We designed different fluorescence polarization (FP) assays dedicated to the detection of small analytes, such as adenosine and tyrosinamide (L-Ym), using DNA aptamers as molecular recognition elements. The strategy was based on two properties of nucleic acid aptamers: (i) the conformational change triggered by the target complexation and (ii) the ability of nucleic acids to form duplex structures with their complementary sequences (CS) [1].

In the direct format, the DNA aptamers were labeled with a fluorophore to use them as signaling probes. The fluorescent DNA probe exhibited an increasing FP (or anisotropy) upon binding to a target molecule, based on the change of the microenvironment around the fluorophore (induced by the conformational change of the aptamer upon target binding) [2,3].

In the indirect format, the fluorescent signaling reporter was the complementary DNA strand of the aptamer. For the labeled-CS probe, FP was sensitive to changes in the rotational motion arising from duplex formation (in the absence of target) or from its dissociation (in the presence of target). Hybridization of the fluorescent CS reporter and the aptamer increased FP as the rotational motion was slowed down due to the increased molecular size. Aptamer-target complexation destabilized the hybridized form, resulting in the release of the free fluorescent reporter which was conveniently detected by measuring the decrease of FP [4].

These two approaches highlighted that the nature of the fluorophore linked to the DNA probe (Texas red or fluorescein) clearly influenced the sensitivity of the analysis. For L-Ym target, the direct format exhibited lower detection limits, from 100 to 280 nM, than the indirect one (about 10 µM). However, in the latter case, comparable detection limit (250 nM) was attained optimizing the length of CS [5]. To conclude, FP aptamer-based bio-analysis provided high throughput, homogeneous, rapid and specific assays for small molecules sensing.

References:


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