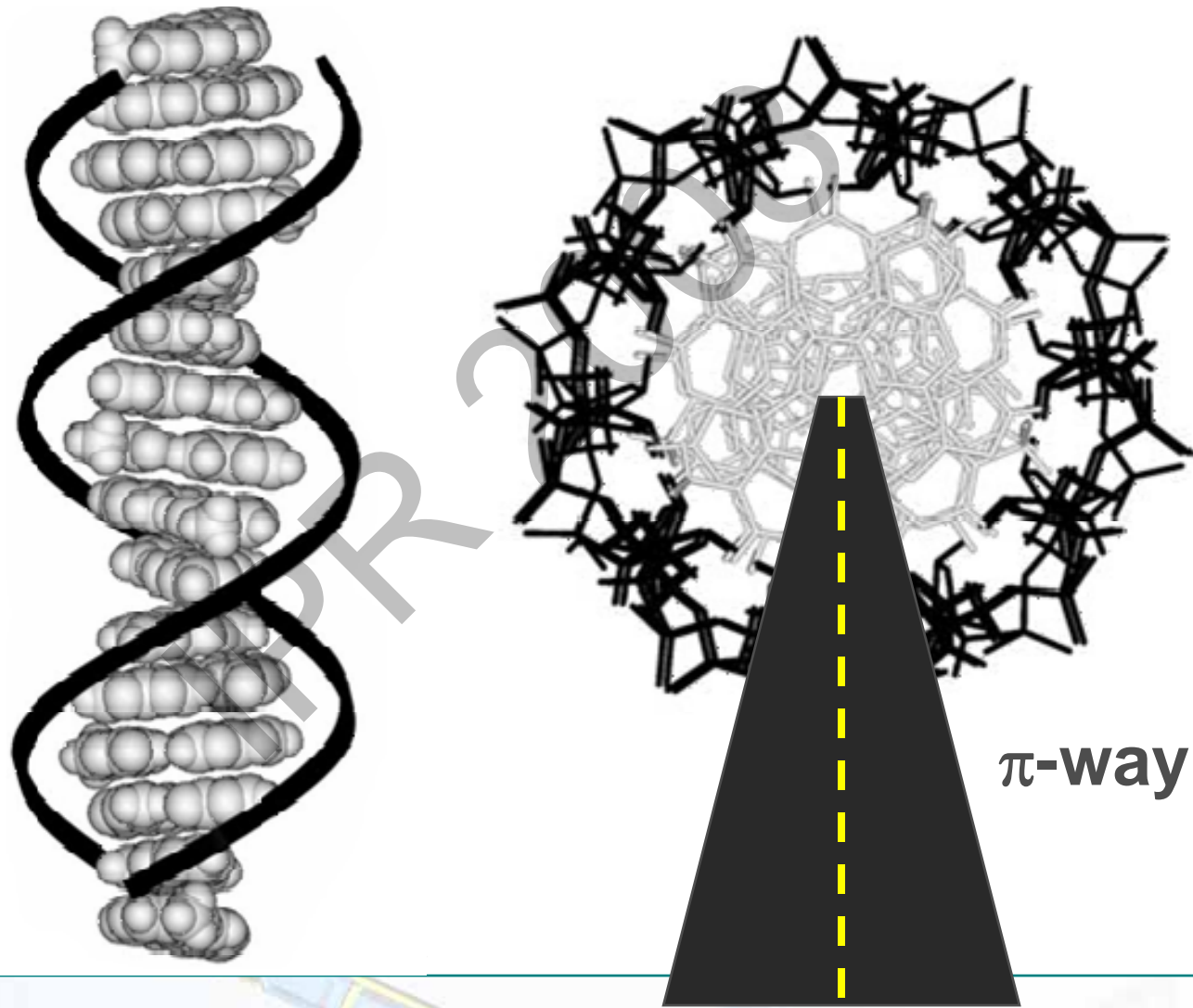


A 3D rendering of a DNA double helix. The structure is shown in a light blue color. The sugar-phosphate backbones are represented by thick, curved ribbons. The nitrogenous bases are shown as flat, pentagonal shapes. A specific sequence of bases is highlighted with yellow outlines: a green pentagon, a red pentagon, and a blue pentagon. Dotted lines connect these highlighted bases, suggesting a path of electron transfer. The background is a light blue gradient with faint, larger-scale DNA helix structures.

Electron Transfer in DNA: A Fluorescence Blob Model Approach

Christine Keyes

DNA Double Helix



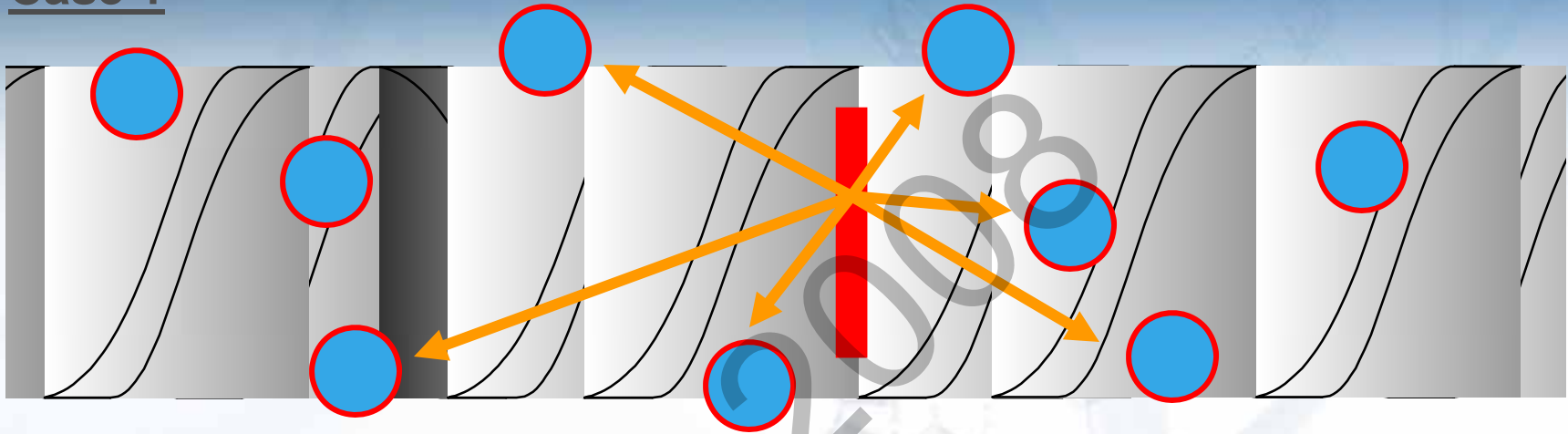


Overview – Electron Transfer in DNA

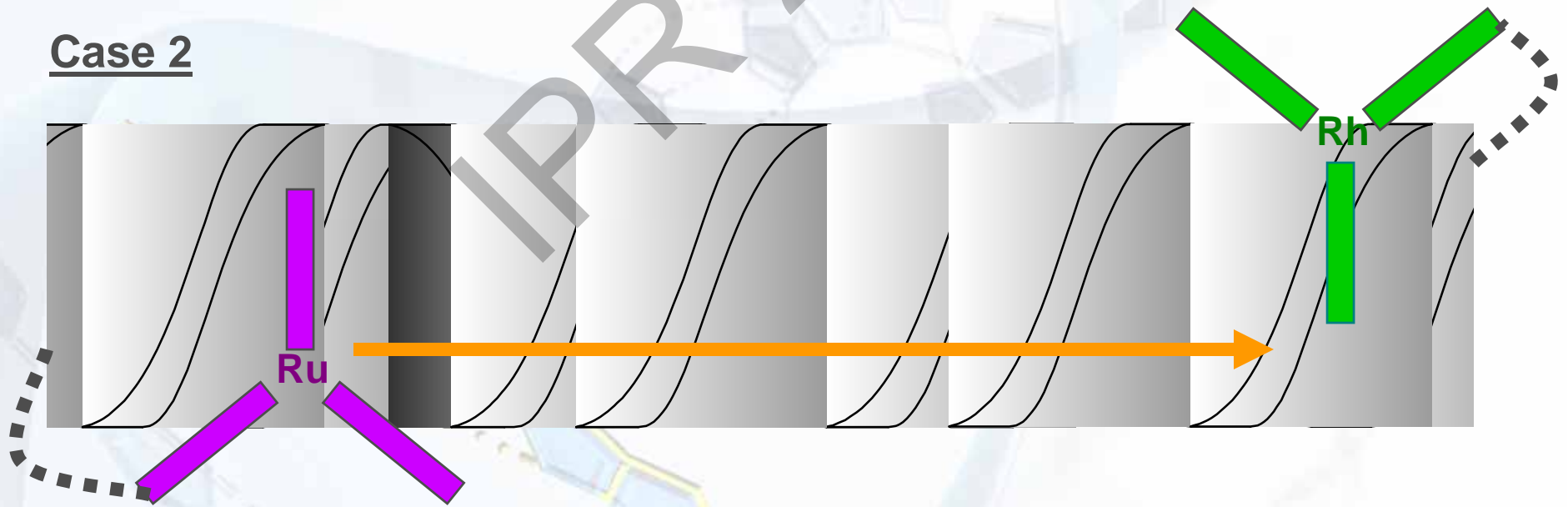
- Two cases:
 1. **Non covalently attached electron donor and acceptor**
 - Unmodified DNA
 - Distance between donors and acceptors is random
 - Fluorescence data analyzed on a case by case basis
 2. **Covalently attached electron donor and acceptor**
 - Known distance between donor and acceptor
 - Fluorescence data yields quantitative values for the rates of electron transfer for a known distance

Overview – Electron Transfer in DNA

Case 1



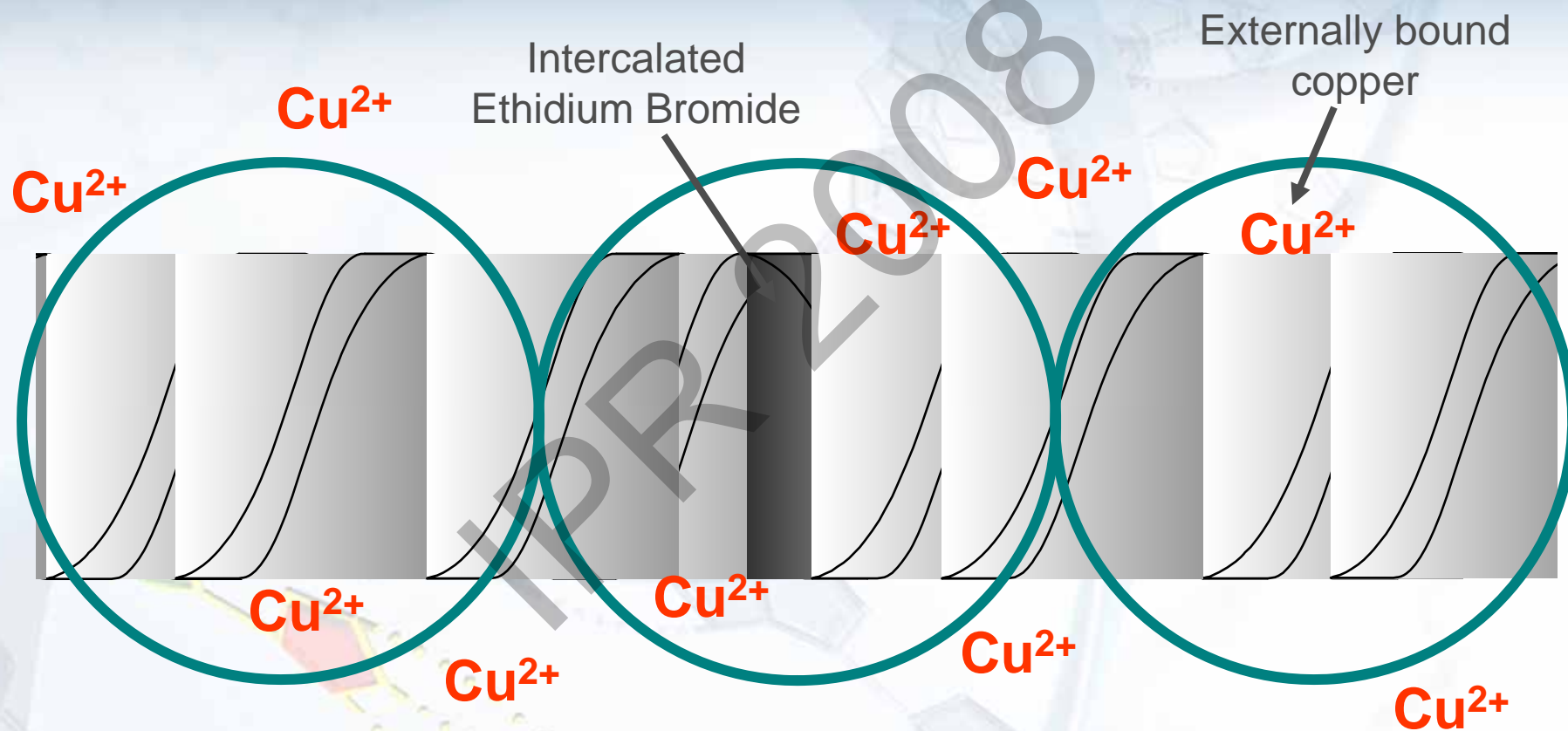
Case 2



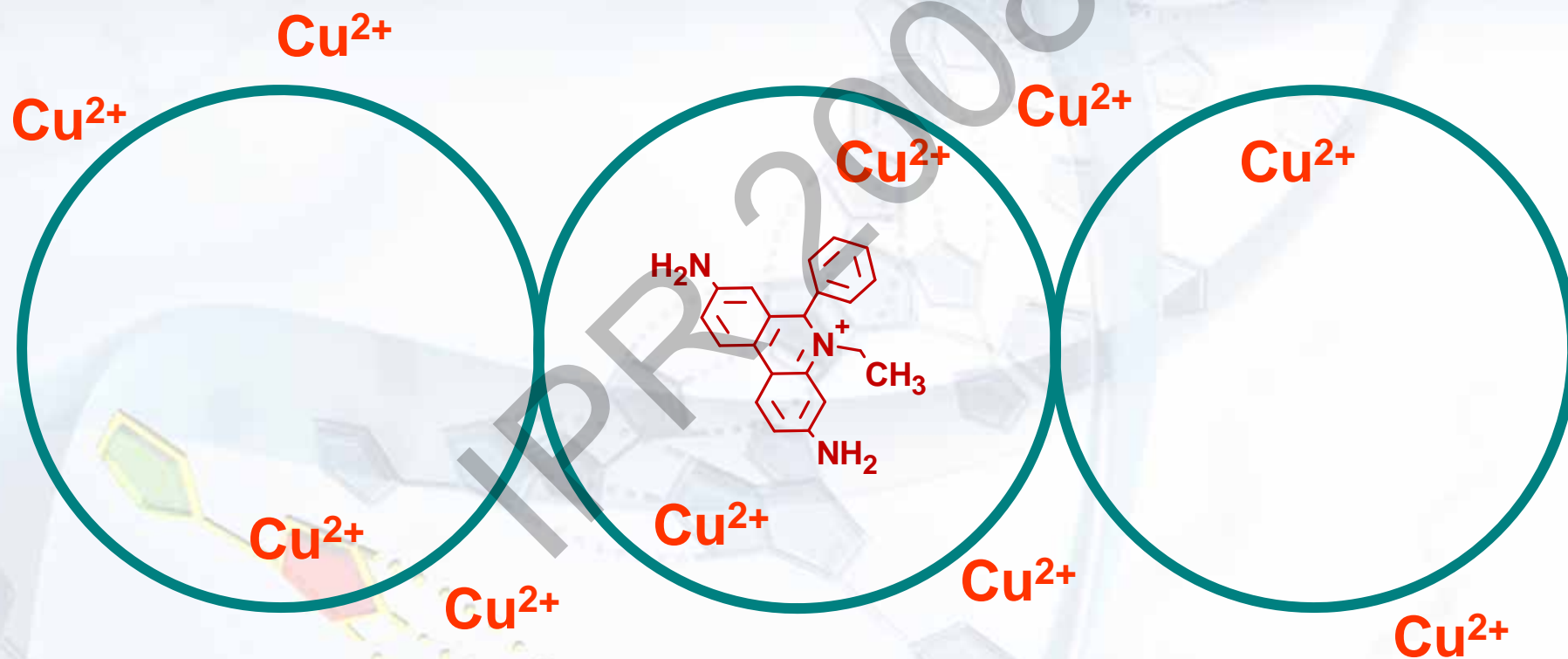
Electron Transfer (ET) in DNA -Previous Studies

Donor and Acceptor	Covalently attached	d [Å]	k_{ET} (s ⁻¹)	Research group	Year
E and Cu ²⁺ , Co ²⁺ , Ni ²⁺	no	7 – 10	10 ⁸	Atherton/Beaumont	1986
E and MV ²⁺	no	-	10 ⁵	Fromherz	1986
E, AO and DAP ²⁺	no	10 - 17	10 ⁸	Harriman	1992
Tethered Ru ^{II} and Rh ^{III} intercalators	yes	> 40	10 ⁹	Barton	1993
Ru ^{II} and Rh ^{III} ribose complexes	yes	27	10 ⁶	Meade	1995
Ru ^{II} and Rh ^{III} intercalators	no	-	10 ¹⁰	Barton	1996
Tethered E and Rh ^{III} intercalators	yes	17 - 36	10 ¹⁰	Barton	1997
Intercalated acridine and guanine	yes	3 - 10	10 ⁵ - 10 ¹⁰	Tanaka	1998
E intercalator and Z	yes	10 - 17	10 ¹²	Barton/Zewail	1999
Daunomycin and Au electrode	yes	82	10 ²	Barton/Hill	2005

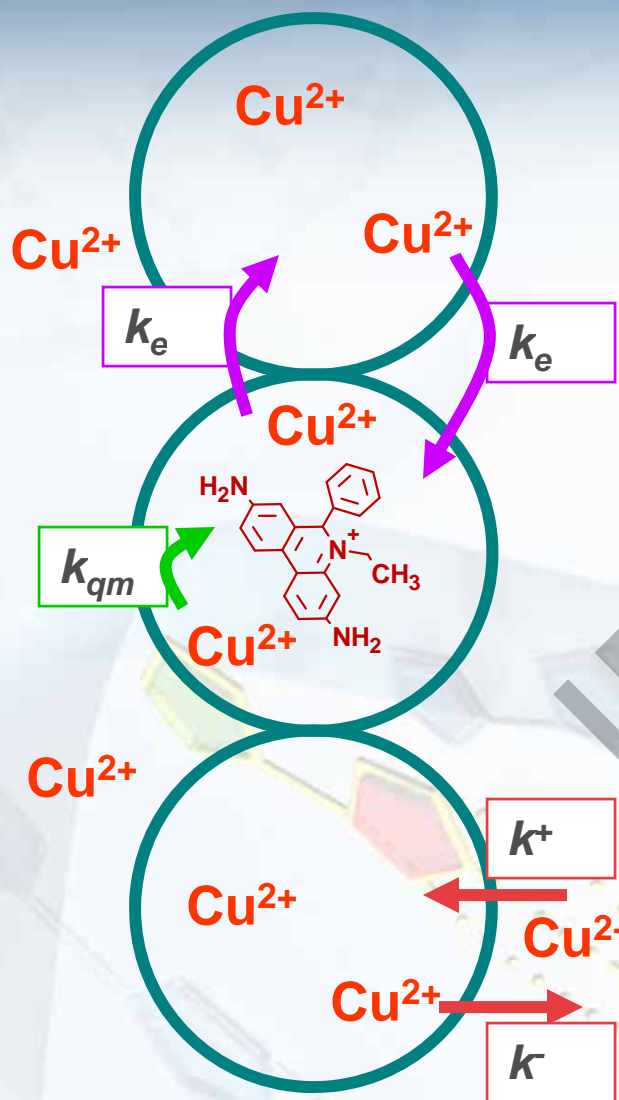
Micellar Approach to ET in DNA



Micellar Approach to ET in DNA

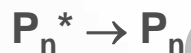


Micellar Approach to ET in DNA



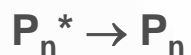
Process

Rate



$$k^o[P_n^*]$$

fluorescence



$$nk_{qm}[P_n^*]$$

quenching



~~$$k^+[P_n^*][Q_a]$$~~

~~quencher adsorption~~



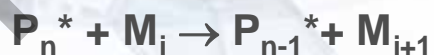
~~$$nk[P_n^*]$$~~

~~quencher desorption~~



$$jk_e[P_n^*][M_j]$$

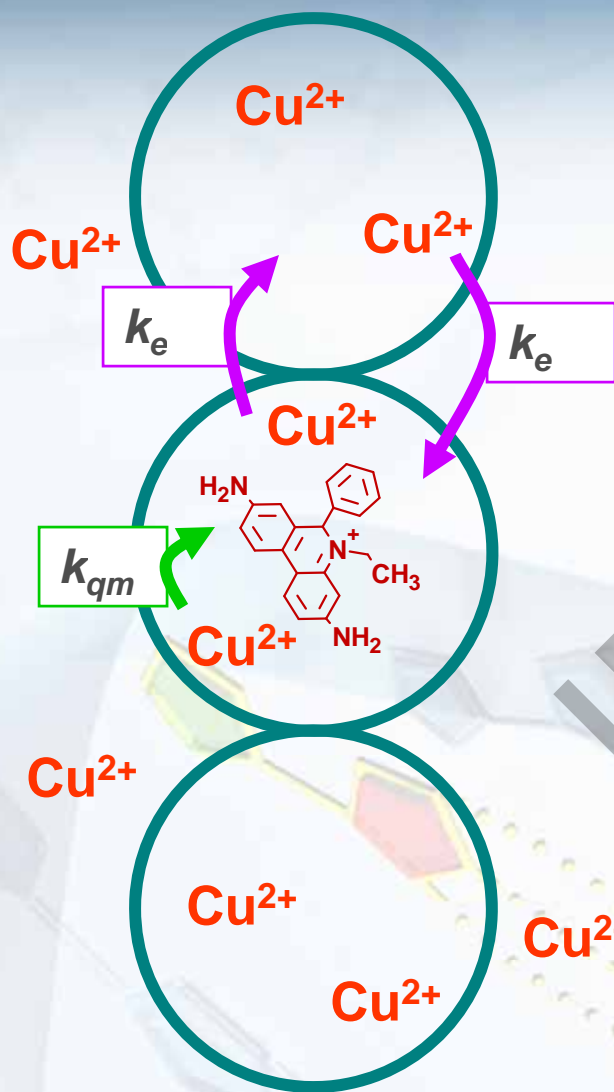
quencher exchange



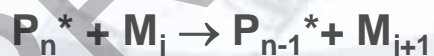
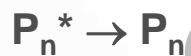
$$nk_e[P_n^*][M_j]$$

quencher removal

Fluorescence Blob Model (FBM): Approach to ET in DNA



Process



Rate

$$k^o[P_n^*]$$

$$nk_{qm}[P_n^*]$$

$$jk_e[P_n^*][M_j]$$

$$nk_e[P_n^*][M_j]$$

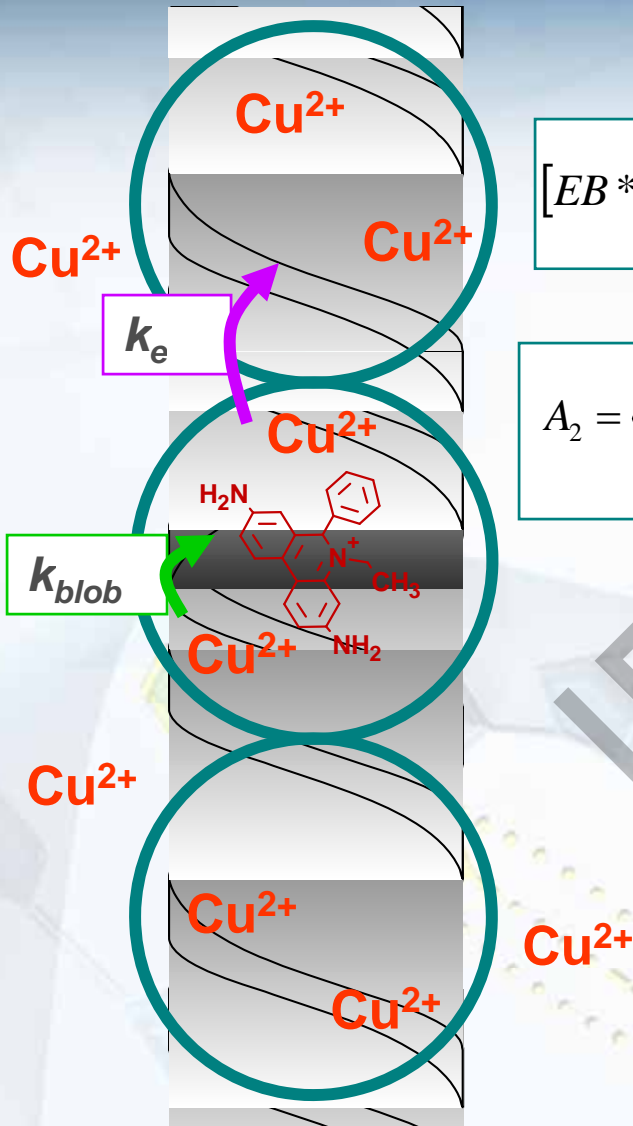
fluorescence

quenching

quencher exchange

quencher removal

Fluorescence Blob Model (FBM): Approach to ET in DNA



$$[EB^*]_t = f_{diff} \exp\left[-\left(A_2 + \frac{1}{\tau_{EB}}\right)t - A_3(1 - \exp(-A_4 t))\right] + f_{fast} \exp(-t / \tau_{fast})$$

$$A_2 = \langle n \rangle \frac{k_{blob} k_e [blob]}{k_{blob} + k_e [blob]} \quad A_3 = \langle n \rangle \frac{k_{blob}^2}{(k_{blob} + k_e [blob])^2} \quad A_4 = k_{blob} + k_e [blob]$$

$\langle n \rangle \rightarrow$ average number of quenchers per blob

$k_{blob} \rightarrow$ quenching rate constant of EB inside a blob

$k_e [blob] \rightarrow k_e$ is the rate constant to exchange a quencher between blobs and $[blob]$ is the local blob concentration



N_{blob} and K

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

$$\langle n \rangle = \frac{[\text{Cu}^{2+}]_b - [\text{Cu}^{2+}]_o}{[blob]}$$

N_{blob} and K

$$\langle n \rangle = \frac{[Cu^{2+}]_b - [Cu^{2+}]_o}{[blob]}$$

Dependant on $[DNA]$

Proportional to N_{blob}



$$[Cu_b^{2+}] = \frac{[Cu^{2+}]}{\frac{1}{K[DNA]} + 1}$$

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

N_{blob} and K

$$\langle n \rangle = \frac{Cu_T^{2+}}{1 + \frac{[DNA]}{KN_{blob}}} - \frac{Cu_o^{2+} N_{blob}}{[DNA]}$$

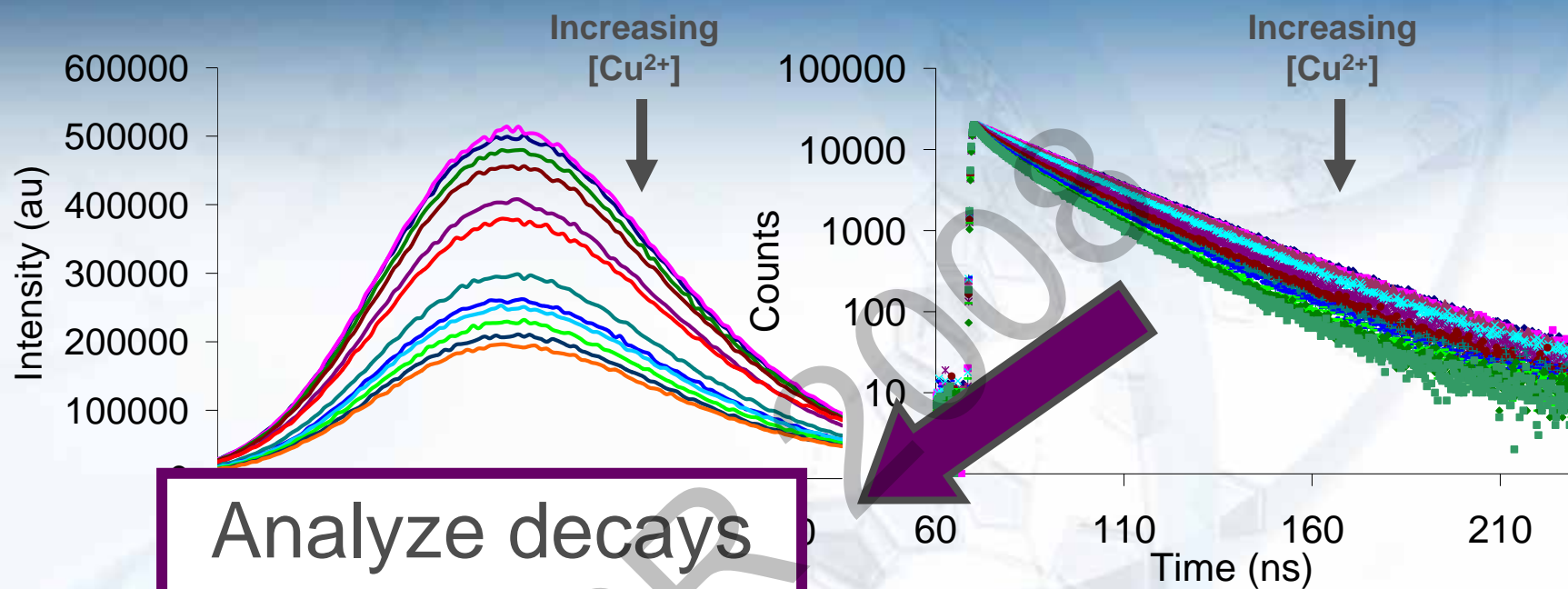
- $\langle n \rangle$ vs. $[Cu^{2+}]_T$ gives:

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

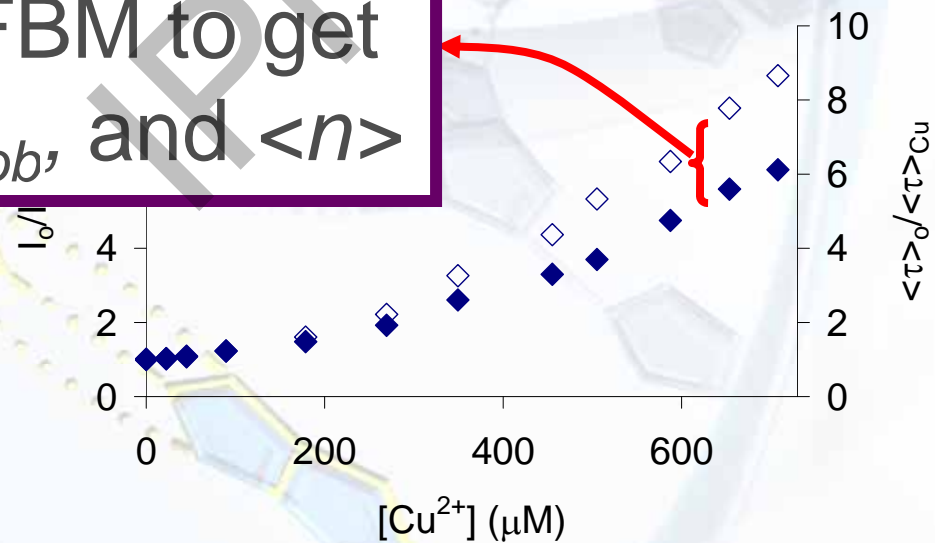
$$\frac{1}{slope} = \frac{1}{KN_{blob}} + \frac{[DNA]}{N_{blob}}$$

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

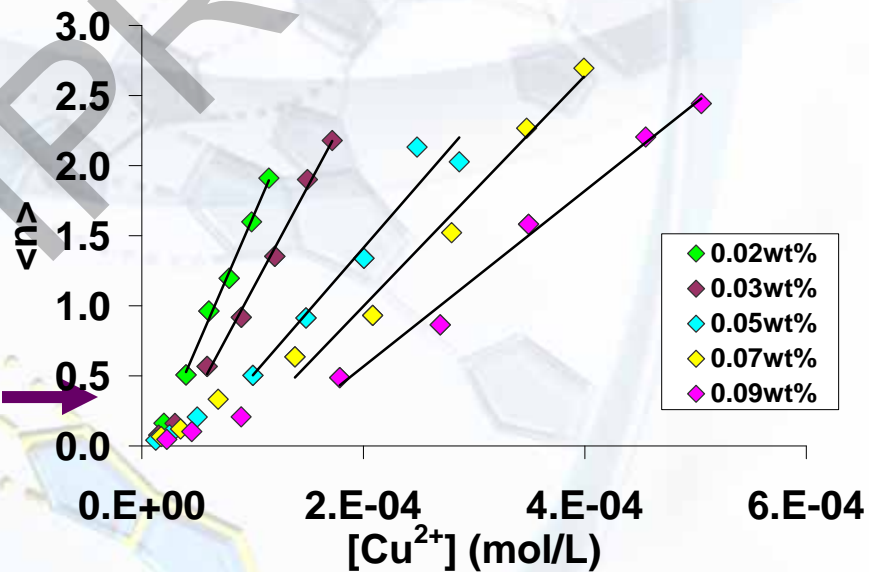
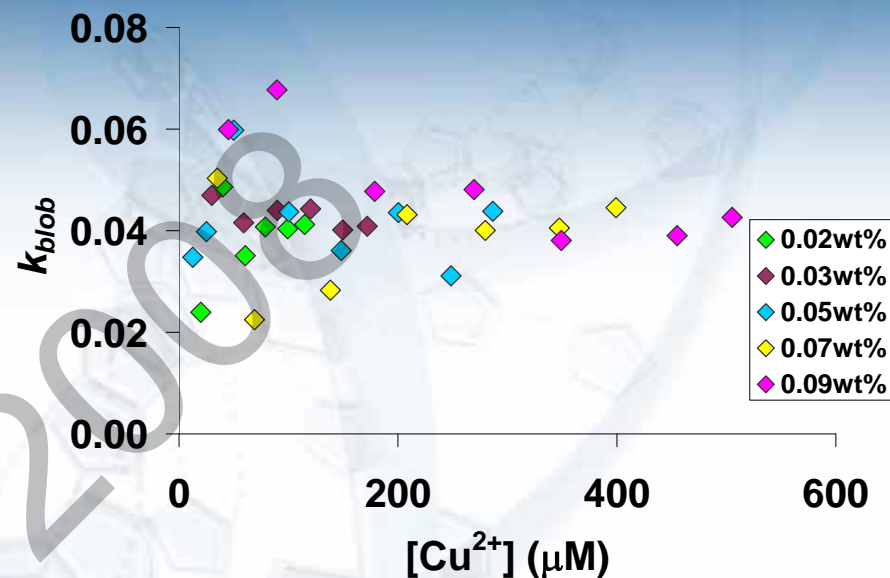
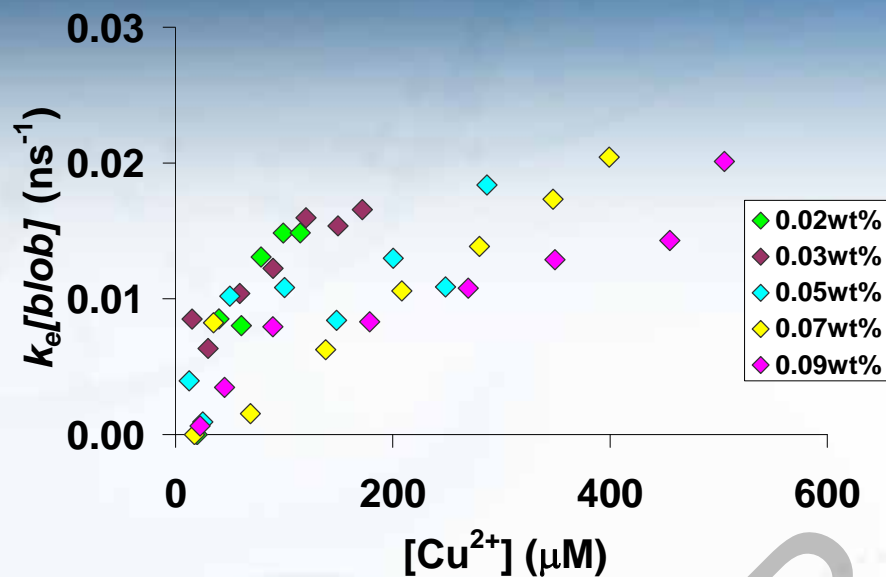
FBM Results



Analyze decays
with FBM to get
 k_e , k_{blob} , and $\langle n \rangle$

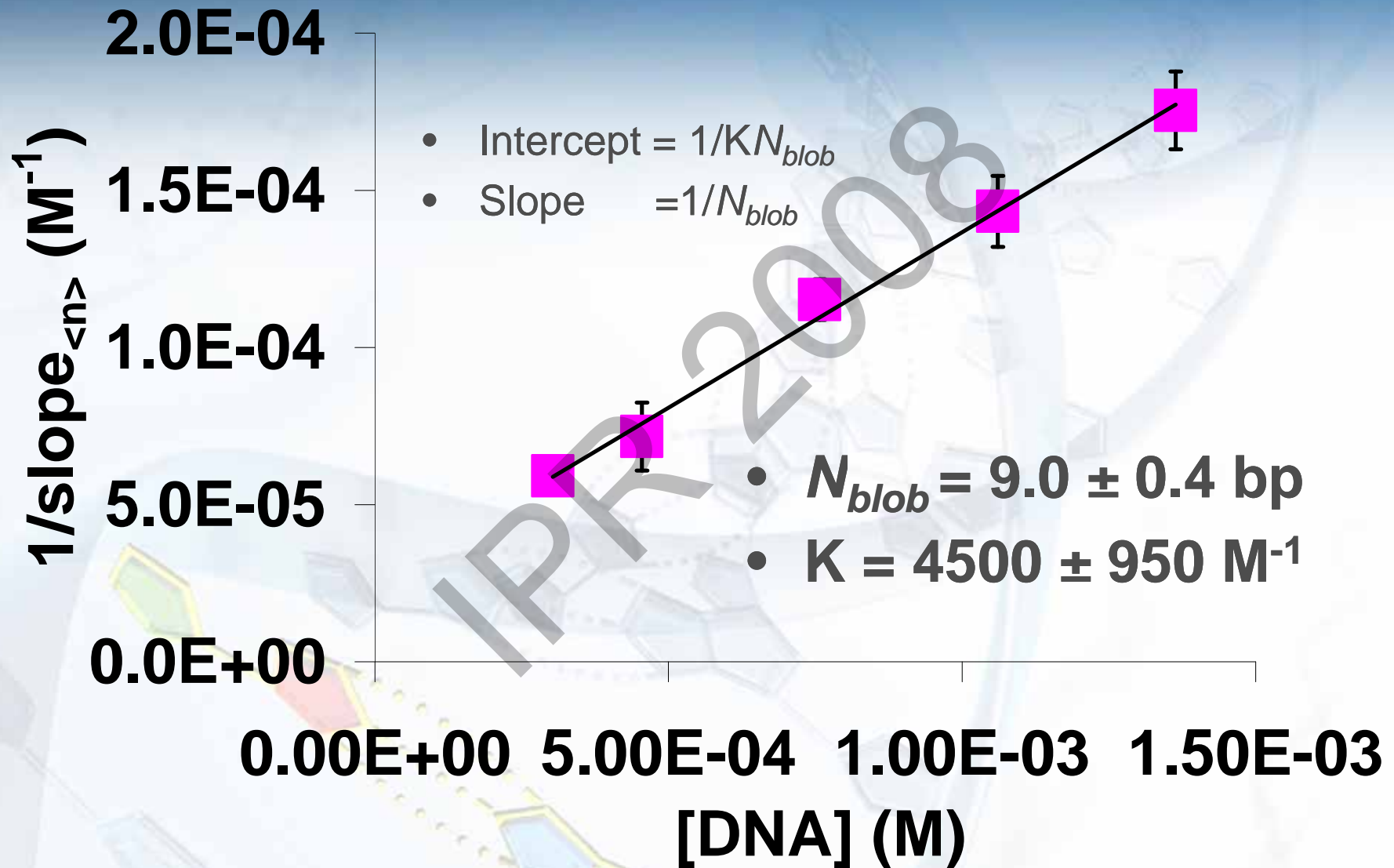


FBM Results

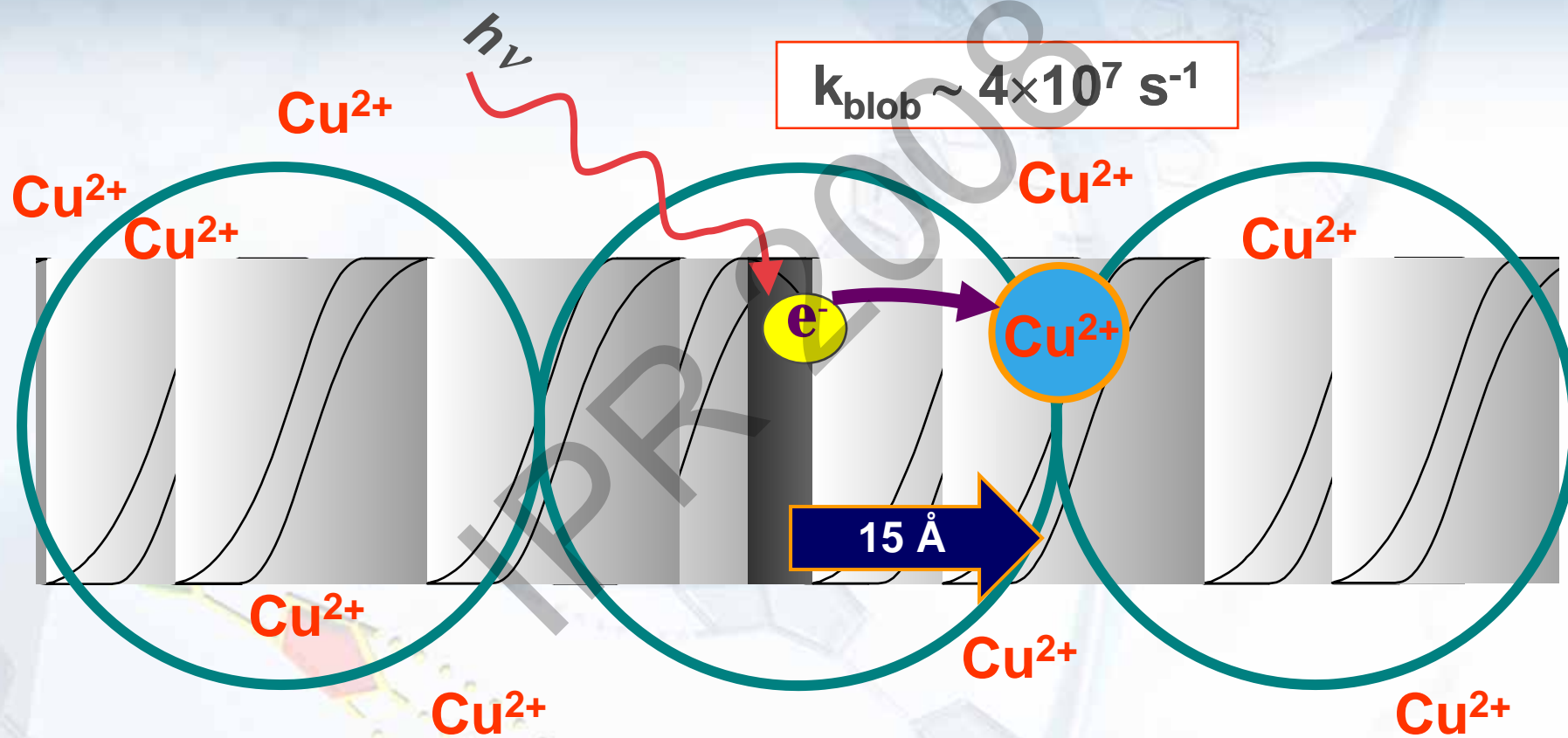


Find slopes beyond $[\text{Cu}^{2+}]$ onset for each [DNA] – plot slope⁻¹ vs [DNA]

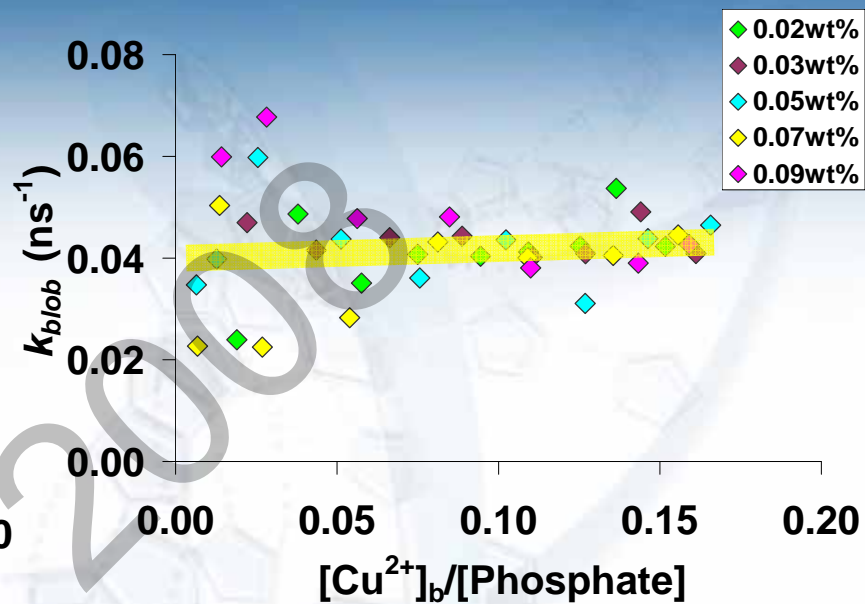
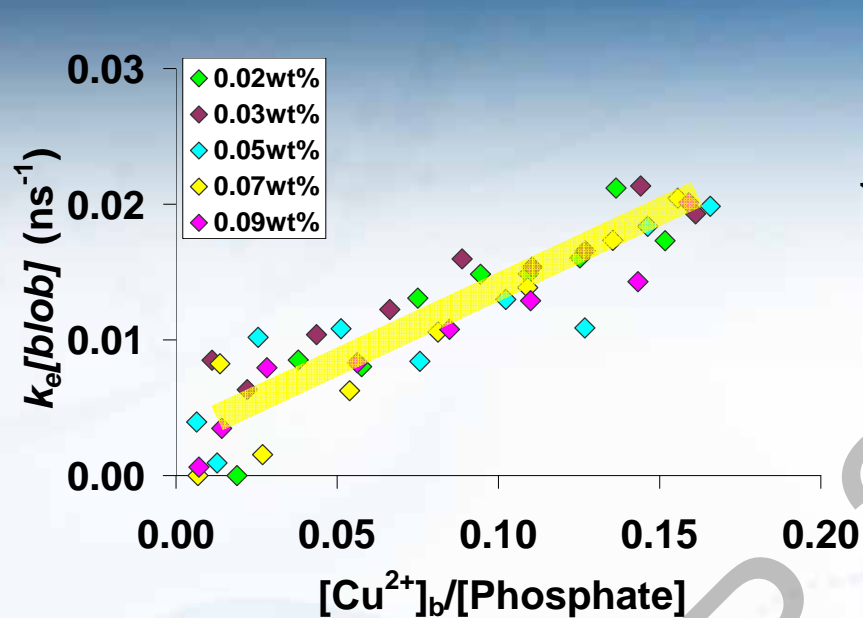
FBM Results – N_{blob} and K



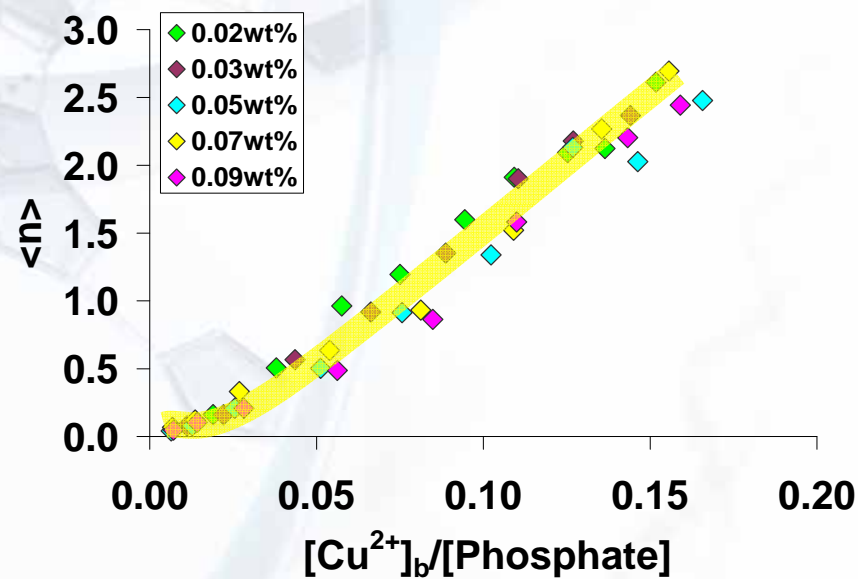
FBM Results



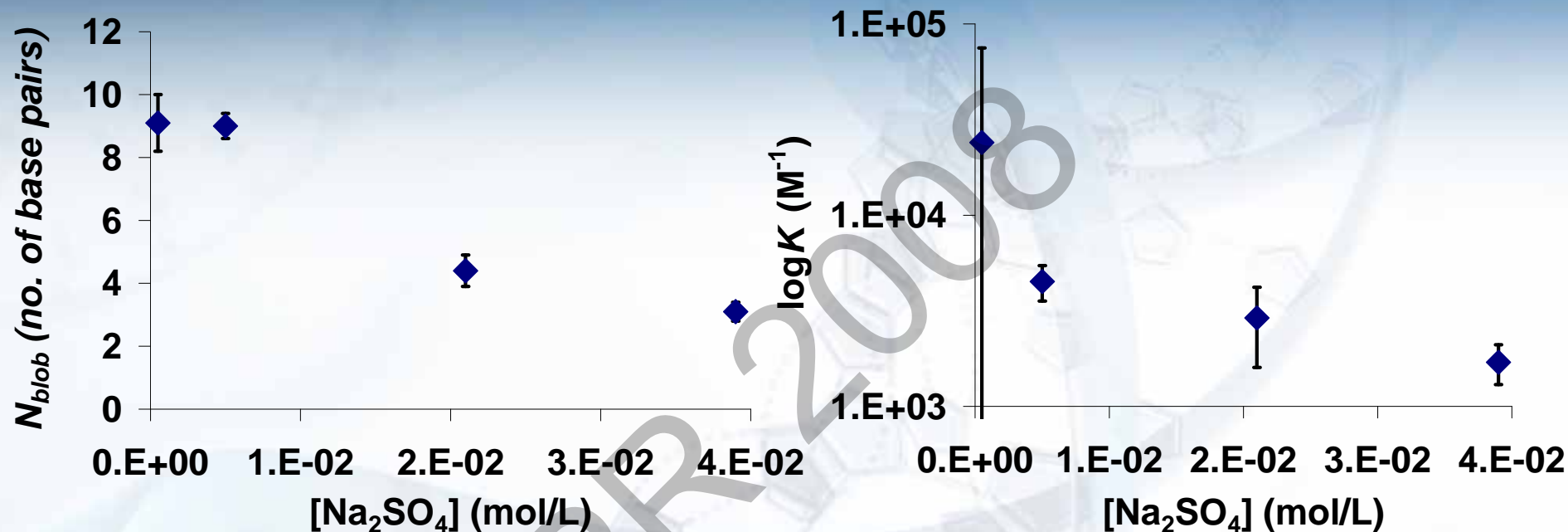
FBM Results



Plot FBM parameters in terms of the concentration of copper bound divided by the DNA concentration



Salt Effect – N_{blob} and K



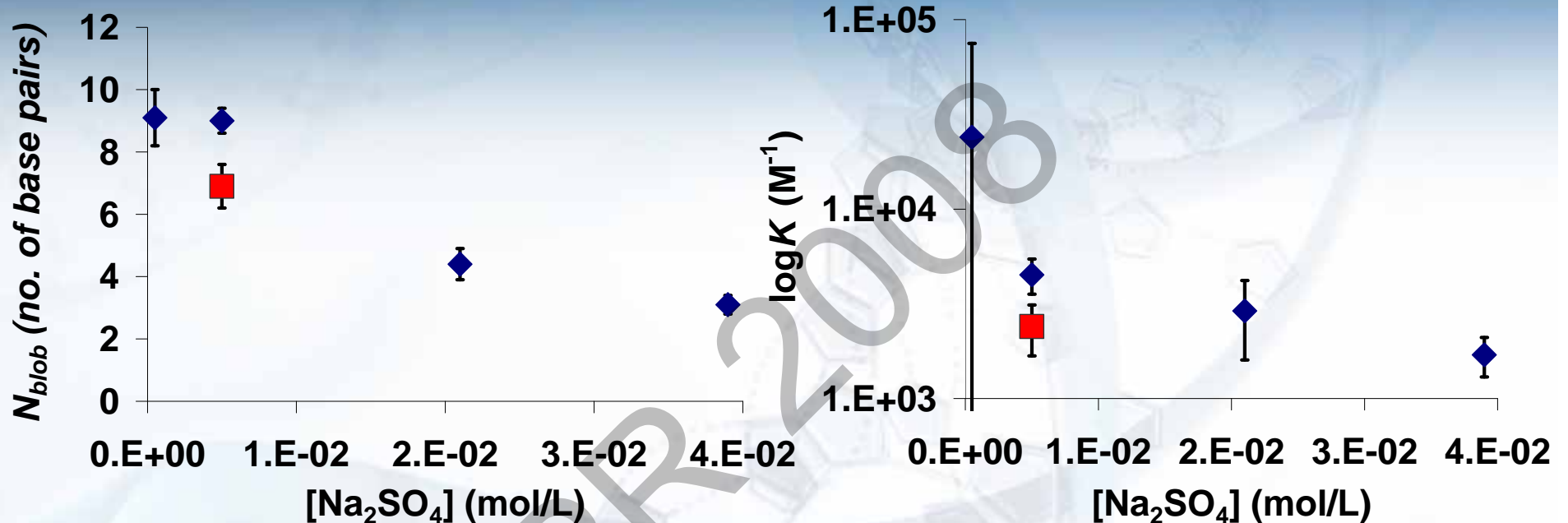
- Binding constant, K , decreases with increasing salt
 - Copper binds less tightly to DNA with increasing Na^+
- N_{blob} decreases with increasing salt
 - Electron transfer is less efficient with increasing Na^+



Viscosity Effect – FBM Parameters

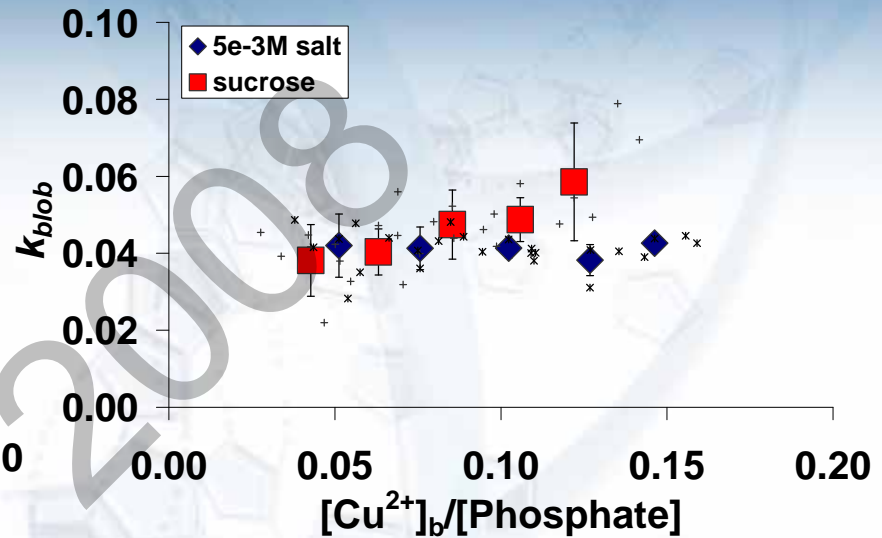
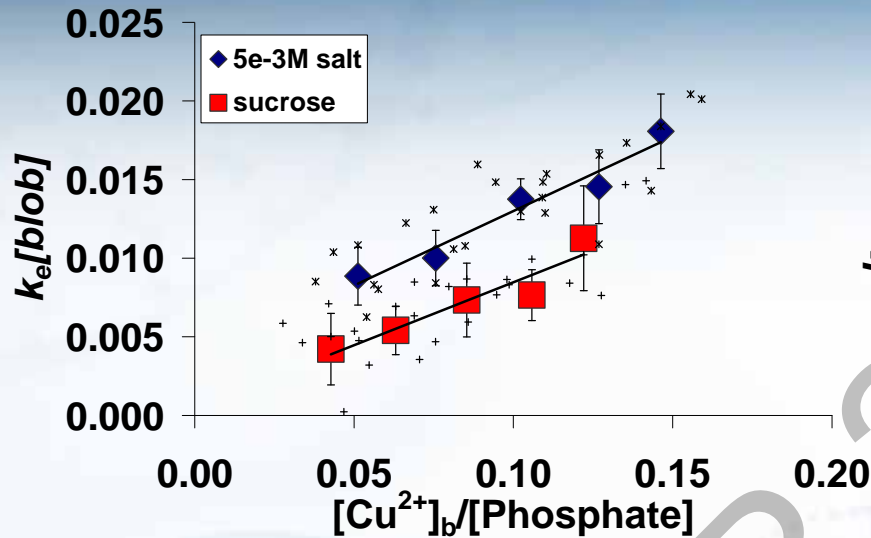
- Obtain N_{blob} and K for solutions containing 50% (w/w) sucrose
- Viscosities:
 - water = 0.89 cP
 - 50% (w/w) sucrose = 9.6 cP
- Is the change in viscosity reflected in the FBM parameters?

Viscosity Effect – N_{blob} and K

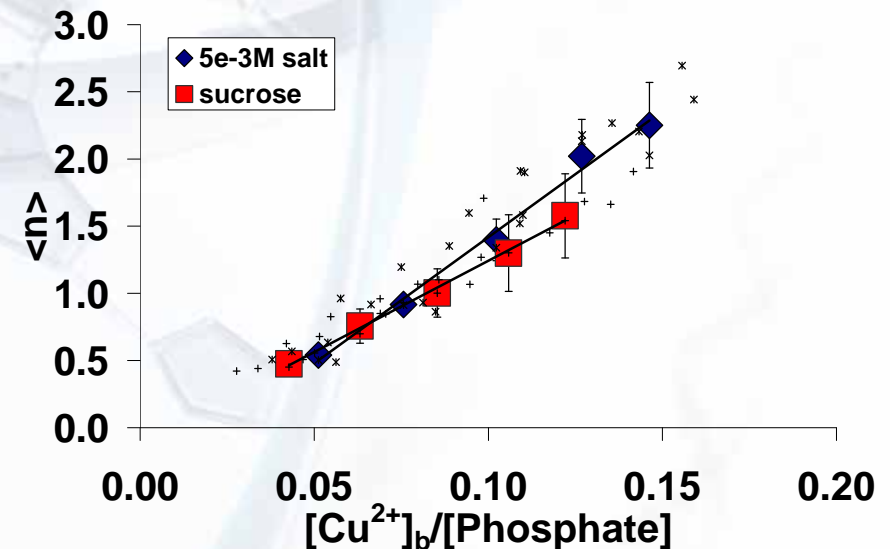


- Binding constant, K , decreases with increasing viscosity
- N_{blob} decreases with increasing viscosity but the effect is much less compared to the addition of salt

Viscosity Effect – FBM parameters



FBM parameters not changed much by viscosity – what happens locally is independent of what happens in the bulk solution

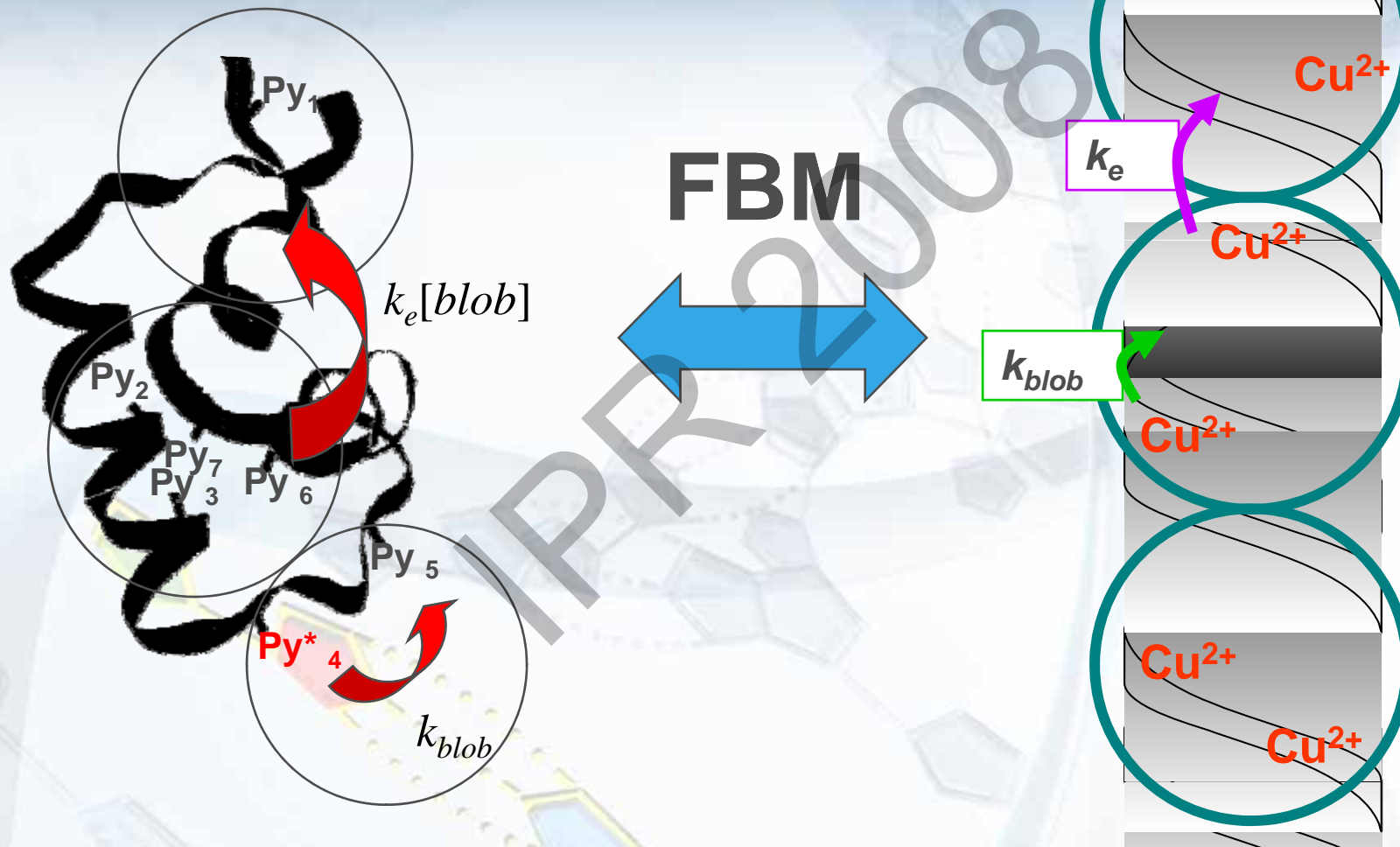




Conclusions

1. Quantitative information on electron transfer was obtained between unspecifically bound donors and acceptors and the analysis was sound no matter what effect was studied
 - At low monovalent salt concentrations electron transfer occurred between intercalated ethidium bromide and externally bound copper over 9 base pairs with a rate constant of $4 \times 10^7 \text{ s}^{-1}$
2. The FBM was used to describe a system where the fluorophore and its quencher have not been covalently attached to a polymer backbone

Conclusions





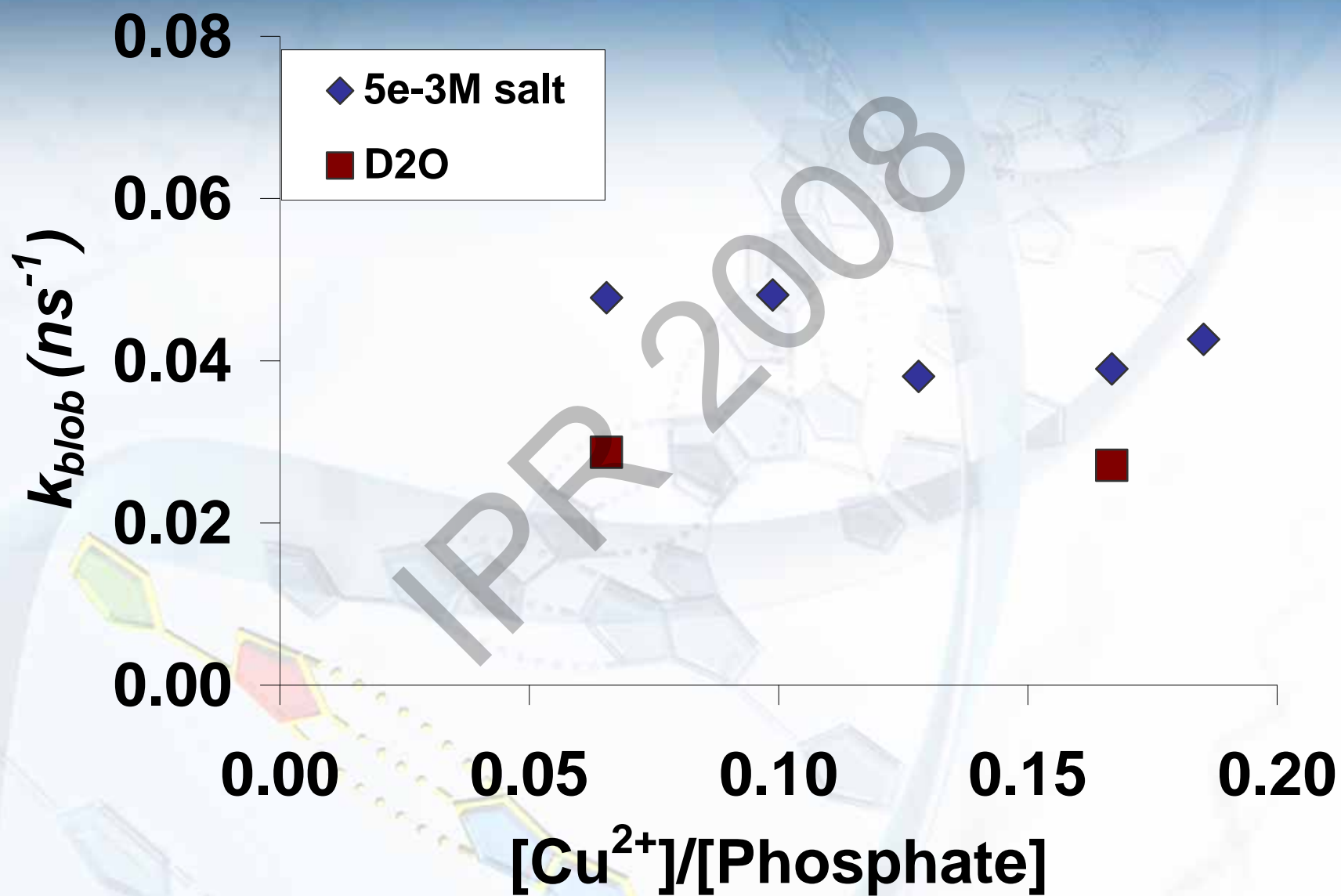
Future Work

- Find the β parameter
 - β is the damping factor exerted by the medium for the electron to pass through from the donor to acceptor site

$$\ln(k_{et}) \propto -\beta r$$

- Change $N_{blob}(r)$ by increasing or decreasing the lifetime of ethidium bromide intercalated in DNA (ex. D_2O)
- Find k_{blob}

Future Work





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

IPR 2008



**THANK YOU
QUESTIONS?**