Small-angle scattering of x-rays (or neutrons) tells us about the size and shape of macromolecules.

\[ Q = \frac{4\pi \sin \theta}{\lambda} \]

Sample - randomly oriented particles

\[ r (\text{Å}) \]

\[ I (Q) \]

Shape restoration
Rigid body modeling

P(r)⇒ probable distribution of inter-atomic distances \((R_g, M, D_{max})\)

3Cp* RNA complex; Claridge et al. (2009) J. Struct. Biol. 166, 251-262
Neutron contrast variation by hydrogen (\(^1\text{H}\))/deuterium (\(^2\text{H}\)) exchange adds a powerful dimension to scattering data from bio-molecular complexes.

**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.
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$_2$O (~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 and molecular crowding

- HCaM measurement was done in 42% D$_2$O 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 $^1$H is a constant with Q.

Note effects of incoherent scattering from $^1$H on backgrounds.
Synaptic Connections & mutations implicated to Autism

Neuroligin – post synaptic extracellular domains

β-neurexin - presynaptic

Stalk region

TMD
P(r) function of NL1-638 shows domain dispositions of the initial homology need refinement

<table>
<thead>
<tr>
<th>Sample</th>
<th>Rg (Å)</th>
<th>Vol (Å³) Experimental</th>
<th>Vol (Å³) Calculated</th>
</tr>
</thead>
<tbody>
<tr>
<td>NL1-638</td>
<td>41.44 ± 0.2</td>
<td>184,172 ± 7,778</td>
<td>199,261</td>
</tr>
</tbody>
</table>

Distance (Angstroms)

P(r) arbitrary units
Shape restoration results using X-ray scattering data from NL1 complexed with $\beta$ neurexin

50% of the reconstructions were similar to the shape shown here, while the other 50% gave shapes that were inconsistent with biochemical data.

To eliminate any uncertainty from the observed degeneracy in the set of shapes that fit the X-ray data, we turned to neutrons.
Solvent matching experiment

NL1 complexed with deuterated β neurexin in ~40% D₂O to solvent match the NL1 in the neutron experiment.
Co-refinement of the β neurexin positions and orientations with respect to NL1 give a model against the X-ray and neutron data gives us a model that we can map autism-linked mutations.

Superposition of SANS scattering and crystal structure for NL-NX

Contrast variation

- To determine the shapes and dispositions of labeled and unlabelled components in a complex
- Requires $\geq 5 \times 200\text{-}300\mu\text{L} (= 1 – 1.5\text{mL})$ of your labeled complex at $\geq 5 \text{ mg/ml}$.
- 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 sensor histidine kinase KinA - response regulator spo0A in *Bacillus subtilis*

- **Failure to initiate DNA replication**
  - **DNA damage**
  - **Change in N₂ source**
  - **Sda**
    - **KipA**
    - **KipI**

- **KinA**
  - **Spo0F**
  - **Spo0B**
  - **Spo0A**

- **Sporulation**

- **Environmental signal**
More of our molecular actors

**KinA**

*Based on H853 Thermotoga maritima*

- **Sda**
- **KipI**
- **Pro**$^{410}$
- **DHp**
- **His**$^{405}$
- **CA**

*Pyrococcus horikoshi*
HK853 based KinA model predicts the KinA
KinA$_2$ contracts upon binding 2 Sda molecules

**KinA$_2$**  \( R_g = 29.6 \) Å, \( d_{\text{max}} = 95 \) Å

**KinA$_2$-Sda$_2$**  \( R_g = 29.1 \) Å, \( d_{\text{max}} = 80 \) Å
Use $R_g$ (from MULCh) for Sturhman analysis

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

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

$R_H = 25.40 \text{ Å}$
$R_D = 25.3 \text{ Å}$
$D = 27.0 \text{ Å}$
Use Compost (from MULCh) to solve for $I(Q)_{11}, I(Q)_{22}, I(Q)_{12}$

$$I(Q) = \Delta \rho_1^2 I_{11}(Q) + \Delta \rho_2^2 I_{22}(Q) + \Delta \rho_1 \Delta \rho_2 I_{12}(Q)$$
Histidine kinase-antikinase, \( \text{KinA}_2^{-2\text{DSda}} \)

Histidine kinase-antikinase, \textit{KinA}_2-2^D\text{Kip}!
Pull down assays and Trp fluorescence show mutation of Pro$^{410}$ abolishes KipI binding to KinA but Sda can still bind.

Trp fluorescence confirms that the C-domain of KipI interacts with KinA.
KipI-C domain has a cyclophilin-like structure

3Å crystal structure
KipI-C domain

Overlay with cyclophilin B
Aromatic side chain density in the hydrophobic groove

The KinA helix containing Pro$^{410}$ sits in the KipI-C domain hydrophobic groove.
A possible role for cis-trans isomerization of Pro$^{410}$ in tightening the helical bundle to transmit the Kipl signal to the catalytic domains?

Or is the Kipl cyclophilin-like domain simply a proline binder?
DHp helical bundle is a critical conduit for signaling.
The N-terminal regulatory domains of the cardiac myosin binding protein C (cMyBP-C) influence motility.
cMyBP-C in Muscle Contraction

- cMyBP-C plays **structural** and **regulatory** roles in striated muscle sarcomeres. However, the specific details of how it interacts with actin and myosin are unclear.
SAXS data + crystal and NMR structures of individual modules show the N-terminal domains of mouse cMyBP-C form an extended structure with a defined disposition of the modules.

Neutron contrast variation on actin thin-filaments with deuterated C0C2 show they bind actin and stabilize filaments.

Mixing mono-disperse solutions of cMyBP-C with actin results in a dramatic increase in scattering signal due to the formation of a large, rod-shaped assembly.
SANS data show regulatory cMyBP-C domains (mouse) stabilise F-actin and provide a structural hypothesis for the observed Ca\(^{2+}\)-signal buffering effect.

Whitten, Jeffries et al. (2008) *PNAS* 105, 18360
SAXS data show significant species differences

**Correlation between % Pro/Ala composition in the C0-C1 linker and heart rate from different organisms** (Shaffer and Harris (2009) *J. Muscle Res. Cell Motil.* 30:303-306.)

Jeffries, Lu et al. (2011) *J. Mol. Biol.* 414, 735-748
SAXS data cannot define relative positions of human C0 and C1.
NMR relaxation data show human P/A_\text{L} is flexible.
2D reconstruction of human C0C1-actin assembly from neutron contrast series consistent with C0 binding with a flexible and extended $P/A_L$.
NMR data identify residues involved in (human) C0-actin interaction
Actin Binding Hot-spots

Shared Actin and Myosin Binding Sites

Lu, Jeffries et al. (2011) *J. Mole. Biol.* 413, 908-913
Switching Facilitated by Flexible P/A Linker Regulated by Phosphorylation?

By combining EM, Crystallography, SANS, SAXS and NMR, we show that

- Human C0C1 interacts with actin specifically and promotes formation of regular assemblies of F-actin decorated by C0C1.
- Human C0 and C1 interact with myosin and actin using a common set of binding determinants.
- NMR and SAXS data indicate that P/A linker is flexible and can facilitate N-terminal domains spanning the interfilament distances.
- The switching could be regulated by phosphorylation of the motif?
Calmodulin: linking cMyBP-C with Ca\textsuperscript{2+} signaling pathways to coordinate phosphorylation events and synchronise the multiple interactions between cMyBP-C, myosin and actin during the heart muscle contraction?

The motif of human cMyBP-C is required for its Ca\textsuperscript{2+}-dependent interaction with calmodulin (CaM).

Ca\textsuperscript{2+}-CaM addition to \textsuperscript{15}N-C1C2 results in significant intensity changes for \textasciitilde66\% of the amide resonance peaks mapped to the structured region of the motif (W322 in the motif disappears on the first addition).

Identification of CaM residues affected by C1C2 binding (NMR intensity changes).
Small-angle X-ray Scattering indicates CaM is in an semi-extended conformation when bound to its binding domain in cMyBP-C (Cpep)
CaM may act as a structural conduit that links cMyBP-C with Ca$^{2+}$ signaling pathways to help coordinate phosphorylation events and synchronise the multiple interactions between cMyBP-C, myosin and actin during the heart muscle contraction.