Supplementary Information

Towards the electrochemical identification of species

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1. Materials

All chemicals were used as received unless otherwise indicated. Zinc percholarte hexahydrate was obtained from Aldrich (Milwaukee, WI, USA). Sodium perchlorate (anhydrous) was purchased from Alfa Aesar (Ward Hill, MA, USA). Potassium ferrocyanide trihydate and EDTA were from EM Science (Darmstadt, Germany). Tris(hydroxymethyl)-aminomethane, ultrapure was from BMD (Indianapolis, IN, USA). Sulfuric acid (95- 98%) and perchloric acid (70%) ware from Caledon Laboratory Chemicals (Georgetown, ON, Canada). Ethanol was obtained from Commercial Alcohols (Brampton, ON, Canada) and freshly distilled prior to use. All solutions were prepared with deionized water (18 MΩcm resistivity) produced by a Millipore Milli-Q water purification system.

2. Instrumentation

SECM experiments were carried out with a CHi-900b scanning electrochemical microscope (CH Instruments, Austin, TX). A standard three-electrode cell was used with the 25 μm diameter Pt tip electrode (RG between 2 and 3), a platinum wire as the counter electrode, and an Ag/AgCl (CH Instruments) as the reference electrode. All measurements were performed in 20 mM Tris-ClO_4 (pH 8.6) containing 1 mM $K_4[Fe(CN)_6]$ and 50 mM $NaClO_4$ as a supporting electrolyte. The tip potential was held constant at E_T = 0.5 V to oxidize $[Fe(CN)_6]^{4-}$ at a diffusion controlled rate. Gold substrates were prepared by thermal e-beam deposition (Western Nanofabrication Facility, ON, Canada). Spotting of the DNA micro-arrays was carried out using a spotting robot (SpotBot, Telechem, Sunnyvale, CA),

3. Preparation of gold substrates

Gold substrates were prepared by thermal e-beam deposition. A custom e-beam deposition system was used (Western Nanofabrication Facility, ON, Canada). The deposition process was carried out at $8 \cdot 10^{-6}$ bar and rate of 0.1 nm/s. First, 5 nm layer of titanium was deposited onto a silicon wafer (device grade purchased from University Wafer, MA, USA), followed by 150 nm layer of gold (99.99% purity). After deposition the gold substrates were cut into 1×1 cm pieces. Prior to use substrates were rinsed with concentrated sulfuric acid, rinsed with copious amounts

of Milli-Q water, sonicated for 5 minutes in 1:1 mixture of freshly distilled ethanol and Milli-Q, then rinsed with copious amounts of ultra pure water and dried in the stream of nitrogen (Praxair, ON, Canada).

4. Oligonucleotides and DNA hybridization

The following DNA strands were used to study the effect of mismatches on electrochemical response.

1: HO-$(CH_2)_6$-S-S-$(CH_2)_6$-
5'-GGT-CAA-CAA-ATC-ATA-AAG-ATA-TTG-G-3'
2: 3'-CCA-GTT-GTT-TAG-TAT-TTC-TAT-AAC-C-5'
3: 3'-CCA-GT***C***-GTT-TAG-TAT-TTC-TAT-AAC-C-5'
4: 3'-CCA-GTT-GTT-TAG-***C***AT-TTC-TAT-AAC-C-5'
5: 3'-CCA-GTT-GTT-TAG-TAT-TTC-***C***AT-AAC-C-5'
6: 3'-CCA-G***C***T-GTT-TAG-TAT-TTC-TA***C***-AAC-C-5'
7: 3'-CCA-GTT-GTT-***C***AG-TAT-***C***TC-TAT-AAC-C-5'
8: 3'-CCA-GT***A***-GTT-TAG-TAT-TTC-TAT-AAC-C-5'
9: 3'-CCA-GT***G***-GTT-TAG-TAT-TTC-TAT-AAC-C-5'
10: 3'-CCA-GT***C***-***T***TT-TAG-TAT-TTC-TAT-AAC-C-5'
11: 3'-CCA-GTT-GTT-TA***T***-***C***AT-TTC-TAT-AAC-C-5'
12: 3'-CCA-GTT-GTT-T***TT***-***C***AT-TTC-TAT-AAC-C-5'

Sequence of the disulfide-labelled bovine CYTbos1 primer strand and unlabeled sequences corresponding to seven common animal species are given below.[1]

a1: HO-$(CH_2)_6$-S-S-$(CH_2)_6$-
5'-CGA-TCA-ATC-CCC-AAC-AAA-CTA-3' (CYTbos1 primer)
a2: 3'-GCT-AGT-TAG-GGG-TTG-TTT-GAT-5' (cattle, *Bos taurus*)
a3: 3'-GC***A-***AGT-TA***A-***GG***A-***TT***A-***TTT-GAT-5' (pig, *Sus scrofa*)
a4: 3'-GC***G-***AG***G-***TAG-GGG-TTG-TTT-GA***A***-5' (chicken, *Gallus gallus*)
a5: 3'-GCT-AGT-TA***A-***GG***A-***TTG-TTT-GAT-5' (buffalo, *Bubalus bubalis*)
a6: 3'-GCT-AGT-TA***A-***GGG-TT***A-***TTT-***A***AT-5' (deer, *Dama dama*)
a7: 3'-GC***A-***AGT-TA***A-***GG***T-***TT***A-***TT***C-***GAT-5' (quail, *Coturnix coturnix*)

a8: 3'-GC***A***-AG***G***-TA***A***-GGG-TTG-TTT-GAT-5' (horse, *Equus caballus*)
Oligonucleotides used in this study were obtained from BioCorp DNA Inc. (Dollard-des-Ormeaux, Quebec, Canada) and were of HPLC purity. Immediately after receiving, oligonucleotides were resuspended in freshly prepared 20 mM Tris-ClO_4 (pH 8.6) and 1 mM EDTA to a concentration of 100μM by incubating at 65°C for 10 minutes. Next, equal volumes of modified strand (disulfide) and unmodified strand were combined, heated to 65°C for 5 minutes, cooled down and kept at room temperature for 2 hours. Resulting hybrid solutions (50 μM duplex) were then divided into smaller aliquots which were stored at -30°C. As necessary sample aliquot s were removed from the freezer thawed and used.

5. Micro-array printing

Printing of the DNA micro-arrays was carried out using a spotting robot (SpotBot3, Telechem, Sunnyvale, CA) equipped with Megasonic Wash Station and a 50 μm pin (SMP2, Telechem). Freshly cleaned gold substrates were placed inside the printing chamber, and humidity inside the chamber was maintained between 85 – 95 % during the printing process. 10 % solution of freshly distilled ethanol was used as wash buffer. Detailed spotting parameters are listed below.
Pin configuration: 1x1
Spot spacing (center to center): 140 μm
Pre-print spots per sample: 10
Sample loading time: 5.0 s
Pre-print time: 0.0 s
Print time: 0.2 s
Number of wash/dry cycles: 5
Wash/dry duration: 2.0 s
Last cycle wash duration: 5.0 s
Last cycle dry duration: 10 s
This procedure allowed for depositing 0.5 nl of respective ds-DNA solutions (total 0.025 pico-moles of duplex) onto gold substrate. After printing was completed the substrates were placed on top of a moist filter paper inside a Petri dish. The Petri dish was then wrapped with Parafilm and incubated for 72 h at 5 °C. This resulted in the formation of films consisting of

ds-DNA and hydroxyalkyl groups tethered to the gold surface. The presence of the hydroxyalkyl groups minimizes non-specific interaction of ds-DNA with the gold surface. Incubation of the samples in Zn^{2+} solution was done directly in the SECM cell. The cell was filled with 0.3 $Zn(ClO_4)_2$ in 20 mM tris-ClO_4 and incubated at 5 °C for 15 h.

6. Preparation of the SECM tip electrodes

SECM probe electrodes used in this work were custom made. Briefly, 25 μm dia. Pt electrodes were obtained by placing 25 μm dia. Pt wire (99.95%, Alfa Aesar, MA, USA) into a micropipette pulled from a glass capillary 1.5/0.84 mm OD/ID (World Precision Instruments, Inc., FL, USA) using the micropipette puller (PP-83, Narishige, Japan). Next, the Pt wire was sealed under vacuum using the heating coil of the puller. The tip of the sealed micropipette was then polished flat to expose the Pt disk. Surface of the electrode was polished using rotating alumina lapping discs (3.0, 0.3 μm, World Precision Instruments, Inc., FL, USA). Finally, the size of the glass sheath surrounding the disc electrode was reduced by manual polishing with a rotating polishing pads (3.0 and 0.05 μm) in order to minimize the electrode RG parameter (the ratio of the radius of the sealing glass to the radius of the Pt wire). The obtained tip electrodes were evaluated under an optical microscope and tested by performing cyclic voltammetry and SECM approach curve experiments. Before use electrodes were cleaned electrochemically by cycling the potential applied to the electrode between the onset of hydrogen and oxygen evolution for minimum of 100 cycles. This procedure allowed obtaining tip electrodes with RG value between 2 and 3.

7. Scanning electrochemical microscopy

Approach curves. All approach experiments were carried out using stepper motor of the CHi-900b (piezo scanner was disabled) with tip electrode biased at 0.5 V vs Ag/AgCl. Typically, tip was approached towards the surface form initial tip-sample separation of 200 μm and stopped when tip current, I_T, increased to 130% ($I_T/ I_{T,\infty} = 1.3$) of its initial value, $I_{T,\infty}$. The approach was performed in 1μm step increments with 0.066667 s per increment. In the case of approach experiments where centers of individual DNA spots were approached the approach was stopped

when tip and sample came in to contact. Then the approach curves were corrected in such way that contacts point corresponds to zero separation distance.

SECM imaging. Imaging was initiated after approaching the sample as described above. The tip electrode was scanned above the sample in amperometric mode with tip potential at 0.5 V. The substrate electrode was not biased. Typically, the first scan was performed using 10 μm step increments (0.066667 s) to quickly locate the DNA array, then fine scan was acquired using 5 μ step increments (0.066667 s).

Determination of tip-sample separation. Positive feedback SECM theory was employed to estimate tip-sample separation, d. For $I_T/ I_{T,\infty} = 1.3$ separation distance was found to be about 15 μm. Fig. S1 shows experimental data recorded on approach towards clean gold surface (×) with 25 μm dia. Pt tip. The solid line represents calculated approach curve which was obtained using positive feedback SECM theory.[2] The fit between experiment and theory is very good, therefore, it was used to determine tip-to-sample separation distance and tip electrode RG factor (RG is defined as ratio of the insulator radius to the disk electrode radius). The best fit was obtained for theoretical curve calculated for RG = 2. Dashed line indicates tip-sample separation distance, d = 15 μm ($I_T/I_{T,\infty} = 1.3$), which was used as scanning distance in all SECM imaging experiments.

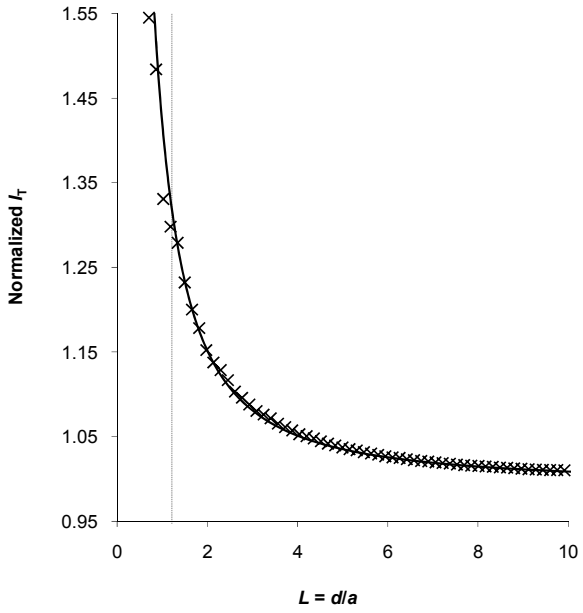

Fig. S1 Normalized approach curve recorded above Au substrate (×). Current normalized by $I_{T,\infty}$ and distance normalized by tip radius, *a*. Solid line represents simulated approach curve for RG = 2. Data obtained in 1 mM $K_4Fe(CN)_6$, 50 mM $NaClO_4$, 20 mM Tris-ClO_4 (pH 8.6), E_T = 0.5 V.

8. Data treatment and presentation

All original approach curve data, after converting to text format, was plotted is MS Excel 2007 and tip-sample separation distance was corrected assuming contact point corresponded to $d = 0$ μm. In all approach curves current was normalized by $I_{T,\infty}$ and distance by *a*.

Original SECM data files were converted to text files (matrix format) and reopend using Gwyddion software (Gwyddion is an Open Source software platform for visualization of scanning probe microscopy data, http://gwyddion.net). SECM images were then visualized using appropriate color map, rotated, flipped horizontally, cropped and exported to a bitmap format. Sample SECM image before visualization in Gwyddion software is shown in Fig. S2.

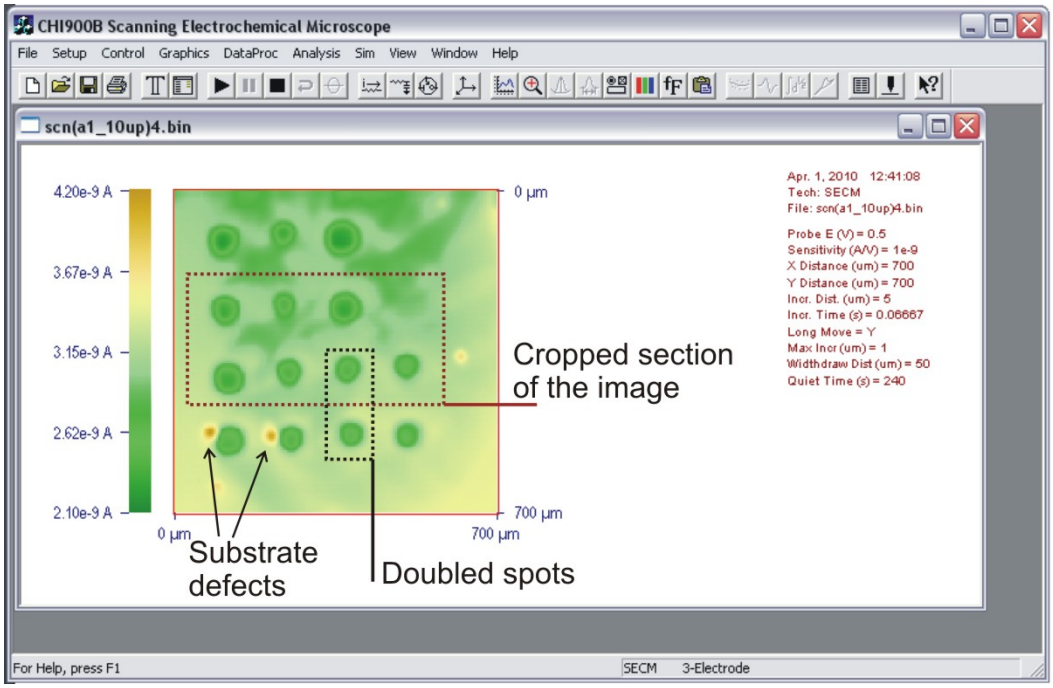

Fig. S2 Capture of the CHi-900b software window showing representative SECM image of the DNA micro-array prepared form animal strands. Array consisted of 7 different ds-DNA samples and each of the strands was spotted twice. Cropped section of the image and substrate defects are indicated in the image.

9. FEM simulation

Theoretical approach curves were obtained by means of numerical simulation. Based on the assumption of irreversible substrate kinetics, a steady-state diffusion problem for SECM geometry was solved numerically in dimensionless form by using the finite element method (COMSOL Multiphysics 3.5a software with Chemical Engineering Module) and following procedures described elsewhere.[3] This numerical simulation approach is advantageous over the frequently used analytical solutions, as it is difficult to accurately describe the complex SECM geometry with an exact analytical solution.[4, 5] SECM geometry used in FEM calculations is shown in Fig. S3.

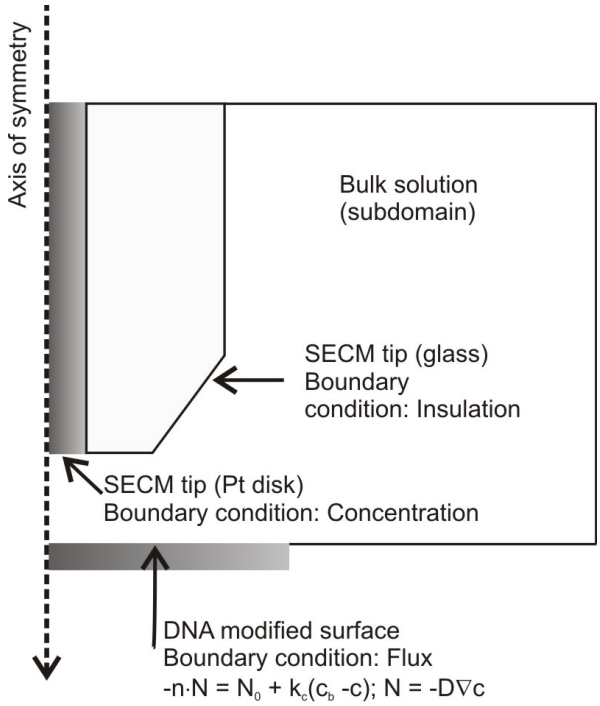

Fig. S3 Geometry of the SECM experiment in cylindrical coordinates used in FEM calculations. Drawing not in scale.

Example of the distribution of the $K_4[Fe(CN)_6]$ concentration in the tip-sample gap predicted by FEM simulation is shown in Fig. s4.

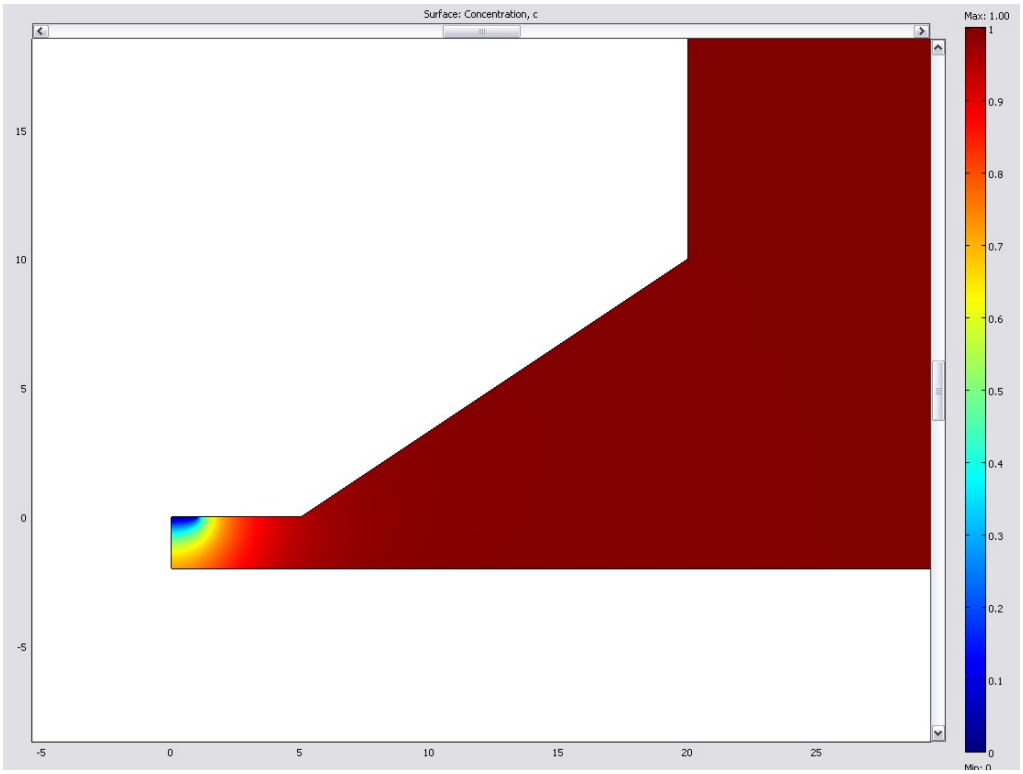

Fig. S4 Concentration distribution $K_4[Fe(CN)_6]$ in tip-substrate gap predicted using FEM simulation. Calculated for $\Lambda = 0.08$ and $L = 2$.

Numerical simulation allowed us to obtain theoretical approach curves for different values of a dimensionless rate constant, Λ, which can be described as:

$$\Lambda = k^0 a/D$$

where k^0 is the apparent rate constant, a is the tip radius and D is the diffusion coefficient. In this case, the apparent rate constant accounts for the combined contributions of electron transfer across the film and penetration of the redox mediator into the film.[4]

Diffusion coefficient for $[Fe(CN)_6]^{3-}$/ $[Fe(CN)_6]^{4-}$ $D = 3.47 \times 10^{-6}$ cm^2s^{-1} was determined from steady-state limiting current as given by:

$$i = 4nFDCa$$

where i is the measured current, n is the number of electrons, F is the Faraday constant, D is the diffusion coefficient and C is the concentration of the redox mediator and a is the radius of the electrode. Modeling the experimental data allowed us to assess the apparent rate constants for electron transfer across the DNA films.

10. Predicted rate constants

Apparent rate constants predicted for model DNA micro-array and micro-array prepared from animal sequences in the absence and presence of Zn^{2+} are summarized in Table S1.

Table S1 Apparent electron transfer rate constants in units of $cm \cdot s^{-1}$ determined in the absence and presence of Zn^{2+} for the model DNA microarray and the array utilizing animal sequences. Errors, SD over 5 replicates.

Strand	$k^0 \cdot 10^{-5}$ (no Zn^{2+})	$k^0 \cdot 10^{-4}$ (Zn^{2+})	Strand	$k^0 \cdot 10^{-4}$ (no Zn^{2+})	$k^0 \cdot 10^{-4}$ (Zn^{2+})
1+2	4.5 ± 0.5	1.2 ± 0.2	a1+a2	1.0 ± 0.1	1.2 ± 0.2
1+3	6.2 ± 0.6	3.7 ± 0.4	a1+a3	1.4 ± 0.1	2.1 ± 0.2
1+4	5.1 ± 0.6	1.4 ± 0.2	a1+a4	1.7 ± 0.2	3.0 ± 0.3
1+5	9.3 ± 0.9	5.1 ± 0.6	a1+a5	2.3 ± 0.3	5.8 ± 0.5
1+6	8.0 ± 0.8	7.1 ± 0.8	a1+a6	1.9 ± 0.2	3.7 ± 0.6
1+7	10 ± 1.2	7.5 ± 0.8	a1+a7	2.8 ± 0.2	7.7 ± 0.5
1+8	8.2 ± 0.9	4.4 ± 0.5	a1+a8	1.2 ± 0.1	1.7 ± 0.2
1+9	7.7 ± 0.8	0.2 ± 0.3	-	-	-
1+10	5.9 ± 0.7	0.2 ± 0.3	-	-	-
1+11	6.4 ± 0.8	0.2 ± 0.2	-	-	-
1+12	7.2 ± 0.8	0.2 ± 0.3	-	-	-

11. References

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