Determination of pH-Ionization Behaviour for a Series of Amphiphilic Polypeptides

Michael Fowler

IPR Annual Symposium

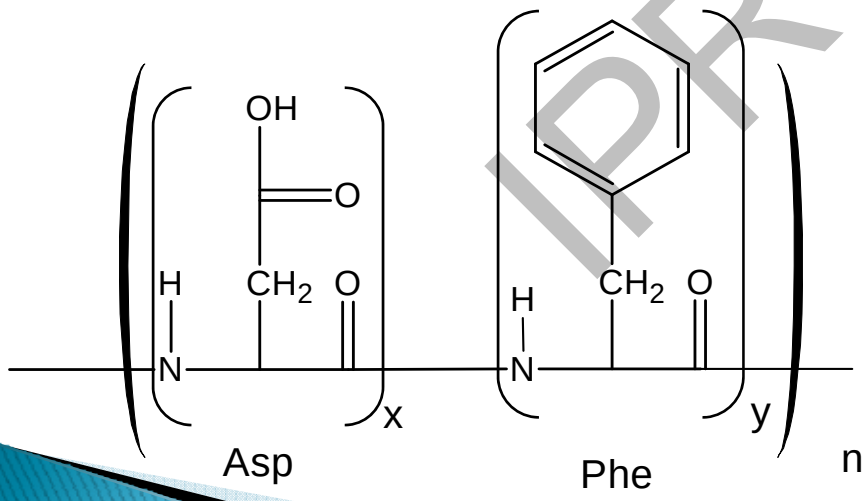
May 13th 2008

Outline

- Purpose
- Introduction
- Experimental
- Results
- Conclusions and Future Work
- Acknowledgements

Purpose

- The goal of this work is to study the effect of pH on the degree of ionization of series of polypeptide polymers containing varying ratios of aspartic acid (Asp) and phenylalanine (Phe).



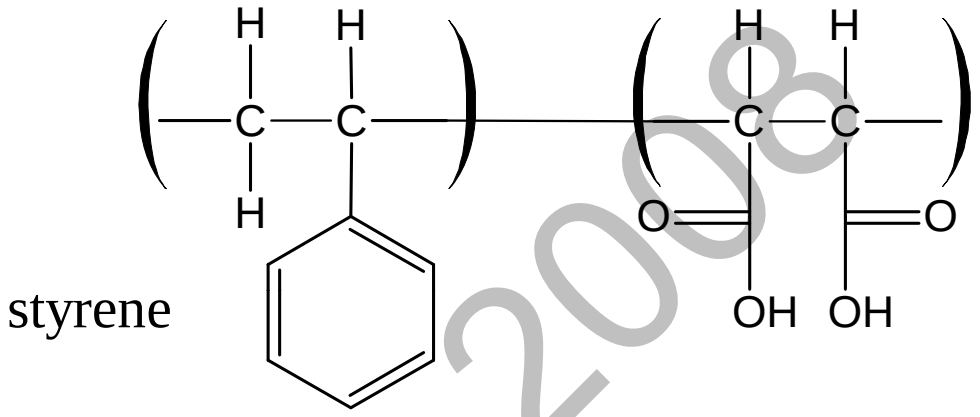
x =	y =
1	3
1	2
1	1
2	1
3	1

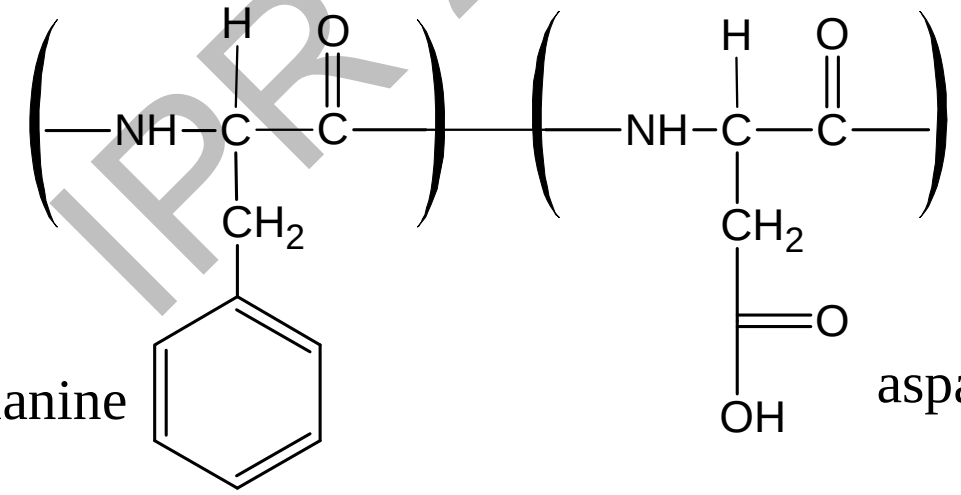
Introduction

- One type of drug delivery system uses amphiphilic copolymers, which contain both hydrophilic and hydrophobic monomers
  - The hydrophobic monomers provide an environment that can solubilize a drug or other active agent
  - The hydrophilic monomers ensure the water solubility of the drug-polymer complex. i.e. polymeric micelles

- The Asp and Phe components of the polymers under study chemically resemble the components of styrene-maleic anhydride (SMA) copolymers.
  - SMA showed promise as a drug delivery system, however its synthetic nature raised concerns over its biocompatability

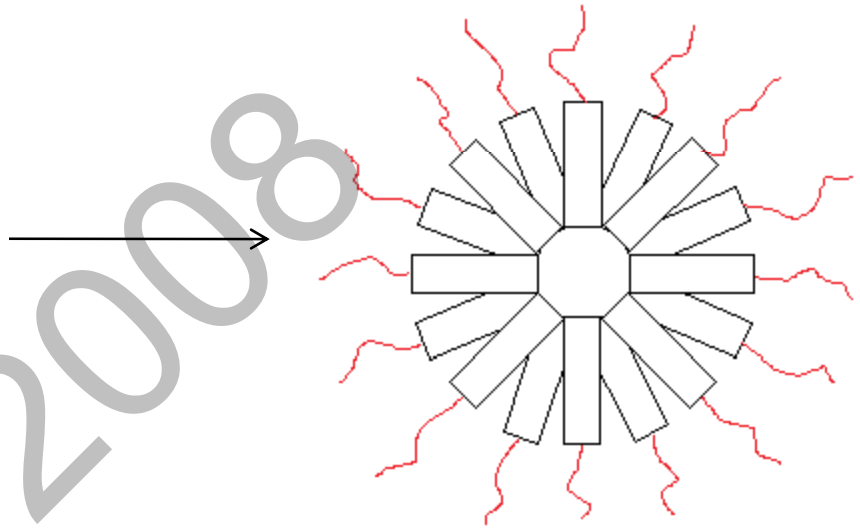
Side Chain Structure

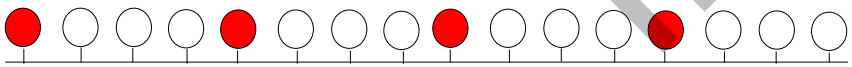




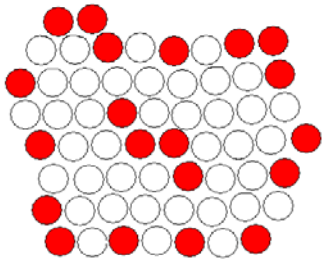
Effect of Sequence on Conformation

Block Copolymer





Sequential Copolymer



= hydrophilic group

= hydrophobic group

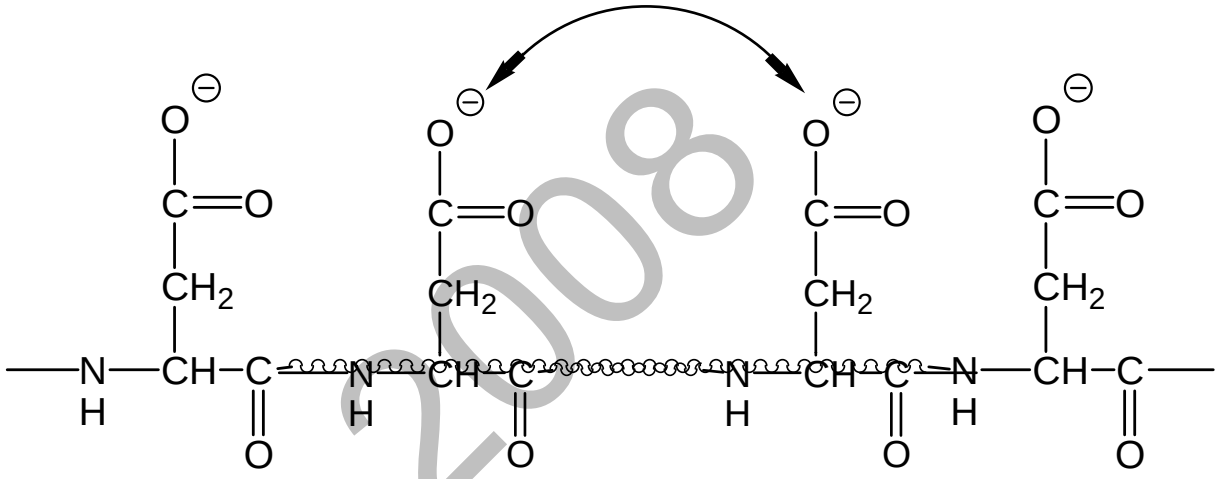
Amphiphilic Polyelectrolytes

- Amphiphilic polyelectrolytes are polymers containing both hydrophilic and hydrophobic monomers
  - In the case of $(Asp_xPhe_y)_n$, the aromatic group of Phe is hydrophobic while the ionized COO^- is strongly hydrophilic

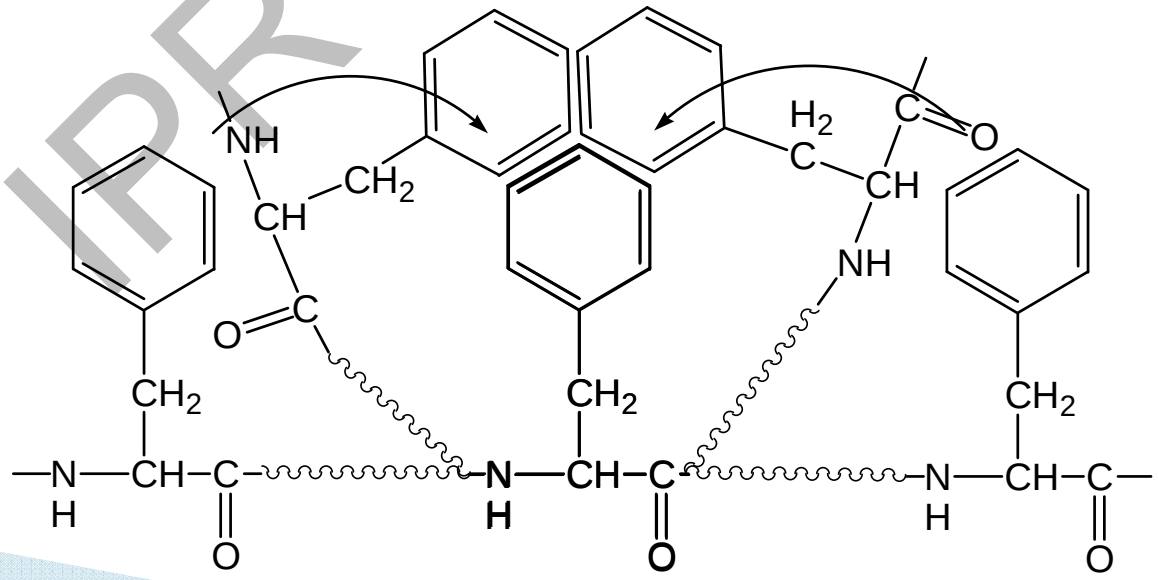
- The presence of these two groups on the same polymer leads to competing effects when in aqueous solution
  - The hydrophobic forces from Phe groups encourage a collapsed conformation, while electrostatic repulsions between COO^- groups encourage a more extended conformation

Competition Between Charge Repulsion and Hydrophobic Effects

Asp



Phe

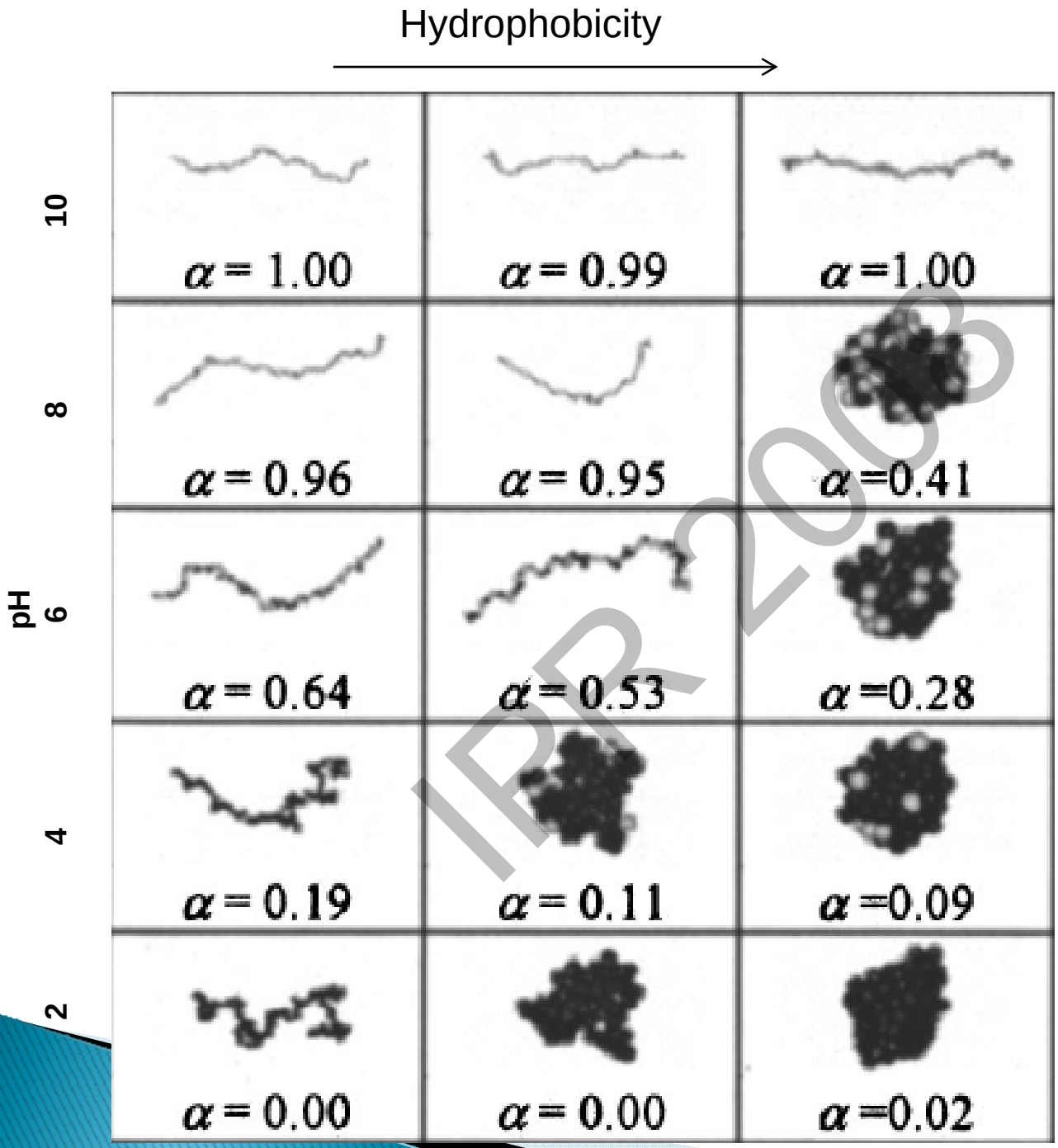


Effect of pH

- Attractive Hydrophobic forces are essentially constant

- Electrostatic repulsive forces depend on pH
  - The fraction of Asp residues in the ionized form (α) is high at high pH; at lower pH α decreases.
  - A lower α value affects the aggregation behaviour and conformation of the polymer, due to increased hydrophobicity and decreased charge repulsion effects from neutral Asp residues

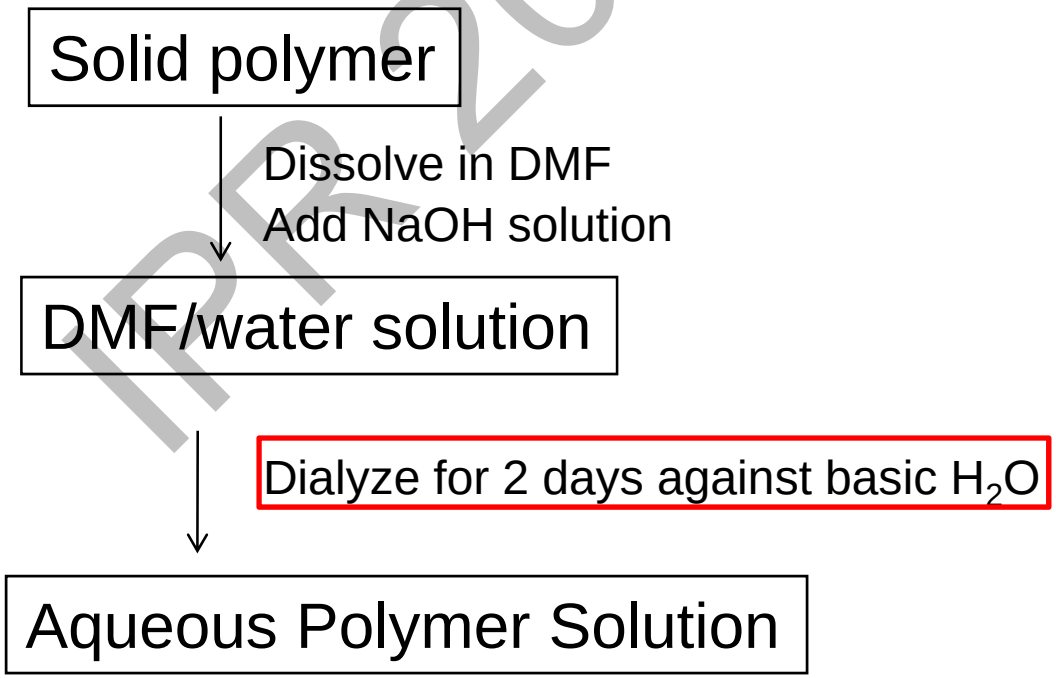
Modelling



Ulrich, S.; Laguecir, A.; Stoll, S.
J. Chem. Phys. ***2005***, 122, 094911

CO_2 Contamination

- The amphiphilic polypeptides under study are insoluble in acidic aqueous solutions, and must be prepared by dialysis under basic conditions using the following general protocol:



- Basic aqueous solutions in contact with the atmosphere absorb CO_2, converting it to bicarbonate (pKa = 6.4, 10.3) which titrates in the same range as polymeric Asp.

- The concentration of Asp residues in a 1 g/L sample solution is on the order of 1-3 mM; the presence of even very small amounts of bicarbonate will give rise to erroneous trends in the titration curve

- To prevent bicarbonate-related errors in the titration curve, all aqueous solutions used during sample preparation must be degassed, and all solutions must be kept out of contact with the atmosphere (dialysis, sample storage, during the titration)

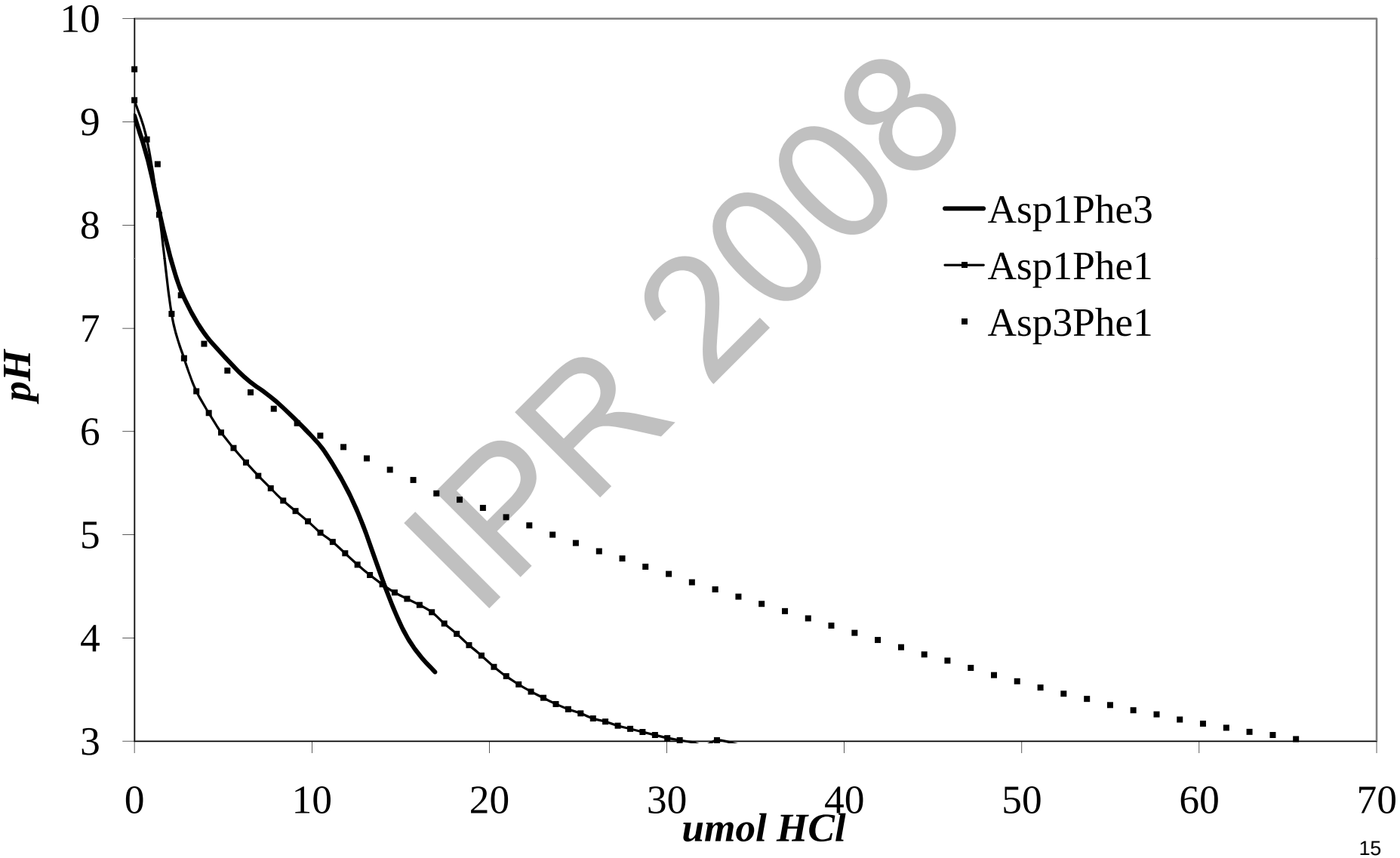
Experimental

- Polypeptide samples for titration were prepared by dissolving in DMF, followed by the dropwise addition of 0.1 M NaOH to 30% v/v. The DMF-water solution was then dialyzed against pH 10.5 unbufferd water under N_2 to prevent CO_2 contamination.

- Aqueous solutions were titrated under a stream of N_2 with 0.1 M HCl, added in 10 μL increments.

Results

Titration of $(Asp_xPhe_y)_n$



Determination of α

$$\alpha = [COO^-] / [COOH + COO^-] \quad (1)$$

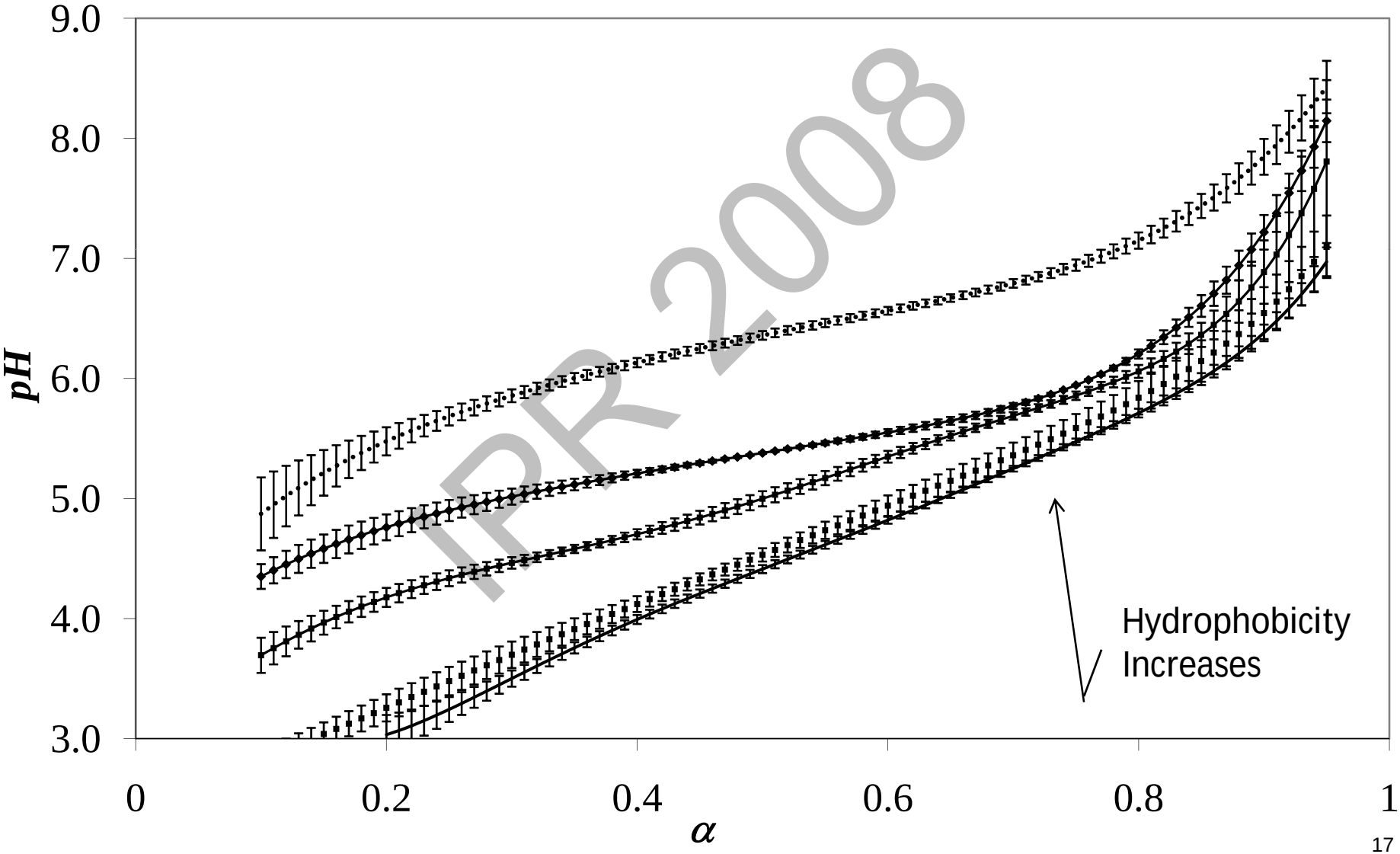
$$[Na^+] + [H^+] = [OH^-] + [Cl^-] + [COO^-] \quad (2)$$

$[H^+]$, $[OH^-]$ – derived from pH

$[Cl^-]$ – derived from μmol HCl added

$[Na^+]$, $[COOH]$ – derived from starting conditions

pH vs α



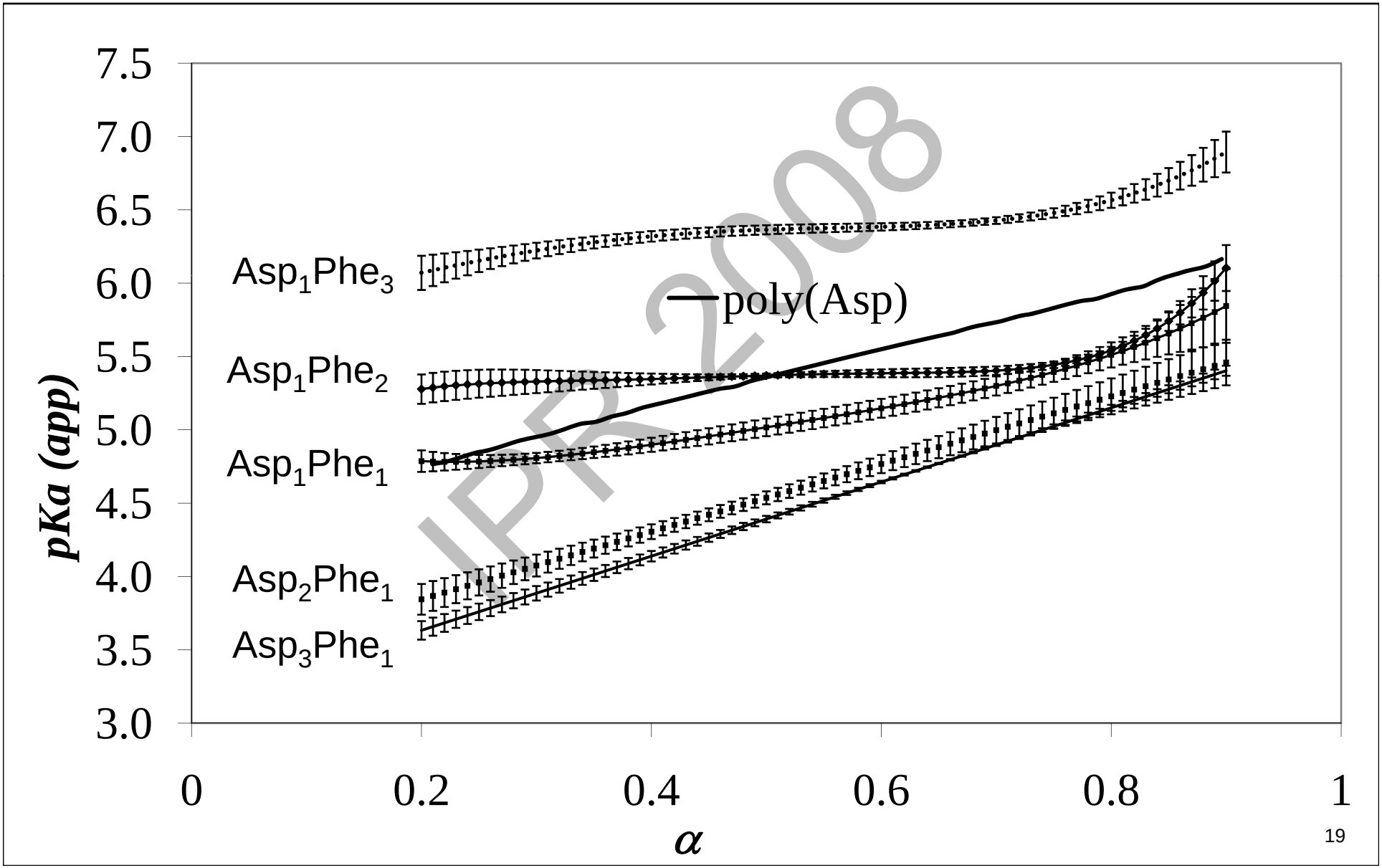
- pH vs α: Some trends visible, but it is difficult to relate to chemical nature of polypeptides

- pK_a vs α: yields more detailed information about the behaviour/environment of acidic monomer residues

- pK_a values for a polypeptide at a given pH may be obtained using the Henderson-Hasselbach equation

$$pK_a = pH + \log([COOH]/[COO^-])$$
$$= pH + \log[(1-\alpha) / \alpha]$$

pK_a vs α



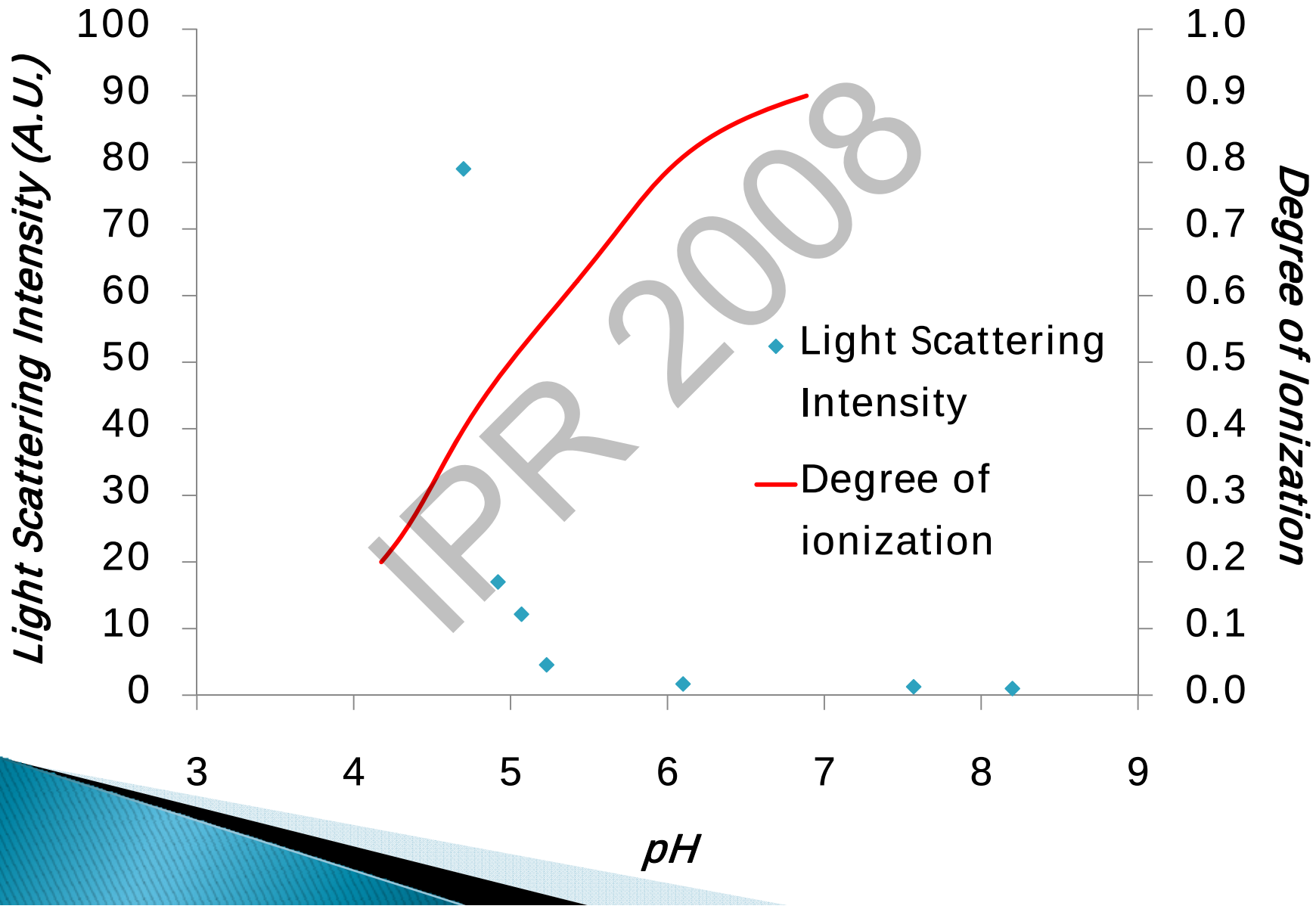
Conclusions

- At lower values of α, the pK_a of hydrophobic polypeptides reaches a plateau, similar to monomeric acid groups
- The pK_a of hydrophilic polypeptides increases steadily as a function of α, which is expected for a polyelectrolyte
- The 1:1 peptide shows hydrophobic-like behaviour at low α, and polyelectrolyte-like behaviour at high α.

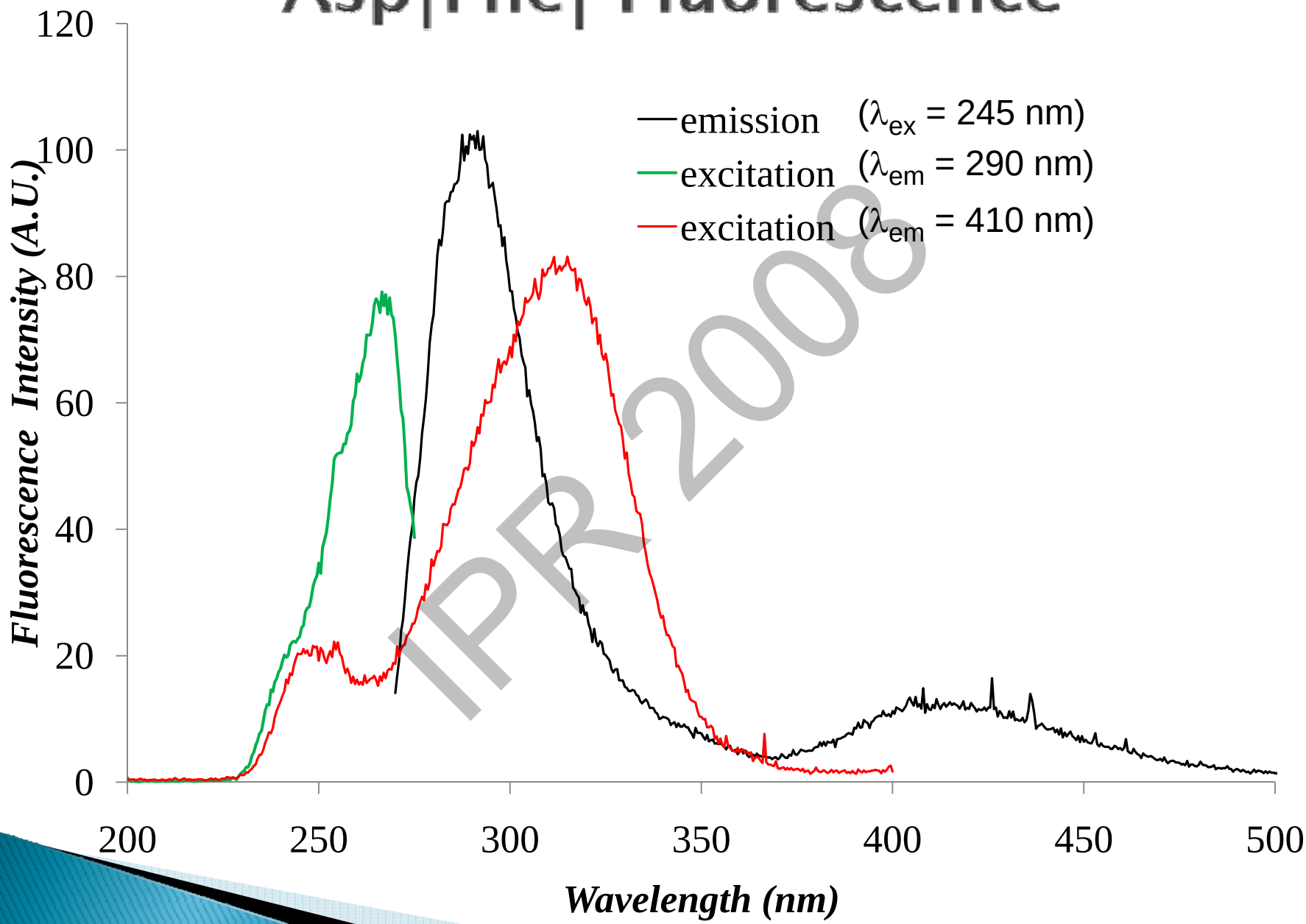
Future Work

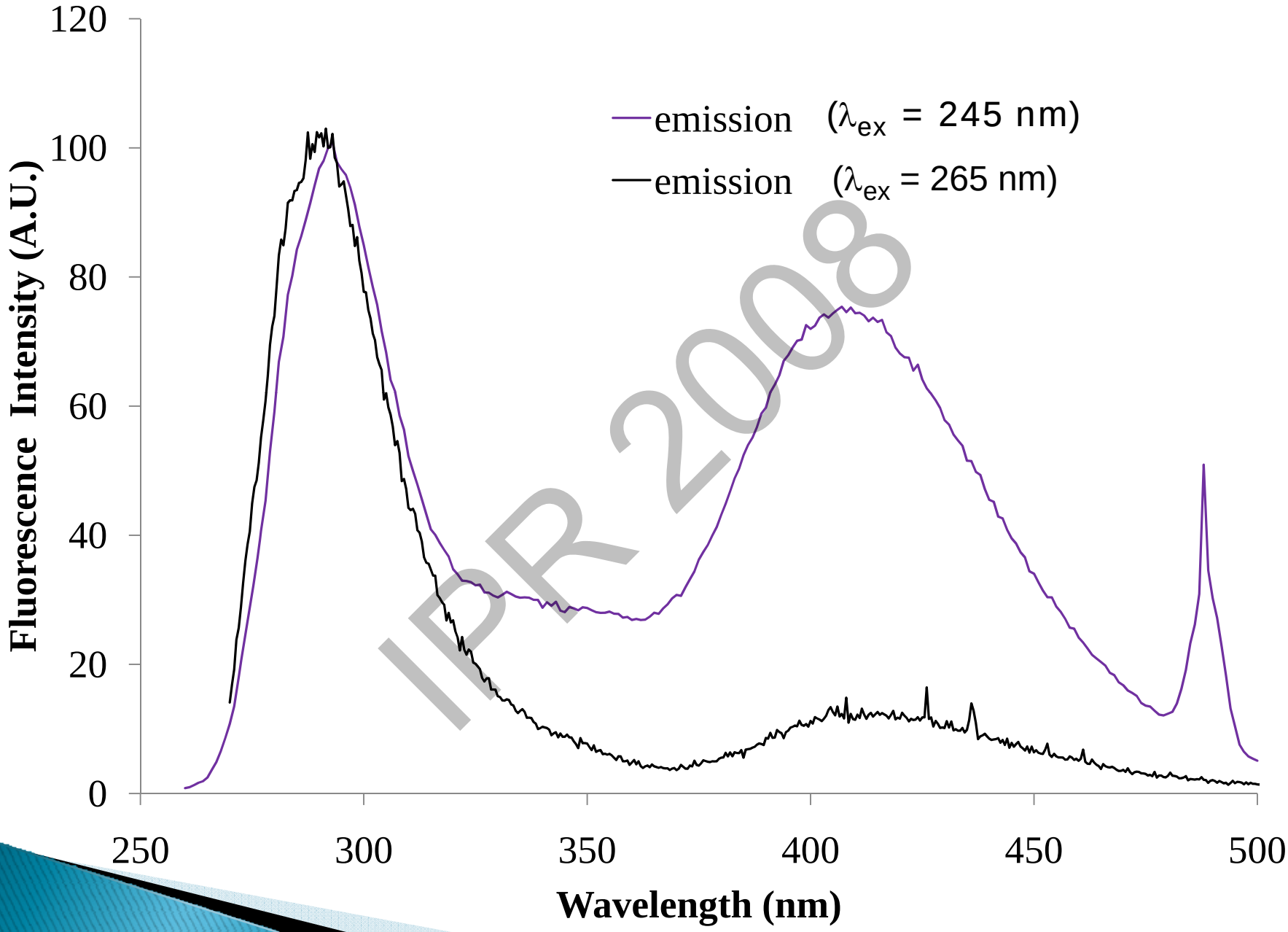
- Prepare aqueous $(Asp_xPhe_y)_n$ solutions and correlate the level of polymer aggregation with pH using light scattering
- Apply fluorescence techniques to further characterize the pH dependence of aggregation using the intrinsic fluorescence of Phe

Light Scattering Intensity

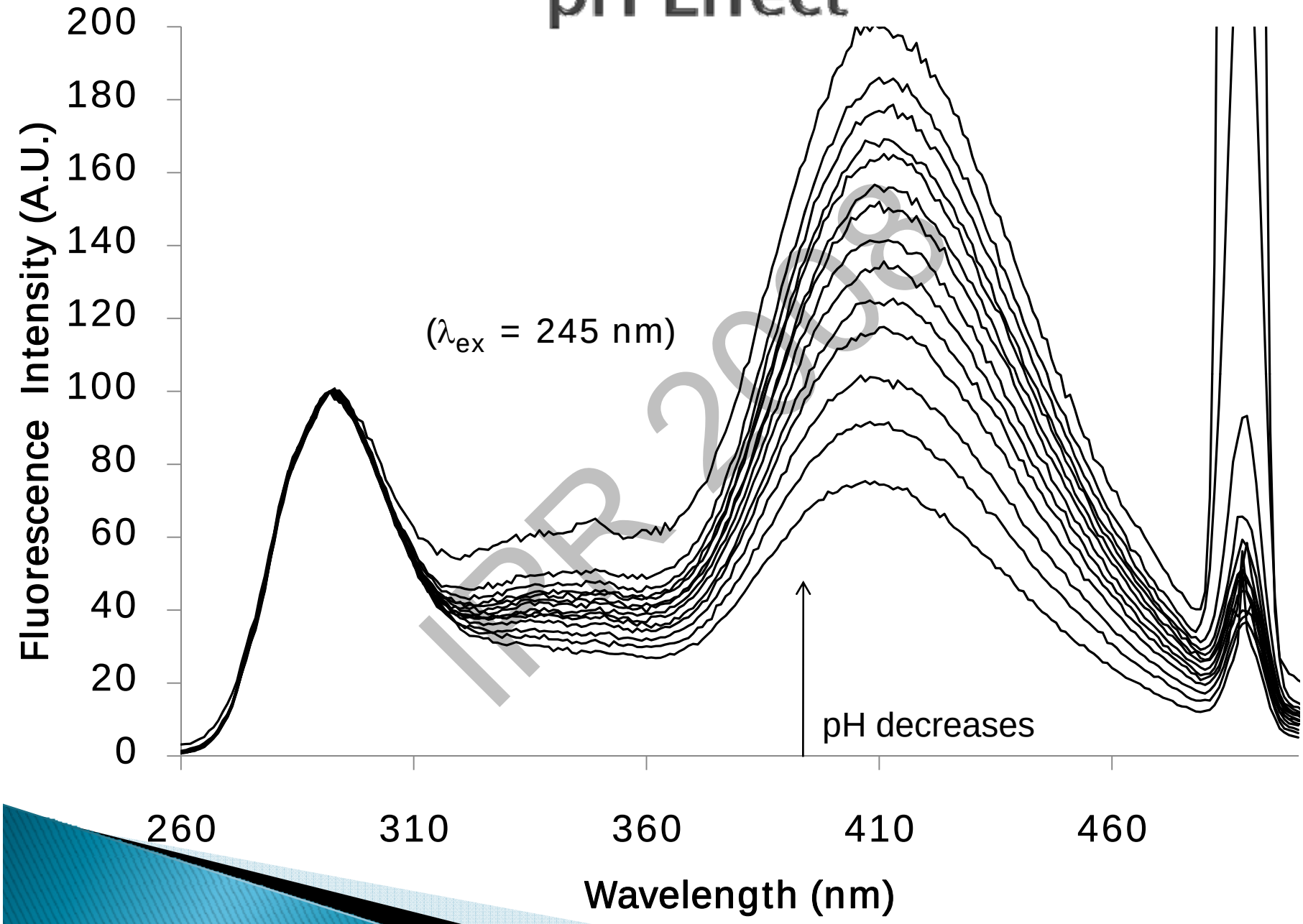


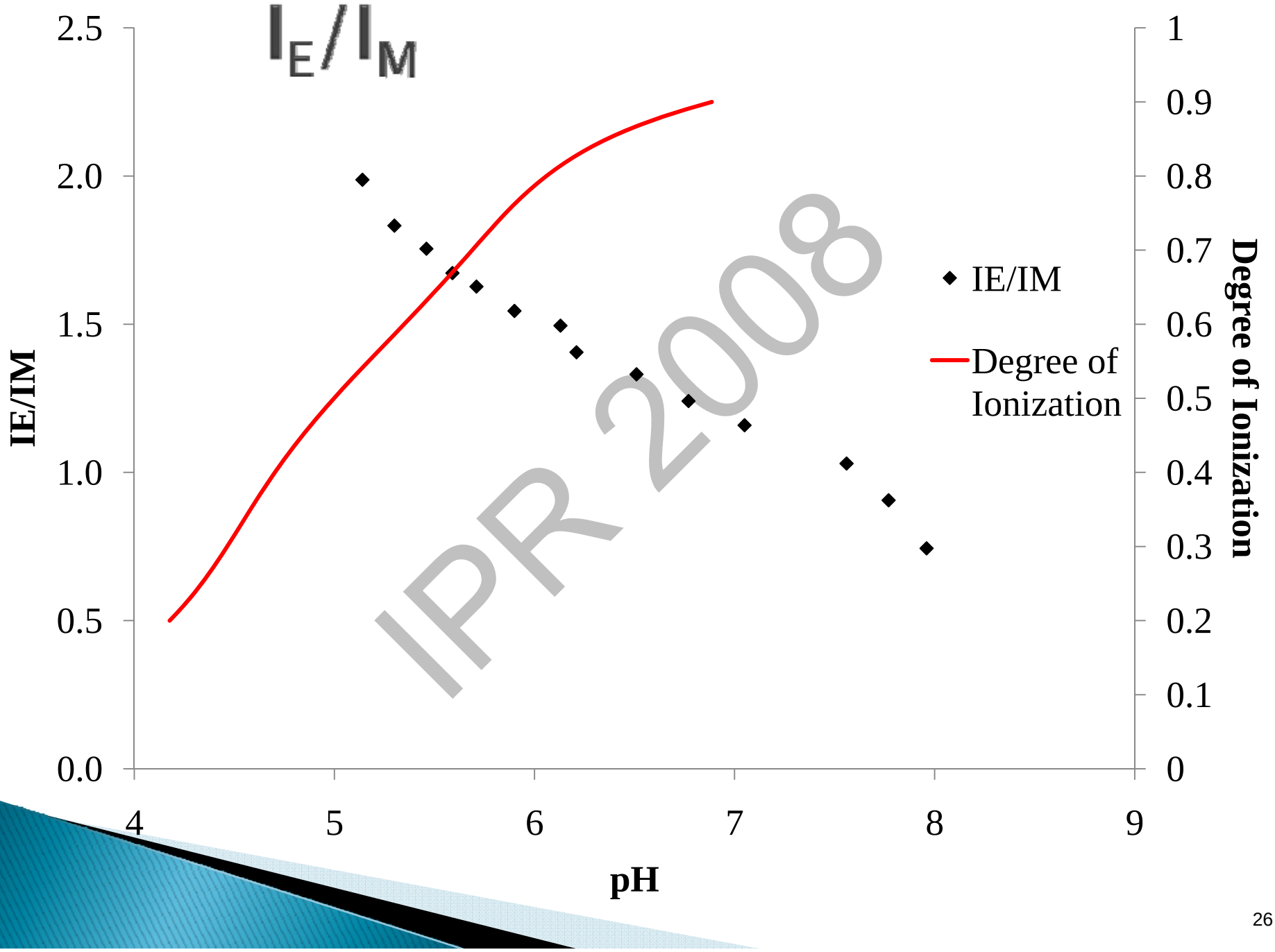
Asp_1Phe_1 Fluorescence




120
100
80
60
40
20
0
Fluorescence Intensity (A.U.)
emission (λex = 245 nm)
emission (λex = 265 nm)
250
300
350
400
450
500
Wavelength (nm)

pH Effect




I_E/I_M
2.5
2.0
1.5
1.0
0.5
0.0
IE/IM
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Degree of Ionization
4
5
6
7
8
9
pH
IE/IM
Degree of Ionization
IPR 2008

Acknowledgements

- Dr. J. Duhamel

- Colleagues from the Duhamel and Gauthier labs, both current and former

Questions?

References:

- Ulrich, S.; Laguecir, A.; Stoll, S. *J. Chem. Phys.* ***2005,*** 122, 094911
- Siddique, B. Ph. D. Thesis, University of Waterloo, Waterloo, 2007.

Additional Note

- Cutoff for pK_a/α plots are not uniform due to inaccuracies in the pH meter
  - At low pH, drift and rounding errors become significant
- These effects were noticed by plotting $\Delta\alpha$ vs μmol HCl

$$\Delta[Na+] + \Delta[H+] = \Delta[OH-] + \Delta[Cl-] + \Delta[COO-]$$

$$\Delta[H+] - \Delta[Cl-] = \Delta[COO-]$$

$(\Delta[H+] - k)$ is proportional to $\Delta\alpha$

Δα vs µmol HCl