Broadening the Scope for Aptamer Binding: *in silico* Prediction of Optimal Aptamer Modifications

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Aptamers are functional nucleic acids that bind to a wide range of targets (e.g., small molecules, proteins, metals, and cells) with high affinity and specificity. They also have a breadth of applications from allosteric inhibitors, conformationally controlled electrical switches and florescent dyes. Aptamers rival antibodies for use in diagnostics due to the ease and low cost associated with large-scale synthesis, as well as their greater chemical stability *in vivo*. Aptamers can be chemically modified to enhance binding affinity, structural stability, melting point, and degradation rate among other physicochemical properties. However, the placement and quantity of modifications for optimal aptamer performance is currently difficult to predict. To optimize aptamer design, highly granular knowledge of how aptamers bind to their target is required. Unfortunately, the chemical synthesis and characterization (enzyme binding, crystallization, structure determination) of many custom aptamer–protein complexes are challenging, costly, and time-consuming processes that are not always successful. By contrast, molecular dynamics (MD) simulations can provide structural insights into the interactions between a wide range of potential modifications and protein targets.

A computational framework using MD simulations to predict aptamer–target binding interactions requires a complete scope to be investigated, including isolated aptamer dynamics and the structure of the bound complex. The present work considers modifications of the well-studied prototypical thrombin binding aptamer (TBA). Specifically, TBA (5'-GG TT GG TGT GG TT GG-3') has previously been modified through incorporation of bulky T analogs, 5-(indolyl-3-acetyl-3-amino-1-propenyl)-2'-deoxyuridine (W) at all T positions, as well as the smaller variant, 5-(methyl-3-acetyl-3-amino-1-propenyl)-2'-deoxyuridine (K), at two T sites. The present work uses long-time scale classical MD simulations starting from unmodified TBA–thrombin crystal structures to rationalize the experimentally reported changes in TBA stability and thrombin binding affinity upon modification at each T position. The excellent correlation between the computed data and experimental information, including data from two crystal structures of W and K-modified TBA bound to thrombin, verify the accuracy of our computational approach. Our new computational formulism can now be applied to understand the behavior of other TBA variants in the absence of experimental data to gain an appreciation for how many different modifications impact aptamer function.