



**Argonne**  
NATIONAL  
LABORATORY

*... for a brighter future*



U.S. Department  
of Energy



THE UNIVERSITY OF  
CHICAGO



**Office of  
Science**

U.S. DEPARTMENT OF ENERGY

A U.S. Department of Energy laboratory  
managed by The University of Chicago

# Scientific Opportunities with Ultrafast SAXS and Requirements

**P. Thiyagarajan**

**T. Irving, D. Tiede, R. Winans**

**IPNS, XSD, CHM Divisions and IIT**

*APS Upgrade Summary Workshop*

*August 10, 2006*

# Structure, Mechanisms and Kinetics of Supramolecular Systems

## ■ Biological Systems

### – Structure

1. Functional Biomacromolecular complexes (conc. 0.02 to 0.1 mg/ml)  
(e.g. circadian rhythm, kinases)

### – Kinetics:

1. Protein and RNA Folding
2. Phase transitions in Membranes
3. Formation of unilamellar vesicles
4. Biomineralization
5. Mechanical behavior of bones
6. Swelling behavior of wood

$\mu\text{s} - \text{s}$  ( $>0.1 \text{ ms}$ )



$\text{ms} - \text{s}$  ( $>0.1 \text{ s}$ )



## ■ Molecular and Supramolecular Systems

1. Solar energy  $\text{ps} - \text{ns}$  ( $>0.1 \text{ ns}$ )
2. Nanocatalysis  $\text{ns} - \text{ms}$  ( $>0.1 \text{ ms}$ )

# TR-SAXS of RNA and Protein Folding Kinetics

## ■ Protein folding

- Early phase of compaction – still contentious – debated – more experiments are needed.
- Still cannot fold proteins using computers – local biases in the early stages are the reason – requires experimental data
- Bigger proteins have multiple phases, some very fast.

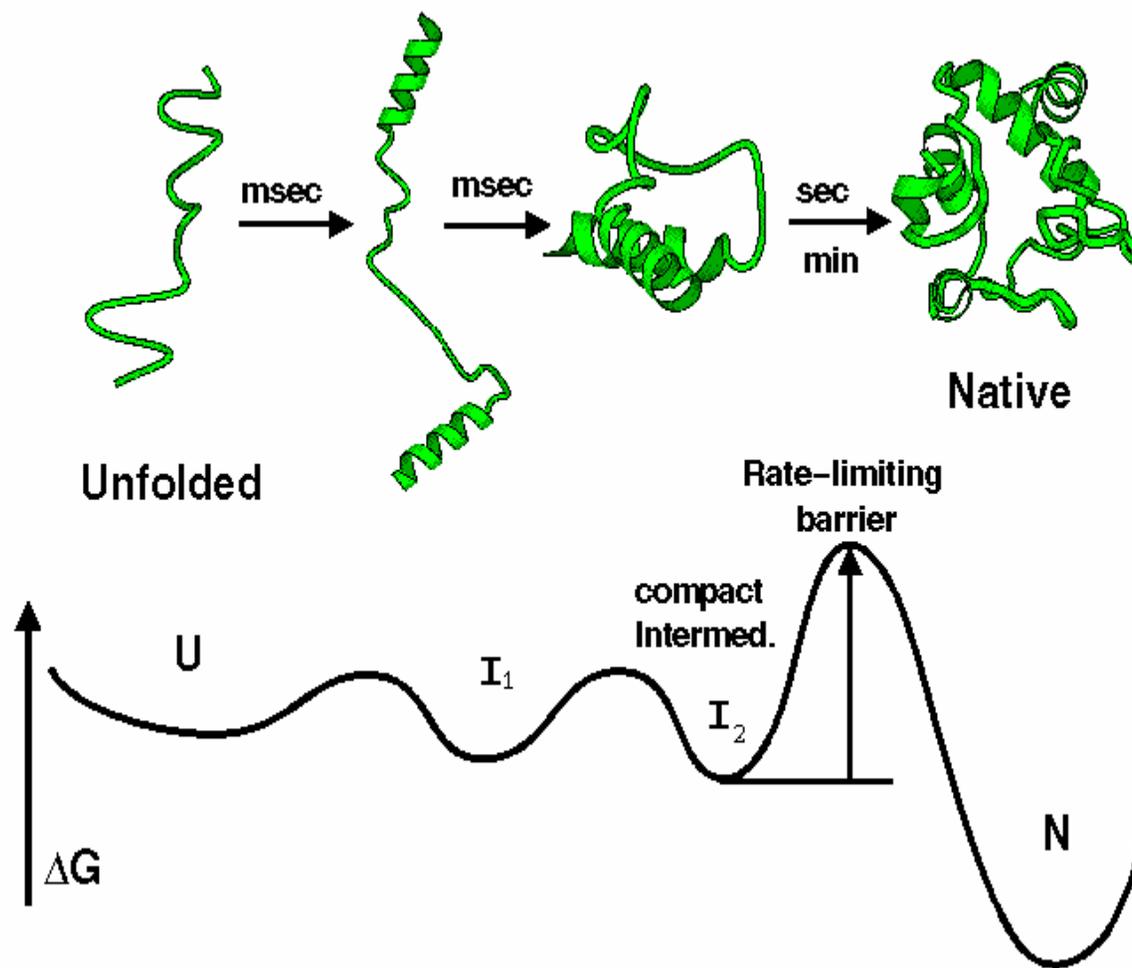
## ■ RNA Folding

- RNA folding has more phases, U- $\rightarrow$ leq is submsec, and then leq- $\rightarrow$ N has multiple phases.
- Comparison of folding pathways for the mesophilic and thermophilic systems- Difference in cooperativity, energetics and kinetics using appropriate mutants.
- Structural details along the folding pathway.

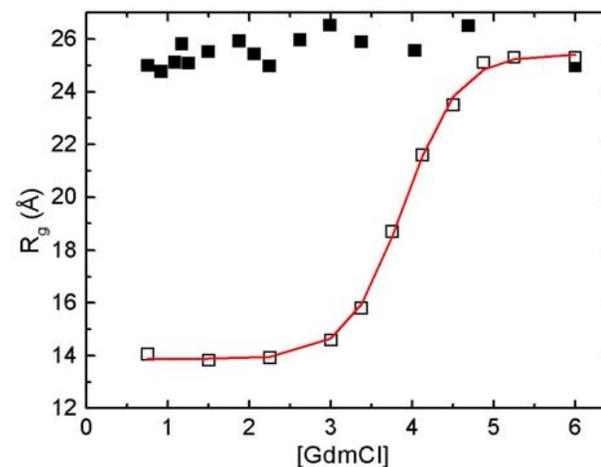
## ■ Will impact folding studies using computer simulation.

- Better Local biases can be used in simulations

## Hypothetical protein folding reaction



## Ubiquitin

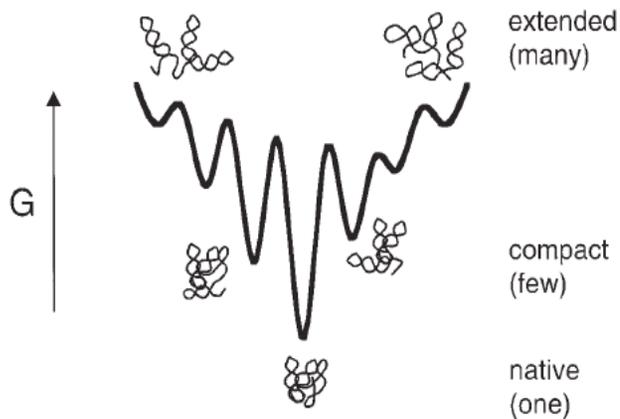


Early collapse is not a  
obligate step (Jacob JMB  
2005)

What are the sequence and time scales of folding events?

# Complex Folding Pathway of Catalytic RNAs

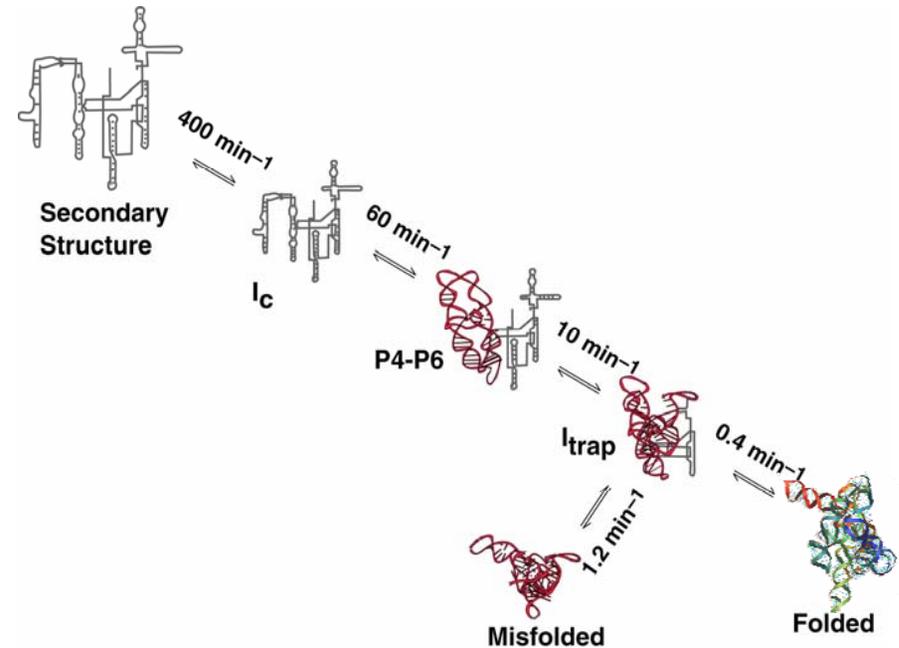
## news and views



**Fig. 1** Hypothetical energy folding landscape of RNA. The graph schematically indicates free energy ( $G$ , vertical axis) as a function of conformation (horizontal axis). A subset of the extended molecules undergoes specific nucleation and collapse to the native structure ( $N$ ). The remaining population becomes trapped in a collection of compact, metastable intermediates ( $I$ ) that correspond to local minima in the rough energy landscape. The intermediates contain many native and some non-native interactions. Transitions from  $I$  to  $N$  cross significant energy barriers and occur close to the native structure.

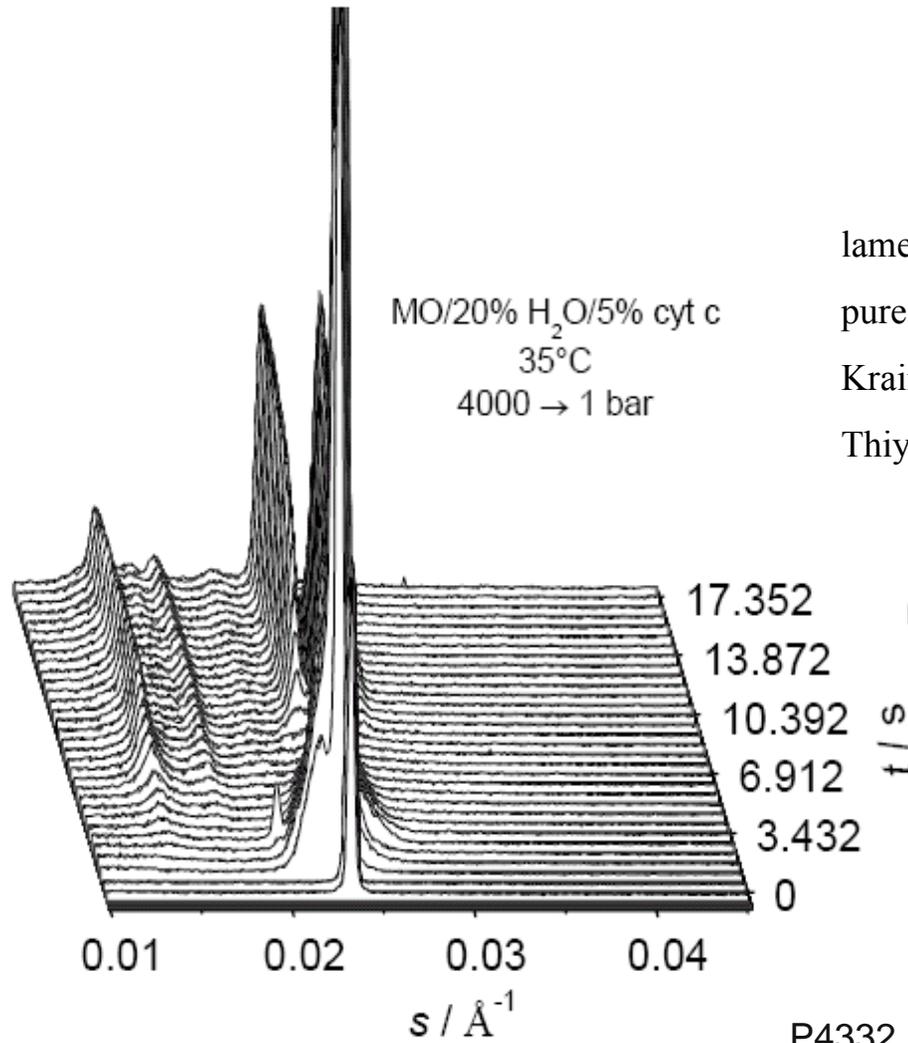
Woodson, Nature Structure Biology 2000

## The *Tetrahymena* ribozyme



Catalytic and S domains of *Bacillus subtilis* RNase P RNA: Early collapse occurs within a few microseconds

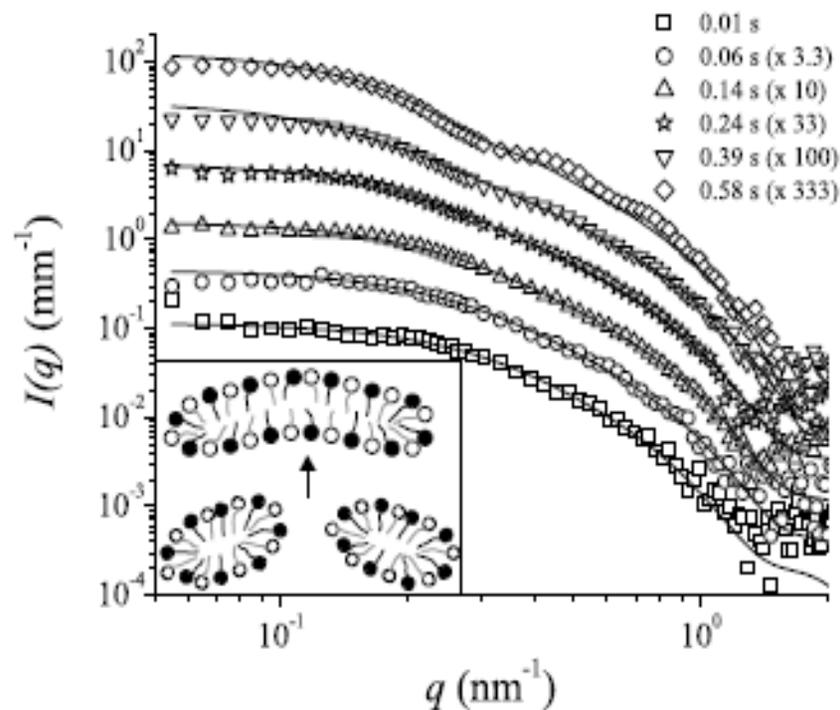
# Kinetics of Phase transitions in Lipid/cholesterol/protein



lamellar-to-cubic and inter-cubic phase transitions of pure monoolein and with cytochrome c  
Kraineva, J, Narayanan RA, Kondrashkina E, Thiagarajan, P., Winter, R. Langmuir 21, 3559, 2005.

P4332, Pn3m, Ia3d and L $\alpha$  phases

## Kinetics of Unilamellar Vesicle Formation



Most cationic systems that form vesicles will show timescales in the submillisecond range

Zwitterionic Tetradecyldimethylamine oxide [C<sub>14</sub>H<sub>29</sub>NCH<sub>3</sub>2O]  
+ anionic lithium perfluorooctanoate (C<sub>7</sub>F<sub>15</sub>COOLi)  
(Weiss et al, PRL 94, 038303, 2005)

# Mechanical Behavior of Biological Nanocomposites

## ■ Mechanism of Bone fracture:

- Mineralized collagen fibers extend only by a fraction of the applied strain, while the rest of the deformation takes place in the glue layer.
- Deformation extremely strain-rate dependent
- Could probe only at low strain rates – weak signal
- **Cannot probe at Strain rates relevant for bone fracture under impact**
- **Shorter data collection times (ms region) necessary to address on how bones fracture in a more direct way.**

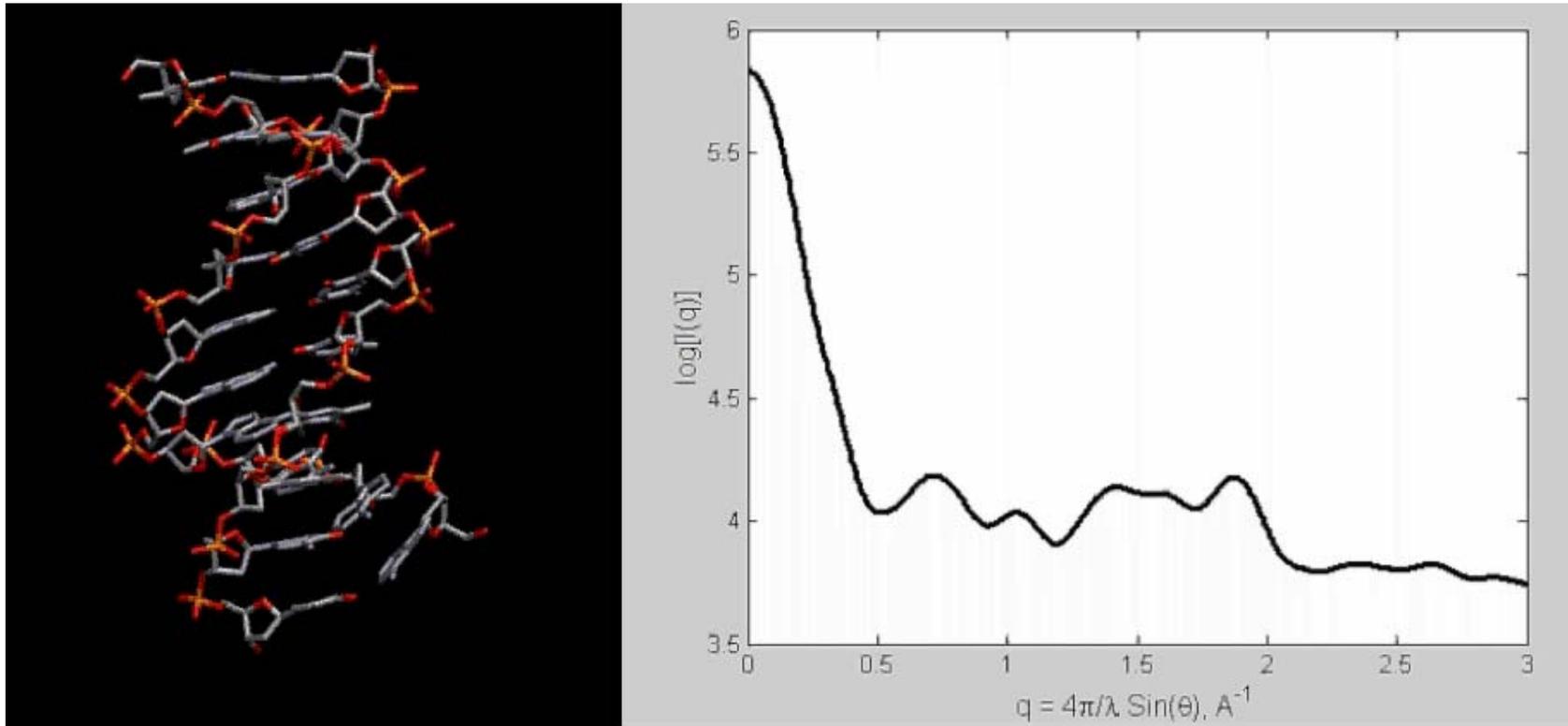
## ■ Wood Cells:

- Reversible swelling behavior of wood cell is poorly understood.
- Kinetics of the deformation of the wood cell of great interest. (Biomimetic)
  - *New insights into the development of growth stresses in trees.*
  - ***will foster research on new bio-inspired hydrogel-based actuators.***

## ■ Not yet possible to perform sub-second SAXD on these systems (signal is weak). New things will come out even in the ms regime.

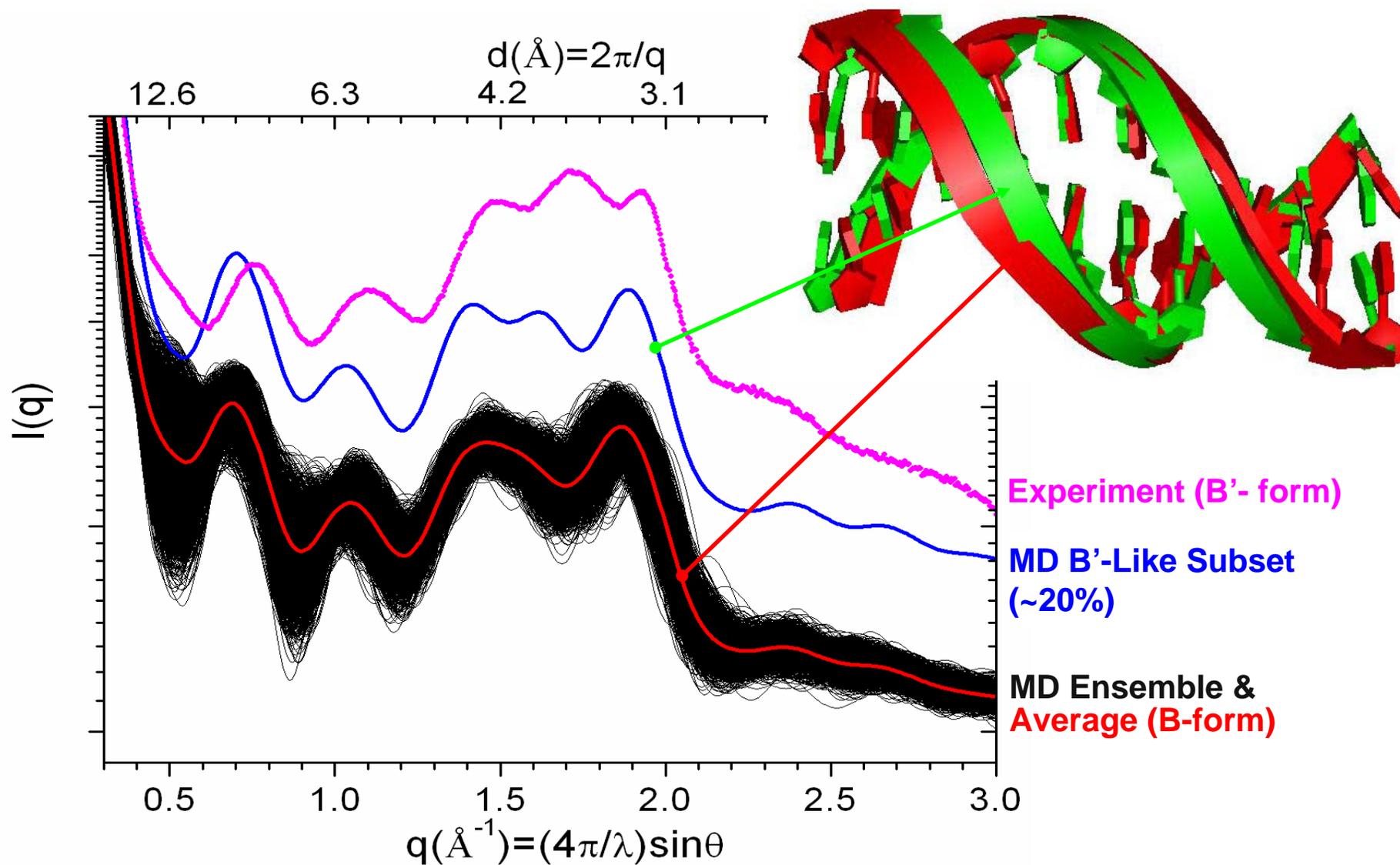
# MD Simulation DNA Conformation Fluctuations: Snapshots at 5 ps Steps

X. Zuo  
D. Tiede



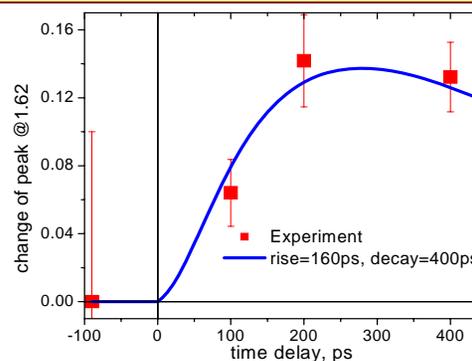
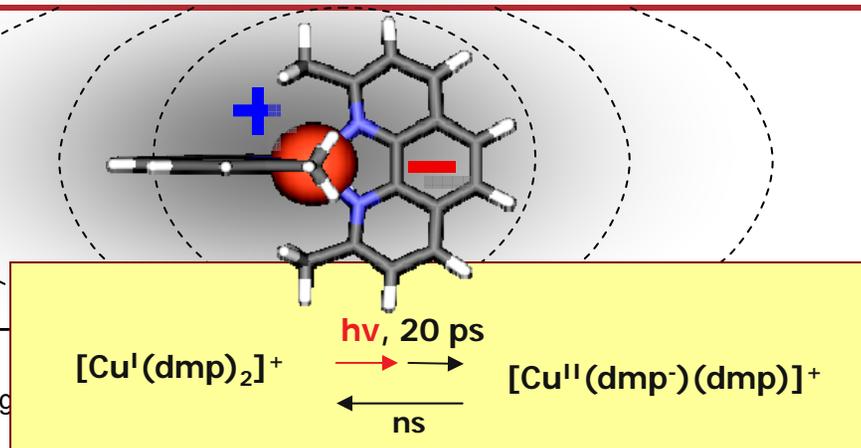
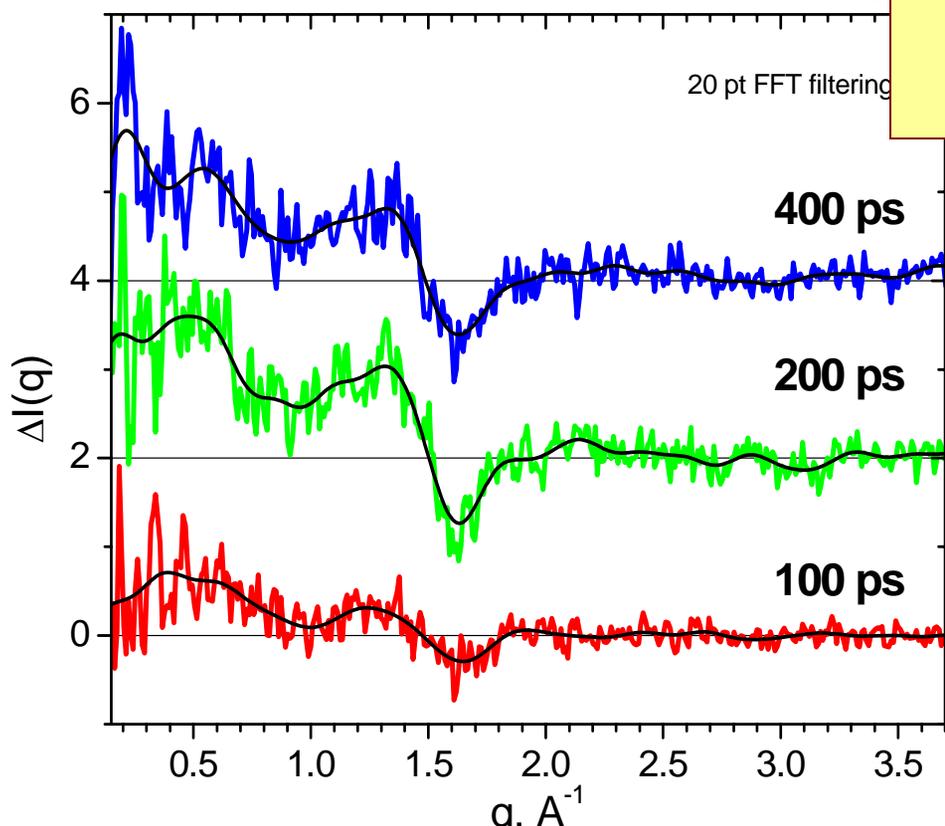
- (Bio) Molecular Dynamics Occurs on ps Time Scale
- X-ray Scattering Resolves Individual Conformers
- Time-resolved Opportunity:
  - i) Single Molecule (LCLS?)
  - ii) Synchronized-Ensemble (Laser induced T-Jumps, charge-transfer, pH-jumps, etc)

# Demonstrated Experimental Capability DNA Conformer Structure Fingerprinting



# Watching Molecular Response to Sudden Perturbation Structure & Energy: A Molecular Tsunami

Scattering Difference Patterns  
 $I(q)$  after laser *-minus-*  $I(q)$  no laser



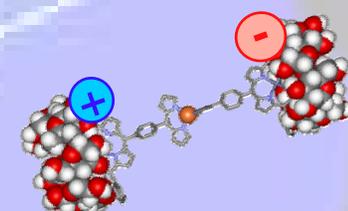
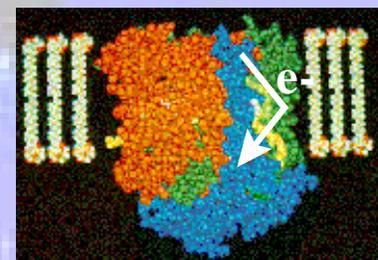
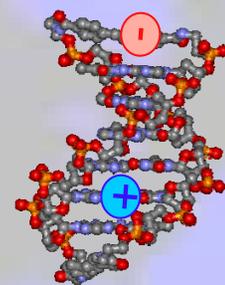
WAXS changes measured with 100 ps time resolution following metal-to-ligand excited-state charge separation in  $\text{Cu}(\text{I})$  (dimethyl-penanthroline)<sub>2</sub>. Following laser-initiated formation of the MLCT state, excited state relaxation which involves dimethylphenanthroline ligand rearrangement to a more planar organization and a solvent coordination at the axial position in about 20 ps. The final  $[\text{Cu}^{\text{II}}(\text{dmp}^-)(\text{dmp})]^+$  charge-separated state lasts for about 1-2 ns. 6/26 – 7/4, 06

# Protein Quakes & Molecular Tsunamis

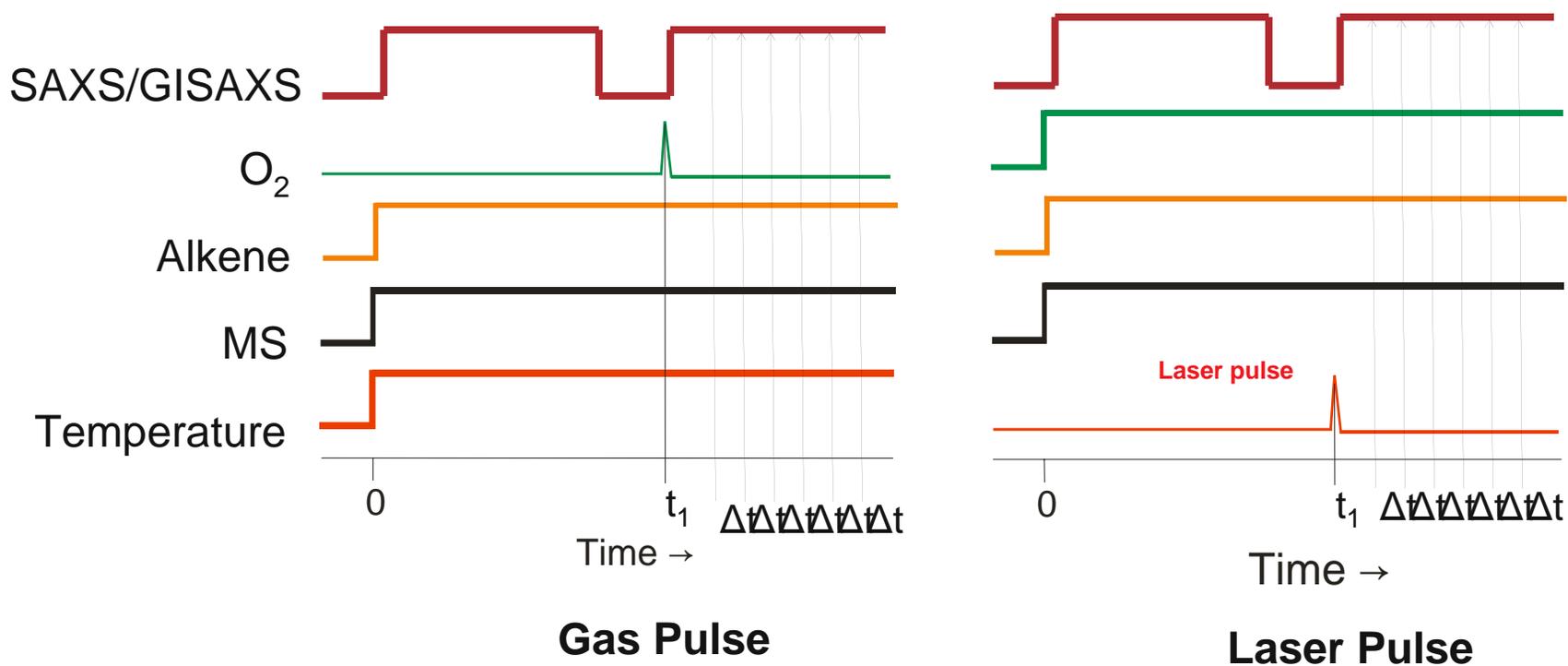
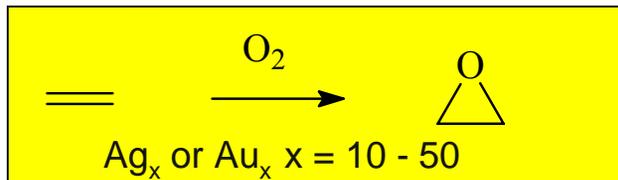
## Molecular Response to Sudden Perturbation of Structure & Energy:

- Electron/Charge Transfer
- Cofactor Conformational Change  
*Cis-Trans*
- Temperature Jump  
*Local- cofactor*  
*Solvent*
- pH Jump

TR-WAXS Technique to Watch  
Ensuing Protein Quakes or  
Molecular Tsunamis



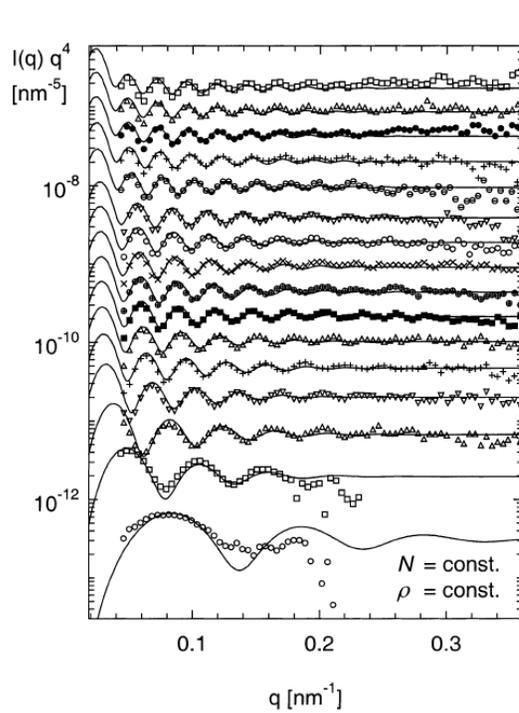
# Time Resolved GISAXS in Nanocatalysis Studies - Perturb – Multi-probe Experiments



In a pulse IR probe study the reaction occurs in a few seconds

in E→EO with oxygen pulses, D. A. Bulushev et al. Applied Catalysis, A:, 123, (2), 301(1995))

# Initial stages of Biomineralization Kinetics

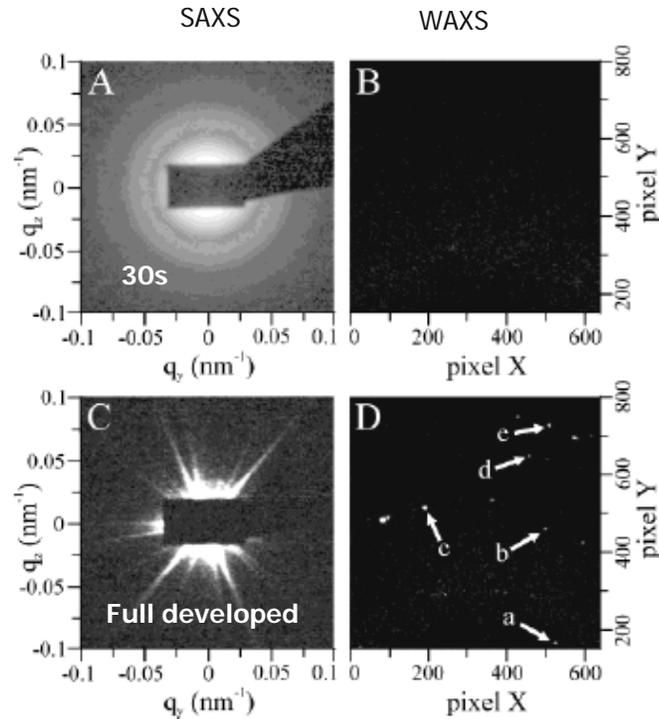


Data shown : 0.5s step

Langmuir (2002), 18(22), 8364-8369

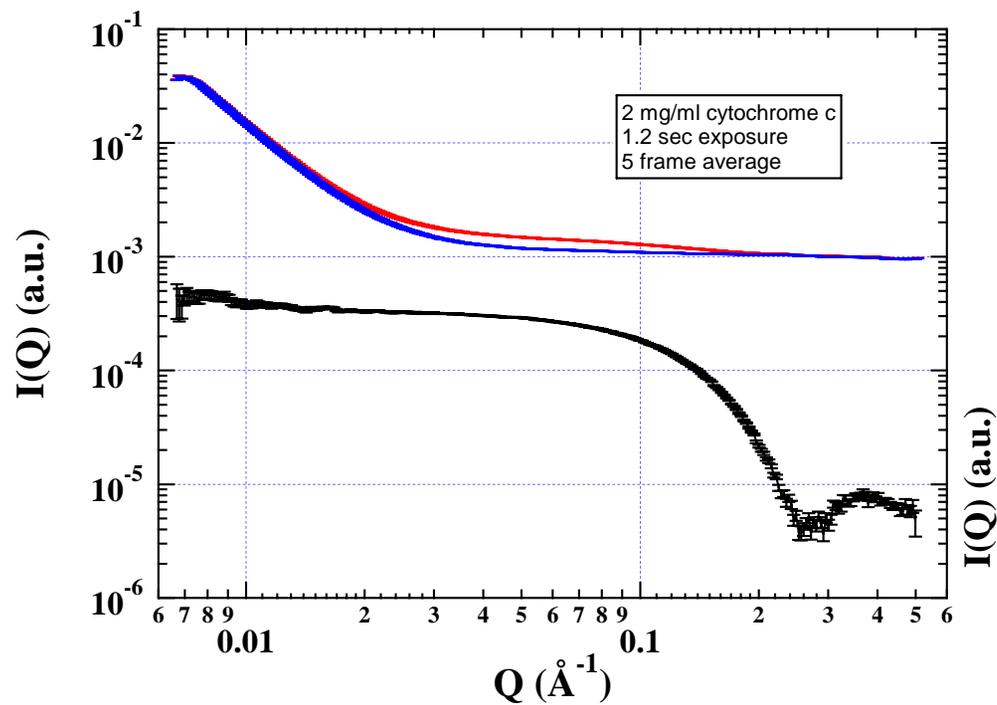
TR SAXS on the unseeded formation and growth of colloidal calcium carbonate particles. Equimolar aqueous solutions of  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  and  $\text{Na}_2\text{CO}_3$  were rapidly mixed in a stopped-flow apparatus

The crystal shape and size in the early stage, (1~50ms) could not be resolved due to limitation in the flux and the speed of detection.

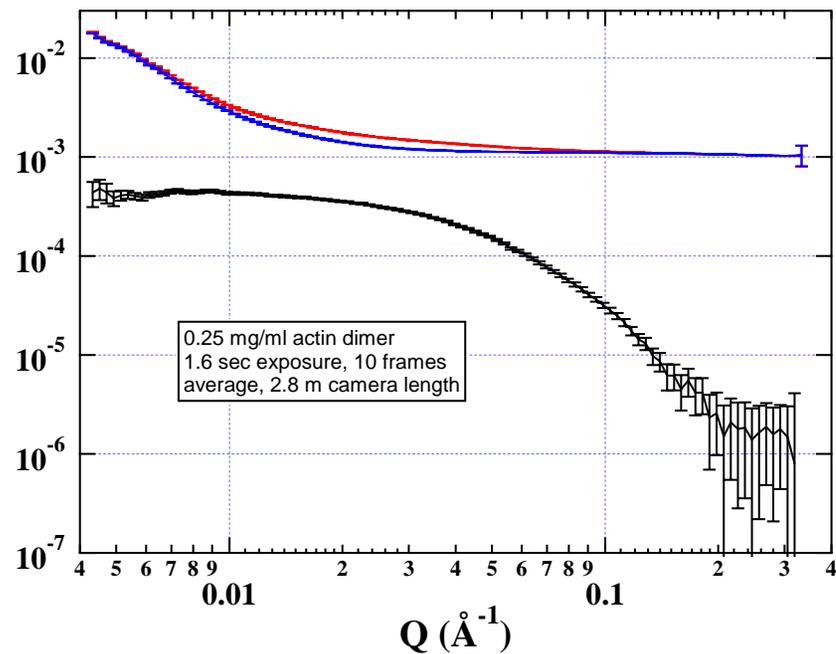


J. Phy. Chem. B. 2003, 107, 5123-5125

# Typical SAXS data of proteins



Wide Q range is necessary



# SAXS of Functional Complexes and TR-SAXS on Kinetics

## Functional Complexes

- FPLC to separate the complexes and bring them to the X-ray window (highly dilute)

## Kinetics

CCD read out time is  $> 1$  sec. we use it as a static device and control the sample

- $> 10$  ms time resolution
  - Continuous flow – changing flow rate with stopped-flow rapid mixer
- $\sim 100$  microsec to ms time resolution
  - Microchannel mixer – flow rate (higher flow rate)
- High sample consumption
  - 2 (5 ms) to 20 mg (500  $\mu$ sec) of sample
  - Faster time resolution will require prohibitively higher amount
  - Not desirable: this impedes science
  - Higher brilliance and faster detectors needed

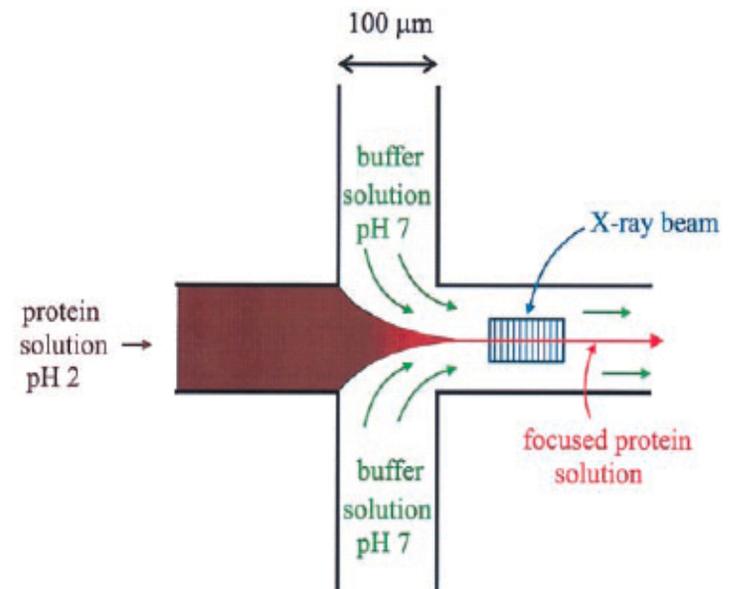


FIG. 1. A schematic of the diffusive mixer.

## Instrument requirements and sample control

- Cleaner beams, less background, more reliable background subtraction
  - our biggest problems.
- Innovation of sample control
  - Ability to selectively obtain data on functional complexes using fractionation
  - Conformational change upon light or T-jump or pH jump, ionic conditions?
  - Stopped-flow
  - Microfluidics
  - Pressure jump
  - Reduction of radiation damage
  
  - Flames
  - Film growth
  - Catalysis

## Area Detector requirements

- High count-rate capability ( $> 1 \times 10^6$  Xph/s/pixel) (single photon counting)
- Fast acquisition ( $\sim$ microsec time domain)
- Histogramming capability of SAXS data for different times (TRSAXS)
- Large pixelated area (200mm x 200mm)
- High dynamic range ( $> 10^6$ ) (Similar to single photon counting)
- High sensitivity ( $\sim 80\%$ )
- High spatial resolution (Low angle  $\sim 60\mu\text{m}$  FWHM)
- Operation in vacuum

Silicon based pixel detectors may be a better choice

## *Computing Needs for Ultrafast SAXS*

- Fast image data reduction
- High-speed and data storage with high-availability
- Grid computing for online analysis capabilities

## *Acknowledgments*

- Tobin Sosnick, U of C
- Martin Egli and Rekha Pattanayek, Vanderbilt U.
- Peter Fratzl, Max Planck Institute, Postdam, Germany
- Thomas Weiss, SSRL
- Byeongdu Lee, APS
- Stefan Vajda, CHM and CNM
- Pete Jemian, APS
- Soenke Seifert, APS
- Klaus Attenkofer, APS
- Liang Guo, IIT, BioCAT
- Jan Hessler, CHM, ANL
- Pete Jemian, APS