Nanopore-based Identification of Histone 3 Post-Translational Modifications

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Post-Translational Modifications (PTMs) of proteins play crucial roles in modulating the function of proteins, which are key players in cellular function and diseases [1]. Specifically, dysregulation of the PTMs on Histone 3 have been linked to disease; for instance, depletion of H3K18-acetylation (H3K18ac) was associated with aggressive cancers, as well as poor patient prognosis with higher chance of tumor recurrence [2-3], and H3K36-acetylation (H3K36ac) reduces DNA damage signaling [4]. With many additional histone PTMs under active investigation, there is a growing demand for detection techniques that offer high sensitivity, reproducibility, and lower costs, compared to traditional mass spectrometry [5]. Here, we address this need with the use of nanopore technology, a single-molecule detection method that is already successful for DNA-sequencing and increasingly explored for protein analysis [5]. Using this approach, we target discrimination of the Histone 3 wild-type (H3wt) from its variants with three PTMs: H3K18ac, H3K36ac and H3K18 with a decanoyl (H3K18dec). The optimization of the experimental process was completed through testing of multiple genetically engineered nanopores by comparing their interaction with the H3wt. This allowed characterization of our different analytes individually, analyzing their dwell times, signal frequencies, and current blockages. We enabled label-free measurements of the H3 protein and discriminated it from its PTMs.

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