Small-Angle X-ray Scattering

Learn the story of your protein

Nozomi Ando @ Cornell — CHESS HP Bio Workshop 2021 — April 29 2021
Structural biology techniques

**Crystallography**

**Cons:**
- must crystallize protein
- crystallization artifacts

**Pros:**
- structure determination can be fast
- still the gold standard for atomic detail


**SAXS**

**Cons:**
- low resolution (10-20 Å)
- interpretation best done in reciprocal space

**Pros:**
- insight into protein behavior in solution
- connects structural data and tells a story


**Cryo-EM**

**Cons:**
- hardware and software barriers
- achieving < 3 Å resolution is not trivial

**Pros:**
- game changer for large and slightly heterogeneous samples

Structural biology techniques

**SAXS**

**SAXS fundamentals:** How to process and interpret data

**Pro tips on sample preparation:** How and more importantly, why?

**Advanced analysis:** Extracting more information from data

*Cons:*
- low resolution (10-20 Å)
- interpretation best done in reciprocal space

*Pros:*
- insight into protein behavior in solution
- connects structural data and tells a story


Nozomi Ando — CHESS HP Bio Workshop 2021
A brief history of SAXS in structural biology

SCATTERING IN SILICA. These 30-year-old photographs show small-angle scattering in silica gel (above) and the lack of such scattering in vitreous silica (below). The difference arises because the gel divides into small particles, whereas the vitreous sample is homogeneous.

—FIG. 2

Guinier, “30 Years of Small-Angle X-Ray Scattering”, Physics Today Nov. 1969
A brief history of SAXS in structural biology

1995: calculation of SAXS profiles from atomic coordinates

1997: ab initio shape determination

A brief history of SAXS in structural biology

1995: calculation of SAXS profiles from atomic coordinates

1997: \textit{ab initio} shape determination


Nozomi Ando — CHESS HP Bio Workshop 2021
A brief history of SAXS in structural biology

1995: calculation of SAXS profiles from atomic coordinates

1997: ab initio shape determination

Fig. 2. Shape determination of the ribonucleotide reductase R1 protein.

Case study: Ribonucleotide reductase

Ribonucleotides → ATP → dATP → Deoxyribonucleotides

ribonucleotide reductase (RNR)

Nozomi Ando — CHESS HP Bio Workshop 2021
Enzyme activity requires two proteins to transiently interact

*E. coli* Class Ia RNR

Class I RNRs require inter-subunit radical transfer.

Nozomi Ando — CHESS HP Bio Workshop 2021
The hunt for the active complex

1961: Discovery of RNR in *E. coli*

1990, 1994: Crystal structures of subunits

1997: SAXS of $\alpha_2$ subunit

$\alpha_2\beta_2$ docking model proposed

2009: Drug-inhibited crystal structure

Nozomi Ando — CHESS HP Bio Workshop 2021
SAXS reveals two complexes and connects structural data

Negative-stain electron microscopy (EM)

Analytical ultracentrifugation (AUC)

Small-angle X-ray scattering (SAXS)

X-ray crystallography

physiological (1 - 2 μM)

[0.15 μM]
[1 - 10 μM]
[2 - 30 μM]
[25 μM]

Nozomi Ando — CHESS HP Bio Workshop 2021

SAXS reveals two complexes and connects structural data

Analytical ultracentrifugation (AUC)

X-ray crystallography

Small-angle X-ray scattering (SAXS)

Negative-stain electron microscopy (EM)

physiological (1 - 2 μM)

[0.15 μM]

2 - 30 μM

1 - 10 μM

25 μM

Nozomi Ando — CHESS HP Bio Workshop 2021

Structure of dATP-inhibited $\alpha_4\beta_4$ complex

Nozomi Ando — CHESS HP Bio Workshop 2021

Structure of dATP-inhibited $\alpha_4\beta_4$ complex

Structure of dATP-inhibited $\alpha_4\beta_4$ complex

3D EM reconstruction

2 asymmetric units

Ed Brignole

Christina Zimanyi

SAXS connects conformational states and data from other techniques

α₂ + β₂ (inactive) ↔ α₂β₂ (active) ↔ α₄β₄ (inactive) ↔ α₈β₈ (inactive)

allosteric regulation of activity

kinetic stabilization during radical transfer

crystallization

Activity regulation in the enzyme family

**dATP** binding to N-terminal **ATP-cone domain** promotes quaternary structures that are incompatible with catalysis.

Emergence of the class Ib RNRs

Class Ib RNRs lost allosteric activity regulation.
Signatures of activity regulation... in a class Ib RNR?

The catch? This is a class Ib RNR.

Nozomi Ando — CHESS HP Bio Workshop 2021

Anion exchange (AEX)-coupled SAXS

scattering images

anion-exchange chromatography-coupled SAXS

Regals: regularized alternating least-squares

Protein peaks:
Background:


Nozomi Ando — CHESS HP Bio Workshop 2021
AEX-SAXS reveals a new oligomeric form

Endogenous ligand (dAMP) binds to truncated N-terminal domain.
Mapping allosteric transitions with SAXS

Step 1: Anion-exchange (AEX-SAXS) + Regals

identify liganded states

Step 2: Titration SAXS + singular value decomposition (SVD)

identify structural transitions

Step 3: Size-exclusion (SEC-SAXS) + evolving factor analysis (EFA)

structural modeling


Discovery of a new inhibition mechanism

From SAXS, we knew:

Endogenous ligand binds 1 site per monomer to produce an I-dimer. dATP converts both the monomer and I-dimer to the same filament.

∴ There are 2 binding sites for dATP & the I-dimer is part of the filament.

2.5 Å crystal structures

4.8 Å cryo-EM structure

4.7 Å cryo-EM structure

SAXS model

An elegant form of convergent allostery

dATP at I-site sequesters $\alpha_2\beta_2$ units into a filament.

**ATP** at I-site acts to siphon the active form of $\alpha_2\beta_2$.

Nozomi Ando — CHESS HP Bio Workshop 2021

Take-home messages

- SAXS

  Low-resolution but provides incredible insight into protein behavior in solution.

  Easy to change solution conditions and do a comprehensive search of conformational space.

  SAXS connects data from other techniques.

  SAXS allows comparative study of protein homologs.

  SAXS tells a story.