Single-cell Protein Sequencing by Utilization of Amino Acid Selective Chemistries and Nanopore Sequencing

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Abstract

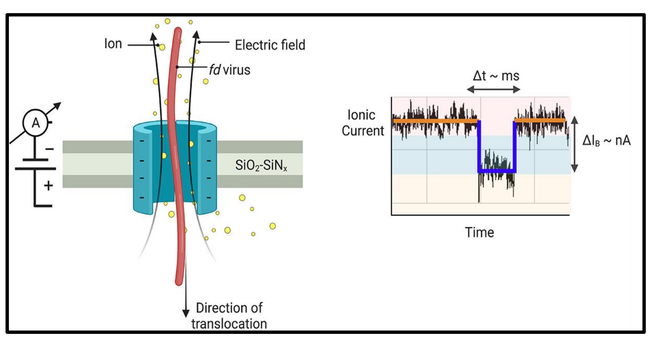
The proteome of a cell provides insight into the current state of activity and is under constant fluctuation due to numerous factors. Understanding the proteome by sensitive quantitative analysis of the proteins present would facilitate a novel view into the cell state. Providing significant benefits to biomarker discovery and molecular diagnostics as accurate quantification is necessary for complex diseases in which protein abundance is in constant flux. However, the current state of the art relies on mass spectrometry which has many drawbacks including a lack of high sensitivity and coverage. Other methods involve labeling amino acid residues with fluorescent molecules followed by subsequent Edman degradations to generate a fluorescence readout. This method lacks throughput and the ability for multiplexed detection. To address this, our lab is creating a new protein sequencing strategy that will use amino acid-selective chemistries along with conjugation to a positively charged peptide. The addition of a positive charge to the amino acid residues allows for detection using a solid-state nanopore, by measuring the change in current as the polypeptide chain is passed through. The current readout is then processed with an algorithm to fill in gaps for unmodifiable amino acids providing a high-accuracy amino acid readout.

Objective

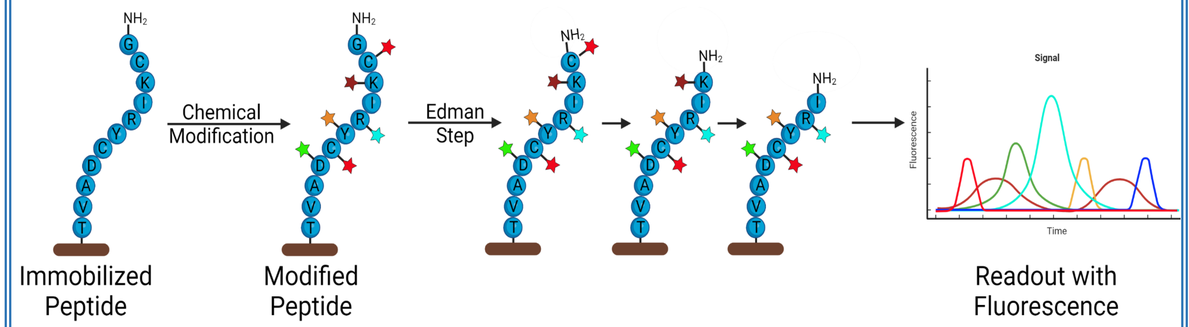
Determine the amino acid sequence at single-cell concentrations using barcoding and nanopore sequencing for the entire proteoform.

Introduction

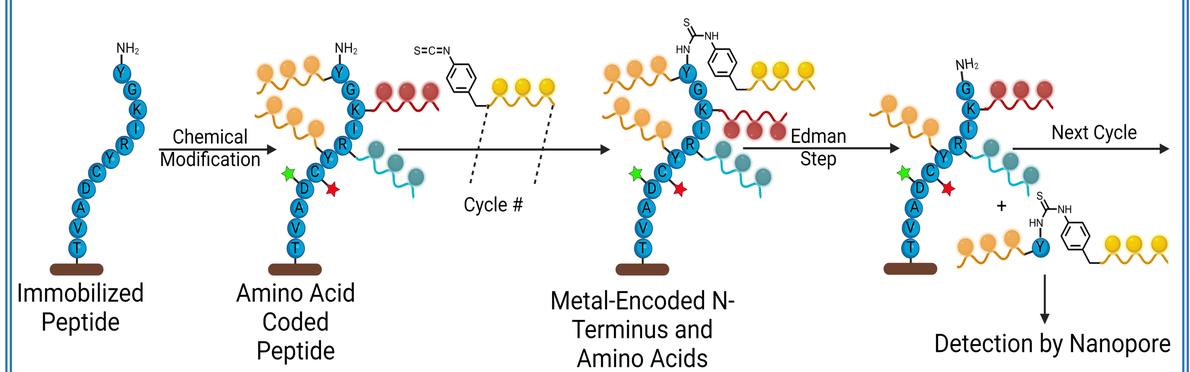
- Current protein sequencing techniques have several drawbacks
- Proteins can serve as biomarkers for disease
- Nanopore sequencing works by detecting a change in current caused by pore blockage
- Nanopores are widely used in DNA sequencing but there are several issues in adapting the technology to protein sequencing



Previous Work

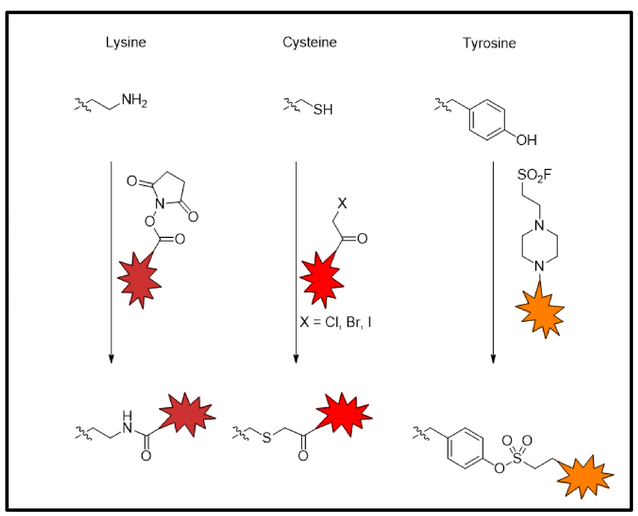


Our Workflow



Improvements

- New workflow moves away from fluorescent sequencing
- Metal barcoding strategy utilizes nanopore and machine-learning algorithms
- Solid-state nanopores are more tunable and durable than biological ones
- Creation of new amino acid-specific bioorthogonal reactions
- Improved identification of post-translational modifications
- Prevents signal loss caused by fluorescent dye degradation during Edman steps



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