**Supporting Information**

*Damköhler Calculation*

For the Damköhler calculation, we set $k_a = 1.984x10^4$ $M^{-1}s^{-1}$ as determined by Lee and coworkers for the S-protein/ACE2 binding pair.[1] $C_{p0}$ was estimated as $4.3x10^{-9}$ $mol*m^{-2}$ by dividing the number of moles of capture protein deposited per test line area ($1x10^{-12}$ $mol*mm^{-2}$) by the internal surface area conversion factor of 1 $mm^2$ = 250 $mm^2$ for 0.45 μm pore size nitrocellulose.[2] The pore radius is $2.25x10^{-7}$ m, and we estimated the diffusivity of a protein to be on the order of $10^{-10}$ $m^2s^{-1}$, since the experimentally determined diffusivity of IgG was found to be $2x10^{-10}$ $m^2s^{-1}$ in nitrocellulose.[3]

*Equation 1 Derivation*

Our system is modeled as a second order reaction for binding of an antigen (A) to a capture probe (P) to form a bound complex (B):

$$A + P \rightleftarrows B$$

where the forward reaction proceeds at rate $k_a$ and the backward reaction proceeds at rate $k_d$. Assuming first-order Langmuir kinetics, the change in $\tilde{C}_b$ over time is described by:[4]

$$\frac{d\tilde{C}_b}{dt} = k_a c_s\left(\tilde{C}_{p0} - \tilde{C}_b\right) - k_d\tilde{C}_b$$

where $c_s$ is the surface concentration of the antigen. By assuming $c_s$ is approximately equal to the initial surface concentration of the antigen ($c_0$) and that no capture probes are bound at $t = 0$ ($\tilde{C}_b(t = 0) = 0$), the first-order linear ordinary differential equation can be solved:

$$\tilde{C}_b^{'}(t) = -(k_a c_0 + k_d)\tilde{C}_b(t) + k_a c_0 \tilde{C}_{p0}$$

$$\tilde{C}_b(t) = exp[-\int_0^t (k_a c_0 + k_d) ds] * 0 + \int_0^t exp[-\int_s^t (k_a c_0 + k_d) du] * k_a c_0 \tilde{C}_{p0} ds$$

$$\tilde{C}_b(t) = \frac{k_a c_0 \tilde{C}_{p0}}{k_a c_0 + k_d}(1 - e^{-(k_a c_0 + k_d)t})$$

By, dividing both sides of the solution by $\tilde{C}_{p0}$, and by dividing both sides of the quotient by $k_d$, this solution simplifies to:[5]

$$\frac{\tilde{C}_b}{\tilde{C}_{p0}} = \frac{\frac{c_0}{K_D}}{1 + \frac{c_0}{K_D}}(1 - \exp\left(-\left(\frac{c_0}{K_D} + 1\right)k_d t\right))$$

As $t \to \infty$, $$\frac{\tilde{C}_b}{\tilde{C}_{p0}} = \frac{\frac{c_0}{K_D}}{1 + \frac{c_0}{K_D}}.$$

*Methods for achieving contact between the Nafion membrane and the nitrocellulose membrane*

The first challenge in developing an ICP-enhanced LFA was to find a method to attach the Nafion membranes to the nitrocellulose membrane. The use of magnets (Figure S1 a, b) resulted in uneven enrichment because the contact between the nitrocellulose membrane and the Nafion was poor. The use of Scotch tape resulted in better enrichment (Figure S1 c, d), but the lack of lamination across the nitrocellulose membrane resulted in evaporation of the solution. Next, we hand-painted Nafion solution onto microfluidic diagnostic tape that laminated the

nitrocellulose membrane (Figure S2 a, b). Improved enrichment was obtained, but the reproducibility of the width and thickness of the Nafion was poor. Finally, we used an automatic lateral flow assay dispenser reagent to dispense the Nafion solution onto the microfluidic diagnostic tape (Figure S2 c, d). Improved enrichment was obtained, and the membranes could be made reproducibly.

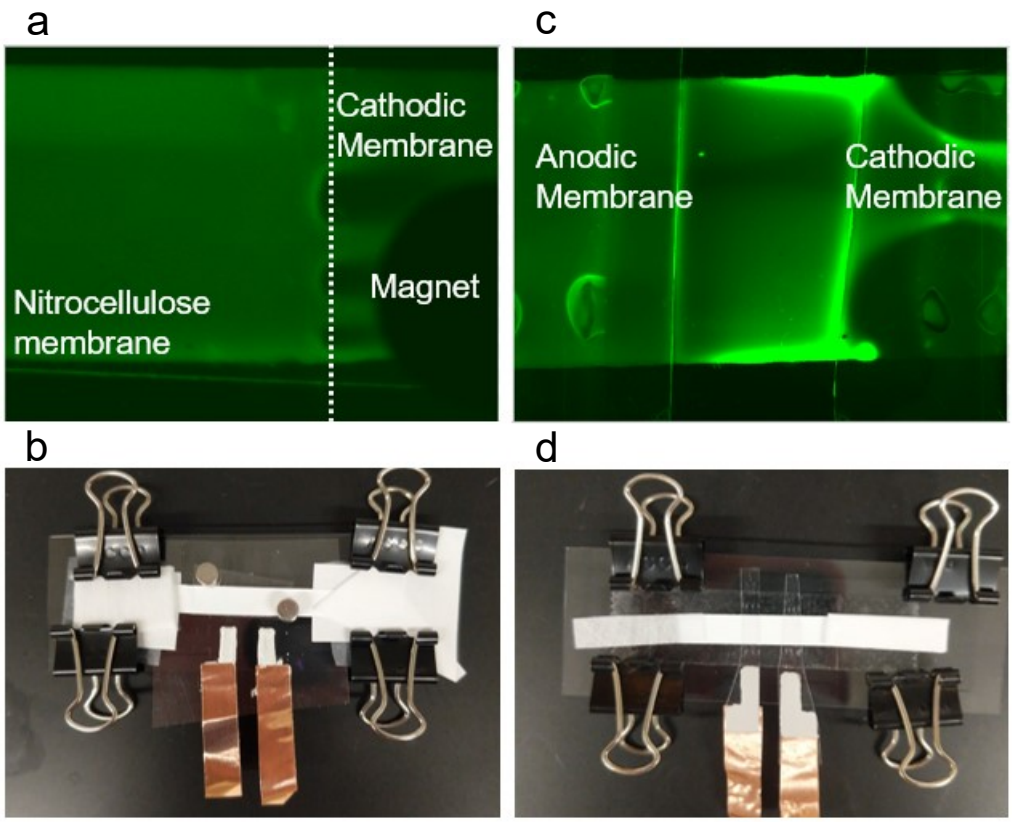



**Figure S1**. Fluorescence micrographs of the enrichment of $BODIPY^{2-}$ with different methods of adhering the Nafion membrane to the nitrocellulose membrane: magnets (a) and Scotch tape (c). Photographs of devices used to enrich $BODIPY^{2-}$ with different methods of adhering the Nafion membrane to the nitrocellulose membrane: magnets (b) and Scotch tape (c).

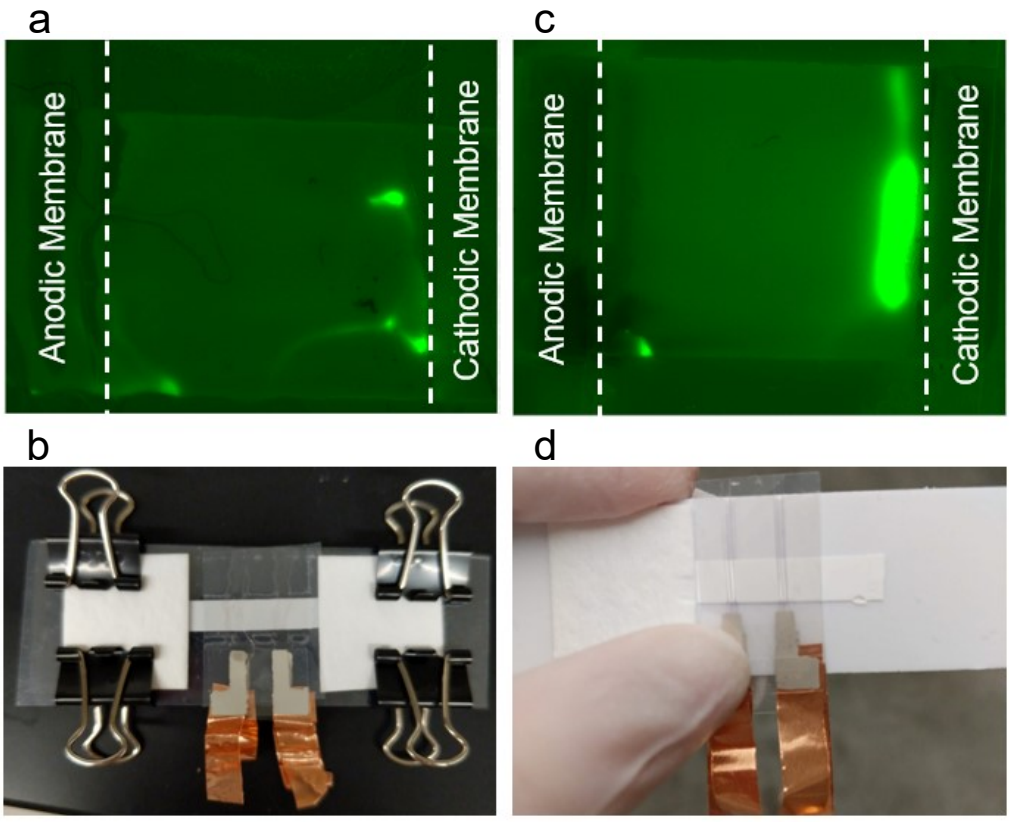
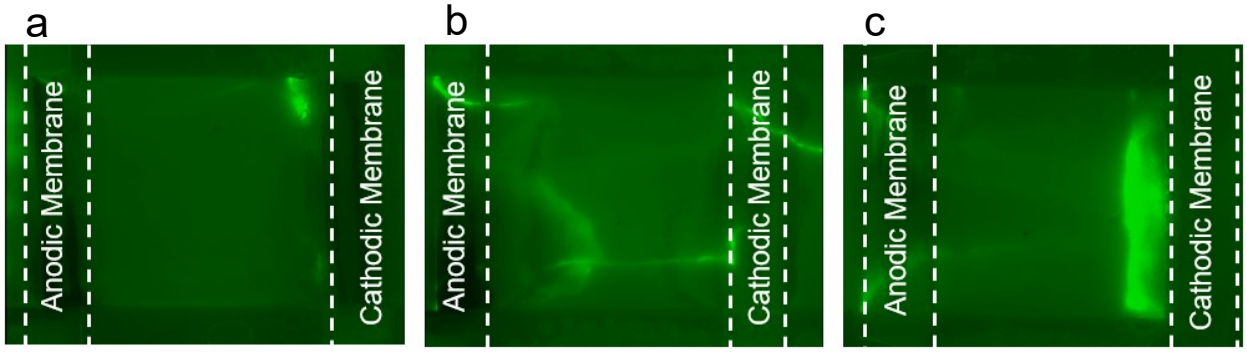



**Figure S2.** Fluorescence micrographs of the enrichment of $BODIPY^{2-}$ with different methods of painting the Nafion membrane microfluidic diagnostic tape: hand painted (a) and dispenser painted (c). Photographs of devices used to enrich $BODIPY^{2-}$ with different methods painting the Nafion membrane microfluidic diagnostic tape: hand painted (b) and dispenser painted (d).

*Enrichment of S-protein with different pretreatment solutions*

To enrich S-protein in a nitrocellulose membrane, the sample pad and nitrocellulose membrane had to be pretreated. The pretreatment solution from Lee and co-workers (composed of 10 mM




2-amino-2-methyl-1-propanol (pH 9.0), 0.5% BSA, 0.5% β-Lactose, 0.05% Triton X-100, 0.05% sodium azide)[1] was used initially (Figure S3 a). The pretreatment solution increased the number of ions, so it was difficult to form the IDZ and IEZ. Consequently, the enrichment of S-protein was poor. Next, a commercially available LFA pretreatment solution, Stabilgaurd,[6] was used (Figure S3 b). Again, the conductivity was too high, so the IDZ was unstable and any enrichment that occurred flowed away. Finally, a solution of 1 mM Pluronic F-127 was used to pretreat the LFA (Figure S3 c). Excellent and stable enrichment was achieved.

**Figure S3**. The enrichment of S-protein with three different pretreatment solutions: the pretreatment solution from Lee and co-workers (a), Stabilgaurd (b), and Pluronic F-127 (c).

*pH dependence of S-protein binding to ACE2 capture probes*

The pH dependence of S-protein binding to the ACE2 test line was investigated. Four different solutions were prepared with pH 2, 5, 7, and 8. S-protein, 0.4 ug/mL, was added to each of the four solutions containing Tris buffer, then added to a nitrocellulose membrane with an ACE2 test line. The fluorescence intensity of the test lines were observed at the same time point (Figure S

4). The best binding of S-protein to the test line is observed at pH 7, while no binding occurred at pH 2 and 5. Minimal binding was observed for the pH 8 solution.

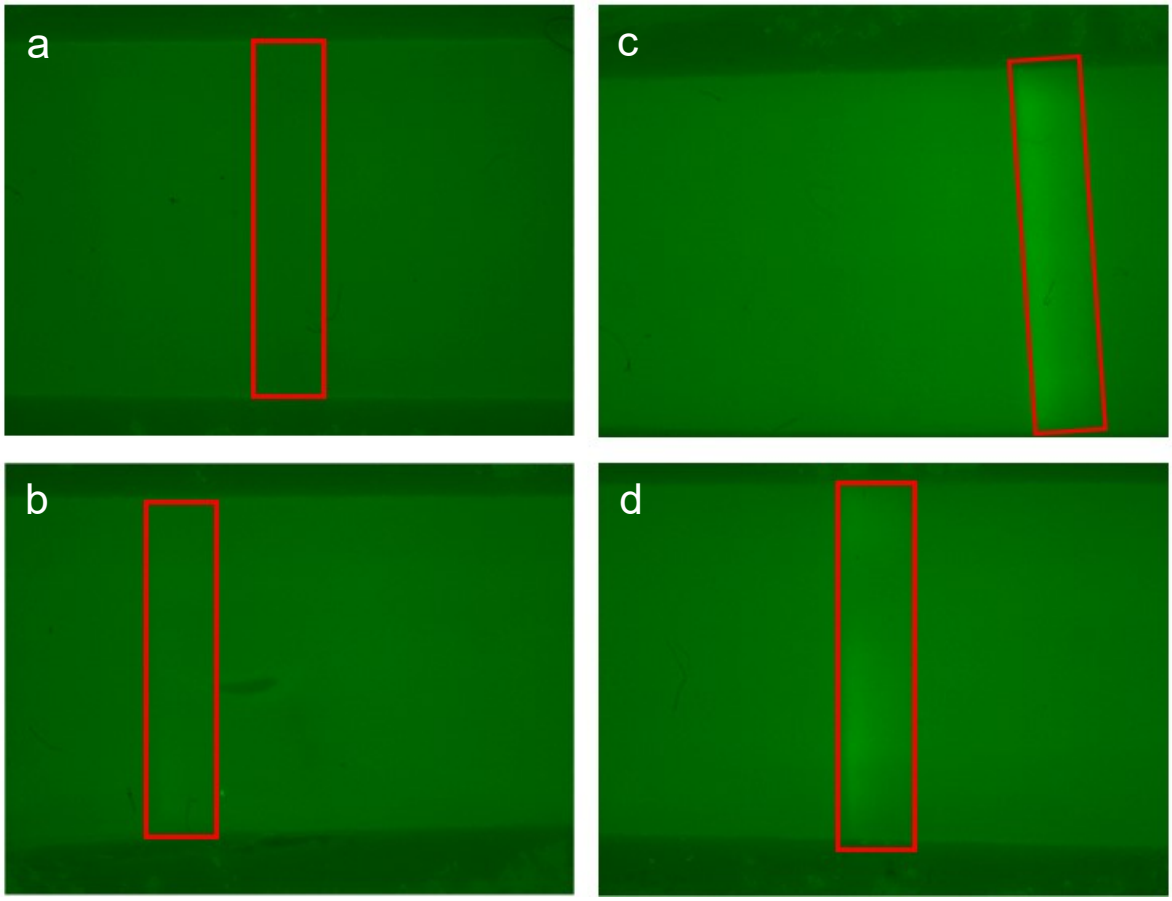



**Figure S4**. Fluorescence micrographs of S-protein added to a nitrocellulose membrane containing an ACE2 test line. Four pH-levels of running buffer (100 mM Tris HCl with 0.05% Tween-20) were tested: pH 2 (a), pH 5 (b), pH 7 (c), and pH 8 (d). The test line is outlined with a red box. The initially concentration of S-protein is the same for each assay, as well as the time that the assay was allowed to incubate before images were taken.

*Changing the running buffer for S-protein enrichment*

The enrichment S-protein was much improved with the Pluronic pretreatment, as can be seen in Figure S5 a. However, the enriched plug of S-protein with Tris running buffer occurs directly

next to the Nafion membrane, which is acidic. Figure S5 b is a fluorescence micrograph of S-protein in Tris running buffer before voltage has been turned on. The S-protein interacts with the Nafion membrane and collects at the cathodic Nafion membrane, until the capillary flow can push it downstream. We were concerned that the Tris buffer did not have a high enough buffering capacity to negate the acidity of the Nafion, so we investigated other buffers: Bis Tris Propane and HEPES buffers. The Bis Tris Propane buffer was too conductive, which lead to poor enrichment (Figure S5 c). However, enrichment with HEPES buffer was good, and the enriched plug did not occur directly next to the Nafion membrane (Figure S5 d). Therefore, HEPES buffer was selected as the running buffer moving forward.

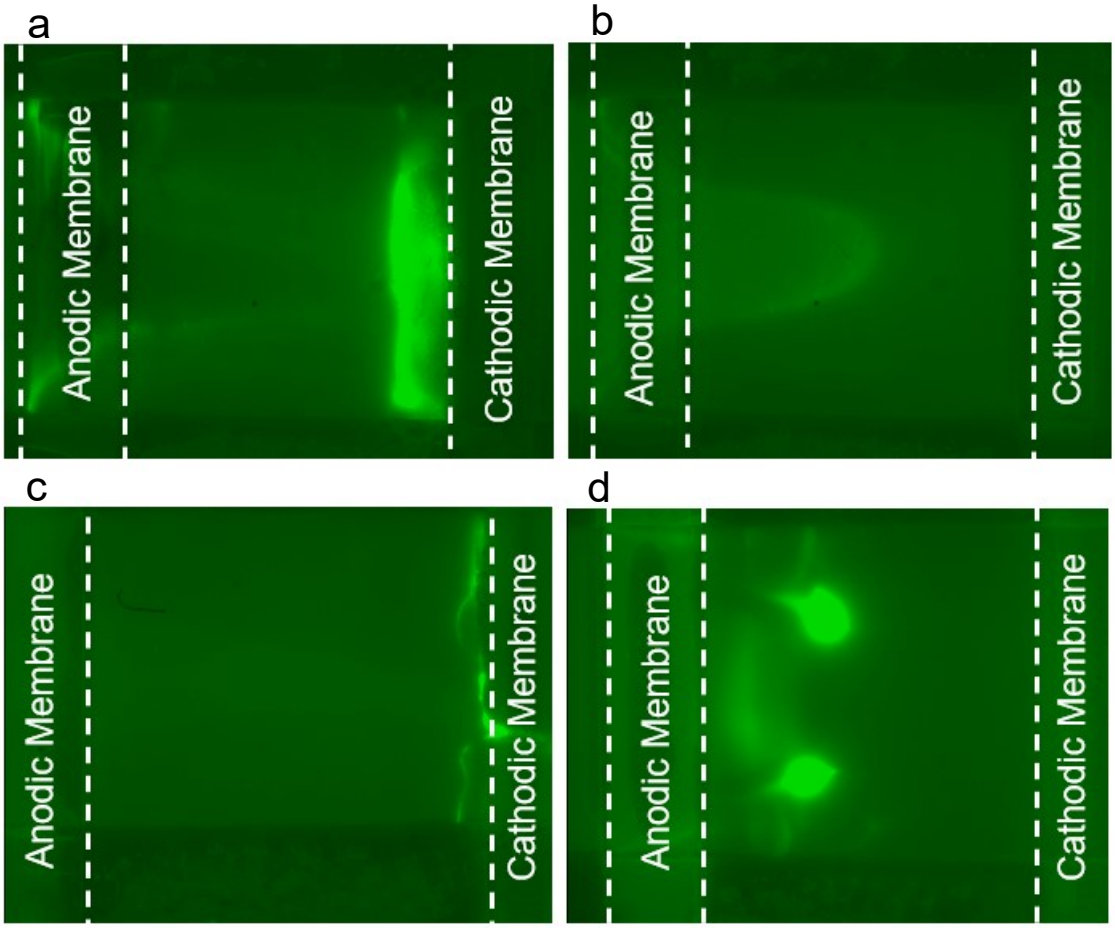



**Figure S5**. Fluorescence micrographs of enrichment of S-protein in Tris buffer (a), Bis Tris Propane buffer (c), and HEPES buffer (d). (b) Fluorescence micrograph of the experiment shown in (a) before the voltage was turned on.

*Current voltages curves of 1 mm wide nitrocellulose membrane*

The current voltage curves of S-protein in HEPES buffer with the 1 mm wide nitrocellulose membrane is shown in Figure S6 a. The onset of enrichment occurred at 13 V, and the resistance of the system, calculated form the slope of the CVC in the Ohmic region, is 3.16 MΩ.

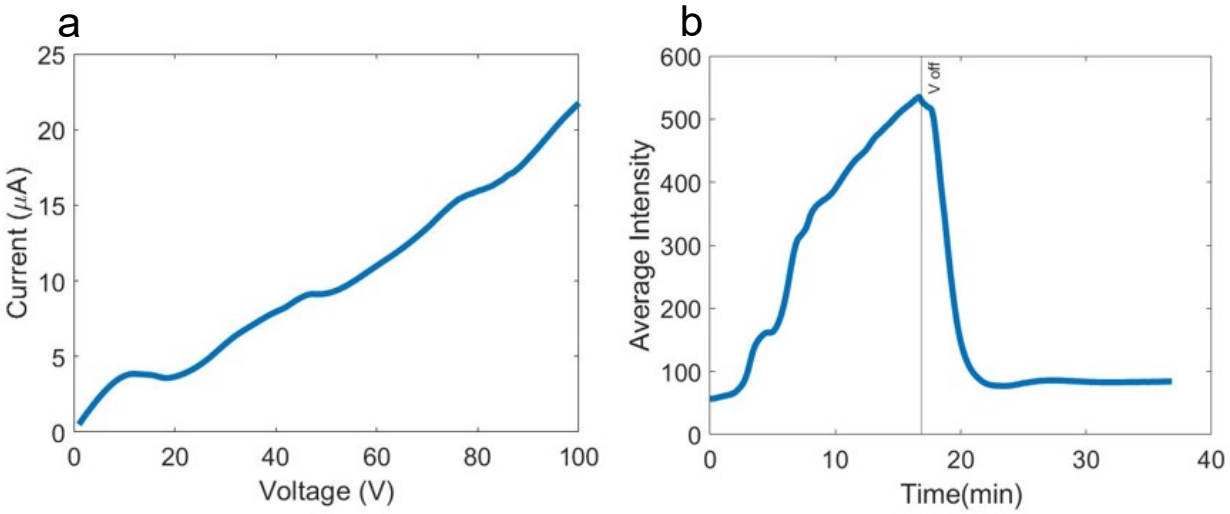



**Figure S6.** The current voltage curve for 1 mm wide nitrocellulose membrane. (a) The current is measured as the voltage is ramped from 1 to 100 V with a rate of 1 V per 10 s. The current represents the average current obtained for each voltage applied. (b) The average fluorescence intensity of a rectangular region containing the enriched plug while the CVCs from (a) were collected.

*Calibrating fluorescence intensity to concentration of S-protein*

To calculate the amount that the S-protein had been enriched during ICP, a calibration curve was obtained comparing the fluorescence intensity of the nitrocellulose membrane with distinct concentrations of S-protein. To create the calibration curve in Figure S7, each concentration of S-protein in HEPES running buffer was added directly to a pretreated nitrocellulose membrane that was sandwiched by the backing card and microfluidic diagnostic tape. The solution was allowed to wick across the membrane for 5 min, until a steady fluorescence intensity over time was observed. The average fluorescence intensity was calculated for each concentration at t = 5

min. The trend,

$average\ fluorescence\ intensity\ =\ 56.0\ *\ S-protein\ concentration\ +\ 31$ was used to calculate the concentration of S-protein in the enriched plug.

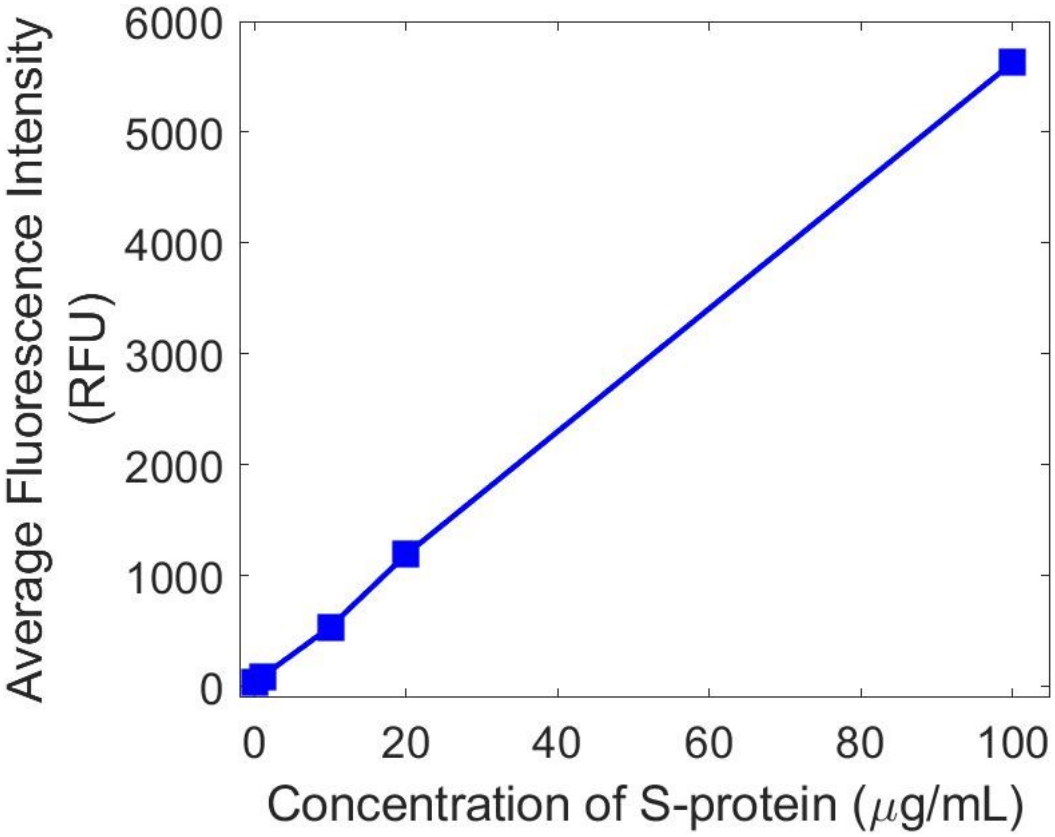



**Figure S7**. Calibration curve obtained comparing the concentration of S-protein in a nitrocellulose membrane to the average fluorescence intensity.

*Calibration curve with fluorescence intensity instead of signal enhancement*

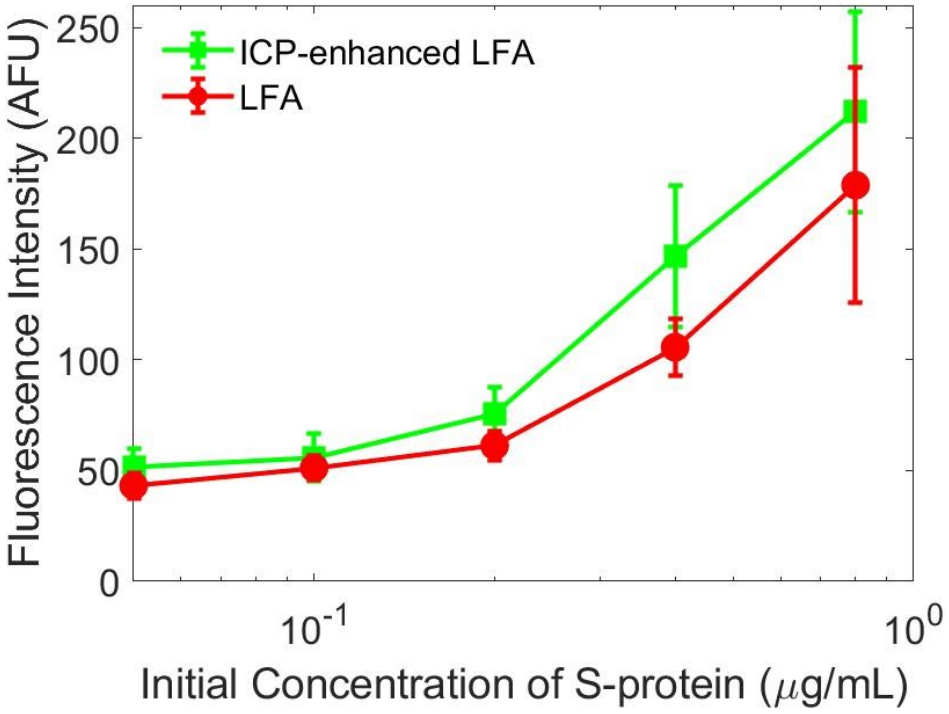



Figure S8. The raw fluorescence intensity data from the calibration curve in Figure 7 (main text) is plotted instead of the signal enhancement.

**References:**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