Small-Angle X-ray Scattering (SAXS)

Naoto Yagi
Hiroshi Sekiguchi
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1. Learning what is SAXS ...and WAXS

Non-crystalline diffraction ... what is diffraction? what is scattering?
Hierarchy in non-crystalline materials
Pitfalls in SAXS

2. Visiting three beamlines: BL40XU, BL40B2, BL45XU

These three SAXS beamlines in SPring-8 have different x-ray sources and optics. To have an actual look at these beamlines is a valuable experience.
BL40XU:
http://www.spring8.or.jp/wkg/BL40XU/instrument/lang-en/INS-0000000353/instrument_summary_view
BL40B2:
http://www.spring8.or.jp/wkg/BL40B2/instrument/lang-en/INS-0000001280/instrument_summary_view
BL45XU:
http://www.spring8.or.jp/wkg/BL45XU/instrument/lang-en/INS-0000000334/instrument_summary_view
BL03XU

3. Understanding optics for SAXS

Using the above three beamlines as examples, designs of SAXS beamlines are explained.
BL40XU: helical undulator --- double focusing mirrors Pink beam!
BL40B2: bending magnet --- double crystal monochromator --- bent cylindrical mirror
BL45XU: tandem vertical undulators --- double crystal diamond monochromator --- double focusing mirrors
BL03XU: undulator --- double crystal Si monochromator --- double focusing mirrors
Other beamlines: BL20XU and beamlines in other facilities.

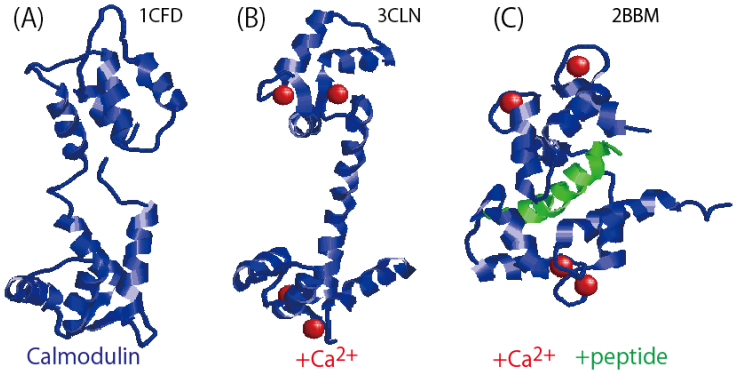
4. Understanding detectors for SAXS

Several different types of detectors are used at the above three beamlines. Apart from basic detectors such as ion chambers, they are all area detectors.
RAXIS: image plate detector
X-ray image intensifier + CCD camera: high sensitivity and fast readout
CMOS flatpanel: solid-state area imager
PILATUS: photon-counting pixel detector

5. Protein solution scattering measurements at BL40B2

A protein solution scattering experiment will be conducted at BL40B2. The target protein is calmodulin, and its conformational change upon binding Ca^{2+} ion and a peptide will be analyzed by SAXS.

Calmodulin (an abbreviation for CALcium-MODULated proteIN) is a calcium-binding messenger protein expressed in most eukaryotic cells. Calmodulin acts as an intermediary protein that senses the calcium level and relays the signal to various calcium-sensitive enzymes, ion channels and other proteins. Calmodulin is a dumbbell-shaped protein composed of two globular domains connected together by a linker (Fig. A). Each domain binds two calcium ions as shown in Fig. B. The linker between the domains is a long alpha helix. Calmodulin typically wraps around its target peptide, with the two globular domains gripping either side of it (Fig. C). Brief introduction of calmodulin is in the following website (Protein Data Bank, Molecule of the Month, http://www.rcsb.org/pdb/101/motm.do?momID=44).



Mastoparan is used as a target peptide for calmodulin in this beamline practical. It is a peptide toxin from wasp venom, and its amino acid sequence is following, Ile-Asn-Leu-Lys-Ala-Leu-Ala-Ala-Leu-Ala-Lys-Lys-Ile-Leu.

SAXS measurement will be obtained under the following condition,

Wavelength of incident X-ray: 1 Å,
Sample-to-detector distance 1150mm,
Exposure time: 30 sec.

Since scattering angle from the sample depends on the size of the molecule and the wavelength of the incident X-ray, the sample-to-detector distance has to be set accordingly.

The exposure time has to be determined by considering the quality of data and the X-ray radiation damage on the sample (X-ray radiation damage is common problem in SAXS measurement of proteins.).

The actual sample-to-detector distance is calibrated by irradiating a reference sample of known structure. We usually used silever behenate as a reference sample (d_{001}= 58.38 Å).

In the first step, we will obtain the scattering from calmodulin solution in the absence of Ca^{2+} ion and mastoparan under different calmodulin concentrations, such as 5.0 mg/ml, 2.5 mg/ml, 1.25 mg/ml, in Buffer A. Scattering from Buffer A will be measured on every calmodulin measurement to obtain the scattering of calmodulin (When we have obtained two experimental scattering profiles, one from the protein solution and one from the buffer, we subtract one from the other to get the scattering of protein only.)

Buffer A
50mM Tris-HCl pH=7.2, 100mM NaCl, 1mM EDTA

Next, we will obtain the scattering from calmodulin in the presence of Ca^{2+} ion and mastoparan under different calmodulin concentrations, such as 5.0 mg/ml, 2.5 mg/ml, 1.25 mg/ml, in Buffer B.

Buffer B
50mM Tris-HCl pH=7.2, 100mM NaCl, 1mM EDTA, 5mM $CaCl_2$, 675μM Mastoparan

Calmodulin, molecular weight=16706 10mg/mL=599μM
Mastoparan, molecular weight =1479 1mg/mL=676μM

6. Practicing data analysis

Introduction to widely used SAXS data processing software (fit2D, PRIMUS, etc.)

Important formulae:

Definition of "q". 2θ is the scattering angle.

$$q = 4\pi \frac{\sin(2\theta/2)}{\lambda}$$

Guinier Plot ... R_g is radius of gyration

$$I(q) \propto \exp(-\frac{q^2 R_g^2}{3})$$

Pair distribution function ... Fourier transform of autocorrelation function

$$P(r) = \frac{r}{2\pi^2}\int_0^\infty I(q) q \sin(qr) dq$$

Scattering from a sphere (radius=R)

$$I(q) = I_e V^2 \rho_0^2 \left[\frac{3[\sin(qR) - (qR)\cos(qR)]}{(qR)^3}\right]^2$$

Scattering intensity

$$I(q) = I_e \left|F(q)\right|^2 = I_e \int_v \rho(r_k) e^{-iq\cdot r_k} dr_k \int_v \rho(r_k) e^{iq\cdot r_k} dr_k$$