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Affiliation

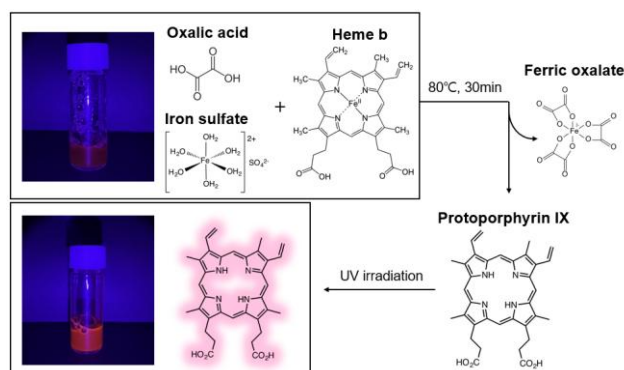
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Title of the presentation

Trace blood detection by inducing fluorescence of hemoglobin  
 via selective iron exclusion

This study proposes a method for detecting trace blood by selectively removing the central Fe from hemoglobin to induce fluorescence. In heme, Fe(II) or Fe(III) is coordinated to a porphyrin ring, and the resulting metal-to-ligand charge transfer (MLCT) promotes non-radiative decay of excited electrons, suppressing porphyrin's intrinsic fluorescence under UV light. To induce fluorescence, human whole blood was treated with chelate (oxalic acid) and reductant (ferrous sulfate) at 80 °C for 30 minutes. Oxalic acid chelated and removed the central iron ion from heme; ferrous ion reduced  $\text{Fe}^{3+}$  to  $\text{Fe}^{2+}$  to inhibit re-metalation, thereby promoting formation of fluorescent protoporphyrin IX. The generated protoporphyrin IX exhibited strong fluorescence under 395 nm UV irradiation, increasing proportionally with oxalic acid concentration. The fluorescence was most clearly visible to the naked eye at an oxalic acid to hemin (a heme substitute) mass ratio of 4500:1 and above 1 mol/L. Furthermore, we found that Fe exclusion and fluorescence generation in solution could be applied to latent bloodstain detection; oxalic acid paste was applied to dried artificial hemoglobin stains, enabling iron removal and fluorescence induction.



**Figure 1. Mechanism of Fluorescence Induction via Iron Exclusion from Heme b in Hemoglobin**