SUPPORTING INFORMATION (SI)

Real-time electrochemical detection of pathogens DNA using electrostatic interaction of a redox probe

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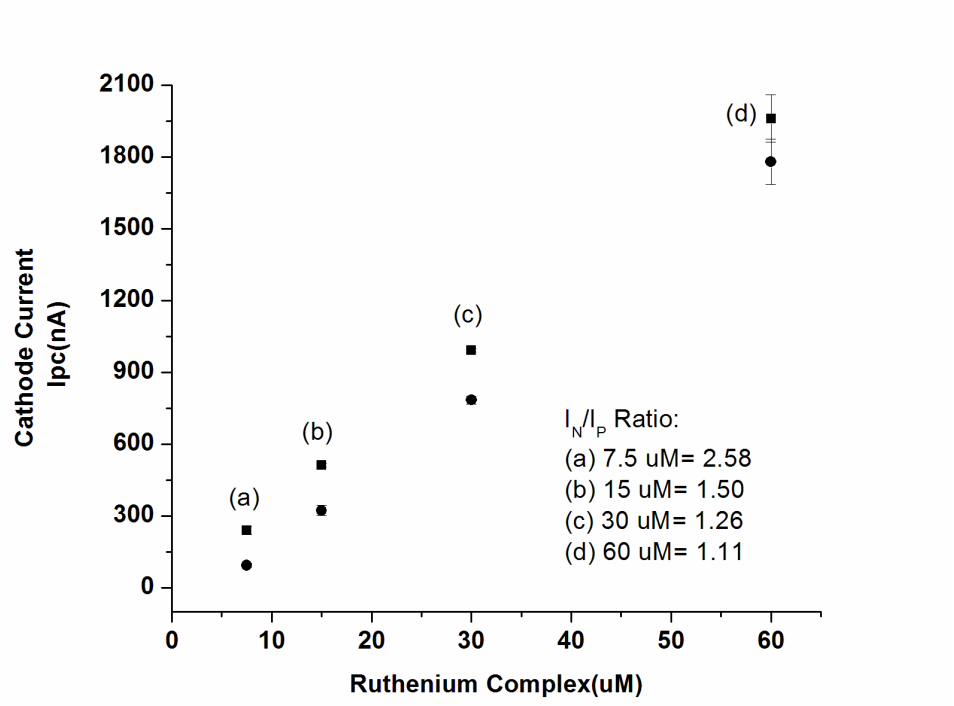
Supplementary Information

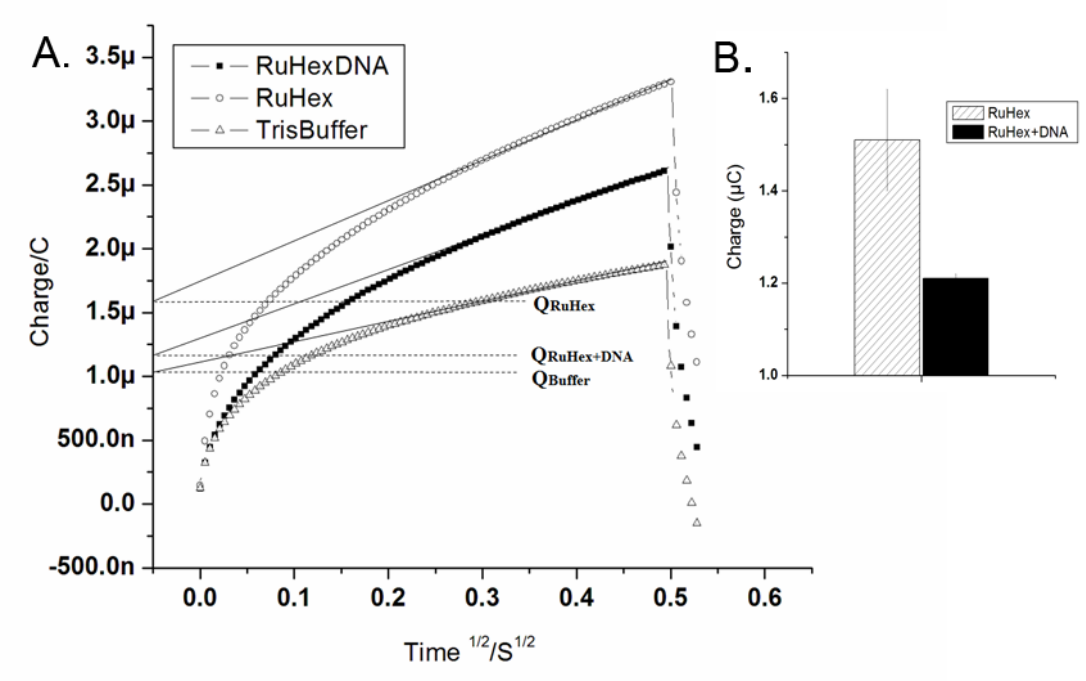
S1.



S1: Optimization of the RuHex concentration (7.5-60 µM) in the presence (P) and absence (N) of dsDNA in 10 mM tris buffer (pH 7.4) using type A electrode. Nevertheless, the 7.5-30 µM RuHex was convenient and reasonable, but we have chosen 15 µM for its appropriate and sufficient signal acquisition without any inhibition of isothermal amplification over time. Error bars indicate the standard deviation of at least three replicated measurements.

Supplementary Information

S2.

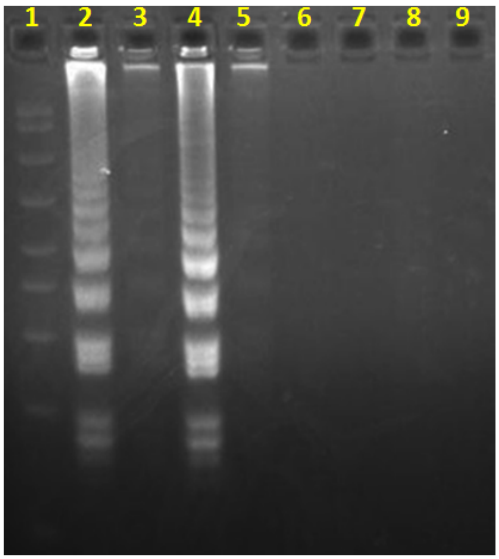


S2: A. Chronocoulometry response curves to observe the signal intensity with RuHex (Q_{RuHex}) and RuHex-dsDNA (10 ng/µL) complex ($Q_{RuHex+DNA}$) in solution using 15 µM of RuHex in Tris-buffer. The dotted lines represent the fit to the data used to determine the intercept at t=0. B. Signal of the electrode in

presence and absence of DNA. The measurement conditions were same as with A. Error bars show the standard deviations of measurements taken from at least three independent experiments.

Supplementary Information

S3.



E.Coli LAMP based amplicons (at 40 min.):

Lane 1: Marker (50-2000 bp)
Lane 2: 1x PC (1000fg/µl)
Lane 3: 25x PC (1000 fg/µl)
Lane 4: 1x PC (100fg/µl)
Lane 5: 25x PC (100fg/µl)
Lane 6: 1x NTC (H_2O)
Lane 7: 25x NTC (H_2O)
Lane 8: 1x NC (Staph100fg/µl)
Lane 9: 25x NC (Staph100fg/µl)

1 ×= Undiluted 5 µL gel loaded LAMP product
25×= 25× diluted 5 µL gel loaded LAMP product

S3: An example of *E. Coli* LAMP based amplicons as observed with 1× and 25× dilution on agarose gel (lane 2-9). Although, 5 µL loaded sample with 25× dilution (lane 3 and 5) shows faint band, but our SWV

based observation shows clear current differences (see fig 4B) at 40 min. to distinguish the same diluted products. The high sensitive end point amplicon detection shows promising impact on our real-time electrochemical sensing with RuHex in the shortest possible time. Staph 100 fg/µL of gDNA was used to show cross reactivity. Agarose gel electrophoresis (2%) was performed with 1×TBE buffer.

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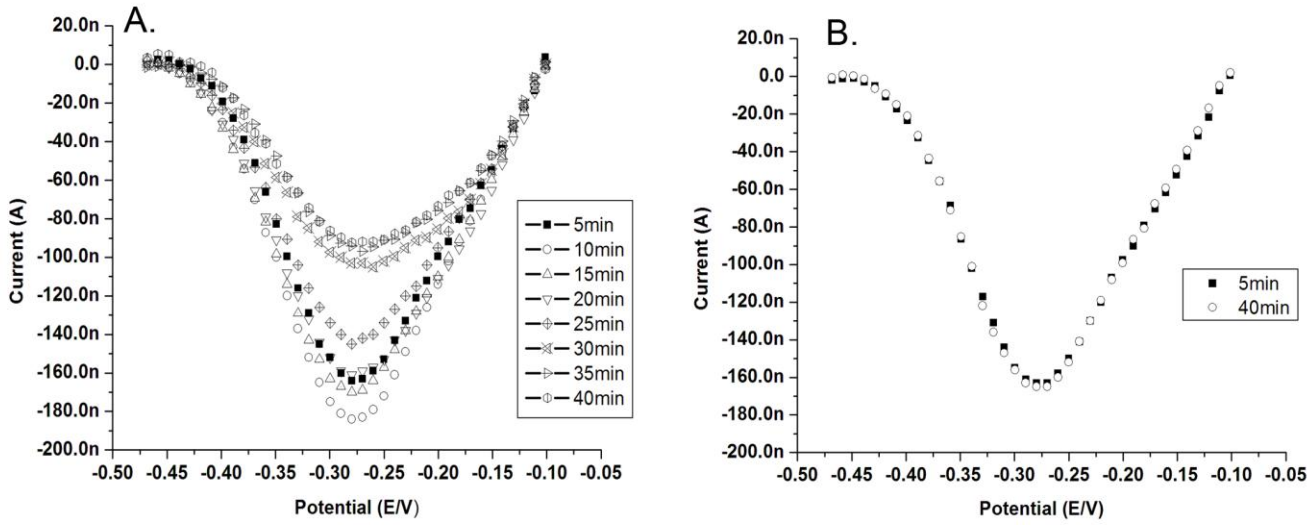
Supplementary Information

S4

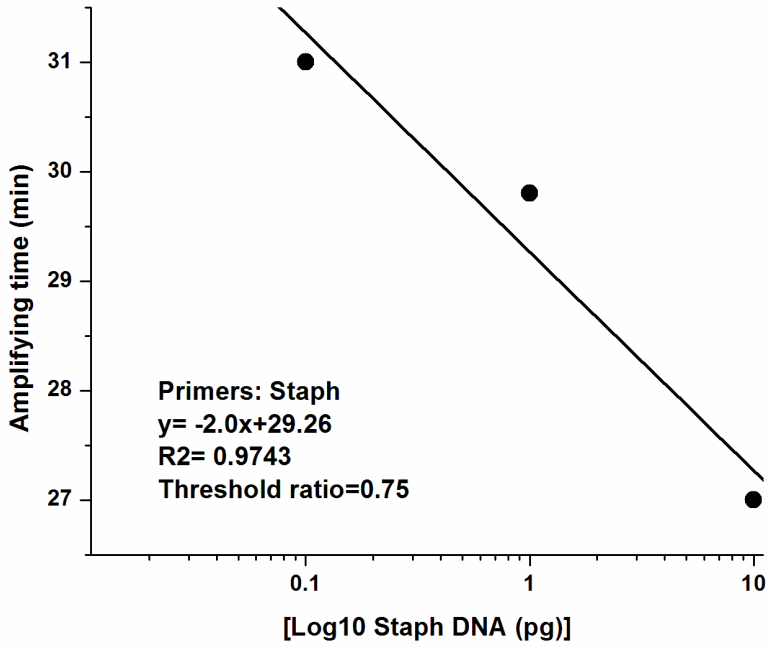


S4: Series of electrochemical SWV cathode peaks (baseline corrected) recorded during the LAMP amplification of (A) 10 pg/µL of *S. Aureus* template gDNA from 5-40 min, and (B) NTC (H_2O), 5 and 40

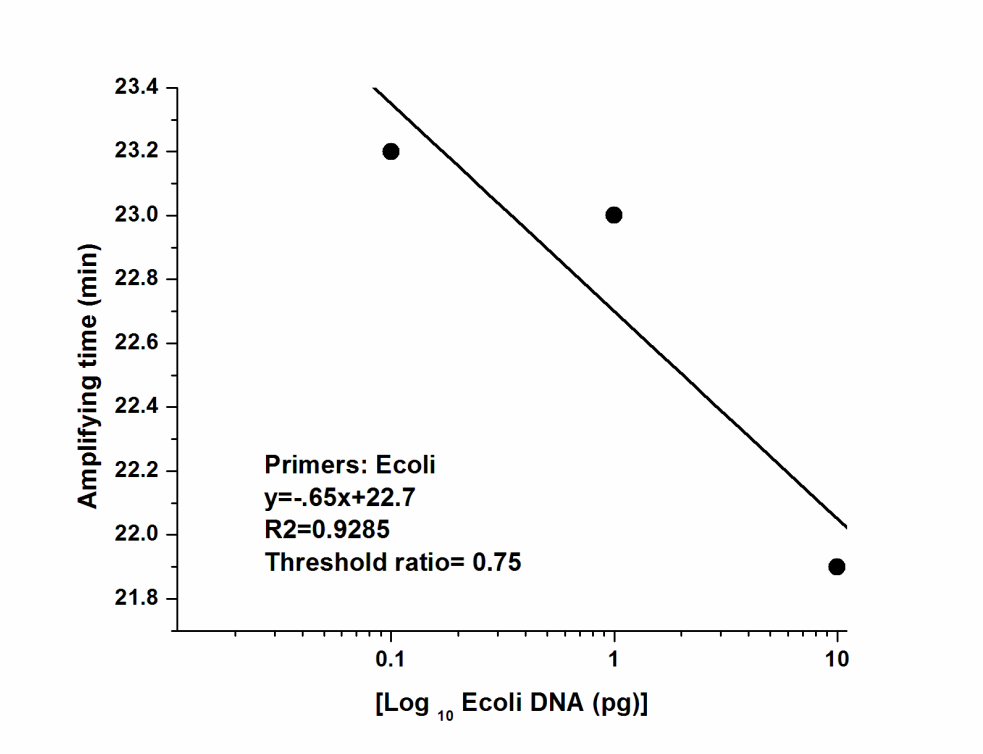
min. The experiments were performed in 50 μL of LAMP solution at 66° C containing 15 μM of RuHex. The SWV curves on figure A were achieved on each 5 min. interval. The electrode type B was used for all real-time measurements, and one electrode was used for multiple scans up to 40 min.

Supplementary Information

S5



S5: The threshold ratio 0.75 is taken while *S. Aureus* specific loop primers were used for 10 pg/μL, 1 pg/μL and 100 fg/μL of template gDNA and that crossed the threshold ratio at 26.2, 30.0 and 31.25 min, respectively. Threshold reaction times are plotted against the $\log_{10}$ of added *S. Aureus* gDNA. Standard curve is calculated and the linear regression line; the equation and R^2 are all shown.



S6: The threshold ratio 0.75 is taken while *E. Coli* specific loop primers were used for 10 pg/µL, 1 pg/µL and 100 fg/µL of template gDNA and that crossed the threshold ratio at 22.3, 23.3 and 24.0 min, respectively. Threshold reaction times are plotted against the $\log_{10}$ of added *S. Aureus* gDNA. Standard curve is calculated and the linear regression line; the equation and R^2 are all shown.

Table S-7 : Isothermal electrochemical sensing of DNA/RNA in recent years using different redox molecules

Samples, sizes	**Target gDNA/Plasmid/µL**	**Copies/ µL**	**Detection time (Min)**	**Redox Type, Amplification Protocol, Voltammetry**
S. Aureus gDNA 2.9 Mb *(Current Study)*	10 pg 1 pg 100 fg	$3.1x10^3$ $3.2x10^2$ 3.1x10	26 28 29	Ruthenium Hexaamine, LAMP, SWV
E. Coli gDNA 4.6 Mb *(Current Study)*	10 pg 1pg 100 fg	$2x10^3$ $2x10^2$ 2x10	21 22 22.5	Ruthenium Hexaamine, LAMP, SWV
E. Coli Plasmid 89 bp[1]	- - -	$5x10^9$ $5x10^6$ $5x10^3$	15 37 48	$Os[(bpy)_2DPPZ$, HDA,SWV
H1N1 Virus, 13000 bases[2]	6.36 ng 636 pg 63.6 pg	$6.79x\ 10^9$ $6.79x10^8$ $6.79x10^7$	17.5 20.2 22.4	Methylene Blue, LAMP, SWV

Table S-8: Detection of salmon dsDNA (data from figure 3) at room temperature

DNA Conc.	RSD (%) of

ng/µL	cathode current value (measured at room temperature), n=3
0	3.71
1	5.01
2.5	5.19
5	2.03
7.5	5.29
10	3.57

Table S-9: Detection of loop amplicon at 65^0 C (for *E. Coli*, data of figure 6 A, 100 fg *E.Coli* DNA was used as target) .

Time of Analysis	RSD (%) of cathode current value (measured at

(min.)	65° C), n=3
5	3.8
10	1.14
15	4.52
20	1.63
25	5.90
30	2.54
35	6.84
40	4.44

References of SI:

1. Nagatani, N.; Yamanaka, K.; Saito, M.; Koketsu, R.; Sasaki, T.; Ikuta, K.; Miyahara, T.; Tamiya, E., *Analyst*. 2011, 21, 5143-5150.

2. Kivlehan, F.; Mavre, F.; Talini, L.; Limoges, B.; Marchal, D., *Analyst*, 2011, 3635-3642.