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Quencher-free fluorescent strategies for aptasensor development

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Nucleic acids have greatly expanded beyond their abilities to store genetic information. Functional nucleic acids can now be created *in vitro* that are capable of binding specific targets with high specificity and sensitivity. These novel classes of biomolecules, termed aptamers, have been employed as versatile tools for the detection of important biological targets for applications in imaging, diagnostics and therapeutics. A common strategy for aptamer diagnostic application is to employ fluorescent dyes that generate a turn-on or turn-off emissive response to target binding. The molecular beacon (MB) approach is popular, and involves the matching of a strongly emissive 5'-end-labeled probe with a 3'-quencher in a duplex or hairpin system. Target binding removes the quencher from the emissive dye to afford a turn-on signal. Although this approach can work efficiently, it requires the presence of an additional modified 3'-fluorescence quencher that is difficult to purify because the failed oligonucleotide sequences also contain the 3'-quencher moiety. In this presentation, quencher-free strategies for aptasensor development will be presented. Fluorescent dyes with emission sensitivity to microenvironment polarity, solvent rigidity or DNA topology have been incorporated into DNA aptamers that bind proteins, small molecule toxins and heavy metals. Dye types utilized in these studies include hemicyanines, BODIPYs and fluorescent DNA base analogs (FBAs). These dyes can be free in solution (label-free) or covalently attached to the DNA aptamer at specific internal locations or at the 5'-end. Strategies to incorporate visible dyes into DNA aptamers using solid-phase DNA synthesis, together with the pros and cons of a label-free versus a label strategy for aptasensor development will be presented.

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