Insulator-Based Dielectrophoretic Manipulation of DNA in a Microfluidic Device

Lin Gan

07/17/2015

Motivation

Photo credit: (1) commons.wikimedia.org (2) h-a-y-s-t-a-c-k.net (3) joshmayo.com

Motivation

Requirements in separation technique

- Low sample volume
- Rapid
- Compatible with analysis methods
- Easy to produce, low cost

Dielectrophoresis (DEP)

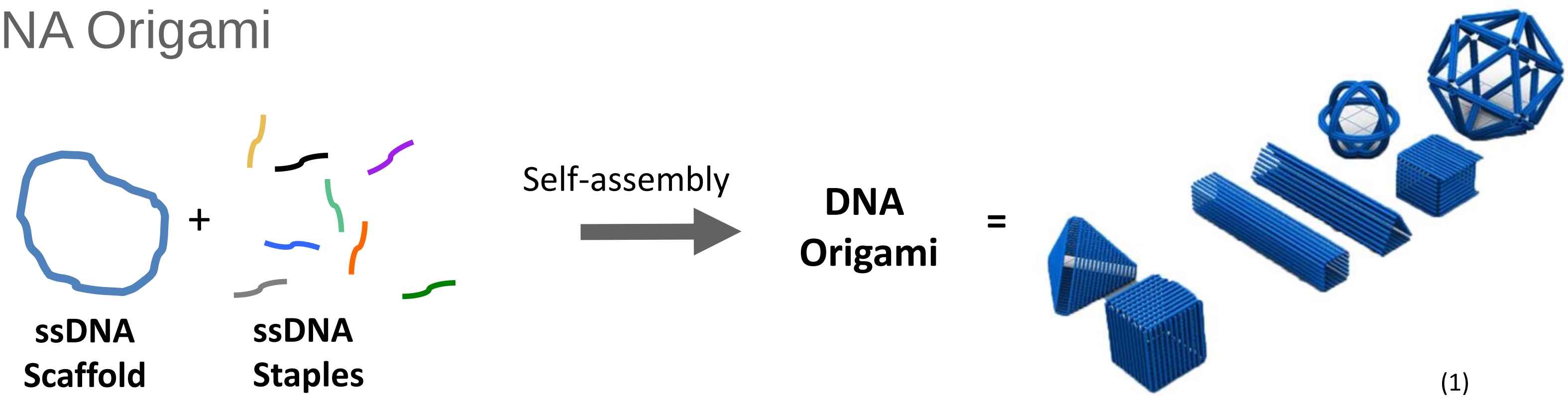
- µL, pM
- Within 1 hour
- Gel free, label free, orthogonal analysis
- Photolithography

Motivation

DNA

Structure known - complementary base pair, conductivity obtained

DNA Origami



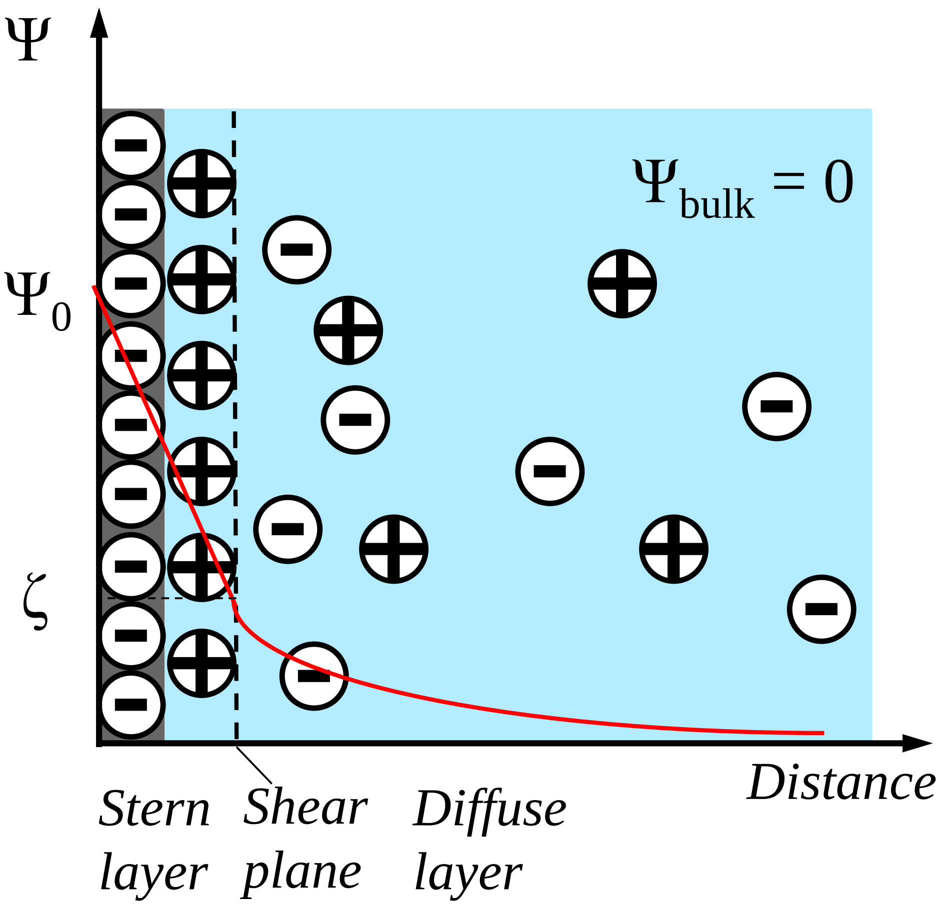
Long ssDNA strand directed by short ssDNA strands to form desired shapes

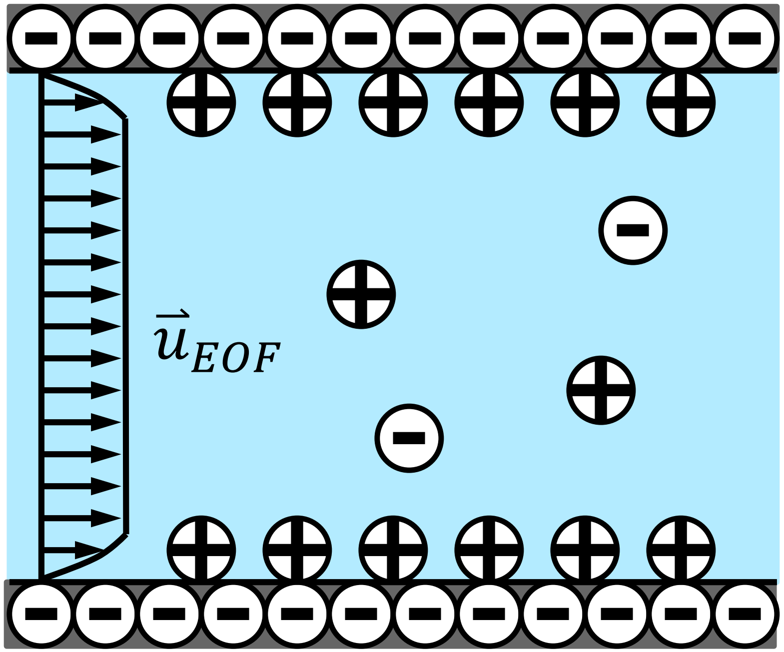
(1) Shih, et al. Struc. Biol. *(*2010);

Outline

- Background
- Device and experimental setup
- Projects
    - DEP manipulation of DNA origamis
    - Polarizability determination of DNA origami
    - Effect of buffer valency in DEP trapping

Background : Electric Double Layer (EDL) and Electroosmosis (EOF)



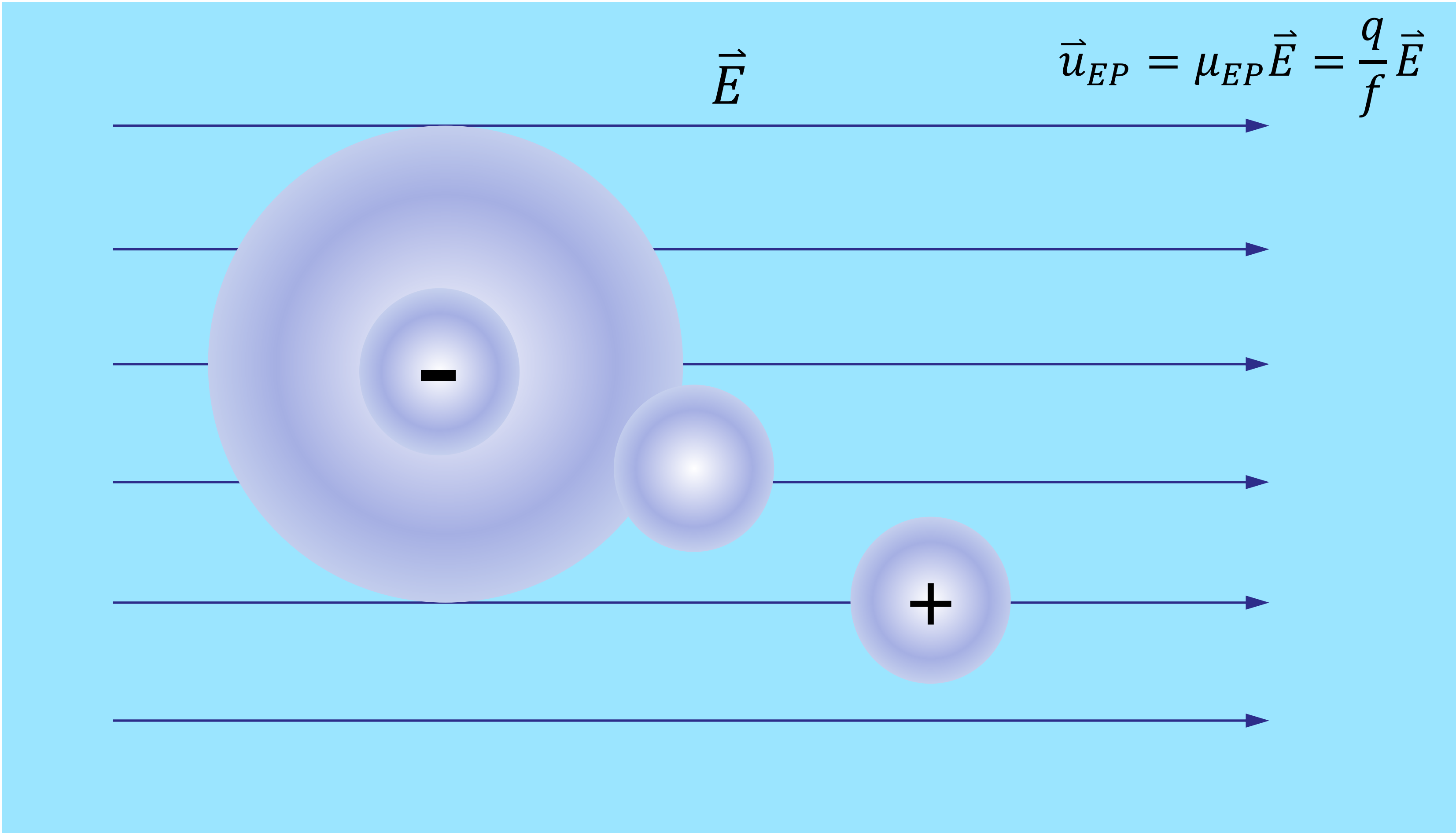


$$\vec{u}_{EOF} = \mu_{EOF}\vec{E}$$

$\vec{E}$ - electric field μ_{EOF} - EOF mobility $\vec{u}_{EOF}$ - EOF velocity

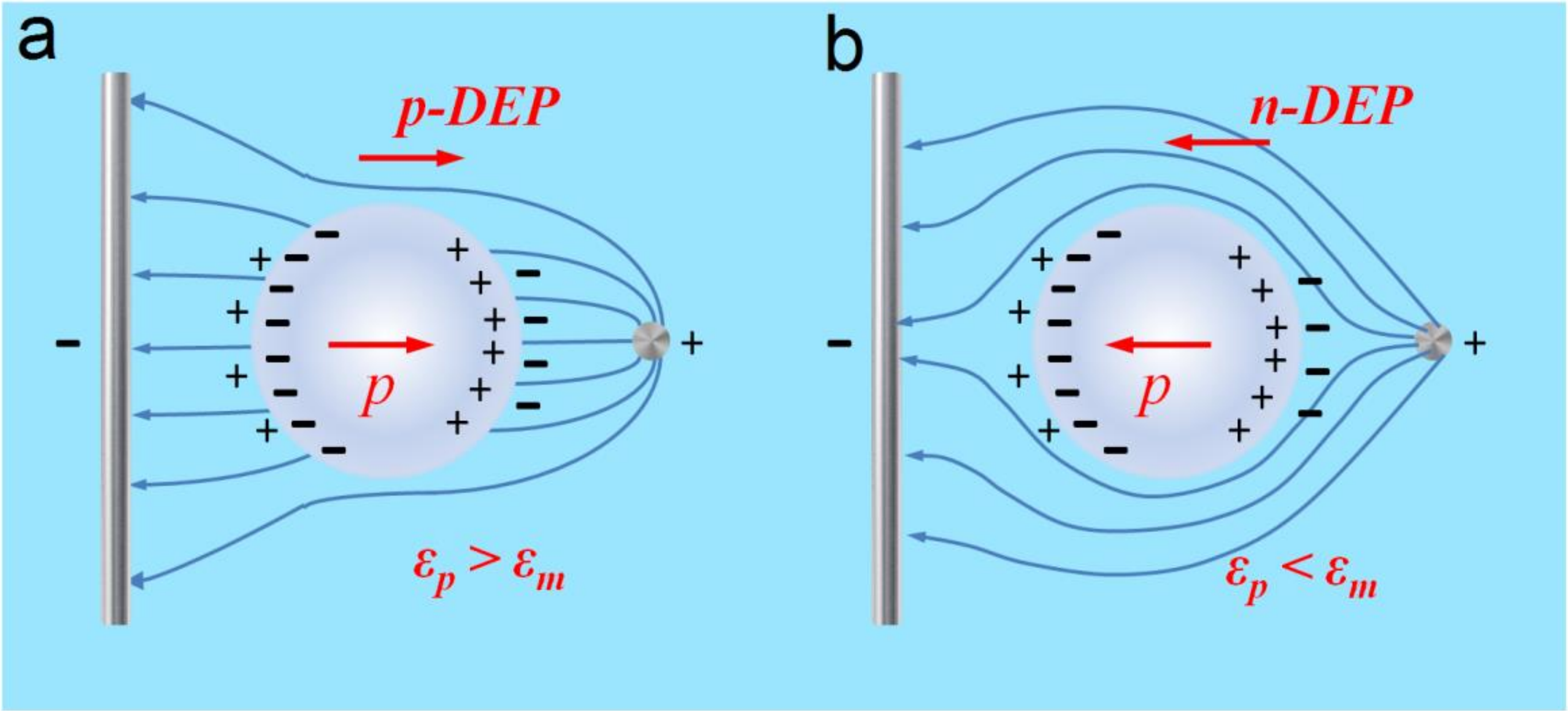
Background : Electrophoresis

Electrophoresis is the movement of dispersed charged particles relative to the surrounding liquid medium under the influence of a spatially uniform electric field



Background : Dielectrophoresis

Dielectrophoresis: The movement of particles in non-uniform electric field.



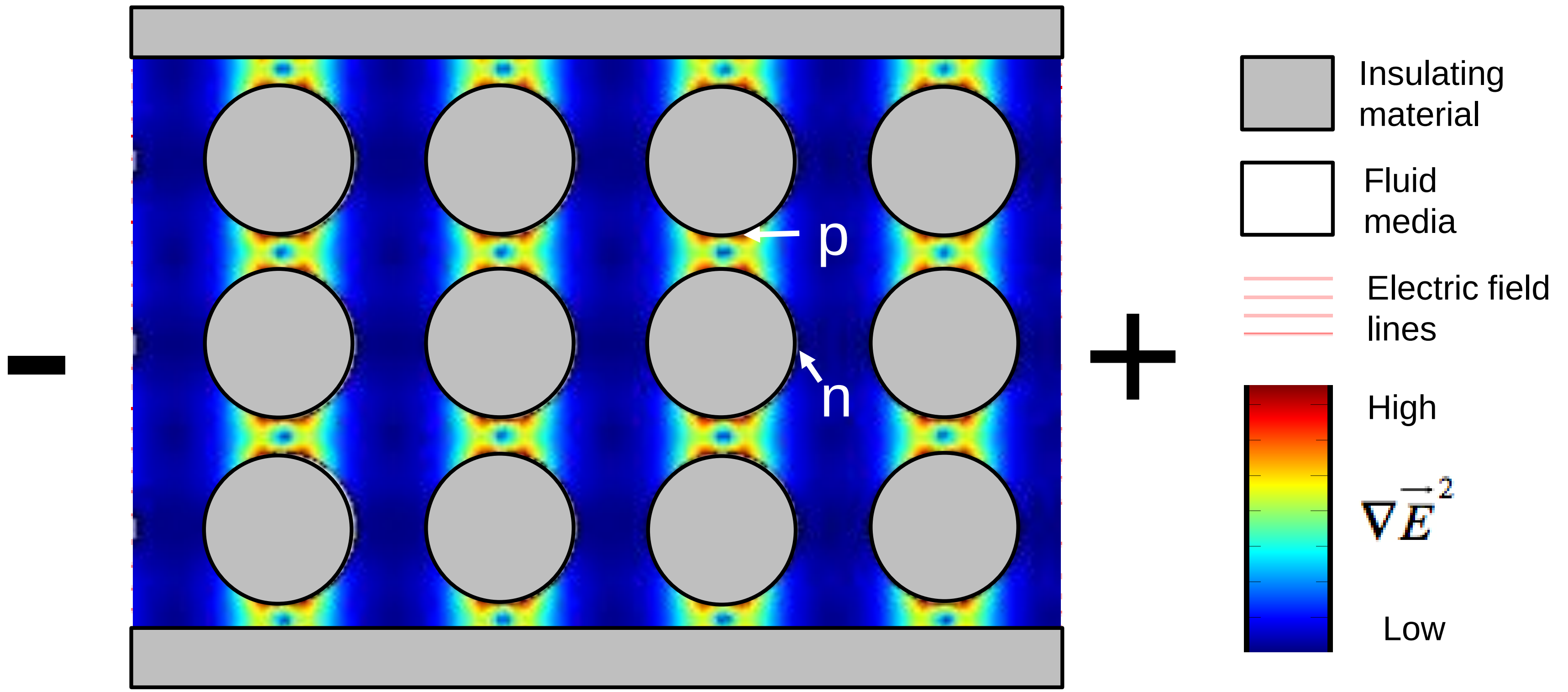
$$\vec{F}_{DEP} = \frac{1}{2}\alpha \nabla \vec{E}^2 \qquad \vec{u}_{DEP} = -\mu_{DEP} \nabla \vec{E}^2$$

α – polarizability, depend on size, shape, conductivity of particle and medium frequency of applied electric field.

Background : Dielectrophoresis

- Generating non-uniform electric field
- insulator-based DEP (iDEP)

Place insulating structures (obstacles) between a pair of electrodes



Background : Dielectrophoresis

Summary of models for DNA for DEP

Short DNA (< 150 bp) ⟶ Stiff rod(1)

Long DNA (>> 150 bp) ⟶ Coiled – sphere(2)

Maxwell-Wagner-O'Konski (MWO) Theory(3)

- Consider polarization occurs due to migration and convection of ions in electric double layer (EDL)
- Suitable for low frequency, thin EDL

Dukhin-Shilov(DS) Theory(3)

- Diffusion layer also affects polarization
- Suitable for high frequency, thin EDL

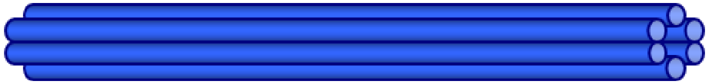
Poisson-Nerst-Plank (PNP) Theory(1, 2)

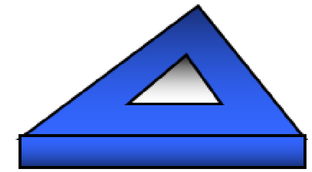
- Suitable for high and low frequency, thick EDL

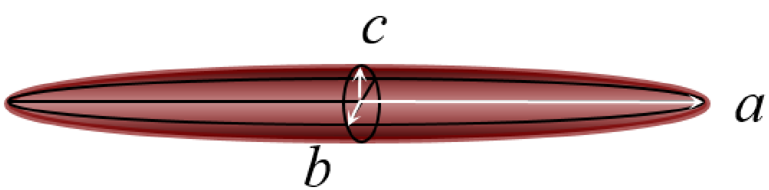
DEP mechanism is still unclear

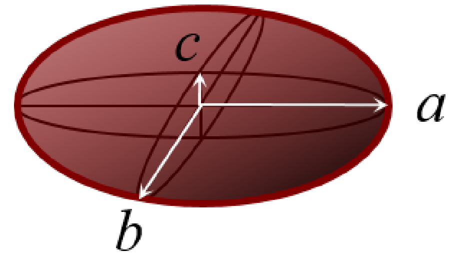
(1) Zhao et al. Langmuir(2008); (2) Zhao et al. Phy. Rev. E (2011); (3) Ermolina et al. J of Colloid & Interface (2005);

DNA Origami and Polarizability Prediction









$$\vec{F}_{DEP} = \frac{1}{2}\alpha\nabla\vec{E}^2$$

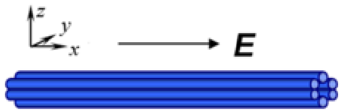
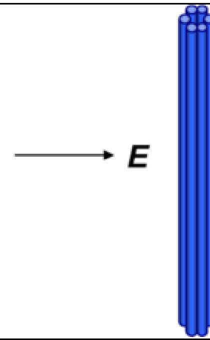
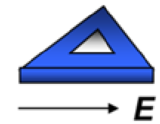
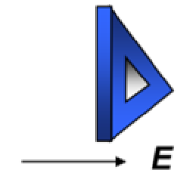
$$\alpha = \frac{8}{3}\pi abc\varepsilon_m \frac{\sigma_p - \sigma_m}{Z\sigma_p + (1 - Z)\sigma_m}$$

$$\mu_{DEP} = \frac{\alpha}{2f}$$

$$f = 6\pi\eta\frac{2}{S}$$

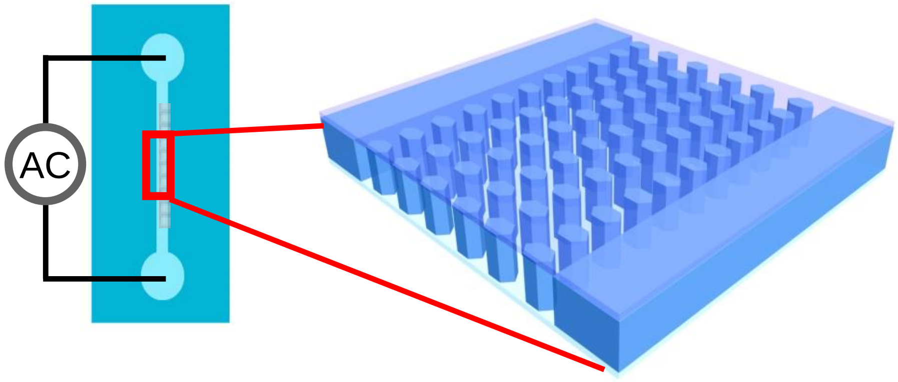
σ	conductivity p particle m medium
Z	depolarization factor
f	friction coefficient
η	viscosity of medium
$\frac{2}{S}$	mean transitional coefficient
a, b, c	dimensions of the particle

DNA Origami and Polarizability Prediction

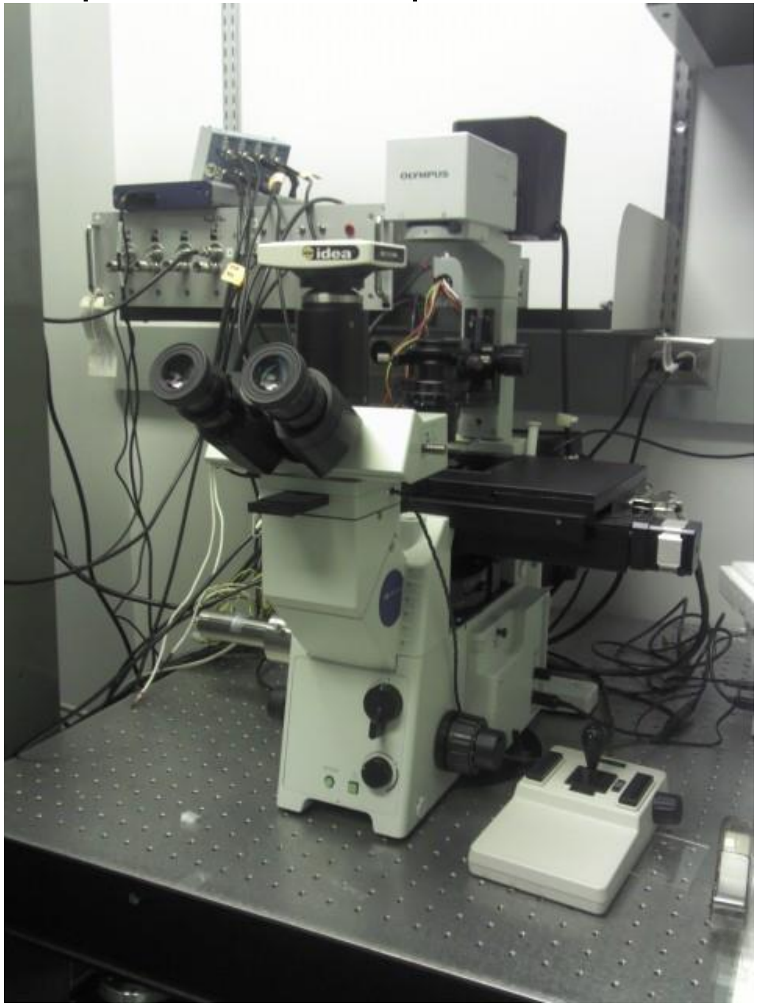
	6HxB (∥)	6HxB (⊥)	Triangle (∥)	Triangle (⊥)
Shape				
Z	$\frac{bc}{2a^2e^3}\left[ln\left(\frac{1+e}{1-e}\right)-2e\right]$ $e=\sqrt{1-\frac{bc}{a^2}}$	$\frac{1}{1-\gamma^2}-\frac{\gamma^{-2}}{4(1-\gamma^{-2})^{-1.5}}\ln\left[\frac{1+(1-\gamma^{-2})^{0.5}}{1-(1-\gamma^{-2})^{0.5}}\right]$ $\gamma=\frac{c}{a}$	$\left(-\frac{\gamma^2}{2M}\right)+\left(\frac{\pi\gamma}{4M^{1.5}}\right)-\left(\frac{\gamma}{2M^{1.5}}\right)\arctan\left(\frac{\gamma^2}{M}\right)$ $M=1-\gamma^2$	$\left(\frac{1}{M}\right)+\left(\frac{\pi\gamma}{2M^{1.5}}\right)-\left(\frac{\gamma}{M^{1.5}}\right)\arctan\left(\frac{\gamma^2}{M}\right)^{0.5}$
S	$S=2\frac{1}{\sqrt{a^2-b^2}}\ln\frac{a+\sqrt{a^2-b^2}}{b}$		$S=\frac{2}{\sqrt{a^2-c^2}}\tan^{-1}\frac{\sqrt{a^2-c^2}}{c}$	
$\alpha\,(F\cdot m^2)$	2.603×10^{-30}	0.014×10^{-30}	3.473×10^{-30}	0.045×10^{-30}
$f\ (kg\cdot s^{-1})$	7.064×10^{-9}	1.528×10^{-9}	1.048×10^{-9}	1.557×10^{-9}
$\mu_{DEP}\ (m^4\cdot V^{-2}\cdot s^{-1})$	1.704×10^{-21}	0.004×10^{-21}	1.657×10^{-21}	0.015×10^{-21}

Trapping Device Set-up

Device



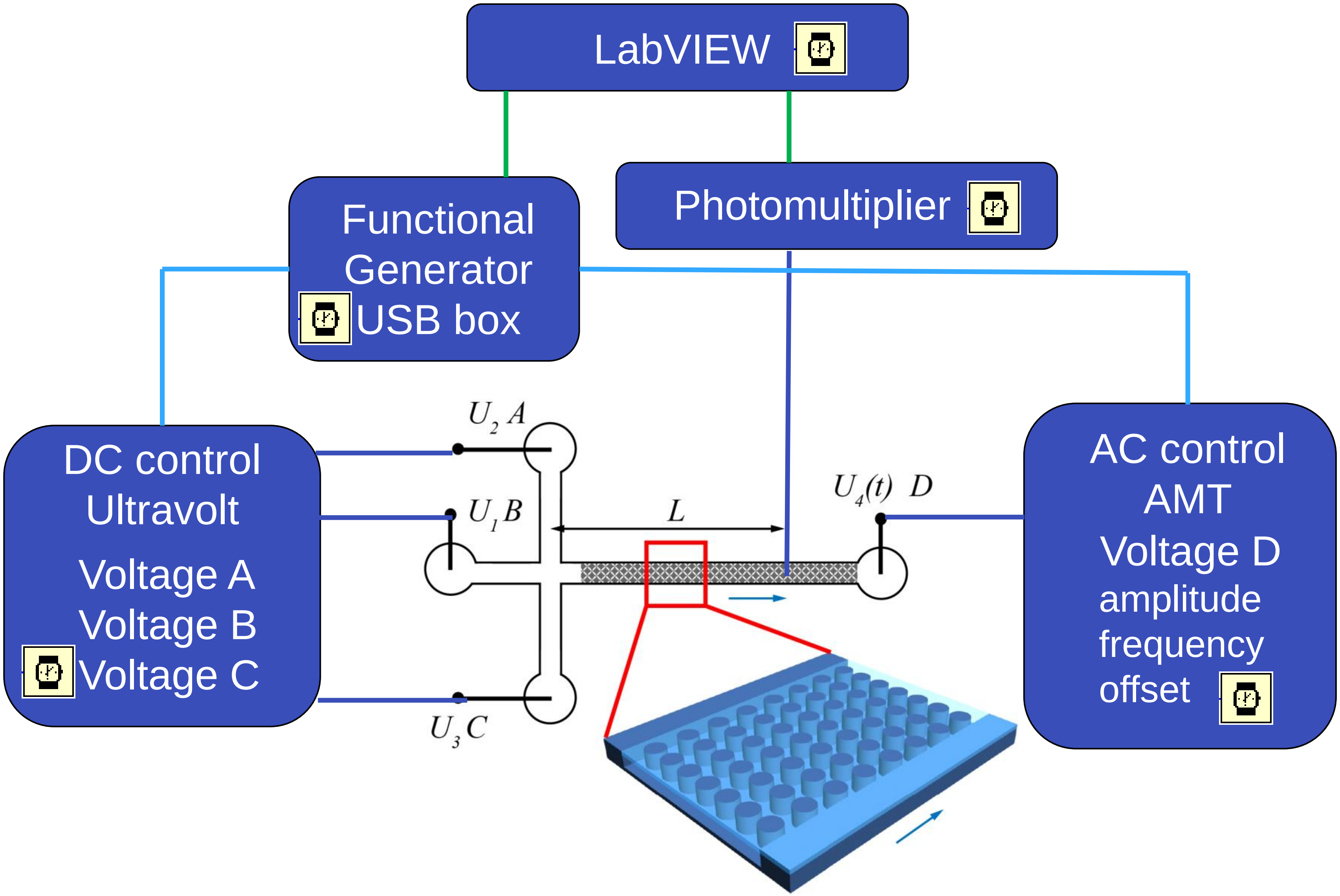
Experimental setup



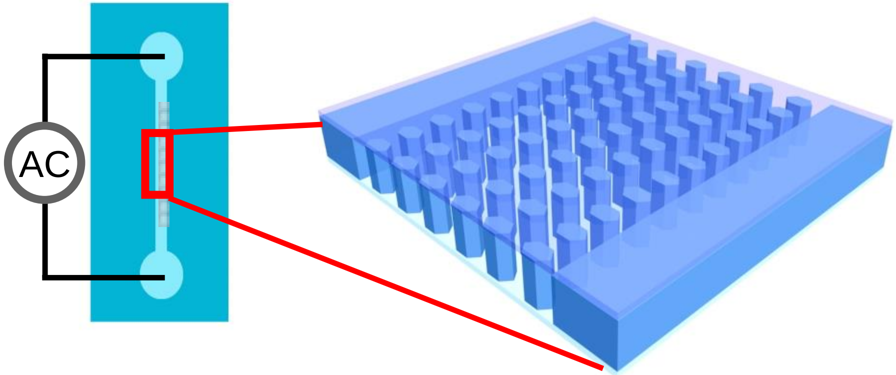
Fluorescence Video Microscope and microdevice.

DNA is labeled with YOYO-1 (λ-Max_{Ex} = 491 nm, λ-Max_{Em} = 509 nm)

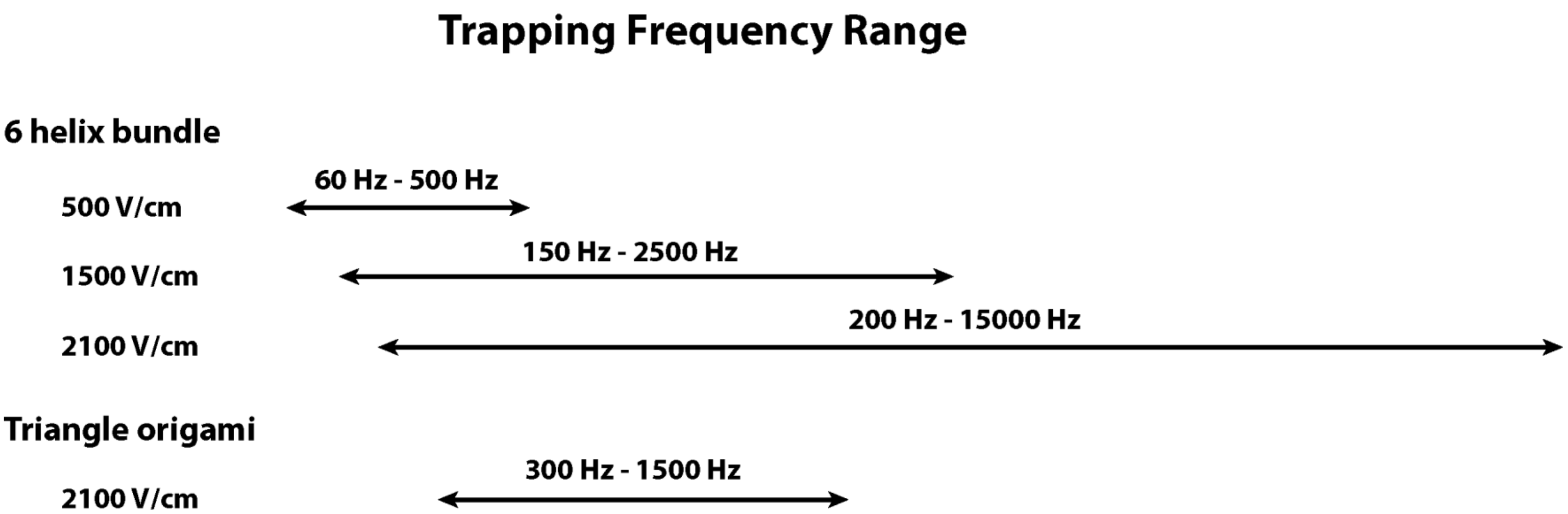
Determination of Polarizability Device Set-up



- Projects
  - DEP manipulation of DNA origamis
  - Polarizability determination of DNA origami
  - Effect of buffer valency in DEP trapping

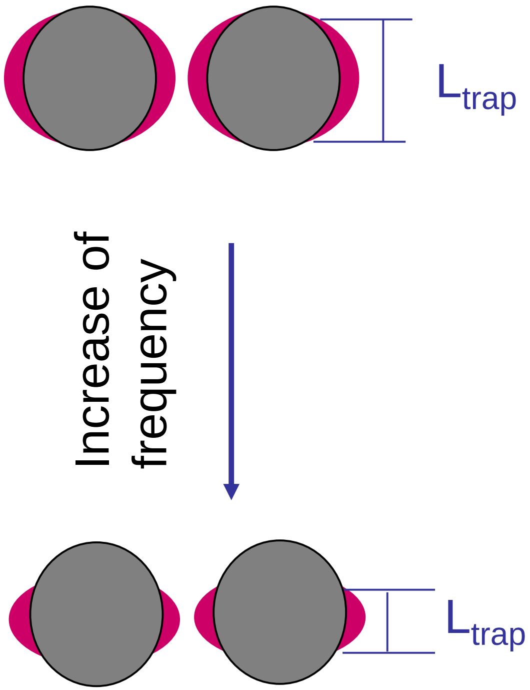


Origami Trapping - Frequency Dependence



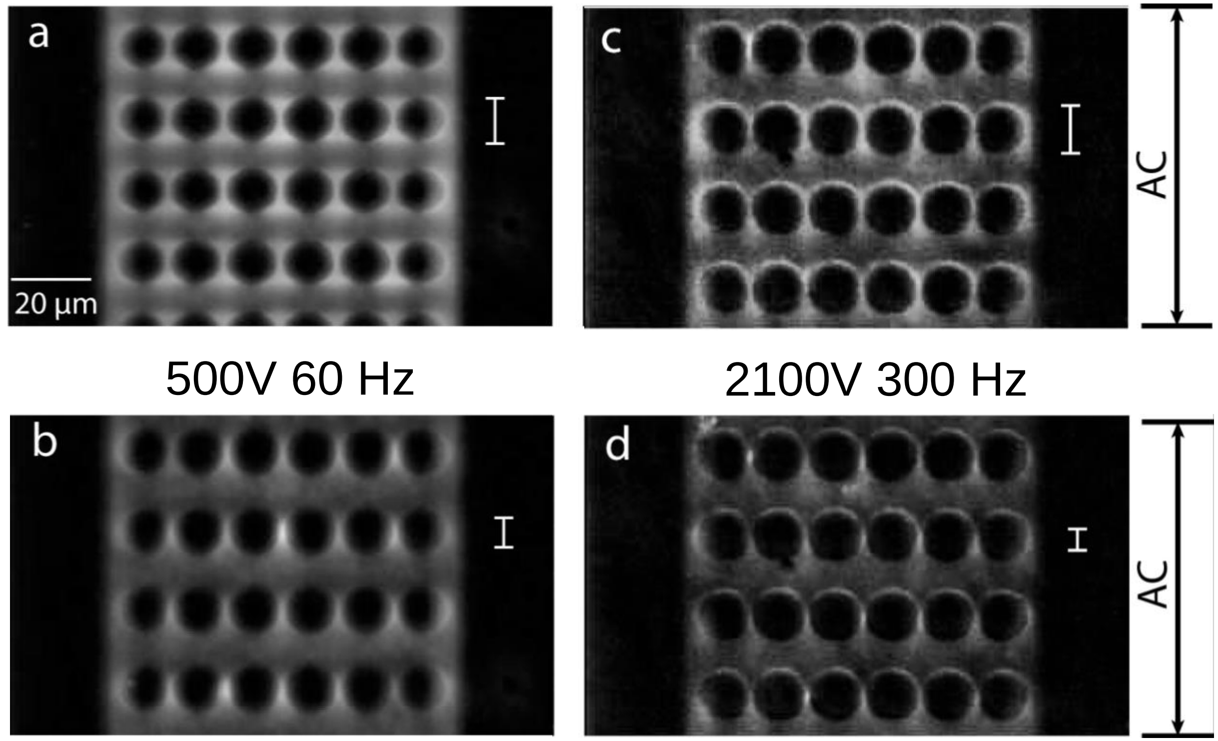
(1) Gan et al. Anal. Chem. (2013)

Origami Trapping - Frequency Dependence



6 HxB Trapping

Triangle Origami Trapping



500V 60 Hz

2100V 300 Hz

500V 400 Hz

2100V 1000 Hz

(1) Gan et al. Anal. Chem. (2013)

Simulation

Convection-diffusion model:

Flux:

$$\vec{j} = -D\nabla c + c(\vec{u}_{EP} + \vec{u}_{EOF} + \vec{u}_{DEP})$$

Steady state:

$$\frac{\partial c}{\partial t} = \nabla \vec{j} = 0$$

$$\vec{F}_{DEP} = \vec{F}_{drag}$$

$$\vec{u}_{DEP} = \frac{\vec{F}_{DEP}}{f} = \alpha \nabla \vec{E}^2 / 2f$$

For an ellipsoid particle,

$$f = 6\pi\eta \frac{2}{S}$$

Take 6Hxb as an example,
Assuming it's parallel to the electric field

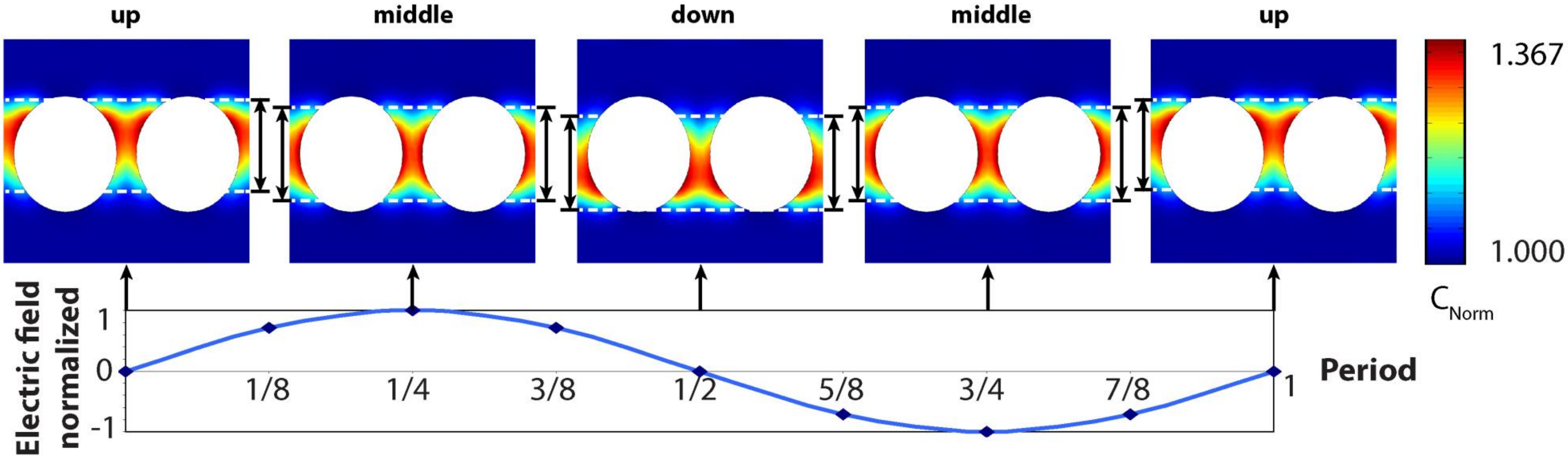
$$S = \frac{2}{\sqrt{a^2 - b^2}} ln \frac{a + \sqrt{a^2 - b^2}}{b}$$

Parameters

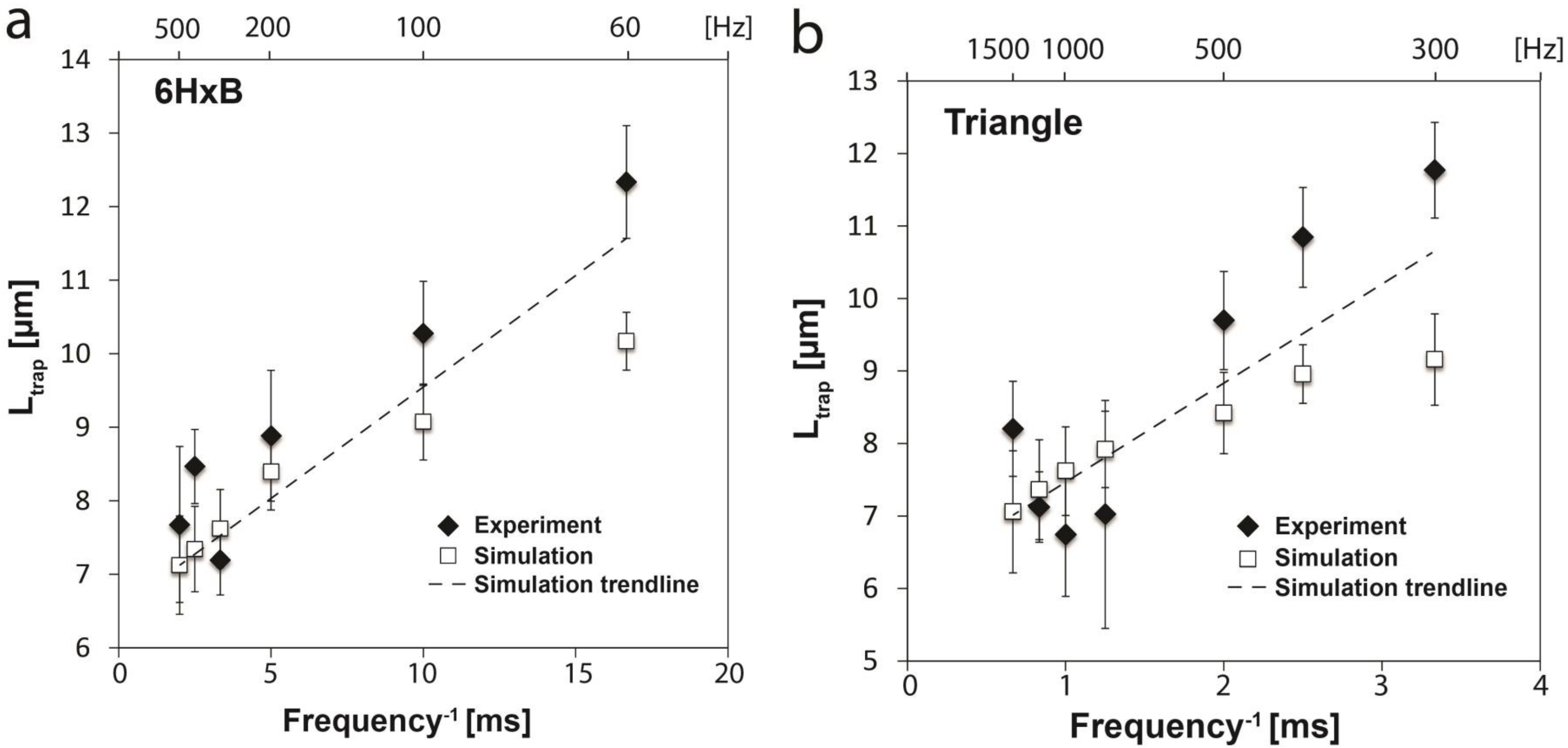
D	3.951 x 10^{-12} m^2/s	diffusion coefficient
f	7.649 x 10^{-10} kg/s	friction factor
μ_{DEP}	2.831 x 10^{-22} $m^4 / (V^2 s)$	DEP mobility
μ_{EP}	3.5 x 10^{-8} $m^2 / (Vs)$	EP mobility(1)
μ_{EOF}	2.2 x 10^{-8} $m^2 / (Vs)$	EOF mobility

(1) Regtmeier et al.Anal. Chem (2007)

Numerical Study – Time dependant concentration profiles



Trapping Distance comparison



$$L_{trap} = \mu_{EP}\boldsymbol{E}t_{half}$$

(1) Gan et al. Anal. Chem. (2013)

Experimental Determination of Polarizability

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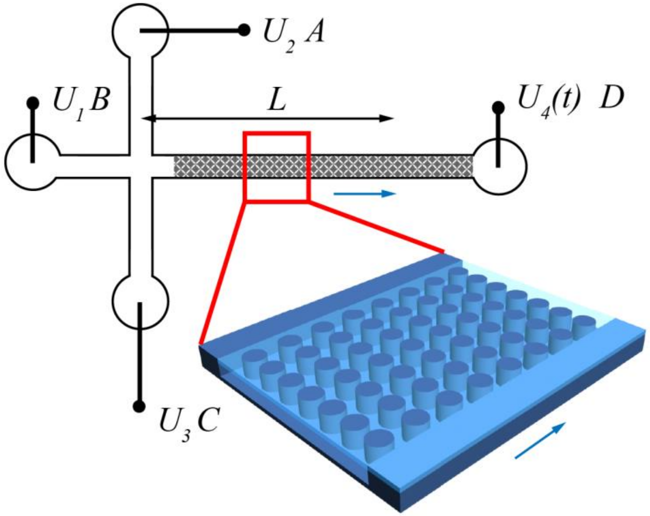
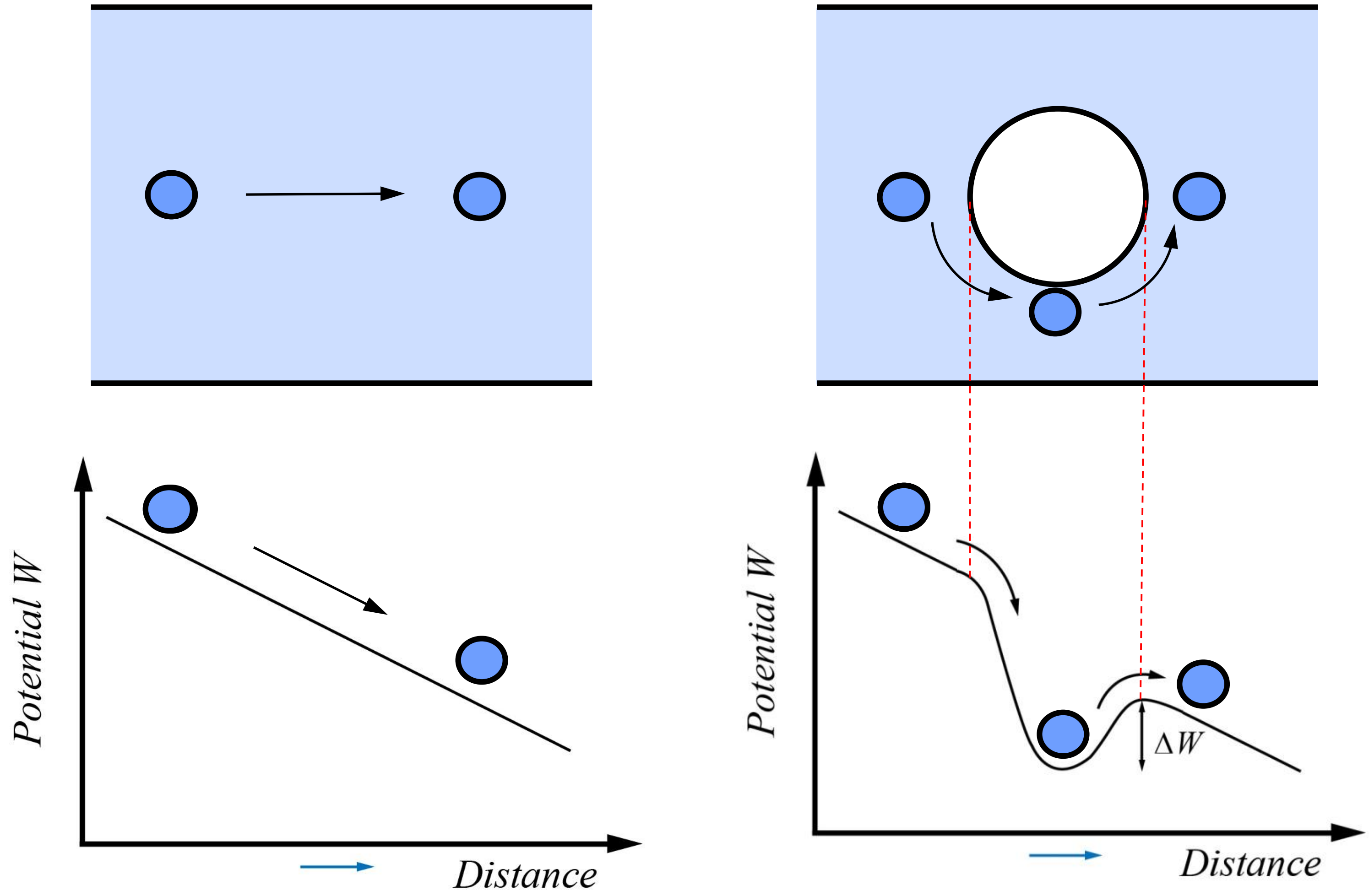
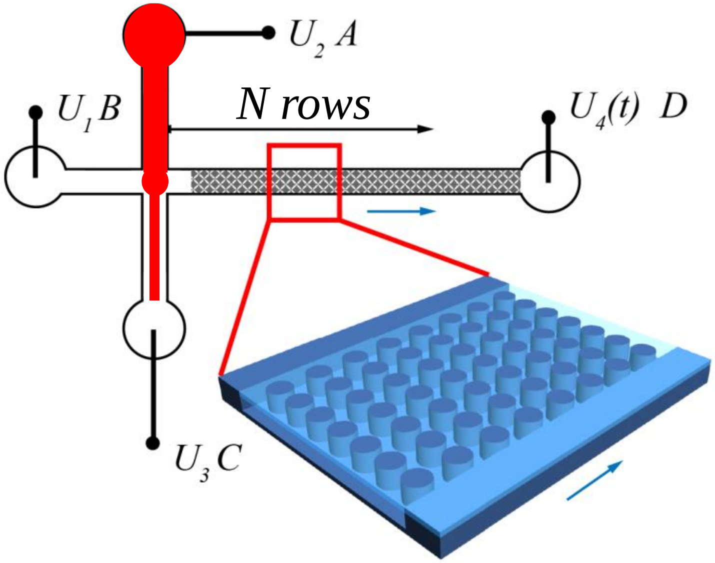


Experimental Determination of Polarizability



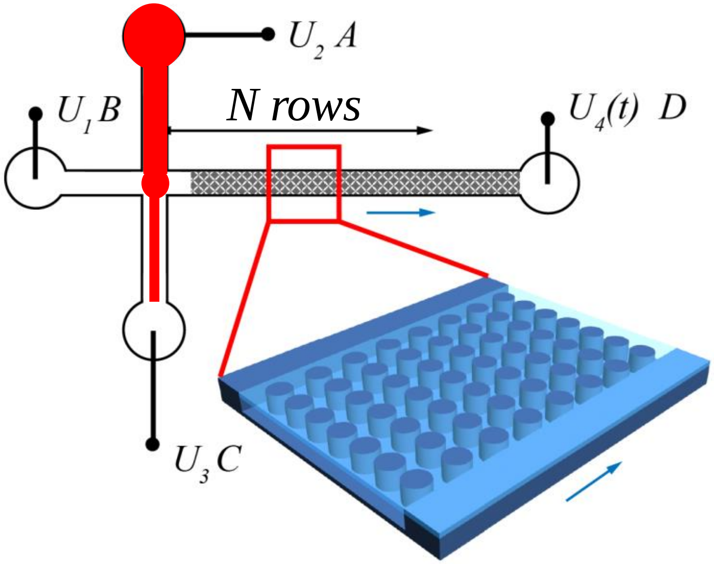
τ Time delay due to DEP trap

With DC only



t_0

AC with DC offset



t

$$\tau = (t - t_0)/N$$

Experimental Determination of Polarizability

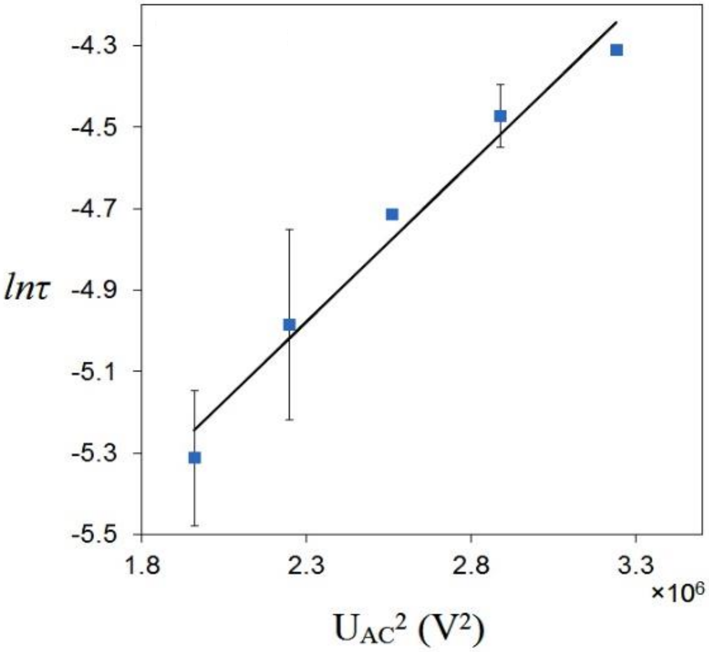
$$\tau = (t - t_0)/N$$

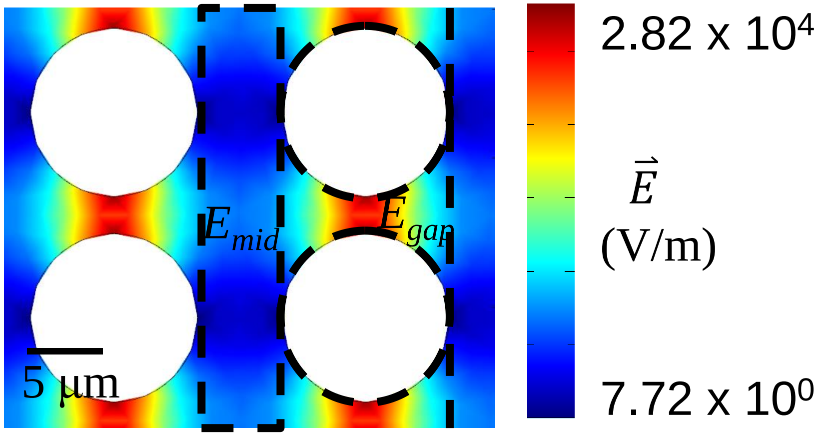
$$\tau = \frac{1}{D} \cdot \left(\frac{kT}{qE}\right)^2 \exp\left(\frac{\Delta W_{DEP}}{kT}\right)$$

$$\Delta W_{DEP} = \frac{1}{2}\alpha \vec{E}^2$$

$$ln\tau = \gamma + c\alpha U_{AC}^2/k\,T$$

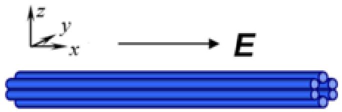
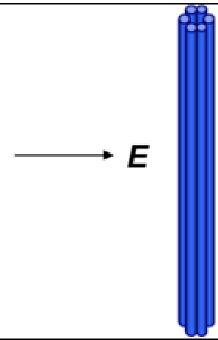
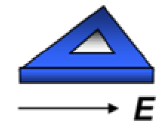
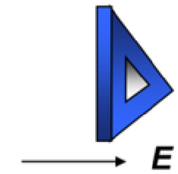
$$c = \frac{1}{2}\frac{E_{gap}^2}{U_{AC}^2}\left(1 - \frac{E_{mid}^2}{E_{gap}^2}\right) = 886.42\ m^{-2}$$





$$\gamma = \ln\left(\frac{1}{D}\right) + 2\ln\left(\frac{k_B T}{qE}\right)$$

Experimental Determination of Polarizability

	6HxB (‖)	6HxB (⊥)	Triangle (‖)	Triangle (⊥)
Shape				
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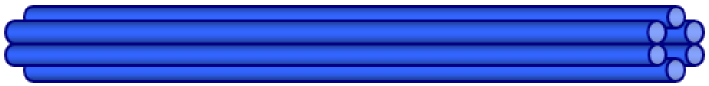
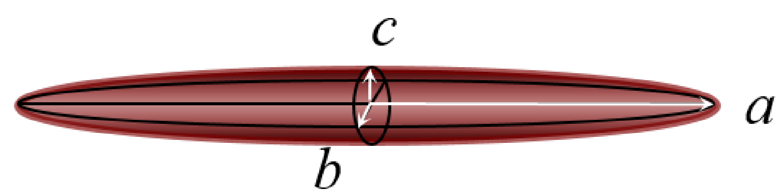
$6HxB$

Determination of origami conductivity

$$\alpha = \frac{8}{3}\pi abc\varepsilon_m \frac{\sigma_p - \sigma_m}{Z\sigma_p + (1-Z)\sigma_m}$$



$$\sigma_{6HxB} = 22.8\ (\pm 3.8)\ S/m$$

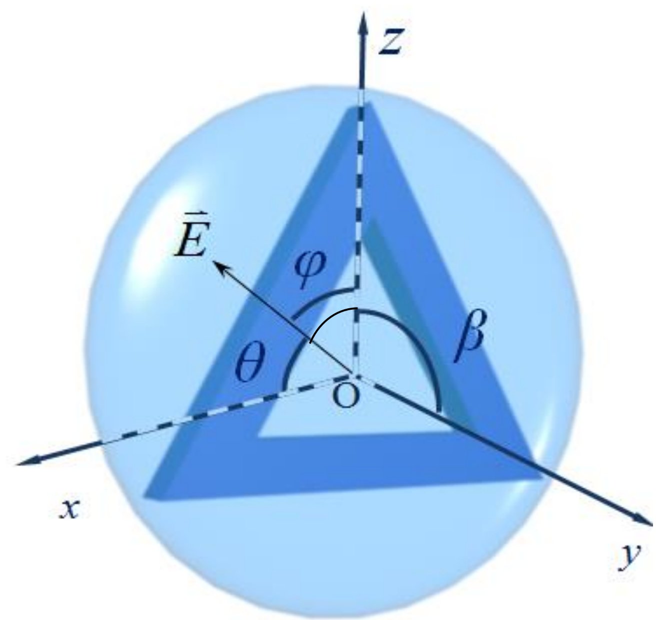


Triangle origami orientation

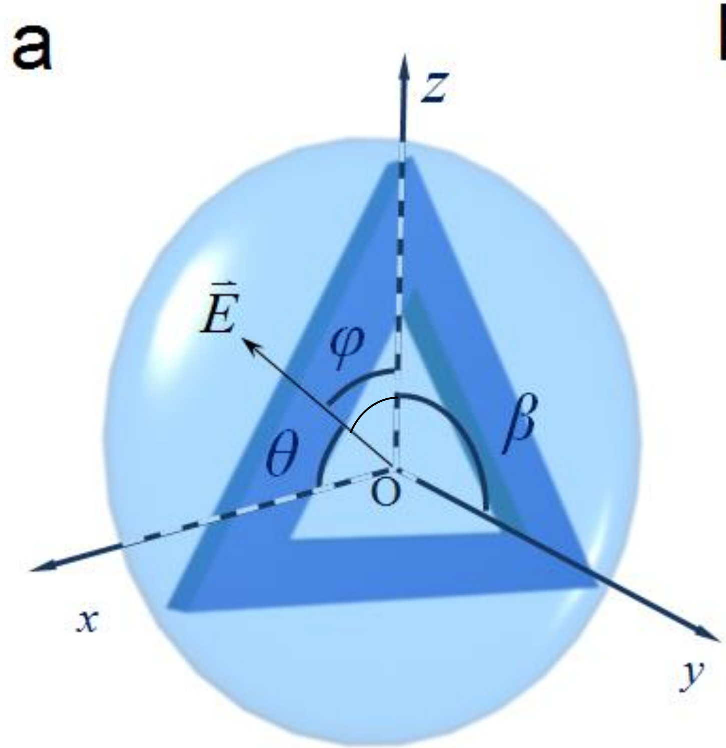
$$\vec{F}_{DEP} = \vec{F}_x + \vec{F}_y + \vec{F}_z$$

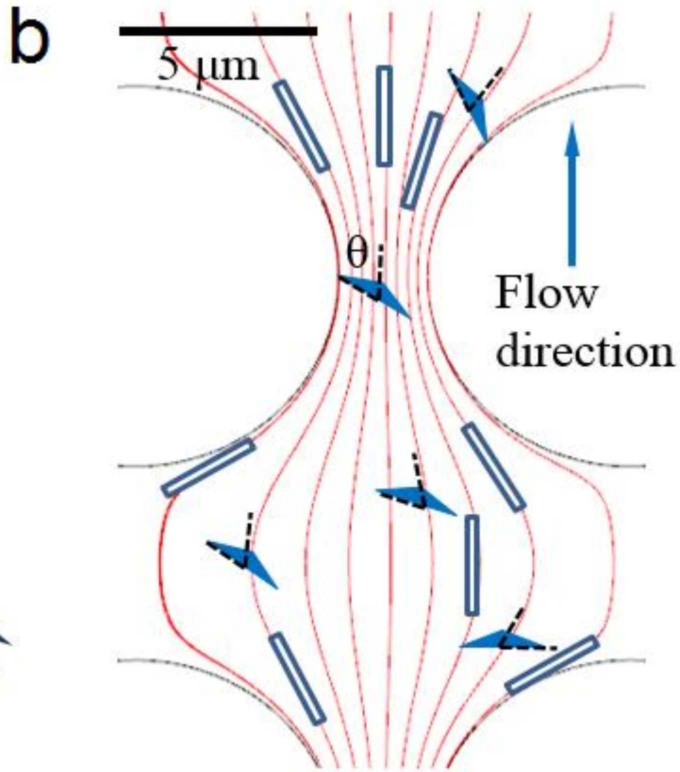
$$|\vec{F}_{DEP}|^2 = |\vec{F}_x|^2 + |\vec{F}_y|^2 + |\vec{F}_z|^2$$

Considering the symmetry of the structure with $\beta = \theta$, the orientation of the triangle origami can be calculated from the vector and geometry relations



Experimental Determination of Polarizability





solution	I	II
θ (˚)	59.4	69.3
φ (˚)	46.1	30.0

- Projects
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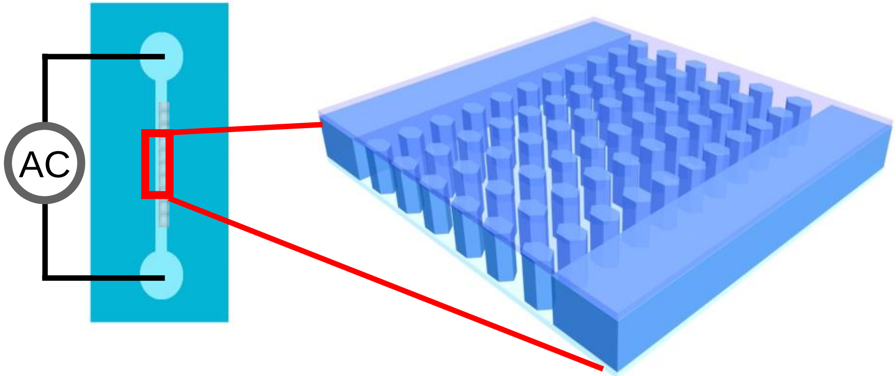


Effect of Buffer Valency Trapping

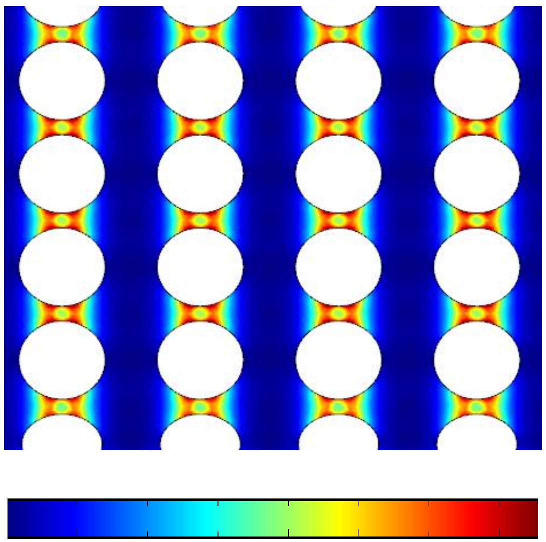
Counterion Condensation (CC) theory

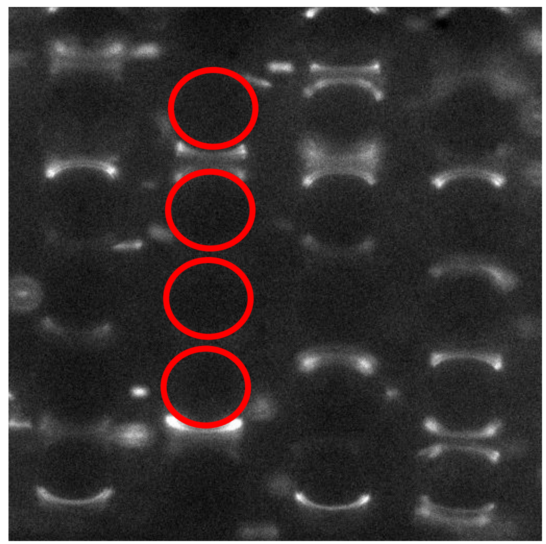
- Manning 1978
- describing the partial neutralization of the charges around DNA as a function of DNA conformation and counterion valence.

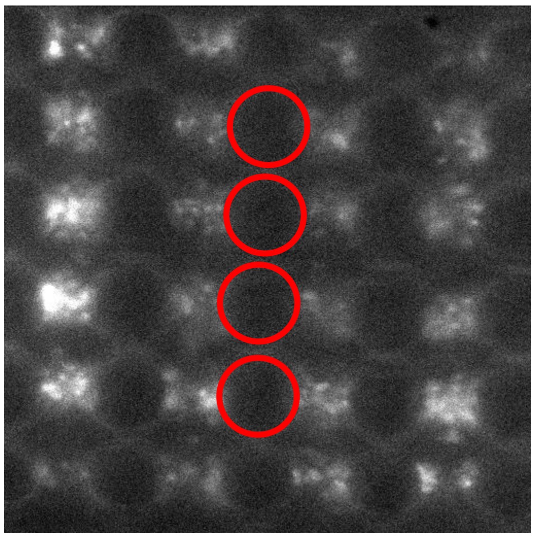
Effect of Buffer Valency Trapping

λ-DNA Trapping

AC ⟷ AC ⟷ AC ⟷







$1.66e^{12}$ $\nabla \vec{E}^2$ $3.77e^{15}$

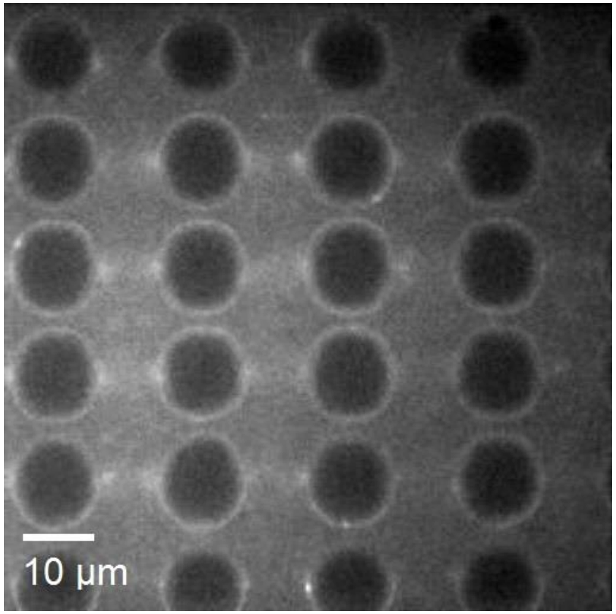
KH_2PO_4/K_2HPO_4 ~ 10 Mm
2000 V 60 Hz

KH_2PO_4/K_2HPO_4 ~ 5 mM, $MgCl_2$ ~ 5 mM
1000 V 60 Hz

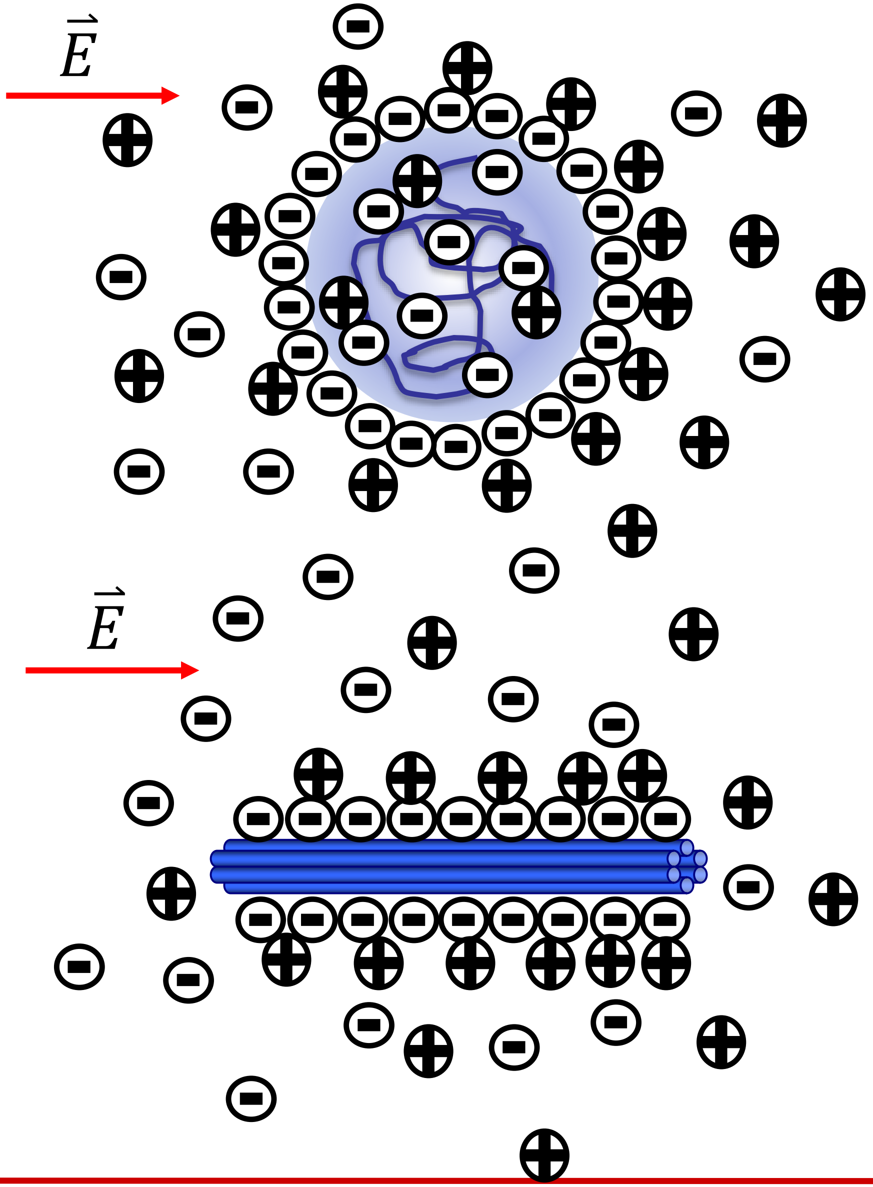
Buffer : pH = 7.0, σ = 0.20 S/m

Effect of Buffer Valency Trapping

6HxB DNA Trapping



KH_2PO_4/K_2HPO_4 ~ 5 mM,
$MgCl_2$ ~ 5 mM
1000 V 40 Hz



Conclusion

- The research projects enrich the study in DEP mechanism for submicron biomolecules
  - Two artificial DNA structures with same scaffold but great topological difference showed distinct DEP trapping behaviors.
  - Simulation model is in good agreement with experiment.
  - The polarizabilities for the two species are experimentally determined by measuring the migration times through a potential landscape exhibiting dielectrophoretic barriers.
  - The orientations of both species in the escape process and were studied suggesting that their diffusion is influenced by alignment with respect to the electric field during the escape process.
  - Buffer valency study reveals that di-valent counterions neutralize the phosphate charge on DNA more efficiently than mono-valent counterions, resulting a difference in the decrease of DNA surface conductivity.

Acknowledgements

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Dr. Alexandra Ros

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