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DNA intercalators tilt, wobble & twirl:

Elucidating DNA overstretching

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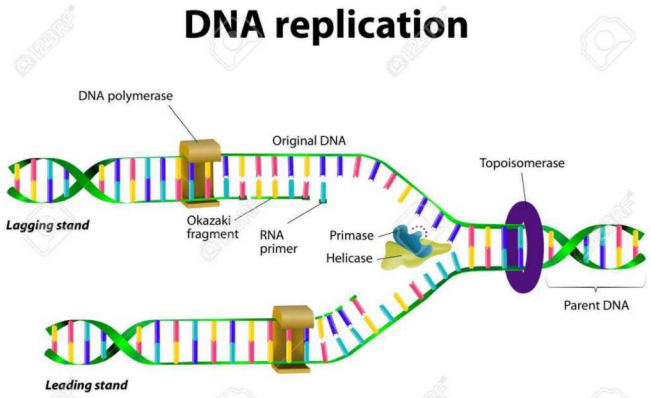
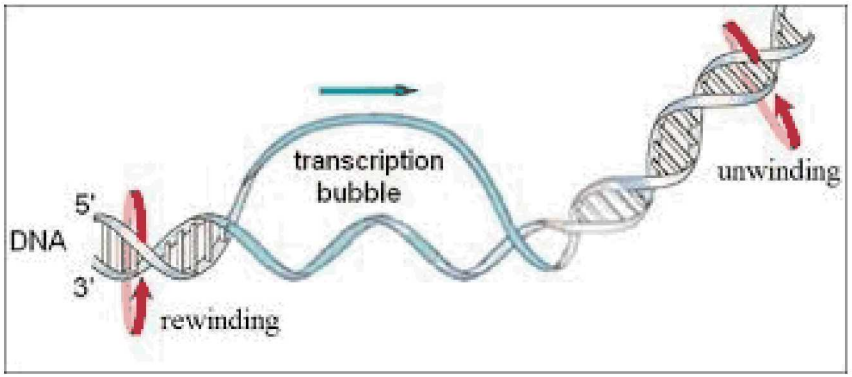
rice Advances

Why study the structure & dynamics of DNA?

- Insight into biological function





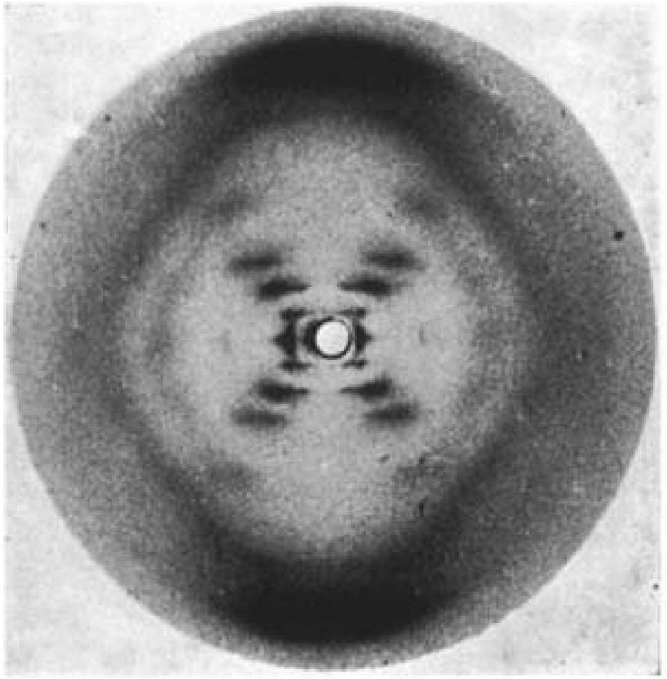
- DNA-based smart materials / DNA origami

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

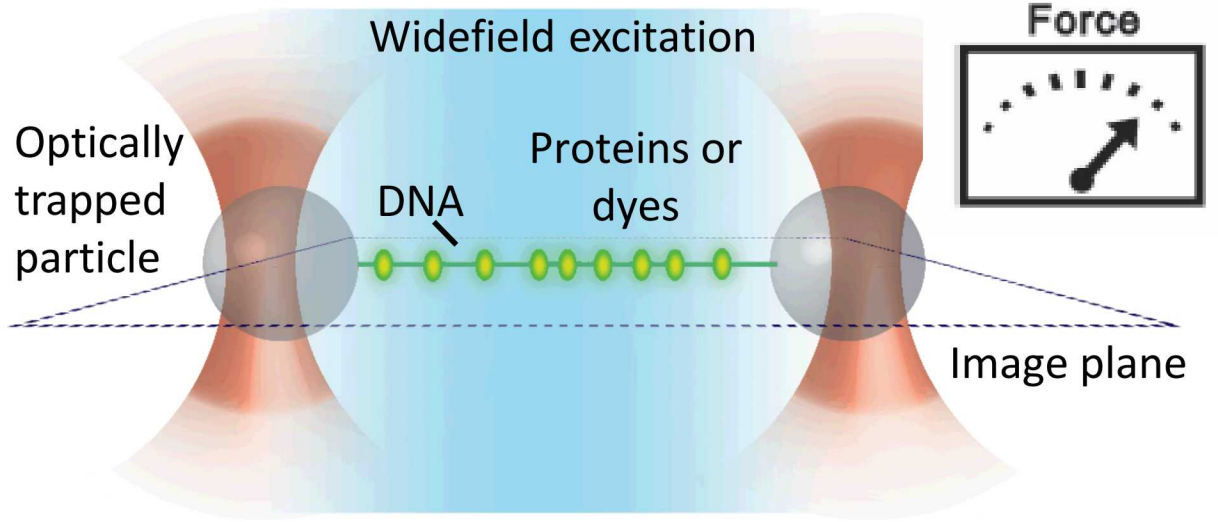
Pros:

- 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).

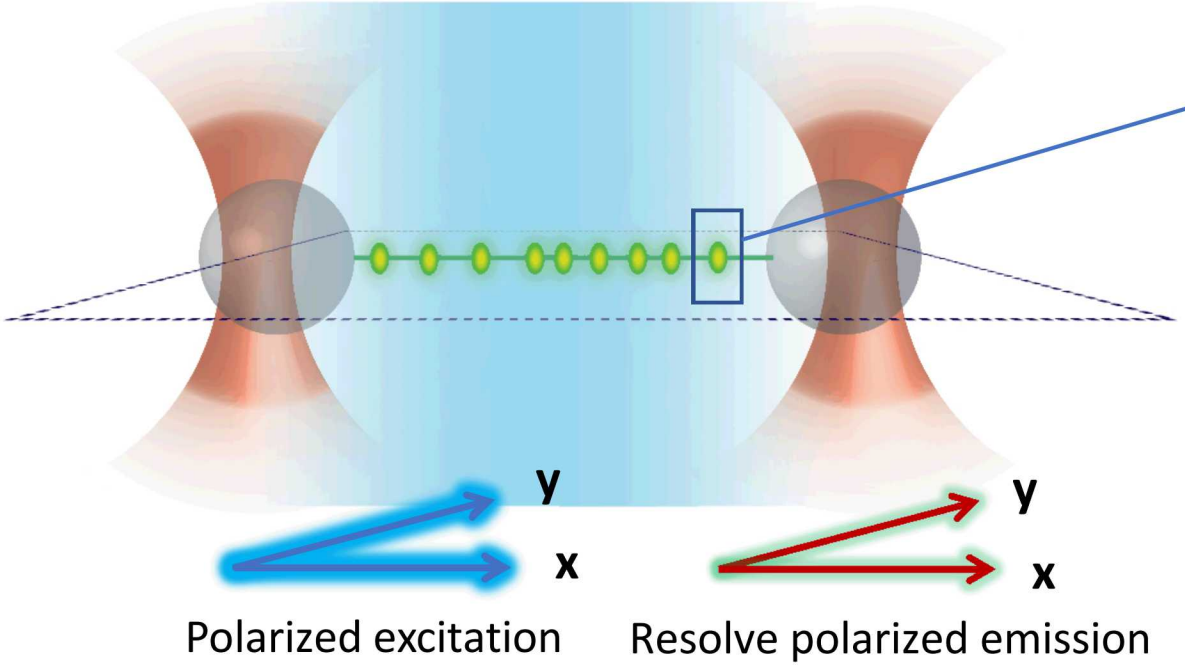
Outline for today:

- Use **fluorescence polarization microscopy** w/ optical tweezers & DNA force spectroscopy

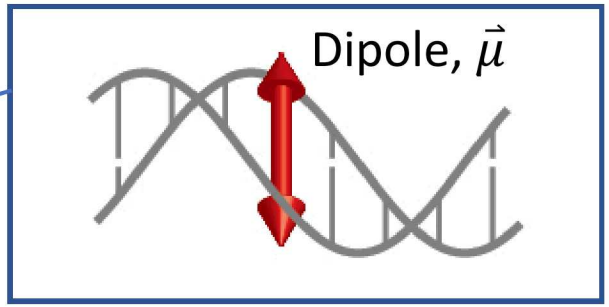
- Gain insight into **S-DNA**, an elusive DNA structure that forms under tension

Refs:
Cluzel et al., *Science* **271**, (1996).
Smith et al., *Science* **271**, (1996).

Fluorescence polarization imaging of stretched DNA



Polarized excitation Resolve polarized emission



Dyes intercalate between DNA base pairs

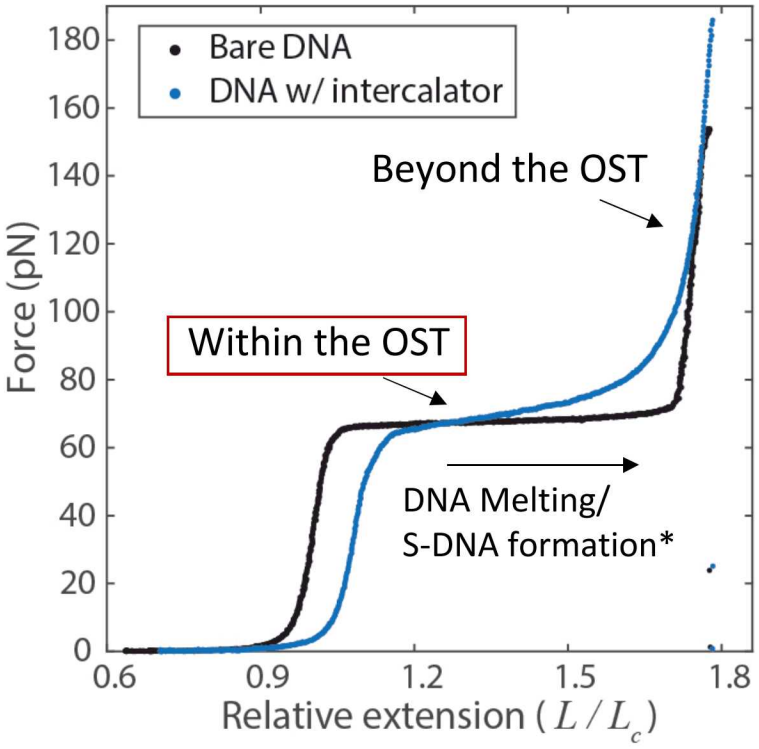
DNA intercalators & overstretching:

Biebricher et al. *Nat. Commun.* **6**, (2015)
Schakenraad et al., *Nat. Commun.* **8**, (2017)

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

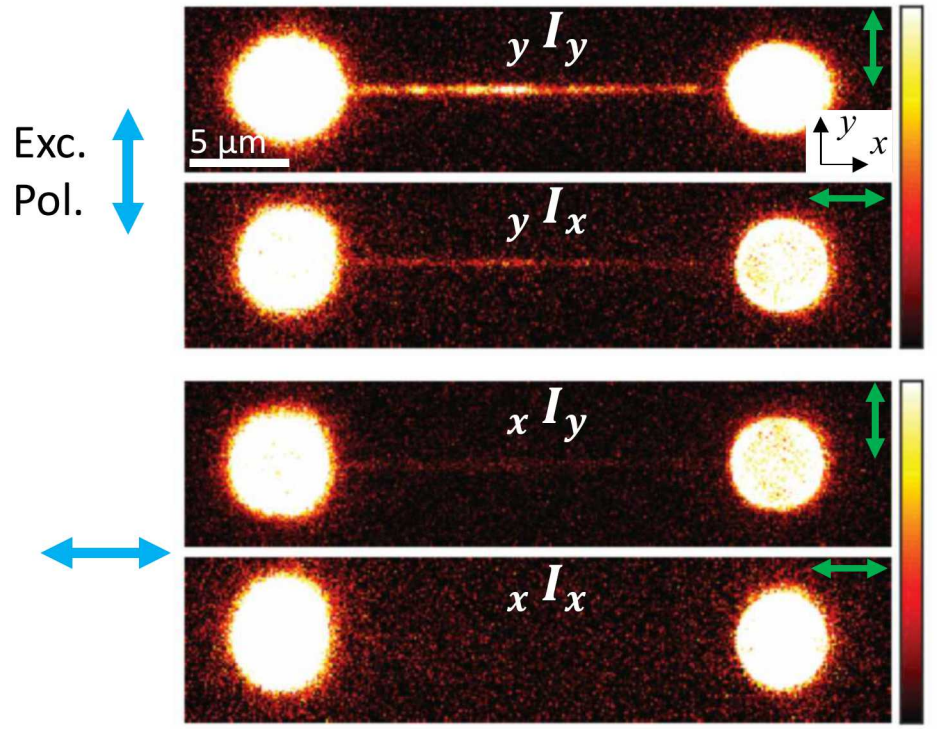
The Overstretching Transition (OST) & Polarization Response

λ-DNA: $L_c = 16.5$ µm

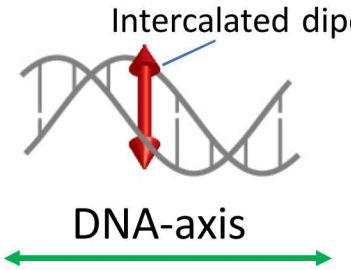


DNA extension: $L/L_c = 1.3$

YOYO-1 labeled DNA



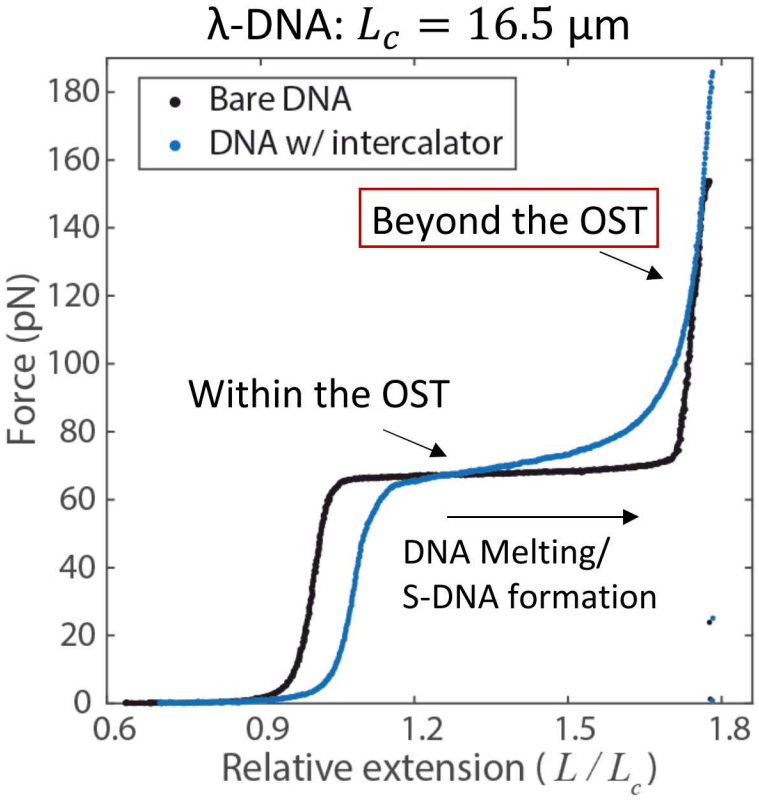
Interpretation: Within the OST, base pairs align intercalators perpendicular to the DNA-axis

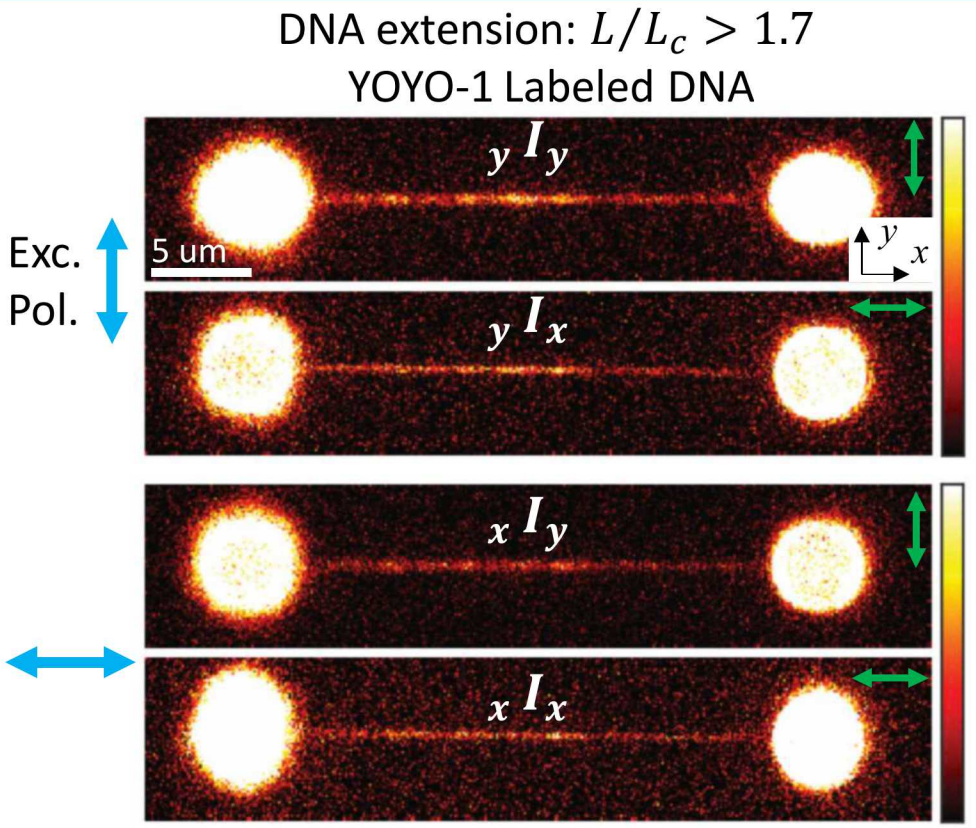


*Refs:

van Mameren et al. *PNAS* **106**, 18231 (2009)
King et al. *PNAS* **110**, 3859 (2013)

The Overstretching Transition (OST) & Polarization Response



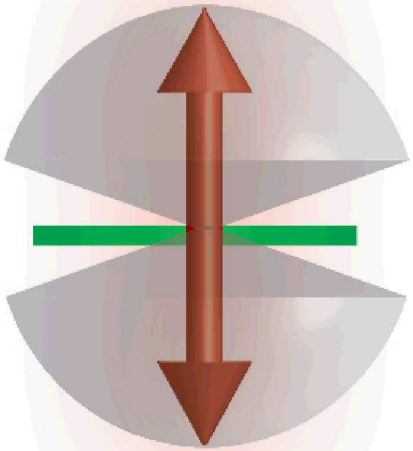


Interpretation: ??
??????????????????

WHAT IS GOING ON?????

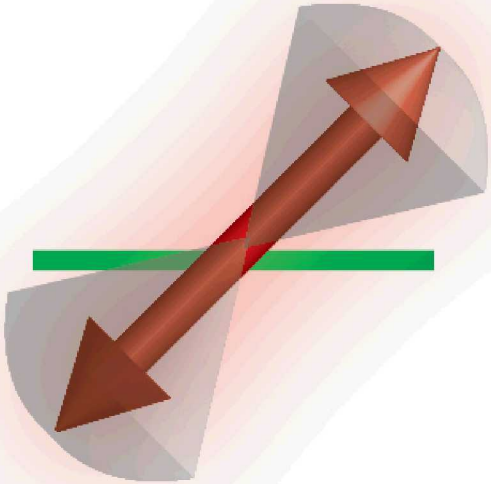
Two hypotheses*:

Wobbly dipoles



Or

Tilted dipoles

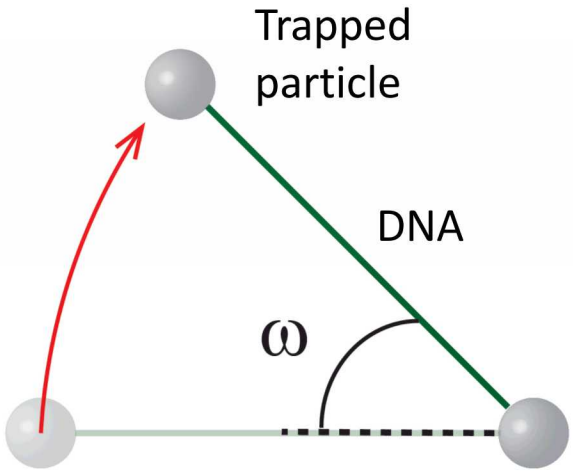


(**Or** some combination of the above)

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

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

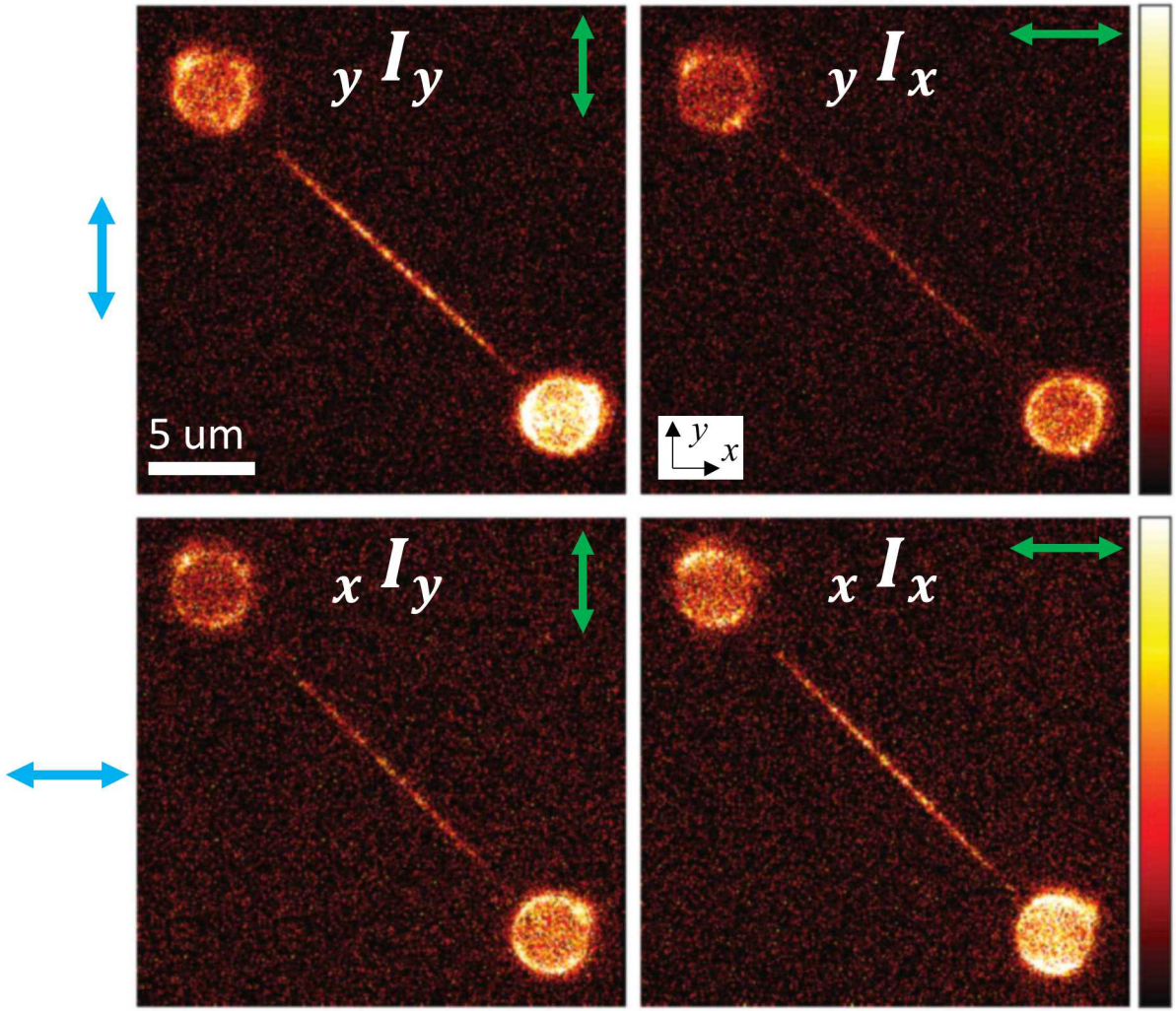
A New Strategy…



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

$\omega = 45°$; $L/L_c > 1.7$

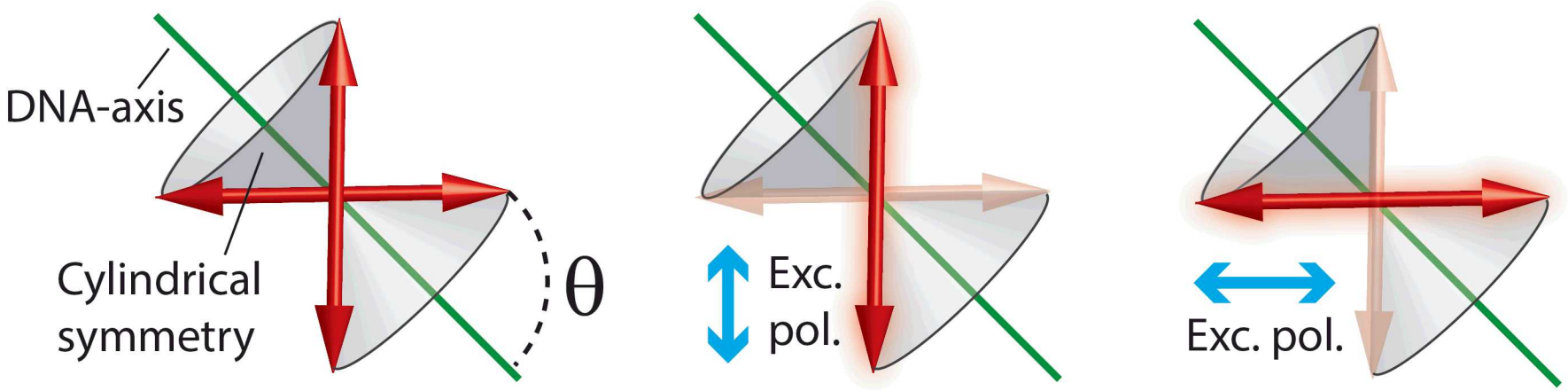
YOYO-1 Labeled DNA



Hypothesis: Tilted dipoles

First, an intuitive explanation:

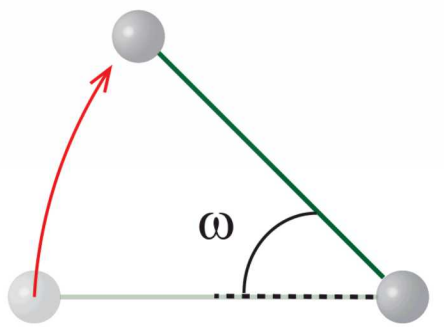
- Assume cylindrical symmetry
- All dipoles tilt by a mean angle θ



- *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:



$$ {}_xP(\omega) = \frac{{}_x I_x - {}_x I_y}{{}_x I_x + {}_x I_y} $$

(x-polarized excitation)

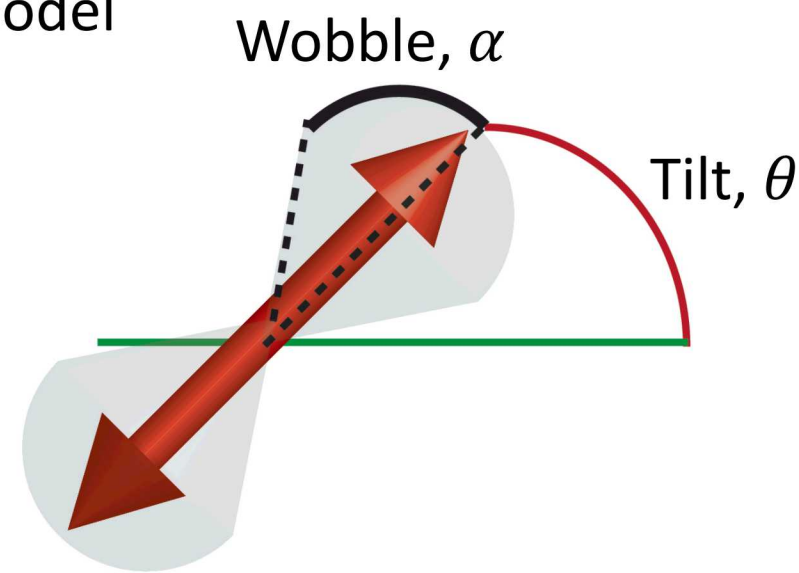
$$ {}_yP(\omega) = \frac{{}_y I_x - {}_y I_y}{{}_y I_x + {}_y I_y} $$

(y-polarized excitation)

Fit polarization data to a theoretical model

Estimate the parameters:

- Mean axial tilt θ
- Wobble-cone α

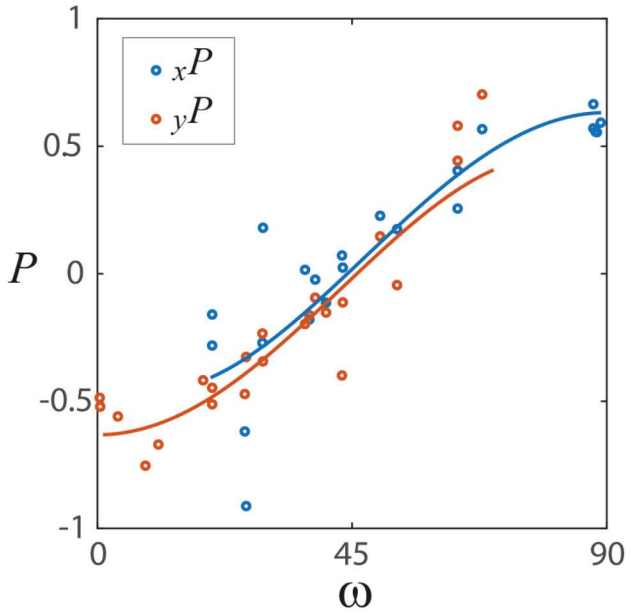


Refs:

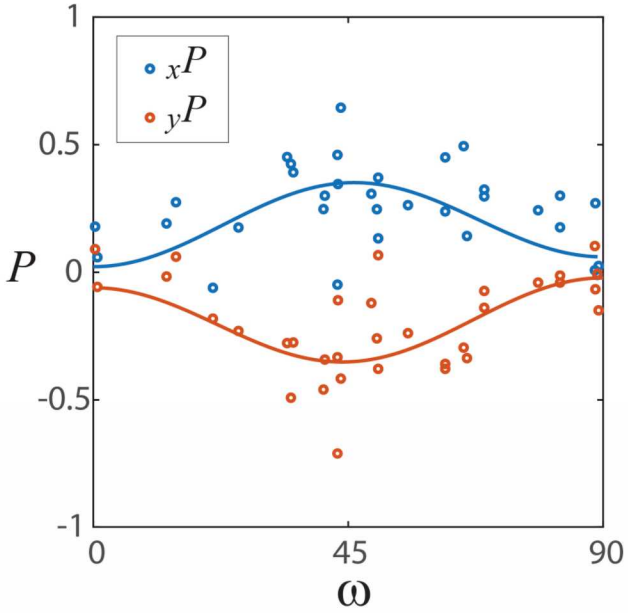
Lew & Backlund et al. *Nano Lett.* **13**, 3967 (2013)
van Mameren et al. *J. Chem. Phys.* **14**, 123306 (2018)
Irving *Biophys. J.* **70**, 1830 (1996)

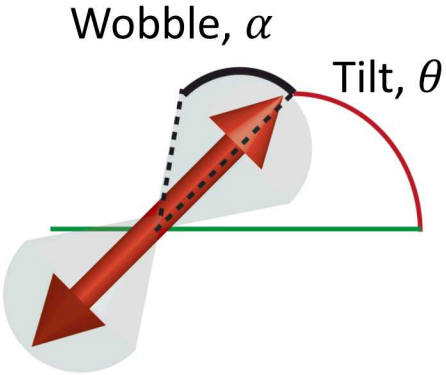
Beyond the OST, intercalators tilt

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



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





*Within OST:

- $\theta = 85.4° \pm 4.0°$
- $\alpha = 29.1° \pm 4.5°$

Beyond OST:

- $\theta = 53.6° \pm 1.4°$
- $\alpha = 39.1° \pm 1.9°$

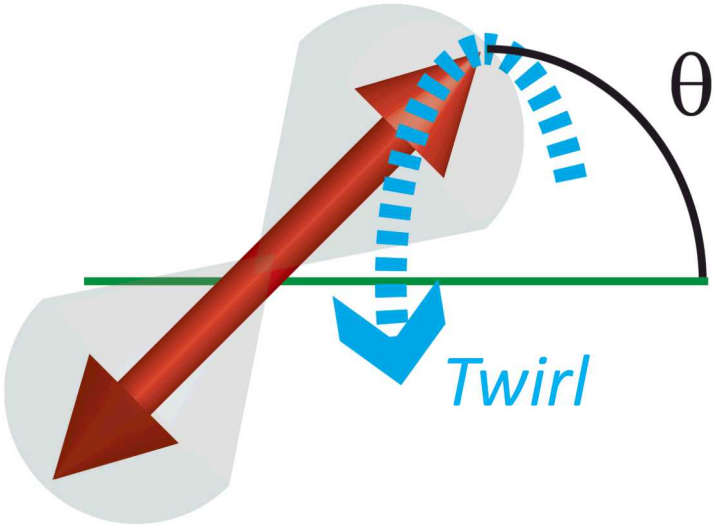
*Compare with:

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:

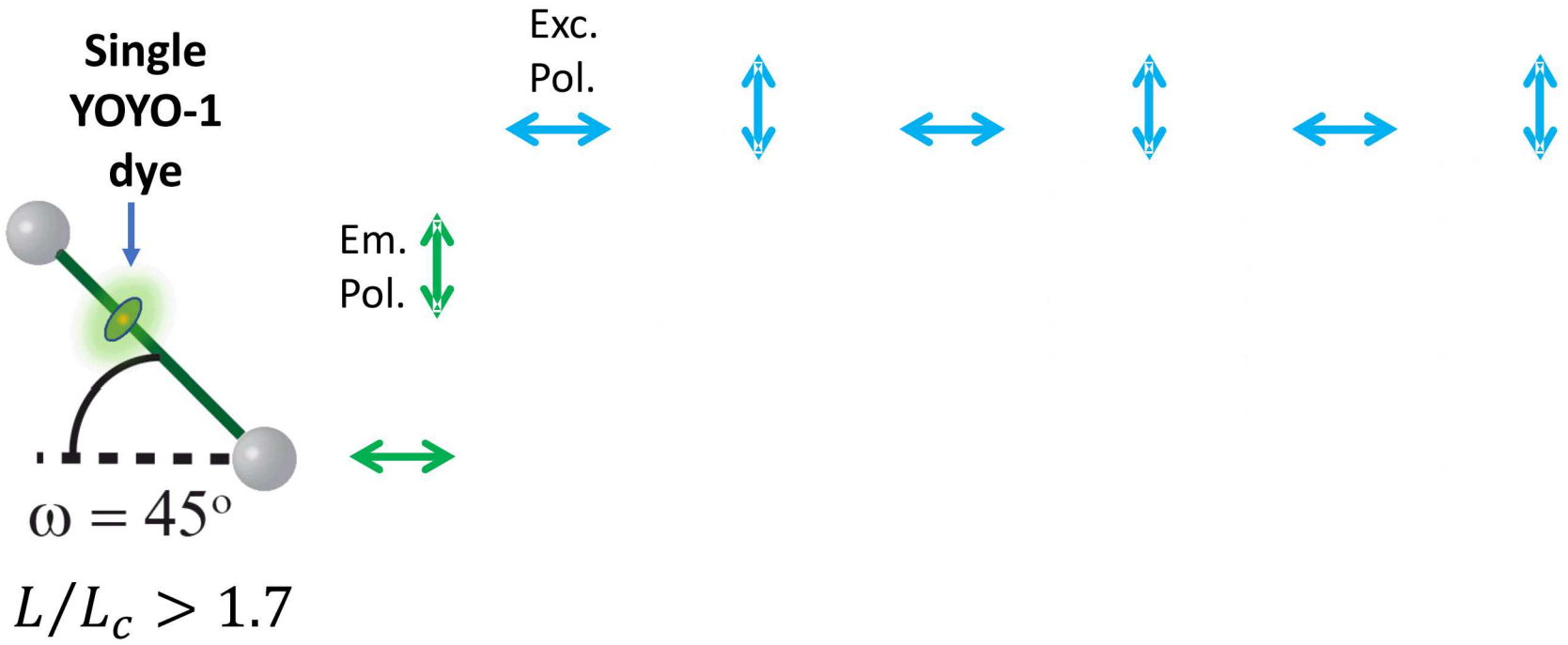
- Could dipoles ‘twirl’ about the DNA-axis?



- Stretched DNA in a high ionic strength buffer (1M NaCl) Beyond the OST. (Favors S-DNA formation.)

- *Observed single binding/unbinding intercalators*

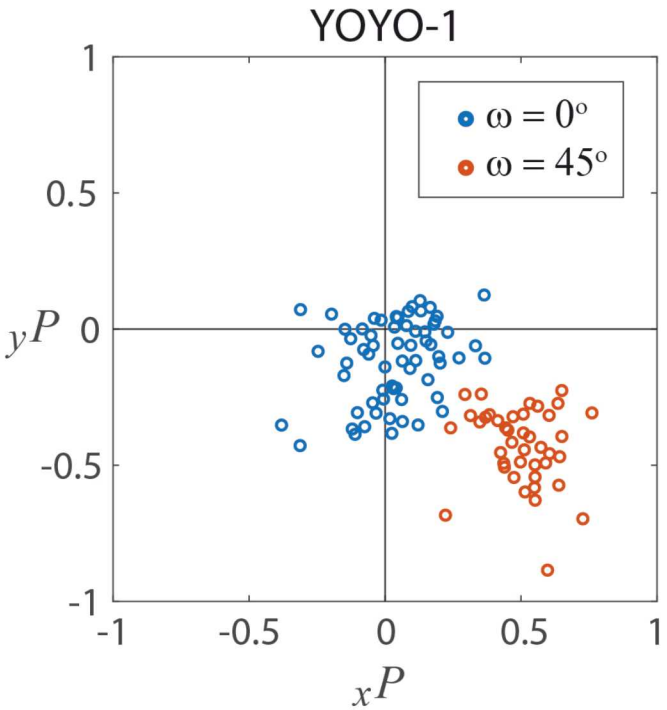
Single-intercalator “polarization switching” beyond the OST

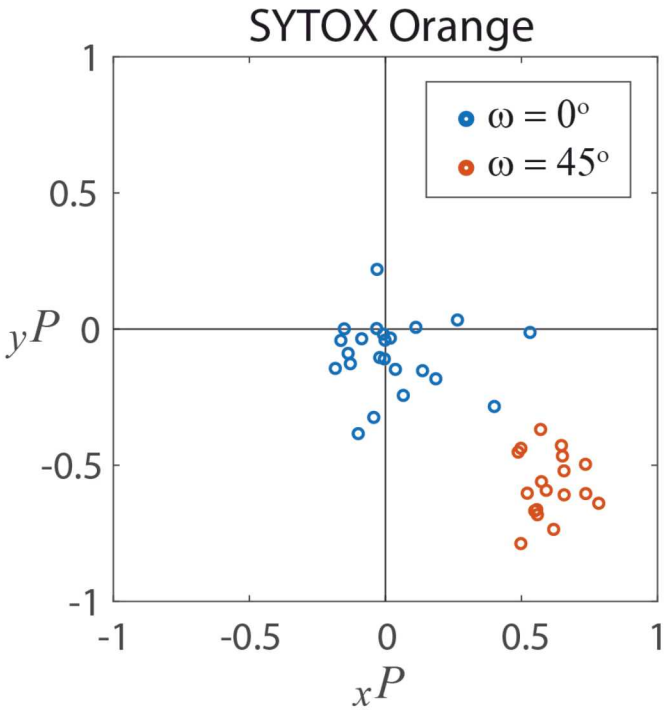


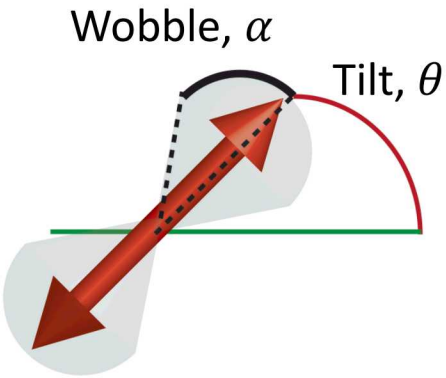
- Excitation polarization switches emission polarization **of a single dye molecule**

Single-intercalator "polarization switching" beyond the OST

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





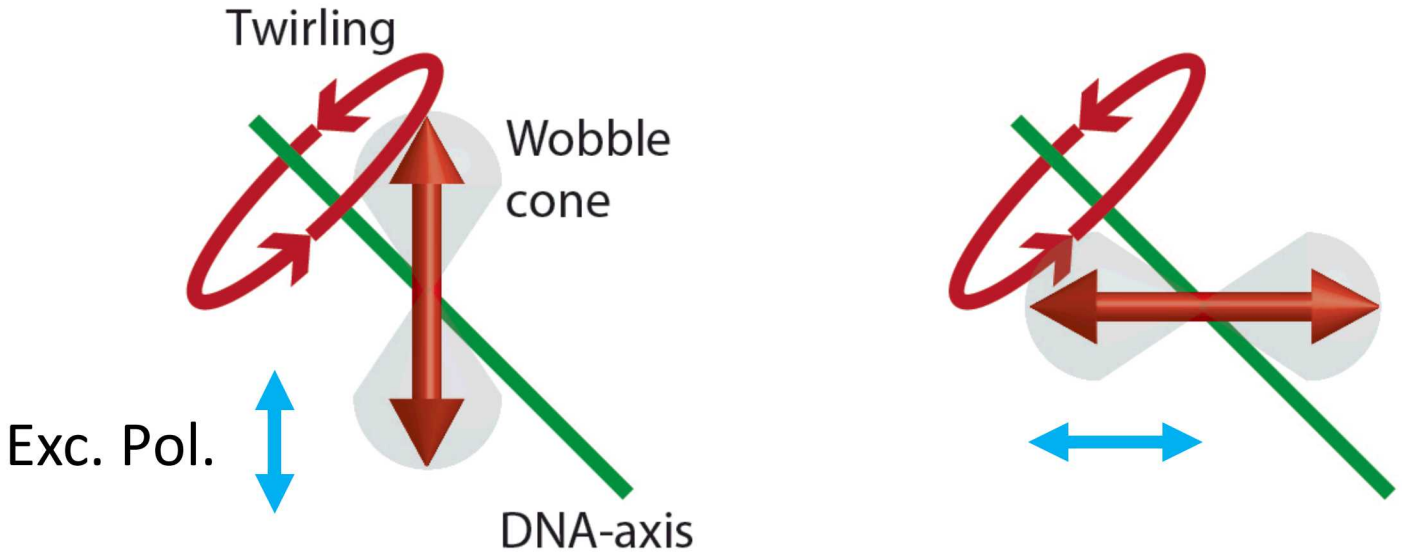


YOYO-1:
- $\theta = 53.9° \pm 0.5°$
- $\alpha = 29.8° \pm 1.1°$

SYTOX Orange:
- $\theta = 53.3° \pm 0.7°$
- $\alpha = 21.4° \pm 1.9°$

Intercalators Twirl

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



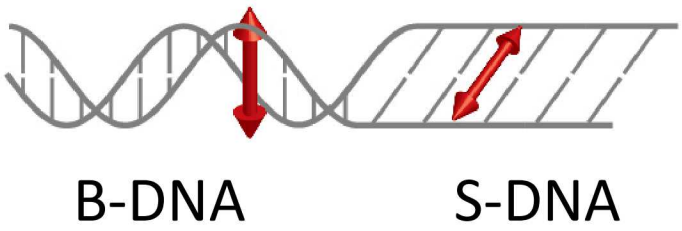
- Dipole twirling due to Brownian twisting of the DNA – rotational correlation time estimated as ~100 nsec*

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

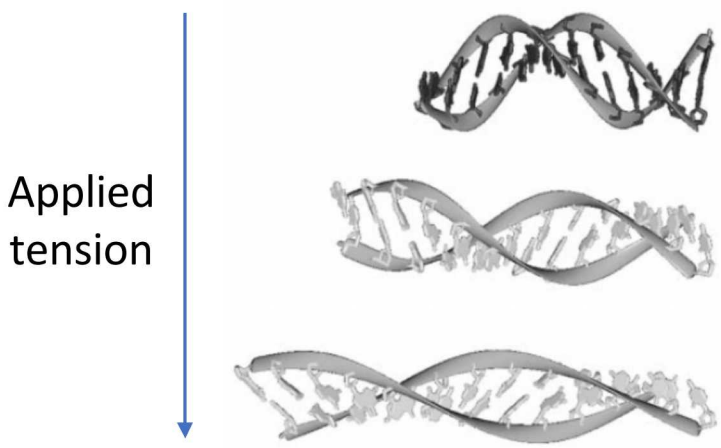
Also see: Yang et al., *Nano Lett.* **18**, (2018). (Rotational diffusion of DNA mixtures)

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

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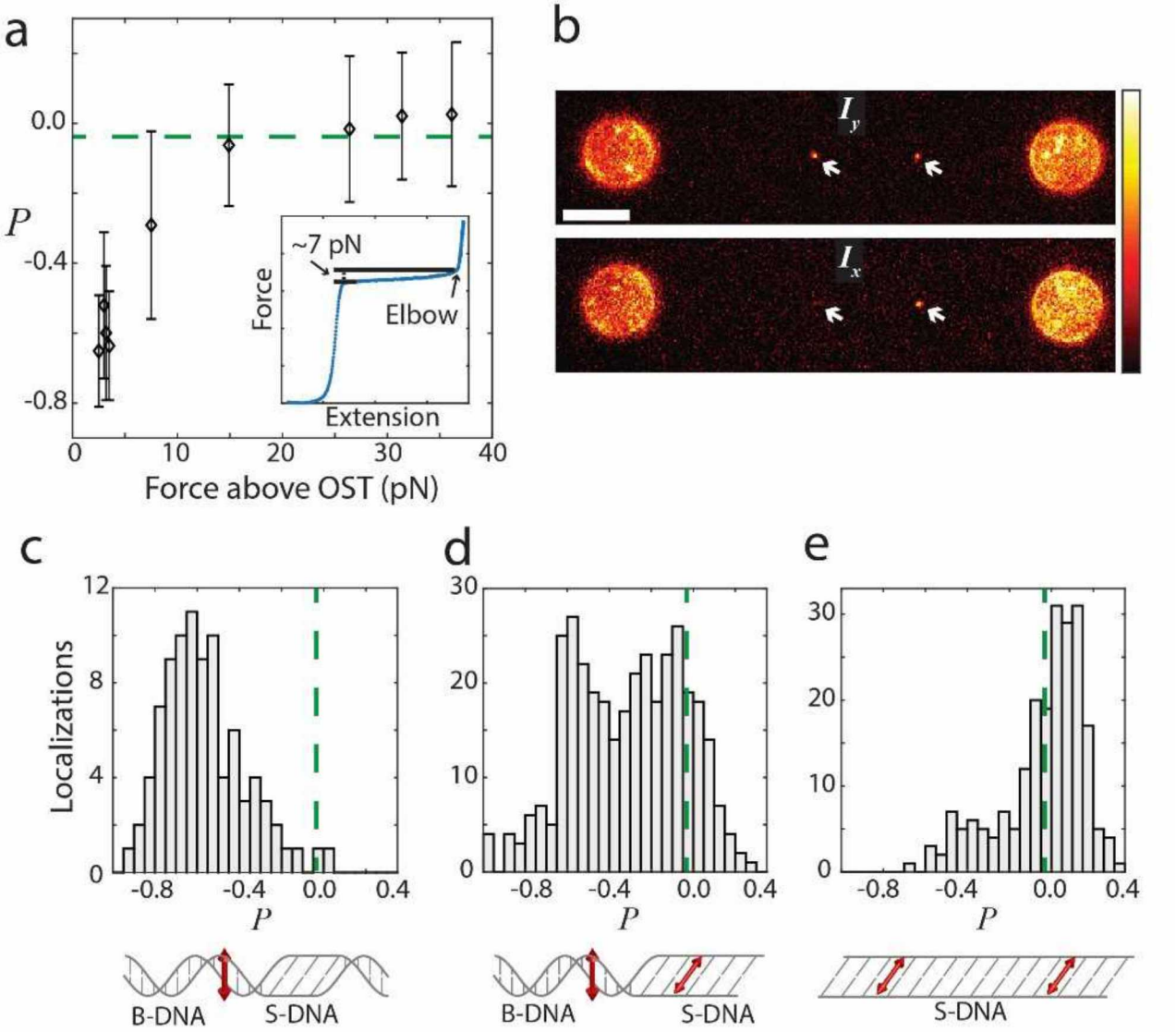
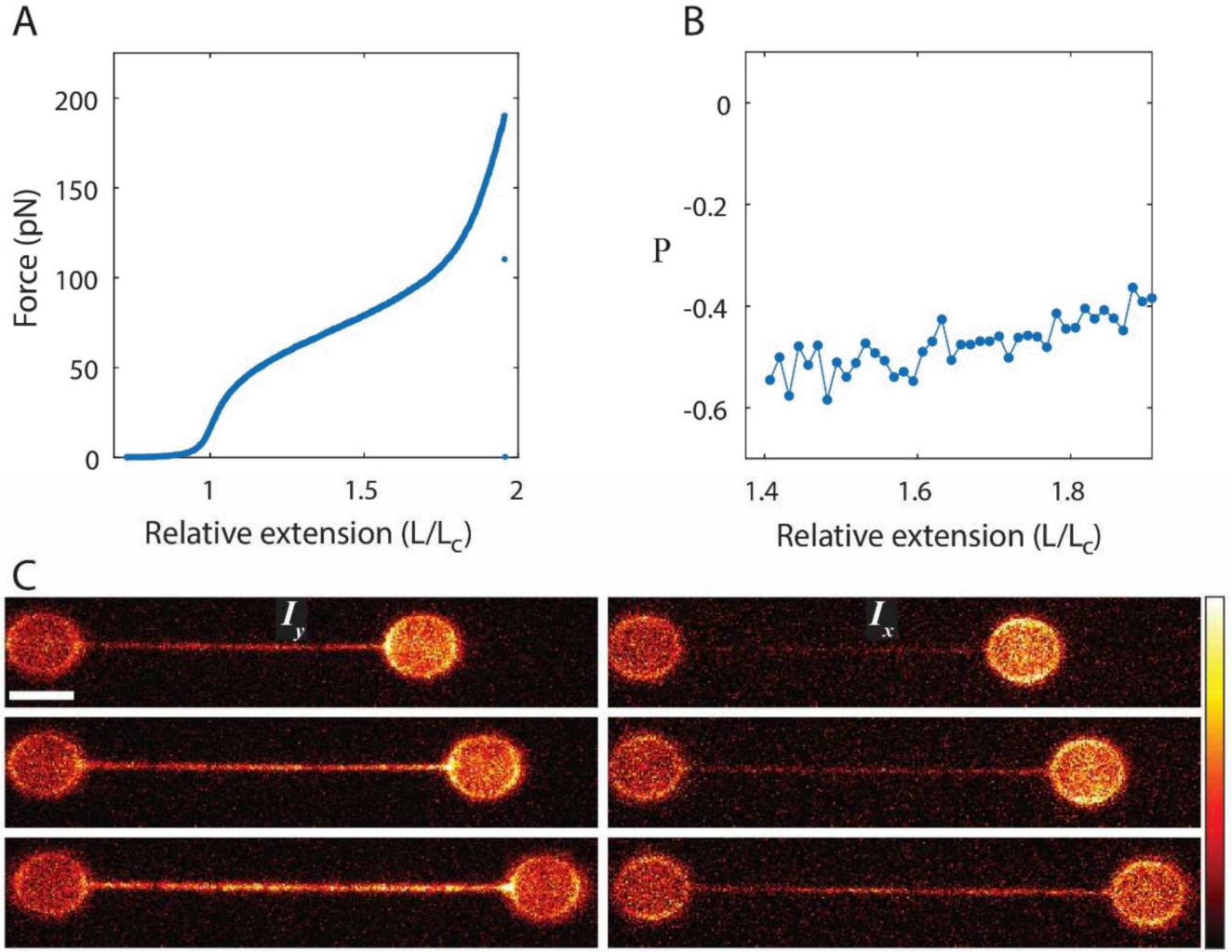
Intercalator tilting occurs abruptly at the end of the OST

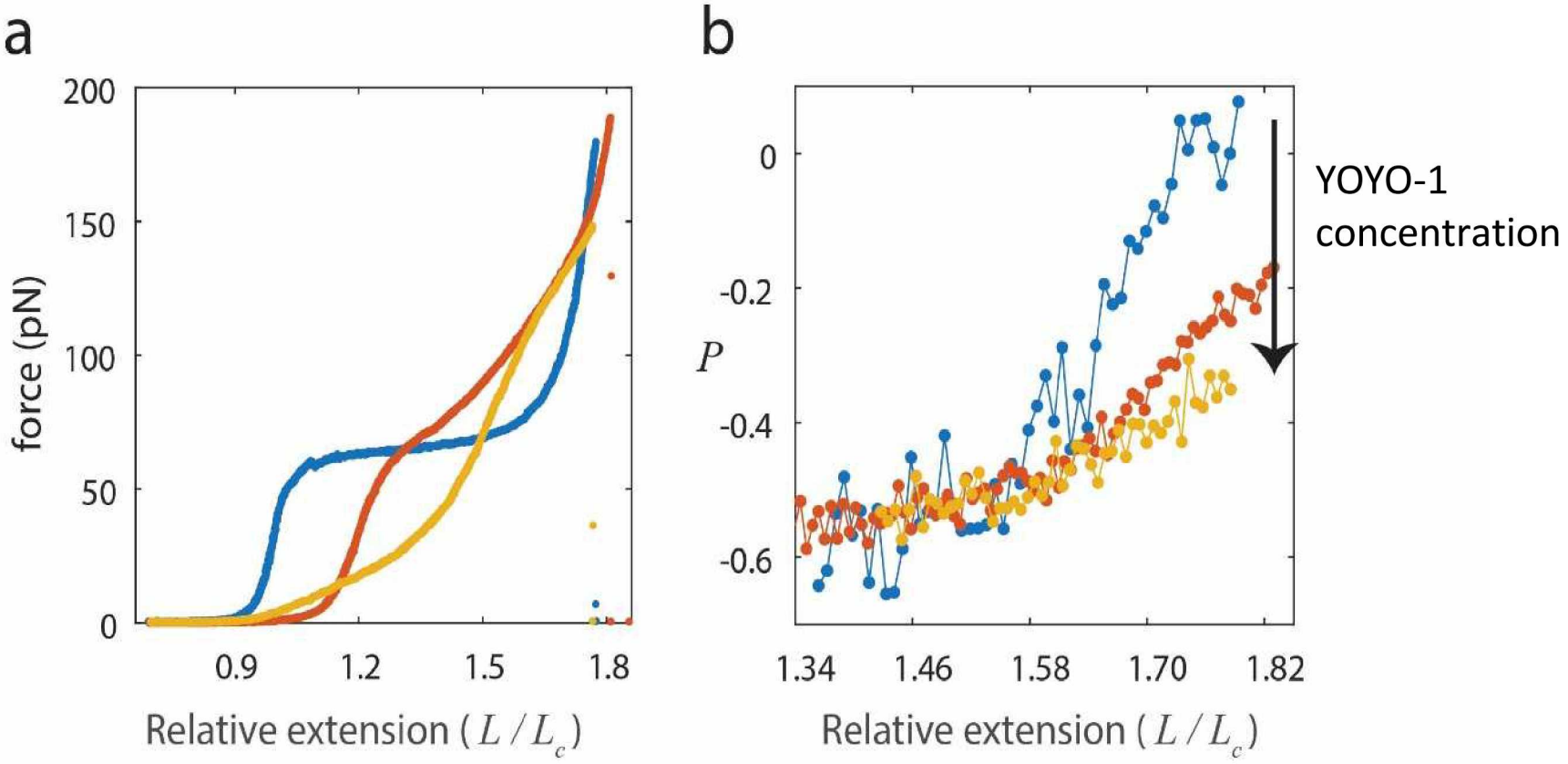


Less depolarization at higher dye concentration (Hyperstretched* YOPRO-labeled DNA)



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

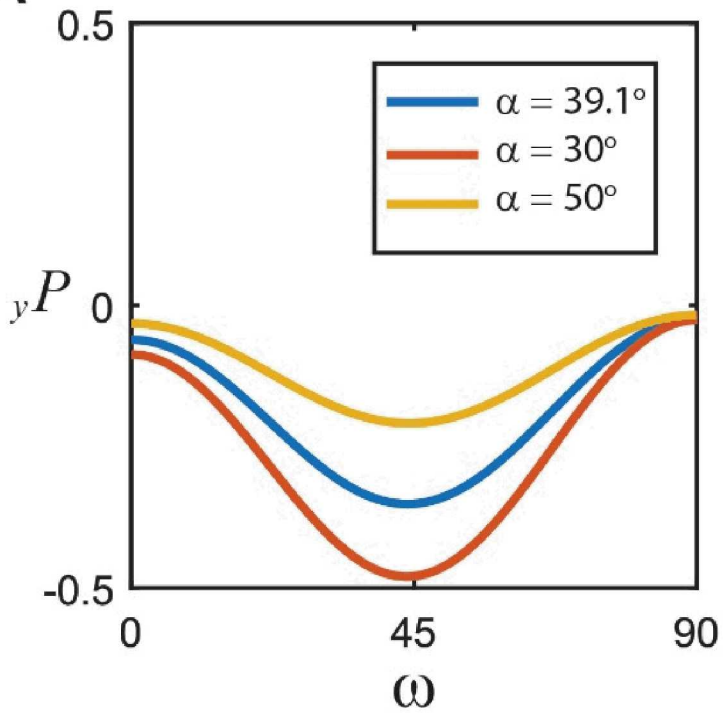
Less depolarization at higher dye concentration (YOYO-1-Labeled DNA, different concentrations)



Distinguishing wobble and tilt

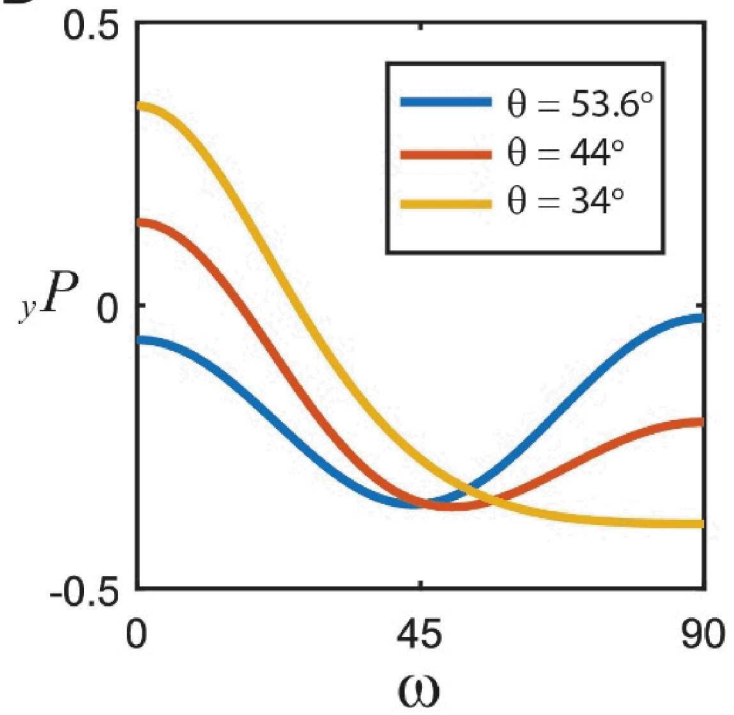
A

- Θ constant, α varied



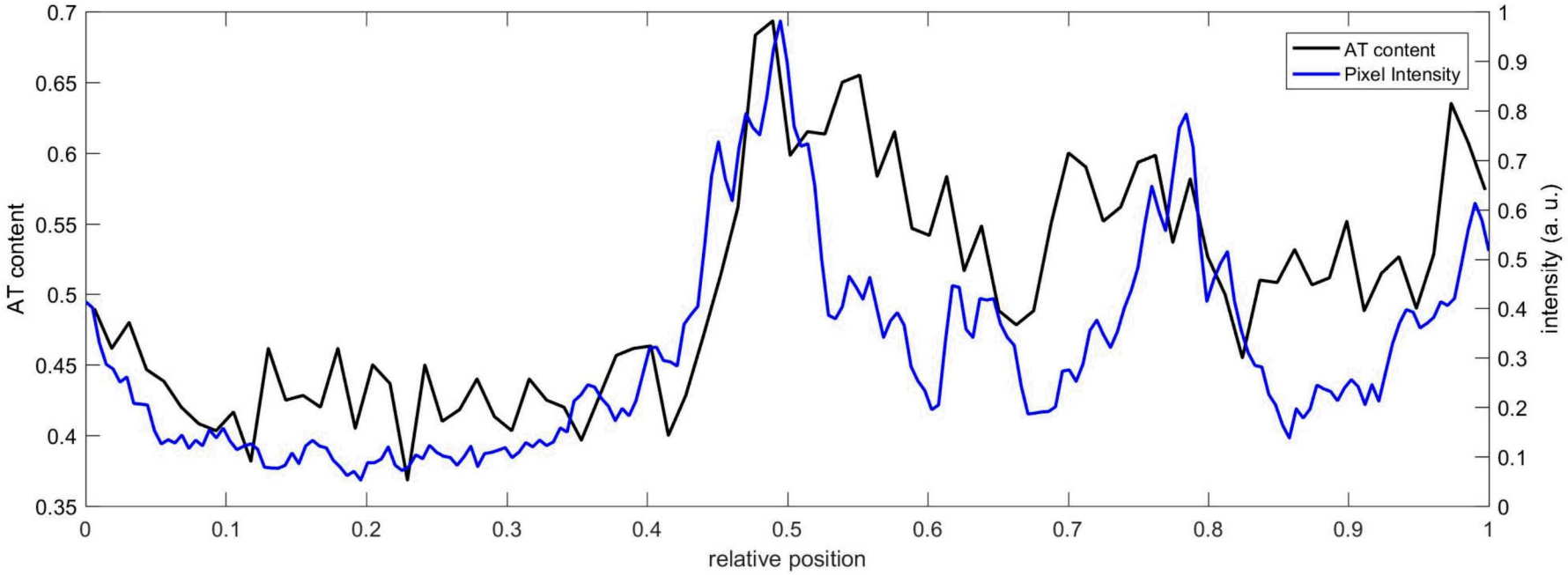
B

- Θ varied, α constant



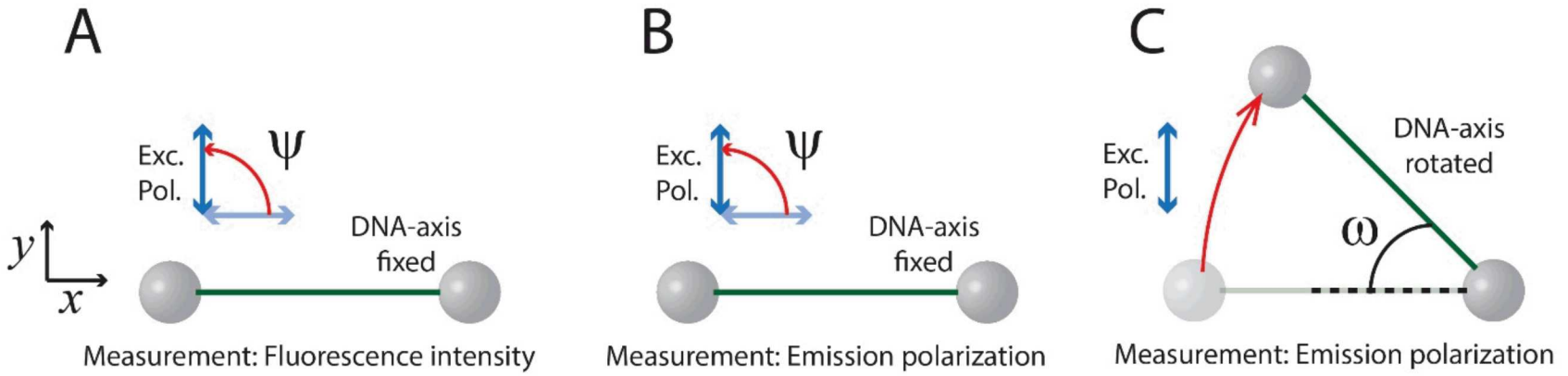
(Simulated polarization ratios)

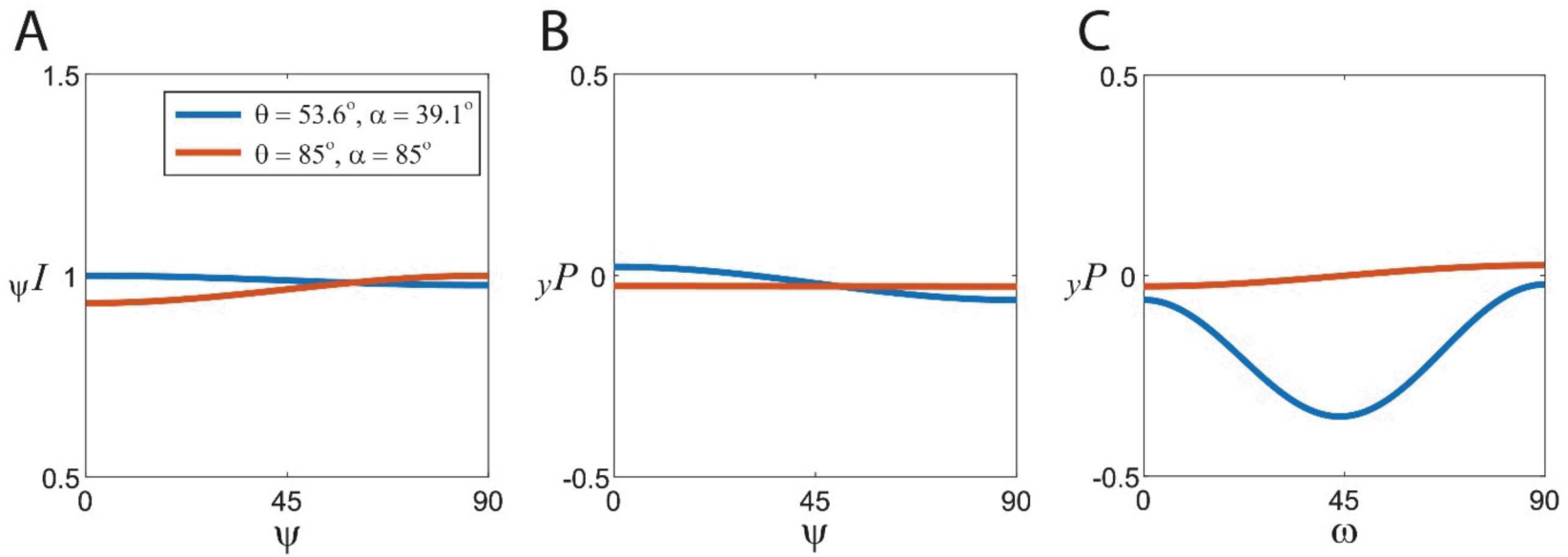
Sequence dependence



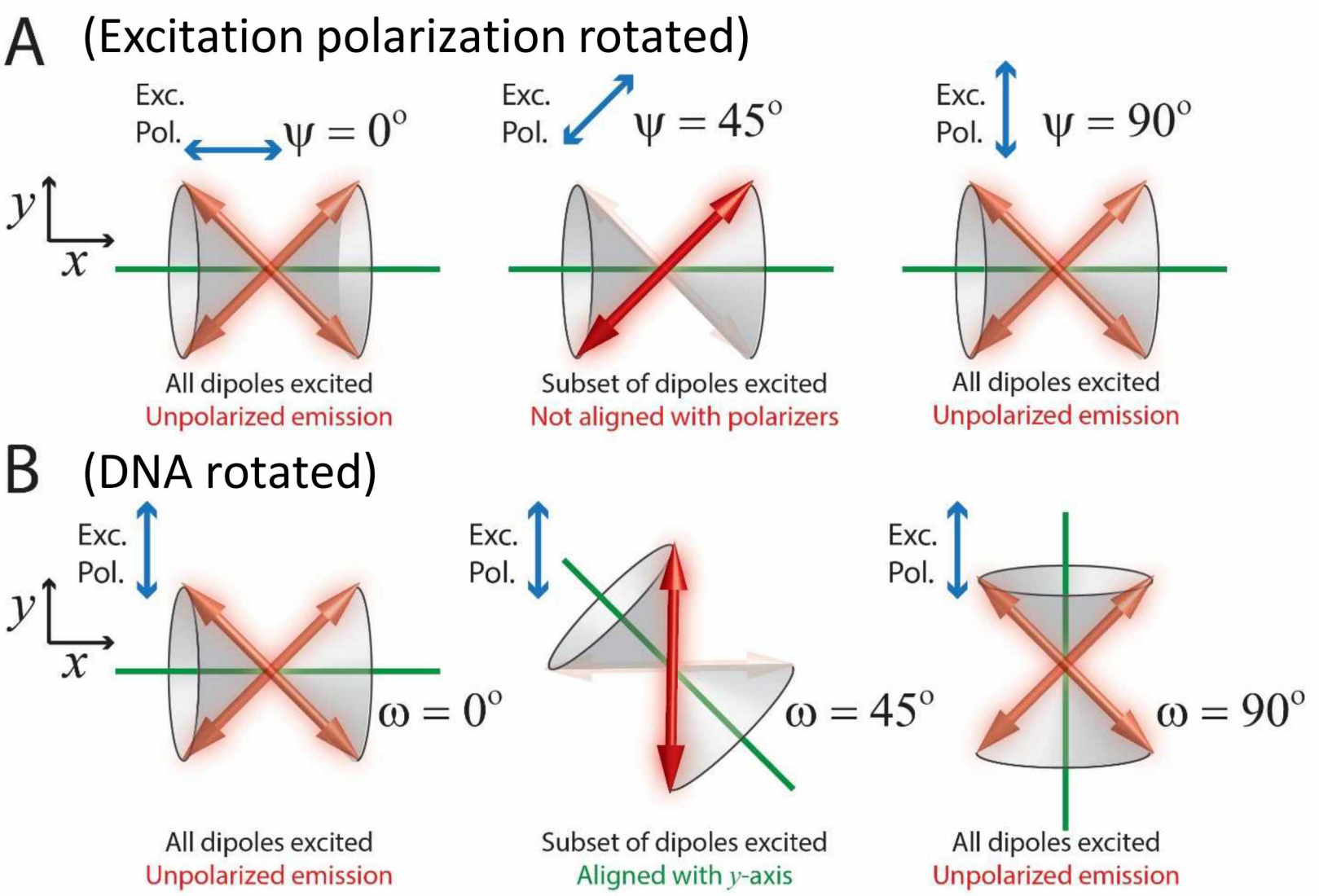
- Summed fluorescence intensity from single molecule binding events
- Dye prefers AT-rich regions of the lambda-DNA construct

Different experimental configurations considered:





Difference between rotating DNA vs. rotating excitation polarization



Effect of energy transfer on estimated tilt and wobble

Relative DNA extension: Within the OST (regime 2)

	θ	α
$\beta = 10°$	$85.4° \pm 3.9°$	$28.3° \pm 4.7°$
$\beta = 20°$	$85.3° \pm 3.6°$	$25.6° \pm 5.2°$
$\beta = 30°$	$85.2° \pm 3.2°$	$20.3° \pm 6.8°$

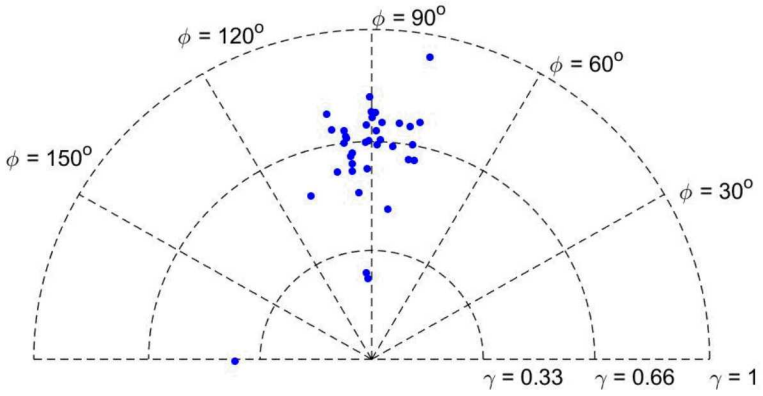
Relative DNA extension: Beyond the OST (regime 3)

	θ	α
$\beta = 10°$	$53.7° \pm 1.4°$	$38.7° \pm 1.9°$
$\beta = 20°$	$54.0° \pm 1.4°$	$37.4° \pm 2.0°$
$\beta = 30°$	$54.5° \pm 1.4°$	$35.0° \pm 2.2°$

Excitation polarization modulation

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

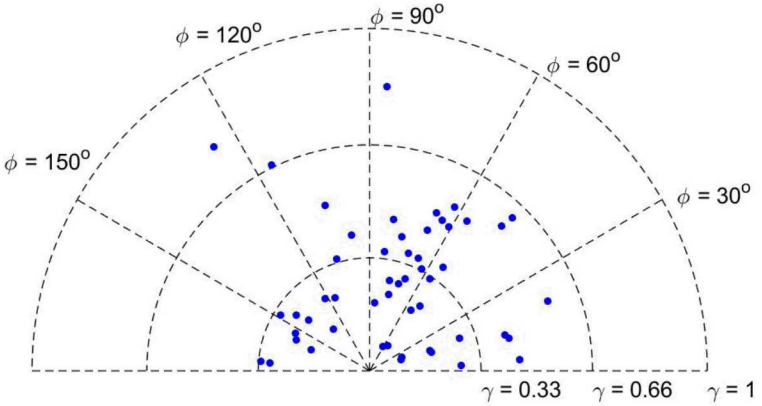
L/Lc ~ 1.3



$\hat{\gamma} = 0.64 \pm 0.13$

$\hat{\phi} = 88.3° \pm 16.4°$

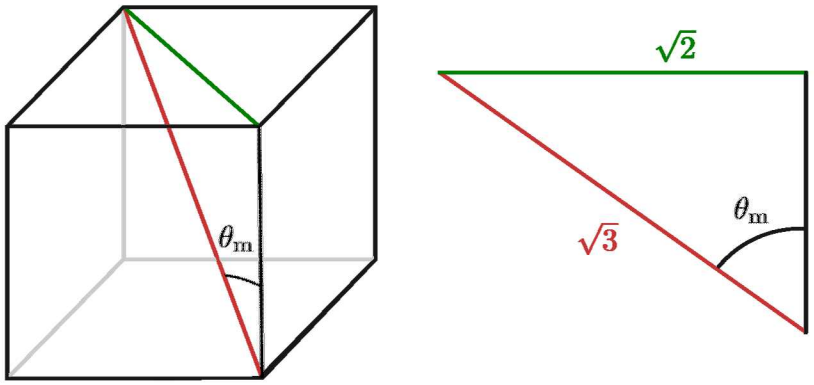
L/Lc > 1.7

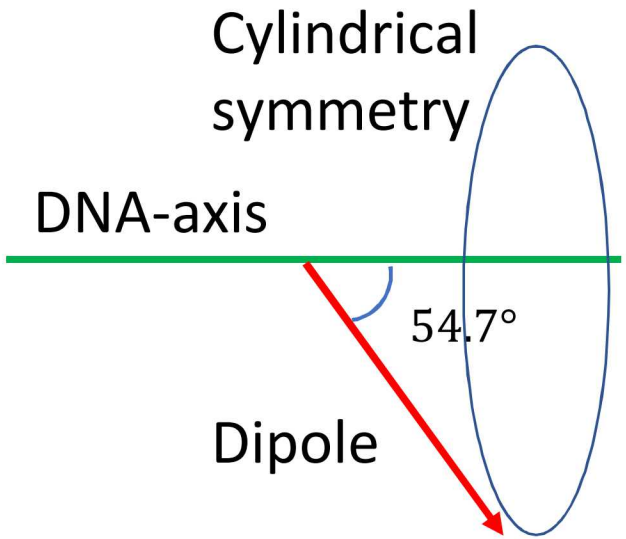


$\hat{\gamma} = 0.35 \pm 0.17$

$\hat{\phi} = 77.6° \pm 46.3°$

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





- 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**