LIGHTNING TALK:

Dr. Charlotte Olagnon

Ultrahigh-throughput droplet-based microfluidic screening to discover new efficient enzymes that convert B type red blood cells to universal O type blood

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Out of all the blood types in the ABO blood group system, only the O type can be universally transfused to any patient. Thus, a substantial supply of O type red blood cells (RBCs) is essential in blood banks. While the cell surface of O type RBCs displays only H-antigen, A and B RBCs each present an additional terminal sugar antigen (GalNAc and Gal, respectively) on the core H-antigen structure. Therefore, conversion of A or B RBCs to O universal type RBCs by removal of the terminal sugars using suitable enzymes, namely glycosidases, has aroused considerable scientific interest over the years. Recently, by screening of a metagenomics library of the human gut microbiome using microtiter plate methodology and sensitive fluorogenic monosaccharide substrates, an efficient two-enzyme system that effected the conversion of A to O RBCs was discovered. This has proved to be a promising candidate for generating O type blood for patient transfusions. Despite the successful achievements in converting A to O type RBCs, advances towards conversion of B to O type blood remained limited due to the low activity of the enzymes available under physiological conditions.

In order to find new enzymes or enzyme classes operating with high substrate specificity on oligosaccharide substrates such as fluorogenic tetra- and pentasaccharide glycosides, much smaller reaction volumes are necessary since multi gram quantities would be required for the plate based approach. Therefore, application of droplet-based microfluidic technologies for screening, which offer considerable minimization of the reaction system defined then by a pico- or nano-liter size water-in-oil droplet, appeared essential.

To this end, a robust system for ultrahigh-throughput droplet-based microfluidic screening has been developed. First, a fluorogenic B antigen tetrasaccharide substrate derivative was efficiently enzymatically synthesized, containing the novel 3-carboxycoumarin, Jericho Blue (JB), a suitable fluorogenic aglycone for droplet-based screening approaches. Then, a coupled enzyme assay was established to identify enzymes with potent α-galactosidase activity on B antigen, and miniaturized. Droplet-based microfluidic screening was developed using an “On Chip” sorter and preliminary studies performed on the metagenomics library of the human gut microbiome. After validation and characterization of the hits, new candidate enzymes have been discovered. These preliminary results pave the way to screening of metagenomic libraries derived from different metagenome sources to increase the chances of novel enzyme hits that convert B type RBCs to universal O type blood, thereby ultimately widening the blood supply.