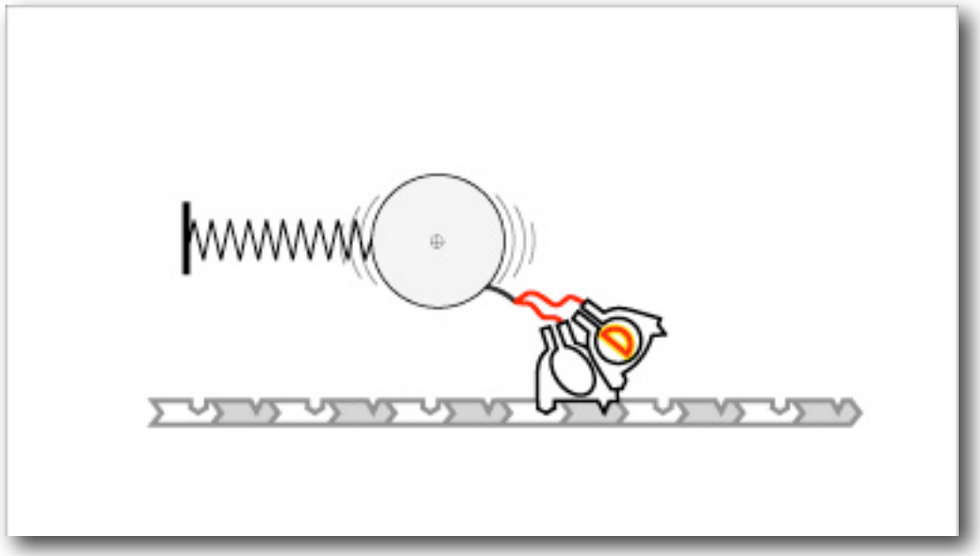
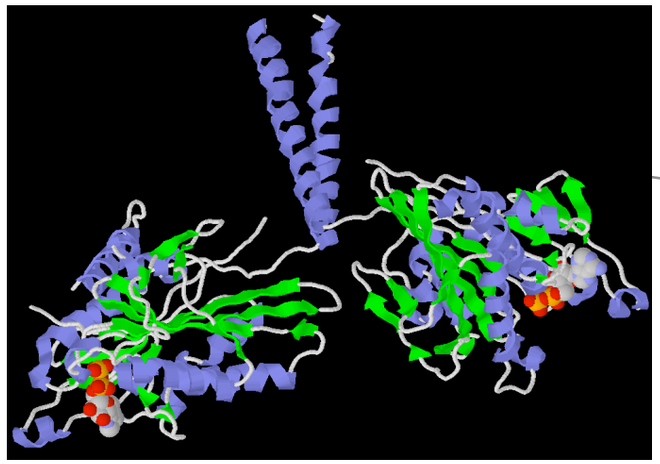
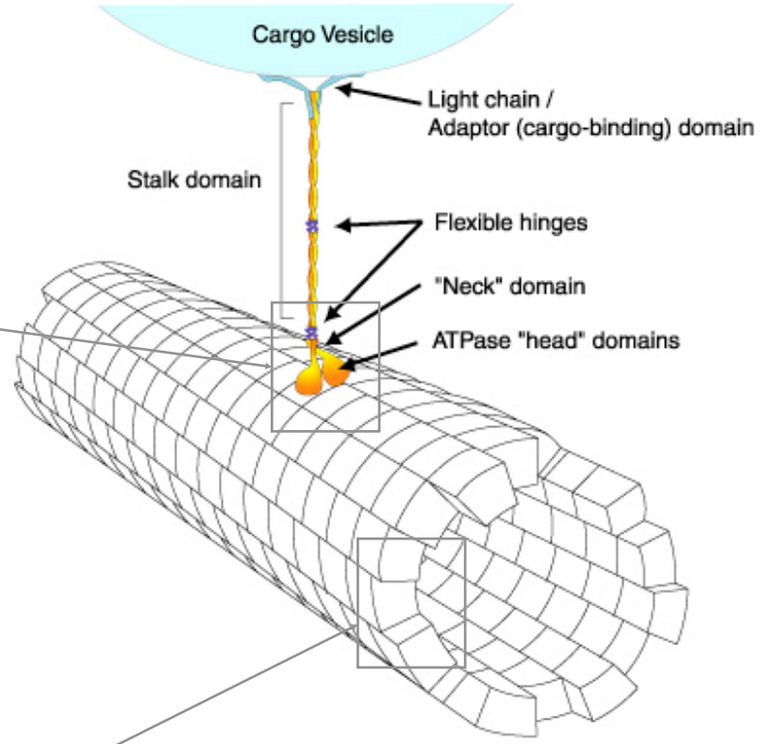
Kinesin stepping mechanism

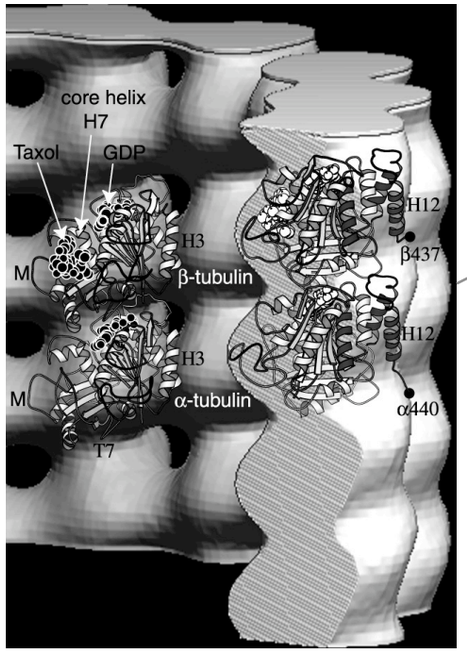


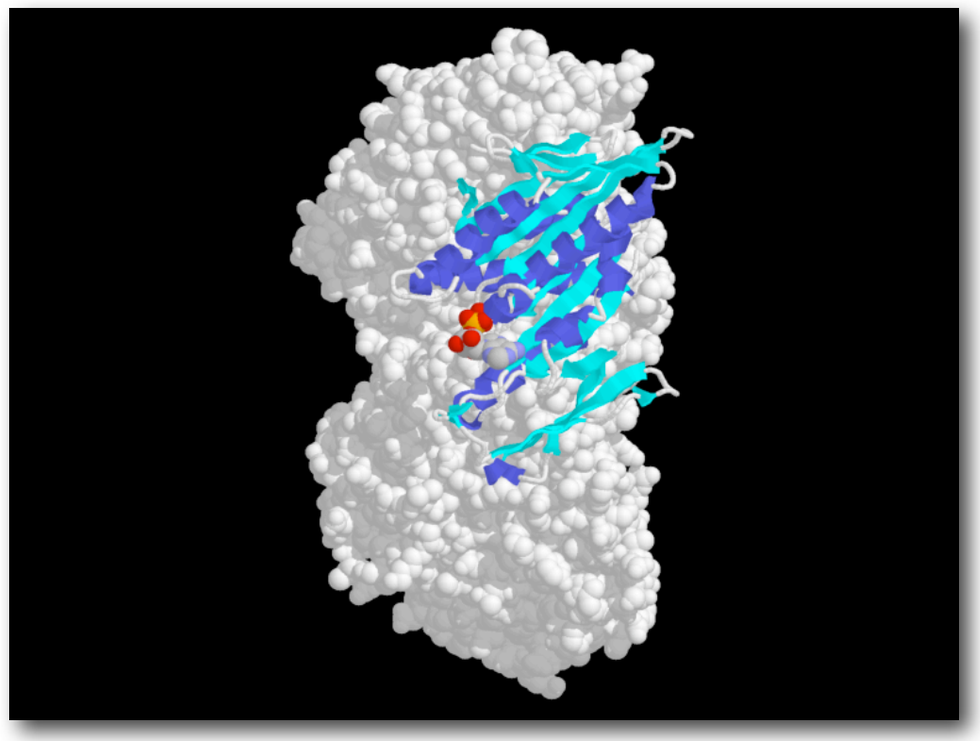
Stepping mechanism of kinesin



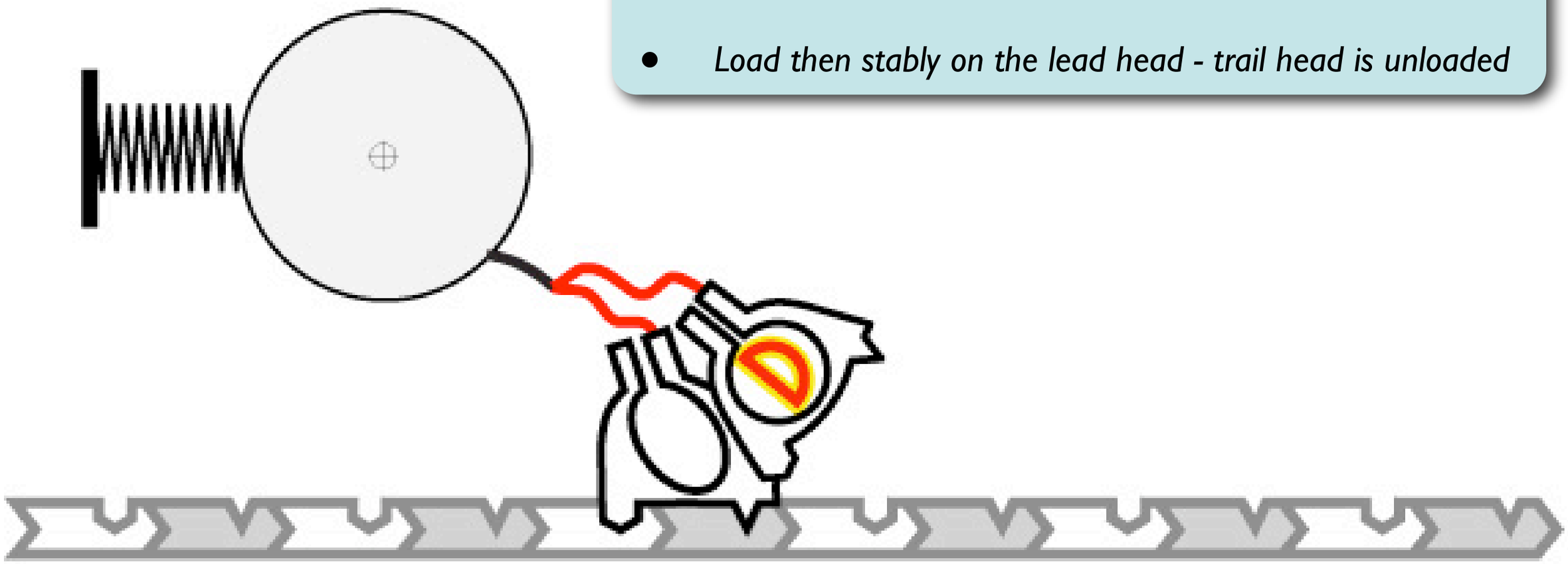


Microtubule dynamics





- *During dwells, one head is bound, the other is parked*
- *ATP binding to the trail head unparks the lead head*
- *Lead head attachment is locked in by ADP release*
- *Load then stably on the lead head - trail head is unloaded*

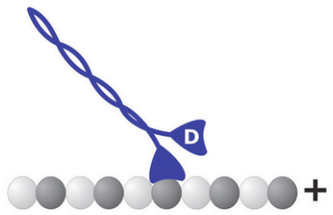


Biochemical kinetics

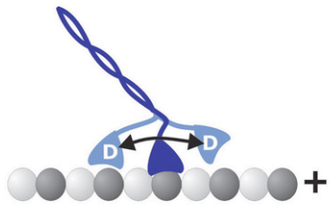
- Binding stoichiometries confirm 1 binding site per heterodimer
- Weak & strong binding states
- Heads trap ADP in absence of microtubules. Microtubule binding activates ADP release ~1000x
- Only one ADP is released when a 2-headed kinesin binds to a microtubule. Release of the second ADP depends on ATP binding.
- Roadblock experiment indicates not much forwards strain sensitivity
- ATP-gated ADP release with tubulin as well as microtubules ..

Cross R.A. (2004)
The kinetic mechanism of kinesin
Trends in Biochemical Sciences **89** 301-309

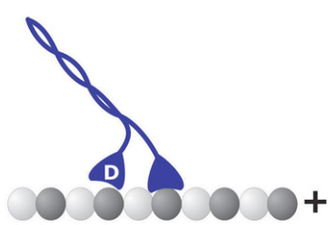
Controversy | *the structure of the waiting state*



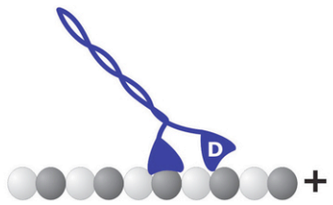
(a) **Detached** and "parked" next to the bound head



(b) **Detached,** diffusing between possible forward and backwards sites.



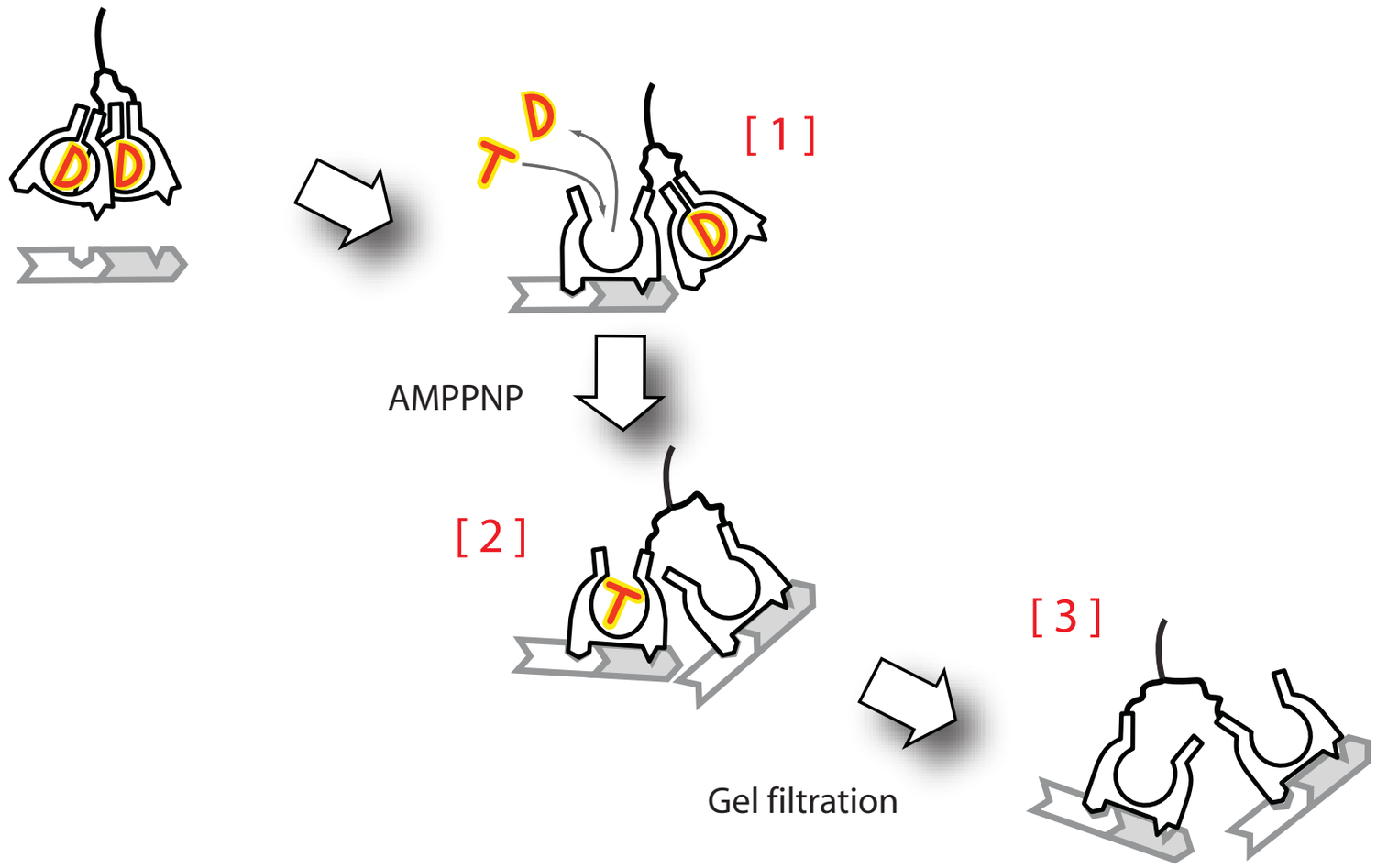
(c) **Weak binding** to the last binding site.



(d) **Weak binding** at the next forward binding site.

Carter N.J. & Cross R.A. (2006)
Kinesin's moonwalk
Current Opinion in Cell Biology **18** 61-67

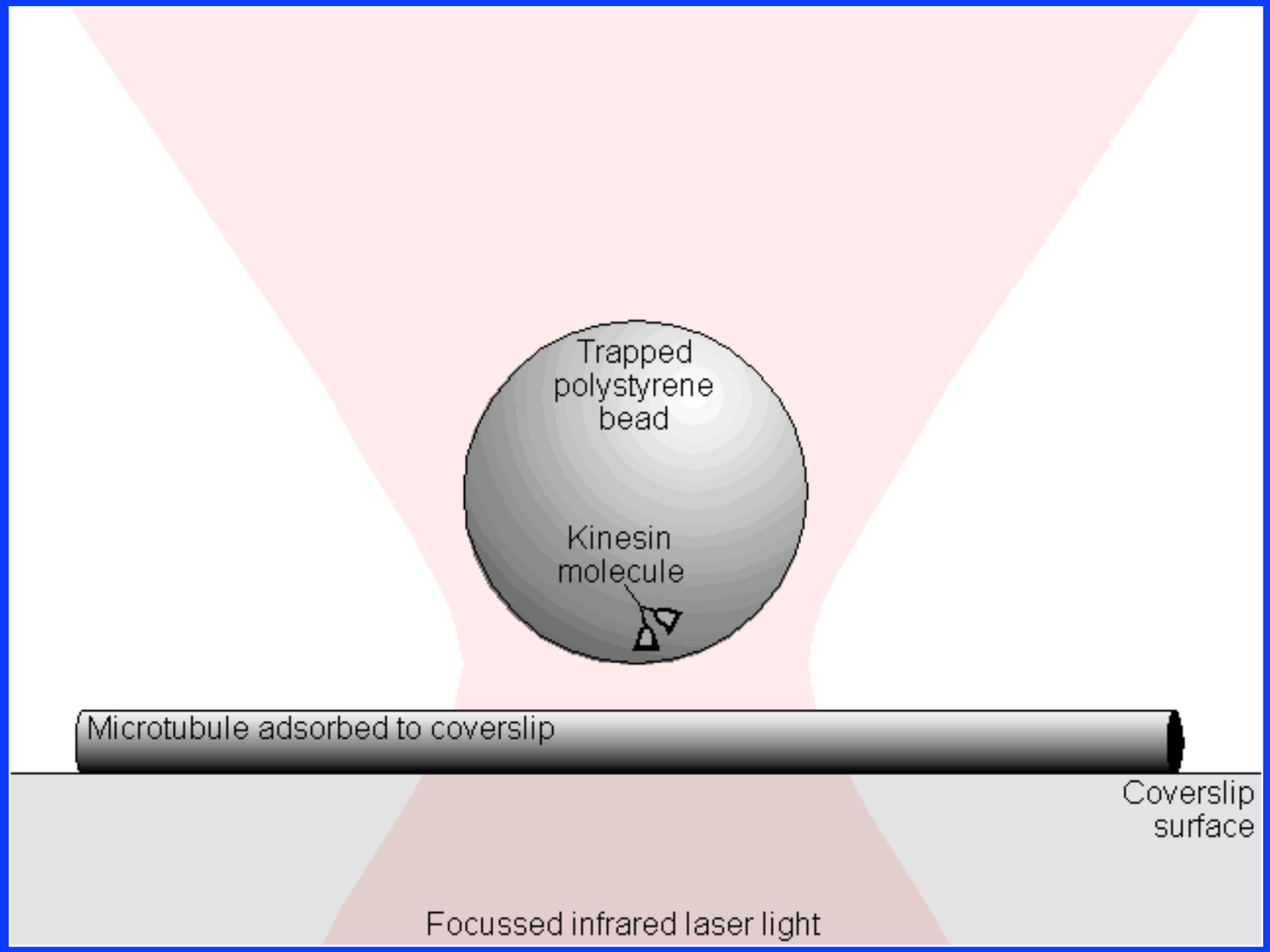
ATP-dependent binding of a second tubulin heterodimer



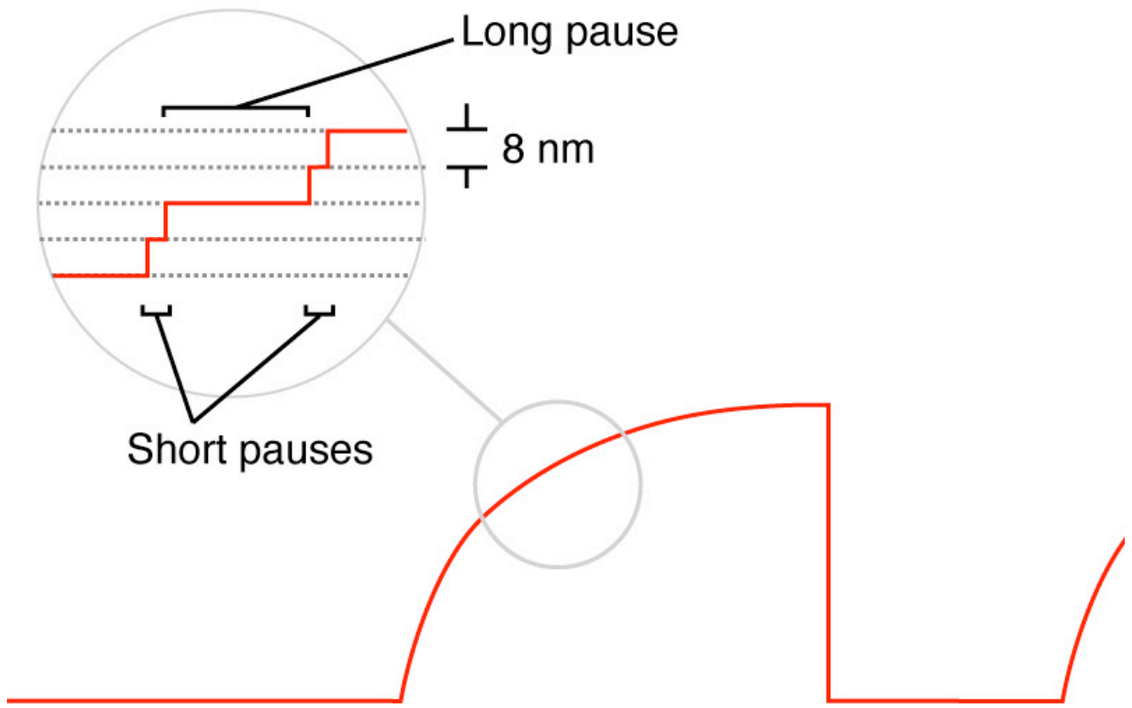
Single molecule mechanics

- 8 nm steps
- Alternate-heads stepping

Single beam optical trap

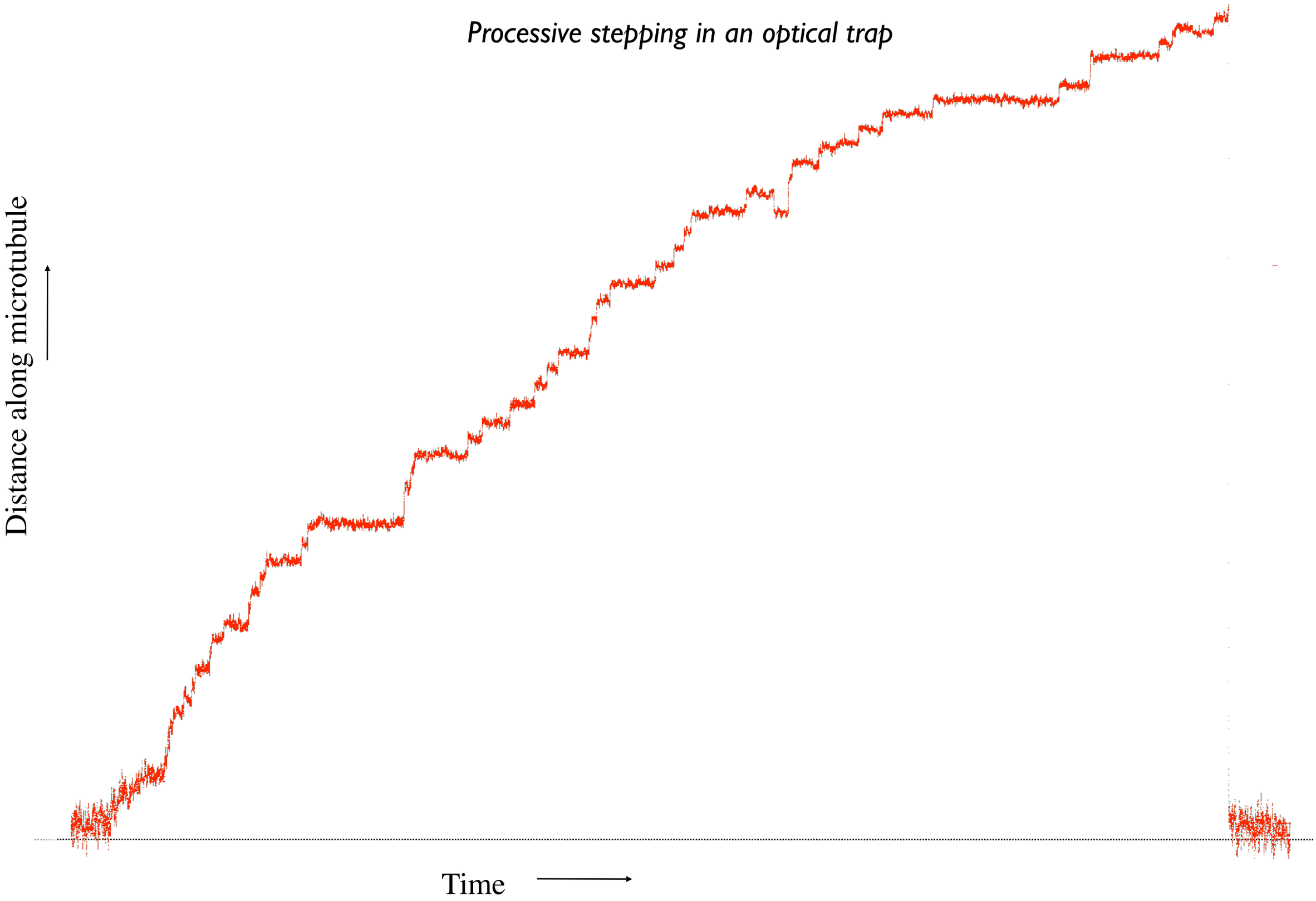


Alternate heads walking action



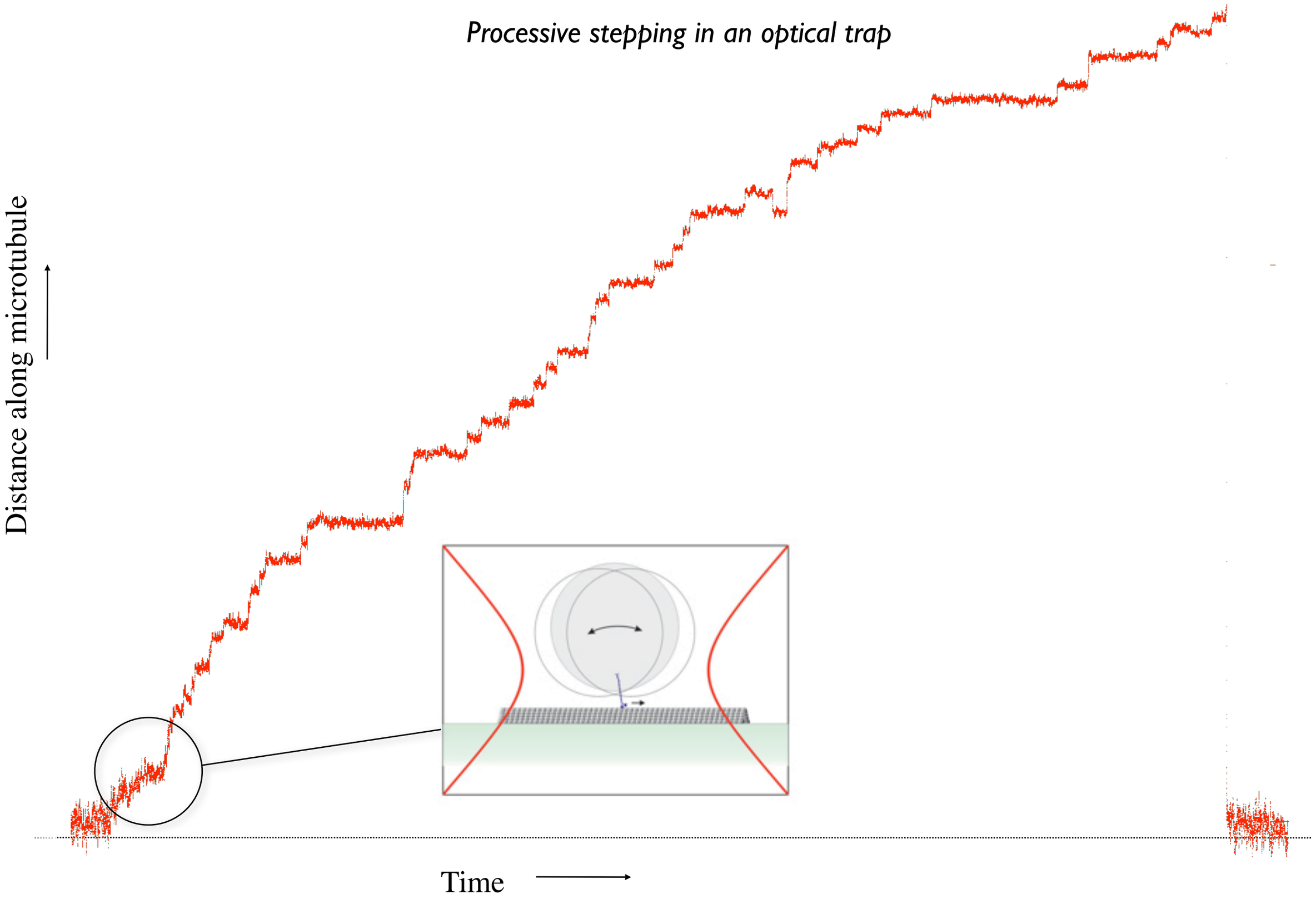
Kaseda et al (2003) Alternate fast and slow stepping of a heterodimeric kinesin molecule *NCB*
Asbury et al (2003) Kinesin moves by an asymmetric hand-over-hand mechanism *Science*
Yildiz et al (2004) Kinesin walks hand over hand *Science*
Higuchi et al (2004) Rapid double 8-nm steps by a kinesin mutant *EMBO J*

Processive stepping in an optical trap



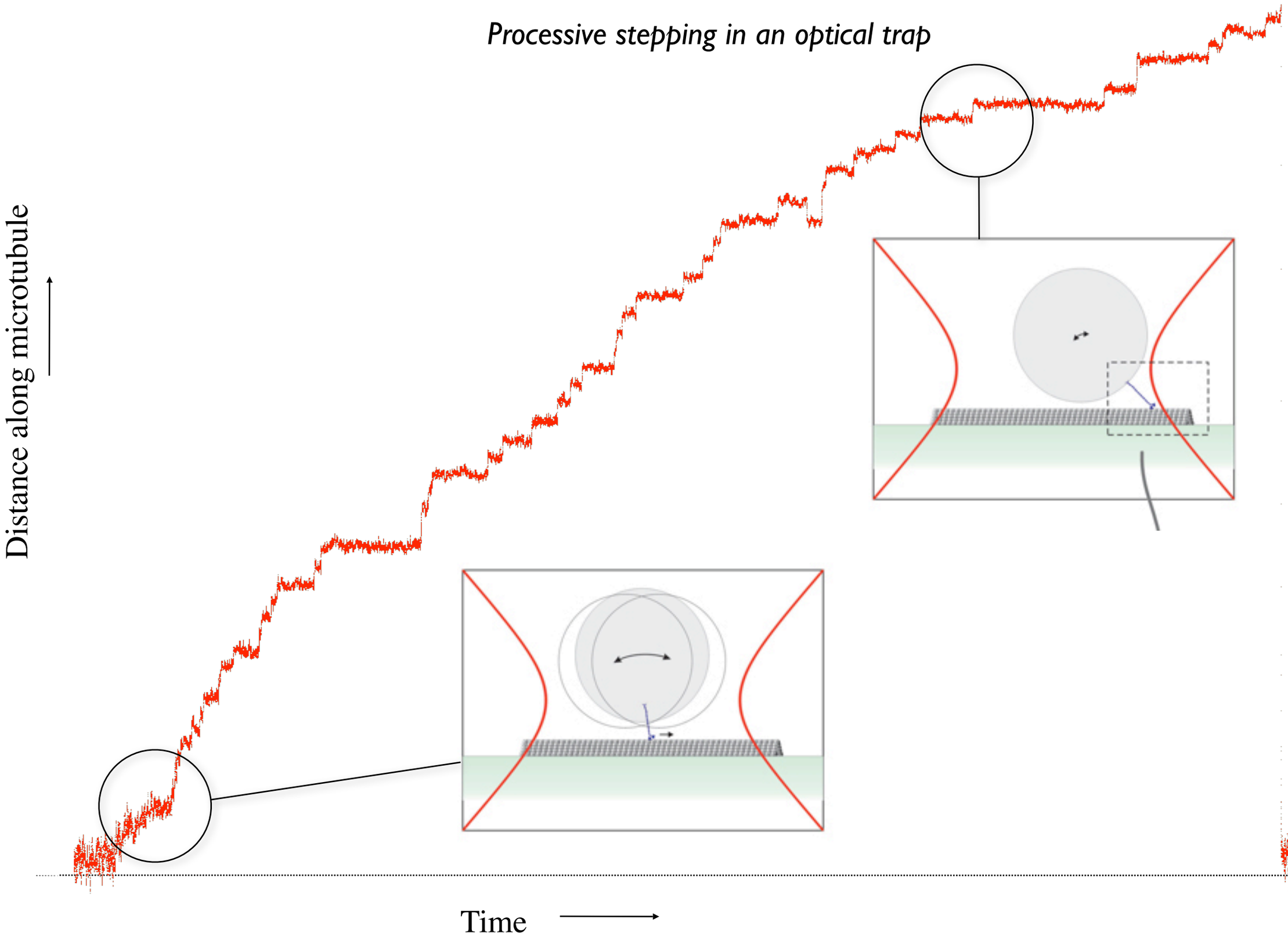
Rat kinesin, 2μM ATP, trap stiffness 0.018pN/nm, 10ms median filter applied.

Processive stepping in an optical trap



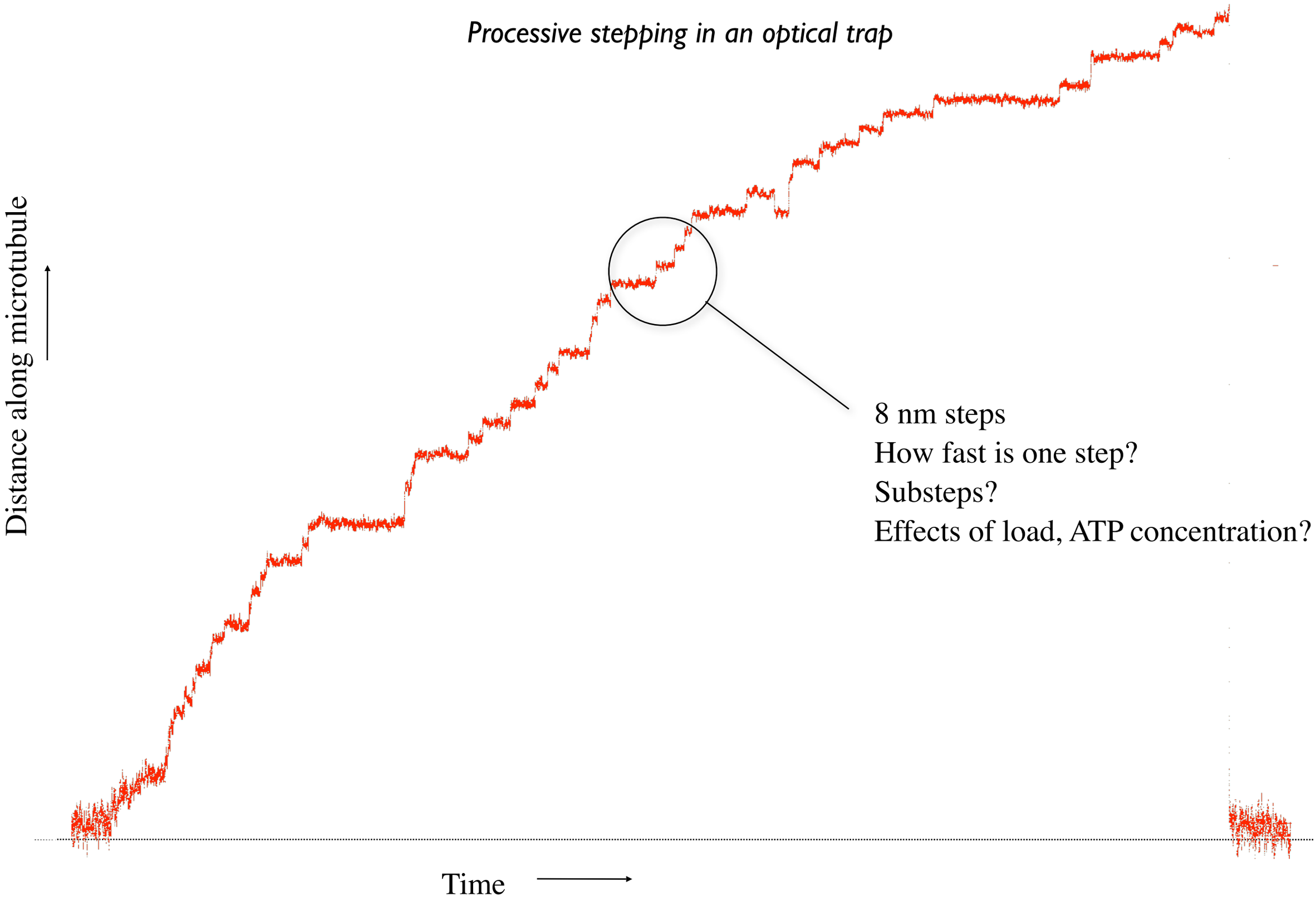
Rat kinesin, 2μM ATP, trap stiffness 0.018pN/nm, 10ms median filter applied.

Processive stepping in an optical trap



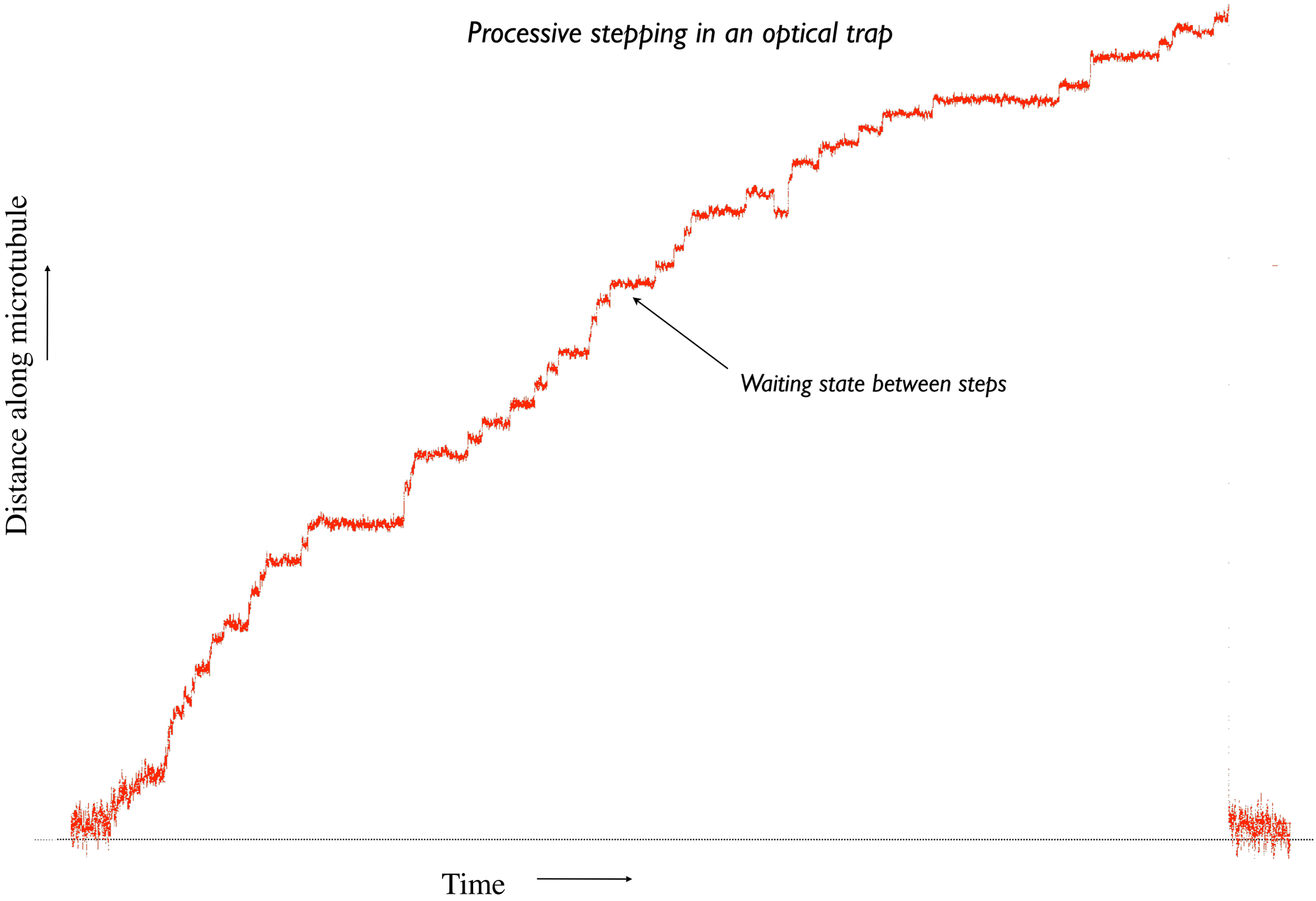
Rat kinesin, 2μM ATP, trap stiffness 0.018pN/nm, 10ms median filter applied.

Processive stepping in an optical trap



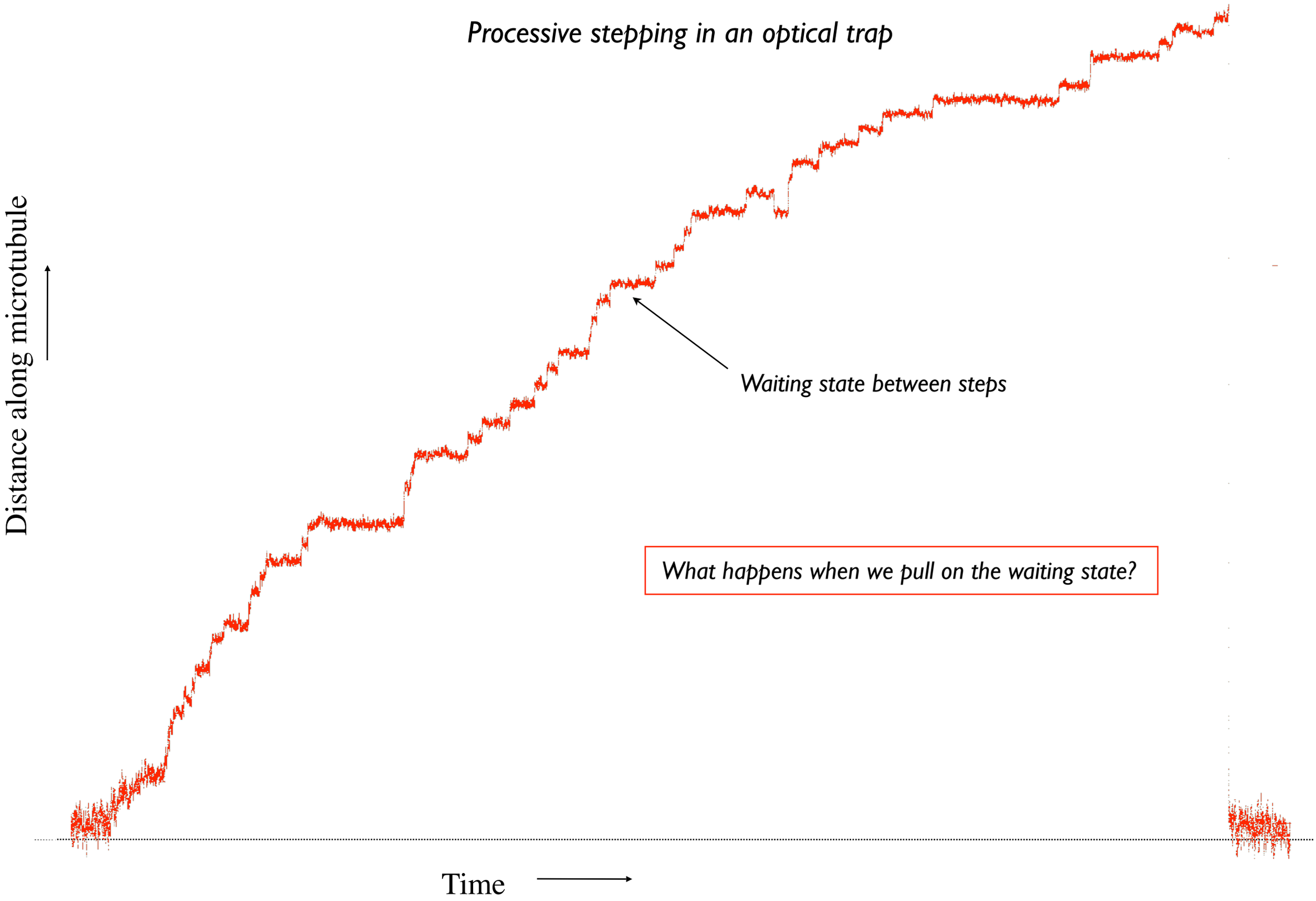
Rat kinesin, 2μM ATP, trap stiffness 0.018pN/nm, 10ms median filter applied.

Processive stepping in an optical trap



Rat kinesin, 2μM ATP, trap stiffness 0.018pN/nm, 10ms median filter applied.

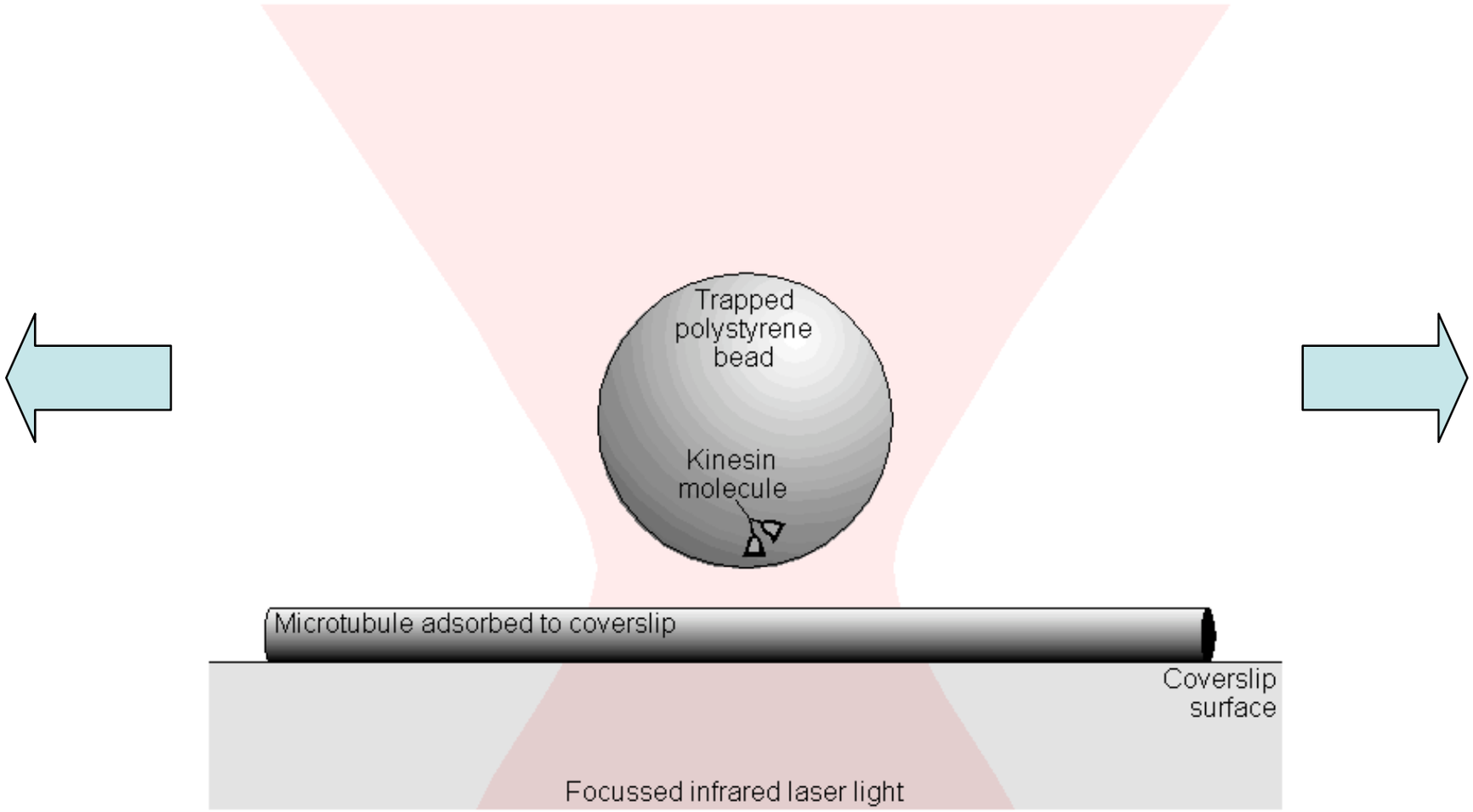
Processive stepping in an optical trap



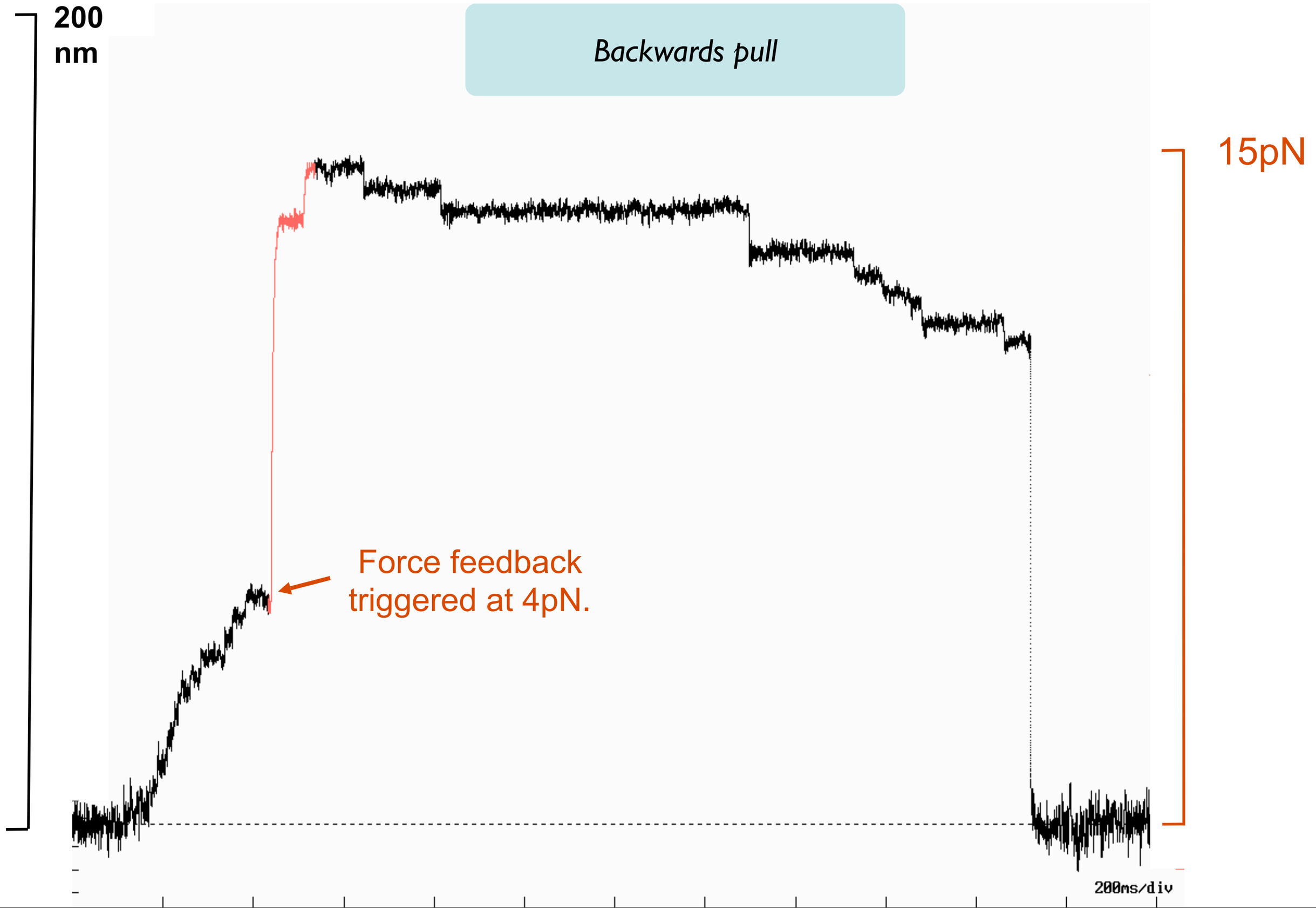
What happens when we pull on the waiting state?

Rat kinesin, 2μM ATP, trap stiffness 0.018pN/nm, 10ms median filter applied.

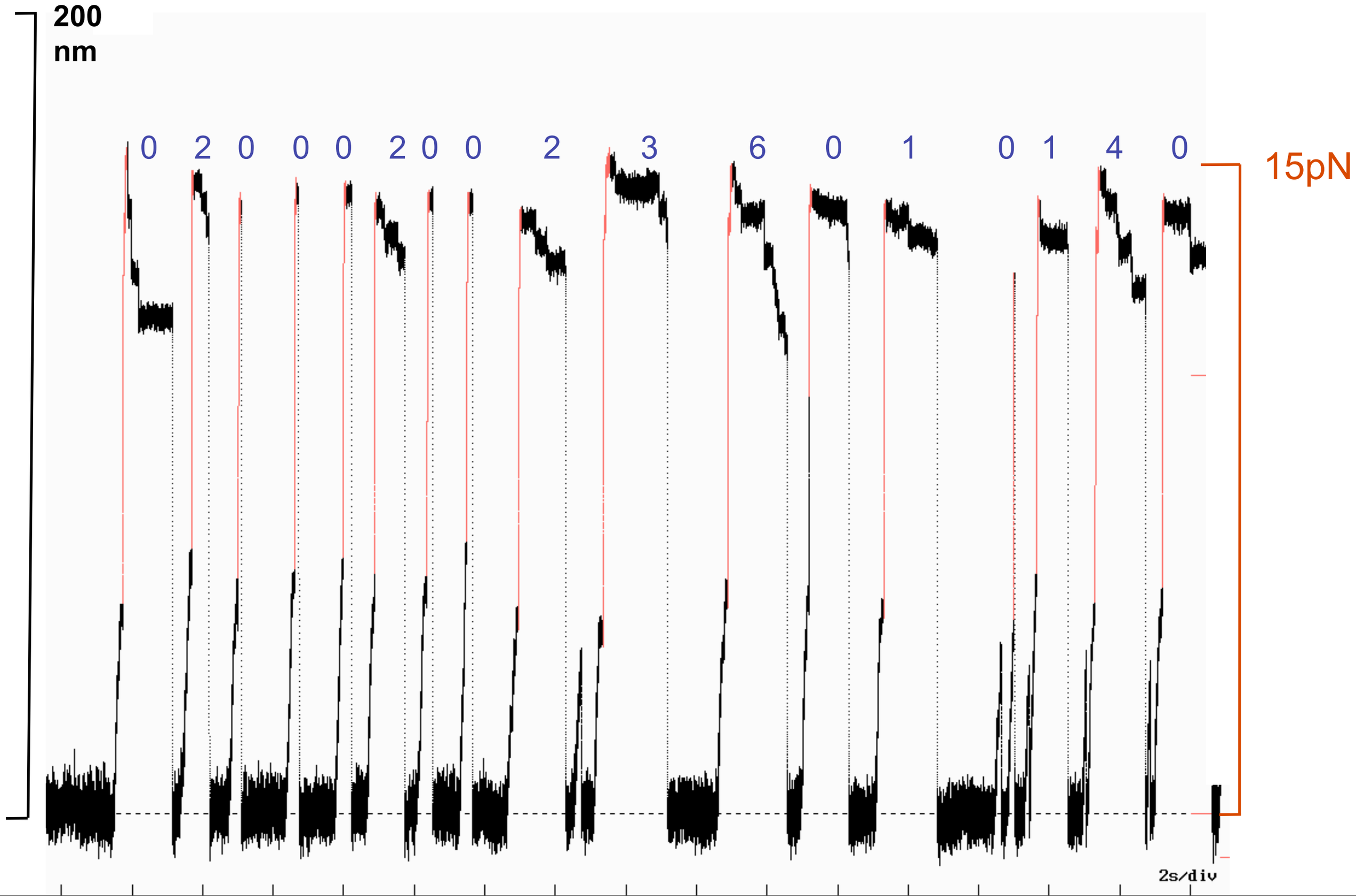
Pull backwards or forwards on walking kinesin molecules..



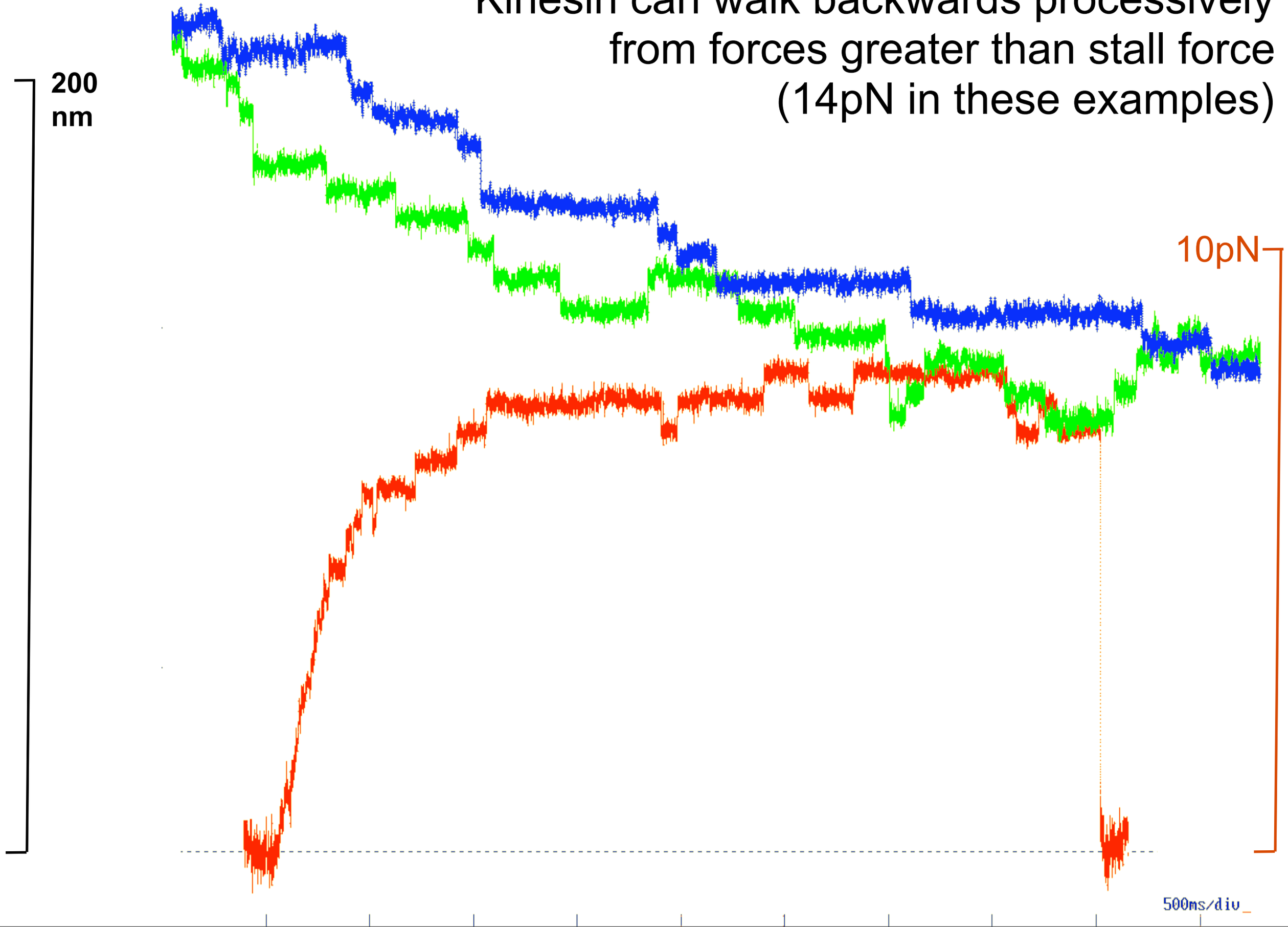
Brief period of force-feedback moves the microtubule (piezo. stage)
till the kinesin is loaded by approximately 14pN.

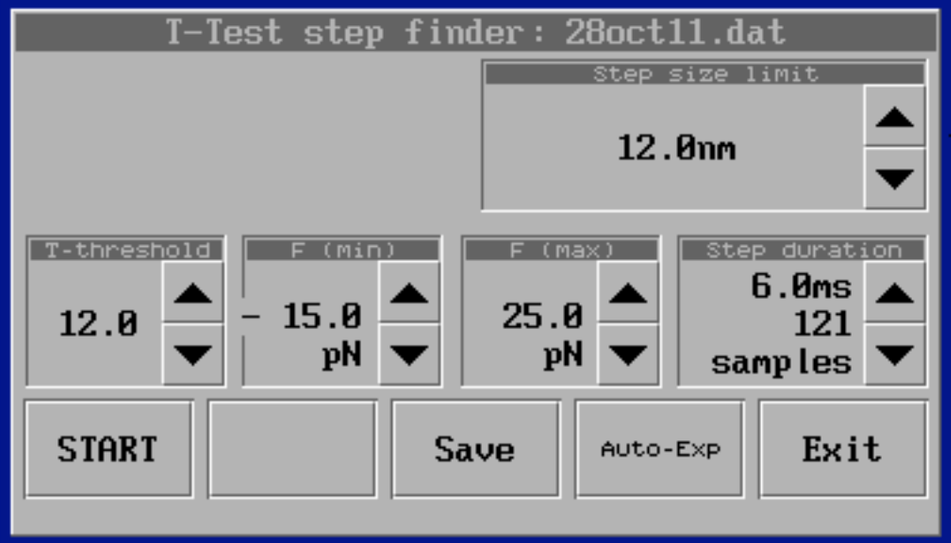


Multiple events. At 14pN, kinesin usually detaches before many back-steps.
(The number of 8nm back-steps, indicated over each event.)



Kinesin can walk backwards processively from forces greater than stall force (14pN in these examples)

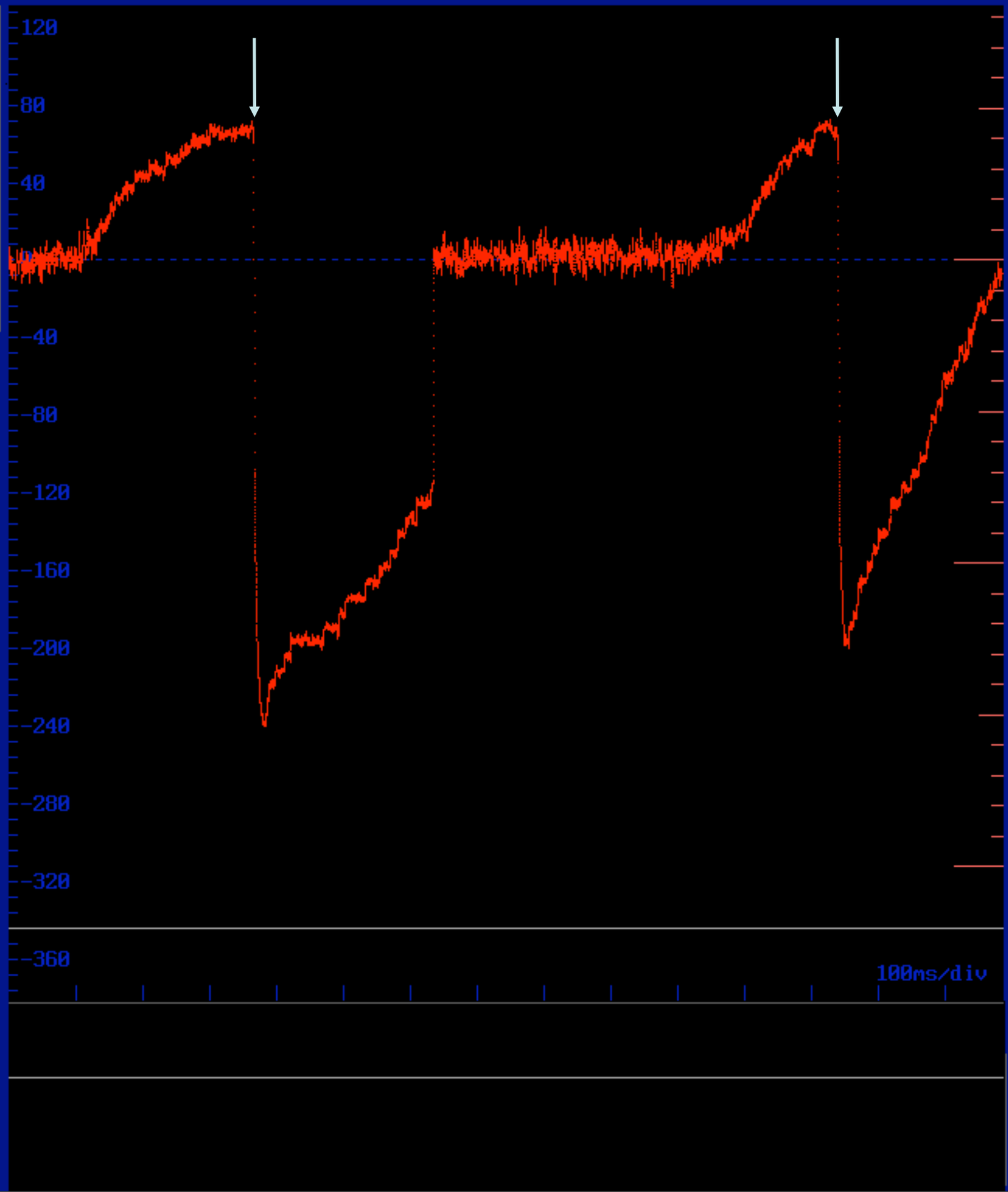




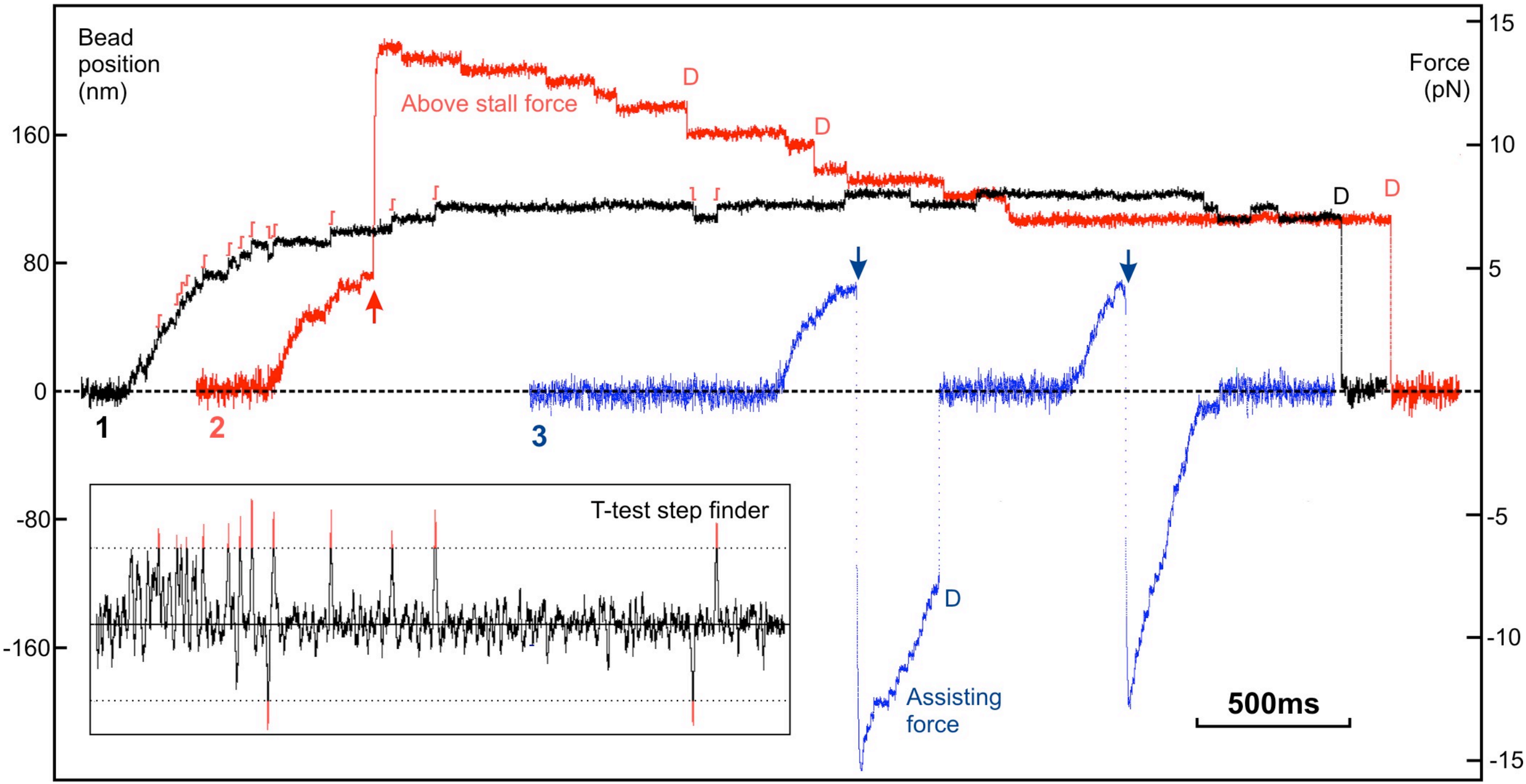
Forwards pull

At 4pN trigger point, the stage is moved until more than 13pN forward load is applied to the kinesin molecule.

(Conditions: *Drosophila* kinesin, 560nm beads, 20kHz sampling, 1mM ATP, K_{trap}=0.06pN/nm)



Kinesin can walk processively backwards from forces > stall force



Individual steps are too noisy - need stepfinder & averaging

1. Scan *t* test through entire data set, mark where value goes over a threshold.
2. Do a global exponential fit across all steps in data set, mark step-origins.
3. Line up the steps and average them together.

Files

Save | Load | 7sep12.dat | C:\data\trap1\nick\2004\ps500\dros-his\

Data display

Plot Width: Samples:262144, 2.62s | Scale: 260nm

Filtering: None | Mean | Median | Kernal — 101 point (1.0ms)

Y - t | 261° | Dots | Offset | Comments | no lines

Analysis

Quadrant Calibration | Step finder
Quadrant linearity | Test signal
Stiffness of trap | Fourier
Trigger | Selection Statistics

Grabber and overlay

Reset mem | Overlay | 1.0 | 1.6 | 2.5 | Grid | Text | Detect Movement | Mem | PZT | Laser

AODs: 0: 8000,8000 — Go.. | +5pN | -5pN | − | + | Funtion | Multi — 1 trap

Piezo: X:0.000µm Y:0.000µm X: 0 Y: 0 — Centre | Func | Trash | M+ | MR

Sampling: Set: 100,000Hz, Desrd: 140,000Hz, ADC:, Range: ± 5.0V — 0h,0h | Save 0zero

Laser: Pwr OFF

0	100	400	900
10	150	500	1000
20	200	600	1100
50	300	800	1200

Focus: ON | Z=0 | Bench mark | Z=1 — 0

Conditions: D 500nm | T 23.5°C
Buffer BRB80-GOC,5mMDTT,1mMATP
Motor Drosophila kinesin (JH)
Users Nick

160
120
80
40
0
-40

200ms/div

14.0Mb (100.0%) 36.7s

Kinesin approaching stall force.

Raw data

(Conditions: *Drosophila* kinesin, 500nm beads, 100kHz sampling, 1mM ATP, K_{trap}=0.054pN/nm)

Files

Save | Load | 7sep12.dat | C:\data\trap1\nick\2004\ps500\dros-his\

Data display

Plot Width: Samples:262144, 2.62s

Scale: 260nm

Filtering: None | Mean | Median | Kernal — 101 point (1.0ms)

Y - t | 261° | Dots | Offset | Comments | no lines

Analysis

Quadrant Calibration | Step finder | Quadrant linearity | Test signal | Stiffness of trap | Fourier | Trigger | Selection Statistics

Sample 1438210
X:41.8nm
Y:132.4nm(7.2pN)
Z:0.00µm
T:14.382s
Laser:1,000mW
Ktrap:0.054pN/nm

Grabber and overlay

Reset mem | Overlay | 1.0 | 1.6 | 2.5 | Grid | Text | Detect Movement | Mem | PZT | Laser

AODs: 0: 8000,8000 — Go.. | +5pN | -5pN | − | + | Funtion | Multi | 1 trap

Piezo: X:0.000µm Y:0.000µm X: 0 Y: 0 — Centre | Func | Trash | M+ | MR

Sampling: Set: 100,000Hz, Desrd: 140,000Hz, ADC:, Range: ± 5.0V | 0h,0h | Save Ozero

Laser: Pwr OFF | 0 | 100 | 400 | 900 | 10 | 150 | 500 | 1000 | 20 | 200 | 600 | 1100 | 50 | 300 | 800 | 1200

Focus: ON | Z=0 | Bench mark | Z=1 | 0

Conditions: D 500nm | T 23.5°C
Buffer BRB80-GOC,5mMDTT,1mMATP
Motor Drosophila kinesin (JH)
Users Nick

160
120
80
40
0
-40

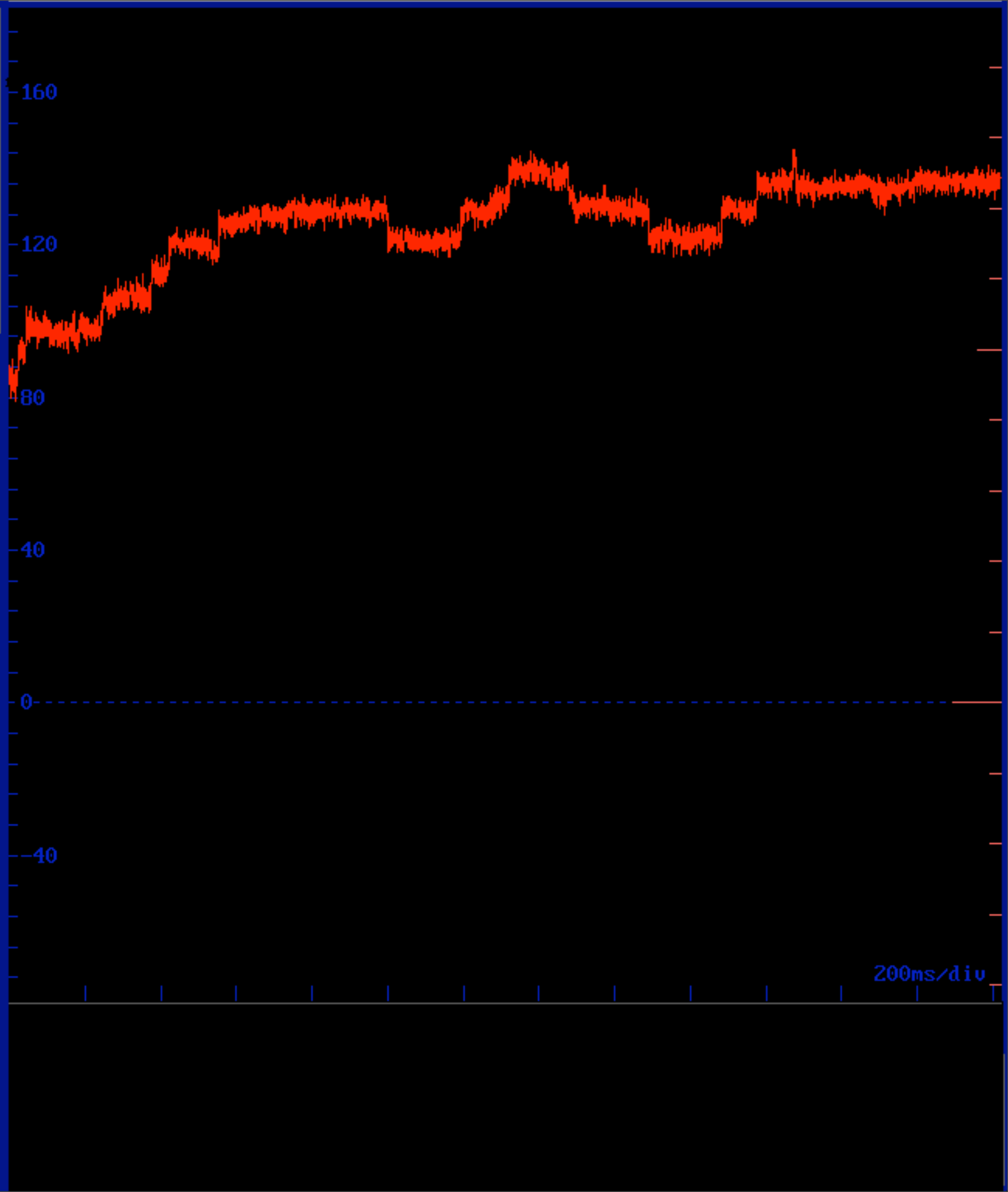
200ms/div

14.0Mb (100.0%)
36.7s

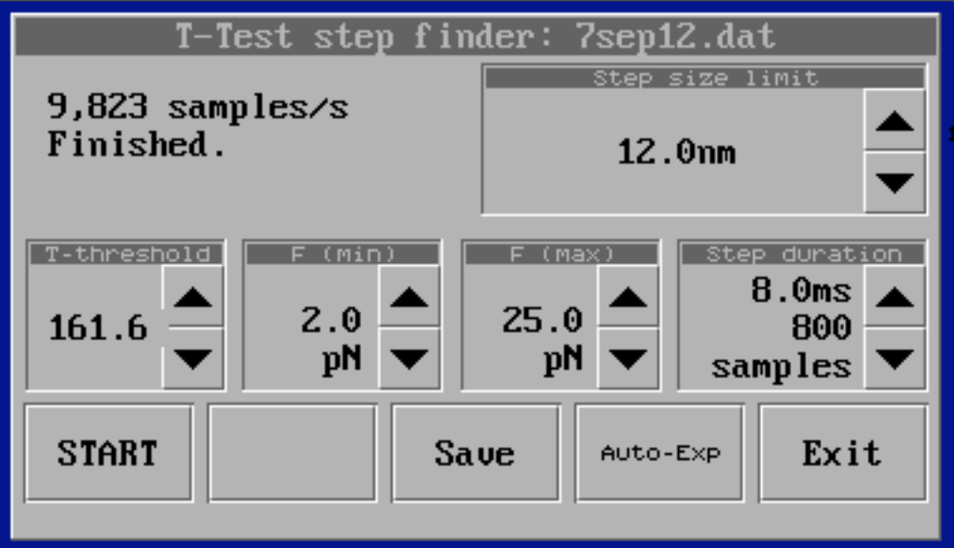
Kinesin approaching stall force.

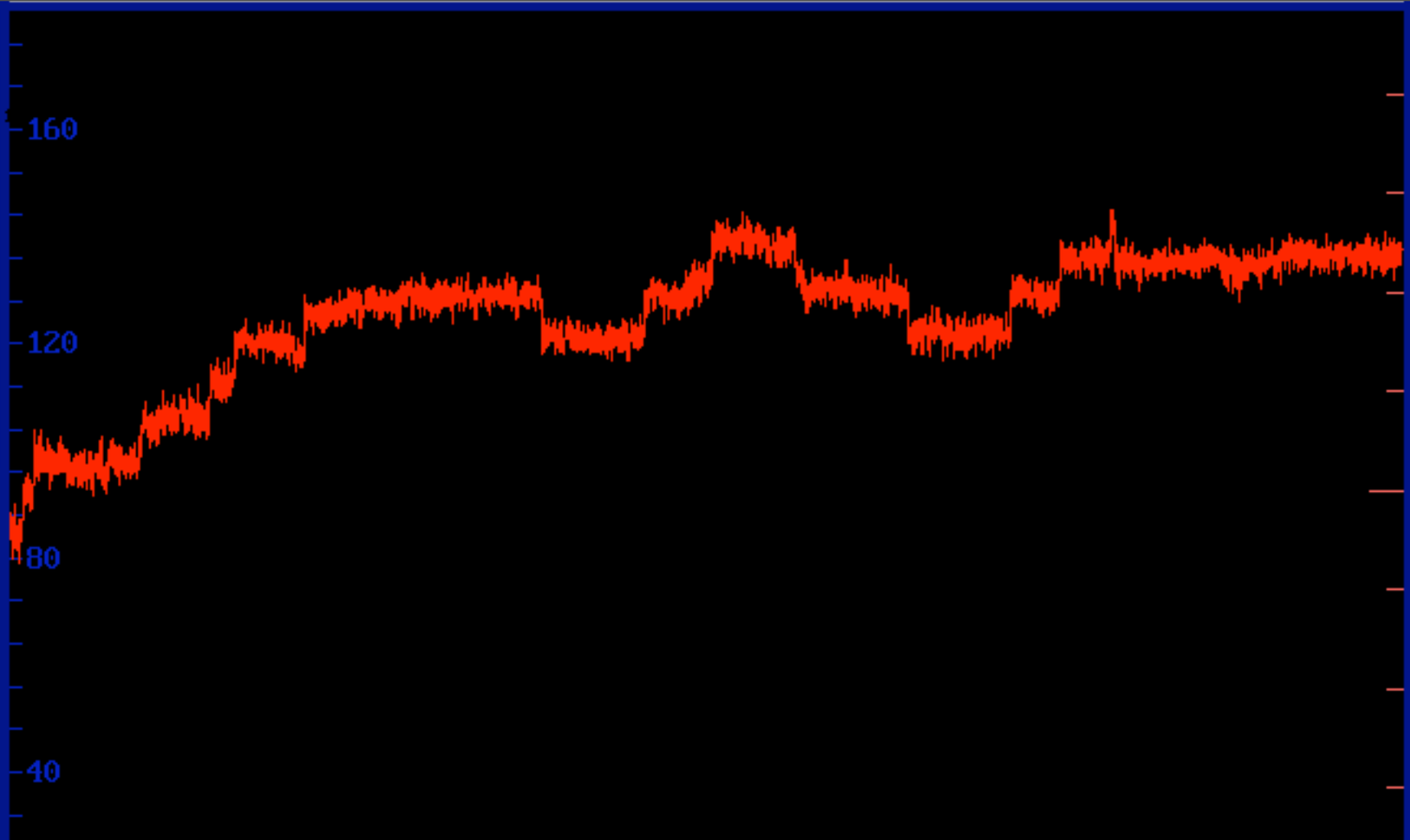
1 ms boxcar filtered

(Conditions: *Drosophila* kinesin, 500nm beads, 100kHz sampling, 1mM ATP, K_{trap}=0.054pN/nm)



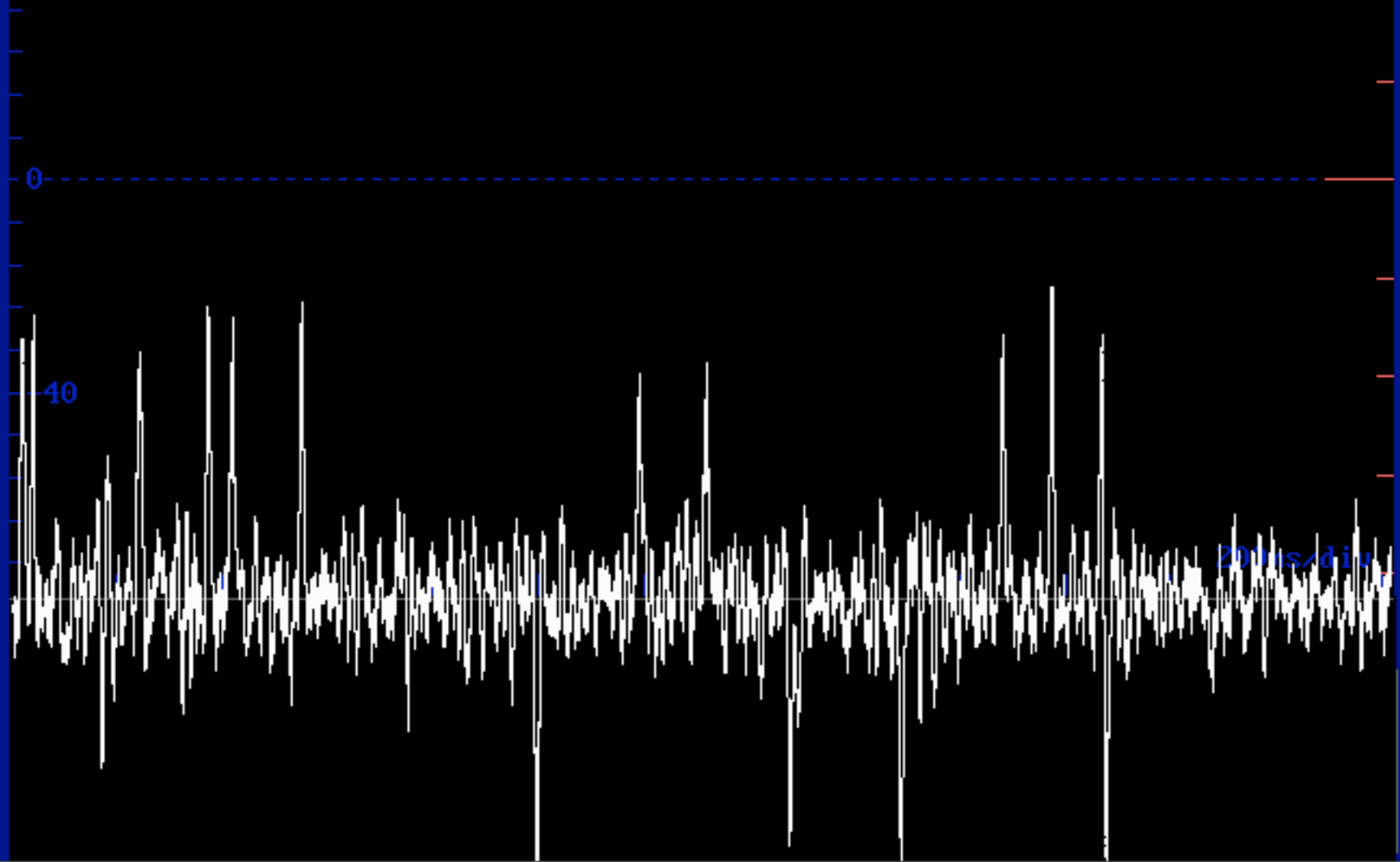
Locating steps automatically…





T-Test applied to the unfiltered data.

Up-spikes for forward steps, down-spikes for back steps.



T-Test step finder: 7sep12.dat

17,005 samples/s
Finished.
Steps: 15

Step size limit
12.0nm

T-threshold
26.0

F (min)
2.0 pN

F (max)
25.0 pN

Step duration
8.0ms
800 samples

START | Save | Auto-Exp | Exit

Fit fore: 107233s-1
back: 68242s-1
1.0ms

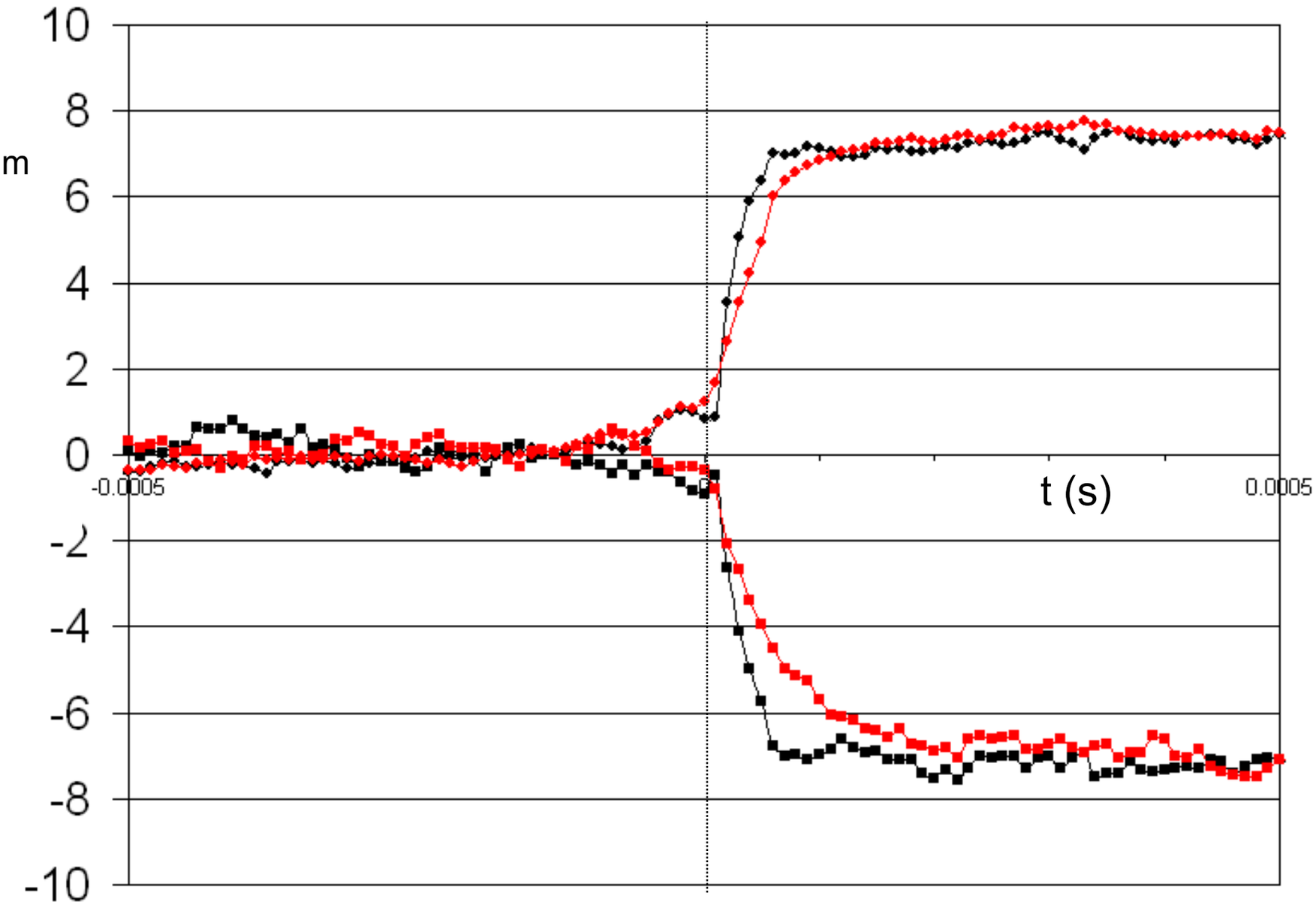
Fore: n = 11, Amp = 7.1nm F=6.3pN
Back: n = 4, Amp = -7.4nm F=7.1pN
16.0ms

160
120
80
40
0
-40

Averaged steps (1ms and 16ms time-scales).

Threshold set to define step positions.

Averaged forward and back-steps from multiple traces



500nm beads
Fore-steps (n=1693)
time const.=15.3µs

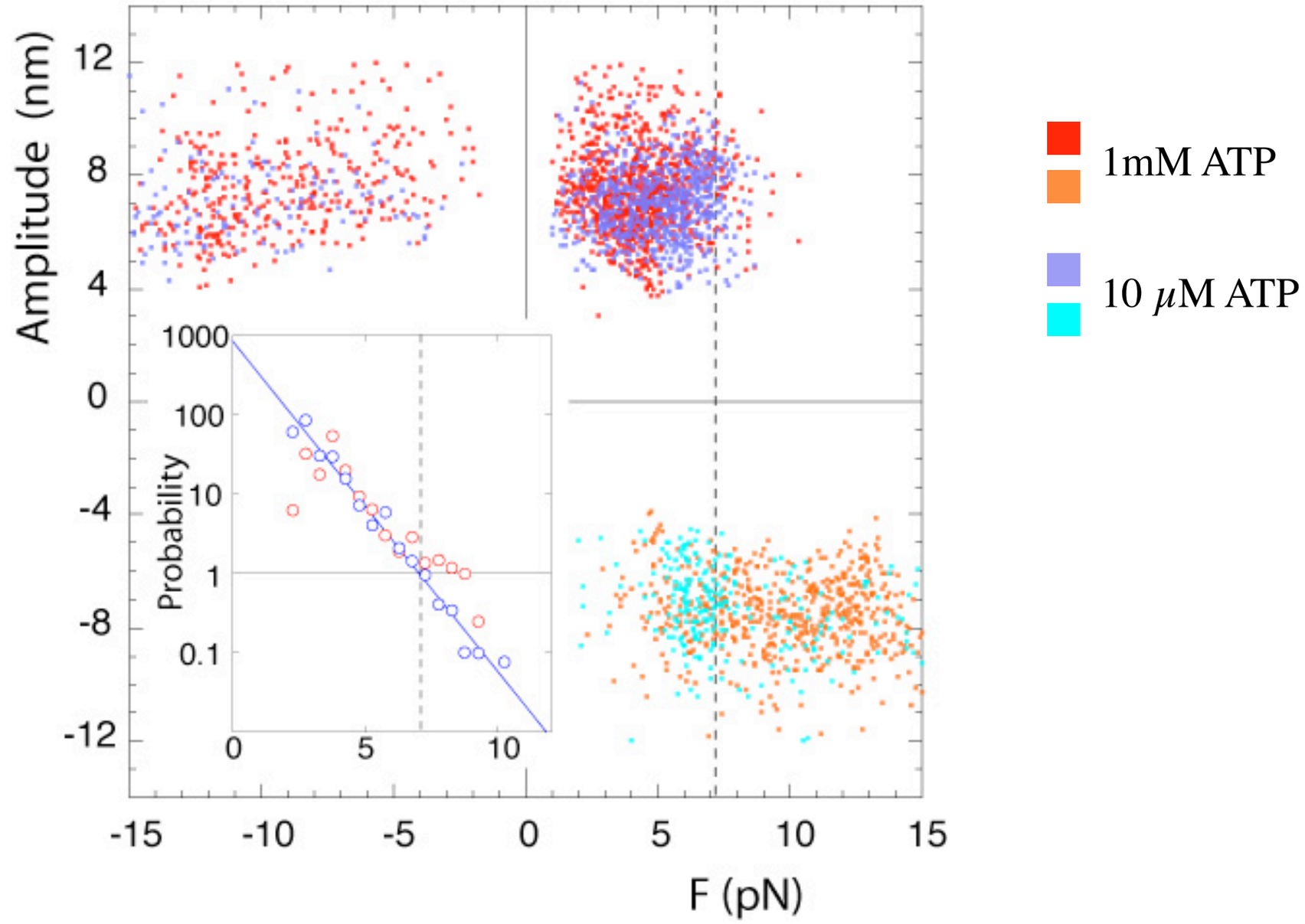
Back-steps (n=316)
time const.=19.4µs

800nm beads
Fore-steps (n=565)
time const.=35.9µs

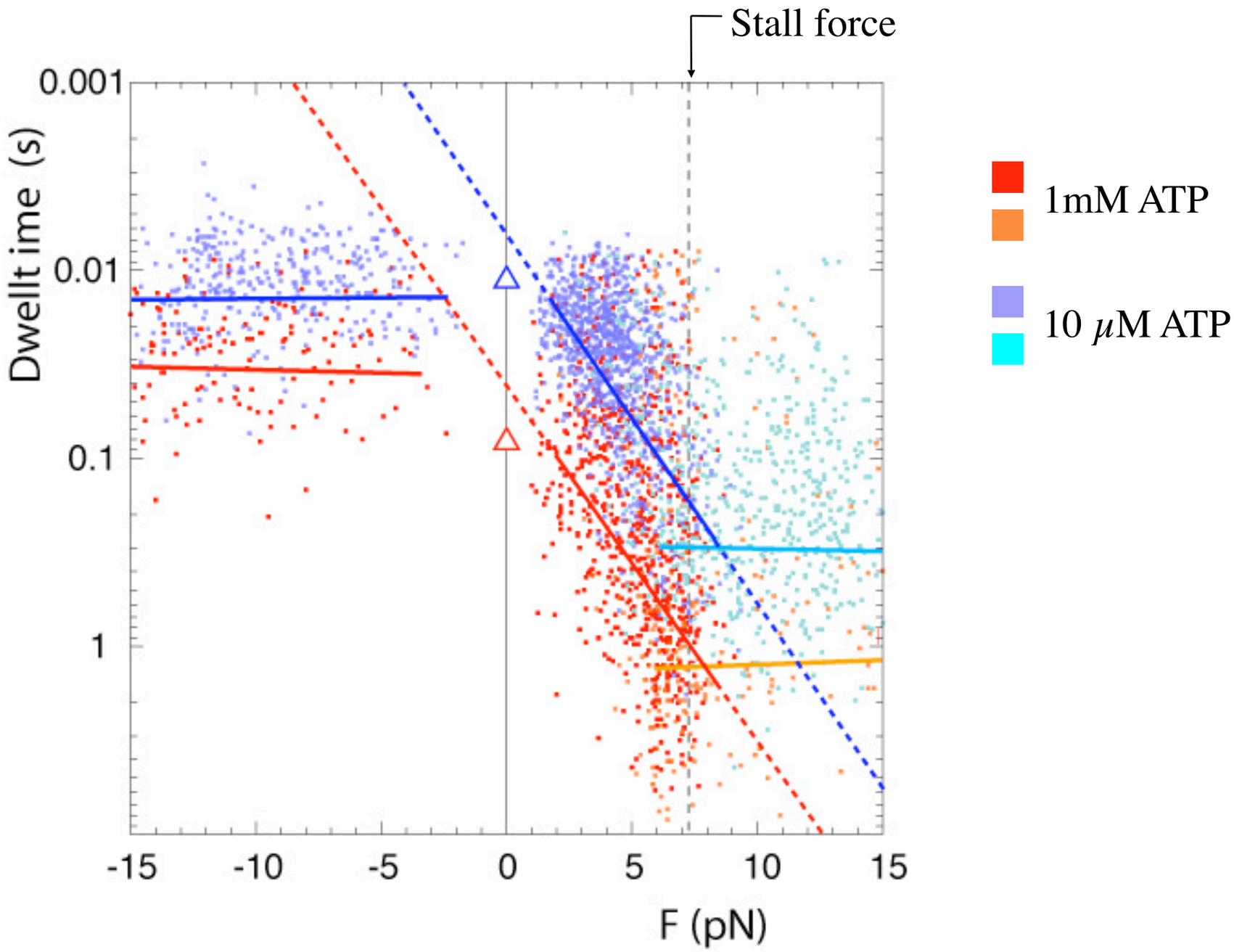
Back-steps (n=68)
time const.=37.3µs

10µs between data points.
Dependence of step duration on bead size

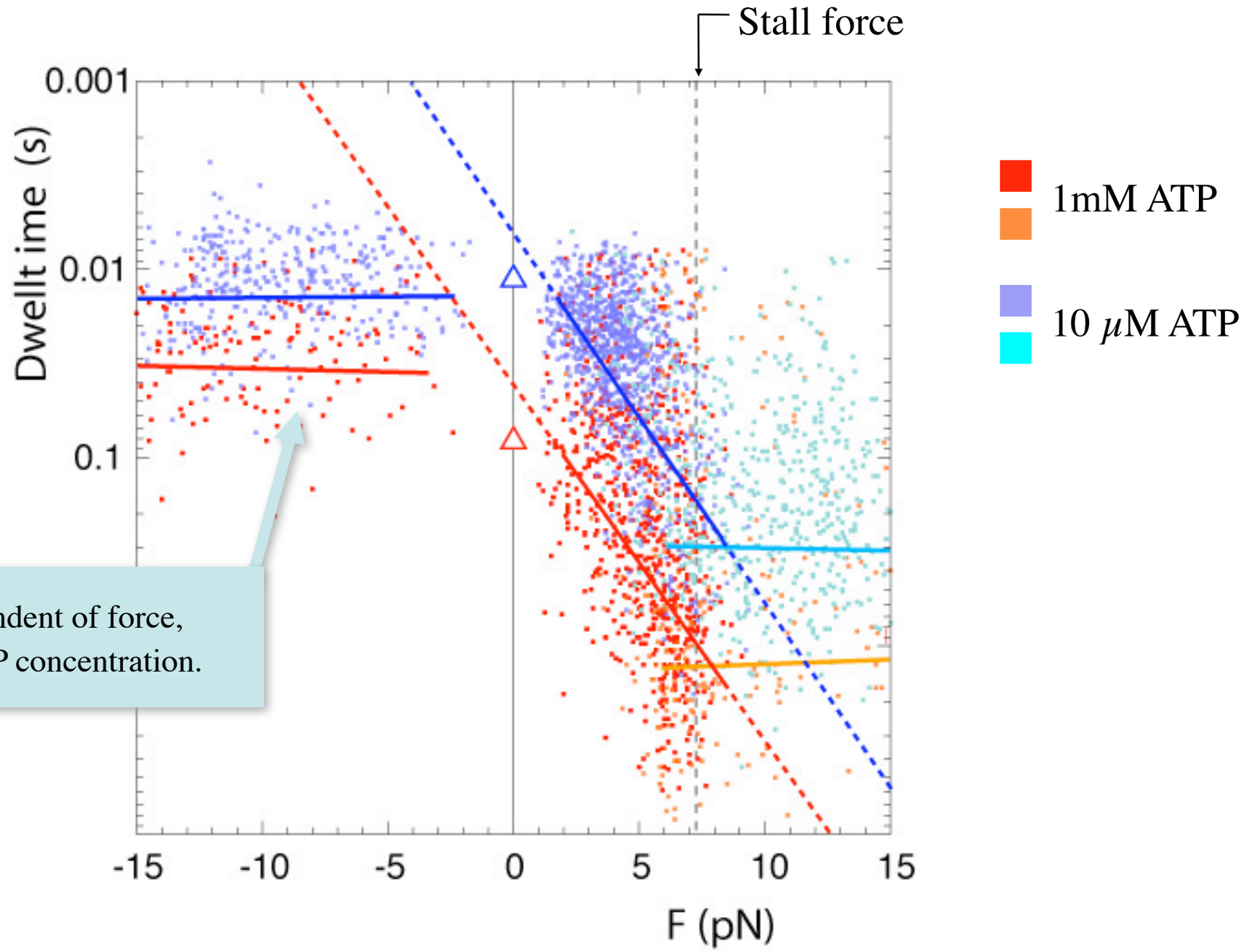
Step amplitudes versus force



Dwell times versus force and [ATP]

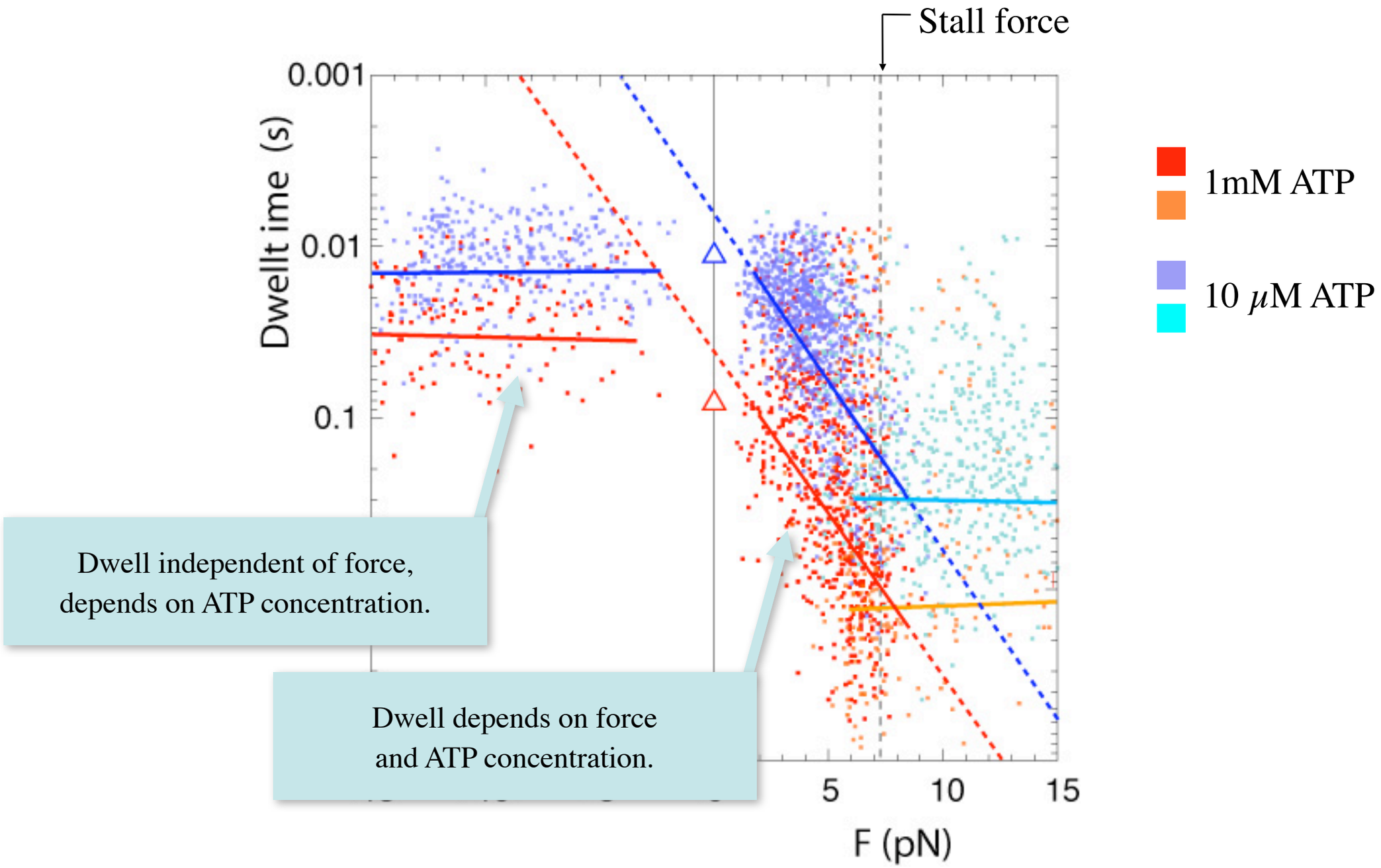


Dwell times versus force and [ATP]

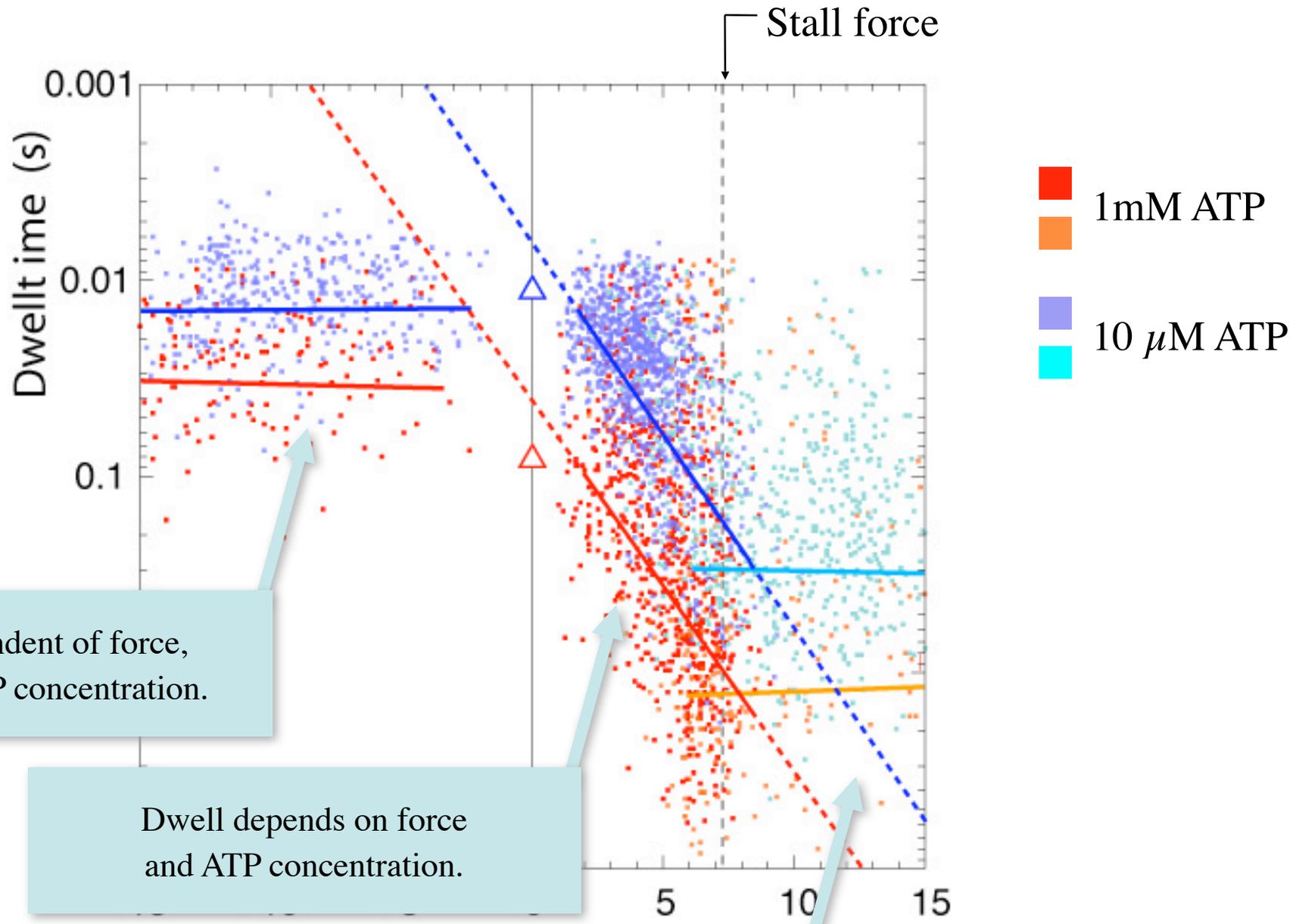


Dwell independent of force, depends on ATP concentration.

Dwell times versus force and [ATP]

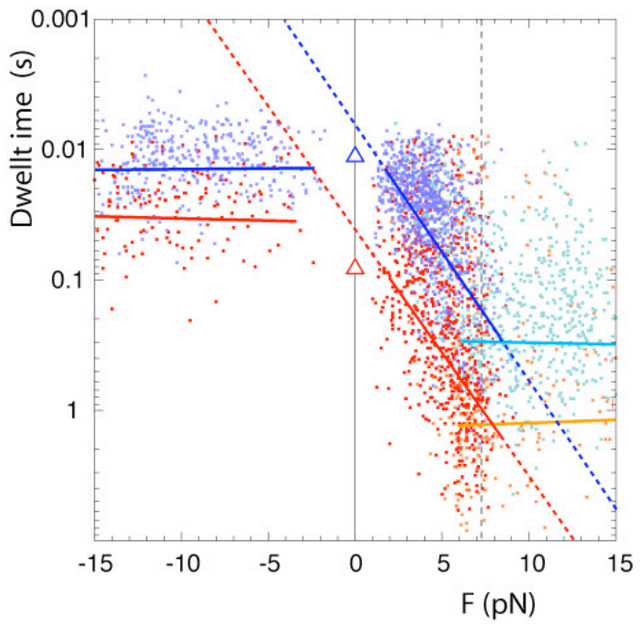


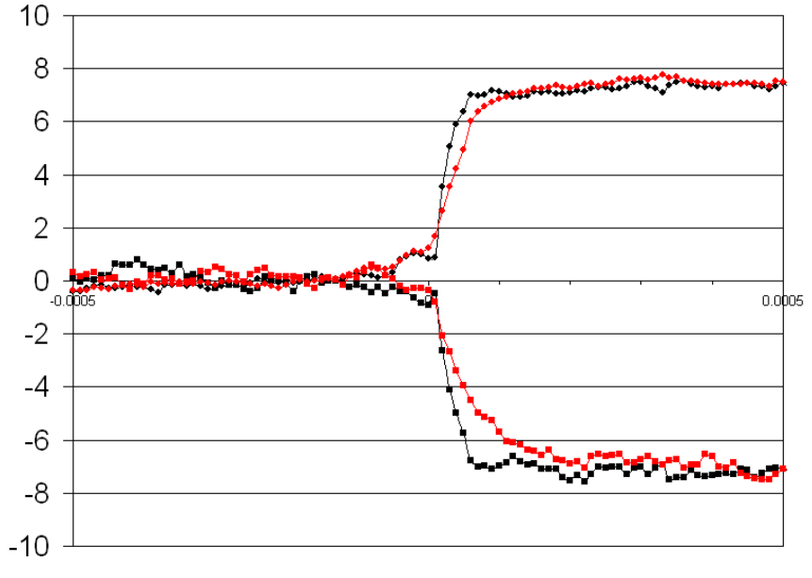
Dwell times versus force and [ATP]

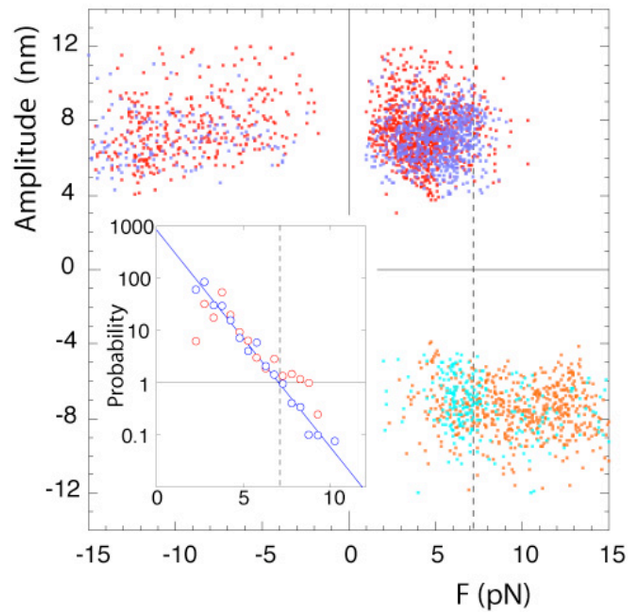


Mechanics of the kinesin step

Nick Carter & Rob Cross

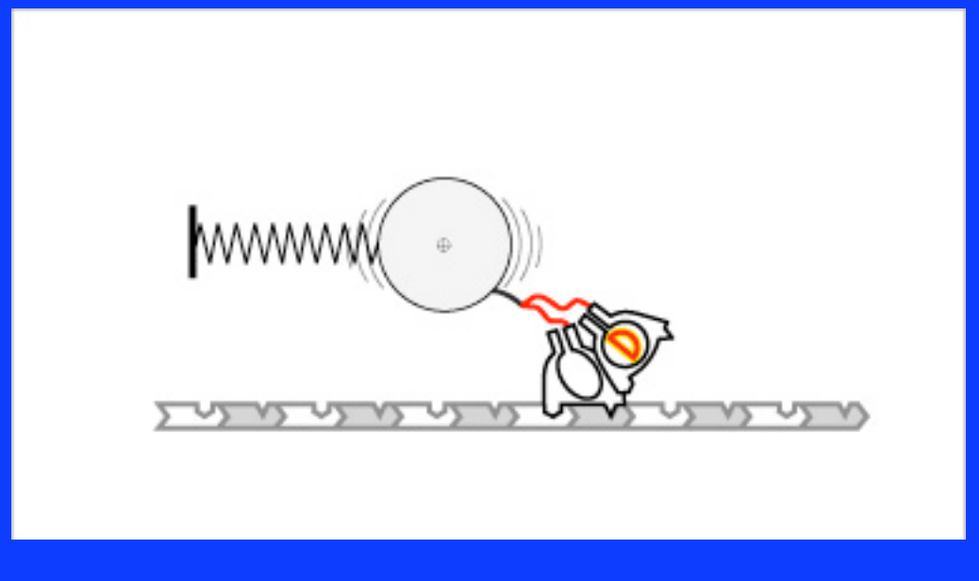






- *Fore and backsteps are single microsecond events: no substeps*
- *At high backwards loads kinesin walks processively backwards*
- *Backsteps require ATP - Dwell-times for backsteps are insensitive to load*
- *Dwell times for forward steps under high backwards load depend exponentially on load*
  Dwells under forward load are insensitive to load.

Mechanics of the kinesin step



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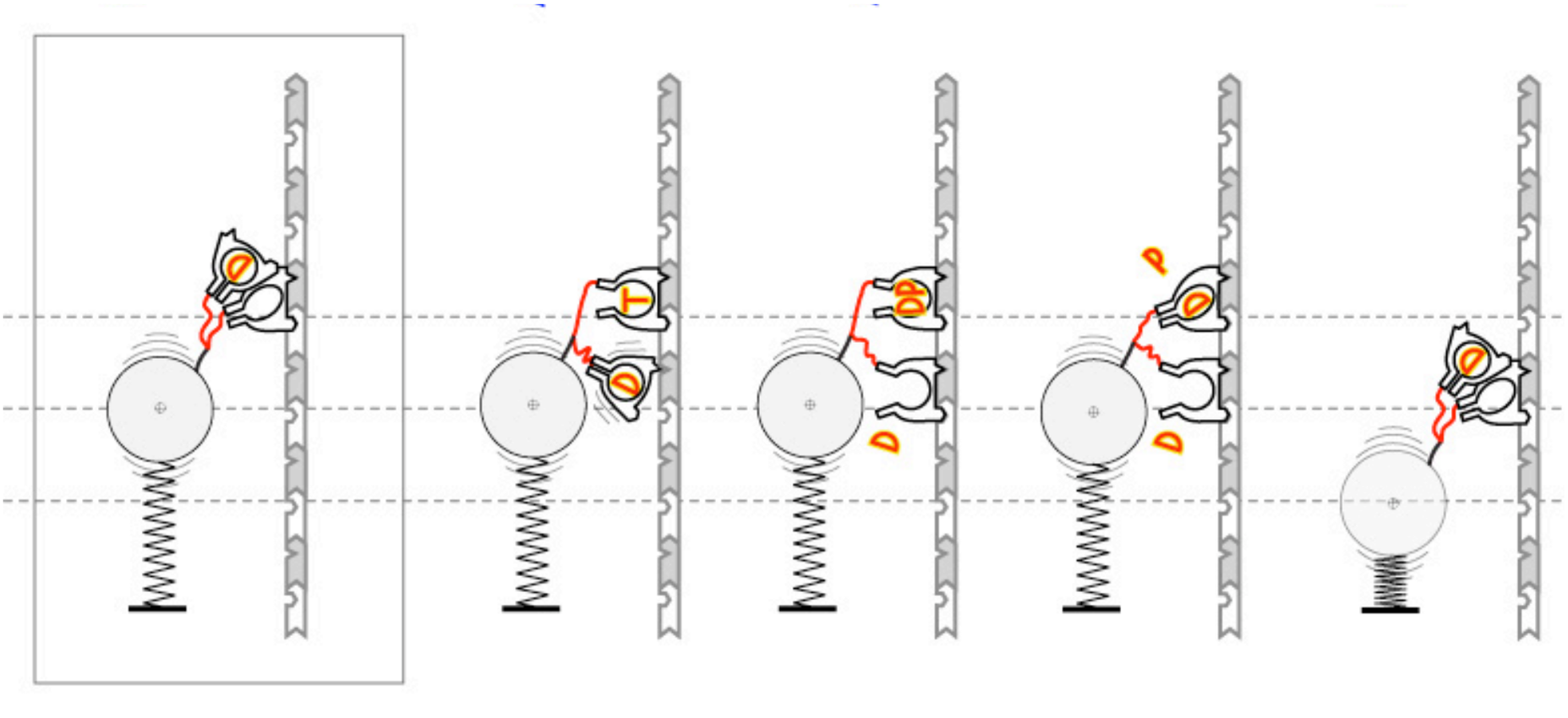
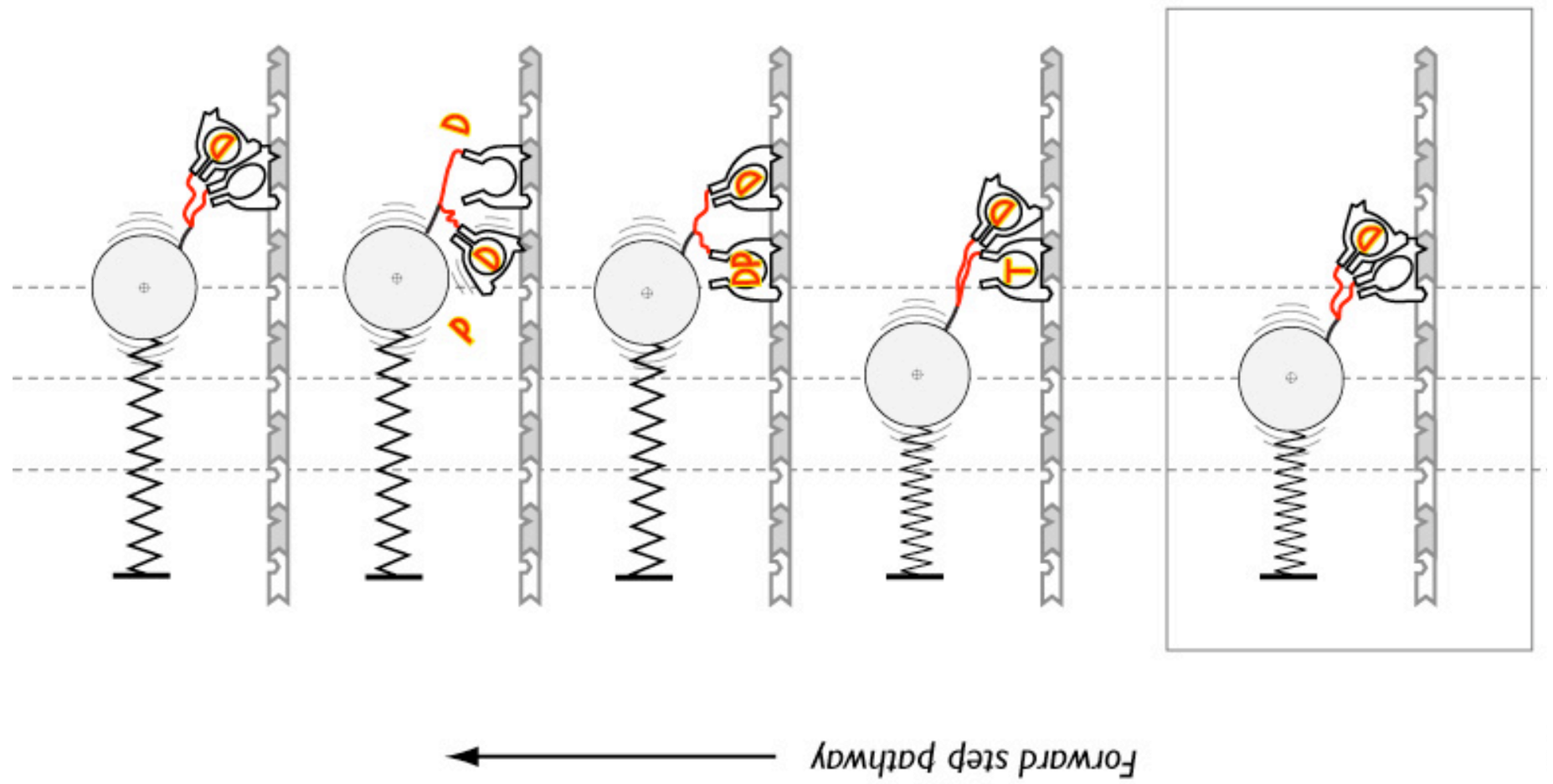

Back step pathway


Forward step pathway

to do ..

http://mc11.mcri.ac.uk/motorhome.html

to do ..

- neck linker docking cycle
- protofilament tracking (straddle/tightrope)
- 2 parked states (?)
- roadblocks
- low-friction attached states
- product rebinding under load

http://mc11.mcri.ac.uk/motorhome.html