Summary: Discussion at Bio-macromolecular Dynamics Workshop

State of the art

Presently versatile instrument suites at large scale facilities - neutrons and X-rays - are available to study biological systems from the macromolecule to the entire cell; for dynamic studies nearly exclusively neutrons serve the purpose; Neutron Spin Echo spectrometers are the flagship instrument with backscattering and time of flight methods supporting it. XPCS barely started as a biological technique to bridge the gap between NS and light scattering. For protein structure determination Synchrotron diffractometers are standard, while neutron instruments are focused on very specific questions like the positions of hydrogen in H-bonds or associated water.
In addition a large suite of complementary non scattering techniques are applied ranging from NMR as the flagship method to single molecule techniques such as FRET or FCS. Furthermore simulations from atomistic to all kinds of coarse graining approaches are essential in analyzing experiments and to solve issues that cannot be tackled directly by experiment. However these simulations need the verification by experiment: "Tune your force fields to fit experiment!" Analytic models are occasionally formulated.
Unfortunately, however, approaches to solve important questions by coordinated efforts using all available techniques are extremely rare.

Important Challenges

As the ultimate great challenge we want to arrive at a complete understanding of the physics and chemistry of the cell from the Angstrom and femtosecond scale to microns and minutes. For this endeavor we want to know:

- Accurate rates under physiological conditions.
- Accurate local concentrations, of metabolites, ions, macromolecules.
- Atomic spatial information, time resolved.
- Driven reactions, accurate binding constants.
- Theories on multiple scales, making it easy to cross (back and forth) scales.

Research has to move from single molecules to the structure and dynamics of ensembles, e.g.:

- We need to understand the effect of crowding on the functioning of bio-macromolecules.
- Liquid-liquid phase separation appears to play a key role.
- Signaling networks need to be unraveled and the mechanism of allostery to be understood.

Rational drug design based on the structure and dynamics of bio-macromolecules will lead to novel drugs and therapies:

- We need to know, where H bonds are, to understand binding, enzyme action etc.
- Water molecules are often bound, so we need to know, where they are. Neutron crystallography is the key for this. Successful experiments need big enough crystals and powerful sources like ESS.
- We need to explore shapes and flexibilities of binding sites and internal dynamics. Neutrons have the potential to provide this information.
- Other important issues are: stapling, gluing, disassembling, allostery. stabilizing proteins, when not tight enough: can we develop "protein glues"? Bind things to hinges. Protein-Protein Interaction modulation (PPI). Strengthening, weakening, etc.
- Vaccine design: E.g. antigen-T Cell receptor interactions. Strength of immune reaction is directly related to the Free Energy of a particular complex. X-rays

combined with neutrons will reveal associated changes of the structure in the complex, etc.
- Drug discovery employing transition states and/or its inhibition: NS can help to identify the existence or lack of suitable structures.
- IDPs can act as signalling hubs/networks, hence they might be good targets to influence cell behaviour.

Needs for the Future

Simulation, modeling and experiment:
A key for future success will be the interrelation of simulation, theory and experiment on the same molecule or molecular ensemble.
- Developments in theory need to comprise statistical mechanics, polymer physics, molecular kinetics, stochastic processes etc.
- At present lots of joint work is going on in this area. How do we go farther? Should we follow the CERN model or the set up at the ILL that have a theory/simulation group at the large-facility? For the breath of biological research a very large effort would be needed in order to make a substantial difference.
- It would be beneficial to merge simulations + NS experiments. We need to combine experiments (e.g. NMR for local interactions, different neutron spectroscopies for fast local and slow global motions, include consistently global conformation from SANS). There is an urgent need that different labs work together.
- Methods need to be developed to integrate SAXS/SANS/Microscopy with simulation. We call for a proof of principle for how such a system would look. However, *"There will never be push button simulation methods"*

Develop user support and interaction at large scale facilities:
- Bio-molecular research concentrates on a small fraction of the instrument suite of a facility (protein-diffractometers, reflectometers, small angle scattering and high resolution spectroscopy). In particular NSE is heavily oversubscribed, so most proposals fail. We need to convince facilities that there's more of a need for this instrument.
- Local cooperation partners and hubs (to avoid moving samples long distances) are important. Ideally we should be able to measure everything at the same place.
- In general, metadata are not handled very well. There's a need to establish standardised data and metadata formats from scattering and other measurements, such that they are easily available. An effort is underway, but there are many issues: only published data? Screening tests? Assign a DOI? Etc, etc, etc.
- We need to get non-scattering biologists on board! NS has quite an activation barrier to getting biologists involved. (E.g. fluorescence techniques accelerated once labeling became easier). Thus, NS providers need to reduce barriers: Meet biologists, where they are, with the experiments that they are already doing!
- Instrument scientists are very busy. It would be helpful to have senior fellows at the facilities to make links between user groups at universities and facilities. They are expected to be knowledgeable about important problems. Instrument scientists can't really be expected to be advocates for scientific uses as well as running the instruments!

- At biological conferences the relevance of NS is not very well appreciated. SAXS is appreciated. SANS is emerging. Need to do something to raise the profile of dynamic studies within the biological science communities.
- It is very difficult, to get larger quantities of bio-molecules such as monoclonal Antibodies mABs or other large molecules. Particular if we don't produce them ourselves. It is even more difficult, to get them deuterated, or from companies (IP agreements, etc) Facilities need to make efforts to provide such molecules. A protein production platform at Lund is being developed. Important to connect users to the materials, and to the simulation tools at the facilities.

Involve new techniques

- Ultrafast spectroscopy field needs to be brought into this community (lots of great work coming out that can be exploited!).
- Microfluidic mixing combing with scattering will help to access new regimes of length and time scales in kinetic experiments.
- Advanced data analysis, and machine learning, should be made more prominent in this community

Teaming up to address large and important problems

Examples could include:

- Collective approaches for drug discovery should be explored; collaboration with pharmaceutical industry could be very profitable.
- Candidate *in vivo* systems should be chosen with the collaboration of biologists and medics, and studied through both *in vivo* and via *in vitro* model studies. Examples could include cell-cell recognition and binding (e.g. T-cells), protein-protein phase separation (p-granules, etc), membrane protein aggregation and signaling pathways. There are many possibilities!

We need to choose systems that can be managed by both sides [to simulate AND measure]. E.g. force fields have a lack of discriminatory data; i.e. the existing data can't distinguish force fields in many cases.

The Role of LINXS

- A need for providing instrument scientists at MAX4 and ESS a scientific home
- Support software development for advanced data analysis
- Links to universities via Masters programs. [note that LINXS will not focus on Masters courses].
- Develop standard proteins to study (e.g. Ig) and methods for allowing many people to work on the same molecules. Get materials, organize teams and round-robin methods;
- For example: define a broad-reaching project with many groups that can catalyse an important flagship project. LINXS can fund post-docs, travel expenses, etc. for such an effort.
    - In 2005 the protein folding community did this (devised target set of experiments and a plan) and this helped the field well.
    - E.g.: have an initial workshop, define systems and techniques, and then move ahead with a larger community.

- Involve industry if possible.
- Can do this with more than one topic of course.