Quiz (2/20/08, Chpt 3, ECB)

1. A chemical process where there is a net gain of electrons is called reduction. A chemical process where there is a net loss of electrons is called oxidation.

2. Enzymes are catalysts, they often lower the free energy of a reaction by favoring a transition state.

3. This nucleotide cofactor prominently featured as an electron carriers are NAD^+ and $NADP^+$.

4. This is the constant at which an enzyme is operating at half of its maximum speed. Km, Michalis-Menten constant

5. The maximum number of catalytic cycles an enzyme can perform per unit time is called the Turnover rate.

FIONA

Fluorescence Imaging with One Nanometer Accuracy

(1.5 nm, 1-500 msec)

Techniques needed

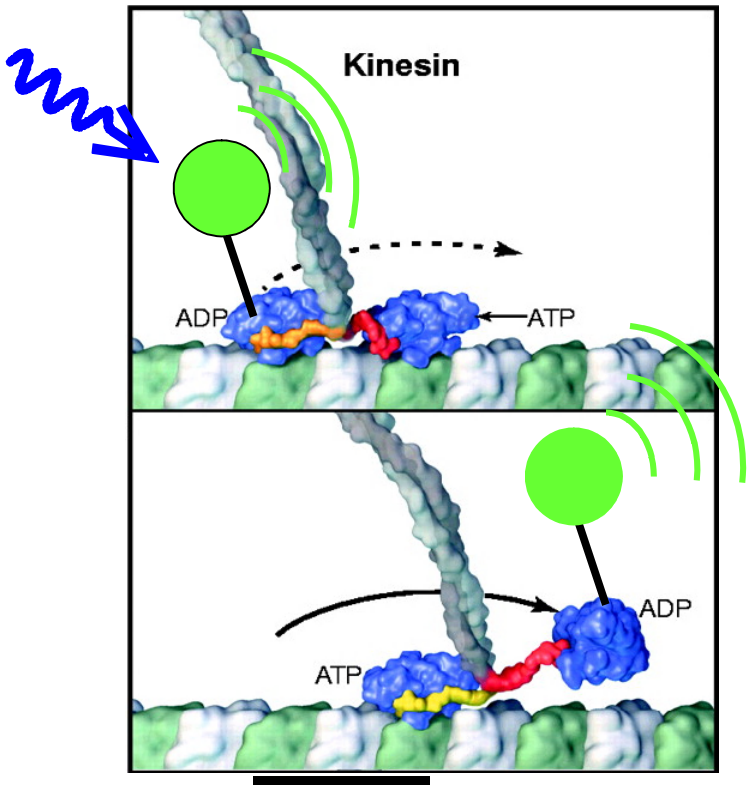
Specificity to look at heads

Nanometer spatial localization

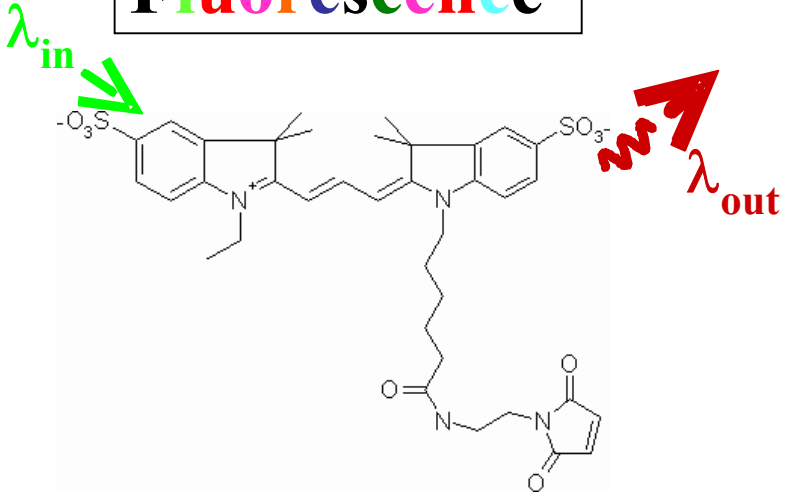
Second temporal resolution

Single Molecule sensitivity

Single Molecule Photostability



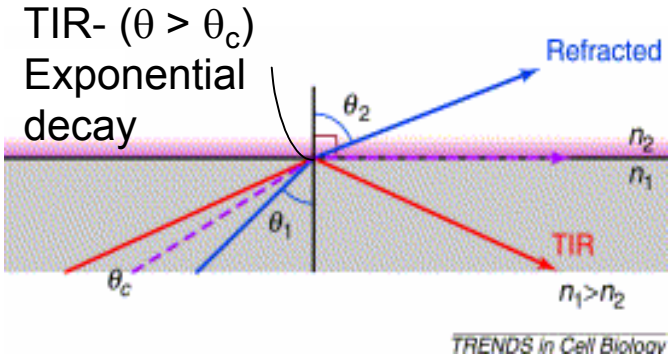
Fluorescence

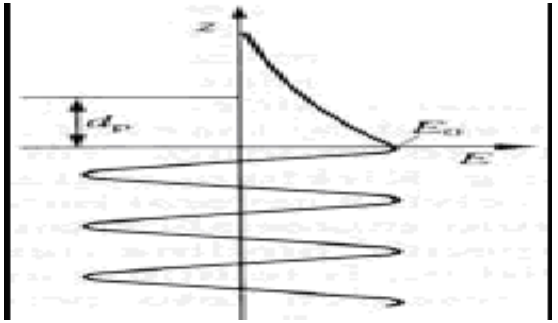


How to get nanometer localization with visible photons?

Imaging Single Molecules with very good S/N

Total Internal Reflection Microscopy





$$d_p=(\lambda/4\pi)[n_1^2\sin^2\theta_i) - n^2_2]^{-1/2}$$

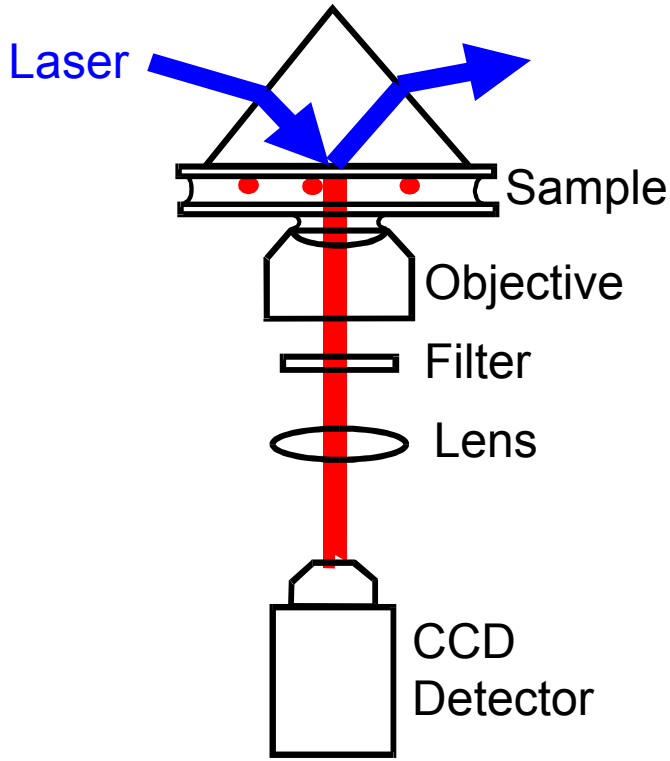
For water (n=1.33) to air (n=1.0): what is TIR angle?

For glass (n=1.5), water (n=1.33):

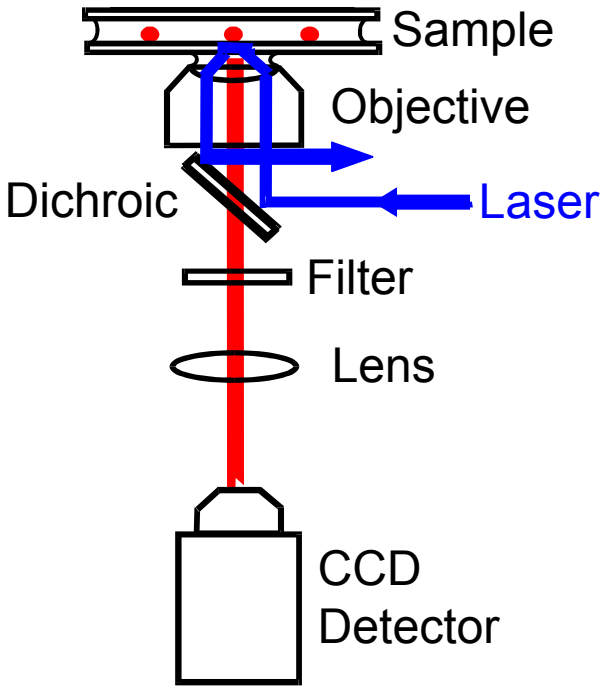
what is TIR angle? >57° what is penetration depth? d_p = 58 nm

With d_p = 58 nm , can excite sample and not much background.

Experimental Set-up for TIR
(2 set-ups)



Wide-field, Prism-type,
TIR Microscope

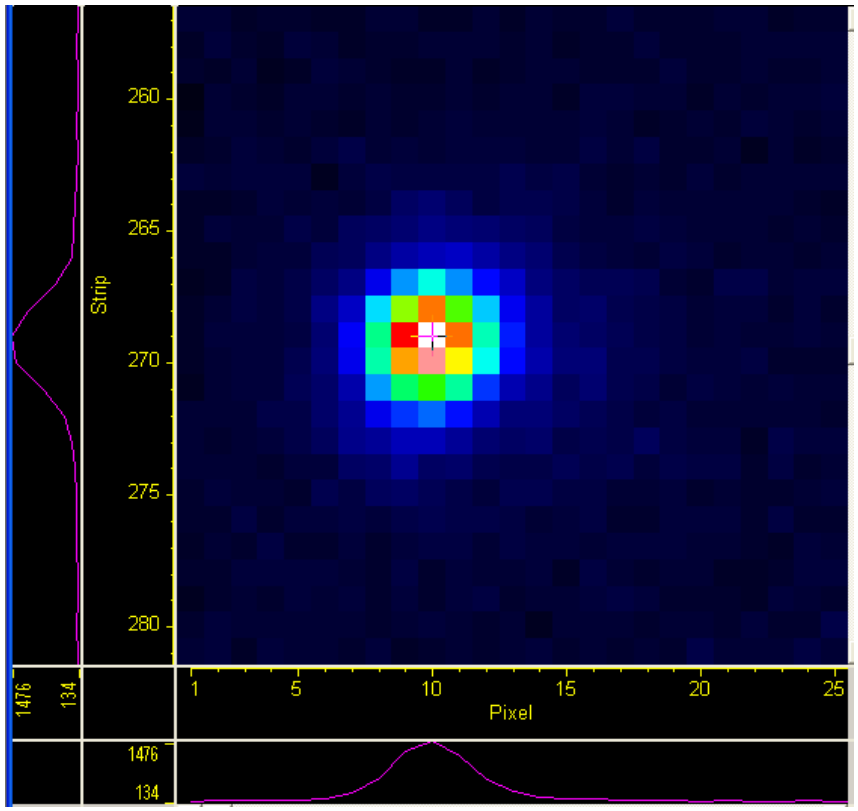


Wide-field
Objective-TIR

In one case, sample is “upside-down.” Does this make a difference? **No!**

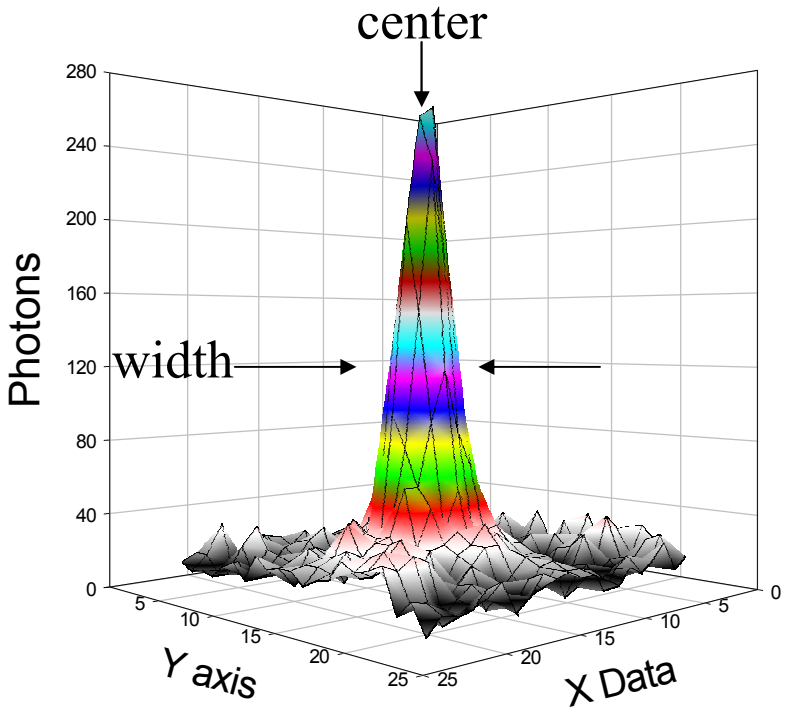
Diffraction limited spot

Width of $\lambda/2 \approx 250$ nm



Accuracy of Center = width/ S-N

= 250 nm / $\sqrt{10^4}$ = 2.5 nm= ± 1.25nm

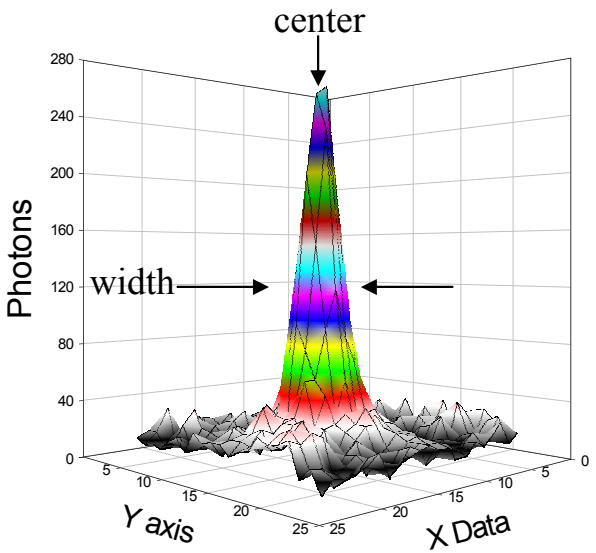


Enough photons (signal to noise)…**Center determined to ~ 1.3 nm**

Dye last 5-10x longer -- typically ~30 sec- 1 min. (up to 4 min)

How well can you localize?

What does it depend on?
(3 things)



1. # of Photons Detected (N)

2. Pixel size of Detector (a)

3. Noise (Background) of Detector (b)
(includes background fluorescence and detector noise)

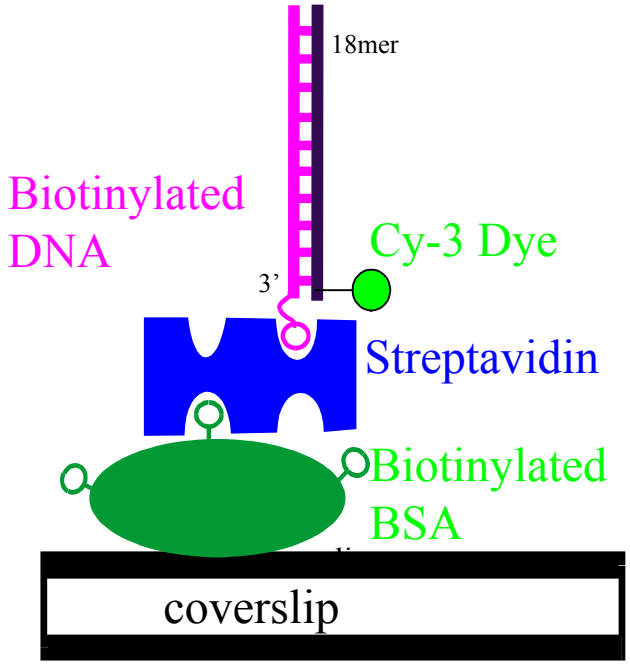
$$\sigma_{\mu_i} = \sqrt{\left(\frac{s_i^2}{N} + \frac{a^2/12}{N} + \frac{8\pi s_i^4 b^2}{a^2 N^2}\right)}$$

derived by Thompson et al. (Biophys. J.).

Experimental Setup: Imaging Single Molecules

Cy3-DNA Immobilized on coverslip

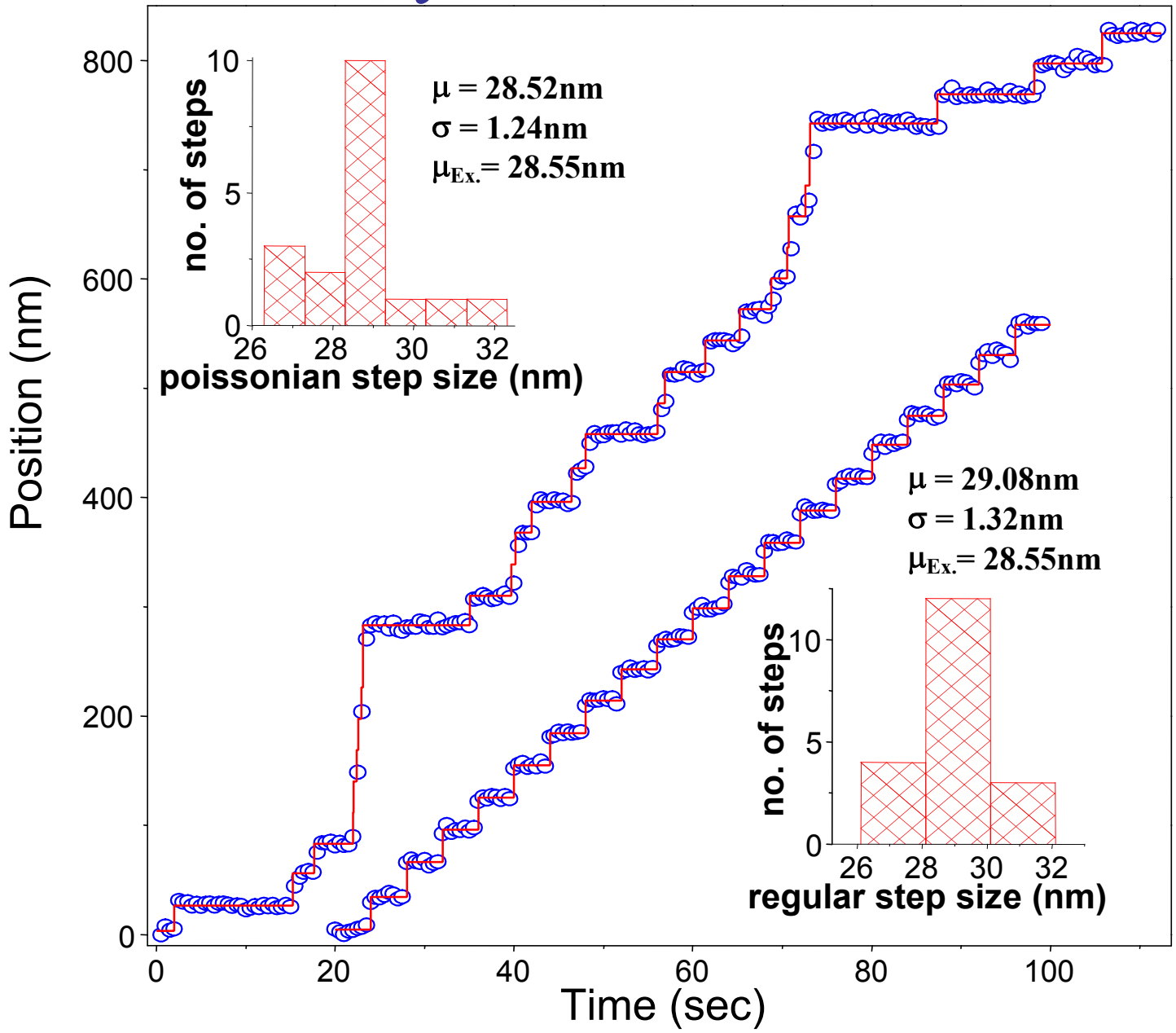
DNA Sample



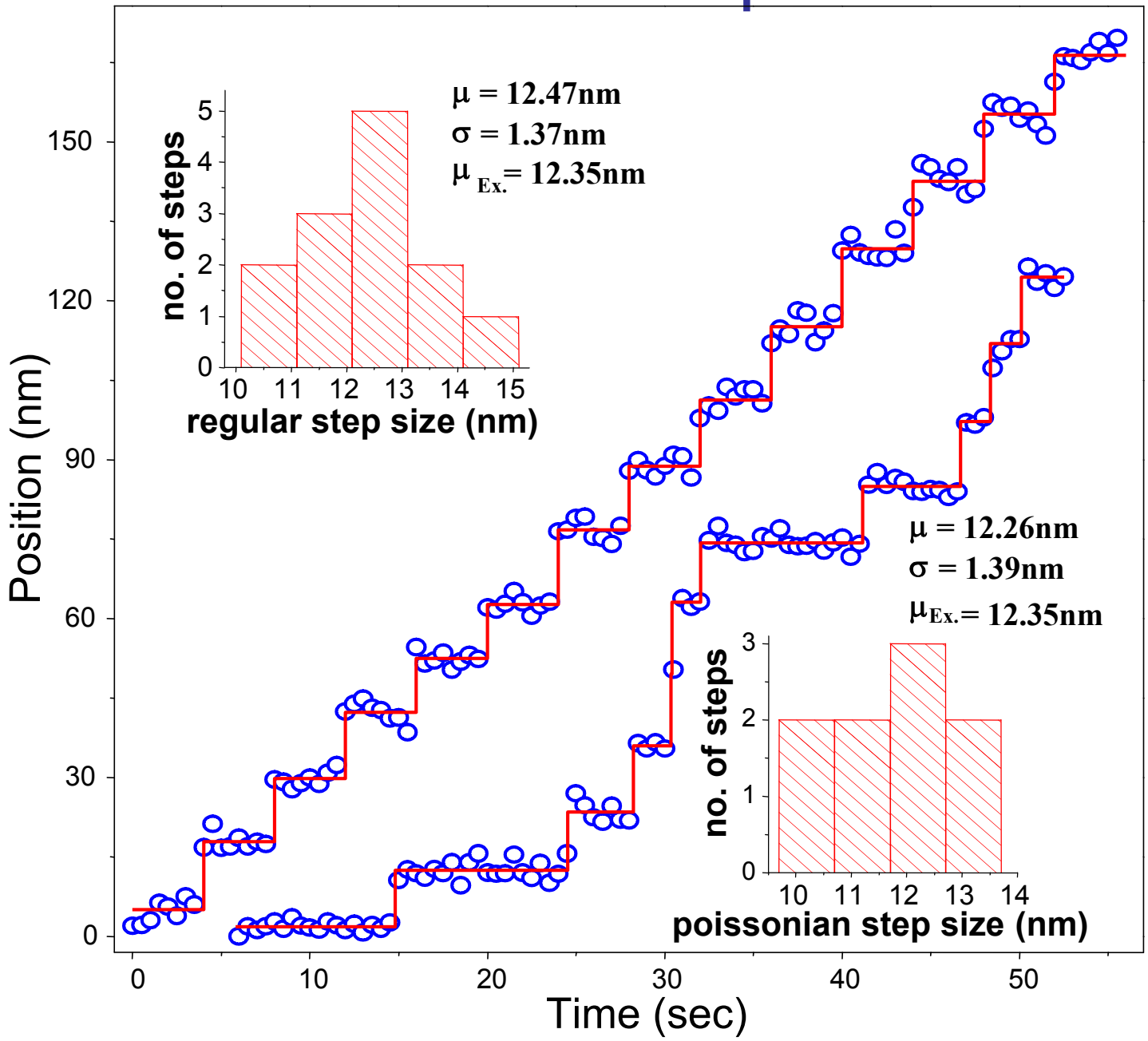
Move stage

In 8 nm, 16 nm, 37 nm increments

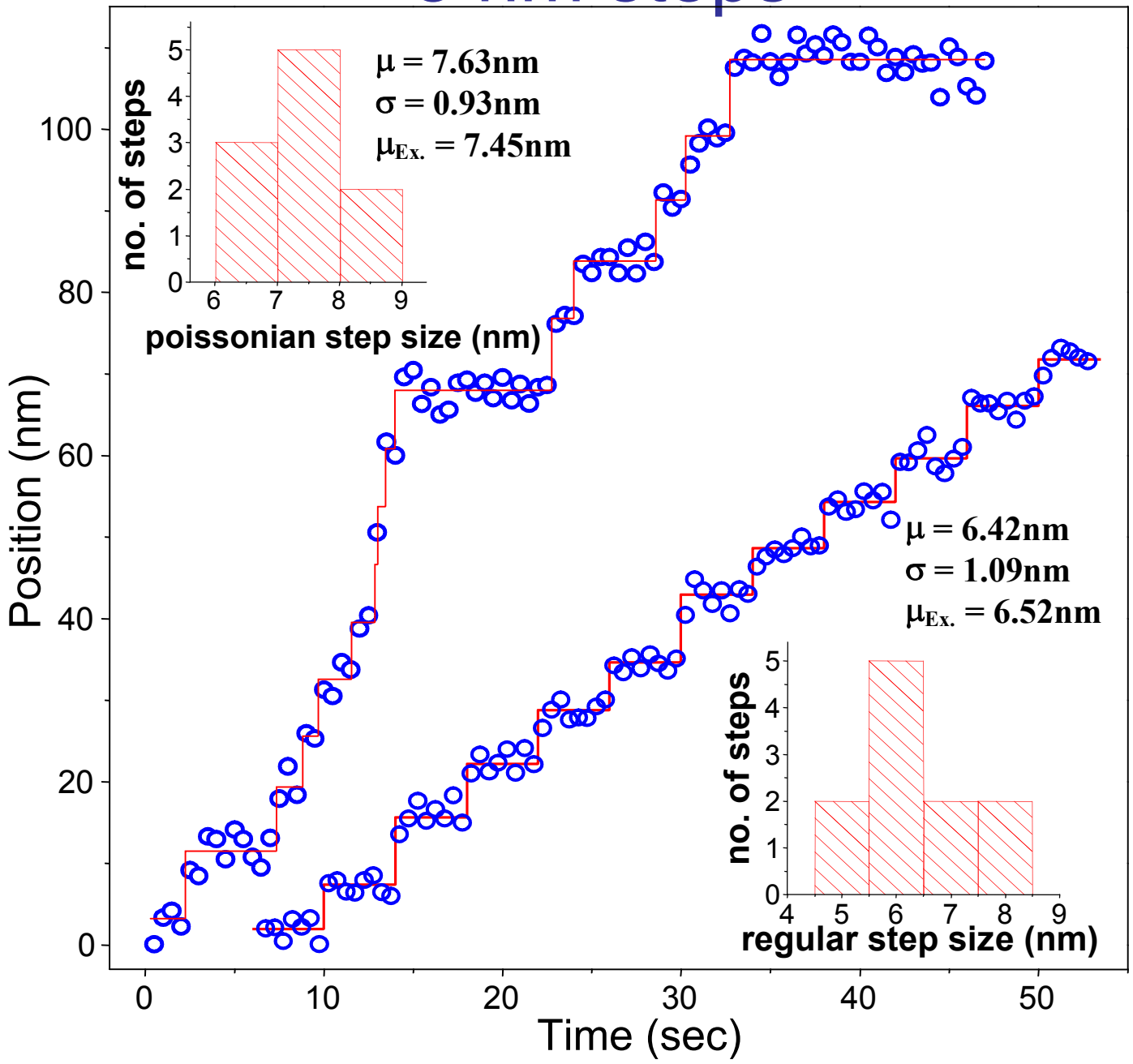
Data: Model System: 30 nm Artificial Steps



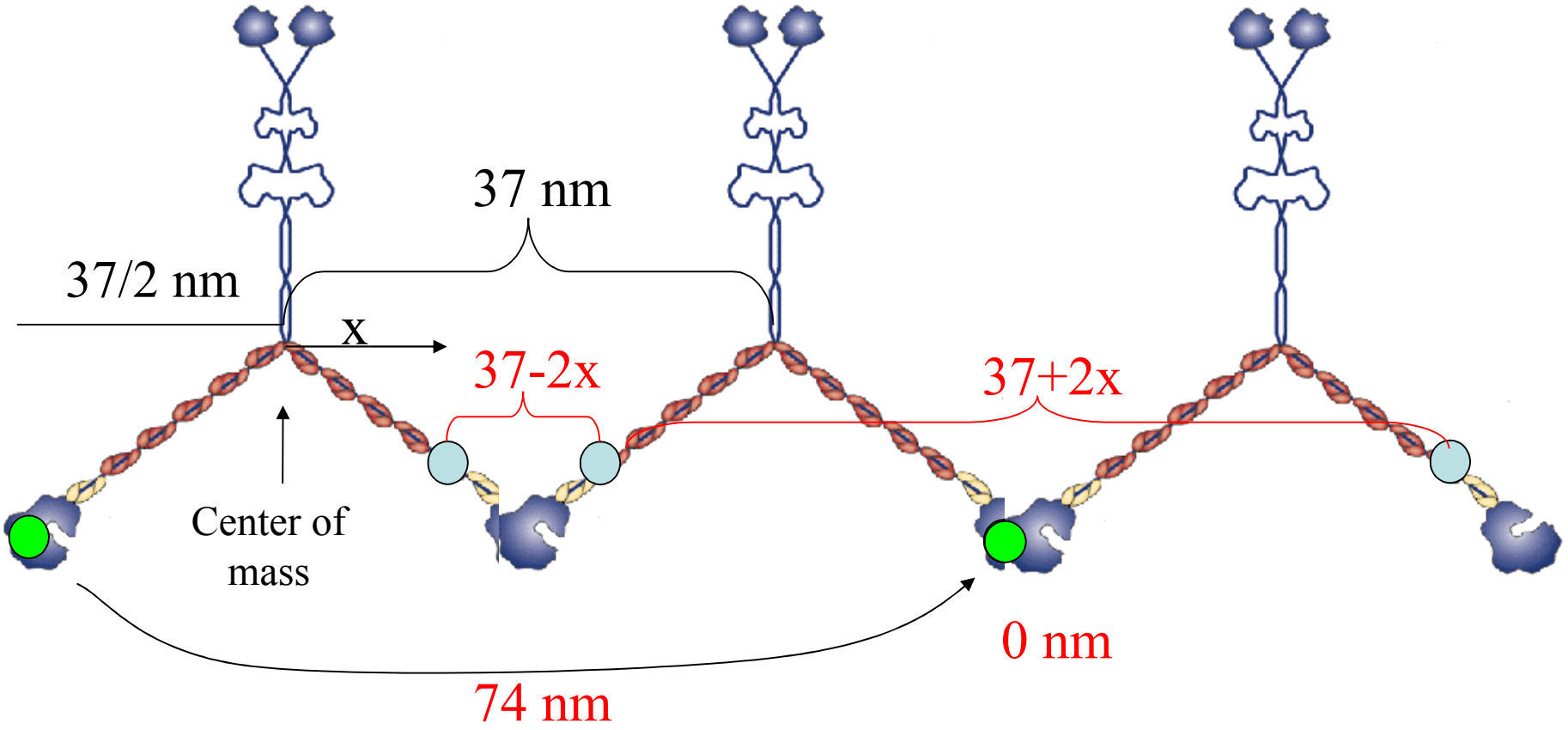
12 nm steps

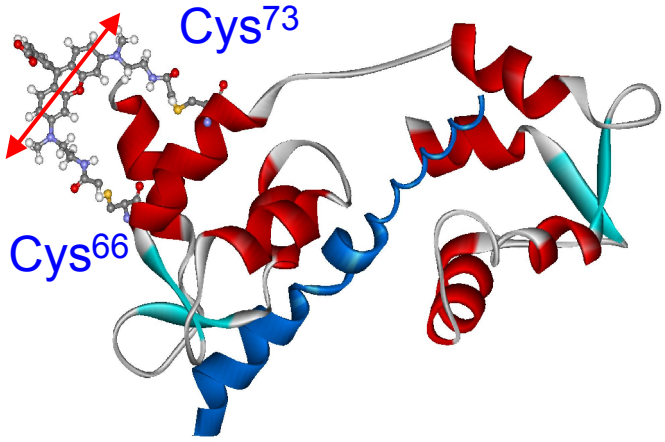

μ = 12.47nm
σ = 1.37nm
μ Ex.= 12.35nm
no. of steps
regular step size (nm)
μ = 12.26nm
σ = 1.39nm
μEx.= 12.35nm
no. of steps
poissonian step size (nm)
Position (nm)
Time (sec)

≈ 8 nm steps


Position (nm)
Time (sec)
0
20
40
60
80
100
0
10
20
30
40
50
μ = 7.63nm
σ = 0.93nm
μEx. = 7.45nm
no. of steps
poissonian step size (nm)
μ = 6.42nm
σ = 1.09nm
μEx. = 6.52nm
no. of steps
regular step size (nm)

Myosin V Labeling on Light Chain: Expected Step Sizes





Expected step size

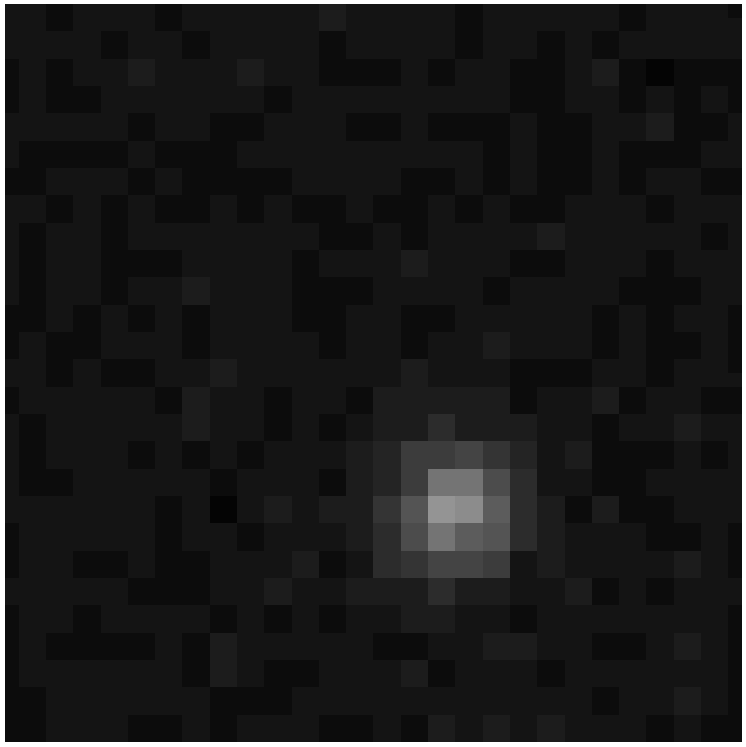
Hand-over-hand:

Head = 2 x 37 nm= 74, 0, 74 nm

CaM-Dye: 37-2x, 37+2x, …

Inchworm: always S_{cm} = 37 nm

A Single Myosin V moving

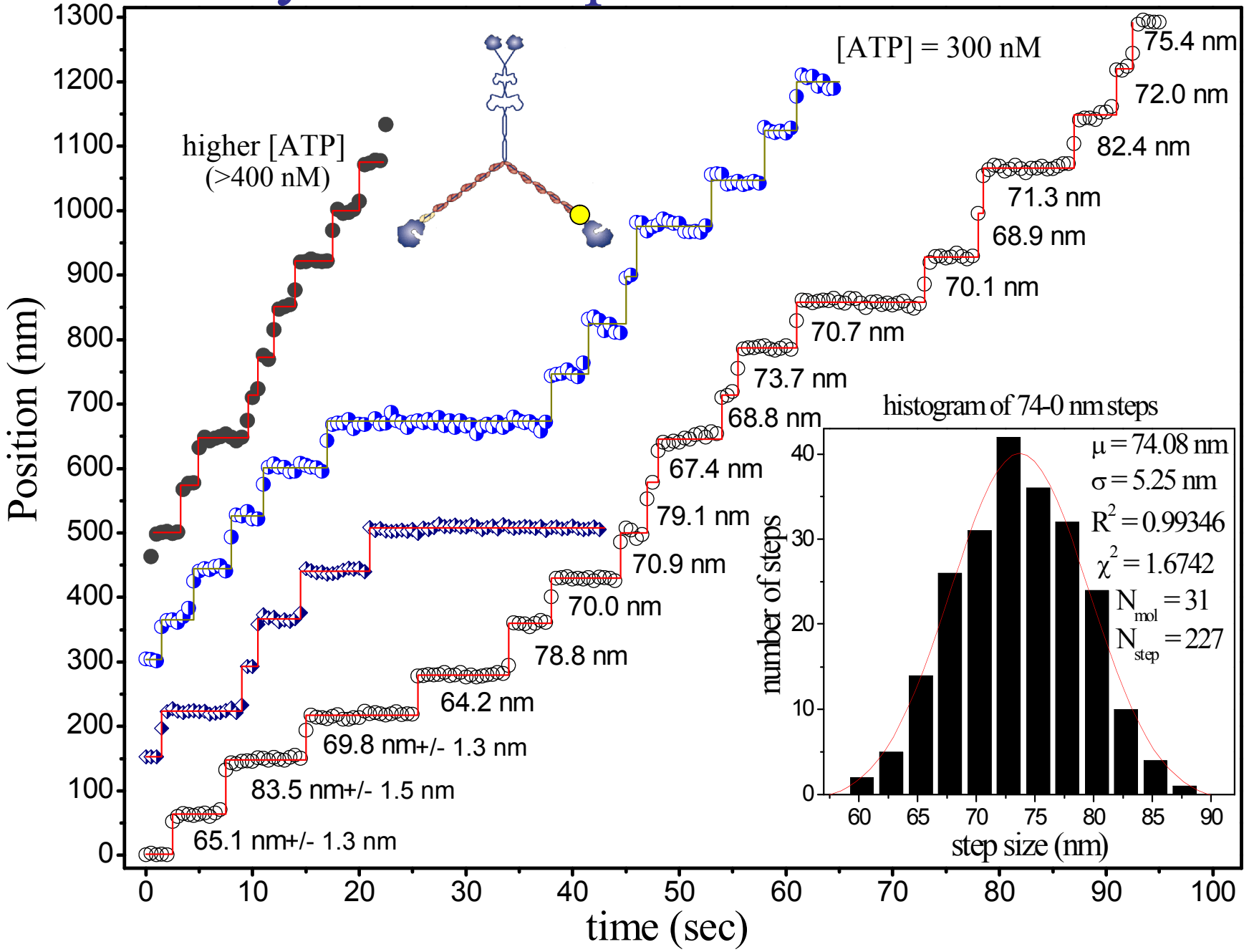


[ATP] = 300 nM (Low)

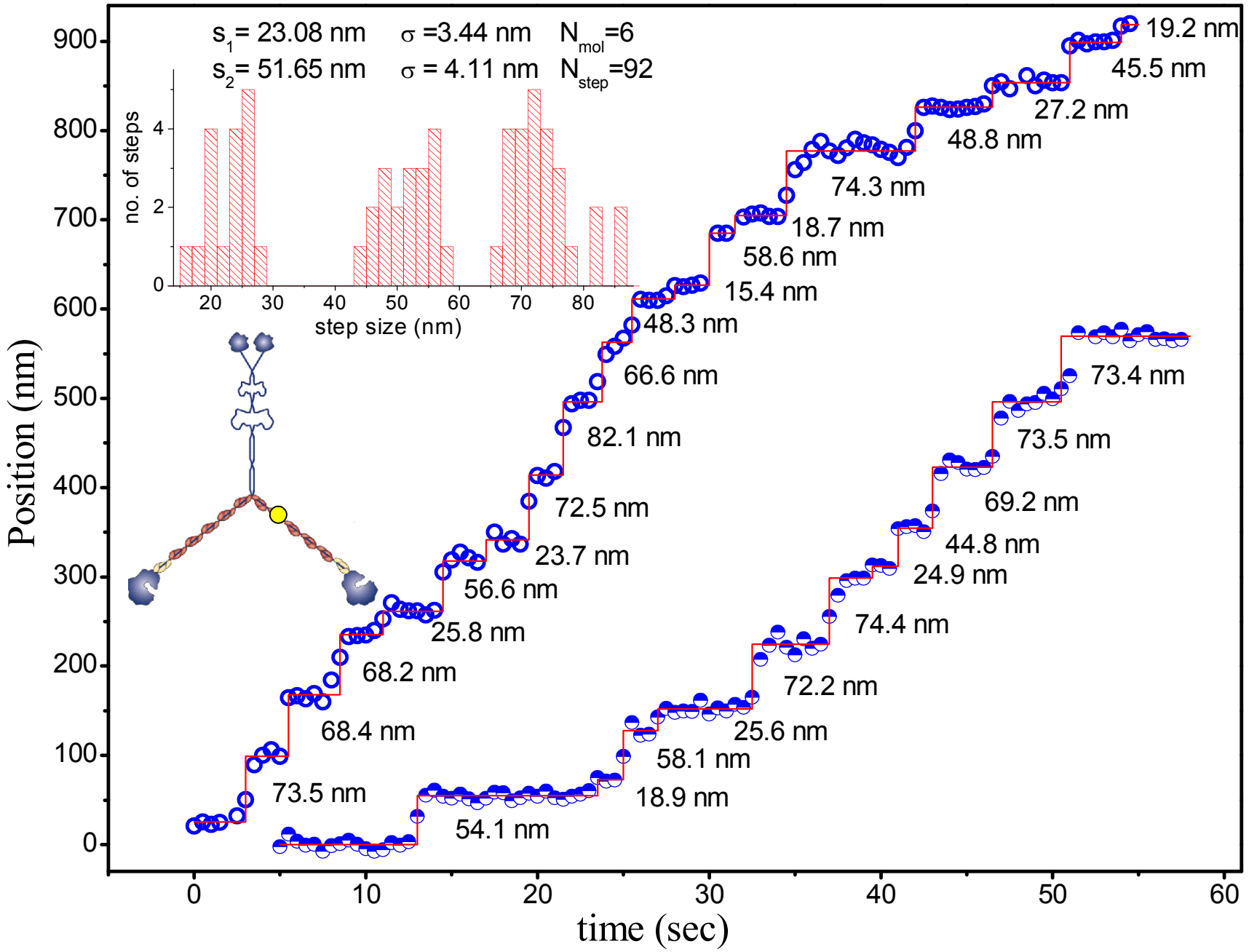
86 nm pixel

37 nm or 74 nm?

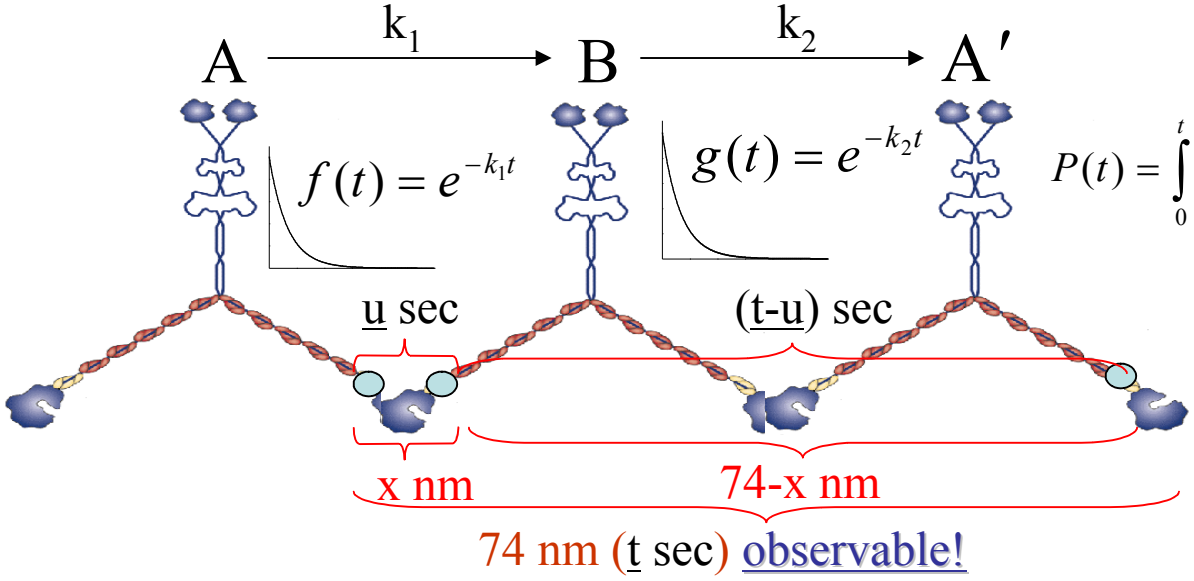
Myosin V steps: 74 nm +/- 5nm



52-23 nm steps



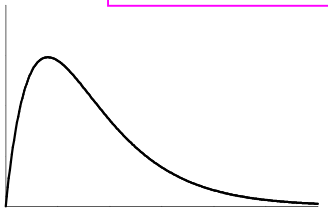
74-0 nm Steps: Detecting 0 nm Intermediate by Kinetics

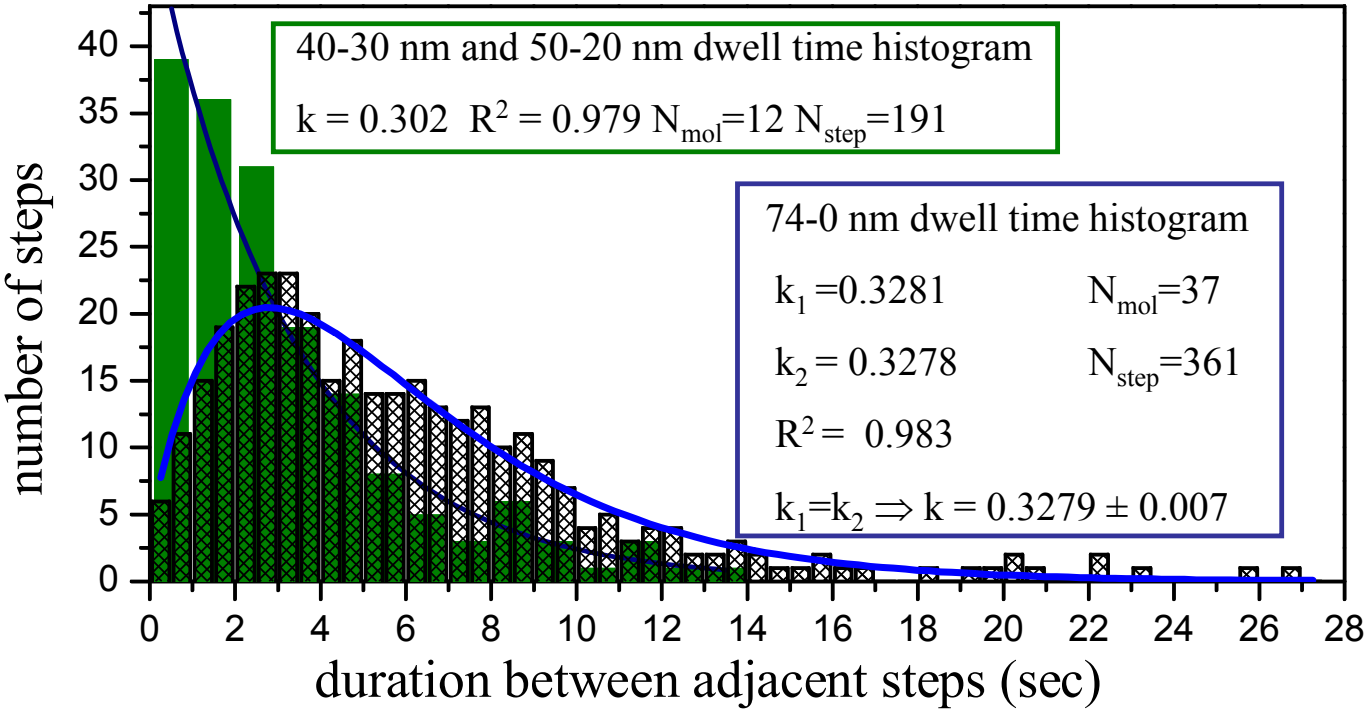


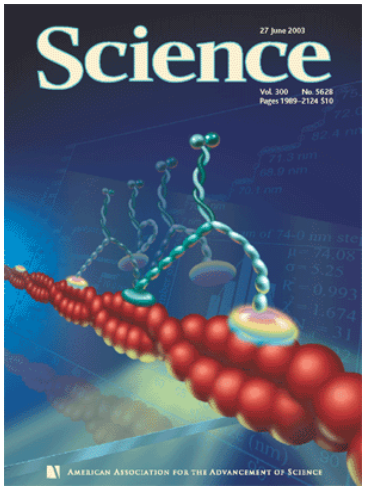
$$A \longrightarrow A'$$

$$P(t) = \int_0^t f(u)g(t-u)du = \frac{e^{-k_2 t} - e^{-k_1 t}}{k_1 - k_2}$$

$$k_1 = k_2 \Rightarrow P(t) = t \cdot e^{-k \cdot t}$$







[ATP] = 300 nM

Class evaluation

1. What was the most interesting thing you learned in class today?

2. What are you confused about?

3. Related to today's subject, what would you like to know more about?

4. Any helpful comments.

Answer, and turn in at the end of class.