SAXS Applications in Structural Biology and Biophysics

Xiaobing Zuo

X-ray Science Division, 12ID-B
Argonne National Laboratory
zuox@anl.gov
Outline

0. Introduction

1. Theory on molecular solution x-ray scattering calculation

2. Molecular structural information from bioSAXS

3. Advanced applications in frontiers of structural biology

4. Time-resolved bioSAXS
0. Introduction

- Bio-SAXS vs material SAXS
- Why BioSAXS for structural biology
Bio-SAXS vs material SAXS

- Sample for BioSAXS:
  - In solution, isotropic
  - High homogeneity in structure
    - High purity, >90-95%
    - Virtually “identical” in size and conformation

- Molecular information from BioSAXS:
  - Scattering signal can be considered as if coming from “a single molecule”
  - Definite molecular size, **NOT size distribution** in *material SAXS*
  - Conformation / Folding state
  - Shape / 3-D low res molecular model
  - Medium-high resolution structure information possible
Why SAXS in structural biology

- Atomic resolution structures from crystallography and NMR still “gold standard” for structural inference

- But, there are limitations
  - Crystallography needs crystals, studies static structures, possibly with artifacts by packing force
  - Solution NMR has size limitation, proteins 30-50kDa, RNA 30-50 nts

- SAXS has virtually no size limitation for biomolecules (1 – 1000nm).
  - Samples can be run in physiological condition
  - SAXS can provide useful, although limited or low res, information on fast time scales
  - Both steady-state structure and kinetics
1. Theory on molecular solution x-ray scattering calculation
   - X-ray wave function
   - Molecular x-ray scattering simulation
   - Available softwares
X-ray scattering pattern arises from interference

- X-ray interacts with/scattered by electrons.
- Scattered X-ray interferes.

Momentum transfer

\[ q = |\mathbf{q}| = \frac{4\pi}{\lambda} \sin \theta \]
X-ray wave function & Scattering amplitude

- X-ray wave function:
  \[ E(t) = E_0 e^{i(\phi_0 + \omega t + \mathbf{k}_0 \cdot \mathbf{r})} \]
  Initial phase: \( \phi_0 \)
  Phase at t: \( \omega t \); \( \omega \): frequency
  Phase at position \( \mathbf{r} \): \( \mathbf{k} \cdot \mathbf{r} \); \( \mathbf{k} \): wave-vector, \( |\mathbf{k}| = 2\pi/\lambda \)

- X-ray scattering **Amplitude** at \( \mathbf{q} \) by a particle at position \( \mathbf{r}_j \):
  \[ A_j(\mathbf{q}) = f \exp(i\mathbf{q} \cdot \mathbf{r}_j) \]
  - For electron: \( f = f_e = 2.8179 \times 10^{-13} \text{cm}^{-1} \)
  - For atoms: \( f = f(q) \) atomic form factor
    - \( f(q=0) = Z \): the total electron of the atom.
    - Atoms with higher \( Z \) are stronger scatterers.

- Scattering amplitude of an ensemble of particles:
  \[ A(\mathbf{q}) = \sum_{j=1}^{n} f_j(q) \exp(i\mathbf{q} \cdot \mathbf{r}_j) = \int V f_e \rho(\mathbf{r}) \exp(i\mathbf{q} \cdot \mathbf{r}) dV \]
X-ray scattering and Debye formula

**In vacuum**, for a molecular with fixed orientation:

Scattering amplitude:

\[ A(q) = \sum_{j=1}^{N} A_j(q) = \sum_{j=1}^{N} f_j(q) \exp(iq \cdot r_j) \]

Scattering intensity:

\[ I(q) = |A(q)|^2 \]

**In solution,**

- **Molecular orientation**
- **In solution, x-ray sees all orientations**

\[ I(q) = \langle I(q) \rangle_{\Omega} \]

average over all orientation

\[ \langle \exp(iq \cdot r) \rangle_{\Omega} = \frac{\sin(qr)}{qr} \]

Loss of the direction the momentum transfer/ angular term

\[ I(q) = \sum_{j} \sum_{k} f_j f_k \frac{\sin(qr_{jk})}{qr_{jk}} \]  

Debye formula

- **Solvent contribution**
Solution x-ray scattering calculation from atomic coordinates

\[ I(q) = \left\langle \left| A_m(q) - A_s(q) + \delta \rho A_I(q) \right|^2 \right\rangle _{\Omega} \]

Scattering intensity - average over all orientations \( \Omega \).

1. Scattering amplitude \textit{in vacuo}

\[ A_m(q) = \sum_{j=1}^{N} f_j(q) \exp(iqr_j) \]

2. Scattering amplitude from the excluded volume using \textit{dummy solvent} approximation

\[ A_s(q) = \sum_{j=1}^{N} g_j(q) \exp(iqr_j) \]

Dummy atom model with Gaussian function type form factor

\[ g_j(q) = G(q) \rho_s V_j \exp\left( -\frac{q^2V_j^{2/3}}{4\pi} \right) \]

Expansion factor

3. Additional scattering from solvent layer

\[ \delta \rho A_I(q) \] where \( \delta \rho \) is the electron density difference between the layer solvent and the bulk solvent and the second term is the form factor of the surface layer envelope.

Solution x-ray scattering simulation programs

  - Most popular for BioSAXS, stand-alone program, fit model to data, fast computational algorithm

  - Use explicit water modeling solvation layer, robust fitting approach

  - A program to compute WAXS

  - Debye-like computation, web server based

- Zuo X, Zhang R, Tiede DM. **SolX**: A computer program for solution molecular x-ray scattering simulations. In preparation, program available on request. (zuox@anl.gov, tiede@anl.gov)
  - Debye-like computation, Windows-based, can handle non-standard atoms/residues, for biomolecules and supramolecules
Structural model verification

Dickerson DNA

Crystal:

- 1BNA
- 355D

NMR Solution:

- 1GIP
- 1DUF
- 1NAJ
- 171D

Zuo & Tiede; JACS, 2005
2. Scattering experiment and data analysis

- bio-SAXS setup
- Solution scattering data
- Structural information in SAXS data
- 3-D structural modeling
A. Scattering experiment setup

SAXS/WAXS setup at 12ID-B at APS
B. Solution X-ray Scattering Data

Scattering is often a high background measurement.
WAXS Examples

- Much information in high angle data
- Molecular signal: 0.1-1%, high background exp
- High flux synchrotron sources necessary

Sample concentration: 2-10mg/m
Data collected at 12ID
Hierarchical structural information in scattering profile

Spatial resolution: \( d = \frac{2\pi}{q} \)

- In various q-regions, one views the molecule at different scale/resolution, see different levels of resolution on the structural details.
- SAXS (0-0.2-0.3 Å\(^{-1}\)): shape, size, inter-particle interactions, etc
- WAXS (> 0.2 Å\(^{-1}\)): internal structural details, of increasing interest
C. Small angle X-ray scattering

measures

size, molecular weight, shape, conformation, inter-particle interactions, ...
**Guinier equation**

Scattering intensity can be expanded in powers of $q^2$:

$$I(q) = I(0) \left[ 1 - \frac{R_g^2 q^2}{3} + kq^4 + \ldots \right]$$

When $q \to 0$,

$$I(q) \approx I(0) \exp \left( -\frac{R_g^2 q^2}{3} \right)$$

$qR_g < 1.3$ for globular; $qR_g < 0.8$ for elongate

$R_g$: radius of gyration  
$I(0)$: forward scattering

$$I(q) = \frac{2I(0)}{q^4 R_g^4} \left( q^2 R_G^2 - 1 + e^{-q^2 R_g^2} \right)$$

$qR_g < 1.4$ for elongate

---

Guinier analysis for compact particles

$I_0 = 0.0034855 \pm 1.4397 \times 10^{-6}$

$R_g = 20.683 \pm 0.012413 \text{ Å}$

$Q_{max} \times R_g = 1.3007$

To get reliable Guinier plot / $R_g$ analysis:

- $q_{max} \times R_g < 1.3$ for globular; $< 0.8$ for elongate
- $q_{min} \leq \frac{\pi}{D_{max}}$
- Multiple ($\geq 5$) data points in linear fashion

---

André Guinier (1911-2000)
Radius of gyration: a size parameter

Radius of gyration \( (R_g) \) is the square root of the averaged squared distance of each scatterer point from the center weighted by excess electron. It is a composite size parameter.

For sample geometric objects:

- **Sphere**
  - (radius \( R \))
  \[
  R_g^2 = \frac{3}{5}R^2
  \]

- **Elliptic cylinder**
  - (semi-axes \( a,b \); height \( h \))
  \[
  R_g^2 = \frac{a^2 + b^2}{4} + \frac{h^2}{12} = R_c^2 + \frac{h^2}{12}
  \]

For a molecule with known model:

\[
R_g^2 \approx \sum_j \frac{\Delta n_j r_j^2}{\sum_j \Delta n_j \text{ excess electrons}}
\]

- \( R_g \) of a molecule in extended conformation is likely larger than that in well-folded conformation.
- \( R_g \) of an oligomer is likely larger than that in molecule in moneric state.
Forward scattering \( I(0) \) measures molecular weight

\[
MW = \frac{N_A I(0) / c}{(\Delta \rho_M)^2}
\]

\( I(0) \) at absolute scale; \( c \) mg/ml

Scattering length density diff: \( \Delta \rho_M = \rho_M - \rho_s \)

\( N_A = 6.02 \times 10^{23} \)

- **Absolute scale:** calibrate with water or other standard
  - Water: \( 1.632 \times 10^{-2} \) cm\(^{-1} \) at 293K
  - For protein on average: \( \Delta \rho_M = 2.086 \times 10^{10} \) cm\(^{-2} \)

\[
MW = 1385 \times \frac{I(0)}{c}
\]

\( MW \) in kDa; \( I(0) \) in cm\(^{-1} \), \( c \) in mg/ml


- **Relative scale:** secondary standard
  - Lysozyme, BSA, etc

\[
MW_p = \frac{I(0)_p / C_p}{I(0)_{st} / C_{st}} \frac{MW_{st}}{MW}
\]

- Standard should have **same nature** of the molecules to determine, and with close MW. Using multiple standards is suggested.
More on MW

- From SAXS profile: web-based program saxsmow

![Graph showing SAXS profile](image)

BSA: 66.5kDa
71.5 kDa

http://www.if.sc.usp.br/~saxs/saxsmow.html

- Some general aspects:
  - Structural factor should be eliminated before using I(0) for molecular weight determination.
  - The errors in MW is about 10%.
  - MW is an important parameter/method for determining oligomeric state of biomolecules in solution because oligomerization is often difficult to determine by other means.
SAXS for determination of oligomeric state

Monomer vs dimer:

Geometric size of an aggregate grows slower than its mass. \( I(0) \) More sensitive than \( R_g \)!

Guinier Plot: interactions & sample condition

Mono-dispersed

Normal / linear

No aggregation

Poly-dispersed aggregates

Curve-up

Repulsion

Curve-down

Structure factor

In solution X-ray scattering, structure factor describes the interactions among macromolecules or particles.

\[ I(q) = P(q) \ast S(q) \]

- \( I(q) \): apparent scattering profile
- \( P(q) \): scattering profile in absence of inter-particle interaction
- \( S(q) \): structure factor

- Structure factor is an undesired effect.
- One should change the buffer condition to reduce or eliminate the inter-particle interactions.
- If the structure factor is inevitable, one should correct it by running samples at a serial of concentrations.

Kratky plot: folding state

Otto Kratky (1902-1995)

Kratky plot: $I(q)q^2$ vs $q$

Scattering profile for a random coil type sample.

Pair distance distribution function (PDDF)

\[ p(r) = \frac{r^2}{2\pi^2} \int_0^\infty q^2 I(q) \frac{\sin qr}{qr} dq \quad \text{--- reverse FT of } I(q) \]

\[ p(r) \sim \sum 1 \times \Delta n(\mathbf{r}_j) \times \Delta n(\mathbf{r}_k) \times r^2 \]

excess electrons of atom \( j \) over solvent

The PDDF of a molecule is the (net-electrons and distance) weighted atom-pair distance histogram.
Information from PDDF

- Largest cord in structure \(D_{\text{max}}\)

- Forward scattering \(I(0)\)

\[
I(0) = 4\pi \int_0^{D_{\text{max}}} p(r) dr
\]

- Radius of gyration – real space

\[
R_g^2 = \frac{\int_0^{D_{\text{max}}} r^2 p(r) dr}{\int_0^{D_{\text{max}}} p(r) dr}
\]

- Can be used to determine \(D_{\text{max}}, I(0),\) size, and shape, etc.
The various dimeric conformations change $p(r)$ and $D_{\text{max}}$ as well.
SAXS & PDDF of various shapes

Small angle X-ray scattering

measures
size, molecular weight, shape, conformation, inter-particle interactions, …

Although important, but limited

had been viewed as a tool of low resolution, as well as low information
D. SAXS for 3D structure reconstruction

Reconstruct 3D structure from 1D SAXS profile is an ill-condition problem
3D shape reconstructions from SAXS data

Obtaining 3D shapes from 1D SAXS data is an ill-defined problem that could be solved by regularizing the fitted models.

Imposing prior restraints on the fitted models such as non-negativity and compactness/connectivity greatly increases solution stability.

Available Programs:
SAXS envelope reveals biological function

A 102 nt element in 3’UTR of turnip crinkle virus mRNA (tcvRNA)
- tcvRNA binds to ribosome and enhances translation.

- tcvRNA has tRNA-like shape!

3. Advanced applications in frontiers of structural biology

- NMR structure refinement for higher accuracy
- Biomolecular complex structure determination
- Structural determination for large RNAs
- Characterization of disordered systems
A. SAXS as global restraint: problem in NMR structures

Problems for NMR structures:
- Constructed from short distance restraints (H---H, <5Å)
- Intrinsically lacking of global dimensional restraints

NMR alone

NMR+SAXS

rmsd: 3.2 Å

| Black: exp data | 23.2 |
| Red: w/o SAXS | 25.1 |
| Blue: w/ SAXS | 23.0 |

SAXS as global restraint: improvement in accuracy

- 1.4 NOEs and 6 RDCs / residue
- Accuracy improvements for both 2-domain and 1-domain geometries
- Validated by later measurement of long-range CH$_3$-CH$_3$ NOEs

SAXS capability available in Xplor-NIH and CNS for proteins and RNAs

B. Complex and SAXS application for its structural determination

- Biomolecules function through interaction, in form of complex
- Structural determination for complexes becomes one central topic in structural biology.

- **Difficulties**
  - Crystallography: crystal; possible irrelevant or false interface
  - NMR: inter-NOE

- **SAXS related methods:**
  - Verify crystallographic complex interface
  - Rigid body modeling
  - Combined use of SAXS and RDC
  - Small angle neutron scattering and scattering contrast variation
  - Multiple phase reconstruction
Complex: Rigid body modeling with SAXS

**Exhaustive search**

**Advantages**
- No distortions
- Enough computing time

**Disadvantages**
- 3D translations + rotations

---

**TABLE 1 Comparison of algorithms for global rigid body modeling**

<table>
<thead>
<tr>
<th>Objects</th>
<th>DIMFOM</th>
<th>GLOBSYMMP</th>
<th>SASREF</th>
<th>BUNCH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Homo- and heterodimers</td>
<td>Symmetric oligomers</td>
<td>Macromolecular complexes</td>
<td>Multidomain proteins;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>with one monomer per</td>
<td></td>
<td>complexes of subunits</td>
</tr>
<tr>
<td></td>
<td></td>
<td>asymmetric part</td>
<td></td>
<td>with missing fragments</td>
</tr>
<tr>
<td>Multiple data sets fitting</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Maximum number of</td>
<td>2</td>
<td>1</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>independent rigid bodies</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Symmetry</td>
<td>P1, P2</td>
<td>P2–P6, P222–P62</td>
<td>P1–P6, P222–P62</td>
<td>P1–P6, P222–P62</td>
</tr>
<tr>
<td>Minimization method</td>
<td>Rolling on the surface</td>
<td>Global grid search</td>
<td>Simulated annealing</td>
<td>Simulated annealing</td>
</tr>
<tr>
<td>Constraints</td>
<td>Symmetry, interconnectivity</td>
<td>Symmetry, interconnectivity</td>
<td>Symmetry</td>
<td>Symmetry, interconnectivity</td>
</tr>
<tr>
<td></td>
<td>—</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Restraints</td>
<td></td>
<td></td>
<td>Interconnectivity, steric</td>
<td>Compactness, steric</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>clashes, pair contacts</td>
<td>clashes, and bond/dihedral</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>angles in DR loops</td>
</tr>
<tr>
<td>Number of target function</td>
<td>4000/0.5</td>
<td>15,000/3</td>
<td>1.2 × 10⁵/50</td>
<td>3 × 10⁵/80</td>
</tr>
<tr>
<td>evaluations/CPU, min</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Complex: combined use of SAXS and RDC

- Residual dipolar coupling (RDC) is an NMR observable, measuring the orientation of a chemical bond.
- RDC also measures the orientation of a molecular fragment or subunit.

**RDC degeneracy:** 4 discrete possible orientations per independent set of RDC data: $(\theta, \phi), (\theta, \phi+\pi), (\pi-\theta, \pi-\phi), (\pi-\theta, 2\pi-\phi)$

- Grid search.
  - 4 possible orientations vs many in RB-rotation
  - Reduce/lift degeneracy in both RDC and SAXS
  - GASR: Global Architecture from SAXS and RDC

GASR program link @ Dr. Y.X Wang website: http://ccr.cancer.gov/staff/links.asp?profileid=5546
The HIV-1 protease is a homodimeric globular protein, with each subunit comprising 99 residues.

<table>
<thead>
<tr>
<th>Structures</th>
<th>Inter-NOE</th>
<th>RDC (Simulated)</th>
<th>SAXS (Simulated)</th>
<th>Backbone RMSD (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NMR (original)</td>
<td>218</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GASR#1</td>
<td>0</td>
<td>0 noise</td>
<td>0 noise</td>
<td>0.21</td>
</tr>
<tr>
<td>GASR#2</td>
<td>0</td>
<td>5Hz noise</td>
<td>0 noise</td>
<td>0.87</td>
</tr>
<tr>
<td>GASR#3</td>
<td>0</td>
<td>5Hz noise</td>
<td>10% noise</td>
<td>1.2</td>
</tr>
</tbody>
</table>

- GASR demonstrates high accuracy in identifying complex interface.
- GASR deals with homo-/hetero-dimer and dual domain proteins.
- The correctness of the GASR structure depends on the quality of experimental data.
- Extra data, such as inter-unit distance restraint, will help.

Small angle neutron scattering (SANS)

- SANS is very similar to SAXS
  - Principle
  - Data analysis

- Difference:
  - Interaction: X-ray with electron, while neutron with nuclei
  - Scattering length: x-ray proportional to Z-number and q-dependent, while more random for neutron scattering and q-independent
  - Available flux: x-ray – high; neutron -- low
  - q-range: x-ray, wide; neutron, narrow
SANS and scattering contrast variation

\[ I(q) = \Delta \rho_1^2 I_{11}(q) + \Delta \rho_2^2 I_{22}(q) + \Delta \rho_1 \Delta \rho_2 I_{12}(q) \]

\[ \Delta \rho(r) = \rho_m(r) - \rho_s(r) \]
NL/NX complex: shape reconstructions from the SAXS data do not yield a unique answer

Contrast-matched neutron scattering of 1H-NL / 2H-NX

in 42% D$_2$O

Nucleic Acids-Protein Complex: multiple phase reconstruction

Protein-rRNA distribution in 70S E.coli ribosome

DAMMIN/F

1 phase vs multiple phase

MONSA

SANS contrast variation data

SAXS

\[ I(s) = 2\pi^2 \sum_{l=0}^{\infty} \sum_{m=-l}^{l} \sum_{k=1}^{4} [\Delta \rho_k A_{lm}^{(k)}(s)]^2 + 2 \sum_{n>k} \Delta \rho_k A_{lm}^{(k)}(s) \Delta \rho_n [A_{ln}^{(n)}(s)]^* \]
C. RNA Biology and SAXS for RNA structure determination

- RNA biology
  - Away beyond protein synthesis: rRNA, mRNA and tRNA

- Difficulty:
  - Difficult to crystallize
  - Size of functional RNA too big for NMR

- Structural feature:
  - Modular, Duplex is the major building block

- SAXS related medium-high resolution methods:
  - SAXS guided structure screening
  - Divide-n-conquer
  - G2G: SAXS+RDC
RNA: filtering structure decoys with SAXS

The MC-Fold and MC-Sym pipeline

Input:
- Sequence
- Restraints if available
- Size limitation: ~120nt

NMR
Lowest-rmsd
Best SAXS-fit

rmsd (vs NMR): 5.6 8.6

RNA: divide-n-conquer

VS Ribozyme 2’ structure

SAXS envelopes

Segment layout

Rigid-body refinement

Lipfert J., et al. (2008), Structure, 16, 1357–1367
http://www.dundee.ac.uk/biocentre/nasg/VS/SAXS_VS/VSmain.html
RNA: combined SAXS and RDC

71-nt Adenine riboswitch (riboA)

Duplexes H1, H2, H3 are in either parallel or anti-parallel fashion

RNA: combined SAXS and RDC


Overall backbone RMSD between the G2G (red) and X-ray crystal (cyan) structures: ~3.0 ± 0.4 Å
D. Structural disorder/flexibility

- Can be detected and analyzed by SAXS

- Detection:
  - \( \text{Rg}, \text{Dmax}, \text{Kratky plot (folding state)} \)

- Ensemble Optimization Method (EOM)
  - Using a small number of conformation represent the entire

- References:
4. Time-resolved bioSAXS
   - Experimental setups
   - An example: RNA folding
Dynamics and Kinetics in Biomolecules

- Time-resolved BioSAXS can cover µs-ms-s and up.
- SAXS measures all atoms.
- Folding, allostery, recognition, etc

TR-BioSAXS: Apparatus

Stopped-flow

Observation Cell
Stop valve
X-rays
Quartz Capillary
Mixing Head
Syringes
Unfolded RNA
Syringe Pump

Roh, et al; JACS, 2010, 132, 10148

- High flux synchrotron x-ray sources are a must for ms scale TRSAXS!

Detectors

Pilatus 100K
Readout: 2.7ms

Pilatus 300K
3.6ms

Data collection time scheme

dead time
Exposure 1ms
Delay > readout

trial#1

trial#2

time

55
TR-BioSAXS: continuous flow for μs

**Turbulent Flow**
- Mylar Film (22 μm Width)
- Image intensifier & CCD
- Syringe Drive
- Mixing Plate (400 μm Width)
- Mixing Point

**Laminar Flow**
- X-ray beam
- Denatured RNA


Flow rate: 200-300 mm/s
Resolution: 70-160 μs

Russell R et al. PNAS 2002, 99, 4266

Mixing through diffusion
Flow rate: 86 mm/s
Resolution: 400 μs
TR-BioSAXS: Example --- Multistage Collapse of a Azoarcus group I Ribozyme

Data/Kratky

Q [Å⁻¹]

analysis

R_g [Å]

0 s
0.6 ms
171 ms
5 s
10 min

extended ensemble
r_p ~ 21 Å

specific collapse (τ ~ 1 ms)

non-specific collapse

conformational search (τ ~ 20 ms)

relaxed coil ensemble (non-specific) r_p ~ 15 Å

native-like
r_p ~ 10 Å

refolding (τ ~ 100 s)

mislabeled

Roh, et al. JACS, 2010, 132, 10148
SAXS Applications in Structural Biology and Biophysics

**Guinier Plot**
- Overall size: $R_g$
- Molecular weight: $I_0$
- Aggregation, Hydration, Ion distribution, etc

**Kratky Plot**
- Folded / unfolded conformation

**Bead model Structure**
- Low Resolution Envelope
- Segmental layout

**SAXS/SANS**
- Structural Info in Real Space: $D_{max}$, Shape, $R_g$, etc

**Caution: Ambiguity!**

**TR-SAXS**
- Global Restraints
- Global Structure of Large RNAs
- Rigid-body Modeling

**Divide-n-conquer**

**Complexes Reconstructed Using sub-units w/ Known Structures**

**Fast structural changes**

**More to come…**

**High Resolution Structures with Accurate Global Shape**

**Unfolded protein**
- Multidomain protein
- Globular protein

**PDDF**
- $D_{max}$

**Notes**
- Saxs/Sans
- Discoveries in Structural Biology and Biophysics
- Overall size: $R_g$
- Molecular weight: $I_0$
- Aggregation, Hydration, Ion distribution, etc

**Folded / unfolded conformation**
Suggested Reading

- **Books:**
  
  

- **Review Articles:**
  
  
  
The End