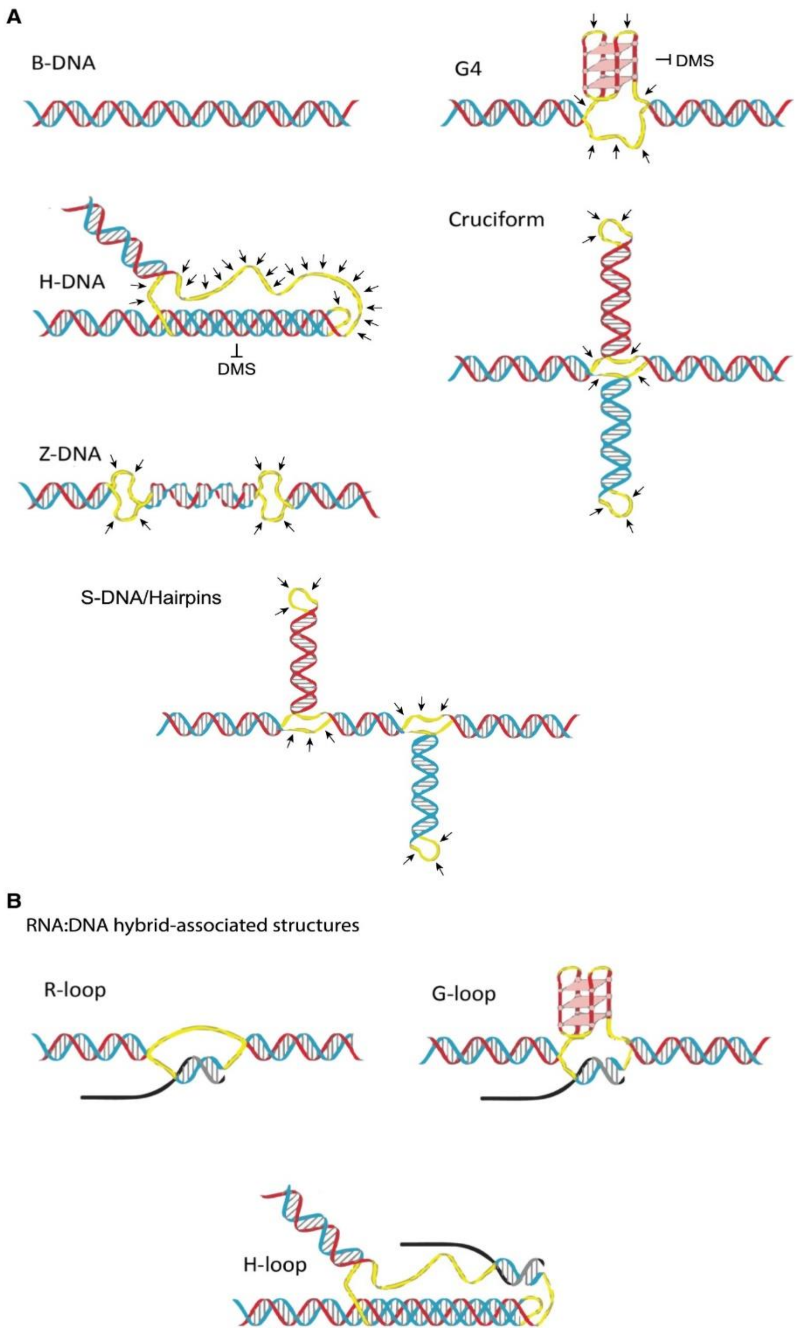
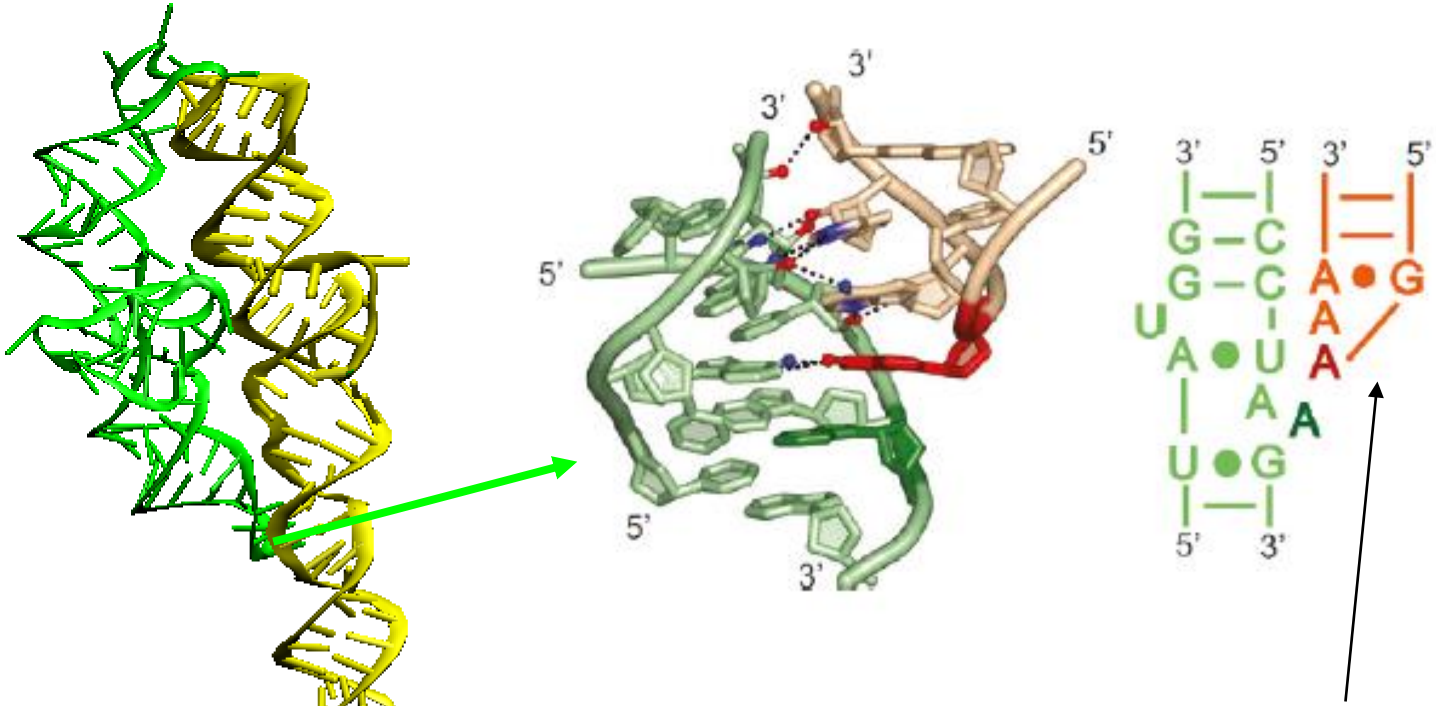
DNA also has different structures!



Detection of alternative DNA structures and its implications for human disease.
Gabriel Matos-Rodrigues et al., 2023. Mol Cell 83:3622-32

Lecture 5. 2023
Dynamics of tertiary interactions

1. TETRALOOP/TETRALOOP RECEPTOR



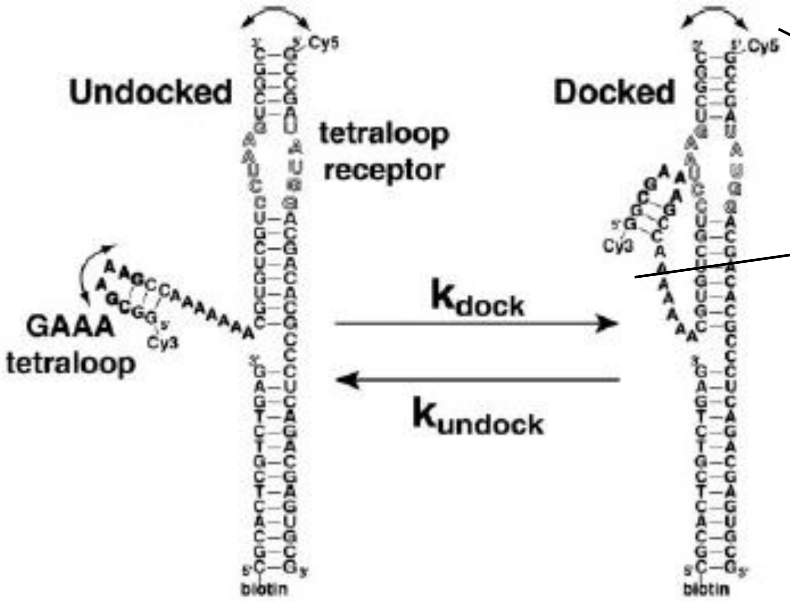
GAAA tetraloop
GNRA

This is a tertiary interaction, one of the RNA:RNA interactions that staple large RNAs together.

How do you study the thermodynamics and kinetics of an RNA tertiary interaction?

Here a tetraloop docks with its receptor. (Note – this is not rigid body docking)

Fluorescence



Cy5 is a FRET acceptor

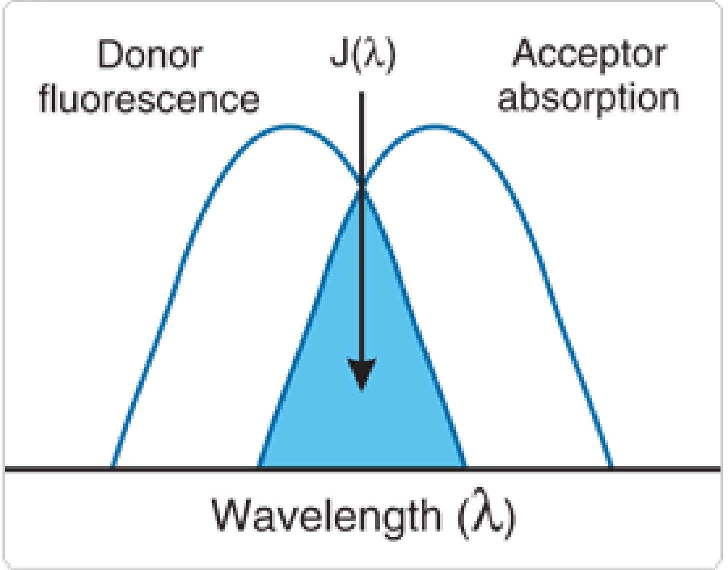
Cy3 is a FRET donor

In the undocked state, the dyes (fluorophores) can be up to 70 Ǻ apart, but in the docked state, their separation is ~35 Ǻ.

Hodak et al., 2005 PNAS 105:10505 – 10510.

FRET: Förster Resonance Energy Transfer

Electronic excitation energy is transferred through transition dipole-dipole interactions, and like any dipole-dipole interaction, it has a distance and orientation dependence.
This is non-radiative transfer.



$$R_O = [8.8 \times 10^{23} \cdot \kappa^2 \cdot n^{-4} \cdot QY_D \cdot J(\lambda)]^{1/6}\ \text{Å}$$

where κ^2 = dipole orientation factor (range 0 to 4; $\kappa^2 = 2/3$ for randomly oriented donors and acceptors)

QY_D = fluorescence quantum yield of the donor in the absence of the acceptor

n = refractive index

$J(\lambda)$ = spectral overlap integral (see figure)

$$= \int \epsilon_A(\lambda) \cdot F_D(\lambda) \cdot \lambda^4 d\lambda \ \text{cm}^3\text{M}^{-1}$$

where ϵ_A = extinction coefficient of acceptor

F_D = fluorescence emission intensity of donor as a fraction of the total integrated intensity

R_o is the distance at which energy transfer is 50% efficient. It varies with dye pair, but typically ranges from 30 – 100 Å

In practice: The efficiency of energy transfer is given by

$$\% \text{ FRET} = 100 \left[1/(1 + (R/R_o)^6) \right]$$

But,
Because we typically don't know the quantum yields of donor and acceptor fluorophore, FRET values are approximated by

$$\% \text{ FRET} = 100 \left[I_A/(I_D + I_A) \right]$$

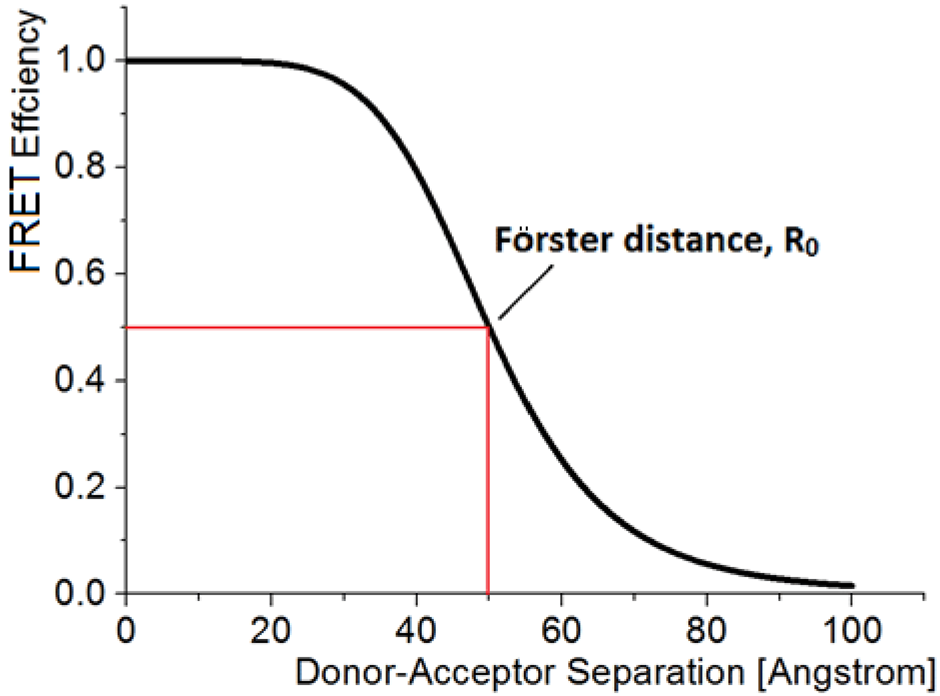
Or alternatively,

$$\text{Efficiency} = I_A/(I_D + I_A)$$

Where I_D is the donor fluorescence and I_A is the acceptor fluorescence

The efficiency of energy transfer is given by

FRET efficiency = $[\ 1/(1 + (R/R_o)^6)]$



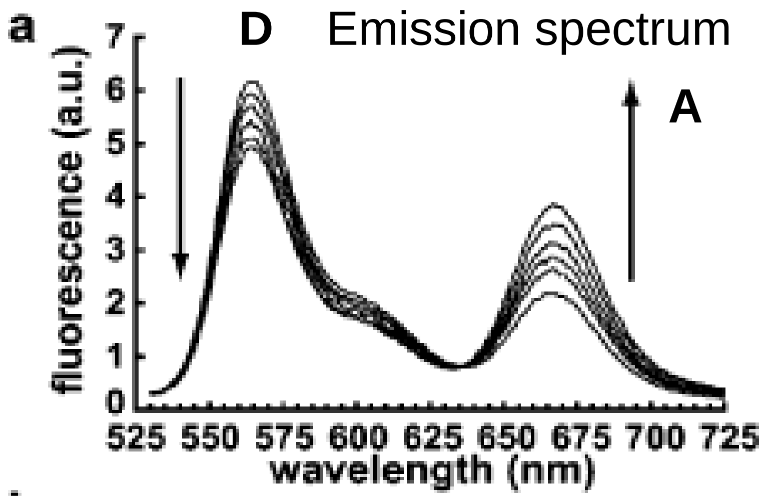
Three important regions of this curve:

1. Where Efficiency = 1
   Donor and acceptor are too close together, there is no distance information.

2. Near the midpoint of the curve where $R = R_o$
   The region most sensitive to distance changes

3. Where Efficiency is near 0.
   Donor and Acceptor are too far apart for transfer.

Note, however, the $1/r^6$ dependence of the signal!



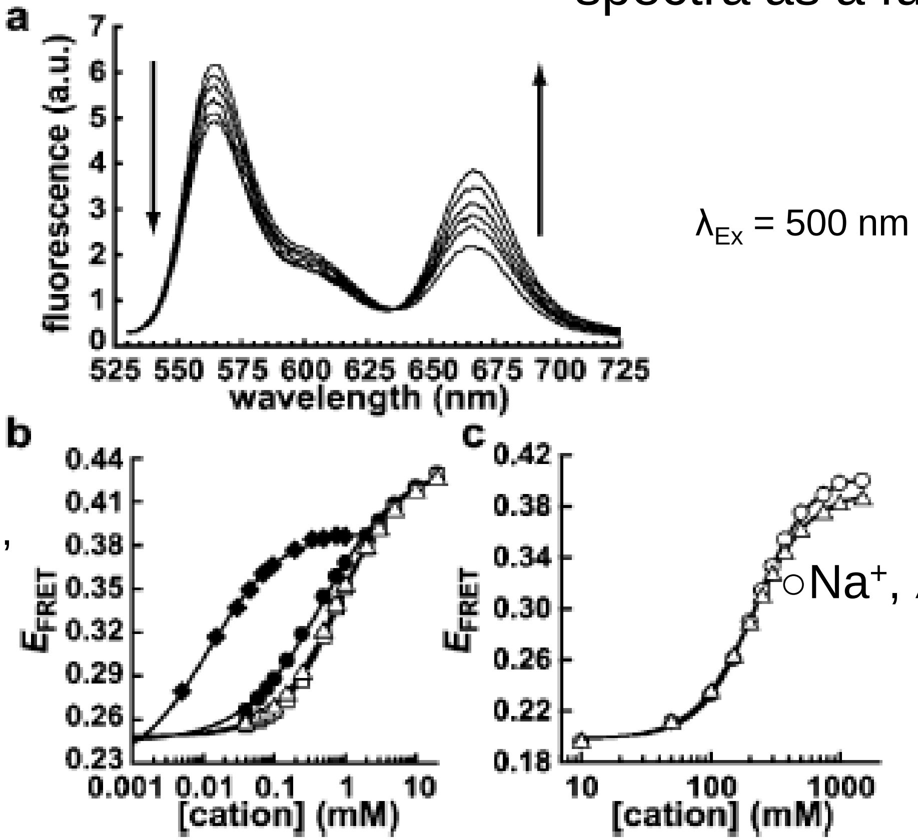
Experiment:
Excite the Donor at a convenient wavelength – one where there is no or very little acceptor absorbance.

If the Acceptor is close to the Donor, the Donor fluorescence will decrease and that of the Acceptor will increase.

Here, a molecule (RNA) is being titrated with a small molecule that results in a change in its overall geometry.

FRET experiments can be done in ensemble mode.

Steady-state Fluorescence
spectra as a function of Mg^{2+}.

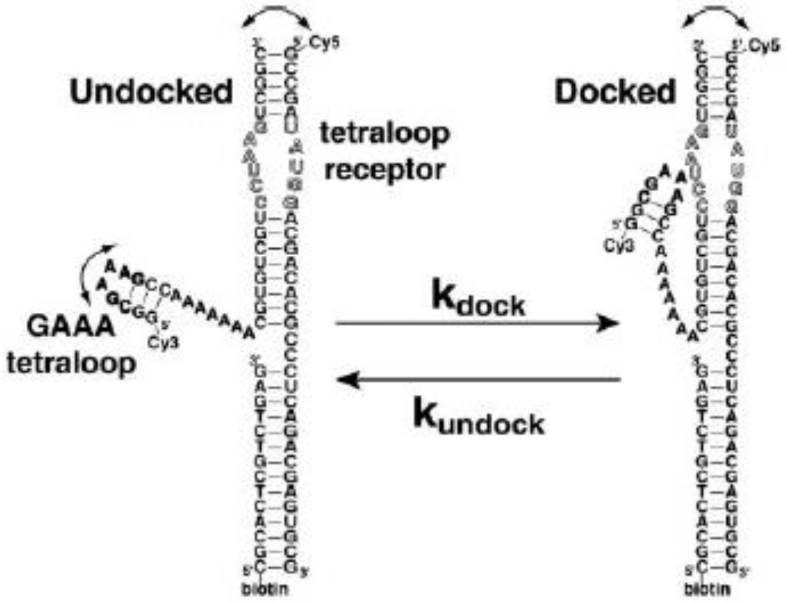


λ_{Ex} = 500 nm

ΔMg^{2+}, □Ca^{2+},
●Mn^{2+},
♦$Co(NH_3)_6^{3+}$

○Na^+, Δ K^+

FIGURE 2: Metal ion-induced docking of the GAAA tetraloop with the receptor. (a) Ensemble fluorescence spectra at Mg^{2+} concentrations between 0 and 10 mM, with fluorescence excited at 500 nm.



Downey et al., 2006 Biochemistry 45:3664-3673.

FRET experiments can be done in ensemble mode.

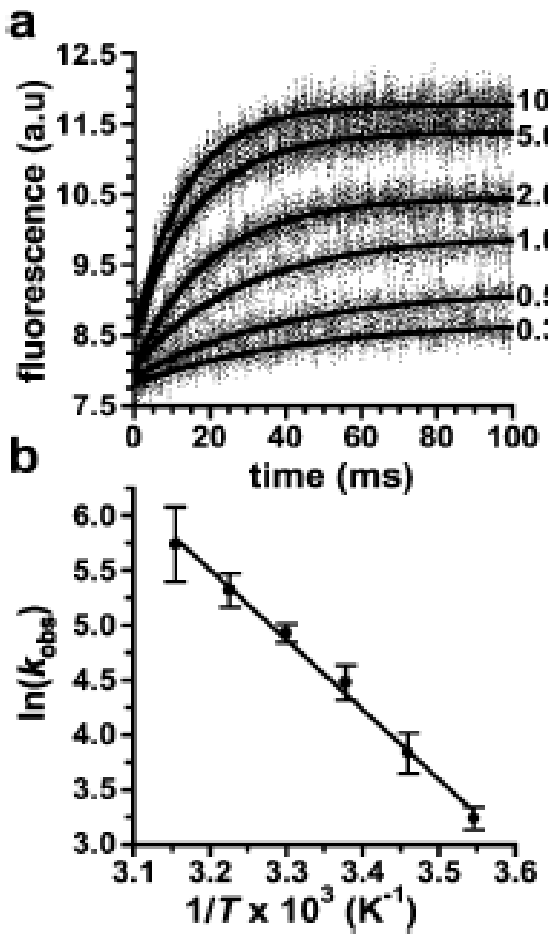


FIGURE 3: Kinetics of GAAA tetraloop−receptor docking from

Mg^{2+}

λ_{Ex} = 500 nm

Kinetics of docking by stopped-flow fluorescence.

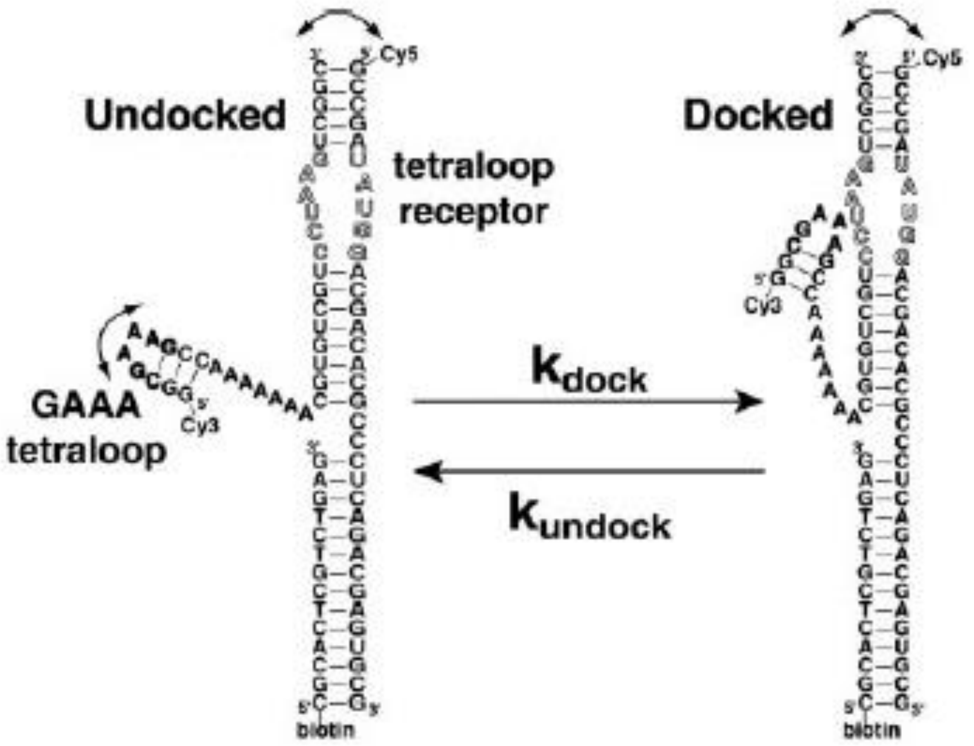
Arrhenius plot for temperature dependence of k_{obs} at 10 mM Mg^{2+}.

E_a (docking) = 12.7 ± 2.6 kcal/mol

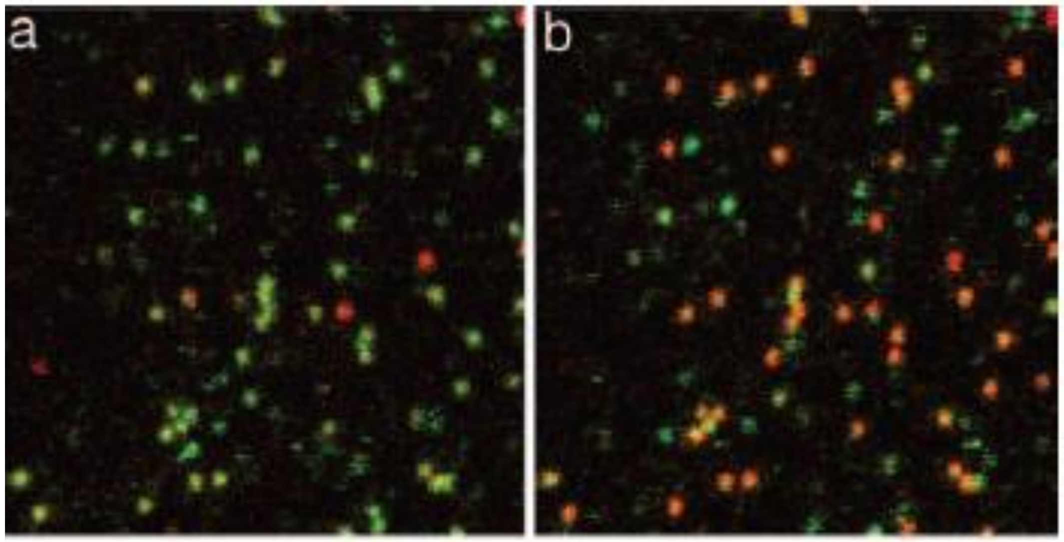
$\ln(k_{obs}) = -(E_a/R)(1/T) + \ln A$

Downey et al., 2006 Biochemistry 45:3664-3673.

FRET experiments can be done in single molecule mode.



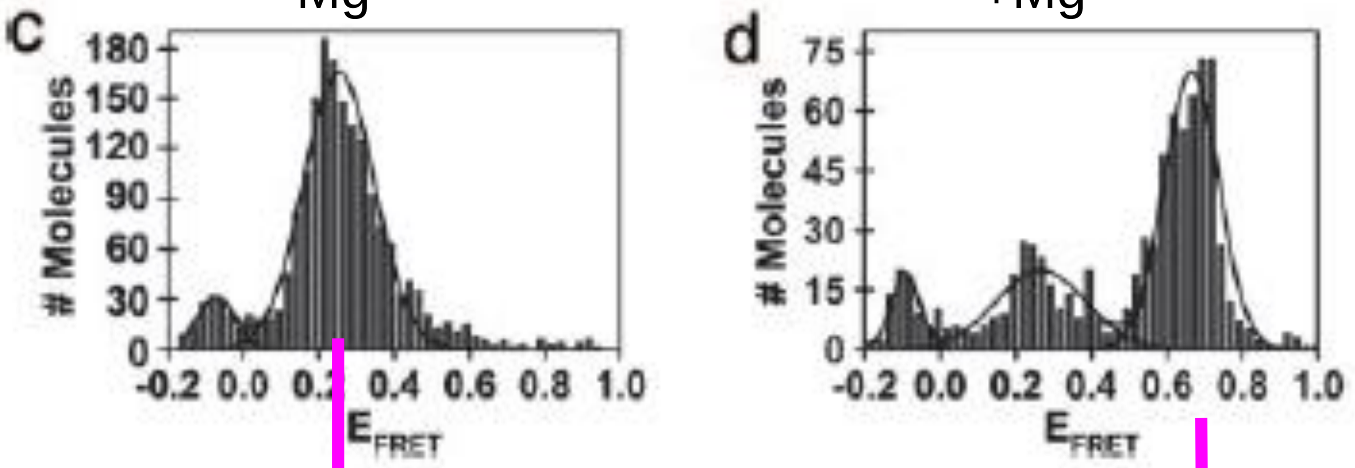
- Mg^{2+} +Mg^{2+}



Green: Cy3 (donor) only
Red: Cy5 (acceptor)

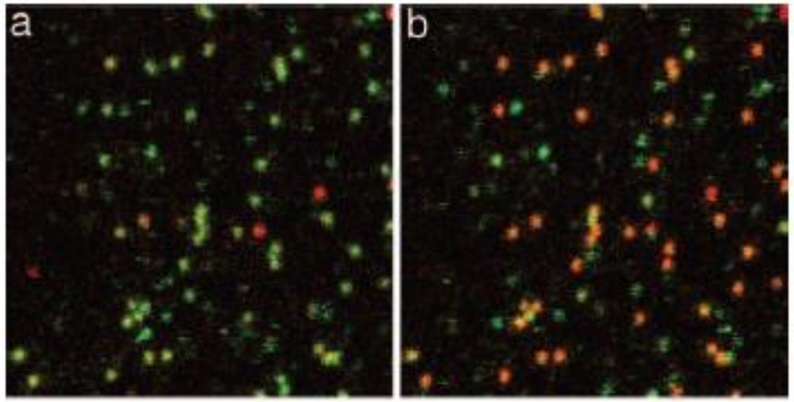
- Mg^{2+}

+Mg^{2+}



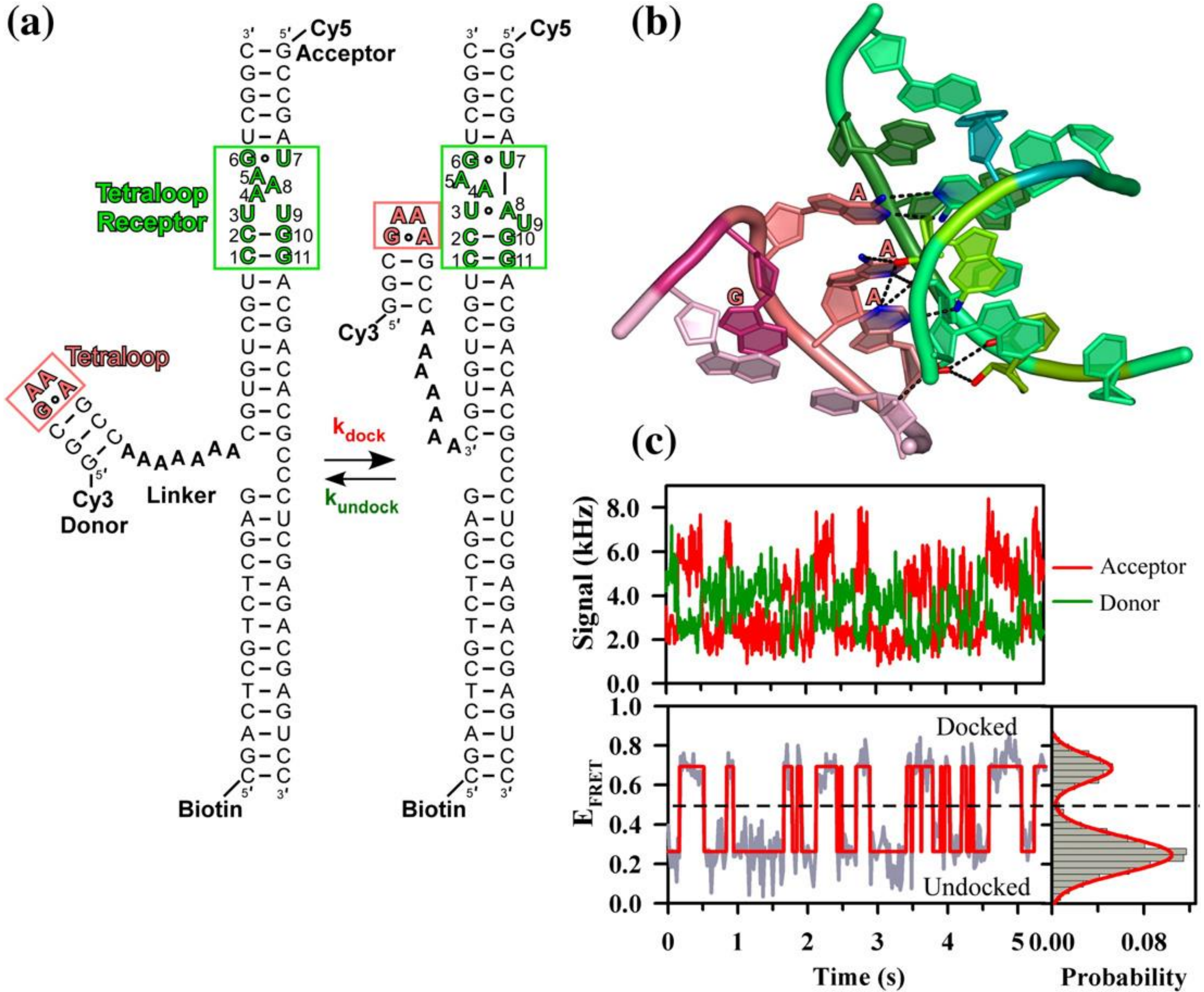
Efficiency = $I_A/(I_D + I_A)$

Consider the value of E and the distribution. What do you conclude?

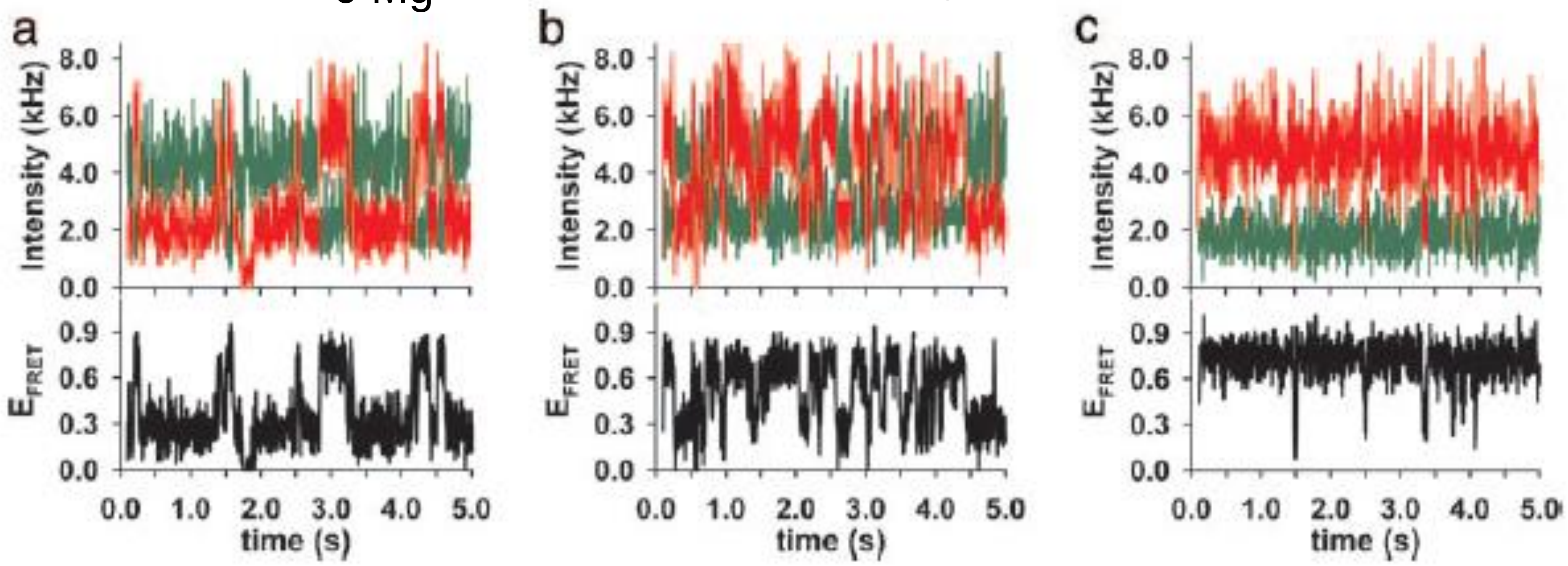


What’s changed?

Single Molecule Time-dependence of docking



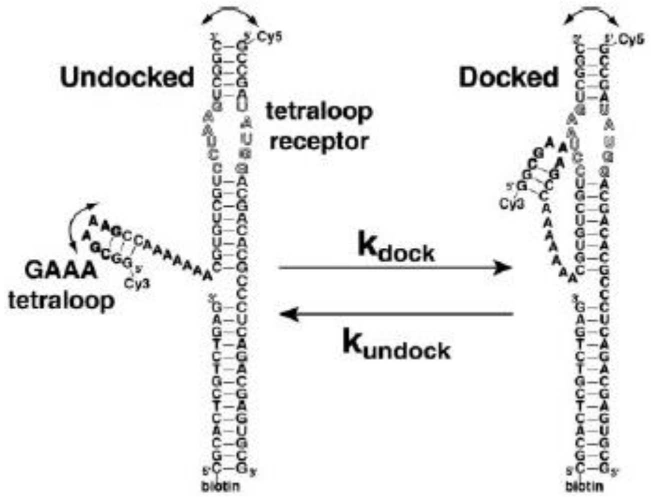
0 Mg^{2+} | 0.5 mM Mg^{2+} | 5 mM Mg^{2+}



Acceptor fluorescence

Donor fluorescence

Efficiency = $I_A/(I_D + I_A)$



What do you think the Mg^{2+} ions are doing?

Table 1. Rate constants and associated equilibrium for docking and undocking at various [Mg^{2+}]

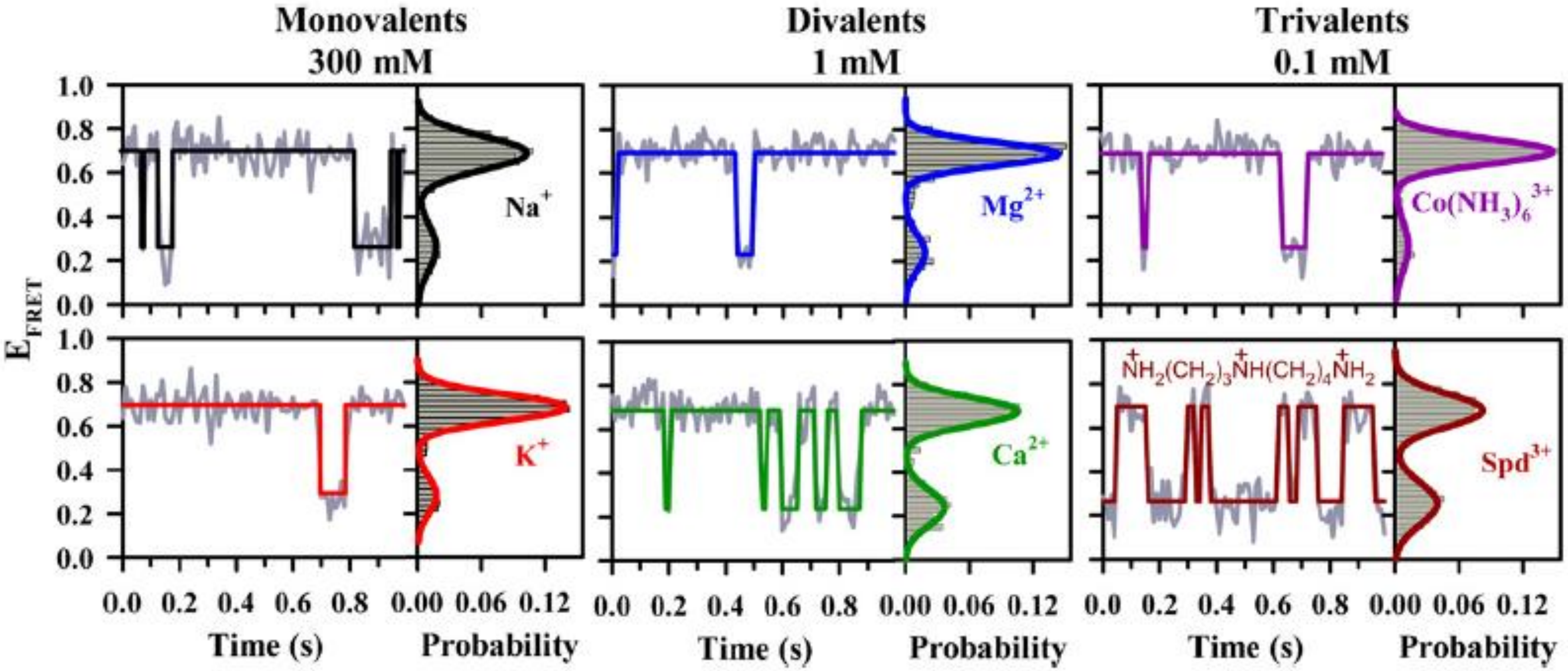
[Mg^{2+}], mM	K_{dock}, s^{-1}	K_{undock}, s^{-1}	K_{dock} (K_{dock}/K_{undock})
≈ 0.0	5.1 ± 0.3	10.3 ± 0.4	0.49 ± 0.04
0.35	10.5 ± 0.2	7.7 ± 0.2	1.36 ± 0.05
0.5	17.7 ± 0.5	6.8 ± 0.2	2.6 ± 0.1
1.0	30.1 ± 1.3	7.2 ± 0.3	4.2 ± 0.2
2.0	38.6 ± 1.3	5.5 ± 0.1	7.0 ± 0.3
5.0	51.2 ± 1.1	4.2 ± 0.2	12.3 ± 0.6
10.0	63.1 ± 1.9	3.3 ± 0.1	19.1 ± 0.9

<u>$\Delta G° = -RT\ln K_{eq}$</u>

- 290 cal/mol
- 790 cal/mol
-1.5 kcal/mol
-2.4 kcal/mol
- 4.1 kcal/mol
- 7.2 kcal/mol
-11.2 kcal/mol

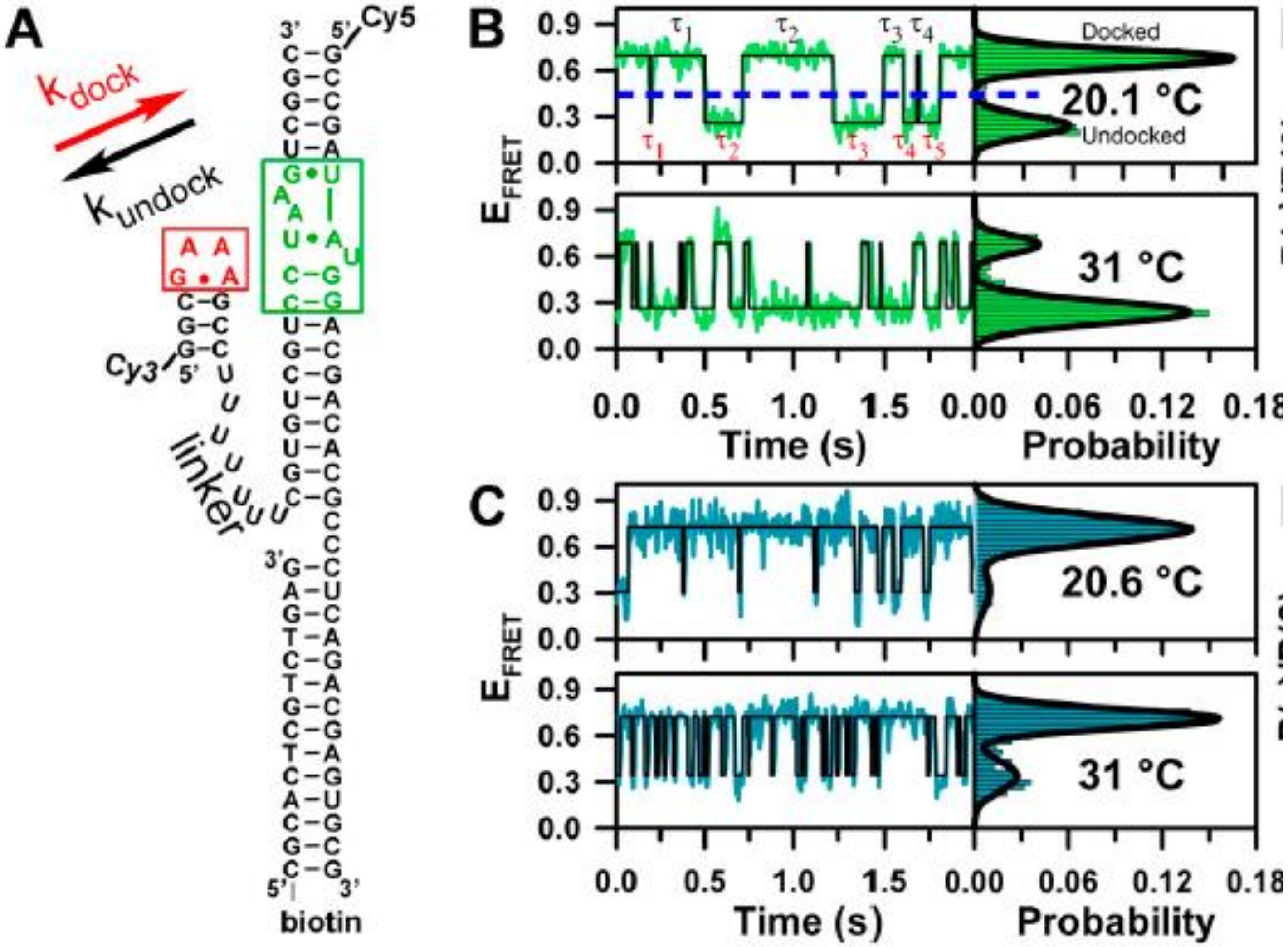
$\Delta G° = -RT\ln Keq$,

T = 22 °C or 295 K,
R = 1.98 cal/K-M
What is the free energy of docking?



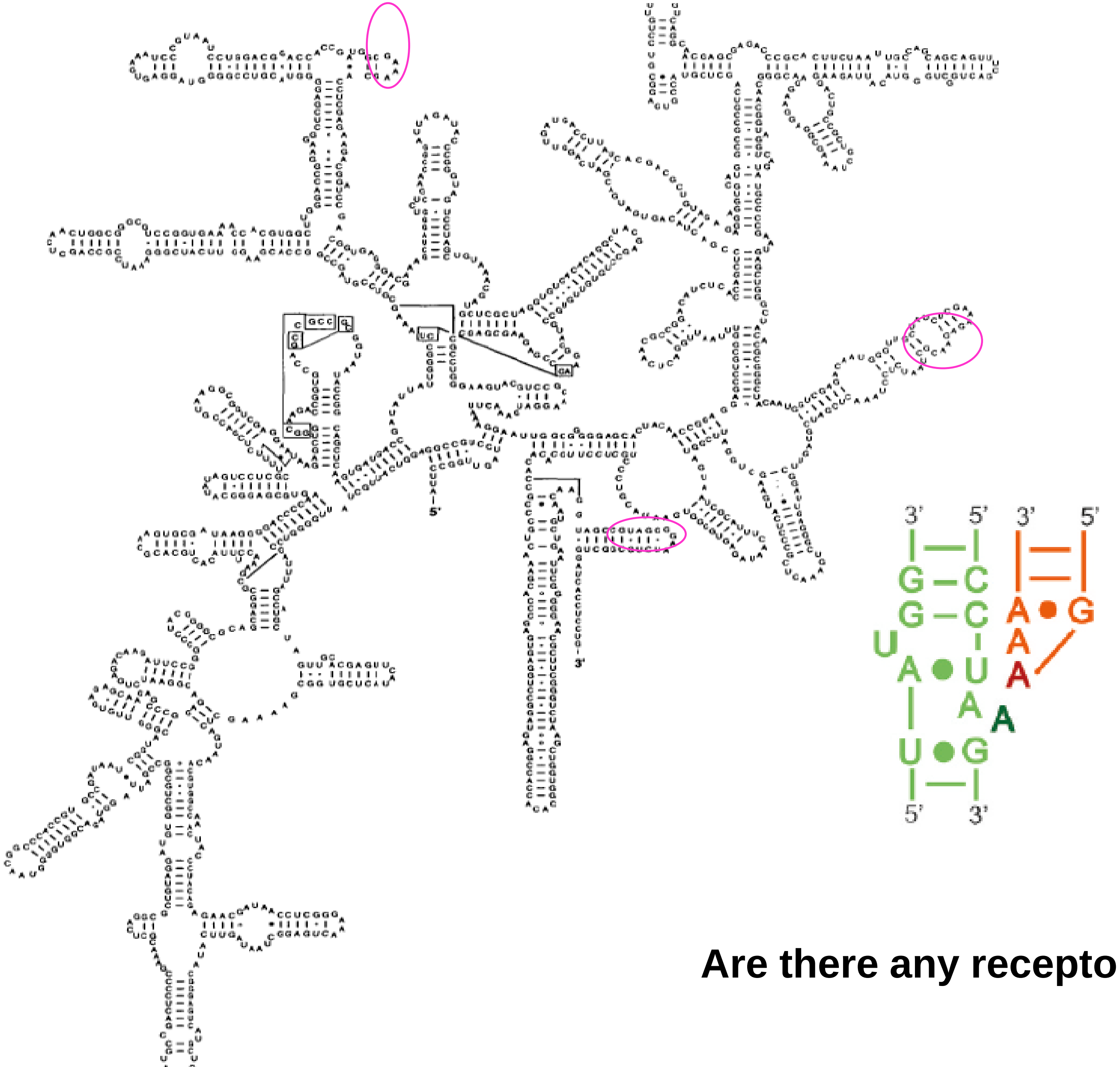
Fiore et al., The Role of counterion valence and size in GAAA tetraloop-receptor docking/undocking kinetics JMB 423 2023 1980216

Same experiment, different linker.
What's the difference?



$0\ Mg^{2+}$, 100 mM KCl

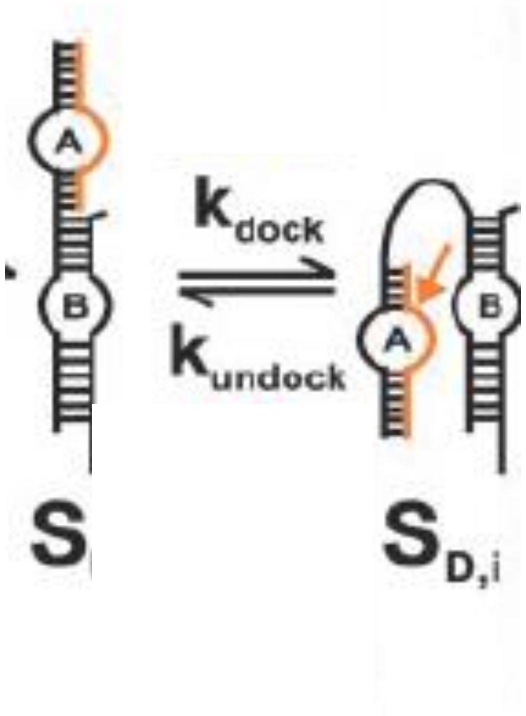
$1\ mM\ Mg^{2+}$,
100 mM KCl

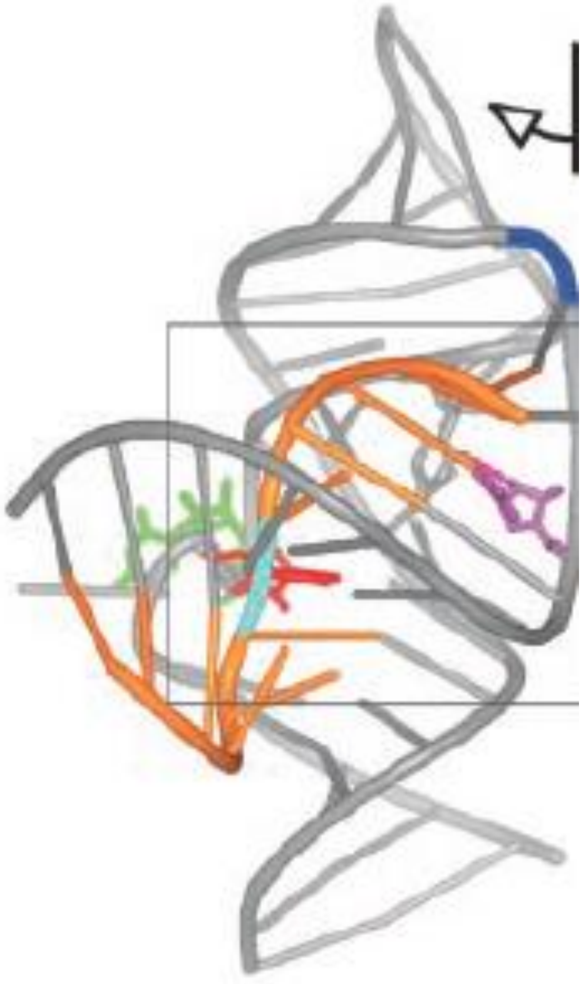


Are there any receptors?

2. Internal Loop:loop interactions

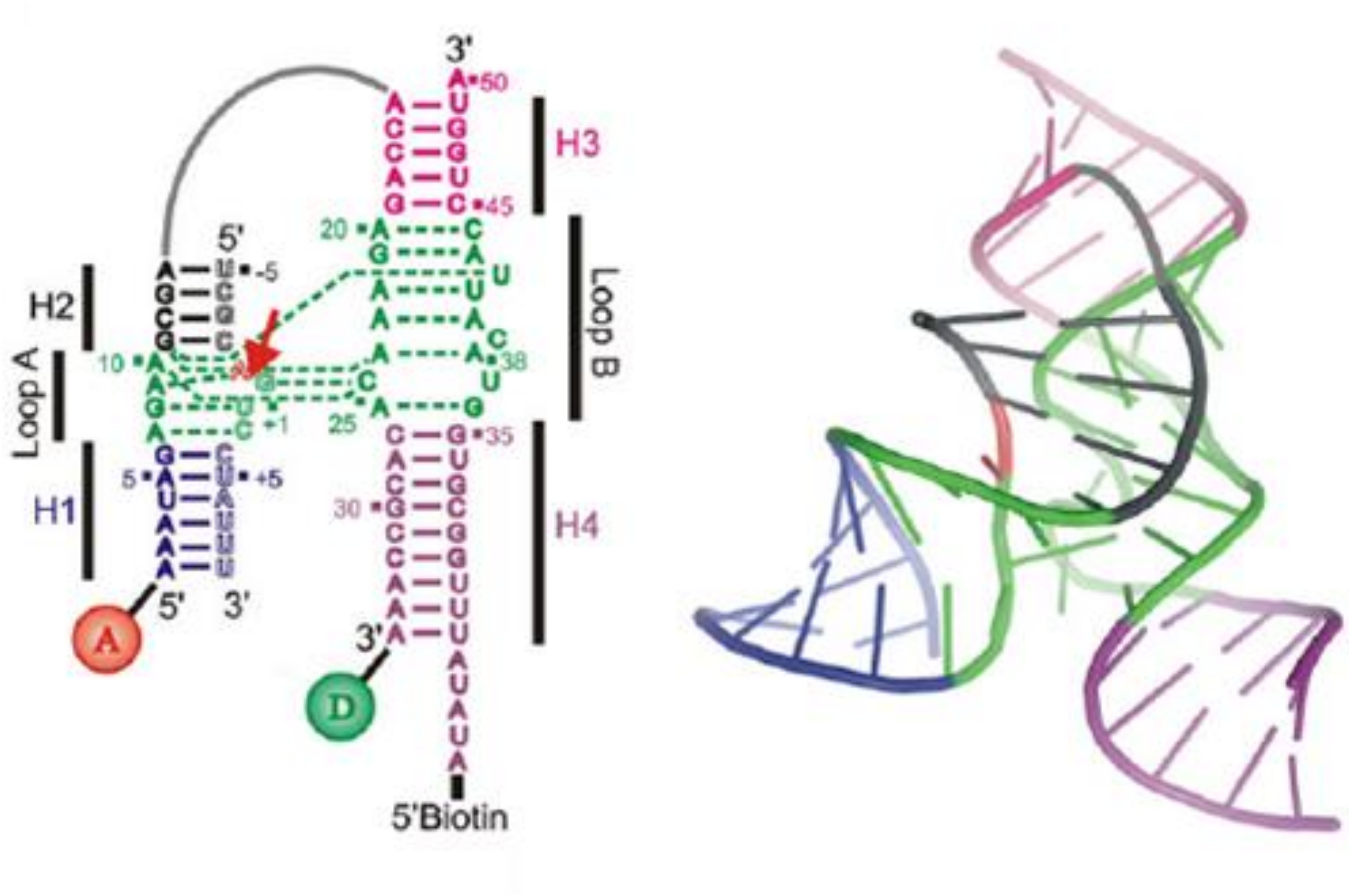
Interactions are exquisitely sensitive to hydrogen bonding





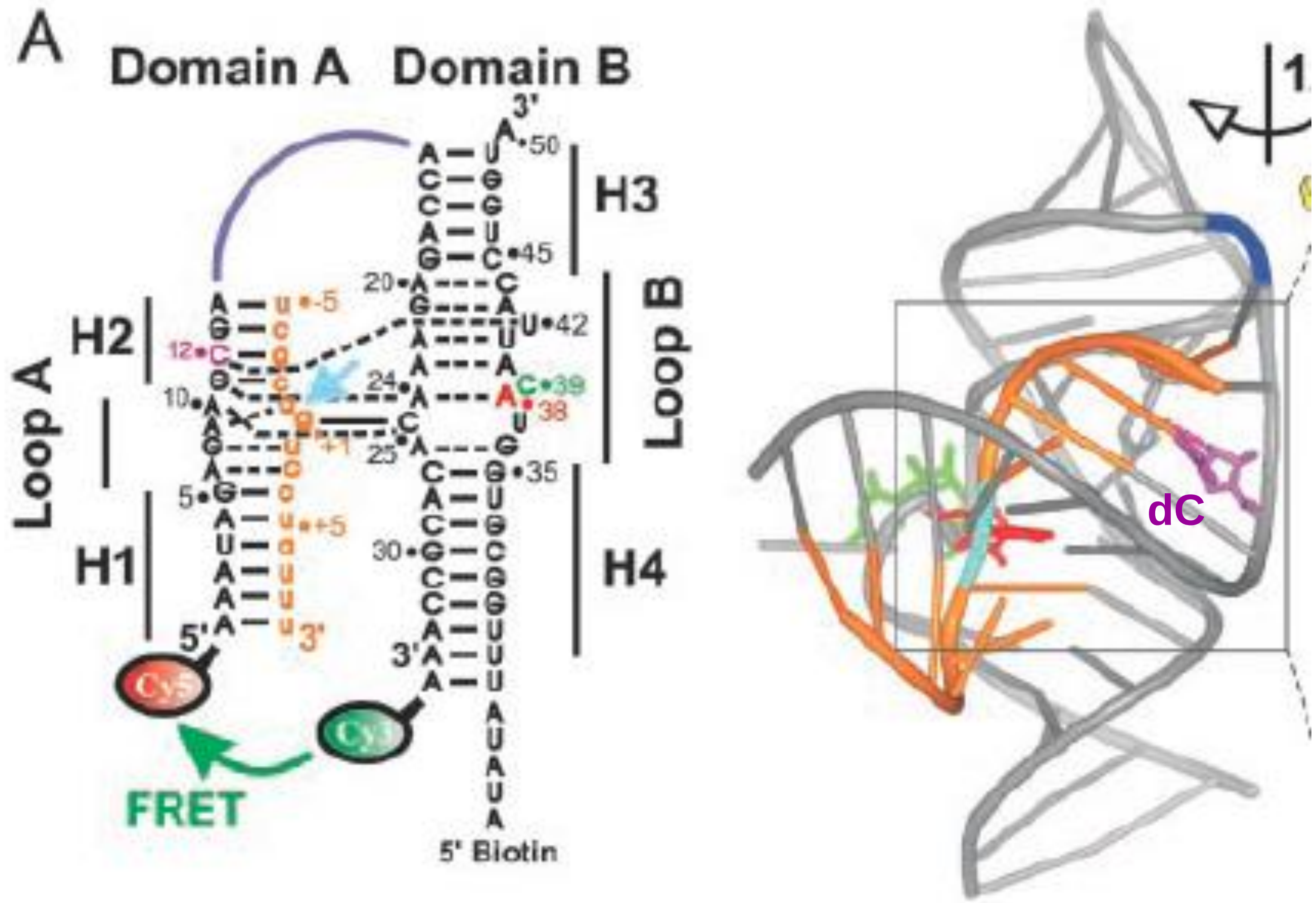
Rueda et al., 2004. PNAS 101:10066-10071

Measure the Dynamics of Docking when there is a conformational change

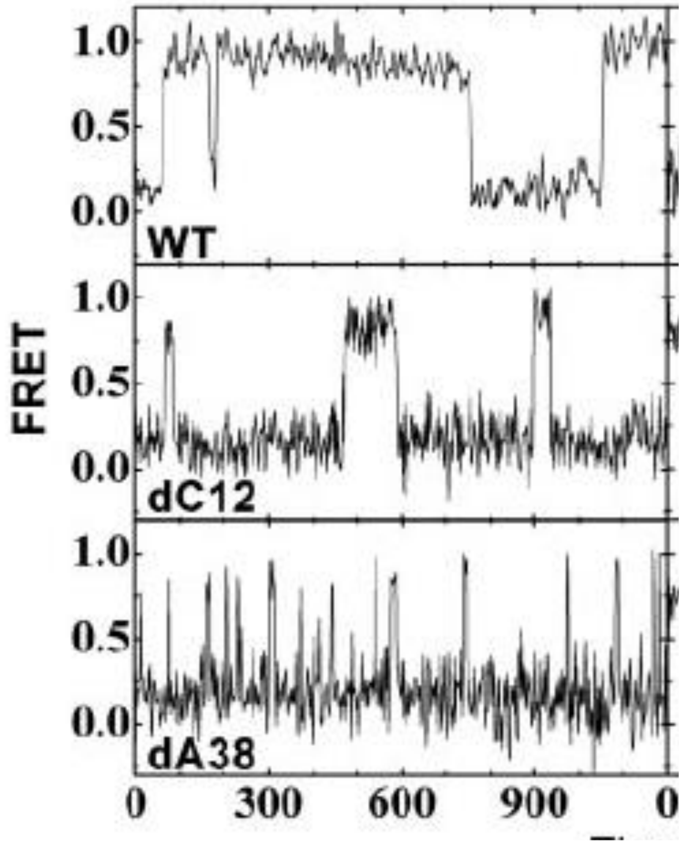


Engineer a three-piece RNA. Tether one strand to glass via biotin/streptavidin.

On one end of the enzyme strand, add a donor and on the other end an acceptor FRET pair.

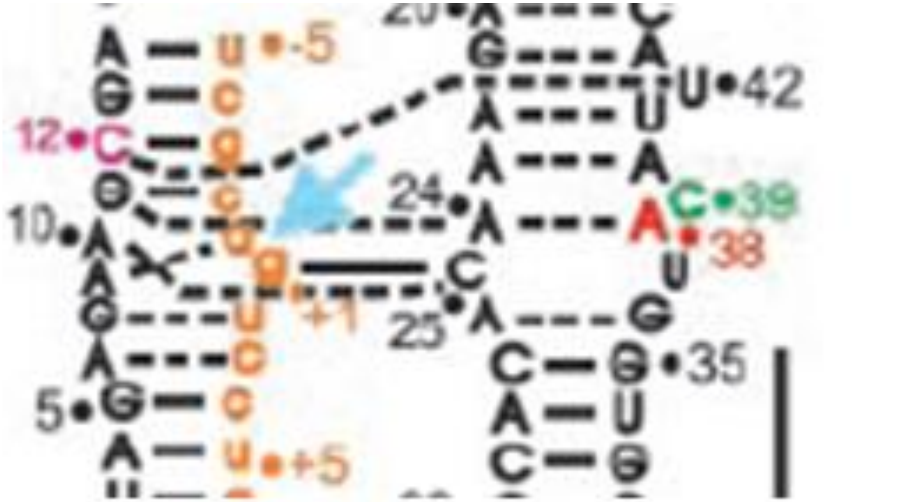


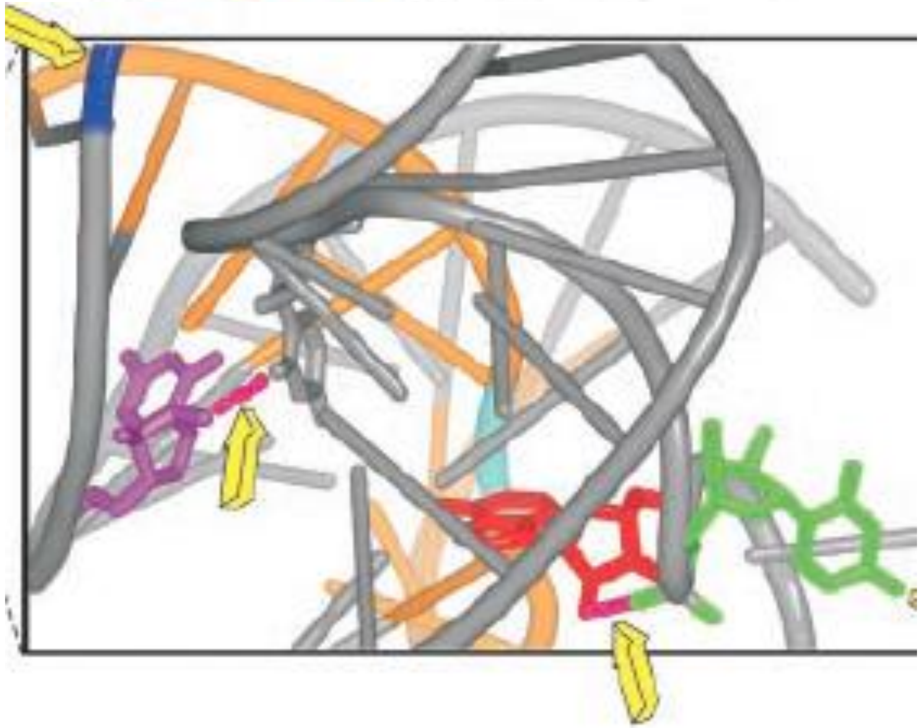
Variant	$k_{dock,obs}$ (s^{-1})
WT	0.014 ± 0.002
dC12	0.033 ± 0.012
dA38	n.d.



Time (sec)

dA38 accelerates undocking, since there is a loss of a hydrogen bond. dC12 destabilizes the docked state





Tertiary interactions fold RNA molecules.

COMMON THEMES

- Role of ions in structure and stability
- Structural dynamics, hydrogen bonding, and water
- Common motifs vs idiosyncratic interactions
- Predictions of tertiary structure