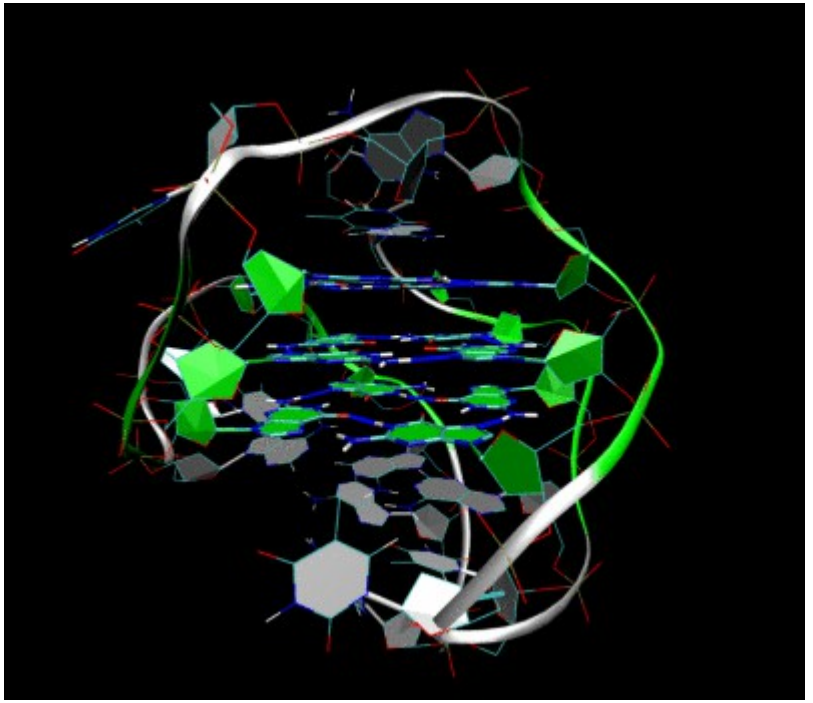
Neutrons in biology: structure and dynamics

Alessandro Paciaroni, University of Perugia



IRIDE photo-production Workshop

10-11 June 2013 *Laboratori Nazionali di Frascati*
Europe/Rome timezone

Driving Forces of Life Sciences

The present major driving forces of life science at the molecular and cellular scale are functional **genomics** and **proteomics**.

Information on the **specific functions** of most if not all proteins encoded in human and other genomes is desirable.

Major obstacles are the **vast complexity** of the individual proteins and the even more delicate **interaction** of different proteins and other biomolecules to form (transient) **functional complexes**.

Neutrons are a unique non-destructive tool for probing precious biological molecular samples

Why do we need to know Protein Structure and Function?

It is currently estimated that there are 25,000–40,000 different genes in the human genome, and with alternative splicing of genes and post-translational modification of proteins the number of functionally distinct proteins is likely to be much higher.

disease mechanisms => immense challenge to modern medicine, as disease may result when any one of these proteins contains mutations or other abnormalities

Protein therapeutics => tremendous opportunity in terms of harnessing protein therapeutics to alleviate disease

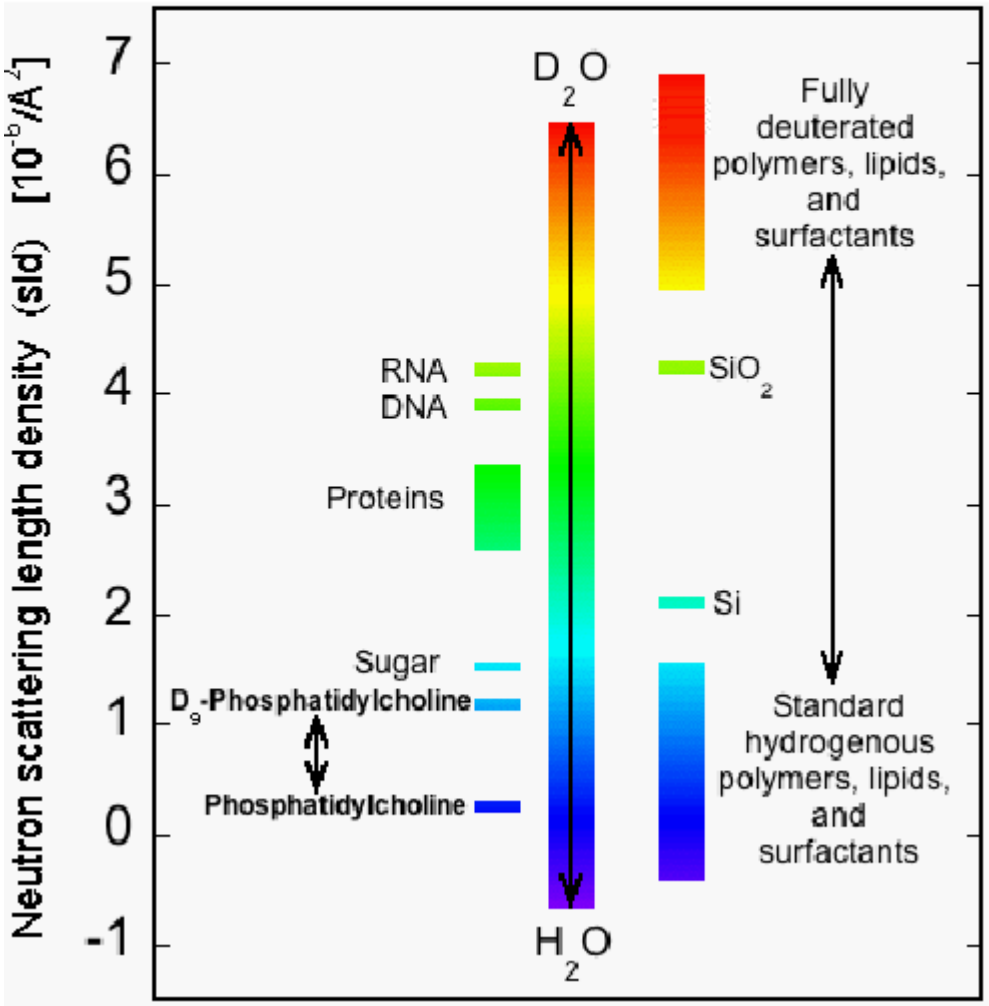
Protein therapeutics => $80 B biopharmaceutical industry (in 2003)

Neutrons provide physiologically relevant, and yet precise, structural data I

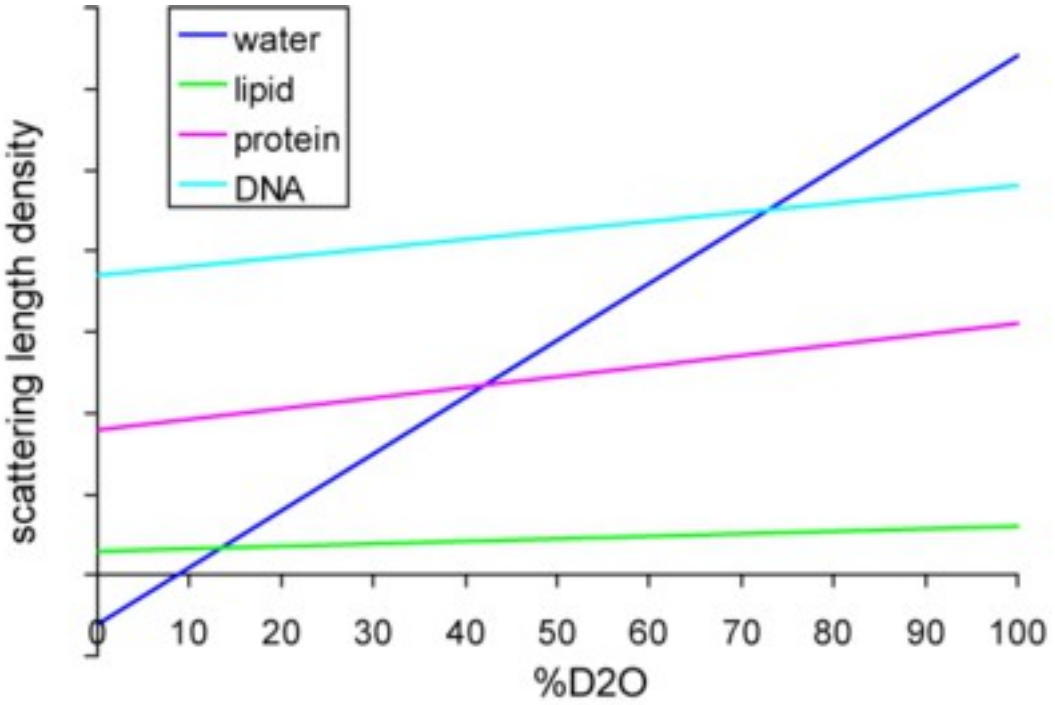
SANS. Focus on structures with nm spatial resolution.

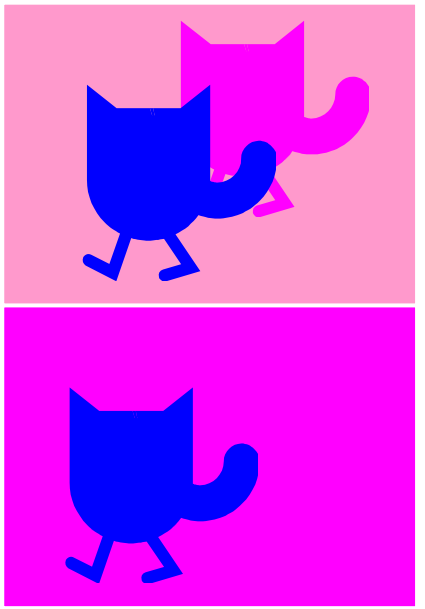
1) Not all proteins crystallize
2) The biological function of most macromolecules involves the formation of large, multi-component complexes.
3) SANS can take advantage of contrast variation by changing the isotopic composition of both the solvent and the individual macromolecular components to elucidate these structures

What neutrons see:
Scattering Length Density



Neutrons provide physiologically relevant, and yet precise, structural data II





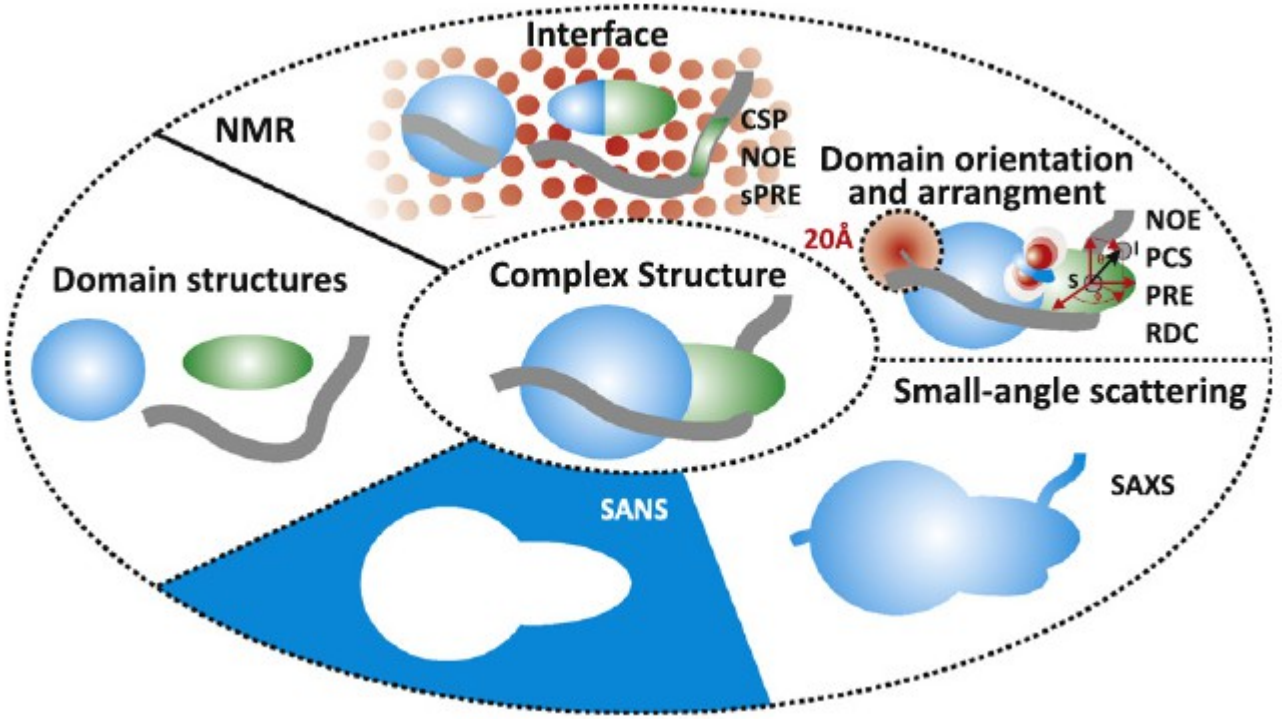
Solvent matching (i.e. matching the scattering density of a molecule with the solvent) allows one to study protein in solvent/co-solvent mixtures and facilitates study of on component by rendering another “invisible.”

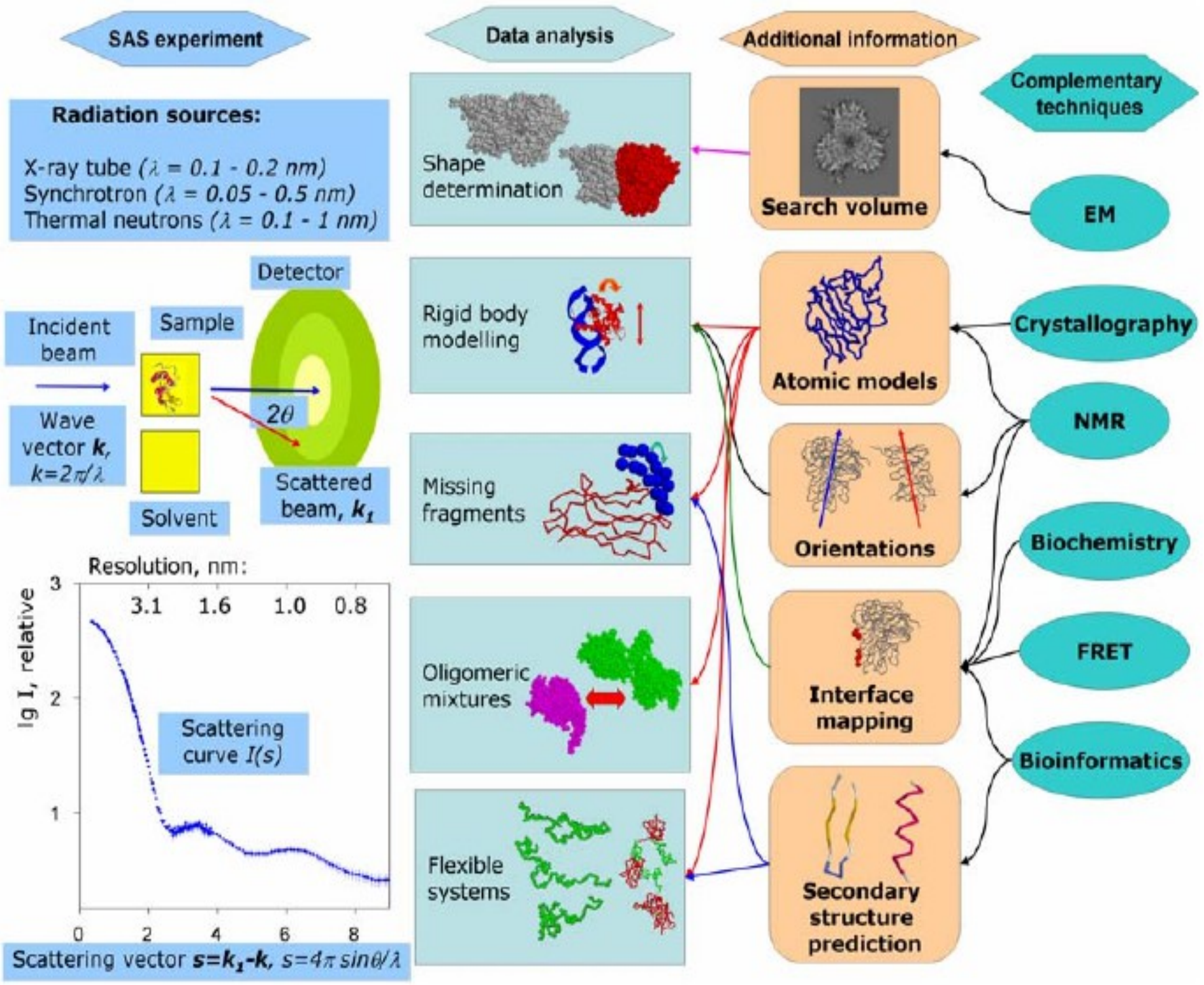
Neutrons provide physiologically relevant, and yet precise, structural data III

The biological function of most macromolecules involves the formation of large, **multi component complexes (post-genomics)**.

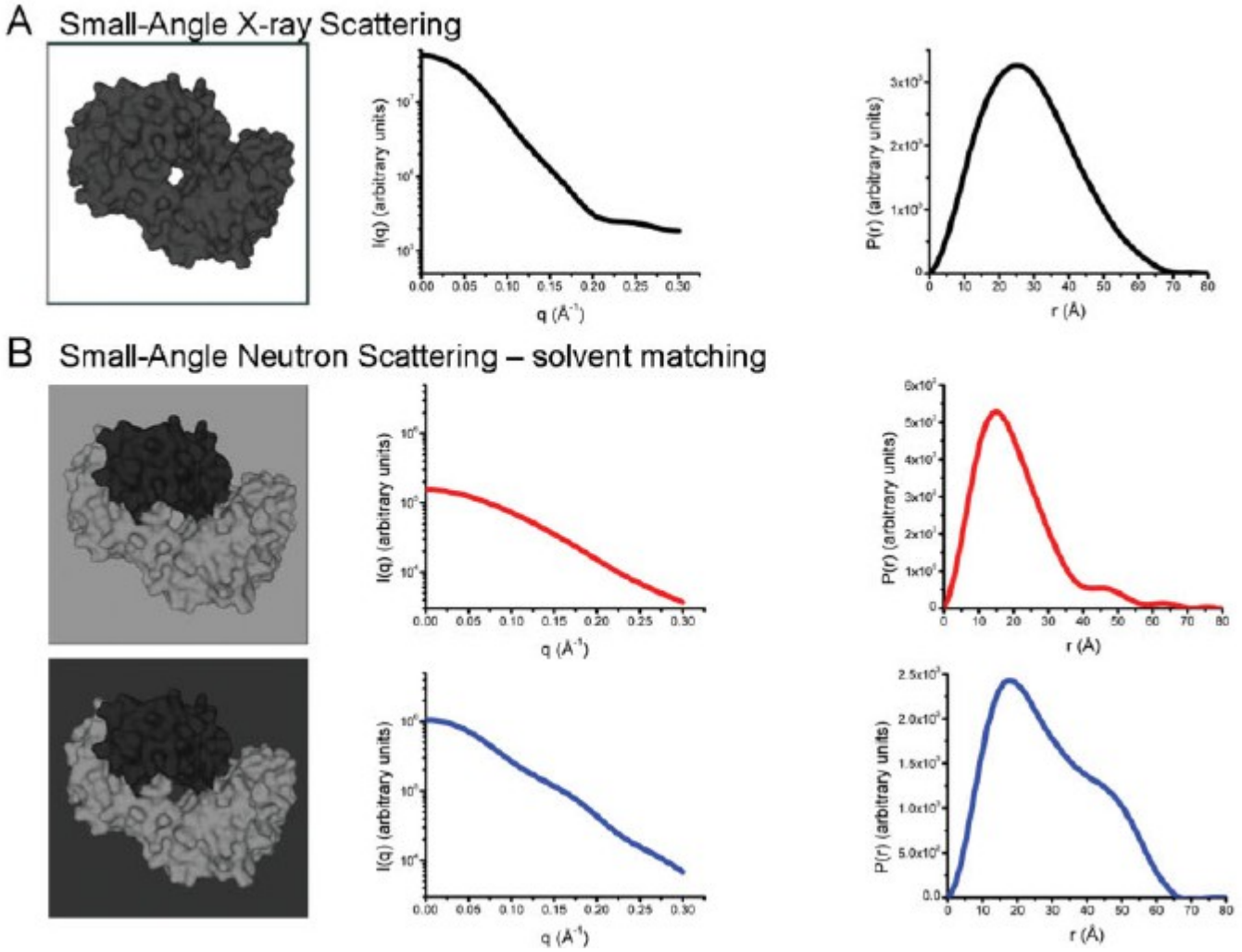
Due to their inherent **flexibility** and **transience** they can often not be crystallised for structure determination.

SANS takes advantage of **contrast variation**



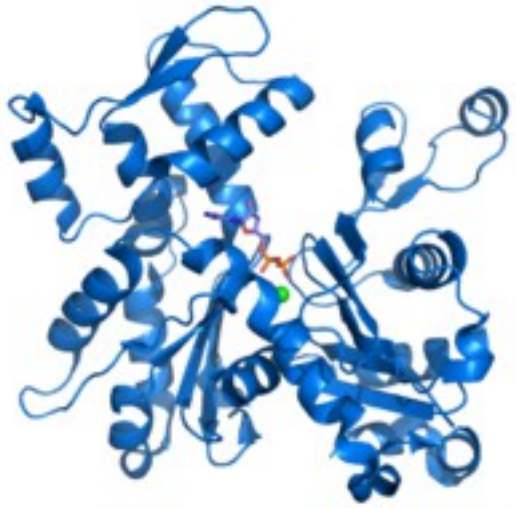

SAS experiment
Data analysis
Additional information
Radiation sources:
X-ray tube (λ = 0.1 - 0.2 nm)
Synchrotron (λ = 0.05 - 0.5 nm)
Thermal neutrons (λ = 0.1 - 1 nm)
Detector
Incident beam
Sample
Wave vector k, k=2π/λ
2θ
Scattered beam, k1
Solvent
Resolution, nm:
3.1
1.6
1.0
0.8
lg I, relative
Scattering curve I(s)
Scattering vector s=k1-k, s=4π sinθ/λ
Shape determination
Rigid body modelling
Missing fragments
Oligomeric mixtures
Flexible systems
Search volume
Atomic models
Orientations
Interface mapping
Secondary structure prediction
Complementary techniques
EM
Crystallography
NMR
Biochemistry
FRET
Bioinformatics

Neutrons provide physiologically relevant, and yet precise, structural data IV

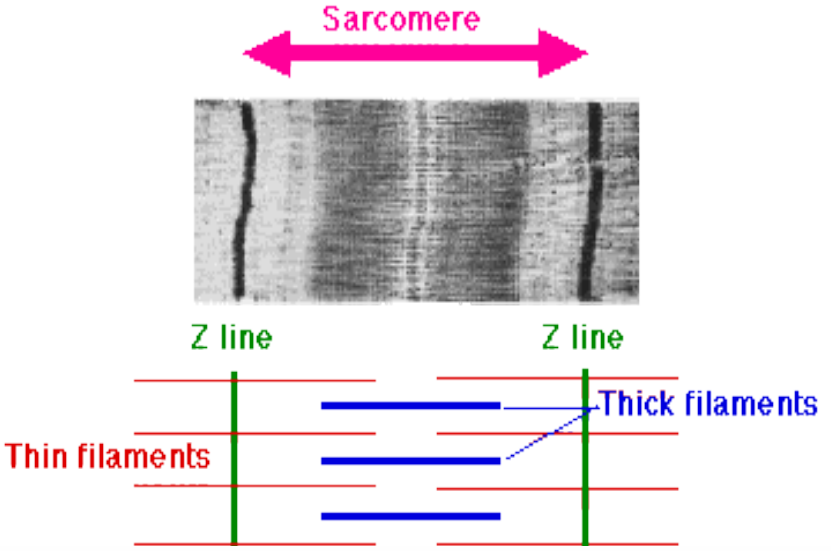


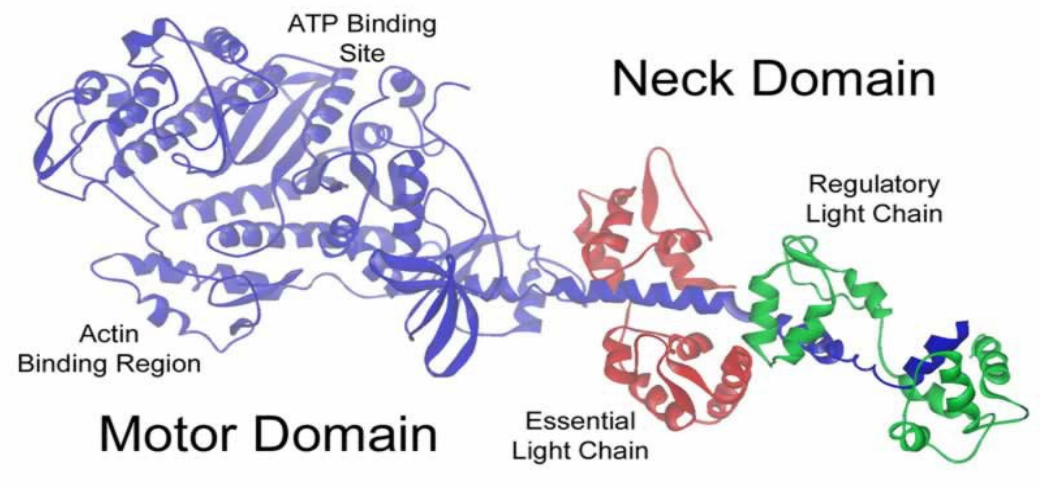
SAS from a complex between deuterated T-cell surface glycoprotein CD1d1 and nondeuterated beta-2 microglobulin.

Neutrons provide physiologically relevant, and yet precise, structural data V



Actin

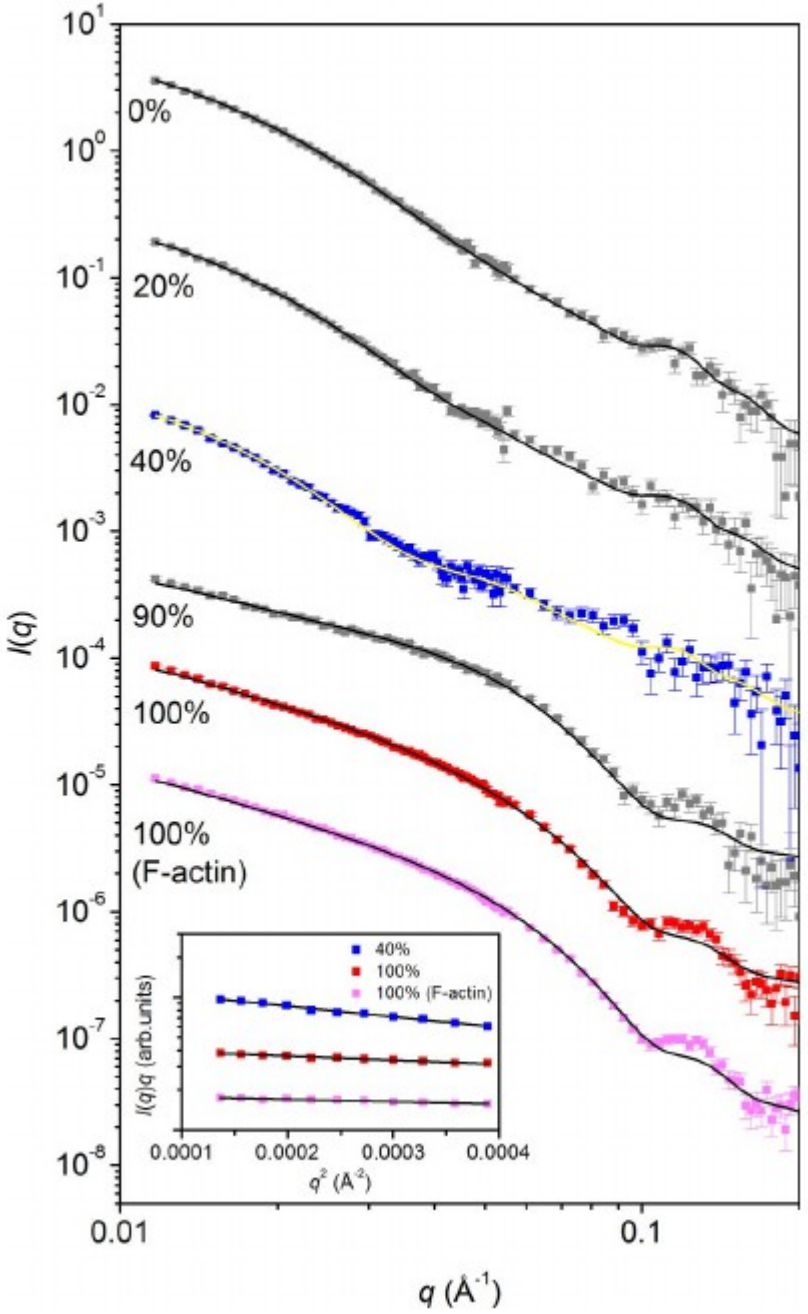




Myosin

Cardiac myosin-binding protein C decorates F-actin:Implications for cardiac function

Cardiac myosin-binding protein C decorates F-actin: Implications for cardiac function



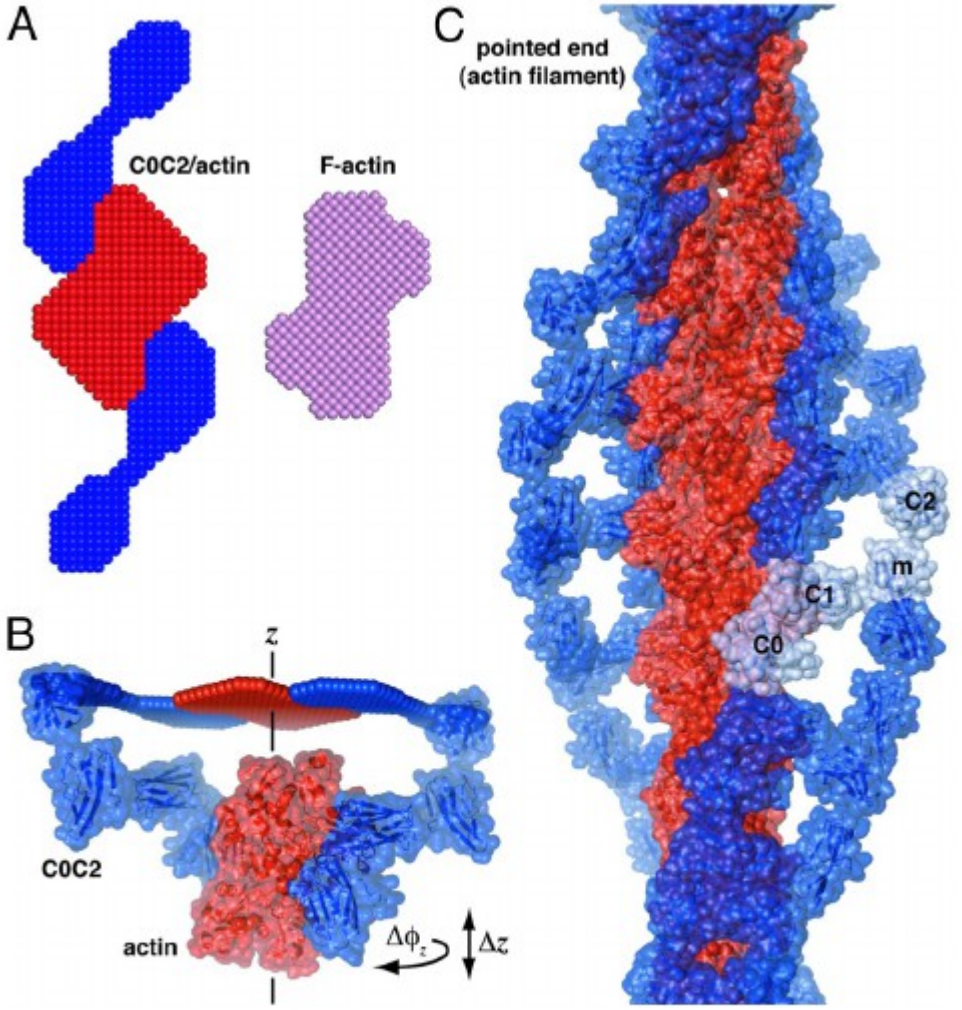


Fig. 3. Models derived from the small-angle neutron scattering data, where the actin component is shown in red, and the C0C2 component is shown is blue. (*A*) Cross-sectional dummy atom model of the C0C2–cardiac actin assembly obtained from optimization against the entire contrast variation series. The cross-sectional model for cardiac F-actin (light magenta) was obtained from optimization against the 3 contrast points measured for F-

Neutrons provide physiologically relevant, and yet precise, structural data

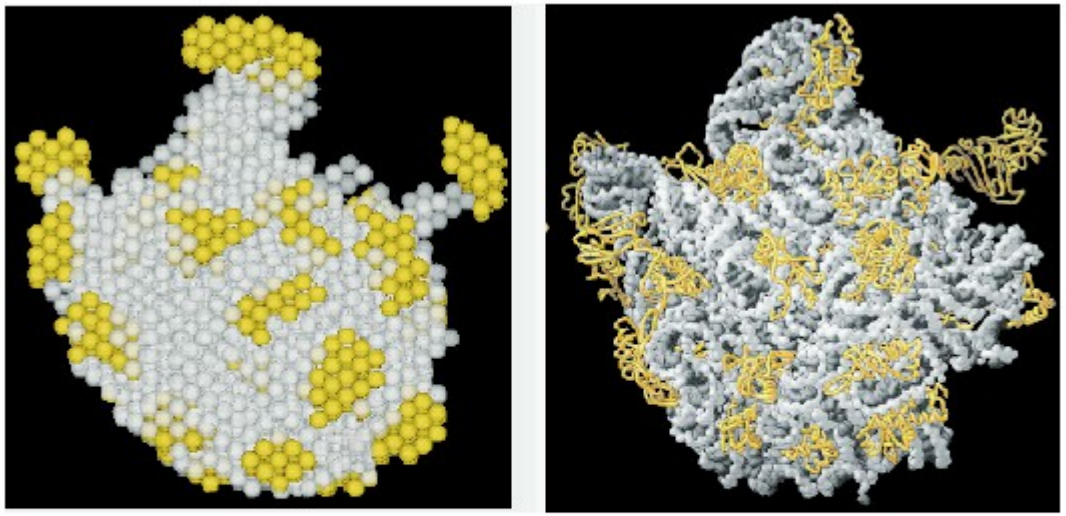
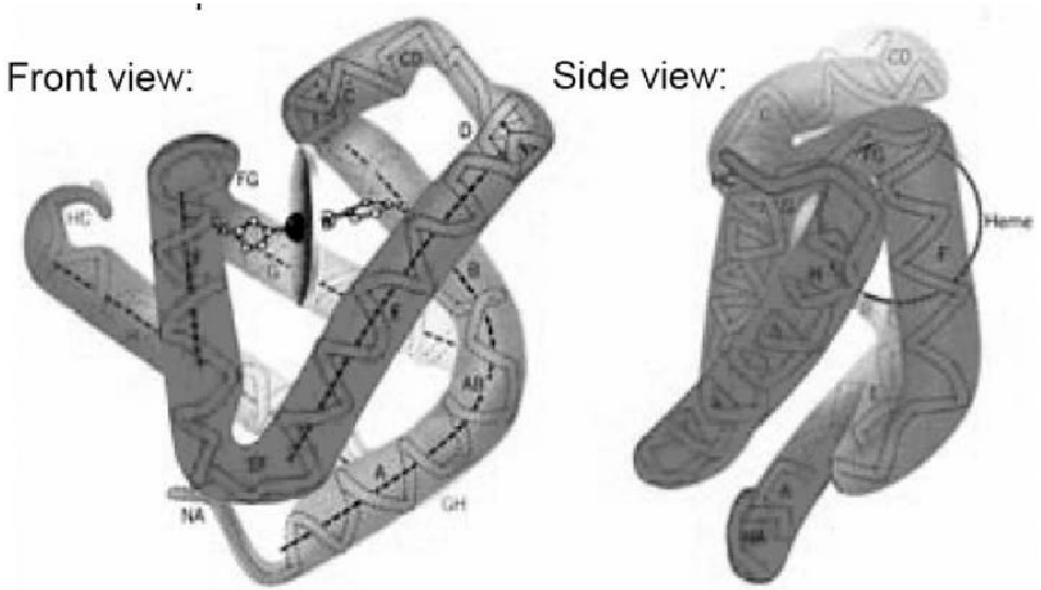


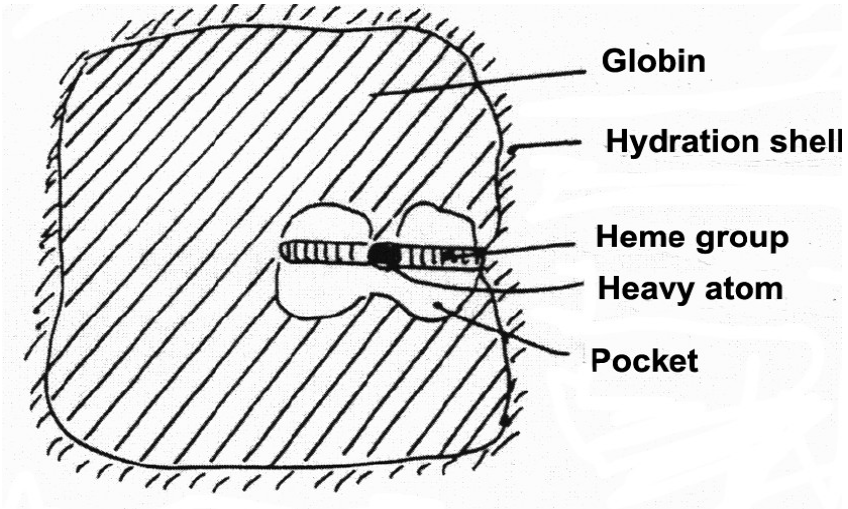
Figure 2: Comparison between the 50S subunit in the map of the 70S *E.coli* ribosome obtained from solution scattering [2,3] (left, resolution 3 nm), and the crystallographic model of the 50S ribosomal subunit *H.marismortui* (right, resolution 0.24 nm, Figure adapted from [4]. Yellow, ribosomal proteins, grey, ribosomal RNA.

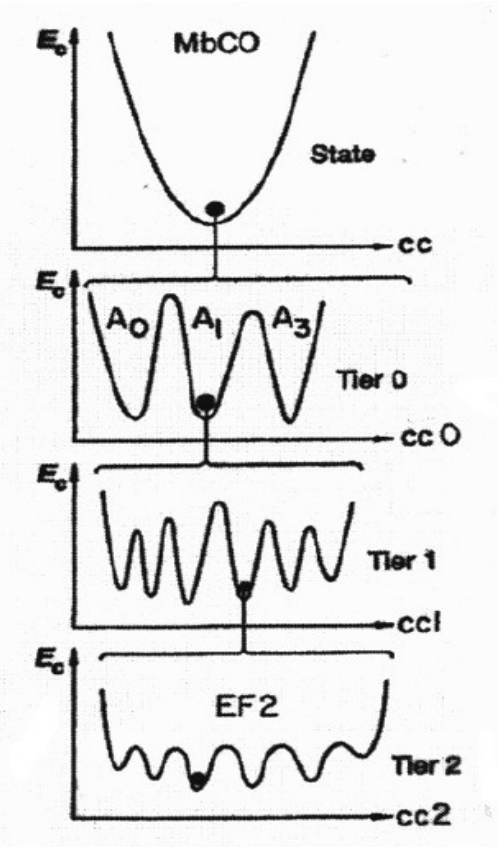
Ribosome: where proteins are synthesized.

Macromolecular dynamics are poorly understood



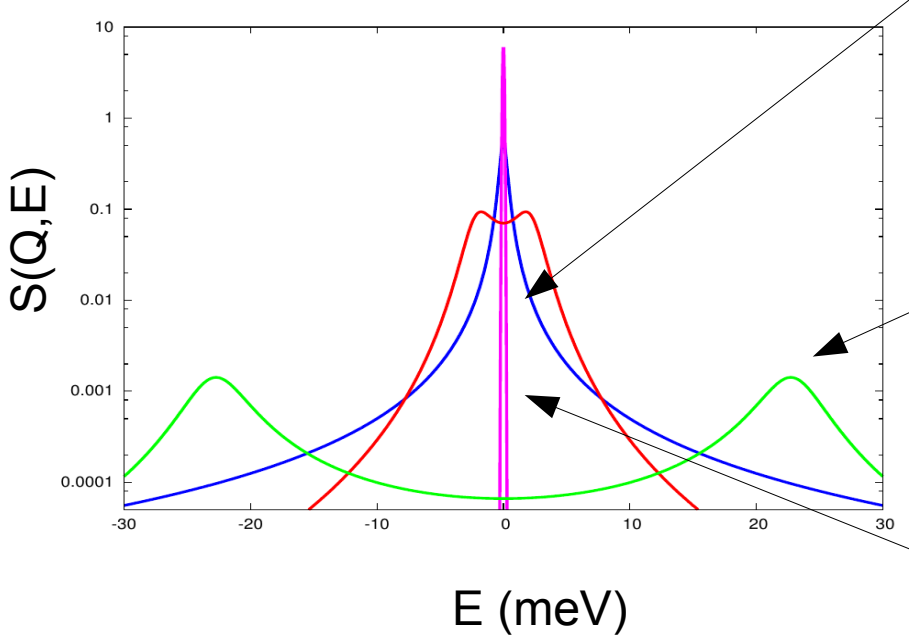
Myoglobin





Protein function and activity, including **enzyme catalysis**, **ligand binding**, **receptor action**, **electron and proton transfer**, are strongly dependent on internal dynamics and conformational fluctuations or rearrangements

Macromolecular dynamics are poorly understood



Quasielastic component: geometry of confined diffusive motions (times and distances)

Inelastic component: vibrational density of states and dispersion curves

Elastic component: Mean square displacements, Debye-Waller factor

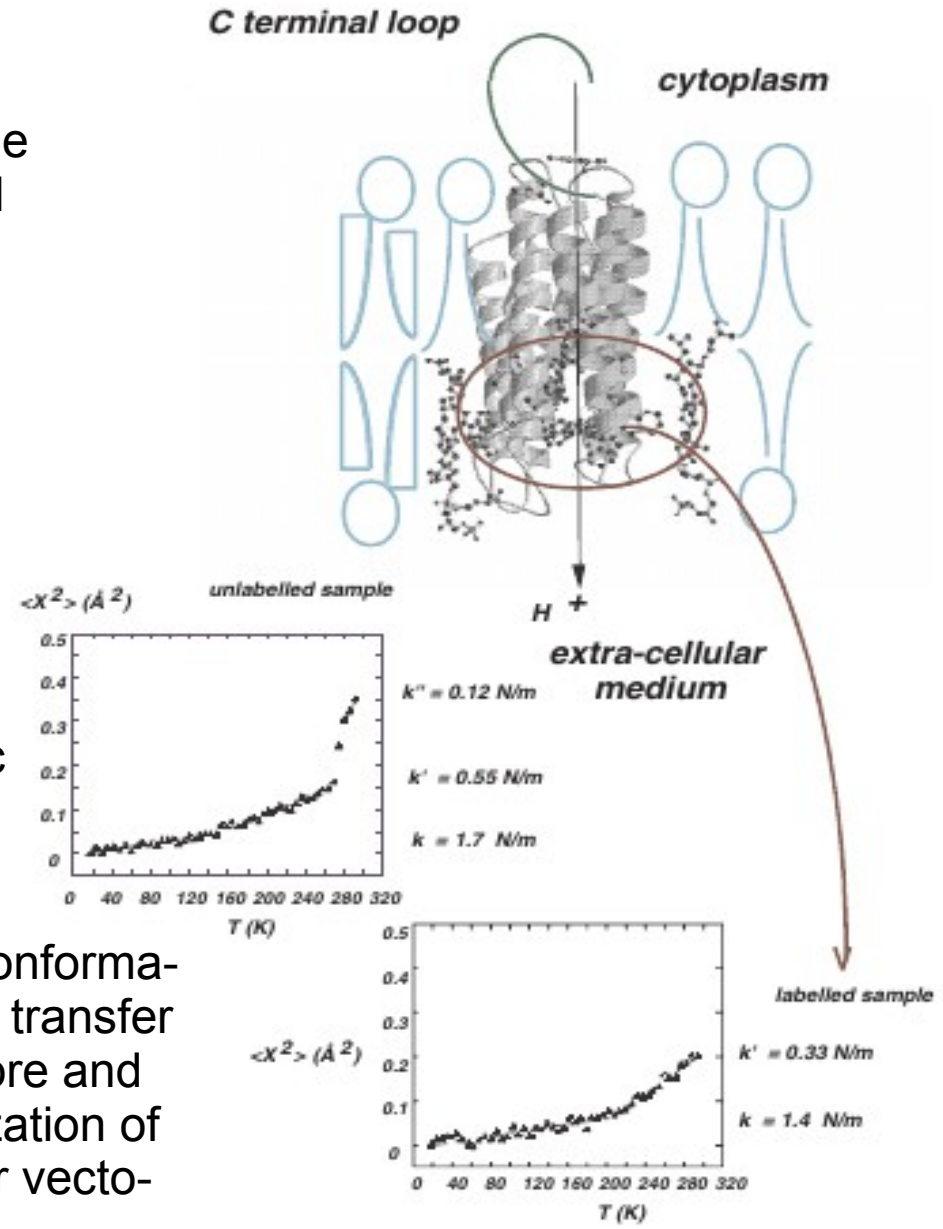
Dynamics-function relation in bacteriorhodopsin

A schematic diagram of the purple membrane from H. salinarum, showing the phospholipid molecules in blue, a bacteriorhodopsin monomer in ribbon mode

Purple membrane is made up of 25% lipids and 75% bacteriorhodopsin, a retinal-binding seven-helix membrane protein that functions as a light-activated proton pump.

Smaller fluctuations in the extracellular half of bacteriorhodopsin than in the cytoplasmic half

The thermally soft cytoplasmic half of bacteriorhodopsin allows the conformational changes associated with proton transfer along its pathway, whereas a stiffer core and extracellular half harness the isomerization of retinal to provide the valve function for vectorial proton transfer



Conclusions

- Neutrons as a gentle probe to biological systems
- Isotopic contrast

- SANS reveals the structure of biological macromolecular complexes
- SANS reveals the interactions in crowded conditions (aggregation)
- INS and QENS reveal dynamics key to biological functionality