

**Reference:** Zhao, Qiang, Li, Xing-Fang, Le, X. Chris. "Aptamer-Modified Monolithic Capillary Chromatography for Protein Separation and Detection," *Analytical Chemistry*, **2008**, 80, Articles ASAP & Supplementary Information.

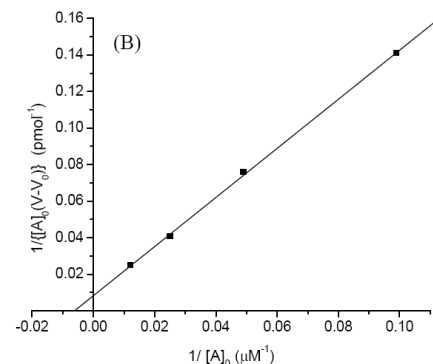
**Bioanalysis and Sample:** The authors describe a procedure to prepare a monolithic capillary column modified with streptavidin and a biotinylated aptamer for cytochrome c. This column can then be used to separate proteins of interest from complex samples as well as determine binding constants, albeit not accurately.

**Importance:** Protein separation and detection is the goal of numerous research groups in the bioanalytical field. Due to their high affinity for targets, aptamers (synthetic strands of RNA or DNA) have been used in the past for affinity based methods for the separation, detection, and purification of their respective target molecules. Previous work has demonstrated the use of both open tubular and packed chromatographic columns for affinity based separations, but both of these have drawbacks. Open tubular columns can be quickly overloaded due to their relative lack of surface area and packed columns suffer from either large dead volumes or high back pressures depending on the size of the particles. Monolithic columns (columns prepared with an *in situ* polymerization) do not exhibit these problems to the extent of the more traditional columns. They are easier to prepare than packed columns and have higher loading capacities than open tubular columns.

**Technique:** After the *in situ* polymerization of the monolith inside the capillary, the column was first modified with streptavidin. A solution of streptavidin was pumped through the capillary for 24 hours. After the streptavidin modification, the target aptamer was immobilized on the surface of the monolith. Prior to immobilization, the cytochrome c aptamer was biotinylated by attaching biotin to the 5' end. The biotinylated aptamer solution was then pumped through the column, allowing the biotin and streptavidin to bind, immobilizing the aptamer. Once the column had been prepared, pressure injections of protein mixtures were made. The polyimide coating on the silica capillary was removed to create a detection window for UV detection done at 214 and 407 nm. Frontal analysis is used to make estimations of dissociation constants as well as calculate the coverage density of the immobilized aptamers.

**Example:** Information gathered from frontal analysis chromatograms of varying concentrations of cytochrome on the aptamer modified monolithic column along with Eq. 1 can be used to generate the plot shown in Figure 1. Both the total number of aptamers ( $B_t$ ) and the dissociation constant ( $K_D$ ) can be calculated from this plot.

$$\frac{1}{[A]_0(V - V_0)} = \frac{K_D}{B_t} \times \frac{1}{[A]_0} + \frac{1}{B_t} \quad \text{Eq. 1}$$



**Figure 1: Plot used to estimate dissociation constants**

**Opinion:** The authors describe an interesting way to prepare a column for the separation of proteins; however their data shows that neither their detection limits obtained nor the estimation of dissociation constants are reliable. Detection limits are above basal levels of the proteins used in the study and the estimation of the dissociation constant of the cytochrome c aptamer was 30 times larger than the currently accepted value. The ability to preconcentrate analytes could prove to be useful, but overall this system in its current format is lacking the reliability necessary for widespread use.