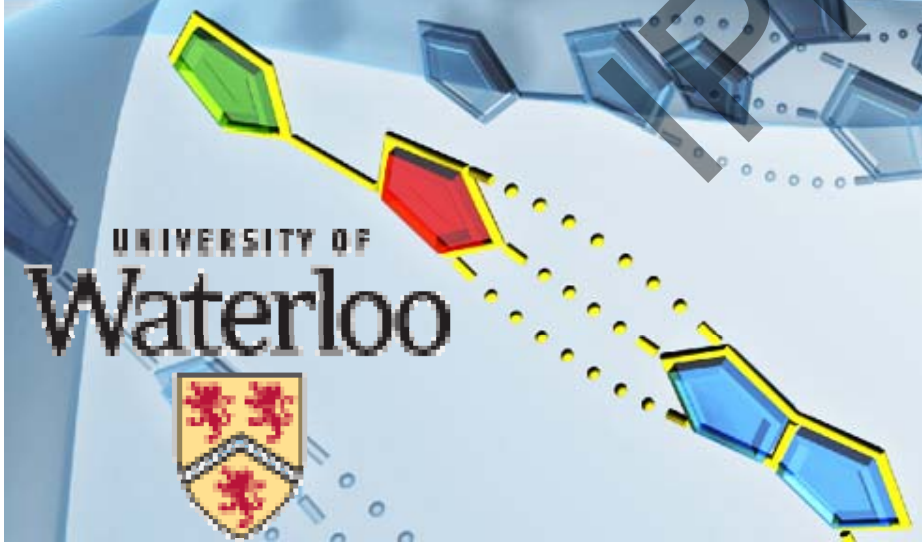
DNA as a Molecular Ruler to Determine the Limiting Length Scale between the Distance of Electron Transfer and Screening Length

Christine Keyes



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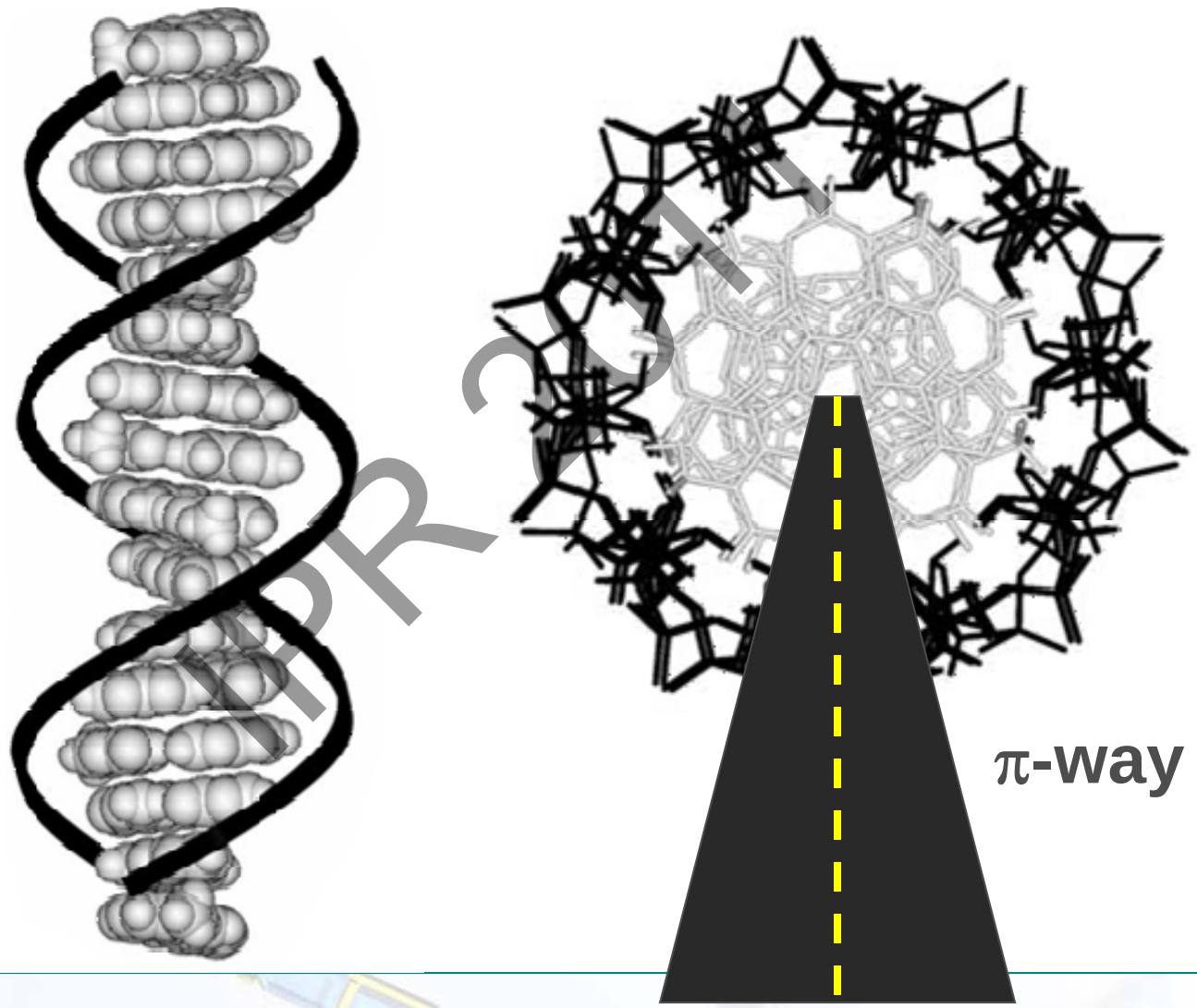
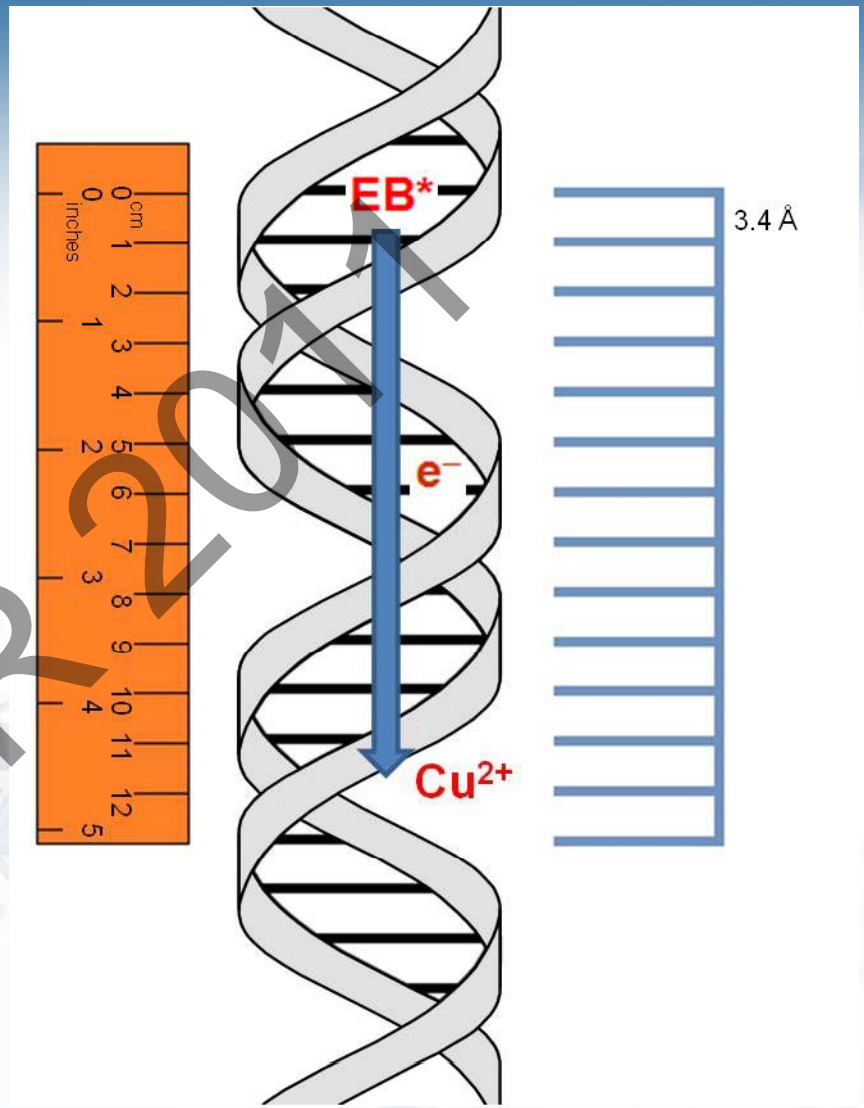
DNA Double Helix

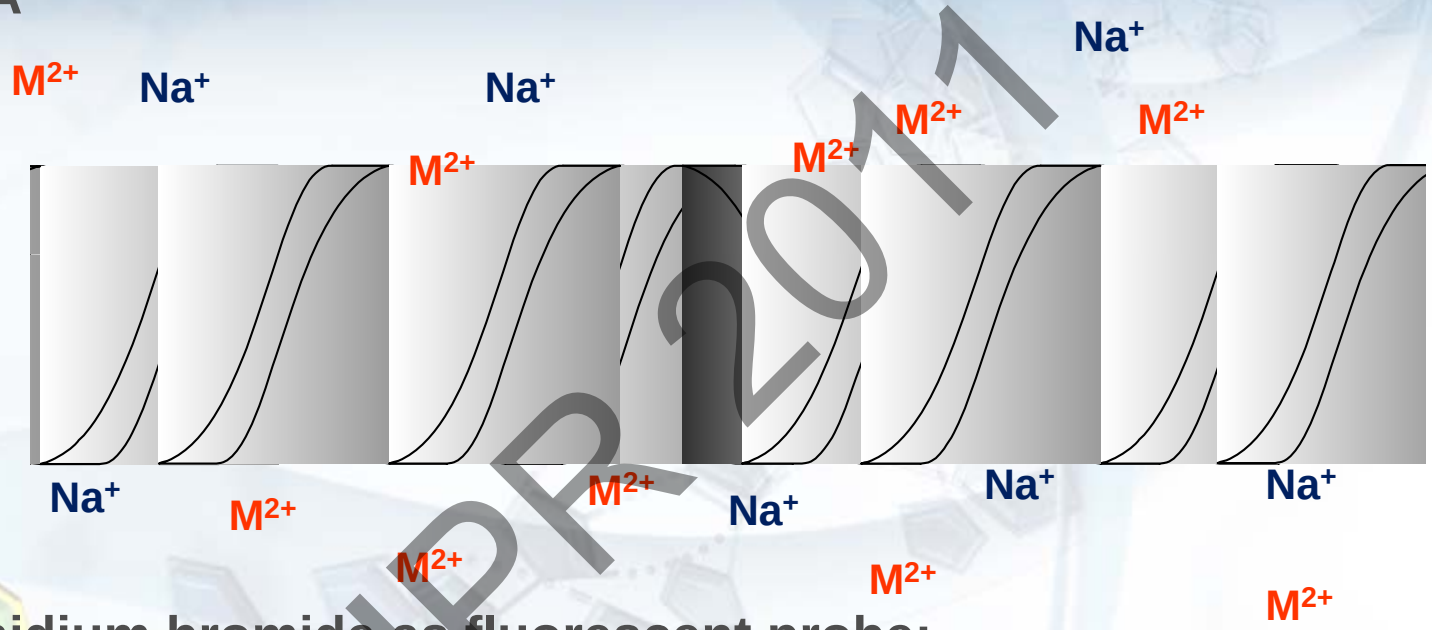


- The structure of DNA is rigid (on the nanometer scale) and well calibrated and can be used to measure distances
- DNA can measure distances of electron transfer



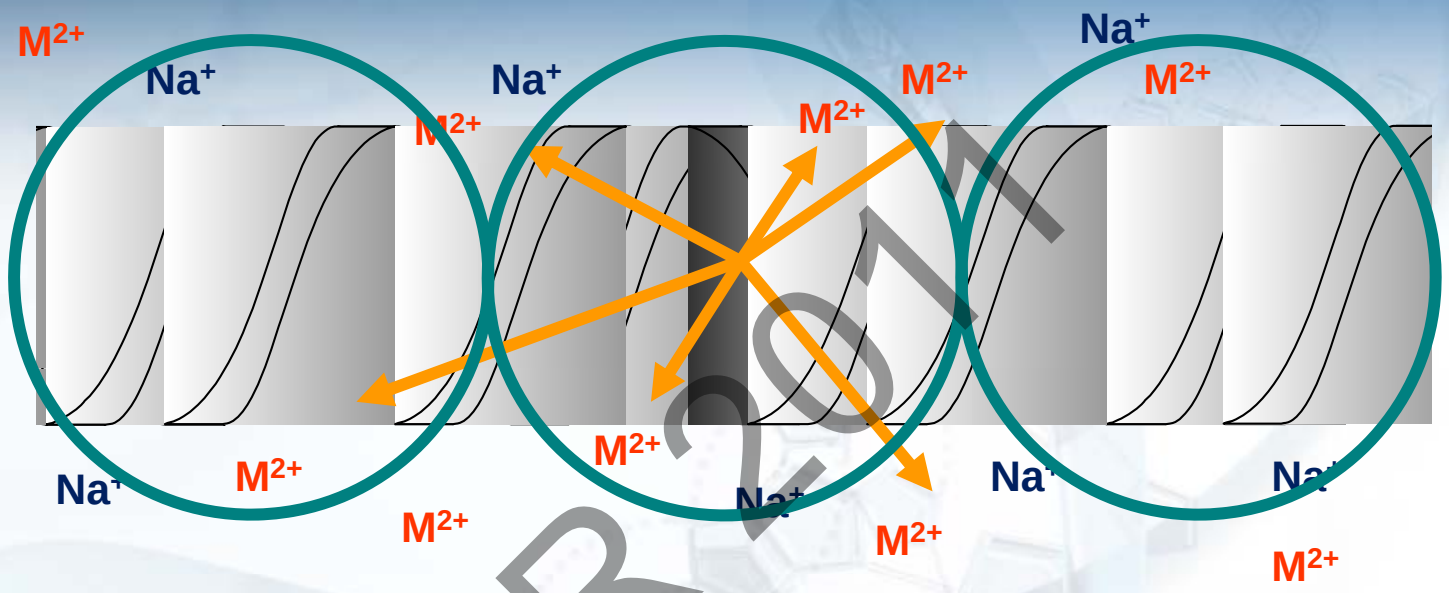
ET in DNA

- Electron transfer occurs in DNA between an excited intercalated ethidium bromide and divalent metal cations externally bound to DNA



- Ethidium bromide as fluorescent probe:
  - Strongly intercalates in DNA
  - Lifetime increases when intercalated in DNA (1.6 ns in pure water vs 23 ns in DNA)
- M^{2+} = Cu^{2+} and the [Cu^{2+}]/[Phosphate] < 0.2 to ensure Cu^{2+} is bound externally to the DNA helix and not to the base pairs

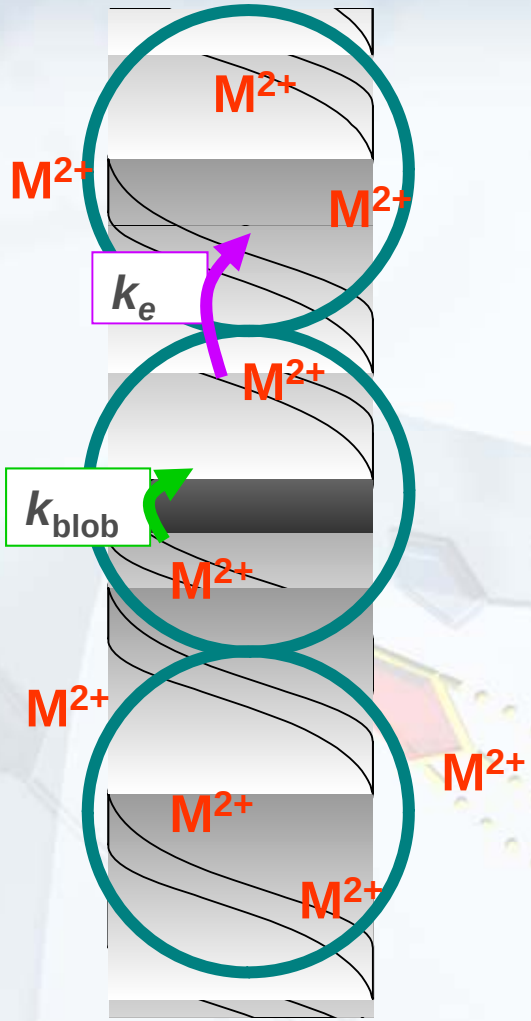
Binding of Divalent Metal Cations to DNA



- Distances spanning the donors and acceptors is random and unknown
- Complicated fluorescence data
  - Fluorescence Blob Model (FBM)
- *blob* – restricted volume where ET can take place due to the electrostatic binding between Cu^{2+} and DNA

FBM Approach to Study ET in DNA

$$[EB^*] = [EB^*]_o \left[f_{slow} \times f(t) \times exp\left[-A_2 t - A_3\left(1 - \exp(-A_4 t)\right)\right] + f_{fast}\, exp\left(-t/\tau_{fast}\right) \right]$$



$$A_2 = \langle n \rangle \frac{k_{blob} k_e [blob]_{loc}}{k_{blob} + k_e [blob]_{loc}} \qquad A_3 = \langle n \rangle \frac{k_{blob}^2}{\left(k_{blob} + k_e [blob]_{loc}\right)^2}$$

$$A_4 = k_{blob} + k_e [blob]_{loc}$$

<n> → average number of quenchers per blob

k_{blob} → quenching rate constant of EB inside a blob

$k_e[blob]_{loc}$ → k_e is the rate constant to exchange a quencher between blobs and $[blob]_{loc}$ is the local blob concentration

N_{blob} and K

- $N_{blob} \rightarrow$ size of a blob in terms of the number of base pairs
- K $\rightarrow$ binding constant of Cu^{2+}
- Find through <*n*> which is given by:

$$< n >= \frac{[M^{2+}]_{bound} - [M^{2+}]_o}{[blob]}$$

N_{blob} and K

$$< n >= \frac{[M^{2+}]_{bound} - [M^{2+}]_o}{[blob]}$$

→ Dependent on [DNA]

→ Proportional to N_{blob}

$$M^{2+}_{free} + DNA \underset{}{\overset{K}{\rightleftharpoons}} M^{2+}_{bound}$$

$$[M^{2+}]_{bound} = \frac{[M^{2+}]_T}{\frac{1}{K[DNA]} + 1}$$

$$[blob] = \frac{[DNA]}{N_{blob}}$$

N_{blob} and K

$$\langle n \rangle = \frac{\left[M^{2+}\right]_T}{\frac{1}{KN_{blob}} + \frac{[DNA]}{N_{blob}}} - \frac{\left[M^{2+}\right]_o N_{blob}}{[DNA]}$$

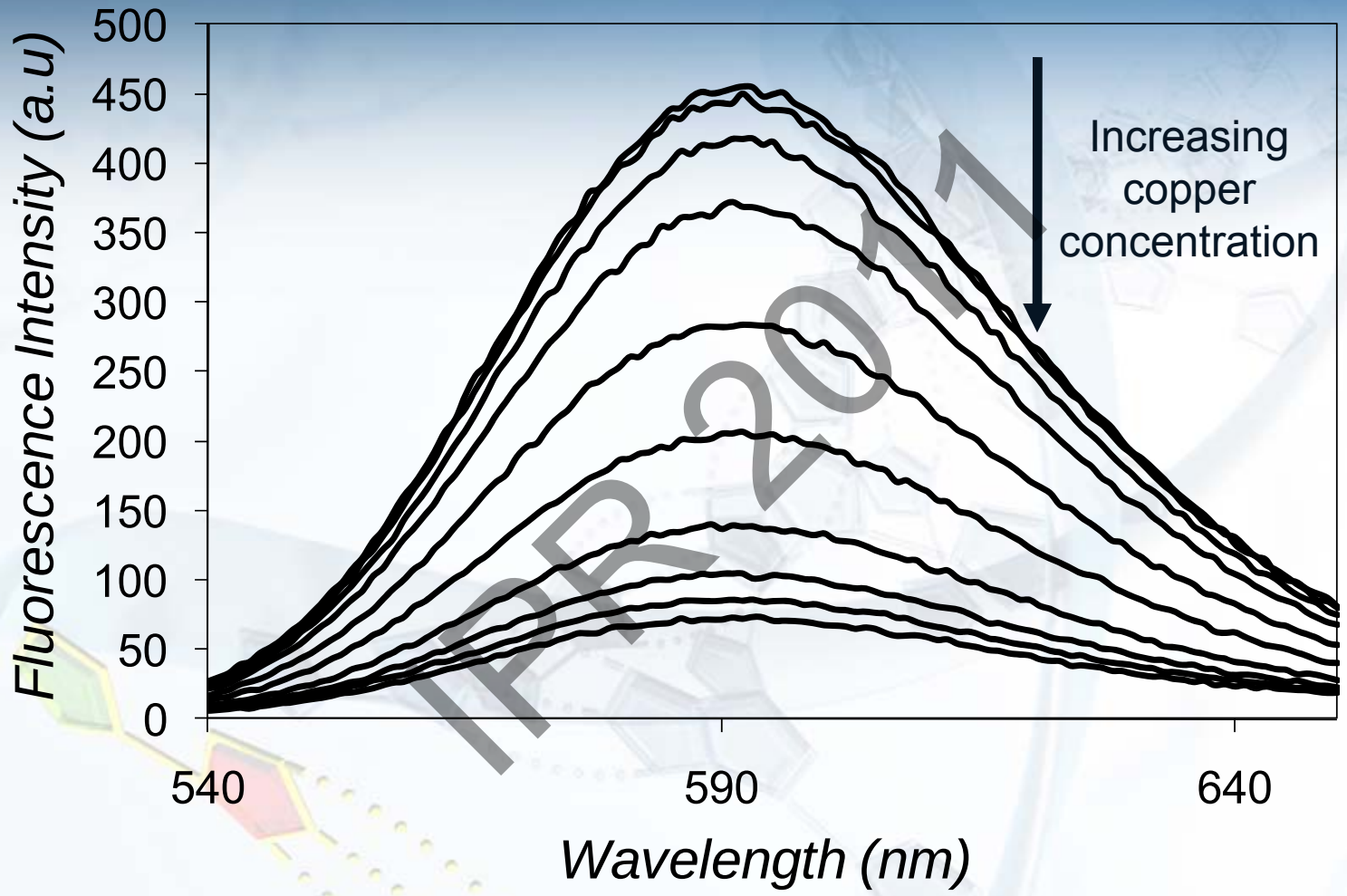
- <n> vs. $[M^{2+}]_T$ gives:

$$slope = \left(\frac{1}{KN_{blob}} + \frac{[DNA]}{N_{blob}} \right)^{-1}$$

$$\frac{1}{slope} = \frac{1}{KN_{blob}} + \frac{[DNA]}{N_{blob}}$$

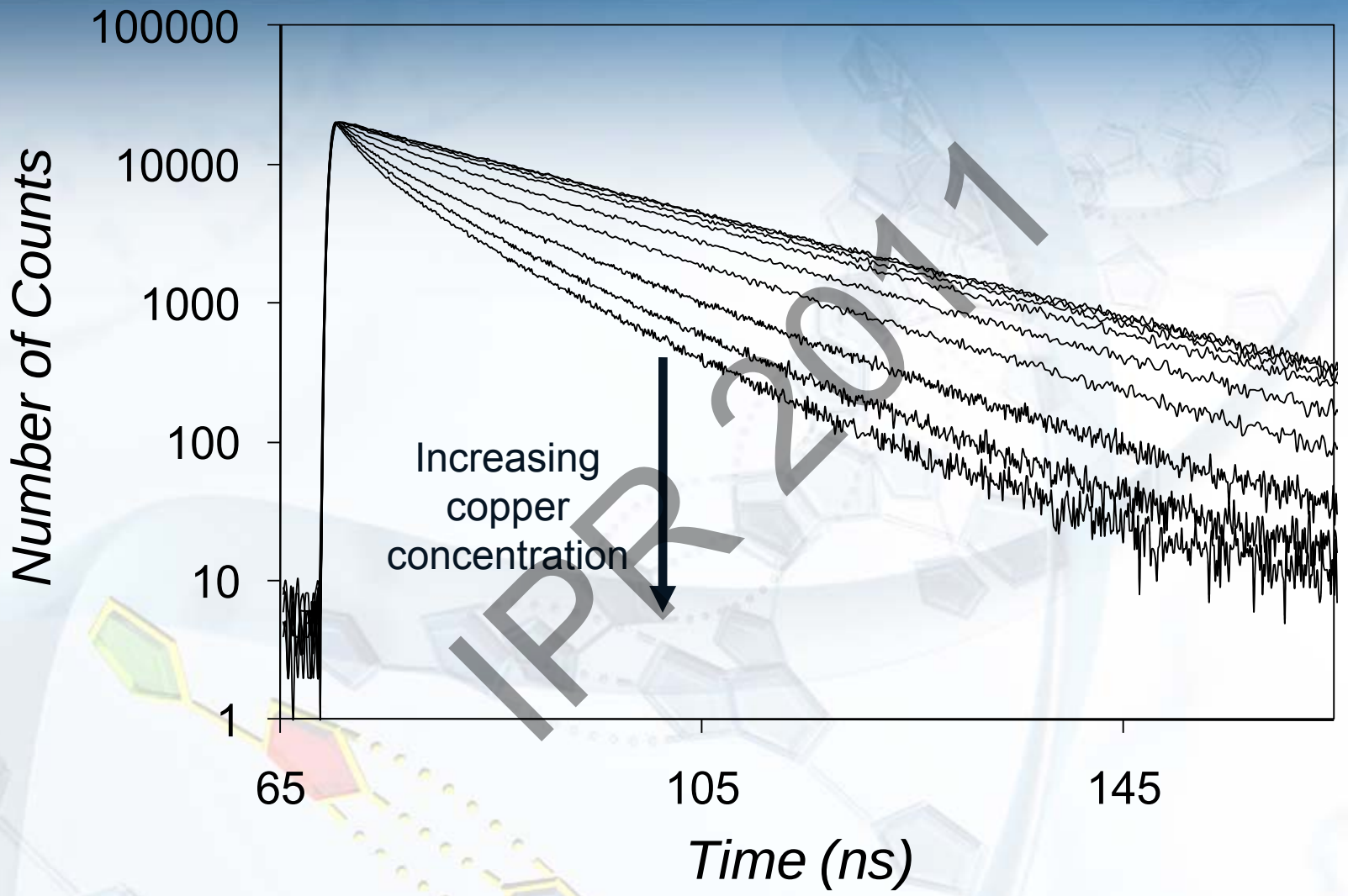
- Intercept = $1/KN_{blob}$
- Slope = $1/N_{blob}$

Steady-State Fluorescence Spectra



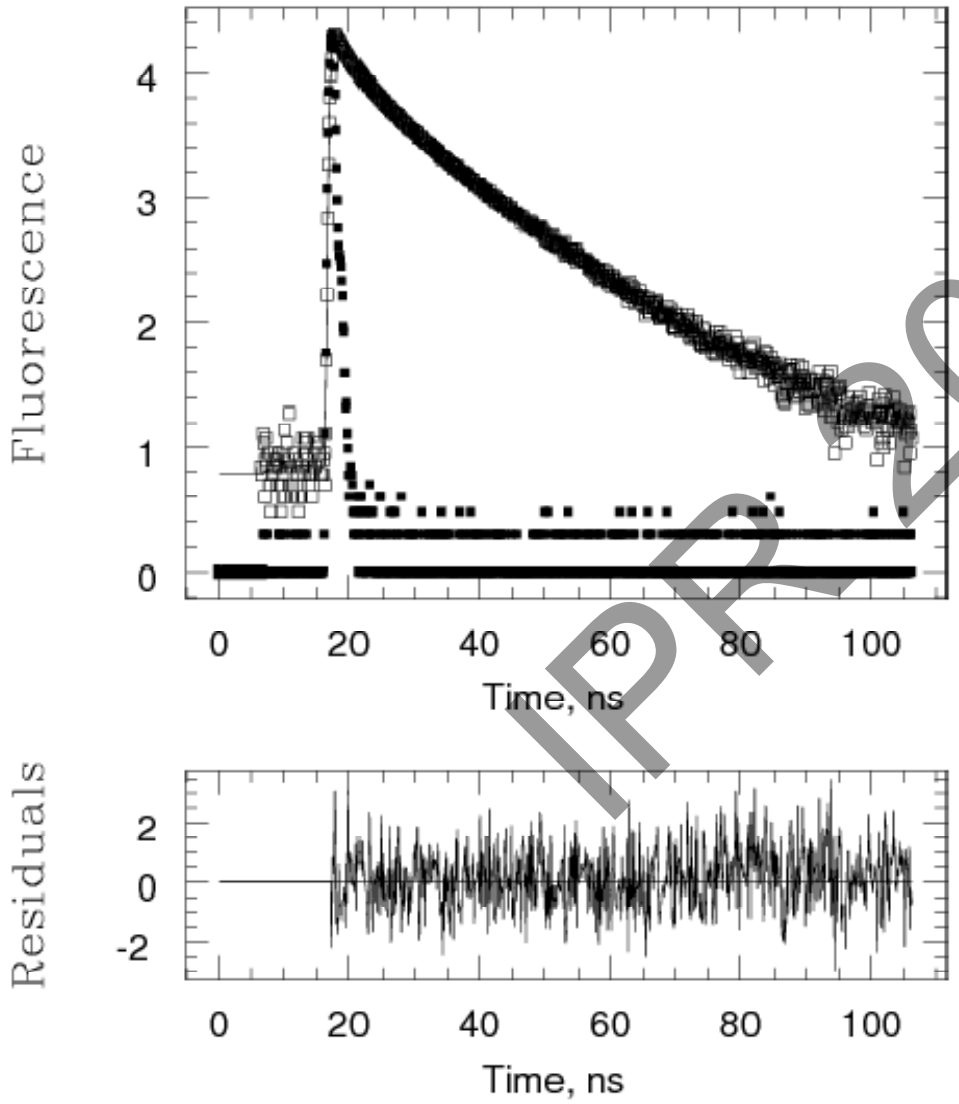
- 0.09 wt% DNA, [EB] = 1×10^{-5} M, [Na_2SO_4] = 5×10^{-3} M, λ_{ex} = 340 nm

Fluorescence Decays



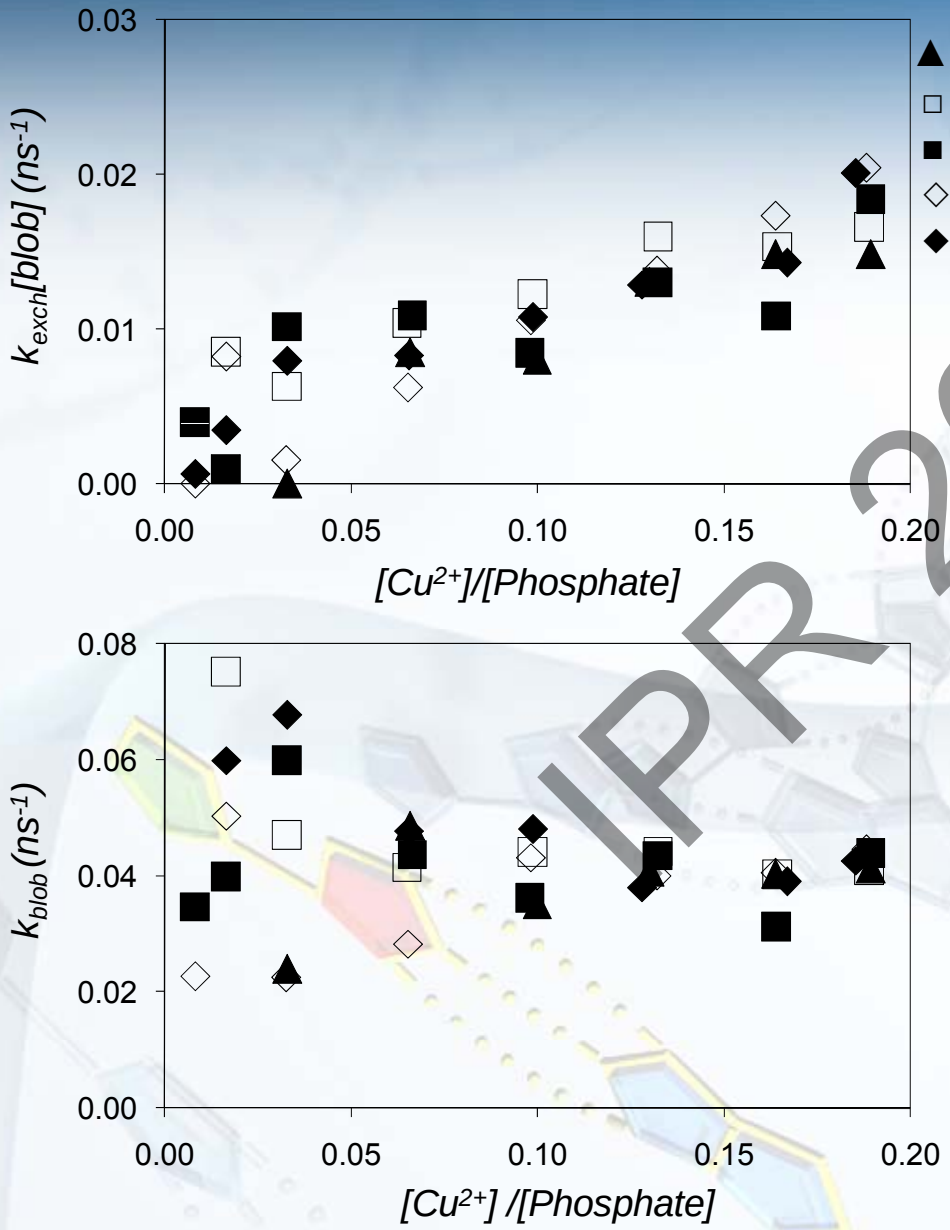
- 0.09 wt% DNA, [EB] = 1×10^{-5} M, [Na_2SO_4] = 5×10^{-3} M, λ_{ex} = 340 nm, λ_{em} = 605 nm

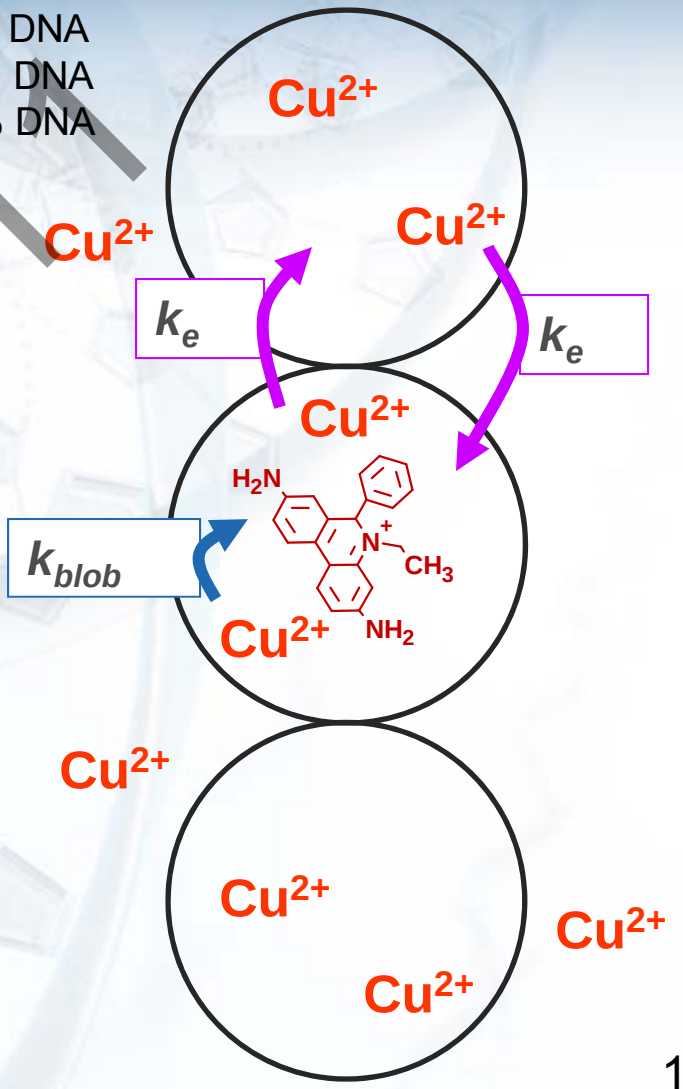
FBM Results



filename = 9D5.18e-4Cu.txt
chisq = 1.16159
tau1 = 0.00000, a1 = 0.00000
exch = 0.02051, a2 = 0.68090
kq = 0.04306, <n> = 2.42642
tau4 = 1.56675, a4 = 0.31910
Background = 5.09836
starting channel = 148

FBM Results





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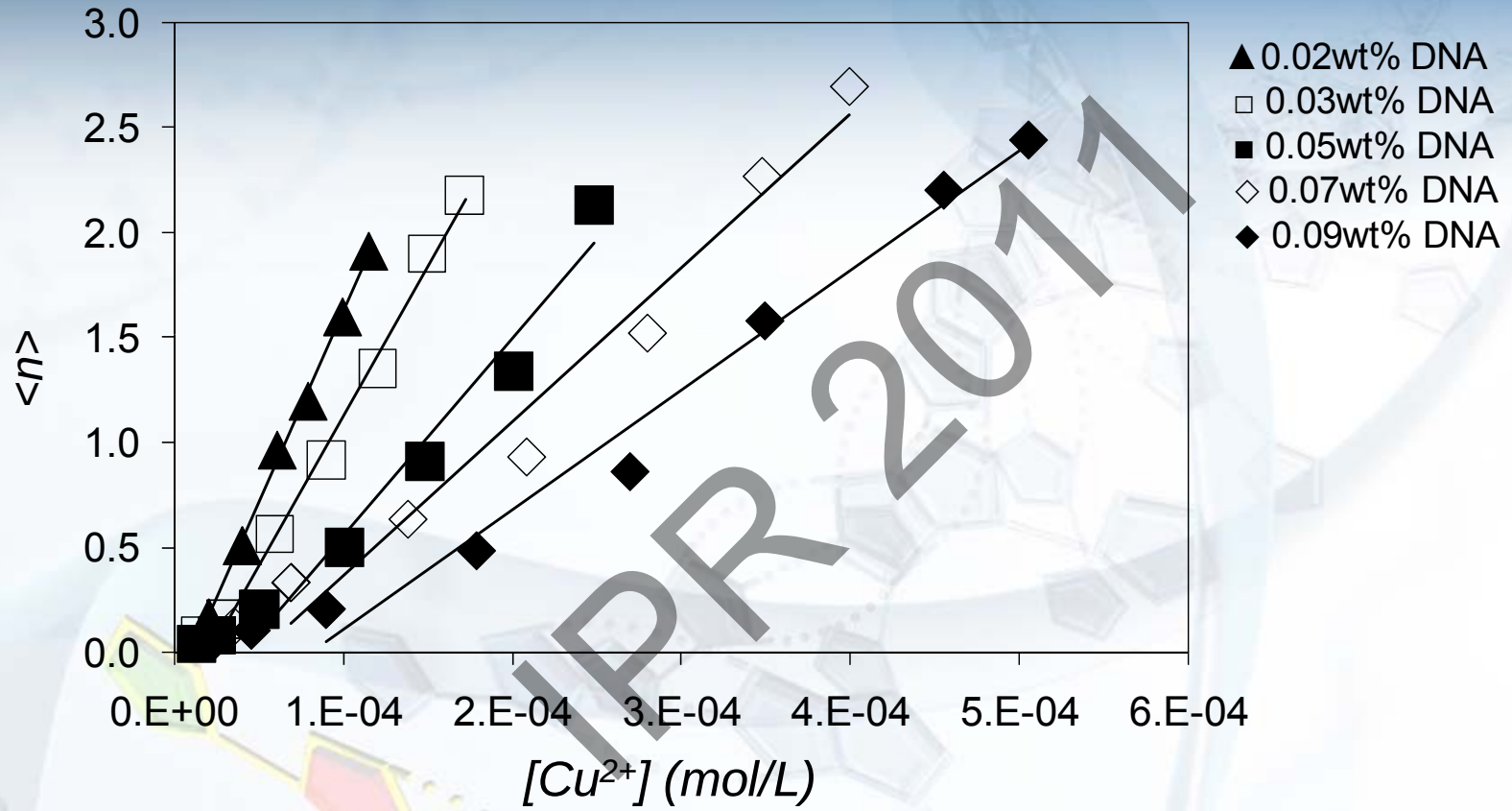
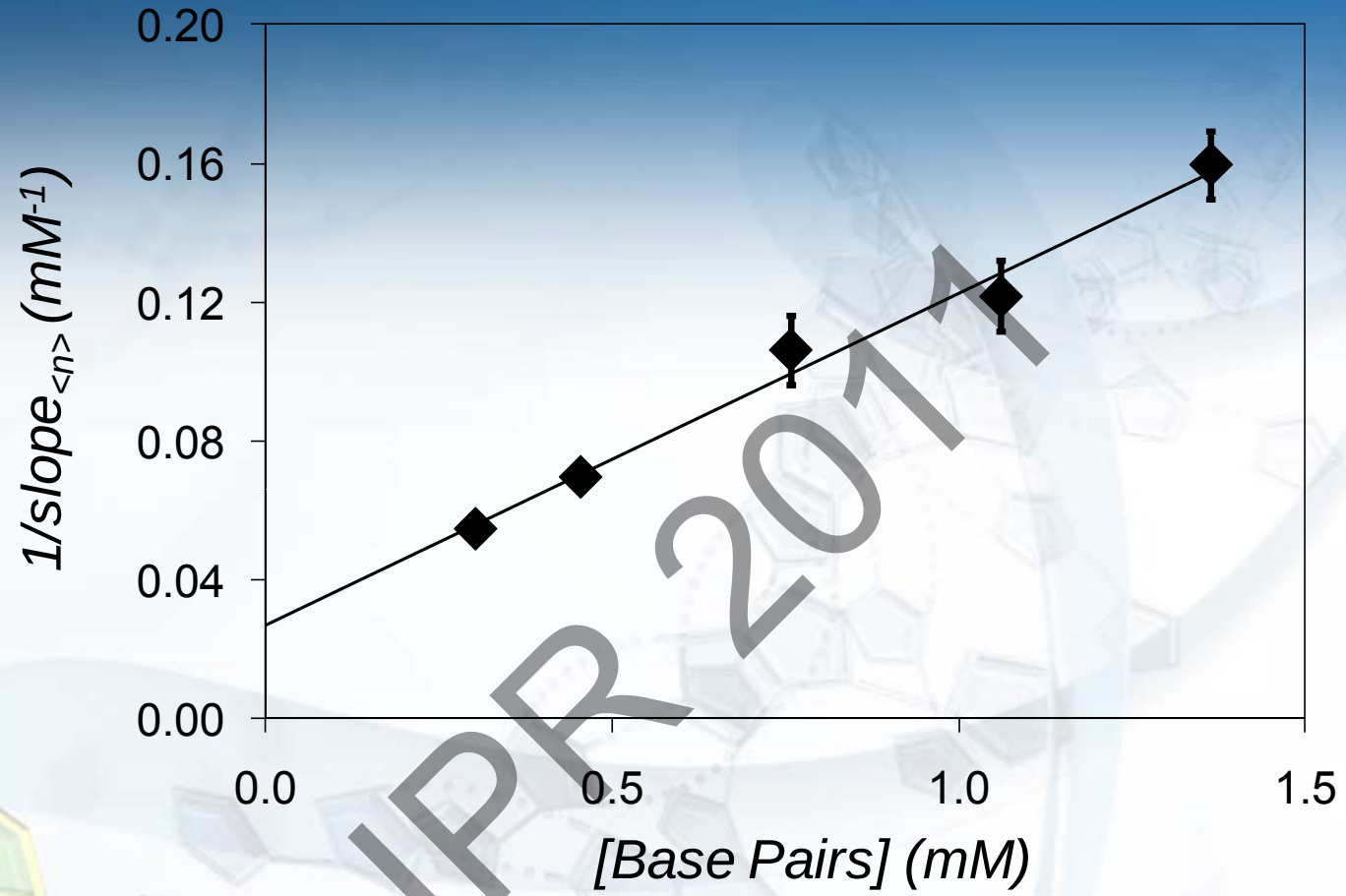


$$< n >= \frac{\left[Cu^{2+}\right]_T}{\frac{1}{KN_{blob}} + \frac{[DNA]}{N_{blob}}} - \frac{\left[Cu^{2+}\right]_o N_{blob}}{[DNA]}$$

$$\frac{1}{slope} = \frac{1}{KN_{blob}} + \frac{[DNA]}{N_{blob}}$$



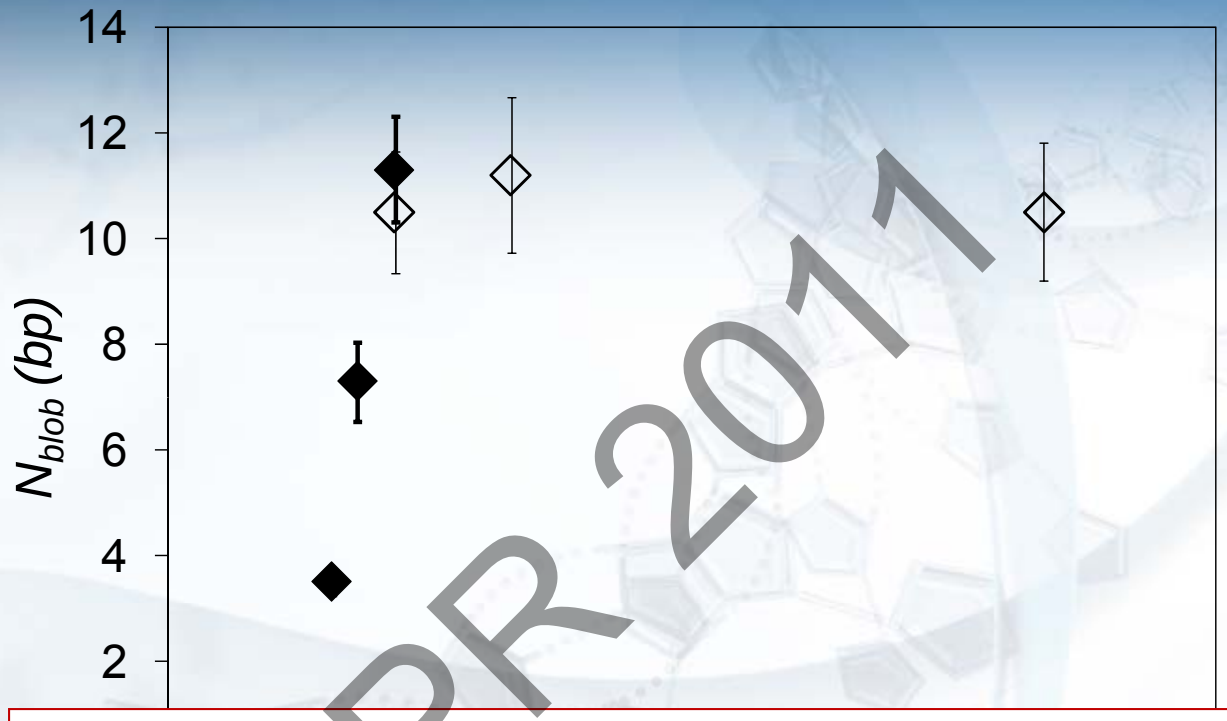
Calf Thymus DNA (15 000 bp):

N_{blob} = 10.5 ± 1.3 bp

K = 3500 ± 1800 M^{-1}

$$\frac{1}{slope} = \frac{1}{KN_{blob}} + \frac{[DNA]}{N_{blob}}$$

FBM does not depend on size of macromolecules as long as the size of the macromolecule is substantially larger than a blob



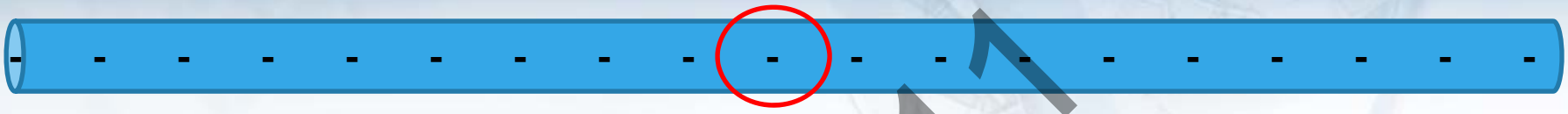
How does $N_{blob}(\infty)$ vary as a function of solution ionic strength, metal cation, and chromophore lifetime?

N_{blob} ~ 10

N_{blob} < DNA size for DNA constructs smaller than 12 bp

Keyes Baig, C.; Duhamel, J. *J. Phys. Chem. B* **2010**, *114*, 13950 – 13960.

Ionic Strength and Polyelectrolytes



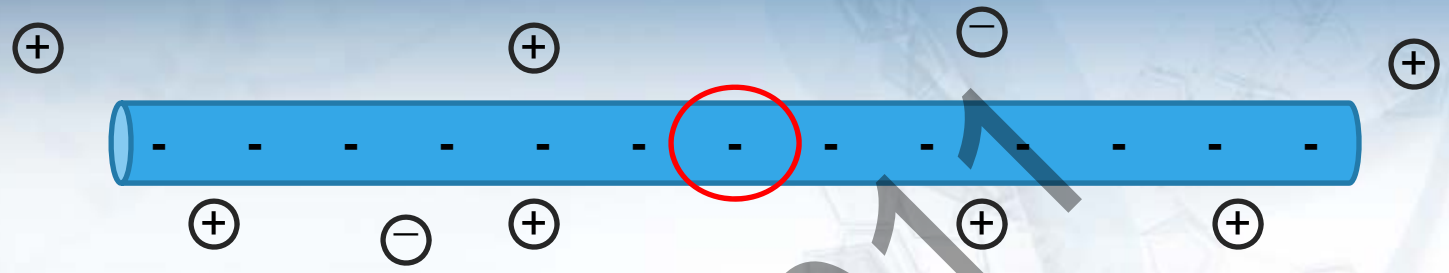
Negatively charged phosphate groups in the backbone are repelled by one another

Added salt screens the repulsion from nearby phosphate groups

Screening length or Debye length is dependent on the ionic strength of the solution

What about the distance of ET? Dependent on ionic strength of the solution?

Ionic Strength and Polyelectrolytes



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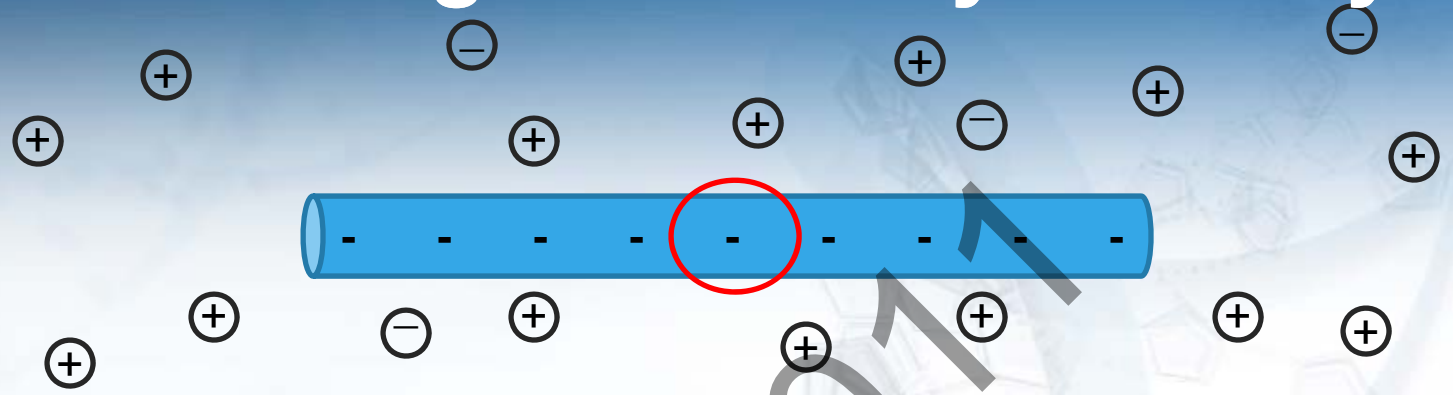

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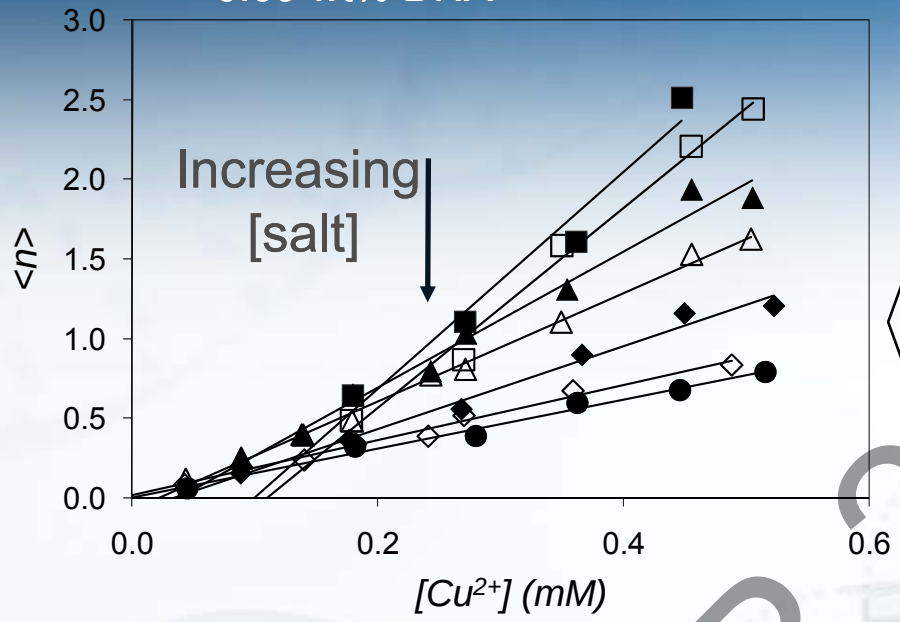
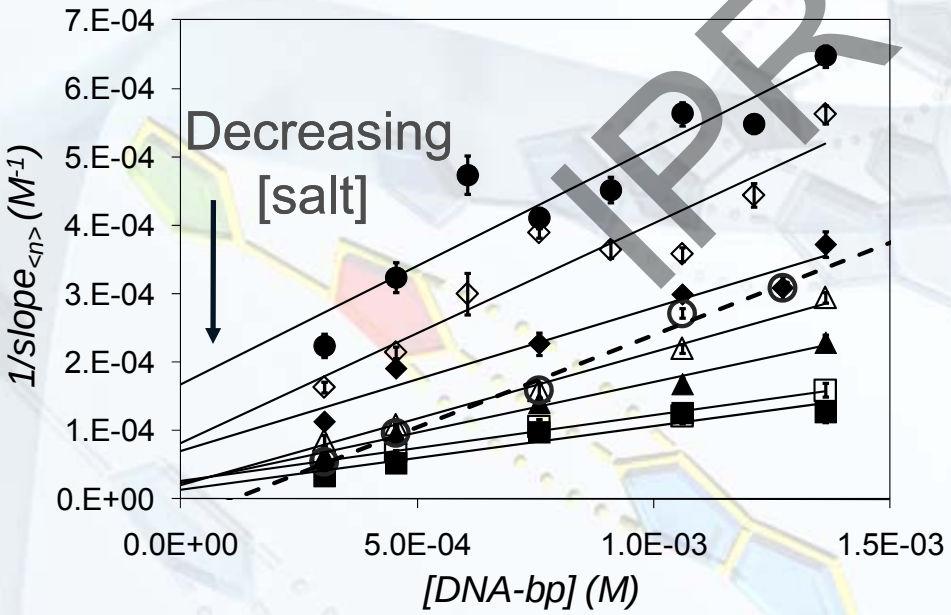
FBM Results

- 0.09 wt% DNA





$$\langle n \rangle = \frac{\left[M^{2+}\right]_T}{\frac{1}{KN_{blob}} + \frac{[DNA]}{N_{blob}}} - \frac{\left[M^{2+}\right]_o N_{blob}}{[DNA]}$$

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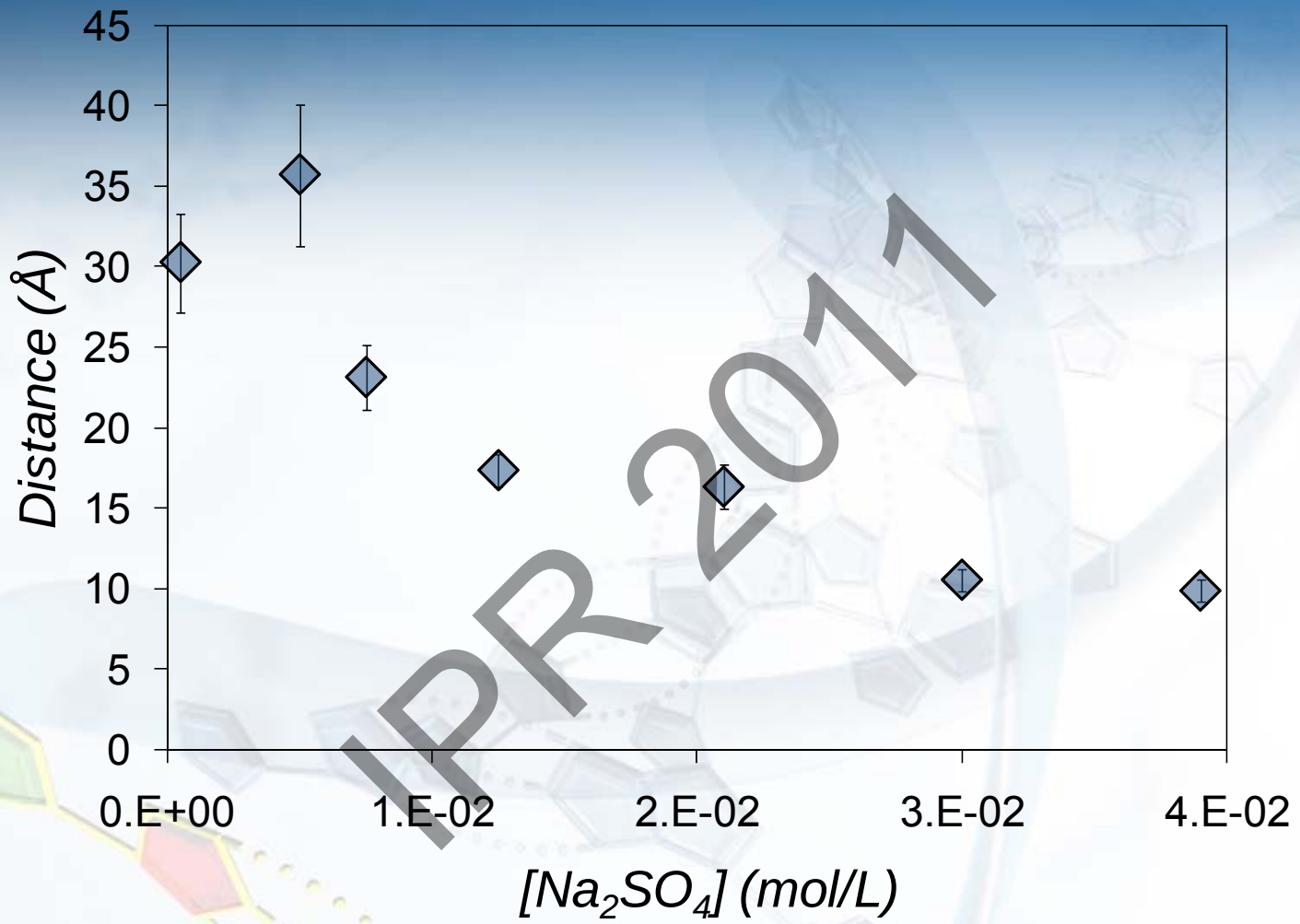
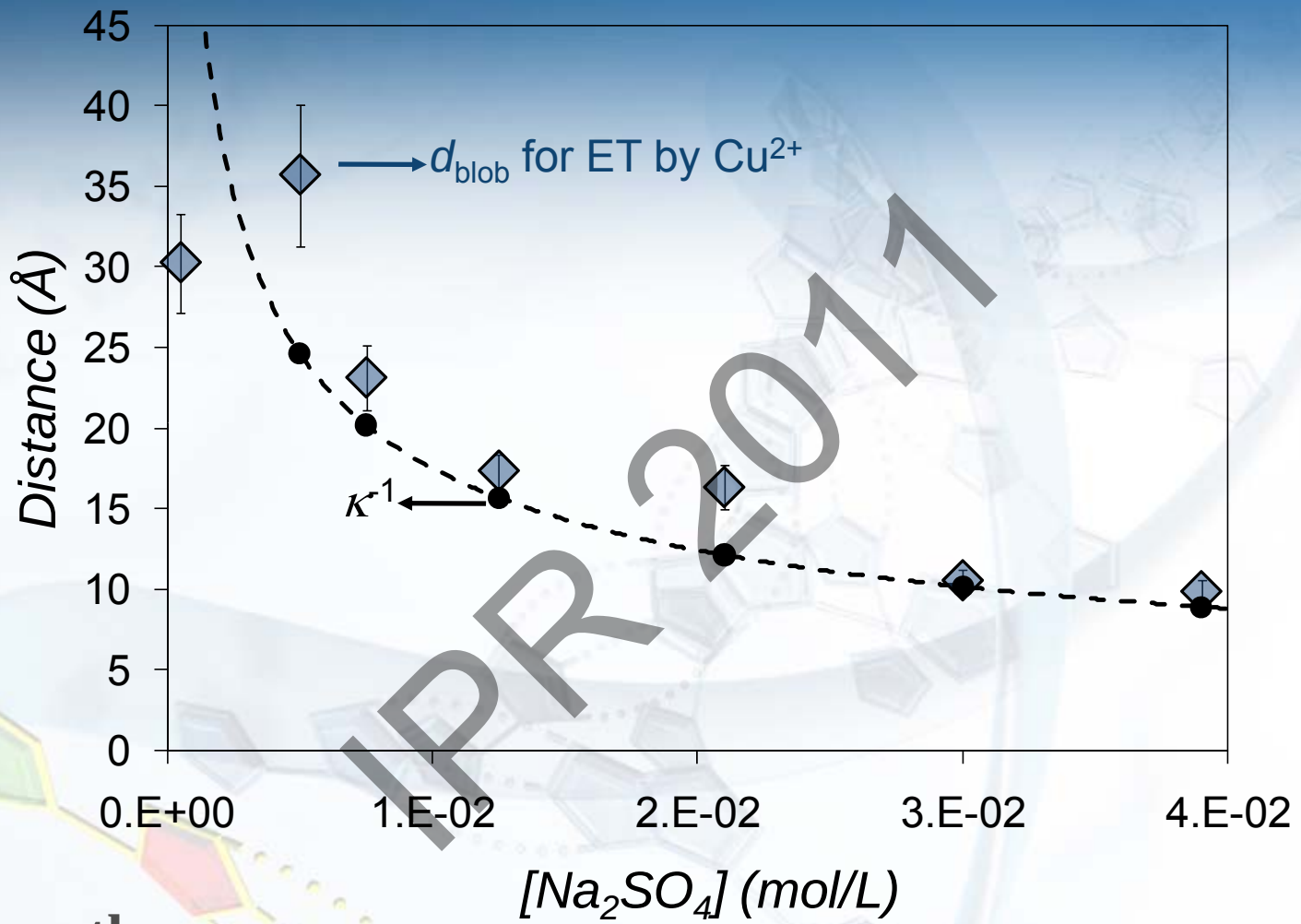


$d_{blob} = N_{blob}$ x 3.4 Å

d_{blob} decreases with increasing ionic strength

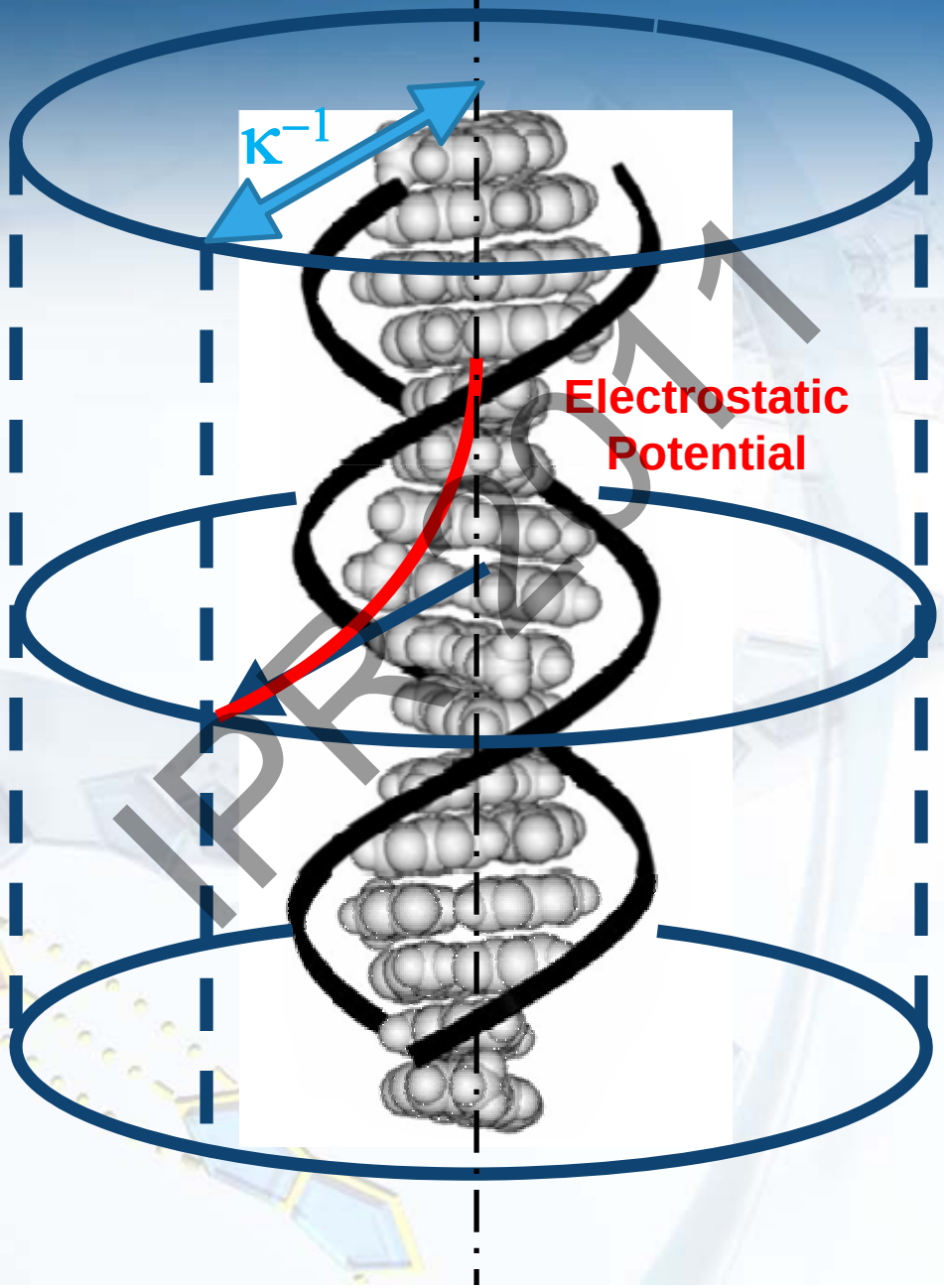
Ionic Strength -N_{blob}



Debye length:

$$\kappa^{-1} = \frac{1}{\sqrt{8\pi b_B N_A I}}$$

Ionic Strength -N_{blob}



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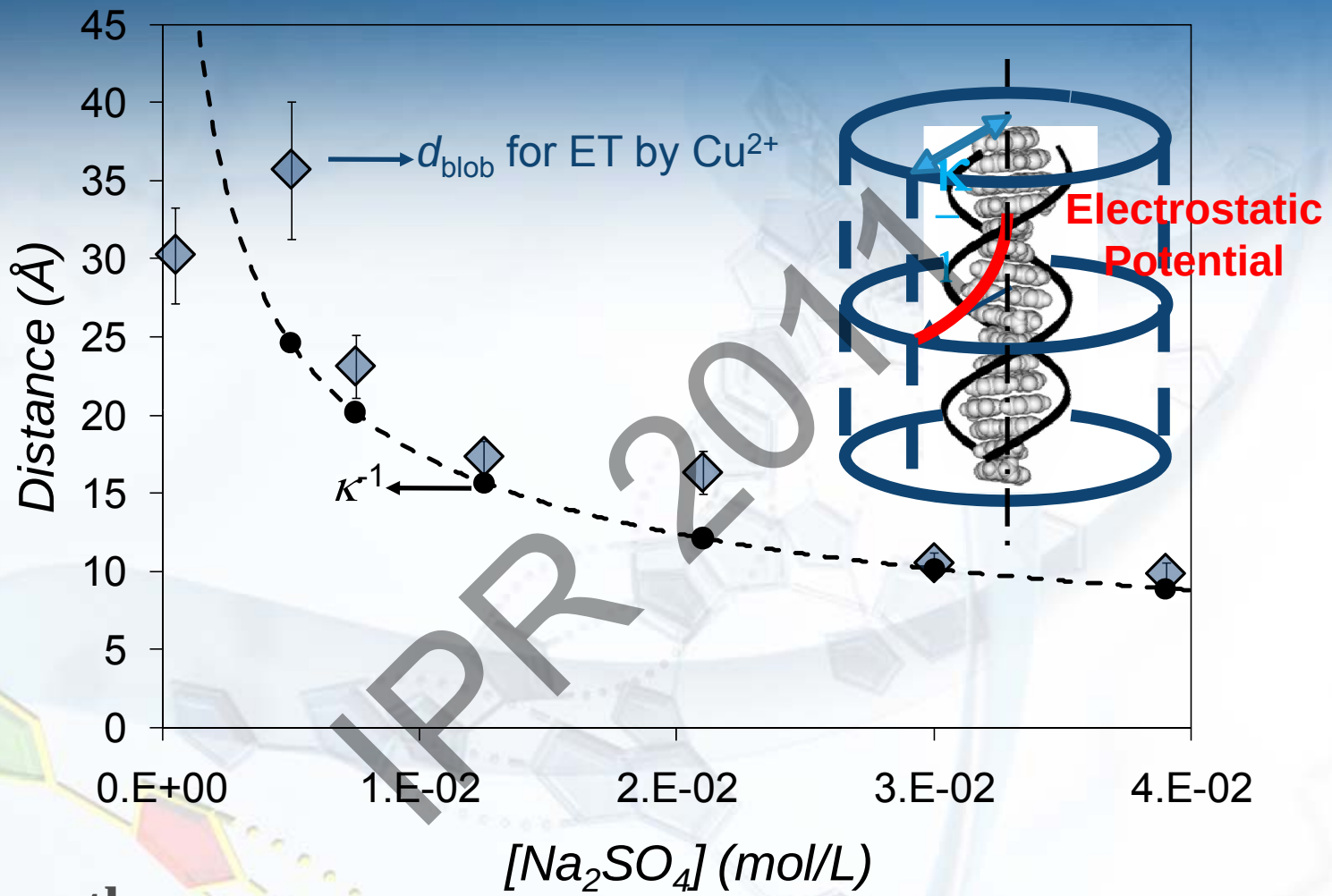

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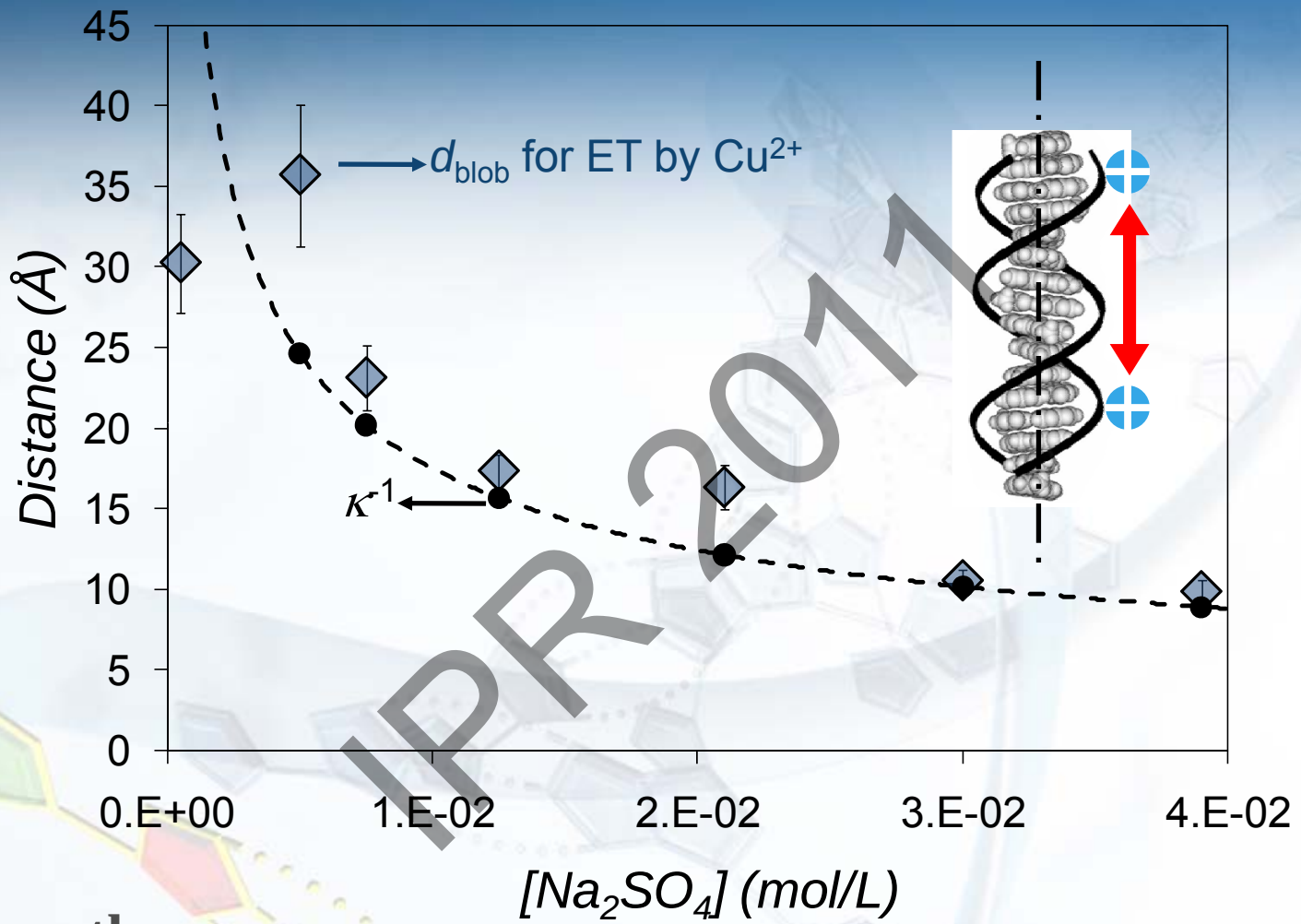
Ionic Strength - N_{blob}



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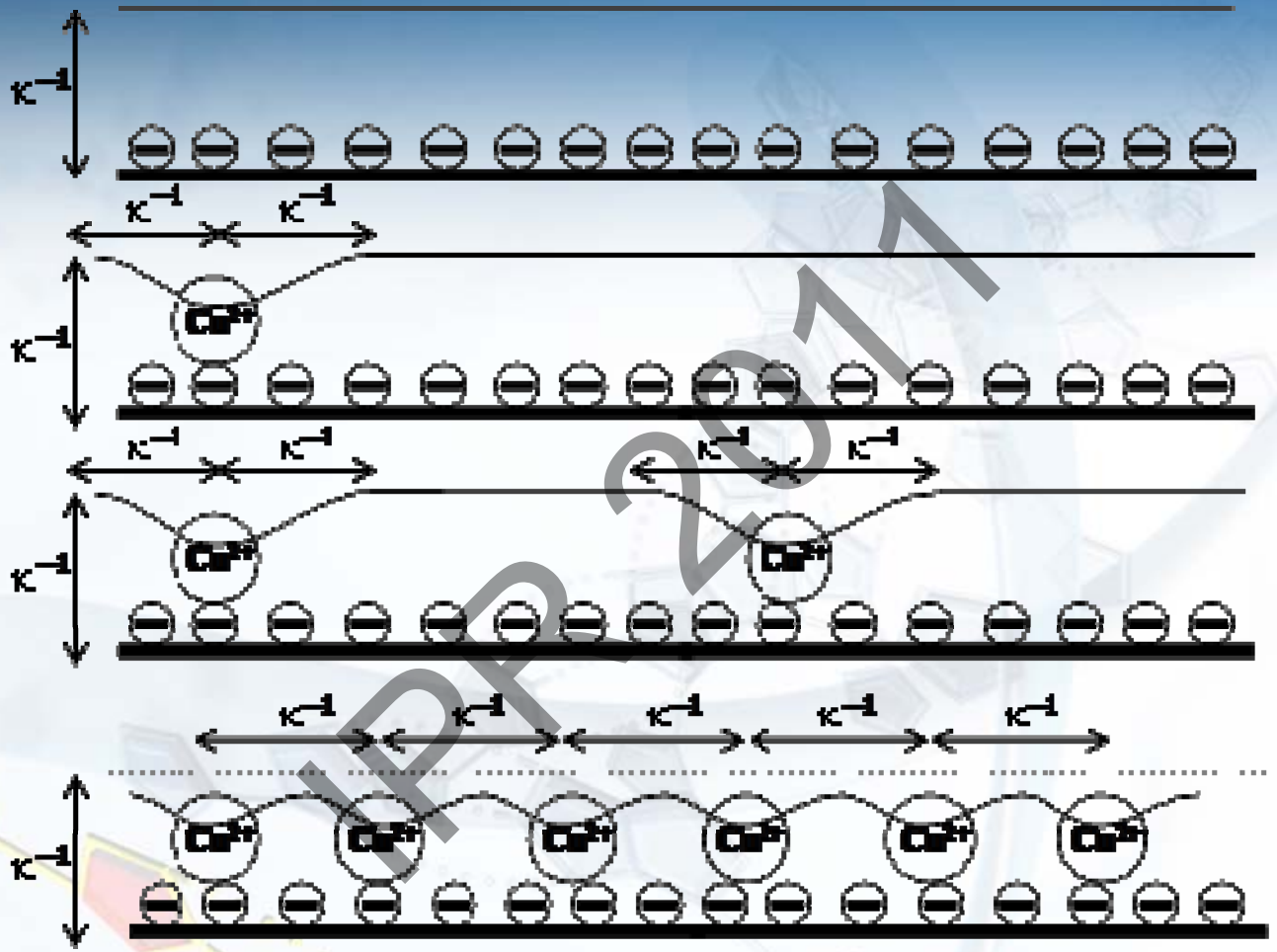
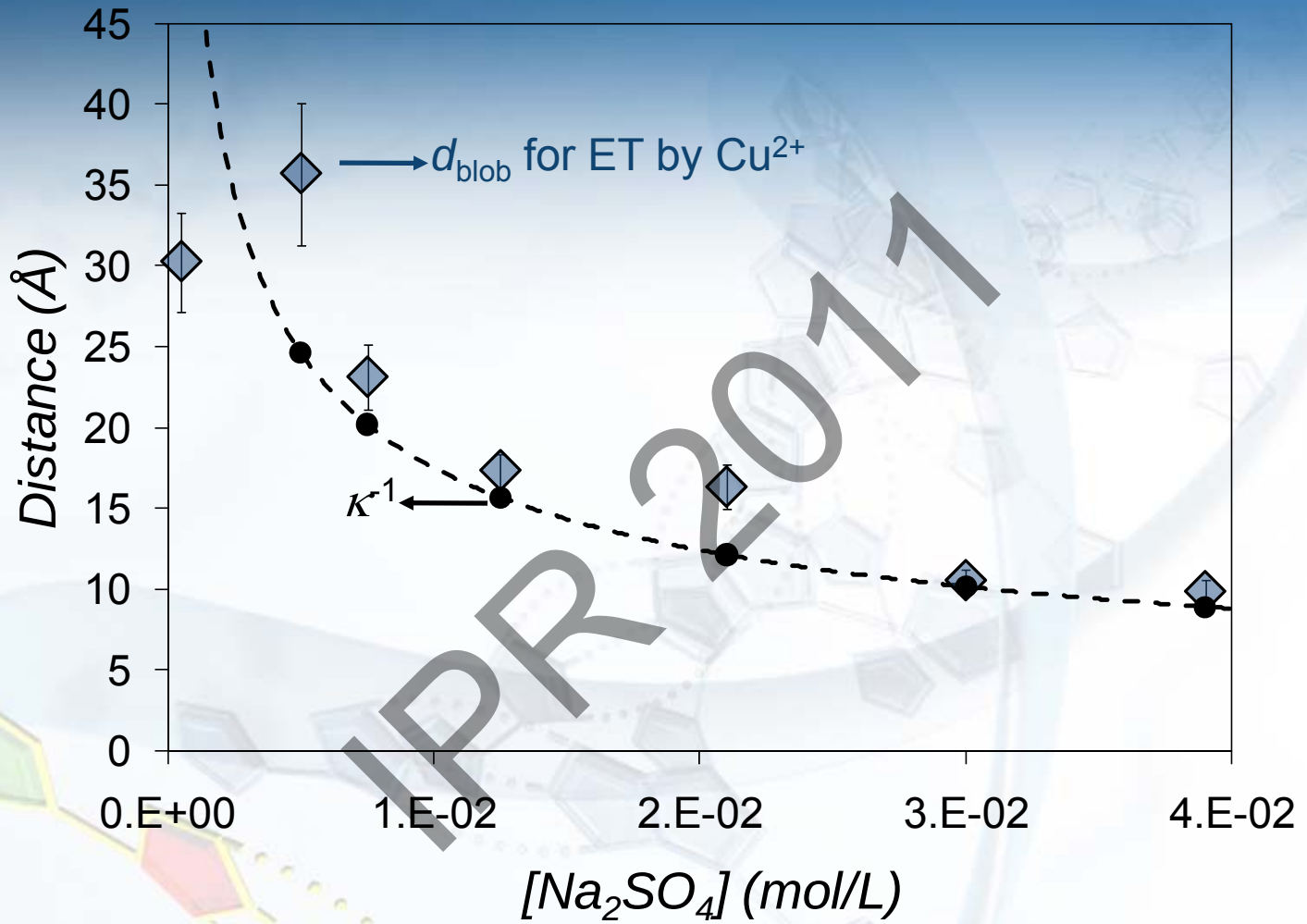
Ionic Strength - N_{blob}



$$\kappa^{-1} = \frac{1}{\sqrt{8\pi b_B N_A I}}$$

d_{blob} should be independent of divalent metal counterion

Ionic Strength - N_{blob}



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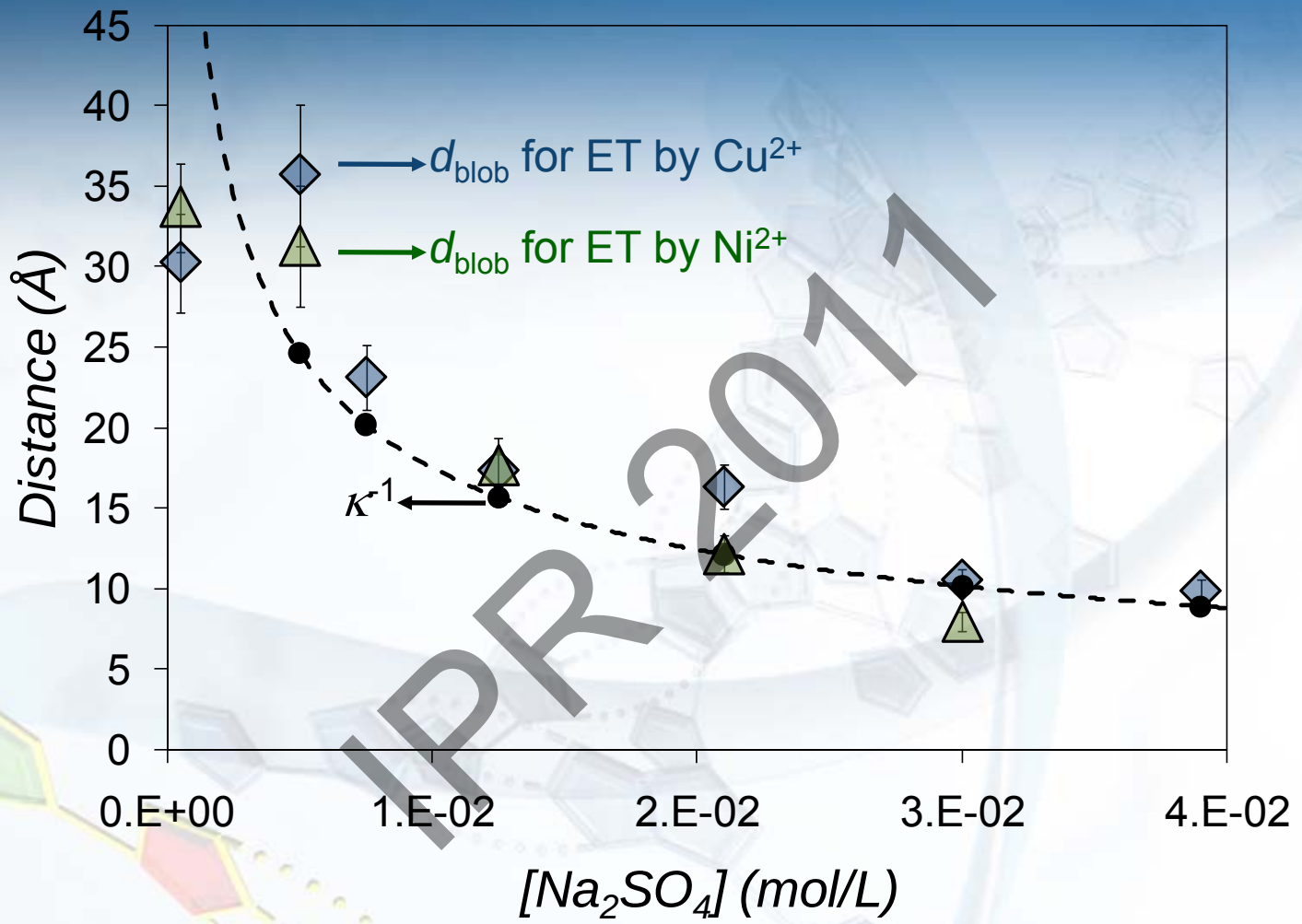
Ionic Strength -N_{blob}



$$\kappa^{-1} = \frac{1}{\sqrt{8\pi b_B N_A I}}$$

d_{blob} is independent of divalent metal counterion

Ionic Strength -N_{blob}

- d_{blob} does not depend on divalent metal cation
- d_{blob} should not depend on the lifetime of the chromophore
- Lifetime of DNA-EB doubles from 23 ns to about 40 ns in D_20 (copper could probe longer distances in D_20)
- d_{blob} (D_20) = 34.7 $\pm$ 4.4 Å
- d_{blob} (H_20) = 35.7 $\pm$ 4.4 Å

Ionic Strength -N_{blob}

- d_{blob} should also not depend on the charge of the metal cation quencher
- Recall:

$$\kappa^{-1} = \frac{1}{\sqrt{8\pi b_B N_A I}}$$

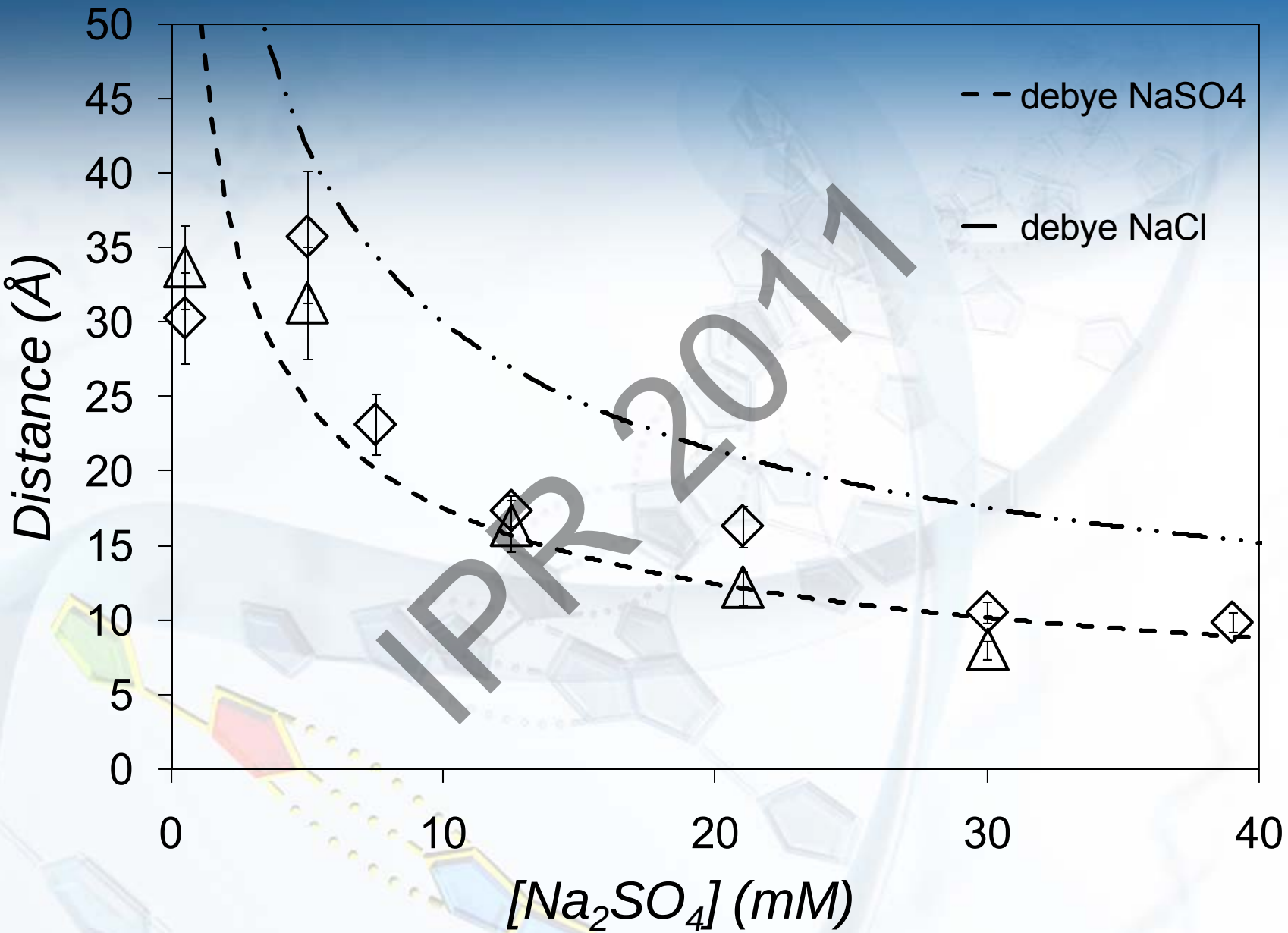
- Ag^+ was tested as a potential quencher of DNA-EB
- Complications arose due to a shifting of the fluorescence maximum

Ionic Strength -N_{blob}

- Ag^+ was tested as a potential quencher of DNA-EB
- Complications arose using silver as a quencher:
  - **Shift in the fluorescence maximum of DNA-EB**
  - **Solubility issues with sulfate as a counterion**
  - **Possible strong binding of silver to he bases of DNA even at low concentrations**

Ionic Strength -N_{blob}

- What happens to d_{blob} for the quenching of DNA-EB by copper cations for a different salt solution?
- A different salt solution such as NaCl yields a different Debye length
- If d_{blob} truly follows the Debye length then an increase in the Debye length should result in an increase in d_{blob}

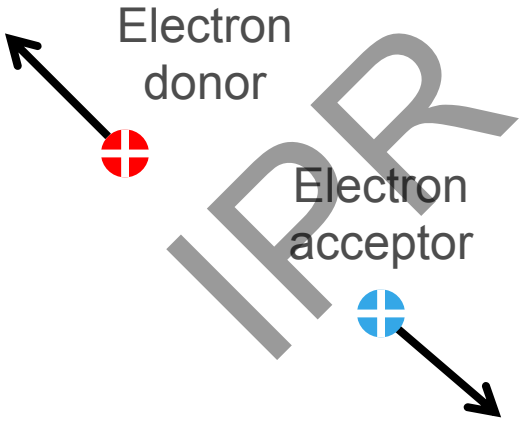

50
45
40
35
30
25
20
15
10
5
0
Distance (Å)
0
10
20
30
40
[Na2SO4] (mM)
debye NaSO4
debye NaCl

Summary

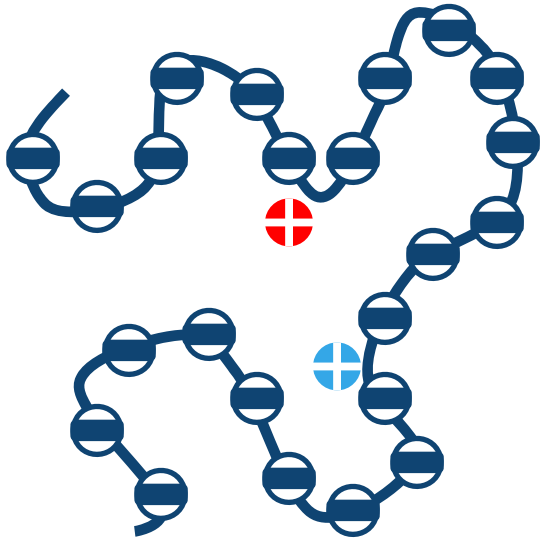
- FBM is successful in analyzing the complicated fluorescence decays acquired for ET between DNA-EB and divalent metal cations randomly distributed around the DNA helix
- $d_{blob} = \kappa^{-1}$ (at high salt) represents an average distance of minimal approach between divalent metal cations bound near the DNA helix

Summary

- The FBM enables one to characterize the complex electron transfer process in polyelectrolytes with a single parameter, namely N_{blob}.



Weak electron transfer



Strong electron transfer

Future Work

- Determine if $d_{blob} = \kappa^{-1}$ (at high ionic strength) for the quenching of DNA-EB by copper cations in NaCl solutions having different ionic strength

Acknowledgements

- Dr. Jean Duhamel
- Manoj Mathew
- Duhamel Lab Colleagues, both current and former
- NSERC


THANK YOU
QUESTIONS?
IPR 2011