Single Molecule Studies of the Initiation of DNA Mismatch Repair

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Today's talk: DNA mismatch repair

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Ruoyi Qiu

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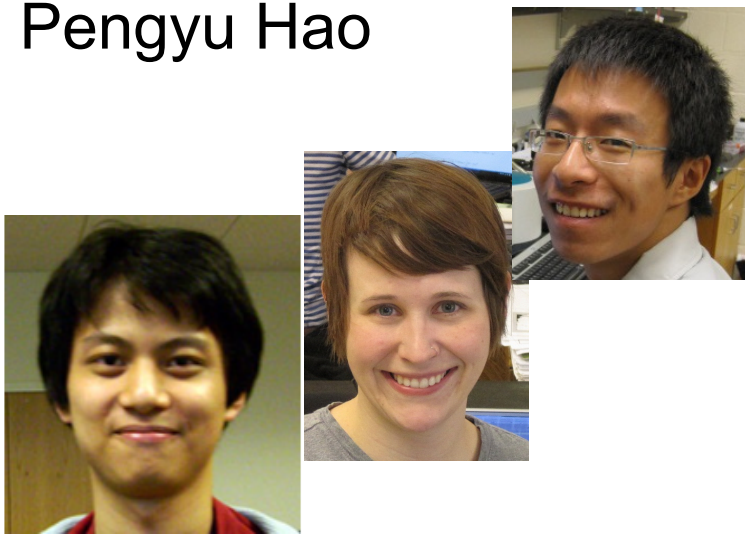
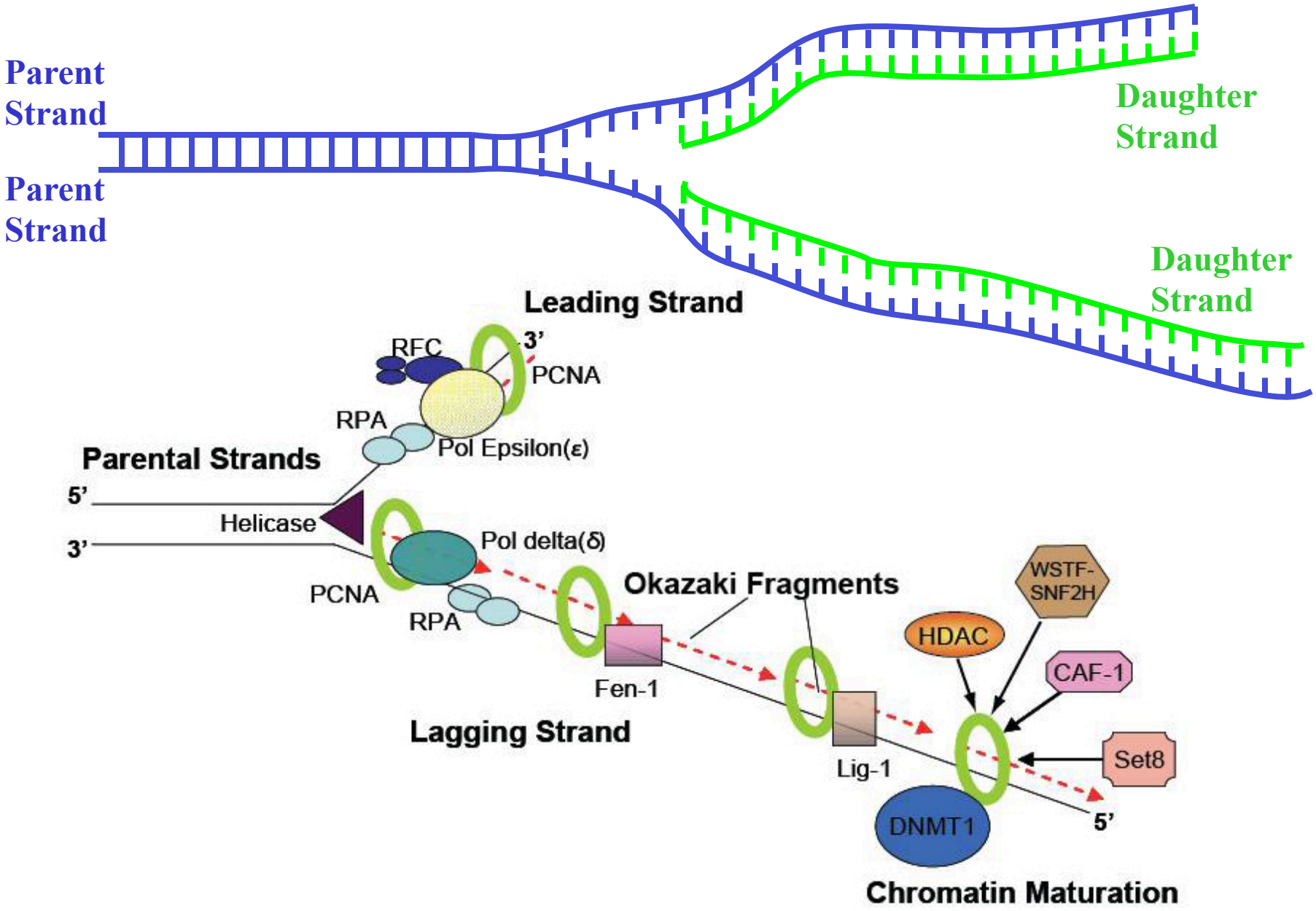
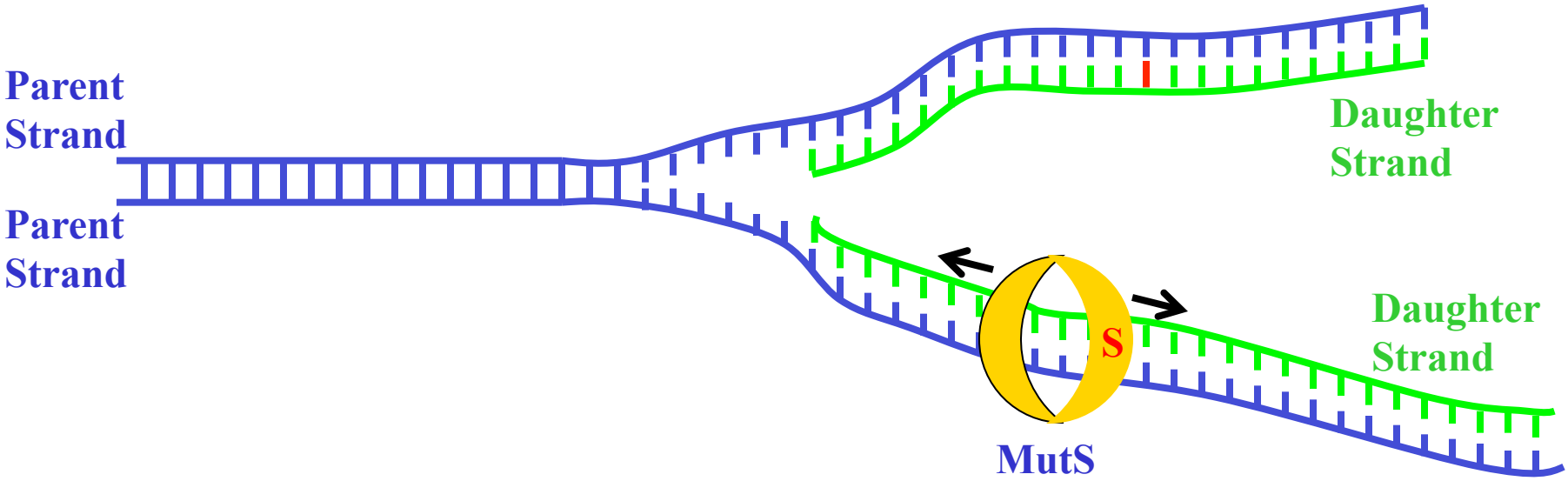
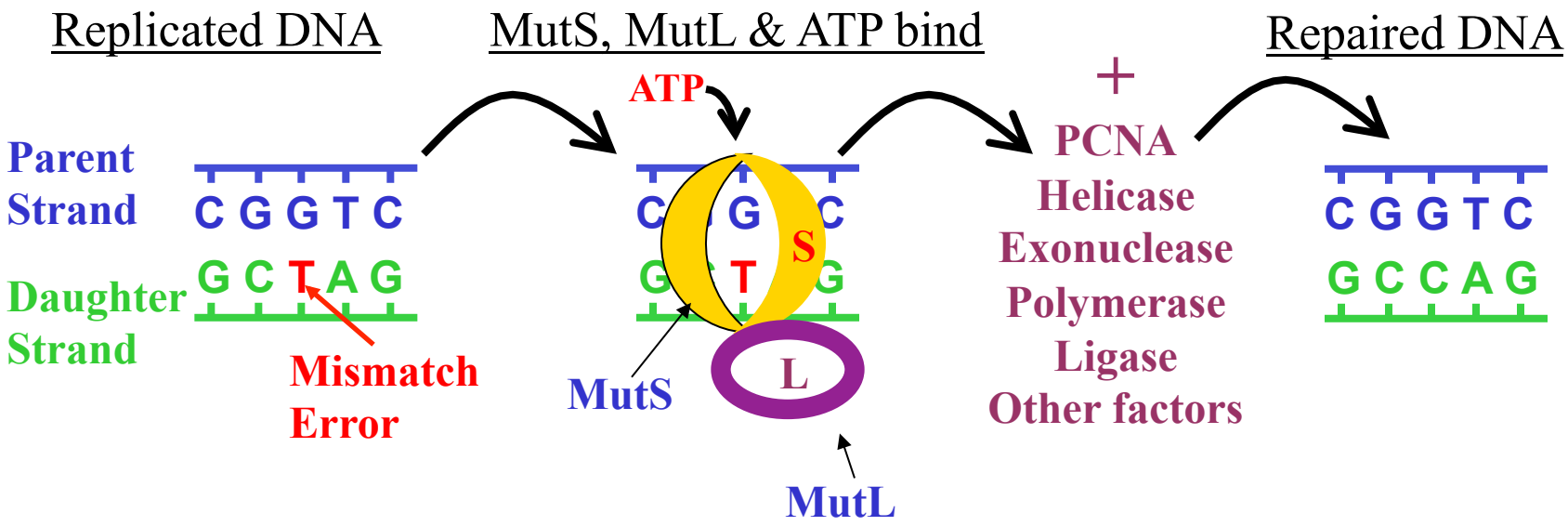


DNA mismatch repair following DNA replication



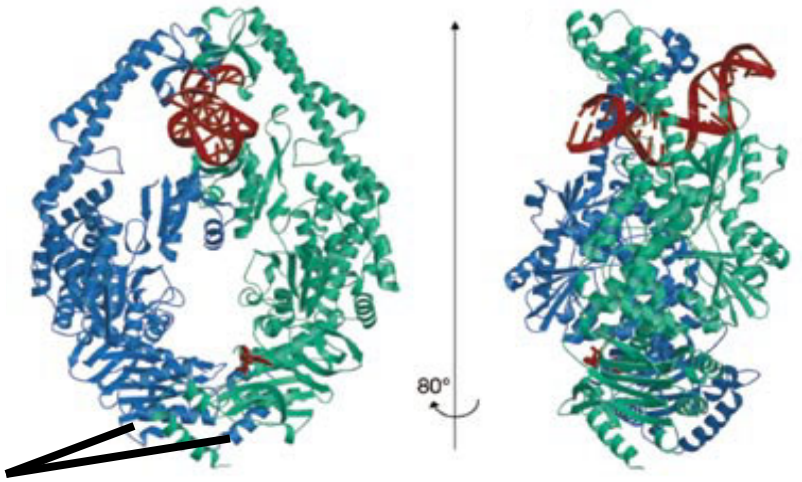
DNA mismatch repair following DNA replication





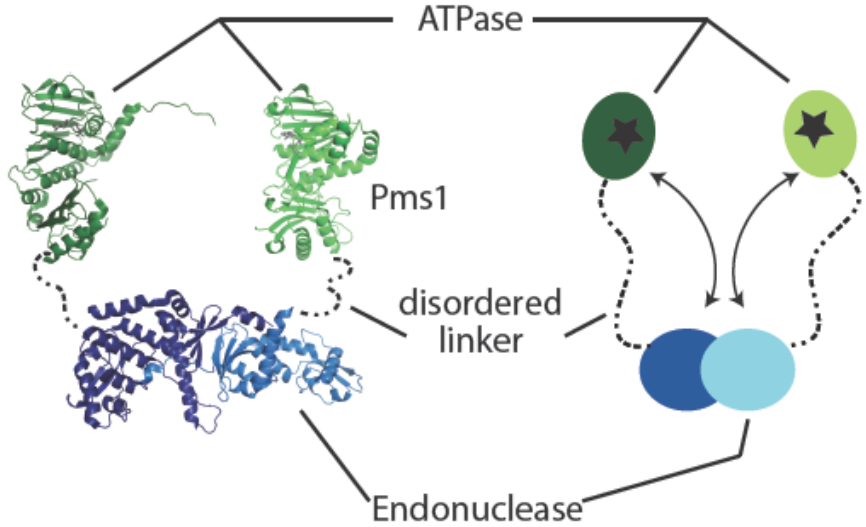
MutS and MutL X-ray Crystallography

E coli:GT



ATPase

Lamers *Nature*, **407**, 711 (2000)

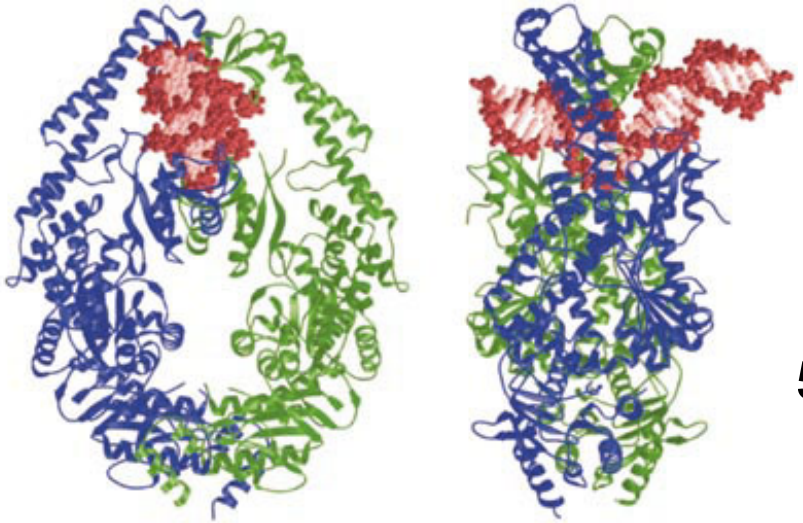


Pillon *Mol Cell* **39**, 145(2010)

MutS

MutL

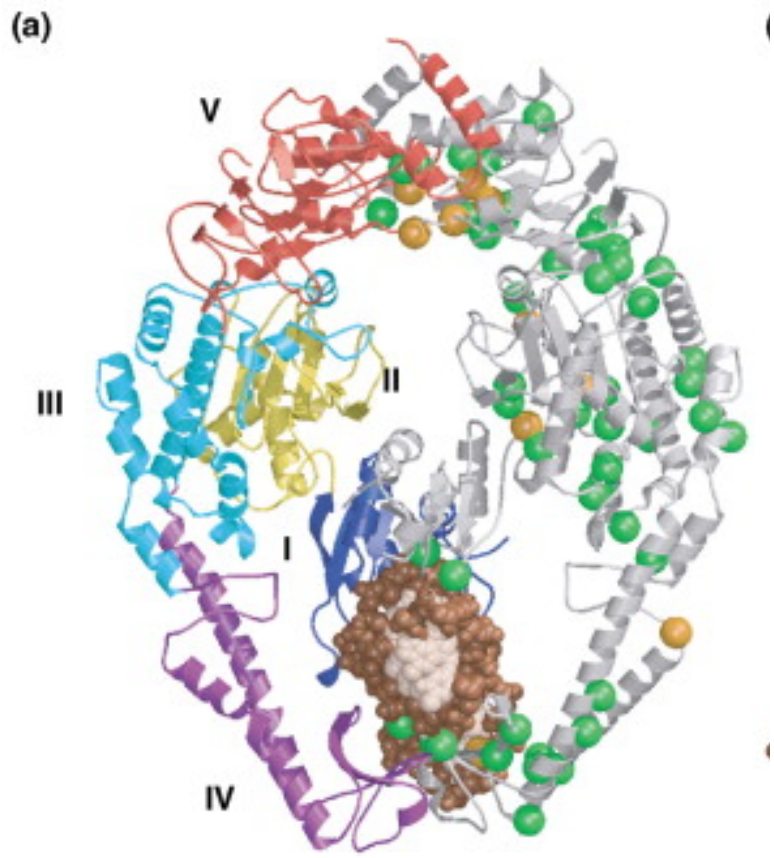
Taq:T-bulge



Obmolova *Nature*, **407**, 703 (2000)

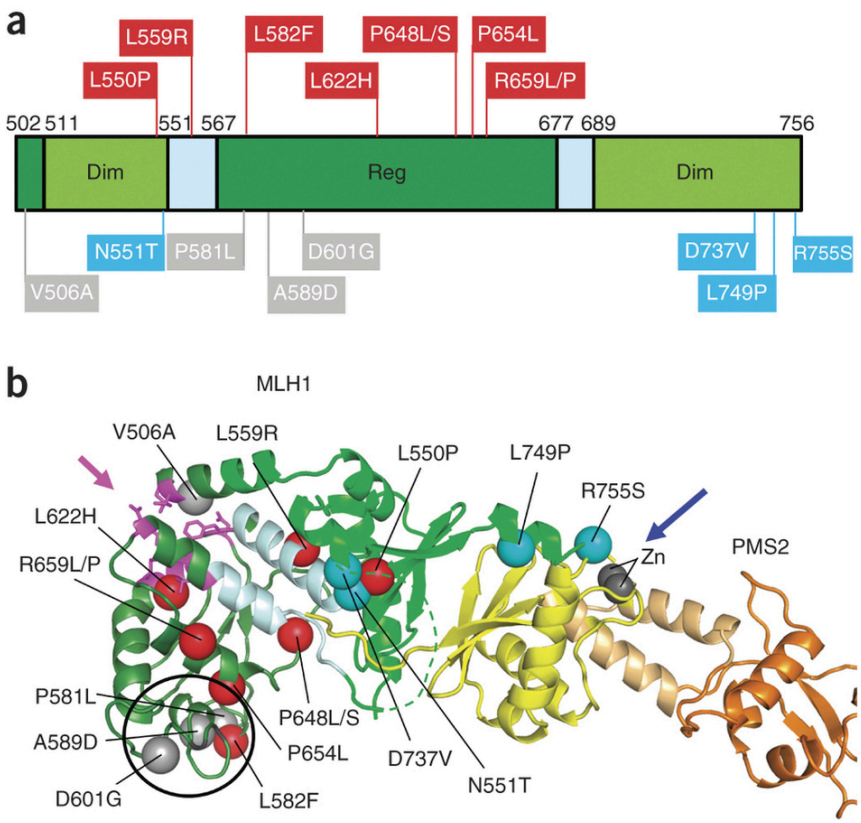
All crystal structures of different MutS homologues bound to at least 5 types of errors or damage all have sharp DNA bends

Cancer-Associated Inherited Mutations of MutS & MutL



DNA Mismatch Repair : The Hands of a Genome Guardian

Structure, Volume 8, Issue 12, 2000, R237 - R241

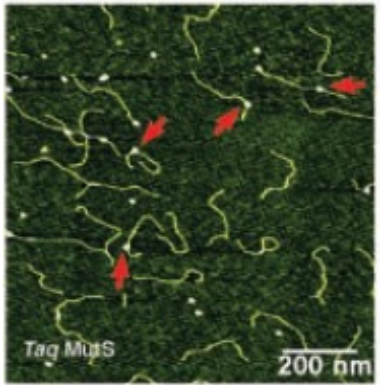


Structure of the MutLα C-terminal domain reveals how Mlh1 contributes to Pms1 endonuclease site

NSMB, **20** 161 (2013)

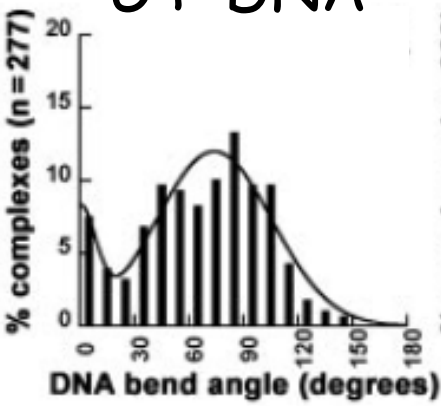
AFM Imaging of MutS

Wang, et al., PNAS, 100, 14822 (2003)

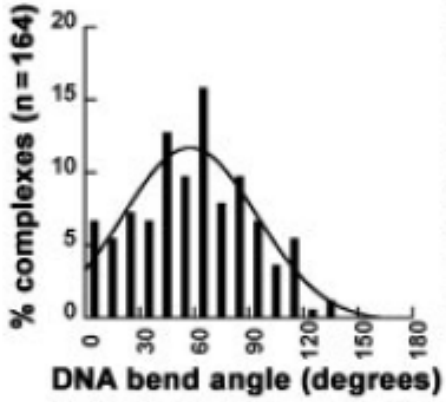


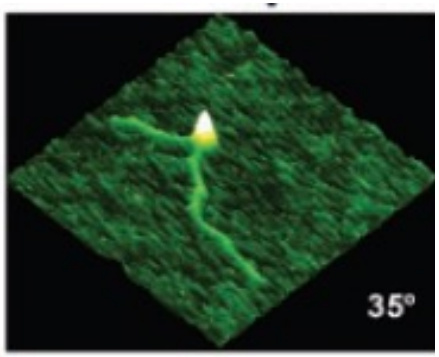
Mismatch DNA

E. Coli GT-DNA

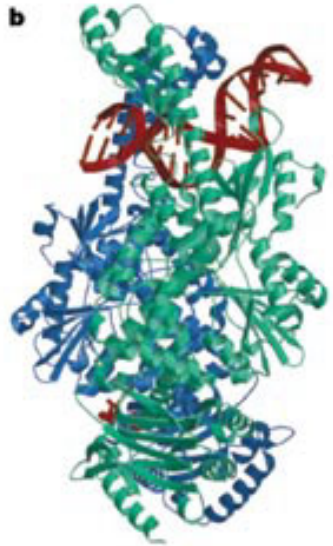


Homoduplex DNA





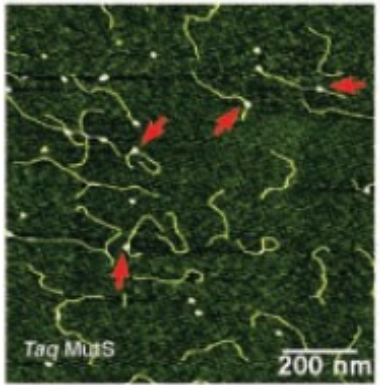
X-ray crystallography sees bent DNA



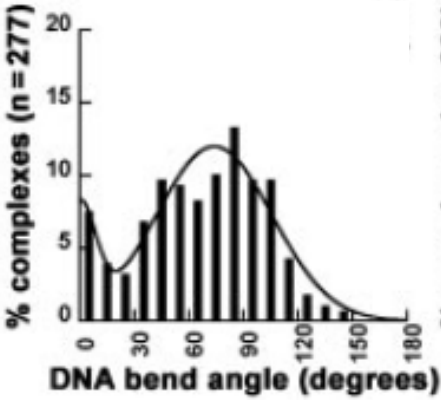
Nature, **407**, 711 (2000)

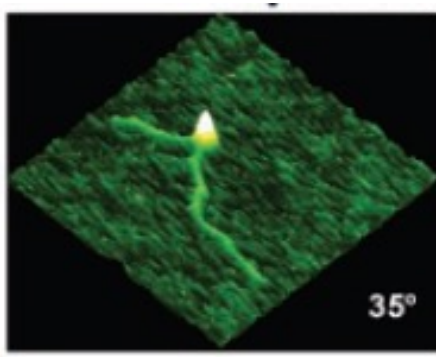
AFM Imaging of MutS

Wang, et al., PNAS, 100, 14822 (2003)



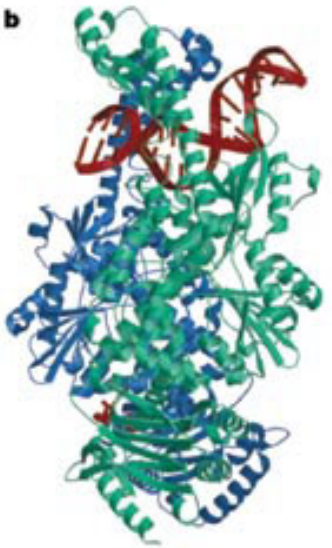
Mismatch DNA





Use Single molecule FRET for:

- **DNA bending dynamics**
- **Conformational changes within MutS**
- **MutS sliding on DNA**



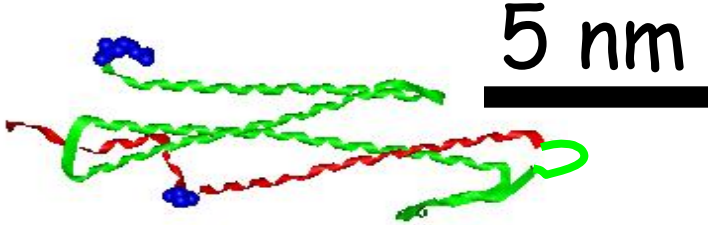
Nature, **407**, 711 (2000)

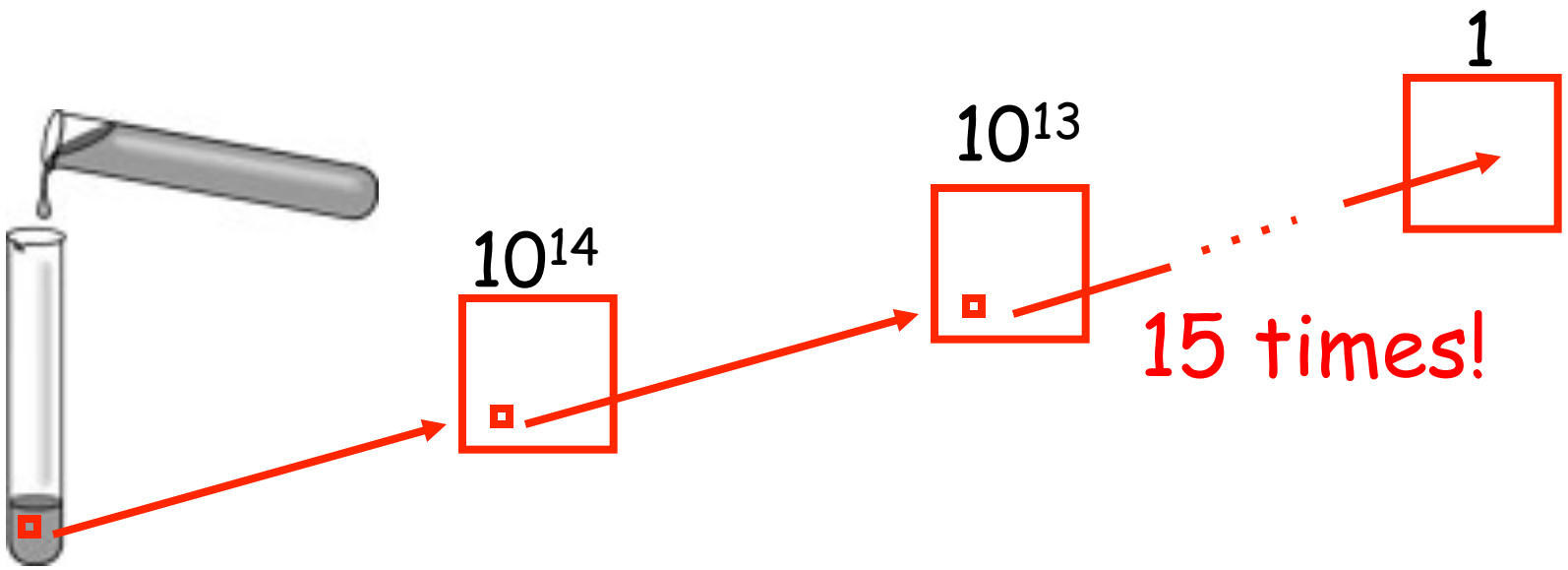
Single molecules

Most samples have lots of molecules

- 1 ml, 1 mM samples have ~10^{15} molecules
- 10 ng plasmid DNA is ~ 10^{11} molecules
- 1 pancreas cell has ~ 10^{6}-10^{7} ribosomes

Single molecules





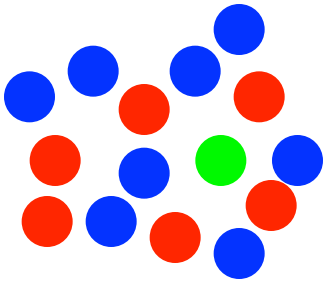
Watching one molecule allows you to see things that are hidden when averaging over 10^{15} molecules

Single molecules - No Averaging

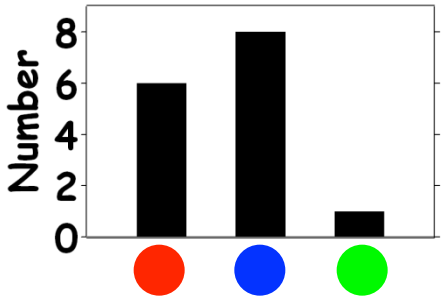
- **Heterogeneous population**

Bulk averaged approach

"Blue + Red = Magenta"



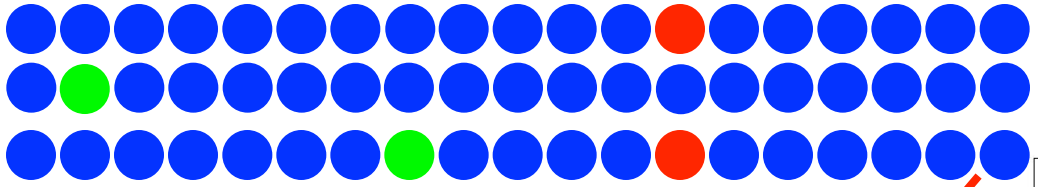
Single molecule approach



- **Rare Transient Events-fluctuations**

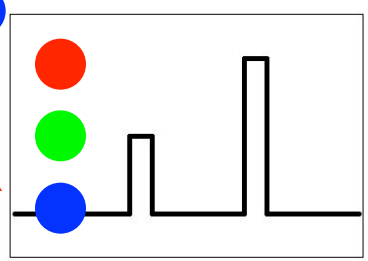
Bulk averaged approach

"It's blue!"



Time

Single molecule approach

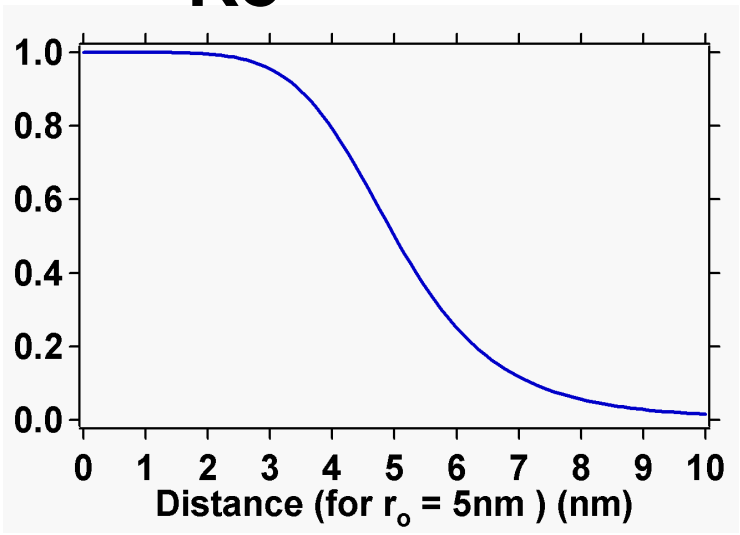


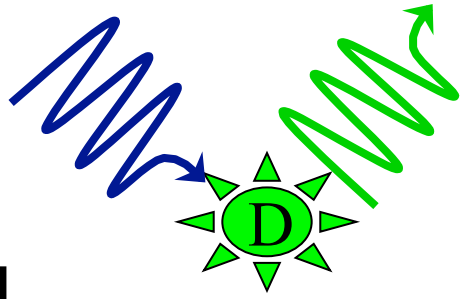
Time

FRET - My Key Tool

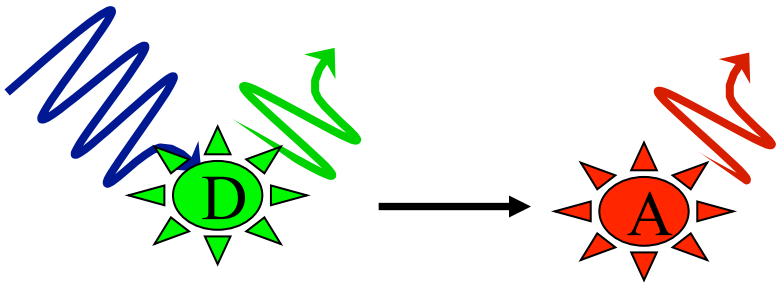
Fluorescence Resonance Energy Transfer (FRET)

$$E = \frac{1}{1 + \left(\frac{d}{Ro}\right)^6} = \frac{Red}{Green + Red}$$

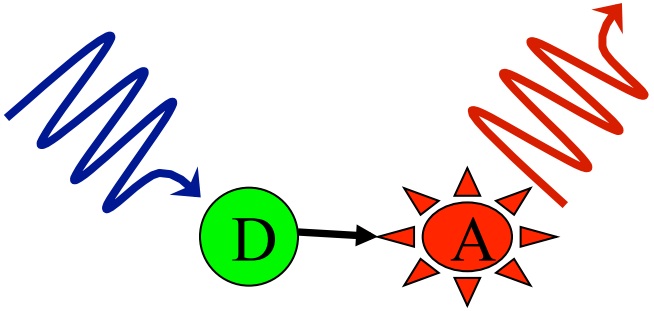




FAR APART (>8 nm) = NO FRET



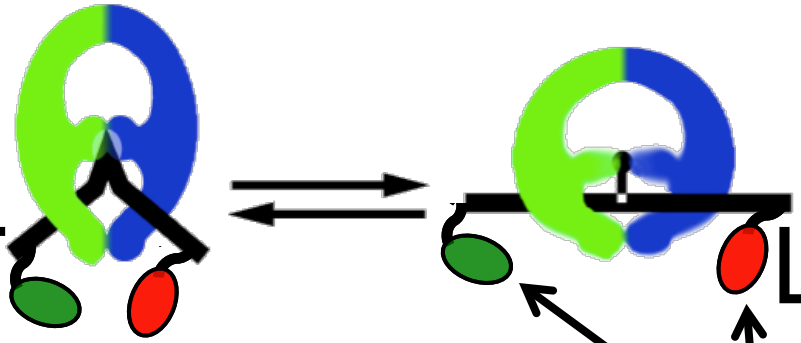
IN BETWEEN (~5 nm) = MEDIUM FRET



CLOSE (<2 nm) = HIGH FRET

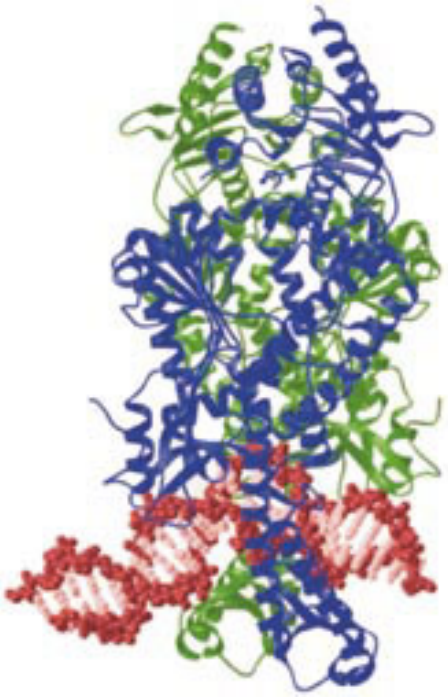
FRET to study mismatch repair

Bent
High FRET



Unbent
Low FRET

Specific dye labels sites for donor & acceptor spanning the DNA mismatch



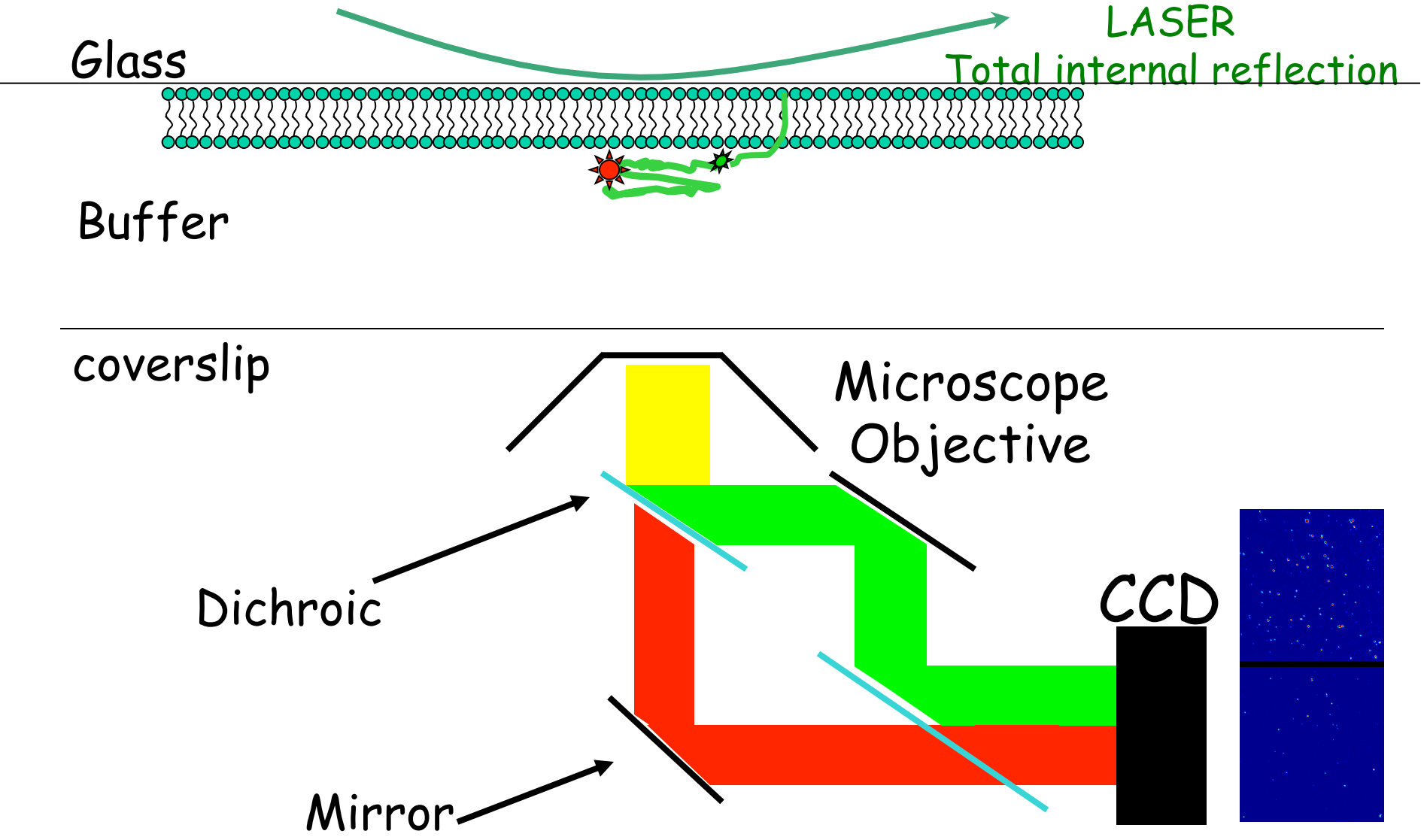
Obmolova *Nature*, **407**, 703 (2000)

We use T. aquaticus MutS

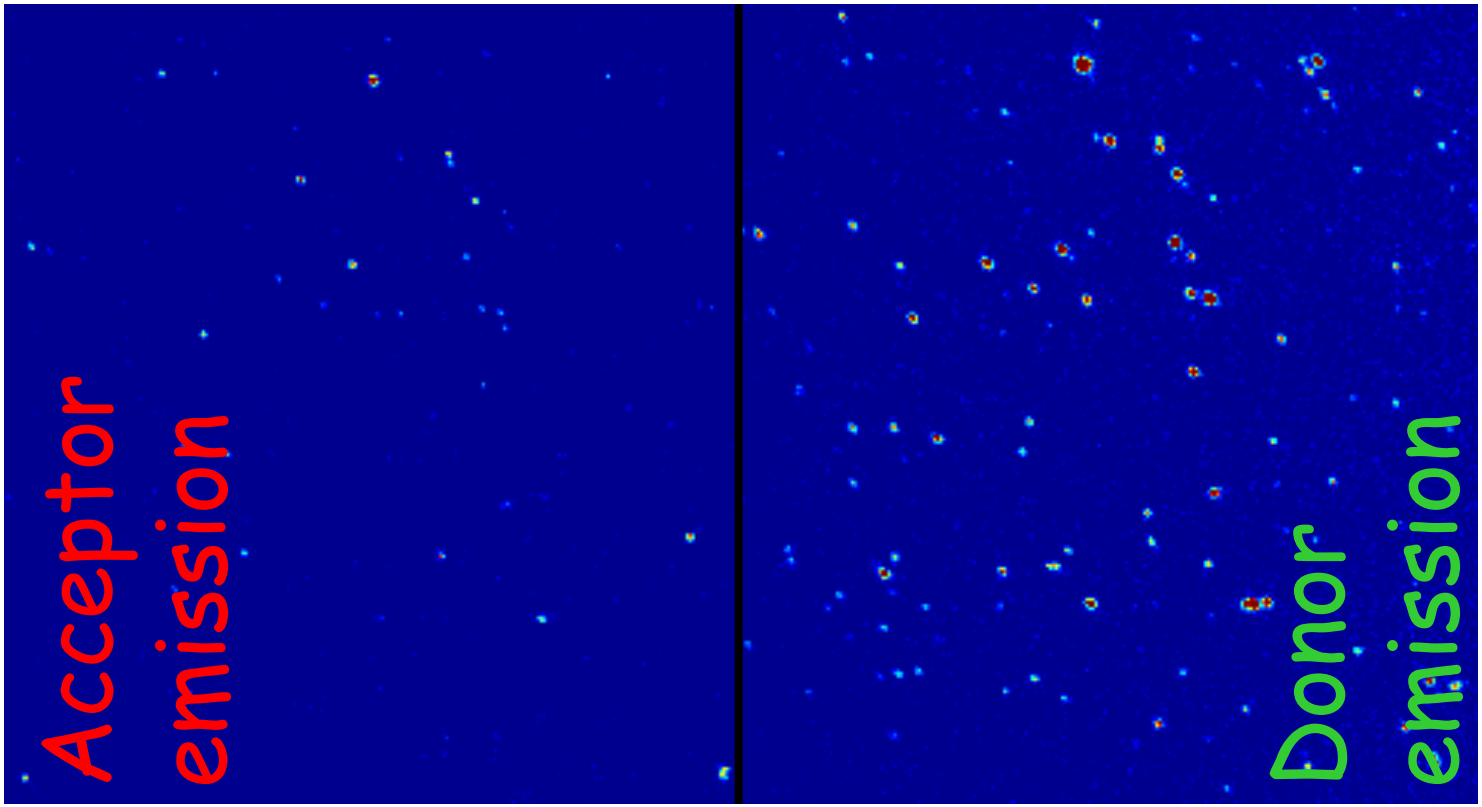
(MMR more like eukaryotic than E coli)

- Taq has no MutH gene
- Taq MutL endonuclease

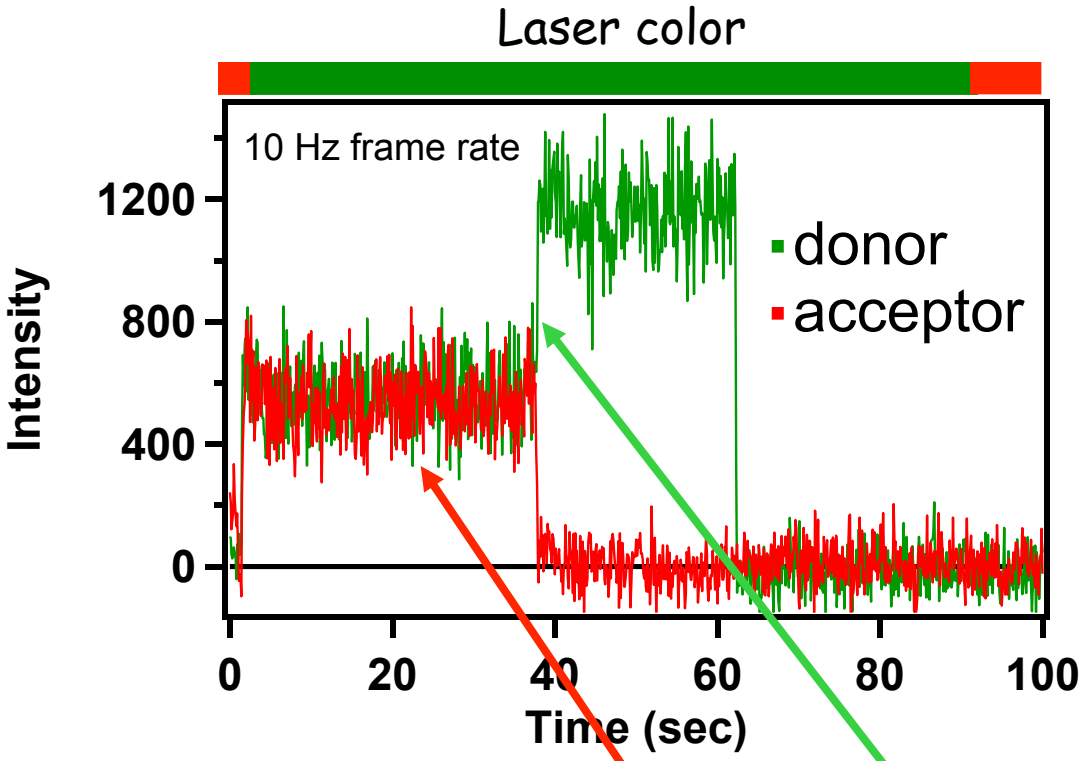
How to measure FRET from single molecules

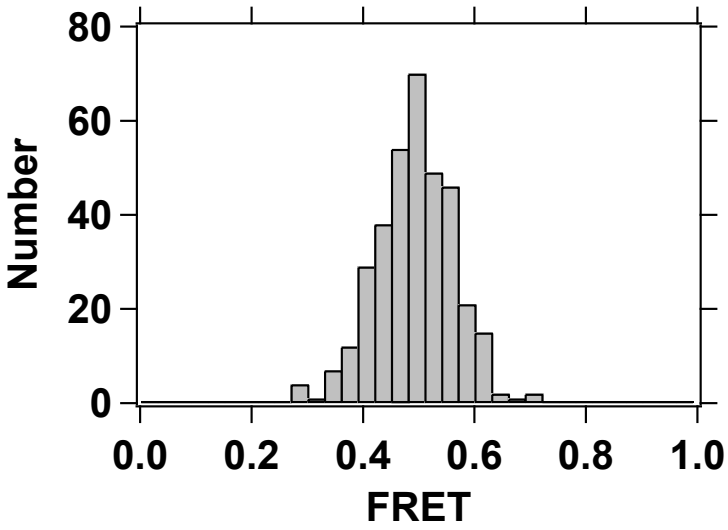


Raw Data Example

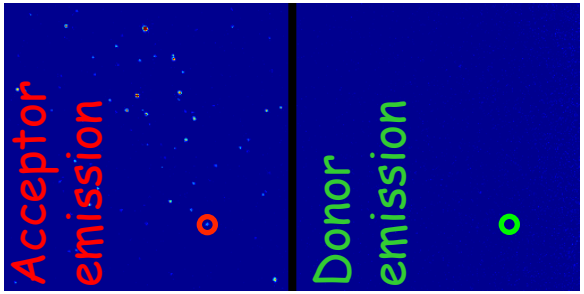


Raw FRET Data Example

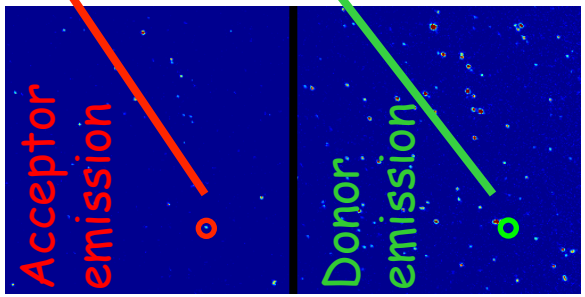




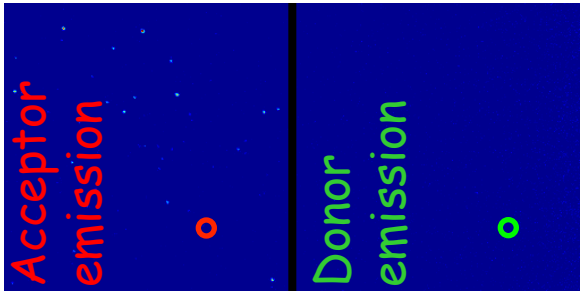
$$FRET = \frac{\mathbf{Red}}{\mathbf{Green} + \mathbf{Red}}$$



Red laser

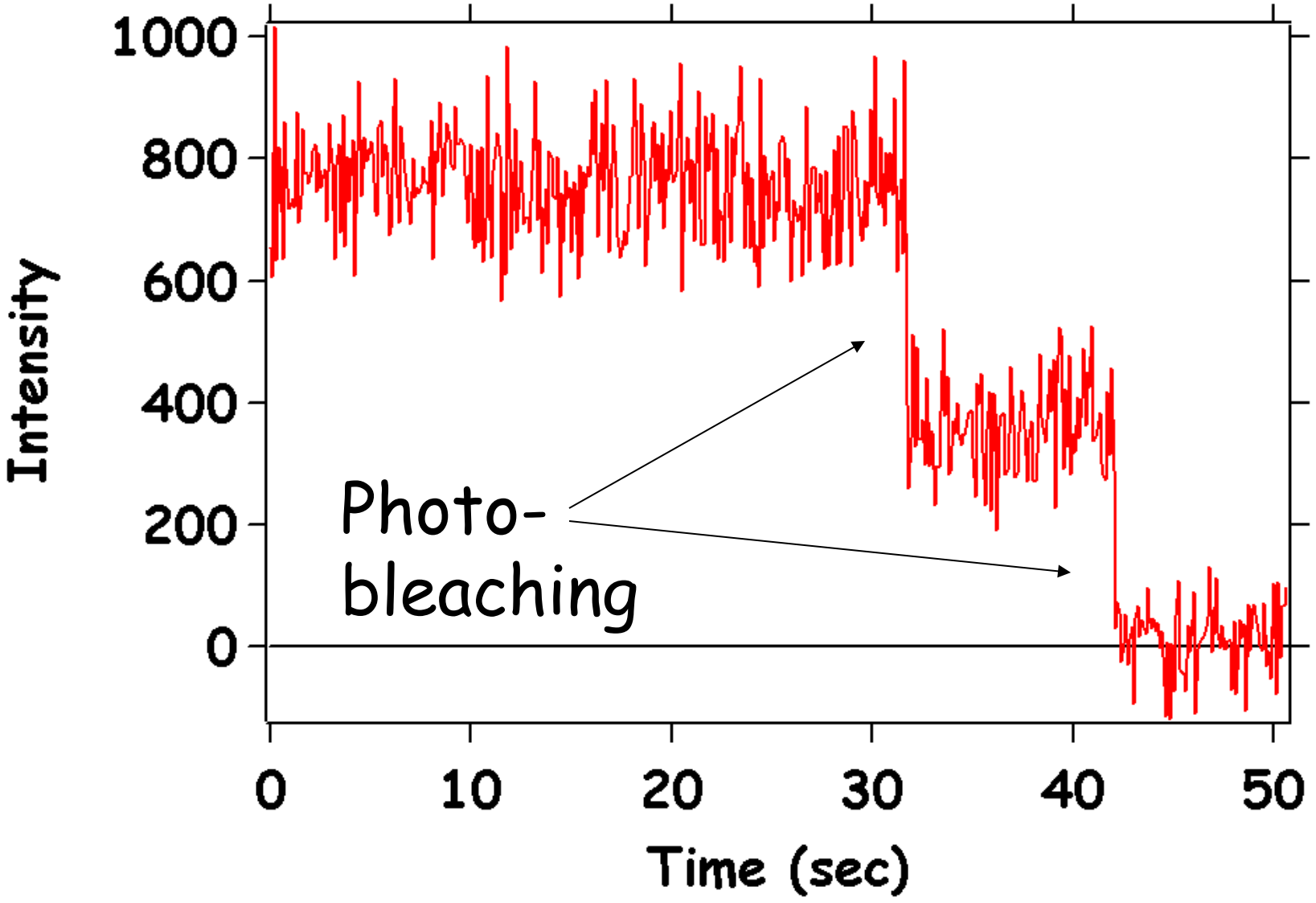


Green laser

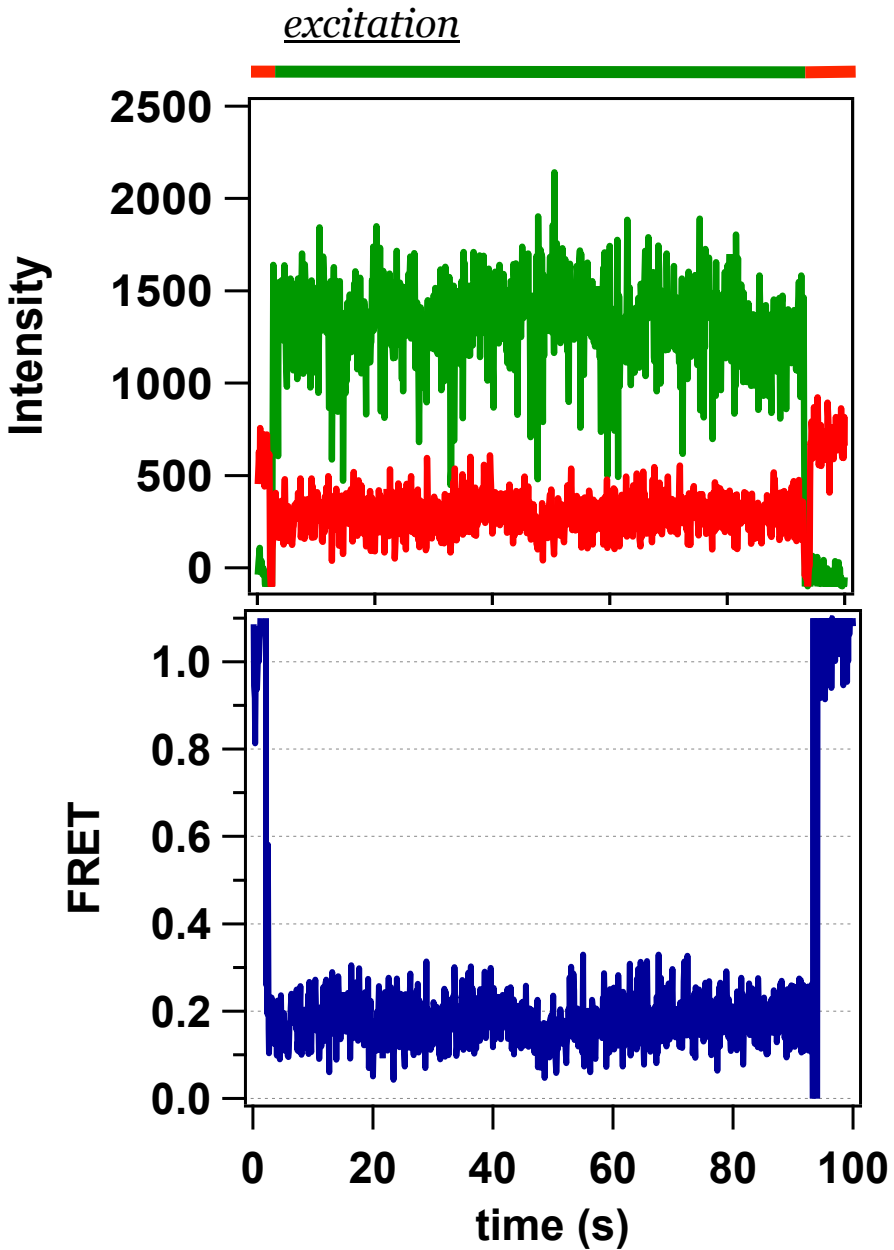


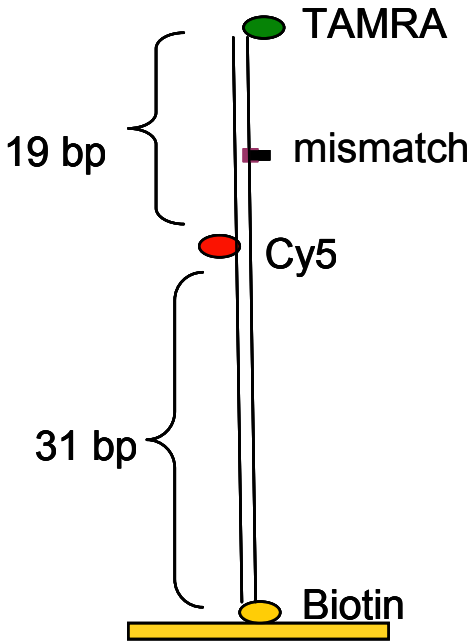
Red laser

2 Single Molecules: Donor only



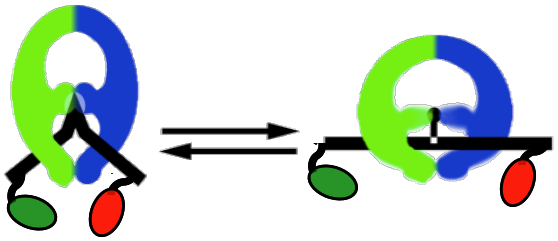
Free DNA before protein



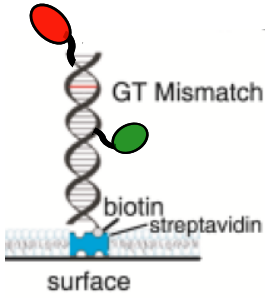


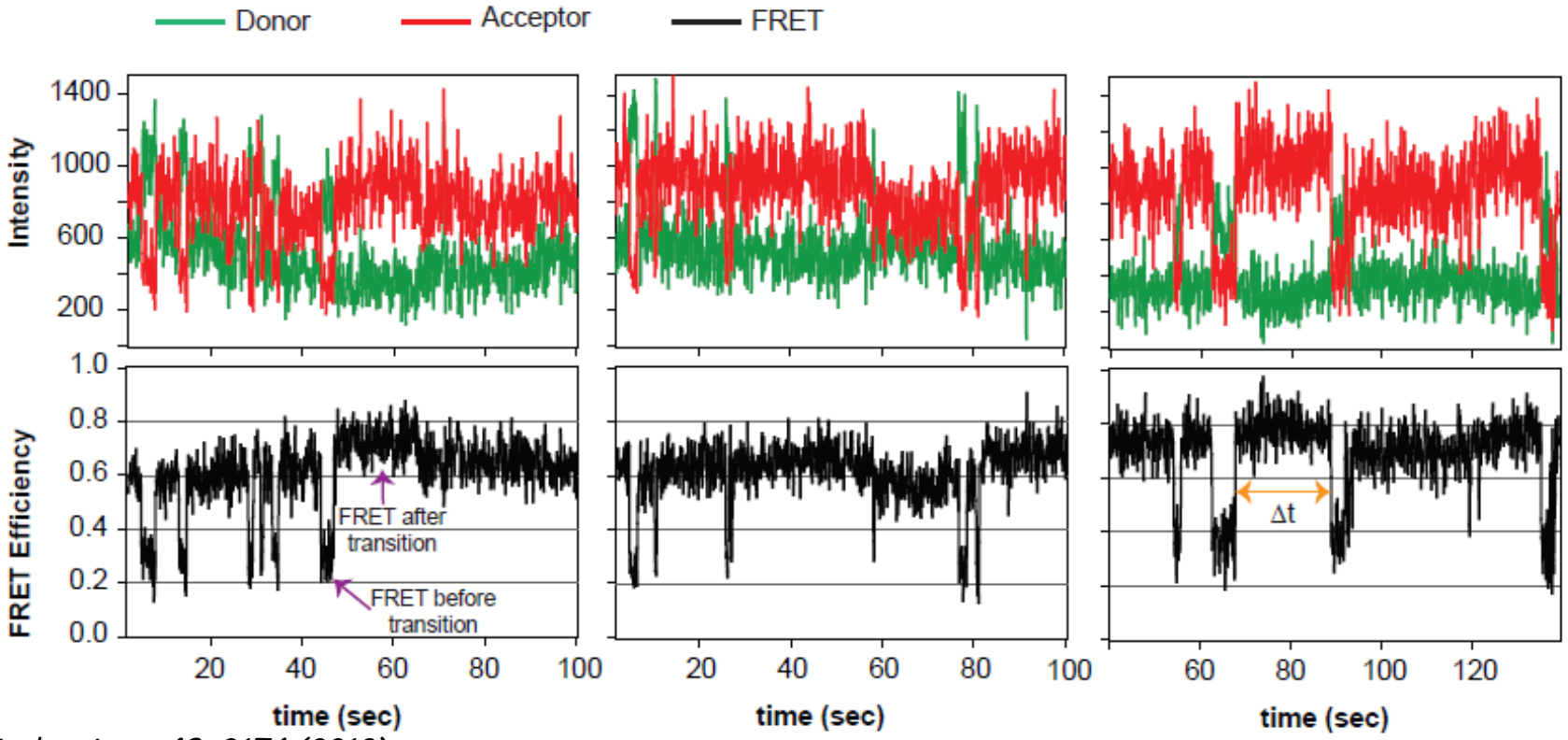
MutS induced DNA bending at a GT mismatch

Bent
High FRET



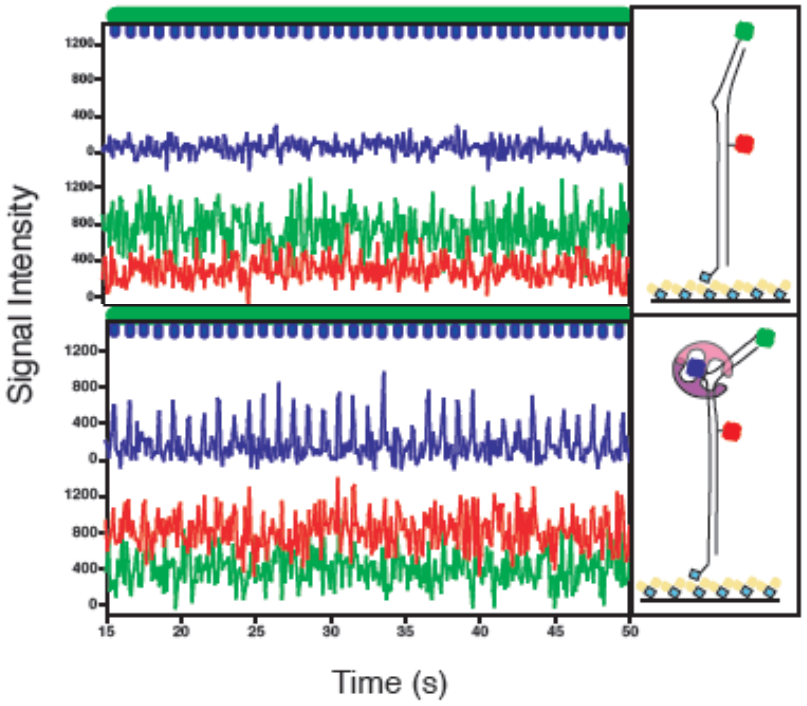
Unbent
Low FRET

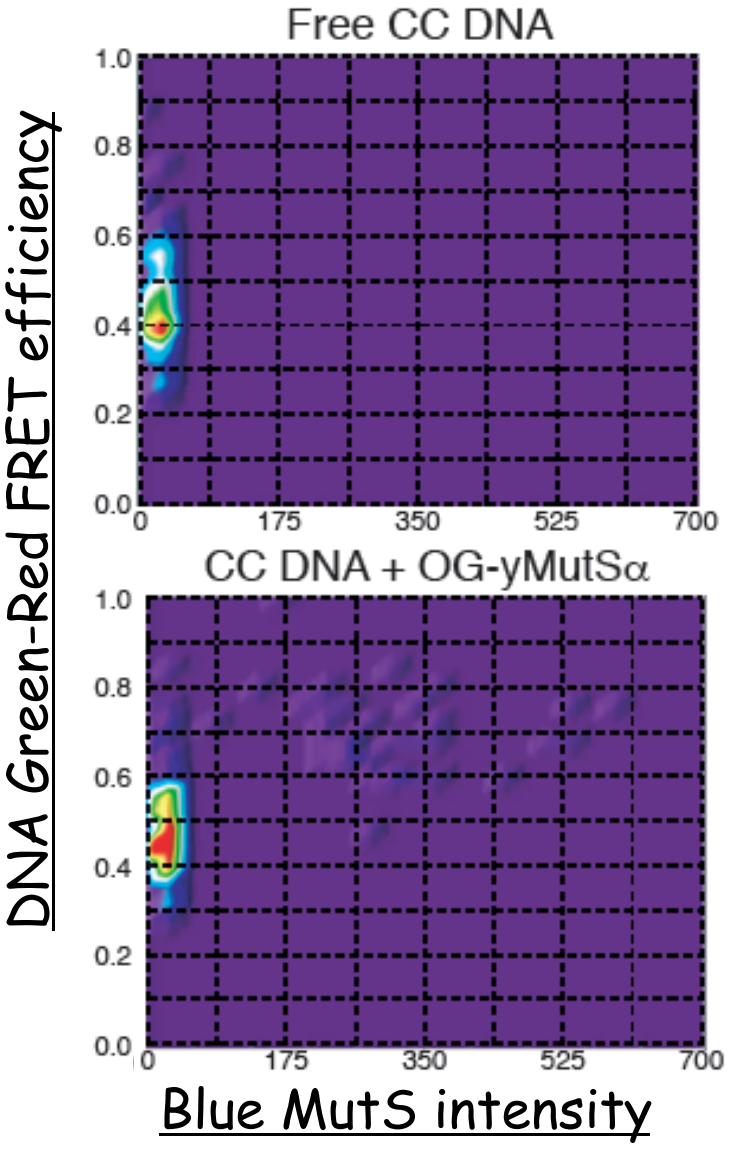




Sass *Biochemistry* ***49***, *3174 (2010)*

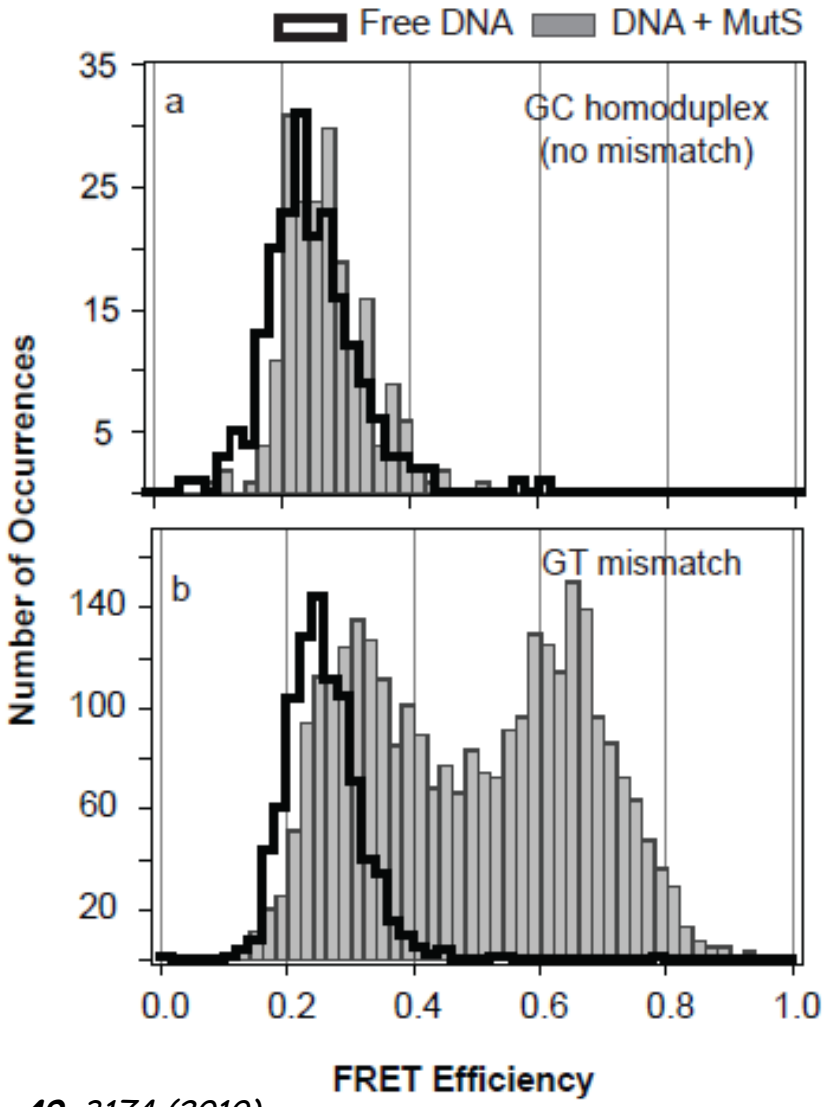
Dye-labeled MutS protein is bound to bent DNA





DeRocco BioTechniques, **49**, 807 (2010)

Population analysis of many molecules

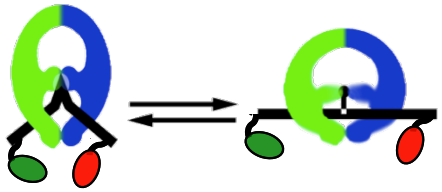


G-C (no mismatch)

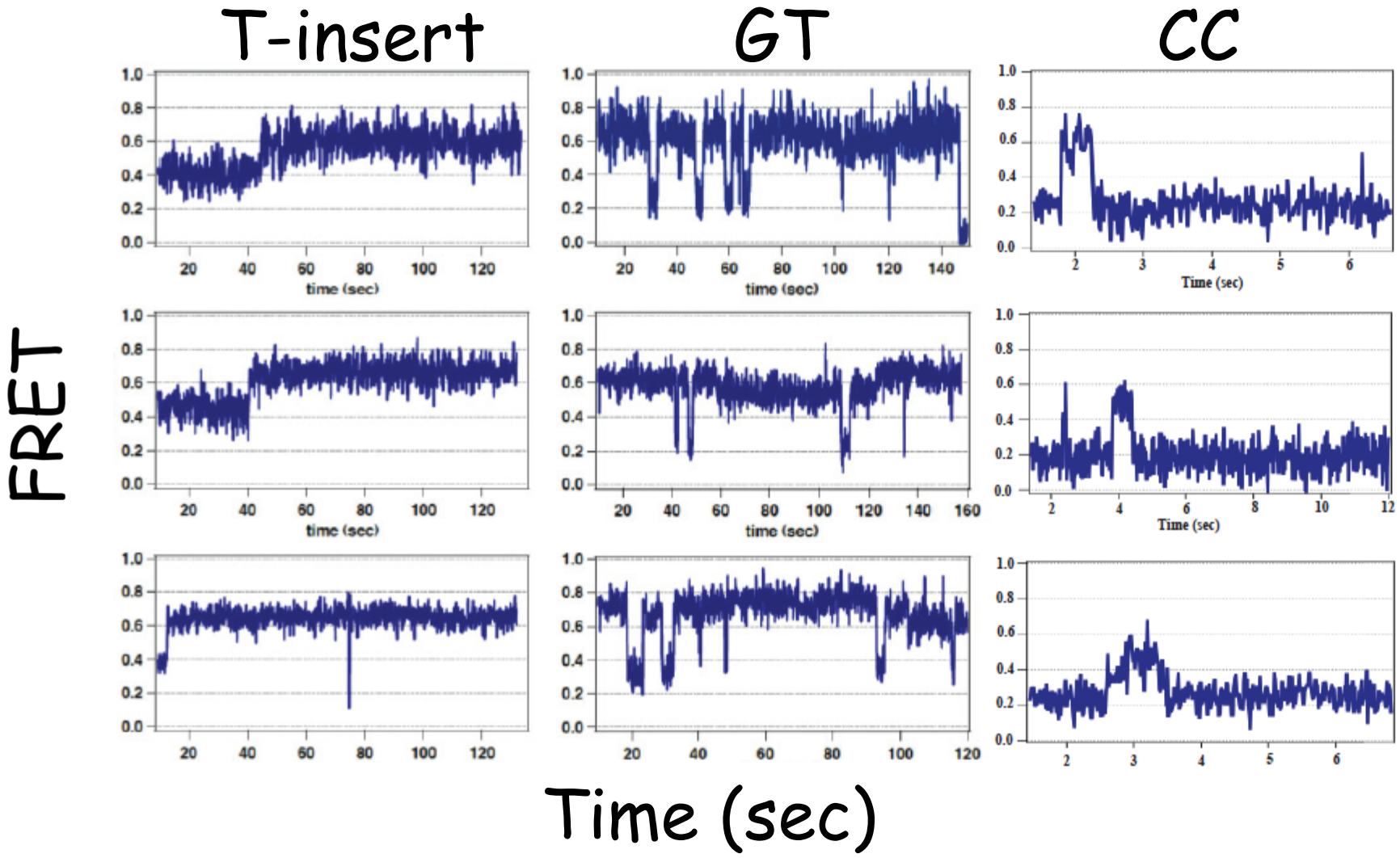
G-T mismatch

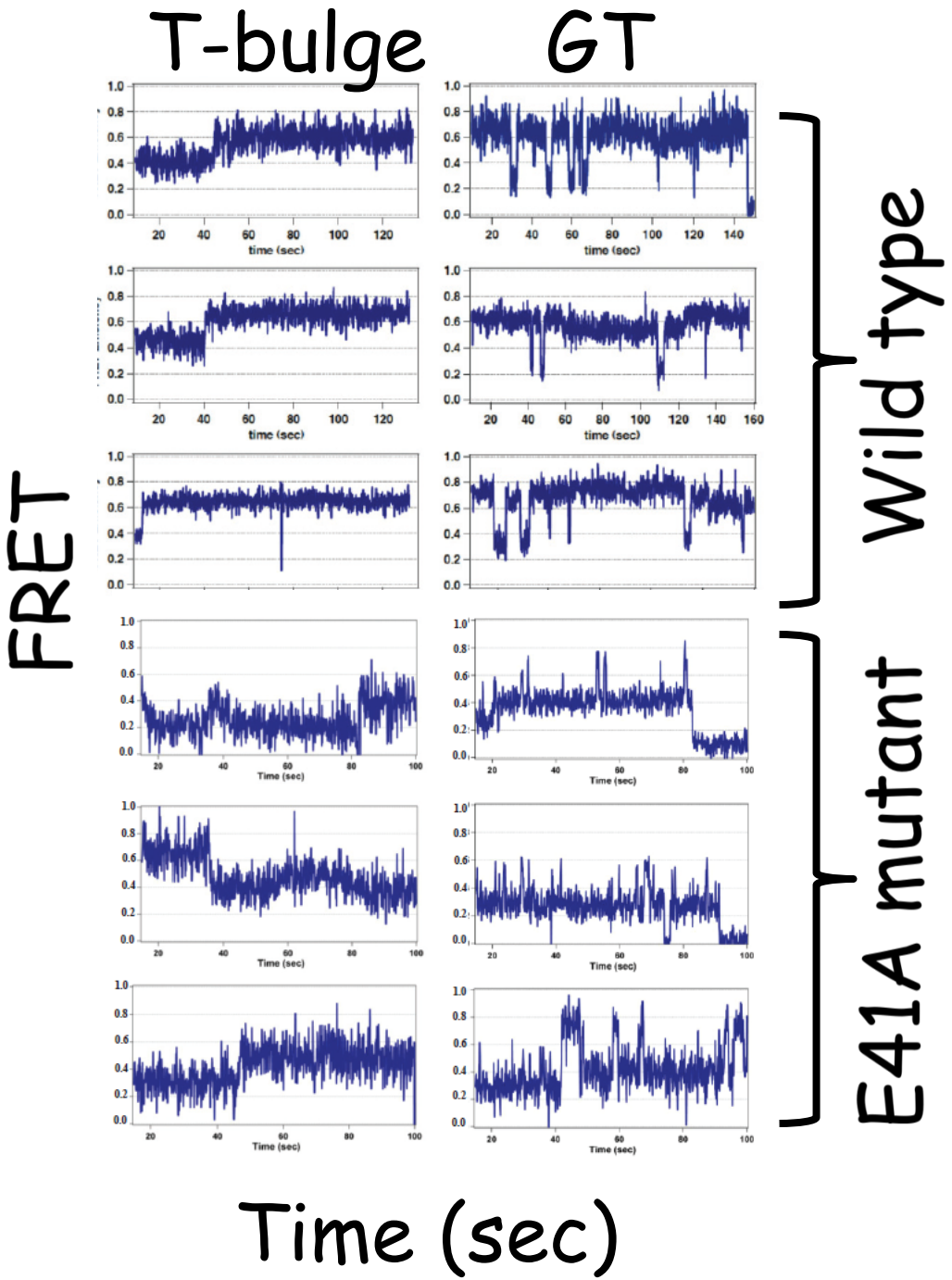
Sass *Biochemistry* ***49***, *3174 (2010)*

DNA bending for different mismatches



DeRocco et al.,
Biochemistry, 53, 2043 (2014)





DeRocco et al.,
Biochemistry, 53, 2043 (2014)

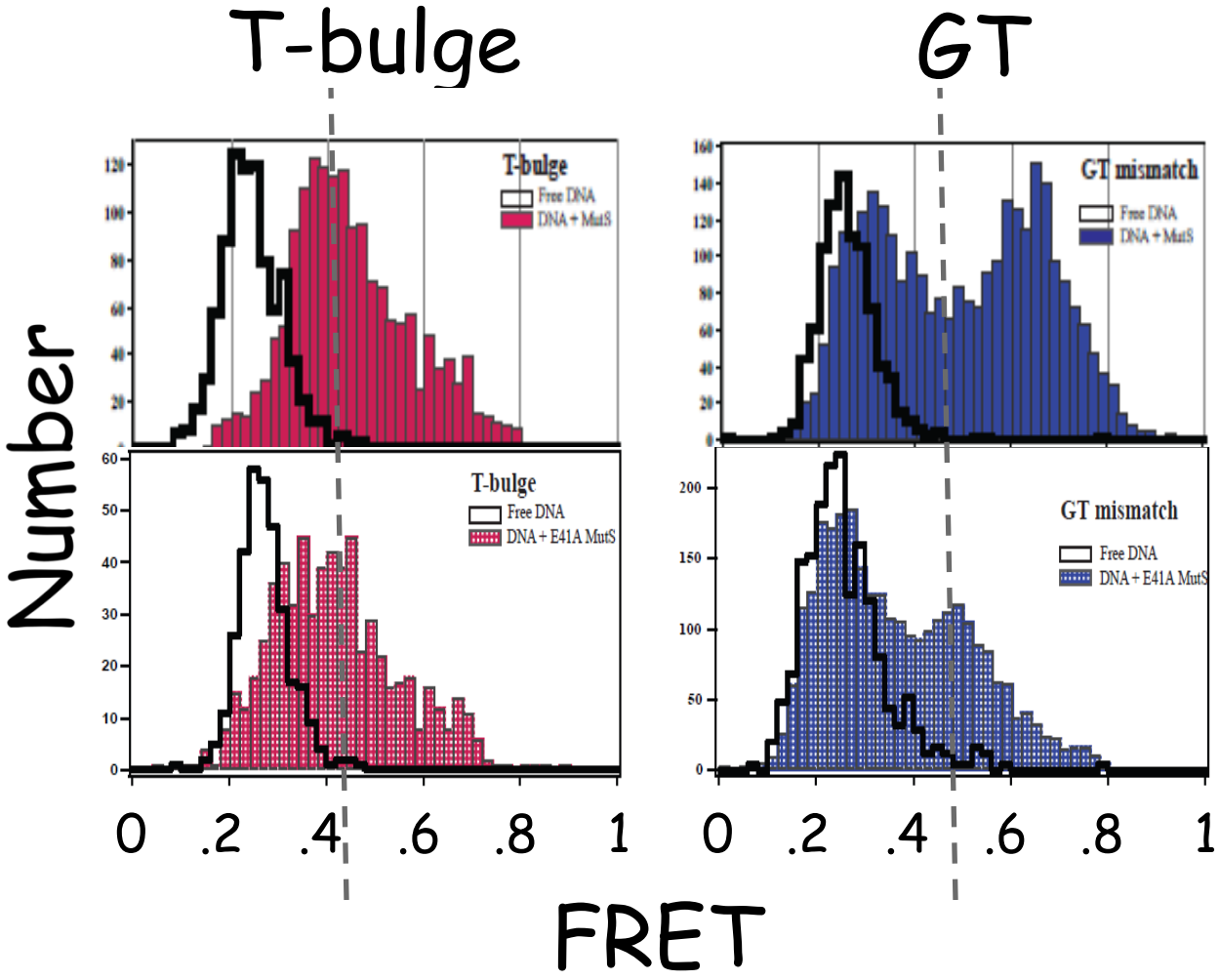
E41A mutant

-T-bulge:

- **more** dynamic
- **same** bending

-GT

- **similar** dynamic
- **lower** bending



DeRocco et al.,
Biochemistry, 53, 2043 (2014)

Wild type

E41A mutant

In vivo E41A-type mutation in yeast:

Mutant repairs T-bulge normally

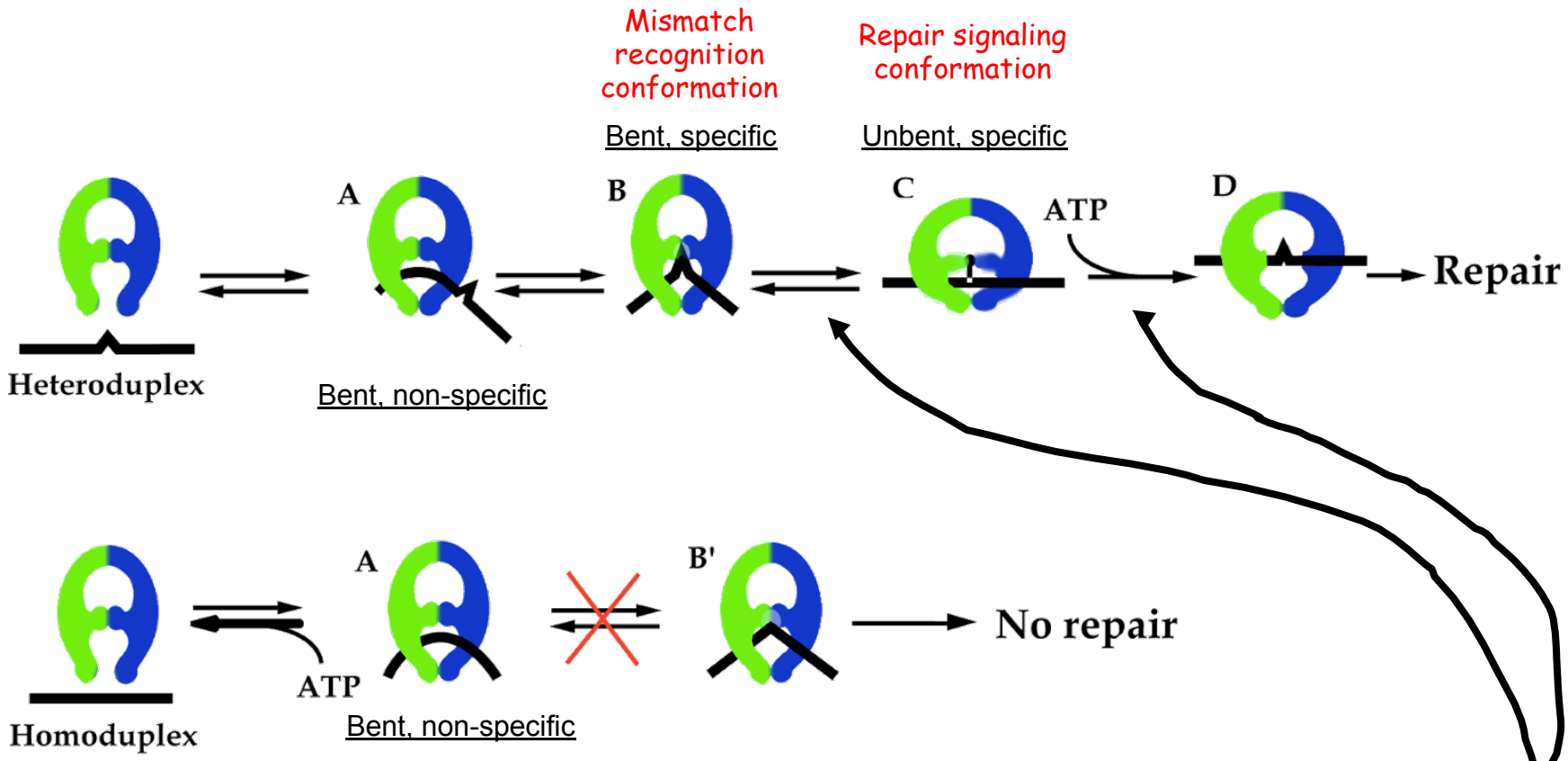
Mutant fails to repair GT – characteristic oxidative stress

Holmes, Scarpinato et al. DNA Repair **6(3): 293-303.** 2007

E41A mutant

- T-bulge:
  - **more** dynamic
  - **same** bending
- GT
  - **similar** dynamics
  - **lower** bending

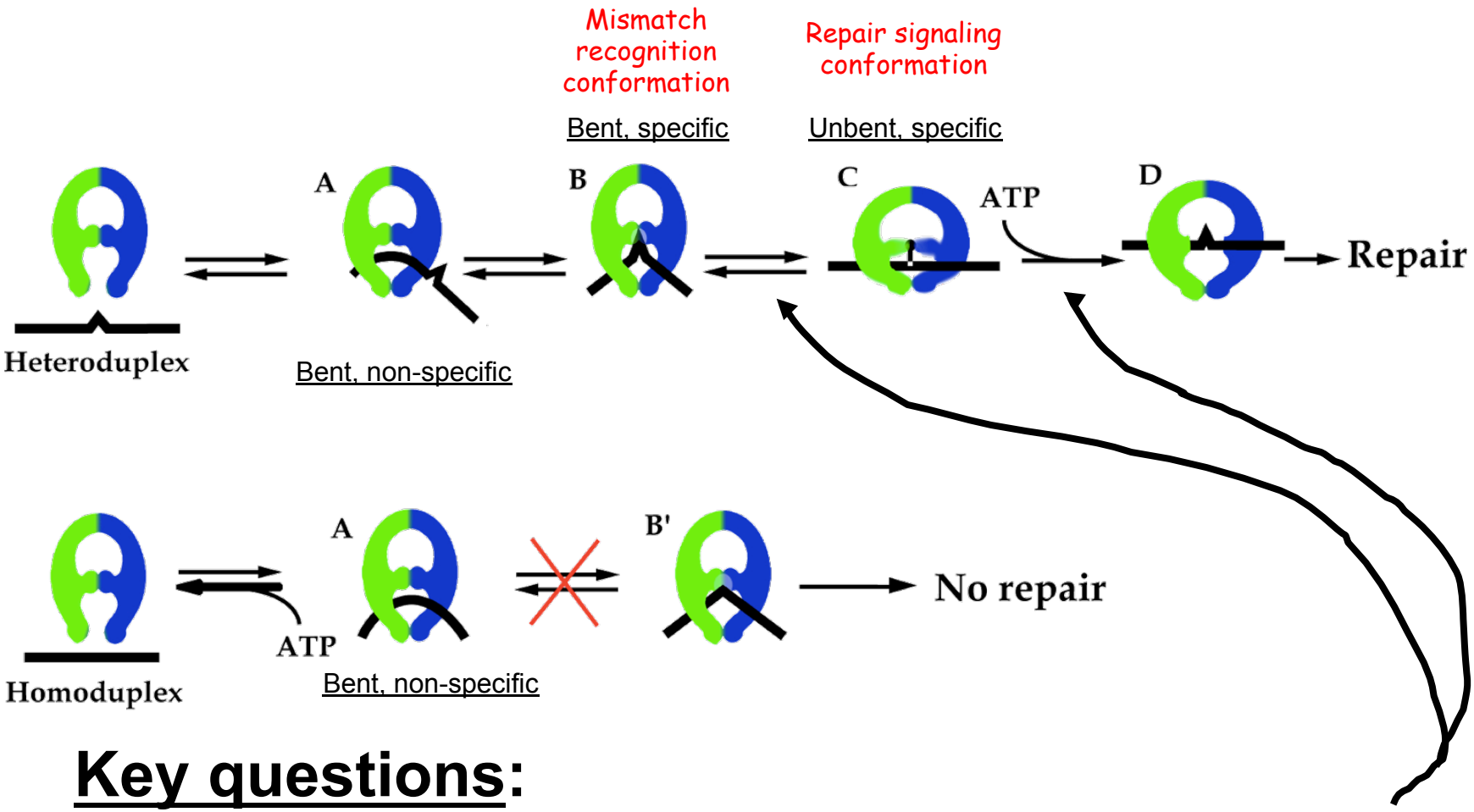
DNA mismatch repair: Bending Model



Different mismatches (GT, CC,etc.) → different state frequencies → different efficiency of repair.

Cancers mutations modifications pattern of states visited

DNA mismatch repair: Bending Model

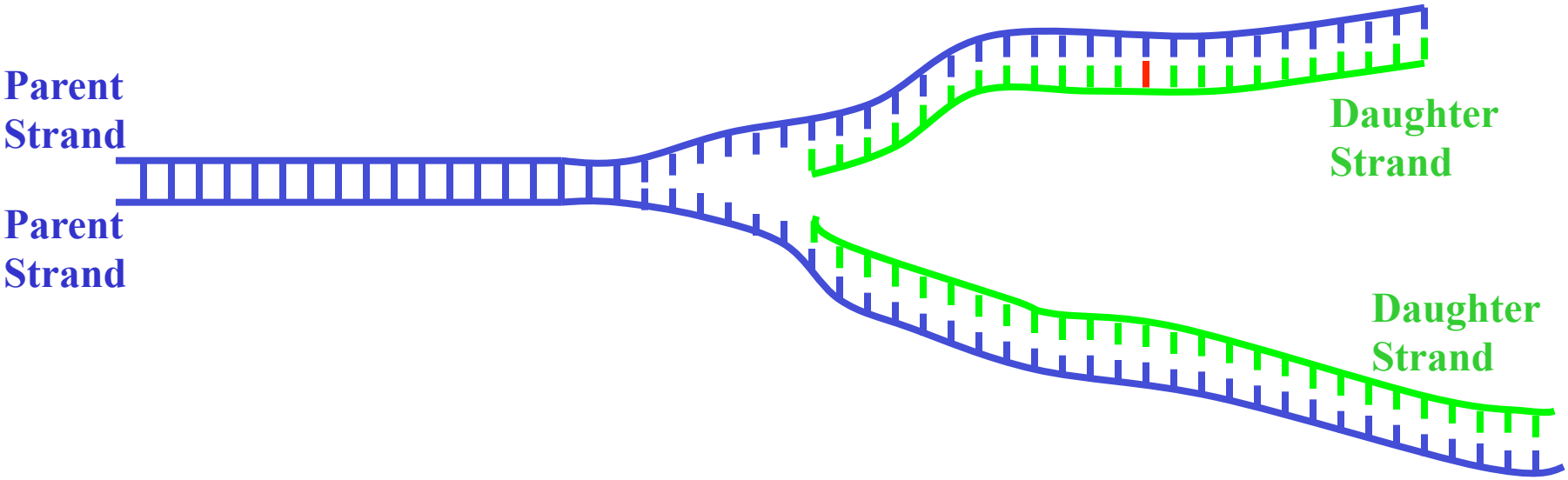


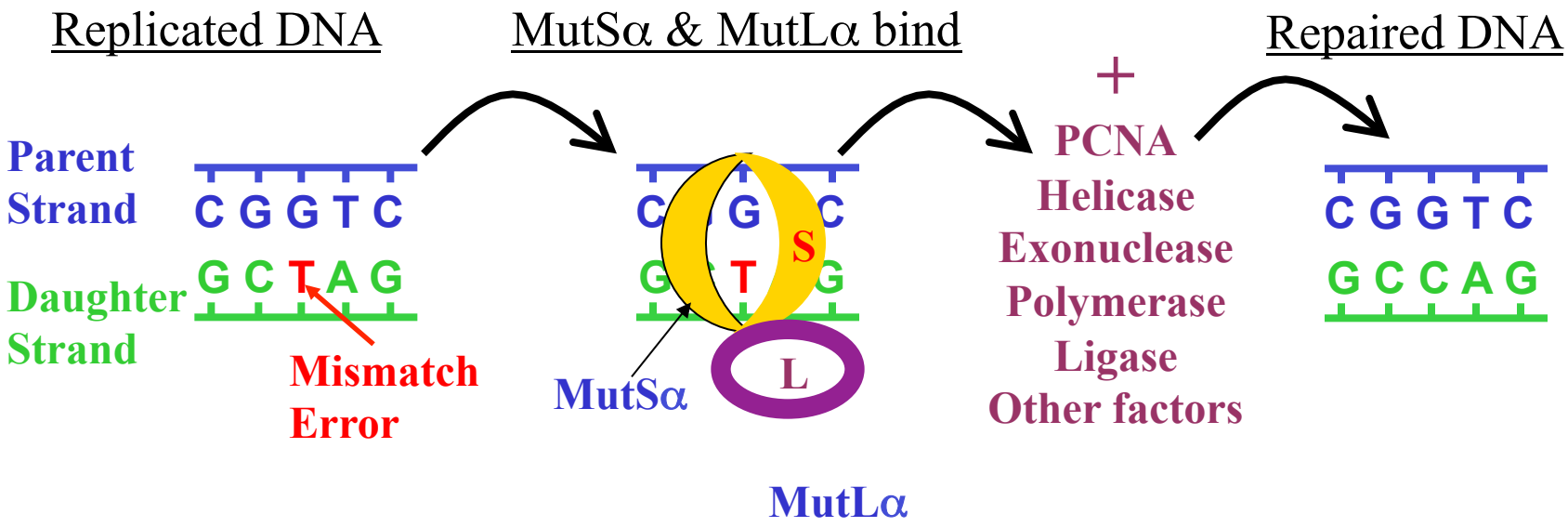
Key questions:

- what are the intermediates at these steps

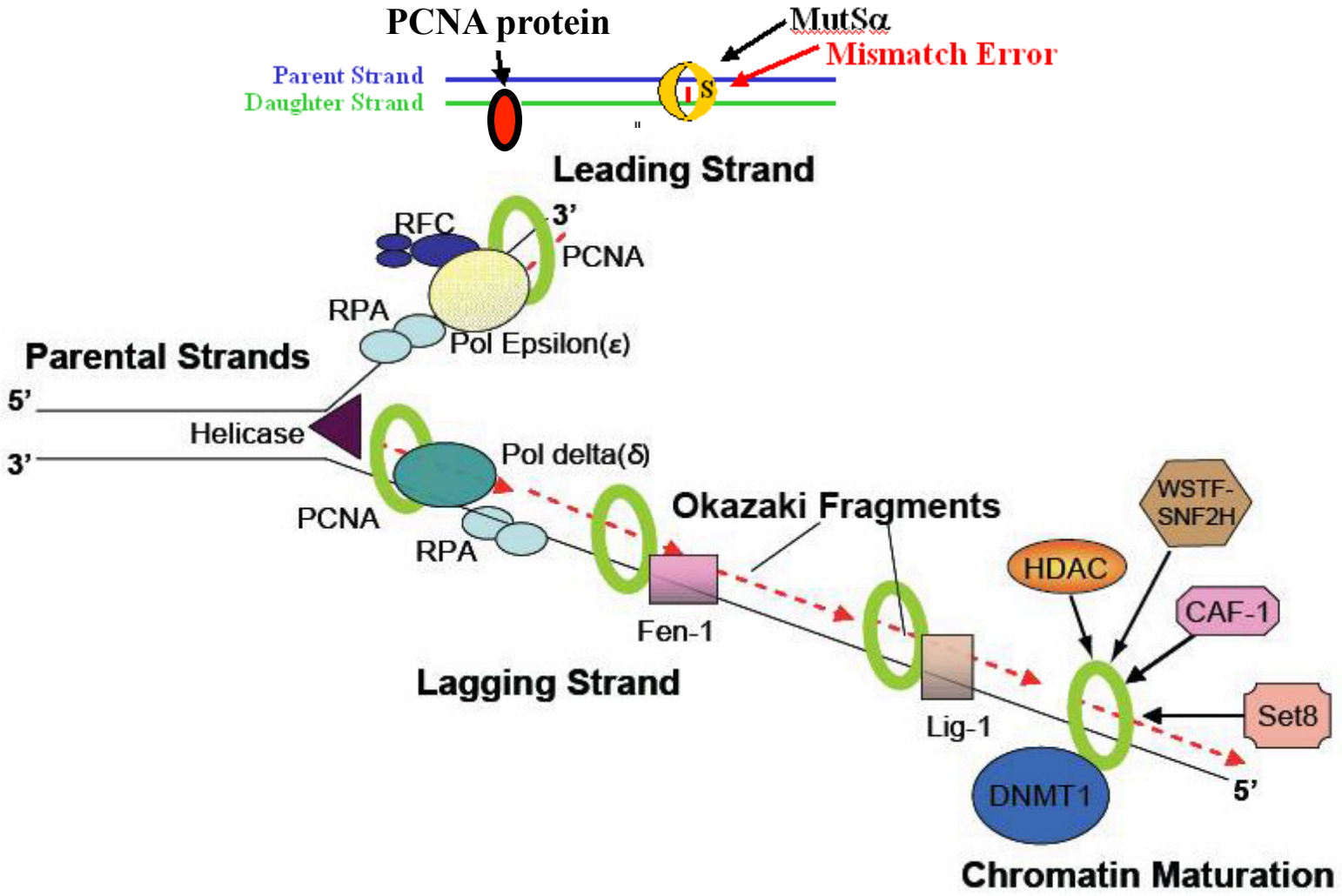
- what is the strand discrimination mechanism

Models of daughter strand identification

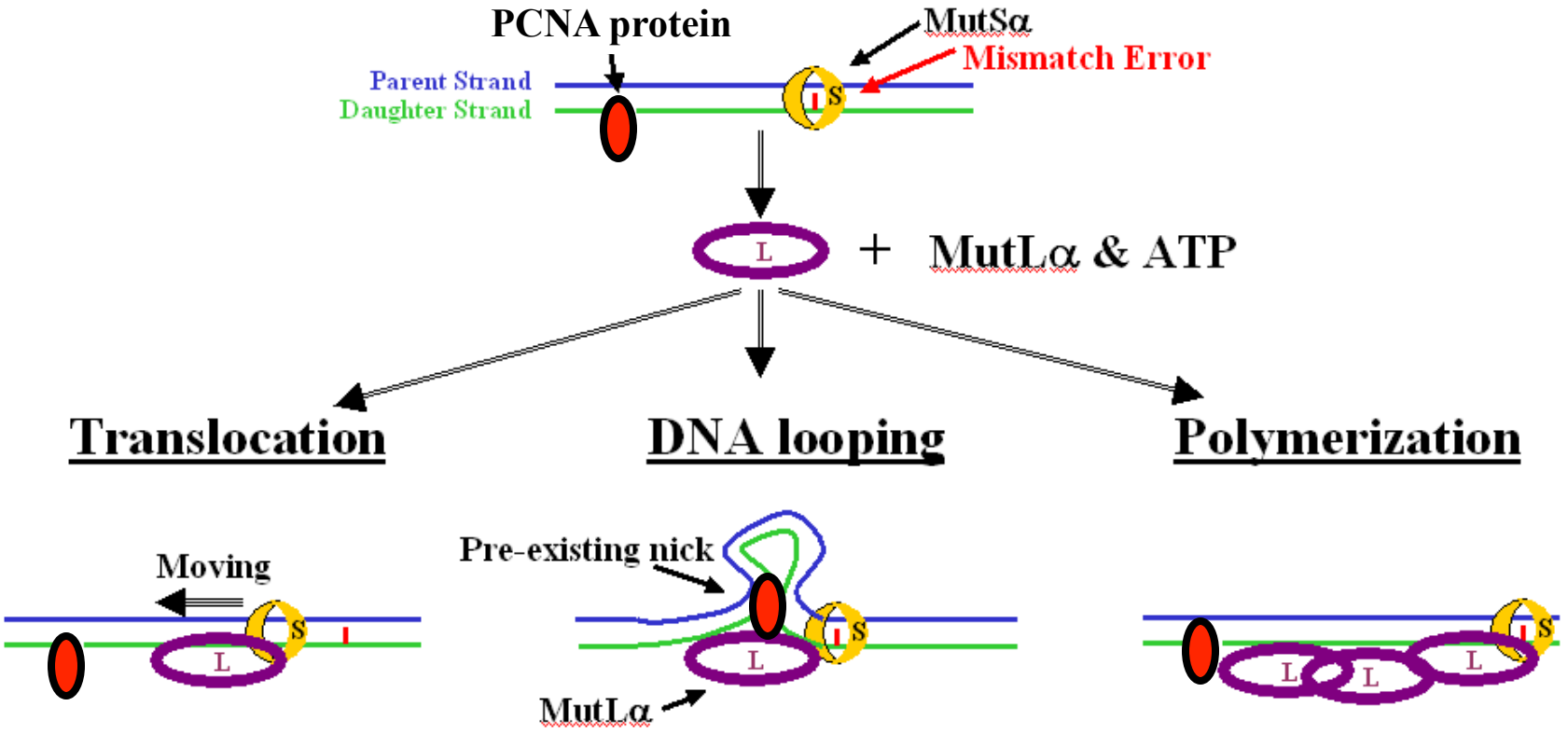




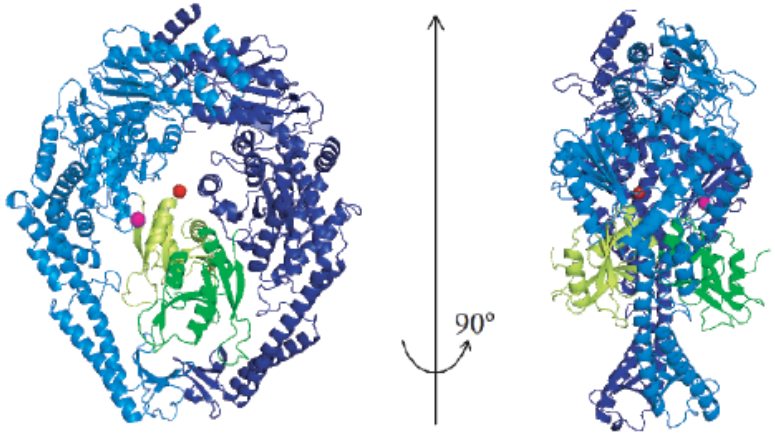
Models of daughter strand signaling in eukaryotic DNA mismatch repair



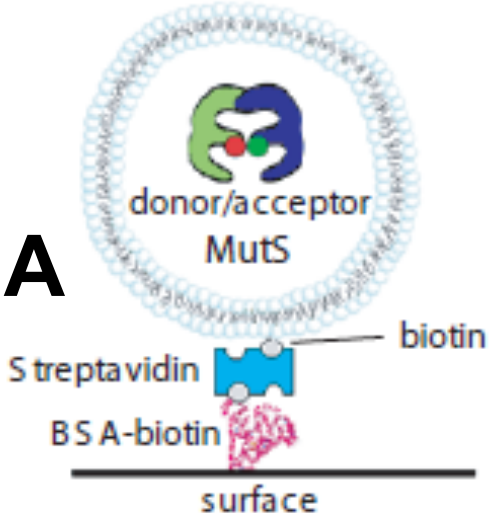
Models of daughter strand signaling in eukaryotic DNA mismatch repair

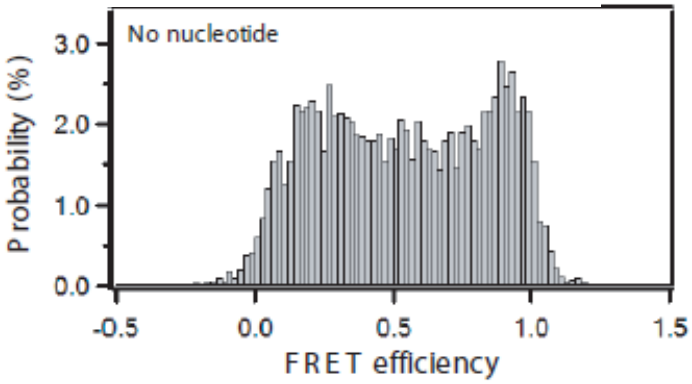


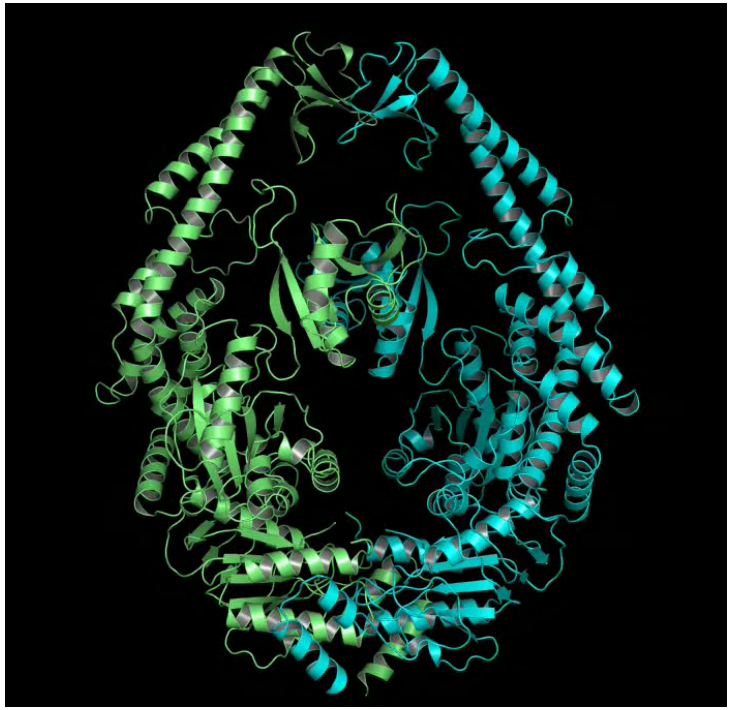
Conformation of free MutS dimer

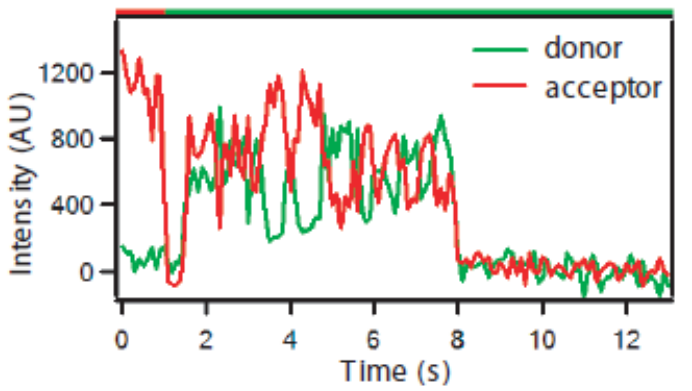


No DNA







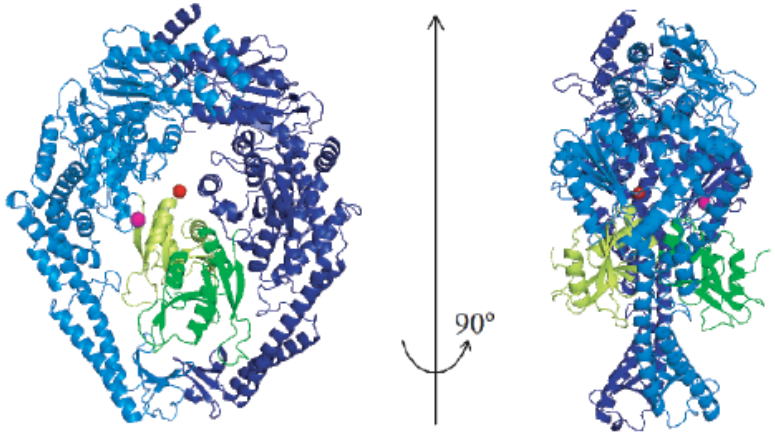


Crystal without DNA

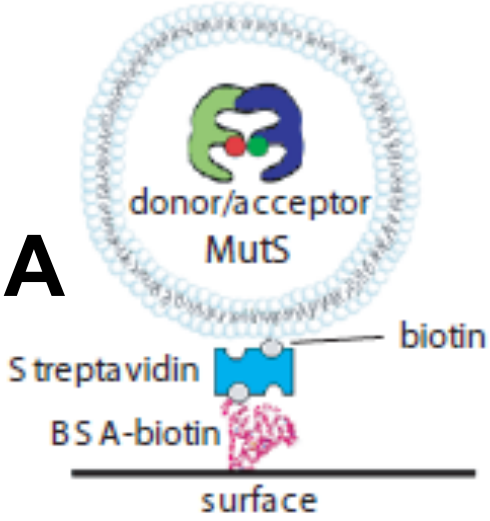
Obmolova et al.
Nature, 407, 703 (2000)

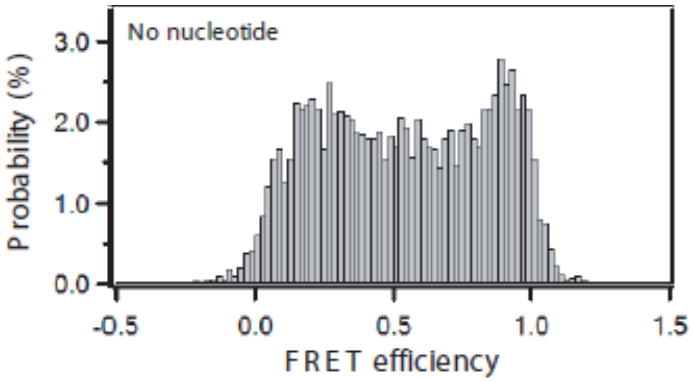
Qiu et al.,
EMBO J, 31, 2528 (2012)

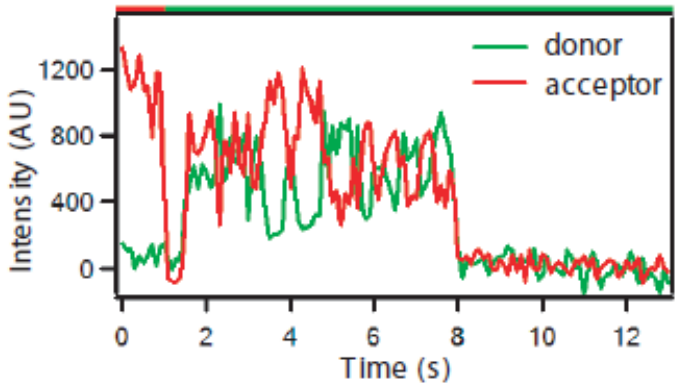
Conformation of free MutS dimer



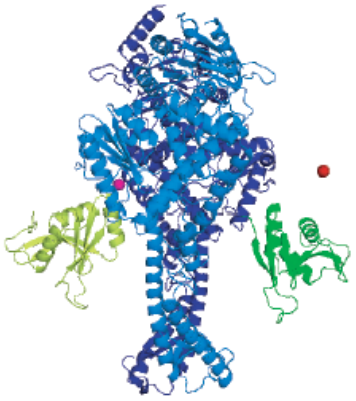
No DNA



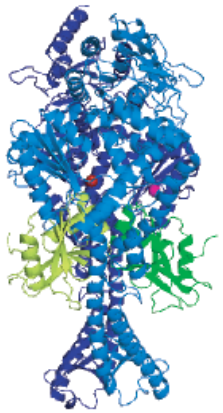




MD simulation

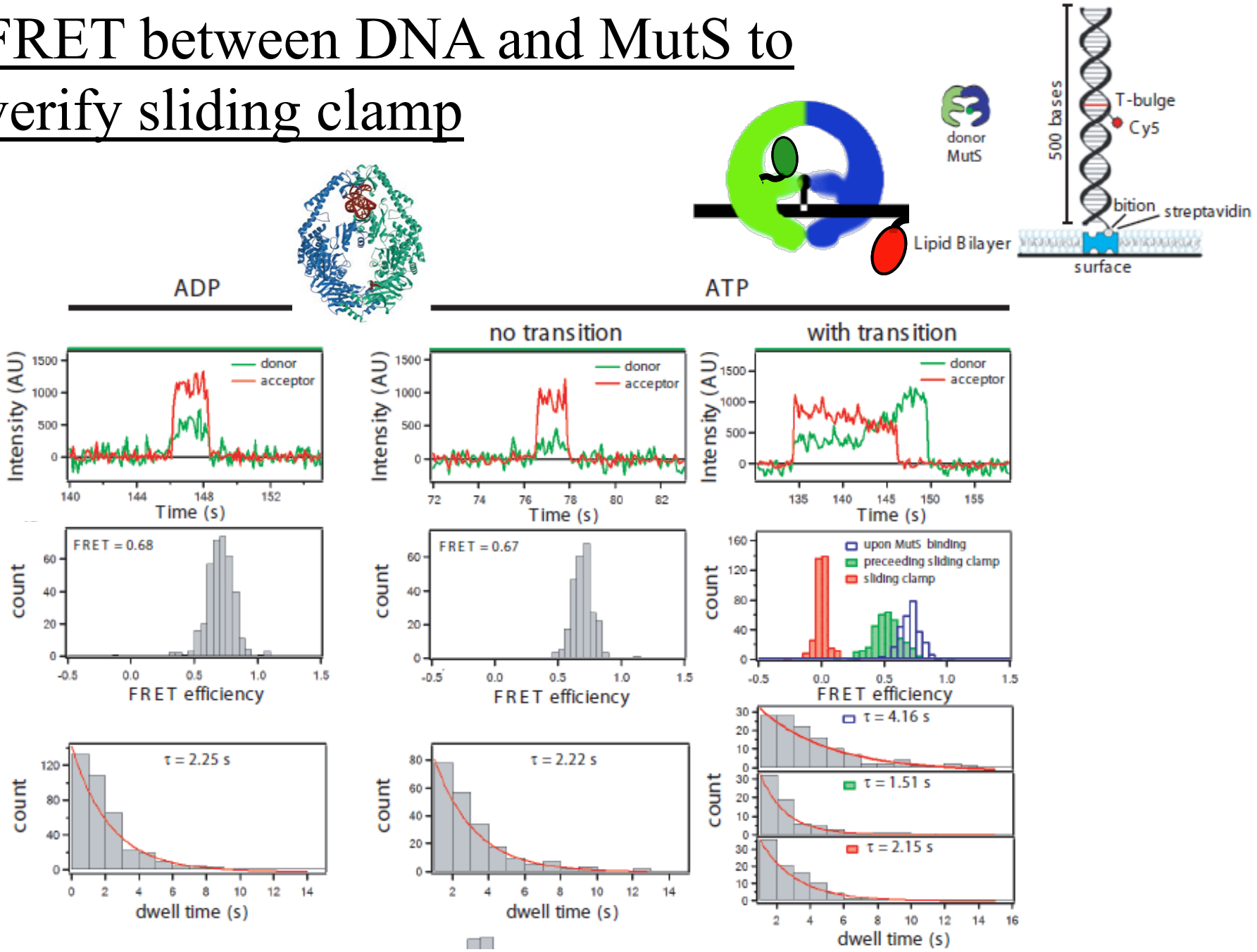


Low FRET



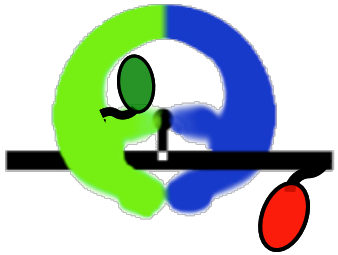
High FRET

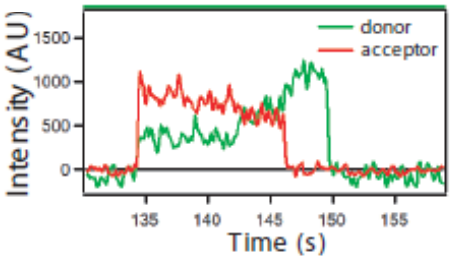
FRET between DNA and MutS to verify sliding clamp

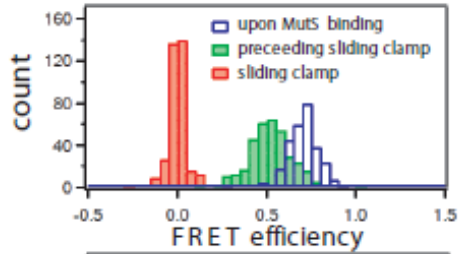


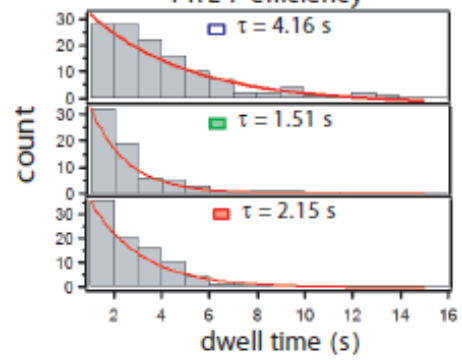
Qiu et al., EMBO J, 31, 2528 (2012)

Same kinetics for FRET between DNA binding domains and FRET to the DNA





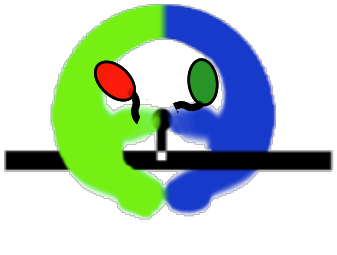


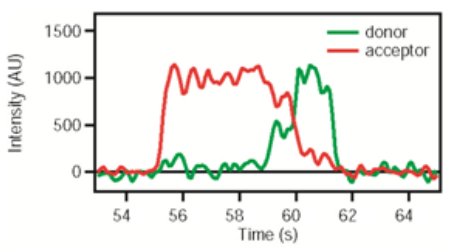


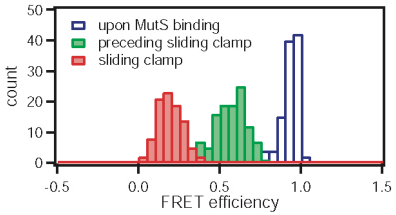
4.2 s

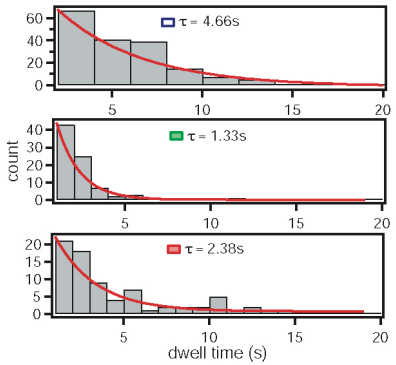
1.5 s

2.5 s









4.7 s

1.3 s

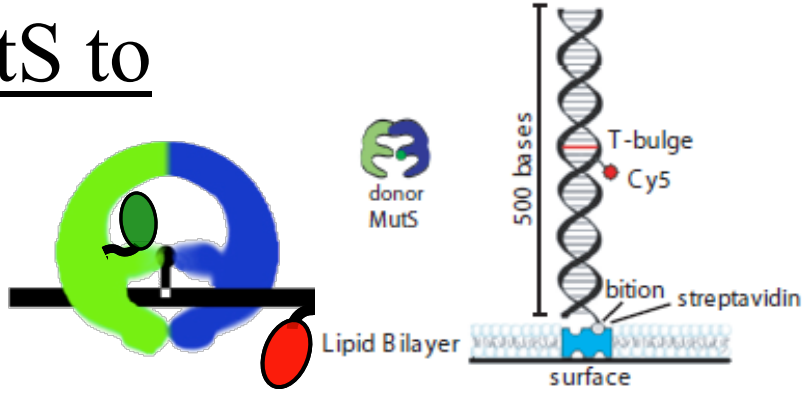
2.4 s

Requires
1) ADP during mismatch binding

AND

2) ATP to activate sliding clamp

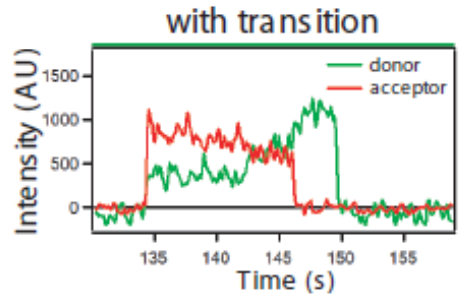
FRET between DNA and MutS to verify sliding clamp

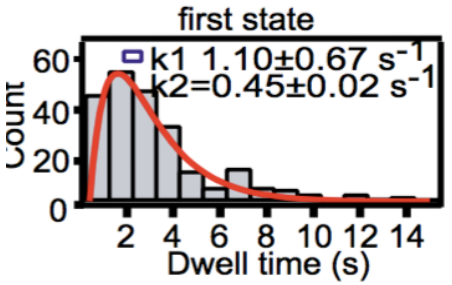


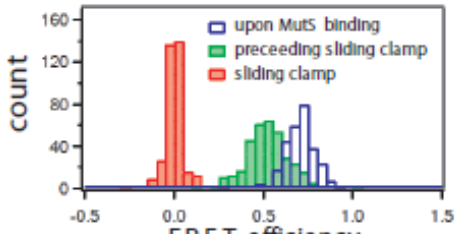
There is a hidden intermediate

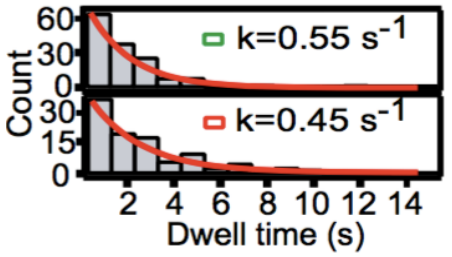
$$A \xrightarrow{k_1} X \xrightarrow{k_2} B,$$

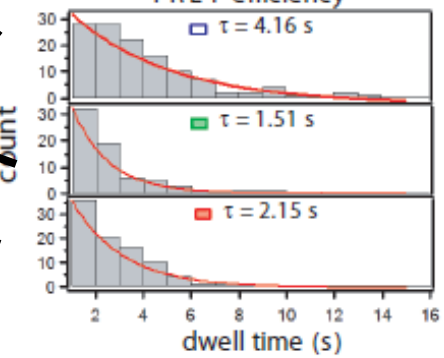
$$\frac{k_1 k_2}{k_1 - K_2}\left(e^{-k_2\tau} - e^{-k_1\tau}\right)$$







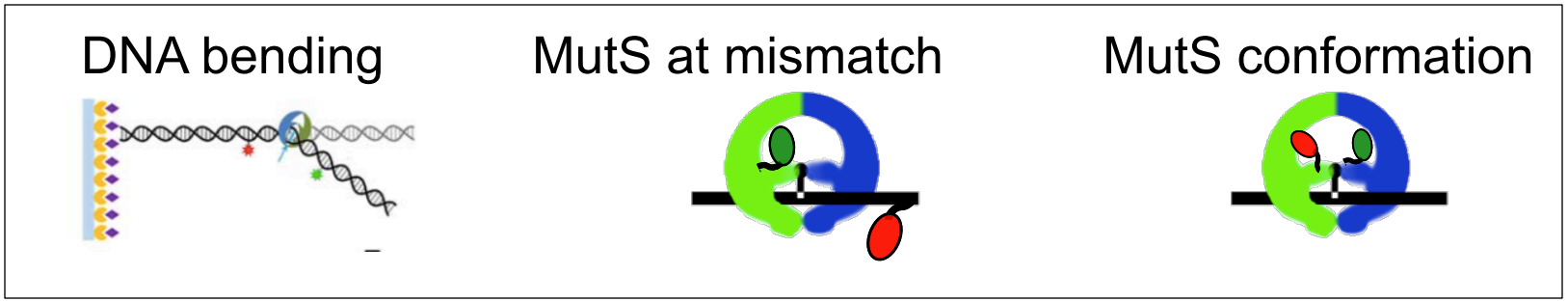




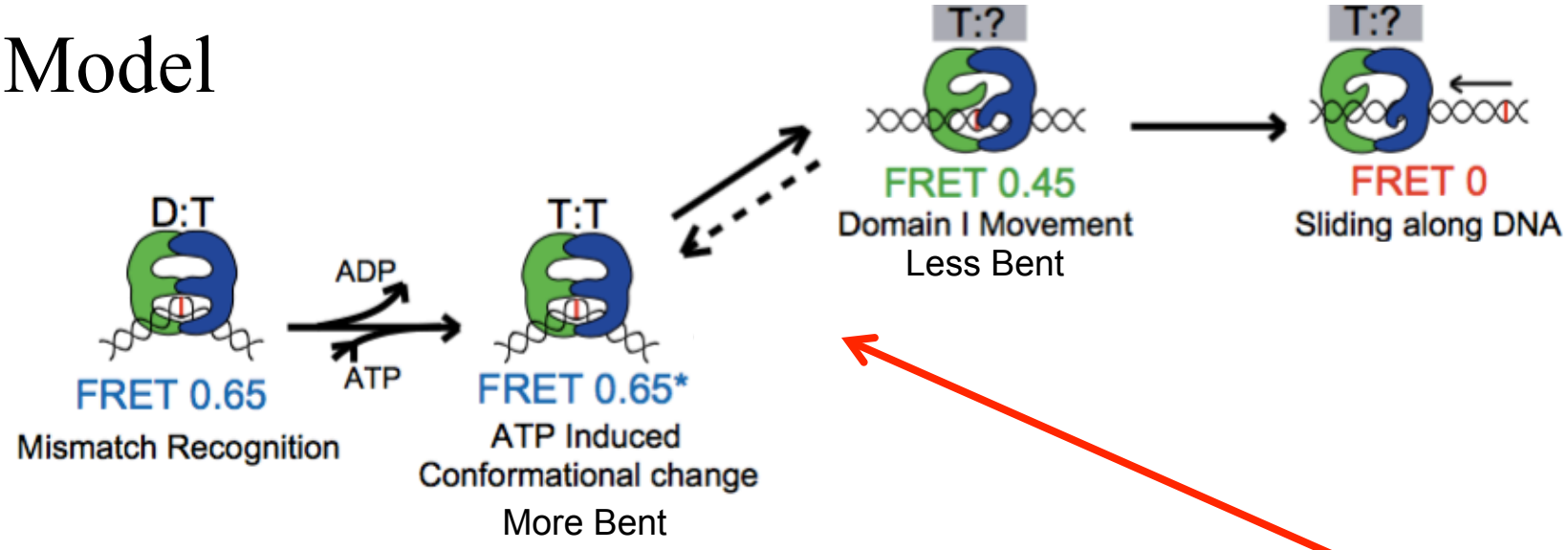
Before sliding clamp
2 FRET states
But
3 kinetic states

Qiu et al., EMBO J, 31, 2528 (2012); Qiu et al., PNAS, 112, 10914 (2015)

Combined results of 3 experiments support a model

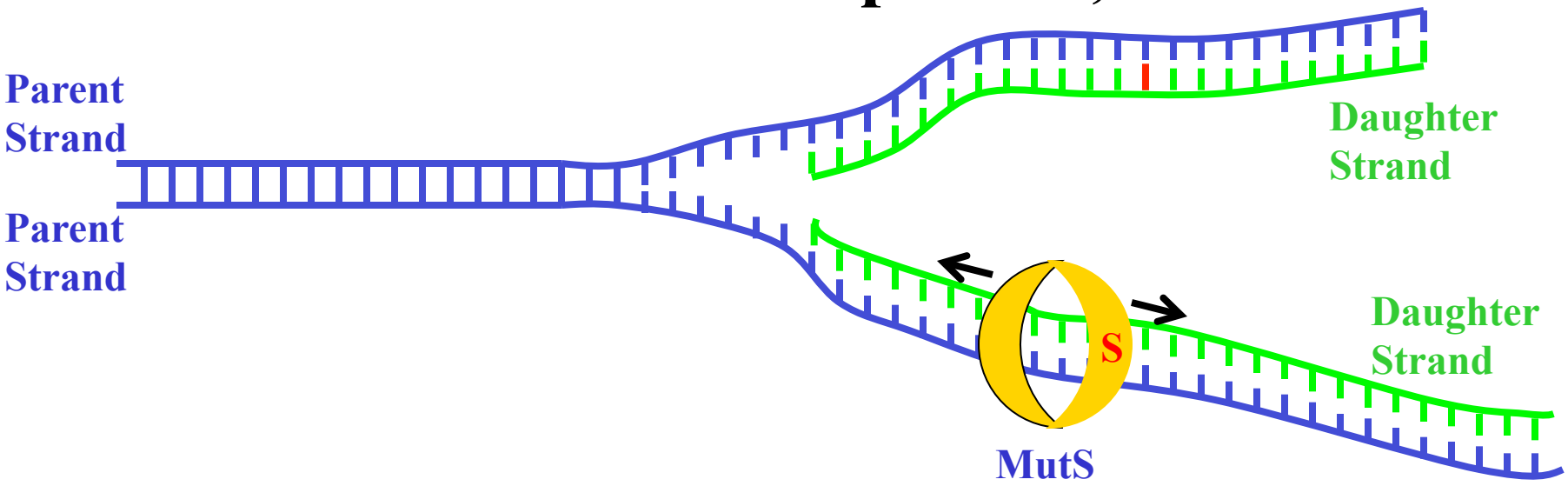


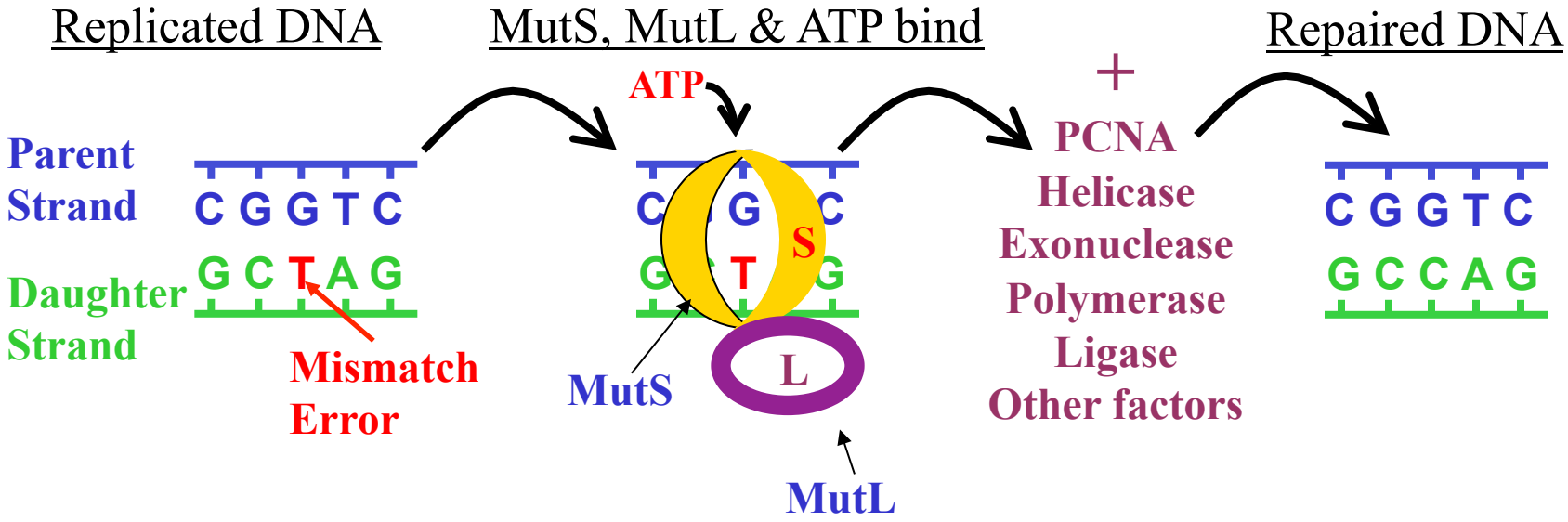
Model



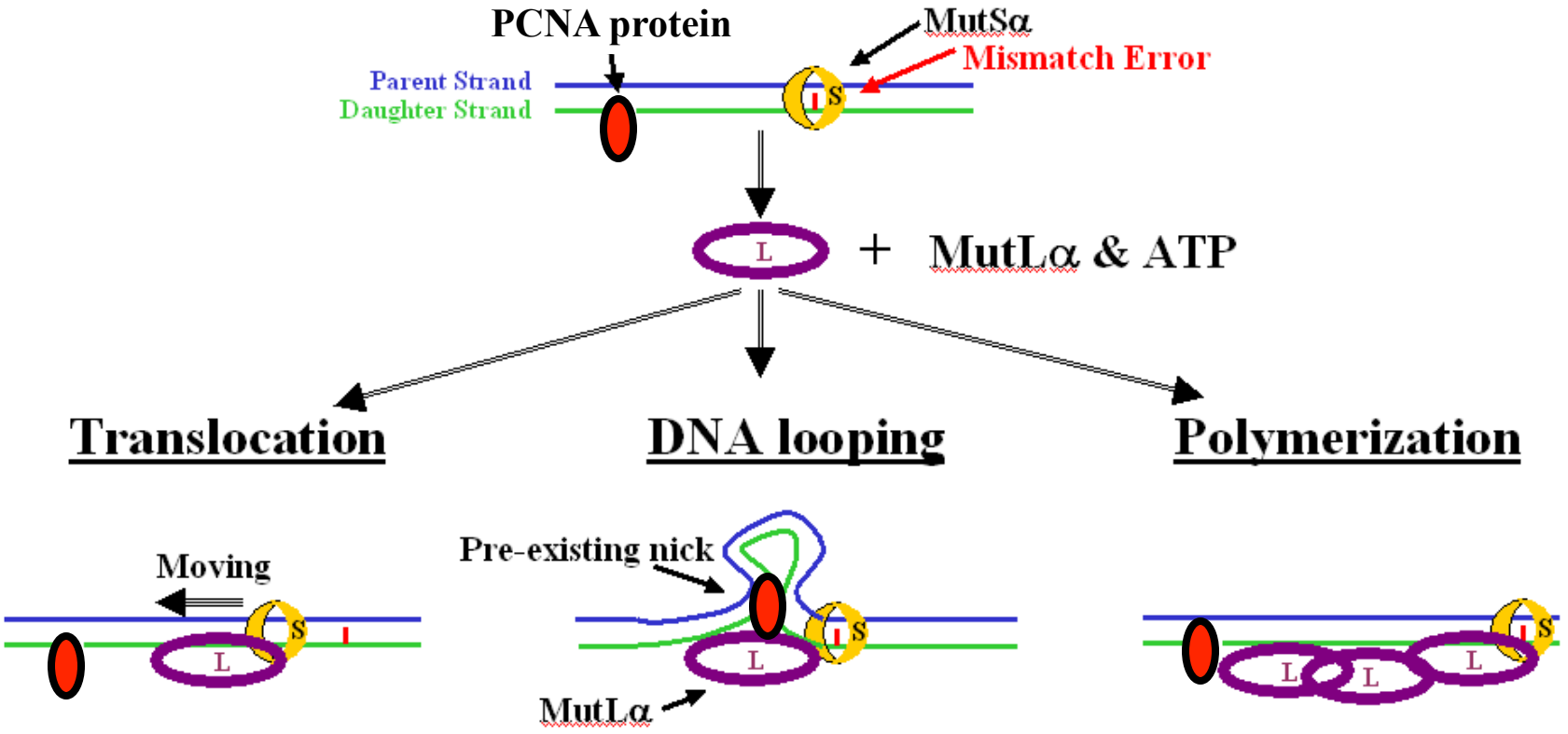
Different mismatches have different dynamics, which controls the efficiency of repair.

What about the next protein, MutL?

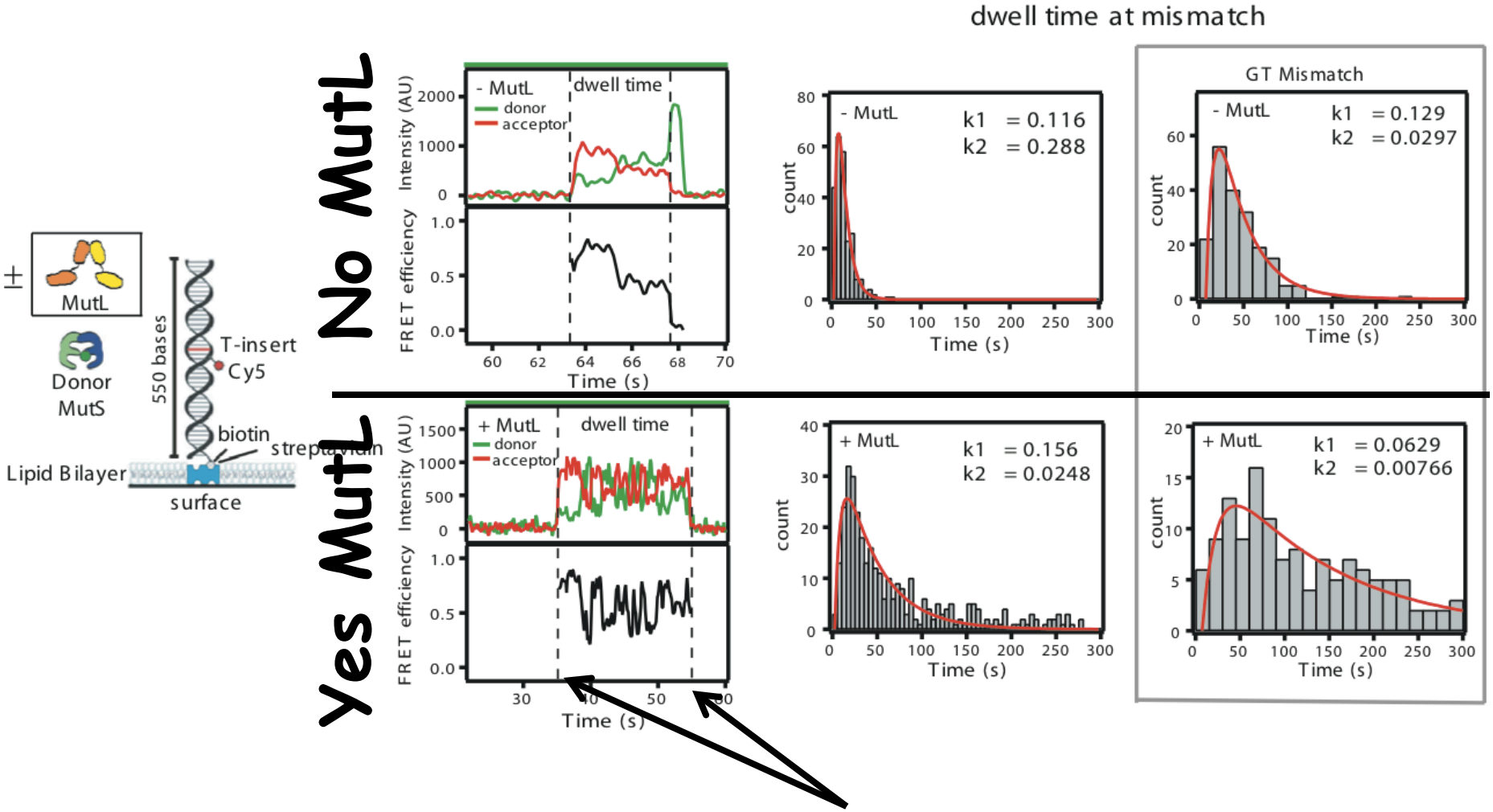




Models of daughter strand signaling in eukaryotic DNA mismatch repair



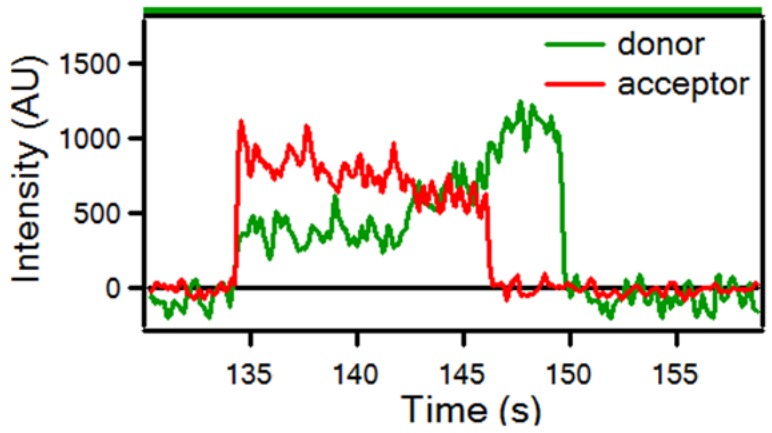
What about the next protein, MutL?



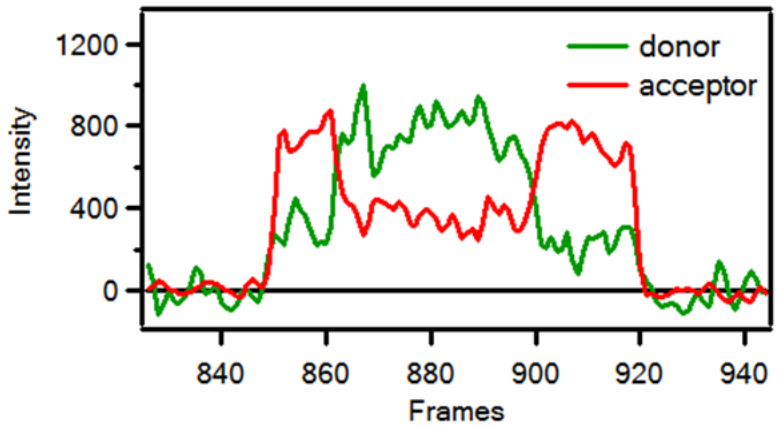
MutL lengthens the dwell time of MutS at mismatch

MutL prevents ATP/mismatch triggered sliding clamp

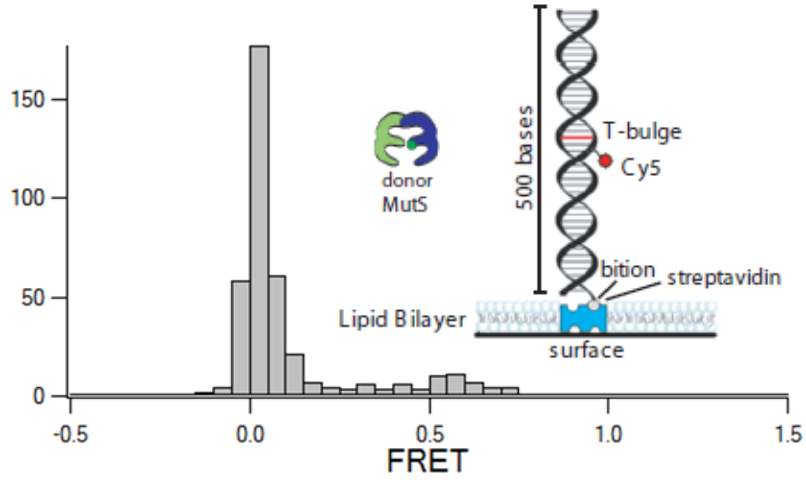
No MutL

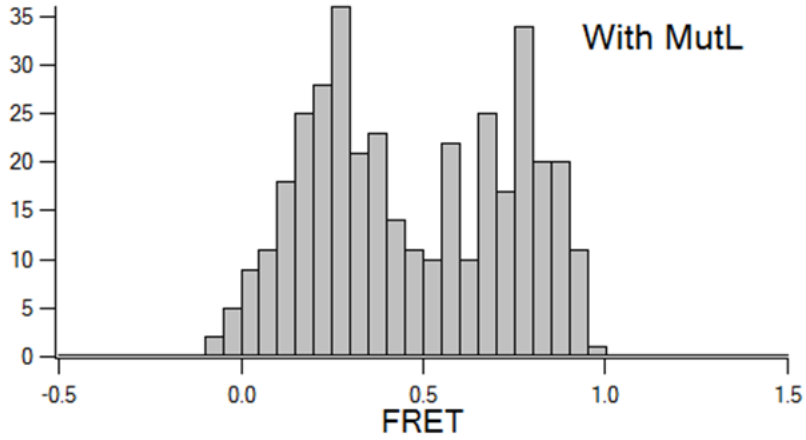


200 nM MutL



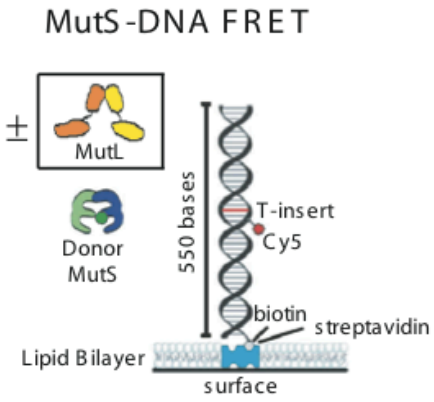
Only events with transitions:
FRET before unbinding

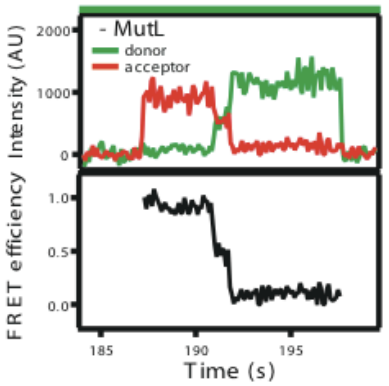


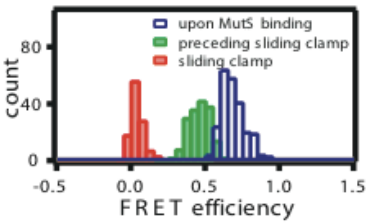


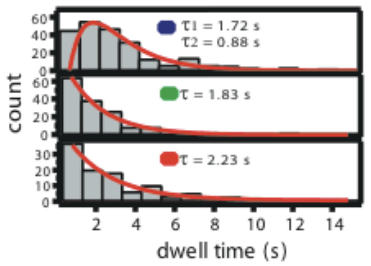
Qiu et al., PNAS, 112, 10914 (2015)

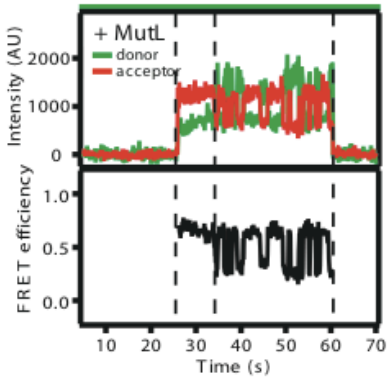
Analysis of intermediate states

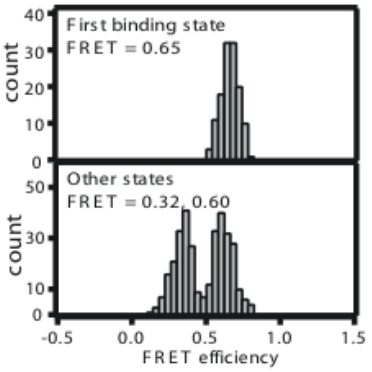


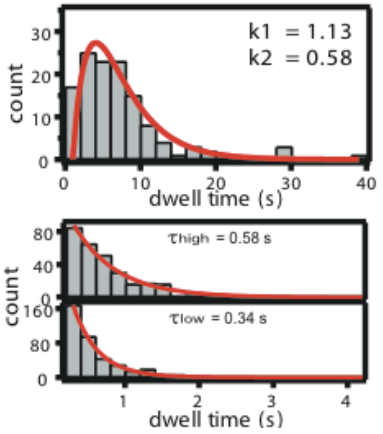








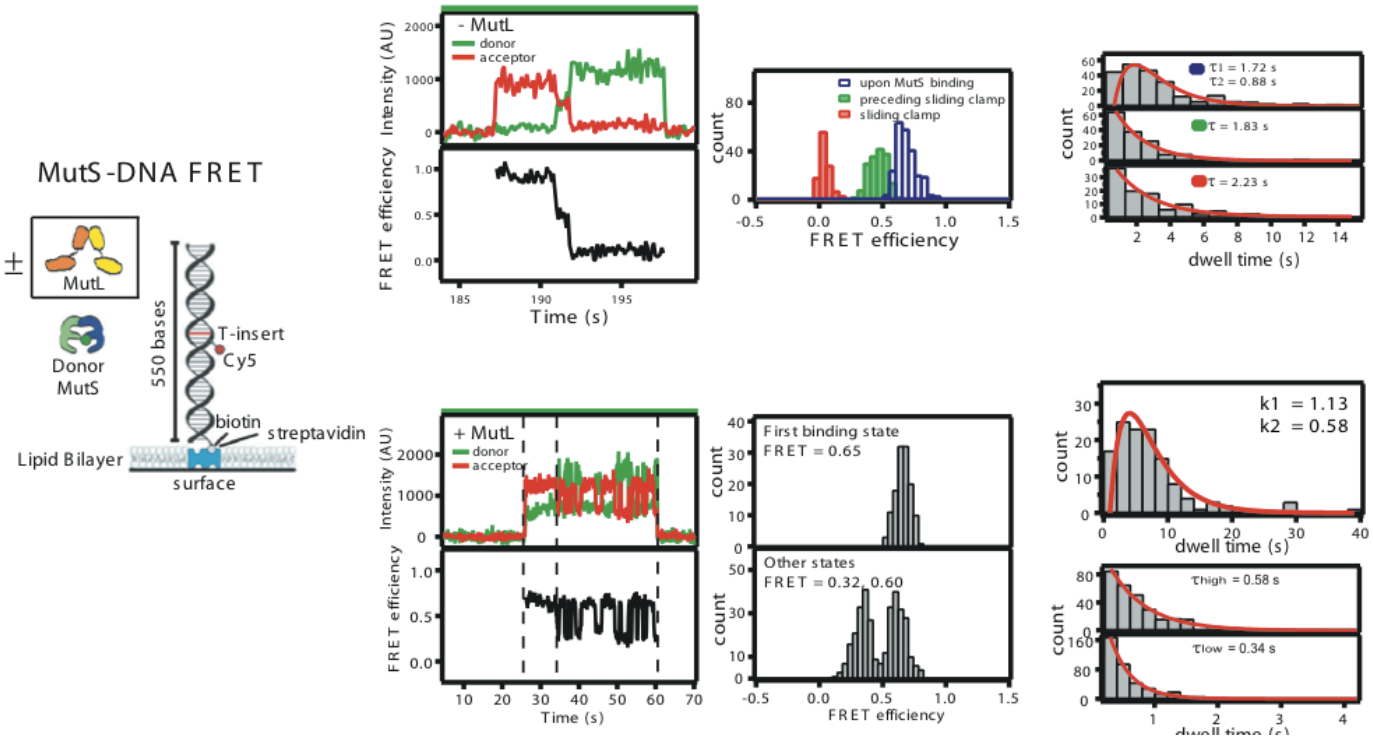


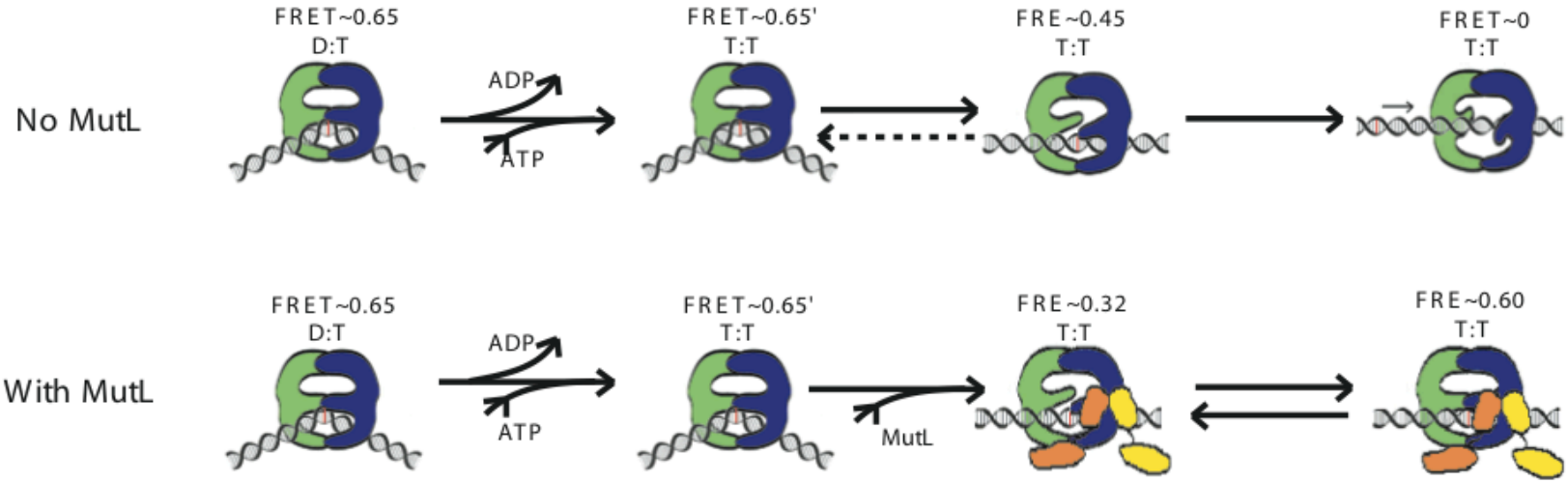


$$A \xrightarrow{k_1} X \xrightarrow{k_2} B,$$

$$\frac{k_1 k_2}{k_1 - K_2}\left(e^{-k_2\tau} - e^{-k_1\tau}\right)$$

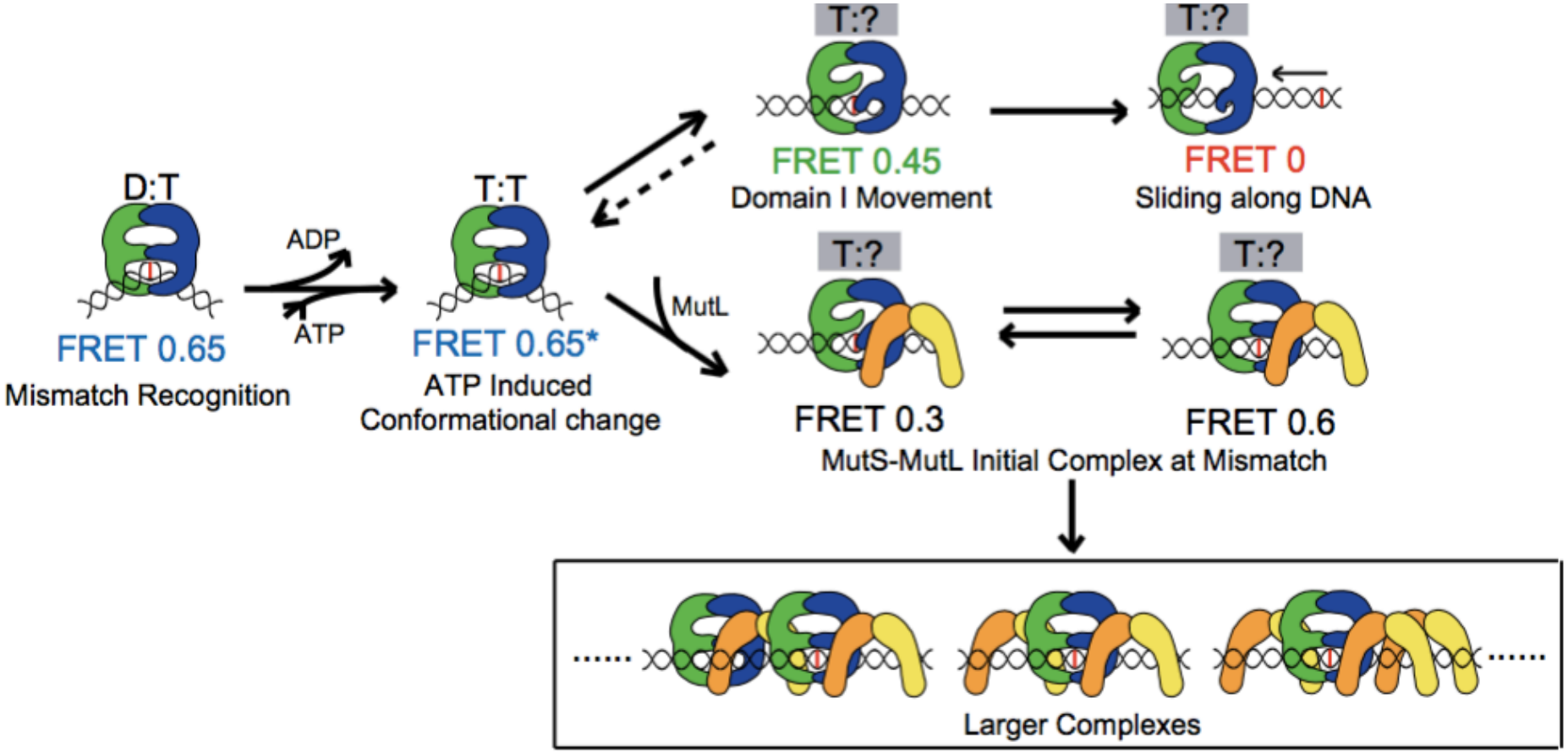
Analysis of intermediate states





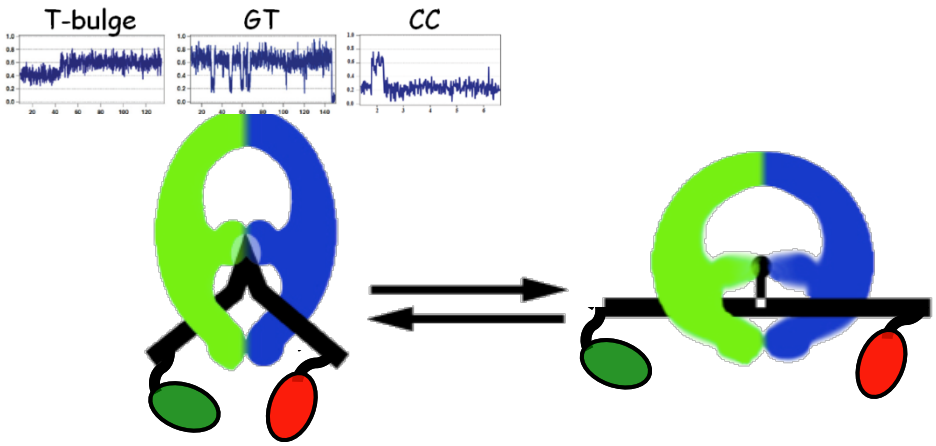
Qiu et al., PNAS, 112, 10914 (2015)

Summary of mismatch repair signaling interactions

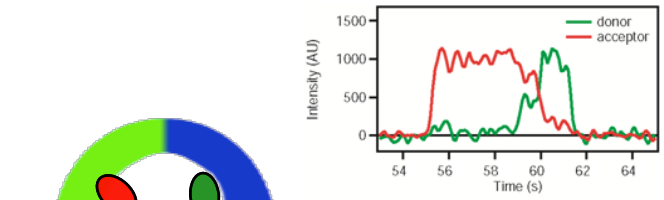


Method Summary: Single molecule FRET reveals dynamics of DNA repair proteins

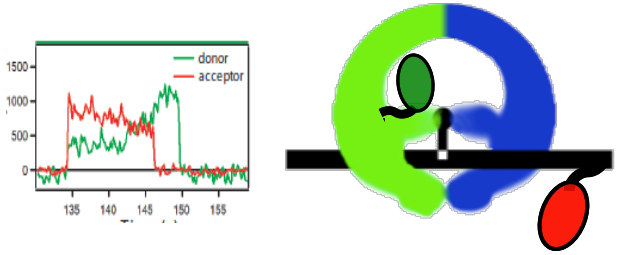
DNA conformation



Protein conformation



Position on DNA relative mismatch

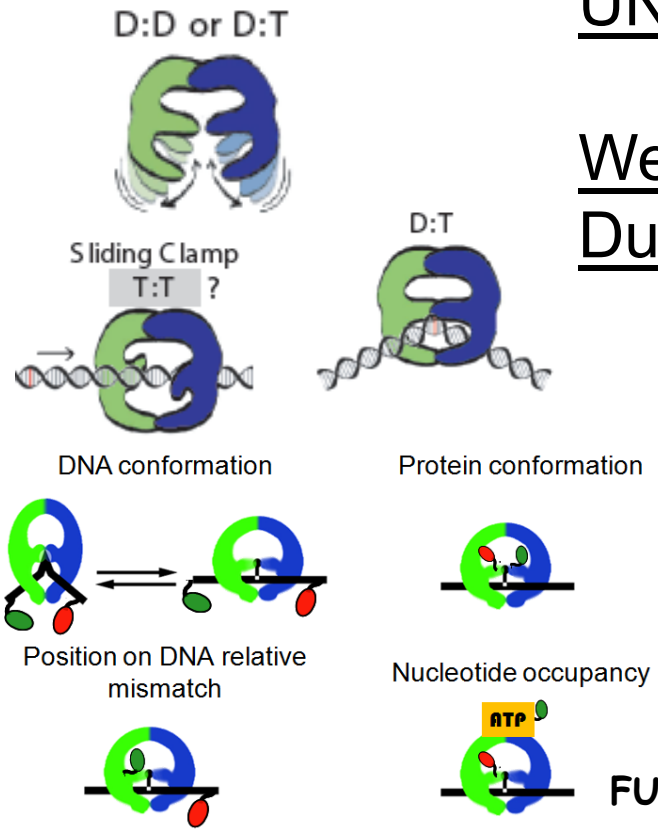


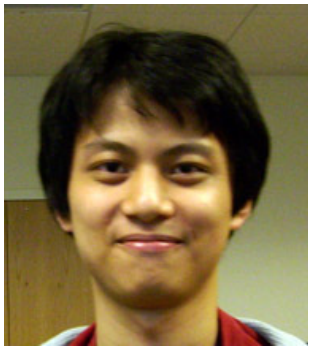
Nucleotide occupancy

Final Thought

- Single molecule FRET reveals dynamic structural rearrangements and multi-molecular interactions during complex, multi-step phenomena

North Carolina State: **Ruoyi Qiu**, Liz Sacho
UNC-CH: **Jake Gauer**, **Vanessa DeRocco**, **Lauryn Sass**, Dorothy Erie
Wesleyan: Manju Hingorani
Duke: Paul Modrich





FUNDING: American Cancer Society

NIH