# Quantitative Base-resolution Sequencing Strategies to Investigate RNA Modifications in Gene Expression Regulation

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The reversible $N^6$-methyladenosine ($m^6A$) methylation regulates messenger RNA (mRNA) fate in various biological processes. Functional characterization of other mRNA modifications, such as pseudouridine (Ψ), 2'-*O*-methylation (Nm), 5-methylcytidine ($m^5C$), and internal $N^7$-methylguanosine ($m^7G$), has been hampered by the lack of the sensitive and quantitative methods that can map these RNA modifications transcriptome-wide. In this talk, based on chemical approaches and enzyme engineering, I will introduce the quantitative sequencing tools to uncover multiple mRNA modifications at base resolution, and to monitor their modification fraction change through misincorporation and deletion signatures. These methods assigned the specific 'writer' protein for the modified sites accurately and facilitated the discovery of 'reader' proteins that reveal the functional roles of these RNA modifications. Besides the RNA modifications on steady-state RNA, I will introduce DAMM-seq to site-specifically detect and quantify multiple RNA methylations simultaneously within mitochondrial nascent RNA, which demonstrated ALKBH7-mediated reversible RNA methylation to regulate the processing and structural dynamics of polycistronic mitochondrial RNAs. All these biotechnologies set the stage to investigate the roles and mechanisms of multiple RNA modifications in gene expression regulation and diverse biological processes.