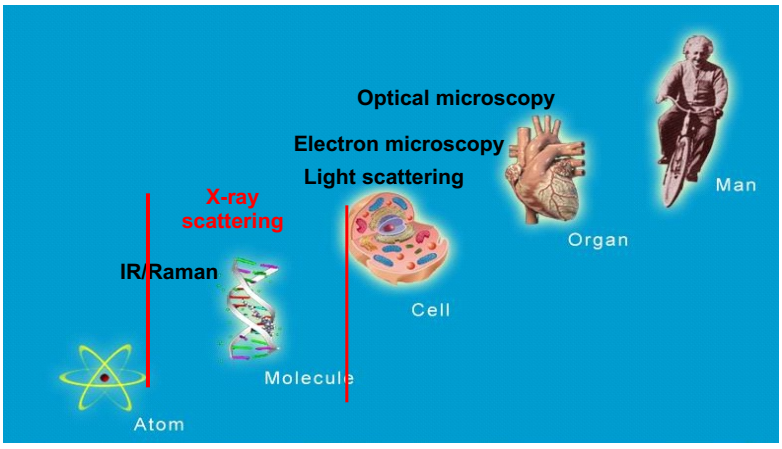
Gormley Lab Group meeting
SAXS Review

Sanjeeva Murthy
October 2, 2024

1

Length scales accessible by different techniques



2

Why SAXS?

Meso- and nano-scale structures

Parameters from SAXS

- Radius of gyration and Dmax
- Porod exponent (structural inhomogeneity - roughness and granularity - fractal dimension)

- Surface area or surface to volume ratio
- Volume of the particle
- Molecular mass (with absolute intensity)
- Pair distribution function (shape and size)

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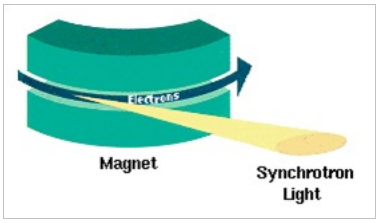
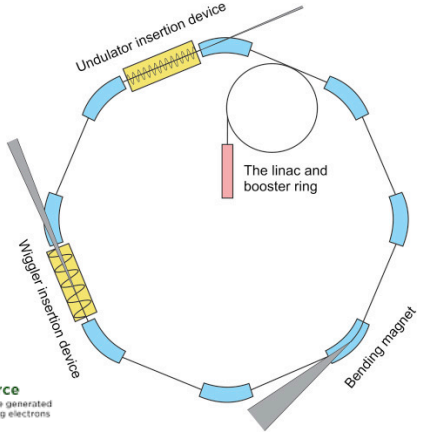
3

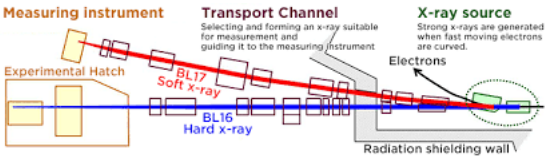
The hardware



https://en.wikipedia.org/wiki/Synchrotron_radiation#/media/File:Syncrotron.png



https://www.sciencedirect.com/topics/physics-and-astronomy/synchrotron-radiation



Sumitomo Electric. Synchrotron Radiation Beamline for ...
https://global-sei.com/company/press/2017/01/prs108.html

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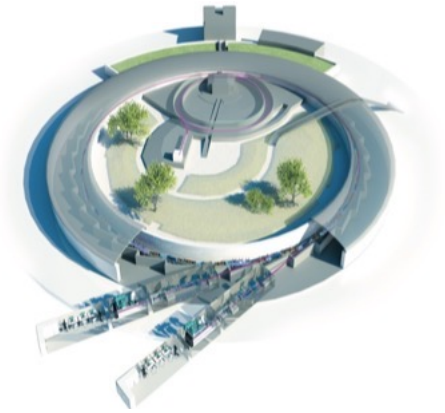
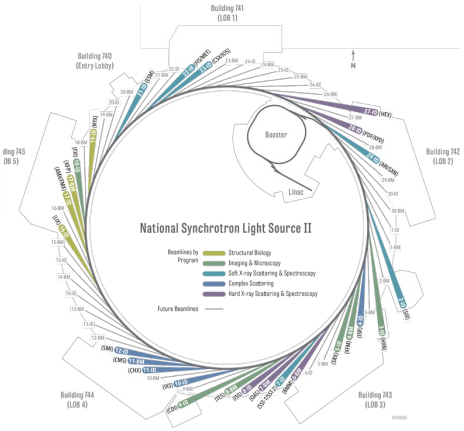
4

The machine





https://www.esrf.fr/about/synchrotron-science/synchrotron

- 66 beam lines around a circumference of 792 m (2598 feet).
- A combination of bending magnets, three-pole wigglers, and insertion devices.
- Wide spectral range: From far infrared (0.1 eV) to very hard x-ray region (>300 keV)

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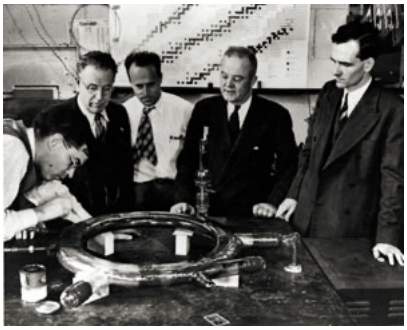
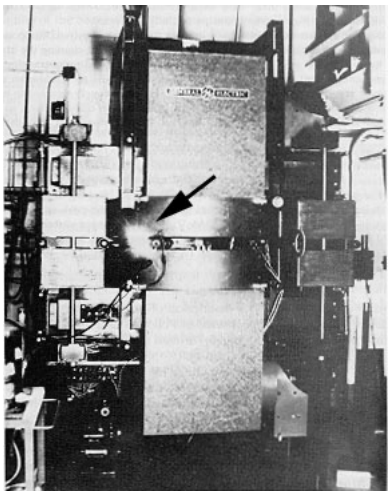
How it all began



https://cerncourier.com/a/synchrotron-radiation-is-brighter-than-ever/

At GE, Pollack got permission to assemble a team to build a 70-MeV electron synchrotron to test the idea.….Langmuir is credited as recognizing it as synchrotron radiation or, as he called it, "Schwinger radiation."



First visual observation of synchrotron radiation at General Electric Research Laboratory in Schenectady, New York, on April 24, 1947.

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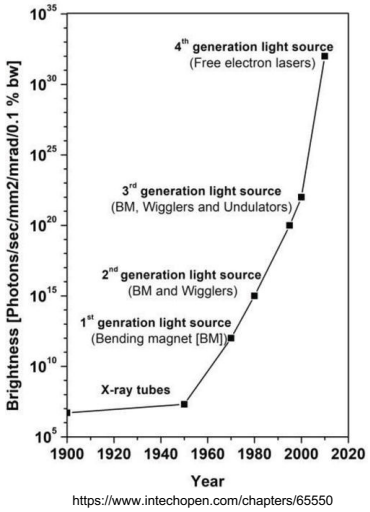
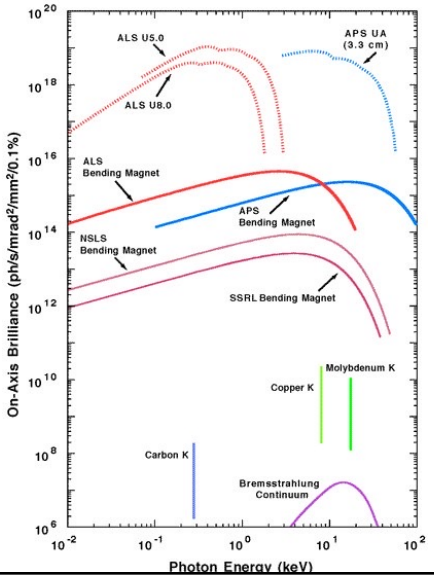
X-ray intensity and spectrum



https://www.intechopen.com/chapters/65550



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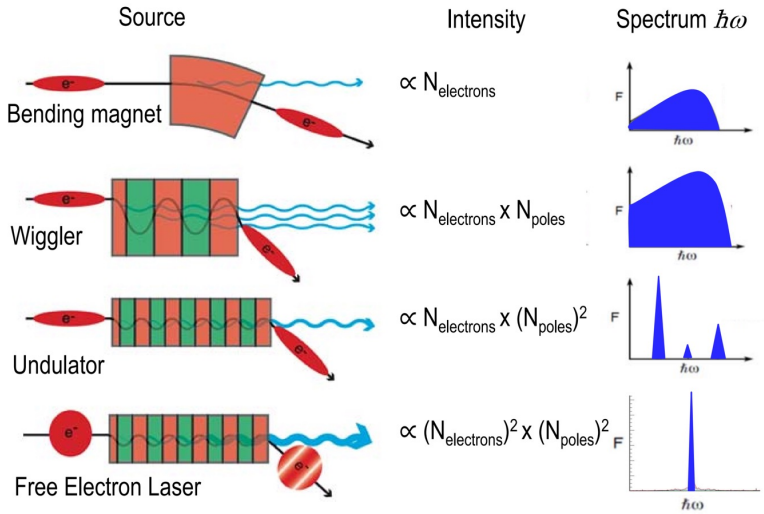
Wiggler, undulator and FEL



https://physics.stackexchange.com/questions/514819/what-is-the-difference-between-an-undulator-and-a-free-electron-laser-fel

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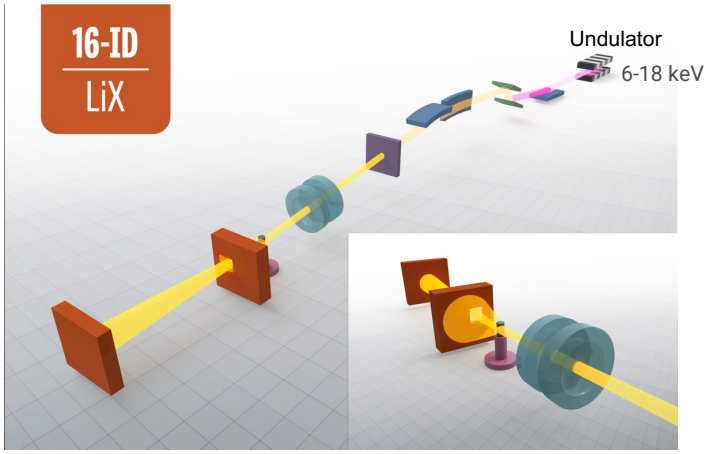
Small-angle beam line



Source: 16ID website

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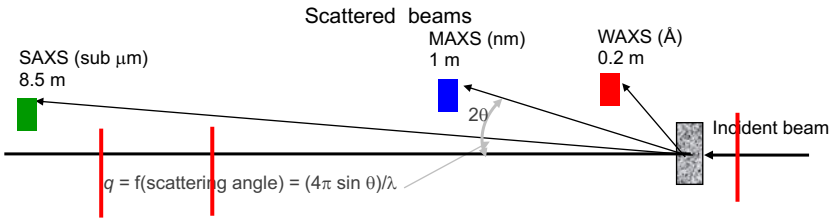
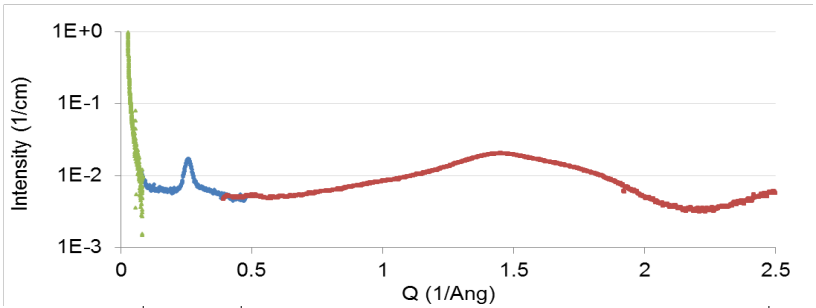
Length scale and scattering angles





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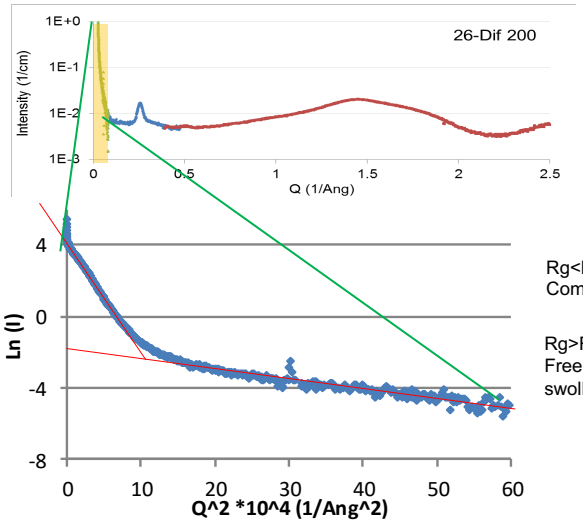
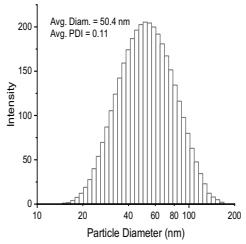
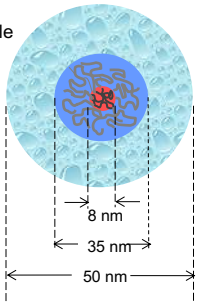
Scattering at low Q's
Size of the nanosphere: Guinier analysis





Rg<Rh
Compact molecule

Rg>Rh
Free draining,
swollen particle



Murthy, Zhang, Borsadia and Kohn
Nanospheres with a Smectic Hydrophobic Core and an Amorphous PEG Hydrophilic Shell: Structural Changes and Implications for Drug Delivery
Soft Matter 14:1327-1335 (2018).

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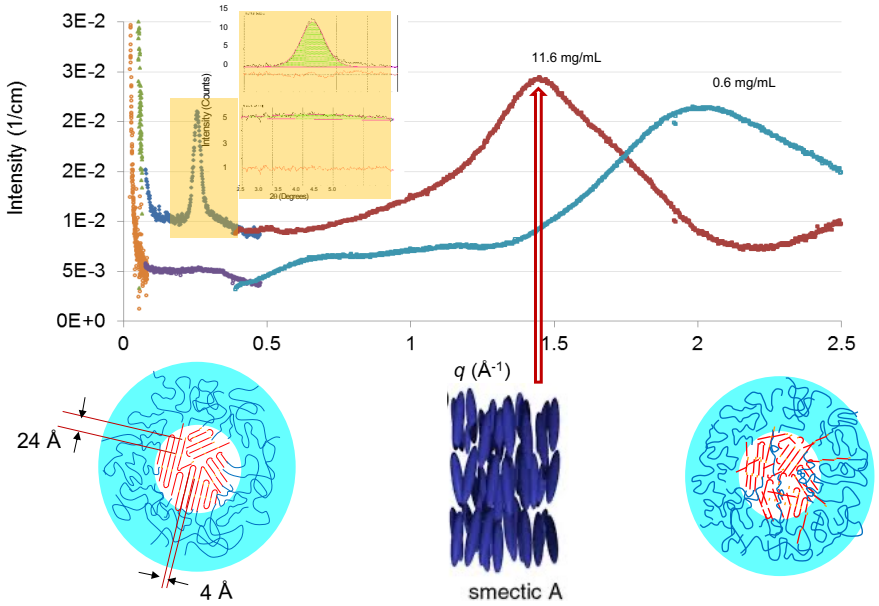
Internal structure of nanospheres



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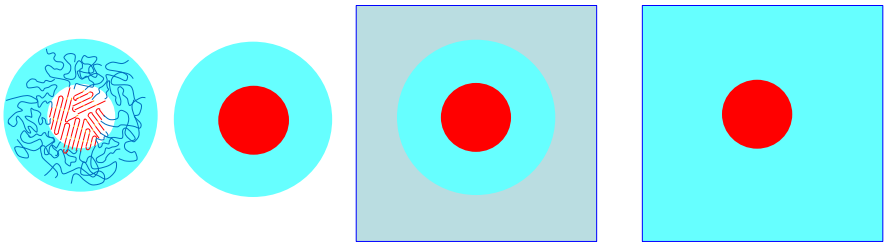
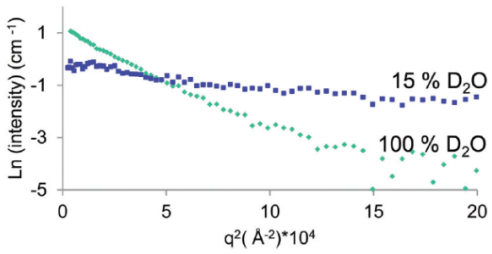
Contrast variation





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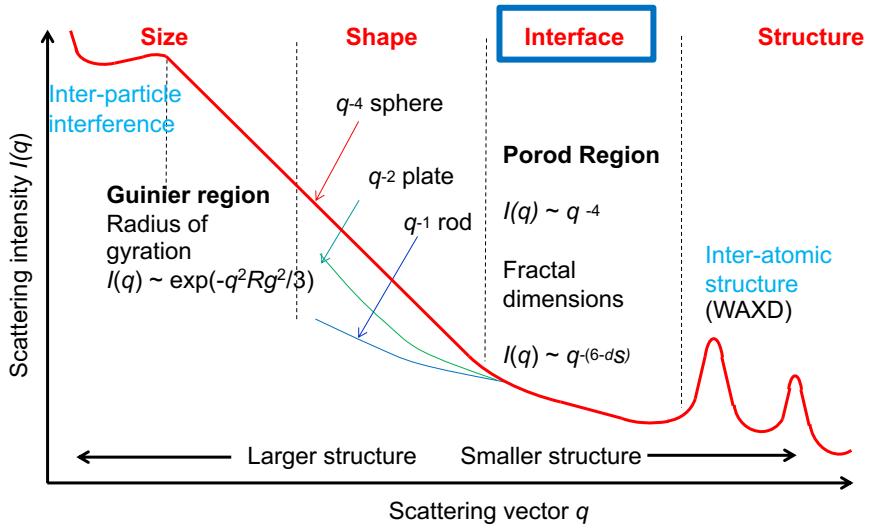
SAXS of particulate systems



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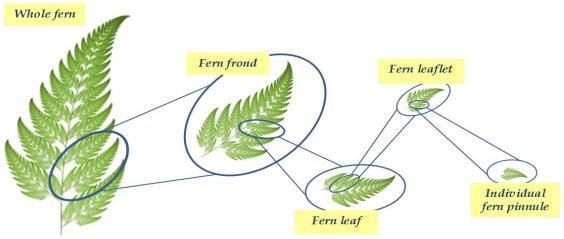
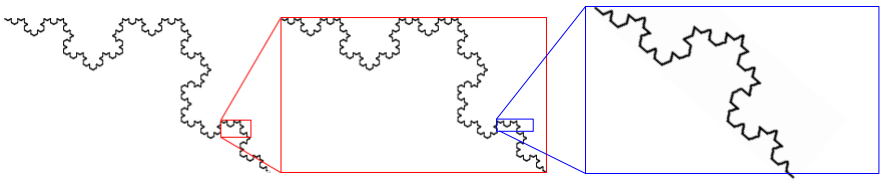
3. Porod exponent: Fractal basics



https://fractalwork.com/julian-wilson-explains-the-nature-of-a-fractal-using-an-example-from-nature/

A **fractal dimension** is an index for characterizing fractal patterns 1.6 -1.8



The Mandelbrot Set—Part I: Fractals | Rhapsody in Numbers (yozh.org)

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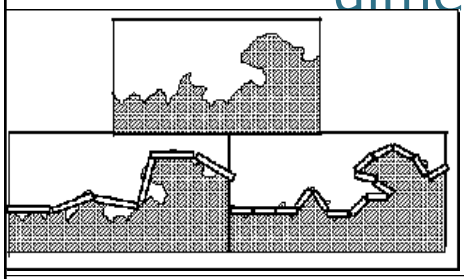
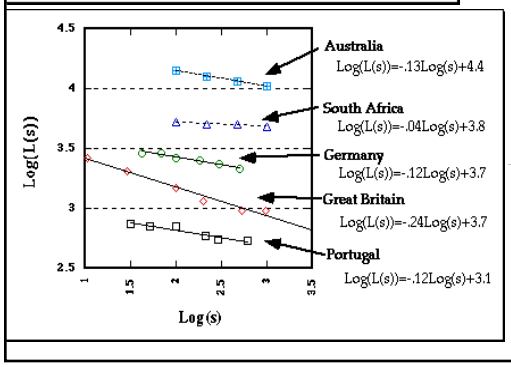
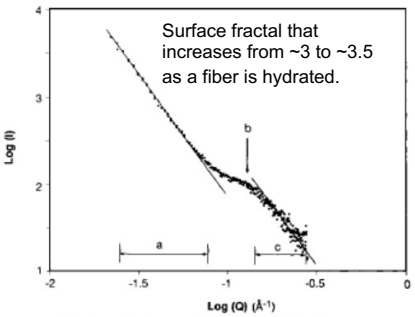
Determining the fractal dimension







Murthy, Bednarczyk, Moore and Grubb, J. Polym. Sci. Poylm. Phys. (1996) 34: 821-835.

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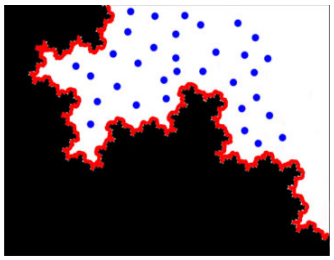
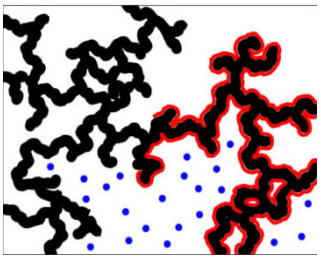
Roughness in 2D and 3D



In a surface fractal only the surface (red) is fractal.



In a mass fractal, the solid (black) is a fractal.

Porod's law: $I(q) \propto q^{-4}$

Valid for two-phase system with sharp boundaries. Diffuse boundaries reduce the exponent.

Surface fractals: $I(q) \propto q^{-(6-D_s)}$ $(2 \le D_s < 3)$

Mass fractals: $I(q) \propto q^{-D_m}$ $(1 \le D_m < 3)$

Space	Surface fractal	Mass fractal
2	$2 < 4\text{-}D_s < 3$	$1 < D_m < 2$
3	$3 < 6\text{-}D_s < 4$	$1 < D_m < 3$
	Rough | Smooth	Unfilled | Filled

Where D_m and D_s are called the mass fractal and surface fractal dimensions, and "6" is replaced by "2d" for a d-dimensional object.

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Porod exponent and fractal dimensions

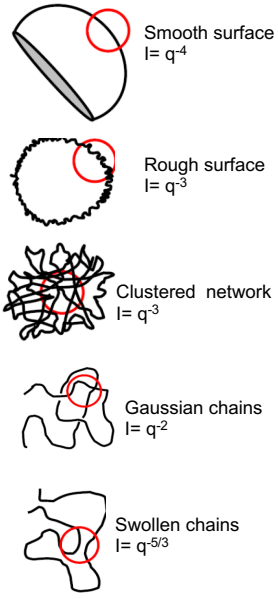
Smooth surface $D_s = 2$
$I = q^{-4}$

Rough surface $D_s = 2.8$
$I = q^{-3.2}$

Mass fractal
$I = q^{-3.5}$

Mass fractal
$I = q^{-1.66}$

Mass fractal
$I = q^{-1}$



Smooth surface
$I = q^{-4}$

Rough surface
$I = q^{-3}$

Clustered network
$I = q^{-3}$

Gaussian chains
$I = q^{-2}$

Swollen chains
$I = q^{-5/3}$

From: fractal mass-surface Ch_33.pdf

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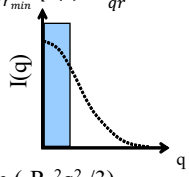
Analysis of SAXS data

SAS: Radius of gyration, R_g

Pair-distance distribution function, $p(r)$

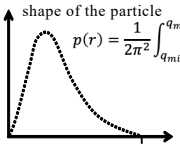
$$I(q) = 4\pi \int_{r_{min}}^{r_{max}} p(r) \frac{\sin(qr)}{qr} dr$$



Fourier Transformation
$p(r)$ = FT ([qr*I(q)]

Probability distribution of distances reflects the size and shape of the particle

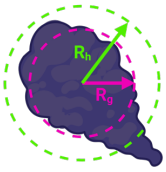
$$p(r) = \frac{1}{2\pi^2} \int_{q_{min}}^{q_{max}} I(q) qr \sin(qr)\, dq$$



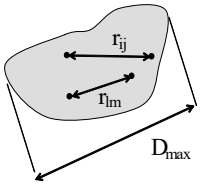
$I(q)=I(0)\exp(-R_g^2 q^2/3)$

Histogram of all such distances

D_{max}



Limited to $q < 1/R_g$



Rg: Depends on particle size and mass distribution and moment of inertia

R_g = Radius of gyration; RMS of all distances from center of mass

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

Radius of a homogeneous sphere whose moment of inertia is same as that of the particle.

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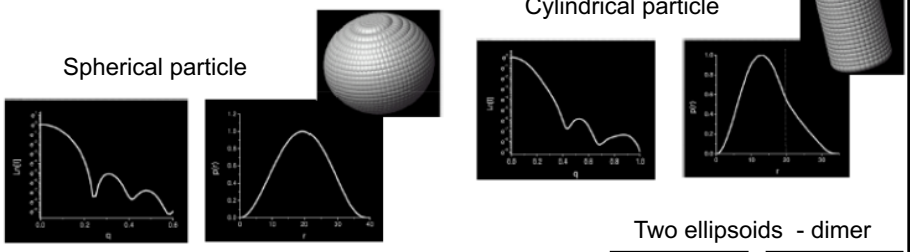
$I(q)$ and $p(r)$ of particles

Spherical particle

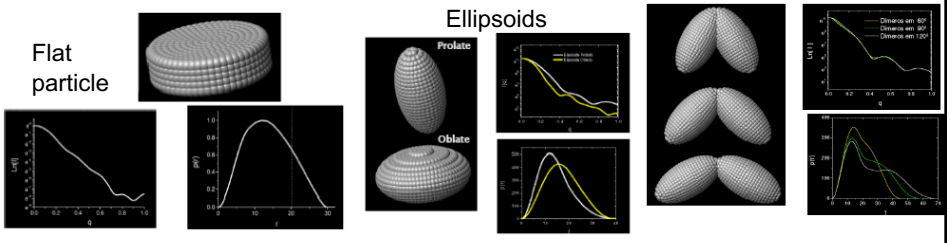
Cylindrical particle



Two ellipsoids - dimer

Flat particle

Ellipsoids



Courtesy of Dr. I.L.Torriani. From: Amemiya and Shinohara. 2011 Cheiron School. University of Tokyo

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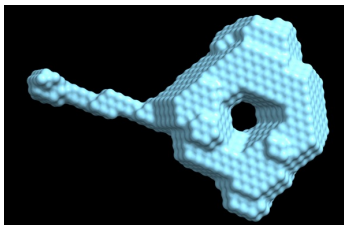
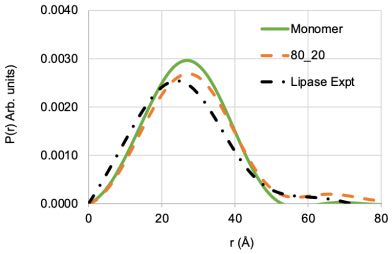
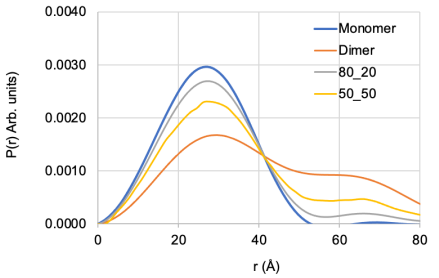
P(r) from Lipase







Data name(s): 240814 lq1 spheres r1.txt, 240823 Mix Mon Dimers p8.txt

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Problems with the data – larger species

Presence of larger species makes the Guinier deviate from a straight line making it difficult to get Rg, the value is ambiguous and not reproducible

Use P(r) functions to get Rg

Uses entire data and do not worry about getting a straight Guinier line

P(r) function has all the information, Rg, Dmax and the conformational details.

Problems with using P(r)

Getting a good P(r) is not easy.

Paring away the long tail at large r in the P(r) function is same as cutting down the low angles in the Guinier.

Data from a mixture of particles (dimers and other aggregates along with monomers) is interpreted as one particle. So, Guinier is still needed.

We need ML tools to automate the analysis

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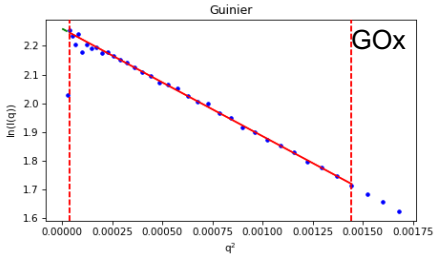
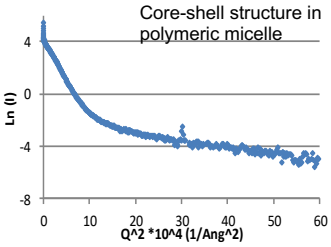
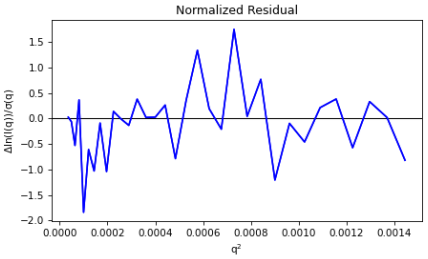
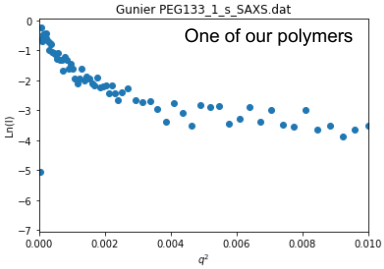
Good, bad and ugly Guinier plots









Polydispersity due to disassembly, partial denaturation and aggregation

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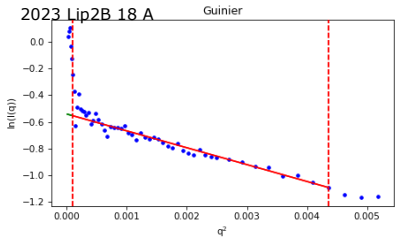
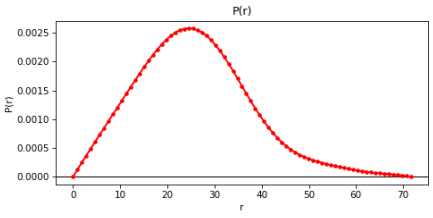
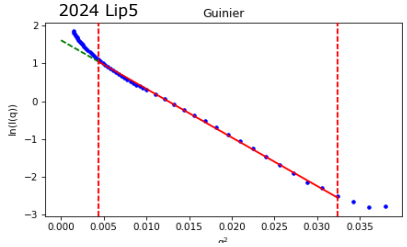
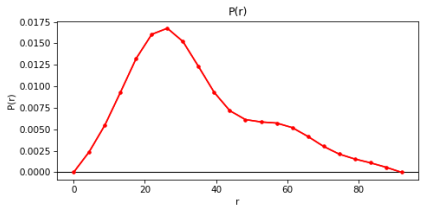
Problems with Guinier plots









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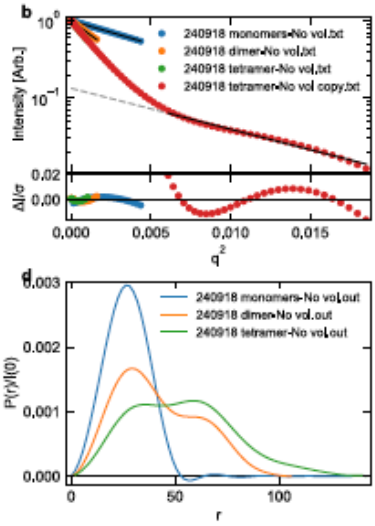
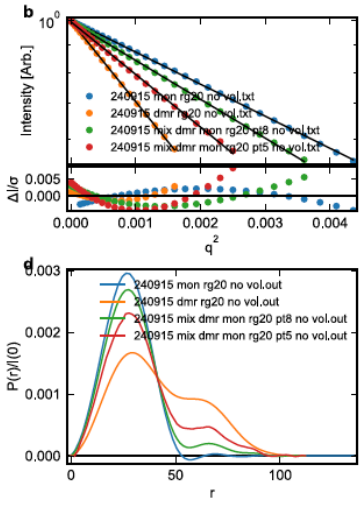
Effect of Dimers and tetramers



240918 monomers-No vol.txt, 240918 dimer-No vol.txt, 240918 tetramer-No vol.txt, 240918 tetramer-No vol copy.txt, 240918 tetramer-No volcopy 2.txt SAXS data overview



240915 mon rg20 no vol.txt, 240915 dmr rg20 no vol.txt, 240915 mix dmr mon rg20 pt8 no vol.txt, 240915 mix dmr mon rg20 pt5 no vol.txt

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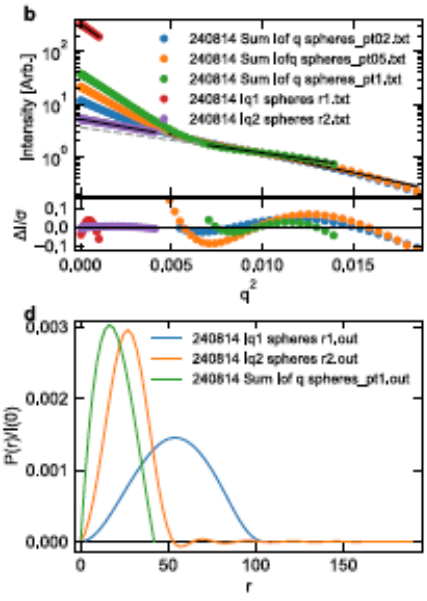
Mixture of two particles



240814 Sum Iof q spheres_pt02.txt, 240814 Sum Iofq spheres_pt05.txt, 240814 Sum Iof q spheres_pt1.txt, 240814 Iq1 spheres r1.txt, 240814 Iq2 spheres r2.txt

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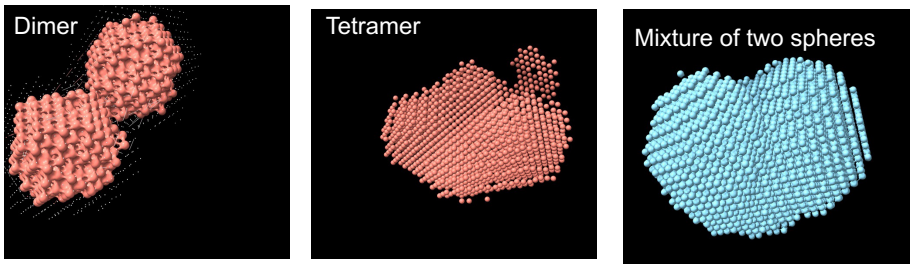
Dimers and monomers



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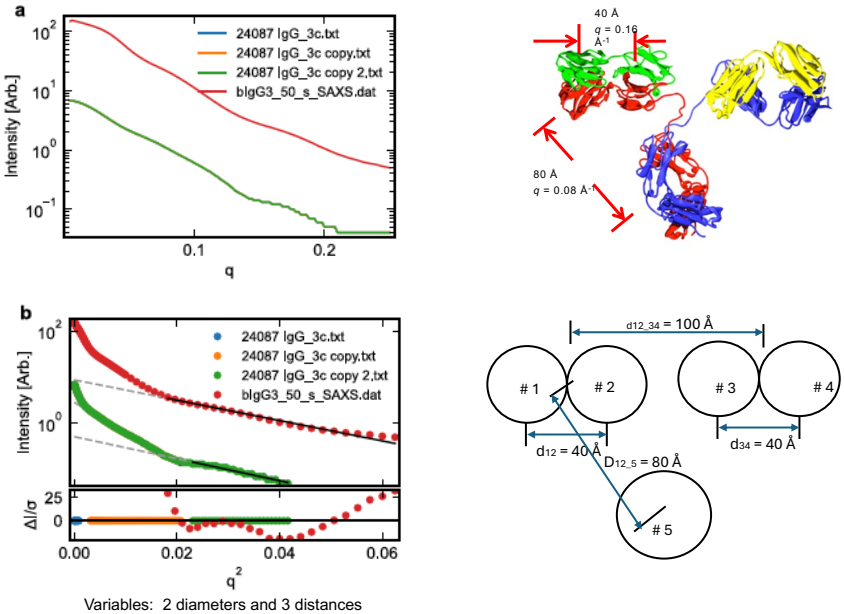
Experimental and simulated data for IgG



Variables: 2 diameters and 3 distances

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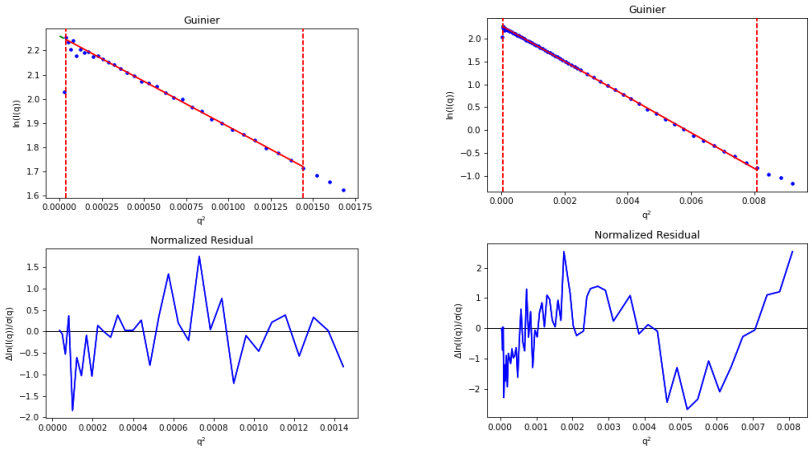
Can we extend Guinier beyond q*Rg = 1.3?



q*Rg =1.3 (current canon)

q*Rg =3.1

Examine SAS-DB files to test these limits

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Three approaches

- Parameterize the scattering curve
  - Calculate Rg, Dmax, Porod exponent, and a compactness parameter (Dmax/Rg?)
- Obtain structural models that fit the data or construct bead models from the P(r) function
  - There is no unique solution
- CREASE: Use Machine Learning to pick the most reasonable bead model and refine this by Molecular Dynamics simulation to derive the structure
  - Reduce bead model to its genetic information – parameters that describe the structure

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Before the SAXS measurements

- Well thought out experiment
- Well characterized solutions
- Minimum 3, preferably at least 5, concentrations

  In the range of 0.1 to 5 mg/mL for lipase

  Up to 150 mg/mL with mAbs
- Make a well plate lay out sheet
- Make a run sheet

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Layout sheet

Plate 1 Layout	1	2	3	4	5	6	7	8	9	Run sheet
A	Buffer_1	Lip14A_N_0.16mg/ml	Lip14B_N_0.08mg/ml	Lip15A_N_0.2mg/ml	Buffer_2	Lip15B_N_0.1mg/ml	Lip18A_N_2.28mg/ml	Lip18B_N_1.24mg/ml	Buffer_3	AB
B	Buffer_4	Lip14A_S_0.16mg/ml	Lip14B_S_0.08mg/ml	Lip15A_S_0.2mg/ml	Buffer_5	Lip15B_S_0.1mg/ml	Lip18A_S_2.28mg/ml	Lip18B_S_1.24mg/ml	Buffer_6	
C	Buffer_7	Lip20A_N_0.48mg/ml	Lip20A_N_0.28mg/ml	BLANK	Buffer_8	BLANK	BLANK	BLANK		CD
D	Buffer_10	Lip20A_S_0.48mg/ml	Lip20A_S_0.28mg/ml	BLANK	Buffer_11	BLANK	BLANK	BLANK		
E	Buffer_13	CR_01_N	CR_02_N	CR_03_N	Buffer_14	CR_04_N	CR_05_N	CR_06_N	Buffer_15	EF
F	Buffer_16	CR_07_N	CR_08_N	CR_09_N	Buffer_17	CR_10_N	BLANK	BLANK		
G	Buffer_19	CR_01_S	CR_02_S	CR_03_S	Buffer_20	CR_04_S	CR_05_S	CR_06_S	Buffer_21	GH
H	Buffer_22	CR_07_S	CR_08_S	CR_09_S	Buffer_23	CR_10_S	BLANK	BLANK		

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Run Sheet

HolderName	Position	SampleName	Sample Concentration	Volume	Exposure	BufferName
SM_AB	1	Buffer_1		45	0.5	
	3	Lip14A_N				Buffer_1
	5	Lip14B_N				Buffer_1
	7	Lip15A_N				Buffer_1
	9	Buffer_2				
	11	Lip15B_N				Buffer_2
	13	Lip18A_N				Buffer_2
	15	Lip18B_N				Buffer_2
	17	Buffer_3				
	2	Buffer_4				
	4	Lip14A_S				Buffer_4
	6	Lip14B_S				Buffer_4
	8	Lip15A_S				Buffer_4
	10	Buffer_5				
	12	Lip15B_S				Buffer_5
	14	Lip18A_S				Buffer_5
	16	Lip18B_S				Buffer_5
	18	Buffer_6				

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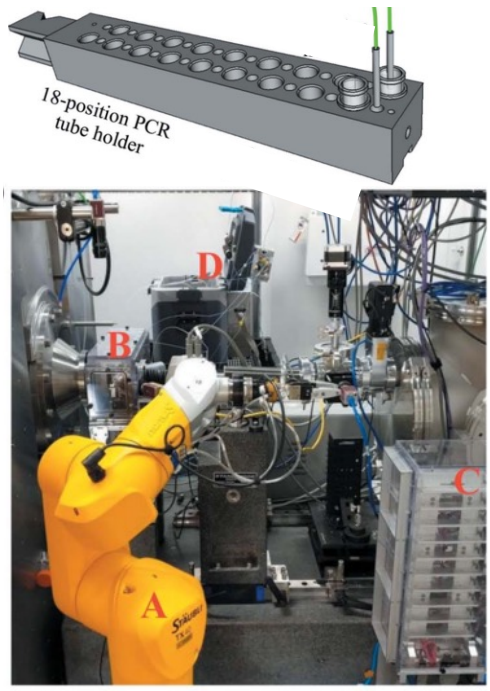
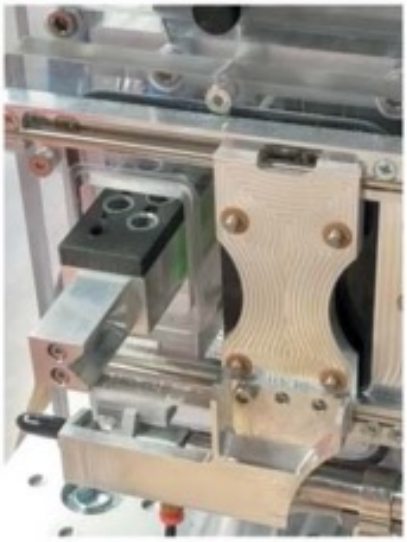
Sample loading





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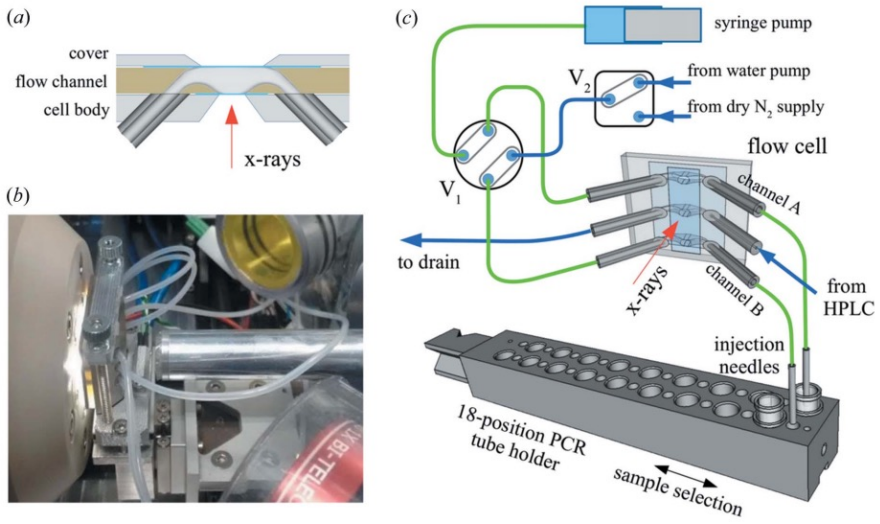
Exposure of the sample to X-rays



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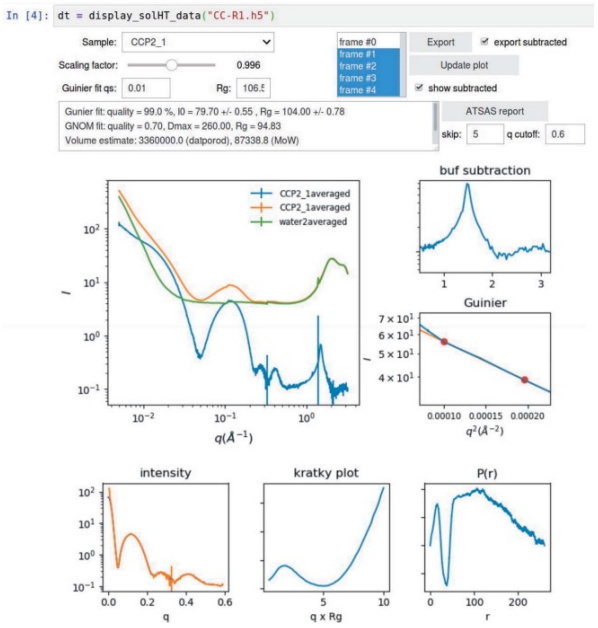
On-line data analysis



Lin Yang … James Byrnes … et al. J. Synchrotron Rad. (2020). 27, 804–812

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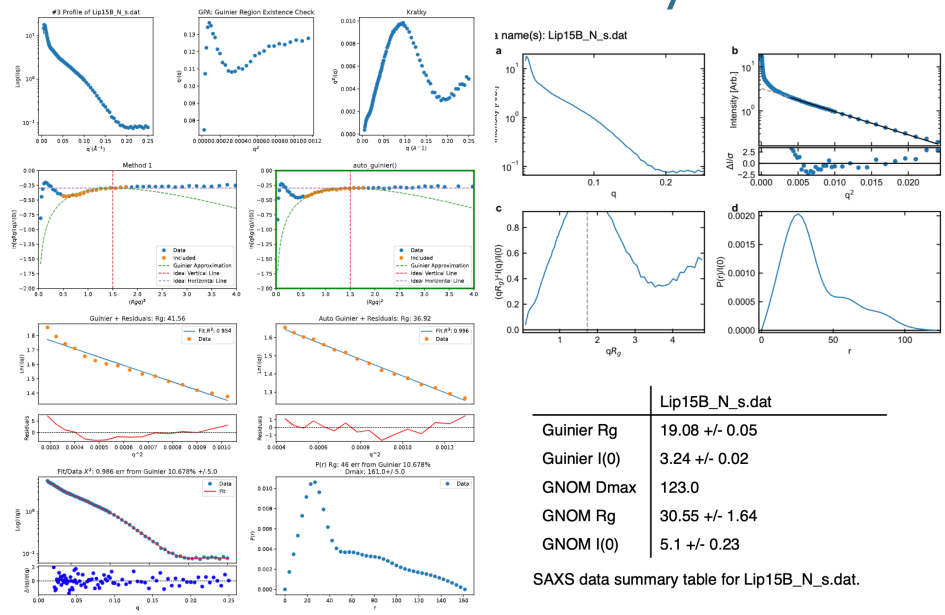
Off-line Data Analysis



	Lip15B_N_s.dat
Guinier Rg	19.08 +/- 0.05
Guinier I(0)	3.24 +/- 0.02
GNOM Dmax	123.0
GNOM Rg	30.55 +/- 1.64
GNOM I(0)	5.1 +/- 0.23

SAXS data summary table for Lip15B_N_s.dat.

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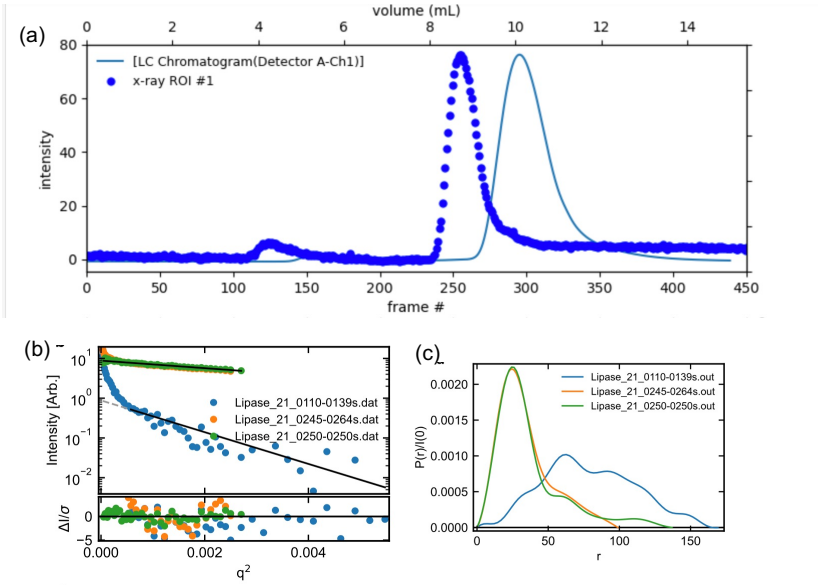
SEC-SAXS
Lipase 21: Strong monomeric peak in DLS



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Summary

- SAXS is a high-throughput biophysical characterization tool for examining macromolecules' size, shape, and structure.
- Advantages include small volumes (~50 mL), the ability to carry out experiments at variable temperatures, and the ability to combine with size exclusion chromatography.
- Expertise is needed to extract the important features that are buried in the data. But it is primed for combining with AI tools and is adaptable to ML techniques.

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