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Biothermodynamics and Drug Design

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The Laboratory of Biothermodynamics and Drug Design (LBDD) was established in 2006 based on the former Laboratory of Recombinant Proteins. The LBDD designs novel chemical compounds for therapeutic purposes. The efficiency of both naturally occurring and synthetic compounds is evaluated by biothermodynamic and structural methods.
The laboratory's personnel consist of five teams according to their research goals and activities:

The Team of Molecular and Cell Biology, headed by Dr. Jurgita Matulienė (Ph. D. in cell biology from the University of Minnesota, USA, 2003), prepares target proteins by gene cloning, expression in *E.coli*, insect, or mammalian cells, and chromatografic purification of large quantities of active proteins sufficient for biothermodynamic measurements of compound interaction with target proteins. Several projects involve the design of mutants and truncated protein domain constructs. Live human cancer cells are cultured for the evaluation of compound anticancer activity. Dr. Vilma Petrikaitė has a Ph. D. in pharmacy and performs compound testing in mice xenografts. The team collaborates with the Laboratory of Immunology and Cell Biology in antibody design and diagnostic markers.

The Team of Organic Synthesis, headed by Dr. Virginija Dudutienė (Ph. D. in organic synthesis from the Vilnius University, 2005), synthesizes compounds that are designed to bind carbonic anhydrases and other drug target proteins. Compounds are designed by computer docking, molecular modeling, and comparison with naturally occurring or previously synthesized compound functional groups. Compound identity and purity is verified by NMR and HPLC-HRMS.

The Team of Biophysics, headed by Prof. Daumantas Matulis (Ph. D. in biochemistry, molecular biology and biophysics from the University of Minnesota, USA, 1998), measures compound binding to target proteins by isothermal titration calorimetry (ITC), fluorescent thermal shift assay (DSF, ThermoFluor®), pressure shift assay (PSA), and conventional enzyme inhibition methods. The team determines the *intrinsic* Gibbs free energies, enthalpies, entropies, heat capacities and volume of binding and measures protein stability in the presence of various excipients.

The Team of Computer Modeling, headed by Dr. Vytautas Petrauskas (Ph. D. in physics from the Vilnius University, 2008), is responsible for the application of computational methods, database management, *in silico* docking of large compound libraries and the analysis of X-ray crystal structures of synthetic compound – protein complexes solved in collaboration with Dr. Saulius Gražulis group at the Laboratory of Protein – DNA interactions. Molecular modelers collaborate with the Laboratory of Bioinformatics and use their methods to model protein structures that are not solved by X-ray crystallogphy. The group, together with several collaborating scientists is developing the software that estimates the energies of compound binding to a protein when only the crystal structure of the free protein is available.

The Team of Amyloid Research. Recently a new team has started upon the return of Dr. Vytautas Smirnovas (Ph. D. from the Technical University of Dortmund, 2007) to Lithuania in 2011. The team is described below.

Research Projects

The Laboratory of Biothermodynamics and Drug Design performs fundamental and applied research focused on protein-ligand interactions and drug design. The state of the art in today's industrial drug design is still based on high-throughput approaches due to the lack of fundamental understanding of physical forces underlying such processes as protein folding and protein-ligand interactions. It is still impossible to predict and computer-model the compounds that would exhibit desired affinity and selectivity profiles towards their target proteins.

Carbonic anhydrases as drug targets

Carbonic anhydrases (CAs), a group of zinc containing enzymes, are involved in numerous physiological and pathological processes, including gluconeogenesis, lipogenesis, ureagenesis, and tumorigenicity. CAs catalyze the conversion of CO_2 to the bicarbonate ion and protons. In addition to the established role of CA inhibitors as diuretics and drugs used to treat glaucoma and high-altitude sickness, it has recently emerged that CA inhibitors could have potential as novel anti-obesity, anticancer, and anti-infective drugs.
There are 12 catalytically active CA isoforms in humans. CAs I, II, III, VII and XIII are cytosolic, CAs IV, IX, XII and XIV

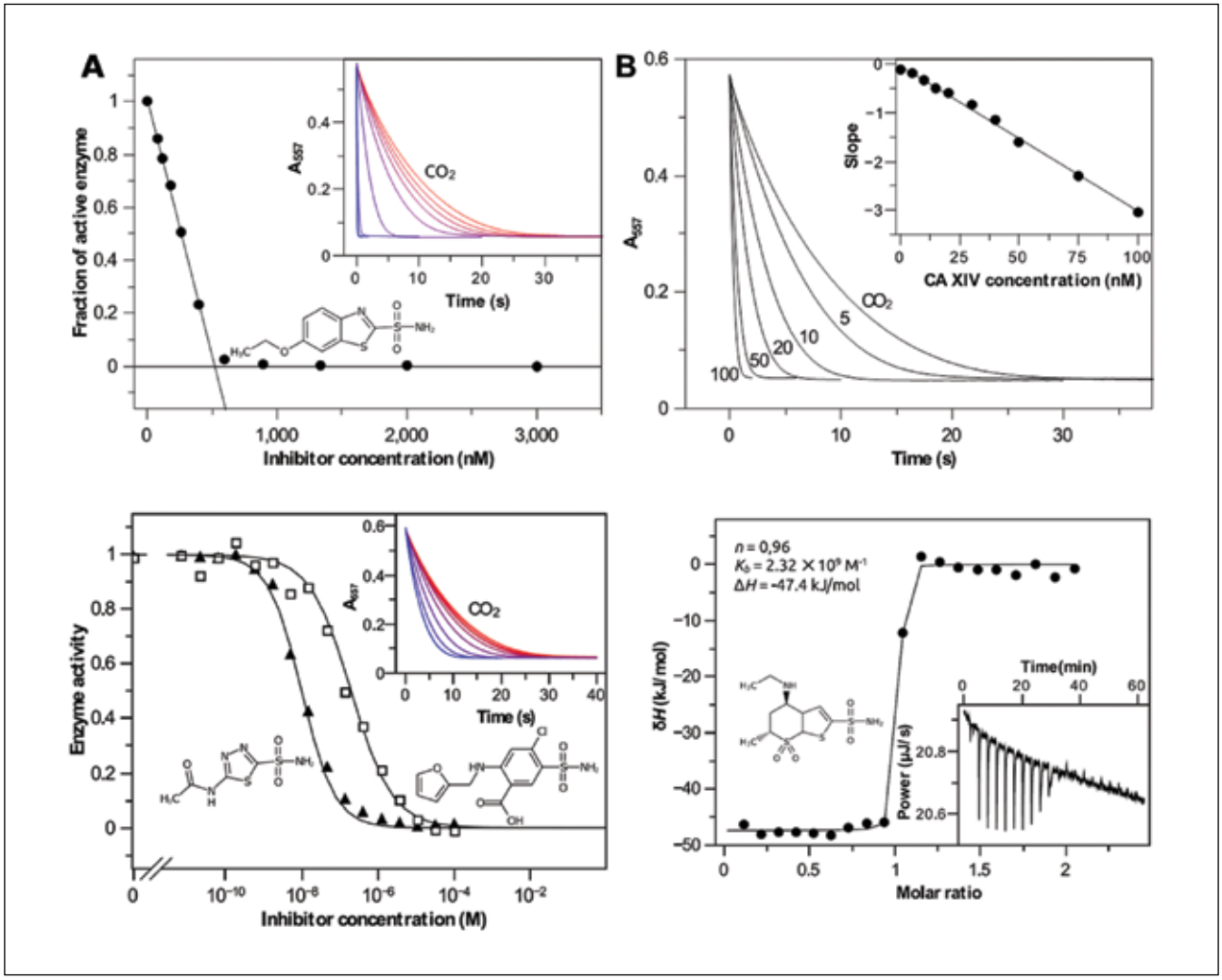


Figure 1. ***Characterization of CA XIV by the stopped-flow activity inhibition assay (A, B), drug dosing curves (C), and the purity demonstration by ITC (D). Published in Juozapaitiene et al 2016.***

are membrane-attached and located on the outside of the cell, CAs VA and VB are found in the mitochondria, and CA VI is the only secreted isoform found in saliva and milk. A number of CA inhibitors, mostly aromatic sulfonamides, have been designed and developed into drugs. However, most inhibitors possess low selectivity towards the target CA isoforms. It is especially important to develop highly selective inhibitors towards the novel anticancer target isoforms, CA IX and XII that are highly over-expressed in numerous tumors and increase cancerous cell survival and metastatic invasiveness.

We have cloned and purified all human CA catalytic domains in bacterial or mammalian cells. Over 700 novel compounds were designed and synthesized that bound CAs with micromolar to picomolar affinities. Six CA isoforms were crystallized in complex with numerous inhibitors and solved to high resolution thus providing structural insight into compound affinity and selectivity. A series of fluorinated CA inhibitors exhibited high affinity and great selectivity towards CA IX isoform. Several other series of compounds were determined to bind various CA isoforms.

However, there are several linked reactions that occur simultaneously with the binding reaction. Such linked reactions greatly influence the observed thermodynamic parameters of binding. For example, affinities are greatly dependent on pH, the enthalpies of binding – on the buffer in solution. Therefore, we determine the intrinsic thermodynamic parameters of binding that are independent of experimental conditions such as buffer and pH and may be directly correlated with the crystal structures.

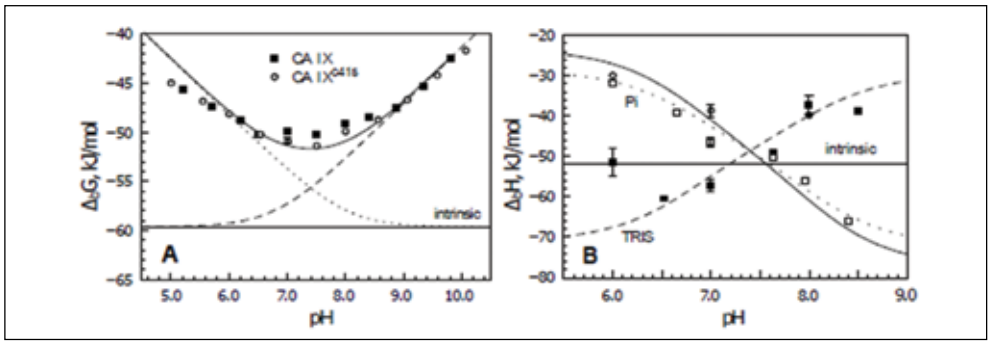


Figure 2. Characterization of CA IX. Binding of ethoxzolamide as a function of pH and determination of intrinsic binding affinity (A), and determination of intrinsic binding enthalpy (B). Published in Linkuviene et al 2016.

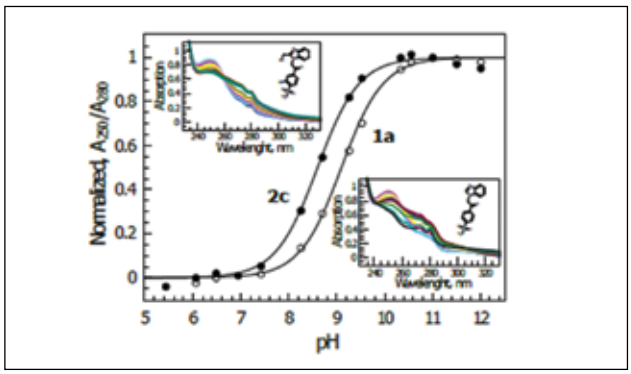
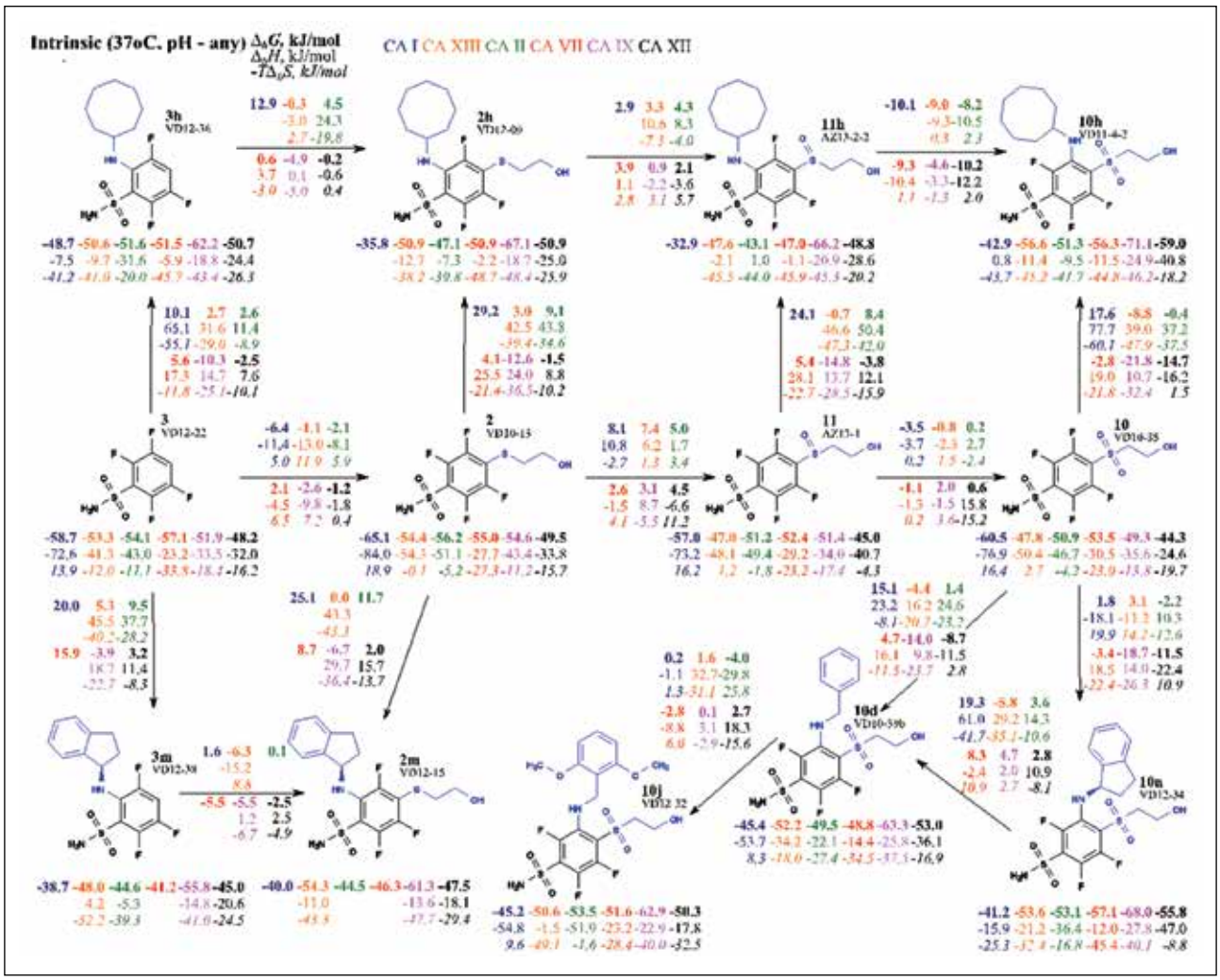


Figure 3. Determination of the inhibitor sulfonamide deprotonation pKa by spectrophotometry. Published in Linkuviene et al, 2016.

Figure 4. A map of intrinsic Gibbs energies and enthalpies of compound binding to 6 CA isoforms. Published in Zubriene et al 2016.



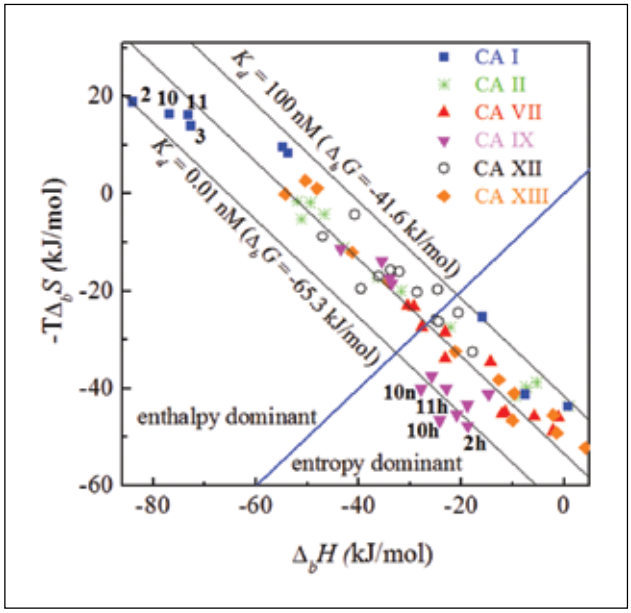


Figure 5. The enthalpy-entropy compensation plot of the fluorinated sulfonamide inhibitor binding to 6 CAs. Published in Zubriene et al 2016.

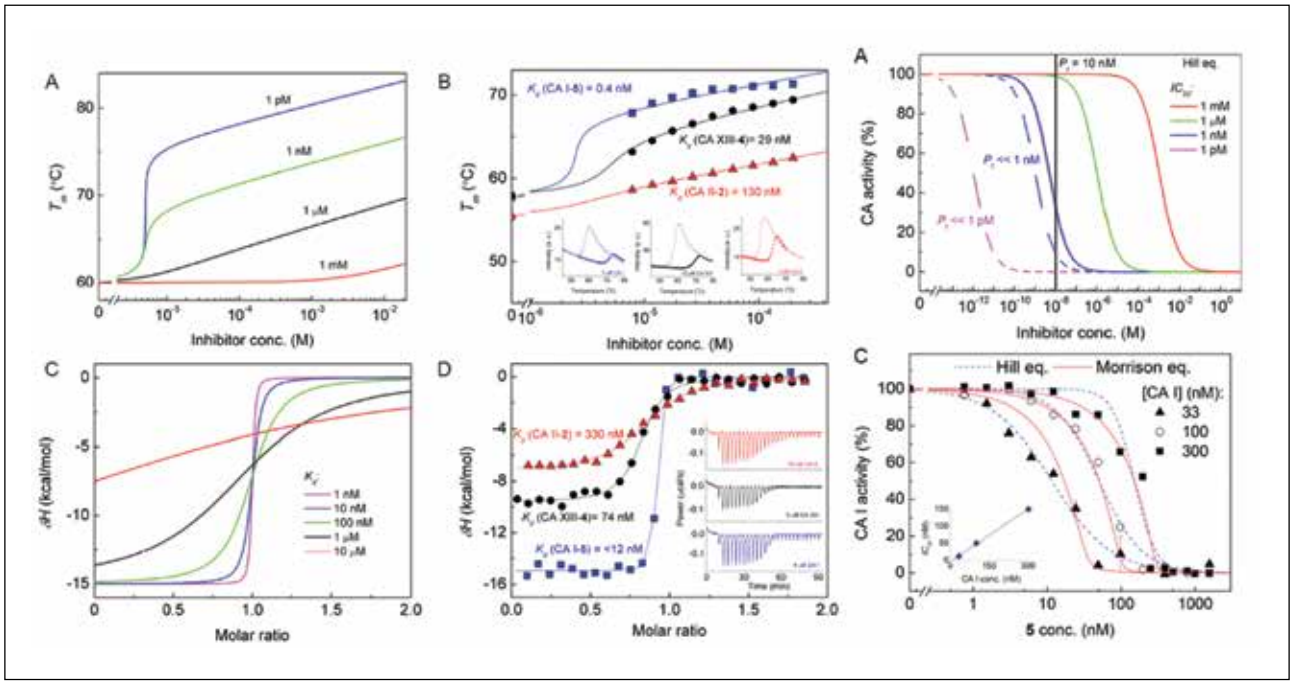


Figure 6. Comparison of theoretical and experimental binding curves of the stopped-flow inhibition assay, isothermal titration calorimetry and thermal shift assay. Published in Smirnoviene et al 2016.

The Team of Amyloid Research

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Protein aggregation into amyloid structure is involved in many diseases, including such neurodegenerative disorders as Alzheimer's and Parkinson's, systemic amyloidoses and even some localized diseases such as type II diabetes or cataracts. There is an increasing evidence of amyloid nature of proteinaceous infectious particles – prions. One of possible ways of prion spreading is self-replication of amyloid-like fibrils, thus there is a chance of all amyloid-associated diseases to be potentially infective.

We study effects of environmental factors such as temperature, pressure, pH, ions, macromolecular crowding, and the presence of different organic solvents, ligands and biomolecules on aggregation kinetics, thermodynamic stability, and structural properties of amyloid-like fibrils. We believe only comprehensive knowledge of all factors may give genuine understanding of mechanisms of amyloid self-replication and thus proteinaceous infectivity.

Services

The LBDD is seeking to license out the compounds described in patents and patent applications. The LBDD is interested in collaborations where our expertise in recombinant protein production and the determination of compound – protein binding thermodynamics and recombinant protein stability characterization could be applied. Protein – ligand binding constants and protein thermal stability profiles at hundreds of conditions may be determined in a single experiment by consuming microgram quantities of protein.

Conferences

The LBDD regularly participates in many international conferences and symposiums, including:

International Conference on the Carbonic Anhydrases.

International Conference on High Pressure Bioscience and Biotechnology.

European Biophysics Congress.

Biophysical Society Annual Meeting.

International Conference of Lithuanian Biochemical Society.

Since 2014 when Prof. Daumantas Matulis became the chairman of the Lithuanian Biochemical Society, we have organized the "XIVth International Conference of Lithuanian Biochemical Society", 2016 06 27 – 30, Druskininkai, Lithuania.

Funding

European Social Fund under the Global Grant Measure

Research Council of Lithuania

Punlications 2015-2016

Journal Articles

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Publications

of The team of amyloid research:

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Patent Applications

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