Session IV, November 21, Thursday

L12

PREAGGREGATION CONFORMATION OF TAU PROTEIN RECOGNIZED BY SPECIFIC ANTIBODIES

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A key yet unresolved question of the pathogenesis of Alzheimer's disease (AD) and other tauopathies is the cause and the mechanism of the transition from the unstructured monomeric tau protein to the insoluble filaments deposited in the brain tissue. In the physiological state, tau protein exists as a conformational ensemble of interconverting structures and on the scale of transition from monomeric through oligomeric and filamentous species we can observe conformations reacting with specific antibodies, mainly with DC11, which is able to specifically discriminate between tau proteins isolated from healthy brain and tau proteins isolated from the brain of AD patient. The antibody recognizes also the recombinant truncated tau proteins up to the shortest fragment tau321-391 [1].

It was found that conformational antibodies DC11 and MN423 have catalytic pro-aggregatory effects in tau aggregation assay, whereas the antibody DC8E8 has inhibitory effects on tau filament formation [2]. This may imply possible mechanism of induction of pathological tau conformation, in which the antibody prepared against pathological tau imprints the pathological conformation into the physiological tau proteins in solution and therefore speeds up the tau aggregation. The information about conformational epitopes of these antibodies is therefore of high significance.

To further uncover the binding mode of the conformational antibody DC11, we have performed NMR epitope mapping using ^{13}C, ^{15}N labelled tau321-391 and tau297-391 (dGAE) and recombinantly prepared Fab fragment of DC11 antibody. The overlay of HSQC spectra showed the region of tau between residues 370-390 to be affected by the binding of DC11, i.e., its C-terminal region. However, previous studies suggest the importance of region 321-325 for the interaction of tau with DC11 antibody. We have further characterized the influence of DC11 Fab binding on the non-epitope tau residues using NMR relaxation measurements of ^{15}N labelled tau dGAE.

We have crystallized Fab fragment of DC11 antibody and attempted to crystallize its complexes with tau peptides tau321-391 and tau371-387. We have also performed SAXS measurements with Fab DC11 and tau proteins tau321-391 and tau297-391 (dGAE).

The results highlight the importance of the R' region of tau, that was recently shown to be important also for tau interaction with microtubules [3]. This sequence forms the interface of rigid filament core and flanking fuzzy C terminal segment in solved tauopathy filaments.

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In the program, this lecture was replaced by contribution of V. Bauerová.

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QUALITY AND INTEROPERABILITY OF STRUCTURAL DATA OF NUCLEIC ACIDS

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The community of structural biologists benefit from the culture to share structural data, widely accepted implementation of the mmCIF dictionary as generally accepted standard how to archive them, and generally well-functioning publicly accessible archive, the PDB. Despite this generally positive situation, there remain serious quality problems. These problems are more serious for nucleic acid structures than for proteins, primarily because there are many fewer high-quality structures [1]. The problems with nucleic acid geometry can be divided into three groups: (i) inconsistently set and applied target values of the valence geometry, bond distances and angles, for nucleotides; (ii) poorly refined backbone geometries; (iii) incompletely and often incorrectly assigned base pairing topologies. In the talk, I introduce web-based tools that address these problems and offer solution to some. The tools are available from our web application dnatco.datmos.org [2]. The web leads you from the Annotation TAB, which offers an overview of the geometry of analyzed structures accessible for a non-expert. The Validation TAB enables an in-depth analysis of nucleic acid structures and expert judgement of their quality. This TAB provides a detailed analysis of NA conformation at the level of dinucleotide. This analysis is based on our original system of the dinucleotide NtC classes [3]. It also offers validation of valence geometry. Soon, we will also provide an integrated tool to analyze base pairs detected in the analyzed structure. Now the web basepairs.datmos.org provides an overview of base pairs in the PDB structures. The TAB Refine offer tools to modify structures during the process of their refinement. The last TAB, Browse, allows the user to view NA structures from various perspectives. Results of most analyses are available for download in several formats.

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This lecture was cancelled.

L14

DYNAMIC THEORY OF PROTEIN CRYSTALLIZATION

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Traditional theory of protein crystallization takes crystallization as a precipitation of ideally dissolved protein molecules from an ideal mother liquor in thermodynamic equilibrium. This gives good results for small organic molecules. However, large surface of protein molecules offers many different adhesion modes and some of them are mutually incompatible in a single crystal. Thus, the protein crystallization is a competitive process between these adhesion modes.

*"**Dynamic theory of protein crystallization (DTPC)**"* respects the fact that the molecularly overcrowded solution near the crystallization conditions contains variety of molecular clusters with adhesion properties different from the "naked molecules". The crystallographer knowing the adhesion properties of clusters can control the adhesion of protein molecules to the surface of growing crystal and increase quality of these crystals simply by adding the required chemicals in the solution. The best results are quarantined by the *„**principle of the dominant adhesion mode (PDAM)**"*. In a simplified form, the criterion for successful crystal growth and increase of crystal quality is the increase of a difference between free energies of the competitive adhesion modes

$$F^{cryst} \sim F^{dominant\ AM} - F^{incompatible\ AM}$$

This offers the crystallographer a rational way not only to grow quality crystals but also to choose the required crystalline form. The new approach changes the situation significantly and leads to enhancement of efficiency and accuracy of all standing crystallization methods. DTPC

with PDAM are general and should be respected by any method of protein crystallization.

Homogeneous crystallization

When the crystallographer knows the rules for formation of these temporary complexes, then he can control preferences of the adhesion modes taking part in the crystal grow. He can decide which of the mutually exclusive protein-protein adhesion modes succeeds and becomes dominant by using his knowledge of the adhesion modes using the ***„protein-surface-active molecules (PSAM)"***. Reach source of the adhesion mode examples is the PDB offering an insight how the ***„protein surface shielding agents"*** work in practice and how the ***„crystal structure forming elements"*** help in finding the best crystal architecture.

Protein crystal can be regarded as a well-defined block of highly concentrated solution (20-70 % water content), where the 3D long-range periodicity is ensured by well-defined intermolecular forces for most atoms in the unit cell. Additives adhering temporally to protein surface in this solution can block some surface that may be decisive for adhesion and deposition of new protein molecules to the surface of growing crystal. The crystallization additives shielding the protein-protein adhesion modes are called "**Protein Surface Shielding Agents (PSSA)**". Their cover usually a large surface on protein surface exposed to solvent protecting thus the specific adhesion modes.

The adhesion potential of good PSSA's is lower than adhesion between protein molecules and thus PSSA's are usually expelled from their position during the crystal growth. However, in some cases they remain built in well-defined positions on the protein surface, so that the PDB gives many examples of their function. Several thousands of examples show the most frequent binding interactions of PEG-based polymers with proteins:

- chelating of several ether oxygens on tips of Lys, Arg, His,
- cations chelated by PEG oxygens bind to carbonyl groups,
- multiple H-bonds in H-bond donor rich grooves,
- combination CH2- interactions and hydrophobic interactions namely in the Trp rich clefts, etc.

Heterogeneous crystallization

Many scientists spend a lot of effort to find crystallization initiators (bio-glass, coarsely wrinkled foils, nano-carbon materials, imprinted polymers, porous Si, hoarse hairs, properly coated nanotubes and nanostructured carbon black, etc.).The principle of dominating adhesion mode explains why these mysterious materials are work. These materials depressions with protein adhesive surface. Specific adhesion of protein molecules leads to their energetically demanding pre-orientation and restricts an access to the adhesive surfaces responsible for incompatible PPAM. The unique PPAMs in the growing crystal nuclei make the nuclei more stable because of lower number of stacking faults. They do not dissolve and can continue to grow even after their release into the seemingly under-saturated bulk solution. Contrary to former mutually contradicting explanation, he DTPC explains all experiments available by now on a unique common basis. The new insight promises better design of crystallization catalyzers promising significant advance in the structure determination by diffraction methods.

L15

MONITORING OF STRUCTURE REFINEMENT PROGRESS WITH PDBCOP

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Structural biology is continuously oriented towards understanding more and more complex structures of proteins, nucleic acids and their complexes. In structure refinement, parameters of tens to hundreds of thousands atoms are modified. Challenging data are frequently recorded at low resolution and low-resolution structure refinement can easily become unstable. The program PDBCOP is designed to reveal instabilities in coordinates and ADPs (Atomic Displacement Parameters).

The program PDBCOP performs quick calculations for common 'Table 1', reports extremes in ADPs, extreme occupancies, generates a sequence of structure model for validation against the original sequence, and compares two PDB files (most likely input and output from structure refinement models). The new version also accepts input in the CIF format.

The original version of PDBCOP by Karla Fejfarová [2] is also still available on the original url https://crysa.fzu.cz/pdbcop/. The new version written in Python3 and also compiled binaries for MS Windows are available on the new address http://kmlinux.fjfi.cvut.cz/~kolenpe1/pdbcop/.

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