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Wide angle X-ray scattering from protein solutions: a new frontier

NSLS-II User Workshop

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U.S. Department
of Energy

UChicago ►
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WHY WAXS?

- Complimentary to NMR and crystallography
 - In native aqueous environment
 - Large scale structural fluctuations
 - Slow motions
- WAXS can be used to characterize conformational changes
 - *larger than those that can be accommodated within a crystal lattice*
 - *in response to ligand binding*
 - *in response to changes in environment*
 - denaturants
 - temperature
 - solvents...
- X-ray solution scattering from proteins may be able to:
 - provide secondary structure information
 - group proteins into fold families
 - distinguish between calculated models of proteins and complexes

Technical Challenges... and our approach

- **Solution scattering from proteins at high q is very weak**
 - Synchrotron sources provide intense, well collimated beams that can be focused without providing excess convergence
- **Presence of strong scattering peak from water and quartz capillary**
 - Small signal on high background
 - Beam positional stability must be extremely good
- **Most proteins are available only in small quantities**
 - Small sample volumes are required
- **Radiation damage may be a problem**
 - Flow cells can minimize x-ray exposure to any one protein
- **Extract information contained in the diffraction patterns**
 - Availability of software to calculate predicted WAXS patterns from crystallographic coordinates (CRYSOL)

Solution Scattering

– a weighted mapping of interatomic vector lengths

- Solution scattering as a function of scattering vector can be expressed in terms of interatomic vectors, r_{ij} , (Debye Formula):

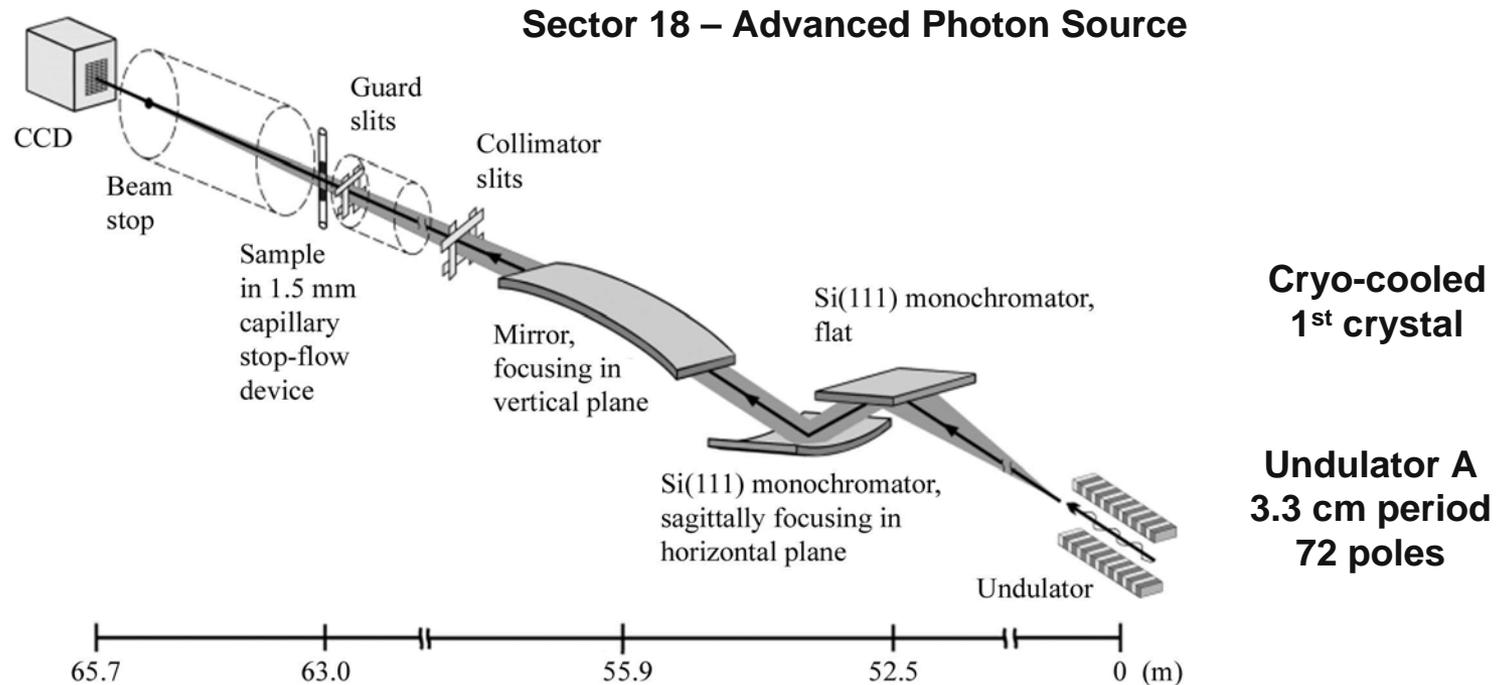
- $$I(q) = \sum I_i(q) + 2 \sum \sum F_i(q) F_j(q) (\sin(qr_{ij})/(qr_{ij}))$$

- SAXS – small q - large r_{ij} – provides information about overall size and shape of the protein
- WAXS – larger q – smaller r_{ij} - provides information about more detailed structure of the protein
- Secondary structures (periodic, repeating features) have characteristic patterns of inter-atomic vector lengths that might be discernable from the solution scattering patterns
- Tertiary structures are well defined arrangements of secondary structures that may also have characteristic patterns
- Protein fold is simply an arrangement of tertiary structures

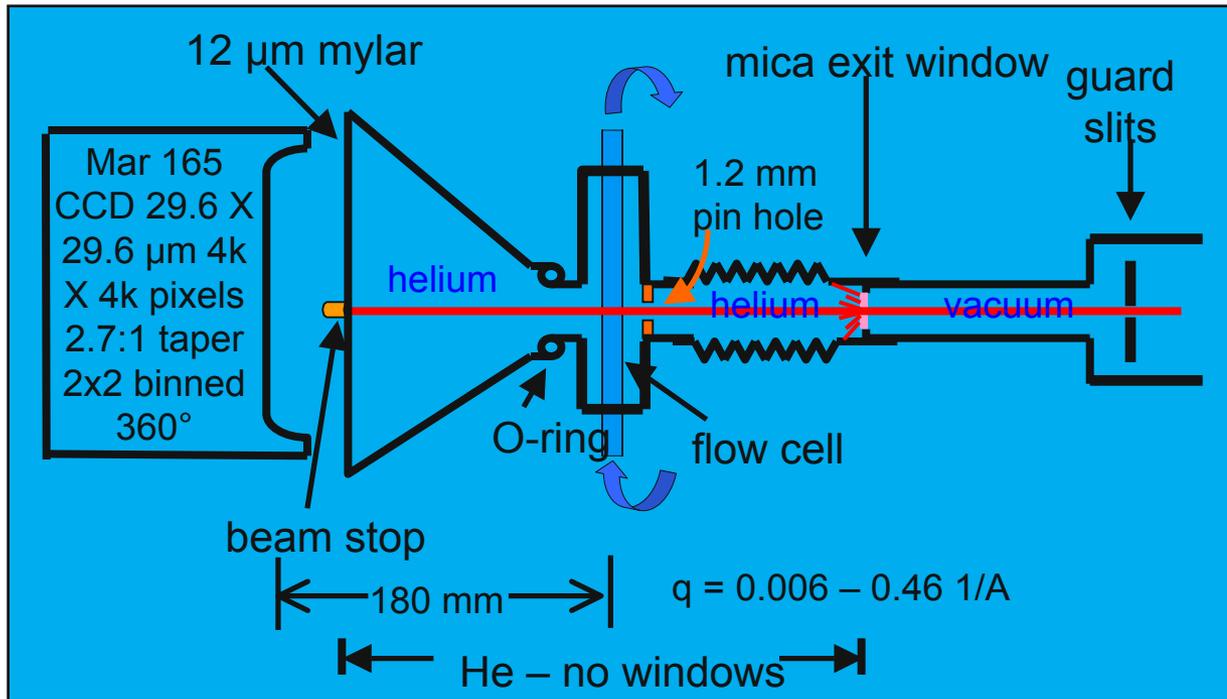
Beamline Configuration – BioCAT

Typical SAXS/WAXS Setup

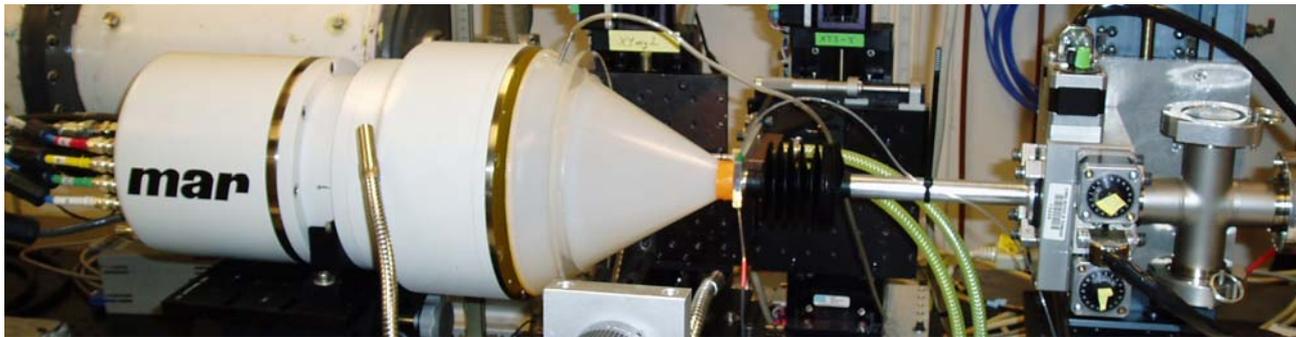
Final beam size: $35\ \mu\text{m} \times 135\ \mu\text{m}$



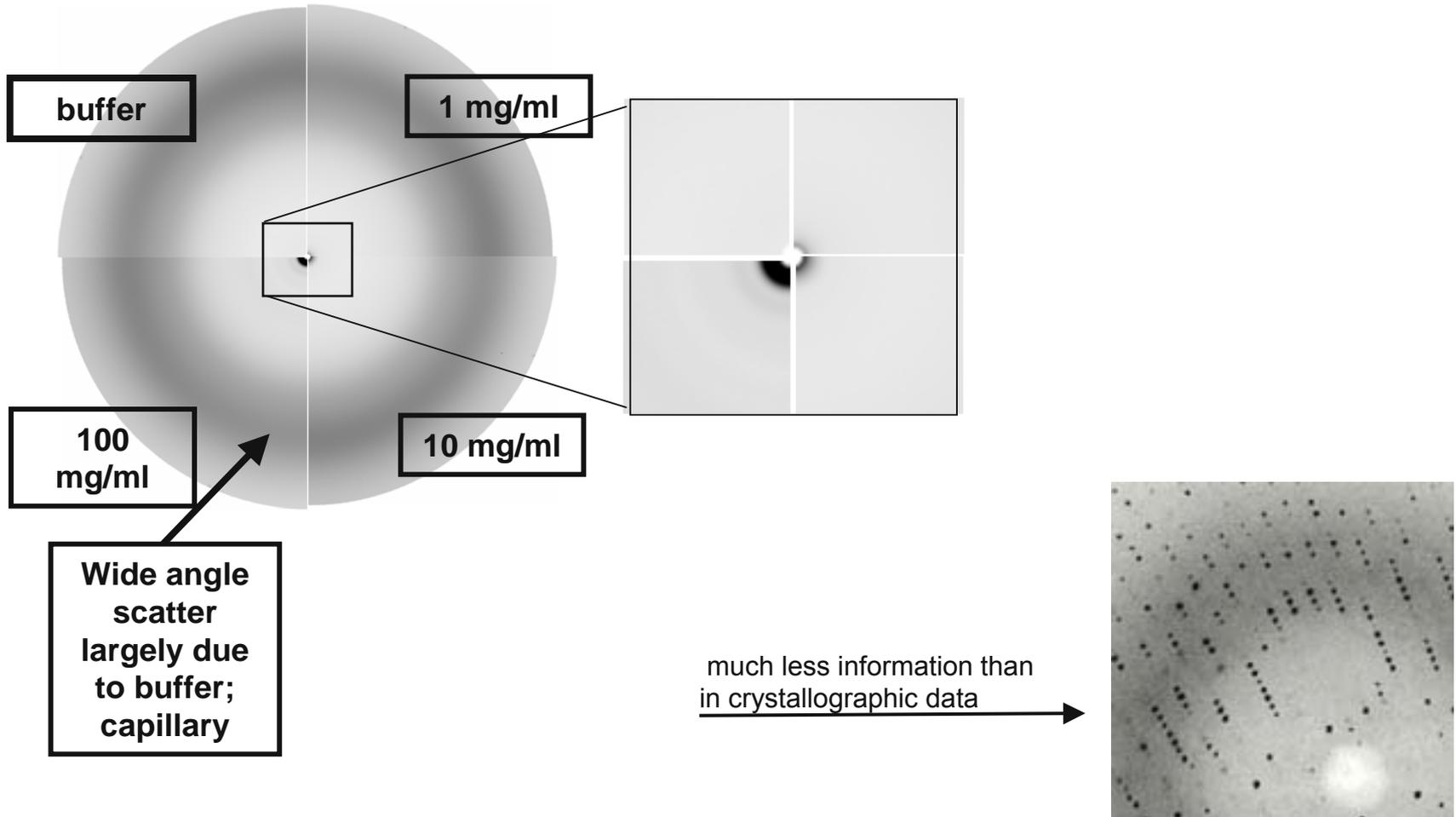
Schematic of Wide Angle Scattering Setup



Fischetti, R. F., Rodi, D. J., Gore, D. B., and Makowski L., Wide angle x-ray solution scattering as a probe of ligand induced conformational changes in proteins. *Chemistry and Biology* (2004) 11: 1-20.

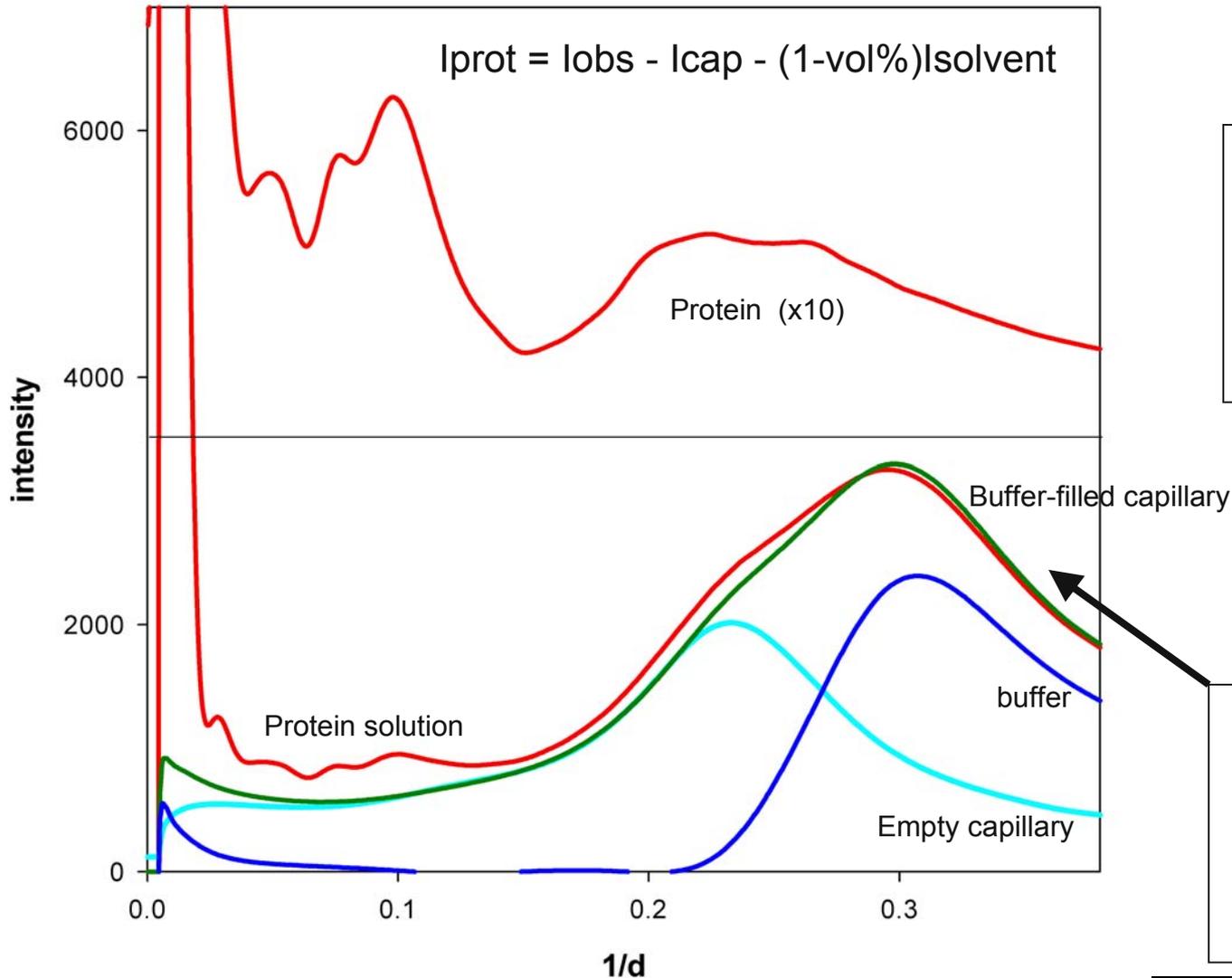


WAXS data is very weak compared to SAXS or crystal diffraction



WAXS from Hb – 150 mg/ml

New data to similar quality at 10 mg/ml



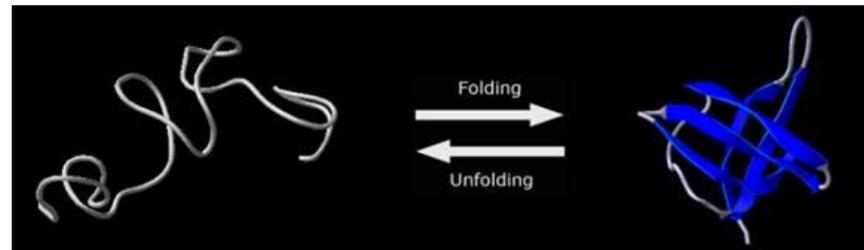
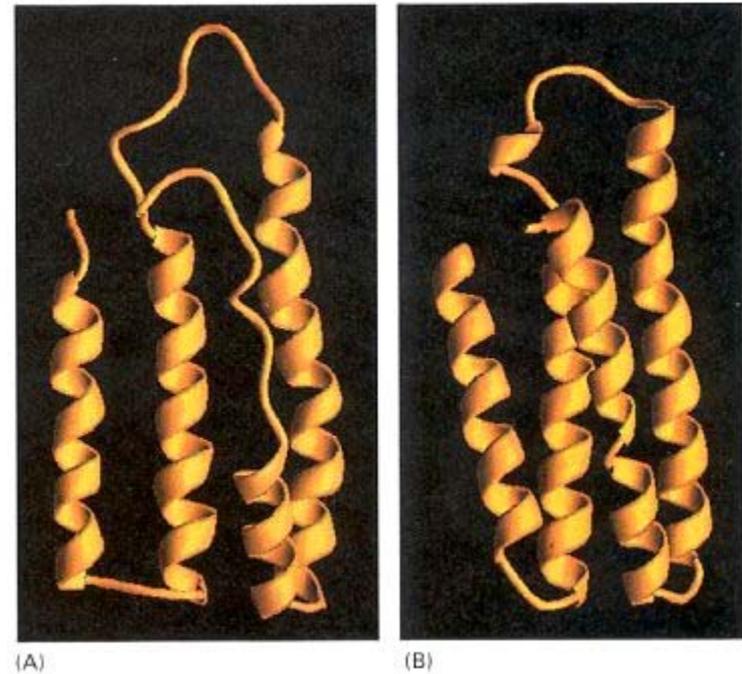
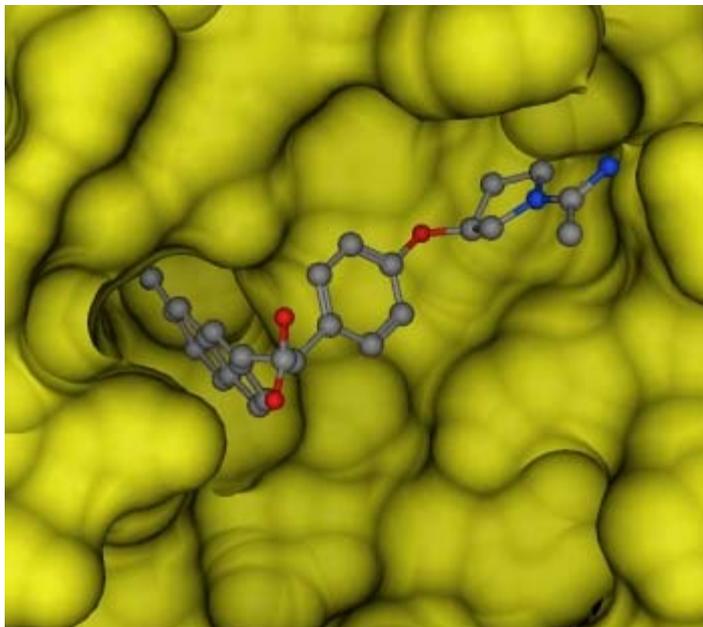
Each data set is composed of scattering from

- (i) Empty capillary
- (ii) Buffer-filled capillary
- (iii) Protein solution-filled capillary

At wide-angles, buffer scatters x-rays more strongly than the protein displacing it in the protein solution !

Characterizing Structural Changes in Proteins in Solution

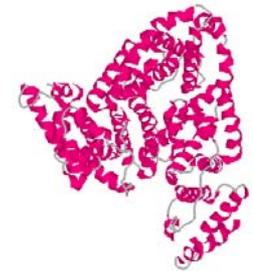
- Denaturants
- Ligand binding
- Molten globules
- Temperature
- Protein concentration



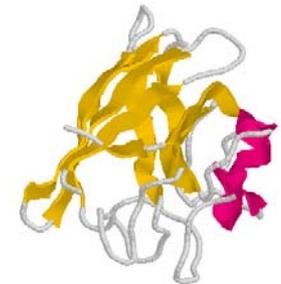
Assessment of α -helix content by WAXS



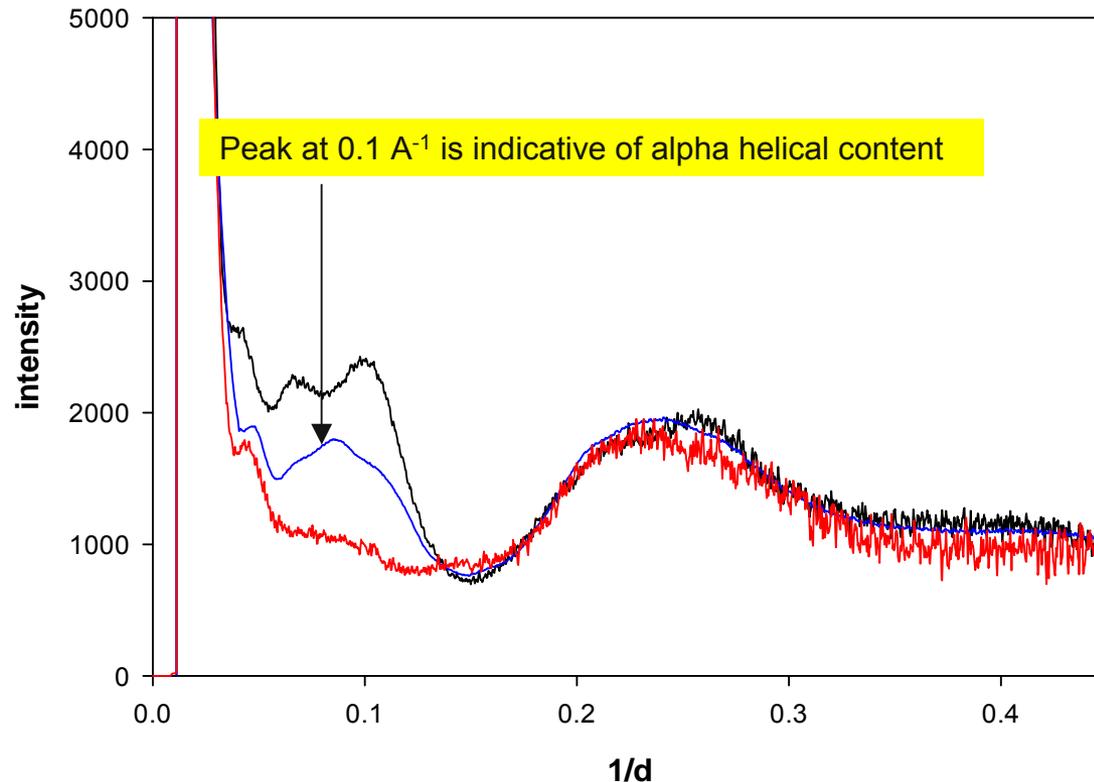
myoglobin
88% α -helical



serum albumin
78% α -helical



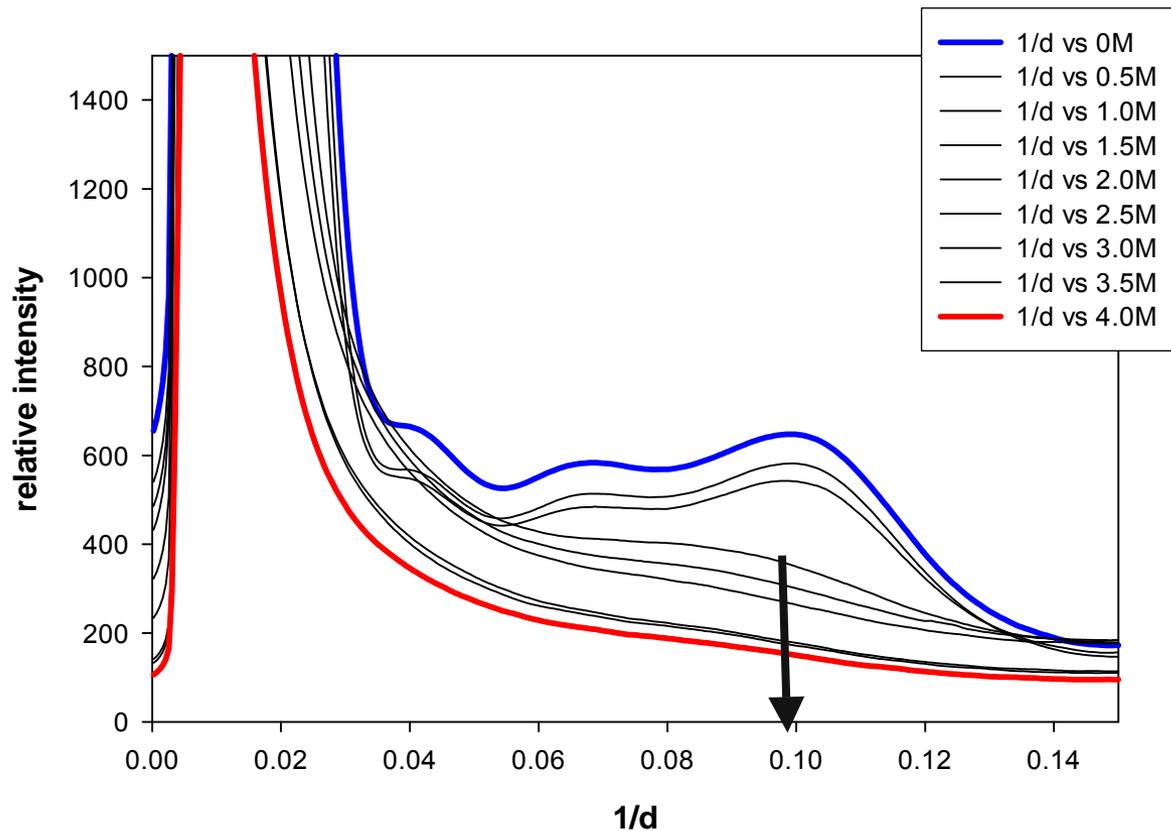
superoxide dismutase
17% α -helical



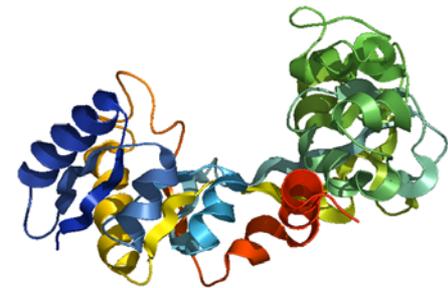
The intensity at a spacing of $(1/d) = 0.1 \text{ \AA}^{-1}$ in computed diffraction patterns from 498 proteins of known structure has a correlation coefficient of **0.32** with the α -helical content of these proteins.

Features in the pattern fade when protein structure degrades

The effect of GuHCl on Mb

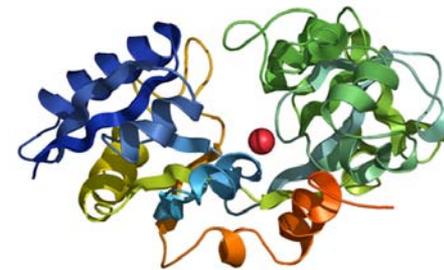


Apo- and Ligand-bound Transferrin



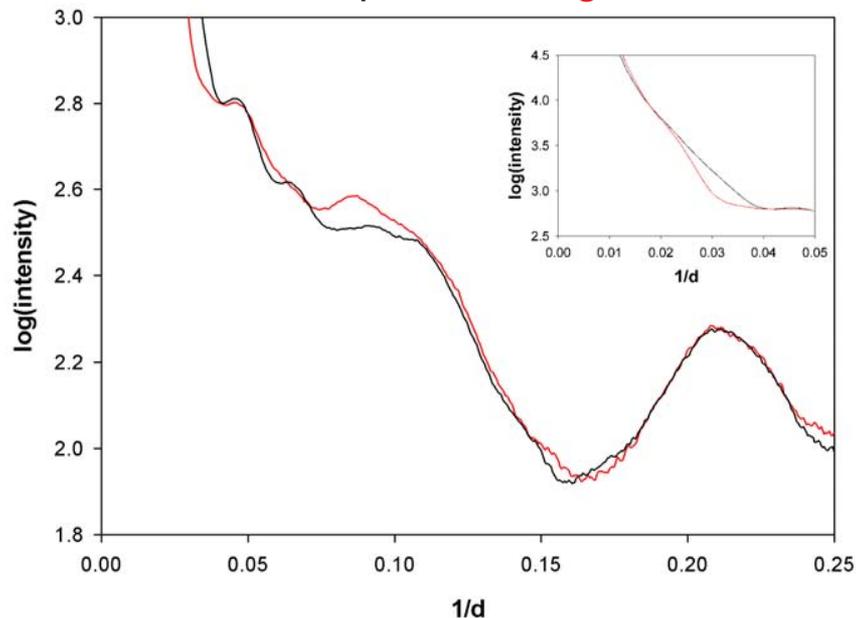
Apo

N-terminal lobe of transferrin in the presence and absence of iron. Conversion from the 'open' apo form to the 'closed' ligand-bound form occurs by bending around a hinge between the two domains in a 'Venus flytrap'-like motion. The N-terminal half undergoes a **63°** rotation of the N2 domain relative to the N1 domain in response to binding .

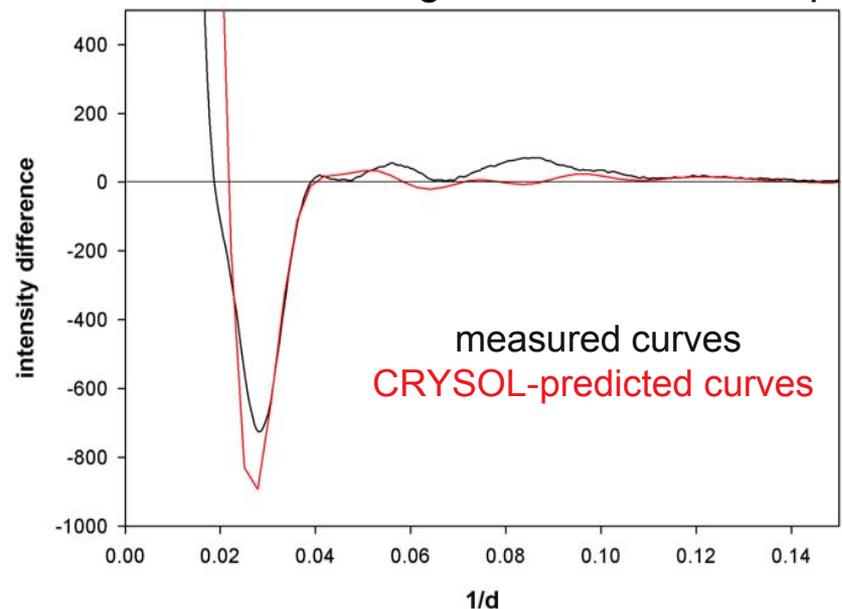


Fe-bound

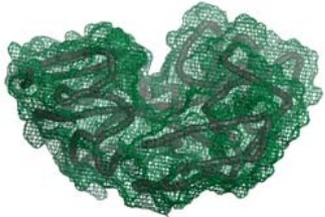
WAXS data apo versus **ligand-bound**



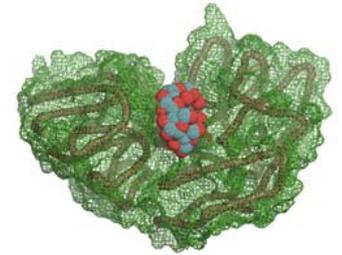
Difference WAXS - ligand-bound minus apo



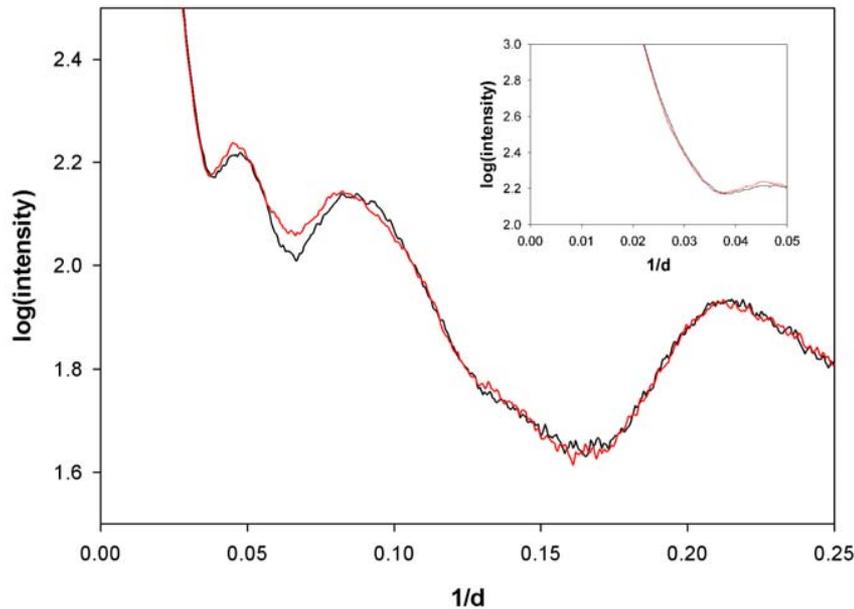
Maltose Binding Protein and Dextrose



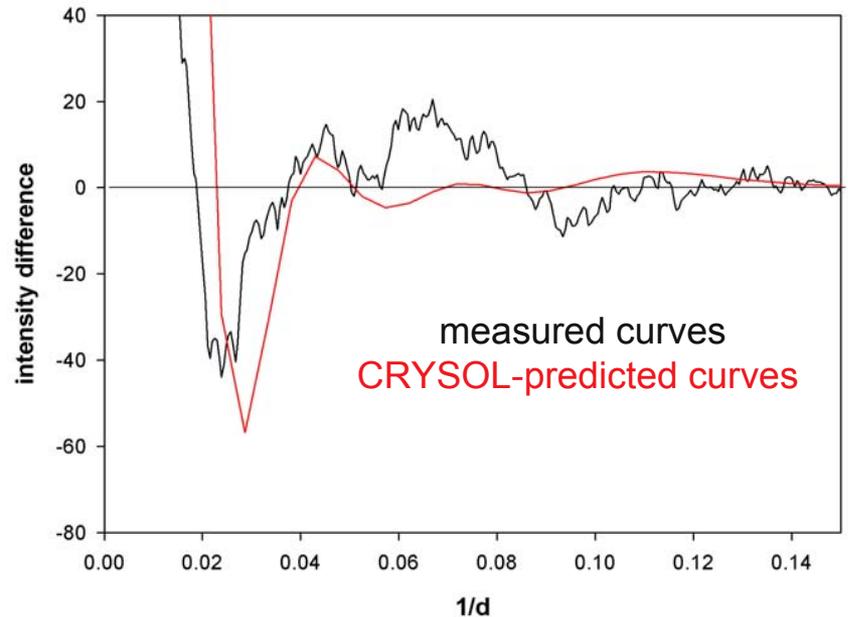
The conformational change between the sugar-bound and apo forms of MBP involves a 35° hinge bending movement accompanied by an 8° anti-clockwise rotational twisting of the smaller N-domain relative to the C-domain, similar to the flytrap movement of transferrin.



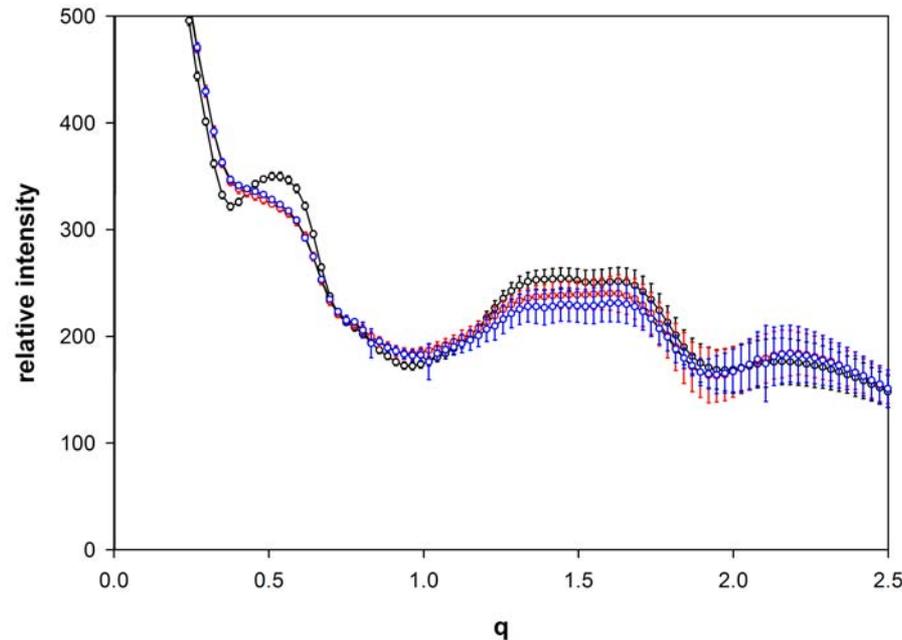
WAXS data apo versus **ligand-bound**



Difference WAXS - ligand-bound minus apo



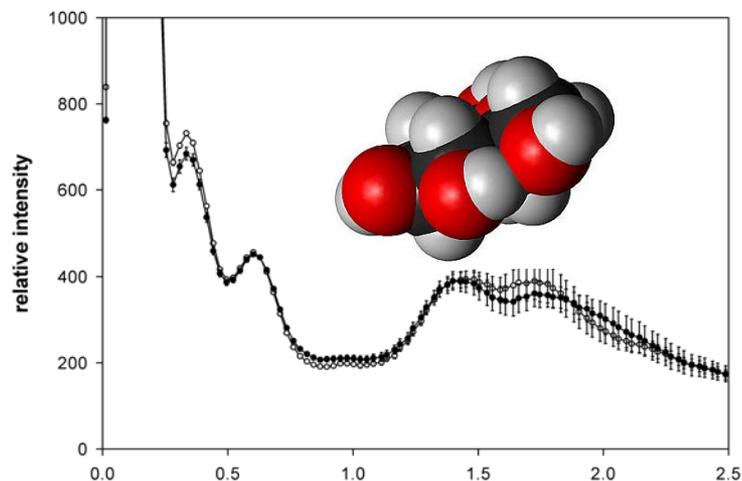
Riboflavin Kinase (RFK,) is an essential enzyme which has been demonstrated to bind its two small molecule ligands at adjacent sites on the surface of the molecule



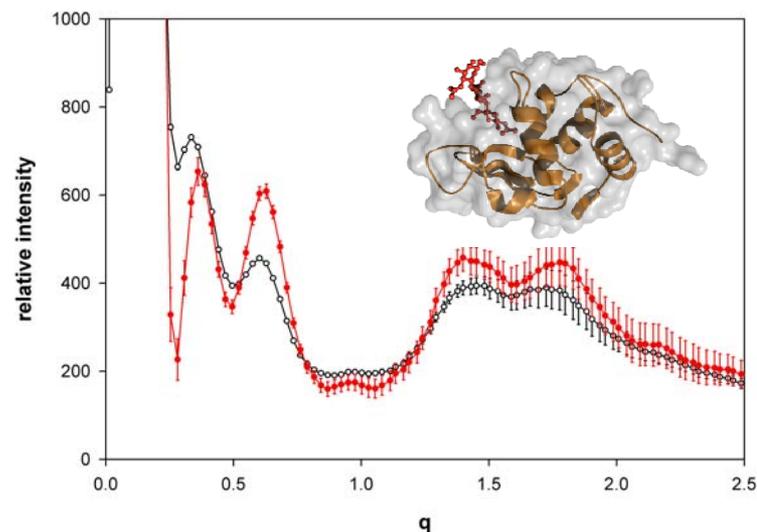
- Each ligand (riboflavin and ATP) modulates the protein in such a manner as to shift a surface flap to a new position. Not only does the addition of each ligand produce a statistically significant change in the scattering profile (reduced chi square, $\chi_{\nu} = 2.94$ for ATP and 2.90 for riboflavin respectively vs. apo RFK normalized for error), but the profiles for ATP and riboflavin are virtually indistinguishable ($\chi_{\nu} = 0.03$ between the two ligand-bound forms).

Ligand binding induced structural changes - lysozyme

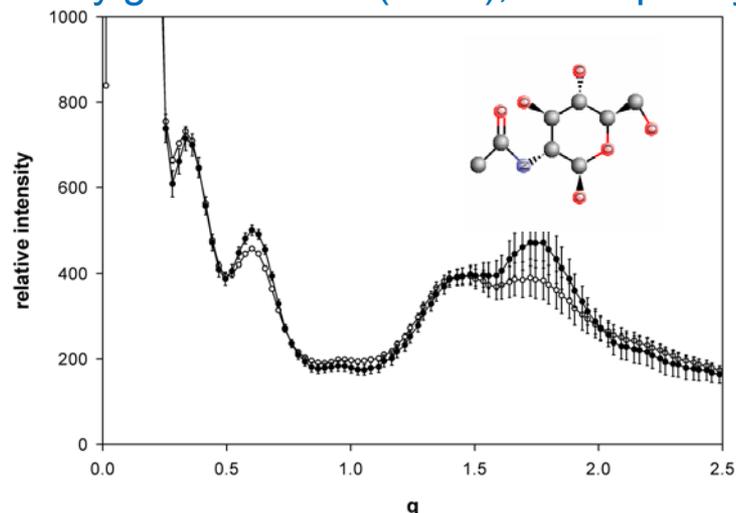
Dextrose – too short to bind



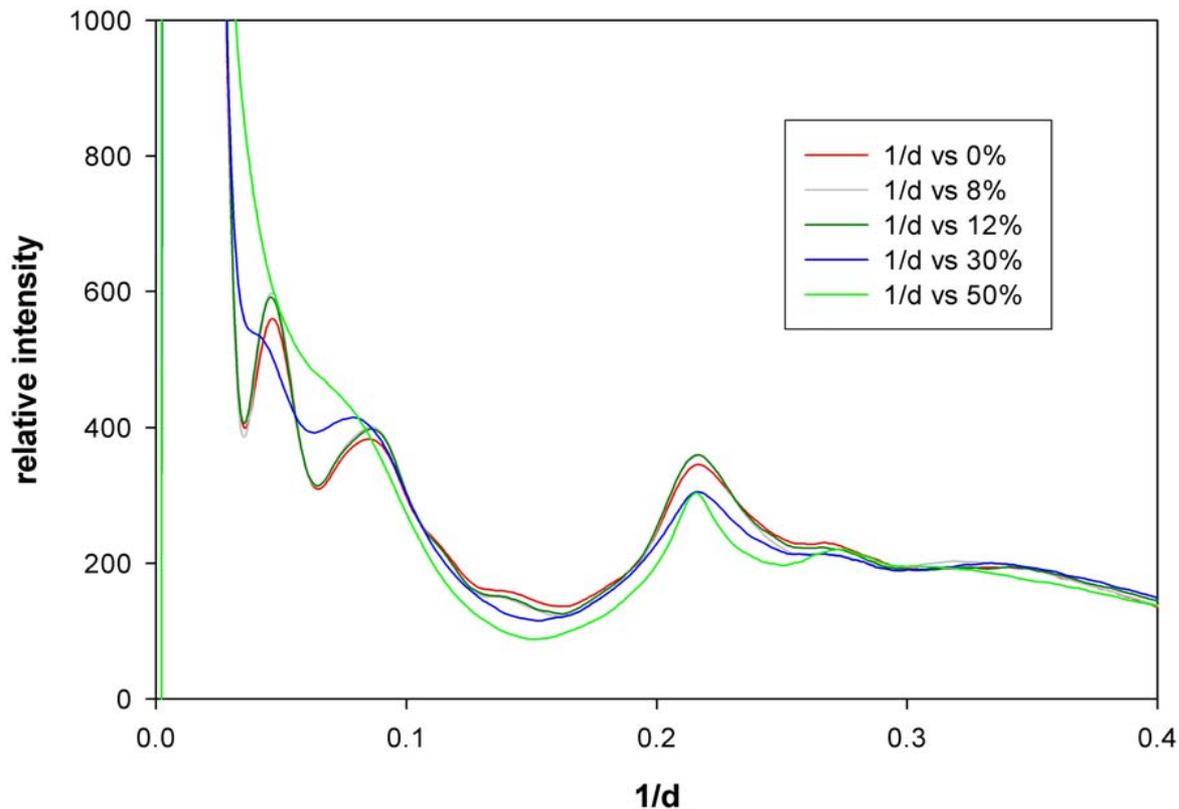
(NAG)₃ , a potent enzyme inhibitor



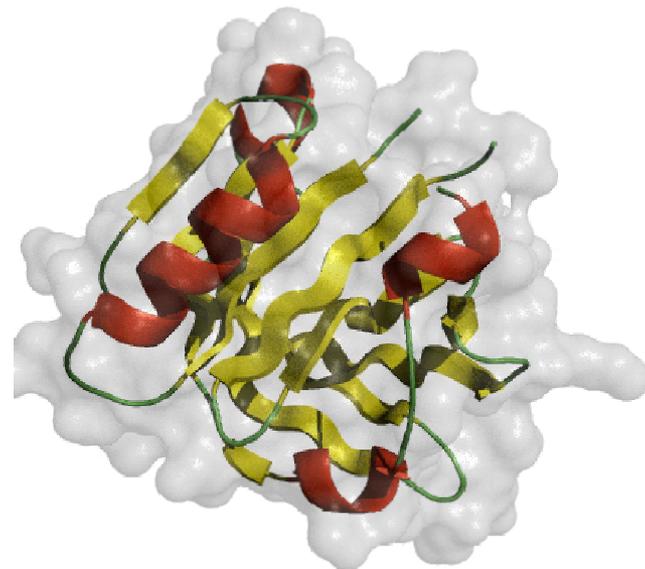
N-acetylglucosamine (NAG), binds poorly



Molten globules of β -lactoglobuli



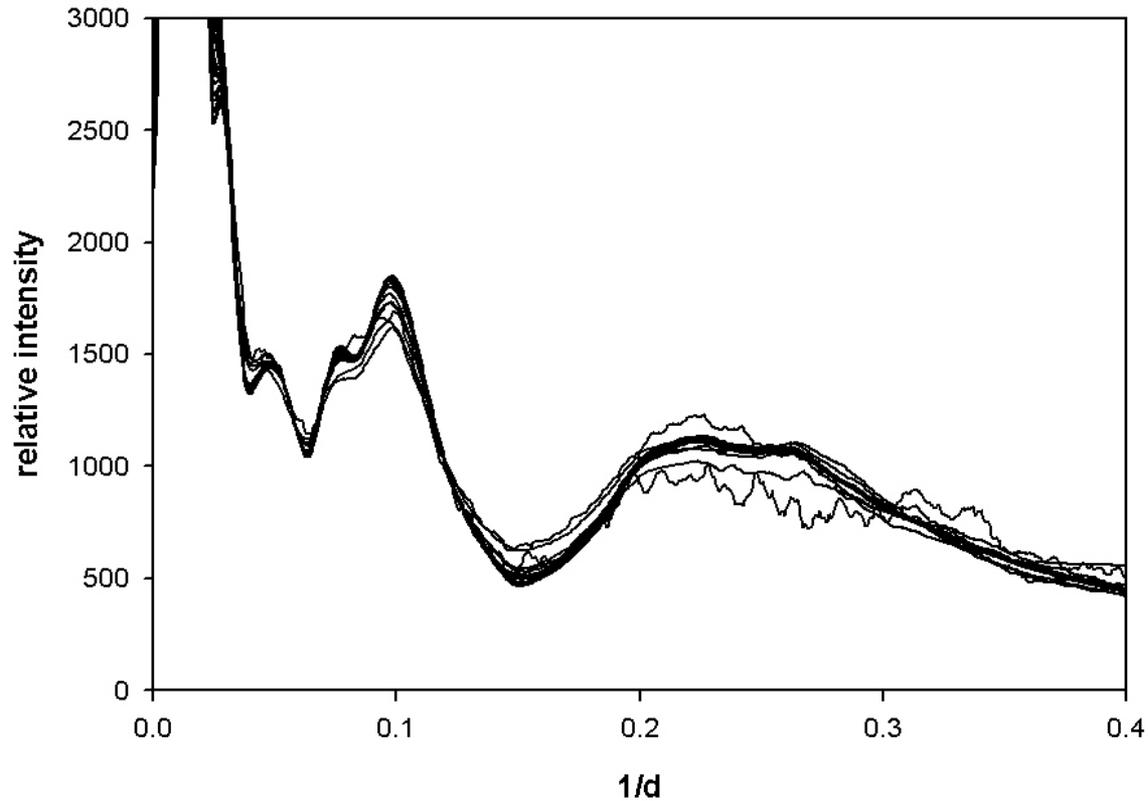
Relatively small changes are observed in intensities at high angles



Forms molten globules at EtOH concentrations of 25-40%

0-12% native dimer
20% native monomer

Concentration series of Hb



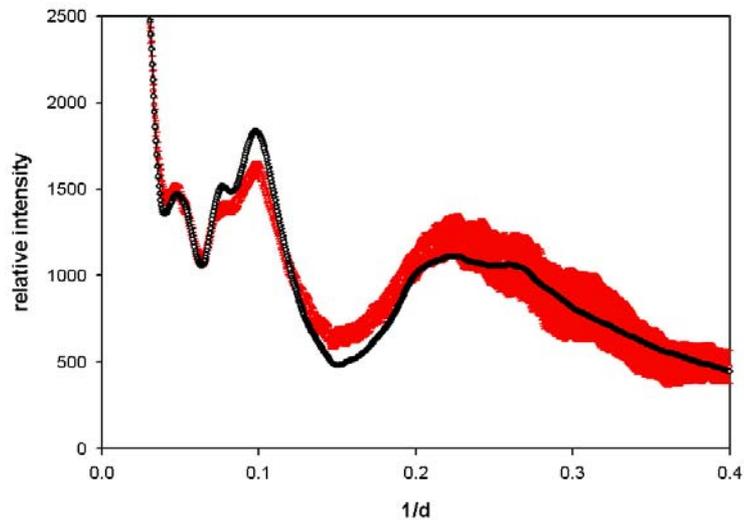
Good data collectable at concentrations down to about 5 mg/ml

20 patterns scaled for concentration and superimposed -
300mg/ml to 2 mg/ml

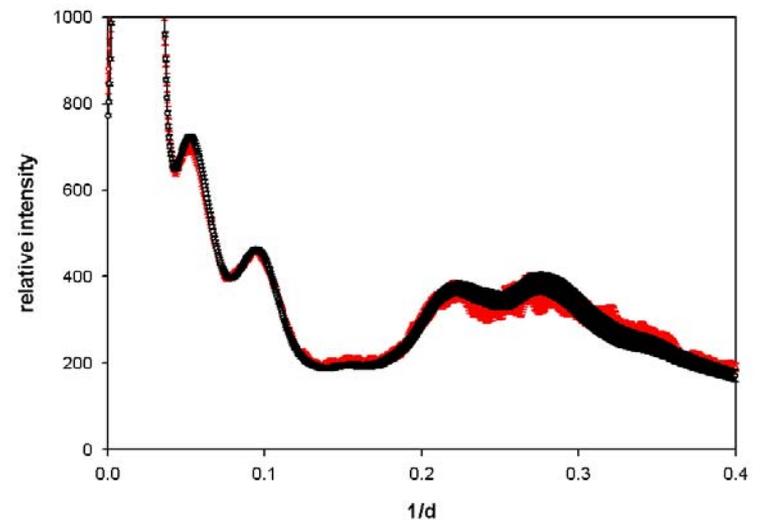
Hb and Lysozyme

49 mg/ml (black) vs 4.1 mg/ml (red)

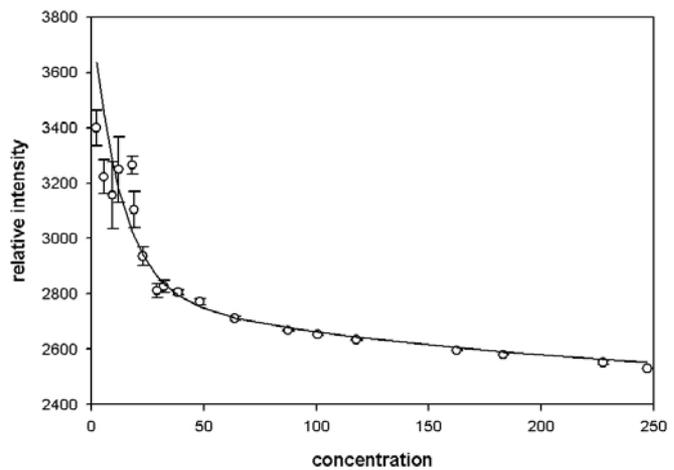
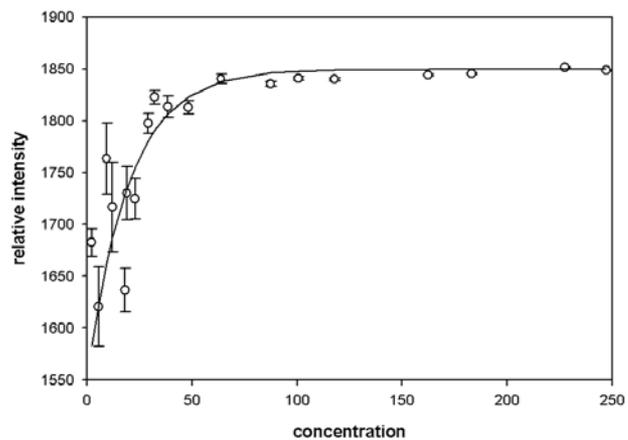
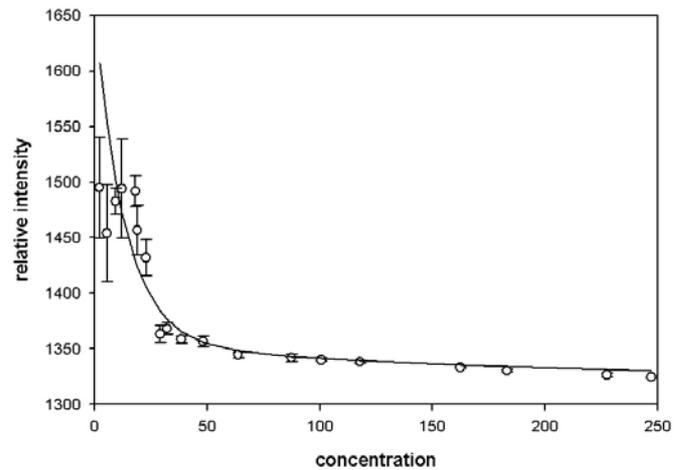
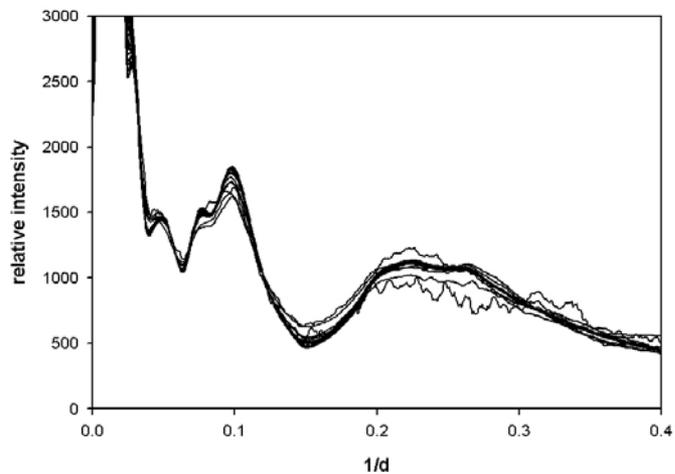
Differences are statistically significant at least to 0.2 \AA^{-1}



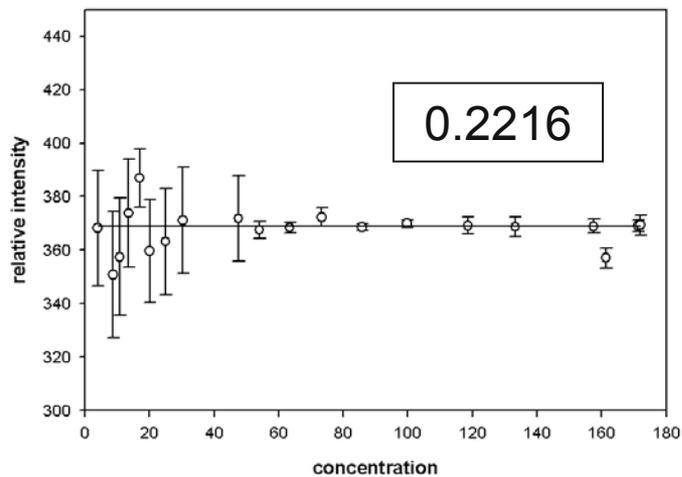
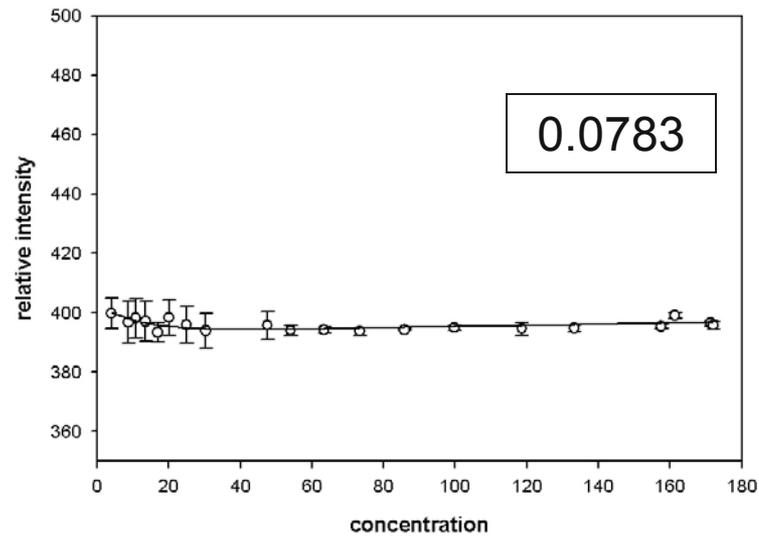
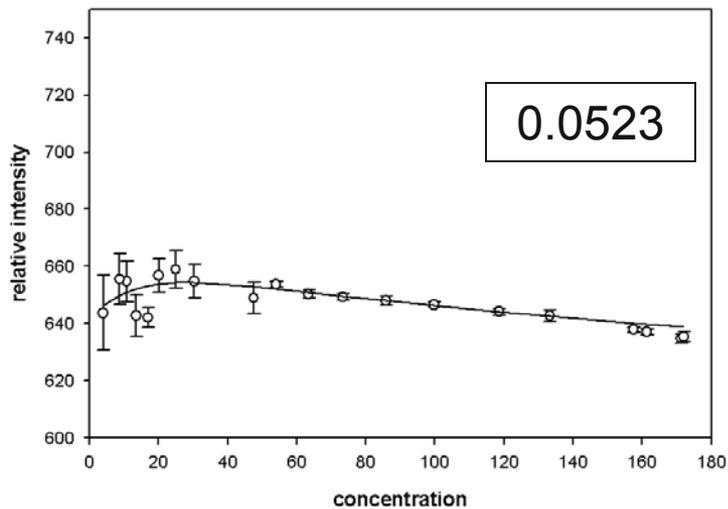
Differences are not statistically significant



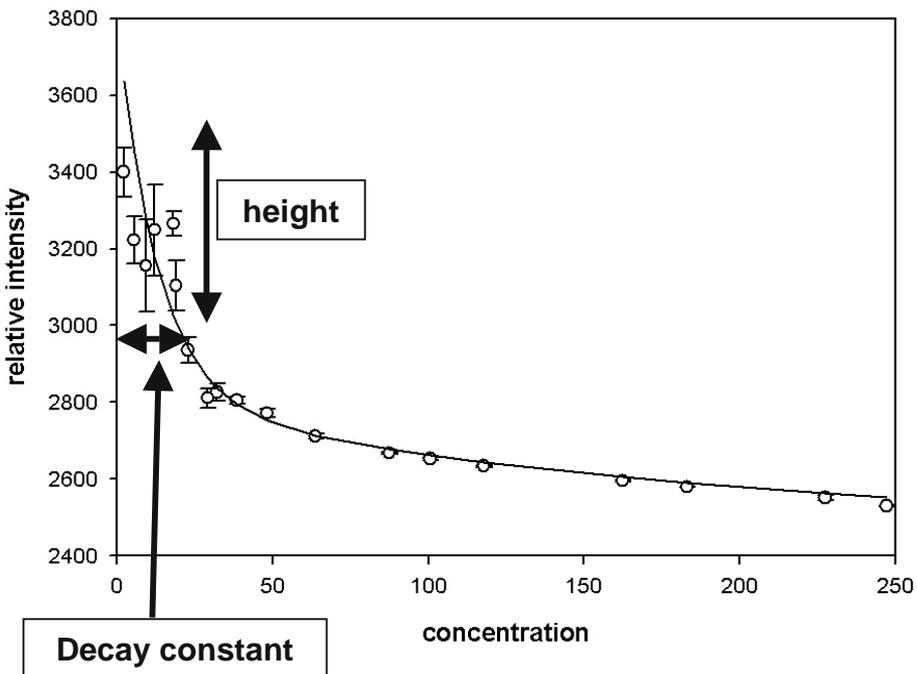
Hb scattering as a function of concentration



lysozyme – scattering at peaks and troughs as a function of concentration



Different proteins undergo different degrees of fluctuation



Protein	(1/d)	height	decay constant
bsa	0.0452	0.0366	11.1000
lys	0.0445	0.0112	16.7000
Avidin	0.0546	0.2077	12.5000
Mb	0.0570	0.1079	14.3000
Hb	0.0415	0.1829	14.3000
Hb (37)	0.0419	0.1092	22.2000

Disorder modeling shows breathing can be interpreted as “rigid body” fluctuations of secondary structures

Conclusions

WAXS data:

- Can be collected to high q without measurable protein degradation
- Contains information pertinent to the secondary and tertiary structure of proteins
- Contains significant fold information
- Is sensitive to small conformational changes induced by ligand binding
- Sensitive to structural changes due to denaturants
- Can provide information on disorder in the protein

Relevance to NSLS-II

Interest in WAXS is growing quickly – new collaborations

- Benoit Roux (Univ. of Chicago), SH2 and SH3 domains of tyrosine kinase
- Tobin Sosnick (Univ. of Chicago), protein folding and intermediates
- Chien Ho (Carnegie Mellon), MAD MAXS (multiwavelength anomalous diffraction, medium angle X-ray scattering) on carboxy-hemoglobin

Future – dynamics and time-resolved capabilities

- One shot experiments
 - 100 msec with APS flux → 100 μ sec with NSLS-II
- Pulse/probe experiments → interesting dynamics