Department of Chemistry Seminar Series – Fall 2020

Fluorescent probes for nucleic acid analysis

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Host: Prof. Fernando Uribe-Romo

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Nucleic acid analysis has benefited from the development of hybridization probes that interrogate nucleic acids in a sequence-specific manner. We have efficiently utilized a split approach for the design of hybridization sensors based on deoxyribozymes and aptamers as scaffolds. In split sensors, analyte-recognizing fragment of the probe is divided into two parts. The two parts need to be simultaneously bound to the target for generation of either fluorescent or colorimetric signal. This approach offers such advantages as excellent selectivity, efficient interrogation of structured nucleic acid sequences, use of a universal signal reporter, and (for fluorescent probes) tolerance to inhibitors in complex mixtures (e.g. cell lysates).

A promising reporter system for hybridization analysis is based on light-up aptamers among which DNA aptamers are preferable over RNA counterparts due to greater stability and lower synthetic cost. We have studied a recently reported light-up DNA aptamer and discovered its ability to bind and increase fluorescence of otherwise non-fluorescent dyes including commercially available auramine O and crystal violet. Based on mutagenesis, spectroscopic and molecular simulation data, we proposed the structure of the aptamer and its dye-binding site. The data can be used in designing aptamer-based probes. Most recently, we discovered the preferential binding of GelRed (a commercial gel staining dye) to singlestranded oligothymidylate sequences, which suggests that poly(dT) may exhibit a regular structure, and may open a route towards designing fluorescent light up sensors.