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Loops, Hairpins and Flipped Bases: A DNA Aptamer that Discriminates *Plasmodium* Lactate Dehydrogenase from the Blind Watchmaker

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Little is known about how aptamers achieve their specificities in binding and discriminating between closely related targets. Under the pretext of investigating the potential for aptamers in malaria diagnostics, here we solve the crystal structure of a new DNA aptamer which was selected and evolved to bind specifically to the *Plasmodium falciparum* lactate dehydrogenase (PfLDH) and not bind to human lactate dehydrogenase. The structure reveals two aptamers bind per *Plasmodium* lactate dehydrogenase tetramer with opposite apical geometry, whereby each aptamer has a distorted hairpin structure. The aptamer comprises a B-helix stem, an asymmetric internal loop involved in target discrimination and an apical loop involved in binding interactions. Each loop contains a critical flipped base. Isothermal titration calorimetry, surface plasmon resonance and electrophoretic mobility shift assay all provide evidence for binding with a dissociation constant in the range 40-70 nM. The structure reveals a unique loop present only in *Plasmodium* lactate dehydrogenase that is not present in any human lactate dehydrogenase that is a crucial determinant of aptamer specificity. The aptamer surprisingly does not inhibit the enzymatic activity of LDH that we explain by aptamer binding opening up the active site increasing substrate accessibility. We also apply the aptamer for LDH detection by conjugating to gold nanoparticles. The study provides a new perspective in aptamer-mediated recognition, and provides visualization of the outcome of the blind in vitro selection and evolution of an aptamer under positive and negative selection pressures (the blind watchmaker). Furthermore, the structure provides a foundation for applying rational design approaches in developing a nucleic acid which may be able to replace antibodies as the underlying mediator of molecular recognition in point-of-care malaria diagnostics.

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