

**Sandia
National
Laboratories**

This paper describes objective technical results and analysis. Any subjective views or opinions that might be expressed in the paper do not necessarily represent the views of the U.S. Department of Energy or the United States Government.

LaB
AMSTERDAM



SAND2019-1546C

DNA intercalators tilt, wobble & twirl: *Elucidating DNA overstretching*

Adam S. Backer, Andreas S. Biebricher, Graeme A. King,
Gijs J. L. Wuite, Iddo Heller & Erwin J. G. Peterman

Adam S. Backer

Harry S. Truman Fellow

Sandia National Laboratories

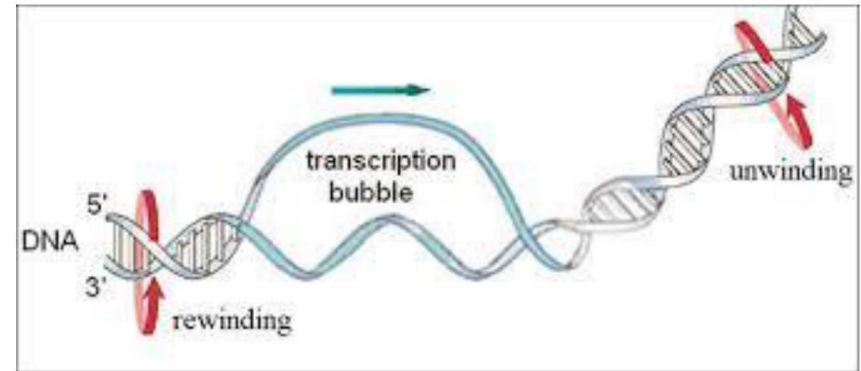
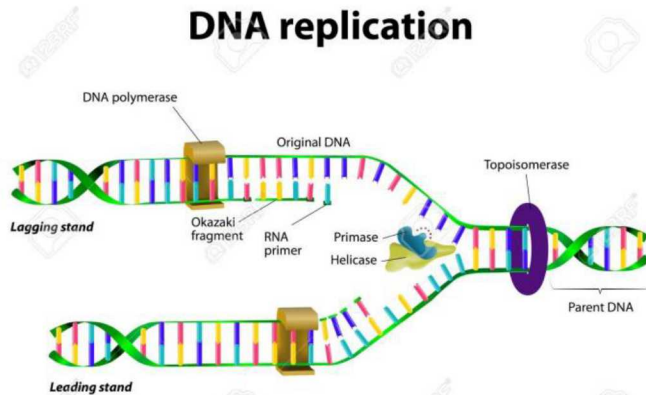
abacke@sandia.gov

Sandia National Laboratories is a multimission laboratory managed and operated by National Technology & Engineering Solutions of Sandia, LLC, a wholly owned subsidiary of Honeywell International Inc., for the U.S. Department of Energy's National Nuclear Security Administration under contract DE-NA0003525.

rice Advances

Why study the structure & dynamics of DNA?

- Insight into biological function



- DNA-based smart materials / DNA origami

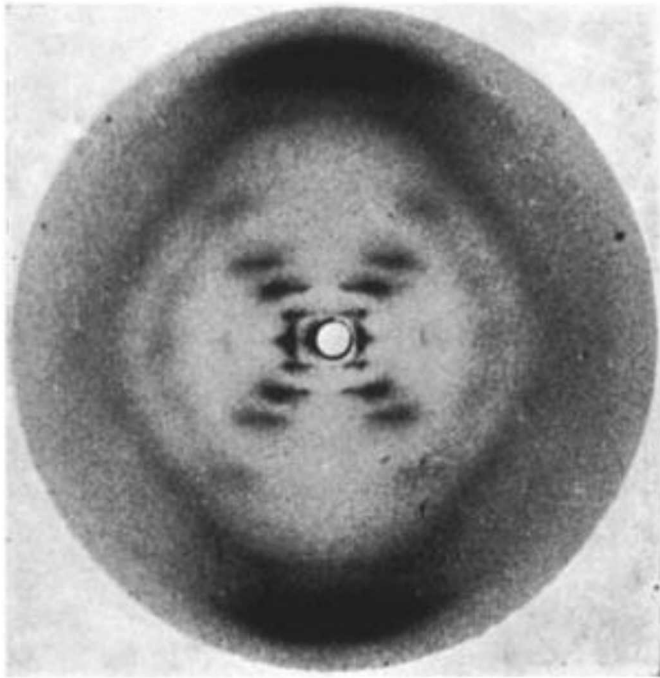
Dietz, Science 2009



Slide credit:
Iddo Heller

Investigating the structure of DNA

X-ray diffraction image: B-DNA



Franklin, Gosling, *Nature* **171**, (1953)

Traditional approaches:

X-ray diffraction, Cryo-EM, NMR

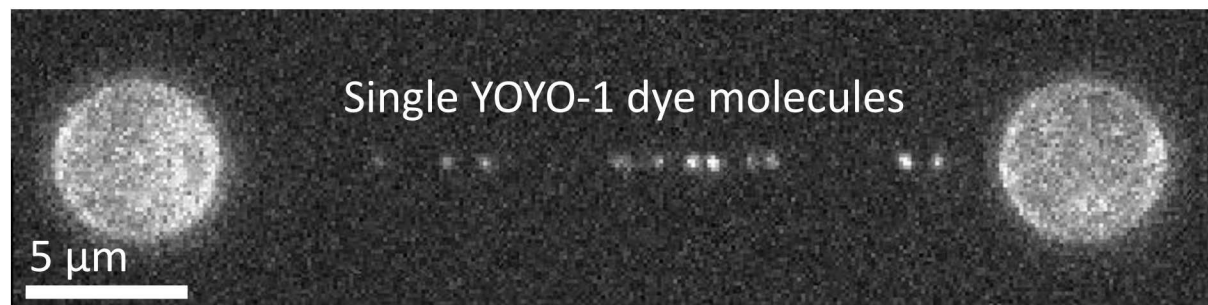
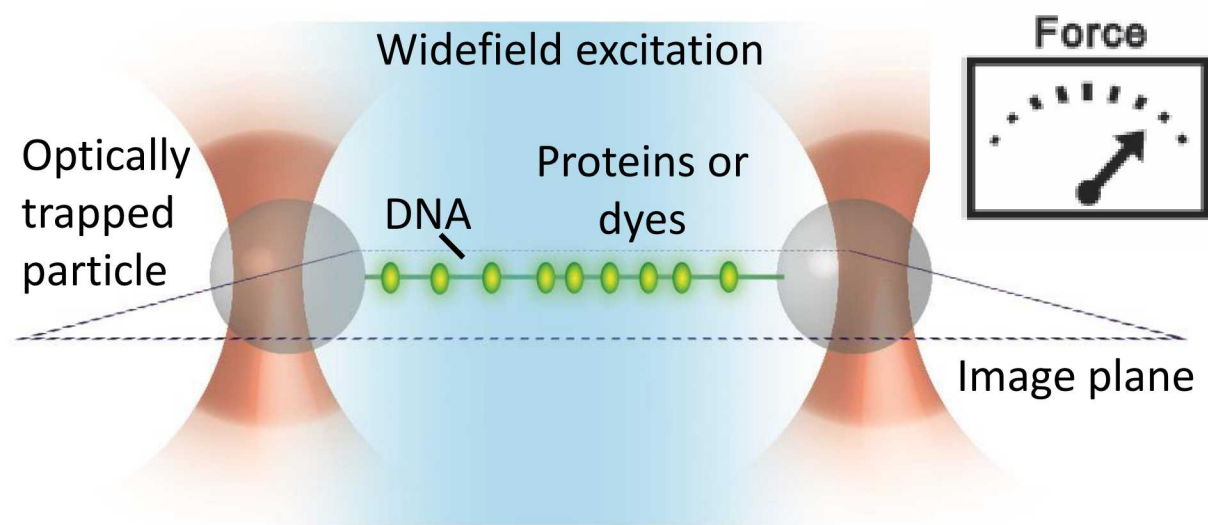
Pros:

- Atomic-scale resolution

Cons:

- Cannot study dynamics (XRD, Cryo-EM)
- Bulk averaging (XRD, NMR)
- Highly perturbative

Combine optical tweezers & fluorescence



Pros:

- Investigate **dynamical properties**
- **Highly specific** labeling
- **Single-molecule** sensitivity

Cons:

- Spatial resolution **limited by diffraction**
(~250 nm)

Refs:

- Heller et al., *Chem. Rev.* **114**, (2014).
- Fazal et al., *Nat. Photonics* **5**, (2011).
- Bustamante et al., *Nature* **421**, (2003).

Outline for today:

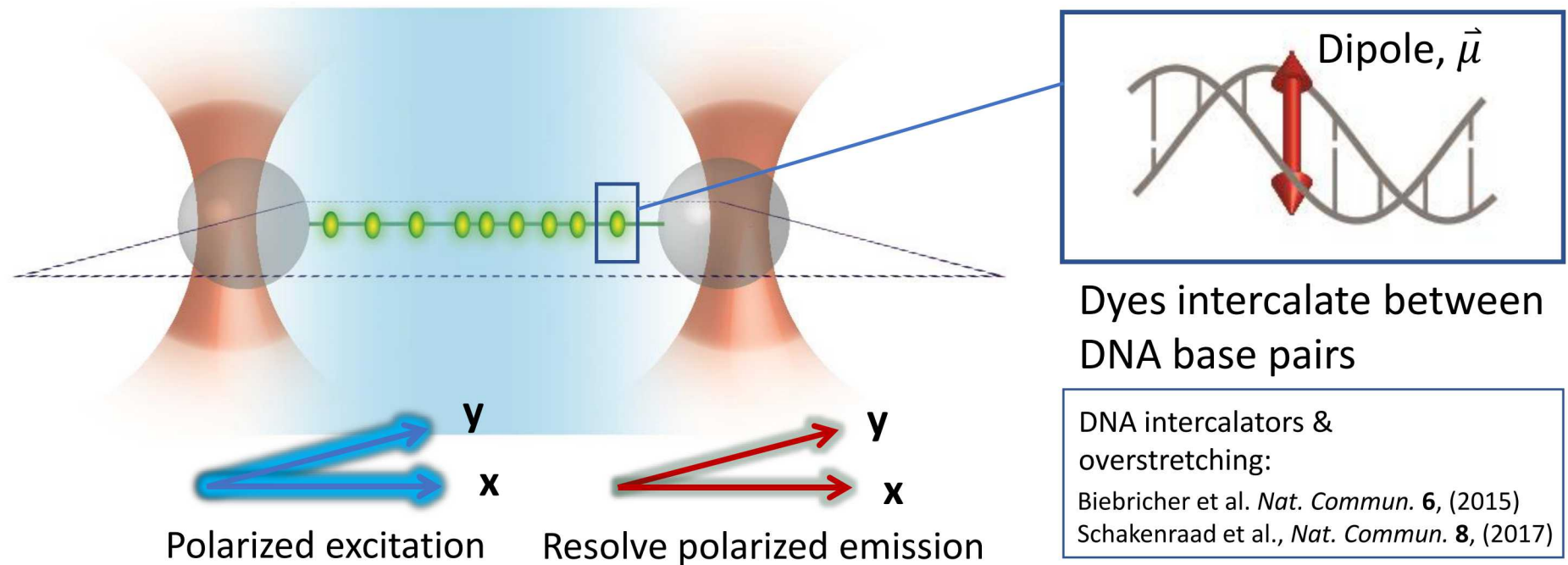
- Use **fluorescence polarization microscopy** w/ optical tweezers & DNA force spectroscopy
- Gain insight into **S-DNA**, an elusive DNA structure that forms under tension

Refs:

Cluzel et al., *Science* **271**, (1996).

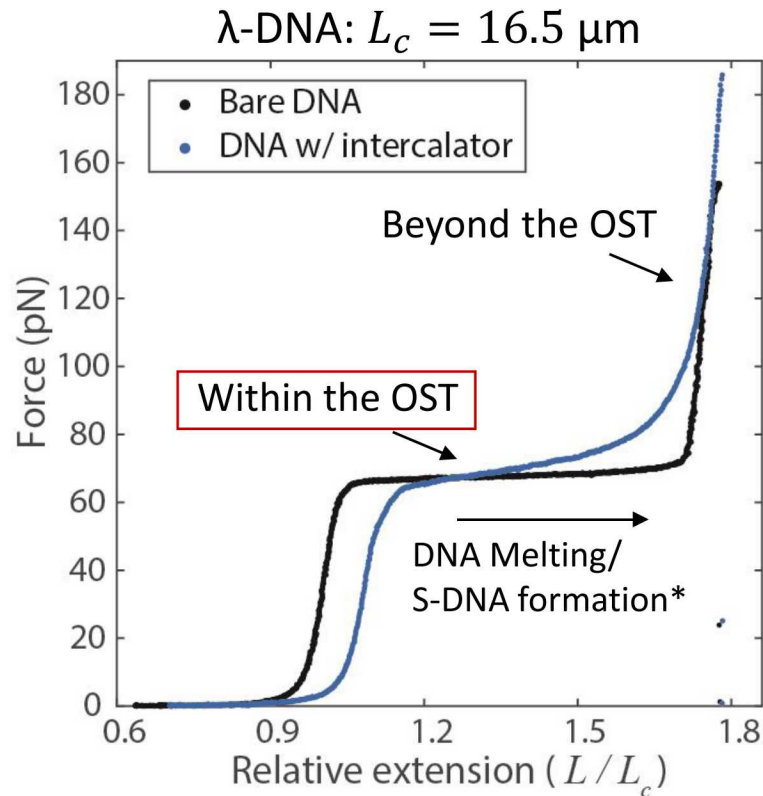
Smith et al., *Science* **271**, (1996).

Fluorescence polarization imaging of stretched DNA

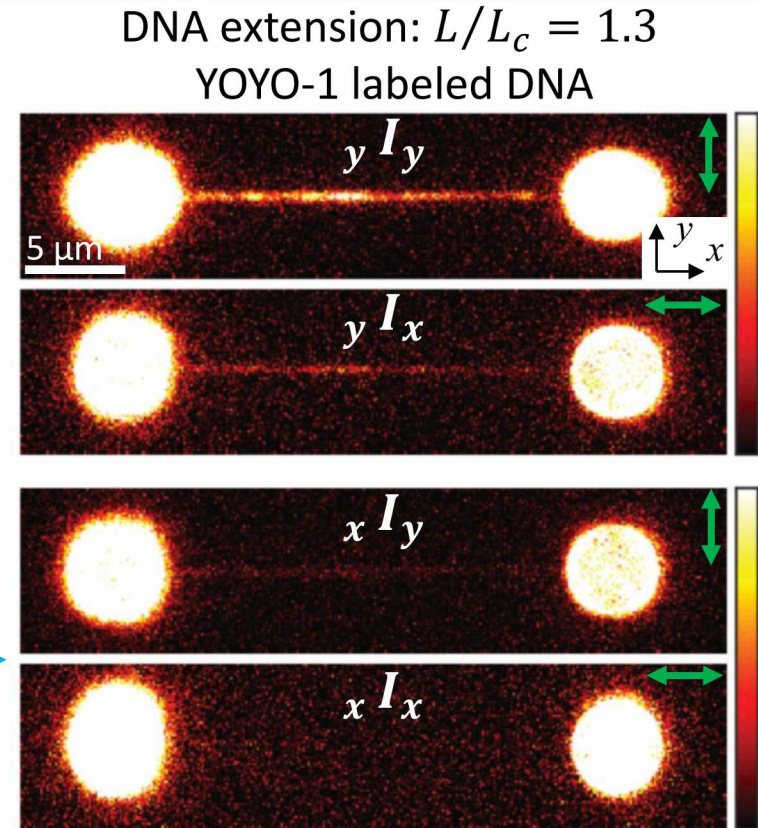


- Polarized light is preferentially absorbed and emitted along dipole axis $\vec{\mu}$
- Use polarization to infer **orientations** of intercalated dyes

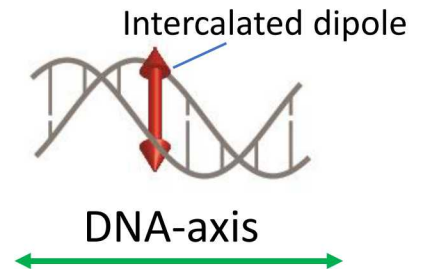
The overstretching transition (OST) & polarization response



Exc.
Pol.



Interpretation: Within the OST, base pairs align intercalators perpendicular to the DNA-axis

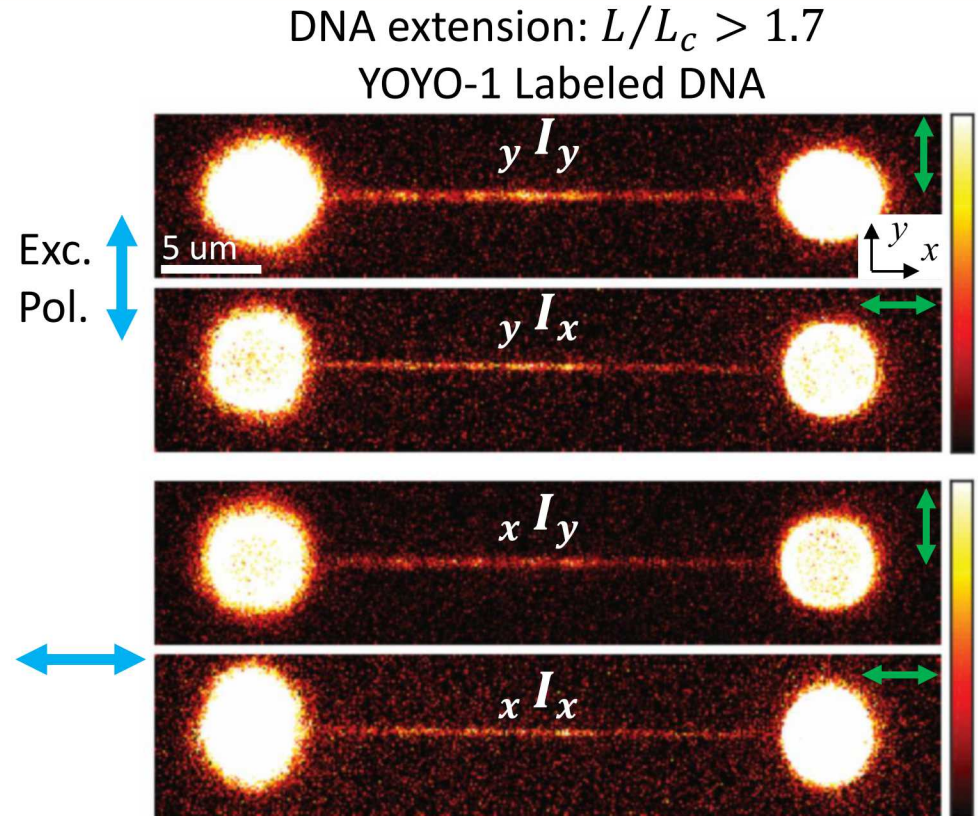
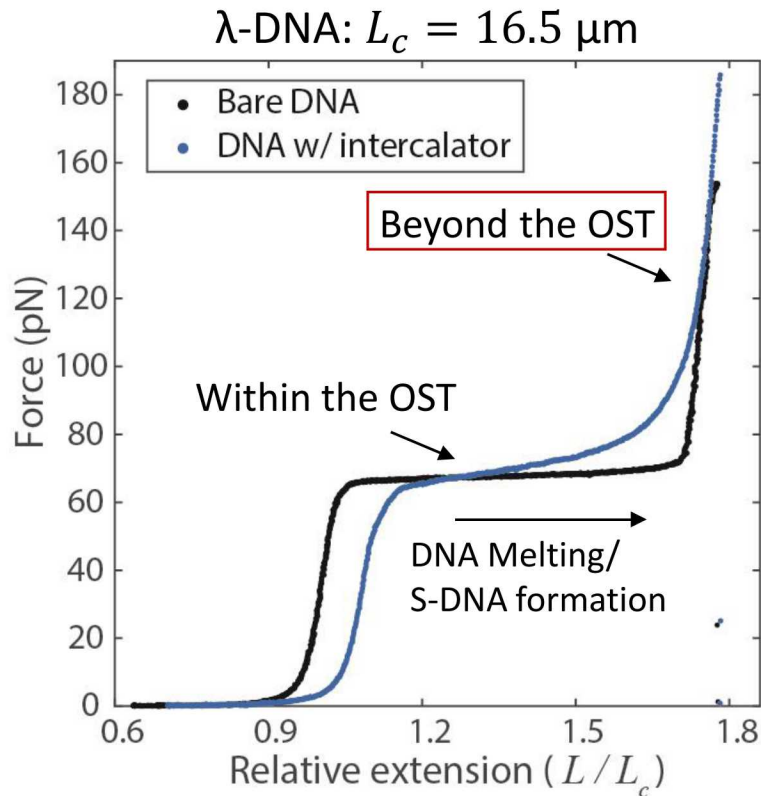


*Refs:

van Mameren et al. *PNAS* **106**, 18231 (2009)

King et al. *PNAS* **110**, 3859 (2013)

The overstretching transition (OST) & polarization response

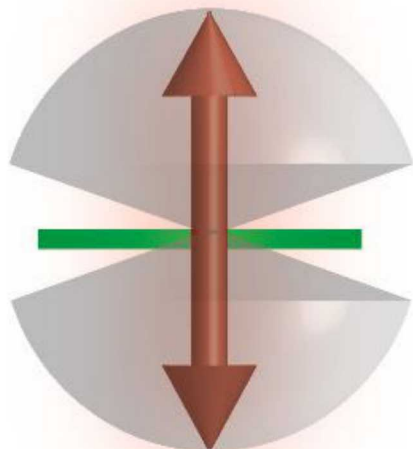


Interpretation: ???
 ?????????????????????????????????????

WHAT IS GOING ON????

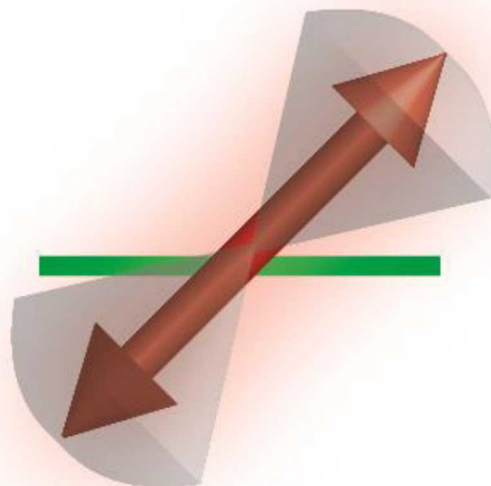
Two hypotheses*:

Wobbly dipoles



Or

Tilted dipoles

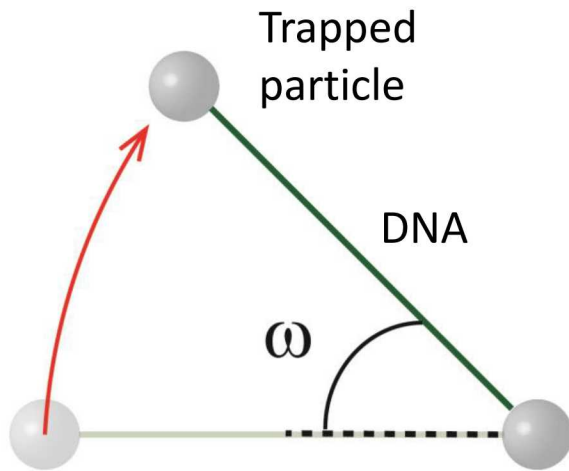


(Or some combination of the above)

- *From previous measurements, not enough info to distinguish wobble from tilt*

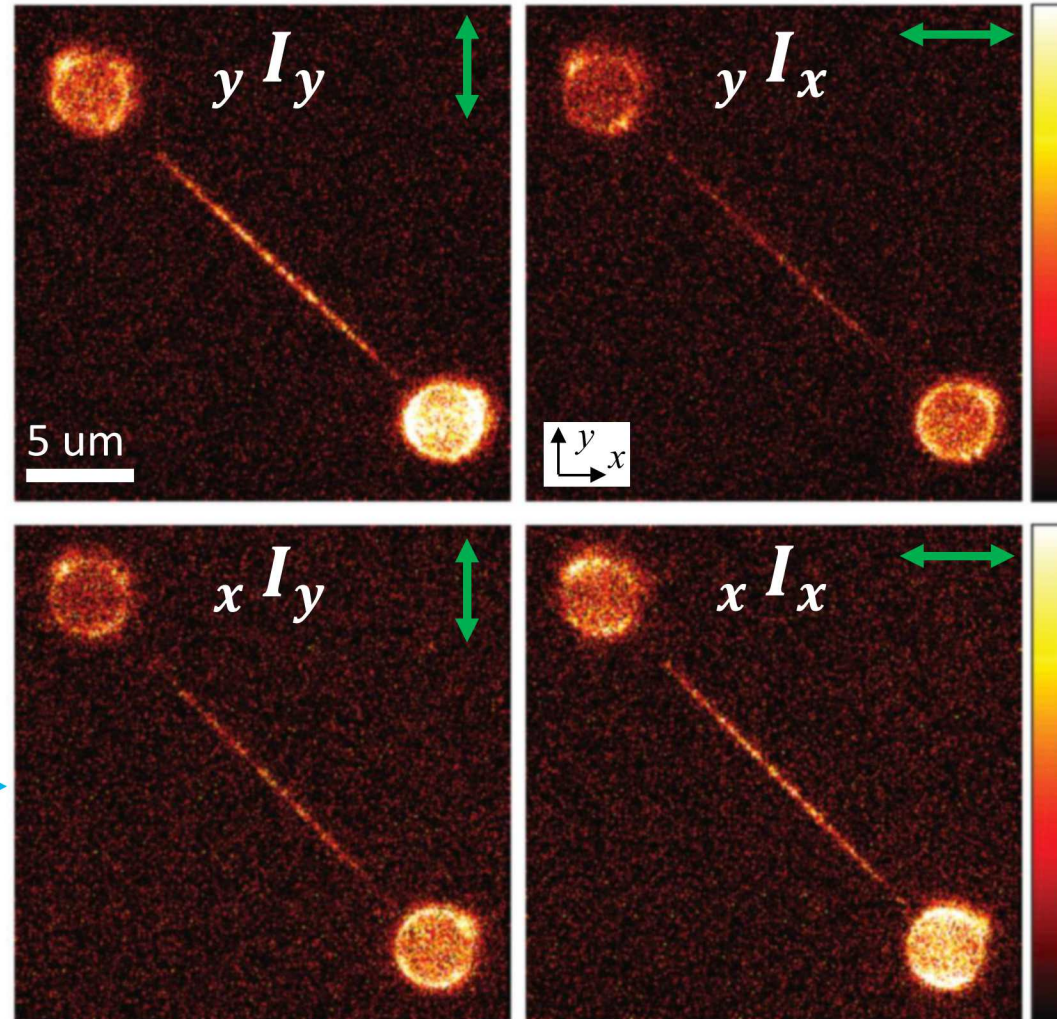
*van Mameren et al. *J. Chem. Phys.* **14**, 123306 (2018)

A new strategy...



- Repeat the experiment, but this time stretch the DNA beyond the OST at an angle ω ...

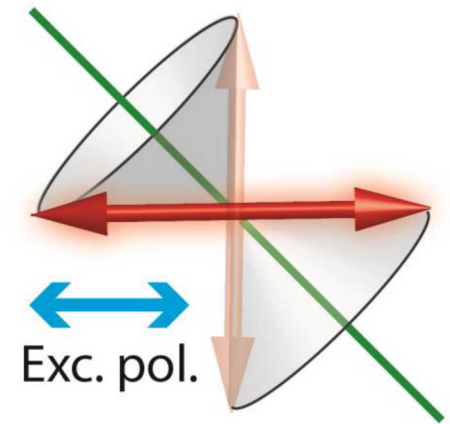
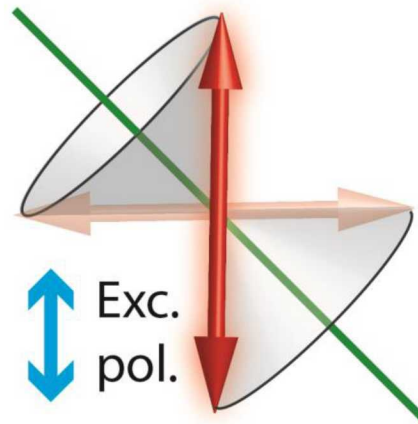
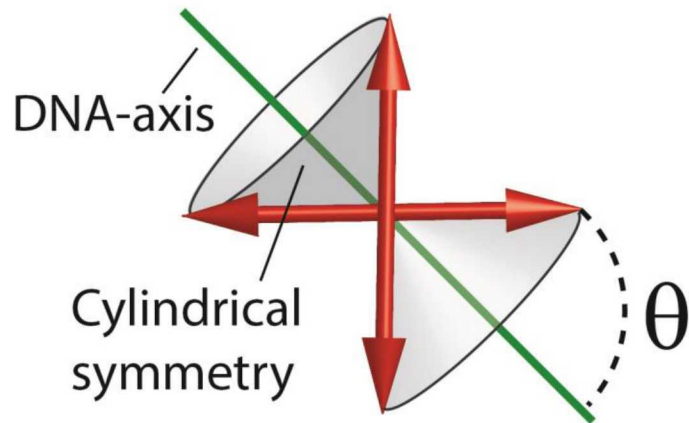
$\omega = 45^\circ$; $L/L_c > 1.7$
YOYO-1 Labeled DNA



Hypothesis: Tilted dipoles

First, an intuitive explanation:

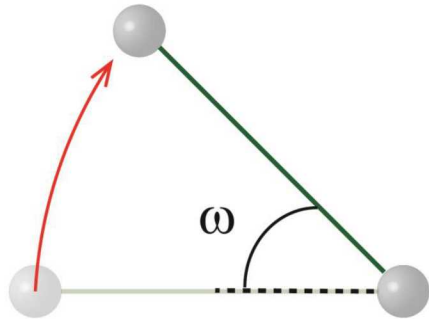
- Assume cylindrical symmetry
- All dipoles tilt by a mean angle θ



- *For particular DNA orientations, different subsets of dipoles are excited*

Quantifying dipole tilt and wobble

Compute fluorescence emission polarization ratios for different DNA orientations ω :



$${}_xP(\omega) = \frac{{}_xI_x - {}_xI_y}{{}_xI_x + {}_xI_y}$$

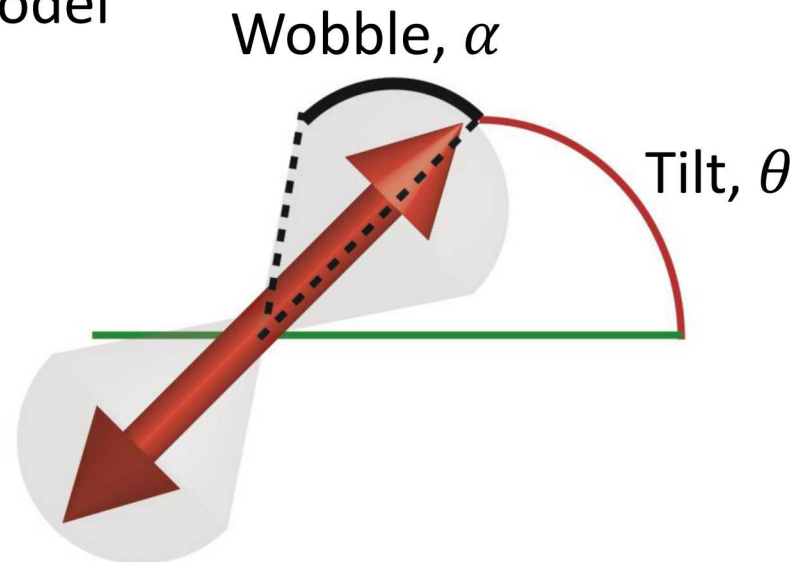
(x-polarized excitation)

$${}_yP(\omega) = \frac{{}_yI_x - {}_yI_y}{{}_yI_x + {}_yI_y}$$

(y-polarized excitation)

Fit polarization data to a theoretical model
Estimate the parameters:

- Mean axial tilt θ
- Wobble-cone α



Refs:

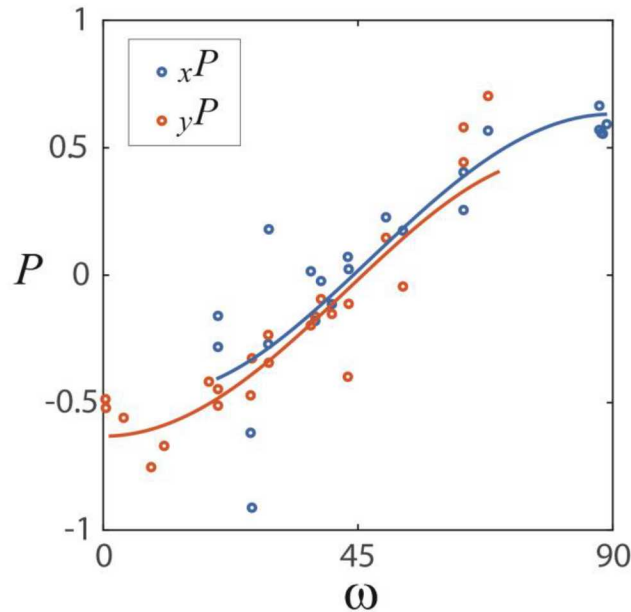
Lew & Backlund et al. *Nano Lett.* **13**, 3967 (2013)

van Mameren et al. *J. Chem. Phys.* **14**, 123306 (2018)

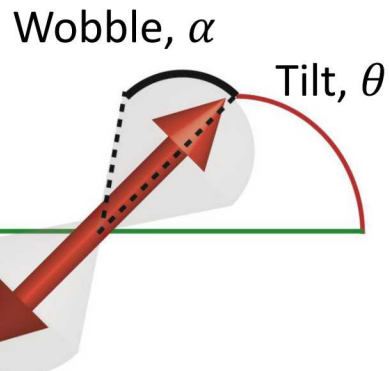
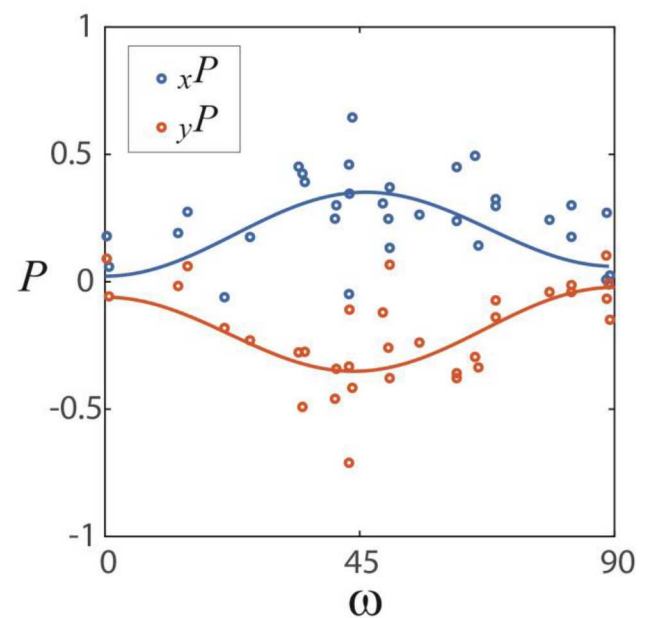
Irving *Biophys. J.* **70**, 1830 (1996)

Beyond the OST, intercalators tilt

Within The OST ($L/L_c = 1.3$)



Beyond The OST ($L/L_c > 1.7$)



*Within OST:

- $\theta = 85.4^\circ \pm 4.0^\circ$
- $\alpha = 29.1^\circ \pm 4.5^\circ$

Beyond OST:

- $\theta = 53.6^\circ \pm 1.4^\circ$
- $\alpha = 39.1^\circ \pm 1.9^\circ$

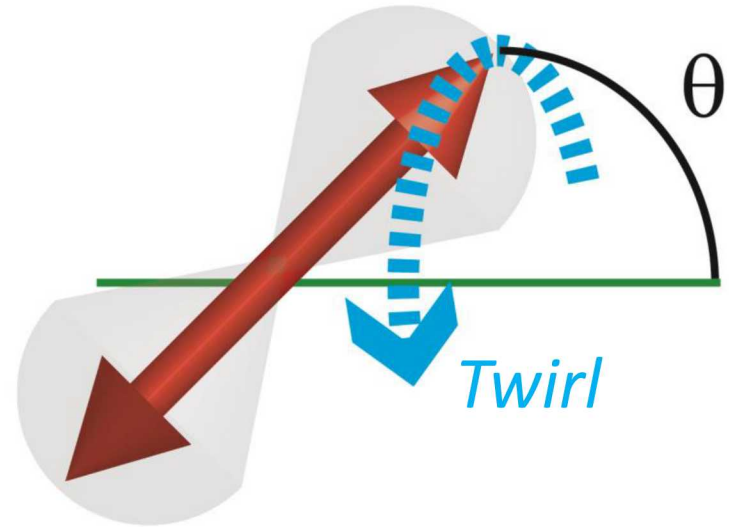
*Compare with:

Cruz et al. *Proc. Natl. Acad. Sci. USA* **113**, E280 (2016); van Mameren et al. *J. Chem. Phys.* **14**, 123306 (2018)

Single-intercalator imaging

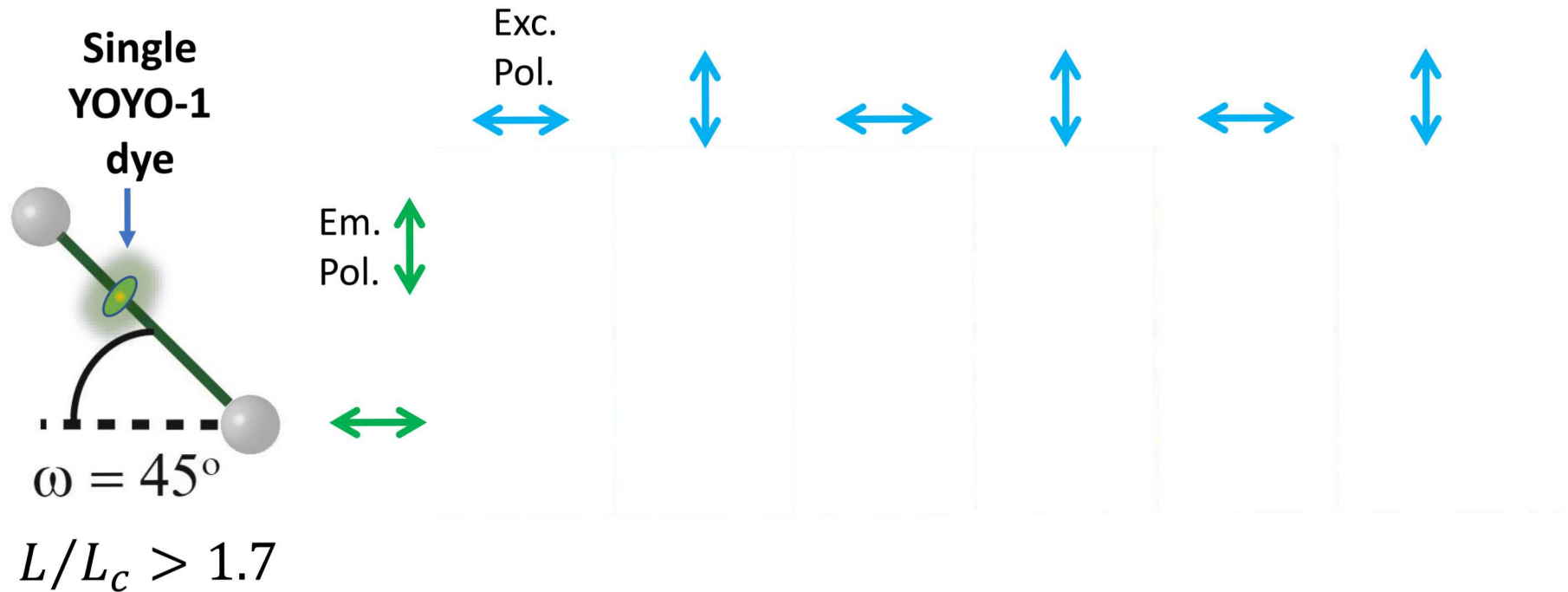
Bulk measurements leave a key question unanswered:

- Could dipoles 'twirl' about the DNA-axis?



- Stretched DNA in a high ionic strength buffer (1M NaCl)
Beyond the OST. (Favors S-DNA formation.)
- *Observed single binding/unbinding intercalators*

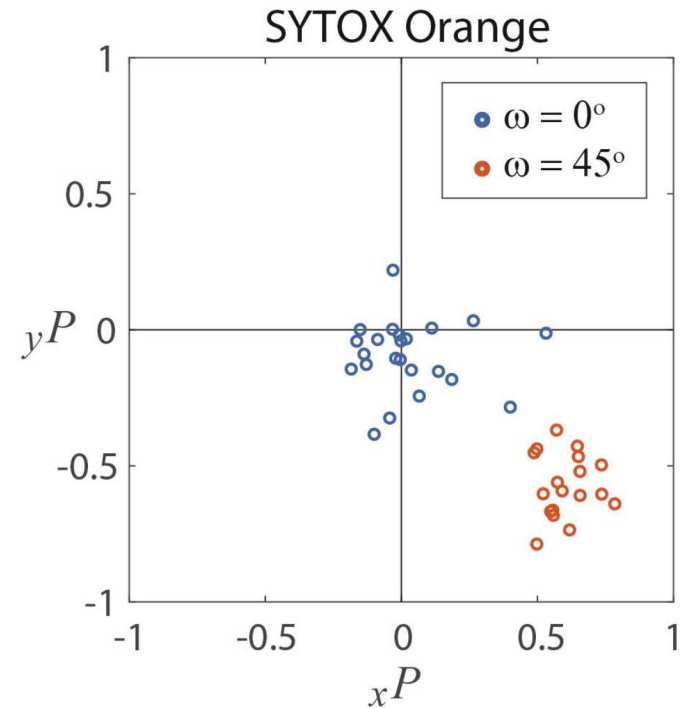
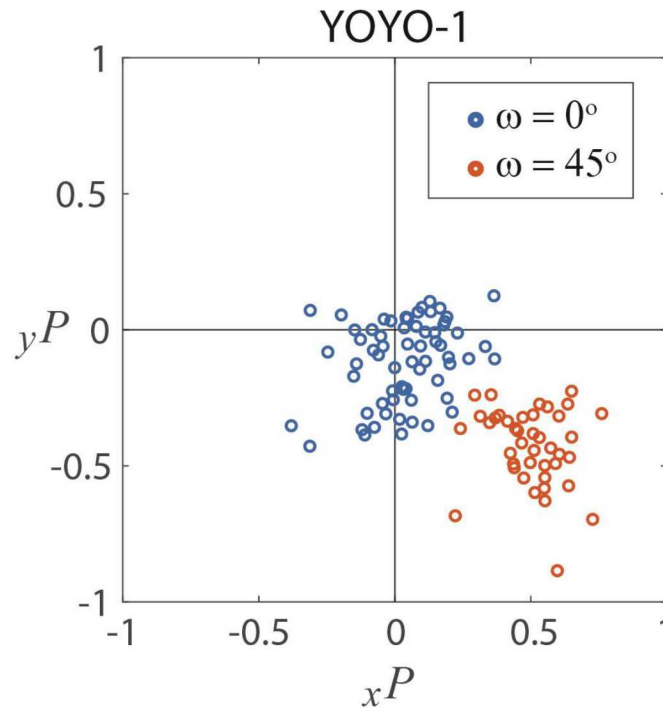
Single-intercalator “polarization switching” beyond the OST



- Excitation polarization switches emission polarization **of a single dye molecule**

Single-intercalator “polarization switching” beyond the OST

- For DNA oriented at $\omega = 45^\circ$, Excitation biases emission



Wobble, α

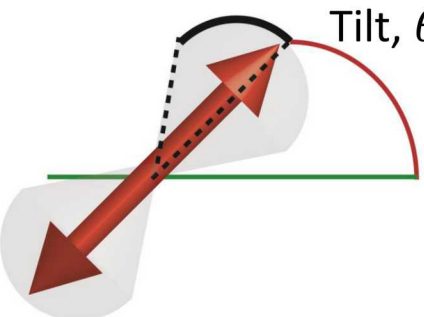
Tilt, θ

YOYO-1:

- $\theta = 53.9^\circ \pm 0.5^\circ$
- $\alpha = 29.8^\circ \pm 1.1^\circ$

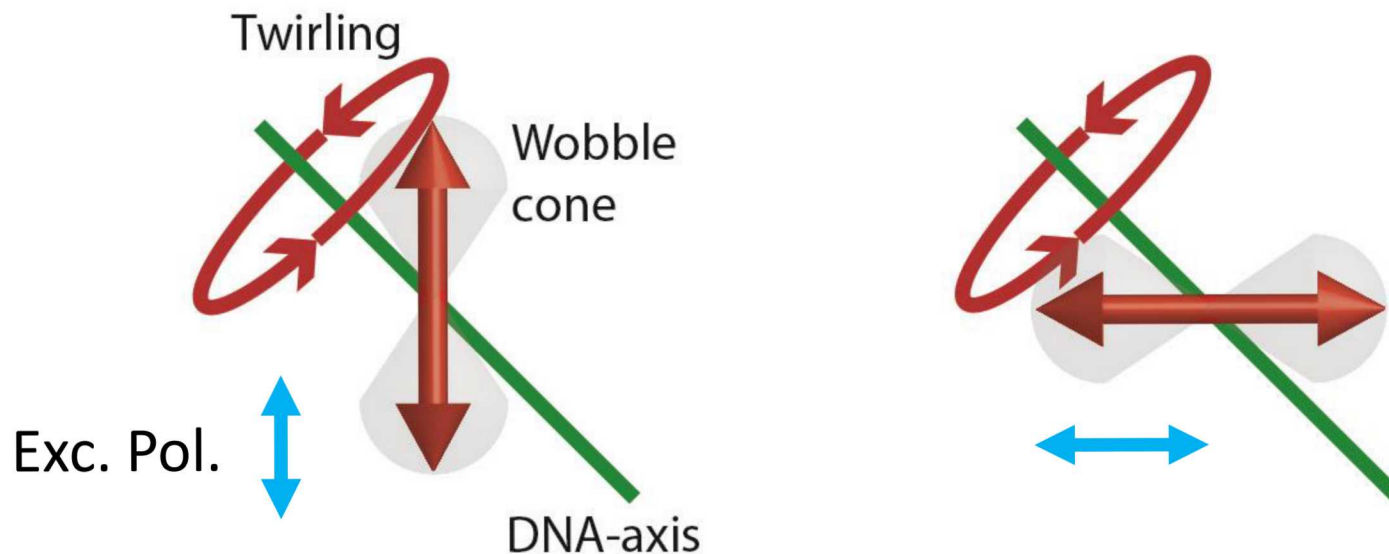
SYTOX Orange:

- $\theta = 53.3^\circ \pm 0.7^\circ$
- $\alpha = 21.4^\circ \pm 1.9^\circ$



Intercalators twirl

- Dipoles twirl at a rate faster than the camera exposure time (1 sec), but slower than the fluorescence lifetime (~ 3 nsec)



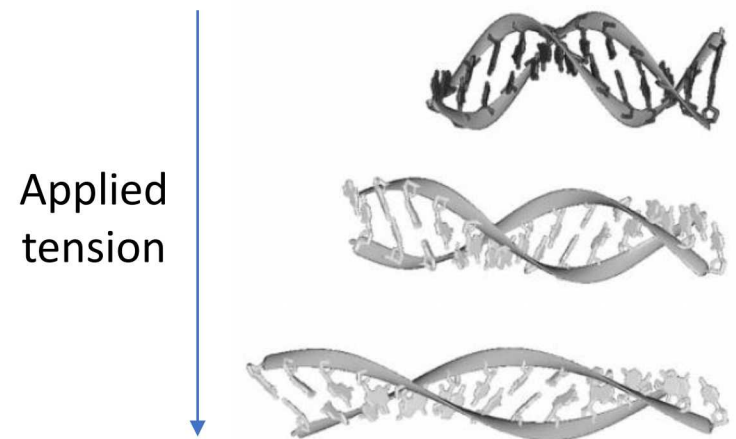
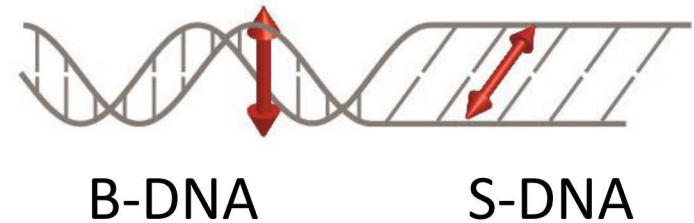
- Dipole twirling due to Brownian twisting of the DNA – rotational correlation time estimated as ~ 100 nsec*

* Barkley and Zimm *J. Chem. Phys.* **70**, (1979).

Also see: Yang et al., *Nano Lett.* **18**, (2018). (Rotational diffusion of DNA mixtures)

Shedding light on S-DNA

- Our data directly shows tilted (dye) dipole orientations in conditions favoring S-DNA
- Theoretical modeling has long predicted that DNA base pairs incline under tension.



Kosikov et al., J. Mol. Biol. **289** (1999)

Acknowledgements

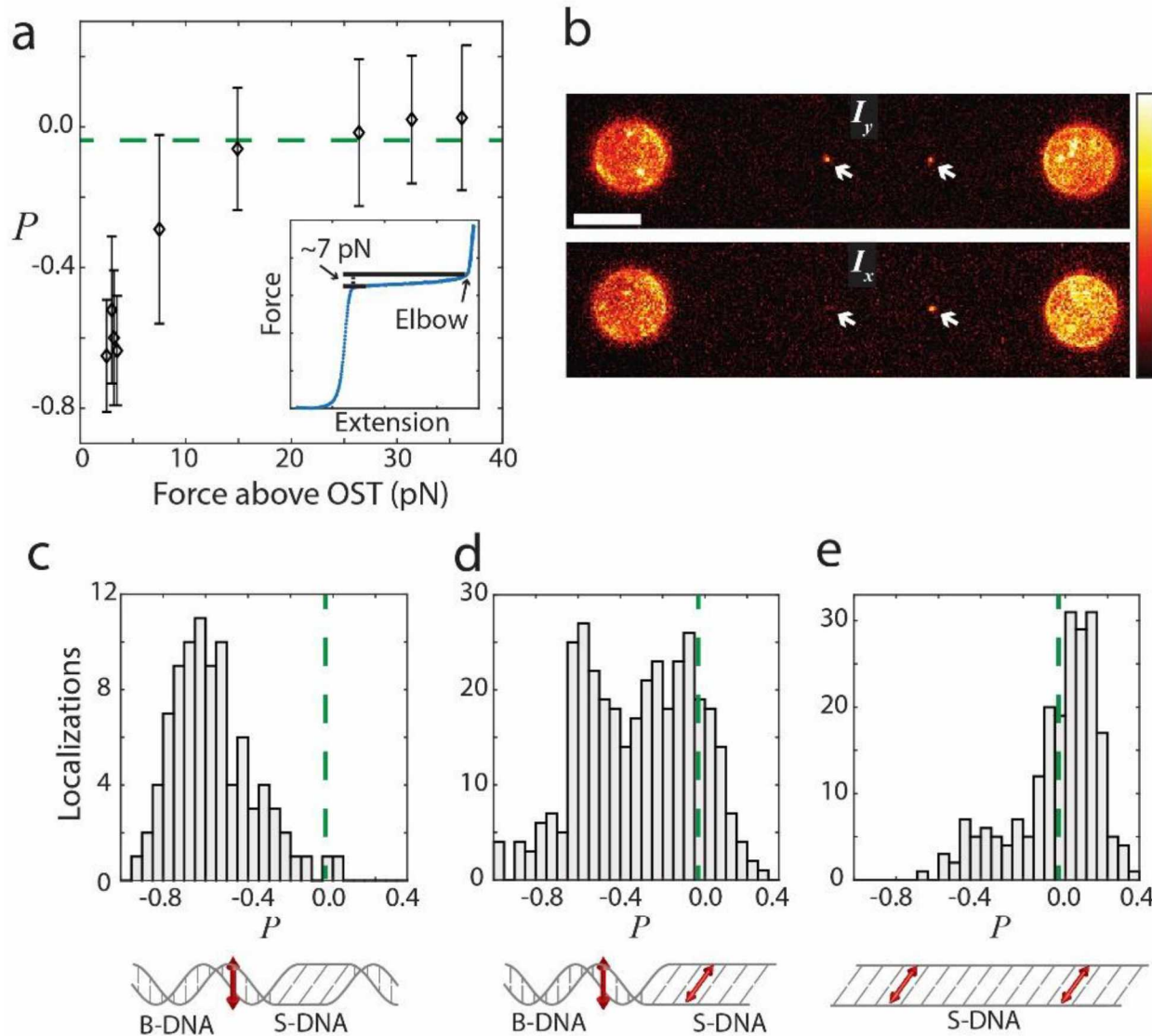
- Sandia LDRD Program
- Harry S. Truman Fellowship
- Wuite, Heller & Peterman Labs (VU)
- LASERLAB-EUROPE Travel grant program



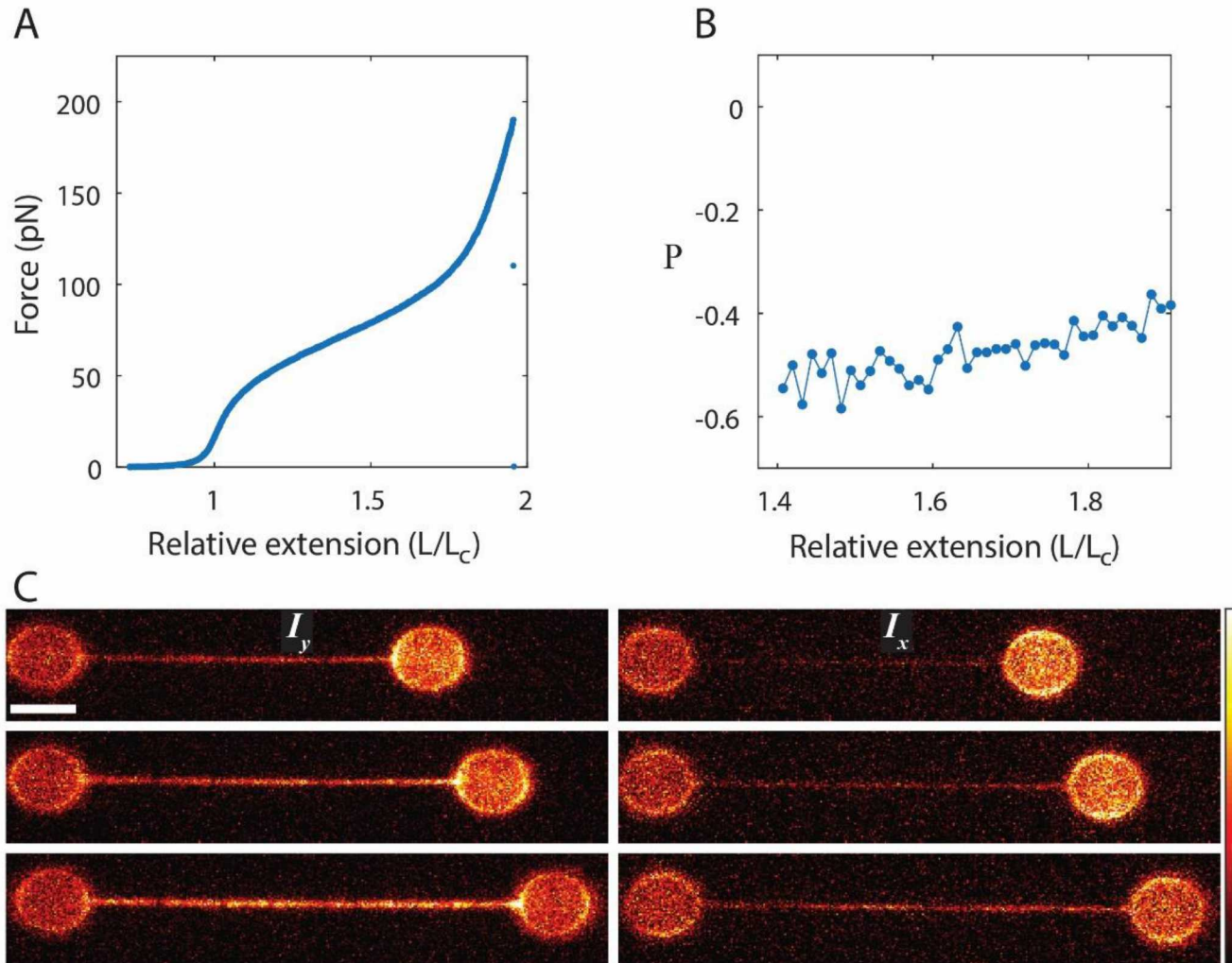
Disclaimer:

This work was supported by the Laboratory Directed Research and Development program at Sandia National Laboratories, a multi-mission laboratory managed and operated by National Technology and Engineering Solutions of Sandia, LLC, a wholly owned subsidiary of Honeywell International, Inc., for the US Department of Energy's National Nuclear Security Administration under contract [DE-NA0003525]. This presentation describes objective technical results and analysis. Any subjective views or opinions that might be expressed in the presentation do not necessarily represent the views of the US Department of Energy or the United States Government.

Intercalator tilting occurs abruptly at the end of the OST

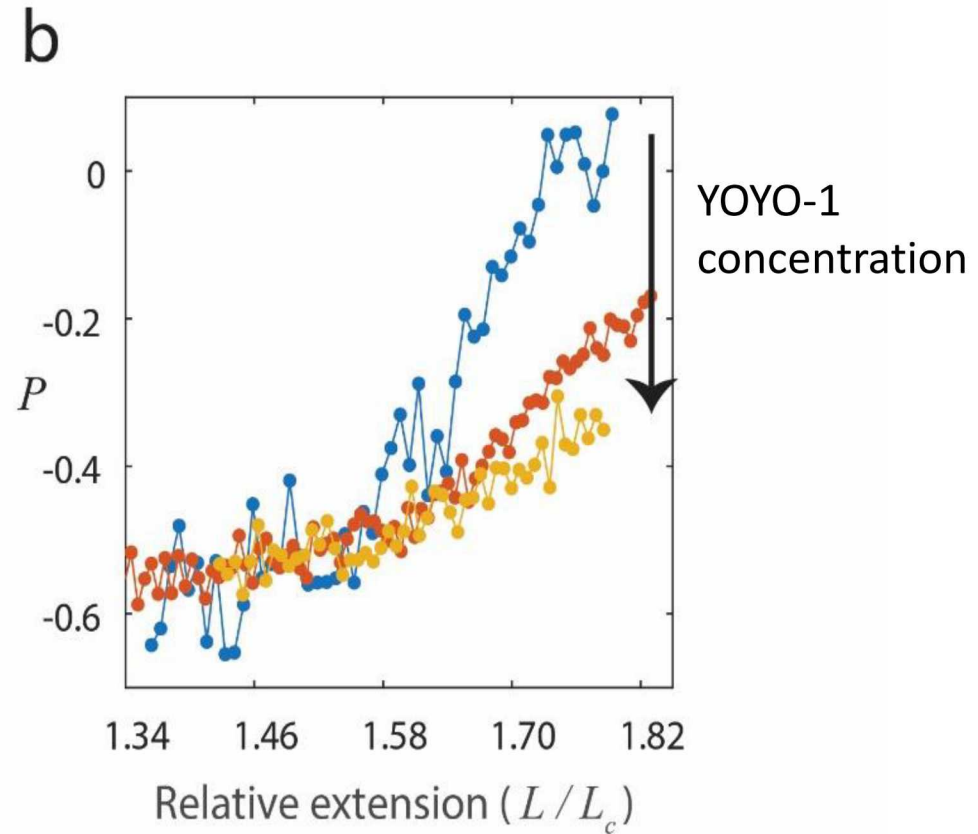
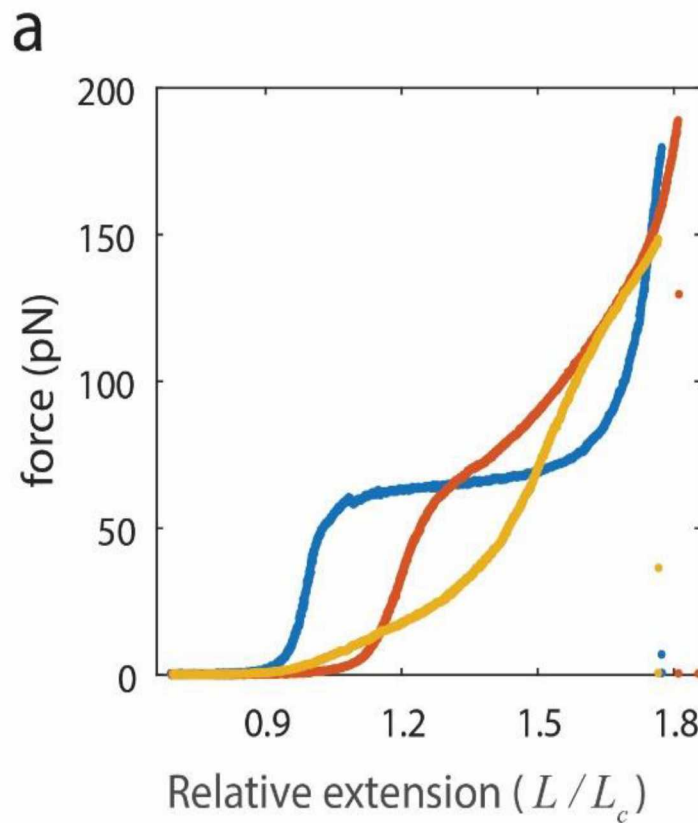


Less depolarization at higher dye concentration (Hyperstretched* YOPRO-labeled DNA)

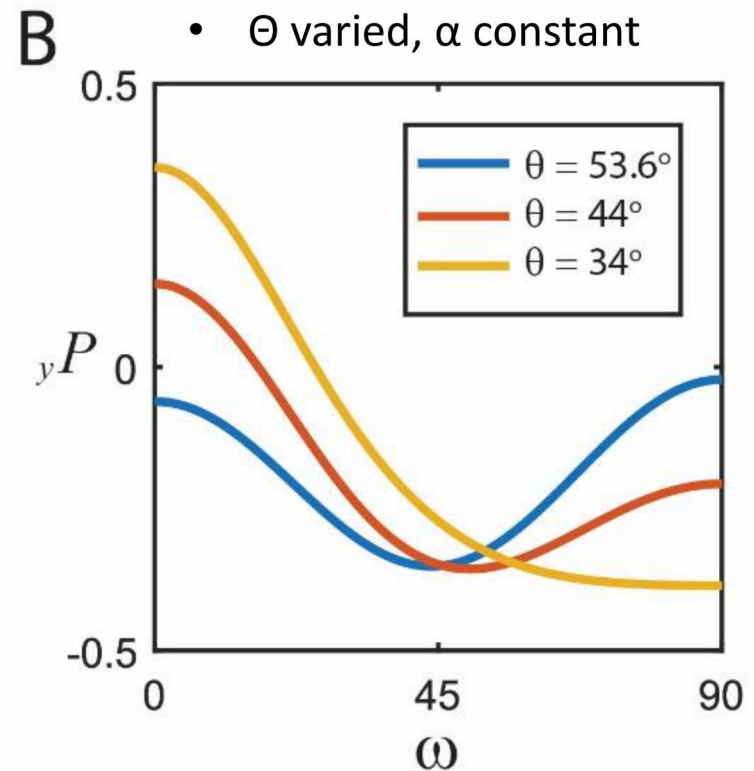
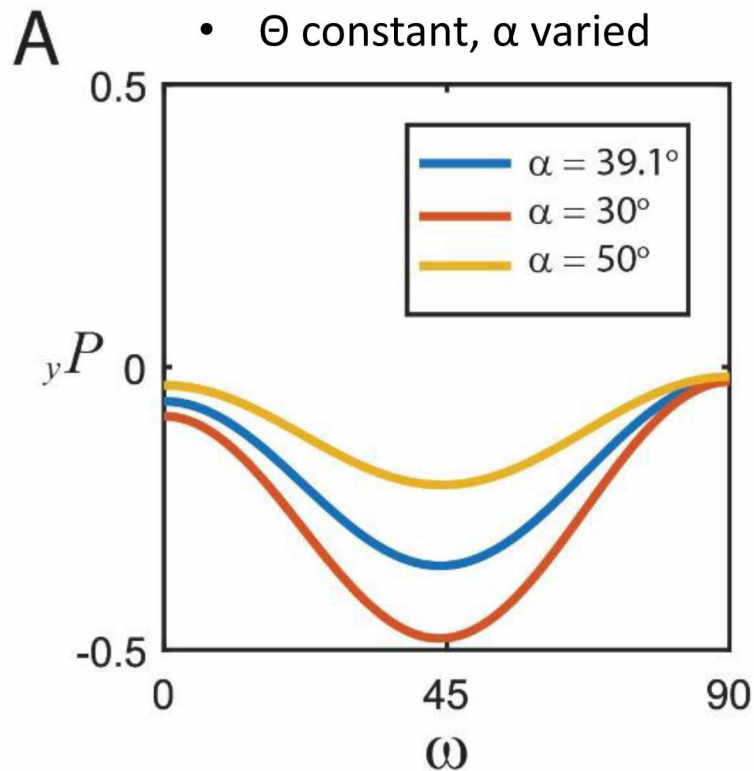


*Schakenraad et al., *Nat. Commun.* **8**, (2017)

Less depolarization at higher dye concentration
(YOYO-1-Labeled DNA, different concentrations)

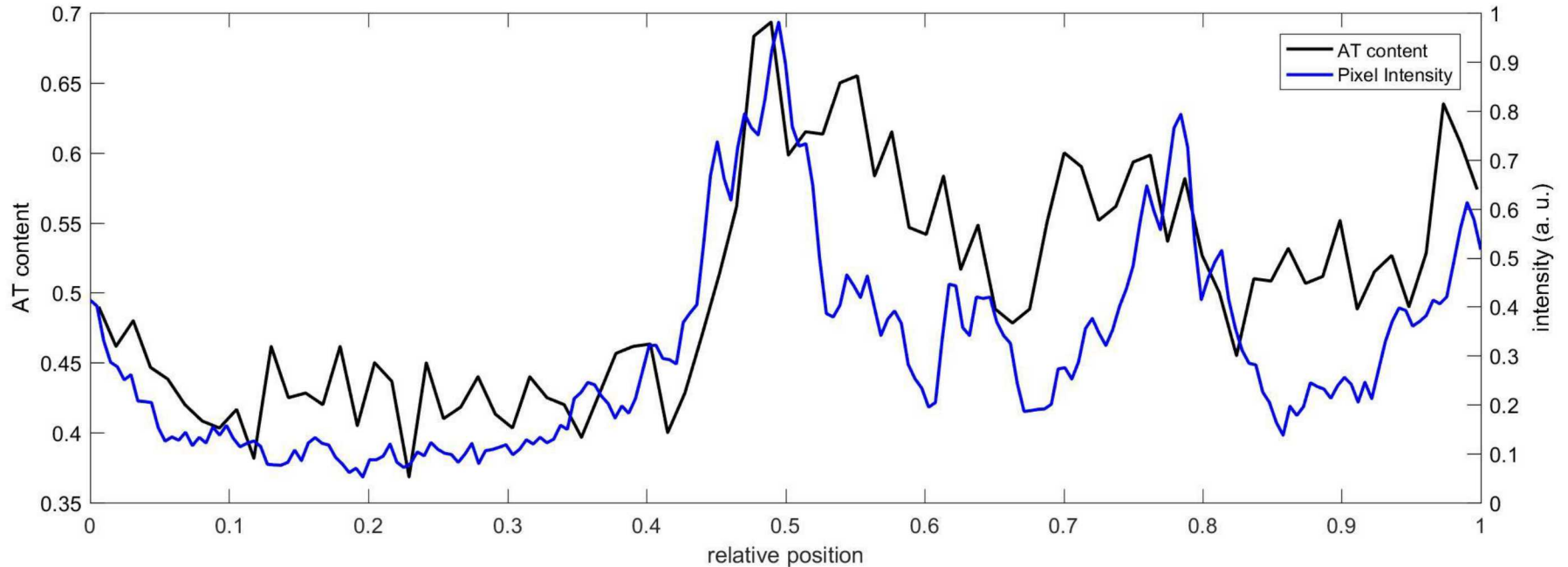


Distinguishing wobble and tilt



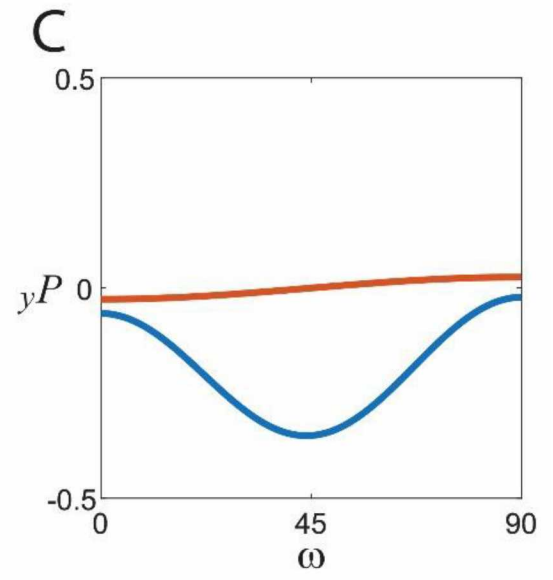
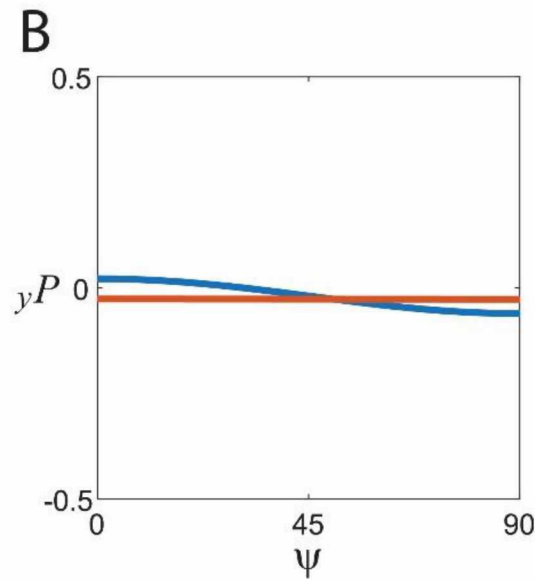
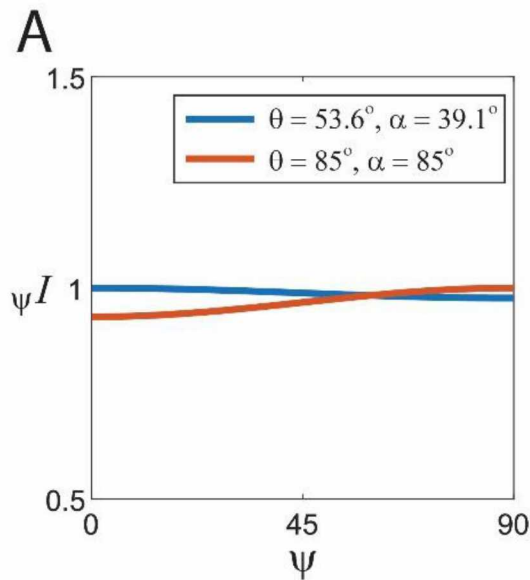
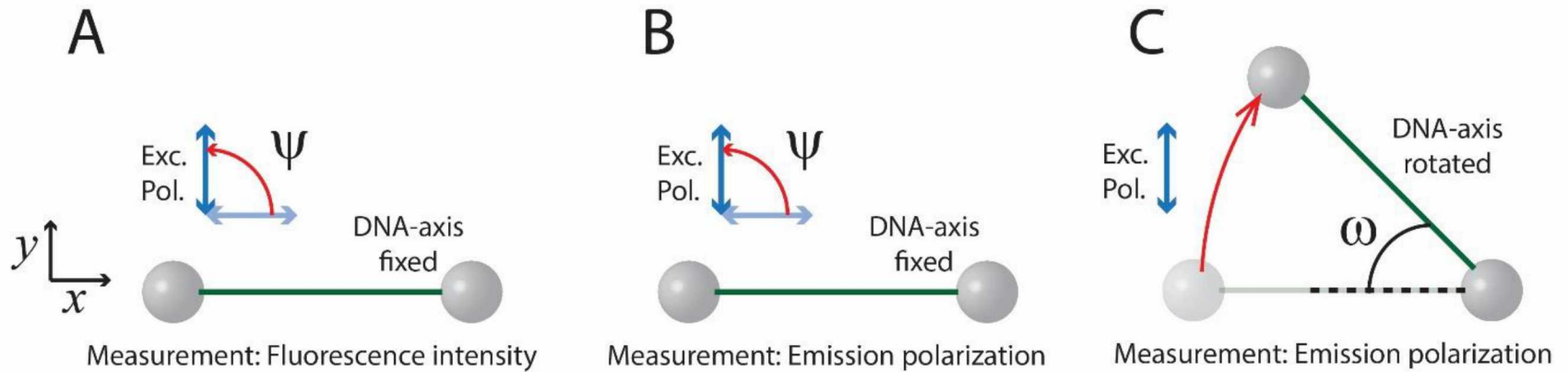
(Simulated polarization ratios)

Sequence dependence



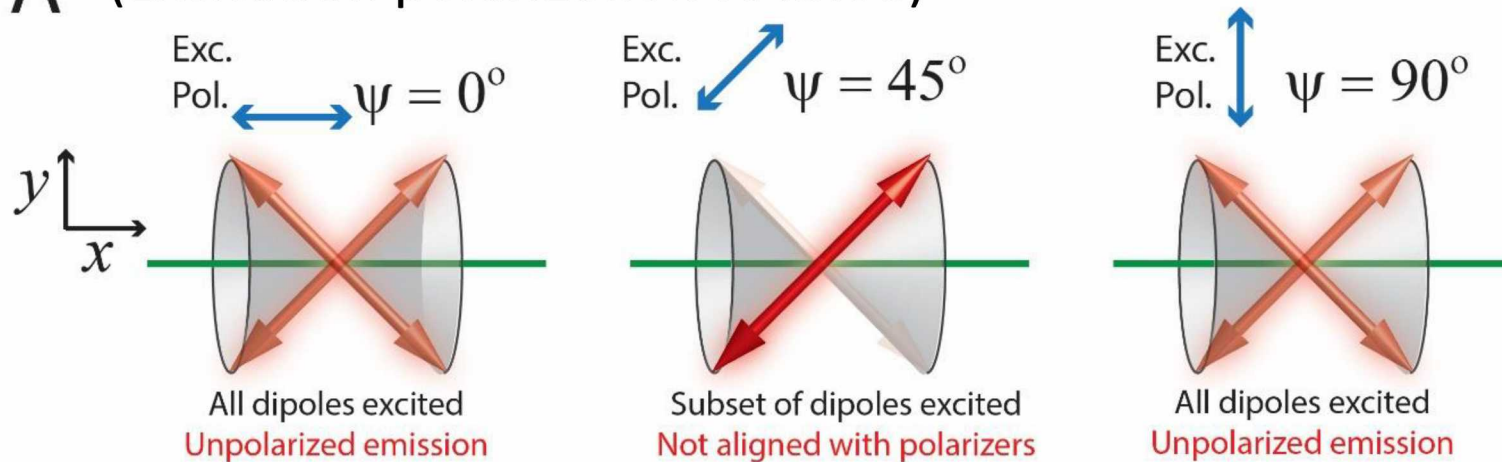
- Summed fluorescence intensity from single molecule binding events
- Dye prefers AT-rich regions of the lambda-DNA construct

Different experimental configurations considered:

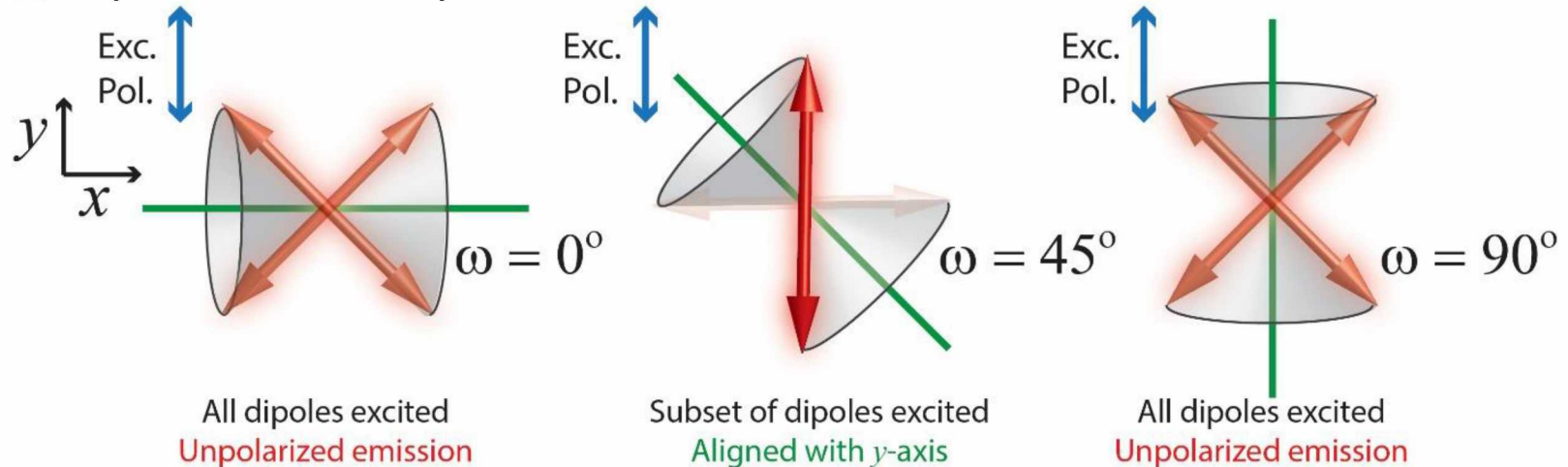


Difference between rotating DNA vs. rotating excitation polarization

A (Excitation polarization rotated)



B (DNA rotated)



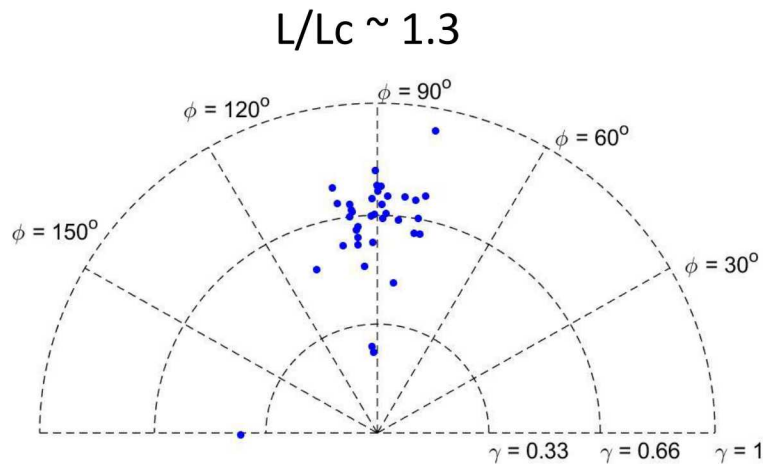
Effect of energy transfer on estimated tilt and wobble

Relative DNA extension: Within the OST (regime 2)		
	θ	α
$\beta = 10^\circ$	$85.4^\circ \pm 3.9^\circ$	$28.3^\circ \pm 4.7^\circ$
$\beta = 20^\circ$	$85.3^\circ \pm 3.6^\circ$	$25.6^\circ \pm 5.2^\circ$
$\beta = 30^\circ$	$85.2^\circ \pm 3.2^\circ$	$20.3^\circ \pm 6.8^\circ$

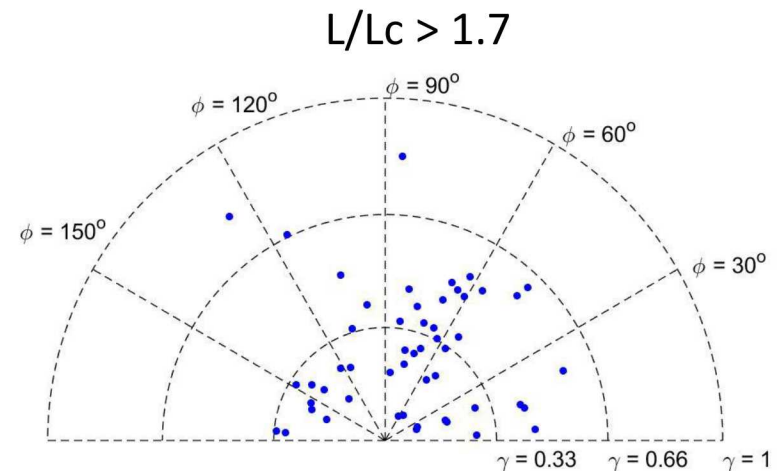
Relative DNA extension: Beyond the OST (regime 3)		
	θ	α
$\beta = 10^\circ$	$53.7^\circ \pm 1.4^\circ$	$38.7^\circ \pm 1.9^\circ$
$\beta = 20^\circ$	$54.0^\circ \pm 1.4^\circ$	$37.4^\circ \pm 2.0^\circ$
$\beta = 30^\circ$	$54.5^\circ \pm 1.4^\circ$	$35.0^\circ \pm 2.2^\circ$

Excitation polarization modulation

- Excited single dye molecules using 6 different excitation polarizations rotated 30 degrees apart.
- Fit intensity data to a sinusoid, extracted modulation depth γ (rotational mobility), and phase ϕ (preferred orientation in x/y plane)

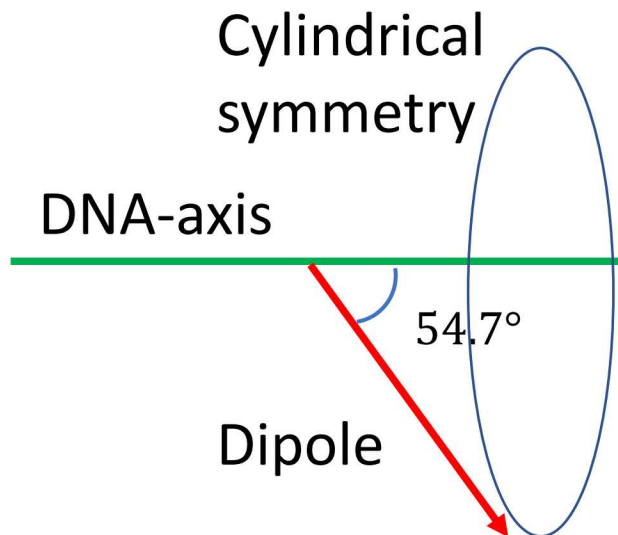
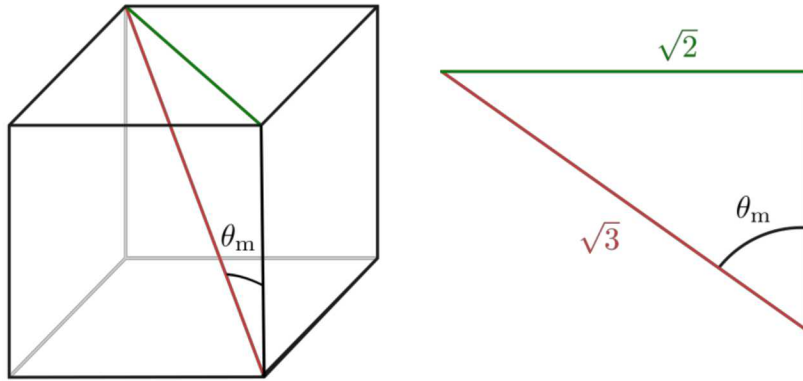


$$\hat{\gamma} = 0.64 \pm 0.13$$
$$\hat{\phi} = 88.3^\circ \pm 16.4^\circ$$



$$\hat{\gamma} = 0.35 \pm 0.17$$
$$\hat{\phi} = 77.6^\circ \pm 46.3^\circ$$

The magic angle: $\theta_m = \tan^{-1}(\sqrt{2}) \approx 54.7^\circ$



- At magic angle, **2nd moments** of dipoles projected along x-, y-, z-axis are equal
- A method that relies **only** on excitation/emission polarization senses only dipole **2nd moments**
- By using excitation **and** emission polarization, can measure **2nd** and **4th moments**