

Supporting information for:

Advances and Challenges in Small-Molecule DNA Aptamer Isolation, Characterization, and Sensor Development

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Table S1. Advantages and limitations of methods used for characterizing aptamer affinity.

Method	Advantages	Main limitations
ITC	Solution-phase binding; label free; provides binding dynamics and stoichiometries	Requires high concentration of aptamer and ligand
SPR	Provides kinetic metrics such as k_{on} and k_{off}	Requires immobilization of either aptamer or target
MST	Applicable to complex matrices	Ligand must be fluorescent, or aptamer needs to be fluorescently labeled
FP	Solution-phase binding	Ligand must be fluorescent, or aptamer needs to be fluorescently labeled; optimization of label position is required; label can alter binding affinity
Bead assay	Simple; does not require specialized instrumentation	Non-specific binding to beads can cause false positives; affinity can be affected by conjugation
Strand displacement	Simple; does not require specialized instrumentation	Only works with stem-loop structured aptamers; requires fluorescent labeling

Table S2. Comparison of sensing mechanisms and signal reporters used in aptamer-based sensors.

	Advantage	Limitation
Sensing mechanism		
Aptamer folding	Rapid; single-aptamer construct	High background; requires careful aptamer engineering
Aptamer assembly	Rapid; low background; compatible with signal amplification	Low affinity of aptamer leads to low sensitivity (but this can be improved with CBSA-based sensors)
Strand displacement	Simple assay development	Low sensitivity
Dye-displacement	High sensitivity; minimum aptamer engineering; label-free	Requires a dye that binds the aptamer and can be displaced by target
Exonuclease digestion	Label-free; generalizable; high sensitivity	Long turn-around time, not ideal for on-site detection due to use of enzymes
Signaling reporter		
Fluorescent label	Sensitive	Requires aptamer labeling; best suited for testing in lab settings
Electrochemical label	Portable; highly selective	Requires aptamer immobilization
Colorimetric - dye	Naked-eye detection feasible	Requires a dye that binds to the aptamer and can be displaced by target; high sensitivity requires instrumentation
Colorimetric - AuNPs	Naked-eye detection feasible	Usually requires aptamer immobilization; low sensitivity