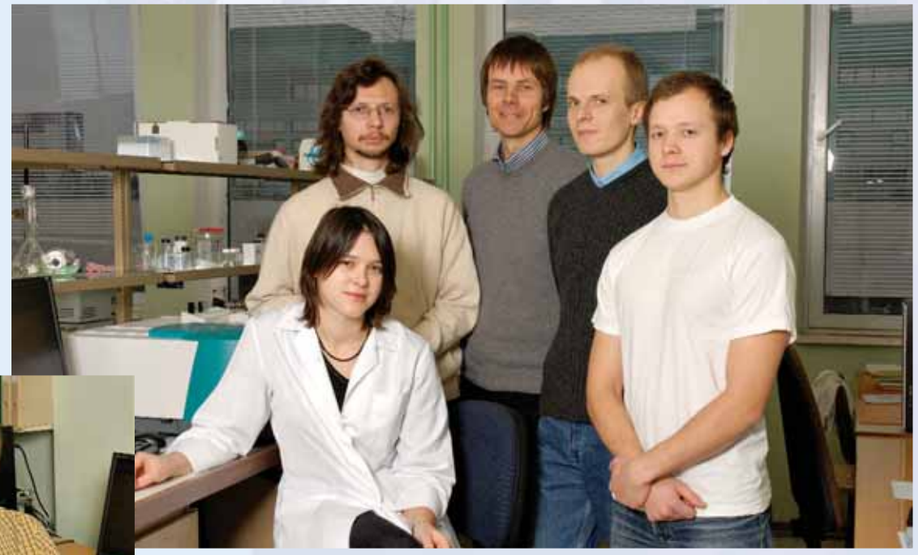
Laboratory of Biothermodynamics and Drug Design



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Institute of Biotechnology
Centre of Excellence

Founded in 2006 instead of the former Laboratory of Recombinant Proteins, the Laboratory of Biothermodynamics and Drug Design (LBDD) seeks to design, synthesize, and characterize novel chemical compounds with anticancer activity. Two classes of compounds were designed in 2006-2007 by structural biothermodynamics methods and experimentally tested by biophysical methods to be active inhibitors of enzymes that are anticancer targets. Two patent applications and several scientific publications were submitted. The advantage of the LBDD is the capability to design compounds in silico and experimentally test them by novel biophysical methods.

The laboratory's personnel is divided into five groups according to their activities:

The Group of Molecular and Cellular Biology is responsible for drug target protein production by cloning the genes of selected target proteins, their expression in E.coli, insect, or mammalian cells, and chromatografic purification of large quantities of active proteins sufficient for biothermodynamic measurements of binding with synthesized ligands. Several projects involve the design of protein domain constructs to alleviate insolubility problems. Protein production often involves their reconstitution and refolding from insoluble inclusion bodies, characterization of protein stability, and the measurement of enzymatic activity.

The Group of Biophysics is responsible for designing thermodynamic models and experimental measurements of chemically synthesized and natural ligand binding to target proteins by the following biophysical techniques: isothermal titration calorimetry (ITC) and protein melting temperature shift (thermal shift, TS, ThermoFluor). The group is equipped by isothermal titration calorimeters, differential scanning calorimeters, temperature-controlled fluorimeters and spectrophotometers. Furthermore, the group studies protein denaturation by high pressure using high pressure fluorimeter.

The Group of Molecular Modeling is responsible for designing thermodynamic models of chemically synthesized ligand binding to target proteins. Molecular modeling of candidate compounds often predicts novel compounds with improved binding capabilities. The group, together with several collaborating scientist 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 Group of Organic Synthesis makes compounds that are designed to bind target proteins either by comparison with compounds of similar chemical structure or by computer simulation and molecular modeling. Special interest and capabilities of the group are in the field of synthesis of compounds with multiple conjugated aromatic heterocycles.

The Group of Industrial Biotechnology was established in 2007 with the start of the Lithuanian National Programme on Industrial Biotechnology (2007-2010). The group investigates technical applications and immobilization of enzymes for the production of biofuels and for starch transformations. Research on metabolic engineering and organic synthesis by fermentation are planned for the nearest future.

Research Projects

Carbonic anhydrases as anticancer targets

Carbonic anhydrases (CAs), a group of ubiquitously expressed 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 anti-obesity, anticancer, and anti-infective drugs (Supuran, 2007).

CAs catalyse a simple reaction – the conversion of CO2 to the bicarbonate ion and protons. There are 14 CA isoenzymes in humans, three of them are inactive CA-like proteins that do not contain zinc and thus do not catalyse the CO2 hydration reaction. Expression of the 11 active CAs is variable in various tissues. The activity of CAs is quite variable too. A number of CA inhibitors, unsubstituted sulfonamides, have already been designed. However, most present inhibitors are insufficiently selective for target CA isozymes, such as hCAIX and hCAXII, anticancer targets.

Here at the LBDD we have designed a novel class of carbonic anhydrase inhibitors that exibit selectivity towards hCAIX over hCAI and hCAII. The inhibitors bind CAs with up to double digit nanomolar dissociation constants and significant selectivity towards specific isozymes.

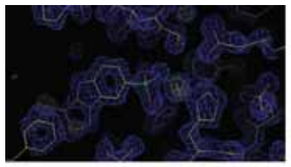

Fig. 1. *General formula of carbonic anhydrase inhibitors and the crystal structure of the inhibitor bound to the active site of hCAII (crystal structure was solved in collaboration with dr. Saulius Gražulis, Laboratory of Protein-DNA Interaction).*

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.

We are interested in the mechanism of Hsp90 action and the thermodynamics of inhibitor binding. A novel group of inhibitors has been designed and synthesized that is similar to radicicol, a well known natural inhibitor of Hsp90.

A novel model based on the thermal shift assay was designed to determine the binding constants of tightly-binding ligands based on the Hsp90-radicicol binding. Upon binding radicicol, Hsp90 exhibits a double thermal transition.

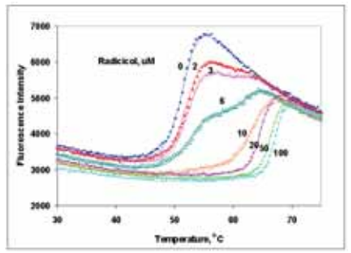

Fig. 2. *Hsp90-radicicol binding by the thermal shift assay*

Characterization of protein stability in the presence of various excipients

To determine the stability profiles of a recombinant protein, we use a miniaturized high-throughput thermal shift assay (also called ThermoFluor®) [Matulis D. et al. Biochemistry, 2005, 44, 5258-66]. The assay measures protein melting temperature by following the fluorescence of probes such as 1,8-anilino naphthalene sulfonate. The following picture shows the stability profile of hCAII in various buffers at various pHs.

The effect of a number of excipients such as osmolytes, organic solvents, amino acids, common metal cations and anions, lipids, and other chemicals and biochemicals on protein stability is tested in a single plate consuming several micrograms of recombinantly produced protein. The assay facilitates the determination of the optimal conditions for storage or other functional assays.

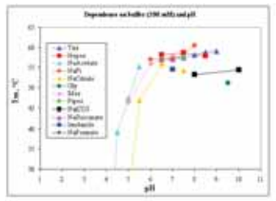

Fig. 3. *Stability profile of hCAII in different buffers at various pHs*

Determination of ligand binding constants by the thermal shift assay

The thermal shift assay allows us to measure ligand binding constants in high throughput while consuming micrograms of protein. Together with the isothermal titration calorimetry, we determine the full thermodynamic picture, including the Gibbs free energy, enthalpy, entropy, and the heat capacity of selected binding reactions. The data is used for the design of more potently binding ligands.

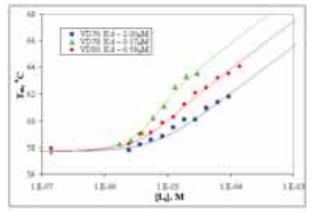

Fig. 4. *Determination of ligand binding constants to hCAXIII.*

Studies of Protein Denaturation by High Pressure

Pressure studies yield a variety of thermodynamic parameters that are unavailable by other methods, such as molar volume, expansion coefficient, and compressibility of the proteins in the native and denatured states. Ligand binding affects protein temperature and pressure stability. The pressure stability information yields novel methods of determining ligand binding equilibria.

Services

The LBDD is interested in the collaborations where our expertise in recombinant protein stability characterization and the determination of ligand-protein binding thermodynamics may be applicable. We can determine target protein thermal stability at hundreds of conditions in a single experiment by consuming microgram quantities of protein.

Collaboration

Prof. Seppo Parkkila, Institute of Medical Technology and School of Medicine, University of Tampere and Tampere University Hospital, Tampere, Finland
Prof. Claudiu Supuran, Universita degli Studi di Firenze, Laboratorio di Chimica Bioinorganica, Firenze, Italy
Prof. Maciej Zylicz, International Institute of Molecular and Cell Biology, Warsaw, Poland
Matthew J. Todd and Barry Springer, Johnson&Johnson, PRD, L.L.C., Philadelphia, USA, have donated ISS high pressure fluorimeter, Microcal MC2 isothermal titration calorimeter, Microcal MC2 differential scanning calorimeter, and protein purification chromatography system
Prof. Cathy Royer, Centre for Structural Biochemistry, Montpellier, France
Dr. Rolandas Meškys, Institute of Biochemistry, Vilnius, Lithuania
Prof. Sigitas Tumkevičius, Vilnius University, Faculty of Chemistry, Vilnius, Lithuania
Prof. Prutenis Janulis, Lithuanian Agricultural University, Kaunas, Lithuania

Grants

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Publications 2006-2007

1. Sereikaite J., **Jachno J.**, Santockyte R., Chmielevski P., Bumelis V. and **Dienys G.** Protein Scission by Metal Ion–Ascorbate System. Protein J., 2006 (25):369-378.
2. **Dudutienė V., Baranauskienė L., Matulis D.**. Benzimidazo[1,2-c][1,2,3]thiadiazole-7-sulfonamides as inhibitors of carbonic anhydrase. Bioorg. Med. Chem. Lett., 2007 (17): 3335-3338.
3. **Zaveckas M.**, Žvirblienė A., Žvirblis G., Chmieliauskaitė V., Bumelis V., Pesliakas H.. Effect of surface histidine mutations and their number on the partitioning and refolding of recombinant human granulocyte-colony stimulating factor (Cys17Ser) in aqueous two-phase systems containing chelated metal ions. J. Chromatogr. B, 2007 (852):409-419.
4. Romaškevič T., Budrienė S., Liubertienė A., Gerasimčik I., **Zubrienė A.** and **Dienys G.** Synthesis of chitosan-graft-poly(ethylene glycol) methyl ether methacrylate copolymer and its application for immobilization of maltogenase. Chemija, 2007 (18):33-38.
5. **Baranauskienė L., Matulienė J.**, and **Matulis D.** Determination of the thermodynamics of carbonic anhydrase acid-unfolding by titration calorimetry. J. Biochem. Biophys. Meth., in press.