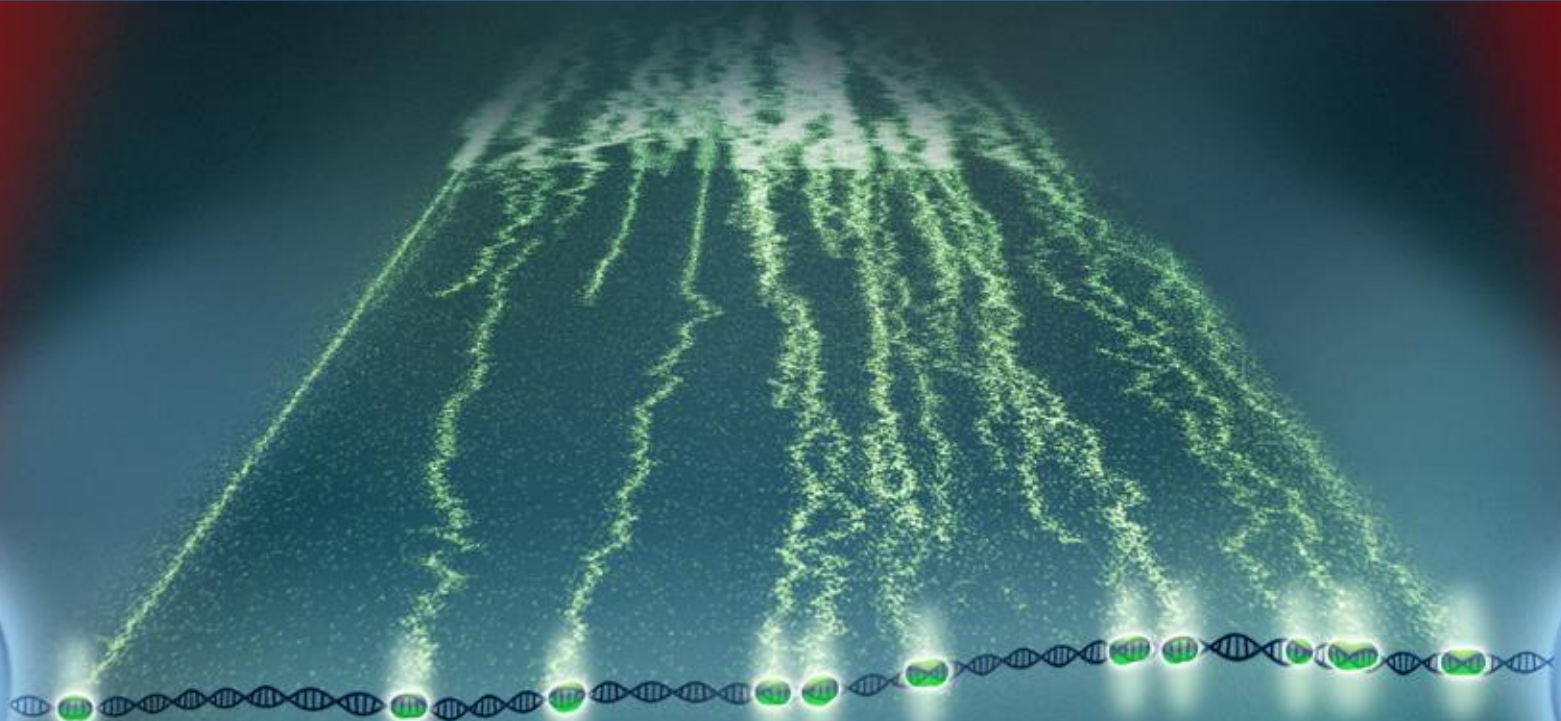


Single-molecule observation and manipulation of complex molecular Interactions



Gijs Wuite

Why manipulate & image single molecules?

Many DNA enzymes are highly dynamic molecular machines

Seeing them directly allows verification of models

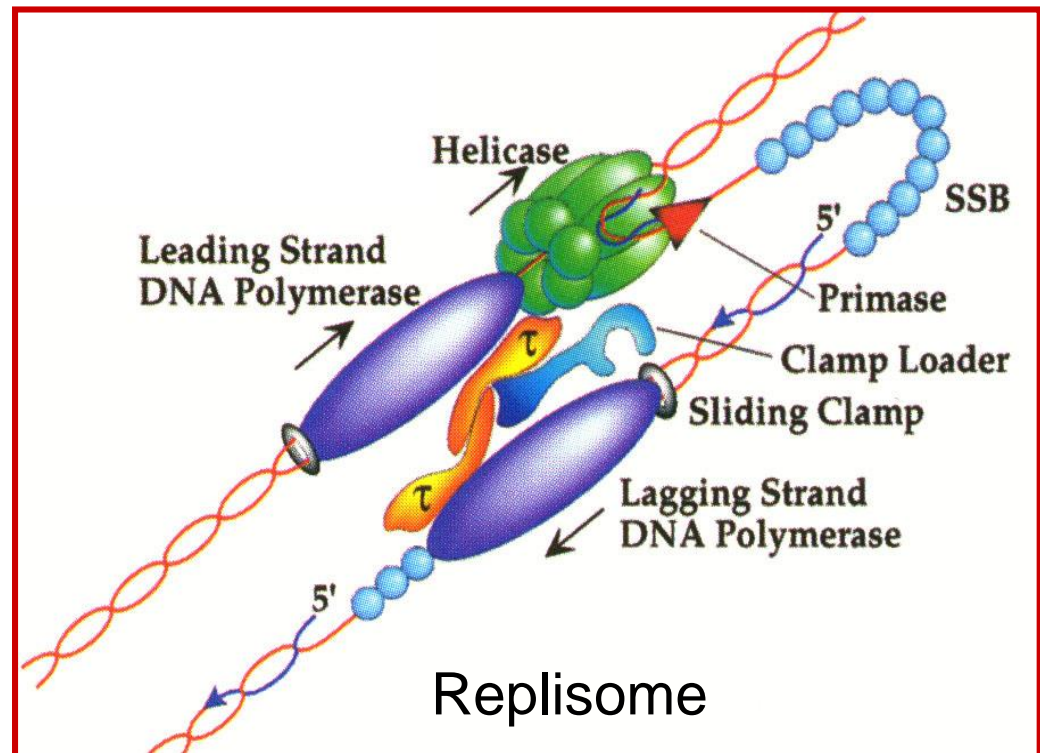
Moreover many use energy to move and manipulate substrates

Trying to understand the connection between chemistry, force generation and movement

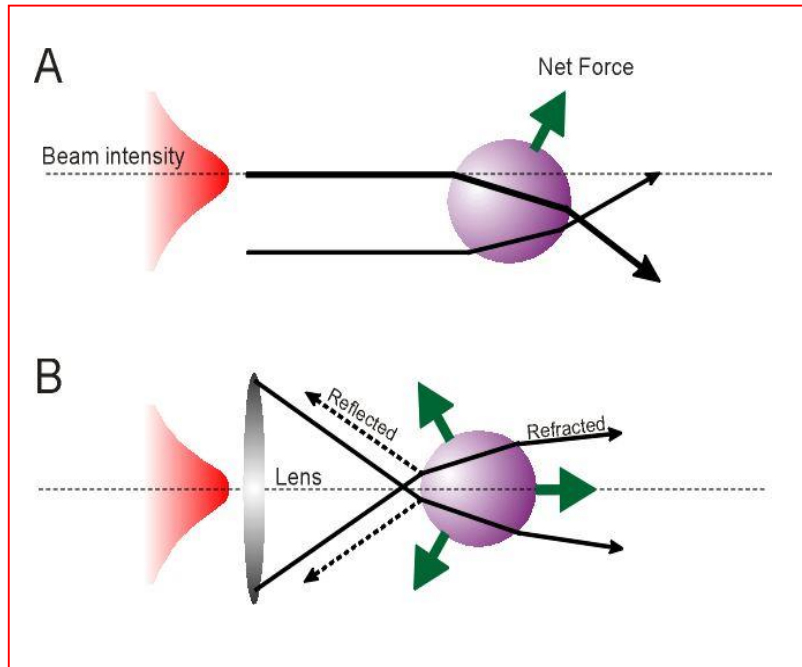
Use force as a variable

Protein interactions:

- (non)-Specific
- Single vs. multiple binding
- Passive vs. active (motors)



Single molecule manipulation with Optical Tweezers

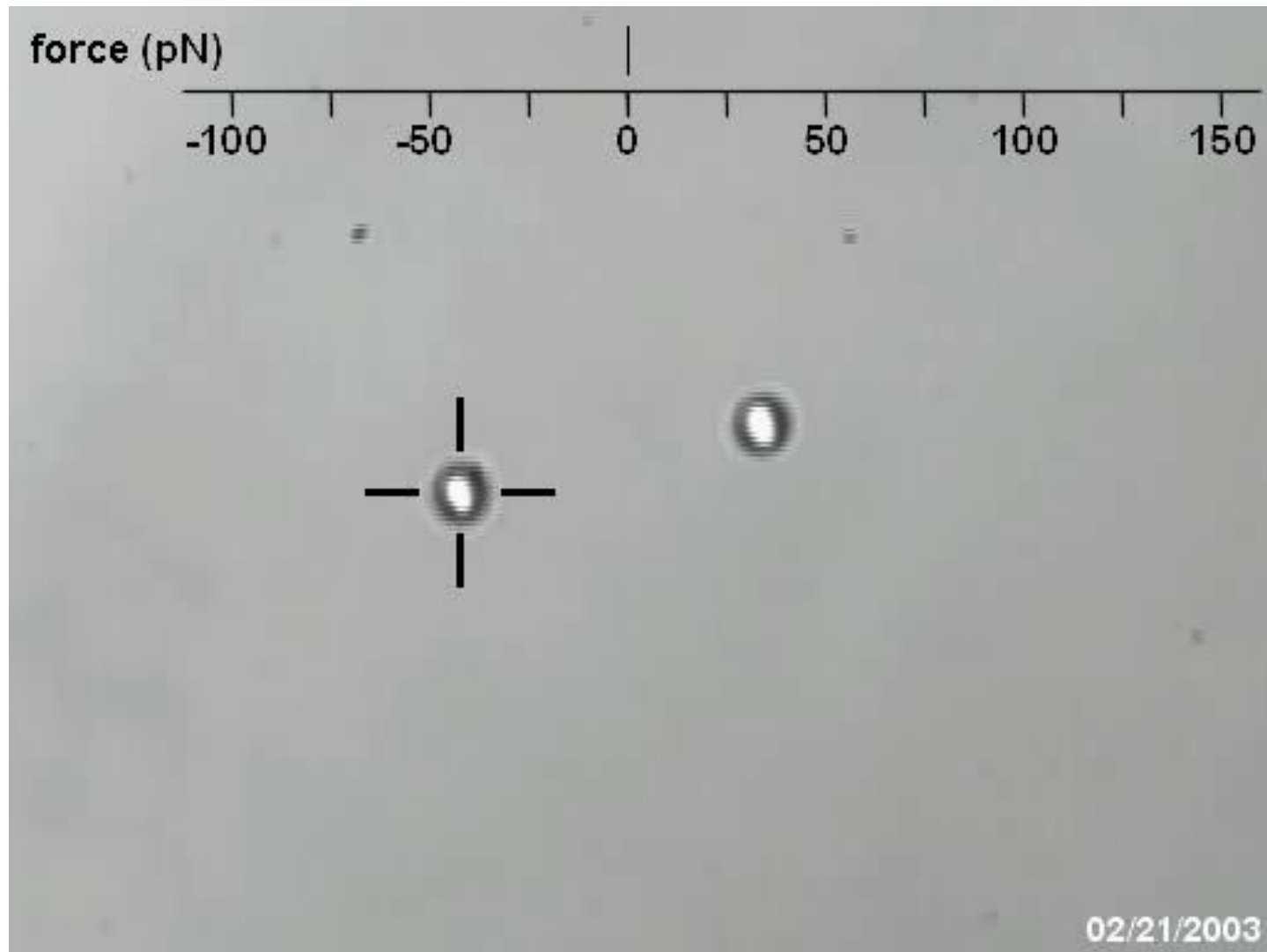


Arthur Ashkin – Nobel Prize
Lauriate 2018

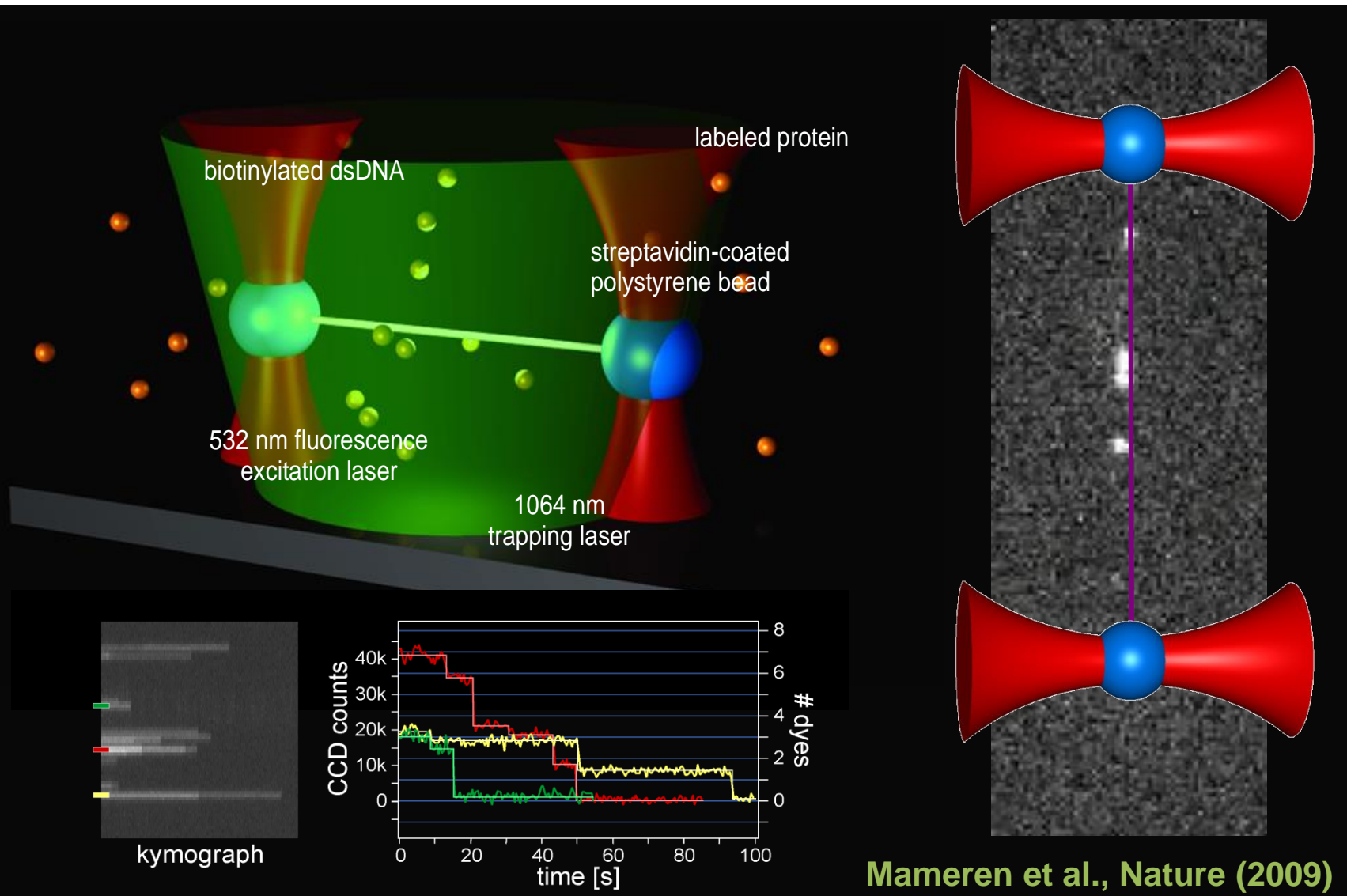


Typical stiffness 100 pN/ μm
Typical forces 0.1-100 pN
Typical distances 1 nm
Typical times 1 ms

Manipulating DNA

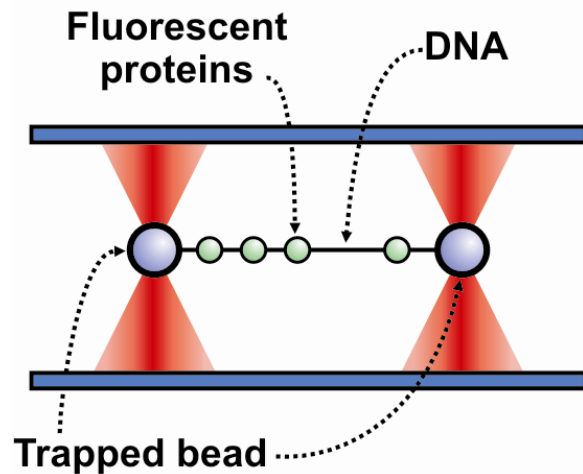


Optical tweezers with Fluorescence

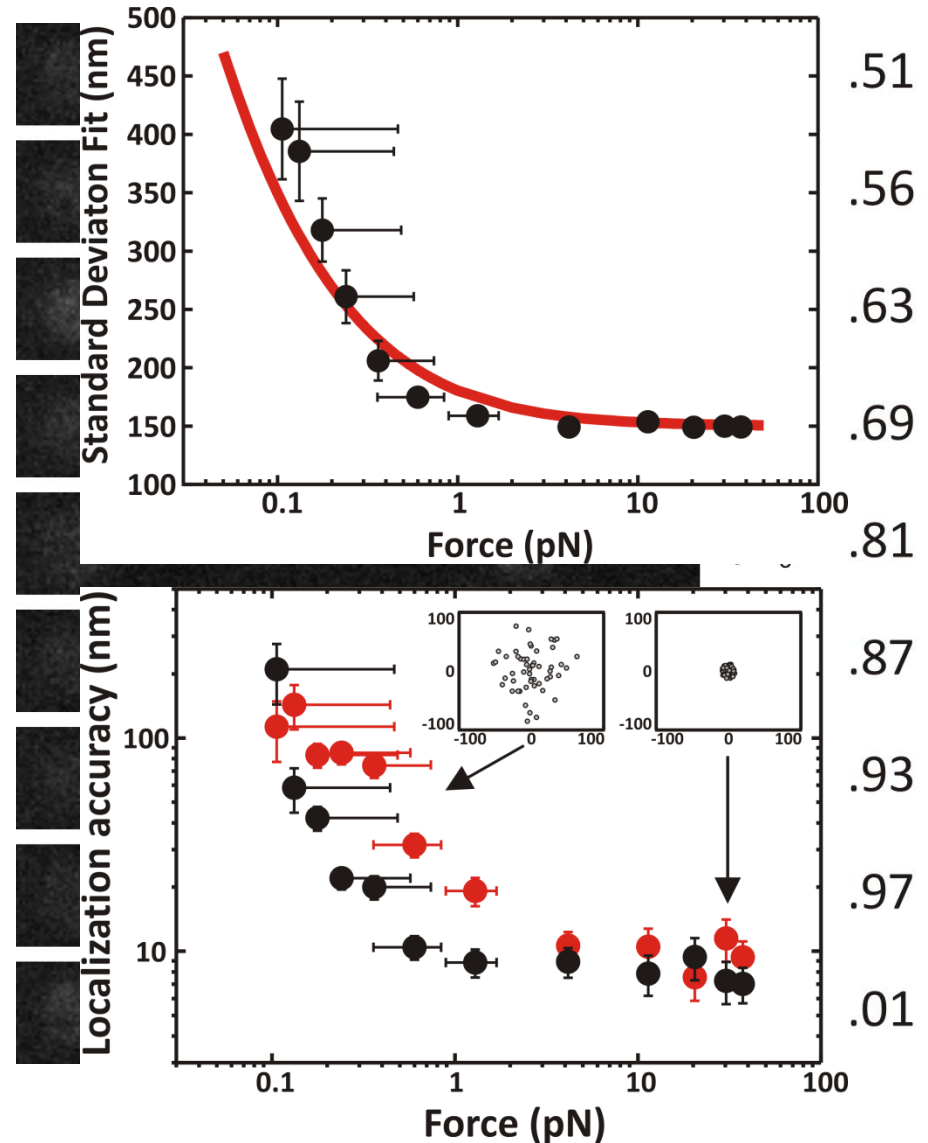


Force control and direct visualization

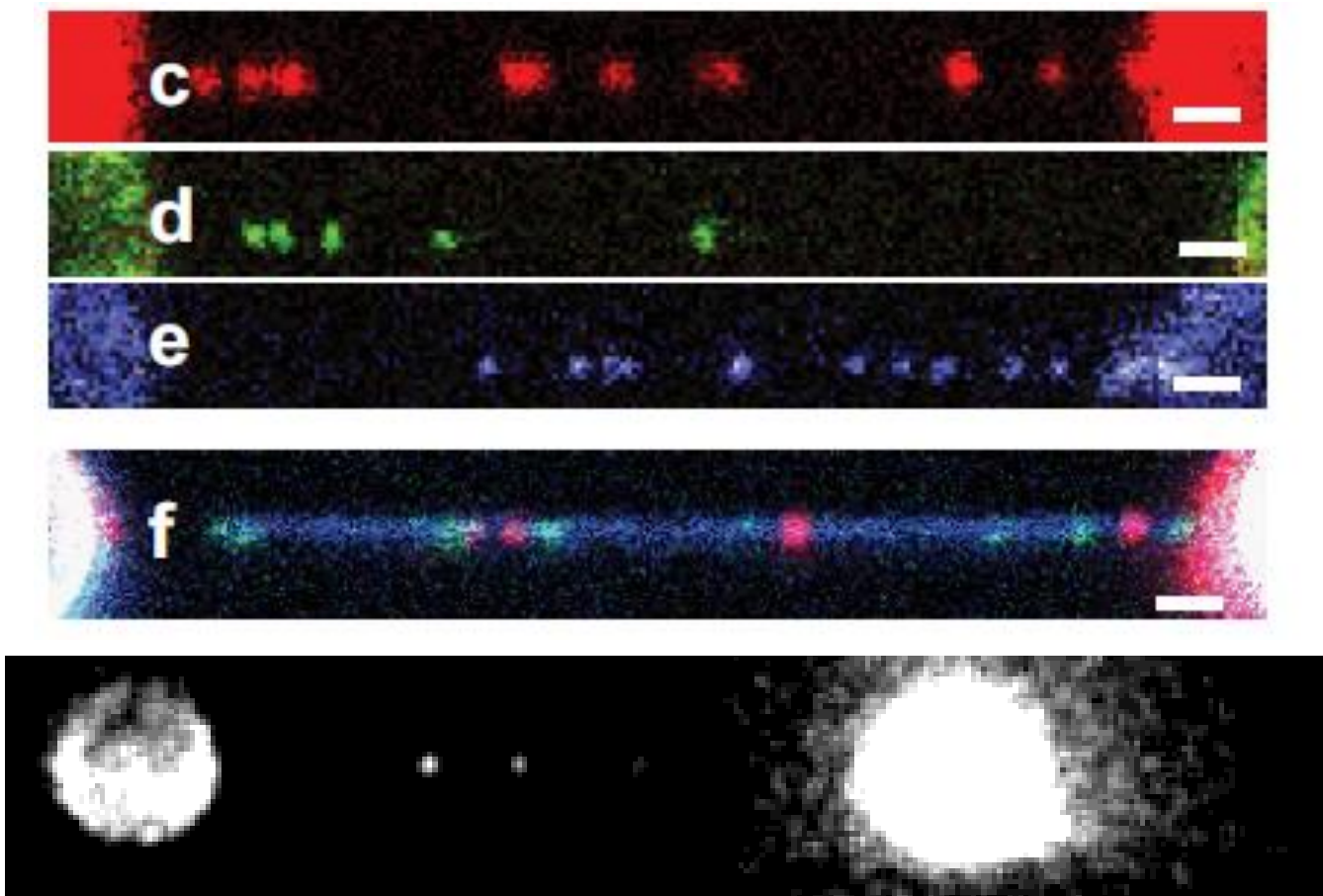
DUAL OPTICAL TRAPS FLOW STRETCHING sm-FLUORESCENCE



8 nm (25 bps) accuracy @ 1 pN
and up!

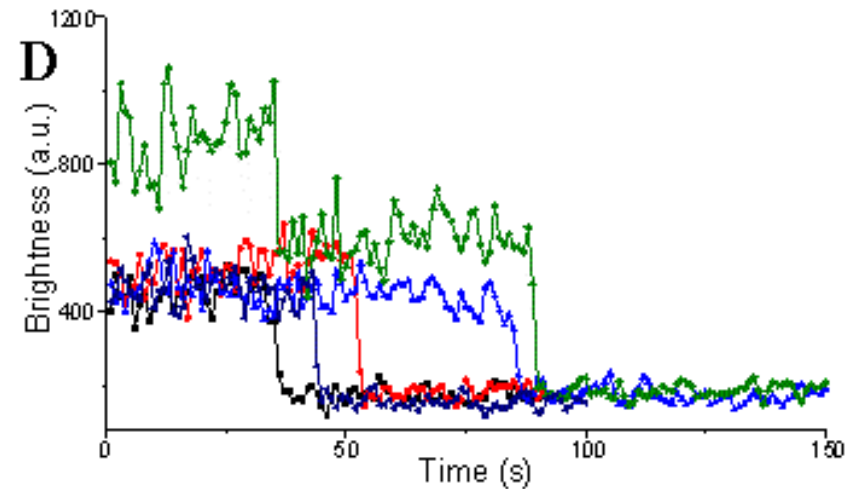
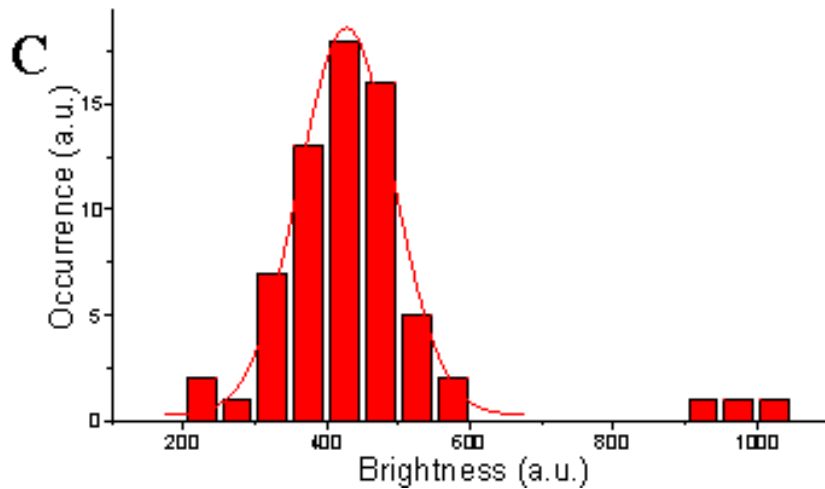
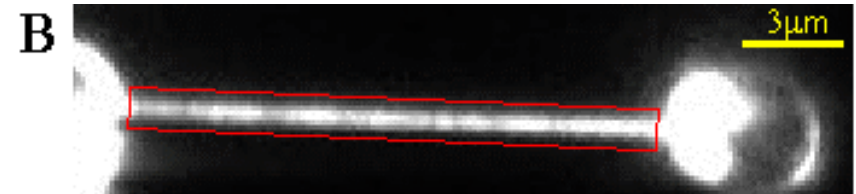


Multi color detection & Real time binding



- Direct observation of multiple proteins on DNA
- Binding in real-time we observe in concentrations below 10 nM
- In confocal mode up to 100 nM

Physical properties of a protein

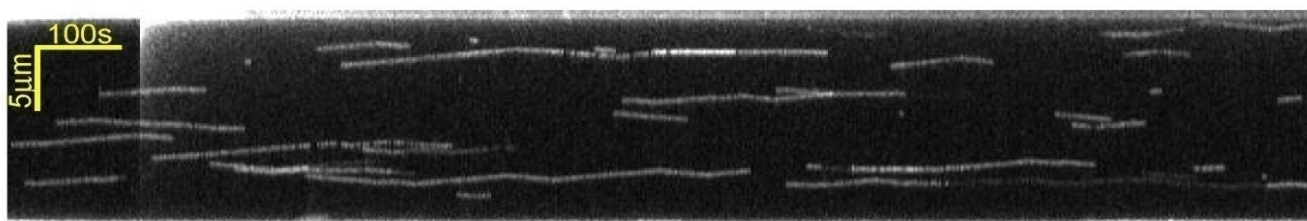


- Single molecule interactions at 50 pM a protein (PICH)
- Interactions predominantly monomers at low concentrations
- Determining the footprint (17 ± 3 bp = ~ 6 nm)

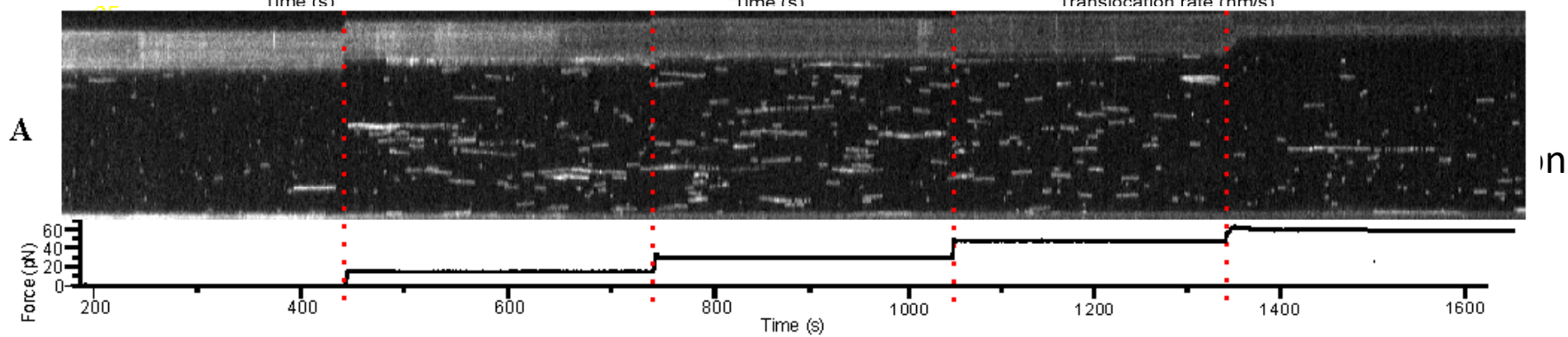
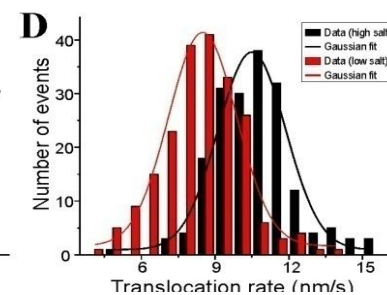
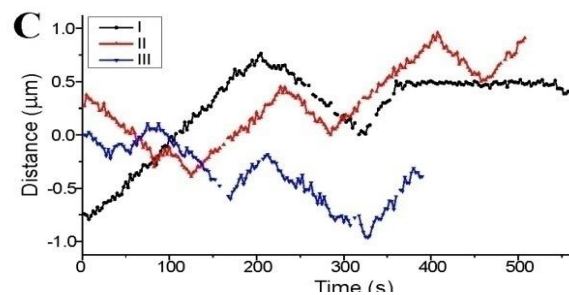
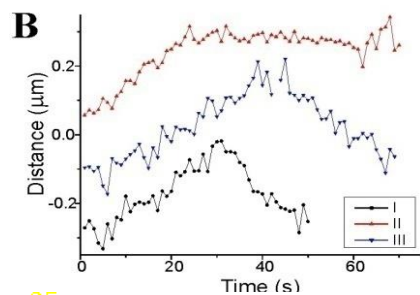
Proteins bind, move and feel the force



100 mM NaCl
2 mM MgCl₂



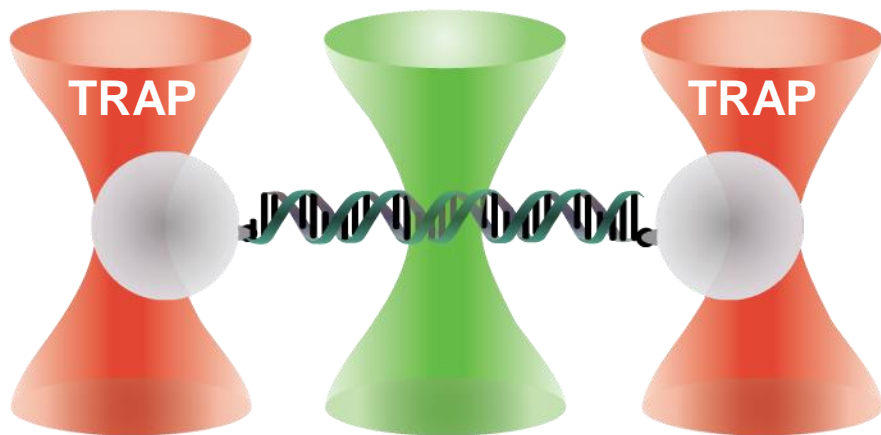
25 mM NaCl
2 mM MgCl₂



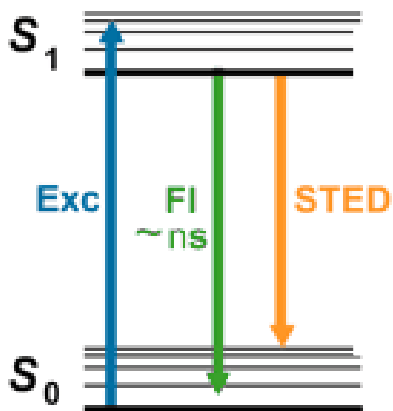
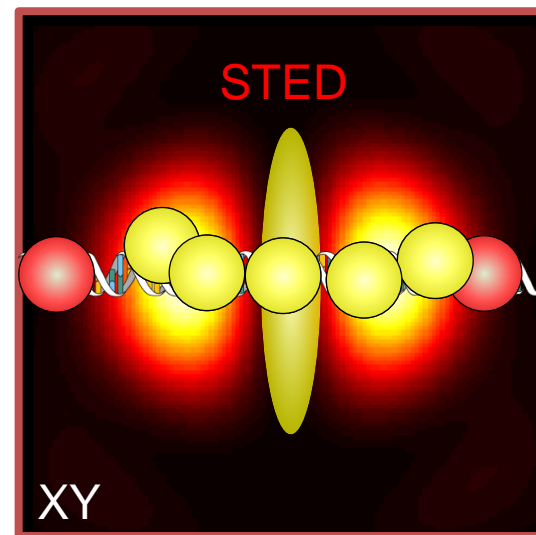
in

Super resolution microscopy

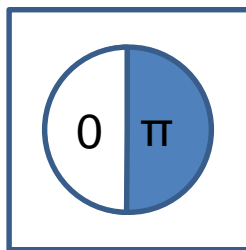
EXC+STED



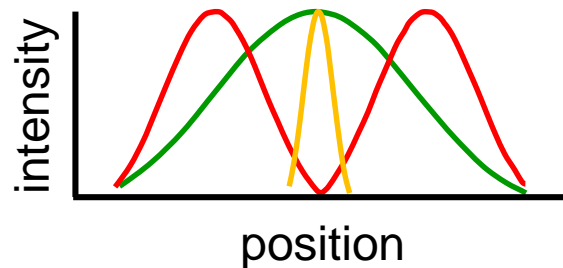
High density **ON** DNA



1D-STED

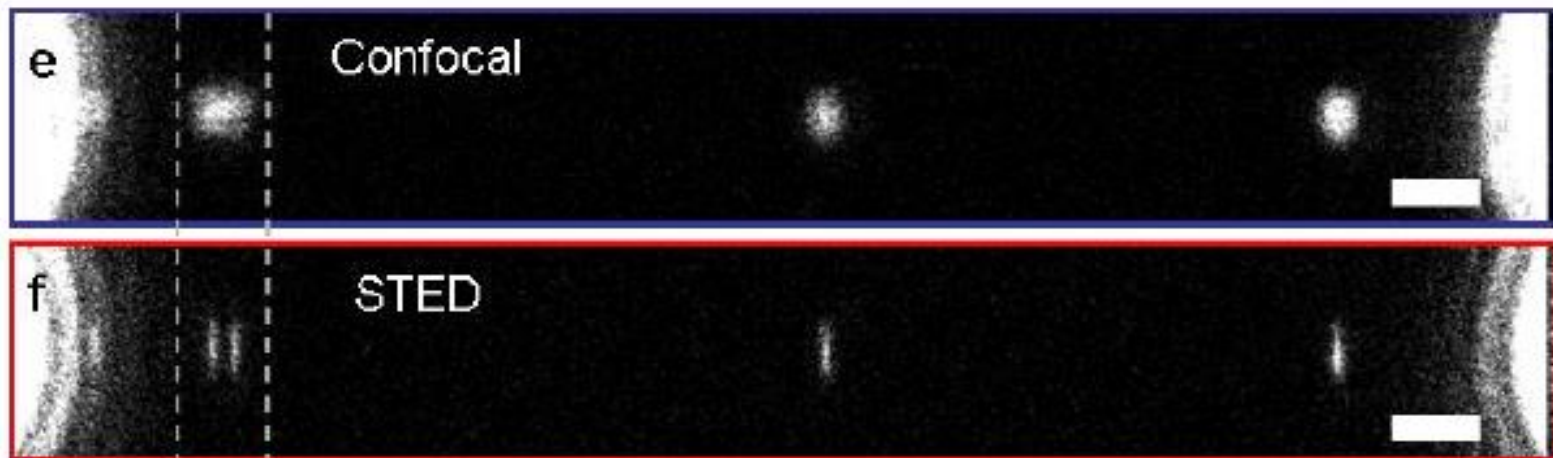
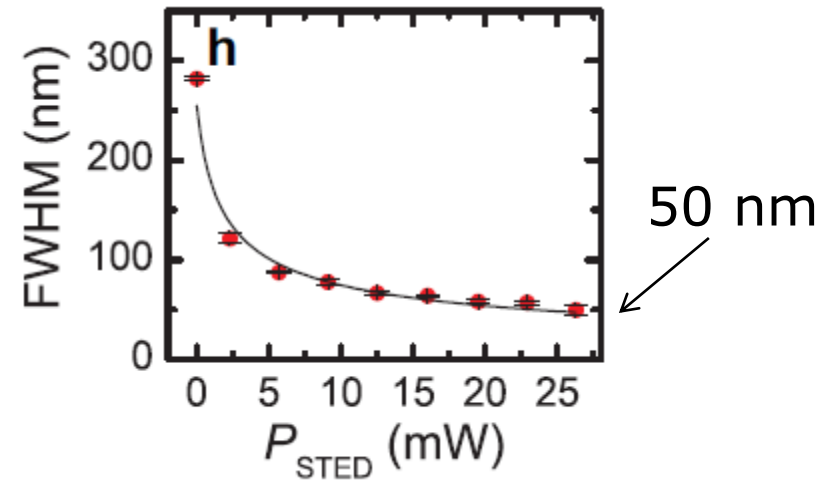
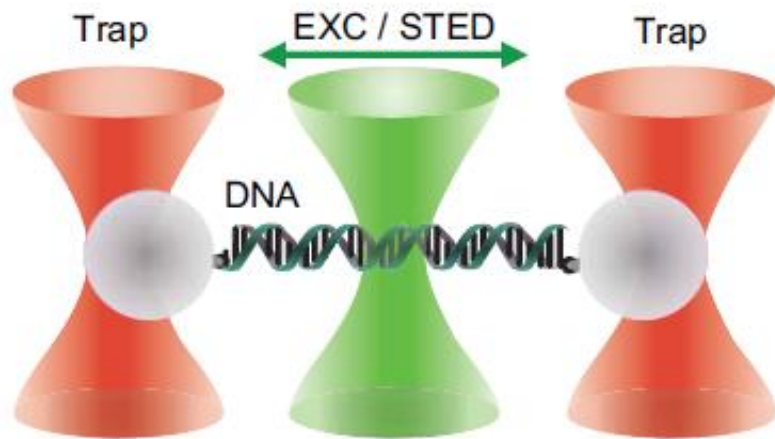


Binary phaseplate

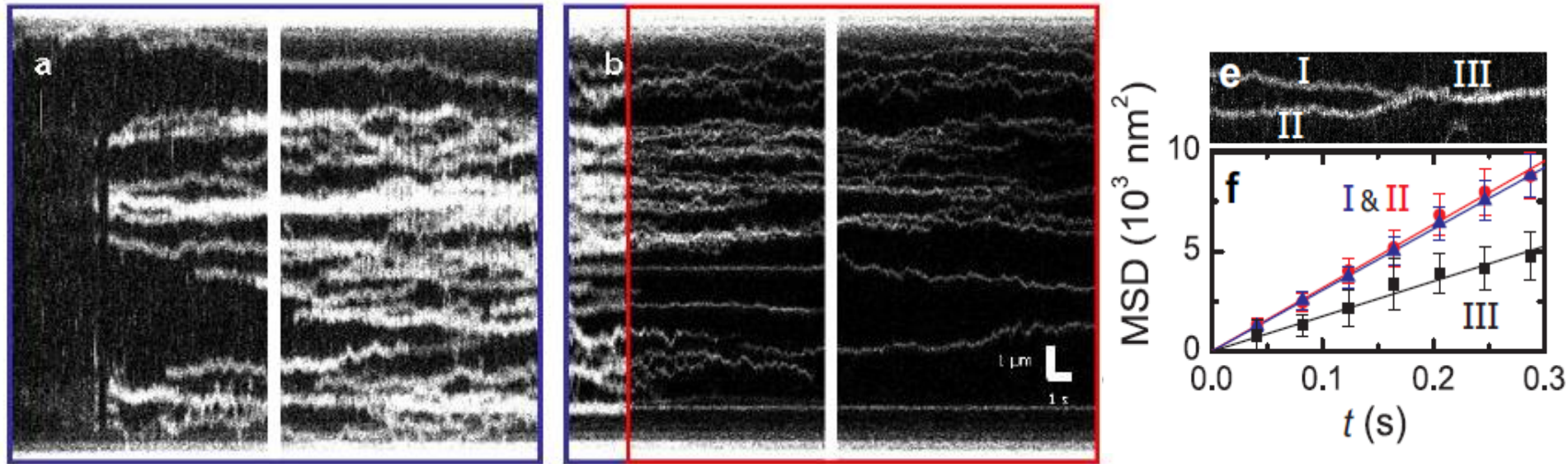


STimulated Emission Depletion

Super resolution (STED) with Trapping



Super resolution on densely coated DNA



- Tracking many proteins at the same time
- Proteins influence each other
- Densely coated DNA biologically more relevant and show different behaviour

Trapping with Fluorescence

With wide-field illumination:

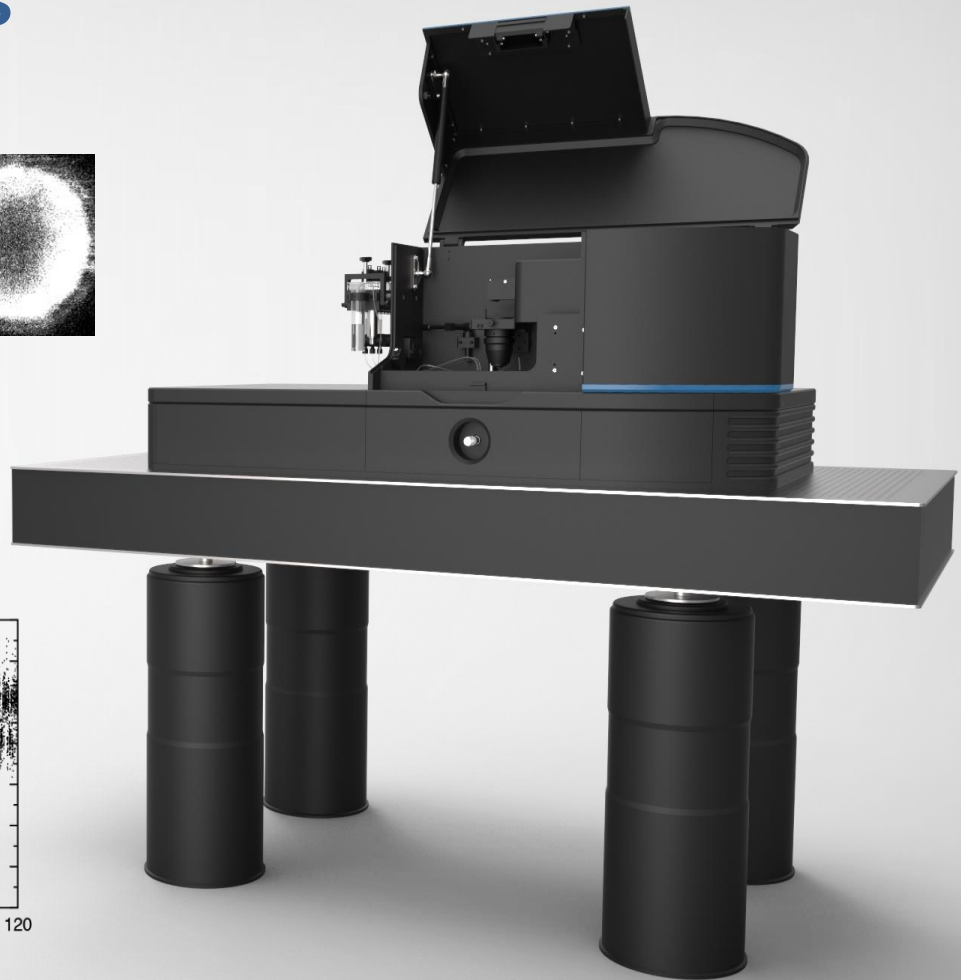
- Localization of single proteins on DNA with 8 nm accuracy
- Footprint & oligomeric state directly measurable
- Observe binding in real-time @ $>[10 \text{ nM}]$
- Multi-color detection: different proteins on DNA can be tracked

With super-resolution:

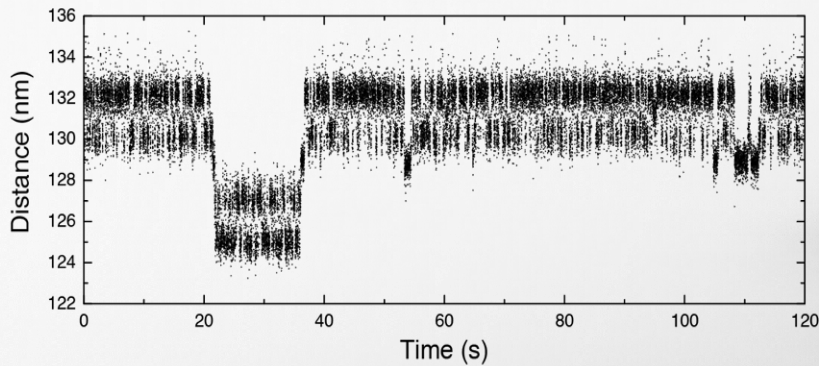
- Tracking proteins in high density on DNA
- Resolution $>50 \text{ nm}$
- Localization 6 nm
- Observe binding in real-time @ $[100 \text{ nM}]$
- Biologically relevant conditions

C-Trap™ Applications

Live observation of Protein – DNA interaction



Best in class force resolution: <0.3 pN @ 200 Hz



Acknowledgements



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