Electronic Supplementary Material (ESI) for Analyst.

Supporting Information for:

Real-Time Visualization of Serine Protease Cleavage of Oligopeptides by Using GGH–Cu^{2+} Complex

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Stability of Cu^{2+} in buffer solutions: To find a working buffer solution, we tested the stability of Cu^{2+} in different buffers. First, copper(II) sulfate was added into deionized water to a final concentration of 200 mM. Next, 1 µL of the above solution was added into 199 µL of buffer solution of PBS (1×), HEPES (10 mM), and Tris (50 mM) to achieve a final concentration of 1.0 mM. Figure S1 shows that in both PBS and HEPES buffer, bluish copper precipitates appear immediately upon addition of Cu^{2+} when the pH is above 8.0. This is caused by the formation of copper(II) hydroxide in alkaline solution. However, in Tris buffer, no copper precipitate is observed because Tris can stabilize the copper ions at high pH. Moreover, precipitates were not observed neither in presence of 50 mM of sodium carbonate nor 50 mM of sodium sulfide in Tris buffer (Figure S2), showing that the protease assay can also be conducted in the presence of these anions.

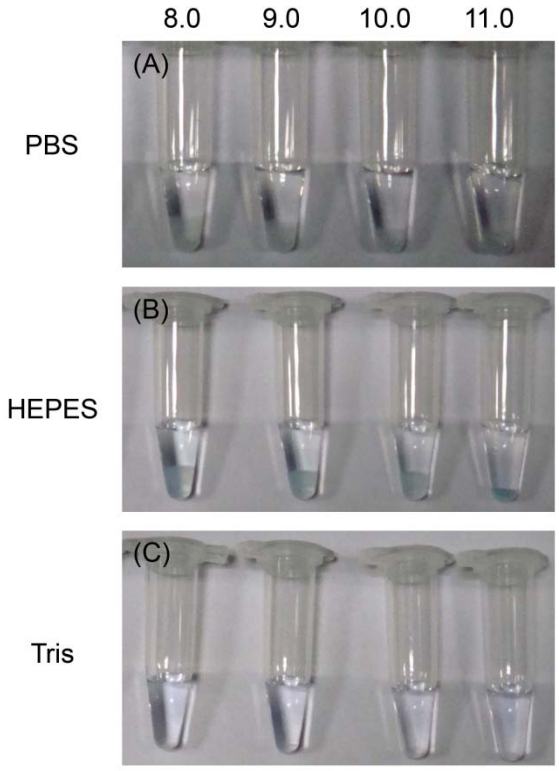

Figure S1. 1.0 mM of Copper(II) sulfate in buffer solutions of (A) PBS, (B) HEPES, and (C) Tris. The pH of the buffer solution is indicated above.

Figure S2. 1.0 mM of Copper(II) sulfate in Tris buffer (50 mM, pH 8.0) containing (A) 50 mM of sodium carbonate, and (B) 50 mM of sodium sulfide.

Absorbance of GGH/Cu^{2+} and P_1/Cu^{2+}: Figure S3 shows that an absorption peak appears at 525 nm after the addition of GGH to Cu^{2+} (1.0 mM each) solution. However, after the addition of **P_1** to Cu^{2+} (1.0 mM each) solution, an adsorption peak appears at 565 nm. This result allows us to determine the GGH concentration independently from the adsorption peak at 525 nm.

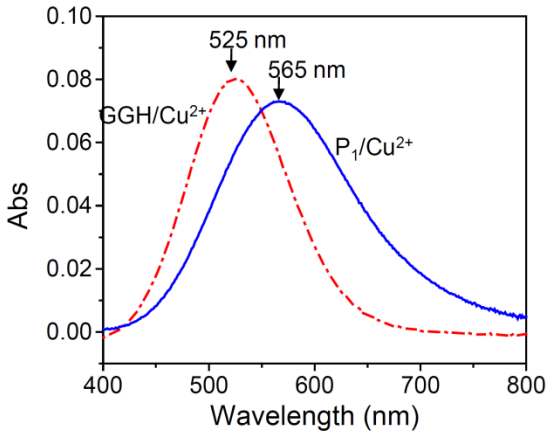

Figure S3. UV-Vis spectra of the metallo-peptide complexes of GGH/Cu^{2+} (dash-dot line) and **P_1**/Cu^{2+} (solid line).

Titration of GGH to P_1/Cu^{2+} mixture: Different concentrations of GGH (0, 0.2, 0.5, 1.0, and 1.5 mM) were mixed with a solution containing mixture of **P_1** and Cu^{2+} (1.0 mM each). Figure S4 shows a shift in the absorption peak from 565 nm to 525 nm with increasing concentration of GGH. This result shows that GGH/Cu^{2+} is more stable than **P_1**/Cu^{2+} complex.

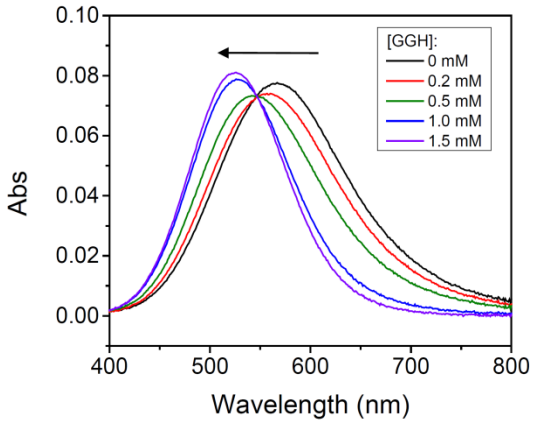

Figure S4. UV-Vis spectra for the titration of GGH to **P_1**/Cu^{2+} mixture.

Calibration of GGH: A calibration curve for GGH concentration was obtained by adding known concentrations of GGH (0, 0.05, 0.25, 0.5, 0.75, and 1.0 mM) into Tris buffer (50 mM, pH 8.0) containing 1.0 mM of Cu^{2+}, as shown in Figure S5.

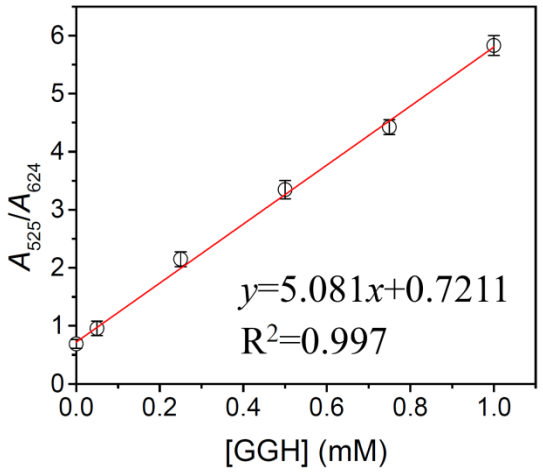

Figure S5. Calibration of normalized absorbance (A_{525}/A_{624}) against GGH concentration. The error bar represents a standard deviation of three parallel experiments.