Dissecting molecular machines: the single-molecule approach

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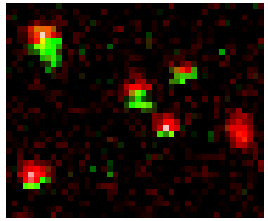
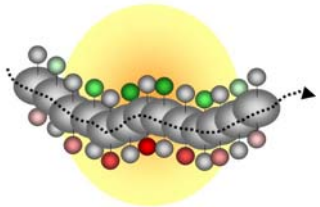
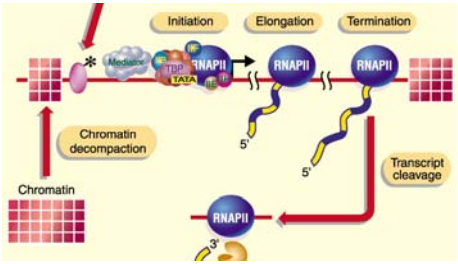
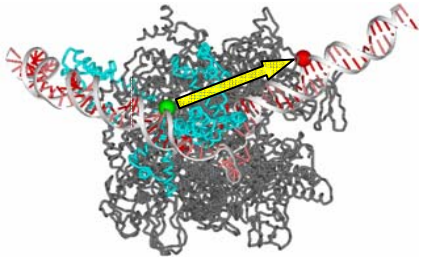




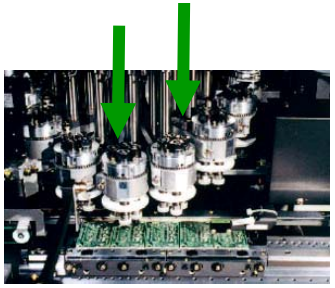




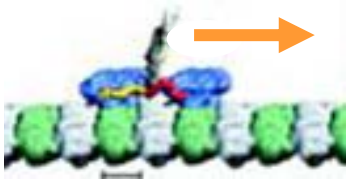
Oxford Statistics, Nov 29 2005

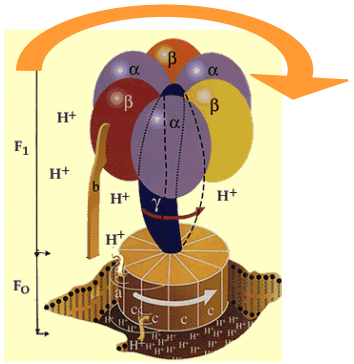
Machines, large and (very) small

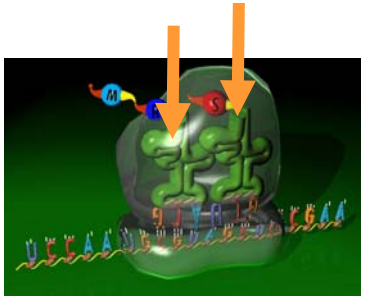
Scale: 1-50 m



Scale: 1-50 nm



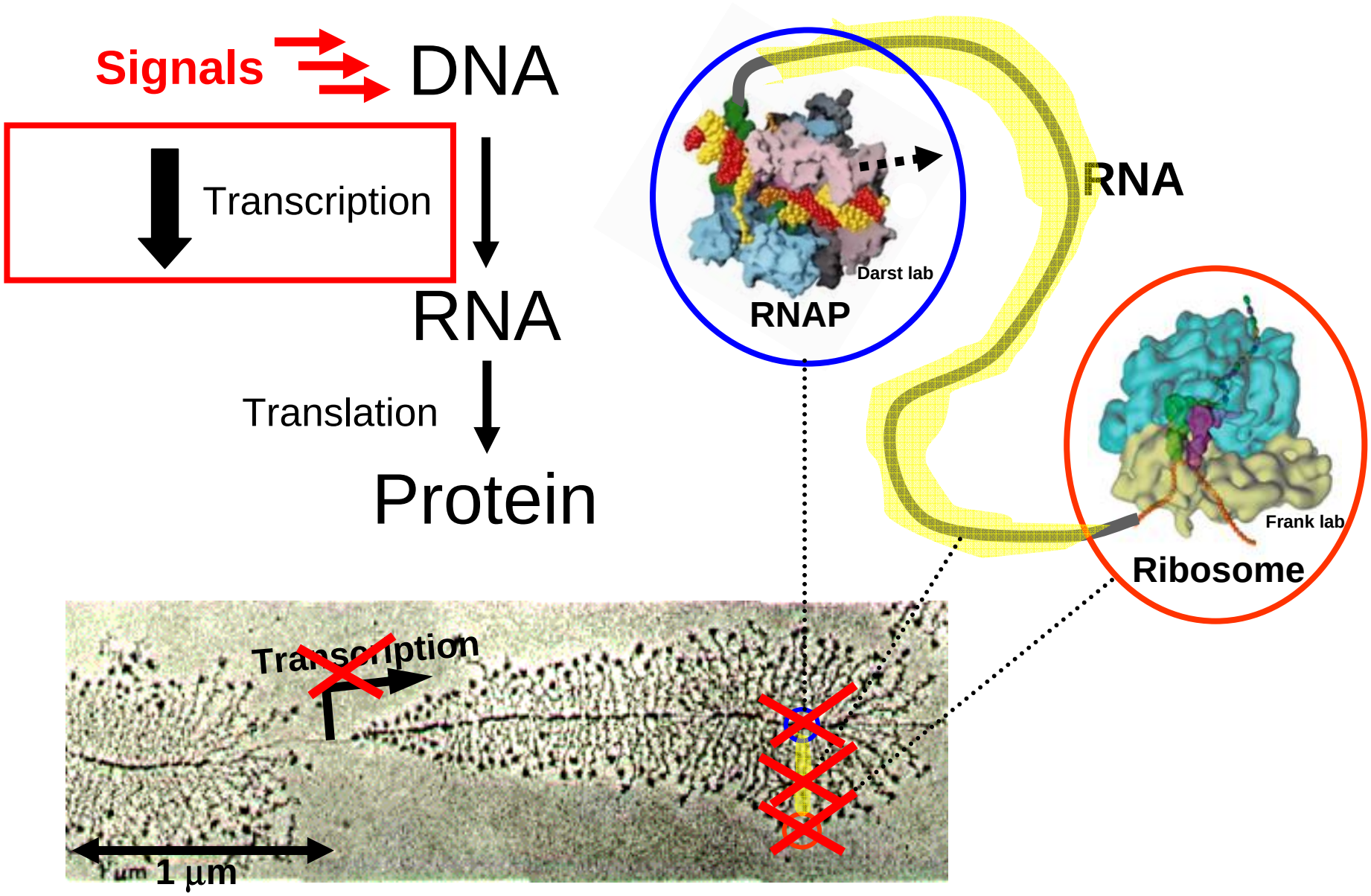




Bionanotechnology:

- **Understand how biomachines function--BIO**
- **Study single biomolecules--NANO**
- **Build machines/devices using bio- components or principles--TECHNOLOGY**

Gene expression: the path from gene to protein



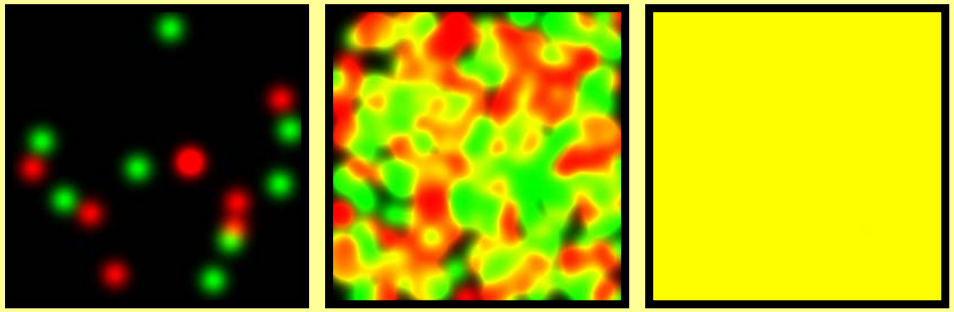
Single-molecule analysis: no averaging!
("one molecule at a time")

Single-molecule methods detect:

Static heterogeneity

-*Non-interconverting, distinct* species within an ensemble (e.g. active/inactive machinery)

- Lost by ensemble averaging

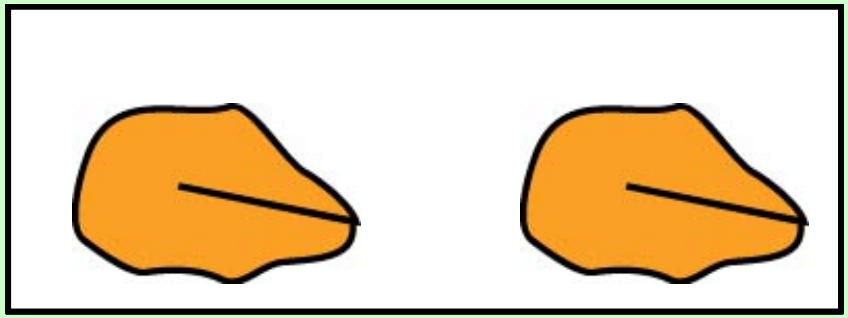


Increasing Concentration →

Dynamic heterogeneity

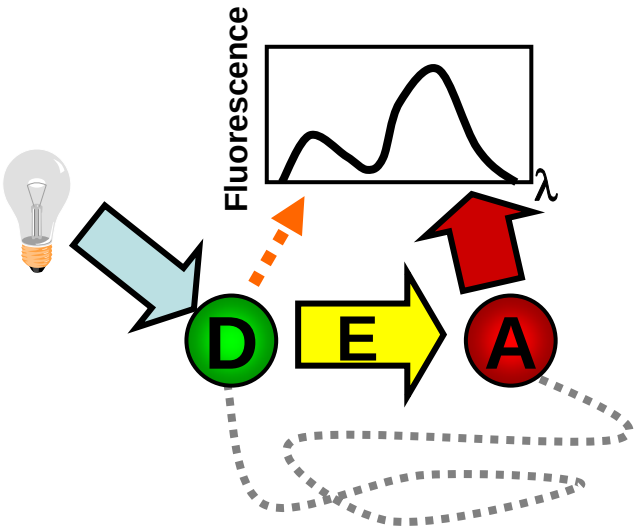
- *Interconverting, distinct* states of a single molecule

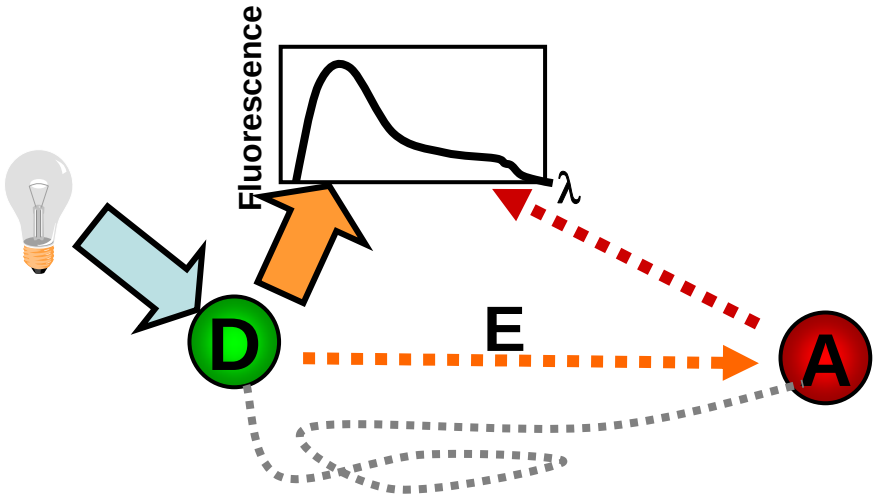
- Lost by ensemble-averaging (due to lack of synchronization)



Fluorescence resonance energy transfer:

A reporter of structure and interactions at the 2-10 nm scale

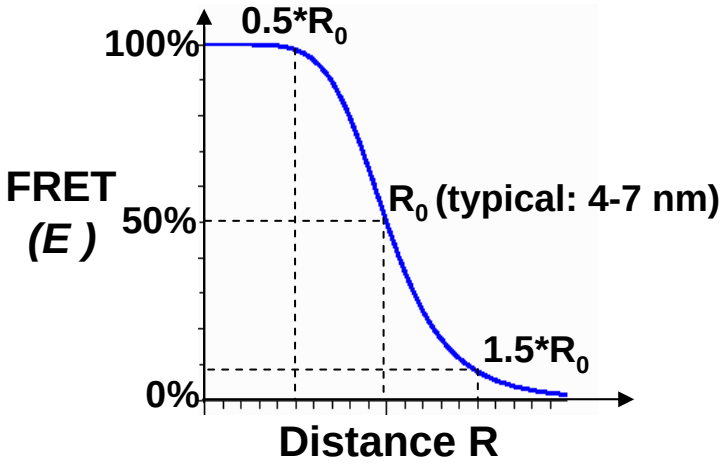




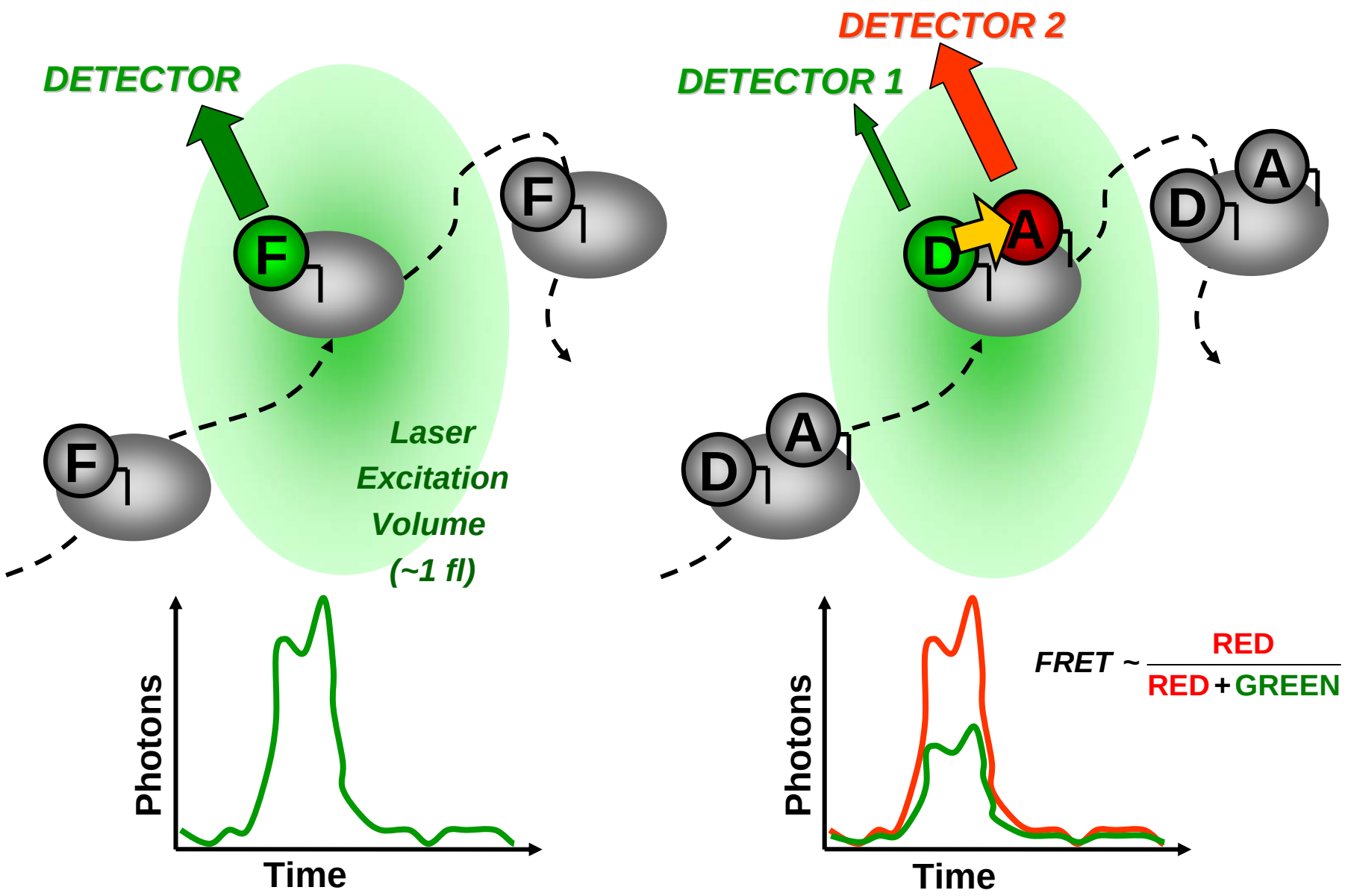
FRET Efficiency, *E*:

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

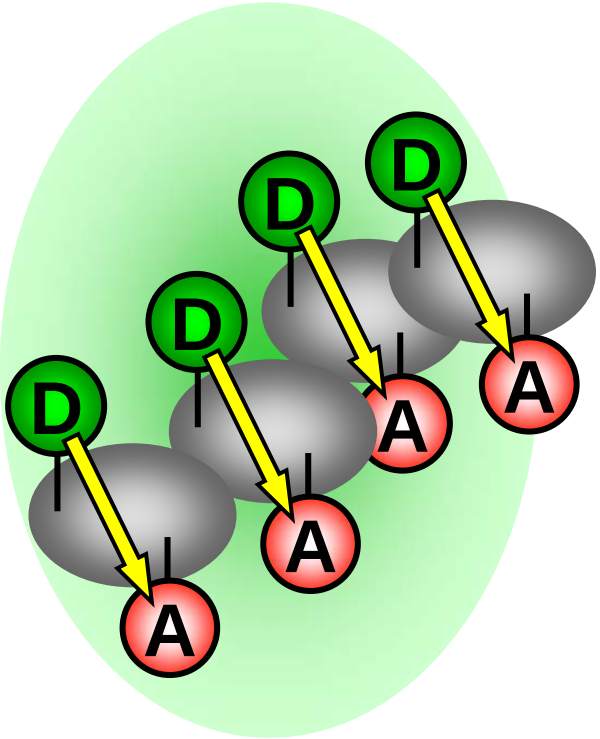
R **= D-A distance**
R_0 **= Förster radius**
(D-A distance where *E* = 50%)



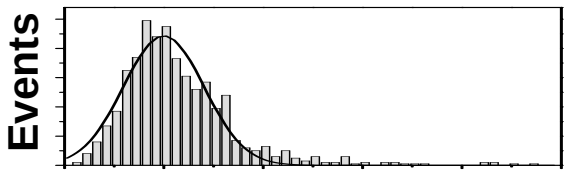
Single-molecule FRET on diffusing molecules



Single-molecule FRET spectroscopy



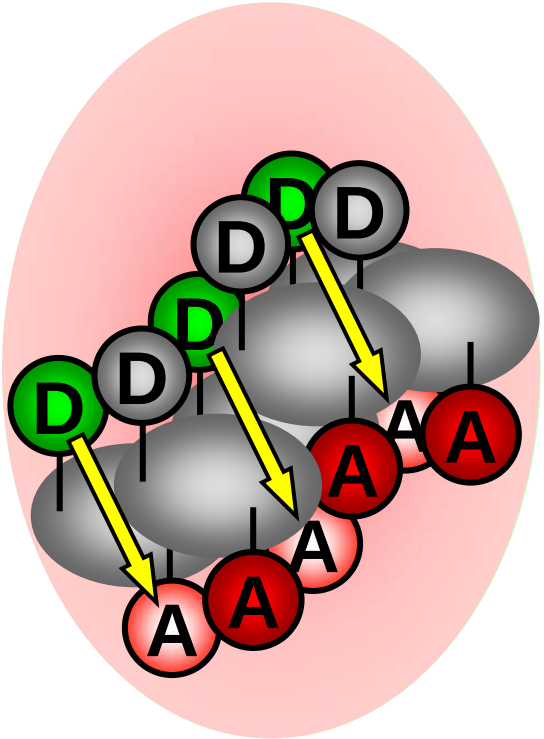
(distance information)

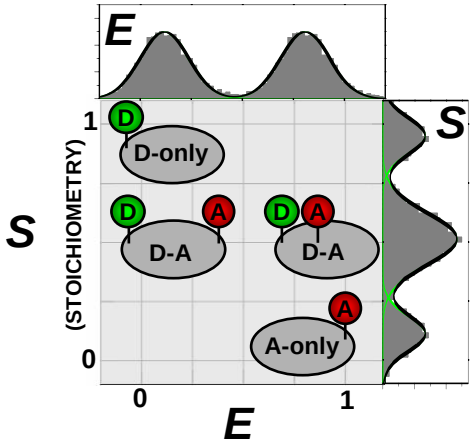


E (FRET efficiency)

Ha *et al*, PNAS, 1996; Deniz *et al*, PNAS, 1999

Alternating-Laser EXcitation (ALEX) Spectroscopy



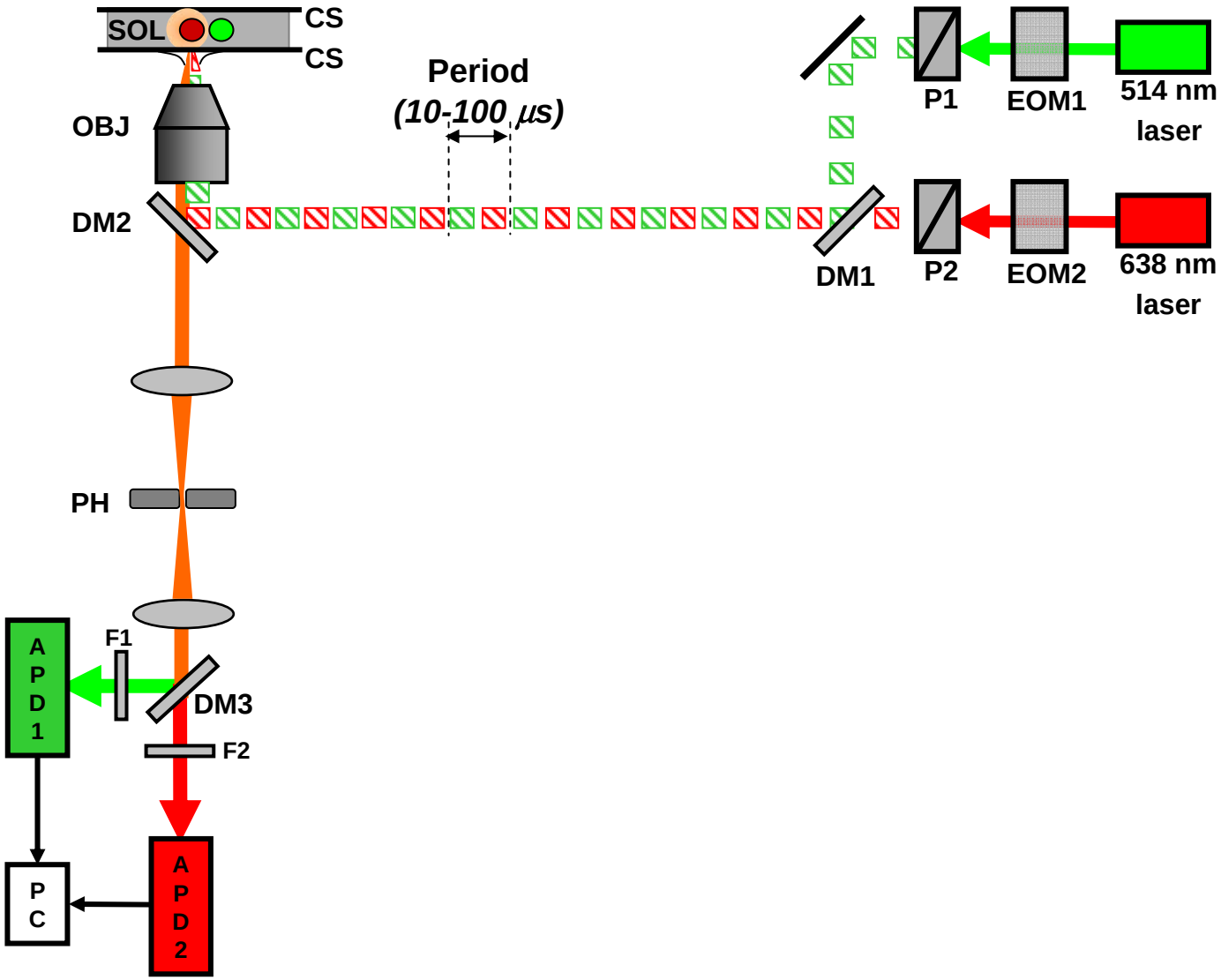


Kapanidis *et al*, PNAS, 2004

Alternating-laser excitation (ALEX) of single molecules

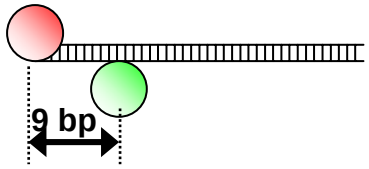
Collaboration with S. Weiss (UCLA)

Alternating-laser excitation microscopy

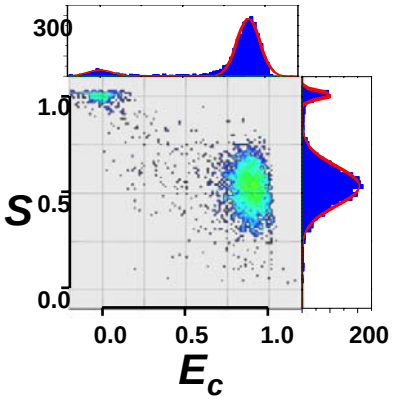


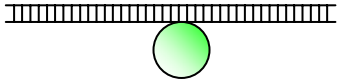
Fluorescence-aided molecule sorting

Model systems: DNA with different interprobe distance and probe stoichiometry

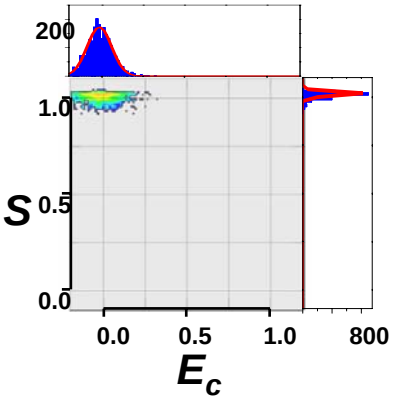


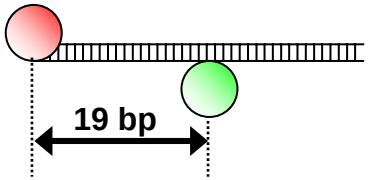
$R_{D\text{-}A}$~ 4 nm:
high FRET



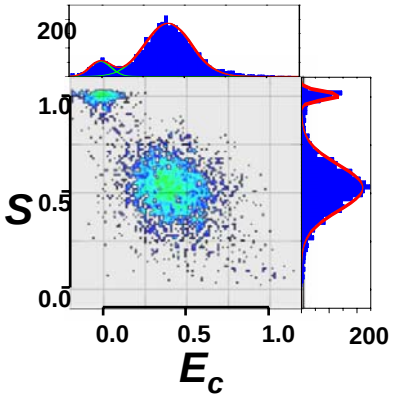


Donor-only DNA

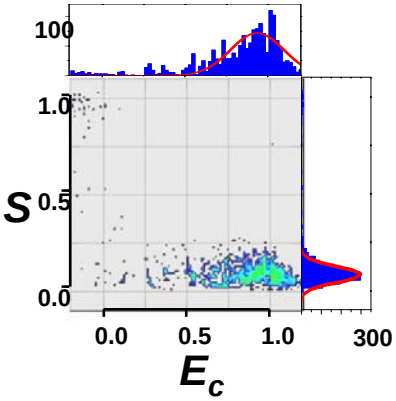


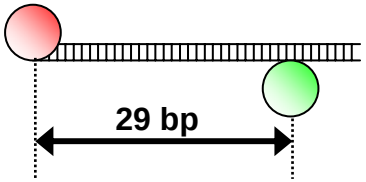


$R_{D\text{-}A}$~ 7 nm:
intermediate FRET

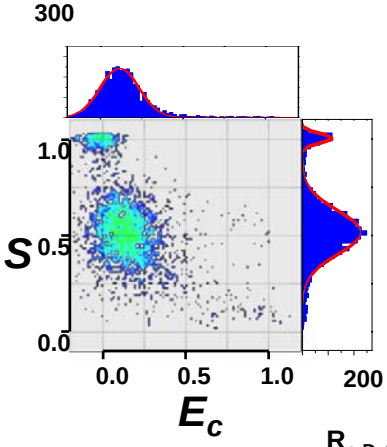


Acceptor-only DNA





$R_{D\text{-}A}$~ 11 nm:
low FRET

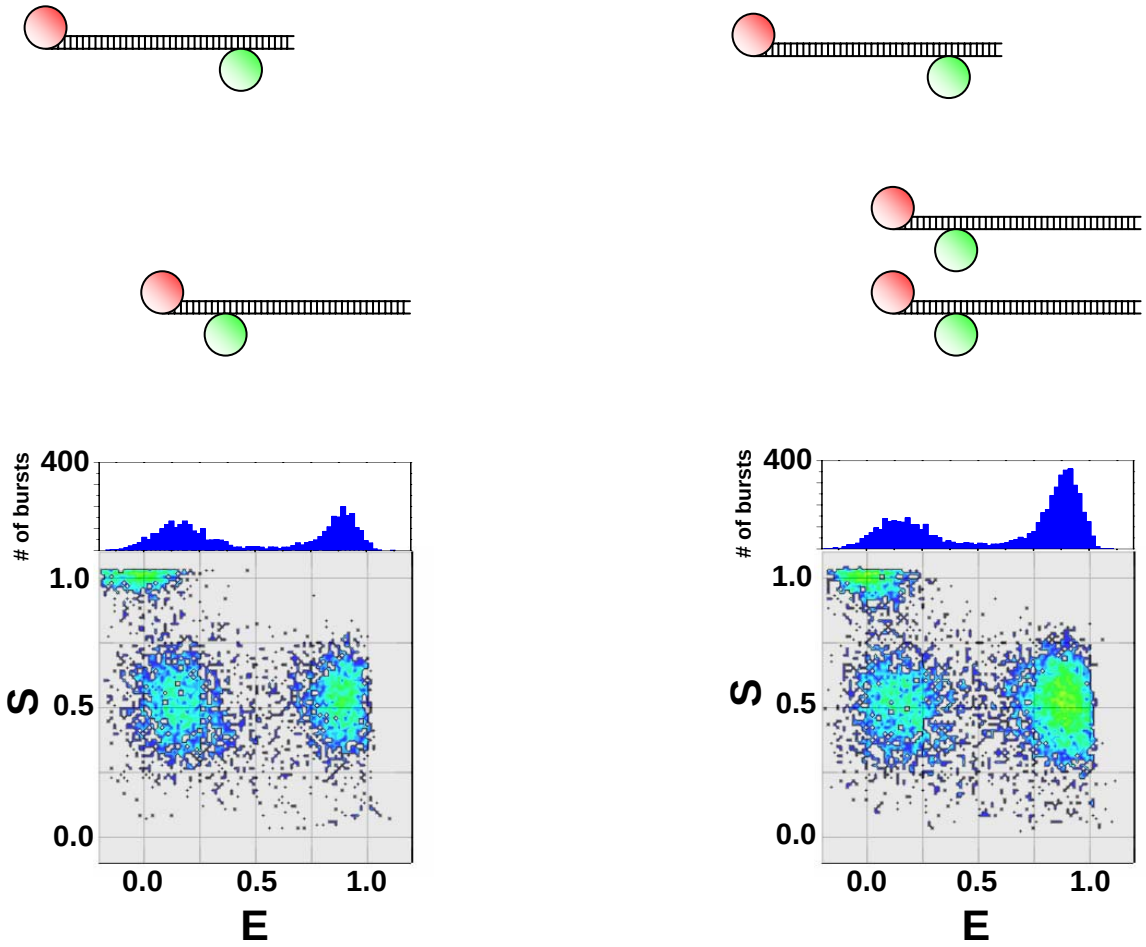


$R_{o,D\text{-}A}$~ 6.9 nm

Quantitation of mixture components

Low-E

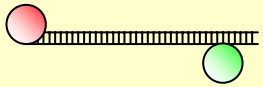
High-E



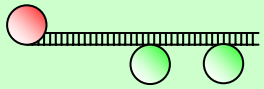
Ratio $\frac{\text{Low-E}}{\text{High-E}}$ **1:0.9** **1:1.8**

- accurate quantitation of species with same diffusion time

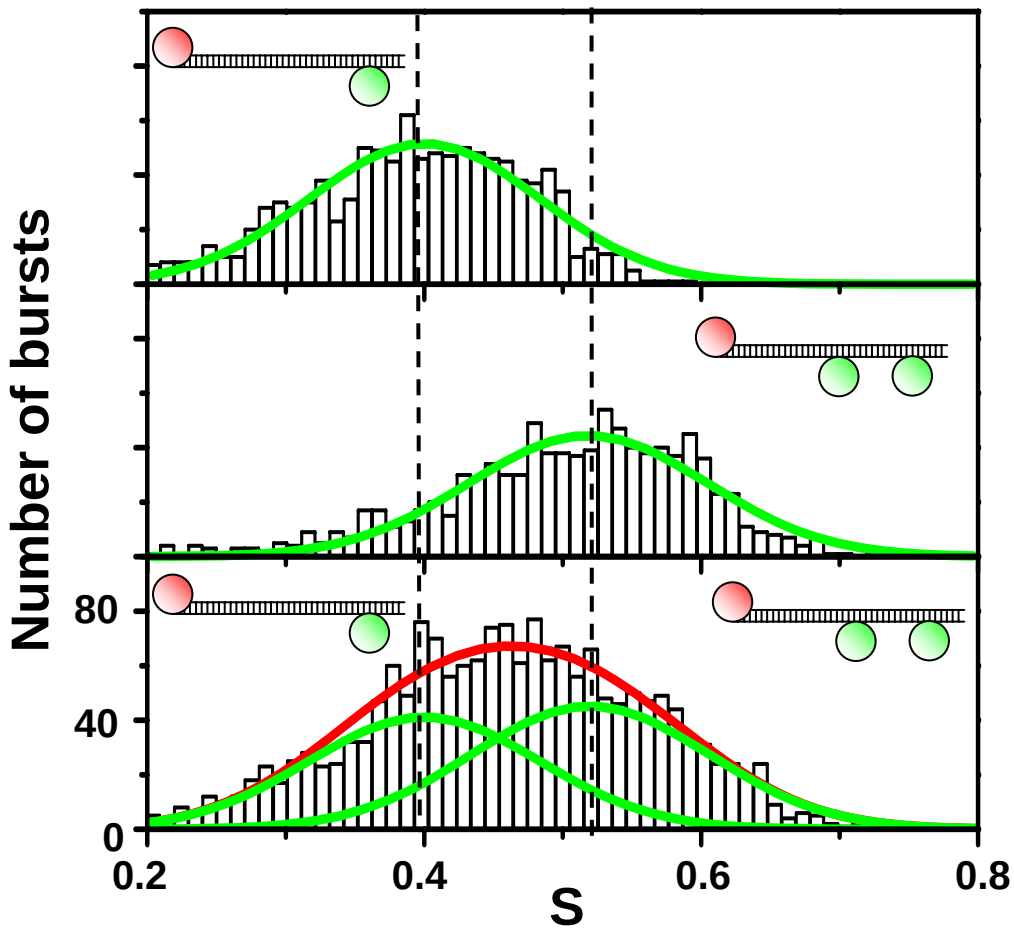
Analysis of complex stoichiometries



$$S = \frac{F_{514ex}}{F_{514ex} + F_{638ex}}$$



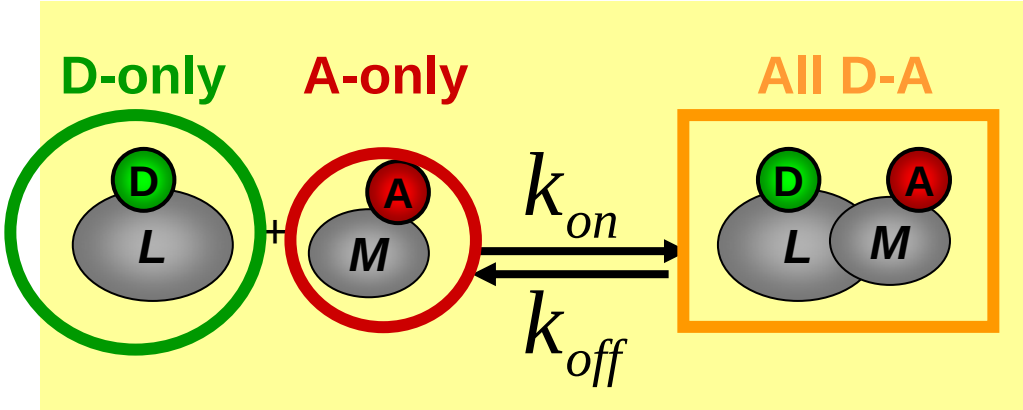
$$S = \frac{2 * F_{514ex}}{2 * F_{514ex} + F_{638ex}}$$



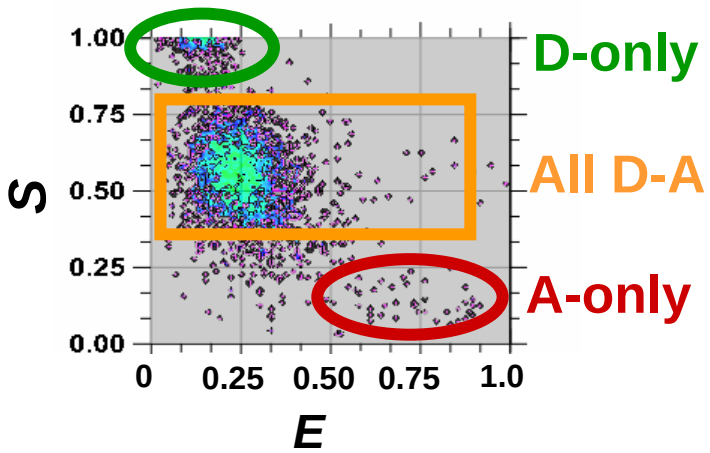
Ratio $\frac{D\text{-}A}{D_2\text{-}A}$ 0.9:1

- can monitor protein dimerization and oligomerization

Monitoring biomolecular interactions

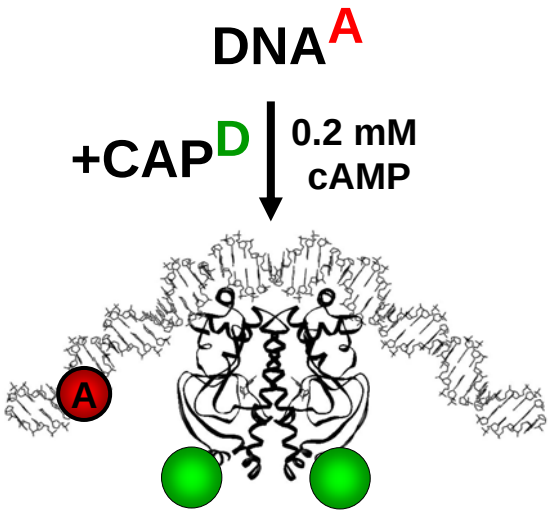


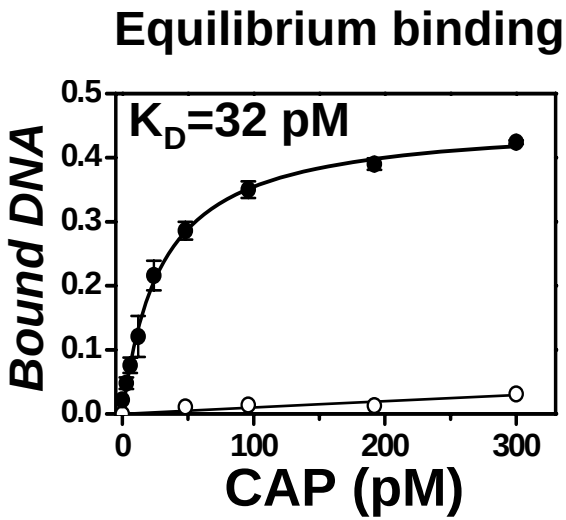
$$K_a = \frac{k_{on}}{k_{off}} = \frac{[ML]}{[M][L]}$$



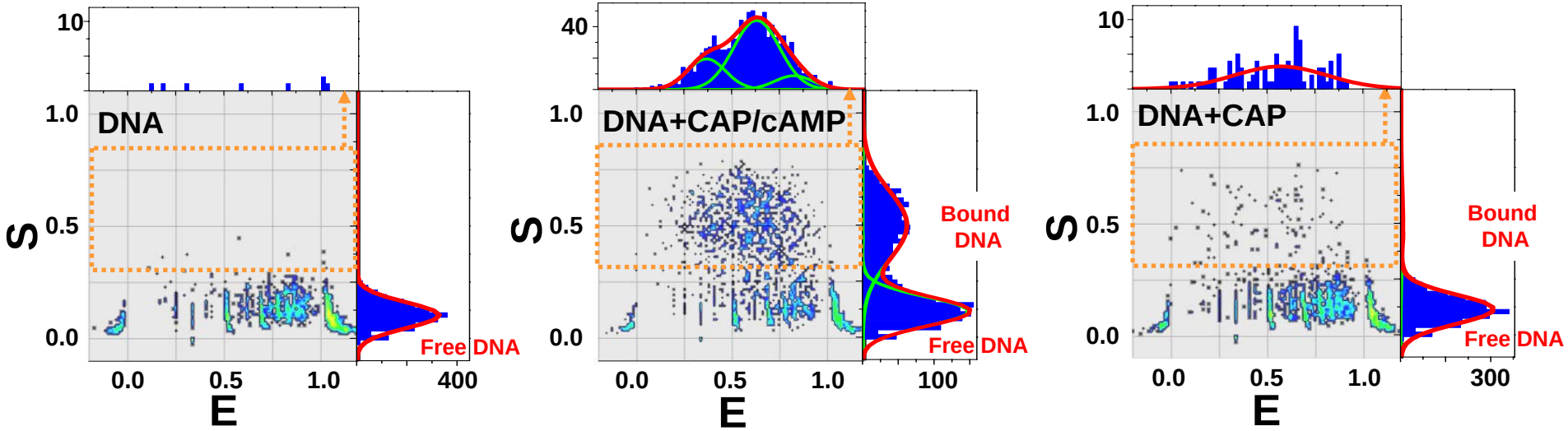
- **Affinity of interaction (K_a)**
- **Association/dissociation kinetics (k_{on},k_{off})**
- **All constants with structural information**
- **Can evaluate effects of unlabeled small molecules (e.g., drug candidates)**

Application: protein-DNA interactions

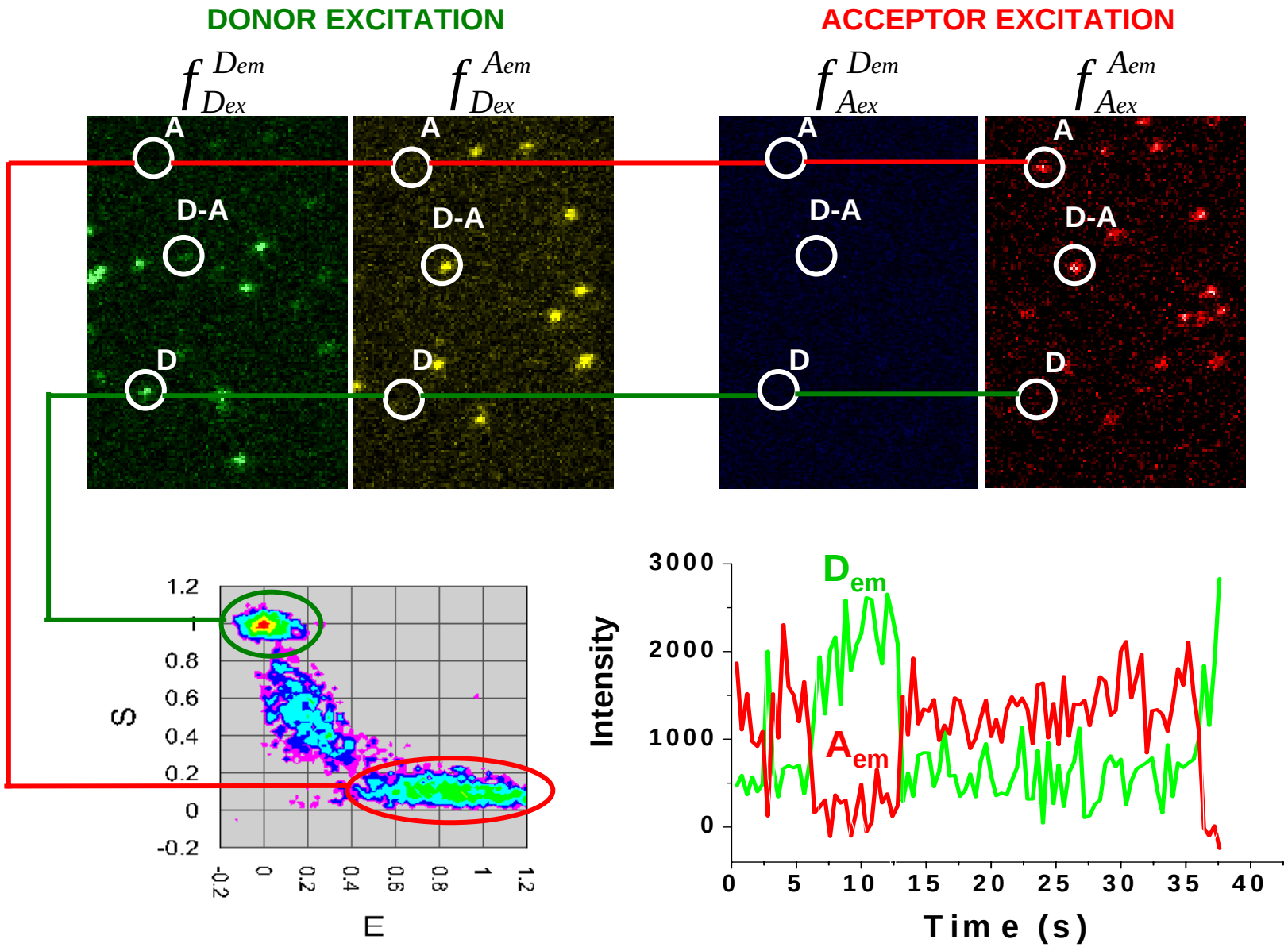




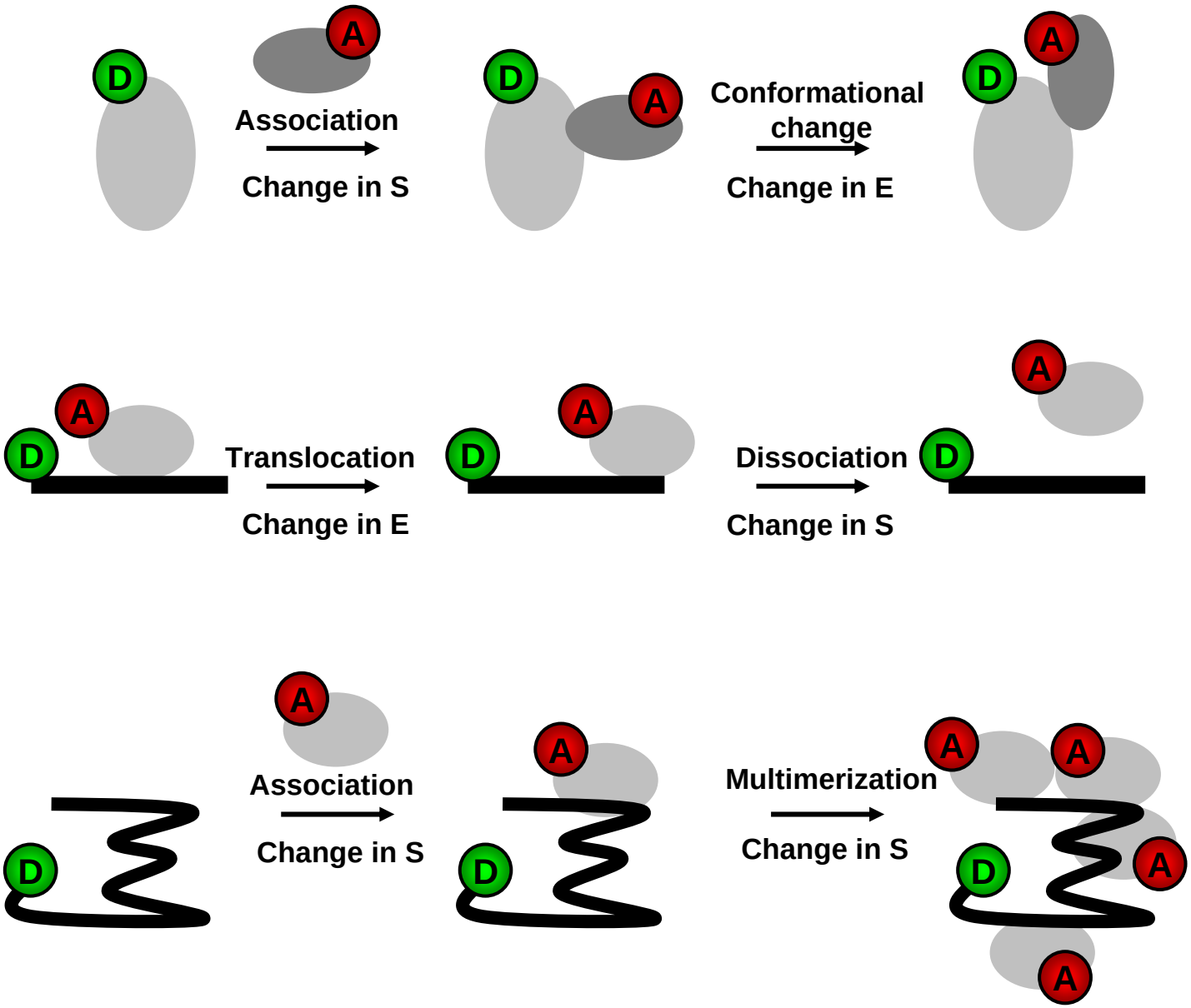
Excellent agreement with ensemble measurements



Sorting of immobilized molecules



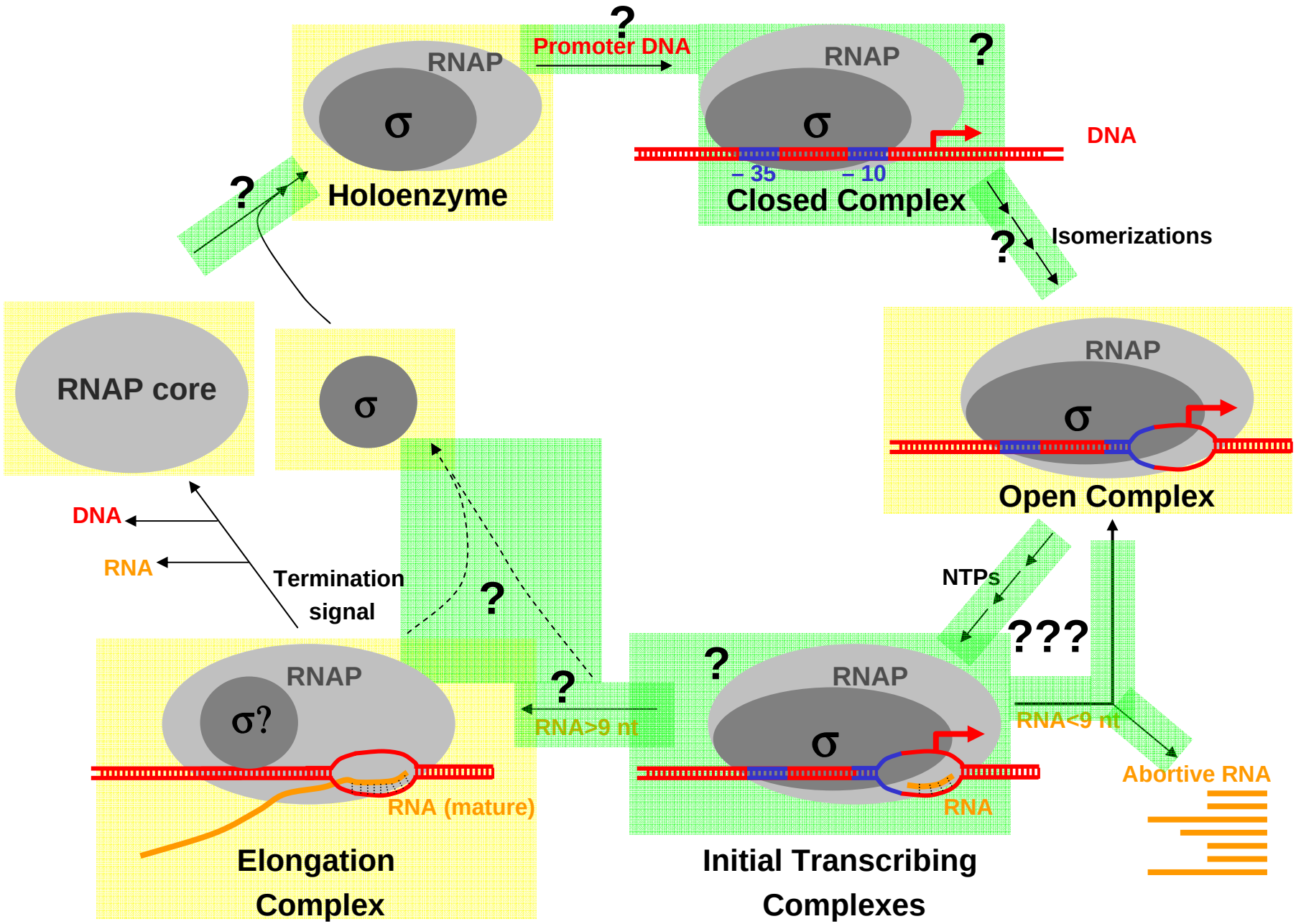
ALEX applications



Mechanism of gene transcription: abortive initiation

Collaboration with S. Weiss (UCLA), R. Ebright (Rutgers)

Bacterial transcription cycle

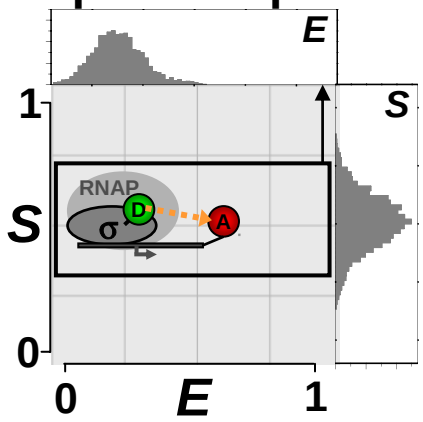


ALEX assay for protein movement and dissociation: σ^{70} release in elongation

LEADING-EDGE FRET ASSAY

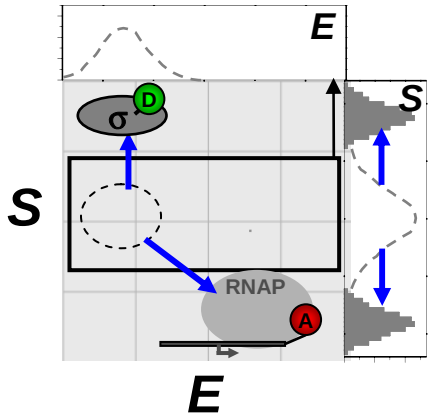
Initiation:

Open complex



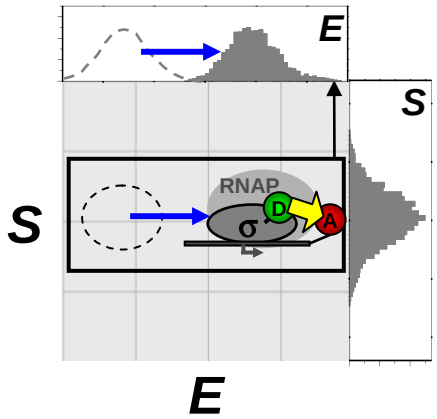
NTPs

Elongation: Release



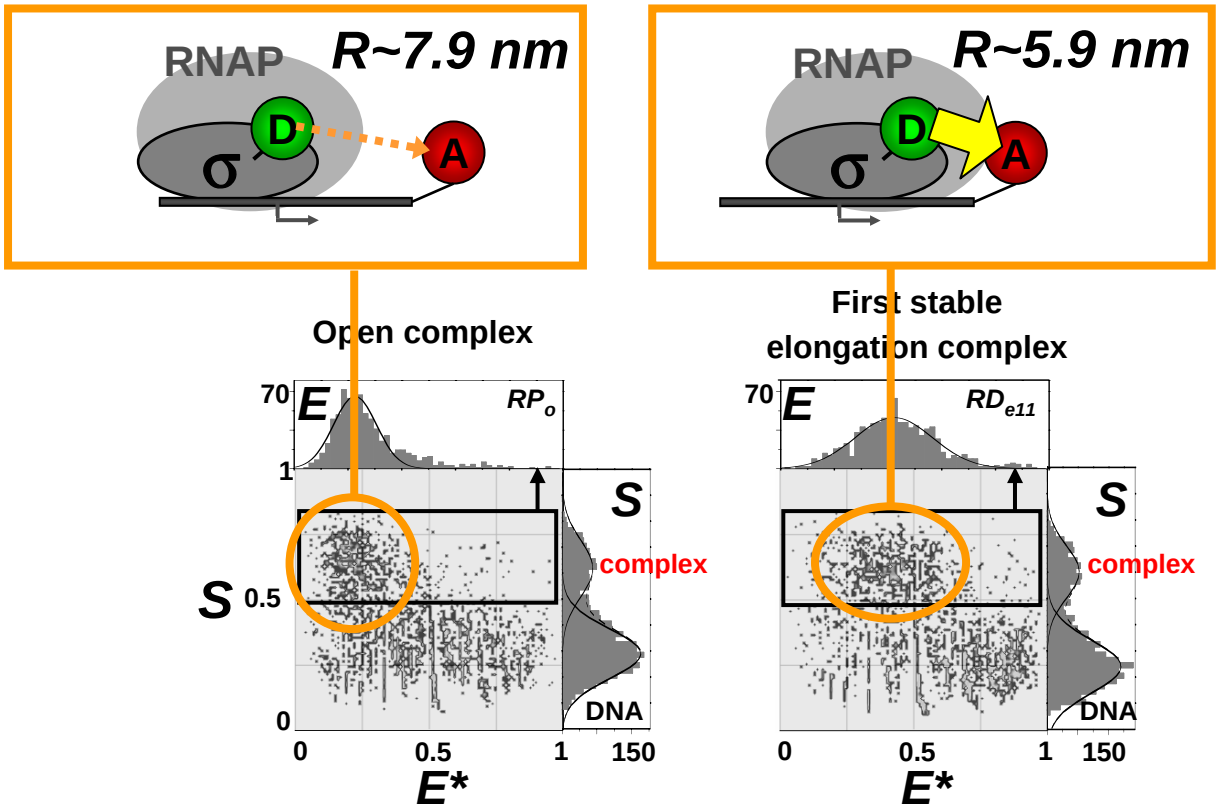
NTPs

Elongation: No release



Studying σ^{70} retention in transcription elongation

~ 2 nm distance change:
equivalent to RNAP
translocation
by 1 turn of DNA

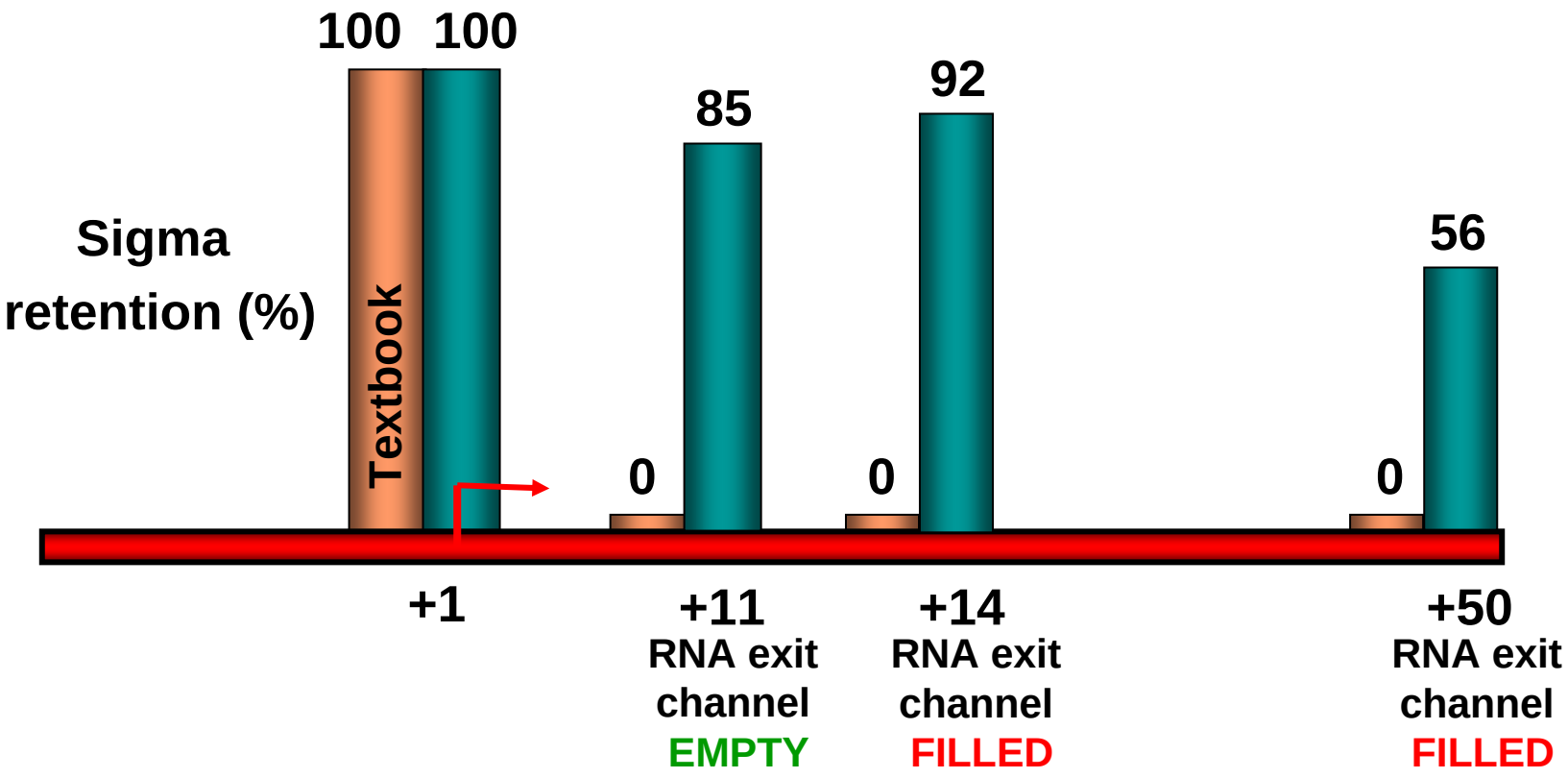


– σ^{70} retention in the first elongation complex

- High translocational activity

Lee, Kapanidis et al. (2005) *Biophys J* 88, 2939; Kapanidis et al. (2005). *Mol Cell* issue of Nov 11

Summary of σ^{70}-retention results



- Stochastic, non-obligatory σ^{70} release during elongation *in vitro*

-Determinants on DNA sequence

-Consistent with recent *in vivo* data