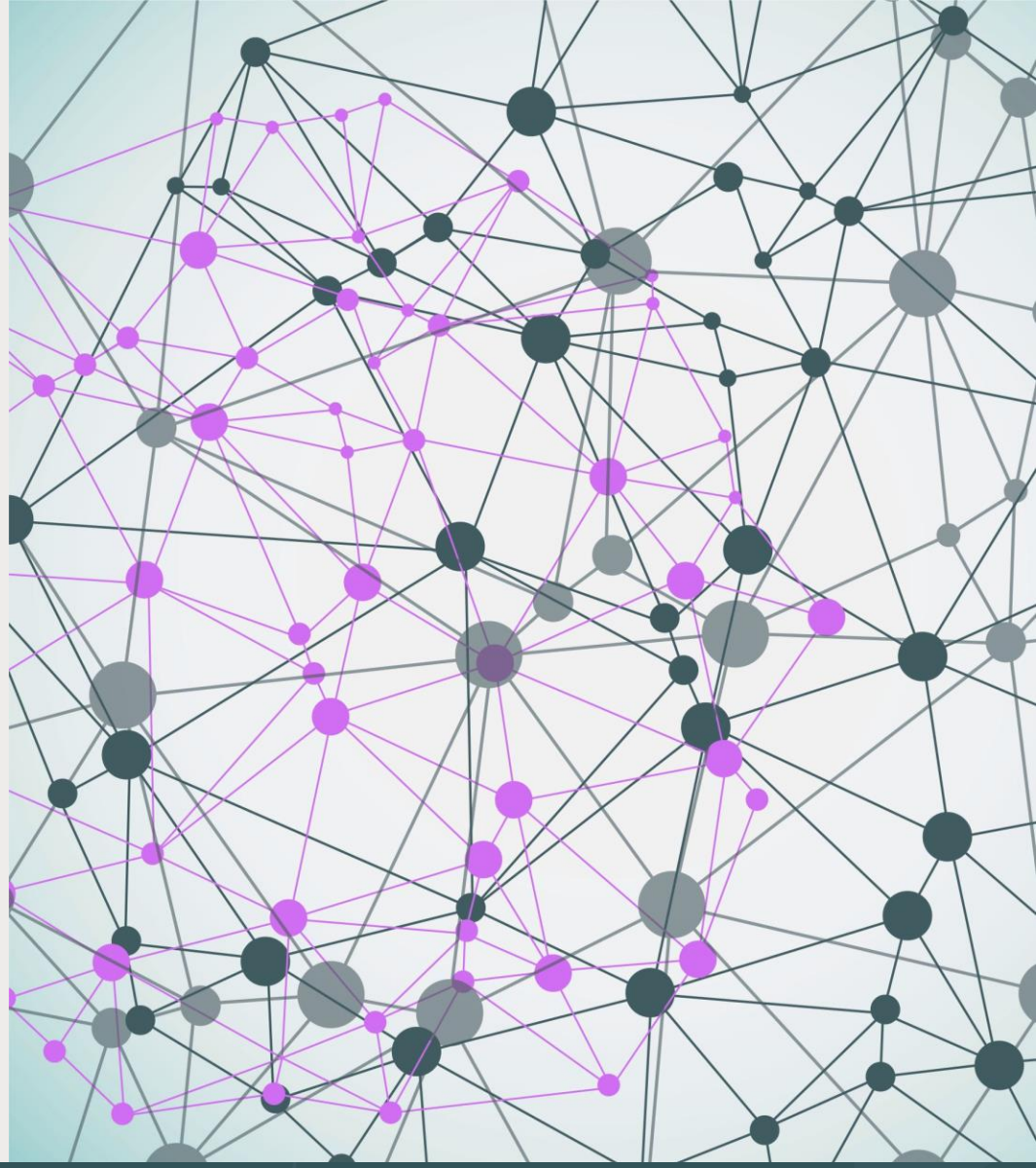
Small-angle scattering for structural biology—Expanding the frontier while avoiding the pitfalls

David A. Jacques & Jill Trewhella



Presented By: Matthew Schwengler

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Overview

Goal

A review intended to, "provide a road map through the small-angle scattering experiment, while also providing a set of guidelines for the critical evaluation of scattering data.

1. Basics	• What is Small Angle Scattering? • Essential Equations • Contrast Matching & Variation
2. Preliminary Checks	• Sample Purity & Concentration • Blanks
3. Data Acquisition	• Effects of Radiation & Concentration • Initial Data Inspection
4. Modeling	• High-Res Validation • Multi-Domain/Subunit Modeling

Basics of SAS – What is Small Angle Scattering?

- A technique meant to provide high-precision information regarding shape and size of a molecule

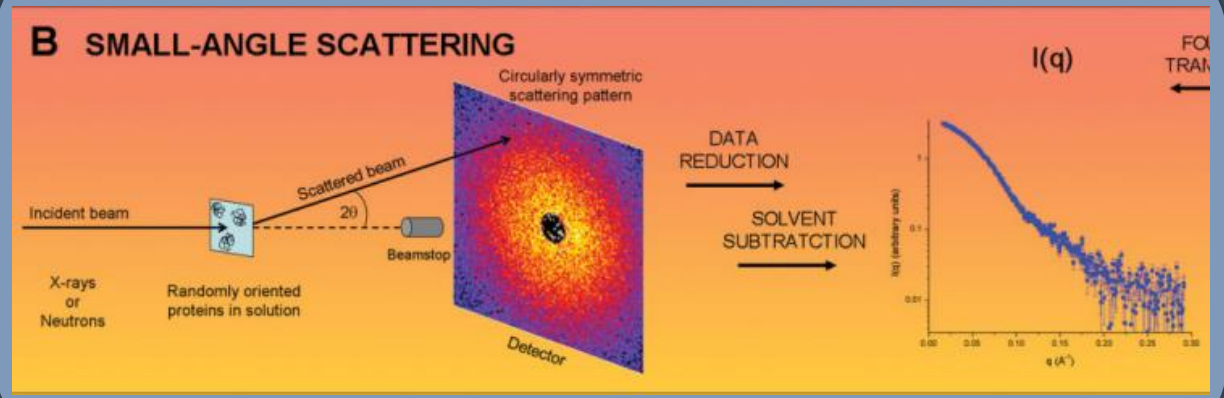
- Collimated beams of x-rays or neutrons illuminate a sample, this light is scattered by the atoms of the sample

- Scattered waves are recorded by a detector

- To make sense of the data, many different forms of editing are employed

X-Ray Sample Amount: >1mg/mL in 5-30µL of solution

Neutron Scattering Sample Amount: >3mg/mL in 150-300µL of solution



Basics of SAS – Essential Equations

Radius of Gyration

$$R_g^2 = \frac{\int P(r) r^2 dr}{2 \int P(r) dr}$$

R_g = Radius of Gyration
P(r) = Interatomic Distance Distribution
r = Frequency of interatomic vector lengths in a protein

Zero-Angle Scattered Intensity Equation

$$I(0) = N(\Delta\rho V)^2 = \frac{C\Delta\rho^2 \nu^2 M}{N_a} = 4\pi \int_0^{D_{max}} P(r) dr$$

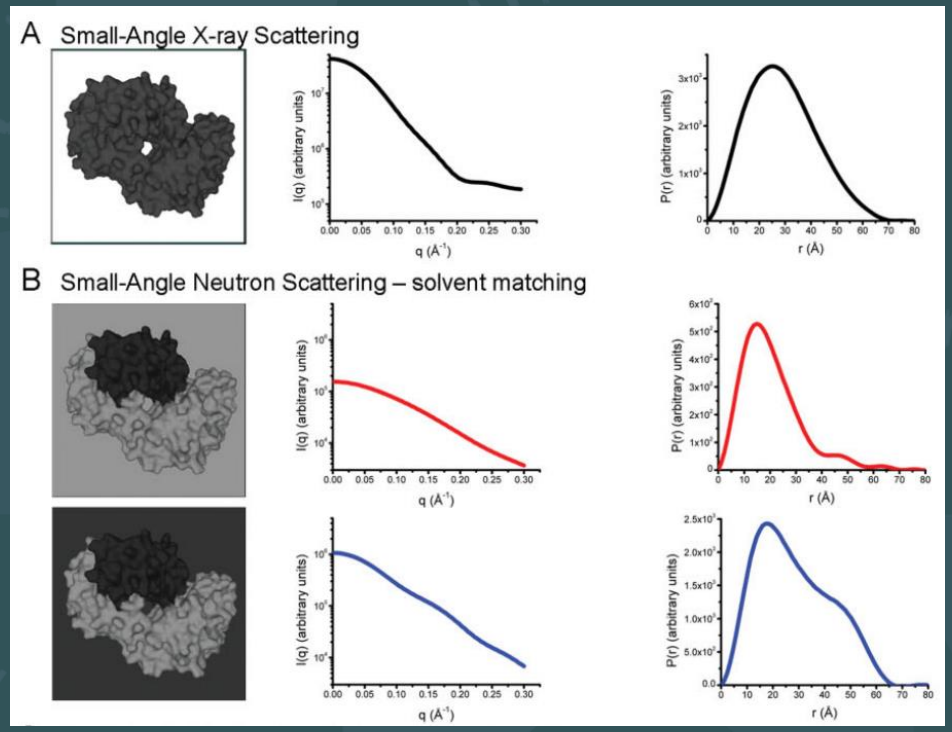
N = Scattering particles/Unit Volume
$\Delta\rho$ = Contrast
V = Particle Volume
C = Mass/Unit Volume
ν = Partial Specific Volume
M = Molecular Weight
N_a = Avagadro's Number
D_{max} = Maximum Linear Distance

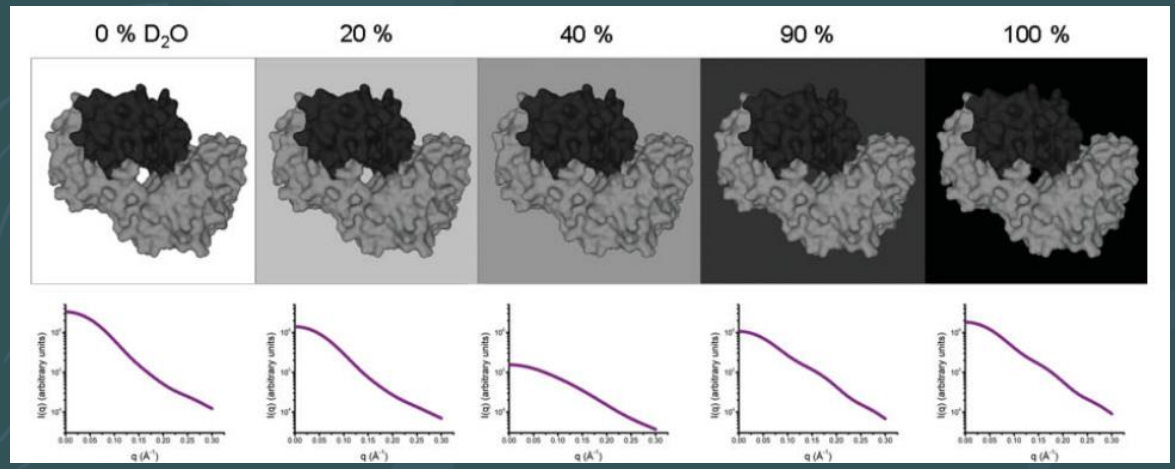
Basics of SAS – Contrast Matching & Variation

- Used primarily in molecular shape studies

- Involves the manipulation of protein and solvent scattering densities

- X-rays vs Neutrons?





Graphs: X-axis = q(A), Y-axis = I(q)

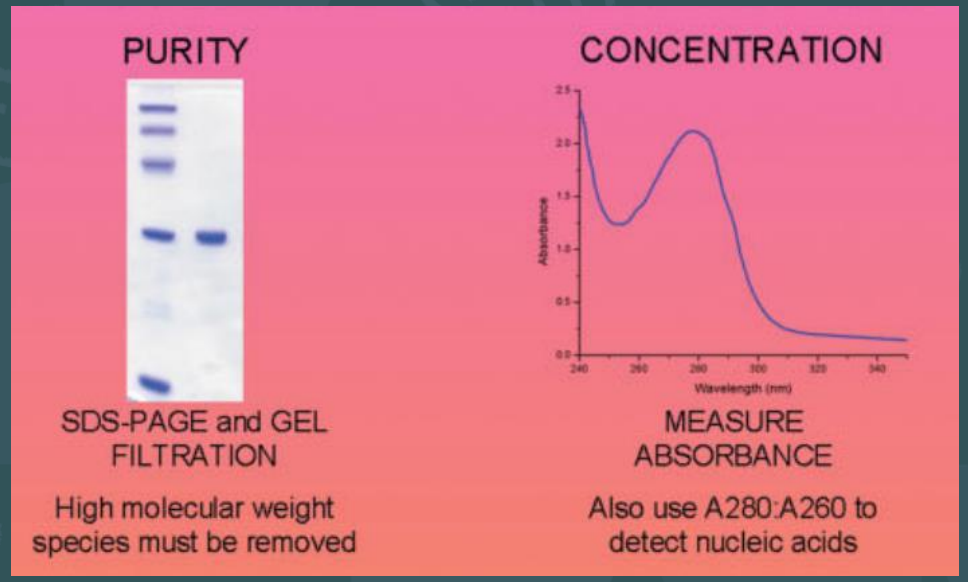
Preliminary Checks

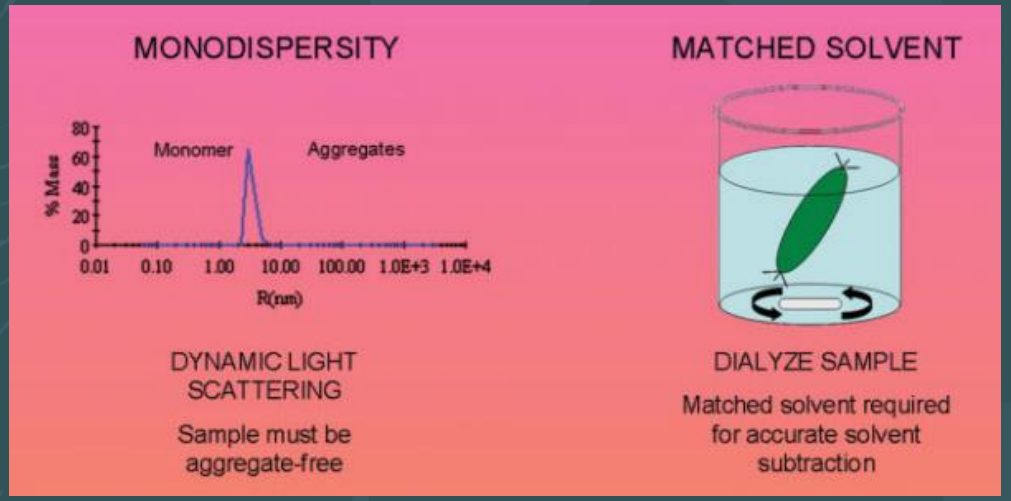
Purity, Monodispersity, & Concentration

- SDS-PAGE
- 280:260nm Absorbance Ratio
- Dynamic Light Scattering
- SEC

Blanks

- Matching solvent blanks are needed to obtain the scattering of the protein
- Dialysis is recommended by the authors to ensure a matched blank





Data Acquisition

Radiation Damage

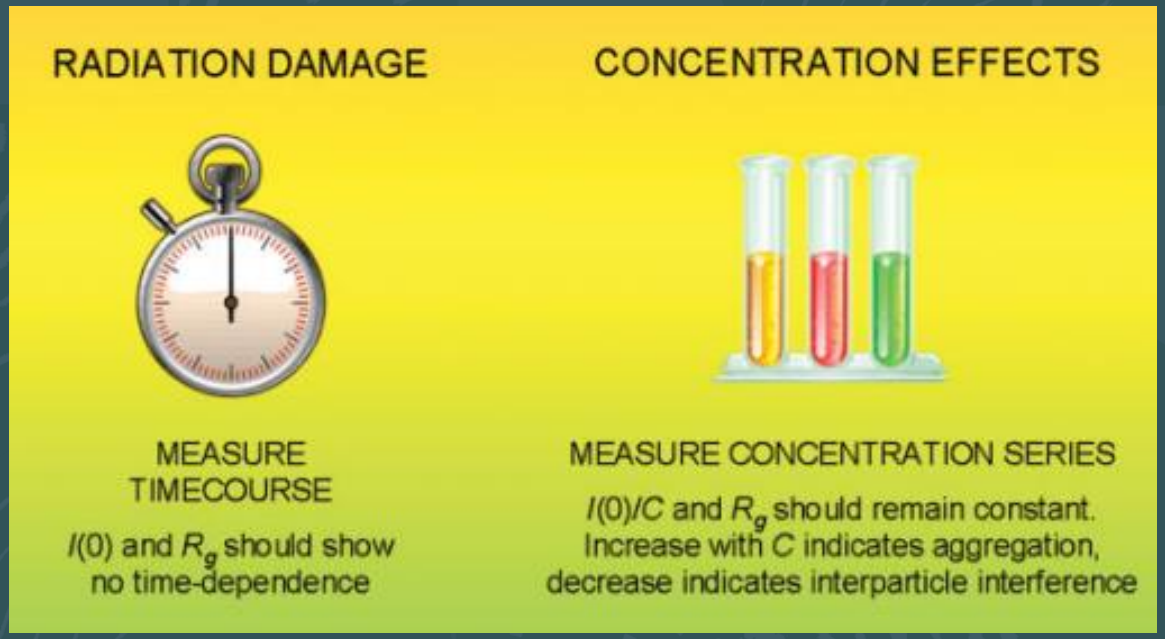
- Bond Breakage & Free Radical Formation
- Radiation Induced Aggregation

Solutions

- Free Radical Absorber in solvent (DTT, Ascorbate)
- Exposure and Result Comparison
- SDS-PAGE

Concentration Effects

- Looking at I(0)/C, C, & Rg can help deduce if there is concentration dependent interference



Data Acquisition

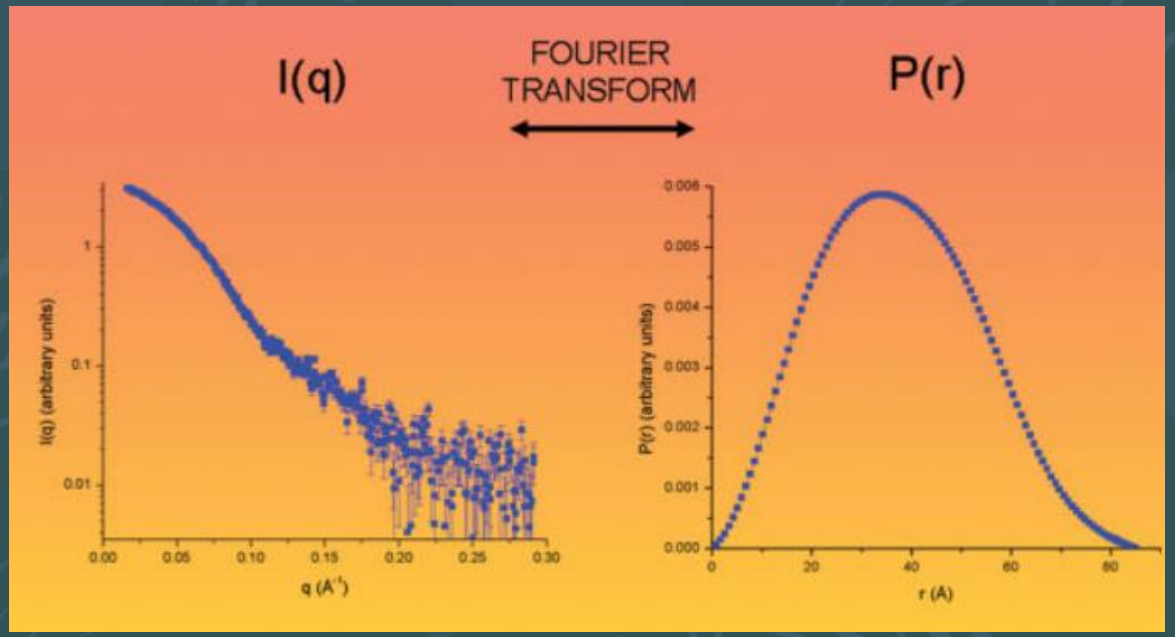
Data Transformation

- Obtained data is in the form of I(q) vs q

- This gives information on the shape, but more is needed

- Fourier Transformation

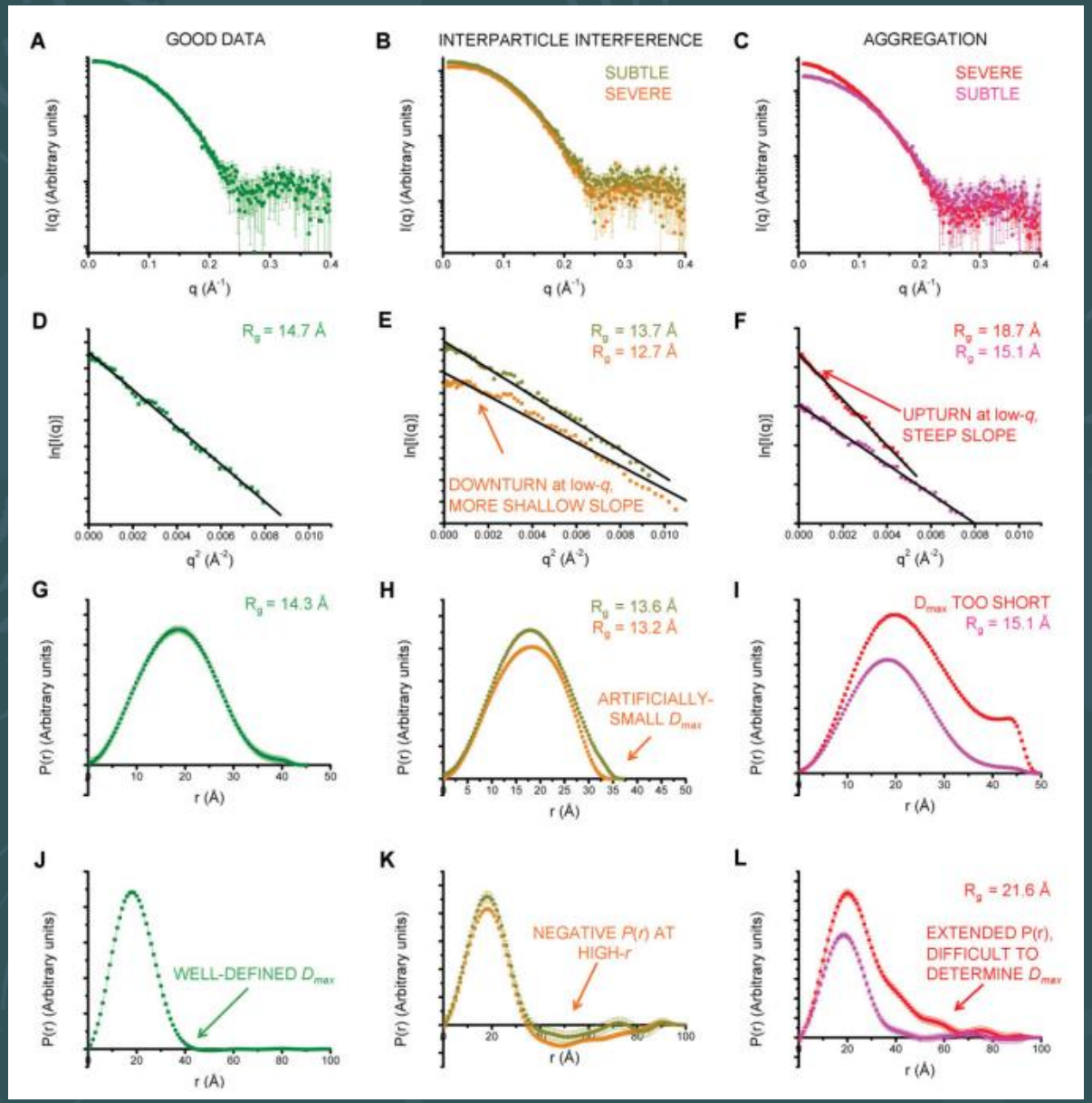
- This gives the function, P(r), the interatomic distance distribution



Data Acquisition

Initial Inspections of Data

- log[I(q)] vs log(q)
- Guinier Plot
- Behaviour of the P(r) function



A-C: log[I(q)] vs log(q) plots
D-F: Guinier Plots
G-L: P(r) Function Plots (J-L have larger x-axis)

Data Acquisition

Standards

- Calibrate I(0) to a scattering standard

- Allows the MW & volume to be obtained from the concentration

Calibration can be done two ways:

1. Comparison with a known particle of similar composition (Protein Standard)
2. An absolute scale using the scattering of water

Recall that:
$I(0) \alpha (\Delta\rho v)^2$

Errors in these values can lead to large MW differences

By rearranging an earlier equation, we get:

$$\mathrm{MW} = \frac{I(0) * N_A}{C(\Delta\rho v)^2}$$

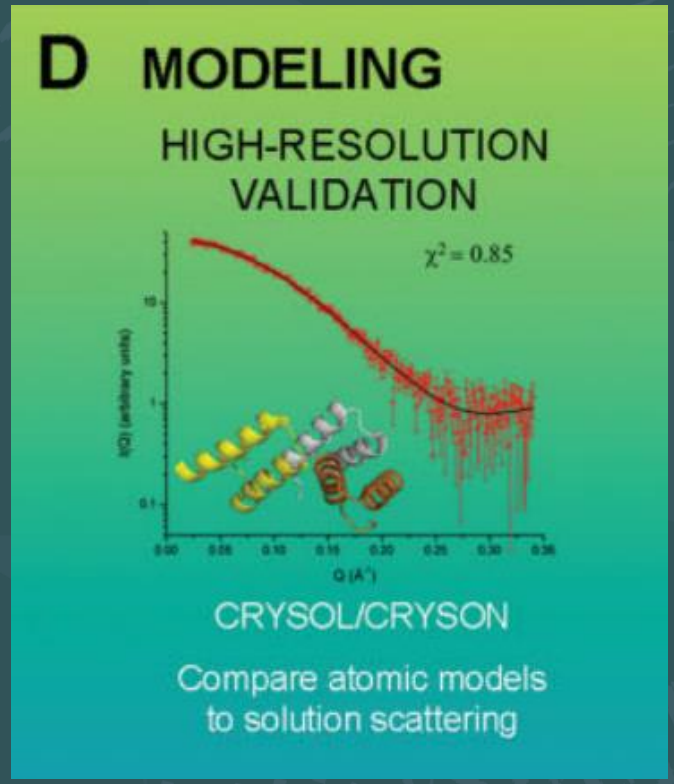
Modeling

High-Resolution Validation

- Theoretical scattering using programs like CRYSOL

- Atomic models can be obtained using x-ray crystallography or NMR, theoretical models using homology modelling

- “scattering data are significantly challenged in proving any model correct, they can unequivocally prove a model wrong or incomplete”



Modeling

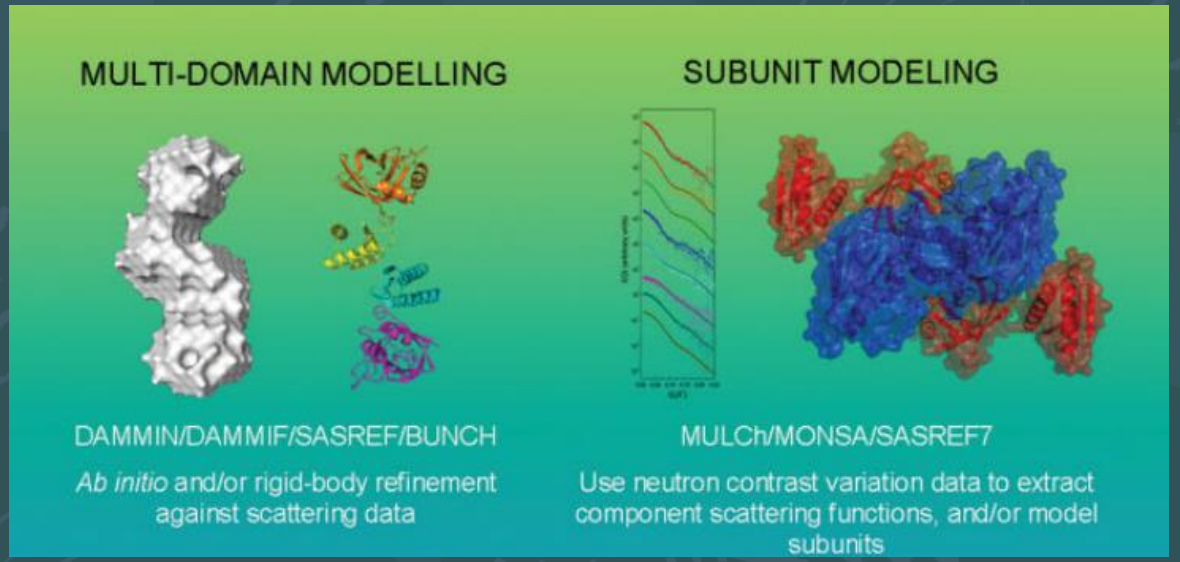
Multi-domain Modeling

- Ability to construct a molecular shape from scattering data alone

- Use *ab initio* algorithms to represent a particle by finite volume elements, employ annealing to fit experimental data

Subunit Modeling

- Used if a conformational change is expected



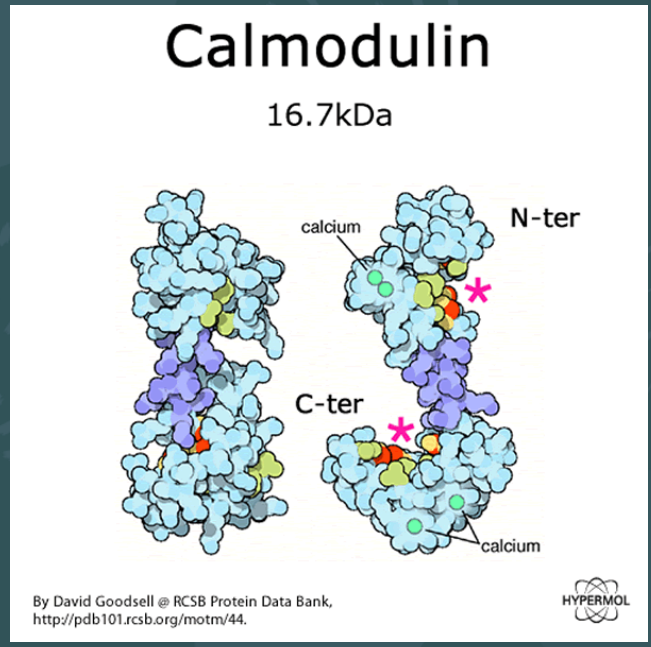
Modeling

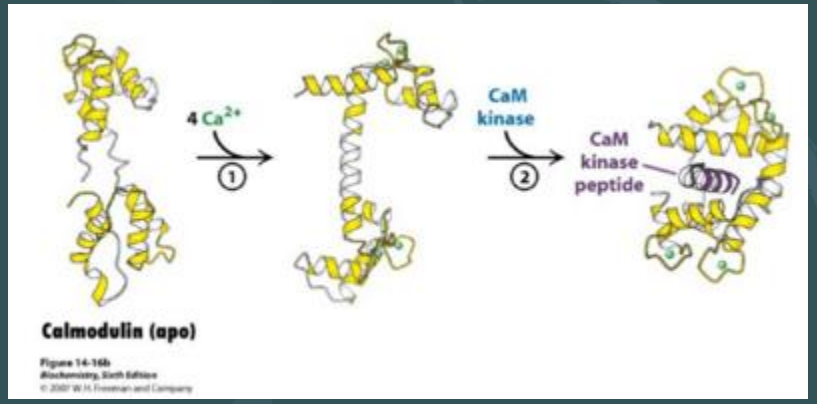
Subunit Modeling Example

- Analyzed the protein, calmodulin

- Found that a dramatic conformational change occurred when it was bound to its target peptide

- Peptide was small enough to not contribute to the scattering profile





Conclusion

SAS experiments must exhibit high standards in quality control and data validation including:

- Detailed reports of data such as the P(r) and Guinier Plots
- Concentration Series
- Normalization of at least one secondary standard

References

- Jacques, D. A., & Trewhella, J. (2010). Small-angle scattering for Structural Biology—expanding the frontier while avoiding the pitfalls. Protein Science, 19(4), 642–657. https://doi.org/10.1002/pro.351

- Hypermol. (n.d.). Calmodulin. https://www.hypermol.com/calmodulin/

- *Calmodulin JMU*. Calmodulin JMU - Proteopedia, life in 3D. (n.d.). https://proteopedia.org/wiki/index.php/Calmodulin_JMU

Questions?