Correlative Tweezers Fluorescence Microscopy:
super-resolution STED-imaging of DNA-protein interactions

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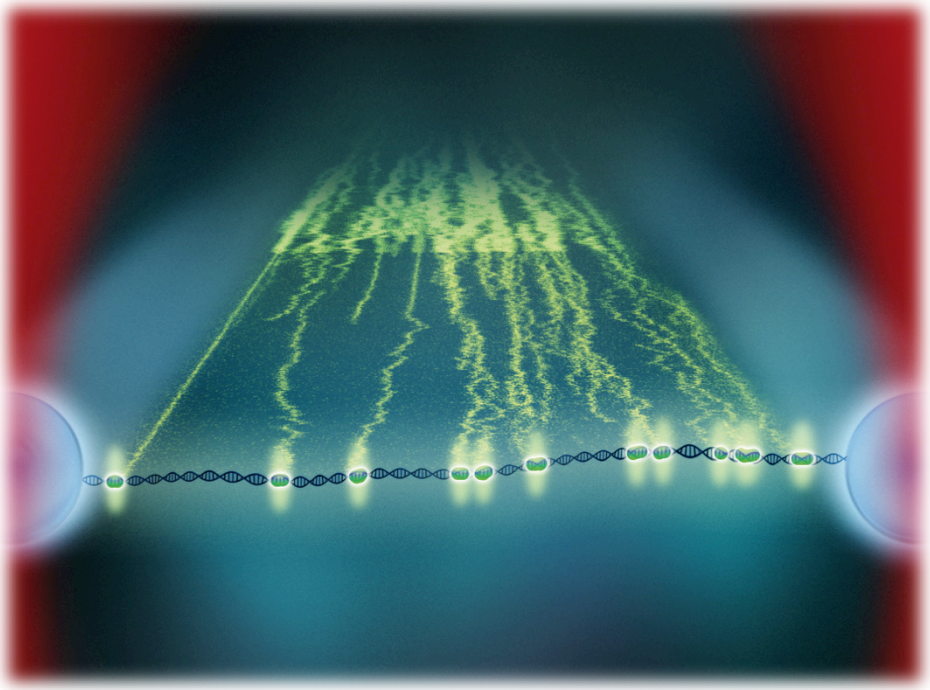

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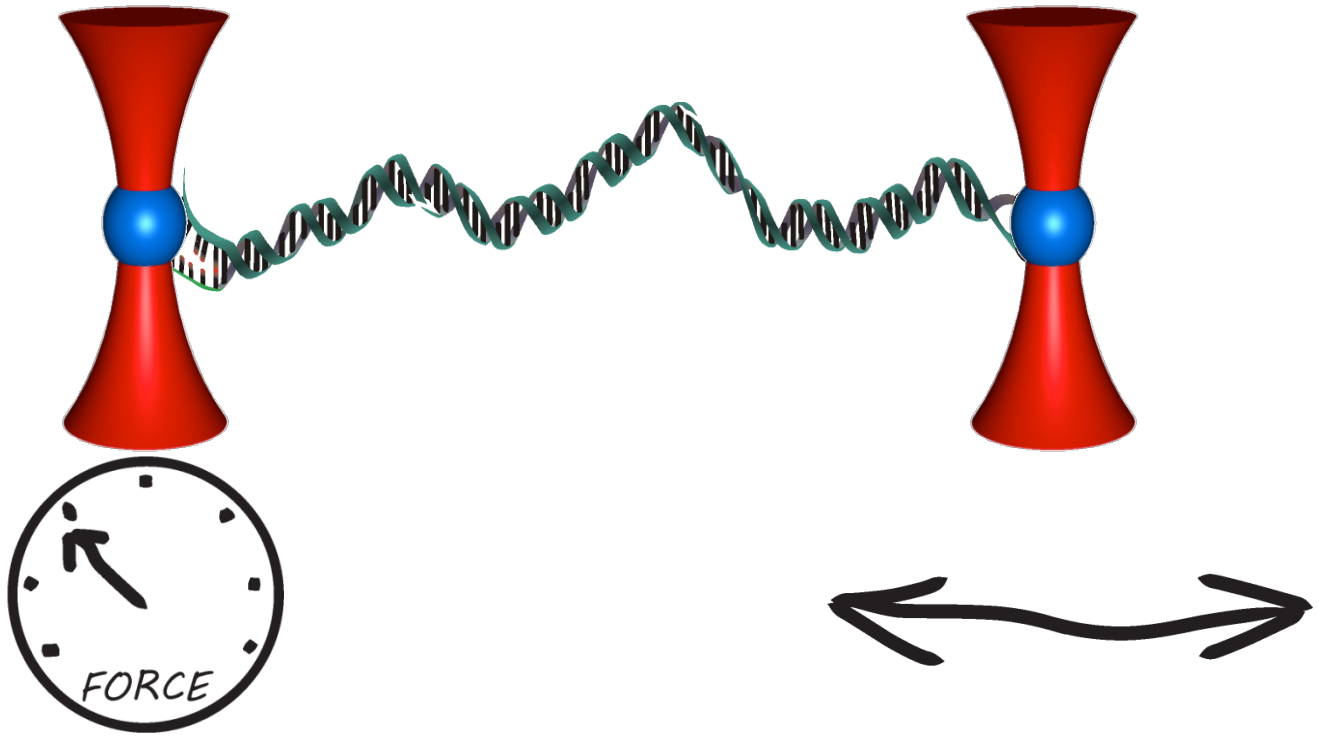
Manipulation of DNA with optical tweezers



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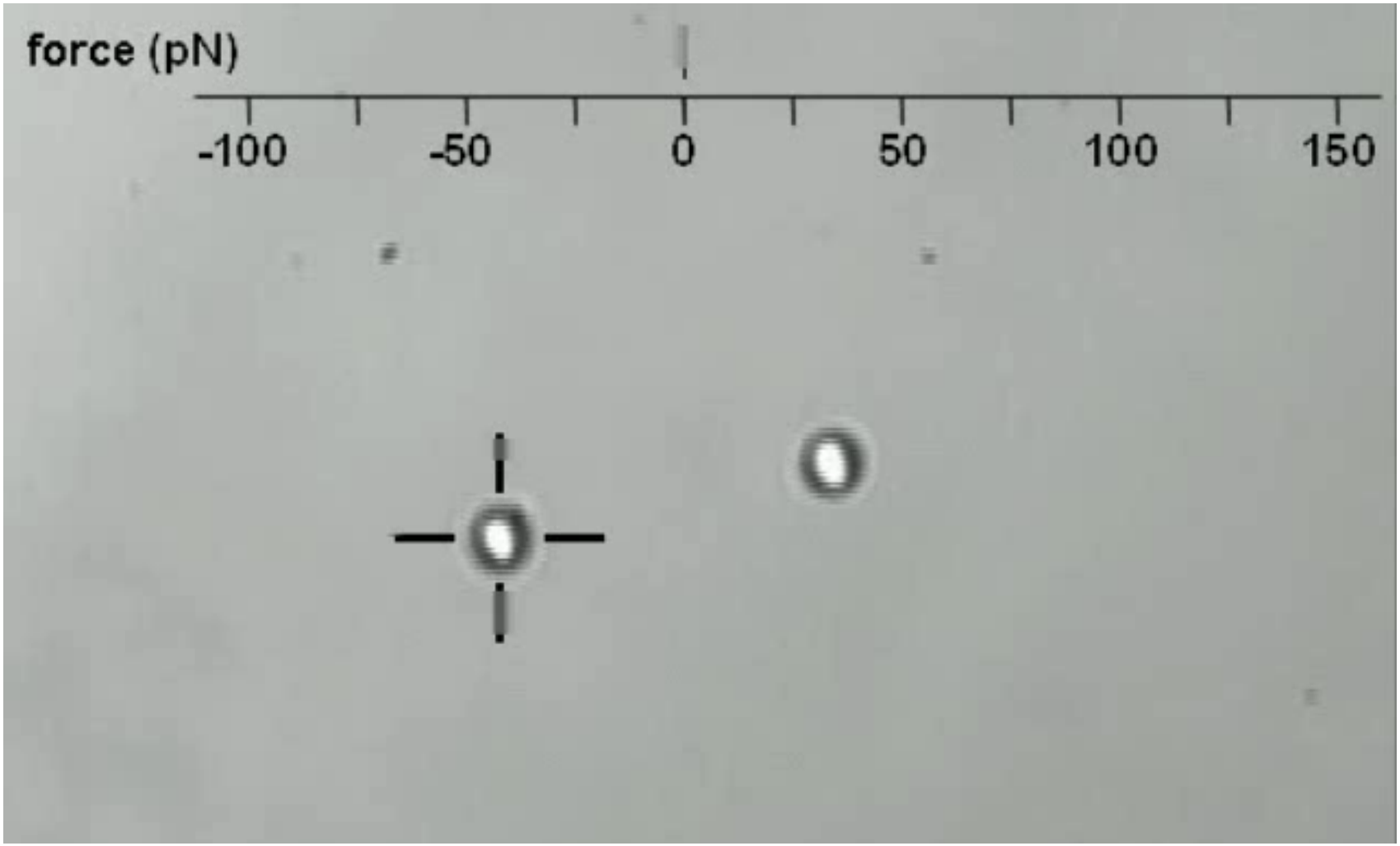

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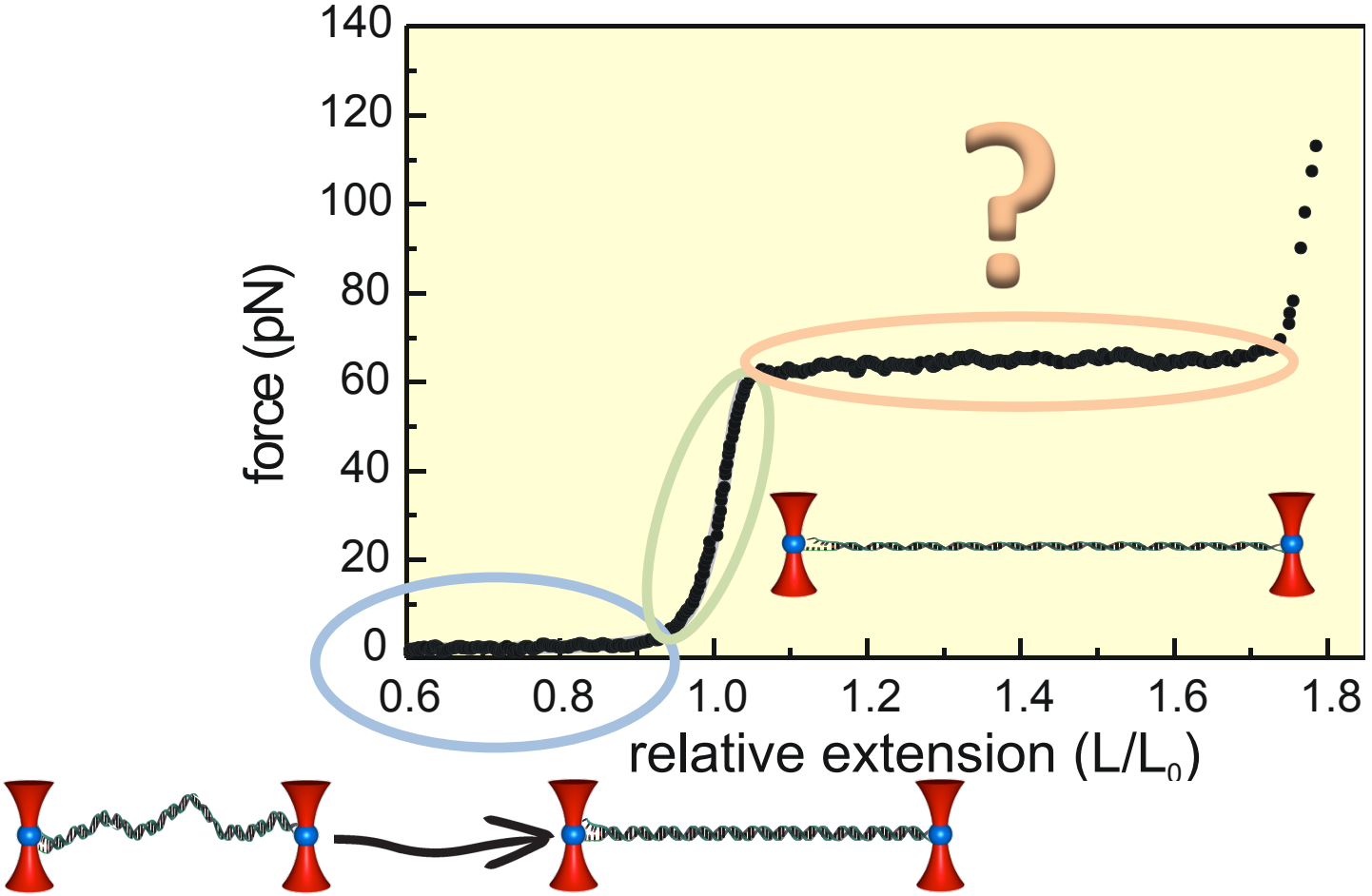
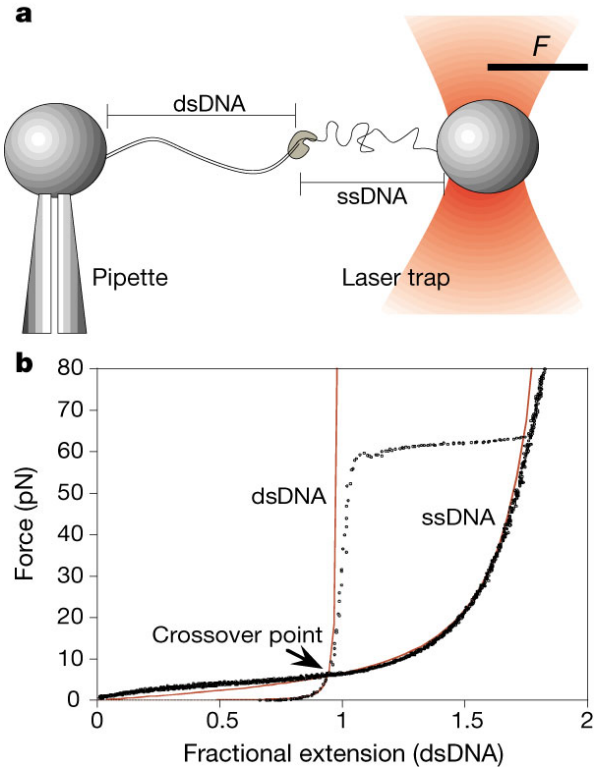


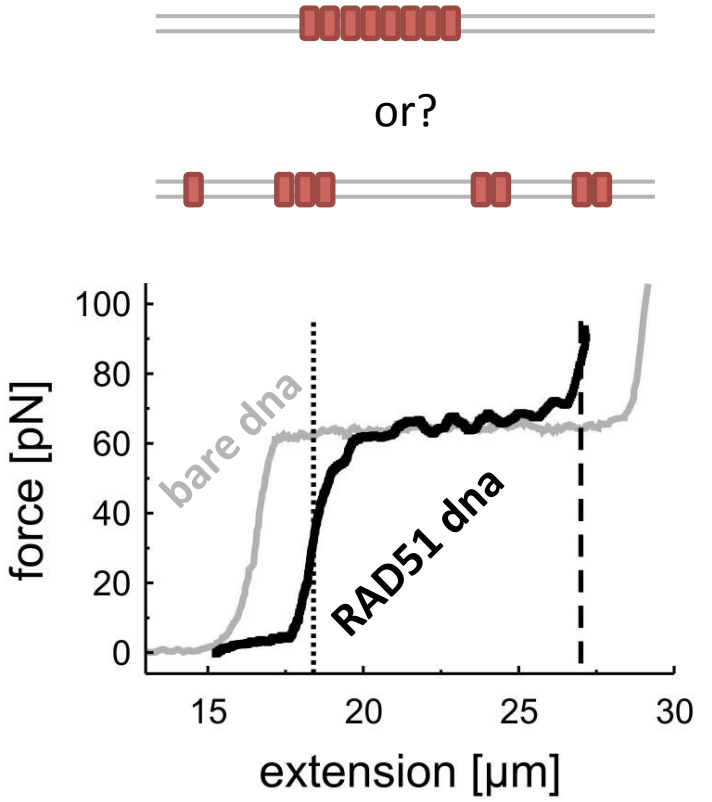
global information on whole DNA molecule

Manipulation of DNA with optical tweezers

Applications



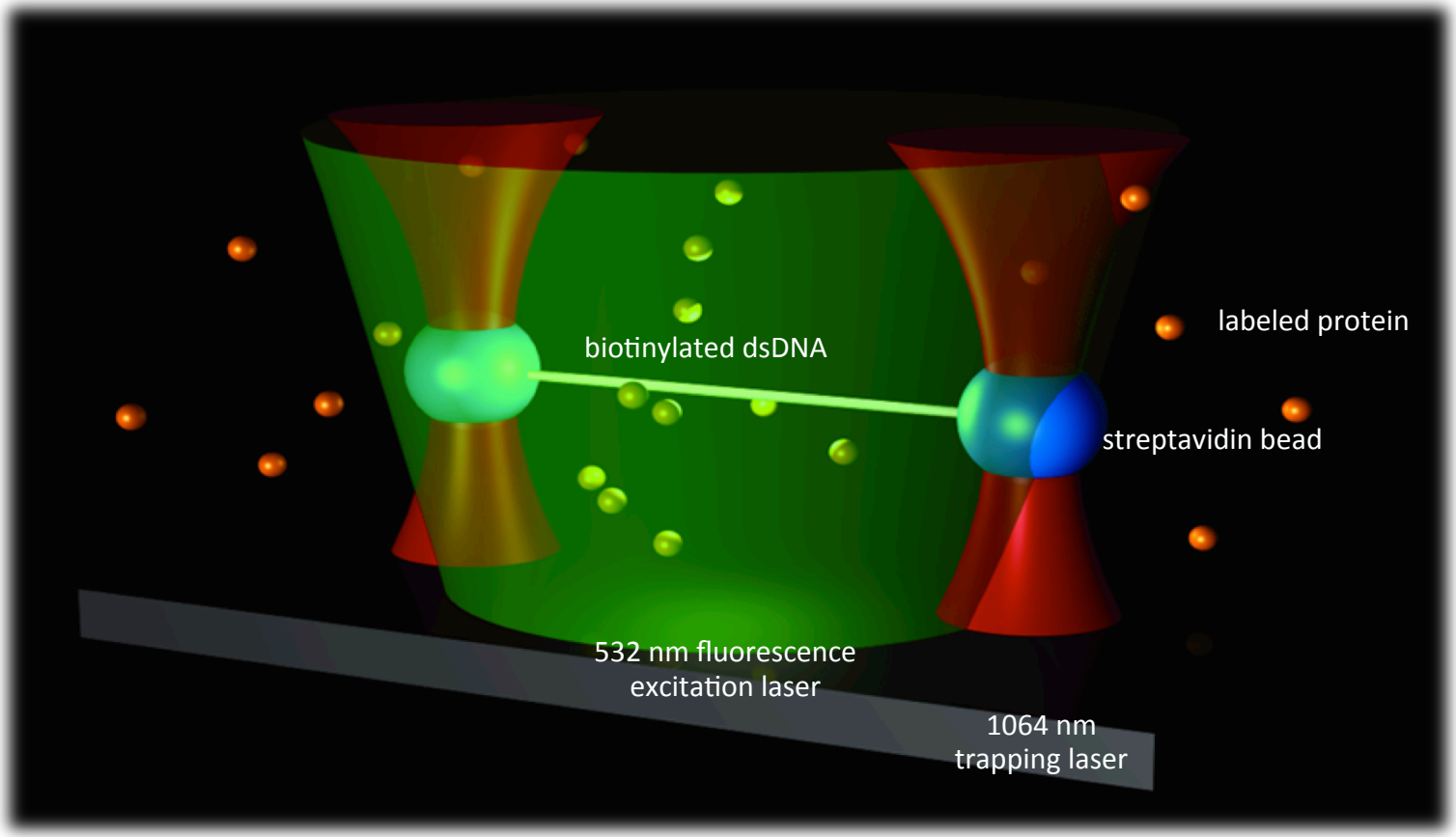
DNAp converting ssDNA in dsDNA
Wuite et al., Nature 2000



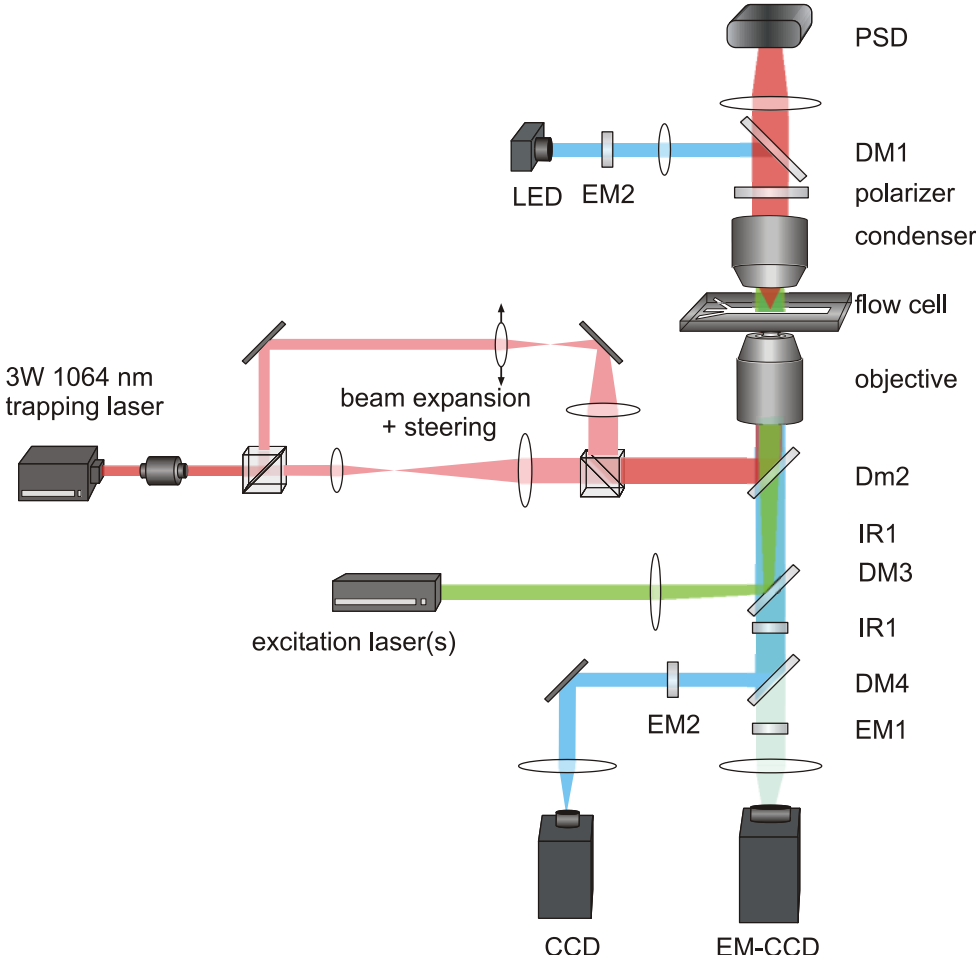
RAD51 binding to dsDNA
van Mameren et al., Biophys J 2006

Heller et al., Chemical Reviews 2014

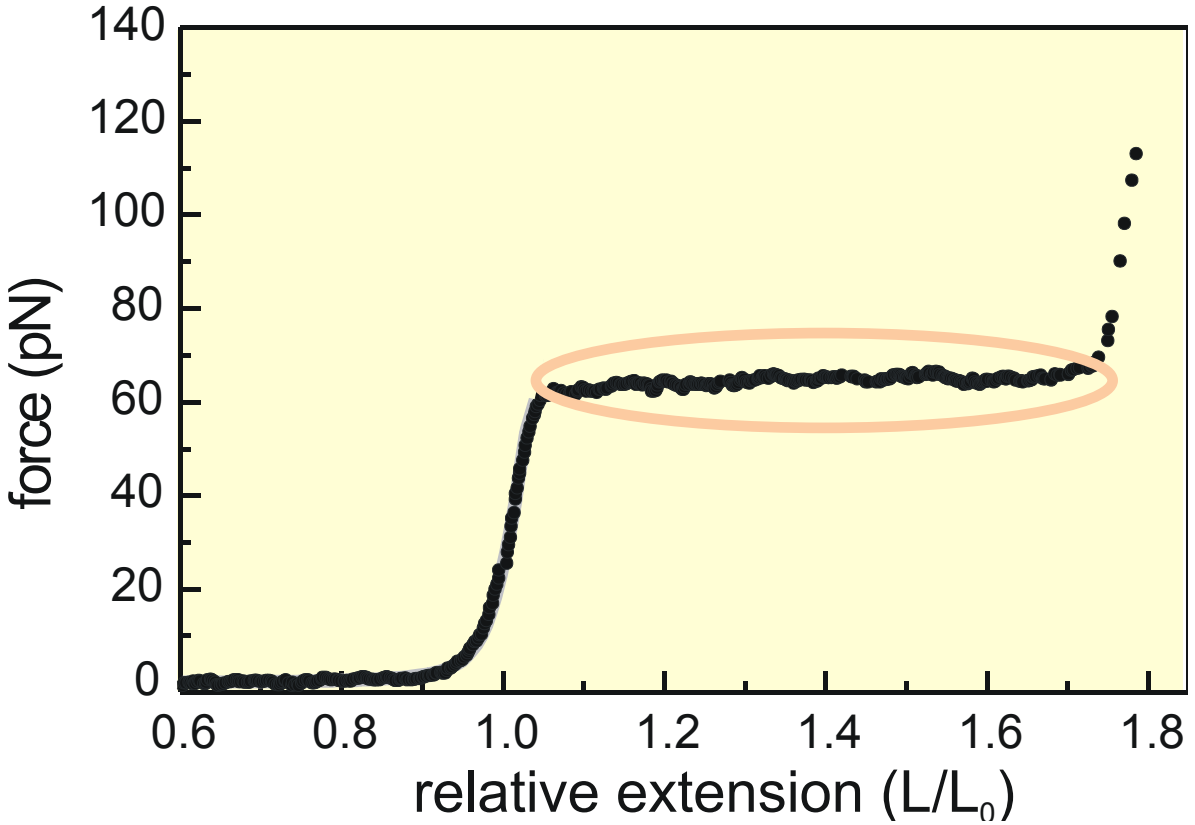
Our approach: combine optical tweezers with fluorescence microscopy



Optical tweezers combined with epi wide-field fluorescence microscopy



Overstretching = force-induced melting*



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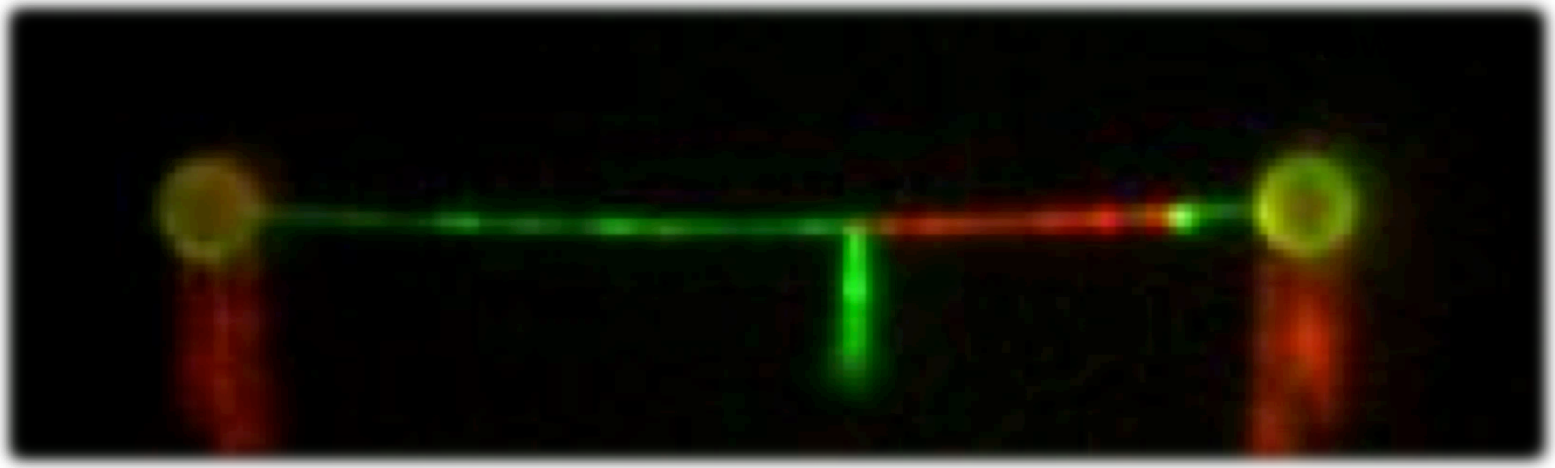
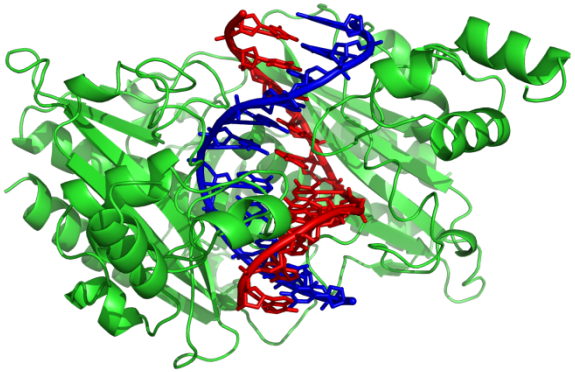


- **green** eGFP-RPA **ssDNA**
- **red** POPO-3 **dsDNA**
- buffer flow stretches frayed ssDNA

van Mameren et al. PNAS 2009

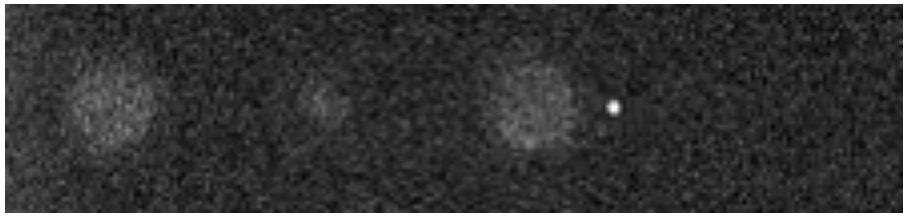
***DNA with free end, at low salt & room temperature**

Single-molecule sensitivity in fluorescence

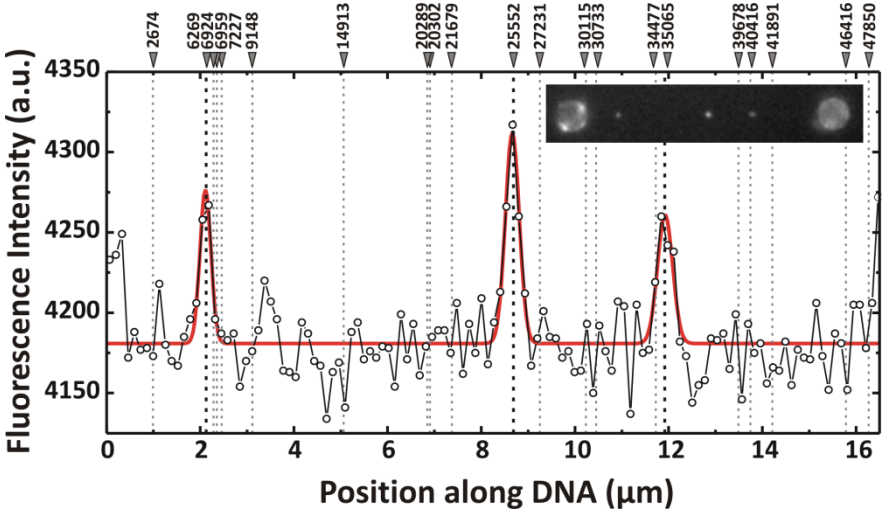


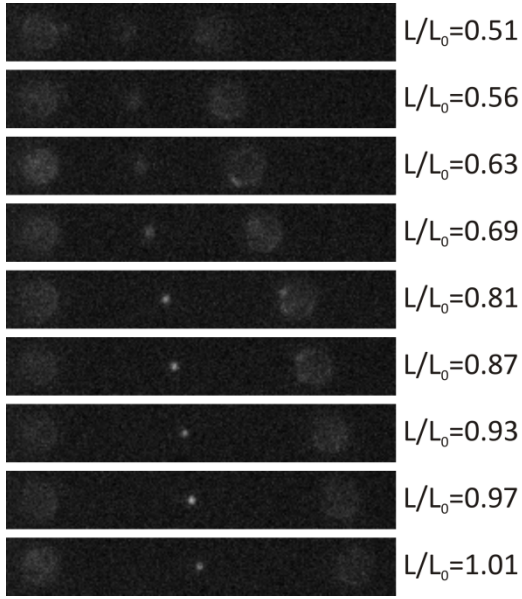
- Alexa555-labeled EcoRV
- Single-step bleaching → single molecules

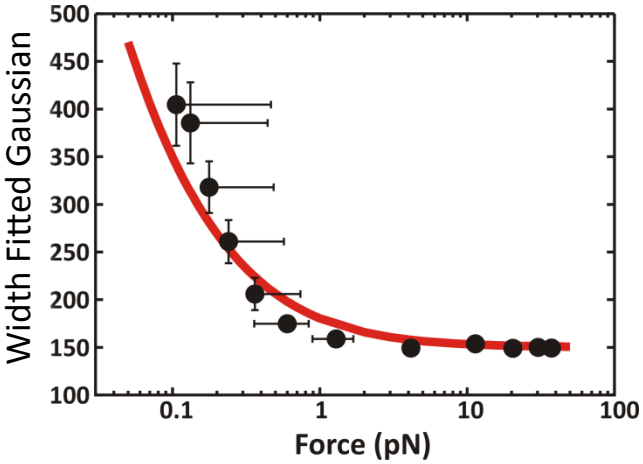
Single-molecule sensitivity in fluorescence

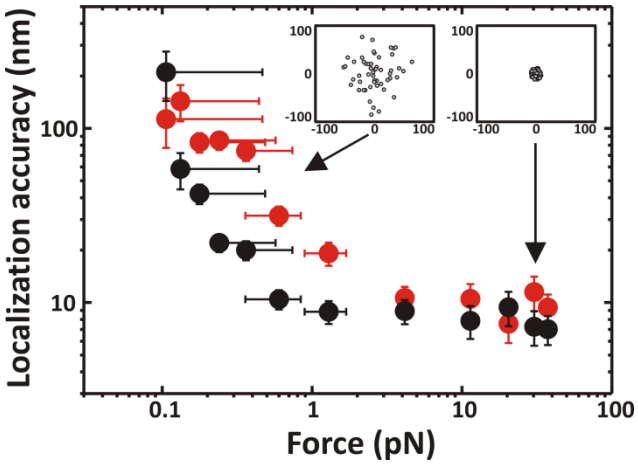


EcoRV binding: error with known sites 14±7nm



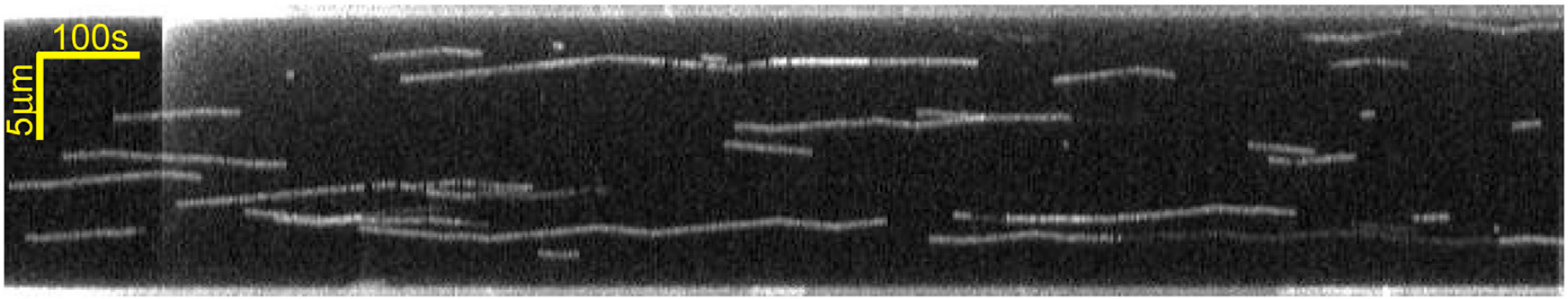






Candelli et al., PCCP 2011

Example: PICH, a DNA translocase specially adapted for processing anaphase bridge DNA

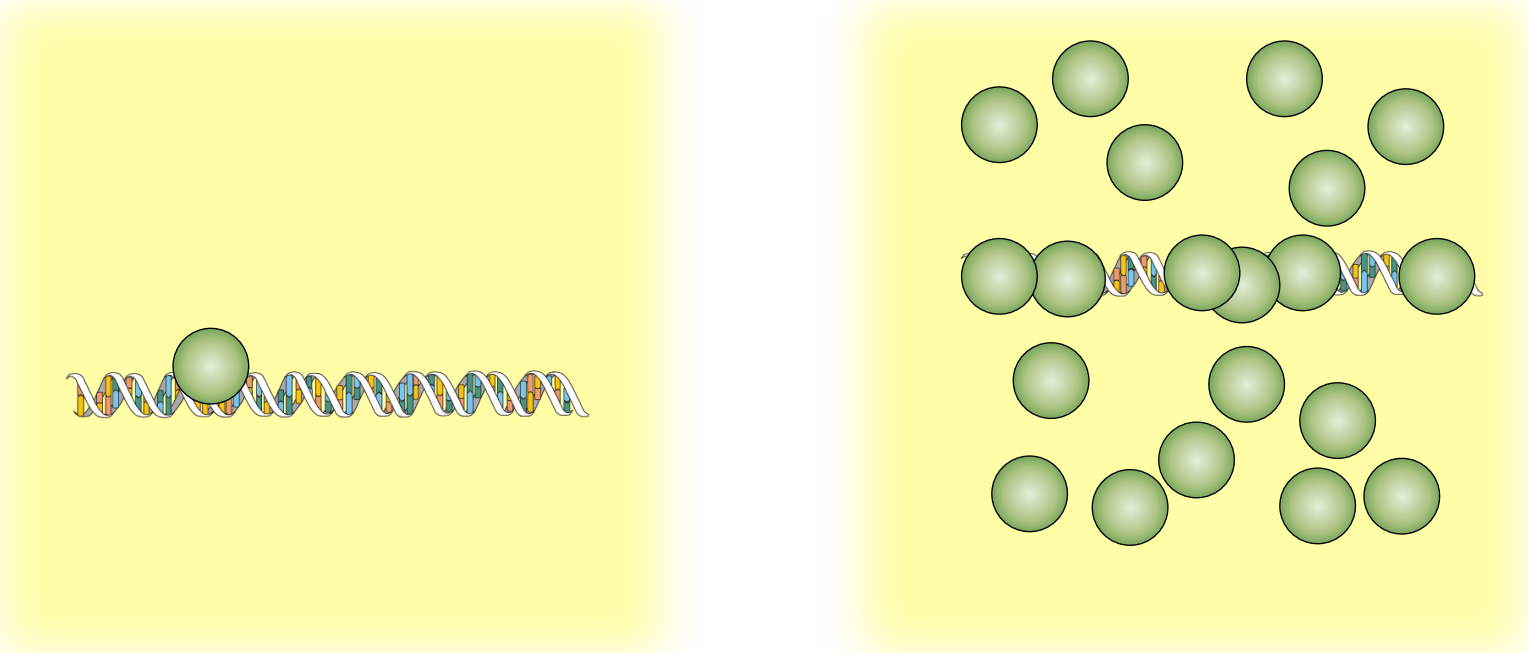


v = 10 nm/s ≈ 30 bp/s

Biebricher et al., Mol. Cell 2013

Limitations

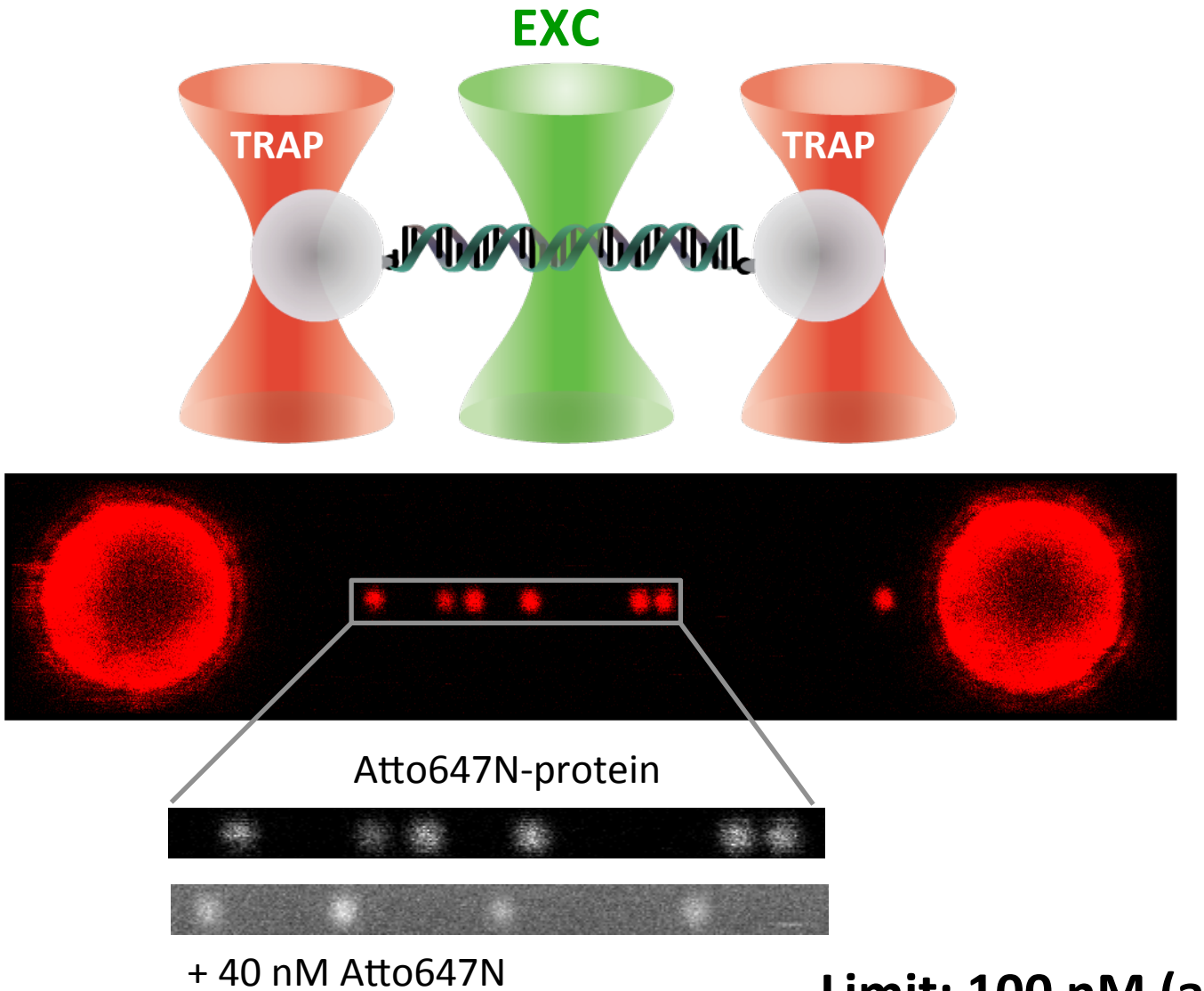
in vitro vs ***in vivo***



1. Low conc. to suppress background
2. Low conc. to observe separate binding

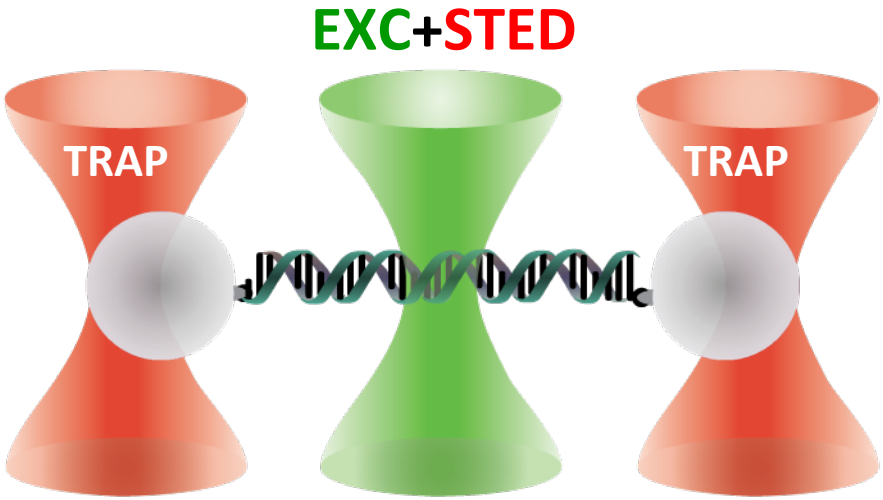
1. High concentration
2. High density on DNA

Confocal fluorescence microscopy

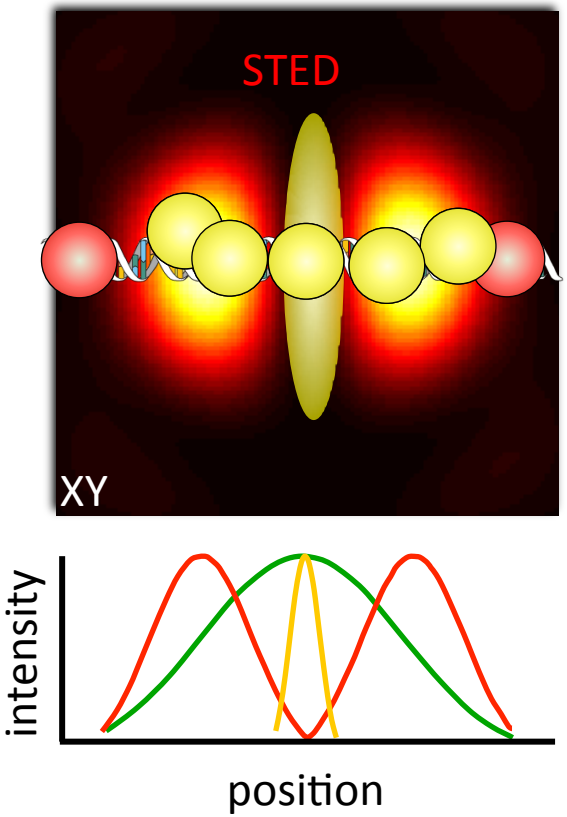


Limit: 100 nM (at 1 ms)

1D stimulated-emission depletion



High density **ON** DNA



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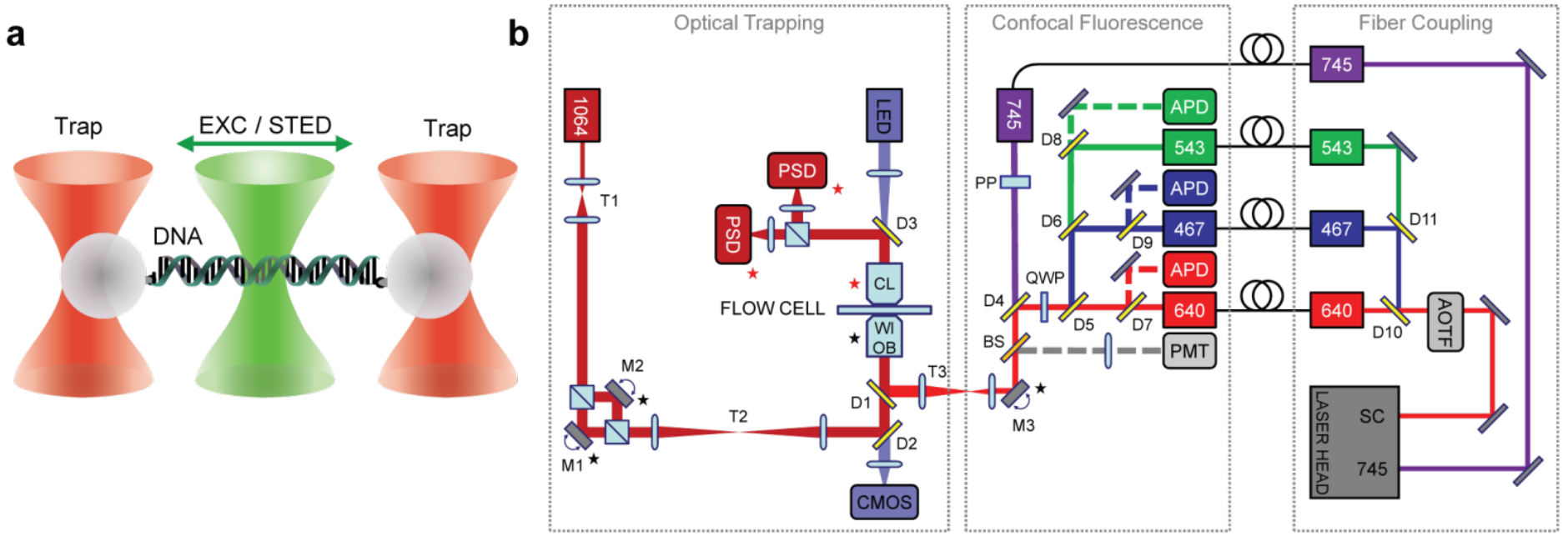

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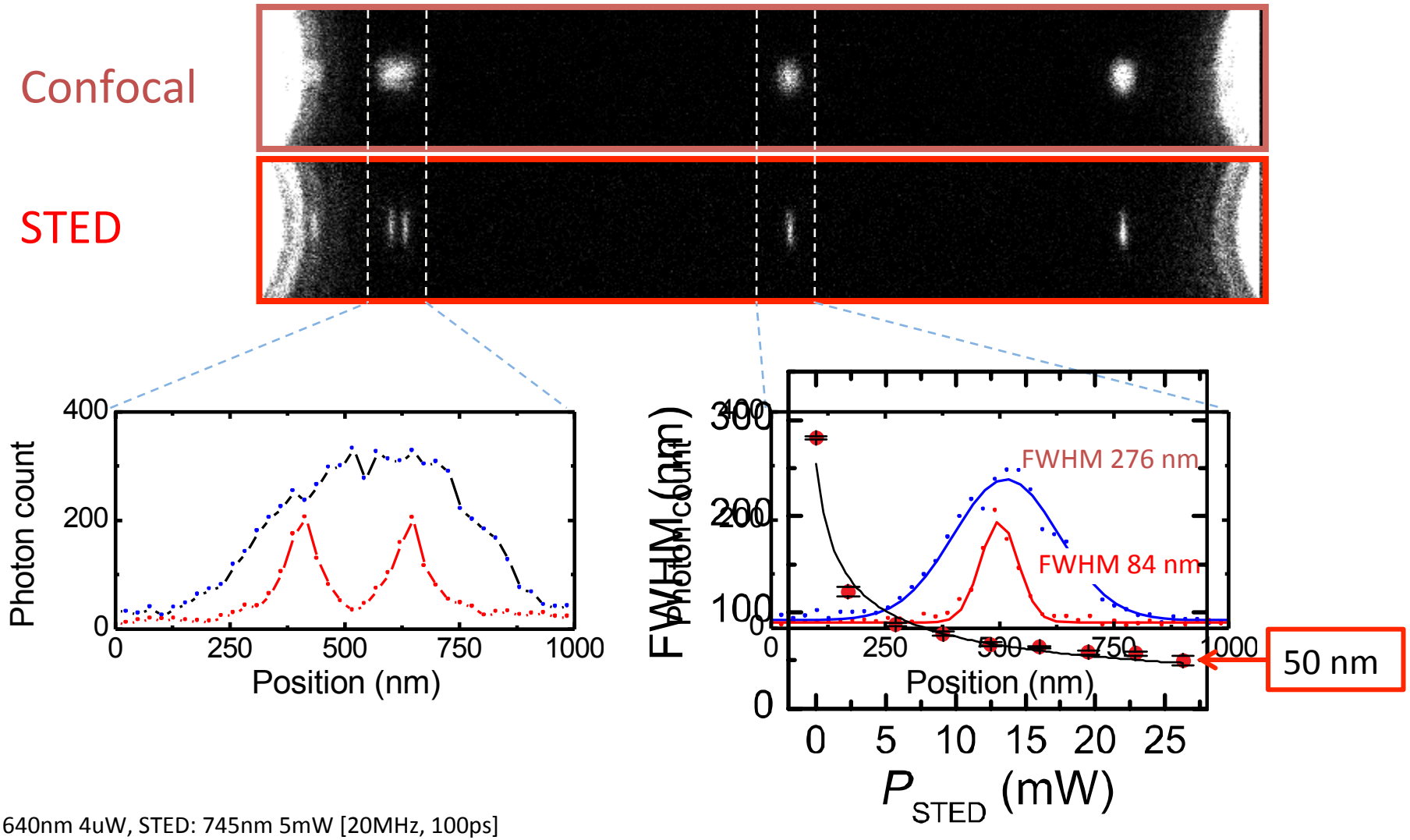
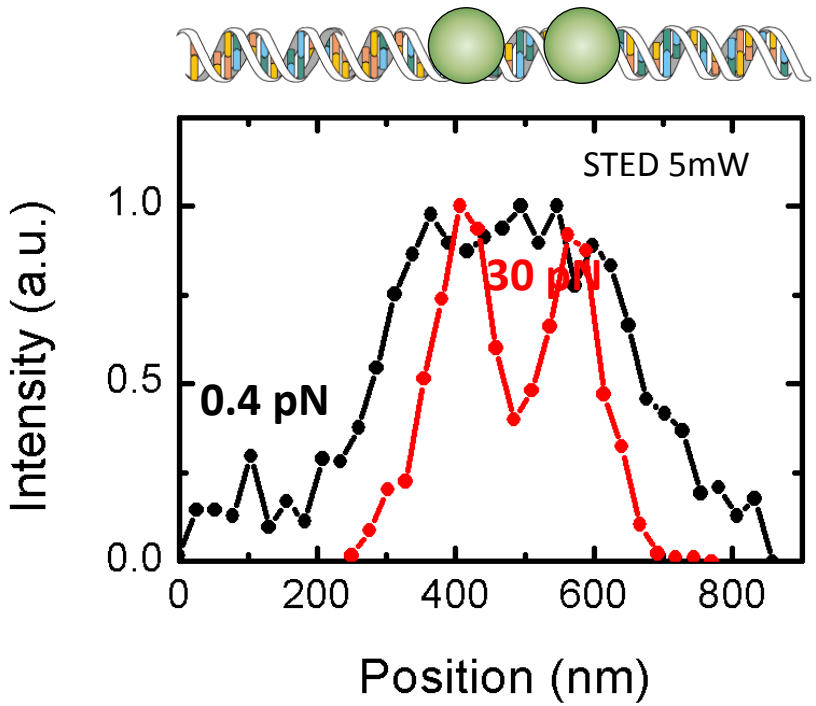
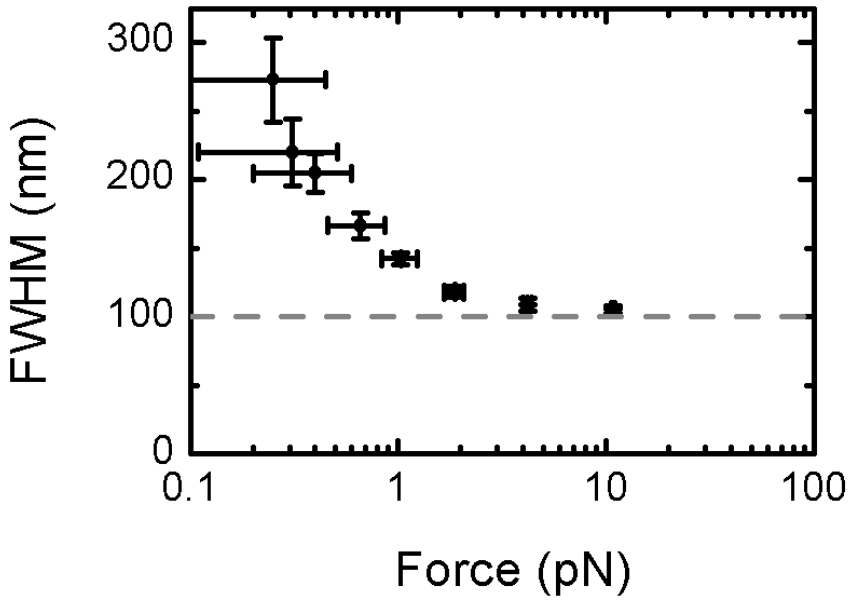


Exc: 640nm 4uW, STED: 745nm 5mW [20MHz, 100ps]

1D stimulated-emission depletion

Point-spread function tension dependent

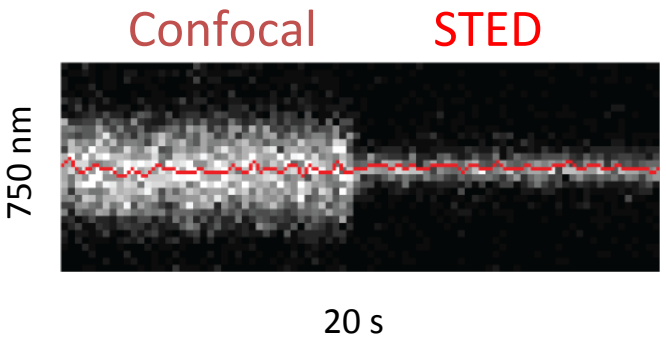


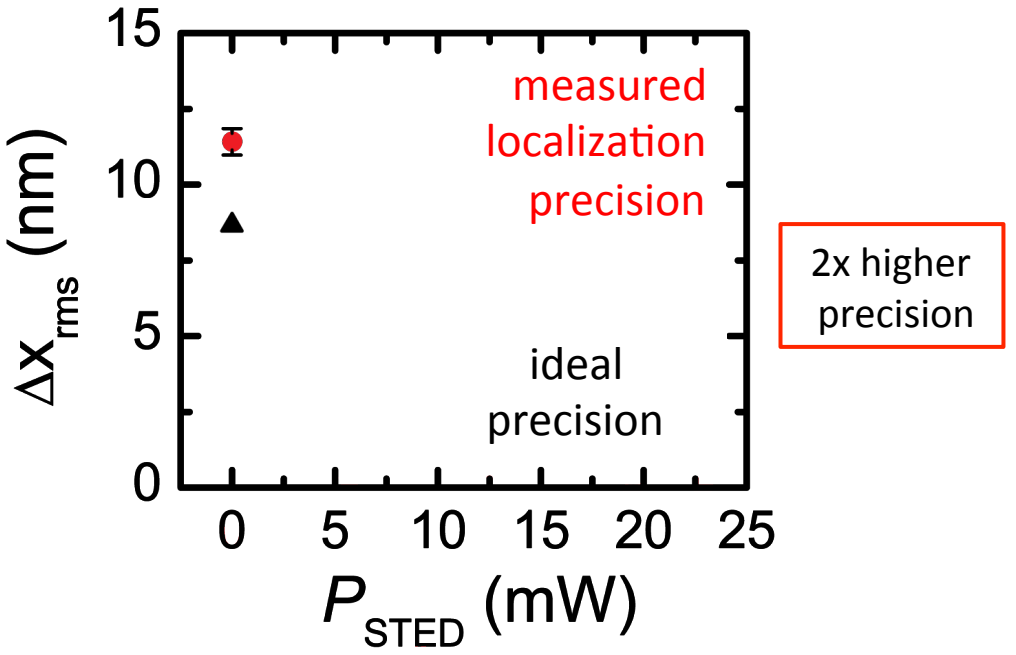


1D stimulated-emission depletion

Localization accuracy

Kymograph





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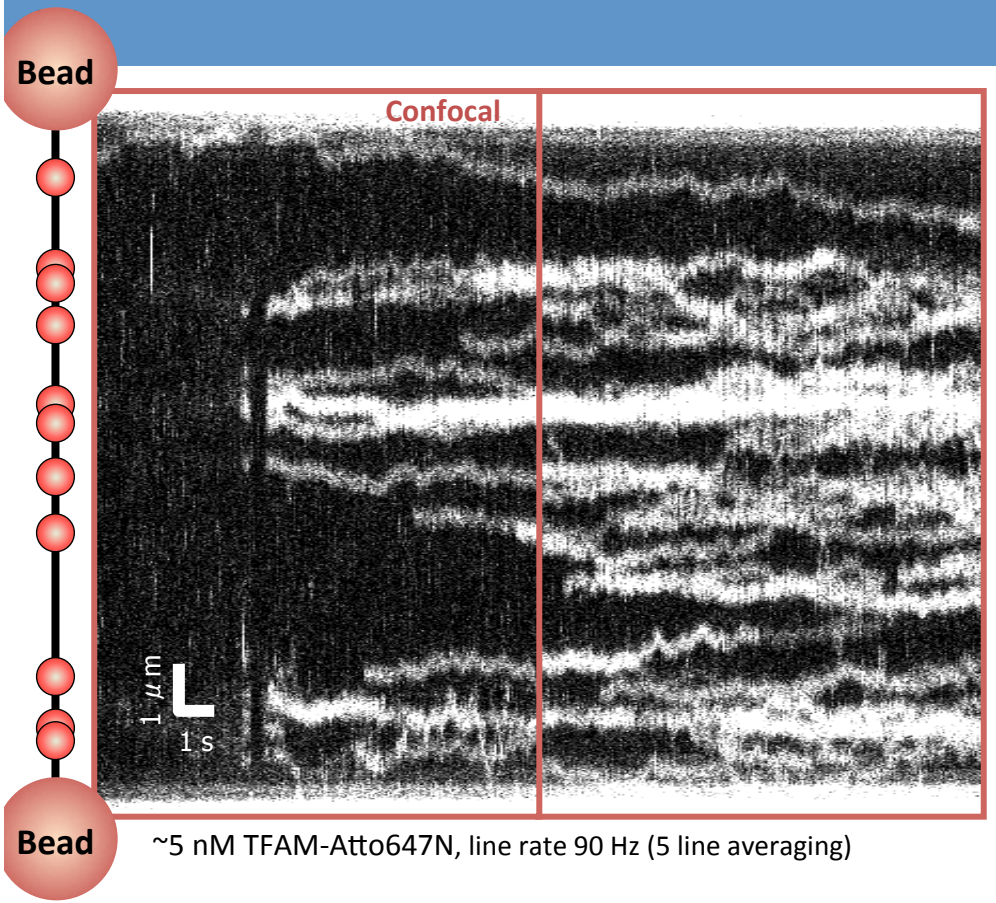
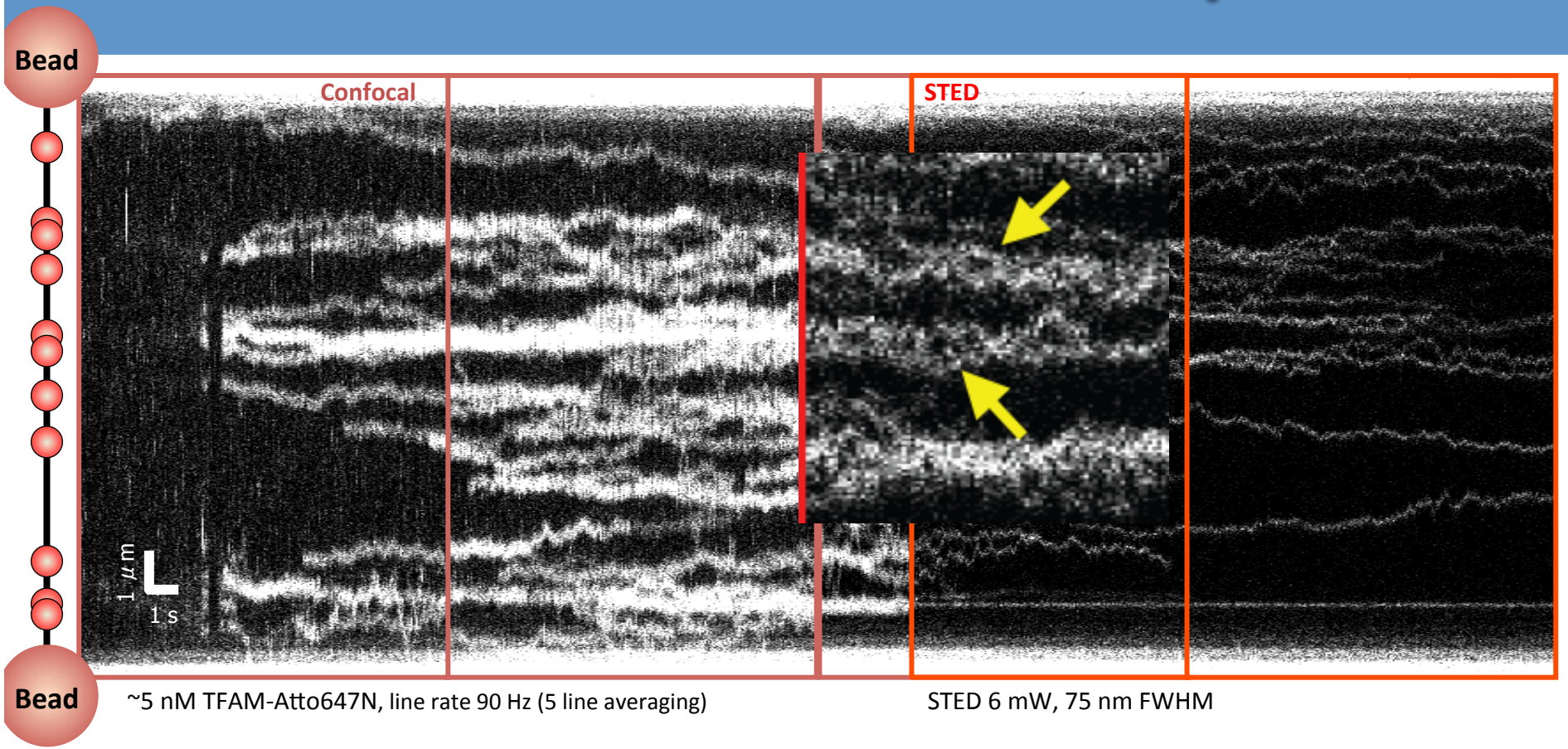


~5 nM TFAM-Atto647N, line rate 90 Hz (5 line averaging)

Application to TFAM (Mitochondrial Transcription Factor A)

1D stimulated-emission depletion

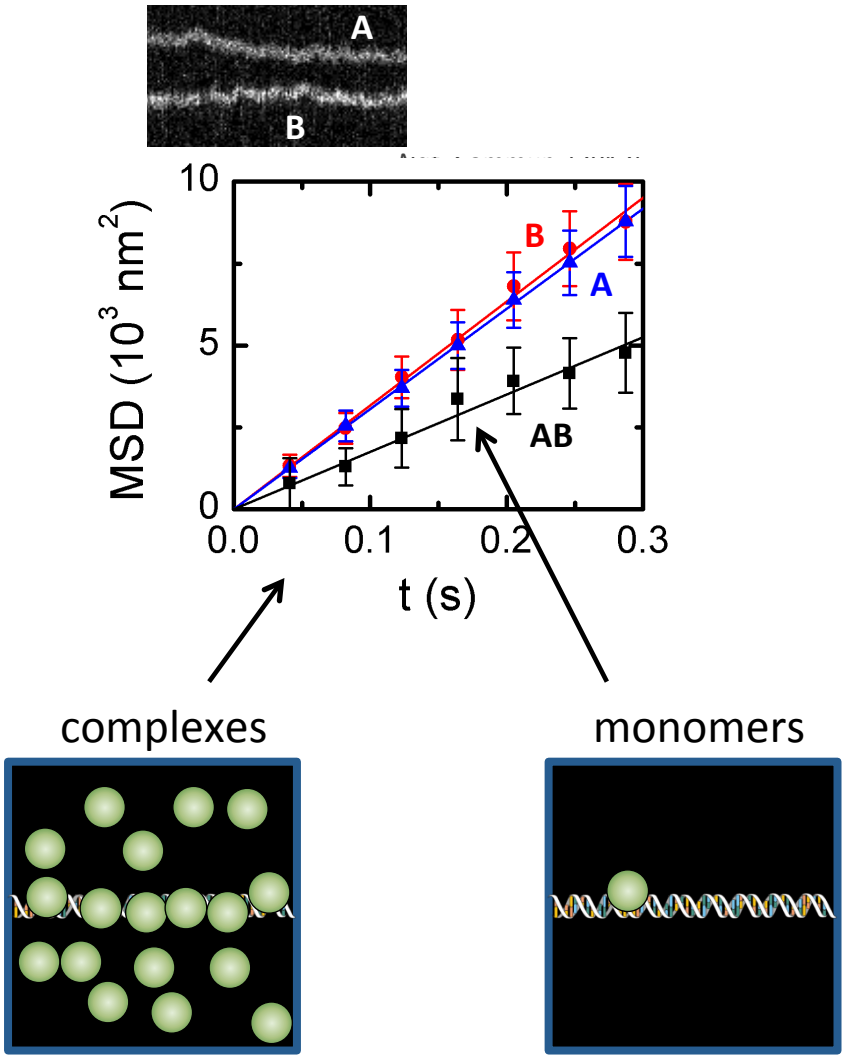


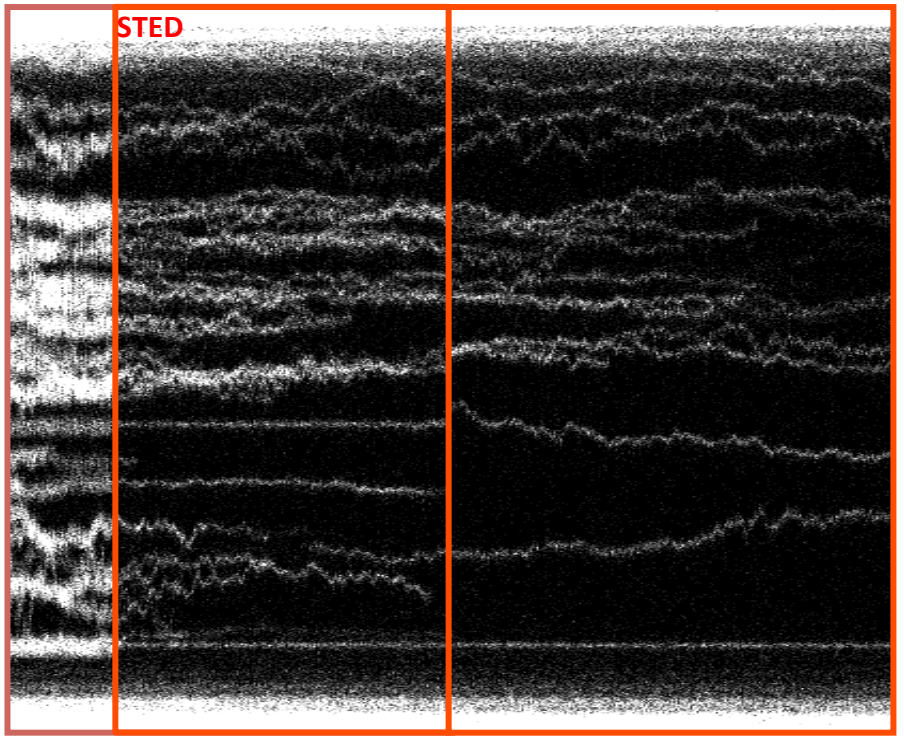
~5 nM TFAM-Atto647N, line rate 90 Hz (5 line averaging)

STED 6 mW, 75 nm FWHM

Application to TFAM (Mitochondrial Transcription Factor A)

1D stimulated-emission depletion





STED 6 mW, 75 nm FWHM

Heller et al., Nature Methods 2013

Conclusions

- Fluorescence microscopy adds local information to tweezers experiments.
- Super-resolution localization accuracy can be achieved using epi wide-field excitation.
- Better suppression of background due to fluorophores in solution using confocal.
- Super resolution (so far down to ~50 nm) using STED, localization accuracy ~7 nm.

LUMICKS

Correlative optical tweezers-fluorescence microscopy systems

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Acknowledgements

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MPI Göttingen	Stefan Hell		
JLU Giessen	Carolin Menges	Wolfgang Wende	

FOM

physics of the genome

NWO
Netherlands Organisation for Scientific Research