Single-molecule sequencing of immunopeptides and antigenic protein markers on the Platinum platform

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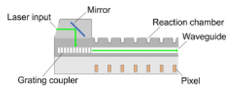
Abstract

- **Motivation**: **A single peptide-MHC complex can activate immune cells**; thus, single-molecule sequencing has the potential to provide the requisite sensitivity to elucidate the immunopeptidome and profile tumor-associated antigens.
- **Benchtop platform**: **Platinum reveals the primary structure of single protein molecules**, interrogating immunopeptides and proteins that elicit immune responses.
- **Platinum workflow**:
  - Derivatized peptides are conjugated via C-termini and loaded into nanoscale reaction chambers of a **semiconductor chip**.
  - During sequencing, **N-terminal amino acid (NAA) recognizers, labelled with different fluorophores**, reversibly bind to cognate NAAs and **generate distinct binding patterns**.
  - **Aminopeptidases** sequentially cleave NAAs to expose subsequent amino acids for recognition. Upon completion of a sequencing run, data is automatically transmitted for **cloud-based analysis**.
  - **Traces are segmented into contiguous groups of pulses, or recognition segments (RSs), each representing interactions between recognizers and NAAs**.
  - The **order of recognizer binding and kinetic properties of RSs are analyzed to generate high-confidence alignments to reference peptides and identify proteins**.
- **Results**: Platinum detects peptides selected from a mixture of viral epitopes that activate T cells.
  - Platinum not only **sequences cytokines** that can remodel the immunopeptidome but also reveal the **constituents of immunoprotein mixtures**.
  - Platinum enables the detection of sites subjected to **post-translational modification (PTM)**, such as citrullination of arginine residues.
- **Summary**: Platinum profiles the amino acid sequence of viral peptides and proteins integral to immune checkpoint.

Next-Generation Protein Sequencing™

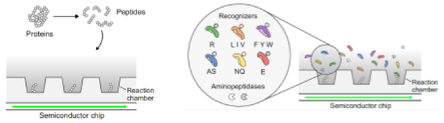
Platinum

Semiconductor chip



- Each reaction chamber is associated with a pixel.
- Semiconductor chip uses a filterless system that excludes excitation light based on photon arrival time.
- Evanescent illumination at reaction chamber bottoms from nearby waveguide.

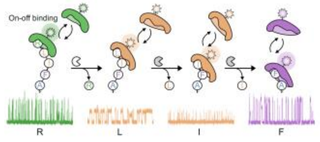
Prepare proteins for sequencing



- Functionalization of peptides at C-terminal lysines enables immobilization to the chip.
- Dye-labeled NAA recognizers and aminopeptidases are added.
- Sequencing process proceeds in real time without fluidic exchange of reagents.

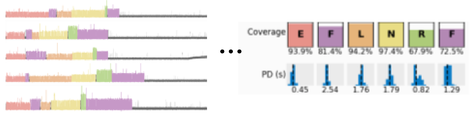
Sequence proteins on Platinum

Recognition segments (RSs)



- **Recognition**: 10s-100s of pulsing events per NAA.
- Fluorescence lifetime differentiates dye-labeled recognizers.
- **Aminopeptidases**: Cleavage events stochastic at the single-trace level.

Analyze protein sequences



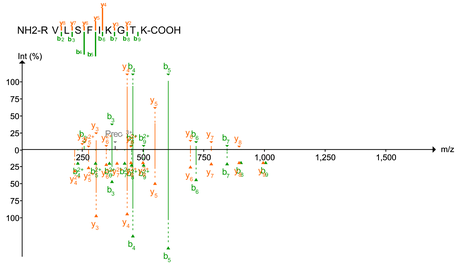
- **Kinetic Signature** summarizes the average sequencing behavior of an ensemble of peptide molecules.
- Sensitive to AAs and variants such as PTMs.
- High-quality reads containing 4 or more RSs and 3 or more unique recognizers are eligible for alignment and aligned to a reference peptide sequence based on kinetic patterns.

Platinum reveals immunopeptides within a mixture of viral epitopes

MHC Class I Control Peptide Pool:

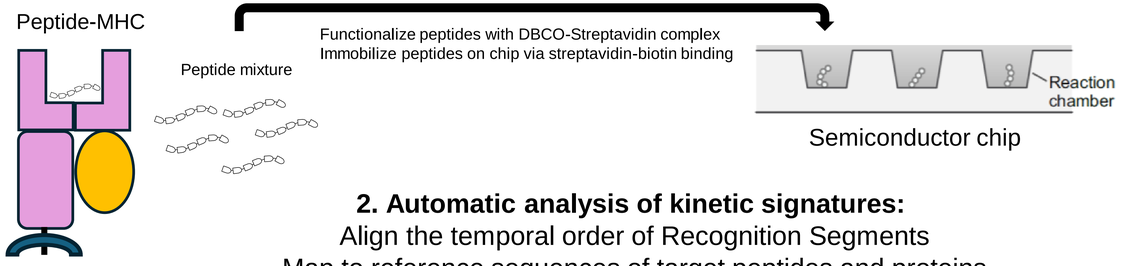
- Mixture of 32 peptides from cytomegalovirus (CMV), Epstein-Barr virus (EBV), and influenza nucleoprotein (NP) virus.
- The pool consists of defined MHC class I-restricted T cell epitopes from these three viruses and can be used as a positive control to stimulate T cells.
- 20 peptides lack lysine (~60% of total peptides), preventing standard peptide derivatization.
- Bioinformatics indicates five sequenceable peptides on Platinum.

Viral peptide epitopes	Source
RVLSFIKGTK	Influenza NP HLA-A3
ILRGSVAHK	Influenza A HLA-A3
RVRAYTYSK	EBV HLA-A3
RLRAEAQVK	EBV HLA-A3
IVTDFSVIK	EBV HLA-A11



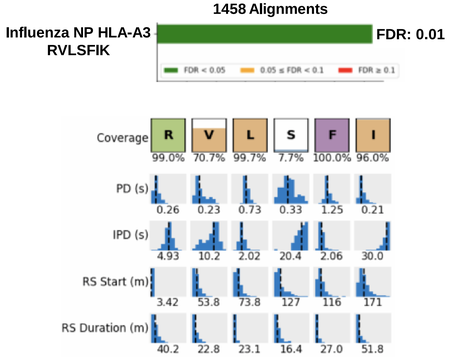
LC-MS/MS identified 27/32 CEF peptides
A spectrum of RVLSFIKGTK peptide
Platinum + LC-MS/MS → complete AA coverage of peptide

1. Elute, prepare, and sequence single peptide molecules on Platinum



2. Automatic analysis of kinetic signatures:

Align the temporal order of Recognition Segments
Map to reference sequences of target peptides and proteins



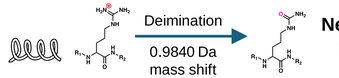
★ Alignments

Coverage (%): Frequency of residue observation.

Pulse duration (PD)/Interpulse duration (IPD):

- Reflects the differences in binding affinity driven by different *dissociation/association* rates for each type of recognizer-NAA interaction.
- Histograms represent the statistical distribution of kinetic data for all the pulses associated with a specific residue. Values reported are the median of the mean PD/IPD for each RS.

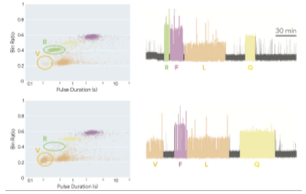
Immunobiology applications



PTMs can form peptide neoantigens → potential therapeutic targets and cancer vaccines

Detection of arginine citrullination from vimentin peptides:

- Increase in PD of neighboring valine residue
- Elimination of arginine recognition



Discern mixtures of signaling immunoproteins

Diverse protein classes:

Growth factors
-CDNF
-FGF2

Cytokines
-IL-4
-IL-6

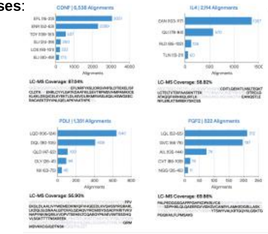
Serum proteins
-HSA
-APOE4

Intermediate filaments
-Vimentin

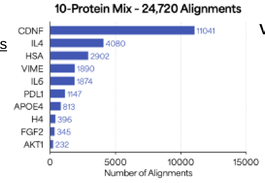
Immunotherapy markers
-PDL1

Nuclear proteins
-Histone H4

Kinases
-AKT1



Version 1 Kit



Version 2 Kit Feb. 2024

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