Scattering of Neutrons: Basics

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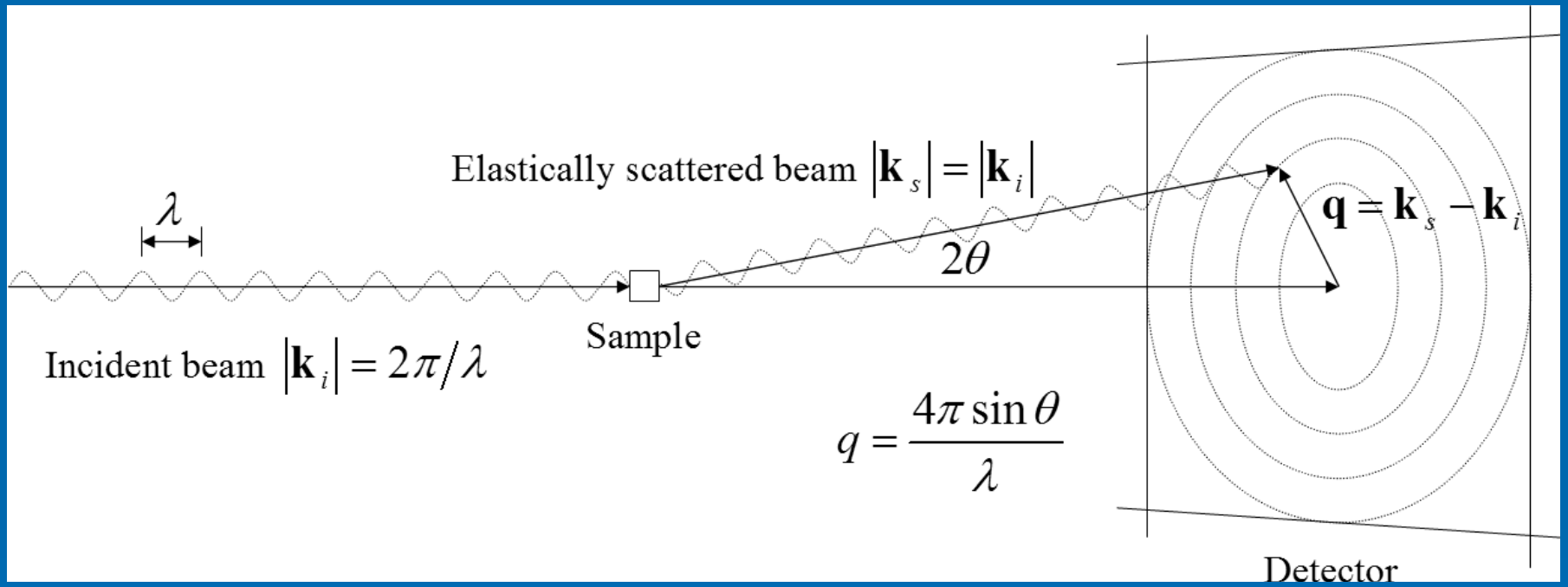
The conceptual experiment and theory is the same for X-rays and neutrons.



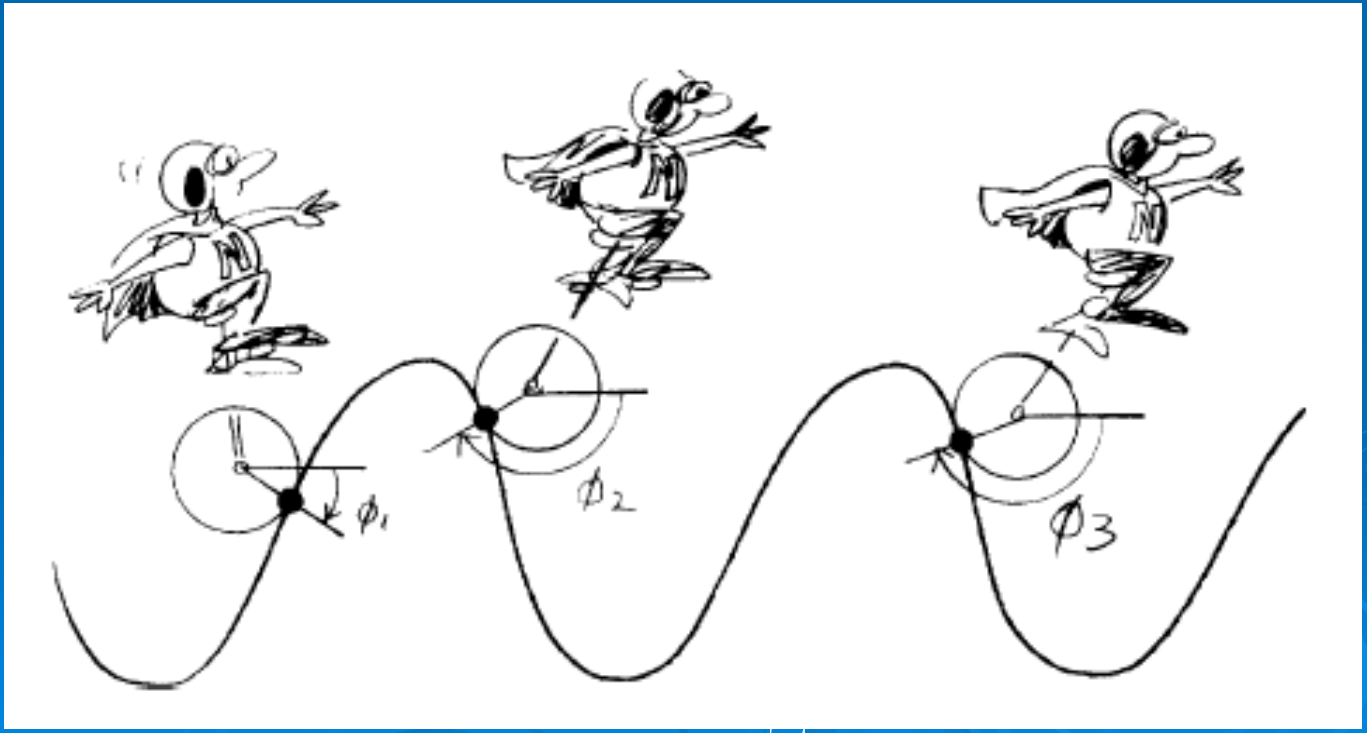
The differences are the physics of the interactions of X-rays (electro-magnetic radiation) versus neutrons (neutral particle) with matter.

Fundamentals

- Neutrons have zero charge and negligible electric dipole and therefore interact with matter via nuclear forces
- Nuclear forces are very short range (a few fermis, where 1 fermi = 10^{-15} m) and the sizes of nuclei are typically 100,000 smaller than the distances between them.
- Neutrons can therefore travel long distances in material without being scattered or absorbed, i.e. they are and highly penetrating (to depths of 0.1-0.01 m for many elements).
- Example: attenuation of low energy neutrons by Al is ~1%/mm compared to >99%/mm for x-rays

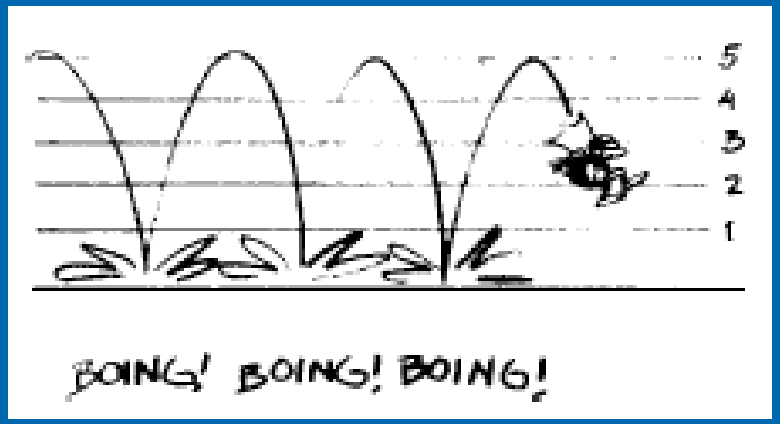
Neutrons are particles that have properties of plane waves

They have amplitude and phase



They can be scattered elastically or inelastically

Elastic scattering changes direction but not the magnitude of the wave vector

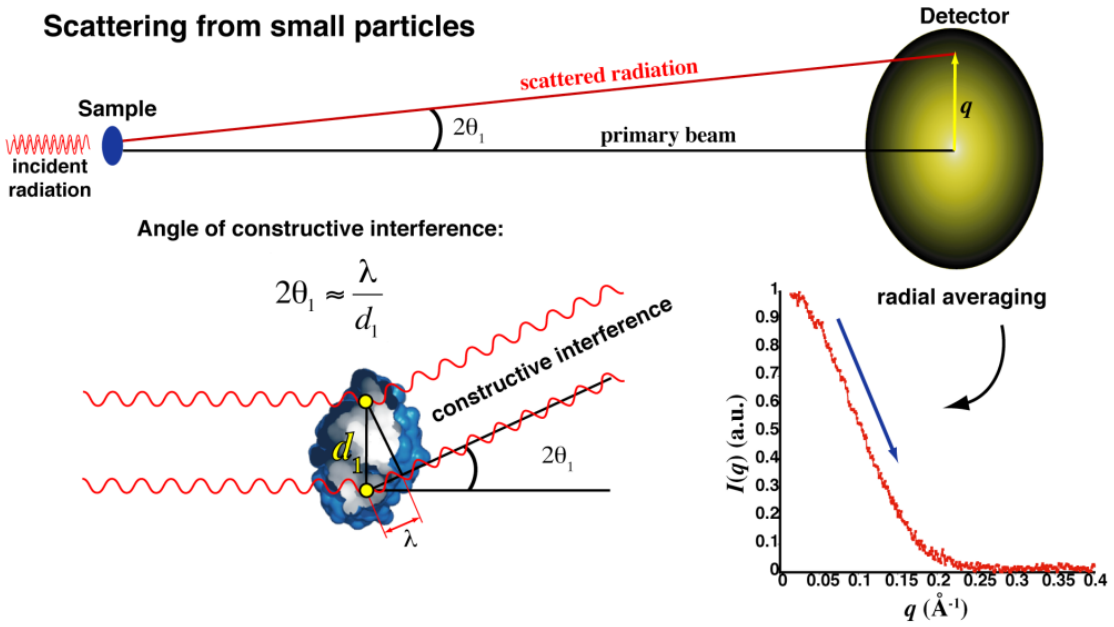


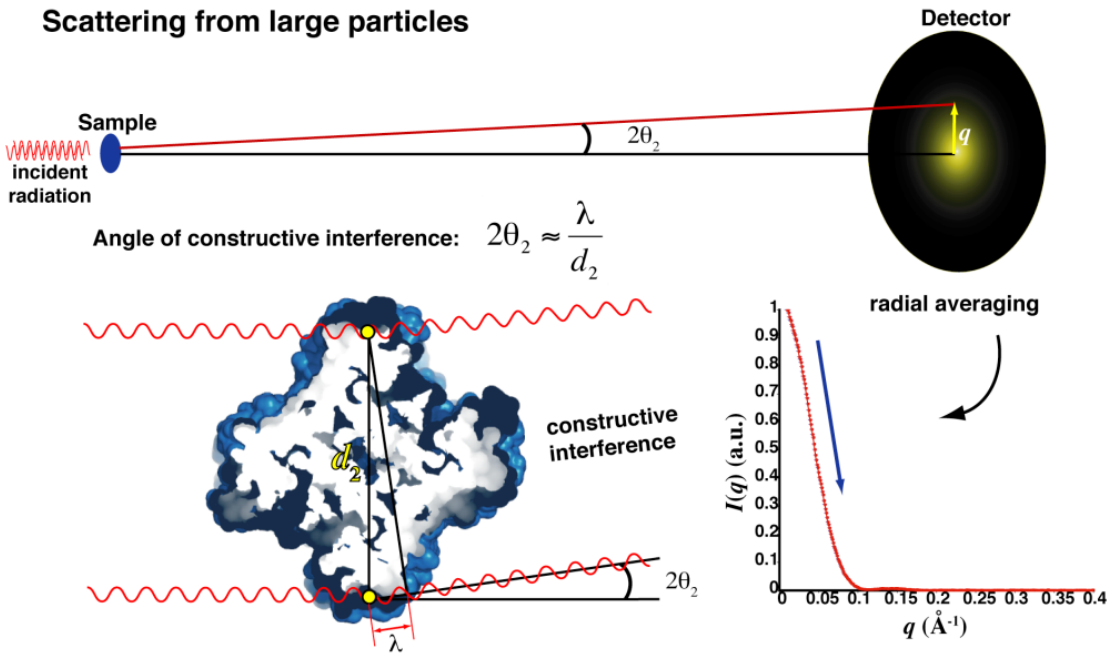
Inelastic scattering changes both direction and magnitude of the neutron wave vector

Coherent scattering is “in phase” and thus can contribute to small-angle scattering. Incoherent scattering is isotropic and in a small-angle scattering experiment and thus contributes to the background signal and degrades signal to noise.

Coherent scattering essentially describes the scattering of a single neutron from all the nuclei in a sample

Incoherent scattering involves correlations between the position of an atom at time 0 and the same atom at time t





Small-angle scattering occurs when a plane wave neutron passes through a particle and interacts with the nuclei in the particle giving rise to scattered wavelets of coherent, elastic scattering that can interfere.

The angle for constructive interference is inversely proportional to the size of the scattering object.

The neutron scattering cross-section is the effective area presented by a nucleus to a neutron

In other words, the cross-section is the probability of a scattering event when a nucleus is exposed to a beam of neutrons.

The neutron scattering cross section is defined as:

$$\sigma = 4\pi b^2$$

and has coherent and incoherent components.

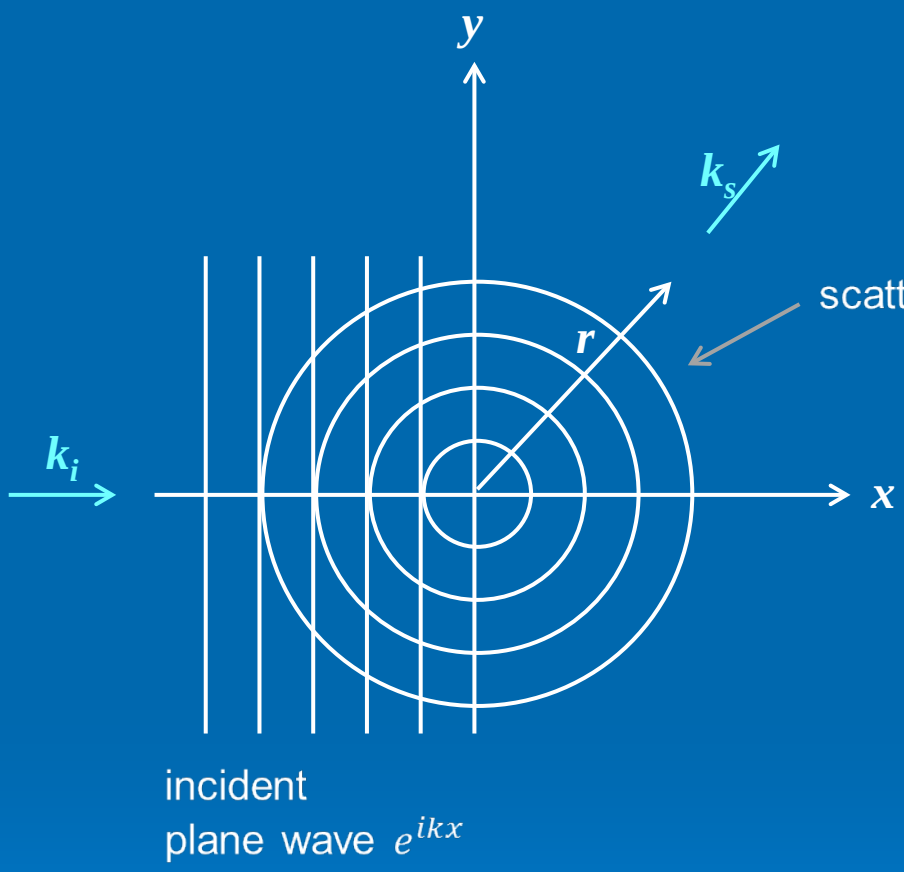
b is referred to as the scattering length; as if *b* were the radius of the nucleus as seen by the neutron.

b depends on the nuclear isotope, spin relative to the neutron & nuclear eigenstate

Among the nuclei commonly found in biomolecules, ^{1}H has the largest $\sigma_{incoherent}$, by a factor of ~40 and is therefore gives rise to a very large background signal

Atom	Nucleus	$\sigma_{coherent}$ (10^{-24} cm)	$\sigma_{incoherent}$ (10^{-24} cm)
Hydrogen	**^{1}H**	**1.8**	**80.2**
Deuterium	**^{2}H**	**5.6**	**2.0**
Carbon	^{12}C	5.6	0.0
Nitrogen	^{14}N	9.4	2.0
Oxygen	^{16}O	4.2	0.0
Phosphorous	^{31}P	5.1	0.2
Sulfur	Mostly ^{32}S	2.8	0.0

Consider the elastic, coherent scattering ($|\boldsymbol{k}_i| = |\boldsymbol{k}_s|$) from a single fixed nucleus with coherent scattering length b at $r = 0$



The scattering signal from an assembly of N atoms is the result of constructive interference of the scattered circular wavelets. The differential cross-section (count rate) per unit solid angle is:

$$\frac{d\sigma}{d\Omega} = \sum_{i,j}^{N} b_i b_j e^{-i\boldsymbol{Q}\cdot(\boldsymbol{r}_i - \boldsymbol{r}_j)}$$

where $\boldsymbol{Q} = \boldsymbol{k}_s - \boldsymbol{k}_i$

Neutron scattering lengths for isotopes of the same element can have very different neutron scattering properties

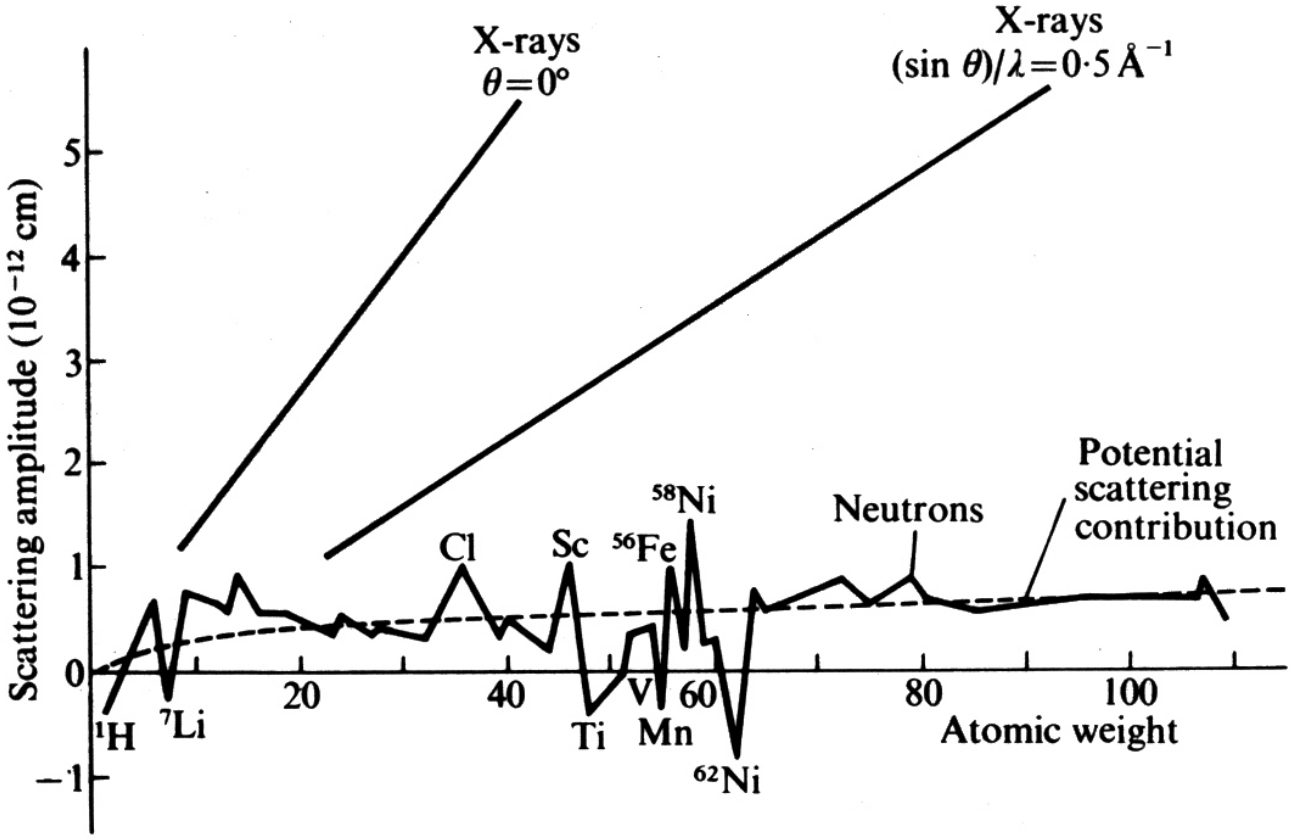


FIG. 22. Irregular variation of neutron scattering amplitude with atomic weight due to superposition of 'resonance scattering' on the slowly increasing 'potential scattering'; for comparison the regular increase for X-rays is shown. (From *Research* (*London*) 7, 257 (1954).)

b values for nuclei typically found in bio-molecules

Atom	Nucleus	(10^{-12} cm)	$f_{x\text{-}ray}$ for $\theta = 0$ in electrons (and in units of 10^{-12} cm)[a]
Hydrogen	**^{1}H**	**-0.3742**	**1.000 (0.28)**
Deuterium	**^{2}H**	**0.6671**	**1.000 (0.28)**
Carbon	^{12}C	0.6651	6.000 (1.69)
Nitrogen	^{14}N	0.940	7.000 (1.97)
Oxygen	^{16}O	0.5804	8.000 (2.25)
Phosphorous	^{31}P	0.517	15.000 (4.23)
Sulfur	Mostly ^{32}S	0.2847	16.000 (4.5)

At very short wavelengths and low Q, the X-ray coherent scattering cross-section of an atom with Z electrons is $4\pi(Zr_0)^2$, where $r_0 = e^2/m_e c^2 = 0.28 \times 10^{-12}$ cm.

For dilute, randomly oriented biomolecules (N biomolecules per unit volume) in solution, the small-angle scattering intensity expressed as an integral is:

$$I(Q) = N\Delta\rho^2 \left\langle \left| \int_V e^{i\vec{Q}\cdot\vec{R}} \right|^2 \right\rangle_\Omega$$

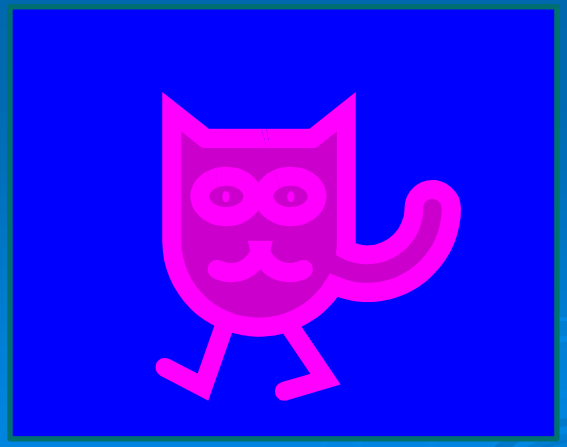
$\vec{R}$ is the vector between scattering centers at positions $\vec{r}_i$ and $\vec{r}_j$. The integration is over all atom pairs in the biomolecular volume V, and the rotational average $\langle \quad \rangle$ is over all orientations.

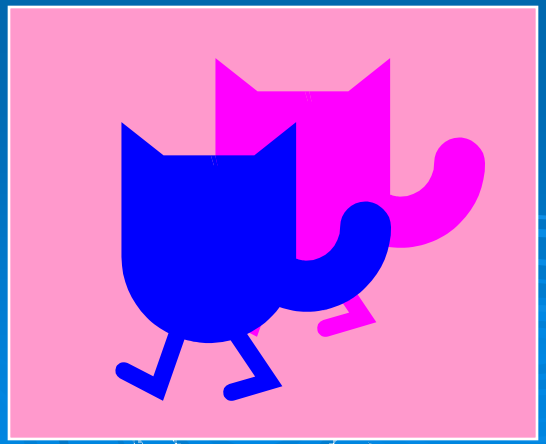
$\Delta\rho$ is the "contrast" = ρ - ρ_s, where ρ and ρ_s are the average scattering length densities/unit volume for the biomolecule and the solvent, respectively. *i.e.* $\sum_i^N \frac{b_i}{V}$

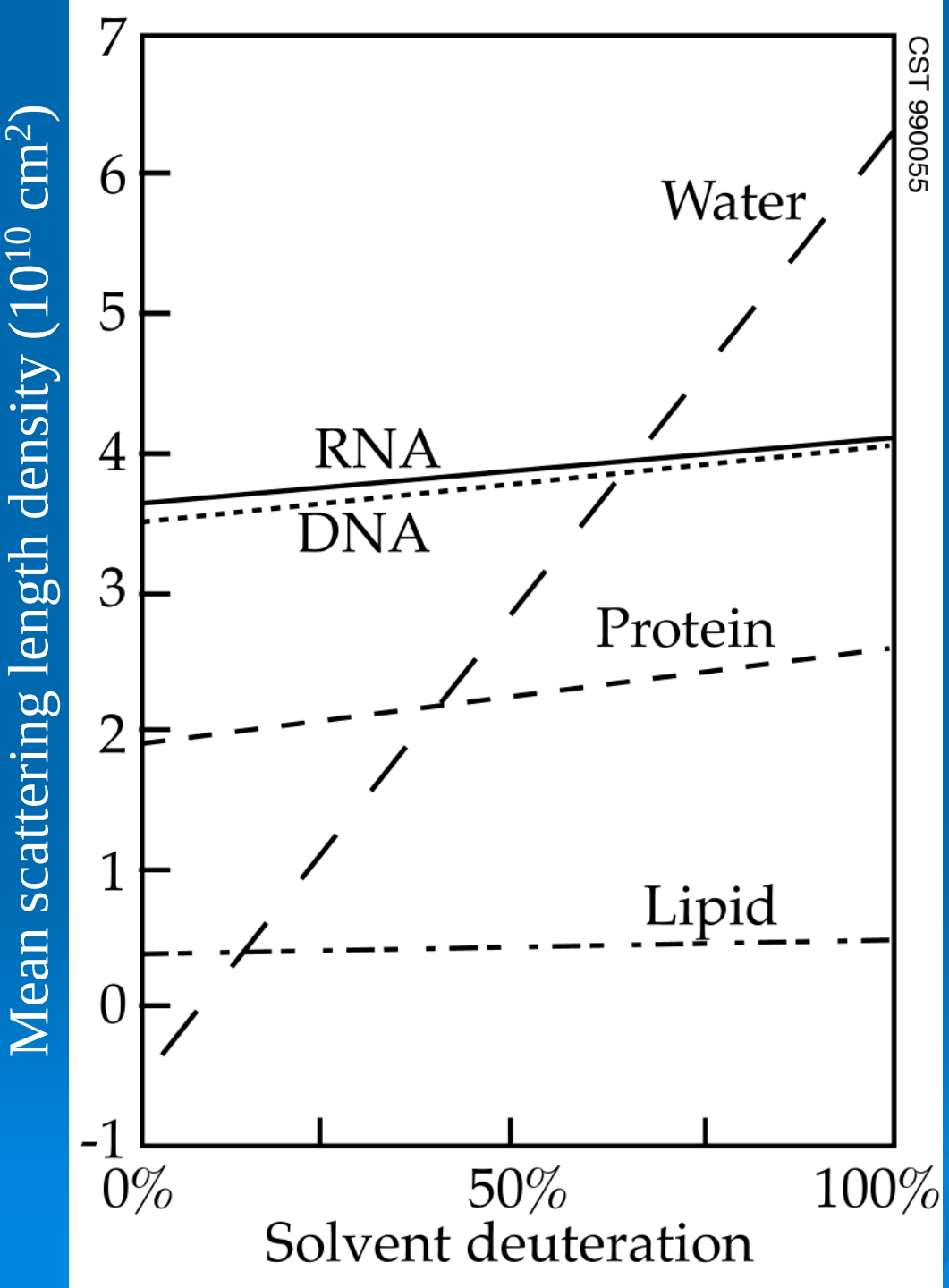
$I(Q)$ is zero if $\rho = \rho_s$

Neutron scattering and contrast variation

- Because the coherent scattering lengths of H (^{1}H) and D (^{2}H) have different signs:
  - By adjusting the H/D ratio in a biomolecule and/or its solvent one can systematically vary ρ and hence contrast.
  - One can obtain information on the internal density fluctuations of a scattering particle.

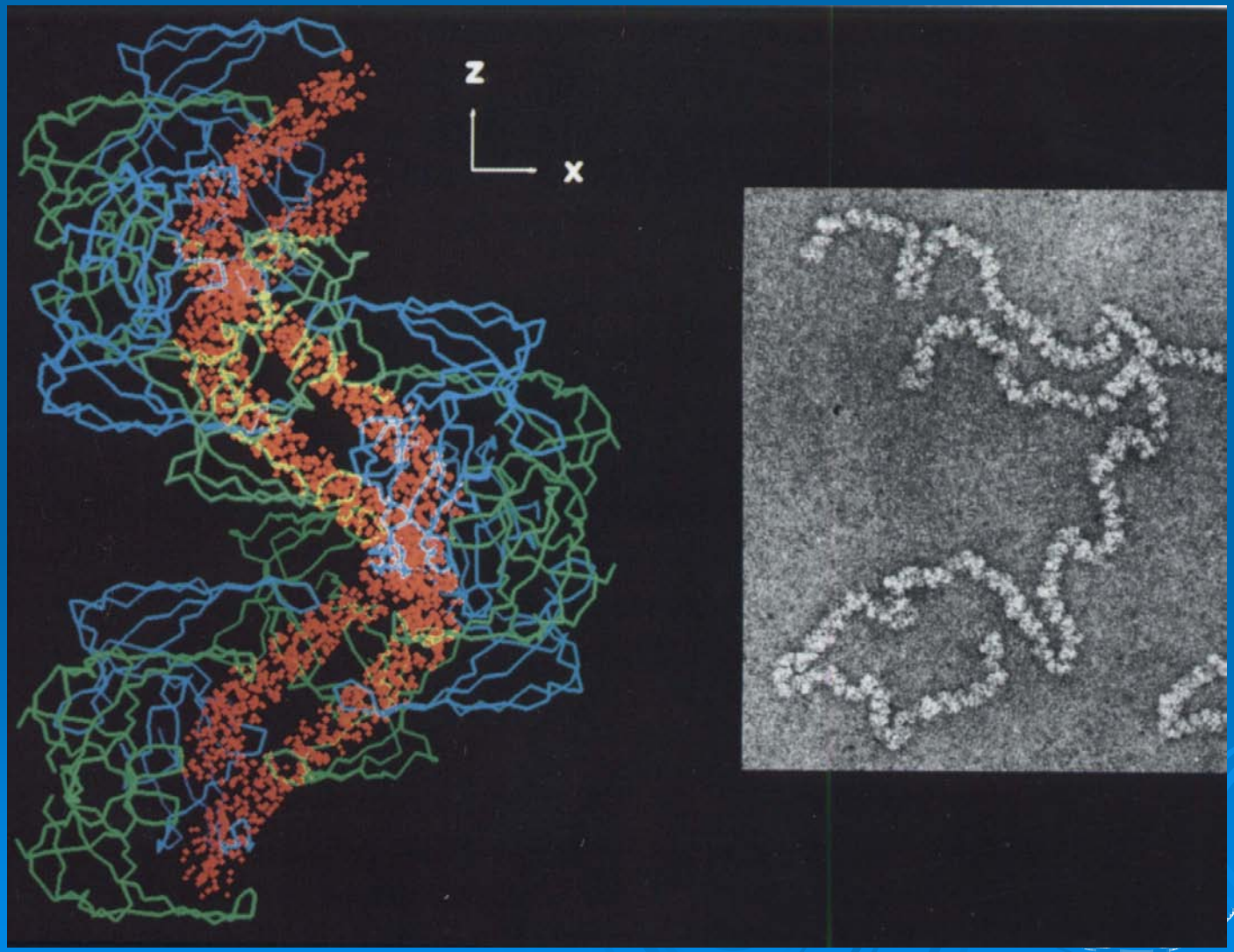






SANS contrast variation can take advantage of the fortuitous fact that the major bio-molecular constituents of have mean scattering length densities that are distinct and lie between the values for pure D_2O and pure H_2O

DNA and protein have inherent differences in scattering density and SANS contrast variation experiments can reveal shapes and dispositions of DNA and protein components.



Olah et al. (1995) "Structures of fd Gene-5 Protein Nucleic-Acid Complexes: A Combined Solution Scattering and Electron-Microscopy Study," *J. Mol. Biol. 249*, 576.

Or one can deuterate on component of a protein complex and reconstitute the complex as a two (or more) scattering density component particle

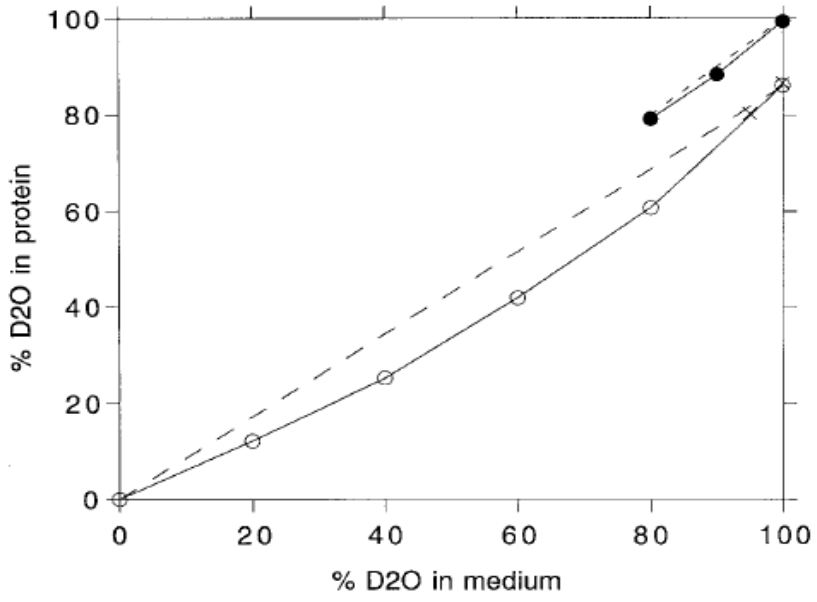


FIG. 1. Fractional deuteration of recombinant proteins in *Escherichia coli.* The deuterium content of purified protein was determined using mass spectroscopy and is presented in % of the chemically nonexchangeable protons. Open circles represent the deuteration of PDF with $[^2H]_2O$ only and filled circles PDF labeling with $[^2H]_2O$ and perdeuterated glucose. Crosses derive from deuteration experiments with two other recombinantly expressed proteins. The dashed lines indicated statistically idealized values.

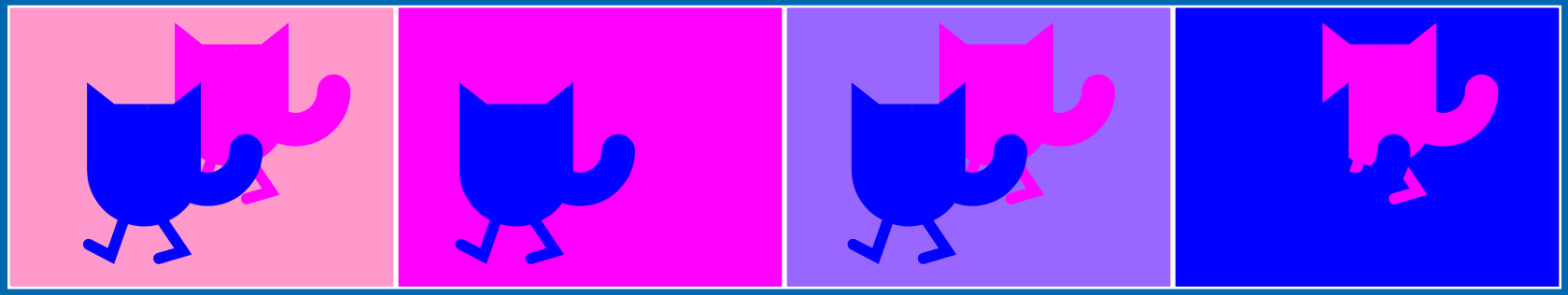
See Leiting, Marsilio and O'Connell "Predictable Deuteration of Recombinant Proteins Expressed in *Escherichia coli*," (1998) *Analytical Biochemistry 265*, 351-355

Incorporation of deuterium **up to 86%** of the chemically non-exchangeable protons can be obtained by using D_2O as the deuterium source. Complete deuteration can only be obtained by addition of perdeuterated carbon source **(glucose or glycerol).**

Use mass spec to determine final deuteration levels.

Planning your SANS experiment

- Evaluate suitability of system and sample conditions required
- Choose your data collection strategy (solvent matching or full contrast variation?)



0% ⟶ Increasing %D_2O in the Solvent ⟶ 100%

Solvent Matching: Manipulation of H:D ratios so the scattering density of one or more components equals that of the solvent and thus becomes invisible

Contrast Variation: Manipulation of H:D ratios so that the contribution of a component to the scattering signal is systematically varied.

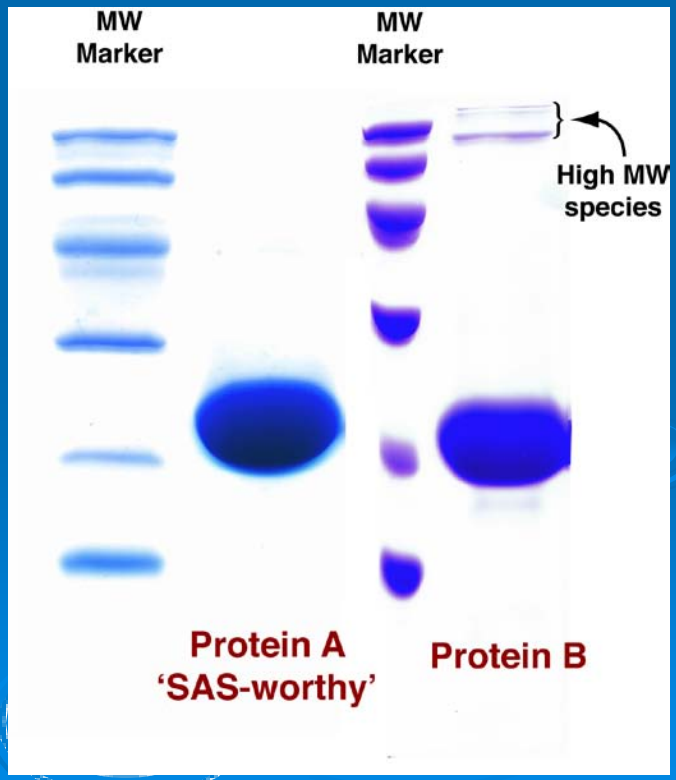
- Determine feasibility and necessary sample conditions; home SAXS instrument hugely beneficial
- Determine how much sample is needed
- Decide on your deuterium labeling strategy
- Determine optimal deuteration levels (solvent and any biomolecular components)
- See MULCh: ModULes for the analysis of neutron Contrast variation data*

http://smb-research.smb.usyd.edu.au/NCVWeb/

*MULCh, Whitten et al, *J. Appl. Cryst.* 2008 41, 222-226

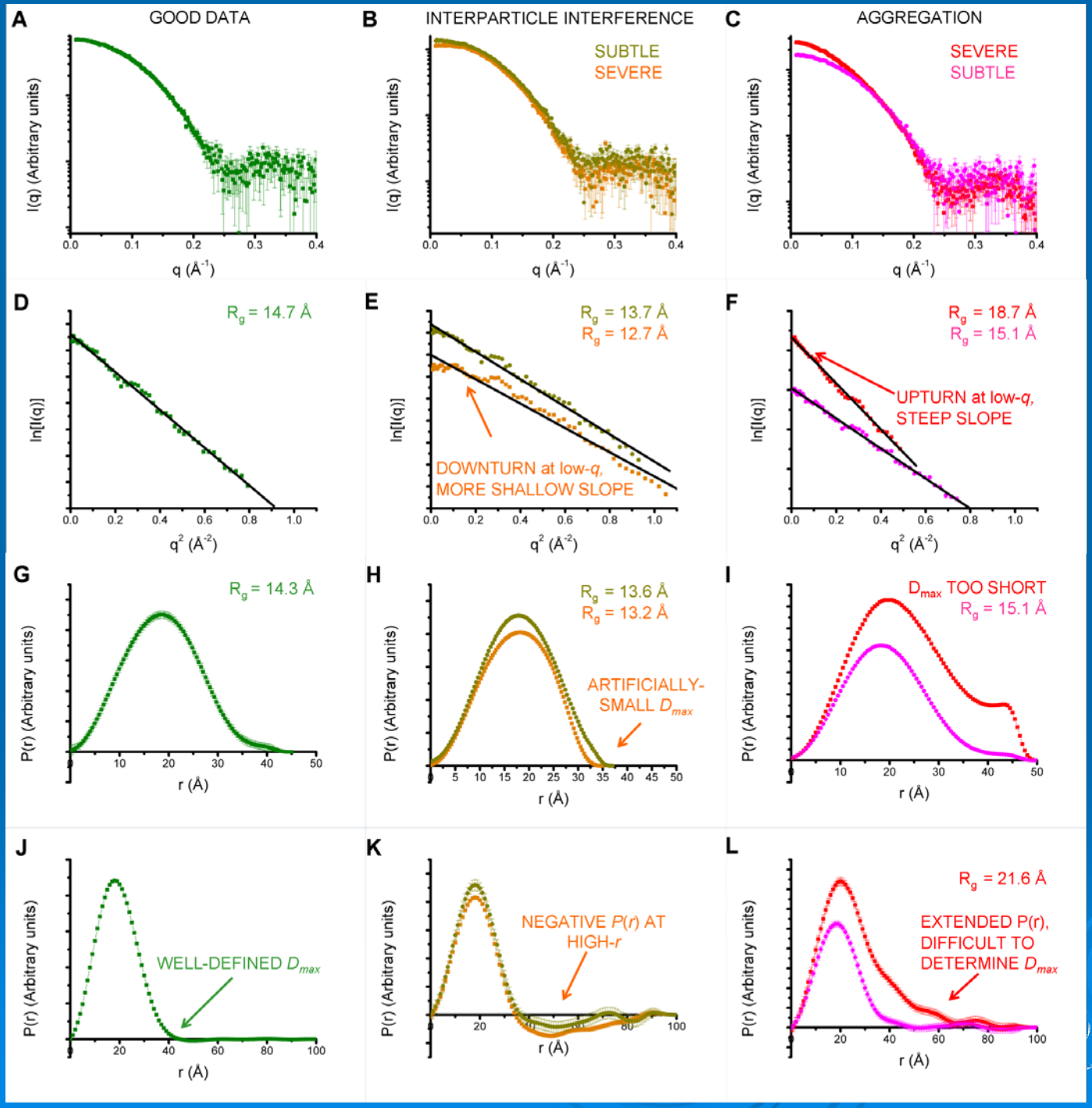
Sample requirements for small-angle scattering determination of particle shape/structure

- Highly purified samples containing mono-disperse, identical particles without significant inter-particle distance correlations (*i.e.*, $S(q) = 1$)
  - Use DLS to evaluate samples for potential aggregates (mass fraction aggregates<0.01%)
  - Use a final gel filtration step in the purification (immediately) prior to measurement to eliminate any aggregates



Preliminary SAXS experiments increase success rate for SANS

- Demonstrate purity and mono-dispersity of samples in H_2O and D_2O.
- Explore the concentration dependence of the small-angle X-ray scattering to determine if the is any inter-particle interference of concentration dependent aggregation.
- If necessary, adjust the solution conditions to avoid the above effects; by changing pH, salt concentration, or decreasing particle concentration.
- Determine the particle mass, molecular volume, and overall shapes of the components and the complex they form (Guinier and $P(r)$ analyses, shape restoration)



Jacques *et al.* (2012)
Acta Cryst. D64, 620.

http://smb-research.smb.usyd.edu.au/NCVWeb/

→ NCVWeb Home
→ *Contrast*
→ *Rg*
→ *Compost*

MULCh: ModULes for the analysis of Contrast variation data

The purpose of this set of programs is to allow the extraction of structural parameters from Neutron Contrast Variation data for two component systems. There are three modules to facilitate this:

1. ***Contrast*: This module determines the contrast ("scattering power" relative to the solvent) for each subunit in the complex for various proportions of D_2O in the solvent. The contrast is important for the subsequent modules, but also helpful in planning and experiment.**
2. *Rg*: Analyses the dependence of the radius of gyration upon contrast. From this analysis information can be extracted relating to the radii of gyration of each subunit and their separation.
3. *Compost*: Decomposes the scattering profiles down to scattering from each subunit, and a cross term, related to the disposition of each.

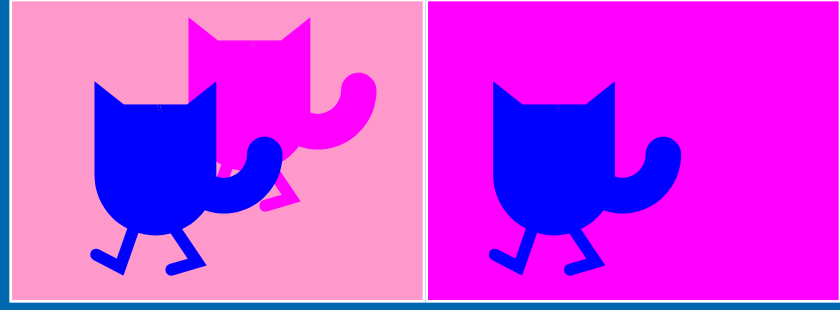
Testing and basic description of the work has been published: A.E. Whitten, S. Cai and J. Trewhella, J. Appl. Crystallogr. If this page is used we ask that you cite that article.

The MULCh manual can be found here

The source code for the modules can be found here

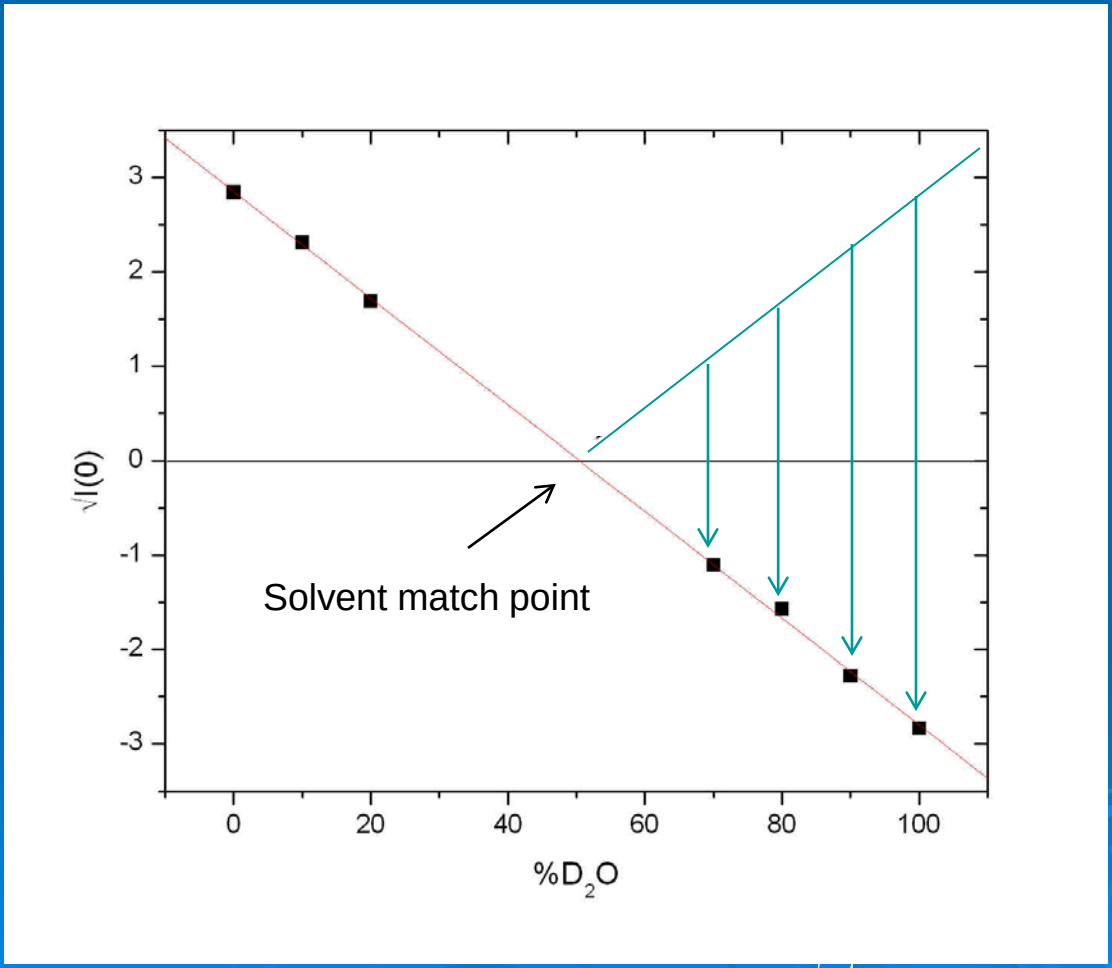
Test data and input files for MULCh can be found here

Solvent matching



- For two scattering density component complexes; internal density fluctuations within each component <<< scattering density difference between them.
- Best used when you are interested in the shape of one component in a complex, possibly how it changes upon ligand binding or complex formation.
- Requires enough of the component to be solvent matched to complete a contrast variation series to determine required %D_2O (~4 x 200-300 μL, ~5 mg/ml) for precise solvent matching.
- Requires 200-300 μL of the labeled complex at 5-10mg/ml.

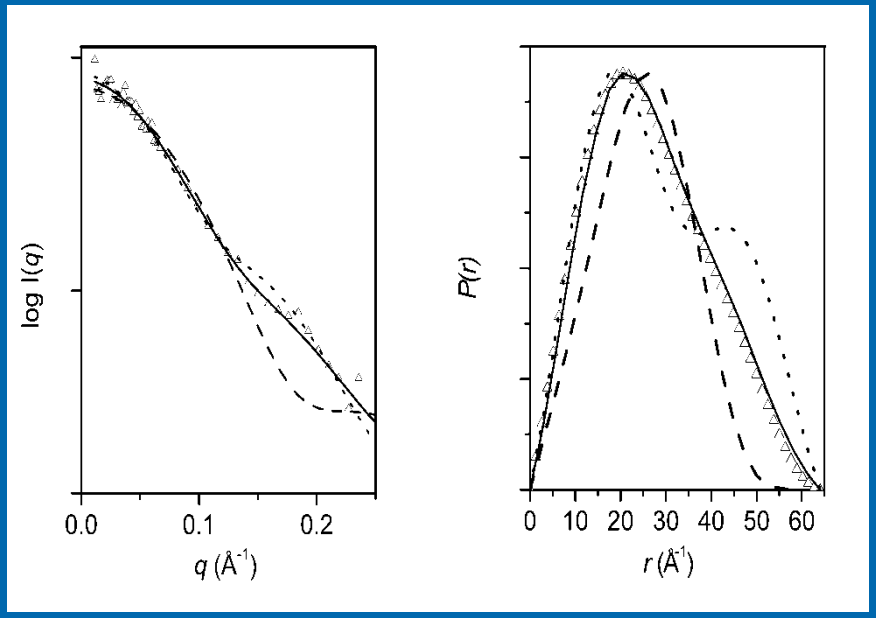
Accurate solvent match point determination is critical

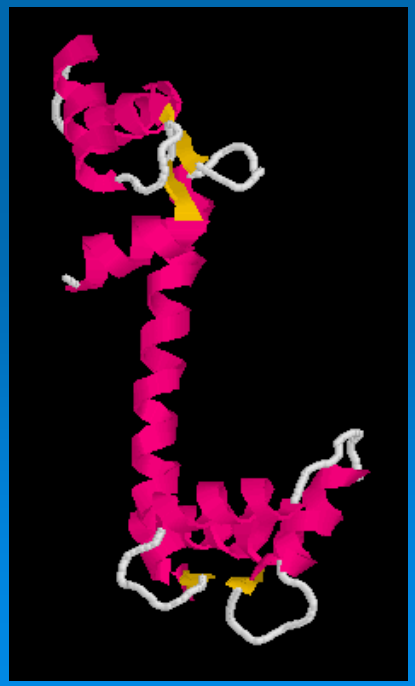


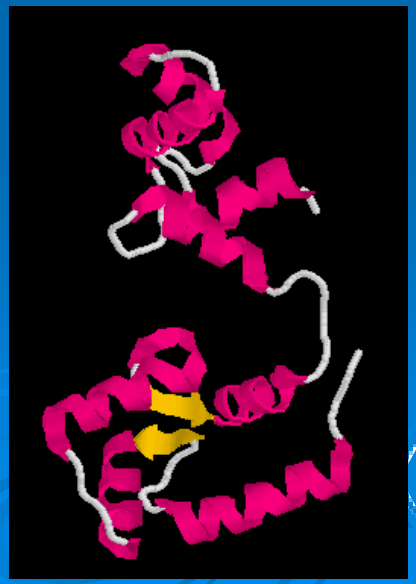
Solvent Matching Experiment

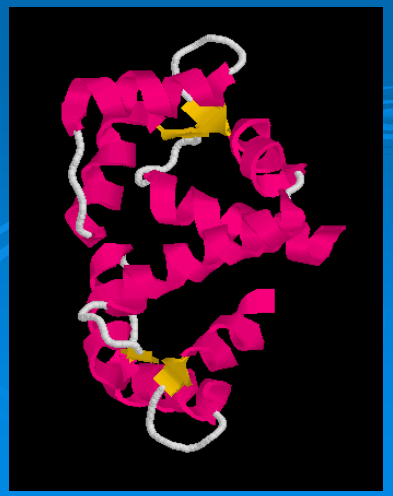
Deuterated calmoduln (^{D}CaM) complexed with the MA protein from HIV-1 measured in 41% D_2O. Only the ^{D}CaM contributes to the scattering and we can distinguish between a fully extended, collapsed or partially collapsed CaM conformation

Taylor et al., *Biophys. J.103*, 1-9, 2012

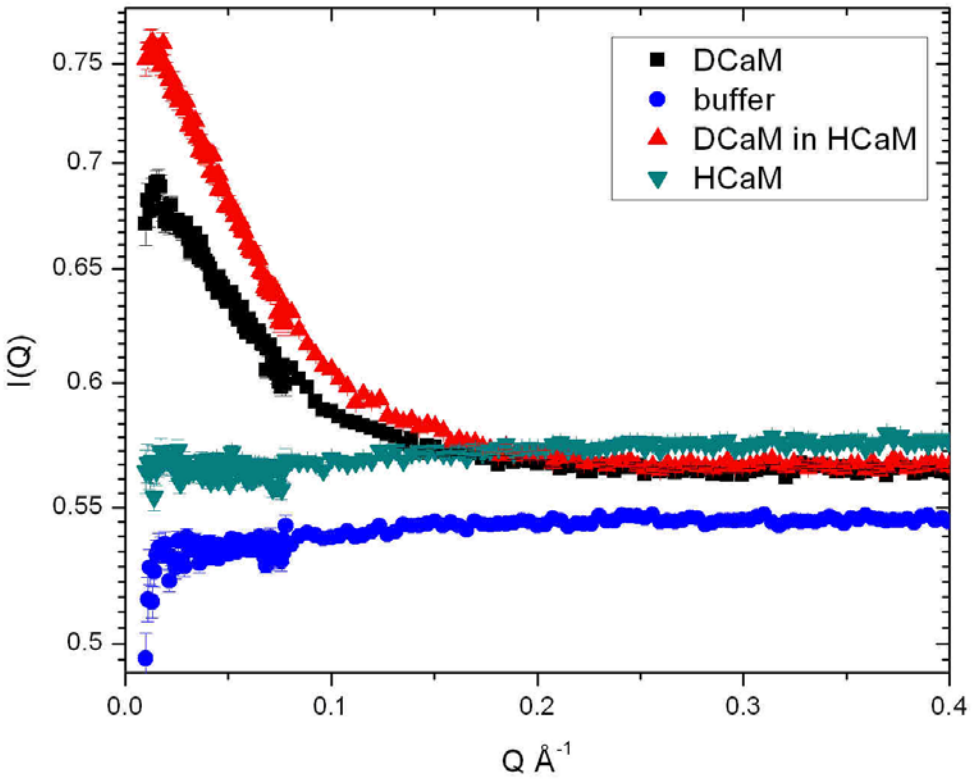








Solvent matching and molecular crowding

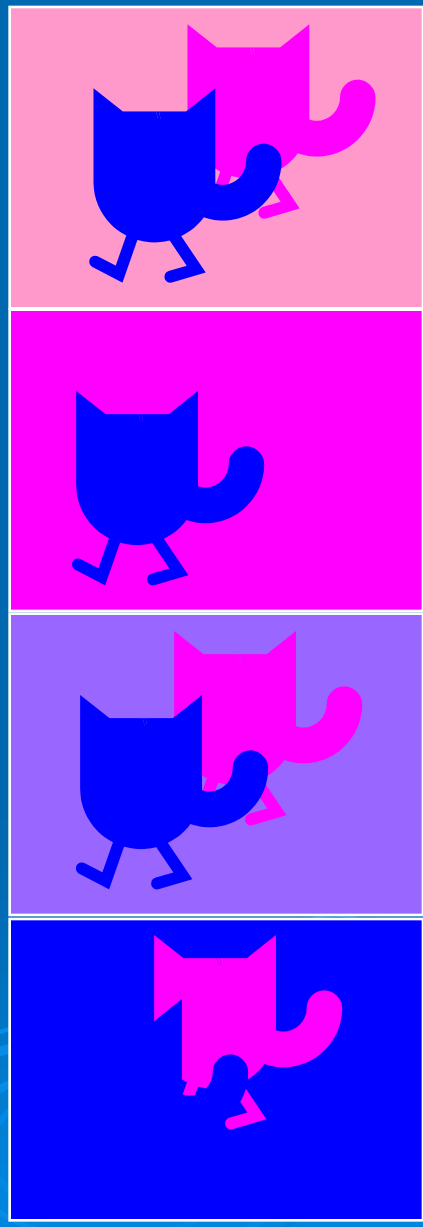


- HCaM measurement was done in 42% D_2O to solvent match the HCaM.
- Objective was to see DCaM in presence of high concentrations of HCaM, but without interference from HCaM
- Incoherent scattering from ^1H is a constant with Q

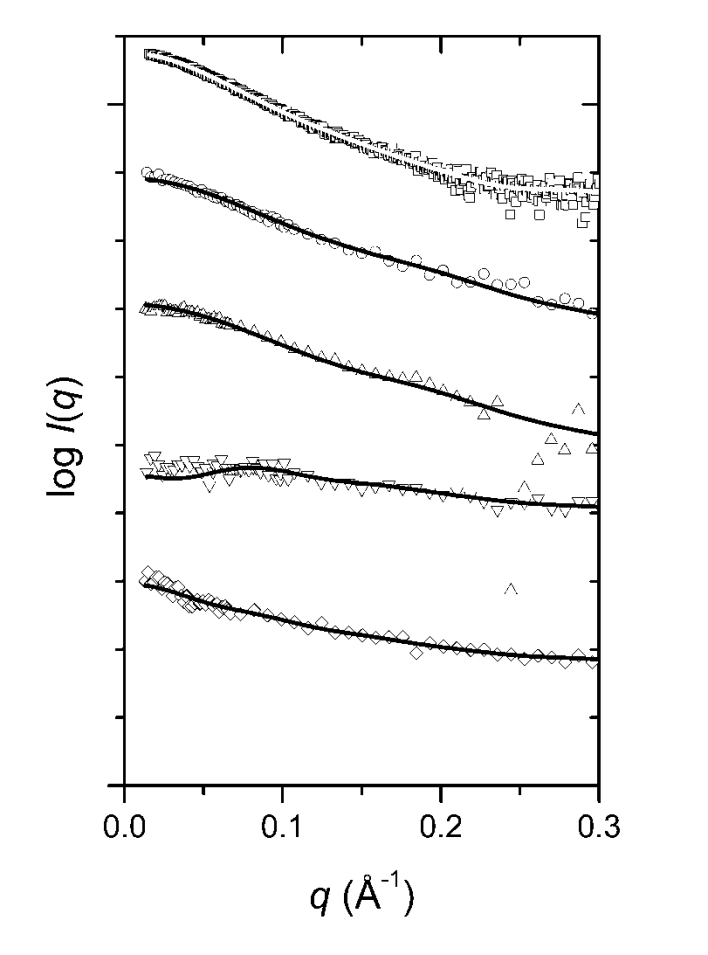
Note effects of incoherent scattering from ^1H on backgrounds

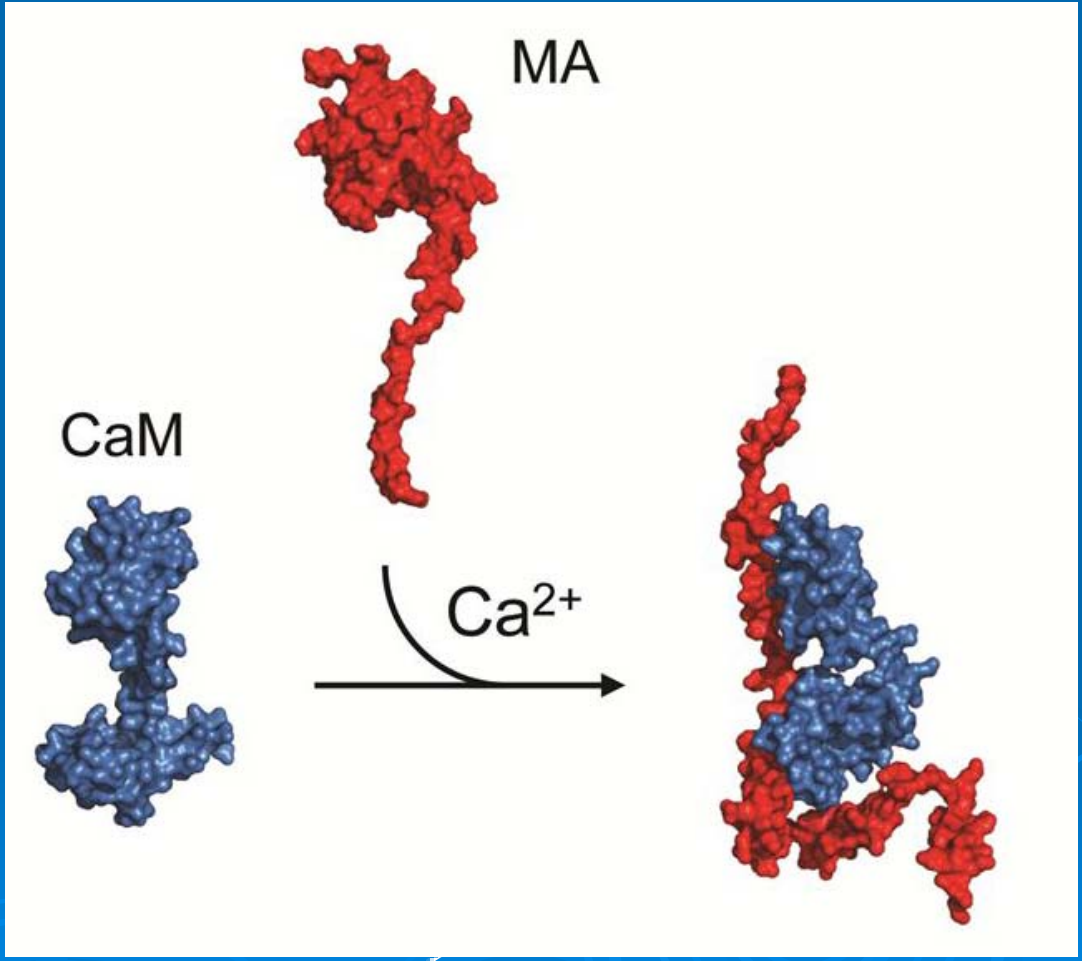
Contrast variation

- To determine the shapes and dispositions of labeled and unlabelled components in a complex
- Requires ≥ 5 x 200-300μL (= 1 – 1.5mL) of your labeled complex at ≥ 5 mg/ml .
- Deuteration level in labeled protein depends upon its size.
  - Smaller components require higher levels of deuteration to be distinguished.
  - Ideally would like to be able to take data at the solvent match points for the labeled and unlabeled components



The host Ca^{2+} receptor CaM binds the multifunctional HIV-1 MA protein and unfolds its N-terminal domain in the presence of Ca^{2+}

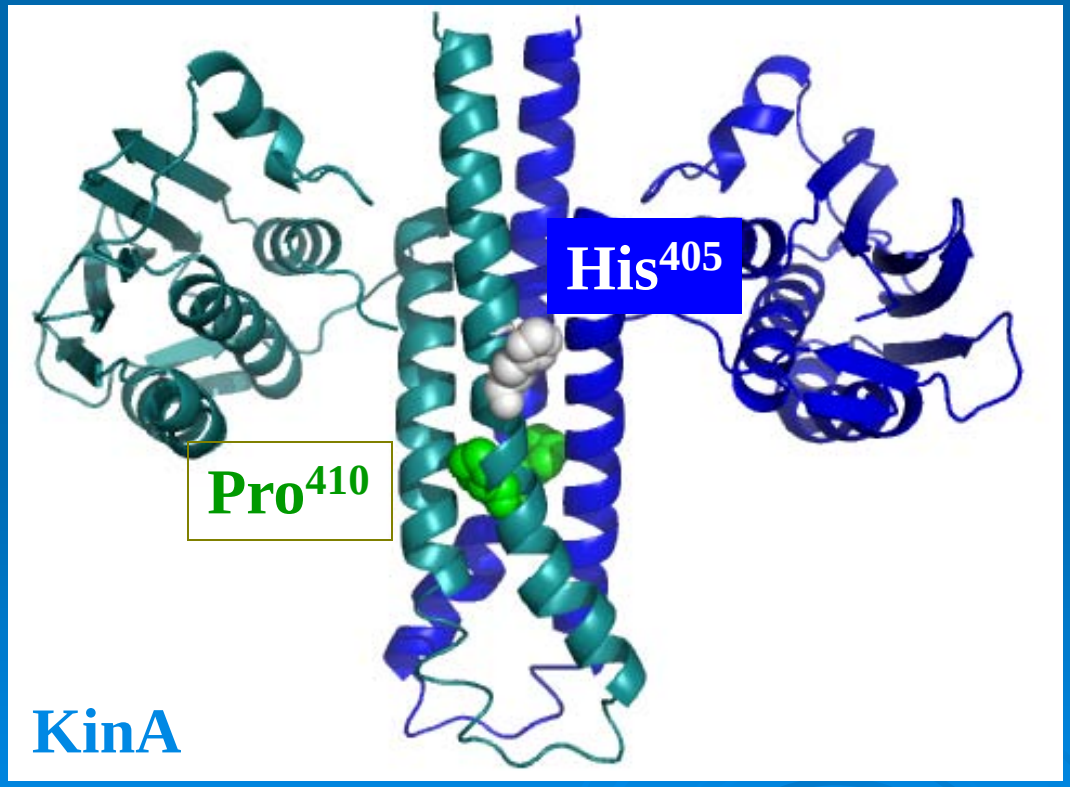


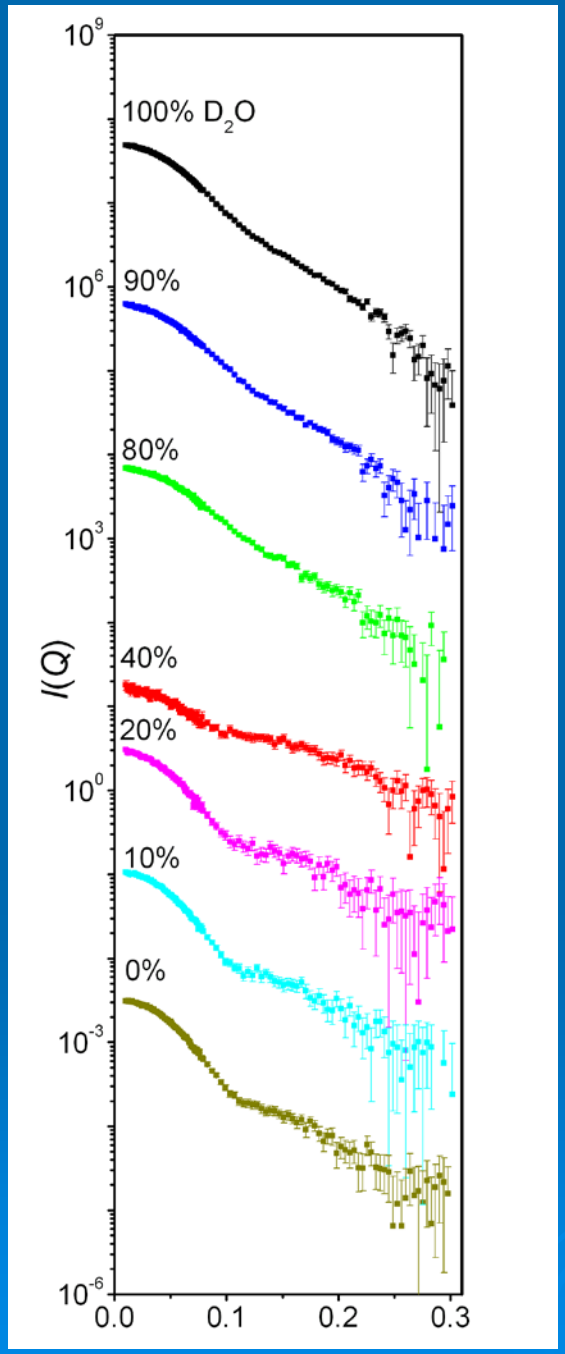


Taylor et al., *Biophys. J.103*, 1-9, 2012

A classic contrast variation experiment

The molecular actors:
a bacterial histidine kinase, KinA, and the protein inhibitor Sda

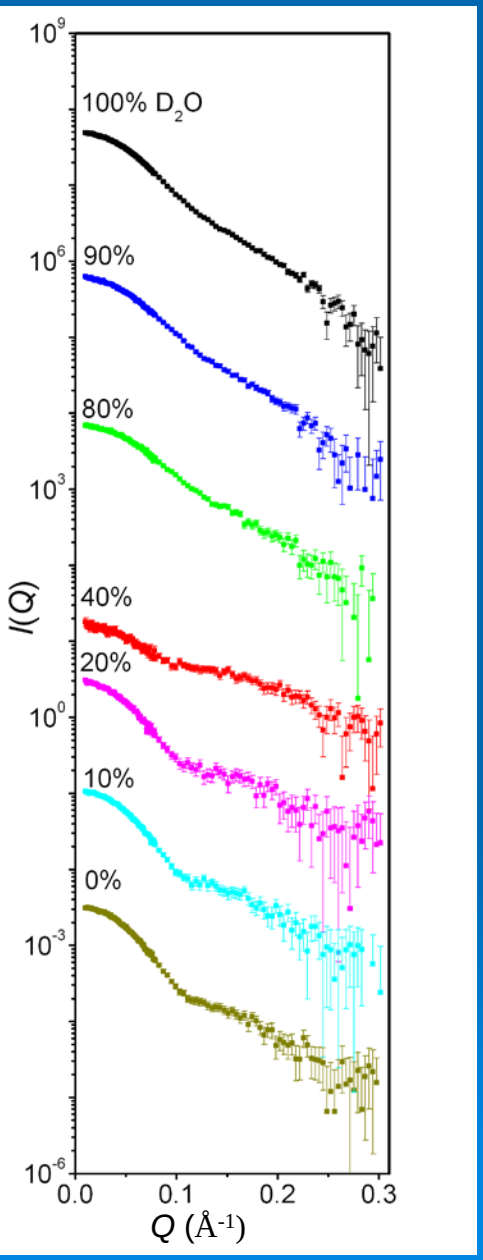




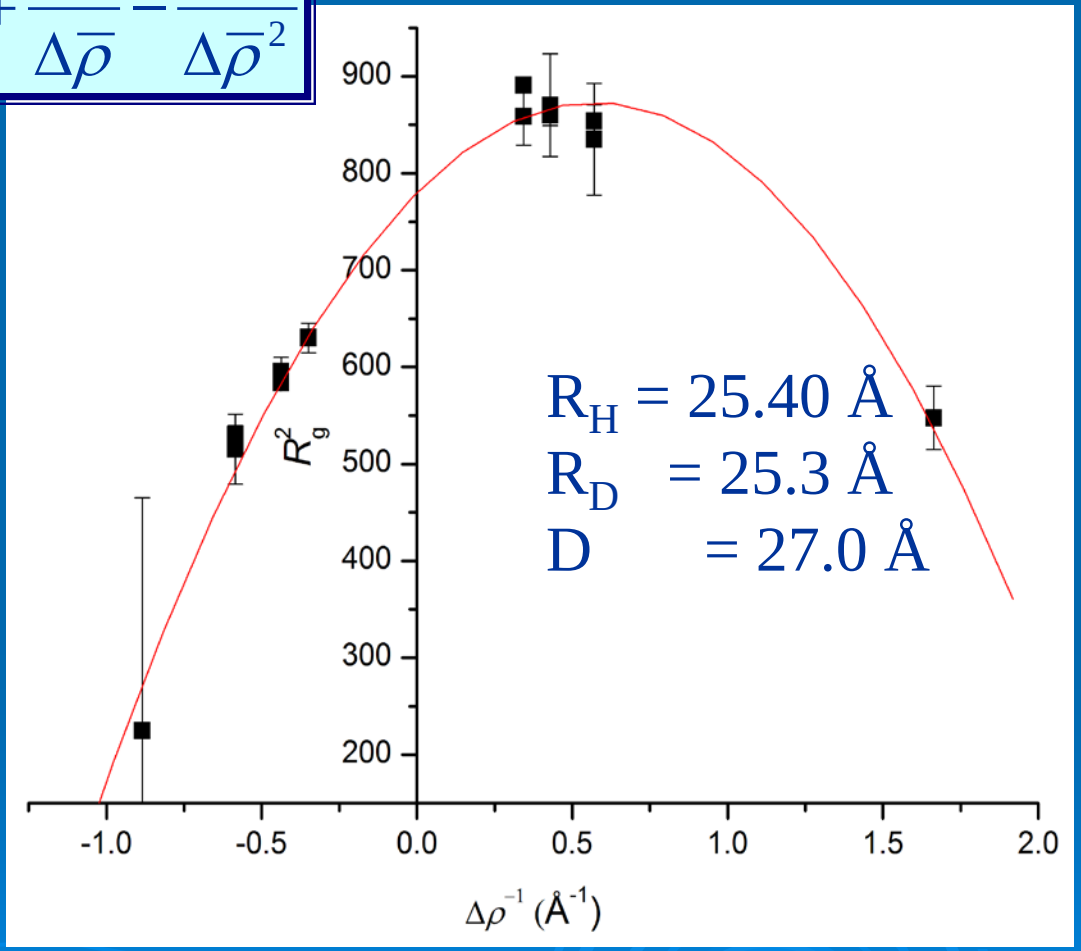
$KinA_2$-$2^{D}Sda$ complex experiment

- Measure sample and solvent blanks at each contrast point (use a broad range of D_2O concentrations)
- Subtract solvent blank data from sample
- Sample to low-q with sufficient frequency to determine large distances accurately (min. 15-20 points in the Guinier region)
- Measure to high enough q to aid in checking background subtraction (q = 0.45 $Å^{-1}$)
- q = 0.01 - 0.45 is typical range for 10-150 kDa particles, usually requires two detector positions

Use *Rg* (from MULCh) for Sturhman analysis

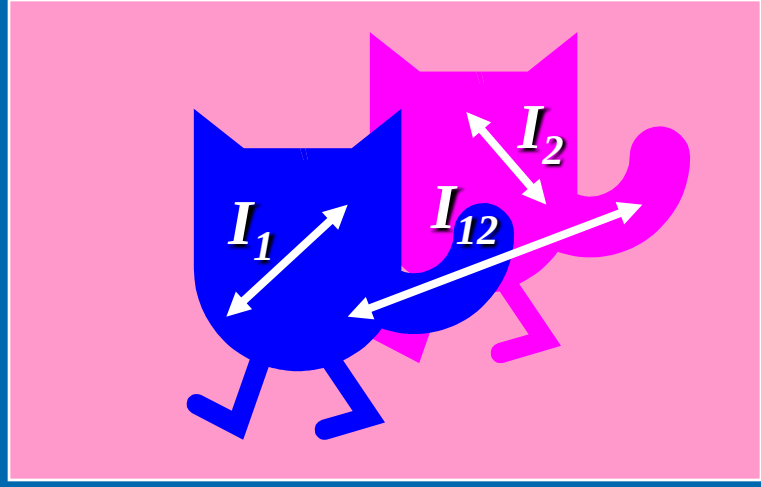


$$R_{obs}^2 = R_m^2 + \frac{\alpha}{\Delta\bar{\rho}} - \frac{\beta}{\Delta\bar{\rho}^2}$$

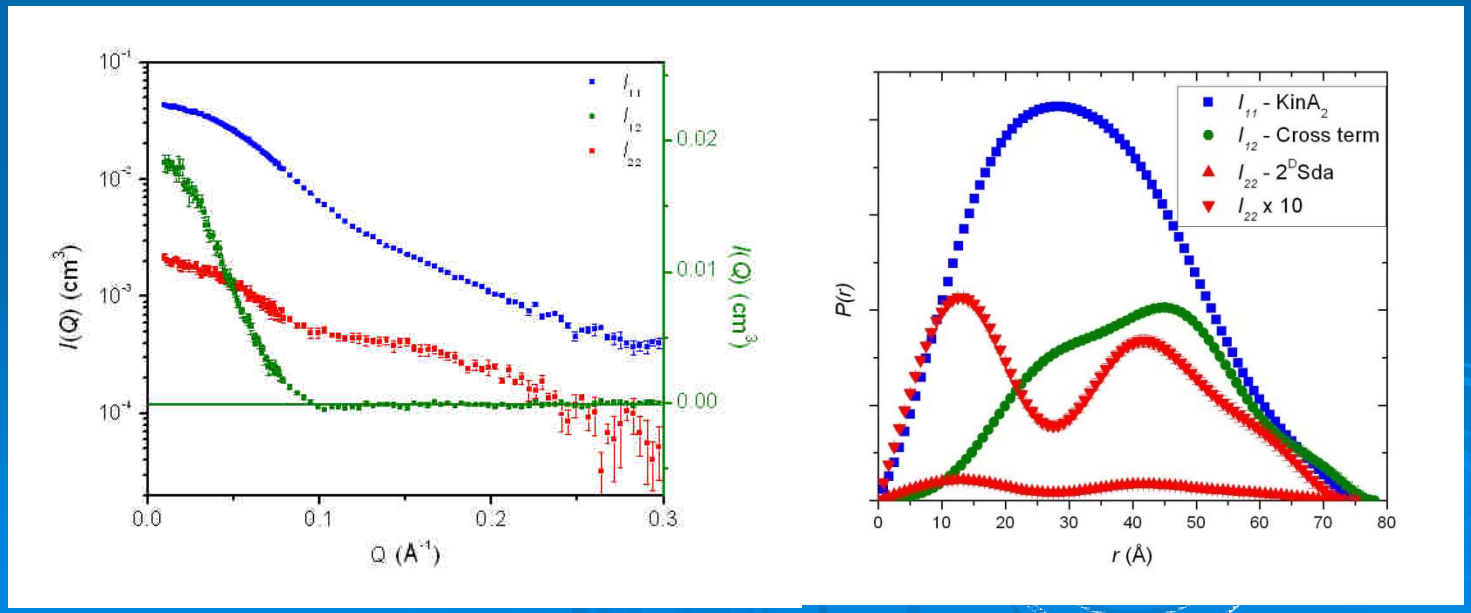


Sign of α indicates whether the higher scattering density object is more toward outside (+) or inside (-)

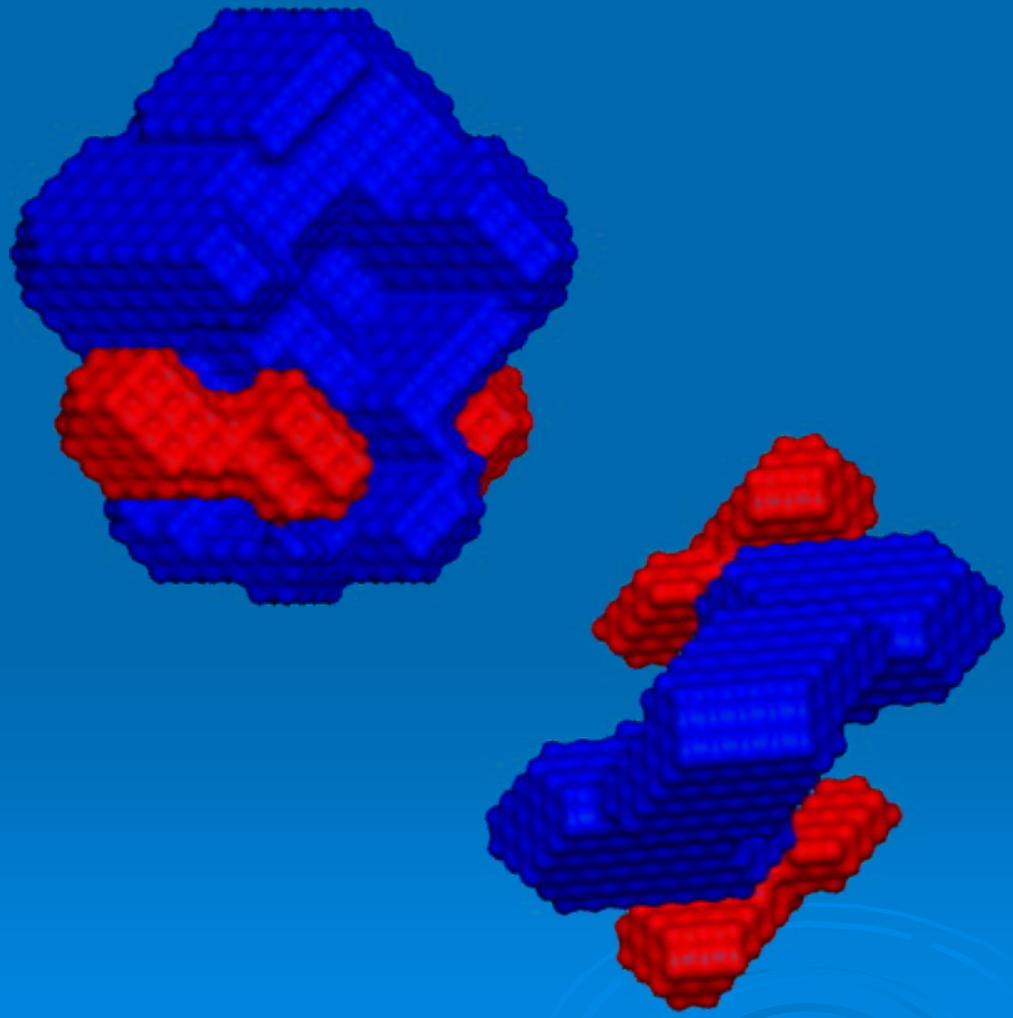
Use *Compost* (from MULCh) to solve for $I(Q)_{11}$, $I(Q)_{22}$, $I(Q)_{12}$

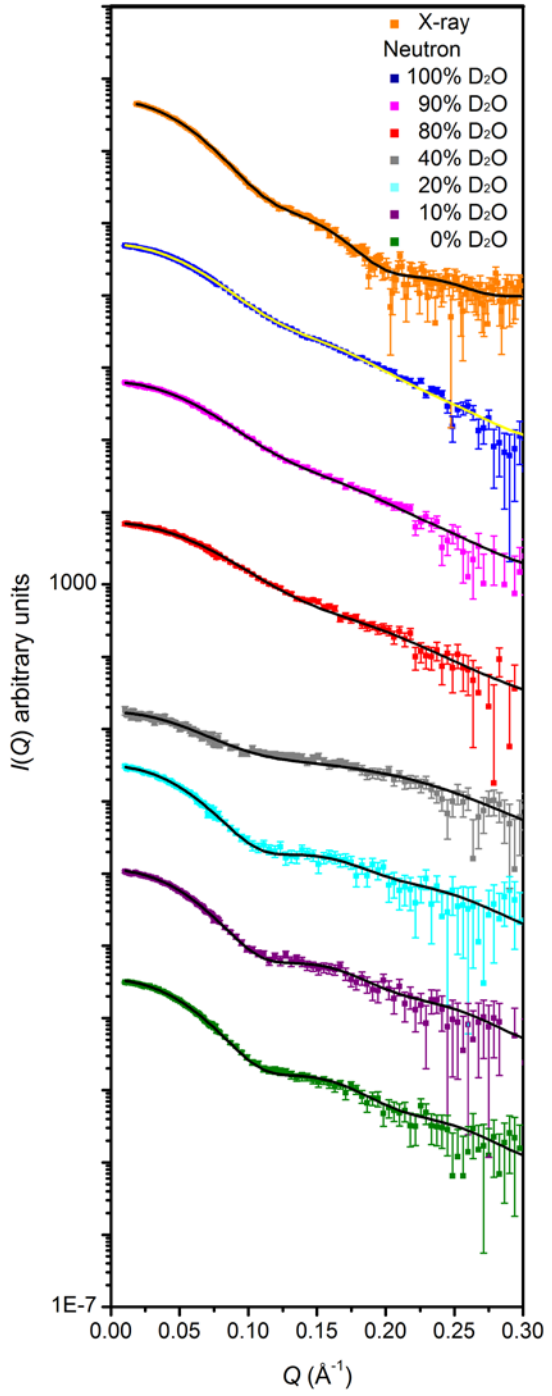


$$I(Q) = \Delta\rho_1{}^2 I_{11}(Q) + \Delta\rho_2{}^2 I_{22}(Q) + \Delta\rho 1 \Delta\rho 2 I_{12}(Q)$$

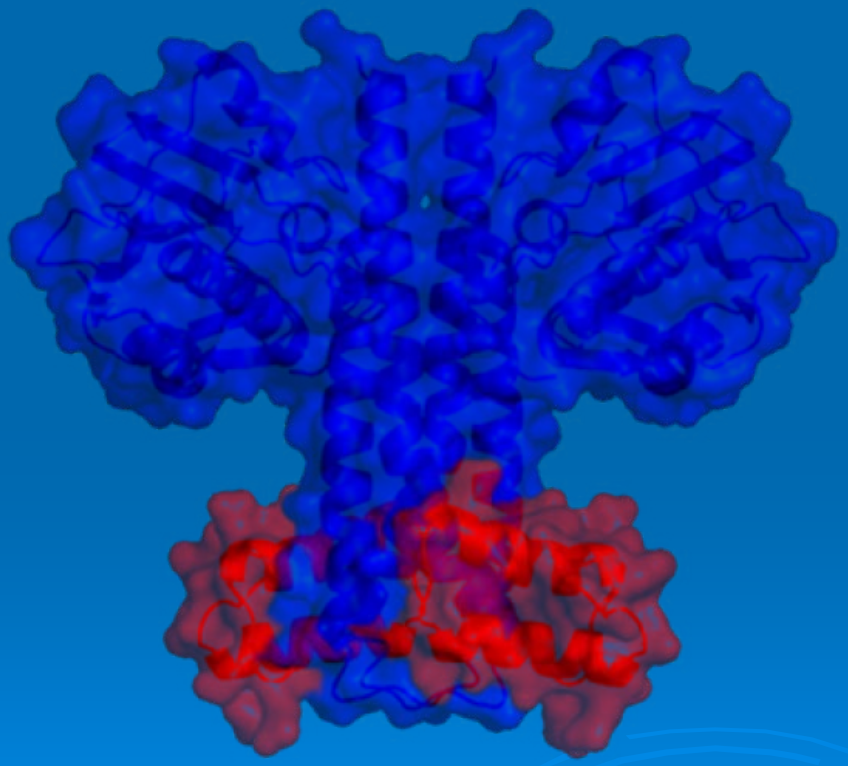


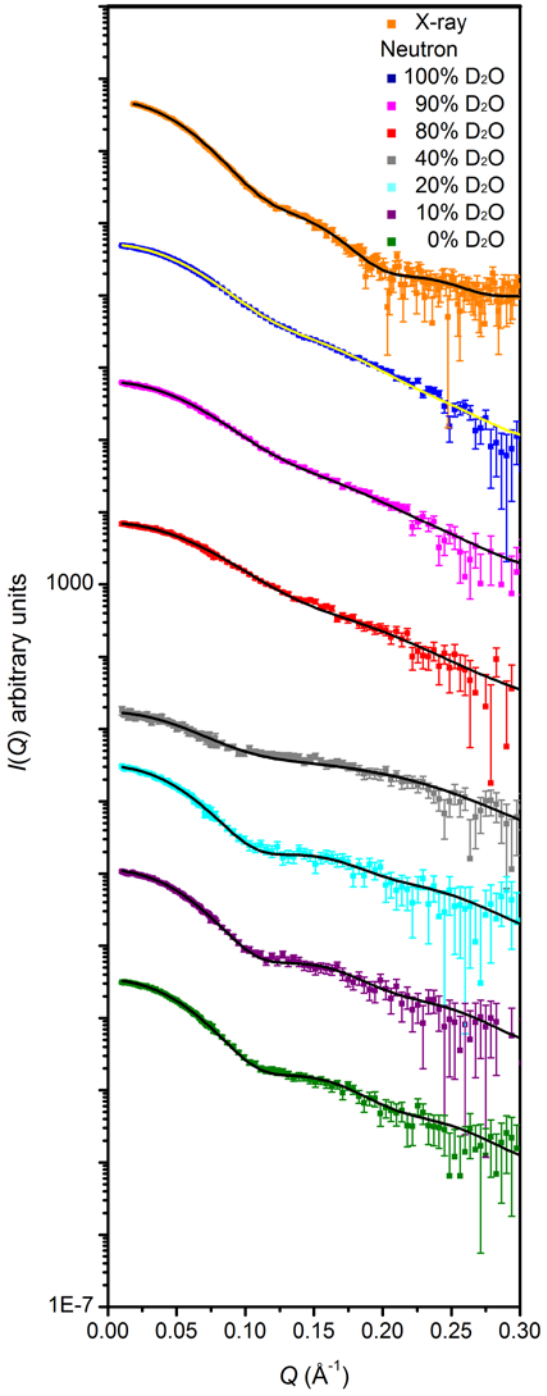
MONSA
3D shape restoration





Use SASREF7 to do rigid body refinement of the components against the scattering data (if you have pdb files for components)





χ^2

1.27

0.97

0.63

0.56

0.76

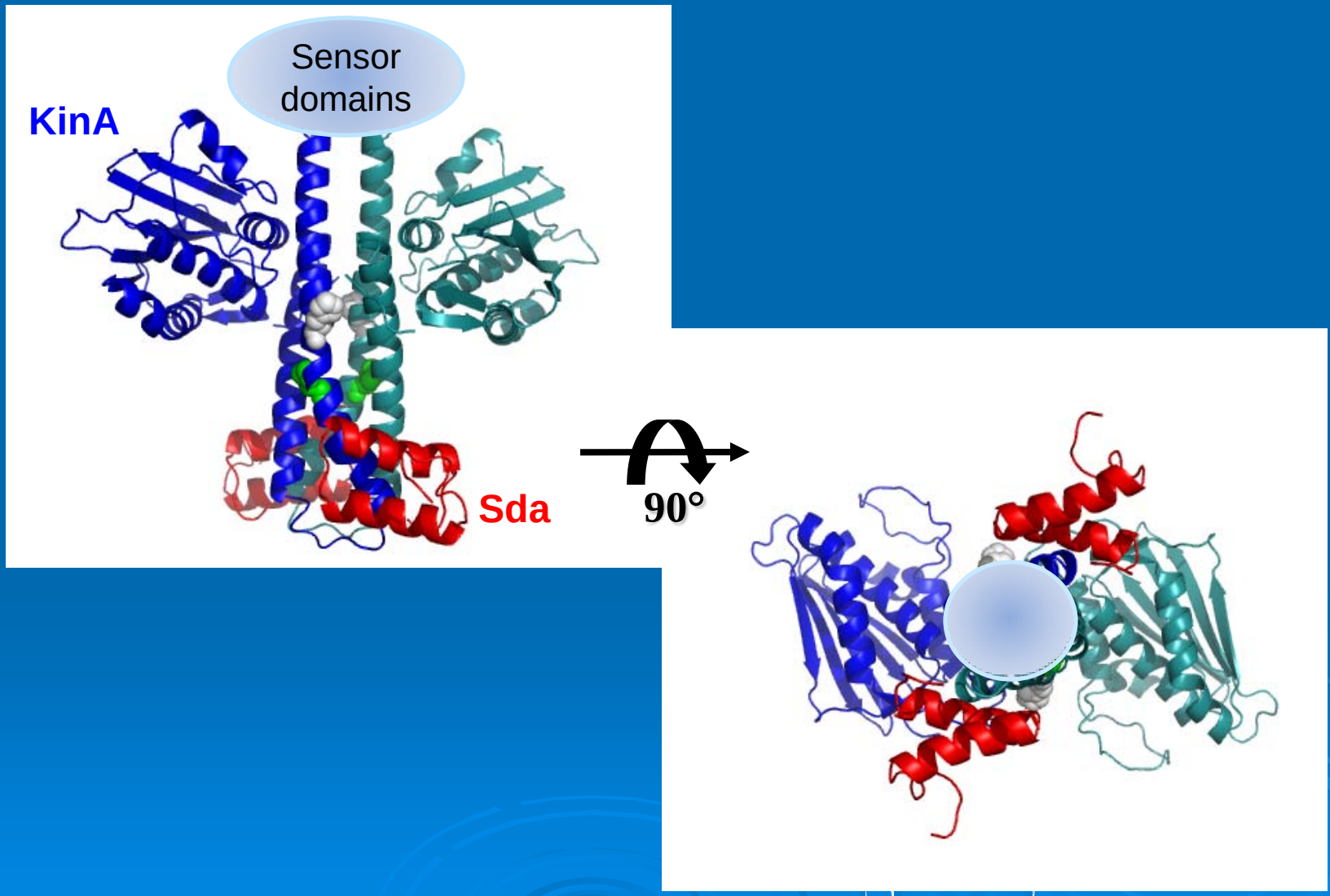
0.92

1.12

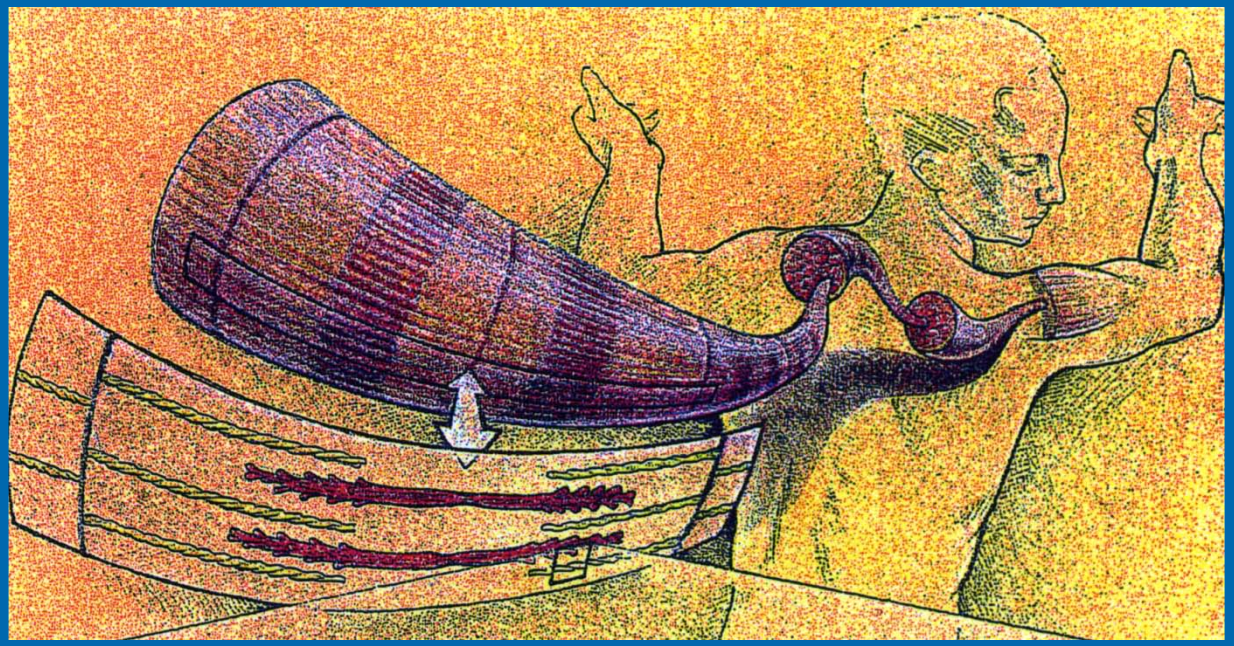
0.95

Whitten et al. (2007) *J. Mol. Biol. 368*, 407-420

Neutrons reveal Sda inhibitor binds at the base of the dimerisation domain that connects to the sensor domains.



Whitten et al., *J. Mol. Biol. 368*, 407, 2007

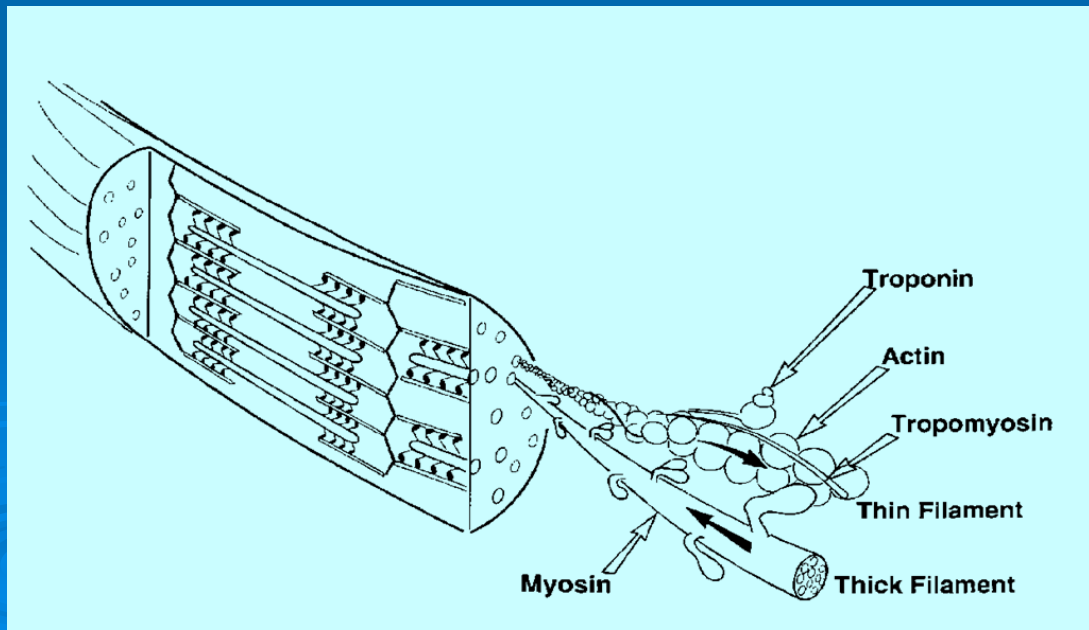


The “sliding filament model” of muscle contraction. Dozens of proteins work in concert to produce the mechanical sliding, with the troponin complex acting as a Ca^{2+} sensitive switch.

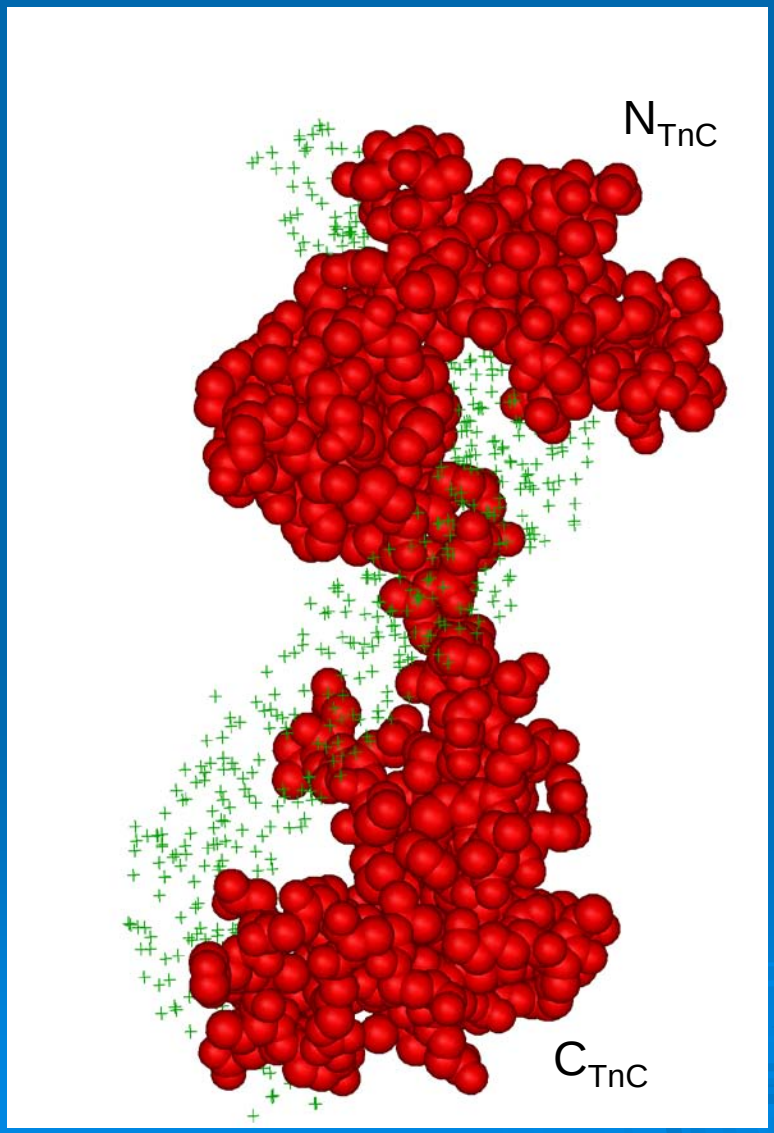
More than 2 components?

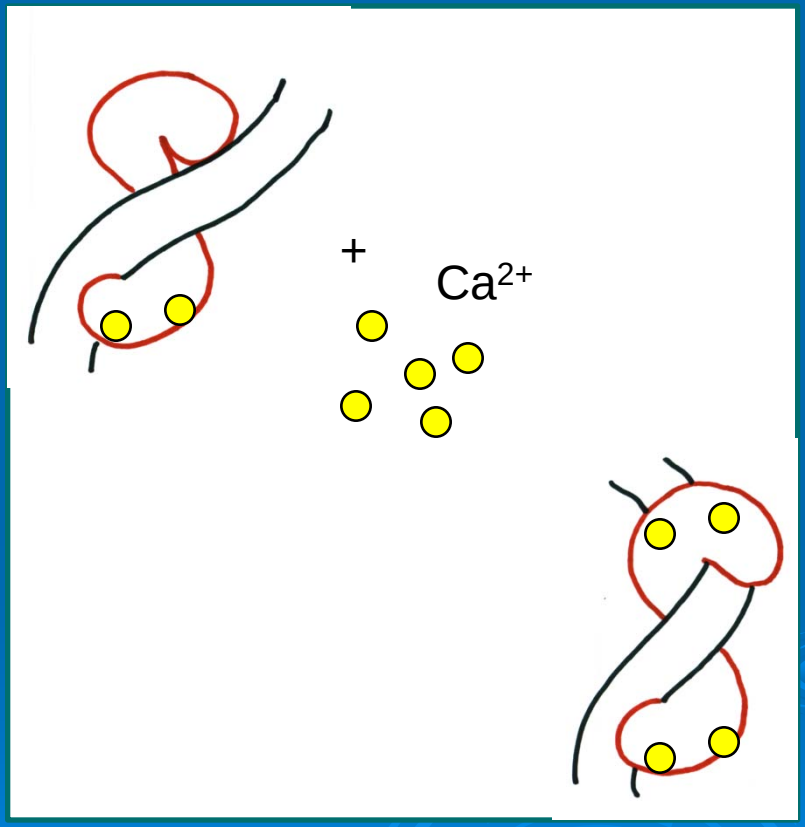
Troponin

- TnC receptor for Ca^{2+} signals
- TnI inhibits cross-bridge interactions between thick & thin filaments
- TnT transmits inhibitory signal along thin filament

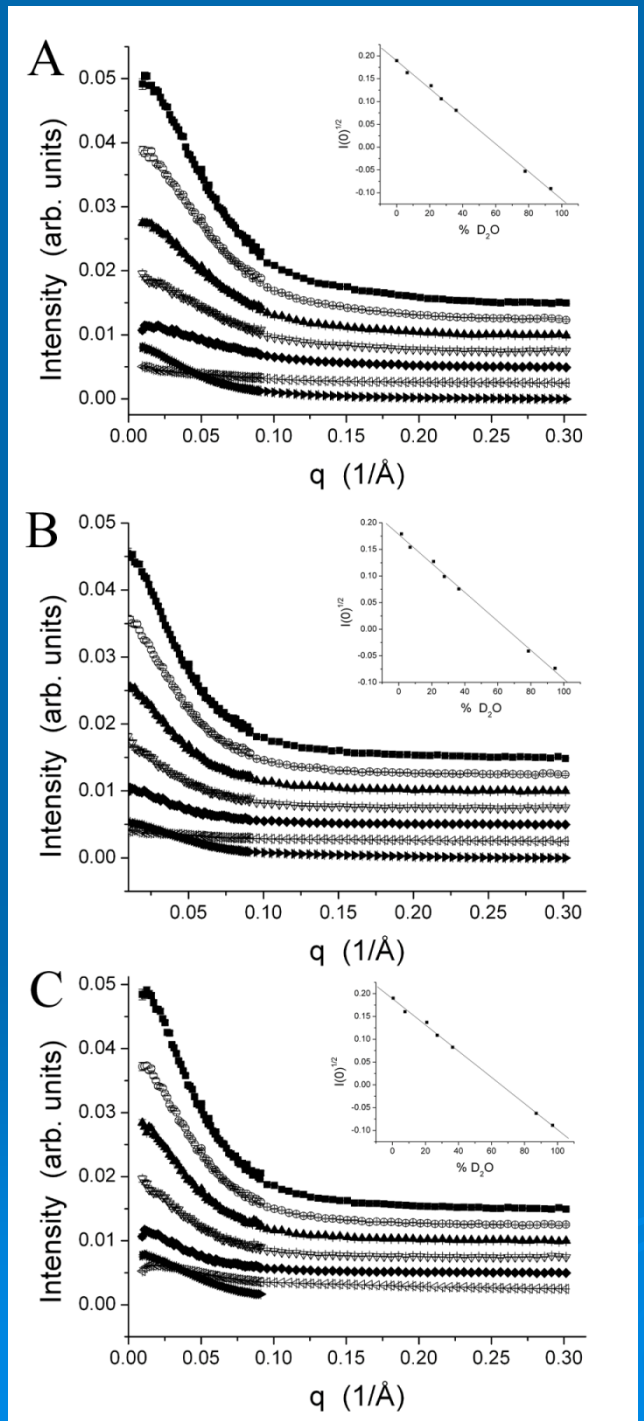


Neutron scattering derived model for the skeletal troponin C-troponin I complex shows troponin I spiraling around troponin C





Olah et al. (1994) *Biochemistry 33*, 8233;
Olah & Trewhella (1994) *Biochemistry 33*, 12800.



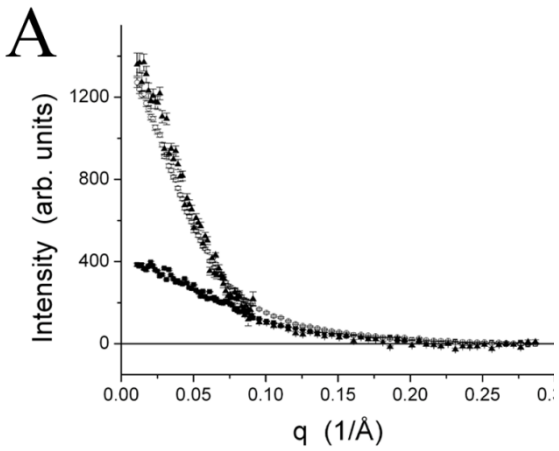
Neutron contrast series from cardiac TnC/TnI/TnT$_{(198\text{-}298)}$ with:

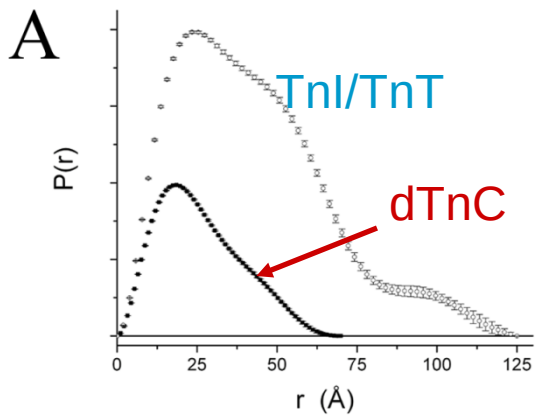
deuterated TnC

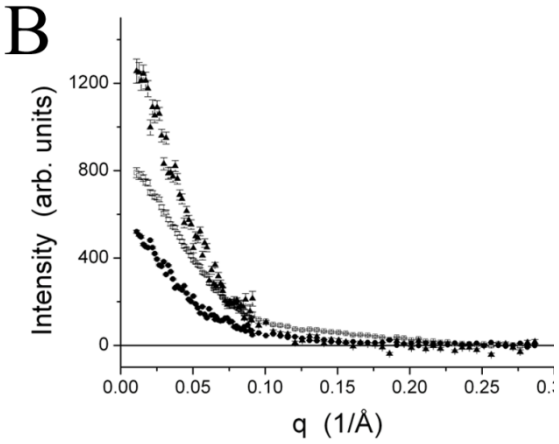
deuterated TnI

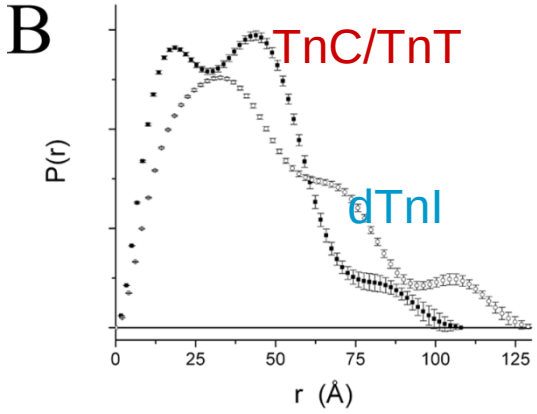
deuterated TnI, phosphorylated (by PKA at Ser^{24} and Ser ^{25})

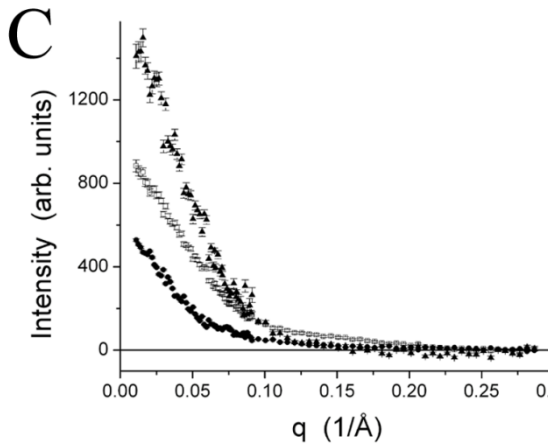
Heller et al. (2003) *Biochemistry 42*, 7790

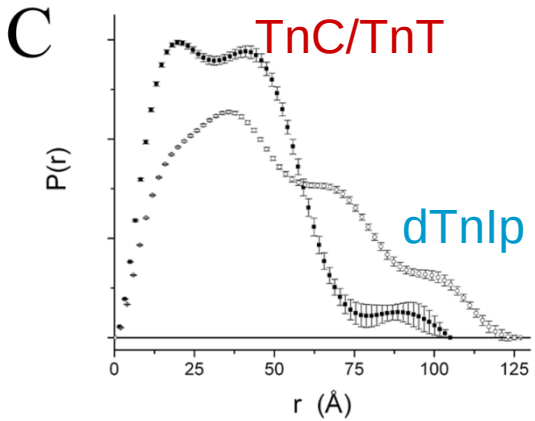

A
Intensity (arb. units)
1200
800
400
0
0.00
0.05
0.10
0.15
0.20
0.25
q (1/Å)

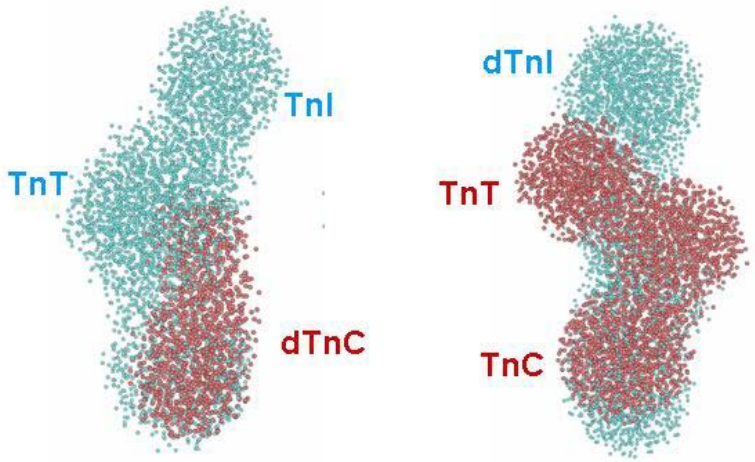

A
TnI/TnT
dTnC
P(r)
0
25
50
75
100
125
r (Å)

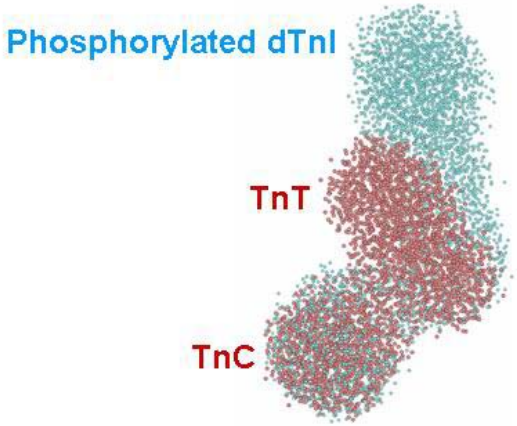

B
Intensity (arb. units)
1200
800
400
0
0.00
0.05
0.10
0.15
0.20
0.25
q (1/Å)


B
TnC/TnT
dTnI
P(r)
0
25
50
75
100
125
r (Å)


C
Intensity (arb. units)
1200
800
400
0
0.00
0.05
0.10
0.15
0.20
0.25
q (1/Å)


C
TnC/TnT
dTnIp
P(r)
0
25
50
75
100
125
r (Å)


TnI
TnT
dTnC
dTnI
TnT
TnC


Phosphorylated dTnI
TnT
TnC

X-ray Sources	Neutron sources[1]
Laboratory sources	Reactors
• Sealed X-ray tubes, low maintenance but practically need a line source geometry for protein work • Rotating Anode source, higher maintenance but provides a point source geometry	• steady state sources of cold[2] neutrons that are collimated to provide a narrow wavelength band ($\Delta\lambda/\lambda$ ~10%)
Accelerator-based synchrotron sources	Accelerator-based spallation sources
• Tunable, high brilliance beams for rapid measurement/low protein concentrations	• pulses of cold[2] neutrons with instruments designed to use time of flight so that all wavelengths in a given pulse can be used - which compensates at least partially for low time-averaged neutron fluxes

[1]Neutron sources are inherently low-intensity sources.

[2]SANS requires 'cold' neutrons; thermal neutrons are passed through a liquid hydrogen moderator to slow them (generally to ~4-6 Å)

Only use neutrons when they can uniquely provide the information you need:

	Brightness (s^{-1} m^{-2} $ster^{-1}$)	dE/E (%)	Divergence ($mrad^2$)	Flux (s^{-2} m^{-2})
Neutrons	10^{15}	2	10 x 10	10^{11}
Rotating anode	10^{16}	3	0.5 x 10	5×10^{10}
Bending magnet	10^{24}	0.01	0.1 x 5	5×10^{17}
Wiggler	10^{26}	0.01	0.1 x 1	10^{19}
Undulator (APS)	10^{33}	0.01	0.01 x 0.1	10^{24}

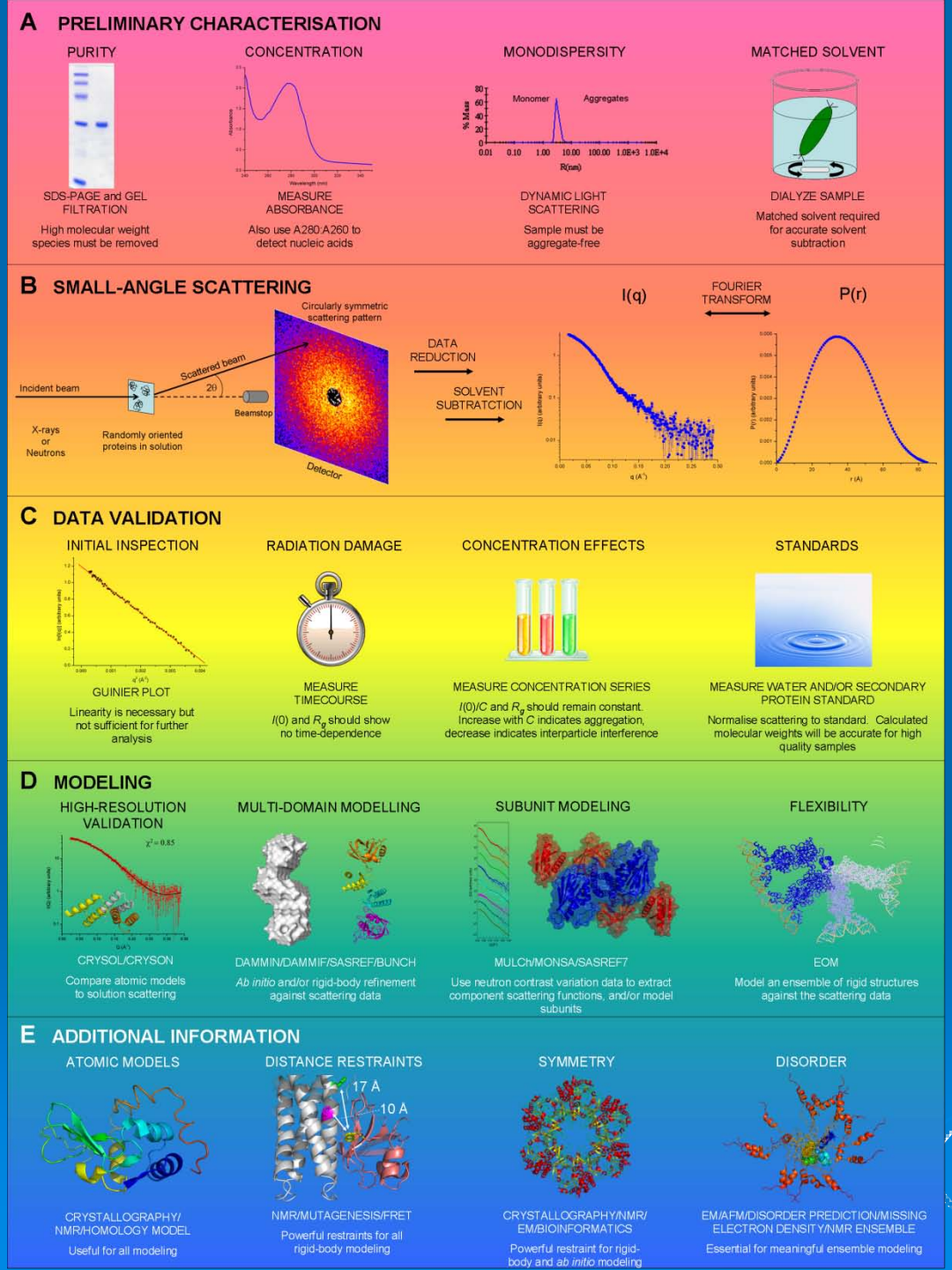
Data from “An Introduction to X-ray and Neutron Scattering,” Roger Pynn

Comparison of some X-ray/neutron sample and measurement parameters			
	X-rays		**Neutrons**
	Lab source	synchrotron	
Sample concentration	2-10 $mg.mL^{-1}$	0.1-1.0 $mg.mL^{-1}$	2-15 $mg.mL^{-1}$
Sample volume	10-50 µL	10-50 µL	100-300 µL
Measurement time	5 - 60 m	seconds	15 - 240 m
Radiation damage	can be significant*	Xrays	non-ionising

*Higher intensity beams increase the risk of radiation damage due to free radical formation, mitigated by flowing samples and using free radical absorbing agents such as DTT, TCEP, ascorbate buffers.

Neutron scattering sample cells

- Helma quartz cells (high precision path-length, suprasil) – need lots of them!
- Banjo-style (280 μL per 1 mm path length) or rectangular (170 μL per 1 mm path length) cells can be used
- Path lengths are only good to 1%, so good idea to measure sample and solvent background in the same cell if practical, but experiment logistics may prohibit that, so calibrate cells?
- High incoherent scattering for ^{1}H means you always want ≤ 1mm $^{1}H_2O$ in the neutron beam to avoid multiple scattering



Jacques & Trewhella (2010) "Small-angle Scattering for Structural Biology; Expanding the Frontier While Avoiding the Pitfalls," *Protein Science 19*, 642-657

Publication guidelines - IUCr

Acta Crystallographica – notes for authors

11.3. Small-angle scattering data

Guidelines for articles reporting structural modelling of small angle scattering may be found at

http://journals.iucr.org/services/sas/.

For articles that present experimental SAS data, the deposition of an ASCII file representing the background-corrected scattering profile(s) with errors is recommended.

draft requirements for presenting biological macromolecule small-angle scattering data

It is not the intention of this document to define a quality requirement for SAS experiments that would be acceptable for publication. Rather, the purpose is to outline the way in which SAS experiments should be presented in order to enable the reader to independently assess the quality of any interpretations made by the authors.

Publication guidelines - commentary

Jacques *et al.* (2012) *Acta Cryst.* **D64**, 620.

scientific comment

Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Publication guidelines for structural modelling of small-angle scattering data from biomolecules in solution

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Correspondence e-mail: mitchell.guss@sydney.edu.au

Small-angle scattering is becoming a mainstream technique for structural molecular biology. As such, it is important to establish guidelines for publication that will ensure that there is adequate reporting of the data and its treatment so that reviewers and readers can independently assess the quality of the data and the basis for any interpretations presented. This article presents a set of preliminary guidelines that emerged after consultation with the IUCr Commission on Small-Angle Scattering and other experts in the field and discusses the rationale for their application. At the 2011 Congress of the IUCr in Madrid, the Commission on Journals agreed to adopt these preliminary guidelines for the presentation of biomolecular structures from small-angle scattering data in IUCr publications. Here, these guidelines are outlined and the reasons for standardizing the way in which small-angle scattering data are presented.

Additional handy references

- Whitten, A. E. and **Trewhella, J.** "Small-Angle Scattering and Neutron Contrast Variation for Studying Bio-molecular Complexes," Microfluids, Nanotechnologies, and Physical Chemistry (Science) in Separation, Detection, and Analysis of Biomolecules, Methods in Molecular Biology Series, James W. Lee Ed., Human Press, USA, Volume 544, pp307-23, 2009.

- Neutron Scattering, A Primer, Roger Pynn, in LASCIENCE Summer 1990 http://www.fas.org/sgp/othergov/doe/lanl/pubs/number19.htm

- Neutron Scattering: Introduction and Neutron Scattering Theory, Roger Pynn http://www.ncnr.nist.gov/summerschool/ss13/pdf/Lecture_1_Theory.pdf

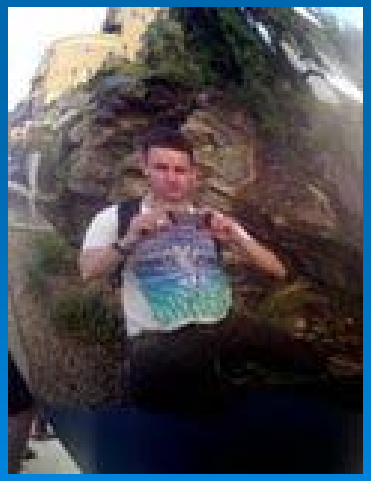
- An Introduction to Neutron and X-ray Scattering http://neutrons.ornl.gov/conf/nxs2009/pdf/IntroductoryLecturesPynn.pdf

- Jacques, D. A., Guss, J. M. and **Trewhella, J.** "Antikinases: their structures and roles in two-component signaling," in Two-component Systems in Bacteria, R. Gross and D. Beier Editors, Caister Academic Press, 978-1-908230-08-9, publication date August 2012.

Andrew Whitten

Cy Jeffries

Neutron Ted



James Taylor

John Chow

David Jacques