미래기반 가속기
전문인력양성 사업단

특론: 가속기 실험실습 I

(NUCE719P-01/PHYS715P-01, 정모세)

소각 X-선 소각 산란 개요 및 활용

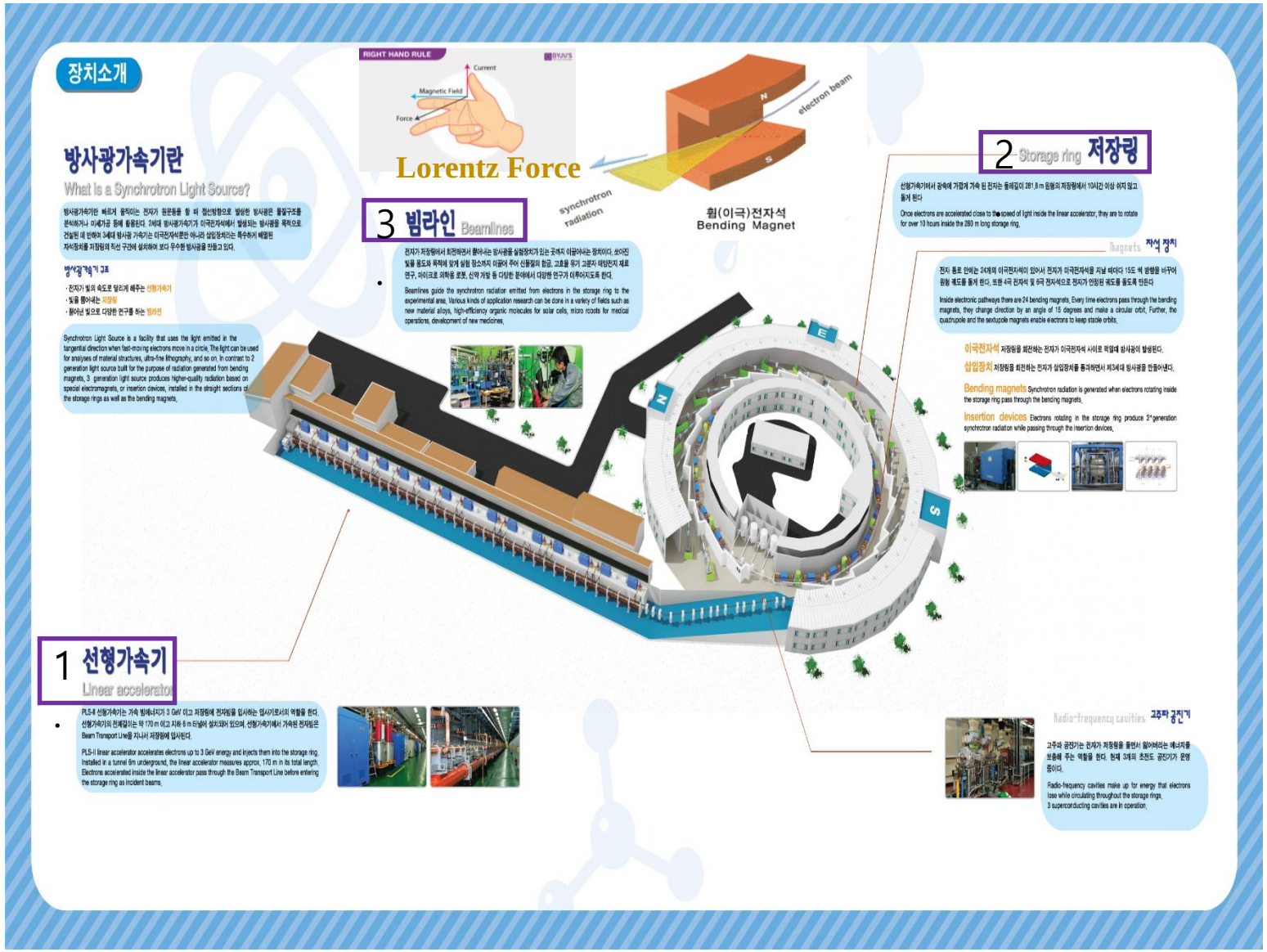
진 경 식

첨단원자력공학부

jinks@postech.ac.kr, PAL 저장링동 224호

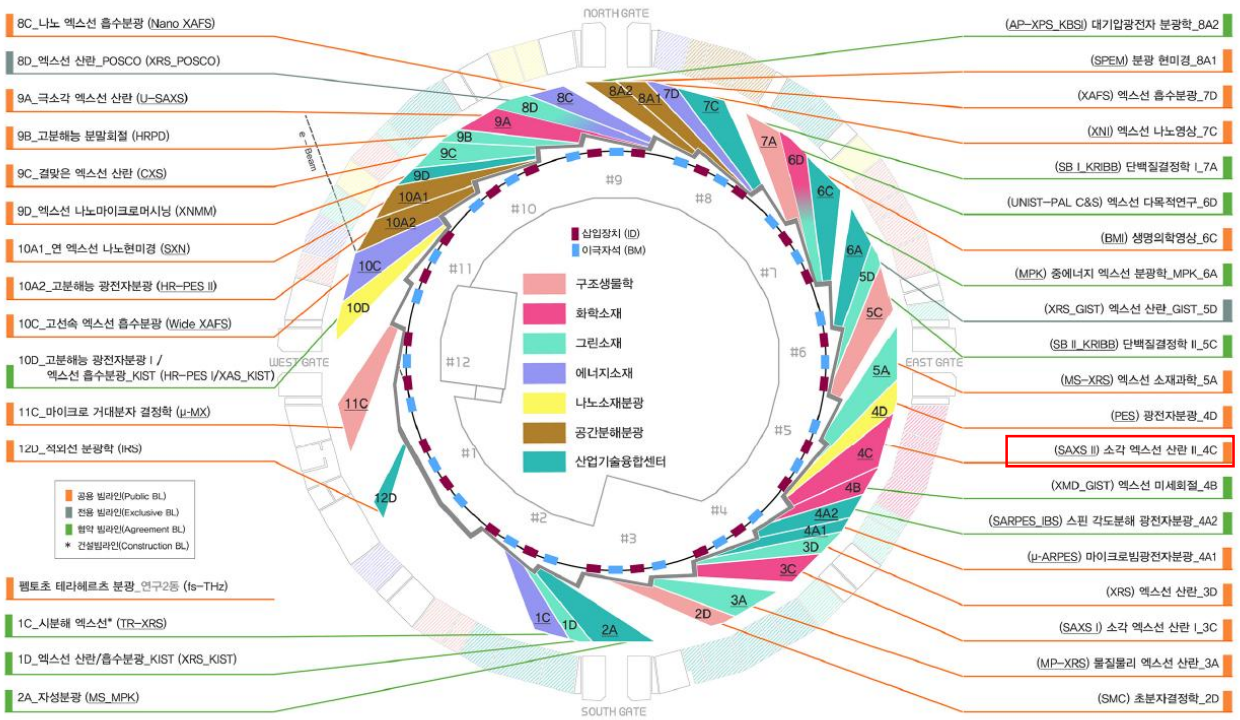
PAL Facility

PAL Facility : PLS-II



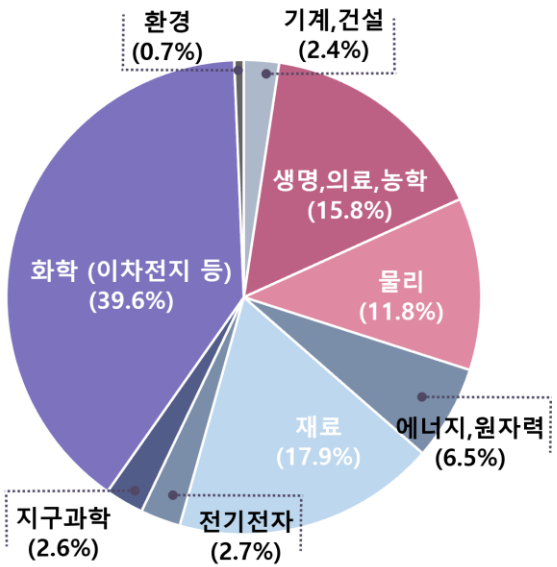
3세대 빔라인 현황 (36기)

Storage Ring
- Electron beam energy: 3 GeV
- Ring current: 400 mA, Top-up mode
- User beamtime: 190 days/year



▸ '22년 배정 기준

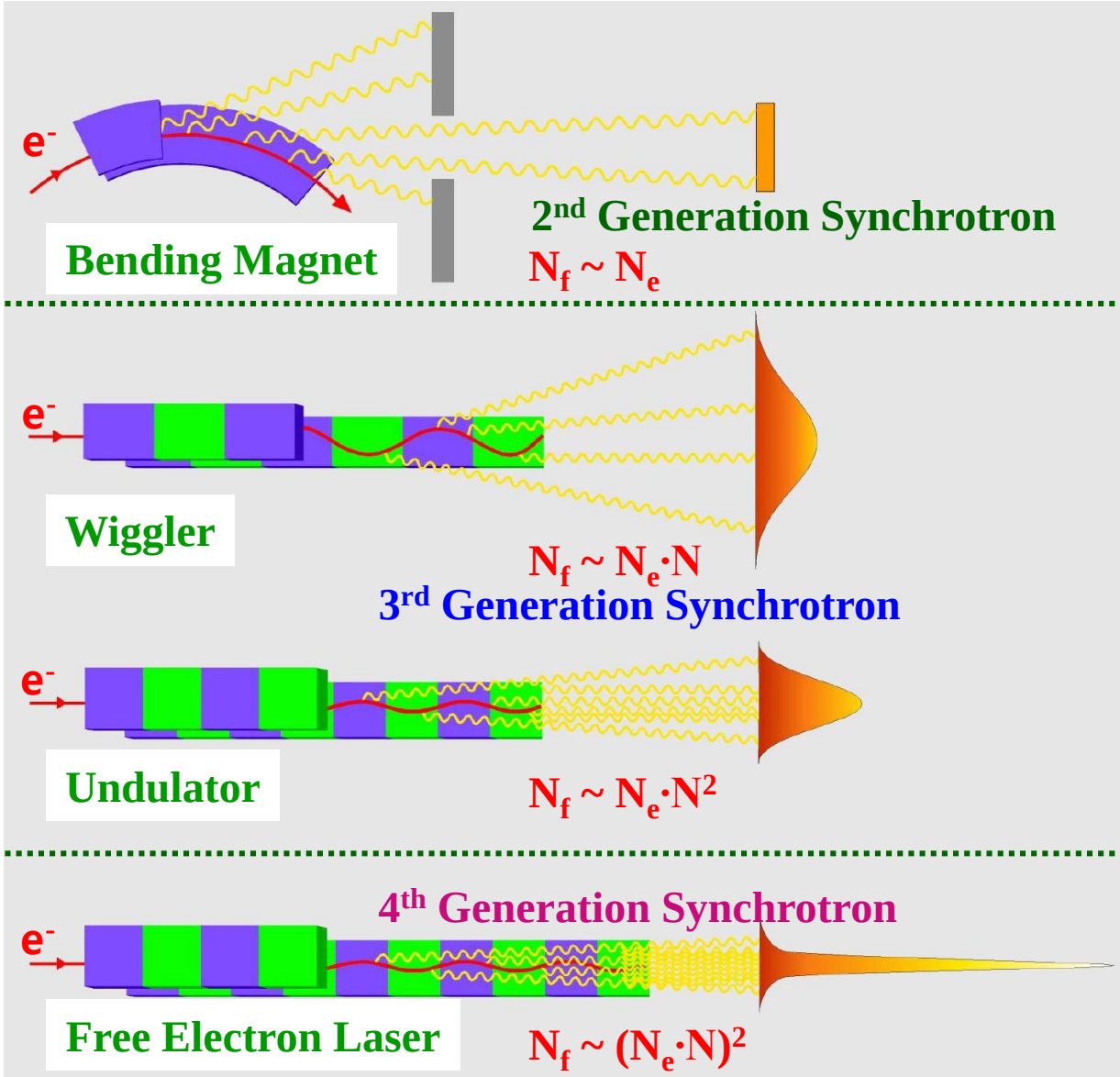
3세대 연구분야



3세대 사용기관 (배정)

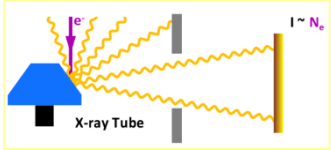
구분	국 내			외국	계
	대학	연구소	산업체		
'21	1,539 (82.3%)	234 (12.5%)	52 (2.8%)	46 (2.4%)	1,871
'22	1,691 (84.2%)	220 (11.0%)	73 (3.6%)	25 (1.2%)	2,009

PAL Facility : Synchrotron Radiation Sources



Synchrotron radiation is produced when particles are accelerated in a storage ring.

N_f : the number of radiated photons
N_e : the number of electrons in the beam
N : the number of undulator periods



Wiggler and undulator - linear magnetic structures - are used to improve the properties of the radiation produced.

Direct descendants of the bending magnets in a storage ring, wiggler and undulator, force the particles to travel along a zig-zag path so that the emitted light waves are superimposed.

These magnetic structures are now used to produce synchrotron radiation in laboratories worldwide. Extremely intense X-ray radiation with laser-like properties is generated by free-electron lasers.

Small-angle X-ray Scattering (SAXS)

- ❖ SAXS is a **fundamental and powerful tool** for structural analyses of polymers, metals, alloys, liquid crystals, and colloidal systems.
- ❖ Synchrotron radiation X-ray sources with a **high-flux photon and low-divergence beam** enable broader application of SAXS, such as time-resolved measurements of powder/film samples in solid and nanoparticles/biomacromolecules in solution.
- ❖ SAXS is becoming a **standard tool** for the structural characterization under carefully controlled conditions, such as pH, ionic strength, chemical reagents, humidity, electric/magnet field, pressure, and temperature.

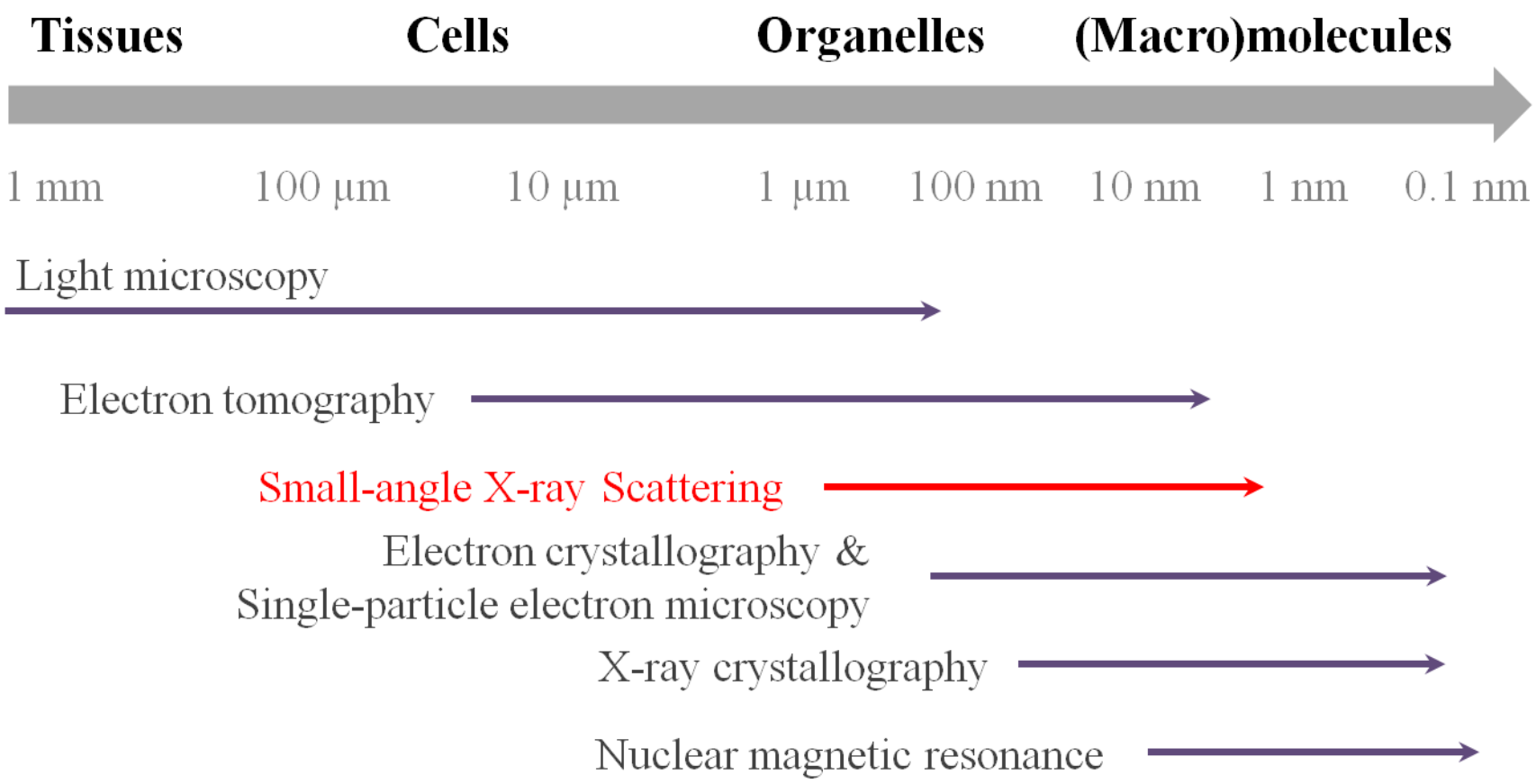
✓ **Beamlines currently in operation at PAL: 3C SAXS I, 4C SAXS II, 6D UNIST-PAL, and 9A U-SAXS**

Applications

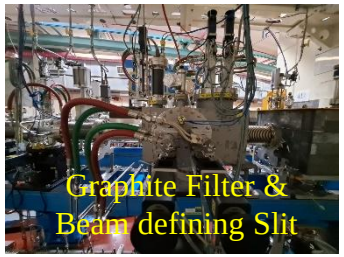
SAXS is used for the determination of nanoscale structure of particle systems

- Energy Material: Organic Solar Cell, Battery
- Flexible Display: Organic Thin-film Transistor
- Ultra Low-k Dielectric Material
- Nano-template Fabrication
- Micro-array Fabrication
- Synthetic Polymer Nanostructure Studies
- Biological Nanostructure Studies (Protein, DNA, Drug Delivery System, etc.)

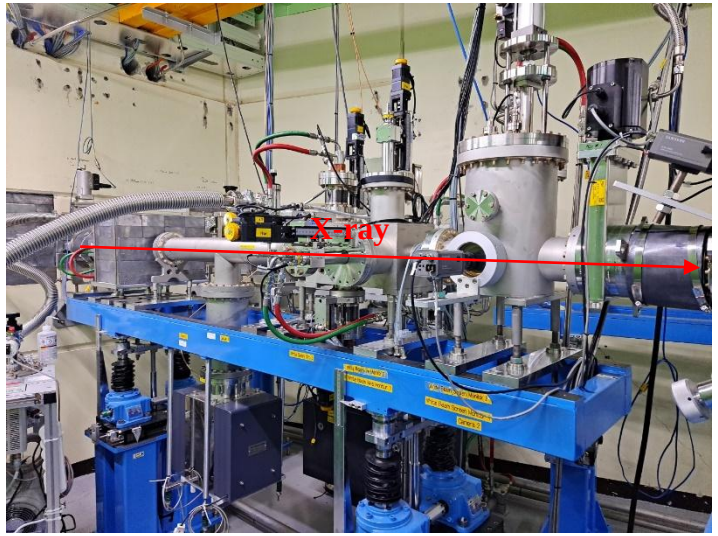
Resolutions

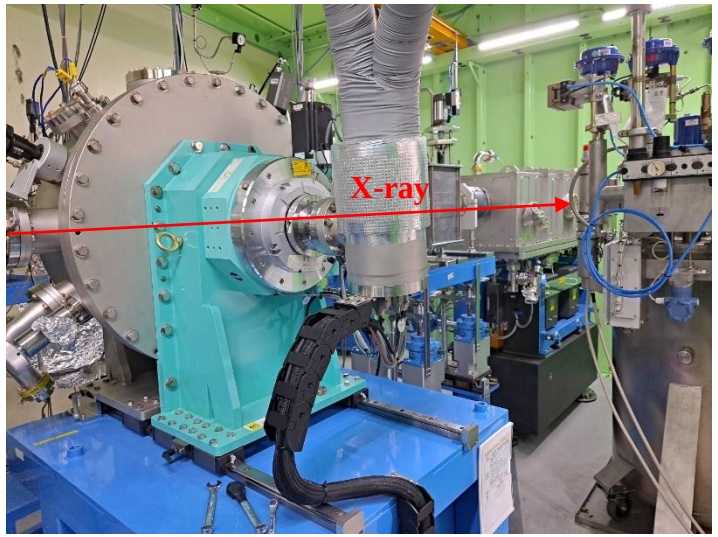


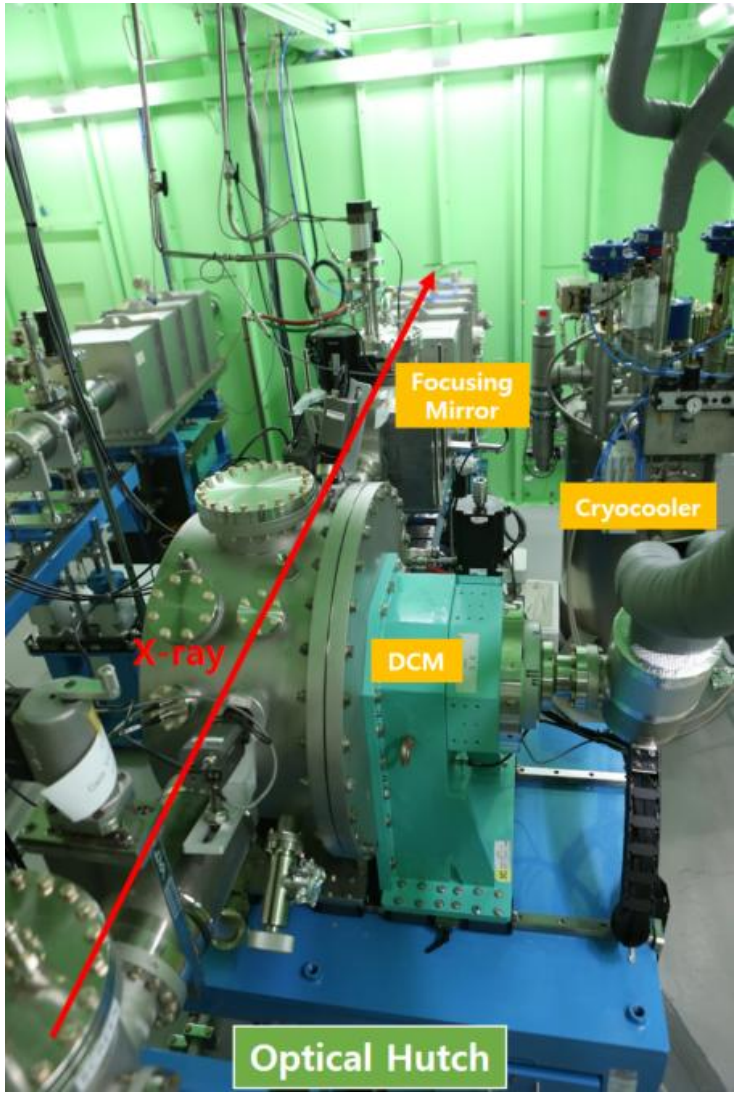
Optical Components in Storage ring



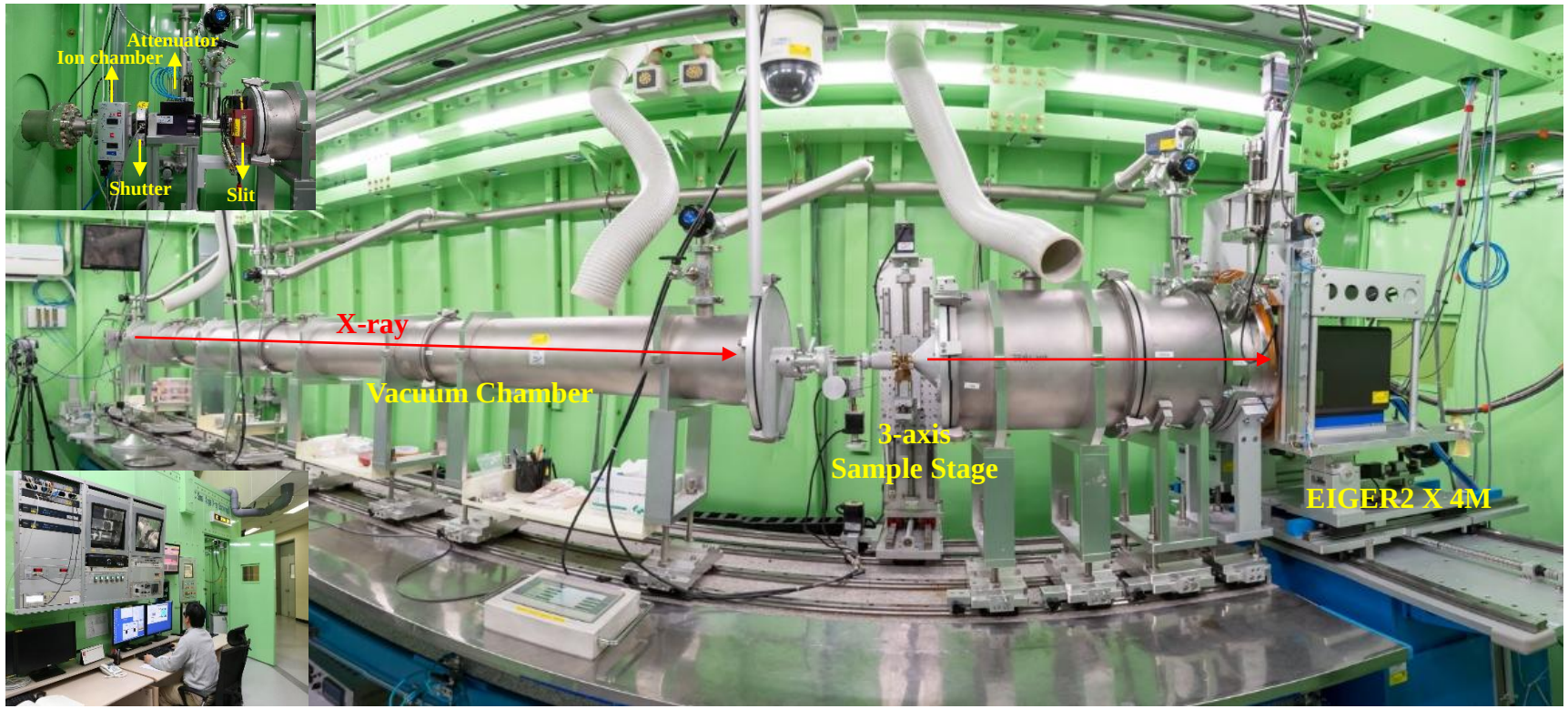
Optical Components in Optical Hutch







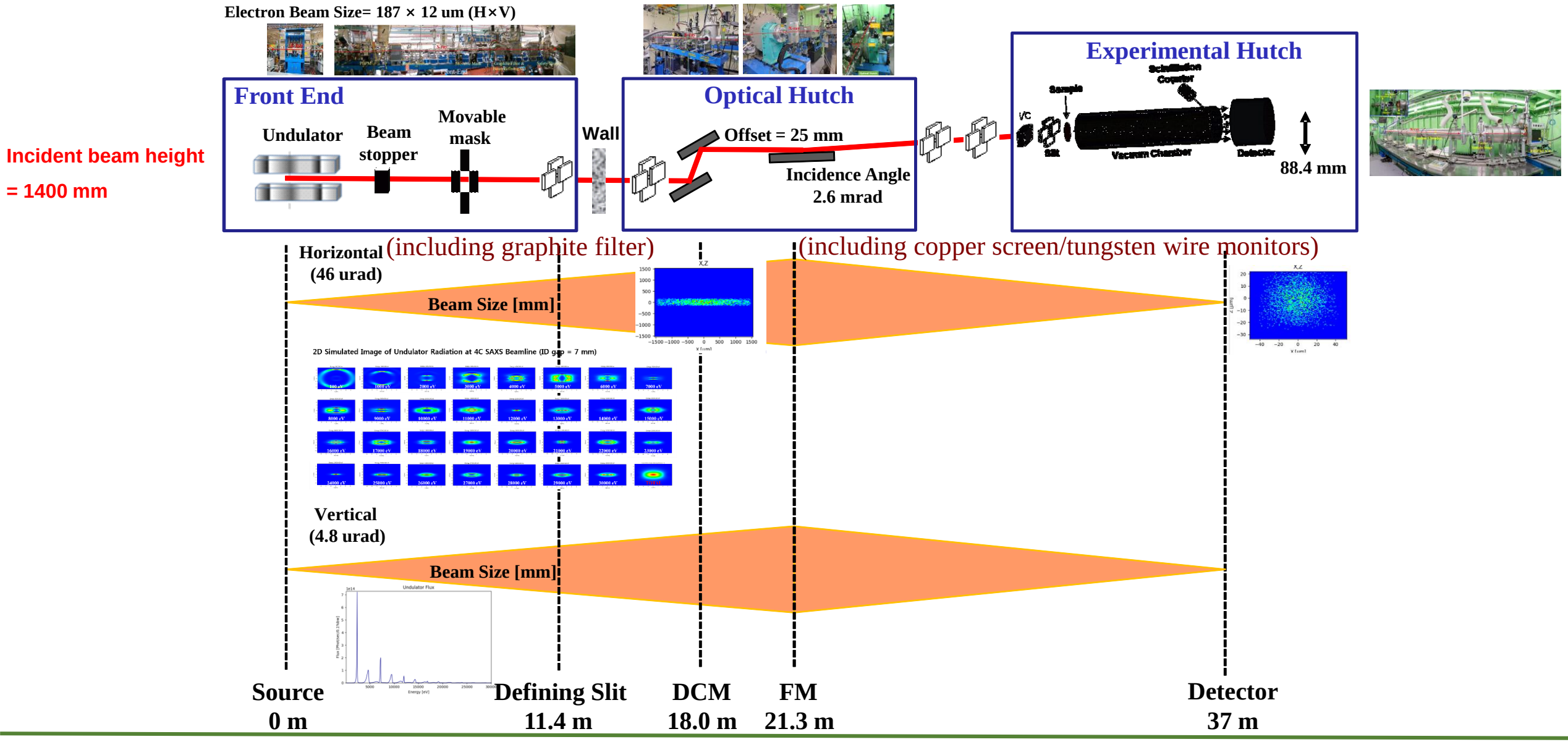
Experimental Components in Hutch



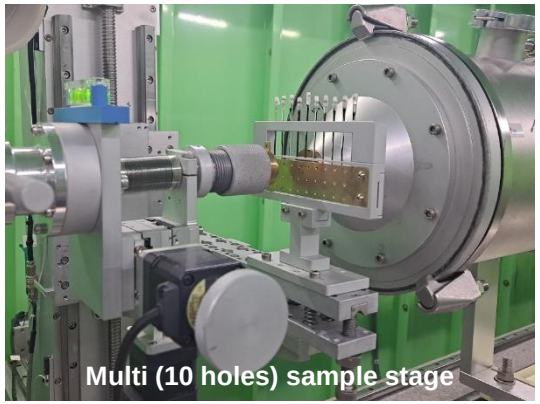
Photograph of optical components in optical hutch and experimental devices in the experimental hutch of the end-station

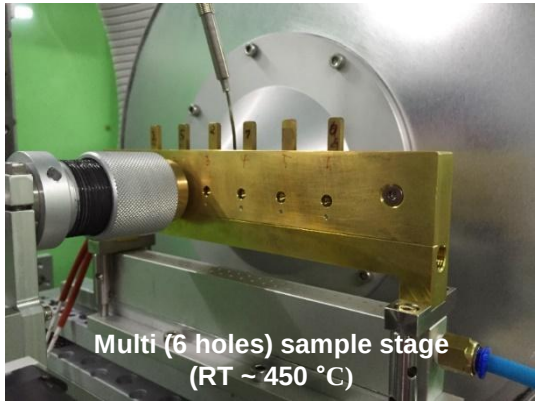
"Small-angle X-ray Scattering Beamline BL4C SAXS at Pohang Light Source II" *Biodesign* **2017**, 5 (1), 24-29

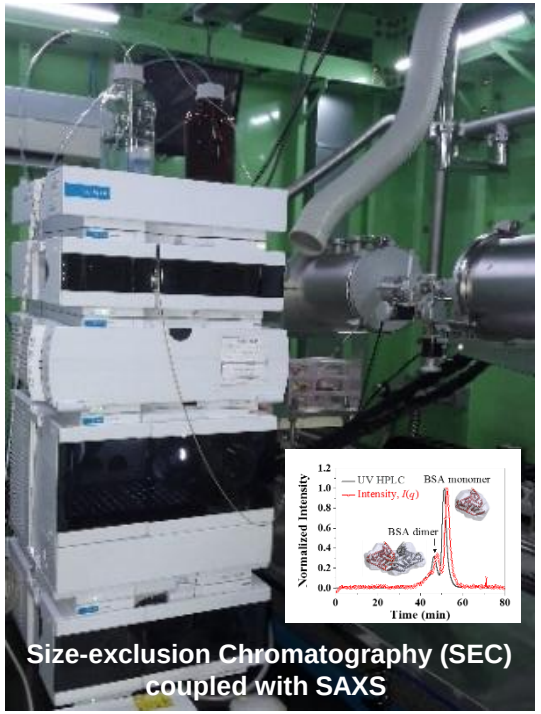
4C SAXS II Beamline Layout



Sample Environments

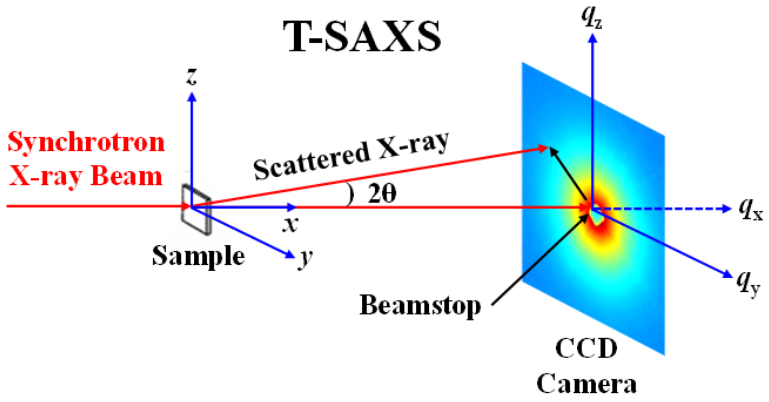


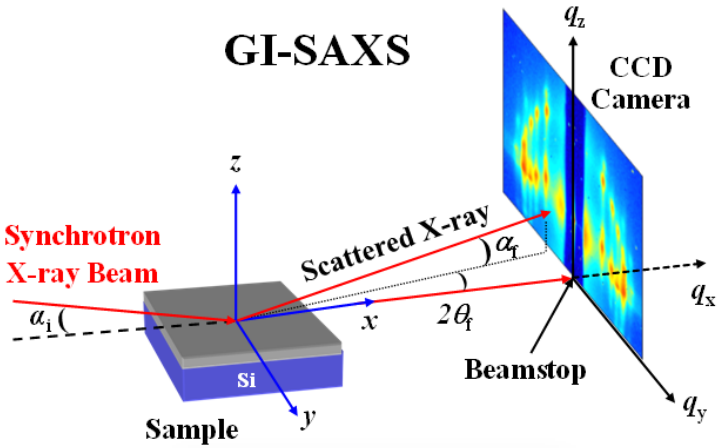


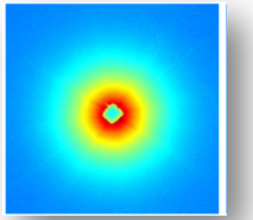


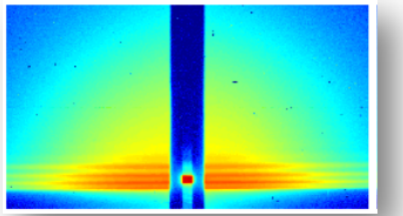
Photograph of experimental sample stages in the experimental hutch of the end-station

Experimental Configuration: Transmission SAXS vs. Grazing-incidence SAXS



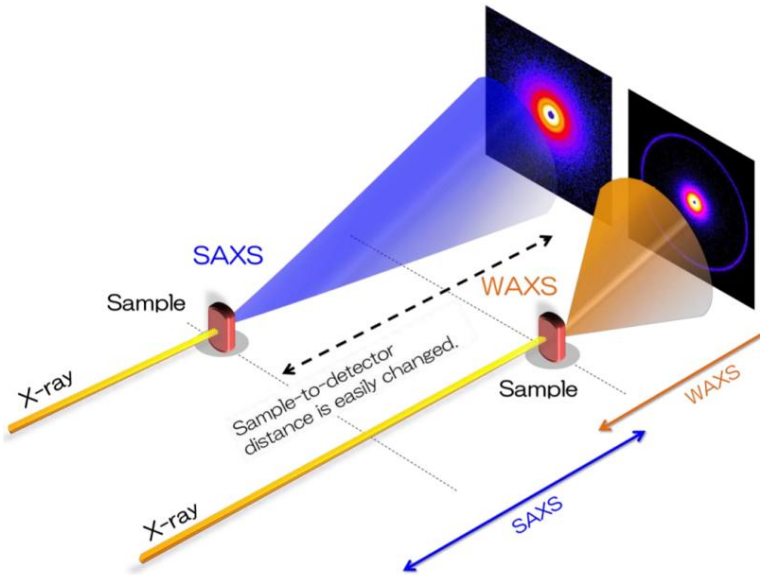






T-SAXS	GI-SAXS
Easy measurement Easy analysis In-plane information Bulk samples & Solution samples	Strong intensity Easy preparation of samples In-plane & out-of-plane information ($q_{//}$, q_z) Thin film samples
Any possible scattering from substrate Transparency of substrate High energy & High flux X-ray beam	Scattering from surface/internal structure Scattering from reflected & transmitted beam Refraction effects Special setup

Experimental Setting: Sample-to-detector distance (SDD)

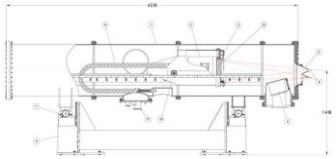


PLS-II 4C SAXS

PETRA-III P12 SAXS

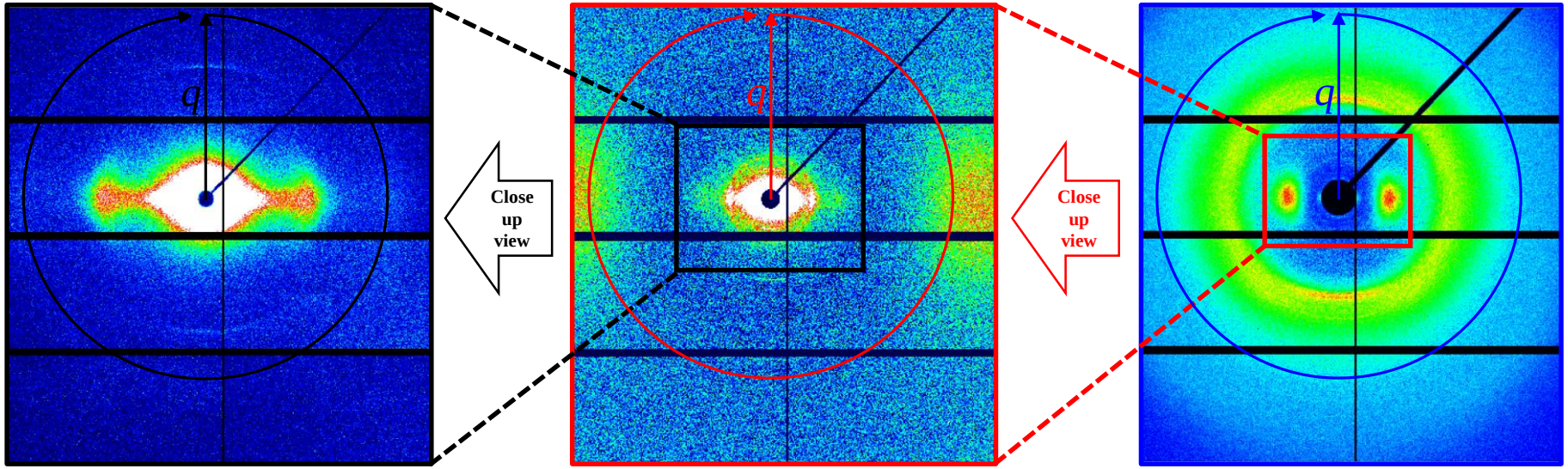
➢ **Variable *q*-range vacuum chamber**

✓ Facilitate rapid sample-to-detector distance (SDD) change



APS 12 ID-B SAXS

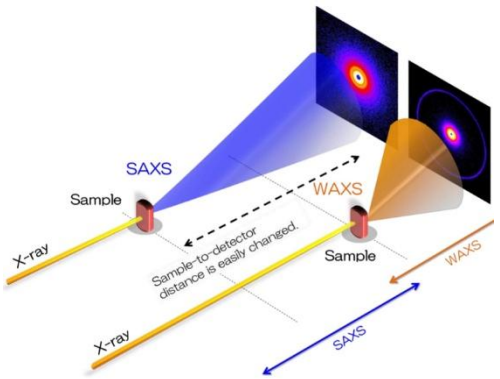
Scattering Patterns of Fiber Strands as a function of SDD

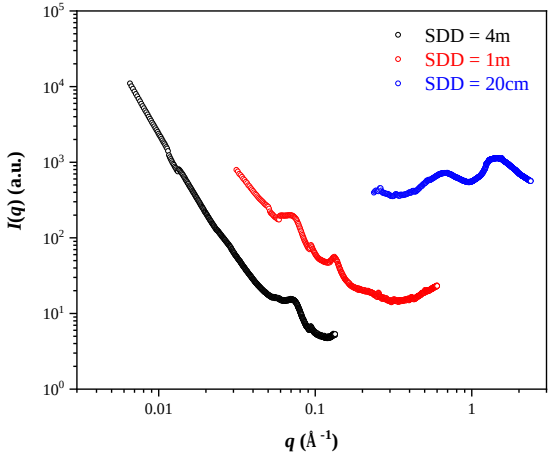


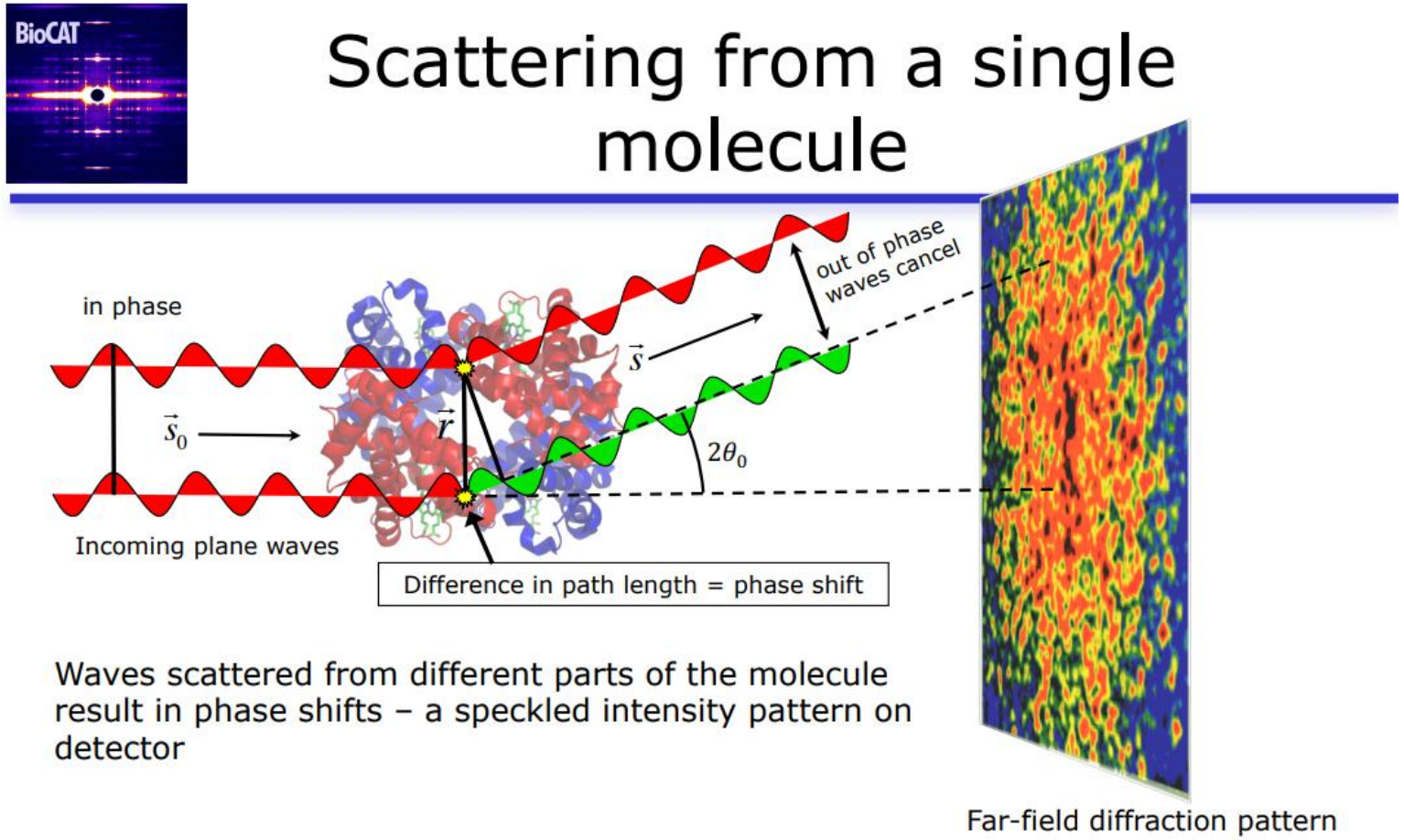
SDD = 4m
(q = 0.0065 ~ 0.1344 Å $^{-1}$)

SDD = 1m
(q = 0.0314 ~ 0.6 Å $^{-1}$)

SDD = 20cm
(q = 0.237 ~ 2.38 Å $^{-1}$)

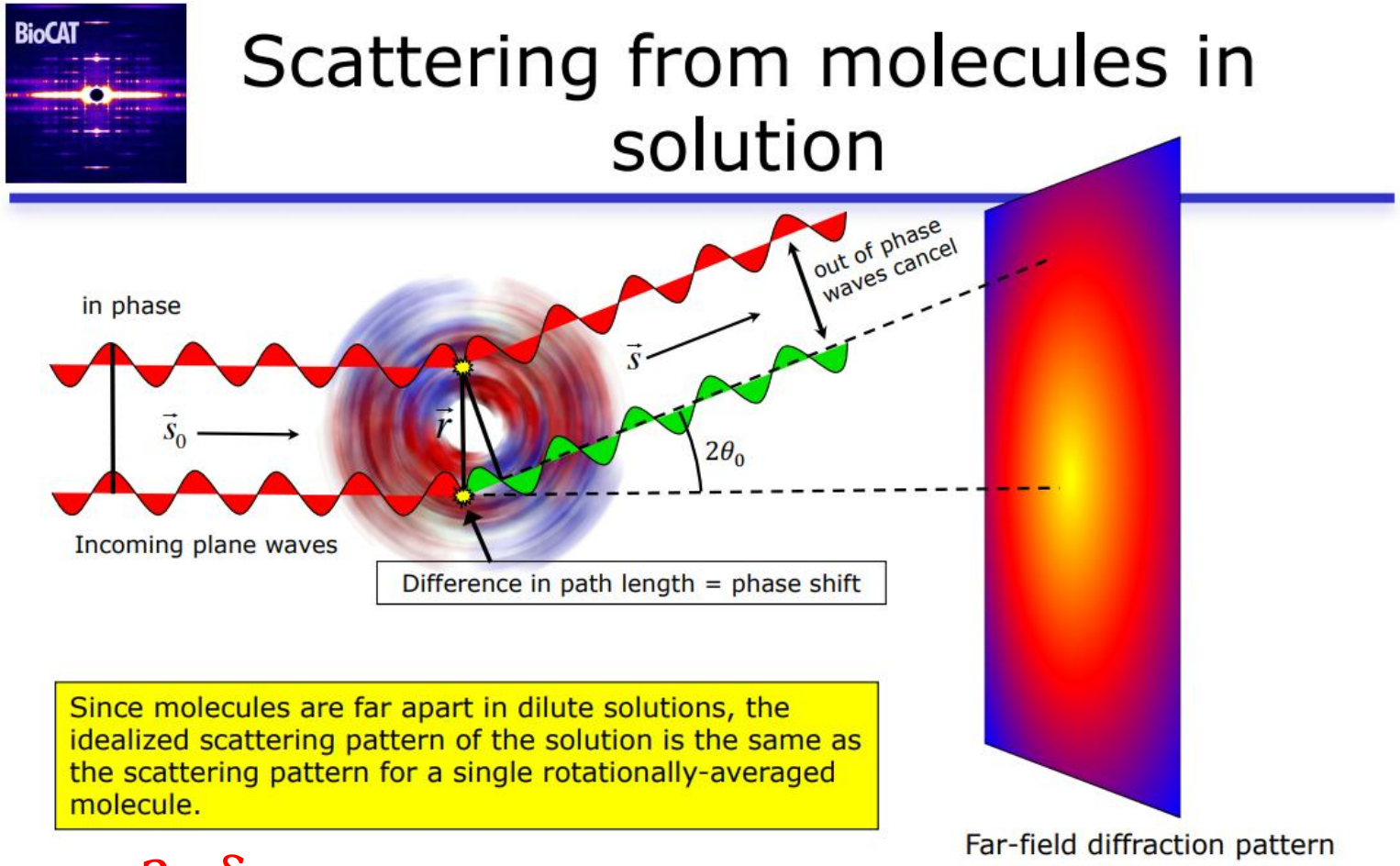






$$\Delta\phi = \frac{2\pi\delta}{\lambda} \qquad A \propto \mathrm{Sum}(An, \Delta\phi) \qquad I \propto A^2$$

Images from Richard Gillilan's BioSAXS Essentials presentation

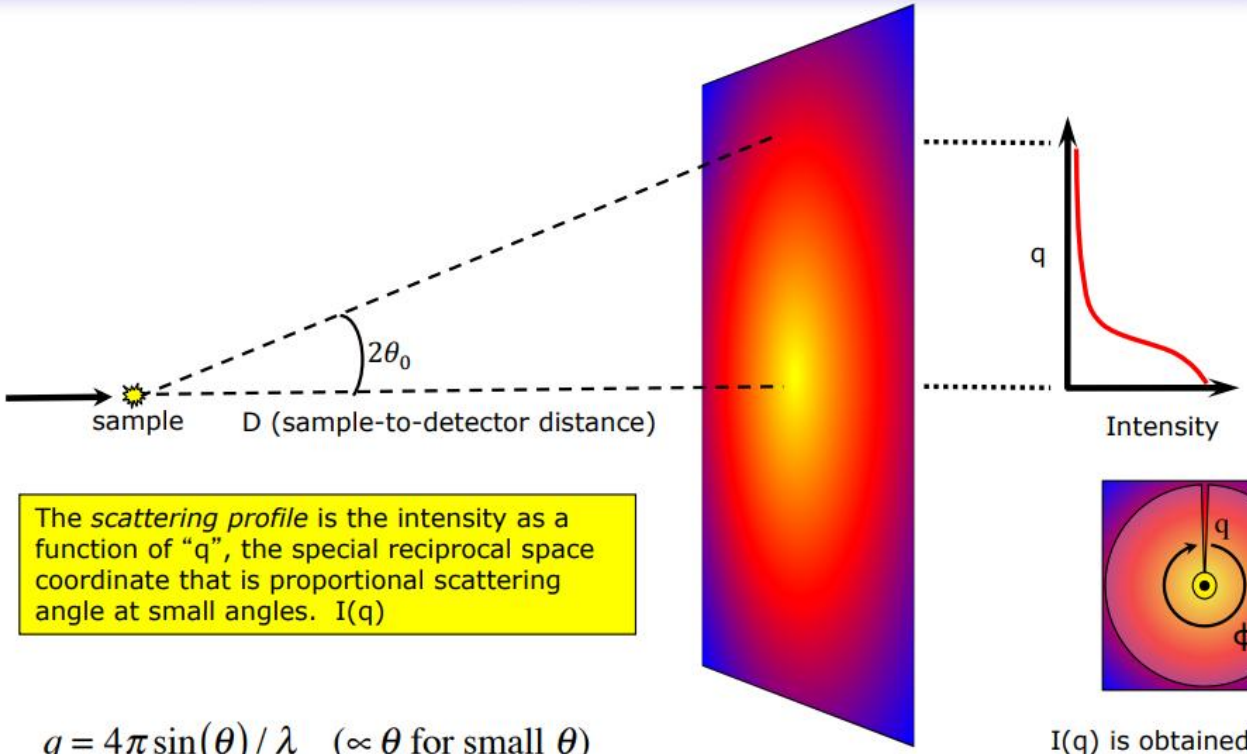


Since molecules are far apart in dilute solutions, the idealized scattering pattern of the solution is the same as the scattering pattern for a single rotationally-averaged molecule.

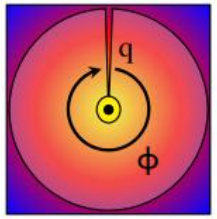
$$\Delta\phi = \frac{2\pi\delta}{\lambda} \qquad A \propto \mathrm{Sum}(An, \Delta\phi) \qquad I \propto A^2$$

Images from Richard Gillilan's BioSAXS Essentials presentation

The scattering profile



The *scattering profile* is the intensity as a function of "q", the special reciprocal space coordinate that is proportional scattering angle at small angles. I(q)



$$q = 4\pi \sin(\theta) / \lambda \quad (\propto \theta \text{ for small } \theta)$$

I(q) is obtained by integrating around the circle. For detectors, the standard deviation of signal σ(q) is also calculated.

Slide from Richard Gillilan's BioSAXS Essentials presentation

Scattering profile

$$I(q) \propto Mc(\rho_1 - \rho_2)^2 |F(q)|^2 S(q)$$

$I(q)$ – Experimental intensity

M – molecular weight

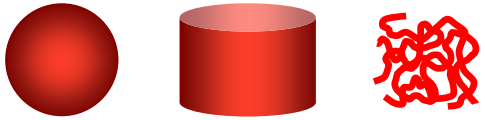
c – concentration

ρ – scattering density (electrons per unit volume)

ρ_1 - particle

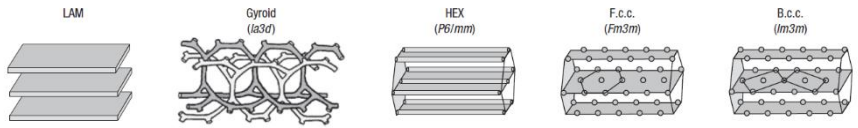
ρ_2 - solvent

$F(q)$ – Form factor, i.e. molecular shape



$S(q)$ – Structure factor, i.e. inter-molecular interaction

≈ 1 for dilute solutions

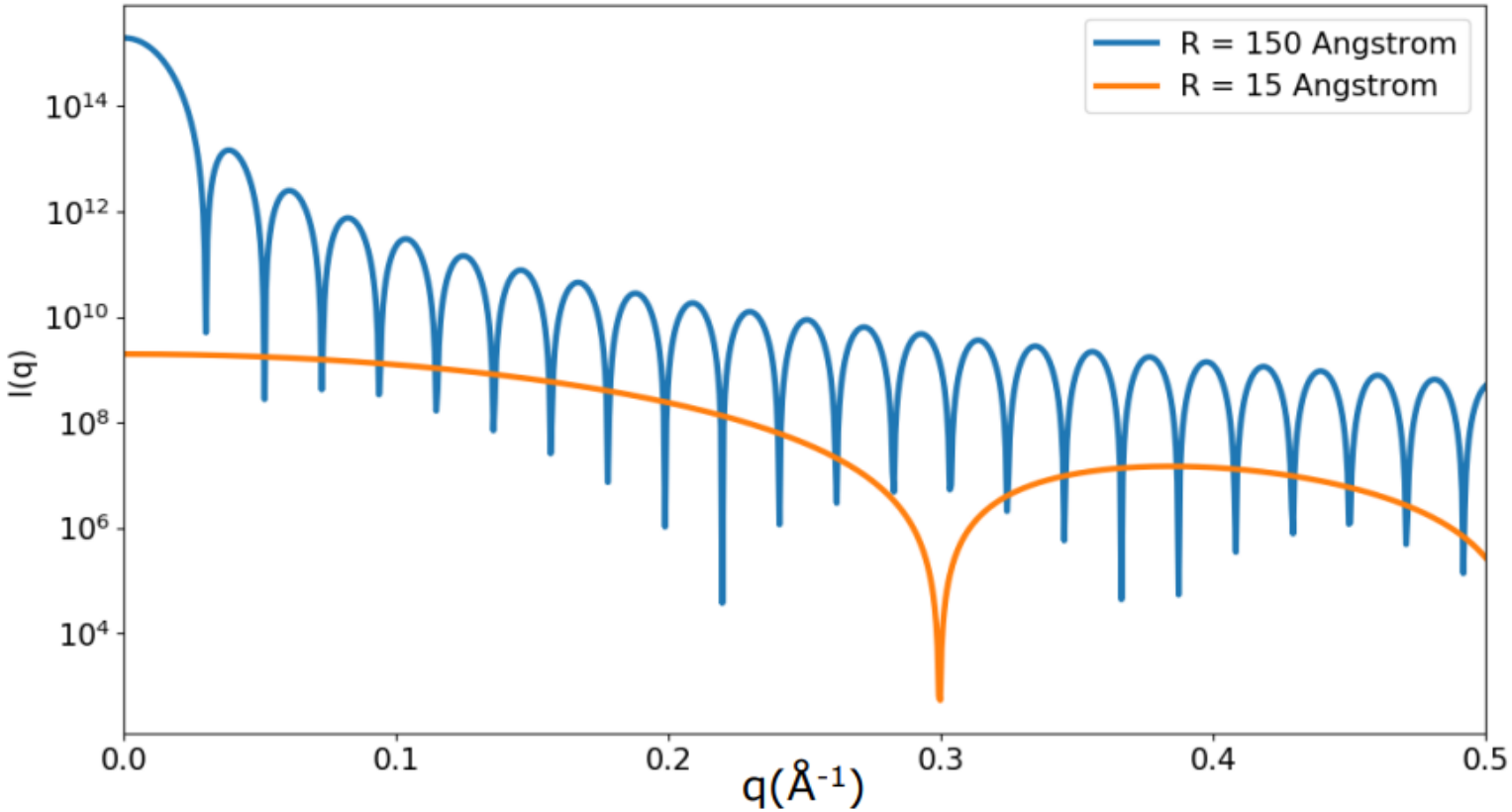


Slide from Jesse Hopkins's BioSAXS Essentials presentation

Scattering profile from a sphere

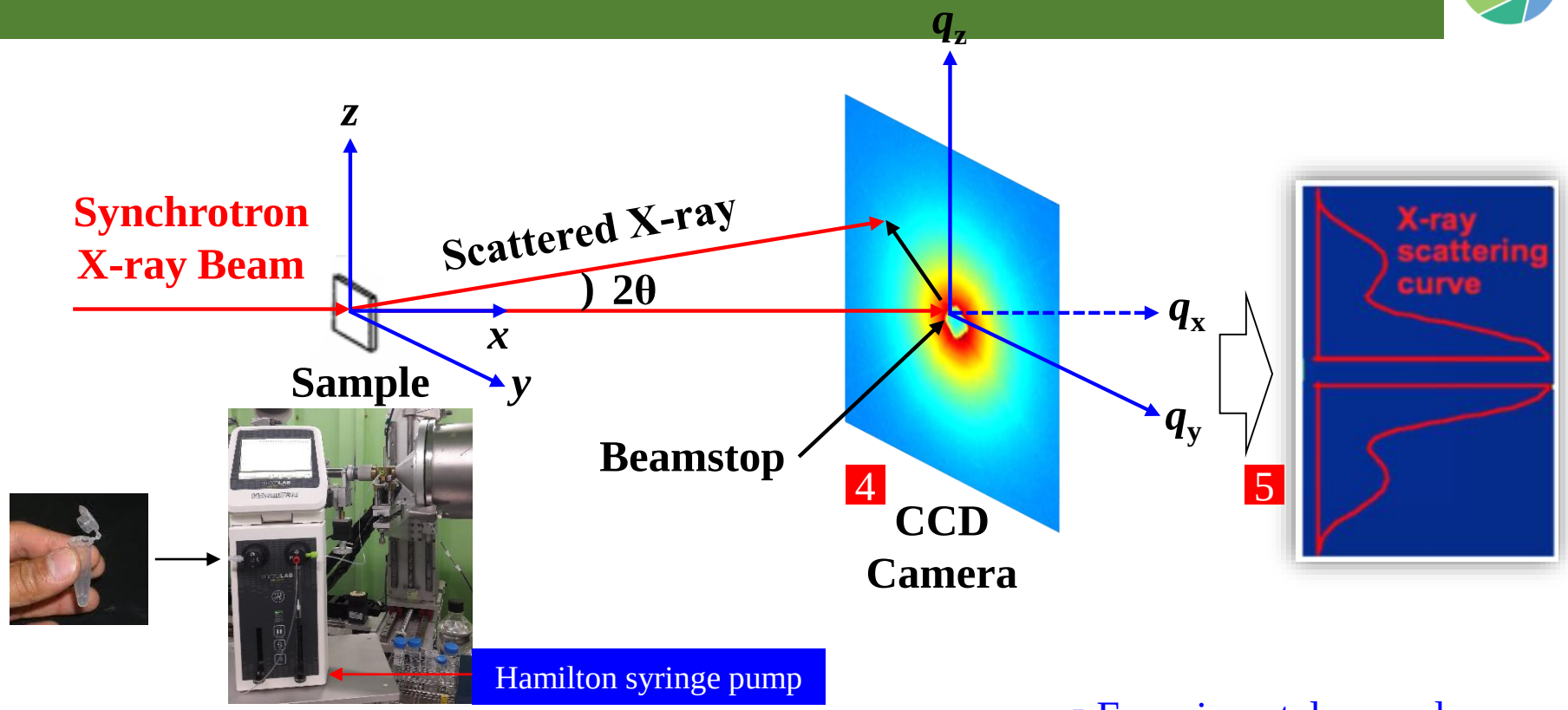
Scattering from a uniform density sphere with radius R:

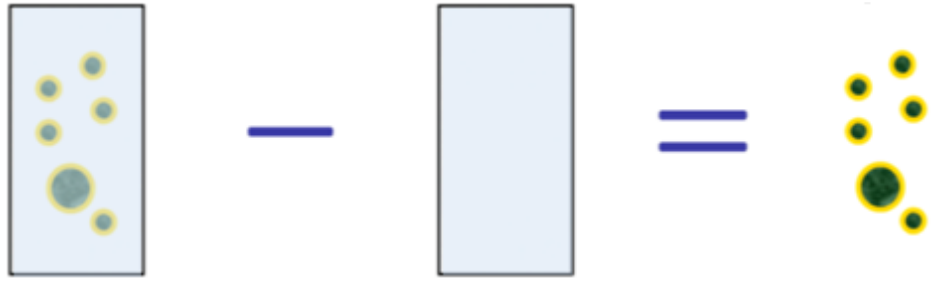
$$I(q) \propto \left(\frac{4\pi}{3}R^3\right)^2 \left(3\frac{\sin(qR) - qR\cos(qR)}{(qR)^3}\right)^2$$



Slide from Jesse Hopkins's BioSAXS Essentials presentation

Experimental Procedure of SAXS





1 $I_{Molecules + Buffer\ solution}(q)$

2 $I_{Buffer\ solution}(q)$

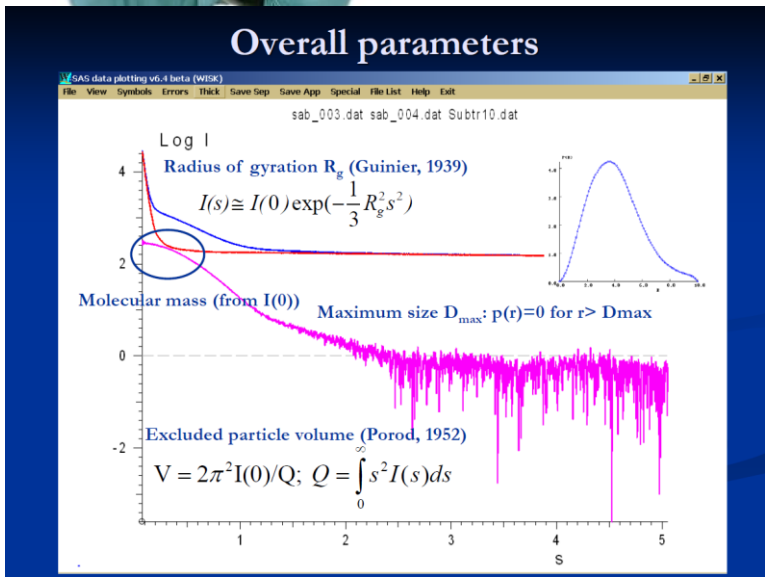
3 $I_{Molecules}(q)$

- Experimental procedures

1. Sample measurement
2. Background measurement
3. Background correction
4. Corrected 2D SAXS image
5. Radial averaged 1D SAXS curve

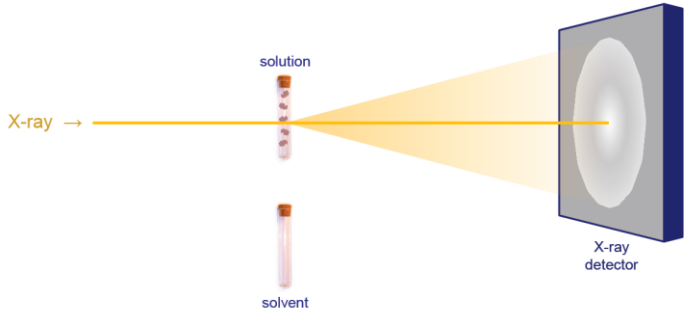
SAXS data processing, and overall parameters

- 3D → 2D → 1D
- Experiment design and data reduction
  - Exposure time
  - Background subtraction
  - Dilution series
- Overall parameters:
  - Guinier analysis:
    R_g, I(0), molecular mass
  - Volume
  - p(r), D_{max}



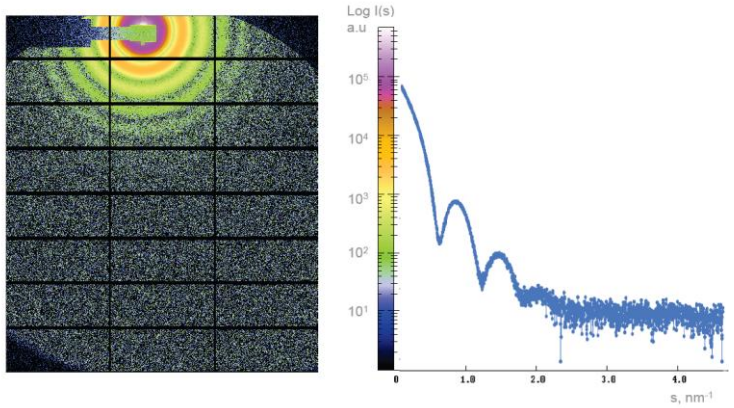
SAXS data processing, and overall parameters

SAXS experiment

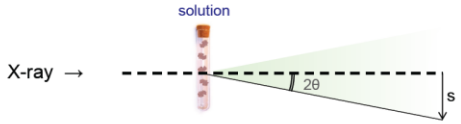


- Few kDa to GDa
- Monodisperse and homogeneous
- Concentration: 0.5–10 mg/ml
- Amount: 10–100 µl

2D → 1D

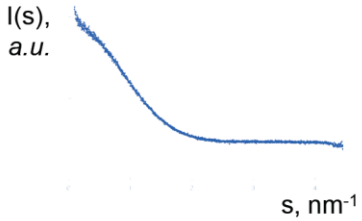


Notations and units



$|s| = 4\pi \sin\theta/\lambda$

2θ – scattering angle
λ – wavelength
s – scattering vector
I(s) – intensity



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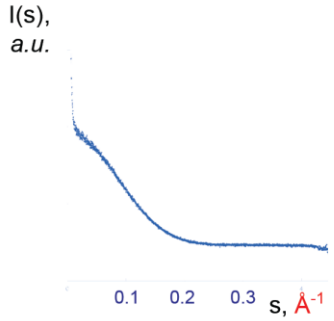


X-rays - Radiation damage!!!

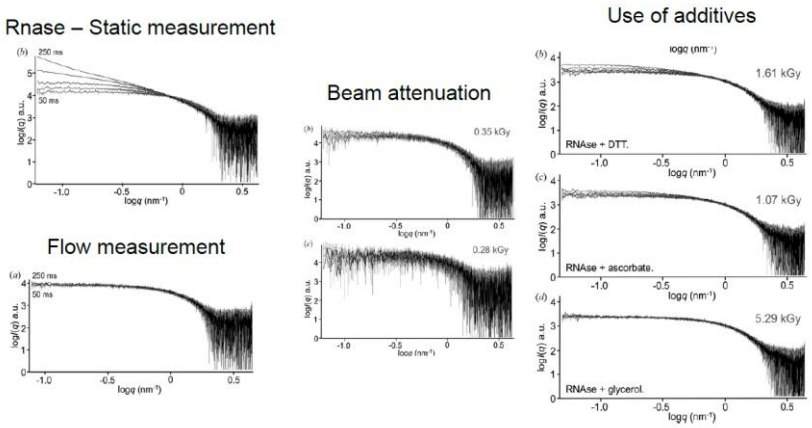
- With intense synchrotron beam: radiation damage:

$$H_2O \longrightarrow H\cdot \quad OH\cdot$$

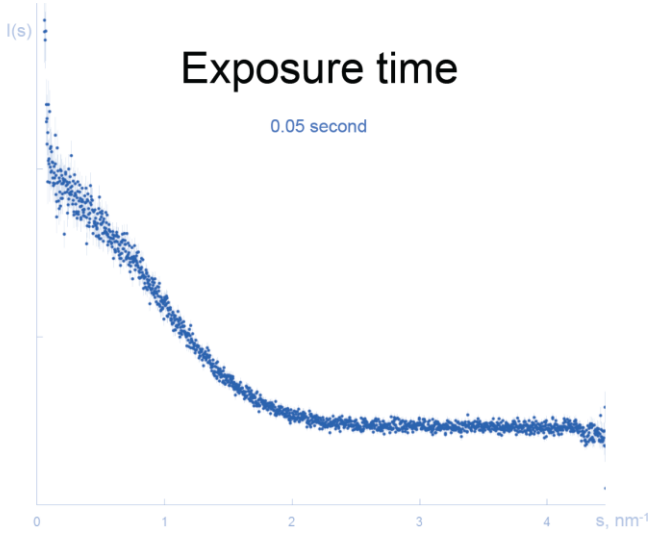
Free radicals: oxidize proteins which leads to their aggregation

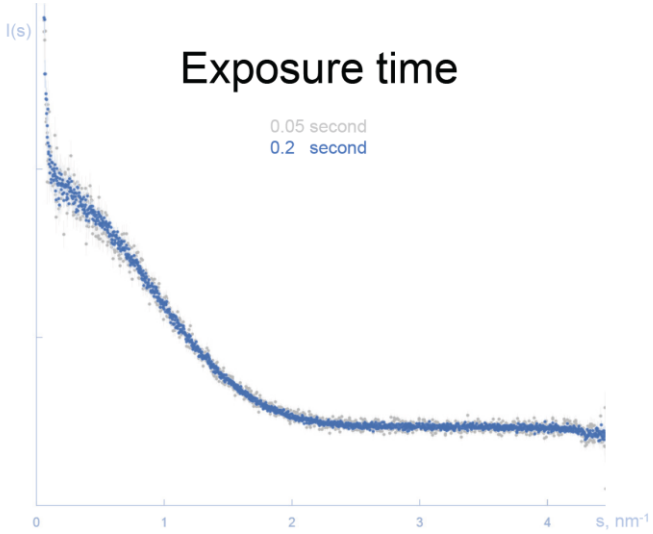
- Monitor radiation damage: collect several frames and compare them.
- Limit the radiation damage

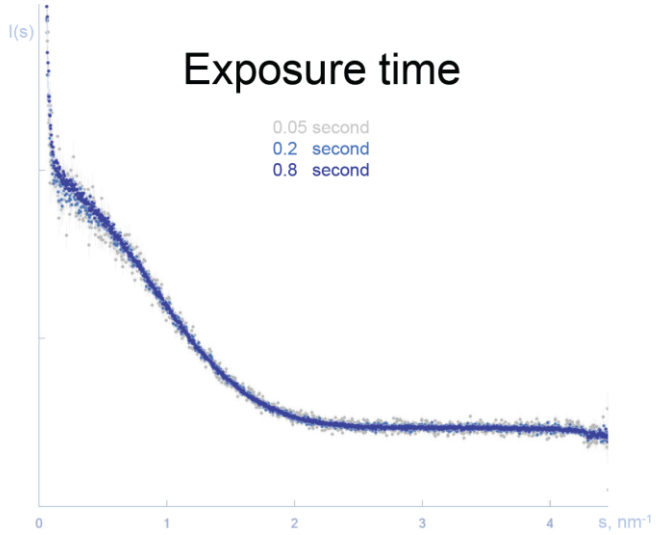
X-rays - Radiation damage!!!

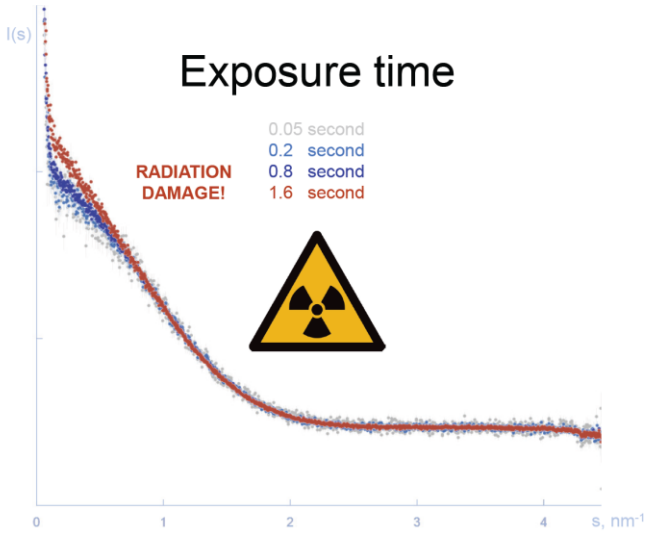


SAXS data processing, and overall parameters

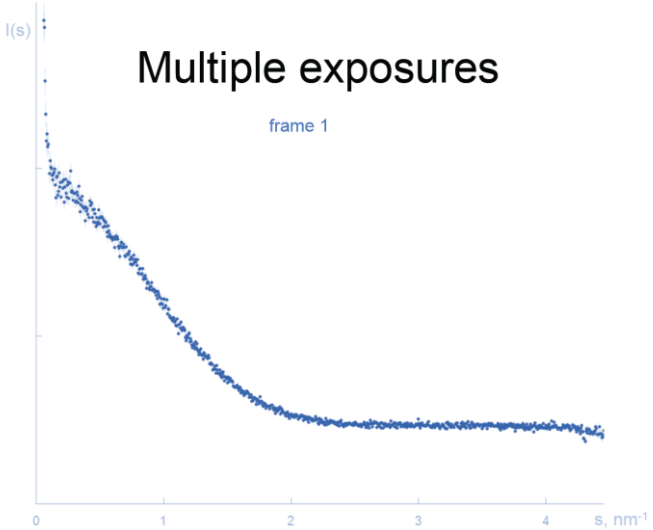


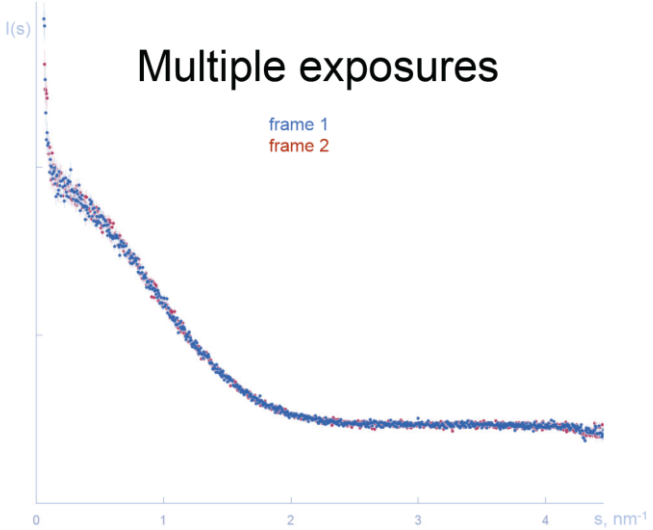


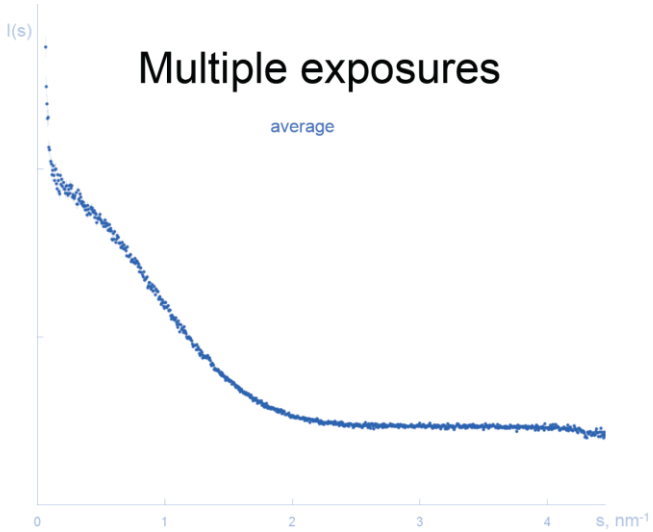




SAXS data processing, and overall parameters



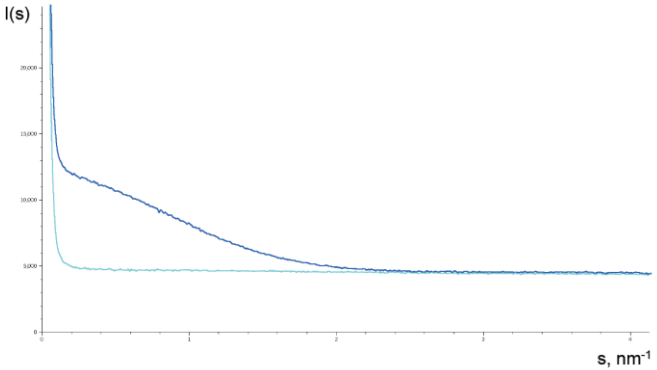




SAXS data processing, and overall parameters

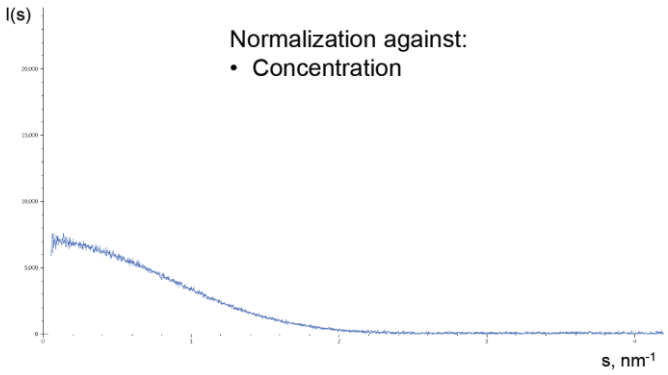
Background subtraction

Solution minus Solvent



Background subtraction

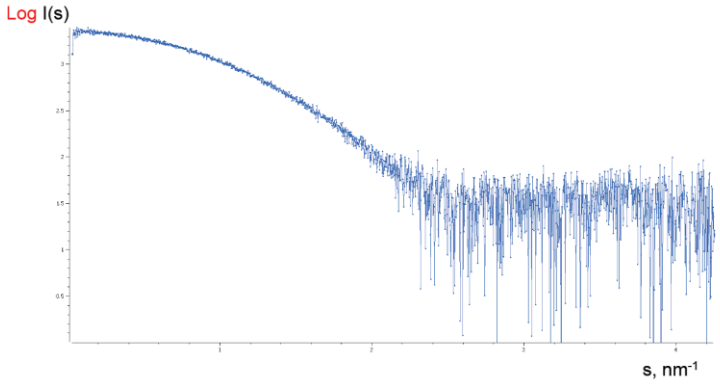
Solution minus Solvent



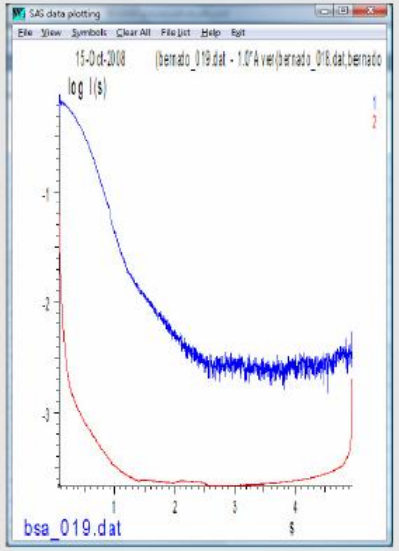
Normalization against:

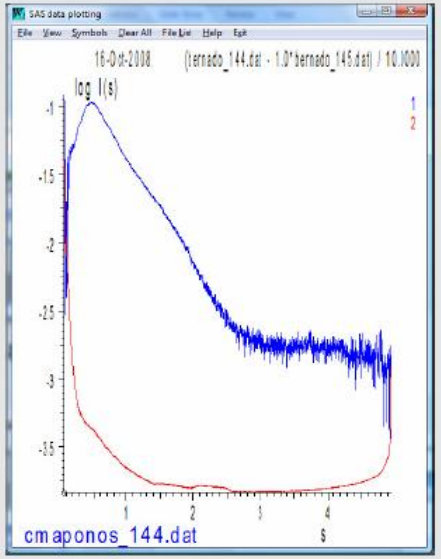
- Concentration

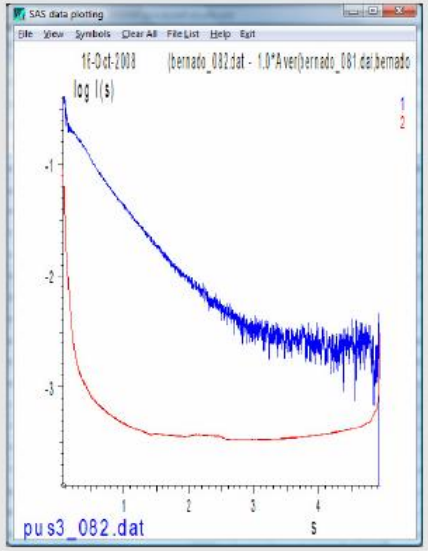
Logarithmic plot

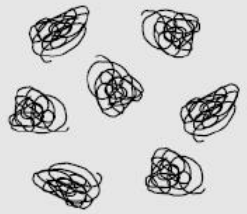


Inter-particle interactions

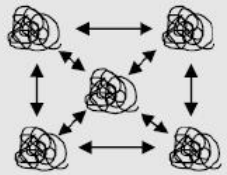




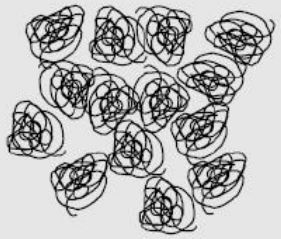




Ideal solution of particles



Repulsive particle interactions



Attractive particle interactions

SAXS data processing, and overall parameters

Radius of gyration (R_g)

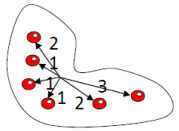
Definition

Measure for the overall size of a macromolecule

Average of square center-of-mass distances in the molecule
weighted by the scattering length density

R_g^2 is the average electron density weighted squared distance of the scatters from the centre of the object

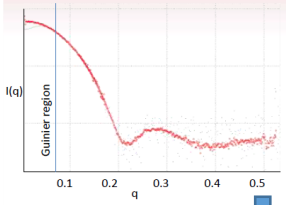
$$R_g^2 = \frac{\int \mathbf{r}^2 \rho(\mathbf{r}) d\mathbf{r}}{\int \rho(\mathbf{r}) d\mathbf{r}}$$



$R_g^2 = (1^2 + 1^2 + 1^2 + 2^2 + 2^2 + 3^2)/6 = 20/6$
$R_g = \sqrt{3.333} = 1.82$

- Solid sphere radius R:
  $R_g = \sqrt{(3/5)}\, R$
- Thin rod length L
  $R_g = \sqrt{(1/12)}\, L$
- Thin disk radius R:
  $R_g = \sqrt{(1/2)}\, R$

The Guinier approximation



The low-q region of the scattering curve is characteristic for the overall dimension of the particle.

$$\lim_{q \to 0} I = I_0 \exp\left(-\frac{1}{3} q^2 R_g^2\right)$$

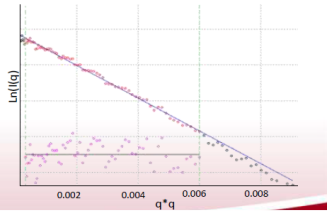
I_0 is proportional to M_w

Radius of gyration: size of the particle

"The Guinier Plot"

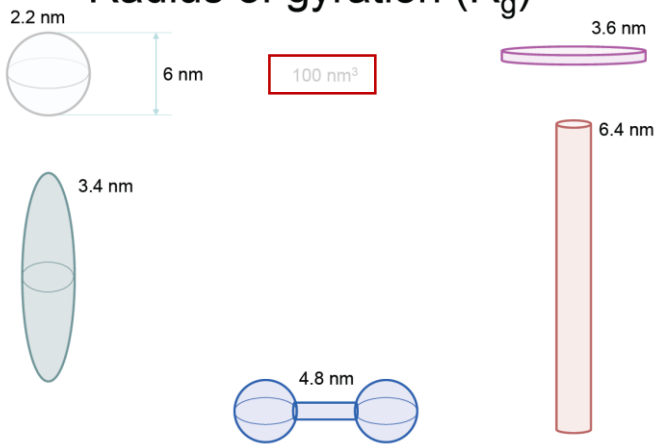
Plot ln I against q^2 →Straight line, slope $-R_g/3$

$$\ln I = \ln I_0 - \frac{1}{3} q^2 R_g^2$$



Deviation from the straight line in the Guinier plot indicate intermolecular interaction or aggregation

Radius of gyration (R_g)



SAXS data processing, and overall parameters

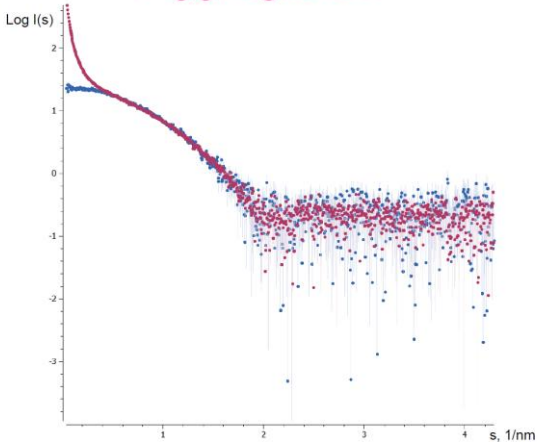
Aggregation

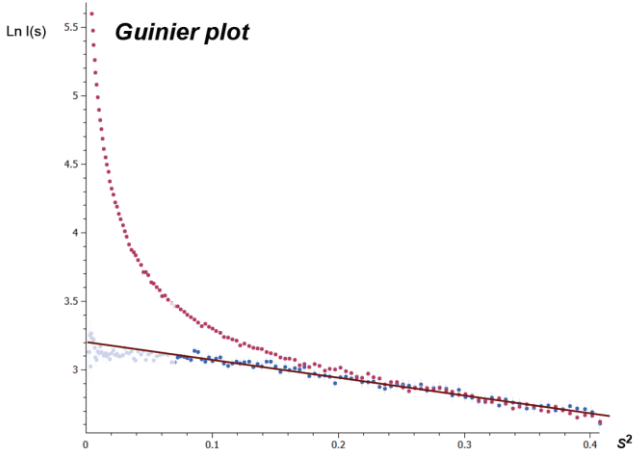
Monodisperse sample

Aggregation

Aggregated sample

Aggregation

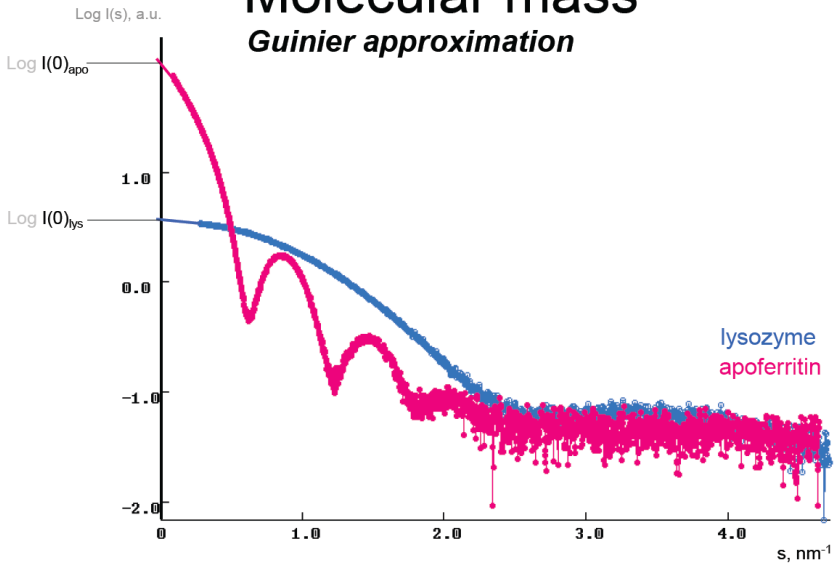




SAXS data processing, and overall parameters

Molecular mass

Guinier approximation



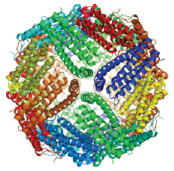
I(0) and Molecular Mass

$$\frac{MM_{sample}}{MM_{BSA}} = \frac{I(0)_{sample}}{I(0)_{BSA}}$$

$$MM_{sample} = I(0)_{sample} \cdot MM_{BSA} / I(0)_{BSA}$$

BSA
R_g = 3.1 nm
I(0) = 11.7 a.u.
MM_{BSA} = 66 kDa

R_g = 1.46 nm
I(0) = 2.68 a.u.
MM = 15.1 kDa



R_g = 6.81 nm
I(0) = 79.45 a.u.
MM = 450 kDa

SAXS data processing, and overall parameters

Porod volume

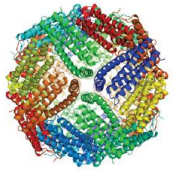
Excluded volume of the hydrated particle

$$V_P = \frac{2\pi^2 I(0)}{\int_0^\infty [I(s) - K_4] s^2 ds}$$

K_4 is a constant determined to ensure the asymptotical intensity decay proportional to s^{-4} at higher angles following the Porod's law for homogeneous particles

Porod law

Excluded volume of the hydrated particle

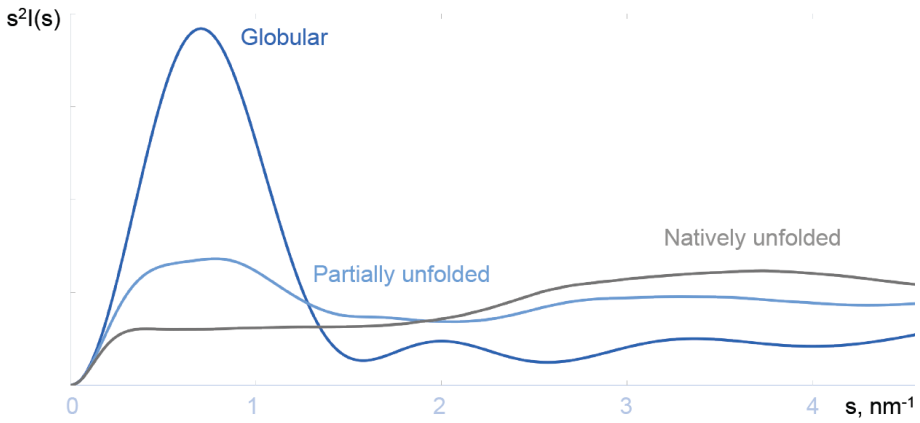


21 nm³	974 nm³
~13 kDa	~610 kDa

SAXS data processing, and overall parameters

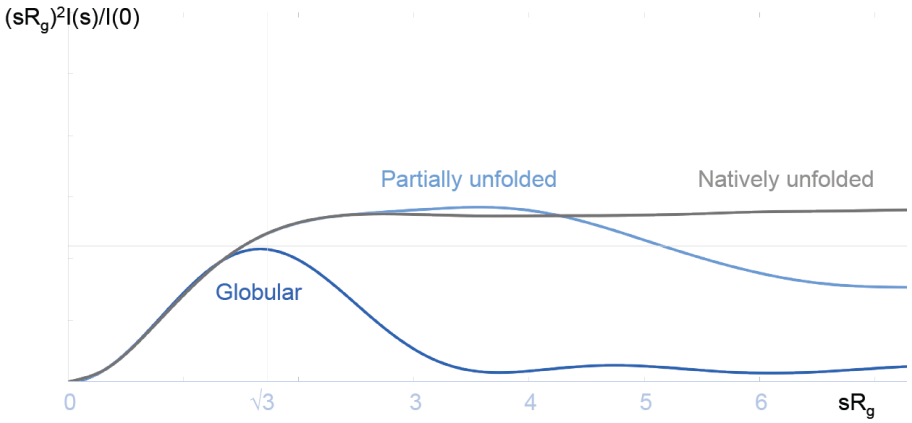
Kratky plot

Patterns of globular and flexible proteins

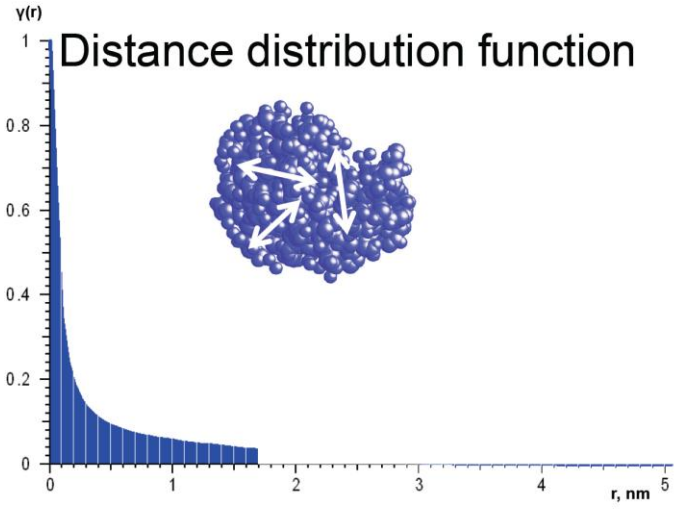


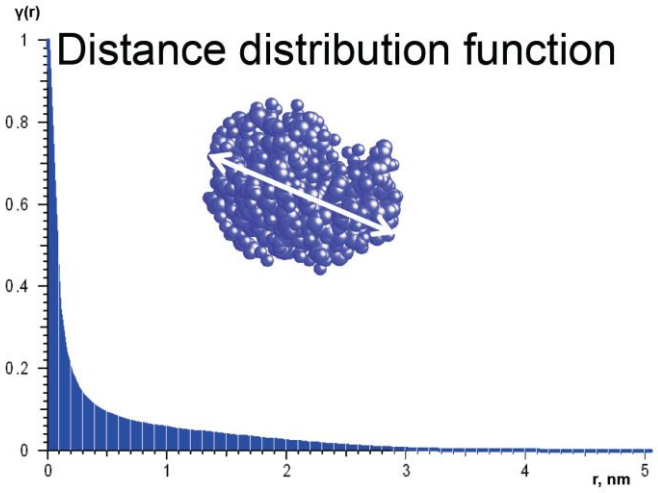
Normalized Kratky plot

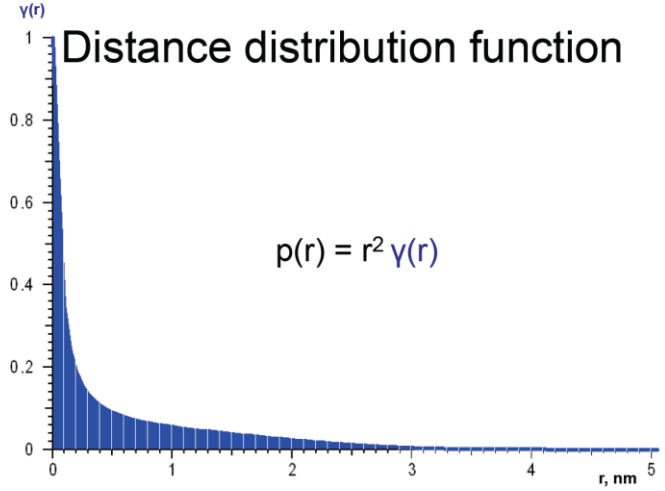
Patterns of globular and flexible proteins

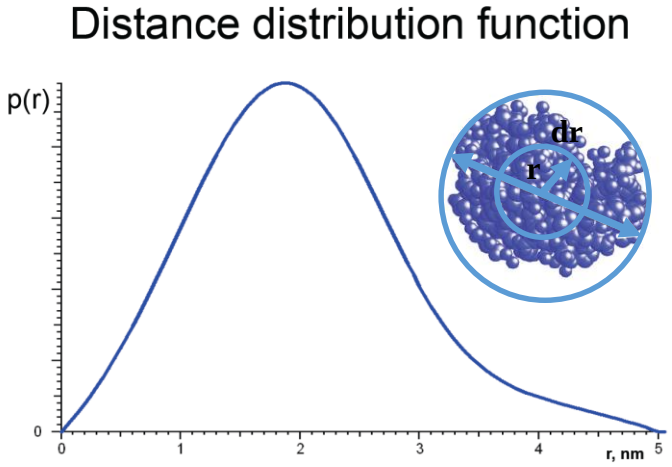


SAXS data processing, and overall parameters



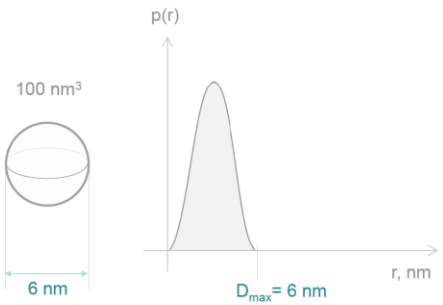




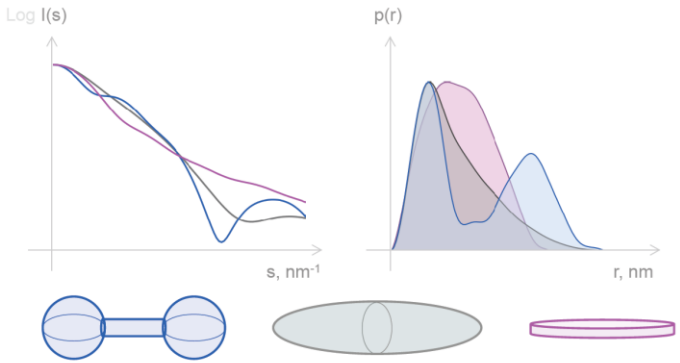


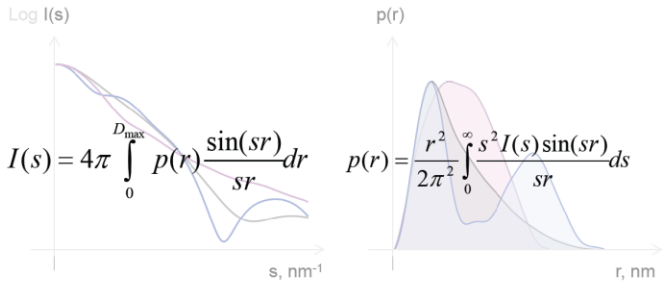
SAXS data processing, and overall parameters

Distance distribution function



Distance distribution function



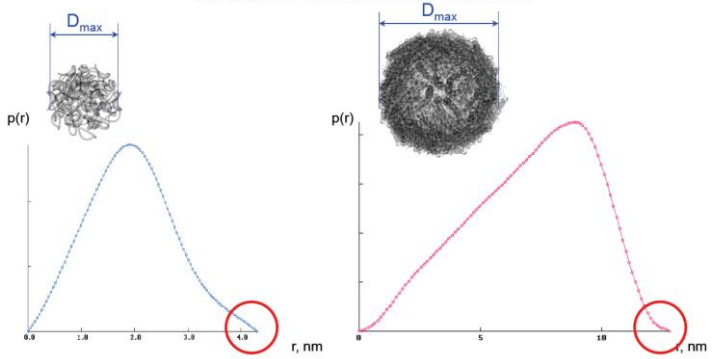


$$I(s) = 4\pi \int_0^{D_{max}} p(r) \frac{\sin(sr)}{sr} dr$$

$$p(r) = \frac{r^2}{2\pi^2} \int_0^{\infty} \frac{s^2 I(s) \sin(sr)}{sr} ds$$

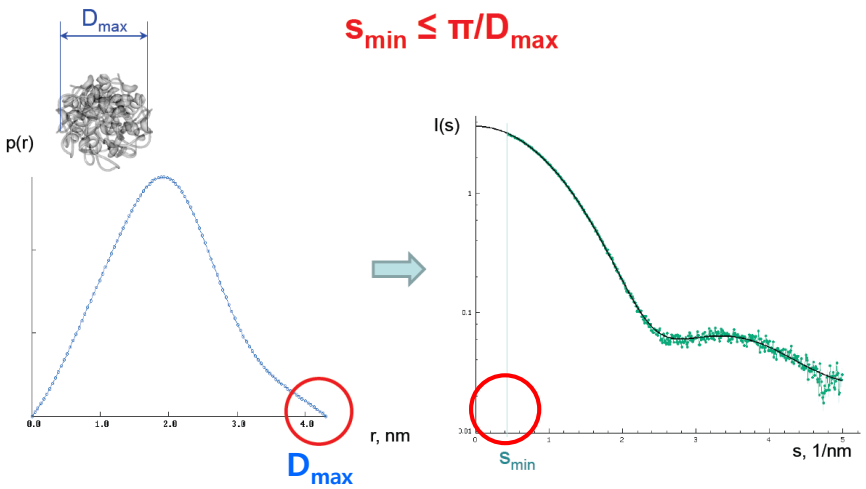
p(r) plot

Distance distribution function

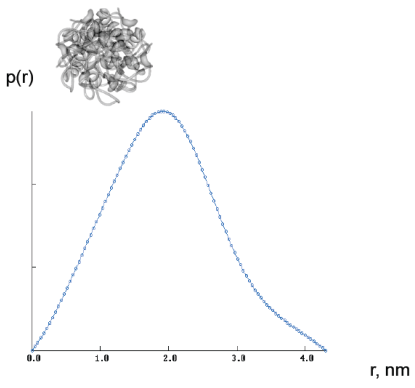


SAXS data processing, and overall parameters

Data quality



R_g and I(0) from p(r)



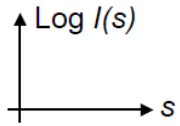
$$I(0) = 4\Pi \int_0^{D_{max}} p(r)dr$$

$$R_g^2 = \frac{\int_0^{D_{max}} r^2 p(r)dr}{2\int_0^{D_{max}} p(r)dr}$$

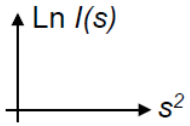
Summary

- Exposure 3D → 2D
- Radial averaging → 1D
- Normalization
- Background subtraction
- Analysis

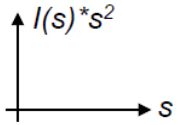
- Log plot



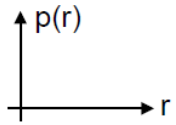
- Guinier plot (R_g, MM)



- Kratky plot (flexibility)



- p(r) plot



Modeling for Nanoparticle and Biomacromolecule (Form Factor)

1. Model Independent Approach: Ab initio methods

- *Monodisperse* systems (size, shape)
- Biomacromolecules (Protein, DNA, RNA) and Self-assembled Nanoparticles

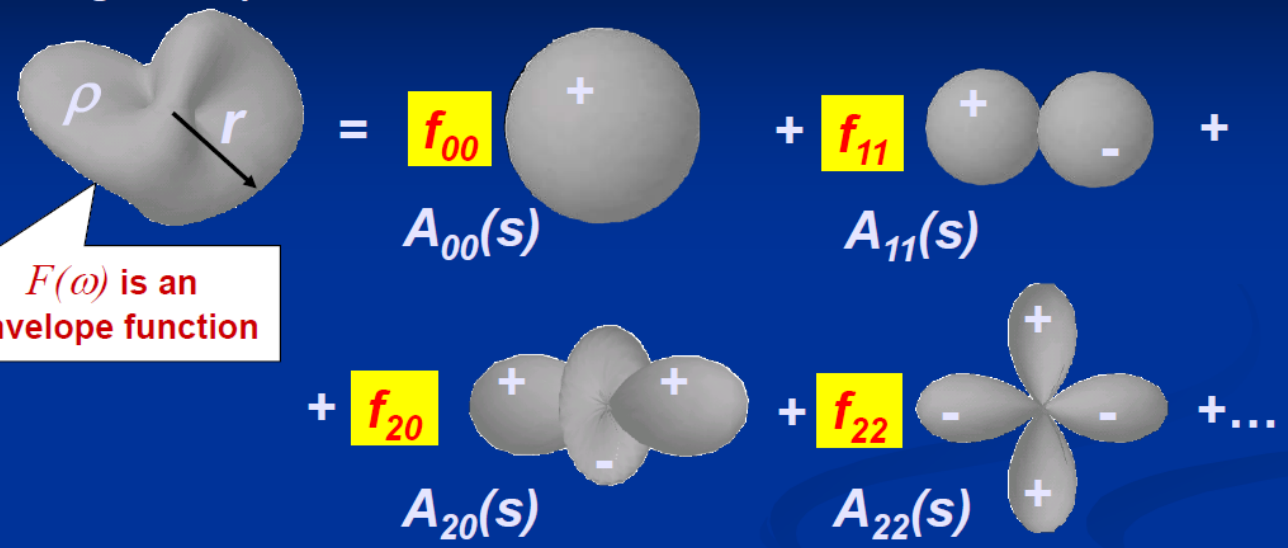
2. Model Dependent Approach: Scatter, SASfit, SASView, etc.

- Monodisperse and *polydisperse* systems (size)
- Self-assembled Nanoparticles

1.1. Model Independent Approach: Ab initio methods

Shape parameterization by spherical harmonics

Homogeneous particle



$F(\omega)$ is an envelope function

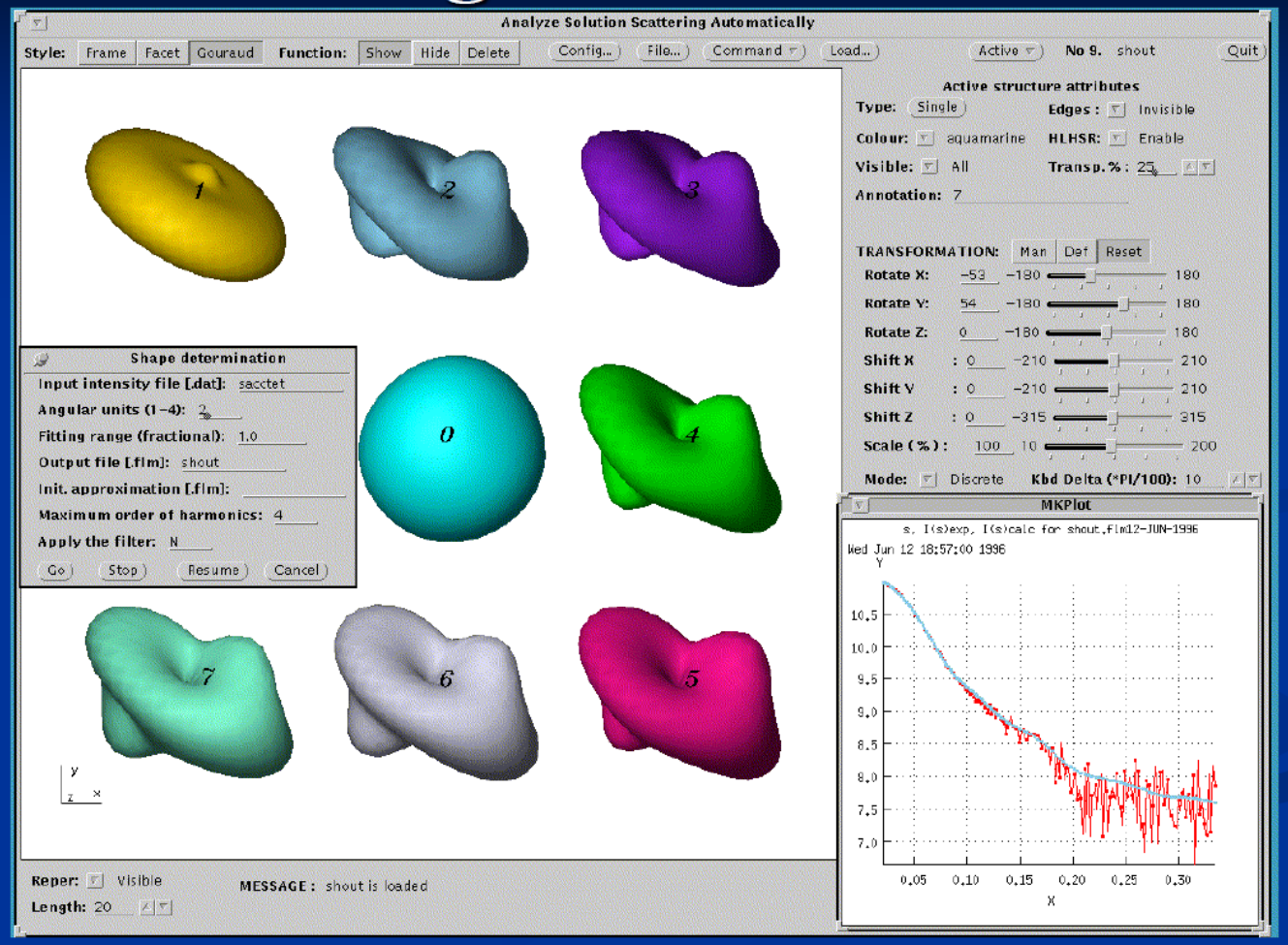
Spatial resolution: $\delta = \pi R / (L+1)$, R – radius of an equivalent sphere.

Number of model parameters f_{lm} is $(L+1)^2$.

One can easily impose symmetry by selecting appropriate harmonics in the sum. This significantly reduces the number of parameters describing $F(\omega)$ for a given L.

1.2. Model Independent Approach: Ab initio methods

Program SASHA

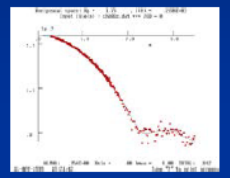


1.3. Model Independent Approach: Ab initio methods

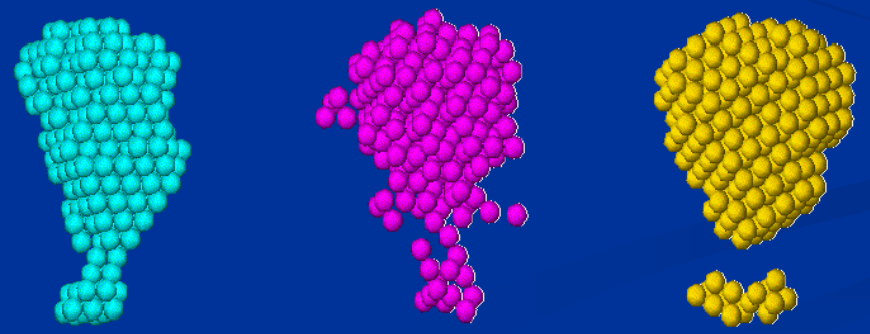
Ab initio program DAMMIN

Using simulated annealing, finds a compact dummy atoms configuration X that fits the scattering data by minimizing

$$f(X) = \chi^2[I_{\exp}(s), I(s,X)] + \alpha P(X)$$



where χ is the discrepancy between the experimental and calculated curves, $P(X)$ is the penalty to ensure compactness and connectivity, $\alpha>0$ its weight.



compact

loose

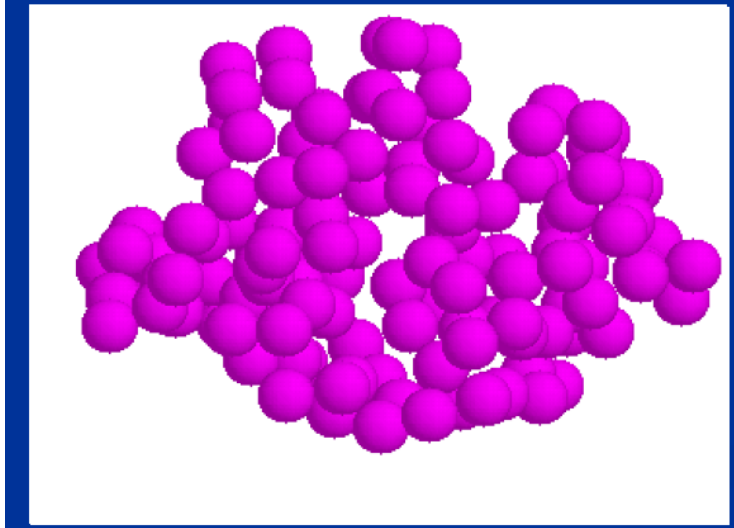
disconnected

1.4. Model Independent Approach: Ab initio methods

Ab initio dummy residues model

- Proteins typically consist of folded polypeptide chains composed of amino acid residues

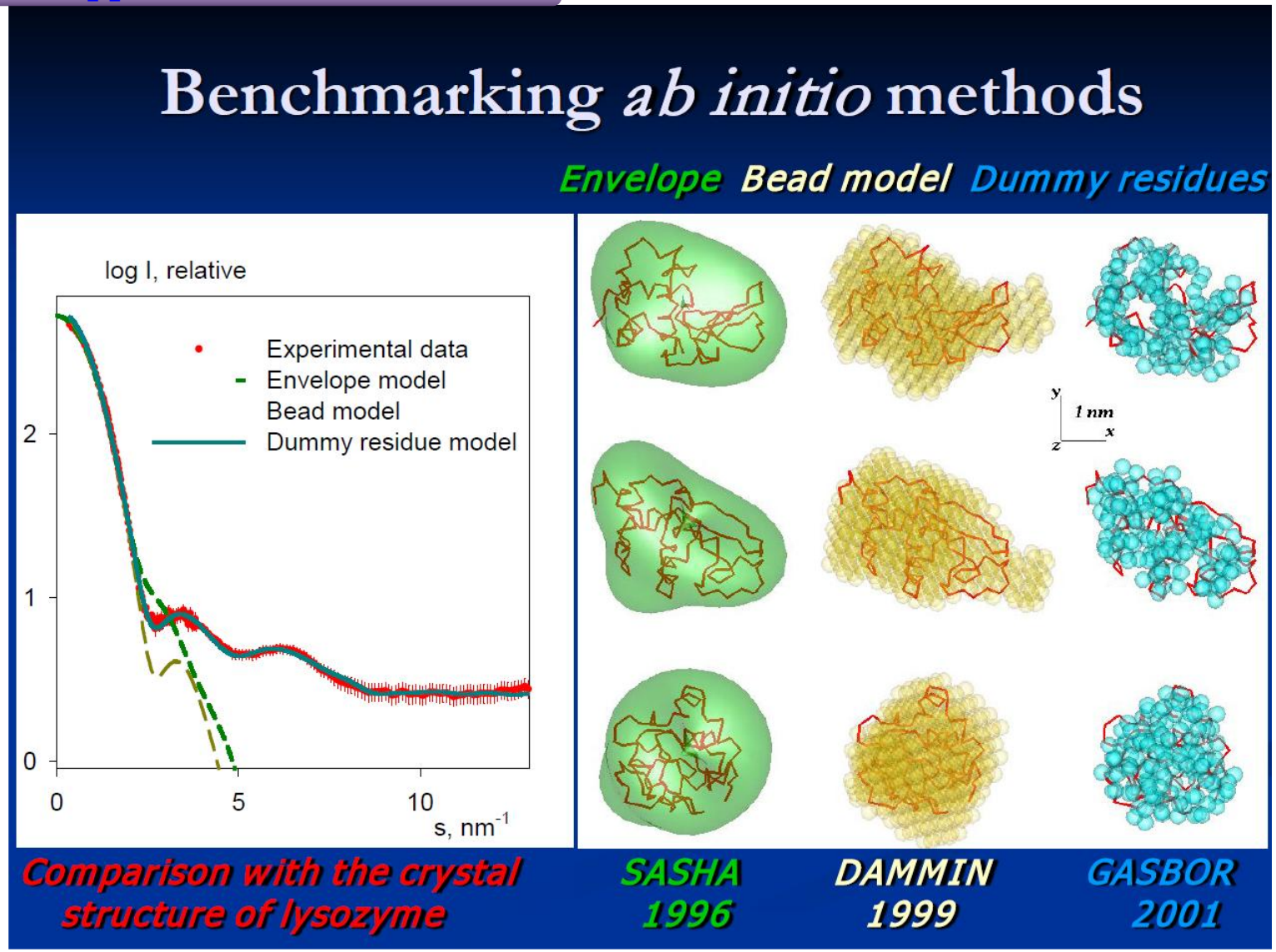
At a resolution of 0.5 nm a protein can be represented by an ensemble of K dummy residues centered at the Cα positions with coordinates $\{r_i\}$



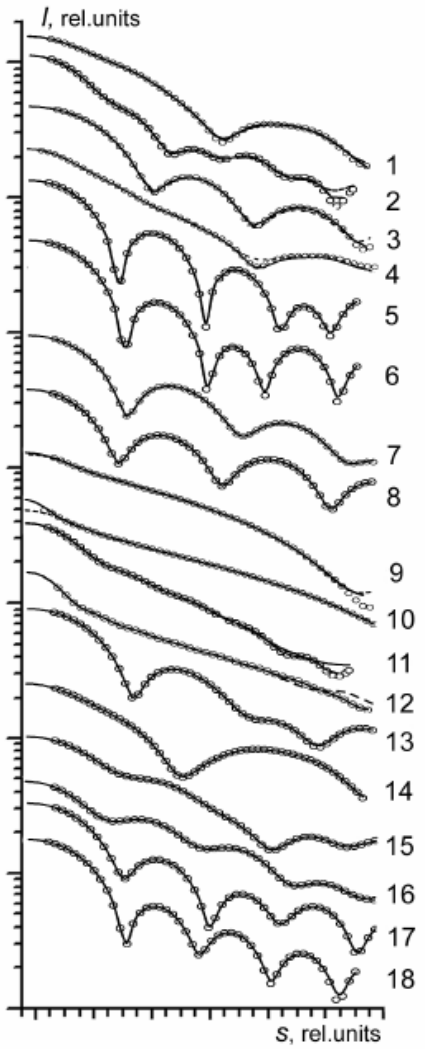
Scattering from such a model is computed using the Debye (1915) formula.

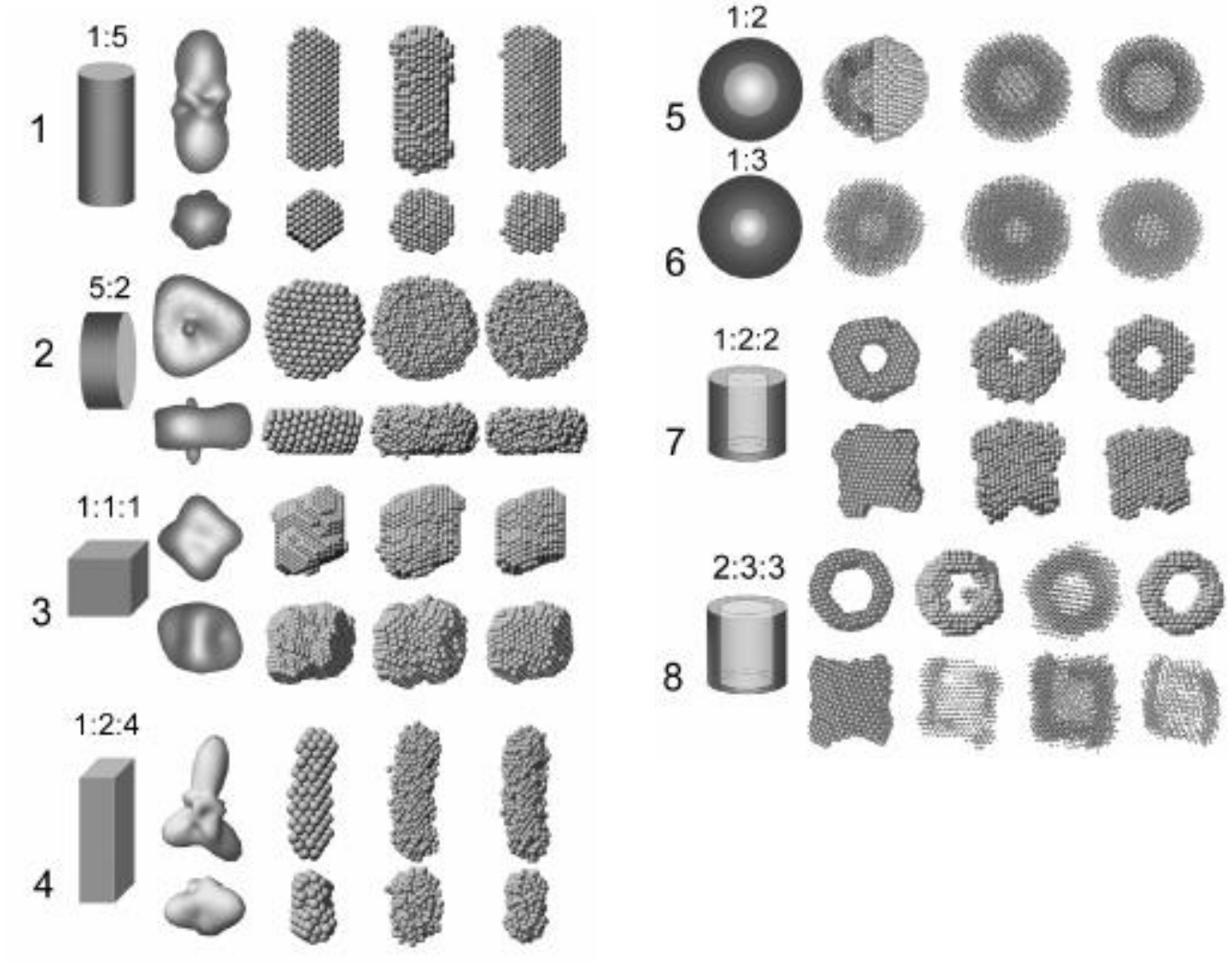
Starting from a random model, simulated annealing is employed similar to DAMMIN

1.5. Model Independent Approach: Ab initio methods



1.6. Model Independent Approach: Ab initio methods

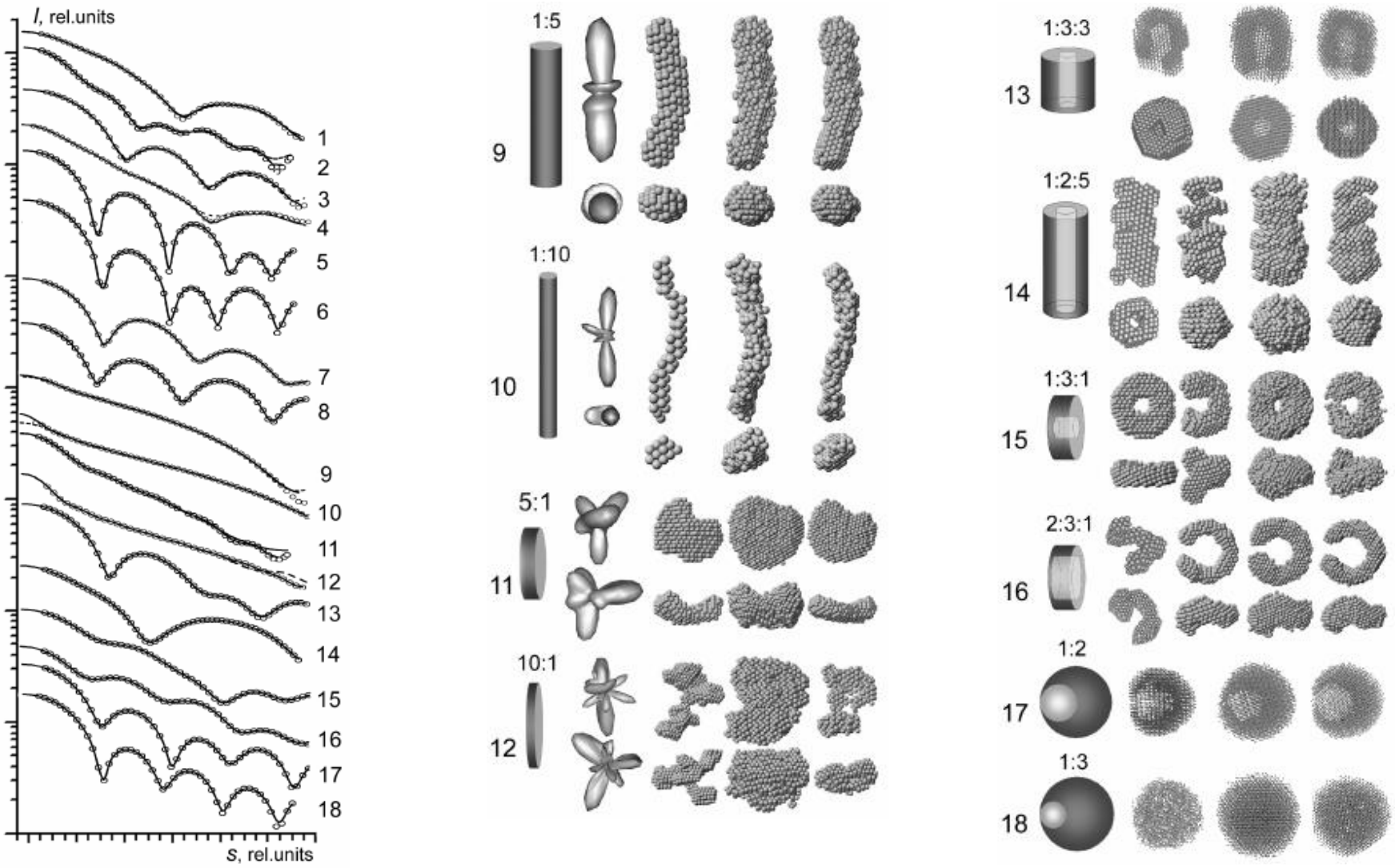




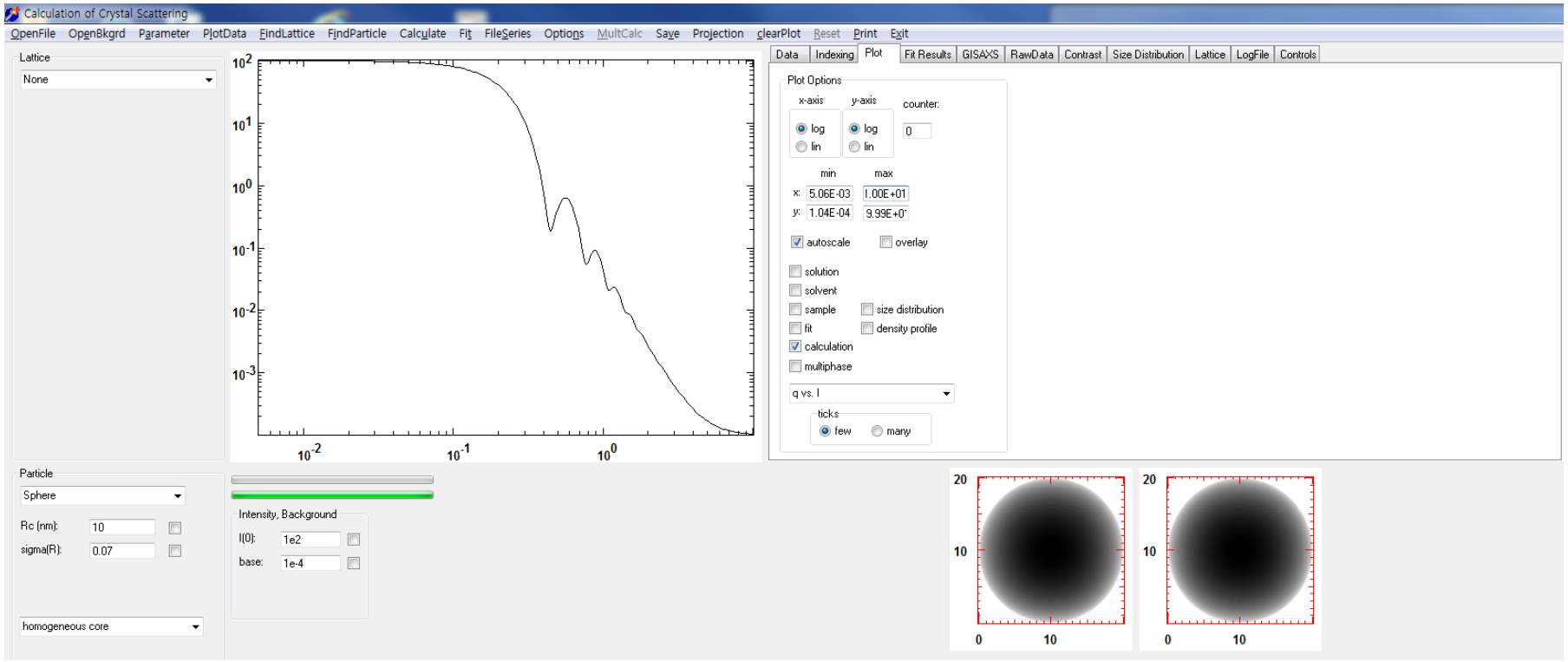
SASHA DAMMIN DAMAVER TSR (total spread region) DAMAVER MPV (most populated volume)

J. Appl. Cryst. **2003.** 36, 860-864

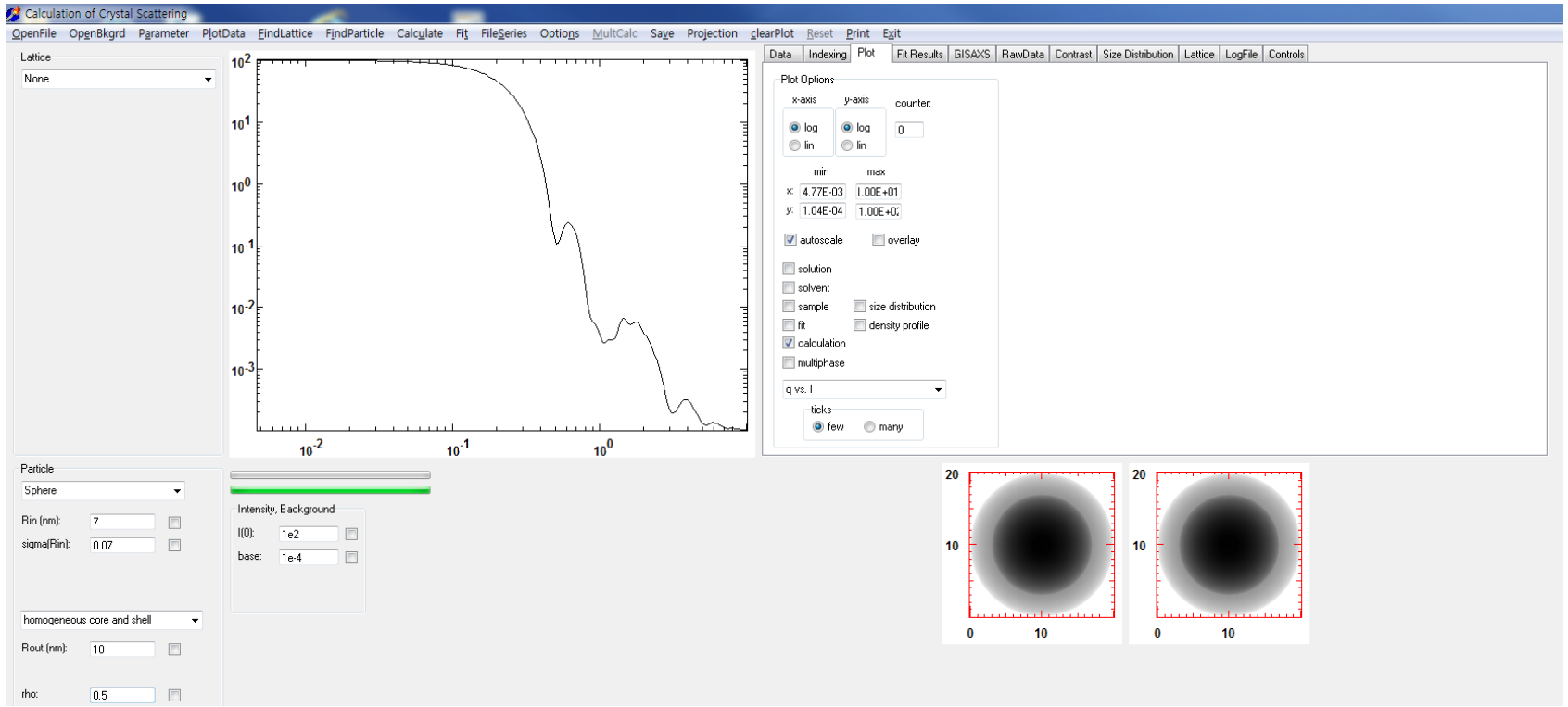
1.7. Model Independent Approach: Ab initio methods



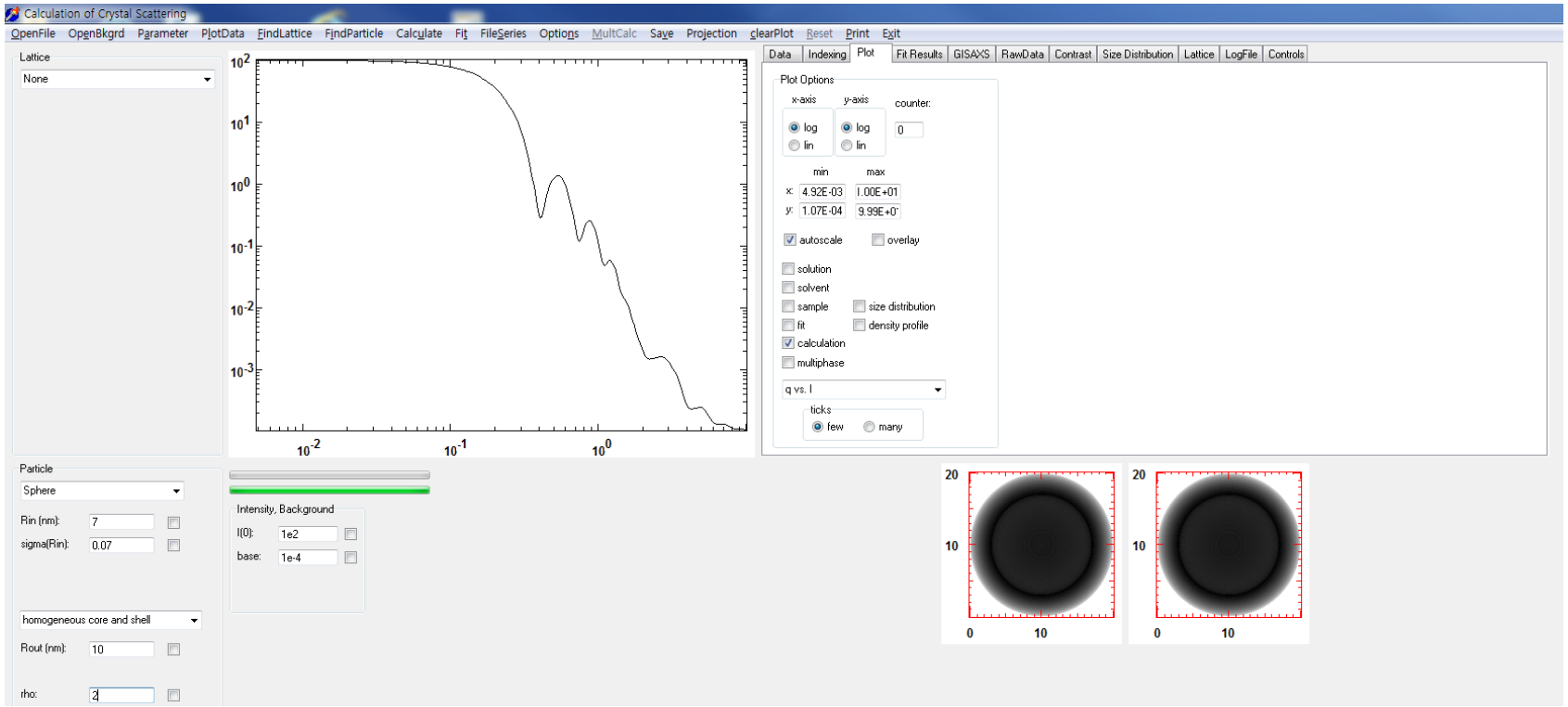
2.1. Model Dependent Approach: Sphere Model



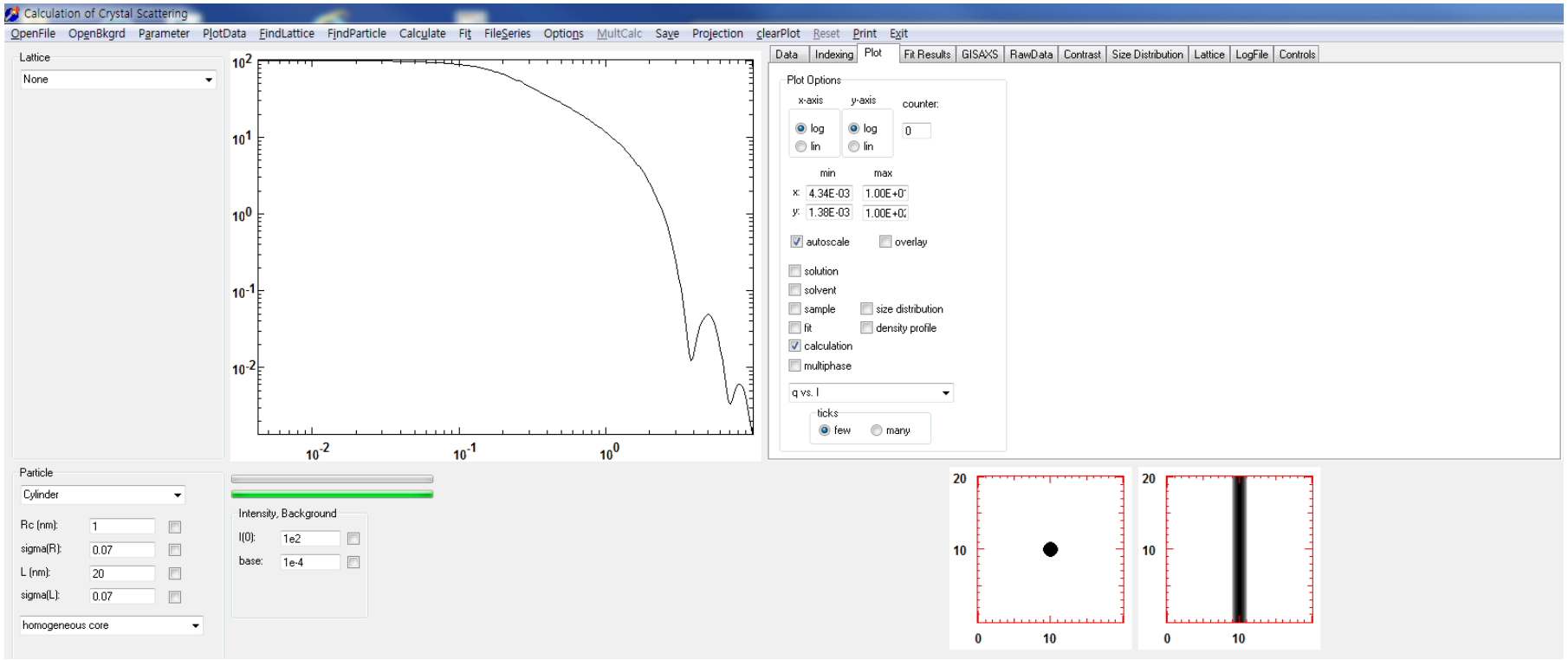
2.2. Model Dependent Approach: Core-shell Model



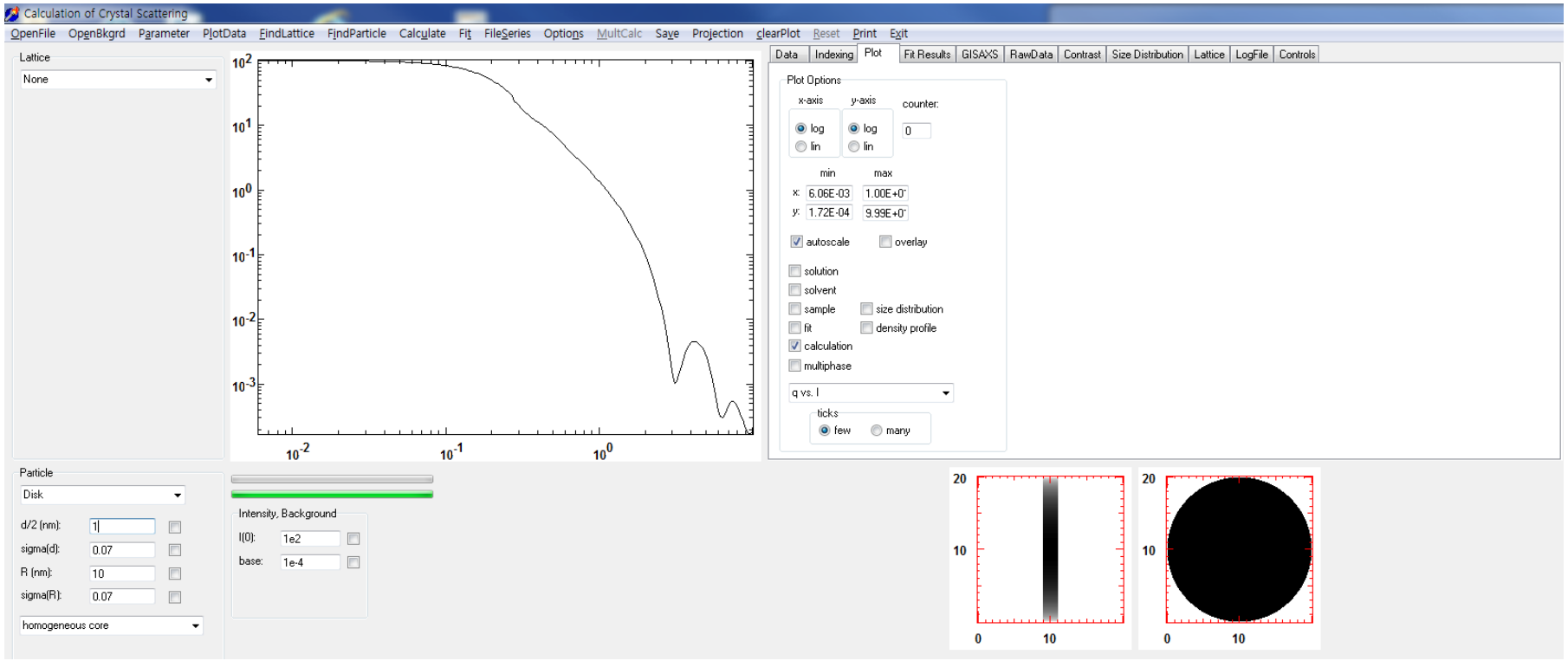
2.3. Model Dependent Approach: Core-shell Model



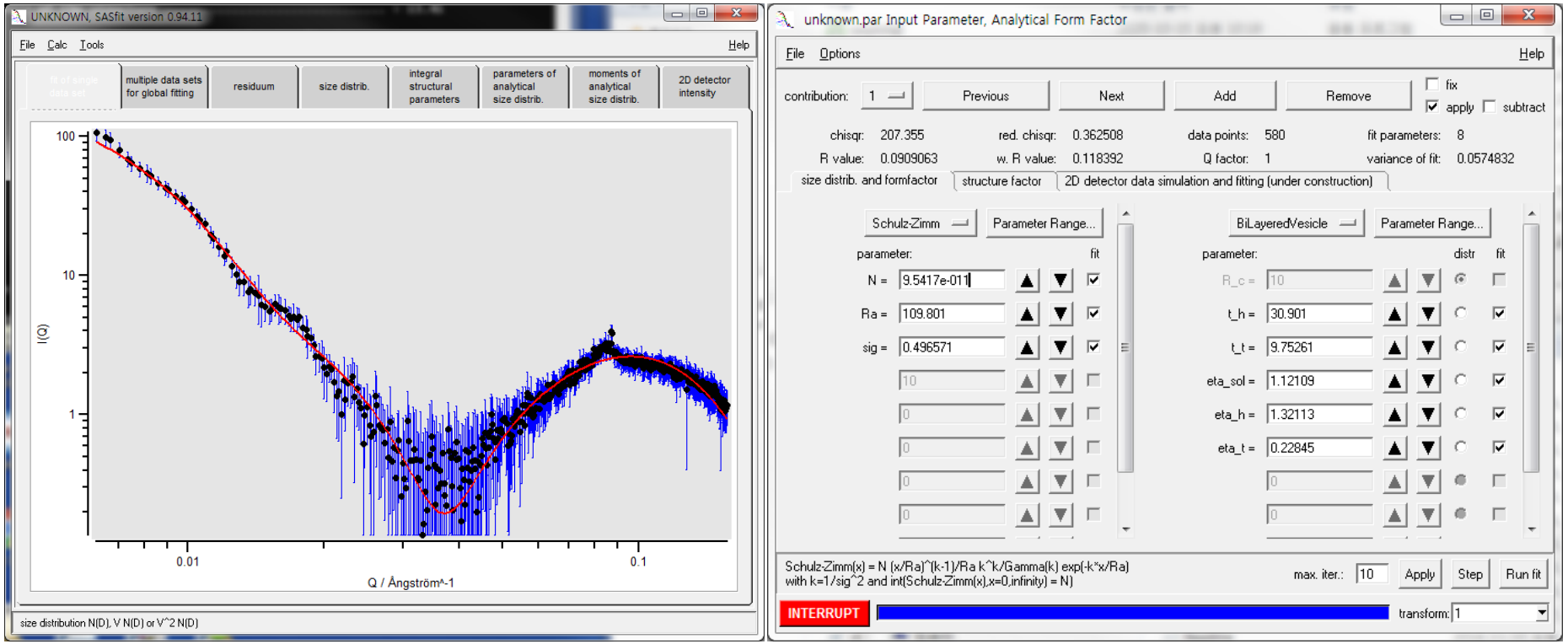
2.4. Model Dependent Approach: Cylinder Model



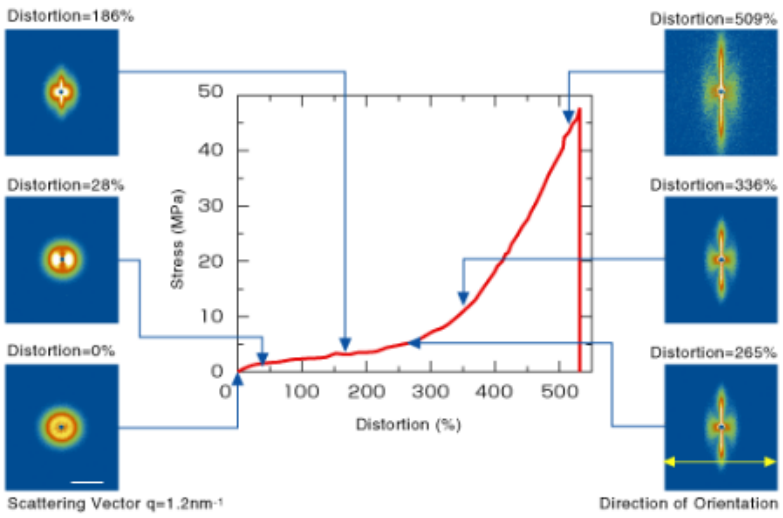
2.5. Model Dependent Approach: Disk Model



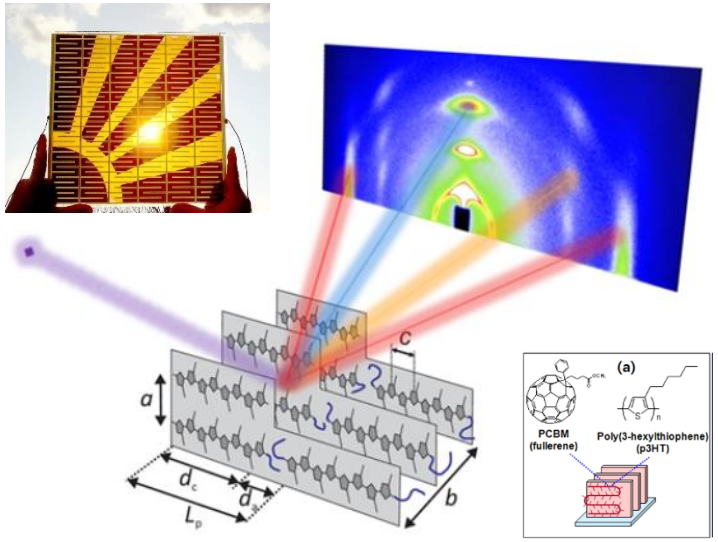
2.6. Model Dependent Approach: Bilayered Vesicle Model



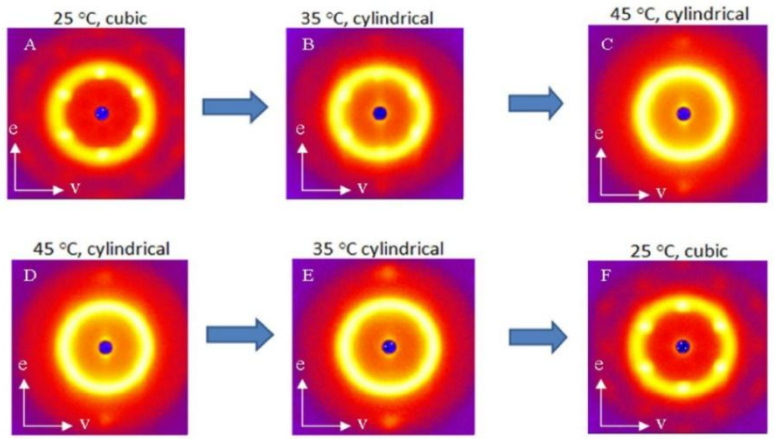
Bilayered vesicle model
(Thickness of outer part of bilayer=3nm, Thickness of inner part of bilayer=1nm, R=10nm)



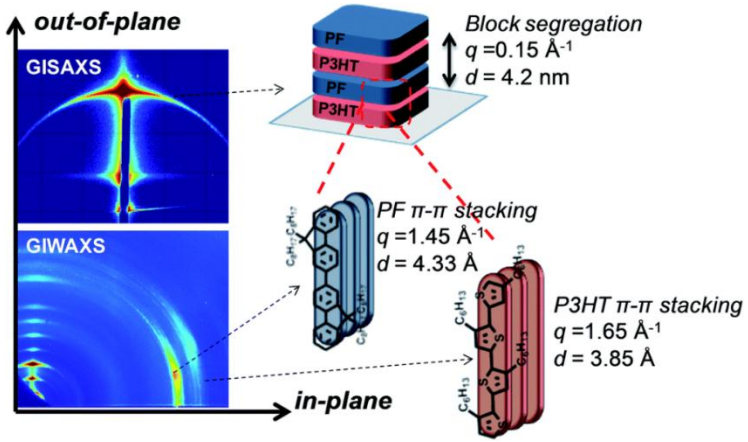
▪ 타이어의 응력-변형률 연구



▪ 태양광 전지 (Organic Solar Cells)

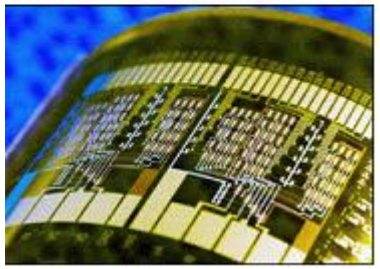


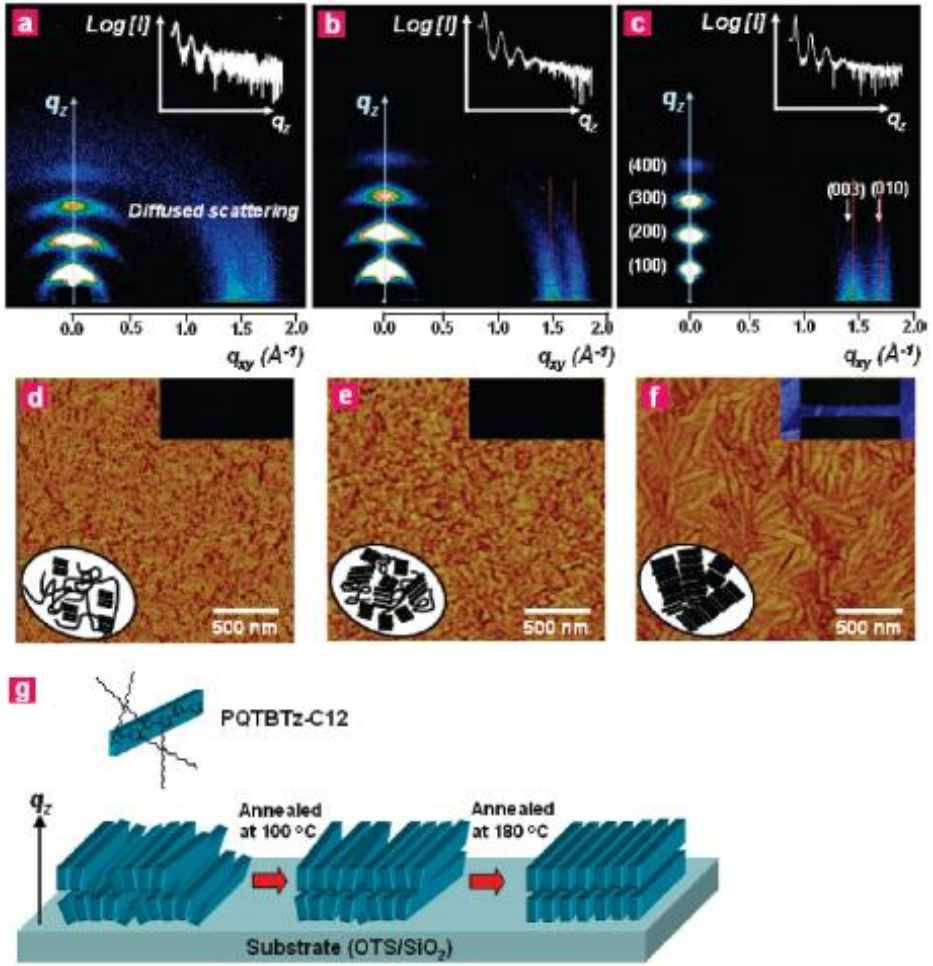
▪ 블록공중합체 고분자의 구조/역학적 이력 연구



▪ 용매 어닐링 처리 액정 고분자의 구조 연구

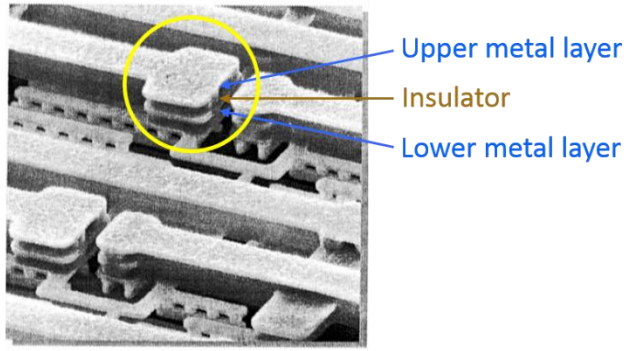
Flexible Display Application: Organic Thin-film Transistor

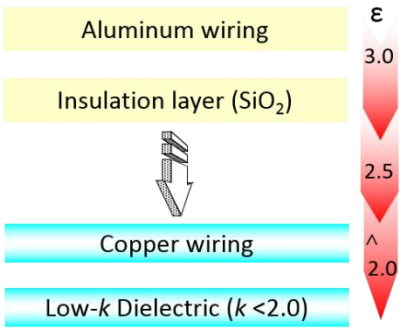


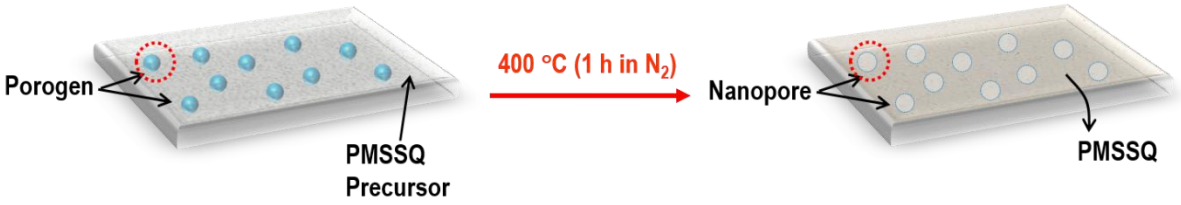


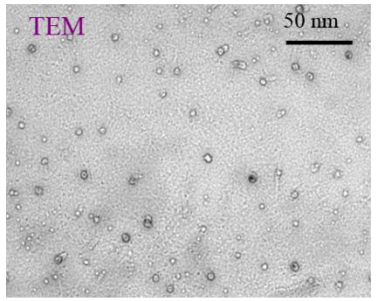
Journal of the American Chemical Society (2009)

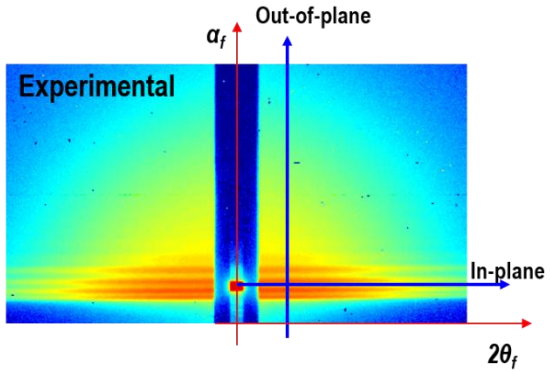
Ultra-low-K Dielectrics in Nano-thin-films





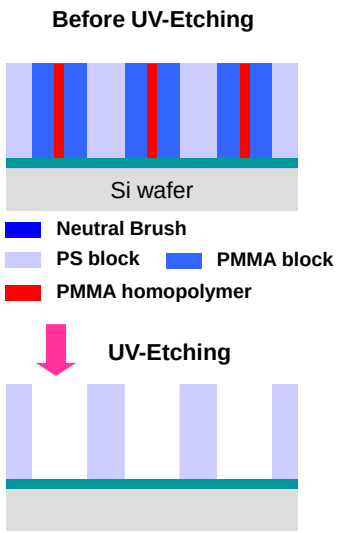


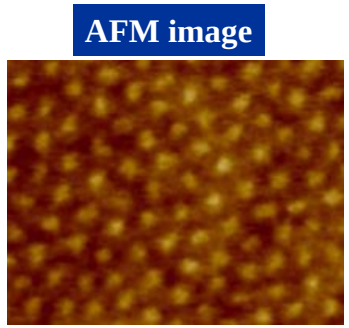


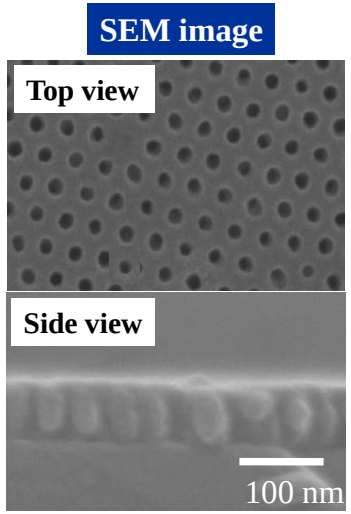


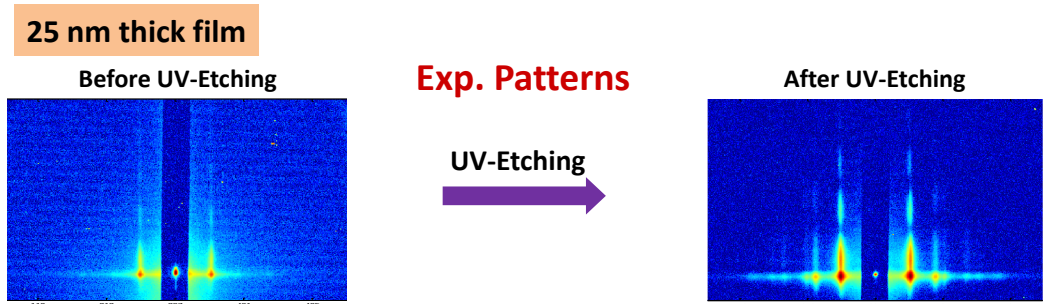
Nat. Mater. 4, 147 (2005), Adv. Mater. 17, 696 (2005), Macromolecules 2005, 38, 4311-4323, J. Appl. Cryst. 2008, 41, 281–291

Nano-templates: Ultra-fine Filter Membranes



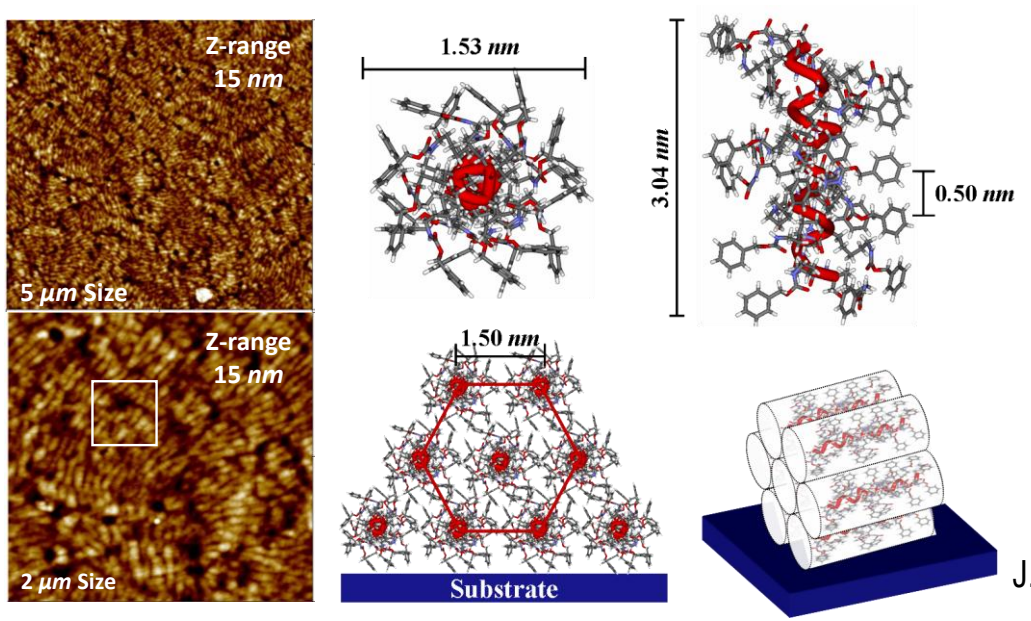




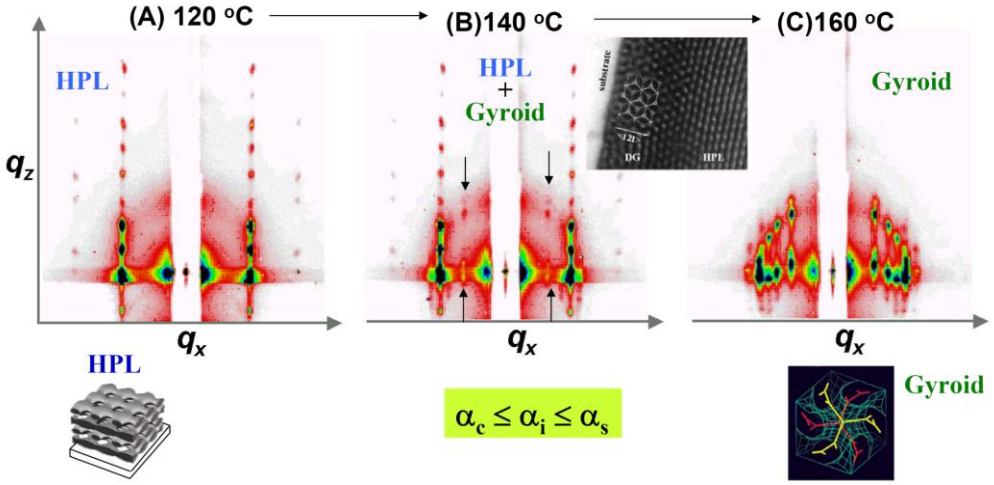


Adv. Funct. Mater., 18, 1371 (2008)

Polymer Nanostructure



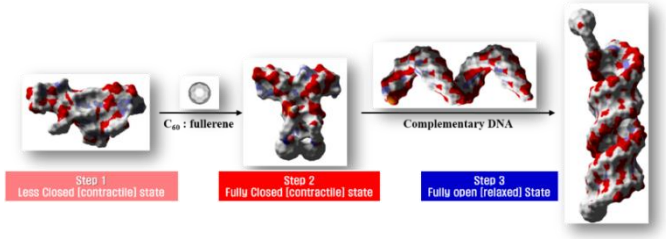
J. Phys. Chem. B, (2008)



Macromolecules, (2005)

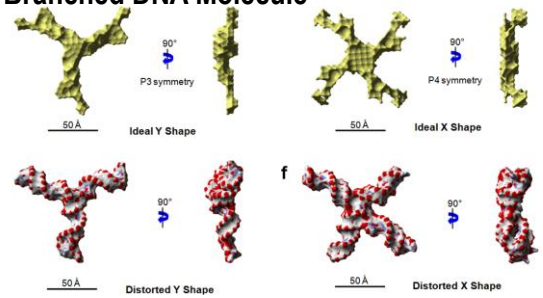
Research Results

1. Proton-sensitive DNA



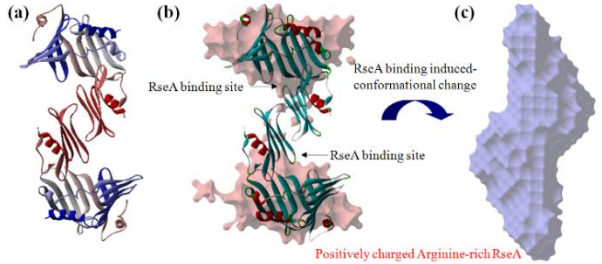
Adv. Mater. 2009, 21, 1907-1910.

2. Branched DNA Molecule



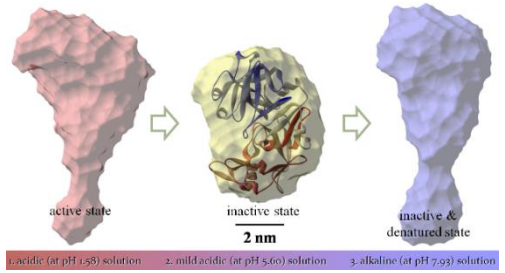
Scientic Reports 2013, 3, 3226

3. Stress-related RseA/RseB Protein



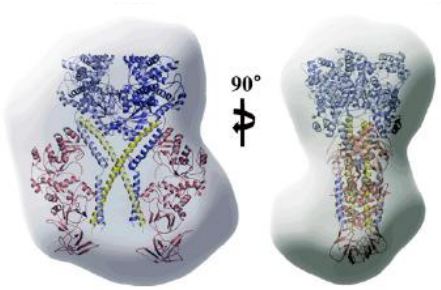
PNAS 2007, 104, 8779-8784.

4. Digestion-related Pepsin Protein



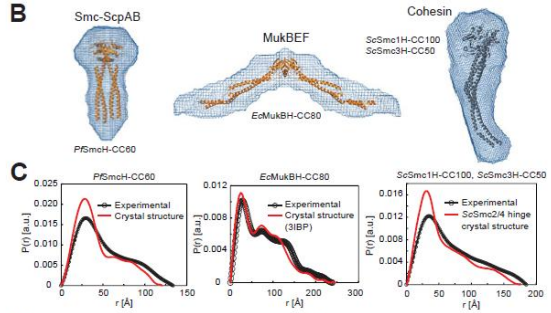
J. Phys. Chem. B 2008, 112, 15821-15827.

5. Mammalian Translation-related Protein



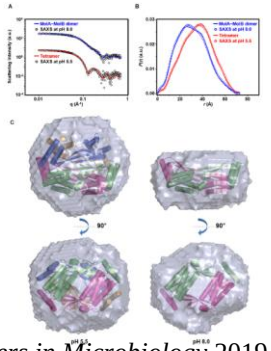
PNAS 2014, 111, 15084-15089.

6. Chromosome-related SMC Protein



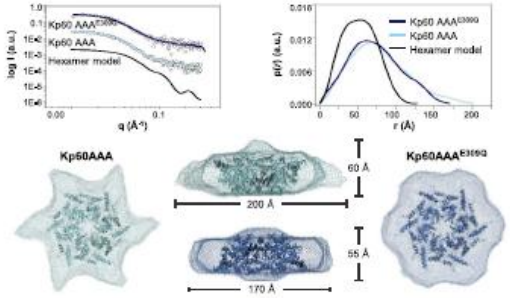
Molecular Cell 2015, 57, 290-303.

7. Pathogenic Bacteria-related Protein



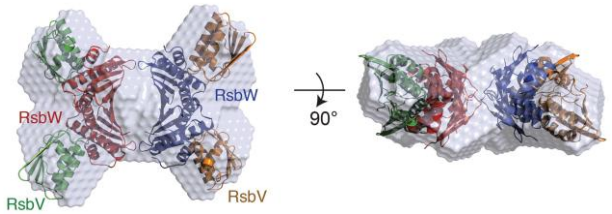
Frontiers in Microbiology 2019, 9, 3329.

8. Microtubule (MT)-severing Enzyme



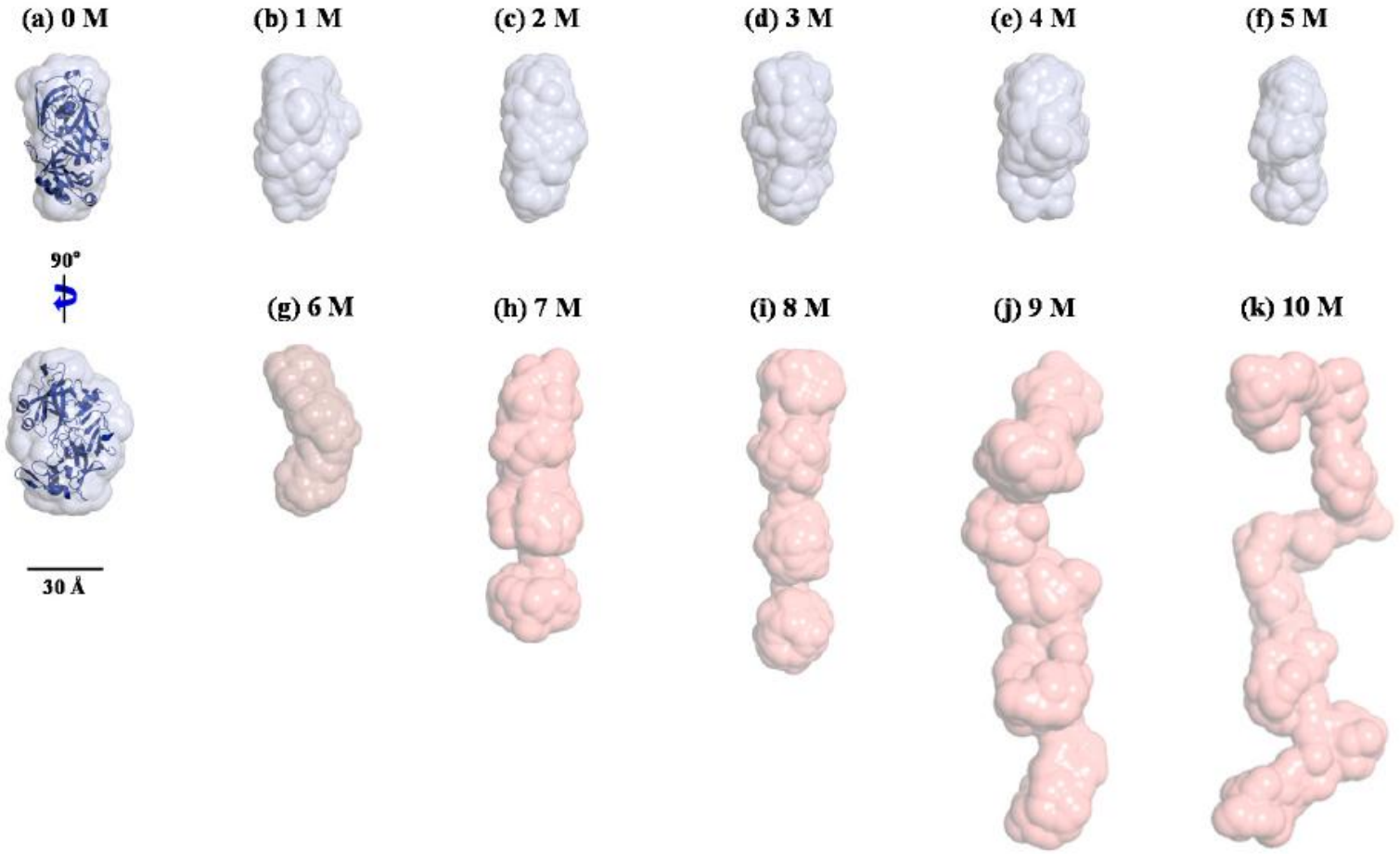
Cell Reports 2019, 26, 1357-1367.

9. Stress-related RseV/RseW Protein



IUCrJ 2020, 7, 737-747.

GASBOR *ab initio* Envelope of Pepsin in Solution



Structural models of pepsin in solution under various denaturing conditions with 0–10 M urea (**a–k**, respectively) using the *ab initio* shape method program GASBOR. Surface rendering in the structural model was achieved using the program PyMOL. For the comparison of overall shapes and dimensions, the ribbon diagrams of the atomic crystal structure of pepsin were superimposed on the reconstructed dummy residues models using SUPCOMB (NSD = 1.802).

"Chemically Denatured Structures of Porcine Pepsin using Small-Angle X-ray Scattering" *Polymers* **2019**, 11(12), 2104

Liquid Crystalline Lipid Nanoparticles

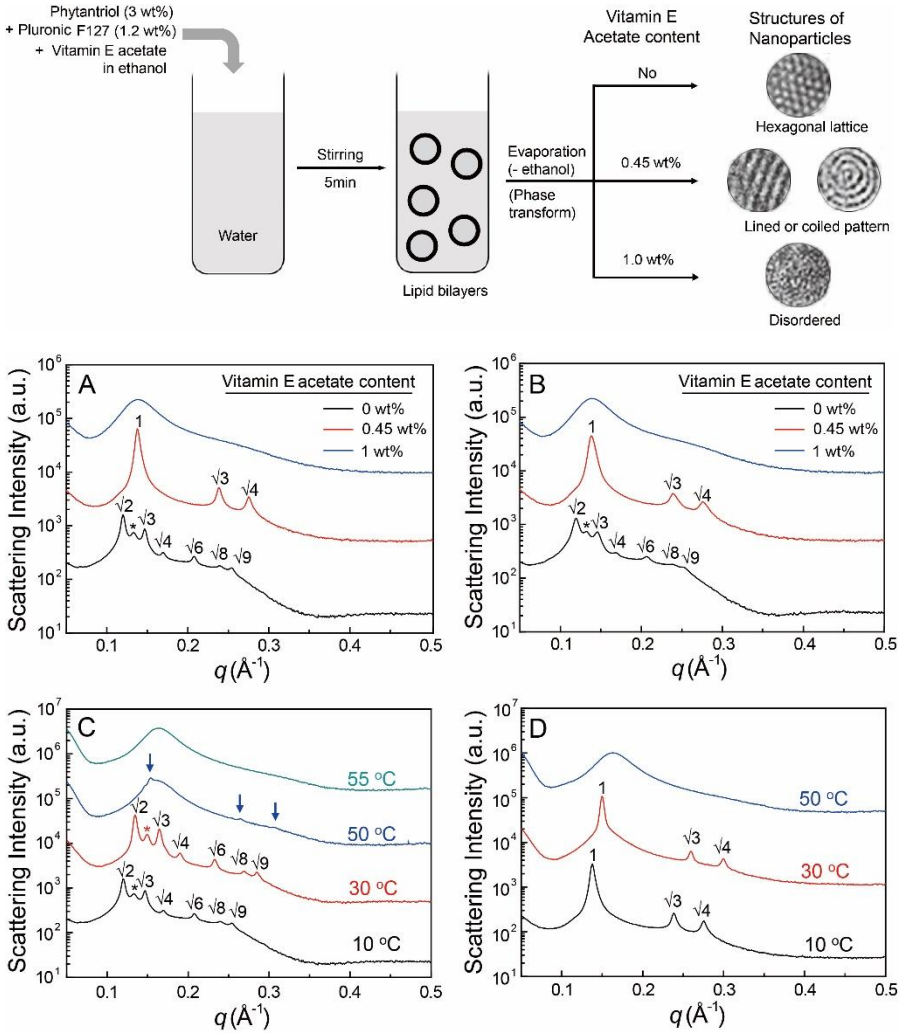


Table 2. Structural parameters obtained from the SAXS data (Figure 5) of the liquid nanoparticles.

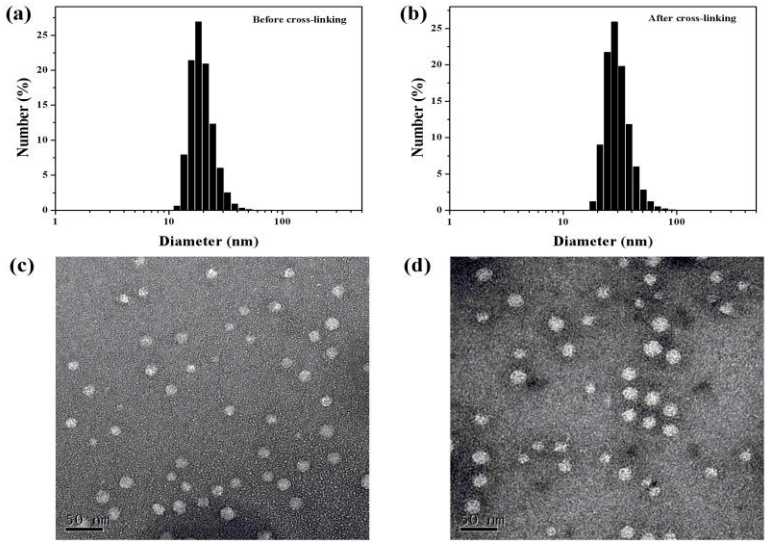
Preparation Method	Vitamin E Acetate Concentration (wt%)[c]		Space Group	Lattice Parameter, a (Å)
Evaporation Method[a]	0	10 °C[d]	*Pn3m* H_2	74.1 ± 0.2 -[e]
		30 °C	*Pn3m* H_2	66.0 ± 0.2 -[e]
		50 °C	H_2 L_2	47.4 ± 0.2 38.9 ± 0.3[f]
		55 °C	L_2	38.3 ± 0.3[f]
	0.45	10 °C	H_2	52.8 ± 0.3
		30 °C	H_2	48.5 ± 0.3
		50 °C	L_2	38.5 ± 0.3[f]
	1.0	10 °C	L_2	45.3 ± 0.3[f]
Conventional Method (Ref. 16)[b]	0	10 °C	*Pn3m* H_2	74.3 ± 0.3 -[e]
	0.45	10 °C	H_2	52.6 ± 0.3
	1.0	10 °C	L_2	45.1 ± 0.3[f]

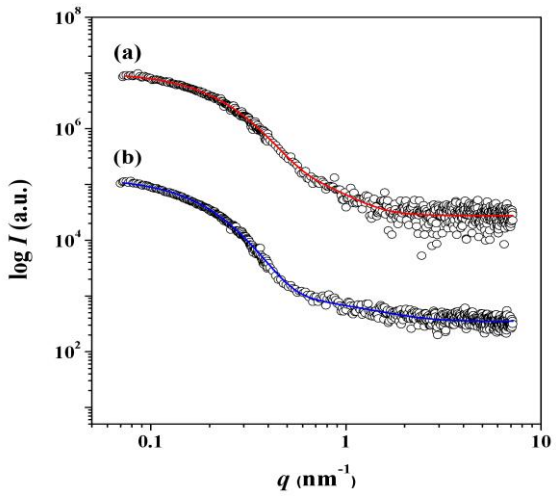
a) The method developed from the present study.
b) The method from Ref. 16.
c) For all the samples, the concentrations of phytantriol and F127 were 3.0 and 1.2 wt%, respectively, in the aqueous dispersion.
d) Measurement temperatures
e) Lattice parameters were not determined due to lack of second and third peaks. See the text for the explanation.
f) The characteristic distance for the L_2 phase ($d = 2\pi/q$) from the observed single broad peak.

Collaboration : Prof. Eun Chul Cho (Amorepacific & Hanyang Univ.)

Do-Hoon Kim, Sora Lim, Jongwon Shim, Ji Eun Song, Jong Soo Chang, Kyeong Sik Jin* and Eun Chul Cho* *ACS Applied Materials & Interfaces* **2015,** 7, 20438-20446

Cross-linked Polymer Micelles



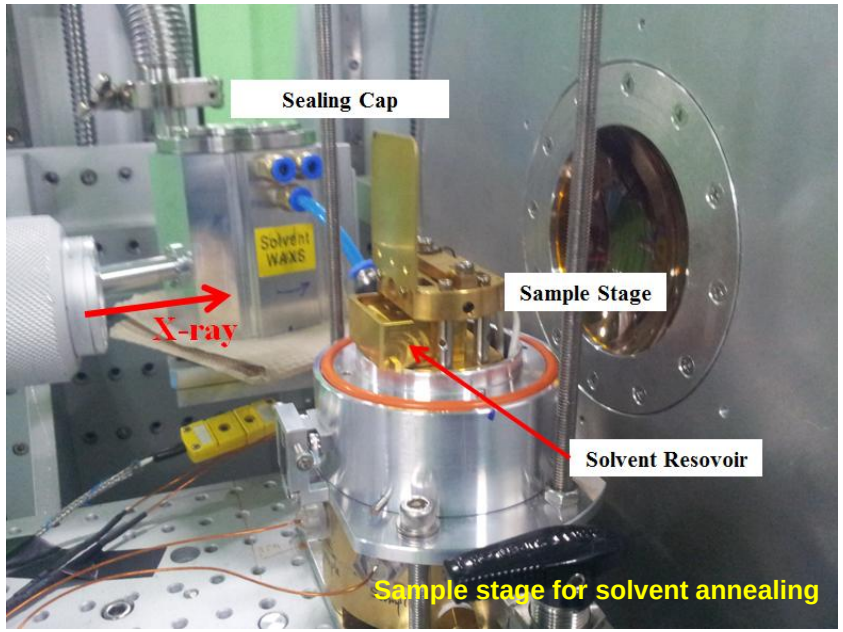


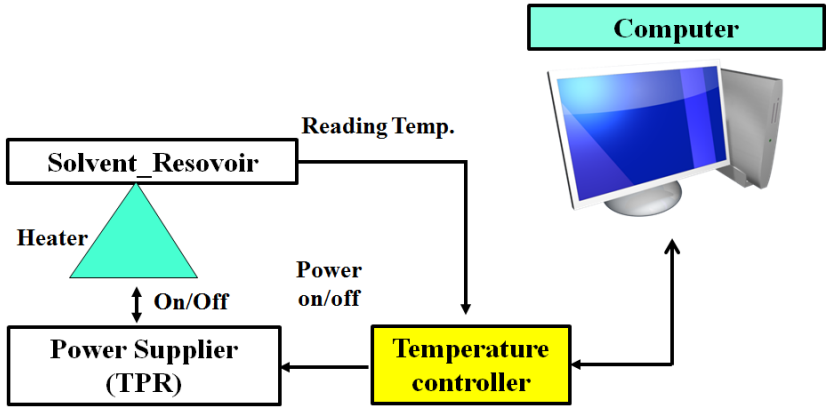
Sample	NCPMs		CPMs	
Model	sphere		sphere	
Type	homogeneous core and shell		homogeneous core and shell	
[a]R_c (nm)	3.220	[b](4.317)	1.460	[b](1.914)
[c]σ_R	0.326	-	0.322	-
[d]$R_{c,\ max}$ (nm)	2.888	[e](3.944)	1.228	[e](1.732)
[f]R_m (nm)	11.90	[g](15.69)	13.70	[g](17.96)
[h]$R_{m,\ max}$ (nm)	10.61	[i](14.48)	12.21	[i](16.40)
[j]Rho	0.106	-	0.019	-

Hyun-Chul Kim*, Kyeong Sik Jin*, Se Guen Lee, Eunjoo Kim, Sung Jun Lee, Sang Won Jeong, Seung Woo Lee, and Kwang-Woo Kim. *Journal of Nanoscience and Nanotechnology* **2016,** 16, 6432-6439.

Collaboration : Dr. Hyun-Chul Kim (DGIST)

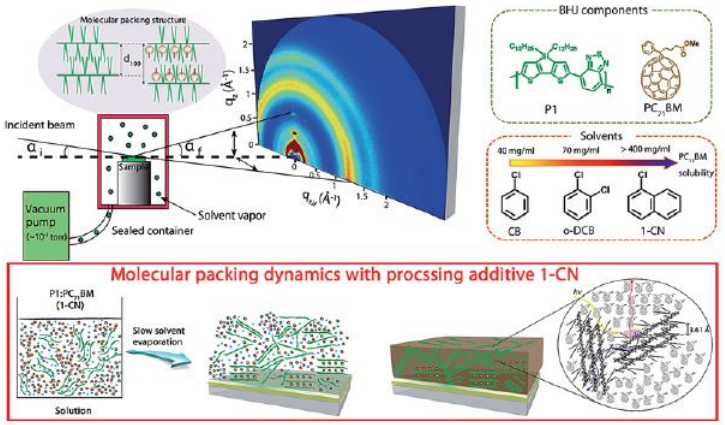
Solvent Vapor Adsorption Device (at 3C BL)





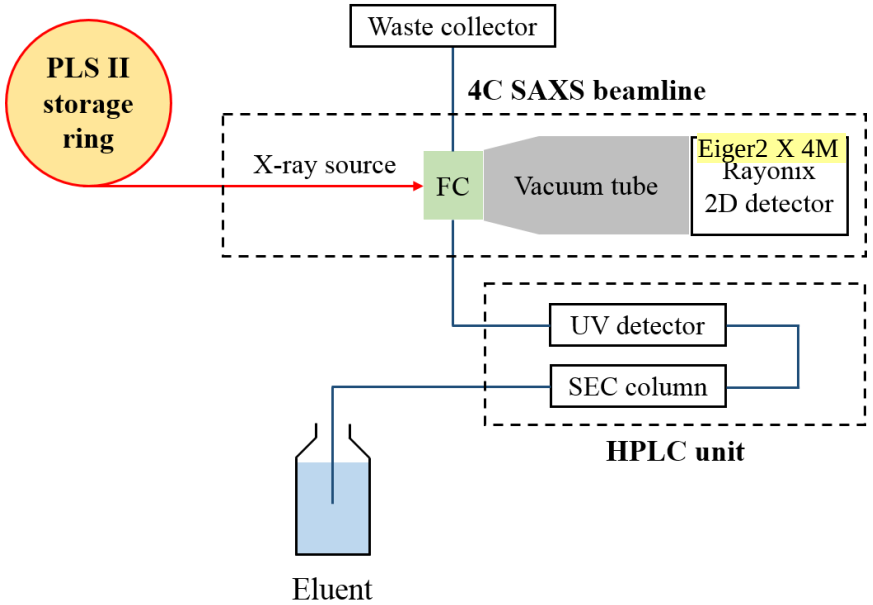
Design Criteria

Temperature range : 20 °C ~ 250 °C
Solvent Reservoir : 20x20x5 mm³

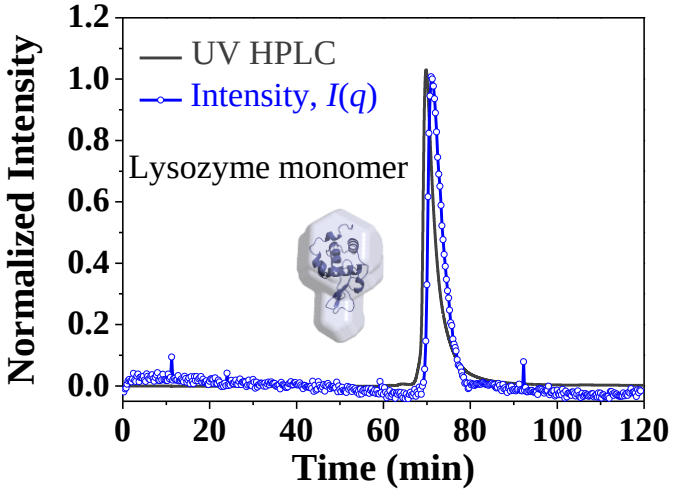


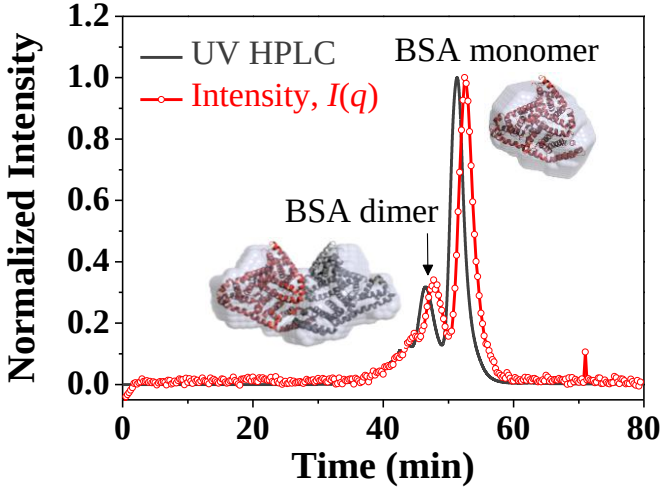
“ In-situ Studies of Molecular Packing Dynamics of Bulk-heterojunction Solar Cells induced by the Processing Additive 1-Chloronaphthalene” **Journal of Materials Chemistry A,** 2015, (DOI: 10.1039/C5TA00833F)

Size-exclusion Chromatography (SEC)-SAXS (at 4C BL)



[Agilent 1260 Infinity HPLC System]

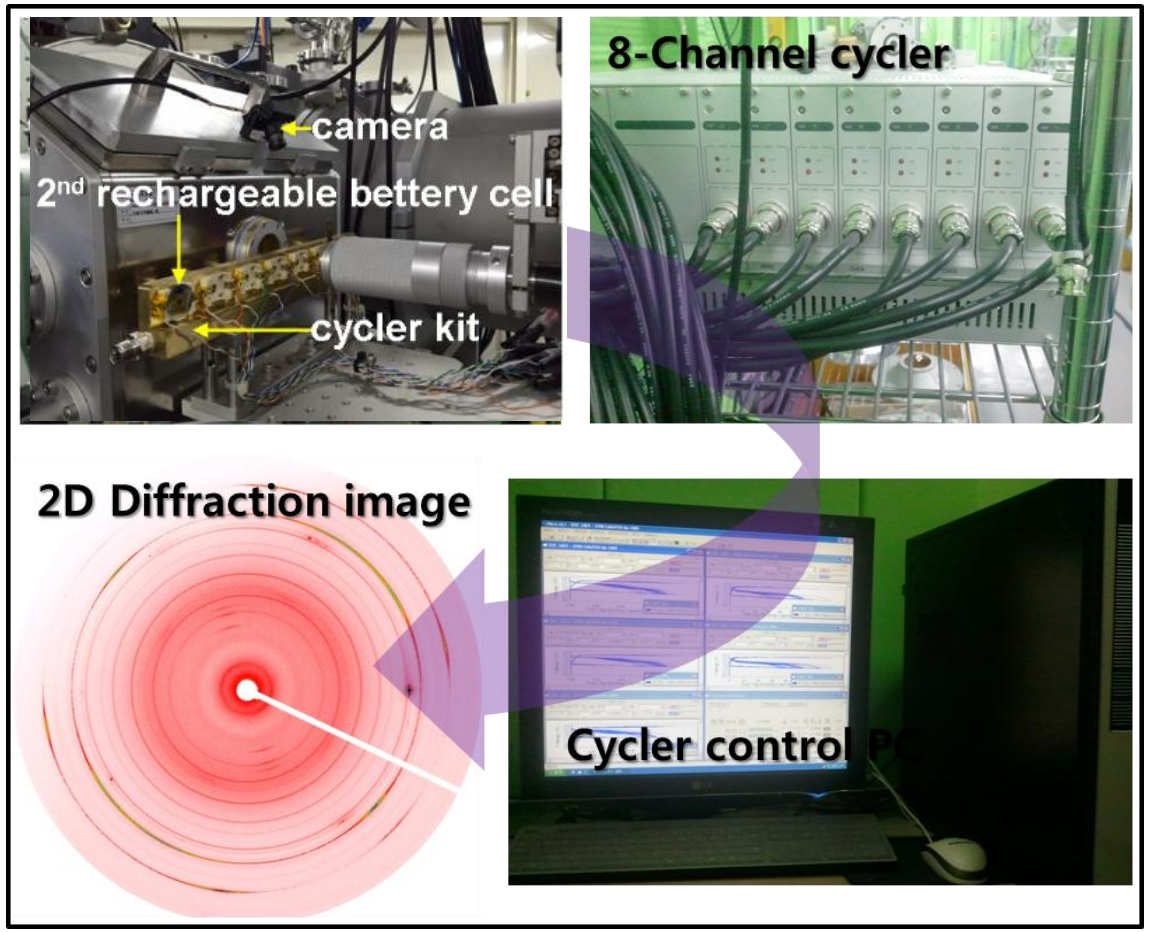


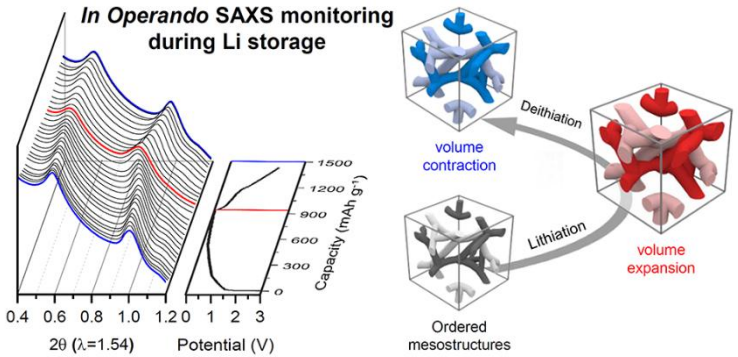


B. Kor. Chem. Soc., (2020)

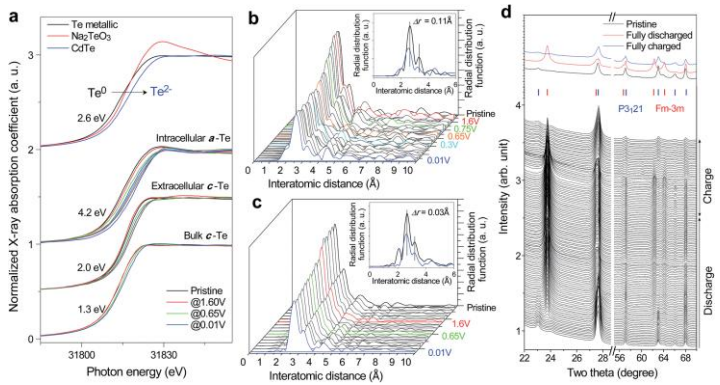
in-situ Experiment (Tr-mode) : Battery Cycling (at 6D BL)

in-situ Cycling : EXAFS, WAXD, SAXS





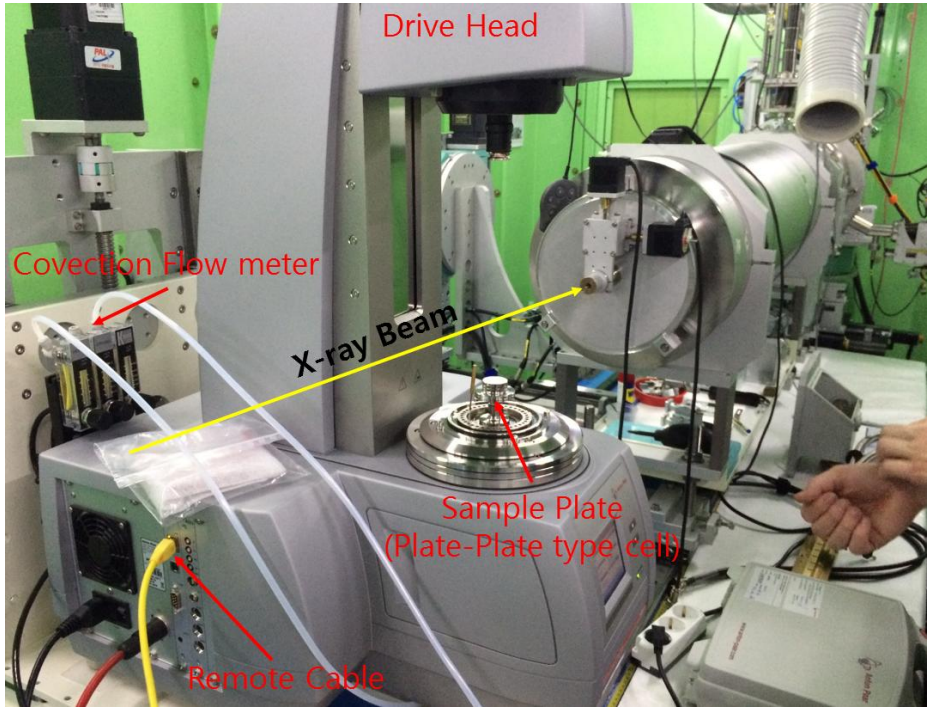
ACS nano 2015, 5, 5470–5477, Y. S. Youn et. Al.

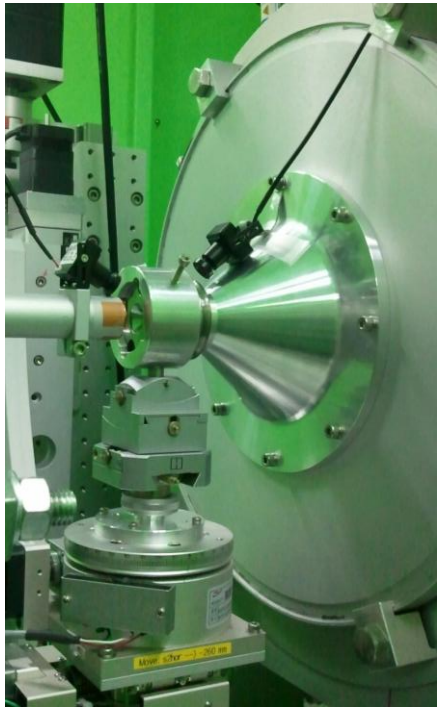


Li-ion storage through anionic redox processes of bacteria-driven tellurium nanorods,

J. Mater. Chem. A, 2015, **3**, 16978, M.G. Kim et. al.

Rheo-, High Pressure-, and Tensile Stress SAXS (at 9A BL)





Thank you for your attention