

Small Angle Scattering for Structural Characterization

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*Basics of small-angle X-ray/neutron scattering (SAXS/SANS)

*Small/Wide angle X-ray scattering (SAXS/WAXS) instrumentation

*Protein solution SAXS







Structure Controls & Civilization

※ Progressive understanding and Controls of Structures
對結構理解與控制的尺度精度
※ 公分(cm) → 厘米(mm) → 微米(micron) → 次微米 (submicron) → 晶體原子結構 (angstrom)
※ 奈米(nm)

The civilization and revolution 當代的文明特徵

※ 石器(stone) → 鐵器 (iron)→ 橡膠(plastic) → 矽晶片 (semiconductor)電子元件

※ 奈米科技(分子科技)(nanotech. & molecular biology) Button up structure

Better understanding and controls are motivated by desiring a better life. But, how to proceed?

Methodology in Structural Understanding

(1) Imaging: light and X-rays (Real space tools)

TEM, SEM, confocal microscopy, XEM Field-Ion microscopy

- (2) Scattering: light, X-rays, neutrons and particles in reciprocal *k*-space or *Q*-space; exp(*ikr-i*\omegat)
- Diffraction for crystal structure (atomic resolution crystallography, powder diffraction)
 Reflection for depth density profile, surface & interface structure of lipid membranes or monolayers
 Small-angle scattering, coherent scattering for non-crystalline structures like proteins in solutions
 Inelastic scattering for structural dynamics, phonons in a liquid crystalline phase







X-ray scattering probes structures from atomic – molecular (micrometer) structures



With elastic X-ray/neutron scattering (no energy loss) $|\vec{k}_f| = |\vec{k}_i| = k = \frac{2\pi}{\lambda}$ $q = 2k = 4\pi \sin\theta/\lambda$

SAXS/SNAS probes structure in the direction of *q*, *scattering wave vector* 2*θ*: *scattering angle*

Typical Q (angle) range in SAXS $0.005 - 0.5 \text{ Å}^{-1} (0.008^{\circ}-8^{\circ})$

Scattering Intensity distribution profile $I(Q)_{abs}$

One-dimensional scattering cross section as a function of Q

$$\frac{d\sigma(Q)}{d\Omega} = \left\langle \left| \int e^{ik \cdot r} V(r) e^{-ik' \cdot r} d^3 r \right|^2 \right\rangle = \left\langle \left| \int e^{iQ \cdot r} V(r) d^3 r \right|^2 \right\rangle$$

$$V(r) = \sum_j b_j \delta(r - r_j) \Rightarrow \text{Scattering Potential (or density distribution of scatterers)}$$

$$I(Q)_{abs} = \frac{d\sigma}{d\Omega} = \left\langle \sum_{i,j} b_i b_j e_{i,j} e_{i,j} \right\rangle \text{Bragg diffraction conditions for 2dsin\theta = n } \lambda$$

$$Q = 4\pi \sin\theta/\lambda$$

 b_j : Interaction strength or scattering amplitude (length) of the scatterer (cross-section) (electrons for X-ray , nucleus for neutrons), *for* the probe particles

Small-angle Scattering: at small angles, $Q(r_i - r_j) \ll 1$, summation of each individual atoms b_i is replaced by an integration for a low-resolution density distribution function $\rho(r)$

$$I(Q) \sim \left| \int \rho(\mathbf{r}) e^{-i\mathbf{Q}\cdot\mathbf{r}} d^3 r \right|^2$$

Experimentally,

l J

molecular resolution

(1) Measured scattering intensity distribution, $I(Q) \Rightarrow I(Q)_{abs}$ (2) From $I(Q)_{abs}$, deduce electron/nuclide density distribution ρ (r)

General relation between the scattering angle (θ or q) and the characteristic length-scale D probed : q (or scattering angle) ~ 1/L



For one-component isotropic colloidal system with scattering basic units (form), the SAXS intensity distribution function can be written as

Io: Zero angle scattering intensity (cross section/contrast) $I_o = CNV_p^2(\rho_{scatt} - \rho_{matrix})^2$ **P(Q)**: Normalized particle structure factor, determined by size and shape of scattering particles $P(Q) = |\int \rho(\mathbf{r})e^{-iQ\cdot\mathbf{r}}d^3r|^2$ **S(Q)**: inter-particle structure factor; determined by particle distribution (i.e. pair-correlation function).

C: concentration of scattering particles N: aggregation number (=1 for no aggregation) V_p : dry volume V for the scattering particles ρ_{scatt} : scattering length density of the scatter (scatter density and scattering length summed from all atoms) ρ_{matrix} : scattering length density of the matrix



SAXS can differentiate size and shape in a nanometer scale



Radius of gyration R_g for particles of irregular shapes - Guinier approximation ($Qr \ll 1$; expansion of exponential term)

 $P(Q) = \left| \int \rho(\mathbf{r}) e^{-iQ \cdot \mathbf{r}} d^3 r \right|^2$

* Particles of irregular shapes (then ignore shape for size only; ρ = constant

 $I(Q) \approx I_o \exp(-Q^2 R_g^2/3) \qquad Q_x^2 X$ $ln(Q) = -(1/3) R_g^2 Q^2$ (Guinier approximation is valid when QR_g <<1) R_g: Radius of gyration

 $R_g = \int \rho(r) r^2 dV / \int \rho dV$ For spheres of uniform density ρ

$$R_g^2 = 4\pi\rho \int_0^R (r^2) r^2 dr / (\rho V) = (3/5)R^2$$

 $R_{\rm g}$ and scattering shape !!

 $Q_x^2 X^2 + Q_y^2 Y^2 + Q_z^2 Z^2 \approx 1/3Q^2 R^2$





For one-component isotropic colloidal system, the SAXS intensity distribution function can be written as

- **Io**: Zero angle scattering intensity $I_o = CN(f \rho_w V_p)^2$
- P(Q): Normalized particle structure factor, determined by size and shape of the scattering particles
- S(Q) : inter-particle structure factor; determined by particle pair-correlation function (particle distribution).

Ordered arrangement of scattering particles in space - Structure factor S(Q)

Colloidal solution containing monodisperse particles: $I(Q) = n_p P(Q)S(Q)$

 $n_p = N/Vs$: Number density of particles

 $P(Q) : \int_{V} \rho(r) d^3r$, Particle form factor (intra-particle interference) S(Q): Structure factor (interference between scattering particles)

 $S(Q) = \frac{1}{N} \left\langle \sum_{i=j}^{N} \sum_{p=1}^{N} e^{i\mathbf{Q}\cdot(\mathbf{R}_{i}-\mathbf{R}_{j})} \right\rangle$ = 1 + n_p $\int_{0}^{\infty} 4\pi r^{2} (g(r) - 1)(\sin(Qr)/Qr)dr$ $g(r) = (1/\langle \rho(\mathbf{r}) \rangle^{2}) \int \rho(\mathbf{r}') \rho(\mathbf{r'-r}) d^{3}r \Rightarrow \text{pair correlation function}$ $[s(0)]^{-1} = (1/k_{b}T)(\partial \Pi/\partial n_{p}) = 1 + 2B_{2}n_{p}; \text{ (related to compressibility)}$ $B_{2} \Rightarrow \text{the second Virial coefficient}$

Structure factor *S*(*Q*) **for hard spheres**

 $S(Q) = 1 + n_p \int_0^\infty 4\pi r^2 (g(r) - 1)(\sin(Qr)/Qr) dr$

g(r) = Pair correlation function for the nanograins (excluded volume effect)Hard sphere : <math>G(Q) = 1/[1 - G(Q)]



S(Q) for more ordered structure Self-assembly of block copolymers for meso-scale (10-100 nm) ordered structures

* P(Q):domain size, shape(sphere, rod, cylinder, etc..)

- S(Q):ordering of domains
 Q-ratio of the scattering peaks
- (1) Periodic structure :
 - 1:2:3:4...;
- (2) Body-centered cubic array : 1: √2 : √3 : √4 : √5;
 (3) Hexagonal array 1: √3 : √4 : √7 : √9 : √12



Extract form factors of NPs & copolymer block from a copolymer/NP composite Copolymer PS-b-P4VP/Nanoparticles complex— ordered meso-scale phase separation



What can be learned from protein solution SAXS: Fold structure and charge interactions of lysozyme



Fractal aggregation of hydrophilic fullerenederivatives $C_{60}(OH)_{18}$ in aqueous solution

*** Pair correlation function**

 $g^{\overline{N}^*}(r) \propto r^{D-d} \exp(-r/\xi)$

S(Q) for fractal structure

$$S(Q) \sim \frac{1}{Q^{D}} \frac{D\Gamma(D-1)}{\left(1 + (Q\xi)^{-2}\right)^{(D-1)/2}} \sin[(D-1)\tan^{-1}(Q\xi)]$$

 $P(Q) \sim 1 \implies I(Q) = P(Q)S(Q) \sim S(Q)$

C (wt %)	I(0) (cm ⁻¹⁾	$R_g(Å)$	$R_g^*(Å)$	ξ (Å)	N	N'
0.625	0.21	22.4 ± 3.0	28.3	13.4	48	40
1.25	0.39	25.6	31.7	15.0	55	54
2.5	1.04	26.9	37.2	17.6	74	80
4.0	3.21	34.3	44.2	20.9	113	125
*10.0	5.18	—	37.4	17.7	—	—



Dummy residues simulation package for protein unfolding structure





Re-calculate SAXS profile

Gnom package

Protein pair correlation function



 C_{α} Bound water Fit p(r) using Gsbor package based on dummy residues

CRYSOL + modified PDB

21

GNOM Program

Transform data to distance distribution function p(r) with GNOM program and do data fit.

PmrA, $D_{max} = 105 Å$



22

Dmitri I Svergun and Michel H J Koch, EMBL Rep. Prog. Phys. 66 (2003) 1735

Distance distribution functions of

typical geometrical bodies.

Available on-site DATA simulation kit with ATSAS developed by the EMBL group



ATSAS 2.5.1 (analysis software)



VMD (molecular graphics software)



VMD 1.9.1

3. Data analysis and Model simulation

• Crysol fitting

Compare (fit) solution structure with PDB crystal structure.



Dammin Program

An ensemble of dummy atom model simulation with P(r) output by GNOM.





GASBOR Output Files:

- 1. log file: contain the same information as the screen output.
- 2. fir file: fit to the raw experimental data
- **3. pdb** file: resulting model in PDB-like format that can be viewed with MASSHA (in ATSAS package) or VMD (molecular visualization program)

• Gasbor Program

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Fitting SAXS data with SASref for relative orientation of two-domain proteins of respective PDB files (CY Chen et al., NSRRC)





Bio-SAXS Check list





EMBL Hamburg BioSAXS team effort in ATSAS software package. The data analysis software can be downloaded from http://www.embl-hamburg.de/biosaxs/atsas-online/download.php

user forum for software problems http://www.saxier.org/forum/viewforum.php?f=5 #Introduction of small angle X-ray
scattering (SAXS)

Synchrotron small/wide angle X-ray scattering (SWAXS) instrument

***SAXS** activities at the NSRRC

Introduction of X-ray source for scattering

Traditional X-ray Source





Advantages of using Synchrotron Radiation X-ray

• High Flux 3x10¹¹ photon/s (time-resolved measurement)

• Tunable wavelength (anomalous scattering for multiphase structure)





2014 Commissioning of Taiwan Photon Source

Taiwan light source TLS (1.5 GeV; 120 m)

Taiwan photon source TPS (3.0 GeV, 548 m)

同步加速器聚頻磁鐵光亮度比較 (TLS, SLS, Diamond, SPring-8, TPS)



Synchrotron SAXS instrumentation

The newly installed BL23A SAXS beamline

Low beam divergence
Wide energy range
High flux and high energy resolution
deflectable beam



BL23A SWAXS beamline: Energy Resolution/Photon Flux regulated by the DCM/DMM



The inner structure of DCM/DMM monochromator.

Performance of DCM/DMM



The SWAXS (synchronized SAXS & WAXS) instrument at BL23A1 Endstation of the NSRRC



Highly automatized BL23A SWAXS instrumental the NSRRC

Collimation stage

Sample stage

Automatic Bellow system for continuous changes in S-D distance under vacuum

Interchangeable CCD/gas detectors

for SAXS

Two linear detectors

for WAXS

Liu, D. G. et al. J. Synchrotron Rad., **2009**, 16, 97. Jeng, U. et al. J. Appl. Cryst. 2010, 43. 110

Feb. 25-26, 2013 Tokai Japan 23A SWAXS Detecting System

MarCCD (SAXS)

Flat Panel (2D WAXS)

Mythen 3K (WAXS-V)

> 1D Gas Detector (WAXS-H)

Pilatus 1M-F (SAXS)

SAXS Dets (1100 – 5000 mm)

Flat Panel (90 – 200 mm)

NSRRC BL23A Small/Wide-Angle X-ray Scattering

1D Dets (400 – 1000mm)

44

Sample Stage (0 mm)

0

tront View/Back View

TIA

Computer controlled sample-to-detector distance (1 - 5 m) with vacuum bellow system

beam energy keV etectable in (complex) hology 100 nm size

Integrated Triggering System for milli-second SAXS/WAXS

Activated by SAXS detecting sys.



Current Detectors

Pilatus 1M-F



Mythen 1k *3



Flat Panel



Pixel size	172 μm	50 μm	50 μm
Format	981 x 1043	1280 x 3 (Linear)	1032 x 1032
Frame Rate	133 Hz	472 Hz	3.3 Hz
	SAXS	1D WAXS	2D WAXS



NSRRC BL23A SWAXS Endstation

Pinhole/slits system

.5m

.3mm

.4mm

Collimation stage with 2 pin-hole slit sets



Sample environments for correlations between structure and property





In-situ LN2 gas cooling process from 320-243 °C Cooling rate 200 °C/min Or water cooling >100 °C/s



Performance for simultaneous ms-SAXS/WAXS for structural evolution

The simultaneous SAXS/WAXS measurements of the melt-crystallization process from 200°C to 86°C of isotactic polypropylene (500 ms resolution). (Applicable to protein crystallization processing?? morphology change before crystallization)



Pilatus

Mythen



W.R. Wu, et.al. ACS Nano 5 , 6233, 2011

Unique Opportunity with the new SWAXS instrument — GISAXS for nanostructures on liquid surfaces



At 25 oC: Lamellar-to-H2D route (GISAXS)

Molar ratio: H2O : HCl : CTABr (61 mM) : TESO = 100: 2: 0.11 : 0.16







SAXS working with complementary tools for Protein folding-unfolding













Prof. Ying-Huang Lai (頑英煌) and his team

Team members / Collaborators



➢ SAXS group members: Chun-Jen Su, Dr. (蘇群仁; 23A beamline manager); Yi-Qi Yeh (葉 奕琪); Liao, Kuei-Fen (廖桂芬); Wen-Bin Su (蘇文斌); Wei-Ru Wu (吳瑋儒);
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