Electronic Supplementary Material (ESI) for ChemComm.


# A photochemical method for determining plasma homocysteine with limited sample processing

**Lovemore Hakuna, Jorge O. Escobedo, Mark Lowry, Aabha Barve, Naneki McCallum and Robert M. Strongin***

## Supplementary Material

**Table of Contents**

**Experimental Methods and Instrumentation**

All chemicals were purchased from Sigma-Aldrich and Fisher Scientific and used without further purification. The 0.45 µm *Single StEP*™ PVDF filter vials were purchased from Thomson Instrument Company. UV-visible spectra were acquired on a UV-Vis Cary 50 (Agilent Technologies). The light intensity was measured with a Melles Griot Broadband Power/Energy Meter 13PEM001. The light source for the photolysis experiments was a ReptiSun™ compact fluorescent lamp (Zoo Med Laboratories Inc.).

**Reduction of Human Blood Plasma**

Reconstituted human plasma is incubated with immobilized TCEP gel in a 1:1 volume ratio at room temperature for 1 h with vortexing. Separation of the reduced plasma from the gel can be achieved by centrifugation at 1000 rpm for 5 min or by filtration using a *Single StEP*™ 0.45 µm PVDF filter vial.

**General procedure for the photolysis of thiols in the presence of viologens**

In a quartz cuvette sealed with a rubber septum (GL14-S, Starna Cells), a solution of viologen (20 or 50 mM in 0.5 M Tris buffer pH 7.0) is deoxygenated by bubbling Ar for 5 min. Human plasma (spiked or non spiked) is added to the cuvette and irradiated for 15 min with a Reptisun™ lamp. Absorption spectra is collected on a UV-Vis Cary 50. The proportion of the plasma solution is 10% of the total volume after being mixed with the viologen solution.

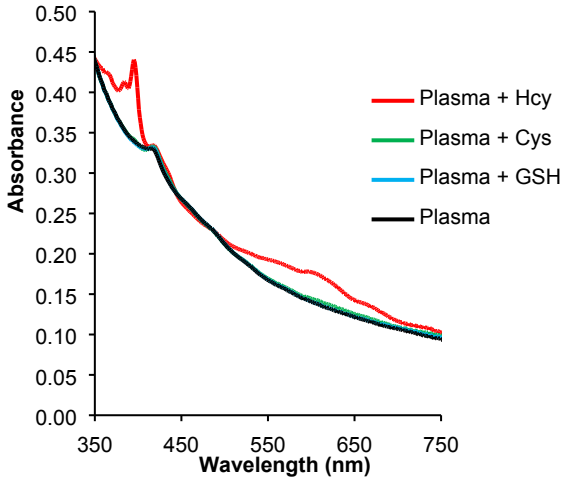


**Fig. S1** Spectral response of $MV^{2+}$ towards various spiked thiols in human blood plasma upon irradiation. Absorption spectra of solutions of $MV^{2+}$ (50 mM) in human blood plasma and 0.5 M Tris buffer (pH 7.0) spiked with 1.5 µM Hcy, 25 µM Cys and 0.6 µM GSH. Plasma (10% v/v) was added to an argon-saturated solution of viologen, thiol and buffer and irradiated for 15 min using a Reptisun™ lamp.

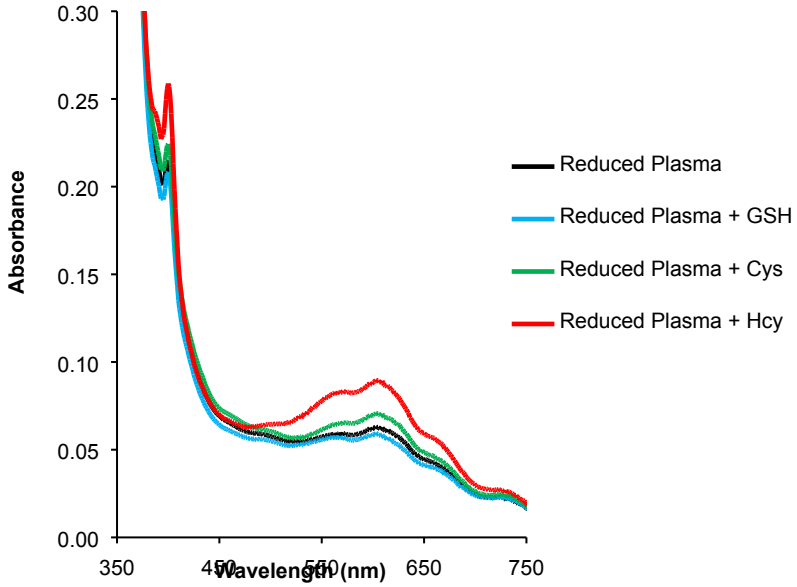


**Fig. S2** Spectral response of $BV^{2+}$ towards various spiked thiols in reduced human blood plasma upon irradiation. Absorption spectra of solutions of $BV^{2+}$ (20 mM) in reduced human blood plasma and 0.5 M Tris buffer (pH 7.0) spiked with 1.5 µM Hcy, 25 µM Cys and 0.6 µM GSH. Plasma was incubated for 1 h with TCEP Gel followed by centrifugation at 1000 rpm for 5 min. The reduced, filtered plasma (10% v/v) was added to an argon-saturated solution of viologen, thiol and buffer and irradiated for 15 min using a Reptisun™ lamp.

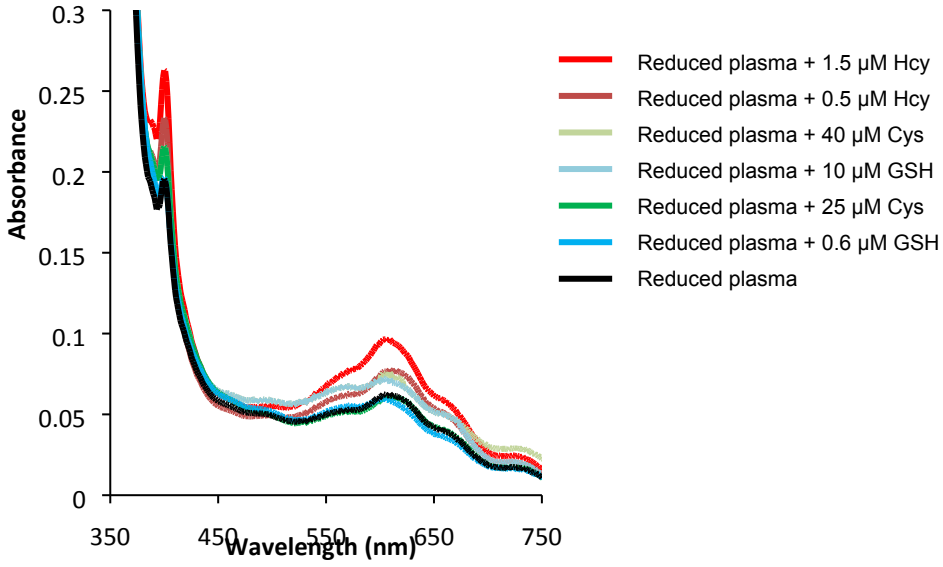


**Fig. S3** Spectral response of $BV^{2+}$ towards various spiked thiols in reduced human plasma upon irradiation. Absorption spectra of solutions of $BV^{2+}$ (20 mM) in reduced human blood plasma and 0.5 M Tris buffer (pH 7.0) spiked with increasing concentrations of thiols. Plasma was incubated for 1 h with TCEP Gel followed by centrifugation at 1000 rpm for 5 min. The reduced plasma (10% v/v) was added to an argon-saturated solution of viologen, thiol and buffer and irradiated for 15 min using a Reptisun™ lamp.