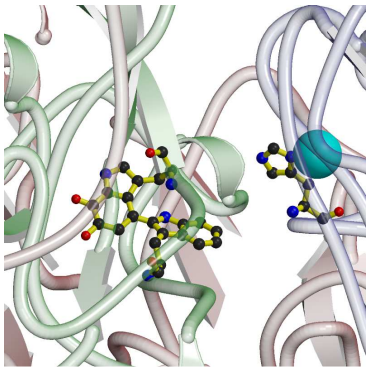
RESEARCH CONFERENCES

ESF-EMBO Symposium
Molecular Perspectives On Protein-Protein Interactions

25-30 May 2013
Polonia Castle in Pultusk, Poland

Chaired by: Prof. Marcellus Ubbink, University of Leiden

Co-chaired by: Gideon Schreiber, Weizmann Institute of Science, Colin Kleanthous, Oxford University



http://www.esf.org/conferences/13407

Highlights & Scientific Report

Conference Highlights

Please provide a brief summary of the conference and its highlights in non-specialist terms (especially for highly technical subjects) for communication and publicity purposes. (ca. 400-500 words)

The ability of proteins to interact fast and specific is a basic feature of the complexity of life. While protein-protein interactions are discussed in many biology meetings, the basic principles governing these interactions deserve a platform of its own. The series of the International Conference on the Molecular Aspects of Protein-Protein Interactions aims to provide this platform. The fourth edition (PPI-4) took place in Pultusk, Poland, from May 25 till May 30, 2013. Previous meetings were held in Eilat, Israel (2005), Dubrovnik, Croatia (2008) and Feliu de Guixols, Costa Brava, Spain (2010).

The meeting brought together 114 scientists from 27 countries, with 24 invited speakers and 21 speakers selected from the abstracts. 64 posters were presented during the two poster sessions.
The oral presentations were divided over 12 sessions, covering a broad range of topics related to protein-protein interactions. The atmosphere was very informal and the set-up of the program, with relatively many short presentations, led to ample scientific discussion.

The meeting has demonstrated the significant progress that is made in understanding protein interactions. Particularly noteworthy are, first, the new insights in large molecular machines (nano-machines), such as the ribosome and the pili production apparatus, which is a consequence of the rapid development of cryo-Electron Microscopy methods. Second, the spectacular results that were reported in the design of new protein interactions by large-scale selection methods illustrate what the field is currently capable of. These methods are well on their way to replace classic monoclonal antibody production for the synthesis of proteins that bind new targets with high specificity.

☐ I hereby authorize ESF – and the conference partners to use the information contained in the above section on 'Conference Highlights' in their communication on the scheme.

Scientific Report

Executive Summary

(2 pages max)

The 4th edition of the International Conference on the Molecular Aspects of Protein-Protein Interactions (PPI-4) took place in Pultusk, Poland, from May 25 till May 30, 2013. Previous meetings were held in Eilat, Israel (2005), Dubrovnik, Croatia (2008) and Feliu de Guixols, Costa Brava, Spain (2010).

The meeting brought together 114 scientists from 27 countries, with 24 invited speakers and 21 speakers selected from the abstracts. 64 posters were presented during the two poster sessions.
The oral presentations were divided over 12 sessions, covering a broad range of topics related to protein-protein interactions.

The atmosphere was very informal and the set-up of the program, with relatively many short presentations, led to ample scientific discussion, which was greatly valued by the participants. The accommodation and organizational support by ESF was also highly appreciated.

The meeting has demonstrated the significant progress that is made in understanding protein interactions. Particularly noteworthy are, first, the new insights in large molecular machines (nano-machines), such as the ribosome and the pili production apparatus, which is a consequence of the rapid development of cryo-Electron Microscopy methods. Second, the spectacular results that were reported in the design of new protein interactions by large-scale selection methods illustrate what the field is currently capable of. These methods are well on their way to replace classic monoclonal antibody production for the synthesis of proteins that bind new targets with high specificity.

Planning for a new edition of the meeting in 2015 has begun.

Scientific Content of the Conference

(1 page min.)

Session 1 – *Theory & Computation*

In this session theoretical approaches to study protein-protein interactions were discussed. Docking approaches to predict protein complex structures as well as to study the process of complex formation itself represent an important part of this research. Specific topics that were discussed were the behavior of proteins in concentrated solutions, such as found inside cells (Rebecca Wade), and comparison between complexes from species to understand the evolution of protein interfaces (Sarah Teichmann).

Session 2 – *Membrane protein complexes*

Membrane protein complexes are particularly difficult to study because for a good understanding they should be studied in their natural membrane environment. Still, major progress is being made in this area with important new understanding of biochemical processes involving PPIs. By combining various biophysical and biochemical approaches a complete picture can be obtained. In particular, the power single-particle cryo-electron microscopy, which has developed fast over the past few years, became visible in several presentations, both in this session and in others. An example is the work discussed on toxin entrance into Gram-negative bacteria, a very complex process in which the toxin recruits various cellular proteins to enable its entrance into the cytosol (Colin Kleanthous).

Session 3 – *Kinetics & Thermodynamics*

The biophysical characterization of protein complexes comprises the fundamental research that helps us understand how the basic biophysical interactions, such as H-bonding, hydrophobic interactions and electrostatics, are employed in the formation of protein complexes. Complexes with different biological roles also use different types of interactions to produce the thermodynamic and kinetic properties required for that function, a theme that returned in the Transient Interactions Session. A very interesting study was presented on the effects of crowing, both in vitro and in vivo (Gideon Schreiber). Contrary to general opinion, it turns out that crowing has little net effect on the thermodynamic properties of the complex, most likely as a consequence of two opposing effects of crowding, the excluded volume effect and the restricted motion.

Session 4 – *Evolution and design*

Much progress has been made in the approaches to make new protein complexes. By directed evolution, with or without help of rational design, it is now possible to obtain a binding partner for any protein, as was illustrated by the presentations of Sachdev Sidhu and Sarel Fleishman. This opens the way to the production of artificial antibodies without the need to produce those in animals. It demonstrates the power of the evolutionary approaches, but at the same time, this result does not imply that protein complex formation is completely understood. On the contrary, it is often not possible to explain why certain residues, sometimes located far from an interface still affect complex formation.

Session 5 – *Signalling complexes*

Signaling processes are essential in all living organisms, because they allow them to react to changes in the environment. The first step is the recognition of the signal molecule by the receptor, which transmits the signal across the cell membrane. Jacob Piehler discussed new approaches to study single receptor molecules embedded within the cell membrane upon addition of the signal molecule. These approaches are based on very advanced fluorescence methods. In this way the multimeric state of receptors as well their movement through the membrane can be followed directly.

Session 6 – *Networks*

In the past decade the development of proteomics techniques has made it possible to produce extensive networks of protein interactions in cells. However, the small overlap between different types of networks suggests that they contain many false positives. Currently, the curation of these networks tries to improve this situation (Shoshana Wodak). Ulrich Stelzl made the very important point in his presentation that post-translational modification (PTM) can readily influence PPIs and therefore the PPI networks. Since the number of PTM per protein can be in the tens and the range of possible PTMs is large, a daunting task is awaiting researchers aiming to understand the complexity of cellular PPIs. Patrick Alloy discussed another, top-down strategy to develop new therapeutics, the application of large-scale systematic literature searches after the effects of existing medicinal drugs to find new applications for them.

Session 7 – *Structural Approaches*

In this session Masaki Nojiri presented crystallography studies on very weak protein complexes, as found between electron transfer proteins and redox enzymes, which are present in nearly all metabolic pathways. Obtaining crystals of such weak complexes is very difficult because these complexes are inherently dynamic, with the encounter complex (an ensemble of weakly bound orientations) representing an important fraction of the complexes (see also the session on weak interactions). Yet, these results demonstrate that it is possible to obtain crystals of the ground

state of such complexes. Dominik Gront showed how far small angle X-ray scattering (SAXS) can be taken to describe the structures of protein complexes. In combination with other techniques SAXS can clearly add valuable information for structure determination.

Session 8 – *Intrinsically unstructured protein complexes*

Many proteins do not assume a well-defined three-dimensional structure, but exist as an intrinsically disordered protein (IDP). This very property often makes them suitable to interact with many partners and also to function as logic gates, interacting in one way with partner A and another way with partner B, such that both are mutually exclusive. IDPs can also fold partly upon binding to a well-define site, while interacting in a transient fashion with its still disordered part elsewhere on the partner protein (Peter Wright, Monika Fuxreiter). Stefan Rüdiger describe the interaction of an IDP, the tau protein and a chaperone protein, which normally functions to help proteins fold into their 3D structure. His advanced NMR studies showed that the interaction covers a large area of the chaperone without hot spots in the interaction site.

Session 9 – *Large multi-protein complexes*

Three presentations showing beautifully the power cryo-EM were given by Stefan Rausner (in the Signaling session) and Bern Bukau and Gabriel Waksman, all working with very large complexes, toxins, the ribosome and the bacterial pilus machinery. By analyzing multiple complexes from different stages of the biochemical process, it becomes possible to get a comprehensive picture of the successive steps in the process. Viviane Richter presented very interesting work on the formation and fission of mitochondria.

Session 10 – *Transient Interactions*

Many protein complexes are formed not with the aim to for a stable, well-defined and tight complex, but to rapidly perform a task and turn-over fast. In particular, complexes of electron transfer proteins are made to be short-lived. Brian Hoffman illustrated this with work on the complex of myoglobin and cytochrome b_5, a complex that is so dynamic that it exists as a pure encounter complex. It was demonstrated that such a complex can be made more specific by rational design of complementary electrostatic charges on the proteins. Marcellus Ubbink presented the description of the encounter complex of plastocyanin and cytochrome *f*, a pair of proteins involved in photosynthesis. An important finding was that this encounter complex is dominated by hydrophobic interactions and not electrostatic forces, which is more usual. Consequently, the distinction between encounter complexes and final, active complex blurs. This leads to new, activationless model for complex formation.

Session 11 – *Emerging techniques*

In this session Peter Crowley described new compounds that may serve as tools to influence PPIs. Calixarenes are complexes that associate weakly but specifically with lysine residues. Crystal structures of cytochrome *c* with several calixarenes bound demonstrated the details of these interactions. Moran Jerabek-Willemsen described the possibilities of microscale thermophoresis, a new approach to determine binding constants of protein complexes.

Session 12 – *Protein-protein interactions in disease and drug development*

Inhibition of PPIs by small molecules remains a challenge but recent work shows that in some cases this can be achieved. Topics discussed in this session included interactions involving ubiquitin-like proteins (Yuan Chen), Hsp90 chaperones (Lynne Regan) and Inhibitor of Apoptosis protein (IAP, Kurt Deshayes).

Forward Look

(1 page min.)

- *Assessment of the results*
- *Contribution to the future direction of the field – identification of issues in the 5-10 years & timeframe*
- *Identification of emerging topics*

Theory

- Docking remains challenging for membrane systems
- Interactions between proteins and lipids needs to be understood better, because they represent great new challenges for PPI (requiring a combination of techniques)
- For the study of interactions of proteins at the membrane/cytosol interface better methods are needed. Microscopy and single-molecule techniques are particularly promising.

Kinetics/Thermodynamics

- More insight into the *in vivo* behaviour of PPI is being obtained.
- Measuring macroscopic parameters in the (sub)cellular environment remains a challenge, like quantifying protein concentration and localization in cells, and also real measures of pH and ionic strength etc in cells.
- The process of dissociation is still a blind spot in our understanding of PPIs

Evolution and Design

- Successful protein binders can now be developed by selection methods.
- But do we understand affinity and specificity? What is the role of water and of avidity in interactions?
- A new issue is the importance of residue co-evolution in design of new PPIs
- The link between theory and design: are we getting closer to link the fields?

Networks

- Many thousands of PTMs lead to extensive and subtle regulation.
- What are the synergies/interactions between PTMs? Antibodies only give information on single sites
- How to interpret network data? What is their reliability?
- Application of large data sets has a great future, e.g. for new therapies

IDPs

- The diverse roles that IDPs play enables them to have more than one function of a protein ('moonlighting')
- Dynamics of IDPs needs to be understood better.
- How do IDPs interact in cells? Conversely, how do they behave on their own?
- Use of NMR *in vitro* is powerful, but other technologies are also needed to study IPDs in cells (single-molecule methods?)

Large complexes

- EM is a very important tool.
- Marriage of small complex methods with large complex structures is required.

- Studies of transient but large complexes (gel-like structures and colloids) may well develop in the near future.
- What is the role of protein-protein interactions in the formation of organelles.

Transient Complexes

- How to describe encounter complexes? Methods & modelling need to be combined but can still not provide a comprehensive picture.
- New methods are required to study large dynamic complexes.

PPI in disease

- A new insight is that interfaces are not generally large or hydrophobic
- Better understanding on transient interactions is required to develop inhibitors of PPIs
- The role of protein-protein interactions in allostery and enzyme behaviour as well as dynamic allostery in PPIs need more attention.

▪ Is there a need for a foresight-type initiative?

No, the field rapidly develops and the directions are clear from the above list.

Business Meeting Outcomes

▪ *Election of the Organising Committee of the next conference*
▪ *Identified Topics*
▪ *Next Steps*

The general opinion was that meetings on this topic are extremely relevant and not covered by other existing meetings. A new edition has been planned for 2015, to take place in Canada. A chair has been proposed. Possibilities for funding will first be researched.

The topics will remain largely the same because the many sessions cover the area well. Two specific topics were suggested to be included:

- More presentations on systems for protein expression and purification.
- The results of free energy lasers for studying dynamics of structures.

Atmosphere and Infrastructure

▪ *The reaction of the participants to the location and the organization, including networking, and any other relevant comments*

The atmosphere was very informal, with a lot of good scientific discussion after the presentations, during the breaks and at the poster sessions. The relaxing environment of Pultusk Castle contributed to that. Participants were very positive about the excellent food, the drinks during the poster session and the very adequate help provided by the ESF representative. There were some complaints about the poor wireless connections to the Internet, both in the rooms and the lecture hall.

X I hereby authorize ESF to publish the information contained in the above Scientific Report on the ESF Research Conferences Webpages. No sensitive or confidential information (see above) has been included in this report

Date & Author:

June 25, 2013 Prof. Dr. Marcellus Ubbink