# Supporting Information

# Bio-Inspired Reaction Enables a Simple and Sensitive Assay for Colorimetric Detection of Methylglyoxal

Shih-Ting Wang, Yiyang Lin, Christopher D. Spicer and Molly M. Stevens*[a]

Department of Materials, Department of Bioengineering and Institute for Biomedical Engineering, Imperial College London, Exhibition Road, London SW7 2AZ, United Kingdom.

## Experimental Section

**Materials.** Gold chloride hydrate ($HAuCl_4 \cdot 3H_2O$), sodium citrate, 3-mercaptopropionic acid, *o*-phenylenediamine, and methylglyoxal solution (40 wt % in water) were purchased from Sigma-Aldrich (UK). Distilled water was purified using Milli-XQ (resistivity ~ 18 MΩ) equipment. All other chemicals were of analytical grade and used as received.

**Instrumentation.** Absorption spectra were recorded on a SpectraMax M5 multimode microplate reader (Molecular Devices Ltd.). Sizes and morphologies of gold nanoparticles (AuNPs) were determined using a JEOL JEM-2100F transmission electron microscope (TEM) with an operating voltage of 200 kV. Element characterisation by energy-dispersive spectroscopy (EDS) was performed with dark field STEM (scanning transmission electron microscopy) mode on the TEM. Dynamic light scattering (zeta sizer nanoseries, Malvern Instruments Ltd.) was performed to characterise the hydrodynamic radius of AuNPs aggregates. Proton nuclear magnetic resonance ($^1H$ NMR) spectra were recorded on a Bruker

AM-400 (400 MHz) All chemical shifts are quoted on the $\delta$ scale in ppm using residual solvent as the internal standard. Coupling constants, *J*, are reported in Hz with the following splitting abbreviations: s = singlet, d = doublet, m = multiplet.

**Synthesis of citrate-stabilised AuNPs**. AuNPs were synthesised according to the procedure of Grabar and colleagues[1]. In brief, 50 mL of $HAuCl_4 \cdot 3H_2O$ (1 mM, 50 µmol) was brought to reflux with vigorous stirring, then 5 mL of sodium citrate (38.8 mM, 194 µmol) was rapidly added to the solution. Stirring was maintained for about 12 minutes, during which time the solution changed from pale yellow to deep red. The AuNP solution was then removed from the heat, and continued to be stirred until it reached room temperature.

**Preparation of AuNPs functionalised with mercaptopropionic acid (MPA).** Functionalisation of the AuNPs was performed by incubating 1 mL of the citrate-stabilized AuNPs prepared above with mercaptopropionic acid sodium (1 mM) overnight at room temperature. The residual ligands were removed by centrifugation at 8000 rpm for 5 minutes at room temperature, and the AuNP pellet was re-suspended in 1 mL water and stored at room temperature.

**Detection of methylglyoxal (MGO).** Typically, MGO at various concentrations (0.05-20 µM) was mixed with OPD at a final concentration of 0.5 µM, in sodium phosphate buffer (30 mM, pH 7.5). The reaction was left in the 96-well plate for 30 minutes before adding 20 µL citrate-stabilised AuNPs to a total solution volume of 200 µL. The assays were performed in two individual wells of a 96-well plate. The absorption spectra of AuNP solution containing different MGO concentration were then recorded by UV-visible spectrometer with a step of 5 nm from 450 nm to 850 nm.

**Detection of MGO with varied concentrations and sizes of AuNPs.** MGO detection with different concentrations of AuNPs was performed by mixing MGO and OPD (final concentration of 1 μM and 0.5 μM, respectively) in sodium phosphate buffer (30 mM, pH 7.5) for 30 minutes before adding 5, 10, or 20 μL citrate-stabilised AuNPs to a total solution volume of 200 μL. The assay with different sizes of AuNPs was performed by adding 60 μL of 10, 15, or 20 nm AuNPs (BBI solutions) to a solution (200 μL) containing MGO and OPD. The absorption spectra were obtained as described above.

**Detection of MGO in pre-treated human serum samples.** Human serum (Sigma) was treated by centrifugation at 10000 rpm (4 °C) with 10 kDa and 3 kDa centrifugal filters sequentially to remove the serum proteins. Further removal of lipids, amino acids and small molecules in serum was performed by incubating the serum with silica powder (Sigma) for 30 minutes followed by centrifugation for 5 minutes at 5000 rpm to remove the precipitates. MGO detection was performed in 30 v/v % pre-treated serum in 30 mM phosphate buffer (pH 7.5), with the OPD-MGO reactions being performed for 20 minutes before addition of the AuNPs.

**Detection of other ketones and aldehydes.** Detection of other physiologically relevant ketones and aldehydes was performed under the same conditions described for MGO, with a fixed concentration of OPD at 0.5 μM, and ketones/aldehydes at 10 μM. Assays were performed in two individual wells of a 96-well plate.

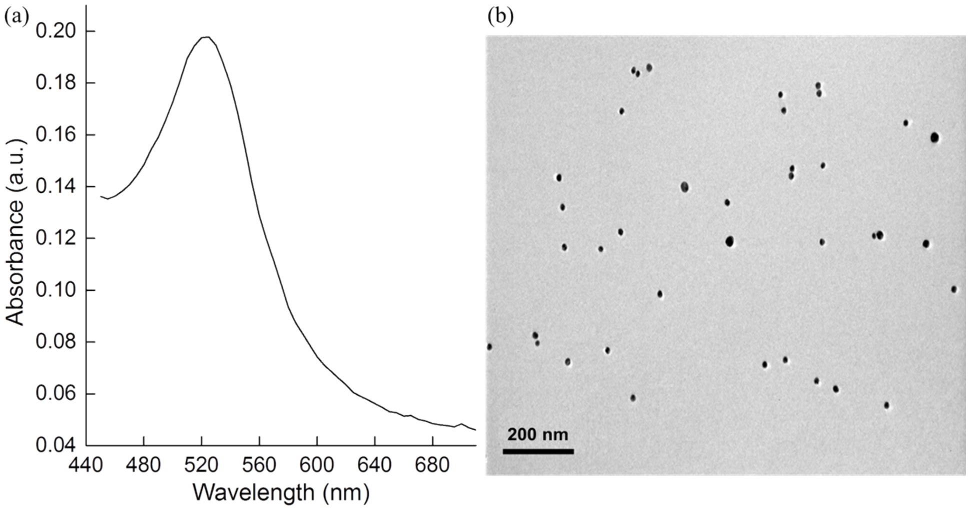

**Fig. S1** (a) UV/vis spectrum and (b) TEM images of citrate-stabilized AuNPs.

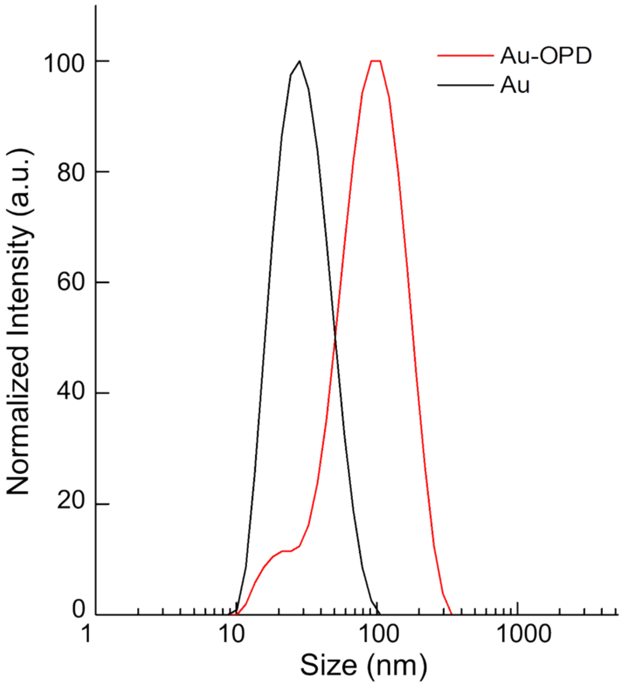

**Fig. S2** Dynamic light scattering (DLS) of citrate-stabilised AuNP solutions with or without 0.5 μM OPD, showing OPD-induced AuNP aggregation and the formation of 120 nm clusters.

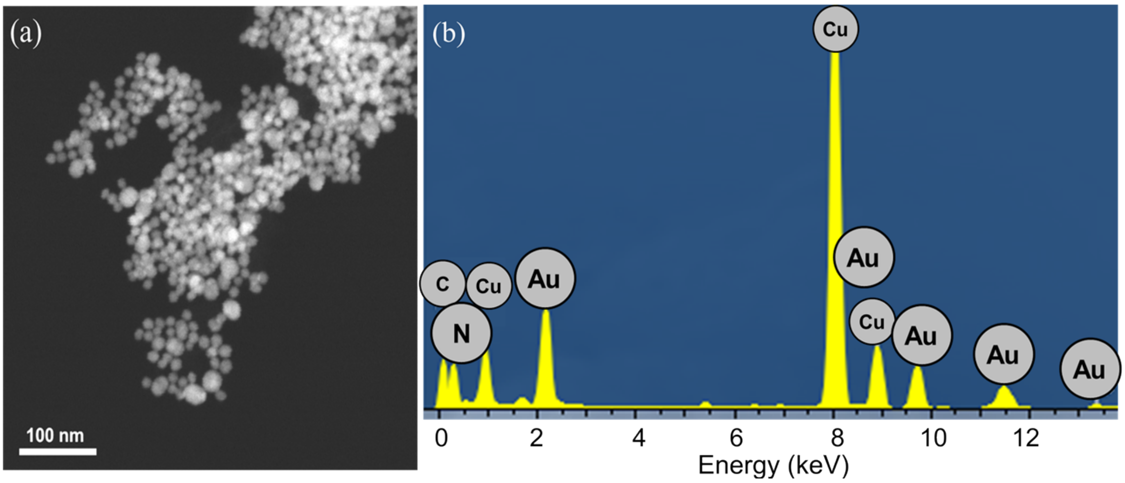

**Fig. S3** (a) Dark-field scanning tunnelling electron microscopy (DF-STEM) imaging of citrate-stabilised AuNP aggregation in the presence of 0.5 µM OPD and (b) the energy dispersive X-spectroscopy showing the binding of OPD to the Au surface.

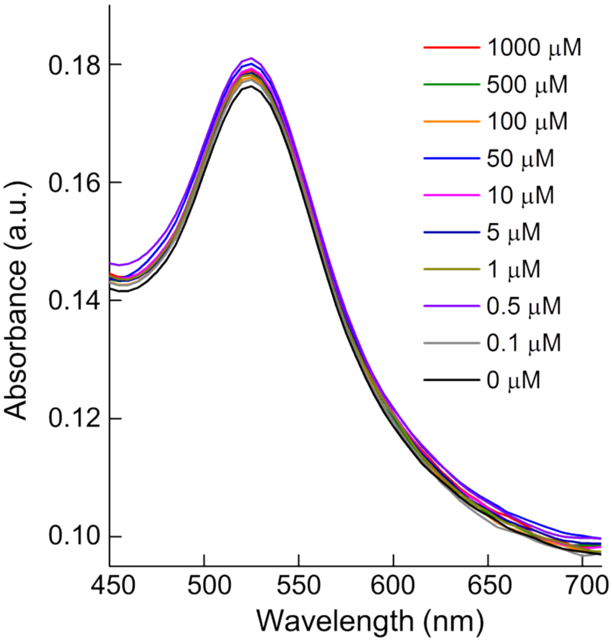

**Fig. S4** UV/vis spectra of 3-mercaptopropionic acid (MPA)-functionalised AuNPs incubated with OPD at different concentrations ranging from 0.1 µM to 1 mM.

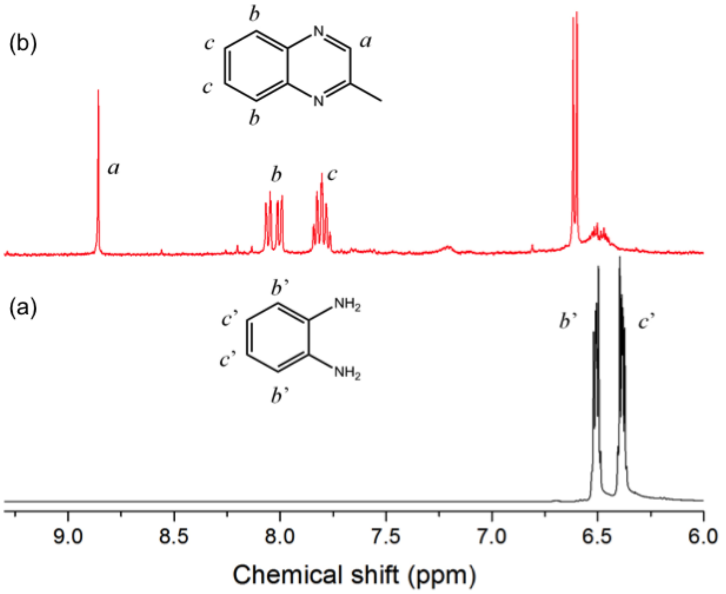

**Fig. S5** $^1$H NMR spectra of (a) OPD (0.3 M, 0.5 mL) in DMSO-$d_6$ and (b) the product formed by incubating MGO (2 M, 0.25 mL) and OPD (0.2 M, 0.25 mL) in DMSO-$d_6$ for two hours, showing the formation of 2-methylquinoxaline. (a) $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ = 6.51 (2H, dd, $J$ = 5.6, 3.5 Hz, Hc'), 6.38 (2H, dd, $J$ = 5.6, 3.5 Hz, Hb') ppm; (b) $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ = 8.82 (1H, s, Ha), 8.03 (1H, dd, $J$ = 7.9, 1.8 Hz, Hb), 7.98 (1H, dd, $J$ = 7.9, 1.8 Hz, Hb), 7.71-7.83 (2H, m, Hc) ppm.[2, 3]

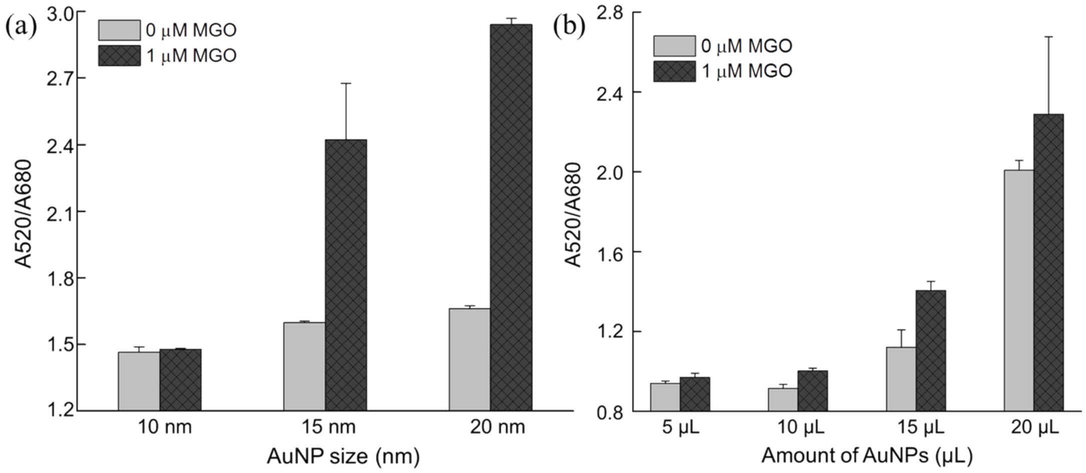

**Fig. S6** (a) AuNP aggregation assay for MGO detection using AuNPs of different sizes. (b) Effect of AuNP concentration on the assay, where 5 µL, 10 µL, 15 µL and 20 µL of AuNPs were used in 200 µL solution. In both experiments, 0.5 µM OPD was reacted with MGO for 30 minutes before addition of AuNPs, and the absorbance ratio was plotted at 520 nm and 680 nm. The assays were performed in three individual wells of a 96-well plate.

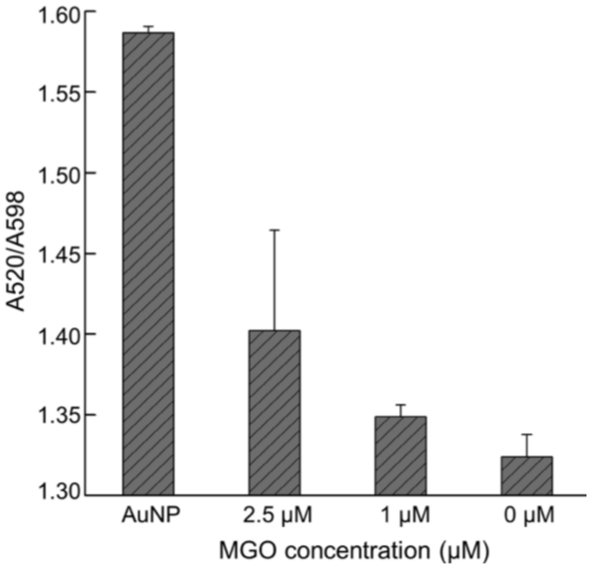

**Fig. S7** MGO detection performed in 30 v/v % pre-treated human serum. The OPD concentration is 1 µM, and the OPD-MGO reaction was undertaken for 30 minutes before the addition of AuNPs. Due to the lower AuNP aggregation in serum samples, the absorbance ratio at 520 nm to 598 nm was plotted against the different MGO concentrations. The assay was performed in two individual wells of a 96-well plate.

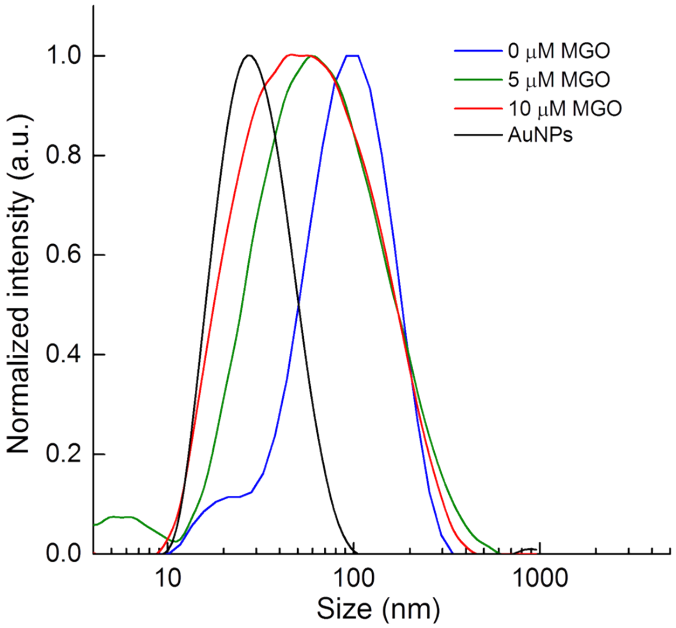

**Fig. S8** DLS measurement of citrate-stabilised AuNP in the presence of 0 μM, 5 μM, and 10 μM MGO.

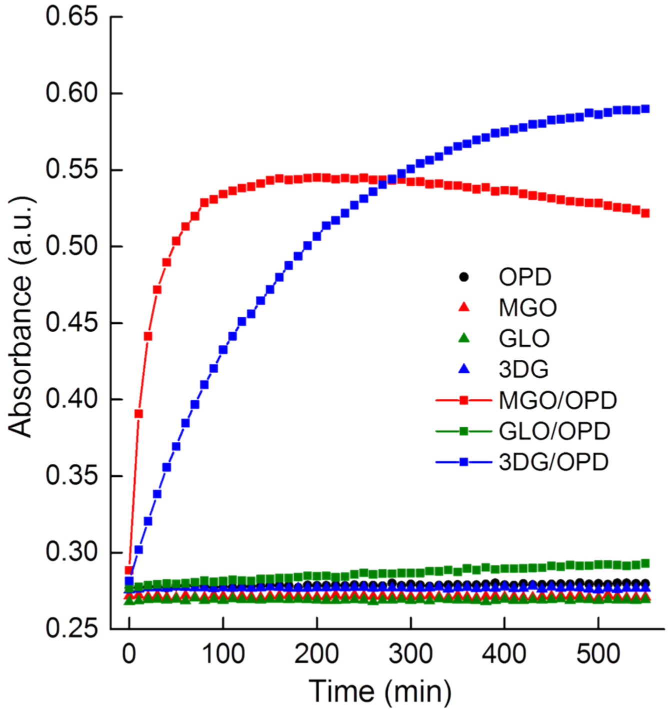

**Fig. S9** UV/vis measurement of the reaction kinetics between OPD and MGO, glyoxal and 3-deoxyglucosone. The concentration of OPD, MGO, glyoxal, and 3-deoxyglucosone was 100 μM and the reaction was performed in a 1:1 ratio. Experiments were conducted in a 96-well plate and the absorbance was recorded at 315 nm every 5 minutes. Conversion to the product (78 % for OPD to MGO reaction, and 18 % for OPD to 3-deoxyglucosone) was calculated by the absorbance at 30 minutes in comparison to the absorbance value after reacting for 550 minutes.

1 K. C. Grabar, R. G. Freeman, M. B. Hommer and M. J. Natan, *Anal. Chem.*, 1995, **67**, 735-743.

2 M. A. del Valle, F. R. Diaz, M. E. Bodini, G. Alfonso, G. M. Soto and E. D. Borrego, *Polym. Int.*, 2005, **54**, 526-532.

3 J. A. Donarski, D. P. T. Roberts and A. J. Charlton, *Anal. Methods*, 2010, **2**, 1479-1483.