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Session I, Monday, September 12

L1

CRYSTALLOGRAPHY BEFORE LAUE'S DISCOVERY

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The Max von Laue's discovery of the diffraction of X-rays by crystals in 1912 and subsequent diffraction experiments of William Henry Bragg and William Lawrence Bragg significantly changed the subject of crystallography towards crystal structure analysis. The lecture focuses on the history of crystallography since the first serious work on geometrical crystallography by Johannes Kepler in 1611 (*A New Year's Gift of Hexagonal Snow*) till the crucial diffraction experiment of Laue, Friedrich and Knipping in 1912. This contribution illustrates the main areas of crystallographic research at that time and important milestones in the scientific progress in crystallography of that time.

Study of crystal morphology has played a central role for many centuries. Nicolaus Steno studied in 17th century quartz crystals and noted that, despite differences in size and crystal habit, the angles between corresponding planes were constant (law of constancy of interfacial angles). This law was repeatedly later restated by Romé de l'Isle and Domenico Guglielmini. Christian Samuel Weiss and Frierich Mohs made also significant contributions. William Henry Miller developed the familiar *hkl* notation for description of crystal faces (around 1839) and constructed the first two-circle goniometer for study of crystal morphology. Victor Mordechai Goldschmidt should be mentioned for his systematic study of crystal morphology and for his well-known *Atlas der Krystallformen* (1913)*;* an extensive collection of crystal drawings**.**

Worth-noting is the building-block theory of crystal structures, introduced by René-Just Haüy in 1822 (*Traité de Cristallographie*), which led directly to the lattice models. Haüy suggested that crystals are composed of array of blocks, called *integral molecules* (i.e. unit-cell in current terminology). He also showed that it is possible to construct different external shapes of crystal by replicating the same basic blocs in different ways. Symmetry was proved to be essential. A few years later, August Bravais correctly derived the 14 three-dimensional lattices that now carry hid name. At the begging of 19th century, the term "*isomorphism*" and "*polymorphism*" was introduced (Romé de l'Isle) and intensively studied (E. Mitscherlich). Paul von Groth – a founder of *Zeitschrift für Kristallographie* (1877) – made a significant contribution to the chemical and physical crystallography at the end of 19th century.

Study of groups and its application to crystallography was a fruitful field of research in the 19th century. It is very interesting fact, that all of the 230 space groups were independently derived by Arthur Moritz Schöenflies, Evgraf Stepanovich Fedorov and William Barlow at the approximately same time in 1980s.

This contribution is partly based on [1-4].

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L2

SOLUTION OF MAGNETIC STRUCTURES

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Structure analysis of standard crystals is becoming more and more a routine task. Data collection with standard laboratory equipment can be done in a few hours for many cases, and the solution by modern methods, as charge flipping [1], might take some minutes at the most. In the last years, similar progress has been achieved for solution and refinement of modulated and composite crystals [2], so that nowadays some of these structures can be solved routinely by non-specialists.

However, new materials exhibiting specific physical properties, as magnetism, electrical conductivity or superconductivity, require more detailed structure analysis. Such compounds must be studied under different conditions, like high pressure and non-ambient temperature, to describe structural changes during phase transitions. For these cases, structure analysis is still challenging and a swiftly developing field.

Phase transitions can lead to the ordering of magnetic moments and/or development of ferroelectric moments in the material. To study magnetically ordered systems neutron diffraction must be used. The solution and refinement of magnetic structures is usually made by decomposition of the magnetic configuration space into basis modes, which transform according to different physically irreducible representations (irreps) of the space group of the paramagnetic phase [3]. Recently it was shown that the direct use of Shubnikov (magnetic) space and superspace groups enables easier handling of non-modulated and modulated magnetic structures as well, and simplifies the algorithms dealing with diffraction data of magnetic structures [4]. For incommensurately modulated magnetic structures this approach may be particularly beneficial, as for instance in the analysis of multiferroic phases [5].

Solution of a magnetic structure starts with symmetry analysis. The program Jana2006 provides a simple procedure for testing different irreps of the parent paramagnetic structure, as follows from the already known nuclear structure, and links them with the corresponding Shubnikov space or superspace groups. The magnetic structure model resulting from the selected magnetic symmetry can be checked in VESTA [6] which is launched by Jana2006, whereas the simulated powder data for a model can be compared against the measured diffraction profile. Jana2006 can also call via internet the recently developed program ISODISTORT [7, 8] which provides similar analysis (although more detailed), and use its results.

As a next step, all the acceptable models from the representation analysis are refined and the solution having the best fit with experimental data can be selected as the final solution of the magnetic structure. However, this process is not necessarily straightforward and, in many cases, different magnetic spin configurations can similarly fit the data. Consequently, other experimental methods like polarized neutron diffraction must be used to identify the correct solution.

The basic idea behind the new option for magnetic structures in Jana2006 lies fully on magnetic and nuclear symmetry, similarly to classical crystallography. Symmetry is applied during data processing (merging symmetry-related reflections for single-crystal data or reducing the generated reflections to the independent ones for powder data) as well as in the calculation of magnetic structure factors. This new approach greatly improves the stability of the refinement and offers a logical path to describe twin domains.

Two procedures are possible for the combination of nuclear and magnetic diffraction. The traditional way used in FullProf [9] is to combine two independent phases. The method preferred in Jana2006 is to use a common structural description for both phases and make the combination of intensities internally, as follows from the calculation of structure factors.

Jana2006 also offers simultaneous refinement against different diffraction experiments. Commensurate and incommensurate phases can be combined as well, as powder and single-crystal data. Therefore, manifold possibilities are available for the refinement of modulated magnetic structures from various experiments.

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Session II, Monday, September 12

L3

DYNAMIC THEORY OF PROTEIN CRYSTALLIZATION AS A TOOL FOR A CONTROL OF PROTEIN CRYSTAL ARCHITECTURE

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Crystallography is very precise and handy method for determination of "small molecular structures". In structure determination of biological macromolecules and their complexes, the processing is much more complex and the precision is much worse. The crystallization process is considered as hardly predictable and thus crystallization trials are based on an expensive random screening of many hundreds of crystallization conditions as a rule. Nevertheless, the success rate of crystallization of the native proteins is still quite low. A deeper understanding the crystallization process seems to be prerequisite for radical improvement of the present status.

Many problems with protein crystallization are caused by obligatory high water content in crystals (50 % on average). Protein molecules remain in crystals highly solvated and are in dynamic equilibrium with solution also in crystalline state. The stability of molecules in crystal is thus ensured only by intermolecular interactions between adhesive patches of neighbor macromolecules forming a rigid 3D scaffold in the crystal. Most of solvent filling 30-80 % of crystal volume remains dynamically disordered ("fluent") and invisible in maps of electron density. Thus, the rigidity and stability of the 3D macromolecular scaffold of crystal is of basic importance, i.e. the design of the **protein crystal architecture** is very important.

Role of PEG in crystallization. The dynamical theory of protein crystallization and knowledge of protein-protein adhesion modes explains why the poly(ethylene glycol)-type polymers (**PEGs) are the most successful precipitants** [1,2]. The reason is that the PEG-type polymers have, in addition to their precipitating effect, also a large scale of **protein-surface active molecules** (PSAM) and **protein-surface shielding agents** (PSSA) properties. Analysis of these properties is described in our former papers [1,2]. Reader can also get his own experience using the tools offered by the database of protein-polymer interactions. He can scan over hundreds selected protein structures and observe readily different the protein-PSAM adhesion modes. The Table with categorized protein-PSAM interactions can serve as a guide to evaluate applicability of the useful adhesion modes (in press).

The analysis of already solved protein structures deposited in the PDB gives the necessary knowledge of adhesion properties of proteins required for planned strengthening the dominant and weakening of other non-compatible adhesion modes in crystallization solution. These studies including a review of protein crystal architecture (in press) can be simplified by the "Database of protein-polymer interactions".

Database of protein-polymers interactions (DPPI) [2] contains about 4000 experimentally observed PEG-protein interfaces. It consists of a set of protein structures crystallizing with PEG-like polymers and useful scripts allowing easy visualization of PEG activities on protein surfaces. Seeing the PEG fragments interfering with protein-protein interfaces, with different types of salts, blocking competitive crystal contacts helps us to understand the dynamic processes during crystal growth and allows rationalization of crystallization screens on molecular basis. The database of protein-polymer interactions is available from hasekjh@seznam.cz.

Observation of adhesion modes between protein and protein surface active molecules led us to formulation of the **dynamic theory of crystallization**, and the **principle of a single dominating adhesion mode**. The lecture summarizes last progress in development of this new approach and shows that a knowledge of the adhesion properties of protein surface-active molecules gives us an efficient tool for crystallizing most of proteins, for growing the desired crystal polymorphs, and for analyzing various protein-protein adhesion modes important in the living nature.

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L4

INFLUENCE OF MUTATIONS ON THE PROTEIN STRUCTURE AND PROPERTIES

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Ribonuclease Sa (RNase Sa) is an extracellular enzyme secreted by *Streptomyces aureofaciens* into the growth medium. It is the smallest member of the microbial T1 ribonuclease family with just 96 amino acids. Crystal structure of RNase Sa has been determined at 1.2 and 1.0 Å resolution [1,2] and a solution structure has been determined using NMR [3]. RNase Sa has proven to be an excellent model for various types of studies including structure-function relationship, mechanism of enzymatic reaction, protein-protein recognition, protein folding, flexibility and conformational stability of globular proteins.

For the better understanding the principles of conformational stability of proteins, single mutations in RNase Sa molecule were designed to remove a limited number of precisely defined hydrogen bonds and the stability of mutant proteins was measured [4,5,6,7]. Crystal structures of mutant proteins were determined in our laboratory at 1.0-1.7 Å resolution and the changes in hydrogen bonding were analyzed. It has been proved that i) intramolecular hydrogen bonds contribute substantially to the protein stability, ii) polar groups burial contribute to protein stability, iii) side chains on the surface of a protein that form intramolecular hydrogen bonds can make significant contributions to protein stability and iv) the effect of a single amino-acid mutation on conformational stability of protein highly depends on the location of the substitution and its environment in the structure.

Mutant proteins originally designed for the stability study have been analyzed also from the point of view of their catalytic properties, crystallizability and structural flexibility. Because of some difficulties at crystallization only eight structures have been published, yet. At present, there are additional six structures solved. Altogether, structures of fourteen mutants has been solved: two mutants of Asn 39, a residue conserved in the microbial T1 ribonuclease family (mutants N39S, N39D), three tyrosine to phenylalanine mutants (Y51F, Y80F, Y86F), four tryptophan mutants (D1W, Y55W, T76W, Y81W), three small polar and nonpolar group mutants (S24A, I71V, T95A) and two charged mutants (D79A, Q94K). Unexpected structural changes in the conformation of the surface loop have been observed in the case of N39S and N39D mutants. Two mutations, Q94K and T76W caused changes in the crystal packing, moreover, the main chain of the Q94K mutant is cleaved in the region of surface loop between Arg63 and Thr64. Structural flexibility has been also studied; the superposition of all mutant structures revealed the close similarity in in hydrophobic core and flexibility on the surface of the molecule, the picture is similar to the superposition of the structures solved by NMR method. Moreover, in some structures alternative conformations of main chain has been observed in various regions. The mutations influenced also the enzymatic activity in spite of the fact that none of mutated amino acid residues is directly involved into the substrate binding or cleaving. Asparagine 39 mutations caused changes in the conformation of the loop which forms a substrate binding pocket which resulted in the significant decrease of the activity to 20-2 %. Tyrosine 86 is positioned at the active site in the close proximity of the catalytic Glu54 and its mutation to phenylalanine led to decreasing the activity to 7 %. On the other hand, mutation of Ile71, which is positioned also very close to Glu54, to leucine, increased the enzyme activity 4 times. All the results of structure-function studies of RNase Sa mutants will be discussed in the presentation.

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L5

STRUCTURE AND GENOME DELIVERY MECHANISM OF *STAPHYLOCOCCUS AUREUS* PHAGE THERAPY AGENT phi812-K1 DETERMINED BY CRYO-ELECTRON MICROSCOPY

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Worldwide occurrence of multidrug-resistant pathogenic bacteria has increased interest in alternative treatments including bacteriophage-based therapy. Bacteriophage phi812 belongs to genus Twort-like virus, subfamily *Spounavirinae* and can infect at least 75 % of Methicilin-resitant *S. aureus* strains (MRSA) and 95 % of Methicillin-sensitive *S. aureus* strains. We have employed cryo-electron microscopy to determine structure and genome delivery mechanism for polyvalent staphylococcal backteriophage phi812-K1. Phi812-K1 has a 90 nm diameter isometric head and 240 nm long contractile tail ended by a double layered baseplate. The tail and baseplate of the native phage are dynamic. Therefore, a divide-and-conquer strategy was employed to separately determine the cryo-EM reconstructions of the individual phage parts. The structure of the icosahedral head could be refined to 5.0 Å resolution and additional sub-averaging within the T =1 6 icosahedral asymmetric unit allowed determination of the major capsid protein to 3.8 Å resolution. The structures of the native tail and baseplate were solved to 8 Å and 12 Å resolution, respectively. In order to examine the mechanism of the infection process, we determined the structure of the phage in the contracted state. The phage head is not altered after the DNA ejection. However, both the baseplate and tail undergo large reorganizations documented in their 6 Å and 8 Å resolution structures. Comparison of the tail and baseplate structures in the native and contracted conformation allowed us to determine the changes accompanying cell wall recognition and binding which is then followed by injection of the bacteriophage genome into the host bacteria.

SL1

CARBORANE AND METALLACARBORANE INHIBITORS OF CARBONIC ANHYDRASE IX, PROMISING COMPOUNDS FOR THERAPY

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We have previously identified metallacarboranes and carboranes as a promising class of specific inhibitors of HIV protease (HIV-PR) and Carbonic Anhydrase IX (CA IX) enzymes [1].

Here we report on recent advances in the molecular design of carborane and metallacarborane inhibitors targeting CA IX isoenzyme. This enzyme, which is associated with solid hypoxic tumors, belongs to newly identified targets for cancer therapy and diagnostics.

The scope of currently available site-directed modifications on various boron cages is overviewed, with an emphasis on the progress in the synthesis of carboranes and metallacarboranes substituted by sulfamide, sulfonamide and other similar groups, *i.e.* functions known to bind tightly to the zinc atom in the active site of CA-IX. The new generations of polyhedral inhibitors of CA-IX, based on the careful selection of boron cages and optimized substitu-

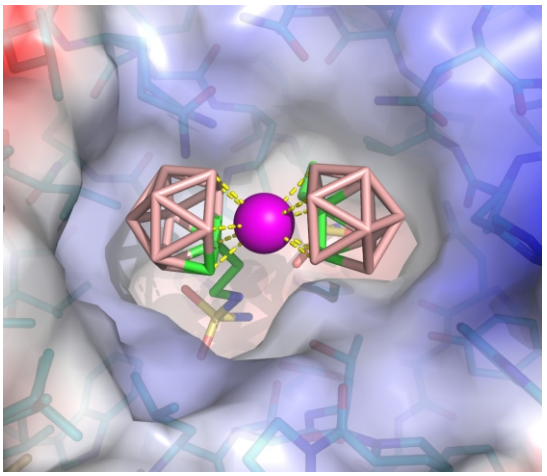

Figure 1. Top view on the 1,1'-dietlylsulfamido substituted cobalt bis(dicarbollide) in the active site of CA-IX enzyme.

tions, exhibit significantly enhanced *in vitro* activities with corresponding Ki values in the range of tenths of pM to several nM. The structure-activity relationship (SAR) observed within a small library of ca. 60 substituted carboranes and metallacarboranes is discussed.

These results are complemented by synchrotron structures of enzyme-inhibitor complexes and by a short overview of pharmacologically relevant factors such as plasma protein binding, cell membrane penetration, and basic results from toxicology and pharmacokinetic studies (mouse model) performed on a panel of the selected inhibitors of CA IX enzymes. Due to promising inhibitory properties, these compounds are thus primarily considered as candidates for drugs applicable in cancer treatment.

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Session III, Tuesday, September 13

L6

NMR AND X-RAYS ARE NOT ENEMIES ANYMORE

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NMR spectroscopy and X-ray crystallography have traditionally been viewed as rivals in three dimensional protein structure elucidation, NMR being less successfull one. The fundamental difference between NMR and X-rays lies is the following: X-rays provides almost direct spatial information about atomic positions, while NMR signal directly encodes frequency of nuclei (more precisely of nuclear spins). After introducing basic physical principles of NMR, the standard pipeline of sample preparation and signal assignment will be explained, with emphasis on description to various useful paramaters that can be extracted without previous structure knowledge, however are very useful in characterizaation of a system under scope. Namely chemical shifts, residual dipolar couplings and paramagnetic relaxation enhancemets. A special attention will be paid to NMR methods for characterization of protein dynamics on various timescales. Specifically the possibility of characterizing the low populated conformational protein states opens a path to a challenging goal of describing protein as a dynamic ensemble of functional states. A real life examples of studies of protein-protein and protein-small molecule interactions will be described and explained.

L7

POZITRONOVÁ ANIHILAČNÍ SPEKTROSKOPIE

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Pozitronová anihilační spektroskopie využívá pozitron jako sondu ke studiu struktury materiálu. V pevných látkách jsou pozitrony anihilovány elektrony a emitované anihilační záření přináší informaci o parametrech anihilačního procesu. Hlavní pozorovatelné jsou doba života pozitronu a Doppleruv posuv energie anihilačních fotonů. Pozitron implantovaný do dokonalého krystalu je delokalizovaný v krystalové mříži a pozitronová hustota má formu modulované rovinné vlny. Defekty krystalické mříže spojené s volným objemem (např. vakance, dislokace, hranice zrn atd.) představují pro pozitron potenciálové jámy a mohou vést k záchytu pozitronu, tj. muže dojít ke vzniku vázaného stavu pozitronu v defektu. Takto zachycené pozitrony mají delší dobu života než pozitrony delokalizované v krystalické mríži. Doba života pozitronu je určena lokální elektronovou hustotou v místě defektu. Každý stav pozitronu v daném materiálu přispívá do spektra dob života pozitronu exponenciální komponentou. Změřením dob života těchto exponenciálních komponent je možné identifikovat typy defektu ve studovaném materiálu. Z intenzit techto komponent lze potom pomocí vhodného modelu určit koncentrace defektu.

V přednášce bude vysvětlen princip pozitronové anihilační spektroskopie a ilustrovány její možnosti na příkladě studia ultra jemnozrnných materiálů připravených silnou plastickou deformací. Budou rovněž zmíněny oblasti kdy se informace získaná pomocí pozitronové anihilacní spektroskopie překrývá s informací o reálné struktuře materiálu získané studiem tvaru rozšíření difrakčních profilů rtg. záření.