Probing Long Range Chain Dynamics of a Polypeptide in Aqueous Solution

IPR 2010

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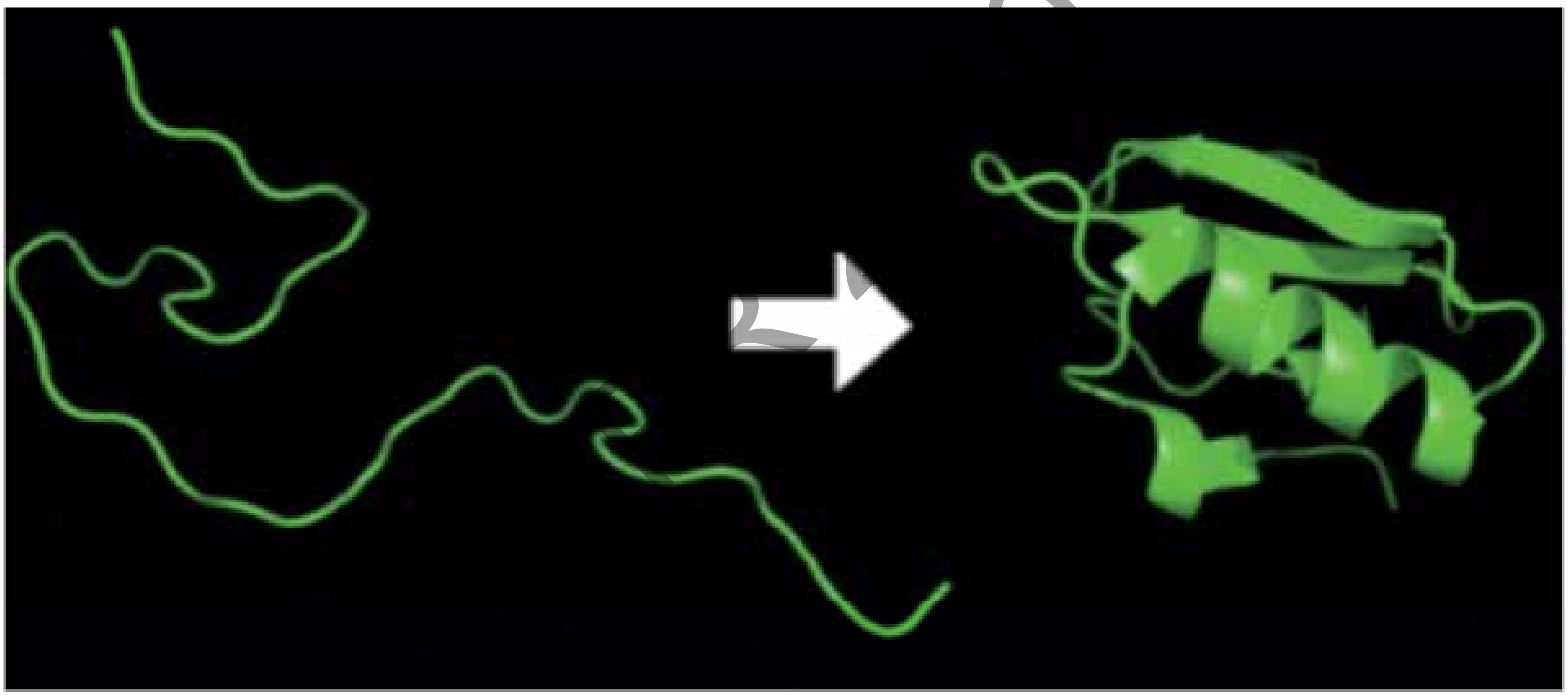
May 11, 2010

Purpose

- Protein folding

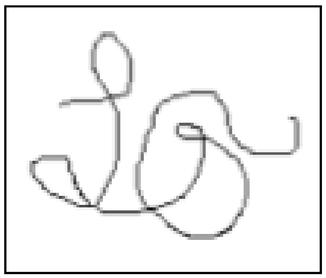


http://www.chemguide.co.uk/organicprops/aminoacids/proteinstruct.html

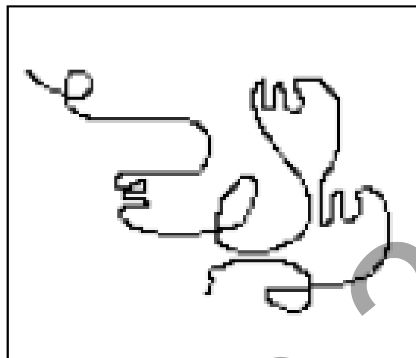
Purpose

Protein Folding

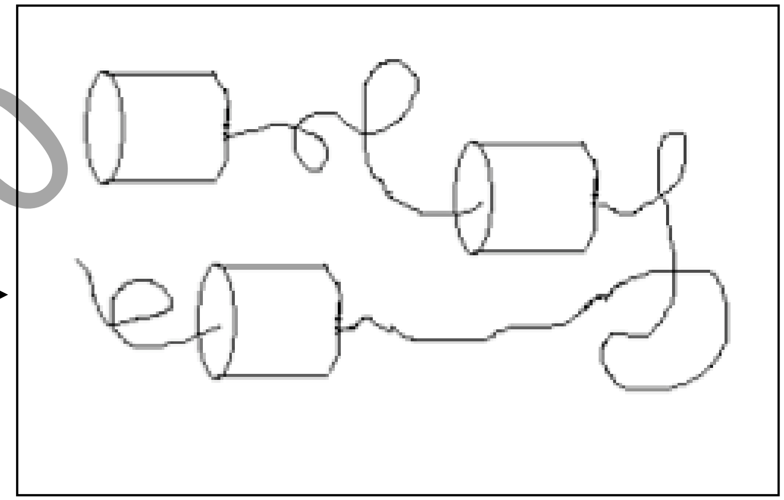
1) Random coil



2) Hydrophobic collapse

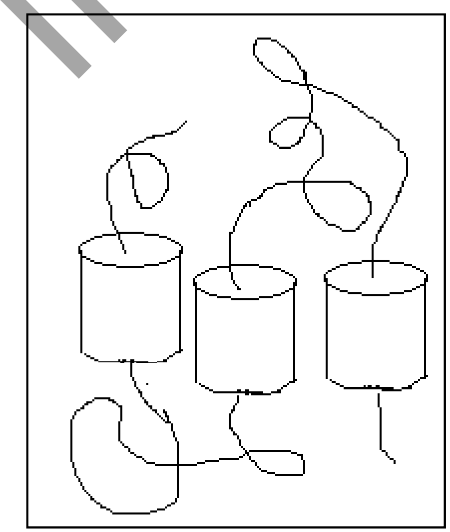


3) Formation of secondary structures



1) → 2) LRPCD

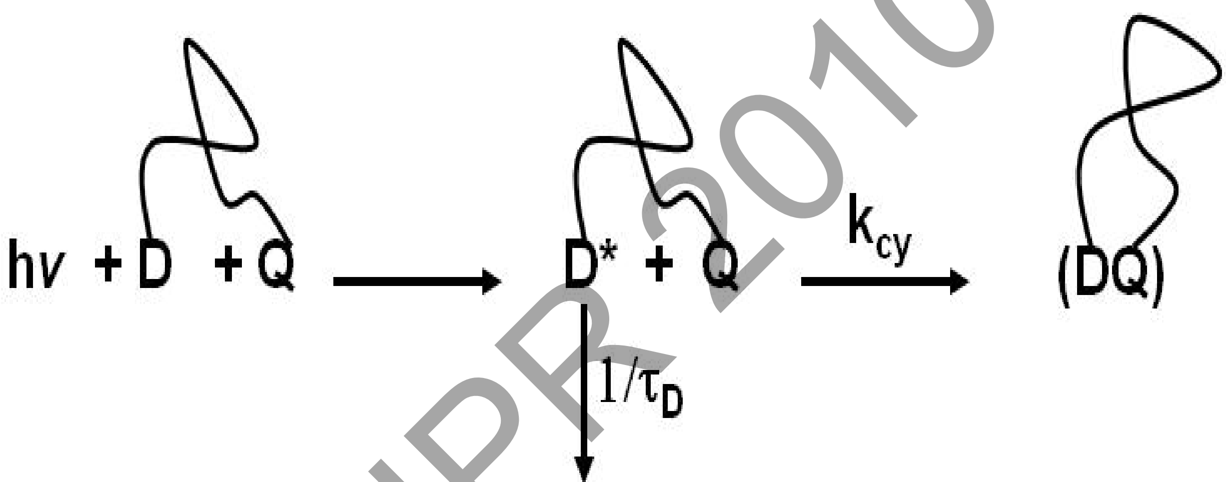
4) Tertiary structure = structure of folded protein



LRPCD = Long range polymer chain dynamics

Fluorescence Dynamic Quenching (FDQ)

Covalent attachment of a luminophore (D) and its quencher (Q) at the ends of a polymer chain



- Measurement of the rate constant, k_{cy}, for quenching of the fluorescing luminophore through end-to-end cyclization (EEC)

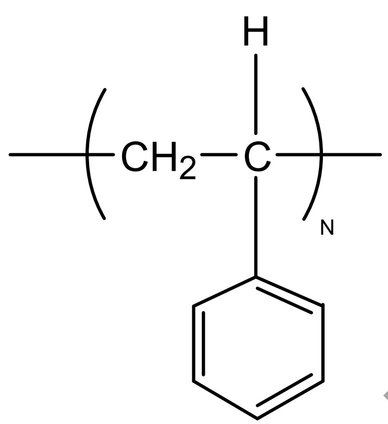
- k_{cy} and chain length (*N*) will give information on the flexibility of the polymer chain

Winnik, M. A. *Acc. Chem. Res*. **1985**, 18, 73-79.

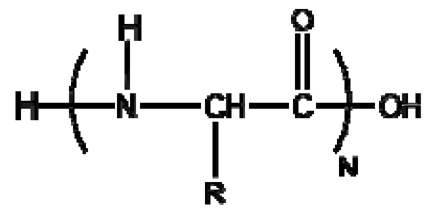
End-to-End Cyclization (EEC)

Limitations

- Bulk of the chain invisible
- Limited to monodisperse chains
- Cyclization rate constant (k_{cy}) is dependent on chain length (N)



N = 100



N = 66

❖ Can not be used for determining LRPCD of a polypeptide

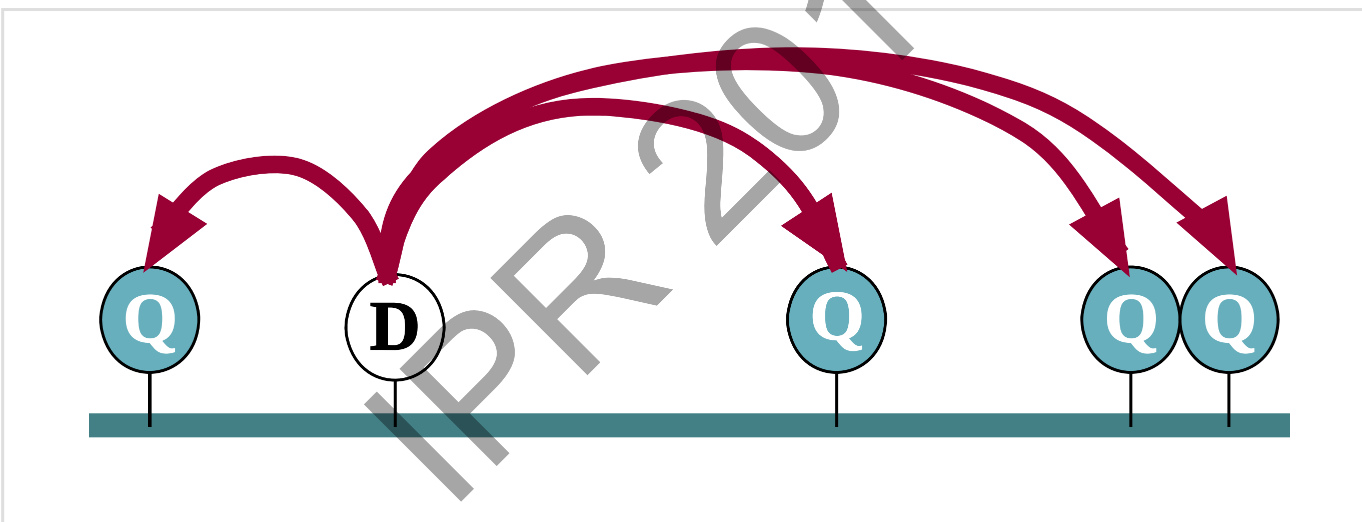
Solution - Random labeling

Winnik, M. A. *Acc. Chem. Res*. **1985**, 18, 73-79.

Random Labeling

A polymer chain is randomly labeled with a luminophore and its quencher

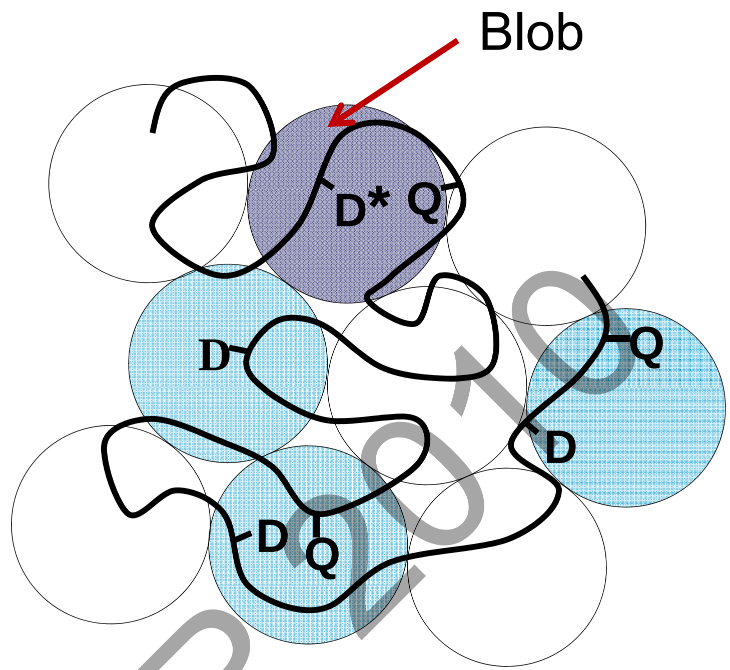
- Distribution of rate constants



- Data analysis becomes complicated

Solution- Fluorescence Blob Model (FBM)

FBM



- k_{blob}= quenching rate constant of an excited luminophore (D) located in a blob containing a single quencher (Q)

- N_{blob} = monomer units/blob (equivalent to N of EEC)

- Product k_{blob} X N_{blob} describes LRPCD (equivalent to k_{cy} of EEC)

Background

FBM

- Previous use in lab
  - Polystyrene
  - Poly(*N*,*N*-dimethylacrylamide) (PDMA)
  - Hydrophobically modified water-soluble polymers
  - Poly(*L*-glutamic acid) (PGA)

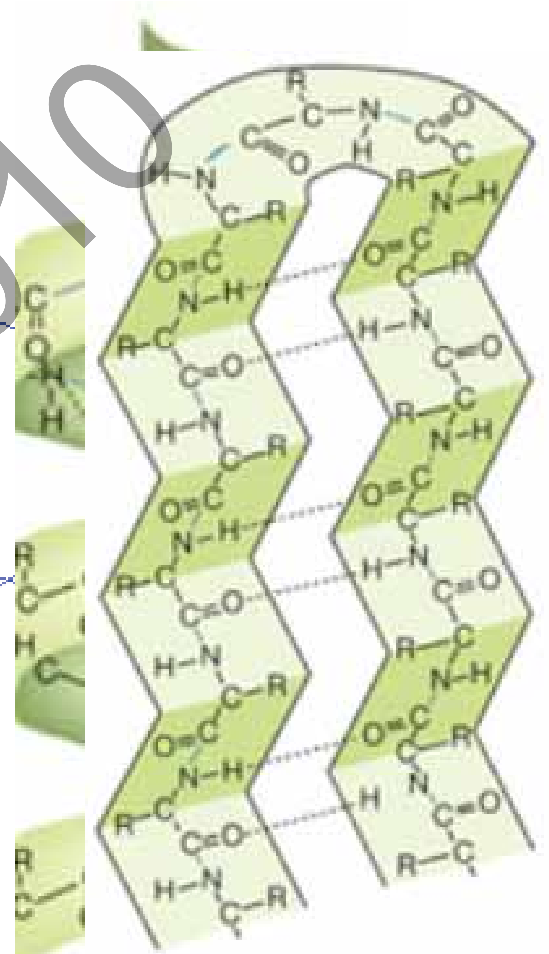
- Present study

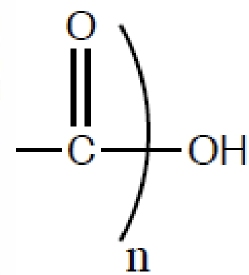
  First time to study the LRPCD of a polypeptide in aqueous solution

Polypeptide of Interest

Poly(*L*-lysine) (PLL)

NH_2 will allow covalent attachment of the luminophore and quencher onto PLL





Three conformations

- Random-coil
- α-helix
- β-sheet

http://www.chemguide.co.uk/organicprops/aminoacids/proteinstruct.html

Luminophore

- Pyrene
  - ☑ Long lifetime (200-400 ns)
  - ☑ Covalent attachment
  - ❑ Water-soluble

✖ **Not a good choice**

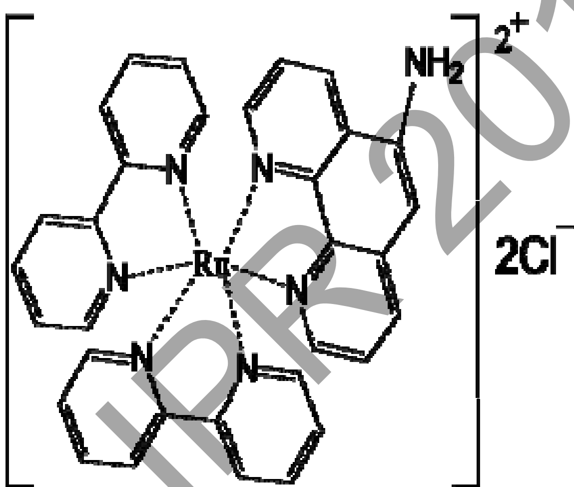
- Ruthenium polypyridine complexes
  - ☑ Long lifetime (~600 ns)
  - ☑ Covalent attachment
  - ☑ Water-soluble

✓ **Good Choice**

Luminophore

$[Ru(bpy)_3]^{2+}$ Popular ruthenium polypyridyl complex

Ruthenium bisbipyridine 5-aminophenanthroline chloride ($RuNH_2$)



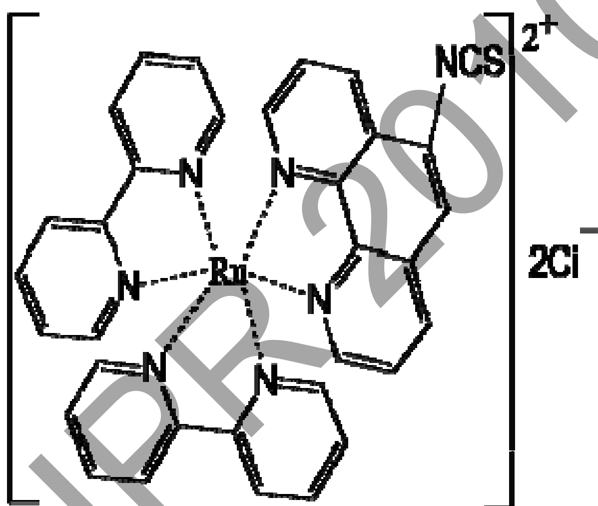
- Phenanthroline ligand allows attachment to a polymer
- Lifetime of ~600 ns in 0.1 M Na_2CO_3 solution
- Water-soluble

Quinn, Cristina. Master's Thesis, University of Waterloo, 2007.

Luminophore

Isothiocyanate derivative of $RuNH_2$

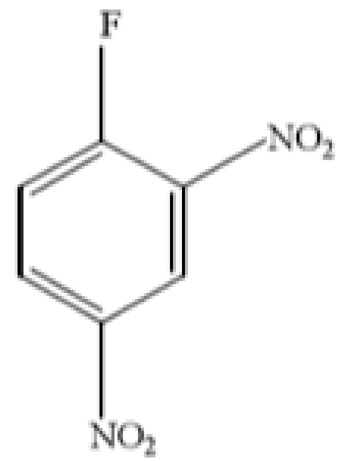
Ruthenium bisbipyridine 5-Isothiocyanatophenanthroline chloride (RuNCS)



NCS group will allow attachment onto the PLL via a thiourea linkage

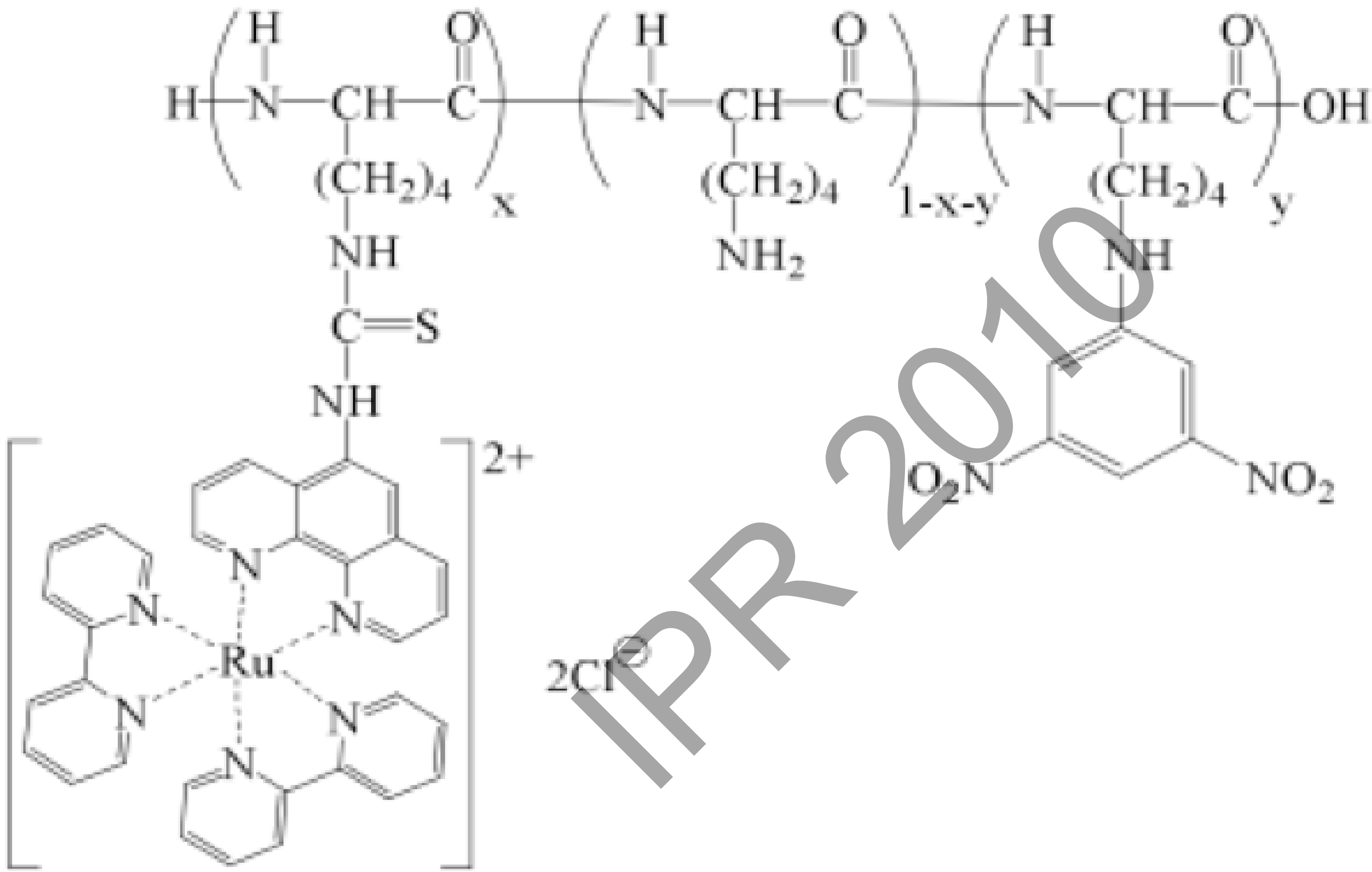
Quencher

- Partially Water-soluble
- Covalent attachment to PLL
- Collisional quenching



1-Fluoro-2,4-dinitrobenzene (FDNB)

Labeled PLL



x = 1%
y = 1-10%

PLL labeled with RuNCS and FDNB via thiourea, and urea linkages respectively

Goals

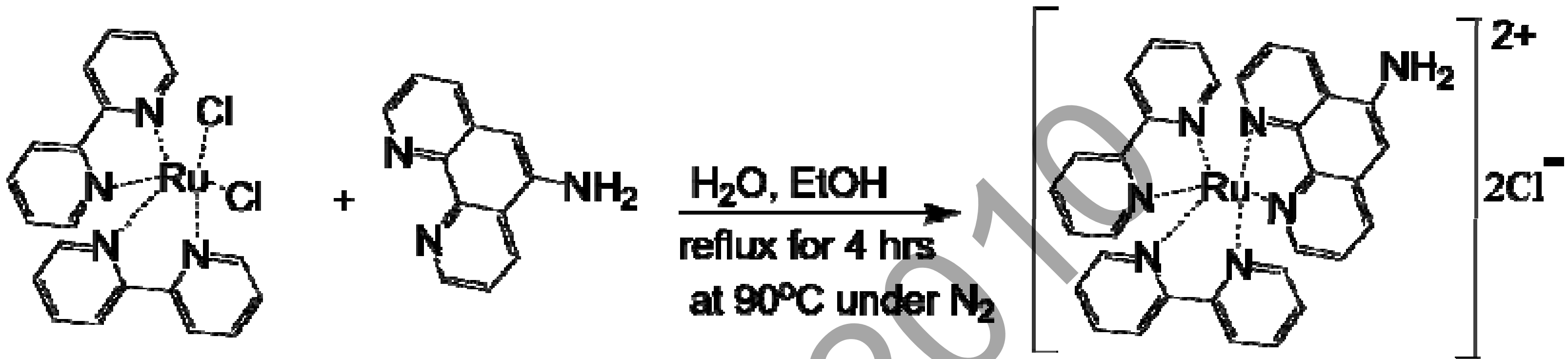
- Synthesis, characterization, and modification of the water-soluble luminophore
- Covalent attachment of the luminophore and quencher to the polypeptide
- Time-resolved fluorescence studies on the solutions of labeled PLL at different pH's
- Analysis of the fluorescence decays by using the FBM to gain information about the LRPCD of a polypeptide as PLL changes conformations

Preliminary Work

1. Synthesis and characterization of $RuNH_2$
2. Conversion of $RuNH_2$ to RuNCS
3. Labeling of PLL with RuNCS
4. Labeling of PLL with FDNB
5. Labeling of RuNCS labeled PLL with FDNB
6. Time resolved fluorescence studies of PLL labeled with RuNCS and FDNB

Preliminary Work

1.Synthesis of $RuNH_2$



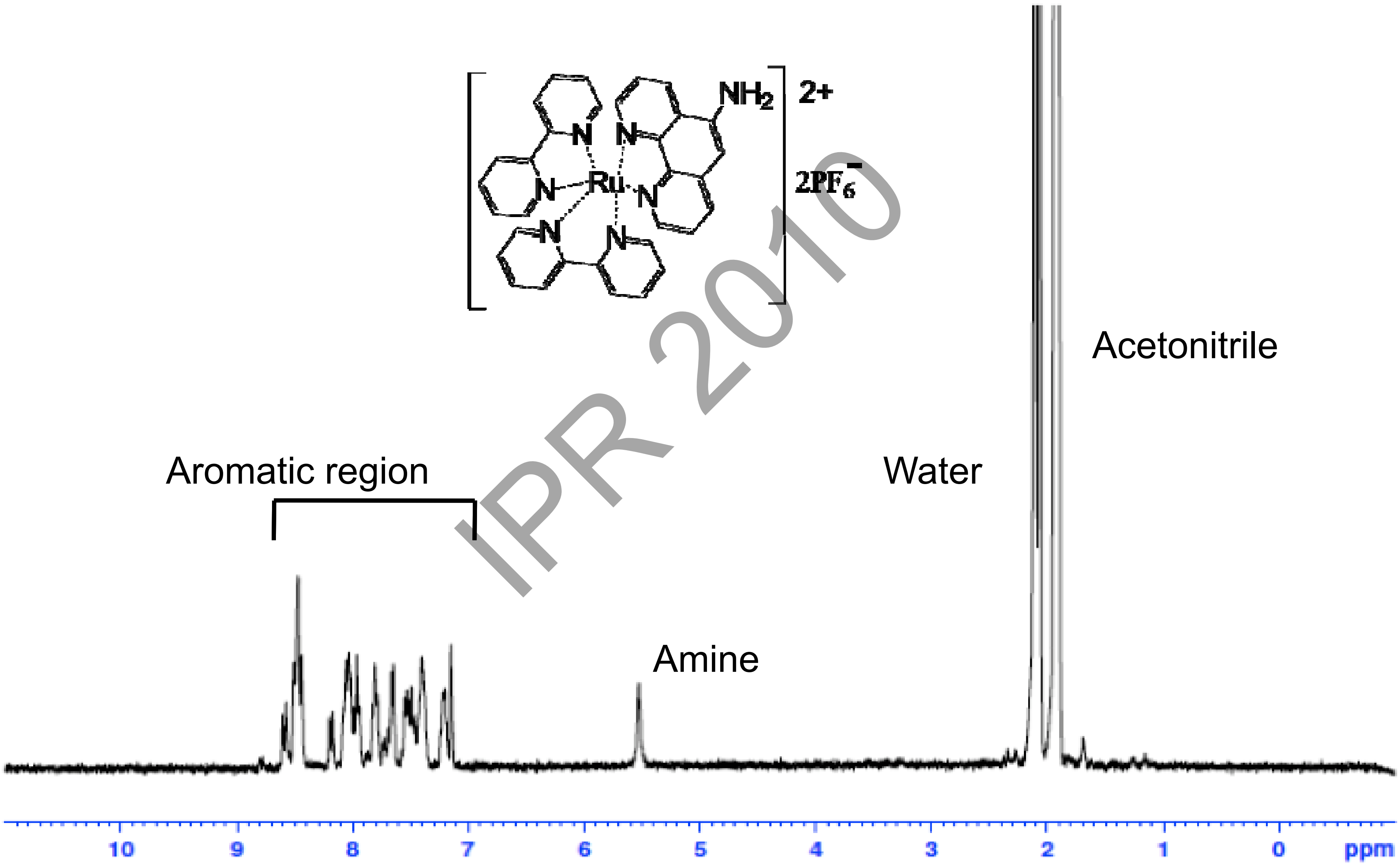
bis(bipyridyl) ruthenium (II) dichloride ($RuCl_2$) + 5–amino–1,10–phenanthroline (5-phen) ⟶ $RuNH_2$

Procedure by Ellis et al., and Quinn.
Purification by column chromatography
87% yield

Ellis, C.D.; Margerum, L.D.; Murray, R.W.; Meyer, T.J. *Inorg. Chem.* **1983**, *22*, 1283–1291.
Quinn, Cristina. Master's Thesis, University of Waterloo, 2007.

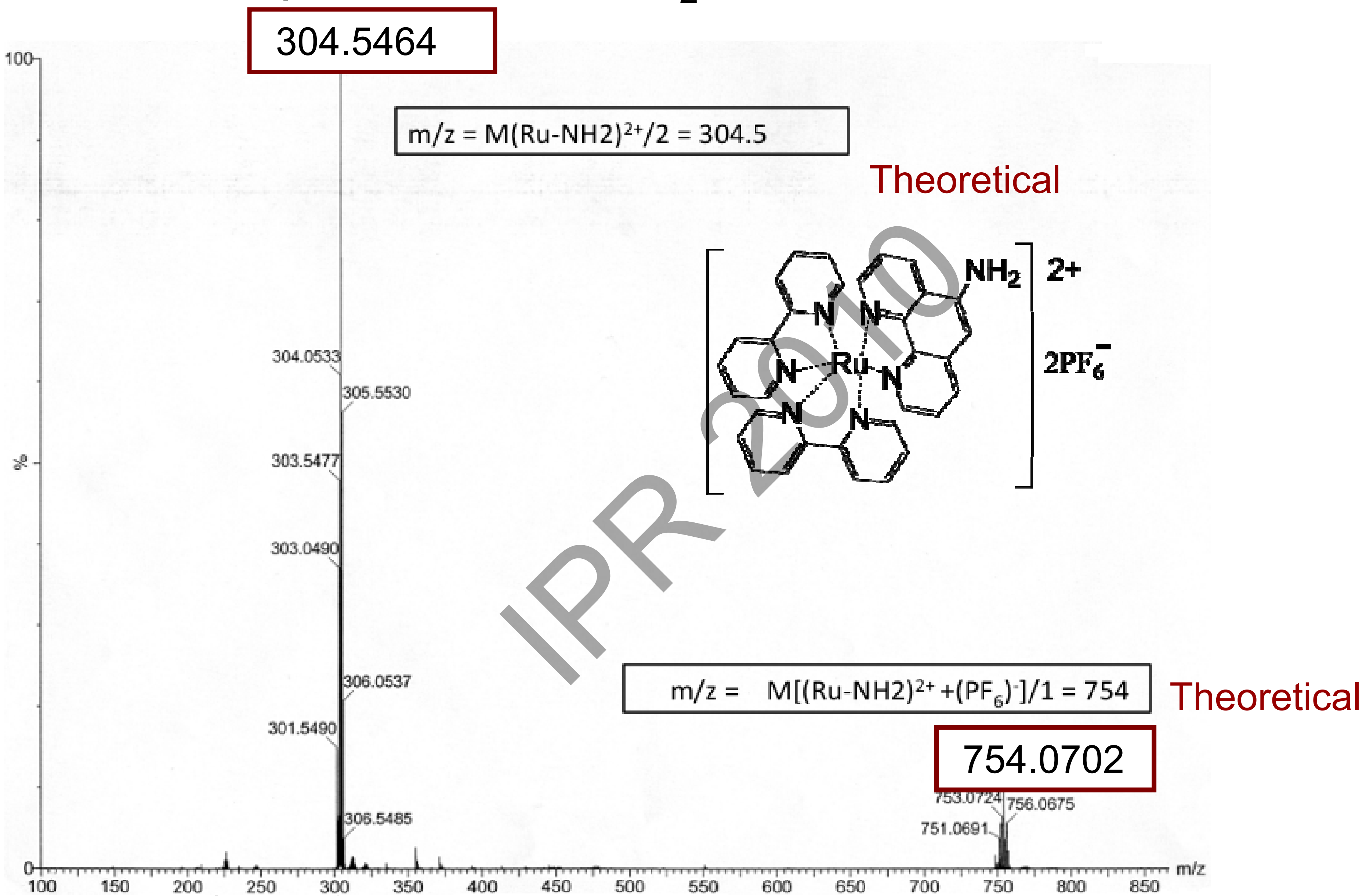
Preliminary Work

^1H NMR of $RuNH_2$ in deuterated acetonitrile

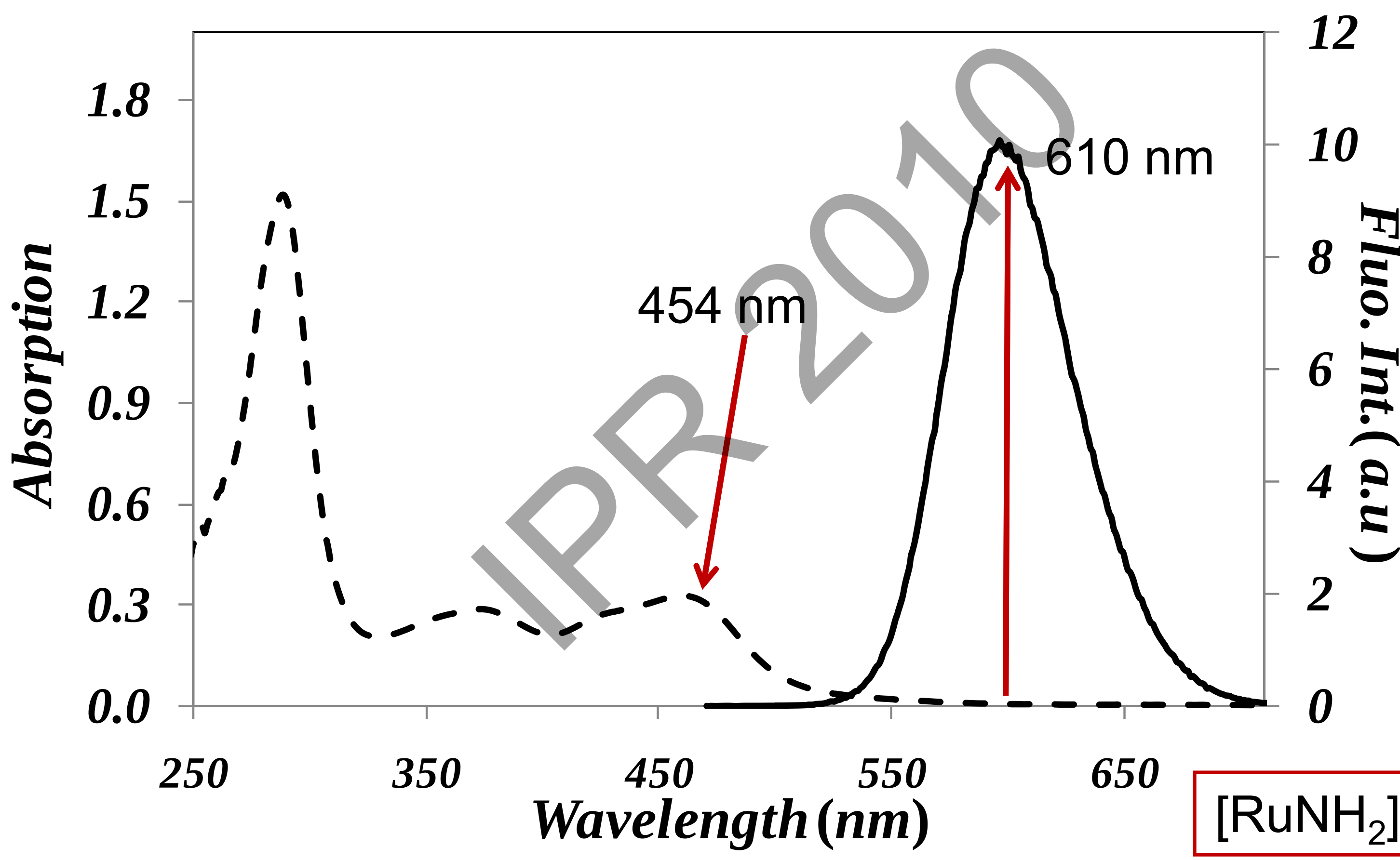


Preliminary Work

Mass spectrum of $RuNH_2$ in 1:1 acetonitrile : water



Absorption and Emission Spectra of $RuNH_2$



[$RuNH_2$] = 20 μM

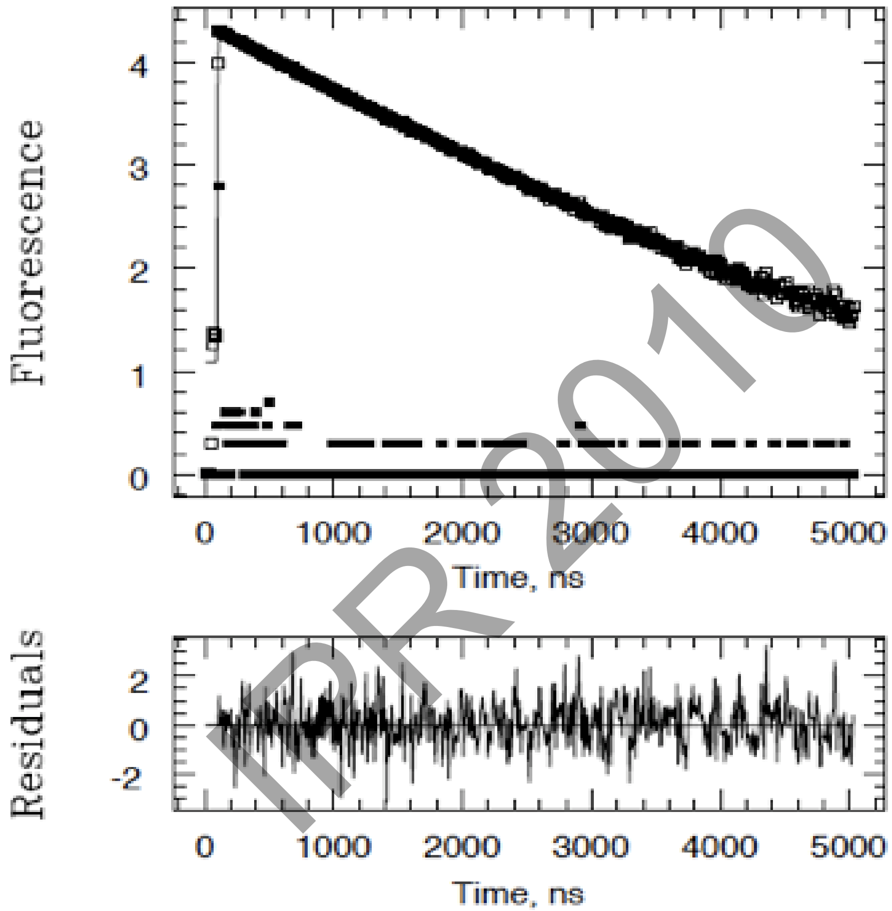
0.1 M Na_2CO_3 solution (pH 9.6)

Ryan, E.; O'Kennedy, R.; Feeney, M.M.; Kelly, J.M.; Vos, J.G. *Bioconjugate Chem.* **1992**, *3*, 285–290.

Preliminary Work

Time-resolved Fluorescence Decay

$\chi^2 = 1.08$
$<\tau>= 667$ ns



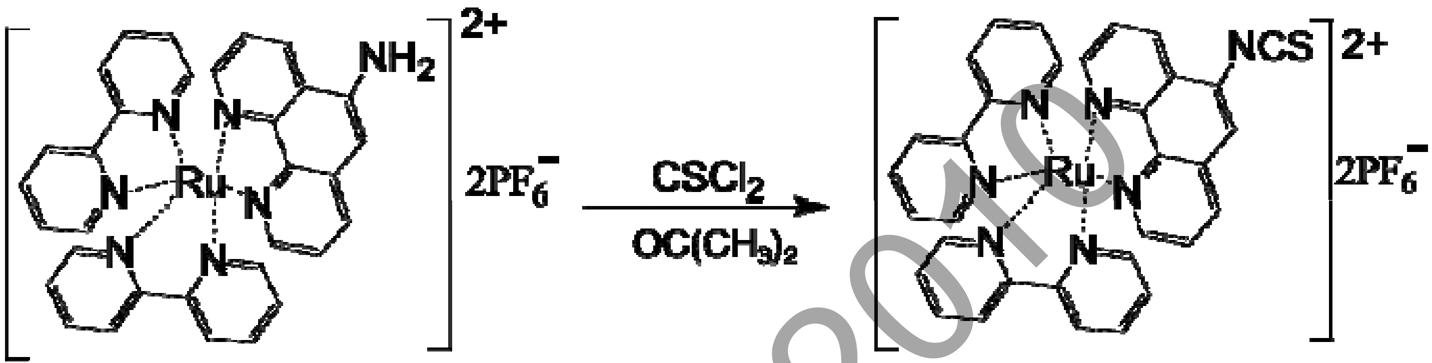
$RuNH_2$

λ_{ex}= 454 nm, λ_{em}= 600 nm

Degassed $RuNH_2$ in 0.1 M Na_2CO_3 solution (pH 9.6)

Ryan, E.; O'Kennedy, R.; Feeney, M.M.; Kelly, J.M.; Vos, J.G. *Bioconjugate Chem.* **1992**, *3*, 285–290.

2. Synthesis of RuNCS

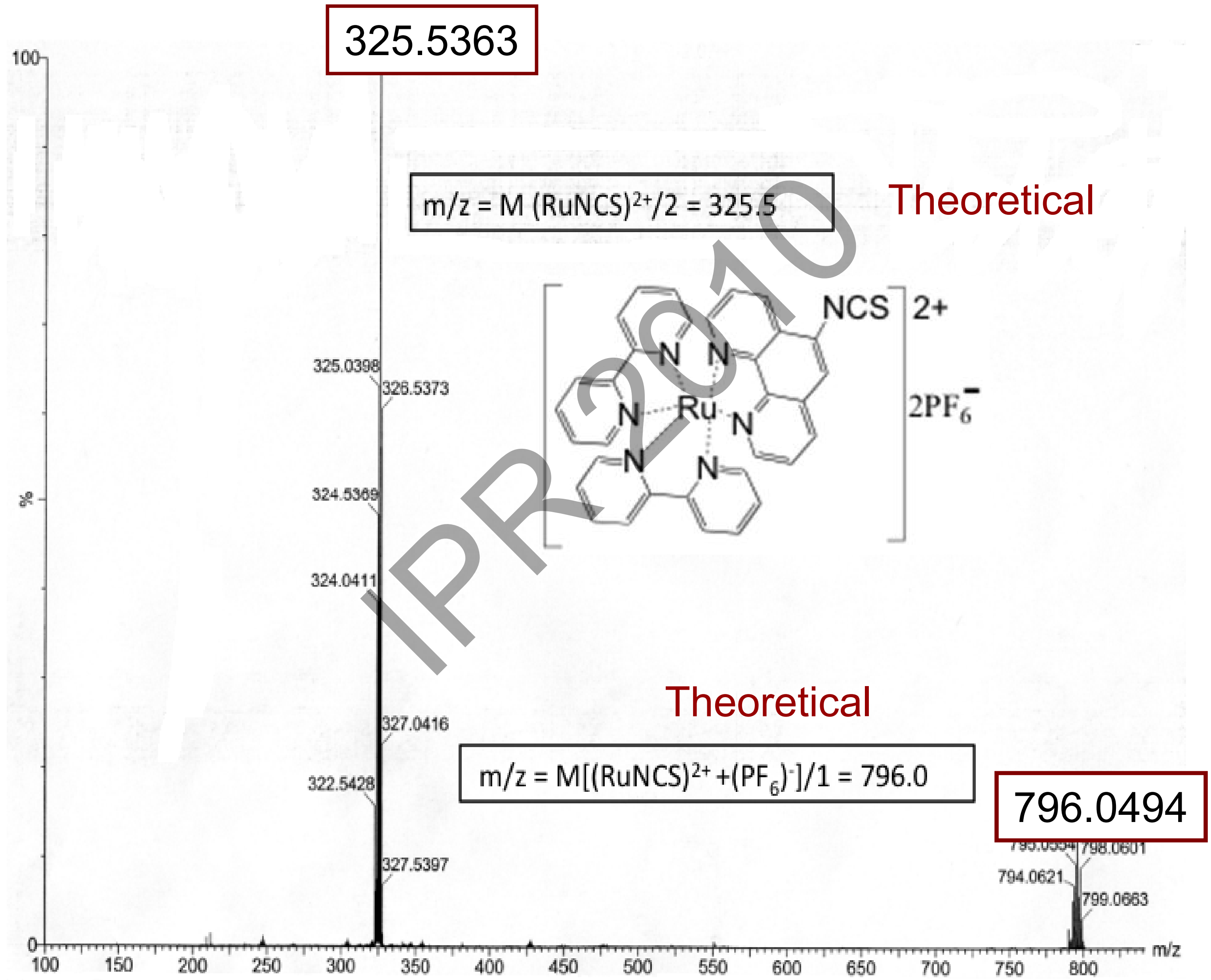


RuNH$_2$ reacted with thiophosgene (in acetone)
Procedure followed by Ryan et al.
Yield 79%

Ryan, E.; O'Kennedy, R.; Feeney, M.M.; Kelly, J.M.; Vos, J.G. *Bioconjugate Chem.* **1992**, *3*, 285–290.

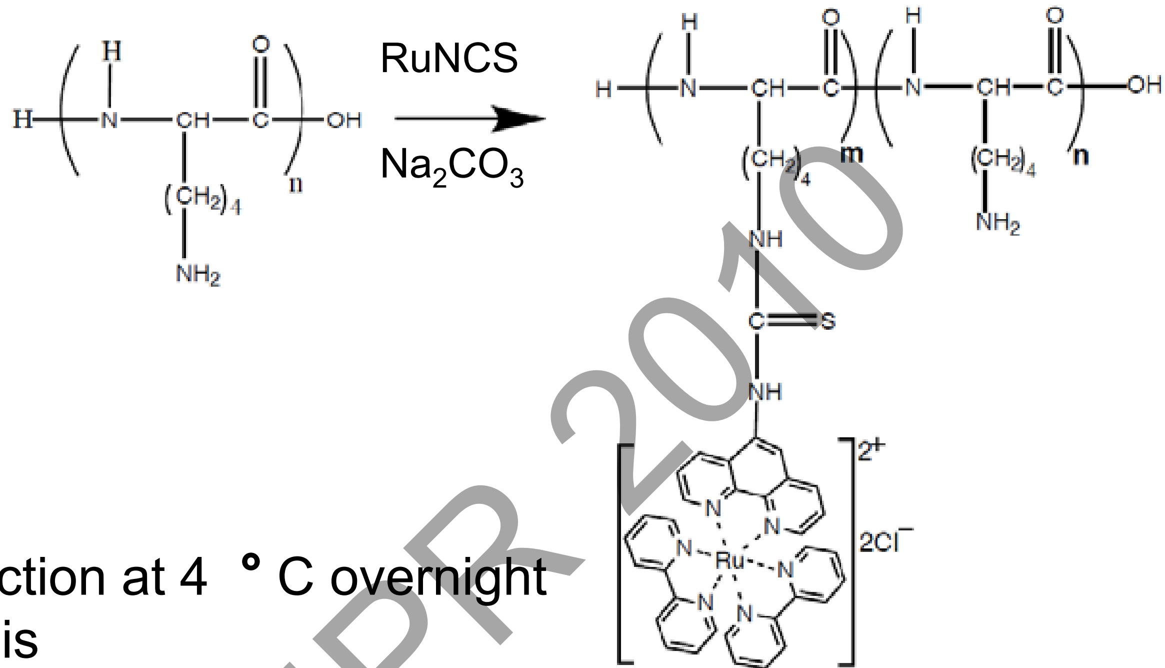
Preliminary Work

Mass Spectrum of RuNCS in 1:1 acetonitrile : water



Preliminary Work

3. Labeling PLL with RuNCS



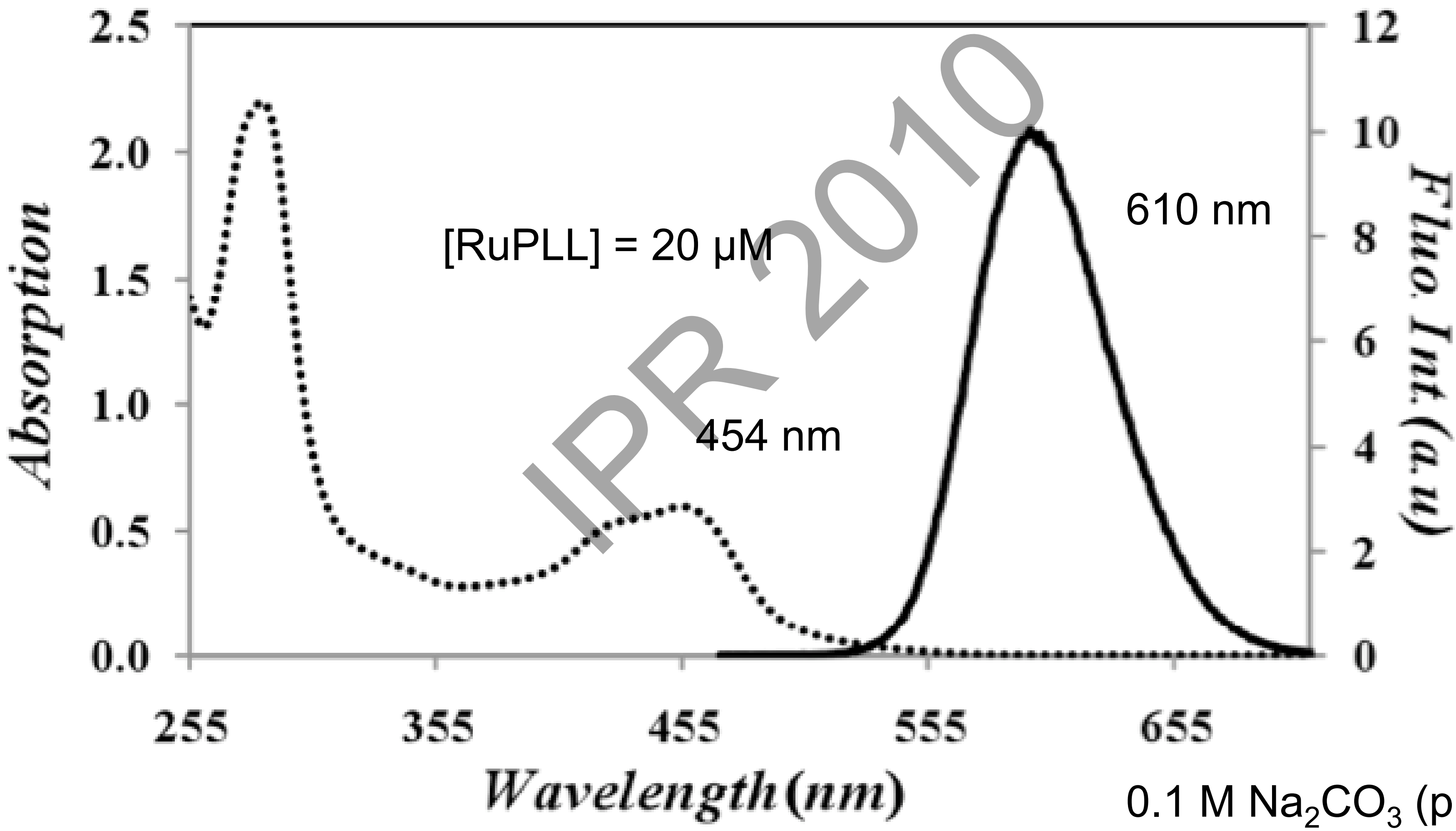
Conjugation reaction at 4 °C overnight
Extensive dialysis

Procedure followed by Ryan et al.

Ryan, E.; O'Kennedy, R.; Feeney, M.M.; Kelly, J.M.; Vos, J.G. *Bioconjugate Chem.* **1992**, *3*, 285–290.

Preliminary Work

Absorption and emission spectra of PLL labeled with RuNCS (RuPLL)

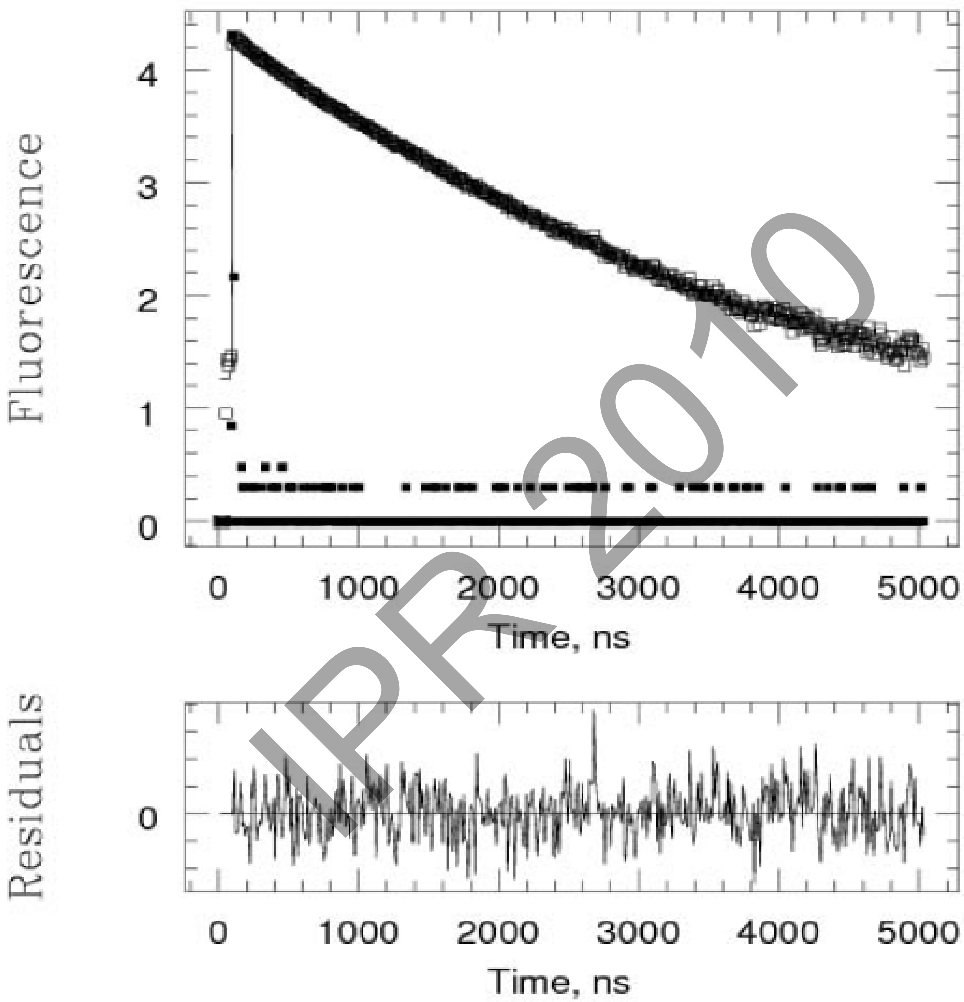


0.1 M Na_2CO_3 (pH 9.6)

Preliminary Work

Time-resolved Fluorescence Decay

χ^2 = 0.971
<τ>= 593 ns

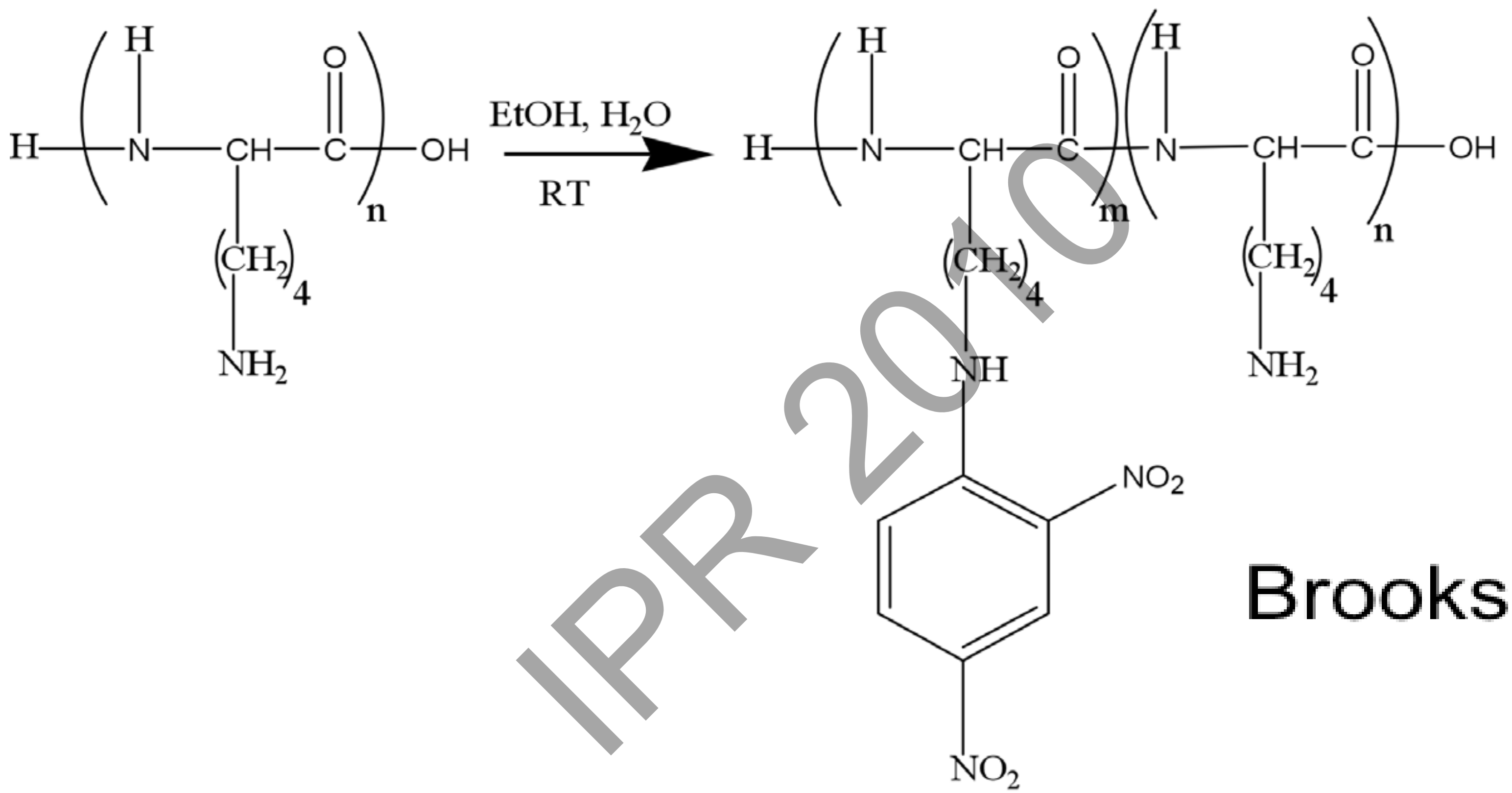


λ_{ex}= 454 nm, λ_{em}= 600 nm

Degassed in 0.1 M Na_2CO_3 solution (pH 9.6)

Preliminary Work

4. Labeling PLL with Quencher



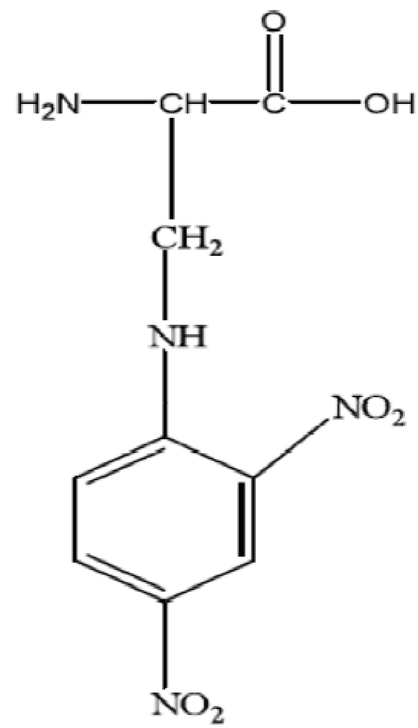
Brooks, 1975

Preliminary Work

4. Labeling PLL with Quencher

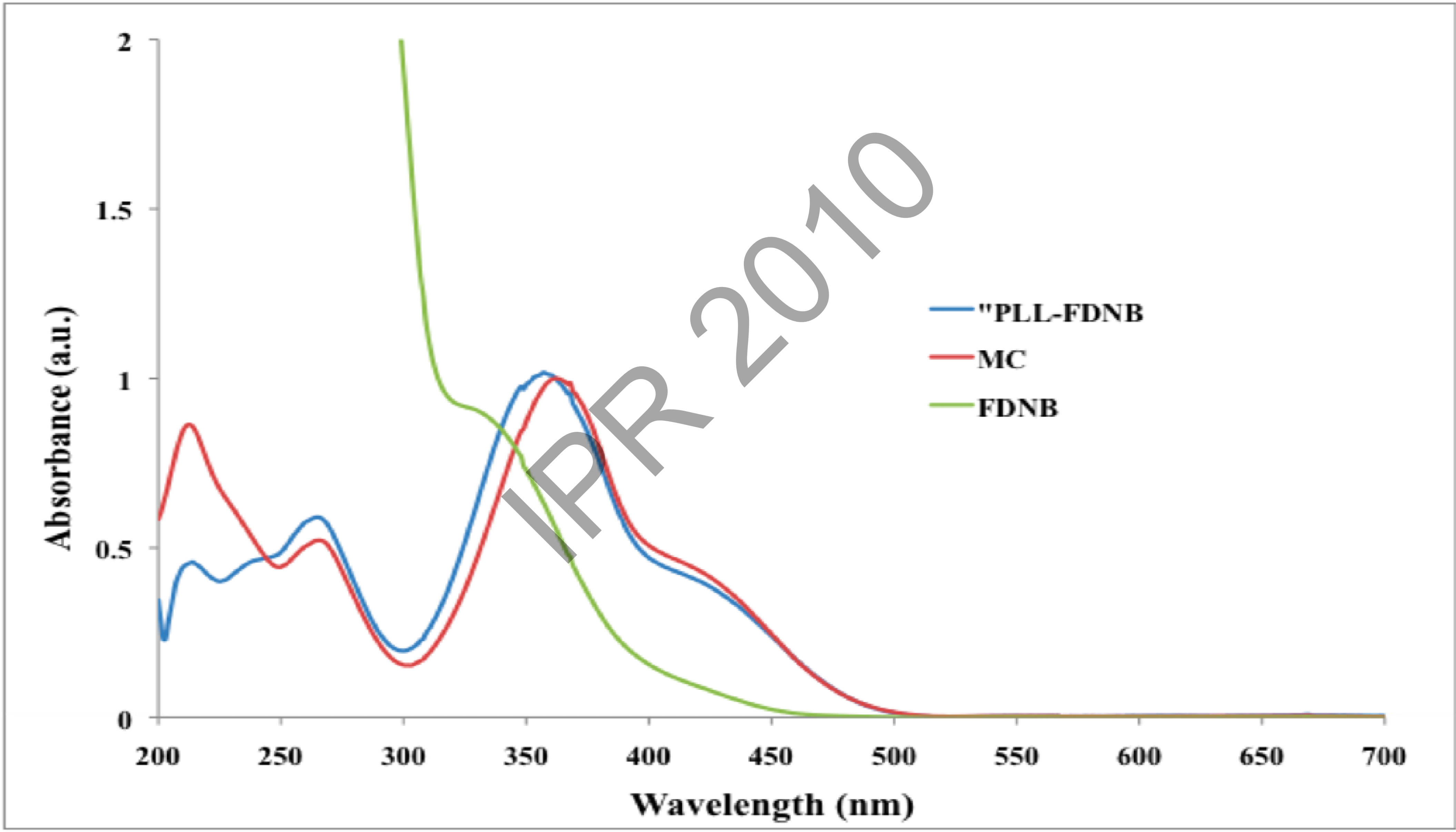
Model Compound

N_ε-DNP-L-Lysine Hydrochloride

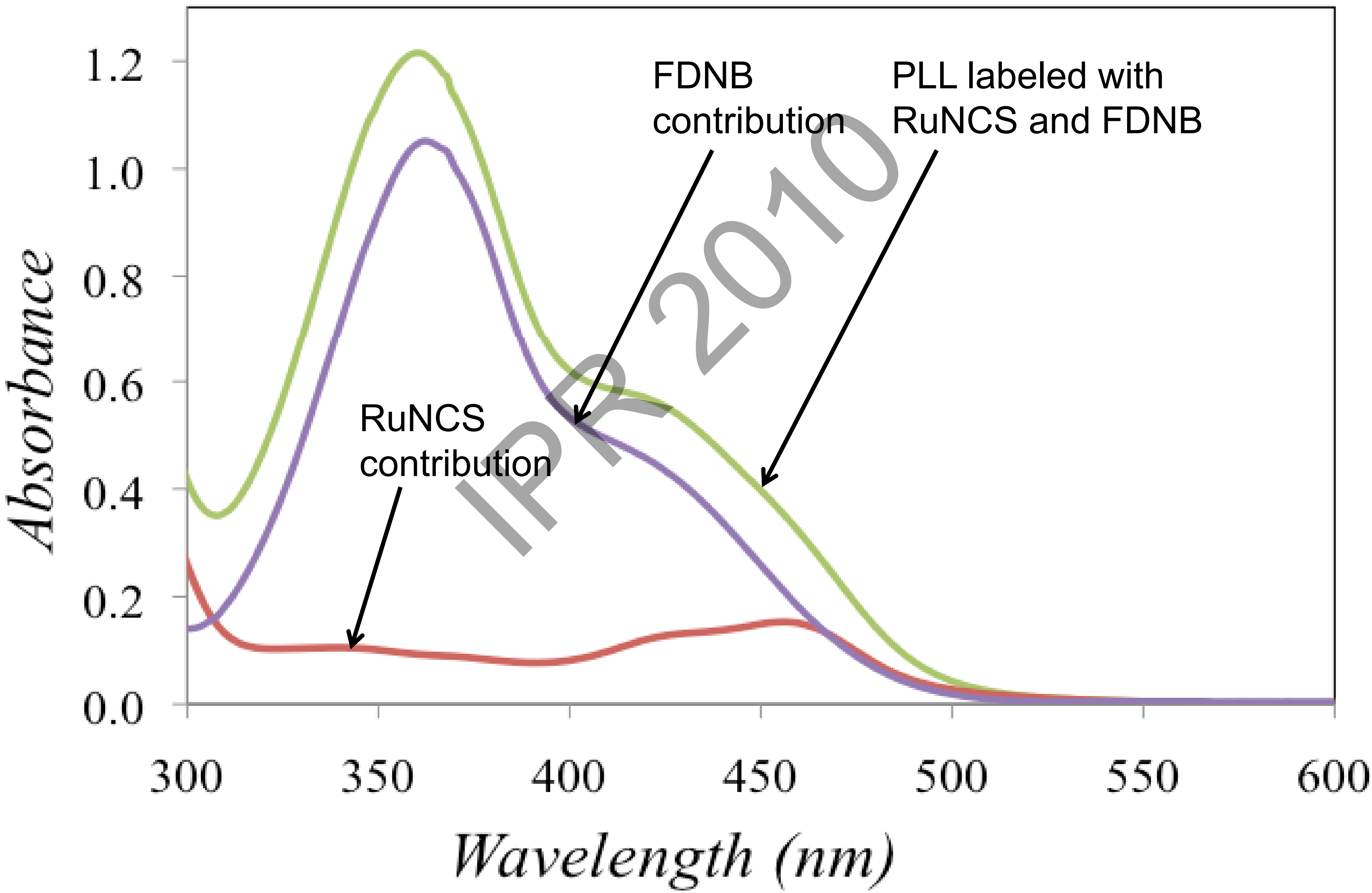


Extinction Coefficient at 362 nm = 15800 $L.mol^{-1}.cm^{-1}$

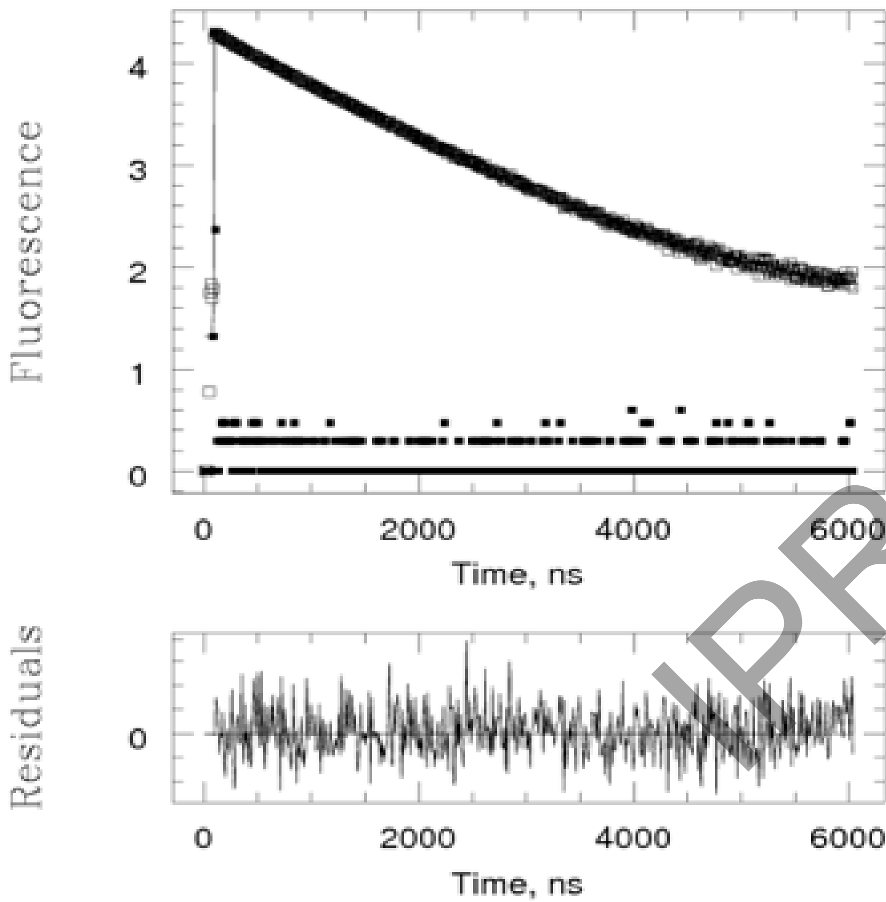
4. Labeling PLL with Quencher



5. PLL labeled with RuNCS and FDNB

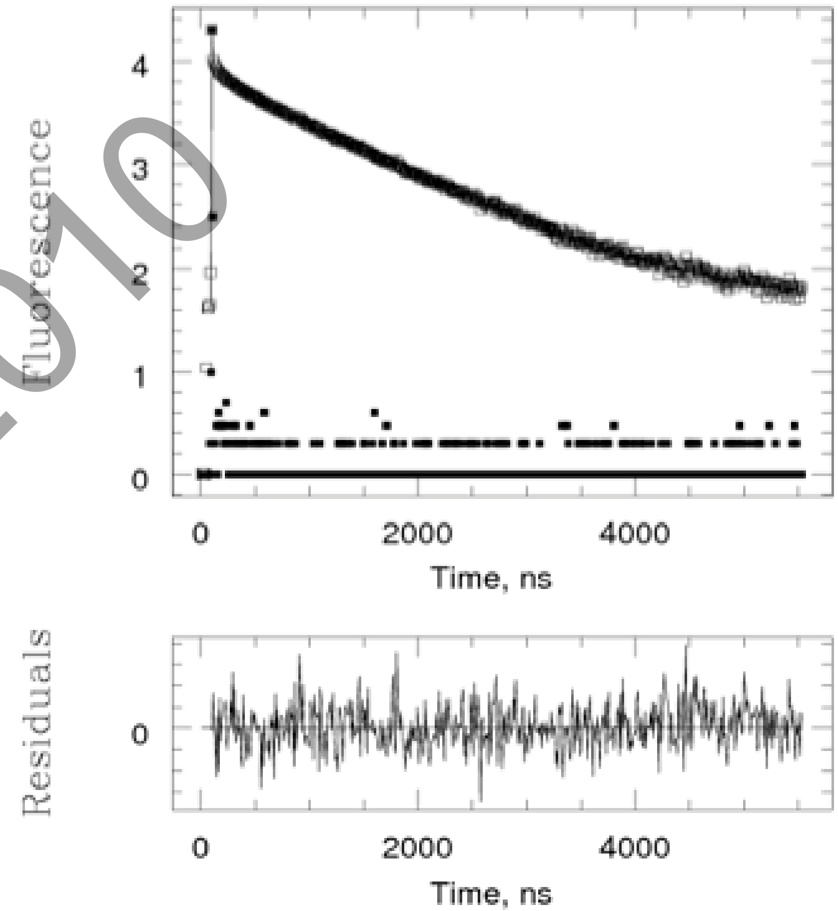


6. Time-resolved Fluorescence Studies



PLL labeled with RuNCS

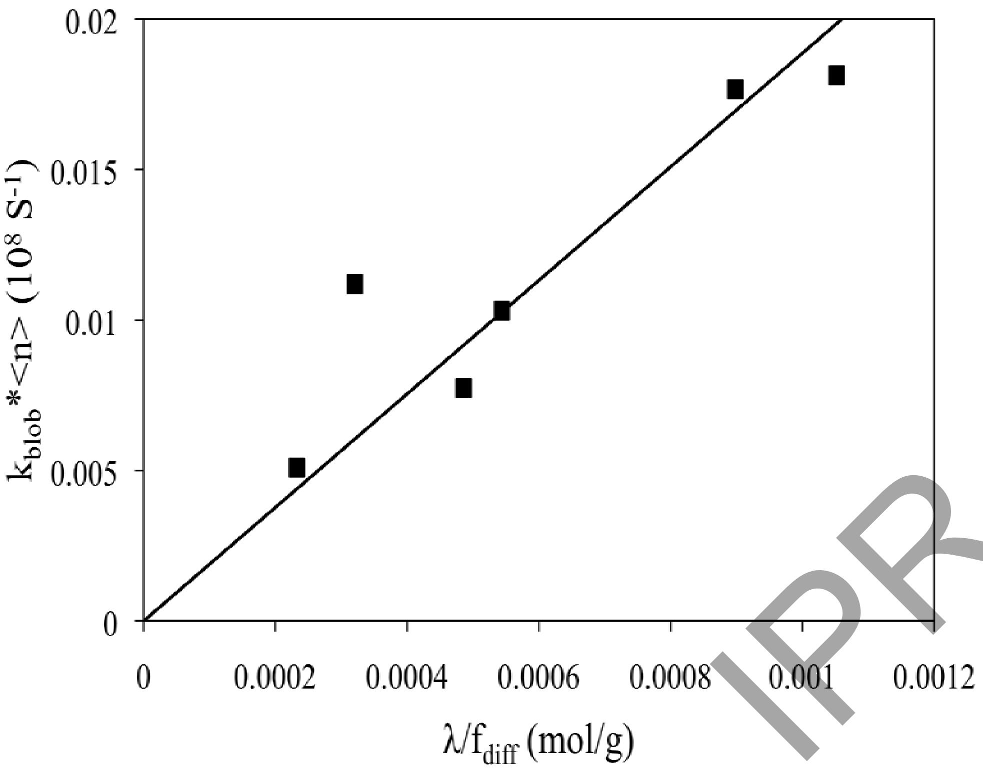
$<\tau> = 760$

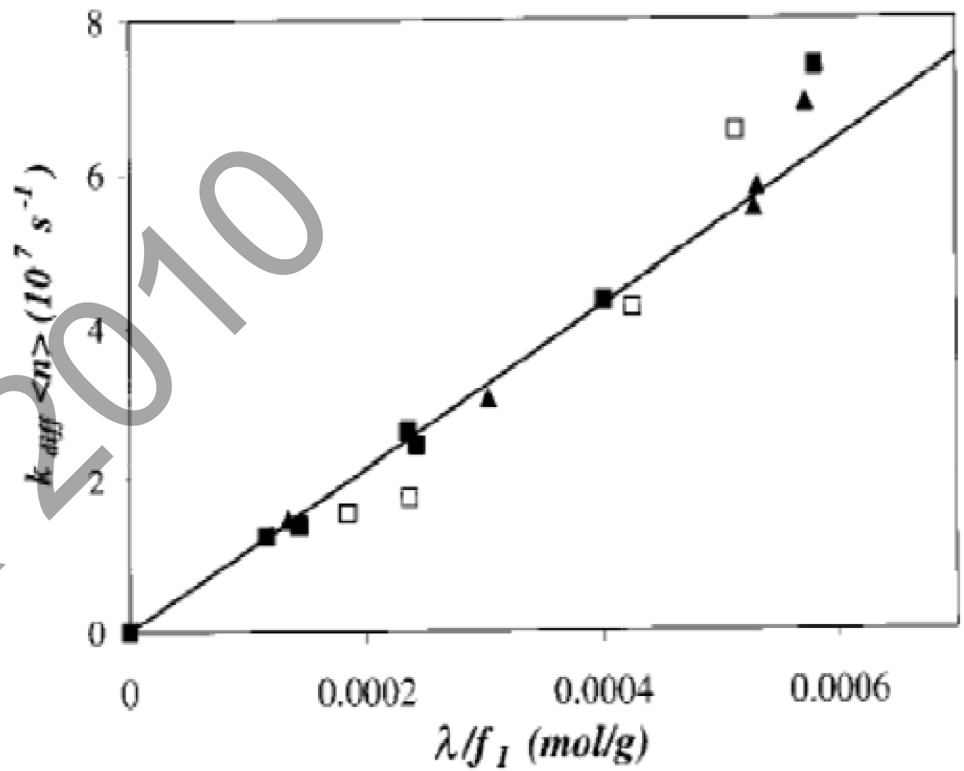


PLL labeled with RuNCS and FDNB

$<\tau> = 590$

6. Time-resolved Fluorescence Studies



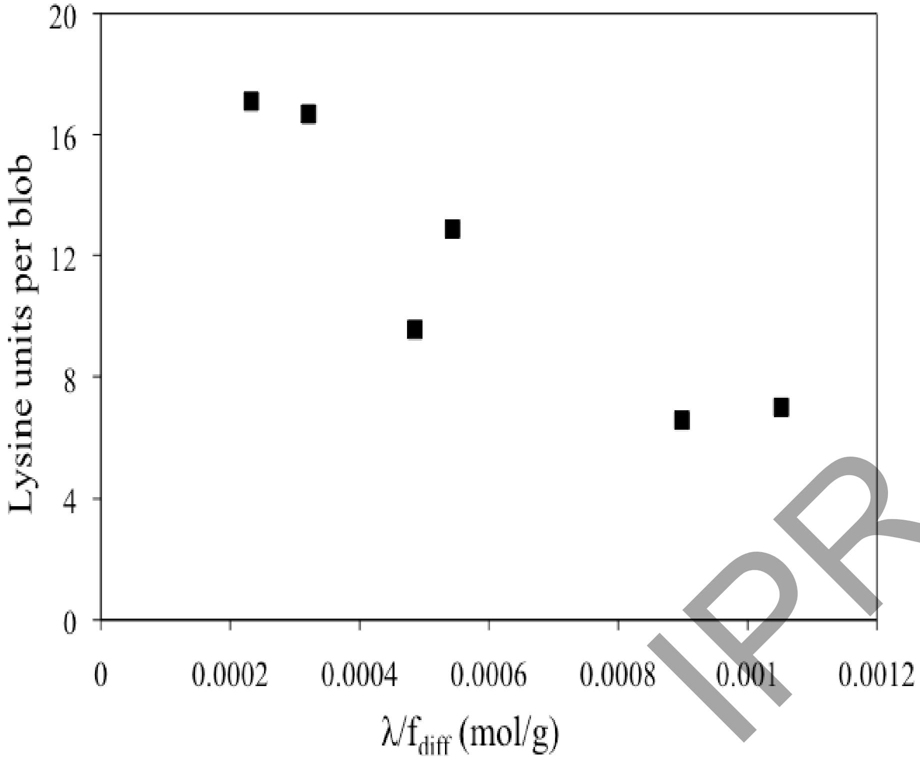


Polylysine labeled with RuNCS and FDNB

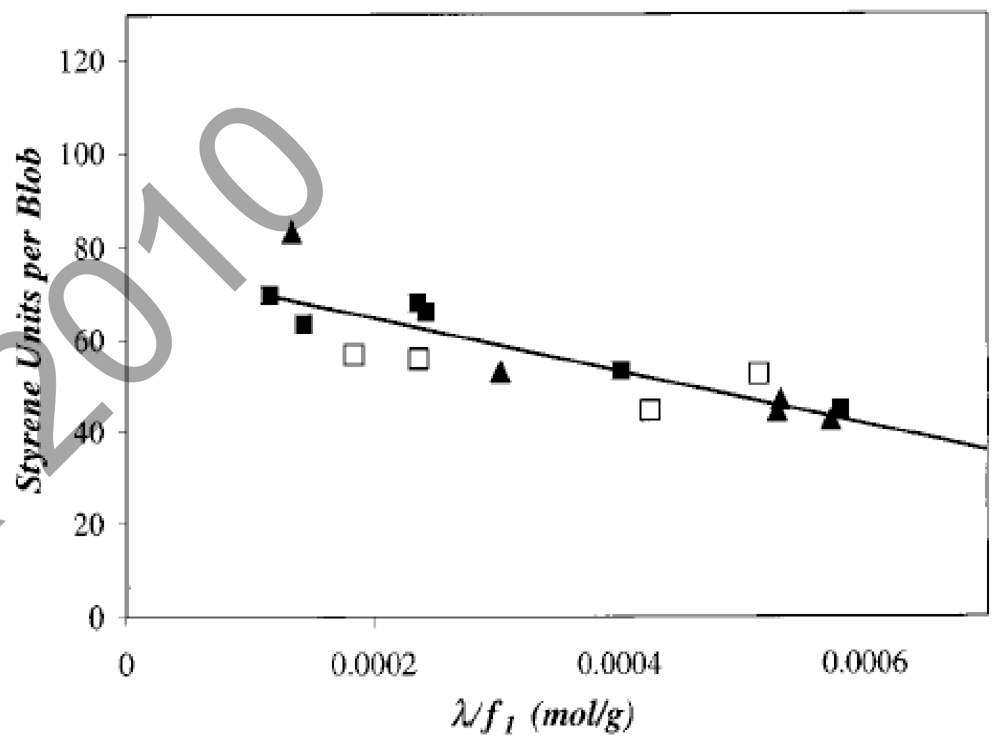
Polystyrene labeled with Pyrene

$$k_{blob} \times <n> \propto \frac{<n>}{V_{blob}} = [Q]_{loc}$$

6. Time-resolved Fluorescence Studies

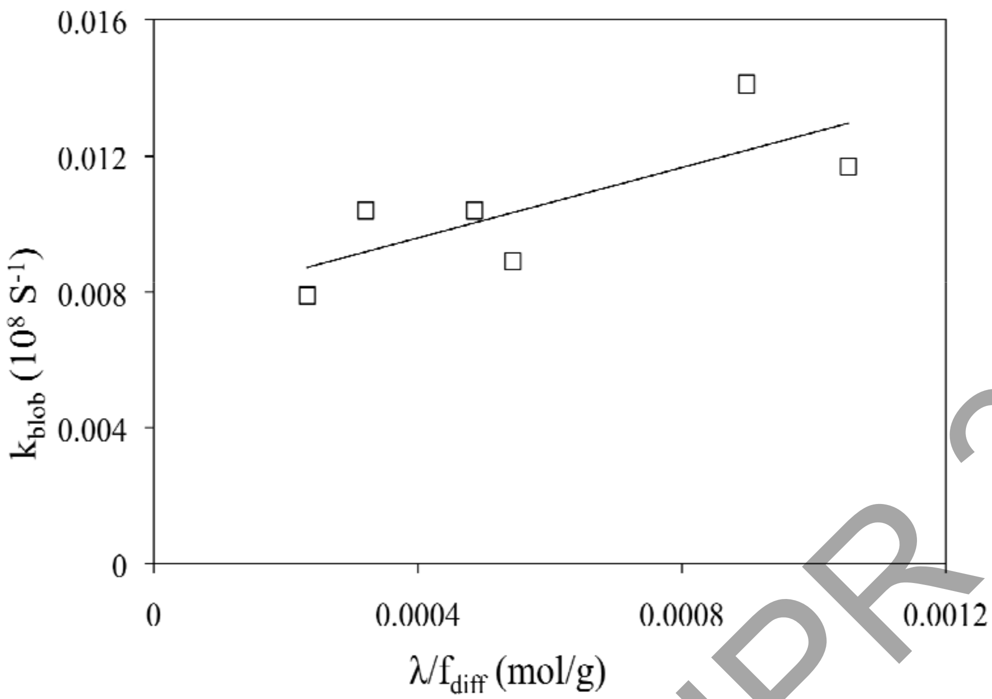


Polylysine labeled with RuNCS and FDNB

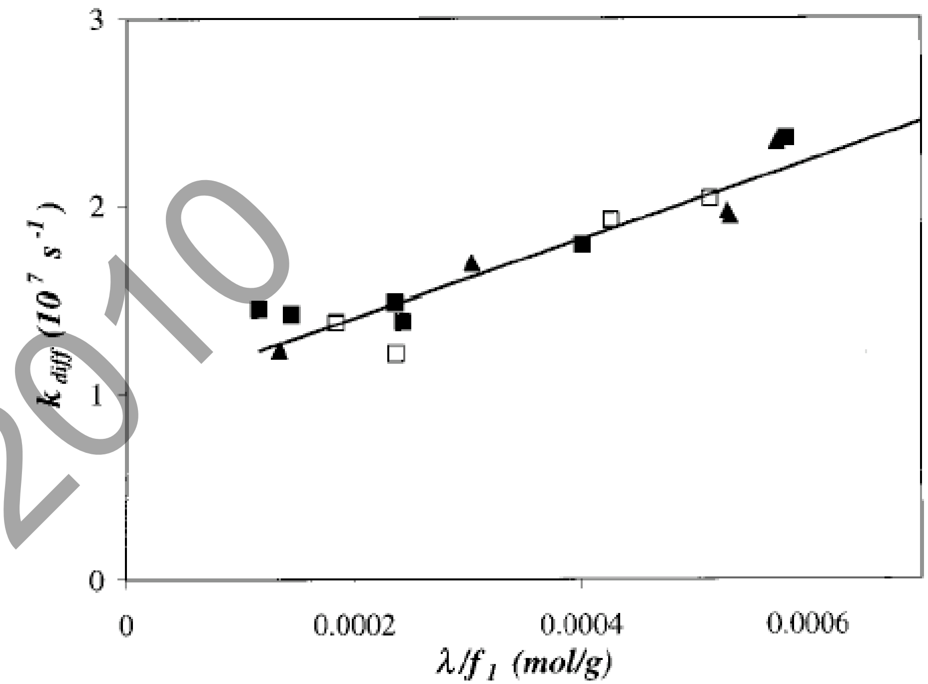


Polystyrene labeled with Pyrene

6. Time-resolved Fluorescence Studies



Polylysine labeled with RuNCS and FDNB

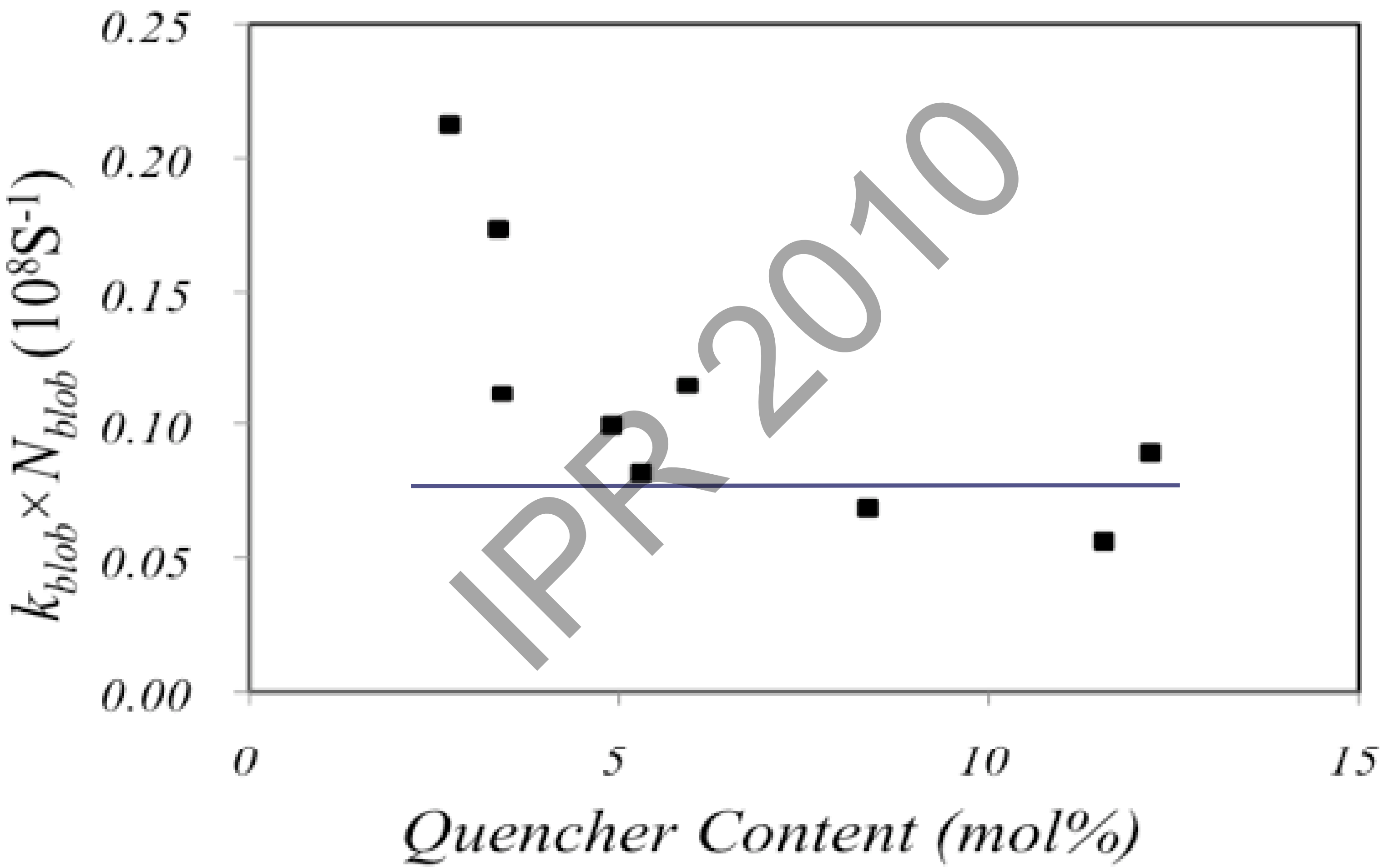


Polystyrene labeled with Pyrene

$$k_{blob} \propto \frac{1}{V_{blob}} \propto \frac{1}{N_{blob}}$$

Preliminary Work

6. Time-resolved Fluorescence Studies



Preliminary Work

6. Time-resolved Fluorescence Studies

Product η 5 k_{blob} 5 N_{blob} 5 n shows internal dynamics of a polymer

Polylysine	**Polystyrene**	**Polyisoprene**
23± 6 × 10^{3} Pa	74 × 10^{4} Pa	244 × 10^{4} Pa

Conclusions

- Successful synthesis and characterization of RuNCS
- Successful attachment of RuNCS and FDNB onto PLL
- Time-resolved fluorescence studies of PLL attached with RuNCS and FDNB
- Agreement of preliminary FBM data with earlier results

Future work

- Obtaining time-resolved fluorescence decays with variation of solution pH

- Analyzing the fluorescence decays with the FBM

Acknowledgements

- Dr. Jean Duhamel
- Dr. Mario Gauthier
- Duhamel & Gauthier groups

Comments/Questions?