Announcements

Assignment due on Wednesday.

I strongly suggest you come and talk to me.

(but don't leave it to the last minute!)

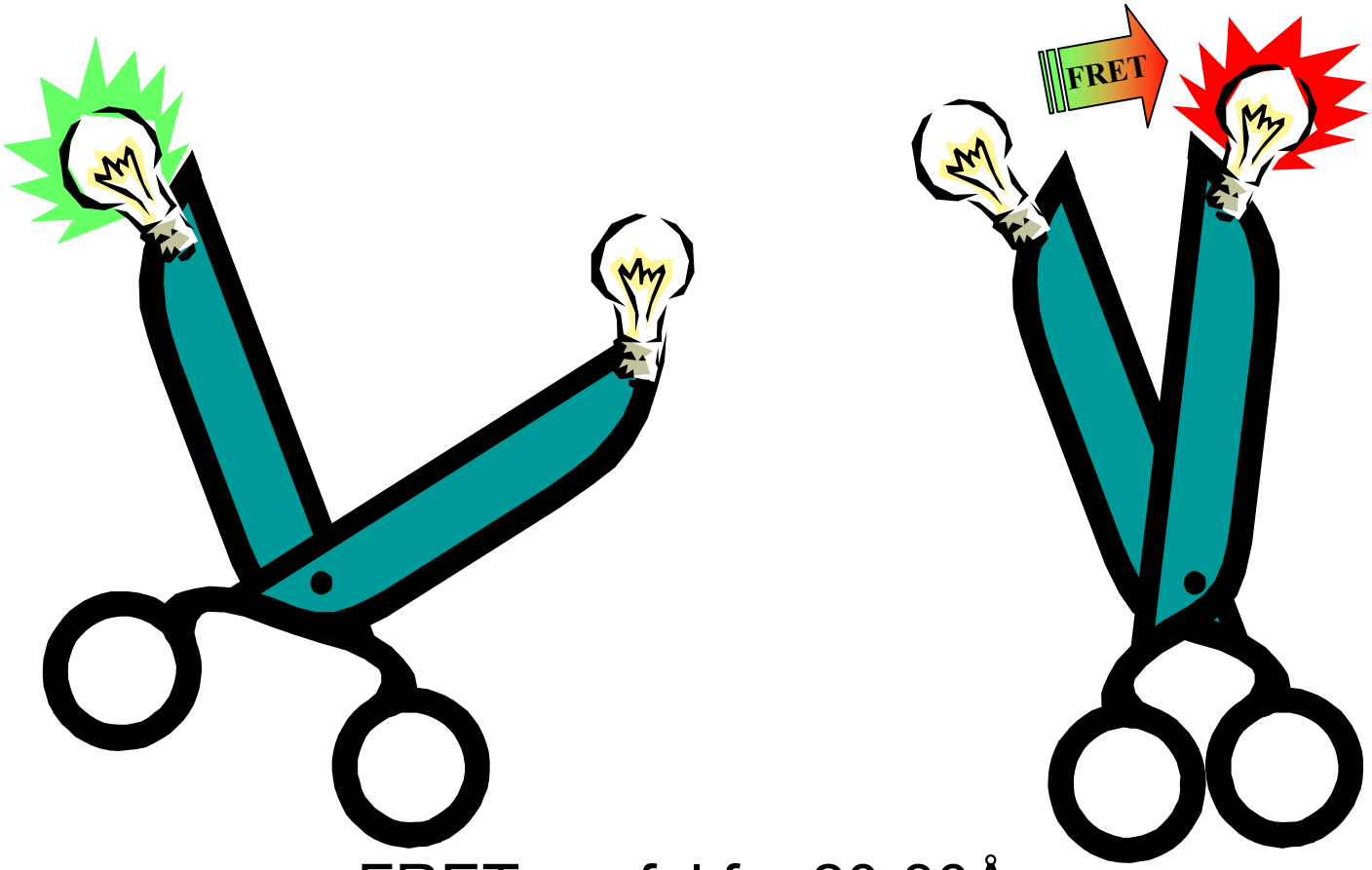
Pick: 1) an article, 2) a review article,
and 3) some general textbook(s)
You will then give a 10 min. talk on (plus 2-3 min. for questions).
This Wednesday, hand in your articles (1 and 2),
plus a ½ - 1 page write up.

1) Why you are interested in it,
2) What you think major technical innovation is,
3) What is main biological point.

March 10th : Mid-term Exam!

March 27th & 28th (evenings) : Your presentations (?)

FRET: measuring conformational changes of (single) biomolecules

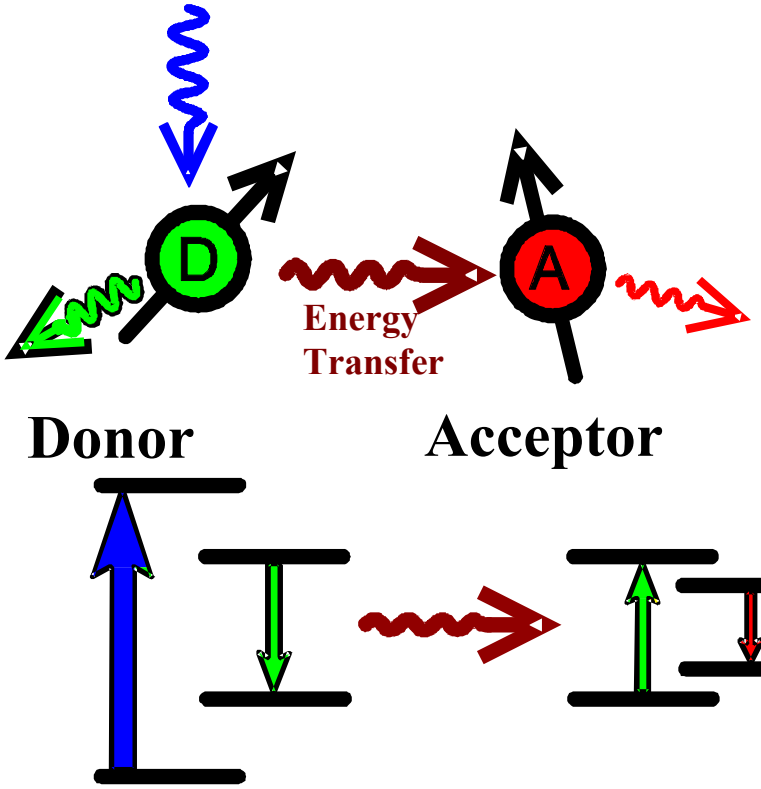


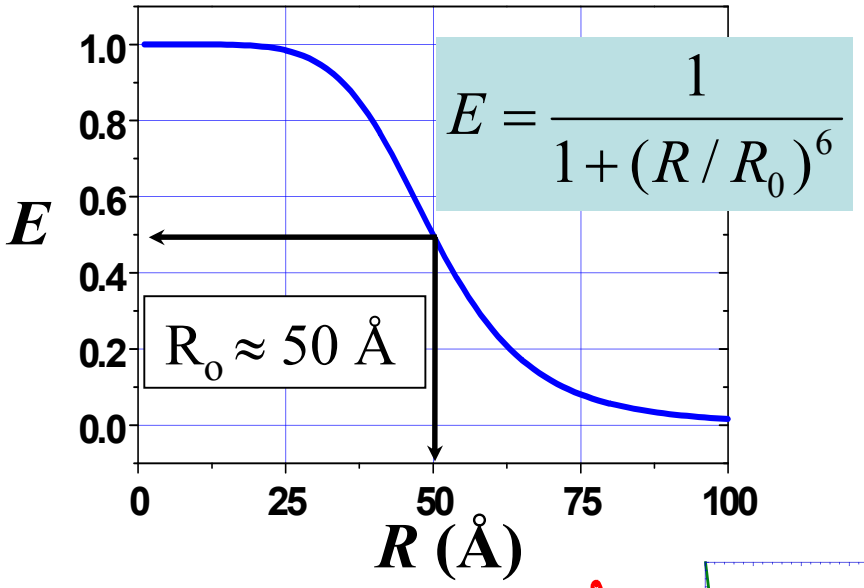
FRET useful for 20-80Å

Distance dependent interactions between green and red light bulbs can be used to deduce the shape of the scissors during the function.

Fluorescence Resonance Energy Transfer (FRET)

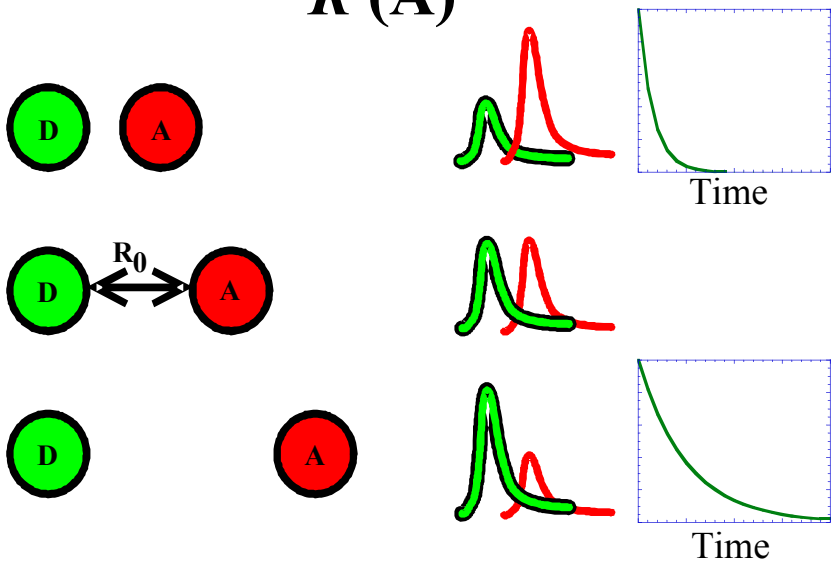
Spectroscopic Ruler for measuring nm-scale distances, binding





Dipole-dipole Distant-dependent Energy transfer

Look at relative amounts of green & red

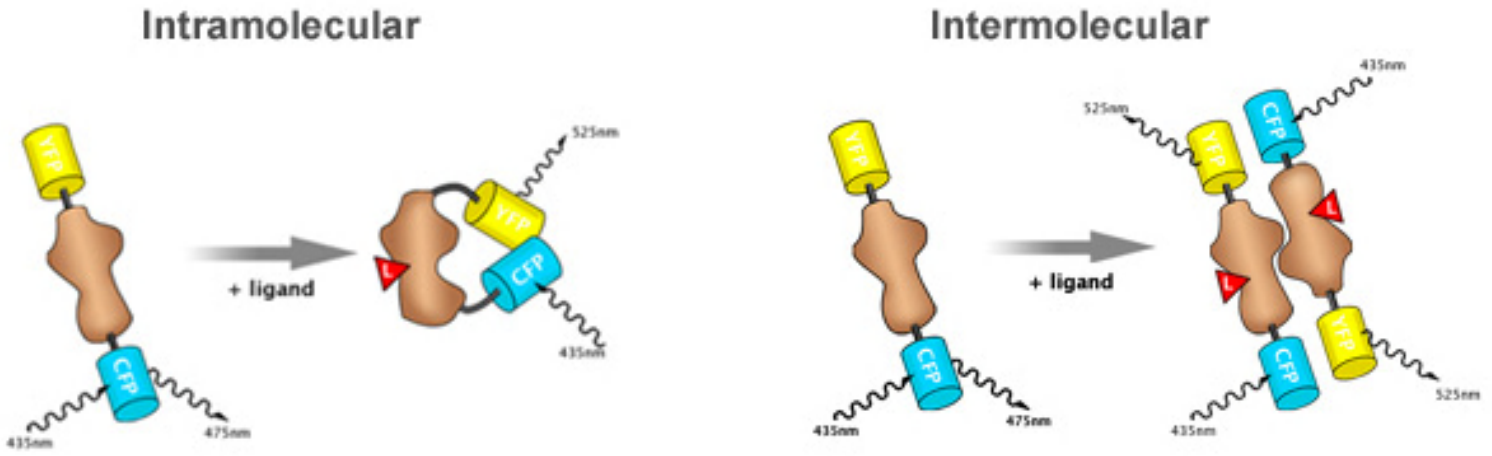


Energy Transfer goes like…

$$E = \frac{1}{1+(R_0/R)^6} \quad \textbf{or} \quad E = \frac{1}{1+(R/R_0)^6} \quad \textbf{?}$$

Take limit…

FRET is so useful because R_o (2-8 nm) is often ideal



Two types of experiments
FRET can be used to study both conformational changes within molecules by tagging one protein with two fluorophores, and to study protein-protein interaction by tagging two proteins with a single fluorophore.

Bigger R_o (>8 nm) can use FIONA-type techniques

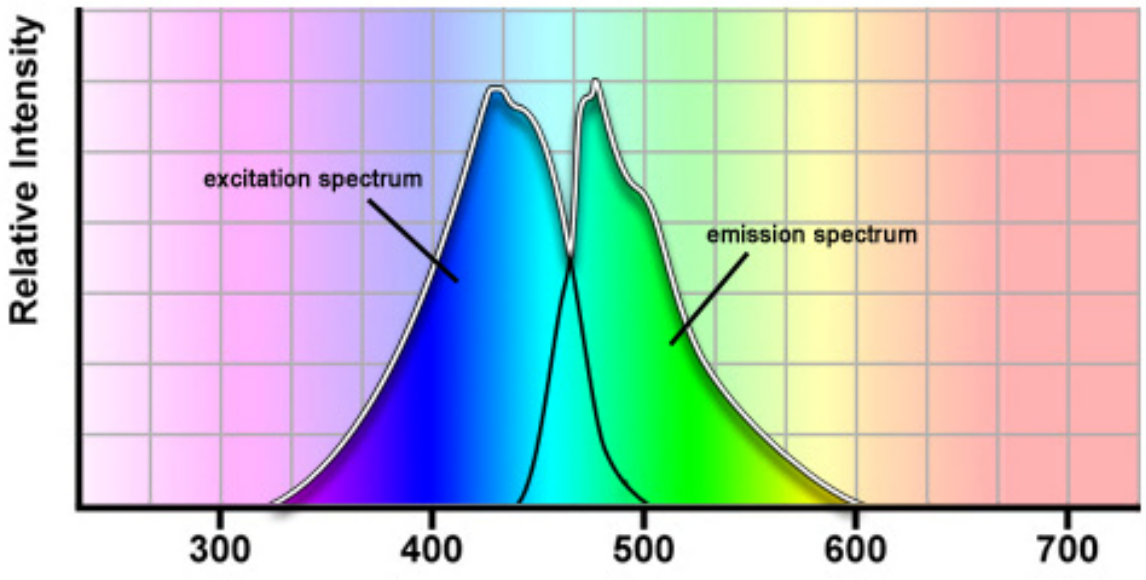
Terms in R_o

$$E = \frac{1}{1+(R/R_0)^6}$$

$$Ro = 0.21\left(Jq_D n^{-4}\kappa^2\right)^{1/6}$$ in Angstroms

- J is the normalized spectral overlap of the donor emission (f_D) and acceptor absorption (ε_A)
- q_D is the quantum efficiency (or quantum yield) for donor emission in the absence of acceptor (q_D = number of photons emitted divided by number of photons absorbed).
- n is the index of refraction (1.33 for water; 1.29 for many organic molecules).
- κ^2 is a geometric factor related to the relative orientation of the transition dipoles of the donor and acceptor and their relative orientation in space.

Donor Emission



CFP spectrum

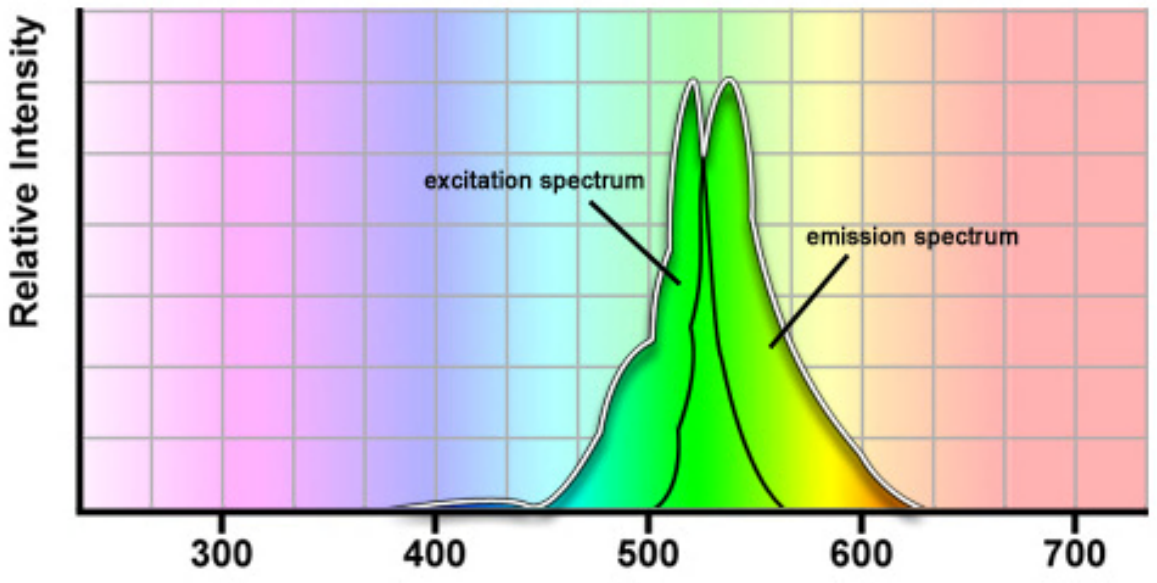
CFP is optimally excited at 434nm and has a peak emission at 477nm.

Click to continue >>

Donor Emission

http://mekentosj.com/science/fret/

Acceptor Emission



YFP spectrum

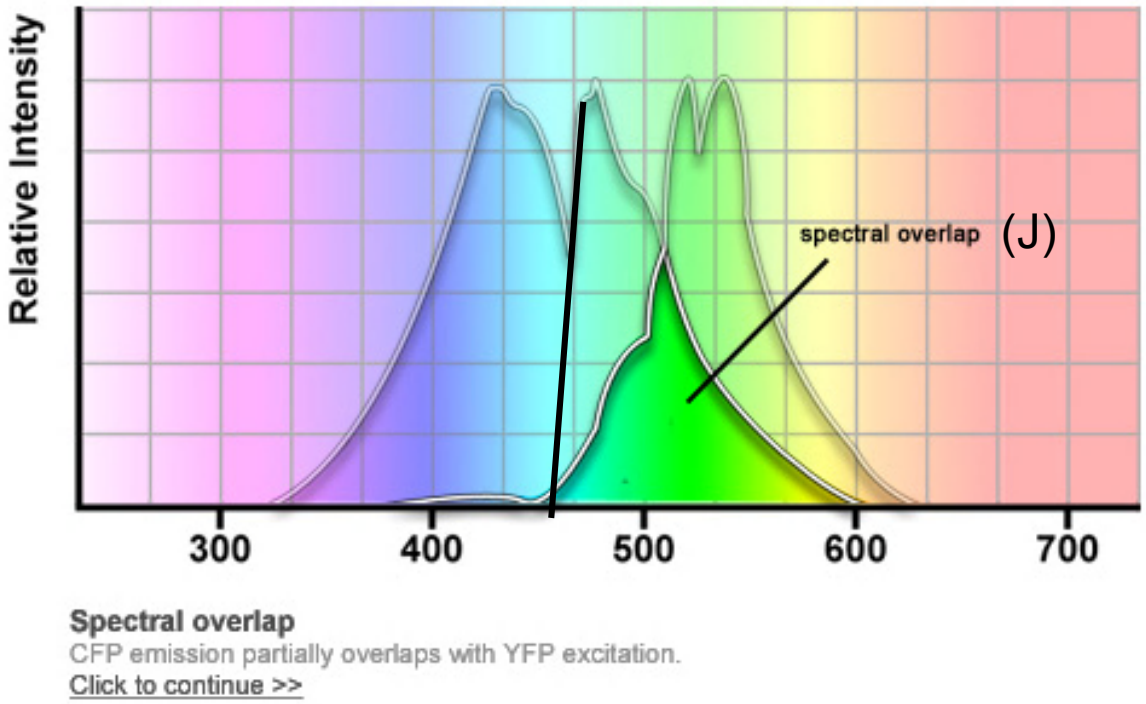
YFP is optimally excited at 513nm and has a peak emission at 527nm.

Click to continue >>

Acceptor Emission

http://mekentosj.com/science/fret/

Spectral Overlap terms

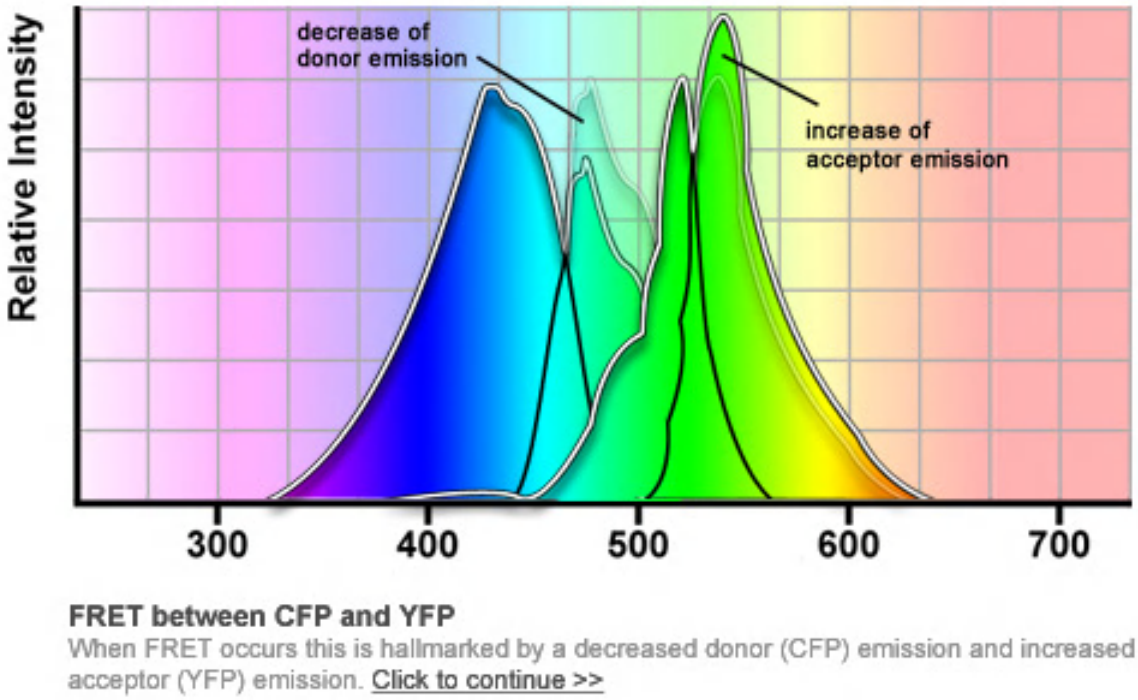


Spectral Overlap between Donor & Acceptor Emission

For CFP and YFP, R_o, or Förster radius, is 49-52Å.

http://mekentosj.com/science/fret/

With a measureable E.T. signal



FRET between CFP and YFP

When FRET occurs this is hallmarked by a decreased donor (CFP) emission and increased acceptor (YFP) emission. Click to continue >>

E.T. leads to decrease in Donor Emission & Increase in Acceptor Emission

http://mekentosj.com/science/fret/

Terms in R_o

$$Ro = 0.21\left(Jq_D n^{-4}\kappa^2\right)^{1/6}$$ in Angstroms

where *J* is the normalized spectral overlap of the donor emission (f_D) and acceptor absorption (ε_A)

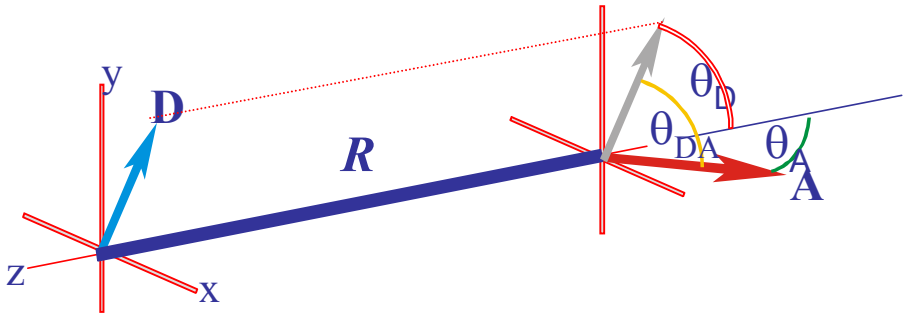
$$J = \frac{\int \varepsilon_A(\lambda)\, f_D(\lambda)\lambda^4\, d\lambda}{\int f_D(\lambda)\, d\lambda} \quad (\text{ in } \underline{\text{M}}^{-1} \cdot \text{cm}^{-1} \cdot \text{nm}^4)$$

(Draw out $\varepsilon_a(\lambda)$ and $f_d(\lambda)$ and show how you calculate J.)

κ^2 : Orientation Factor

The spatial relationship between the DONOR emission dipole moment and the ACCEPTOR absorption dipole moment

$$\kappa^2 = (\cos\theta_{DA} - 3\cos\theta_D \cos\theta_A)^2$$



($0 < \kappa^2 > 4$)
κ^2 often = 2/3

where θ_{DA} is the angle between the donor and acceptor transition dipole moments, θ_D (θ_A) is the angle between the donor (acceptor) transition dipole moment and the R vector joining the two dyes.

κ^2 ranges from 0 if all angles are 90°, to 4 if all angles are 0°, and equals 2/3 if the donor and acceptor rapidly and completely rotate during the donor excited state lifetime.

✓ **κ^2 is usually not known and is assumed to have a value of 2/3 (Randomized distribution)**

How to measure Energy Transfer

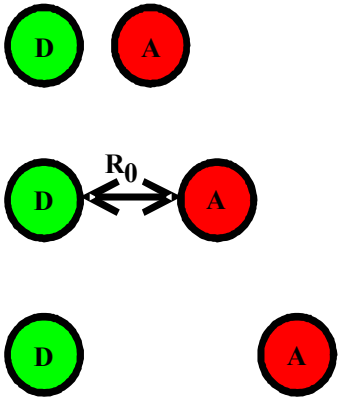
Donor intensity decrease, donor lifetime decrease, acceptor increase.

E.T. by changes in donor.

Need to compare two samples, d-only, and D-A.

$$E = \left(1 - \frac{I_{D_A}}{I_D}\right)$$
$$= 1 - \frac{\tau_{D_A}}{\tau_D}$$

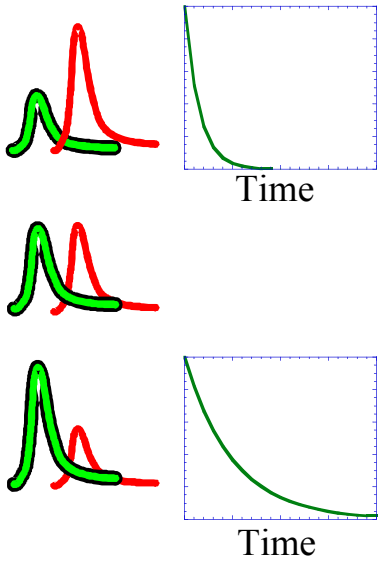
Where I_{D_A}, τ_{D_A} are the donor's intensity, and excited state lifetime in the presence of acceptor, and I_D, τ_D are the same but without the acceptor.



E.T. by increase in acceptor fluorescence and compare it to residual donor emission.

Need to compare one sample at two λ and also measure their quantum yields.

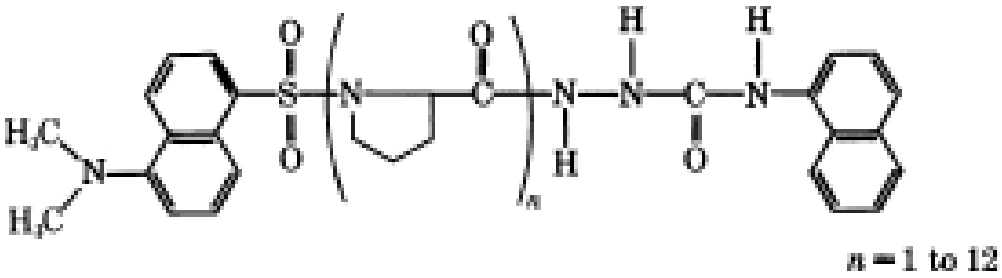
$$E = \frac{\frac{I_{A_D}}{q_A}}{\frac{I_{D_A}}{q_D} + \frac{I_{A_D}}{q_A}}$$



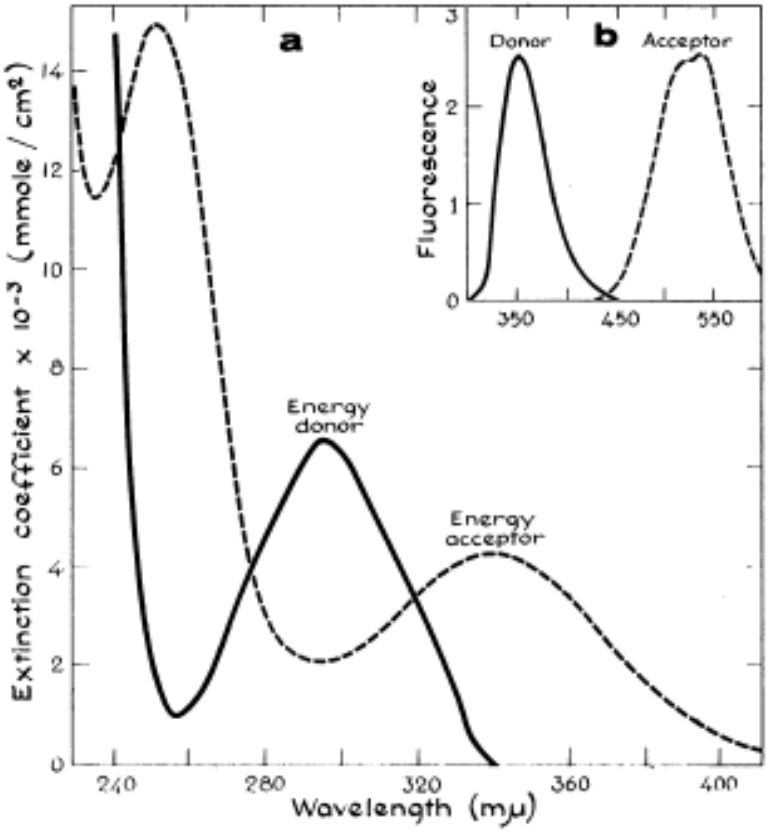
Example of Energy Transfer

Stryer & Haugland, PNAS, 1967

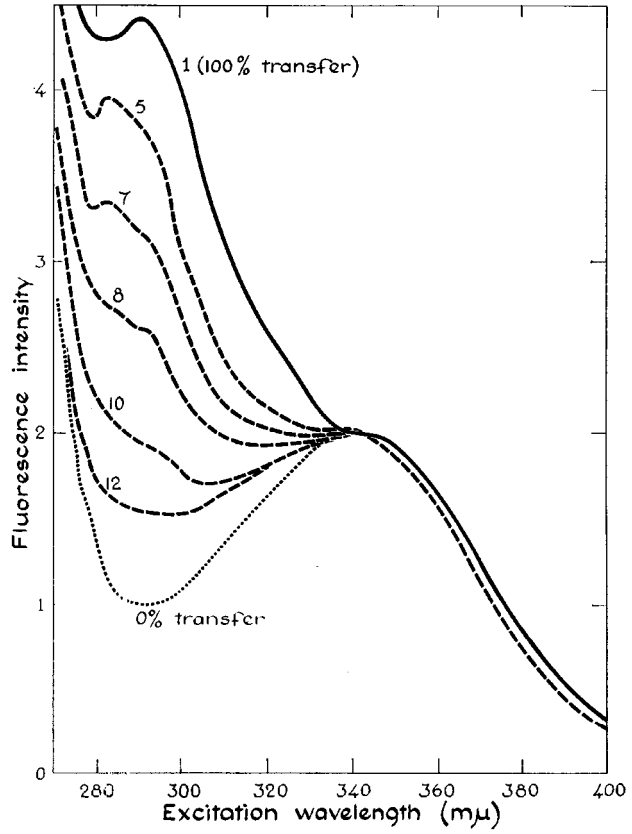
Donor Linker Acceptor

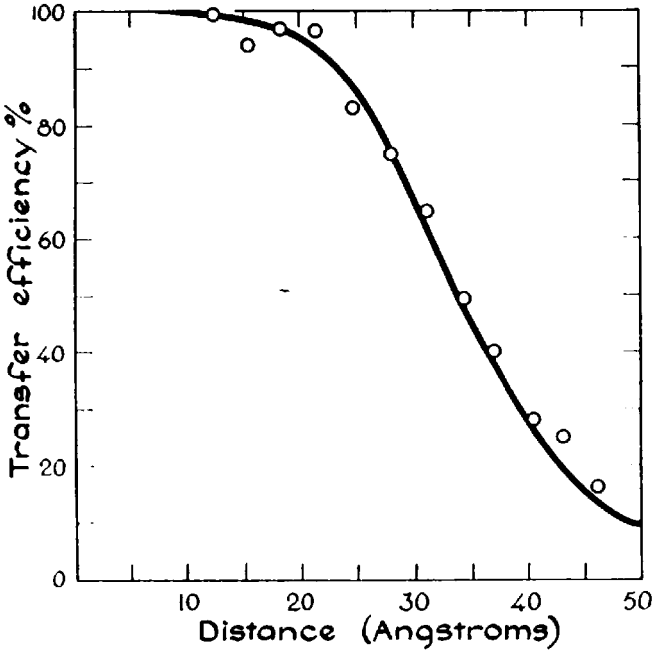


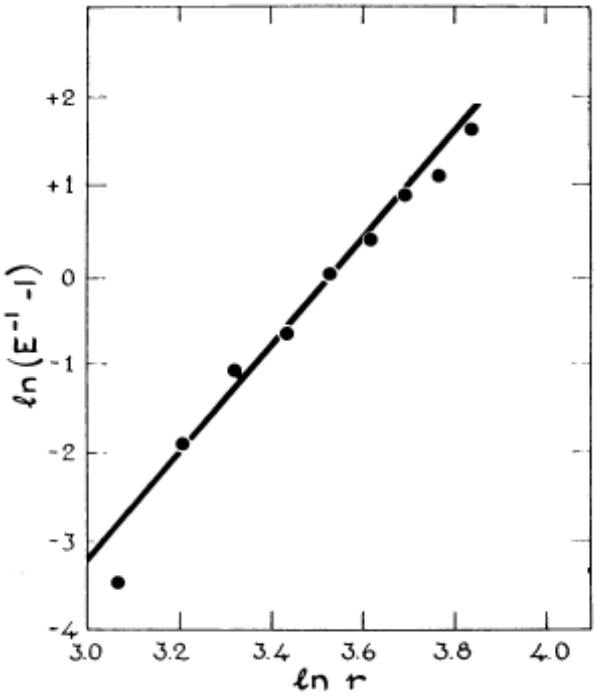
Spectral Overlap



Energy Transfer

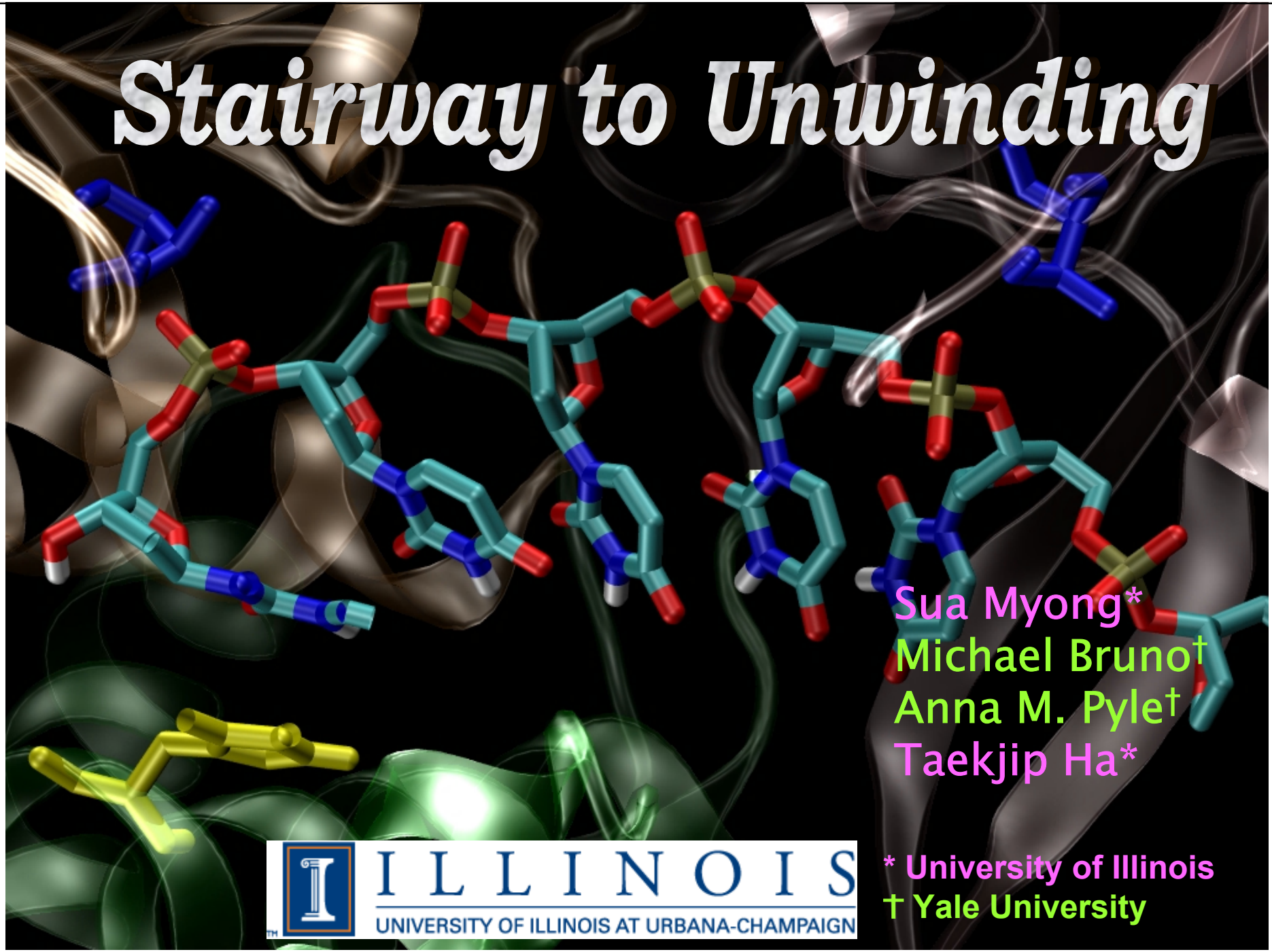






Stryer & Haugland, PNAS, 1967

Stairway to Unwinding



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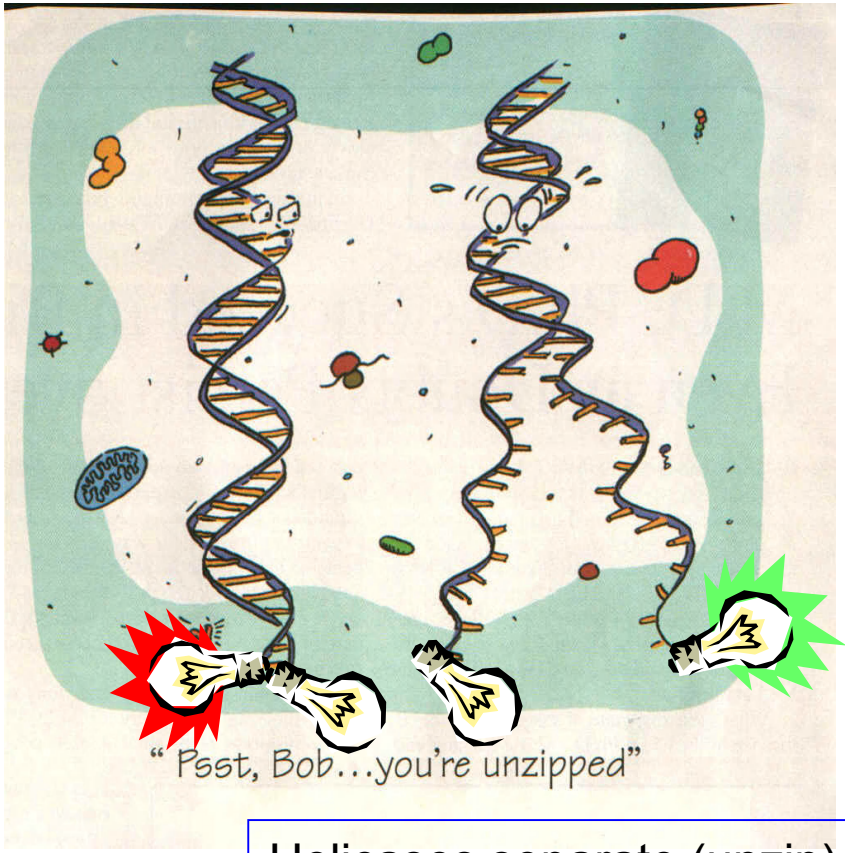
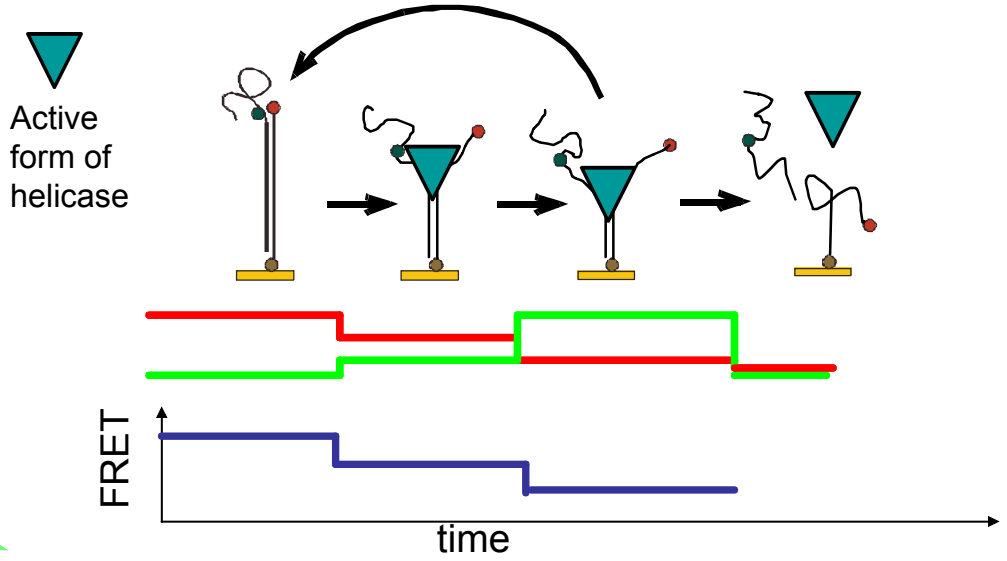
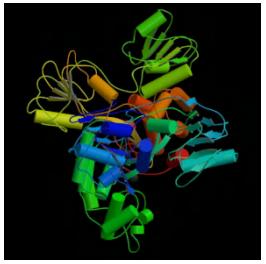
Unzipping mystery of helicases





Ha et al, Nature (2002)

Helicases separate (unzip) two DNA strands. We can measure very transient intermediates during unzipping using single molecule techniques.



About HCV NS3 helicase

1. Hepatitis C Virus (HCV) is a deadly virus affecting 170 million people in the world, but *no cure or vaccine*.

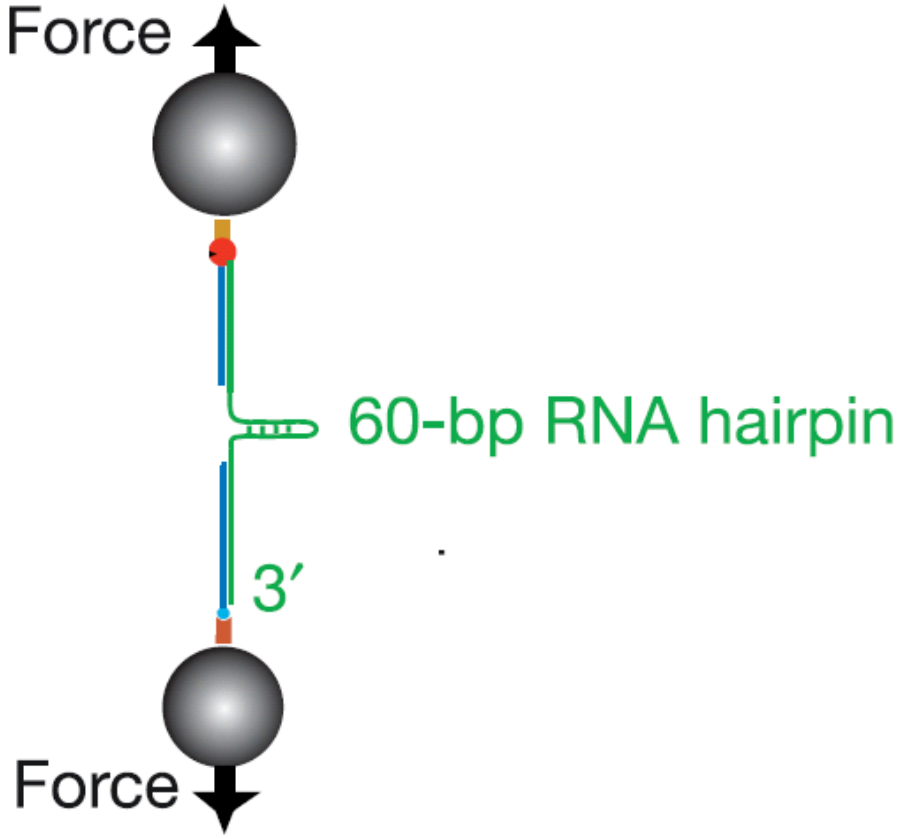
2. Non-Structural protein 3 (NS3) is essential for viral replication.

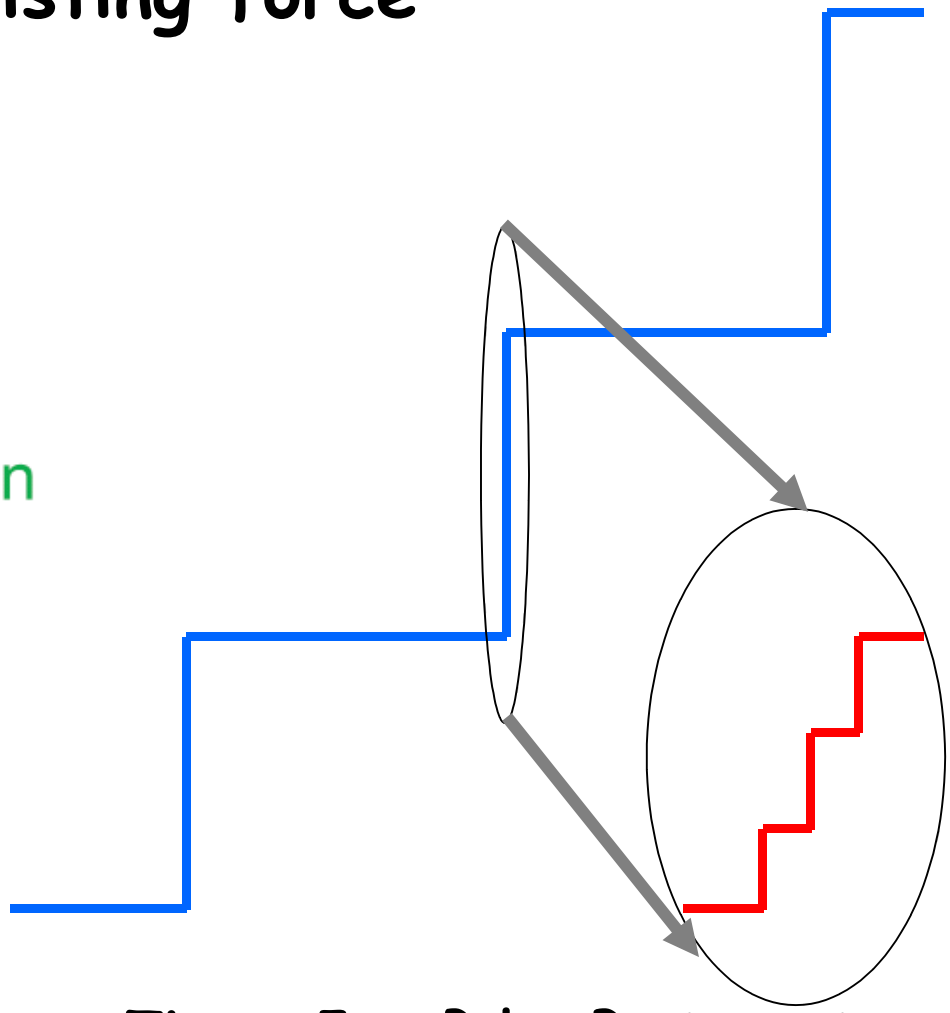
3. NS3 is composed of serine protease and a helicase domain

4. NS3 unwinds both RNA and DNA duplexes with 3' overhang

5. In vivo, NS3 may assist polymerase by resolving RNA secondary structures or displacing other proteins.

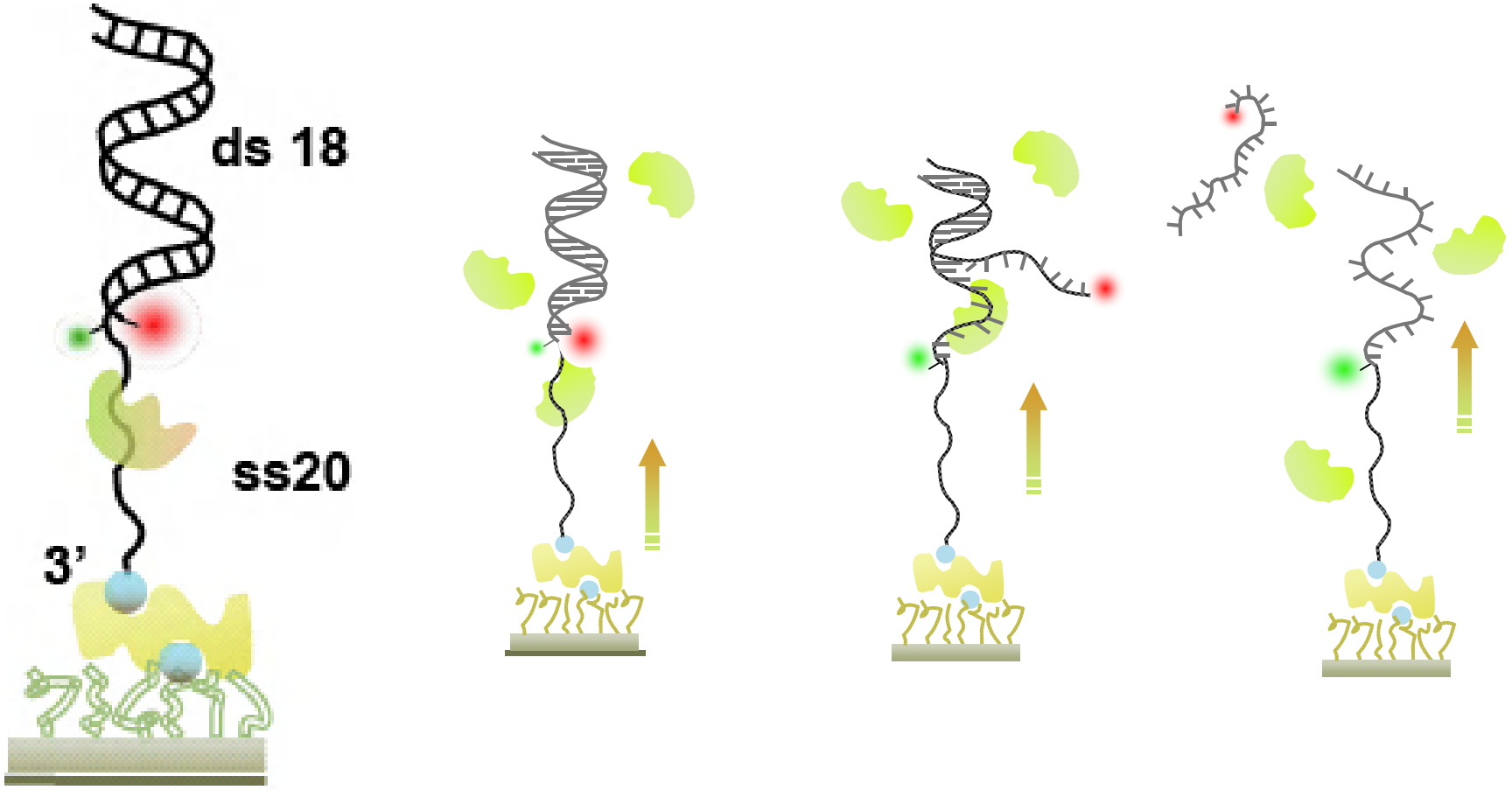
Steps (~11 bp) and substeps (2-5 bp) in NS3 catalyzed RNA unwinding under assisting force





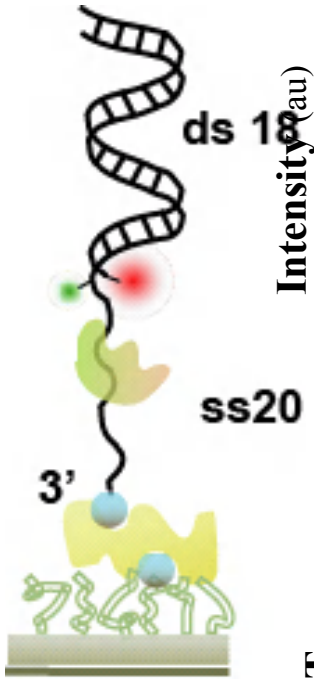
Dumont, Cheng, Serebrov, Beran, Tinoco Jr., Pyle, Bustamante. Nature (2006)

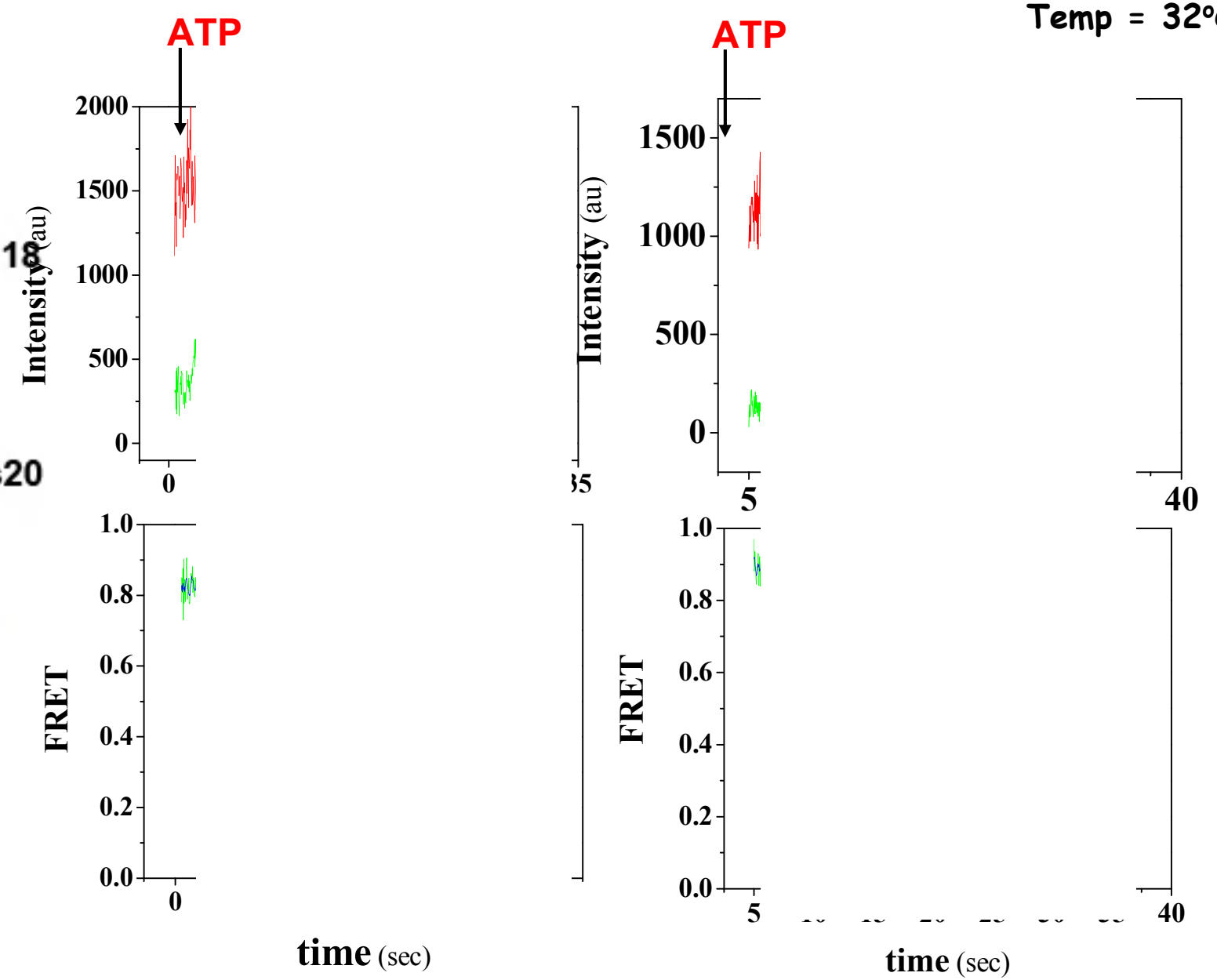
Watching Helicase in Action!



SIX steps in 18 bp unwinding

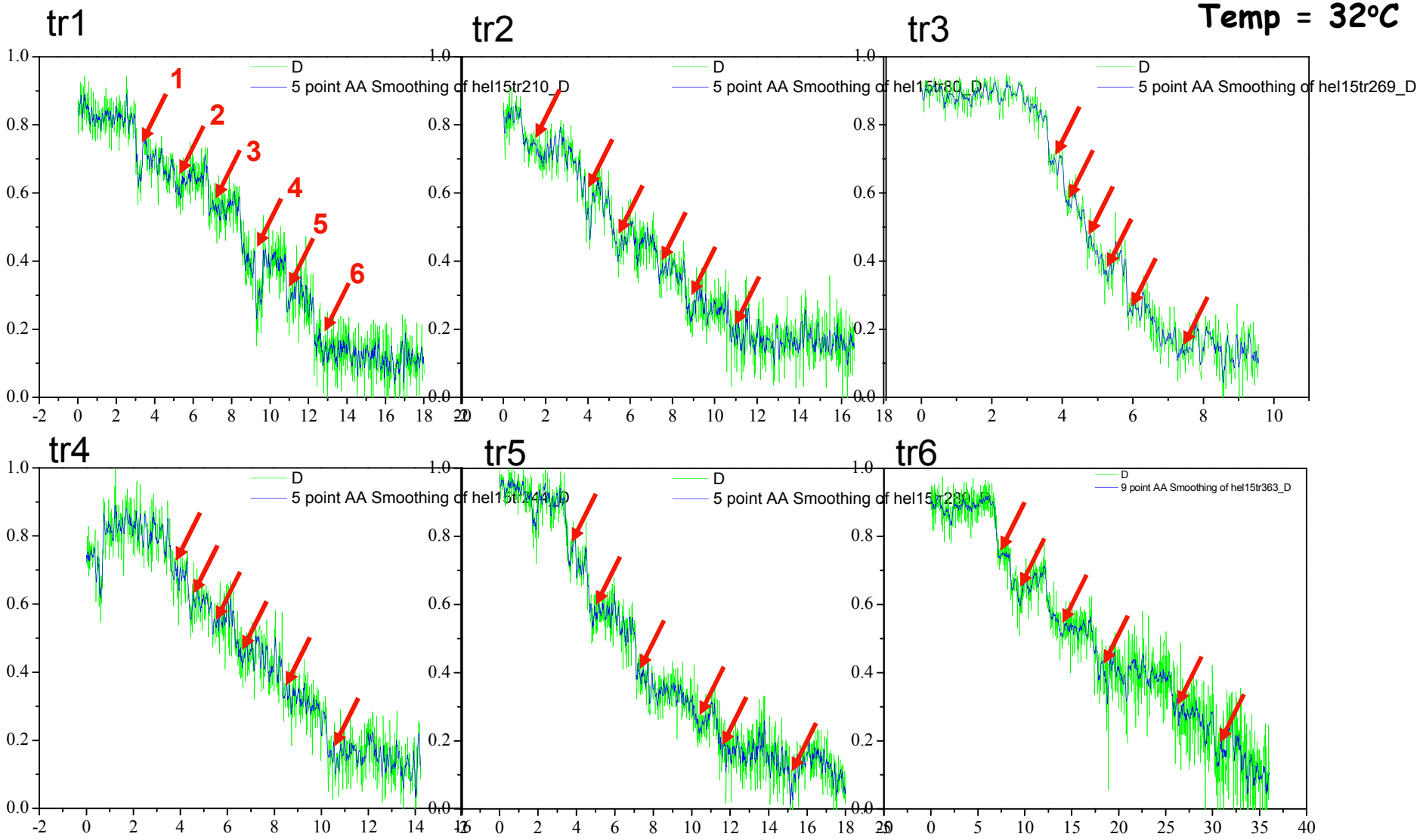
[NS3]=25nM
[ATP]=4mM
Temp = 32°C





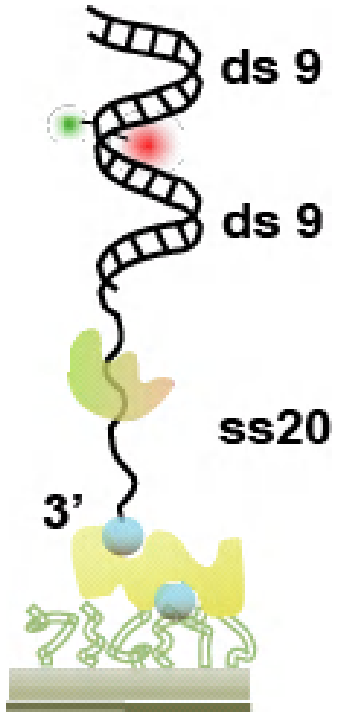
More traces with Six steps

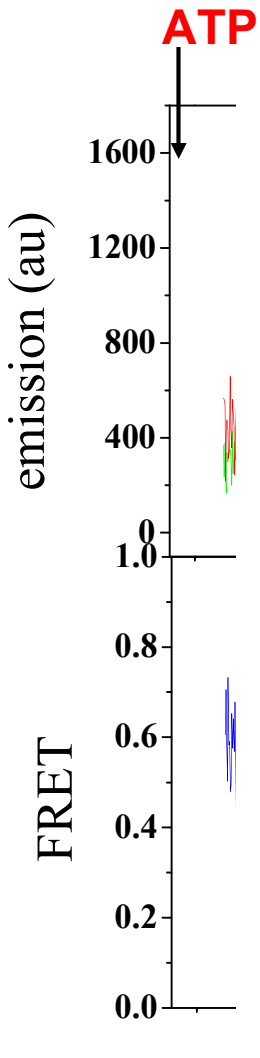
[NS3]=25nM
[ATP]=4mM
Temp = 32°C

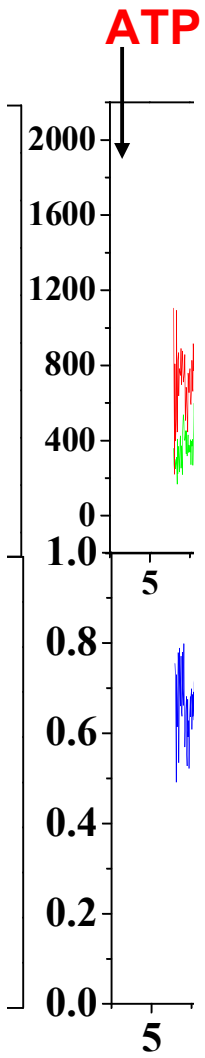


THREE steps in 9 bp unwinding

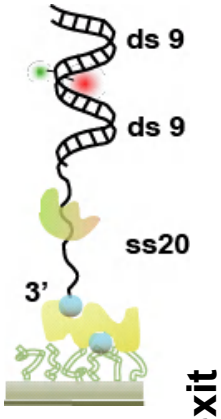
[NS3]=25nM
[ATP]=4mM
Temp = 32°C

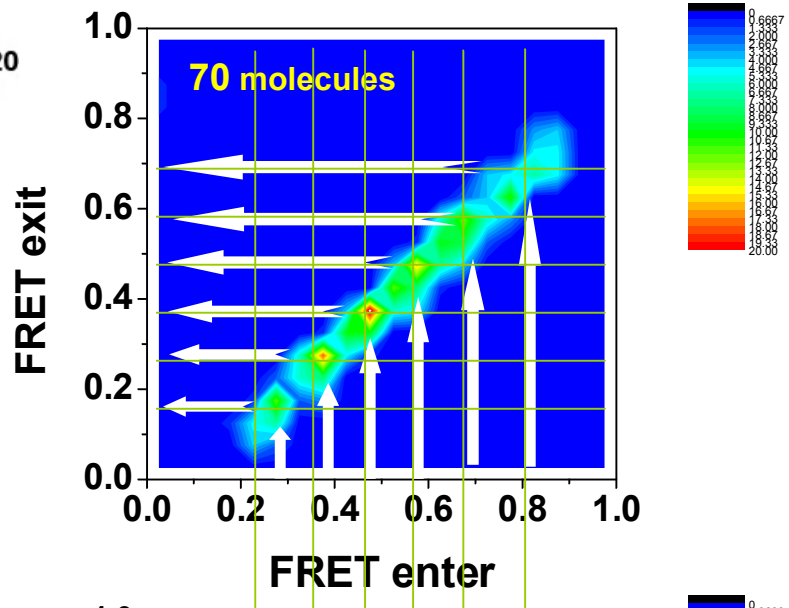


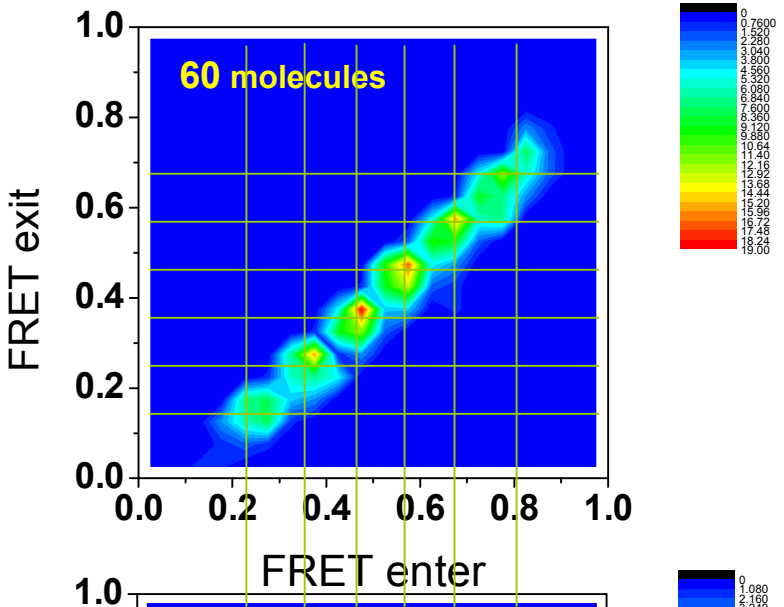


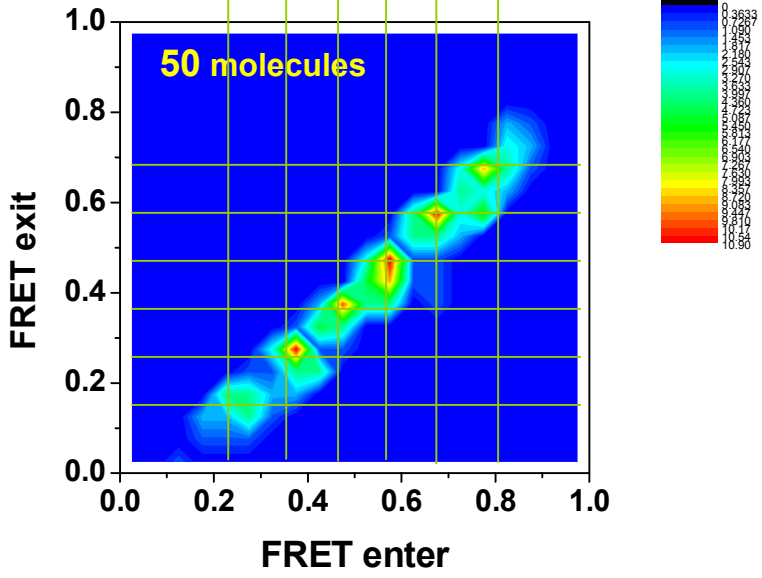


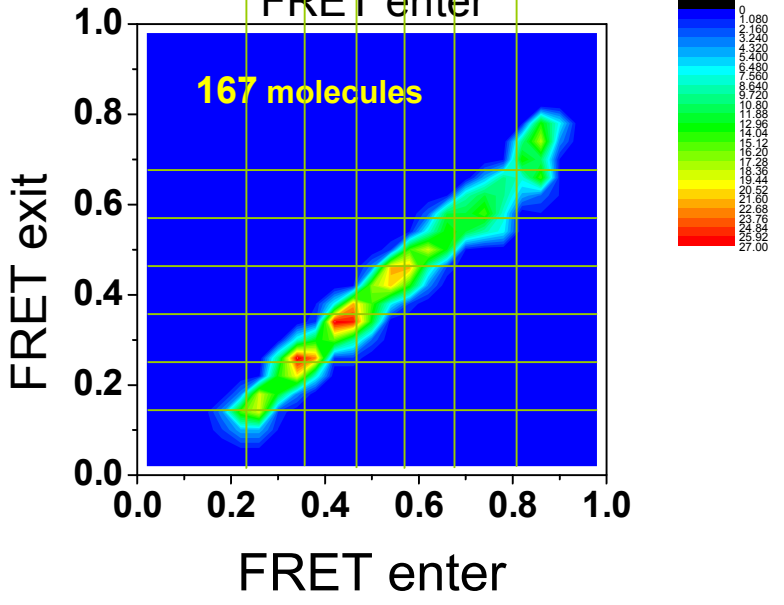
Transition Density Plot (6 steps)

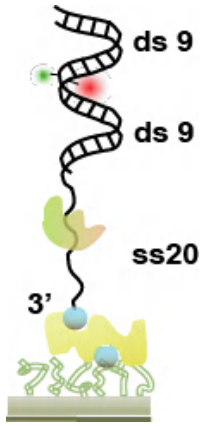




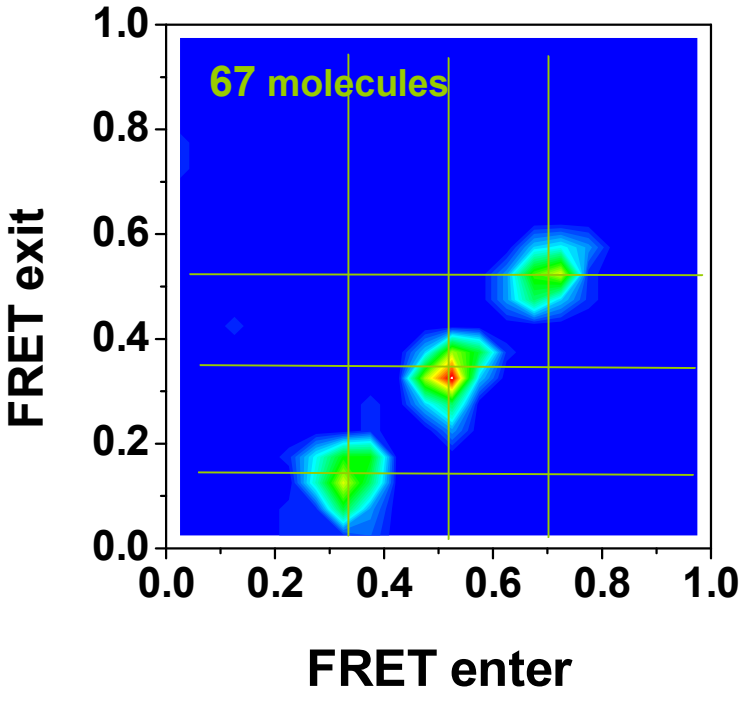


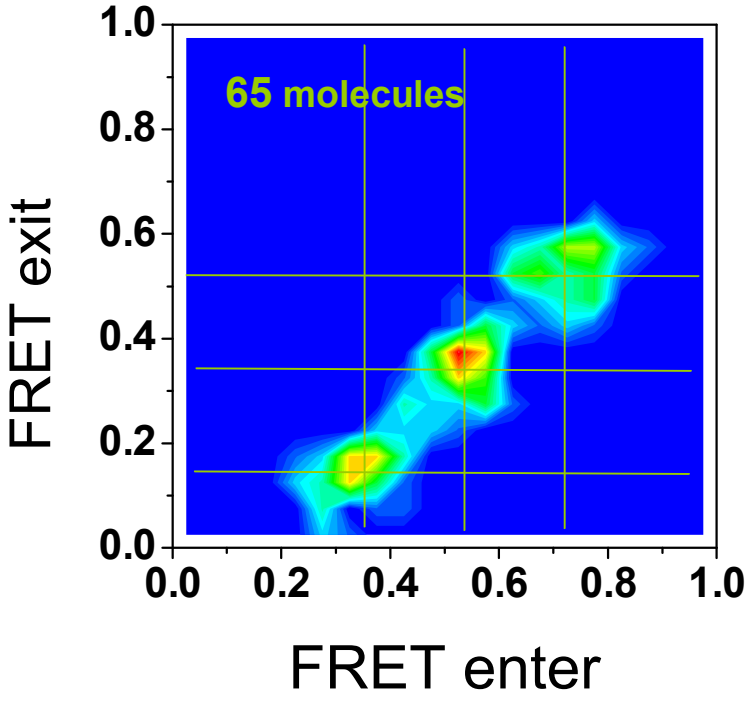






Transition Density Plot (3 steps)



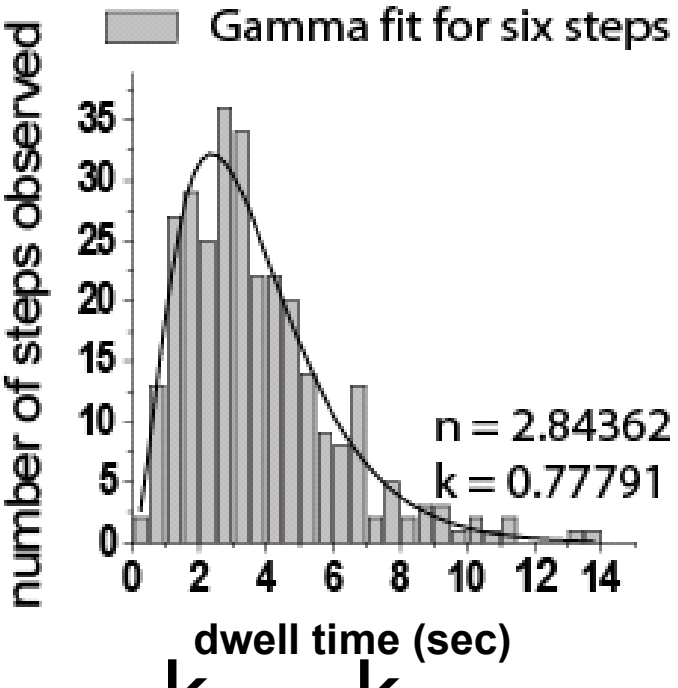


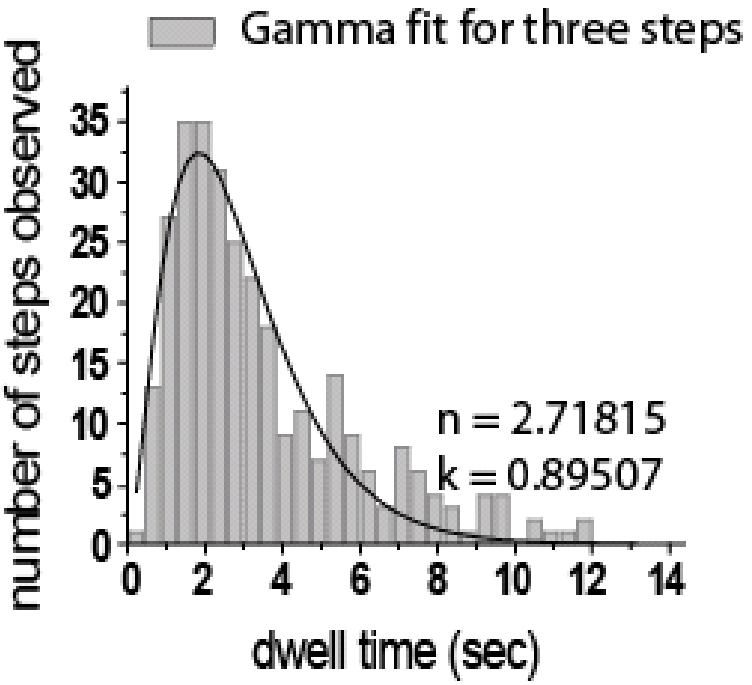
Is each step due to one rate limiting step i.e. one ATP hydrolysis?

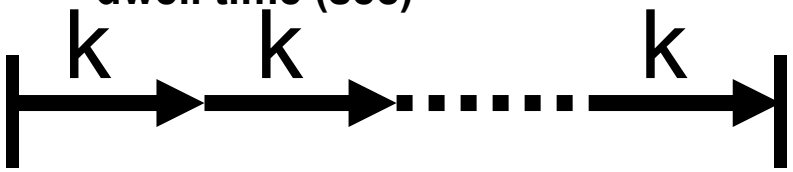
-> Build dwell time histograms

Non-exponential dwell time histograms

Smallest substep = Single basepair !



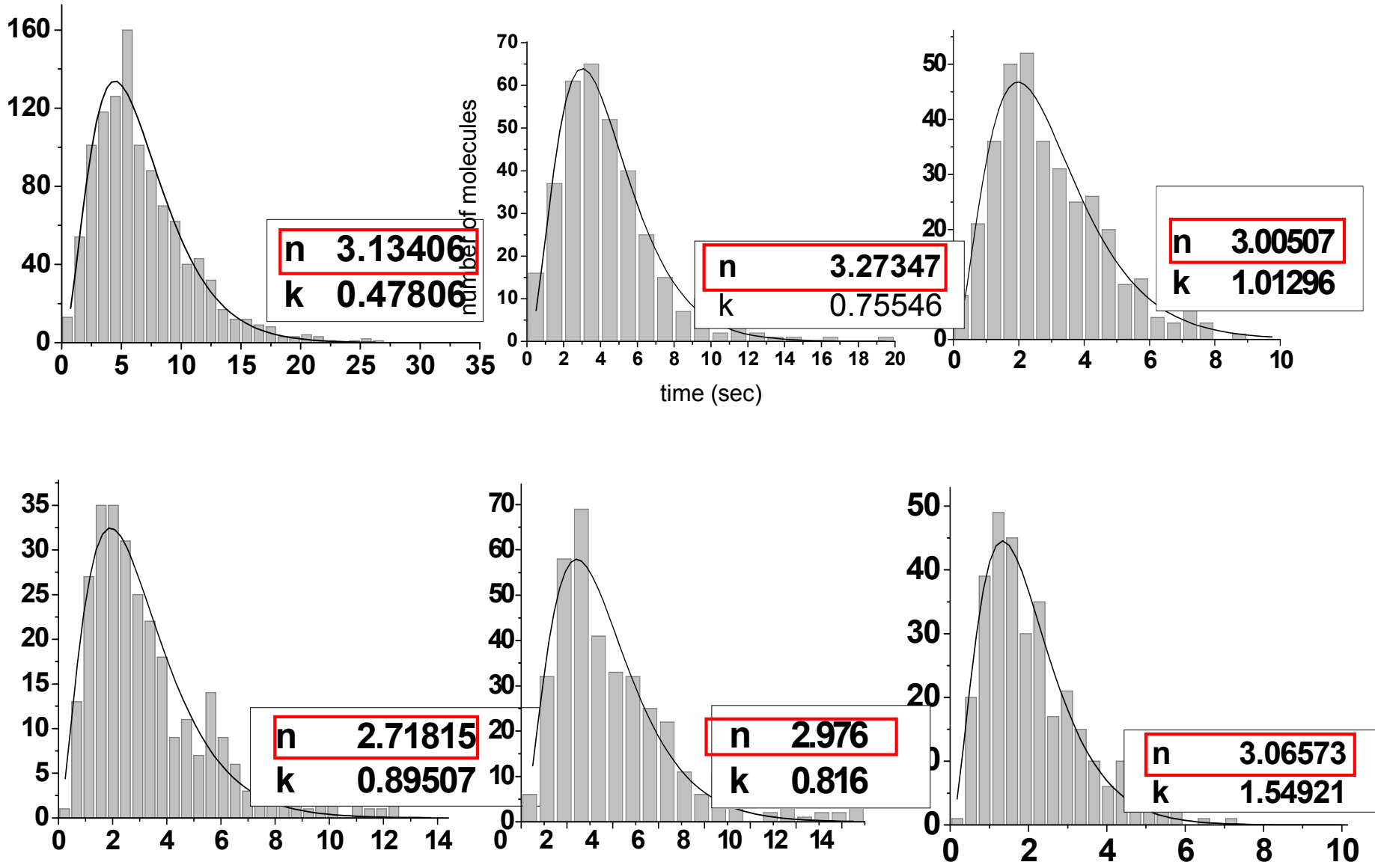




$$t^{n-1}e^{-kt}$$

n irreversible steps

Independent measurements yield n value close to 3



A model for how NS3 moves (next lecture)

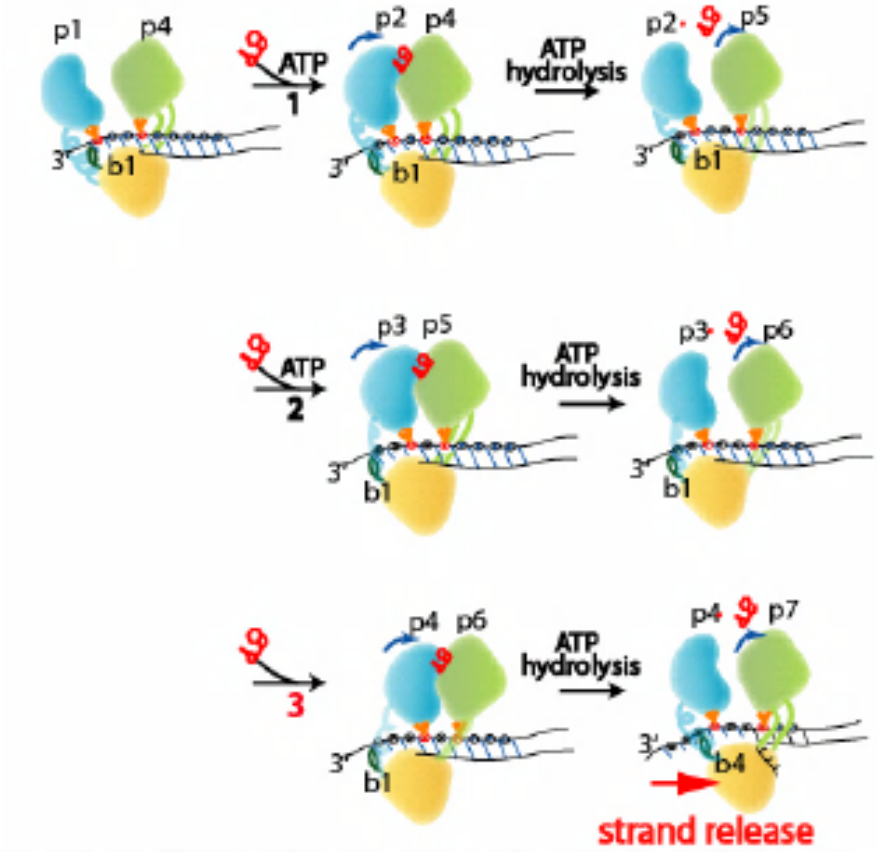


Fig. S9 Based on our data and the previously reported structure of NS3 we propose an unwinding mechanism involving three base pair step composed of three individual steps of one base pair. Domains 1 and 2 colored blue and green respectively, position themselves 3 nt away from each other via two threonine residues interacting with two phosphates reperesnted by two orange protrusions at protein-DNA interface. The two domains move in concerted manner by closing and opening as ATP binds and ADP is released thereby translocating one base toward 5' end (movement denoted by p1 p2 etc). Such single base translocation continues three times while domain 3 remains base stacked through tryptophan 501 (dark green hexagon) generating strain on itself. This spring-loaded domain 3 moves in a burst of 3 nt unzipping three base pairs as a result. The displaced strand gets loaded on domain 3 and maintains contact with the enzyme while it undergoes further unwinding, possibly forming a loop around it.

Why are threonine's involved?

What's the evidence for this?

Read original article!

Myong et al, Science,2007

A movie for how NS3 moves (next lecture)

We made a little movie out of our results and a bit of imagination. The two domains over the DNA move in inchworm manner, one base at a time per ATP while the domain below the DNA stays anchored to the DNA through its interaction, possibly the tryptophan residue. Eventually, enough tension builds and DNA is unzipped in a three base pairs burst.

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.