UCF Biophysics Group Seminar

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Interaction of DNA with Fluorogenic Dyes

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ABSTRACT

Nucleic acid-binding fluorogenic dyes are commonly used in research practice for cell staining, visualizing DNA/RNA in gel, qPCR assays and other biosensing application, as well as intracellular RNA tracking. Most applications rely on interactions of the dyes with nucleic acids in a sequence-non-specific manner. The dyes can interact with double-stranded DNA, for example, via minor- or major-groove binding, or intercalation between the base pair planes. At the same time, some dyes may still tend to interact with nucleic acid sequences rich in particular nucleotides. Recently, we have discovered the preferential binding of GelRed (a commercial gel staining dye) to single-stranded oligothymidylate sequences. Based on pH and ionic strength dependences, electrostatic interactions with oligonucleotide backbone and hydrogen bonding between thymine and exocyclic amino groups of the dye were proposed as likely stabilizing forces in the dye-oligothymidylate complex.

Sequence-specific binding of fluorogens to nucleic acids often requires selection of these DNA/RNA sequences from a random nucleic acid library via Systematic Evolution of Ligands by EXponential Enrichment (SELEX) technique. The resultant light-up aptamers are capable of increasing fluorogens' fluorescence up to hundred- or even thousand-fold. RNA light-up aptamers are promising tags for RNA monitoring in live cells, while DNA light-up aptamers offer label-free advantages for fluorescent bioassays. An example of such dye-aptamer systems is dapoxyl binding aptamer (DAP). We have discovered promiscuity of DAP interactions with environment-sensitive dyes. Based on mutagenesis, biochemical and spectroscopic data, we have proven formation of a guanine-quadruplex structure for DAP, which is essential for the aptamer's ability to enhance dyes' fluorescence.