Joint Application of SAXS and SANS

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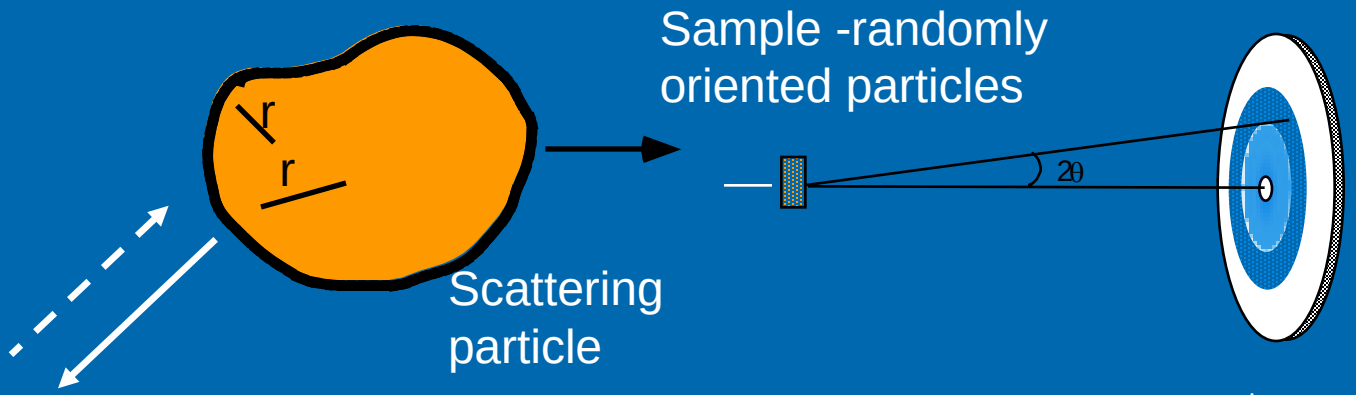
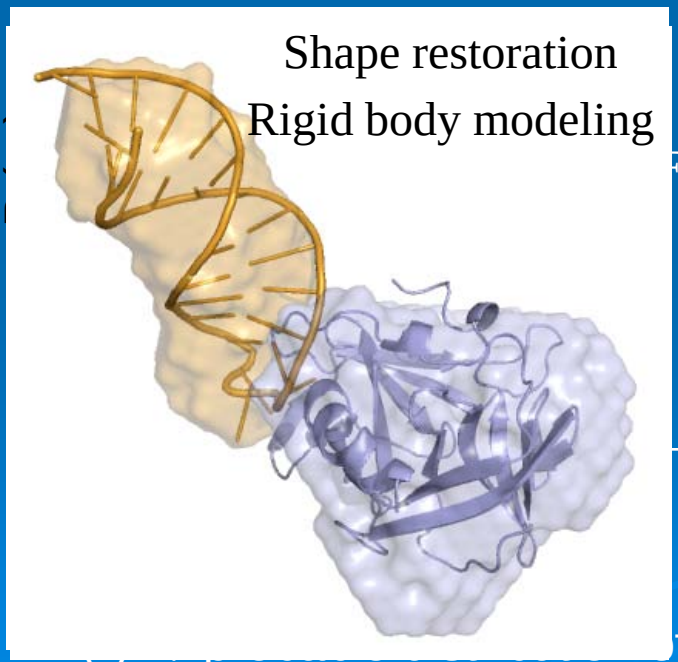
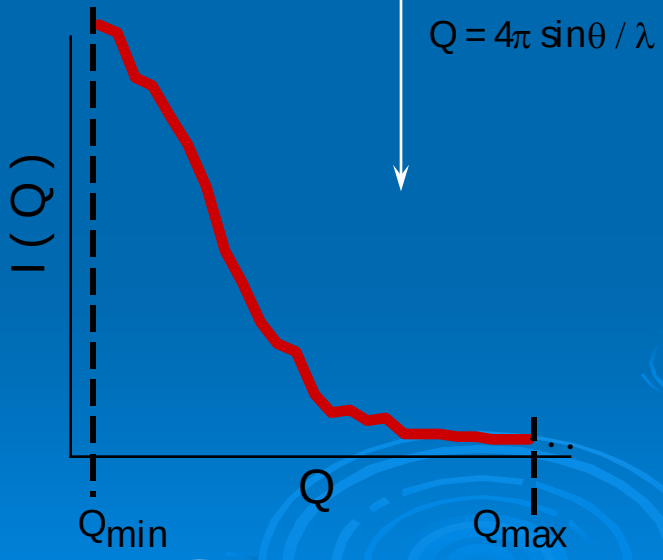
University of Sydney

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





Fourier transform



f

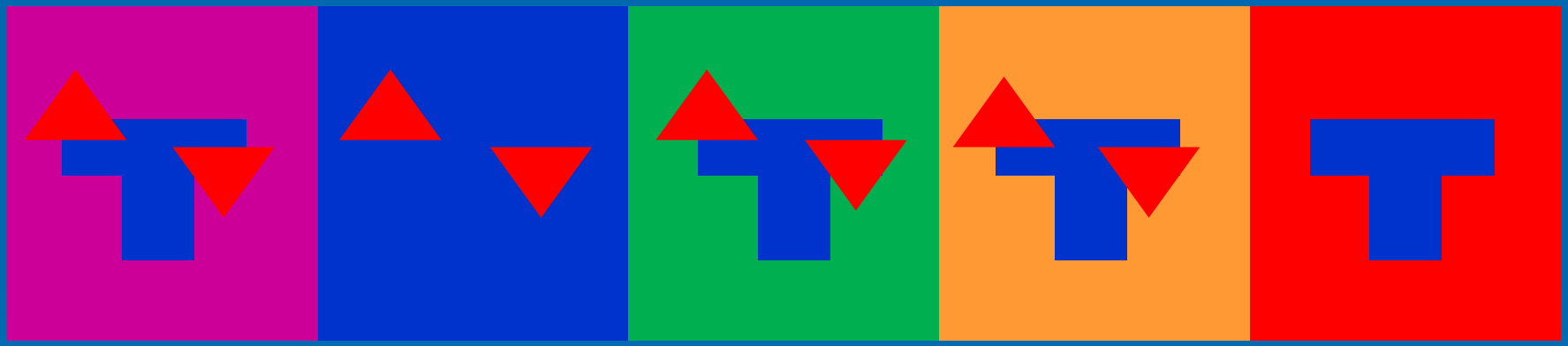
inter-atomic distances (R_g, M, D_{max})

3C^{protease}-RNA complex; Claridge et al. (2009) *J. Struct. Biol. 166*, 251-262

Some review:

- SAS data represent a time and ensemble average of randomly oriented structures; the rotational-averaging of 3D structures yields a 1D profile
- The SAS experiment is conceptually simple, but practically demanding; both instrumentally and with respect to samples
- SAS data primarily tell us about shape; as shapes become more complex, different shapes can yield the same scattering profile
- *Nonetheless, certain parameters, can be determined both accurately and precisely* (R_g, *Molecular mass*, distance distributions over a wide range 5 – 1000's Å) *and 3D models developed or tested against SAS data can advance our understanding of bio-molecular structure/function relationships*

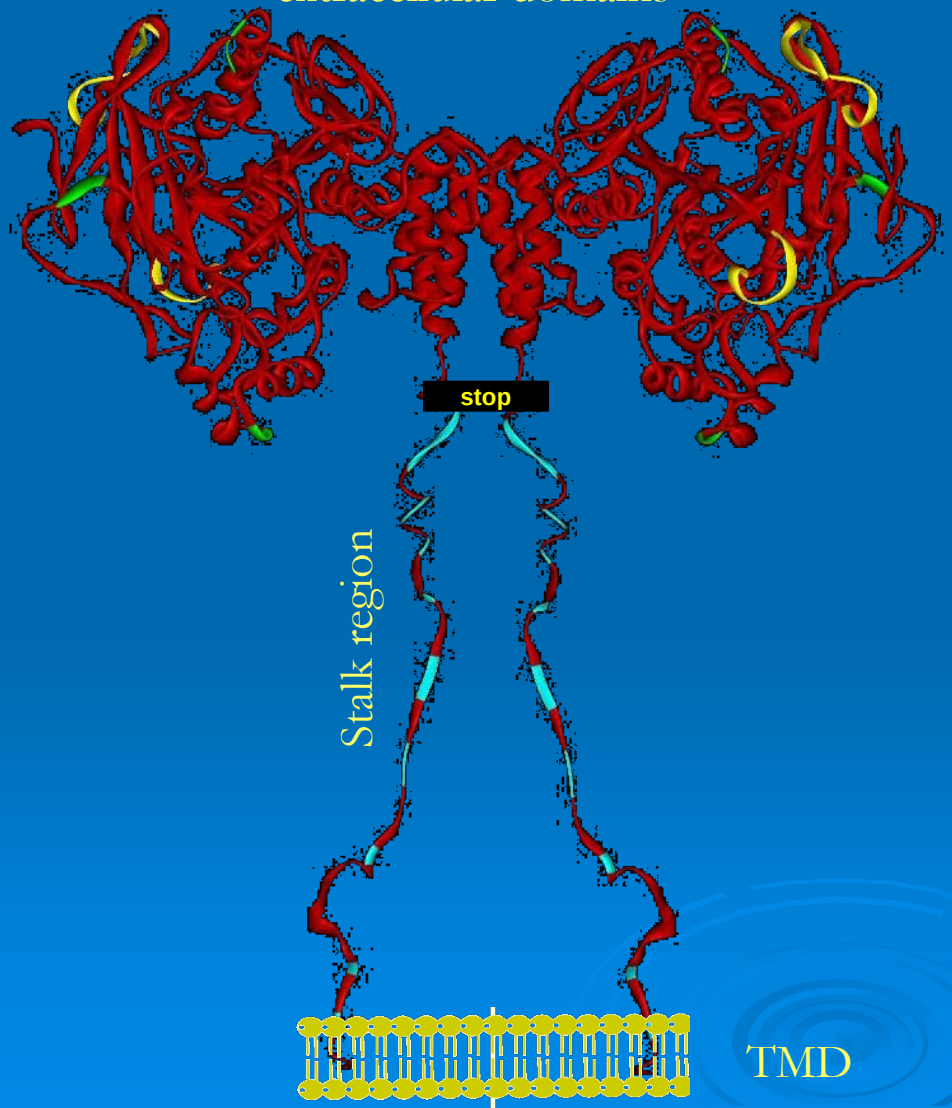
Neutron contrast variation by **hydrogen (^{1}H)**/**deuterium (^{2}H)** exchange adds a powerful dimension to scattering data from bio-molecular complexes



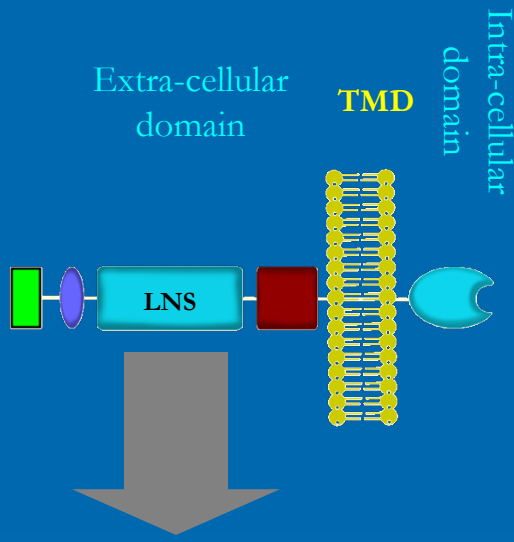
Synaptic Connections & mutations implicated to Autism

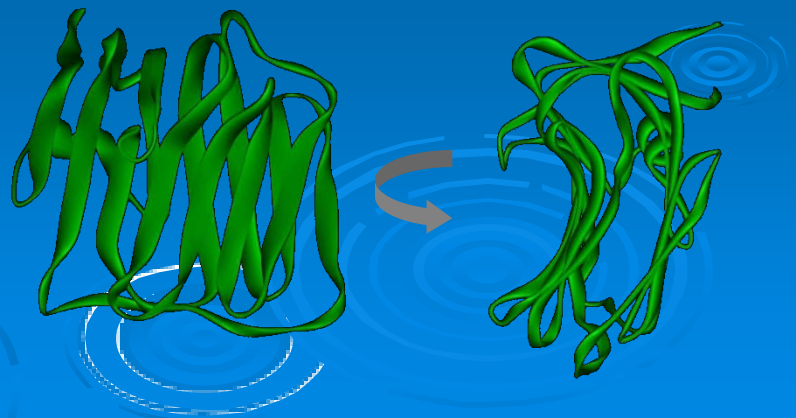
Neuroligin –post synaptic

extracellular domains



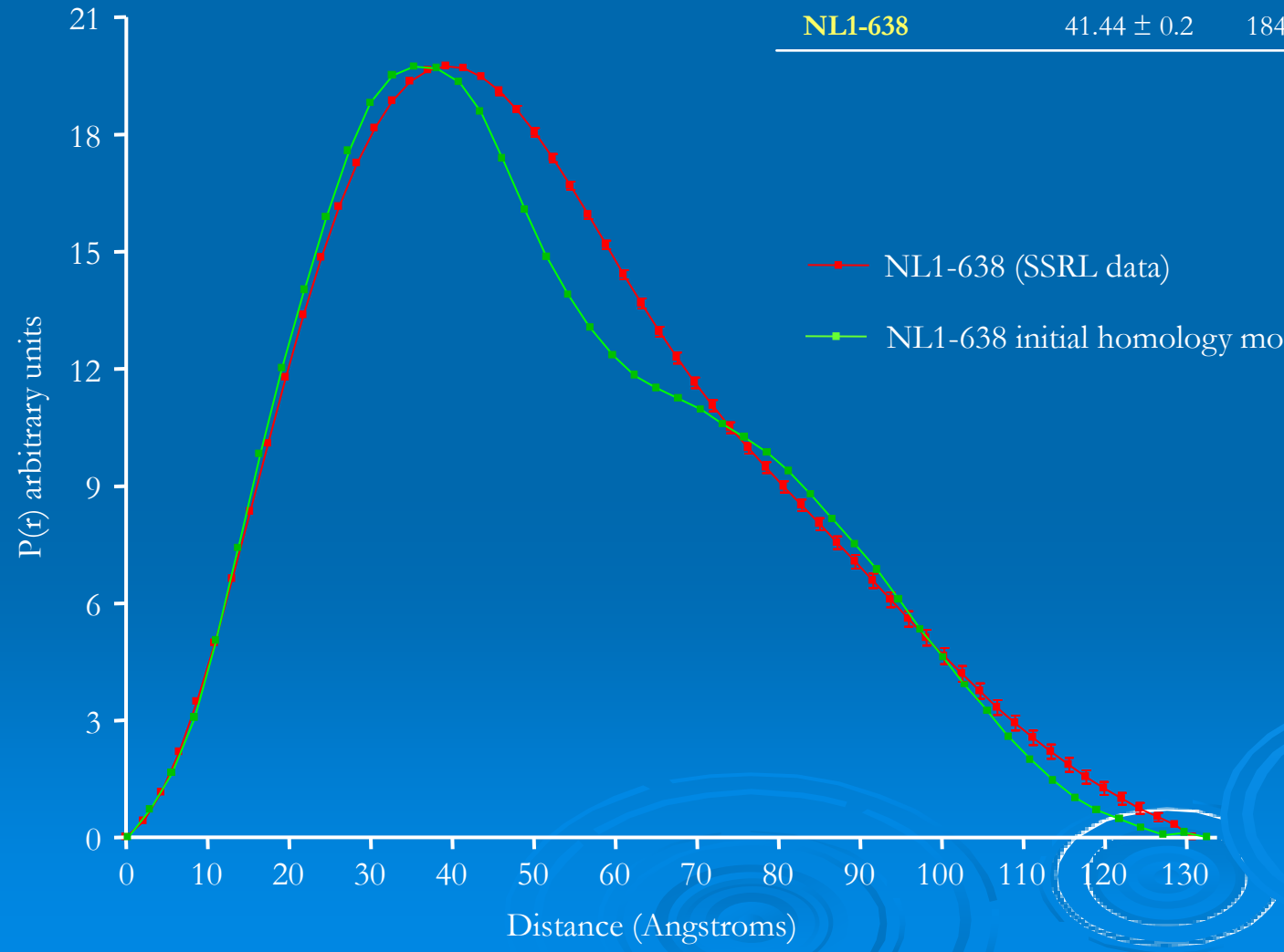
β-neurexin - presynaptic

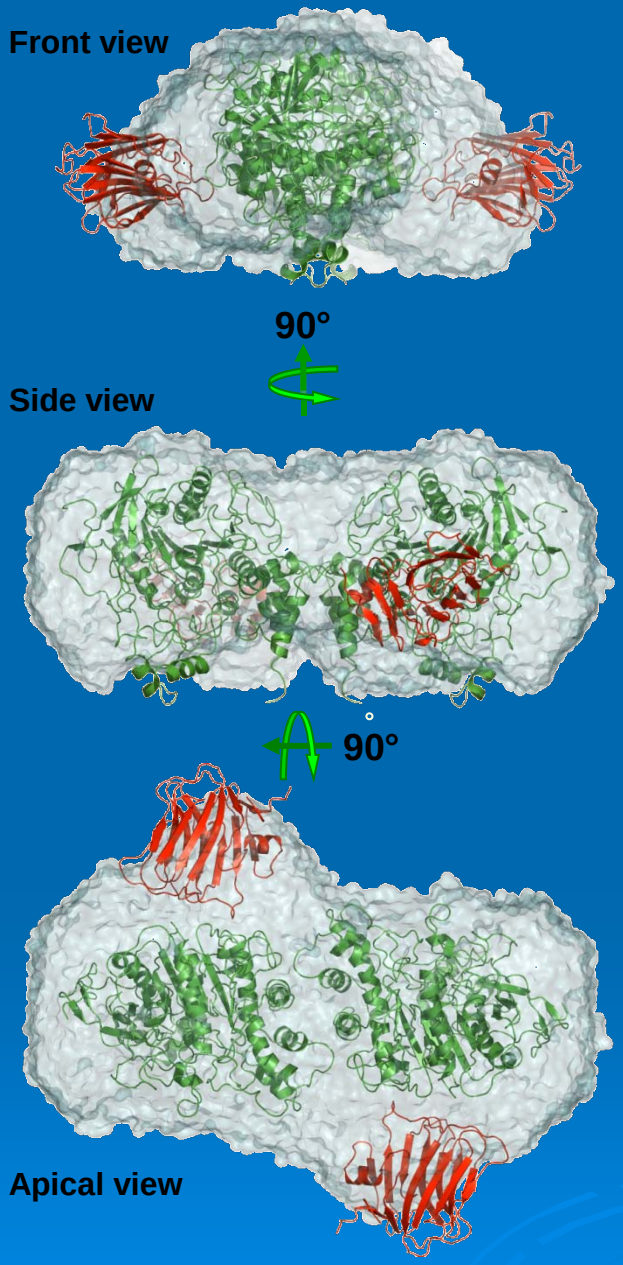




P(r) function of NL1-638 shows domain dispositions of the initial homology need refinement

Sample	Rg (Å)	Vol (Å^3) Experimental	Vol (Å^3) Calculated
NL1-638	41.44 ± 0.2	184,172 ± 7,778	199,261

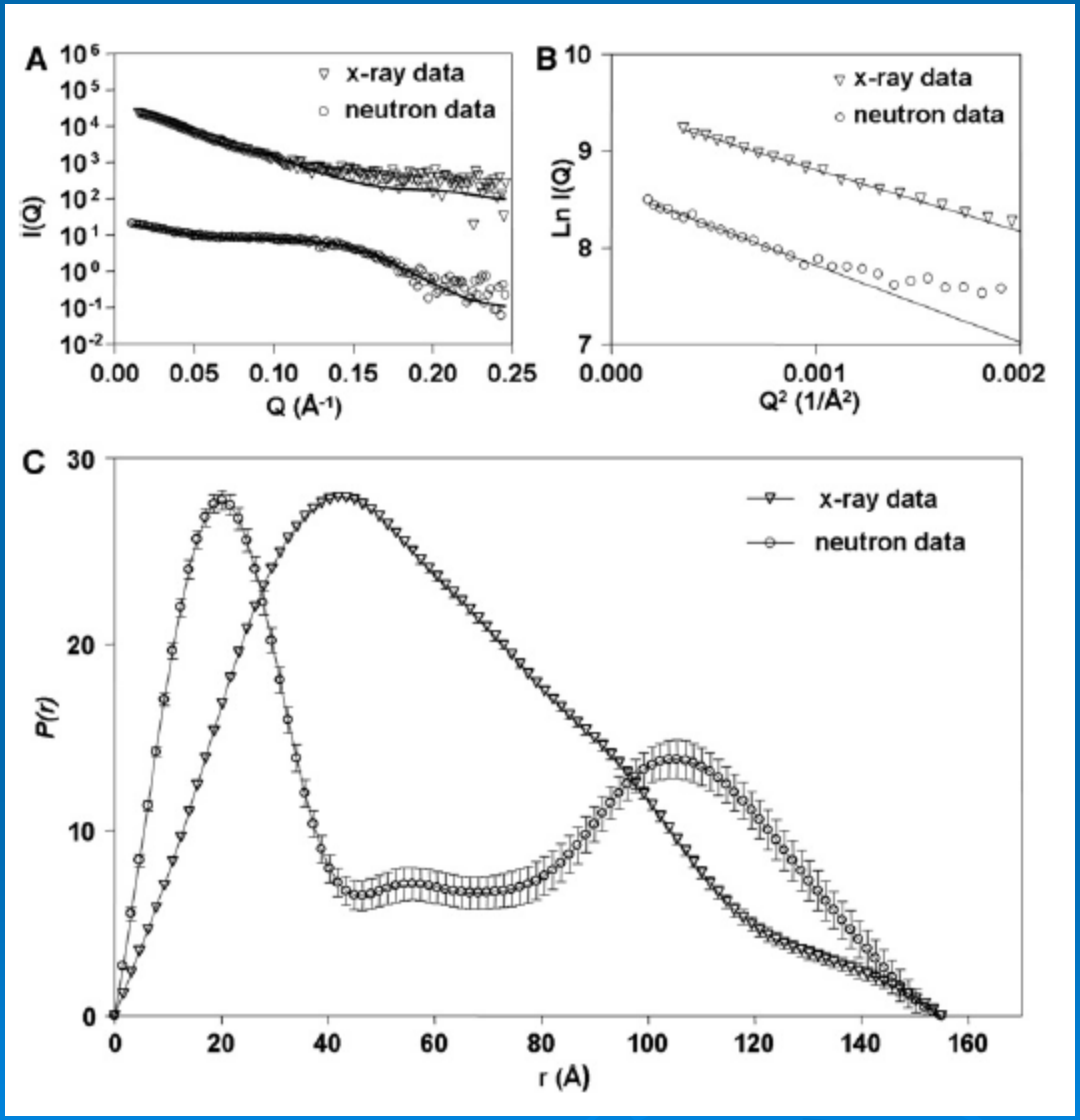




Shape restoration results using X-ray scattering data from NL1 complexed with β neurexin

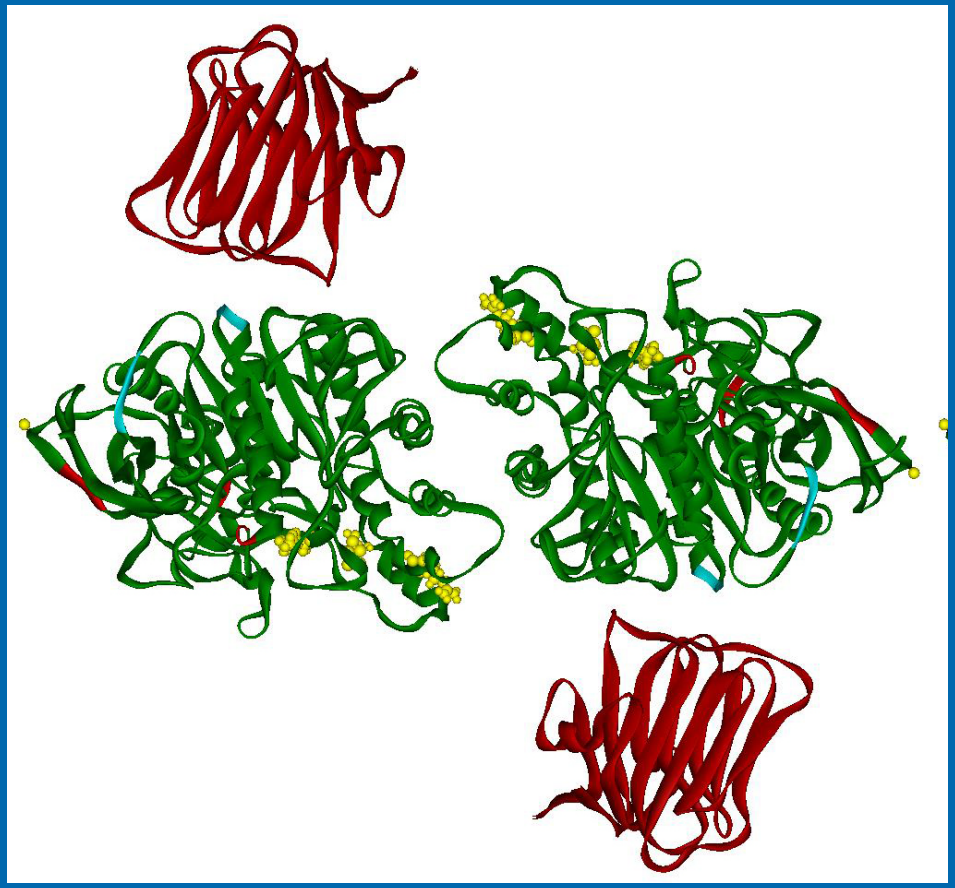
50% of the reconstructions were similar to the shape shown here, while the other 50% gave shapes that were inconsistent with biochemical data.

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

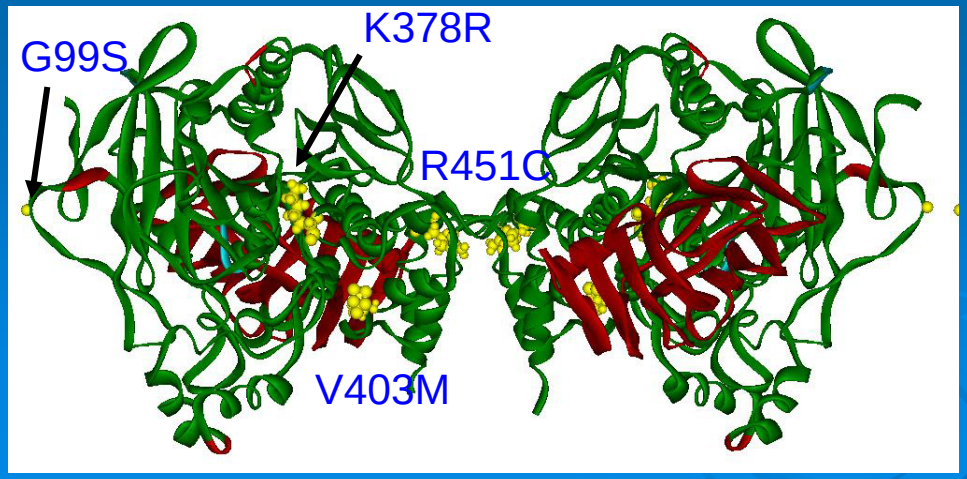


Solvent matching experiment

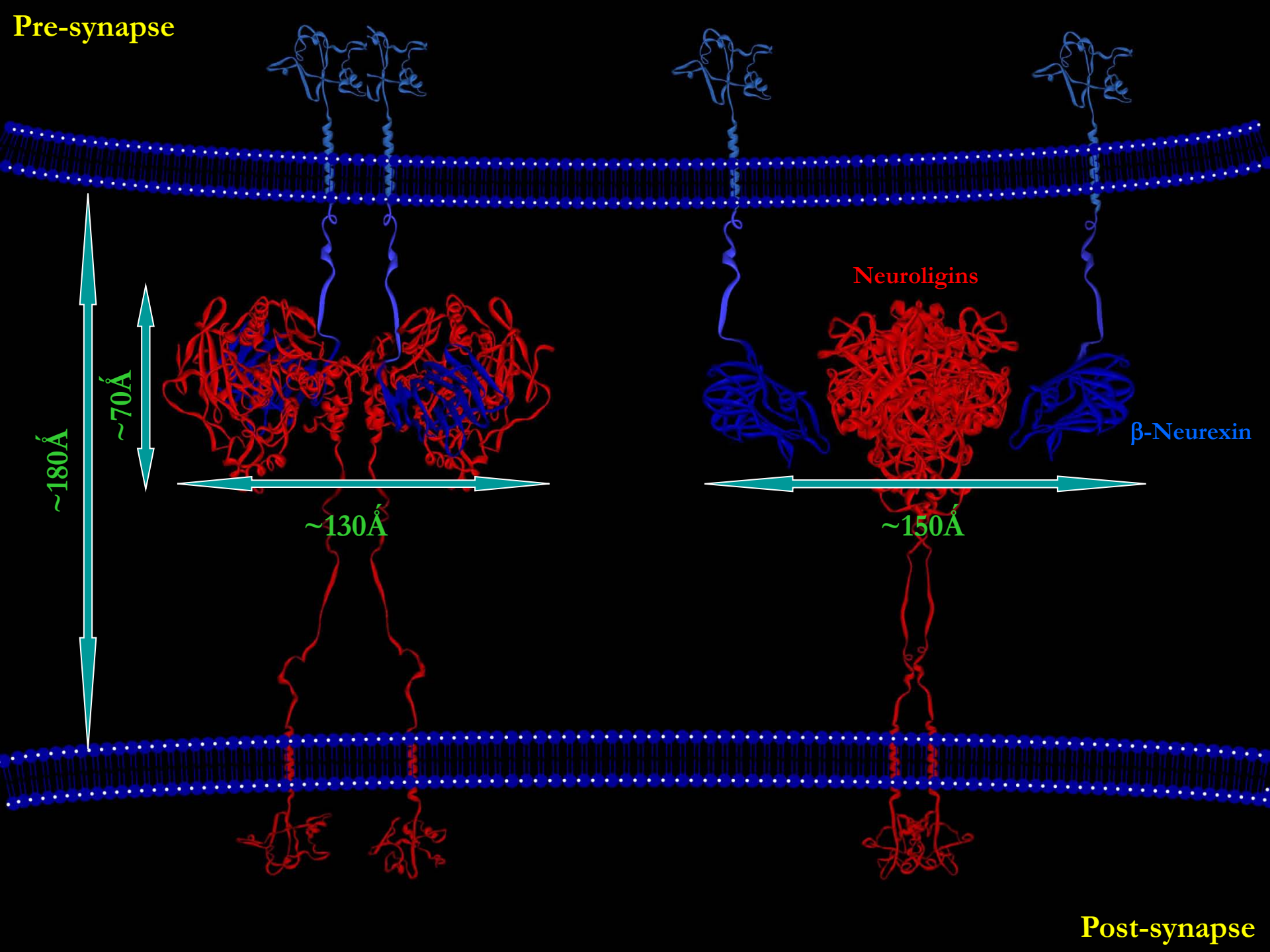
NL1 complexed with deuterated β neurexin in ~40% D_2O to solvent match the NL1 in the neutron experiment.



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



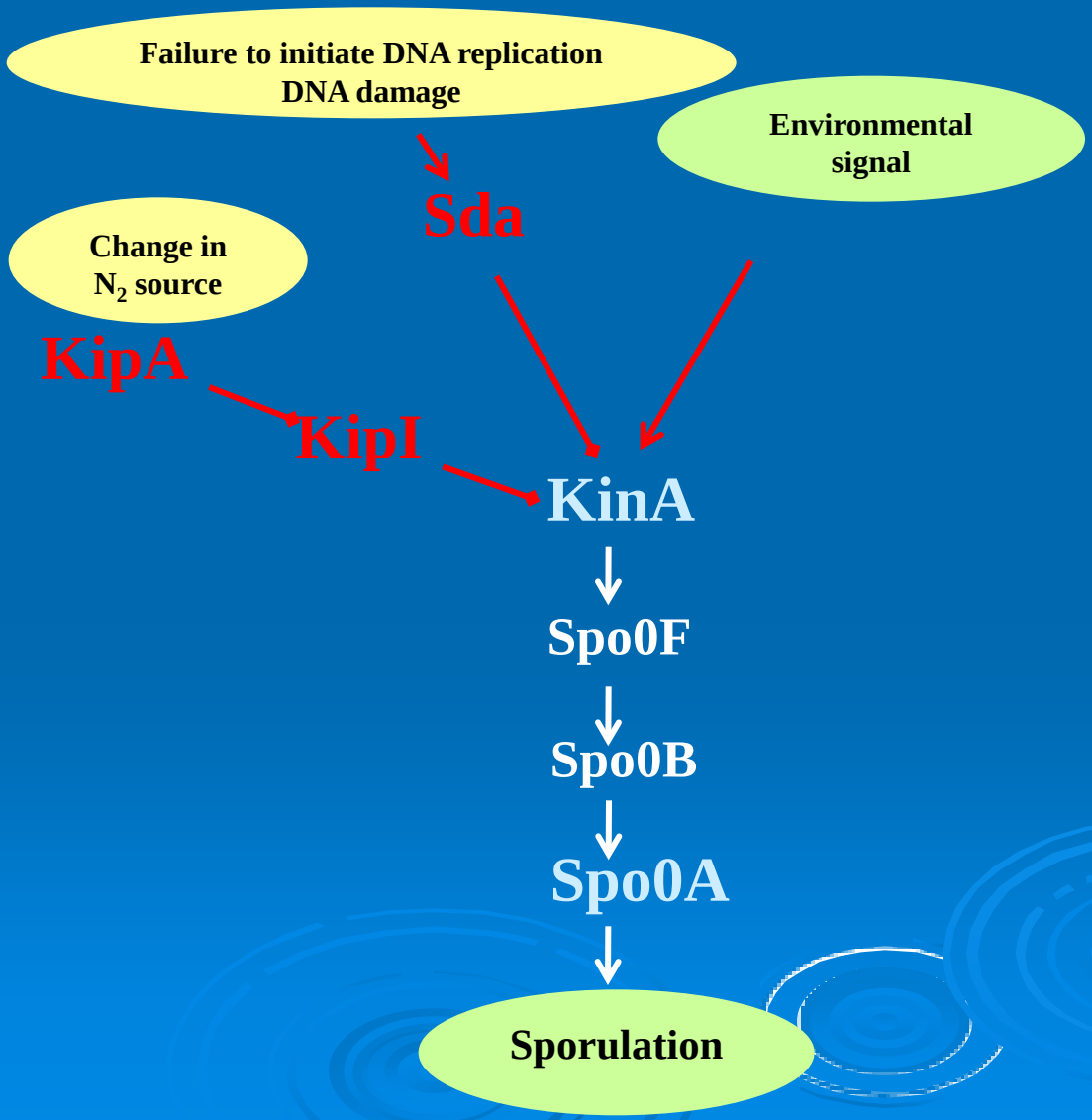
Comoletti, Grishaev, Whitten et al. *Structure 15*, 693-705, 2007.


Pre-synapse
~180Å
~70Å
~130Å
Neuroligins
β-Neurexin
~150Å
Post-synapse

Superposition of SANS scattering and crystal structure for NL-NX

Crystal Structure (3BIW) Arac et al. (2007) *Neuron* *56*, 992-1003

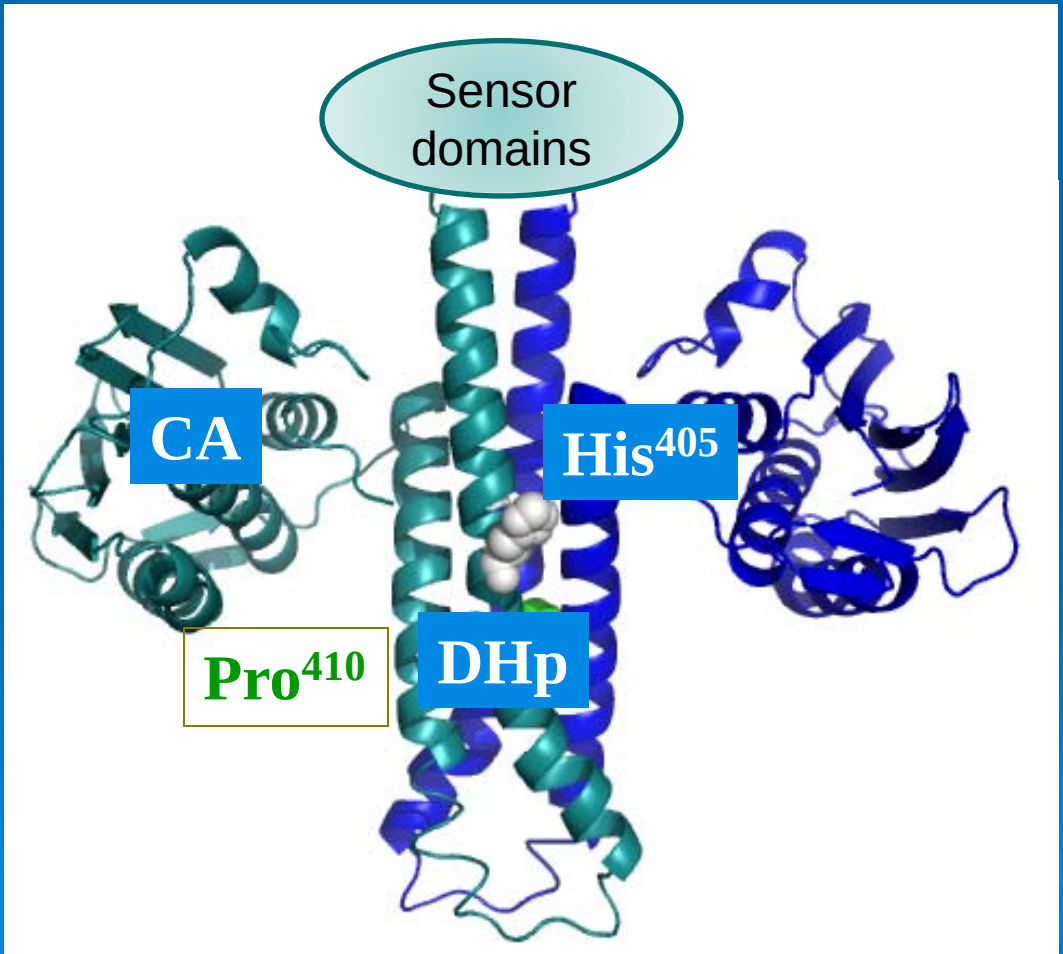
The sensor histidine kinase KinA - response regulator spo0A in *Bacillus subtilis*



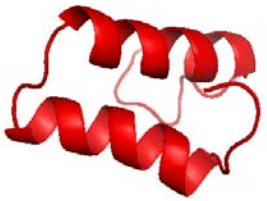
More of our molecular actors

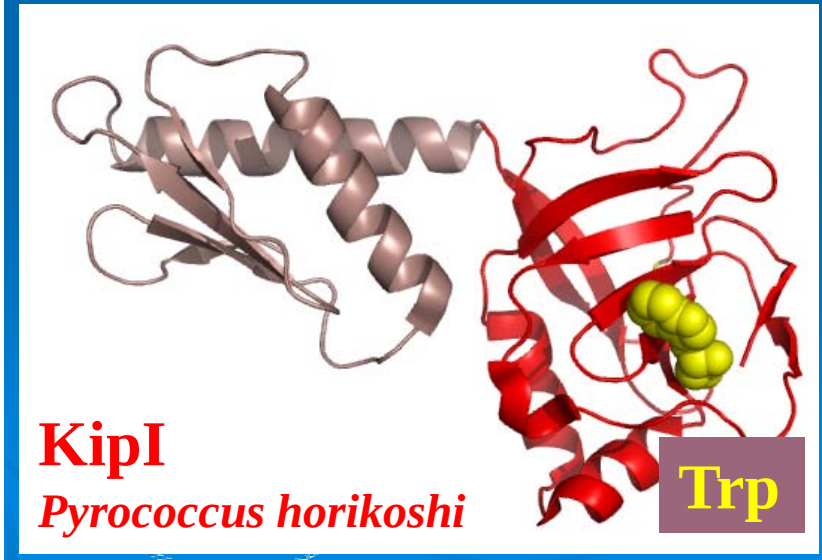
KinA

Based on H853 Thermotoga maritima

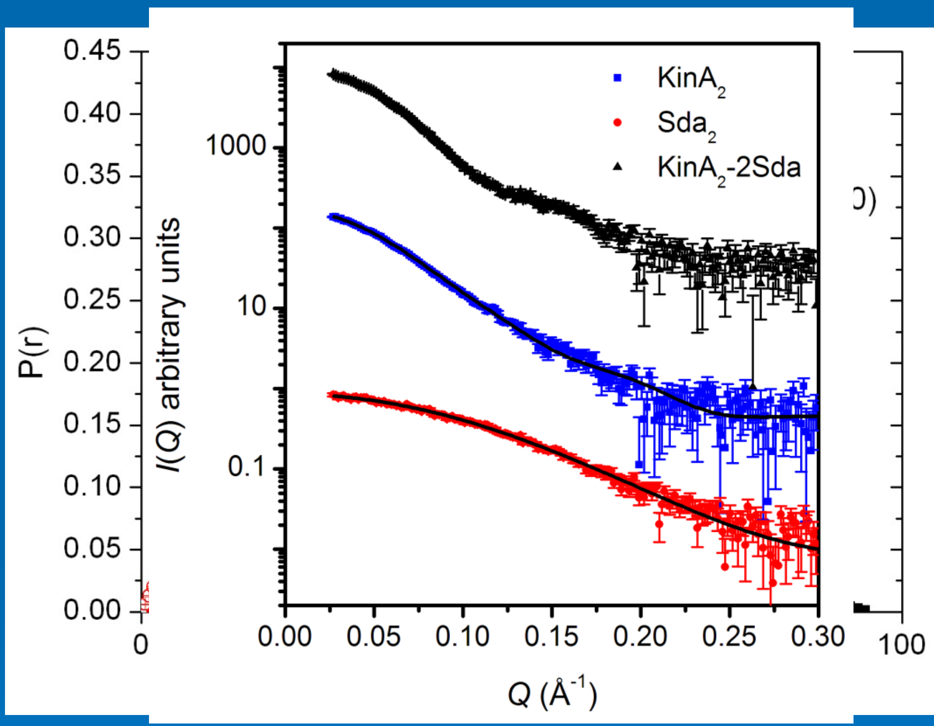


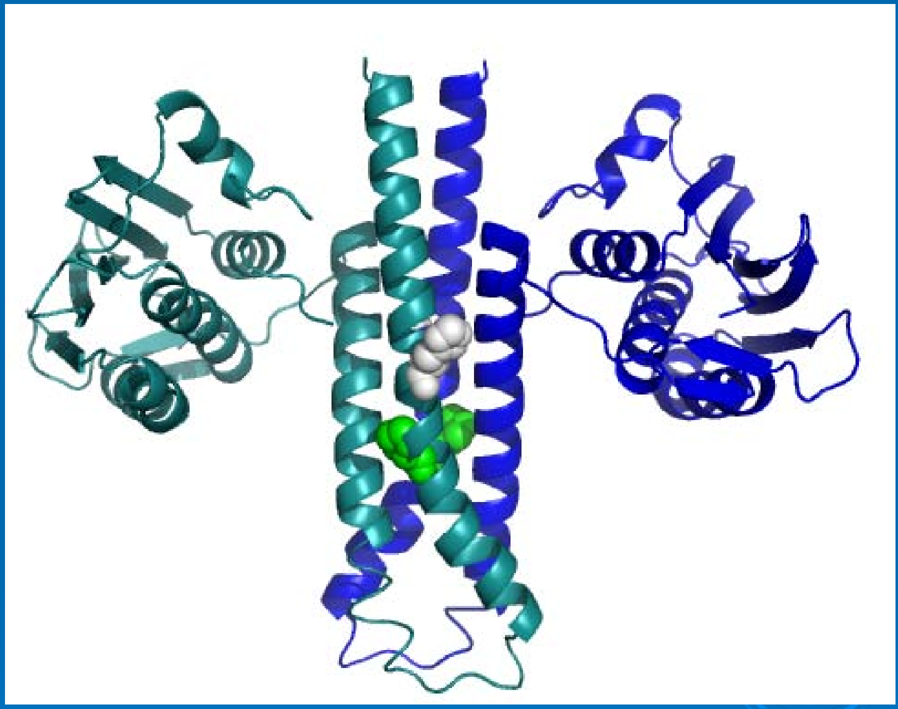
Sda





$KinA_2$ contracts upon binding 2 Sda molecules
HK853 based KinA model predicts the SAXS data



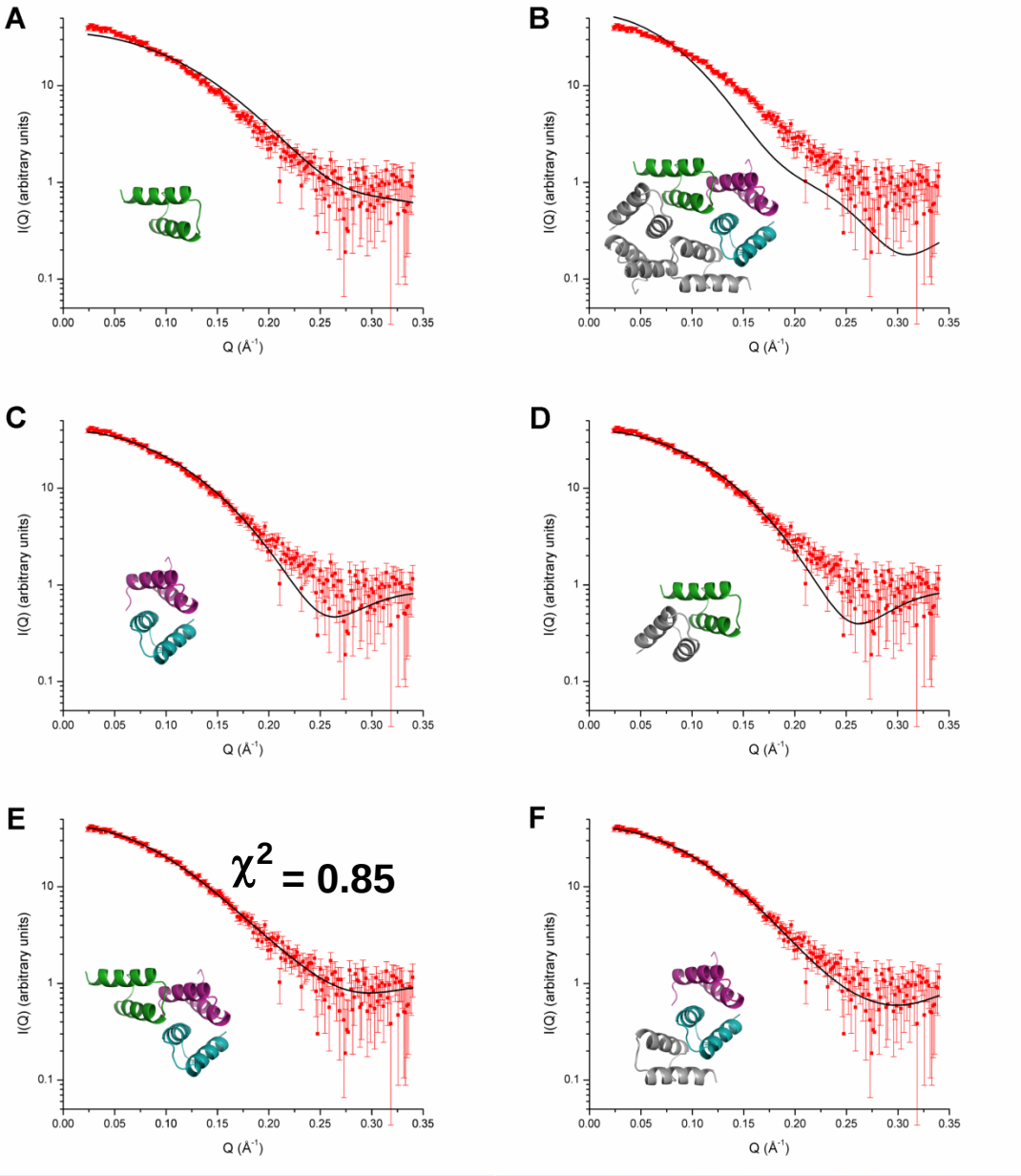


$KinA_2$ R_g = 29.6 Å, d_{max} = 95 Å

$KinA_2$-Sda_2 R_g = 29.1 Å, d_{max} = 80 Å

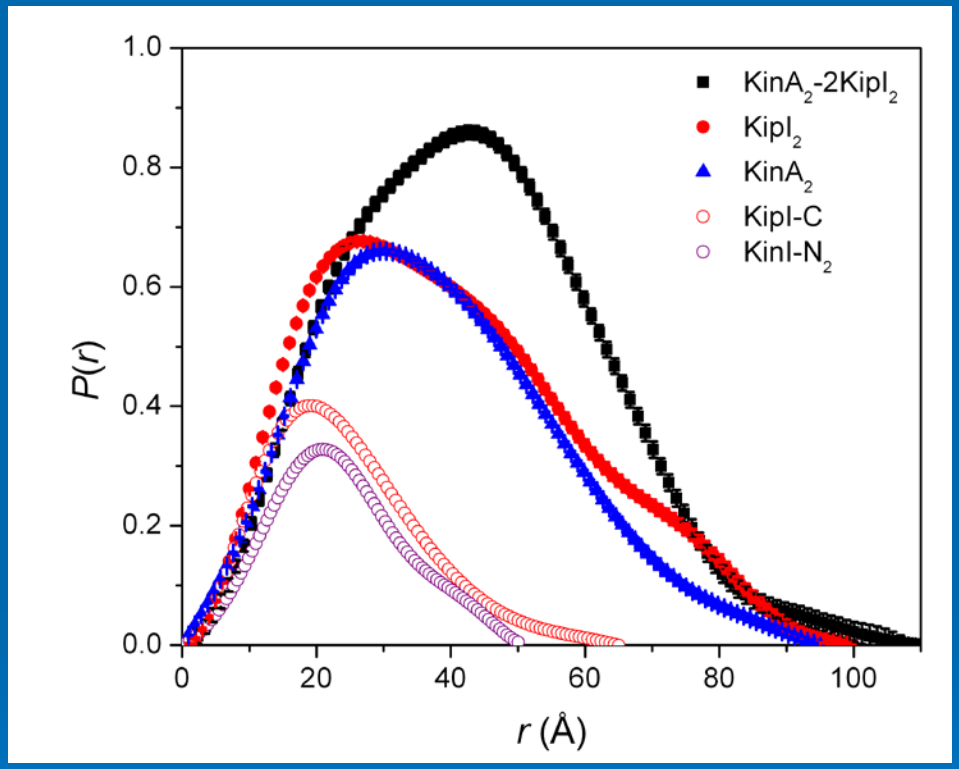
Sda? R_g = 15.4 Å, d_{max} = 55 Å

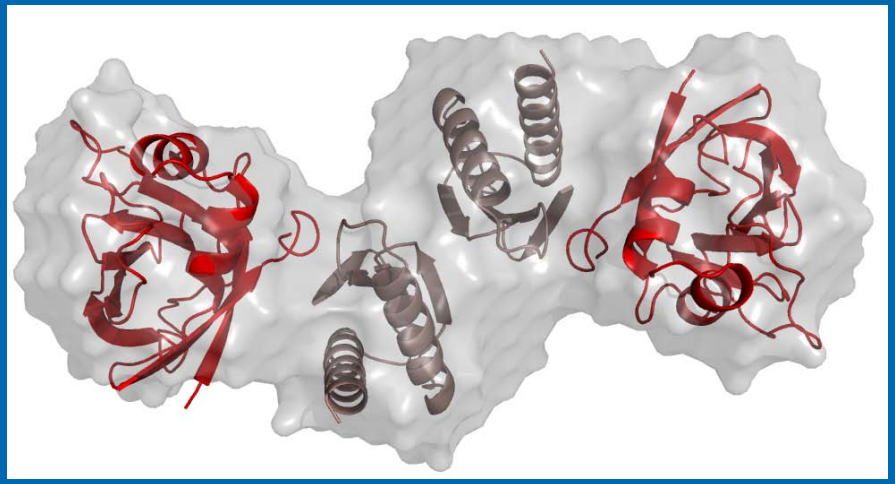
Unexpected result: Sda is a trimer in solution



Jacques, et al "Crystal Structure of the Sporulation Histidine Kinase Inhibitor Sda from *Bacillus subtilis* – Implications for the Solution State of Sda," *Acta D65*, 574-581, 2009.

KipI dimerizes via its N-terminal domains and 2 KipI molecules bind $KinA_2$



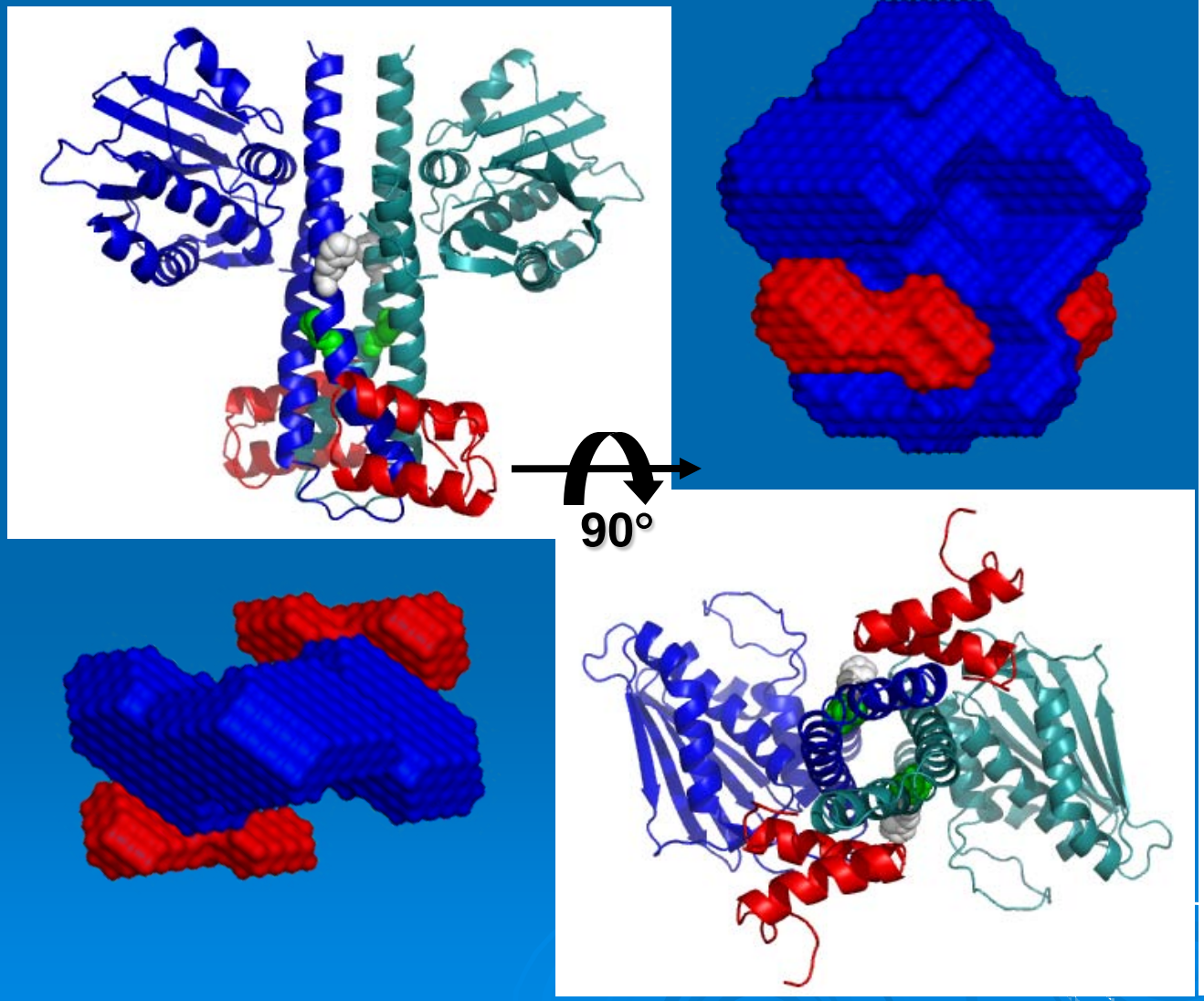


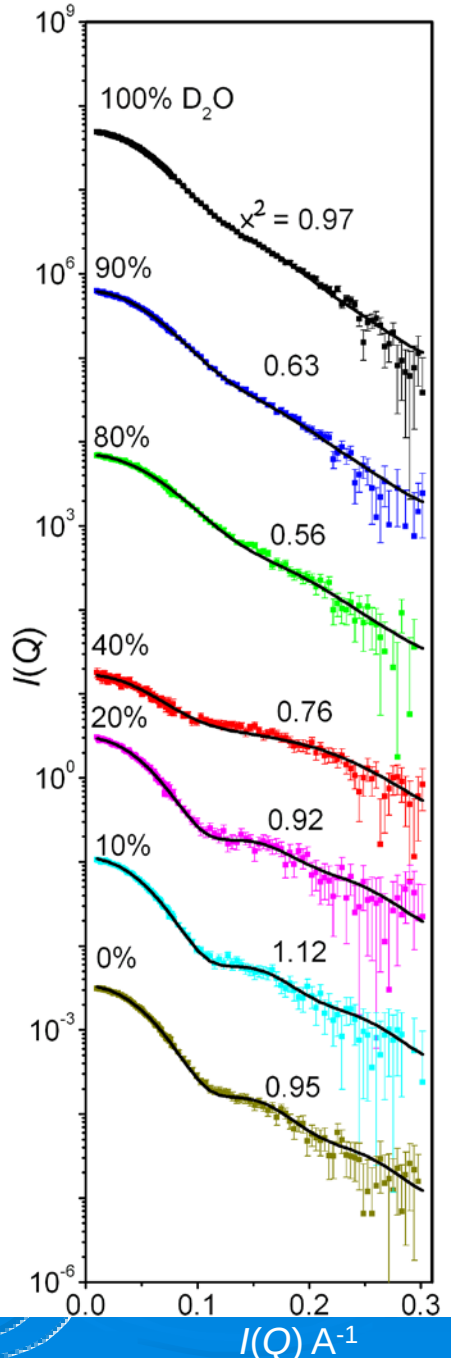
$KipI_2$ R_g = 31.3 Å, d_{max} = 100 Å

$KinA_2$ R_g = 29.6 Å, d_{max} = 80 Å

$KinA_2$-2KipI R_g = 33.4 Å, d_{max} = 100 Å

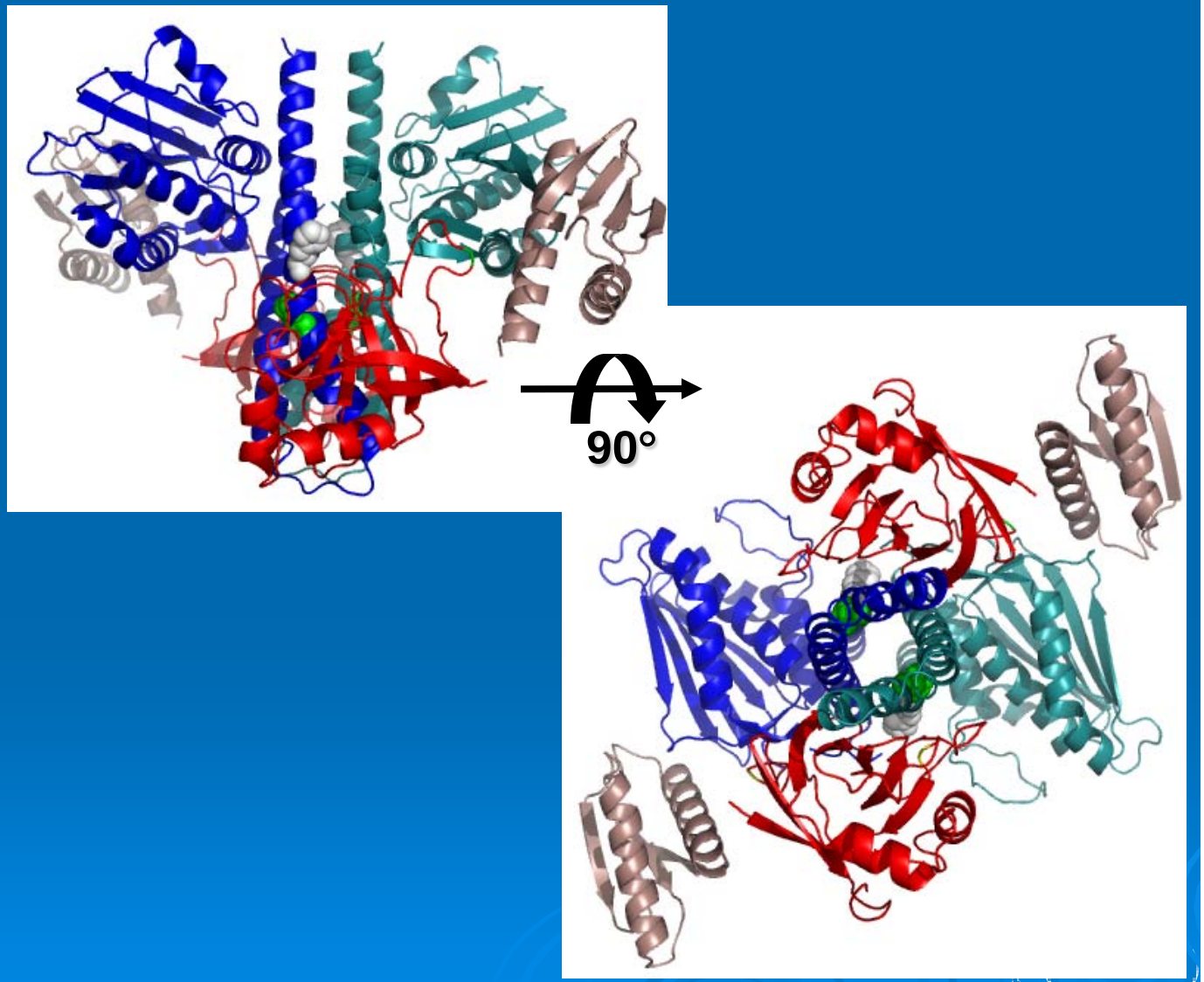
Histidine kinase-antikinase, $KinA_2$-$2^{D}Sda$

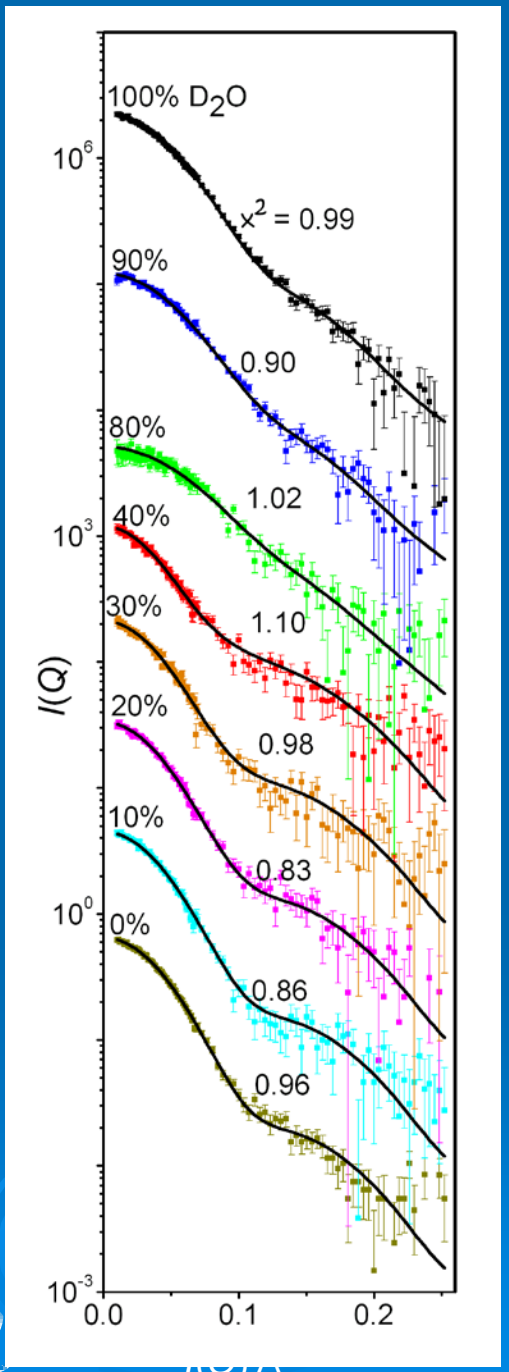




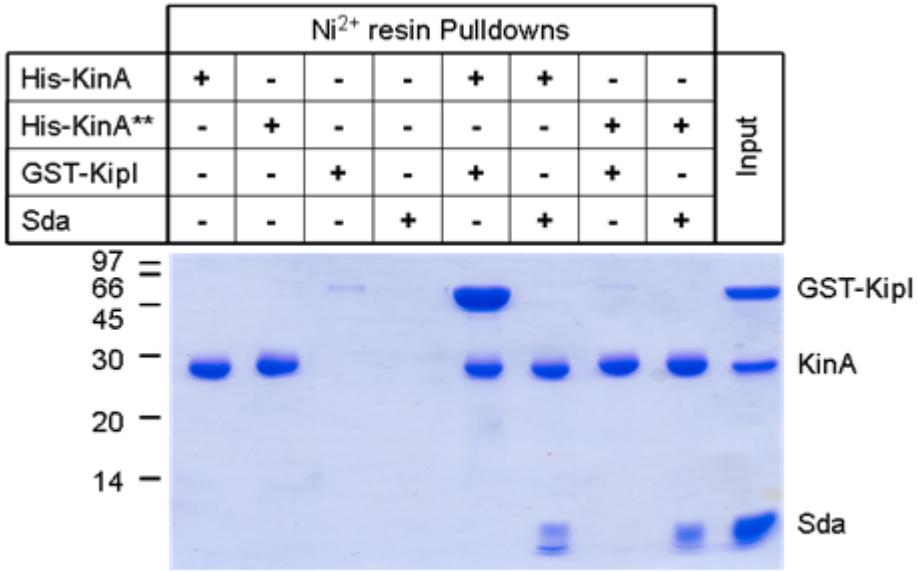
Whitten, Jacques, Langely et al., *J. Mol.Biol. 368*, 407, 2007

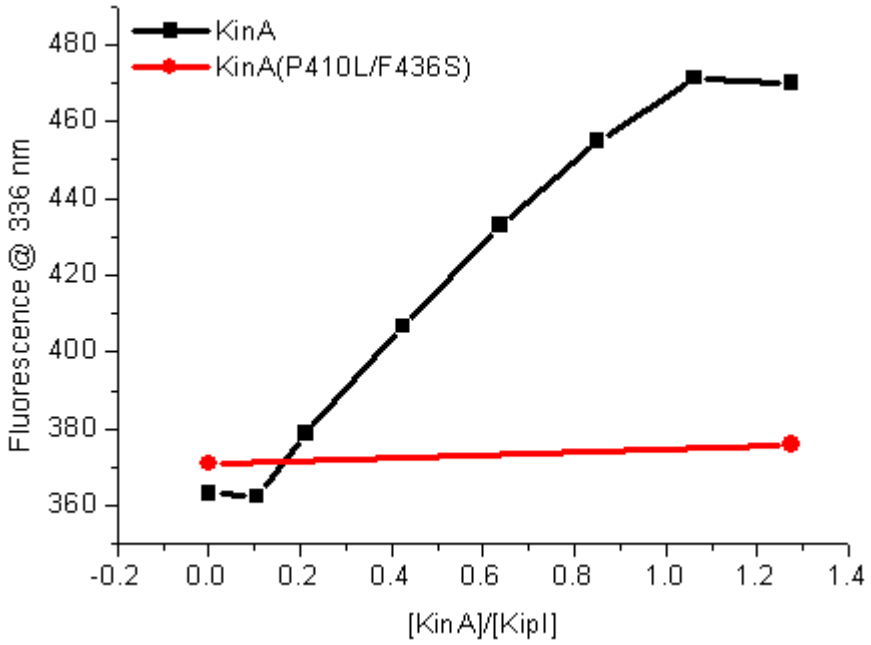
Histidine kinase-antikinase, $KinA_2$-$2^{D}Kip$!





Jacques, Langely, Jeffries et al, in press *J. Mol.Biol.* 2008

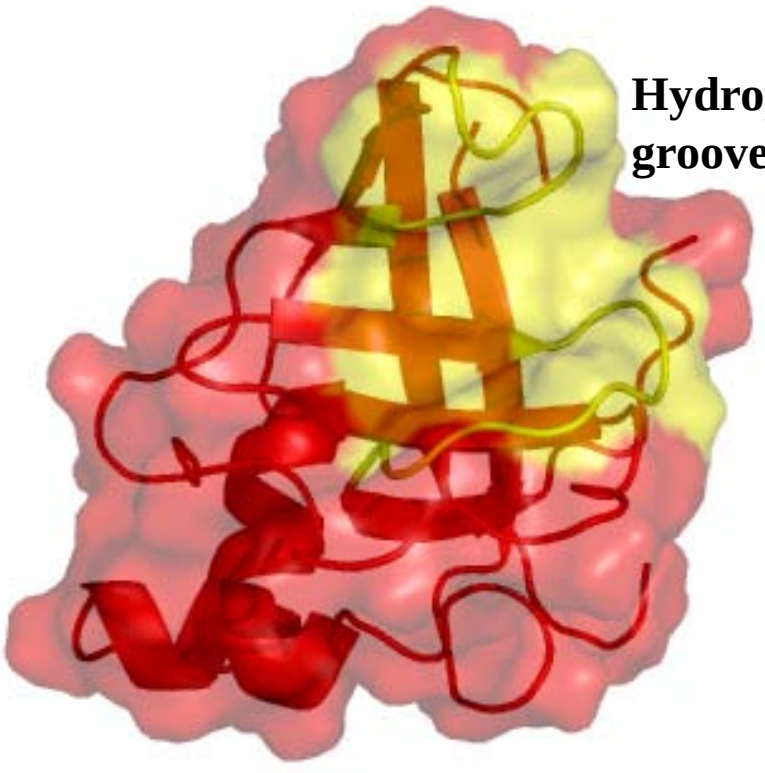




Pull down assays and Trp fluorescence show mutation of Pro^{410} abolishes KipI binding to KinA but Sda can still bind.

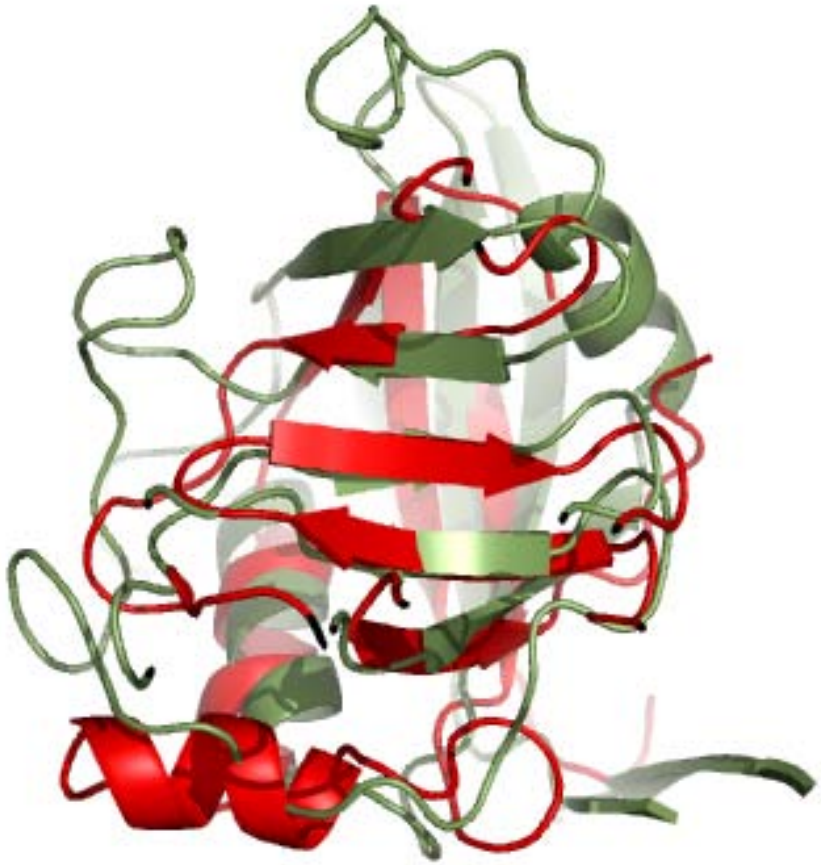
Trp fluorescence confirms that the C-domain of KipI interacts with KinA

KipI-C domain has a cyclophilin-like structure



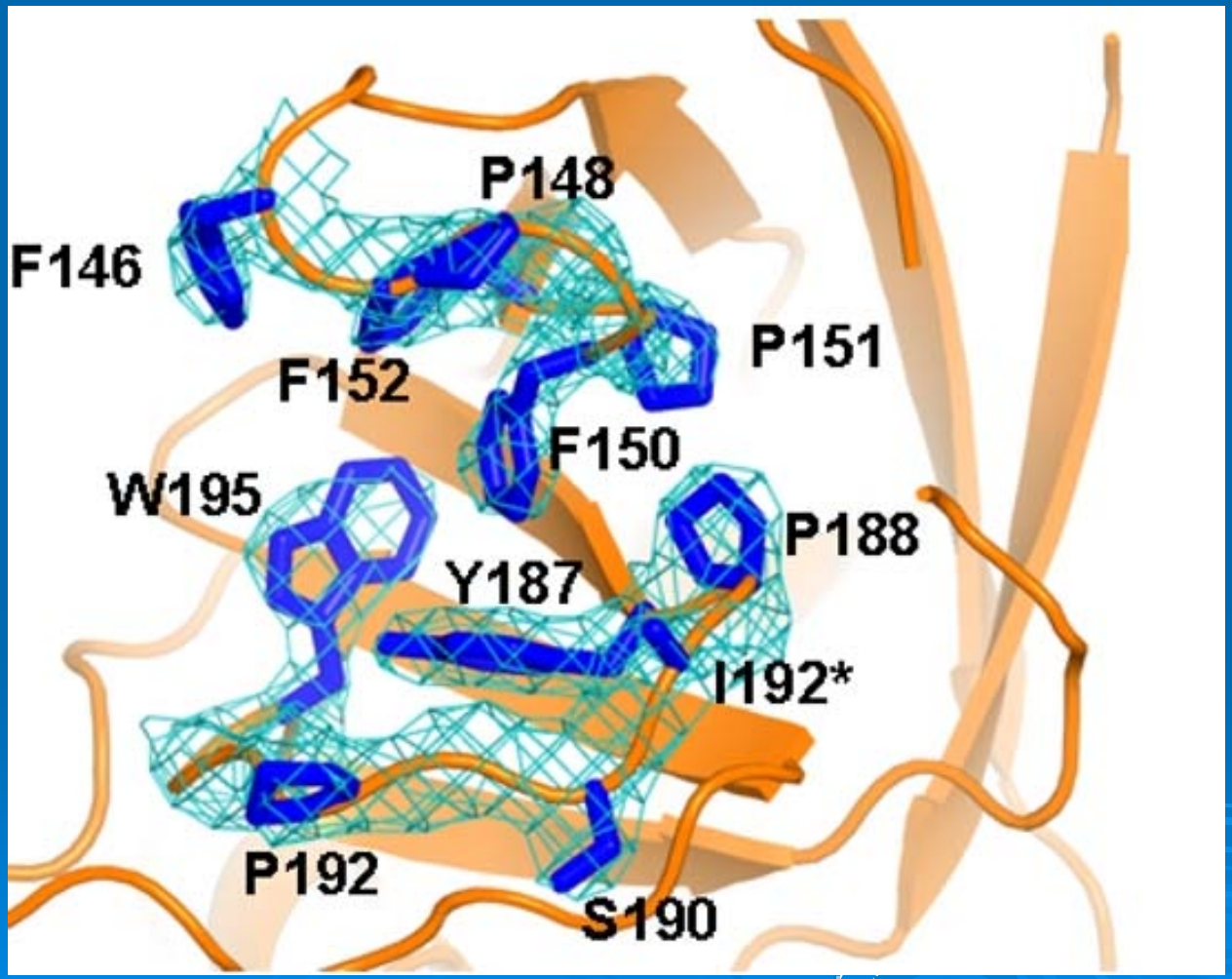
Hydrophobic groove

3Å crystal structure
KipI-C domain



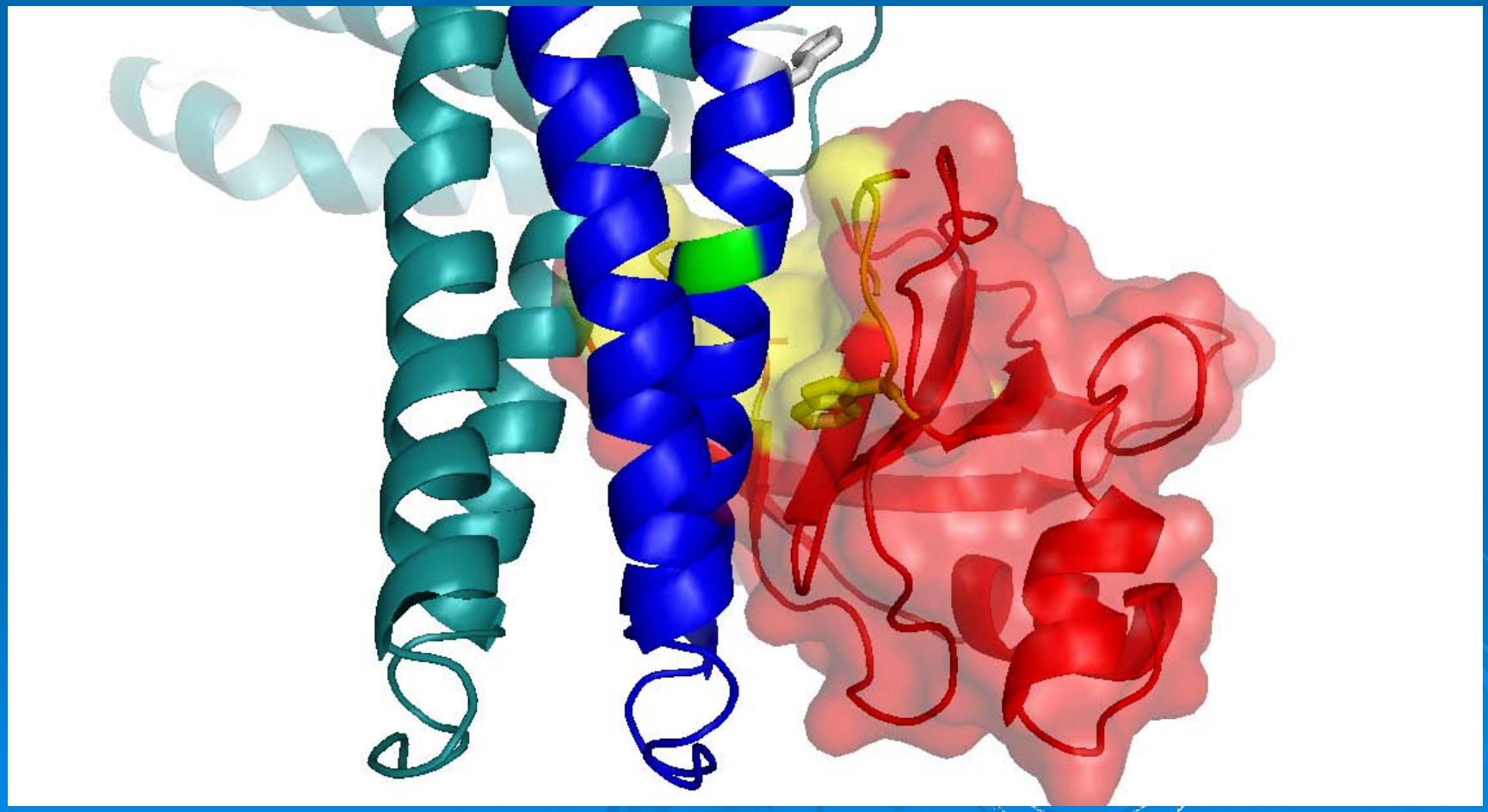
Overlay with cyclophilin B

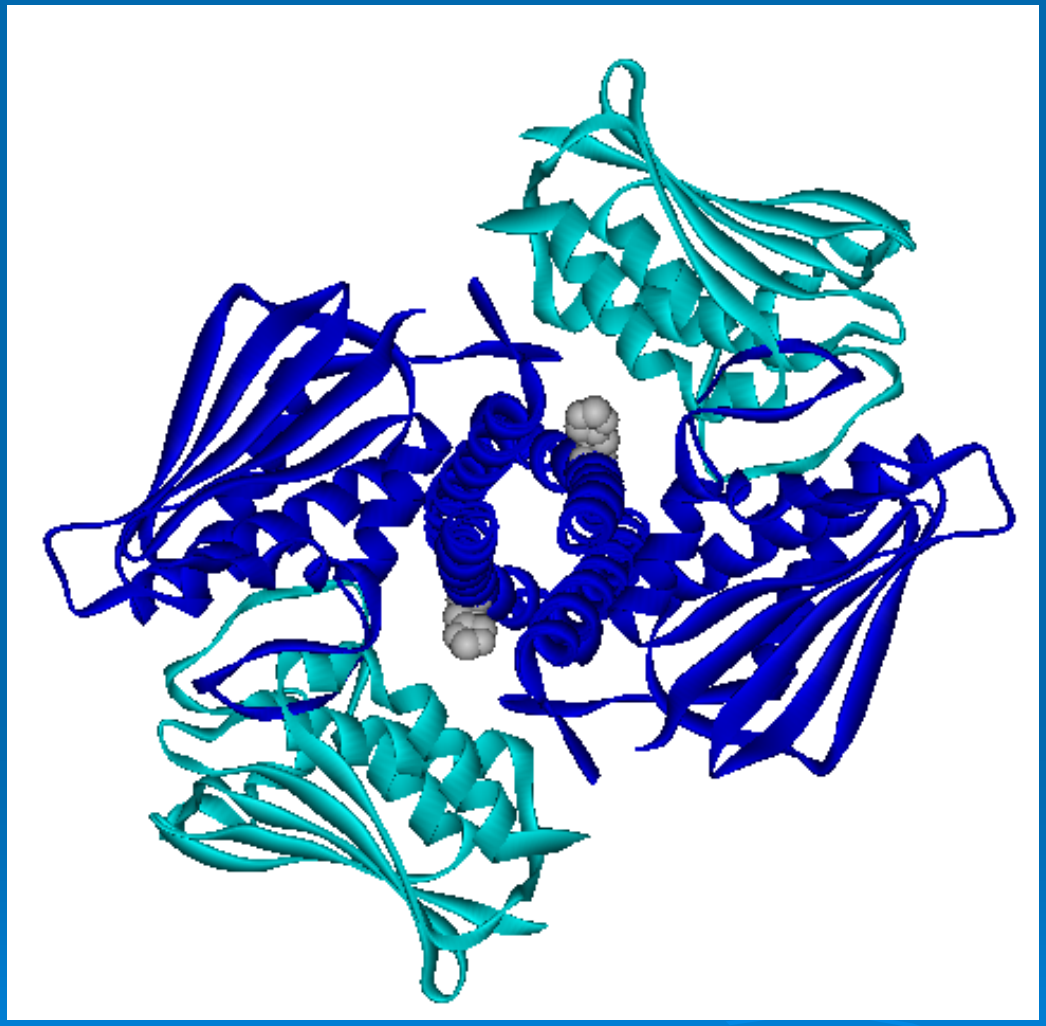
Aromatic side chain density in the hydrophobic groove



Jacques, Langely, Jeffries et al, in review *J. Mol.Biol.* 2008

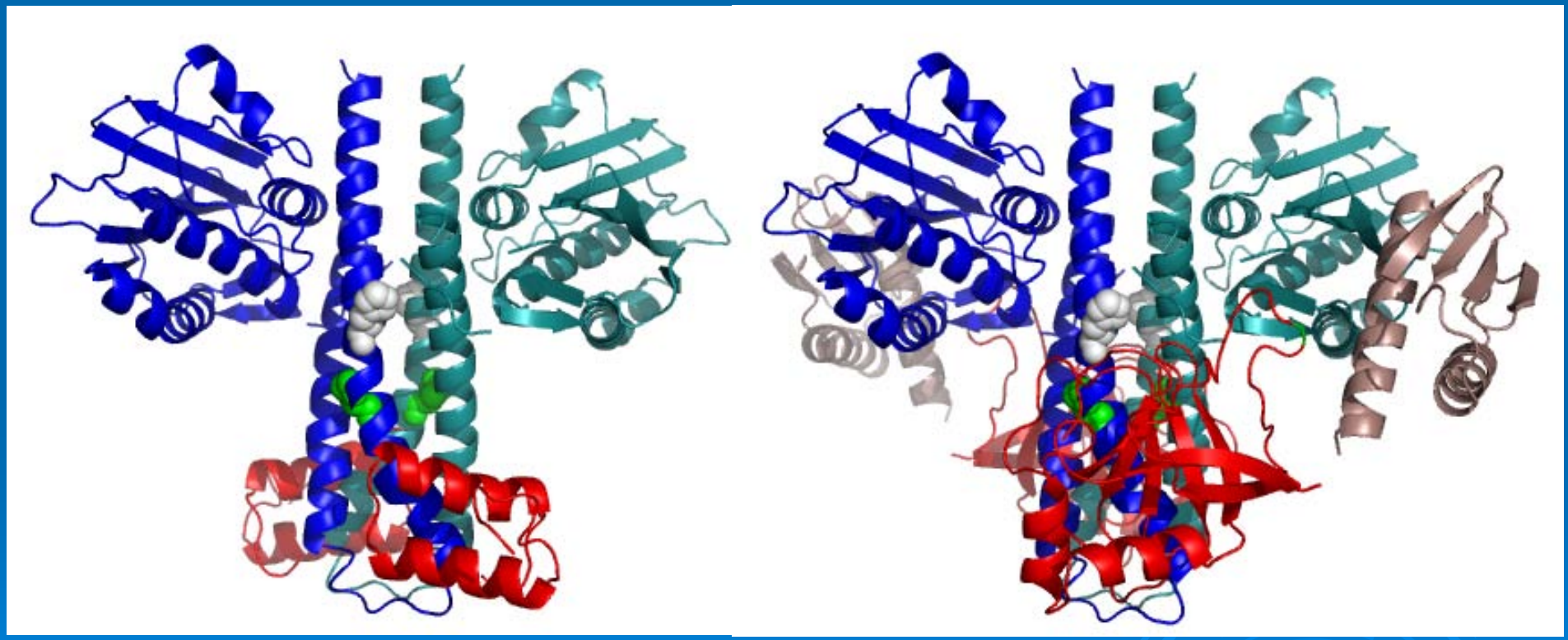
The KinA helix containing Pro^{410} sits in the KipI-C domain hydrophobic groove





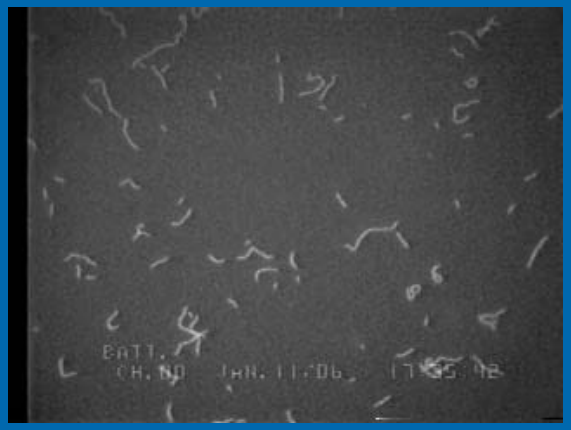
A possible role for *cis-trans* isomerization of Pro^{410} in tightening the helical bundle to transmit the KipI signal to the catalytic domains?

Or is the KipI cyclophilin-like domain simply a proline binder?

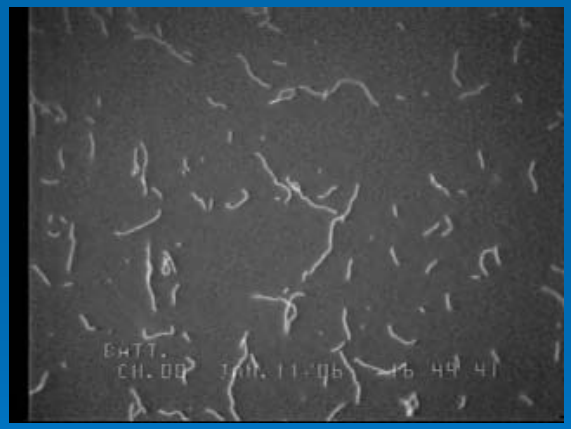


DHp helical bundle is a critical conduit for signaling

The N-terminal regulatory domains of the cardiac myosin binding protein C (cMyBP-C) influence motility



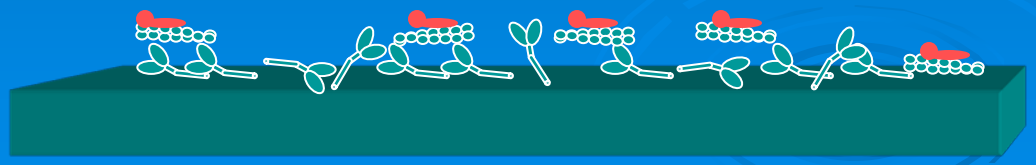
High Ca^{2+}



Low Ca^{2+}

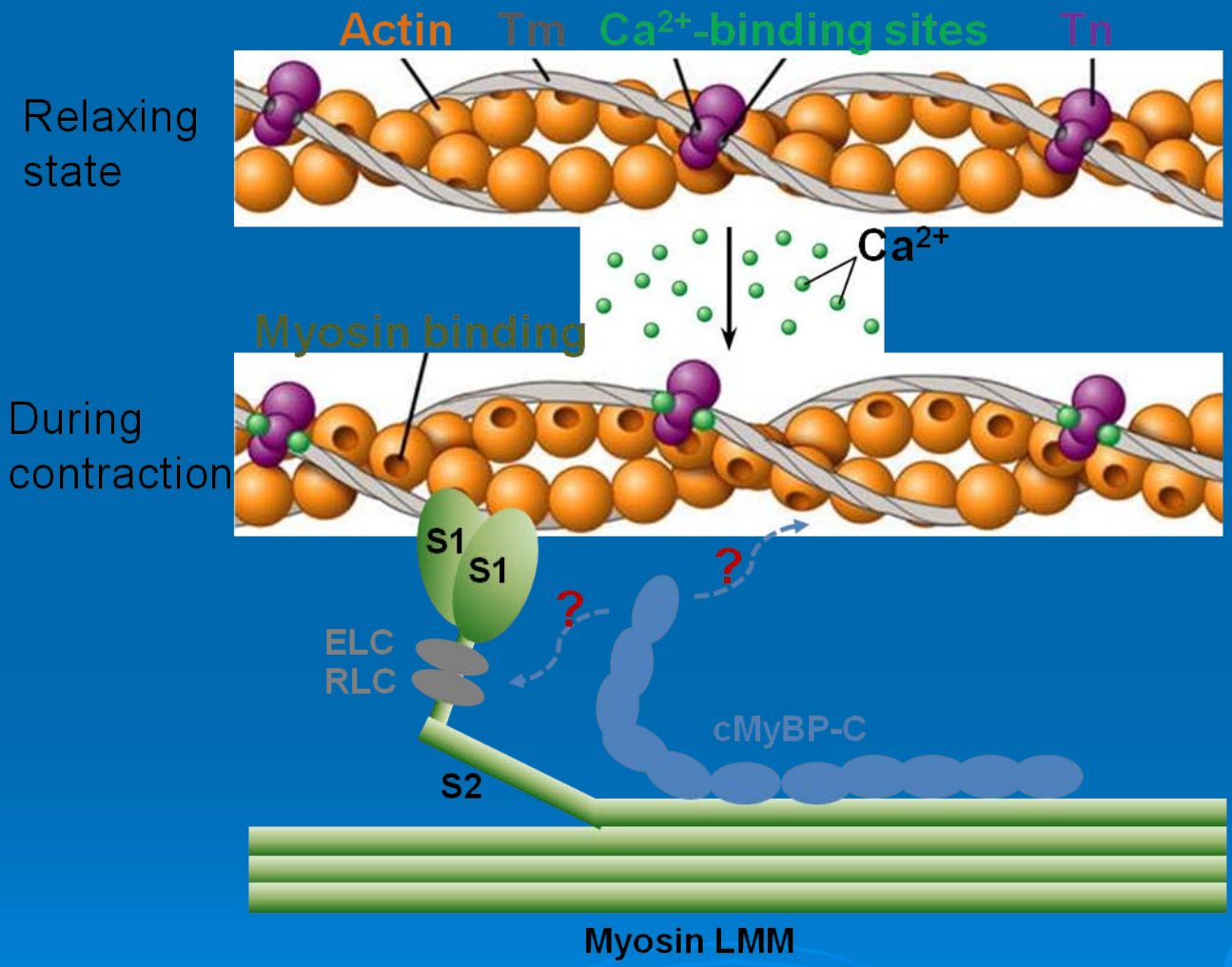
Controls

+cMyBP-C reg domains

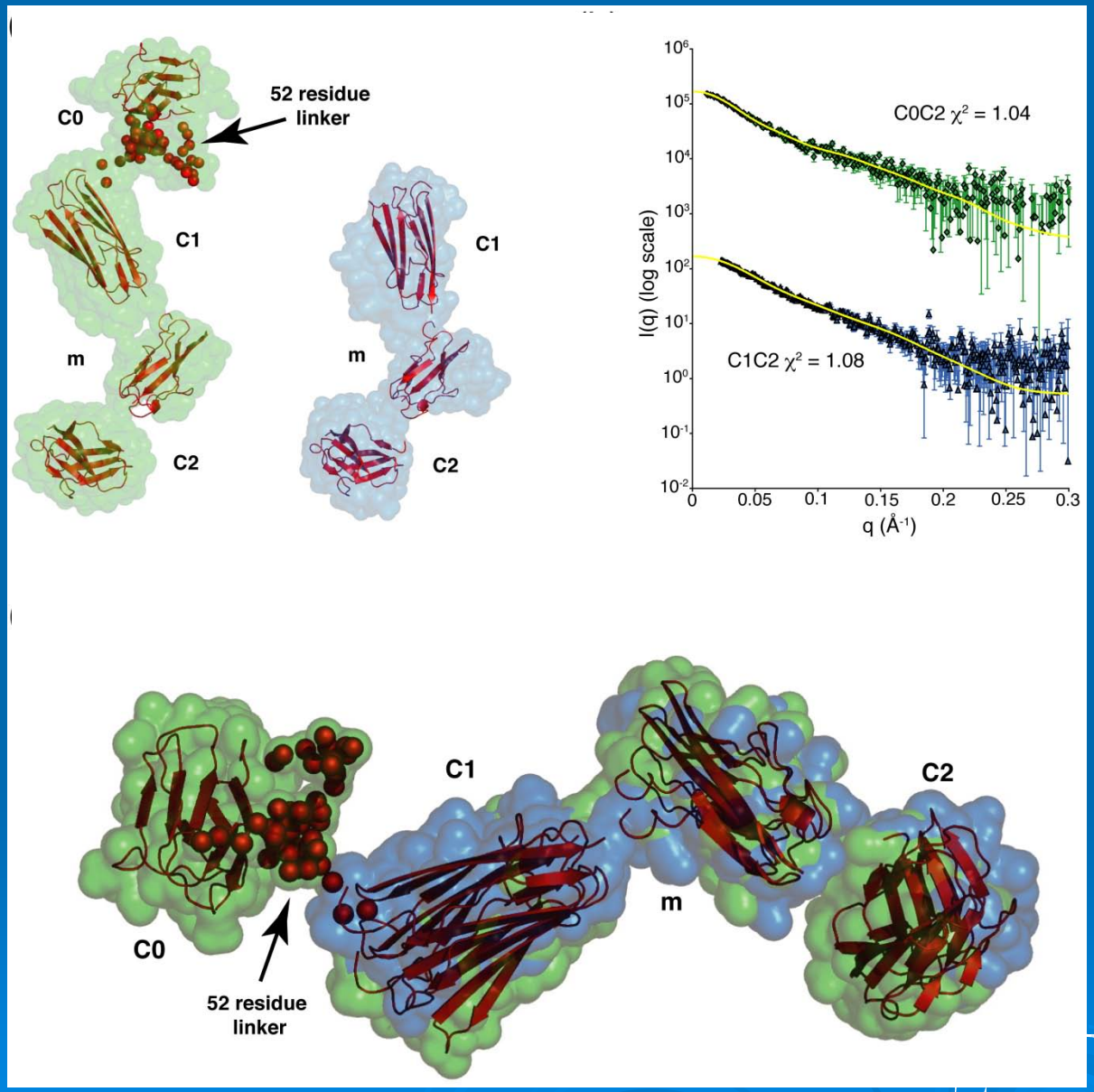


movies courtesy of Samantha Harris, UC Davis

cMyBP-C in Muscle Contraction

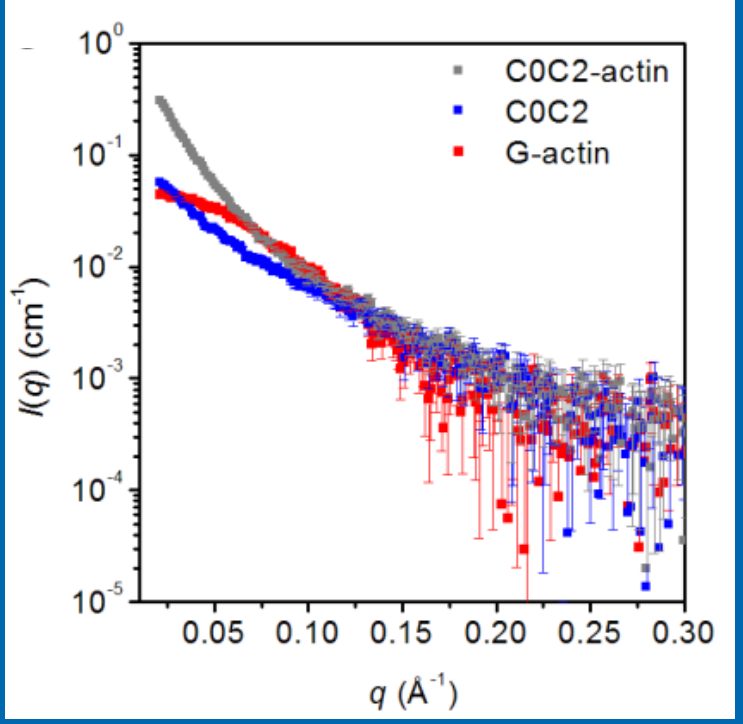


- cMyBP-C plays **structural** and **regulatory** roles in striated muscle sarcomeres. However, the specific details of how it interacts with actin and myosin are unclear.

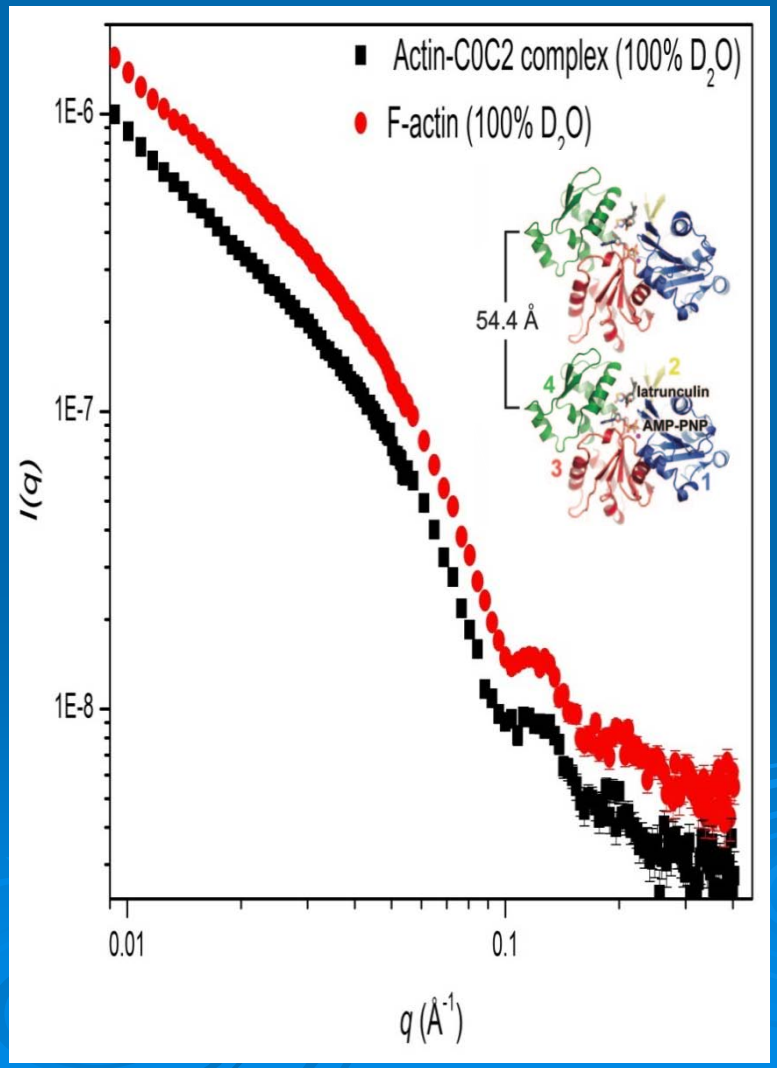


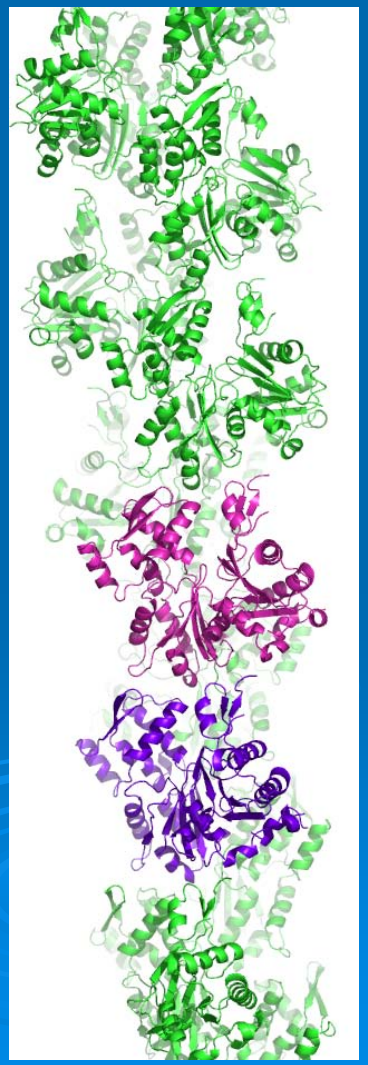
SAXS data + crystal and NMR structures of individual modules show the N-terminal domains of mouse cMyBP-C form an extended structure with a defined disposition of the modules

Jeffries, Whitten et al. (2008)*J. Mol. Biol. 377*, 1186-1199

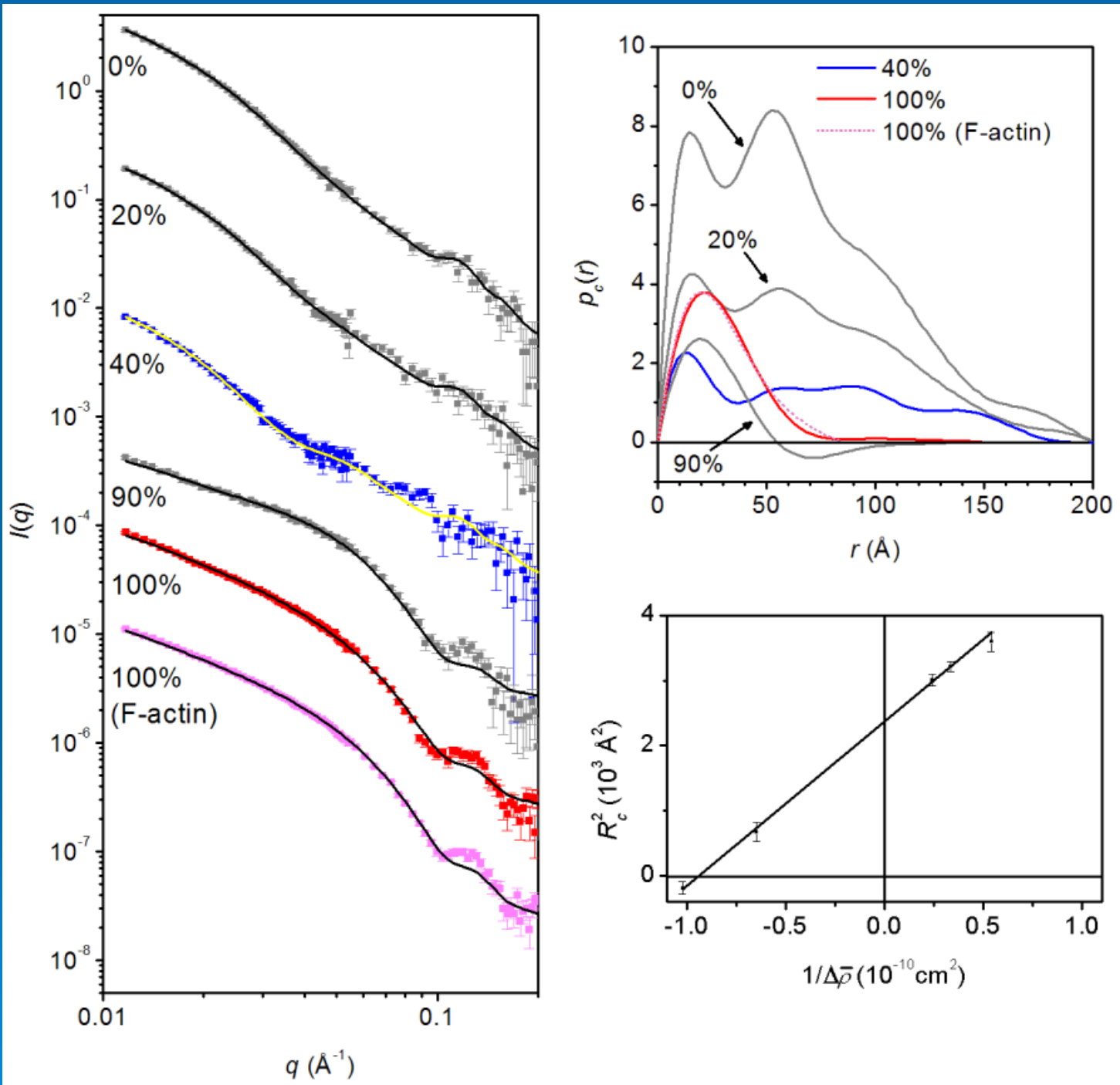


Mixing mono-disperse solutions of cMyBP-C with actin results in a dramatic increase in scattering signal due to the formation of a large, rod-shaped assembly

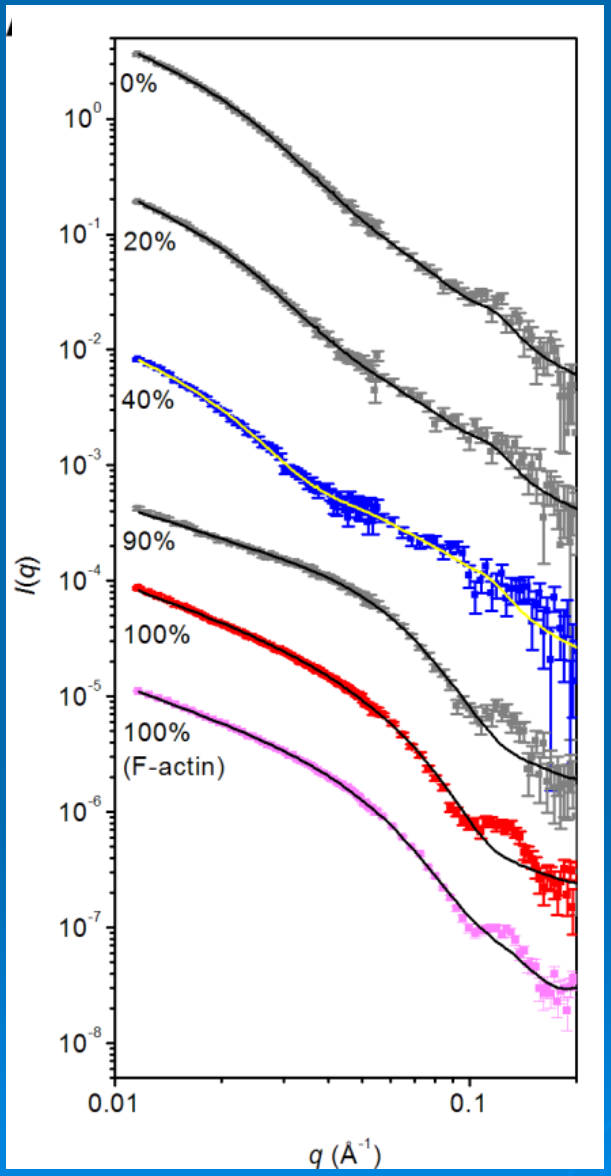


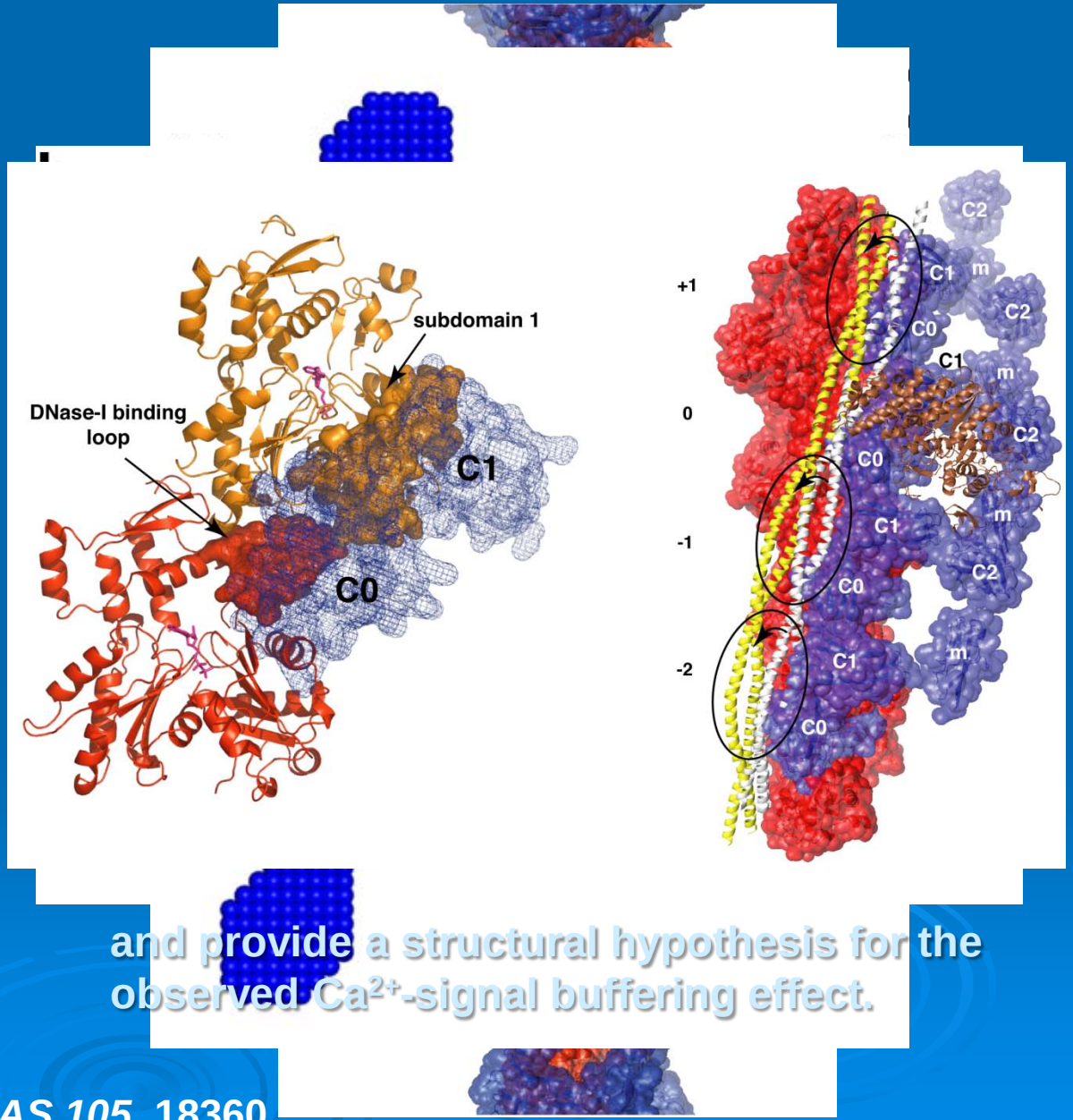


Neutron contrast variation on actin thin-filaments with deuterated C0C2 show they bind actin and stabilize filaments


0%
20%
40%
90%
100%
100% (F-actin)
I(q)
q (Å-1)
0.01
0.1
40%
100%
100% (F-actin)
0%
20%
90%
pc(r)
r (Å)
0
50
100
150
200
Rc2 (10³ Å²)
1/Δρ̄ (10-10 cm²)
-1.0
-0.5
0.0
0.5
1.0

SANS data show regulatory cMyBP-C domains (mouse) stabilise F-actin

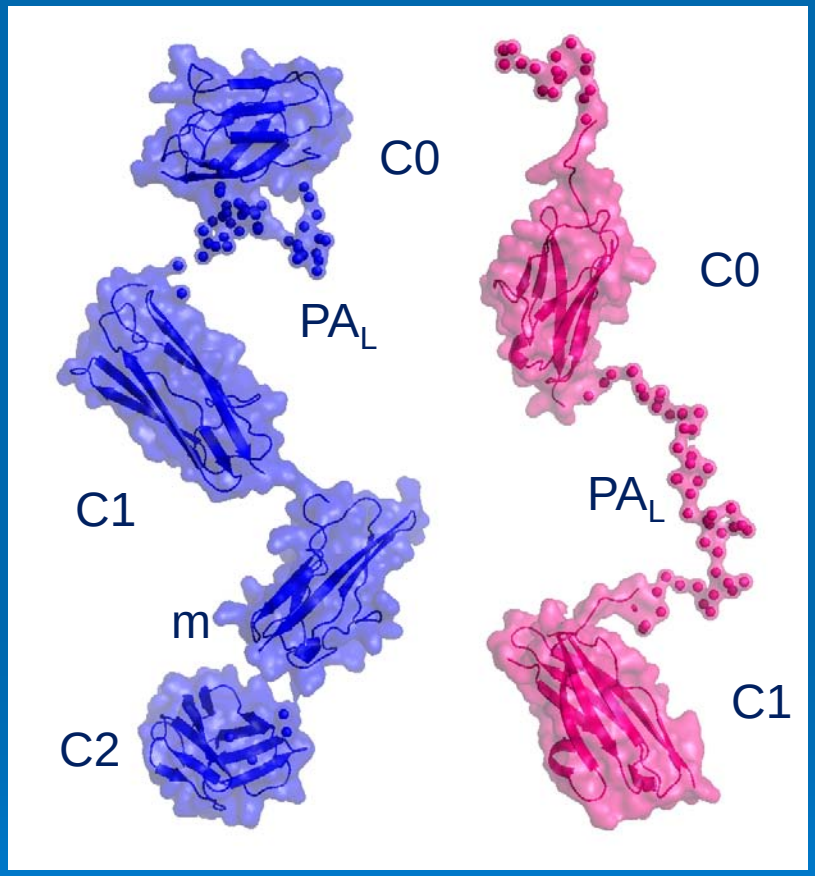




and provide a structural hypothesis for the observed Ca^{2+}-signal buffering effect.

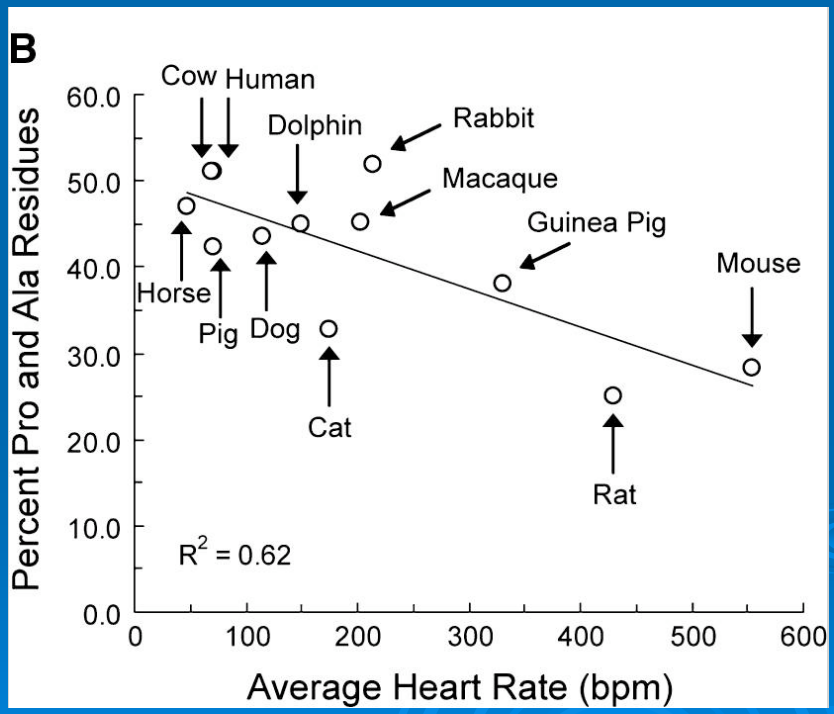
Whitten, Jeffries et al. (2008) *PNAS 105*, 18360

SAXS data show significant species differences



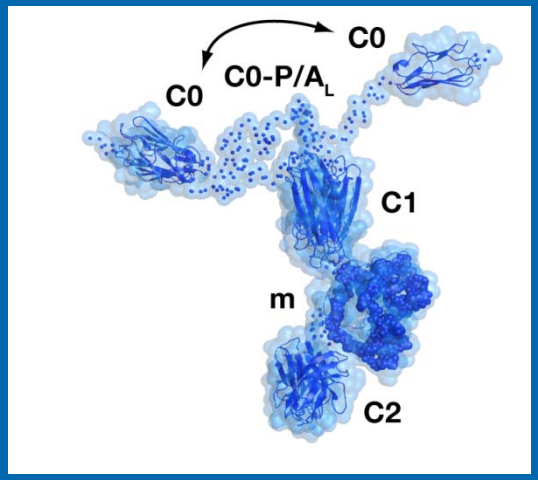
mouse **human**

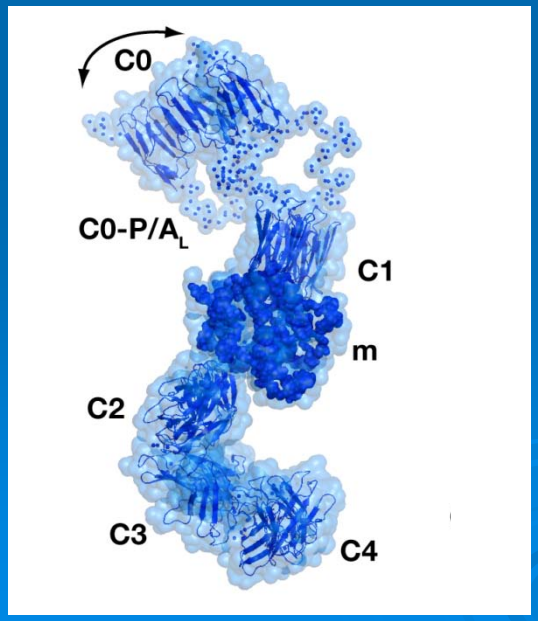
Correlation between % Pro/Ala composition in the C0-C1 linker and heart rate from different organisms (Shaffer and Harris (2009) *J. Muscle Res. Cell Motil.* 30:303-306**.)**

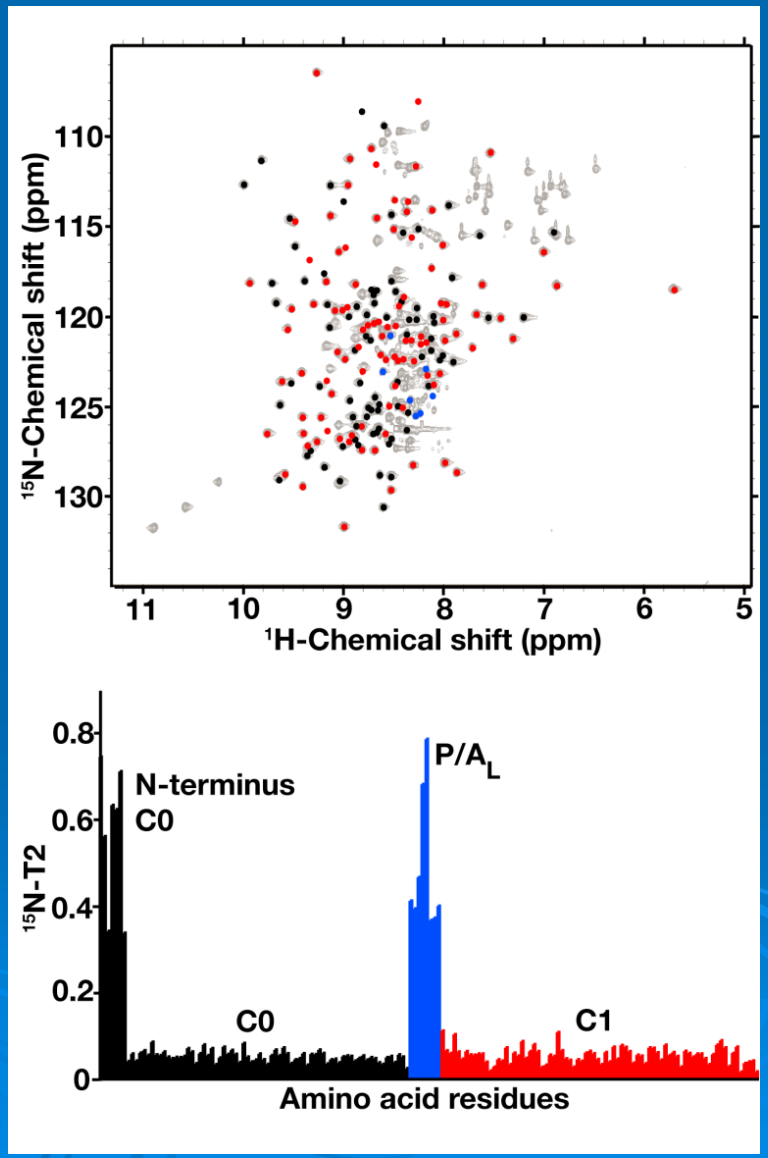


Jeffries, Lu et al. (2011) *J. Mol. Biol. 414*, 735-748

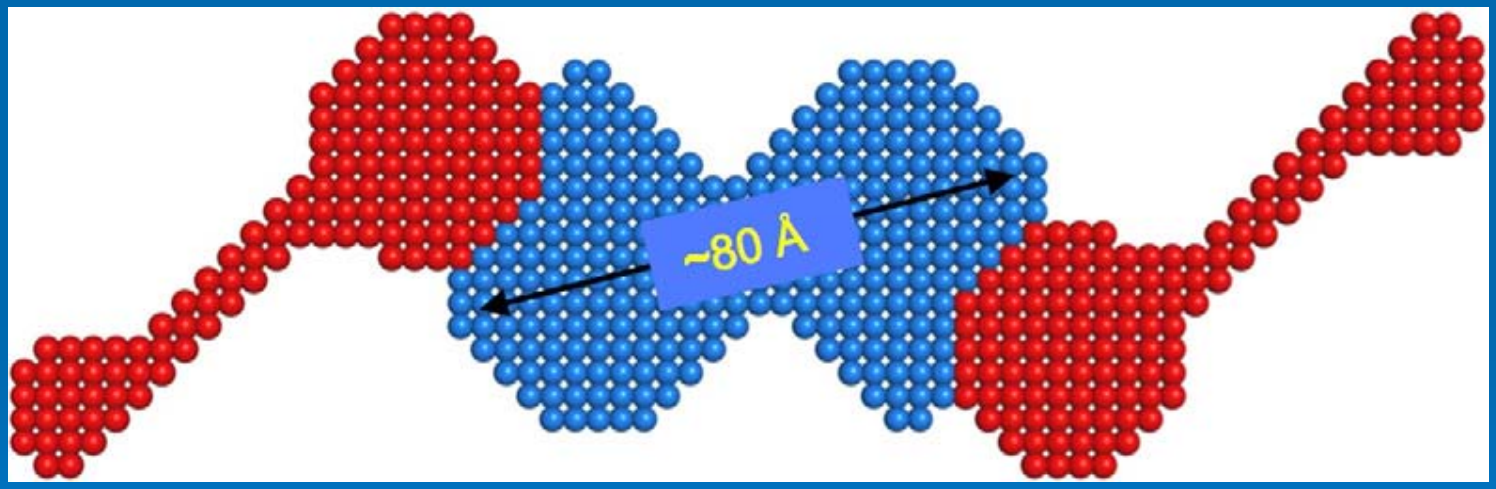
SAXS data cannot define relative positions of human C0 and C1
NMR relaxation data show human PA_L is flexible





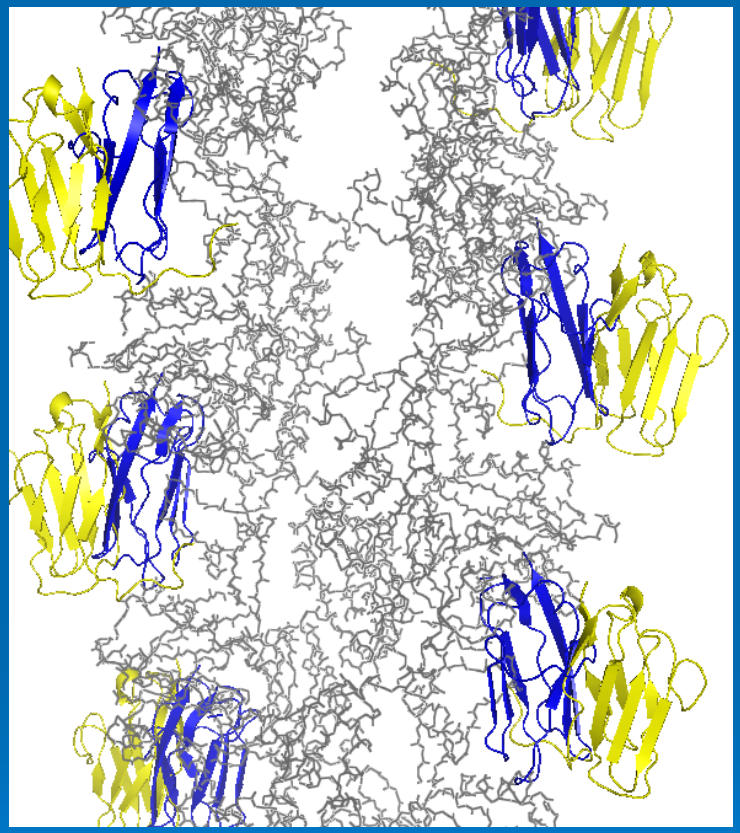


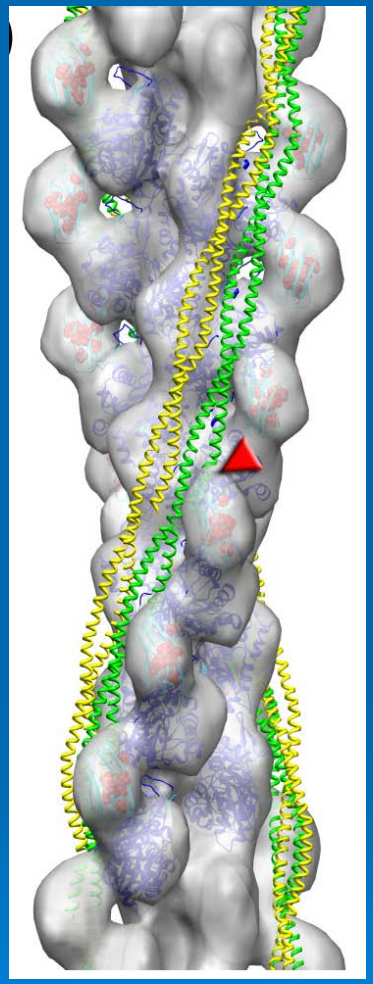
2D reconstruction of human C0C1-actin assembly from neutron contrast series consistent with C0 binding with a flexible and extended P/A_L

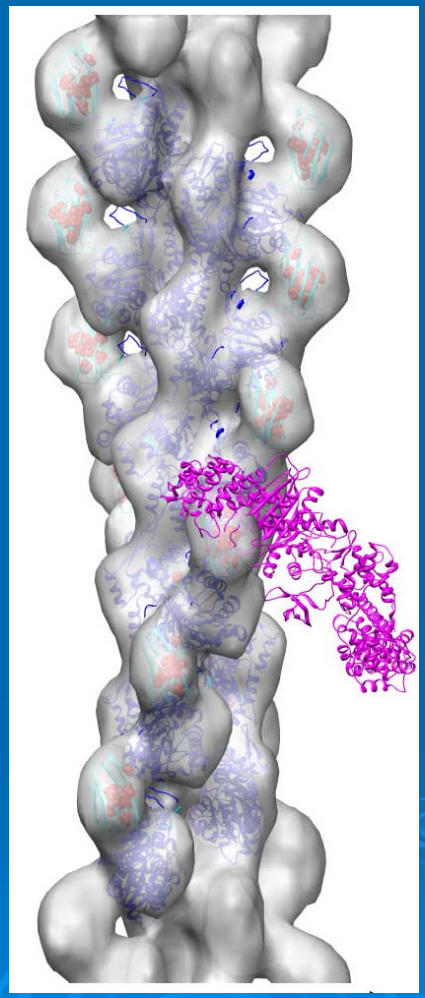


Lu et al., *J. Mol. Biol. 413*, 908-913, 2011

Human EM and mouse SANS comparison

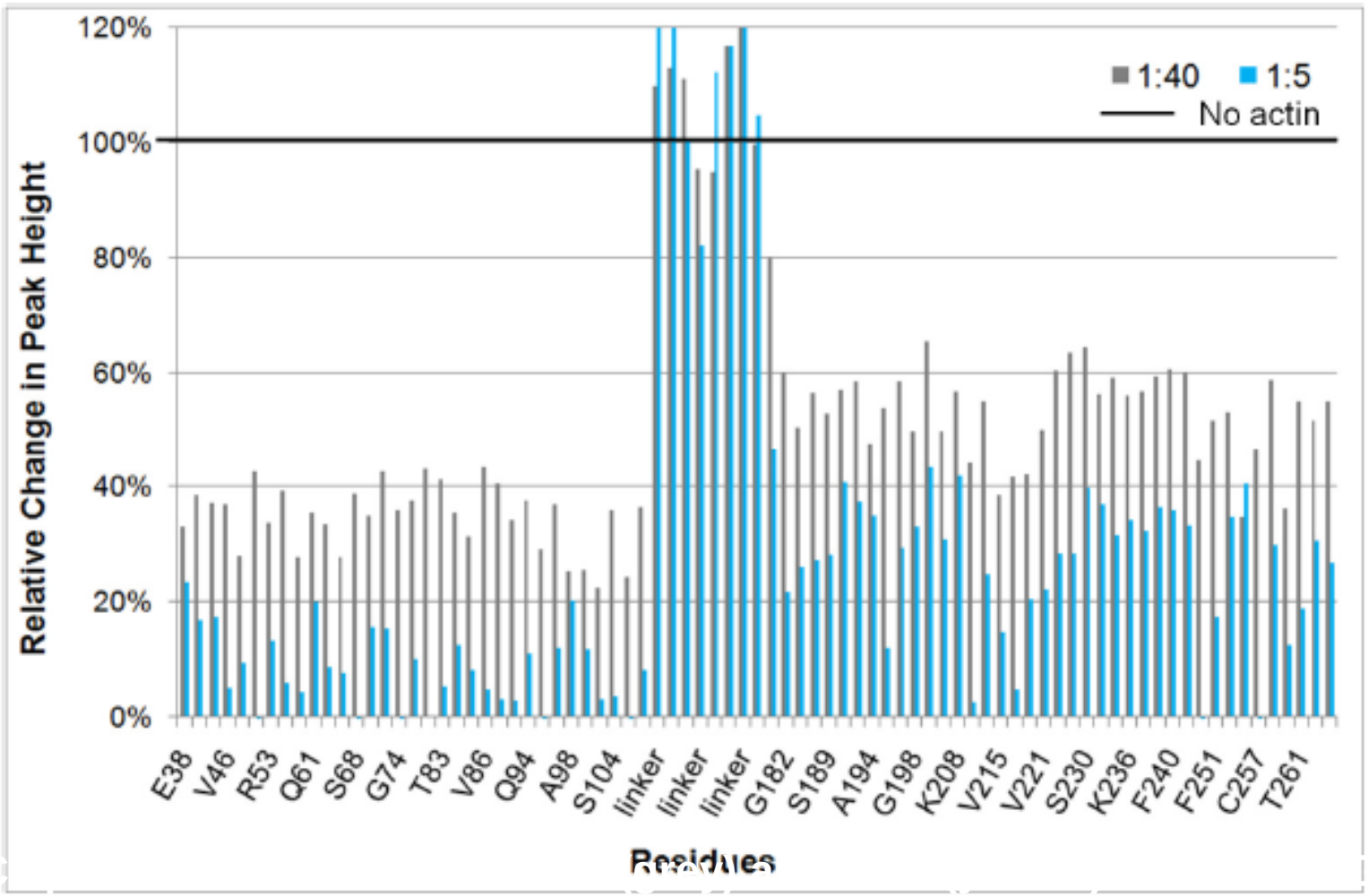




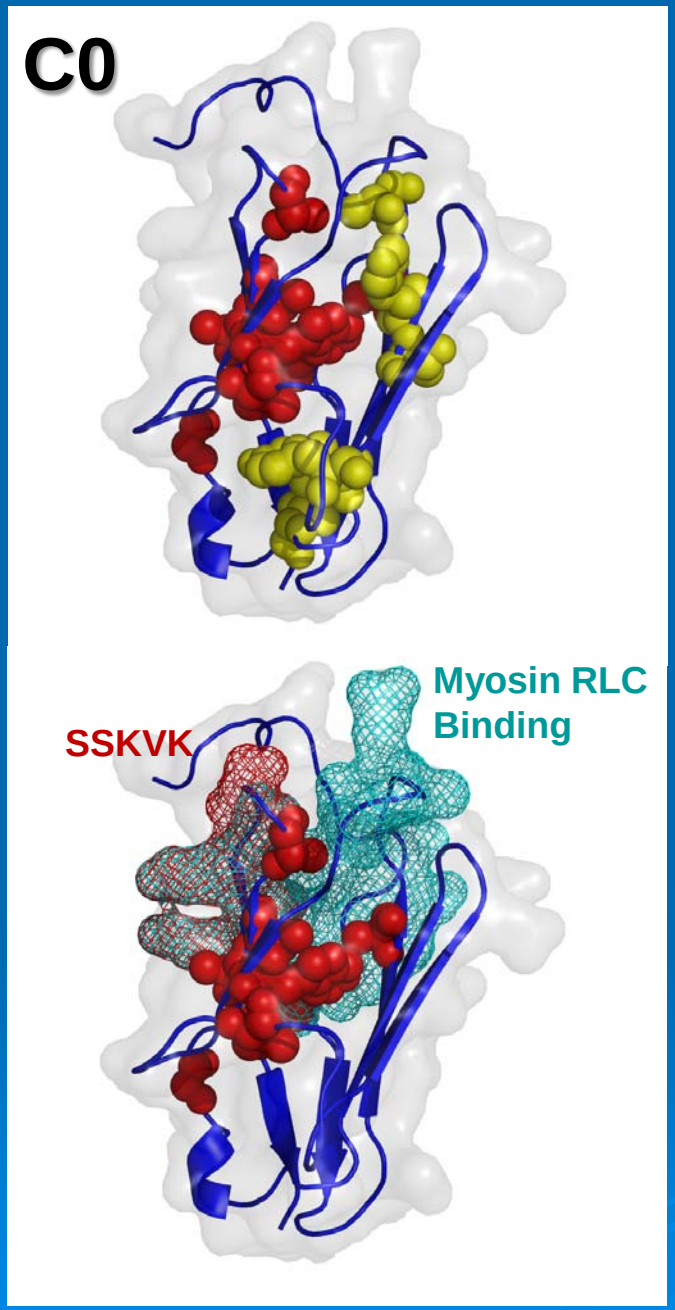


Orlova, Galkin, Jeffries, Egelman, Trewhella (2011) *J. Mol. Biol. 412*, 379-386

NMR data identify residues involved in (human) C0-actin interaction



HS ctin



Actin Binding Hot-spots

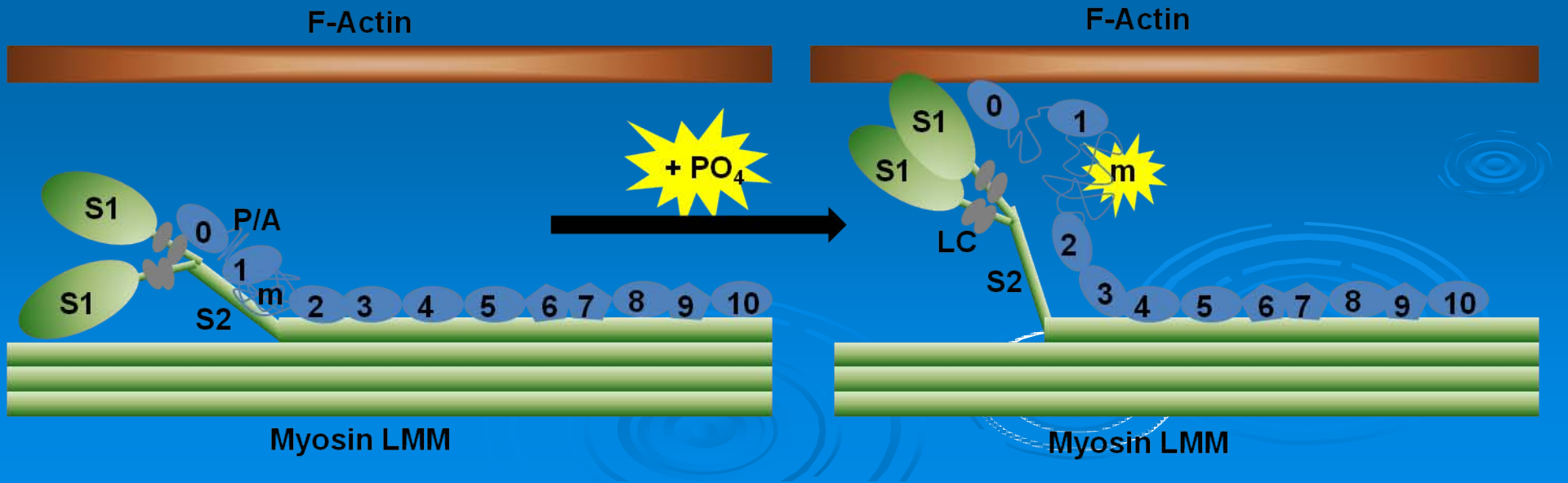
Shared Actin and Myosin Binding Sites

Lu, Jeffries et al. (2011) *J. Mole. Biol. 413*, 908-913

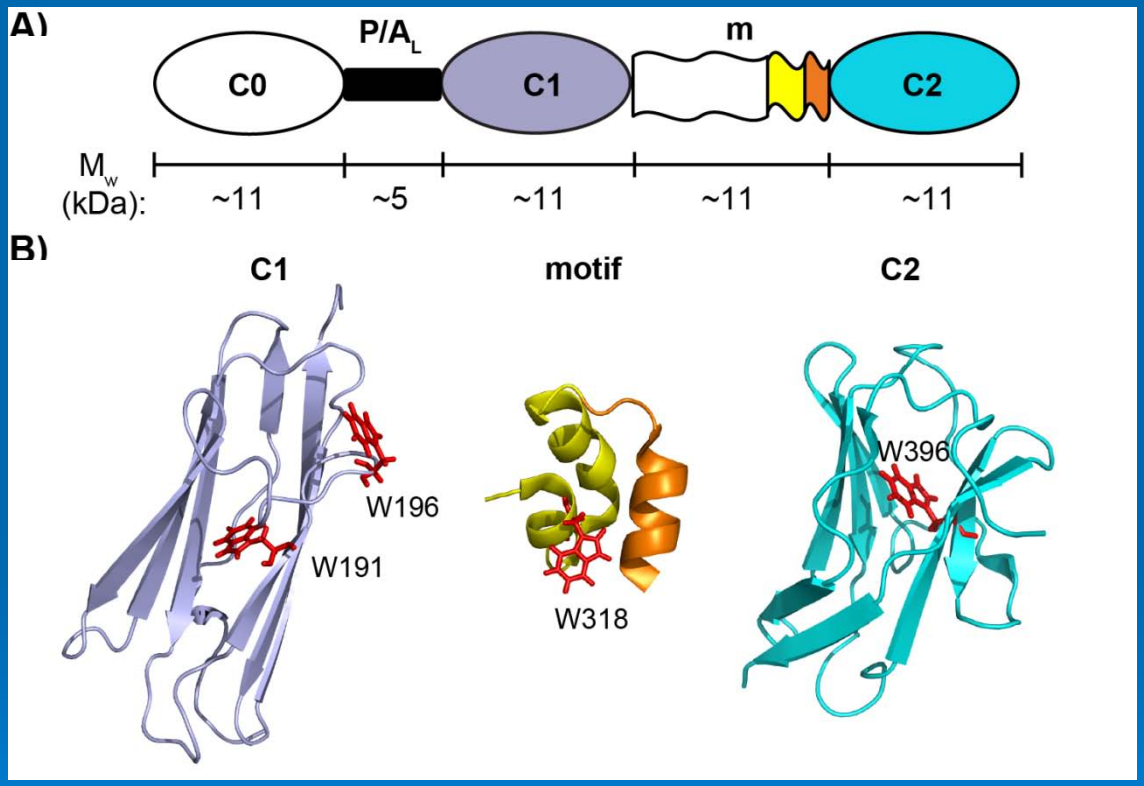
Switching Facilitated by Flexible P/A_L Regulated by Phosphorylation?

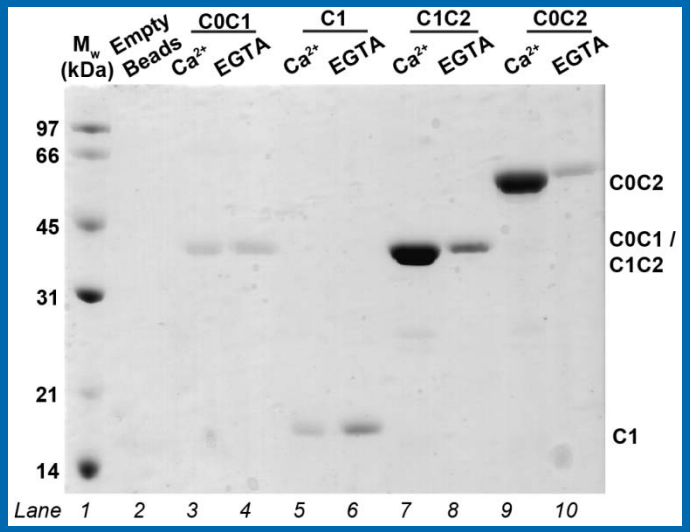
By combining **EM, Crystallography, SANS, SAXS** and **NMR**, we show that

- 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?



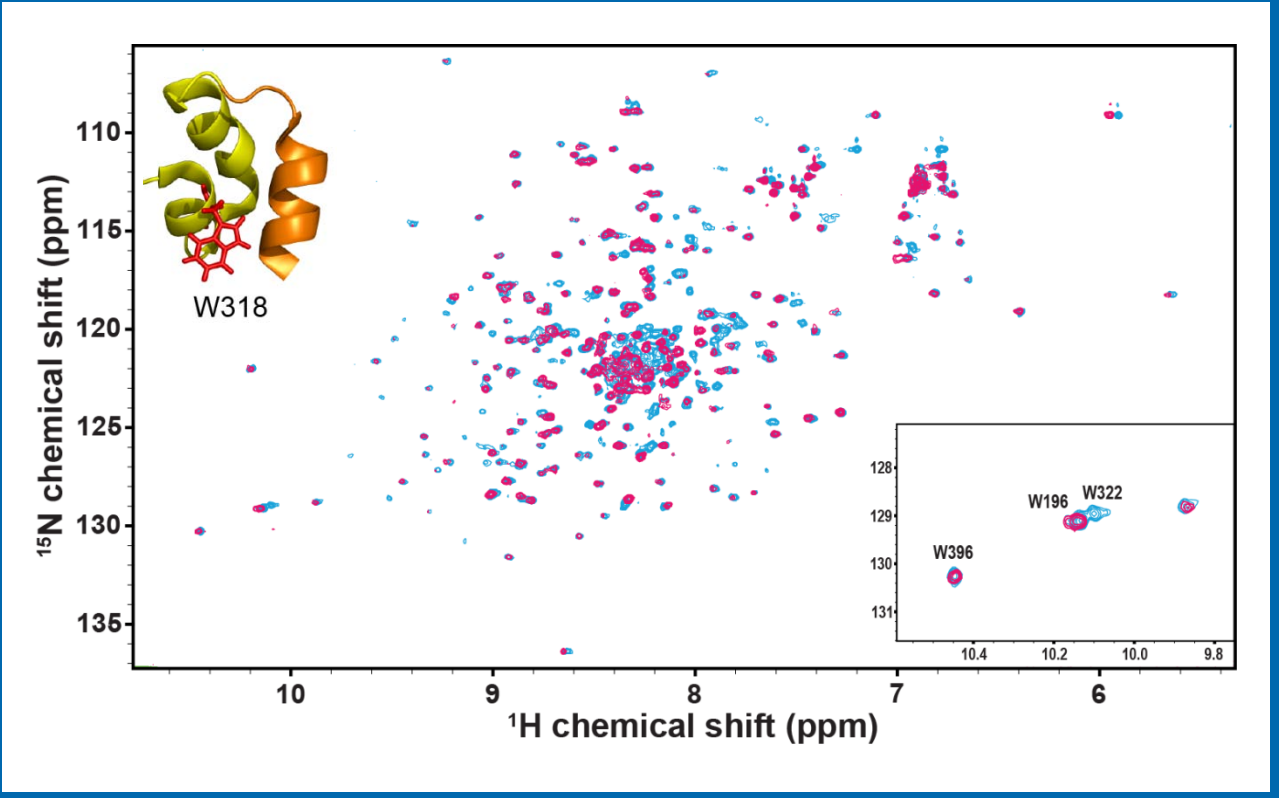
Calmodulin : linking cMyBP-C with Ca^{2+} signaling pathways to coordinate phosphorylation events and synchronise the multiple interactions between cMyBP-C, myosin and actin during the heart muscle contraction?





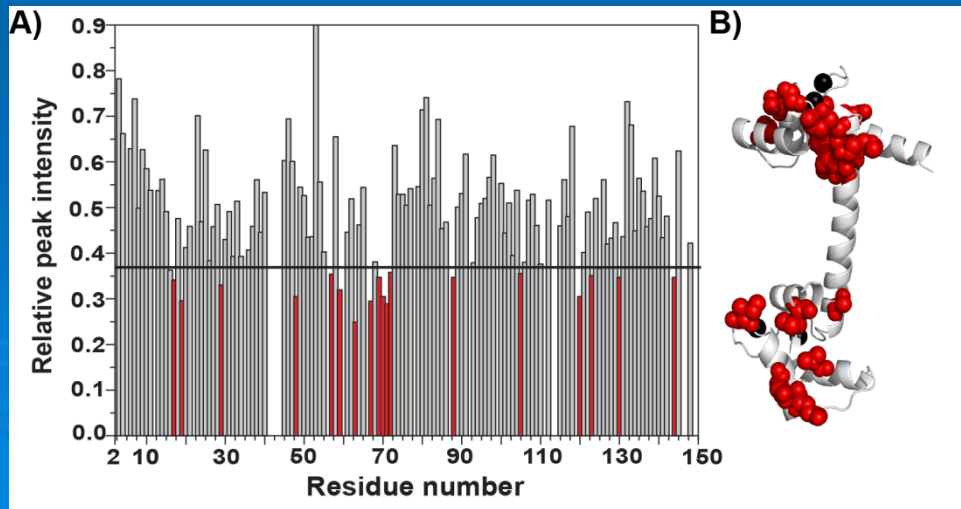
The motif of human cMyBP-C is required for its Ca^{2+}-dependent Interaction with calmodulin(CaM)

Lu, Jeffries et al. (2012) *J. Biol. Chem. 287*, 31596-607

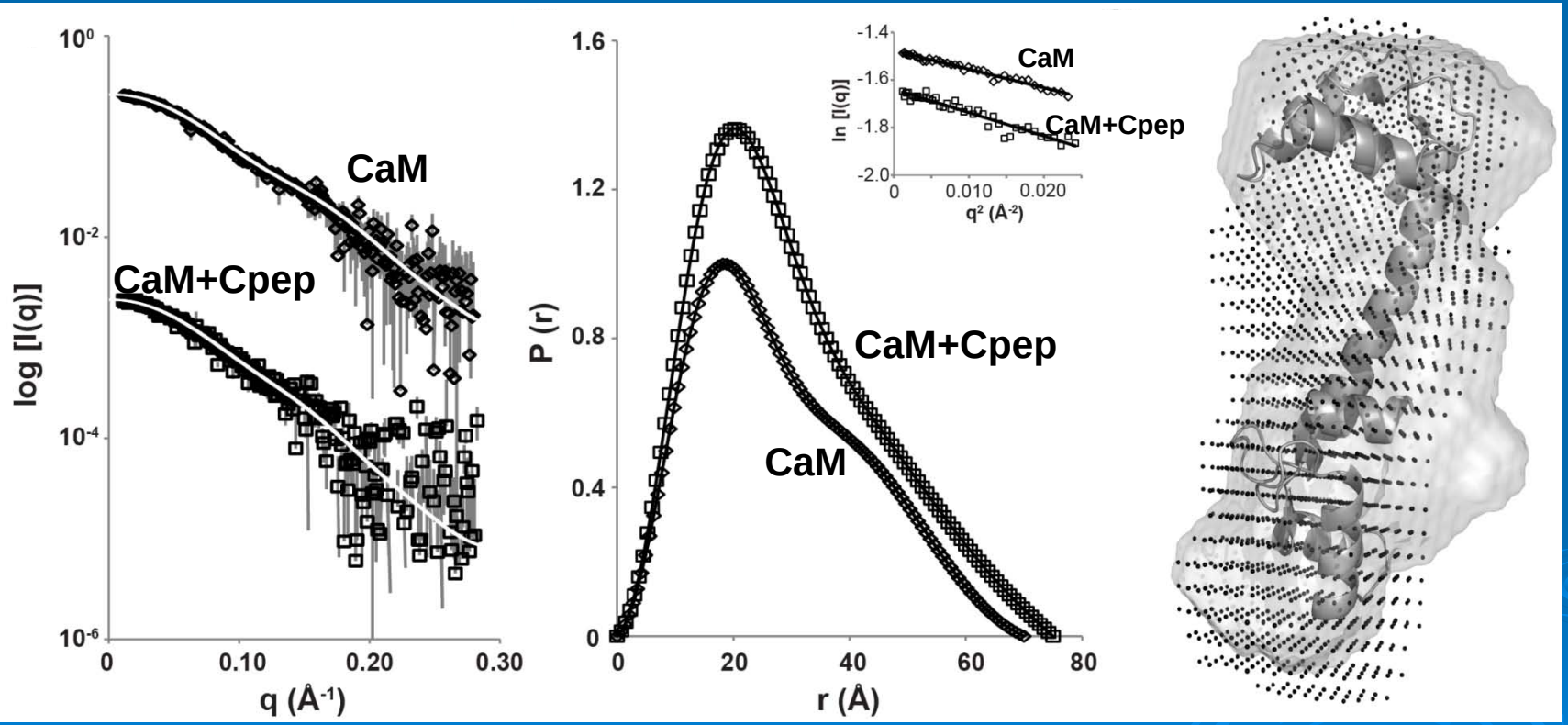


Ca^{2+}-CaM addition to ^{15}N-C1C2 results in significant intensity changes for ~66% of the amide resonance peaks mapped to the structured region of the motif (W322 in the motif disappears on the first addition).

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

James Taylor

Yanling Lu

Cy Jeffries

Neutron Ted

Andrew Whitten

John Chow

David Jacques

Tchau meus amigos brasileiros - a gente se vê