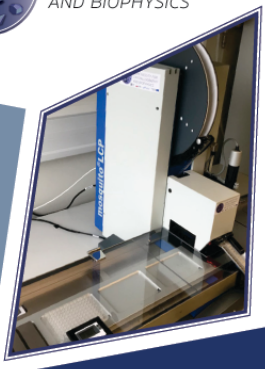
UNIWERSYTET WARSZAWSKI

CORE FACILITY FOR CRYSTALLOGRAPHY AND BIOPHYSICS

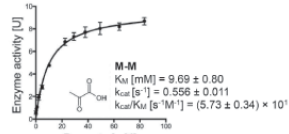
The Core Facility for Crystallography and Biophysics (CFCB) at the Biological and Chemical Research Centre, University of Warsaw was established by the project "Core facility for crystallographic and biophysical research to support the development of medicinal products" funded by the TEAM-TECH Core Facility programme from the Foundation for Polish Science, under the supervision of Prof. dr hab. Krzysztof Woźniak (crystal.chem.uw.edu.pl) and dr Jan Kutner (Deputy Manager).

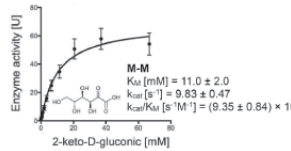


The mission of the CFCB is focused on analysis of proteins and small chemical copounds leading to crystallization trials for academic and commercial users. The CFCB will enable the study of challenging biochemical and pharmaceutical problems, with emphasis on drug development and collaborations with the research groups and companies.

The equipment available in CFCB can be used in a variety of advanced analyses and methods, in order to help with research projects requiring structural information. Some methods are more suited to the type of target molecules: proteins or small molecule compounds, belonging to the "BIO" or "CHEM" pipelines, respectively, whereas others can be used for both biological and chemical research (e.g. in house X-ray diffractometers). Most methods are used as stand-alone services with a separate dedicated report to the customer, or combined into a larger comprehensive service. In both cases please contact as by e-mail or by offer request form.

Biomacromolecules and complexes ("BIO" pipeline)	
Method or service	**Report to Customer**
Ligand and Protein Binding Assays	
MicroScale Thermophoresis (MST) - Ligand or protein partner binding *Thermo Fluor Assay* (TSA) - Buffer and additive selection - Ligand binding assay *Enzymatic activity assays and readouts* - Protein activity or concentration - Ligand binding *SEC-MALLS* - Protein partner binding - Protein-detergent complexes	Best protein construct Suggested buffer for sample stabilization Suggested Ligand Choice Suggested Detergent Ligand or protein partner Binding (K_d) Protein activity (V_{max}, K_m, k_{cat}…)
Quality Check and Optimization	
SDS-PAGE analysis (1 D or 2 D) *Spectrophotometer plate reader* *Dynamic Light Scattering* - Protein sample dispersity	Concentration Purity Homogeneity
SEC-MALLS - Aggregation assay - Oligomerization state assay	Molecular weight estimate Homogeneity Complex formation
Crystallization Trials	
Initial screening and optimization Custom or commercial condition sets Hanging/sitting drop, sandwich plates, LCP Incubation at different temperatures Additive screens Seeding Soaking with heavy atoms for phasing *Customer-provided compound library* Soaking or co-crystallization *Visualization and crystallization drop scoring*	*Crystallization conditions report* Buffer composition Crystal morphology (including photographs) Temperature Additives
Diffraction and Data Collection	
In-house Diffraction Data Collection Fishing, freezing, mounting Testing crystal diffraction quality Dataset collection for suitable crystals	Crystals X-ray diffraction quality All or preliminary diffraction images and statistics Full diffraction dataset if collected successfully
External Synchrotron Trip Shipping and measurement, phasing	
Model Building	
Structure solving and model building (crystallographic computing, bioinformatics)	Final model file and statistics Model deposition (PDB) if not confidential
Sample and Data Storage	Samples, crystals or data can be handed over

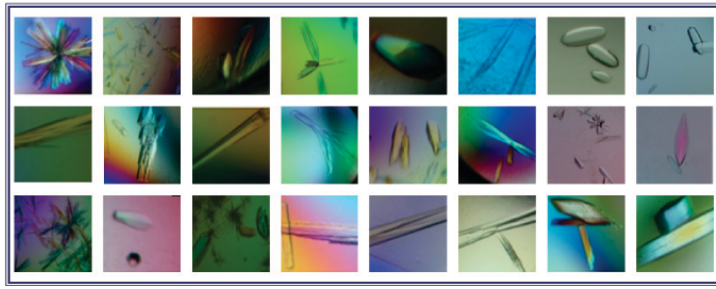




Comparison of kinetic graphs and parameters of *Sm*GhrA and *Sm*GhrB in reaction with different substrates with NADPH as a cofactor.*

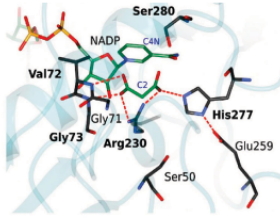
Protein crystals

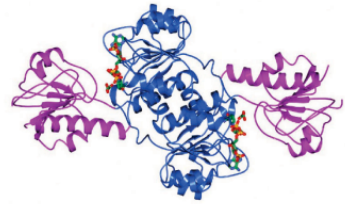
M. Kisiała
Unpublished data



*Sm*GhrB+NADP+2-Keto-D-gluconate*

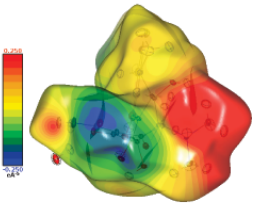
*J. Kutner et al.
Biochemistry
2018, (6), pp 963–977



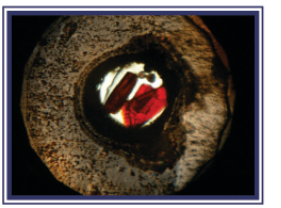


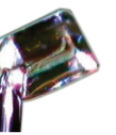
Sm*GhrB+NADPH+Oxalate

Small Molecule Compounds ("CHEM" pipeline)	
Method or service	**Report to Customer**
Phase Transitions (Solid state transformations)	
TDS investigations DSC studies Thorough elucidation of the thermodynamic properties of the various forms	TDS or DSC data Interpretation of results
Polymorphism Analysis	
Preliminary studies Propensity screen including robotic high-throughput screening *Profile Study* (detailed test of various conditions for crystallization process) Solvent effects (polar, nonpolar), Temperature, Kinetics vs. thermodynamic forms, Pseudopolymorphism (hydration, solvation) Melting points *Amorphous Screens* (analysis of different forms induced by various factors) Solvent type, slow and fast evaporation, Slow and fast crystallization, Thermal and physical (e.g. grinding) effects	Thermodynamic stability of forms and hydrates (solvates) with a reasonably high probability while keeping the experimental effort limited First insight into complexity of the polymorphism for a given substance Complete profiling of the polymorphic behavior Amorphous screening report
Crystallization Trials	
Initial screening and optimization Crystallization via: batch, solvent evaporation, in-gel, concentration or diffusion gradient, … Incubation at different temperatures Different solvents and their mixtures Additives Homo- and heteroseeding Solid state grinding approach *Visualization and crystallization drop scoring*	Temperature effect Type of solvent Composition of mixtures Grinding conditions Crystal shape (including photographs) Characterization of all relevant polymorphs
Diffraction Data Collection	
In-house diffraction data collection *Synchrotron trips and Neutron sources* Setting data collection strategy Data collection and processing	Copies of the data strategy and processing files Crystals X-ray diffraction quality All or preliminary diffraction images and statistics Full diffraction dataset if collected successfully
Model Building	
Structure solving and model building Analysis of residuals Applying methods beyond IAM (if feasible)	Crystal Information File (CIF) and statistics Files for full final refinement (ins and hkl files) Model deposition (CSD and/or ICSD)
Sample and Data Storage	Samples, crystals or data can be handed over



Experimental Hirshfeld surface of doxycycline**

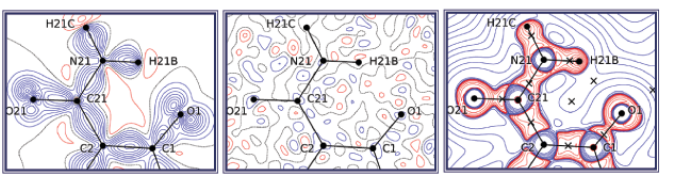


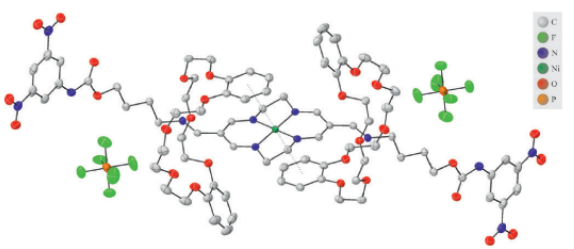


Crystals in a Diamond Anvil Cell and single crystal**

Electron distribution maps for doxycycline amide moiety**

** D. Tchoń and A. Makal
Unpublished data





Rotaxane - Displacement ellipsoids are drawn at the 50% level, Ni···π interactions are represented by dashed lines

K. Woźniak, Unpublished data

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