Ternary Surface Monolayers for Ultrasensitive (Zeptomole) Amperometric Detection of Nucleic-Acid Hybridization without Signal Amplification

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Table S-1. Oligonucleotides used in this study.

Oligonucleotide	Sequence (5'→3')
Thiolated Capture Probes (SHCP)	
EC SHCP	Thiol-TAT TAA CTT TAC TCC
UNI SHCP	Thiol-GTT CCC CTA CGG TTA CCT T
Detector Probes (DP)	
EC FITC-DP	CTT CCT CCC CGC TGA-FITC
UNI FITC-DP	GTT ACG ACT TCA CCC CAG-FITC
*Complementary EC target**	TCA GCG GGG AGG AAG GGA GTA AAG TTA ATA
Non-complementary EC target	CTG GGG TGA AGT CGT AAC AAG GTA ACC GTA GGG GAA C
2-Base mismatched EC	TCA GCG GGG AGG AAG GGA GTC AAG TGA ATA
3-Base mismatched EC	TCA ACG AGG AGC AAG GGA GTA AAG TTA ATA

*The sequence of the 30-mer complementary DNA EC target is a copy of partial region of the *E. coli* 16S rRNA gene (position 432-461 according to the 5'→3' nucleotide sequence).

The *E. coli* probe pair (EC SHCP and EC FITC-DP) was designed to be fully complementary to both synthetic target EC DNA and the partial region of the *E. coli* 16S rRNA targets.[S1]

The universal probe pair (UNI SHCP and UNI FITC-DP) detects *Citrobacter freundii*, *Enterobacter aerogenes*, *Escherichia coli*, *E. faecium*, *E. cloacae*, *Enterococcus faecalis*, *Proteus mirabilis*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Morganella morganii*, *Staphylococcus aureus*, *S. saprophyticus.*[S1]

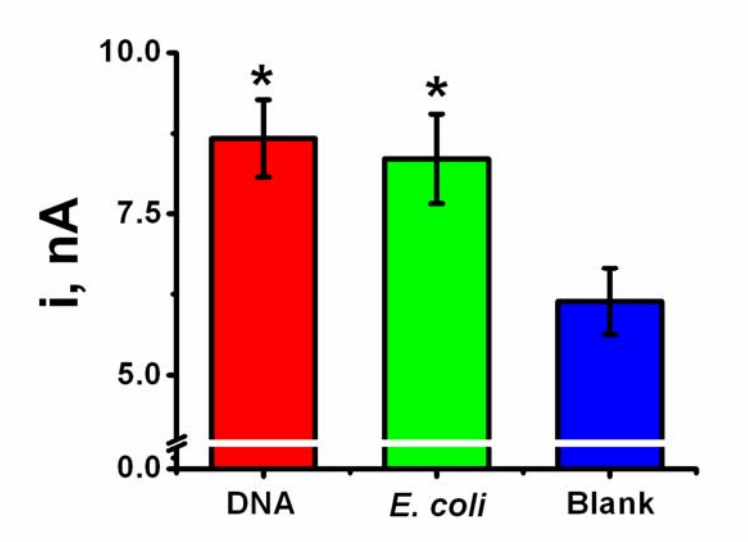

Figure S-1. Detection limit of the assay for EC target DNA and *E. coli* rRNA. Column graph corresponding to the chronoamperometric signals obtained after hybridization with 10 fM of target EC DNA (red column), 1 CFU/sensor (green column) and 0 nM target DNA (blue column). Error bars estimated from five parallel experiments. *Indicates a significance value of $P < 0.005$. Chips modified with the EC SHCP/DTT + MCH monolayer.

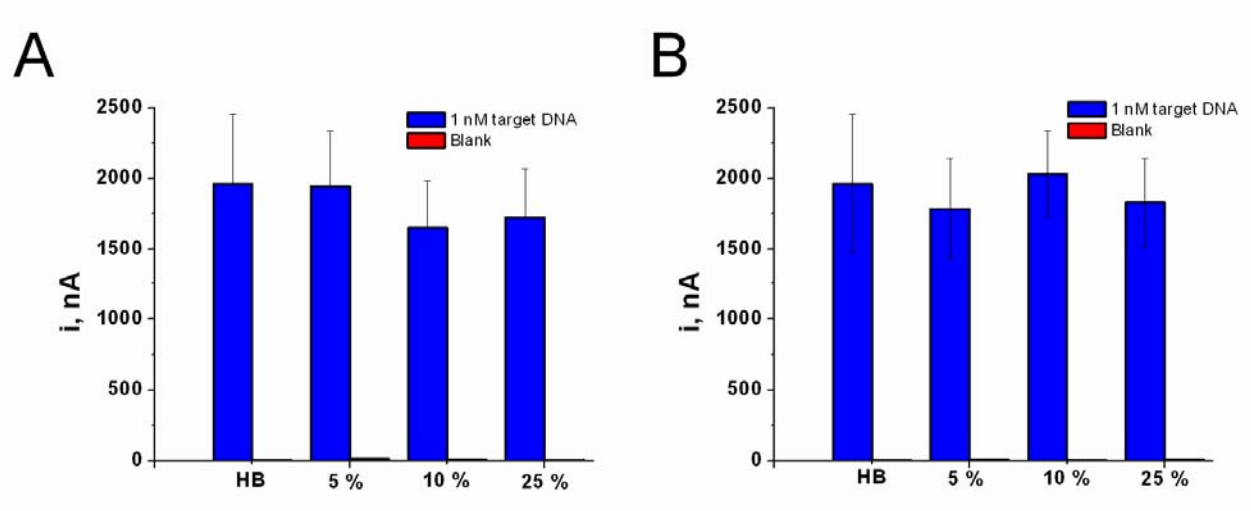

Figure S-2. Non-fouling behavior of the EC SHCP/DTT+MCH-based sensor. Comparison of the detection of 1 nM target DNA (blue columns) and the corresponding blank (0 target DNA) signals (red columns) spiked in pure buffer (HB) and human-serum (A) and human-urine (B) samples diluted to different percentages by HB buffer.

Application for identification of real uropathogenic clinical isolates. Clinical isolates of the following uropathogens were obtained from the UCLA Clinical Microbiology Laboratory with approval of the UCLA Medical Institutional Review Board and undoubtedly identified by conventional culture-based assays: *Escherichia coli* (EC139, EC103, EC71 and EC28), *Klebsiella pneumoniae* (KP295 and KP243), *Pseudomonas aeruginosa* (PA291 and PA98), *Enterobacter aerogenes* (EA368 and EA99), *Proteus mirabilis* (PM291 and PM351), *Serratia marcescens* (SM070 and SM068), *Enterobacter hormaechei* (EH367 and EH151) and *Acinetobacter baumannii* (AB1505 and AB028). The isolates were received in vials containing brucella broth with 15% glycerol (BBL, Maryland) and were stored at -70°C. Overnight bacterial cultures were freshly inoculated into Luria broth (LB) and grown to logarithmic phase as measured by the optical density at 600 nm. Concentrations in the logarithmic-phase specimens were determined by serial plating, typically yielding 10^7 to 10^8 bacteria/ml. The uropathogens grown in LB were stored as frozen pellets at -70°C until the time of experimentation.

The new interface was applied to the determination of these 18 clinical isolates. For these analysis, both the UNI and EC capture probes (defined in Table S1) were tested in duplicates and each clinical isolate pellet was lysed by resuspension in 10 μL of 1 M NaOH and incubation for 5 min. A 50 μL aliquot of both detector probes (EC and UNI FITC-DP, 0.25 μM) in HB was added to this 10 μL bacterial lysate and only 4 μL of the resulting 60 μL lysate-probe mixture were cast on each capture-probe modified sensor and incubated for 15 min, followed by the same capture of Anti-FITC-HRP and the electrochemical detection steps, described in the DNA hybridization assay section.

As illustrated in Figure S-3 the evaluation with 18 uropathogenic clinical isolates has revealed that the new platform (based on EC SHCP/DTT + MCH monolayer) is highly specific in identifying and discriminating only the *E. coli* isolates (EC139, EC103, EC71 and EC28) from closely related uropathogens. As expected, for the species specificities of the capture probe used[S1] all clinical isolates tested showed hybridization signals at the UNI SHCP-modified platform.

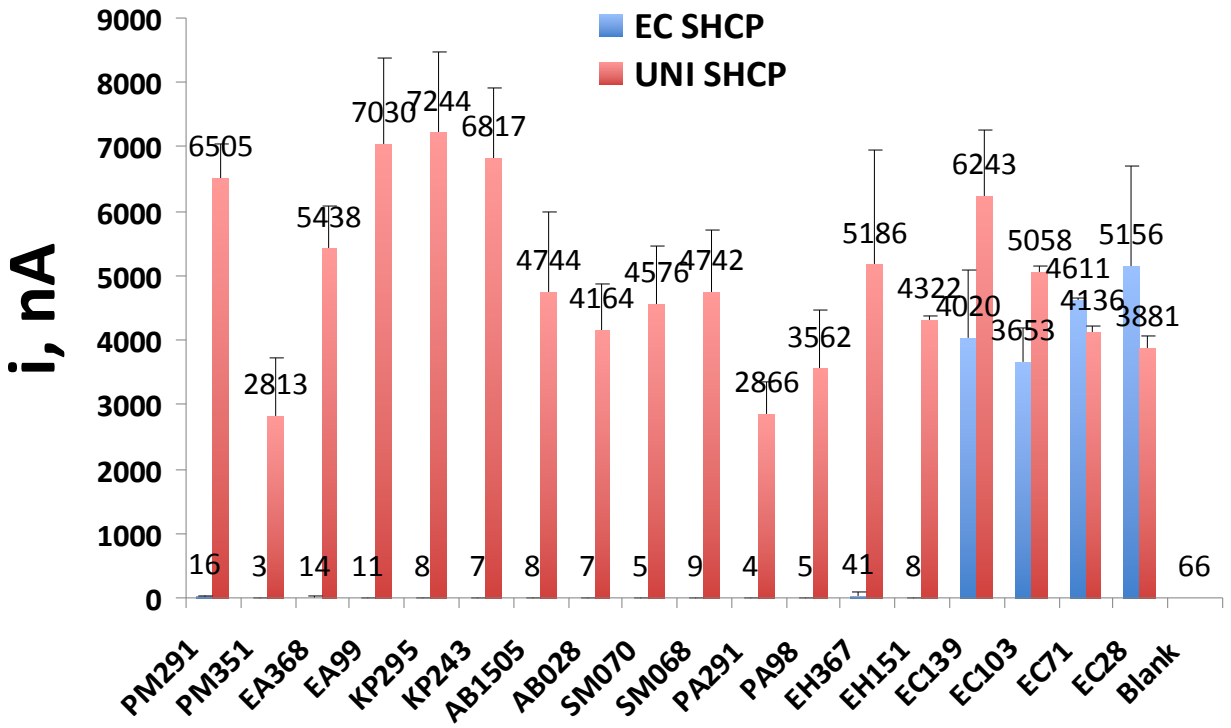

Figure S-3. Column graph corresponding to the chronoamperometric signals obtained using the ternary monolayer sensor interface after hybridization with 0 nM target DNA (Blank) and each of the 18 clinical isolates tested. Results obtained over chips modified with EC SHCP/DTT + MCH monolayers (blue columns) or UNI SHCP/DTT + MCH monolayers (red columns). Error bars estimated from two parallel experiments.

REFERENCE

(S1) Liao, J.C.; Mastali, M. ; Gau, V.; Suchard, M. A.; Møller, A. K.; Bruckner, D. A.; Babbitt, J. T.; Li, Y.; Gornbein, J.; Landaw, E. M.; McCabe, E. R. B.; Churchill, B. M.; Haake, D. A. *J. Clin. Microb.* **2006**, *44*, 561-570.