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Supporting Information

A Biomimetic Phosphatidylcholine-Terminated Monolayer Greatly Improves the In Vivo Performance of Electrochemical Aptamer-Based Sensors

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Materials and methods.

Materials. PC-terminated hexane thiol was synthesized according to previous literature. [1] Kanamycin was purchased from Gold Biotechnology (MO, USA) and used as received without further purification. Bovine whole blood was purchased from Hemostat Laboratories (CA, USA) and used as is. Circulator pump for whole blood experiments was purchased from Cole-Parmer (IL, USA). Gold wire (0.2 mm diameter) and tungsten wire (0.2 mm diameter) for electrode fabrication were purchased from Sigma-Aldrich and Goodfellow Cambridge Limited, respectively. Conductive silver epoxy adhesive was obtained from ITW Chemtronics. Teflon tubes used as insulation were purchased from Zeus Industrial Products. Oligonucleotides were synthesized by LGC Biosearch Technologies, purified by C18 HPLC and PAGE, confirmed by HPLC profile and mass spectrometry. The sequences used in this study were:

Doxorubicin aptamer:

5'-HO-$(CH_2)_6$-S-S-$(CH_2)_6$-ACCATCTGTGTAAGGGGTAAGGGGTGGT-$(CH_2)_7$-(MB)-3'

Aminoglycoside aptamer (C6-linker or C11-linker aptamer with n being either 6 or 11, respectively):

5'-HO-$(CH_2)_n$-S-S-$(CH_2)_n$-GGGACTTGGTTTAGGTAATGAGTCCC-$(CH_2)_7$-(MB)-3'

Sensor Fabrication. Gold wire electrodes were made using the following procedure:

the wire is composed of two parts: gold wire (5-6 mm, 0.2 mm diameter) and tungsten wire (8 cm, 0.2 mm diameter). These two components were first cold soldered using electrically conductive silver epoxy adhesive. The tungsten wire was then insulated using a heat shrinkable teflon tubing leaving the gold wire exposed for DNA modification and 1 cm tungsten wire at the other end for connection to the potentiostat. Following the insulation step, the gold portion of these was electrochemically roughened in order to increase the surface area. Briefly, the sensors were immersed in 0.5 M sulfuric acid and rapidly pulsed between $E_{initial}$ = 0.0 V to E_{high} = 2.0 V vs Ag/AgCl for 400,000 times with each pulse being of 2 ms duration. All the probe oligonucleotides were dissolved with Tris buffer to a final concentration of 100 μM, aliquoted and stored at -20°C ready for use. To fabricate our sensors, the freshly cleaned electrodes were immersed in 200 nM DNA solution for 1 hr at room temperature, which was previously prepared by incubating a solution of 100 μM thiolated DNA and 20 mM tris-(2-carboxyethyl) phosphine hydrochloride (1 : 200) for 1 hour at room temperature, and further diluted by phosphate buffered saline (137mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, pH 7.0). The resulting sensors were washed with deionized water and then incubated in 20 mM 6-mercaptohexanol solution overnight at 4°C. The functionalized sensors were then rinsed with pure water prior to use.

Electrochemical measurements Electrochemical measurements were performed at room temperature using a CHI630C potentiostat with a CHI684 Multiplexer (CH

Instruments, Austin, TX) and a standard three-electrode cell containing a platinum counter electrode and an Ag/AgCl (3M NaCl) reference electrode. Square wave voltammetry (SWV) was performed using a potential window of -0.1 to -0.4 V (versus Ag/AgCl), 1 mV potential step, 50 mV amplitude and 60 Hz frequency. For the stability experiments in flowing whole blood, the sensors were scanned every 10 s. All the experiments were conducted in a closed-loop system with a continuous flow of whole blood (1 mL/s) using a circulator pump to mimic circulation in the vasculature.[2] The sensors after target response experiments were regenerated by washing with fresh blood (3×).

Optical imaging All optical imaging data were obtained via Microscope Eclipse E600 (Nikon) with a 20× objective. Gold films were used for optical imaging measurements other than our wire electrodes because the roughness of the wire surface rendered it difficult to see blood cell adhension. All the samples were prepared by standard protocol as sensor fabrication, followed by incubation in whole blood for 12 hr. After incubation, the samples were rinsed by H_2O for three times and incubated in water for 10 min before imaging.

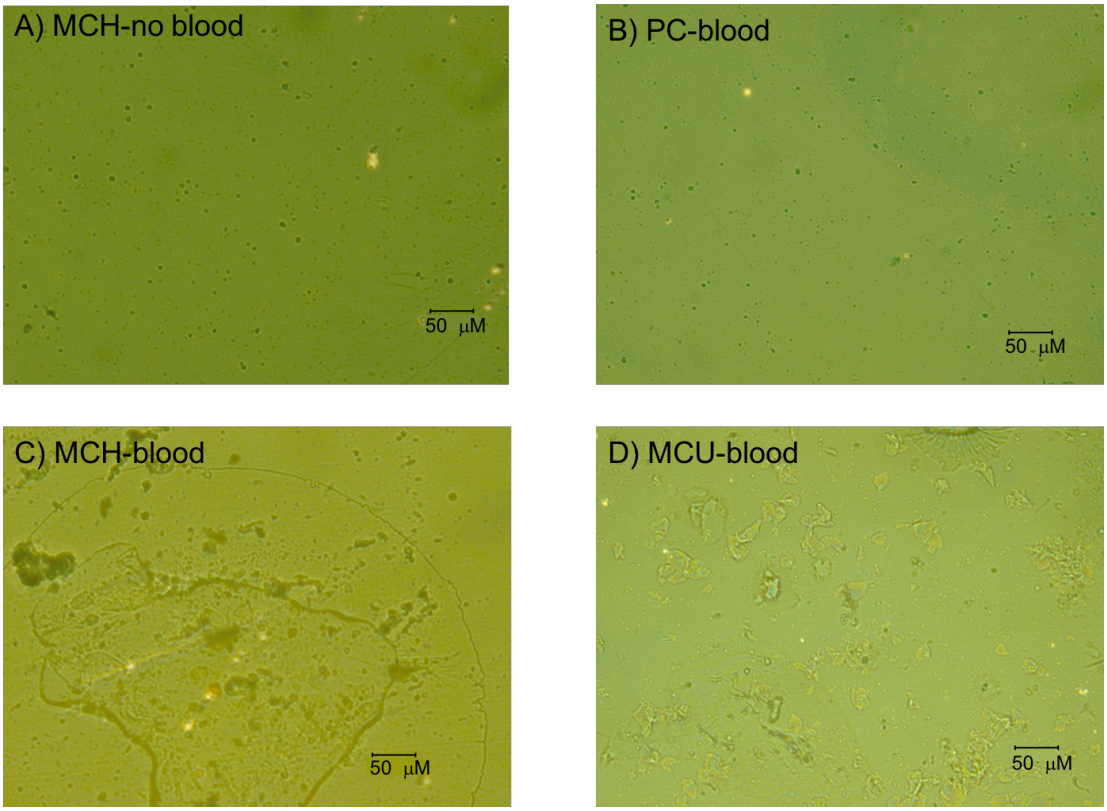

Figure S1. Optical micrographs of flat gold surfaces indicate the fouling resistance of PC SAMs. Shown are a gold film coated with a MCH monolayer prior to incubation in whole blood as well as PC-, MCH-, MCU-monolayers after incubation in whole blood for 12 hr. We employed flat gold films here rather than our wire electrodes because the roughness of the wire surface rendered it difficult to see blood cell adhesion.

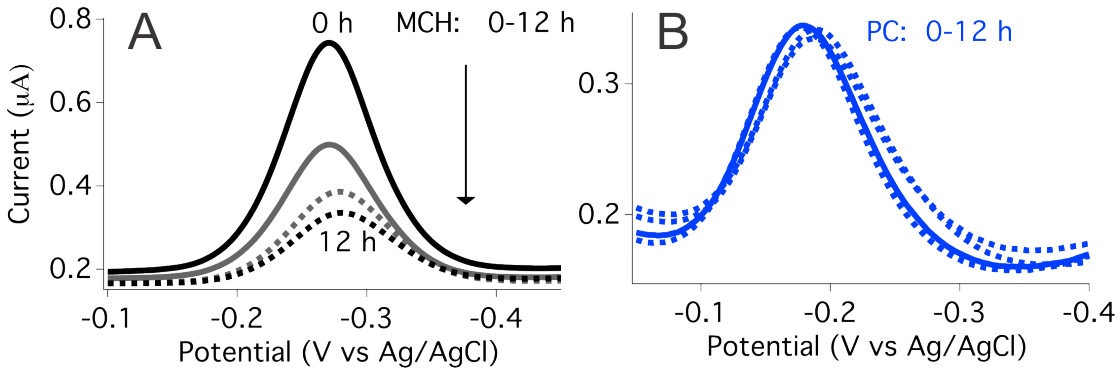

Figure S2. A biomimetic self-assembled monolayer (SAM) improves the performance of a doxorubicin-detecting E-AB sensor in vitro in flowing whole blood. (A) Voltammograms collected from MCH-based sensors placed in flowing whole blood in vitro in the absence of target illustrate the dramatic drift seen under these conditions. (B) PC-based sensors, in contrast, are quite stable under the same conditions.

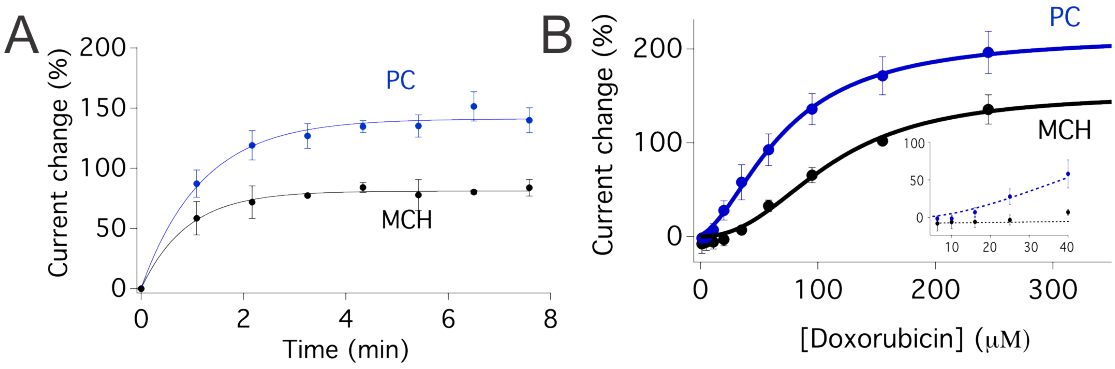

Figure S3. The target response of doxorubicin-detecting sensors in whole blood. (A) Both PC-based and MCH-based doxorubicin sensors respond rapidly to their target, with 90% of the signal change occurring within ~2 min. (B) Equilibrium binding curves indicate that PC-based sensors respond to target with somewhat greater signal gain. The error bars shown in this figure and the following figures represent the standard deviation of at least three measurements obtained from independently fabricated sensors.

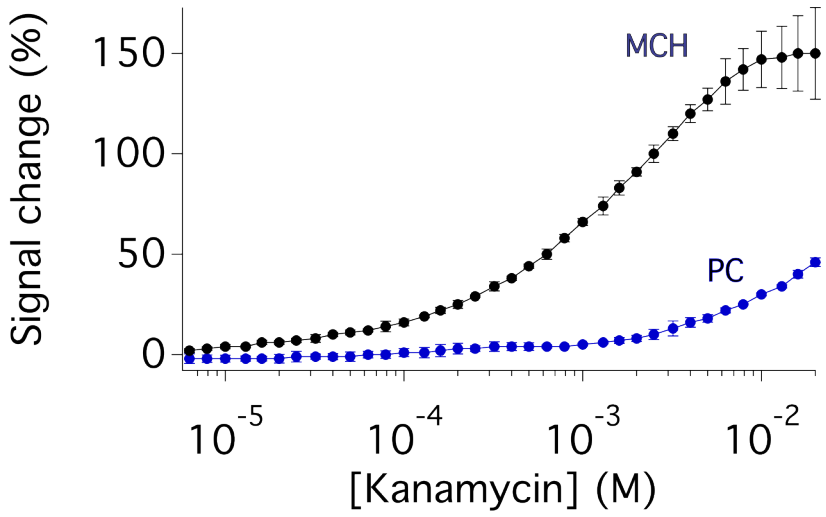

Figure S4. Dose-response curve of an E-AB kanamycin-detecting sensors built using a C6 linker on the aptamer. The performance of such a PC-based sensor is poor relative to that of the analogous MCH-based sensors, presumably because the stem of this aptamer, which is involved in target binding, is buried in the thick PC monolayer.

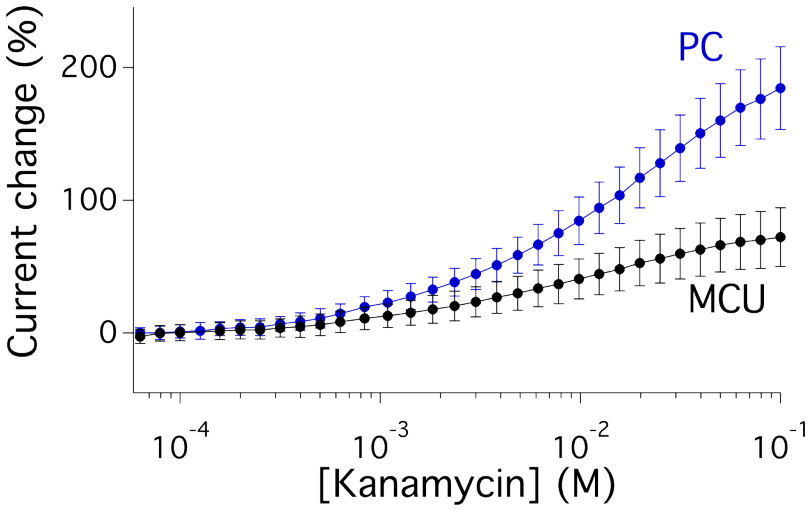

Figure S5. Dose-response curve of an E-AB kanamycin-detecting sensors built using a C11 linker on the aptamer. The performance of such a PC-based sensor is significantly better than that of the analogous MCH-based sensors (see discussion in text).

Reference:

1. Goda, T.; Tabata, M.; Sanjoh, M.; Uchimura, M.; Iwasaki, Y.; Miyahara, Y. *Chem. Commun.* **2013**, *49*, 8683-8685.
2. H. Li, N. Arroyo-Currás, D. Kang, F. Ricci, K. W. Plaxco, *J. Am. Chem. Soc.* **2016**, *138*, 15809-15812.