Session VIII - Thursday, June 23

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PAIRED REFINEMENT AND CRYSTAL ANISOTROPY

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Anisotropy in diffraction qualities of crystals may represent a serious threat to the structure determination process. When the differences in various directions of the reciprocal space exceed the range of 0.5Å resolution, problems with phasing may arise, and the structure refinement process is frequently unstable. Such difficulties also appear for structures with high resolution. Although a limited number of tools for the data analysis is available, the current praxis is not standardized and needs thorough revision.

We analyzed diffraction data from a crystal of an engineered protein binder with a promising application as a protein therapeutics [1]. The protein crystallized in space group $I4_122$. The initial diffraction data quality indicators of the data processed the standard way suggested the high-resolution diffraction limit at 2.9 Å. However, a combination of data processed with *STARANISO* [2] and paired refinement with *PAIREF* [3] showed a possible extension of the diffraction limit to 2.6 Å in the direction along the *l* axis. Calculated electron density display moderate improvement and easier interpretation for some side chains.

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This work was supported by MEYS CR (projects CAAS – CZ.02.1.01/0.0/0.0/16_019/0000778) from the ERDF fund and by the GA CTU in Prague (SGS22/114/OHK4/2T/14).

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STRUCTURE DATABASE OF ORGANIC POLYMERS AND THEIR INTERACTIONS WITH BIOMACROMOLECULES

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The Cambridge Structure Database of Organic and Organo-Metalic Compounds (CSD) **[1]** in its 2022 version) can be searched by the keyword "polymer". It results in a large number of *organo-metalic polymers*, i.e. the crystalline structures in which organic molecules are inter-connected by metal bridges to form the "infinite" 1D, 2D, or 3D networks passing through the whole crystalline blocks. These crystals are typically regular and the corresponding clear diffraction pattern allows reliable and precise structure determination required for deposition into the CSD.

However, in the case of the *classical organic polymers*, the preparation of the high quality crystalline samples is extremely difficult namely because of polydispersity and extremely long times required to achieve the equilibrium state. Diffraction quality is thus often very low. The experimental structures are often inaccurate and require theoretical re-modelling. Roughly, a half of the structure determinations do not satisfy requirement for deposition in the CSD. Some synthetic or natural polymers can be found also in the ***Crystallography Open Database [2]***. However, about half of the published structures are not present in these databases**.** This is the reason why the ***Polymer Structure Database (POLYBASE-2011)*** collecting all available organic polymers **[3]** was prepared. The new ***POLYBASE-2022*** version will be completely re-cured now.

Hydrophilic polymers are often used as precipitants for crystallization of bio-macromolecules. It is the reason why the ***Database of Protein-Polymer Interactions (DPPI-2011)*** **[3]** was formed. The contemporary ***DPPI-2022 version*** contains 3667 PDB structures of bio-macromolecules (proteins and nucleic acids). The structures collected from the ***RCSB server*** **[4]** experimentally confirm complexation of poly(ethyleneglycol) chains (at least four monomers in length) at the protein surface.

Because many of these proteins are complexed with more polymer chains, the ***DPPI-2022*** contains several thousand experimentally verified interactions of hydrophilic organic polymers bound directly on the surface of protein molecules. Visual inspection of the ***DPPI-2022*** provides surprisingly high number of various types of protein-polymer interactions. Classification of these interactions is a useful background for explaining the success of poly(ethyleneglycol)-type polymers in many economically important applications in the industry, science, medicine and pharmaceutics.

The ***Polymer Structure Database (POLYBASE)*** and the ***Database of Protein-Polymer Interactions (DPPI)*** are presently updated and will be available on request in their new versions by the end of 2022.

The research was supported by the project CZ.02.1.01/0.0/0.0/15_003/0000447 from the ERDF.

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THE UNIQUE STRUCTURE OF THE CELL WALL BINDING DOMAIN OF PHAGE ENDOLYSIN

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Bacteriophage-encoded endolysins, enzymes showing bacteriolytic activity, are of growing interest for their applications as enzybiotics in veterinary and/or human medicines and various field of biotechnology, e.g. food safety. Bacteriophage BFK20 is a lytic phage of *Brevibacterium flavum* CCM 251 (gram positive corynebacteria), industrial producer of L-lysine. The genome of the bacteriophage BFK20 has been sequenced and analyzed (EMBL accession no. AJ278322) [1]. The gene product of ORF24´ was identified as endolysin gp24´ (UniProt ID Q9MBI0), an enzyme necessary for cell lysis and release of mature phage particles from the infected bacterial cells. The protein is composed of two domains, a catalytic domain exhibiting N-acetylmuramoyl-L-alanine amidase activity, and a cell wall binding domain (gp24BD), which are connected by a proline-rich linker. The individual domains were cloned separately and the cell wall binding capability of the C-terminal region (81 aa) was proved experimentally [2]. The whole protein as well as individual domains were crystallized, but only crystals of individual gp24BD were obtained. Needle shaped crystals belonging to hexagonal space group P622 were grown overnight. Crystals diffracted to 3.2Å resolution using synchrotron source of radiation, but were twinned and not suitable for structure determination. Later, the crystals recrystallized directly in the crystallization drop. The newly obtained crystals diffracted to 1.4Å resolution using home diffractometer. They belonged to tetragonal space group $P42_12$. The same protein samples stored for several weeks at 4 °C crystallized directly in the form of tetragonal crystals. Tetragonal crystals were dissolved and the protein analysis showed random proteolysis and protein shortening by 8 amino acid residues.

The 1.4Å resolution data set was used for structure solution by the direct method using the program ARCIMBOLDO-LITE [3]. The overall structure revealed very loose bundle of three -helices. The asymmetric unit contains one protein molecule. The crystal symmetry gives four molecules in the unit cell forming very compact tetramer. Based on the PISA prediction the tetramer is stable also in solution. The oligomers have also been experimentally detected, thus one can speculate about the oligomeric state as biologically active unit. Closer inspection revealed the amphipathic nature of the helices and the tetrameric coiled-coil structure. An electron density was found in the tetramer cavity to which metal and chlorine ions were modelled. The coiled-coil structure is interrupted by a loop (one from each molecule). Between each two neighbour molecules a molecule of glycerol was identified sitting mostly at the loop. Molecular docking experiments performed using a rhamnose molecule supported the idea that glycerol maps the binding site which may be large enough to accommodate a substrate composed of several monomers. The gp24BD is unrelated to any of the known cell wall binding domains of phage endolysins by the amino acid sequence and also by the structure; this is the first evidence of the endolysin binding domain showing the coiled-coil structure. BFK20 phage endolysin binds to the cell walls of corynebacteria in a highly specific manner, unfortunately, the specific composition and structure of their cell walls and the exact peptidoglycan substrate of gp24BD have not yet been determined. To answer the

questions concerning the functioning of the binding domain and the whole endolysin, further work is needed, especially the structure of the whole molecule and the complexes with possible ligands should be solved.

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Session IX - Thursday, June 23

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XRPD AS A POWERFUL TOOL FOR STUDY OF PAINTED ARTWORKS

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Laboratory X-ray powder diffraction is a very effective and non-destructive method for direct phase analysis of paint layers usually consisting of complicated mixtures of pigments, binders, dyes, fillers and/or degradation products. While a conventional Bragg-Brentano set-up allows direct non-invasive analysis of smaller painted objects, e.g. miniature portraits, a micro-diffraction mode plays a substantial role in the analysis of samples (usually smaller than 1 mm) taken from paintings. The application of mineralogical analysis for study of provenance and technology of late Gothic/early Renaissance painting materials as well as examples of uncovered degradation products will be presented. The methodological pros and cons will be also discussed.

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DETERMINATION OF STRUCTURE OF SMALL PARTICLES

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We are producing and studying Magnetic Nanoparticles (MNPs) due its applications in biomedicine. The suitable size have to be comparable to biological entities (cells, proteins, and genes), controllable transport of MNPs in human body (drug delivery). The purpose of our study is produce particles for their ability to generate heat when an AC magnetic field is applied (magnetic hyperthermia). In particular, magnetic hyperthermia therapy is based on the fact that some types of cancer cells are more sensitive at temperature 41-45 °C than the healthy cells and that the required heat can be produced by MNPs. Nowadays majority in-field investigations are based on *in vitro* or *in vivo* animal model, but also, in the case of iron oxide based MNPs, this approach is used at the clinical level [1]. The heating ability of MNPs is dependent on morphology, microstructural and magnetic properties of MNPs, but also related to the amplitude and frequency of an applied magnetic field. In that sense, during the last years the synthesis methods have been intensively developed in order to control particle size distribution, surface effects and the degree of interparticle interactions, so that magnetic properties favourable for particular application could be successfully tailored. Although the tons of studies was published, there is huge confusion and misunderstanding in terms such as

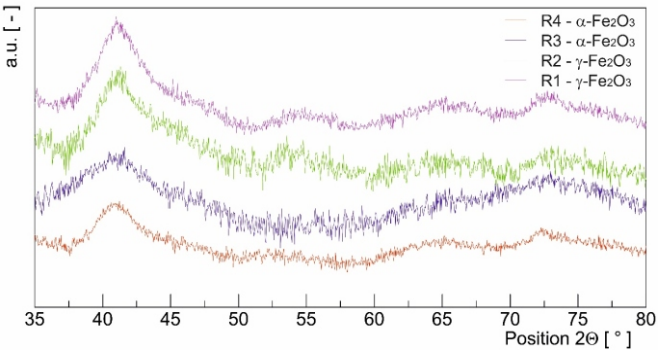

Figure 1. X-ray pattern of tiny particles.

particle, crystalline and grain size, which influenced the main characteristic – the magnetic properties. The confusion is originated by very wrong understanding of analytical method which are applied. For our purpose of magnetic hyperthermia, we are looking for magnetite Fe_3O_4 and maghemite $-Fe_2O_3$.

The standard method of determination of phase composition by X-ray powder diffraction (XRD) is not very helpful (see Fig. 1) in our case it produced results on the range