CARNEGIE MELLON UNIVERSITY
BME 2024 SPRING SEMINAR SERIES

Developing tools for single-molecule sequencing and imaging of RNA modifications

PRESENTED BY

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SCHEDULE

Doherty Hall (DH) 2315

Thursday,
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(11:00-12:00PM)

Mammalian cells expend large amounts of energy into generating >100 different enzyme-mediated RNA chemical modifications that can change the base-pairing, RNA secondary and tertiary structures, or recruitment of RNA binding proteins among many functions. Pseudouridine modified mRNAs are more resistant to RNAse-mediated degradation and have the potential to modulate immunogenicity and enhance translation in vivo, as demonstrated by the recent mRNA vaccines. However, we have yet to understand the precise biological function of pseudouridine on mRNAs due to a lack of tools for their direct detection and quantification.

We have recently developed an algorithm for identifying psi sites directly on mammalian mRNA transcripts using nanopore sequencing. We achieve this by exploiting systematic base-calling errors that occur at psi sites as a function of deviations in the current signals for k-mers as well as long, synthetic mRNA controls bearing psi. We have created a critical list of “ground truth”, psi sites, and have also uncovered previously unreported, psi sites. We then use our algorithm to classify types of pseudouridine hyper-modification that may occur on mRNAs: Type 1 is mRNA sites with high occupancy; type 2 is mRNAs that may have >1 pseudouridine on a single read. Using our algorithm and pipeline we have observed that 1. Pseudouridine sites in the human transcriptome may be conserved or differentially expressed across cell types, and 2. Pseudouridine modifications are dynamically regulated in response to cellular state. These insights can guide future designs on mRNA therapeutics.

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