



IPR

Institute for Polymer Research

Characterization of polypeptide aggregates in aqueous solution

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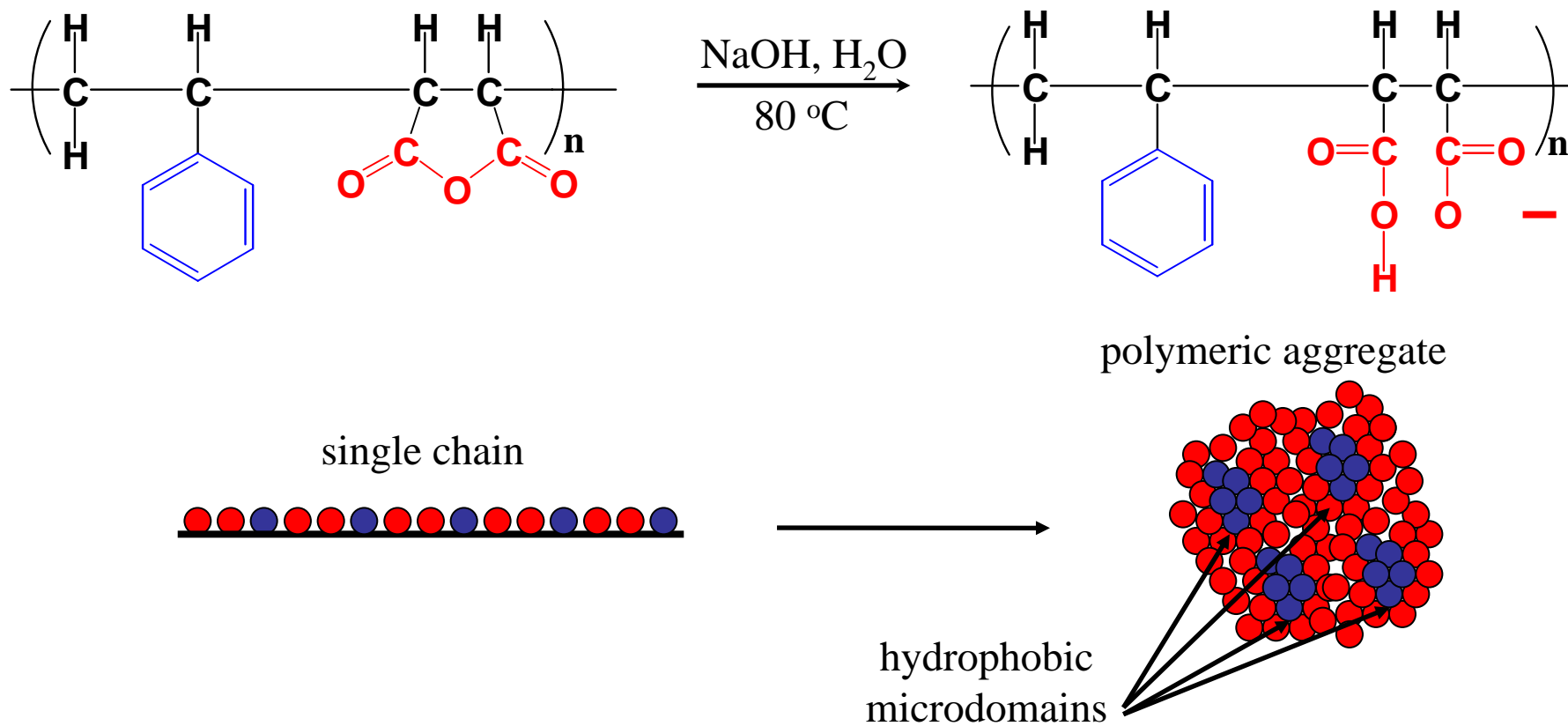
May 16th 2006
28th Annual IPR Symposium

University of Waterloo

Outline

- Introduction & project objective
- Synthesis of polypeptides
- Characterization of polypeptides using:
 - Circular Dichroism
 - Dynamic Light Scattering
 - Fluorescence energy transfer and fluorescence quenching
 - and Transmission Electron Microscopy (TEM)
- Conclusions
- Acknowledgements

Styrene Maleic Anhydride Copolymers (SMA)



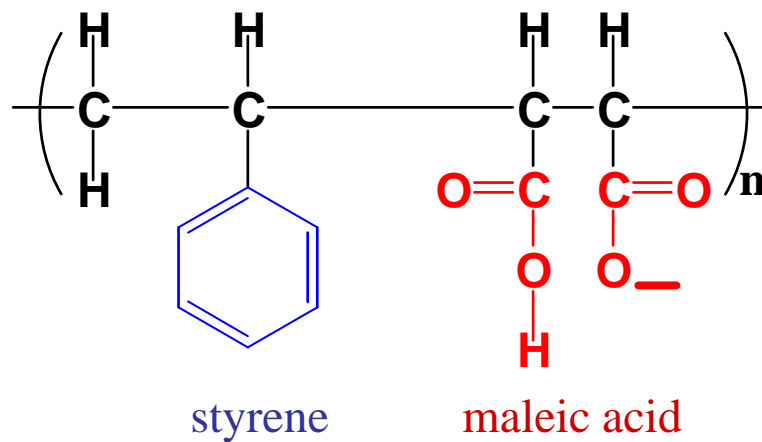
SMA aggregates can dissolve large amounts of hydrophobic compounds (pyrene, anthracene, phenanthrene, perylene) and release them over periods of minutes to hours in a manner which can be controlled by fine-tuning the sequence and the ionization level of the polymer.

Duhamel, J. et al *Langmuir* **2002**, *18*, 3829-3835.

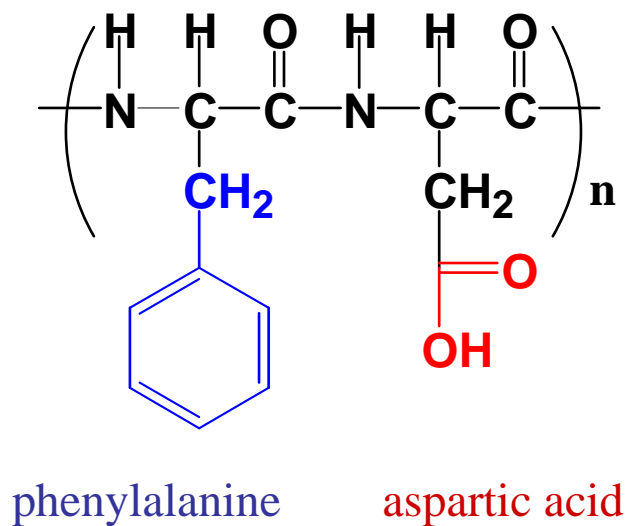
Unfortunately, SMA is not biocompatible.

A Polypeptide Analogue of SMA

SMA



Poly(AspPhe)



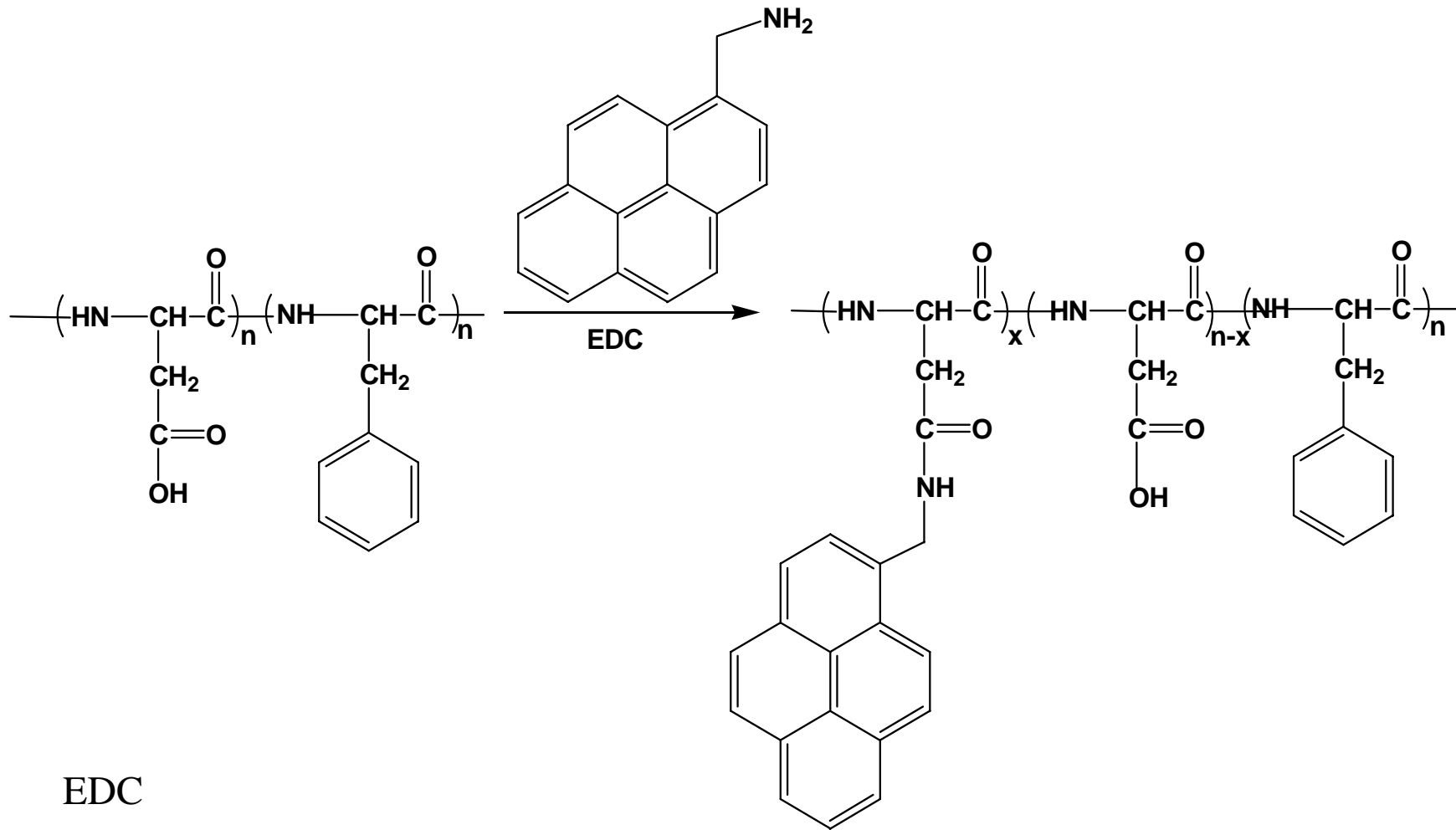
Polypeptides Prepared

0.	Poly(Asp)	Poly(Asp)
1.	$-(\text{Asp}(\text{OH})\text{Asp}(\text{OH})\text{Asp}(\text{OH})\text{Phe})_n-$	Asp_3Phe_1
2.	$-(\text{Asp}(\text{OH})\text{Asp}(\text{OH})\text{Phe})_n-$	Asp_2Phe_1
3.	$-(\text{Asp}(\text{OH})\text{Phe})_n-$	Asp_1Phe_1
4.	$-(\text{Asp}(\text{OH})\text{PhePhe})_n-$	Asp_1Phe_2
5.	$-(\text{Asp}(\text{OH})\text{PhePhePhe})_n-$	Asp_1Phe_3

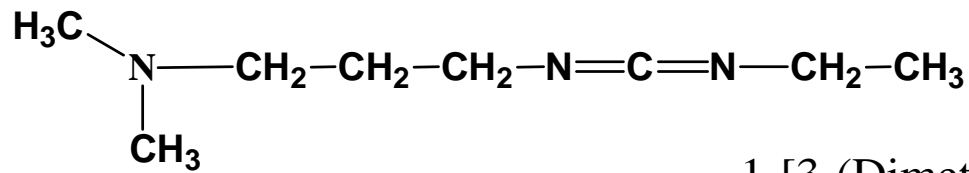
Increasing Hydrophobicity

The structure of each Asp_xPhe_y monomer was checked by ^1H NMR and mass spectrometry.

Pyrene Labeling



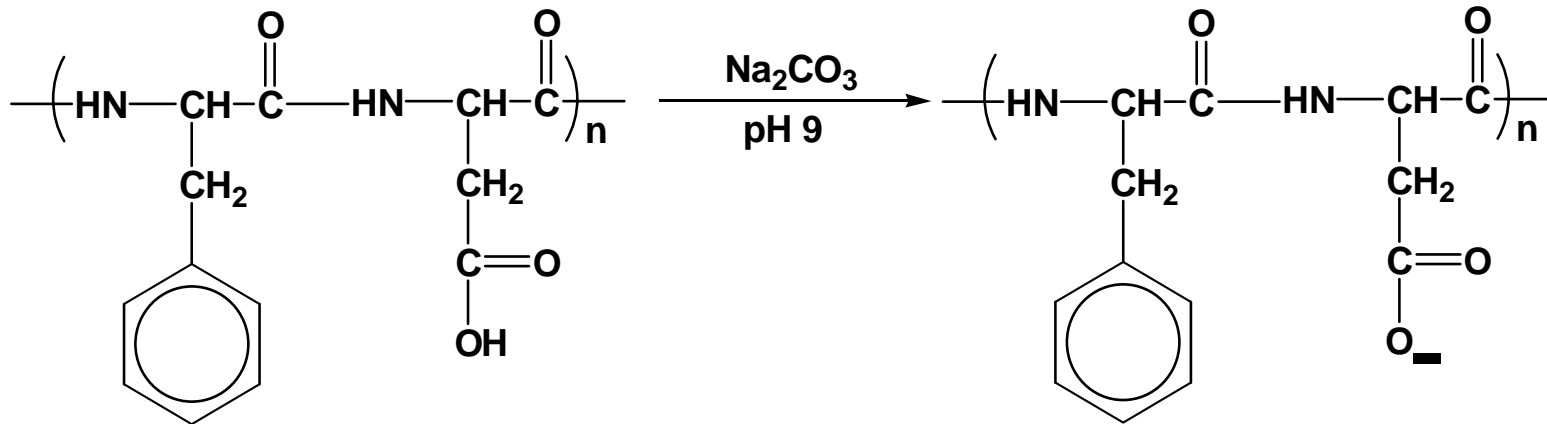
EDC



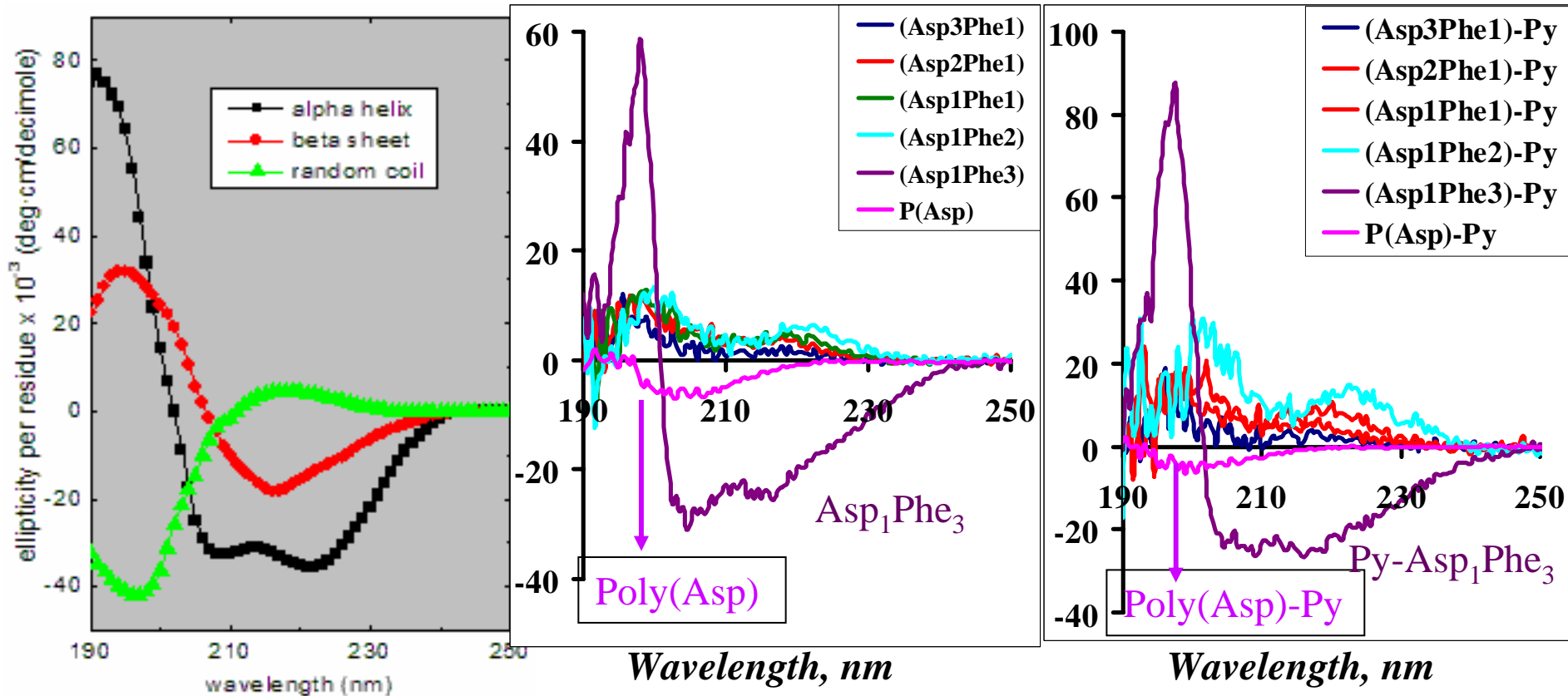
1-[3-(Dimethylamino)propyl]-3-ethylcarbodiimide

Sample Preparation

To a solution of 100 mg of polypeptide in 10 ml of DMF, a known quantity of 0.1 N NaOH was added slowly with stirring. This solution was then dialyzed against a solution of 0.01 N Na₂CO₃ at pH 9 for two days. The resulting polypeptide solution had a concentration between 1-2 mg/mL.



Circular Dichroism



Random Coil
Undefined conformation
Alpha-helix

poly(Asp)
poly(Asp₃Phe₁)
poly(Asp₁Phe₃)

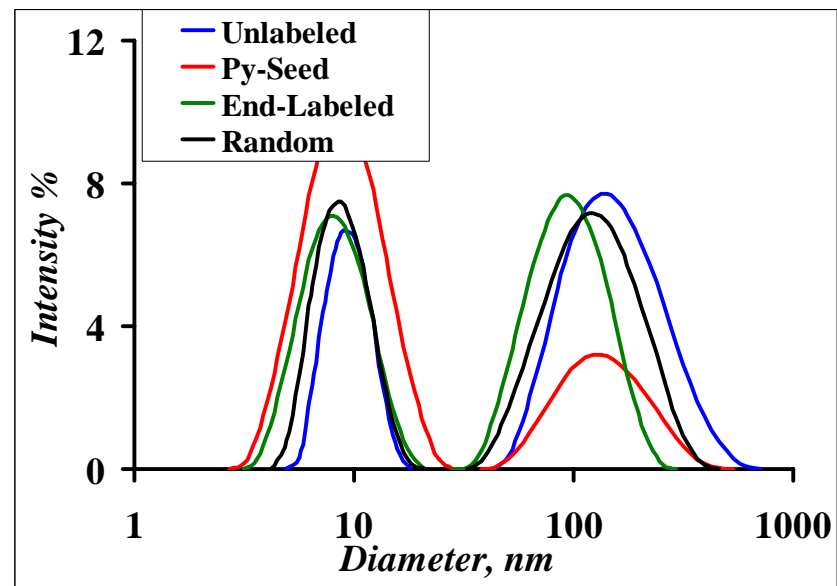
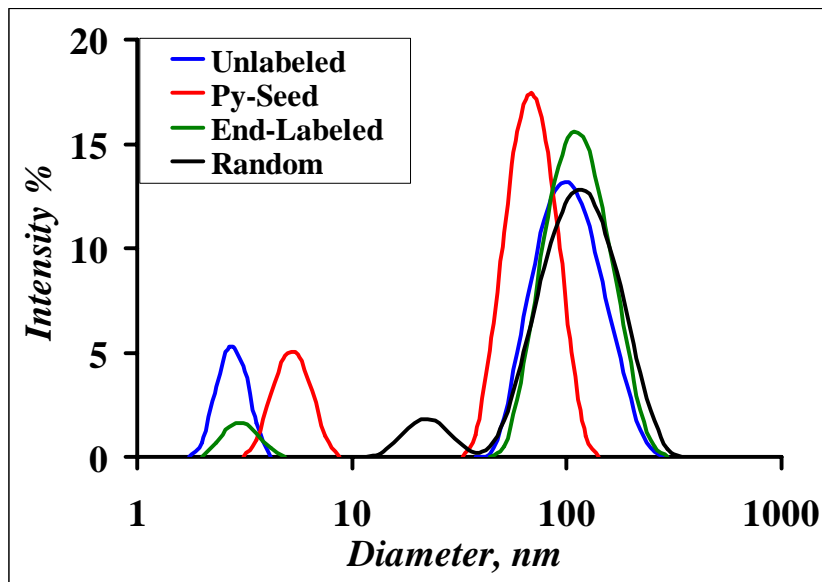
poly(Asp₂Phe₁)

poly(Asp₁Phe₁)

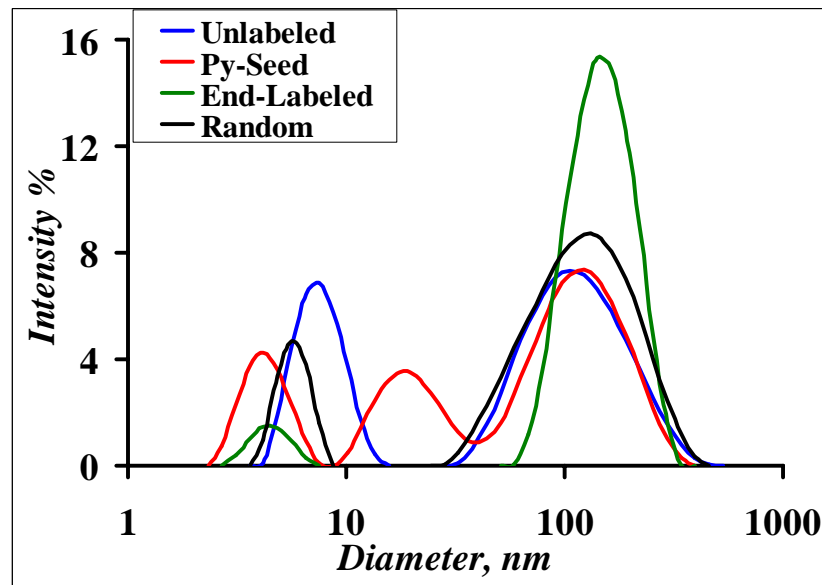
poly(Asp₁Phe₂)

There is little difference between the unlabeled polypeptides and the pyrene labeled polypeptides.

Size Determination by Dynamic Light Scattering



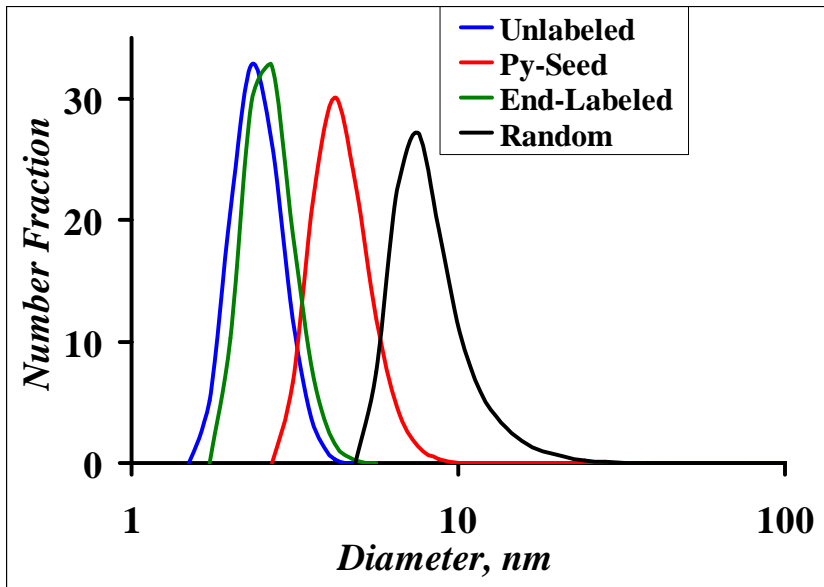
Asp₃Phe₁



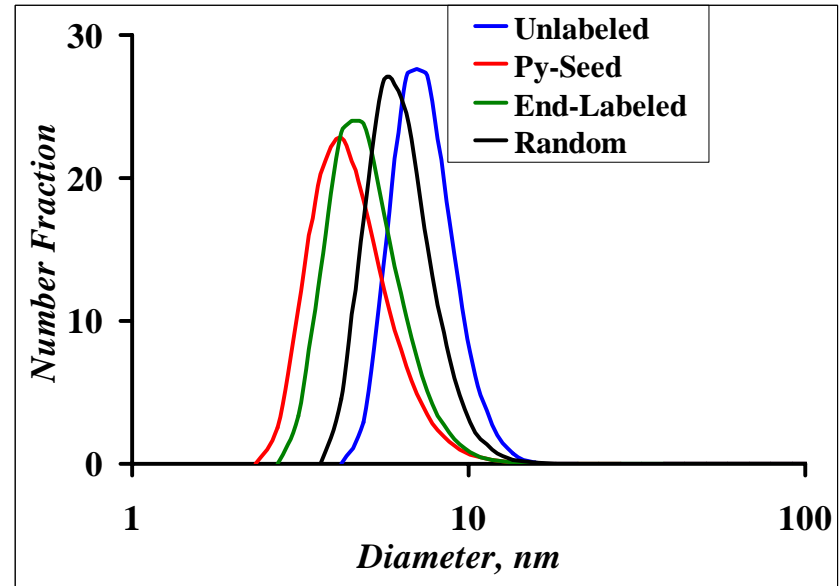
Asp₁Phe₃

Asp₁Phe₁

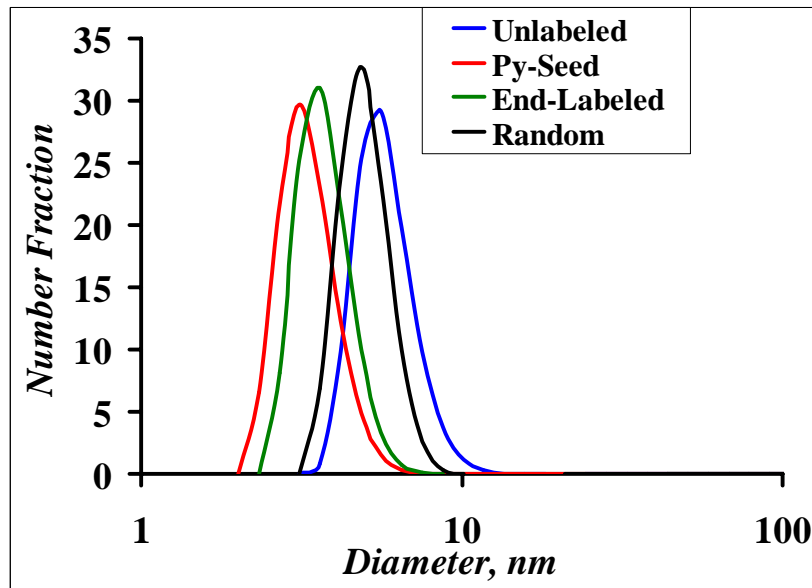
Size Determination by Dynamic Light Scattering



Asp₃Phe₁

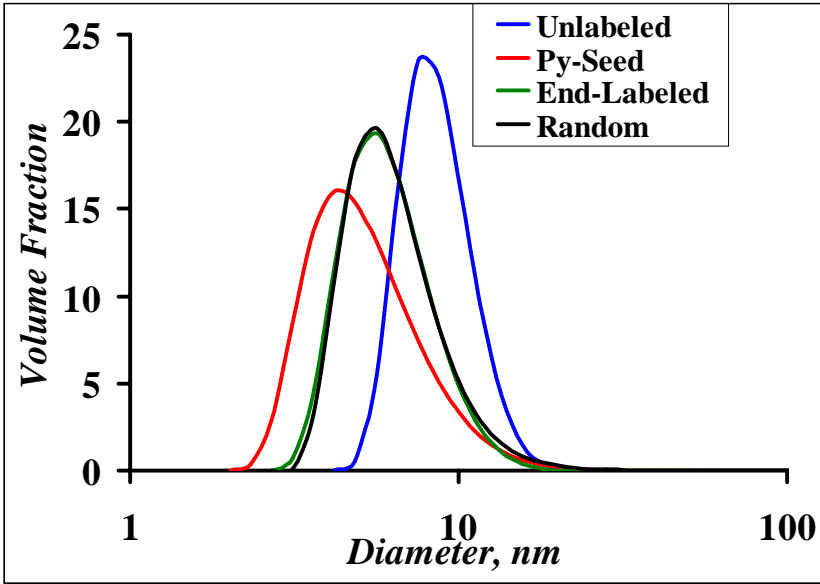
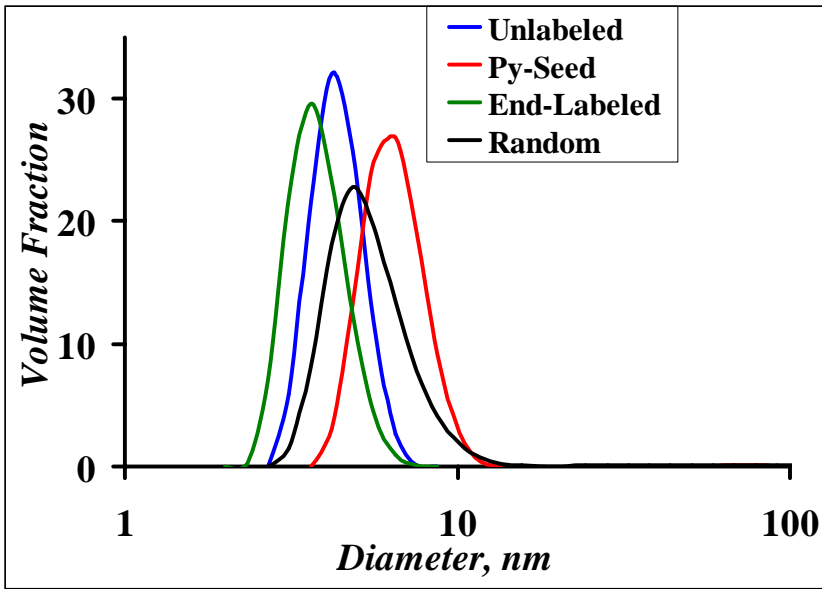


Asp₁Phe₃

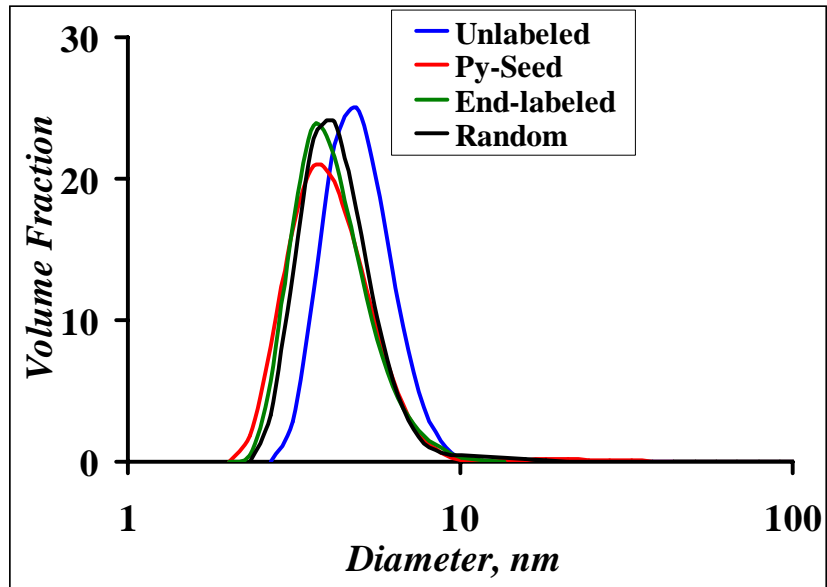


Asp₁Phe₁

Size Determination by Dynamic Light Scattering



Asp₃Phe₁

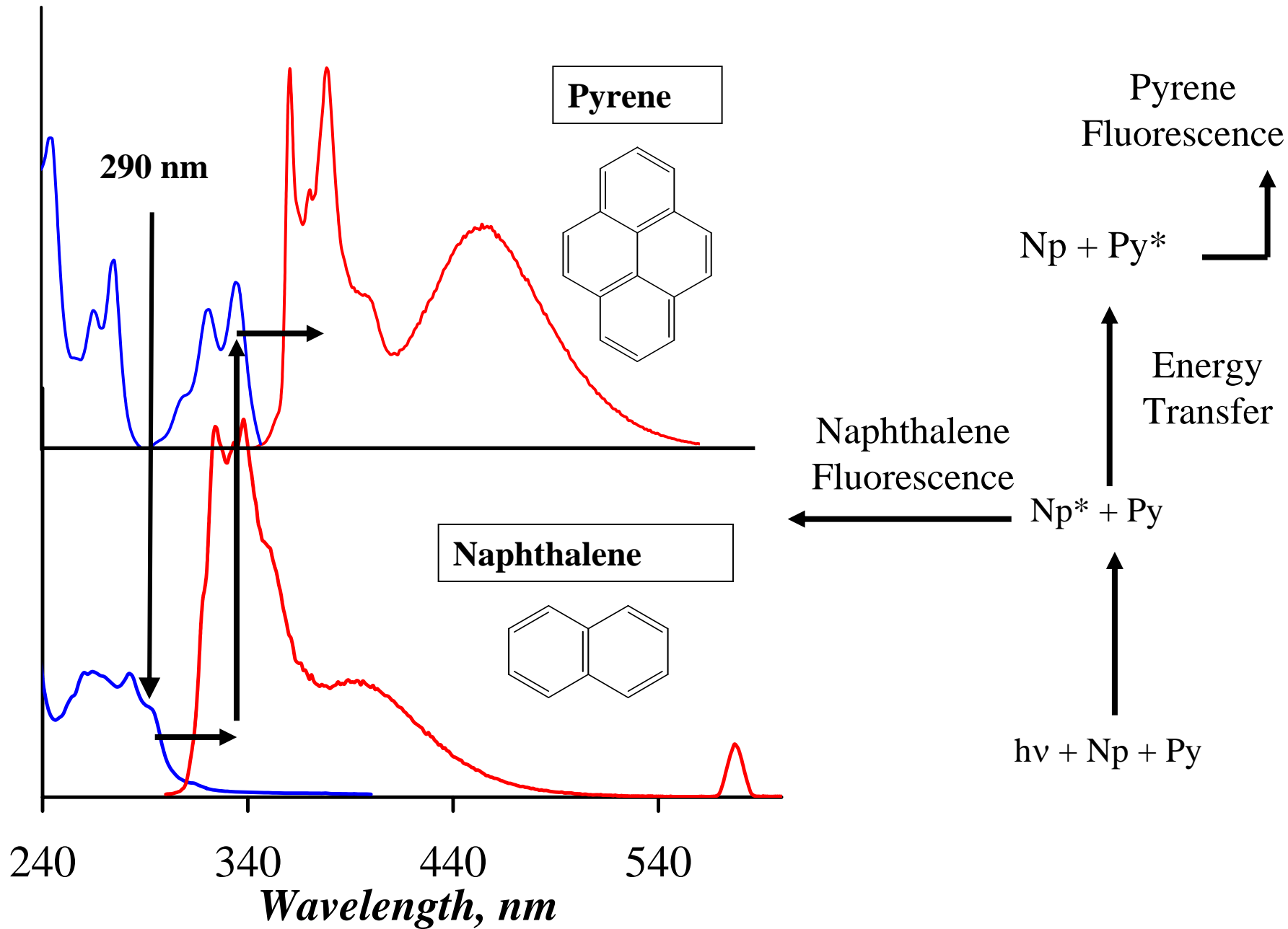


Asp₁Phe₃

Asp₁Phe₁

Energy Transfer Studies

-Absorption
-Emission

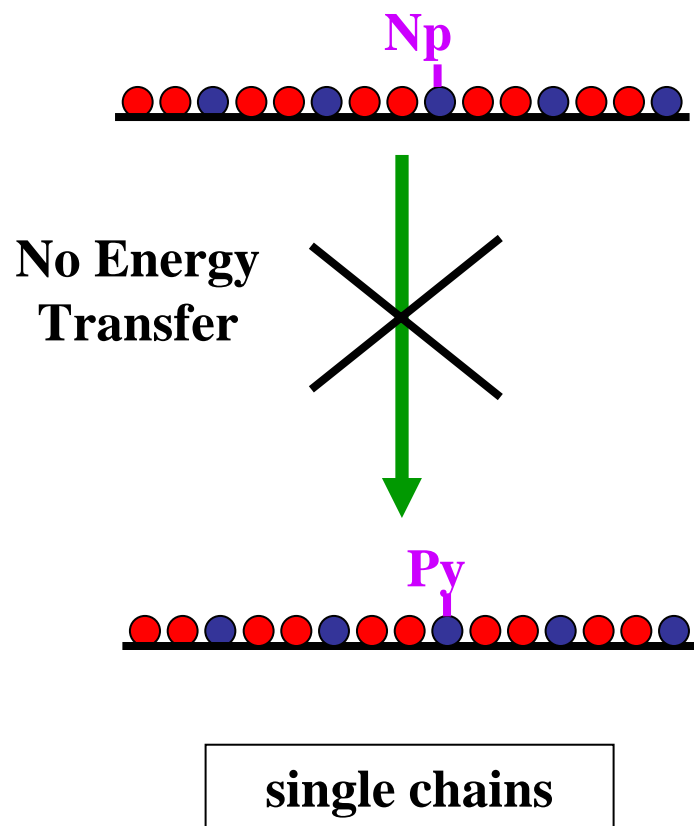


Energy Transfer Studies

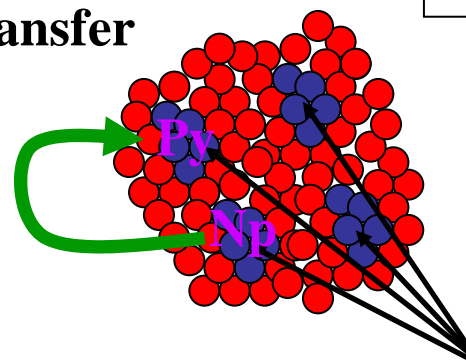
$$k_{ET} = \left(\frac{R_o}{r} \right)^6 \frac{1}{\tau_{Np}}$$

$r \gg R_o$ then $k_{ET} \rightarrow 0$

$R_o(Np,Py) \sim 25 \text{ \AA}$



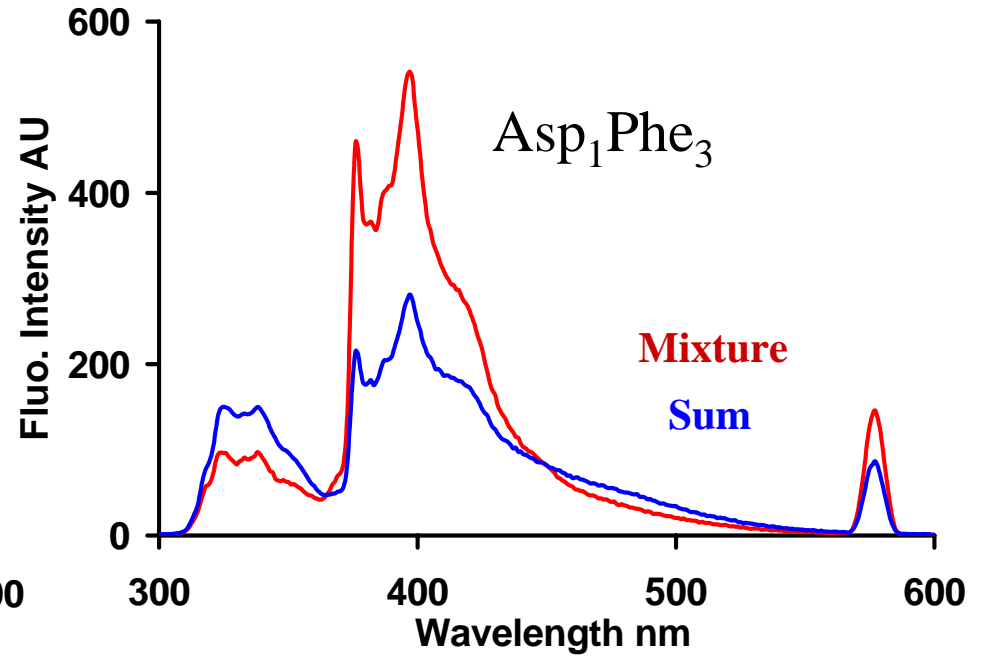
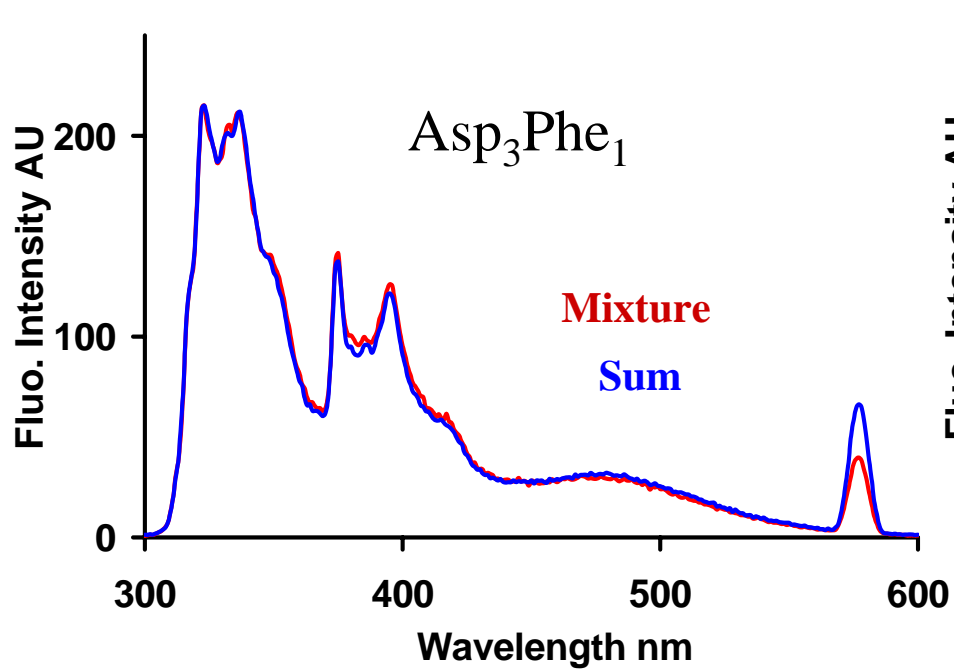
Energy Transfer



polymeric aggregate

hydrophobic microdomains

Energy Transfer Studies



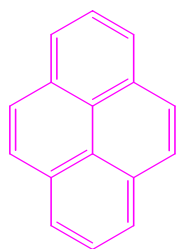
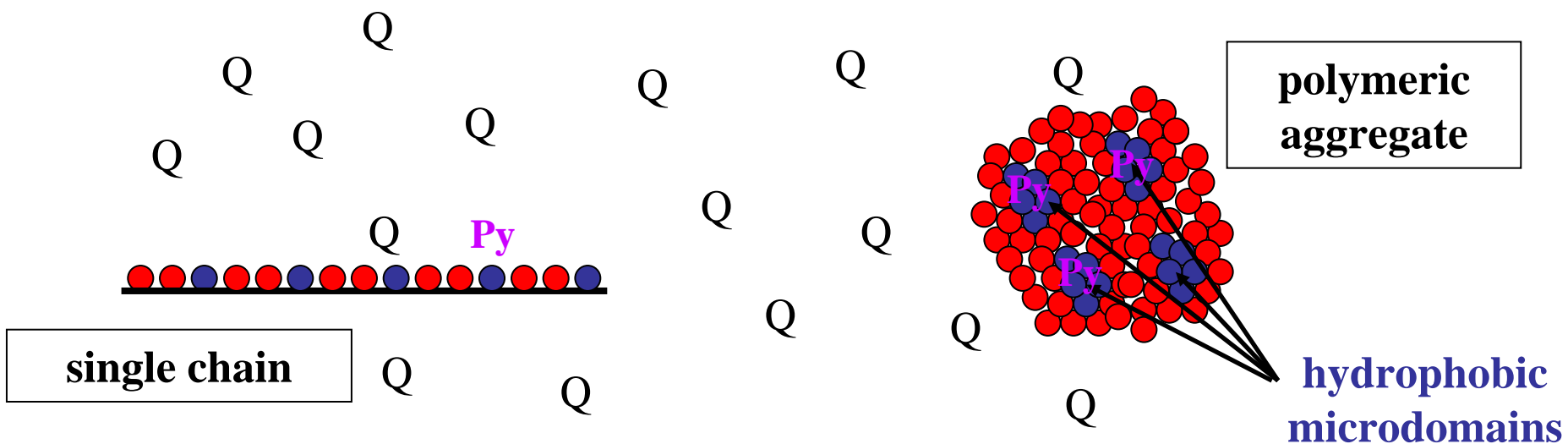
Energy Transfer Efficiency

$$E = 1 - \frac{I_{N_p} / [N_p]}{I^0_{N_p} / [N_p]}$$

Aggregation

Sequence	Efficiency %
Asp ₃ Phe ₁	0
Asp ₂ Phe ₁	0
Asp ₁ Phe ₁	0
Asp ₁ Phe ₂	16
Asp ₁ Phe ₃	34

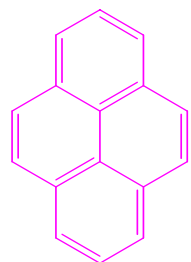
Fluorescence Quenching Measurements



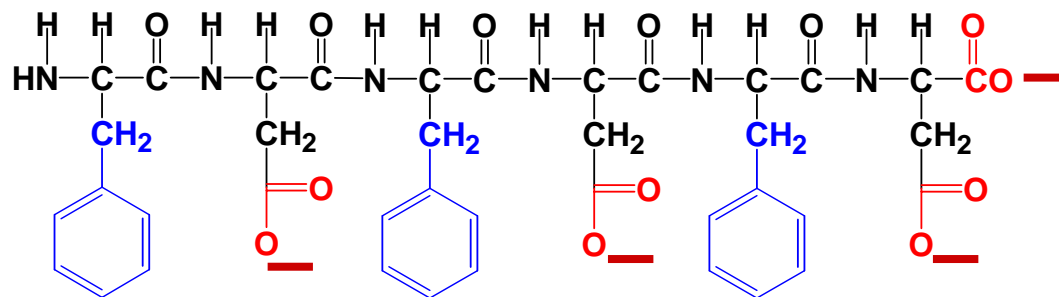
Pyrene = Py

H_3CNO_2 Nitromethane = Q

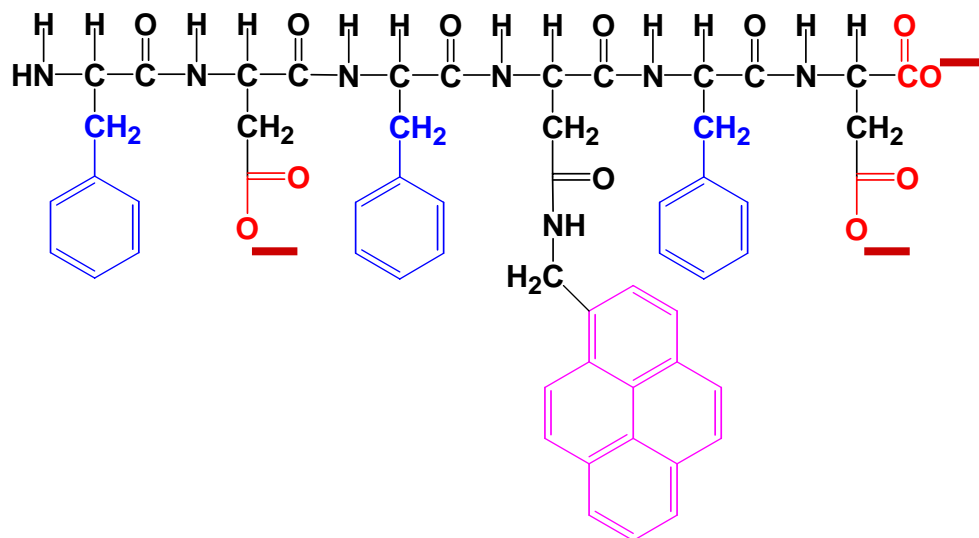
Single chain	→	efficient quenching
Polymeric aggregates	→	protective quenching



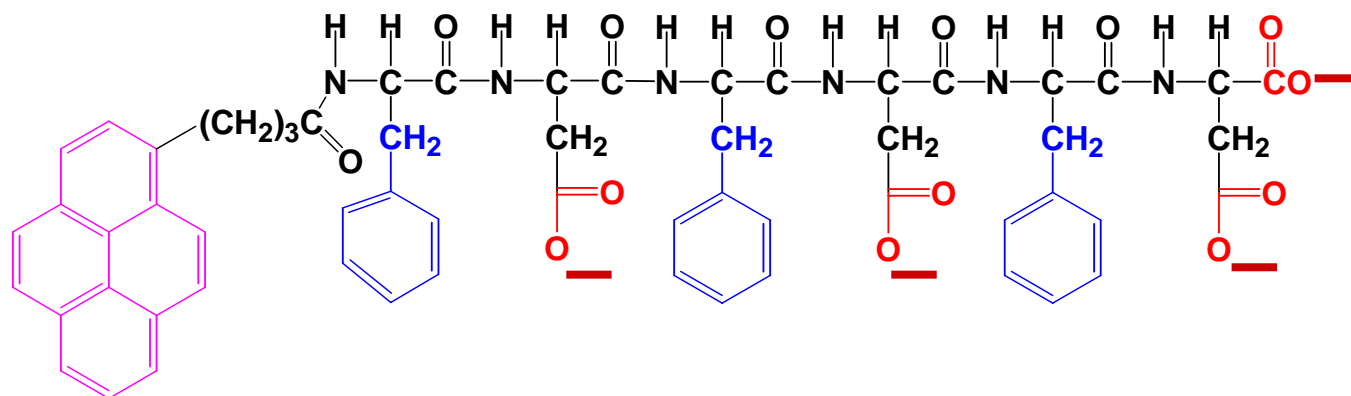
+



“Pyrene used as a probe”



Polypeptide randomly labeled with pyrene

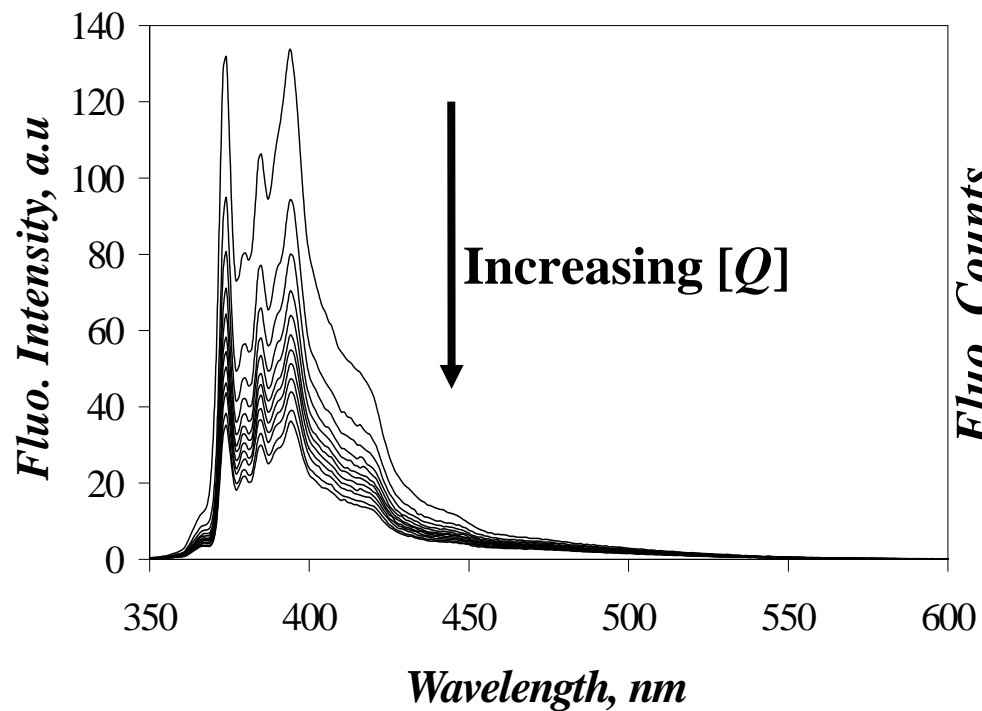


Polypeptide end-labeled with pyrene

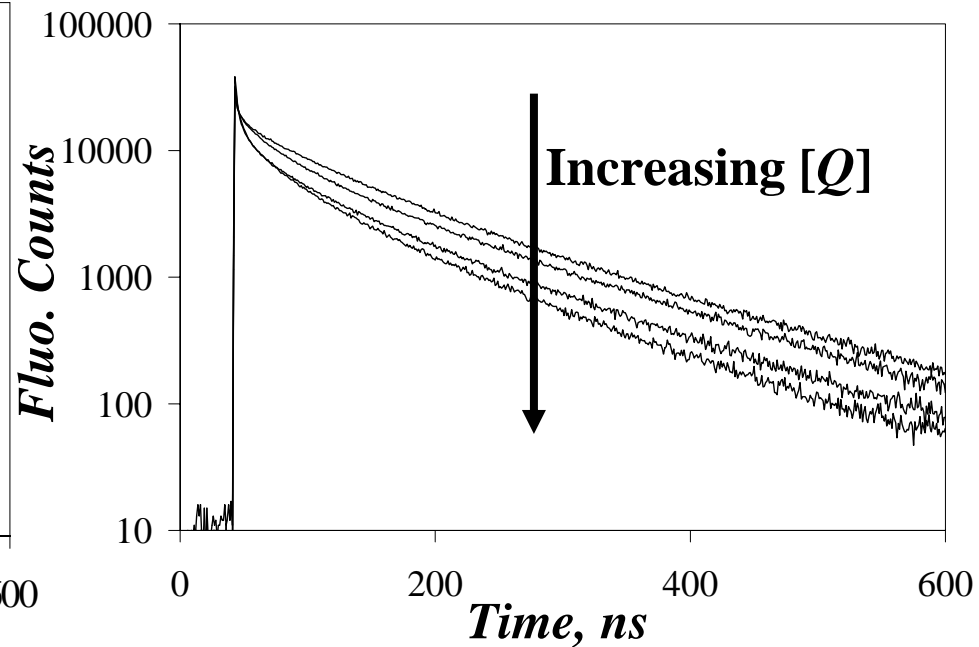
Fluorescence Quenching Measurements

“Pyrene Used as a Probe”

Steady-State Fluorescence



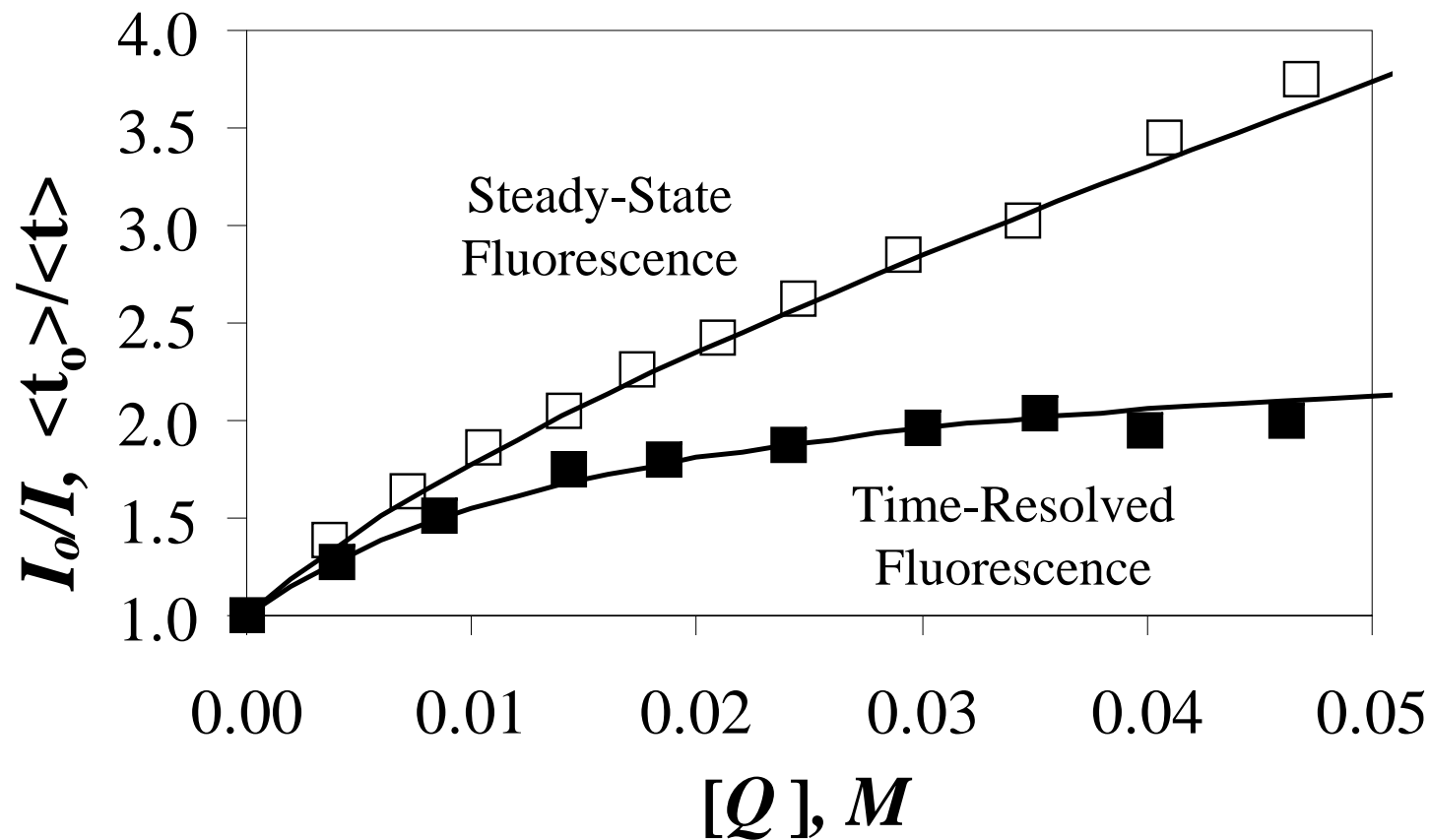
Time-Resolved Fluorescence



Fluorescence Quenching Measurements

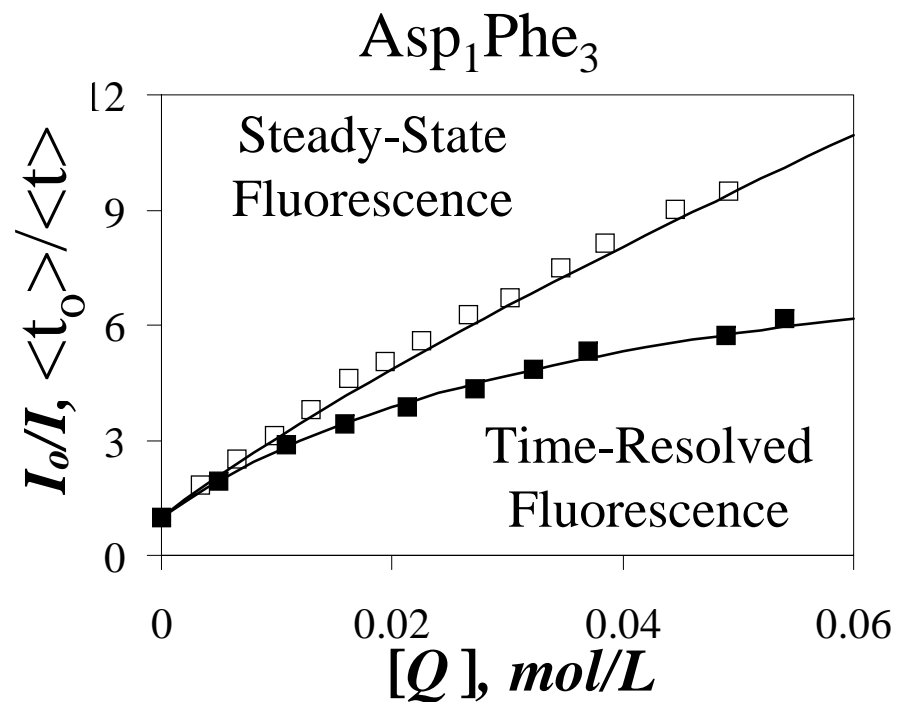
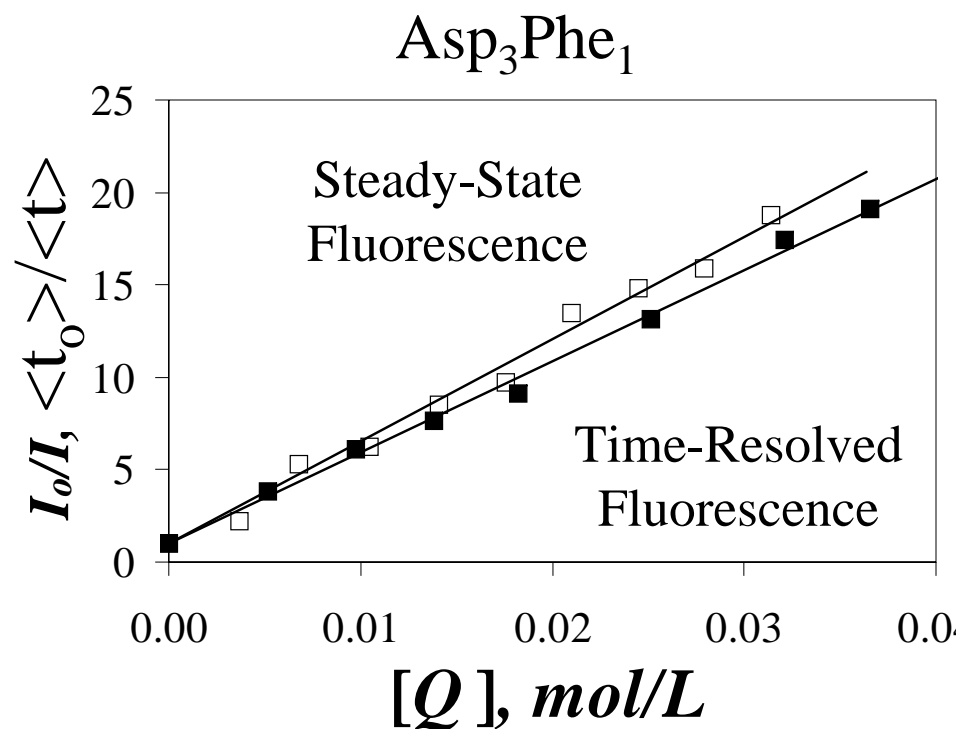
“Pyrene Used as a Probe”

$$\frac{\tau_0}{\tau} \times 1 + k_q \tau_0 [Q]$$



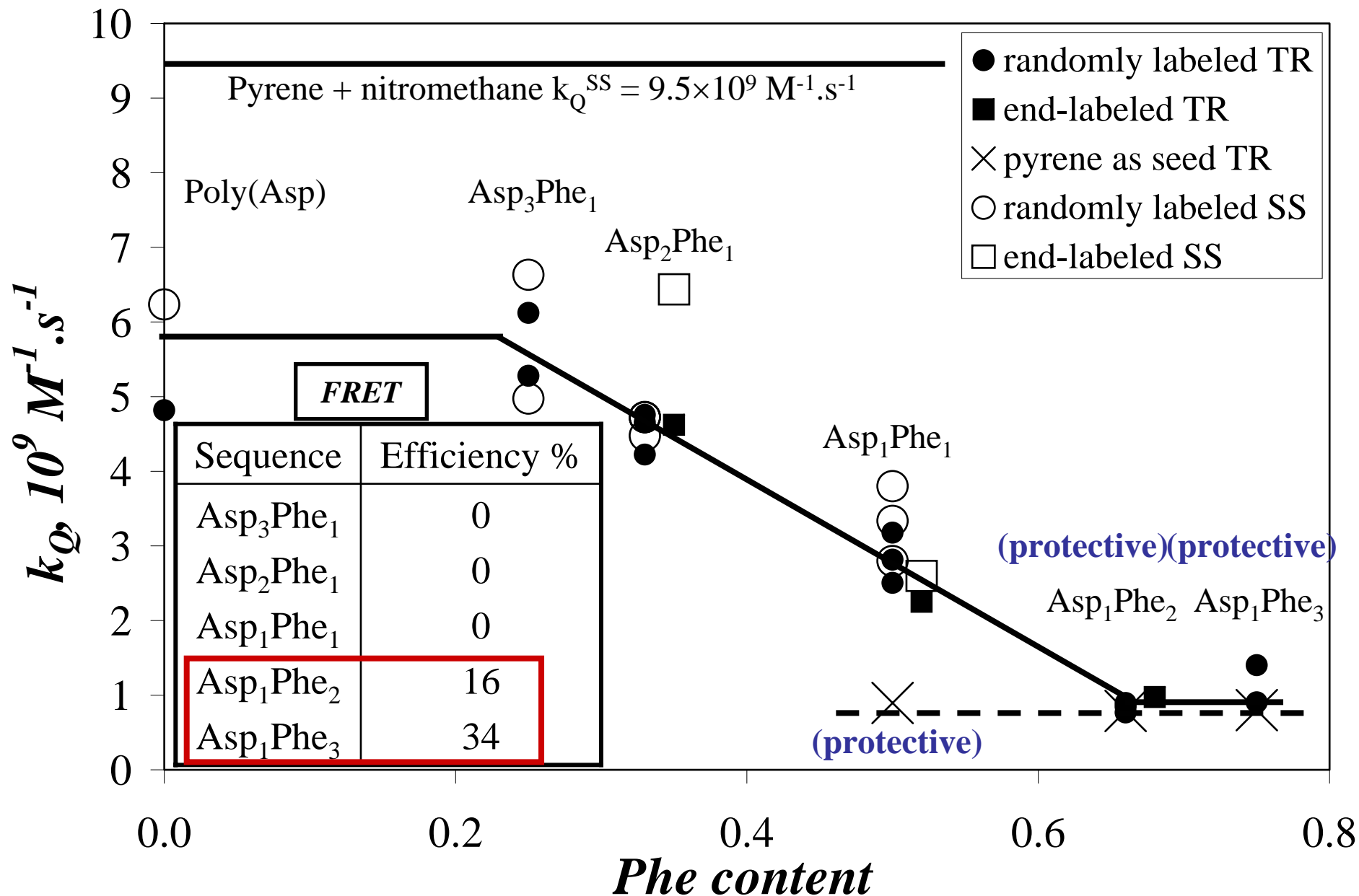
Fluorescence Quenching Measurements

Polypeptides Randomly Labeled with Pyrene



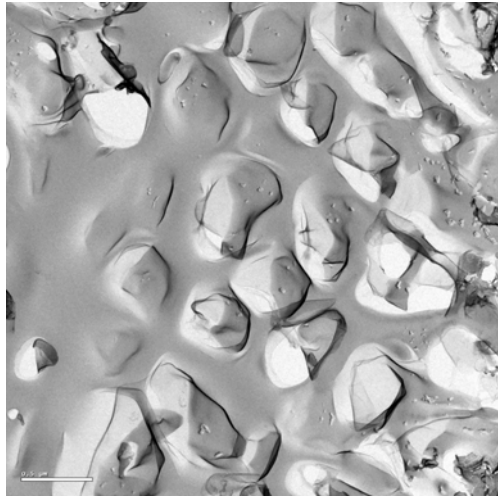
Asp ₃ Phe ₁	Diffusional Quenching
Asp ₁ Phe ₃	Protective Quenching

Quenching of Pyrene Labeled Polypeptides



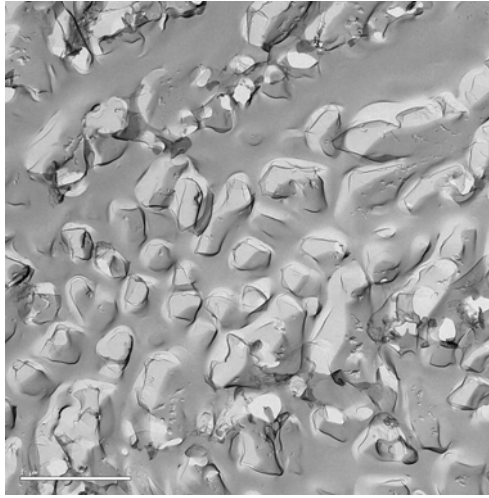
Freeze Fracture TEM

Asp₃Phe₁



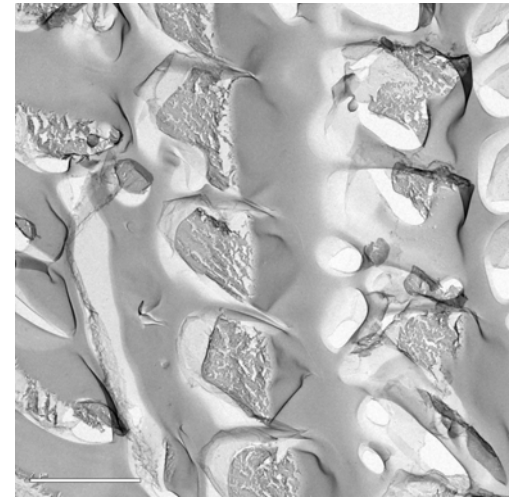
~0.5 μ m

Asp₁Phe₁



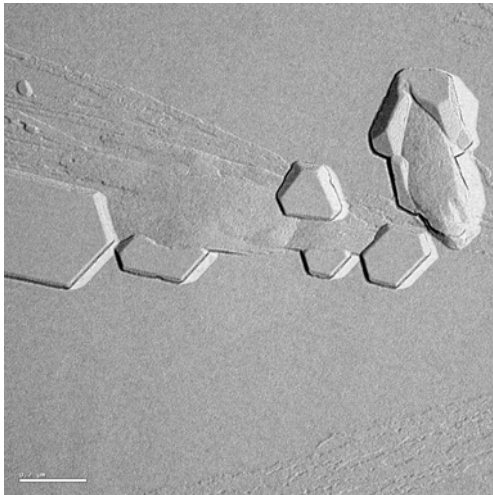
~0.2-1 μ m

Asp₁Phe₃



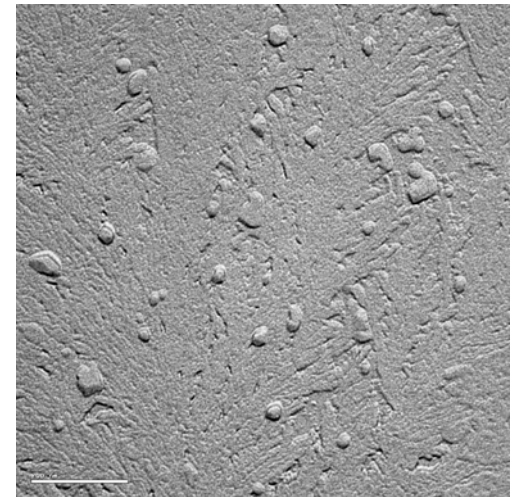
~2 μ m

TEM Images



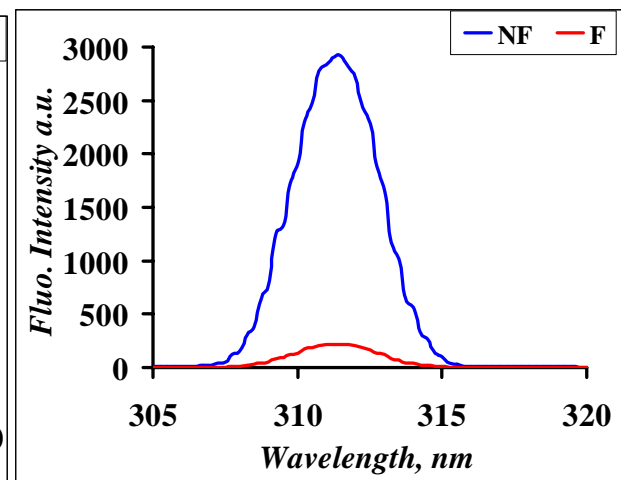
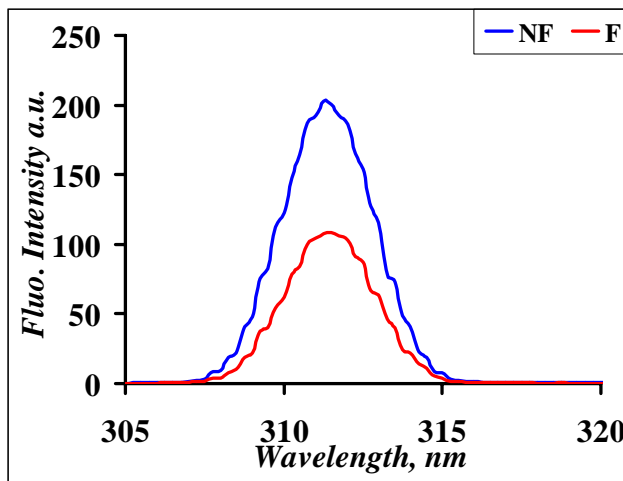
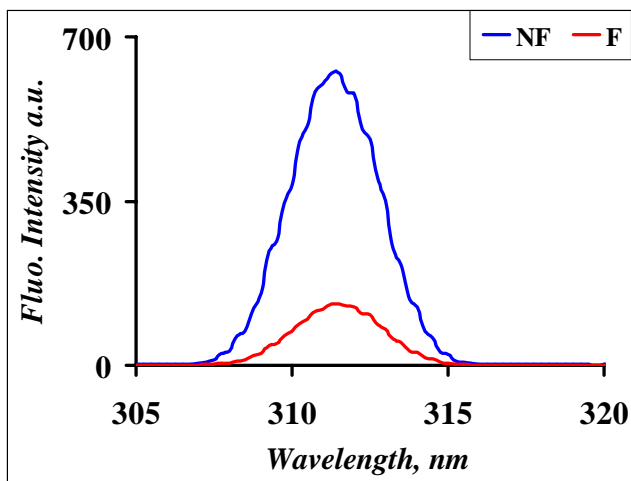
~0.2-1 μ m

~50-100 nm

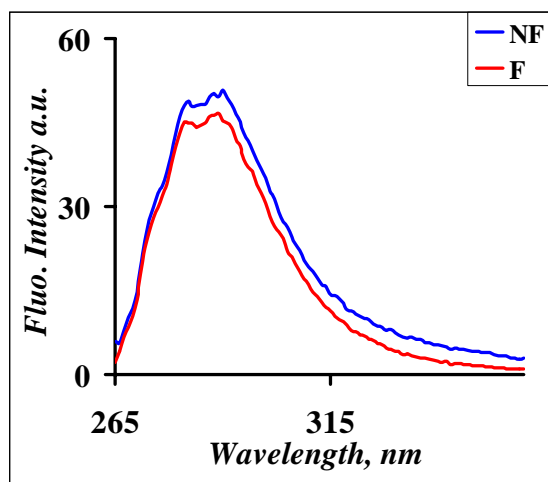


Light Scattering/Fluorescence Emission

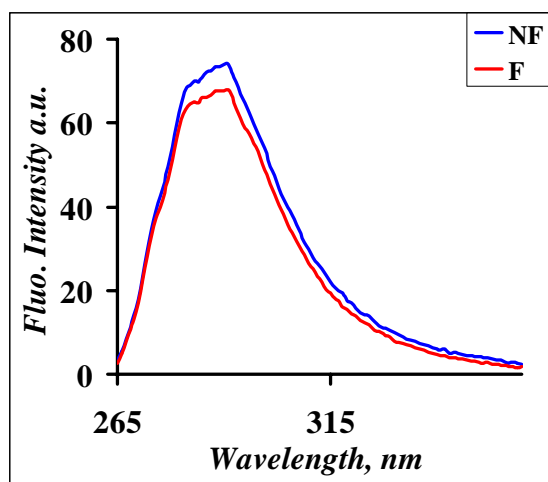
Polypeptide aggregates



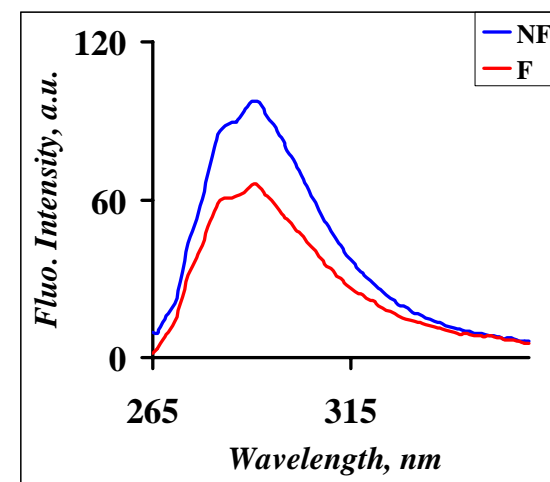
Asp₃Phe₁



Asp₁Phe₁



Asp₁Phe₃



Conclusions

- 1) Five polypeptides having well-defined Asp_xPhe_y sequences with increasing Phe content were prepared.
- 2) The properties of the polypeptides in solution were characterized by Circular dichroism, dynamic light scattering, fluorescence energy transfer, fluorescence quenching and TEM.
- 3) According to the FRET experiments, intermolecular associations occur between polypeptides having high Phe contents.
- 4) Pyrene-labeled polypeptides exhibit protective quenching when the Phe content is high.
- 5) Polypeptide aggregates can be visualized by TEM images.

Acknowledgements

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