SUPPLEMENTARY MATERIAL

An electrochemical biosensor utilizing a cobalt-based DNA-binding metallo-intercalator to amplify pathogenic nucleic acid detection

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Supplementary Material

Figures

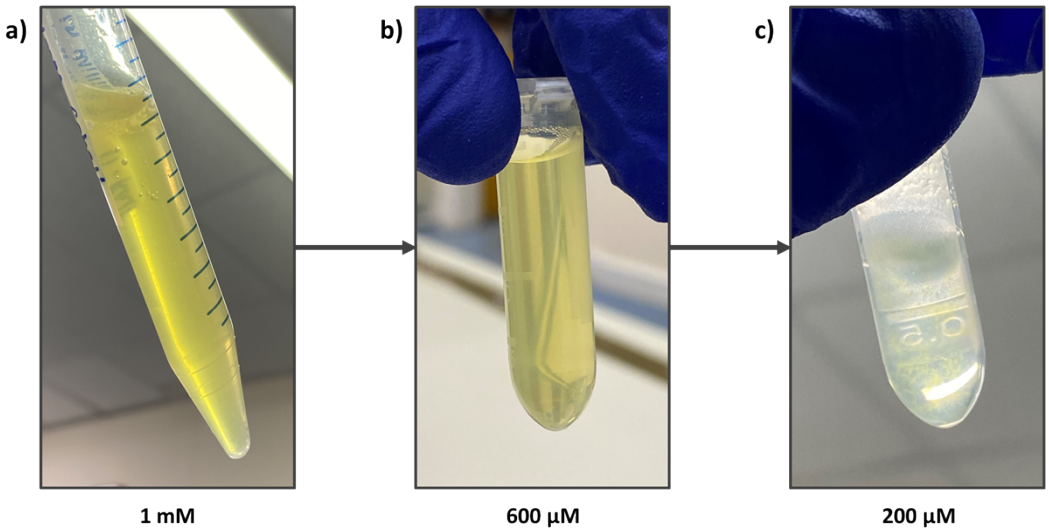

Fig. S1 Dissolution of $[Co(GA)_2(aqphen)]Cl$ in MilliQ and 50 mM PB + 100 mM K_2SO_4. Dilution involved a) re-suspension of $[Co(GA)_2(aqphen)]Cl$ in MilliQ at a concentration of 1 mM, followed by b) dilution in MilliQ to 600 µM and finally c) dilution to 200 µM working concentration in 50 mM PB + 100 mM K_2SO_4.

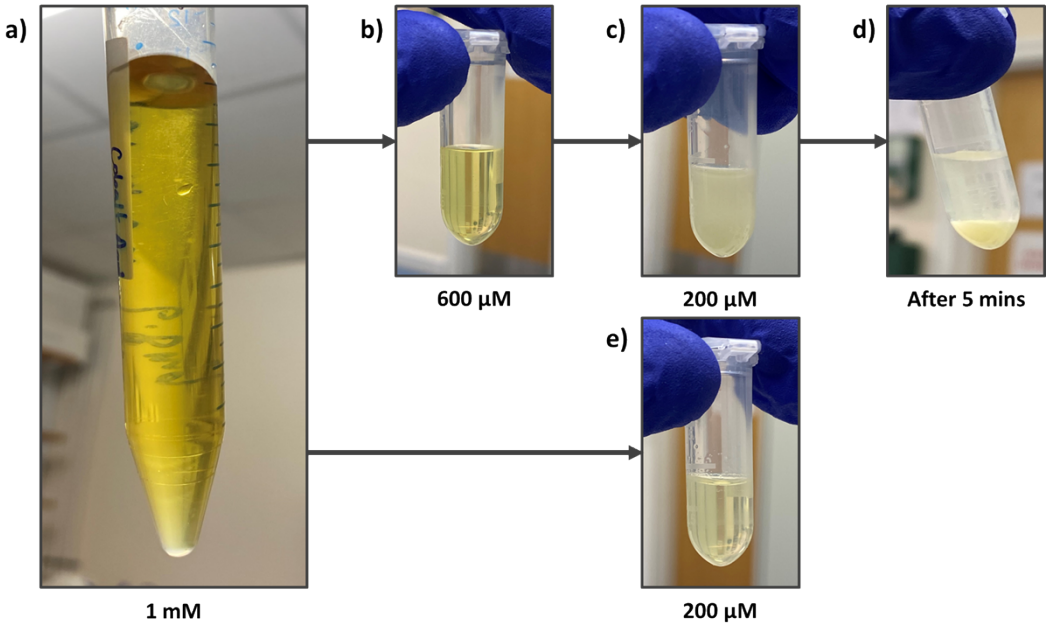

Fig. S2 Dissolution of $[Co(GA)_2(aqphen)]Cl$ in DMSO and 50 mM PB + 100 mM K_2SO_4. Two different dilution methods were tested: first, a) $[Co(GA)_2(aqphen)]Cl$ was dissolved in 100% DMSO to 1 mM and sonicated at 70 °C for 2 hours, b) diluted to 600 µM in 100% DMSO and c) diluted to a final working concentration of 200 µM in 50 mM PB + 100 mM K_2SO_4. d) shows aggregation and settling of $[Co(GA)_2(aqphen)]Cl$ after 2 minutes. The second dilution method involved a) $[Co(GA)_2(aqphen)]Cl$ dissolved in 100% DMSO and sonicated at 70 °C for 2 hours followed by e) dilution to 200 µM directly in 50 mM PB + 100 mM K_2SO_4.

The dose-response curve for label-free detection of ssDNA was fitted with a non-linear regression curve for specific binding following the equation:

$$Y = d + \frac{a - d}{1 + (\frac{x}{c})^b}$$

Where *d* is the maximum value obtained, *x* is the concentration of the target, *c* is the point of inflection and *b* is the hillslope. Using this model, the dose-response curve was fit, and an R^2 value of 0.9611 was given suggesting a strong correlation (Fig. S3). The IC50 value was observed to be 43.76 nM and the hillslope to be 0.6447 which is usual for a negatively cooperative binding relationship (Table S1).

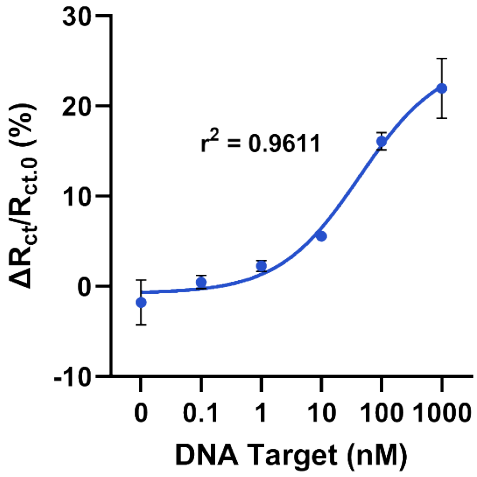

Fig. S3 Charge transfer resistance $\Delta R_{ct}/R_{ct.0}$ dose-response curve versus DNA target oligonucleotide concentration for detection of Escherichia coli O157:H7 ssDNA in 50 mM PB + 100 mM K_2SO_4 containing 2 mM $[Fe(CN)_6]^{3-/4-}$ ($n \geq 3$).

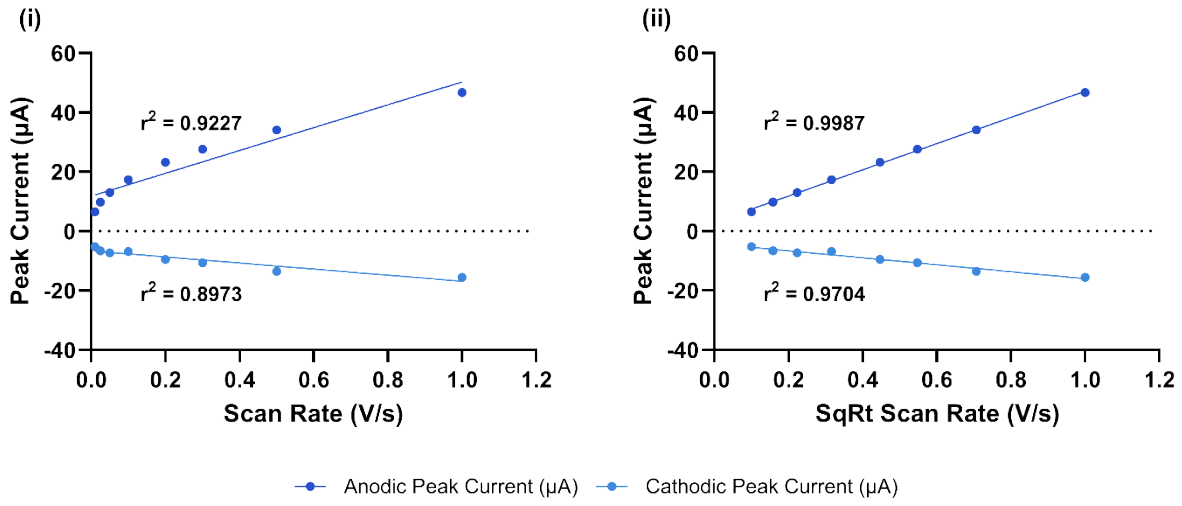

Fig. S4 Cyclic voltammetry data showing the relationship between peak current and (i) scan rate or (ii) the square root of scan rate for the redox process of $[Fe(CN)_6]^{3-/4-}$ (n ≥ 3).

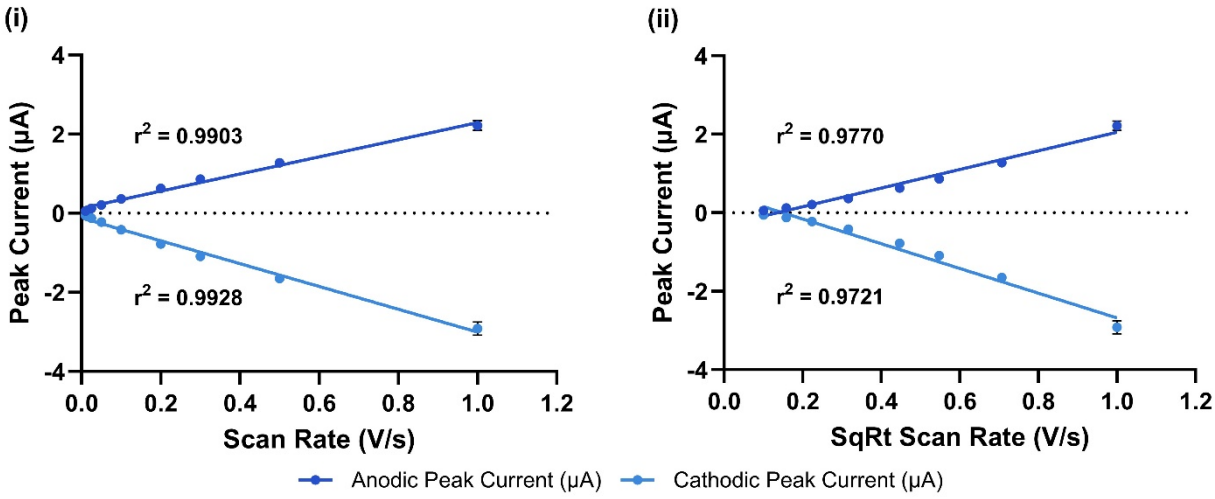

Fig. S5 Cyclic voltammetry data showing the relationship between peak current and (i) scan rate or (ii) the square root of scan rate for the redox process of $[Co(GA)_2(aqphen)]Cl$ (n ≥ 3).

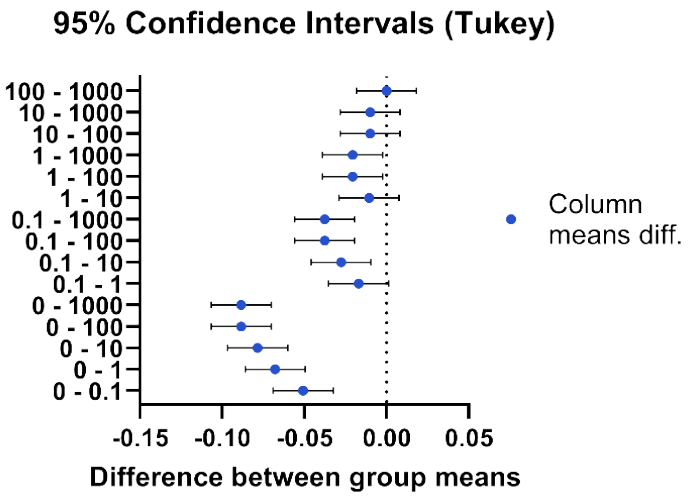

Fig. S6 Multiple comparisons confidence interval (95%) plot of the ordinary one-way ANOVA from the dose-response of co-incubated target ssDNA with $[Co(GA)_2(aqphen)]Cl$.

Tables

Table S1 Non-linear fitting of the unlabeled dose-response for E. coli DNA detection using faradaic EIS in 2 mM $[Fe(CN)_6]^{3-/4-}$.

Sigmoidal, 4PL, X is log(concentration)	
Best-fit values	
Top	25.19
Bottom	-0.7801
LogIC50	1.641
HillSlope	0.6447
IC50	43.76
Span	25.97
95% CI (profile likelihood)	
Top	21.08 to 48.08
Bottom	-3.896 to 0.8634
LogIC50	1.330 to 2.966
HillSlope	0.2964 to 1.074
IC50	21.39 to 924.9
Goodness of Fit	
Degrees of Freedom	20
R squared	0.9611
Sum of Squares	72.26
Sy.x	1.901
Number of points	
# of X values	24
# Y values analyzed	24

Table S2 Non-linear fitting of the labeled dose-response for E. coli DNA detection using DPV in 100 mM PB with 200 mM $[Co(GA)_2(aqphen)]Cl$ intercalation.

Sigmoidal, 4PL, X is log(concentration)	
Best-fit values	
Top	0.4480
Bottom	-99.93
LogIC50	-11.40
HillSlope	0.3242
IC50	3.955e-012
Span	100.4
95% CI (profile likelihood)	
Top	0.4405 to 0.4578
Bottom	???
LogIC50	??? to -1.535
HillSlope	0.2295 to 0.4444
IC50	??? to 0.02918
Goodness of Fit	
Degrees of Freedom	14
R squared	0.9648
Sum of Squares	0.0006158
Sy.x	0.006632
Number of points	
# of X values	18
# Y values analyzed	18

Table S3 Multiple comparisons ordinary one-way ANOVA carried out for the labeled dose-response for E. coli DNA detection using DPV in 100 mM PB with 200 mM $[Co(GA)_2(aqphen)]Cl$ intercalation.

Number of families	**1**
Number of comparisons per family	15
Alpha	0.05

Tukey's multiple comparisons test	**Mean Diff.**	**95.00% CI of diff.**	**Below threshold?**	**Summary**	**Adjusted P Value**	
0 vs. 0.1	-0.05067	-0.06896 to -0.03237	Yes	****	<0.0001	A-B
0 vs. 1	-0.06767	-0.08596 to -0.04937	Yes	****	<0.0001	A-C
0 vs. 10	-0.07833	-0.09663 to -0.06004	Yes	****	<0.0001	A-D
0 vs. 100	-0.08833	-0.1066 to -0.07004	Yes	****	<0.0001	A-E
0 vs. 1000	-0.08833	-0.1066 to -0.07004	Yes	****	<0.0001	A-F
0.1 vs. 1	-0.01700	-0.03530 to 0.001295	No	ns	0.0742	B-C
0.1 vs. 10	-0.02767	-0.04596 to -0.009372	Yes	**	0.0028	B-D
0.1 vs. 100	-0.03767	-0.05596 to -0.01937	Yes	***	0.0002	B-E
0.1 vs. 1000	-0.03767	-0.05596 to -0.01937	Yes	***	0.0002	B-F
1 vs. 10	-0.01067	-0.02896 to 0.007628	No	ns	0.4162	C-D
1 vs. 100	-0.02067	-0.03896 to -0.002372	Yes	*	0.0240	C-E
1 vs. 1000	-0.02067	-0.03896 to -0.002372	Yes	*	0.0240	C-F
10 vs. 100	-0.01000	-0.02830 to 0.008295	No	ns	0.4804	D-E
10 vs. 1000	-0.01000	-0.02830 to 0.008295	No	ns	0.4804	D-F
100 vs. 1000	0.000	-0.01830 to 0.01830	No	ns	>0.9999	E-F

Test details	**Mean 1**	**Mean 2**	**Mean Diff.**	**SE of diff.**	**n1**	**n2**	**q**	**DF**
0 vs. 0.1	0.3577	0.4083	-0.05067	0.005447	3	3	13.16	12
0 vs. 1	0.3577	0.4253	-0.06767	0.005447	3	3	17.57	12
0 vs. 10	0.3577	0.4360	-0.07833	0.005447	3	3	20.34	12
0 vs. 100	0.3577	0.4460	-0.08833	0.005447	3	3	22.94	12
0 vs. 1000	0.3577	0.4460	-0.08833	0.005447	3	3	22.94	12
0.1 vs. 1	0.4083	0.4253	-0.01700	0.005447	3	3	4.414	12
0.1 vs. 10	0.4083	0.4360	-0.02767	0.005447	3	3	7.184	12
0.1 vs. 100	0.4083	0.4460	-0.03767	0.005447	3	3	9.780	12
0.1 vs. 1000	0.4083	0.4460	-0.03767	0.005447	3	3	9.780	12
1 vs. 10	0.4253	0.4360	-0.01067	0.005447	3	3	2.770	12
1 vs. 100	0.4253	0.4460	-0.02067	0.005447	3	3	5.366	12
1 vs. 1000	0.4253	0.4460	-0.02067	0.005447	3	3	5.366	12
10 vs. 100	0.4360	0.4460	-0.01000	0.005447	3	3	2.596	12
10 vs. 1000	0.4360	0.4460	-0.01000	0.005447	3	3	2.596	12
100 vs. 1000	0.4460	0.4460	0.000	0.005447	3	3	0.000	12