Supporting Information

'Dispersible Electrodes': A Solution to Slow Response Times of Sensitive Sensors

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S1. Experimental Materials and Methods

Materials

Chemical	Abbreviated	Grade	Supplier
Polyethylene(imine), Branched, 25 kDa	PEI	-	Aldrich, Sydney
3-mercaptopropionic acid	MPA	> 99.0 %	Aldrich, Sydney
2-(N-Morpholino)ethansulfonic acid hydrate	MES	> 99.5 %	Sigma, Sydney
N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride	EDC	> 98.0 %	Fluka, Sydney
N-Hydroxysuccinimide	NHS	> 99.0 %	Fluka, Sydney
Copper(II) sulfate pentahydrate	$CuSO_4 \cdot 5H_2O$	> 98.0 %	Sigma, Sydney
Gold(III) chloride trihydrate	$HAuCl_4 \cdot 3H_2O$	> 99.9 %	Aldrich, Sydney
GlyGlyHis	GGH	-	Sigma, Sydney

Instrumentation

Transmission electron microscopy (TEM) images were obtained using a Philips CM 200 TEM operating at 200 kV. X-ray photoelectron spectroscopy (XPS) was performed on a Thermo Scientific VG-ESCALAB 220-iXL spectrometer with a monochromated Al KR source (1486.6 eV). The spectra were accumulated at a takeoff angle of 90° and analyzer pass energy of 20 eV. Inductively coupled plasma mass spectrometry (ICP-MS) was performed using a Perkin Elmer ELAN 6100 ICP-MS. Cyclic voltammetry (CV) and square wave voltammetry (SWV) measurements were performed using an Autolab PGSTAT 302N potentiostat.

Synthesis of Fe_3O_4-Au_{coat} (For detailed characterization see [1])

The 50 nm (average face-centered diagonal) cubic Fe_3O_4 cores were precipitated by mixing 0.7 g of $FeSO_4$ with 80 mL of distilled water followed by the addition of 10 mL of 2.0 M KNO_3 and

10 mL of 1.0 M NaOH in an oxygen-free environment. The initially formed $Fe(OH)_2$ was heated at 90 °C in the presence of varying concentrations of PEI solution (2 g/L) for 2 h, during which $Fe(OH)_2$ was oxidized to Fe_3O_4 nanoparticles with PEI self-assembled on its surface. Particles were magnetically separated from reaction mixture by placing a neodymium disk magnet (25 mm diameter) magnet below the reaction vessel (200 mL beaker) for 5 min to capture all magnetic particles before discarding the reaction solution. The collected Fe_3O_4−PEI particles were rinsed 5 times with Milli-Q water and suspended in 80 mL of Milli-Q water, yielding a suspension of Fe_3O_4 (2.4 g/L, pH 7).

A 2 mL volume of the above Fe_3O_4−PEI suspension was first sonicated for 2 min with an ultrasonic probe (Misonix S4000 Sonicator) and then stirred for 2 h with 90 mL of 2 nm colloidal gold particles. The colloidal gold particles were produced from the $NaBH_4$ (0.075%) reduction of 1% $HAuCl_4$ in 38.8 mM sodium citrate. The Fe_3O_4−PEI−Au_{seed} particles were magnetically separated from excess Au colloid solution. The particle surfaces were then functionalized again with PEI by heating in a 80 °C oven for 1 h in the presence of a 5 g/L PEI solution followed magnetic separation and dispersion in 20 mL of Milli-Q water.

Au shells were grown by iterative reduction of $HAuCl_4$**.** 20 mL of Au-seeded Fe_3O_4 NPs were mechanically stirred with 110 mL of 0.01 M NaOH (pH ~11.5). A first iteration of 0.5 mL of 1% $HAuCl_4$ was added along with 0.75 mL of 0.2 M $NH_2OH.HCl$ followed by 0.5 mL of 1% $HAuCl_4$ and 0.25 mL of 0.2 M $NH_2OH.HCl$ for the subsequent iterations. A total of up to five iterations were made, with 10 min in between iterations. The Fe_3O_4−Au_{coat} particles were then magnetically separated and dispersed in 20 mL of Milli-Q water.

Step-wise Attachment of GlyGlyHis to Fe_3O_4-Au_{coat}

In the first step, 10 mL of Fe_3O_4−Au_{coat} NPs (4.8 mg) was added to 50 mL of 0.01 mol/L of 3-mercaptopropionic acid (MPA) solution (75% ethanol, 25% MQ-water). The mixture was sonicated for 1 min with an ultrasonic probe to evenly disperse the particles in MPA; the mixture was then stirred with a glass stirrer for 4 hr.

After 4 hr, the particles were magnetically separated from the MPA solution and rinsed 3 times with MES buffer (pH 6.8) to remove unbound MPA molecules. The Fe_3O_4−Au_{coat} NPs were then separated from the wash solution and 20 mL a freshly prepared solution of 0.02 mol/L EDC and 0.004 mol/L NHS in 0.1 mol/L MES buffer (pH 6.8) was added to the particles to activate the

carboxyl groups of the immobilized MPA. The particles were sonicated for 30 s with an ultrasonic probe and stirred with a glass stirrer for 45 min.

After 45 min, the particles were magnetically separated from the EDC/NHS solution and rinsed once with MES buffer (pH 6.8). The Fe_3O_4–Au_{coat} NPs were then separated from the wash solution and 5 mL of 10 g/L GlyGlyHis was added for attachment to the nanoparticles. The particles were rolled gently with the peptide solution for 4 hr to allow for binding onto the particles. After 4 hr the Fe_3O_4–Au_{coat}-GGH NPs were magnetically separated from solution, rinsed with MES buffer and dispersed in 5 mL of Milli-Q water with an ultrasonic probe.

The attachment of GlyGlyHis to the gold-coated nanoparticles is illustrated in Figure S1.

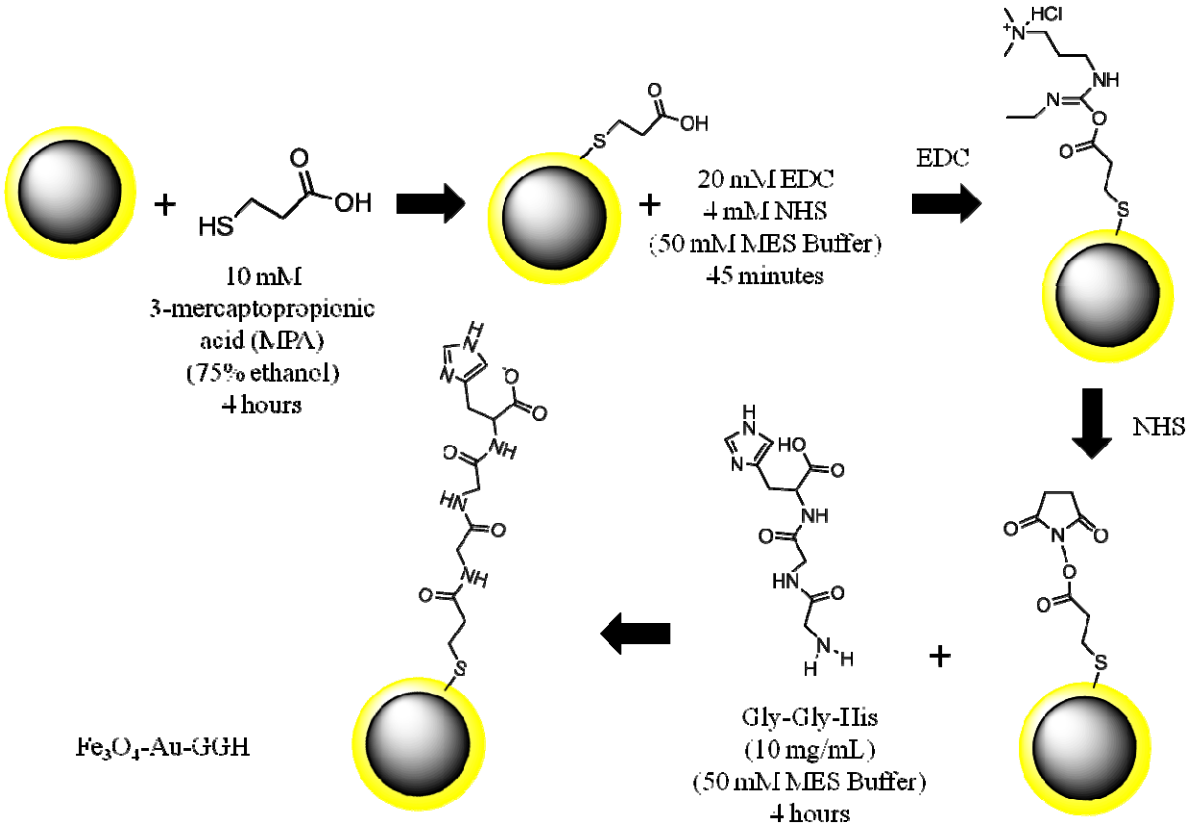
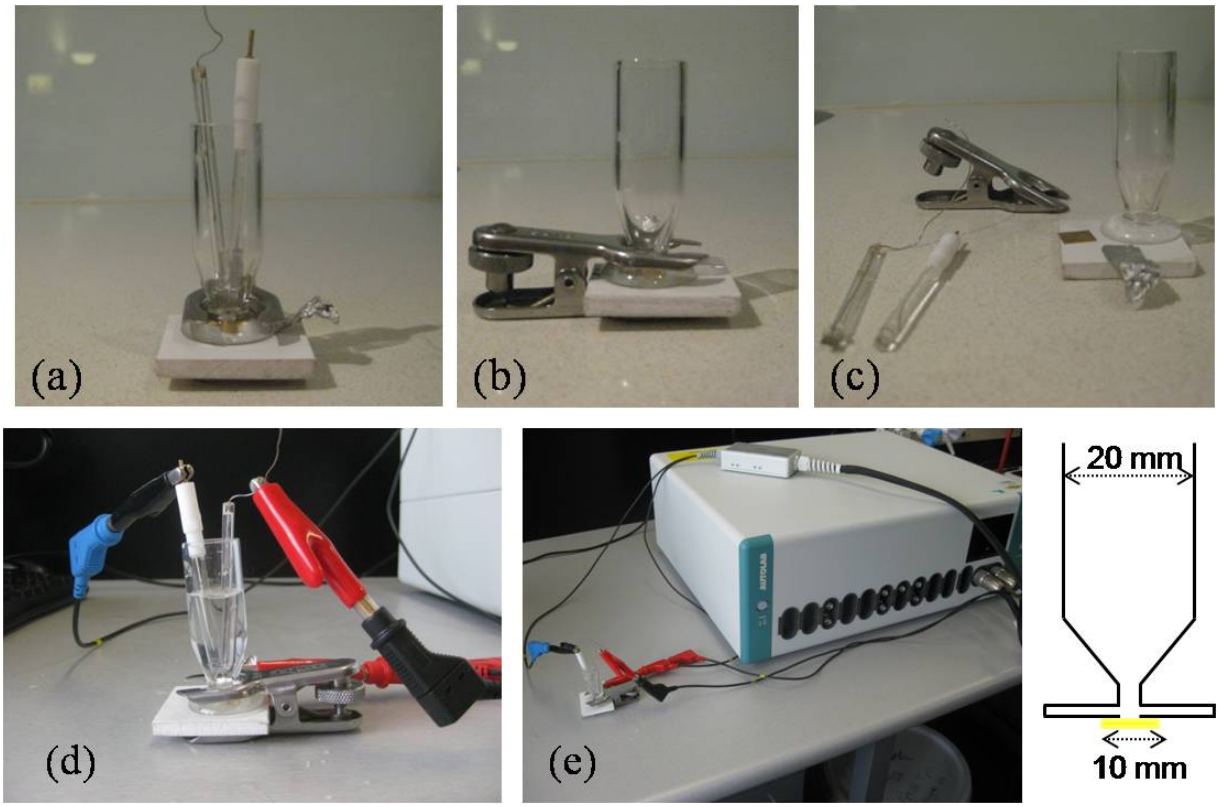


Figure S1. Step-wise attachment of GlyGlyHis to Fe_3O_4-Au_{coat}

Copper Detection Measurements

All glassware was rinsed with 6 mol/L HNO_3 and then thoroughly with Milli-Q water to avoid metal contamination. A 0.1 mol/L stock solution containing Cu^{2+} ions was prepared by dissolving $CuSO_4$. Dilute copper solutions were prepared by serial dilution with 50 mM

ammonium acetate buffer (pH 7.0) to the appropriate concentrations. Polystyrene containers (Bacto, Sydney, Australia) were used to store dilute copper solutions to minimize copper adsorption onto the side walls of containers.



The electrochemical cell utilized in this work consists of:

1. 10 mm by 10 mm gold foil
2. 30 mm by 30 mm polyethylene base
3. Glass tube (20 mm dia.) with tapered and flanged end
4. Ball and joint clamp
5. Platinum wire counter electrode
6. Ag/AgCl reference electrode

Gold foil cleaning:

Before each set of experiments, the gold foil electrode was cleaned by polishing the surface with alumina powder and soaking for 1 hour in piranha solution (50% conc. H_2SO_4, 50% H_2O_2). This was followed electrochemical cleaning by holding the potential of the gold foil in 0.1 M $HClO_4$ for 30 s to remove any metal contaminants.

Pre-measurement:

The clean gold foil is then camped below the glass cell using a ball and joint clamp (c). The electrodes are connected to the potentiostat and placed within the glass cell (d).

Measurement procedure:

For each detection measurement, 2 mL of copper solution was added to electrochemical cell. (i) 0.5 mL (0.5 mg) of Fe_3O_4−Au_{coat}-GGH NPs were then added to the electrochemical cell and (ii) allowed to mix for 5 min (incubation period). After 5 min, (iii) a magnetic field was applied at the base of the cell for 2 min using a neodymium disc magnet (6 mm radius, 6 mm thickness, AMF Magnetics, Sydney, Australia). The applied magnetic field attracts the particles onto a gold macroelectrode reference and counter electrodes were immersed in the solution and electrochemical measurements were performed (either cyclic voltammetry or square wave voltammetry, Autolab PGSTAT 302N).

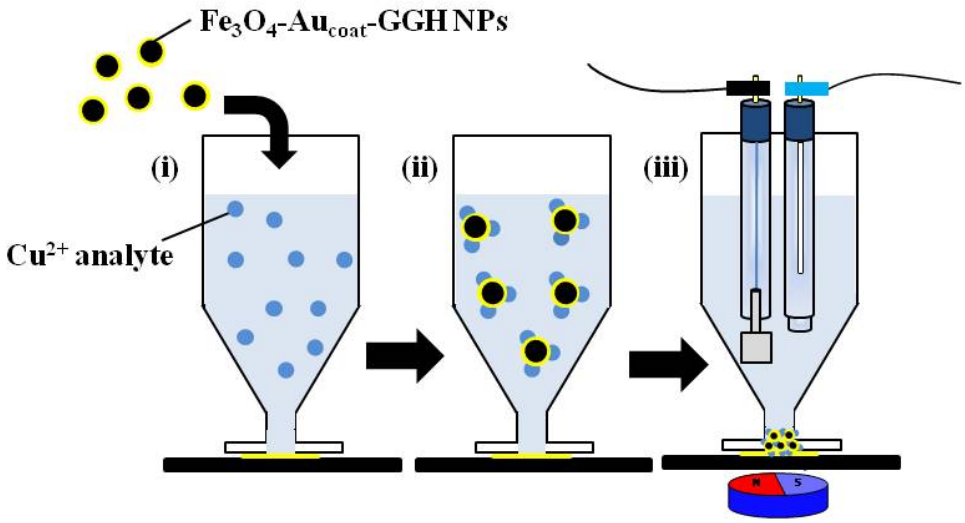


Figure S2. Schematic illustration of the copper detection measurements

S2. Transmission electron microscopy (TEM) charaterisation of synthesized Fe_3O_4-Au_{coat} nanoparticles

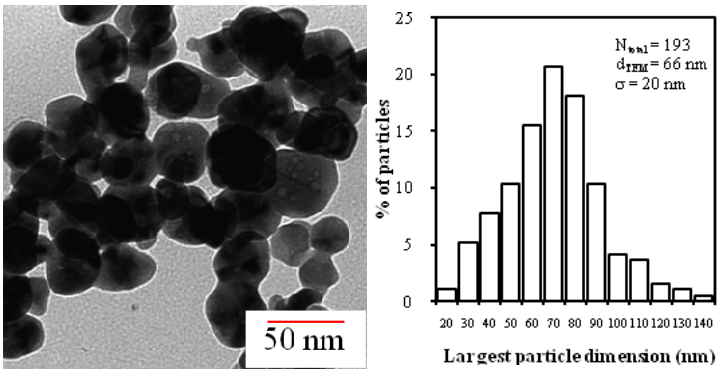


Figure S3. TEM image of Fe_3O_4−Au_{coat} particles with histogram of particle distribution (TEM counting)

S3. X-ray photoelectron spectroscopy (XPS) measurements to confirm attachment of functional molecules

The modification of the gold surface with MPA can be confirmed via the emergence of several characteristic peaks in the high resolution spectra of the S 2p and C 1s regions (Figure S4a and S4b). Analysis of the S 2p region (Figure S4a) indicated the presence of two doublets with the main doublet (solid line) occurring at 162.0 eV which was attributed to MPA molecules chemisorbed on gold and indicates the formation of a thiolated species, while the minor peak (dotted line) can be attributed to physisorbed MPA molecules.[2] Further evidence of the successful attachment of MPA on the Fe_3O_4-Au_{coat} nanoparticle was the emergence of peaks centered at 288.8 eV and 284.7 eV which were attributed to the presence of carboxyl and methylene groups respectively.[3]

Activation of the MPA modified Fe_3O_4-Au_{coat} nanoparticles with EDC/NHS resulted in the emergence of a peak in the N 1s region of the spectra, which was not observed previously (Figure S4c). Peak fitting yielded three components at binding energies of 399.7, 400.7 and 402.1 eV which were attributed to the secondary, tertiary amines of EDC and the NHS ester respectively. Finally, the successful attachment of GlyGlyHis molecules onto the particles could be confirmed by analysis of the N 1s region of the spectra after the particles had been mixed with peptide (Figure S4d). Comparing Figure S4c and S4d, it can be seen that the peak at binding energy 402.1 eV attributed to the NHS ester was no longer detectable after peptide coupling. Peak fitting yielded three peaks at 399.8, 400.7 and 401.5 eV. The peak at 399.8 eV was attributed to the C=N nitrogen of the imidazole side chain of histidine, while the peak at 401.5 eV was attributed to the C=N nitrogen of the imidazole ring.[3] The peak at 400.7 eV was assigned to amide nitrogens.

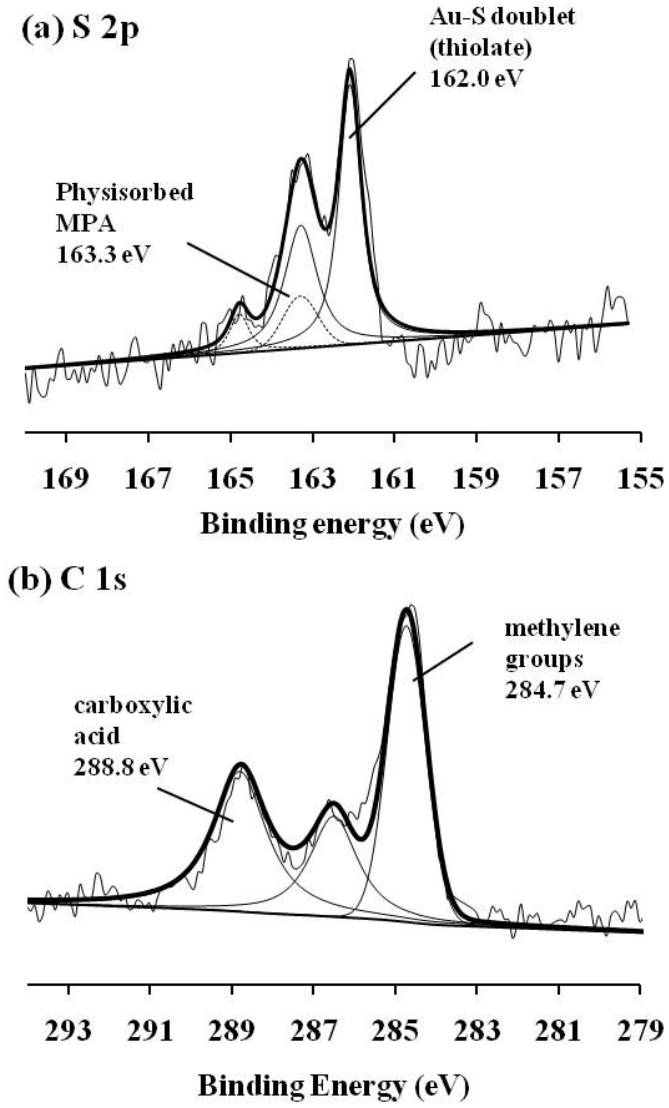


Figure S4(i). XPS spectra showing the surface chemistry of Fe_3O_4-Au_{coat} nanoparticle surfaces during the functionalization process (a) S 2p region after MPA attachment, (b) C 1s region after MPA attachment,

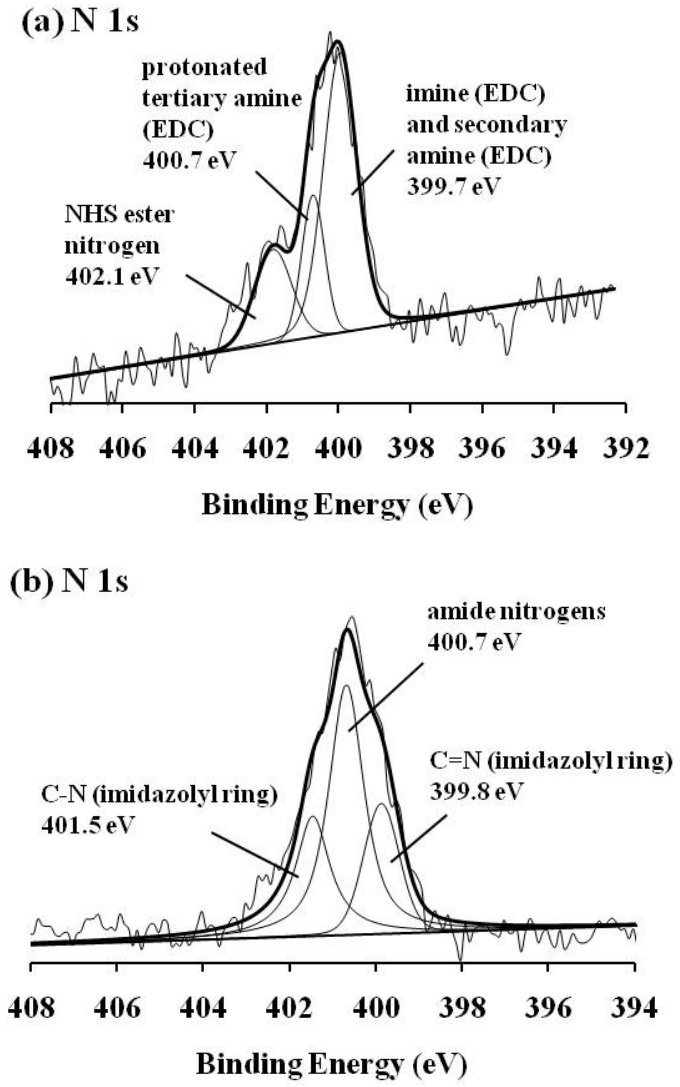


Figure S4(ii). (a) N 1s region after EDC activation, (b) N 1s region after GlyGlyHis attachment.

S4. Inductively Coupled Plasma - Mass Spectrometry (ICP-MS) measurements to determine Fe_3O_4-Au_{coat}-GGH nanoelectrodes copper capture performance

ICP-MS was used to demonstrate that a much higher percentage of the available analyte is brought to the macroelectrode surface compared to gold disc electrodes. ICP-MS, as the standard analytical method for detecting low concentrations of Cu^{2+}, has a typical detection limit of 0.1 ppb. To ascertain the extent of copper captured by the nanoparticle electrodes, 3.6 mg of Fe_3O_4-Au_{coat}-GGH nanoelectrodes were added to 50 mL of 1.0 ppb of $CuSO_4$ solution (Figure S6).

After allowing 10 minutes for the nanoelectrodes to capture the Cu^{2+}, the nanoelectrodes were magnetically separated from the $CuSO_4$ solution. ICP-MS analysis of the solution showed copper to be undetectable in the solution (Figure S5). This indicates that more than 90% of the Cu^{2+} was captured by the nanoparticles. In contrast, with a planar electrode, there was no change in the amount of copper from the initial Cu^{2+} concentration detected by ICP-MS, after exposing the GlyGlyHis modified electrode to the Cu^{2+} solution; thus indicating the planar electrodes captured only negligible amounts of the Cu^{2+}.

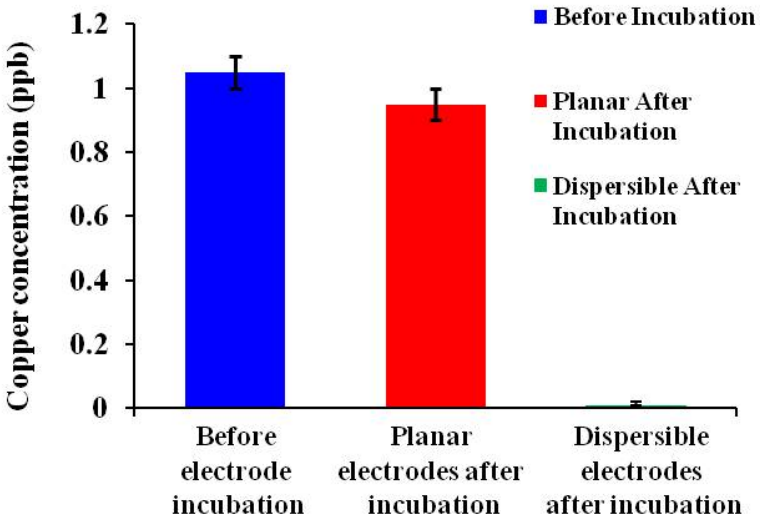


Figure S5. ICP-MS measurements of copper content before and after incubation with Fe_3O_4-Au_{coat}-GGH nanoelectrodes.

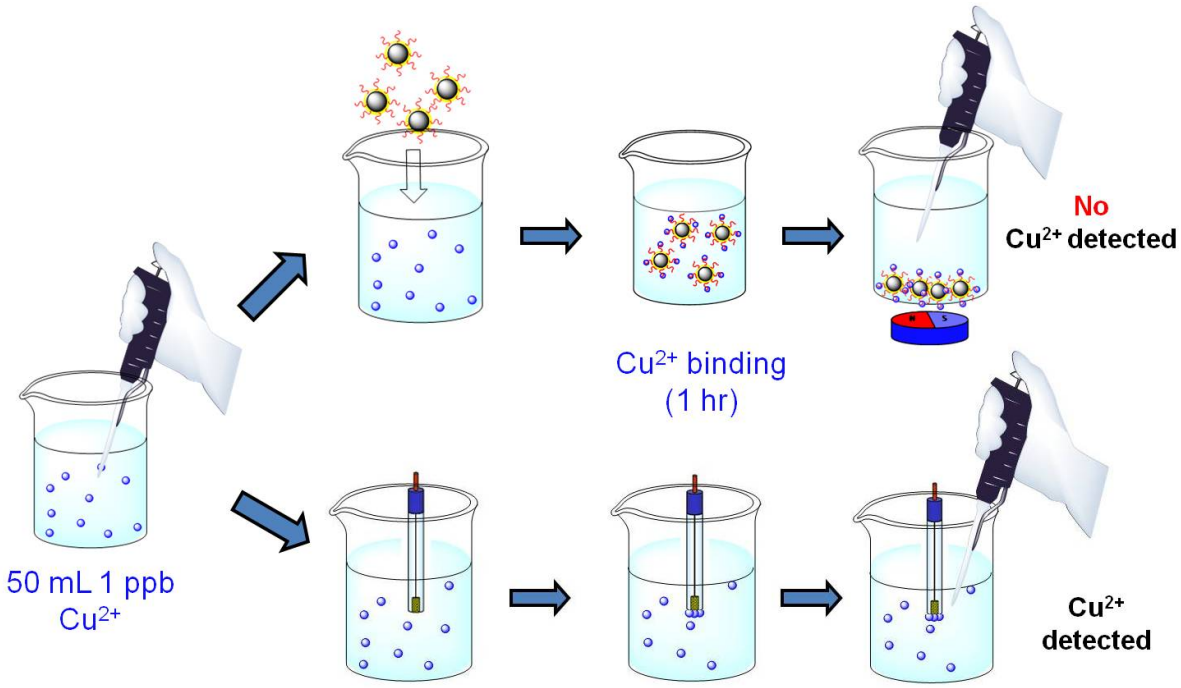


Figure S6. Schematic illustration of the copper capture measurement.

S5. Square Wave Voltammetry (SWV) to determine current response to different copper concentrations

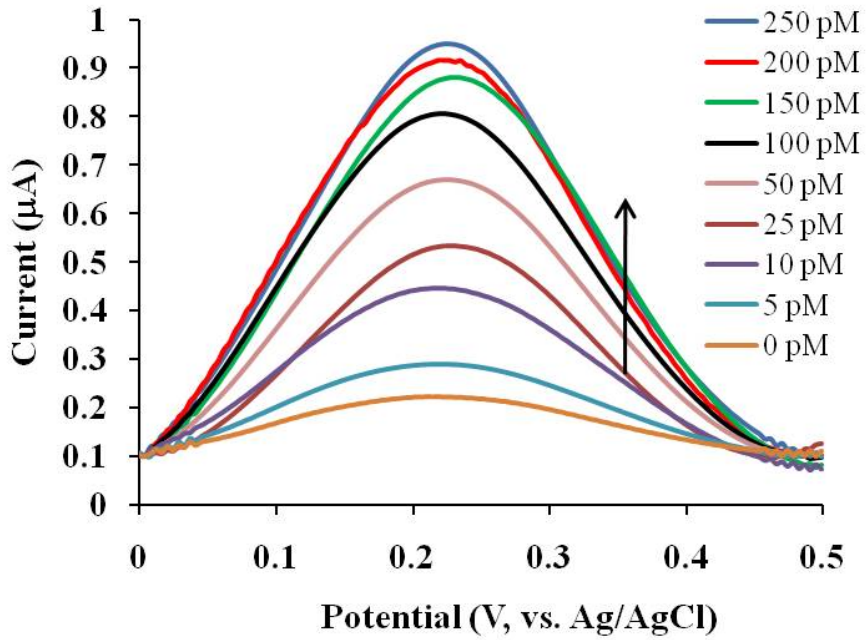


Figure S7 Square wave voltammograms showing current response of the nanoelectrode system as a function of copper concentration.

In order to determine the electrochemical response of the nanoelectrode systems, square wave voltammograms were performed. It can be seen in Figure S7 that increases in copper concentration in solution resulted in increases to the current response measured. This increase is as result of an increased number of Cu^{2+} ions being captured by the nanoelectrodes. The lowest detectable concentration of copper was found to be 2 pM which represents a more than 1000-fold decrease on previously reported limits.[4]

S6. Control Experiments

Control experiments were performed to compare the performance of the dispersible nanoelectrode system against (1) a similarly functionalised stationary macro electrode and (2) a stationary electrode modified with similar functionalised gold coated magnetic nanoparticles (see schematic provided in Figure S8). These control electrode constructs allow us to determine if Fe_3O_4-Au_{coat}-GGH nanoelectrodes will be advantageous.

- Construct (**1**) was made via the same process as the nanoelectrodes as described in detail in the supporting information section. Briefly, a 1 cm^2 piece of polished gold foil is functionalized with mercaptopropionic acid. This is followed by GGH attachment via carbodiimide coupling.
- Construct (**2**) was made by immobilizing Fe_3O_4-Au_{coat}-GGH nanoparticles onto the gold foil. This was done by allowing Fe_3O_4-Au_{coat}-GGH nanoparticles to settle on the gold foil surface under and applied magnetic field.

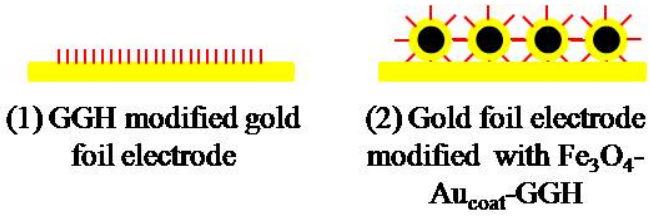


Figure S8. Control electrode constructs for comparison with Fe_3O_4-Au_{coat}-GGH dispersible electrodes system.

Figure S9 below shows square wave voltammograms of the responses of the three different electrode systems to samples of 2 mL of 50 pM Cu^{2+}. It can be seen that at this concentration, no response to the Cu^{2+} is observed for the control electrode constructs. A significant response is observed for the dispersible electrodes system. This highlights the fact that at such low Cu^{2+} concentrations, the ability for the electrodes to actively seek the analyte is vital to enabling detection at pM concentrations.

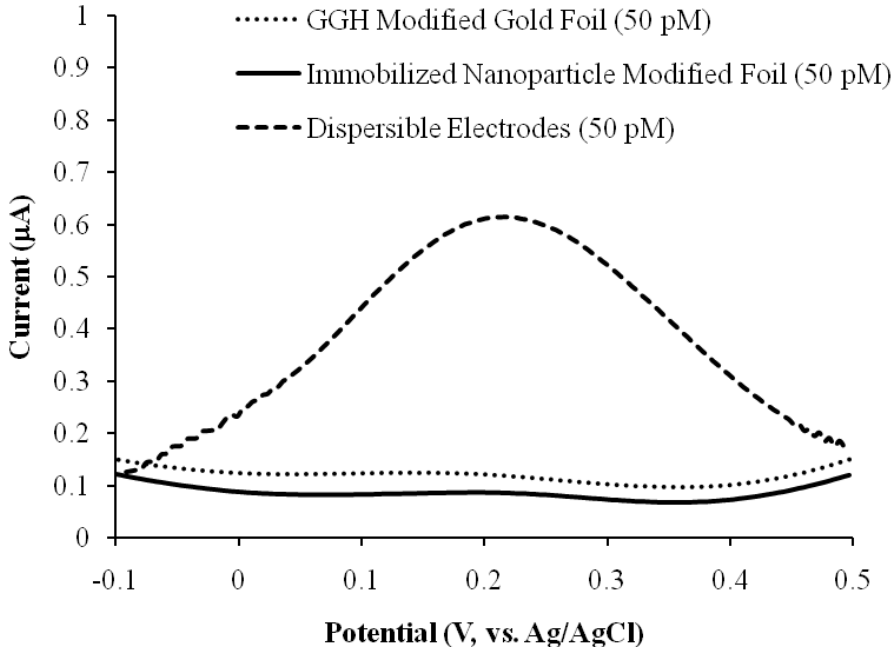


Figure S9. Current response of various control electordes to 50 pM Cu^{2+}

The system effectively requires us to think about the analysis in terms of amount of analyte rather than the concentration. So, here increased volume at the same concentration means more analyte, and hence high analytical signal. This is demonstrated below with two control experiments. The first control involved determining the response of the nanoelectrodes to different sample volumes where the concentration of the analyte was the same as seen in Figure 3 of the manuscript..

A second control investigated the response of a 100 pM 2 mL Cu^{2+} sample and compared the current response to the same sample diluted by 2 times (i.e. 50 pM 4 mL Cu^{2+}). It can be seen from Figure S10 below that the response of the two samples is roughly equivalent. This provides further confirmation that it is the total number of Cu^{2+} captured that determines current response.

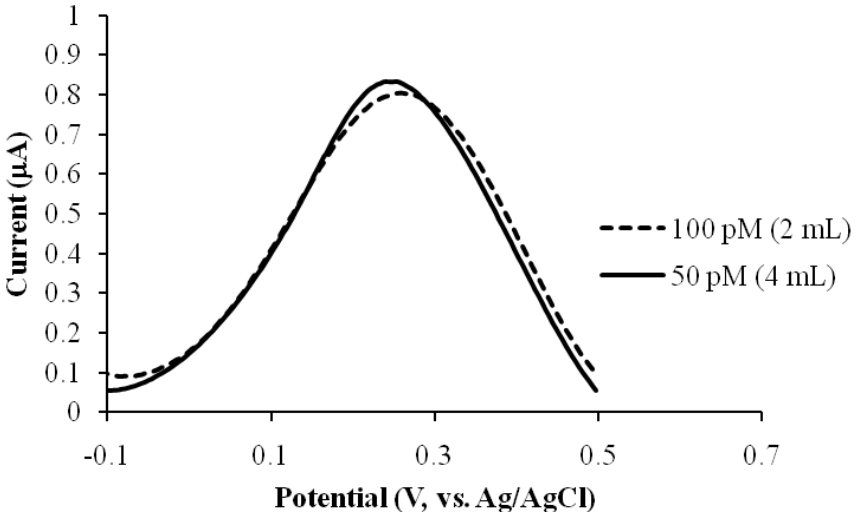


Figure S10. Curent response of a 100 pM sample and a 2x diluted sample

Again this control highlights why the way we think about an analytical system must change when disucssing this system. The amount of particles returned to the macroelectrode is far less important than one might think. This is demonstrated with a control experiment where the number of nanoelectrodes attracted onto the macro-electrode for detection is varried. It can be seen from Figure S11 below that although different amounts of particles were added, the current response is the same within experimental uncertainty.

This indicates that because the dispersible nanoelectrodes are able to capture all Cu^{2+} in the sample, after being drawn to the macro-electrode the same current response is observed. So the requriement for the system to be insensitive to the loss of a few nanoparticles is for the nanoparticle binding sites to essentially be in excess relative to the amount of analyte.

Considering this concept was developed for detecting low concentrations of analyte this is not a difficult criteria to fulfill. If however, the particles bearing the few analytes are lost then of course the analytical signal will be influenced. This is where sampling comes in, and in this case the solution is to increase the sample volume as shown above. Again, increasing sample volume for improved analytical signal is counter intuitive when compared with planar sensing systems. This is important because it shows that as long as a sufficient number of nanoelectrodes are used to capture the analyte, the response remains consistent.

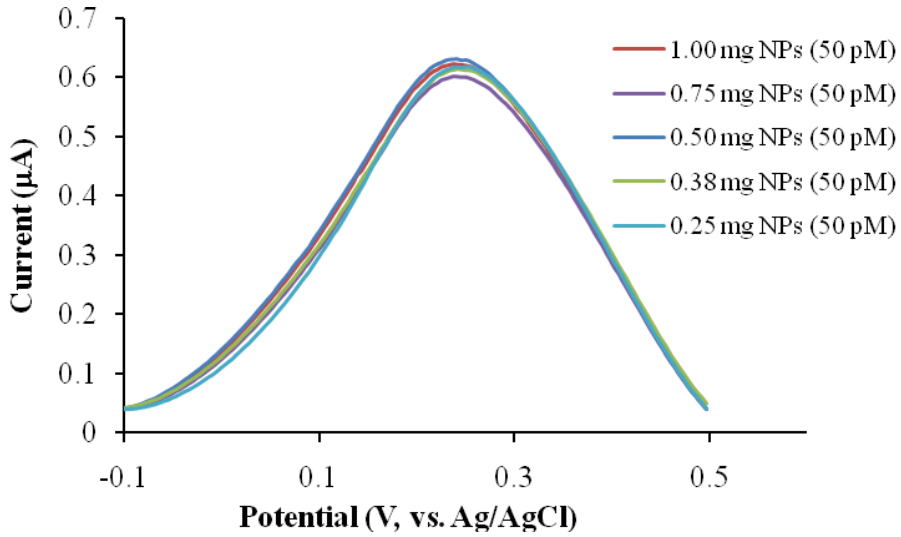


Figure S11. Current response of a 2 mL 50 pM Cu^{2+} sample using different amounts of Fe_3O_4-Au_{coat}-GGH.

References

1. I. Y. Goon; L. M. H. Lai; M. Lim; P. Munroe; J. J. Gooding; R. Amal, *Chem. Mater.* **2009**, 21, 673-681.
2. G . Gonella; O. Cavalleri; S. Terreni; D. Cvetko; L. Floreano; A. Morgante; M. Canepa; R. Rolandi, *Surf. Sci.* **2004**, 566-568, (Part 1), 638-643.

3. E. Chow; E. L. S. Wong; T. Bocking; Q. T. Nguyen; D. B. Hibbert; J. J. Gooding, *Sens. Actuators, B* **2005**, 111-112, 540-548.
4. Liu, G. Z.; Nguyen, Q. T.; Chow, E.; Bocking, T.; Hibbert, D. B.; Gooding, J. J. *Electroanalysis* **2006**, *18*, 1141-1151.