Bio-QCr: Employing the Full Potential of 3D Electron Diffraction in Macromolecular Crystallography Using Quantum Crystallography

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Project goal. Each living entity is made up of proteins, nucleic acids, lipids, carbohydrates, metabolites, and water. These molecules, although made of a limited set of atoms, differ in the number of atoms and the way in which these atoms connect to create a three-dimensional structure. The aim of the project is to improve experimental determination of 3D structures of biological macromolecules. Knowing the atomic and electronic structure of biomacromolecules is very important for understanding their function.

Three-dimensional electron diffraction (3DED, microED) enables the determination of the structure of macromolecules at the near atomic or atomic resolution basing on crystals smaller than 1 μm. 3DED is an emerging new technique in structural biology, offering unique capabilities comparing to traditional X-ray diffraction (XRD) and novel single-particle cryogenic electron microscopy (cryoEM). However, the analysis of 3DED data is complicated and it still poses a number of challenges. One of them is the possibility to include in the analysis the effect of interactions between the atoms in the macromolecules. This part of structural analysis belongs to the area of quantum crystallography (QCr). The goal of this project is to deeply integrate the world of QCr with structural biology, and make it possible to access the quantum-crystallographic information obtained from very small crystals of biomacromolecules.

Reasons for attempting a particular research topic. Accurate structural descriptions of biomacromolecules are essential for advancing our knowledge of biology, medicine, biotechnology and agriculture. They are crucial to understand the processes of life at the level of single molecules and atoms. They find applications in structure-function correlation studies; elucidation of mechanisms of cellular processes, enzymatic reactions, and signaling pathways; rational drug design and developments; design enzymes for industrial purposes, *etc*.

The essence of 3DED, XRD or cryoEM experiments is to observe how a beam of electrons or X-rays scatters on the tested sample. By analyzing the obtained scattering image (diffraction in the case of crystals), we can reconstruct how the atoms of our sample were arranged in space. To proper analyze the image, we need to model how each atom scatter the beam. We call these models "scattering factors". The QCr approaches have been used for many years in X-ray crystallography to, among others, provide better models of scattering factors. Aspherical atom scattering factors were introduced to XRD replacing the conventional spherical independent atom model (IAM) of scattering. Also in electron crystallography focusing on small molecules, aspherical approach named TAAM, based on multipolar representation of electron density and MATTS databank, was recently tested with promising results.

Electron scattering is much more sensitive to the effects of redistribution of atoms' electron density between interacting atoms than X-ray. This is because the electrons interact with sample's electrostatic potential, which depends on a delicate balance between the negative charge of the atom's electron density and the positive charge of the atom's nucleus, unlike X-rays interacting only with electron density. The sensitivity is most apparent at low scattering angles (in low resolution ranges). Diffraction experiments for macromolecular crystal probe the low angle scattering much better than for small molecules. We hypothesize that combination of aspherical scattering factors with 3DED data for biomacromolecules leads to better quality of structural information, and for 3DED data of exceptionally high quality it is even possible to extract from them quantitative information about the fine details of electrostatic potential of biomacromolecules.

Description of research. We will implement aspherical scattering factors approach into commonly used biomacromolecular crystallographic software in order to perform refinements on large amounts of biomacromolecular diffraction data of varying resolution and quality, taking advantage of other methodologies supporting biomacromolecular refinements developed over the years and already implemented. We will quantify the extent to which aspherical scattering models are superior to the IAM for biomacromolecular crystallography and define their areas of applicability. We will answer the question whether currently available experimental 3DED data show greater sensitivity to scattering models than XRD. We also plan to collect new 3DED data and apply the developed methods to interesting scientific problems like mechanisms of photosynthesis, in particular electron transfer in ferredoxins or light energy transfer via chlorophyll in antenna complexes.

Substantial results expected. As a result, we will broadly open standard biomacromolecular 3DED and XRD crystallographic experiments to QCr approaches and take standard biomacromolecular crystallography from the level of mere analysis of atomic connectivity to a higher level of assessment of the electrostatic properties and electronic structure of biomacromolecules. The project will significantly affect the entire life sciences.