Electronic Supplementary Information

# An Amplification-Free Ultra-Sensitive Electrochemical CRISPR/Cas Biosensor for Drug-Resistant Bacteria Detection

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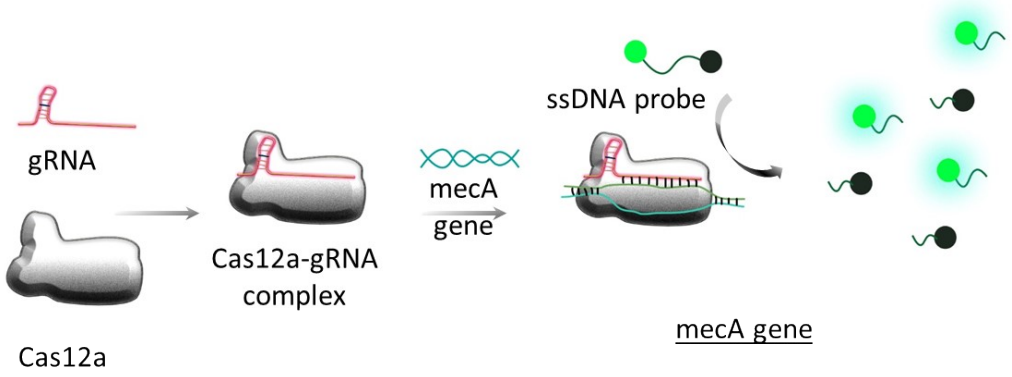


**Scheme S1** Schematic of the fluorometric assay for the CRISPR/Cas biosensor.

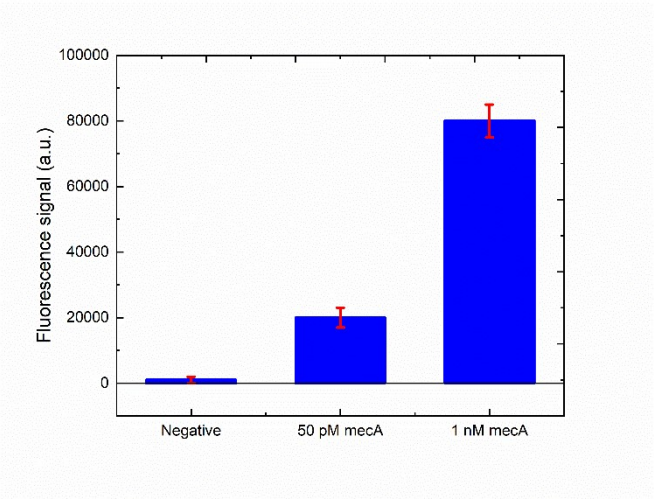


**Figure S1**. Fluorometric signal of 1 nM and 50 pM *mecA* compared to the negative control (i.e. without the *mecA* gene target).

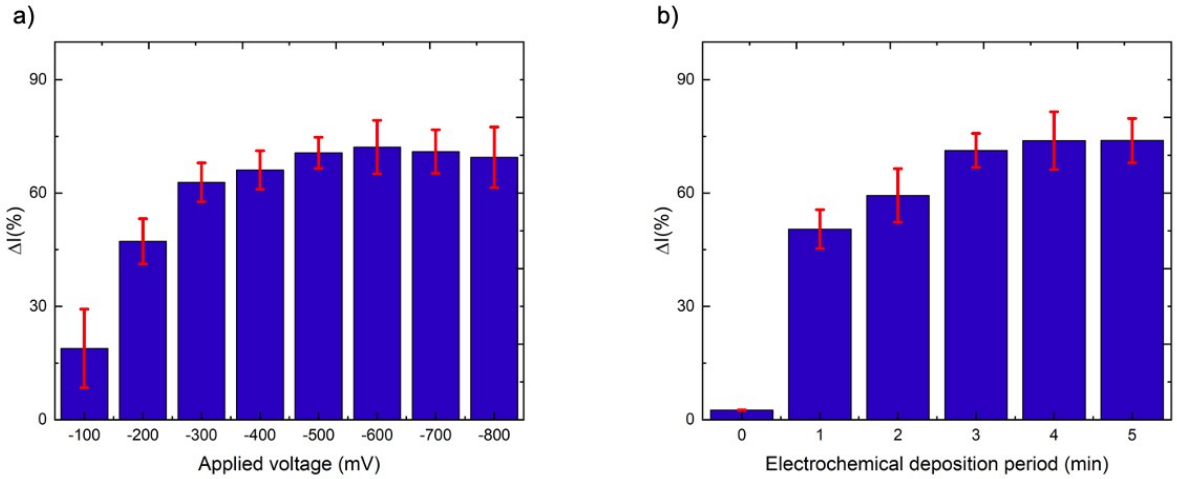


**Figure S2** Electrochemical reduction optimization for E-Si-CRISPR as function of (a) applied voltage (-100 to -800 mV), and (b) electrodeposition time (0 to 5 minutes).

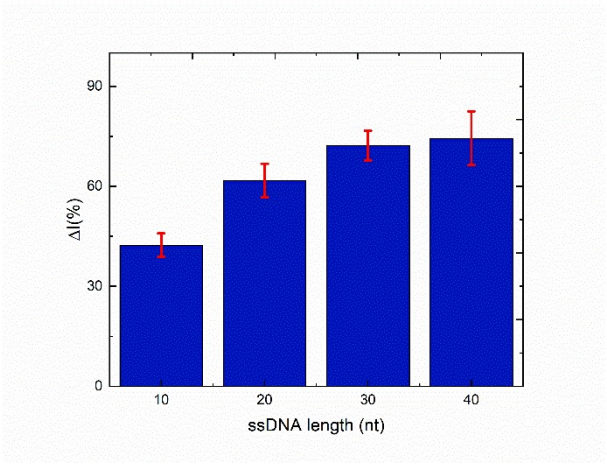


**Figure S3** Thiolated ssDNA length optimization on the electrode surface for E-Si-CRISPR, studied from 10 to 40 nt.

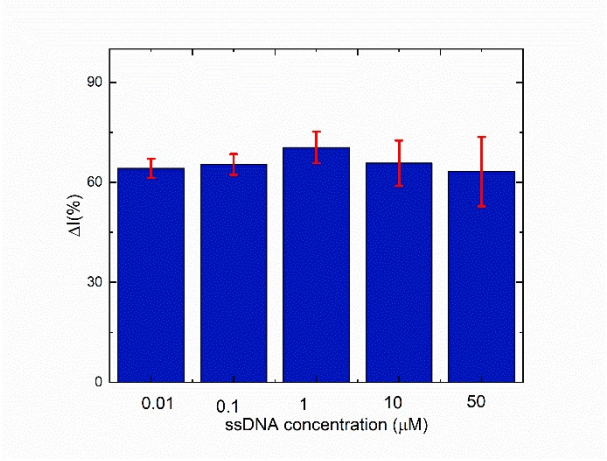


**Figure S4** Thiolated ssDNA concentration optimization on electrode surface for E-Si-CRISPR, studied from 0.01 to 50 μM. The ssDNA was modified and the surface passivated by MCH later.

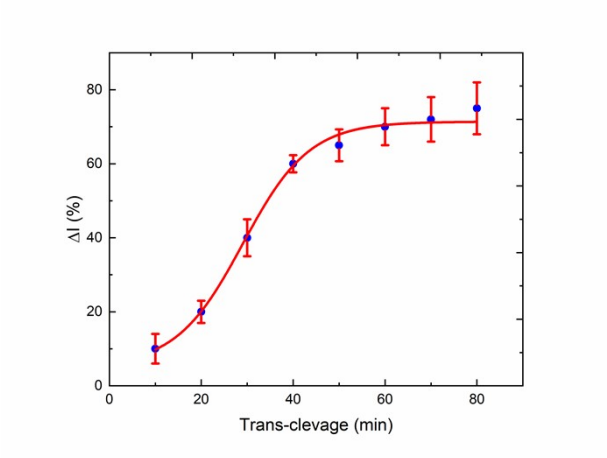


**Figure S5** Trans-cleavage period optimization of the lysed MRSA for E-Si-CRISPR, studied from 10 to 80 minutes.

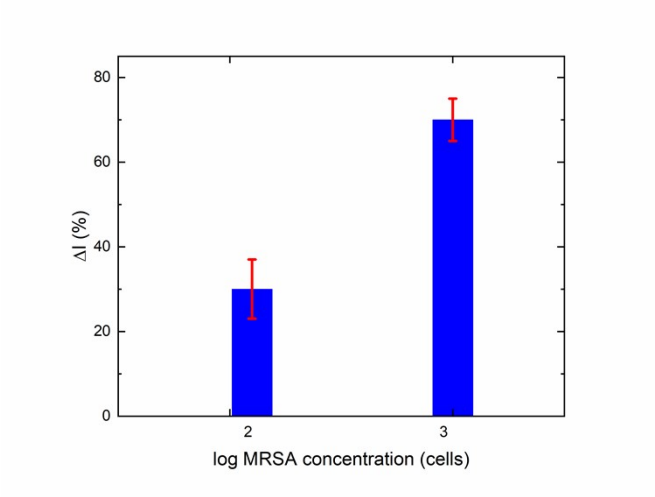


**Figure S6** Electrochemical signal of spiked MRSA cells in human serum with concentration of ca. $10^2$ and $10^3$ cells per detection using optimized condition as used in Figure 5b.

**Table S1**. PCR primers for *mecA* amplification, and the oligonucleotides for E-Si-CRISPR

| Name | Oligonucleotide Sequence |
|---|---|
| LF | 5' AGATTGGGATCATAGCGTCAT 3' |
| LB | 5' TTGAGGGTGGATAGCAGTACC 3' |
| up mec A | 5'-TCT TCA TGT TGG AGC TTT TTA TCG TAA A-3' |
| down mecA | 5'-TTT ACG ATA AAA AGC TCC AAC ATG AAG A-3' |
| mmPAM | 5' TCTTCATGTTGGAGCTTTTTATCG TATA-3' |
| mmT1 | 5' TCTTCATGTTGGAGCTTTTTATCT TAAA-3' |
| mmA5 | 5' TCTTCATGTTGGAGCTTTTAATCG TAAA-3' |
| mmG10 | 5' TCTTCATGTTGGAGGTTTTTATCG TAAA-3' |
| 10nt | 5' AAAAAAAAAA-3' |
| 20nt | 5' AAAAAAAAATTAAAAAAAAA-3' |
| 30nt | 5' AAAAAAAAAAAAAAATTTAAAAAAAAAAAA-3' |
| 40nt | 5' AAAAAAAAAAAAAAAAAAAATTAAAAAAAAAAAAAAAAAA-3' |
| FAM Probe | FAM-TTAATT-BHQ1 |
| gRNA | 5'-uaa uuuu cau cua agu gua gaucga uaa aaa gcu cca aca ug-3' |

**Table S2**. A comparison of reported electrochemical techniques for CRISPR/Cas biosensors.

| Year | Enzyme | Signal Amplification | Target | LoD | Electrochemical method | Assay Time (h) | Ref |
|---|---|---|---|---|---|---|---|
| **2019** | Cas12a | - | HPV-16 | 50 pM | SWV | 2 | 1 |
| **2019** | Cas13a | HRP/AntiFAM | miRNA-19b | 10 pM | CA | 4 | 2 |
| **2021** | Cas13a | Catalytic hairpin assembly | miRNA-21 | 2.6 fM | DPV | 2 | 3 |
| **2020** | Cas12a | Catalytic hairpin assembly | HPV-16 and -18 | 30 pM | DPV | 1.5 | 4 |
| **2021** | Cas12a | Rolling circle amplification | ATP | 0.46 pM | SWV | 5 | 5 |
| **2021** | Cas13a | Catalytic hairpin DNA circuit | NSCLC-related RNAs | 0.5 fM | SWV | 1 | 6 |
| **2021** | Cas12a | Recombinase-assisted amplification | Listeria monocytogenes | 0.68 aM | SWV | 1.5 | 7 |
| **2021** | Cas12a | - | MRSA | 3.5 fM | SWV | 1.5 | This work |

*SWV: square wave voltammetry, CA: chronoamperometry, DPV: differential pulse voltammetry*

**References**


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