

## Online Affinity Micro Free-Flow Electrophoresis for the Continuous Monitoring of Insulin via a Competitive Immunoassay

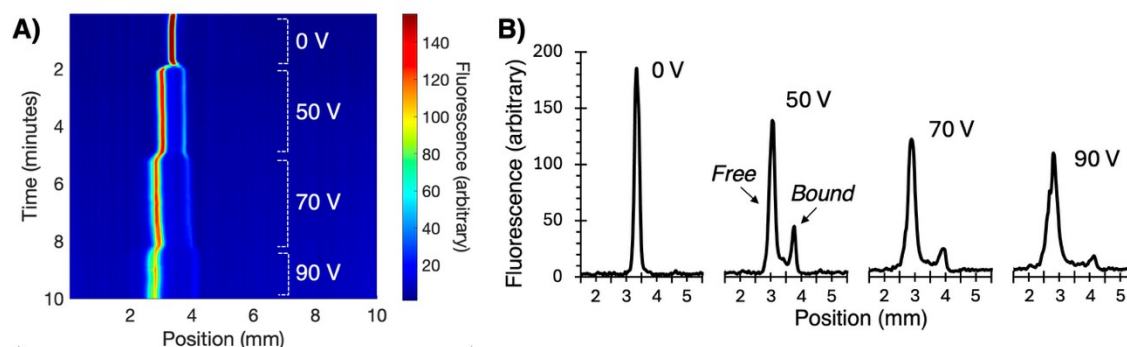
<sup>a</sup>Department of Chemistry, University of Minnesota, 207 Pleasant Street SE, Minneapolis, Minnesota 55455, United States.

<sup>b</sup>Department of Integrative Biology and Physiology, University of Minnesota Medical School, University of Minnesota, 2231 6<sup>th</sup> Street SE, Minneapolis, MN 55455, United States

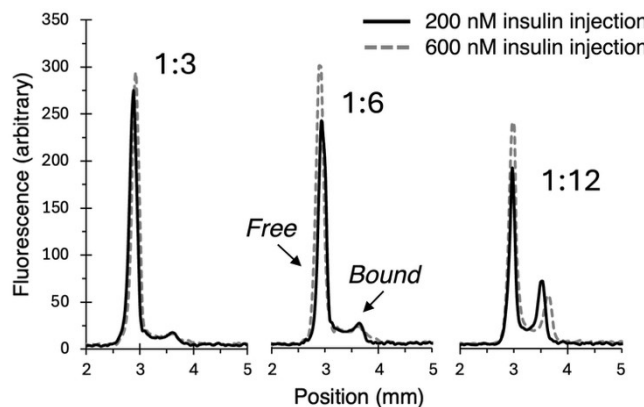
\* Corresponding author, bowser@umn.edu

Gretchen S. Burke,<sup>a</sup> Seokwon Jo,<sup>b</sup> Emilyn U. Alejandro,<sup>b</sup> and Michael T. Bowser<sup>\*a</sup>

### Supplementary Information



**Figure S1.** Voltage optimization for the online  $\mu$ FFE immunoassay separation. Separation of free and bound insulin-FITC following online mixing of 50 nM insulin-FITC with varying antibody concentration: 150 nM (1:3 ratio), 300 nM (1:6 ratio), or 600 nM (1:12 ratio), during two insulin injections of 200 nM (black line) or 600 nM (dotted grey line), no BSA. Continuous scans at each voltage in (B). Cropped linescans display 3 mm of the 10 mm separation space.



**Figure S2.** Affinity reagent ratio optimization for the online  $\mu$ FFE immunoassay separation. Separation of free and bound insulin-FITC following online mixing of 50 nM insulin-FITC with varying antibody concentration: 150 nM (1:3 ratio), 300 nM (1:6 ratio), or 600 nM (1:12 ratio), during two insulin injections of 200 nM (black line) or 600 nM (dotted grey line), no BSA. 1:6 insulin-FITC:antibody ratio resulted in highest sensitivity to unlabeled insulin. Cropped linescans display 3 mm of the 10 mm separation space.