# Serbian Biochemical Society Eighth Conference

with international participation

University of Novi Sad – Rectorate Hall 16.11.2018. Novi Sad, Serbia

"Coordination in Biochemistry and Life"

### Foreword

Dear Colleagues

Welcome to the 8<sup>th</sup> Conference of the Serbian Biochemical Society, entitled "Coordination in Biochemistry and Life".

The title of this year's Conference refers to an important place of coordination chemistry in biochemistry and biomedicine, but also to a need to coordinate the efforts towards new knowledge with fellow scientists from other fields in order to reach more. The collaboration within FEBS3+ (Croatia, Hungary, Slovenia, and Serbia) Meeting Programme continues with the invited lecture of our dear colleague Tantos Ágnes from Research Center for Natural Sciences, Budapest, Hungary. For the first time we have 'Diaspora Lecture' that will be delivered by Miloš Filipović, a top 'product' of Serbian biochemistry who is now affiliated at the Université de Bordeaux. We have more than forty PhD students from Serbia, Hungary, and Belarus with poster presentations, and for the first time the Conference is held outside the capital. It believe that we are getting better each year, and that we are prepared for future challenges.

I would like to express my gratitude to the members of the Scientific Board who suggested lecturers, to all respected colleagues who accepted the invitation, and to our dear hosts from the University of Novi Sad.

Editor of the Proceedings Ivan Spasojević

# Production, purification and structural characterisation of recombinant BanLec-Bet v 1

## Isidora Protić-Rosić<sup>1\*</sup>, Milica Popović<sup>1</sup>, Uroš Anđelković<sup>2</sup>, Marija Gavrović-Jankulović<sup>1</sup>

<sup>1</sup>Department of Biochemistry, Faculty of Chemistry, University of Belgrade, Belgrade, Serbia

<sup>2</sup>Institue of Chemistry, Technology and Metallurgy, University of Belgrade

\*e-mail: i.proticrosic@gmail.com

The sublingual route of allergens administration in allergen-specific immunotherapy (ASIT) is proven to be a successful way to treat patients with respiratory allergy. The trend of replacing natural extracts with purified recombinant allergens is growing. Although the purified allergens themselves are not good immunogens, the combined vector systems and adjuvans can improve their immunogenicity <sup>1</sup>. Cell surfaces are decorated by different glycan structures, so the lectins specific for these glycans can be used to deliver particular therapeutic to target specific tissue <sup>2</sup>. Banana lectin (BanLec) is mannose-specific protein which belongs to the subfamily of Jacalin-related lectins <sup>3</sup>. Apart from its characteristic to bind glycans, BanLec also modulates immune cells *in vitro* <sup>4</sup>. On the other hand, Bet v 1 (*Betula verrucosa*) is the major birch pollen allergen. T-cell epitops are distributed over almost entire protein structure <sup>5</sup>.

In the study the recombinant BanLec-Bet v 1 construct is designed, produced by the recombinant DNA technology, purified and characterized by classical biochemical methods for the application in the ASIT of birch pollen allergy.

The expression of newly designed BanLec-Bet v 1 was performed in *E. coli* BL21 (DE3). After expression the protein was found in the inclusion bodies from which it was extracted with 4 M urea solution. After renaturation, affinity chromatography (Sephadex G-75 superfine) was used for protein purification. Biochemical characterization of the chimera was performed by: SDS PAGE electrophoreses, CD spectroscopy and mass spectrometry. Biological activity of the construct was confirmed by binding of BanLecBet v 1 to a horseradish peroxidase glycoprotein in ELISA. Purified BanLec-Bet v 1 showed molecular mass of 32 kDa. CD spectra of the recombinant construct revealed well defined secondary structures with predominant beta sheets (41.2%). By mass spectrometry 51.8% of the BanLec-Bet v 1 primary structure was confirmed.

Biologicaly active recombinant BanLec-Bet v 1 was produced by the recombinant DNA technology. Further *in vitro* and *in vivo* studies will evaluate immunomodulatory potential of BanLec-Bet v 1 for application in ASIT.

#### Acknowledgements

This study was supported by the Ministry of Education, Science and Technological Development (Grant No. 172049)

### References

- 1. Tourdot S, et. al. Efficacy of sublingual vectorized recombinant bet v la in a mouse model of
- birch pollen allergic asthma. Vaccine 2013;31:2628-37. Dimitrijevic R, Jadranin M, Burazer L, Ostojic S, Gavrovic-Jankulovic M. Evaluation of the thermal stability and digestibility of heterologously produced banana lectin. Food Chem 2. 2010;120:1113-8.
- 3. Dimitrijevic R, Stojanovic M, Micic M, Dimitrijevic Lj, Gavrovic-Jankulovic M. Recombinant banana lectin as mucosal immunostimulator. J Funct Food 2012;4: 636-41.
- 4. Sansone A, Sansone M, Dias C, Nascimento J. Oral administration of banana lectin modulates cytokine profile and abundance of T-cell populations in mice. Int J Biol Macromol 2016;89:19-24.
- 5. Gajhede M, et al. X-Ray and NMR structure of Bet v 1, the origin of birch pollen allergy. Nat Struct Biol 1996;3:1040-5.