Investigating *xpt* G-Riboswitch Folding Using FRET

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Riboswitches are untranslated mRNA domains that function as regulatory elements by modulating gene expression. Specific metabolite binding and proper aptamer domain folding are critical for expression regulation. Several riboswitches have been characterized, including riboswitches that bind guanine and adenine. High-resolution structures of these riboswitches reveal that the aptamer domains of the *B. subtilis xpt* G-riboswitch and the *ydhL* A-riboswitch differ by just one nucleotide, which forms a Watson-Crick base pair with the bound metabolite. Additionally, an important interaction between loops 2 and 3 (L2 and L3 below) forms as the riboswitch adopts a folded structure that resembles a tuning fork. This observation implies that there may be a distance change between the prongs (L2 and L3) of the tuning fork during folding.

Fluorescence resonance energy transfer (FRET) is an ideal tool for monitoring these types of conformational changes because of its sensitivity to changes in chromophore distances. In addition to ensemble (bulk-phase) FRET, single-molecule FRET allows for the characterization of rare or unsynchronized folding events. We have site-specifically labeled small RNAs with fluorescent probes and assembled the fragments by ligation into the full-length *xpt* G-riboswitch aptamer domain for ensemble FRET. Each riboswitch construct includes an RNA extension for hybridization, allowing for single-molecule FRET studies, which require immobilization to a surface. We are using FRET to probe conformational changes by monitoring metabolite- and Mg^{2+}-dependent folding of the *xpt* G-riboswitch.