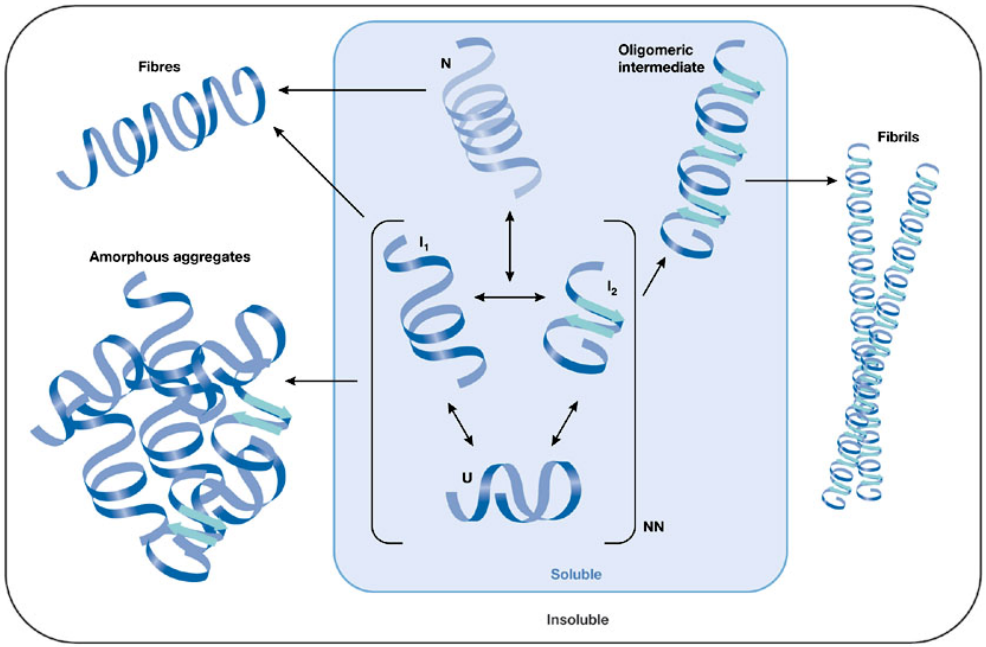
Aggregation of α-Chymotrypsinogen A in Aqueous Solutions

Aaron Aziz
Advisor: Dr. Yun Liu
Colleague: Dr. Jiang Du

National Institute of Standards and Technology
Technology Administration, U.S. Department of Commerce

What is Protein Aggregation?



- It is a broad term used to define a type of protein self assembly
- Classified as native or nonnative proteins
- Can include both soluble and insoluble protein aggregates
- Typically these aggregates are unfavorable products

Figure 1. Schematic diagram of protein aggregation pathways.

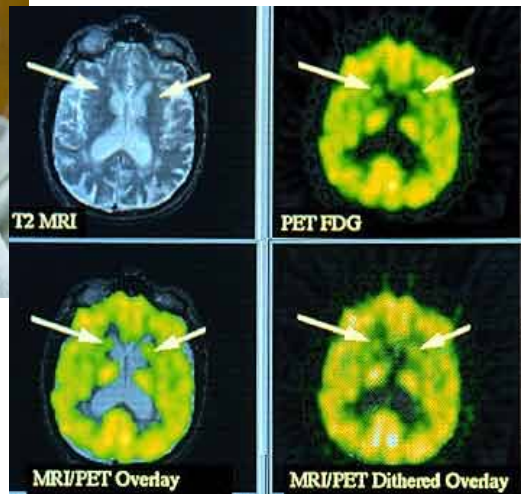
Quality control of protein folding in extracellular space. J. J. Yerbury.

Why is it important?

1. Bio-processing operations

2. Pharmaceuticals

3. Debilitating diseases



4. Cytotoxicity

NIST

Our study

- α-Chymotrypsinogen A

Goals:

1. Aggregation dynamic of α-Chymotrypsinogen A
2. Broaden this understanding to other proteins
3. Apply to real world problems

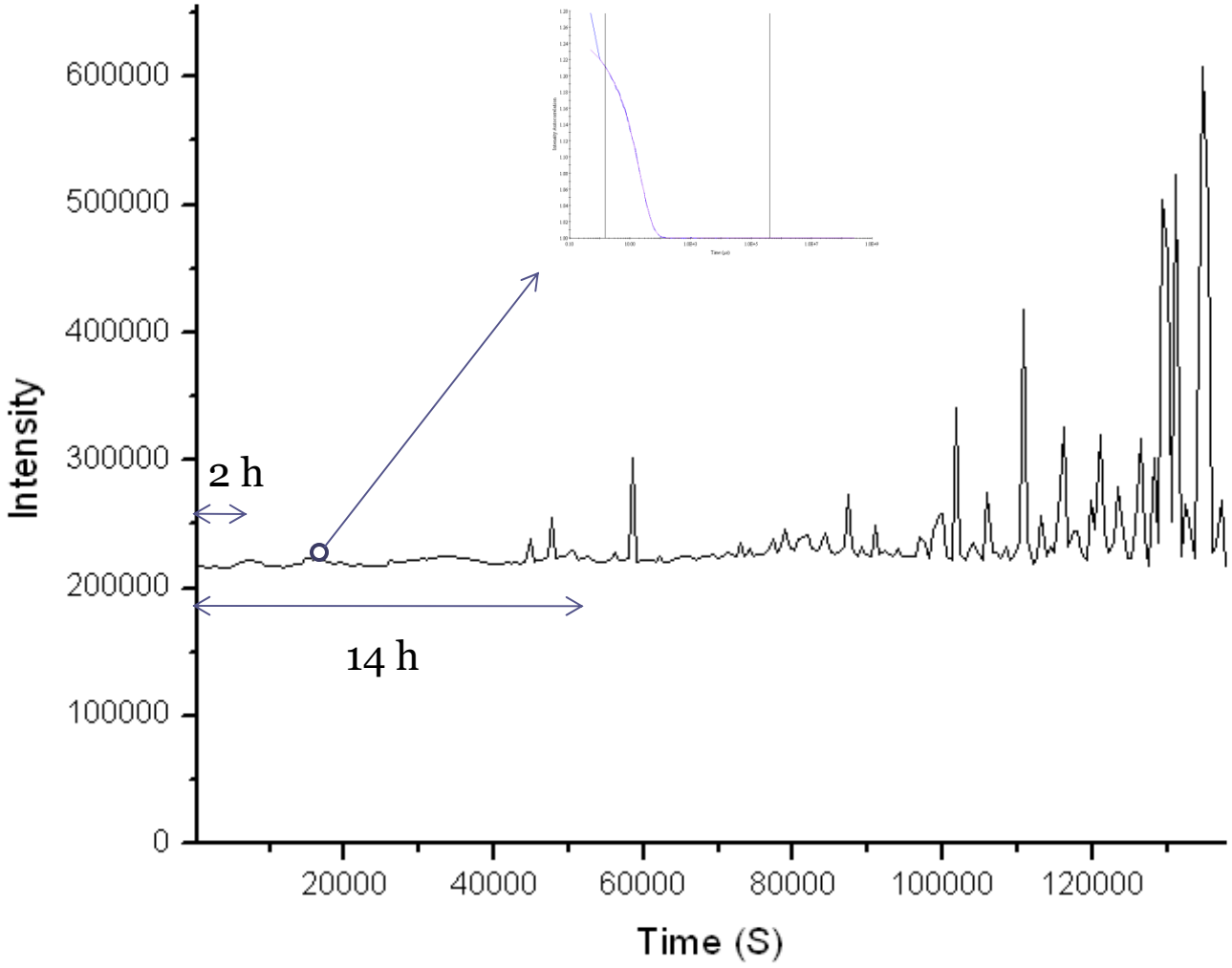
Techniques:

1. Dynamic Light Scattering
2. Circular Dichroism
3. Small-Angle Neutron Scattering

"…30% of the original protein sample aggregated in a period of 2 hours into clusters comprising four or more molecules each."

NIST

Dynamic Light Scattering Results



5 mg/ml pH=7.3

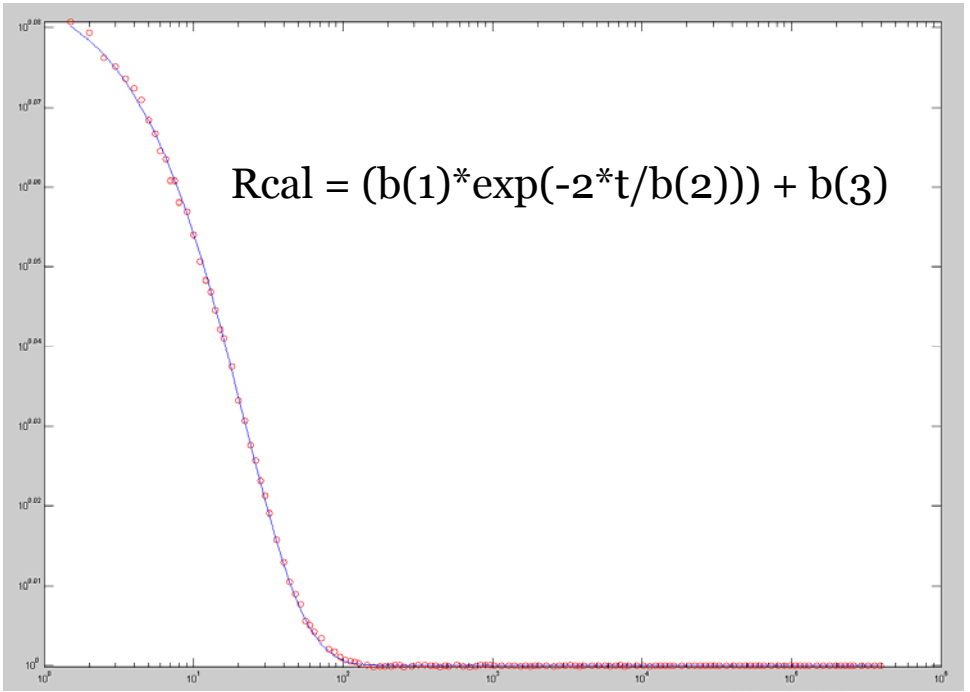
Decay Curve Fitting Results

$$I_2(t) = A(e^{-2t/\tau}) + B$$

```
clc;
clear;
h = fopen('testdata.txt', 'r');
data = fscanf(h, '%g %g', [4 inf])';

t = data(:,1);
Rexp = data(:,2);
loglog(t,Rexp,'ro');
hold on

bo = [.22 40 1];
b = lsqnonlin(@expfunc, bo,[],[],[],data)

Rcal = (b(1)*exp(-2*t/b(2))) + b(3);
plot(t,Rcal,'b');

k = 2*pi/(782.7e-9/1.33);
q = (sqrt(2)*k);
D = 1/((b(2)*(10^-6))*(q^2));
a = (1.3806503e-23*298.15)/(6*pi*8.94e-4*D)
```

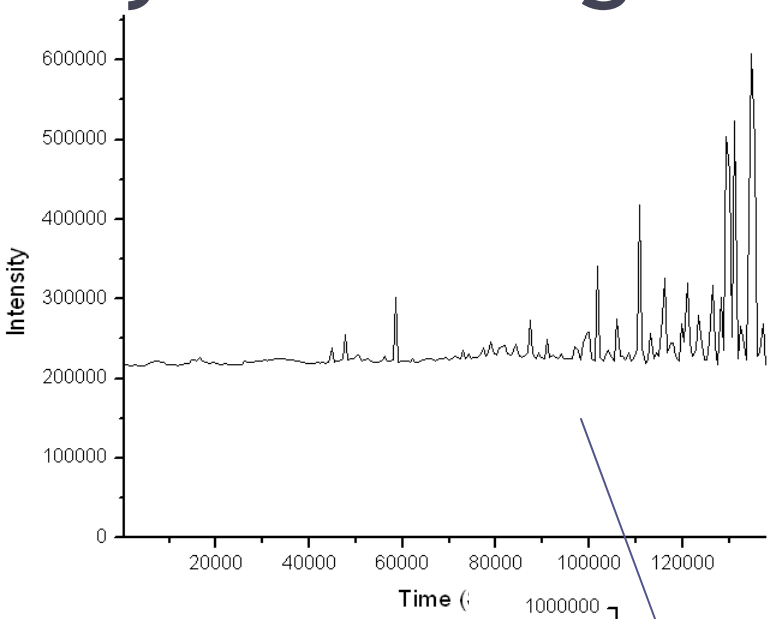
Hydrodynamic Radius:

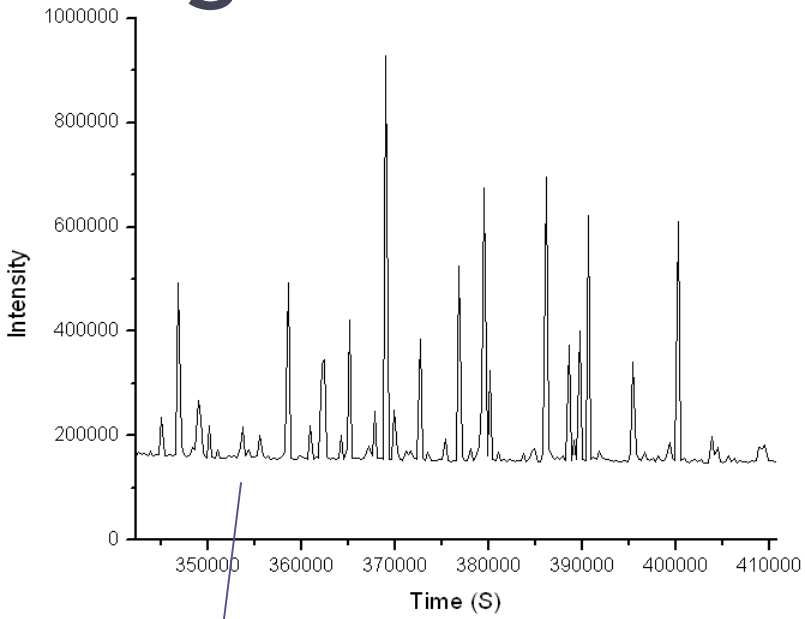
a = 2.2293e-009 = 2.23 nm

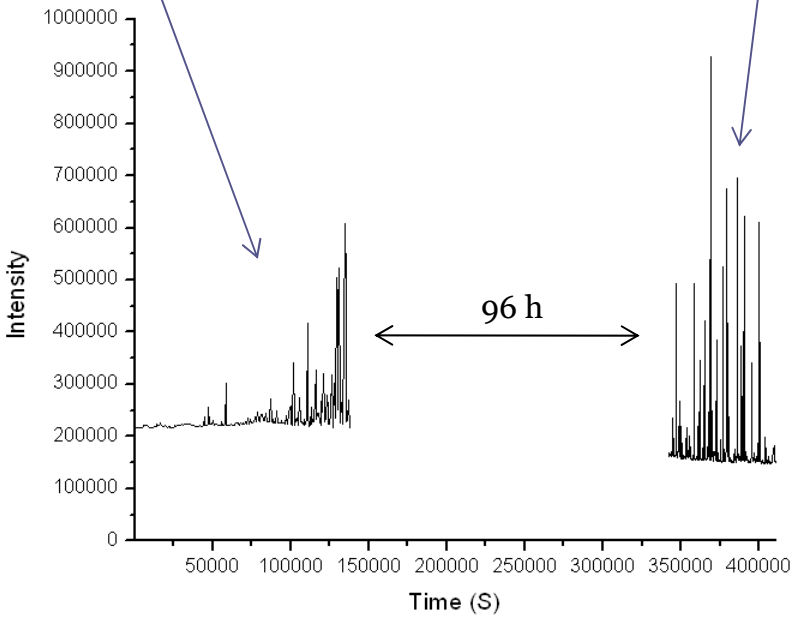


NIST

Dynamic Light Scattering Results



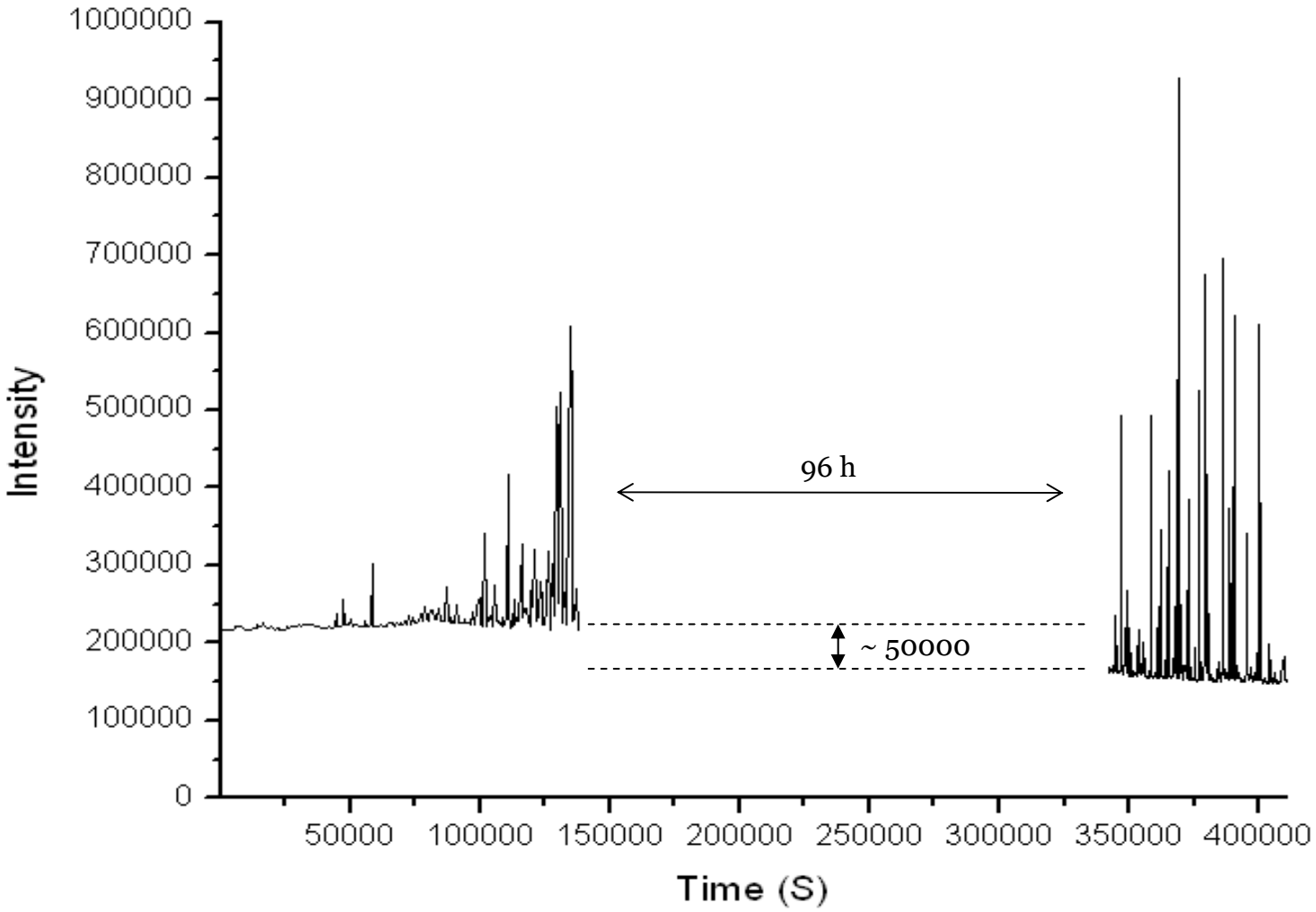




96 h

5 mg/ml pH=7.3

Dynamic Light Scattering Results

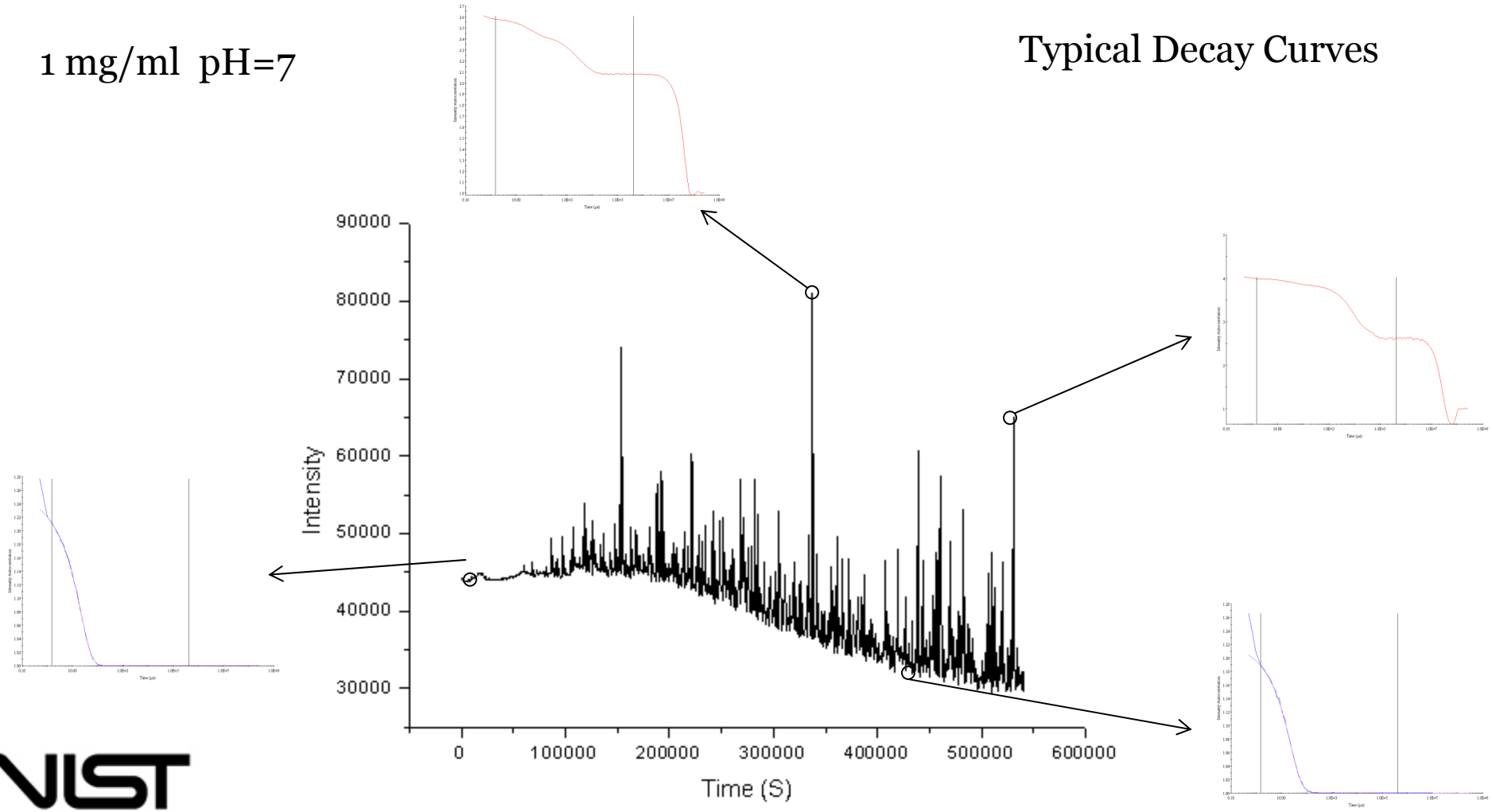


5 mg/ml pH=7.3

Dynamic Light Scattering Results

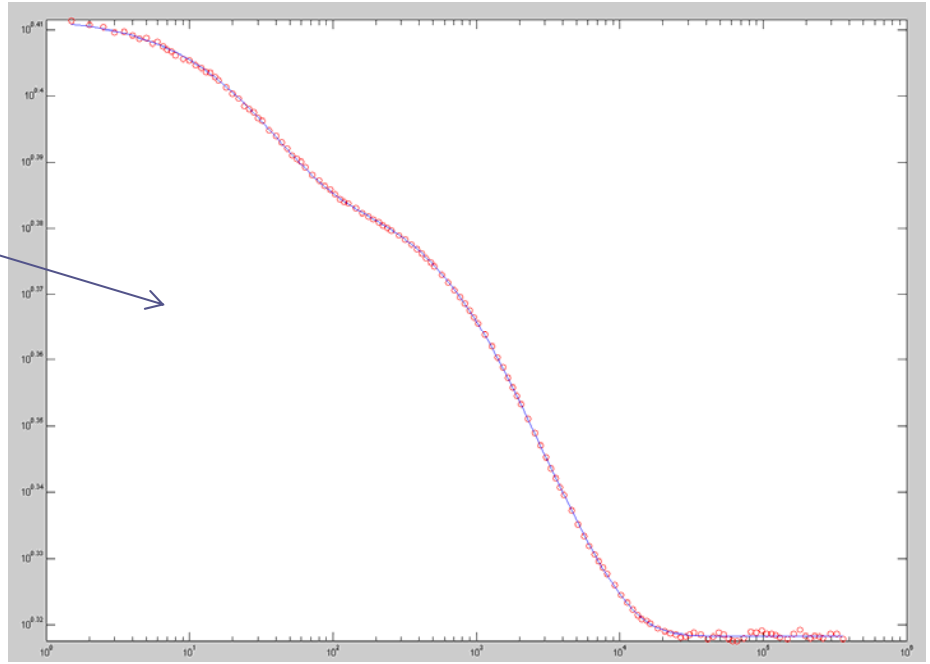
1 mg/ml pH=7

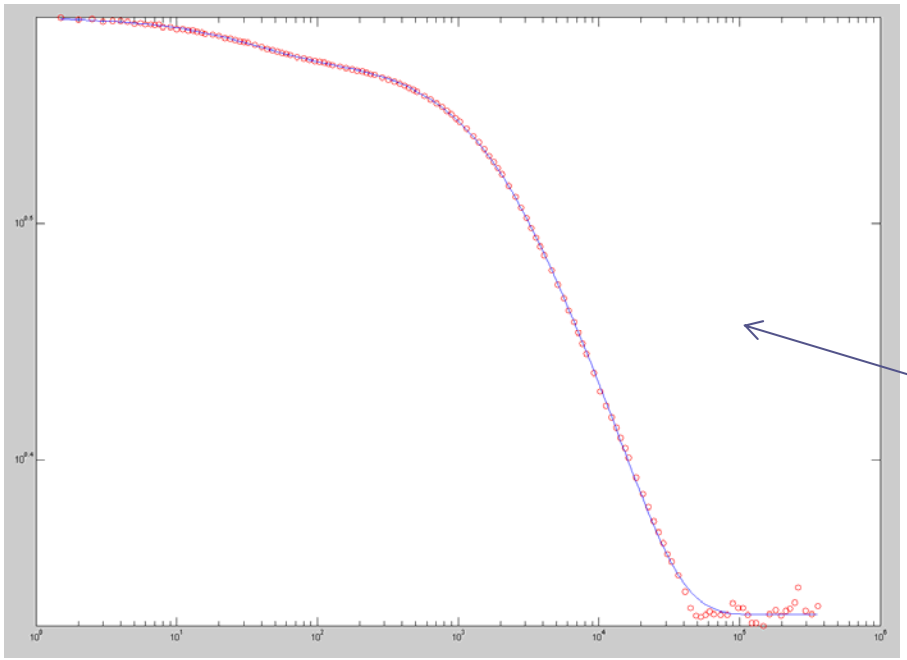
Typical Decay Curves



Decay Curve Fitting Results

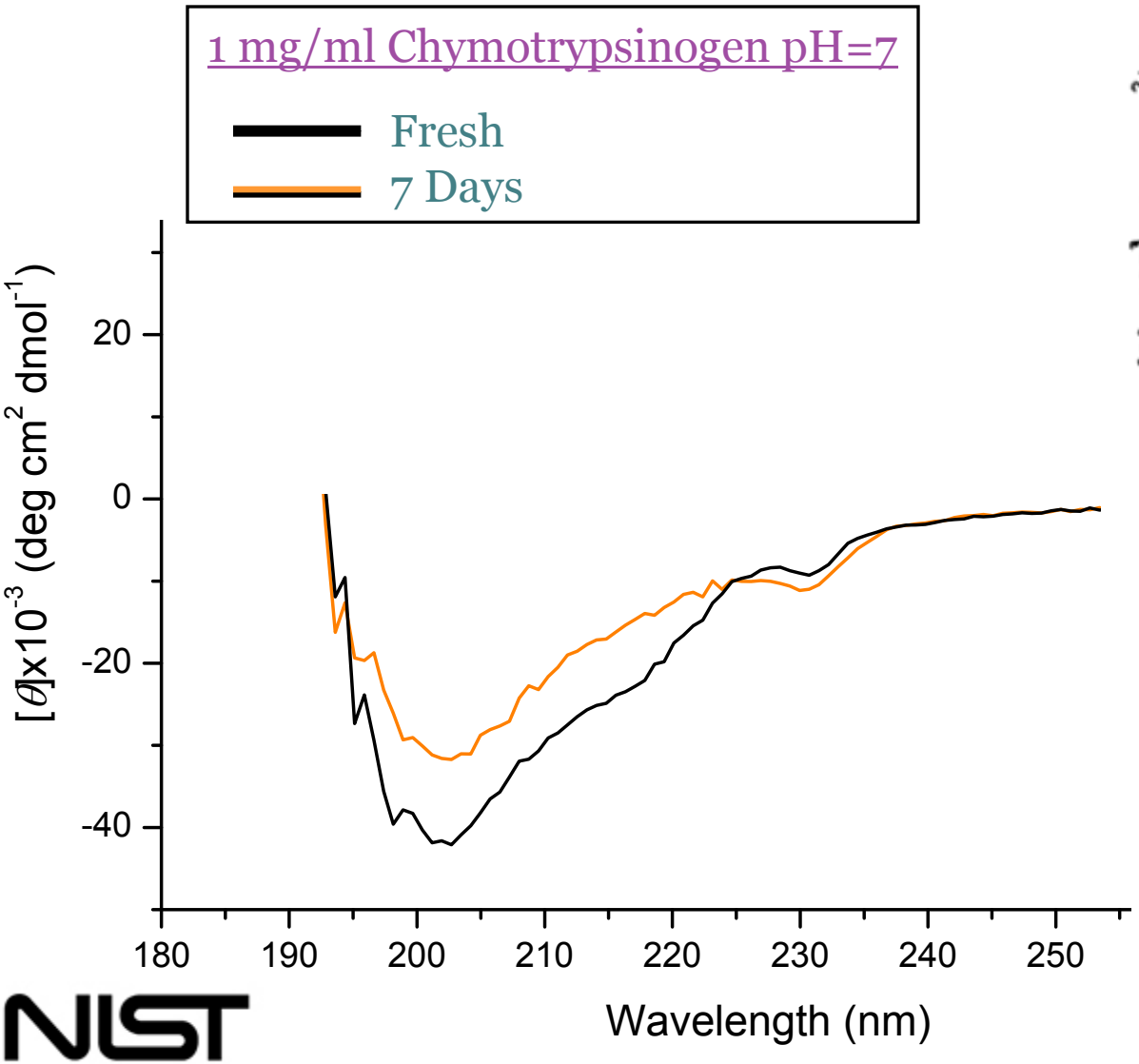
a1 = 2.2110e-009 = 2.21 nm
a2 = 9.3650e-008 = 93.7 nm
a3 = 8.3982e-007 = 840 nm





a1 = 1.9266e-009 = 1.93 nm
a2 = 1.9271e-007 = 193 nm
a3 = 2.4384e-006 = 2.44 μm

Circular Dichroism



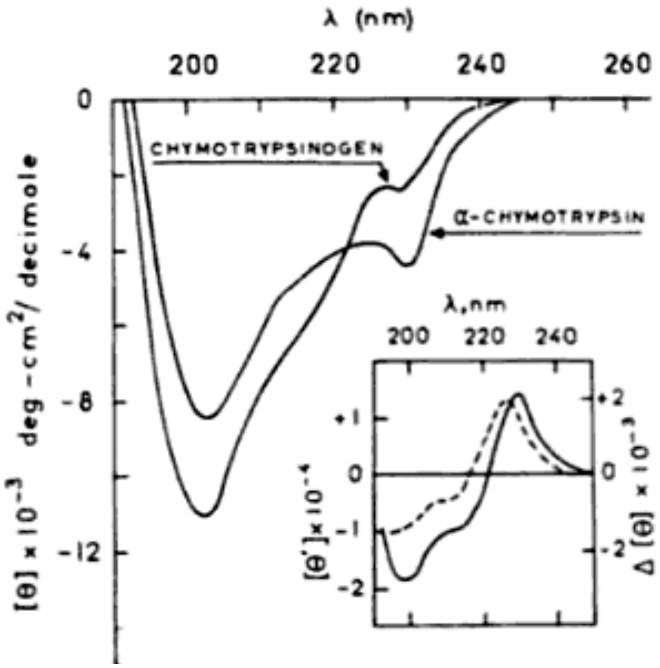
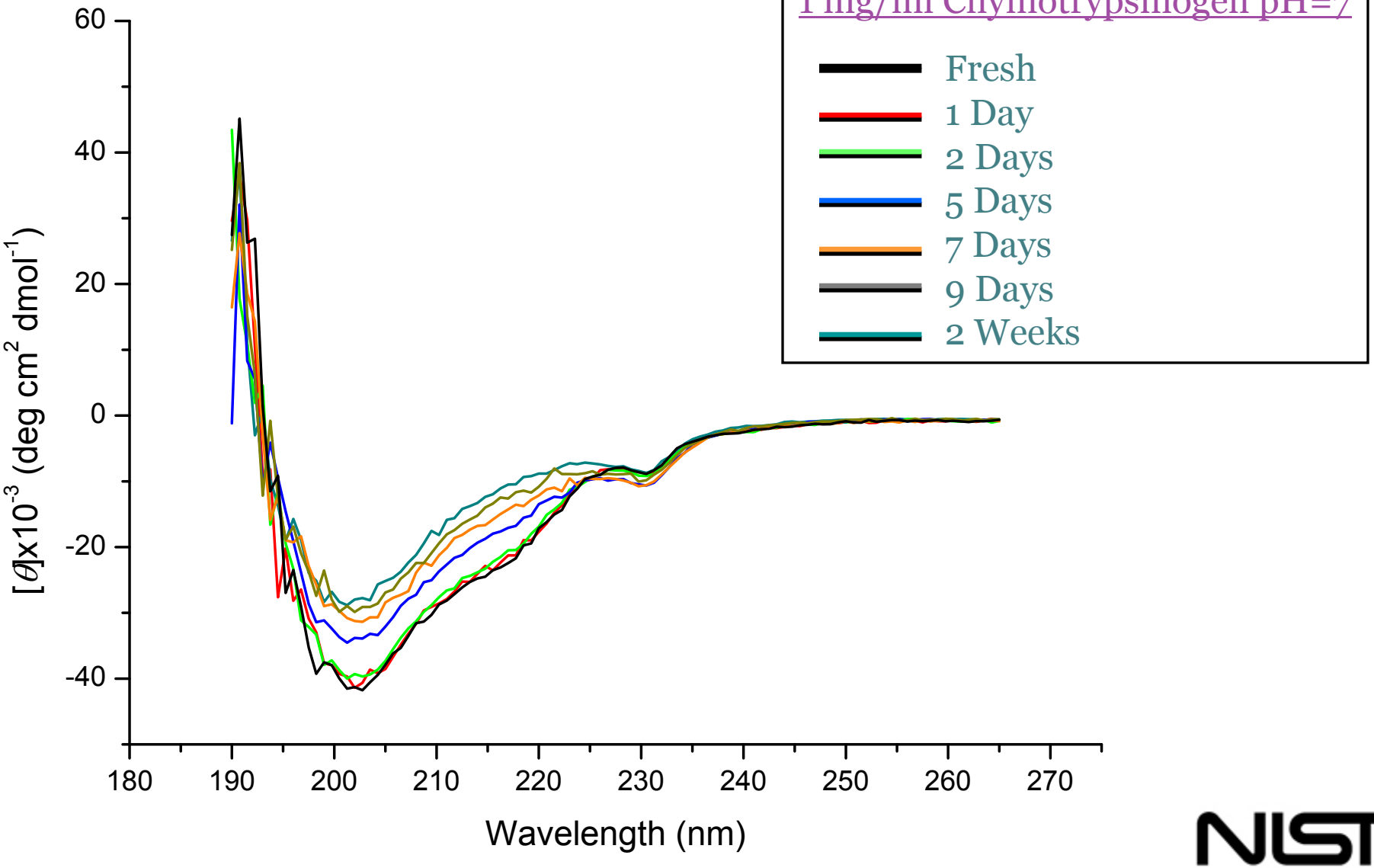


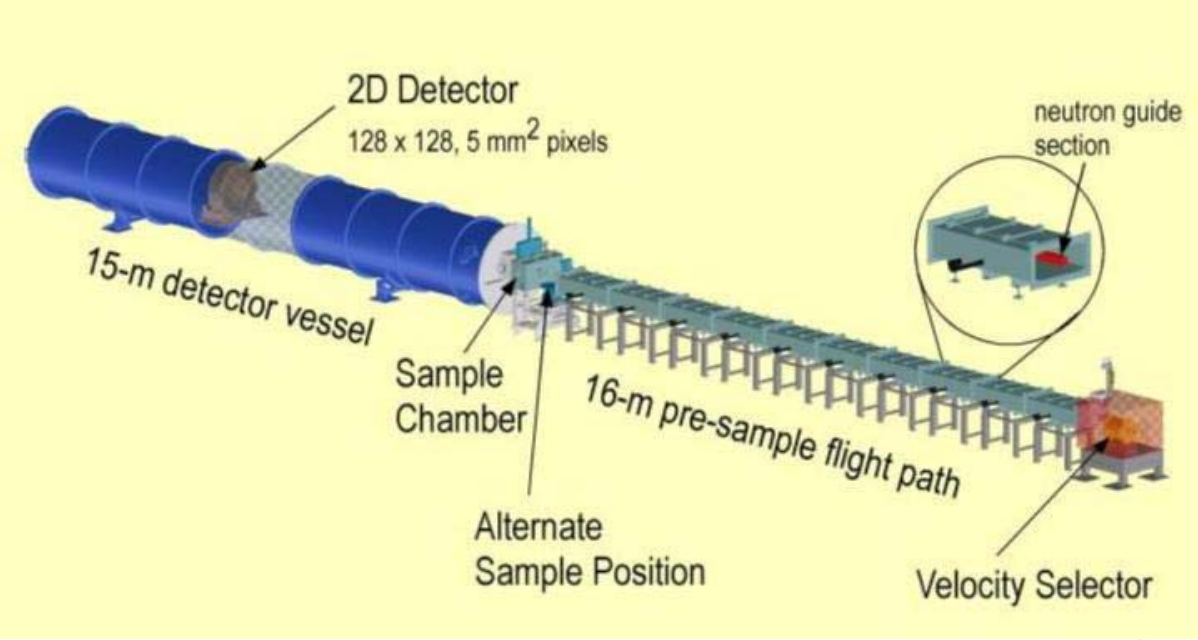
Figure 5. Far-UV CD spectra of chymotrypsinogen and α-chymotrypsin at pH 7. The inset shows the difference spectrum (chymotrypsinogen – chymotrypsin) as the solid curve (right ordinate) and the CD spectrum of *N*-acetyl-L-tryptophanamide as the dashed curve (left ordinate). The data on chymotrypsin(ogen) are from unpublished work of M. J. Gorbunoff and S. N. Timasheff, and those for $AcTrpNH_2$ are from Shiraki (1969). (Reprinted with permission from Cantor and Timasheff, 1982, by permission. © 1982, Academic Press, Inc.)

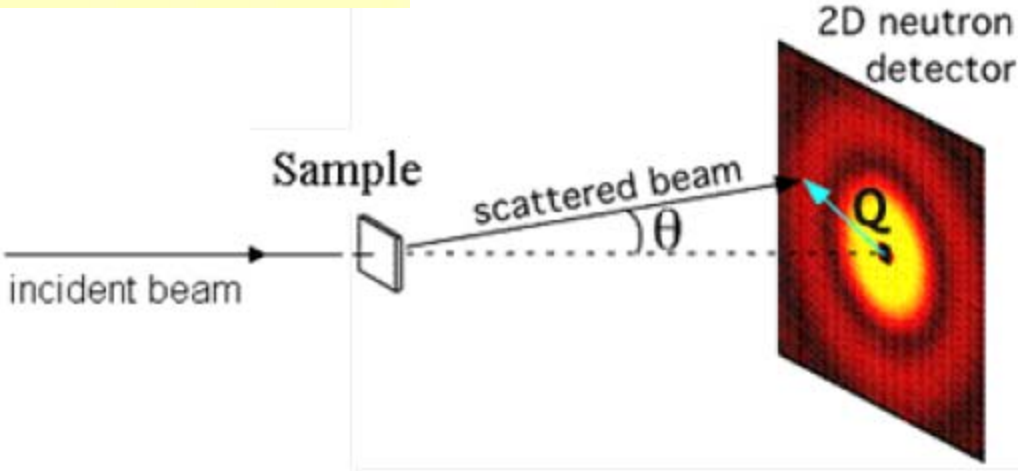
NIST

Circular Dichroism



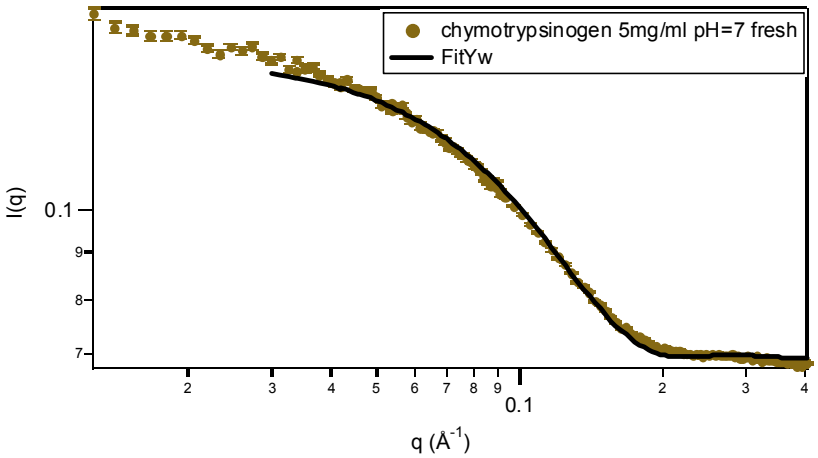
Small-Angle Neutron Scattering (SANS)



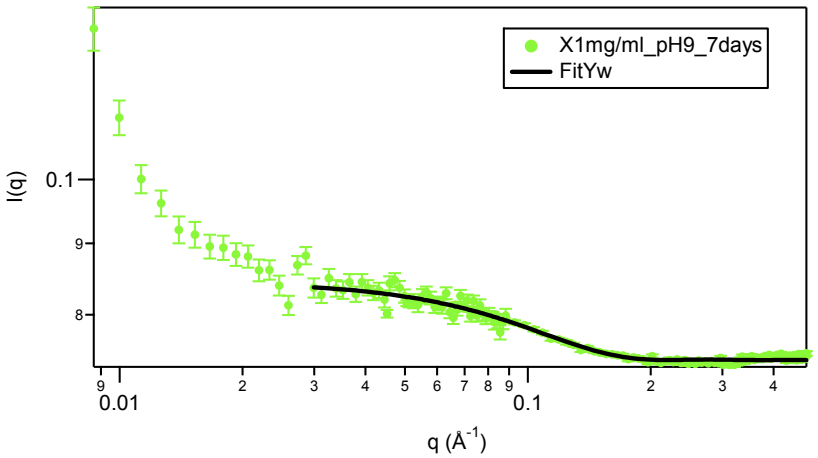


NIST

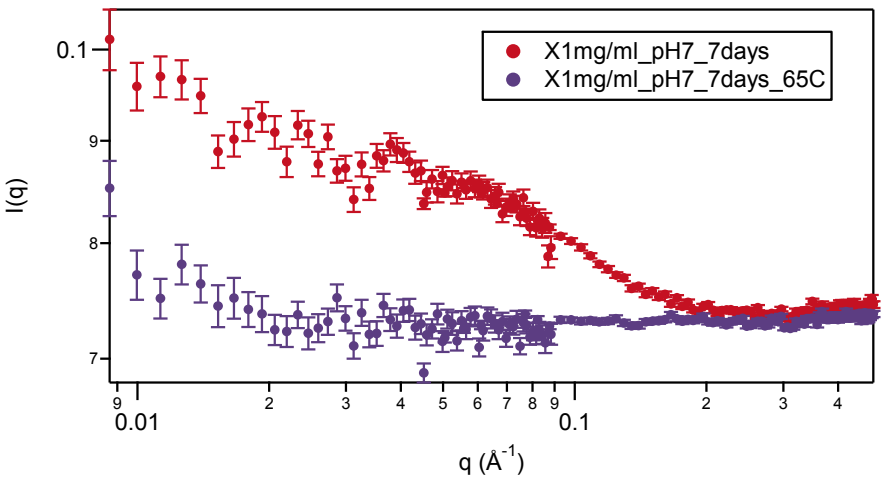
Small-Angle Neutron Scattering (SANS)

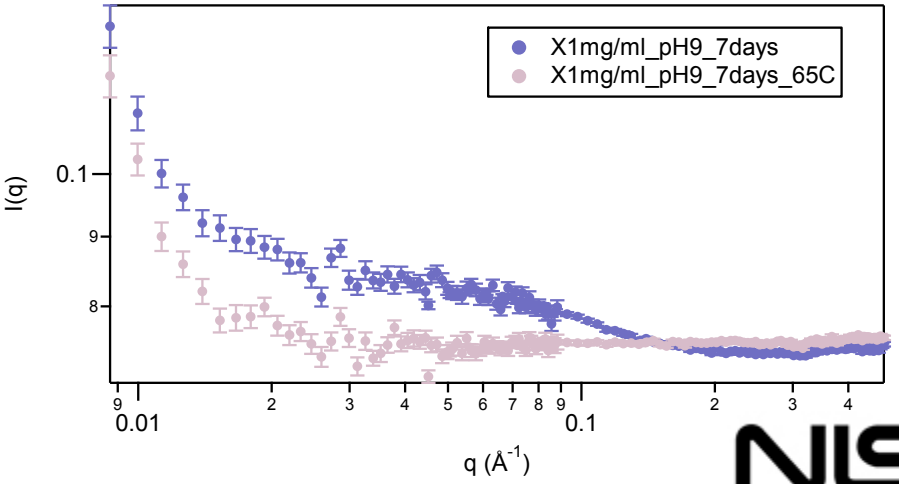


Smeared Sphere Model
Radius = 2.0 nm

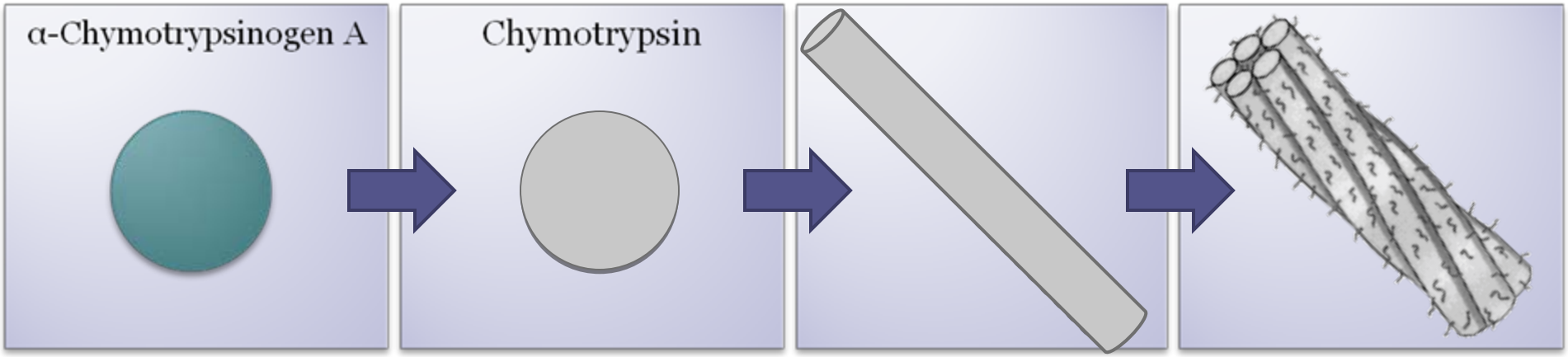


Smeared Sphere Model
Radius = 2.1 nm





Conclusion



- Aggregate
  - Linear semi-flexible polymer chain
  - Does not precipitate

Future Plans

- Chymotrypsin Testing
- Trypsin Inhibitor
- Vary pH
  - pH = 3
  - pH = 9
  - pH = 11

Acknowledgements

Yun Liu
Jiang Du
Paul Butler
Andrea Hamill
Christopher Roberts
NIST
SURF/NCNR

NIST

Thank You!

¿Questions?

References

Velev, O. D, and E. Kaler, and A. Lenhoff. (1998). Protein Interactions in Solution Characterized by Light and Neutron. *Biophysical Journal*, *75* pp. 2682-2697.

Yerbury, J. J, and E. Stewart, and A. Wyatt, and M. Wilson. (2005). Quality control of protein folding in extracellular space. *EMBO Reports*, *6* (12), pp. 1131-1136.

NIST

Materials

- α-chymotrypsinogen
- deionized water from a Millipore Milli-Q system (solutions for DLS)
- NaCl (used to adjust the electrolyte concentration)
- 10 mM citrate buffer (0.01 mol/L)
  deionized water (1 L)
  citric acid (192.14 x 0.01= 1.9214 g)
- 0.1 M NaOH (0.1 mol/L)
  deionized water (0.1 L)
  NaOH (40.01 x 0.01= 0.4001 g)
- 0.1 M HCl
  dilute from concentrated HCl solution