Abstract title: Exploration of Ribosome Structure-function relationships by systematic introduction of Pseudouridines and 2'O-methyl groups at novel ribonucleotides

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Ribosomes are multi-subunit molecular machines essential for mediating mRNA translation within cells. The functional centers of rRNAs are decorated with evolutionarily conserved modifications which are catalyzed by RNA modifying enzymes directly and/or guided by several small nucleolar rRNAs (snoRNAs) which confer sequence specificity of the modification. rRNA modifications studied extensively to date have been functionally implicated in altering ribosome biogenesis, pre-rRNA processing and anomalous translational activity. However, the range of different functions conferred to ribosomes by various modifications studied extensively, remain yet to be interrogated systematically. Pseudouridines and 2'O-methylations are the most abundant rRNA modifications. Our study aims to systematically introduce these modifications at novel ribonucleotide positions not fixed by evolution using designer H/ACA and C/D box snoRNAs. We will generate different pools of designer H/ACA and C/D box snoRNAs to allow modification of all available novel sites on yeast rRNA. This will result in both gain-of-function and loss-of-function phenotypes. We aim to examine the consequences of the artificially introduced modifications on yeast growth, bulk translation, and translation fidelity by using a combination of biochemical as well as custom designed NGS platforms complemented with structural analysis to decipher the functional implications of induced modifications of rRNAs. Our study aims to integrate these approaches along with custom designed computational pipeline(s) to identify and quantify different modifications on rRNA(s) of yeast which will lay the foundation to understand the impact of rRNA modifications on ribosome structure-function and further widen the scope to interrogate modifications on other RNA species.