Electronic Supplementary Information

Rapid SOMAmer-based detection of C-reactive Protein using isotachophoresis and an ionic spacer

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Contents

Section S1. Initial placement of the probe in the channel

The placement of the target and probe molecules in the channel is an important decision in the assay design. When working with targets that have relatively low or unknown mobilities (such as proteins), we generally recommend that the target be initially loaded into the LE. A target with a mobility lower than that of the TE and placed in the TE neither focuses in ITP nor encounters ITP-focused probes. Placing such a low-mobility target in the LE ensures that the target molecules encounter ITP-focused probe, even if the free target molecules do not focus in ITP.

The placement of the higher-mobility probe is more interesting. In this analysis, we only consider placing the probe molecules in either the LE or TE buffers. As described in the main text, the spacer molecules are included in the TE buffer. We note that it is possible to include probe molecules in both buffers. Given a maximum concentration of probe in both buffers, doing so can maximize the number of molecules entering the ITP zone and thus maximize reaction speed-up. However, probe molecules can be expensive and should thus only be placed where they will be most effective.

The key term influenced by the initial loading of the probe is the probe flux term, expressed as $\dot{N}_{P,LE}$ and $\dot{N}_{P,spacer}$ when placed in the LE or spacer, respectively. $\dot{N}_{P,LE}$ and $\dot{N}_{P,spacer}$ are given by:

$$\dot{N}_{P,LE} = 1 - \frac{\mu_P}{\mu_{LE}} \tag{1}$$

$$\dot{N}_{P,spacer} = \left(\frac{\mu_P}{\mu_{spacer}} - 1 \right) \frac{\mu_{CI} - \mu_{LE}}{\mu_{CI} - \mu_{spacer}} \frac{\mu_{spacer}}{\mu_{LE}} \frac{c_{LE}}{c_{TE}^{well}} \tag{2}$$

A key parameter, therefore, in deciding on the placement of the probe, is the ratio between the two flux terms, defined as:

$$\phi = \frac{\dot{N}_{P,LE}}{\dot{N}_{P,spacer}} = \frac{\left(\mu_{LE} - \mu_P\right)\left(\mu_{CI} - \mu_{spacer}\right)}{\left(\mu_P - \mu_{spacer}\right)\left(\mu_{CI} - \mu_{LE}\right)} \frac{c_{TE}^{well}}{c_{LE}} \tag{3}$$

For $\phi \approx 1$, the rate at which the probe enters the ITP zone is not greatly dependent on its initial placement. However, for $\phi >> 1$, placing in probe in the LE leads to a greater flux of probe into ITP. Similarly, for $\phi << 1$, reaction kinetics are improved by placing the probe in the TE. In Fig 2, we see the dependence of ϕ on the mobilities of the probe and spacer for equimolar concentrations of LE and TE. We applied eq. 3 for the assay described in this paper, using SPRESSO[1] to obtain the mobilities of the LE (μ_{LE} = -79 × 10^{-9} $m^2V^{-1}s^{-1}$), spacer (μ_{spacer} = -17 × 10^{-9} $m^2V^{-1}s^{-1}$), and counterion (μ_{CI} = 20 × 10^{-9} $m^2V^{-1}s^{-1}$), and an approximation of probe mobility based on published values (μ_P = -30 × 10^{-9} $m^2V^{-1}s^{-1}$).[2] Using equal TE and LE concentrations, we found that $\phi \approx 1.5$ for our assay chemistry. We note that a secondary advantage of placing the probe in the LE is the lack of background noise behind the trailing ITP peak, due to the continuous accumulation of fluorescent probe from the TE reservoir.

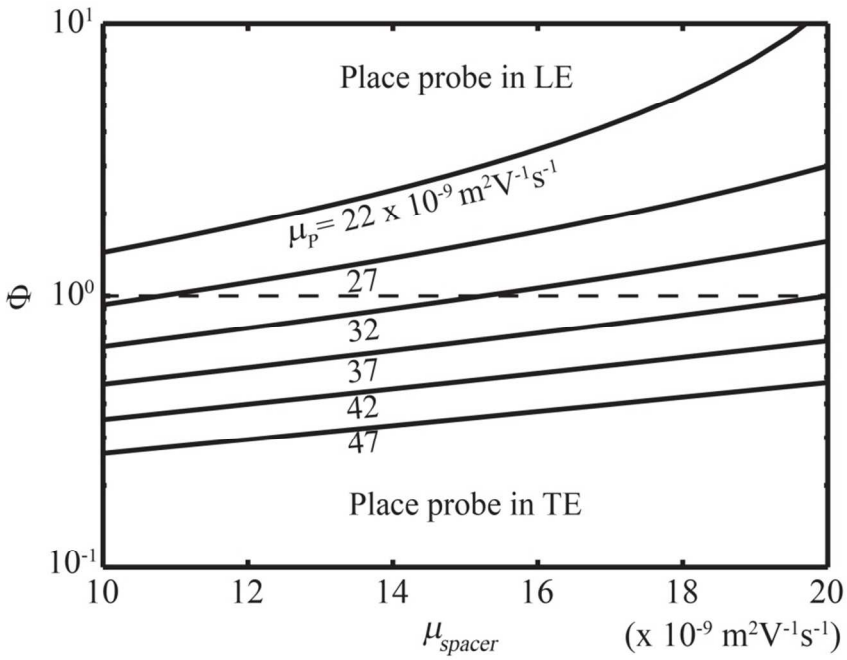

Figure S1. Results from the analytical modeling showing the ratio of probe flux to the ITP reaction zone from a starting position in the LE and TE, respectively. This ratio is plotted for various spacer and probe mobilities. We find that for values of $\phi >> 1$, including the probe in the LE results in greater flux into the ITP reaction zone and thus accelerated reaction kinetics. This effect is reversed for low values of ϕ. We conclude that, when the probe and spacer have similar mobilities, the probe should be placed in the LE. In this calculation, we assume equimolar LE and TE concentrations, as well as anE mobility of -79 x 10^{-9} m^2/V.s (that of the commonly used Cl^-) and a counterion mobility of 20 x 10^{-9} m^2/V.s (that of Imidazole, a common counterion).

Section S2. Effect of Z-block on non-specific interactions

Z-block is a synthetic oligodeoxynucleotide containing modified dU nucleotides synthesized at SomaLogic. Z-block is described briefly in Kraemer at al.[3] We used this reagent in our experiments in order to reduce non-specific interactions in the assay. To demonstrate its efficacy, we used bovine serum albumin (BSA) as a non-specific target with and without Z-block. We measured the ratio of bound SOMAmer to total SOMAmer, and plotted the results in Figure S2. We found a 50-fold reduction in non-specific binding between the CRP SOMAmer and BSA in the presence of 10 μM Z-block. We hypothesize that Z-block can play a significant role in the extension of this assay to complex samples with many proteins.

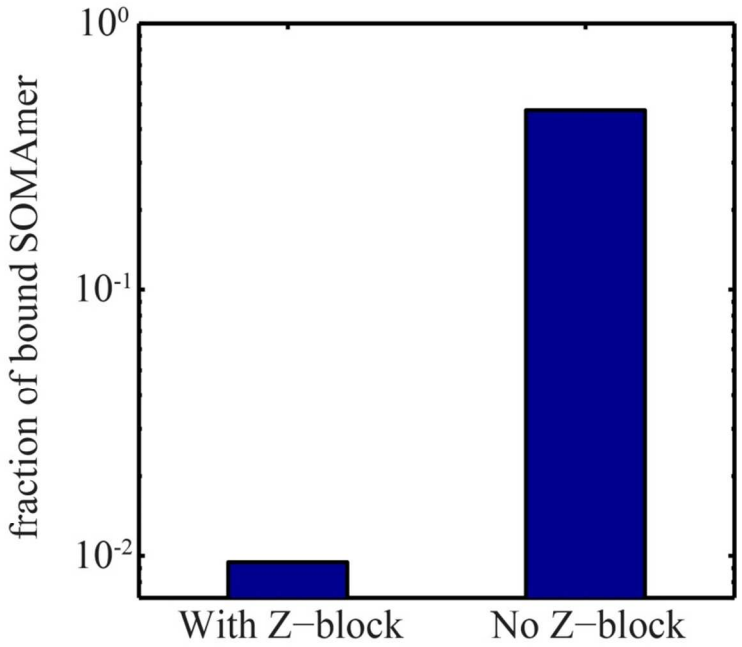

Figure S2. Quantification of the effect of Z-block on the non-specific interactions between SOMAmer and BSA. We performed two sets of experiments with 180 nM SOMAmer and 7.5 μM BSA. In the first set (left), we included 10 μM Z-block and found that the ratio of bound to total SOMAmer was 0.01. In the second set (right), we did not include any Z-block and found the same ratio to be 0.48, a 50-fold increase in non-specific interactions.

Section S3. Raw data from data experiments

We include a bar graph of the raw data from the experiments we used to build the titration curve. We note that even at protein target concentrations exceeding the SOMAmer concentration 10-fold, the fraction of bound SOMAmer does not reach unity. We suspect that this is due to observed protein aggregate formation among other possible factors.

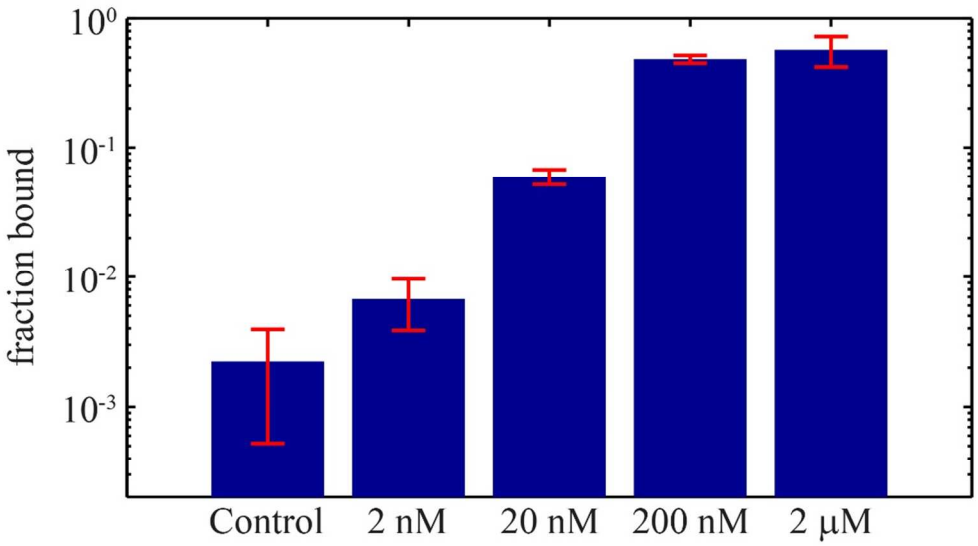

Figure S3. Raw data from the assay experiments for the detection of CRP protein using CRP-specific SOMAmer. For all experiments, we fix the SOMAmer concentration at 180 nM and vary CRP concentrations. Control here describes a buffer that contains no CRP protein. The fraction of bound SOMAmer does not reach unity even at the highest protein target concentrations. We attribute this to protein aggregate formation and other factors.

Section S4. Simplified model under abundant probe assumption

We present the simplified model reflecting the assumption that probe molecules are abundant relative to target molecules. In this case, we can neglect the reaction terms in the probe concentration equations. The result of this simplification propagates to the other equations describing the concentration of free target and the number of molecules of probe-target complex.

In the downstream LE region:

$$\begin{cases} c_{P,down} \approx c_P^0 \\ \dfrac{\mathrm{d}c_{T,down}}{\mathrm{d}t} = -k_{on} c_P^0 c_{T,down} \\ \dfrac{\mathrm{d}N_{PT,down}}{\mathrm{d}t} = k_{on} c_P^0 c_{T,down} A(L_0 - U_{ITP} t) \end{cases} \tag{4}$$

In the ITP zone:

$$\begin{cases} \dfrac{\mathrm{d}c_{P,ITP}}{\mathrm{d}t} = \dfrac{U_{ITP}}{\delta} \eta_{P,LE} c_P^0 \\ \dfrac{\mathrm{d}c_{T,ITP}}{\mathrm{d}t} = -k_{on} c_{P,ITP} c_{T,ITP} + \dfrac{U_{ITP}}{\delta} \eta_{T,LE} (c_{T,down} - c_{T,ITP}) \\ \dfrac{\mathrm{d}N_{PT,ITP}}{\mathrm{d}t} = k_{on} c_{T,ITP} c_{P,ITP} (\delta A) \end{cases} \tag{5}$$

Section S5. Experimental results from serum experiments

We explored the feasibility of our assay in a complex sample by spiking CRP target into 20x-diluted serum sample. The experimental protocol for these experiments is similar to the one presented for a simple buffer sample. We present the experimental results from these experiments in Figure S4. We achieved a limit of detection of 25 nM in 20x-diluted serum, which translates to 500 nM in an undiluted serum sample. While this sensitivity is not clinically-relevant for cardiovascular risk assessment, it is relevant for identifying bacterial and viral infections.[4]

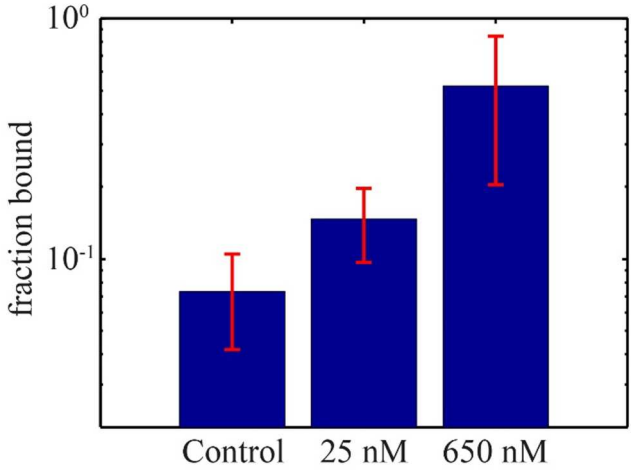

Figure S4. Experimental data from CRP assay in diluted serum sample. For all experiments, we use a SOMAmer concentration of 180 nM and vary CRP concentrations. We dilute serum samples 20-fold in LE buffer. We then spike CRP target into the mixture. We observe significantly higher background signal in the negative control case. We attribute this increase in signal to non-specific interactions between the SOMAmer and other serum proteins.

We also note that using 0.2% Triton X-100 led to decreased aggregate formation than 0.02% or 0.12% Tween-20. We hypothesize that this is due to Triton X-100 being a harsher detergent than Tween-20, and thus was more successful in solubilizing serum proteins. However, we still observed significant aggregates regardless of the surfactant chosen.

References

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