Developing a high-throughput biochemical method to measure and predict RNA folding stabilities in cells

The regulatory function of RNA to specify gene expression is guided by the interactions it makes —with itself by folding into structures and with proteins—that influence downstream events. Genomic approaches have provided strong evidence for the importance of RNA structure in function and regulation, but the tools we use to predict what RNAs are structured are based on *in vitro* measurements, and there is strong evidence supporting extensive unfolding of RNAs in cells. To understand the complex gene regulation program and to ultimately modulate gene expression for therapeutic intervention, we need predictive models for RNA structure and interactions—for an arbitrary RNA sequence—and we need to be able to predict their functional outcomes.

To address these questions requires systematic and quantitative approaches that extend beyond traditional functional genomics. Accordingly, we have developed high-throughput cellular biochemistry (HTCB) to bring biochemistry to genomic scale in cells. Using a designed library that varies individual RNA features that contribute to folding combined with DMS-MaPseq chemical probing, we measured the thermodynamic stability of thousands of RNA structures *in vitro* and in cells. Our method allows for the quantification of differences in RNA structural ensembles in and out of cells, of the extent cellular unfolding, and of deficiencies in standard computational models used to predict RNA structures. With this, we can establish a comprehensive model for RNA folding in cells that is predictive, testable, and can be extended to relate the energetics of RNA structure to downstream effects on gene expression.