

Summary of PhD thesis

INVESTIGATION OF INTERACTIONS AND REACTIONS OF PROTEINS AND CARBOHYDRATES USING ISOTHERMAL TITRATION CALORIMETRY

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I. Introduction and objectives

Investigation of carbohydrates and carbohydrate modifying enzymes has long history in the University of Debrecen. In our research group we deal with *in vitro* investigation of enzymes, which directed inhibition could be effective in the prevention and therapy of diabetes and obesity in the future. Accordingly, the results written in my dissertation related to this topic.

As a result of the continuous development of instrumental analytics, such classical methods as calorimetry started to spread again. Since Lavoisier made the first calorimeter, this technique underwent an enormous development. Nowadays, the modern, automatable microcalorimeters are able to detect the smallest heat changes occur in physical or chemical reactions, even in very low volumes. The main application area of microcalorimetry is the investigation of molecular interactions, but it is also used to determine the main kinetic parameters in enzyme catalysed reactions or examine the metabolic activity of living cells.

Isothermal titration calorimetry (ITC) accurately measures the heat changes between the reference and sample cell in an adiabatic environment. During each injections small aliquots of ligand are added to the sample cell, where the interaction is formed generating heat change. Since every physical or chemical change is followed by heat change, ITC can be considered as universal detector. Under optimal conditions, in a single binding study not only the thermodynamic parameters (ΔH , ΔG , ΔS) but also the stoichiometry of the reaction and the strength of binding (K_D , K_A) can also be determined. In enzyme kinetic studies constants of Michaelis equation v_{max} and K_M can be obtained.

Several ITC based methods have been published to get enzyme kinetic information, in which the raw data obtained with single or multiple injection methods are evaluated in different ways. Nevertheless, in certain cases (e.g. badly soluble substrates) these methods cannot provide adequate information. If the sample amount is limited, the long optimisation procedure also reduces the chance of successful measurement.

In our research we would have liked to develop and apply a new universal ITC-based method using the combination of the published single and multiple injection ones, which eliminates the disadvantages of the earlier methods, and can be used economically for both enzyme kinetic and inhibition studies. In our examinations a well-known reaction, the human salivary alpha-amylase catalysed hydrolysis, was used as a model system. To test the universality of the applied method such reactions were investigated, which can hardly be determined using other techniques.

We would have liked to take advantages of the classical application of ITC, thus protein-carbohydrate interactions and binding properties of enzymes and inhibitor compounds were also investigated.

II. Applied methods and equipments

The used substrates and enzymes were purchased from Sigma Aldrich and Serva. Since the commercial porcine pancreatic alpha-amylase (PPA) contained significant amount of undesired other compounds and human salivary alpha-amylase was stabilized with ammonium-chloride, both enzymes were purified using Amicon 30K (Millipore, MA, USA) centrifugal filter unit.

The applied ITC instrument was a MicroCal ITC200 microcalorimeter (Northampton, MA, USA) equipped with sample cell (204 μ l) and injector (40 μ l). The obtained raw data were evaluated with the Origin software supplemented with ITC evaluation upgrade.

To verify the results obtained with ITC method, HPLC and spectrophotometric measurements were carried out using chromophore containing substrates. The first attempt of investigation of compounds got from our German collaborators (Freie University, Berlin) was also performed using UV-VIS spectrophotometer.

HPLC measurements were performed with Agilent Infinity HPLC (Agilent, Santa Clara, CA) system equipped with manual sampler, degassing module and diode array detector. Evaluation of kinetic data was based on the change of area of the main product. Spectrophotometric measurements were carried out at 400 nm with a double-beam, JascoV550 spectrophotometer (Jasco Co., Easton, MD, USA) at 37 °C using GalG₂CNP (Sorachim SA, Lausanne, Switzerland) substrate. Initial reaction rate was determined from the slope of the initial phase of kinetic curve, for which Jasco Spectra Manager software was applied. Kinetic and dose-response curves were fitted with Grafit (Erithacus Software Ltd, Horley, UK) and Origin (Northampton, MA, USA) softwares.

To the inhibition studies the aqueous infusions from dried plant parts purchased from Herbária Kft were prepared. The composition of infusions with MALDI-TOF MS (Bruker, MA, USA) was analyzed. The water-soluble antioxidant capacity was measured with ACW method using PHOTOCHEM[®] equipment (Analytik Jena AG, Jena, Germany) and the official ACW kits. The obtained curves were evaluated with PCLsoft (Analytik Jena AG, Jena, Germany) software.

III. New scientific results

1. I determined the binding affinity, stoichiometry and binding properties of different antigen-antibody, macromolecule-ligand and enzyme-inhibitor complexes using isothermal titration calorimetry (ITC).

The classical application of ITC is to determine the binding properties, stoichiometry and thermodynamic parameters of interacting molecules based on the heat change occurs during the binding. The most prevalent use is the examination of antigen-antibody, macromolecule-ligand and receptor-ligand interactions.

1.1. I determined the binding constant and stoichiometry of the antigen binding of a specific human serum albumin binding monoclonal antibody using ITC.

Albumin is the most abundant protein in human serum. The high albumin concentration has a significant disturbing effect on the examination of other serum proteins. An albumin specific antibody has been produced, which bound to TiO₂ resin could selectively remove albumin from the serum samples. The stoichiometry of antigen-antibody binding was assessed with capillary zone electrophoresis (CZE) and ITC. Both measurements resulted in 1:2 ratio that means one antibody binds two antigen molecules. Affinity capillary electrophoresis (ACE) and ITC were used to get the binding constants. Obtained K_i values were 4.45×10^{-8} and 1.08×10^{-7} M determined with ACE, and 2.74×10^{-8} and 1.04×10^{-7} M in the case of ITC.

1.2. I verified and characterised the interaction between an anticoagulant carbohydrate derivative and a positively charged γ -cyclodextrin using ITC.

Fondaparinux is an anticoagulant synthetic pentasaccharide derivative that selectively inhibit Xa factor. The excretion occurs in kidneys after 17-21 hours, but in certain cases the shorter elimination would be necessary. Complex formation with a cationic derivative of γ -cyclodextrin is a possible way to accelerate the excretion. In our investigation the strength of fondaparinux – cationic γ -cyclodextrin interaction was measured

On the basis of the obtained dissociation constant ($K_D = 125 \pm 7 \mu\text{M}$), cationic γ -cyclodextrin (γCD^+) is able to bind fondaparinux, but the strength of the interaction is significantly weaker than the strength of interaction between fondaparinux and factor Xa ($K_D = 32 \text{ nM}$). Binding is getting remarkably weaker in the presence of NaCl in concentration dependent manner, thus the suspected chelating activity will not work in the human blood stream. I also investigated the fondaparinux binding properties of a same functional groups containing maltooctaose (G8^+) and a γ -cyclodextrin (γCD). The first can bind fondaparinux with low affinity ($K_D = 3.57 \text{ mM}$), but at γCD no any interaction was detected. . Based on our results (Table 1)

both electrostatic interactions and the hydrophobic cavity of (γ CD⁺) are needed to the binding. The thermodynamic parameters show that the binding is an exothermic, both enthalpy and entropy driven reaction, in which electrostatic interactions and desolvation also take part.

Table 1: Dissociation constants and thermodynamic parameters of fondaparinux binding of positively charged oligosaccharides

	FP+ γ CD ⁺	FP+G ₈ ⁺	FP+ γ CD
K_D (mM)	0,125	3,57	
ΔH(cal/mol)	-1686	-4422	Unmeasurable
TΔS (cal/mol)	-3844	948	
ΔG (cal/mol)	-5530	-3473	

- In combination with the advantageous properties of earlier described ITC-based methods, I worked out a new technique, which is suitable for both enzyme kinetics and inhibition measurements.**

All the earlier published single and multiple injection methods often take a long time, the optimisation is complicated and the evaluation of the obtained results is difficult. These disadvantages can be eliminated with the new method, where the determination of initial reaction rate is fast due to the single injection. During the discontinuous measurement, the addition of one small aliquot of enzyme solution makes the method economic and the reaction rate can be determined right after the injection. One measurement takes about 2-3 minutes.

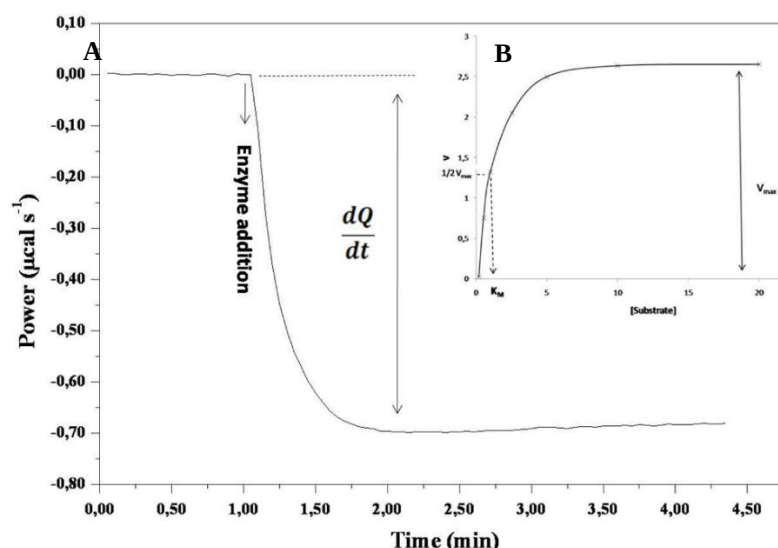


Figure 2: A: Determination of K_M with ITC-based method. A: Thermogram and its evaluation used to measure reaction rate. B: Michaelis-Menten curve fitted to data obtained at HSA catalysed maltoheptaoside hydrolysis reaction

The system can be easily optimised following a simple approach. Fitting the appropriate curve to the obtained data points, kinetic parameters, IC_{50} and K_i can be calculated.

2.1. Using the HSA catalysed hydrolysis reactions, I ascertained the main parameters of the measurement.

Investigating the reaction of human salivary α -amylase and maltoheptaose or starch, I set up the parameters needed to evaluate and reproduce data, namely the volume and concentration of injected enzyme, rate of injection, stirring speed and applied reference power. With the optimisation of the above mentioned parameters I managed to set up a fast, ecologic and sensitive method, which can be applied in broad range of (enzyme-substrate) systems including some, which cannot or just hardly examine using other methods.

2.2. I justified the obtained results using three independent methods and with comparison of these with data from the literature.

To check the results obtained with ITC I applied parallel spectrophotometric and HPLC measurements. In these tests, chromophore group containing substrates (GalG₂CNP and CNP-G₇) were used. The data obtained with ITC are in good agreement both with my parallel results and data from the literature. Differences in the magnitude of data were not experienced in the parallel measurements.

Table 2: K_M values (mM) of HSA-catalysed hydrolysis of CNP-G₇ and GalG₂CNP obtained with parallel methods in comparison with data from the literature.

Substrate	ITC	UV-VIS	HPLC	Literature
GalG ₂ CNP	2.8	1.0	ND	0.9 ¹
CNP-G ₇	0.6	ND	0.9	0.3 ²

3. Taking advantages of the universality and robustness of the technique I examined such enzyme reactions, which are hardly investigated with other methods.

The sensitive detection of substrates and products is a pivotal point in the investigation of carbohydrate modifying enzymes. Since ITC measures the heat change occurring during the reaction, some problems of other methods can be

¹ Kandra L, Zaj  cz   , Remenyik J, Gy  m  nt G. Kinetic investigation of a new inhibitor for human salivary α -amylase. Biochemical and biophysical research communications. 2005;334:824-828.

²Ramasubbu N, Ragunath C, Sundar K, et al. Structure-function relationships in human salivary α -amylase: role of aromatic residues. Biologia 2005;16:47–56.

eliminated, therefore we are able to examine the enzyme with the natural substrate, in circumstances close to the native ones.

3.1. I compared the main kinetic parameters of free and chromophore containing oligosaccharide hydrolysis reactions catalysed by human salivary α -amylase.

To get comparable kinetic parameters, the use of same conditions are critical. Using the developed method I ascertained the kinetic parameters of HSA catalysed hydrolysis reactions of free and chromophore group containing saccharides from a short one (GalG₂CNP) via oligomers (G₃-G₈) until the amylose, starch azure and natural polymer starch substrate. The K_M values present that the aromatic chromophores facilitate the reaction due to the interactions with aromatic residues in the active site of the enzyme. Furthermore, G₇ and longer oligomers are able to model properly the HSA catalysed starch hydrolysis reaction.

Table 3: Summary of K_M values of HSA-catalysed hydrolysis reactions on free and chromophore-containing maltooligomer substrates measured by ITC

	G ₃	G ₄	G ₆	G ₇	G ₈	Amylose	Starch
mM	3.4	2.4	1.4	1.2	1.1		
mg/mL	1.7	1.6	1.4	1.4	1.4	1.4	1.4
	GalG ₂ CNP	CNP-G ₄	CNP-G ₆	CNP-G ₇	CNP-G ₈		Starch azure
mM	2.9	2	1	0.6	0.6		
mg/mL	1.9	1.6	1.1	0.8	0.9		0.7

Besides the above written kinetic studies I investigated the effect of heat treatment on the digestibility of starch using HSA and PPA. I concluded that cooking improves the digestibility of starch based on the obtained rate values.

3.2. I compared the PPA and HSA catalysed starch and starch azure hydrolysis reactions and based on the results I suggest the modification of the official recommendations.

Starch azure is an alternative choice between the natural starch substrate and the well detectable modified maltooligomer derivatives. Substrate inhibition was observed at the HSA and PPA catalysed starch azure hydrolysis, which can alter the measurement even at the concentration range recommended by the manufacturer (Sigma). Fitting curve to the data using Haldane equation (Figure 2B), I determined the K_i values both PPA ($K_i = 16.4$ mg/mL) and HSA ($K_i = 49$ mg/mL), respectively. On the basis of the above written effect of heat treatment and substrate inhibition, the modification of official method about the kinetic investigations with starch azure was suggested.

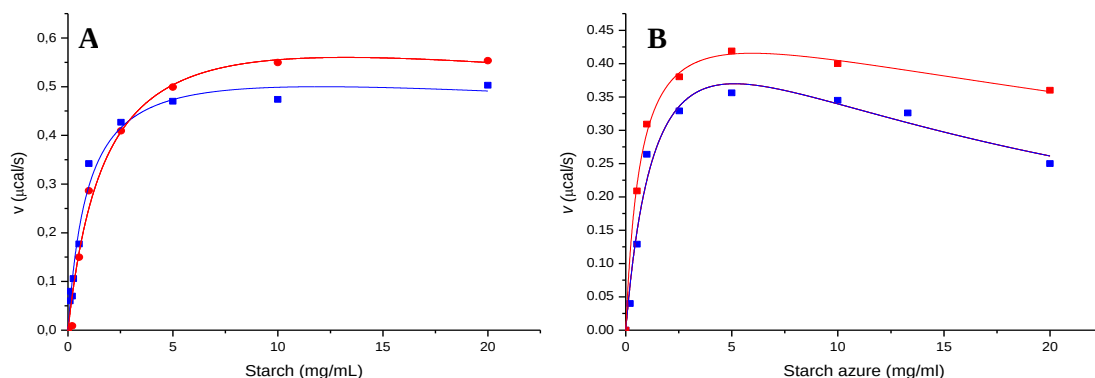


Figure 2: Substrate concentration dependence of rate values of starch (A) and starch azure (B) HSA (upper curve) and PPA (lower curve) catalysed hydrolysis reaction.

3.3.I verified the suitability of our method to investigate enzyme reactions on hardly soluble and gelatinous substrates using an industrial enzyme mixture.

Since inhomogeneity, high viscosity and intense colour do not disturb the measurement, the ITC-based method is suitable for investigation enzyme reactions working on badly soluble, viscous and/or colourful substrates solutions. I investigated the substrate concentration dependence of a general and an industrial pectinase (Pectinex ULTRA AFP) in highly viscous, gelatinous solutions. Both enzyme products contain multiple enzyme activity, thus to compare these I determined the apparent K_M values (K_M') at two different temperatures. The industrial enzyme product contains cellulose, which is in synergism with pectinase. Therefore, I investigated the cellulose activity using the same conditions and determined the K_M' values in the same two temperatures. The results are summarised in Table 4, which show well the effect of temperature on the K_M 's and reaction rate values. K_M values of enzyme reactions are generally increases at higher temperature, indicating that the conformation changes caused by the intensified thermal motions make the substrate binding weaker.

Table 4. K_M' and v_{max}' values of degrading reactions catalysed by pectinase and cellulase from Pectinex Ultra AFP (25 and 45°C)

	Temperature	Cellulase	Pectinase
K_M' (mg/mL)	45 °C	18.3±2.5	10.1±3.5
	25 °C	3.9± 0.4	2.1±0.3
v_{max}' (μcal/sec)	45 °C	4.2±0.4	5.4±0.1
	25 °C	0.6±0.1	1±0.3

4. I verified that the method is suitable for investigate enzyme inhibition properties of complex solutions and drug-like compounds.

A potential opportunity in diabetes therapy is the inhibition of carbohydrate digesting enzymes, but the available drugs have several undesirable side-effects. The research for new natural and synthetic components is continuous, with which the same effect can be achieved or which are applicable as complementary therapy.

4.1. Using a well-known glycoside hydrolase inhibitor and parallel measurements, I confirmed that the ITC method can be used in inhibition studies.

Acarbose is the active ingredient of commercially available Glucobay pills prescribed to heal diabetes. It inhibits carbohydrate degrading enzymes (α -glucosidase, α -amylase) hence it can reduce the releasing and absorbing amount of glucose. Using GalG₂CNP-, CNP-G₇-, starch-HSA and starch-PPA systems I determined the IC₅₀ values and compared with data from the literature, which were in good agreement. In case of starch-HSA and starch-PPA systems I determined the K_i values with Dixon plot, which were 9 μ M and 14 μ M, respectively. I also measured the binding constant of acarbose with ITC, which was 0.85 $\pm$ 0.56 μ M at acarbose-PPA system.

4.2. I measured the water soluble antioxidant capacity and α -amylase inhibition properties of crude water extracts made of sixty spice and herbal medicine.

Several medicinal plants are used in folk medicine to regulate blood sugar level. I investigated the HSA inhibition potential of 60 plant extracts using ITC and 0.14% starch substrate. The results (Figure 3) show that the leaves of different types of berries, green tea, cinnamon and allspice contains such active ingredients, which have significant α -amylase inhibition activity (IC₅₀ < 1.2 mg mL⁻¹).

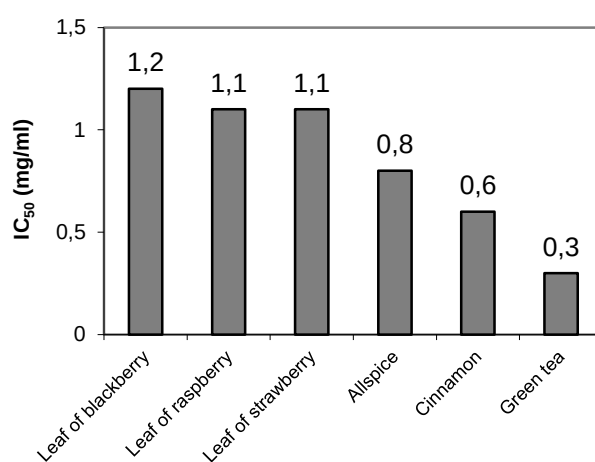


Figure 3. IC₅₀ values of plant extracts having the best inhibitor potency on HSA catalysed starch hydrolysis reaction

With ACW method I measured the water soluble antioxidant capacity of each extract relative to ascorbic acid standard. Between the obtained IC₅₀ and antioxidant capacity values a weak correlation was recognised, which may be caused by the phenolic components identified on the mass spectra, as well.

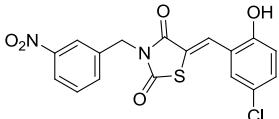
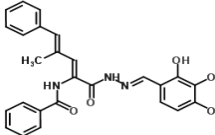
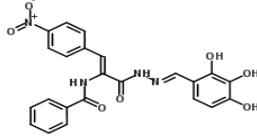
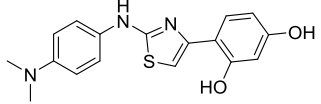
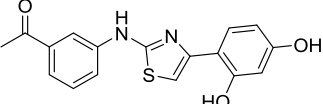
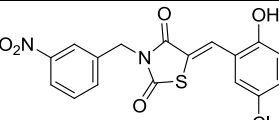
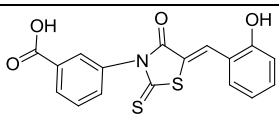
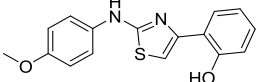
4.3.I applied the ITC method to examine HSA inhibition efficiency of badly soluble, apolar compounds in an organic solvent containing aqueous system.

Water miscible organic solvent in ITC measurement can be applied only with caution due to their low boiling point, volatility and the remarkable heat change occurs during the mixing.

In cooperation with a research group from the Freie Universität I tested several potential α -amylase inhibitor using spectrophotometry and ITC. The investigated ~50 compounds were selected after docking studies. The majority of these were badly soluble in water, thus the inhibition studies were carried out in 2% DMSO containing buffer in two stages. In the first step I measured the inhibition efficiency of each compounds on 5 nM HSA catalysed short chromogen substrate (3.75 mM GalG₂CNP) hydrolysis with spectrophotometer. The compounds showed good inhibition were tested further with ITC using 0.14 % starch substrate.

8 out of 50 compounds presented significant inhibition on GalG₂CNP substrate (IC₅₀ $\leq$ 100 μ M), but every eight showed slight effect using starch substrate. These results summarised in Table 5 show that short, chromophore containing substrates used in clinical diagnostics for activity measurements do not serve reliable information about the efficiency of the inhibitor in natural circumstances.

Table 5. Comparison of IC_{50} values of chosen drug-like inhibitors measured on GalG₂CNP and starch substrates

Investigated inhibitor		IC_{50} (μ M)	
No	Structure	GalG ₂ CNP (UV-VIS)	Starch (ITC)
18a		85	>650
20a		70	>400
20h		100	N.D.
26		100	>800
27		100	>1000
30		50	>300
31		62	>400
38		40	>400

IV. Possible utilisation of the results

With our project we expanded the techniques available for the researchers take part in carbohydrate chemistry and biochemistry research. Due to the high price and the limited application, ITC is not a routine procedure, but the number of publications deal with ITC is steadily increasing.

Based on our results this method exceeds the classic applications, which is proved by the growing number of studies focus on development of enzyme kinetic methods using ITC. The reason is the universality of the method, hence in enzyme kinetics the sensitive detection of substrates or products means one of the main problems. The method we described could be a useful alternative to replace other ITC-based methods, which application is often difficult and the optimisation is unnecessarily long. This technique is robust enough to be applicable in the case of inhomogeneous enzymatic systems and it can takes the enzyme kinetic and inhibition studies more flexible, time-saving and cost-efficient. Moreover, this method provides an opportunity to test the enzymes on their own natural substrates, which studies serve more reliable information about the efficiency of inhibitors in the natural circumstances. Comparison of IC_{50} values obtained with short chromogen substrate and starch reflects well this difference.

The results of antigen-antibody assay contribute to the development a new human serum albumin binding chromatography column facilitating the investigation of low-concentration proteins isolated from human serum. In hope of another new application was carried out the binding measurement with cationic cyclodextrin derivatives. The obtained results will redound the design of new, more effective, complexing compounds.

Publications

(Publications related to the thesis)

Melinda Andrási, Gábor Lehoczki, Zoltán Nagy, Gyöngyi Gyémánt, András Pungor, Attila Gáspár: **A comparative study of capillary electrophoresis and isothermal titration calorimetry for the determination of binding constant of human serum albumin to monoclonal antibody.**

Electrophoresis, **2015**;36: 1274–1281. (IF.: 2,482)

Jamil Al-Asri, Erika Fazekas, Gábor Lehoczki, Andrej Perdih, Cornelia Görick, Matthias F. Melzig, Gyöngyi Gyémánt, Gerhard Wolber, Jérémie Mortier. **From Carbohydrates to Drug-Like Fragments: Rational Development of Novel α -Amylase Inhibitors.**

Bioorganic&medicinal chemistry, **2015**;23: 6725-32. (IF: 2,923)

Gábor Lehoczki, Kármén Szabó, István Takács, Lili Kandra & Gyöngyi Gyémánt. **Simple ITC method for activity and inhibition studies on human salivary α -amylase.**

Journal of Enzyme Inhibition and Medicinal Chemistry. **2016**;6:1648-53. (IF: (2015) 3,428)

Jamil Al-Asri, Gyöngyi Gyémánt, Erika Fazekas, Gábor Lehoczki, Matthias F. Melzig, Gerhard Wolber, Jérémie Mortier. **α -Amylase Modulation: Discovery of Inhibitors Using a Multi-Pharmacophore Approach for Virtual Screening**

ChemMedChem. **2016**. DOI: 10.1002/cmdc.201600427 (IF: (2015) 2,98)

Publications do not related to the thesis

Tamás Gáll; Gábor Lehoczki; Gyöngyi Gyémánt; Tamás Emri; Zsuzsa M. Szigeti; György Balla; József Balla; István Pócsi. **Optimization of desferrioxamine-E production by *Streptomyces parvulus*.**

Acta Microbiologica et Immunologica Hungarica **2016**;63:475–489. (IF: (2015)0,568)

Virág Kiss, Gábor Lehoczki, Katalin Ósz. **Mathematical description of pH-stat kinetic traces measured during photochemical quinone decomposition**

Photochemical and Photobiological Sciences **2016**. DOI: 10.1039/C6PP00333H ((IF: (2015) 2,235)

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Lehoczki Gábor, Gyémánt Gyöngyi. **ITC: Új alternatíva enzim kinetikai vizsgálatokhoz**

Tavaszi szél konferencia 2014. március 21-23., Debrecen

Gábor Lehoczki, Gyöngyi Gyémánt. **Application of microcalorimetry for inhibition study of HSA**

Annual meeting of the Working Committee for Carbohydrates, Nucleic Acids and Antibiotics of the Hungarian Academy of Sciences, 2014. május 21-23. Mátraháza

Lehoczki Gábor, Szabó Kármén, Takács István, Gyémánt Gyöngyi. **Gyors ITC módszer enzimreakciók vizsgálatához**

Doctoral Workshop 2015. október 10-11., Pécs

Gábor Lehoczki, KláraPető, István Takács, Gyöngyi Gyémánt, Renátó Kovács. **Anti-amylase and antifungal effect of common herbs and spices.**

8th Central European Conference "Chemistry towards Biology" 2016. augusztus 28 – szeptember 1., Brno

Gábor Lehoczki, Kármén Szabó, Gyöngyi Gyémánt. **Screening and classifying plant derived inhibitors for carbohydrate metabolism enzymes.**

Annual meeting of the Working Committee for Carbohydrates, Nucleic Acids and Antibiotics of the Hungarian Academy of Sciences, 2017. május 31- június 2., Mátraháza

Posters related to the thesis

Gábor Lehoczki, Gyöngyi Gyémánt. **Kinetic investigation of human salivary amylase using microcalorimetry**

11th International Interdisciplinary Meeting on Bioanalysis, 2014. október 20-22., Brno

Melinda Andrási, Gábor Lehoczki, Zoltán Nagy, Gyöngyi Gyémánt, András Pungor, Attila Gáspár. **Determination of Binding Constant of Human Serum Albumin to Monoclonal Antibody using Capillary Electrophoresis and Isothermal Titration Calorimetry.**

10th Balaton Symposium on High-Performance Separation Methods, 2015. szeptember 2-4., Siófok

Gyöngyi Gyémánt, Judit Remenyik, Gábor Lehoczki, Lóránt Jánossy, Lili Kandra. **20 years of studies on human salivary amylase. Overview of methodology and results**

Debreceni Szénhidrát Kollokvium 2015 – Lipták András Emlékkonferencia, 2015. nov. 6-8.



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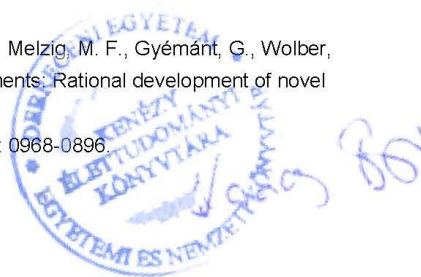
DEENK/260/2017.PL
PhD Publikációs Lista

Candidate: Gábor Lehoczki
Neptun ID: A3POII
Doctoral School: Doctoral School of Chemistry
MTMT ID: 10054367

List of publications related to the dissertation

Foreign language scientific articles in international journals (4)

1. **Lehoczki, G.**, Szabó, K. A., Takács, I., Kandra, L., Gyémánt, G.: Simple ITC method for activity and inhibition studies on human salivary α -amylase.
J. Enzym. Inhib. Med. Chem. 31 (6), 1648-1653, 2016. ISSN: 1475-6366.
DOI: <http://dx.doi.org/10.3109/14756366.2016.1161619>
IF: 4.293
2. Al, A. J., Gyémánt, G., Fazekas, E., **Lehoczki, G.**, Melzig, M. F., Wolber, G., Mortier, J.: α -amylase modulation: discovery of inhibitors using a multi-pharmacophore approach for virtual screening.
ChemMedChem. 11 (21), 2372-2377, 2016. ISSN: 1860-7179.
DOI: <http://dx.doi.org/10.1002/cmdc.201600427>
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3. András, M., **Lehoczki, G.**, Nagy, Z., Gyémánt, G., Pungor, A., Gáspár, A.: A comparative study of capillary electrophoresis and isothermal titration calorimetry for the determination of binding constant of human serum albumin to monoclonal antibody.
Electrophoresis. 36 (11-12), 1274-1281, 2015. ISSN: 0173-0835.
DOI: <http://dx.doi.org/10.1002/elps.201400513>
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4. Al, A. J., Fazekas, E., **Lehoczki, G.**, Perdihi, A., Görick, C., Melzig, M. F., Gyémánt, G., Wolber, G., Mortier, J.: From carbohydrates to drug-like fragments: Rational development of novel α -amylase inhibitors.
Bioorg. Med. Chem. 23 (20), 6725-6732, 2015. ISSN: 0968-0896.
DOI: <http://dx.doi.org/10.1016/j.bmc.2015.09.007>
IF: 2.923





List of other publications

Foreign language scientific articles in Hungarian journals (1)

5. Gáll, T., **Lehoczki, G.**, Gyémánt, G., Emri, T., Máthéné Szigeti, Z., Balla, G., Balla, J., Pócsi, I.:
Optimization of desferrioxamine E production by *Streptomyces parvulus*.
Acta Microbiol. Immunol. Hung. 63 (4), 475-489, 2016. ISSN: 1217-8950.
DOI: <http://dx.doi.org/10.1556/030.63.2016.029>.
IF: 0.921

Foreign language scientific articles in international journals (1)

6. Kiss, V., **Lehoczki, G.**, Ősz, K.: Mathematical description of pH-stat kinetic traces measured
during photochemical quinone decomposition.
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IF: 2.344

Total IF of journals (all publications): 16,188

Total IF of journals (publications related to the dissertation): 12,923

The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of Web of Science, Scopus and Journal Citation Report (Impact Factor) databases.

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