Supplementary Information

A sensitive dual colorimetric and fluorescence system for assaying the activity of alkaline phosphatase that relies on pyrophosphate inhibition of the peroxidase activity of copper ions

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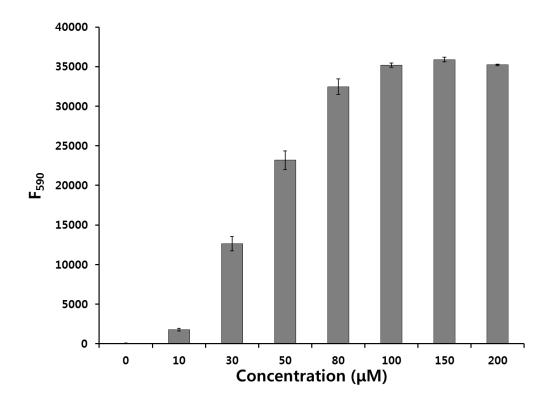


Figure S1. Fluorescence intensities ($\lambda_{max} = 590 \text{ nm}$) of oxidation products formed by reaction of AUR with varying concentrations of Cu²⁺. The final concentrations of AUR substrate and H₂O₂ are 50 μ M and 1 mM, respectively.

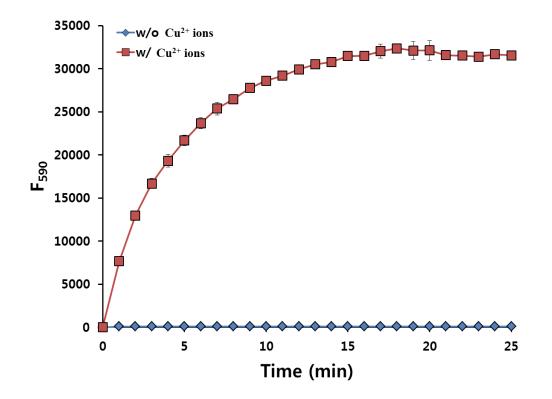


Figure S2. Time dependent fluorescence intensities ($\lambda_{max} = 590 \text{ nm}$) of reaction mixtures containing AUR in the absence (blue) and presence (red) of Cu²⁺ ions. The final concentrations of Cu²⁺, AUR substrate and H₂O₂ are 100 μ M, 50 μ M and 1 mM, respectively.

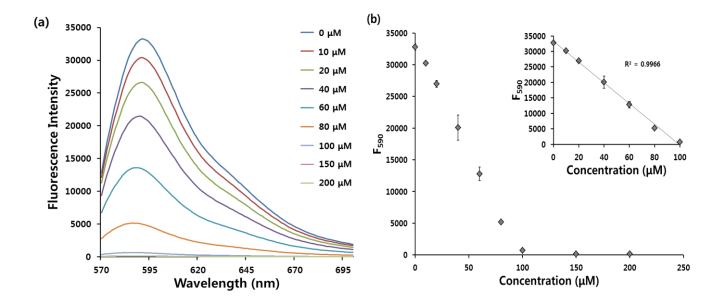


Figure S3. The enlarged version of Figure 3.

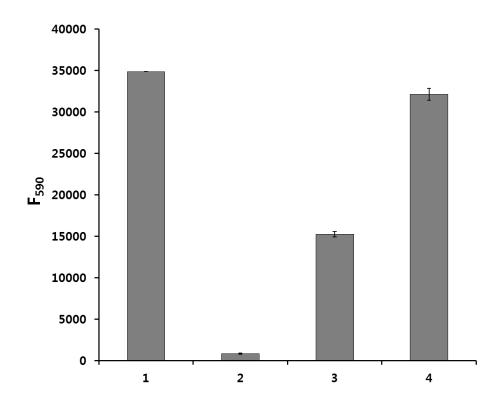


Figure S4. Fluorescence intensities from oxidation products formed by reaction between AUR and Cu^{2+} after the addition of PPi and ALP. (1) and (2) are fluorescence signals obtained by using free Cu^{2+} and Cu^{2+} in the presence of PPi, respectively. (3) and (4) are fluorescence signals obtained by using Cu^{2+} in the presence of PPi and ALP. Cu^{2+} is first added to the PPi solution followed by the addition of ALP (3). Alternatively, ALP is first added to the PPi solution followed by the addition of Cu^{2+} (4). The final concentrations of Cu^{2+} , PPi, AUR substrate and H_2O_2 are 100 μ M, 100 μ M, 50 μ M and 1 mM, respectively.

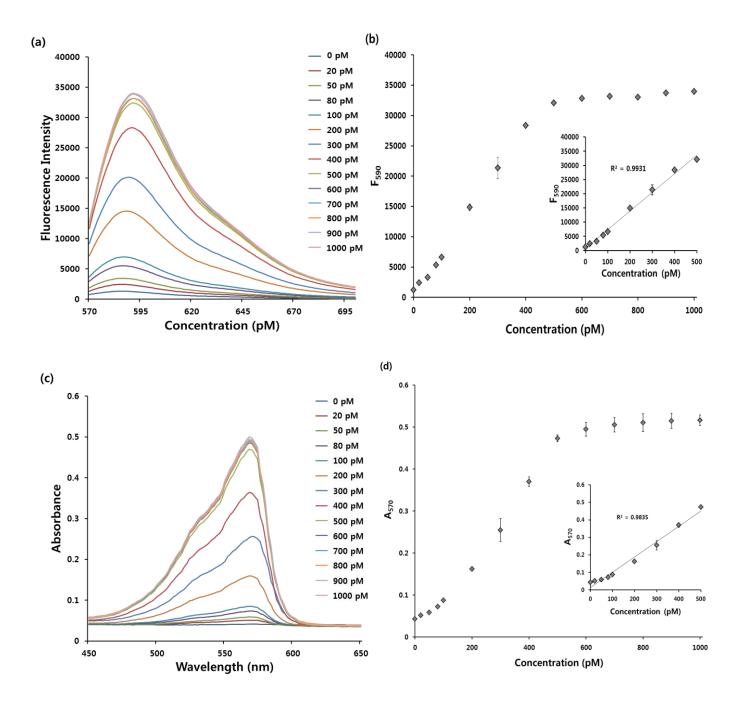


Figure S5. The enlarged version of Figure 5.

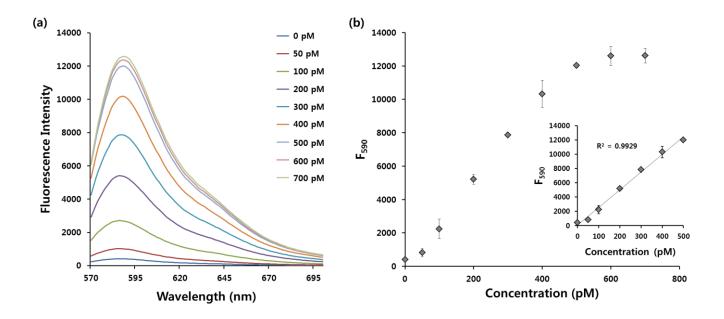


Figure S6. (a) Fluorescence emission spectra of the AUR oxidation products formed by reaction with Cu^{2+} in the presence of diluted human serum spiked with varying concentrations of ALP. (b) ALP concentration dependent changes of fluorescence intensities at 590 nm. Inset: The linear range of the plot between F_{590} and ALP concentration (0–500 pM) in diluted human serum. The final concentrations of Cu^{2+} , PPi, AUR substrate and H_2O_2 are 100 μ M, 100 μ M, 50 μ M and 1 mM, respectively.