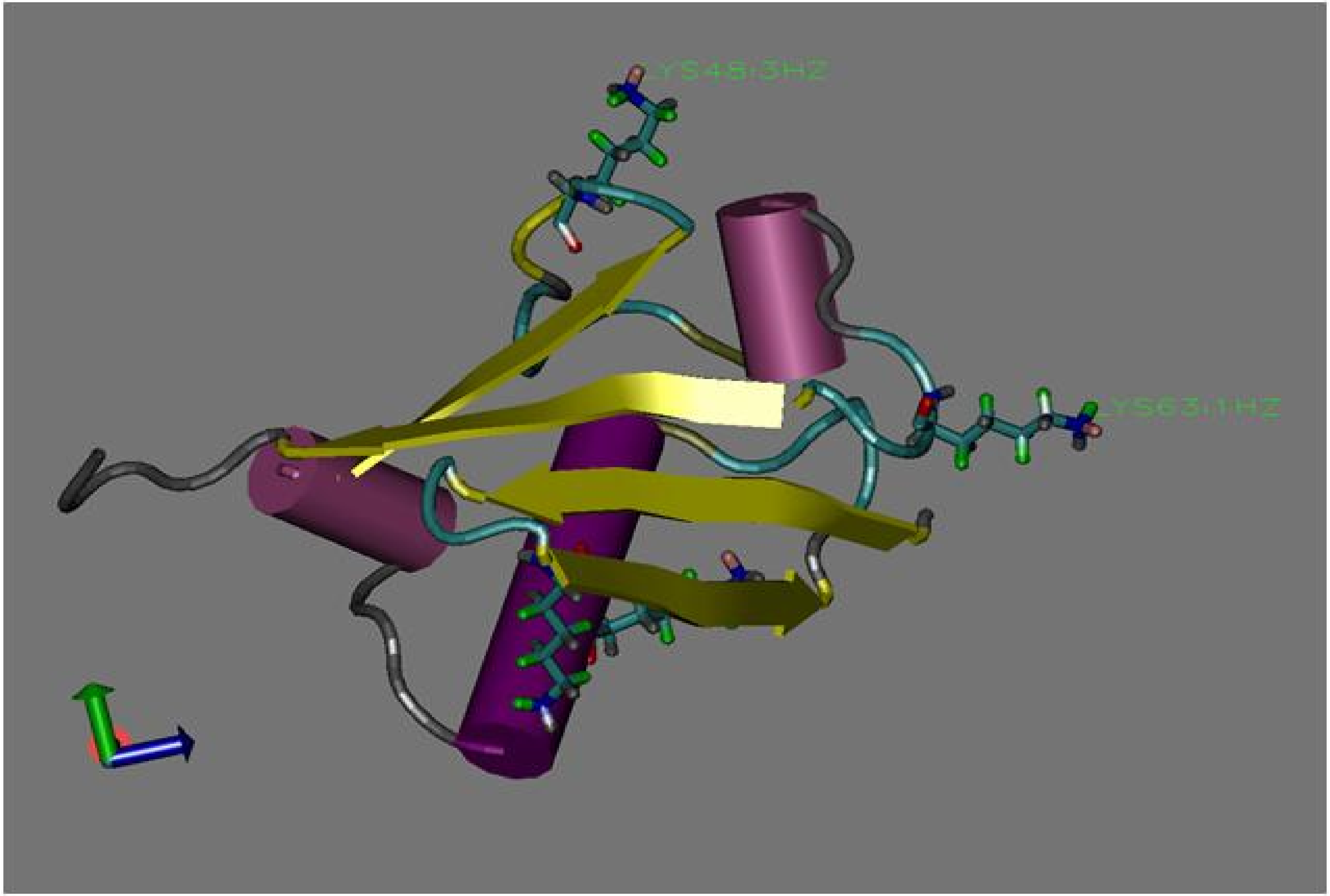
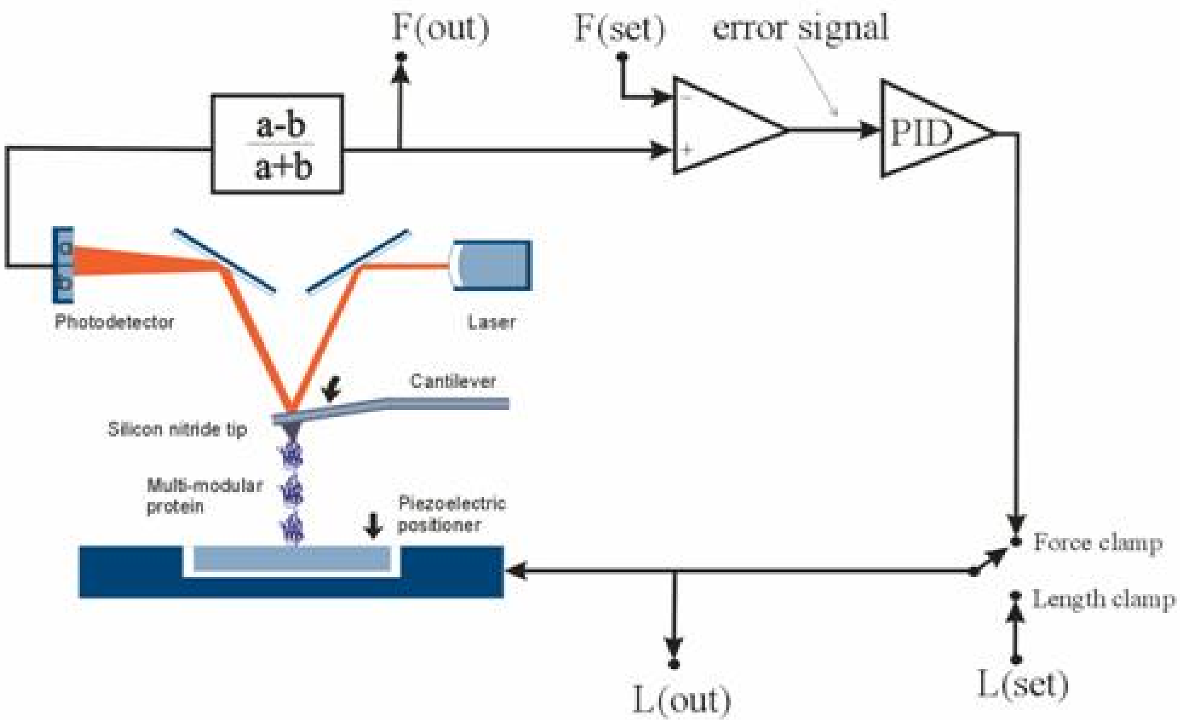
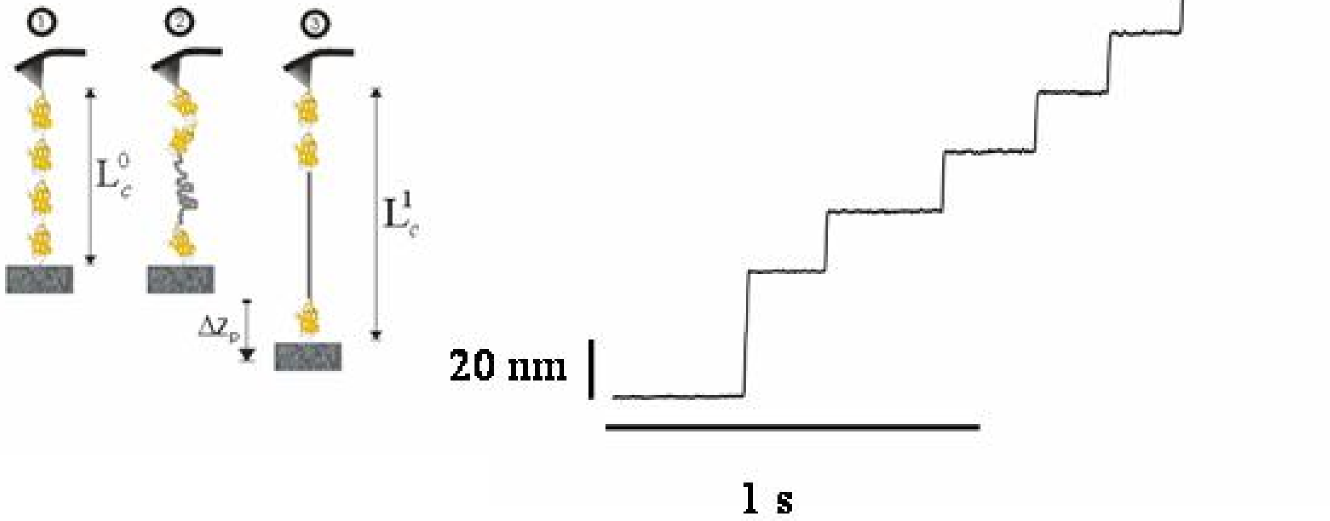
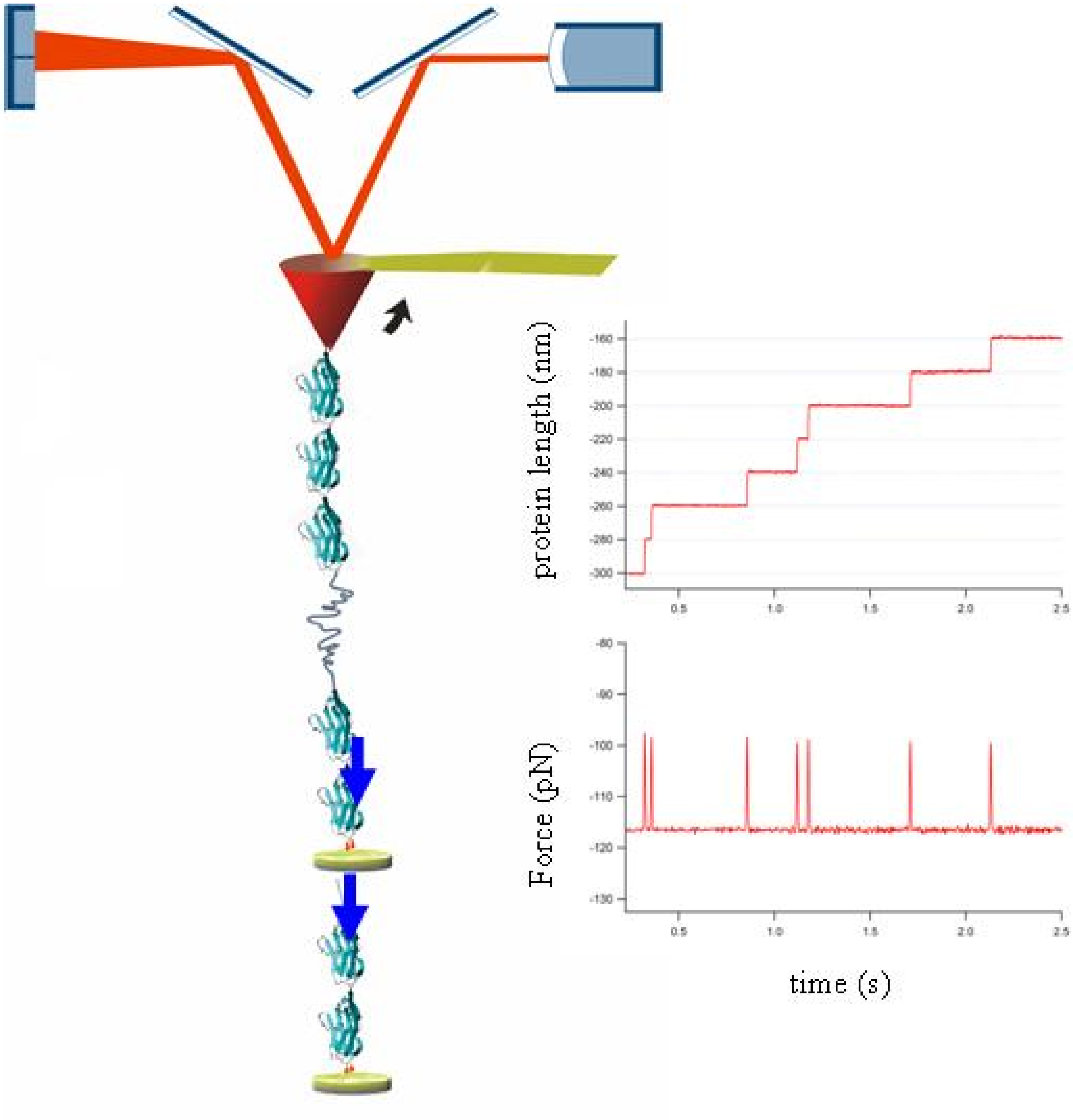
Biological Sciences, Columbia University, New York
and
Physics Department, Ludwig-Maximilians-University
Munich

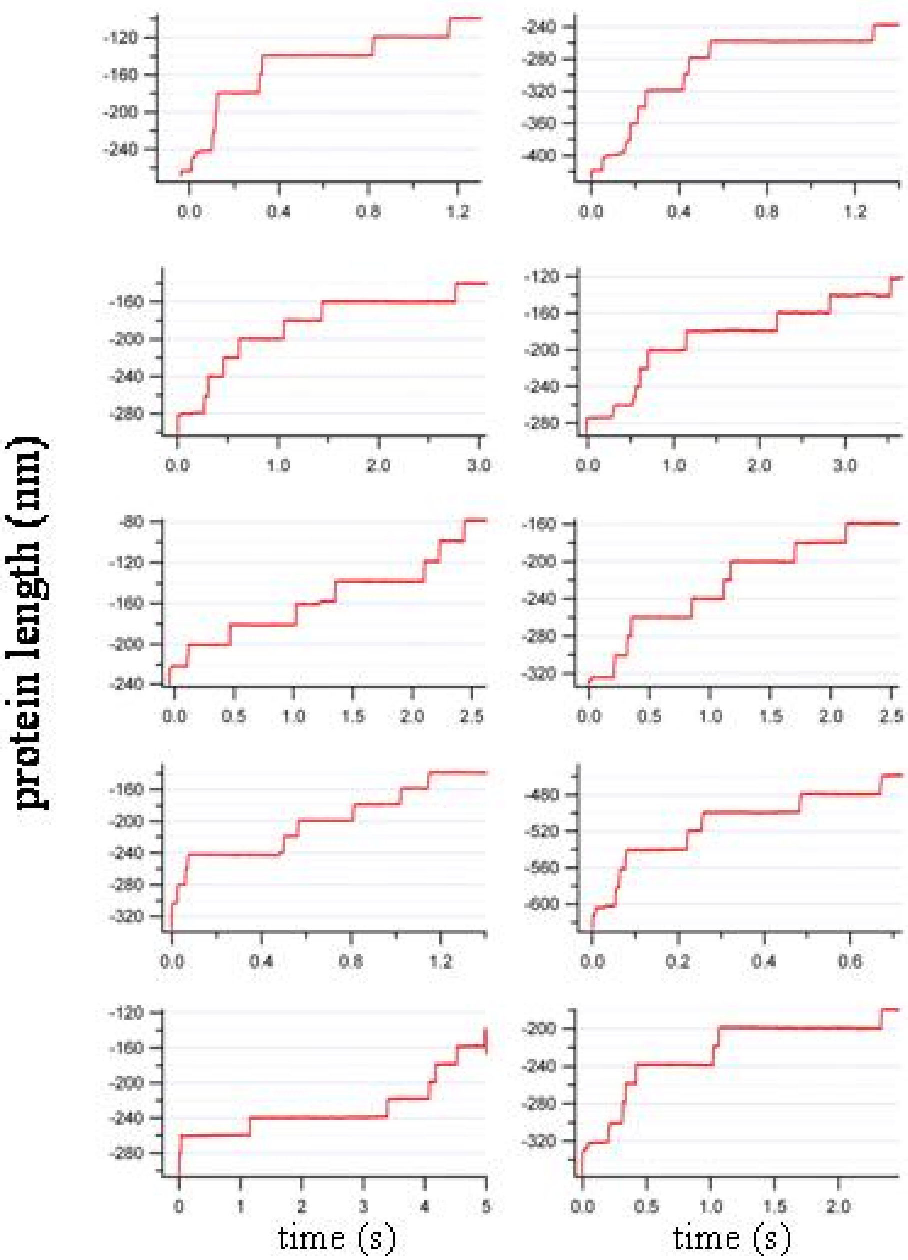

YS48:3HZ
LYS63:1HZ


F(out)
F(set)
error signal
a-b
a+b
−
+
PID
Photodetector
Laser
Cantilever
Silicon nitride tip
Multi-modular protein
Piezoelectric positioner
Force clamp
Length clamp
L(out)
L(set)

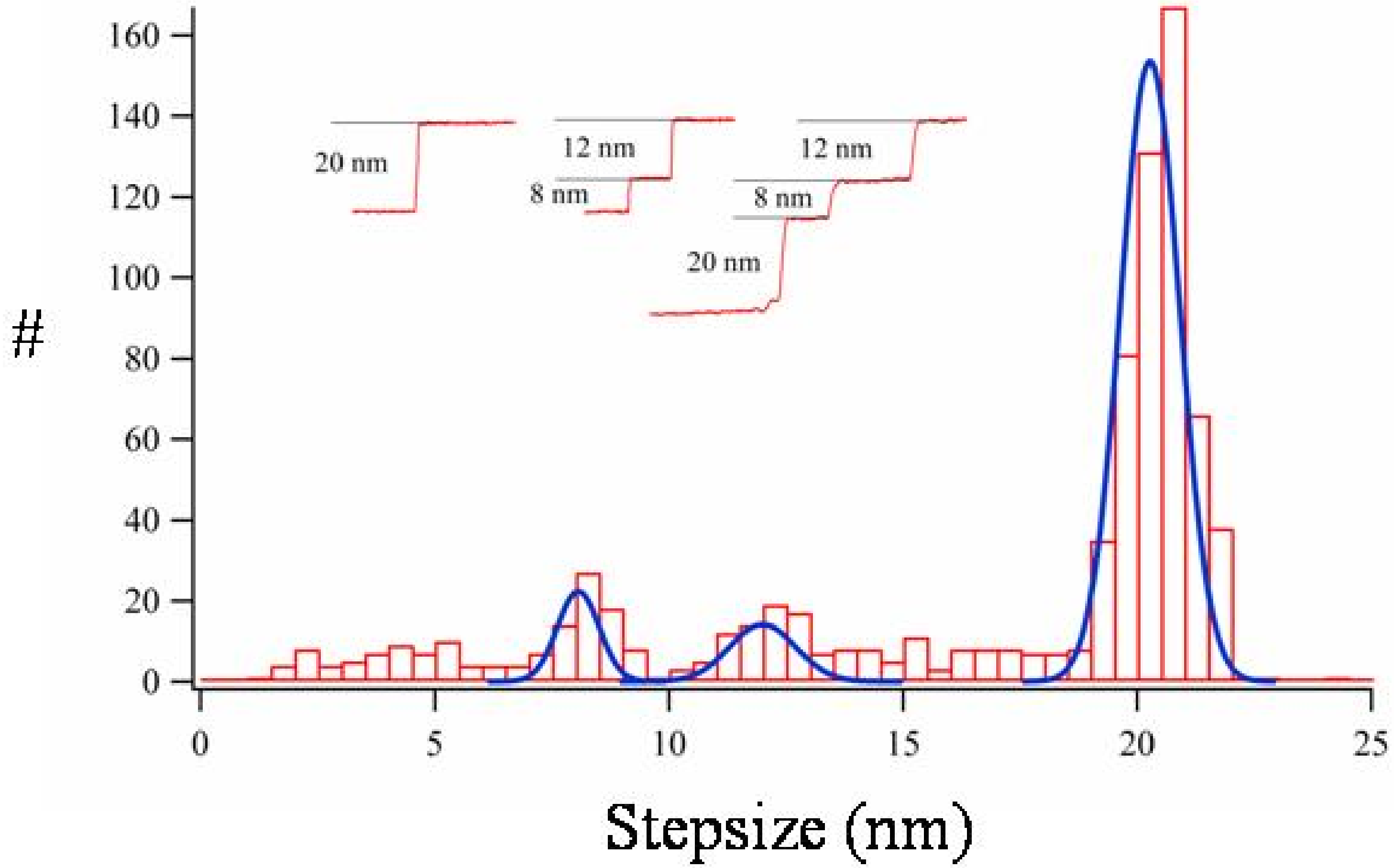

①
②
③
L_c^0
L_c^1
ΔZ_p
20 nm
1 s

Force-clamp

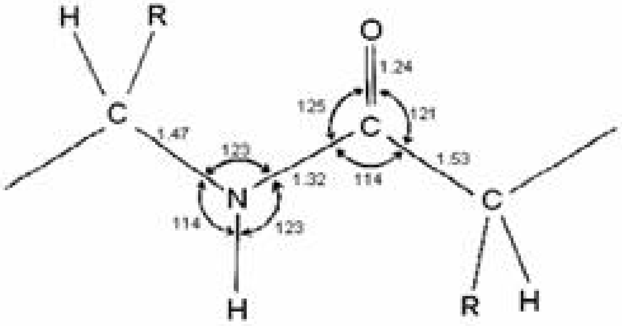

protein length (nm)
-160
-180
-200
-220
-240
-260
-280
-300
0.5
1.0
1.5
2.0
2.5
Force (pN)
-80
-90
-100
-110
-120
-130
0.5
1.0
1.5
2.0
2.5
time (s)

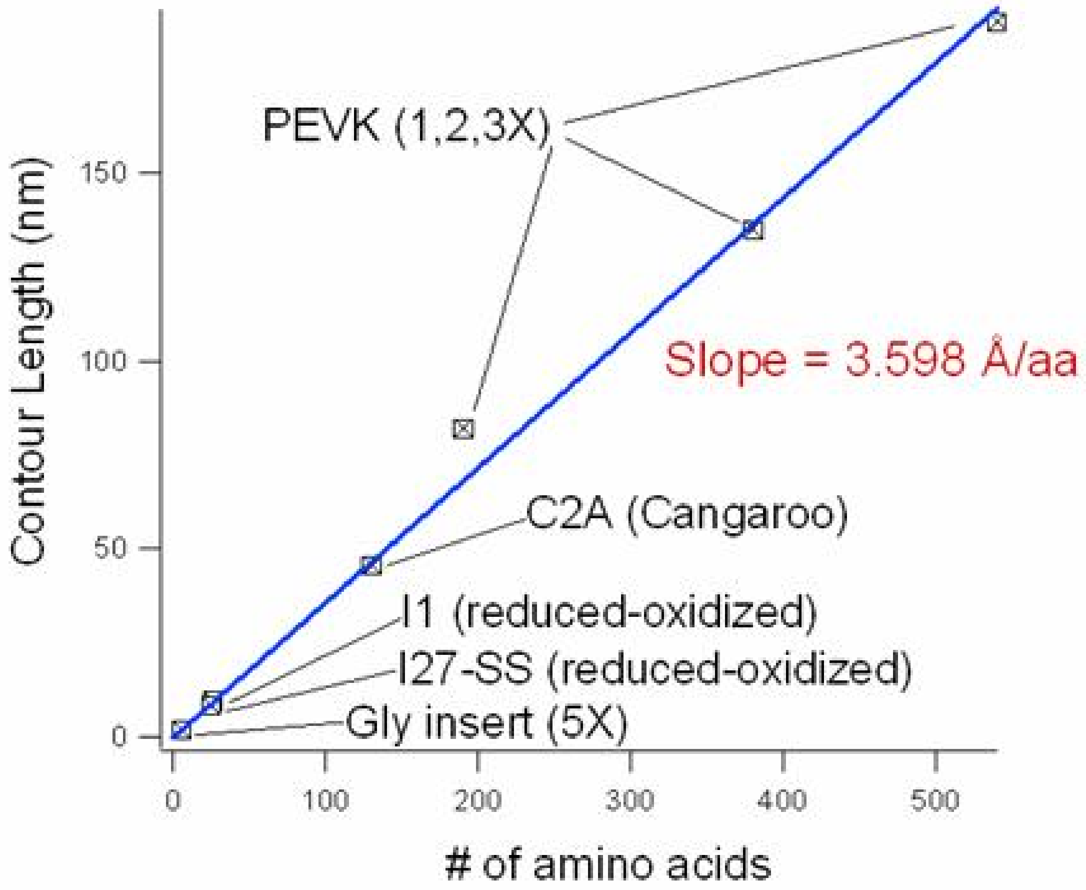

protein length (nm)
-120
-160
-200
-240
0.0
0.4
0.8
1.2
-240
-280
-320
-360
-400
0.0
0.4
0.8
1.2
-160
-200
-240
-280
0.0
1.0
2.0
3.0
-120
-160
-200
-240
-280
0.0
1.0
2.0
3.0
-80
-120
-160
-200
-240
0.0
0.5
1.0
1.5
2.0
2.5
-160
-200
-240
-280
-320
0.0
0.5
1.0
1.5
2.0
2.5
-160
-200
-240
-280
-320
0.0
0.4
0.8
1.2
-480
-520
-560
-600
0.0
0.2
0.4
0.6
-120
-160
-200
-240
-280
0
1
2
3
4
5
time (s)
-200
-240
-280
-320
0.0
0.5
1.0
1.5
2.0
time (s)

Igor Demonstration of actual data

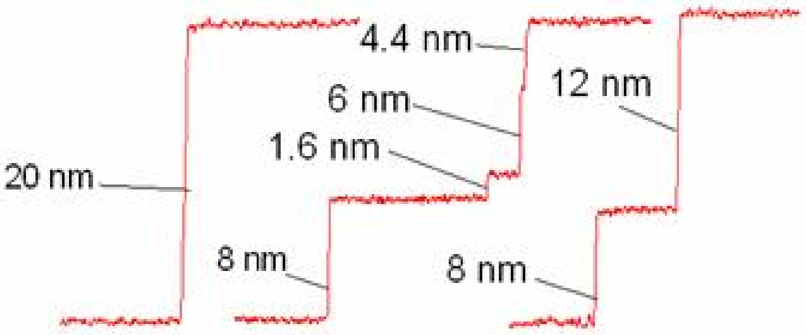


#
160
140
120
100
80
60
40
20
0
0
5
10
15
20
25
Stepsize (nm)
20 nm
12 nm
8 nm
12 nm
8 nm
20 nm


H
R
C
1.47
123
N
114
123
H
1.32
125
C
O
1.24
121
114
1.53
C
R
H

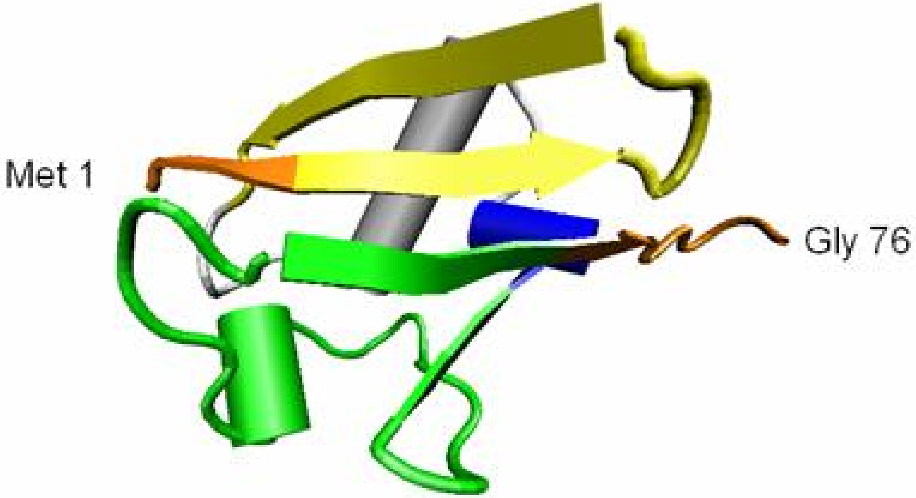

PEVK (1,2,3X)
Slope = 3.598 Å/aa
C2A (Cangaroo)
I1 (reduced-oxidized)
I27-SS (reduced-oxidized)
Gly insert (5X)
Contour Length (nm)
0
50
100
150
0
100
200
300
400
500
# of amino acids

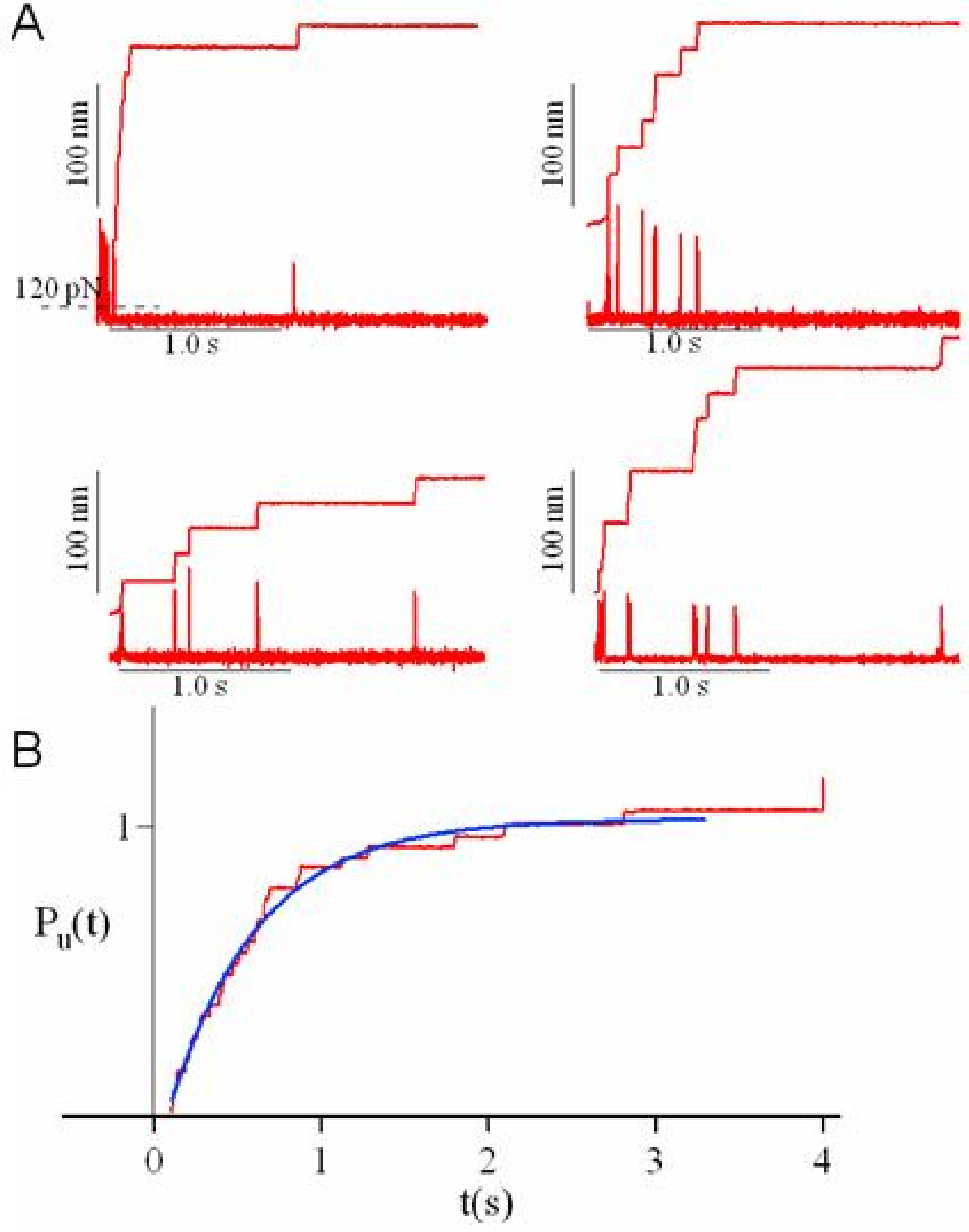
at F>> we get a contour length increment of 3.6 Å/aa, however, at F=100 pN the WLC model predicts 3 Å/aa



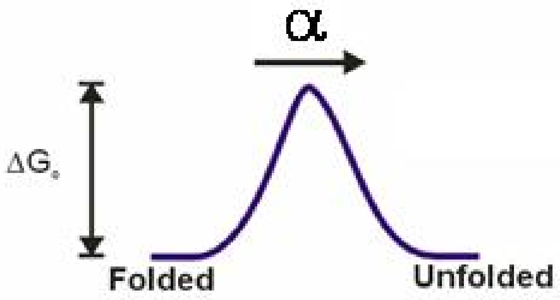
linker = 9 aa (1-3;71-76,orange)
8 nm = 27 aa (43-69,green)
1.6 nm = 5 aa (38-42,blue)
6 nm = 20 aa (18-37,grey)
4.4 nm = 15 aa (4-17,yellow)

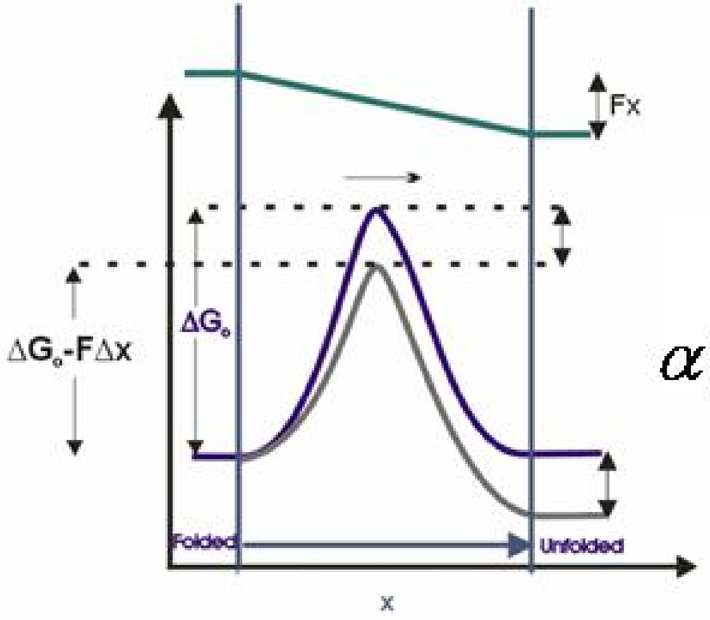
20 nm = 67 aa



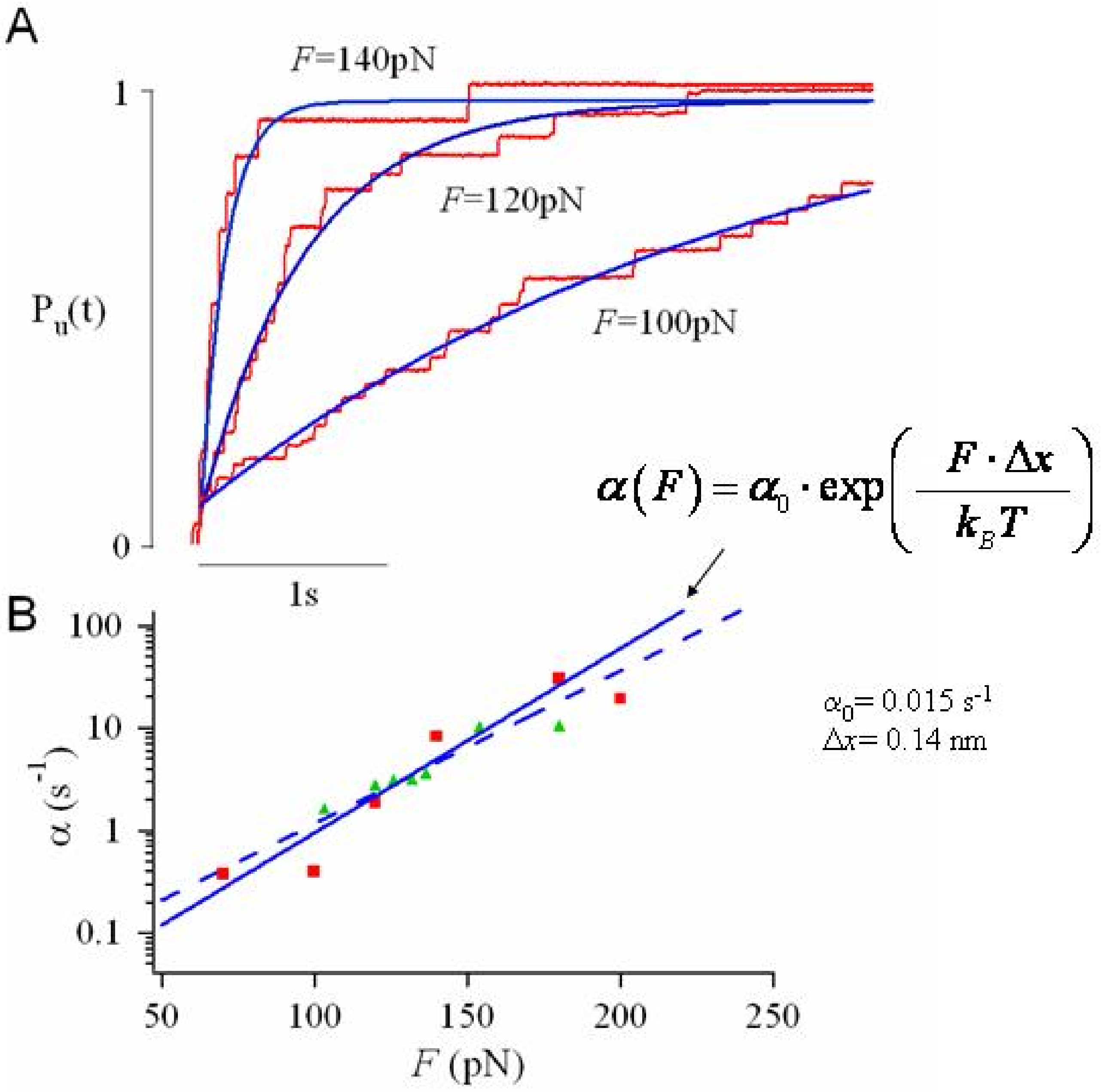

A
100 nm
120 pN
1.0 s
100 nm
1.0 s
100 nm
1.0 s
100 nm
1.0 s
B
1
P_u(t)
0
1
2
3
4
t(s)

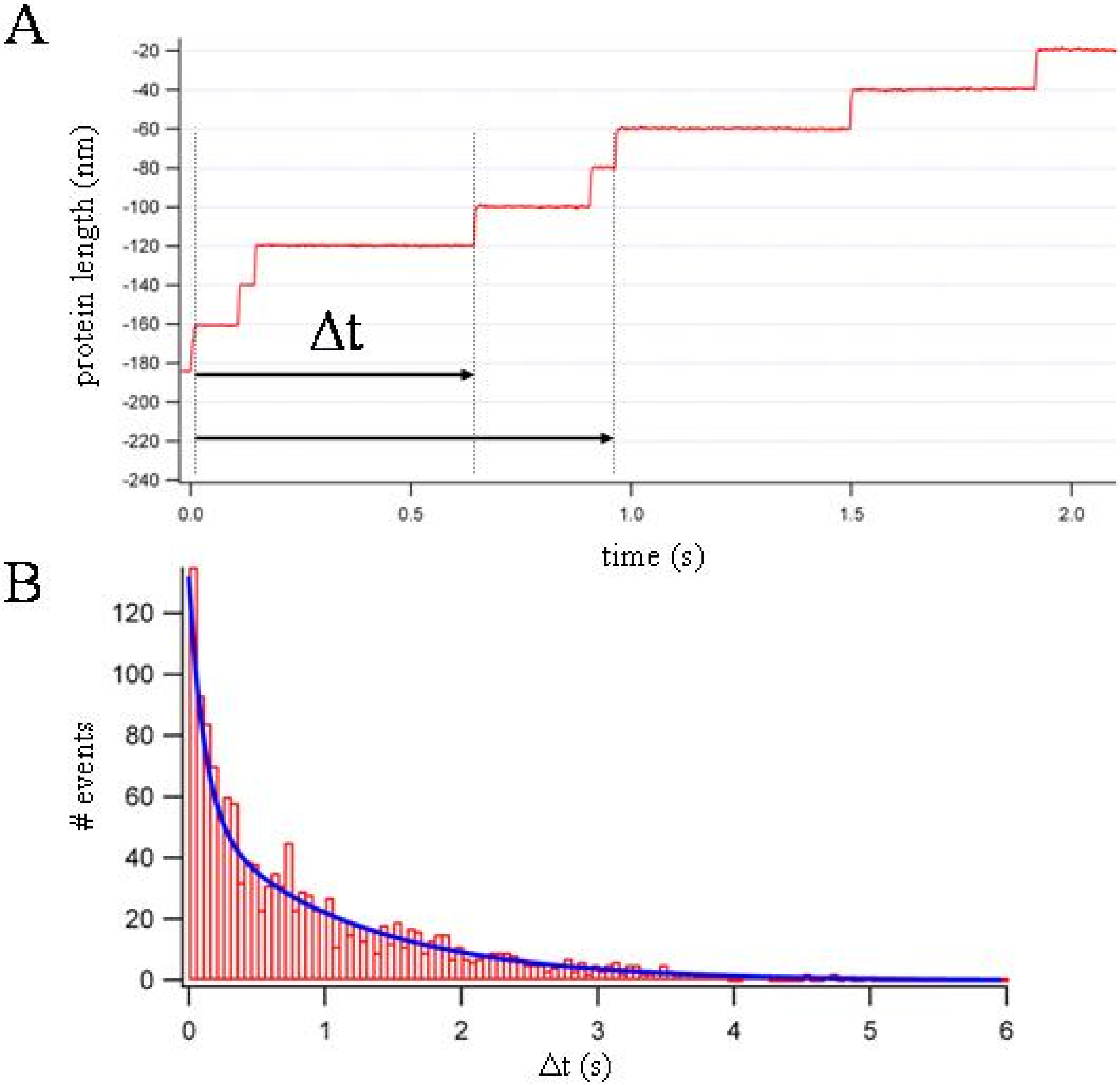
Effect of an external force on a rate constant

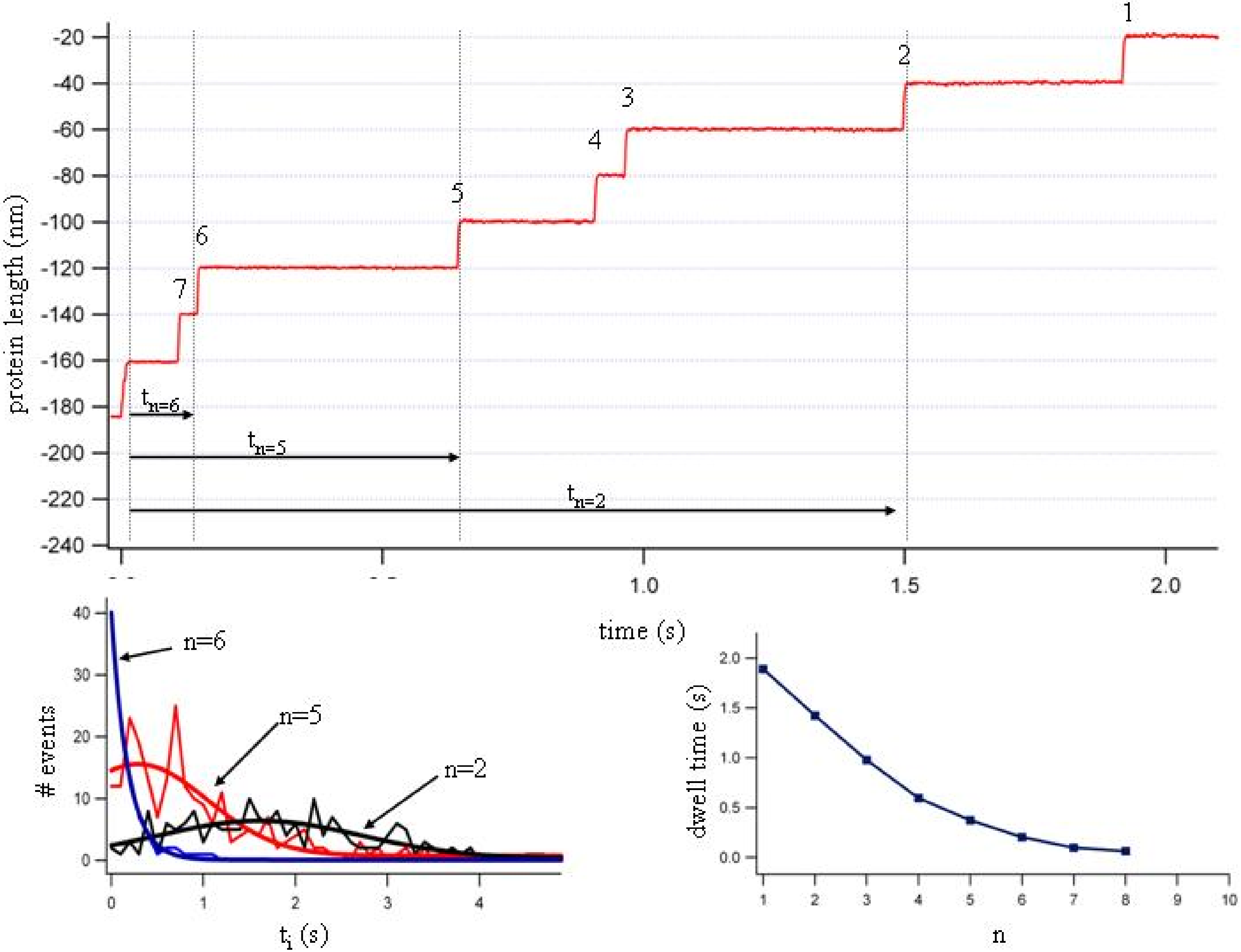




$$\alpha(F) = Ae^{-\frac{\Delta G}{k_B T}} e^{\frac{F\Delta x}{k_B T}}$$


A
F=140pN
F=120pN
F=100pN
1
0
$P_u(t)$
1s
$$\alpha(F) = \alpha_0 \cdot \exp\left(\frac{F \cdot \Delta x}{k_B T}\right)$$
B
100
10
1
0.1
$\alpha\ (s^{-1})$
50
100
150
200
250
F (pN)
α_0= 0.015 s^{-1}
Δx= 0.14 nm


A
protein length (nm)
-20
-40
-60
-80
-100
-120
-140
-160
-180
-200
-220
-240
Δt
0.0
0.5
1.0
1.5
2.0
time (s)
B
events
120
100
80
60
40
20
0
0
1
2
3
4
5
6
Δt (s)



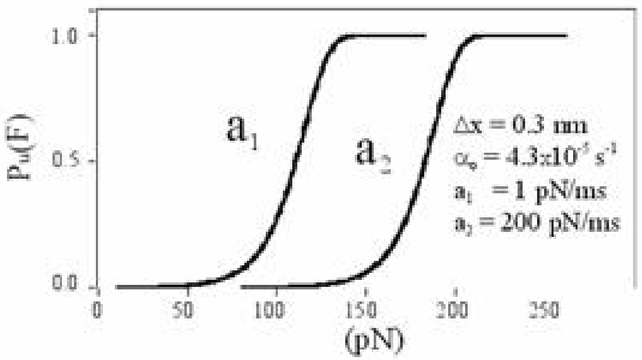
protein length (nm)
-20
-40
-60
-80
-100
-120
-140
-160
-180
-200
-220
-240
1
2
3
4
5
6
7
$t_{n=6}$
$t_{n=5}$
$t_{n=2}$
1.0
1.5
2.0
time (s)
events
40
30
20
10
0
n=6
n=5
n=2
0
1
2
3
4
t_i (s)
dwell time (s)
2.0
1.5
1.0
0.5
0.0
1
2
3
4
5
6
7
8
9
10
n

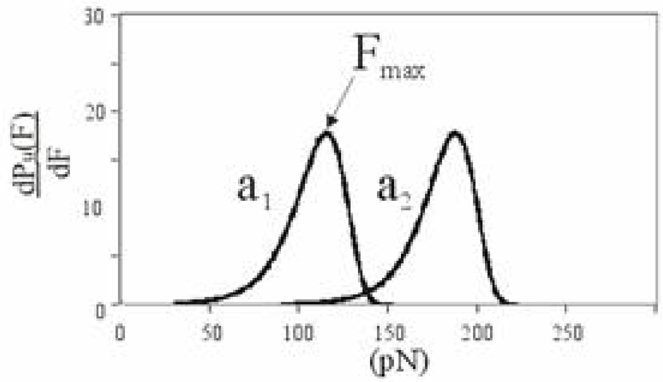
(1) $dP_u = \alpha(t)(1 - P_u(t))\, dt$

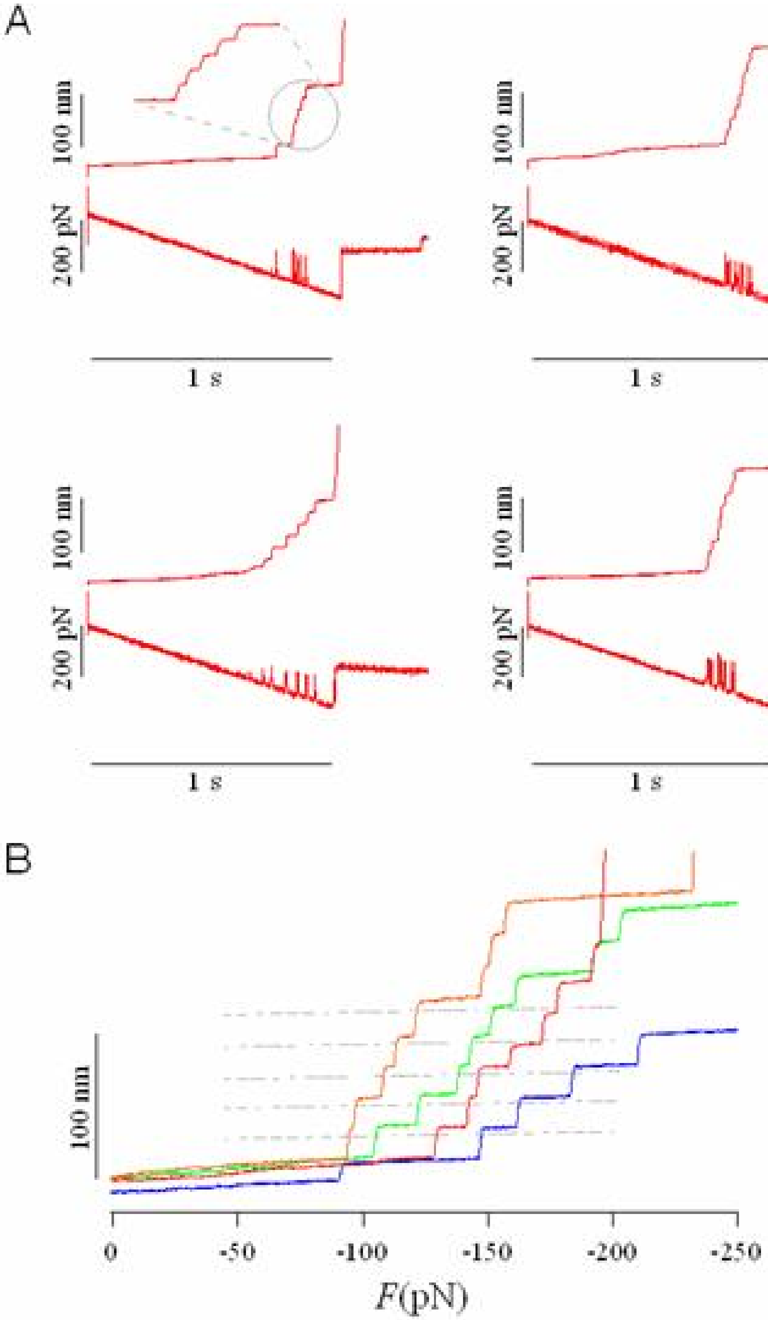
$F(t) = a\,t$

(2) $$P_u(F) = 1 - e^{-\frac{\alpha_o}{a}\int_0^F e^{\frac{f\cdot\Delta x}{kT}}\,df}$$

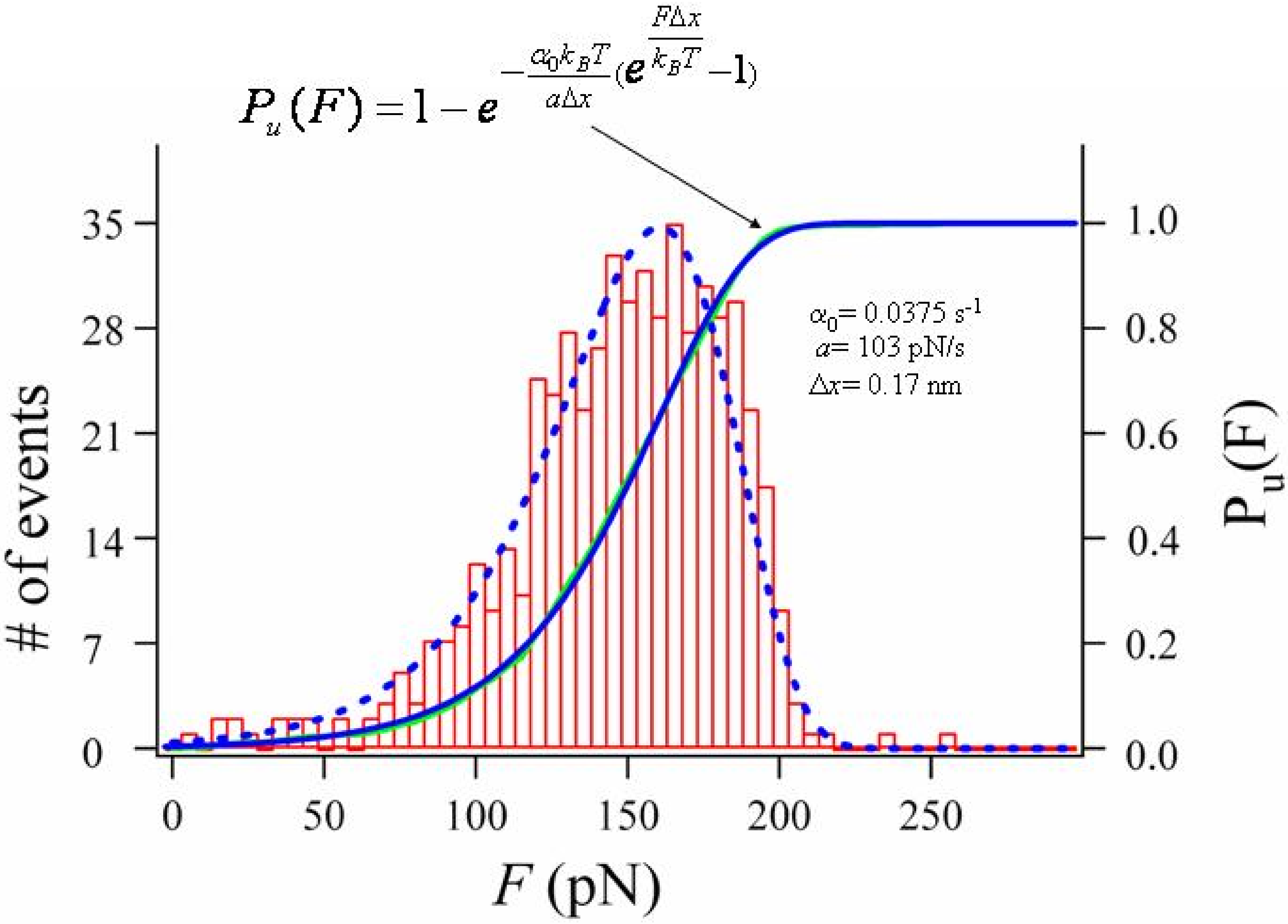
(3) $$\frac{dP_u(F)}{dF} = \frac{\alpha_o}{a}\cdot e^{\frac{F\cdot\Delta x}{kT}}\cdot e^{-\frac{\alpha_o\cdot kT}{\Delta x\cdot a}\left[e^{\frac{F\cdot\Delta x}{kT}}-1\right]}$$






A
100 nm
200 pN
1 s
100 nm
200 pN
1 s
100 nm
200 pN
1 s
100 nm
200 pN
1 s
B
100 nm
0
-50
-100
-150
-200
-250
F(pN)

Igor Demonstration of actual data



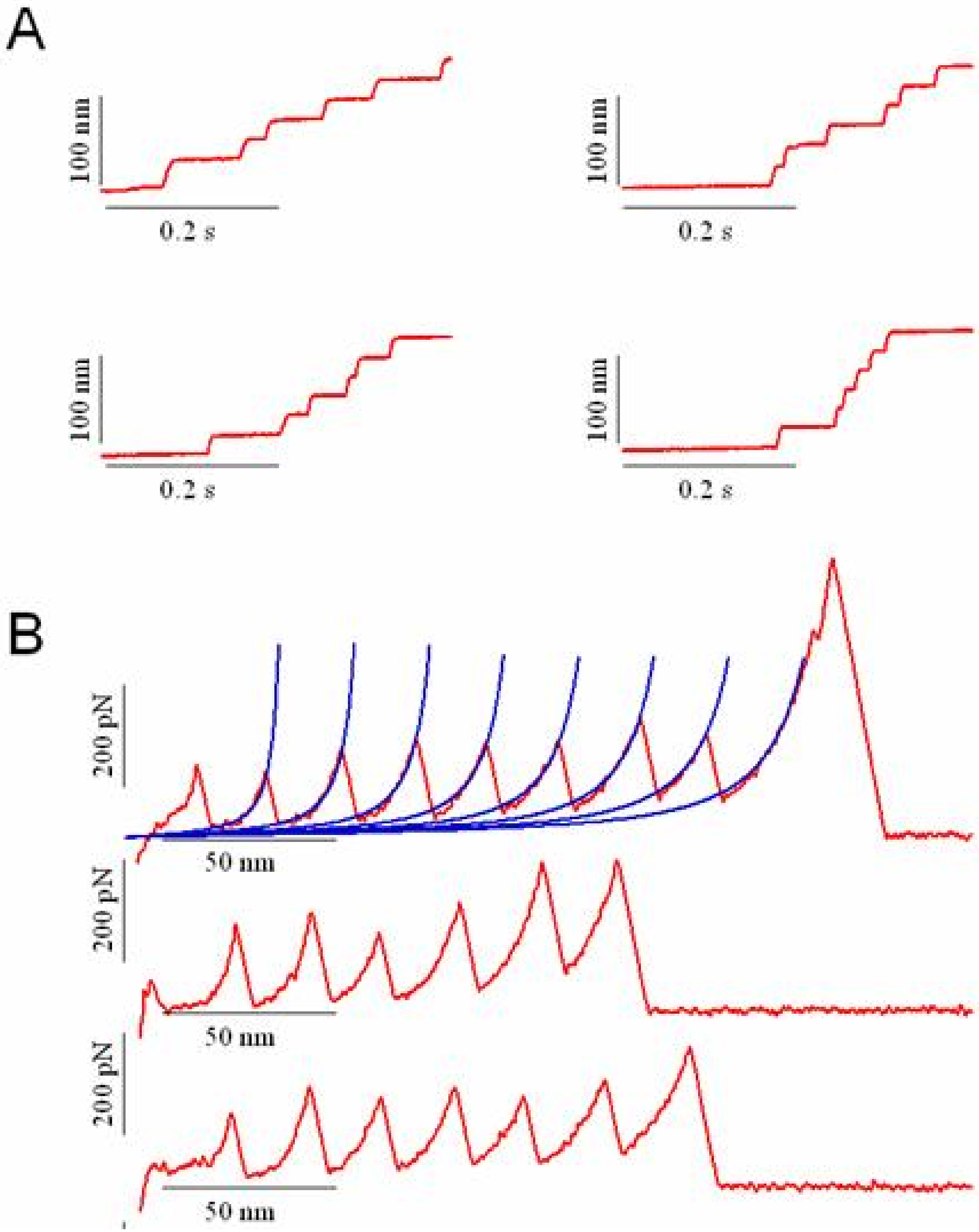
$$P_u(F) = 1 - e^{-\frac{\alpha_0 k_B T}{a\Delta x}\left(e^{\frac{F\Delta x}{k_B T}}-1\right)}$$

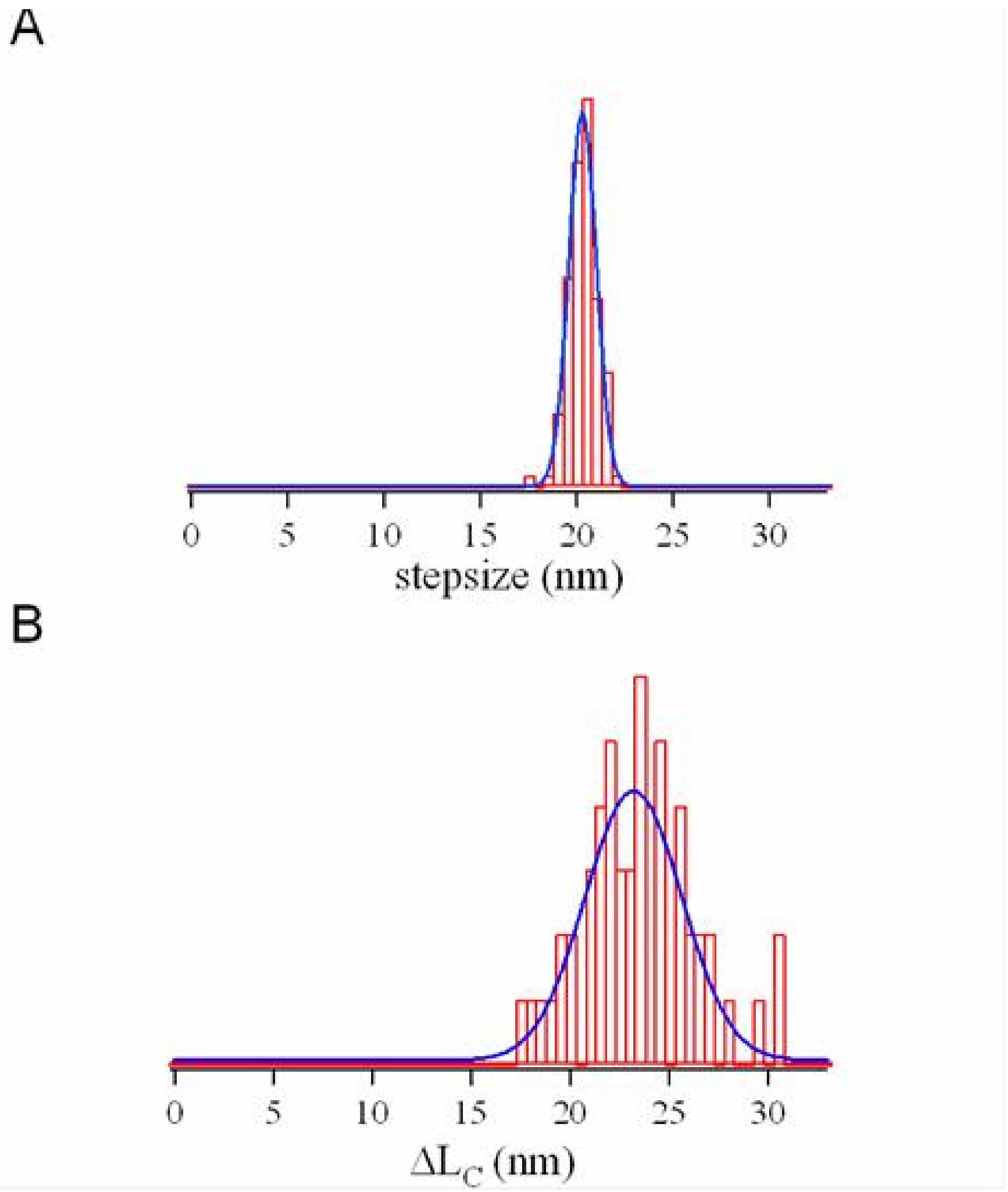
$\alpha_0 = 0.0375\ s^{-1}$
$a = 103$ pN/s
$\Delta x = 0.17$ nm

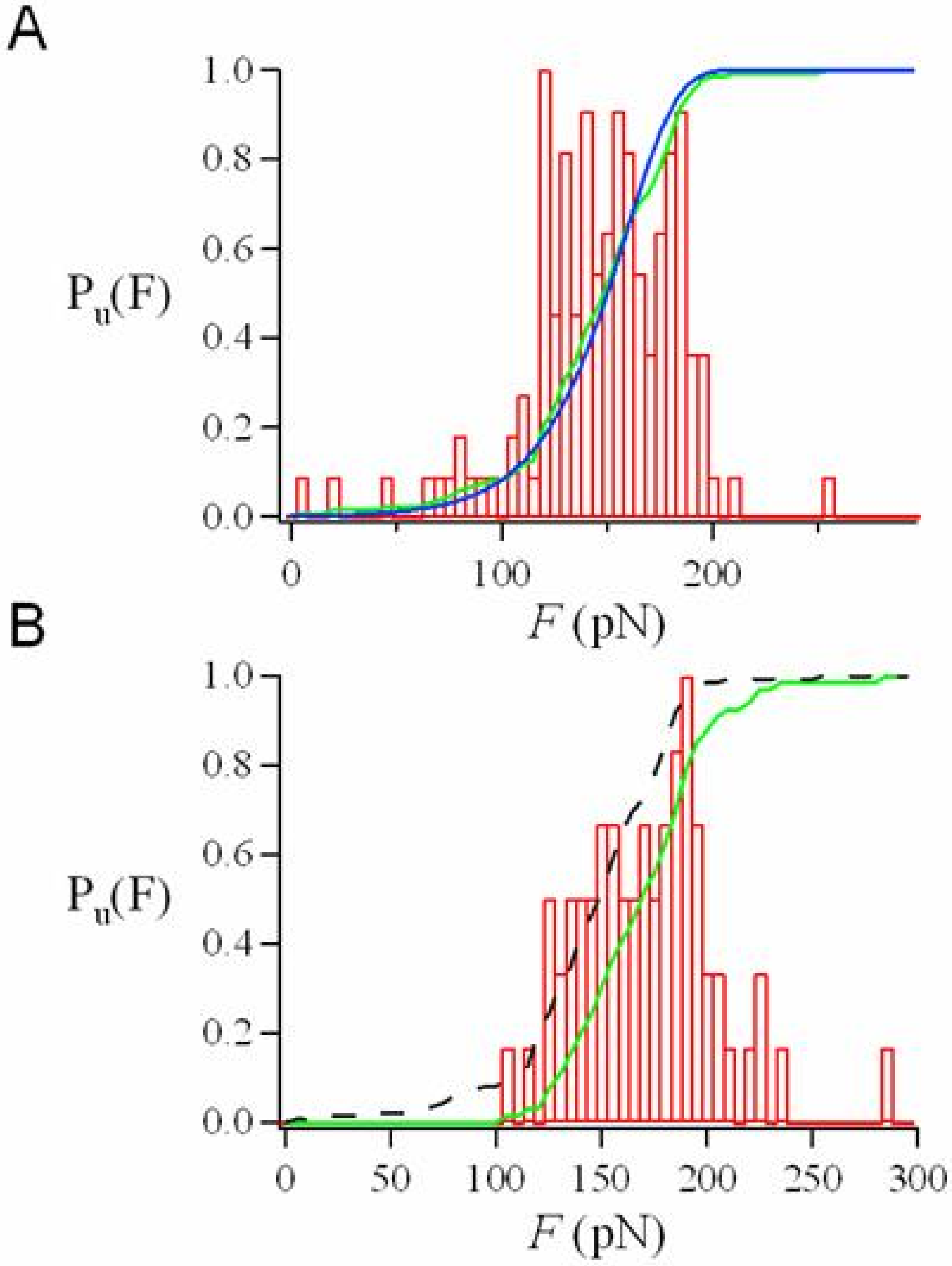
Sawtooth patterns *vs* Staircases

Michael Schlierf and
J.M. Fernandez

Biological Sciences, Columbia University, New York
and
Physics Department, Ludwig-Maximilians-University
Munich


A
100 nm
0.2 s
100 nm
0.2 s
100 nm
0.2 s
100 nm
0.2 s
B
200 pN
50 nm
200 pN
50 nm
200 pN
50 nm

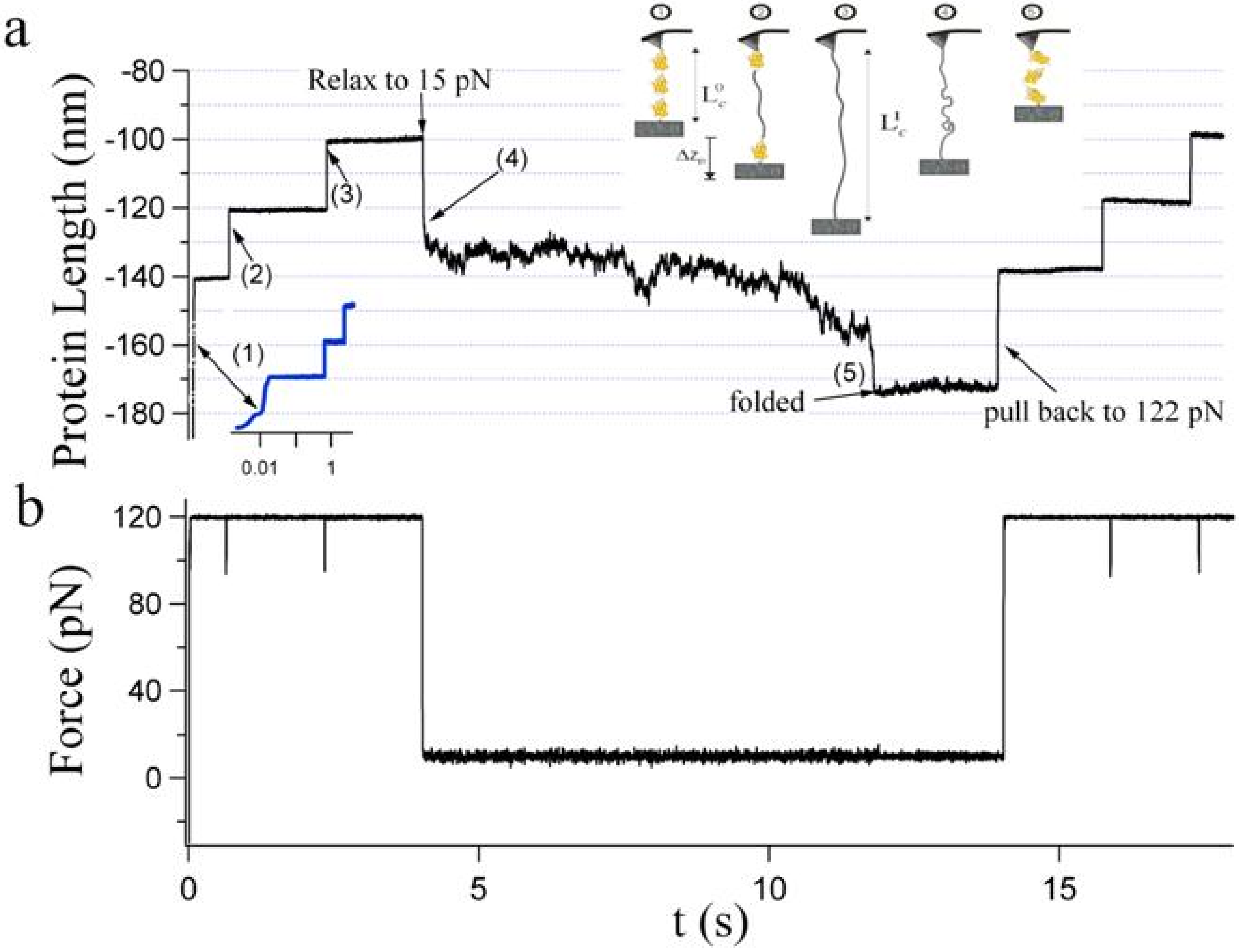

A
0
5
10
15
20
25
30
stepsize (nm)
B
0
5
10
15
20
25
30
ΔL_C (nm)

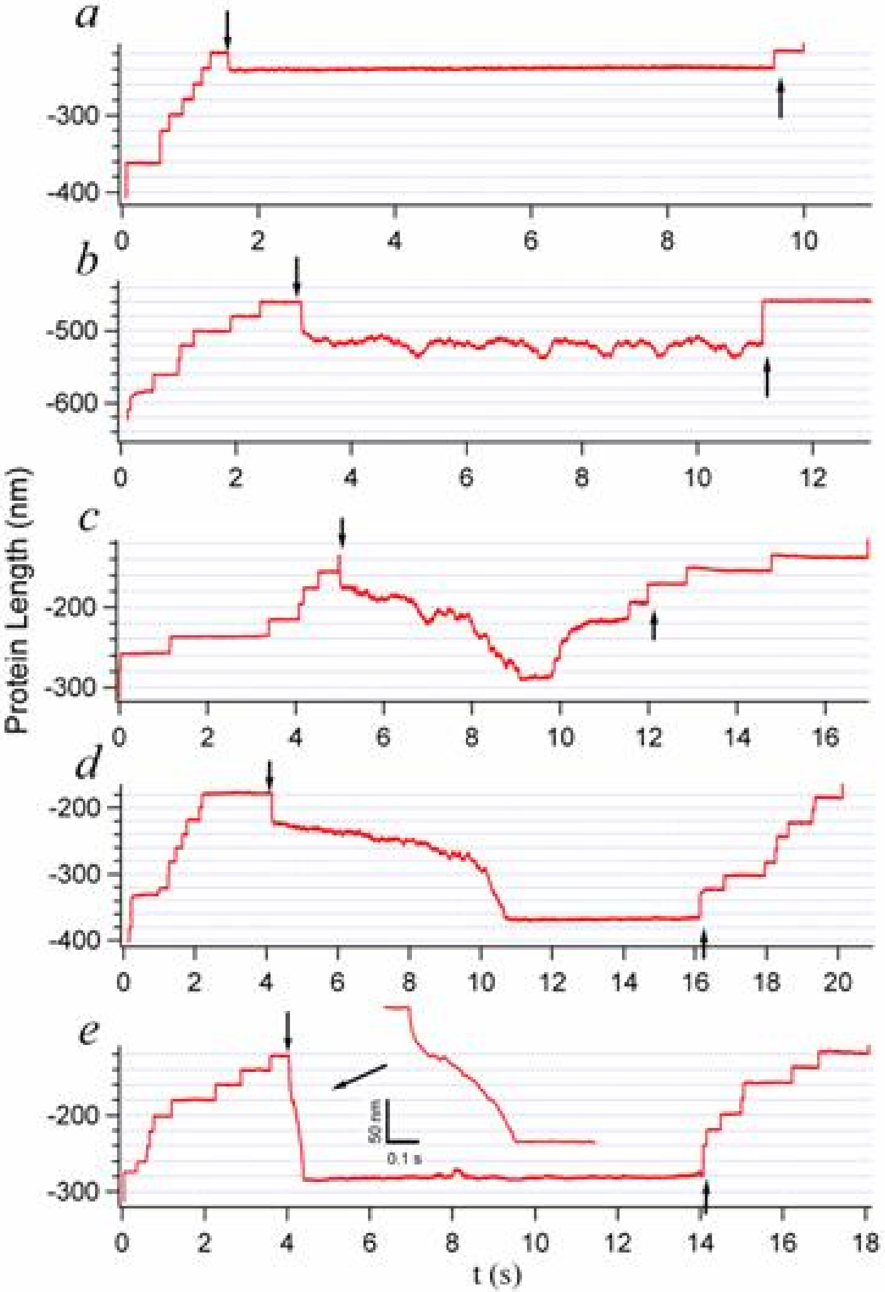

A
P_u(F)
1.0
0.8
0.6
0.4
0.2
0.0
0
100
200
F (pN)
B
P_u(F)
1.0
0.8
0.6
0.4
0.2
0.0
0
50
100
150
200
250
300
F (pN)

The folding trajectory of ubiquitin captured with force clamp spectroscopy

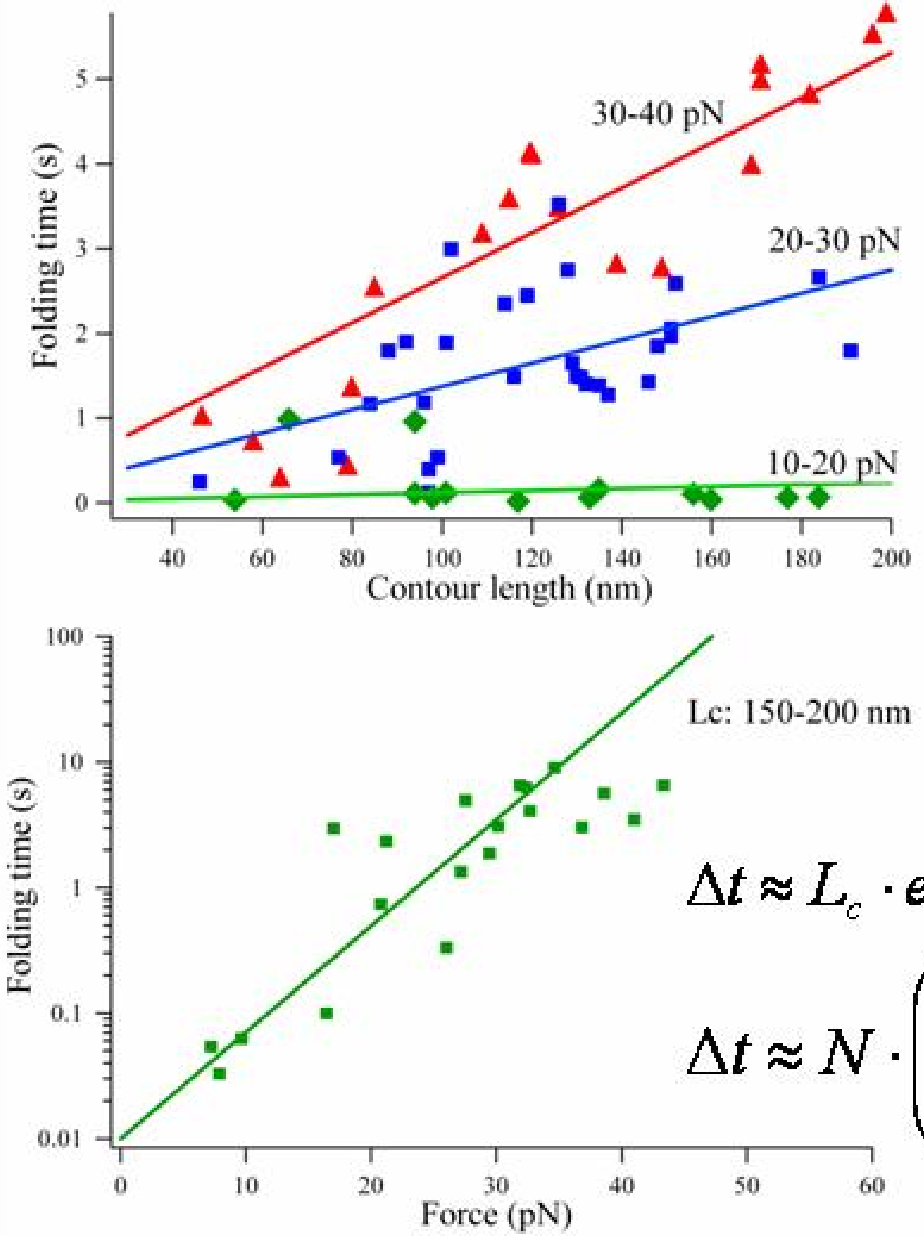
Hongbin Li and J.M. Fernandez

Biological Sciences, Columbia University, New York


a
Protein Length (nm)
-80
-100
-120
-140
-160
-180
Relax to 15 pN
(1)
(2)
(3)
(4)
(5)
folded
pull back to 122 pN
0.01
1
L_c^0
Δz_p
L_c^1
b
Force (pN)
120
80
40
0
0
5
10
15
t (s)


a
-300
-400
0
2
4
6
8
10
b
-500
-600
0
2
4
6
8
10
12
c
-200
-300
0
2
4
6
8
10
12
14
16
Protein Length (nm)
d
-200
-300
-400
0
2
4
6
8
10
12
14
16
18
20
e
50 nm
0.1 s
-200
-300
0
2
4
6
8
10
12
14
16
18
t (s)

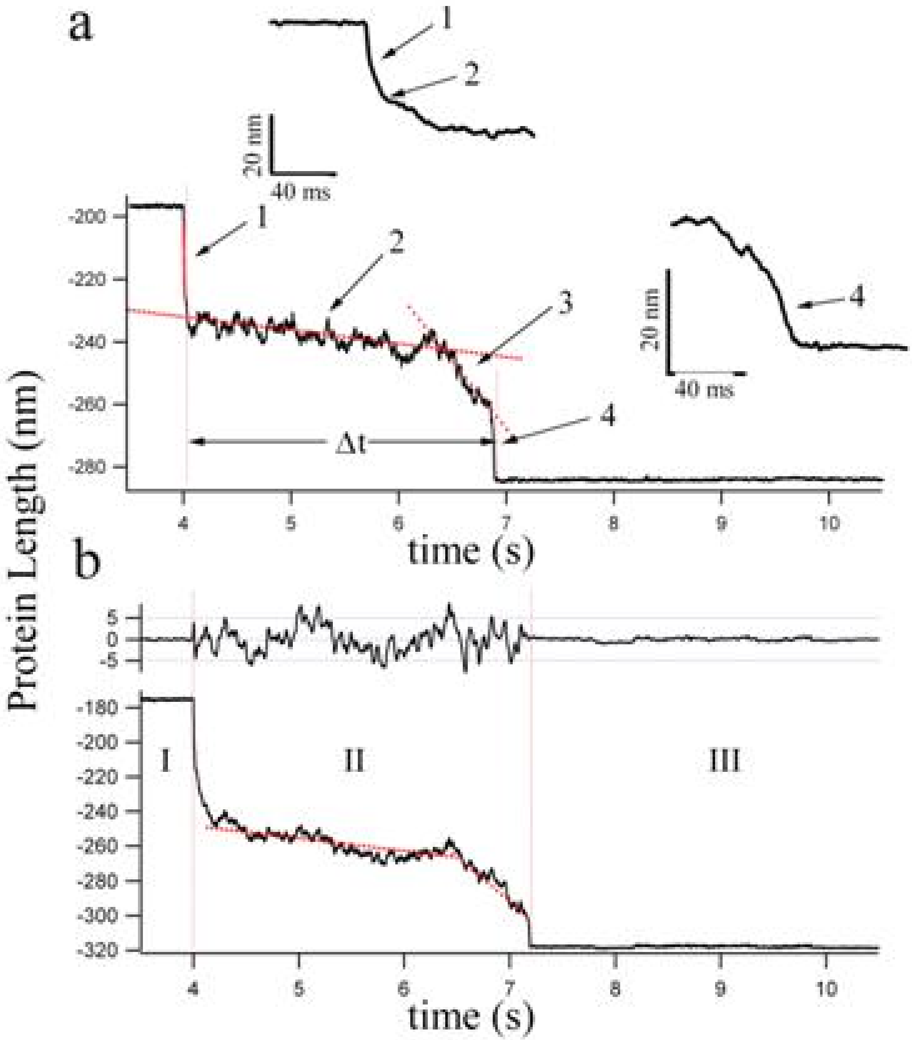
Igor Demonstration of actual data

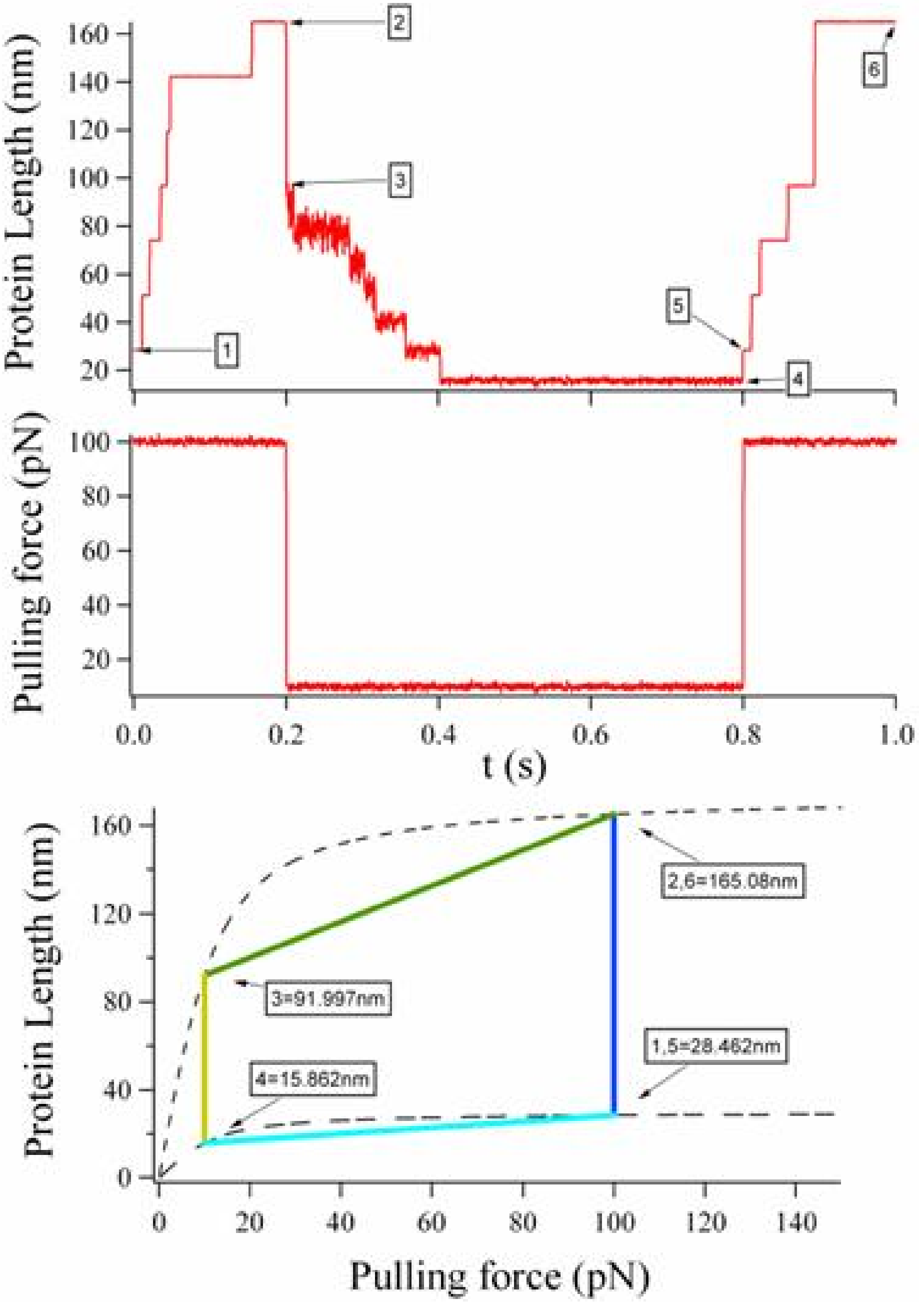


$$\Delta t \approx L_c \cdot e^{F \cdot 0.2} \cdot 6 \times 10^{-5}$$

$$\Delta t \approx N \cdot \left(\frac{F_{\Theta}}{F_{\Theta} - T} \right)^{6} \cdot \tau_0$$

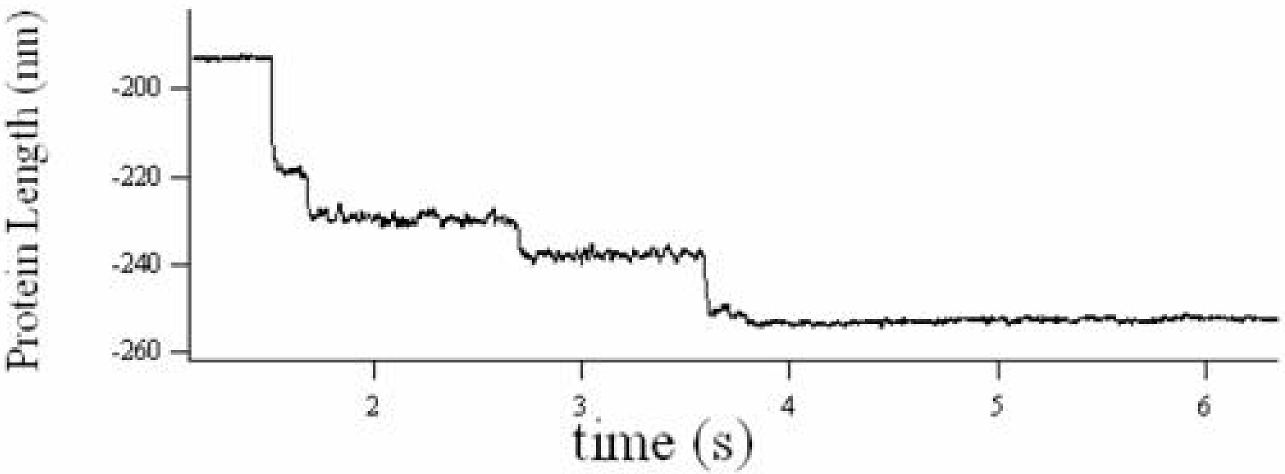
Figure 4


a
1
2
20 nm
40 ms
-200
-220
-240
-260
-280
1
2
3
4
Δt
20 nm
40 ms
4
4
5
6
7
8
9
10
time (s)
b
Protein Length (nm)
5
0
-5
-180
-200
-220
-240
-260
-280
-300
-320
I
II
III
4
5
6
7
8
9
10
time (s)

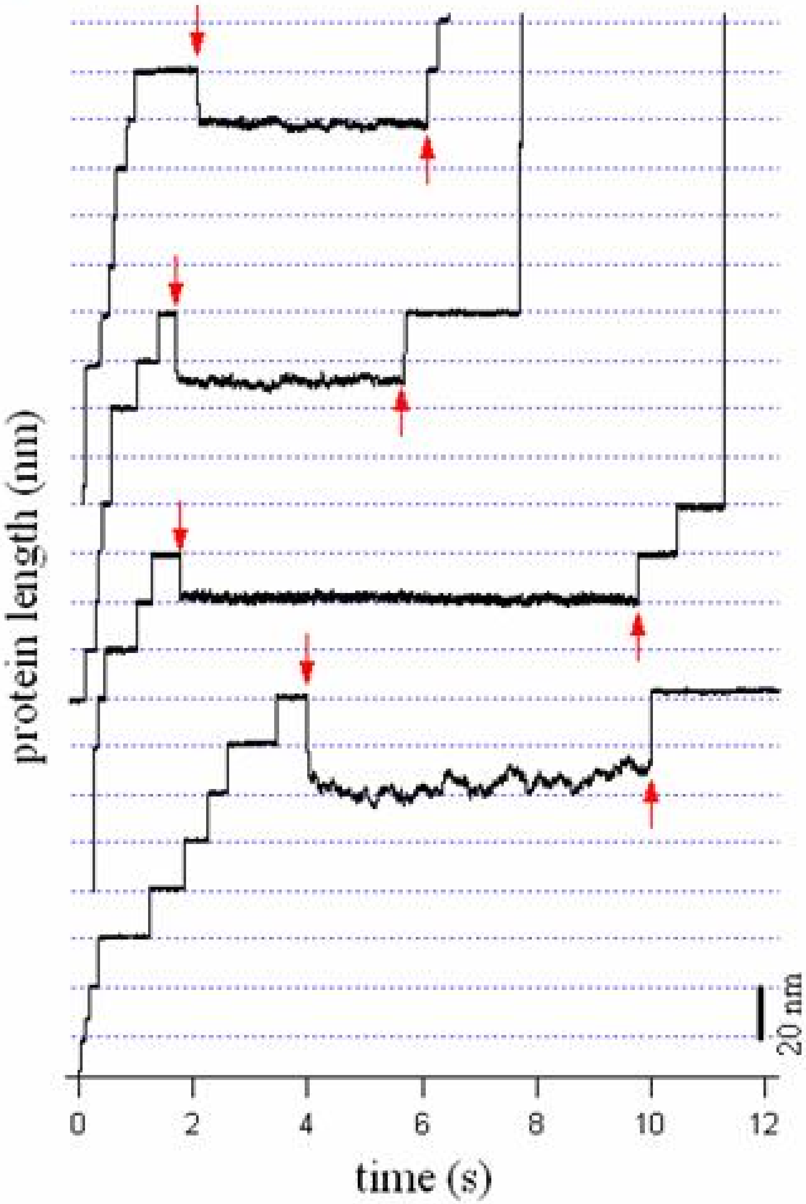

Protein Length (nm)
160
140
120
100
80
60
40
20
1
2
3
4
5
6
Pulling force (pN)
100
80
60
40
20
0.0
0.2
0.4
0.6
0.8
1.0
t (s)
Protein Length (nm)
160
120
80
40
0
2,6=165.08nm
3=91.997nm
4=15.862nm
1,5=28.462nm
0
20
40
60
80
100
120
140
Pulling force (pN)

Monte Carlo Demonstration

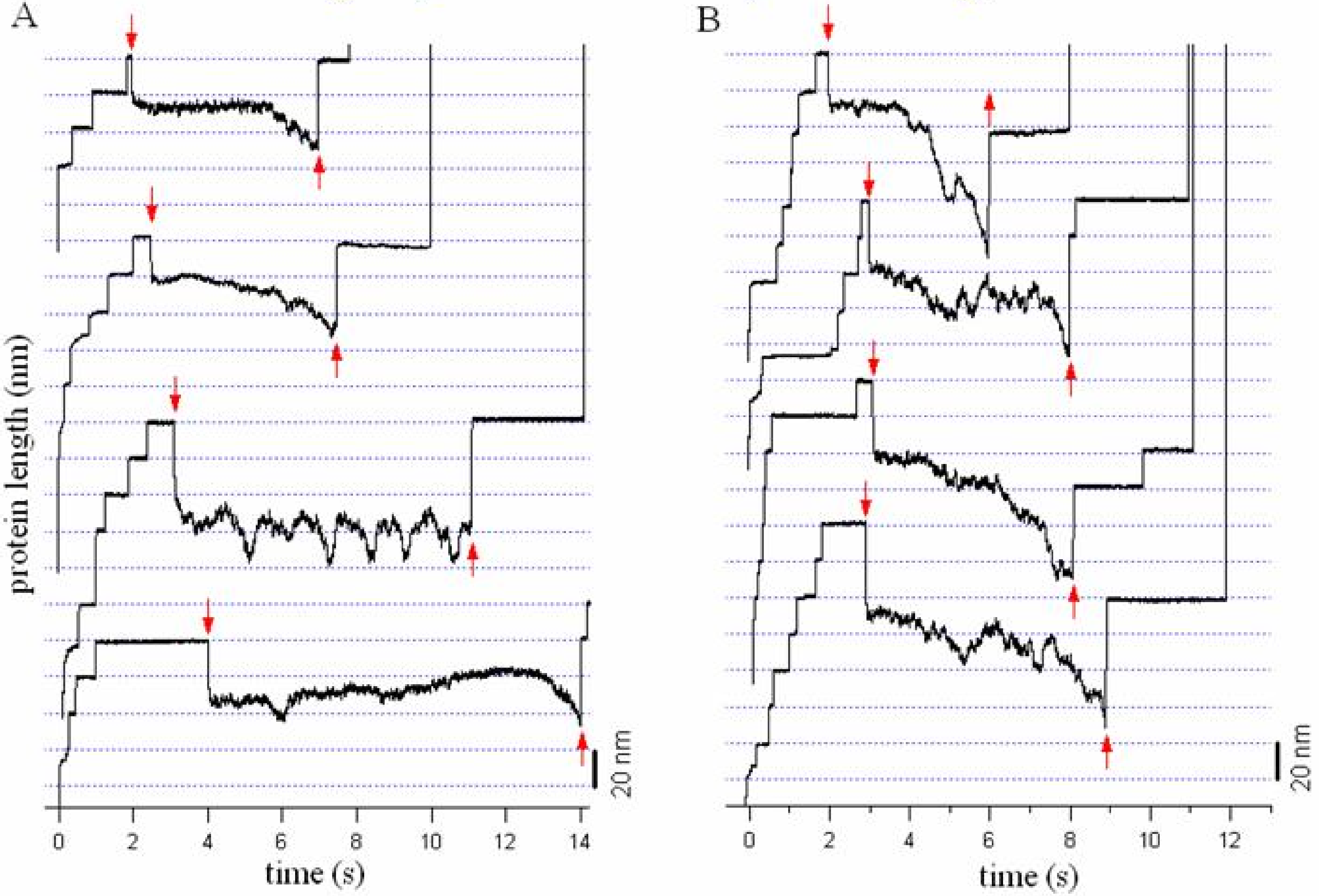
The only case where we have observed a ubiquitin folding trajectory composed of step-like events.

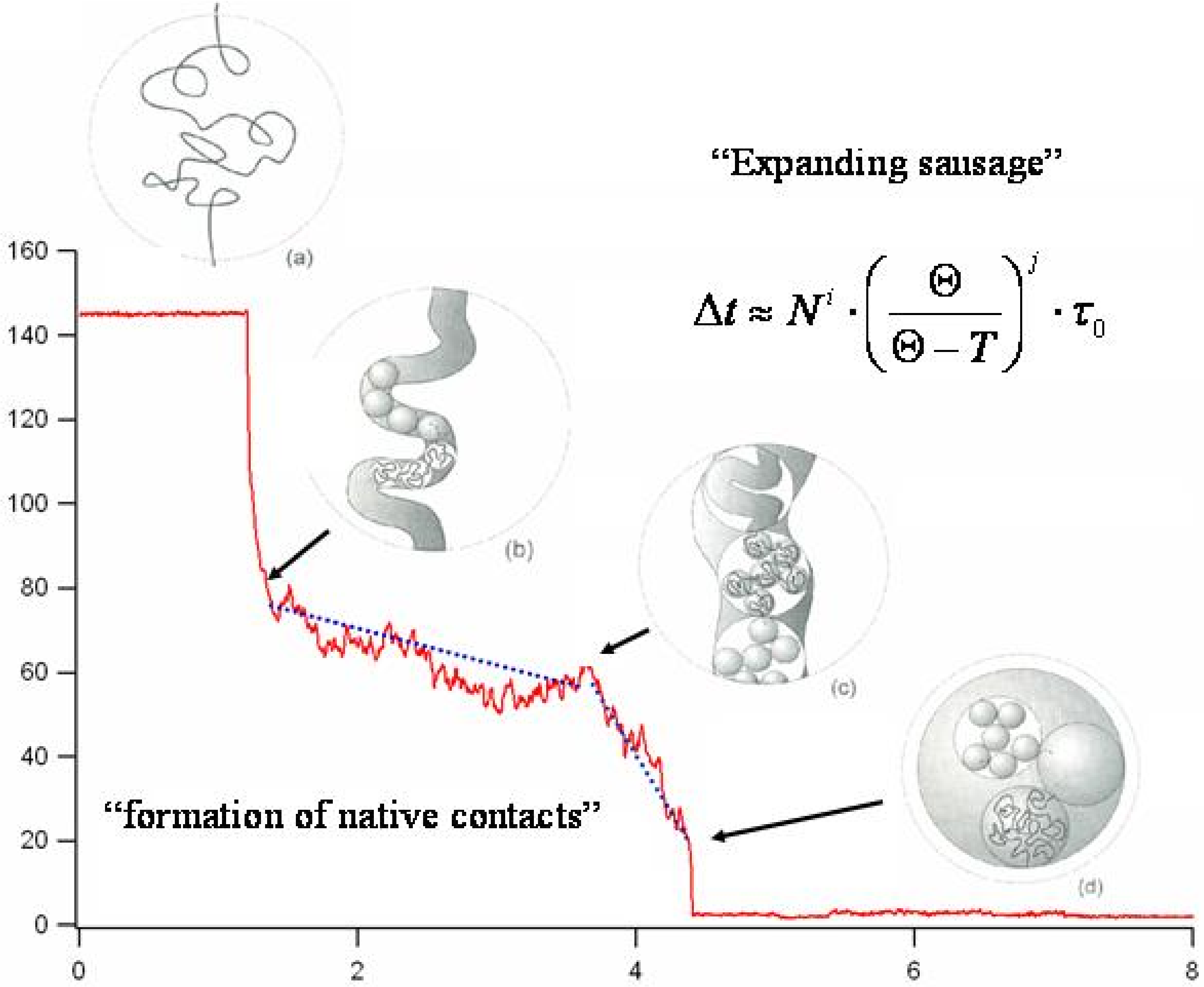


Folding trajectories interrupted at stage #2



Folding trajectories interrupted at stage #3





"Expanding sausage"

$$\Delta t \approx N^{i} \cdot \left(\frac{\Theta}{\Theta - T}\right)^{j} \cdot \tau_0$$

"formation of native contacts"

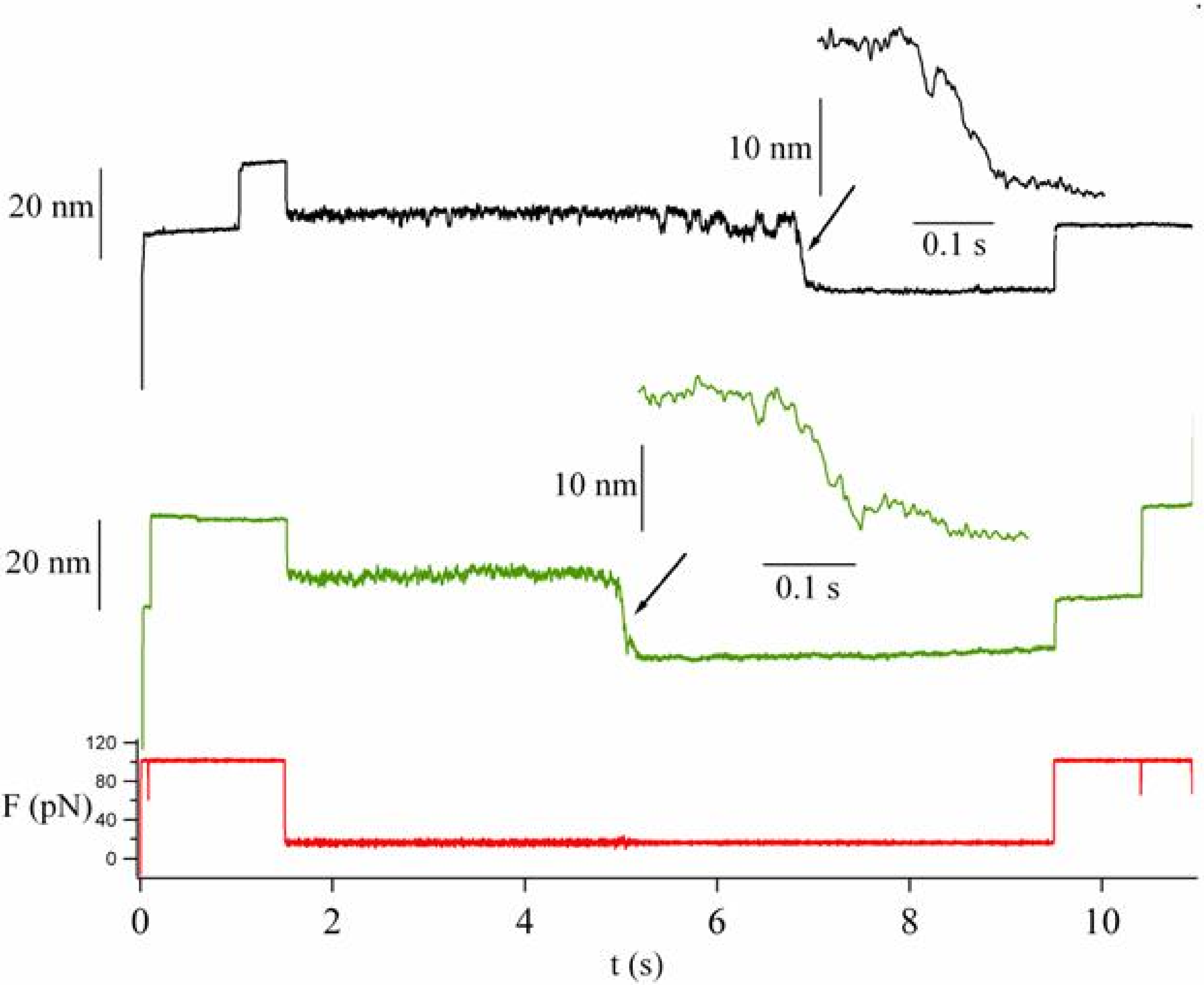
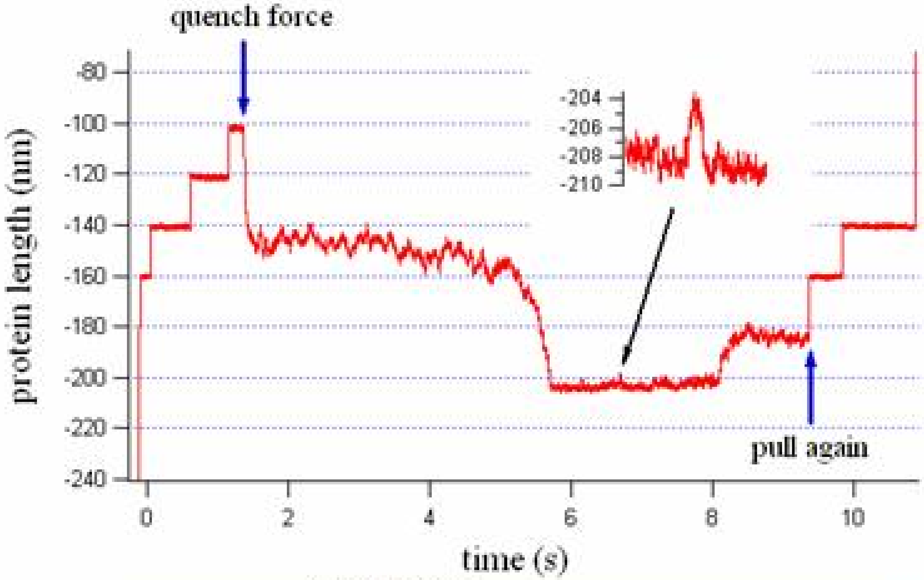
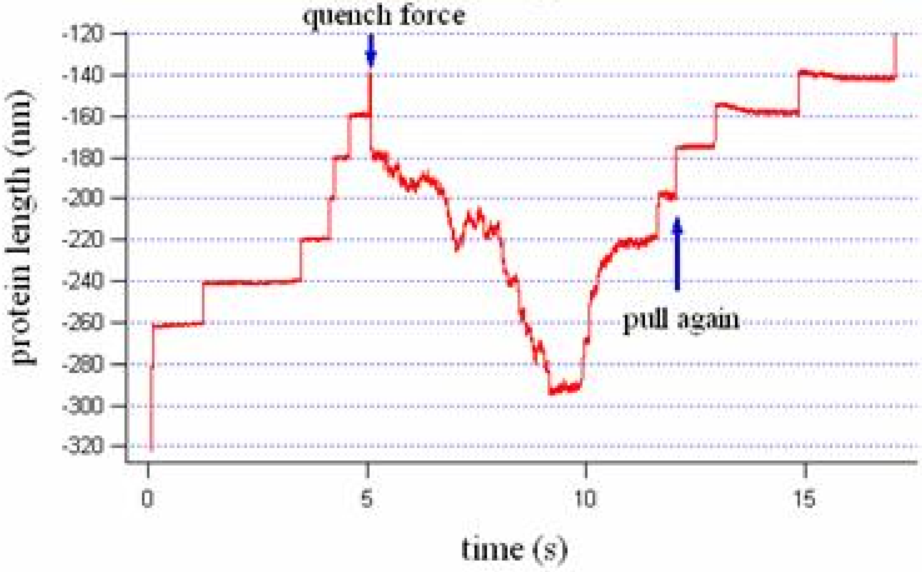


Figure 5

folding near equilibrium





Conclusions

1.- Force clamp spectroscopy can measure the complete folding trajectory of a protein.

2.- In contrast to expectations, the folding trajectory of ubiquitin does not show steps and appears continuous with distinct stages tha are marked by large fluctuations.

3.- The total duration of a folding trajectory is dependent both on the stretching force and the contour length of the unfolded protein.

4.- Folding trajectories cannot be described by a state diagram and appear to have many of the properties of polymer collapse

5.- Examination of these trajectories provides a new benchmark to understand protein folding.