Supplementary information

An Unexpected Use of Ferrocene. A Scanning Electrochemical Microscopy Study of a Toll-Like Receptor Array and Its interaction with *E.coli*

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Experimental conditions

Materials. Gold/Silicon (Au/Si) was prepared by the Nanofabrication facility at University of Western Ontario (London, ON). Phosphate buffered saline (PBS) buffer (pH ~ 7.4) and aminoferrocene were bought from Sigma-Aldrich (Oakville, ON). Potassium ferrocyanide and sodium perchlorate was purchased from EM Science (Billerica, MA) and Alfa-Aesar (Ward Hill, MA) respectively. The recombinant mouse TLR1 (1476-TR-050), recombinant human TLR4/MD-2 (3146-TM-050/CF) and recombinant mouse TLR5 Fc Chimera (7915-TR-025) were obtained from R&D Systems (Minneapolis, MN). All aqueous solutions were prepared using deionized water (Millipore Milli-Q; 18 MΩ•cm resistivity). All reagents were used as received with no further modification unless otherwise stated within this manuscript. Milli-Q water was used throughout this study for all purposes including electrochemistry, sample solutions and rinsing. 1-Lipoic acid n-hydroxysuccinimide ester (LPA) and 2-aminoethylferrocenylmethylether were synthesized following published protocols[1-2].

Preparation of Au/Si modified with LPA. LPA solution (2mM) was prepared by dissolving the LPA into anhydrous ethanol. The Au/Si pieces (1 cm × 1 cm) were immersed in LPA ethanol solutions for 48 hours at 277 K, then removed, rinsed with ethanol thoroughly and blown dried using a stream of nitrogen gas.

Preparation of TLR1, TLR4, TLR5 and *Escherichia Coli K12* solutions. TLR1, TLR4 and TLR5 solutions were prepared according to the manufacturer's instruction by dissolving the receptors in PBS buffer (pH~7.4) and stored at 277 K. All TLR concentrations are 100 μg/ml. *Escherichia coli K12 (E. coli K12)* culture was provided by University of Toronto Scarborough Biology Teaching Laboratory. The bacteria were washed and resuspended into PBS buffer

(pH~7.4). The concentration of *E. coli K12* was calibrated using light scattering. The final concentration used in the experiment was 5×10^8 CFU/ml.

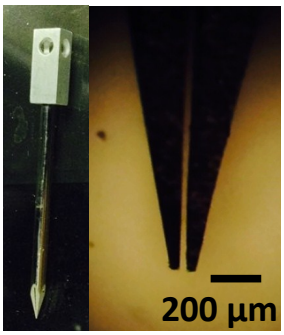

Figure S1. 946MP2 pin used in Arrayit Spotbot 3.

Preparation of microarrays of TLR1, TLR4 and TLR5. An Arrayit Spotbot 3 (Sunnyvale, CA) equipped with Megasonic Wash Station was used for creating the microarrays. Prepared TLR1, 4, and 5 solutions were loaded into the cells of the Arrayit microplates (Sunnyvale, CA). Deionized water was used as the wash buffer for the 946MP2 pin (Sunnyvale, CA), which is shown in Figure S1. The humidity was maintained at 85 – 95% during the spotting process. The detailed spotting conditions are listed below:

Pin configuration: 1x1

Spot spacing (center to center): 150 µm (TLR5) and 200 µm (TLRs)

Pre-print spots per sample: 10

Sample loading time: 10.0 s

Pre-print time: 0.0 s

Print time: 1.0 s

Number of wash/dry cycles: 5

Wash/dry duration: 3.0 s

Last cycle wash duration: 5.0 s

Last cycle dry duration: 10.0 s

After printing was completed the substrates were placed on top of a PBS moistened filter paper (pH~7.4) inside a Petri dish. The Petri dish was then wrapped with parafilm and incubated for 48 h at 278 K.

The substrates were then removed and rinsed thoroughly using deionized water and blown dried using nitrogen gas. The substrates were modified in the following step by immersion in 25 mM of 2-aminoethylferrocenylmethylether- or aminoferrocene-PBS solutions (pH~7.4) for 1 hour at 277 K, then removed, washed using deionized water and blown dried with a stream of nitrogen gas.

Scanning electrochemical microscopy (SECM) measurement. SECM experiments were carried out with a CHI-900b (CH Instruments, Austin, TX) at room temperature in an electrochemical cell using a three-electrode configuration. A platinum (Pt) wire, an Ag/AgCl/3.0M KCl electrode and a Pt SECM tip were fitted as the respective counter electrode, reference electrode and working electrode. Modified Au/Si substrates were mounted in the cell and used without any bias during the experiment. The SECM probe electrode was custom-made by sealing a 25 μm diameter Pt wire (99.95%, Alfa Aesar, MA, USA) into a micropipette, which is 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)[3]. The electrode was polished carefully to RG~5 using alumina lapping discs (3.0, 0.3 and 0.05 μm, World Precision Instruments, Inc., FL, USA). The electrode was cleaned before each experiment by sonication in water/ethanol (50:50) for 10 mins and running cyclic voltammetric scans in acid (H_2SO_4, pH~1) between 0 and 1.4 V for 100 cycles at scan rate of 0.5 V/s. The solution for the SECM measurement contained 2 mm $K_4[Fe(CN)_6]$ aqueous solution as the redox probe and 50 mM $NaClO_4$ as the supporting electrolyte. A steady current is obtained prior to each approach curve measurement or imaging. The imaging was carried out with 5 μm increment steps (0.066667s) at an applied potential of 0.5 V. The modified Au/Si substrates were not biased during the measurement.

COMSOL Multiphysics. The experimental approach curves were normalized to the steady-state current before fitting them against theoretical curves generated using COMSOL Multiphysics software.[4-6] The theoretical curves were calculated using means of numerical simulation with assumption of irreversible substrate kinetics. The steady-state diffusion for the SECM experiment was solved in dimensionless form using COMSOL Multiphysics. The simulation was carried out using consistent tip geometry and RG ratio to the experimental set up.

Subsequently, the reaction kinetics for the modified surfaces was estimated. The continuous and dashed lines are the approach curves shown in the Figure 1. b) are calculated using known values for the dimensionless rate constant (Λ). The normalized distance (L) is the ratio of the tip/substrate separation (d/a) to the tip radius. Rate constant, k^0, plots in Figure 1. c) are for the surface following each step of the surface modification process. These rate constants were calculated using dimensionless rate constants Λ values estimated by contrasting the experimental approach curve data against the calculated approach curves.

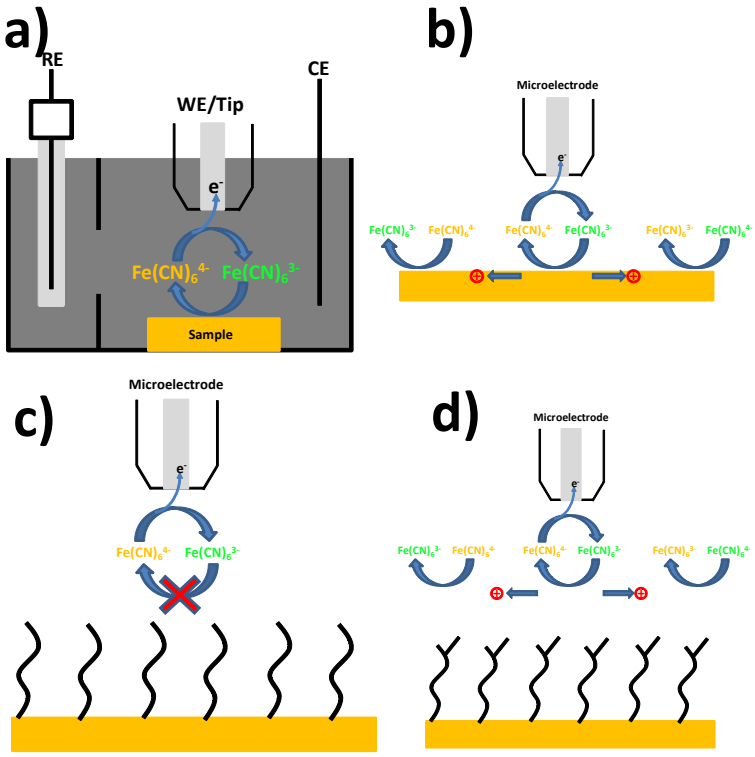

Figure S2. a) An illustration of the SECM set up; mechanisms of b) positive current feedback on the bare gold surface; c) negative current feedback on the gold surface modified with LPA; and d) positive current feedback on gold modified with LPA and a ferrocene derivative.

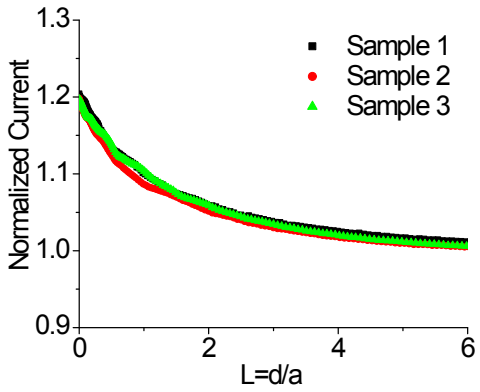

Figure S3. Three approach curves obtained from three samples of surfaces modified with aminoferrocene.

Reference:

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