## Enhancement of latent fingermarks on non-porous surfaces using anti-L-amino acid antibodies conjugated to gold nanoparticles

Xanthe Spindler,\*<sup>a,c</sup> Oliver Hofstetter,<sup>b</sup> Andrew M. McDonagh,<sup>a</sup> Claude Roux<sup>a</sup> and Chris Lennard<sup>c</sup>

<sup>a</sup> Centre for Forensic Science, University of Technology Sydney, Sydney, NSW, Australia
<sup>b</sup> Department of Chemistry and Biochemistry, Northern Illinois University, DeKalb, Illinois 60115, USA
<sup>c</sup> National Centre for Forensic Studies, University of Canberra, Canberra, Australia

## SUPPLEMENTARY INFORMATION

## Synthesis of anti-L-amino acid antibody reagent

16 nm gold-citrate nanoparticles were prepared from tetrachloroauric acid according to the methods outlined in J. Turkevich, P. Stevenson and J. Hillier, Discussions of the Faraday Society, 1951, 11, 55-75. Anti-L-amino acid antibodies were conjugated to the gold-citrate nanoparticles using two methods detailed below:

- 1. An aliquot of 0.75 mg/mL anti-L-amino acid antibody solution (246 µL) in magnesiumfree phosphate buffered saline (PBS) was added to gold-citrate nanoparticle solution (692  $\mu$ L), mixed and incubated at room temperature for 15 minutes. The sample was centrifuged and washed three times with cold 2-Morpholinoethanesulfonic acid monohydrate (MES) prior to reconstitution.
- 2. Ethanolic 10 mM 23-Mercapto-3,6,9,12-tetraoxatricosanoic acid (HS-OEG<sub>3</sub>-COOH) solution (76.9  $\mu$ L, 0.77  $\mu$ mol) was added to gold-citrate nanoparticle solution (692  $\mu$ L) and incubated for six hours at room temperature. The solution was centrifuged for 10 washed twice with 0.01 minutes and Μ MES buffer. Aqueous N-(3dimethylaminopropyl)-N'-ethyl-carbodiimide hydrochloride/N-hydroxysuccinimide (EDC/NHS) solution (0.01 M, 2.0 µL) was added to the HS-OEG<sub>3</sub>-COOH coated nanoparticles and allowed to incubate for 2 minutes at room temperature before centrifuging as before and resuspending in MES buffer. Anti-L-amino acid antibody solution (246  $\mu$ L) was added and allowed to incubate at room temperature for 2 hours, centrifuged and washed as before.

The anti-L-amino acid antibody reagent solutions were stored at 4° C until used. Solution UV-visible spectroscopy using an Agilent 8435 UV-visible spectrophotometer indicated that the solutions had a stable storage period of three weeks before aggregation of the nanoparticles occurred, characterised by broadening and red shifting of the absorbance maximum from 526 nm to 650 nm. Solutions were centrifuged and resuspended in 20 µL of water and diluted to 1 mL with acetone. The reagent was immediately applied to the fingermark to minimise denaturation of the antibodies and aggregation of the reagent.

**Qualitative macroscopic assessment of anti-L-amino acid antibody reagent performance** Six sets of fingermarks from three male donors were collected on cleaned aluminium foil and stored at ambient laboratory conditions for two weeks. The samples were bisected and each half treated with one of the following formulations:

- 1. Anti-L-amino acid antibodies in PBS;
- 16 nm gold-citrate nanoparticles in deionised water prepared according to J. Turkevich, P. Stevenson and J. Hillier, *Discussions of the Faraday Society*, 1951, 11, 55-75;
- 3. 16 nm gold nanoparticles capped with HS-OEG<sub>3</sub>-COOH;
- Anti-L-amino acid antibody-nanoparticles prepared following protocol 1 in the previous section (based on D. Pissuwan, C. Cortie, S. Valenzuela and M. Cortie, *Gold Bulletin*, 2007, 40, 121-129);
- Anti-L-amino acid antibody-nanoparticles prepared following protocol 2 in the previous section (based on C. Cao and S. J. Sim, *Biosensors and Bioelectronics*, 2007, 22, 1874–1880).

The samples were developed with these solutions and visualised in the same manner as detailed in the Communication.



**Fig. S1** A qualitative assessment of the performance of the anti-L-amino acid antibody reagent on 2 week old bisected latent fingermarks. (a) free anti-L-amino acid antibodies, (b) gold-citrate nanoparticles, (c) gold nanoparticles capped with HS-OEG<sub>3</sub>-COOH, (d) anti-L-amino acid reagent prepared using method 1, and (e) anti-L-amino acid reagent prepared using method 2.

## Scanning electron microscopy

In order to verify the observations recorded by fluorescent macro photography, single latent fingermarks from a female donor were deposited on a clean silicon wafer and dried in a

desiccator at 22° C for one week. The fingermarks were subsequently treated with formulations 2-5 from the previous section, omitting the secondary fluorescent tagging step.

The treated samples were visualised using a Zeiss field emission gun scanning electron microscope (FEGSEM) with an accelerating voltage of 5.0 kV, aperture of 30  $\mu$ m and 4 mm working distance. Each sample was viewed at 200x and 200,000x magnification, the horizontal widths of field (HWOF) of each magnification are given in Figures S2-5. An Oxford INCA microanalysis system was used to analyse the elemental composition of the observed particles and the fingermark deposits to which they had adhered. An untreated fingermark was used as a negative control.

All of the samples visualised by FEGSEM showed varying levels of ridge detail erosion due to the application of the reagent (Figures S1-S4). Small clusters of nanoparticles were observed across each ridge – with the highest density occurring at the pores – in three of the four samples. The anti-L-amino acid antibody reagent synthesised following the procedure by Pissuwan *et al.* produced the greatest number of high density nanoparticle clusters, despite producing similar luminescence and ridge detail during fluorescent imaging. The cause of observed difference is still under investigation.

Minimal ridge detail was observed under low magnifications for the fingermark treated with HS-OEG<sub>3</sub>-COOH-capped nanoparticles, which agreed with the results obtained by fluorescent imaging. The loss of ridge detail, hence analytes, in this case may have been due to the capping agent solubilising the fingermark emulsion or due to a subsequent reaction between the free carboxylic acid and various fingermark constituents.

Supplementary Material (ESI) for Chemical Communications This journal is (c) The Royal Society of Chemistry 2011



**Fig. S2** FEGSEM images of a latent fingermark treated with 16 nm gold-citrate nanoparticles at (above) 200x magnification (HWOF 1.80 mm) and (below) 200,000x magnification (HWOF 1.80 μm).



**Fig. S3** FEGSEM images of a latent fingermark treated with 16 nm HS-OEG<sub>3</sub>-COOH-capped gold nanoparticles at (above) 200x magnification (HWOF 1.80 mm) and (below) 10,000x magnification (HWOF 36.0 μm).

Supplementary Material (ESI) for Chemical Communications This journal is (c) The Royal Society of Chemistry 2011



**Fig. S4** FEGSEM images of a latent fingermark treated with anti-L-amino acid antibody-reagent synthesised by the Pissuwan *et al.* method (16 nm particle size) at (above) 200x magnification (HWOF 1.80 mm) and (below) 200,000x magnification (HWOF 1.80 μm).



**Fig. S5** FEGSEM images of a latent fingermark treated with anti-L-amino acid antibody-reagent synthesised by the Cao & Sim method (17 nm particle size) at (above) 200x magnification (HWOF 1.80 mm) and (below) 200,000x magnification (HWOF 1.80 μm).