Report on the Lorentz workshop 'Proteins and Beyond' (12-16 October 2015)

The aim of the workshop was to discuss the future of protein research. The central questions to be addressed were: Has the reductionist approach to study isolated proteins with advanced biophysical methods been exhausted and should the future aim be to study the protein only in its native environment, such as the living cell? What are the next challenges in this field?

We had indicated in the application that this workshop would be considered a success if a) a number of new challenges in protein research for the coming decade had come into sight and b) technique-oriented researchers would have contemplated their methods in light of these new research challenges. A set of challenges and questions would be formulated as the outcome of the discussions.

We brought together scientists from a wide range of areas, both technique and research question oriented and from industry as well as academia. The speakers had tuned their presentations remarkably well to the broad background of the audience and as a result the participants were informed about the latest developments in a range of areas. The term 'reductionism' was central in many discussions. The **general consensus** was that in-cell protein research is becoming very powerful in visualisation of proteins and thus in the study of their function in the cellular context, yet in-vitro studies at the atomic level remain essential for a) structural understanding at the atomic level and b) hypothesis formulation for cellular studies. The first atomic level studies of proteins in the cell are appearing, yet they are very far from what can be achieved using isolated proteins.

The workshop ended with a public session, attended by about 150 people, with three helicopter view presentations on the developments in amyloid research, DNA repair and molecular simulations of biochemical structures and processes. In the introduction by the chair and the following panel discussion conclusions from the workshop were presented to the public.

The organisation of the workshop was excellent. The atmosphere was very informal from the start and everything ran very smoothly. The ample time for discussion avoided pressure on the program and was used flexibly to enable certain conversations to continue longer than planned and to allow participants who were not scheduled as speakers to make additional points.

Each session led to the formulation of research questions or directions of research. The final set is listed below. It does not pretend to be a comprehensive agenda of protein research. It should be considered as food for thought for people in the field and it also helps to identify areas of interest for young researchers starting in protein research.

FROM MOLECULAR LANDSCAPES TO CELLULAR LANDSCAPES

Technical developments:

Promising techniques:

1. CryoEM is rapidly developing and will be combined more with (super-resolution) fluorescence, leading to structures of ever larger supramolecular complexes being determined.
2. Cellular protein research is using the microscope as an analytical tool, producing very large data sets (e.g. fluorescent cell movies). For extraction of useful quantitative data closer collaboration with bioinformaticians is required.

3. Crosslinking entire cell lysates to identify new contacts between proteins followed by MS analysis presents a new, promising way for interactomics.
4. Dynamics of proteins can be studied at timescale of ps – seconds by combining biophysical methods, enabling much better descriptions of protein's energy landscapes.
5. Organoids offer much better context to study proteins in situ, in particular with CRISPR/Cas tools for genetic modification.

Required for the future progress:

6. Better fluorescent probes (more stable, genetically introduced, switchable, smaller) required.
7. Protein purification methods need to be faster, more robust and automated. We also need methods to stabilize proteins for medicinal use.
8. We need course grained systems for modelling of cellular processes.
9. Bioinformatics should better integrate existing omics datasets (datamining).
10. Alternatives for radioactive labelling (e.g. pulse chase) are required. Can isotope labelling / unnatural aa help, together with MS?
11. Parameters need to be identified that link in-vitro and in-cell properties of proteins.

Biological Questions (from larger to smaller scale):

1. How do cells divide?
2. What are the molecular mechanisms of aging?
3. What are the molecular mechanisms of cancer development?
4. Can we predict how drugs affect cellular stress response networks?
5. Can we understand stochastic properties of molecular biology and their ramifications?
6. How do proteins fold in the cell? Which roles do chaperones play?
7. How do glycans influence folding and function of proteins?
8. How do membranes affect protein folding and function and how do proteins affect membranes?
9. How do IDPs (intrinsically disordered proteins) behave and function in cells and what are their roles in disease?
10. Can we comprehend the versatility of post-translational modifications (in time)?
11. What are the energy landscapes of proteins and what is the functional reason that they are so complex? Which of the states and motions are relevant for the function?