TEL AVIV UNIVERSITY

The Center for Nanoscience and Nanotechnology

Small Angle X-ray Scattering (SAXS): from Theory to Application

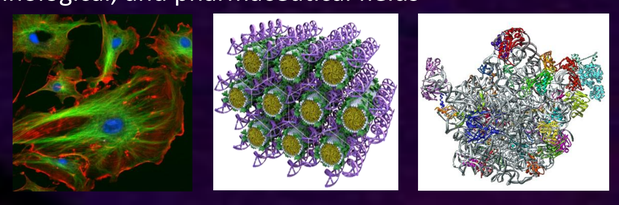
Rona Sharabani[1,2], Adi Laser-Azogui[2,3], Roy Beck[2,3,*]

[1] School of Chemistry, [2]The Center for Nanoscience and Nanotechnology, [3]School of Physics and Astronomy, Tel Aviv University

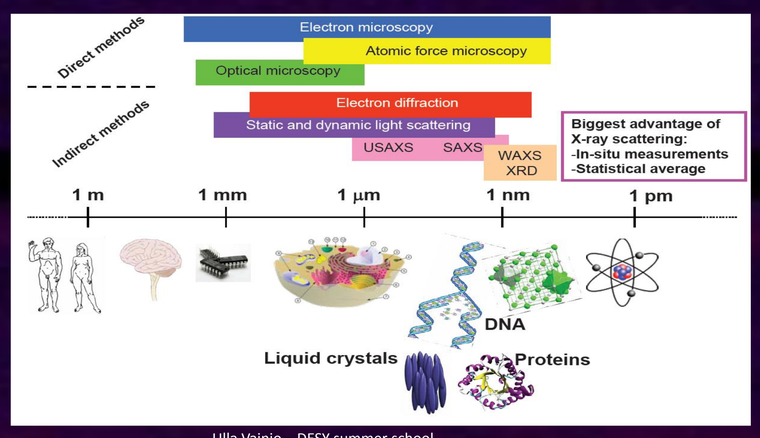
[*]roy@post.tau.ac.il

Introduction

- Conformational changes within a molecule, or formation of macromolecular and supramolecular complexes, are propagated through various length scales (0.1-100nm)
- The shape of a supramolecule is determined by its physical properties and interactions, and is therefore responsible for its biological functions
- The mechanistic understanding of such complexes will allow usage of structure-function correlation in the medical, biotechnological, and pharmaceutical fields



Determining the structure of matter



Ulla Vainio – DESY summer school

SAXS in comparison to other methods

Conventional structural methods to study the building blocks of biological molecules include:

- **Crystallography**: Protein crystallography provides high resolution atomic structures. Crystal packing limits domain movements, so flexible regions are often invisible in the electron density or removed to enable crystallization
- **NMR**: provides atomic resolution information of proteins in solution, but is limited by the size of the proteins studied
- **EM**: provides high resolution but requires dehydrated or frozen samples.
- **Optical Microscopy**: very low spatial resolution and often requires fluorescence tags

Scattering Theory experiments

X-ray diffraction:

When matter is irradiated by an X-ray beam, one of the primary interactions is the elastic scattering of X-rays by electrons.

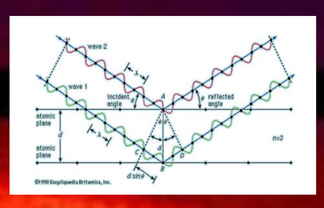
X-ray is scattered by spatially correlated electron pairs constructively and destructively interferences, according to Bragg's low: $n\lambda = 2d \cdot sin\theta$

λ – wavelength of the incident beam

d – spacing between planes (electron pair distance)

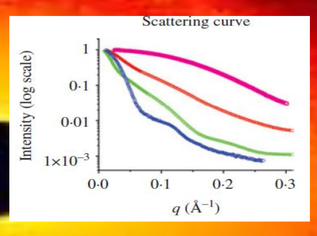
θ – angle between the incident ray and the scattering planes

n – an integer



Solution scattering:

- X-ray scattering is proportional to the electron density of the macromolecule, $\rho(r)$, and is the Fourier transform of the spatially averaged auto-correlation function of the electron density: $I(q) = 4\pi \int_0^{D_{max}} \langle \rho(\vec{r}) \cdot \rho(-\vec{r}) \rangle \frac{\sin(qr)}{qr} r^2 dr$



I – intensity

$\rho(r)$ – electron density

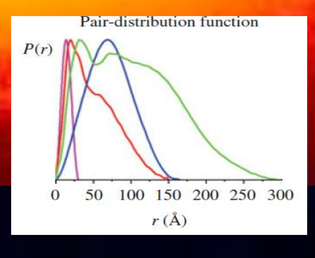
D_{max} – maximum dimension of the macromolecule

$\vec{r}$ – the vector describing the spatial position

q – scattering vector

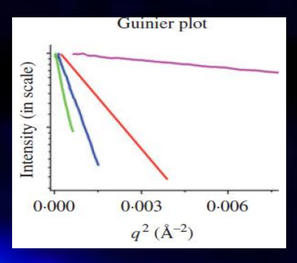
- The spatially averaged auto correlation function of the electron density multiplied by r^2 is called the pair distribution function or $P(r)$:

$$P(r) = r^2 \langle \rho(\vec{r}) \cdot \rho(-\vec{r}) \rangle \; ; \; P(r) = \frac{r}{2\pi^2} \int_0^\infty I(q) q sin(qr) dq$$



- The radius of gyration (R_g), is the square root of the average squared distance of each scatterer from the particle center. At low resolution, the scattering can be described by the Guinier approximation:

$$I(q) = I(0) \exp\left[-\frac{q^2 R_g^2}{3}\right] \quad ; \quad \frac{I(0)}{c} = \frac{N_A M_w}{\mu^2}(1 - \rho_0 \psi)^2$$



$I(0)$ – direct beam intensity

c – concentration of the target particle; $c = N\mu m/N_A$ where N is the number of particles

M_w – molar weight

N_A – Avogadro's number

ρ_0 – average electron density of the

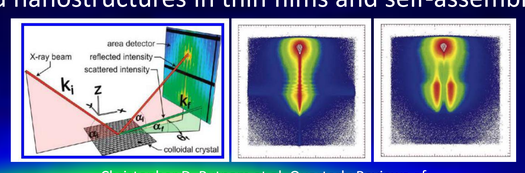
ψ – ratio value of the volume of the particle to its number of electron

μ – the ration of the molecular weight to the number of electron

The Guinier plot of the $\log(I(q))$ against q^2 will give a straight line from which R_g and $I(0)$ can be extracted. On an absolute scale, $I(0)$ is the square of the number of electrons in the scatterer and is unaffected by particle shape and is useful for molecular weight determination. Since $I(0)$ depends on the square of the number of electrons (molecular weight), SAXS is particularly sensitive to the assembly state of the scatterers.

Surface scattering:

Grazing incidence Small X-ray Scattering (GISAXS) is a powerful tool to study nanostructured surfaces, combing the accessible length scale of SAXS and the surface sensitivity of grazing incidence radiation. Application range from characterization of quantum dot array, self-organized nanostructures in thin films and self-assembly on nanoscale length.



Christopher D. Putnam et al, Quarterly Reviews of Biophysics 40, 3 (2007), 191-285

GISAXS advantages:

- ✓ GISAXS does not require any specific sample preparation
- ✓ The film thickness may range from a few nm to several 10nm such thin films are still penetrated by X-ray beam
- ✓ The film surface, the film interior, as well as the substrate film interface are all accessible. By varying the incidence angle the various contributions can be identified
- ✓ The limitation of conventional SAXS with respect to extremely small sample volumes in thin film geometry are overcome

Interacting supramolecules:

Scattering profile can be described by two functions: Form Factor, FF(q), and Structure Factor, SF(q):

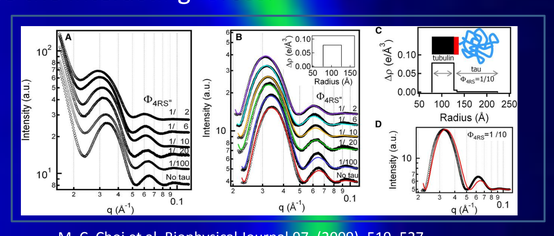
$$I(q) = \left(FF(q) \cdot SF(q)\right)^2$$

Form factor – structural information about the individual building block

Structure factor – information about the organization of the building blocks in a lattice

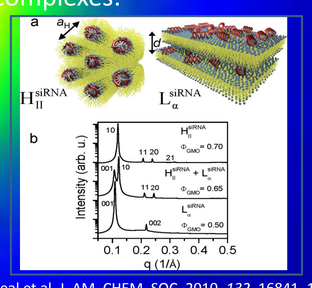
From fitting the scattering intensity curves one can solve the structures and deduce the interacting forces.

Example for structure factor study – microtubules structure in solution:



M. C. Choi et al. Biophysical Journal 97, (2009), 519–527

Example for Structure factor study – siRNA / Lipids complexes:



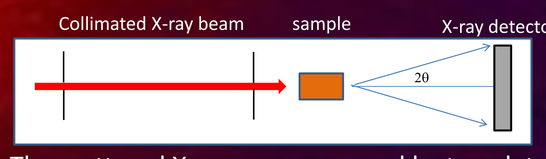
C. Leal et al. J. AM. CHEM. SOC. 2010, *132*, 16841–16847

SAXS major advantages

- ✓ Nondestructive
- ✓ Variable sample states, e.g. solution, powder, gel….
- ✓ Does not require tagging
- ✓ Minimal sample preparation
- ✓ Large length span (1-100nm): SAXS characterizes shape and conformation for variable-sized macromolecular complexes
- ✓ Measurements can directly define the global shape and conformation

Experimental system

- Monochromatic (Cu (K_α), 8KeV) ultra-focused X-rays beam is collimated by hybrid slits
- The X-ray beam go through a vacuum flight tubes to minimize excess scattering



- The scattered X-rays are measured by two detectors aligned in parallel. This setup enables simultaneous detection of both SAXS and wide angle X-ray scattering (WAXS), allowing extended measurable length-span
- The system is fully motorized and computer controlled, which allows remote control and easy operation

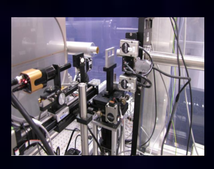
System components

X-ray Source: GeniX (Xenocs) low divergence system combines -

(1) a micro focus sealed tube X-ray source

(2) 3D multilayer optics to deliver an intense beam with minimum divergence in both vertical and horizontal planes

The **sample holder** is fully motorized and designed with several experimental configurations including capillaries, powders, solutions, grazing surfaces, temperature control and others



When the X-ray beam hits the sample it scatters. Our system consists of two different, fully motorized, two-dimensional **detectors** to simultaneously measure WAXS and SAXS (MAR345 and Pilatus 300K, respectively).

The distance between the sample and the detectors can be altered between 0.2 to 2.5 meters, which gives us correlation measurements in the range of 0.3-120nm.

Something about the detectors

Pilatus 300:

Operates in "single photon counting" mode. The X-rays are directly transformed into electric charge and processed in the CMOS readout chip. This has no dark current and readout noise. The detector area is 83.8x106.5mm² with 172x172μm² pixels

MAR345 image plate detector:

Consists of a scanning unit which holds the image plate and the equipment to read and erase the plate.

The detector stores an X-ray image on a phosphor screen. After exposure is completed, the plate is rotated and a scanning unit moves from the edge to the center of the plate and reads the data in a spiral format.

When the scanning is completed the scanning head is withdrawn and 3 strong lights are turned on to erase the plate. The plate diameter is 345mm with 100x100μm² pixels