

Unstructured Proteins: biophysical characterization and biological significance

(Conference on Intrinsically Unfolded Proteins (IUP), Budapest, May 20-24, 2007)

Until the recent time a paradigm that proteins can be functional only after folding into a defined three-dimensional structure seemed undisputable. A huge mass of data questioning this dogma has appeared during the last ten years. There were discovered proteins that function without assuming a stable tertiary structure, and exist in solution in a state traditionally thought as being denatured. It was Dr. Peter Wright (The Scripps Research Institute, CA, USA), who boldly stepped forward in 1999 with the suggestion of a possibility that some proteins can naturally function without folding into the tertiary structure. It came as a complete surprise that 70% of all eukaryotic proteins have unstructured regions. As a consequence of such ubiquity of either partial or complete lack of structure, the problem of studying these proteins, development of analysis methods either *in vivo*, *in vitro* or *in silico*, nomenclature issues, *etc.* gain significant importance.

IUP – first international conference on unstructured proteins – was organized by the laboratory of Dr. P ter Tompa (Institute of Enzymology, Hungarian Academy of Sciences, Budapest) with Conference Centre of Central European University as its venue. The importance of the Conference topic was confirmed by a sheer number of participants: 160 vs. 60 expected by the organizers.

An opening talk was given by Dr. Keith Dunker (Indiana State University, USA). Commencing from the history of the field, he communicated that the first ideas and experiments showing the possibility of a protein functioning without stable defined tertiary structure appeared as early as 1935! A per cent of completely or partially unstructured proteins in eukaryotic proteomes is high (40 and 70% respectively), while prokaryotic proteins have more “modest” unstructuredness. The main emphasis of the talk was on the link between lack of structure and protein function (unstructured proteins interact with many partners more often which is important for signal transduction inside a cell); these proteins are hubs in interactomes; alternatively spliced proteins are often unstructured; these proteins are extremely important for drug design, but are very difficult to deal with due to ineffectiveness of traditional docking and ligand libraries screening software. Prof. Dunker addressed a terminological issue as well: he believes that a traditional term “induced fit”, which characterizes action mechanism of unstructured proteins, is unacceptable and advocates instead the term “coupled binding and folding”.

The session of biophysical characterization of intrinsically unstructured proteins started with the plenary lecture of Dr. Peter Wright, who emphasized the importance of NMR as a main biophysical technique to study IUPs, which form a certain structural continuum.

Dr. Philipp Selenko (Harvard Medical School, MA, USA) demonstrated abilities of his unique method of *in vivo* NMR for the analysis of protein disorder inside a living cell. *Xenopus* oocytes are inoculated with labeled protein and spectra are recorded. This method confirms existence of IUPs *in vivo*.

Professor Laurence Barron told about rarely used method of Raman optical activity (ROA), which he applies in his lab at University of Glasgow, UK to the analysis of secondary structure content and determination of a protein disorder degree.

Dr. George Rose (John Hopkins University, MD, USA) presented a time scale and entropic cost of transition between folded and unstructured state of a protein.

Dr. Bojan Zagrovic (ETH, Zürich, Switzerland) used molecular dynamics technique for the analysis of a conformational ensemble assumed by intrinsically unstructured protein in solution. He utilized possibilities of "Folding at home" GRID project to compute thousands of trajectories.

On the session devoted to the link between intrinsic lack of structure, protein function and diseases Dr. Vladimir Uversky (Indiana State University, IN, USA) lectured about importance of IUPs for medicine. Many proteins linked to genetic disorders are completely or partially unstructured. Completely unstructured protein alpha-synuclein, associated with Parkinson's disease can serve as a classical example. Noteworthy, a characteristic feature of many IUPs is stabilization of their secondary structure with increase of temperature or pH!

Dr. Eckhard Mandelkow (Max-Planck-Institut, Germany) devoted his talk to unstructured protein tau, which causes Alzheimer disease.

Dr. Gary Daughdrill from State University of Idaho, Moscow, ID, USA reported results of his study of conformational ensembles of p53 transactivation domain by NMR and molecular dynamics simulation. From the analysis of amino acids contact maps he calculated a set of most statistically probable conformational ensembles.

A theme of plenary lecture given by Emily Dimmer (Wellcome Trust Genome Campus, UK) was quite distinct from the others. Presenting the Gene Ontology project (GO) she spoke about the importance for scientific community to partake, and make an effort to the necessary terminology standardization (there are about ten different

terms solely for the phenomenon of protein structural disorder solely).

Dr. Ronald Wetzel (University of Pittsburgh School of Medicine, PA, USA) showed the results of grafting (he used this very term!) and interaction of disordered repetitive sequences of Huntington protein – a cause of Huntington disease.

On the session devoted to bioinformatics characterization of disorder groups of Istvan Simon (Institute of Enzymology, Budapest), Robert Esnouf (University of Oxford, UK), Oxana Galtzitskaya (Institute of Protein, Pushchino, Russia), Szuszanna Dosztanyi (Institute of Enzymology, Budapest), Israel Silman (Weizmann Institute of Science, Jerusalem, Israel), and Monika Fuxreiter (Institute of Enzymology, Budapest) presented their results.

The research of David Jones (London University College, London, UK), who is one of the founders and active participants of CASP experiment (Critical Assessment of Techniques for Protein Structure Prediction, by the way, CASP now has a section devoted to intrinsically unstructured proteins), is especially noteworthy. His prediction method is based on Dr. Vapnik's Support Vector Machines (SVM) and gives significant results.

Ada Yonath from Weizmann Institute of Science, Jerusalem, Israel started a session on disorder in protein-protein and protein-RNA interactions by giving a brilliant talk about hypothetical protein disorder of ribosomal proteins. The audience was enchanted by a short animated film, based on real experimental data and computer modeling, which demonstrated the mode of work of several ribosomal proteins in the context of a full ribosome.

Dr. Piter Tompa showed his results suggesting that partially unstructured regions can serve as weak protein degradation signals.

Dr. Gennady Verkhivker (The University of Kansas and University of California, San Diego) analyzed strengths and limitations of computer docking approach to the design of ligands interacting with unstructured proteins.

Institute of Molecular Biology and Genetics of NAS Ukraine (Kyiv) presented three posters by O. Novosylna et al. The importance of intrinsic disorder

for normal and oncogenic isoforms of eEF1A, A. Kornelyuk et al. Conformational flexibility of unstructured interdomain linker in mammalian tyrosyl-tRNA synthetase studied by MD simulation, and F. Tereshchenko et al. Dynamics of cytokine-like C-terminal domain of mammalian tyrosyl-tRNA synthetase at 315K: a transient disorder or a molten globule?. These works generated a significant interest.

Talks in couloirs and poster session gave the opportunity to establish contacts with many scientists working in this domain of science.

We would like to thank the organizer of the conference Dr. Peter Tompa and his group – Denes Kovacs and Ezster Házzy for a rare in recent years faultless organization of the meeting.

Köszönöm szépen! – Thank you very much!

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