ESF Brainstorm session SONS/DYNA
Brussels, Belgium
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Physical techniques to uncover the physics of chromatin; Single molecule studies

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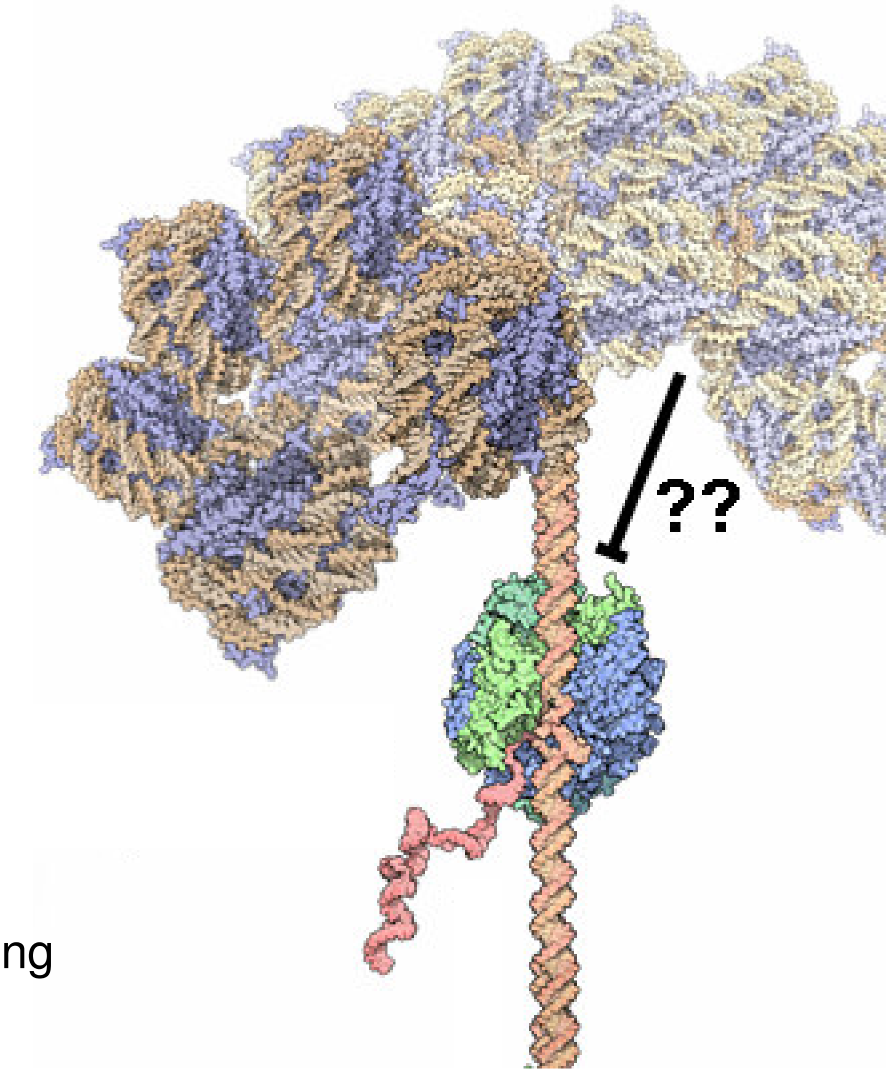
Condensation vs. transcription…

Objective: To obtain ***microscopic*** data on the ***physical properties*** of chromatin and to challenge structural and ***dynamic*** models of chromatin



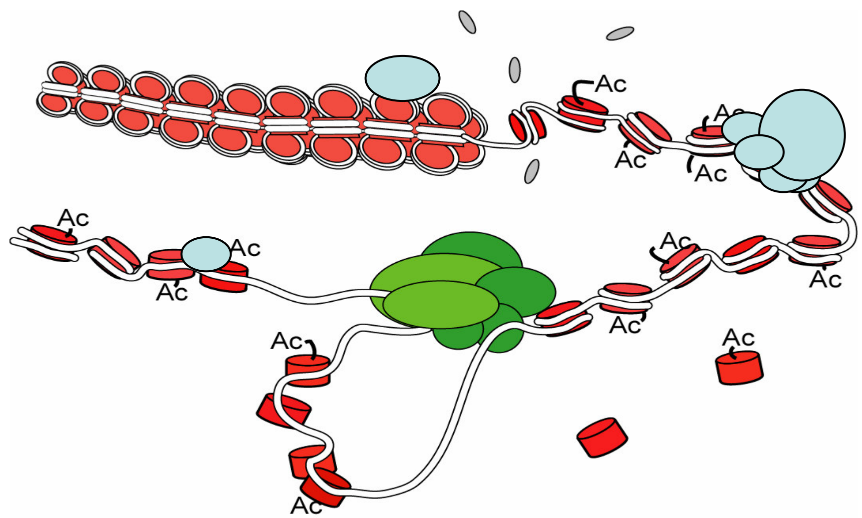
How about:

- ❑ histone modifications?
- ❑ ATP-dependent remodellers ?
- ❑ non-uniform linker length?
- ❑ linker histones?
- ❑ DNA sequence dependent positioning
- ❑ …

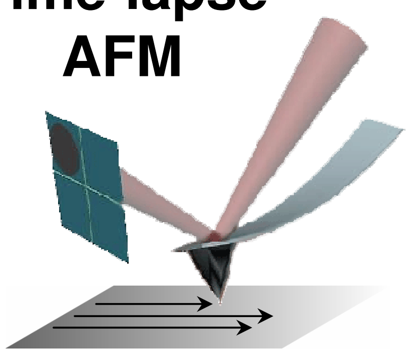
Compilation of illustrations by David S. Goodsell, The Scripps Research Institute pdb molecule of the month

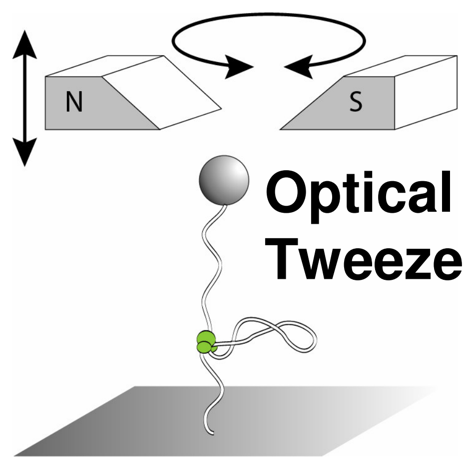
Experimental challenges of studying chromatin structure:

- Highly heterogeneous
- Highly dynamic
- nm – sub-micrometer range
- Many species of proteins involved

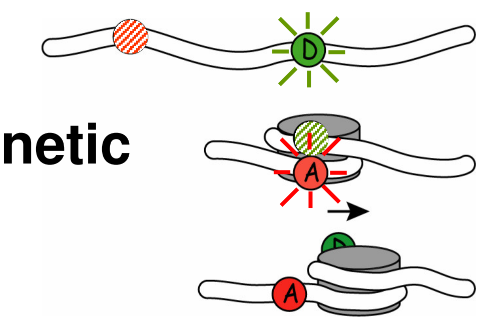


Time-lapse AFM





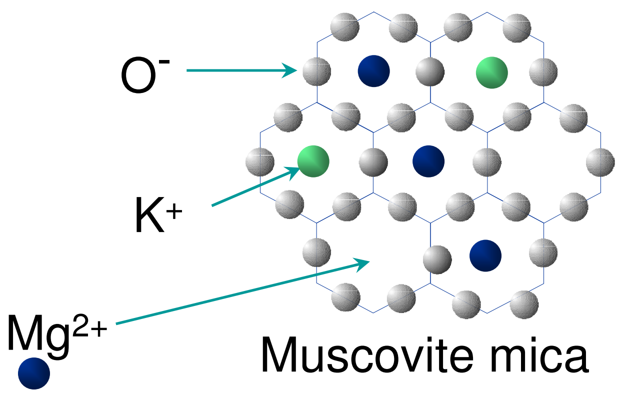
Optical / Magnetic Tweezers



spFRET

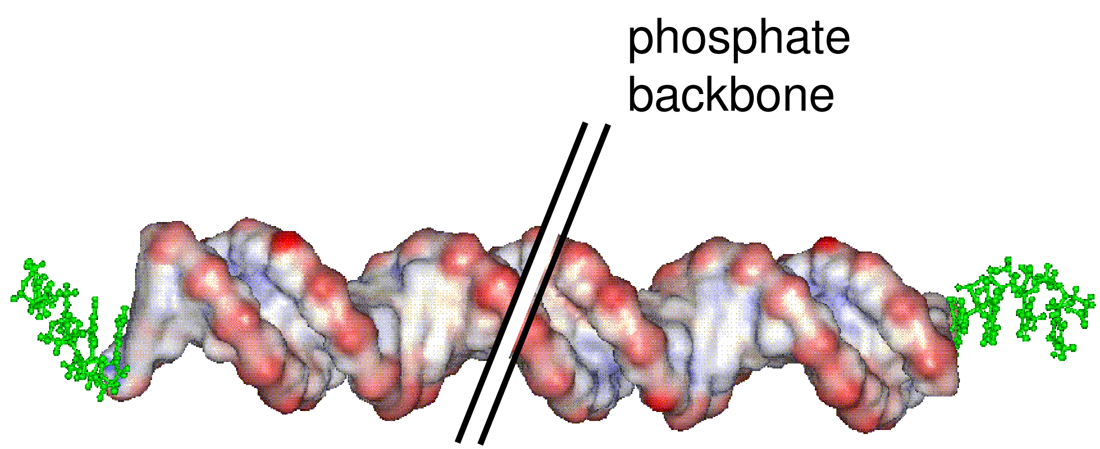
Sample preparation for DNA imaging

Adsorption to an atomically flat solid support

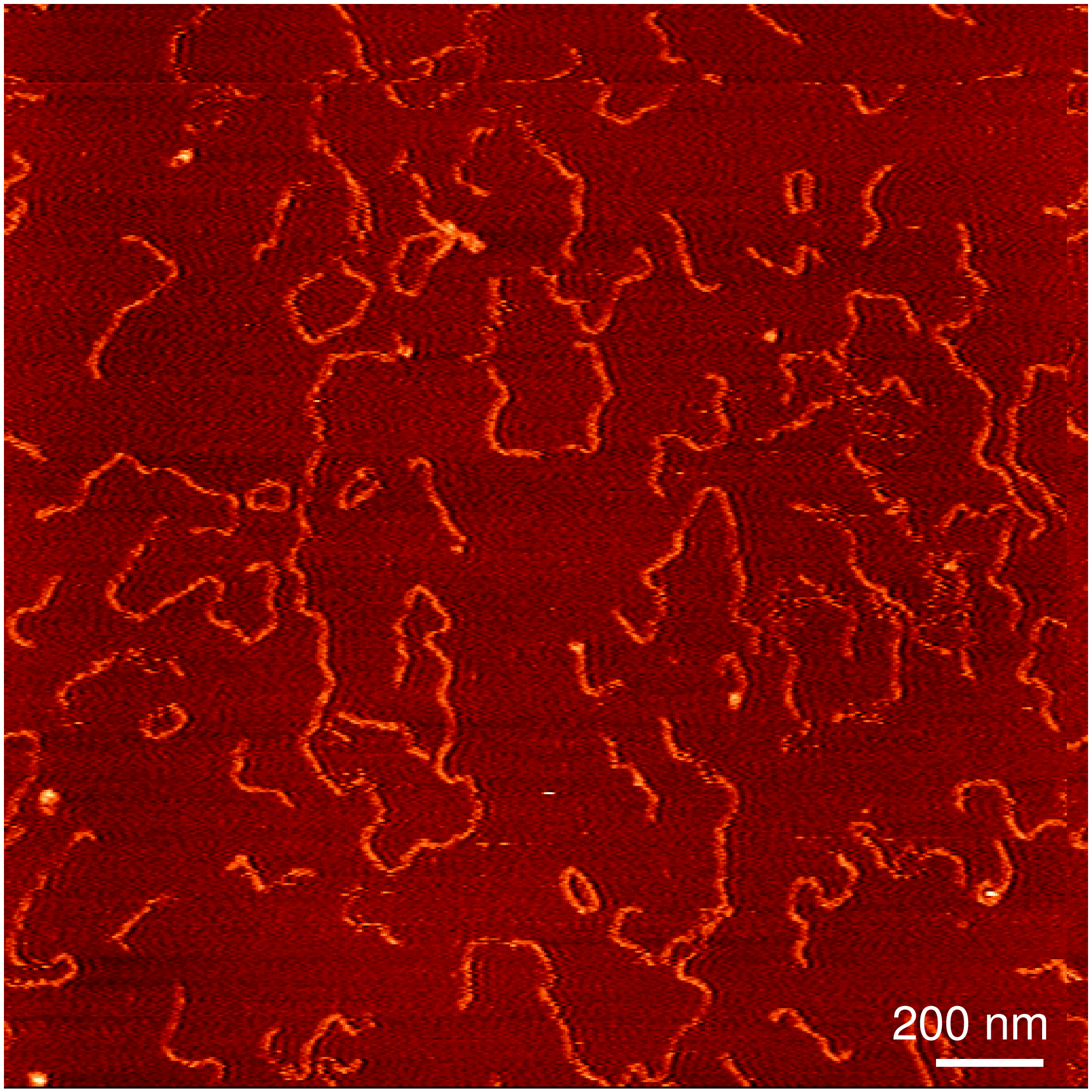


- K+ dissociates from the mica surface
- Mg 2+ acts as a bridge between the two negatively charged surfaces

- 1000 bp dsDNA
- EcoRI cut: AATT sticky ends

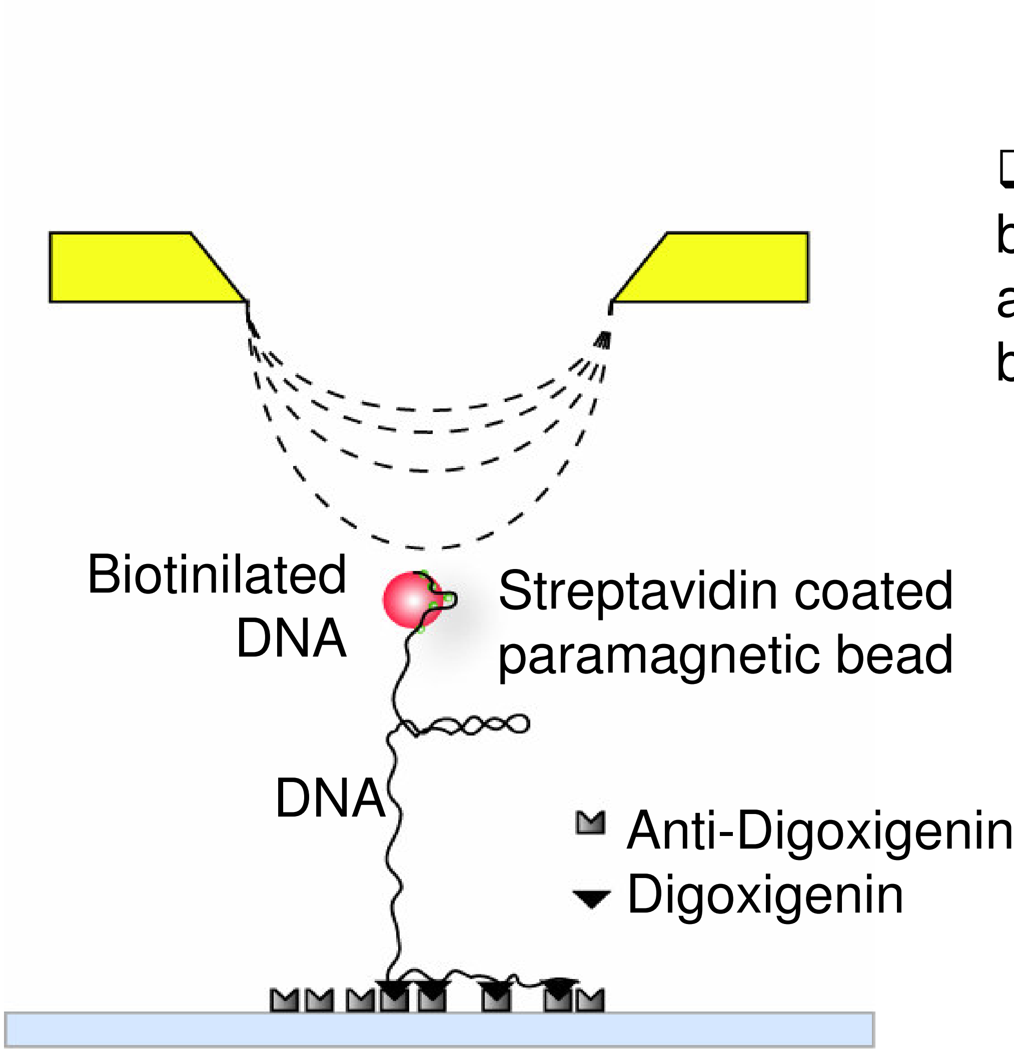


AFM in buffer



Van Noort et al. Biophys J. 1998

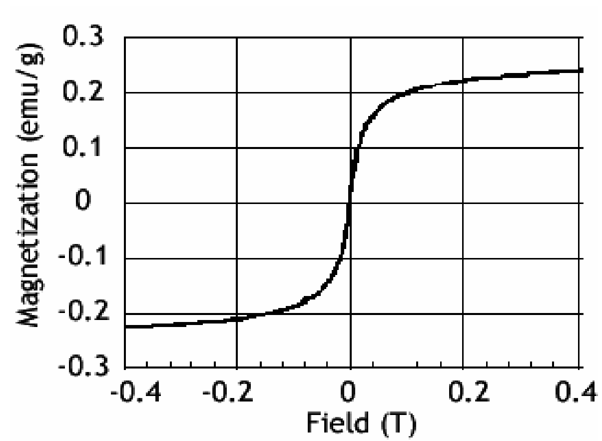
Magnetic tweezers



- ❑ By moving the magnets both the force and the twist of a single DNA molecule can be controlled:

$$\text{Force} = M \bullet \nabla H$$

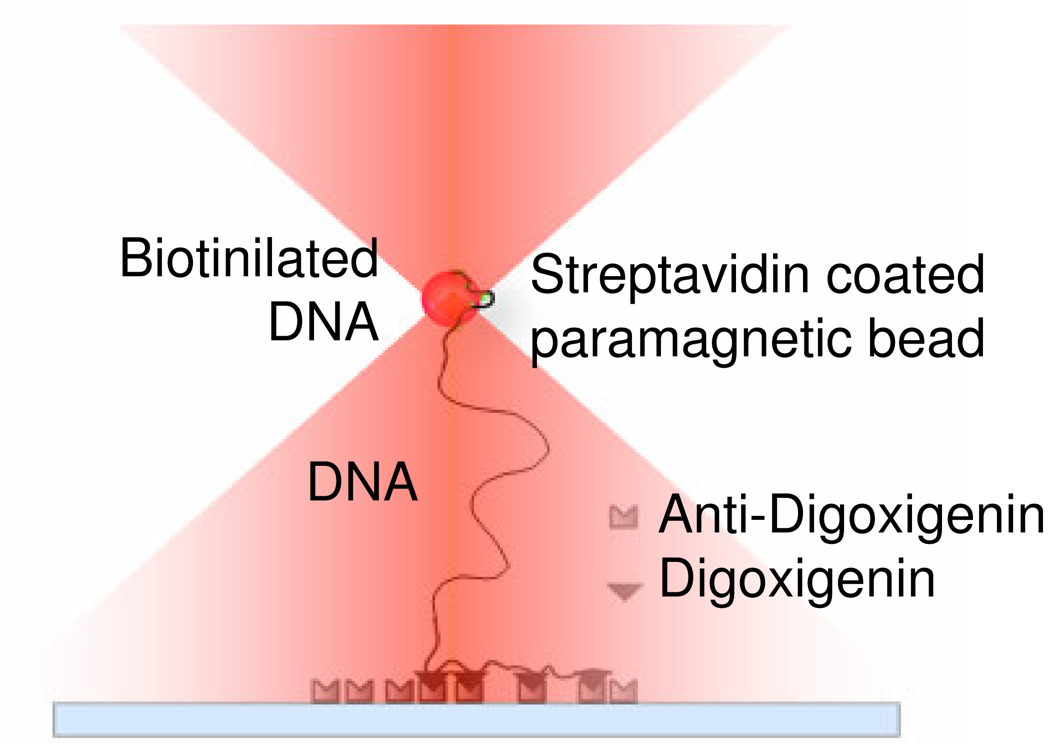
$$\text{Torque} = M \otimes H$$

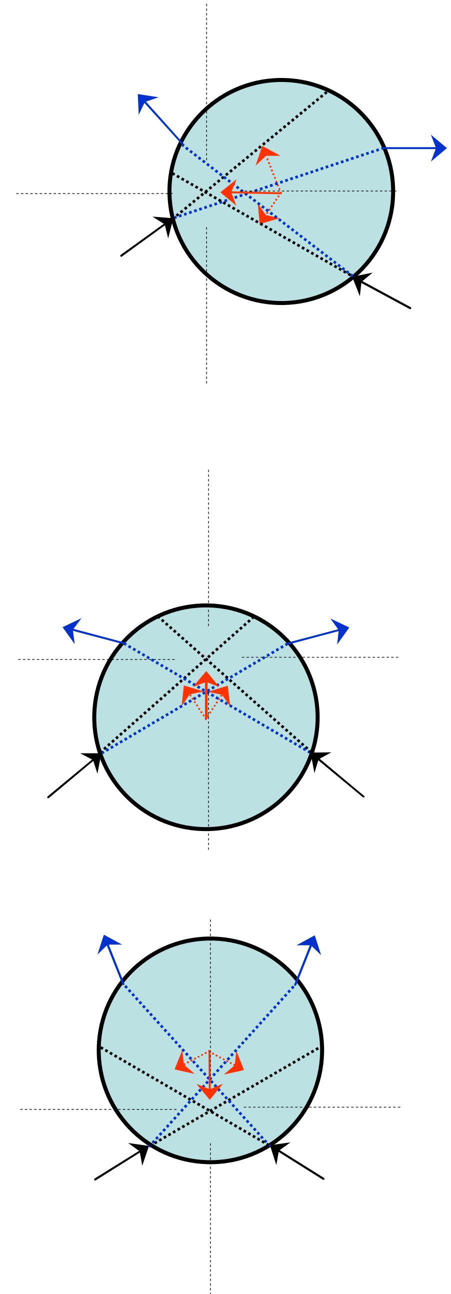


Optical Tweezers

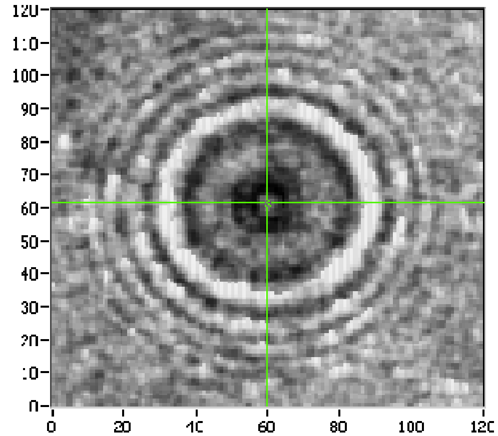
- ❑ A strongly focused laser traps high-refractive index micron-sized beads

- ❑ The bead/DNA can be manipulated with nm accuracy by moving the focus or the slide

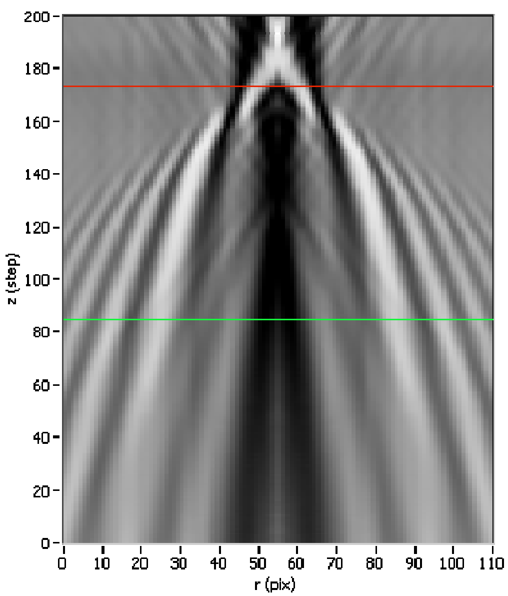


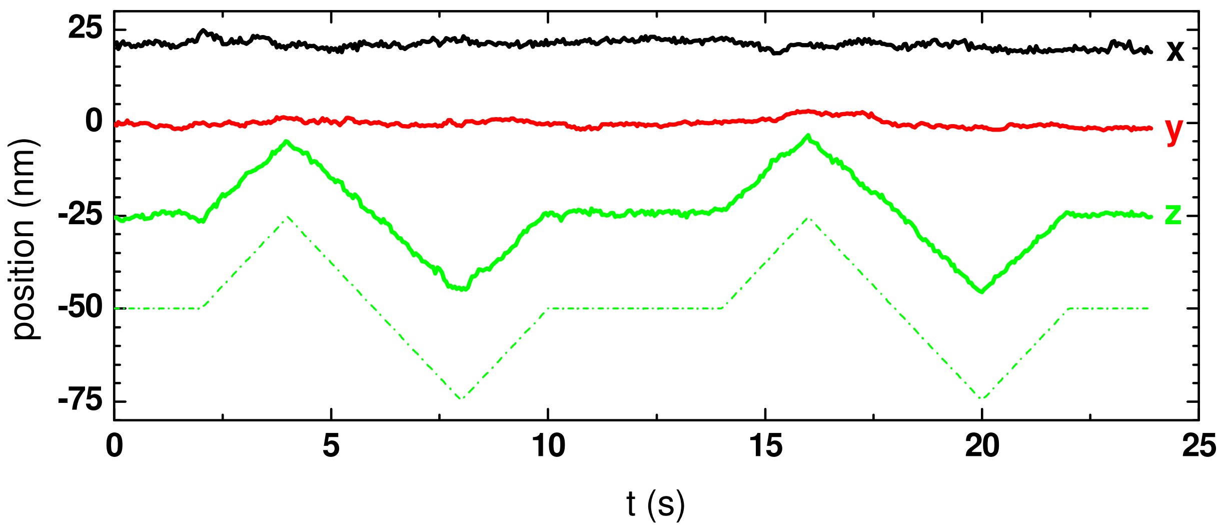


Bead Detection

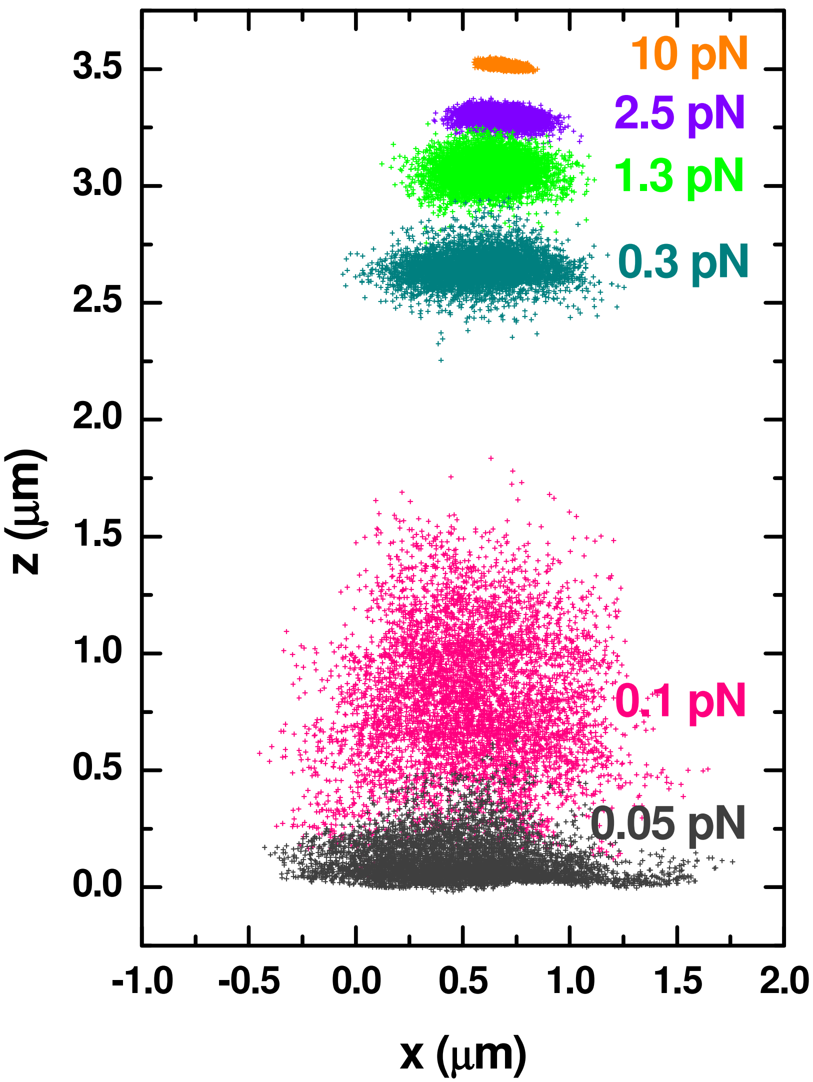


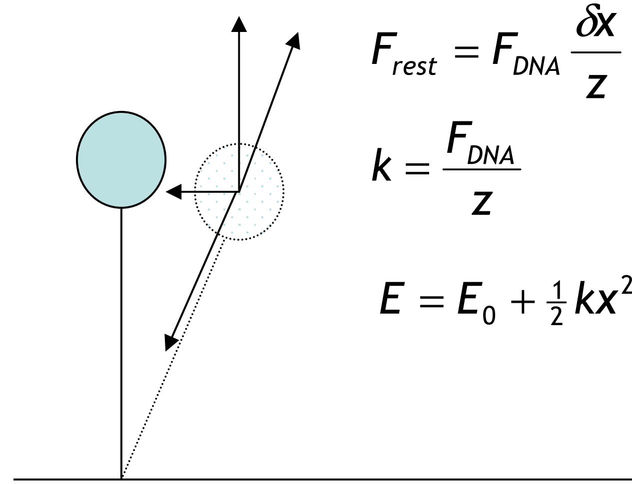
- xy position by video microscopy and image processing
- Create a look up table by scanning in the z-direction and calculating a radial profile
- Calculate the z position by comparing the current radial profile with the LUT
- Accuracy ~ 5 nm (@ 120 Hz)





Force measurements





$$F_{rest} = F_{DNA}\frac{\delta x}{z} = \frac{F_{DNA}}{z}\delta x = k\delta x,$$

$$k = \frac{F_{DNA}}{z}$$

$$E = E_0 + \tfrac{1}{2}kx^2 + \ldots$$

Equipartition theorem:

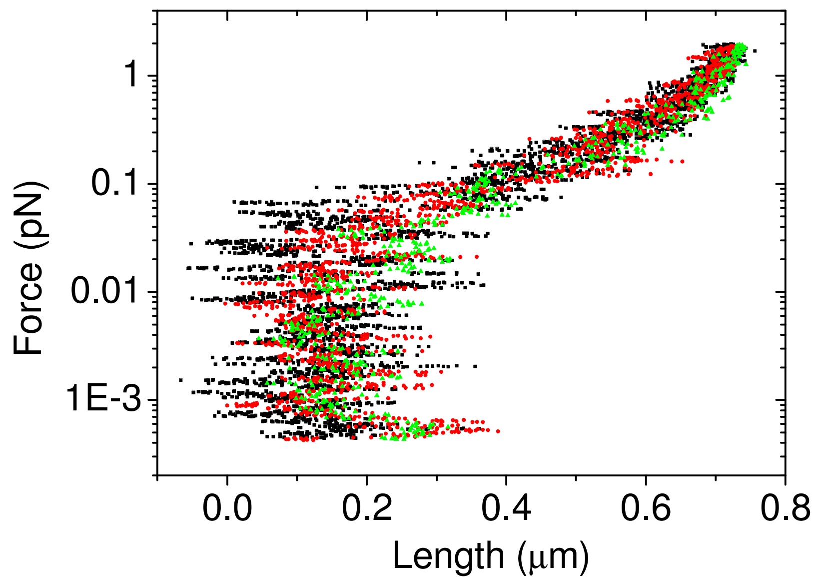
$$E = \tfrac{1}{2}k_bT = \tfrac{1}{2}kx^2$$

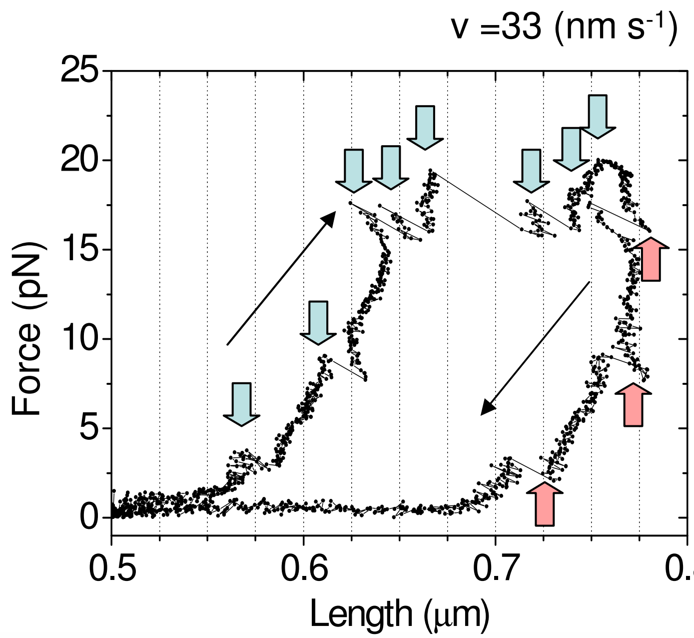
$$F = \frac{k_bTz}{\delta x^2}$$

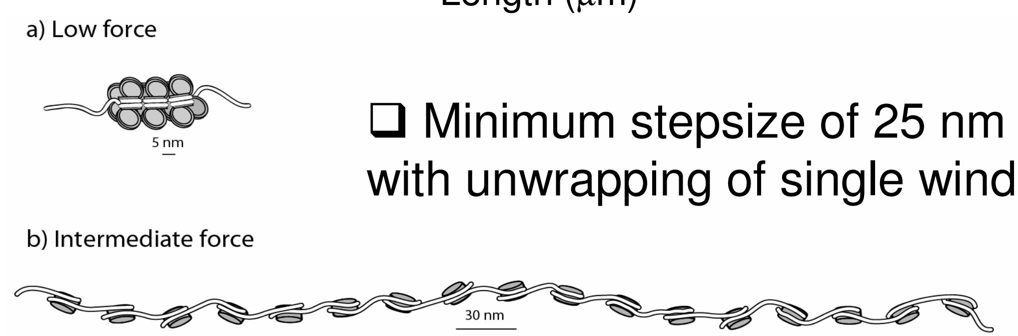
$$\tau = \frac{12\pi^2\eta Rz}{F}$$

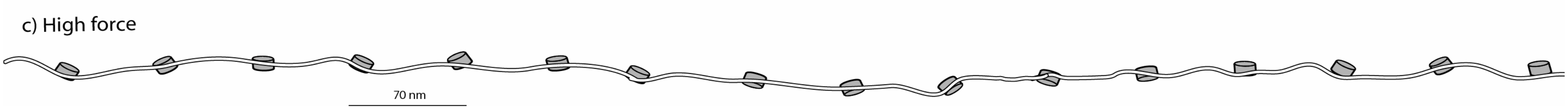
Strick et al. 1996 Science

Tweezers manipulation of Chromatin

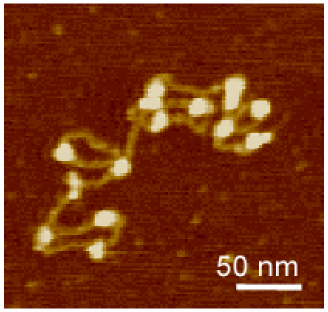




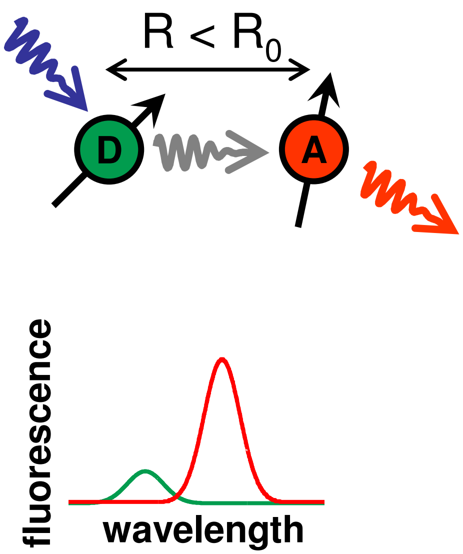


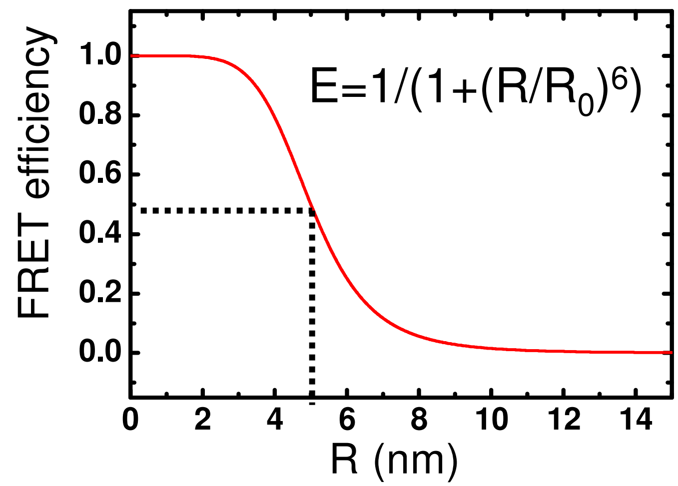


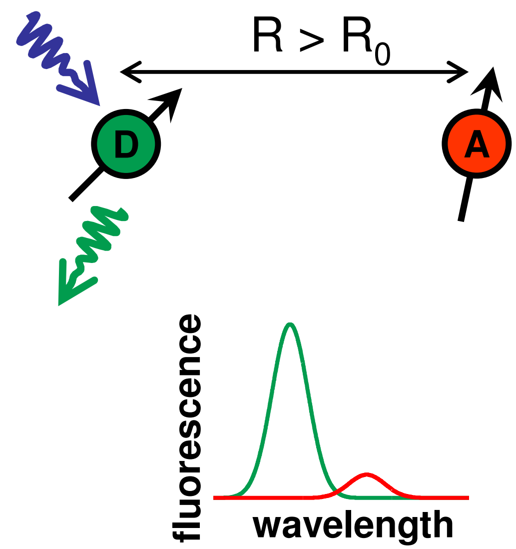
- Minimum stepsize of 25 nm is consistent with unwrapping of single wind



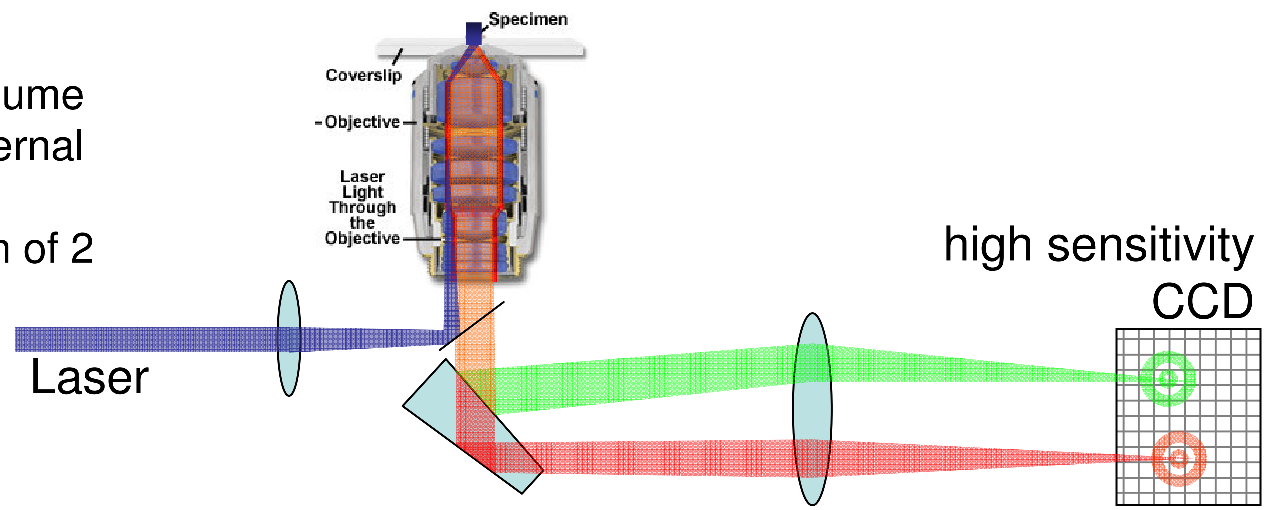
single pair FRET



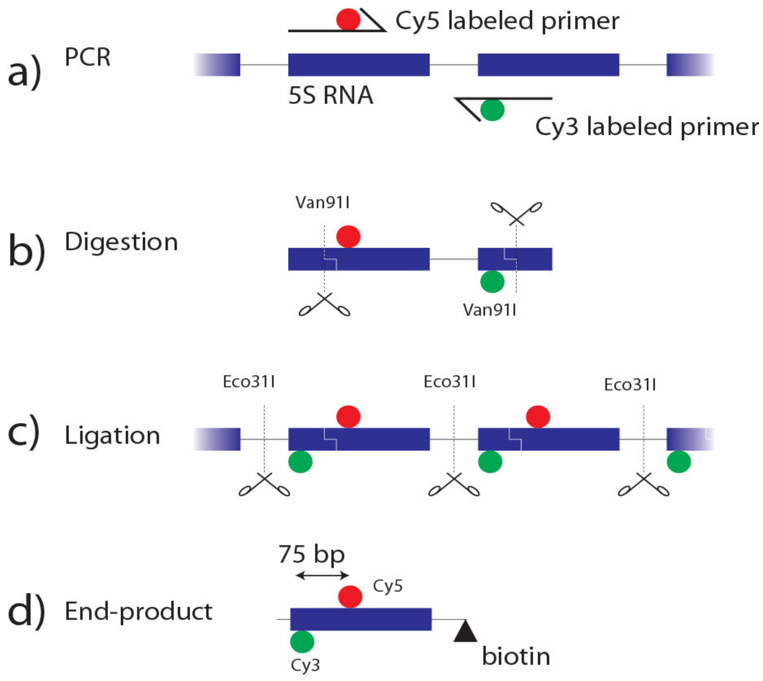


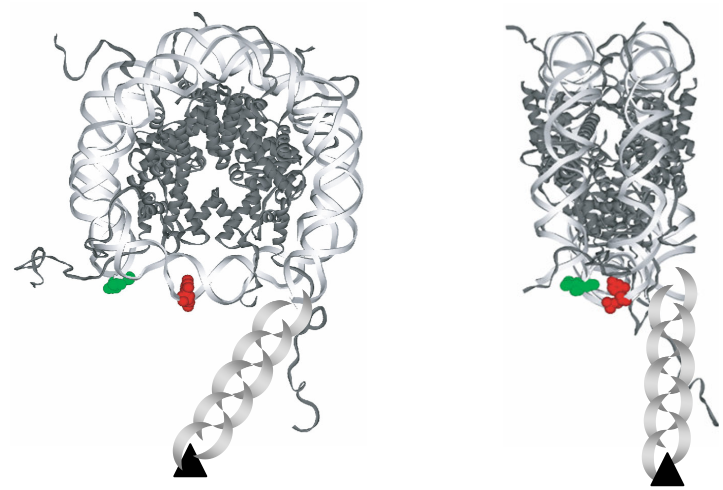


- ❑ Reduced illumination volume (=background) by Total Internal Reflection (TIR)
- ❑ Simultaneous acquisition of 2 channels by wedge



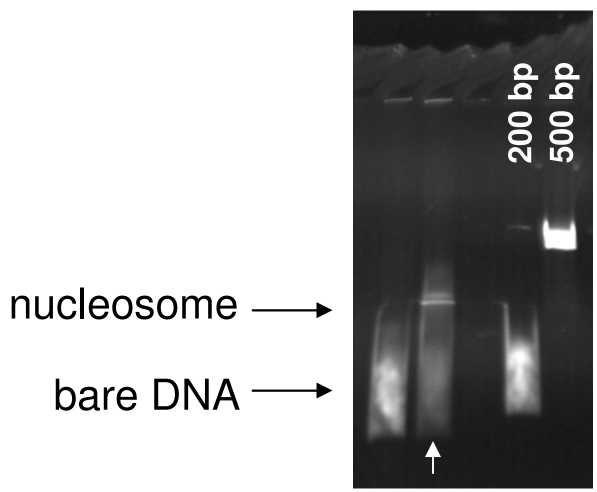
Assembly of a triple-labeled nucleosome

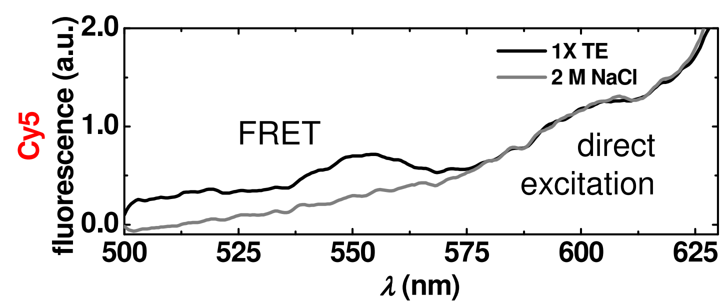




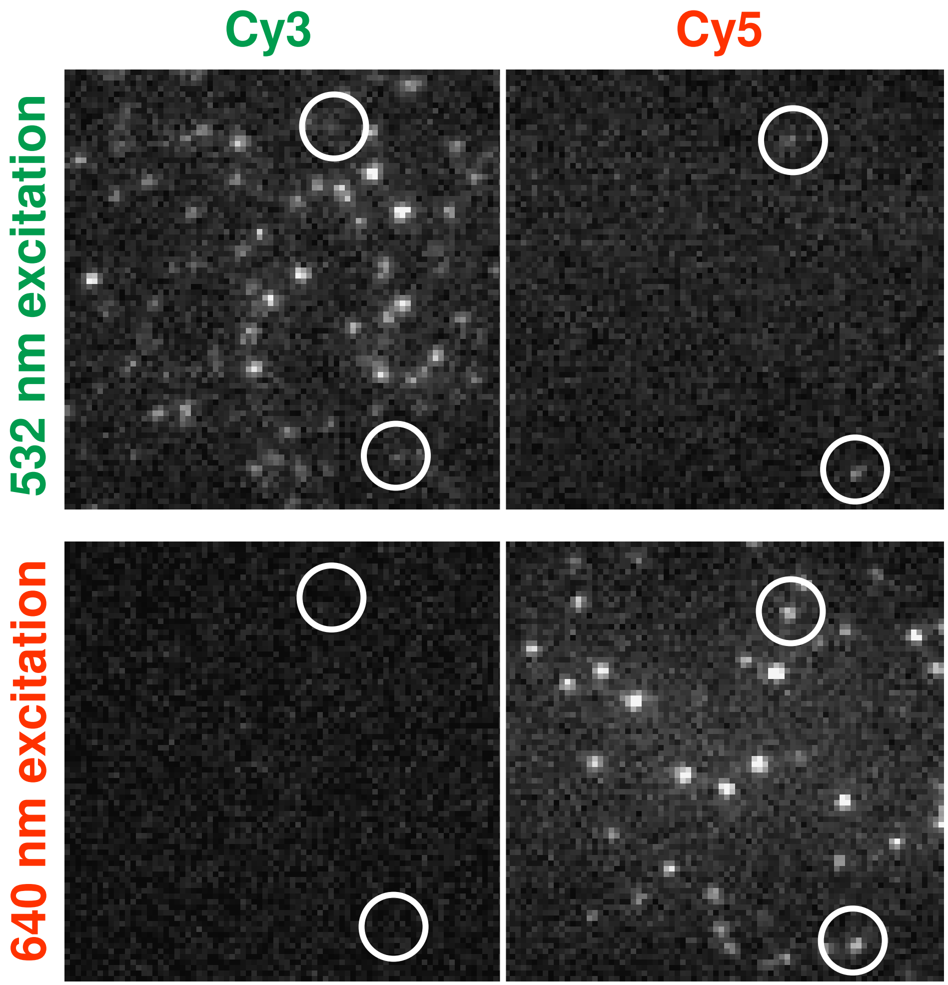
Coarse bulk characterization:

- ~50% reconstituted nucleosome
- ~30% FRET efficiency



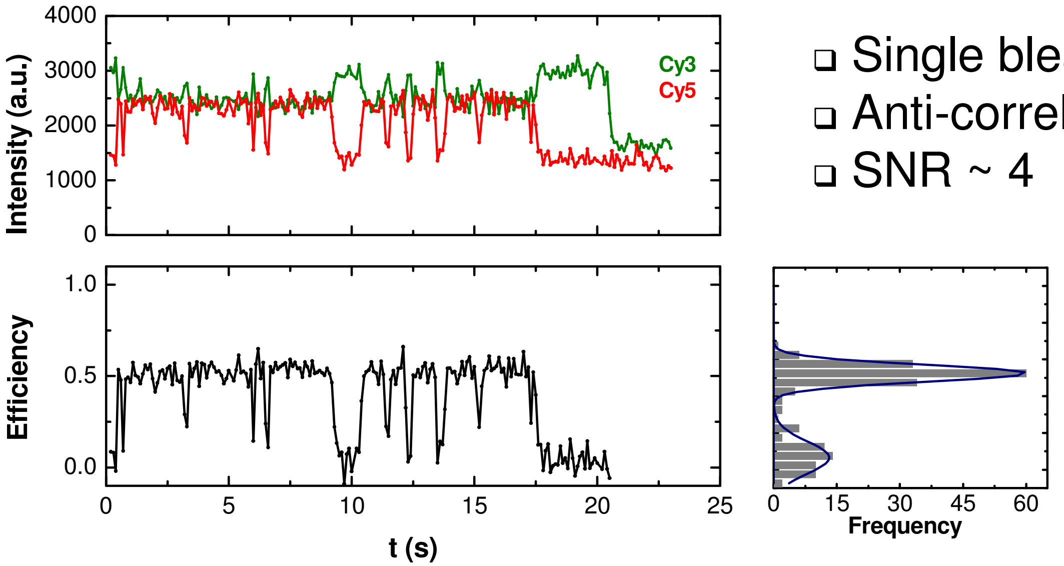


TIRF microscopy spFRET

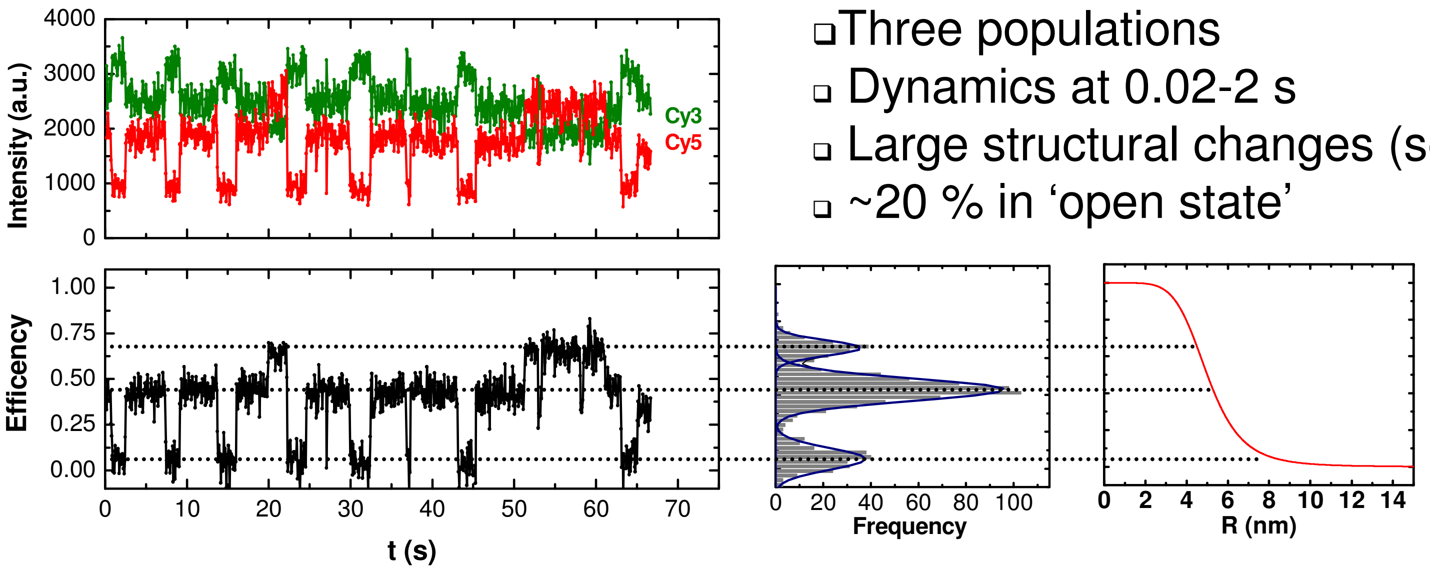


- Individual molecules can be distinguished
- Positions of Cy3 and Cy5 correlate
- Only a small fraction (~7%) shows FRET
- Without 3 mM $MgCl_2$ only 1% FRET

spFRET dynamics



- ❑ Single bleaching events
- ❑ Anti-correlated fluorescence intensity
- ❑ SNR ~ 4



- ❑ Three populations
- ❑ Dynamics at 0.02-2 s
- ❑ Large structural changes (several nm's)
- ❑ ~20 % in 'open state'

Challenges

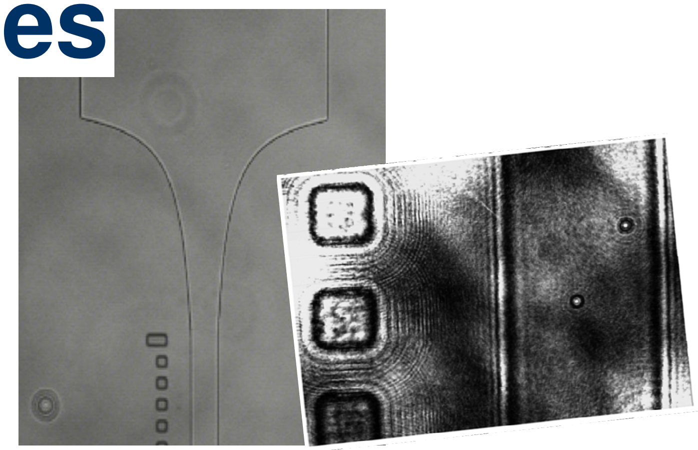
- ❑ Chromatin is highly heterogeneous and it's dynamic folding is now known to be a dominant factor in gene regulation
- ❑ Physical models of chromatin dynamics are only marginally supported by experimental results

- ❑ Who, When, Where, What …. How????

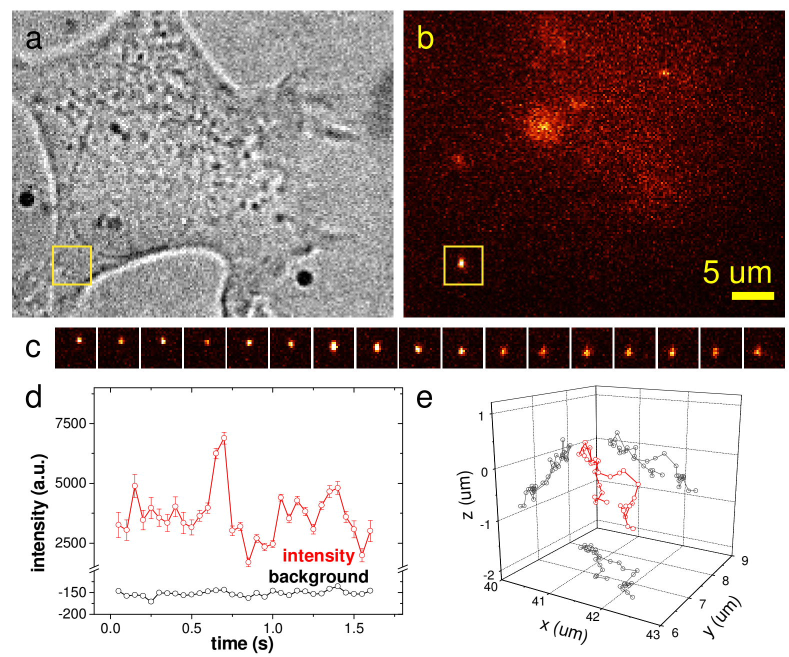
Approach:

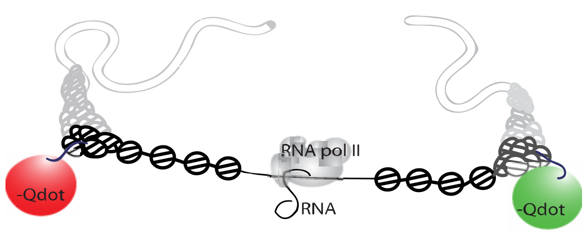
- ❑ Identification of relevant factors
- ❑ Nano-scale visualization in intact cells
- ❑ Purification of natively assembled intact structures
- ❑ Structural and dynamical analysis @ single molecule/nm level
- ❑ Assembly in vitro
- ❑ Educated modifications to test or modify functionality

Opportunities



- ❑ Smart surface modifications
- ❑ Patterning of functional surfaces
- ❑ Integration with micro-fluidics
- ❑ Combinations of functionalities
- ❑ High throughput devices
- ❑ In vivo tracking of single molecules









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