

UNIVERSITY OF RHODE ISLAND
Department of Chemistry

SEMINAR

Room 234 Pastore Hall
3:00 p.m, Monday, Feb. 15, 2016

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"Simple and Accurate
Mass Spectrometric Method for
Sequencing microRNA"

HOST

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Simple and Accurate Mass Spectrometric Method for Sequencing microRNA Biomarkers

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Abstract

The increasing interest in microRNA (miRNA) as diagnostic biomarkers or potential drug targets has raised the demand for more accurate miRNA detection. One way to improve the accuracy is by using mass spectrometry (MS) to measure miRNA directly. Matrix-assisted laser desorption/ionization (MALDI) MS stands apart from other MS techniques due to the fact that a MALDI matrix is required for sample preparation. In this study, by exploiting the acidity of MALDI matrix and its mixing with miRNA prior to MS measurements, a simple method to generate RNA sequencing ladders is developed. The method utilizes the MALDI matrix to hydrolyze RNA at high temperature. The resulting sequencing ladders are ready to be measured without any desalting. By using MALDI SpiralTOF MS, the monoisotopic mass of each RNA fragment was measured. The RNA sequence was determined by sequentially comparing nucleotide compositions that were calculated from measured monoisotopic masses. The use of nucleotide compositions to assist the spectral interpretation has the advantages on distinguishing the complementary sequencing ladders, and allows the nucleotide identity at each position to be crosschecked multiple times. Together with the analysis of both complementary sequencing ladders, 100% sequence coverage and sequence accuracy were achieved in a blinded study.