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# Synchrotron and ligand binding studies in drug design

# Význam synchrotronových měření a vazby ligandů v návrhu léčiv

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**Summary**

Structural studies of macromolecular interactions performed at synchrotron radiation sources represent the key experiment in rational drug design. Here, two case studies are reported: glutaminyl cyclase from *Drosophila melanogaster* and mutant variants of bovine cationic trypsin.

**Souhrn**

Strukturní studie makromolekulárních interakcí na zdrojích synchrotronového záření představují klíčový experiment v procesu návrhu nových léčiv. Tato práce se zabývá dvěma případy analýzy interakcí na modelových systémech: glutaminyl cyklasou z octomilky obecné a mutovanou formou hovězího trypsinu.

**Klíčová slova**

Rentgenové záření, synchrotron, makromolekula, ligand

**Key words**

X-ray, synchrotron, macromolecule, ligand

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# 1 Introduction

Macromolecular crystallography plays an irreplaceable role in modern design and development of novel drugs. The method enables visualization of macromolecules in atomic details. Concepts of experimental methods utilize observation of binding modes of small-molecule ligands and of potential structural changes induced by the macromolecule:ligand interaction.

The whole process of crystallographic structure analysis of a macromolecular sample consists of crystallization, X-ray diffraction experiment, data processing, structure determination, refinement, and structure validation. Although the method in general is very robust, current development of experimental approaches and improvement of instrumentation, mainly X-ray detectors, leads to a broadened spectrum of applications.

The crucial step in the structure determination process is crystallization of a macromolecule. Availability of high resolution diffracting crystals with an accessible active site is important for structure-based drug design. In some cases, searching for several crystallization conditions is necessary (see Section 2.1).

In many aspects, modern in-house X-ray generators have almost reached the capacity of synchrotron radiation sources. However, the difference between them and the most brilliant synchrotron beamlines remains remarkable. Standard in-house data collection lasts several hours, whereas the usual data collection on the P13 beamline at PetraIII (Hamburg, Germany) lasts several minutes. The speed of the

measurement allows fast data collection of tens to hundreds of macromolecule:ligand interactions. Today, the diffraction measurement is usually much shorter than the evaluation of the experimental data. Ligand misinterpretation is also not an uncommon feature.

A major disadvantage of in-house X-ray generators is the fixed wavelength of the primary beam. Tunability at synchrotron sources is frequently used for experimental phasing leading to structure determination. This case is not discussed in the thesis.

One of the most promising methods of discovering new medications is fragment-based drug discovery. It is based on testing and searching for interactions between the library of small chemical fragments and a macromolecule. Structural knowledge at high resolution allows synthesis of new and more complex drugs that are composed of a combination of observed and chemically linked fragments that bind with a higher affinity.

Current developments of the experiments and data processing methods utilize marginal advances in observation and evaluation of ligand binding. However, the state-of-the-art approaches are not optimal yet and still offer enough opportunity for further improvement (see Chapter 3).

# 2 Experimental section

Current studies provide evidence for an involvement of glutaminyl cyclase in Alzheimer's disease. Orally available inhibitors of coagulation factor Xa represent therapeutics regulating the blood hemostasis system.

## 2.1 Glutaminyl cyclase

Human glutaminyl cyclase (QC) catalyzes the formation of pyroglutamic acid at the N-terminus of a variety of peptides and proteins. A number of studies has indicated its potential role in Alzheimer's disease. To fully understand the molecular mechanism of QC catalysis, structures of the enzyme from various organisms were determined.

In our case, crystal structures of two QC species from *Drosophila melanogaster* (*Dm*) – fruit fly model – in complex with the high potent inhibitor PBD150 were published first (Koch, et al., 2012). To further increase the impact of the model system, the crystal structure of *Dm*QC in space group *I*4 was published (Kolenko, et al., 2013). Altogether, three various crystallization conditions leading to high quality diffracting crystals are available for testing, analysis, and validation of novel QC inhibitors. All three crystallization conditions lead to various accessibility of the active site (see Table 1, Figure 1). This may result in elimination of some candidates for the structural studies.

**Table 1:** Crystal forms of *Dm*QC.

| PDB code | Space group | Active site accessibility |
|---|---|---|
| 4F9U | $P2_1$ | fully available |
| 4F9V | $P6_5$ | restricted by symmetry related molecule |
| 4FWU | $I4$ | partially restricted |

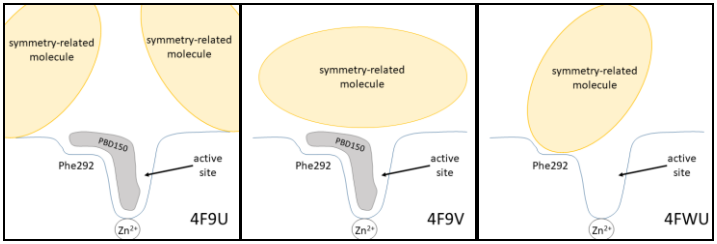


**Figure 1:** Schematic representation of the accessibility of the active site of individual crystal forms of *Dm*QC (PDB codes at the right bottom).

## 2.2 Inhibition of mutant variants of bovine cationic trypsin

Prediction of ligand affinity based on structural knowledge is difficult for the myriad of competing microscopic processes that contribute to ligand:macromolecule interactions. We analyzed interactions of a series of inhibitors with four mutant variants of bovine cationic trypsin onto which the ligand-binding site of factor Xa has been grafted (Tziridis, et al., 2014). Two major motivations for the research were: factor Xa is the target molecule for the design of drugs regulating the blood hemostasis system, *e.g.* treatment of thrombosis, and in general, grafting of an active site on a crystallizable structure homologue is a method to use in case of a non-crystallizable target molecule.

The overall understanding of ligand:macromolecule interactions requires extensive experimental analysis. We determined 21 crystal structures of various inhibitor:mutant combinations. Despite conservative mutations of two residues that are not directly contributing to the inhibitor:macromolecule interaction, significant differences in inhibitor potency towards the mutant variants were observed. These observations show that our understanding of the binding process and its conversion to computational estimation remains incomplete. Our data should contribute to fine-tuning of computational methods in drug design and highlight the importance of experimental approaches despite the significant improvement of current computational protocols.

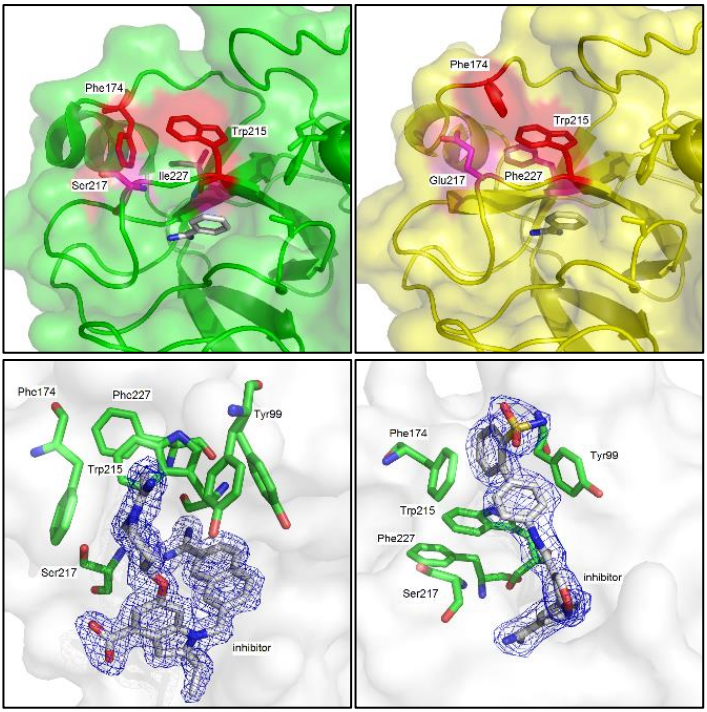


**Figure 2:** Detailed view of the active site and ligand:macromolecule interactions. The loop containing residue Phe174 may adopt *up* (left upper panel - PDB code 3PLB) or *down* (right upper panel – PDB code 3UY9) conformation. Experimental electron densities (2m$F_o$-D$F_c$ at 1σ, blue) of the two cationic bovine trypsin inhibitors (lower left panel - PDB code 3UPE; lower right panel – PDB code 3UQV).

# 3 Future prospectives and plans

In the near future, X-ray crystallography will still play the key role in rational drug design. The general preciseness of individual atom position determination cannot be recently reached by any other method of structural biology. Moreover, continuous development of instrumentation and experimental methods facilitates more precise and more sensitive observation of weak ligand binding. Additionally, continual development of crystallization methods can be expected.

Not only experimental methods are under current development. Improved protocols for data processing and evaluation are available (Sauter, Hattne, Grosse-Kunstleve, & Echols, 2013). Researchers tend to use improved criteria for high resolution data cut-off (Karplus & Diedrichs, 2012). Moreover, open-access data archives provide loads of raw experimental data for potential reevaluation (Editorial, 2016). Apparently, further automation in data processing and validation can be expected.

Concerning ligands, new computational methods, *e.g.* polder map (Liebschner, et al., 2017), enable observation and validation of weaker binding and/or low occupied small-molecule ligands. Such progress in experimental observation can be seen in Figure 3 (unpublished results).

Observation of weak ligand binding represents a great challenge for improvement in macromolecular crystallography. In case of received funding, I propose the development of a *composite polder map* that would reflect weak ligand binding around the whole macromolecule, not

only close to a specific site as it is available in current protocols.

Another promising method of ligand validation is PanDDA that combines multiple observation of a ground state and ligand bound states (Pearce, et al., 2017).

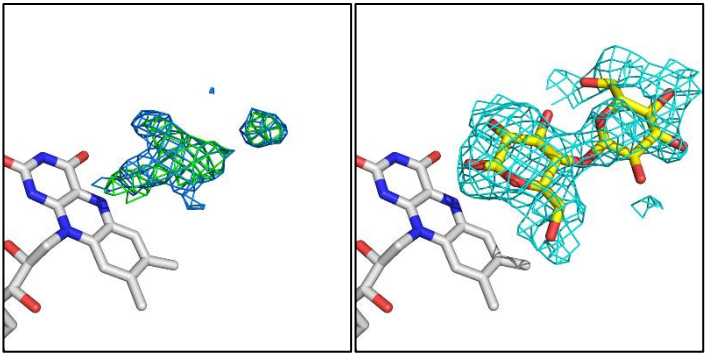

**Figure 3:** Differences between the standard electron density map calculation (left panel, 2mFo-DFc in blue, mFo-DFc in green) and polder map calculation (right panel, polder map in cyan). Standard protocols did not reveal weak binding of a ligand into the active site, whereas polder map strengthened the observation to identify the interaction (unpublished results).

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## 5.2 List of publications

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