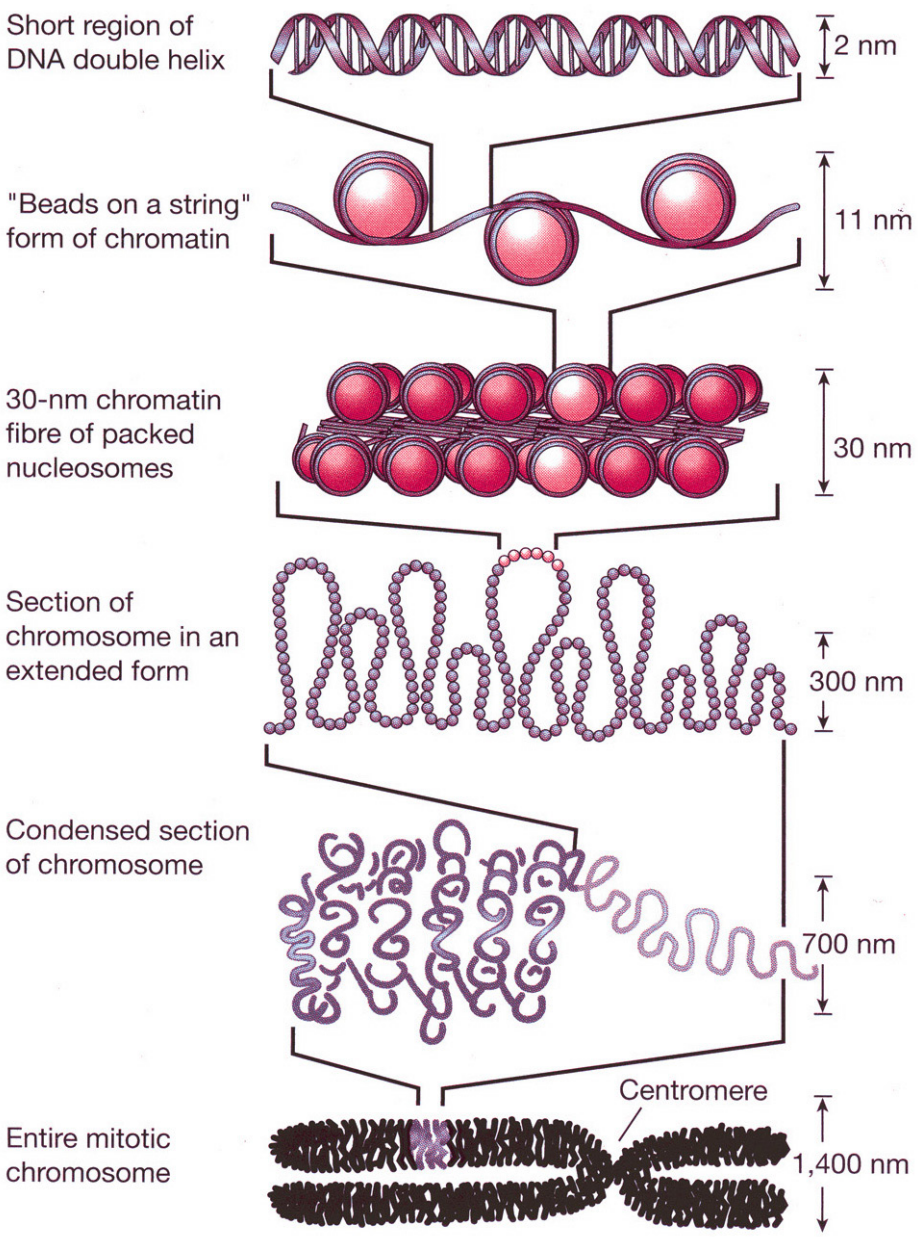
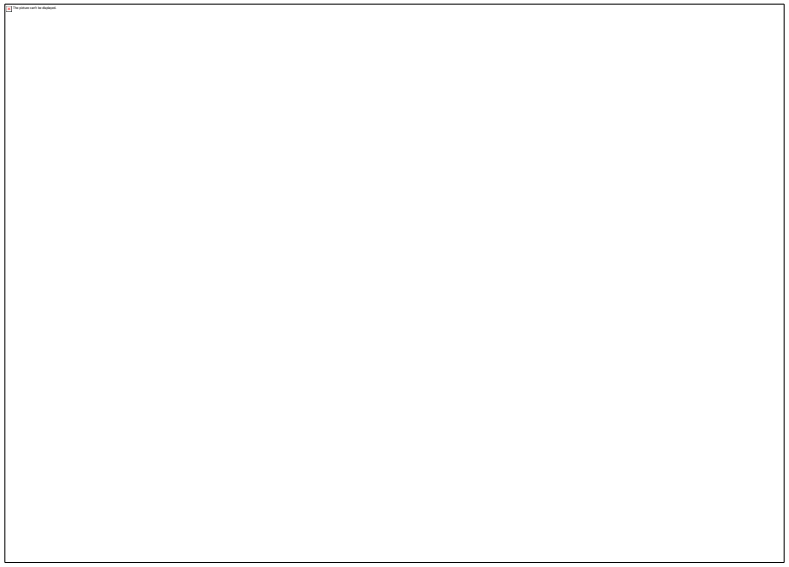
Dynamics of nucleosomal DNA

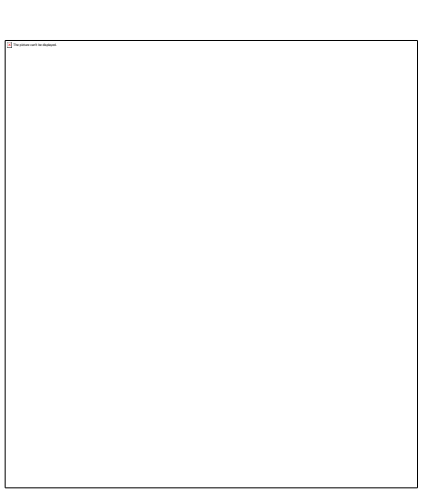


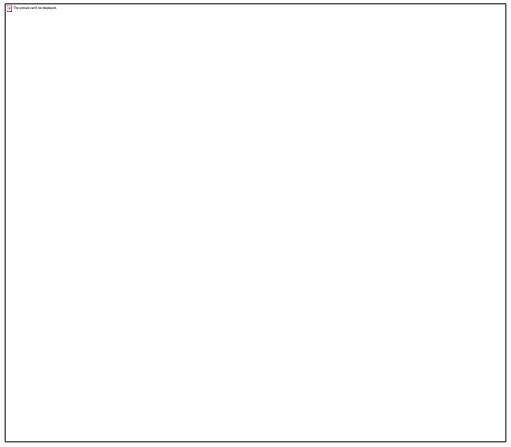
Felsenfeld, G. & Groudine, M. (2003), *Nature* 421: 448-453

NF-KB; Chen et al., Nature 391: 410, 1998

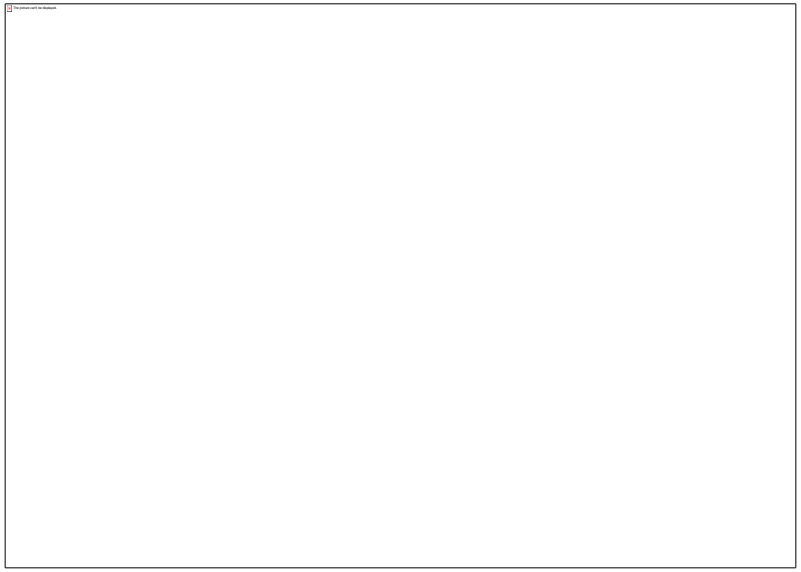


GCN4; Ellenberger et al., Cell 71: 1223, 1992



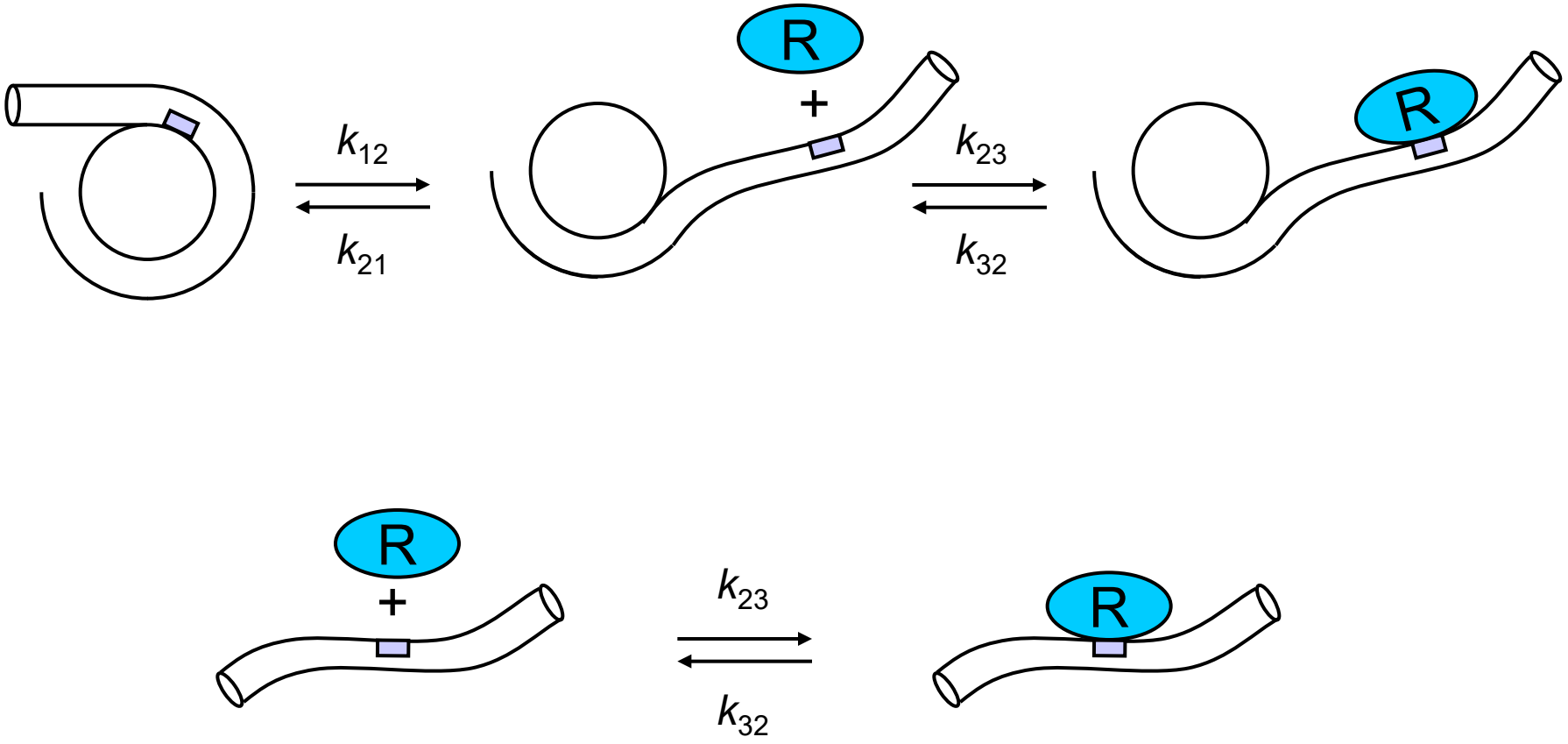


Zif268; Pavletich & Pabo, Science 252: 809, 1991



Bam H1; Newman et al., Science 269: 656, 1995

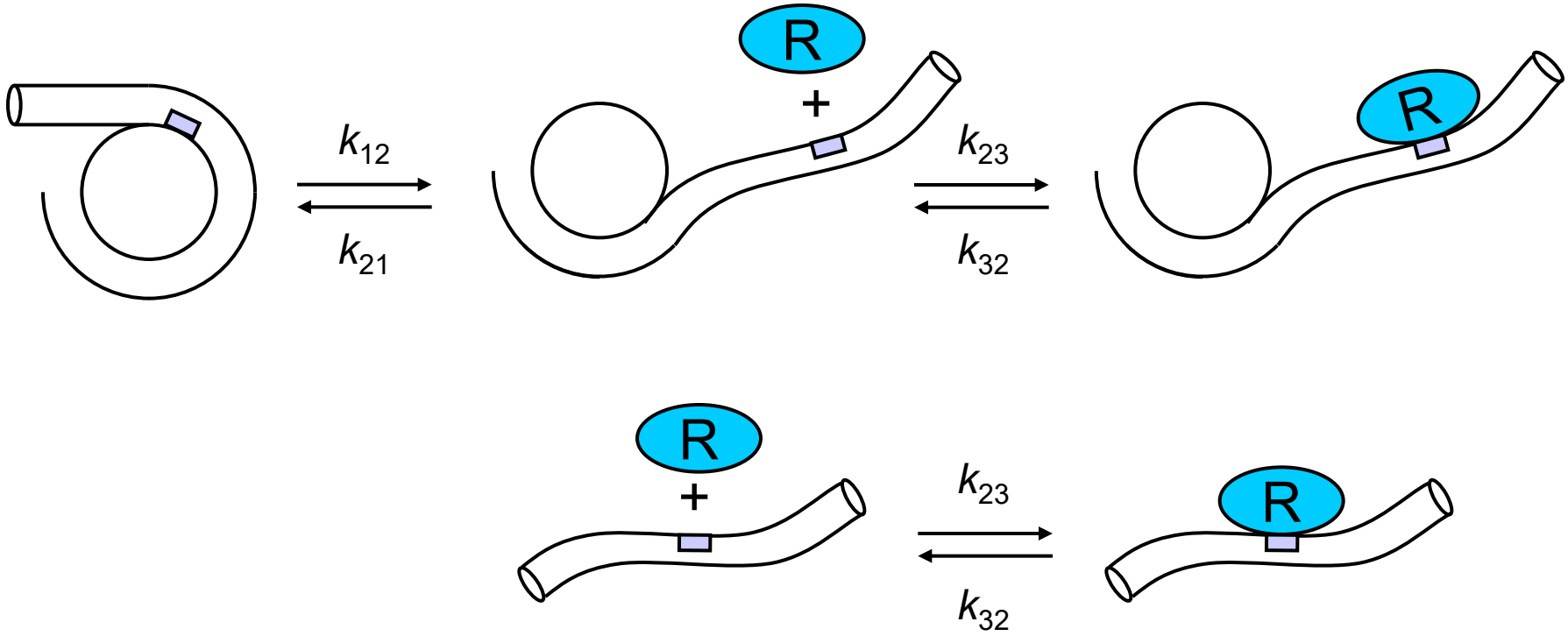
A model for the spontaneous accessibility of nucleosomal DNA target sites



Polach & Widom, 1995

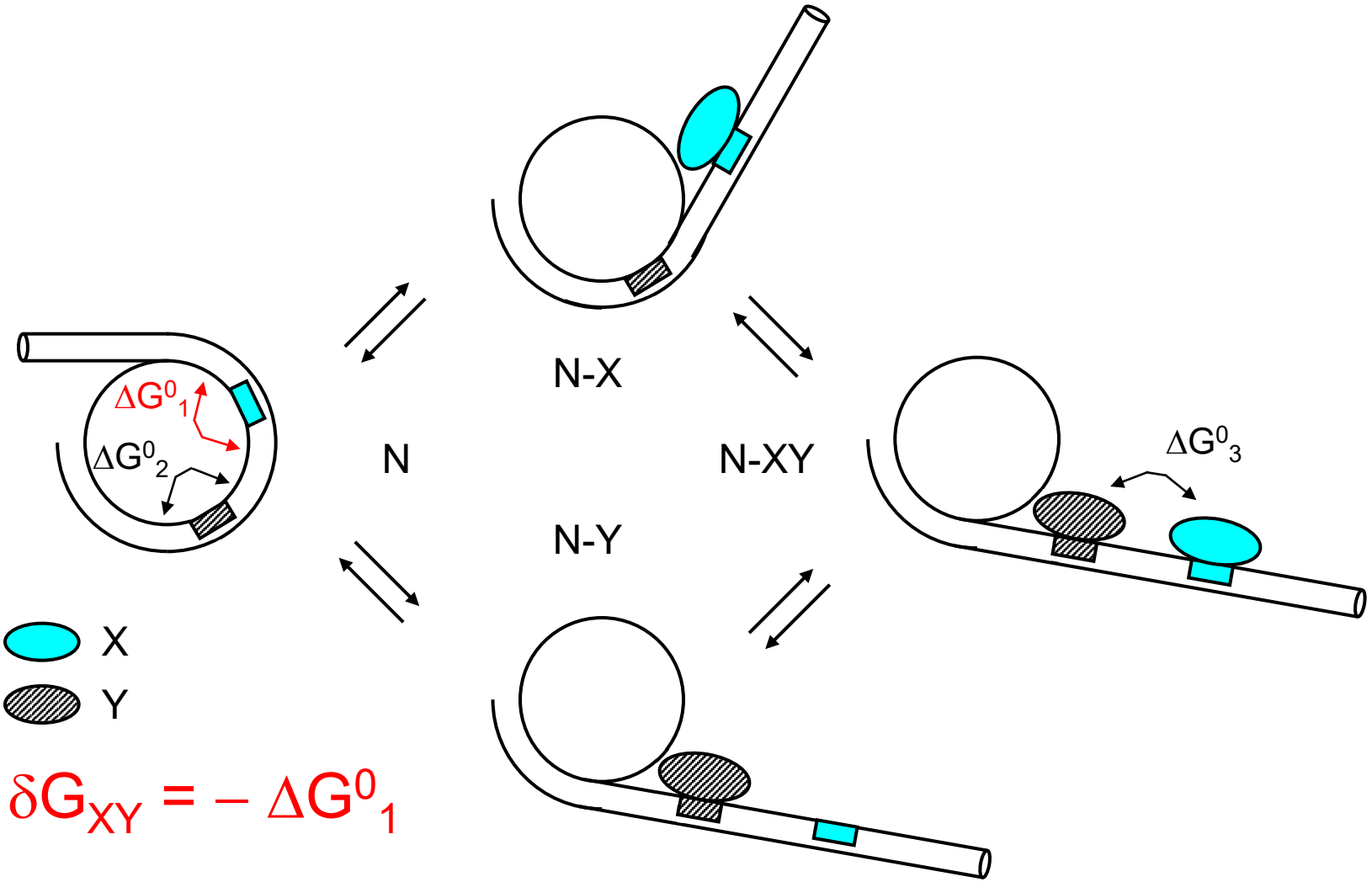
Any protein can bind to DNA in a nucleosome, and we can predict its affinity

$$K_{d,nucleosome} = K_{d,naked\ DNA} / K_{eq}^{conf}$$



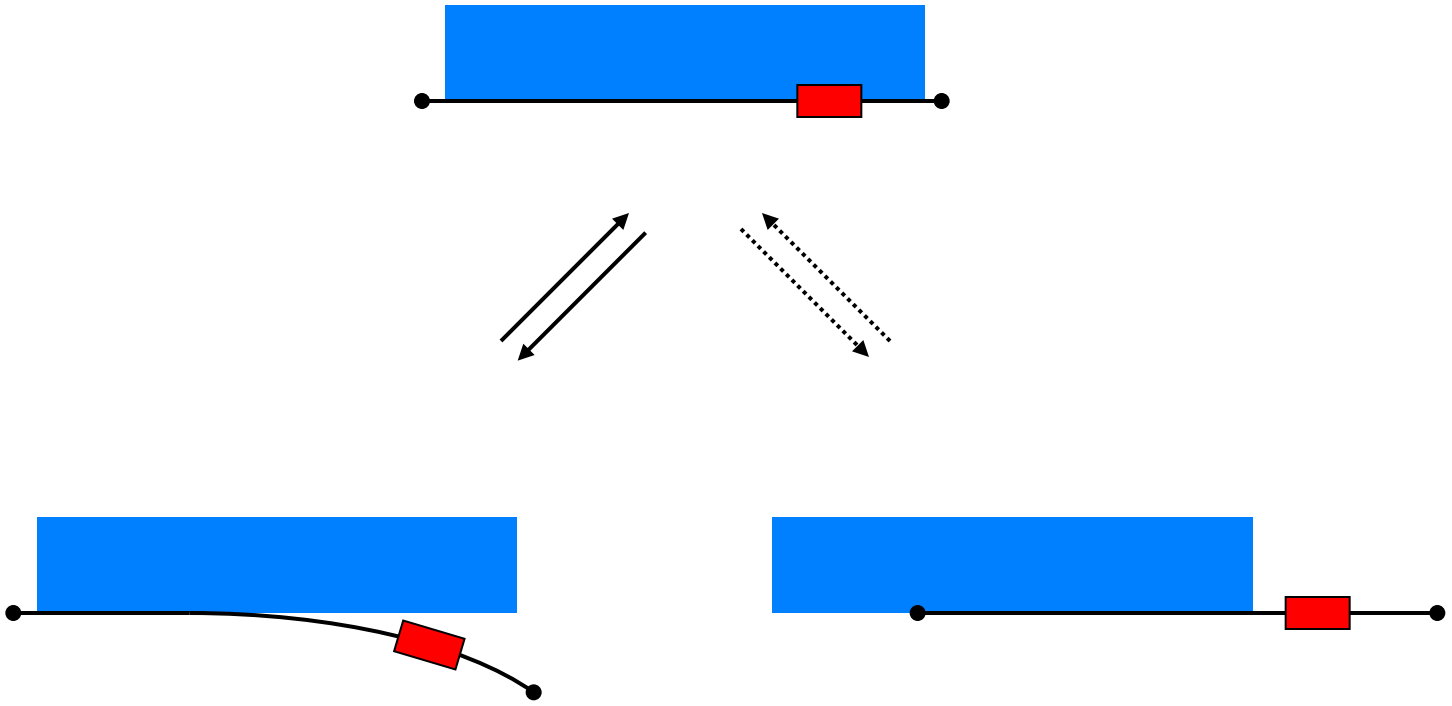
Polach & Widom, 1995

Cooperative invasion of a nucleosome by collaborative competition with histone octamer



Polach & Widom, 1996
Miller & Widom, 2003

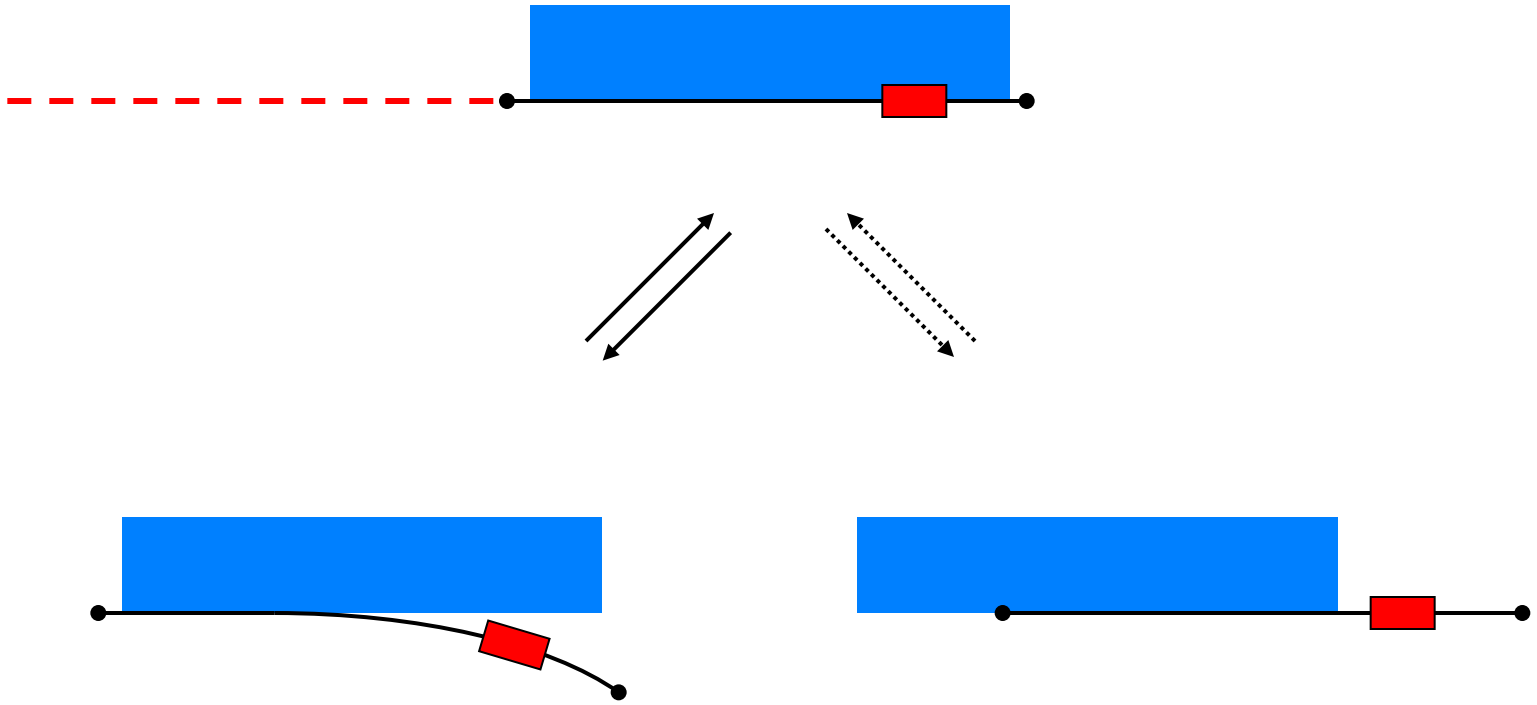
Uncoiling *vs* sliding for a mononucleosome



Energetic costs are similar

Anderson et al., 2002

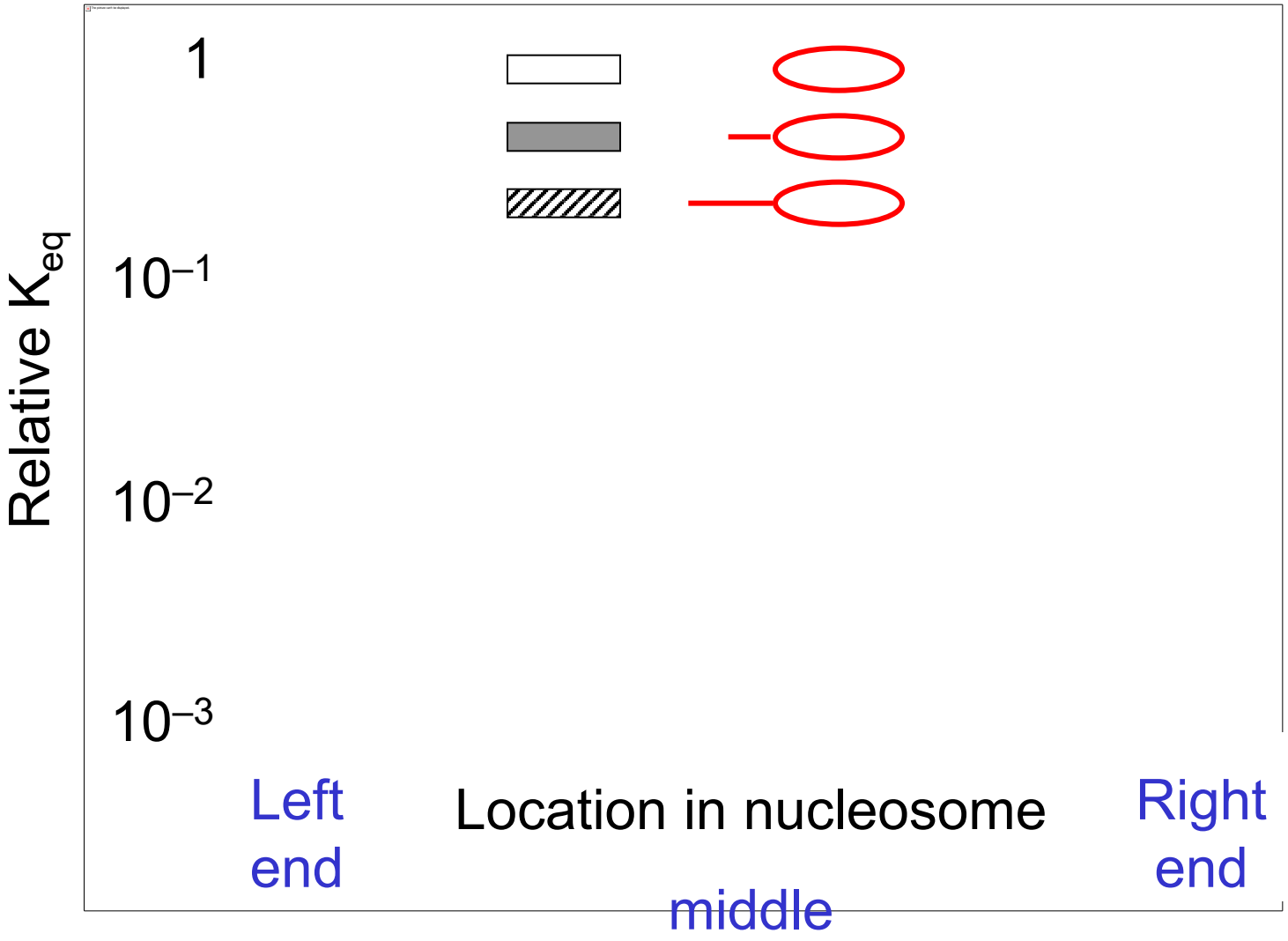
Test of uncoiling *vs* sliding for a mononucleosome



•Extra DNA favors mobility but does not affect uncoiling

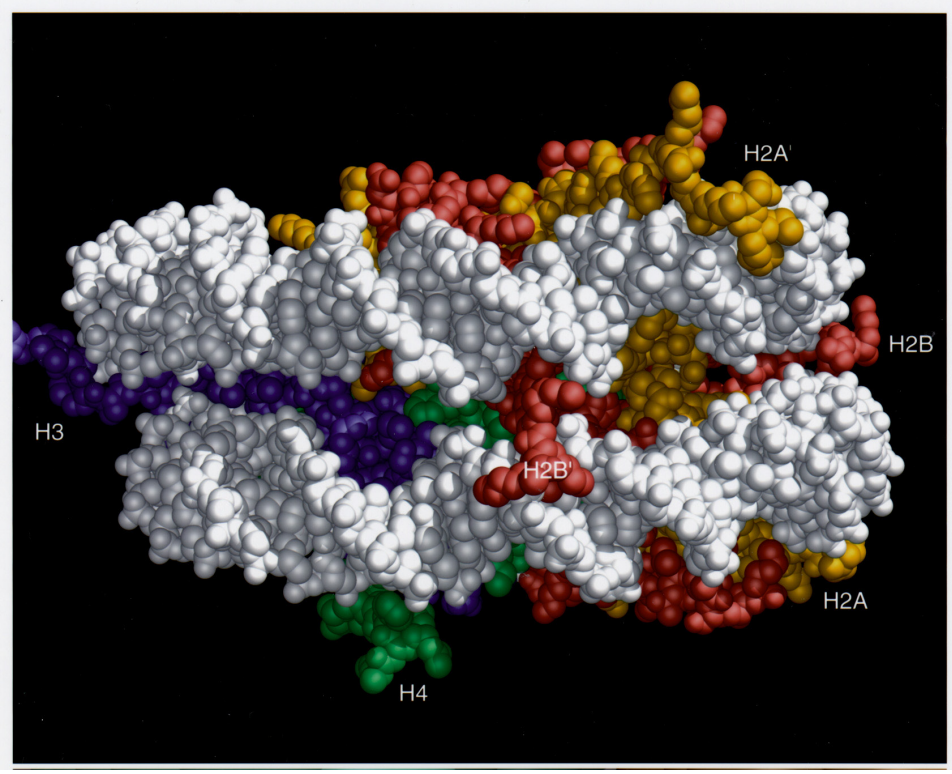
Anderson et al., 2002

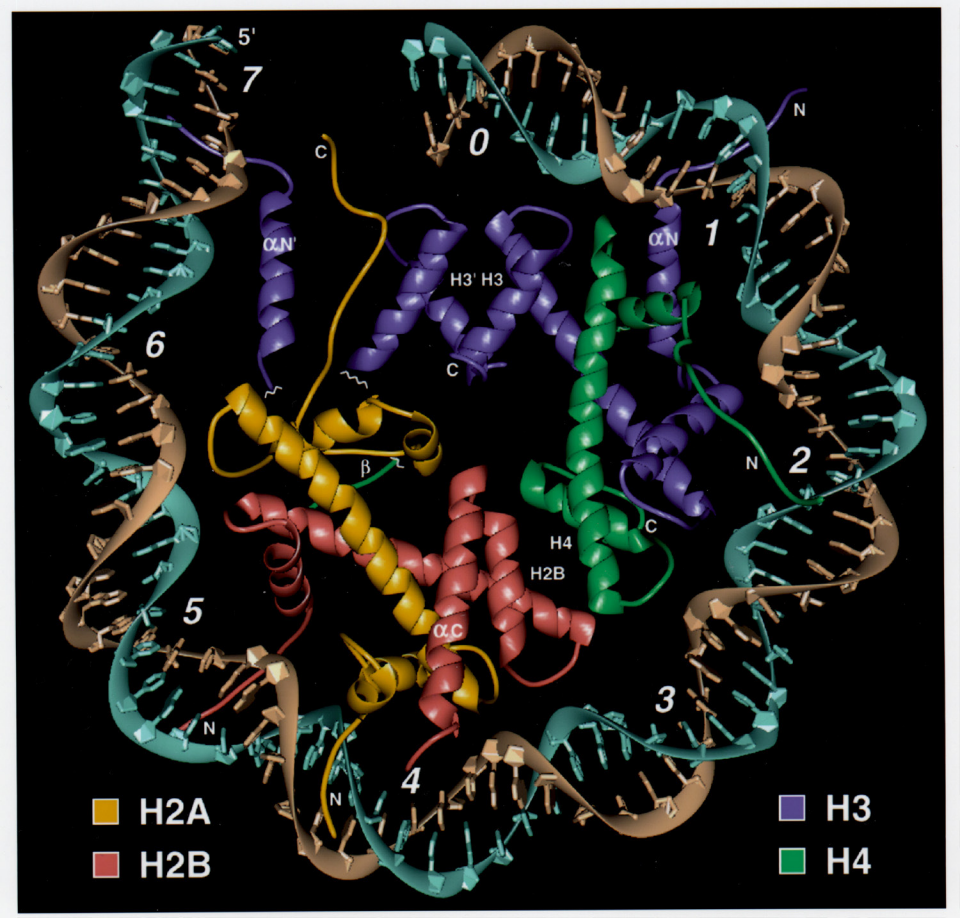
Site exposure occurs without nucleosome sliding



Anderson et al., 2002

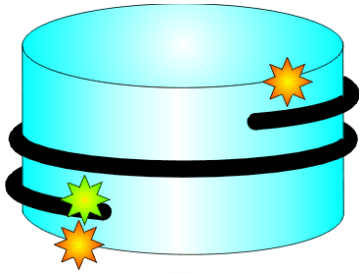
Site exposure via stepwise unwrapping from one end of the nucleosomal DNA

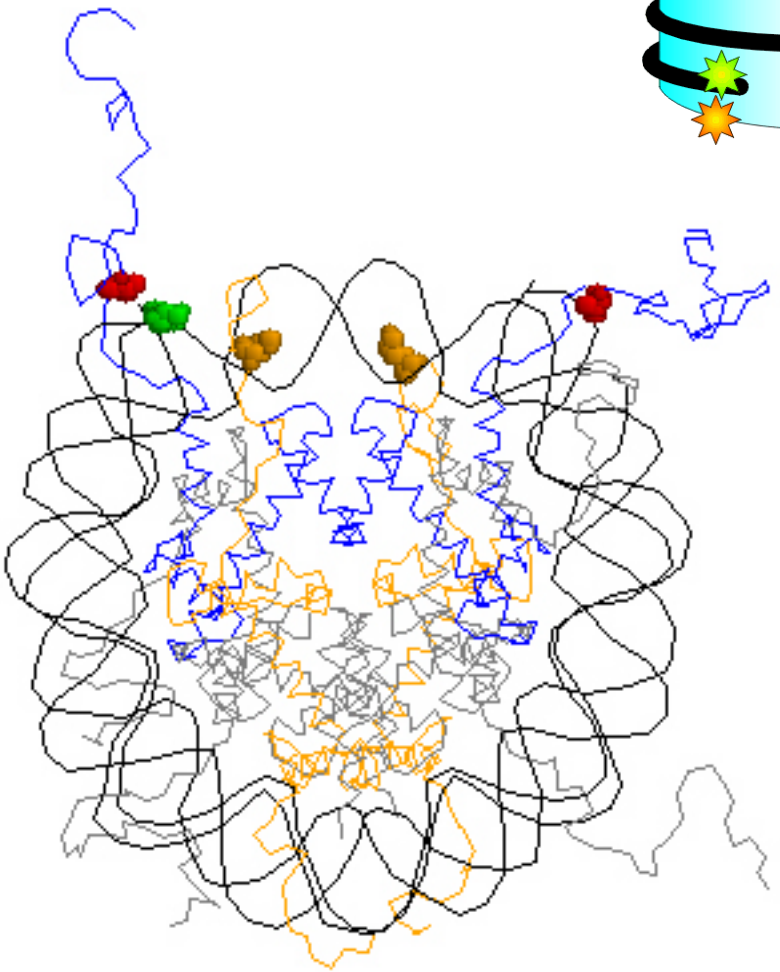


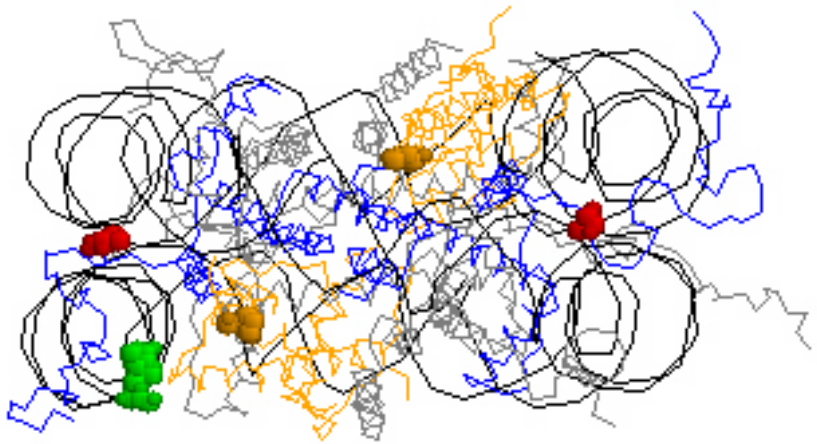


Luger & Richmond, 1997

FRET systems for analysis of nucleosome dynamics



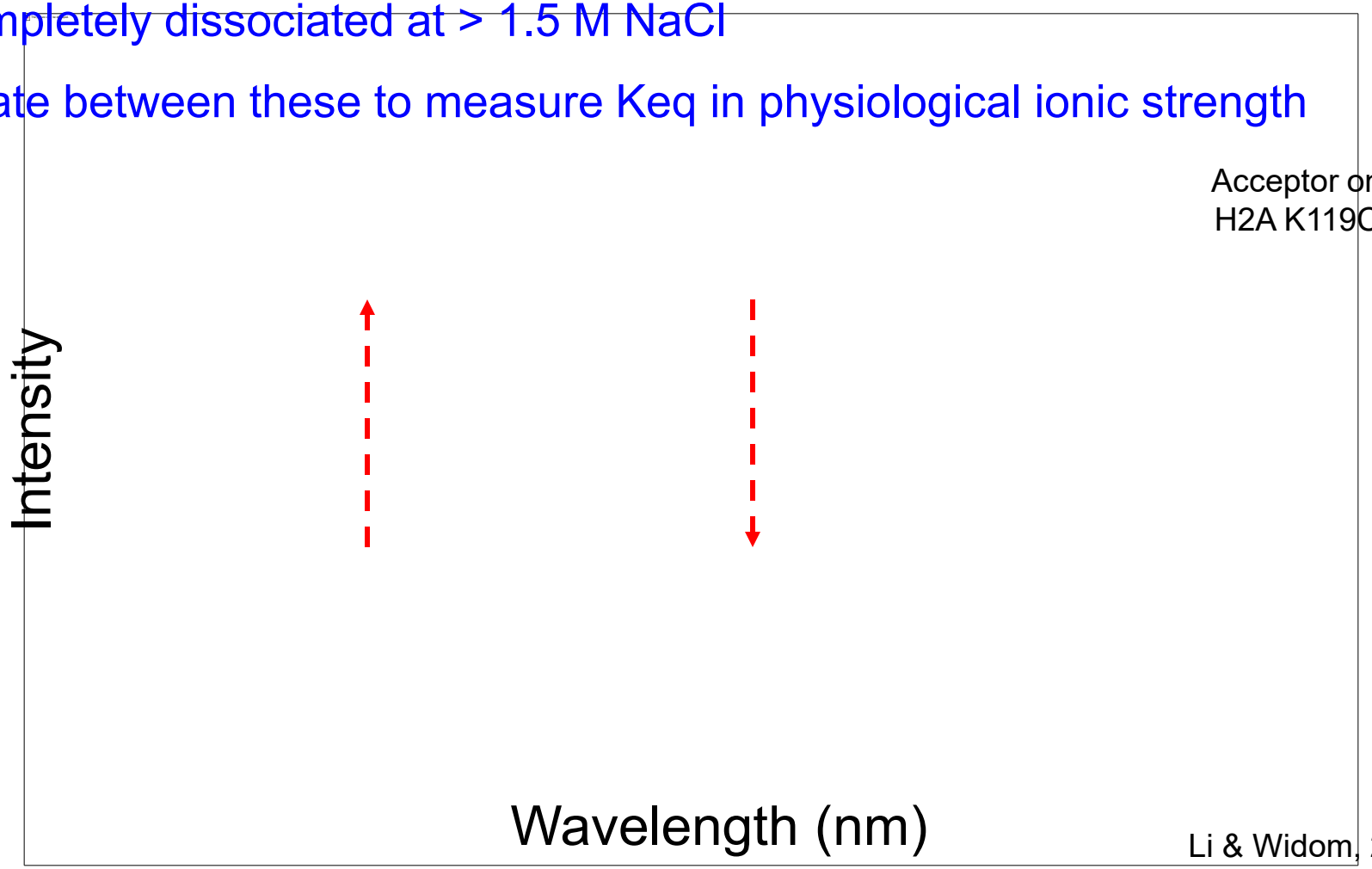




Li & Widom, 2004

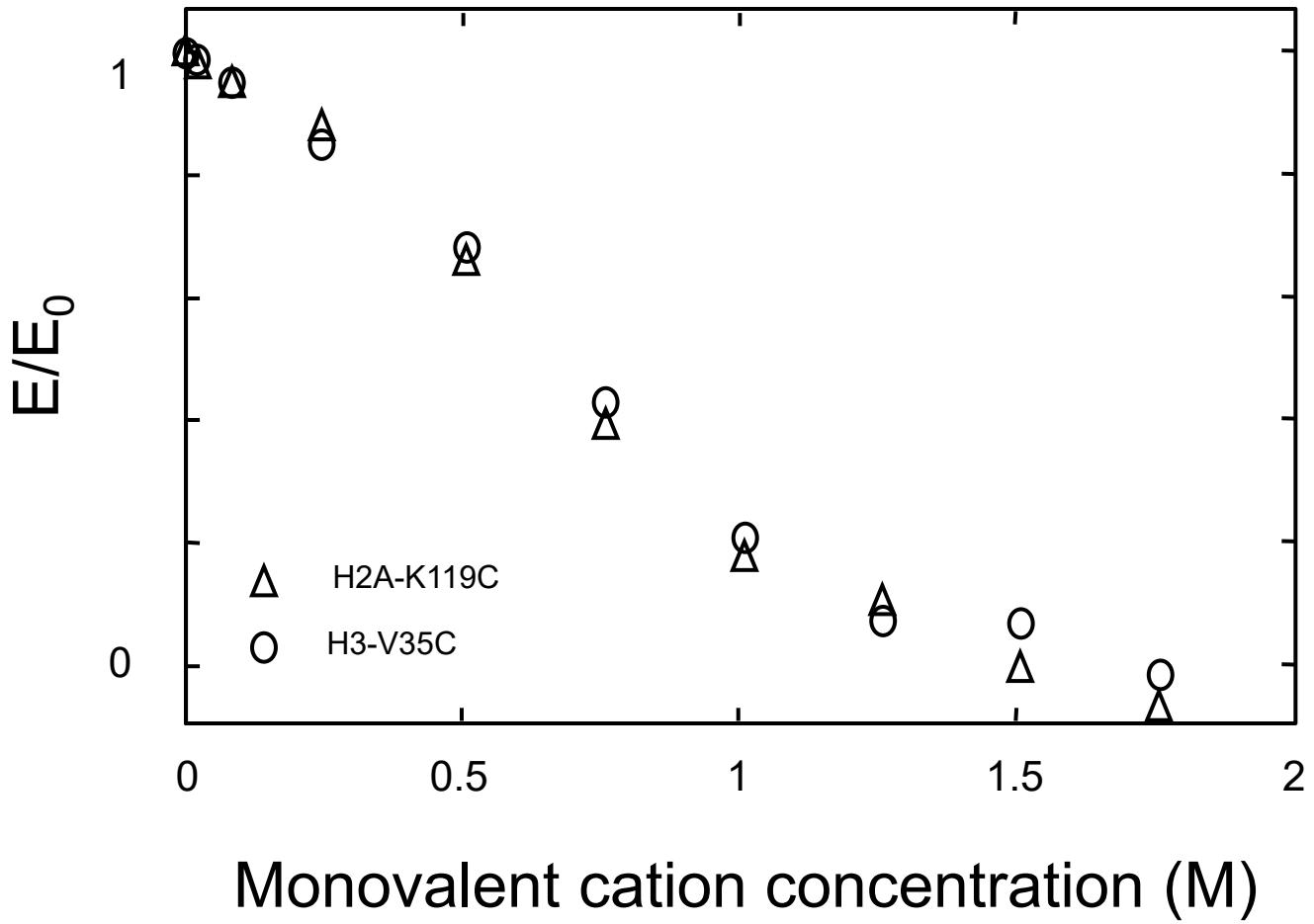
Nucleosome conformational fluctuations in physiological ionic strength solution

- Artificially stable wrapping in sub-physiological ionic strength
- Completely dissociated at > 1.5 M NaCl
- Titrate between these to measure Keq in physiological ionic strength



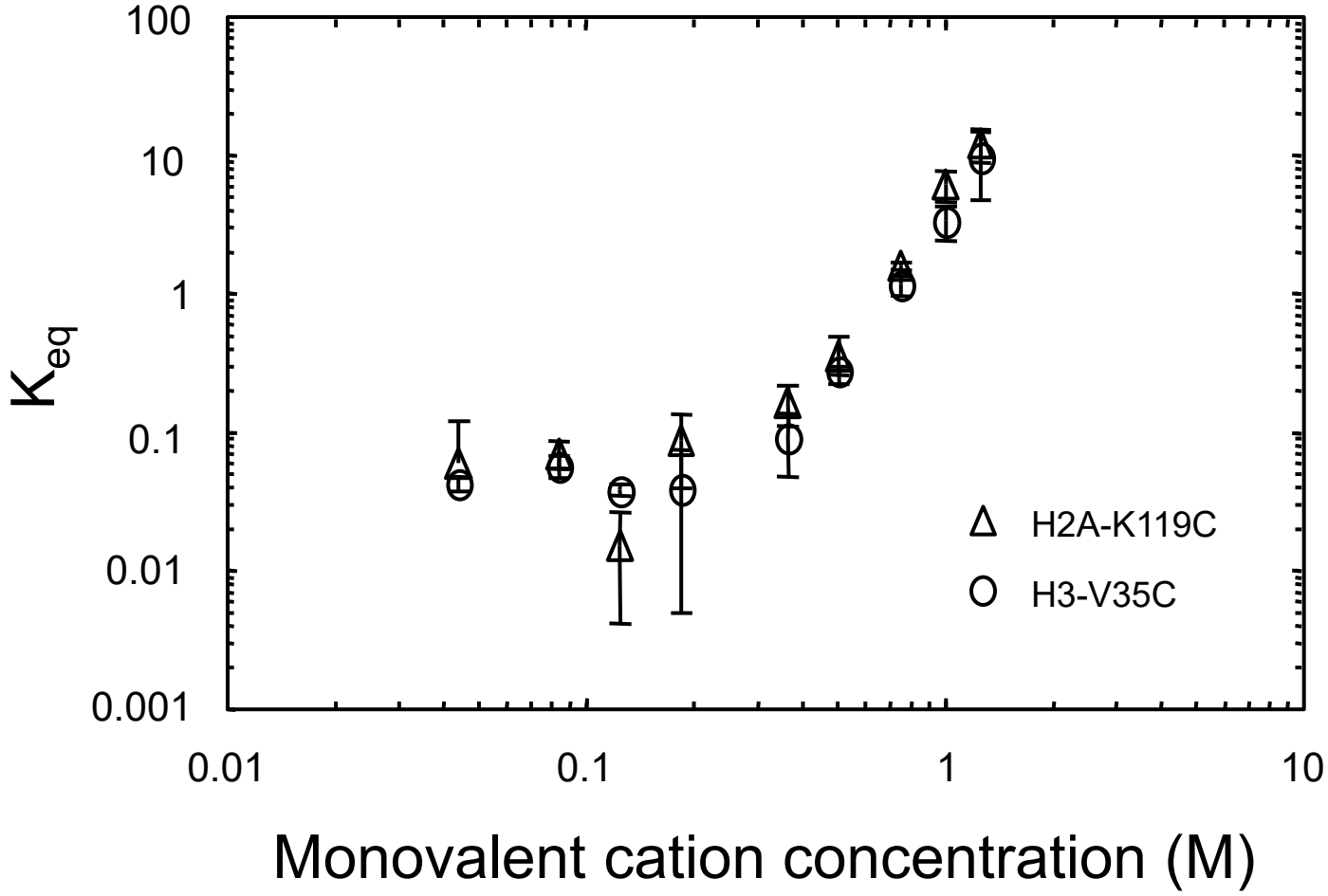
Li & Widom, 2004

FRET efficiency *vs* monovalent cation concentration



Li & Widom, 2004

Equilibrium constant for DNA unwrapping *vs* monovalent cation concentration



Li & Widom, 2004

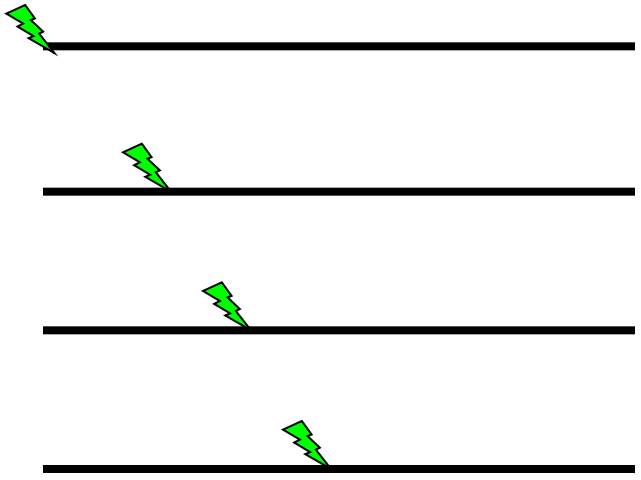
Site exposure at internal nucleosome target sites: Cy3-labeled DNA constructs

Cy3 601–147

601–147 LexA L Cy3 35

601–147 LexA L Cy3 57

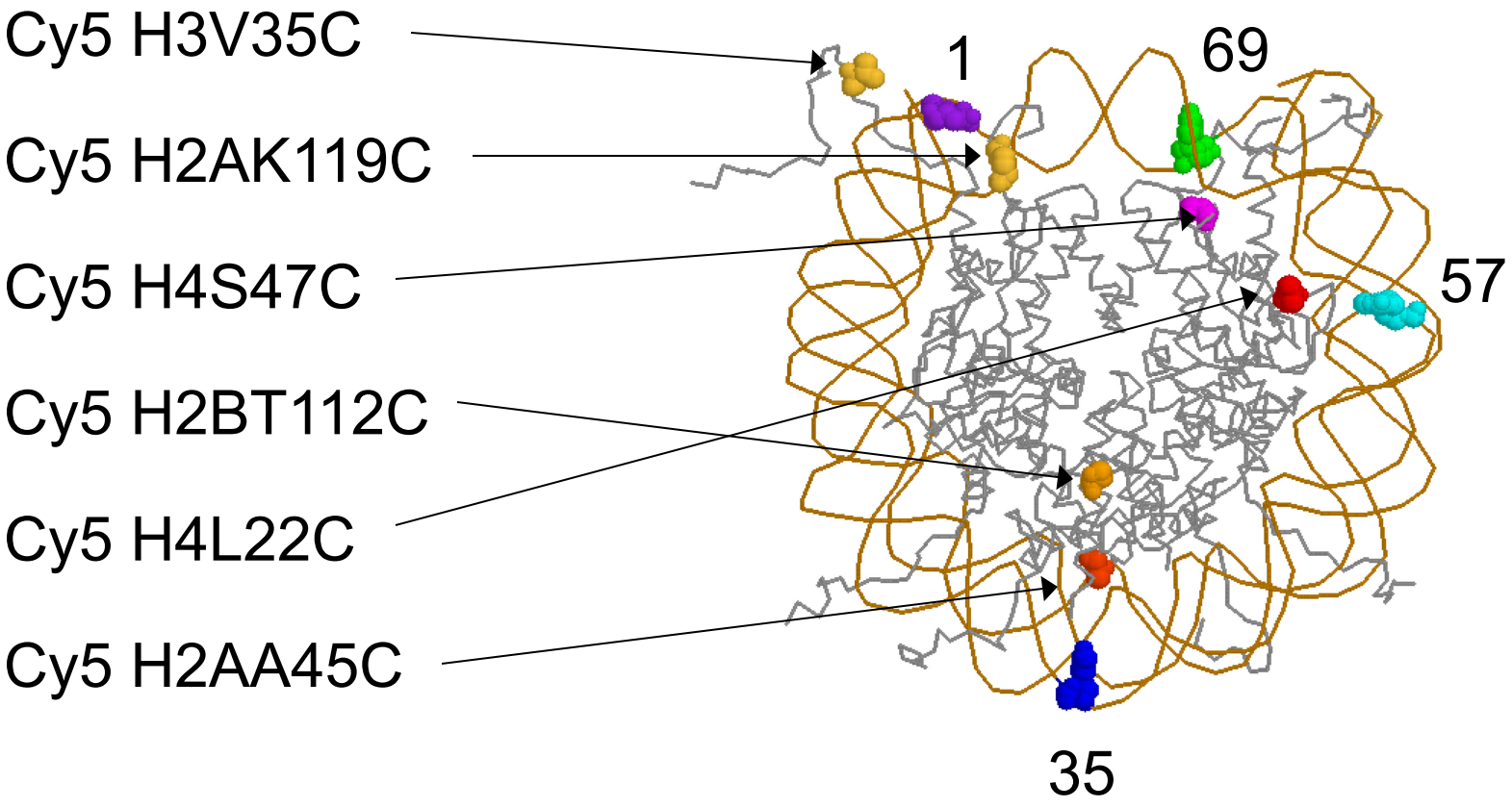
601–147 Cy3 69



Cy3 fluorescence donor

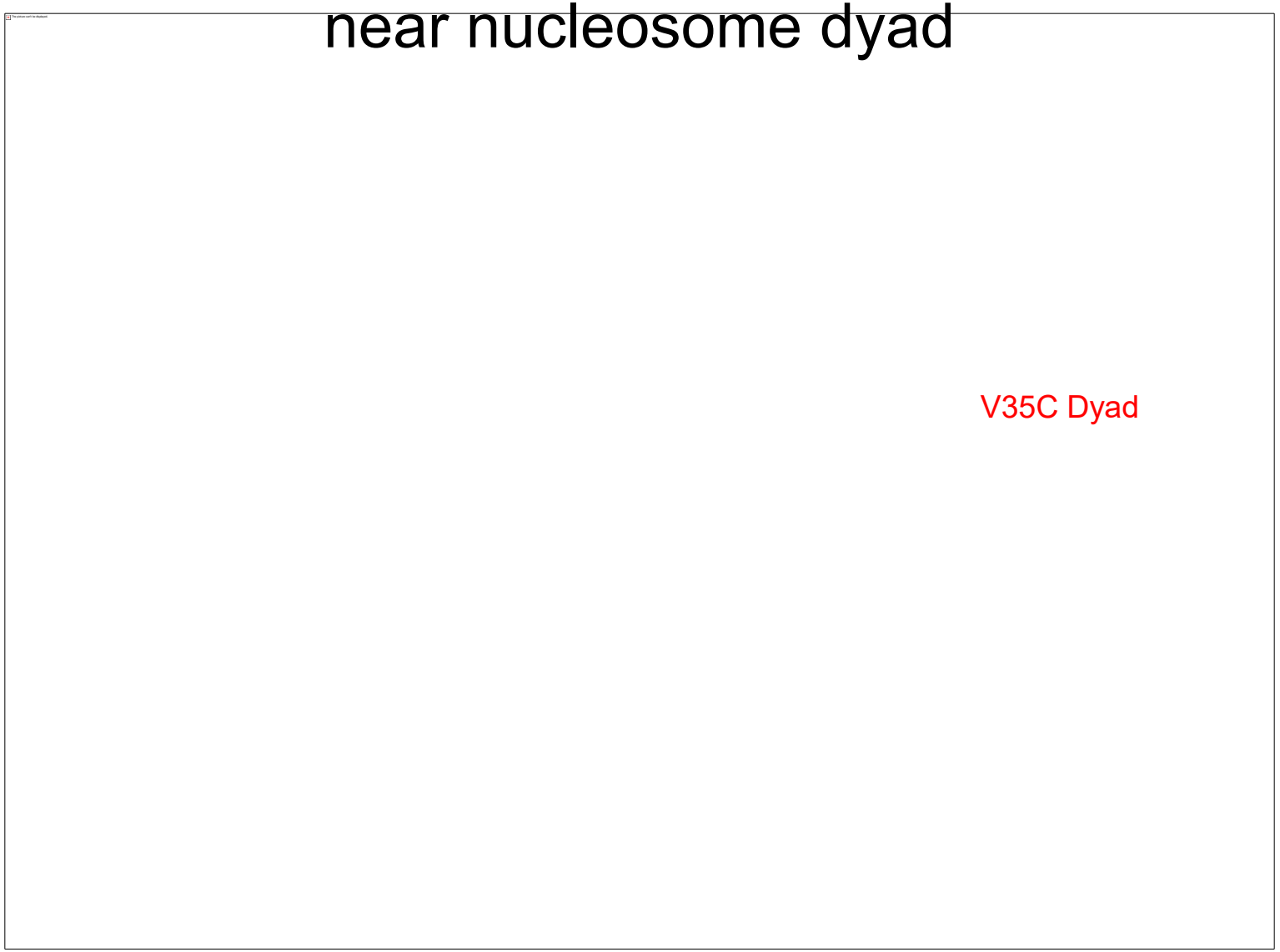
Hannah Tims

Site exposure at internal nucleosome target sites: Cy5-labeled histone octamers and Cy3-labeled DNA

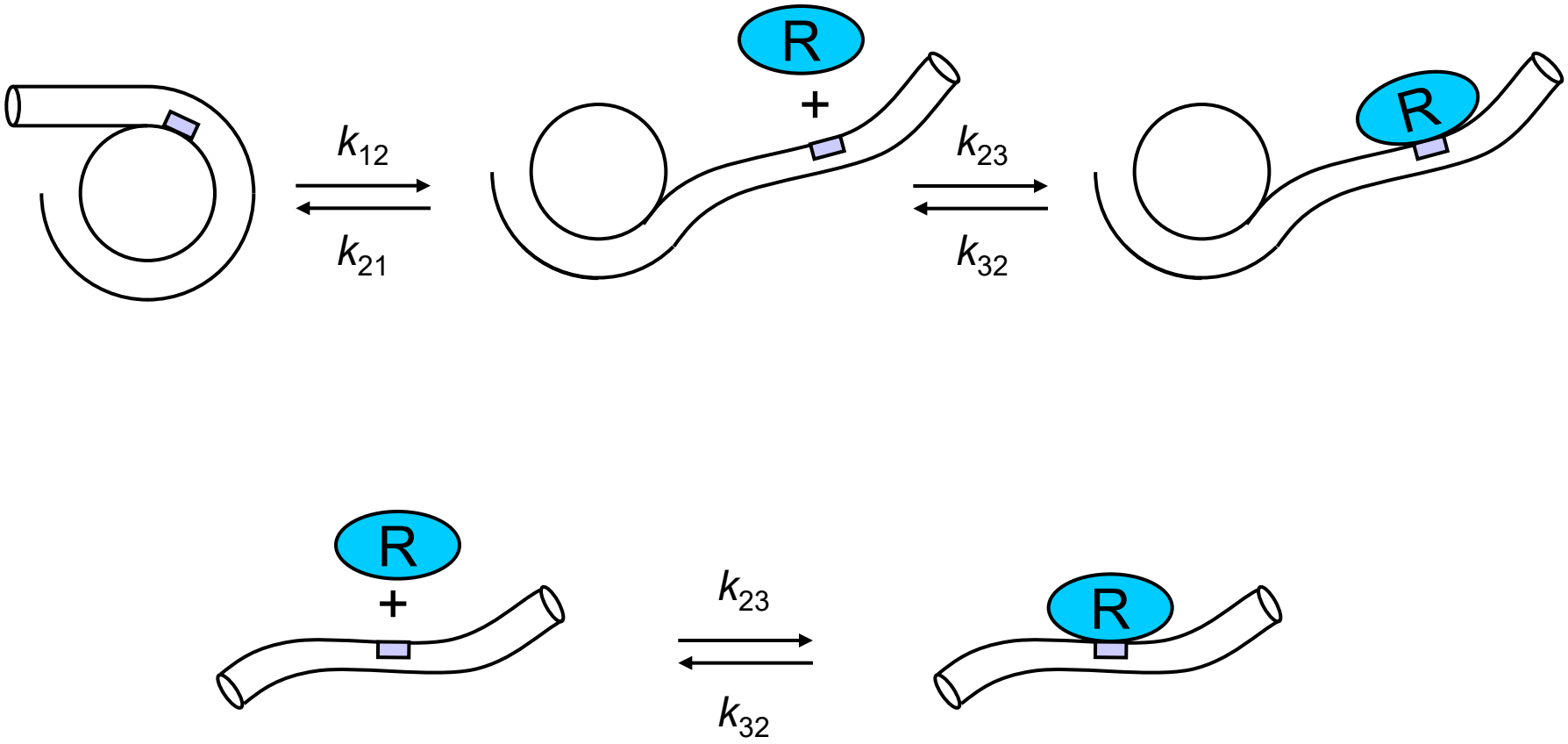


Hannah Tims

Reduced equilibrium constant for site exposure near nucleosome dyad



Hannah Tims



Polach & Widom, 1995

DNA constructs for LexA protein binding

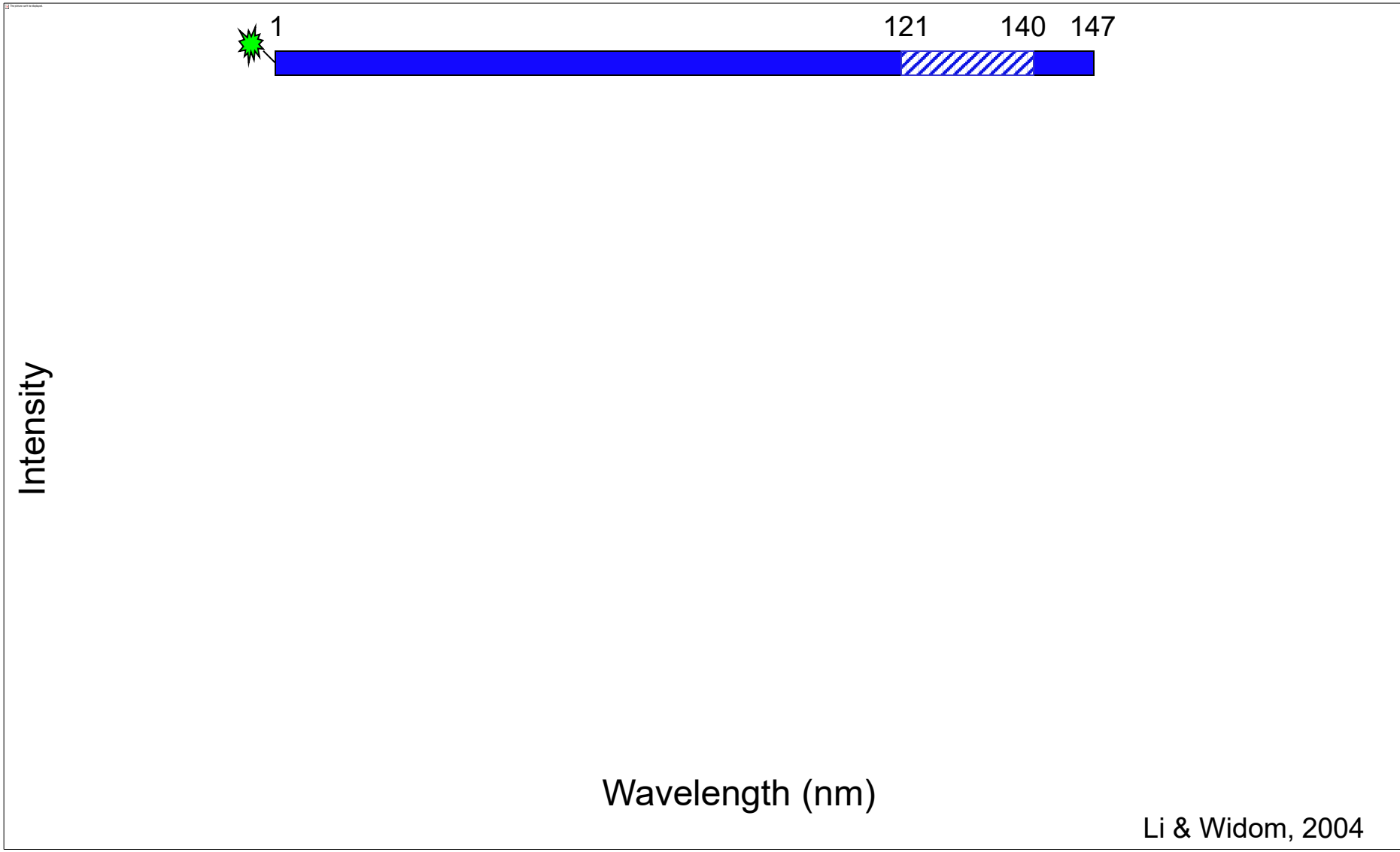
601-147:

LexA147-left:

LexA147-right:

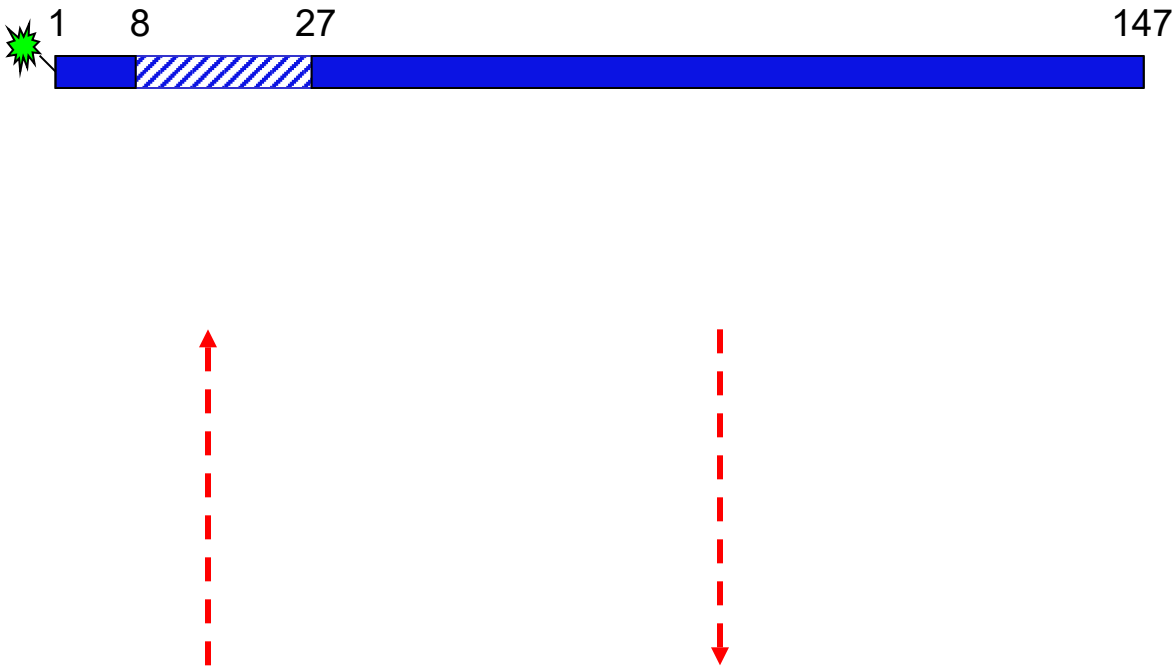
Li & Widom, 2004

No change in FRET when LexA binds to DNA end opposite fluorescence donor



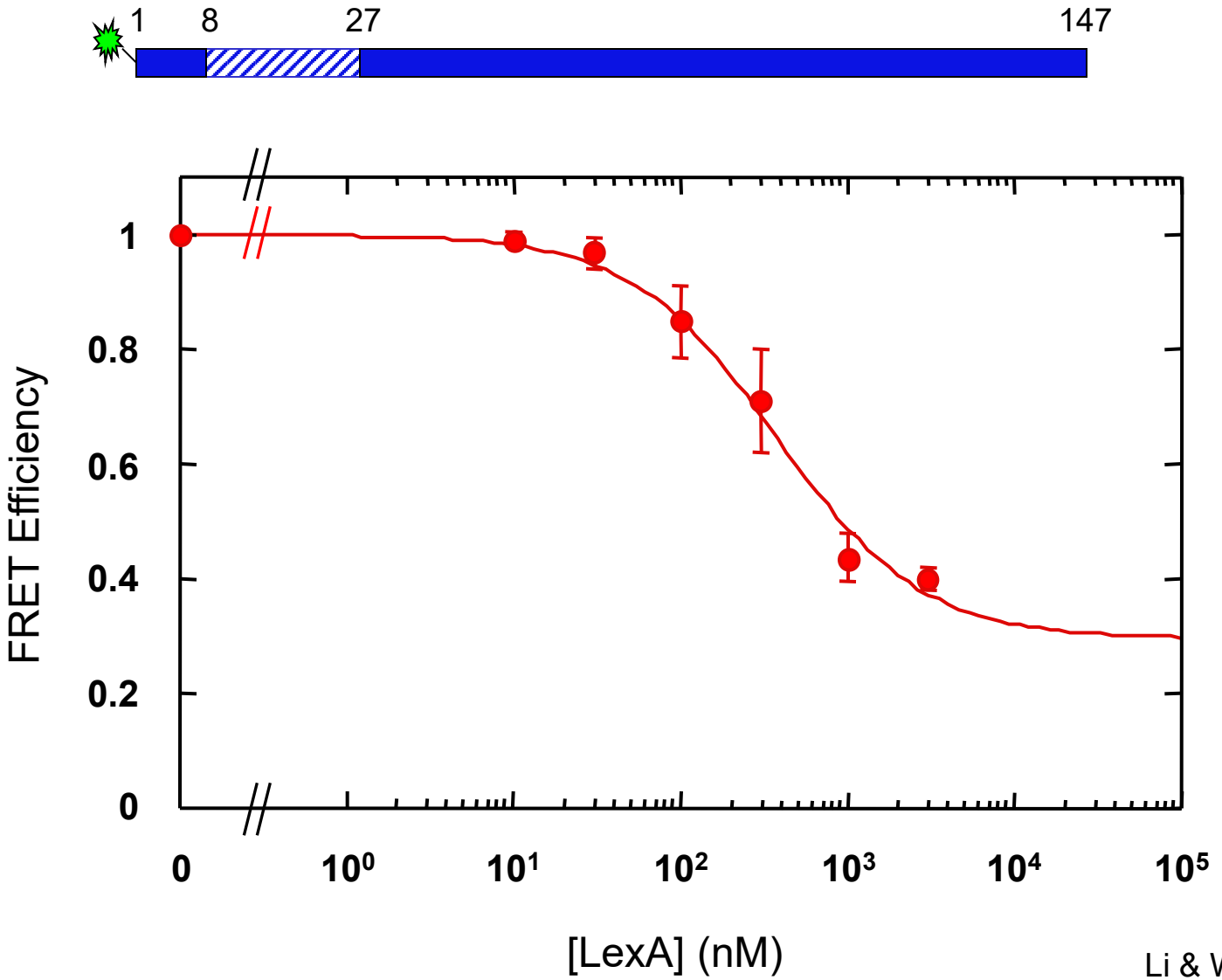
Li & Widom, 2004

DNA unwrapping detected by FRET when LexA binds near fluorescence donor



Li & Widom, 2004

Nucleosome conformational change driven by LexA binding near fluorescence donor



Li & Widom, 2004

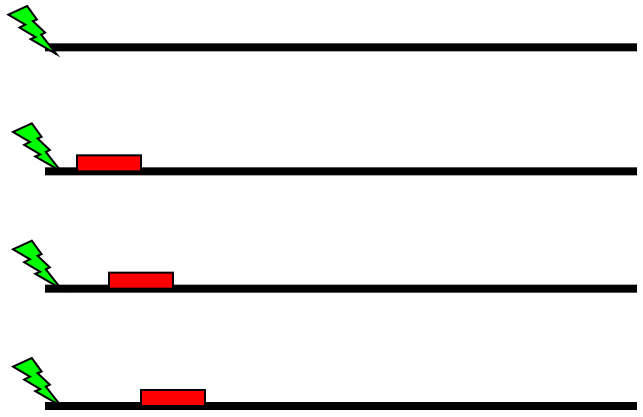
Site exposure at internal nucleosome target sites

Cy3 601–147

Cy3 601–147 LexA L

Cy3 601–147 LexA 17

Cy3 601–147 LexA 27

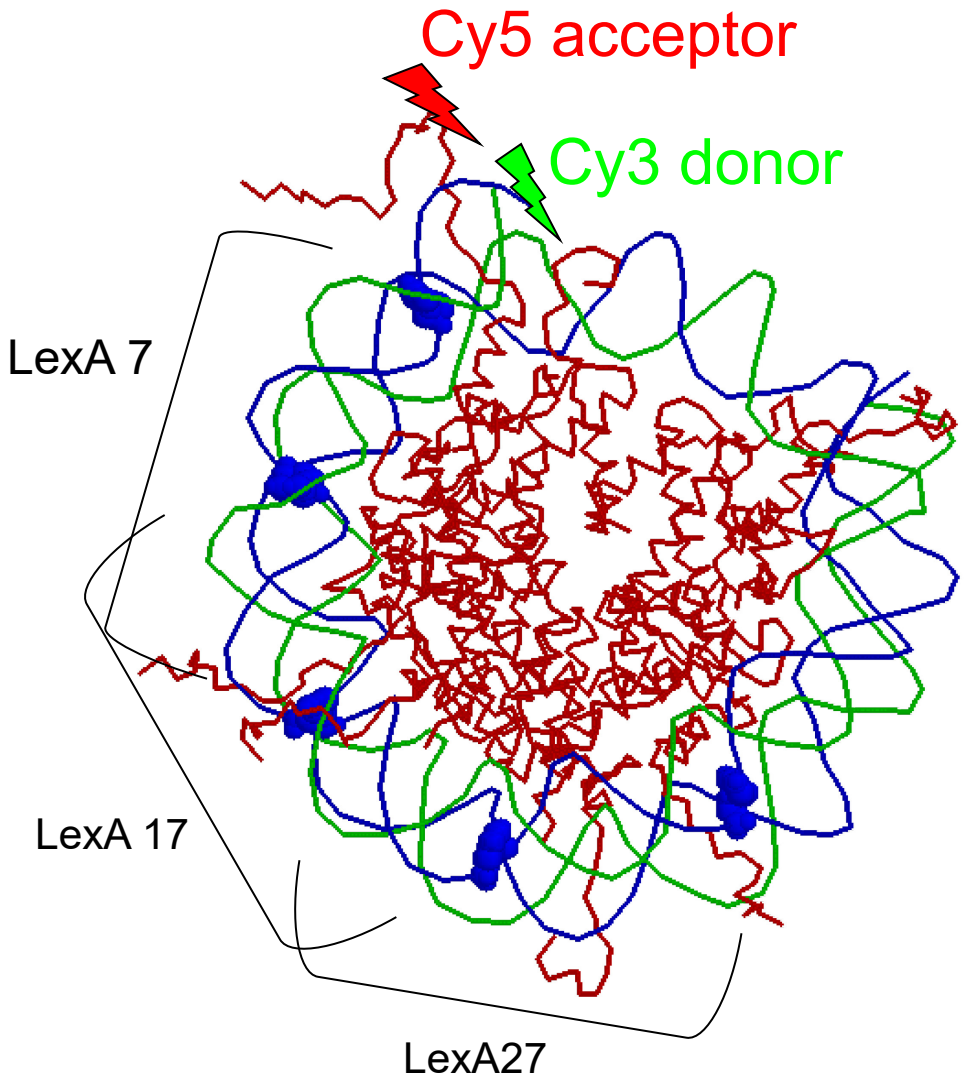


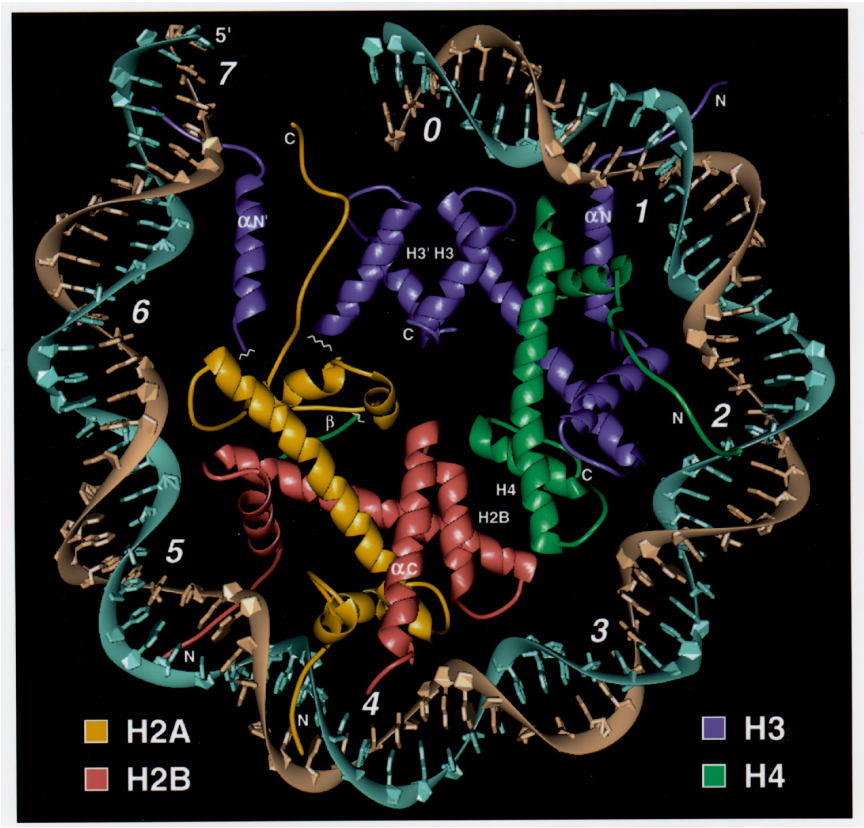
Cy3 fluorescence donor

LexA binding site

Hannah Tims

Protein binding to internal DNA target sites





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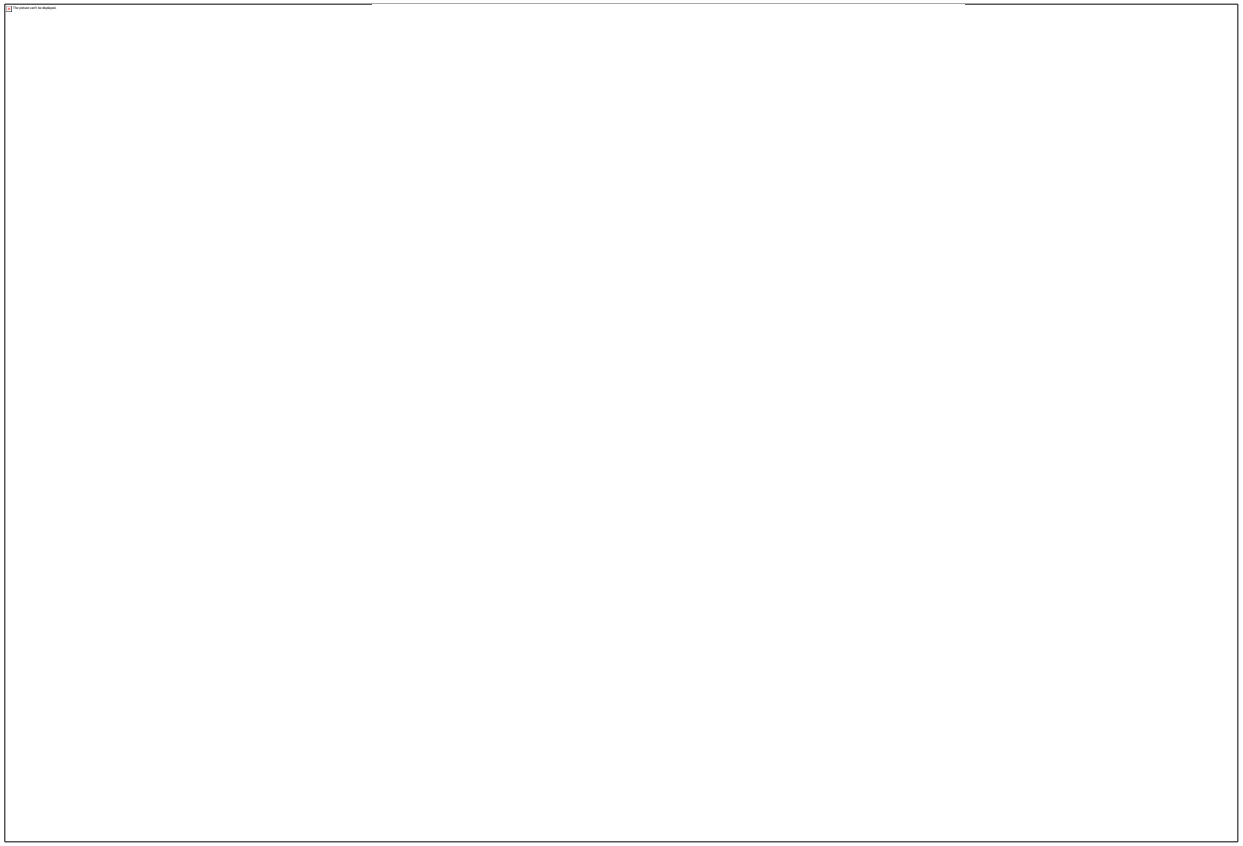
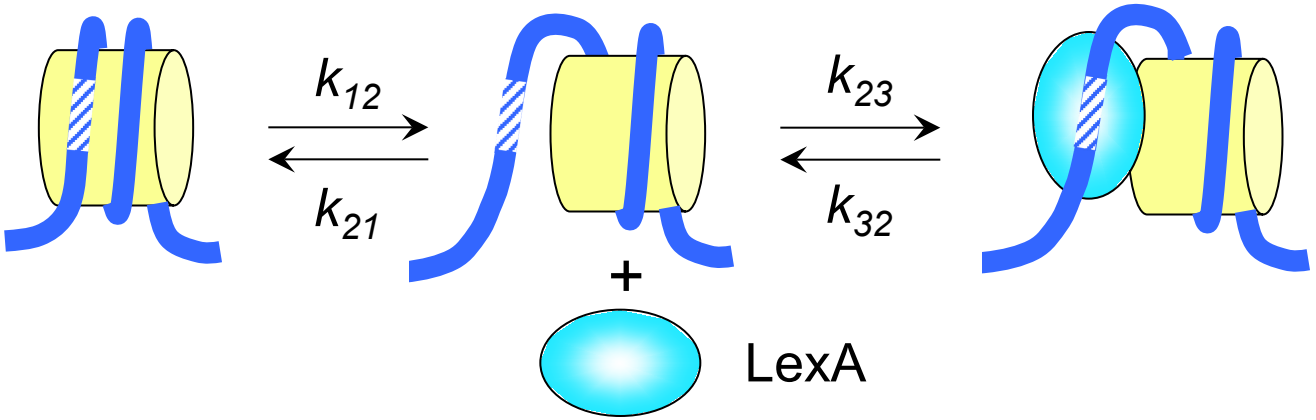
Sites further inside the nucleosome are less accessible (more costly) for protein binding



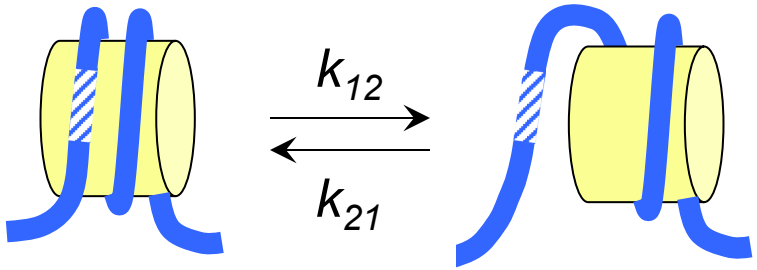
Hannah Tims

Two assays for the rates of site exposure and re-wrapping in nucleosomes

Stopped-flow FRET

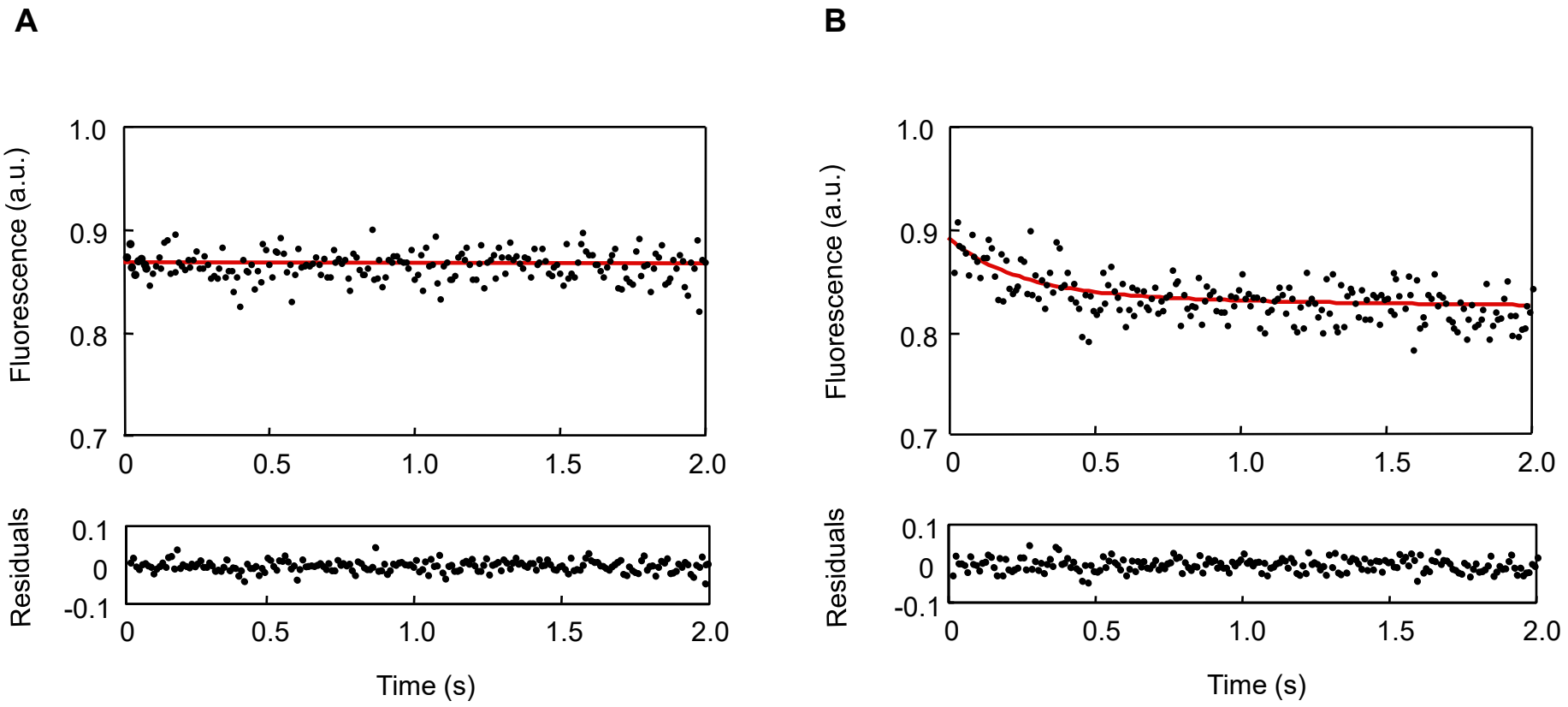


FRET-FCS



Li, Levitus, Bustamante, & Widom, 2005

Stopped-flow analysis of LexA binding to buried nucleosomal target site

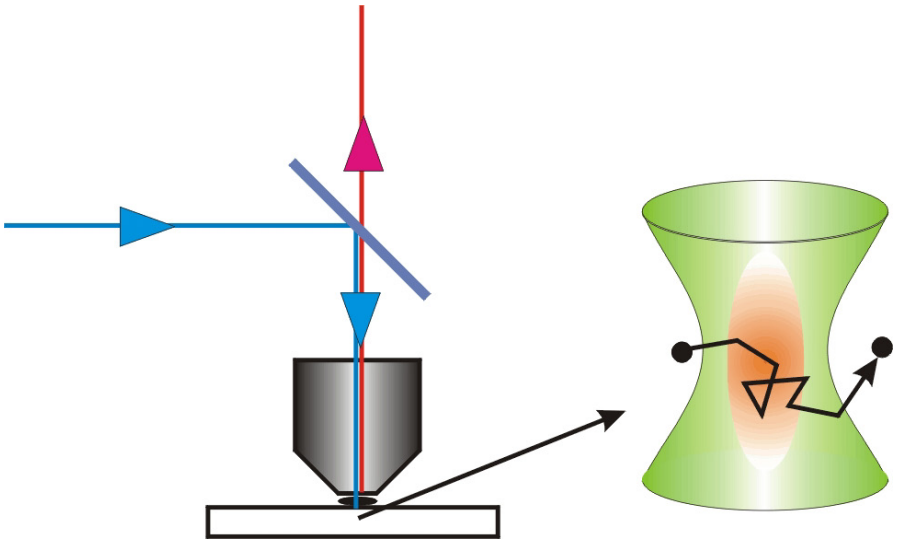


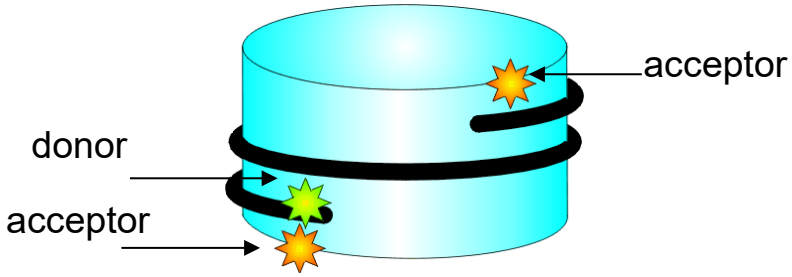
Mock reaction (no LexA)

200 nM LexA

Li, Levitus, Bustamante, & Widom, 2005

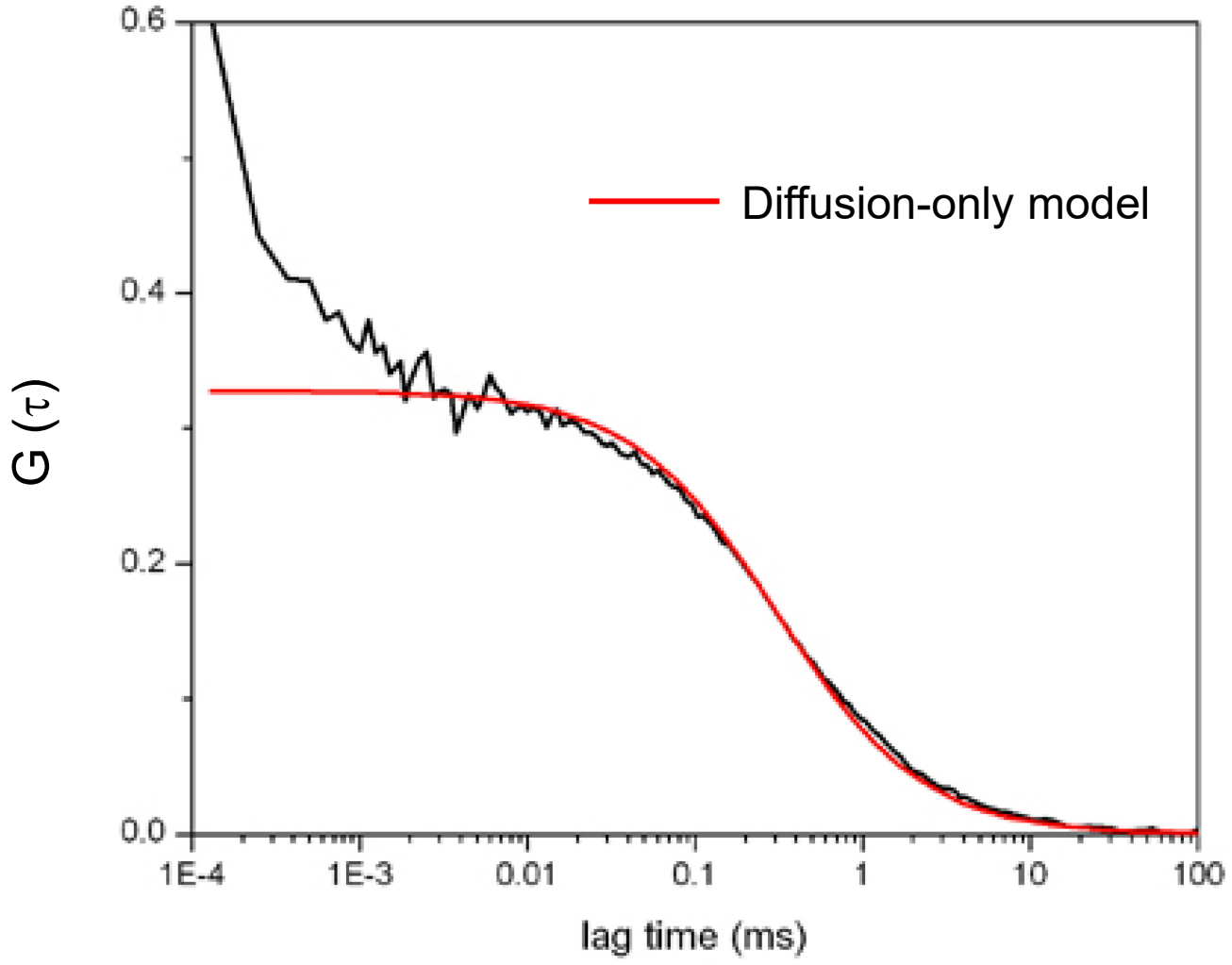
Nucleosome dynamics analyzed by fluorescence correlation spectroscopy





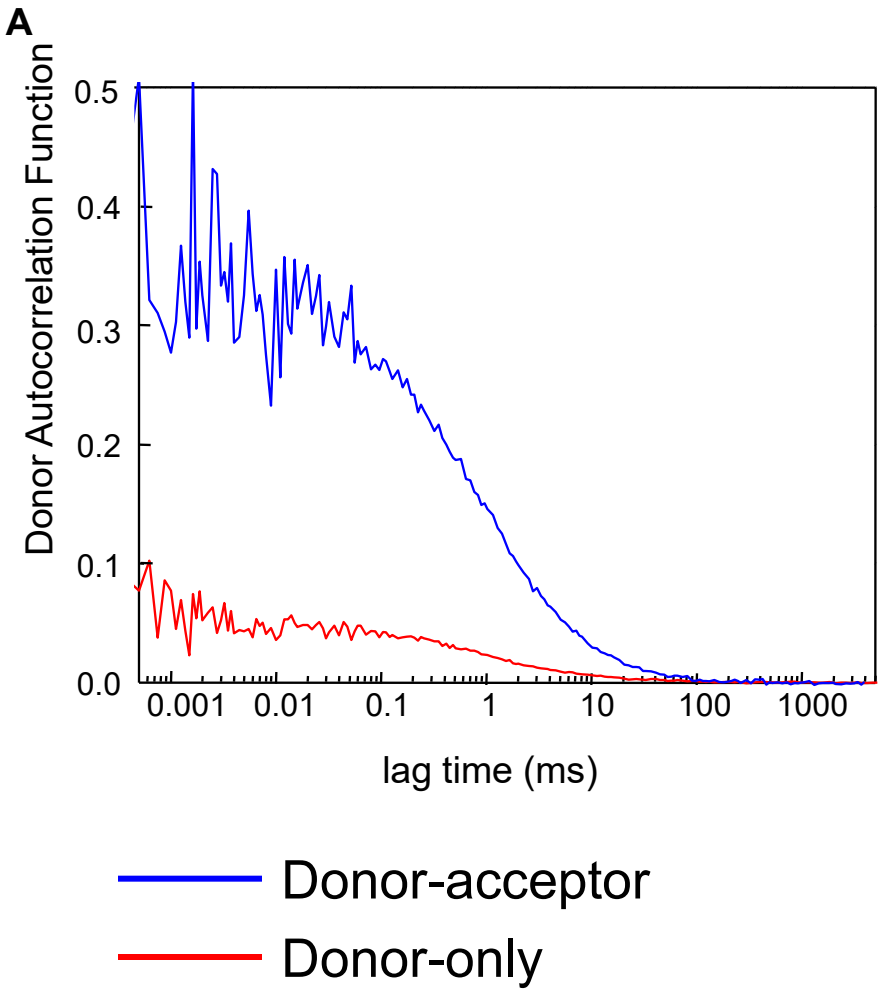
Li, Levitus, Bustamante, & Widom, 2005

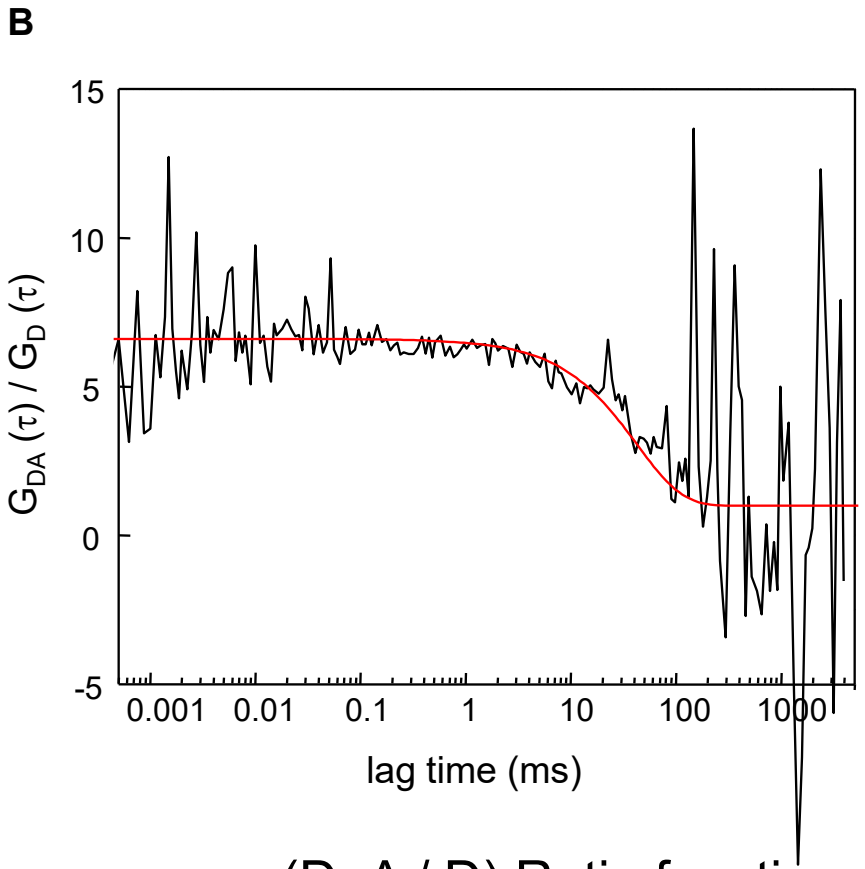
FCS analysis of nucleosomes labeled with donor-only



Li et al., 2005

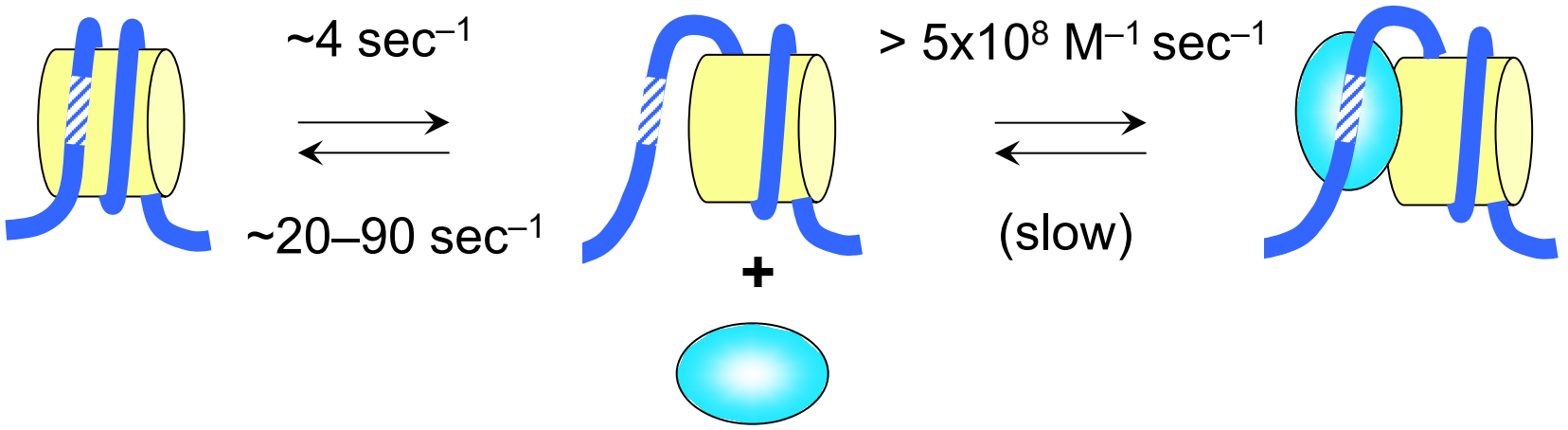
Nucleosome dynamics analyzed by fluorescence correlation spectroscopy





Li, Levitus, Bustamante, & Widom, 2005

Rapid spontaneous site exposure in nucleosomes

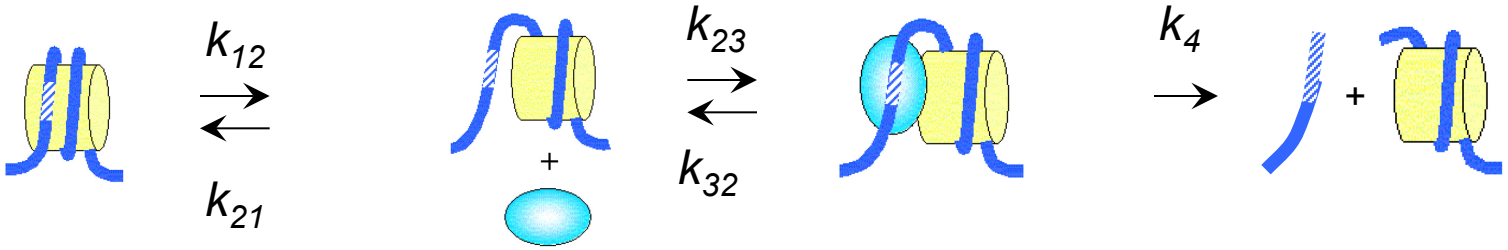


- Explains how remodeling factors can be recruited to particular nucleosomes on a biologically relevant timescale
- Sets tight limits to kinetic efficiency in regulatory protein binding
- Suggests that the major impediment to polymerase elongation is re-wrapping of the nucleosomal DNA

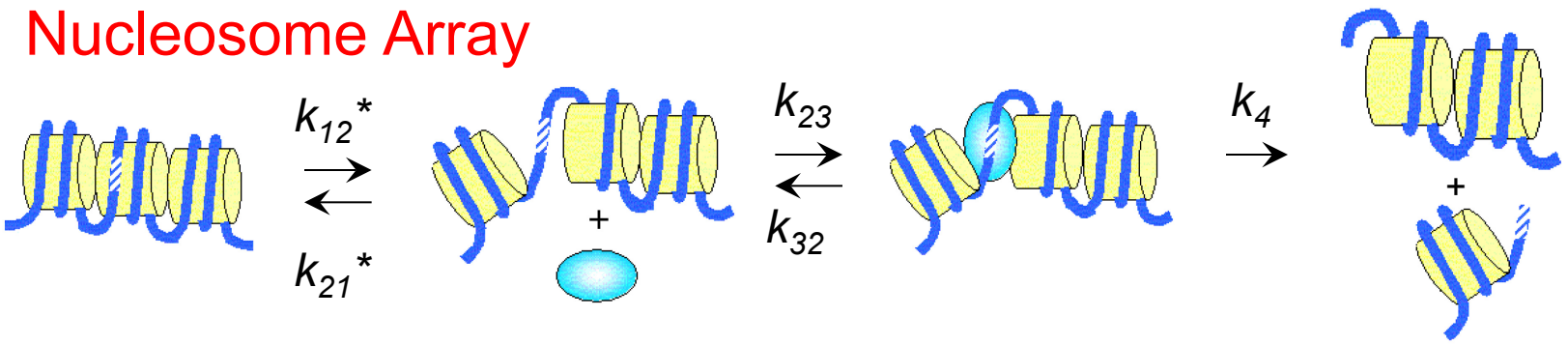
Li, Levitus, Bustamante, & Widom, 2005

Site exposure in long chains of nucleosomes

Single Nucleosomes

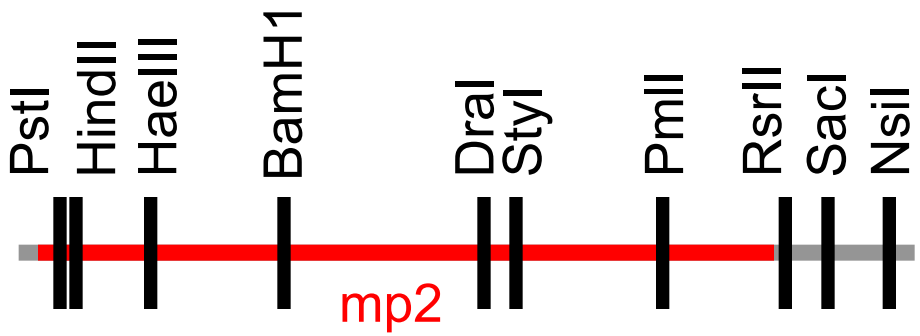


Nucleosome Array

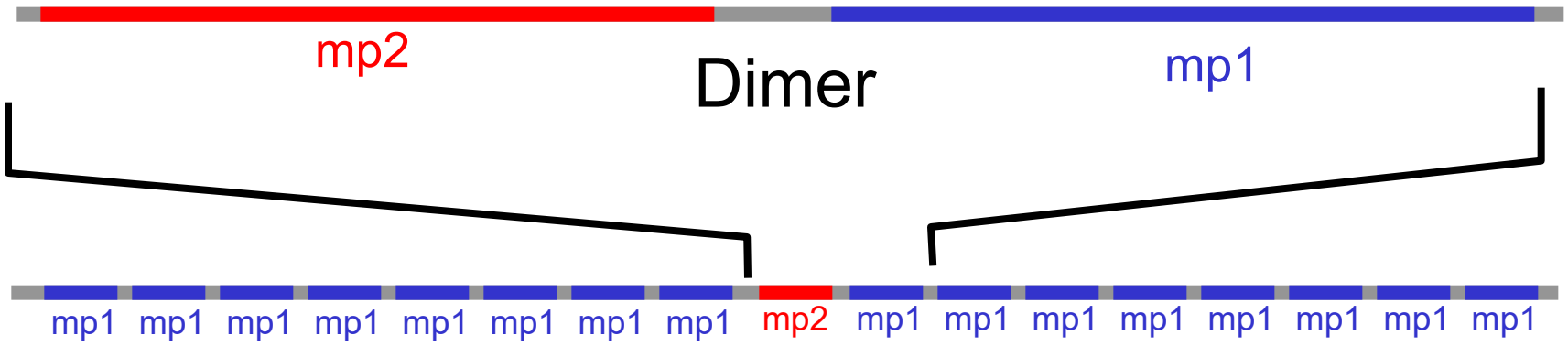


Michael Poirier

Site exposure in long chains of nucleosomes



Monomer



Dimer

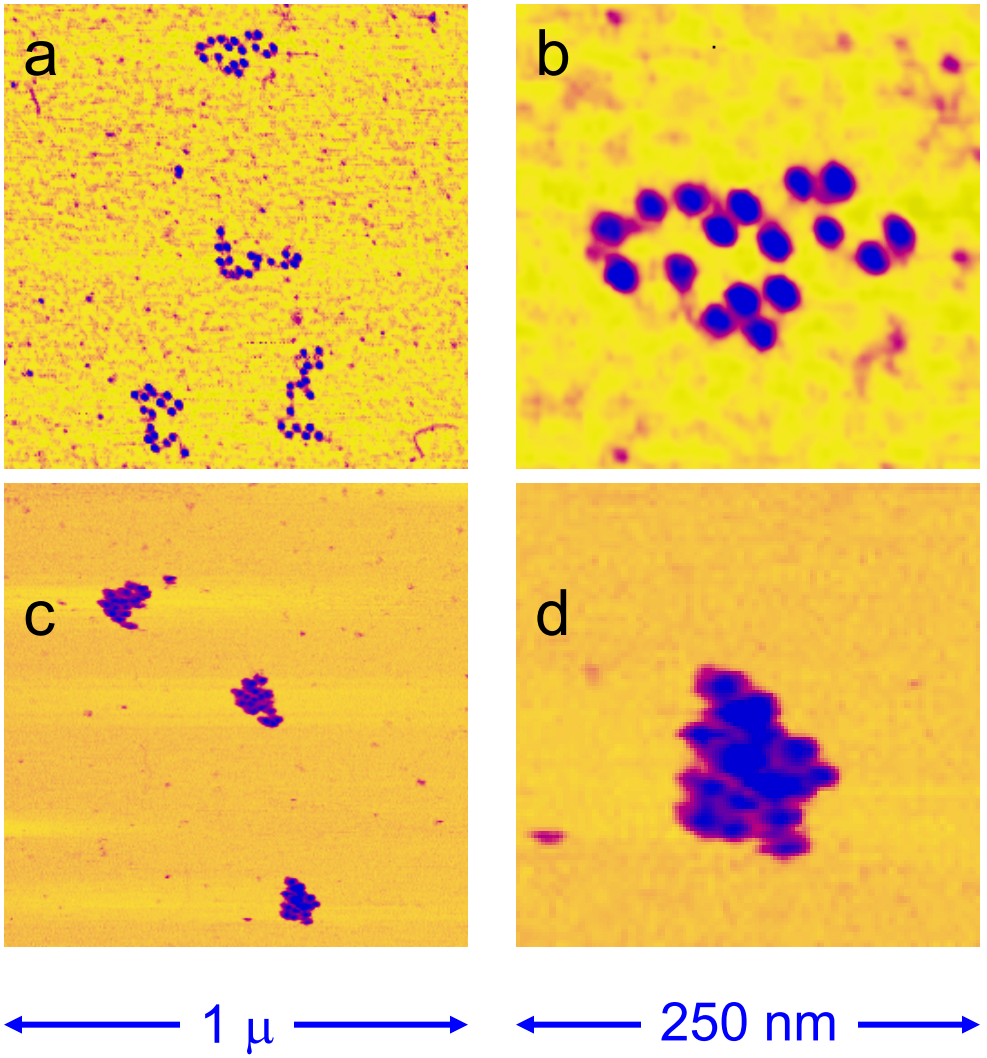
Heptadecamer

Michael Poirier

Cation-dependent folding of 17-mers analyzed by AFM

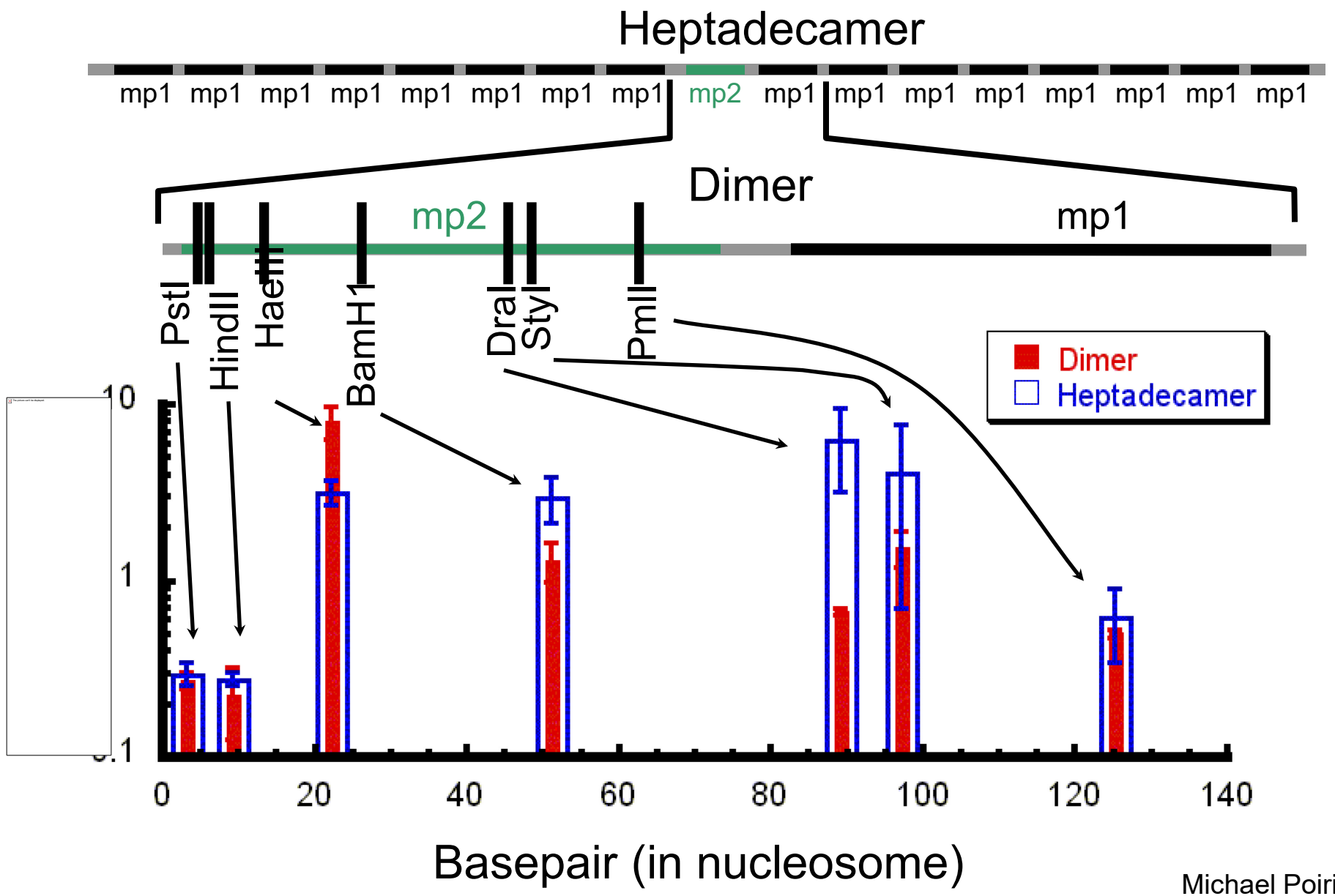
Extended
(low [NaCl])

Compact
(+ Mg^{2+})



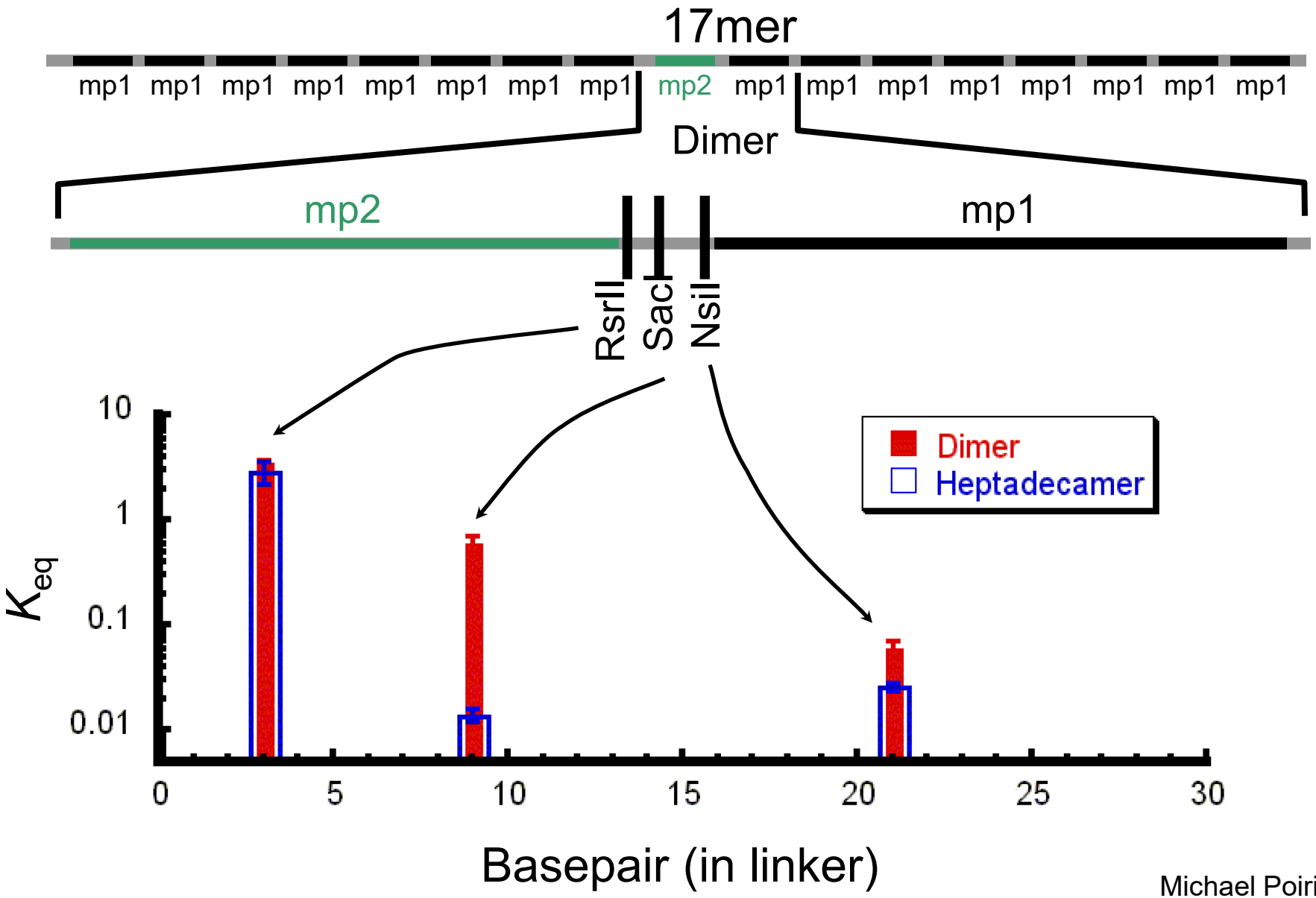
Poirier, Bussiek, Langowski, & Widom

Nucleosomal site accessibility in a chromatin fiber



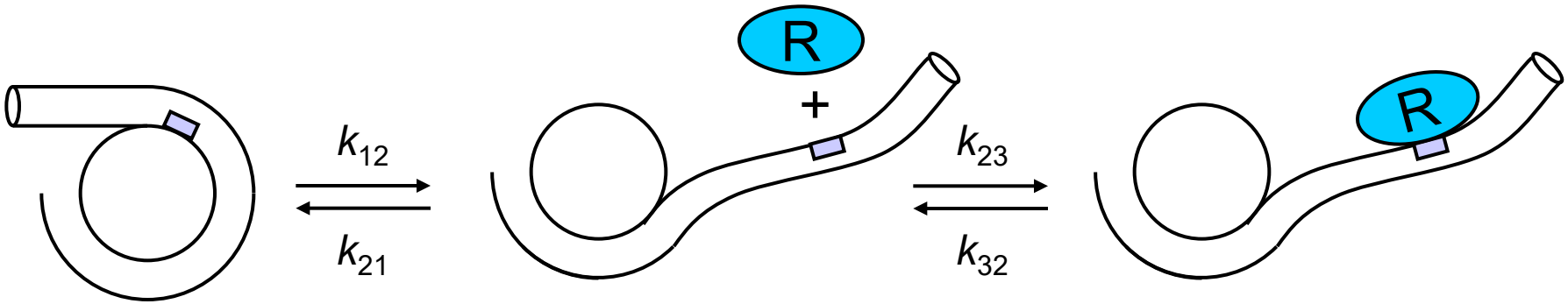
Michael Poirier

Site accessibility in linker DNA



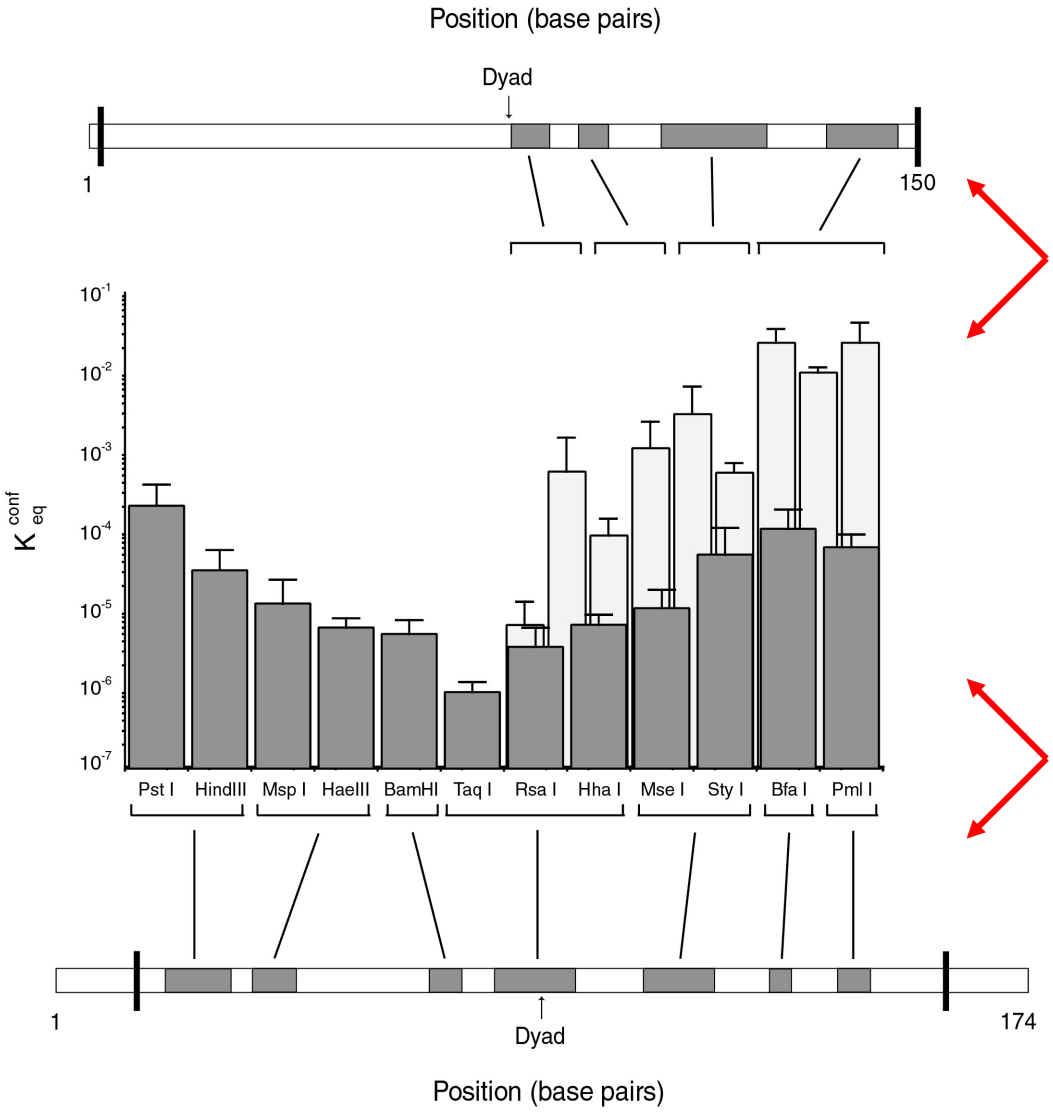
Michael Poirier

Site exposure equilibrium constants depend on the affinity of histone-DNA interactions



Anderson & Widom, 2000
Widom, 2001

Site exposure equilibrium constants depend on the affinity of histone-DNA interactions

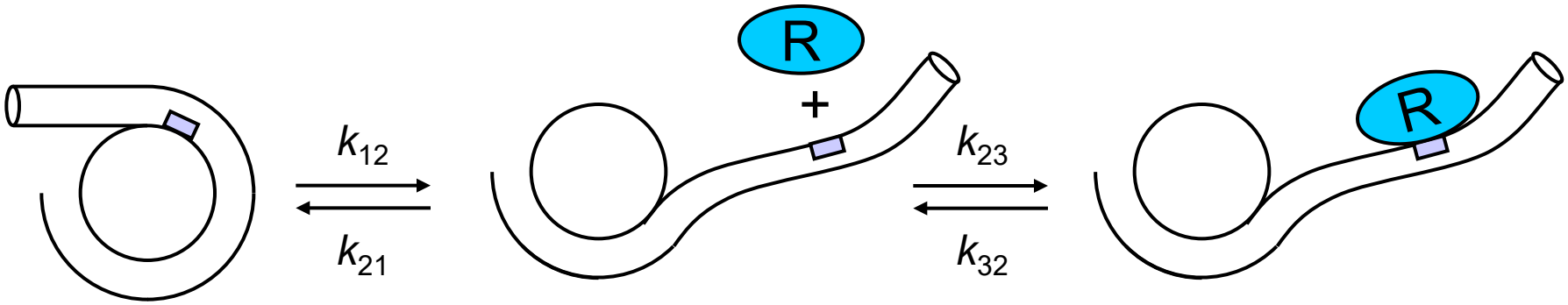


5S rRNA gene DNA

SELEX Non-natural high affinity DNA

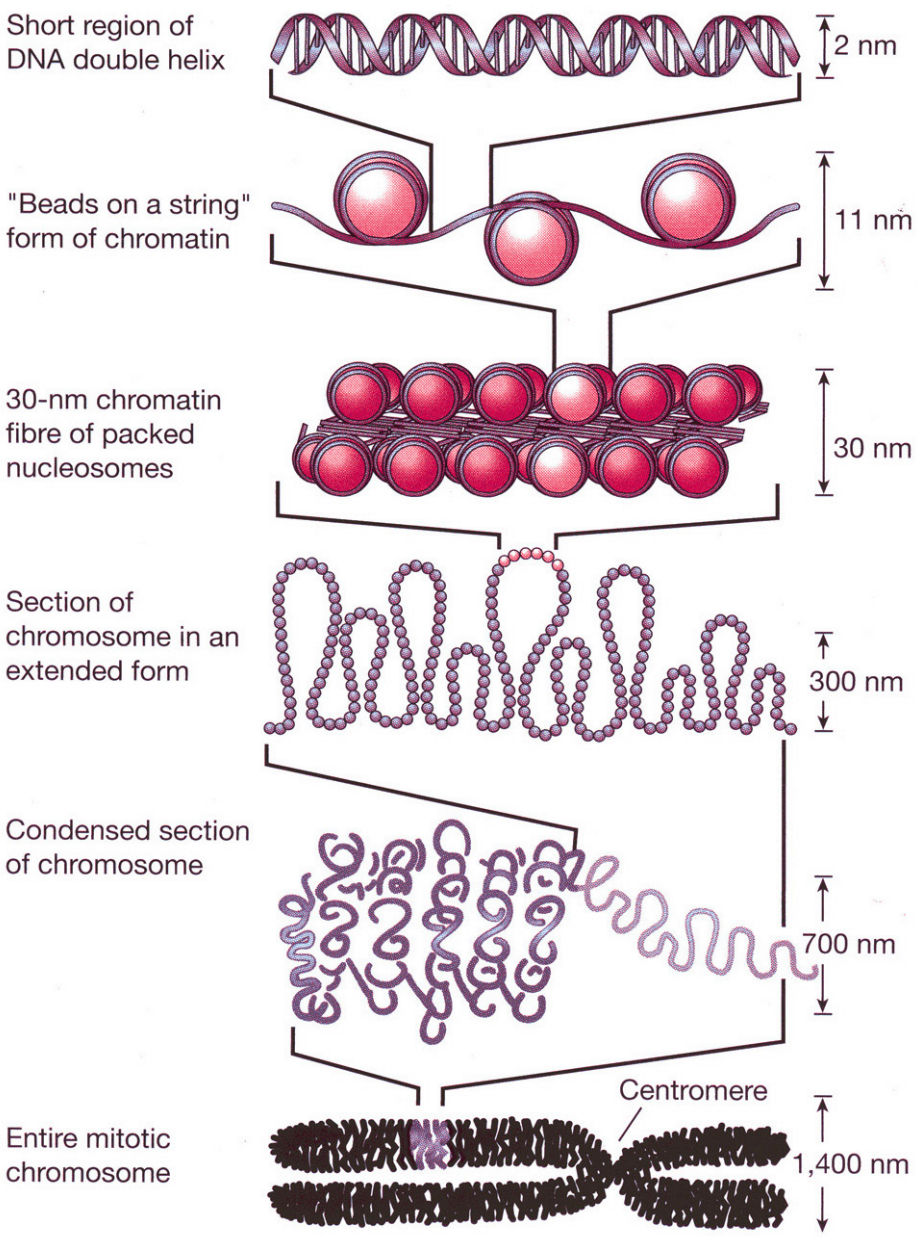
Anderson & Widom, 2000
Widom, 2001

Site exposure equilibrium constants depend on the affinity of histone-DNA interactions



Therefore, the equilibrium locations of nucleosomes along DNA depend on the local affinities of histone-DNA interactions

Anderson & Widom, 2000
Widom, 2001
Segal et al., 2006



Felsenfeld, G. & Groudine, M. (2003), *Nature* 421: 448-453

Acknowledgements

Nucleosome dynamics

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Michael Poirier
Karissa Fortney
Hannah Tims
Georgette Moyle