Studying the Interactions of a Gemini Surfactant with DNA by Fluorescence

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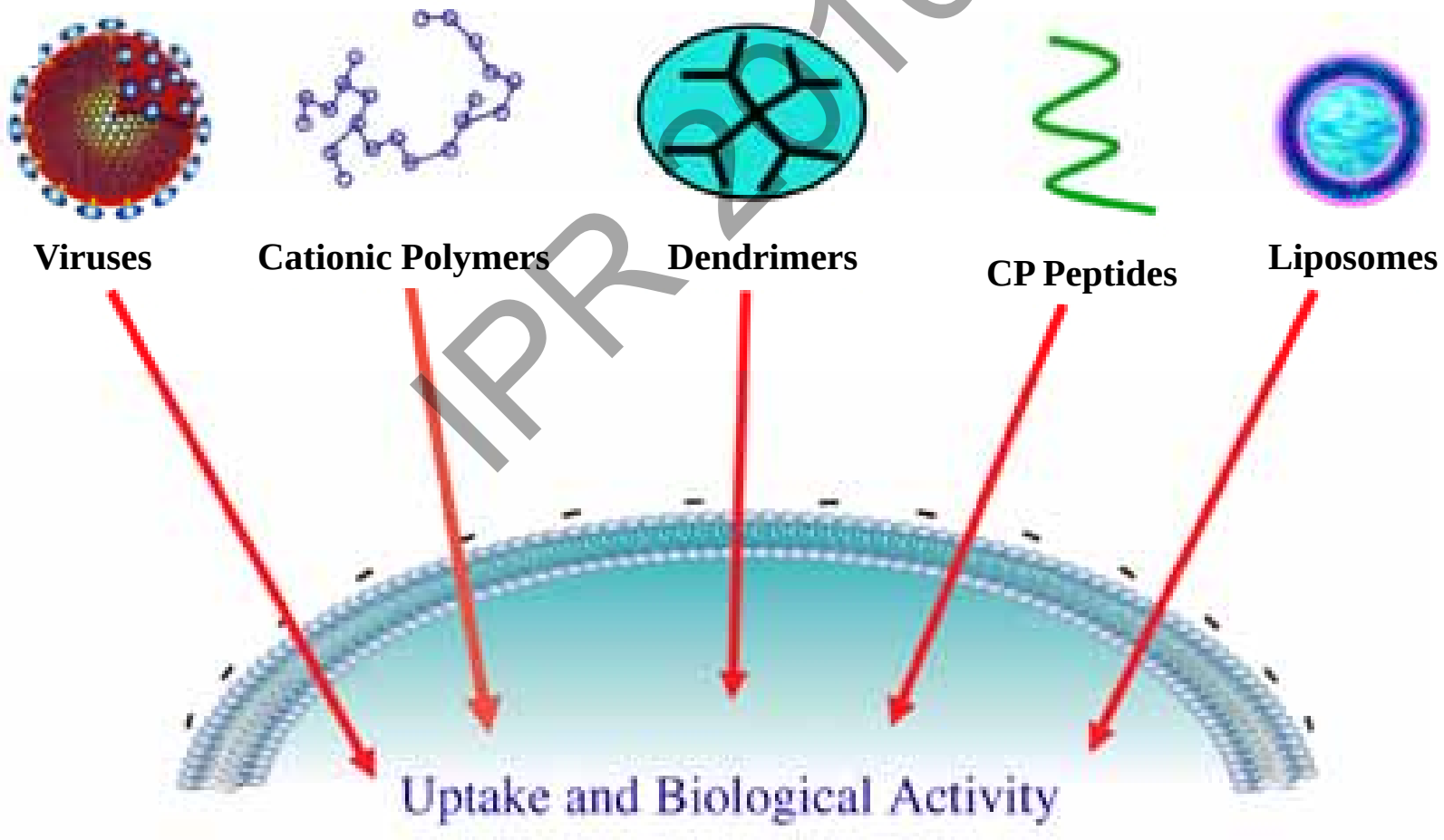
Outline

- **Introduction**
  - **Gemini surfactants in gene delivery**
  - **Study the associations of a pyrene labeled gemini surfactant by fluorescence**
    - **With and without DNA**
- **Results**
  - **Global analysis fits of the fluorescence decays of the pyrene monomer and excimer**
- **Conclusions**

Gene Delivery

- **Types of delivery vectors**
  - **2 Classes: Viral and Non-Viral**



Akhtar, S., *Gene Therapy*, 13, **2006**, 739 – 740.

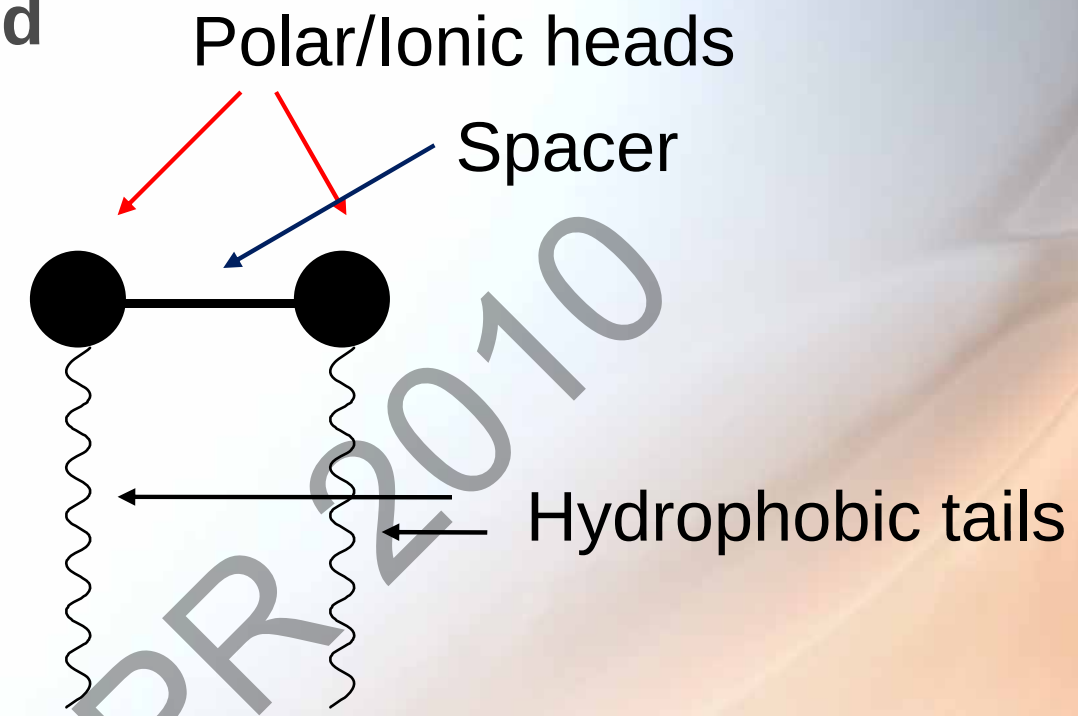
Gene Delivery Vectors

	VIRAL	NON-VIRAL
Advantages	▪Protects gene against degradation ▪High transfer efficiency	▪ Non-toxic, non immunogenic ▪ Not limited in the size of gene they can encapsulate ▪ Relatively cheap
Disadvantages	▪Viral capsids have the potential to generate severe immune response ▪Limited in the size of the gene they can encapsulate	▪ Low efficiency of transfection

Gao, X., Kim, K, Liu, D., *The AAPS Journal*, 9, **2007**, 92 – 104.
Wettig, S. D., Verrall, R. E., Foldvari, M., *Current Gene Therapy*, 8, **2008**, 9 – 23.

Gemini Surfactants

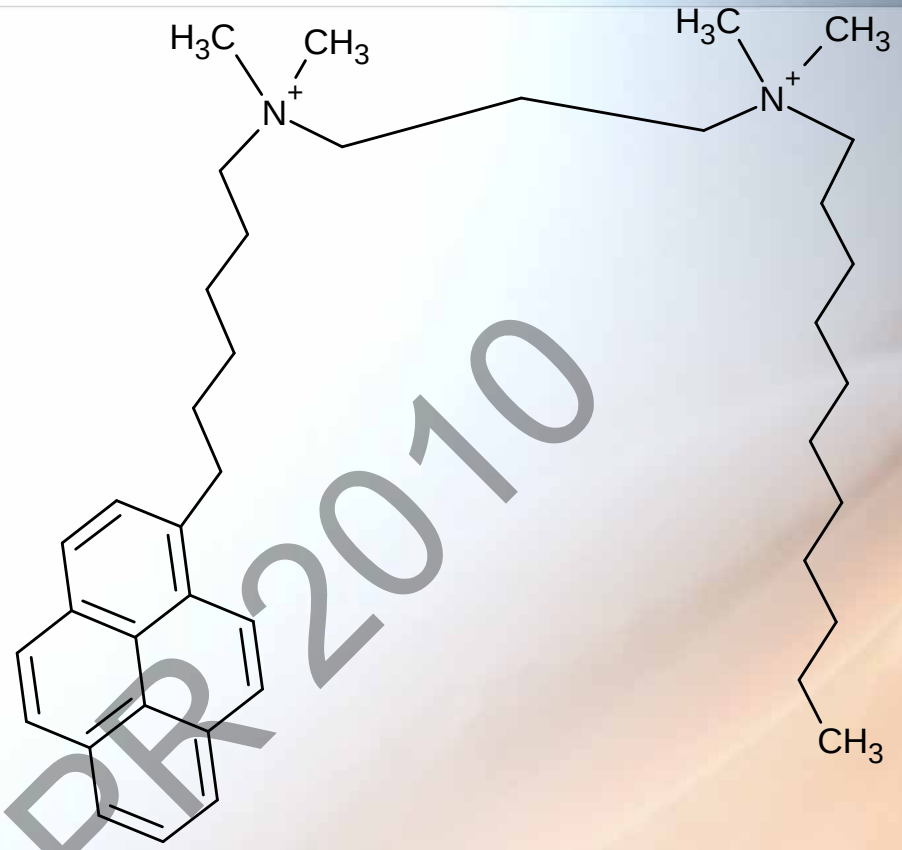
- **Contain 2 head and 2 tail groups that are chemically linked**



- **Spacer: short or long methylene groups, rigid (stilbene), polar (polyether), and non polar (aliphatic, aromatic).**
- **Gemini surfactants can also be asymmetrical.**

Pyrene Labeled Gemini Surfactant

[Py-3-12]

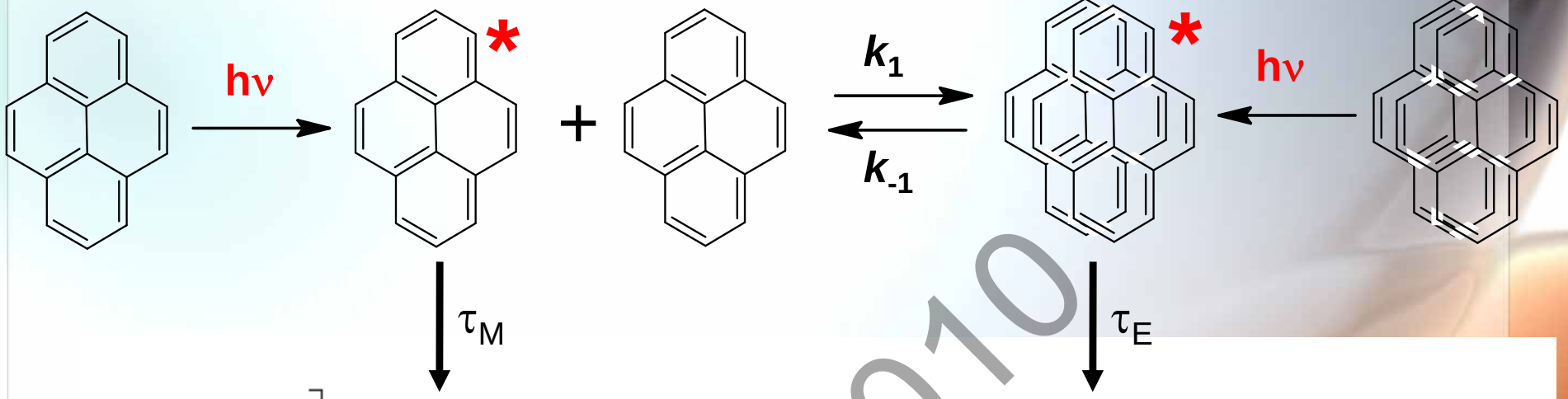


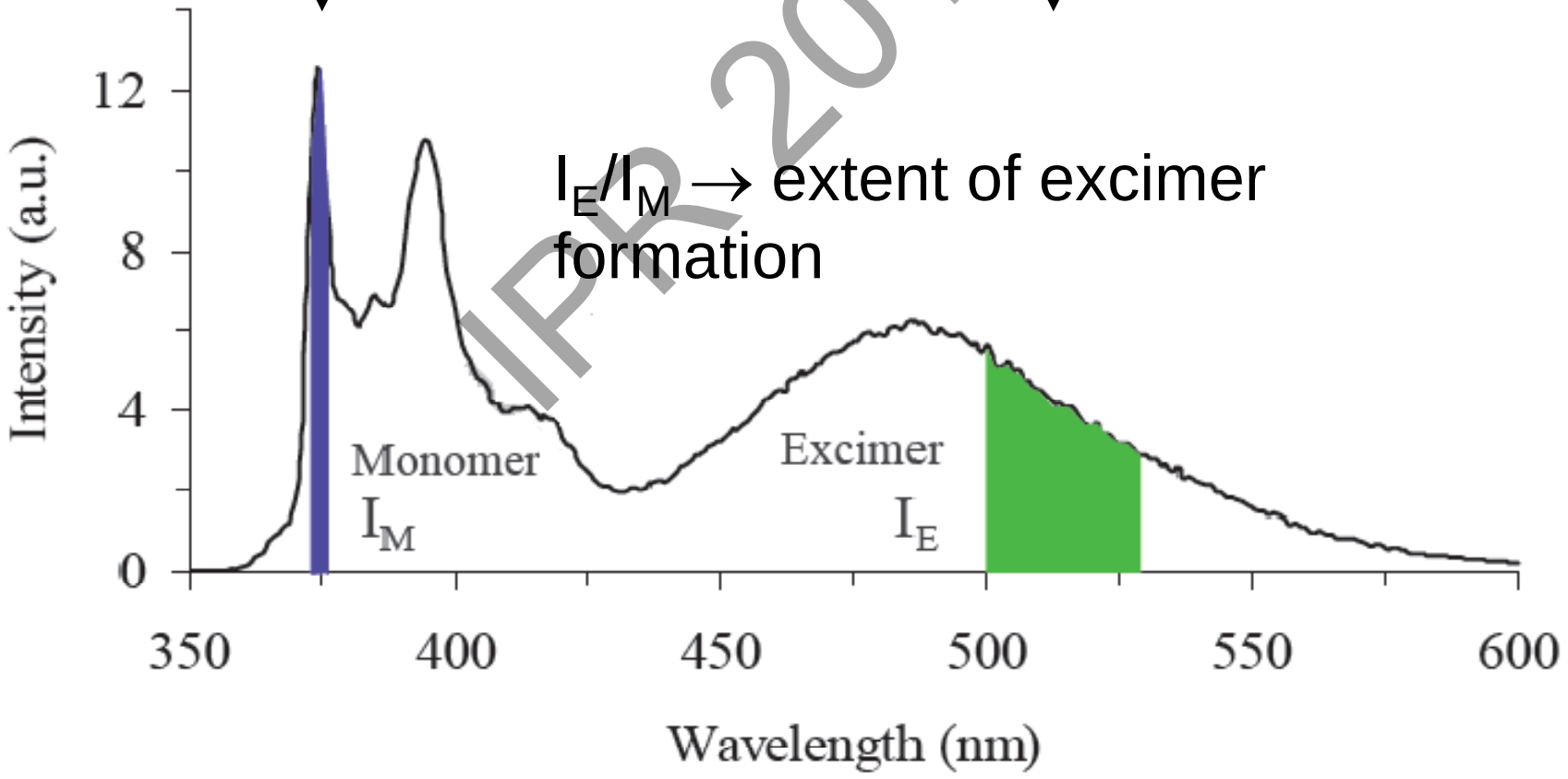
- **Gemini surfactant labeled with pyrene – study the gemini surfatcant complexes with DNA in solution by fluorescnece**
  1) **Characterize the micellization without DNA**
  2) **Characterize the complexes with DNA**
- **Given to us by Dr. Shawn Wettig from the Dept. of Pharmacy at UW – CMC = 0.22 mM**

Why Pyrene?

- **Pyrene – has many interesting photophysical properties**
- **Pyrene's ability to form an excimer (excited dimer)**
- **Used to study the association of pyrene-labeled macromolecules in aqueous solution**

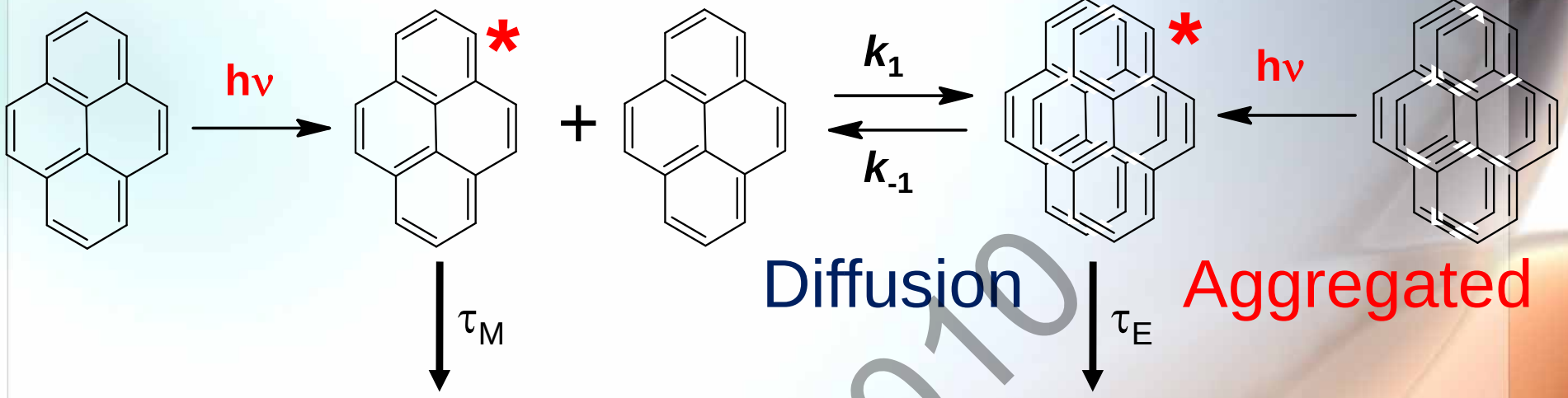
Pyrene - Fluorescence



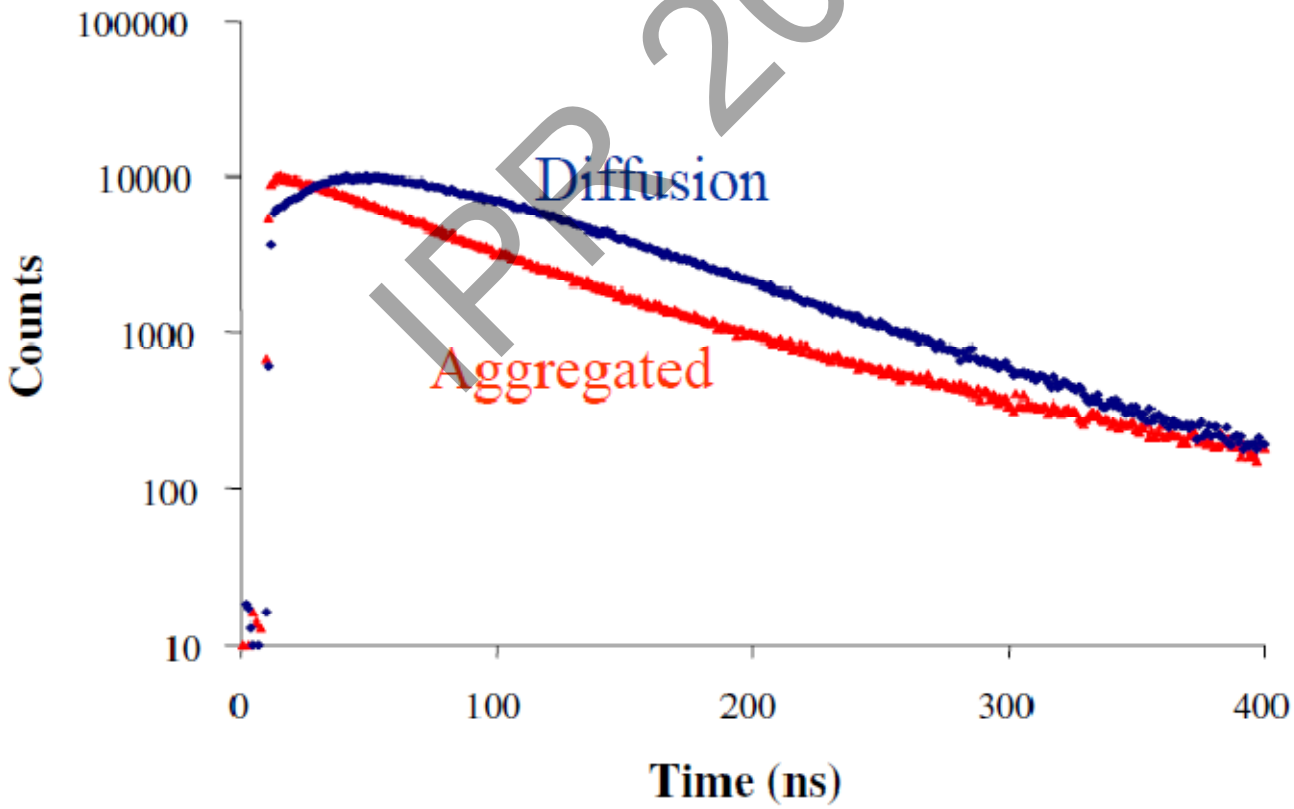


$I_E/I_M \rightarrow$ extent of excimer formation

Pyrene – Excimer Decays

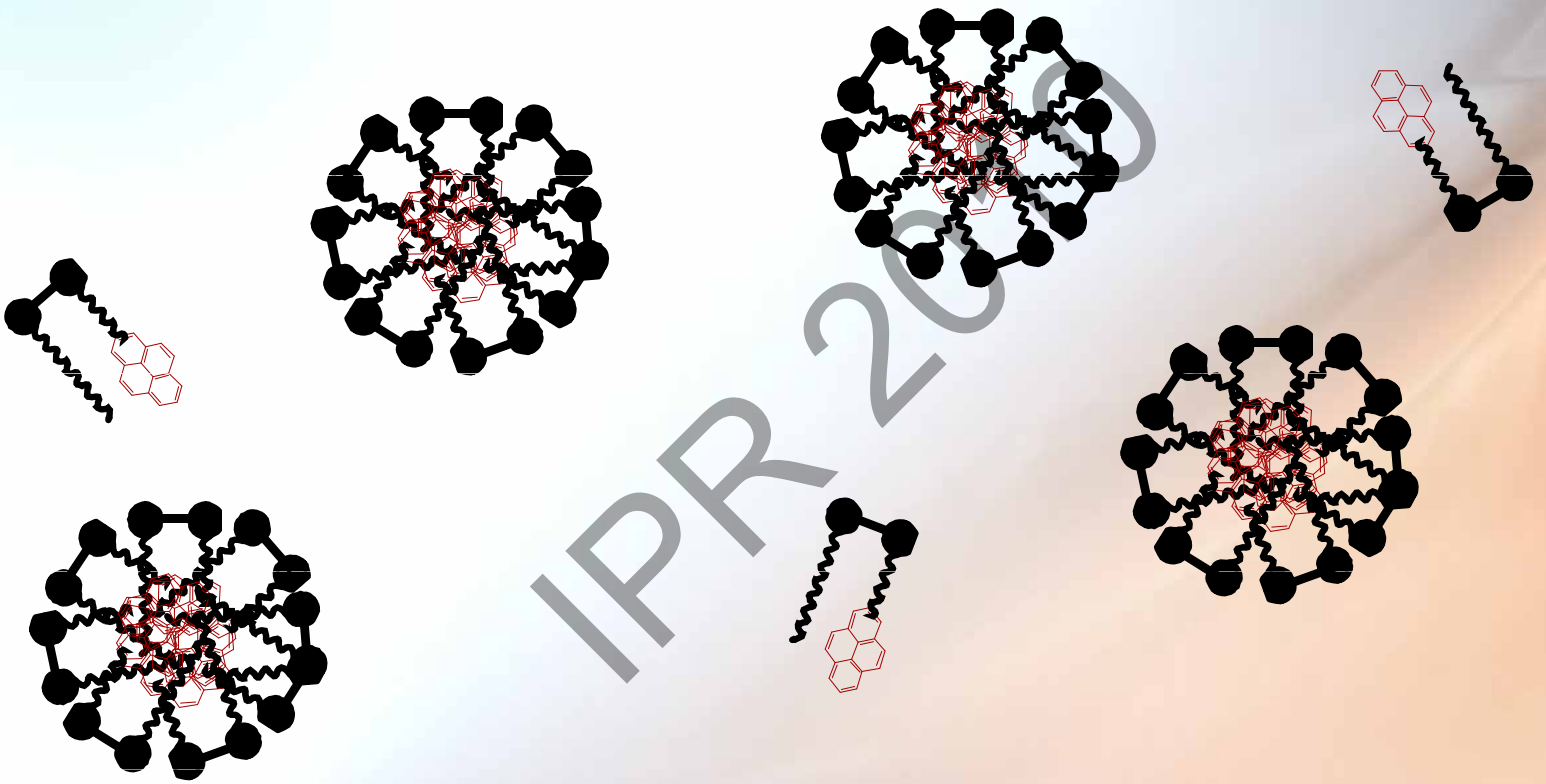


τ_M Diffusion τ_E Aggregated



Pyrene Labeled Surfactant

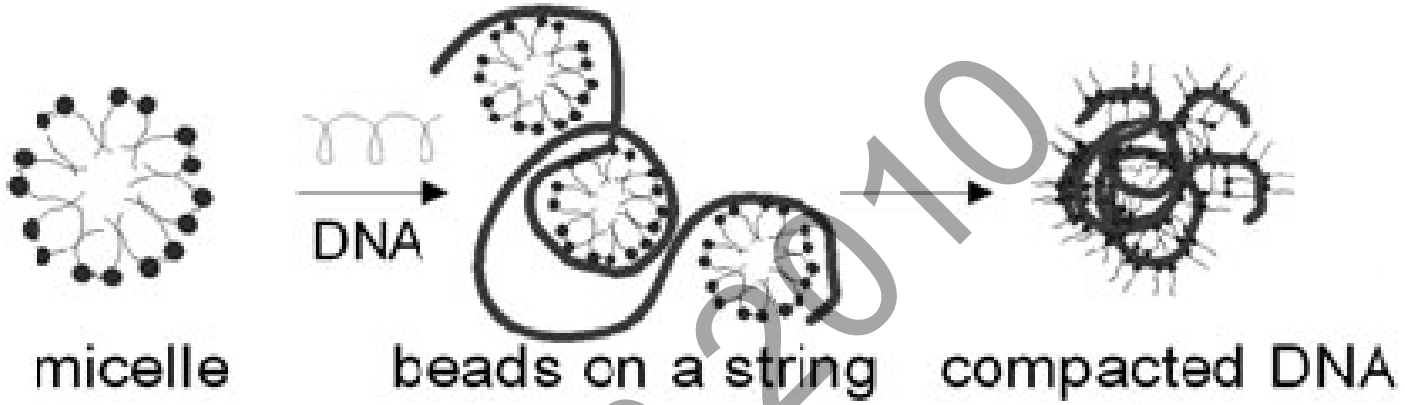
- **Without DNA**



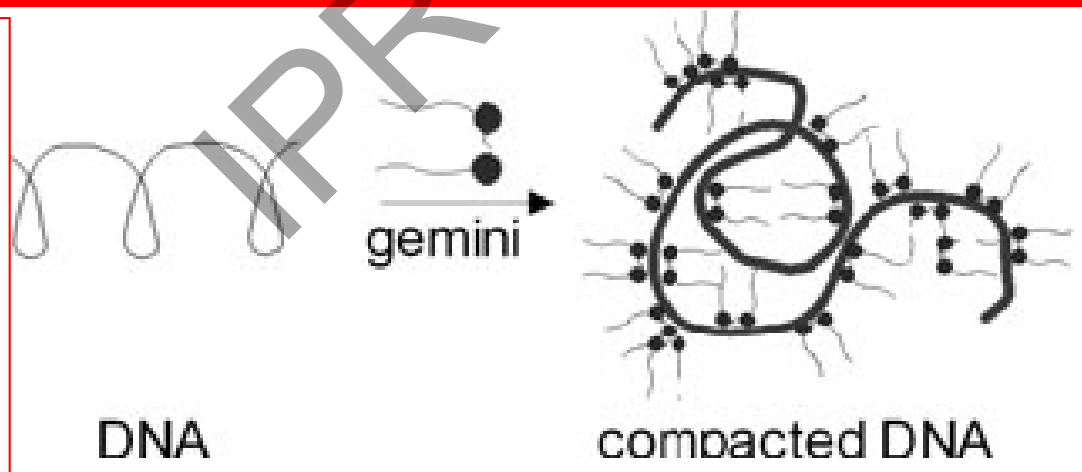
- **Fluorescence data gives us information about the CMC**

Pyrene Labeled Surfactant

- **With DNA**

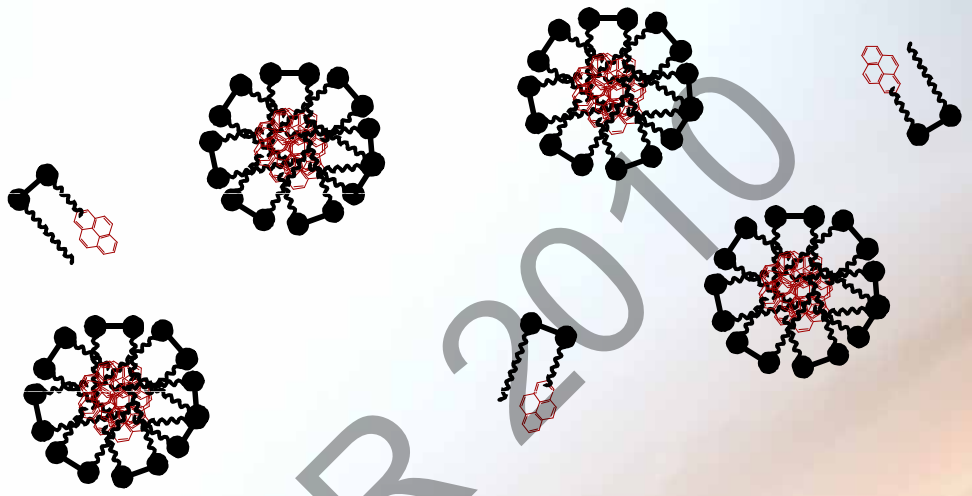


Excimer formation is due to complexation with DNA and not from micellization of the gemini surfactant



Based on AFM results

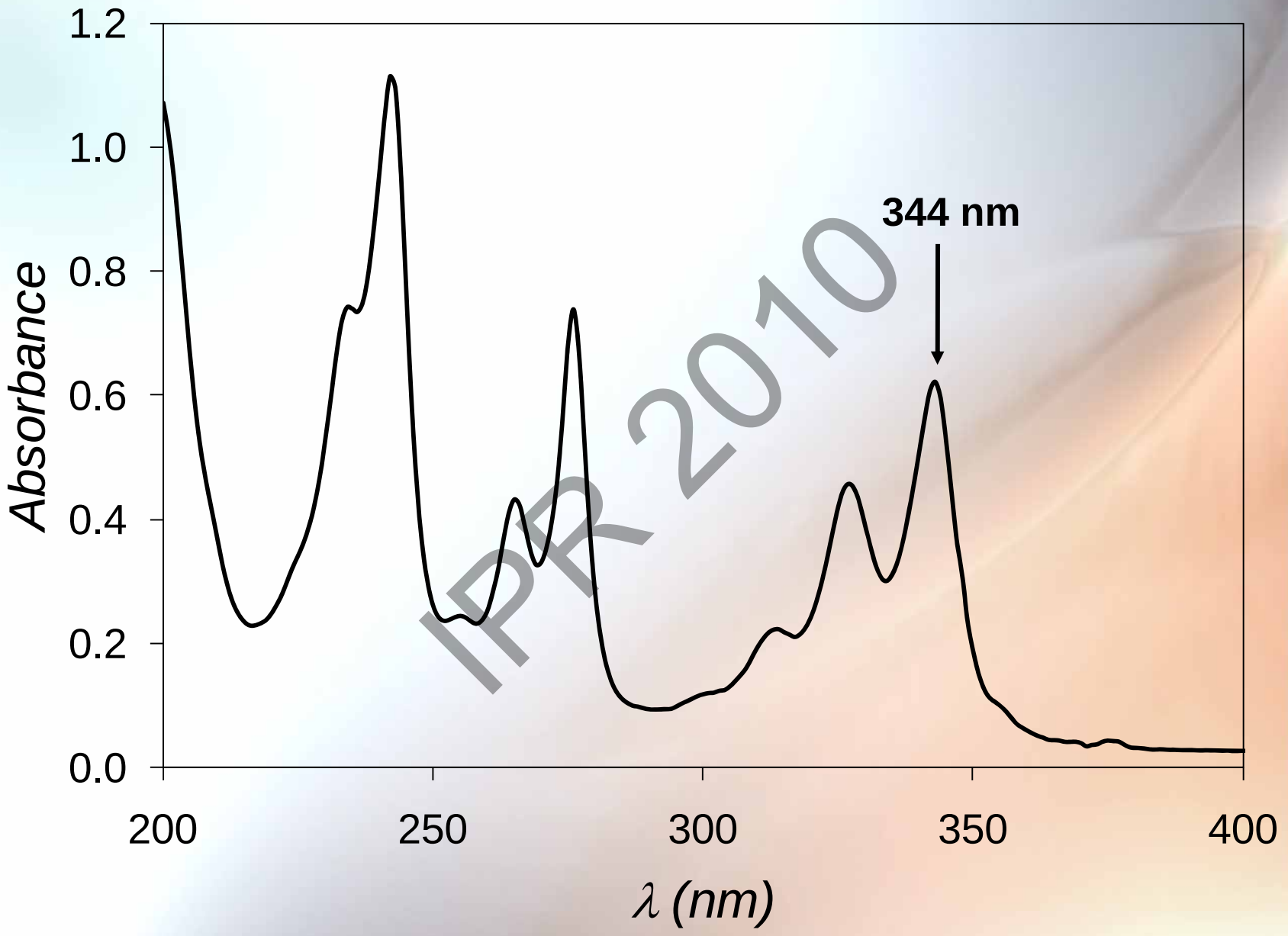
Wang, C., Li, X., Wettig, S. D., Badea, I., Foldvari, M., Verrall, R. E., *Phys. Chem. Chem. Phys.* **2007**, 9, 1616 – 1628.



RESULTS

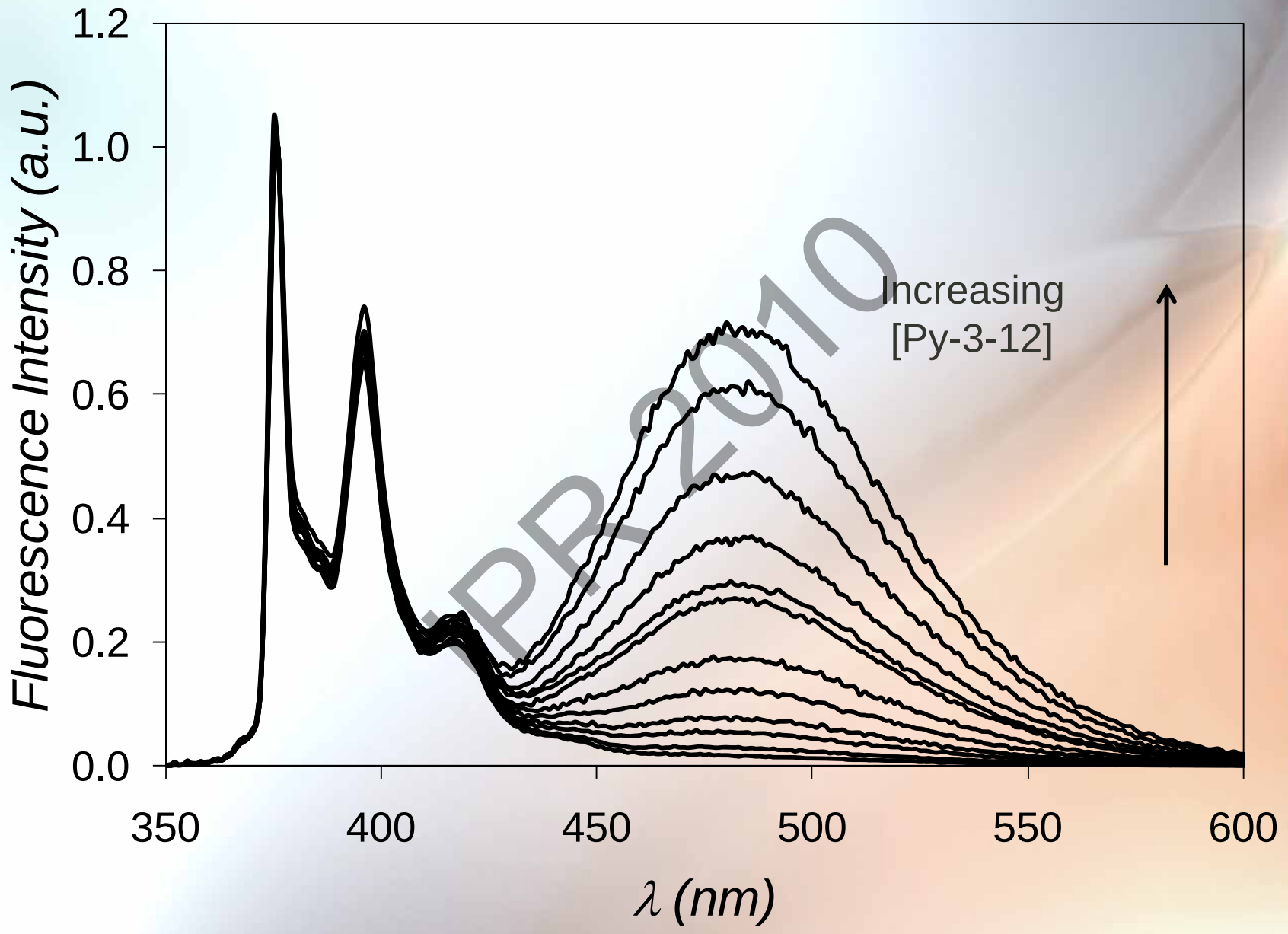
Micellization of Py-3-12 Without DNA

Absorbance Spectrum



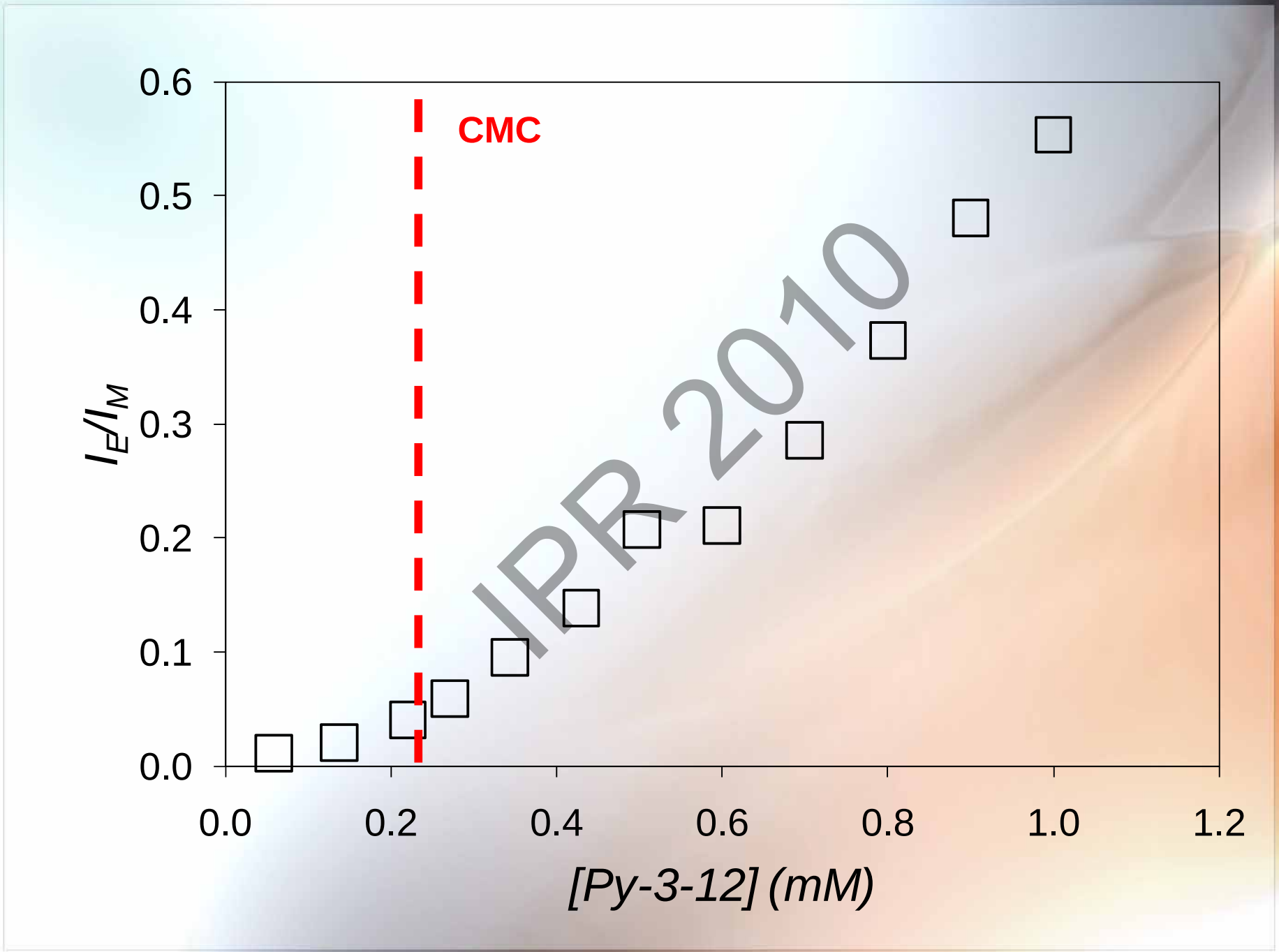
[Py-3-12] = 0.02 mM

Steady State Fluorescence

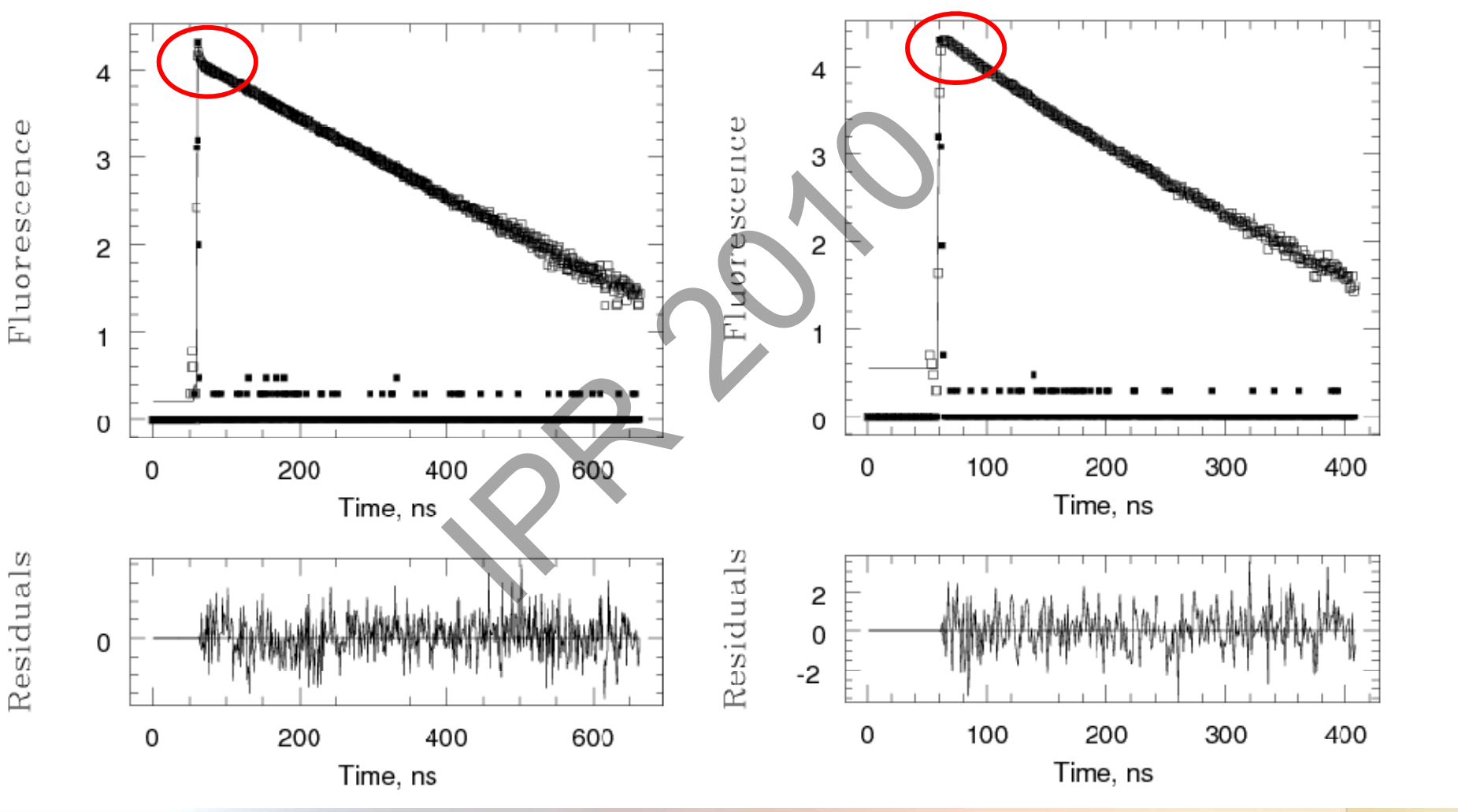


Normalized @ 375 nm

Steady State Fluorescence



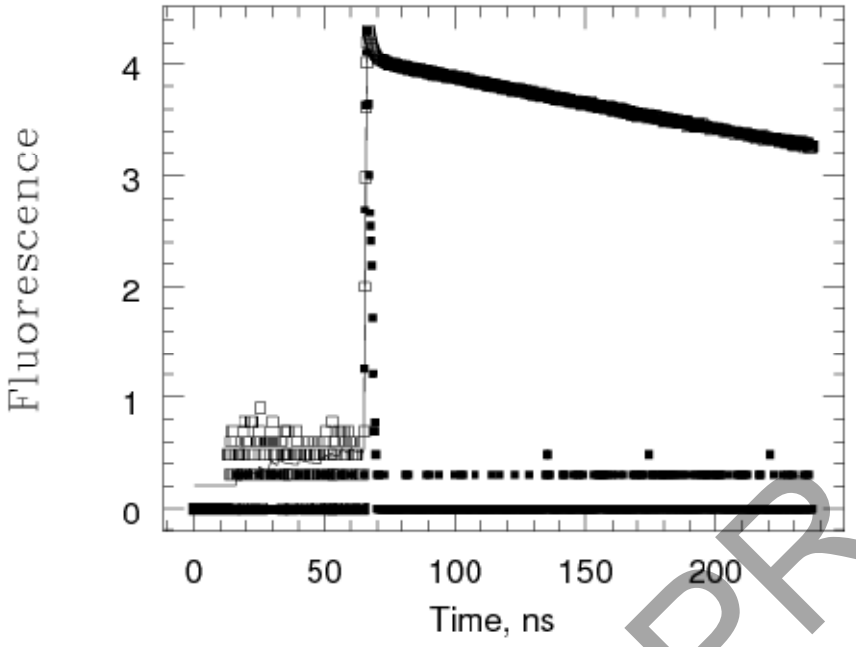
Time Resolved Fluorescence Decays

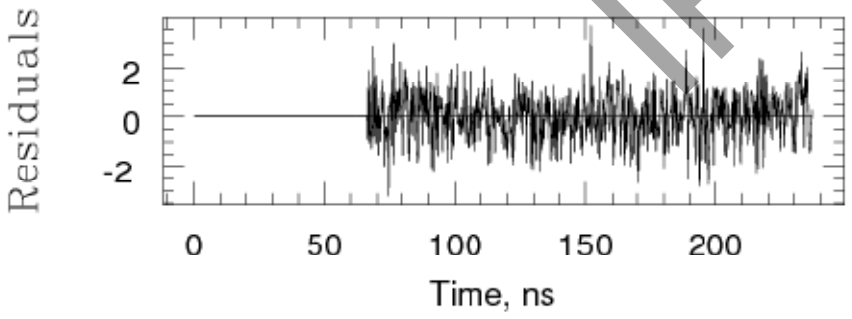


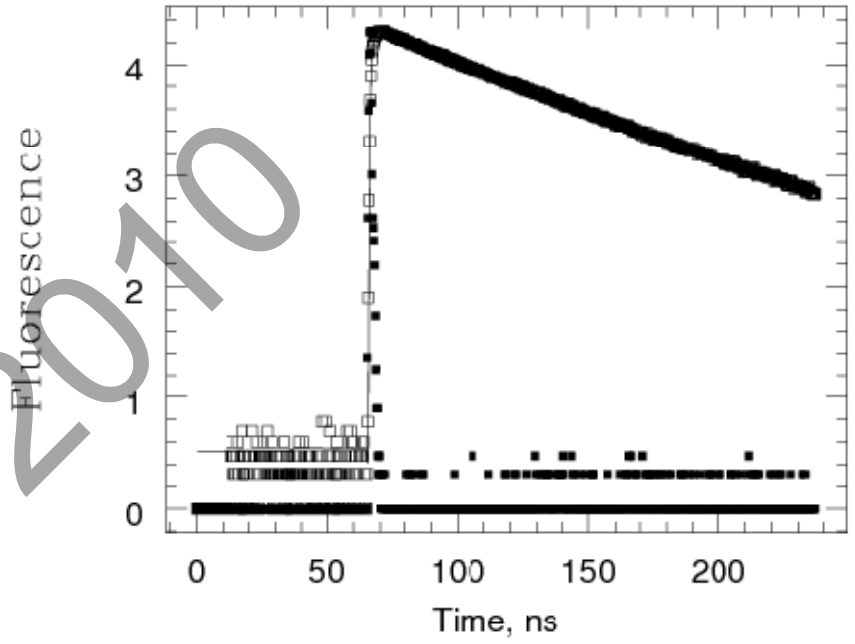
Time per Channel = 1.02 ns /channel → equivalent to short decay time

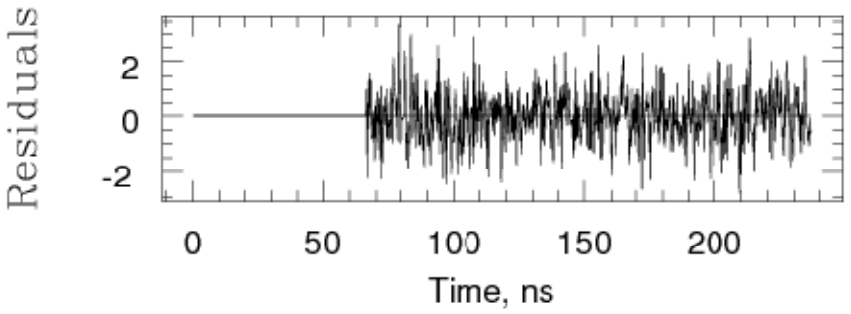
Time Resolved Fluorescence Decays

Time per Channel = 0.24 ns /channel







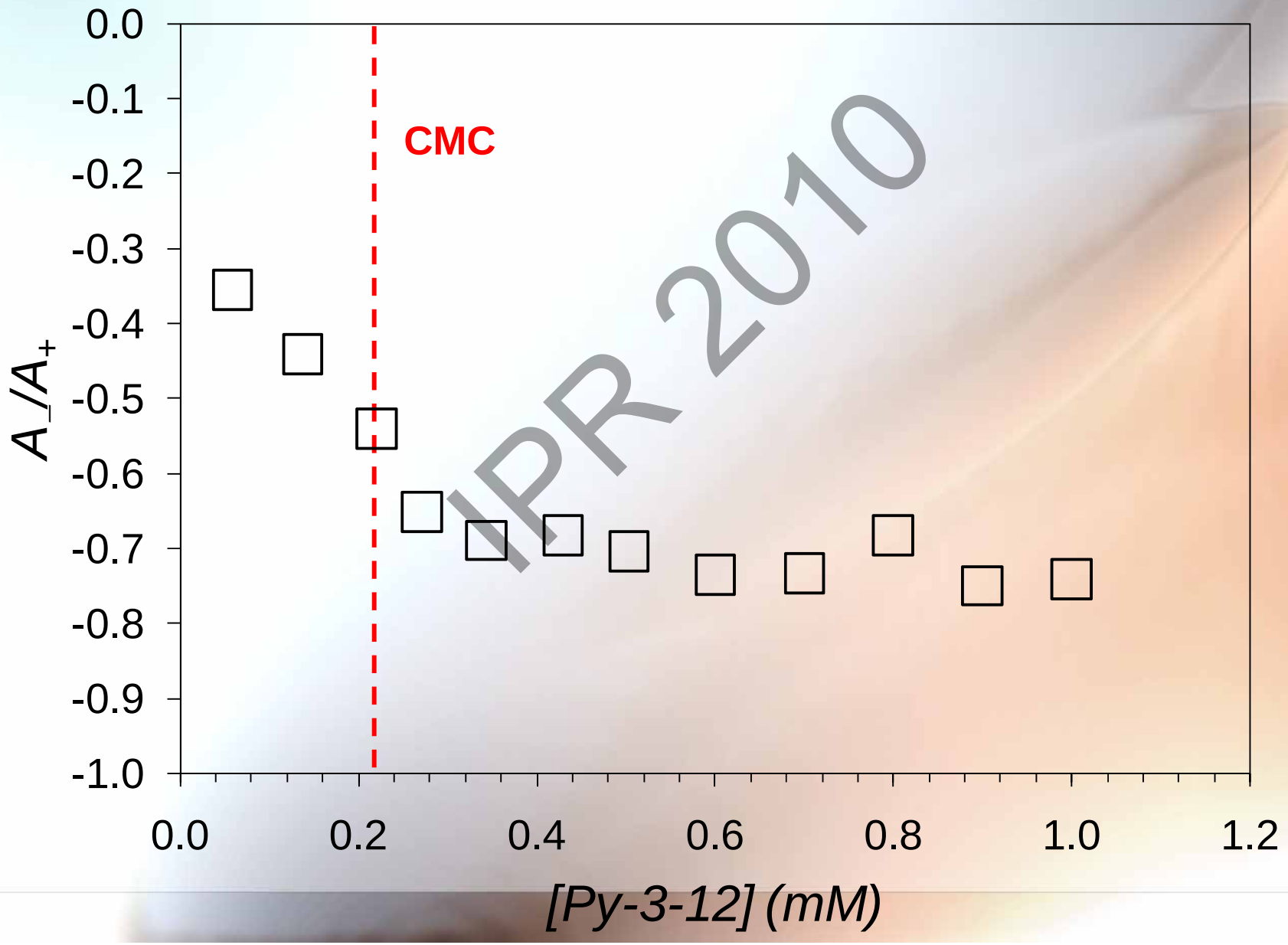


$$I(t) = A_1 e^{-t/\tau_1} + A_2 e^{-t/\tau_2} + A_3 e^{-t/\tau_3}$$

$A_-/A_+ \rightarrow$ -1 excimer formed via diffusion $a_-/a_+ \rightarrow 0$ ground state dimers

Time Resolved Fluorescence Decays

$A_-/A_+ \rightarrow -1$ excimer formed via diffusion $A_-/A_+ \rightarrow 0$ ground state dimers

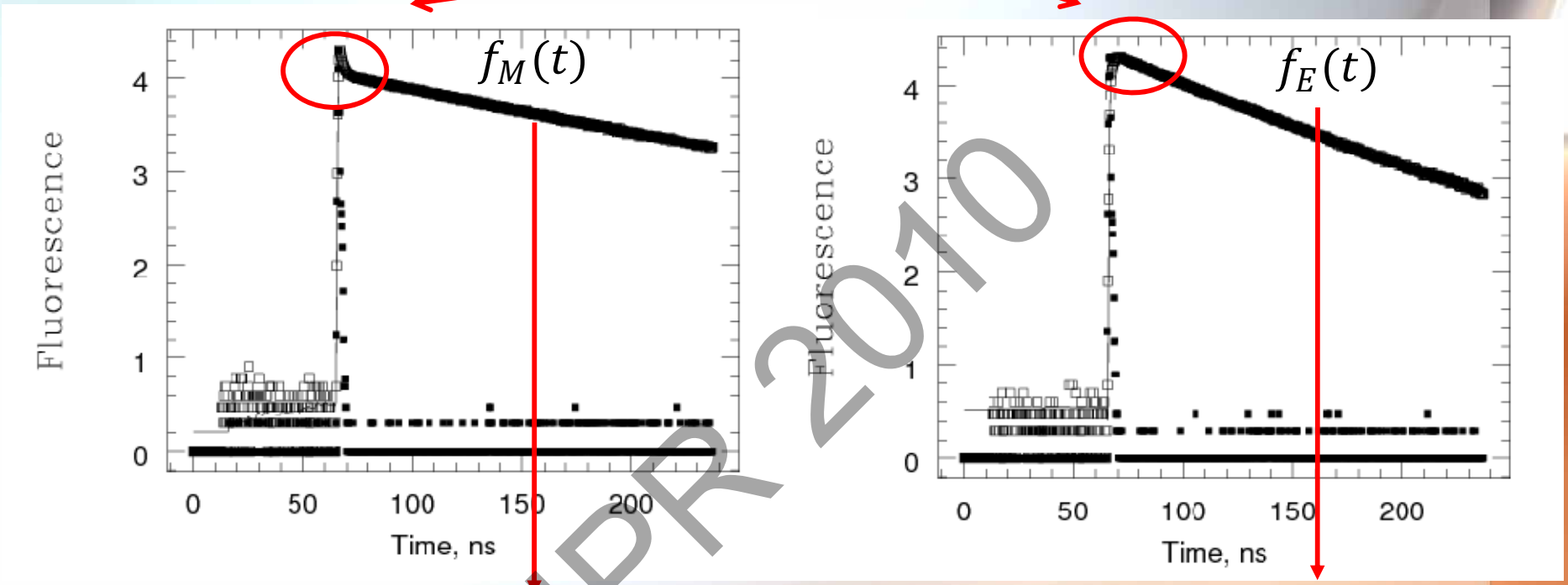


Global Analysis

- **Excimer formation via diffusion**
  - **Kinetics of monomer consumption and excimer formation are coupled**
  - **Decay times in the monomer and excimer (rise time) are found to be the same when fit with a sum of exponentials**
  - **Fit the monomer and excimer globally by ensuring some of the decay times are held the same in the monomer and excimer decays**
  - **Increases the accuracy on the parameters retrieved from the fluorescence decays**

Time Resolved Fluorescence Decays

e^{-t/τ_1}



$a_1 e^{-t/\tau_{M1}} + a_2 e^{-t/\tau_{M2}}$

$a_{E0} e^{-t/\tau_{E0}} + a_D e^{-t/\tau_D}$

Lifetime of pyrene monomer ~ 97 ns

Impurtiy ~ 20 - 6 ns (3%)

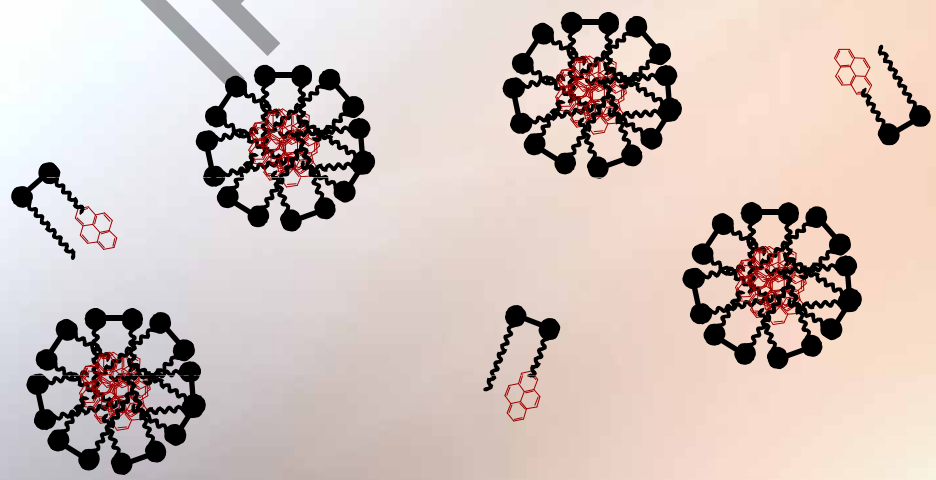
pyrenes that form excimer (proper stacking)

pyrenes that form long lived excimer (improper stacking)

Time per Channel = 0.24 ns /channel

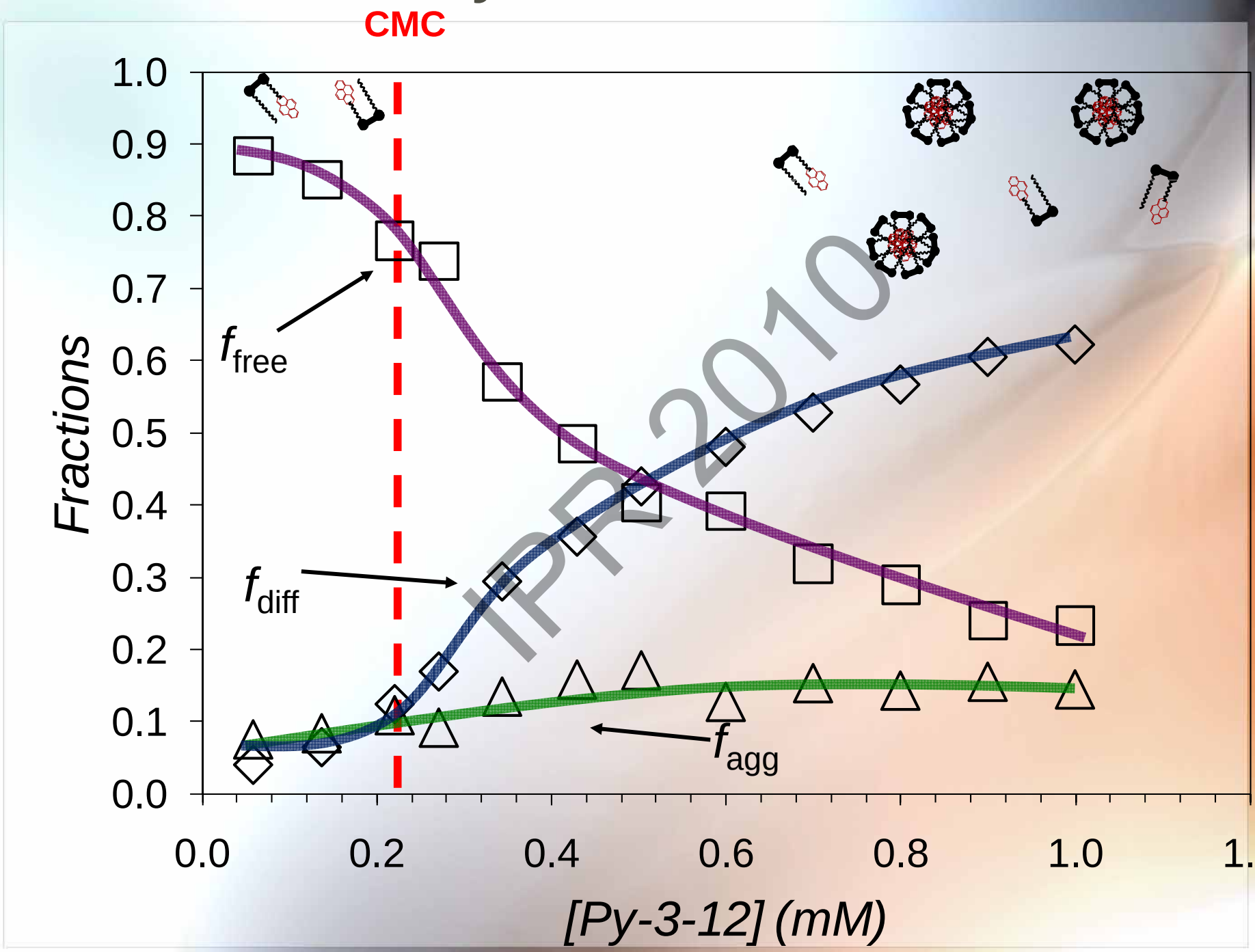
Global Analysis

- f_{free} – fraction of free pyrene
- f_{diff} – fraction pyrene that forms excimer via diffusion
- f_{E0} – fraction of preassociated pyrenes that form excimer (proper stacking)
- f_D – fraction of preassociated pyrenes that form long lived excimer (improper stacking)
- $f_{agg} = f_{E0} + f_D$

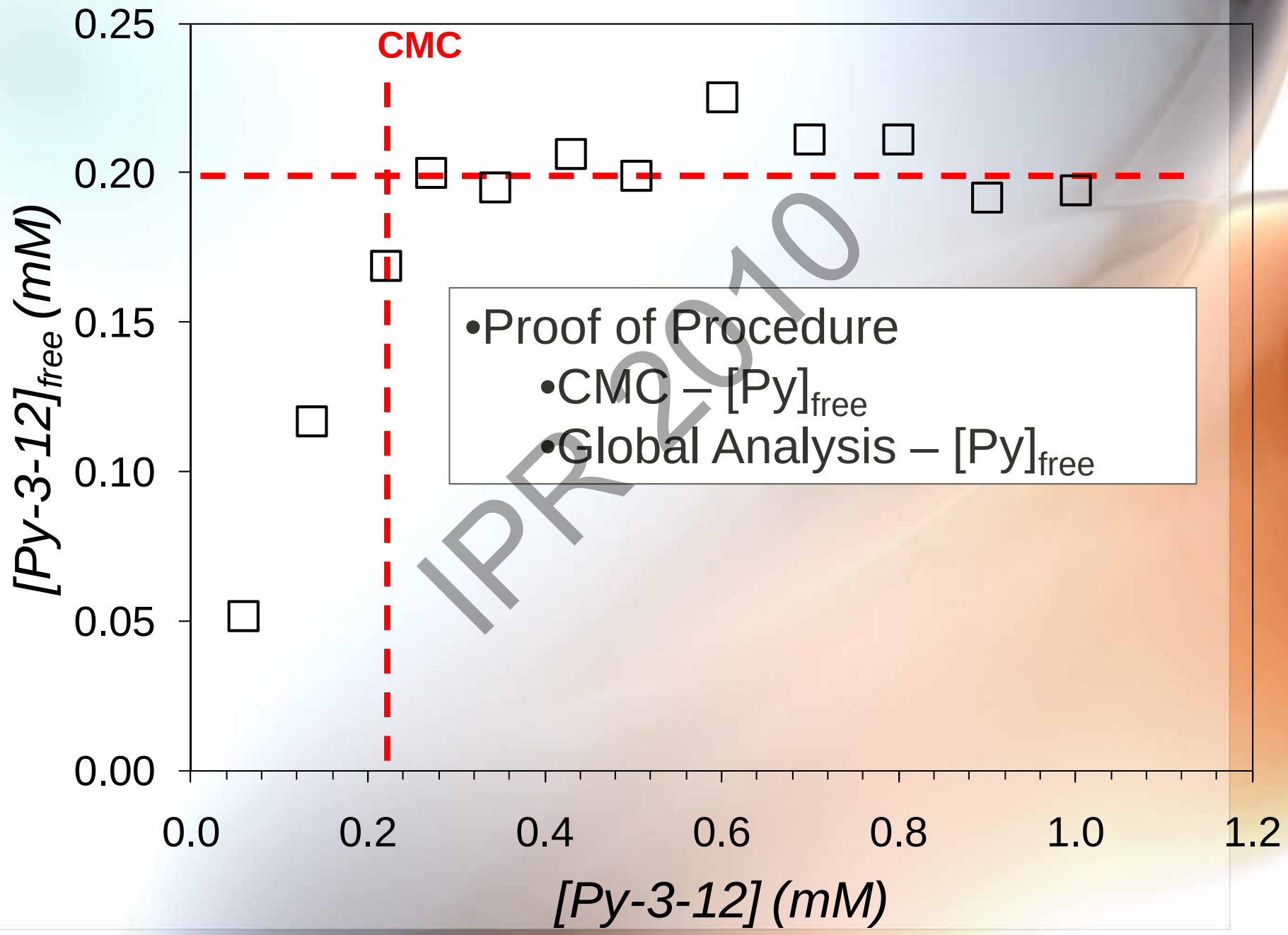


Siu, H., Duhamel, J., *Macromolecules*, 37, **2004**, 9287 – 9289.

Global Analysis



Global Analysis



Global Analysis – Calculation of $(I_E/I_M)^{SPC}$

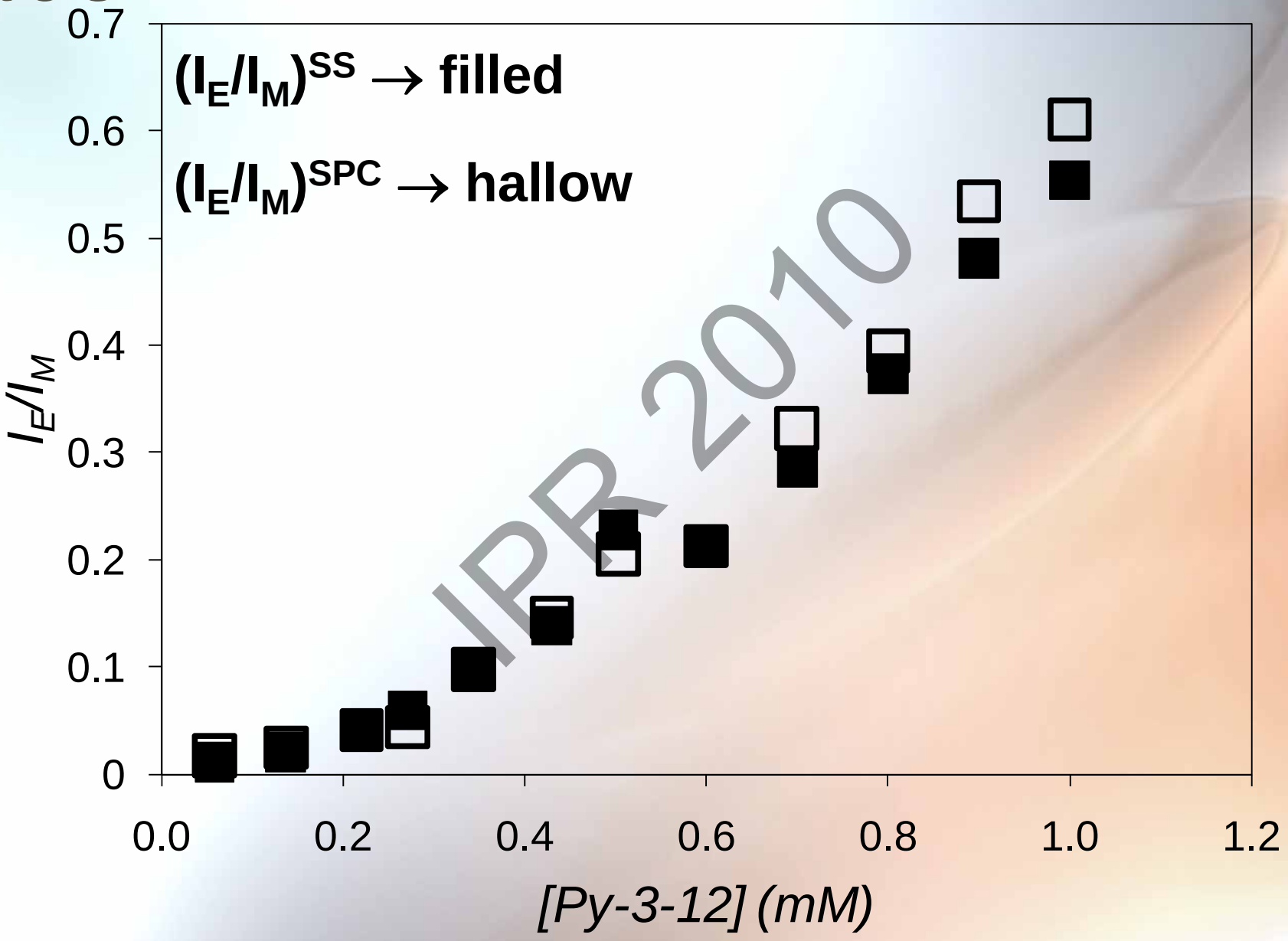
$$I_M = \int_0^\infty [Py^*]_t \, dt = [Py]_o \left[f_{diff} \sum_{i=1}^{n} a_i \tau_i + f_{free} \tau_M \right]$$

$$I_E = \int_0^\infty [E^*]_t \, dt$$

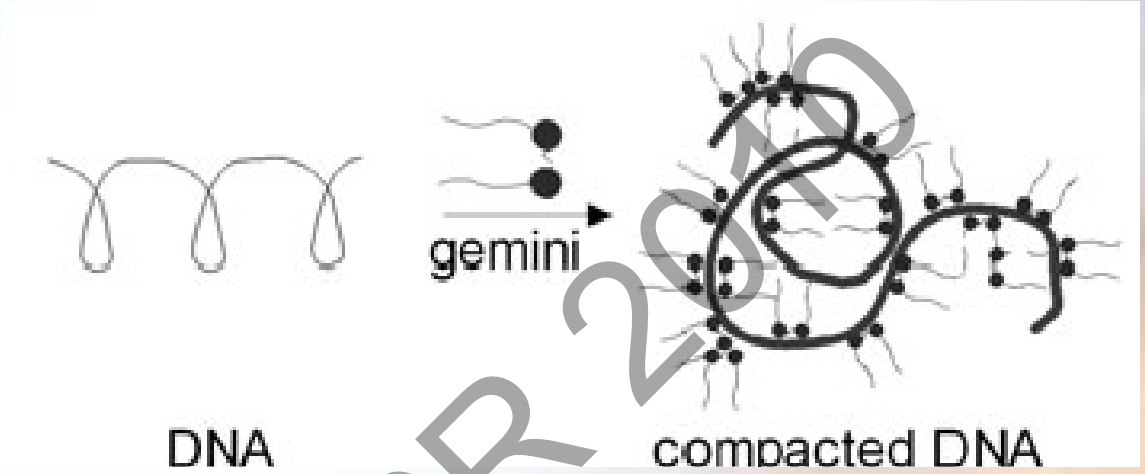
$$= [Py]_o \left[-f_{diffE0} \sum_{i=1}^{n} a_i \frac{\frac{1}{\tau_i} - \frac{1}{\tau_M}}{\frac{1}{\tau_i} - \frac{1}{\tau_{E0}}} \tau_i + \left(f_{E0} + f_{diffE0} \sum_{i=1}^{n} a_i \frac{\frac{1}{\tau_i} - \frac{1}{\tau_M}}{\frac{1}{\tau_i} - \frac{1}{\tau_{E0}}} \right) \tau_{E0} \right.$$

$$\left. - f_{diffD} \sum_{i=1}^{n} a_i \frac{\frac{1}{\tau_i} - \frac{1}{\tau_M}}{\frac{1}{\tau_i} - \frac{1}{\tau_D}} \tau_i + \left(f_D + f_{diffD} \sum_{i=1}^{n} a_i \frac{\frac{1}{\tau_i} - \frac{1}{\tau_M}}{\frac{1}{\tau_i} - \frac{1}{\tau_D}} \right) \tau_D \right]$$

$(I_E/I_M)^{SS}$ versus $(I_E/I_M)^{SPC}$; front-face



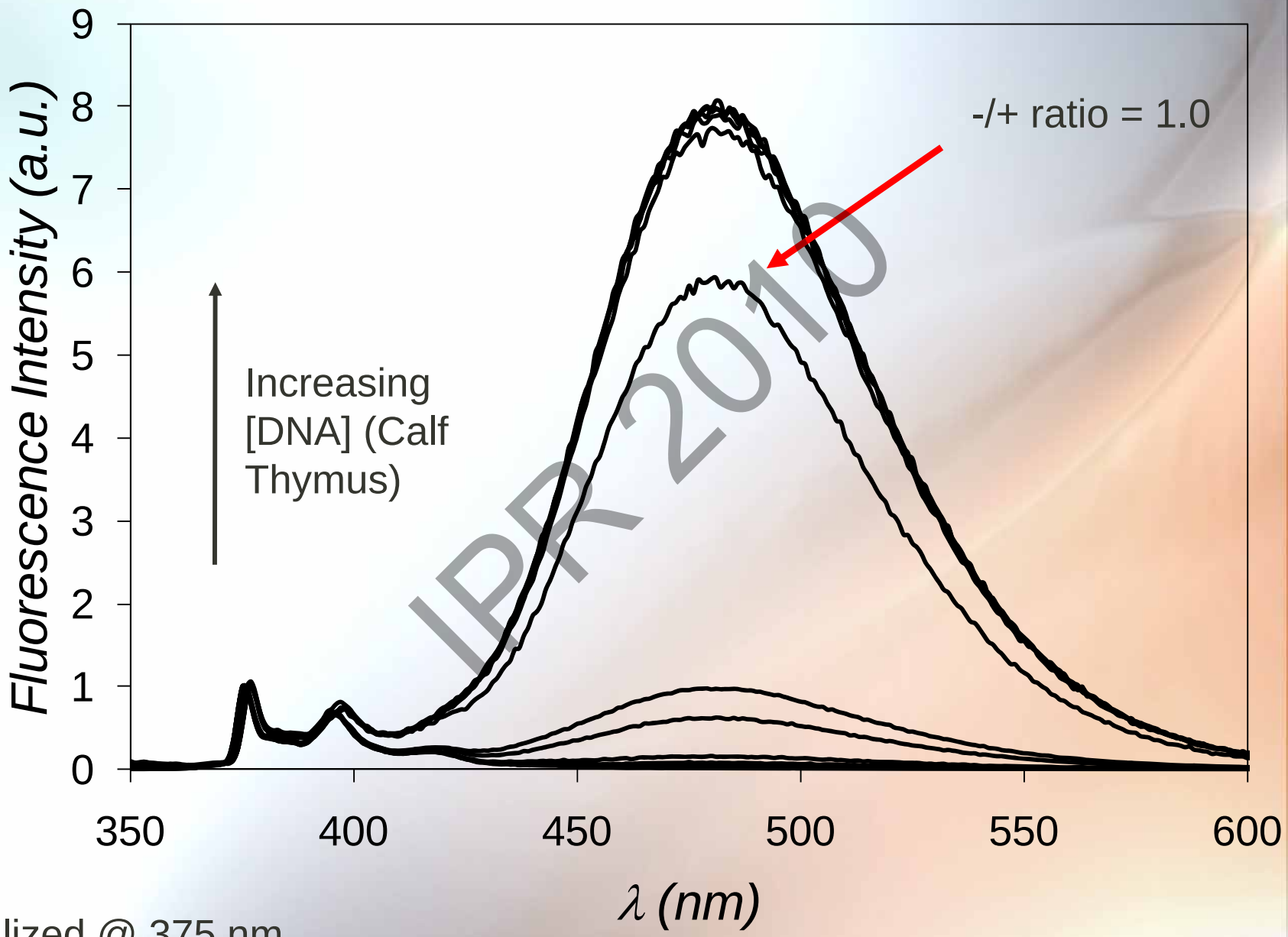
SPC trend scaled to SS trend



RESULTS

Complexation of Py-3-12 With DNA

Steady State Fluorescence

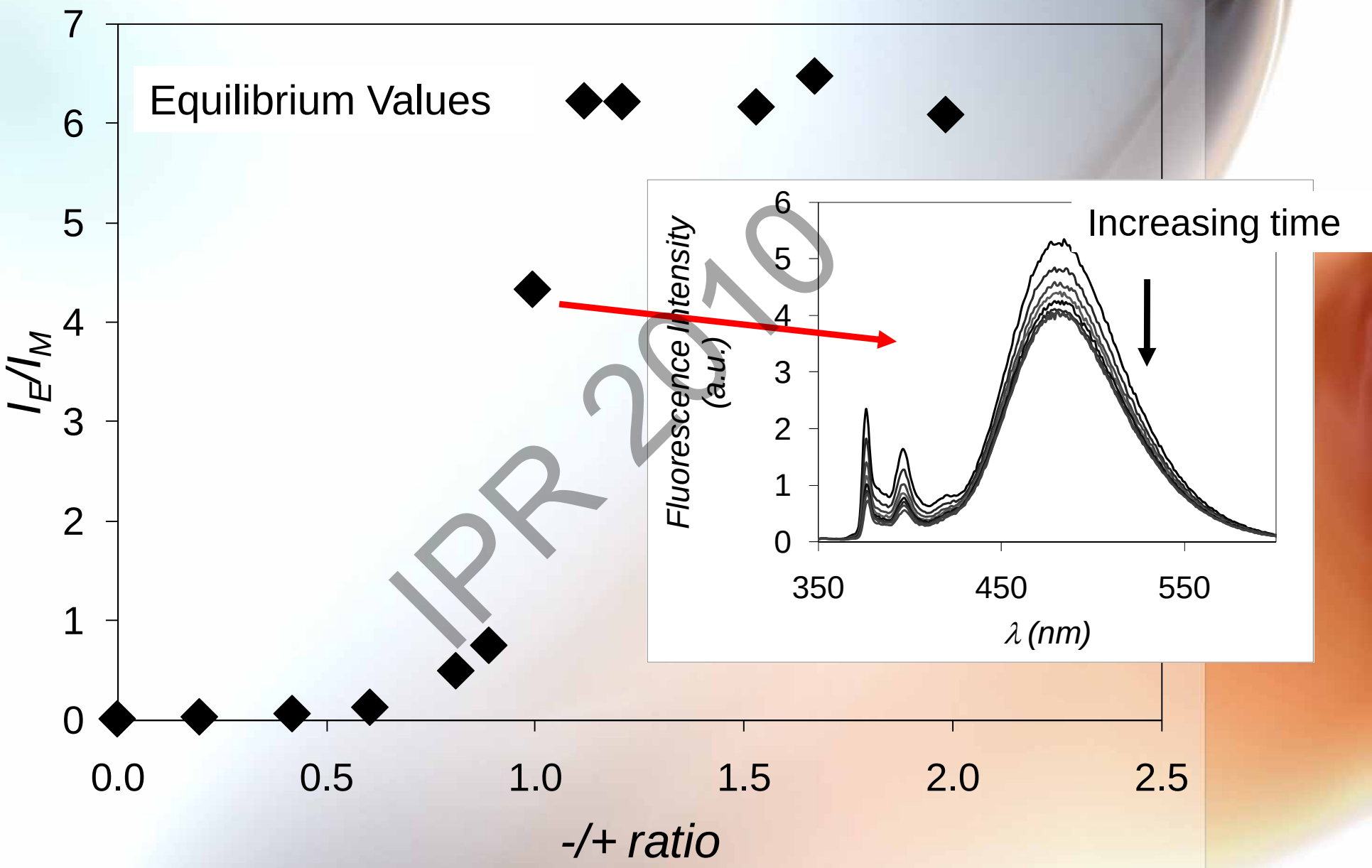


Normalized @ 375 nm

[Py-3-12] = 1e-4M

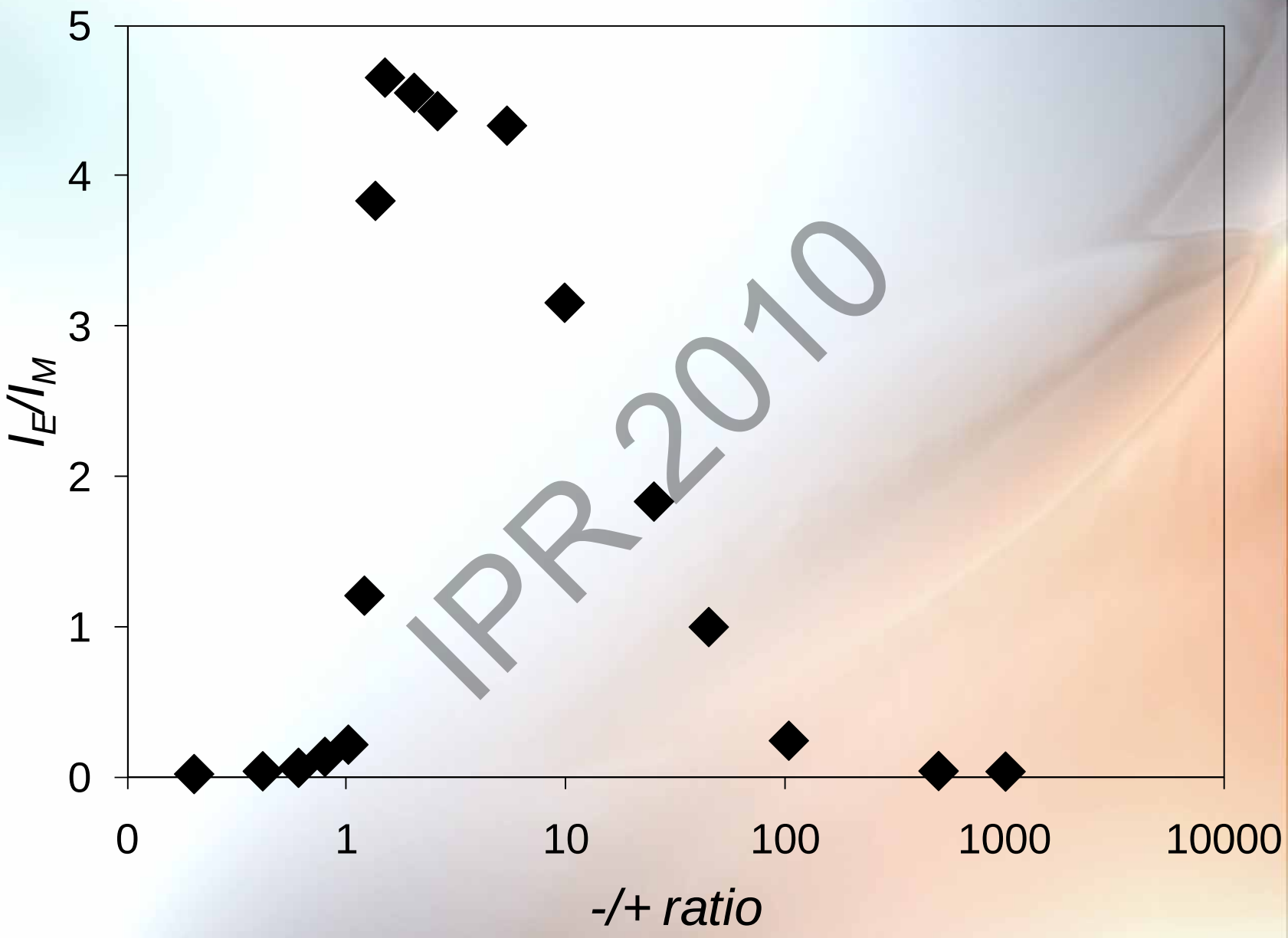
-/+ ratio → [DNA-bp]/[Py-3-12]

Steady State Fluorescence



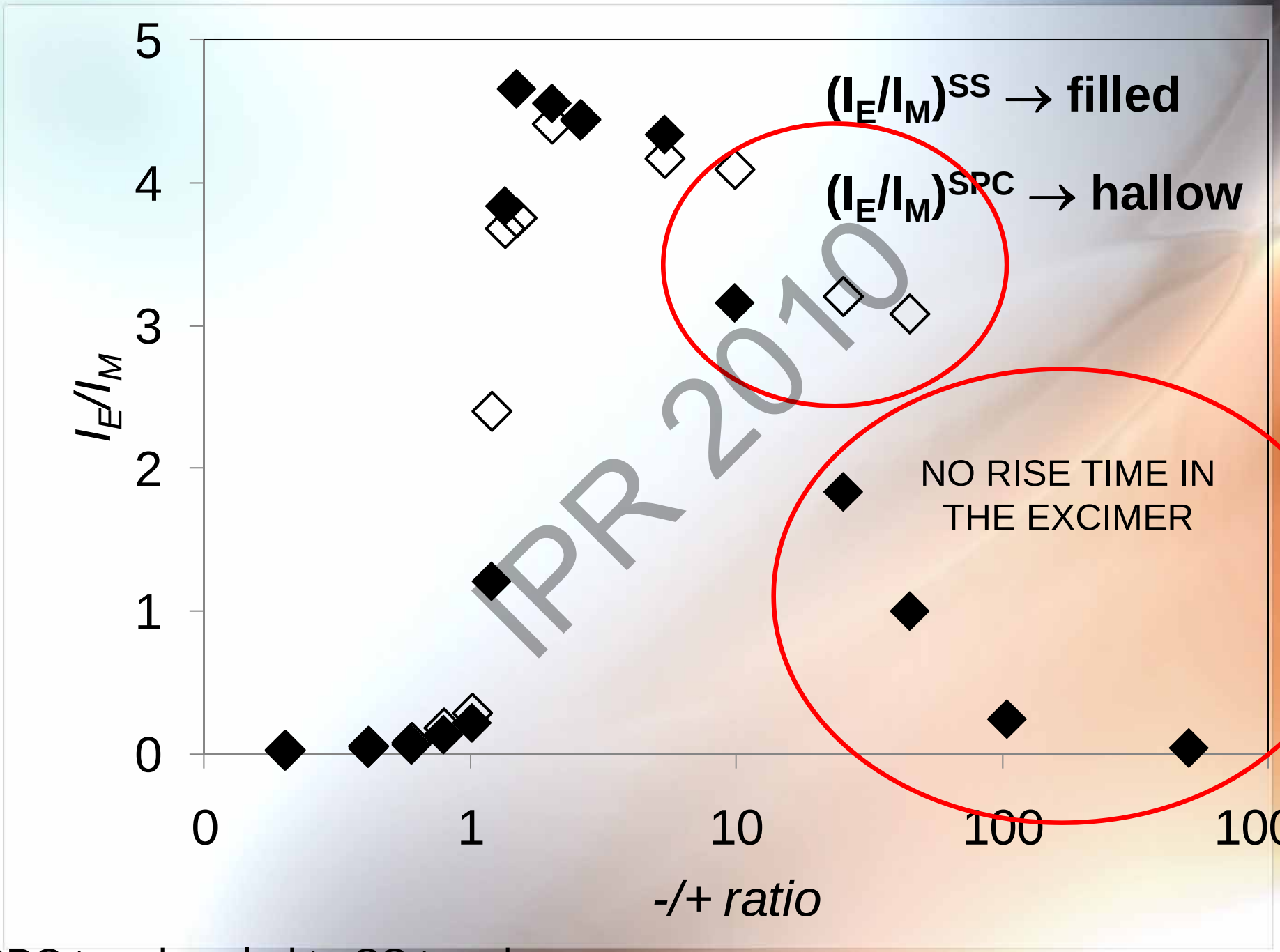
- **Can we add enough DNA separate the surfactant molecules?**
- **$I_E/I_M \rightarrow 0$?**

Steady State Fluorescence



Sheared Calf Thymus DNA – reduce viscosity at high -/+ ratios

$(I_E/I_M)^{SS}$ versus $(I_E/I_M)^{SPC}$



SPC trend scaled to SS trend

Global Analysis