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Plenary Lecture to Celebrate the International Year of Crystallography

CHALLENGES OF VIRUS CRYSTALLOGRAPHY

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The crystallographic analysis of crystals of virus particles was begun, in hope, before computers were invented, but the analysis of virus structures at high resolution came much later, after all sorts of technical advances. The technical advances in structural biology continue at a breathtaking rate and viruses still provide challenges that stimulate these advances. I will chart some of the recent developments and indicate what might happen next. I will do this in the context of our attempts to understand whole virus particle structure and function sufficiently deeply that we can, with the aid of the latest technology, and with suitable computational tools, control conformational transitions for therapeutic purposes. To this end we are currently targeting a number of small RNA viruses and I will hope to convince you that we are now at the stage where complete virus particles are excellent targets for rational, structure-guided, drug and vaccine design.

L1

2014 – INTERNATIONAL YEAR OF CRYSTALLOGRAPHY

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The year 2014 has been declared by the United Nations as the International Year of Crystallography (IYCr2014). It commemorates the centennial of the birth of X-ray crystallography, thanks to the work of Max von Laue and William Henry and William Lawrence Bragg. The year 2014 also commemorates the 50th anniversary of another Nobel Prize, that awarded to Dorothy Hodgkin for her work on vitamin B12 and penicillin. One aim of the Year is to promote education and public awareness through a variety of activities. Crystallographers are active in more than 80 countries, 53 of which are members of the International Union of Crystallography. There is a need to broaden the base of crystallography.

Among justification of the declaration of the IYCr by General Assembly of the United Nations (July 3, 2012) we can find for example this:

"Stressing that education about and the application of crystallography are critical in addressing challenges such as diseases and environmental problems, by providing protein and small molecule structures suited for drug design essential for medicine and public health, as well as solutions for plant and soil contamination,

Considering that the impact of crystallography is present everywhere in our daily lives, in modern drug development, nanotechnology and biotechnology, and underpins the development of all new materials, from toothpaste to aeroplane components,

Considering also the significance of the scientific achievements of crystallography, as illustrated by twenty-three Nobel Prizes awarded in the area, and that crystallography is still fertile ground for new and promising fundamental research,

Considering further that 2014 marks the centenary of the beginning of modern crystallography and its identification as the most powerful tool for structure determination of matter,

Recognizing the leading role of the International Union of Crystallography, an adhering body of the International Council for Science, in coordinating and promoting crystallographic activities at the international, regional and national levels around the world."

There many activities during the whole year and around the World (http://www.iycr2014.org/) including the Czech and Slovak Republics (http://www.xray.cz/iycr/). Starting

with opening ceremony in Paris (January 20-21), they often focus on presenting wide scope of modern crystallography going from protein crystallography to materials science. It is important in many different fields – chemistry, physics, and mineralogy. In last years, popular applications can be found in forensic science, archeology, and art (both analysis of work of art and analysis of symmetry in art). The analysis of the so-called real structure of the matter, in particular thin films, that includes crystal lattice defects, strains, residual stresses, textures, lattice misfits etc. is crucial also for technological development.

Recently, the first X-ray diffraction experiment was performed on another planet – Mars and subsequent analysis revealed the presence of clay minerals, in addition to minerals found in basalt. The age of the rocks showed that Mars hosted wet environments more recently that previously thought. The minerals found are compatible with an environment that was potentially habitable to life with near-neutral pH and moderate temperature. The interaction of mantle minerals such as olivine with water and CO_2 produces simple prebiotic organic compounds. H_2 deliberated in these water-rock reactions can act as an energy source for early chemolithotrophic life.

Since the conferences includes many contributions on protein crystallography, this short contribution will be devoted to the IYCr and more to the importance of crystallography as typically interdisciplinary field.

L2

INTERNATIONAL APPRECIATION OF X-RAY CRYSTALLOGRAPHY

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The General Assembly of the United States greatly appreciated the contribution of crystallography to development of the mankind at its 121st Plenary Meeting. In the Resolution 66/284 adopted on July 3, 2012, the General Assembly proclaimed that the scientific field of crystallography substantially changed the quality of life of the whole mankind in the last century and still has a tremendous potential for future generations. It proclaimed the year 2014 as the Year of crystallography (**IYCr)** and asked the member states to support further development of this fascination science.

The International Union for Crystallography (**IUCr)** as a member of the International Council of Scientific Unions (**ICSU**) was invited to organize actions to support the related activities in 2014 (www.iycr2014.org). Czechoslovakia was in 1948 one of the five founding members of the IUCr. Therefore the Czech and Slovak Crystallographic Association and the Czech and Slovak National Committees of the IUCr take the responsibility to organize the actions in our country (http://www.xray.cz).

One of the main reasons for the proclamation of the International Year of Crystallography was an unusually high number of Nobel prices in crystallography. The IUPAC Commission recognized 23 Nobel prices as prices uniquely related to crystallography. The commission for Nobel prices was first established by the Swedish Academy of Sciences in 1901. The first price in the category physics was awarded to W.C.Röntgen. In the next 113 years, 29 Nobel prices were closely related to crystallography.

My search through the history of Nobel prices identified ***11 prices*** awarded directly for the ***Development of the Diffraction Methods*** and development of necessary instrumentation, other ***7 prices*** were closely related to the ***Applications of Diffraction Methods in Chemistry, and 11 Nobel prices*** were for ***Applications of Diffraction Methods in Structure Biology.***

The results of the search are summarized in Table 1. The Table 2 shows the frequencies of Nobel prices related to crystallography summarized each twenty years since 1901. The 20 years rates of Nobel prices plotted in Figure 1 show a great development of diffraction methods in the first half of 20 century and the new revival in the last 40 years. The methodical development induced continuous not ending sequence of new discoveries in chemistry and biological sciences. The frequency of all Nobel prices related to crystallography (full black line) still grows and nowadays one can generalize that new ***Nobel prices related to crystallography can be expected each two or three years***. This is the rationale of the message contained in the Resolution of the General Assembly, that crystallography

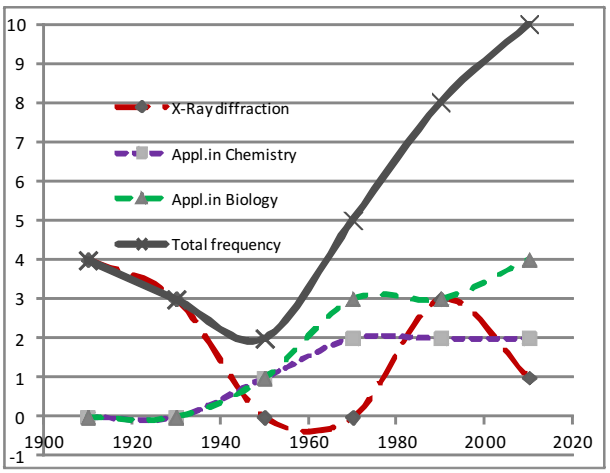

Figure 1. Plots of the 20 years frequencies of Nobel prices related to crystallography. The last point of the "Total frequency line" was linearly extrapolated from 13 years average to the 20 years average.

Table 1. List of Nobel prices closely related to crystallography sorted by the year of approval.

2013 Chemistry M. Karplus, M. Levitt, and A. Warshel DM Appl.in Chemistry
Development of multi-scale models for complex chemical systems

2012 Chemistry R. J. Lefkowitz and B. K. Kobilka DM Appl.in St.Biology
Studies of G-protein-coupled receptors

2011 Chemistry D. Shechtman Diffraction methods
Discovery of quasicrystals

2010 Physics A. Geim and K. Novoselov DM Appl.in Chemistry
Experiments regarding the two-dimensional material grapheme

2009 Chemistry V. Ramakrishnan, T.A. Steitz and A. E.Yonath DM Appl.in St.Biology
Studies of the structure and function of the ribosome

2006 Chemistry R. D. Kornberg DM Appl.in St.Biology
Studies of the molecular basis of eukaryotic transcription

2003 Chemistry P. Agre and R. MacKinnon DM Appl.in St.Biology
Discoveries concerning channels in cell membranes

1997 Chemistry P. D. Boyer, J. E. Walker and J. C. Skou DM Appl.in St.Biology
Elucidation of the enzymatic mechanism of ATP synthesis and of ion-transporting enzyme

1996 Chemistry R.Curl, H. Kroto and R. Smalley DM Appl.in Chemistry
Discovery of the fullerene form of carbon

1994 Physics C. Shull and N. Brockhouse Diffraction methods
Neutron diffraction

1992 Physics G. Charpak Diffraction methods
Discovery of the multi wire proportional chamber

1991 Physics P.-G. de Gennes DM Appl.in Chemistry
Methods of discovering order in simple systems can be applied to polymers and liquid crystals

1988 Chemistry J. Deisenhofer, R. Huber and H. Michel DM Appl.in St.Biology
Determination of the three-dimensional structure of a photosynthetic reaction centre

1985 Chemistry H. Hauptman and J. Karle Diffraction methods
Development of direct methods for the determination of crystal structures

1982 Chemistry A. Klug DM Appl.in St.Biology
Development of crystallographic electron microscopy and the structure of NA-protein complexes

1976 Chemistry W. N. Lipscomb DM Appl.in Chemistry
Structure of boranes

1972 Chemistry C. B. Anfinsen DM Appl.in St.Biology
Folding of protein chains

1964 Chemistry D. Hodgkin DM Appl.in St.Biology
Structure of many biochemical substances including Vitamin B12

1962 Physiology or Medicine F. Crick, J. Watson and M. Wilkins DM Appl.in St.Biology
Helical structure of DNA

1962 Chemistry J. C. Kendrew and M. Perutz DM Appl.in St.Biology
Studies of the structures of globular proteins

Table 1. List of Nobel prices closely related to crystallography sorted by the year of approval (continuation).

1954 Chemistry L. C. Pauling — DM Appl.in Chemistry
Nature of chemical bond and structure elucidation of complex substances

1946 Chemistry J. B. Sumner — DM Appl.in St.Biology
Discover, that enzymes can be crystallized

1937 Physics C. J. Davisson and G. Thompson — Diffraction methods
Experimental discovery of the diffraction of electrons by crystals

1936 Chemistry P. J. W. Debye — Diffraction methods
Investigations on dipole moments and X-ray and electron diffraction in gases

1929 Physics L.-V. de Broglie — Diffraction methods
The wave nature of the electron

1917 Physics C. G. Barkla — Diffraction methods
Discovery of the characteristic Röntgen radiation by some elements

1915 Physics W. H. Bragg and W. L. Bragg — Diffraction methods
The use of X-rays to determine crystal structure

1914 Physics M. Von Laue — Diffraction methods
Diffraction of X-rays by crystals

1901 Physics W. C. Röntgen — Diffraction methods
Discovery of X-rays

has still a high potential for future generation of young scientists and better life of mankind.

The contribution gives also a short review of the 54 years of Macromolecular Crystallography, shows backgrounds of several studies awarded by Nobel price, and gives some estimates of potential fields where the next Nobel prices might be expected.

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Table 2. The 20 years frequencies of Nobel prices related to crystallography. The last point of the "Total frequency line" was extrapolated from the 13 years average to the 20 years average.

Year	Diffraction	Chemistry	Biology	Total
1900-1920	4	0	0	4
1920-1940	3	0	0	3
1940-1960	0	1	1	2
1960-1980	0	2	3	5
1980-2000	3	2	3	8
2000-2013	1	2	4	7
Total	11	7	11	29

L3

USER CONSORTIUM OF SERIAL FEMTOSECOND CRYSTALLOGRAPHY - SLOVAK INVOLVEMENT

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Structure determination at synchrotrons often follows years of tremendous effort to grow large protein crystals that are well enough ordered to diffract to high resolution within the limited exposure to avoid radiation damage. There are no guarantees that the crystal observed under the polarizing microscope, and which appears to be a "good" crystal, will actually diffract.

X-ray free-electron lasers are opening up unique opportunities to image biological materials at high resolution. The high-intensity ultra-short pulses provided by these sources enable us to overcome radiation damage limits by outrunning the structural degradation that inevitably occurs on exposure of soft matter to ionizing radiation [1]. The experience at LCLS (Linac Coherent Light Source, Stanford, USA) showed that most nano- and microcrystalline protein samples that have been tried have diffracted, and usually to higher resolution than has been observed from synchrotron studies on macroscopic crystals, in test cases where these large crystals were available. High-resolution diffraction has been obtained from integral membrane proteins, including G-protein coupled receptors, in the form of sub-micron crystals carried in a lipidic cubic phase matrix. LCLS produces pulses of X-rays more than a billion times brighter than the synchrotron sources which are also based on large electron accelerators.

X-ray free-electron lasers are opening up unique opportunities to image biological materials at high resolution. The high-intensity ultra-short pulses provided by these sources enable us to overcome radiation damage limits by outrunning the structural degradation that inevitably occurs on exposure of soft matter to ionizing radiation [1]. This central idea makes many new techniques possible for the study of reproducible (and quasi-reproducible) structures such as macromolecules and viruses. These methods include single-particle diffractive imaging of viruses [2], and the extension of protein crystallography to smaller and smaller crystal sizes (avoiding the years of development often required to grow large crystals) [3]. All current evidence points to the fact that serial femtosecond crystallography (SFX) could have a remarkable and profound impact on the field of structural biology. Not only does SFX overcome the crystallization bottleneck, but data can be collected very rapidly (several minutes to collect about 10 000 images for complete 3D atomistic reconstruction) and over a wide range of temperatures. Indeed, measurements can most easily be carried out at room temperature. In conventional synchrotron-based macromolecular crystallography, samples are usually frozen to reduce radiation damage, and the crystallization process usually, but not always, allows only a single protein conformation to be studied. The results from other techniques, such as cryo-electron microscopy and atomic force microscopy, make it increasingly clear that this shortcoming of MX is limiting our view of protein interactions. Recent macromolecular crystallography studies at room temperature has shown that flash cooling to reduce radiation damage can bias hidden structural ensembles in protein crystals and remodel the conformational distribution of 35% of side-chains, while eliminating the packing defects necessary for functional motions. Thus room temperature measurements can reveal motions crucial for catalysis, ligand binding and allosteric regulation [4].

SFX technique is also a natural fit for time-resolved measurements at femtosecond timescales. Despite valuable progress in the time-resolved protein crystallography, what is urgently needed is a time-resolved technique which can image individual proteins at sub-nanometer resolution in three dimensions, in their native environment, unaffected by damage from the imaging radiation. Only this technique offers this possibility.

The European XFEL brings a unique capability of over a 200 increase in pulse repetition rate compared with the LCLS, which will vastly increase the efficiency of the method, reducing the time required to carry out a measurement and reducing the quantity of protein required to obtain a structure. The potential user community for SFX is extremely large, and encompasses much of the user base for synchrotron macromolecular crystallography stations around the world. In fact the user base is potentially much larger than that, since this method vastly increases the number of samples that now can be analyzed. Taken together, X-ray FELs have the potential to profoundly impact the field of structural biology. The Slovak scientists are ready to participate in this exciting initiative. There is a plan for the structural biology community participation, specifically to set up a protein quality pre-screening centre at IMB SAS. Further Slovak involvement in User consortium of SFX at XFEL experimental station in Hamburg will be discussed.

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L4

METHODS OF STRUCTURAL BIOLOGY OF INSTRUCT AVAILABLE TO CZECH RESEARCHERS

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The membership of the Czech Republic in the European infrastructure for integrative structural biology Instruct brings previously unprecedented possibilities for Czech structural biologists as for access to the cutting edge technologies and expertise in the field. Instruct centres provide open access based on peer-reviewed application process to four major groups of methods and technologies: Sample preparation, Preliminary characterization, Structural analysis and Data analysis.

Sample preparation platforms enable more complicated or challenging projects with necessity e.g. of mammalian or baculovirus expression to be performed in an environment which enables wider screening of expression constructs and conditions. For research groups lacking characterization techniques several centres providing access to different characterization techniques, such as analytical ultracentrifugation, calorimetry, circular dichroism, surface plasmon resonance and other are available. These techniques can be directly used in connection with some protein expression platforms to ease the process of target molecules production in a desired state.

The major group of offered technologies covers all the currently available structure analysis techniques for biological molecules, including microscopy techniques, X-ray crystallography, solution and solid state NMR, mass spectroscopy techniques, electron paramagnetic resonance, and advanced mass spectrometry approaches.

A simple application is required to be submitted for each project. After evaluation and depending on its result access is granted to perform the required experiments at the requested centres of Instruct. Partial coverage of expenses on the side of the applicant is expected.

Detailed information about Instruct activities, calls for projects, the individual centres and their technologies can be found at http://www.structuralbiology.eu.

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