Is the 17BETA-Estradiol Aptamer Able To Be Modified Using 3-Cyanovinylcarbazole Photo-Cross-Linking To Achieve Site-specific Intra-molecular Binding at the Eighth and Twenty-Ninth Positions, and Will the Modified 17BETA-Estradiol Aptamers Still Bind to the Target 17BETA-Estradiol Hormone?

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17β-estradiol (E2) is a steroidal estrogen used in agriculture to promote animal growth and increase milk yield cows, and is the most common endocrine disrupting chemical (EDC) found in wastewater effluent. Aptamers offer a potential solution for on-site E2 detection, promising cost-effective manufacturing. However, their efficacy under adverse conditions is crucial to withstand the field testing environment. Through the use of 3-cyanovinylcarbazole (CNVK) photo-cross-linking modification to achieve site-specific intra-molecular binding, I hypothesized that the stability of the aptamer structure could be improved. Aptamers characterized for E2 detection were modified in multiple positions with CNVK. The first test was performed using gel electrophoresis to determine if cross-linking was achieved. Once this was verified, binding affinity of the modified aptamers was tested using isothermal titration calorimetry (ITC). Gel electrophoresis confirmed that cross-linking occurred at the eighth position of the E2 aptamer. Testing through the ITC machine suggested an improved binding affinity, however not enough material was recovered after purification to run more than two ITC trials. The experiment partially supported the idea that CNVK modification can improve the binding affinity of the E2 aptamer, and through additional trials, has the potential to strongly support it. There are huge implications here for more accessible field testing, and next steps would be to start exposing the aptamers to adverse conditions and see how well the structure is maintained.