Progress report

6/10-8/10

(Last slide that presented at 2012/06/10)

Continu...

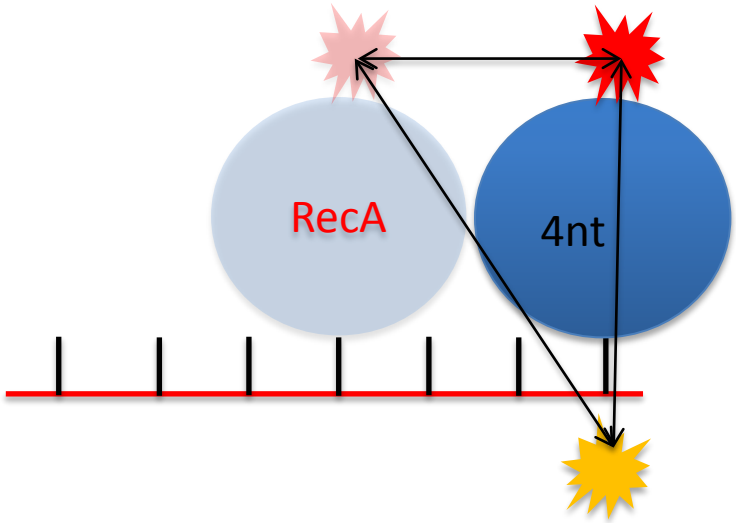
Observe Cy3-Cy7 and Cy3-Cy5 under 3 colors FRET configuration. ✗

Analyse the datas by Matlab (IDL is just for 2 colors) ✓

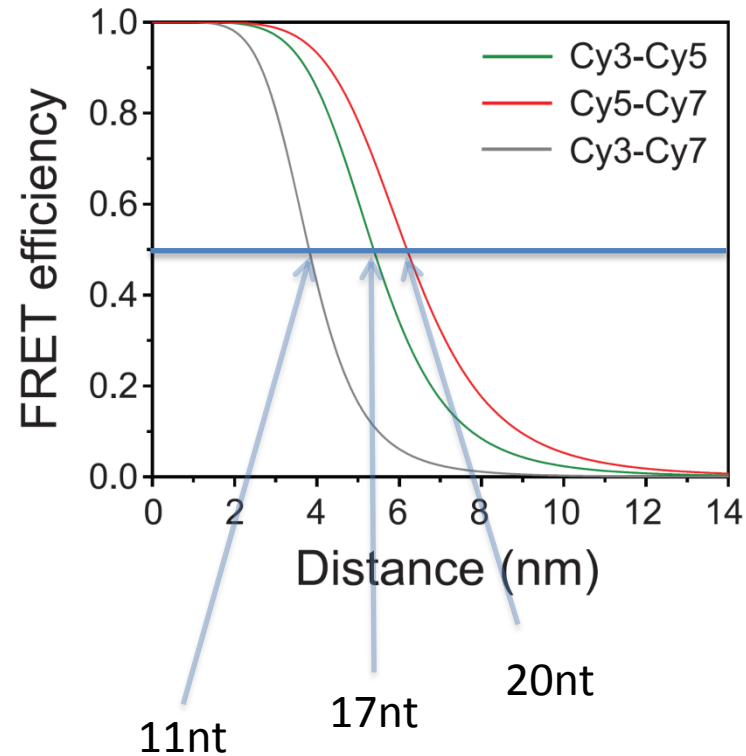
Labeling RecA protein ✓

Measure 3 colors FRET ✗

My goals



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



R0 values of

Cy3-Cy5 (green): 5.4nm

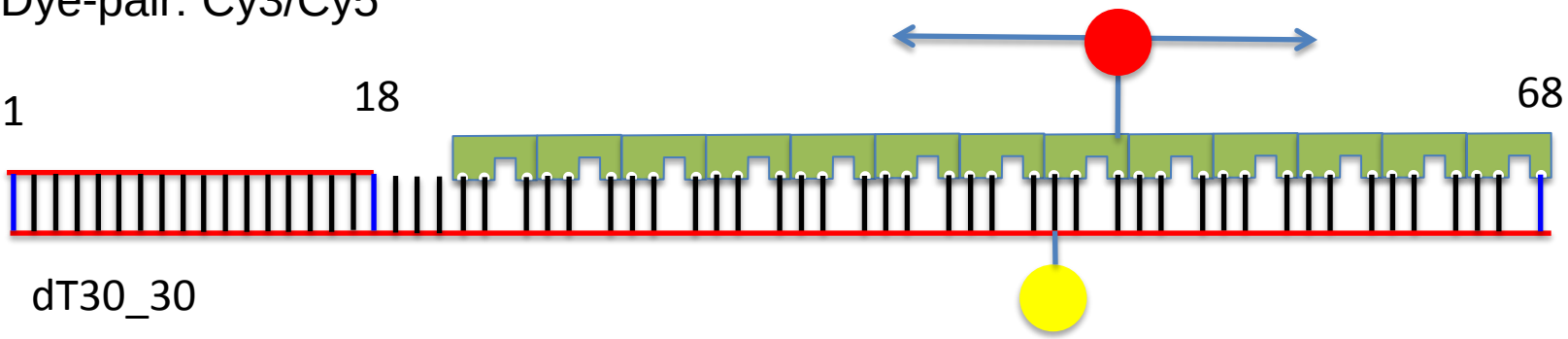
Cy5-Cy7 (red): 6.2nm

Cy3-Cy7 (gray): 3.8-nm

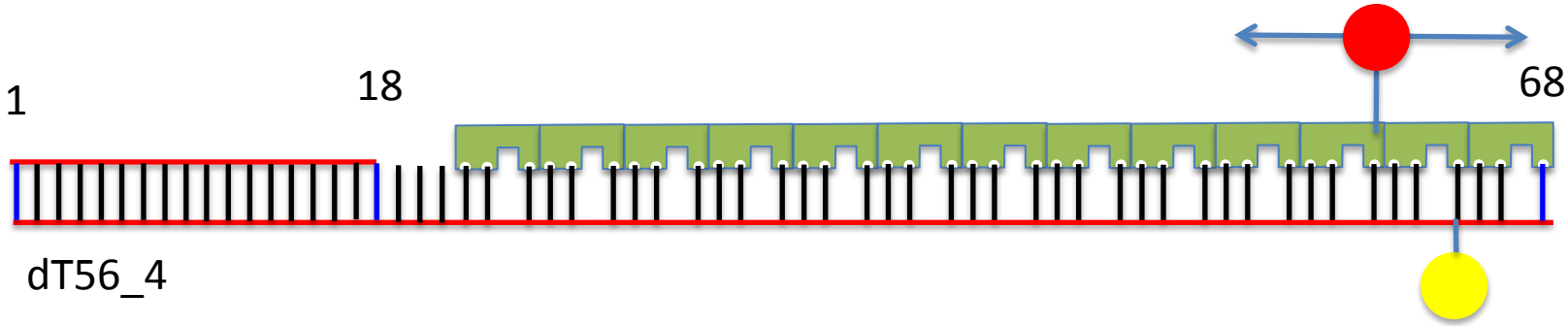
Assume that 1nt~3.5A^0 → Expected value of E is 0.9

My plan

Dye-pair: Cy3/Cy5



dT30_30



dT56_4

Things to do

Labeling the monofunction DNA with donor (Cy3)

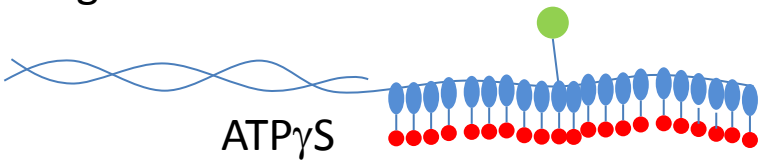
dT30_30

dT56_4

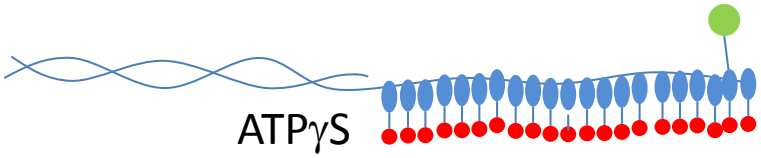
Labeling RecA protein with acceptor (Cy5)

Investigate the fluorescence of only donor as the reference data

Investigate the case that the filament of labeled RecA are formed on the DNA

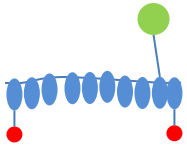


ATPγS



ATPγS

Very high FRET is expected

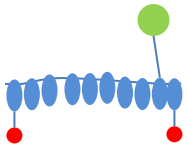


ATPγS

Investigate the case that the filament of RecA are formed on the DNA with very few labeled RecA are random distributed

N(free RecA):N(labeled RecA)

Investigate the displacement of RecA on st DNA

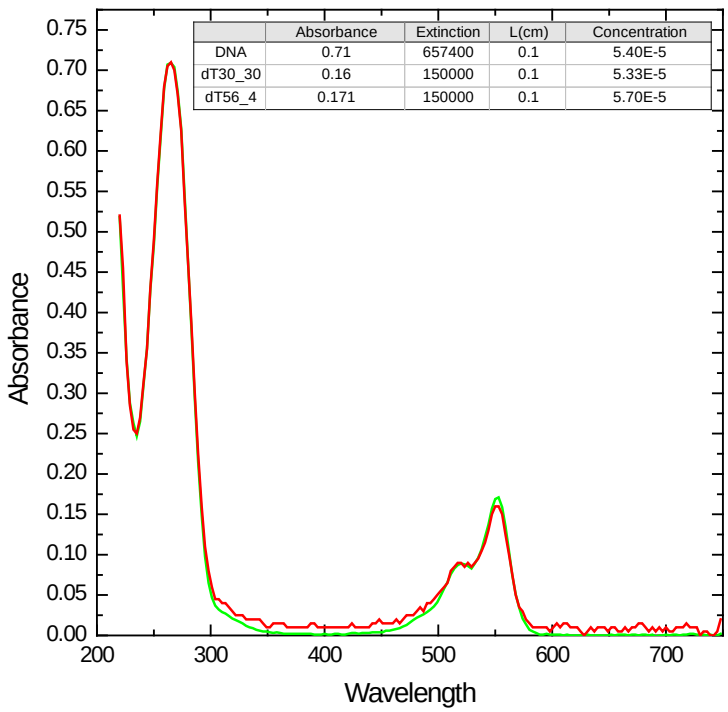


ATP

Progress

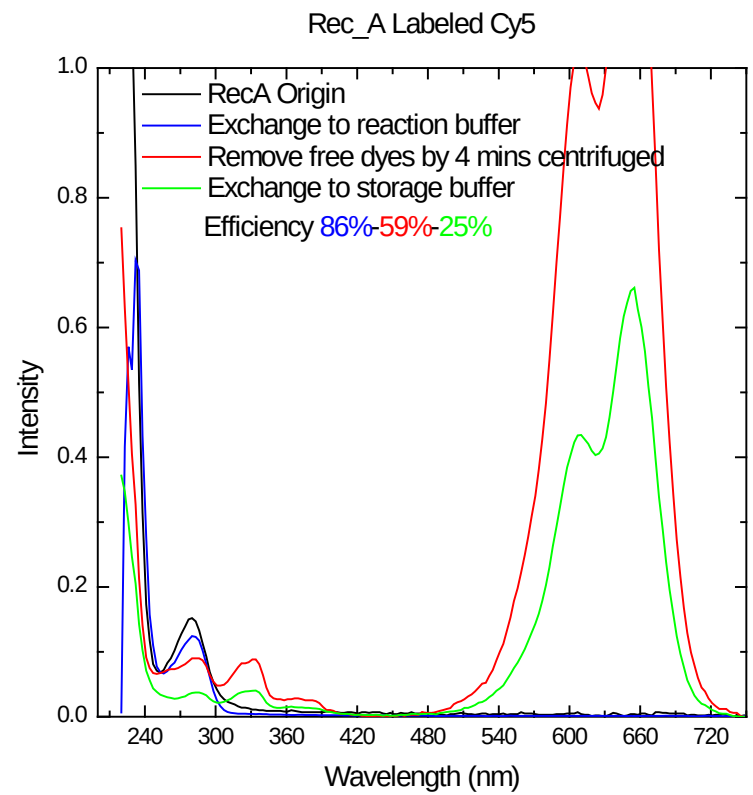
Labeling the monofunction DNA with donor (Cy3)

Labeling RecA protein with acceptor (Cy5)



	Absorbance	Extinction	L(cm)	Concentration
DNA	0.71	657400	0.1	5.40E-5
dT30_30	0.16	150000	0.1	5.33E-5
dT56_4	0.171	150000	0.1	5.70E-5

Labeling the monofunction DNA with donor (Cy3)



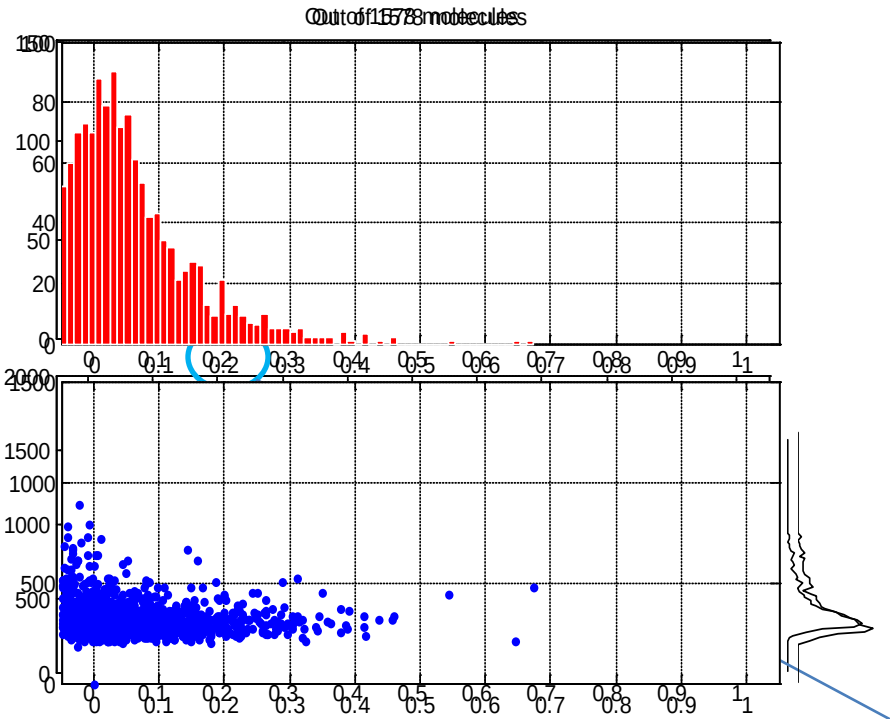
	Absorbance	Extinction	L	Concentration
RecA	0.152	27000	0.1	5.62963E-5
RecA(labeled)	0.09	27000	0.1	3.33333E-5
Cy5(filtered)	1.4995	250000	0.1	5.998E-5
RecA(Final)	0.037	27000	0.1	1.37037E-5
Cy5(Final)	0.6615	250000	0.1	2.646E-5

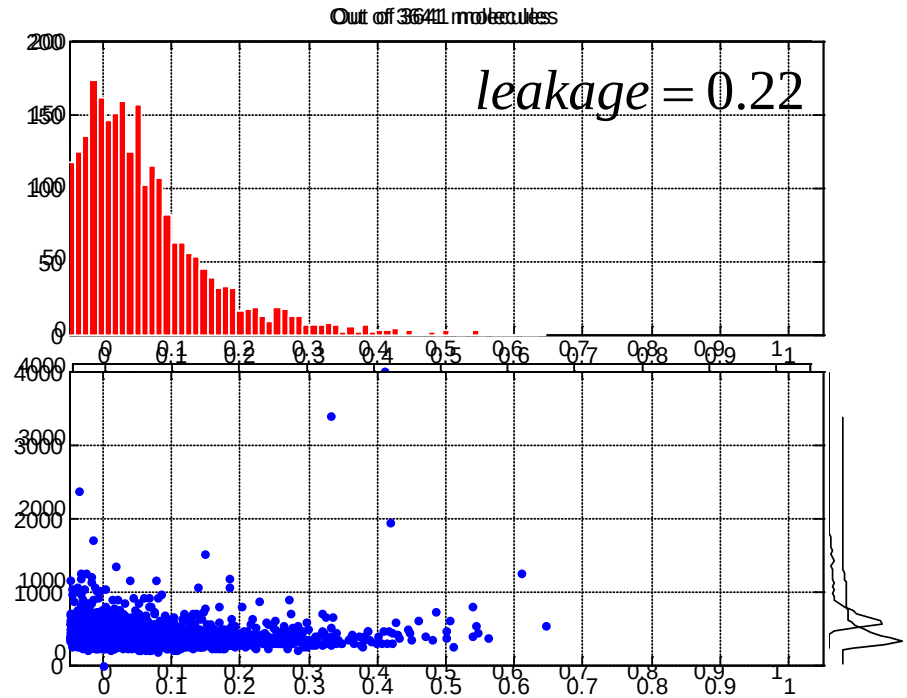
Labeling RecA protein with acceptor (Cy5)

Progress

Investigate the fluorescence of only donor as the reference data

Leakage and background removal





$$E = \frac{I_a}{I_a + I_d} \qquad I_a = 0 \Leftrightarrow E = 0 \qquad E = \frac{I_a + leakage * I_d}{I_a + leakage * I_d + I_d}$$

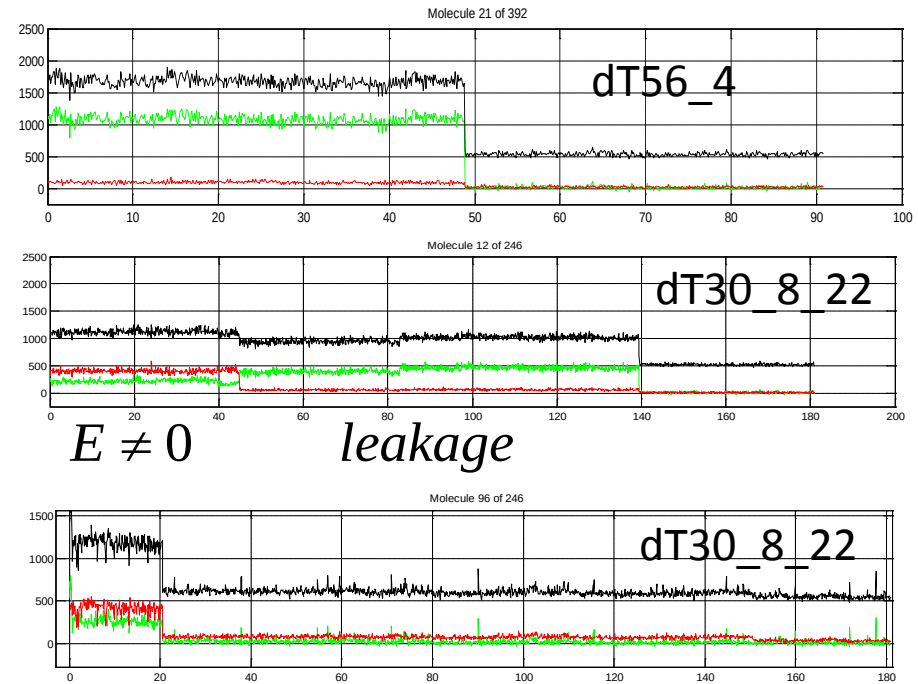
Progress

Investigate the fluorescence of only donor as the reference data

Leakage and background removal

$$E = \frac{I_a + leakage * I_d}{I_a + leakage * I_d + I_d}$$

Is enough ?



$E \neq 0$ *leakage*

$$I_a^{'} = I_a + \alpha_1 I_d + \alpha_2 (I - I_d) + \alpha_3 C$$

$$I_d^{'} = I_d + \beta_1 I_a + \beta_3 C$$

leakage

Direct excitation

Background

$$E \sim \frac{I_a^{'} - (\alpha_1 - \alpha_2) I_d^{'} - C_1}{(1-\beta_1) I_a^{'} + (1 - \alpha_1 + \alpha_2) I_d^{'} - C_2}$$

Assume that I is a constant, C_1 and C_2 include several factors such as I, C and parameters α,β

This factor plays the same role with the leakage in the equation above. But there is another constant value need to be subtracted

Progress

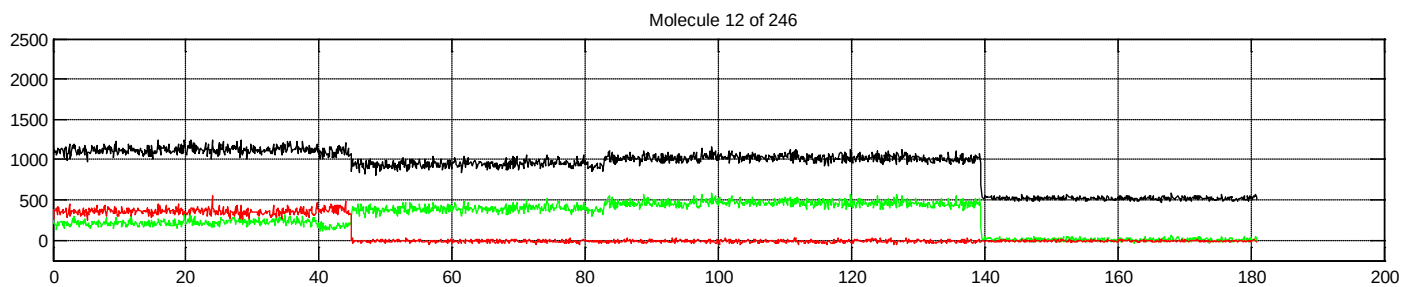
Investigate the fluorescence of only donor as the reference data

Leakage and background removal

$$E \sim \frac{I_a^{'} - (\alpha_1 - \alpha_2) I_d^{'} - C_1}{(1-\beta_1) I_a^{'} + (1-\alpha_1+\alpha_2) I_d^{'} - C_2}$$

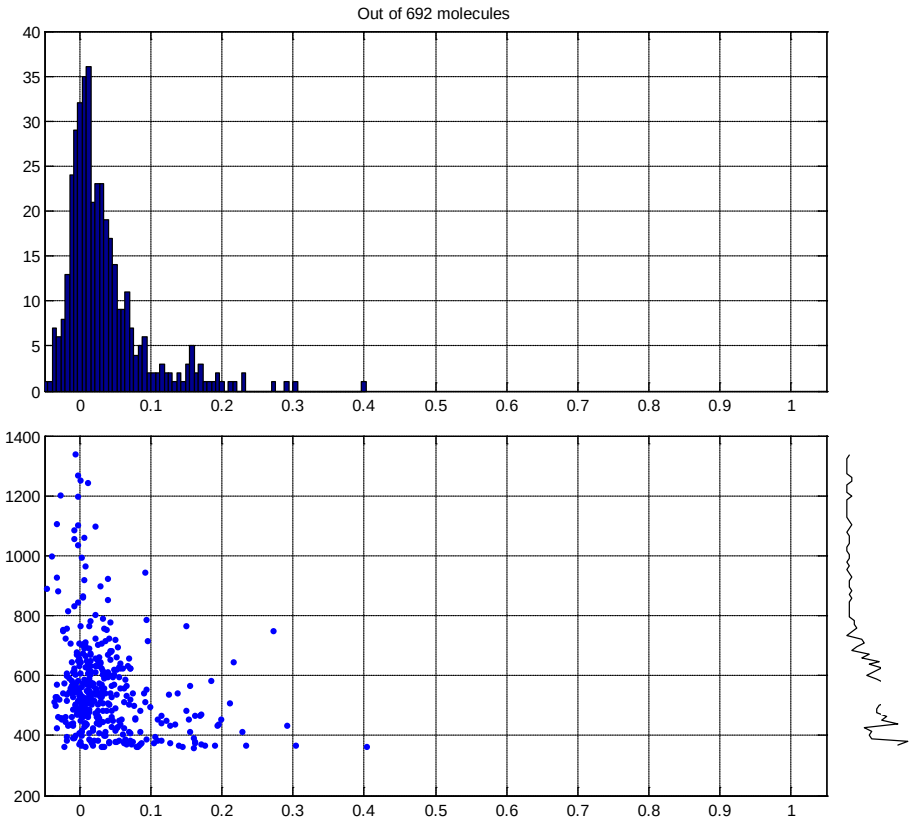
$$E = 0 \Rightarrow I_a^{'} = \alpha I_d^{'} + C$$

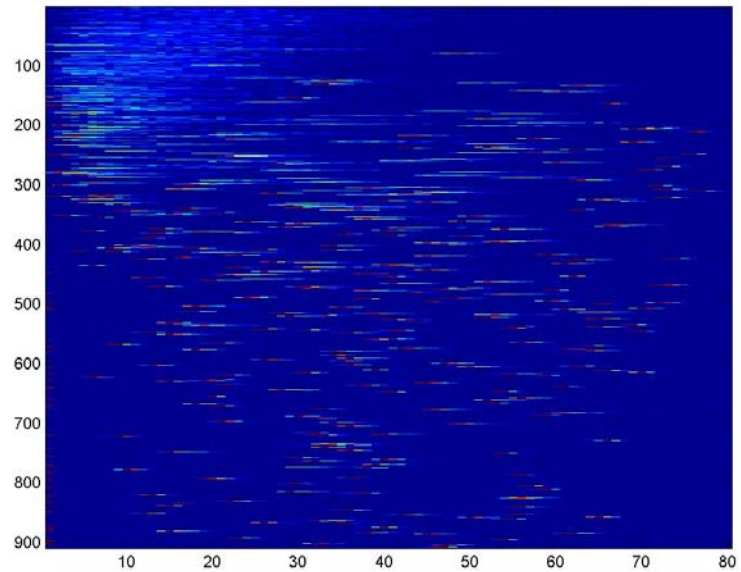
Fit the zero-FRET experiment one can have (α, C)



Progress

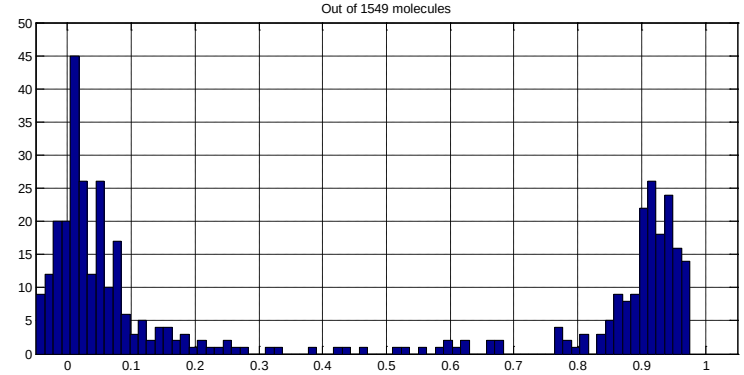
Investigate the fluorescence of only donor as the reference data

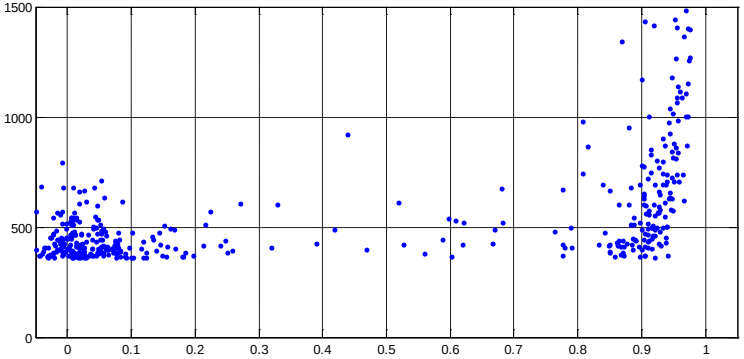


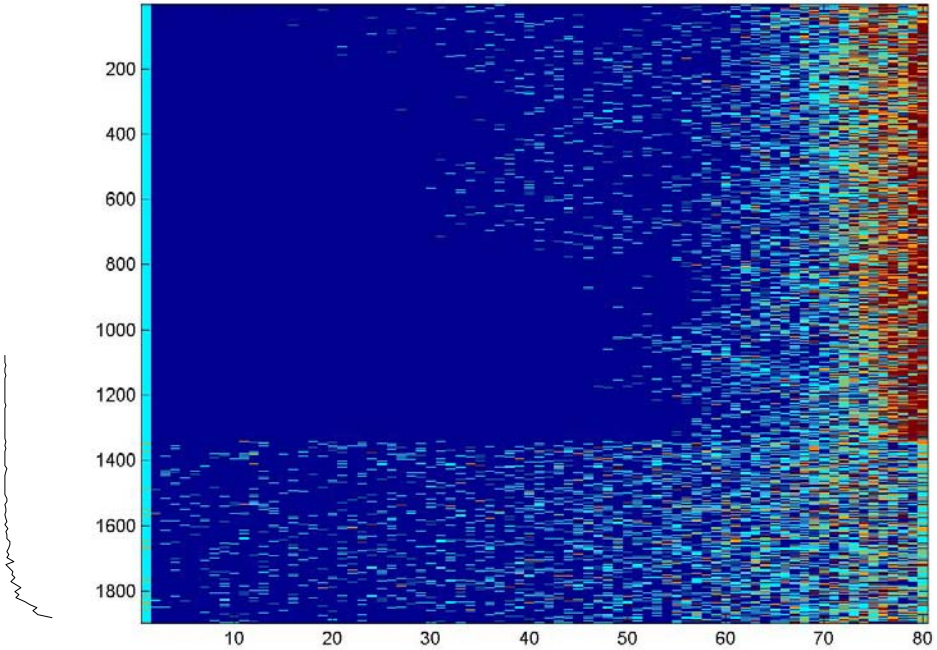


Progress

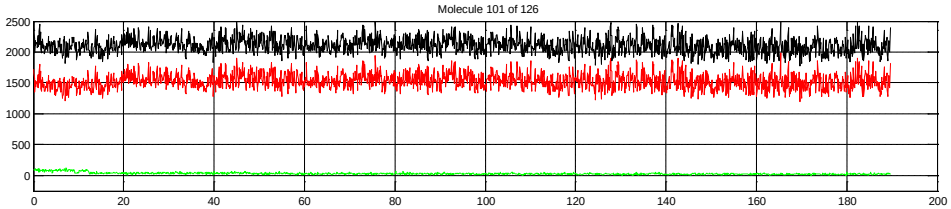
Investigate the case that the filament of labeled RecA are formed on the DNA

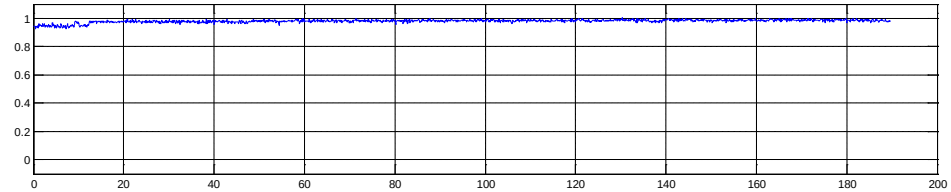






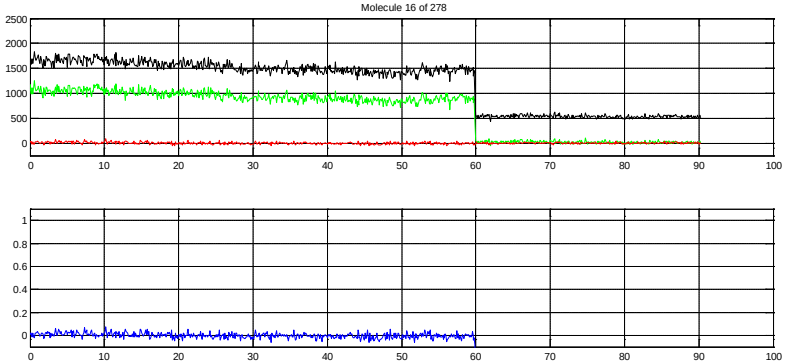
High FRET observed

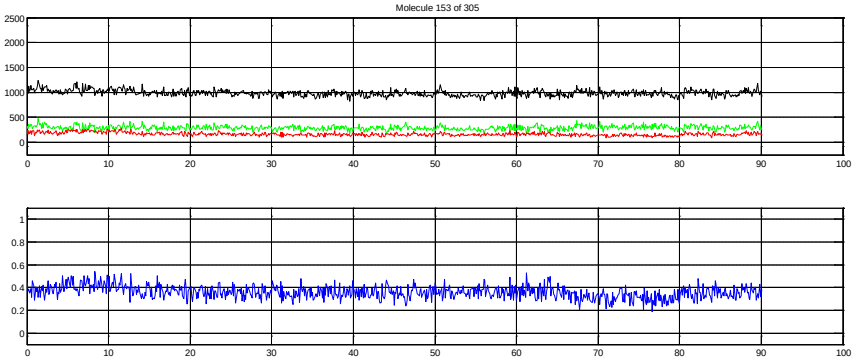


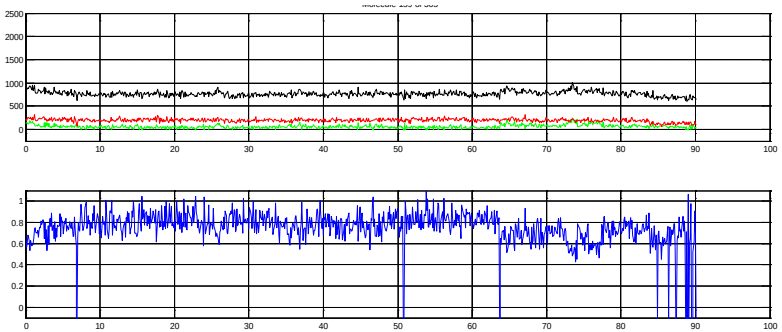


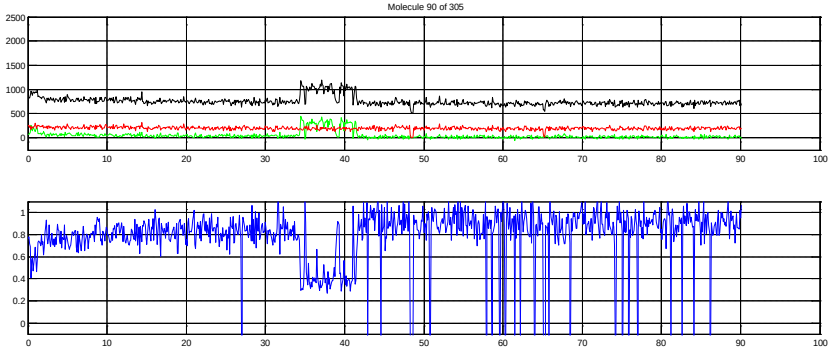
Progress

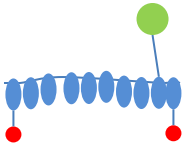
Investigate the case that the filament of RecA are formed on the DNA with very few labeled RecA are random distributed







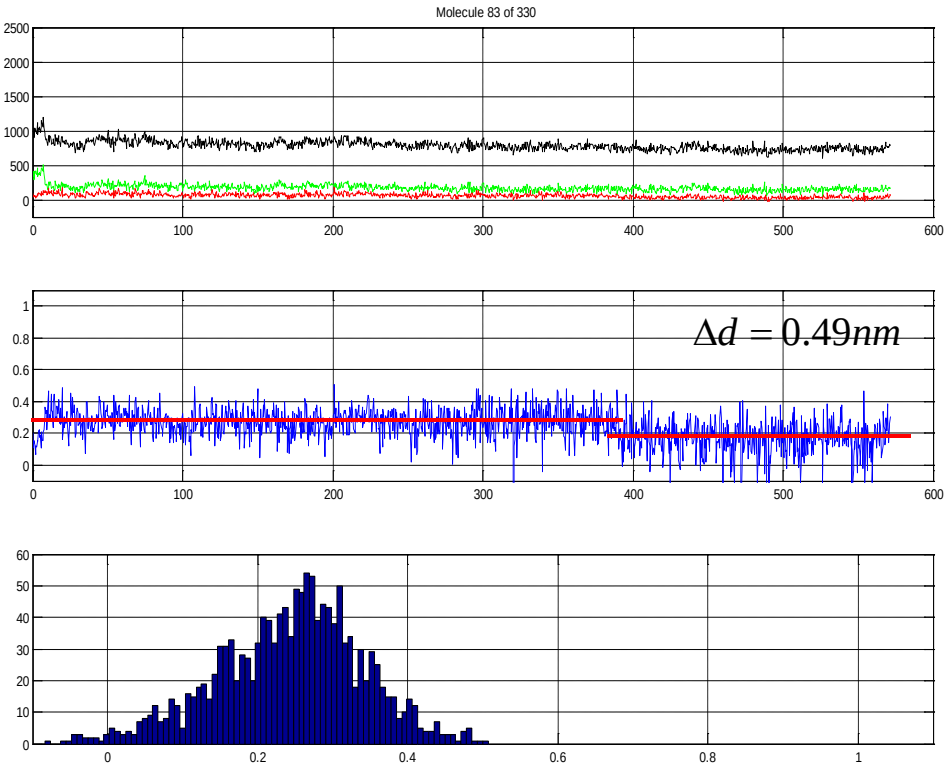


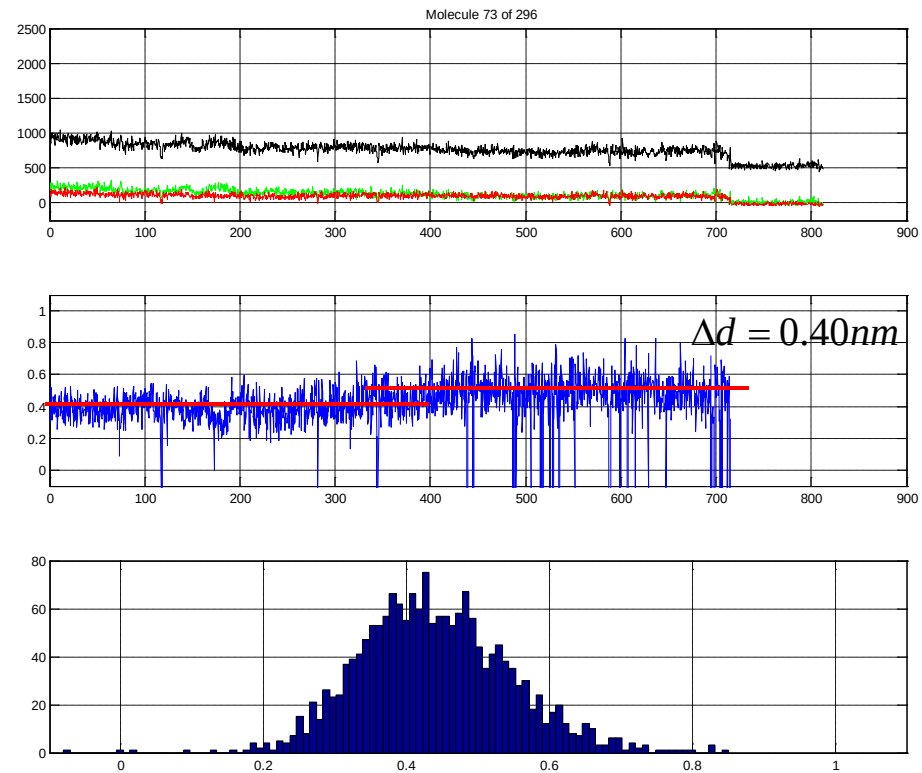


ATPγS

Progress

Investigate the displacement of RecA on st DNA



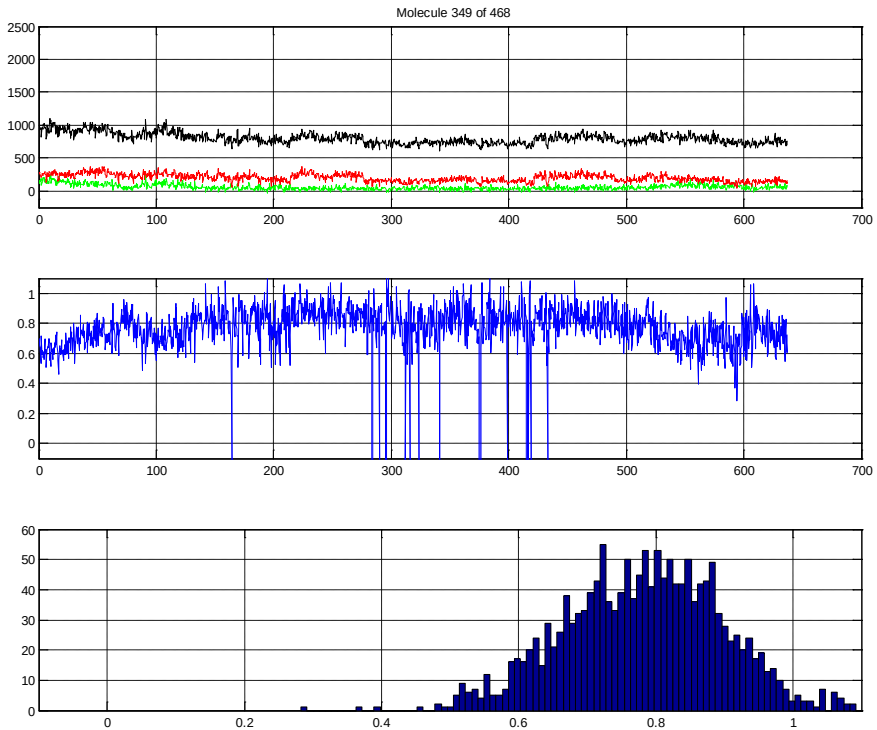


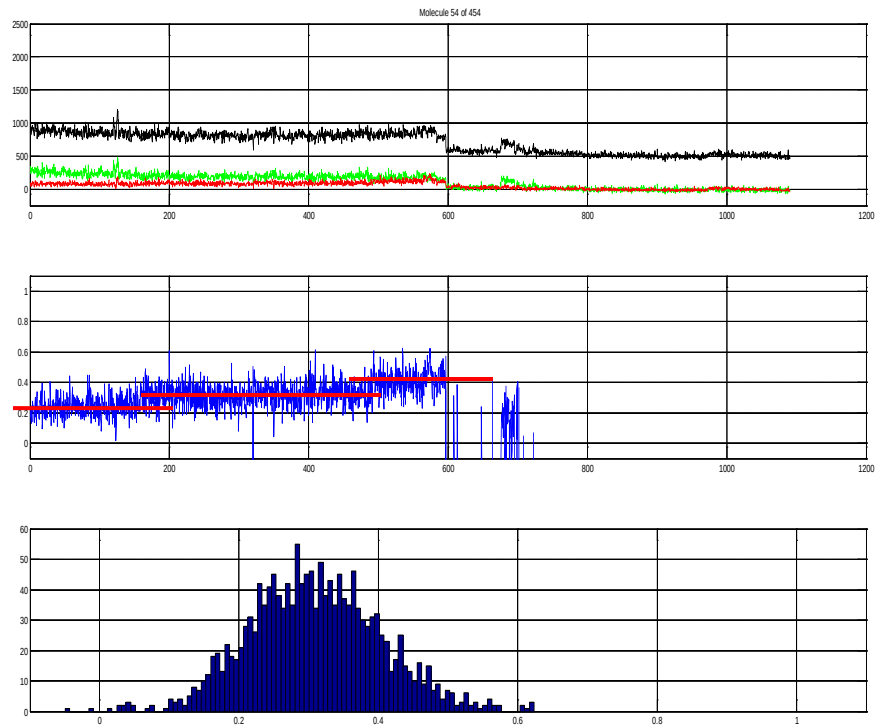
dT30_30 First occupied by RecA filament in ATP
Then, 0.5ul 5nM RecA_Cy5 in ATP was introduced

The displacement is approximately equal to the length of a nucleotide !!!

Progress

Investigate the displacement of RecA on st DNA

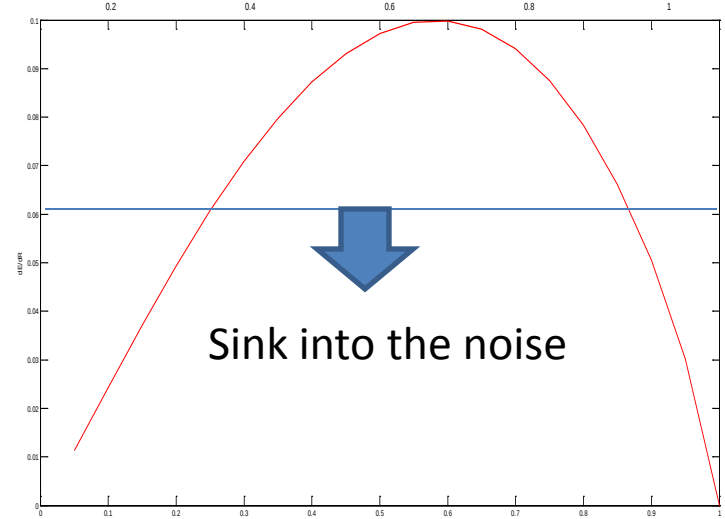




dT56_4 First occupied by RecA filament in ATP

Then, 0.5ul 5nM RecA_Cy5 in ATP was introduced

$$\Delta E = \frac{3.5}{\frac{R_0}{6}\left(\frac{1}{E}-1\right)^{-5/6} E^{-2}}$$



Further plan

-Looking for the optimize ratio between labeled RecA/free RecA in random distributing experiment

- Work with Matlab analyzing program in order to improve the analyzing tools (SNR, observing time etc…)

- Increase the stability of FRET (photostabilities of donor, acceptor. Reproductivity of optical system (leakage, Offset value etc…)

Have a nice weekend !