Electronic Supplementary Material (ESI) for Analyst.

A simple assay for glutathione in whole blood

Lovemore Hakuna, Brandon Doughan, Jorge O. Escobedo, and Robert M. Strongin*

Supplementary Material

Table of Contents

General Methods and Instrumentation

All chemicals were purchased from Sigma-Aldrich or Fisher Scientific and used without further purification. Porcine blood was purchased from Lampire Biological Laboratories. The 0.45 μm *Single StEP*™ PVDF filter vials were purchased from Thomson Instrument Company. UV-visible spectra were acquired on a Cary 50 UV-Vis spectrophotometer. Fluorescence spectra were collected on a Cary Eclipse (Agilent technologies) fluorescence spectrophotometer with slit widths set at 5 nm for both excitation and emission, respectively. The voltage of the photomultiplier was set at 550 V and pH measurements were carried out with an Orion 410A pH meter.

Blood sampling, storage and extraction procedures.

Commercial pig blood (30 μL) is spotted on filter paper and dried for 24 h. The dried blood is extracted into a small volume of buffer (0.6 mL, 50 mM phosphate buffer pH 7.4).

Reduction of oxidized glutathione

The extracted blood in buffer is incubated with immobilized tris(2-carboxyethyl)phosphine (TCEP) gel (1:1 v/v) at rt for 1 h with gentle shaking. Separation of the reduced plasma from the gel is achieved by filtration using a *Single StEP*™ 0.45 μm PVDF filter vial.

Fractionation of the blood extract

The reduced sample is passed through a PD MiniTrap™ G-25 Sephadex™ column. Fractions of 0.3 mL are collected. Fractions 1 to 3 contain proteins and Hb, fractions 4 and 5 contain the native GSH.

Fluorescence detection of GSH

Acrylate probe **1** (2.5 μM) is added to the fractionated solutions in 2.0 mM CTAB media buffered at pH 7.4 (phosphate buffer, 50 mM). Spectra (UV-Vis and Fluorescence) were collected immediately upon addition of the probe.

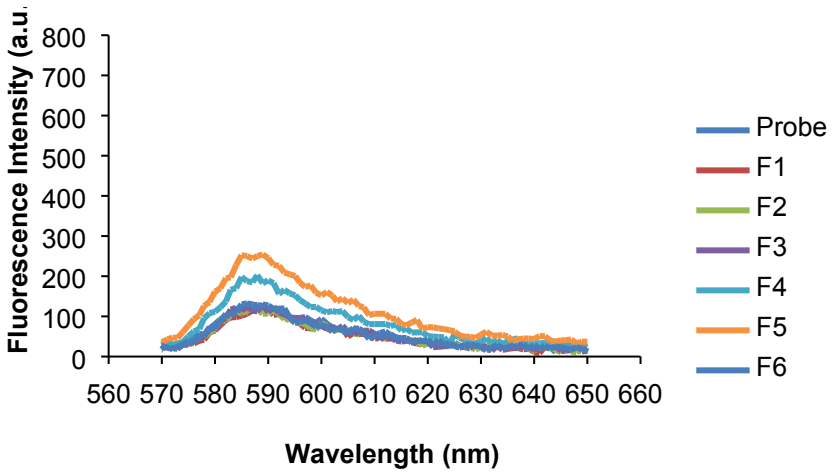

Fig. S1 Spectral response of probe **1** alone and in the presence of fractions F1-F6 at pH 7.4. Fluorescence spectra (λex = 565 nm) of probe (2.5 μM) in solutions of GSH fractions and 2.0 mM CTAB media buffered at pH 7.4 (phosphate buffer, 50 mM). Spectra were taken immediately upon addition of the probe.

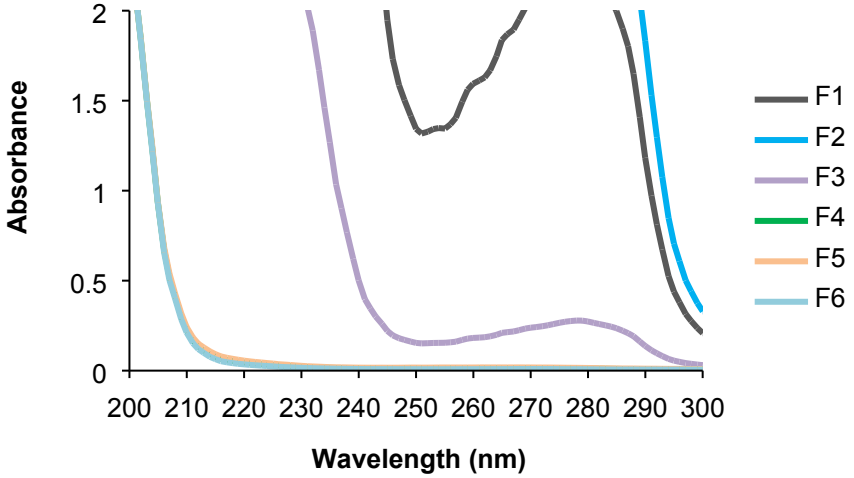

Fig. S2 Spectral response of HSA fractions at pH 7.4. Absorption responses of HSA fractions in phosphate buffer (50 mM) at pH 7.4 Fractions were collected and immediately analyzed for absorption.

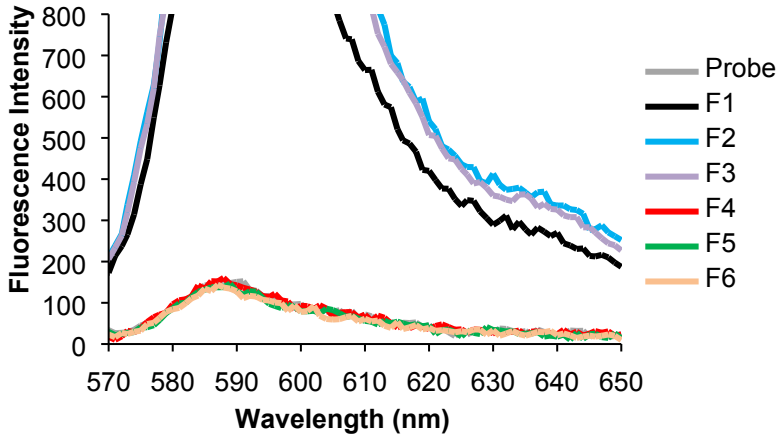

Fig. S3 Spectral response of probe **1** towards various Hb fractions at pH 7.4. Fluorescence spectra (λex = 565 nm) of probe (2.5 μM) in solutions of DBS fractions and 2.0 mM CTAB media buffered at pH 7.4 (phosphate buffer, 50 mM). Spectra were taken immediately upon addition of the probe.