sensors

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Article: Supplementary Information

Engineering the Signal Resolution of a Paper-based Cell-free Glutamine Biosensor with Genetic Engineering, Metabolic Engineering, and Process Optimization

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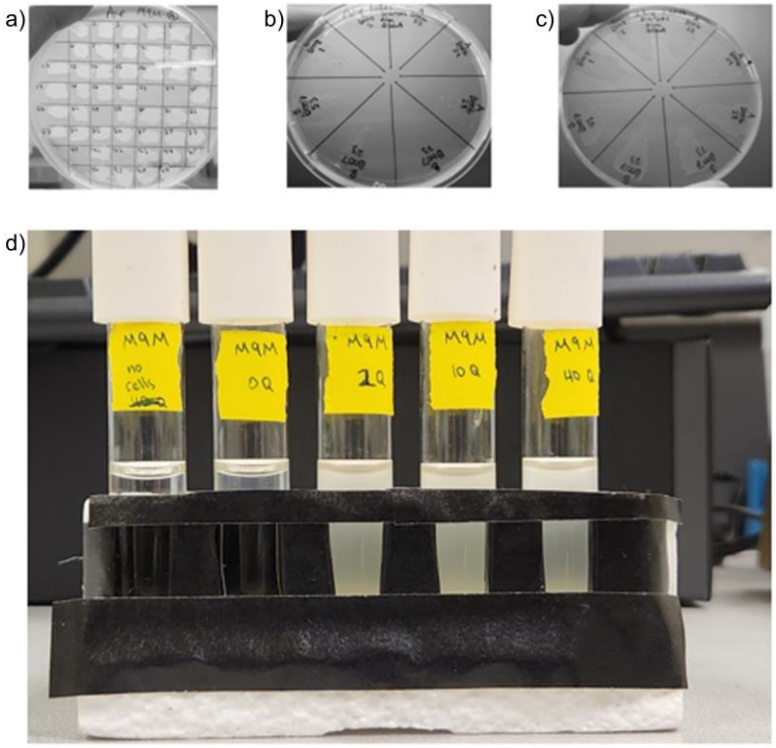

Figure S1: BL21 Star DE3 Δ*lac* Δ*glnA* glutamine auxotroph phenotype. To isolate the auxotrophic colonies after the MAGE mutagenesis protocol, individual colonies were isolated on LB culture plates. Colonies were then numbered and patched onto squares of a minimal media plate (**a**). The colonies corresponding to the cell patches that failed to grow were patched again on minimal media plates whereon they did not grow (**b**), and patched again on standard LB plates whereon they did grow for further propagation (**c**). (**d**) An original uncropped photograph for Figure 2 showing the test tube rack apparatus used for photographing 5 mL liquid *E. coli* cultures in test tubes, which were incubated at 37 °C and 280 RPM and briefly placed in the test tube rack for photographing at indicated time points.

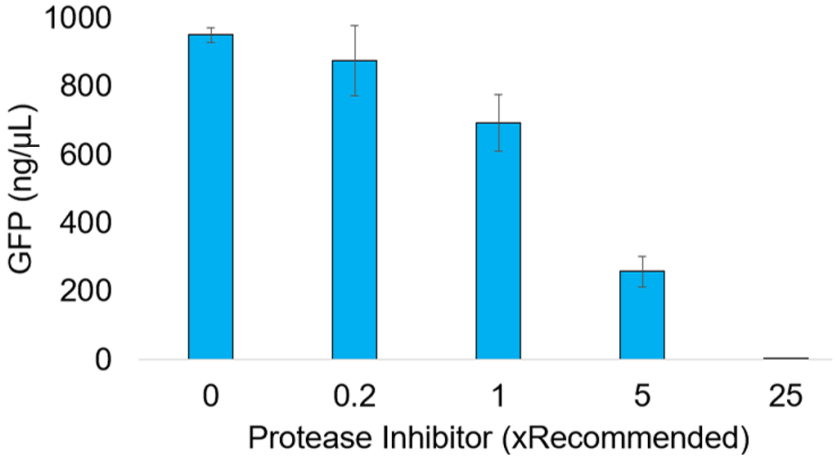

Figure S2: Protease inhibitor toleration of cell-free protein synthesis. The cell-free sfGFP production yields in the presence of varying concentrations of protease inhibitor cocktail, given as normalized multipliers relative to the concentration recommended by the manufacturer for cell lysate.

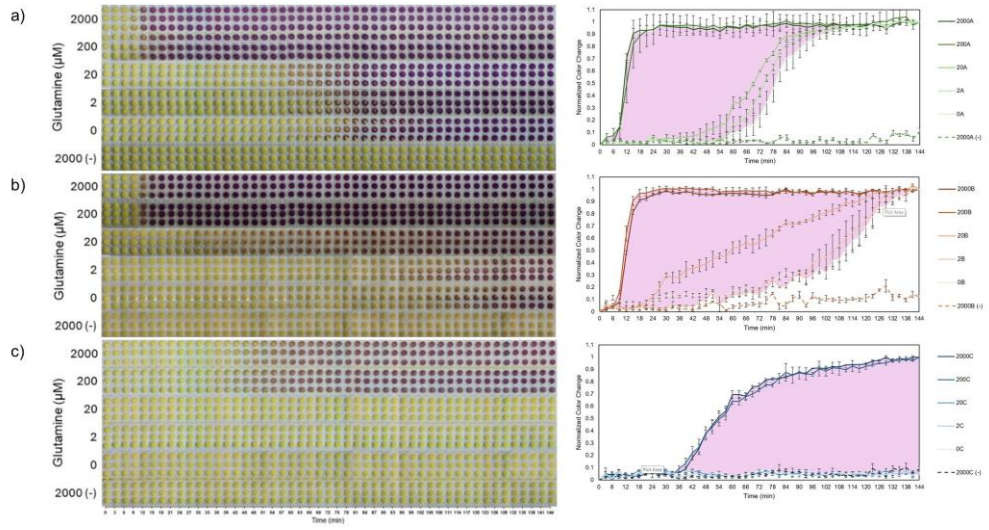

Figure S3: Visual and quantitative paper-based, cell-free glutamine biosensor results. Based on the signal-to-background ratio and signal strength tradeoff shown in Figure 1d, the following three biosensor formulations were engineered into a lyophilized, colorimetric, paper-based format and tested with different glutamine concentrations. (**a**) Δ*glnA* knockout extract with no additives. (**b**) Δ*glnA* knockout extract with MSO and protease inhibitor added. (**c**) Filtered Δ*glnA* knockout extract with MSO and protease inhibitor. Glutamine concentrations (in μM) are indicated on the axis or legend. The quantitative sensor responses are shown on the normalized plots to the right, where the region between the maximum signal and assay background is shaded.

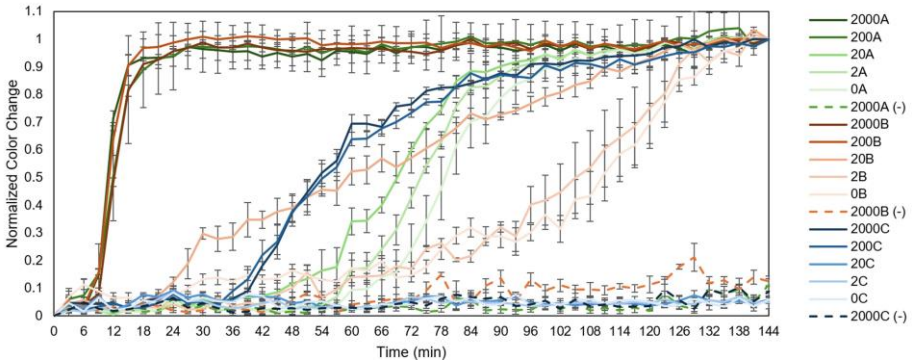

Figure S4: A superimposed quantitative comparison of all tested conditions reported in Figure S3.

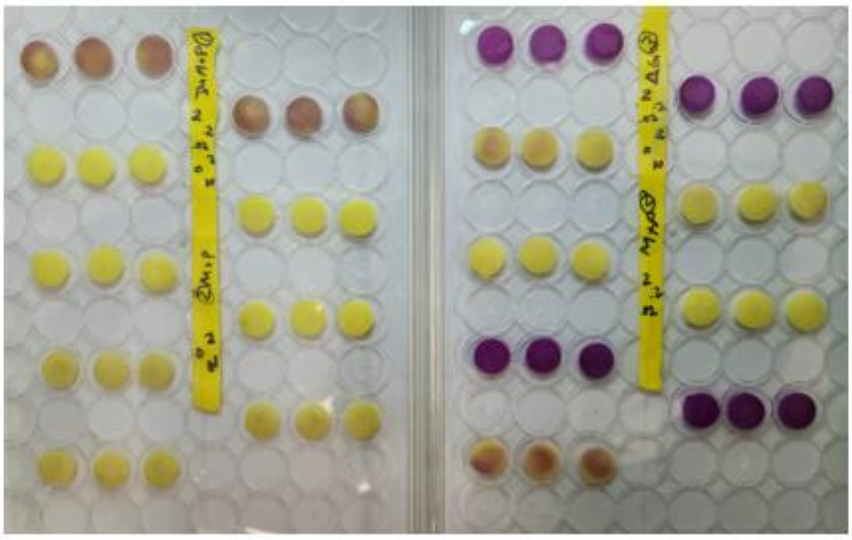

Figure S5: An original uncropped photograph showing the glutamine biosensor. Repeat images of the tests were captured every 3 minutes. To create Figure 2 and Figure S3, these images were cropped individually for each set of triplicates using Adobe Lightroom, and the images were oriented in rows to show the time-dependent glutamine outcome for each concentration tested.

Figure S6: DNA sequences used in this work.

***lacZ* Single Stranded Oligo (26 bp deletion)**

GCTGCGCTGGAGTGACGGCAGTTATCTGGAAGATCAGGATATGTGTCTCGTT-GCTGCATAAACCGACTACACAAATCAGCGATTTCCATG

***glnA* Single Stranded Oligo (10 bp deletion)**

CACGTCACTATCCCTGCTCATCAGGTGAATGCTGAATTCTCAAAATGTTTGAC-GGCTCCTCGATTGGCGGCTGGAAAGGC

MAGE plasmid pJG1219

GGTACCAAATTCCAGAAAAGAGGCCTCCCGAAAGGGGGGCCTTTTTTCGTTTTG
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