2nd FoodEnTwin Workshop
Experimental animal models for food and environment
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Book of Abstracts

Session 1: Mouse and rat models for food allergy

EoodEnTwin highlights **Tanja Cirkovic Velickovic** **University of Belgrade**

The objective of FoodEnTwin (http://horizon2020foodentwin.rs/) is to create a networking collaboration among the University of Belgrade – Faculty of Chemistry (UBFC) and its Center of Research Excellence for Molecular Food Sciences (CoE MFS) and four high renowned institutes from Sweden (Karolinska Institutetl), Austria (Medical University of Vienna) and Belgium (KULeuven and Ghent University) providing a unique opportunity for UBFC and its partners to increase their scientific excellence and visibility, technology innovation capacity and enable frontier research at the crossroad of food, agriculture, chemistry, nutrition and environmental sciences by the infusion of –Omics technologies and experimental animal models. The project focuses on the key target actions of twinning of research activities through networking, training and a lecturing program resulting in a roadmap for a future collaboration, organization of four public Summers Schools, internal and external expert-driven Academia-Industry meetings, two workshops, and finally, bringing the European Food Chemistry conference (EuroFoodChem) in 2021 to the UBFC in Serbia to increase the UBFC, the Serbian and the European visibility in the fields of food sciences. The scientific topic addresses the major challenge of how environmental pollution affects the food we eat at the molecular level. The project will have a significant societal impact. Our dissemination approach will present our networking ideas to a broad public, from experts, the science community and industry stakeholder organization, to interested, non-professionals, making society more aware of the impact that environment has on food and the importance of new approaches in food, nutrition and environmental sciences. The aim of this 3-year project is to use cutting-edge -omics technologies (proteomics, transcriptomics, digestomics, allergomics, metalomics and lipidomics) and experimental animal models to address the challenges in food, nutrition and environmental sciences in a way that enables the creation of a pan-European research network through the twinning research activities in this project..To achieve the objectives of the FoodEnTwin project, the consortium partners have implemented a comprehensive set of measures within the project's key work packages (WPs): Short term staff exchanges; (WP1), Training workshops, and summer schools; (WP2), Dissemination and outreach. (WP3)

Rat models of food allergy **Katrine Lindholm Bøgh** **Danish Food Institute**

In this lecture, I will present an overview of the different parameters that you will need to consider when designing animal experiments. I will give examples of the impact of factors related i) to the proteins, such as dose-response relationship, protein preparation and processing, ii) to the host such as stain, gender and disease status, iii) to the environment such as diet, microbiome and housing condition, and iv) to the experimental design, such route of administration, use of adjuvant and end-point analyses. Allergenicity assessment of novel foods is a difficult task, and not animal models have been validation for such allergenicity assessment. This lecture will provide an example of how animal models can be used in the evaluation of the *de novo* sensitising as well as in cross-reaction capacity. Finally, as brief overview of how animal models can be used for preclinical assessment of new prevention and treatment strategies within food allergy.

Mouse models of food allergy **Karine Adel-Patient** **CEA**

In this lecture, we will have a view of mouse models of food allergy. which are not restricted to gastrointestinal sensitization, then mimicking the real life conditions. I'll present some of our own models and experiences, placing them in other published preclinical and clinical data.

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Bovine γ-Globulin and Lactoperoxidase as major milk allergens among a mammalian meat allergic population

Marija Perusko **University of Belgrade**

Objective. Mammalian meat allergy (MMA) is a severe form of food allergy with delayed symptoms where the IgE antibodies are directed against a carbohydrate epitope, galactose-α-1,3-galactose (α-Gal). Many MMA patients report allergic symptoms upon consumption of milk or dairy products. The aim of the project was to investigate the allergenicity of bovine milk proteins in a MMA population. Material and Methods. Adults with diagnosed MMA (n = 34) were recruited and their sIgE levels to α-Gal, beef and milk were analyzed by ImmunoCAP. Milk proteins were assayed by immunoblot and inhibition ELISA for the presence of the α-Gal epitope and for the binding to mammalian meat allergic patients' IgE. Additionally, capacity of whole milk and milk proteins to activate basophils of MMA patients was tested. Results. Thirty-three out of 34 MMA patients were IgE positive to milk, and their IgE levels to milk were lower than those to α-Gal or beef. Significant correlations between IgE levels to milk and α-Gal (rs=0.55, $P < 0.001$), as well as between milk and beef (rs=0.77, $P < 0.001$) were observed. Immunoblot analysis of milk proteins revealed bovine γ-globulin (BGG) as α-Gal carrier. Other tested milk proteins, α-lactalbumin, β-lactoglobulin, α-casein, β-casein and κ-casein were negative for the presence of α-Gal epitope. BGG was also shown to bind IgE of MMA patients in immunoblot analysis. ELISA experiments showed that whey proteins, BGG and also lactoperoxidase (LPO) exerted a dose-dependent inhibition of MMA patients' IgE binding to α-Gal indicating presence of the α-Gal epitope in these proteins. Importantly, activation of MMA patient's basophils by milk, BGG and LPO was demonstrated. Conclusions. BGG and LPO were identified as α-Gal carrying proteins in milk that bound IgE antibodies and furthermore activated basophils of mammalian meat allergic patients. The study highlights the importance of milk as an allergenic food source among the MMA population. Acknowledgments: This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, grant number 172024 and by EAACI (MP was awarded a Research Fellowship). The project leading to this application has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 810752.

Development of an immune-polymerase chain reaction for detection and quantification of shellfish tropomyosin

Mirjana Radomirovic **University of Belgrade**

Food allergies represent important health problem in industrialized countries, with seafood being recognized as one of the 8 most common sources of allergens. While there are several proteins that have been linked to shellfish allergy, tropomyosin accounts for majority of diagnozed ingestion-related shellfish allergies. Presence of even traces of allergens in food can be a serious health hazard to consumers, which is why proper labeling of food products by food manufacturers is of critical importance for sensitized persons. On the other hand, development of reliable, specific and sensitive methods for detection and quantification of allergens in food products is of the high importance as well. The objective of this study was to develop highly sensitive immuno-polymerase chain reaction (immuno PCR) method for the detection and quantification of shellfish tropomyosin in food samples. Immuno PCR method couples standard sandwich enzyme-linked immunosorbent assay (ELISA) format with real time PCR. Monoclonal antibody was used as capture antibody, while biotinylated polyclonal antibody served as detection antibody. Reporter biotinylated DNA was coupled to detection antibody via streptavidin and subsequently amplified and quantified by real time PCR. Tropomyosin was quantified using highly purified natural shrimp tropomyosin as standard. The results were compared to standard sandwich ELISA. This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, grant number 172024. The project leading to this application has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No 810752.

Discrete Hf18 metal-oxo cluster as a nanozyme for site-specific protein hydrolysis

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Effective heterogeneous catalysts for the controlled transformation of large and complex biomolecules are rare and challenging to develop. In particular, selective hydrolysis of proteins by non-enzymatic catalysis is difficult to achieve, yet it is crucial for many modern applications in biotechnology and proteomics. Herein we report that discrete hafnium metal-oxo cluster ($Hf_{18}O_{10}(OH)_{26}(SO_4)_{13}(H_2O)_{33}$) ($Hf_{18}$), which is centred by the same hexamer motif found in many MOFs, acts as a heterogeneous catalyst for the rapid hydrolysis of horse heart myoglobin (HHM) protein in low buffer concentrations. Remarkably, among 154 amino acids present in the sequence of HHM, strictly selective cleavage at only 6 solvent accessible aspartate residues, Asp5, Asp21, Asp45, Asp110, Asp123 and Asp142 was observed. Mechanistic experiments suggest that the hydrolytic activity is likely derived from the synergistic actuation of Hf^{4+} Lewis acidic sites and the Brønsted acidic surface