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SEMINAR OF THE DEPARTMENT OF BIOTECHNOLOGY, UNIVERSITY OF RIJEKA

Time: **Tuesday, December 10th, 2019 at 1 pm**
Location: **Room O-30, Radmile Matejčić 2 (University Departments)**

Tomislav Čaval, PhD
Post-doc
Utrecht University

Title: Native Mass Spectrometry of Glycoengineered Biotherapeutics

Abstract: Over 80% of approved biotherapeutics are glycosylated representing together a +200 billion $ market. Recombinant production of biotherapeutics (monoclonal antibodies, Erythropoietin (EPO), Lysosomal replacement enzyme…) in mammalian cells is limited by the cells capacity for N-glycosylation, and production of glycoproteins with robust, desirable homogeneous glycoforms remains a challenge. Recently, a wide-ranging knockout system targeting glycosyltransferase genes in CHO cells was reported in order to produce homogenous EPO. However, as EPO can exhibit hundreds of glycoproteoforms, thorough means are needed to assess the glycosylation profile in each engineered variant. In this talk, I will demonstrate how we use high-resolution native mass spectrometry to measure the glycoproteoform profiles of 24 glycoengineered EPO variants. The observed glycosylation profiles revealed co-occurrence of hundreds of variants ranging from EPOs exhibiting heterogeneous, tetra-antennary, polyLAcNAc elongated N-glycans all the way to EPOs with homogeneous, bi-antennary N-glycans. I will also focus on analysis wild type EPO and two glycoengineered EPO variants, mgat5 KO and *mgat 4A/4B* KO, which result in tri-antennary N-glycans stemming from the loss of β6- and β4-branch from tetra-antennary N-glycans, respectively. Additionally, I will introduce a novel classification system based on a unique mass, charge and intensity glycoproteoform profiles of each of 24 EPO variant, we classify them according to similarities in glycosylation profiles. This automated classification distinguishes EPO variants based on glycan branching, elongation, and sialylation, which are all crucial for biotherapeutic efficacy. Finally, additional examples focusing on glycoengineering of monoclonal antibodies and lysosomal replacement enzymes in order to improve their functionality will be presented.

Host: Dr. Ivana Munitić, Laboratory of Molecular Immunology, Department of Biotechnology, University of Rijeka