

## PS.100

**Single-Molecule Peptide Sequencing for Direct Detection of MHC Presented Neoantigens**

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Major histocompatibility complex I (MHC-I) molecules present short peptides from both self and non-self proteins to cytotoxic T cells. Neoantigens, which are peptide sequences containing somatic mutations, are promising targets for tumor immunotherapy and are commonly predicted using whole exome sequencing data. Directly identifying presented neoantigens would be highly beneficial for developing personalized patient therapies. Although mass spectrometry (MS) can directly identify tens of thousands of endogenously processed and presented peptides, its sensitivity is insufficient for detecting rare, low-abundance peptides on cell surfaces. To address this limitation, we explored a novel single-molecule peptide detection method specifically for mutated peptide sequences. In this approach, peptides are identified using their Raman scattering spectrum as a unique vibrational ‘fingerprint’ of the molecule. Silicon nanophotonic chips are designed to strongly amplify Raman scattering, enabling few-to-single molecule sensitivity. First, we obtained the Raman spectra of 40 peptide sequences from MC38 cells, including 20 wild-type and 20 mutated sequences. A neural network model was used to train a classifier capable of distinguishing peptide signals based on their amino acid composition. Importantly, we observed noticeable differences in the Raman spectra caused by single amino acid point mutations, showing that mutated peptides can be distinguished from their wild-type counterparts with over 96% average accuracy. We also demonstrated how peptides can be sequenced on-chip, using the dynamic changes in peptide Raman scattering during residue cleavage as an indication of peptide sequence. Then, we mixed known quantities of the 20 mutant peptides in a wildtype background. We could detect mutated peptides with >80% accuracy, provided no more than twenty distinct peptides bind to each sensor; the accuracy improves to >90% for ten or less peptides on each sensor. Subsequently, we searched for unique mutated fingerprints within an endogenous immunopeptidome isolated from IFN $\gamma$ -stimulated MC38 cells. MHC peptides were fractionated using high-performance liquid chromatography and printed onto our nanophotonic chip using picoLiter droplet ejection. The Raman signal of individual sensors was collected, showing the presence of mutated peptide sequences as well as self-peptides. These findings provide compelling initial evidence for the potential of single-molecule sequencing techniques in the field of antigen presentation.

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## PS.101

**Structural role of fucosylated and galactosylated N-glycans in the enzymatic activity of bovine intestinal alkaline phosphatase**

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Bovine intestinal alkaline phosphatase (biALP), a membrane-bound plasma metalloenzyme, maintains intestinal homeostasis, regulates duodenal surface pH, and protects against infections caused by pathogenic bacteria. It is a dimeric glycoprotein, consisting of two identical subunits containing Zn<sup>2+</sup> and Mg<sup>2+</sup> in the active site. The *N*-glycans of biALP regulate its enzymatic activity, protein folding, and thermostability, but their structures are not fully reported. In this study, biALP-containing gel pieces (2 × 2 mm) were excised from each electrophoresis lane, de-stained, and treated with PNGase F solution. The released *N*-glycans were purified from impurities using a graphitized carbon cartridge and derivatized with procainamide through reductive amination. The structures and quantities of the *N*-glycans of biALP were analyzed by liquid chromatography electrospray ionization-high energy collision dissociation tandem mass spectrometry. In total, 48 *N*-glycans were identified and quantified, comprising high-mannose [6 *N*-glycans, 33.1% (sum of relative quantities of each *N*-glycan)], hybrid (6, 11.9%), and complex (36, 55.0 %) structures [bi- (13, 26.1 %), tri- (16, 21.5 %), and tetra-antennary (7, 7.4 %)]. These included bisecting *N*-acetylglucosamine (33, 56.6 %), mono- to tri-fucosylation (32, 53.3 %), mono- to tri- $\alpha$ -galactosylation (16, 20.7 %), and mono- to tetra- $\beta$ -galactosylation (36, 58.5 %). No sialylation was identified. *N*-glycans with non-bisecting *N*-acetylglucosamine (9, 10.3 %), non-fucosylation (10, 13.6 %), non- $\alpha$ -galactosylation (26, 46.2 %), and non- $\beta$ -galactosylation (6, 8.4 %) were also identified. The activity (100 %) of biALP was reduced to 37.3 ± 0.2% (by de-fucosylation), 32.7 ± 2.9 % (by de- $\alpha$ -galactosylation), and 0.2 ± 0.2 % (by de- $\beta$ -galactosylation), comparable to inhibition by 10<sup>-4</sup> to 10<sup>1</sup> mM EDTA, a biALP inhibitor. These results indicate that fucosylated,  $\alpha$ -galactosylated, and  $\beta$ -galactosylated *N*-glycans are essential components for the full activity of biALP. Notably, when  $\beta$ -galactosylation was removed, the enzymatic activity of biALP was reduced by 98.8%, equivalent to treatment with 10<sup>1</sup> mM EDTA. This study is the first to identify 48 diverse *N*-glycan structures and quantities of bovine as well as human intestinal ALP and to demonstrate the importance of the role of fucosylation and galactosylation for maintaining the activity of biALP.

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