Nanopore Detection of the MELAS A23243 Single Nucleotide Mutation In Mitochondrial tRNA

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Intracellular RNA molecules adopt a wide variety of tertiary structures. While canonical structural features for a class of RNA molecules can be inferred from bulk measurements, structural variations within a class are extremely difficult to detect. For example, the number of unique sequences of eukaryotic transfer RNAs (tRNAs) can be ~100 (excluding post-transcriptional modification variants), although only a few tRNA structures are available. A method capable of detecting subtle differences in these molecules may help elucidate the role of structural differences and unique epigenetic modifications in tRNAs. In this work, we electrophoretically drive individual tRNA molecules through ~3nm diameter pores to study their deformation kinetics during translocation. We find that each tRNA studied exhibits unique translocation kinetics, which we attribute to variations in their mechanical properties. Implementation of machine learning algorithms reveal a multidimensional set of parameters that can be used to ascertain the identity of a tRNA from a single ionic current pulse with 70-90% accuracy. Our results pave the way for a deeper understanding of RNA tertiary structure and its dependence on posttranscriptional modifications. As a case study we extend our nanopore analysis to mitochondrial tRNAs that are affected by a pathogenic mutation. Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) is the most common mitochondrial genetic disease. More than 80% of all cases of MELAS are caused by a single A-G mutation in mitochondrial tRNALeu(UUR), a transfer RNA encoded for by the MT-TL1 gene. Using solid-state nanopores we show that this single-nucleotide substitution causes misfolding of tRNALeu(UUR). We also show that a single highly conserved post-transcriptional methylation greatly enhances the ability of the tRNA to fold. This effect is shown in both the standard folding of the wild-type tRNA as well as the misfolding of the mutant tRNA. Both the effect of the pathogenic mutation, as well as the single methylation, are able to be easily and quickly detected using our solid-state nanopore system. This suggests that nanopore technology can be used to develop a rapid diagnostic tool for genetic mutations that effect tRNA, requiring very little starting material (<1,000 tRNA molecules).

References

[1] R. Y. Henley, B.A Ashcroft, I. Farrell, B.S Cooperman, S.M Lindsay, & M. Wanunu, *Nano letters* ***16*****(1)**, 138 (2016)