Macromolecular Crystallography in the Laboratory

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Introduction

Macromolecular crystallography (MX) is the most powerful method for determining the three-dimensional structures of biological macromolecules. It tends to achieve the highest-resolution information and gives most reliable structures, while not suffering from limitations on sample size - as long as crystals are available. Most diffraction data are collected at synchrotron beamlines with high photon flux, powerful automation, fast detectors, expert processing pipelines and highly experienced staff.

These days, nearly two thirds of all structures are determined with PILATUS and EIGER HPC detectors (Figure 1). Their advantageous properties, such as noise-free readout and maintenance-free operation, make these detectors attractive to academic laboratories as well. In many cases, laboratory diffractometers equipped with PILATUS and EIGER detectors are useful for much more than optimizing crystallization and cryo-cooling conditions before data collection at a synchrotron. This is particularly true for drug design projects that require a quick turn-around in response to newly synthesized compounds and for structural enzymology work where data on mutants are used to create new mutations to probe the active site. Here we show recent results in these two fields of structural biology.

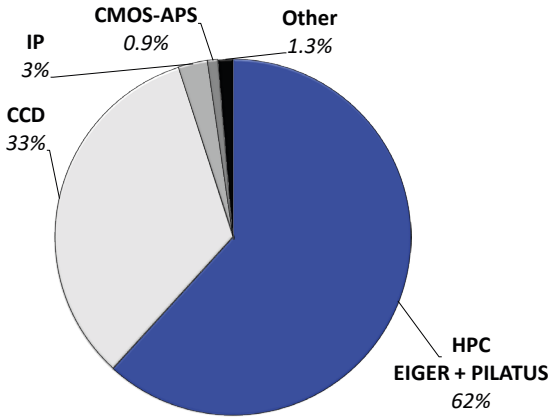

Figure 1. Contribution of detectors to PDB entries released in 2019. Nearly two thirds of all structures were solved with data collected with PILATUS and EIGER HPC detectors. Legacy detectors based on CCDs and image plates (IP) make up most of the rest. Competing technology such as CMOS-APS plays no role.

Application Note

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Application Note

Drug Development

Rational design of antisickling agents

Sickle cell disease is a hereditary blood disorder associated with anemia, infections, episodes of pain, and delayed growth and development. In patients, red blood cells are deformed into the shape of a sickle. The disease is caused by mutations in the gene coding for beta-globin, one the two components of the hemoglobin tetramer. The laboratory of Prof. Martin Safo at Virginia Commonwealth University studies the structure-function relationship of hemoglobin with the goal of developing antisickling agents.

In tight collaboration with crystallographers, medicinal chemists design ligands that are tested for their pharmacology *in vivo* and *in vitro*. Structures of hemoglobin cocrystallized with promising candidates are quickly solved from diffraction data collected with Prof. Safo's EIGER R 4M detector (Figure 2). Being able to react quickly to new information is one of the key advantages of having medicinal chemistry and structural biology in close proximity. Instead of waiting for synchrotron time, Prof. Safo's group can solve structures in a couple of days.

References and published structures

1. T. M. Deshpande *et al., Acta Cryst D.* **74**, 956–964 (2018), doi:10.1107/S2059798318009919.

2. A. Nakagawa *et al., Molecular Pharmaceutics.* **15**, 1954–1963 (2018), doi:10.1021/acs.molpharmaceut.8b00108.

3. P. P. Pagare *et al., Bioorganic & Medicinal Chemistry.* **26**, 2530–2538 (2018), doi:10.1016/j.bmc.2018.04.015.

6DI4, 6BNR, 6BWP, 6BWU (EIGER R 4M).

Structural biology in the pharmaceutical industry

Powerful laboratory diffractometers are essential for pharmaceutical research, development and quality control during production. They allow for all aspects of the experiment to be under full control and for decisions to be made quickly. Among others, Astex Pharmaceutical in the UK, Array Biopharma in the US, and Novo Nordisk in Denmark are all productive with state-of-the-art diffractometers with EIGER R 1M or PILATUS3 R 1M detectors.

References and published structures

1. J. B. Fell *et al., ACS Med. Chem. Lett.* **9**, 1230–1234 (2018), doi:10.1021/acsmedchemlett.8b00382.

2. T. D. Heightman *et al., J. Med. Chem.* **62**, 4683–4702 (2019), doi:10.1021/acs.jmedchem.9b00279.

3. A. M. Kidger *et al., Molecular Cancer Therapeutics,* online first (2019), doi:10.1158/1535-7163.MCT-19-0505.

4. A. Oddo *et al., Biochemistry.* **57**, 4148–4154 (2018), doi:10.1021/acs.biochem.8b00105.

6N2K, 6N2J, 5OTX, 5OTW, 5OTV, 5OTU (PILATUS3 R 1M)
6QMC, 6QMK (EIGER R 1M)

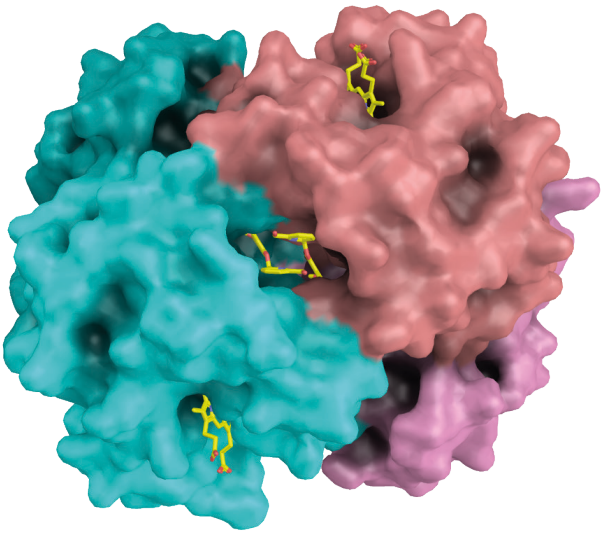

Figure 2: The antisickling agent SAJ-009 binds at the interface between two alpha-globin chains. The alpha-globins are shown in cyan and salmon. Heme groups bind to pockets in the alpha-globin chains (top right and bottom left).

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Fighting Trypanosoma diseases

Sleeping sickness, Chagas' disease, and leishmaniasis pose serious health problems in developing countries. All three diseases are caused by trypanosomes, protozoans characterized by a unique trypanothione redox system. Prof. Emil Pai's laboratory at the University of Toronto studies trypanothione reductase inhibitors, an attractive class of ligands inactive against organisms with conventional glutathione redox systems.

Starting from a previously reported structure, Prof. Pai's group developed the strongest competitive trypanothione reductase inhibitor reported to date. The new inhibitor binds into a large active site that is simultaneously occupied by a buffer molecule retained from protein purification (Figure 3). Thanks to the small pixels and the single-pixel point spread of EIGER R 1M, data collection was straightforward despite the large unit cell of the crystal (118 x 118 x 225 Å^3). Combining the two ligands into one even more tightly binding lead compound is an attractive goal for further research.

References and published structures

1. R. De Gasparo *et al., Chemistry – A European Journal.* **25**, 11416–11421 (2019), doi:10.1002/chem.201901664.

6OEZ (EIGER R 1M)

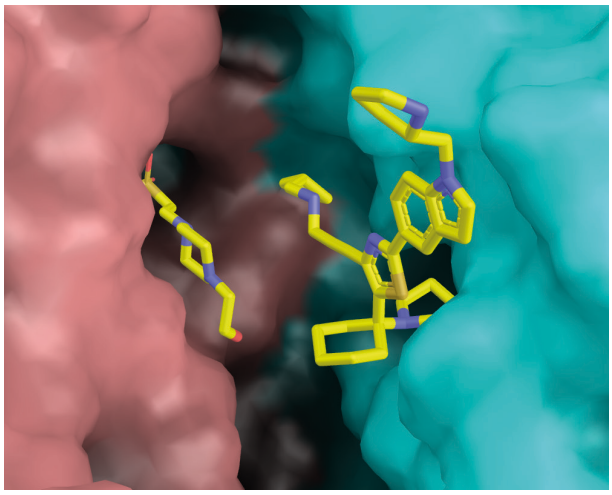

Figure 3: Ligand (+)-2 binds at the dimer interface of trypanothione reductase (TR). The two TR protomers are shown in salmon and cyan, respectively. On the left of the binding pocket, an accidentally bound HEPES molecule can be seen.

Structural Enzymology

Catalytic mechanism of L-asparaginases

L-asparaginases are enzymes that catalyze the hydrolysis of the amino acid asparagine to aspartate. They are clinically important for the treatment of selected leukemias and lymphomas. Dr. Alexander Wlodawer's laboratory at the National Cancer Institute in Frederick studies the catalytic mechanism of L-asparaginases.

Understanding of the mechanism depends on the correct interpretation of all components of the active site. Half a dozen crystal structures determined in Dr. Wlodawer's laboratory from data collected on EIGER R 4M detectors show how earlier structures were misinterpreted as containing native ligands when the observed electron density can be explained better with an extraneous ligand that bound to the enzyme during crystallization (Figure 4).

References and published structures

1. J. Lubkowski *et al., Protein Science.* **28**, 1850–1864 (2019), doi:10.1002/pro.3709.

2. J. Lubkowski *et al., Scientific Reports.* **9** (2019), doi:10.1038/s41598-019-46432-0.

6PA2, 6PA4, 6PA5, 6PA6, 6PA8, 6PA9, 6PAA, 6PAB, 6PAC, 6PAE, 6NX6, 6NX7, 6NX8, 6NX9, 6NXA, 6NXB (EIGER R 4M).

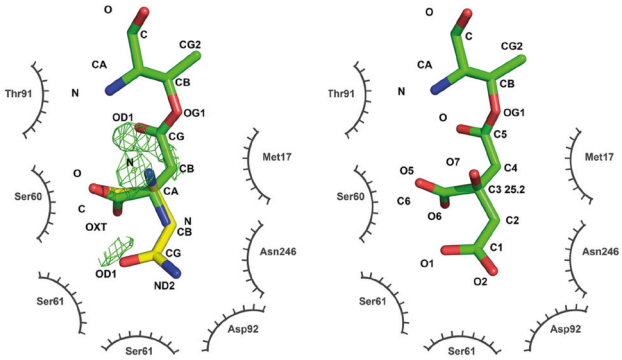

Figure 4: Active site of L-asparaginase. An older structure (PDB code 2HIM, left) was interpreted as showing aspartate covalently bound to threonine 14 (green, catalytic intermediate) with a substrate asparagine overlaid (yellow). Difference density indicates problems with the model. The new structure (PDB code 6NXC, right) reinterprets the ligand density as a covalently bound citrate from the crystallization buffer. No difference density remains after refinement. Figure modified from Lubkowski et al. 2019 (Reference 2, CC BY 4.0).

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Catalytic mechanism of nitrite reductases

The group of Dr. Svetlana Antonyuk and Prof. Samar Hasnain (Figure 5) at the University of Liverpool has long studied the chemistry of nitrite reductases. These proton-coupled redox enzymes contain multiple metal centers and are thus susceptible to radiation damage.

Recent work in the lab combines experiments at a variety of radiation sources to elucidate the catalytic pathway of nitrite reductases. Of particular importance were experiments at an X-ray free electron laser to show the undamaged active site, at a neutron source to show the protonation states of the catalytic residues, and at the laboratory diffractometer to determine the resting states at different pH values.

References and published structures

1. T. P. Halsted *et al., IUCrJ.* **6**, 761–772 (2019), doi:10.1107/S2052252519008285.

2. T. M. Hedison *et al., ACS Catalysis.* **9**, 6087–6099 (2019), doi:10.1021/acscatal.9b01266.

6GTL, 6GTN, 6GTI, 6GTK, 6QPU (EIGER R 4M).

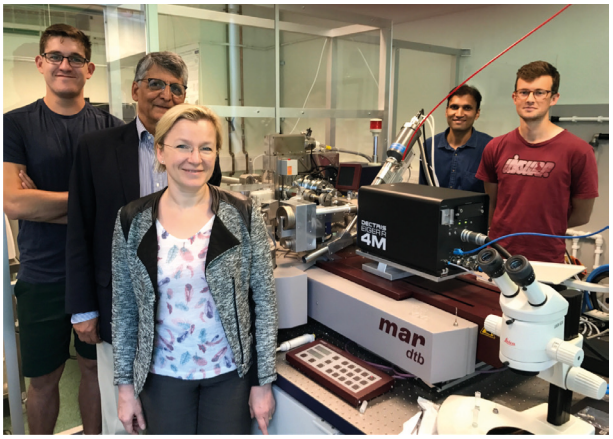

Figure 5: The group of Dr. Antonyuk and Prof. Hasnain in front of their upgraded diffractometer. The EIGER R 4M is mounted on a mardtb (marXperts, Germany).

Conclusion

Detector upgrades have helped academic and industrial laboratories answer biological questions more rapidly and make better use of their synchrotron time. A diffractometer with a state-of-the-art detector is an excellent tool for quickly assessing ligand binding in drug discovery projects and determining the influence of individual side chains on the chemistry of an enzyme. You can solve structures by native SAD and judge the effects of pH and temperature changes on your structure. A modern diffractometer is also indispensable for the training of the next generation of crystallographers.

A laboratory diffractometer can be much more than a screening tool for finding best crystallization and cryo-protection conditions. You can obtain publication-quality structures in the time it takes to send crystals to a synchrotron. This will be especially important in the next few years when many synchrotrons undergo upgrade projects with extended shutdown periods. Accelerate your crystallography by upgrading your existing source with a new detector or by getting a new diffractometer. One possible partner is marXperts, who upgraded the diffractometer in Liverpool with an EIGER R 4M detector (Figure 5).

Application Note

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