Session 2: Unstructured proteins in health and disorders

Lectures

L2.1

Intrinsically disordered proteins in the norm and pathology

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Intrinsically disordered proteins (IDPs) lack stable tertiary and/or secondary structure under physiological conditions *in vitro*. They are highly abundant in nature and have a very broad functional repertoire which complements functions of ordered proteins. Often, intrinsically disordered proteins are involved in regulation, signaling and control pathways. Functions of IDPs may arise from the specific disordered form, from inter-conversion of disordered forms, or from transitions between disordered and ordered as well as between ordered and disordered conformations. The choice between these conformations is determined by the peculiarities of the protein environment, and many IDPs possess an exceptional ability to fold in a template-dependent manner. These proteins are often key players in protein-protein interaction networks and are highly abundant among hubs. Regions of mRNA which undergo alternative splicing code for disordered proteins more often than they code for structured proteins. This association of alternative splicing and intrinsic disorder helps proteins to avoid folding difficulties and provides a unique mechanism for developing tissue-specific protein interaction networks. IDPs are tightly controlled in the norm by various genetic and non-genetic mechanisms. Alteration in regulation of this disordered regulators are often detrimental to a cell and many IDPs are associated with a variety of human diseases, such as cancer, cardiovascular disease, amyloidoses, neurodegenerative diseases, diabetes, and others. Therefore, there is an intriguing interconnection between intrinsic disorder, cell signaling and human diseases. Pathogenic IDPs, such as α-synuclein, tau protein, p53, BRCA1 and many other disease-associated hub proteins represent attractive targets for drugs modulating protein-protein interactions. Several strategies have been elaborated for elucidating the mechanisms of blocking of the intrinsic disorder-based protein-protein interactions.

L2.2

Single-molecule spectroscopy of intrinsically disordered proteins

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In contrast to folded proteins, intrinsically disordered proteins (IDPs) lack a stable three-dimensional structure but, nevertheless, are involved in many biological processes and have a crucial role in various human diseases, including degenerative neuropathies, amyloidosis, and cancer.
I will show how advanced single-molecule spectroscopy in combination with Förster resonance energy transfer (FRET) can be used to investigate the large structural heterogeneity and pronounced dynamics of IDPs by mapping intramolecular distance distributions and quantifying the global relaxation dynamics of the chain in the sub-microsecond range. In combination with concepts of polymer physics, single molecule FRET experiments have allowed us, e.g., to explain the effect of electrostatics on the expansion of IDPs and to identify the contribution of internal friction to unfolded-state dynamics.
Here I will focus on the use of single-molecule FRET spectroscopy to probe the contribution of charge distribution and metal ion interactions on three different highly acidic IDPs: starmaker, starmaker-like, and prothymosin alpha. Interestingly, for all cases, we observe that divalent ions induce more collapsed conformations than monovalent ions at the same ionic strength, indicating preferential interactions of the sequences with calcium and magnesium compared to sodium and potassium and suggesting a common principle underlying the interaction of counterions with charged residues. The experimental observations can be rationalized in terms of a polyelectrolyte model that takes the competing adsorption of monovalent and divalent ions into account explicitly and allows the fractions of free and bound ions to be estimated. The preferential condensation of divalent ions on negatively charged residues may be an important first step in the assembly of biominerals.

L2.3

NMR spectroscopy methods for investigations of Intrinsically Disordered Proteins

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In the last decade, intrinsically disordered proteins (IDPs) or protein regions (IDPRs) have found general interest in molecular biology research. Intrinsic protein disorder is now recognized as one of the key features for a large variety of cellular functions, where structural flexibility presents a functional advantage in terms of binding plasticity and promiscuity. The inherent structural flexibility of IDPs, requires the application of appropriate experimental methods, since X-Ray crystallography cannot access the distribution of conformational states of disordered proteins. In contrast, NMR spectroscopy has been developed into a powerful structural biology technique that offers unique opportunities for structural and dynamic studies of IDPs with the atomic resolution and allows to characterize their conformational space and derive dynamic models. A fundamental problem in the structural characterization of IDP is the definition of the conformational ensemble sampled by the polypeptide chain in solution. Often the interpretation relies on the concept of "residual structure" or "conformational preference". Contrary to well-folded globular proteins, the peculiar properties of IDPs introduce additional challenges that need to be overcome to obtain NMR spectra of sufficient quality and resolution. The significant conformational dynamics exhibited by IDPs leads to severe average of chemical shifts, which are mostly determined by the amino acid chemical composition and by the primary sequence of protein backbone, while contributions deriving from the three-dimensional structure are missing. At the same time, the dynamic behavior leads to favorable relaxation properties, and allow long evolution periods within NMR pulse sequences. The recording time of sufficiently resolved multidimensional spectra is often very long due to the sampling limitations. Recently, several novel 4-6D pulse sequences are proposed. The new experiments employ non-uniform sampling that enables achieving high resolution in indirectly detected dimensions. The new experiments facilitate NMR investigations of intrinsically disordered proteins.

Oral presentations

O2.1

The F domain of Aedes aegypti 20-hydroxyecdysone receptor (EcR) exhibits unique characteristics of intrinsically disordered proteins (IDPs)

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Intrinsically disordered proteins (IDPs) represent a unique class of proteins that do not possess a stable three dimensional structure under physiological conditions [1]. Their structural intrinsic disorder is crucial for the biological functions of these proteins. Due to their unique sequence composition and characteristics, IDPs participate in many regulatory biological processes, including cell signaling [2]. In our preliminary extensive studies, we characterized for the first time the C-terminal region (F domain) of 20-hydroxyecdysone receptor (EcR) from Aedes aegypti (AaFEcR) – a mosquito vector of dengue and Zika [3]. The knowledge about a molecular function and structure of the F domain remains unknown. Because of the absence of vaccine or efficient cure against the mosquito-borne diseases, detailed biochemical and structural characterization of AaFEcR is crucial for understanding the molecular function of EcR and a crucial role the protein plays in the 20-hydroxyecdysone-based signaling pathways, including vitellogenesis in Aedes aegypti [4]. In order to perform the initial biochemical and structural investigations of the recombinant AaFEcR, we established an efficient purification procedure using IMAC and gel filtration. Its molecular weight was confirmed by ESI-TOF MS. Using circular dichroism (CD) spectroscopy we investigated the residual secondary structures present in AaFEcR as well as the influence of trifluoroethanol (TFE) and guanidinum chloride (GdmCl) on the structure-forming potential of AaFEcR. With size-exclusion chromatography (SEC) we established the atypical value of Stokes radius and elution volume, which is more than 1.5 times larger and ca. 1.2 times smaller, respectively, in comparison with a globular protein with the same molecular mass. Our data from the experimental results and the bioinformatics analysis shows that AaFEcR belongs to a family of IDPs and possesses a putative pre-molten globule (PMG) shape.

References:

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Posters

P2.1

Active "domain" of intrinisically disordered Starmaker protein

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Biomineralization is a process by which living organisms form crystals. Proper polymorph, size and shape of biominerals are controlled by organic compounds, mainly by proteins. Starmaker (Stm) protein from inner ear of *Danio rerio* fish, is engaged in otoliths biomineralization. Otoliths are calcium carbonate biominerals responsible for perception of sound and balance. Stm belongs to the group of intrinsically disordered proteins (IDPs). Stm is an extremely acidic protein with two characteristic regions: four highly conserved internal repeats and a region rich in serine and aspartic acid residues. It is believed that this acidic region might be responsible for biomineralization.

In our study we compared the influence of two Stm fragments on biomineralization process. For this purpose two fragments were obtained in *E. coli* cells: Stm1-231 containing four conserved tandem repeats and Stm232-603 with a region rich in serine and aspartic acid residues. Results of biomineralization tests have shown that Stm232-603 is crucial for biomineralization activity. This fragment controls shape and size of calcium carbonate crystals. Moreover analysis of phosphorylation of Stm fragments by casein kinase 2 (CK2) has shown that only region 232 - 603 was phosphorylated. Circular dichroism spectra and gel filtration experiments indicated that both fragments are disordered.

Our results indicate that despite the disordered nature of both fragments, their biomineralization activity is different and they are differently phosphorylated.

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P2.2

Role of the unstructured fragment of human topoisomerase I in kinase activity

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Human topoisomerase I is a partially disordered protein with dual enzyme activity. Firstly, it relaxes supercoiled DNA. Secondly, to acts as a kinase phosphorylating splicing factors – SR proteins. Whereas first activity is well known, including solved crystal structure of topoisomerase I in complex with DNA, second activity remains poorly understood. Only model of topoisomerase – SR protein interaction was based on biochemical studies and molecular dynamic simulations (Ishikawa, 2012). Possibly, important role of the unstructured part of the protein [1-433] in intermolecular interaction results in difficulties with obtaining crystal structure of the complex. Interestingly, unstructured fragment of the protein [1-433] can bind SR proteins with similar affinity as full-length molecule (765 aa). Here we present results of studies on topoisomerase I interaction with one of the SR proteins – SRSF1 using biochemical and biophysical methods, such us HDX-MS (Hydrogen-Deuter eXchange Mass Spectrometry) and NMR spectroscopy.

Reference:

Ishikawa T *et al* (2012) *Biochemistry* **51**: 1803–1816.

P2.3

Vascular-endothelial growth factor and gelatinases in patients with varicose veins

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Chronic venous insufficiency is an important medical problem in developed countries. Increased blood pressure in the varicose veins (VV) can contribute to the overexpression of matrix metalloproteinases (MMPs), affecting the endothelium, smooth muscle and extracellular matrix proteins of the vein wall. Moreover, hypoxia and inflammation occurring within the VV wall contribute to the increased expression of vascular endothelial growth factor (VEGF) in the tissues, that stimulate the expression of MMPs. VEGF plays an important role in maintaining the integrity of blood vessel walls and during the process of angiogenesis. Despite many studies on the pathogenesis of VV, the mechanisms leading to the formation of the disease are still not fully understood.

The aim of this study was to analyze the concentration of gelatinases (MMP-2 and MMP-9), and VEGF in the VV wall and in the plasma of patients with VV. Sixty five patients aged from 22 to 70 years (49 women, 16 men) with VV classified as C2 according to CEAP criteria, were enrolled into the study. The material consisted of: the varicose great saphenous vein obtained during the surgery, and the blood of patients taken from the antecubital vein. Great saphenous VV was removed using Babcock method (stripping). The sex and age matched control groups were enrolled; healthy vein wall samples were obtained from patients, who underwent surgery coronary artery bypass grafting (control No.1, n=10);and blood was collected from healthy individuals (control No.2, n=20). The concentration of gelatinases and VEGF were measured by enzyme immunoassays. The values were expressed as mean ± SD or median and 1st-3rd quartiles.

A significant increase in MMP-9 level (11.20 ng/mg of protein ±1.09) and VEGF (41.06 ng/g of protein, 38.4-42.71) in VV wall compared to control No.1 (MMP-9: 9.98 ng/mg of protein ±1.21, p0.05; VEGF: 26 ng/g of protein, 24.49-27.89) was oserved. A positive correlation between the concentration of VEGF vs. MMP-2 (p=0.03, r=0.27) was found in the VV wall. However, no correlation was found between the concentration of VEGF, and MMP-9 (p=0.4, r=0.11) in the VV wall. No statistical differences between MMP-9, MMP-2 and VEGF levels in plasma of VV patients compared to controls were noticed.

Overexpression of MMP-9 and VEGF in the VV wall may contribute to the spreading of inflammatory process and suggests the intense remodeling of extracellular tissue within the VV wall.

P2.4

Structure and function studies of RNA-binding proteins with FAST motifs and a RAP domain

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The FASTK family (Fas-activated Serine/Threonine Kinase) contains six human proteins which localize to the mitochondria and have been functionally linked to cellular respiration and with a rare mitochondrial disease. While human FASTK was initially annotated as an atypical Ser/Thr kinase later studies dispute this annotation [1]. Structurally, FASTKD proteins contain an N-terminal mitochondrial targeting signal, a pair of FAST motifs and a C-terminal RAP domain (Fig. 1).The N-terminal part is predicted to be highly globular but with small disordered regions. The FAST motifs are putative RNA binding domains with a novel α-helical repeat fold that has no sequence similarity to any other known helical repeat motifs. Interestingly, the RAP domain is found in many members of the recently identified class of octotricopeptide repeat (OPR) proteins, which are abundant in plants and green algae and is believed to play a role in chloroplast RNA biology [2]. The OPR proteins have been shown to bind RNA with preference for some substrates [3], but their structure or RNA binding specificity is unknown. The RAP domain is also overrepresented in Plasmodium, and hence structural information of this domain is relevant to the field of malaria. The obtained structures and functions of these proteins may have relevance to drug design therapeutic strategies, particularly of cancer and inflammation and will likely reveal new folds of RNA binding domains thus contributing to the general knowledge of the rules that govern RNA recognition. Our project aims to provide for the first time structural and novel biochemical information about the relatively understudied FASTK family.

References:

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P2.5

Backbone Resonance Assignment of Tau3x Protein Using New 4D Experiments with Carbonyl-Carbonyl TOCSY

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Nuclear Magnetic Resonance (NMR) spectrscopy is widely used in the studies of intrinsically disordered proteins (IDPs). The resonance assignment in the NMR spectra, a necessary prerequisite for any further NMR studies, in the case of IDPs often appears to be a challenging task due to a severe overlap of resonances in the acquired spectra.

New 4 dimensional experiments, exploiting tocsy type carbonyl-carbonyl coherence transfer using MOCCA-XY16 mixing block (Felli IC *et al* (2009) *J Biomol NMR* **43**:187, Furrer J *et al* (2004) *J Magn Reson* **166**: 39–46), providing valuable CON connectivities as well as easy proline residues resonance assignment is proposed. This techniques allowed full backbone resonance assignment of Tau3x protein (354 a.a.).

All spectra were nonuniformly sampled to provide high resolution in all indirectly sampled dimensions and were processed using Signal Separation Algorithm (Stanek *et al* (2012) *J Magn Reson* **214**: 91). All processing software used is free to use for academic users and is available on http://nmr.cent3.uw.edu.pl/software.

P2.6

Nucleoplasmin-like FKBP39 from *Drosophila melanogaster* is a partly disordered tetramer

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Regulation of insect development and growth although controlled by only two hormones is a complex process. Beside decades of intensive studies there are still many unrevealed aspects. Especially intriguing is the cross-talk of hormonal signalling pathways. FKBP39 was shown to be a key regulator of a dynamic multiprotein complex which crosslinks the two signalling pathways. Nevertheless, neither the structure nor the exact mechanism of action has been revealed. In this work we performed detailed structural analysis of full-length FKBP39 to bring us closer to understanding its role. A range computational methods had predicted partial disorder of FKBP39 conformation, what was further confirmed by biophysical methods. We also found that full-length FKBP39 oligomerise *via* its N-terminal domain which shows a high homology with nucleoplasmin. Importantly, we found that nucleoplasmin-like domain form tetramer. This is the first report of an alternative, other that pentameric arrangement of nucleoplasmin. Molecular modelling revealed a dynamic, very unique structure of FKBP39, where one end forms a stable core and catalytic domains are linked to it by disordered, highly flexible chains. These results provide new insights regarding the conserved structure of nucleoplasmin core domains and provide a potential explanation for the importance of the tetrameric structural organization of full-length nucleoplasmins.

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P2.7

TROSPA from *Ixodes ricinus* — the first intrinsically disordered protein involved in vector-pathogenic microbe recognition

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Currently, the only identified protein involved in tick colonization by *Borrelia* pathogens is the tick receptor for Outer surface protein A (TROSPA). TROSPA is localized in the tick gut epithelium and is capable of recognizing and binding the Outer surface protein A (OspA) from *Borrelia* via an unknown mechanism. The TROSPA-OspA interaction is indispensable for *Borrelia* transmission by ticks. Based on the earlier report by Pal *et al*. [1] as well as on our latest observations, we assumed that TROSPA might be a unique example of an intrinsically disordered protein that adapts its structure to a potential ligand. To verify this hypothesis, we performed detailed studies on recombinant TROSPA from *Ixodes ricinus* using both experimental and computational approaches. Regardless of the method used, we observed that the polypeptide chain of TROSPA has very poor secondary structure. In addition, the collected SAXS data indicated that this protein is highly extended and exists in solution as an ensemble of numerous conformers. All of these features are commonly considered to be the hallmarks of Intrinsically Disordered Proteins (IDPs). The disordered nature of TROSPA may explain why a wide spectrum of *Borrelia* species are capable of colonizing the tick gut. To learn more about the function of TROSPA and OspA, we monitored the response of these proteins to a changing environment. We discovered that both proteins undergo a specific structural switch at pH 7.4 and temperature close to 37°C, i.e. at the physiological conditions of their interaction. We also proposed a putative mechanism for the TROSPA-OspA interaction.