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The Laboratory of Biothermodynamics and Drug Design (LBDD) was founded in 2006 in the place of the former Laboratory of Recombinant Proteins. The LBDD designs novel chemical compounds with anticancer activity. The efficiency of both naturally occurring and synthetic compounds is evaluated by structural biothermodynamics and molecular modelling methods.

The laboratory's personnel consist of five teams according to their research 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) produces drug 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 binding chemical compounds. Several projects involve the design of protein domain constructs. Live human cancer cells are cultured for the evaluation of compound anticancer activity.

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 drug target proteins. Compounds are designed by computer docking, molecular modelling, and comparison with naturally occurring or previously synthesized compound functional groups. The special interest and capabilities of the group are in the field of synthesis of compounds with multiple conjugated aromatic heterocycles.

The Team of Biophysics, headed by Dr. Daumantas Matulis (Ph. D. in biochemistry, molecular biology and biophysics from the University of Minnesota, USA, 1998) measures compound/ligand binding to target proteins by isothermal titration calorimetry (ITC), thermal shift assay (ThermoFluor®), and pressure shift assay (PSA). The team performs the characterization of protein stability in the presence of various excipients and the measurement of enzymatic activity.

The Team of Computer Modelling is responsible for the *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 in the Laboratory of Protein – DNA interactions. Molecular modelling of candidate compounds often predicts novel compounds with improved binding capabilities. The group, together with several collaborating scientists is developing the software that estimates the energetics of ligand binding to a protein when only the crystal structure of free protein is available.

The Team of Applied Biocatalysis, headed by Inga Matijošytė (Ph. D. in biochemistry and biocatalysis from Delft University, The Netherlands, 2008), established in 2007 in conjunction with the start of the Program on the Development of Industrial Biotechnology in Lithuania, applies enzymes as biocatalysts in organic synthesis to achieve desired conversions. Team's research is directed towards the search for enzymes with new functionalities and their development towards applied biocatalysts.

Research Projects

Several protein targets have been selected for the investigation of protein – ligand binding thermodynamics and the design of novel ligands with desired properties. A family of human carbonic anhydrases, heat shock proteins, and several signal-tranducing proteins were chosen as anticancer drug targets.

Novel methods and thermodynamic approaches are being used and developed in the laboratory. Detailed thermodynamic description of natural compound – protein interaction provides clues to improved ligand affinity and specificity. In addition to the Gibbs free energy, enthalpy, entropy, and the heat capacity, the laboratory studies the volume and compressibility of the protein – ligand interactions.

The laboratory has been recently invited to write a review chapter on the thermal shift assay in Royal Society of Chemistry Biomolecular Sciences No.22 book (Cimmperman and Matulis, 2011).

Carbonic anhydrases as anticancer drug targets

Carbonic anhydrases (CAs), a group of zinc containing enzymes, are involved in numerous physiological and pathological processes, including gluconeogenesis, lipogenesis, ureagenesis, tumorigenicity and the growth and virulence of various pathogens. In addition to the established role of CA inhibitors as diuretics and antiglaucoma drugs, it has recently emerged that CA inhibitors could have potential as novel antiobesity, anticancer, and anti-infective drugs (Supuran, 2007). CAs catalyse a simple reaction – the conversion of CO_2 to the bicarbonate ion and protons. There are 12 catalytically active CA isoenzymes in humans. A number of CA inhibitors, mostly unsubstituted sulfonamides, have already been designed. However, most present inhibitors are insufficiently selective for target CA isozymes, such as hCAIX and hCAXII, anticancer targets.

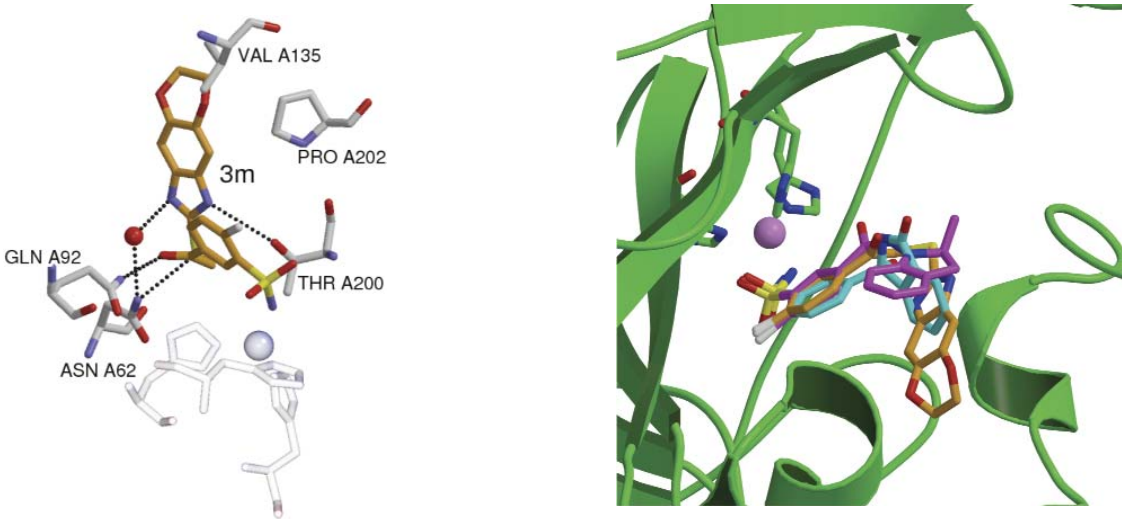

Figure 1. Left panel. View of compound **3m** bound in the active center of hCA II. The Zn atom, His94, His96, and His119 are shown as transparent, inhibitors are shown in orange. Right panel. Superposition of the hCA II–indapamide (magenta), hCA II-chlorthalidone (cyan) with hCA II – **3m** (orange). Zinc ion is shown as pink sphere, His94, His96, His119, and protein secondary structures are in green. The crystal structure was solved in collabora-tion with dr. Saulius Gražulis group, Laboratory of Protein-DNA Interactions (Capkauskaite et al, 2010).

Here at the LBDD we have cloned and purified most soluble CAs and truncated versions of CAs with removed transmembrane domains. Laboratory participated in the characterization of hCA IX (Hilvo et al, 2008). The organic synthesis team together with collaborators designed and synthesized over 200 novel compounds that bind CAs with submicromolar affinity. Several novel groups of CA inhibitors exibited high affinity and appreciable selectivity towards selected CA isozymes (Dudutiene et al. 2007; Baranauskiene et al. 2010; Sudzius et al. 2010; Capkauskaite et al. 2010).

Inhibition of Hsp90 chaperone

Heat shock protein 90 (Hsp90) is a molecular chaperone that is responsible for the correct folding of a large number of proteins. Client proteins of Hsp90 include many overexpressed oncogenes that are critical for the transformed phenotype observed in tumours.

Our laboratory is interested in the mechanism of Hsp90 action and the thermodynamics of inhibitor binding. Thermodynamics of a natural compound radicicol binding to human Hsp90 alpha and beta isozymes and yeast Hsc82 was studied by isothermal titration calorimetry, thermal shift assay, and the pressure shift assay. These studies provided an unusual and detailed picture of Hsp90 inhibitor binding energetics. Radicicol bound Hsp90 with exceptionally large

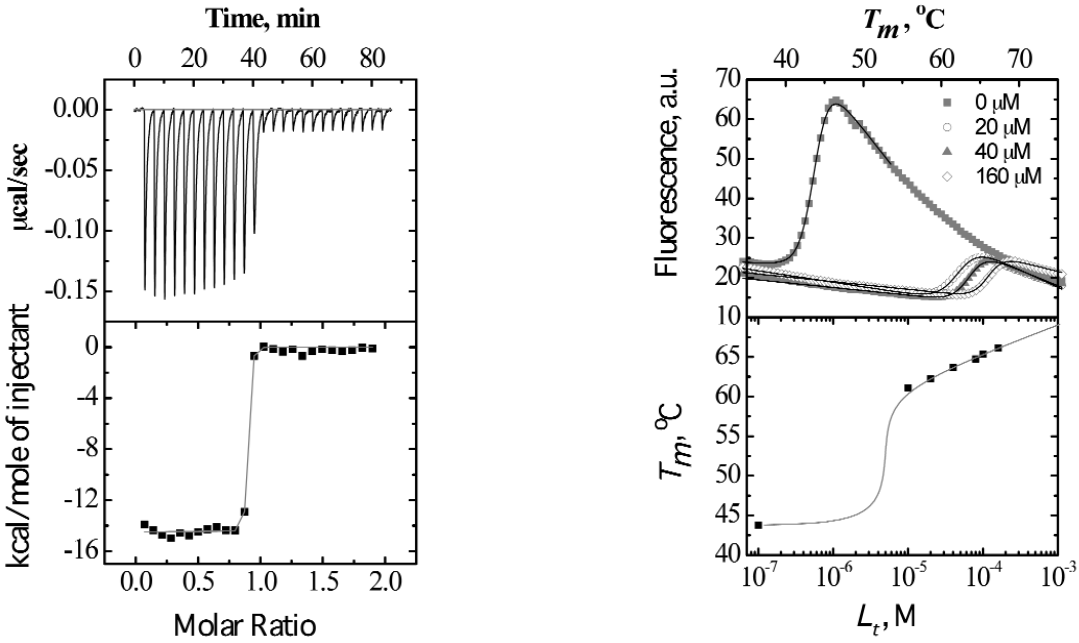

Figure 2. Radicicol binds Hsp90 with high affinity in the order of 100 pM K_d. Titration calorimetry (graphs on the left) yield a stepwise curve – too steep to determine the Kd. Thermal shift assay (graphs on the right, upper panel – fluorescence dependence on temperature at various added ligand concentrations, lower panel – T_m dependence on added ligand concentration) provides quite precise measurement of the binding constant.

exothermic enthalpy and volume of binding (Zubriene et al 2009, Zubriene et al. 2010, Toleikis et al, submitted).

A novel group of inhibitors has been designed and synthesized based on similarity to radicicol and several inhibitors designed by Vernalis, UK. Our series of inhibitors were significantly easier to synthesize and possessed comparable activity towards HeLa and osteosarcoma cells (Cikotiene et al, 2009). Structure – thermodynamics analysis provided further insight into the mechanism of such inhibitor binding to Hsp90.

Ligand binding equilibria by protein high pressure denaturation

The volume changes accompanying ligand binding to proteins are thermodynamically important and could be used in the design of compounds with specific binding properties. Measuring the volumetric properties could yield as much information, as the enthalpic properties of binding. Pressure-based methods are significantly more laborious than temperature methods and are underused. The pressure shift assay (PressureFluor, analogous to the ThermoFluor, thermal shift assay) uses high pressure to denature proteins. The PressureFluor method was used to study the ligand binding thermodynamics of Hsp90. Ligands stabilize the protein against pressure denaturation, similar to the stabilization against temperature denaturation.

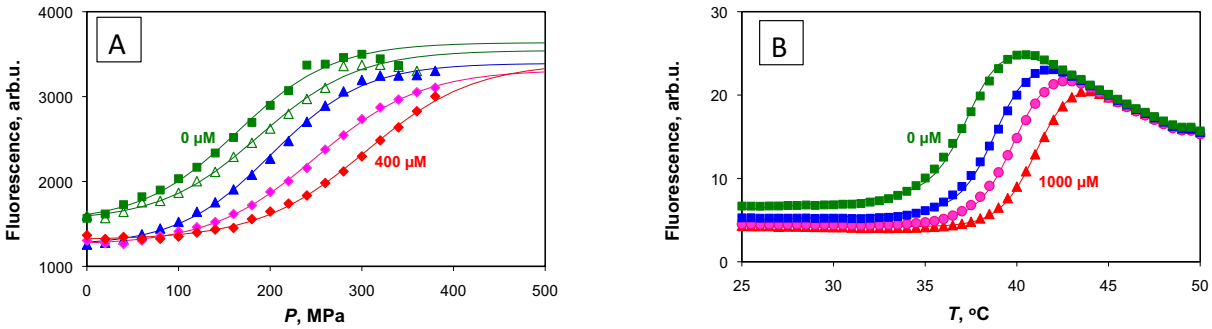

Figure 3. Pressure shift assay (panel A) and thermal shift assay (panel B). Elevated pressure or temperature causes protein denaturation. Addition of a ligand of an increasing concentrations increases protein melting pressure and melting temperature.

Applied biocatalysis projects

The limited number of suitable and well characterized biocatalysts delays the progress in the application of enzymes in the synthesis of compounds for materials, pharmaceuticals and chemicals. The Team of Applied Biocatalysis seeks to identify biocatalysts with novel activities by the three most common ways: screening for enzymes (environmental samples, enzyme and strain collections, expression databases), development of biocatalyst (directed evolution, genetic engineering, development of analytical systems) and the application of biocatalysts (immobilization, recycling, proof of principle, activity/selectivity, stability, and reaction media).

The research focuses on biocatalytic systems employing lipolytic, hydrolytic, proteolytic and oxidative enzymes. We strive to meet scientific challenges in combination with application-oriented research.

Services

The LBDD is seeking to license out the compounds described in patent applications. The LBDD is also interested in the collaborations where our expertise in 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.

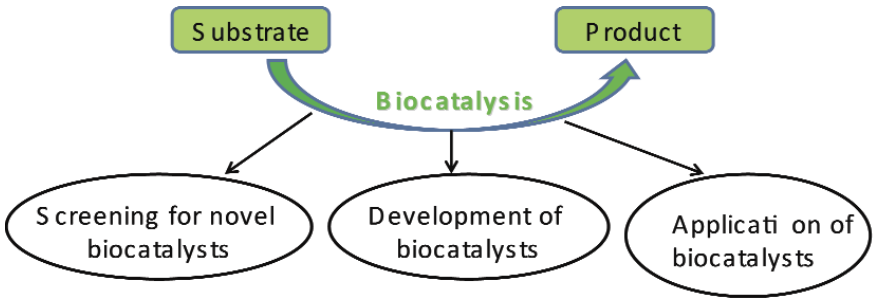

Figure 4. Reseach areas of the Team of Applied Biocatalysis

CONFERENCES AND COLLABORATION

The LBDD regularly participates in many international conferences and symposiums, including:
International Conference on the Hsp90 chaperone machine.
International Conference on the Carbonic Anhydrases.
International Conference on High Pressure Bioscience and Biotechnology.
Biothermodynamics Symposium.
European Biophysics Congress.
Biophysical Society Annual Meeting.
Gibbs Conference on Biothermodynamics.
International Conference of Lithuanian Biochemical Society.

The LBDD has ongoing collaborations with a number of research laboratories and industry worldwide, including:
Institute of Medical Technology, University of Tampere, Finland.
University of Florence, Italy.
International Institute of Molecular and Cell Biology, Warsaw, Poland.
Pharmaceutical Research and Development, L.L.C., Johnson&Johnson, USA.
Centre for Structural Biochemistry, Montpellier, France.
Institute of Organic Synthesis, Riga, Latvia.
Institute of Organic Chemistry, University of Tubingen, Germany.
Chemistry Centre of Madeira, University of Madeira, Portugal.
Cancer Research Centre, University of Edinburgh, UK.
University of Bristol, UK.
Delft University of Technology, Netherlands.
Faculty of Chemistry, Vilnius University, Lithuania.
Faculty of Natural Sciences, Vilnius University, Lithuania.
Institute of Biochemistry, Vilnius, Lithuania.
Lithuanian University of Agriculture, Kaunas, Lithuania.
AB "Amilina", Panevėžys, Lithuania.
UAB „BIOK", Vilnius, Lithuania.
UAB „Biocentras", Vilnius, Lithuania.
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