Design, validation, and application of an enzyme coupled hydrogen sulfide detection assay

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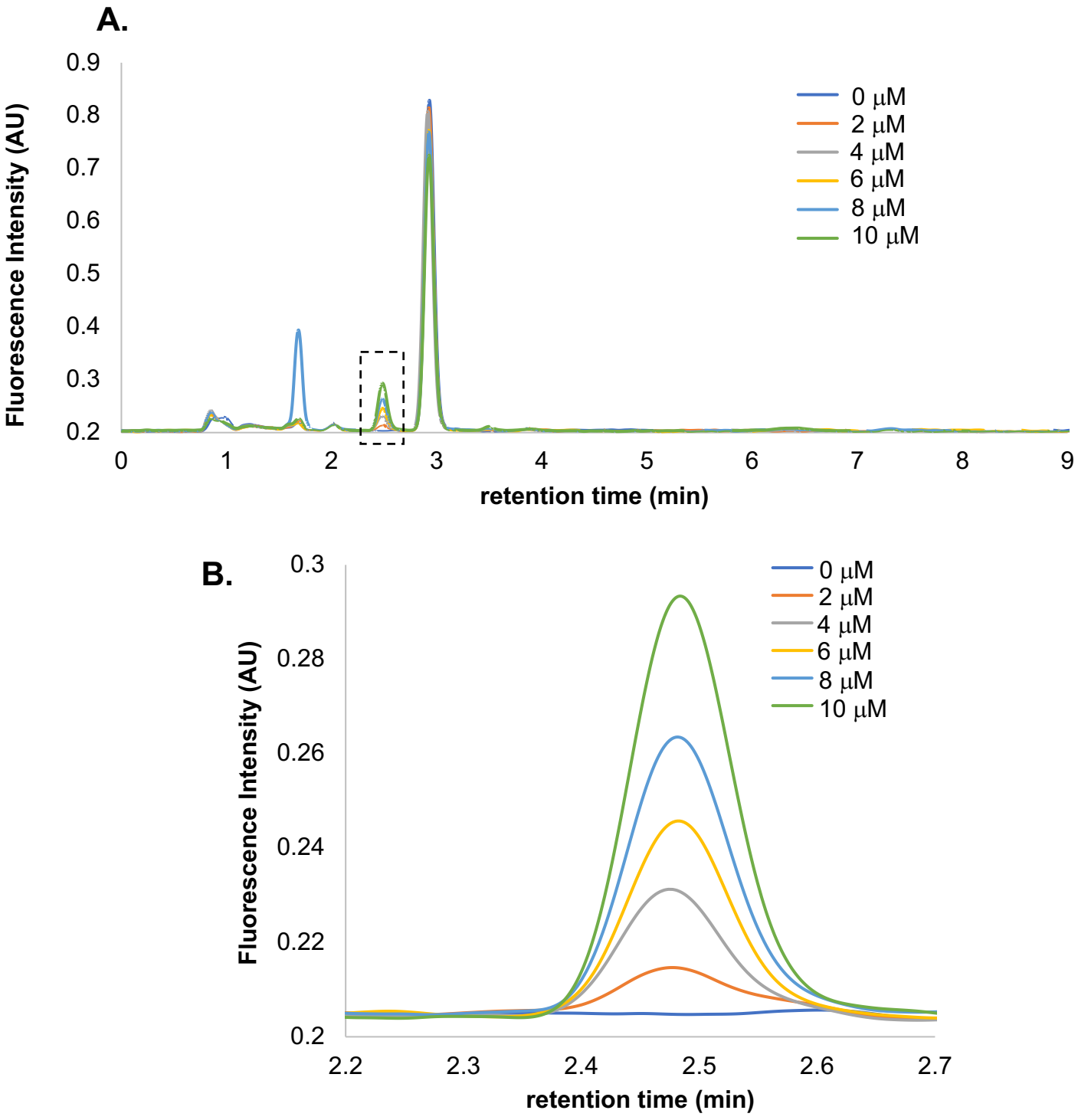
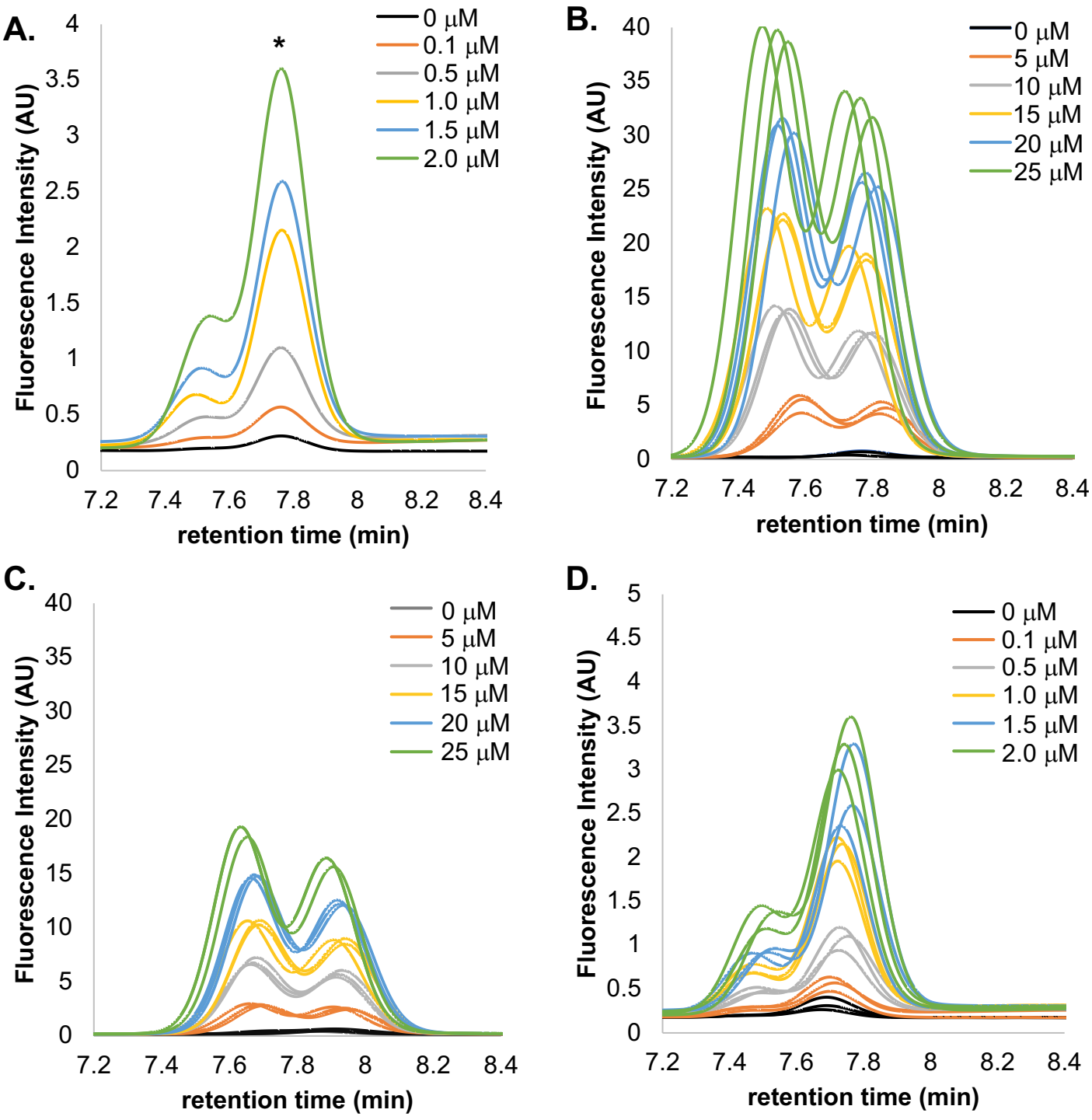


Figure S1: HPLC chromatogram of CysM derived L-cysteine in 65:35 10 mM TCA (pH 1.5):acetonitrile. The formally two-hump L-cysteine peak has collapsed down to one singular peak with a retention time of 2.49 minutes. A) Entire un-cropped HPLC chromatogram and B) zoomed in area of L-cysteine-DMM peak.


A.
Fluorescence Intensity (AU)
retention time (min)
0 μM
0.1 μM
0.5 μM
1.0 μM
1.5 μM
2.0 μM
*
B.
Fluorescence Intensity (AU)
retention time (min)
0 μM
5 μM
10 μM
15 μM
20 μM
25 μM
C.
Fluorescence Intensity (AU)
retention time (min)
0 μM
5 μM
10 μM
15 μM
20 μM
25 μM
D.
Fluorescence Intensity (AU)
retention time (min)
0 μM
0.1 μM
0.5 μM
1.0 μM
1.5 μM
2.0 μM

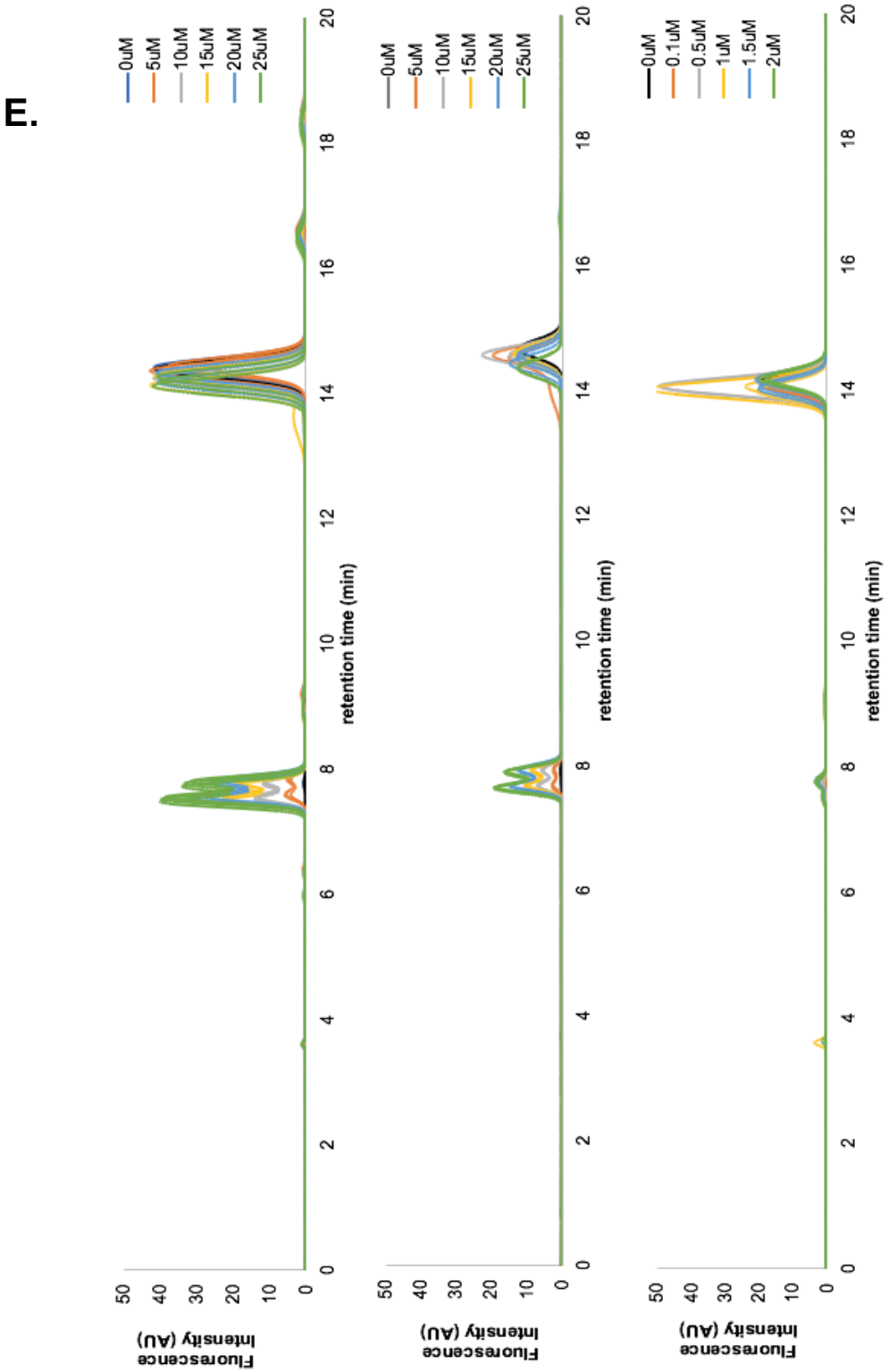


Figure S2: Lower Na_2S and unaligned L-cysteine calibration curves. A) 0-2 µM Na_2S + 60 µM CysM + 1 mM OAS, B) L-cysteine, C) 0- 25 µM Na_2S + 60 µM CysM + 1 mM OAS, D) 0-2 µM Na_2S + 60 µM CysM + 1 mM OAS, E) Un-cropped HPLC traces encompassing entire 20-minute run (left to right: 0- 25 µM L-cysteine + DMM, 0- 25 µM Na_2S + 60 µM CysM + 1 mM OAS, and 0-2 µM Na_2S + 60 µM CysM + 1 mM OAS).

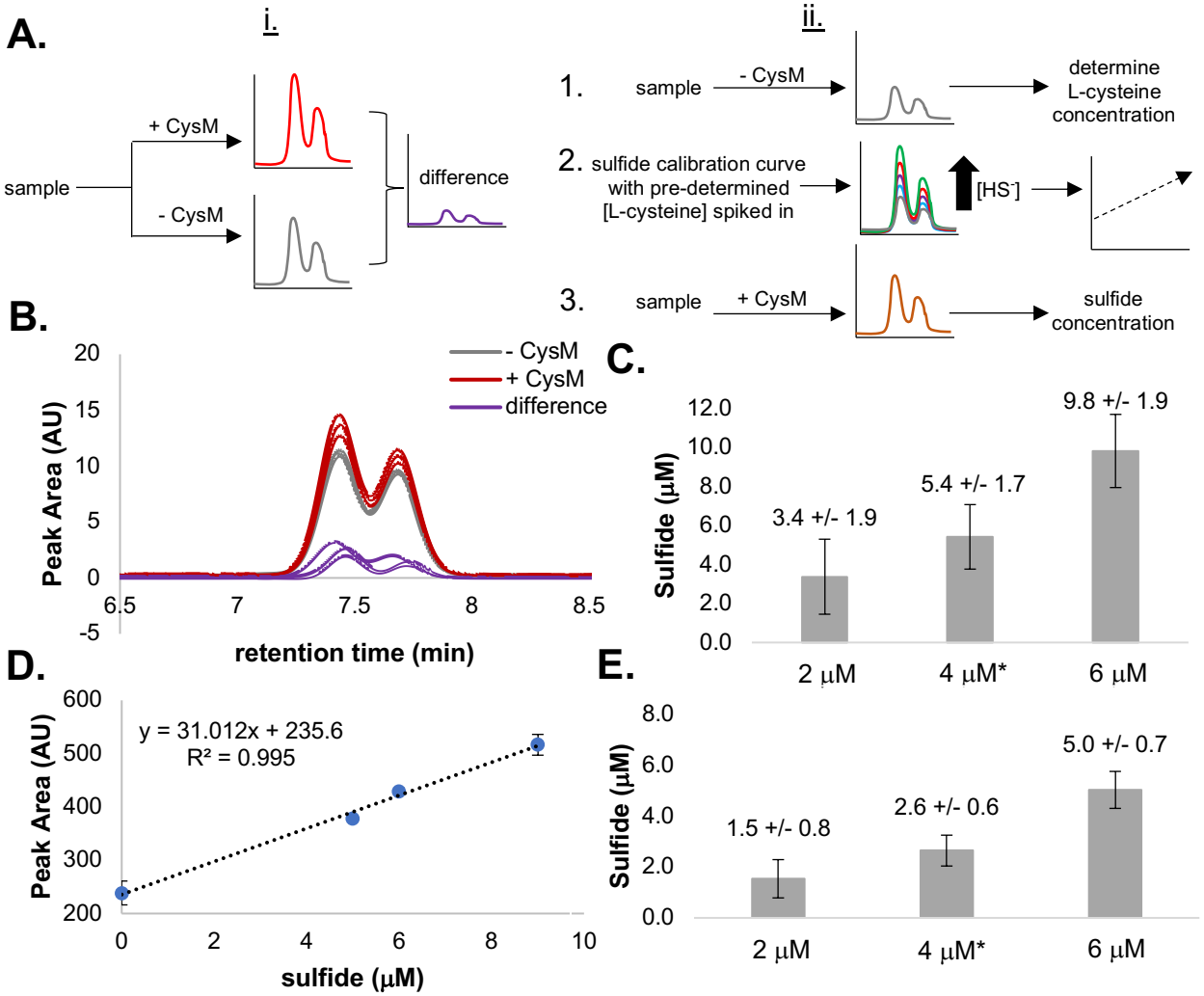


Figure S3: H_2S detection in the presence of L-cysteine. A) Flow chart highlighting the two experimental procedures used to determine sulfide concentrations in the presence of endogenously supplied L-cysteine. i (left) Samples are either treated or not treated with CysM, and the HPLC differential peak area measured and used to determine the L-cysteine concentration produced as a result of CysM-catalyzed sulfide conversion. ii (right) For this method, non-treated samples are first used to determine the concentration of L-cysteine in the samples in question. L-cysteine is then spiked into sulfide calibration curve samples, which is used to generate a new calibration curve under the pre-determined L-cysteine levels. This calibration curve is then used to determine the sulfide concentration in the original samples after CysM treatment. B) Representative trial of H_2S quantification determined by method Ai for the addition of 2 μM H_2S in the presence of 10 μM L-cysteine. For un-treated samples, 1.5 μL degassed ddH_2O was added in lieu of 2 mM CysM. C) Quantification of H_2S in Figure S3B using method Ai. D) H_2S calibration curve generated in the presence of 10 μM L-cysteine according to method ii. E) H_2S levels determined by the calibration curve shown in Figure S3C. All samples were prepared in triplicate with +/- the standard deviation represented as error bars. (*5 μM and 6 μM H_2S samples in Figure S3D and 4 μM sample in Figure S3C and D were prepared in duplicate due to CysM precipitation during sample preparation).

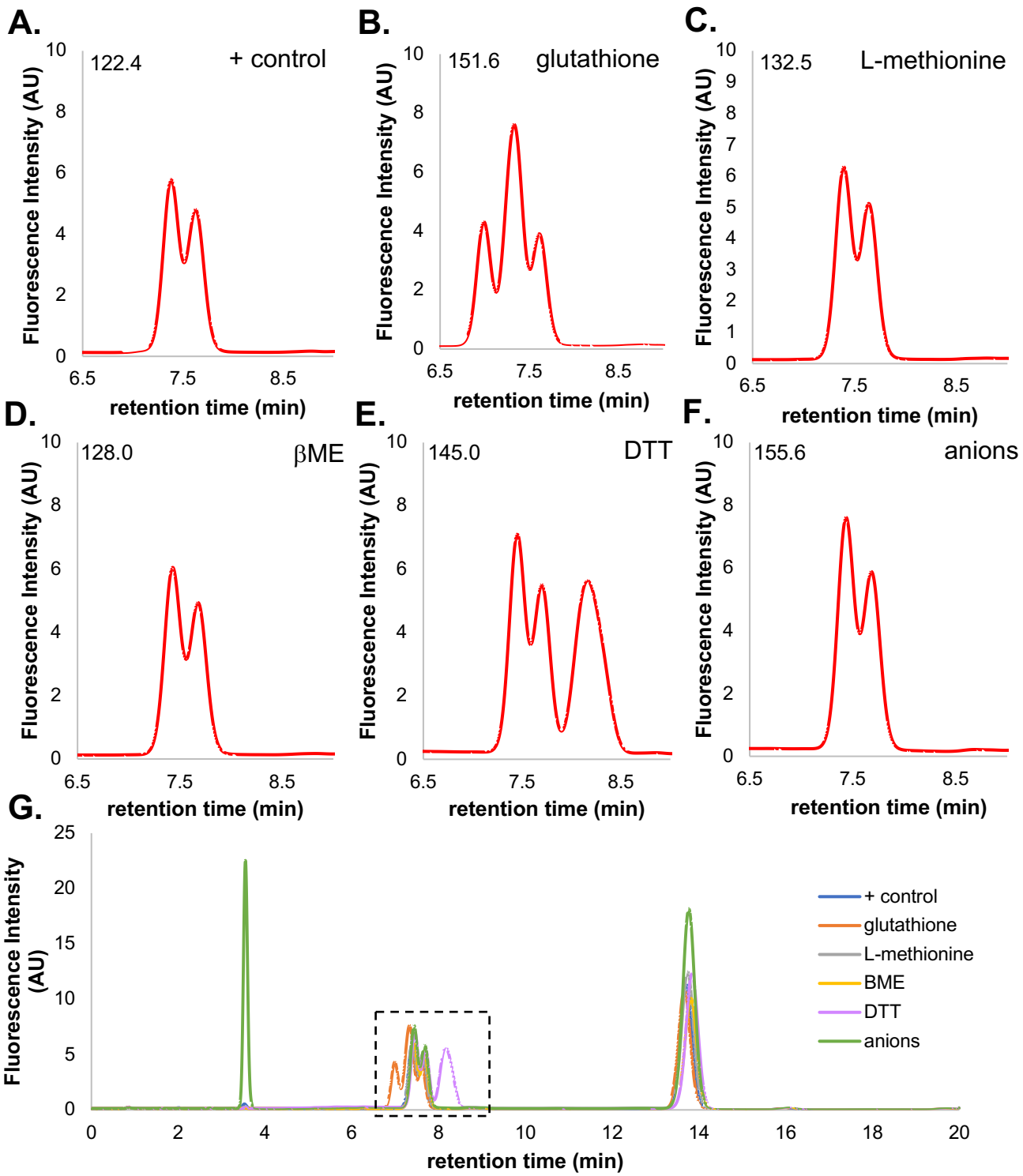


Figure S4: CysM interference assay. For each sample, 6 µM H_2S was incubated with 60 µM CysM and 1 mM OAS in the presence of A) buffer (+ control), B) 5 µM glutathione, C) 5 µM L-methionine, D) 5 µM βME, E) 5 µM DTT and F) anion mixture (100 µM sodium fluoride, sodium citrate, sodium bromide, sodium phosphate, sodium nitrate, sodium sulfite, sodium thiocyanate and sodium carbonate supplemented with 10 µM EDTA). G) Un-cropped HPLC chromatographs of SI Figure S4A-F. Peak areas for each sample is listed to the top left in each chromatograph.

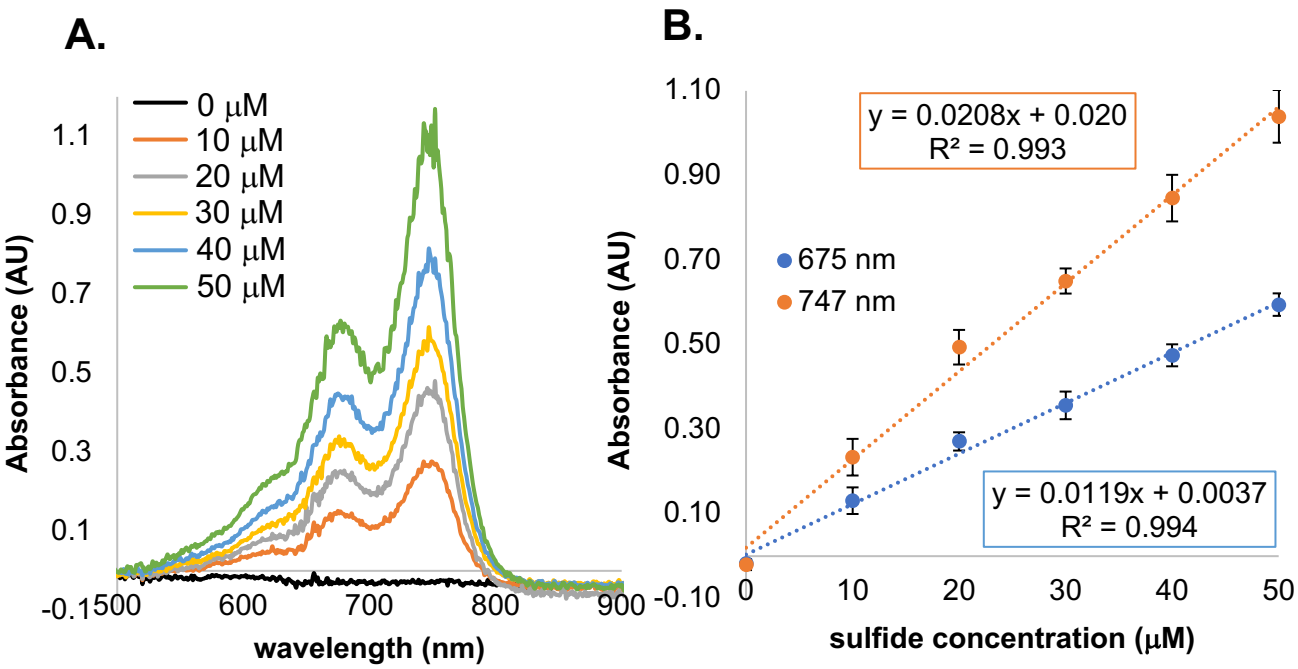


Figure S5: H_2S quantification using the Methylene Blue colorimetric assay. A) Representative UV-Vis spectra of 0 – 50 µM sodium sulfide standards, and B) calibration curves plotting sulfide concentration versus average absorbance intensity at 675 nm (blue) and 747 nm (orange). Each sulfide standard was preprared and measured in triplcate with +/- the standard devation plotted as the error bars.

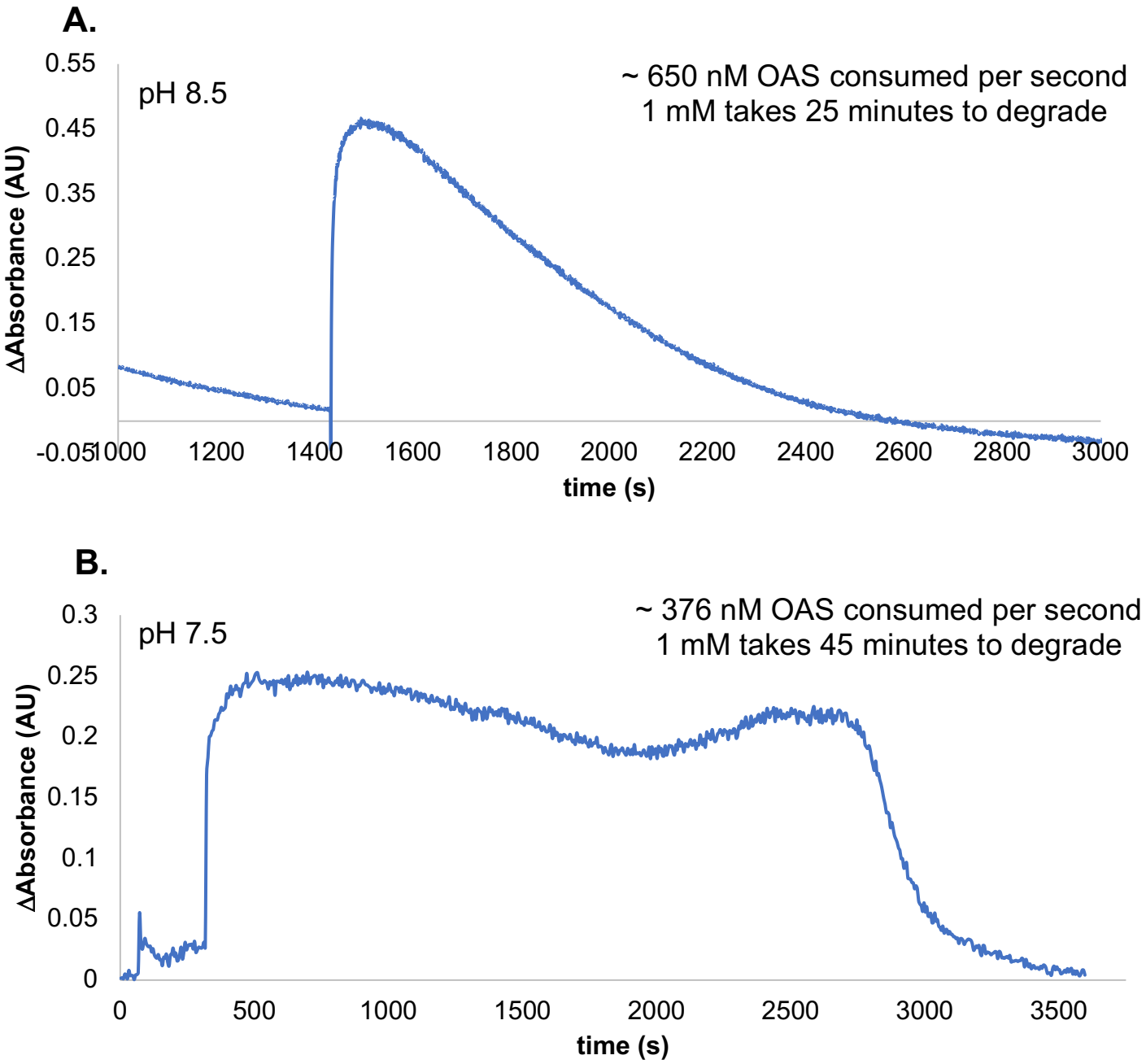


Figure S6: Decay rate of OAS by CysM at (A) pH 8.5, and (B) pH 7.5. For both conditions, 60 µM CysM in XLB was incubated with 1 mM OAS at 25°C with continuous stirring. Absorbance readings at 470 nm were taken every 5 seconds until OAS was fully degraded. Samples were blanked against 60 µM CysM in 50 mM Tris, 150 mM sodium chloride as the specified pH prior to OAS addition.

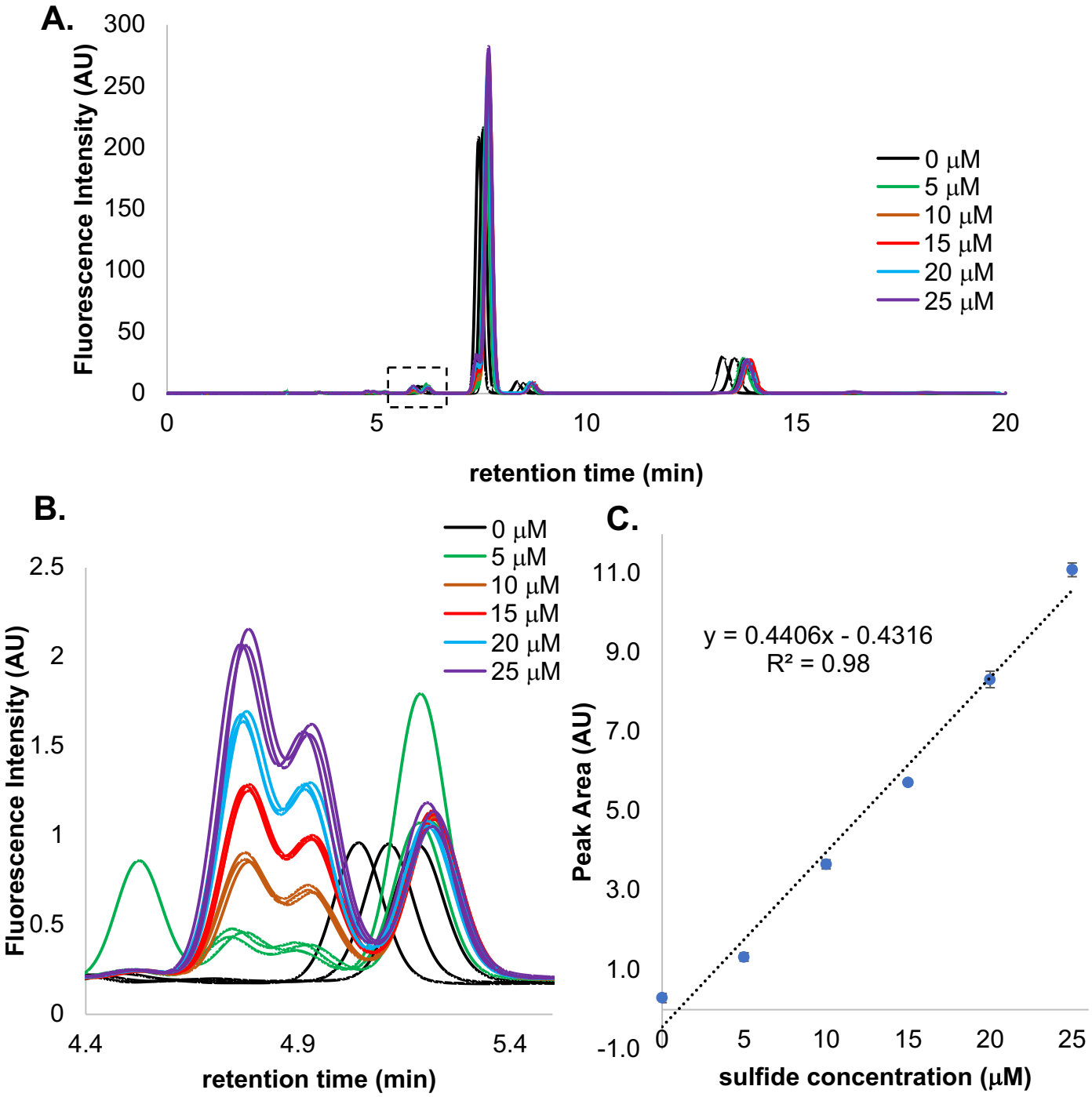


Figure S7: CysM produced L-cysteine-DMM stability over 7 days. Samples were incubated for 7 days at room temperature in the dark, and re-run on the HPLC under identical conditions as described in the Methods section. A) Uncropped HPLC chromatograms, B) zoomed in region of L-cysteine-DMM peaks, and C) calibration curve plotting peak area versus Na_2S concentration.