Institute for Polymer Research
27th Annual Symposium

Symposium documents for

Mark Ingratta

Abstract

Presentation

Fluorescence Study of the Effect of Side-Chain Length on the Side-Chain Dynamics of an Alpha-Helical Polypeptide

Mark Ingratta
Department of Chemistry, University of Waterloo

Developing an understanding of the physical principles that control the folding of proteins has been the focus of attention of numerous research laboratories throughout the world. The results of these studies have led to simulations that model the folding of proteins based on some fundamental assumptions. For instance, the "diffusion-collision model" assumes that secondary structure elements diffuse until they collide and adhere to form the tertiary structure of the protein.[1] The general consensus is that the tertiary structure of a protein results from interactions between the side-chains of secondary structural elements which guide the α–helices and β–sheets into their final three-dimensional arrangement that constitutes the protein structure. In view of the key role played by the side chains of secondary structures in the last steps of the protein folding pathway, experimental methods are required that can characterize the volume probed by the side chains of a secondary structure.

This study represents an attempt at achieving this goal by using fluorescence to characterize the volume probed by the tip of the side-chain of a poly(glutamic acid) (PGA) α–helix, which is used as an example of secondary structure. In these experiments, the fluorescent pyrene probe is attached randomly along the PGA α–helix via two linkers of different length. To increase the linker-length, the alkyl spacer connecting the probe with the PGA side chains is increased from a methylene to a tetramethylene linker. This increases the side chain length between the probe and the peptidic backbone from 5 to 8 atoms.

When a polymer is randomly labeled with pyrene, the dynamics of encounter between any two pyrenes is controlled by the chain length spanning and flanking them. Consequently, randomly labeled polymers exhibit a distribution of chain lengths between any two pyrenes which results in a complicated distribution of rate constants. The blob model is a tool used to circumvent this complication. It works by dividing the polymer coil into blobs, where a blob is the volume probed by an excited dye during its lifetime. In so doing, the focus of the study shifts from the whole polymer chain down to one blob and the dynamics of the chain located inside a

blob are characterized. The motions and volume probed by the side chains having different length will be characterized using the fluorescent blob model.

Pyrene was chosen as a dye to study the dynamics of polypeptides in solution by time-resolved fluorescence, because of its relatively long lifetime and high quantum yield. The natural fluorescence lifetime of pyrene when attached to a polymer is in the 200-300 ns range, depending on the solvent and polymer. Pyrene can be excited at around 340 nm, and emits in the blue region of the visible spectrum around 375 nm as a monomer. If it encounters another ground state pyrene while excited, it forms an excimer species which decays with a lifetime of about 50 ns with an emission centered in the green region of the visible spectrum (430 to 600 nm). Figure 1 displays the fluorescence spectra of pyrene labeled PGA (Py-PGA). As the pyrene content of Py-PGA increases, more excimer is being generated and the emission centered at 480 nm increases.

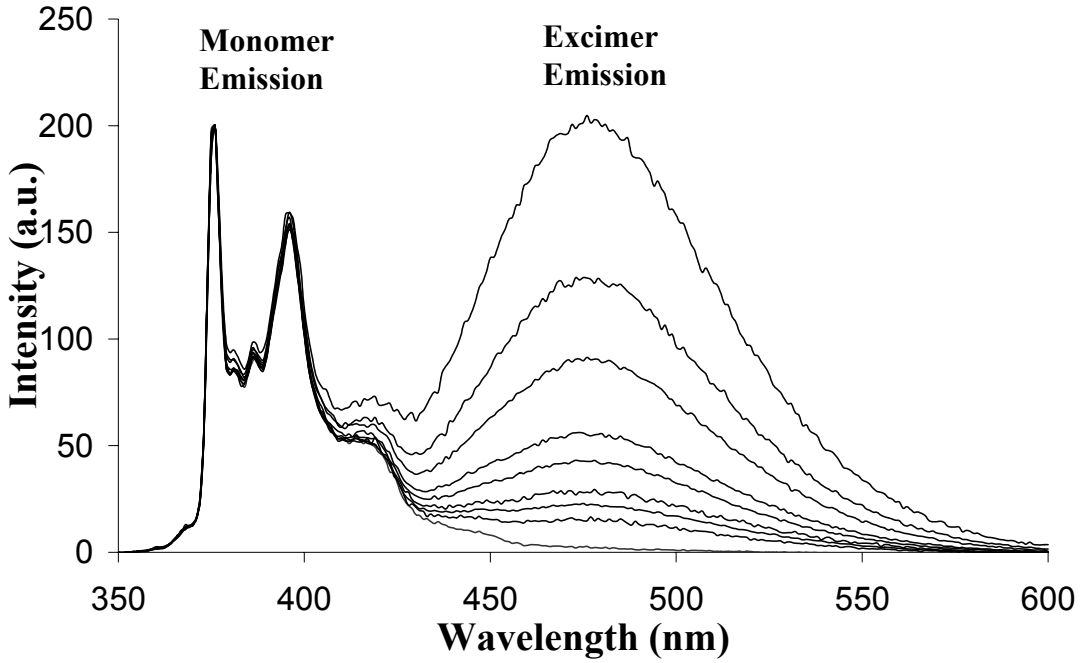


Figure 1: Fluorescence spectra of PGA labeled with increasing amounts of 1-pyrenemethylamine. Pyrene content decreases from top to bottom: 15 – 0.4 mol%.

Three parameters must be defined when using the blob model. They are k_{blob}, $\langle n \rangle$ and $k_e[blob]$. A blob is defined by the volume probed by an excited chromophore during its lifetime. For a pyrene labeled polymer, the rules set out by the blob model impose that an excited pyrene not leave a blob, but a ground state pyrene can diffuse between blobs at a certain rate. The exchange rate between blobs is k_e, while the blob concentration within the polymer coil is [*blob*]. Since the encounter between an excited pyrene and a ground state pyrene results in the formation of an excimer and the accompanying disappearance of the excited pyrene, pyrene is its own quencher. Thus, the number of quenchers per blob is equal to the number of pyrenes per blob and is referred to as $\langle n \rangle$. Finally, the rate constant for excimer formation within a blob is k_{blob}. N_{blob} represents the monomer units that an excited pyrene can probe during its lifetime. The fluorescence decays are used to obtain these characteristics about the blobs of pyrene labeled PGA. The value of N_{blob} for a series of pyrene labeled PGAs is obtained by extrapolating a plot of N_{blob} vs pyrene content to zero pyrene content as shown in Figure 2.

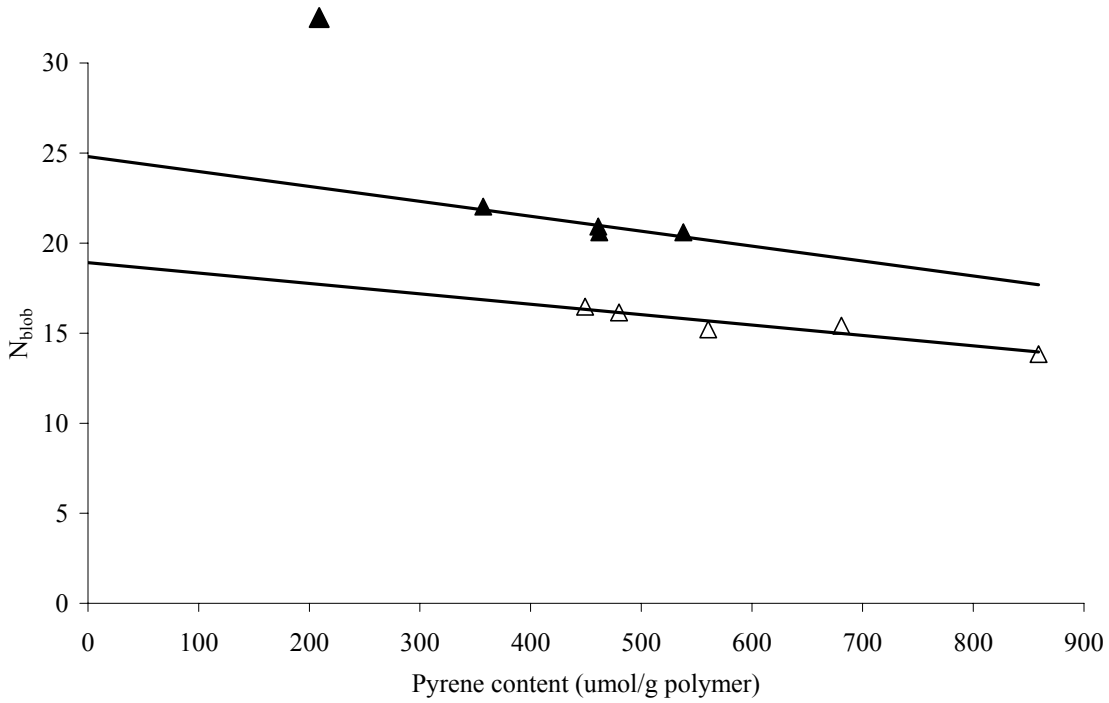


Figure 2: N_{blob} vs. Pyrene content for PGA labeled with 1-pyrenemethylamine (Δ) and 1-pyrenebutylamine ( ) with a pyrene lifetime of 150 and 155 ns respectively.

Two of the most important characteristics obtained from the blob model are k_{blob}, and N_{blob}, which give information on the rate of encounter and volume probed by the dye within its lifetime. Since the blob size is related to the lifetime of the chromophore, a longer lived pyrene will probe a larger volume of polymer coil. For this reason, it is necessary to control the lifetime of the dye by using an external quencher. The chosen quencher is nitromethane and has been used similarly in previous work on pyrene labeled poly(*N, N*-dimethylacrylamide).[2] In DMF, PGA labeled with 1-pyrenemethylamine, (PGA-PMA), has a lifetime of 215 ns, while PGA labeled with 1-pyrenebutylamine, (PGA-PBA) has a lifetime of 155 ns. Thus, by adding nitromethane to a PGA-PMA solution, the lifetime of PGA-PMA can be decreased to equal that of PGA-PBA. The results are shown in Figure 2, where the fluorescence decays of PGA-PMA and PGA-PBA were acquired.

In addition to comparing PGA-PMA and PGA-PBA at 150 ns, fluorescence decays were acquired for pyrene lifetimes ranging from 50 ns up to 215 ns. The results obtained from the analysis of the decays clearly show that the longer linker is able to probe a larger PGA segment, even as the lifetime of pyrene is decreased. Within each series, N_{blob} changes little with the lifetime of pyrene as shown in Figure 3.

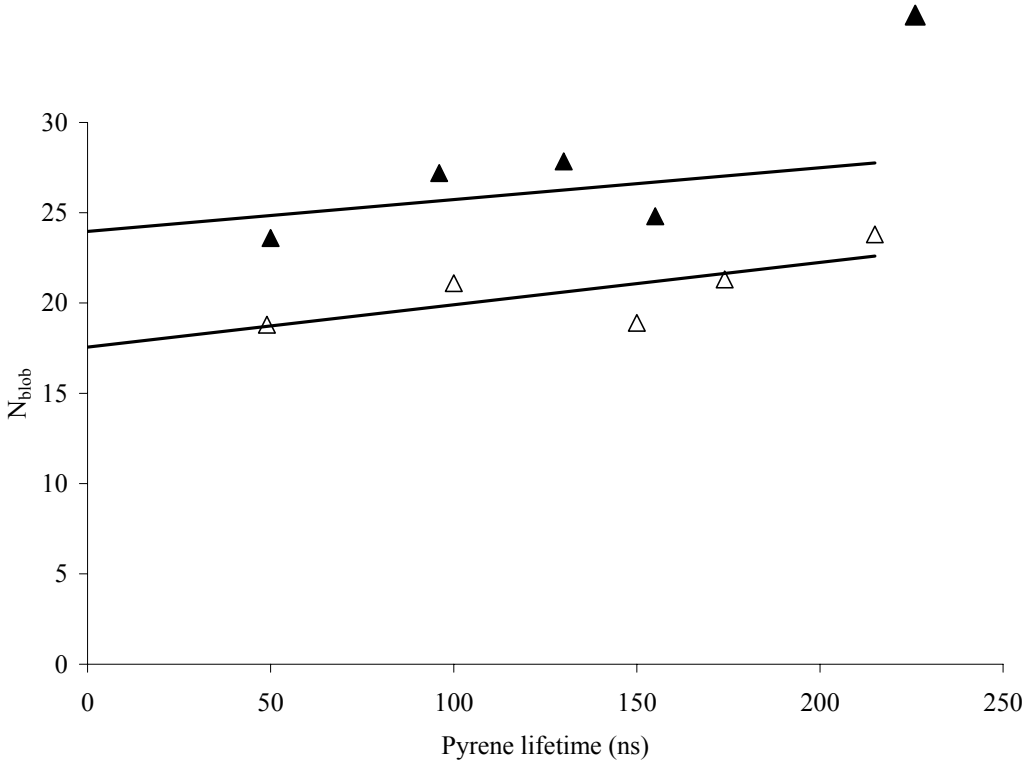


Figure 3: N_{blob} vs the lifetime of pyrene for PGA-PMA (Δ) and PGA-PBA ( )

The rate constant for excimer formation, k_{blob}, can also be compared between the two PGAs. As the lifetime is decreased, k_{blob} increases. This is because the side chain is probing a smaller volume so that the encounters between pyrenes occur at a faster time scale. The physical volume probed by the side chain, V_{blob}, can be compared by looking at k_{blob}^{-1}, which is proportional to V_{blob}.[2] The longer side chain is shown to probe a larger volume over the range of lifetimes as expected. This is shown in Figure 4.

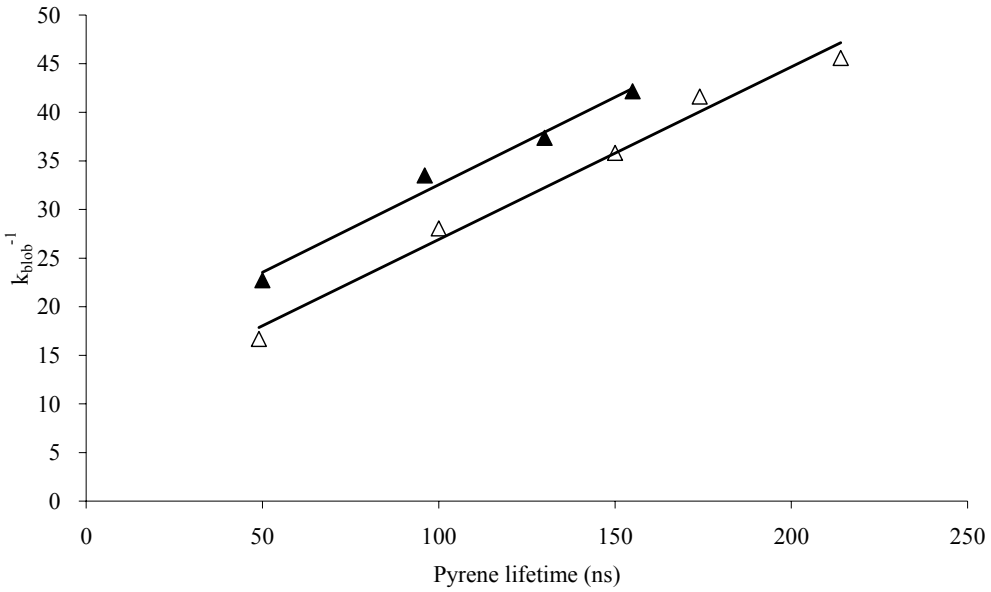


Figure 4: k_{blob}^{-1} vs. the lifetime of pyrene for PGA-PMA (Δ) and PGA-PBA (▲)

Interestingly, although N_{blob} changes little over the time scale probed, V_{blob} increases approximately 3 times over the range studied. This implies that as pyrene is given more time to probe its surroundings, it stretches into the solvent perpendicularly to the helix axis.

Using the fluorescence blob model, an α-helical PGA with two different side chain lengths were quantitatively analyzed and compared. It was found that extending the length of the side chain leads to pyrene probing a larger volume. This is the first step illustrating that the blob model can be used to characterize the volume probed by the side chain of a structured protein or polypeptide.

---

[1] Karplus, M., Weaver, D.; *Protein Science* **1994**, *3*, 650-668.
[2] Kanagalingham, S., Spartalis, J., Cao, T., Duhamel, J., *Macromolecules* **2002**, *35*, 8571-8577.

A Study of Polypeptide Side-Chain Dynamics using Fluorescence

Mark Ingratta

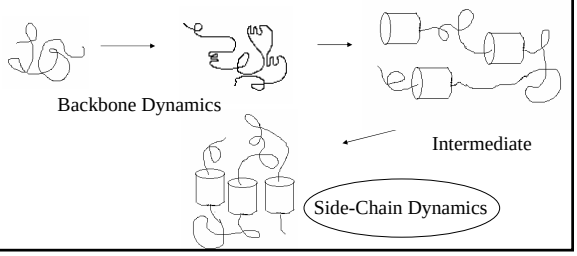
Chemistry Department
University of Waterloo
IPR Symposium
May 18, 2005

Outline

- Purpose
- Pyrene and the Fluorescence Blob Model
- Characterization of Pyrene Labeled Poly(glutamic acid)
- Results
- Future Work

Purpose

- Study dynamics of polypeptides in solution
- Contribute to a better understanding of protein folding



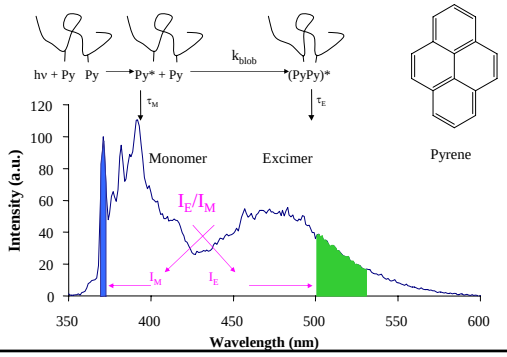
Purpose

- Study the side-chain dynamics of polypeptides in solution using fluorescence
- Side-chain interactions are thought to have an important role in protein folding

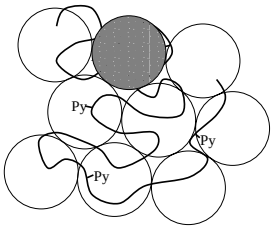
How?

- Use the Fluorescence Blob Model to analyze the time scale of diffusional motions of side-chains of different lengths
- As the side chain becomes longer, it should probe a larger volume around the backbone
- Chromophore of choice: Pyrene

Pyrene Fluorescence



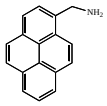
Polymer ⟶ Blobs



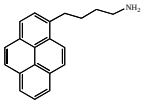
k_{blob} = rate constant for excimer formation by diffusion
$\langle n \rangle$ = number of ground state pyrenes per blob ➞ quenchers per blob

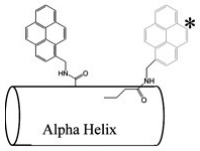
$k_e[blob]$ = rate of pyrene exchange between blobs × blob concentration per polymer coil
N_{blob} = units / blob

Explore the Effect of Side-Chain Length



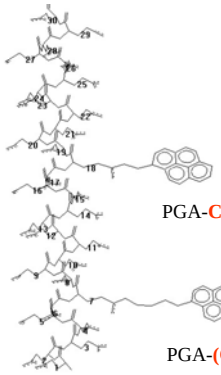
Vs.





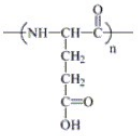
$PyCH_2NH_2$ $Py(CH_2)_4NH_2$

- Vary the length of the linker attached to the chromophore
- Chromophore attached to a well defined, stable structured polypeptide, poly(glutamic acid)



PGA-CH_2Py

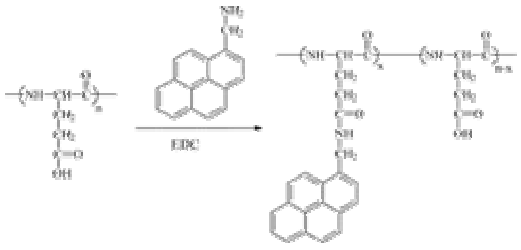
PGA-$(CH_2)_4$Py



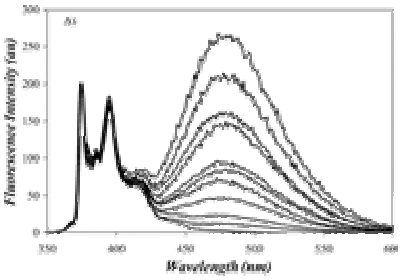
Poly(glutamic acid) = PGA

Characterization of Pyrene-Labeled Poly(glutamic acid)

Pyrene Labeling



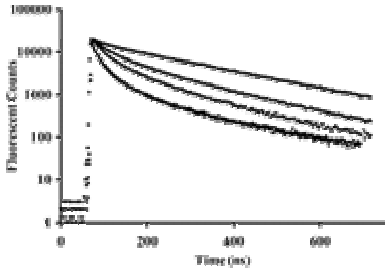
Steady-State Fluorescence



Steady-State Fluorescence spectra of Py-PGA with increasing pyrene content in µmol/g.

Duhamel, J.; Kanagalingam, O'Brien, T. J.; Ingratta, M. W.; *Journal of the American Chemical Society*, **2003**, *125*, 12810-12822.

Time-Resolved Fluorescence



Fluorescence decays of the pyrene monomer for Py-PGA.

Duhamel, J.; Kanagalingam, O'Brien, T. J.; Ingratta, M. W.; *Journal of the American Chemical Society*, **2003**, *125*, 12810-12822.

Volume Control

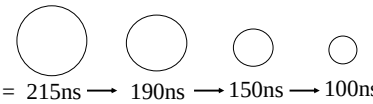
- The lifetime of Pyrene can vary depending on its connectivity to a polymer
- Because blob size is related to the lifetime of the chromophore, a longer lived pyrene will probe a larger volume.

PGA CH_2Py; τ_o = 215ns

PGA $(CH_2)_4$Py; τ_o = 155ns

Volume Control

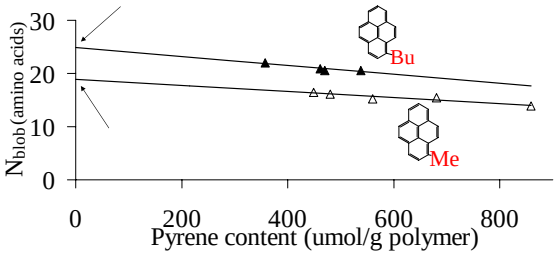
- Therefore, we must be able to control the lifetime of the pyrene probe.
- We do this by using Nitromethane, a well known quencher of pyrene fluorescence.
- For Example:



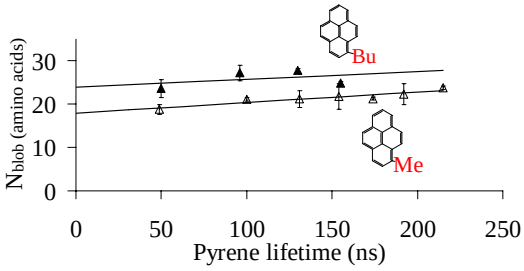
PGA CH_2Py; τ_o = 215ns ⟶ 190ns ⟶ 150ns ⟶ 100ns

Results

PGACH_2Py and PGA$(CH_2)_4$Py at a Pyrene Lifetime ~ 155ns

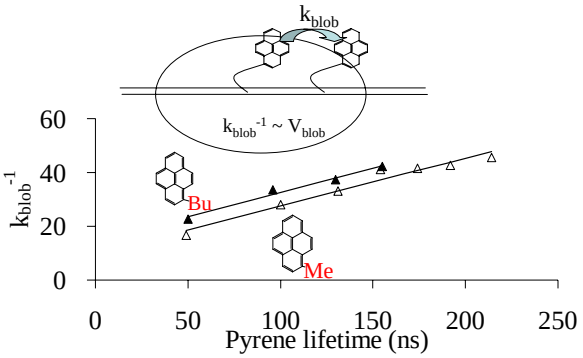


N_{blob} vs. Lifetime



N_{blob} changes ~20% over the 50ns – 200ns range studied

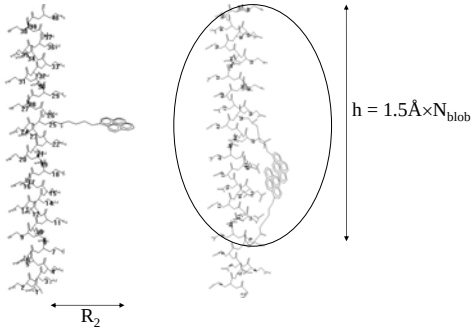
Volume Probed vs. Lifetime



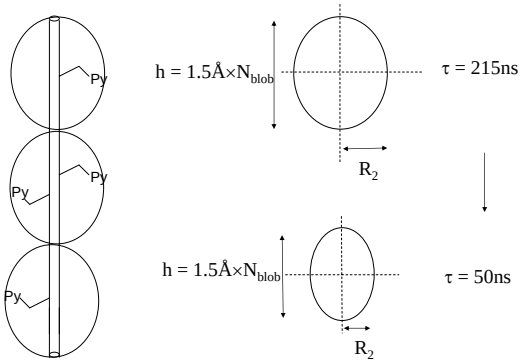
Volume Probed

- N_{blob} decreases slowly with a decreasing lifetime, but V_{blob} decreases nearly 3 times over the same range!
- How? Why?

Volume of a PGA blob



Volume of a PGA blob

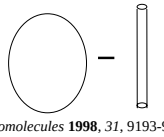


Finding the Volume

- Use an empirical equation derived for a pyrene labeled PEO system*, based on the diffusion of free pyrene:

$$k_{blob} = \frac{2}{3}\frac{kT}{\eta}\frac{1}{V_{blob}}$$

- In this case, use $V_{blob} = \frac{4}{3}\pi\frac{h}{2}R_2^2 - \pi R_1^2 h$

V_{blob} = 

*Lee, S.; Duhamel, J. *Macromolecules* **1998**, *31*, 9193-9200.

Finding the Volume

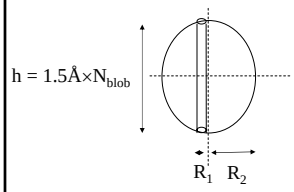
- *Complication*: The equation is based on free pyrene in solution, not attached to a chain.
- *Solution:* Use a constant to accommodate for the difference in geometry between a freely diffusing probe and a probe tethered to a rod.

$$k_{blob} = \frac{2}{3}\frac{kT}{\eta}\frac{1}{V_{blob}} \longrightarrow k_{blob} = \frac{CT}{\eta}\frac{1}{V_{blob}}$$

Therefore

$$k_{blob} = \frac{CT}{\eta}\frac{1}{\frac{4}{3}\pi\frac{h}{2}R_2^2 - \pi R_1^2 h}$$

Volume of a PGA blob



$$k_{blob} = \frac{CT}{\eta}\frac{1}{\frac{4}{3}\pi\frac{h}{2}R_2^2 - \pi R_1^2 h}$$

Step I: Find C using the R_2 and R_1, 15Å and 2.25Å respectively, from modeling

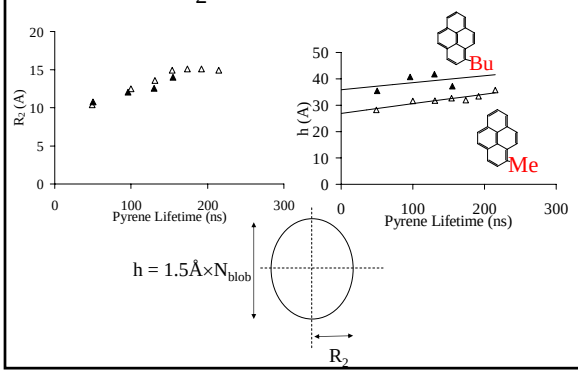
Step II: Using C, determine R_2 for all lifetimes for PGACH_2Py and PGA$(CH_2)_4$Py

Finding C using PGACH_2Py

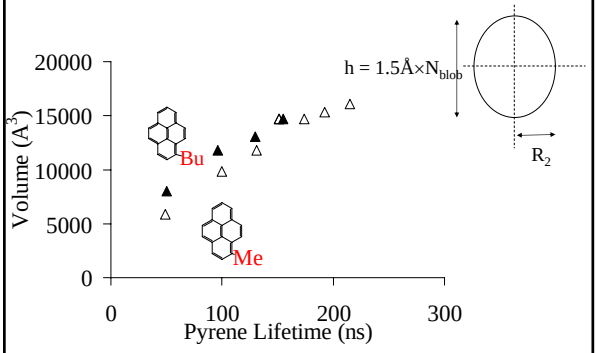
τ (ns)	k_{blob} (×10^{7}s^{-1})	N_{blob} (a.a.)	h (Å)	C*T/η (×10^{11}JPa^{-1})
215	2.2	23.8	35.7	3.6
192	2.3	22.3	33.4	3.5
174	2.4	21.3	31.95	3.5
154	2.4	21.8	32.7	3.6
131	3.0	21.2	31.8	4.3
100	3.6	21.1	31.65	5.2
49	6.0	18.8	28.2	7.7

$$k_{blob} = \frac{CT}{\eta}\frac{1}{\frac{4}{3}\pi\frac{h}{2}R_2^2 - \pi R_1^2 h}$$

R_2, h vs. Lifetime



Calculated Volume vs. Lifetime



Summary of Results

- Value of C×(T/η) for free pyrene (2/3k×(T/η)) is 3.5×10^{12}JPa^{-1}, a factor of 10 larger than the experimental value for our tethered pyrene, 3.5×10^{11}JPa^{-1}.
- For PGA$(CH_2)_4$Py, the lifetime is too short to see the plateau; therefore at 155ns, it has not reached its full volume potential.
- For PGACH_2Py, the length the side chain can probe into solution, R_2, initially increases with time then becomes constant!

Conclusions and Future Work

- Using the Fluorescence Blob Model, we can predict the volume probed by a side chain in a given amount of time.
- Continue to extend the linker series, i.e. 5, 8, 11 atom linkers to establish a trend for the volume probed by a side chain.
- These trends could be extrapolated to account for real amino acid side chains participating in protein folding.

Acknowledgements

Prof. Jean Duhamel and the
Duhamel Lab Group

The Institute for Polymer Research