THEME 6: RNA REGULATION IN EUKARYOTES

One of the hallmarks of eukaryotic gene expression is the extensive use of post-transcriptional, RNA-based regulatory strategies. In eukaryotes, nearly all mRNAs are produced as precursor molecules that must first be capped, edited to remove introns, cleaved, polyadenylated, associate with a large cohort of proteins and nucleic acids that bind the ORF as well as untranslated regions, and even modified to incorporate particular nucleobase modifications. Together these post-transcriptional modifications can determine not just what and how many proteins are produced by a transcript but even the transcript's localization or stability inside the cell. In this module we will explore two cellular processes responsible for RNA-based regulation: the removal of introns by the spliceosome and the silencing of transcripts by RNAi. We will use a combination of *in vitro* single molecule FRET analysis of RNA dynamics, MD simulations of components of the pre-mRNA splicing machinery, and visualize aspects of RNAi in mammalian cells by single-molecule FISH.

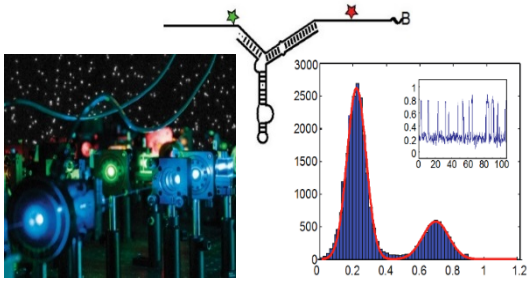
Students will participate in the following 2 modules:

MODULE 1: EXAMINATION OF SPLICEOSOMAL RNA FOLDING BY SINGLE-MOLECULE FRET & MOLECULAR DYNAMICS

Part 1: smFRET measurement of RNA folding

Laboratory: **Aaron Hoskins** (U Wisconsin Biochemistry)

Experiments will address how spliceosomal RNAs change structure in response to other nucleic acids, ligands, and salt conditions. These structural dynamics will be observed in real-time using single-molecule FRET (smFRET). Students will learn how to work with nuclease-sensitive RNAs *in vitro*, carry out smFRET experiments using a TIRF microscope, and analyze single molecule data using available software.

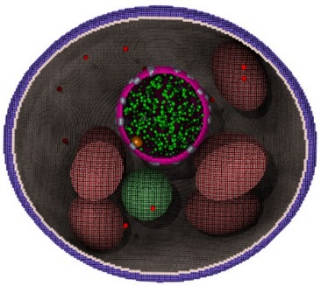


Photo/Artwork Robin Davies (U Wisconsin)
Hoskins lab (unpublished)

Part 2: Simulation of Spliceosome

Laboratory: **Zan Luthey-Schulten** (UIUC Chemistry)

Students will be introduced to computational methods including the structure and sequence analysis program, Visual Molecular Dynamics (VMD), and the modeling program, Nansocale Molecular Dyanmics (NAMD), through lectures and hands-on simulation tutorials using a new QwikMD tool with example systems. Students will apply QwikMD to study RNA and protein components of the human spliceosome to inform what properties of RNA contribute to structural dynamics of these complexes.

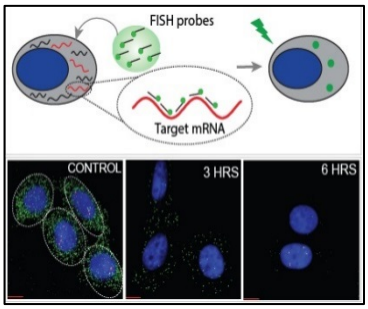


Luthey-Schulten lab (unpublished)

MODULE 2: RNA INTERFERENCE IN MAMMALIAN CELLS MEASURED BY SINGLE-MOLECULE FISH

Laboratory: **Sua Myong** (Johns Hopkins University Biochemistry & Biophysics)

In this module, students will be introduced to single-molecule Fluorescent In-Situ Hybridization (smFISH) method for quantitative measurements of RNA interference (RNAi) through hands-on experiments. Students will monitor changes in Lamin A transcript as a function of silencing time. Different structures of small interfering RNAs (siRNA) will be applied to cells to compare silencing efficiency. Cell imaging will be performed with epifluorescence microscope and the data analyzed by FISH-QUANT program.



Myong lab (unpublished)