Small-Angle X-ray Scattering (SAXS)

SPring-8/JASRI

Naoto Yagi

Wikipedia

Small-angle X-ray scattering (SAXS) is a small-angle scattering (SAS) technique where the elastic scattering of X-rays (wavelength 0.1 - 0.2 nm) by a sample which has inhomogeneities in the nm-range, is recorded at very low angles (typically 0.1 - 10˚). This angular range contains information about the shape and size of macromolecules, characteristic distances of partially ordered materials, pore sizes, and other data. SAXS is capable of delivering structural information of macromolecules between 5 and 25 nm, of repeat distances in partially ordered systems of up to 150 nm. USAXS (ultra-small angle X-ray scattering) can resolve even larger dimensions.
SAXS and USAXS belong to a family of X-ray scattering techniques that are used in the characterization of materials. In the case of biological macromolecules such as proteins, the advantage of SAXS over crystallography is that a crystalline sample is not needed. NMR methods encounter problems with macromolecules of higher molecular mass (> 30000-40000). However, owing to the random orientation of dissolved or partially ordered molecules, the spatial averaging leads to a loss of information in SAXS compared to crystallography.

“scattering” vs. “diffraction”

scattering

X-ray changes its direction by interaction with a non-periodic material

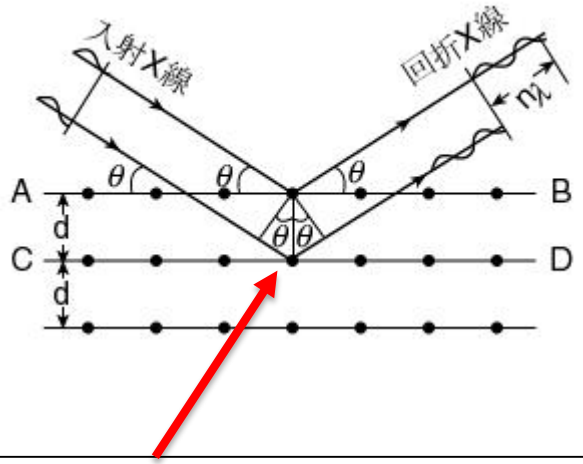
diffraction

X-ray changes its direction by interaction with a periodic material

However, these definitions are not always obeyed.

Crystal does not *diffract* X-ray

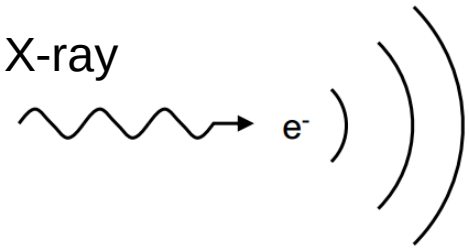
Bragg reflection --- not a reflection



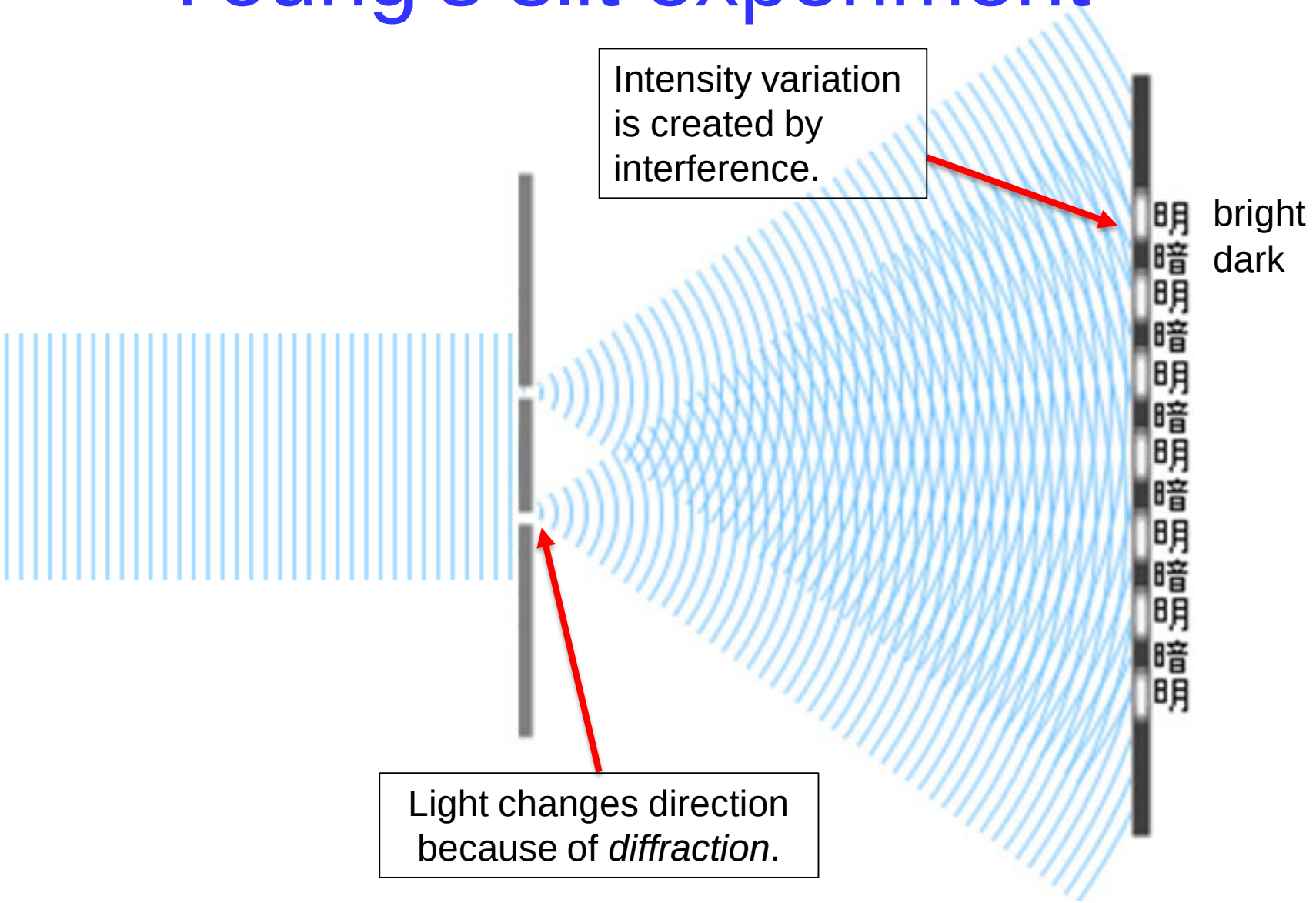
Scattered X-rays form a pattern on the detector because of interference.

An X-ray changes its direction because of Thomson scattering

Diffraction is not involved.



Young's slit experiment



“Diffraction” in a dictionary

Webster's 1913 Dictionary

Diffraction, n. (Opt.)

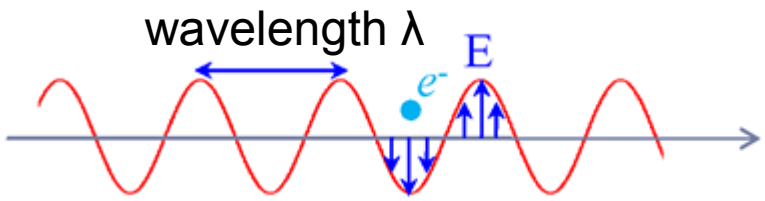
The deflection and decomposition of light in passing by the edges of opaque bodies or through narrow slits, causing the appearance of parallel bands or fringes of prismatic colors, as by the action of a grating of fine lines or bars.

> Remarked by Grimaldi (1665), and referred by him to a property of light which he called diffraction.
>
> --Whewell

Crystals do NOT diffract X-rays

Thomson scattering

X-ray is a travelling wave

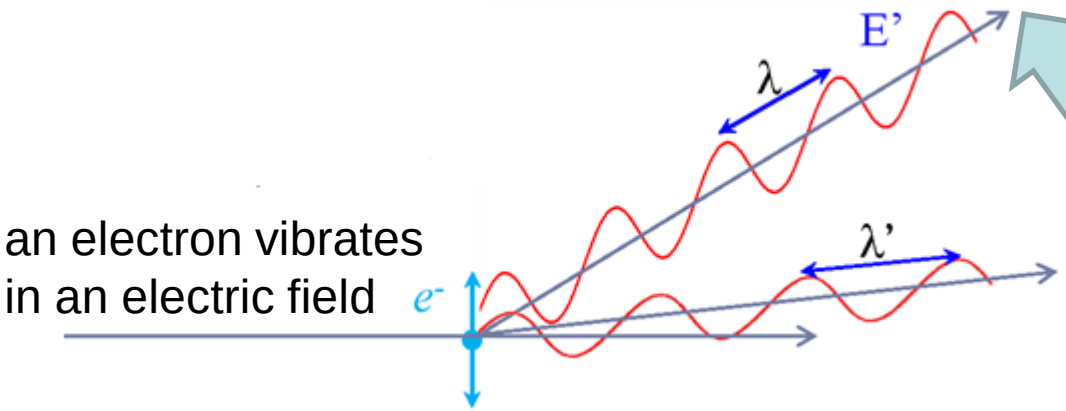


Electric field by an X-ray

$$E(t) = E_0 e^{-i(2\pi\omega t + \phi_0)}$$

X-ray with the same wavelength

same wavelength = elastic scattering

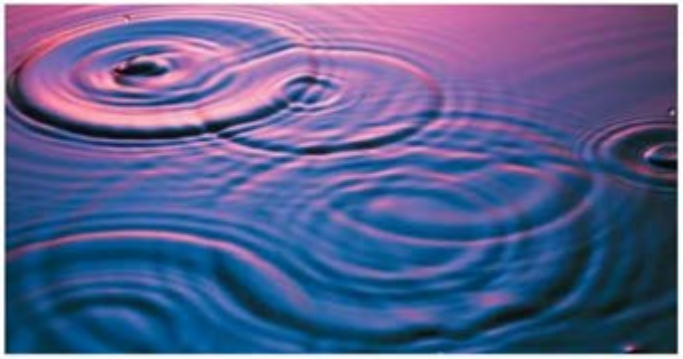


an electron vibrates in an electric field

This is the scattered X-ray

Thomson scattering takes place in all directions, although the intensity depends on the direction.

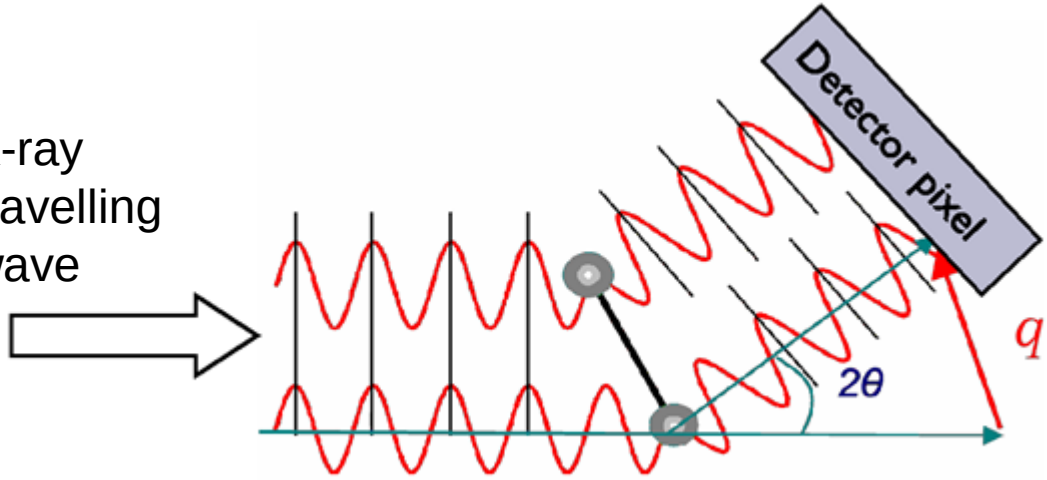
“Interference” is the key phenomenon



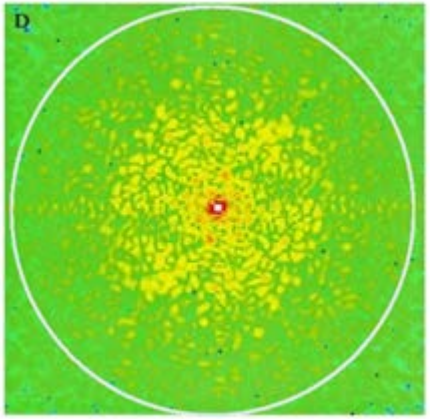
“interference” of waves

At a large distance scattered waves interfere with each other.

X-ray travelling wave



two electrons



an interference pattern is not always periodic.

Scattering from two electrons

path difference

$$\Delta = \vec{k} \cdot \vec{r} - \vec{k_0} \cdot \vec{r} = \vec{S} \cdot \vec{r}$$

scattering vector $\vec{S} = \vec{k} - \vec{k_0}$

phase difference in radian

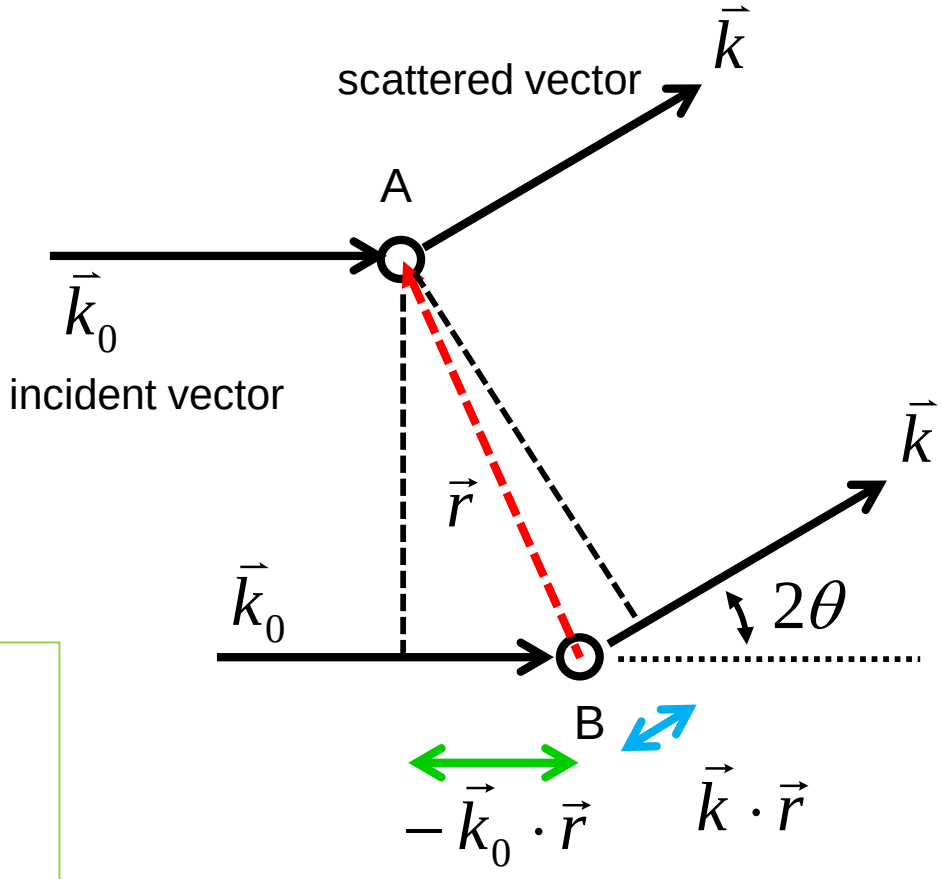
$$\delta\phi = 2\pi\Delta / \lambda$$

superposition of waves at the detector

$$E(t) = E_0(e^{-i(2\pi\omega t)} + e^{-i(2\pi\omega t + \delta\phi)})$$

with N electrons

$$E(t) = E_0 e^{-2\pi i\omega t} \sum_{j=1}^{N} e^{-i\delta\phi_j}$$



detectors measure energy

$$I = \left(E(t)\right)^2$$

Comparison with Bragg refelction

condition of constructive interference is the same phase

$E(t) = E_0(e^{-i(2\pi\omega t)} + e^{-i(2\pi\omega t + \delta\phi)})$

phase difference

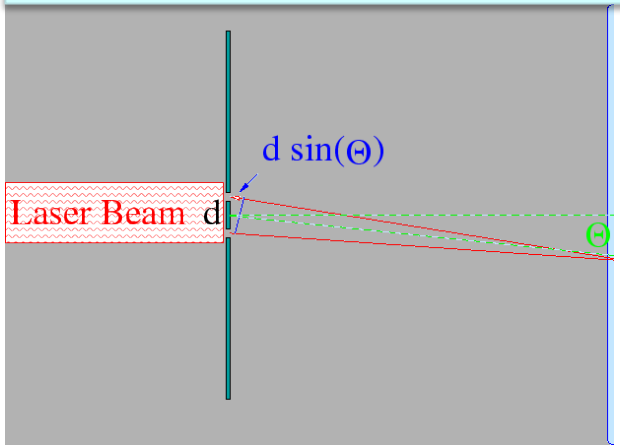
$\delta\phi = 2\pi\Delta / \lambda$

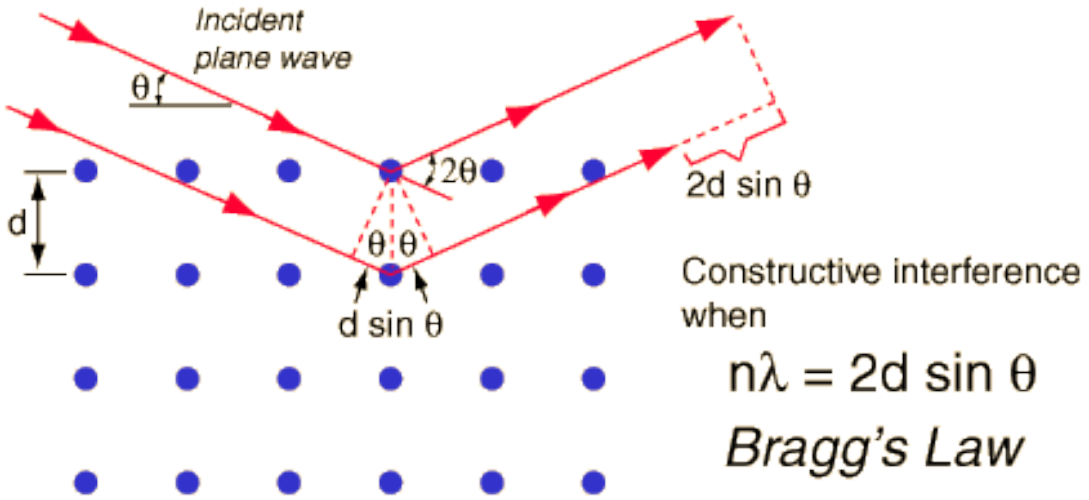
when this is an integer multiple of 2π, constructive interference takes place.

constructive interference occurs when the path difference is a multiple of wavelength

This also applied to Bragg reflection

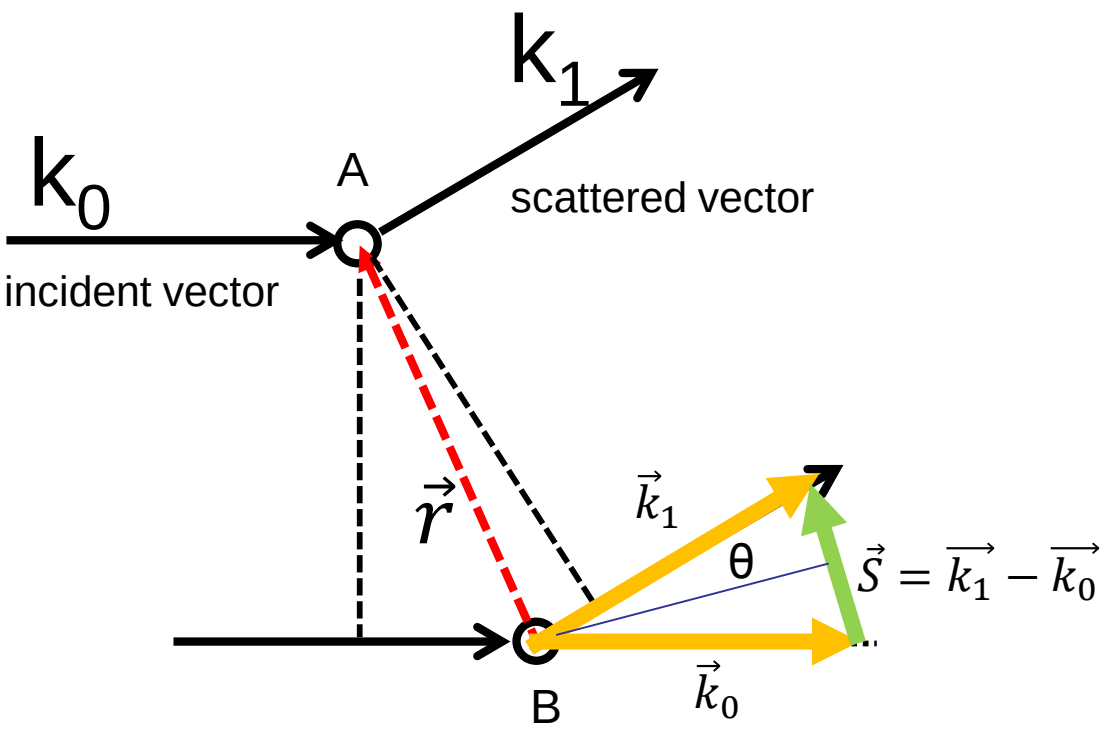
This also applied to a slit diffraction experiment.





Relation to the scattering angle

definition of q



When B is the origin,
$\vec{r}$ is the coordinate of the electron

Q (Momentum transfer)

$$\vec{q} = \left(\frac{2\pi}{\lambda}\right)\vec{S}$$

$$q = |\vec{q}| = \frac{4\pi \sin\theta}{\lambda}$$

path difference

$$\delta\phi = 2\pi\Delta/\lambda = 2\pi\vec{S}\cdot\vec{r}/\lambda = \vec{q}\cdot\vec{r}$$

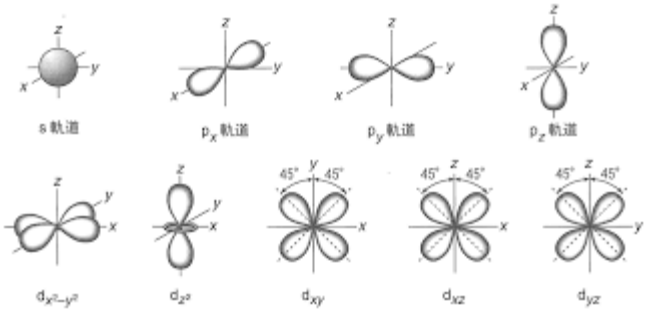
with N electrons

$$E(t,\vec{q}\,) = E_0 e^{-2\pi i\omega t}\sum_{j=1}^{N} e^{-2\pi i\omega t}$$

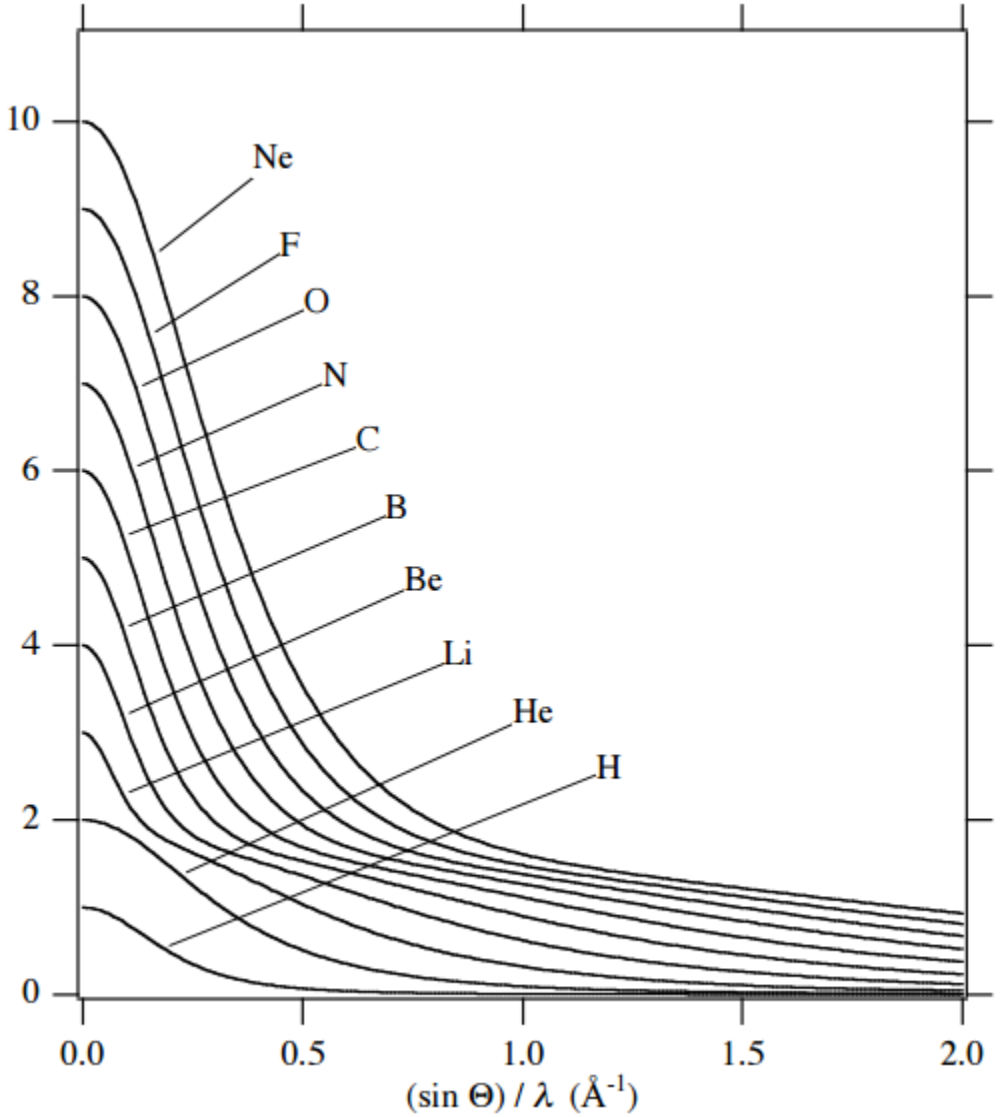
$$= E_0 e^{-2\pi i\omega t}\sum_{j=1}^{N} e^{-\vec{q}\cdot\overrightarrow{r_j}}$$

Atomic scattering factor

distribution of electrons differs in each element



Assumption:
- influence by nucleus is ignored
- nucleus is too heavy to scatter X-rays



Scattering from a molecule

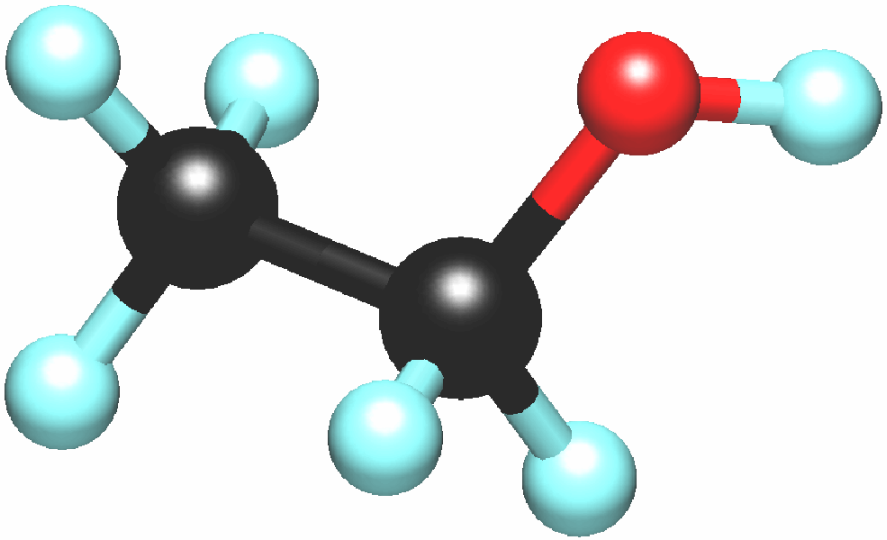
With N electrons

$$E(\vec{q}) = E_0 \sum_{j=1}^{N} e^{-i\vec{q}\cdot\vec{r}_j}$$

With N atoms

$$\mathrm{F}(\vec{q}) = \sum_{j=1}^{N} f_j \; e^{-i\vec{q}\cdot\vec{r}_j}$$

f_j atomic scattering factor
r_j coordinate of the atom



Scattering and interference due to a crystal

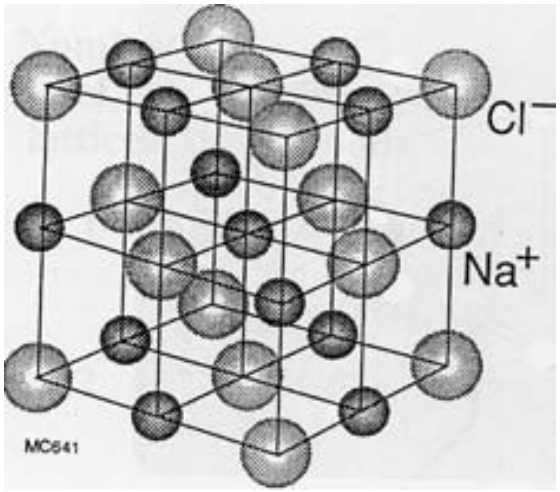
In an atomic crystal

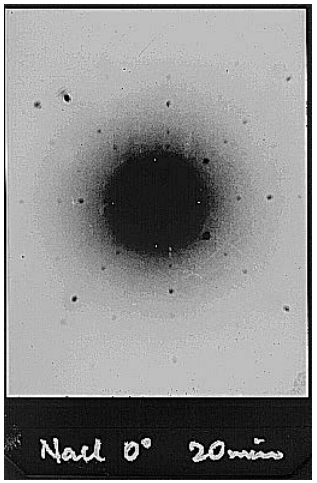
$$E(\vec{q}) = \sum_{j=1}^{N} f_j \; e^{-i\vec{q}\cdot\vec{r}_j}$$

Fourier transform

$$F(\omega) = \int_{-\infty}^{\infty} f(t) e^{-i\omega t} dt$$

In a crystal, r_j is a periodic. Thus, at a particular q, $q \cdot r_j$ is always a multiple of 2π, causing constructive interference. This is Bragg reflection.





Scattering and interference from a non-crystalline material (with a random orientation)

electron density distribution

$$I(\vec{q}) = F^2(\vec{q}) = \left| \int \rho(\vec{r}) e^{-i\vec{q}\cdot\vec{r}} \right|^2$$

This is Fourier transform. Thus, reverse Fourier transform is possible, but the phase is not available.

average over all directions

$$I(\vec{q}) = \langle I(\vec{q}) \rangle_\Omega \qquad \langle exp(i\vec{q}\cdot\vec{r}) \rangle_\Omega = \frac{\sin qr}{qr}$$

in a centrosymmetric object, the density ρ is a function of radius r, ρ(r).

$$F(q) = \sum_j (4\pi r^2 dr)\rho(r)\langle exp(i\vec{q}\cdot\vec{r}) \rangle_\Omega = 4\pi \int \rho(r) r^2 \frac{\sin(qr)}{qr} dr$$

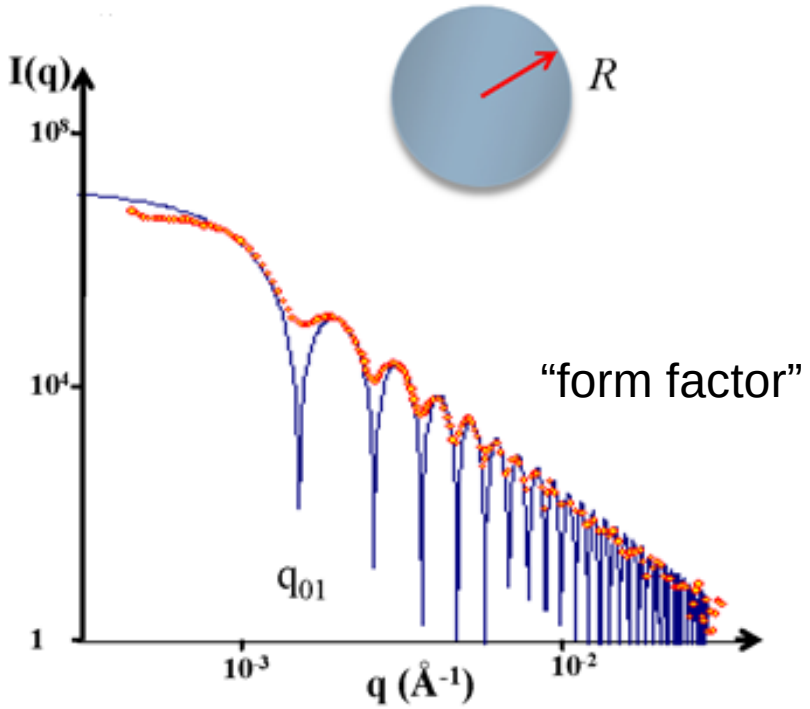
all electrons in a shell with radius r

In the case of a solid sphere

a homogeneous spherical particle (radius R)

$$F(q) = \frac{3(\sin(qR) - qR\cos(qR))}{(qR)^3}$$

$$I(q) = F^2(q) = \left(\frac{3(\sin(qR) - qR\cos(qR))}{(qR)^3}\right)^2$$



Non-Crystalline Diffraction (NCD)

- NCD includes all interactions between non-periodic materials and X-rays.

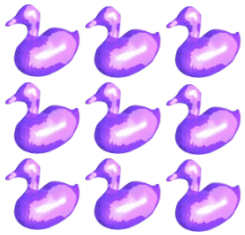
- Diffraction and scattering are the same phenomenon in principle, diffraction being a special case of scattering

- Since a single-crystal diffraction is a special case, it is not included in NCD.

NCD in material science

- Most materials around us are non-crystalline.
- Why crystalline materials are so important?
  -- Proteins: atomic structure cannot be obtained without crystallization
  -- Crystalline materials have unique characteristics
- Metallic materials are not single crystals.
- Non-crystalline materials are not simple in structure (form factor vs. structure factor)
  -- hierarchical structure

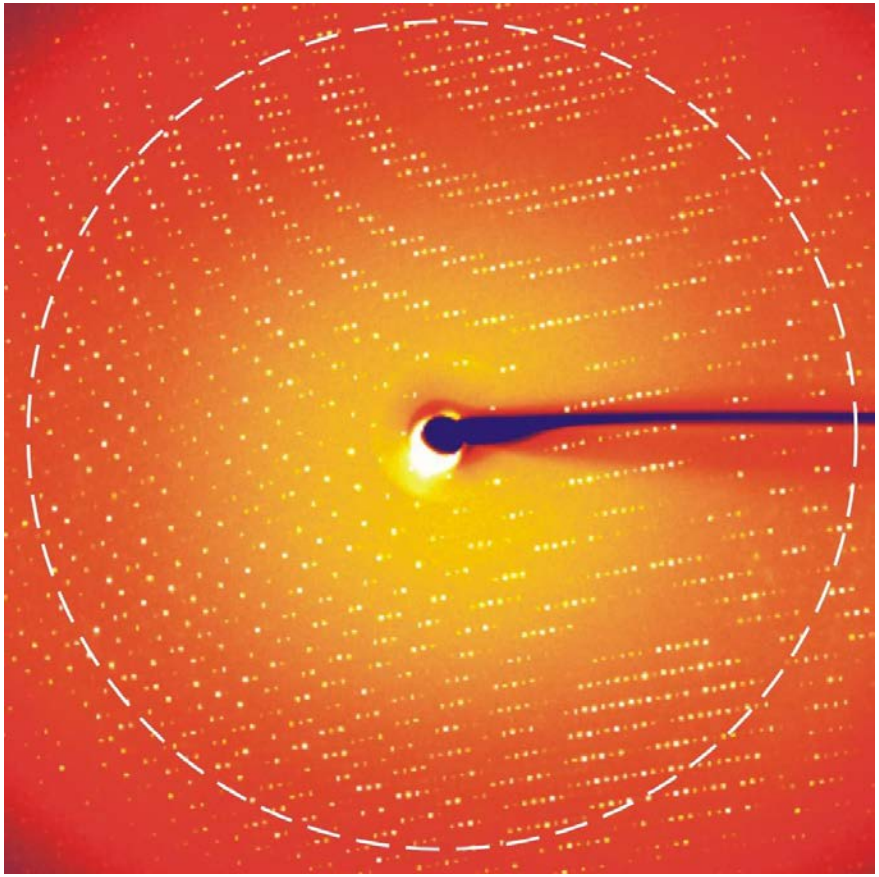
Form and structure factors

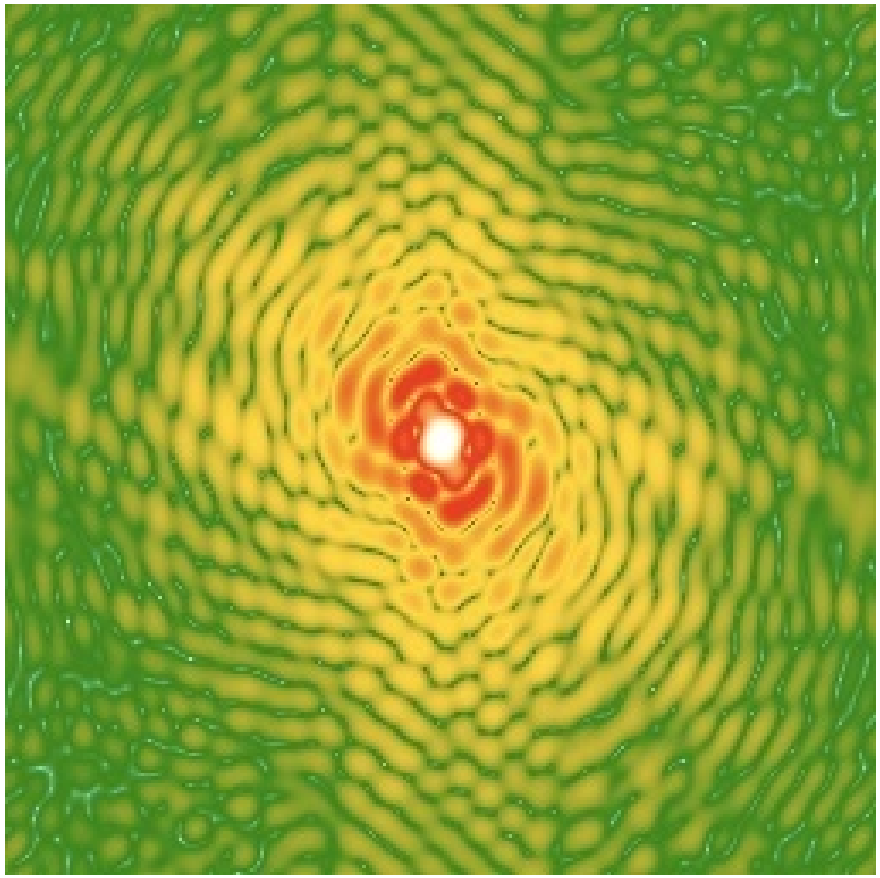


Bragg diffraction

form factor × structure factor

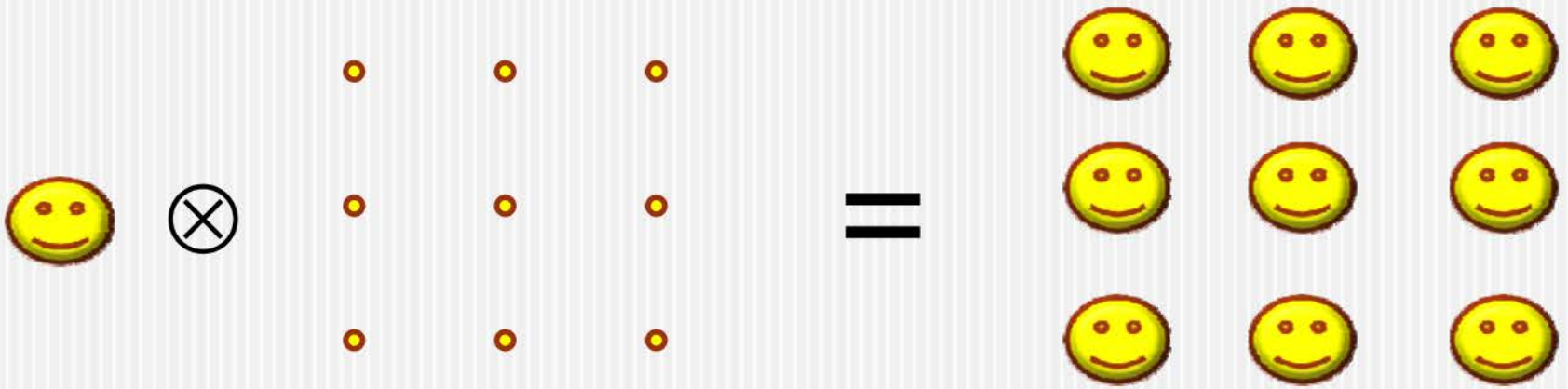
“speckle pattern”
form factor (with fixed orientation)



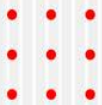


by Prof. Nakasako

Form and structure factors



Form factor – information about the individual building block



Structure factor – information about the lattice

$$I(q) = \int F(x)F^*(x) \otimes \sum \delta(x - x_n) e^{-i\vec{q}\cdot\vec{x}} d\vec{x}$$

$$= |F(q)|^2 \cdot \underbrace{\int \sum \delta(x - x_n) e^{-i\vec{q}\cdot\vec{x}} d\vec{x}}_{S(\vec{q})}$$

$$F(\vec{q}) = \iiint_V \Delta\rho(\vec{x}) e^{-i\vec{q}\cdot\vec{x}} dxdydz$$

$$S(\vec{q}) = \int \sum \delta(x - x_n) e^{-i\vec{q}\cdot\vec{x}} d\vec{x}$$

Hierarchy

- At different size scales, there are different structures.
- Small angle scattering: large structure
- In the case of protein solution scattering
  Small-angle: shape of molecules
  Medium-angle: domain structure
  Wide-angle : secondary structure

Protein solution scattering

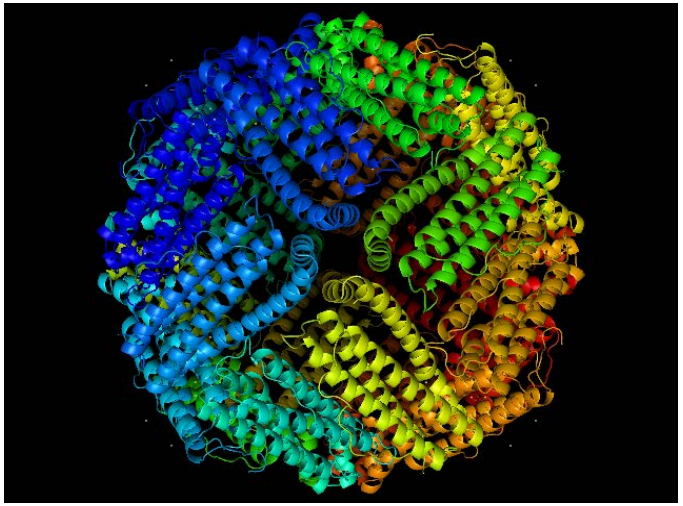
- All particles have the same structure
- All particles are random in orientation
- There is no interaction between the particles.

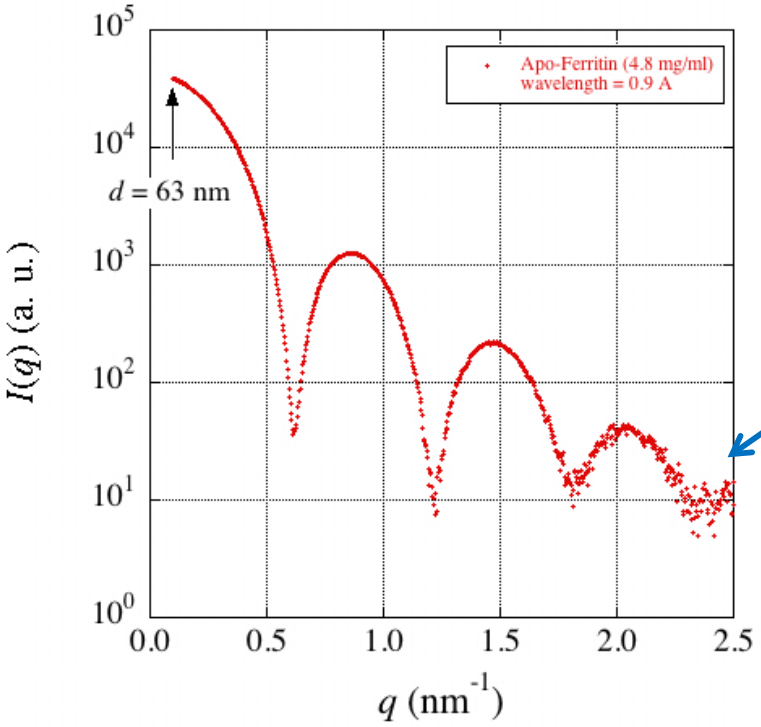
- This is an ideal and special case of small-angle scattering.

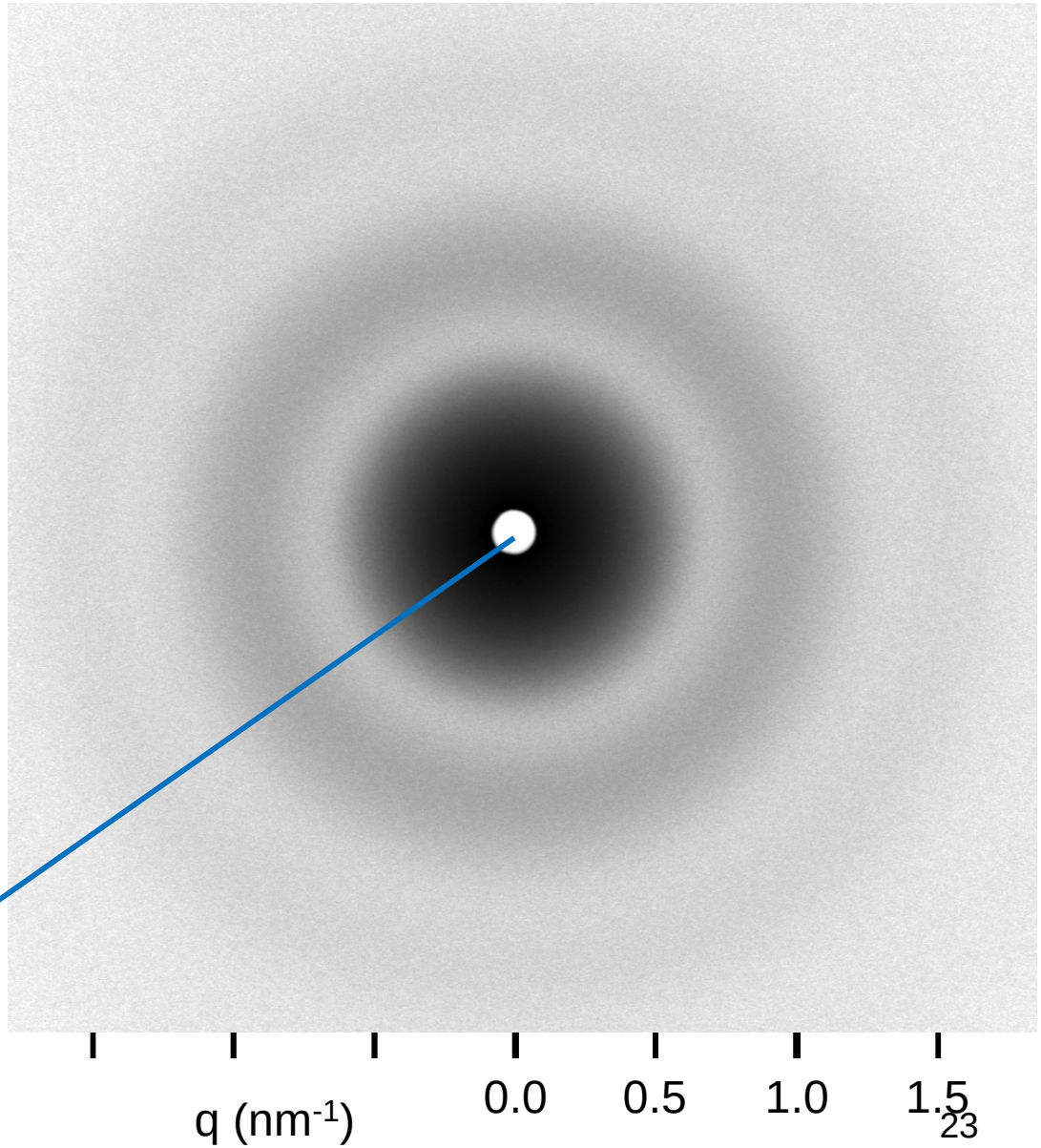
SAXS from protein solution is an average

by Prof. Nakasako

Apo-Ferritin, Mw. 480 kDa

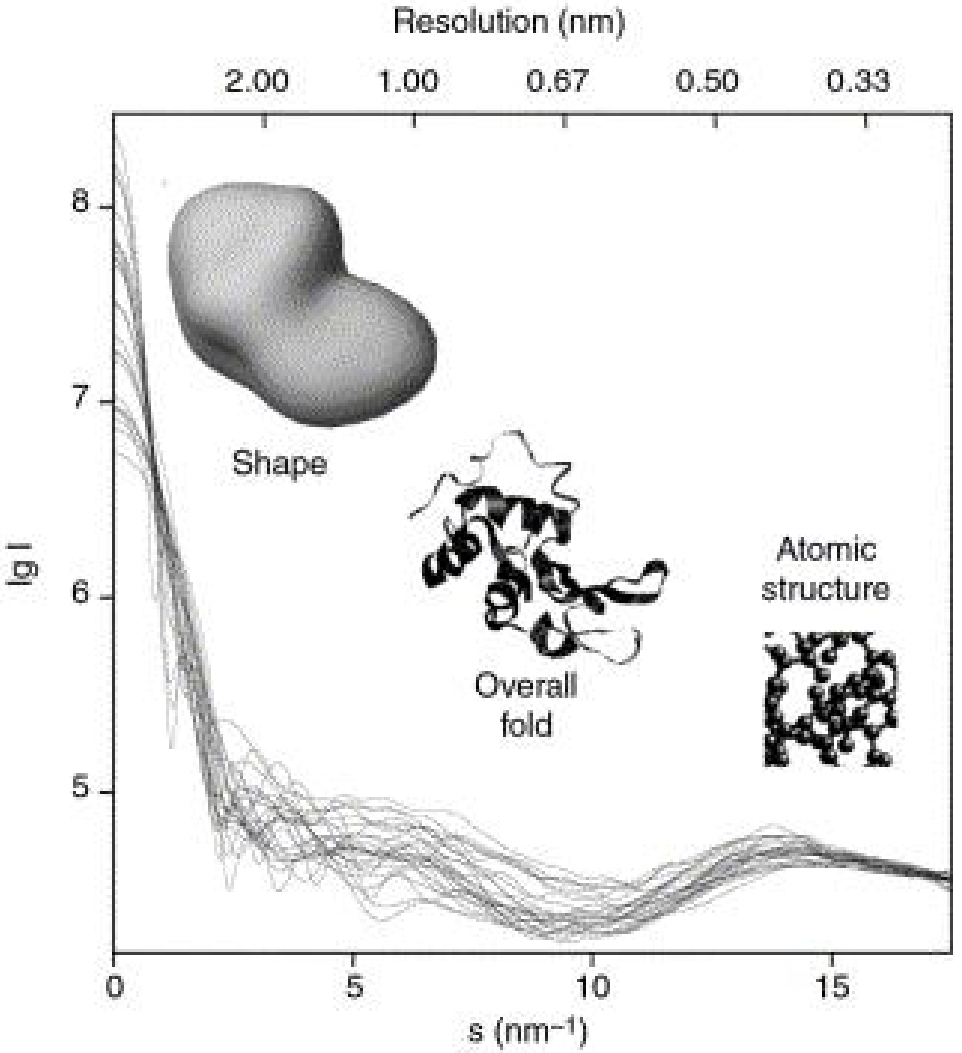




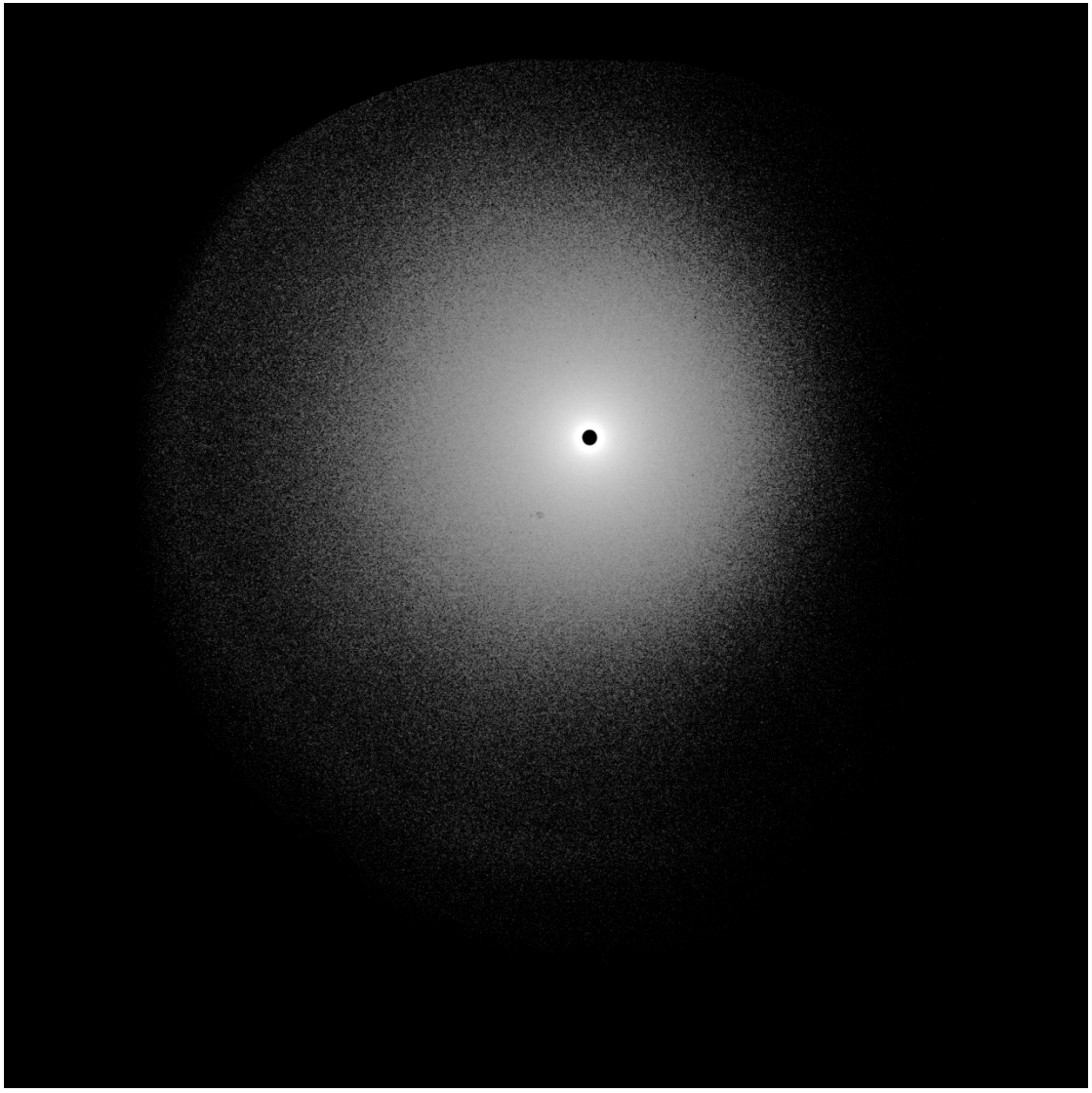


SPring-8, BL45 HP

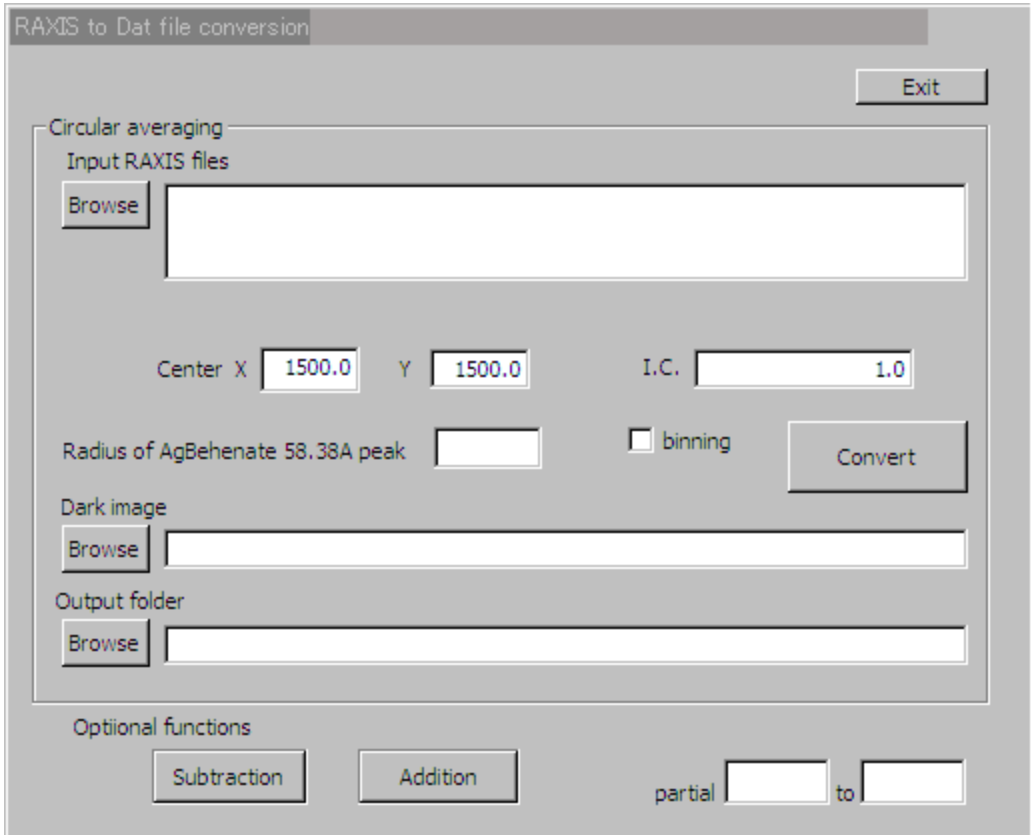
Meaning of the scattering curve



Raw data



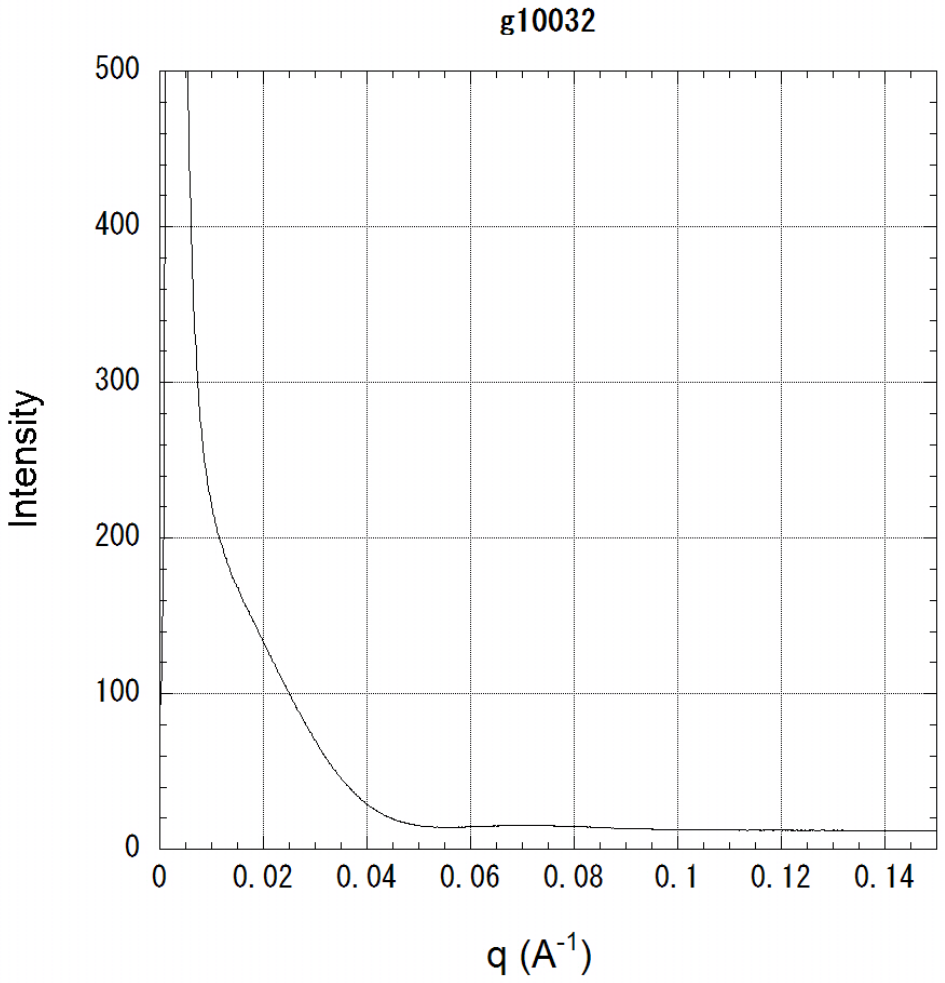
saxs8



Circular-averaging: obtain a one-dimensional profile from a two-dimensional scattering pattern.

Calibration of *q* is done with the known spacing of AgBehenate (58.38Å)

Circular-averaged data



$2\,d \sin\theta = \lambda \qquad 1/d = 2 \sin\theta / \lambda$

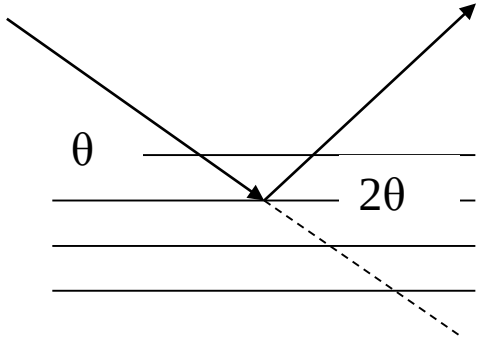
$\mathbf{q = 4\pi \sin\theta / \lambda}$ (=h)

$q = 2\pi / d$

----- $q = 6 / d$

----- $d = 6 / q$

$S = 1 / d$ or $s = 4\pi \sin\theta / \lambda$

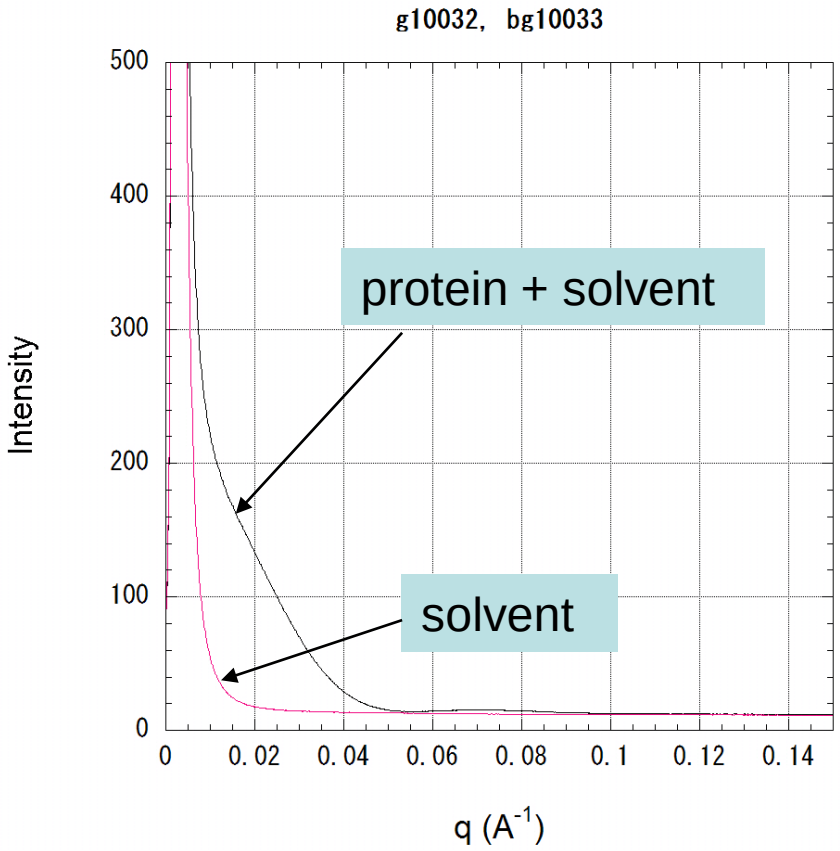


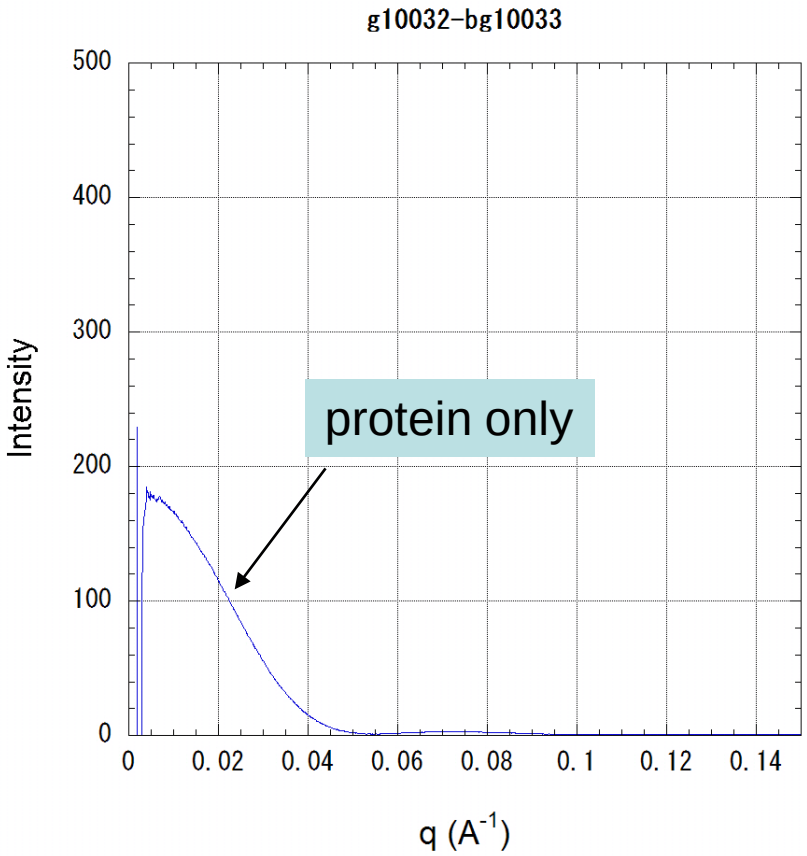
Scattering from protein

- Subtract scattering from the solvent (buffer)
  $I_{Protein} = I_{Sample} - I_{Buffer}$
- Absorption by protein is so small that it can be neglected.

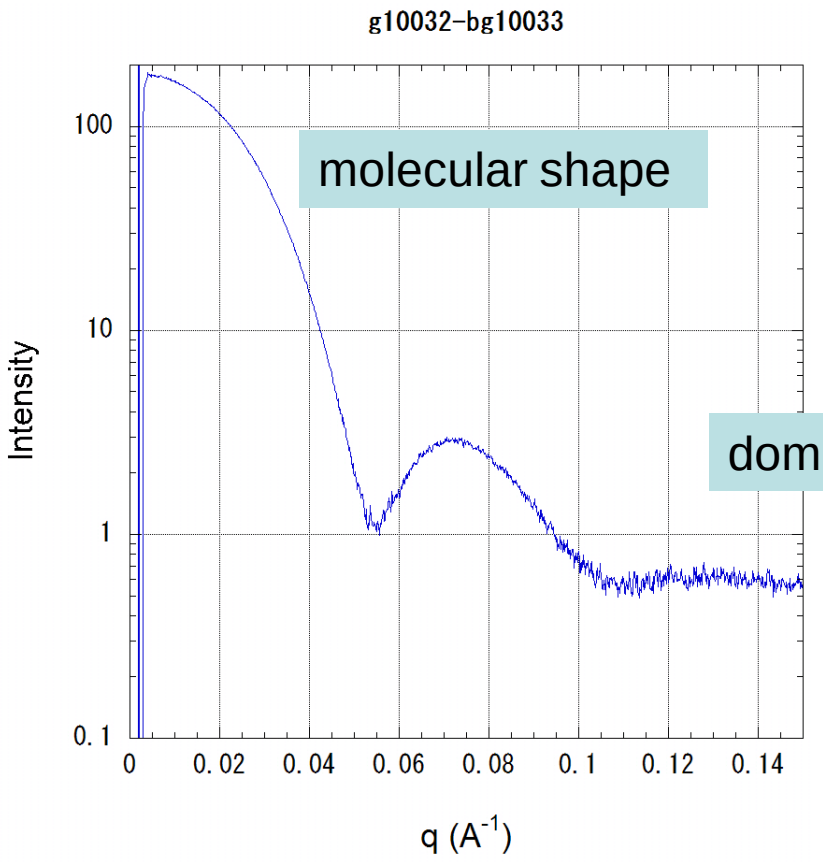
Scattering from protein and solvent must be measured under the same conditions (same exposure time, same sample cell).

Subtraction of solvent scattering





Scattering from protein



molecular shape

domain structure

secondary structure

All analysis will be made on this profile.

It is recommended to measure this with different protein concentrations.

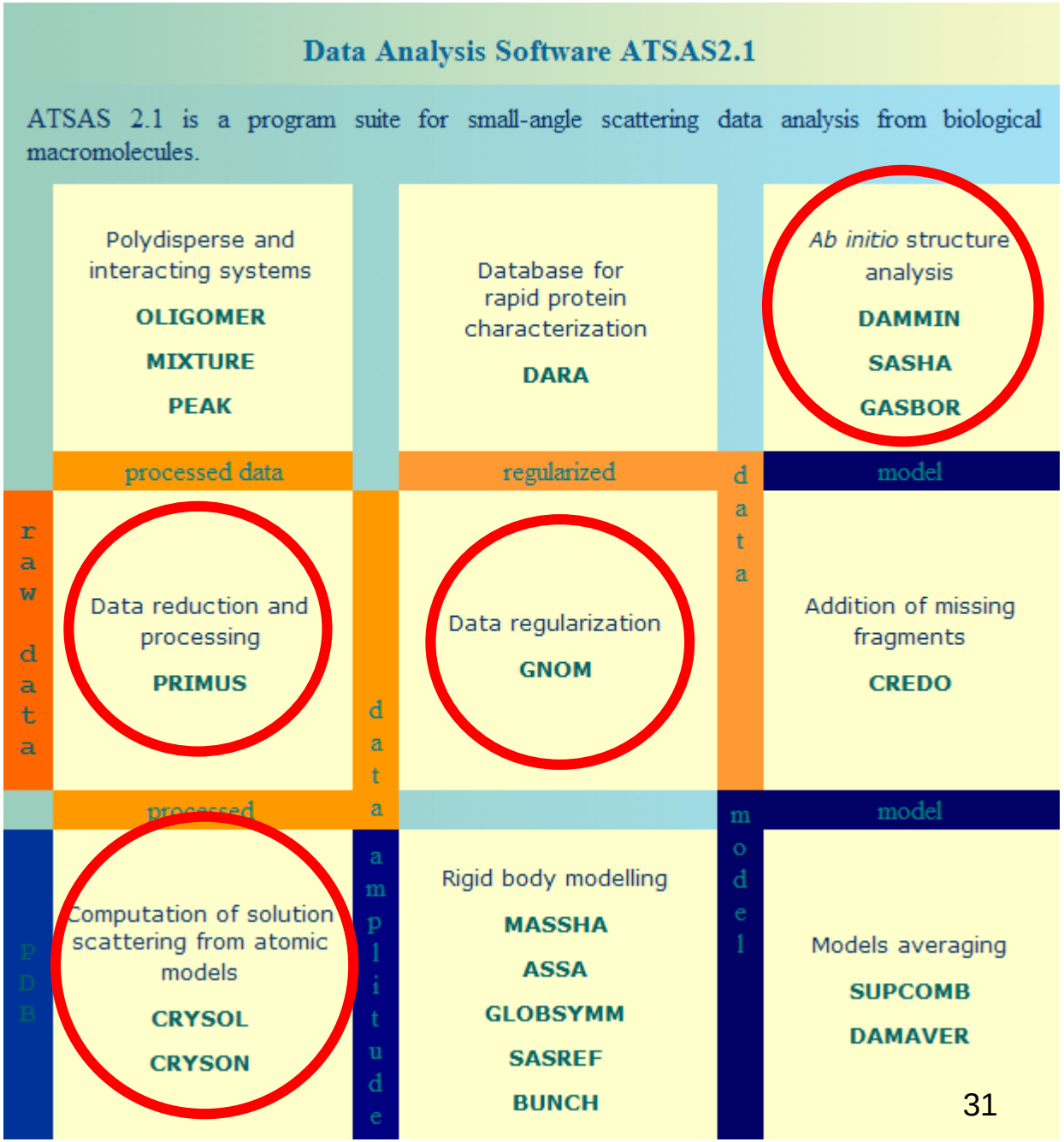
Software: ATSAS

Downloadable

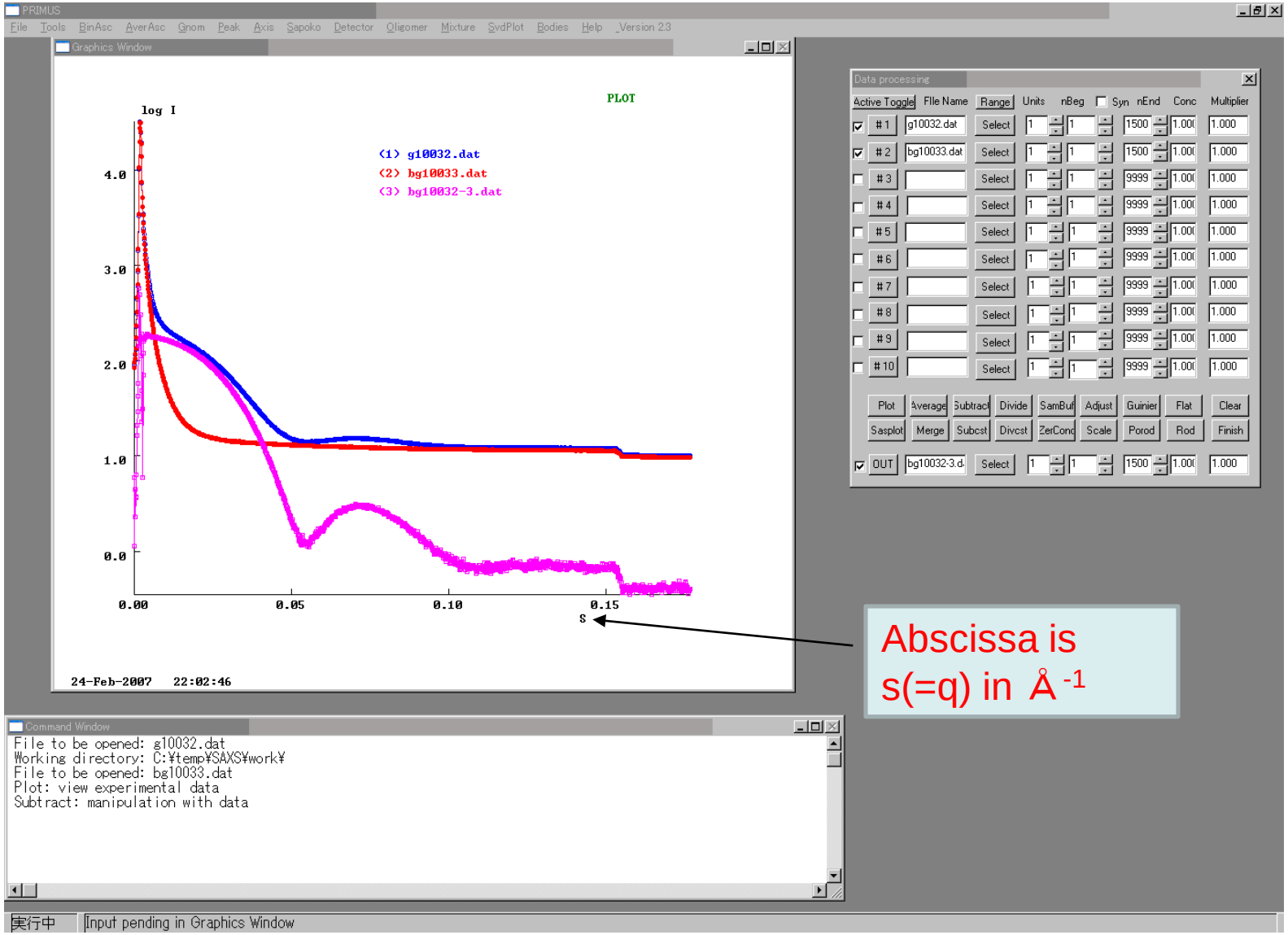
World-standard?

With English manual

http://www.embl-hamburg.de/ExternalInfo/Research/Sax/download.html



PRIMUS



Abscissa is s(=q) in $Å^{-1}$

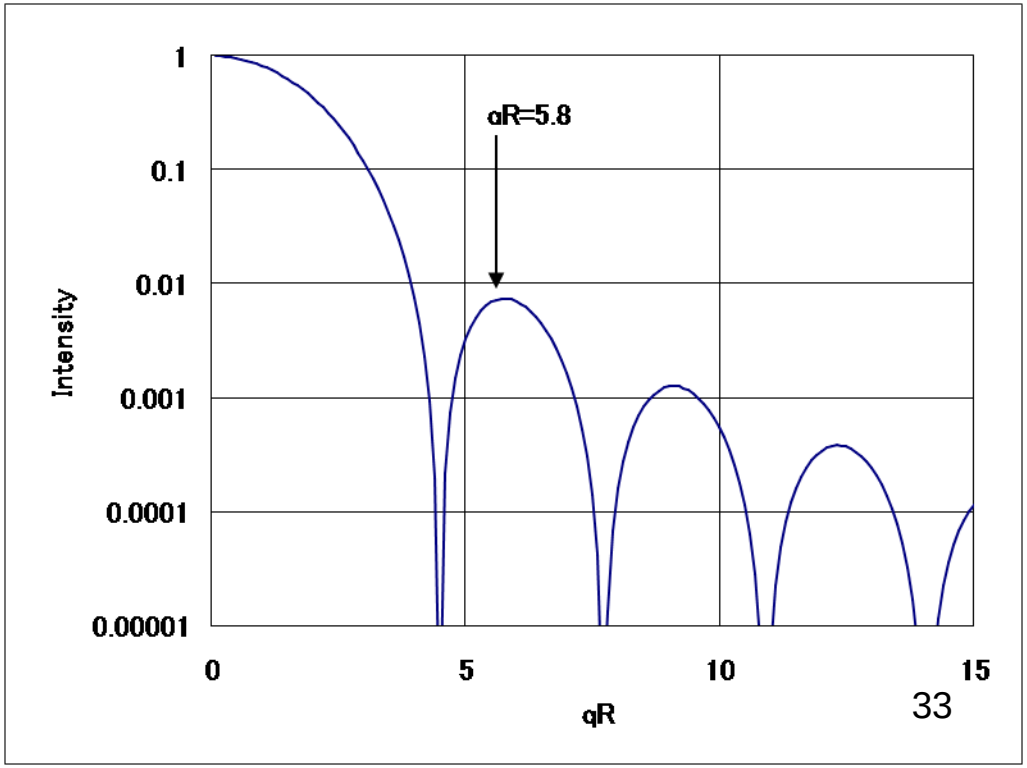
If the protein is a sphere

Scattering from a sphere with radius R

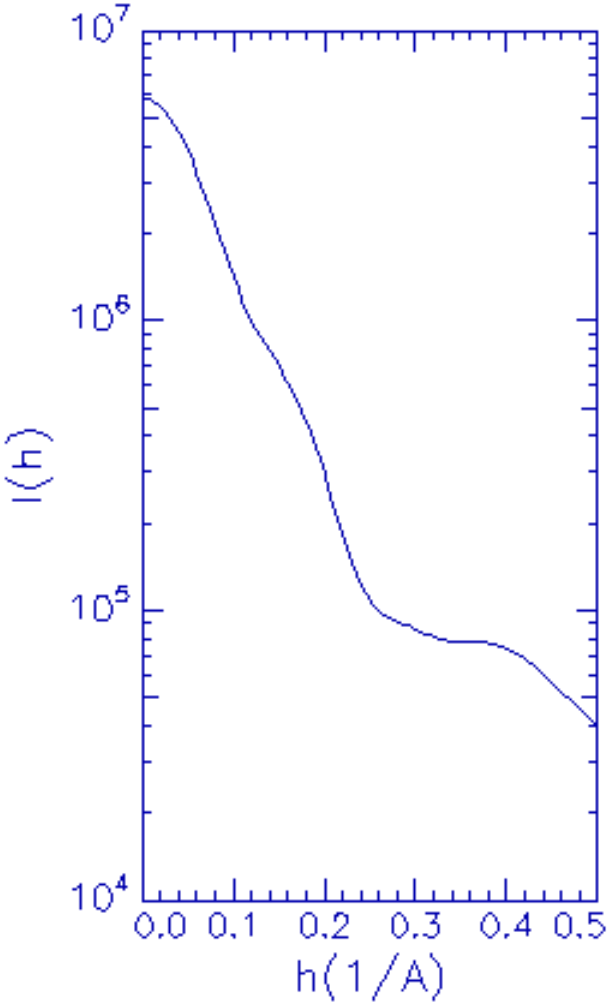
$$I(q) = I_e V^2 \rho_0^2 \left[\frac{3[\sin(qR) - (qR)\cos(qR)]}{(qR)^3} \right]^2$$

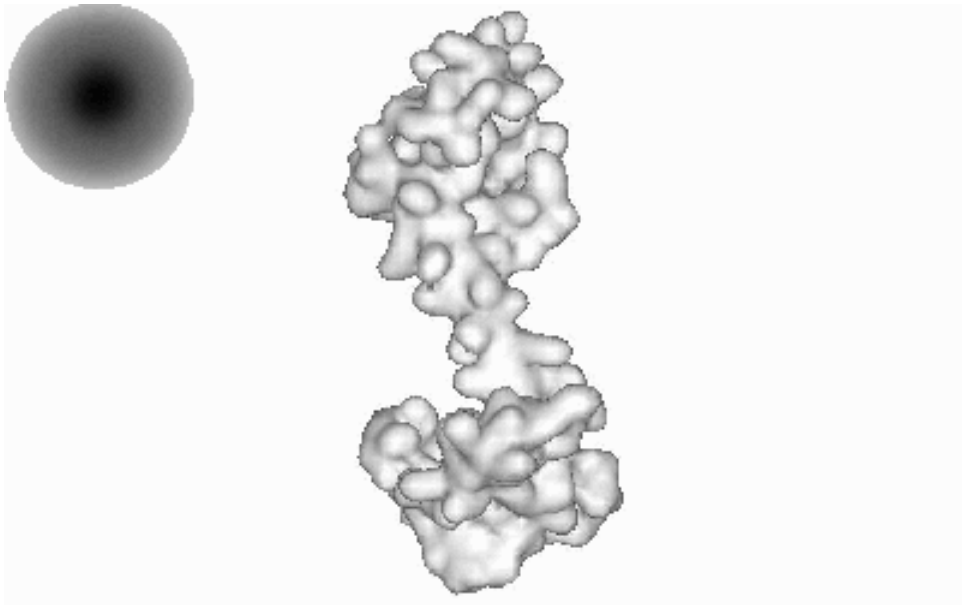
If sphere, radius can be obtained from the peak position.

In the data in the previous slide, the peak is at q=0.07 Å^{-1} and thus R is 83Å.



Calmodulin





http://scattering.tripod.com/

Radius of gyration : Rg

- $R_g^2 = \frac{\int \rho(r) \cdot r^2 \cdot dV}{\int \rho(r) \cdot dV}$

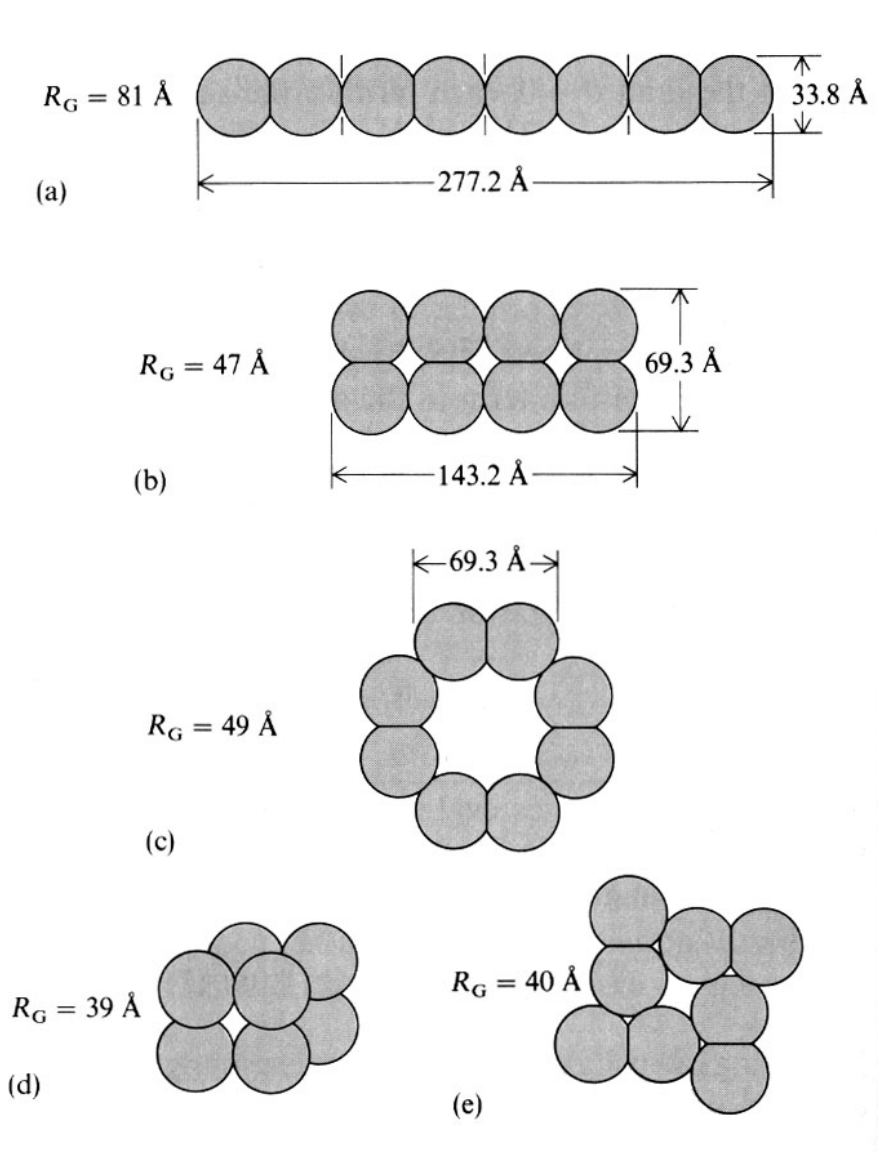
- $I(q) = N_p n_e^2 \exp\left(-\frac{R_g^2}{3} q^2\right)$ approximation

  i.e. near the origin, the intensity distribution is Gaussian

- ln(I(q)) vs. q^2 Guinier plot

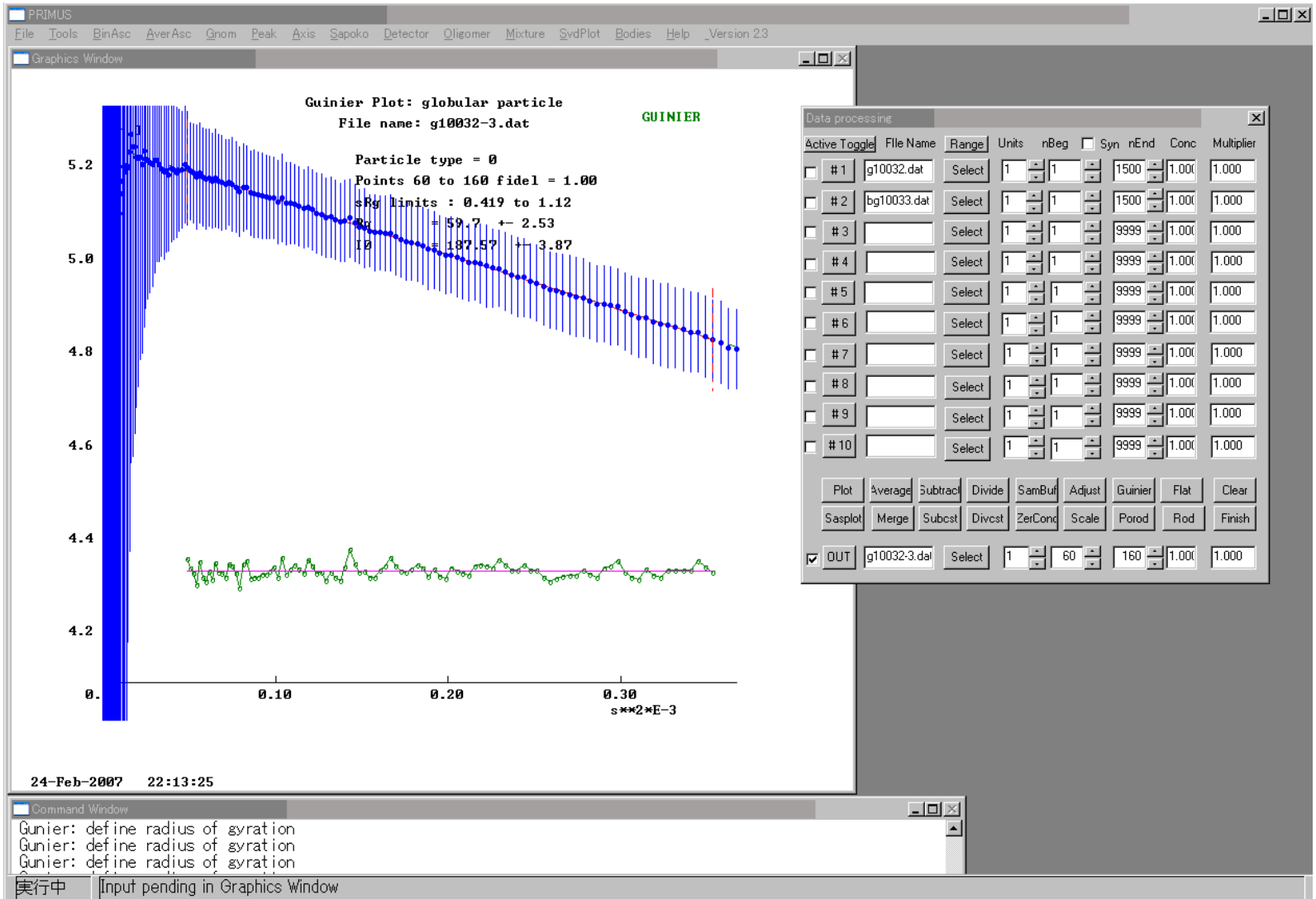
Radius of gyration

Rg depends on the shape.

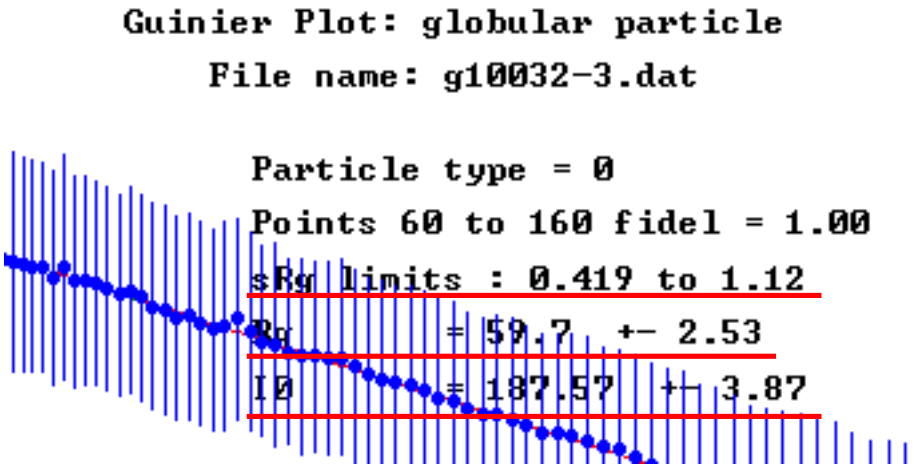


Rg “Guinier”

$$I(q) = N_p n_e^2 \exp\left(-\frac{R_g^2}{3} q^2\right)$$

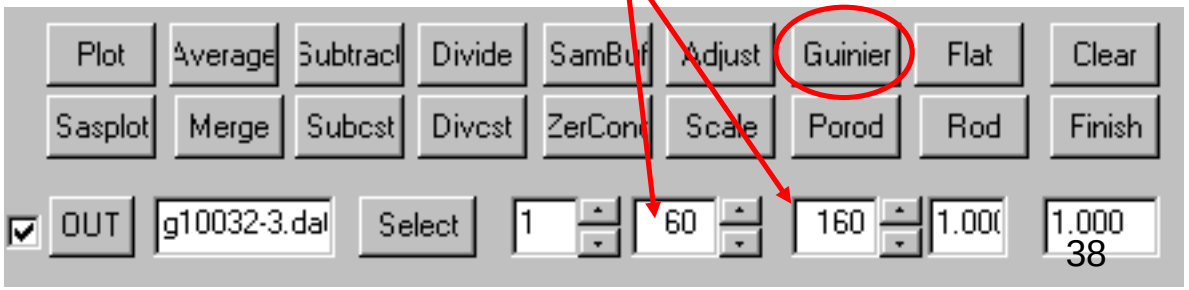


The region where the Guinier approximation is valid



$R_g q_{max} < 1.3$

region to be fitted



Radius of gyration of a sphere

$$R_g^2 = \frac{\int \rho(r) \cdot r^2 \cdot dV}{\int \rho(r) \cdot dV}$$

- $R_g = R\sqrt{3}/5$ spheroid $R_g = \sqrt{(2a^2+b^2)}/5$

  If sphere, radius R can be calculated from R_g

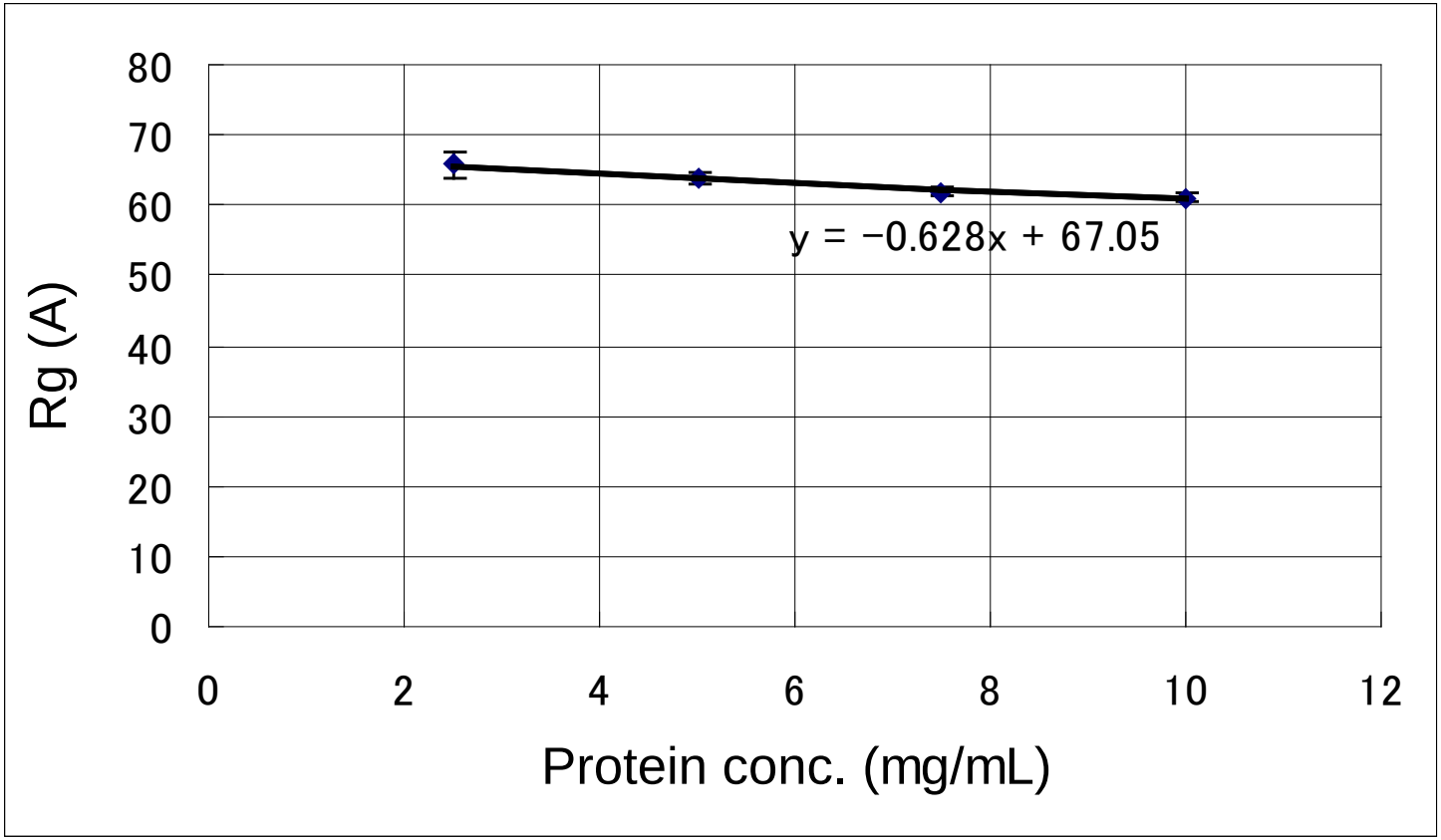
- R_g =59.7 Å gives R=77 Å

  close to 83 Å obtained from the peak position

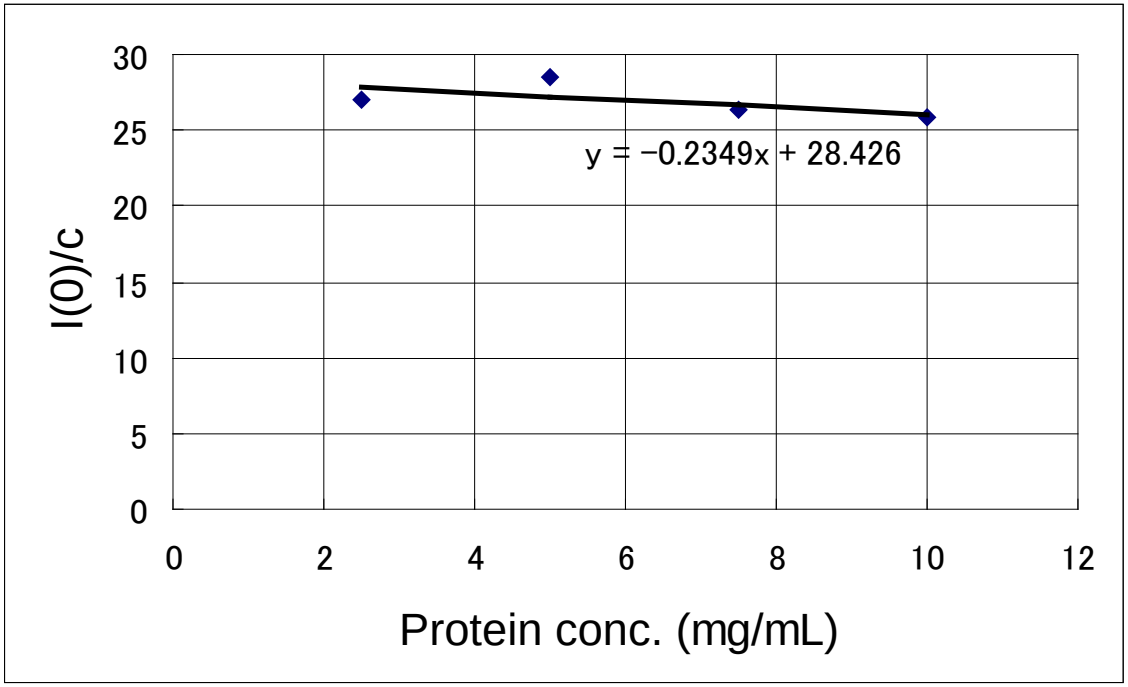
  →This protein can be approximated as a sphere with a radius of about 80 Å.

Dependence on protein concentration

Ideally, infinite dilution



I(0) scattering at the origin



I(0)/c is proportional to the molecular weight.

Molecular weight can be obtained by comparison with a standard protein.

p(r) function

- pair distance distribution function
- Linear self-convolution of electron density
- *p(r)* is uniquely determined by the protein structure.
- *p(r)* is obtainable from the SAXS profile.

$$p(r) = 4\pi \int_0^\infty I(q) qr \sin qr \, \mathrm{d}q$$

PRIMUS

↓

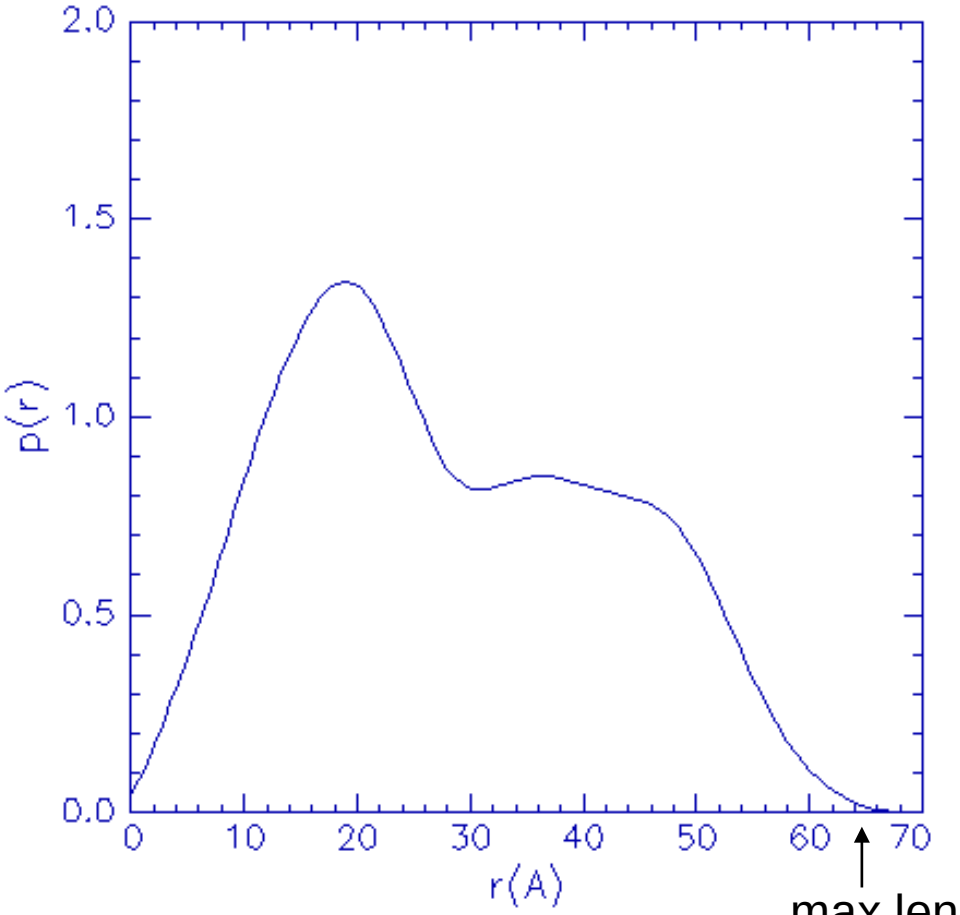
GNOM

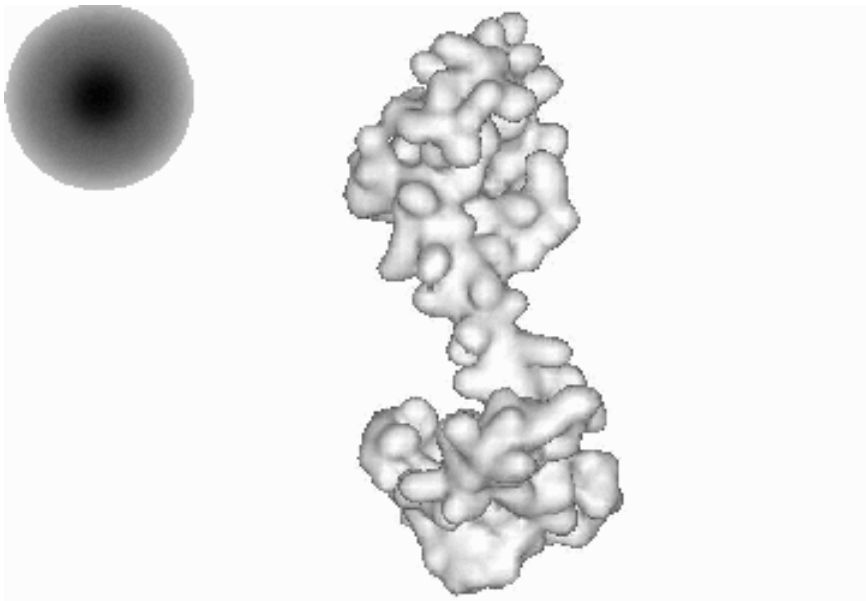
↓

Calculate with a given R_{max}

Calmodulin

p(r) function





http://scattering.tripod.com/

A hypothesis that can be tested by a SAXS measurement

- Find out a difference in structure.
- Obtain R_g and p(r) from a crystal structure and compare with the measurement.
- Test a hypothetical structure.

Calculate SAXS from atomic coordinates

C R Y S O L Version 2.6 -- 26/01/05

-- Program started at 15-Oct-2006 14:17:29—

-------- Real space resolution and grid --------

Maximum order of harmonics ..... : 50
Order of Fibonacci grid ..... : 18
Total number of directions ..... : 4182

---------- Reciprocal space grid ----------

in s = 4*pi*sin(theta)/lambda [1/angstrom]
Maximum scattering angle ..... : 2.000
Number of angular points ..... : 201

--- Structural parameters (sizes in angstroms) ---

PDB file name ..... : pdb2bni.ent
Number of atoms read ..... : 1035
Number of discarded waters ..... : 55
Geometric Center: -5.452 24.939 39.648
Center of the excess electron density: 0.043 0.084 -0.120
Electron Rg : 16.40 Envelope Rg : 16.67
Shape Rg : 16.43 Envelope volume : 0.2084E+05
Shell volume : 0.1176E+05 Envelope surface : 3303.
Shell Rg : 21.05 Envelope radius : 32.30
Shell width : 3.000 Envelope diameter : 59.99
Molecular Weight: 0.1478E+05 Dry volume : 0.1791E+05
Displaced volume: 0.1855E+05 Average atomic rad.: 1.623
Number of residuals : 128

-- No data fitting, parameters entered manually --

Solvent density ..... : 0.3340
Contrast of the solvation shell ..... : 3.000e-2
Average atomic radius ..... : 1.624
Excluded Volume ..... : 1.855e+4
Average atomic volume ..... : 17.92
Radius of gyration from atomic structure
Rg (Atoms - Excluded volume + Shell) ..... : 17.28
Rg from the slope of net intensity ..... : 17.71
Average electron density ..... : 0.4263

---------- Output files ----------

Coefficients saved to file 2bni01.flm
CRYSOL data saved to file 2bni01.sav
Intensities saved to file 2bni01.int
Net amplitudes saved to file 2bni01.alm

C R Y S O L Version 2.6 -- 26/01/05 ---- terminated at 15-Oct-2006 14:21:28--

Limitation of protein SAXS

Atomic structure cannot be obtained uniquely.

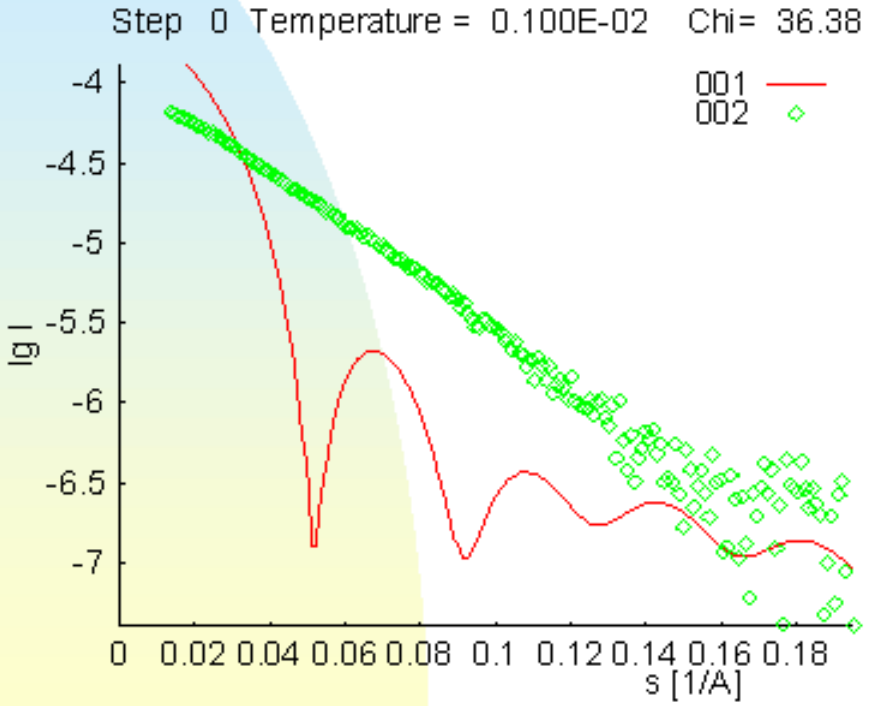
(1)Combine with other information (crystal or NMR structure)

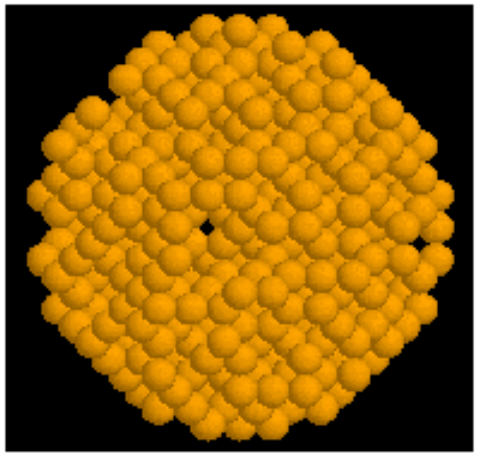
(2)Use of physical constraints --- *ab initio*

ab initio modeling

DAMMIN, GASBOR

S1 shape reconstruction

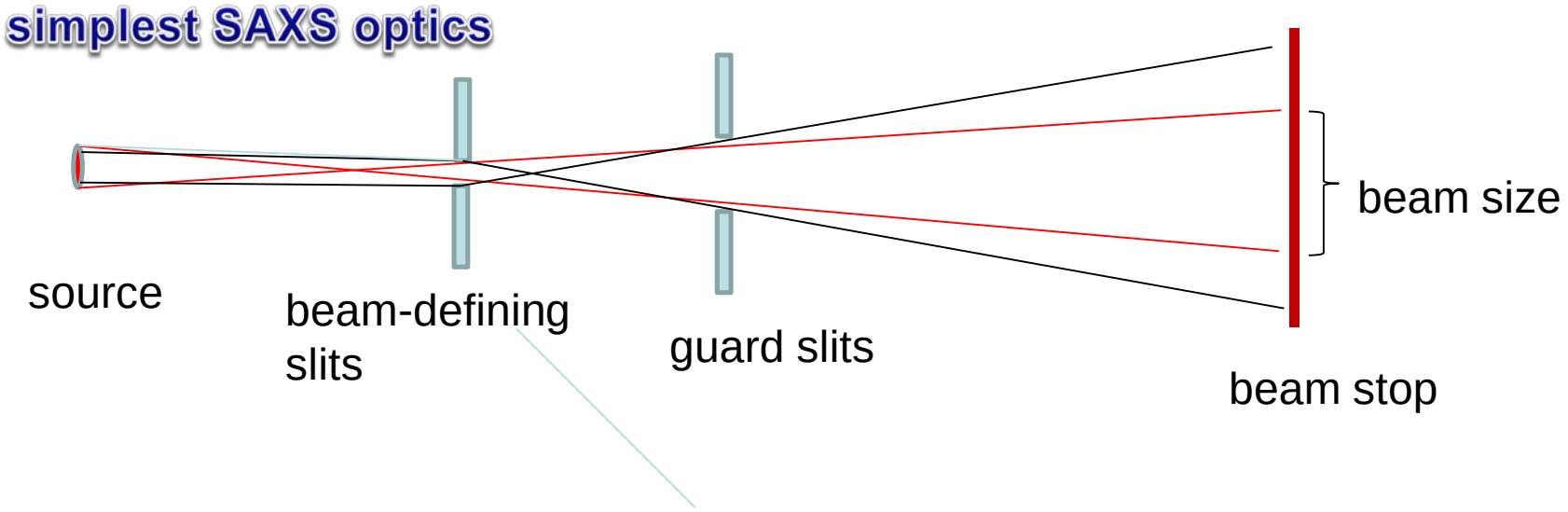




http://scattering.tripod.com/

Optics for SAXS

- Basically a two-slit system
- A focusing optics is desirable for intensity



SAXS beamlines at SPring-8

	X-ray source	optics	Energy (keV)
BL40B2	bend magnet	double-Si(111) + bent cylindrical	7 ~ 18
BL08B2	bend magnet	double-Si(111) + bent cylindrical	7 ~ 18
BL20XU	linear horizontal undulator	double-Si(111) or Si(511)-(333)	8 ~ 113
BL45XU	linear vertical undulator	double-diamond (111) + double-mirror	13.8 (tunable)
BL40XU	helical horizontal undulator	double-mirror	8 ~ 16.5 (pink beam)
BL03XU	linear horizontal undulator	double-Si(111) + double-mirror	6 ~ 35