Hybrid methods using SAXS

Dmitri Svergun
Approaches in structural biology

Individual methods

Hybrid approach

Remember:

SAXS/SANS

are most effective in combination with other methods!
High brilliance beamlines dedicated to SAXS

- **P12 at Petra-3:** About $10^{13}$ photons/seconds in 200*120 µm² (FWHM)
- Energy between 4 and 20 keV (0.6 to 3 Å wavelength)
- Sample-detector distance between 1.5 and 6 m (SAXS/WAXS)
- Typical frame rate: 50 msec

Full automation of the measurements and analysis

Robotic sample changer and on-line SEC/SAXS with MALLS/DLS/RI
Synchrotron beamlines dedicated to or having a significant proportion of biological solution SAXS

- SAXS/WAXS Beamline, Australian Synchrotron, Melbourne, Australia
- SAXS/D, SSRL Beamline 4-2, SLAC, USA
- SAXS endstation at the SIBYLS Beamline, ALS, Berkeley, USA
- SAXS1/2 beamlines at Brazilian Synchrotron Light Laboratory, Brazil
- ID02 SAXS/WAXS/USAXS beamline, ESRF, Grenoble, France
- BM29 BioSAXS Beamline, ESRF, Grenoble, France
- SWING Beamline at Synchrotron SOLEIL, Saint-Aubin, France
- P12 Beamline at DESY (PETRA III), Hamburg, Germany
- cSAXS beamline, SLS, Villigen, Switzerland
- G1 beamline (SAXS/BioSAXS/GISAXS), CHESS, Cornell University, USA
- 4C beamline at PAL (SAXS II), POSTECH, Pohang, South Korea
- 12-BM, 18-ID (BioCAT), Argonne National Laboratory, USA
- BL45XU (RIKEN Structural Biology I) at SPring-8, Japan
- B21 BioSAXS beamline, Diamond, Oxford, UK
- TP25A SAXS beamline, Taiwan Light Source (TLS), Taiwan

Laboratory instruments for BioSAXS (Anton Paar, Bruker, Rigaku, Xenocs)
Advanced methods for SAXS data analysis

Employed by over 14,000 users worldwide

Data processing and manipulations
Rigid body refinement

Ab initio modeling suite
Analysis of mixtures

SAS dissemination and model deposition

The remarkable progress in biological SAXS is prompted by:
- dedicated BioSAXS instruments
- novel analysis methods
- dissemination and standardization efforts
The major problem of SAS

As the scattering data is one-dimensional, reconstruction of 3D models is always ambiguous.
Modern life sciences widely employ hybrid methods.
Hybrid use of SAXS in structural biology

Radiation sources:
- X-ray tube \( (\lambda = 0.1 - 0.2 \text{ nm}) \)
- Synchrotron \( (\lambda = 0.05 - 0.5 \text{ nm}) \)
- Thermal neutrons \( (\lambda = 0.1 - 1 \text{ nm}) \)

Data analysis
- Shape determination
- Rigid body modelling
- Missing fragments
- Oligomeric mixtures
- Hierarchical systems
- Flexible systems

Additional information
- Homology models
- Atomic models
- Distances
- Orientations
- Interfaces

Complementary techniques
- MS
- EM
- Crystallography
- NMR
- Bioinformatics
- Biochemistry
- AUC
- FRET
- EPR

Scattering curve \( I(s) \)
Resolution, nm:
- 3.1
- 1.6
- 1.0
- 0.8

\[ 2\theta \]
\[ k, \quad k=2\pi/\lambda \]
\[ \text{Incident beam} \rightarrow \text{Sample} \rightarrow \text{Detector} \]
\[ \text{Wave vector} \quad k, \quad k=2\pi/\lambda \]
\[ \text{Scattered beam}, \quad k_1 \]

Sample
Solvent
Incident beam
Wave vector \( k, \quad k=2\pi/\lambda \)
Scattered beam, \( k_1 \)
Detector

Resolution, nm:
- 3.1
- 1.6
- 1.0
- 0.8

\( \lg I, \text{relative} \)
\( s, \text{nm}^{-1} \)

\( 02468 \)

\( 1 \)

\( 2 \)

\( 3 \)

\( \text{Scattering curve } I(s) \)

Resolution, nm:
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\( \text{Sample} \rightarrow \text{Solvent} \rightarrow \text{Incident beam} \rightarrow \text{Wave vector} \rightarrow \text{Detector} \rightarrow \text{Scattered beam, } k_1 \)

Reduction sources:
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Examples of hybrid SAXS applications

**SAXS(oshop)** allows for a very effective hybrid modeling by utilizing the scattering data together with a number of structural, biophysical, biochemical and computational approaches

- Macromolecular crystallography (MX)
- Nuclear magnetic resonance (NMR)
- (Cryo)-electron microscopy (EM)
- Fluorescence resonance energy transfer (FRET)
- Biochemistry (labelling, cross-linking)
- Biophysical methods (AUC, DLS, MALLS, CD)
- Computational simulations and docking
Hybrid SAXS/MX

Most popular SAXS shopping tool

MX:
- provides high resolution models of individual subunits, domains or components
- gives possible interfaces of oligomers

SAXS:
- allows one to validate MX models in solution
- gives oligomeric composition
- yields low resolution quaternary structure
- provides information on flexibility and visualizes disordered portions
Hybrid rigid body modelling of multidomain/subunit complexes

A tyrosine kinase MET (118 kDa) consisting of five domains

Program SASREF

Single curve fitting with distance constraints: C to N termini contacts

Catalytic core of E2 multienzyme complex is an irregular 42-mer assembly

The E2 cores of the dihydrolipoyl acyl-transferase (E2) enzyme family form either octahedral (24-mer) or icosahedral (60-mer) assemblies. The E2 core from Thermoplasma acidophilum assembles into a unique 42-meric oblate spheroid. SAXS proves that this catalytically active 1.08 MDa unusually irregular protein shell does exists in this form in solution.

A truncated construct WbdD\textsuperscript{1-459} is monomeric. For the construct WbdD\textsuperscript{1-556} MX yields an active trimer but AAs 505-556 are not seen in the crystal.

SAXS \textit{ab initio} shape reveals that the C-terminal is further extended. A rigid body model was constructed using coiled-coil C-terminal and refining the position of the catalytic domains.

\textit{In vivo} analysis of insertions and deletions in the coiled-coil region revealed that polymer size is controlled by varying the length of the coiled-coil domain.

Crystal structures of substrate-bound chitinase from *Moritella marina* and its structure in solution

Chitinases break down glycosidic bonds in chitin and only few crystal structures are reported because of the flexibility of these enzymes.

The dimeric crystal structure (at BESSY) of chitinase 60 from *M. marina* (MmChi60) contains four domains: catalytic, two Ig-like, and chitin-binding (ChBD). SAXS (at EMBL) demonstrates that MmChi60 is monomeric and flexible in solution. The flexibly hinged Ig-like domains may thus allow the catalytic domain to probe the surface of chitin.

Hybrid SAXS/Biochemistry/Bionformatics

A special SAXSoshopping art usually performed together with MX or NMR

Biochemistry:
- provides possible interfaces in complexes e.g. by site-directed mutagenesis or cross-linking

SAXS:
- Makes the complexes

Bioinformatics:
- Constructs possible complexes, refines the SAXS models
Structural bases for the function of frataxin

Reduced levels of frataxin, an essential protein of yet unknown function, cause neurodegenerative pathology. Its bacterial orthologue (CyaY) forms functional complexes with the two central components to iron–sulphur cluster assembly: desulphurase Nfs1/IscS, scaffold protein Isu/IscU.

SAXS: free IscS is dimeric, free IscU and CyaY are monomeric

Ab initio and rigid body models of complexes: IscU binds on the periphery of IscS dimer, CyaY binds close to the dimerization interface

Structural bases for the function of frataxin

The SAXS-derived models were validated by NMR by measuring spectral perturbation of $^{15}$N labelled CyaY titrated with IscS and further with IscU to up to a 1:1:1 molar ratio. The surface of interaction on IscS was validated by mutations of the residues possibly affecting interaction with CyaY.

Hybrid SAXS/EM

Has now significantly changed because of the resolution revolution in cryo-EM

**EM:**
- provides overall shapes of the macromolecular complexes
- now, also gives (near) atomic structures of frozen samples

**SAXS:**
- may be used to correct the contract transfer function
- can validate EM models in solution
- can use EM structures to look at structural transitions
Study of 70S ribosome *E.coli*

- Molecular mass 2.3 Mda, diameter about 27 nm
- Two unequal subunits, small (30S) and large (50S)
- 30S: 21 individual proteins (TP30)+16S RNA (RNA30)
- 50S: 34 individual proteins (TP50) + 5S RNA+23S RNA (RNA50)
Contrast variation

Yields additional information about shape and internal structure:

- by changing the solvent density and/or
- by selective labeling of specific structure fragments

The use of contrast variation in SAS

Consider a particle consisting of two distinct components with contrasts $\rho_A$ and $\rho_B$. Scattering from such a particle is

$$I(s, \rho_A, \rho_B) = \rho_A^2 I_A(s) + \rho_A \rho_B I_{AB}(s) + \rho_B^2 I_B(s)$$

where $I_A(s)$ is the scattering from component A
$I_B(s)$ is the scattering from component B
$I_{AB}(s)$ is the cross term

If one performs a series of measurements with different $\rho_A$ and $\rho_B$, it is possible to recover partial scattering intensities and thus get additional structural information.
Variation of solvent density

**X-rays:**

*Addition of sucrose or salts*

- RNA, 550 e/nm³
- 60% sucrose, 430 e/nm³
- Protein, 410 e/nm³
- H₂O, 344 e/nm³

**Neutrons:**

*H₂O/D₂O mixtures*

- D-Protein, 130% D₂O
- D-RNA, 120% D₂O
- D₂O, 6.38×10¹⁰ cm⁻²
- H-RNA, 70% D₂O
- H-Protein, 40% D₂O
- H₂O, -0.59×10¹⁰ cm⁻²
Contrast variation on hybrid ribosomes

0% D₂O

40% D₂O

70% D₂O

Protonated 70S ribosome, HH30+HH50

Hybrid 70S with 23S RNA deuterated, HH30+HD50
Scattering data from hybrid ribosomes

Contrast variation by solvent exchange

- HH30+HH50, DD30+HH50, DH30+HH50
  in 0, 35, 50, 75, 100% D$_2$O  
  15 curves
- HH30+DD50 in 0, 35, 50, 75% D$_2$O  
  4 curves
- DH30+DD50 and HH30+DH50
  in 0, 40, 60, 100% D$_2$O  
  4 curves
- HH30 and HH50 in 0, 100% D$_2$O  
  4 curves
- DD30 and DD50 in 0% D$_2$O  
  2 curves

Spin-dependent contrast variation data

- HH30+DD50, DD30+HH50, DH30+DH50
  Polarization = 0 and 1  
  2 curves
- *X-ray scattering curves* from 70S, 30S and 50S  
  3 curves

**TOTAL** 42 curves
Search volume for the 70S ribosome

- Yellow pixels: cryo-EM model of Frank et al. (1995)
- Red and blue circles: dummy atoms belonging to the 30S and 50S subunits, respectively

Number of atoms $M=7860$
Packaging radius $r_0=0.5 \text{ nm}$
Ribosomal data fitted

Neutrons

X-rays


*J. Biol. Chem.* **275**, 14432-14439
A protein-RNA map in the 70S ribosome *E. coli*
Solution *versus* crystal

3 nm resolution neutron scattering model of the 50S subunit in the 70S ribosome *E.coli* (Svergun & Nierhaus, May 2000)

0.24 nm resolution crystallographic model of the 50S subunit *H.marismortui* (Steitz group, August 2000)
Solution versus crystal

3 nm resolution model of the 30S subunit in the 70S ribosome *E.coli* (Svergun & Nierhaus, May 2000)

0.33 nm resolution model of the 30S subunit *Th. Thermophilus* (Yonath group, September 2000)
Solution structure of ribosomal release factor RF1

- Cryo-EM: extended; spans the distance between the ribosomal decoding and peptidyl transferase centers
- Crystal: compact, does not span this distance

In solution, EG makes an L-shaped assembly with subunit-C. This model is supported by the EM showing three copies of EG, two of them linked by C. The data further indicate a conformational change of EGC during regulatory assembly/disassembly.

Inactivation of hematopoietic CSF-1 signaling by the viral decoy receptor BARF1

Hematopoietic human colony-stimulating factor 1 (hCSF-1) is essential for immunity against viral and microbial infections and cancer. The human pathogen Epstein-Barr virus secretes a protein BARF1 that neutralizes hCSF-1 to achieve immunomodulation.

Using MX, EM and SAXS, BARF1 is demonstrated to be flexible and to bind the dimer interface of hCSF-1 locking the latter into an inactive conformation. This suggests a new viral strategy paradigm coupling sequestration and inactivation of the host growth factor to abrogate cooperative assembly of the cognate signaling complex.

Membrane proteins (MPs) were always desired but challenging targets for SAXS studies.

With the on-line SEC-SAXS one can now separate mixtures of protein-loaded and free detergent micelles significantly improving the data quality.

Still, the inherent polydispersity of the detergent environment makes the modeling difficult. Further, detergents may lead to delipidation, instability and to loss of function of the MPs.

A saposin-lipoprotein nanoparticle system, Salipro, is an adaptable nanoscale scaffold system which allows for the reconstitution of membrane proteins in a lipid environment that is stabilized by a scaffold of saposin proteins. (Frauenfeld et al. (2017) Nat. Meth. 13, 345-351)
Stabilization of MPs in salipro NPs

- On their own, Saposin A/lipid NPs form discoidal 8-10 nm particles

- Importantly, salipro NPs can adapt to MPs of various sizes and architectures making the NPs a highly versatile solubilization tool.
Mechanosensitive T2 ion channel protein

The proteins solubilized in Salipro NPs form clearly smaller particles compared to DDM and do not display «micellar» features. Direct shape determination of T2 yields results agreeing well with cryo-EM structure of TRPV1 ion channel (Liao et al (2013), Nature, 504, 107)

A. Flayhan, H. Mertens, Y. Blimke, M. Molledo, D. Svergun, C. Löw (2017), accepted
Hybrid SAXS/FRET

Not yet broadly used but very promising

**FRET:**
- provides estimates of distances between the labelled parts of macromolecules

**SAXS:**
- uses these distances for model building or for validation of the models
Architecture of nuclear receptor heterodimers on DNA direct repeat elements

Nuclear hormone receptors (NHRs) control numerous physiological processes through the regulation of gene expression. SAXS, SANS and FRET were employed to determine the solution structures of NHR complexes, RXR–RAR, PPAR–RXR and RXR–VDR, free and in complex with the target DNA.

Ab initio and rigid body models of NHRs complexed with direct repeat elements

Architecture of nuclear receptor heterodimers on DNA direct repeat elements

NHR-DNA complexes show extended asymmetric shape and reveal conserved position of the ligand-binding domains at the 5′ ends of the target DNAs. Further, the binding of only one coactivator molecule per heterodimer, to RXR’s partner, is observed.

The models and the polarity of RXR–RAR–DR5 and RAR–RXR–DR1 were validated using neutron scattering and FRET.

Resolving a SAXS/FRET controversy for IDPs

Chemically denatured proteins and intrinsically disordered proteins (IDPs) populate heterogeneous conformational ensembles in solution. SAXS measures their average size as the radius of gyration ($R_g$). Single-molecule FRET (smFRET) provides the mean dye-to-dye distance ($R_E$) for proteins with fluorescently labeled termini. Several studies reported inconsistencies between SAXS and smFRET on native and chemically denatured IDPs.

**SAXS:** $R_g$ only marginally changes upon chemical denaturation of an IDP

**smFRET:** $R_E$ significantly increases when an IDP is chemically unfolded suggesting that a native IDP is in a “collapsed” state

Resolving a SAXS/FRET controversy for IDPs

These differences were typically attributed to the influence of the fluorescent labels for FRET and/or to the higher concentrations and averaging for SAXS. A collection of ten labelled (FRET) and labelled/unlabeled (SAXS) IDPs, with the numbers of residues ranging from 36 to 176 was measured in native and chemically denatured states. The contributions of dyes and concentration effects were shown to be minimal. The discrepancy between SAXS and FRET was still clearly observed.

![Graph showing relative change (IDP-denatured)]
Resolving a SAXS/FRET controversy for IDPs

SAXS provides not just the $R_g$ but also the overall shape!

In the above normalized plot A, a map of “asphericity” is given: the more to the right, the more anisometric the average shape is (given the same $R_g$).

Red: native IDP

Blue: chemically denatured IDP

The native IDP ensembles populate more isometric states compared to the unfolded IDPs
Resolving a SAXS/FRET controversy for IDPs

Atomistic simulations of the IDPs were conducted using the CAMPARI force-field with ABSINTH implicit solvation shell. The ensembles were reweighted to agree with the FRET and SAXS observations, and the chemically denatured ensembles clearly displayed more anisometric appearance. Therefore the observed increase in $R_E$ is simply a consequence of higher anisometry of the chemically unfolded IDPs compared to natives.

Hybrid SAXS/NMR

**The most natural SAXS/ma*shopping:** both techniques are applied to solutions in similar conditions and are highly complementary. Both can be used for flexible systems

**NMR:**
- provides local information and is sensitive to orientations of elements (RDC)

**SAXS:**
- provides global information and is most sensitive to movements of elements
Dynamics and function of the C-terminus of the *E. coli* RNA chaperone Hfq

The hexameric Hfq (HfqEc) is involved in riboregulation of target mRNAs by small trans-encoded RNAs. Hfq proteins of different bacteria comprise an evolutionarily conserved core, whereas the C-terminus is variable in length.

By bioinformatics, NMR, synchrotron CD and SAXS the C-termini are demonstrated to be flexible and to extend laterally away from the hexameric core. The flexible C-terminal moiety is capable of tethering long and structurally diverse RNA molecules.

DsrA domain II bound to the RNA chaperone Hfq

A small regulatory RNA (DsrA) associates with the RNA chaperone Hfq and requires this protein for regulation of target E. coli rpoS mRNA, encoding the stationary phase sigma-factor. Previous studies suggested that the hexameric E. coli Hfq (HfqEc) mostly binds sRNAs on the proximal site.

NMR data: superposition of the $^{1}$H-$^{15}$N HSQC spectra of Hfq$_{Ec65}$ RNA free form (blue) and in complex with DsrA$_{34}$ (red) and chemical shift differences. The residues with differences above the threshold are coded red in the Hfq$_{Ec65}$ model

DsrA domain II bound to the RNA chaperone Hfq

SAXS on truncated and full length Hfq complexes reveals 1:1 complexes with a limited flexibility of sRNA, allowing one to visualize the sRNA conformational space.

Conformational activation of C-terminal tunes the Ca\textsuperscript{2+}-binding affinity of S100A4

S100A4 is associated with increased metastasis properties. Its interactions to binding partners (e.g. p53, annexin etc) are mediated by Ca\textsuperscript{2+}-induced conformational changes, which open up a hydrophobic cleft on the surface. S100A4 has an unstructured C-terminal, which may interact with the cleft.

SAXS, NMR and ITC were employed to analyze Ca\textsuperscript{2+}-induced changes on the wild type protein and its C-terminal deletion mutant. SAXS and NMR indicate that the C-terminus is extended when Ca\textsuperscript{2+} is bound, while no effect is observed on the mutant. The results are further rationalized by MD simulations.

Hybrid SAXS user projects at the EMBL

- Surface protein SASG

- kLANA/DNA complexes
  - Ponnusamy et al. NAR (2015)

- Transcription factor heterodimer
  - Matiasen et al. FEBS J (2016)

- Folded RTX Domain of CyaA

- SaThiM from vitamin B1 synthetic pathway

- Bivalent binding to BET bromodomains

- Tungstate transporter protein TupA

- Chromatin remodeling enzyme Chd1

- Transcription factor heterodimer

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The perspectives of integrative modelling

• More and more complicated questions are posed and more complex systems are studied, and synergistic use of complementary information is a must.
• Integrative modelling is the most promising way for characterizing complicated systems over broad ranges of sizes, spatial and temporal resolutions.
• Infrastructural European initiatives promote integrative structural studies and SAXSoshopping is one of the major players in the field of hybrid modelling.
iNEXT – Infrastructure for Structural Biology

Infrastructure for NMR, EM & X-rays for Translational research

www.inext-eu.org

iNEXT expands the availability of structural biology services to new communities of users, and in particular to scientists with backgrounds other than structural biology, including from SMEs

- Six NMR centres
  - Berlin, Brno, Florence, Frankfurt, Lyon, Utrecht (coordinator)

- Six synchrotron sites
  - Diamond, EMBL-HH, EMBL-GR, ESRF, Lund, Soleil

- Five EM facilities
  - Brno, Diamond, EMBL-HD, Leiden, Madrid

- Protein interactions in vivo and in vitro
  - Amsterdam (deputy coordinator), EMBL-HD

- Research Partners
  - Aarhus, Lund

- Training centres
  - Brno, Budapest, Lisboa, Oulu, Patras, Rehovot

- ESFRI Projects
  - ESS, Instruct (and EuroBioImaging, EU-Openscreen affiliated)

23 partners; started September 1, 2015; synchrotron access as of March, 1, 2016

There are also access possibilities for non-EU users!
Future directions of biological SAXS

- Structural methods, especially MX, now face the ‘resolution revolution’ challenge in cryo-EM
- For SAXS, traditional studies of static overall (quaternary) structure and especially structural transitions will stay important thanks to the speed and automation of modern SAXS.
- Other directions utilizing the unique capabilities of SAXS as a structural technique (and combined with other methods in hybrid applications) are expected to play an increasing role in future
Future directions of biological SAXS

- Combinatorial high throughput ligands/additives screening
- The use of WAXS to assess tertiary structure and transitions
- Studies of equilibrium mixtures and membrane proteins, especially in combination with online SEC-SAXS and biophysical analysis
- Flexible fragments and entire macromolecules, including intrinsically disordered proteins
- Time-resolved studies of processes, from sub-ms ((un)folding) to hours (oligomerization, fibrillation)
Some words of caution

- Thoroughly characterize your samples PRIOR TO doing SAS!
- Try to utilise other methods but NEVER trust them blindly!
- Always check integral parameters BEFORE 3D modelling!
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http://www.embl-hamburg.de/biosaxs

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- S100A4: G. Katona (Gothenburg University)
- IDPs: E. Lemke (EMBL, Heidelberg)
- Hfq/DsrA: K. Djinovic-Carugo (Vienna University)
- Tyrosine kinase Met5: E. Gherardi (Pavia University)
- Frataxin: A. Pastore (NIMR MRC, London)
- V-ATPase stator: J. Fethiere (University of Montreal)
- Nuclear receptor: D. Moras (CNRS, Strasbourg)
- Ribosome: H. Stuhrmann (GKSS, Geesthacht), K. Nierhaus (MPIMG Berlin)
- Saposin: C. Loew (EMBL Hamburg)
- WbdD: J. Naismith (St Andrews University)