Fluorescent DNA-Binding Proteins for Single Molecule Visualization

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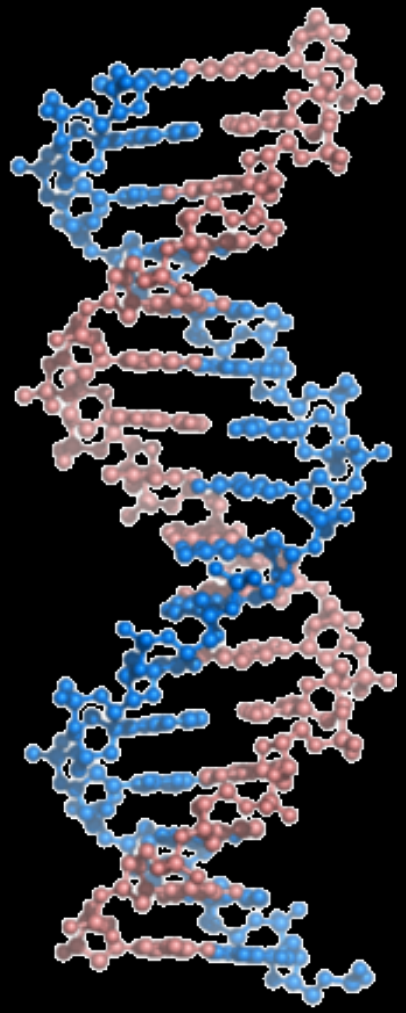
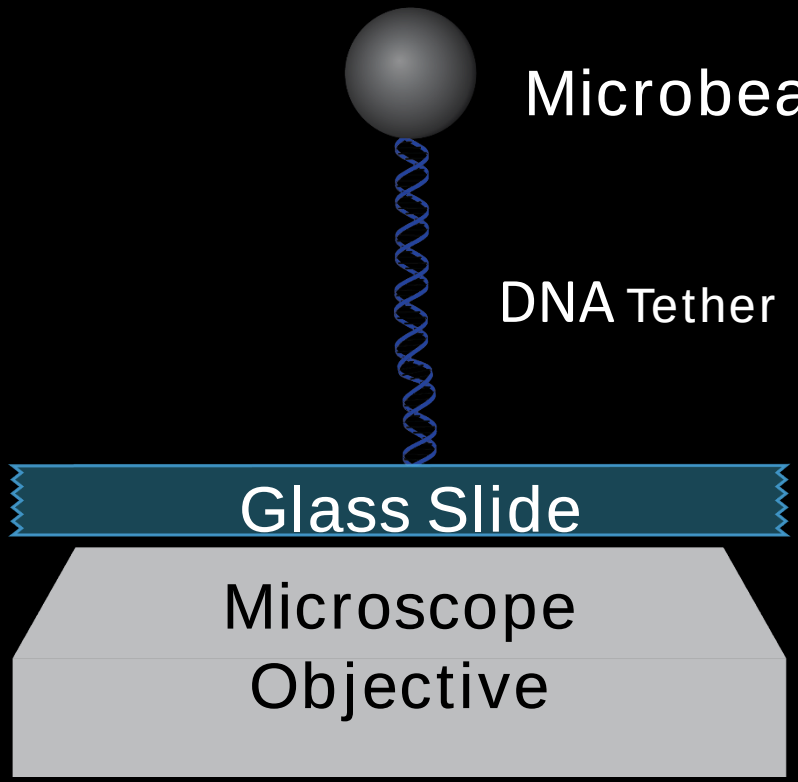
DNA Physical Properties

- Involved in vital processes of transcription and replication
- Double helix ladder shape of DNA gives rise to unique mechanical properties
- Excess deformation prevents biological function



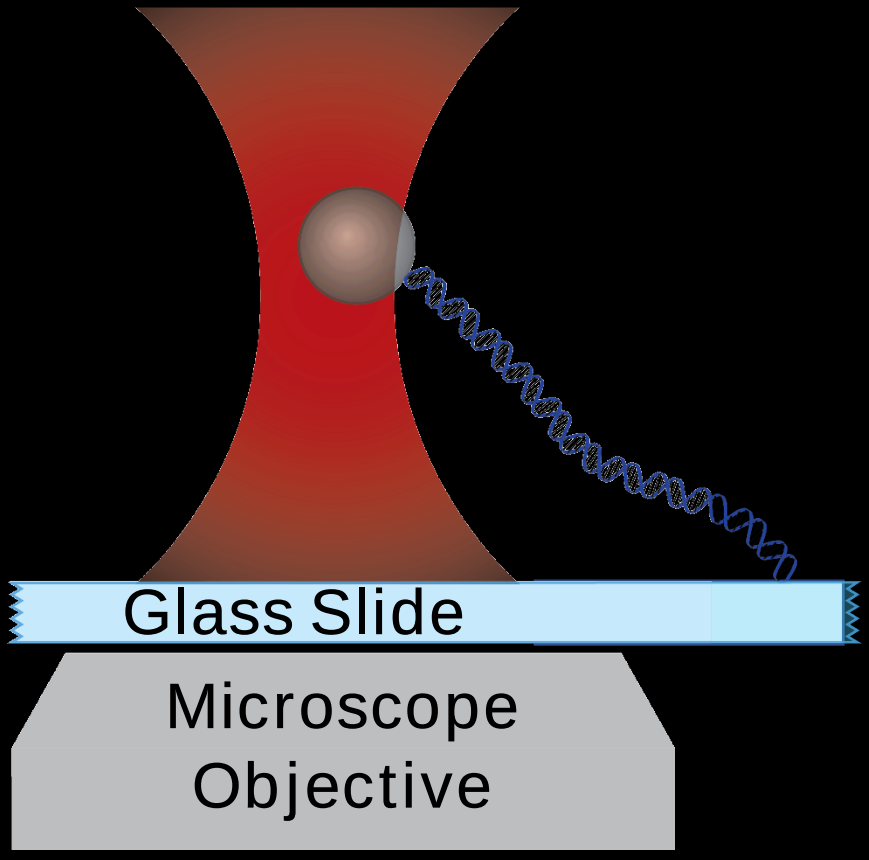
Dr. Kalju Kahn. Department of Chemistry and Biochemistry. UC Santa Barbara. Introduction to Visualization of Biological Molecules.

Single-Molecule Techniques

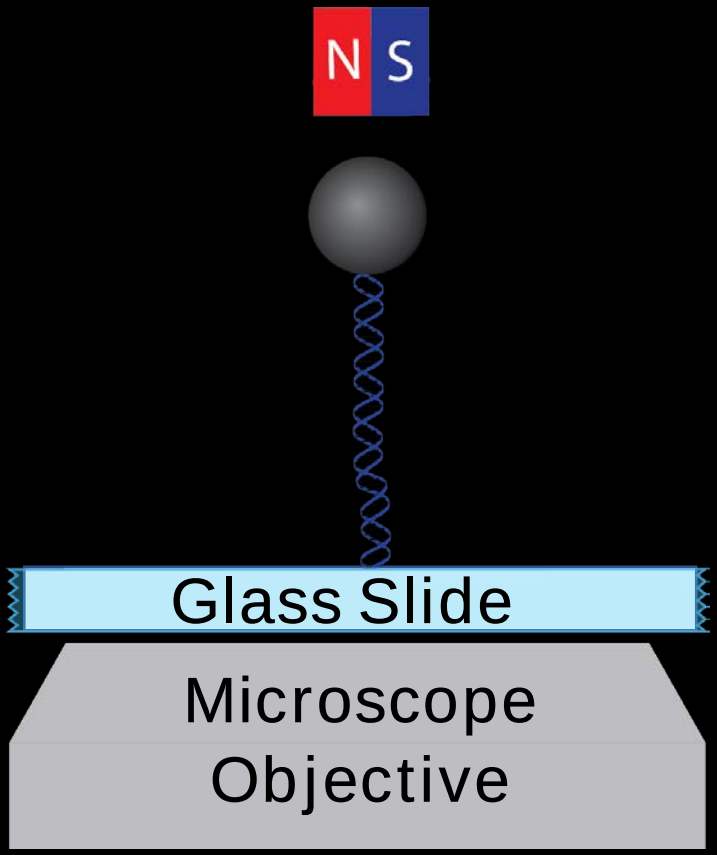


Single-Molecule Techniques

Optical Tweezers



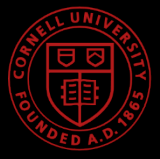
Magnetic Tweezers



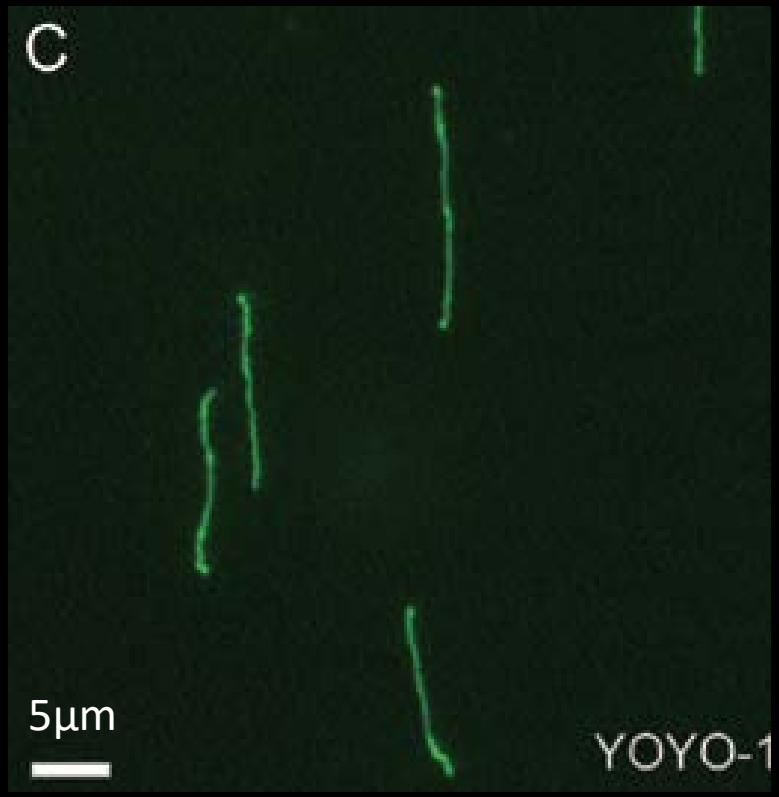
Single Molecule Biophysics of DNA

Single Molecule Techniques allow investigation of:

- Mechanical Properties of DNA
- Physical changes of DNA in different environments or processes
- Interactions between molecular motors and DNA



Visualizing DNA



Fluorescent molecules allow direct visualization of whole DNA strand

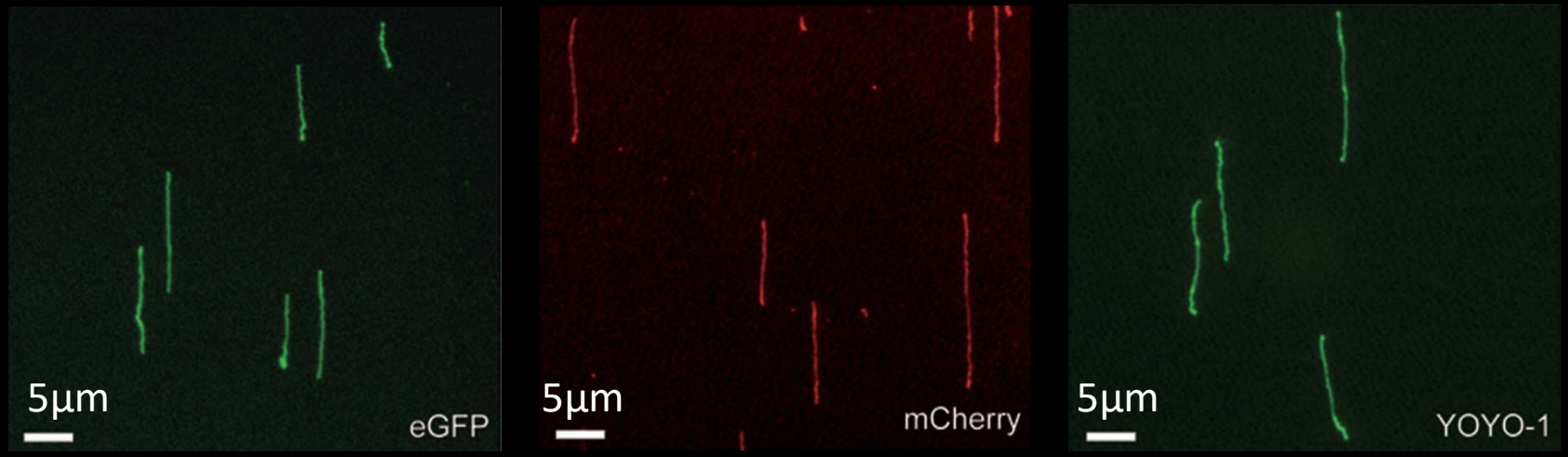
Many fluorescence stains influence mechanical properties of DNA

Lee, S., Oh, Y., Lee, J., Choe, S., Lim, S., Lee, H. S., Jo, K., Schwartz, D. C. (2015) *Nucleic Acids Research 2016, Vol. 44, No. 1.*

Fluorescent DNA-Binding Proteins (FDBP)

Proteins

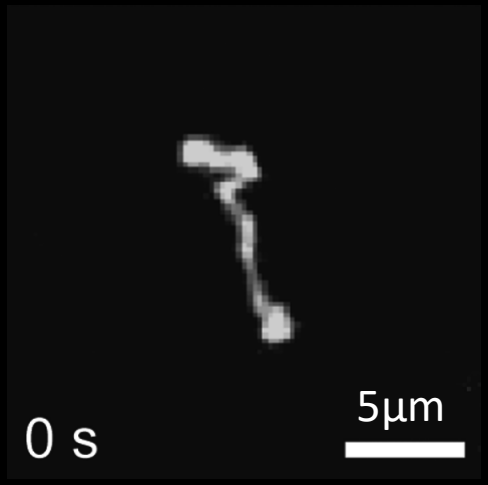
Common Stain



Fluorescent DNA-Binding Proteins can stain similarly to other highly used fluorophores without the adverse effects

Lee, S., Oh, Y., Lee, J., Choe, S., Lim, S., Lee, H. S., Jo, K., Schwartz, D. C. (2015) *Nucleic Acids Research 2016, Vol. 44, No. 1.*

Organic Stain



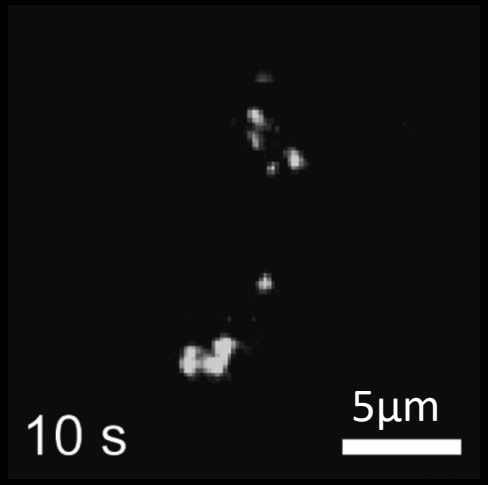
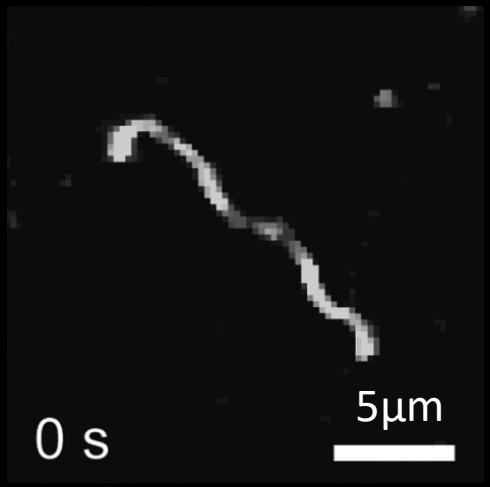


Photo-cleaved

FDBP



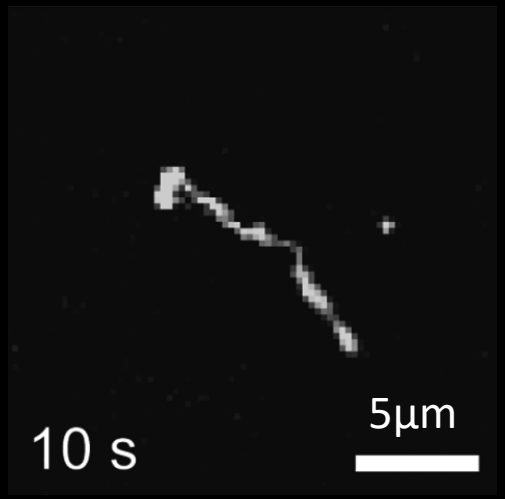
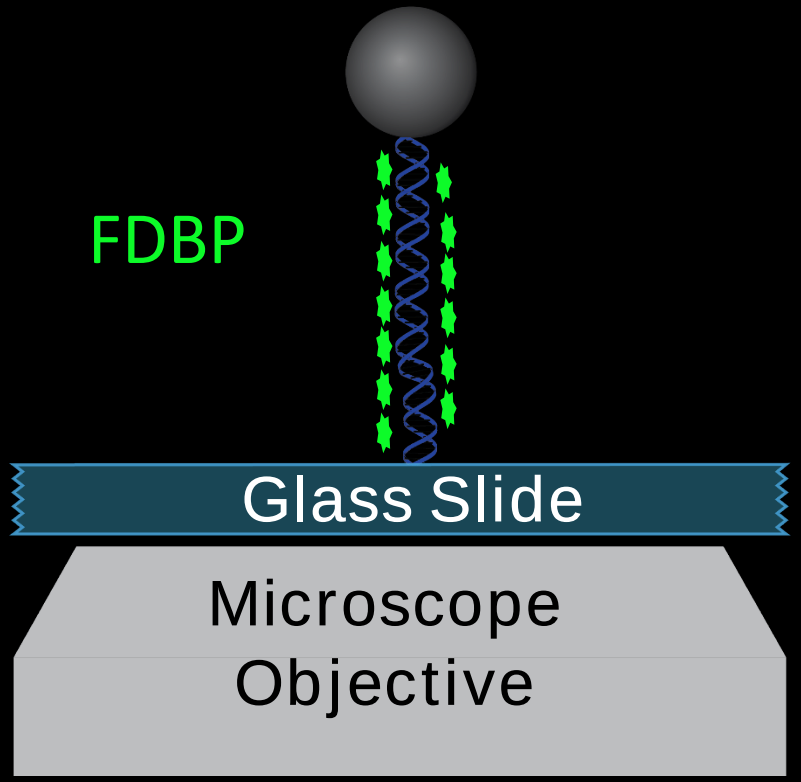


Photo-stable

Lee, S., Oh, Y., Lee, J., Choe, S., Lim, S., Lee, H. S., Jo, K., Schwartz, D. C. (2015) *Nucleic Acids Research 2016, Vol. 44, No. 1.*

Project Goal

Investigate how fluorescent DNA-binding proteins can enhance single-molecule manipulation studies



Purify Fluorescent DNA-Binding Proteins

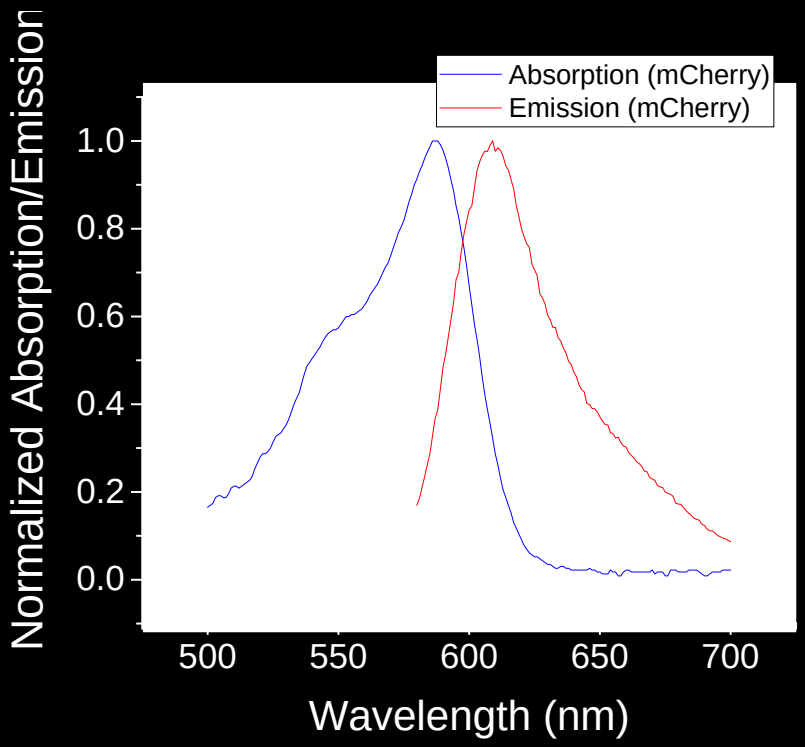
Characterize Binding and fluorescence

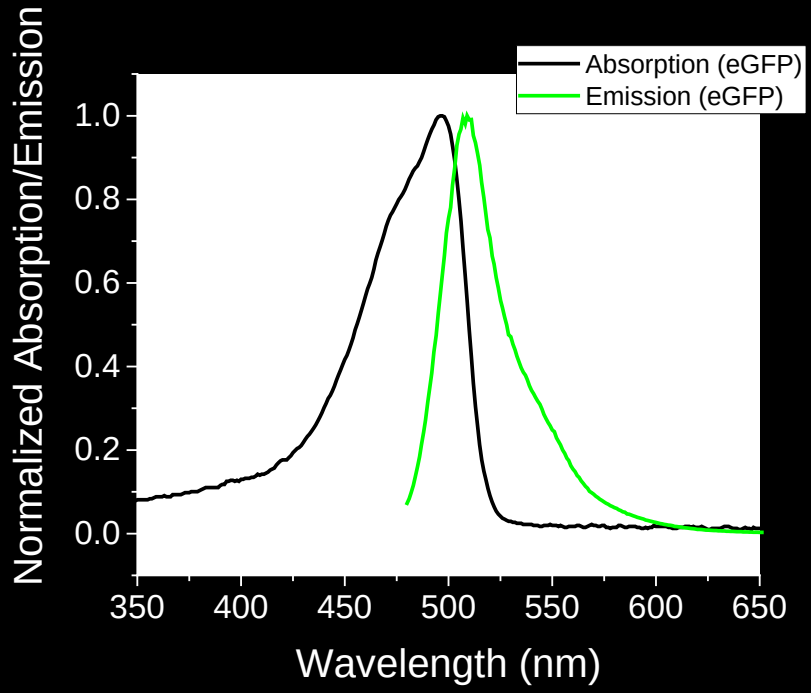
Use FDBPs in single-molecule techniques

Purifying FDBPs

- Purified by transforming competent *E. coli* cells
  - Followed protocol from S. Lee, et al., Nuc. Acids Research 44, e6 (2016).

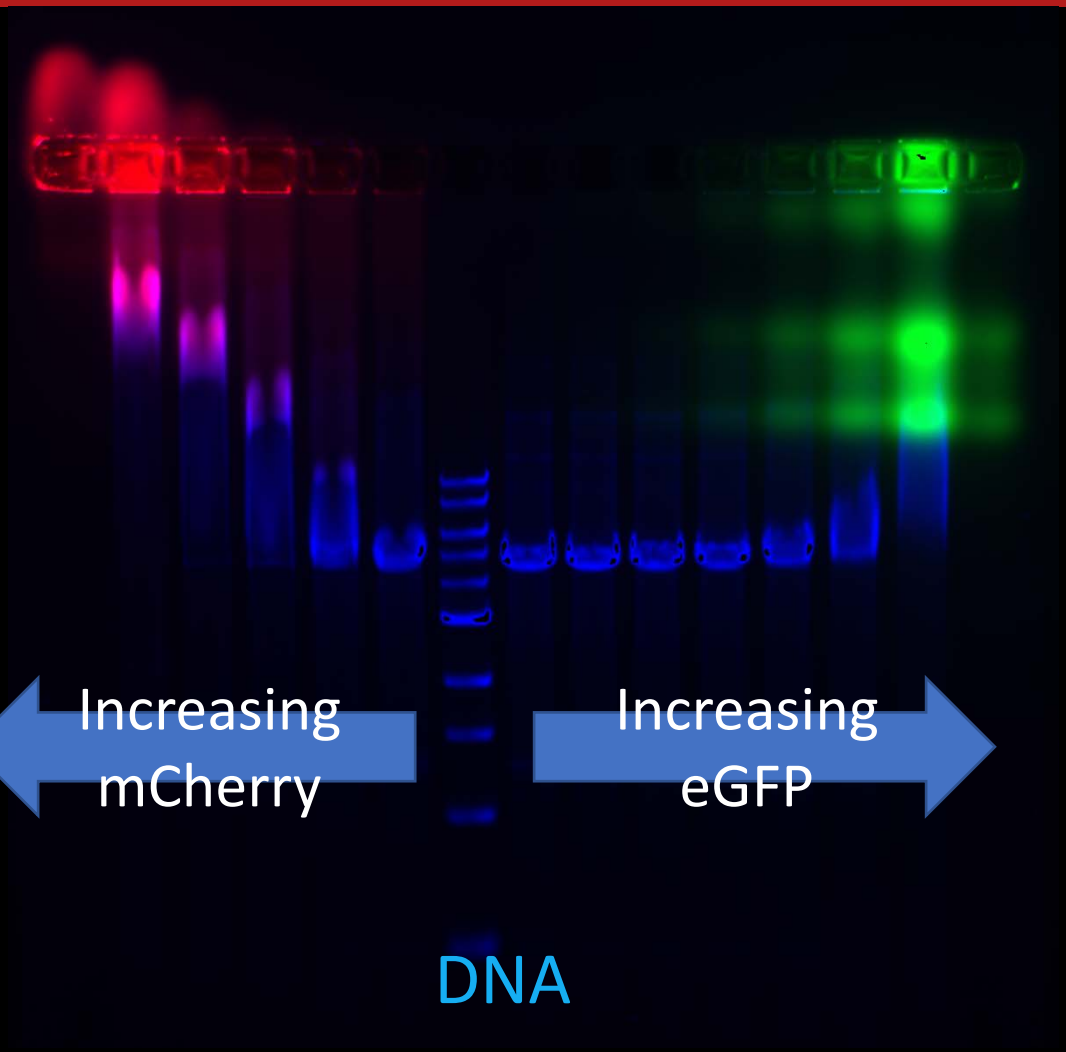
Characterizing Fluorescence



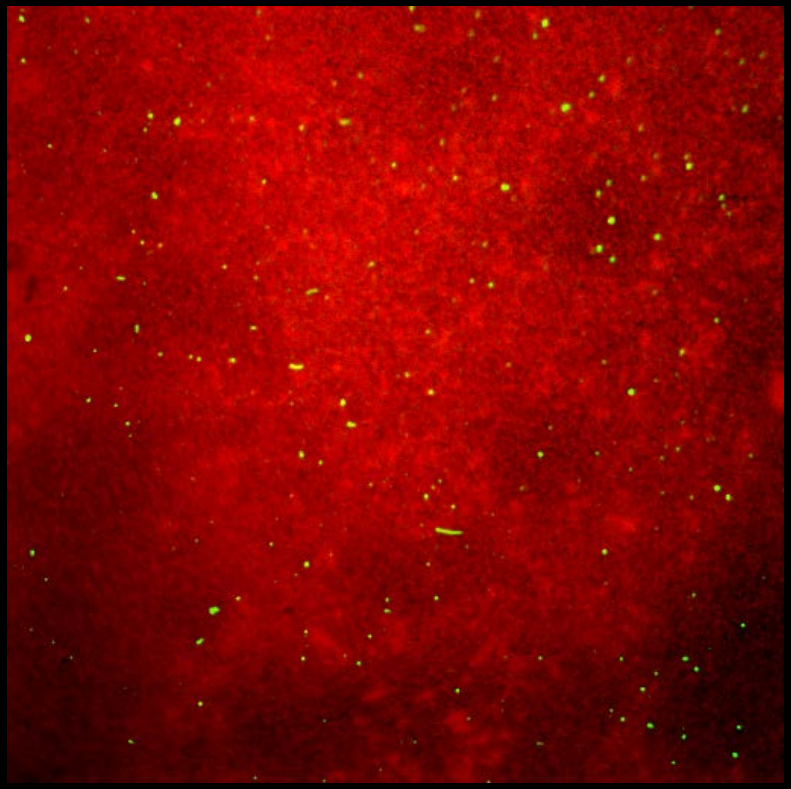


Both FDBPs exhibit expected absorbance/emission spectra

Characterizing Binding



Attempting Single-Molecule Visualization



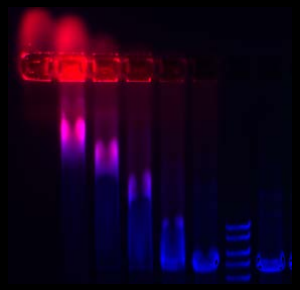
Red:
mCherry DNA-BP

Green:
SYTOX DNA stain

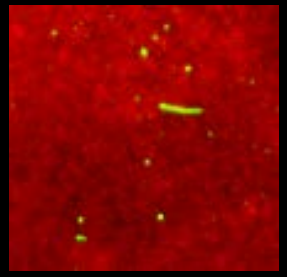
- Green and Red should overlap visibly to form yellow spots
- Needs optimization for practical usage

Conclusions

Fluorescent DNA-binding proteins were purified



Purified proteins bind DNA



Single-molecule chamber conditions require optimization for effective use

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