"Studying protein clusters in solution using SANS and Rheometry"

Dr. Yun Liu, Dr. Aaron Eberle and Navjot Kaur
NIST Center For Neutron Research, Gaithersburg, Maryland

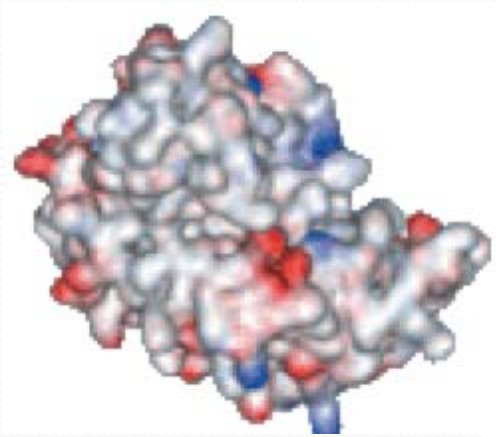
August 5th, 2010

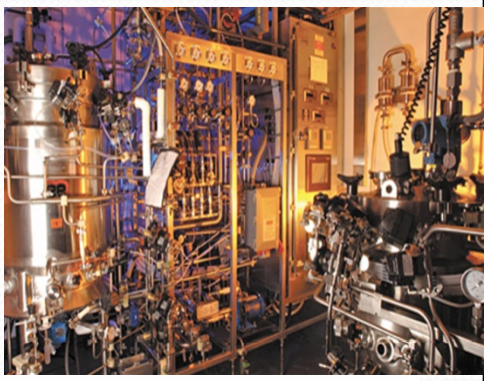
Outline of the Presentation

- Introduction and Applications of Protein
- Problem – Finite size clusters or aggregates of protein
- Our Study – Lysozyme Protein
- Filtering Effect
- Effect on Structure as a function of concentration of added NaCl salt using SANS
- Joint study of concentrated lysozyme protein solution using both SANS and rheometer.
- Future Plans

Introduction and Applications of Protein

- What are Proteins?
- Bio-processing Operations
- Pharmaceutical Industries
- Protein Supplements



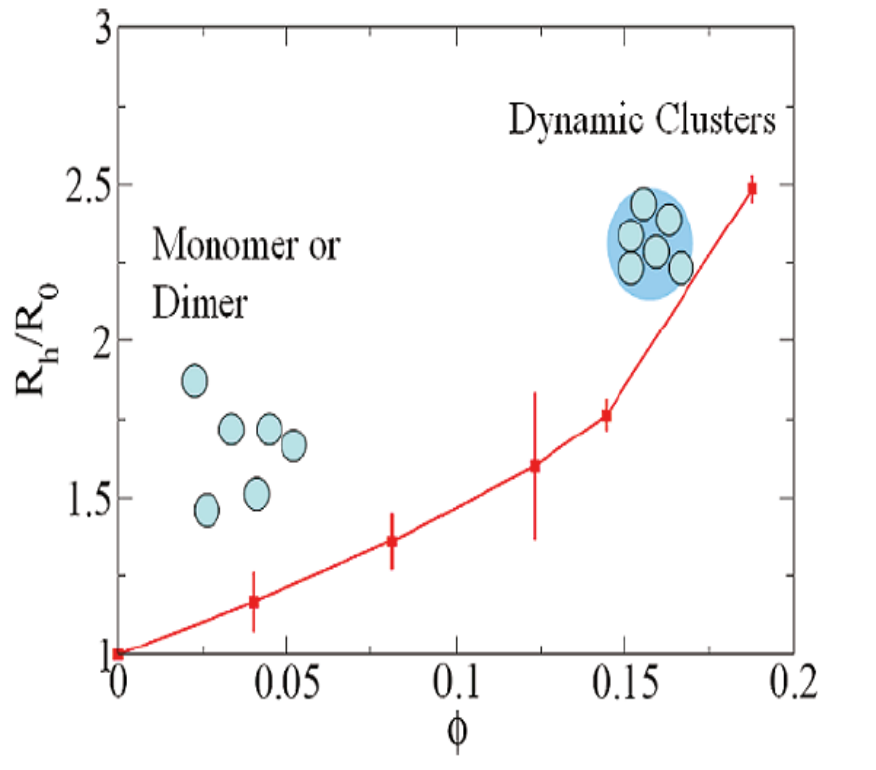


Problem – Finite size clusters or aggregates of protein

- What is Protein Aggregation?
  - Protein self-assembly – include both reversible and irreversible protein aggregates

- Protein Deposition Diseases
  - Alzheimer's Disease

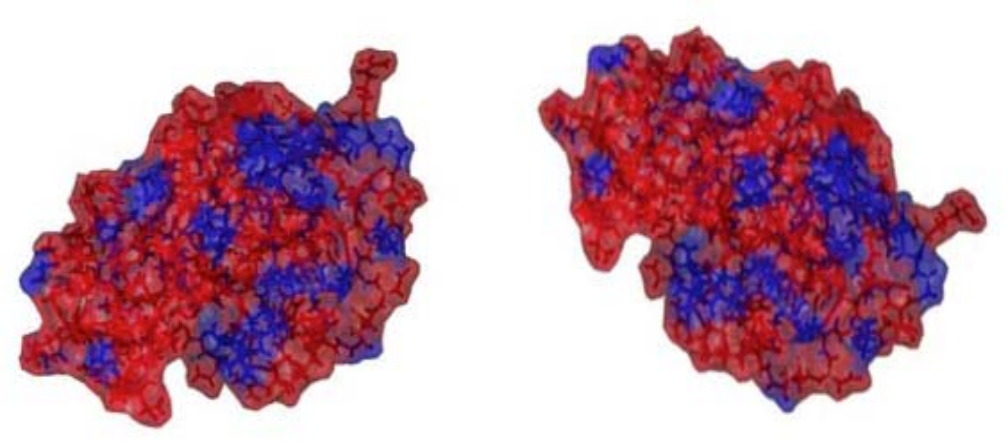
- Protein Drugs
  - Lyophilization



Source: Anthony L Fink (1998). Protein aggregation: folding aggregates, inclusion bodies and amyloid. Folding and Design, 3(1), R9-R23 and Dr. Yun Liu (2010). Dynamic Clusters in Lysozyme Protein Solution

Our Study

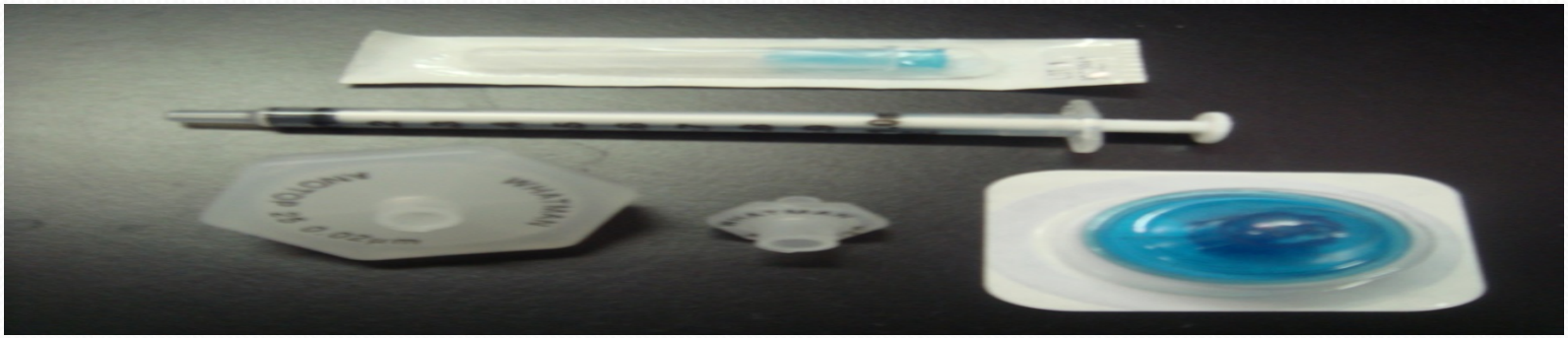
- Lysozyme Protein (extracted from chicken egg white)

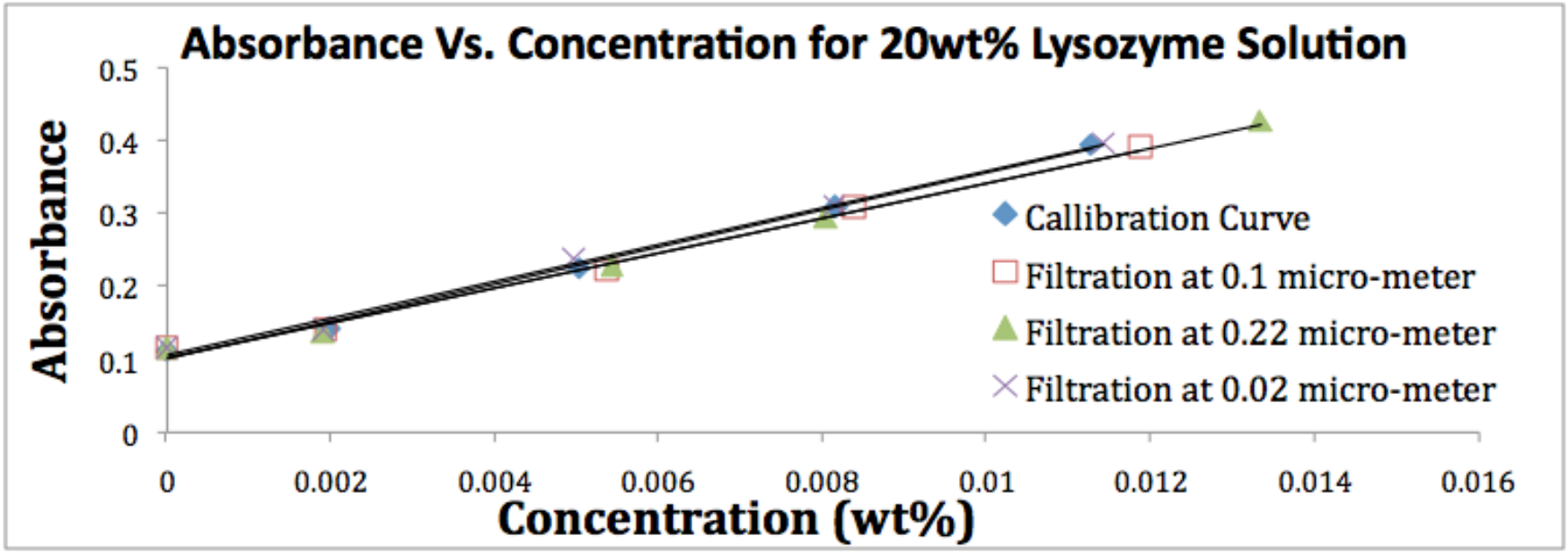


Lysozyme Protein

- Objectives
  - Filtering Effect
  - Effect on structure as a function of concentration of added NaCl salt
  - Bulk flow properties

UV-Spectrophotometry – Filtering Effect

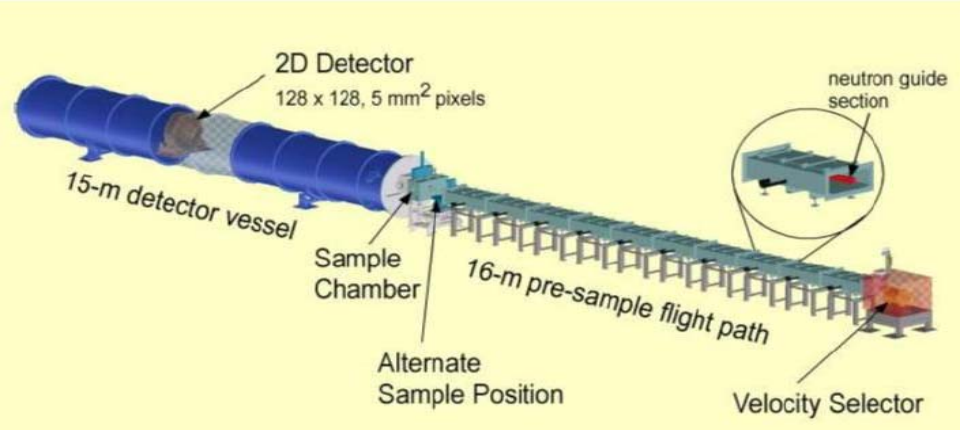


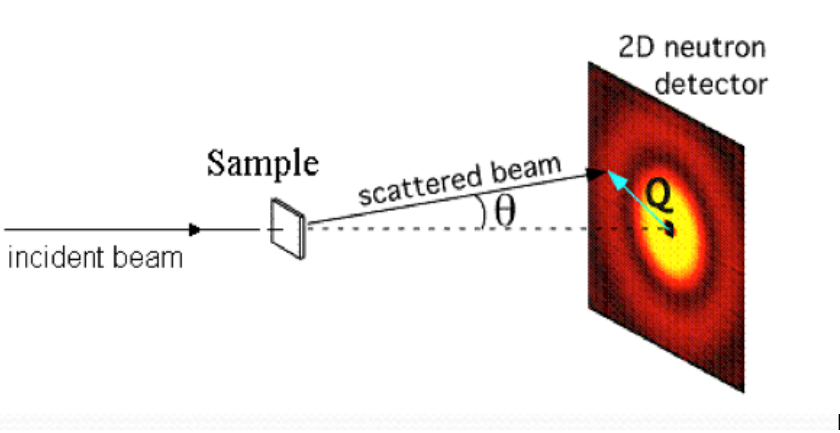


UV-Spectrophotometry Results – Filtering Effect

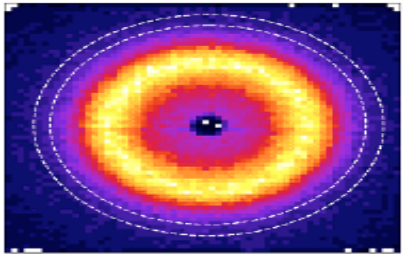
UV-Results for 20wt% Lysozyme Solution						
Filter Size (μm)	S_c	S_d	C	C*	C**	Relative Diff.
0.1	25.495	464.15	19.423	18.20553	1.2174695	0.062681846
0.22	25.495	465.9	19.423	18.27417	1.1488286	0.059147845
0.02	25.495	489.9	19.423	19.21553	0.2074675	0.01068154
UV-Results for 10wt% Lysozyme Solution						
Filter Size (μm)	S_c	S_d	C	C*	C**	Relative Diff.
0.1	25.411	246.07	9.95761	9.683602	0.2740084	0.027517488
0.22	26.88	263.9	10.0125	9.817708	0.1947917	0.019454848
0.02	25.411	248.63	9.95761	9.784345	0.1732646	0.017400223

SANS-Small Angle Neutron Scattering





2D image

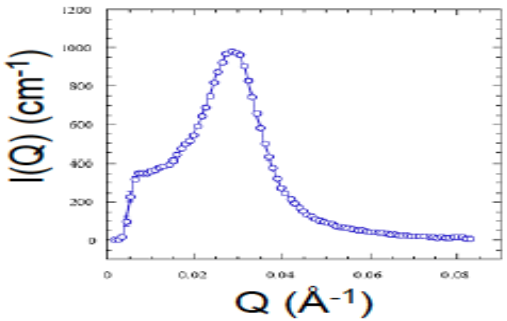


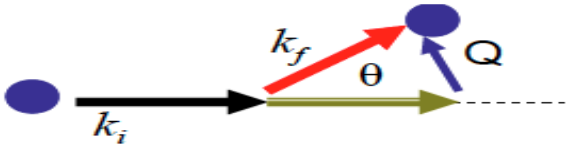
$I(Q_x,Q_y)$: intensity distribution

Annulus average

$$I(Q) = A \times P(Q) \times S(Q)$$

1D data

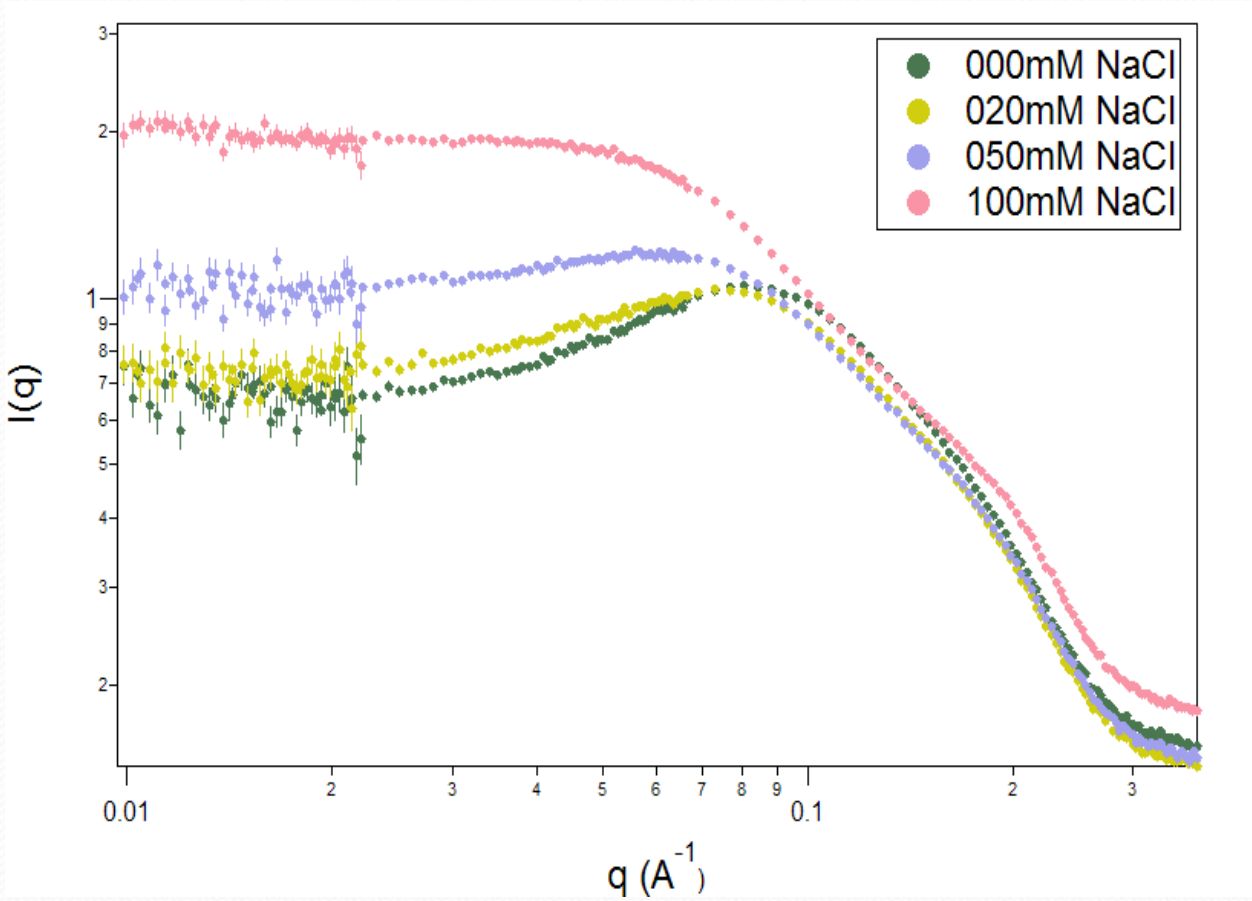


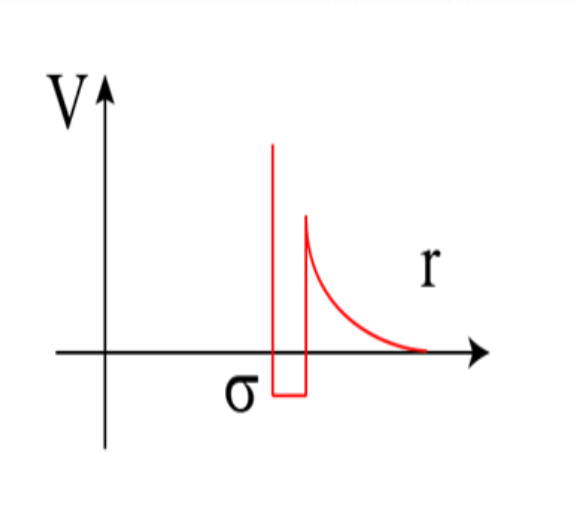


Source: Dr. Yun Liu, *Tutorial Session, ACNS, 2008, Santa Fe.*
Colloidal Interactions in Solution at NIST-NCNR

NG-7 SANS Results

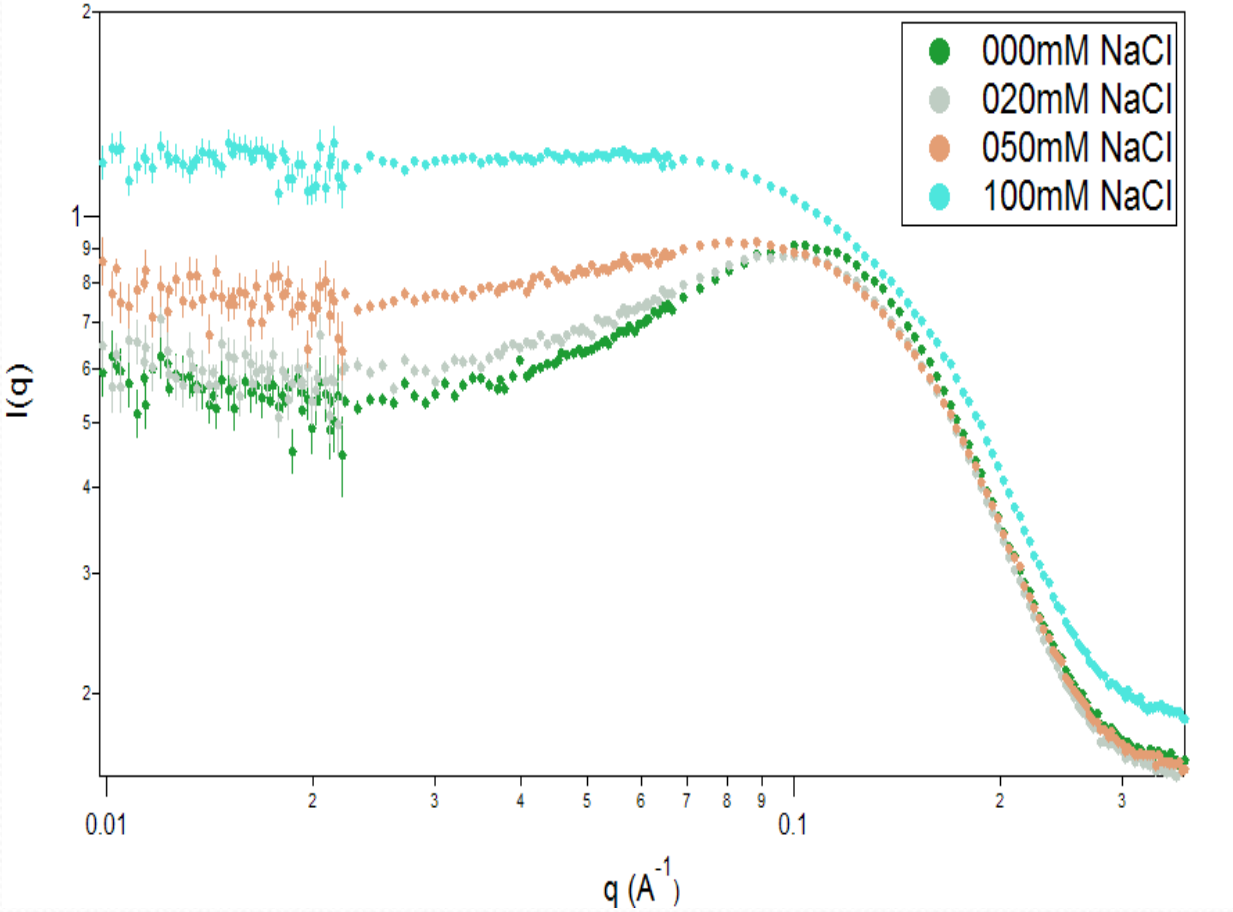
Effect of Concentration of added NaCl salt on Structure of 20wt% Lysozyme Protein Solution at T=10°C

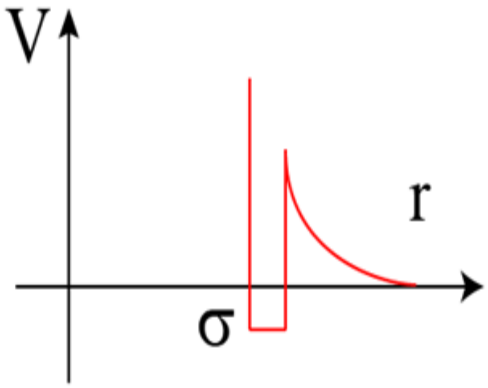




NG-7 SANS Results

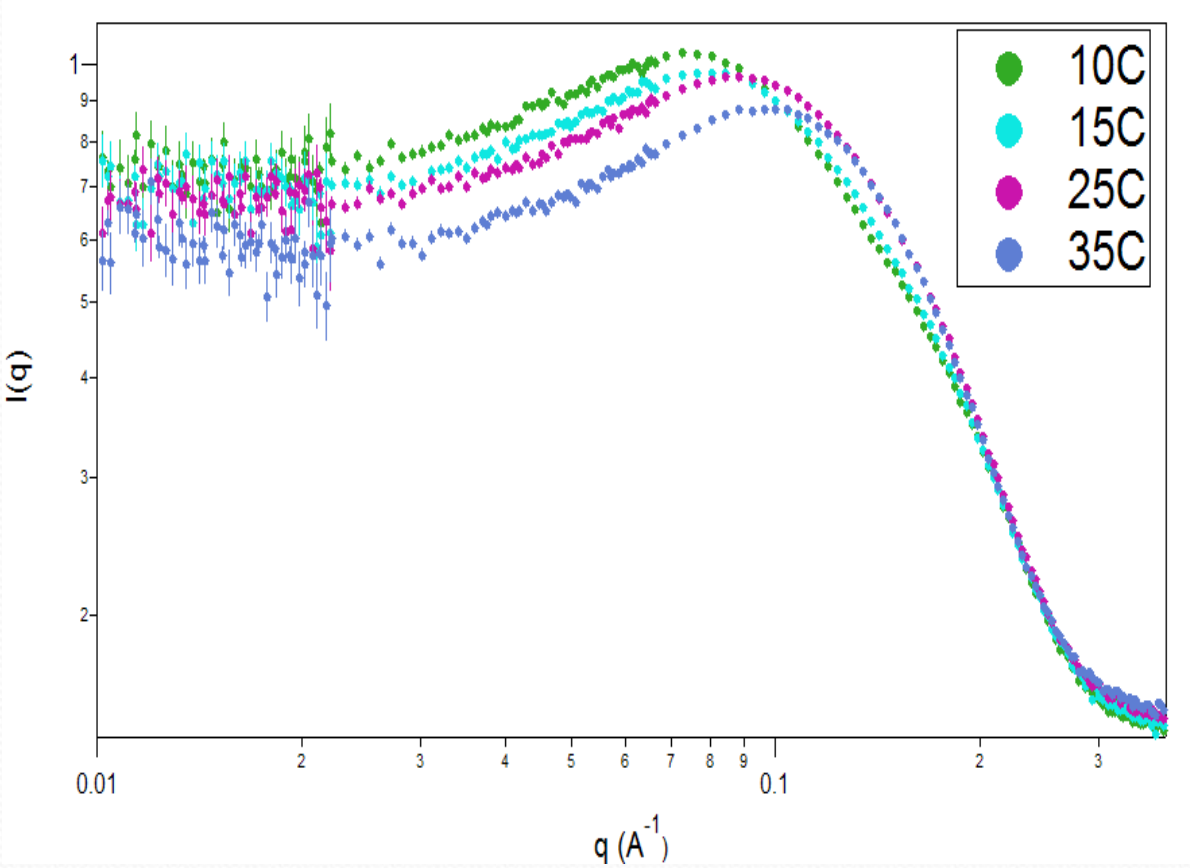
Effect of Concentration of added NaCl on Structure of 20wt% Lysozyme Protein Solution at T=35°C Cont'd

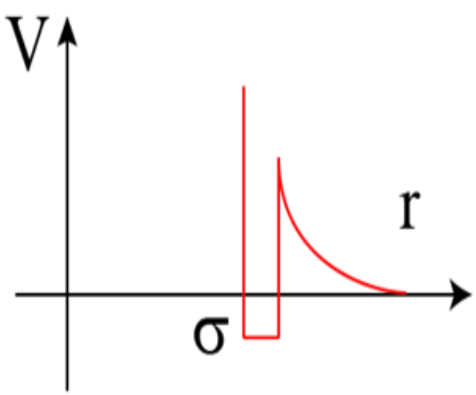




NG-7 SANS Results

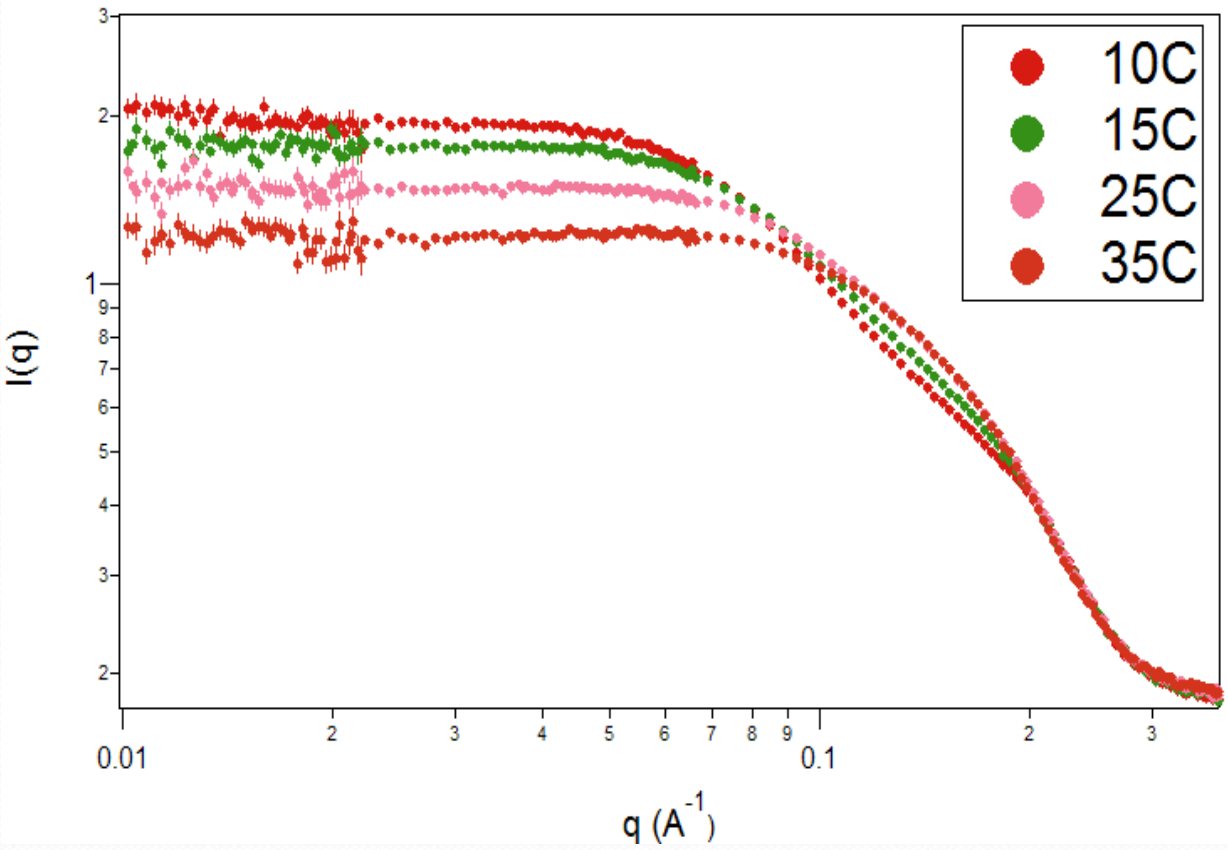
Effect of Temperature on Structure of 20wt% Lysozyme Protein Solution at 20mM NaCl

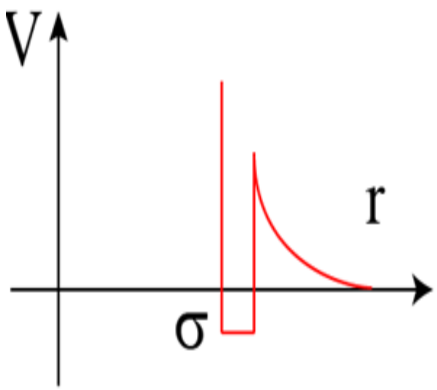




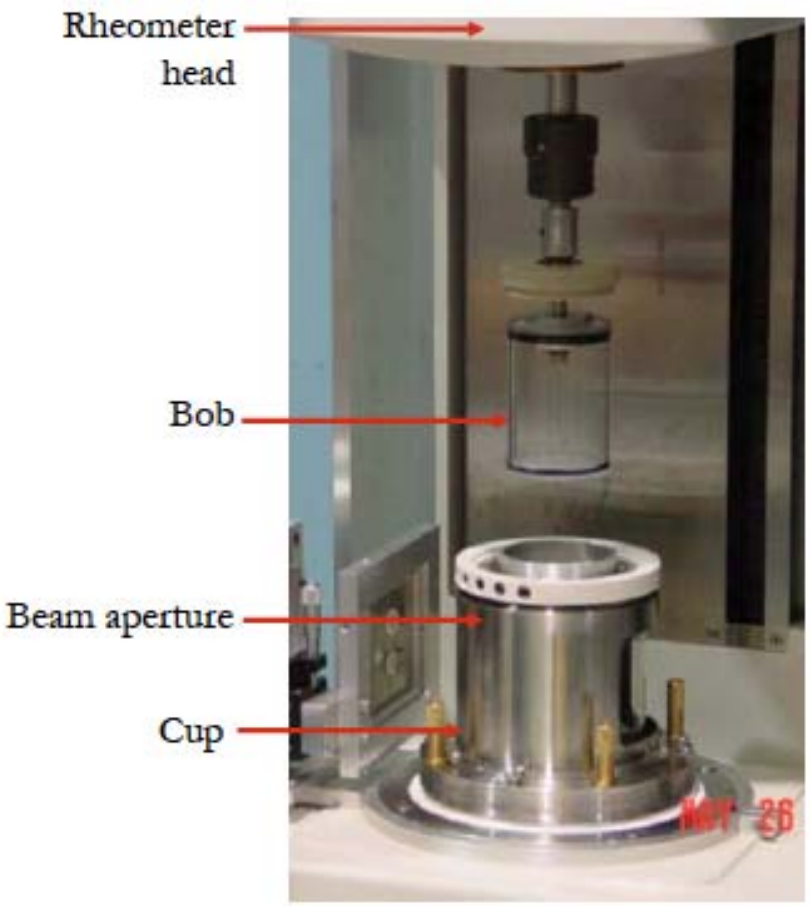
NG-7 SANS Results

Effect of Temperature on Structure of 20wt% Lysozyme Protein Solution at 100mM NaCl

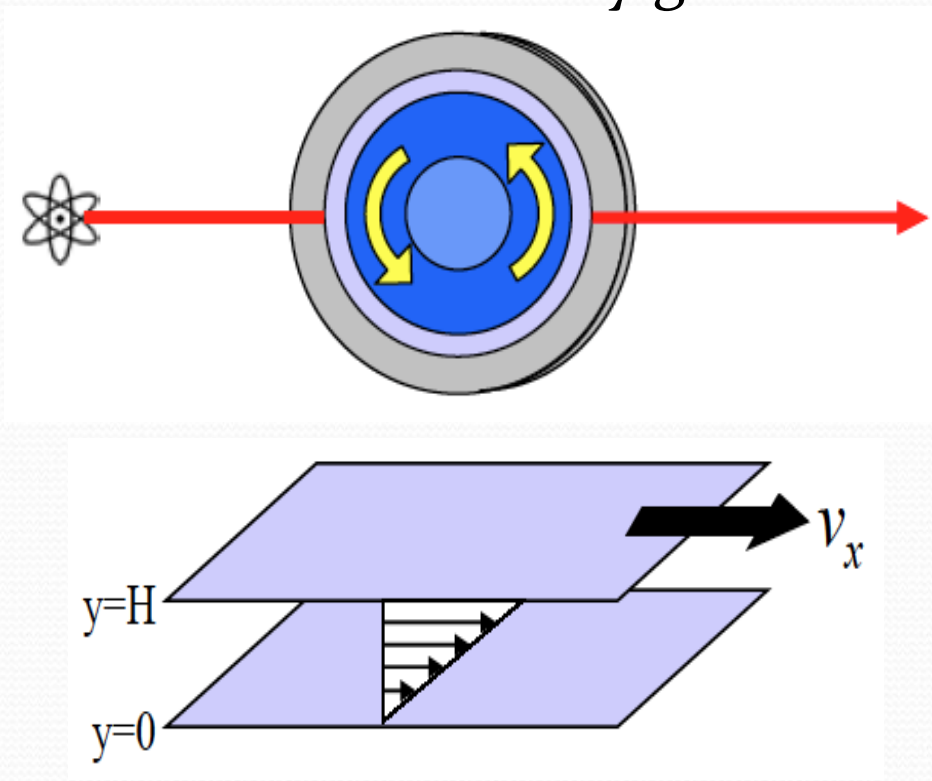




Physica Rheometer (RheoSANS) - Bulk Flow Properties



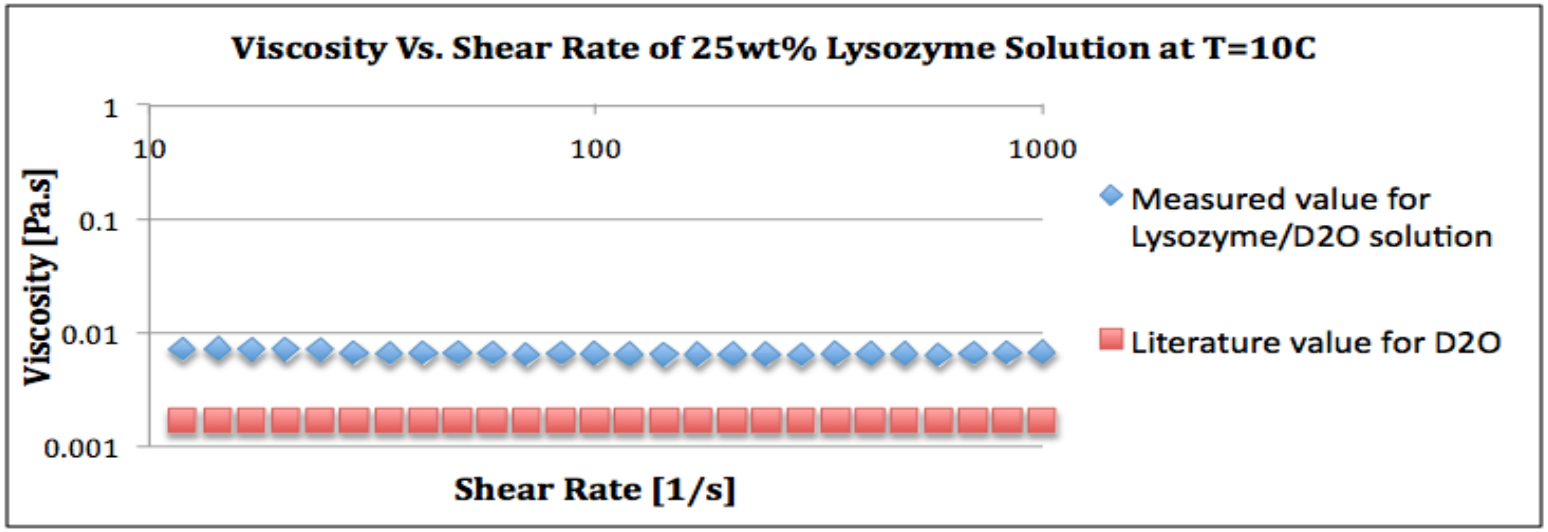
Shear Rate – Velocity gradient



Source: Matt Helgeson (2010), SANS Under Deformation and Flow: Principles and Measurements, MIT. Retrieved from http://www.ncnr.nist.gov/summerschool/ss10/pdf/Talk_Helgeson.pdf

NG-3 RheoSANS Results

Bulk Flow Properties



Viscosity of D_2O and D_2O/Lysozyme Solution at T=10°C

μ_{D_2O}	$\mu_{D_2O/Lysozyme}$	$\mu_{Relative}$
0.00167	0.0077385	4.63

Modified Einstein's Equation

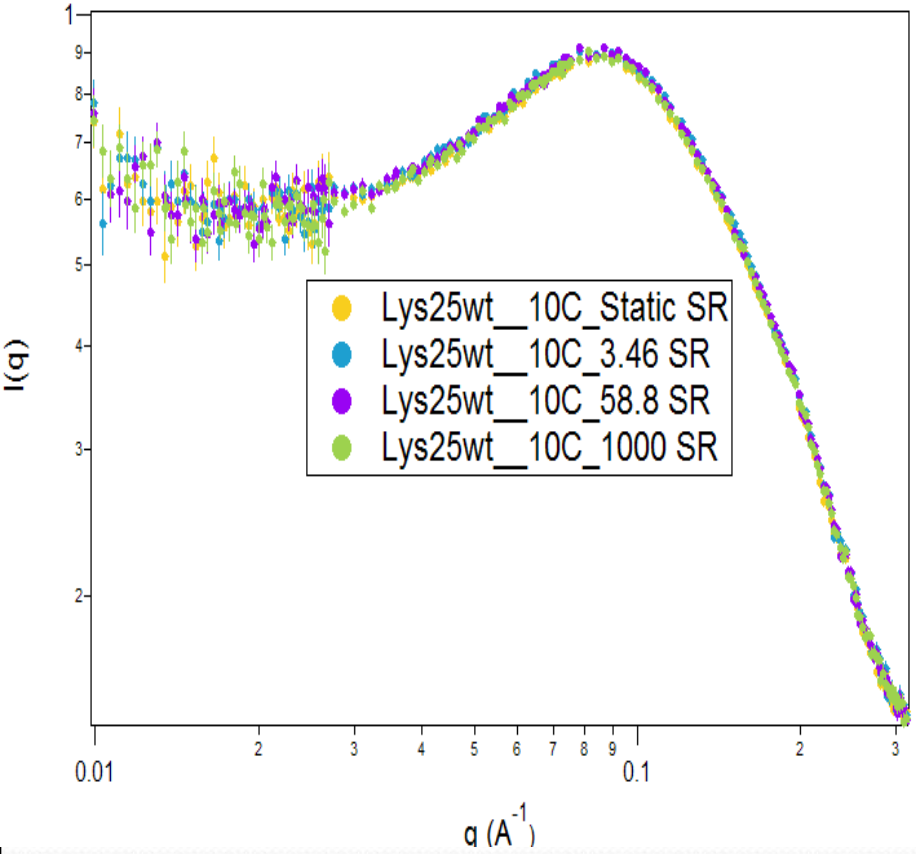
$$\mu_{Relative} = 1 + 2.5\phi + 5.912\phi^2$$

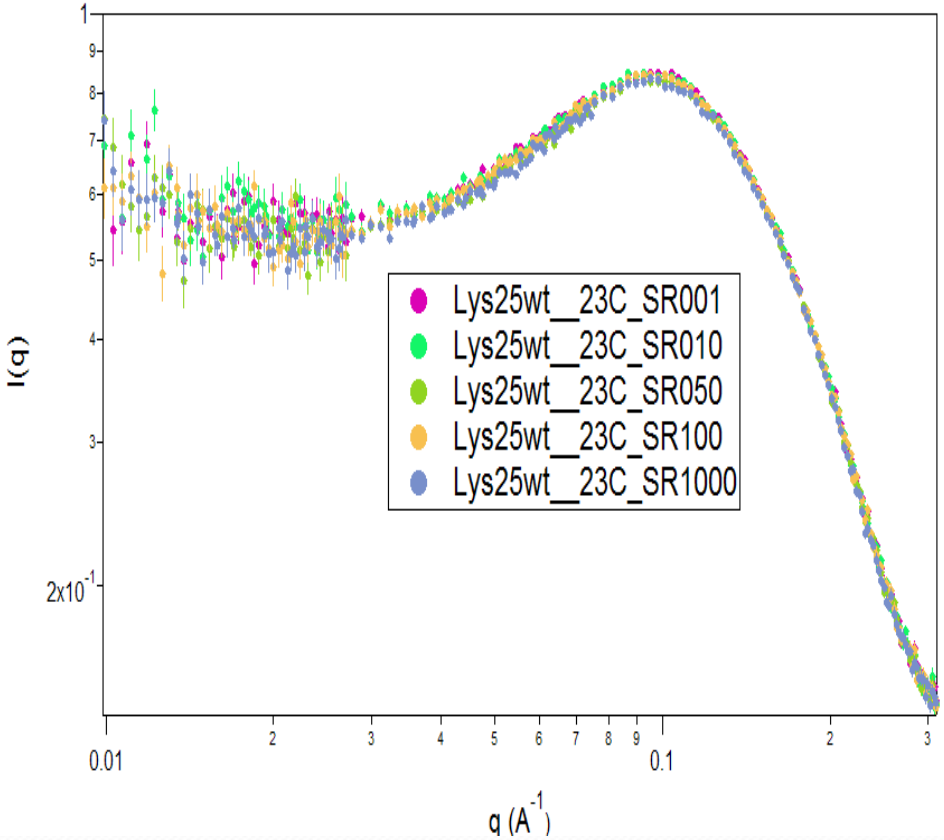
$$\mu_{Relative} = 1.73$$

Source: Frank J. Millero et al., Density and Viscosity of Deuterium Oxide Solutions from *5-70°C*.

NG-3 RheoSANS Results

Effect of Shear Rate on 25wt% Lyso at T=10°C and T=23°C





Conclusion

- No dramatic change in concentration of lysozyme solution (Filtration)

- Inter-protein structure does depends on temperature and concentration of NaCl salt
  - Temperature –Attraction reduced (less intensity)
  - Concentration of NaCl salt – Repulsion reduced (enhanced intensity)

- Viscosity of 25wt% lysozyme solution is independent of shear rate at T=10°C and omM NaCl salt (Newtonian Fluid)

- Structure of 25wt% Lysozyme is independent of shear rate

Future Plans

- Filtering effect
  - Different type of salts
  - Type of buffer

- Rheology
  - Try different conditions where lysozyme solution is Non-Newtonian Fluid

- SANS
  - Quantitative Analysis of SANS Curve to understand the inter-protein potential

Acknowledgements & Questions

- Dr. Yun Liu
- Dr. Aaron Eberle
- Dr. Paul Butler
- Dr. Yuying Gosser
- Dr. Julie Borchers
- NCNR Staff
- NIST SURF Organizers

Any questions?

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

CHRNS NIST