Applications of SANS to Strategic Problems in Biology

(Oak Ridge October 2005)

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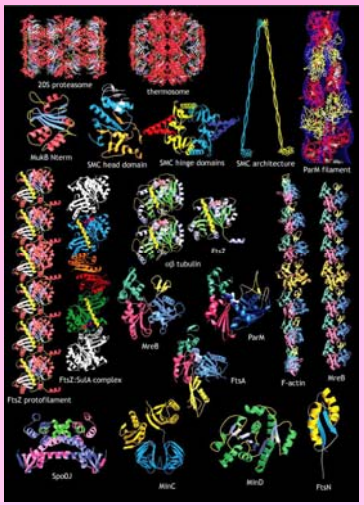
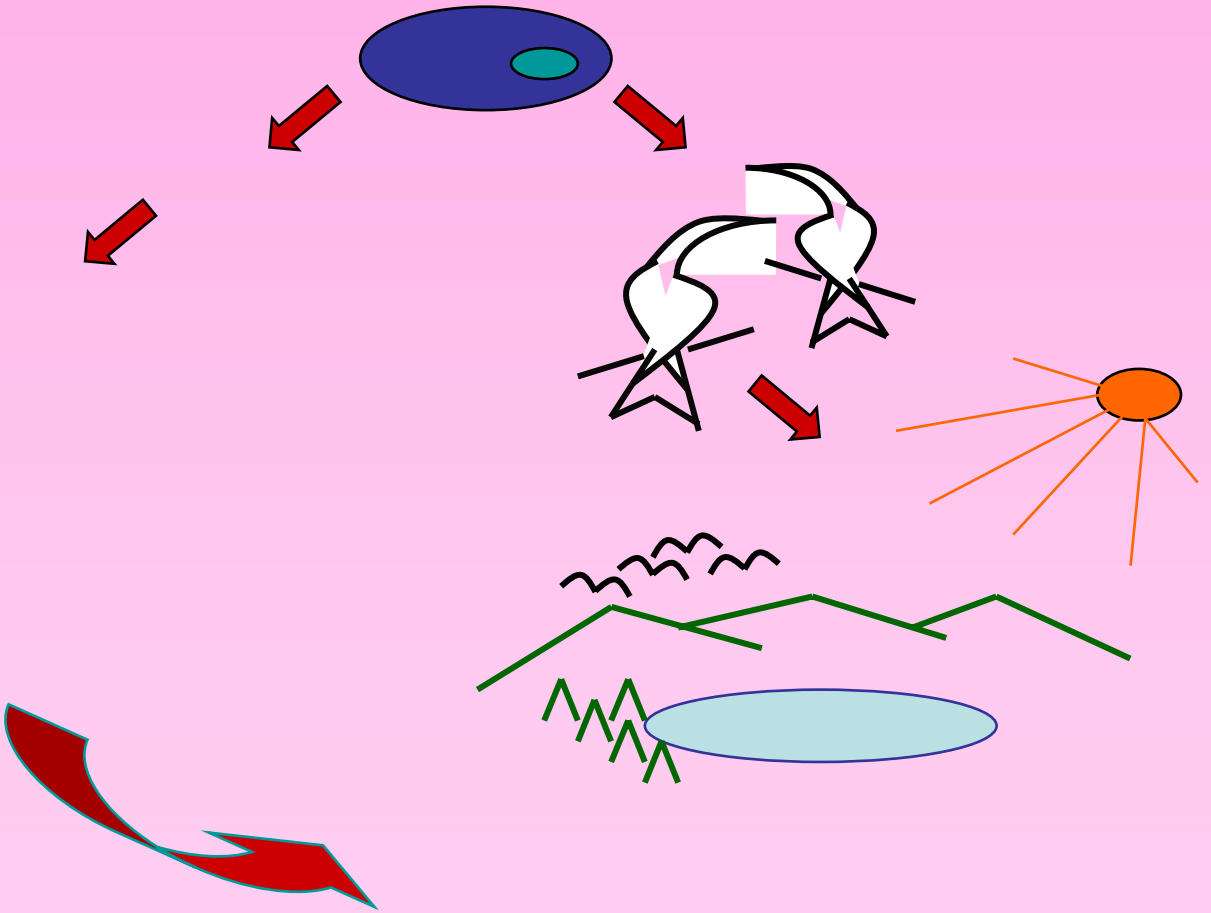
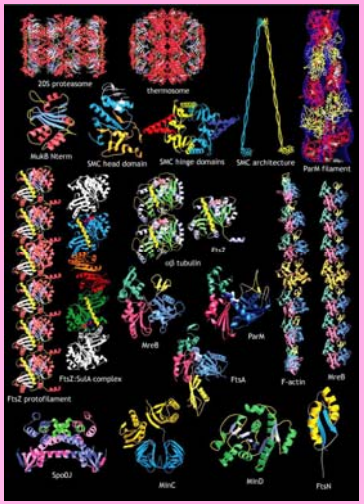
There are important areas and developments in biology
in which SANS
can advance our understanding significantly !

Length Scales in Biology







Structure at the macromolecular level

Molecular biology and biochemistry:
HIGH resolution structures (chemistry)

Xray crystallography... SR, small crystals, high throughput ...
NMR ... EM ...
Neutron crystallography... H-atoms

Structure at the macromolecular and supramolecular level

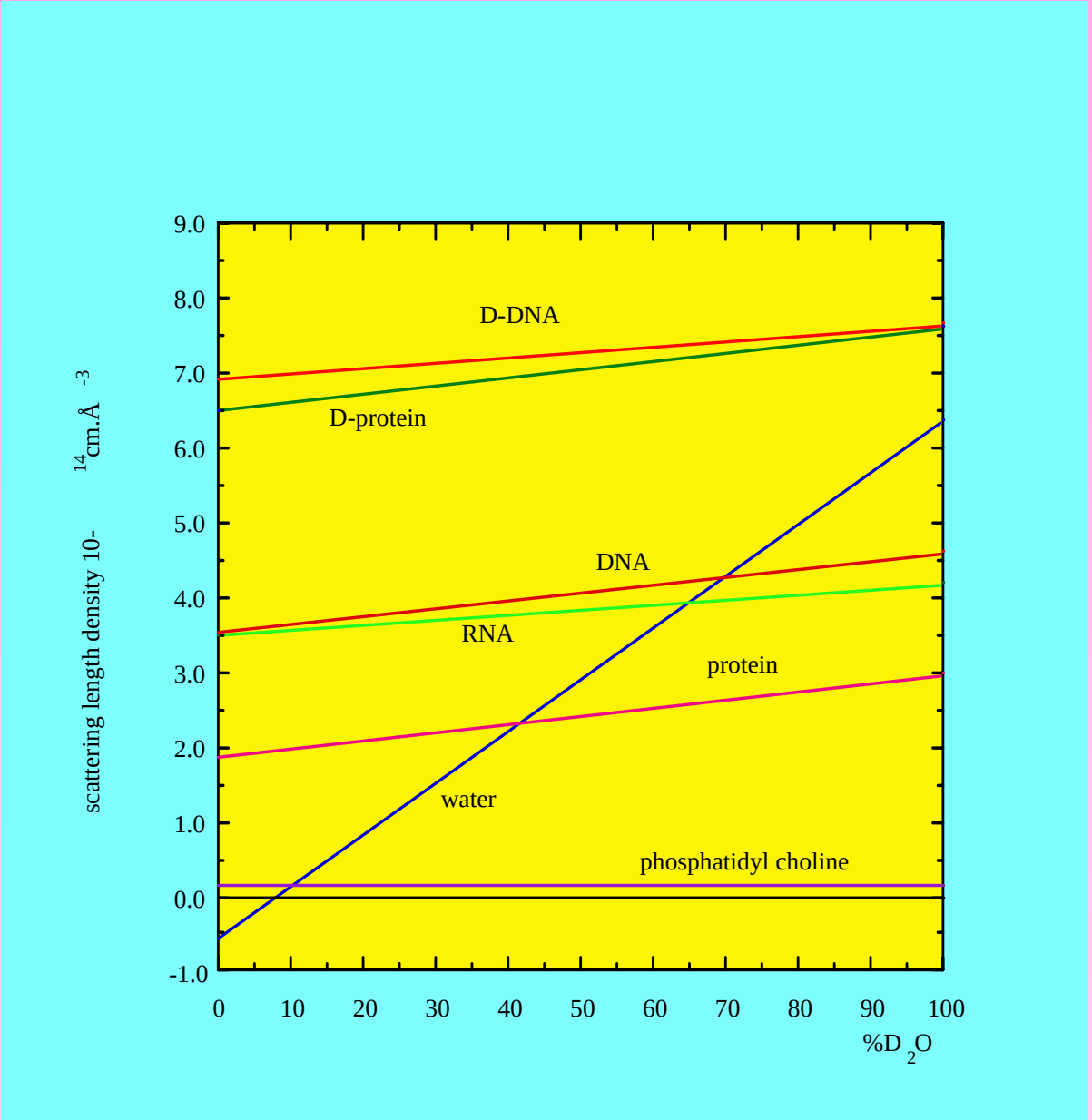
Molecular biology and biochemistry:
***LOW** resolution structures*

Bad reputation : mainly because of early X-ray work !

Briefly : chemistry acts as an important check on a HIGH resolution structure. There is no such check at LOW resolution, and many structures were proposed that were proved wrong when high resolution became available...

Because of the power of H-D contrast variation
THERE HAS NEVER BEEN A WRONG
LOW RESOLUTION NEUTRON STRUCTURE
(to my knowledge)

Contrast variation for biological macromolecules



BUT

Are LOW resolution structures interesting ?
or just insufficient information on systems that
‘have not yet been crystallised’ ?

The example of the Ribosome !

SANS

is a powerful LOW resolution structure method that can provide unique information on a diversity of biological systems

SANS

could be applied to systems 'before a crystal structure is available'

but
it is most powerful on
dynamic interacting or inherently disordered systems
for which at best high resolution structures would be
available only for individual components...

***Also,** it now seems that a large proportion of genomes represents*
'disordered' proteins that take up partial structuring only
when in interaction with other partners

More than thirty years of Neutrons in Biology

A non-exhaustive list of SANS and membrane diffraction publications from the seventies and eighties

Work in its time ?
Work before its time ?

Proteins

Satre M., Zaccai G. (1979). Small angle neutron scattering of Escherichia coli BF1-ATPase. *FEBS Lett.* 102:244-248.

Meyer J., Zaccai G. (1981). Neutron small angle scattering of the Mo-Fe protein (nitrogenase) from Chlostridium pasteurianum. *BBRC.* 98:43-50.

Klein G., Satre M., Zaccai G., Vignais P. (1982). Spontaneous aggregation of the mitochondrial natural ATPase inhibitor in salt solutions as demonstrated by gel filtration and neutron scattering. *Biochim. Biophys. Acta.* 681:226-232.

Dupuis A., Zaccai G., Satre M. (1983). Optical properties and small angle neutron scattering of bovine heart mitochondrial oligomycin sensitivity conferrring protein. *Biochemistry.* 22:5951-5956.

Lehmann M. S., Zaccai G. (1984). Neutron small-angle scattering studies of ribonuclease in mixed aqueous solutions and determination of the preferentially bound water. *Biochemistry.* 23:1939-1942.

Zaccai G., Bunick G.J. & Eisenberg H. (1986). Denaturation of a halophilic enzyme monitored by small-angle neutron scattering. *J.Mol. Biol.* **192**: 155-157.

Zaccai G., Wachtel E. & Eisenberg H. (1986). Solution structure of halophilic malate dehydrogenase from small-angle neutron and X-ray scattering and ultracentrifugation. *J. Mol. Biol.* **190**: 97-106.

Calmettes P., Eisenberg H., Zaccai G. (1987). Structure of halophilic malate dehydrogenase in multimolar KCl solutions from neutron scattering and ultracentrifugation. *Biophysical chemistry.* 26:279-290.

Protein - DNA interactions

Charlier M., Maurizot J.C., Zaccai G. (1980). Neutron scattering studies of lac repressor. *Nature.* 286:423-425.

Charlier M., Maurizot J.C., Zaccai G. (1981). Neutron scattering of lac repressor: a low resolution model. *J.Mol.Biol.* 153:177-182.

Charlier M., Maurizot J. C., Zaccai G. (1983). Non specific binding of lac repressor to DNA. II. A small angle neutron scattering study. *Biophys. Chem.* 18:313-322.

Krueger S., Zaccai G., Wlodawer A., Langowski J., O'Dea M., Maxwell A., Gellert M. (1990). Neutron and light scattering studies of DNA gyrase and its complex with DNA. *J. Mol. Biol.* 211:211-220.

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Protein - RNA

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Cuillel M, Zulauf M, Jacrot B. (1983) Self-assembly of brome mosaic virus protein into capsids. Initial and final states of aggregation. J Mol Biol. 1983 Mar 15;164(4):589-603.

Jacrot B, Chauvin C, Witz J. (1977) Comparative neutron small-angle scattering study of small spherical RNA viruses. Nature. 266(5601):417-21.

Giegé R., Jacrot B., Moras D., Thierry J.C., Zaccai G. (1977). A neutron investigation of yeast valyl-tRNA synthetase interaction with tRNAs. *Nucl. Ac. Res.* 4:2421-2428.

Dessen P., Blanquet S., Zaccai G., Jacrot B. (1978). Antico-operative binding of initiator transfer RNA Met to methionyl-transfer RNA synthetase from Escherichia coli: Neutron scattering studies. *J. Mol. Biol.* 126:293-313.

Serdyuk I. N., Zaccai G., Spirin A. S. (1978). Globular conformation of some ribosomal proteins in solution. *FEBS Lett.* 349-352.

Zaccai G., Morin P., Jacrot B., Moras D., Thierry J.C., Giege R. (1979). Interactions of yeast valyl-tRNA synthetase with RNAs and conformational changes of the enzyme. *J. Mol. Biol.* 129:483-500.

Serdyuk I. N., Grenader A. K., Zaccai G. (1979). Study of the internal structure of E. coli ribosomes by neutron and X-ray scattering. *J. Mol. Biol.* 135:691-708.

Serdyuk I. N., Shpungin J. L., Zaccai G. (1980). Neutron scattering study of the 13S fragment of 16S RNA and its complex with ribosomal protein S4. *J. Mol. Biol.* 153:177-182.

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Protein RNA (more)

Dessen P., Fayat G., Zaccai G., Blanquet S. (1982). Neutron scattering studies of the binding of initiator tRNA Met to Escherichia coli trypsin-modified methionyl-tRNA synthetase. *J. Mol. Biol.* 154:603-613.

Dessen P., Zaccai G., Blanquet S. (1982). Neutron scattering studies of Escherichia coli tyrosyl-tRNA synthetase and of its interaction with $tRNA^{tyr}$. *J. Mol. Biol.* 159:651-664.

Giegé R., Lorber B., Ebel J.P., Moras D., Thierry J.C., Jacrot B., Zaccai G. (1982). Formation of a catalytically active complex between tRNAAsp and aspartyl-tRNA synthetase from yeast in high concentrations of ammonium sulphate. *Biochimie.* 64:357-362.

Li Z.Q., Giege R., Jacrot B., Oberthur R., Thierry J.C., Zaccai G. (1983). Structure of phenylalanine-accepting transfer ribonucleic acid and of its environment in aqueous solvents with different salts. *Biochemistry.* 22:4380-4388.

Dessen P., Zaccai G., Blanquet S. (1985). Methionyl-tRNA synthetase from E. coli: direct evidence for exchange of protomers in the dimeric enzyme by using deuteration and small angle neutron scattering. *Biochimie.* 67:637-641.

Antonsson B., Leberman R., Jacrot B., Zaccai G. (1986). Small-angle neutron scattering study of the ternary complex formed between bacterial elongation factor Tu, guanosine 5'-triphosphate, and $valyl\text{-}tRNA^{val}$. *Biochemistry.* 25:3655-3659.

Zaccai G., Xian S.Y. (1988). Structure of phenylalanine-accepting transfer ribonucleic acid and of its environment in aqueous solvents with different salts. *Biochemistry.* 27:1316-1320.

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Membranes and membrane proteins

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Zaccai G. & Gilmore D. J. (1979). Areas of hydration in the purple membrane of halobacterium halobium: a neutron diffraction study. *J. Mol. Biol.* **132**: 181-191.

Engelman D.M. & Zaccai G. (1980). Bacteriorhodopsin is an inside-out protein. *Proc. Natl. Acad. Sci. (USA)* **77**: 5894-5898.

Block M., Zaccai G., Lauquin G., Vignais P. (1982). Small-angle neutron scattering of the mitochondrial ADP/ATP carrier protein in detergent. *Biochemical and biophysical research communications.* 109:471-477.

Gogol E. P., Engelman D. M., Zaccai G. (1983). Neutron diffraction analysis of cytochrome b5 reconstituted in deuterated lipid multilayers. *Biophys. J.* 43:285-292.

Rogan P. K. & Zaccai G. (1981). Hydration in purple membrane as a function of relative humidity. *J. Mol. Biol.* **145**: 281-284.

Trewhella J., Anderson S., Fox R., Gogol E. P., Khan S., Engelman D.M. & Zaccai G. (1983). Assignement of segments of the bacteriorhodopsin sequence to positions in the structural map. *Biophys. J.* **42**: 233-241.

Membranes and membrane proteins (more)

Jubb J., Worcester D., Crespi H. & Zaccai G. (1984). Retinal location in purple membrane of halobacterium halobium: a neutron diffraction study of membranes labelled in vivo with deuterated retinal. *The EMBO journal* **3**(7): 1455-1461.

Trewhella J., Popot J.L, Zaccai G. & Engelman D. (1986). Localization of two chymotryptic fragments in the structure of renatured bacteriorhodopsin by neutron diffraction. *The EMBO journal* **5**: 3045-3049.

Zaccai G. (1987). Structure and hydration of purple membranes in different conditions. *J. Mol. Biol.* **194**: 569-572.

Dencher N. A., Dresselhaus D., Zaccai G. & Büldt G. (1989). Structural changes in bacteriorhodopsin during proton translocation revealed by neutron diffraction. *Proc. Natl. Acad. USA* **86**: 7876-7879.

Popot J. L., Engelman D. M., Gurel O. & Zaccai G. (1989). Tertiary structure of bacteriorhodopsin Positions and orientations of helices A and B in the structural map determined by neutron diffraction. *J. Mol. Biol.* **210**: 829-847.

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The direction of Biology at the beginning of the 21st Century

André Guinier died in Paris at the beginning of July 2000, only a few weeks after it was announced in the press that a human genome had been sequenced.

End of an era . . . beginning of a new era . . .

‘… first, that we have made incredible progress in deciphering what we know today about protein assemblies; and second, that we still have an enormous amount to learn. Thus, for example, our current drawings of the structure of the nuclear pore complex seem reminiscent of the sketches of houses drawn by young children, and they probably bear similar relation to the real thing…

… research in the post-genome-sequencing era, when most advances will come from successfully dissecting complicated in vitro systems composed of pure components… Here a deep understanding of the key constraints on the system imposed by thermodynamic and kinetic factors, as well as the ability to use new developments in chemistry and physics as appropriate tools, will often be vital for success…’

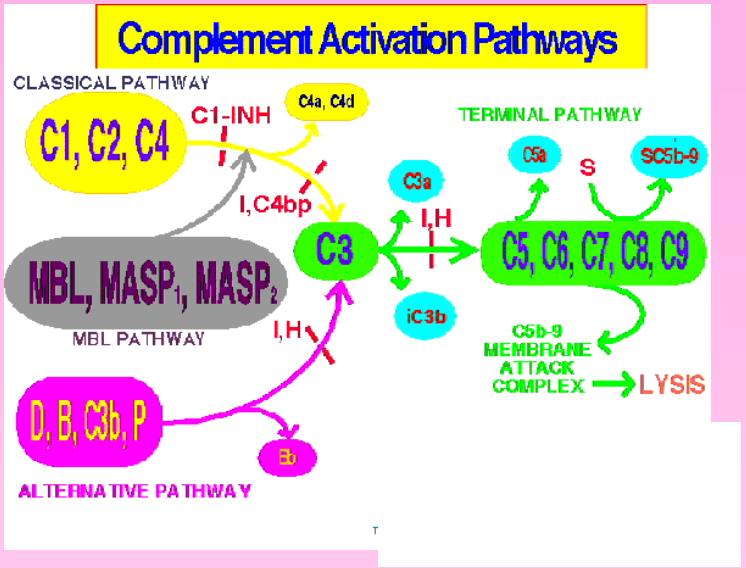
Alberts (1998) *Cell* 92, 291-294

Perhaps more slowly than he intended in 1998,
structural Biology is moving in the direction Alberts predicted,
and in a Preview (SAXS and the Working Protein)
of the February 2005 issue of *Structure* with the cover title
'Macromolecular Assemblies Highlighted'
we can read:

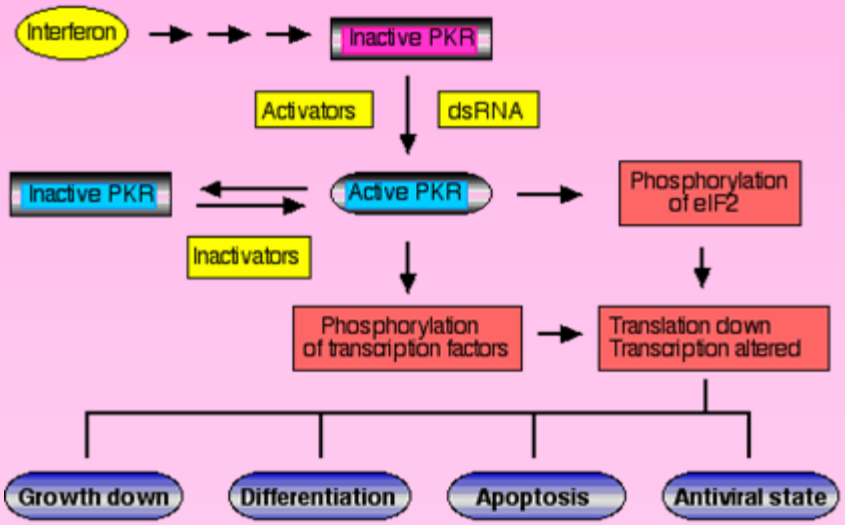
'… In this issue … Davies et al., 2005 present shape reconstructions for the molecular motor p97 using small angle X-ray scattering (SAXS) …
This work emphasizes the emerging potential of SAXS for visualizing the workings of biological machines in solution…'

Nagar and Kuryan (2005) *Structure* 13, 169-173

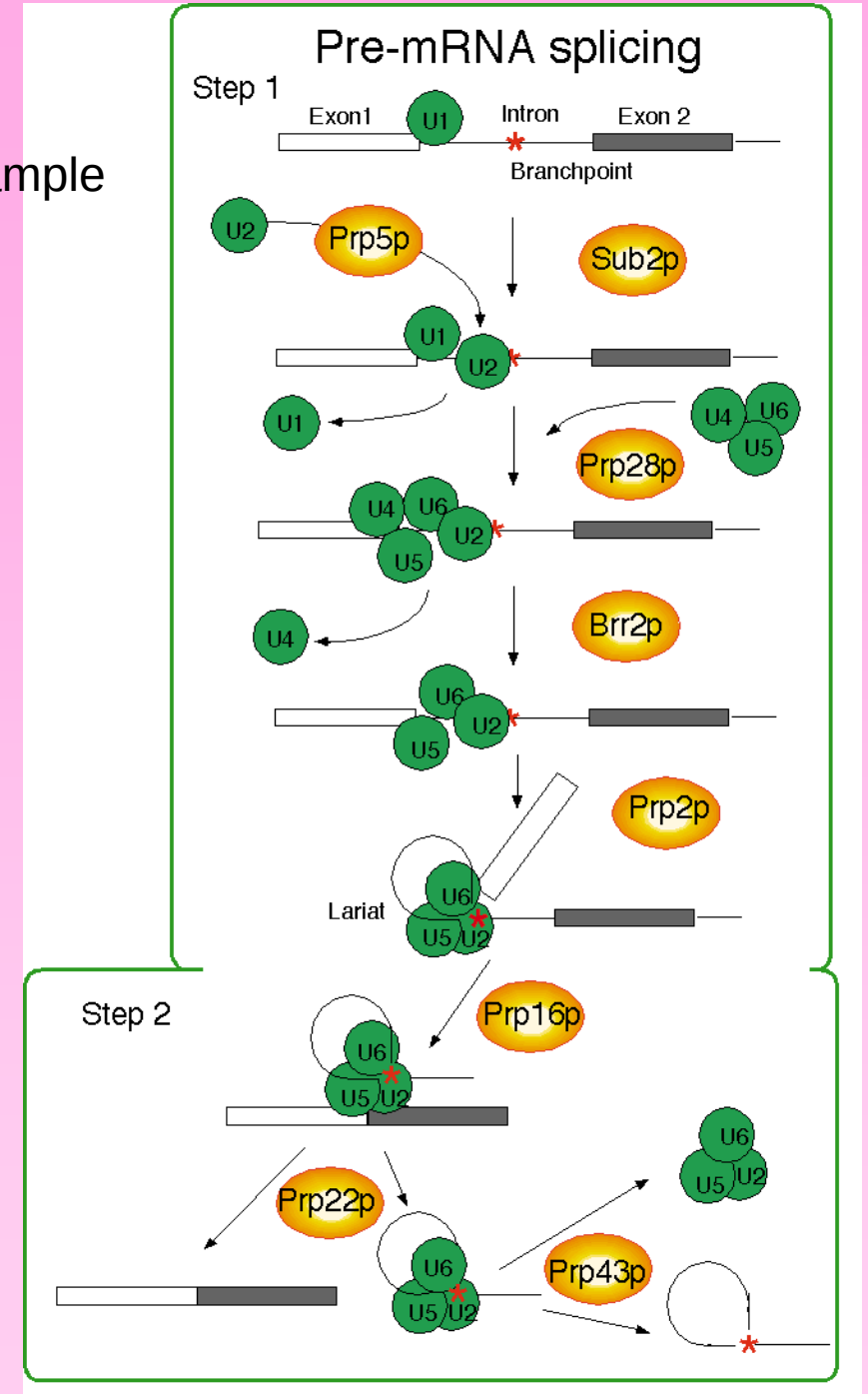
Examples of biochemical ‘cartoons’ of important interacting systems in immunology and the response to viral infection; there are structures available for some of the components but little is known about the structures of the complexes



Interferon activation pathway (PKR)



Another cartoon example



The spliceosome

The emphasis of modern structural biology is gradually changing towards making use of the thousands of high-resolution structures still being solved now by crystallography and NMR in order to address the question of how they act in dynamic molecular assemblies.

In this context, the information that can be obtained from the study of macromolecular complexes *in solution*, under various solvent conditions including those that mimic physiological conditions, is essential.

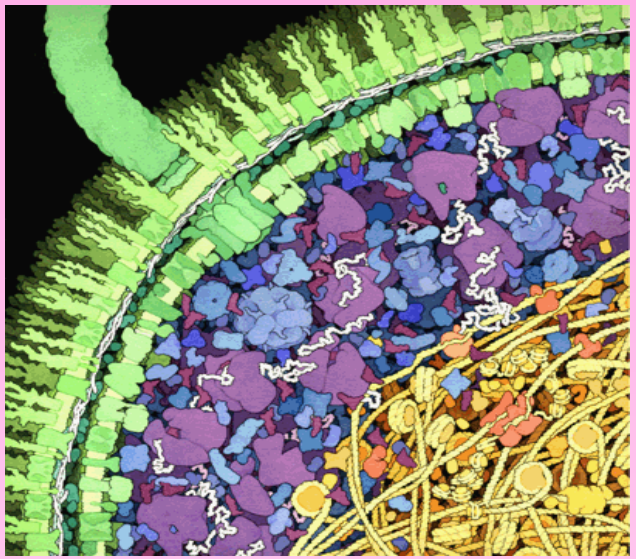
For these studies **SANS** has strong advantages !

The two axioms of Cell theory (Schleiden, Schwann, Remak, Virchow) remain true today

1) *The cell is the organisational unit of all life forms*

2) *Cells arise only from other cells*

The Cell, secret of Life



D.S. Goodsell

We can manipulate genetic information by sequencing cutting sewing bits of DNA together but when we want it expressed efficiently we have to introduce it into a living cell

True for a single protein expressed in a bacterial cell or for Dolly the sheep, which came from an egg cell !

We do not know how to 'make' a cell and DNA doesn't either !

Broken Symmetry

Anderson (1975), Science

Each level of organisation has its own properties and laws

Quantitative differences lead to qualitative differences

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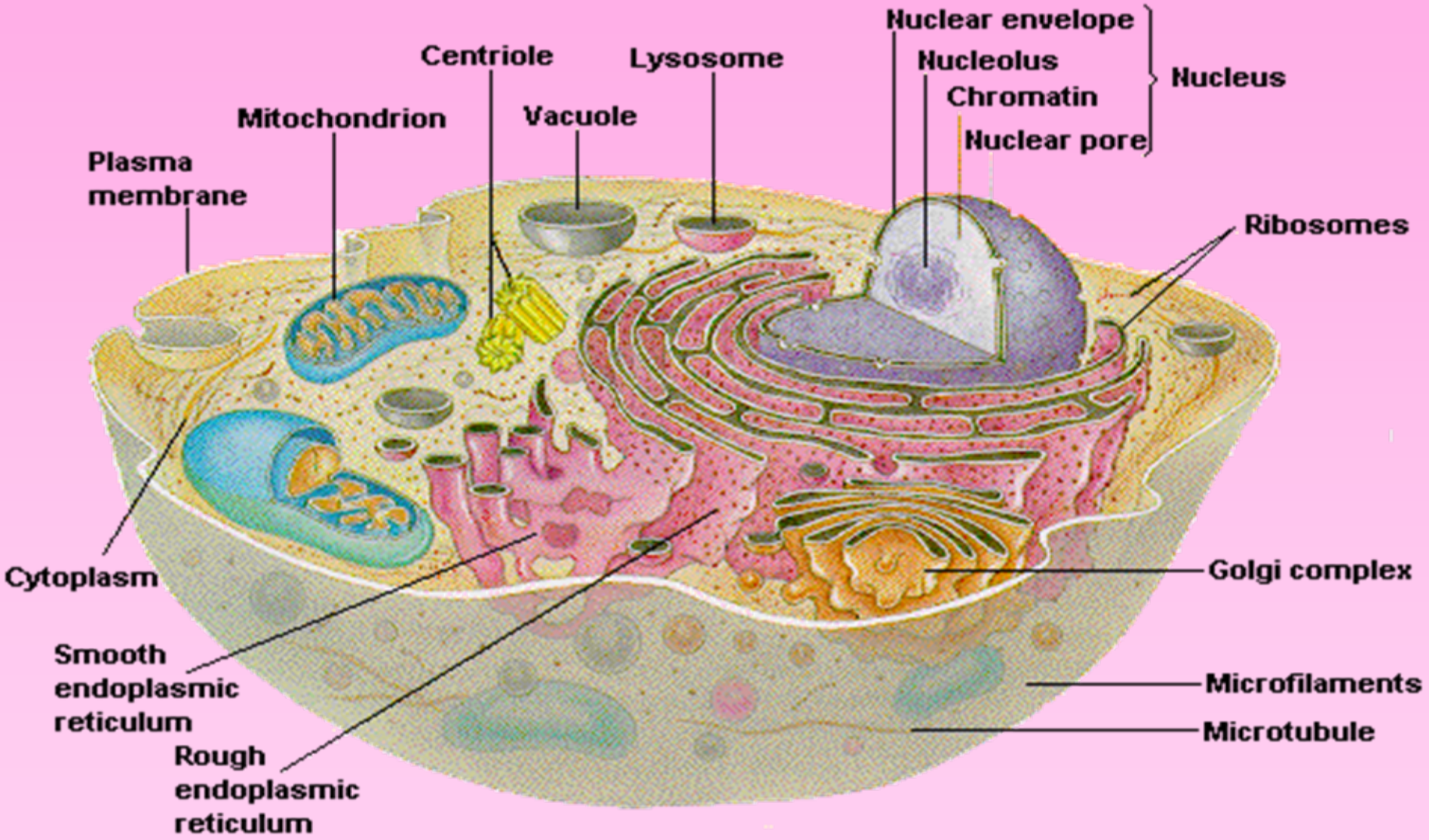
What are the interactions governing the dynamic molecular assemblies in the cell environment ?

These have to be studied as themselves and cannot be predicted from the high resolution structures of their components or even from their time-averaged or static structure !

A eukaryotic cell



The living Cell

An open thermodynamic system
in constant activity, even when it is not dividing

Membrane proteins: differences in chemical potential
DNA repair mechanisms
RNA synthesis and processing
Protein synthesis and processing
Internal membrane traffic
Cytoskeleton dynamics

and and and . . .

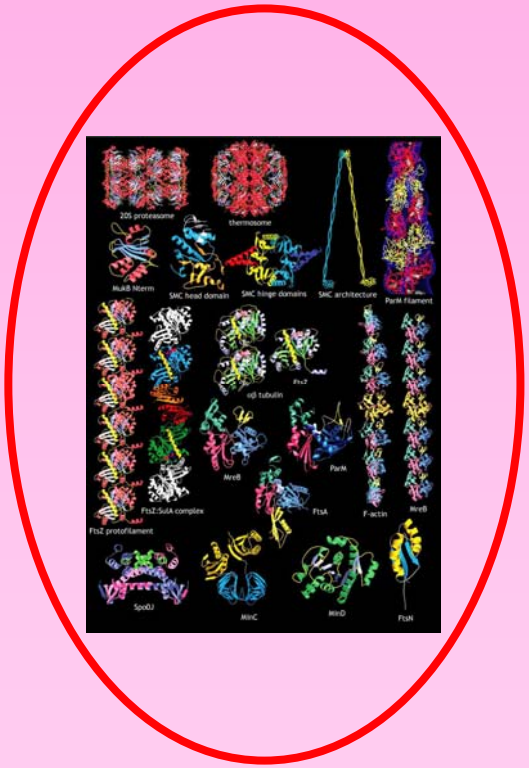
All working in a coordinated synergistic way !

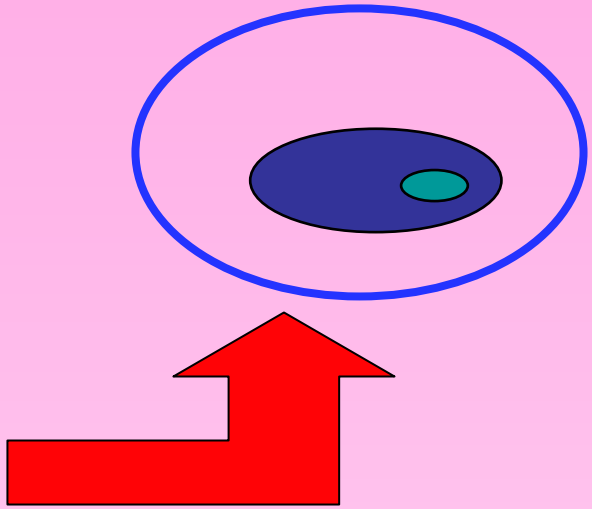
Cells in a human being turn over ~ 20 kg of ATP molecules per day

. . .

Les devises Shadok

IL VAUT MIEUX POMPER MÊME S'IL NE SE PASSE RIEN QUE RISQUER QU'IL SE PASSE QUELQUE CHOSE DE PIRE EN NE POMPANT PAS.





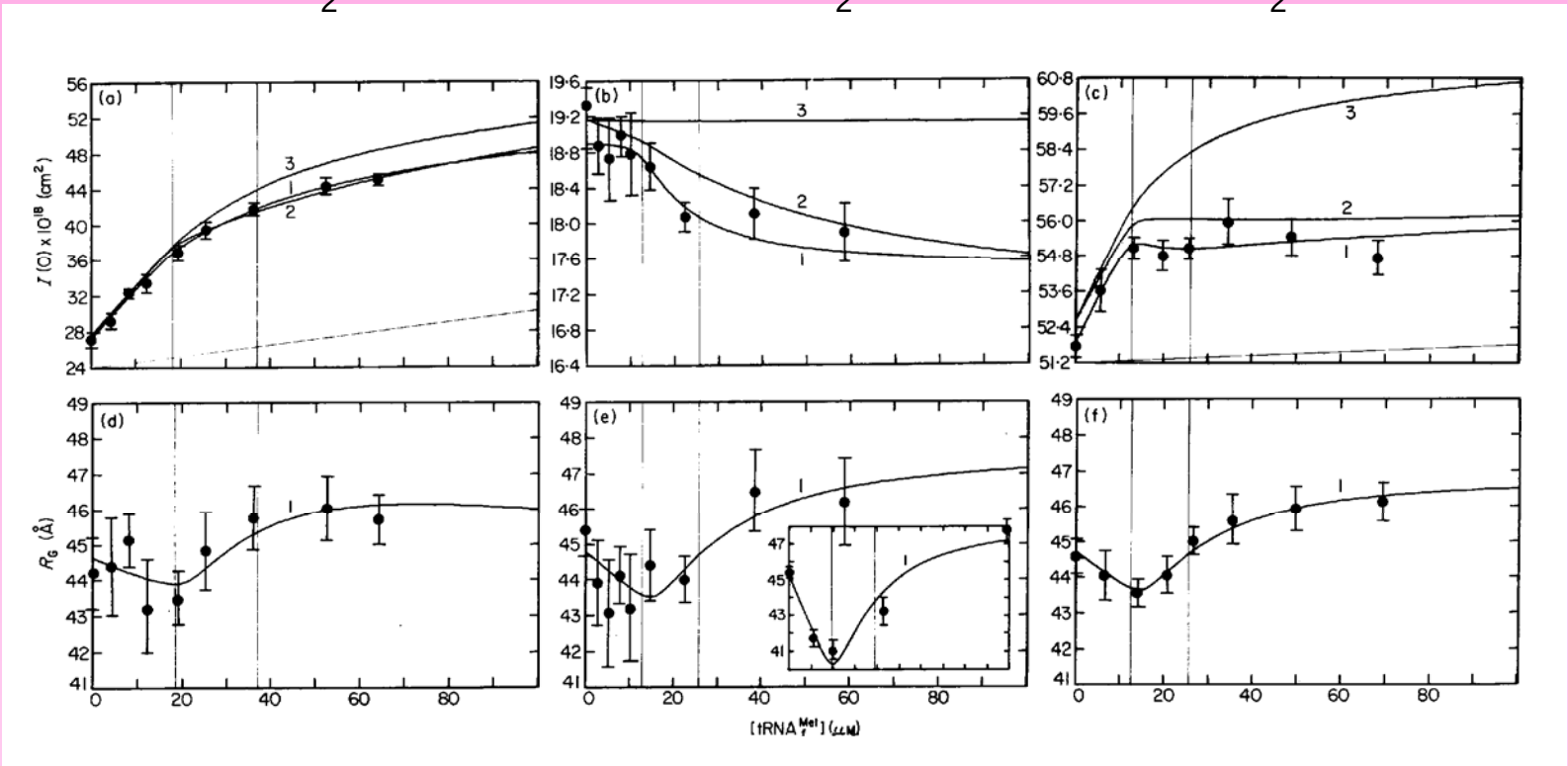
BIOCHEMISTRY !

BIOCHEMISTRY !!

BIOCHEMISTRY !!!

Work before its time ? Dessen et al. (1978): Contrast variation and tRNA in-beam titration to solve the Structural aspects of the antico-operative binding of initiator transfer tRNA-Met to methionyl-transfer RNA synthetase from Escherichia coli

H_2O 77% D_2O D_2O



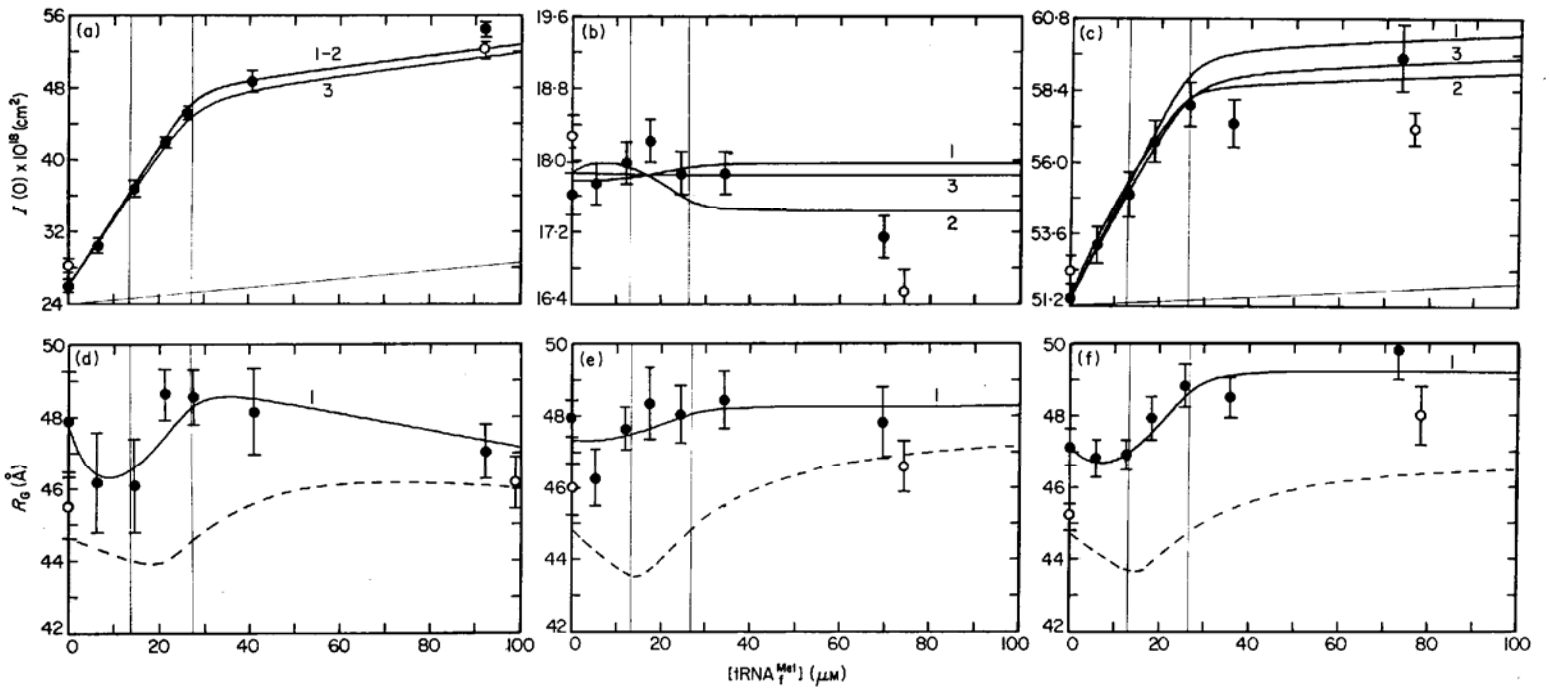
10 mM $MgCl_2$

in H_2O: both visible tRNA>protein

in 77% D_2O: tRNA invisible

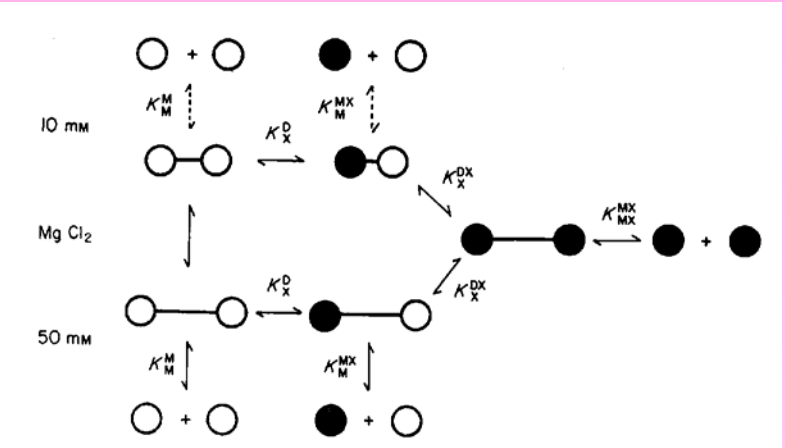
in D_2O: both visible protein>tRNA

H_2O 77% D_2O D_2O

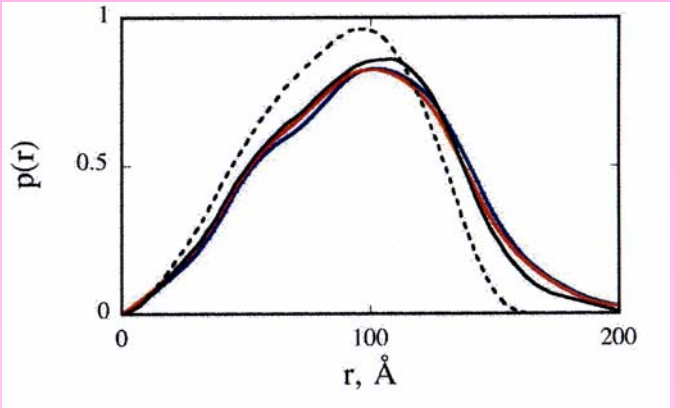


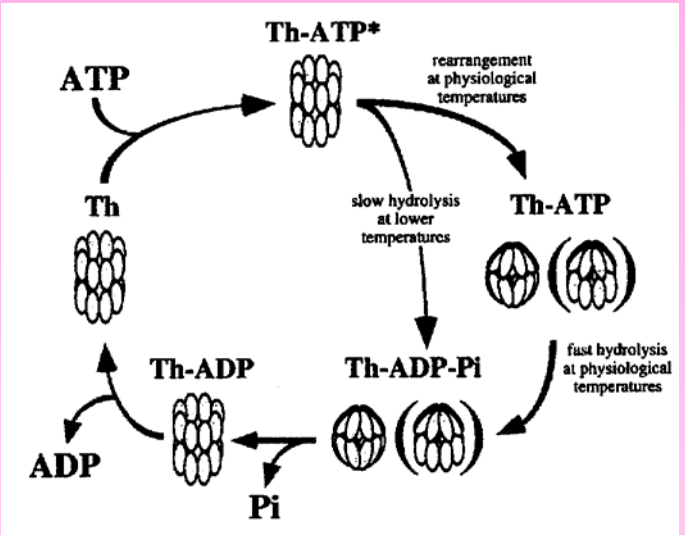
50 mM $MgCl_2$

The interaction model



Gutsche et al. 2001 showed by SANS how the thermosome adopted two different structures in its activity cycle; the ‘round’ structure had been seen by crystallography and the ‘long’ one had been seen by em but it took the SANS experiment to explain the apparent discrepancy and to solve the structural activity cycle of the complex





Troponin Team

SANS + SAXS

Robert Mendelson + Deborah Stone UCSF
Peter Timmins ILL, Grenoble
Theyencheri Narayanan, ESFR, Grenoble

Modelling

William King + Paul Curmi UNSW

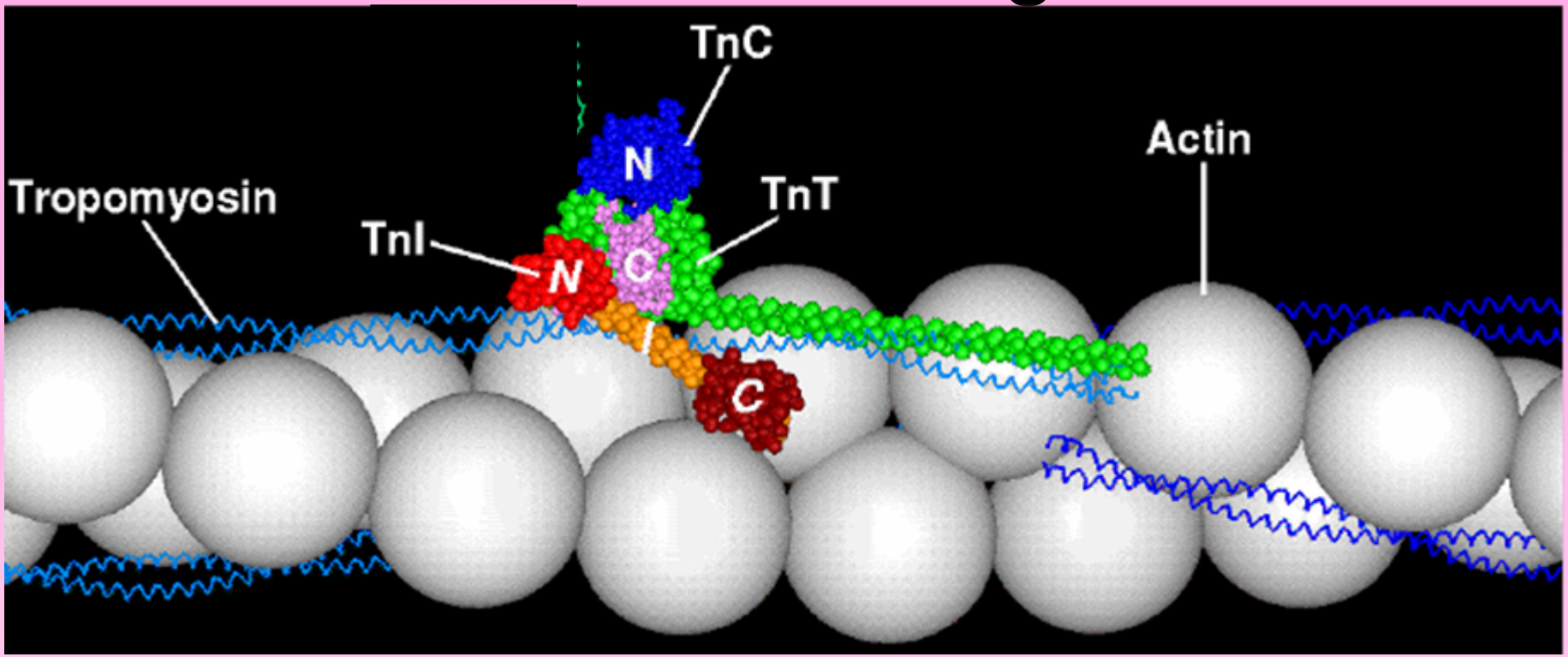
X-ray Crystallography

Maia Vinogradova + Robert Fletterick UCSF

EM

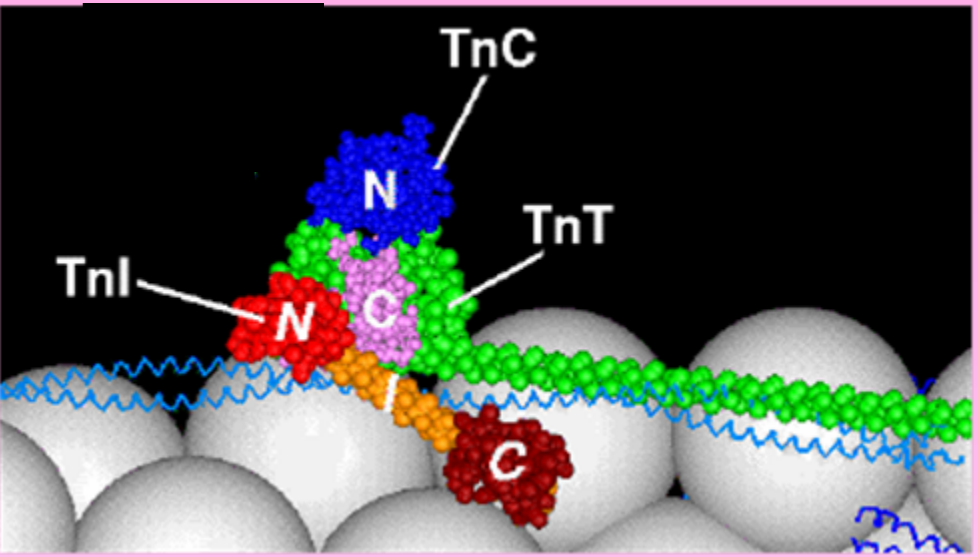
Alnoor Pirani, Victoria Hatch + William Lehman Boston U.
Roger Craig U. Massachusetts
Chen Xu Brandeis U.
Larry Tobacman U. Illinois

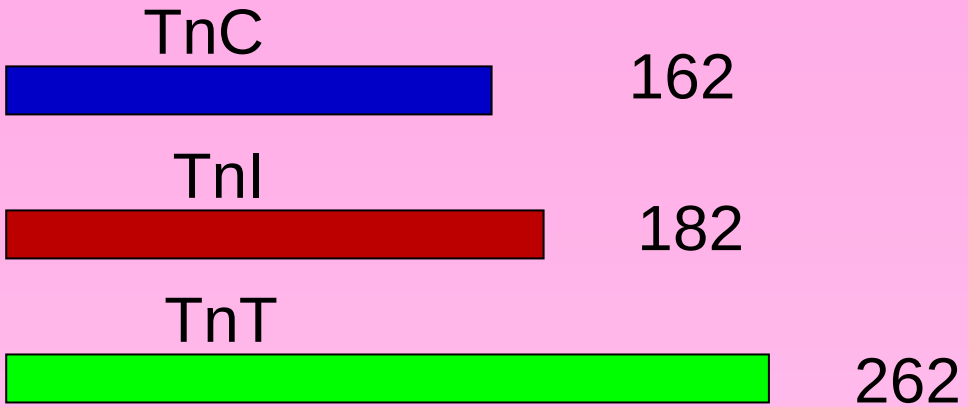
Striated muscle regulation



- Thin filament based
- Tropomyosin: homodimeric coiled coil
- Troponin: heterotrimeric Ca^{2+} regulated switch

Troponin: Ca^{2+} switch





Three subunits:

- TnC - Ca^{2+} sensor - CaM superfamily
- TnI - “inhibitory subunit” - binds actin
- TnT - Tm binding subunit

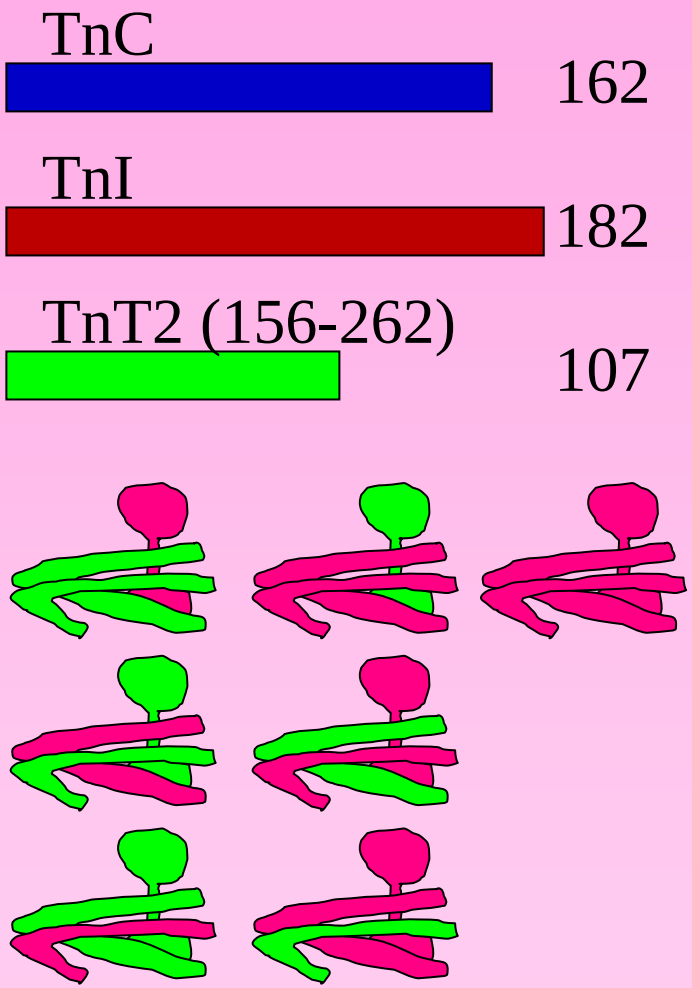
Aim of the project

- Determine the structure of Tn
- Determine the structural changes in Tn $\pm Ca^{2+}$
- Determine the mechanism of regulation

At the start of the work

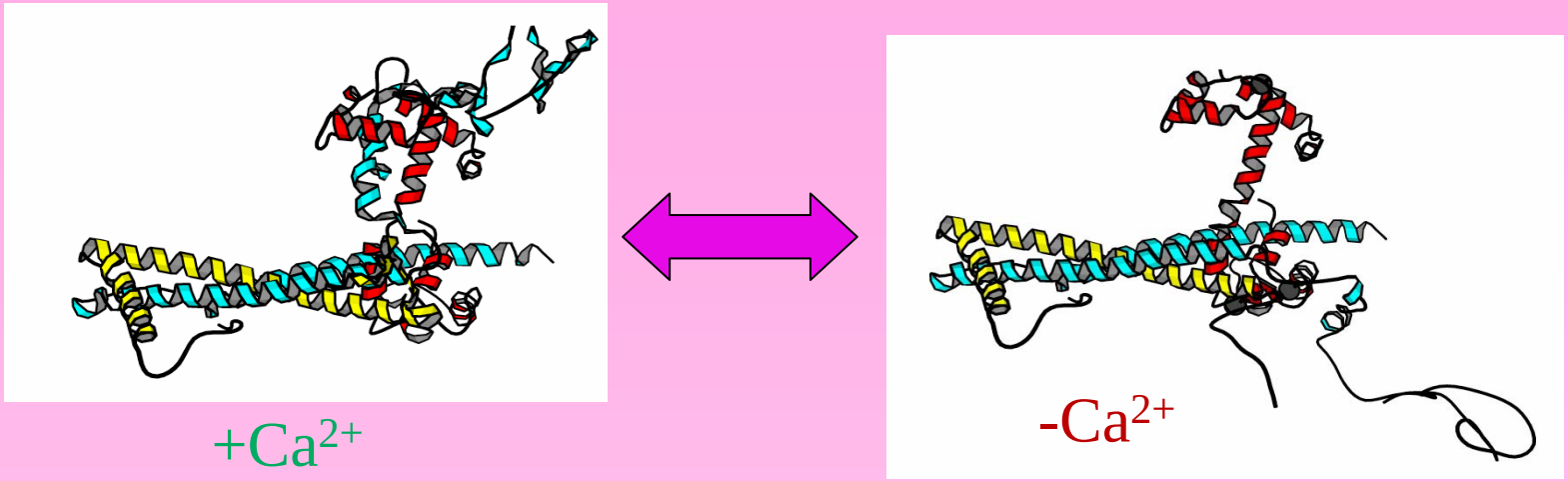
- TnC crystal structures
- SANS data: TnC & TnC.TnI
- Many unsuccessful attempt to crystallise Tn
- No reliable structural information on TnI & TnT

The Proteins



- Chicken skeletal muscle
  - *E. coli* expression
- TnT2
  - No aggregation/dimerisation
- Mix 3 subunits, denature-renature => Tn complex
- All combinations of deuterated/protonated subunits produced

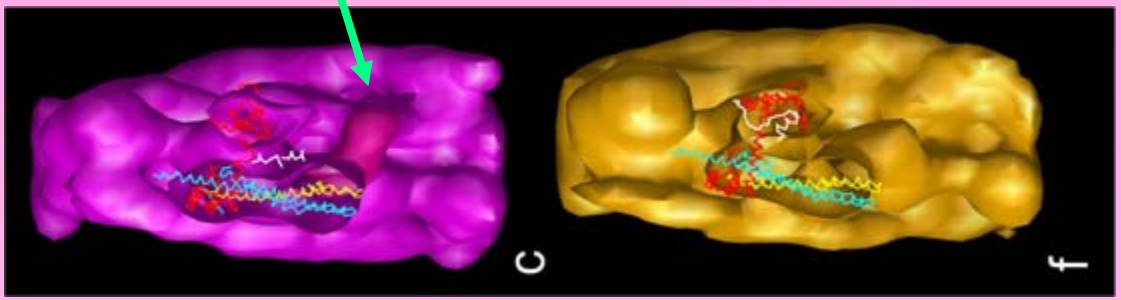
Conclusions: Tn structure



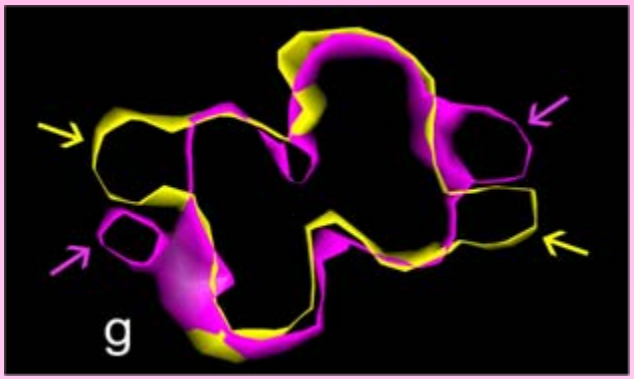
- In skeletal Tn, TnC maintains a dumbbell conformation
- C-TnI has a ball & chain architecture
- Large structural change in Tn due to release of C-TnI in -Ca^{2+} state
- C-TnI ball interacts with actin

Conclusions: Thin filament

C-TnI Ball

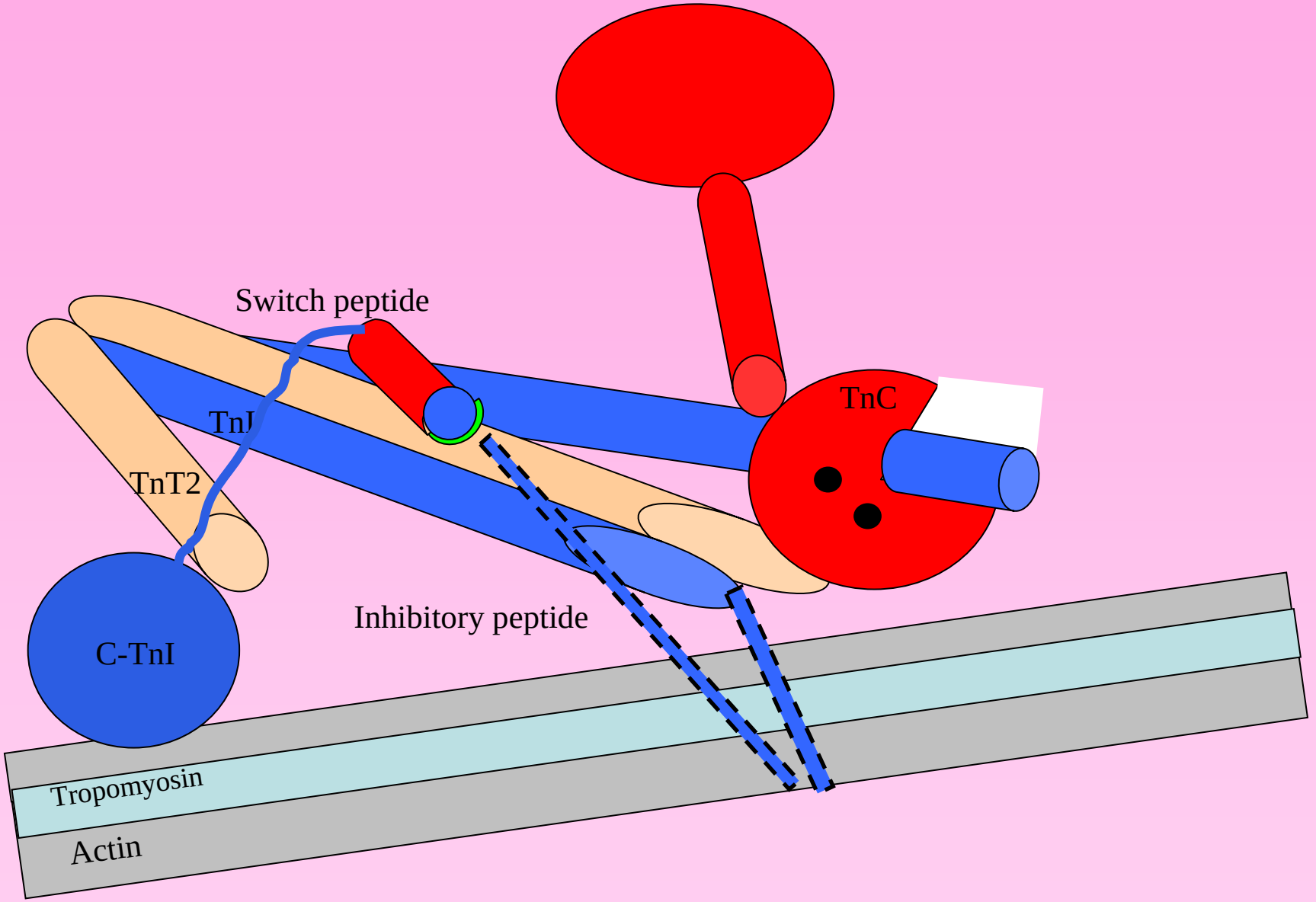


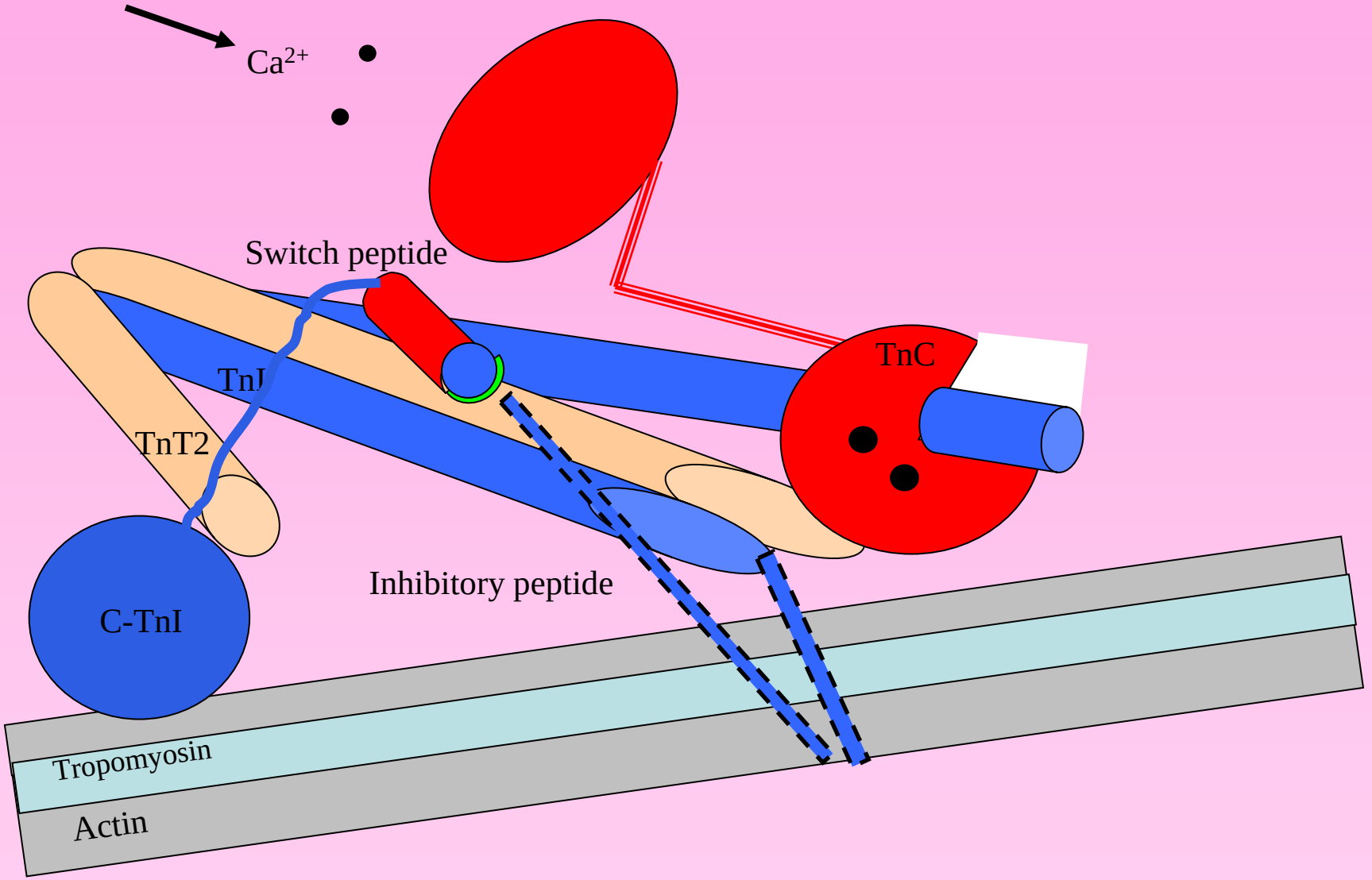
$-Ca^{2+}$ $+Ca^{2+}$

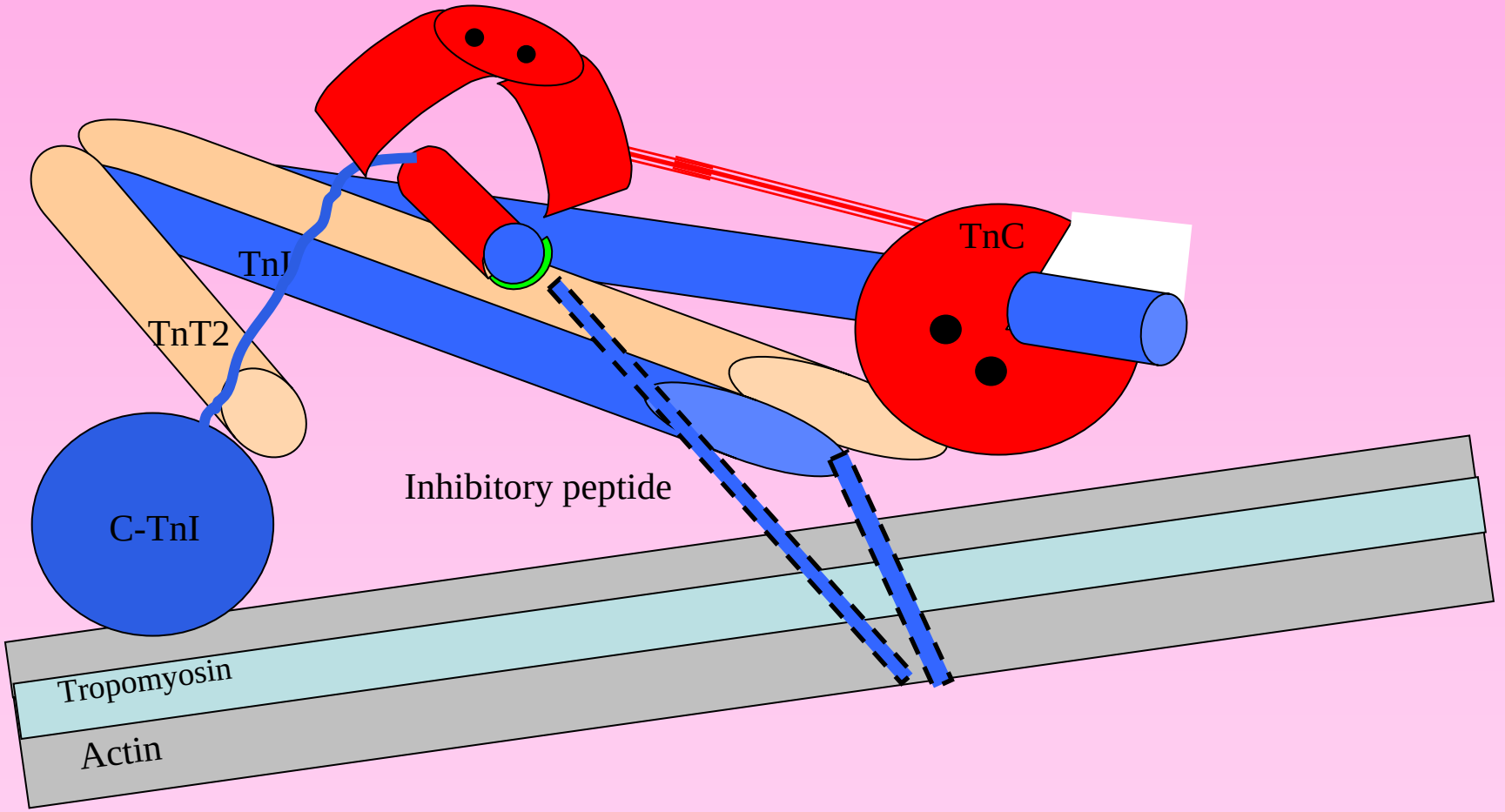


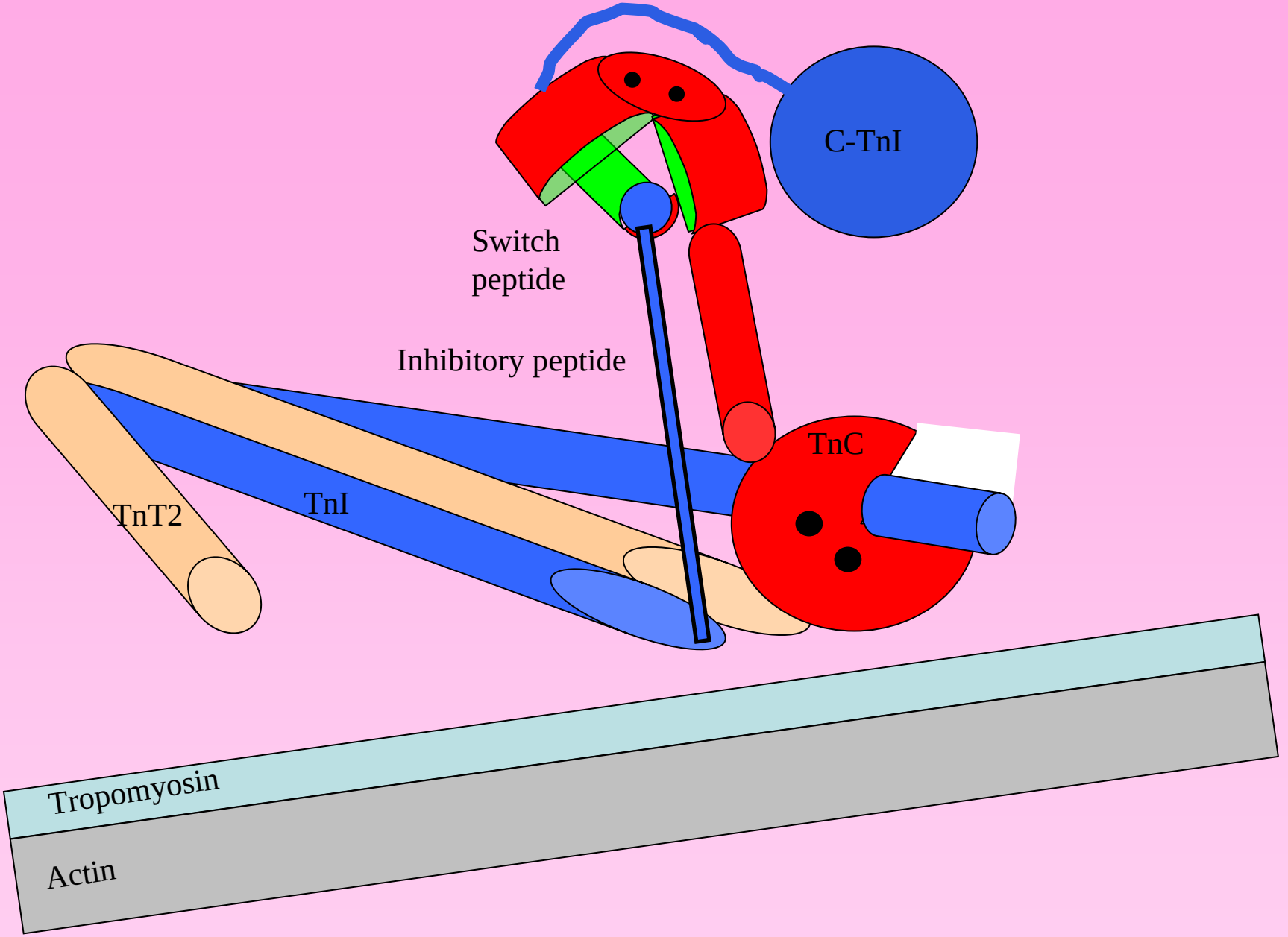
Tm movement

- C-TnI ball visible on actin in $-Ca^{2+}$ state
- Tn position unchanged wrt actin $\pm Ca^{2+}$
- Tm proximal to Tn in $-Ca^{2+}$ state
- Tm moves away from Tn in $+Ca^{2+}$state


Switch peptide
TnC
TnI
TnT2
C-TnI
Inhibitory peptide
Tropomyosin
Actin


Ca2+
Switch peptide
TnC
TnI
TnT2
C-TnI
Inhibitory peptide
Tropomyosin
Actin


TnC
TnI
TnT2
C-TnI
Inhibitory peptide
Tropomyosin
Actin


C-TnI
Switch
peptide
Inhibitory peptide
TnC
TnI
TnT2
Tropomyosin
Actin

and so onwards into the Future . . .

EuroBioSANS

An informal network of scientists from GKSS, HMI, ILL, Juelich and LLB to promote SANS in Biology.

Considerable effort has already been invested at the different reactor centres to open up to ‘biology’ but the community has been less responsive than other scientific disciplines, and clearly a more proactive approach is essential.

Neutron sources are not in competition with each other but on the contrary will all profit from a successful re-emergence of SANS in biology. In this sense EuroBioSANS can be used to help direct projects to the appropriate centre based for example on time availability, geographical or special expertise considerations.

The human base of expertise (SANS scientists who can talk to and understand biochemists and their special needs) must be broadened by encouraging SANS scientists to implicate themselves in the biochemical aspects of a study beyond the SANS experiments themselves. And for successful experiments there should be good Biochemistry laboratory support as close as possible to the SANS stations.

SANS and SAXS

Concentrations ~mg/ml

Better for sample access ('open' quartz cuvette - sample volume ~100 μl)

Better at low *Q*

Better for absolute scale

In H_2O: solvent scattering density ~zero (no specific volume effects)

In D_2O: contrast variation and high signal/noise; low bg (0.1 mg/ml concentration feasible)

Better for solvent conditions (not sensitive to high salt conc etc.)

No radiation damage

Concentrations ~mg/ml

Sample in a capillary - (measurements have been made with as little as 20 μl)

Better at high *Q*

Lower background than for neutron scattering from H_2O. Higher than for neutron scattering from D_2O

Better for kinetics (shorter exposure times)

Significant radiation damage - (flow cells etc to reduce this increase significantly the sample volume required)

Five questions for the setting up of EuroBioSANS

1) Do we agree to put forward a unified front to encourage SANS experiments at our respective reactor centres by Biologists who have little or no neutron expertise but potentially exciting projects on biologically important complexes and their interactions?

2) What human base of expertise (SANS experts who can talk to and understand biochemists and their special needs) is required and how can we strengthen it, considering we are all already very busy with our own projects and instrument responsibilities?should we start by organising a workshop?

3) How can we improve the Biology support around the SANS stations in terms of clean laboratory space, spectrophotometers. cold rooms, etc.?

4) Should we think in terms of unifying analysis methods and programs at the various centres and implementing novel approaches such as the Svergun SAS-crystallography suite for example?

5) And perhaps most difficult and important of all to tackle: can we come up with an acceptable demand to our bosses for block-allocation of measuring time for such experiments that takes into account the flexibility and easy-accessibility that are required for success?

SANS without equations

Problems that can be solved:

1) What is the effective association state (is it a monomer, a dimer...) of a protein or other macromolecule in solution or within a membrane environment?
2) What is its shape or conformation (is it compact and globular or ellipsoidal, long and narrow, or flat and broad, star-shaped or branched… is its structure in solution similar to its crystal structure…)?
3) Do different macromolecules in solution interact to form a complex or not?
4) How do the answers to points 1, 2, 3, vary as a function of solvent conditions (pH, salt, ligand, temperature…), are there modifications in association state or conformational changes?
5) The method of *contrast variation* in small angle neutron scattering allows one to render *visible* only one component within a complex structure. It is then possible to address questions 1 to 4 for individual components within, for example, a macromolecular machine made up of various proteins, a protein nucleic acid complex interaction, or a membrane protein in a lipid or detergent environment.

An important point is that the answers to the different questions above are obtained independently of each other.

Practical requirements for a SAS experiment:

1) On the order of 100μl of solution containing a few mg/ml of macromolecule.
2) An accurate measurement of the macromolecular concentration (in mg/ml).
3) Access to a neutron small angle camera and competent help to plan the experiment

Conceptions and misconceptions

Sample

SANS does not need:

Large crystals

Deuterated macromolecules

Massive amounts of material

Special buffer conditions

Capillary sample containers

A typical sample is a ~100 microlitre solution of a few mg/ml
(therefore ~0.1 mg of macromolecule)
In an easily accessible quartz cuvette
(good for in-beam titrations or adjustment of solution conditions)

Experiment

Exposure times are reasonably short
(a measurement takes ~ minutes or ~ an hour depending on the instrument)
The effective intensity is not <<<< for SAXS with SR
(large λ, λ^3 effect, large beam cross-section, long instruments compensate very effectively)
There is no radiation damage

A consensus for action (1)

We created EuroBioSANS, an informal network of scientists from GKSS, HMI, ILL, Juelich and LLB to promote SANS in Biology.

Considerable effort has already been invested at the different reactor centres to open up to ‘biology’ but the community has been less responsive than other scientific disciplines, and clearly a more proactive approach is essential.

Joe Zaccai is prepared to tour Biology laboratories in Europe to publicize what SANS can do but the reactor centres and instruments must also be prepared to accommodate a user community with very special needs.

It is proposed to create a EuroBioSANS web page in order to promote the technique and centralise useful information, with illustrative examples of previous studies - a SANS ‘primer’ - a ‘checklist interview’ for biological sample preparation, data collection and analysis - a description of data analysis steps and programs - contact addresses for ‘responsible scientists’ at each reactor centre - up-dated information about test-time, fast access etc. - links to the different reactor centre and instrument web pages etc.

A consensus for action (2)

The human base of expertise (SANS scientists who can talk to and understand biochemists and their special needs) must be broadened by encouraging SANS scientists to implicate themselves in the biochemical aspects of a study beyond the SANS experiments themselves. And for successful experiments there should be good Biochemistry laboratory support as close as possible to the SANS stations.

It may not be realistic to unify analysis methods and programs at the various centres in the short term but we should remember that the implementation of common analysis programs at all synchrotron beam lines for protein crystallography has been an important achievement for the efficiency of that user community.

For the present, it was agreed to work within the existing proposal system at each institute, with a special emphasis that test time for feasibility studies should be allocated within reasonably short delays. EuroBioSANS centres are not in competition with each other but on the contrary will all profit from a successful re-emergence of SANS in biology. In this sense EuroBioSANS can be used to help direct projects to the appropriate centre based for example on time availability, geographical or special expertise considerations.

A consensus for action (3)

The setting up of a flexible test-time system is essential for the success of EuroBioSANS. Test time is not a luxury but a necessity. Most first SANS experiments on a biological system from a group that has no previous neutron expertise require test time for the definition of correct experimental conditions, with follow-up measuring time within a reasonable delay.

It was decided to circulate a standard sample in the different reactor centres, in order to calibrate the measuring time needed at each SANS instrument,