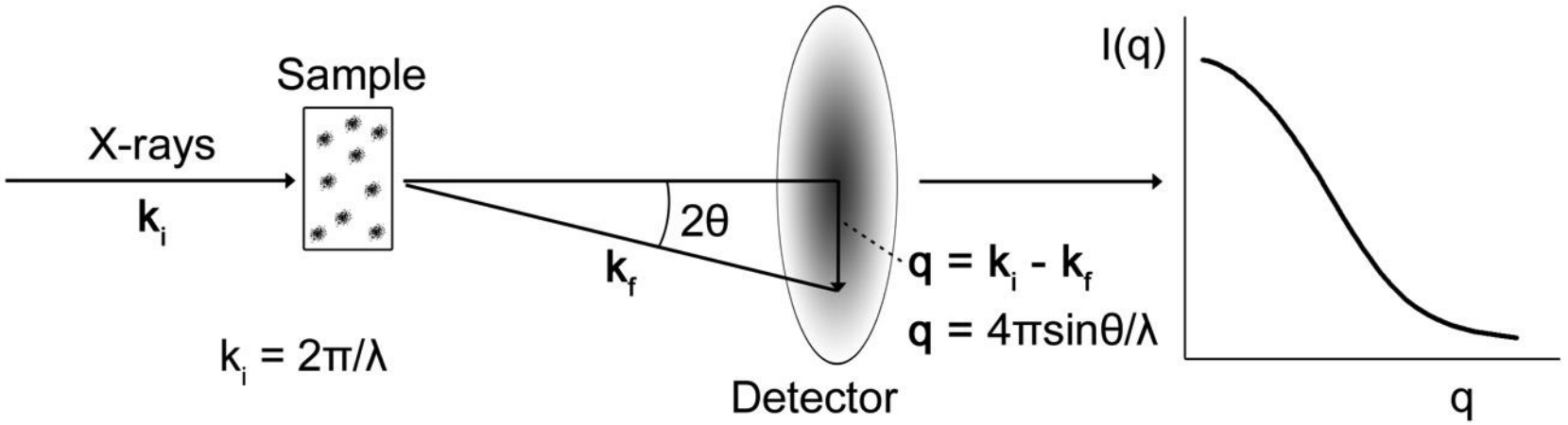
Practicing safe SAXS

(or what goes on behind the beamstop)

Small Angle X-ray Scattering (SAXS)

- SAXS is a low resolution technique (don't expect too much)
- It is a solution technique that does not require a crystal (and can be used to guide crystallization)
- It is sensitive to the sample state and can be used characterize that state (globular, unfolded etc.)
- It is sensitive to all the conformations of the sample (dynamics)
- With a given a model, theoretical SAXS data can be generated (it can be used to rule out incorrect hypothesis or model individual components into a complex)
- SAXS can provide a low resolution envelope of the volume of the sample in solution (but may not).

Schematic of SAXS experiment



$$I(q) = \int 4\pi r^2 \cdot \bar{\rho}^2(r) \cdot \frac{\sin qr}{qr}\, dr$$

SAXS is *deceptively* simple

Small Angle X-ray Scattering (SAXS)

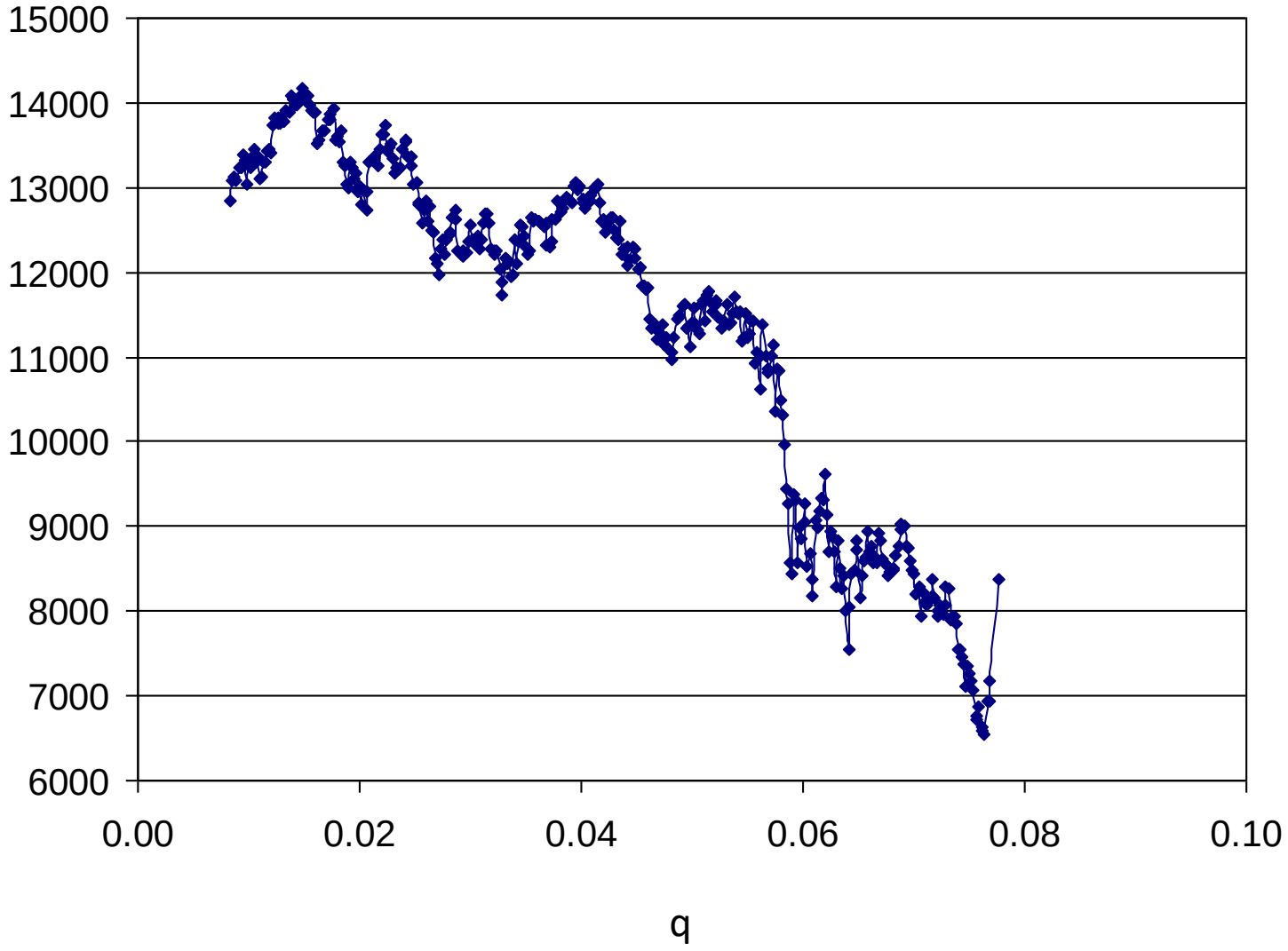
Four main uses:

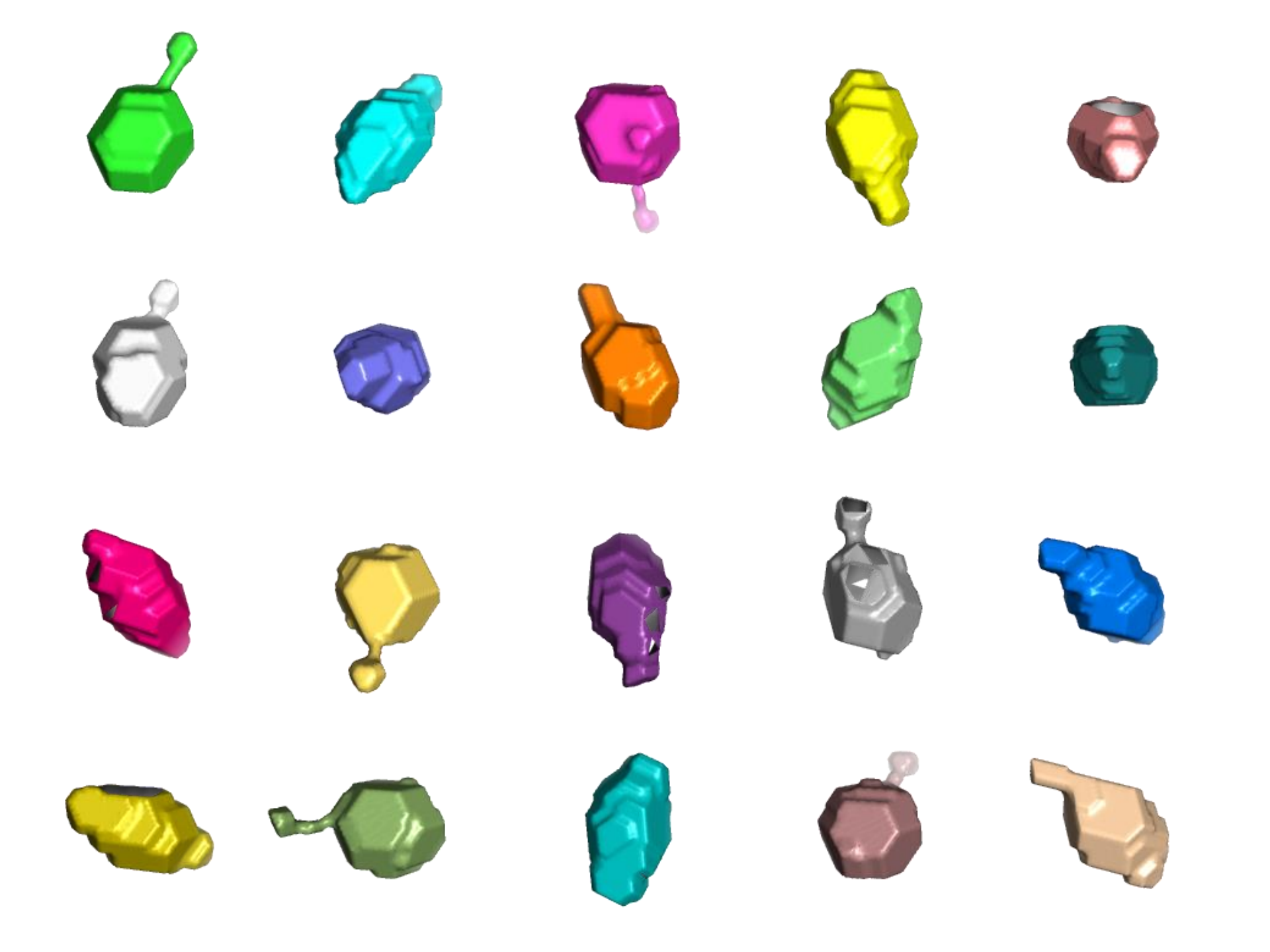
1. Characterization
2. Hypothesis generating or testing
3. Constraining other data
4. Generating a molecular envelope

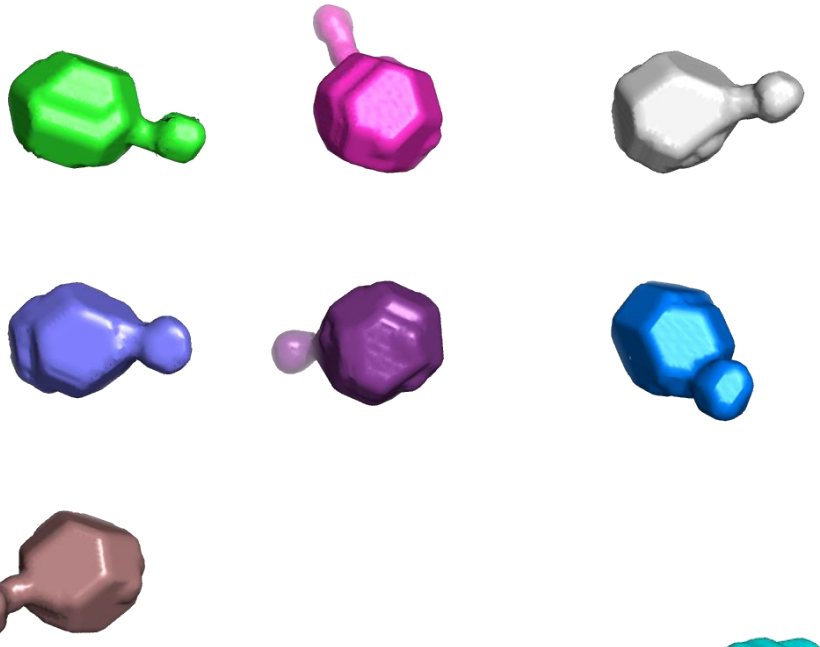
All of which need good data.

However, unlike crystallography, bad data can still give a result.

Lets take some '*scattering*' data



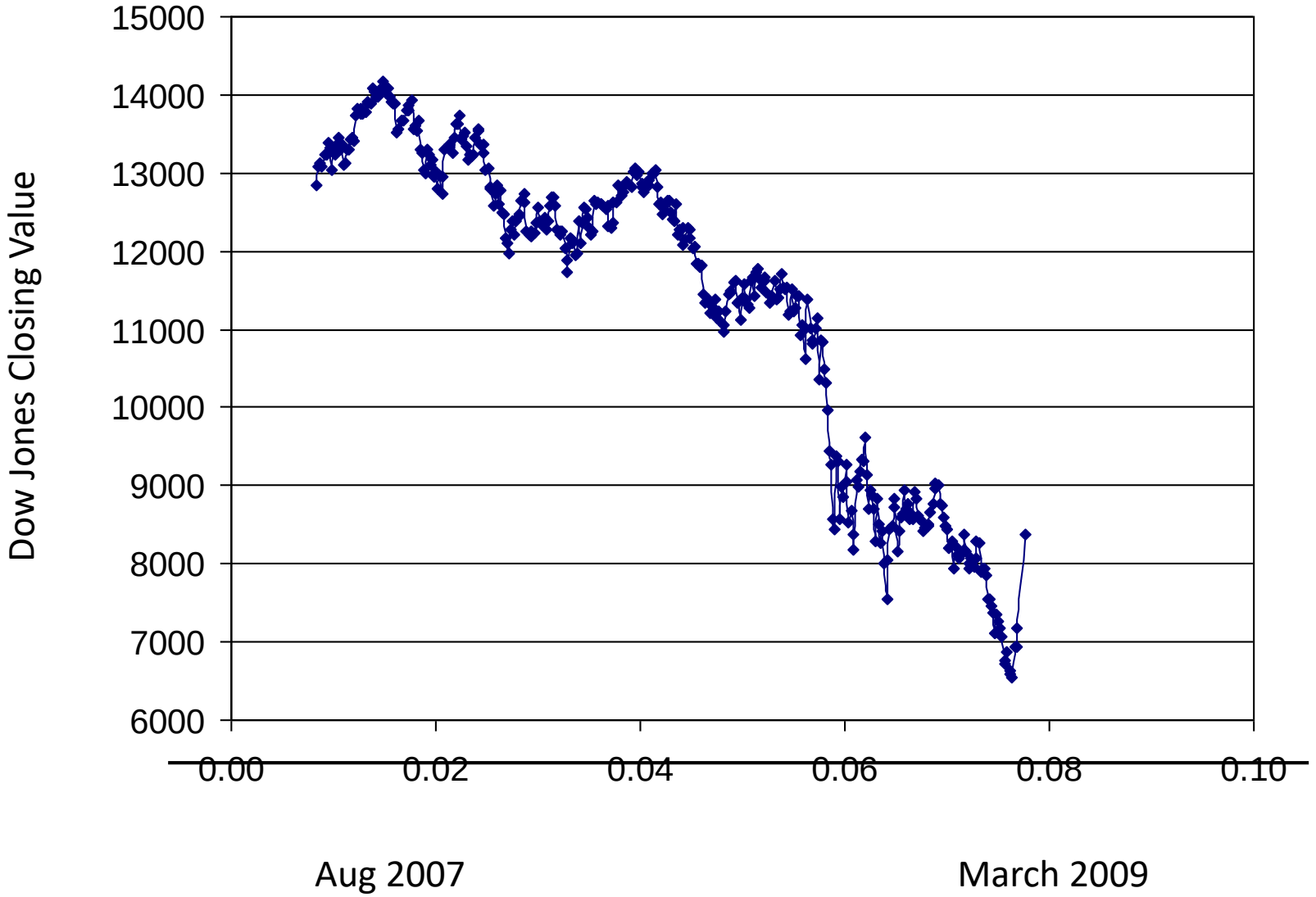




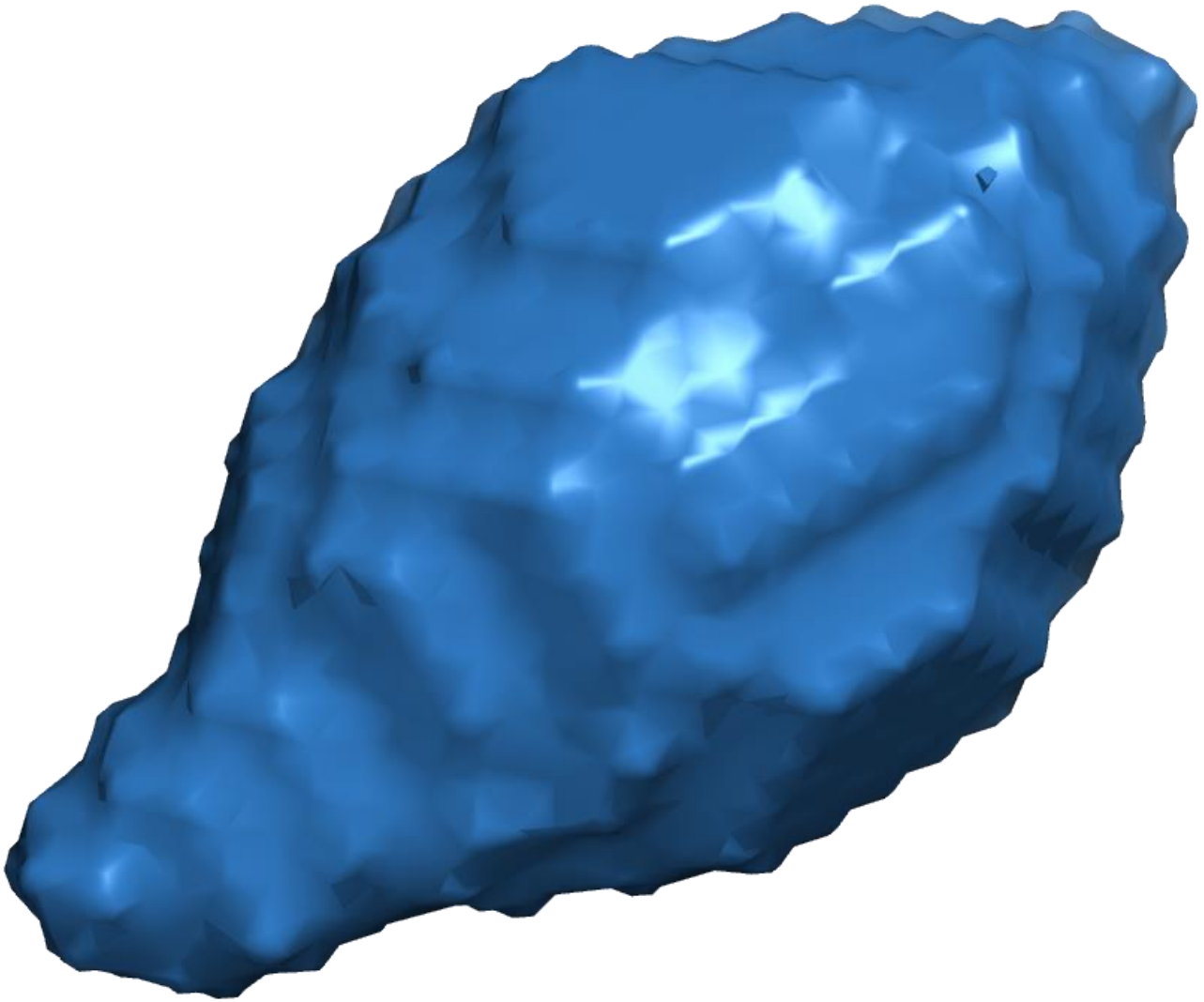
Actually two populations

Both are correct, i.e. they explain the scattering data

The Dow Jones Closing Values from 2007 to 2009



This is the molecular envelope of the recession, not a protein



NSD = 0.613, 20 reconstructions

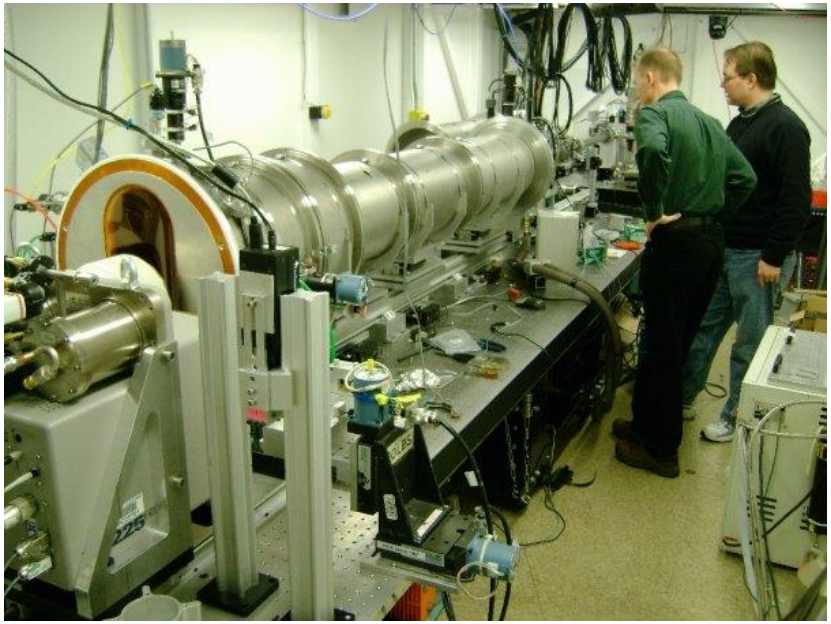
An envelope can be calculated even if it is not SAXS data

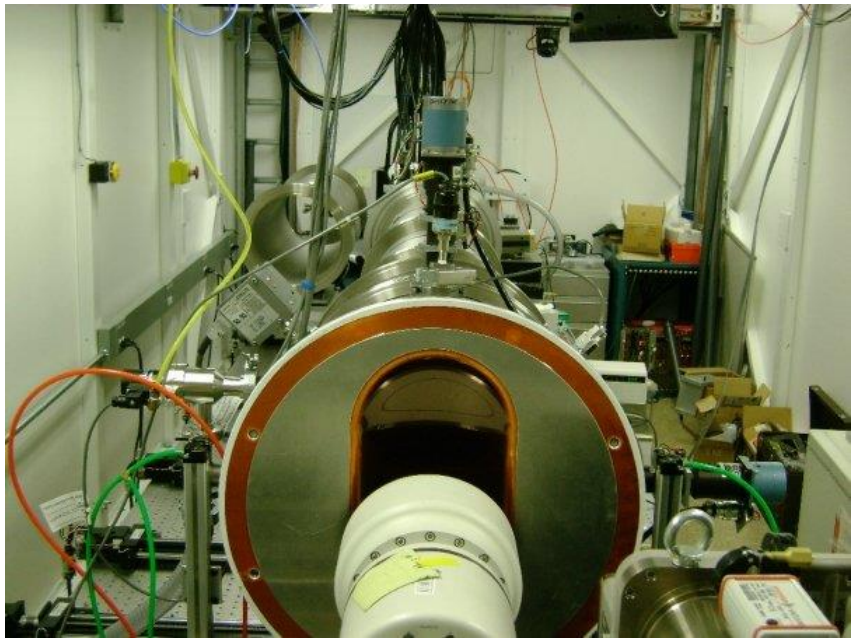
Bad data can still give a result!

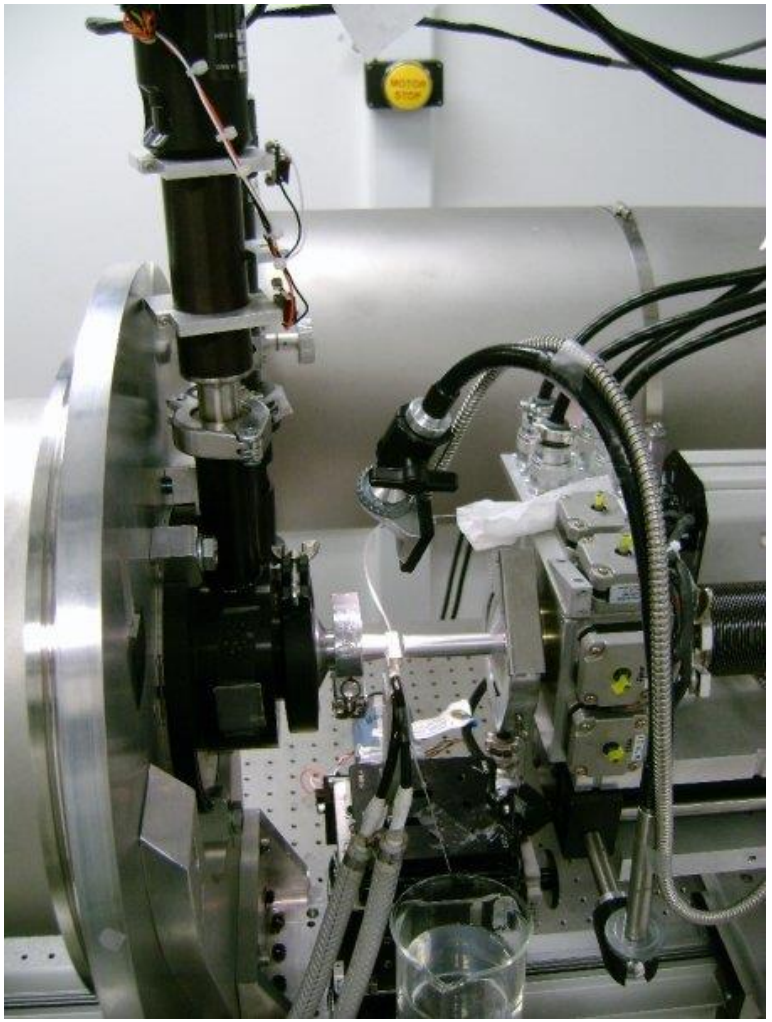
Basic Assumptions in analyzing SAXS data

- A single species exists in solution
- The sample minus the buffer equals the buffer
- That there is no inter-particle interaction
- That there is no radiation damage

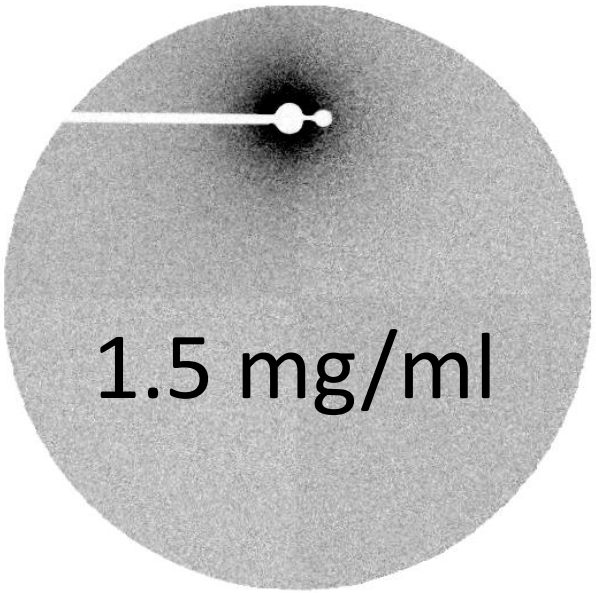
SAXS is *deceptively* simple; the self deception comes with failure to achieve these criteria

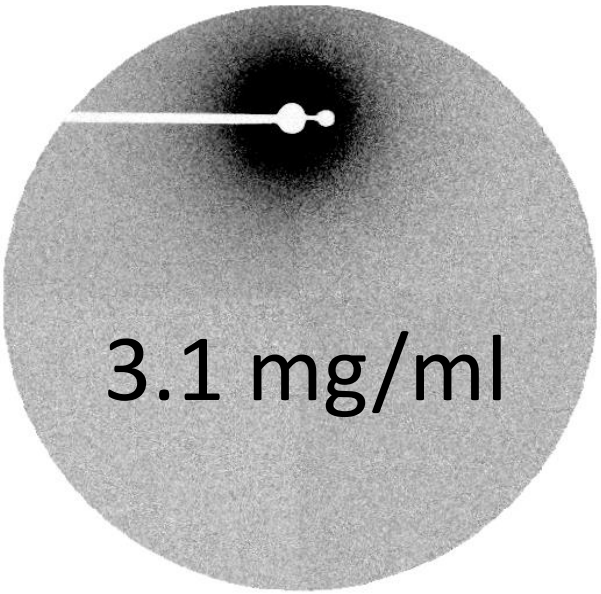


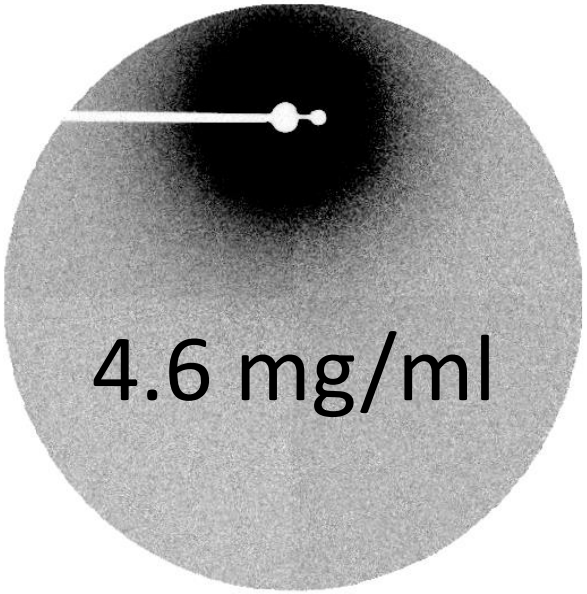


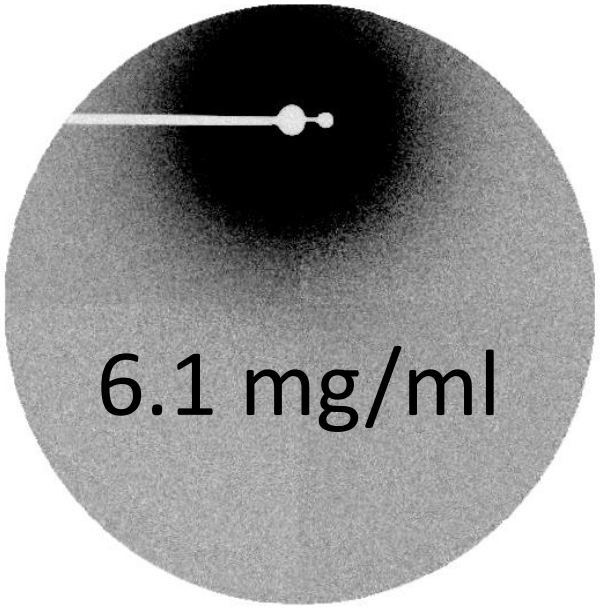


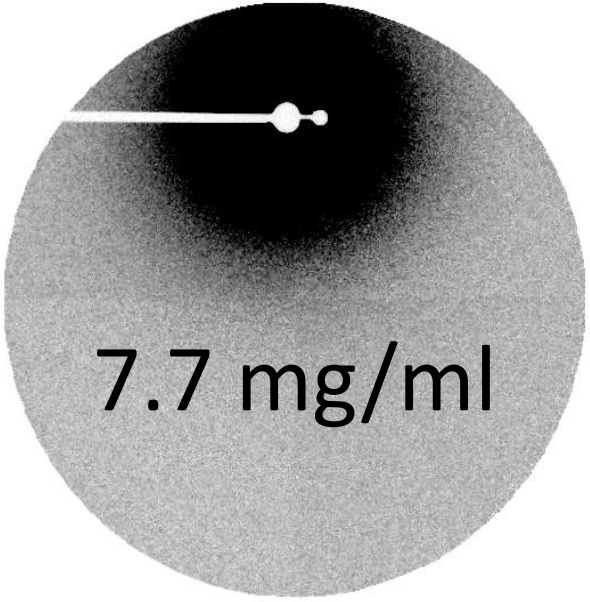
Beamline 4-2 SSRL

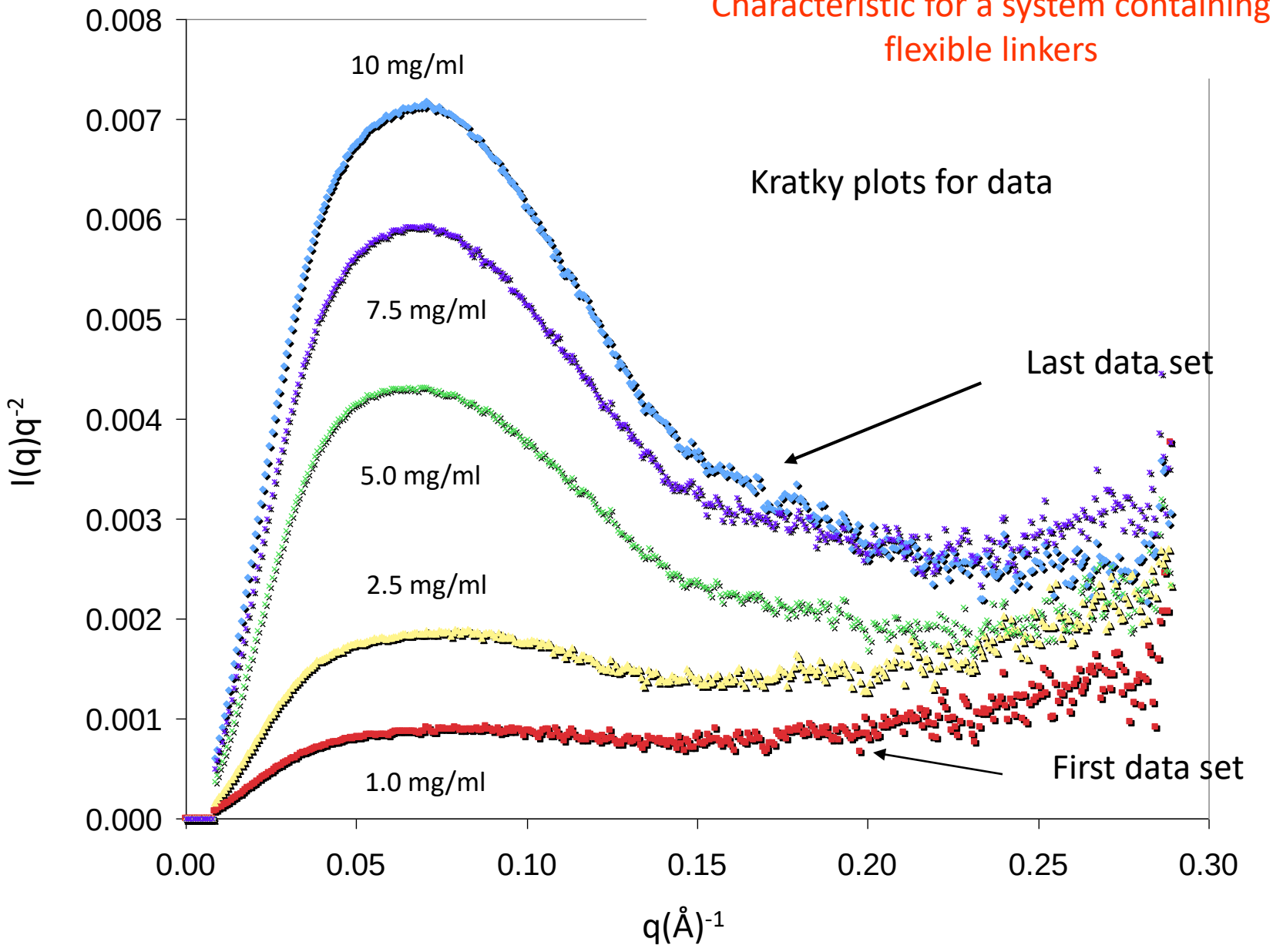

1.5 mg/ml


3.1 mg/ml


4.6 mg/ml

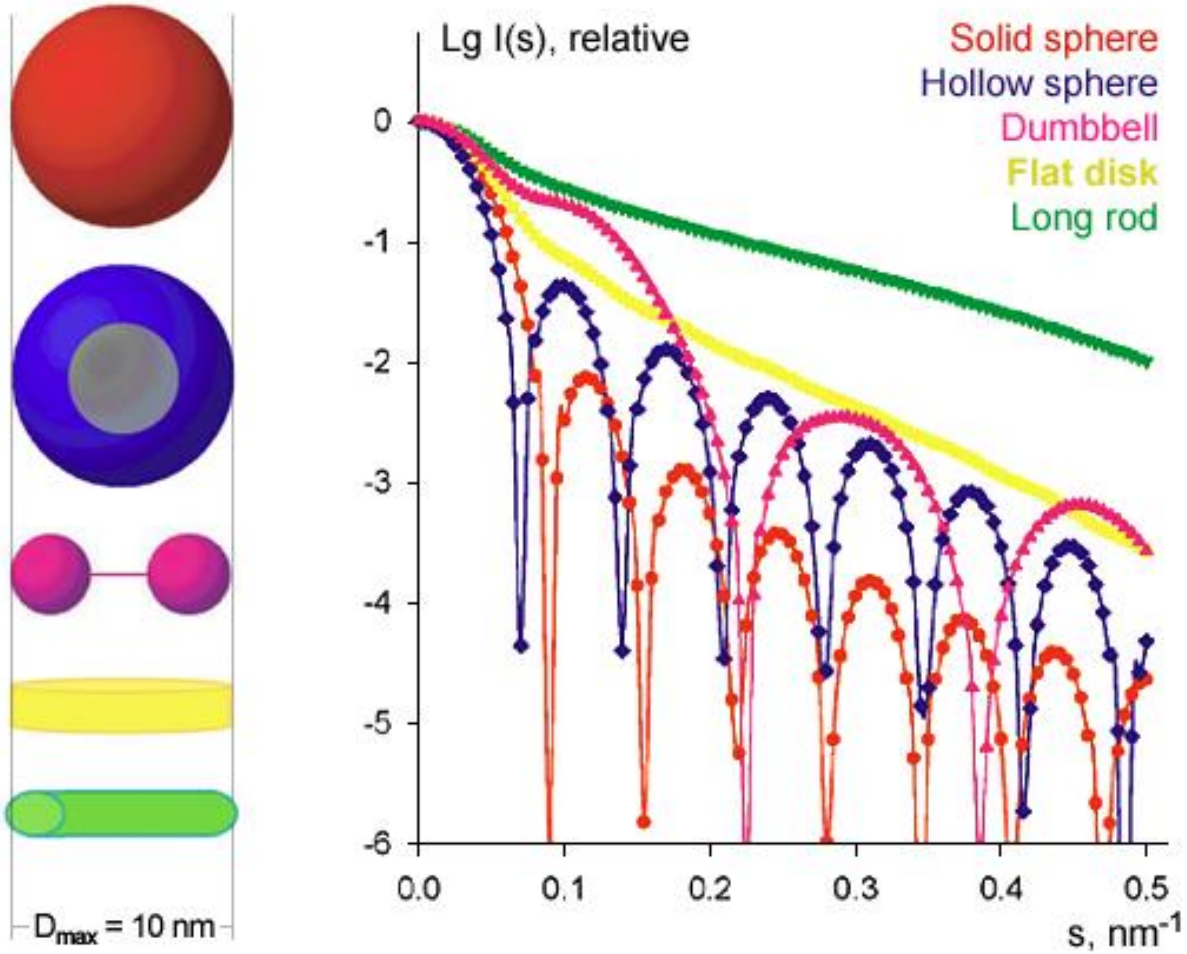

6.1 mg/ml


7.7 mg/ml

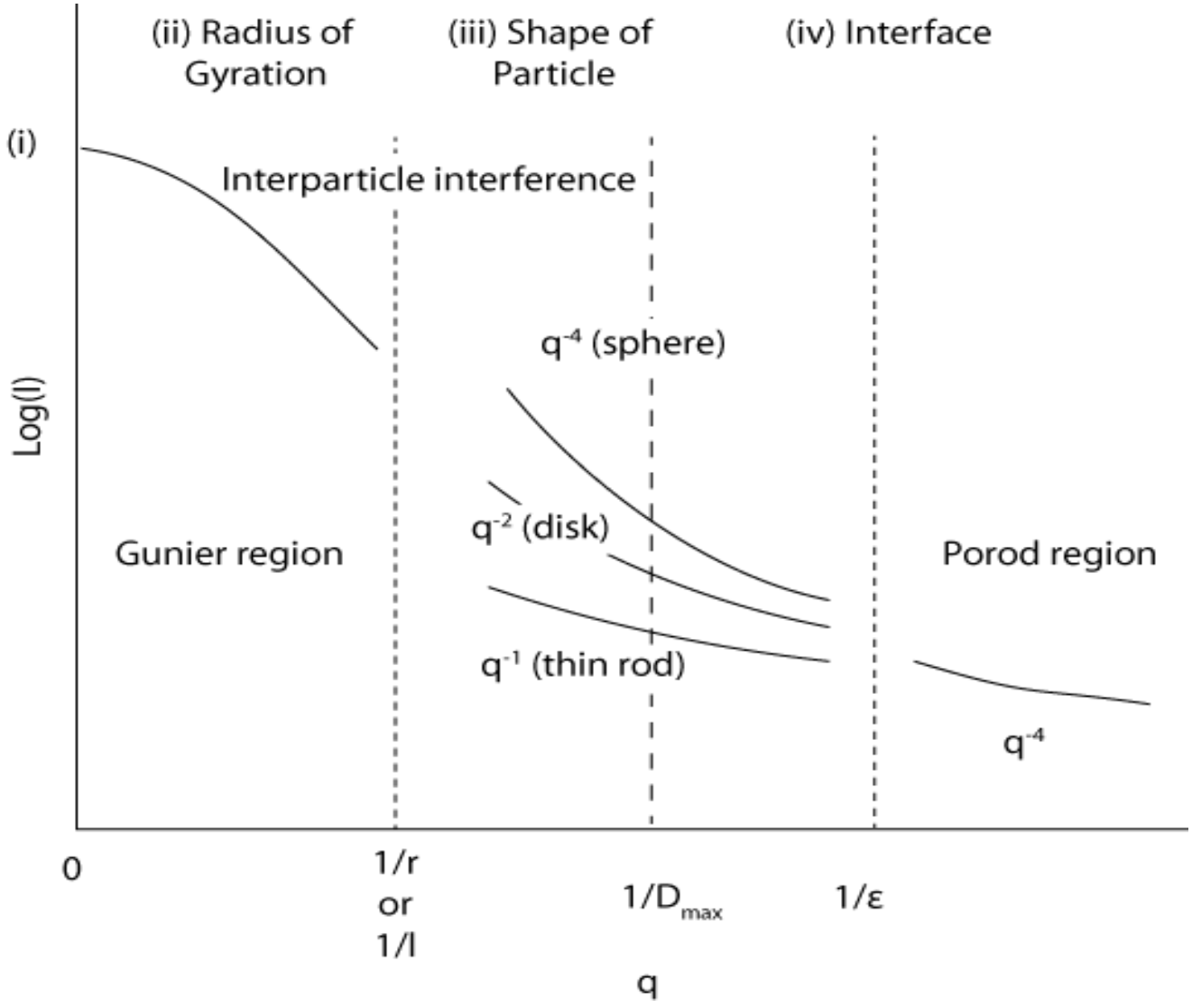

Characteristic for a system containing flexible linkers
Kratky plots for data
10 mg/ml
7.5 mg/ml
5.0 mg/ml
2.5 mg/ml
1.0 mg/ml
Last data set
First data set
I(q)q-2
q(Å)-1
0.000
0.001
0.002
0.003
0.004
0.005
0.006
0.007
0.008
0.00
0.05
0.10
0.15
0.20
0.25
0.30

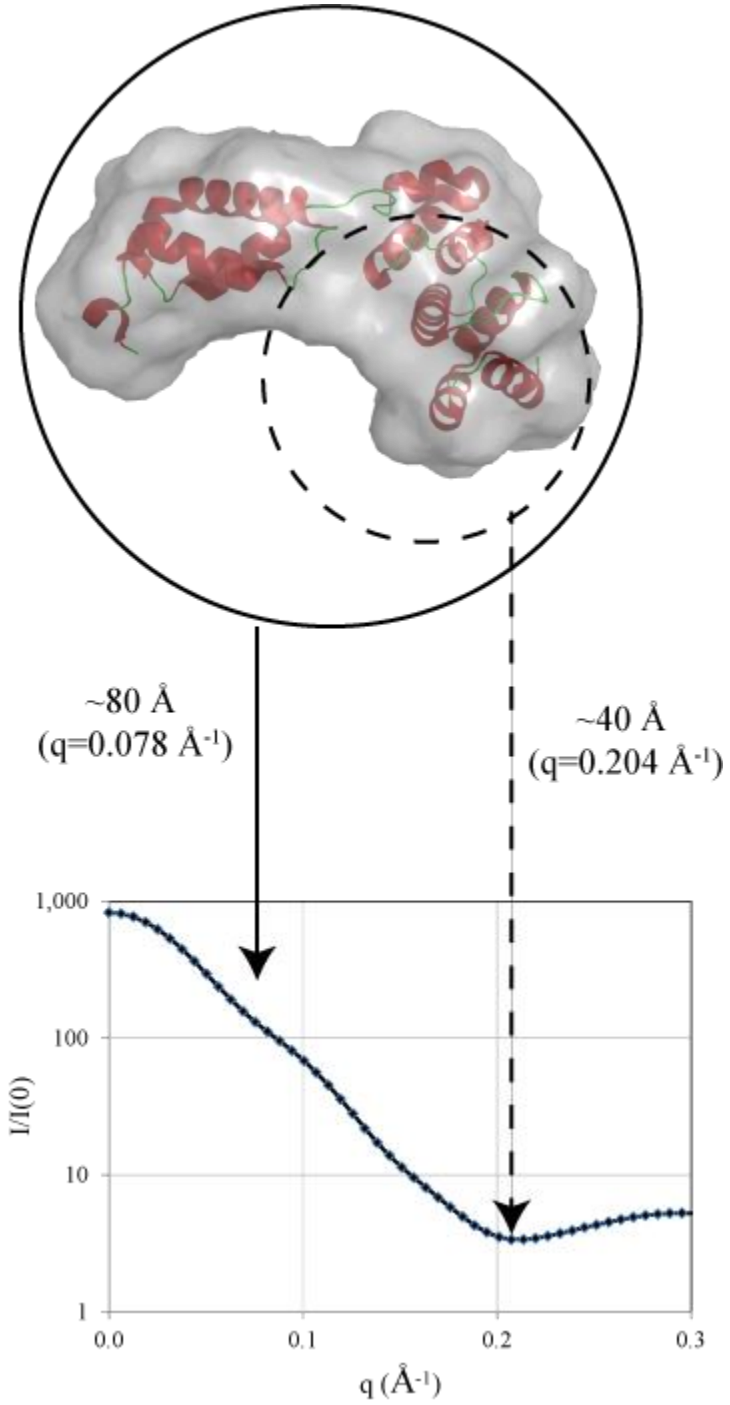
Theory: Scattering Curve

Scattering Data



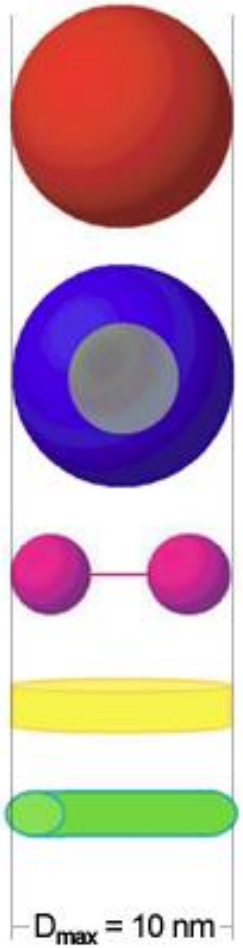
From: Small-angle scattering studies of biological macromolecules in solution, Svergun and Koch, Rep. Prog. Phys., 1735-1782 (2003)

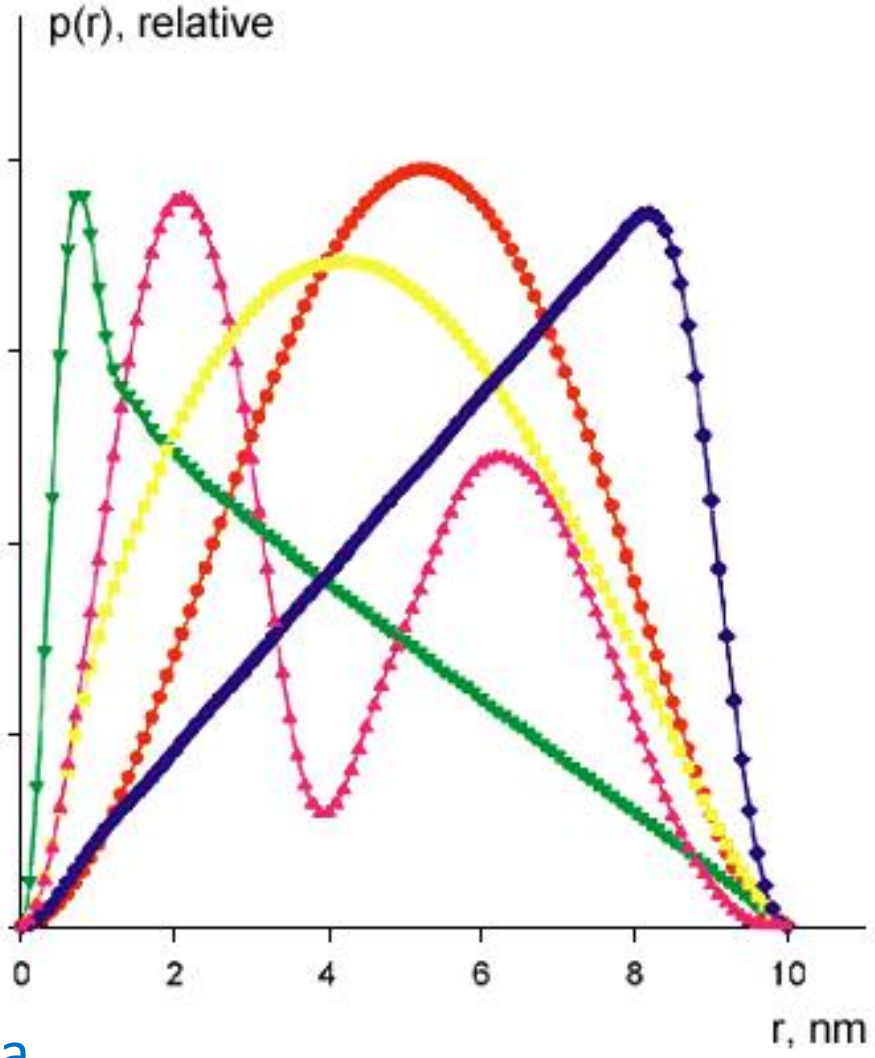

(ii) Radius of Gyration
(iii) Shape of Particle
(iv) Interface
(i)
Interparticle interference
Log(I)
q^-4 (sphere)
q^-2 (disk)
Gunier region
Porod region
q^-1 (thin rod)
q^-4
0
1/r or 1/I
1/D_max
1/ε
q


~80 Å
(q=0.078 Å-1)
~40 Å
(q=0.204 Å-1)
1,000
100
10
1
I/I(0)
0.0
0.1
0.2
0.3
q (Å-1)

Theory: Fourier transform of Scattering Curve (Pair distribution function)

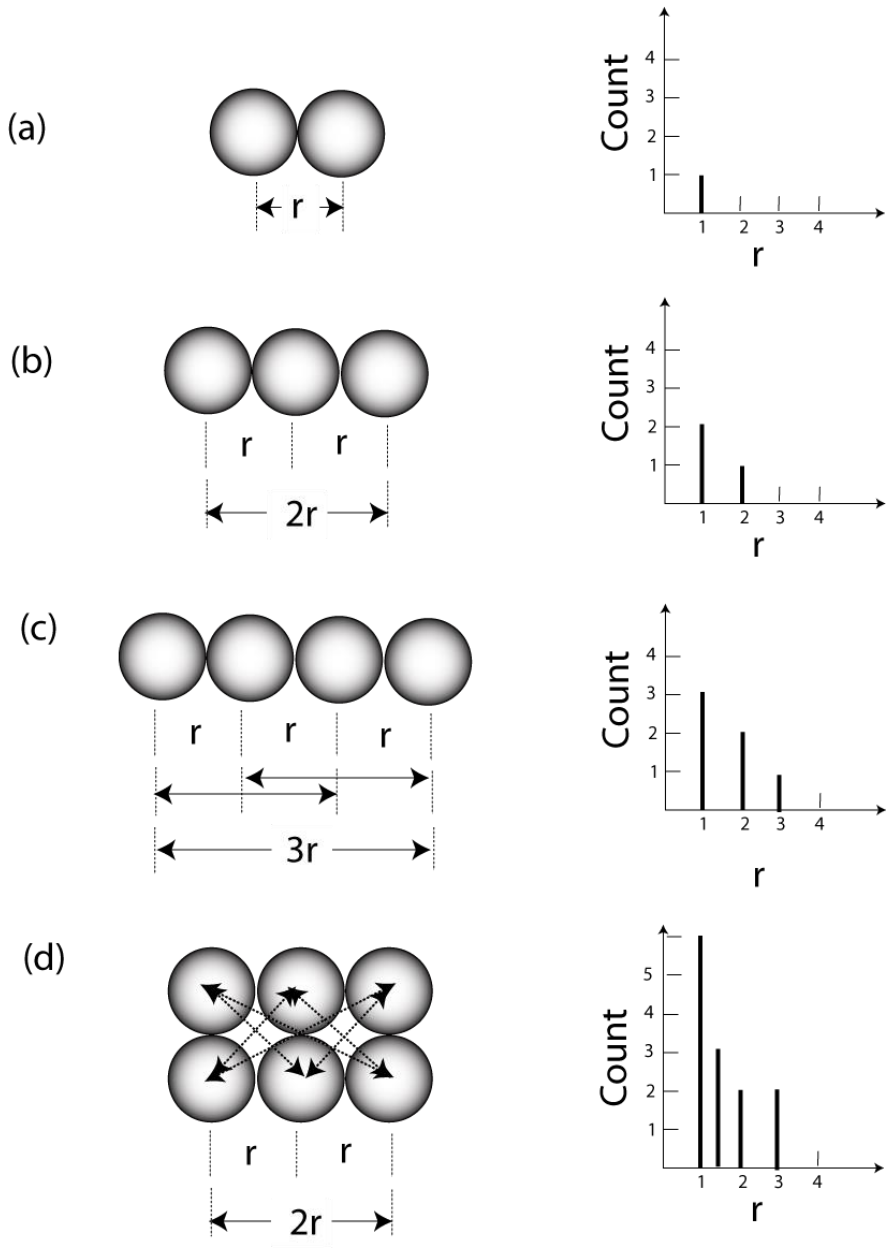
Pair distribution function



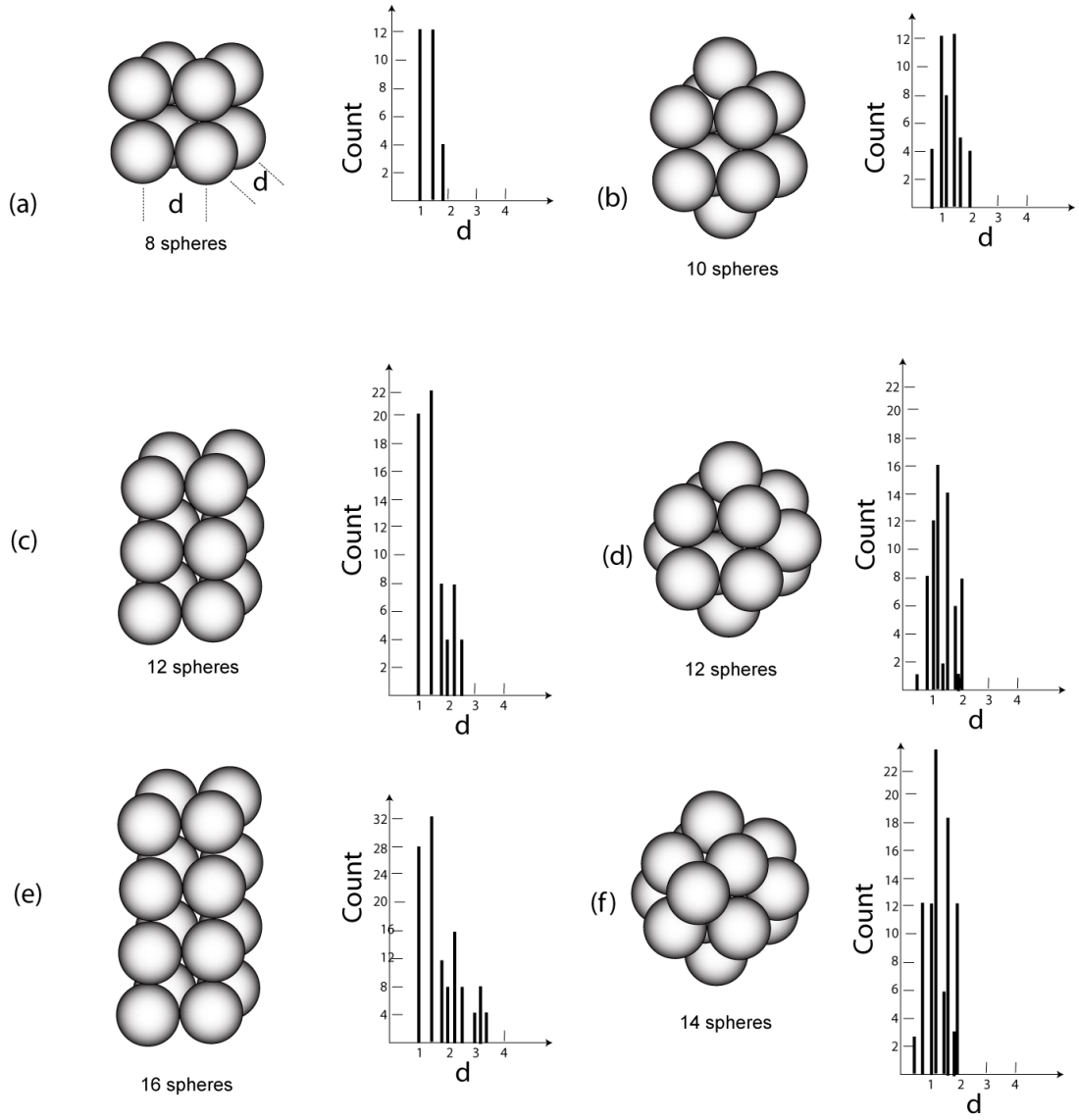


Fourier transform of data.

From: Small-angle scattering studies of biological macromolecules in solution, Svergun and Koch, Rep. Prog. Phys., 1735-1782 (2003)

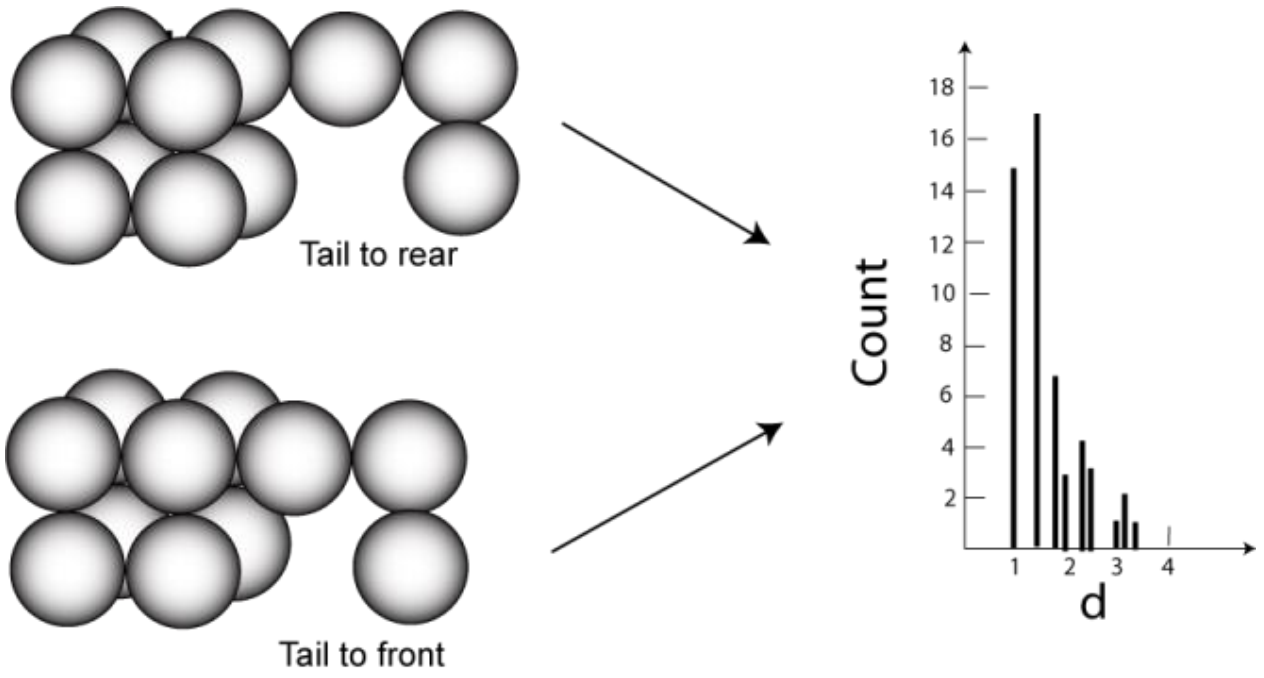


The pair distribution function, P(r), can be thought of as a summation of the interatomic scattering vectors



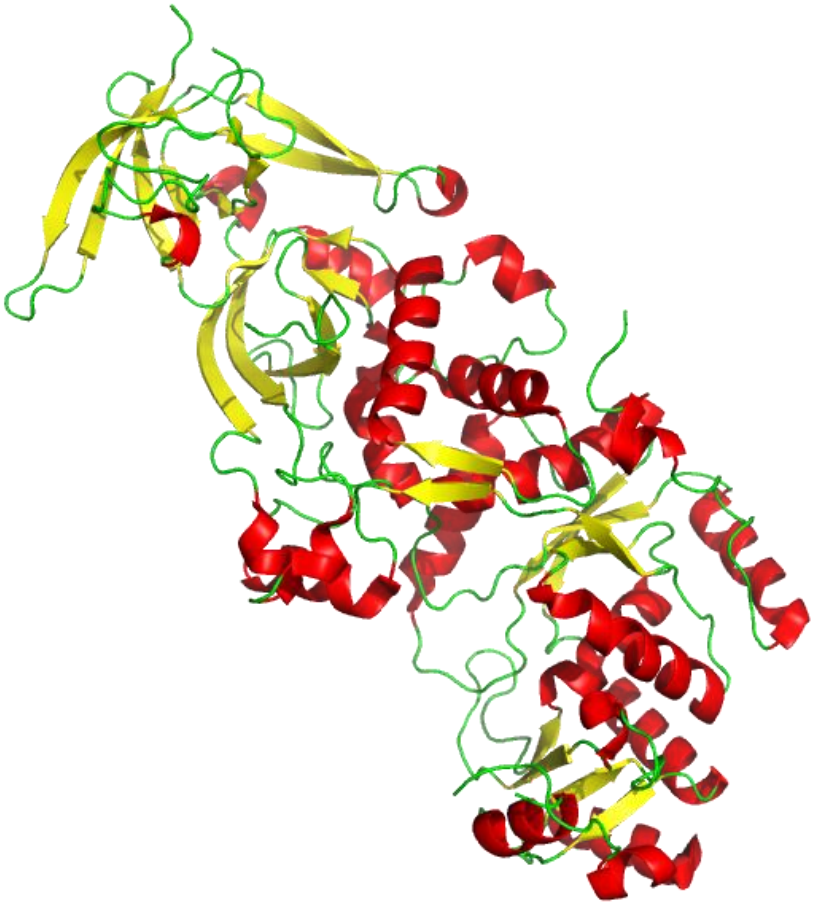
Longer samples will have a wider distribution of scattering vectors while more compact ones will have a narrow distribution but a higher magnitude.

Theory: Data is underdetermined

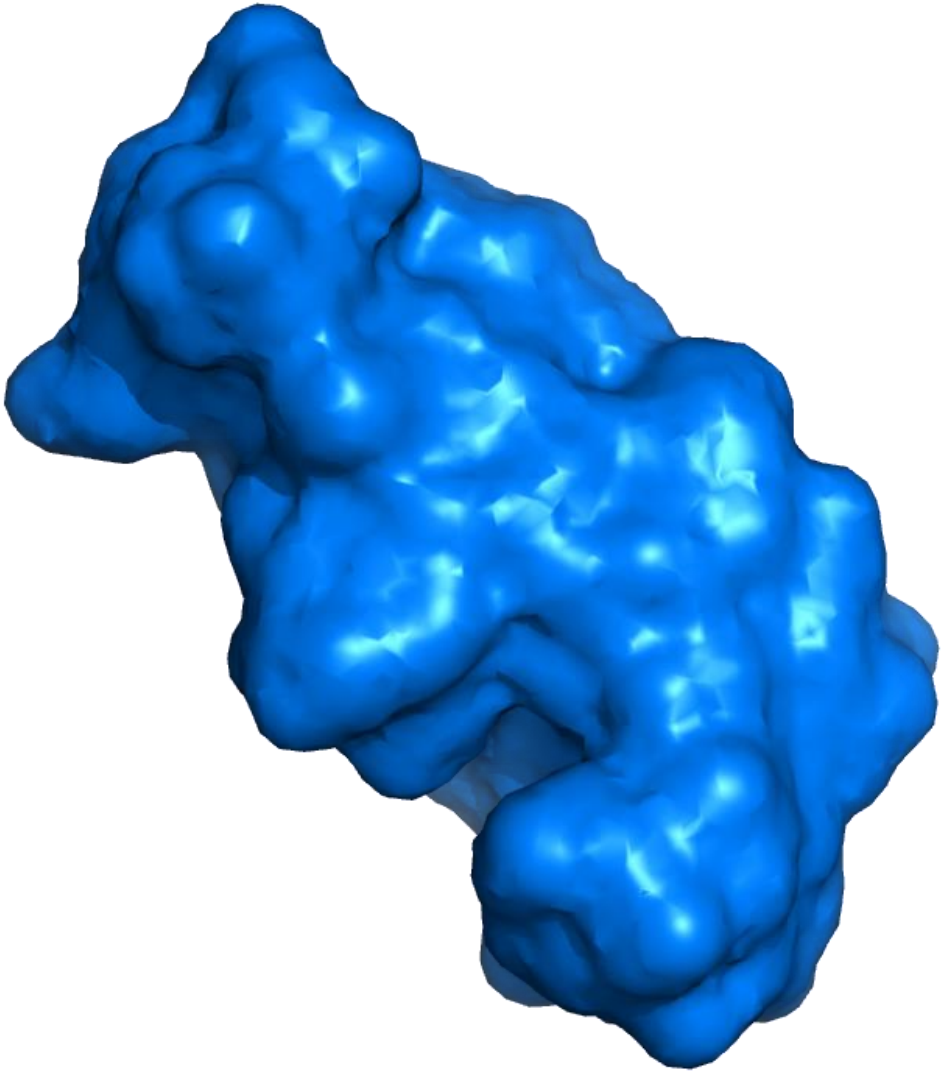

Tail to rear
Tail to front
Count
18
16
14
12
10
8
6
4
2
1
2
3
4
d

Theory: Information Content in SAXS compared to crystallography

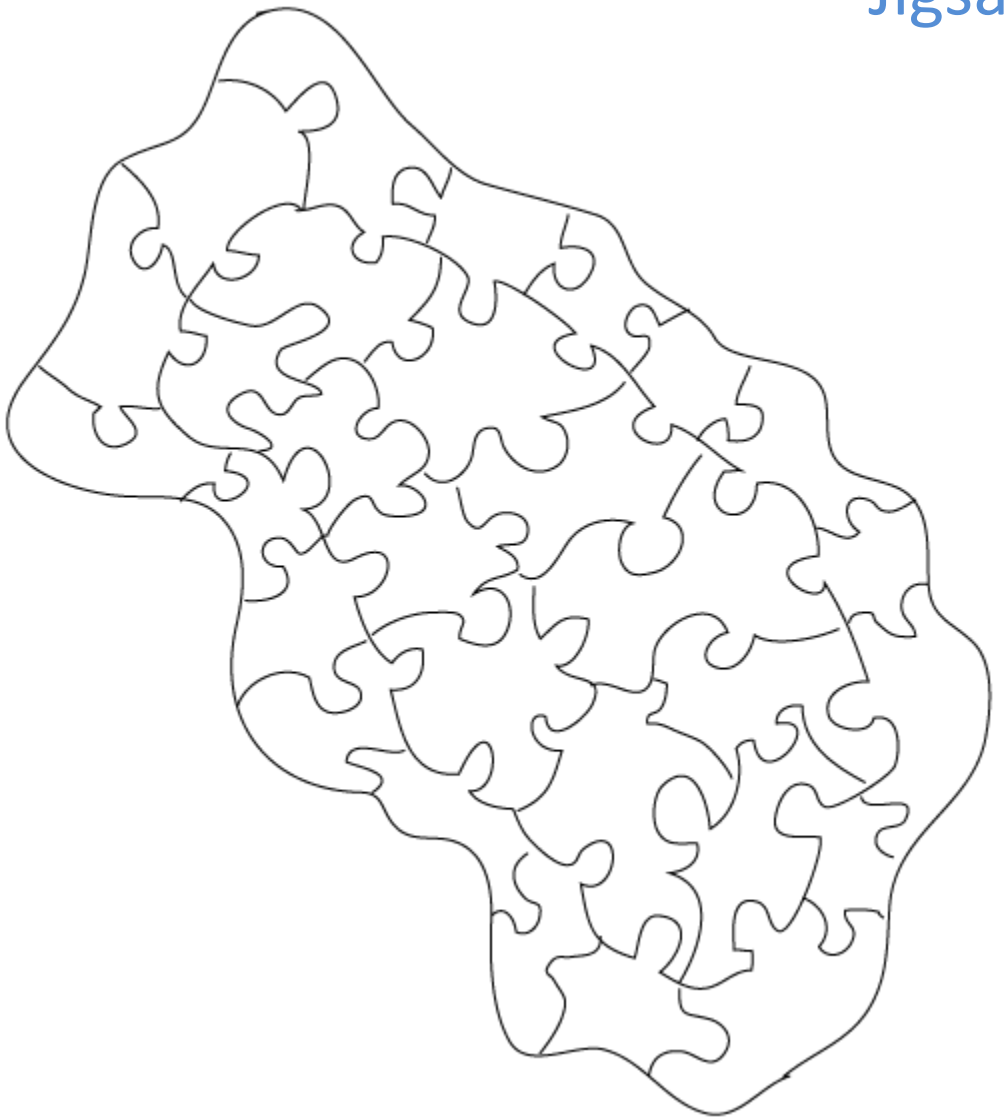
Crystallography



Envelope

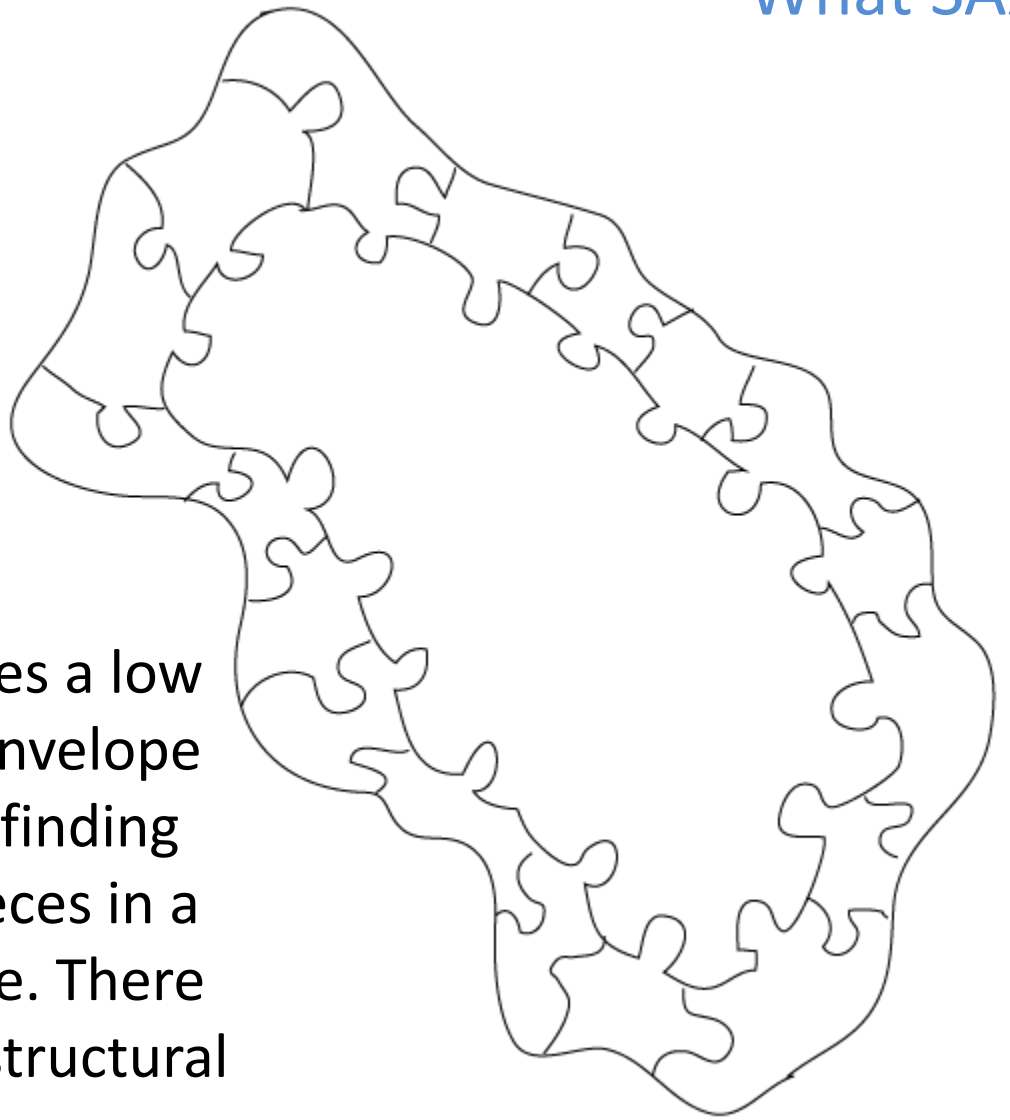


Jigsaw analogy



Switch to 2D for ease of illustration

What SAXS provides



SAXS provides a low resolution envelope … similar to finding the edge pieces in a jigsaw puzzle. There is no other structural information

Low resolution but high quality

SAXS scattering set can be entirely specified by *n* points where:

$$I(q) = \sum_{n=1}^{\infty} I\left(\frac{n\pi}{d_{max}}\right) \cdot \frac{sin(q\ d_{max} - n\ \pi)}{q\ d_{max} - n\ \pi}$$

The number of points is typically on the order of 10 to 20 depending on d_{max} but the scattering curve is continuous. Each point is significantly oversampled resulting in high quality data.

SAXS provides little information content but the quality of the information that is there is high

Basic Assumptions revisited …

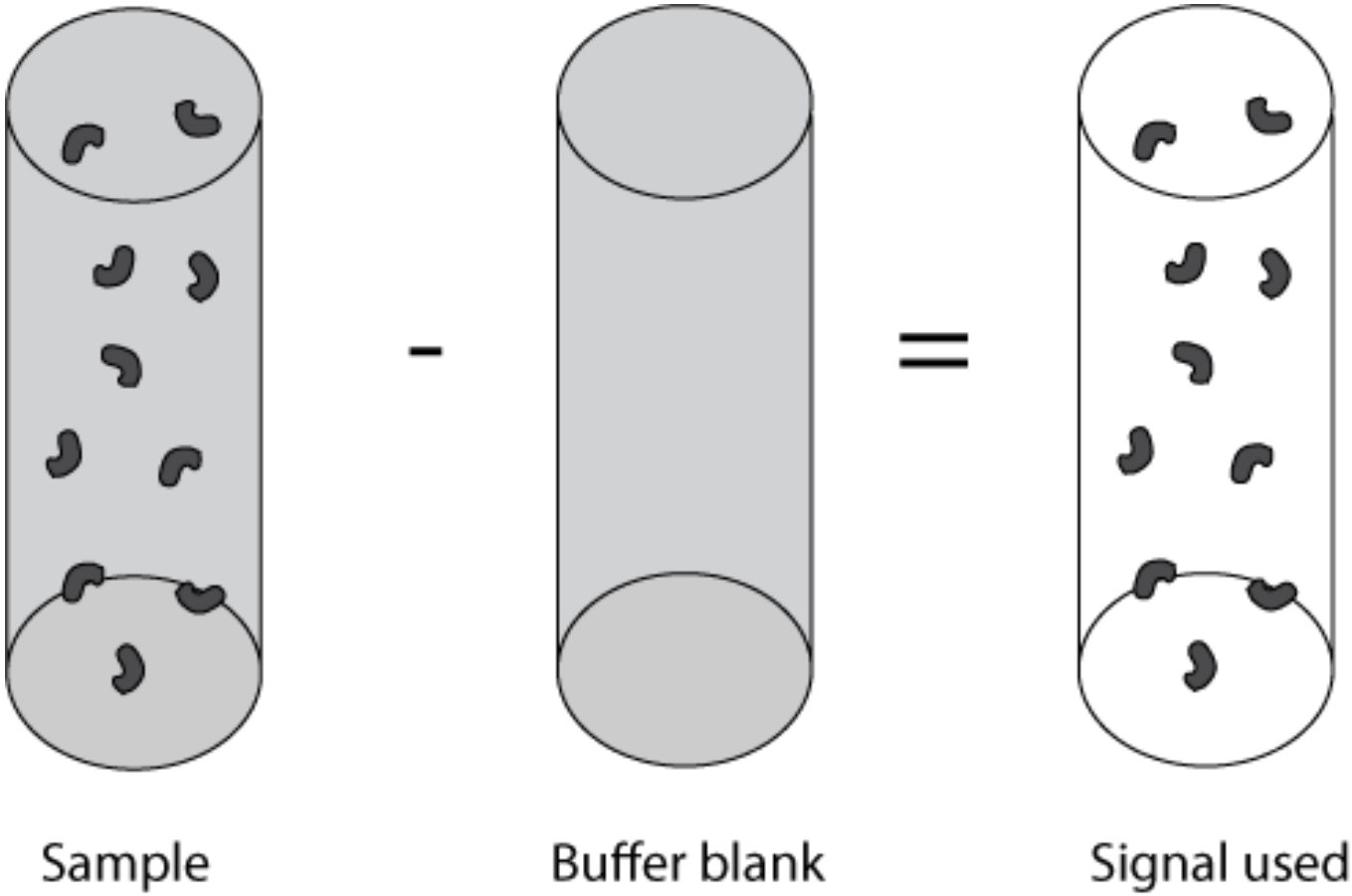
- A single species exists in solution
- The sample minus the buffer equals the buffer
- That there is no inter-particle interaction
- That there is no radiation damage

How do we ensure these?

Does a single species exists in solution?

- Before the experiment
  - Biochemical analysis
  - Dynamic light scattering
  - Native gel
  - Gel filtration (best)
- During the experiment
  - On-line filtration
- Initial data analysis
  - Molecular weight
  - Evidence for concentration dependent effects (oligomer formation)

The basic SAXS experiment …

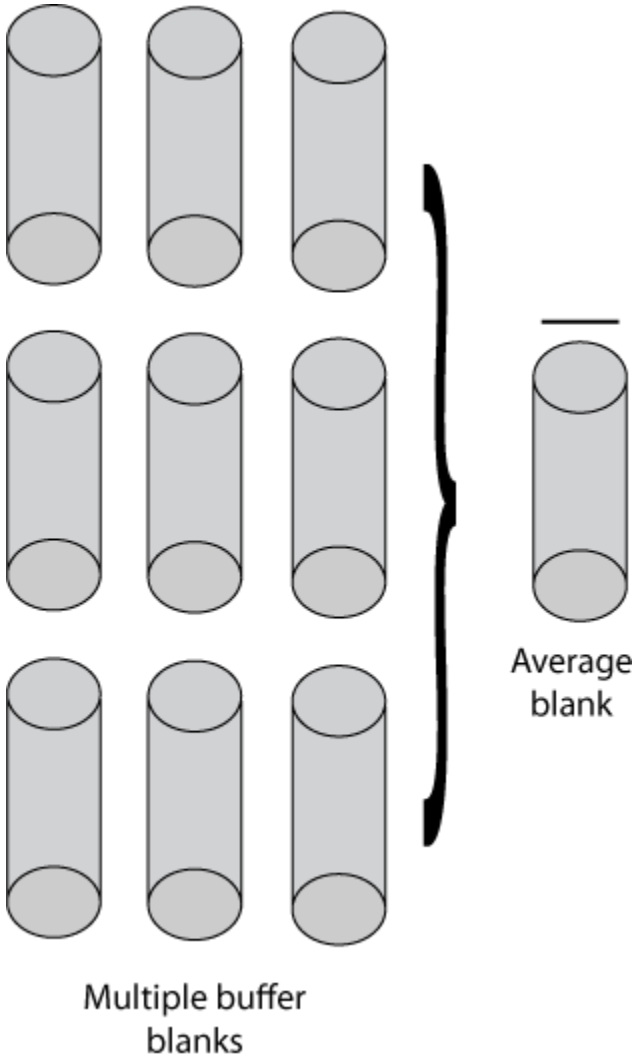


Is the sample minus the buffer just the buffer?

- Before the experiment
  - Use dialysis buffer if possible
  - Avoid generation of ‘artificial buffer’
  - Try several buffers
  - If new, work with the beamline scientist
- During the experiment
  - Monitor the buffer subtraction, does it make sense?
- Initial data analysis
  - Molecular weight
  - Evidence for concentration dependent stoichometry

Is there any inter-particle interaction?

- Before the experiment
  - Use dialysis buffer if possible
  - Avoid generation of ‘artificial buffer’
  - Try several buffers
  - If new, work with the beamline scientist
- During the experiment
  - Monitor the buffer subtraction, does it make sense?
- Initial data analysis
  - Molecular weight
  - Evidence for concentration dependent stoichiometry

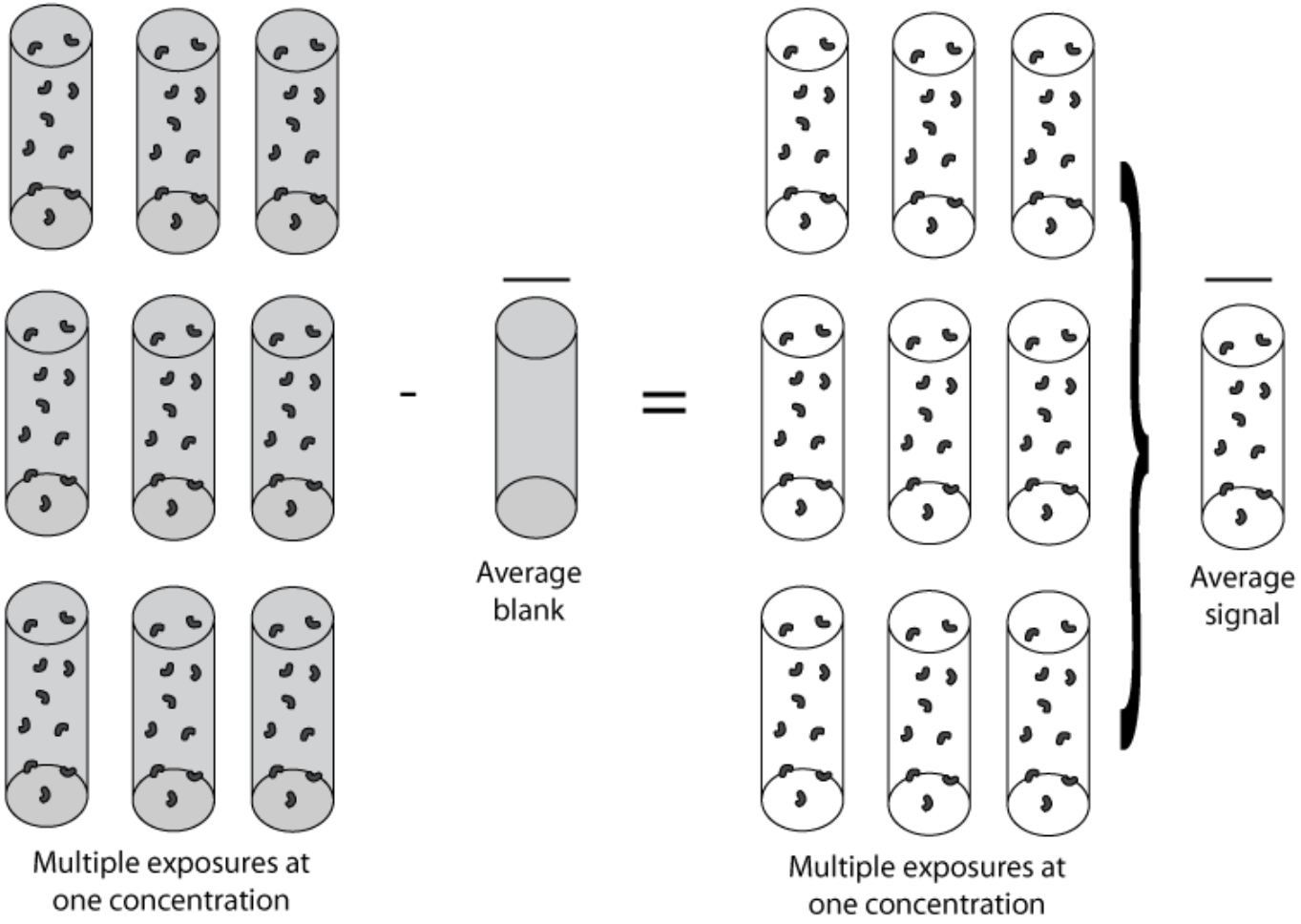


There are multiple ways of doing a SAXS experiment.

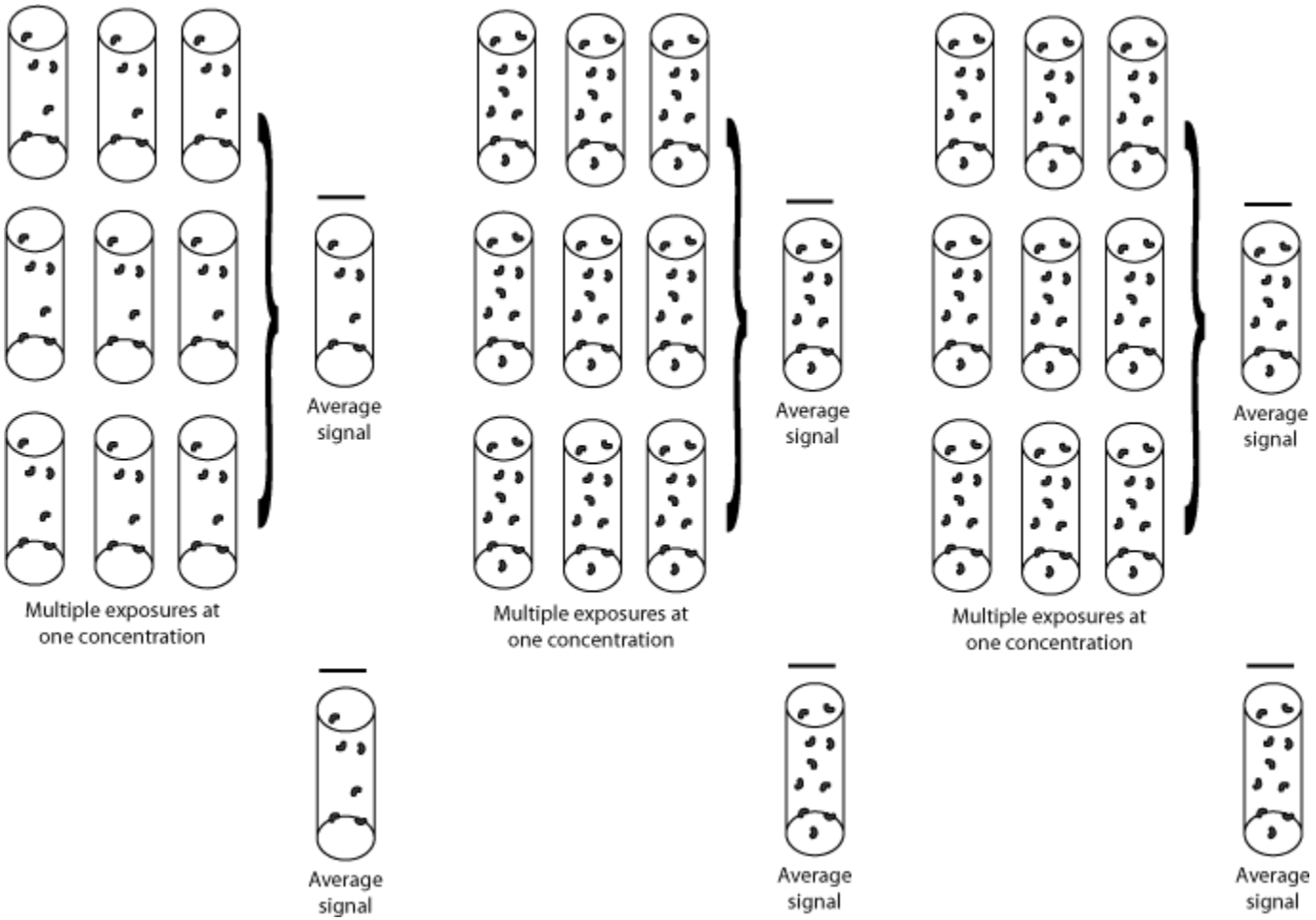
The buffer blank is key to all of them and should be accurately measured.

One approach, used by us, is to measure it multiple times before and after the experiment. The individual scattering profiles are compared, averaged and compared between first and last.

Sample – buffer, multiple times …



Multiple concentrations (at least 3) …

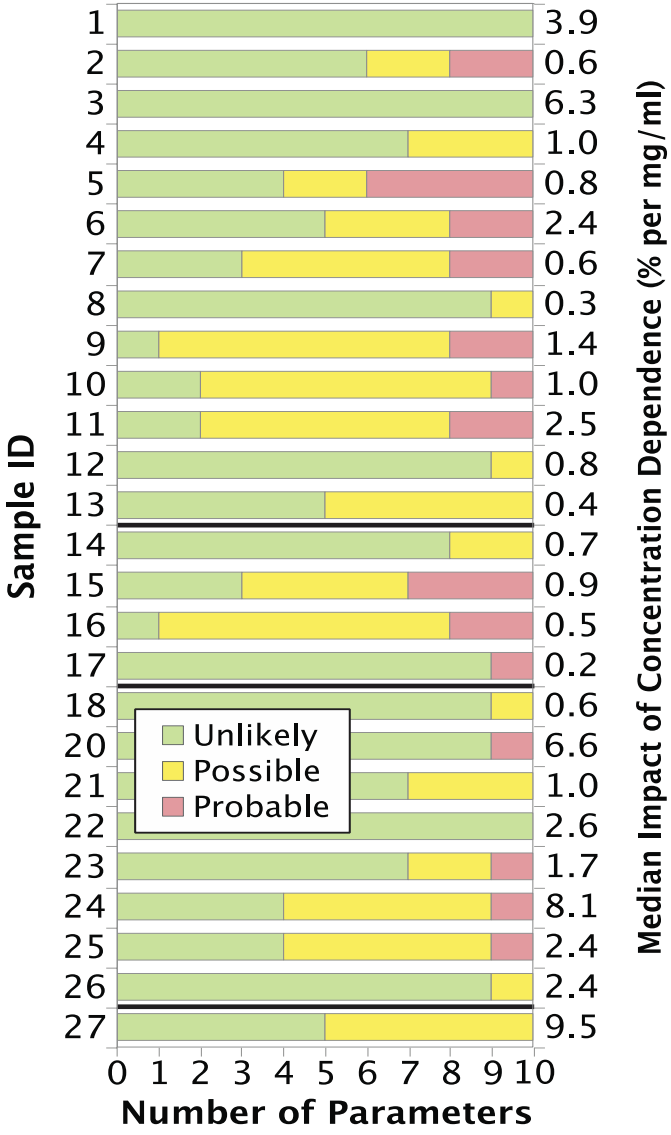


Checking the data

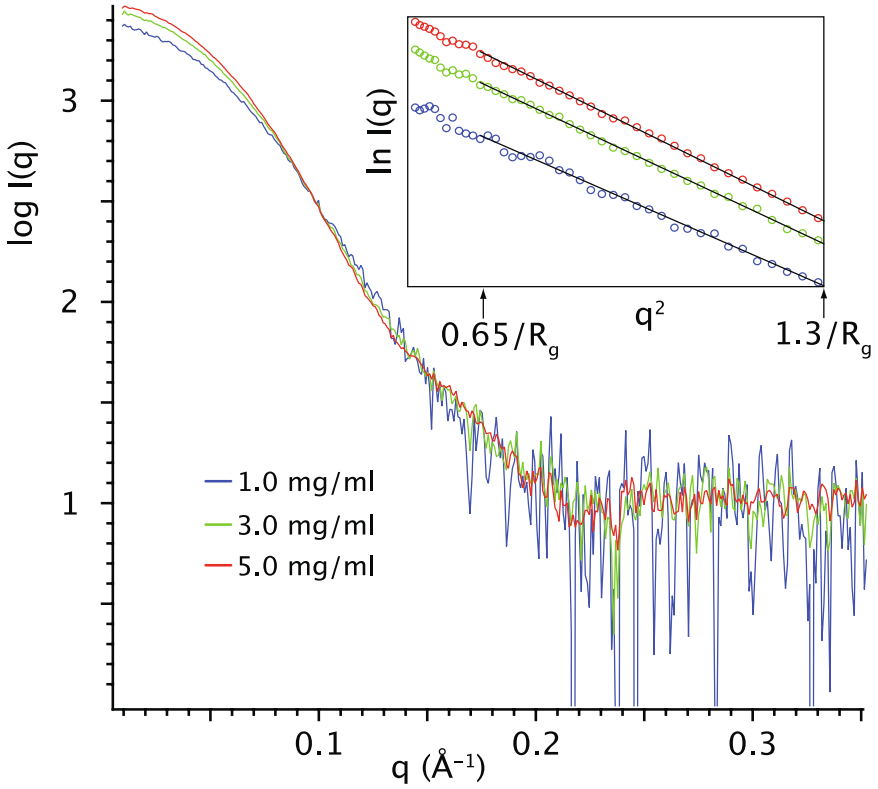
- Radiation damage:
  - Each shot at a single concentration should agree with the first.
- Inter-particle attraction/repulsion
  - The information in SAXS comes from shape, not absolute intensity. Scaled profiles should exactly overlay with no inter-particle effects.

Many, many things not detailed

- Molecular weight determination through I(0)
- Radius of gyration
- Maximum particle dimension measurement (not estimate)
- Globularity/unfoldedness through Kratky plot
- Interparticle effects through Gunier analysis
- Dynamics
- Others



Correlation Frequency Plot for Concentration Dependence Analysis.



Concentration dependence detected for sample 11. Scattering profiles for the lowest (blue), middle (green), and highest (red) concentrations are shown after scaling. The increase in slope and intercept of the data at the low-q region as a function of concentration reflect an increase in the size of the particle.

What question do you want answered?

Defining the question is fundamental to reliable conclusions.

- Types of questions:
  - Characterize the sample:
    - Do I have a globular well folded protein?
    - Is it a monomer or oligomer?
    - Do solution conditions cause gross structural changes?
    - Is there any evidence of dynamics?
  - Make use of the pair distribution or envelope:
    - Invalidate models (it is possible to generate an accurate profile given a structural model). Note, if the model profile fits the data it does not necessarily mean that the model is correct!
    - Place known sub-components of the structure.
    - Identify envelopes of unstructured regions or where a structured region of an unknown structure interacts.
    - etc.

SAXS is a powerful and complementary technique

… but it needs to be used with care

Three talks to follow:

1. Javier Perez - SAXS in the very difficult case of a membrane system with detergents
2. Frank Gabel, IBS - SAXS, SANS and NMR hybrid approaches to complex systems
3. Adam Round, ESRF - Making SAXS easy for you – automated data collection.

Acknowledgements

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Thank you and questions?

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