DATA FROM THE FIELD ON BIOMOLECULAR INTERACTIONS

Minisymposium

"Jožef Stefan"
Institute
Ljubljana, Slovenia

Virtual event, 25th November 2021

Minisymposium

Data from the field on biomolecular interactions

Organizer

Department of Biotehnology, Jožef Stefan Institute

Nanotemper

Editors

Prof. dr. Janko Kos

Dr. Ana Mitrović

Technical editor

Dr. Ana Mitrović

Issued by

Department of Biotehnology, Jožef Stefan Institute, Ljubljana, Slovenia

Online edition

http://www.sbd.si/dokumenti/Nanotemper_minisymposium_book_of_abstracts1.pdf

Ljubljana, 25th November 2021

CIP – Kataložni zapis o publikaciji

Narodna in univerzitetna knjižnica, Ljubljana

Overview

Title: Data from the field on biomolecular Interactions

Date: Thursday, November 25, 2021

Time: 10:00 AM Central European Time

Duration: 1 hour, 30 minutes

Summary

Join us for a virtual meeting and find out how Nanotemper's users from Slovenia are using our technologies for measuring molecular interactions. Three different speakers, three different topics, reveal how you can study binding affinity in varied systems.

Agenda

10:00 -10:05 Introduction

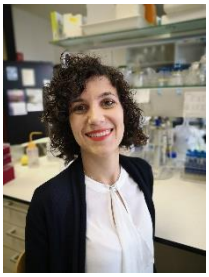
10:05 - 10:25 **Evaluation of new inhibitors of cathepsin V as potential antitumor agents.** Dr. Ana Mitrović, Department of Biotechnology, Jožef Stefan Institute, Ljubljana, Slovenia

10:30 - 10:50 **The binding of small inhibitor 2-hydroxyquinoline to paraoxonase 1: A comparison of spectroscopic and MST data.**
Boštjan Petrič, Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana, Slovenia

10:55 - 11:15 **Introduction of Lanthanide ion into the active site of calcium dependent enzyme.**
Dr. Ajda Taler Verčič, Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana, Slovenia

11:20 - 11:30 Q&A and Concluding remarks

Speakers



Dr. Ana Mitrović
Postdoctoral Researcher
Department of Biotechnology, "Jožef Stefan" Institute, Ljubljana, Slovenia

Ana Mitrović is postdoctoral researcher at Department of Biotechnology, "Jožef Stefan" Institute, Ljubljana, Slovenia. Her research interests are cysteine cathepsins cancer, especially in cancer stem cells and epithelial-mesenchymal transition, and identification and characterization of their new inhibitors for improvement of antitumor therapy. During her research career she contributed to several scientific papers, for her work she received awards and presented her work as lectures and posters at international conferences.

Boštjan Petrič
Young Researcher
Faculty of Medicine, University of Ljubljana

Boštjan Petrič is a Young Researcher at the Faculty of Medicine in Ljubljana. The focus of his research work is the connection between the enzyme paraoxonase 1 and different forms of dementia, as well as the development of improved methods for determining enzyme-kinetic parameters from progress curves.

Dr. Ajda Taler-Verčič
Postdoctoral fellow
Institute of Biochemistry and Molecular Genetics, Medical Faculty, University of Ljubljana, Slovenia

Dr. Ajda Taler-Verčič is a postdoctoral fellow at the Institute of Biochemistry and Molecular Genetics at the Medical Faculty at the University of Ljubljana, Slovenia. She is working in the group for Enzyme research. She received her PhD in 2014 at The Jožef Stefan International Postgraduate School. Her research is focused on proteins, from structural, functional and pathological aspects.

Evaluation of new inhibitors of cathepsin V as potential antitumor agents

Ana Mitrović[1], Emanuela Senjor[1,2], Marko Jukić[2], Lara Bolčina[1,2], Mateja Prunk[1], Matic Proj[2], Milica Perišić Nanut[1], Stanislav Gobec[2], Janko Kos[1,2]

[1]Department of Biotechnology, Jožef Stefan Institute, Jamova 39, 1000 Ljubljana, Slovenia
[2]Faculty of Pharmacy, University of Ljubljana, Aškerčeva cesta 7, 1000 Ljubljana, Slovenia

Cathepsin V is a human lysosomal cysteine peptidase highly related to cathepsin L. However, it differs in tissue distribution, binding site morphology, substrate specificity, and function. In physiological conditions expression of cathepsin V is manly limited to thymus, testis and corneal epithelium, while its elevated levels have been associated with various pathological processes, including cancer. Considering its role in pathological processes, targeting cathepsin V with selective inhibitors opens new opportunities for therapeutic treatments. Therefore, to extend the number of cathepsin V inhibitors, interactions of small molecular compounds from commercial libraries with cathepsin V were evaluated by molecular docking and subsequent biochemical evaluation. Inhibition of cathepsin V with selected compounds was evaluated by using enzyme kinetics and microscale thermophoresis. Moreover, the effect of compounds was evaluated *in vitro* functional assays of processes in which cathepsin V is known to play an important role such cell proliferation, degradation of elastin and effect of cytotoxicity of immune cells. During this study we identified ureido methylpiperidine carboxylate derivatives as the most potent inhibitors of cathepsin V with the significant effect on processes of tumor progression where cathepsin V is involved.

The binding of small inhibitor 2-hydroxyquinoline to paraoxonase 1: A comparison of spectroscopic and MST data

Boštjan Petrič[1], Marko Goličnik[1] and Aljoša Bavec[1]

[1]University of Ljubljana, Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, Vrazov trg 2, SI-1000 Ljubljana, Slovenia

Paraoxonase 1 (PON1) is an important mammalian enzyme, present in blood and several other bodily fluids, usually as part of the HDL complex. It is a promiscuous enzyme whose function can be investigated in vitro with several different types of substrate, while in vivo its main function is considered to be antioxidative, i.e. the breakdown of oxidized lipids. To investigate the enzyme's properties, recombinant soluble forms (rePON1) are often preferred, such as the G2E6 version, first described by Aharoni et al. (2004). One promising avenue of study are specific inhibitors of PON1, among which the small molecular inhibitor 2-hidroxyquinoline (2HQ) is probably best known. We measured the binding constant Kd for the binding of rePON1 to 2HQ with MST, and then compared these results with the constant of inhibition (Ki). To measure Ki, we used the classical enzymatic approach of spectroscopically measuring time-concentration progress curves for the reaction in the absence and presence of the inhibitor. For analysis of the progress curves, we then used the program iFIT (available at http://www.i-fit.si), developed by our group. The program is based on an algorithm for removing unnecessary data points from progress curves, described by Stroberg and Schnell (2016).

Sources:

- Aharoni, A., Gaidukov, L., Yagur, S., Toker, L., Silman, I., & Tawfik, D. S. (2004). Directed evolution of mammalian paraoxonases PON1 and PON3 for bacterial expression and catalytic specialization. Proceedings of the National Academy of Sciences of the United States of America, 101(2), 482–487
- Stroberg, W., & Schnell, S. (2016). On the estimation errors of KM and V from time-course experiments using the Michaelis–Menten equation. Biophysical Chemistry, 219, 17–27.

Introduction of lanthanide ion into the active site of calcium dependent enzyme

Ajda Taler-Verčič[1], Janez Smerkolj[1], Aljoša Bavec[1], Marko Goličnik[1]

[1]Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

Calcium binding proteins are involved in several biological processes. Lanthanide ions La3+ such as Tb3+ and Eu3+ have similar coordination properties to Ca2+ in protein environment, nevertheless their binding affinities to structurally different sites can be variable. Furthermore, some binding sites have even higher affinity to La3+ than to Ca2+ ions (Ye, Lee et al. 2005, Tang, Deng et al. 2020).
We are establishing the protocol to evaluate the exchange of Ca2+ ion with the La3+ ion inside the active site of model enzyme. The binding of alternative ion is assessed by the impact on protein stability; the site specific binding is probed by enzyme kinetics; and the overall structure preservation is evaluated by reverse process – restoring the enzyme activity after re-addition of Ca2+ into the active site of an enzyme.

Tang, S., X. Deng, J. Jiang, M. Kirberger and J. J. Yang (2020). "Design of Calcium-Binding Proteins to Sense Calcium." Molecules 25(9).
Ye, Y., H. W. Lee, W. Yang, S. Shealy and J. J. Yang (2005). "Probing site-specific calmodulin calcium and lanthanide affinity by grafting." J Am Chem Soc 127(11): 3743-3750.