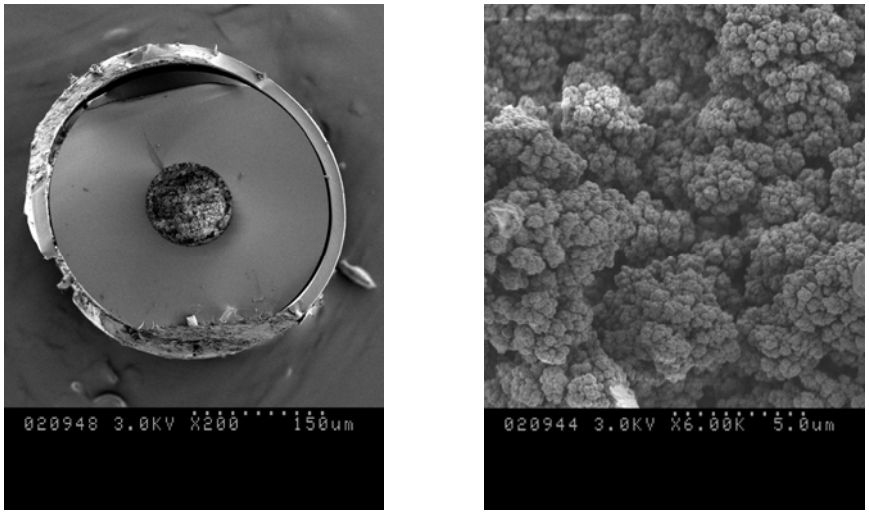
Supporting Information

Aptamer-Modified Monolithic Capillary Chromatography for Protein Separation and Detection

Qiang Zhao, Xing-Fang Li, X. Chris Le*

Division of Analytical and Environmental Toxicology, Department of Laboratory Medicine and Pathology, University of Alberta, Edmonton, AB, Canada T6G 2G3



(A) (B)

Figure S1. Characterization of a capillary monolithic column by scanning electron microscopy. (A) cross section of the capillary monolithic column; (B) structure of monolith inside the capillary.

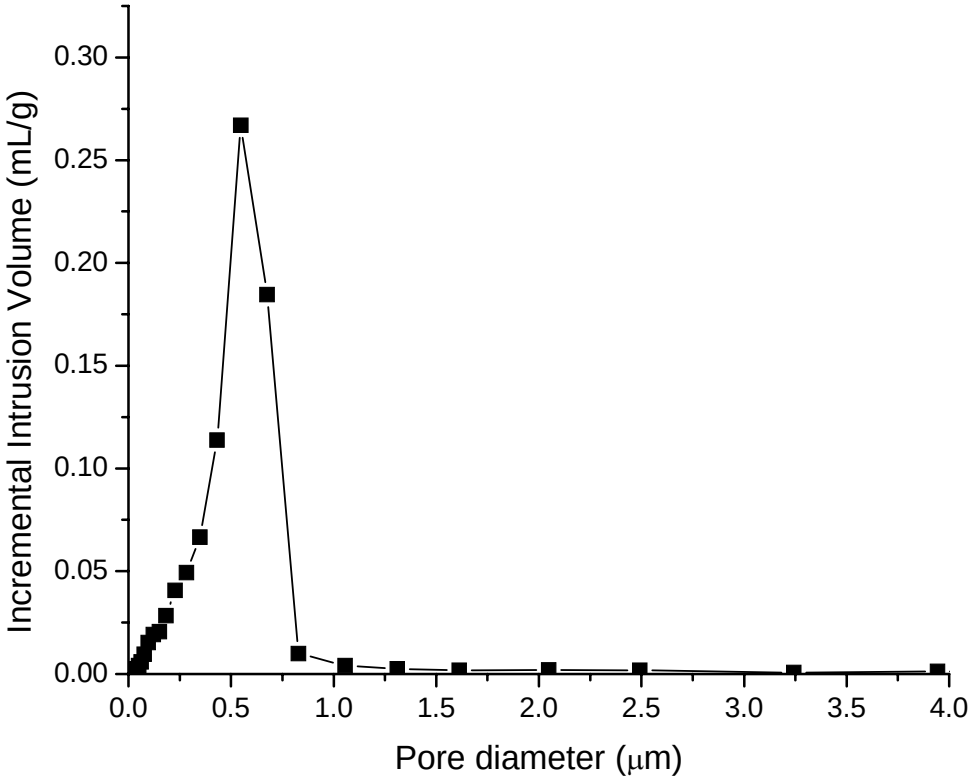


Figure S2. Pore size distribution of monolith, measured by mercury intrusion porosimetry.

Determinations of K_d and the amount of the aptamers on the capillary monolithic column.

K_d values can be expressed by the following equation:[1, 2]

$$K_d = \frac{[A][B]}{[AB]} = \frac{[A]_0\{[B]_0 - [A]_0(V - V_0)/V_c\}}{[A]_0(V - V_0)/V_c} = \frac{B_t}{V - V_0} - [A]_0 \qquad (1)$$

Where [A] is the concentration of unbound target protein (cytochrome c), [B] is the concentration of the unbound aptamer, [AB] is the concentration of the aptamer-target complex, $[A]_0$ is the initial concentration of the applied target, and V is the volume of this solution needed to saturate the column, $[B]_0$ is the concentration of aptamers immobilized onto the column, V_0 is the volume required to elute an unretained molecule, V_c is the total volume of the monolithic column, and B_t is the total number of aptamers immobilized on the column ($B_t = [B]_0 V_c$). Equation (1) can be rearranged to:

$$\frac{1}{[A]_0(V - V_0)} = \frac{K_d}{B_t} \times \frac{1}{[A]_0} + \frac{1}{B_t} \qquad (2)$$

Therefore, B_t and K_d can be obtained from the intercept ($1/B_t$) and the slope (K_d/B_t) of a plot of $1/[A]_0(V\text{-}V_0)$ versus $1/[A]_0$.

Reference

1. Arnold, F. H.; Schofield, S. A.; Blanch, H. W. *J. Chromatogr.* **1986**, *355*, 1–12.

2. Kasai, K.; Oda, Y.; Nishikata, M.; Ishii, S. *J. Chromatogr.* **1986**, *376*, 33–47.

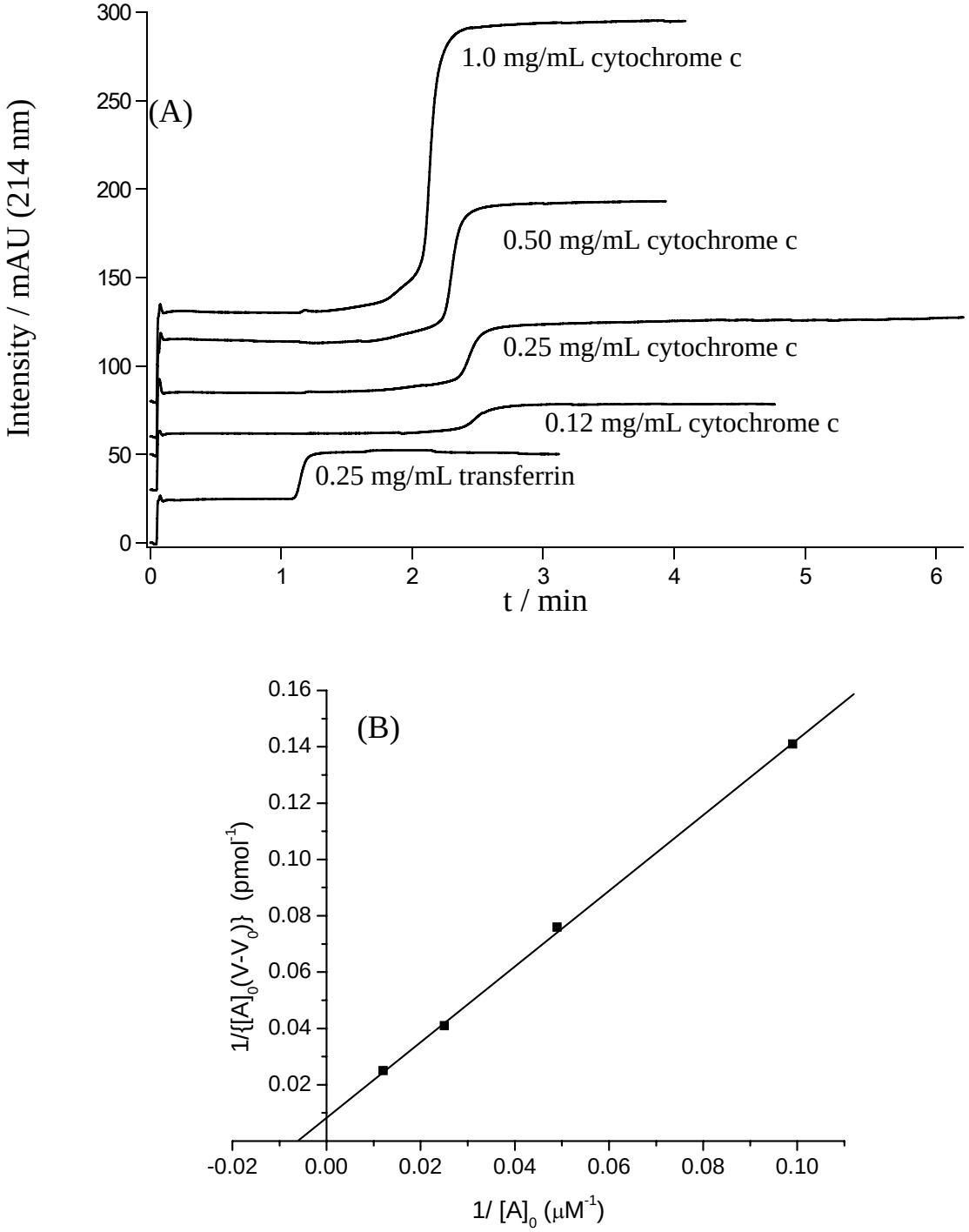


Figure S3. (A) Frontal chromatograms of transferrin and cytochrome c in a mobile phase containing 50 mM Tris-HCl, 20 mM KCl, and 120 mM NaCl (pH 7.4). (B) The plot of $1/[A]_0(V\text{-}V_0)$ versus $1/[A]_0$ based on the data obtained from elution profiles (A) using various concentrations of cytochrome c.

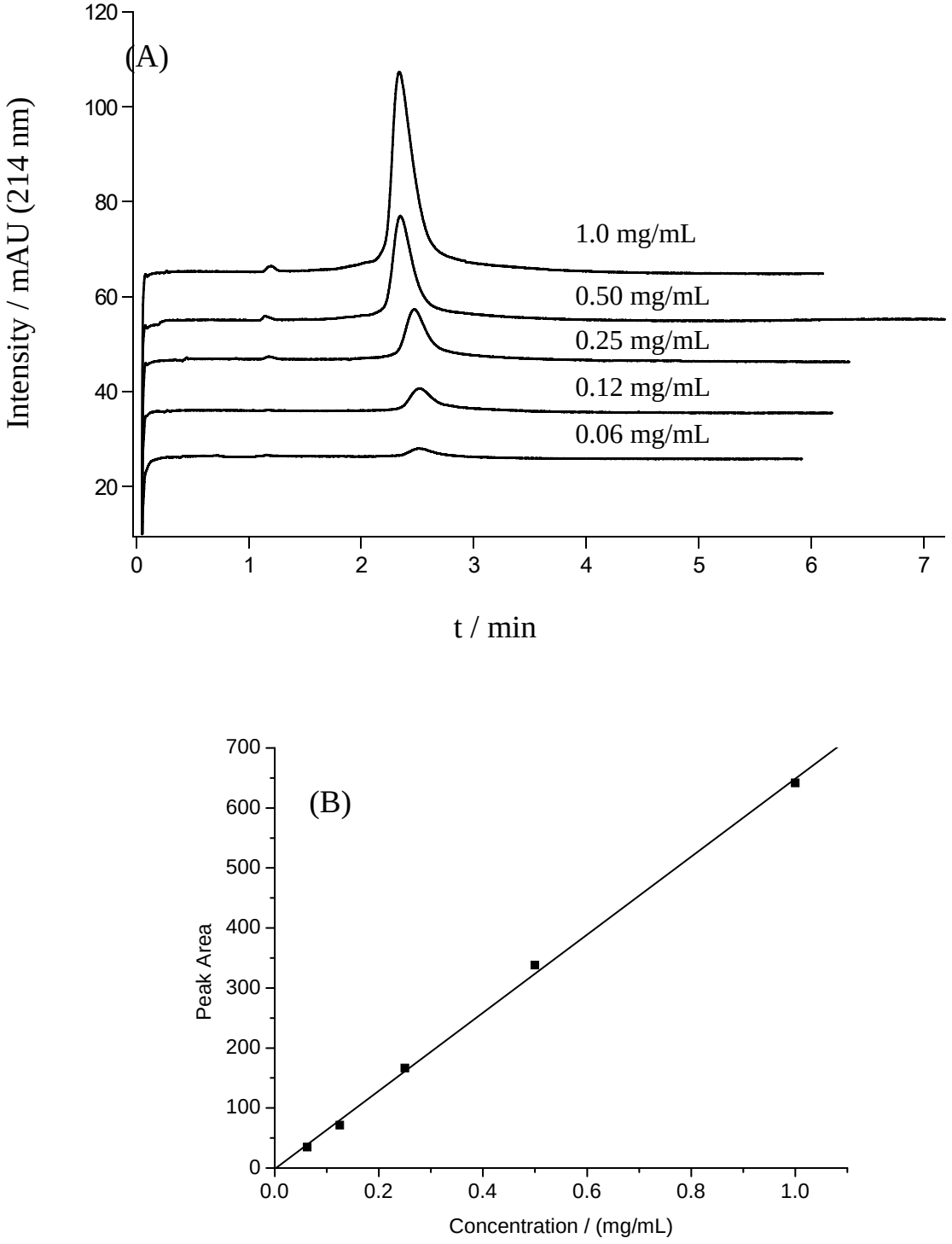


Figure S4. Detection of cytochrome c. (A) Chromatograms of cytochrome c (0.06, 0.12, 0.25, 0.5 and 1 mg/mL). Injection time was 5 seconds with an applied pressure of 8 bar. (B) Relationship between the concentration of cytochrome c and the peak area.

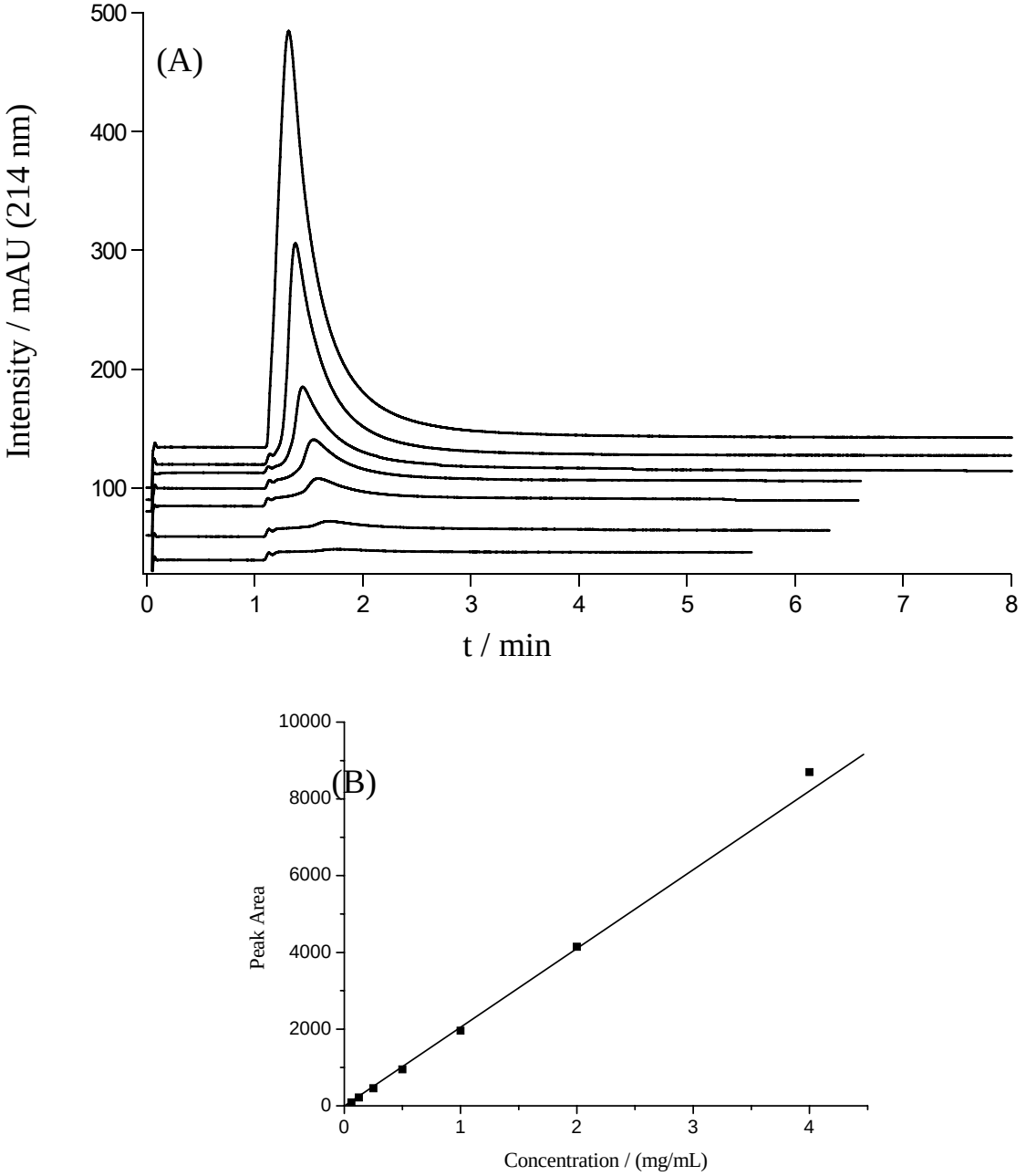


Figure S5. Detection of thrombin. (A) Elution chromatograms of thrombin. Thrombin was injected at a pressure of 8 bar for 10 seconds. It was eluted with a mobile phase containing 50 mM Tris-HCl, 20 mM KCl, and 600 mM NaCl (pH 7.4) after running for 5 min with a mobile phase containing 50 mM Tris-HCl, 20 mM KCl, and 120 mM NaCl (pH 7.4). From the bottom trace to top trace, the corresponding concentration of thrombin was 0.06, 0.12, 0.25, 0.50, 1.0, 2.0 and 4.0 mg/mL, respectively. The recording of the chromatogram traces started immediately after switching to the mobile phase containing 600 mM NaCl. (B) Relationship between the peak area and the concentration of thrombin.

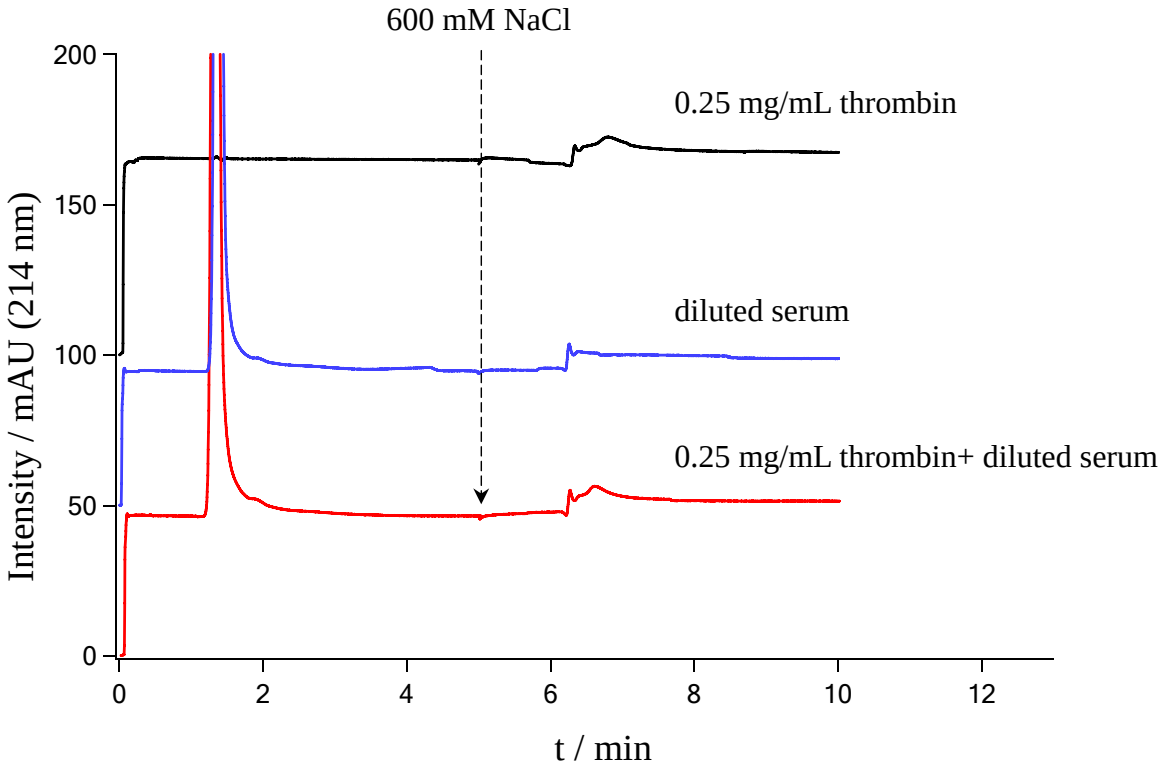


Figure S6. Detection of thrombin spiked in a 10-time diluted human serum sample. Samples were injected at a pressure of 8 bar for 5 seconds. Thrombin was eluted with a mobile phase containing 50 mM Tris-HCl, 20 mM KCl, and 600 mM NaCl (pH 7.4) after running for 5 min with a mobile phase containing 50 mM Tris-HCl, 20 mM KCl, and 120 mM NaCl (pH 7.4).