Electronic Supplementary Material (ESI) for Chemical Science.

Supporting Information

Rapid and ultrasensitive electrochemical detection of circulating tumor DNA by hybridization on the network of gold-coated magnetic nanoparticles

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Experimental Procedures

Materials

Oligonucleotides were synthesized by Biosearch Technologies (Petaluma) and Integrated DNA Technologies (IDT) (Iowa, USA) and presented with detailed sequences in Table S1 and Table S2. Tris(2-carboxyethyl) phosphine hydrochloride (TCEP), 6-mercapto-1-hexanol (MCH), sodium chloride (NaCl), potassium ferricyanide (III) ($K_3[Fe(CN)_6]$), Dulbecco's phosphate-buffered saline were acquired from Sigma-Aldrich. Sodium phosphate monobasic (NaH_2PO_4), sodium phosphate dibasic (Na_2HPO_4) and ethanol were purchased from Chem-Supply Pty. Ltd. Milli-Q water (~18 MΩ cm, Milli-pore, Australia) was used to prepare experimental solutions. Blood used in this experiment was purchased from Australia Red Cross from a healthy donor.

Apparatus

Transmission electron microscopy (TEM) images were taken using a FEI TECNAI G2 microscopy. A CHI660E electrochemical workstation (CHI Instruments, Inc.) was used to measure square wave voltammograms. A three-electrode system was equipped for electrochemical measurements, using a bare gold foil as the working electrode (WE), Ag/AgCl (3.0 M KCl) as the reference electrode (RE) and platinum wire as the counter electrode (CE).

Procedures

Surface modification of gold foil

The gold foil was polished by alumina polishing powders with the particle size of 1.0, 0.3 and 0.05 μm, respectively. After ultrasonically washing in Milli-Q water, absolute ethanol, and Milli-Q water successively, the electrode was dried by nitrogen. Prior to the incubation, 50 μL of 4 μM MB-DNA were reduced with 2 μL of 1 mM TCEP for 1 h. The thiolated MB-DNA monolayer on gold foil was prepared by incubating the cleaned gold electrode in the solution of MB-DNA for 8 h. The above steps were performed in the dark as MB is mildly light-sensitive. Then 40 μL of 2 mM MCH was mixed with 8 μL of 10 mM TCEP for 1 h followed by immersing the gold substrate into the reduced MCH solution for 3 h. Before electrochemical measurement, the gold foil was rinsed thoroughly by Milli-Q water.

Surface modification of Au@MNPs with probe MB-DNA.

Prior to the modification, the disulfide bond of the probe MB-DNA was reduced by adding 1 mM TCEP to 100 nM thiolated MB-DNA, wrapping with aluminum foil and incubating for 1 h at room temperature. To immobilize MB-DNA on the surface of Au@MNPs, redispersed 10 μL Au@MNPs were added into the freshly prepared 100 nM thiolated MB-DNA solution and incubated while mixing gently in a rotating wheel, followed by four dropwise additions of 1 M NaCl solution to obtain a final concentration of 0.15 M and incubating on a rotating wheel overnight. The MB-DNA-Au@MNPs were separated from the supernatant solution by 5 min centrifugation at 5000 rpm. The modified nanoparticles were rinsed twice using phosphate buffered saline solution and discarding the supernatant. In addition, MB-DNA-Au@MNPs were kept in a fridge at 4 ^oC prior to use, which ensures that nanoparticles could remain unchanged for a typical storage time of 2 weeks.

Hybridization procedure

Different concentrations of target DNA solution were prepared by serial dilution of stock DNA target of complementary, single-mismatched, and non-complementary sequences. To have the MB-DNA-Au@MNPs hybridize with target DNA, 100 μL of target DNA in appropriate concentrations was added to the freshly prepared MB-DNA-Au@MNPs and the mixture was kept for 20 min in the rotating wheel in room temperature. Then, the hybridized MB-DNA-Au@MNPs were separated from the supernatant solution by applying a centrifugation step first (5000 rpm for 5 min) and then removal of the supernatant by pipette with a magnet placed at the bottom of the sample tube. The hybridized MB-DNA-Au@MNPs were rinsed twice with phosphate buffered saline solution, discarding the supernatant.

Electrochemical measurement

Signal changes corresponding to specific target were calculated with background-subtracted SWVs: change in current = ($I_{ssDNA\text{-}Au@MNPs} - I_{dsDNA\text{-}Au@MNPs}$) / $I_{ssDNA\text{-}Au@MNPs}$ * 100% (where $I_{ssDNA\text{-}Au@MNPs}$ = current before hybridization, $I_{dsDNA\text{-}Au@MNPs}$ = current after hybridization).

Table S1. The oligonucleotides involved for the detection of short strand (22 nucleotide) DNA.

DNA (short strand)	Sequence
Probe MB-DNA	5'-HS-$(CH_2)_6$-TCA ACA TCA GTC TGA TAA GCT A-$(CH_2)_6$-MB-3'
Probe DNA (pDNA)	5'-HS-$(CH_2)_6$-TCA ACA TCA GTC TGA TAA GCT A-$(CH_2)_6$-3'
Complementary DNA target	5'-TAG CTT ATC AGA CTG ATG TTG A-3'
Single mismatched DNA target	5'-TAG CTT ATC AAA CTG ATG TTG A-3'
Non-complementary DNA strand	5'-TCT TCT TCT GTC TGT TTT GCT T-3'

Note: The above custom oligonucleotides were purchased from Biosearch Technologies (Petaluma).

Table S2. The oligonucleotides involved for the detection of long strand (101 nucleotide) DNA.

DNA (long strand)	Sequence
Probe MB-DNA (22 nt, immobilized on the surface of Au@MNPs, targeting 3' end of target ctDNA)	5'-HS-$(CH_2)_6$-GCT TAC TTT GTT ACA GGC GGT G-$(CH_2)_6$-MB-3'
Probe MB-DNA (22 nt, immobilized on the surface of Au@MNPs, targeting the middle of ctDNA)	5'-HS-$(CH_2)_6$-AAC AAG ATC CAG AAG AGG AAA A-$(CH_2)_6$-MB-3'
Target DNA (101 nt)	5'-TTT TAC AAT TTG CTT ACT GTG CCC ATT ATG AAA ATG CAT CTT TTC CTC TTC TGG ATC TTG TTT TGC TTT GCT GTA GCC ACA CCG CCT GTA ACA AAG TAA GC-3'
Single mismatched target DNA (101 nt)	5'-TTT TAC AAT TTG CTT ACT GTG CCC ATT ATG AAA ATG CAT CTT TTC CTC TTC TGG ATC TTG TTT TGC TTT GCT GTA GCC ACA CCG CCT GGA ACA AAG TAA GC-3'

Note: The above custom oligonucleotides were purchased from Integrated DNA Technologies (IDT) (Iowa, USA).

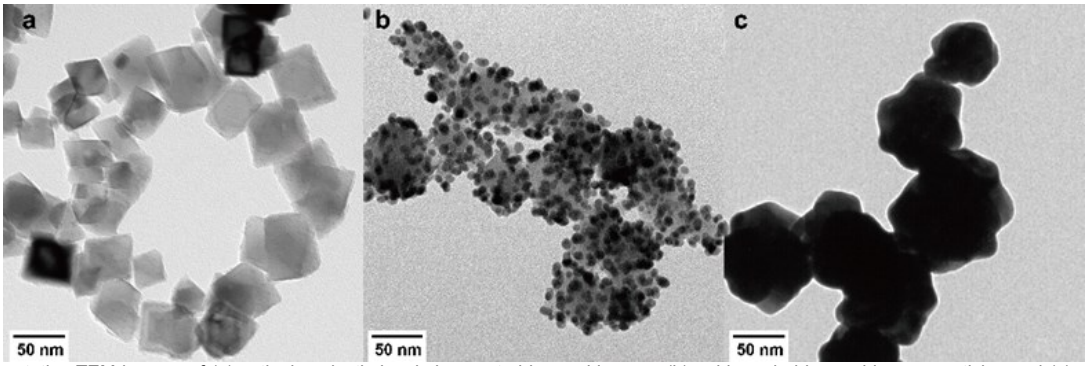

Fig. S1 Representative TEM images of (a) cationic polyethyleneimine coated iron oxide core, (b) gold-seeded iron oxide nanoparticles and (c) gold-coated iron oxide nanoparticles (Au@MNPs). The final concentration of Au@MNPs was 1.56×10^{10} particles/mL measured by inductively coupled plasma optical emission spectroscopy (ICP-OES).

The characterization information of Au@MNPs according to the MIRIBEL principle[1] are included in Table S3. According to our previous work[2], the suspension of Au@MNPs could be collected by a magnet in 2 min, showing the strong magnetism of Au@MNPs. Although the Au@MNPs are not superparamagnetic because of the particle size (>10 nm), the magnetic remanence of the Au@MNPs allows them to be easily redispersed in the absence of magnetic field.

Table S3. Characterization of Au@MNPs.

Question	Y/N	Reference
1.1 Are extensive and clear instructions reported detailing all steps of synthesis and the resulting composition of the nanomaterial?	Y	2, 3, 4
1.2 Is the size and shape of the nanomaterial reported?	Y	2, 3
1.3 Is the TEM characterization of the nanomaterial reported?	Y	2, 3, 4, 5
1.4 Is the superconducting quantum interference device (SQUID) magnetometry curves of the nanomaterial reported?	Y	2
1.5 Is the dynamic light scattering (DLS) measurement of nanomaterial without PEI and with PEI functionalization reported?	Y	2
1.6 Is the UV-vis spectra of the nanomaterial reported?	Y	2
1.7 Is the aggregation stability of nanomaterial reported?	Y	2
1.8 Is the thickness of the estimated nanoshell reported?	Y	2
1.9 Is the size dispersity or aggregation of the nanomaterial reported?	Y	2
1.10 Is the density (mass/volume) of the nanomaterial reported?	Y	2
1.11 Is the Scanning Transmission Electron Microscope /Energy Dispersive Spectroscopy elemental maps of nanomaterial reported?	Y	2
1.12 Is the estimated number of cores within a nanomaterial reported?	Y	2
1.13 Is the XPS characterization of the nanomaterial reported?	Y	2
1.14 Is the XPS characterization of the surface modification of nanomaterial reported?	Y	3, 4, 5
1.15 Is the SEM characterization of the nanomaterial deposited on electrode surface reported?	Y	4, 6
1.16 Is the AFM characterization of the nanomaterial deposited on electrode surface reported?	Y	6
1.17 Is the optical imaging of the nanomaterial deposited on electrode surface reported?	Y	6
1.18 Is the electrochemical resistance characterization of bare gold electrode with and without deposited nanomaterial reported?	Y	6
1.19 Is the amount of any drug loaded reported? 'Drug' here broadly refers to functional cargos (e.g., proteins, small molecules, nucleic acids).	Y	3, 4, 5, 7
1.20 Is the targeting performance of the nanomaterial reported, including amount of ligand bound to the nanomaterial if the material has been functionalized through addition of targeting ligands?	Y	3, 4, 5, 7
1.21 Were characterizations performed in a fluid mimicking biological conditions?	Y	7
1.22 Are details of how these parameters were measured / estimated provided?	Y	7

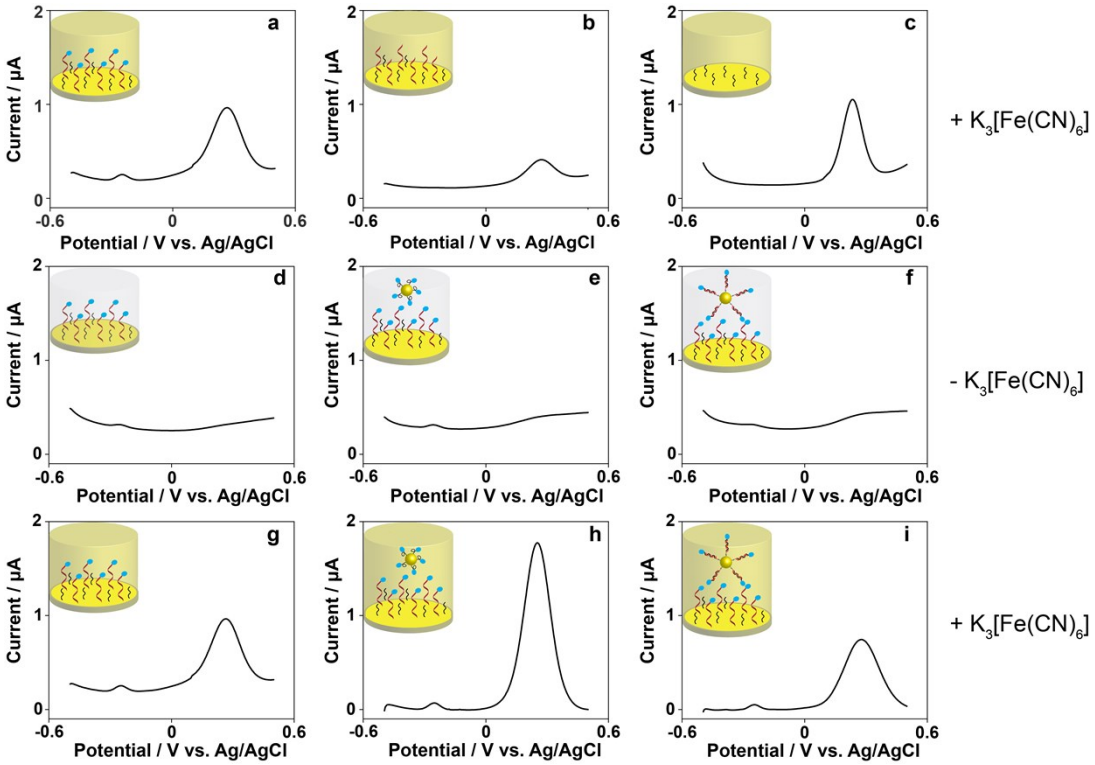

Fig. S2 The importance of the presence of $K_3[Fe(CN)_6]$ in the detection solution for signal amplification. SWVs of (a) MB-DNA/MCH/Au foil, (b) DNA/MCH/gold foil and (c) MCH/gold foil in 0.5 mM potassium ferricyanide in phosphate buffered saline (pH 7.4). SWVs of (d) gold foil pre-modified with thiolated MB-DNA and MCH, (e) before and (f) after exposure of the MB-DNA-Au@MNPs to 100 µL 20 nM complementary target DNA, then nanoparticles were magnetically collected on the surface of gold foil. Electrolyte solution was phosphate buffered saline (pH 7.4). SWVs of (g) gold foil pre-modified with thiolated MB-DNA and MCH, (h) before and (i) after exposure of the MB-DNA-Au@MNPs to 100 µL 20 nM complementary target DNA, then nanoparticles were magnetically collected on the surface of gold foil. Electrolyte solution was 0.5 mM $K_3[Fe(CN)_6]$ in phosphate buffered saline (pH 7.4). The frequency and pulse amplitude of square wave voltammograms were 2 Hz and 25 mV, respectively.

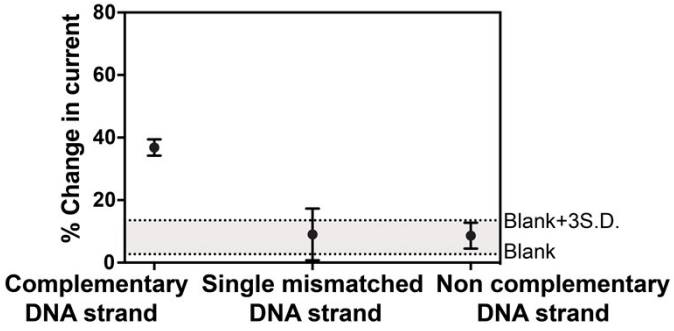

Fig. S3 The hybridization-induced change of SWVs for the investigation of sensor specificity by exposing the sensor to 100 µL 2 pM DNA strands (22 nucleotides) of complementary, single mismatched (AC mismatch in the middle position) and non-complementary sequences. The resultant decreases in current were (36.8±2.6)%, (9.0±8.2)% and (8.7±4.2)% observed for 2 pM complementary, single mismatched and non-complementary DNA strands, respectively. Two dash lines are provided to indicate the levels of the average blank and the three times standard deviation above the averaged blank. Error bars represent standard deviations from 5 independent measurements.

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