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Seminar

Fluorescence Dynamics Reveal the mechanism of Riboswitches

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Riboswitches are 5'-untranslated part of mRNA that regulate gene expression through control of transcription and translation. Small molecule-modulated riboswitches have two domains, one (called aptamer domain) that binds the small molecule (i.e. a specific metabolite molecule) and another (called expression platform) that interacts with the transcription/translation machinery. Binding of metabolite to the aptamer domain enables the action of the expression platform. Another class of riboswitches called RNA thermometers control translation in a narrow range of temperatures presumably by temperature-dependent changes in their folded structure and dynamics of mRNA segments.

With the aim of understanding the mechanism of action of riboswitches, we have labeled RNA switching sequences site-specifically by replacing adenine with the fluorescent analog, 2-aminopurine (2-AP) throughout the RNA length one nucleotide at a time. We then performed pico-second time-resolved fluorescence spectroscopic measurements under a variety of conditions including RNA bound to ribosome. Fluorescence parameters such as fluorescence lifetime, bimolecular quenching constant and fluorescence depolarization time were monitored.

Our results show several interesting aspects associated with the mechanism of action of riboswitches. (i) Aptamer fold is very similar even before the arrival of the ligand (in contrast to ligand-driven folding); (ii) Ligand binding leads to enhanced compactness and narrowing of conformational flexibility indicating 'conformational selection' hypothesis of ligand binding to the aptamer domain; (iii) RNA constructs having both the aptamer domain and expression platform undergoes significant changes in structure following ligand binding and (iv) the mechanism of RNA thermometer involves ribosome-mediated melting of RNA hairpin sequestering the ribosome binding domain of RNA.

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11:30 AM (Tea/Coffee at 11:15 AM)

Seminar Hall, TCIS