Electronic Supplementary Information (ESI)

Highly sensitive electrochemical genosensors based on metallo-porphyrin labelled DNA

Iwona Grabowska,[a] Daniel G. Singleton,[b] Anna Stachyra,[c] Anna Góra–Sochacka,[c] Agnieszka Sirko,[c]Włodzimierz Zagórski–Ostoja,[c] Hanna Radecka,*[a] Eugen Stulz,*[b] and Jerzy Radecki*[a]

[a] Institute of Animal Reproduction and Food Research, Polish Academy of Sciences, Tuwima 10, 10-747 Olsztyn, Poland. E-mail: *j.radecki@pan.olsztyn.pl*; Phone: +48-895234612, Fax: +48895240124.
[b] School of Chemistry and Institute for Life Sciences, University of Southampton, Highfield, Southampton SO17 1BJ, UK. E-mail: *est@soton.ac.uk*; Phone: +44-2380599369.
[c] Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Pawińskiego 5A, 02-106 Warsaw, Poland.

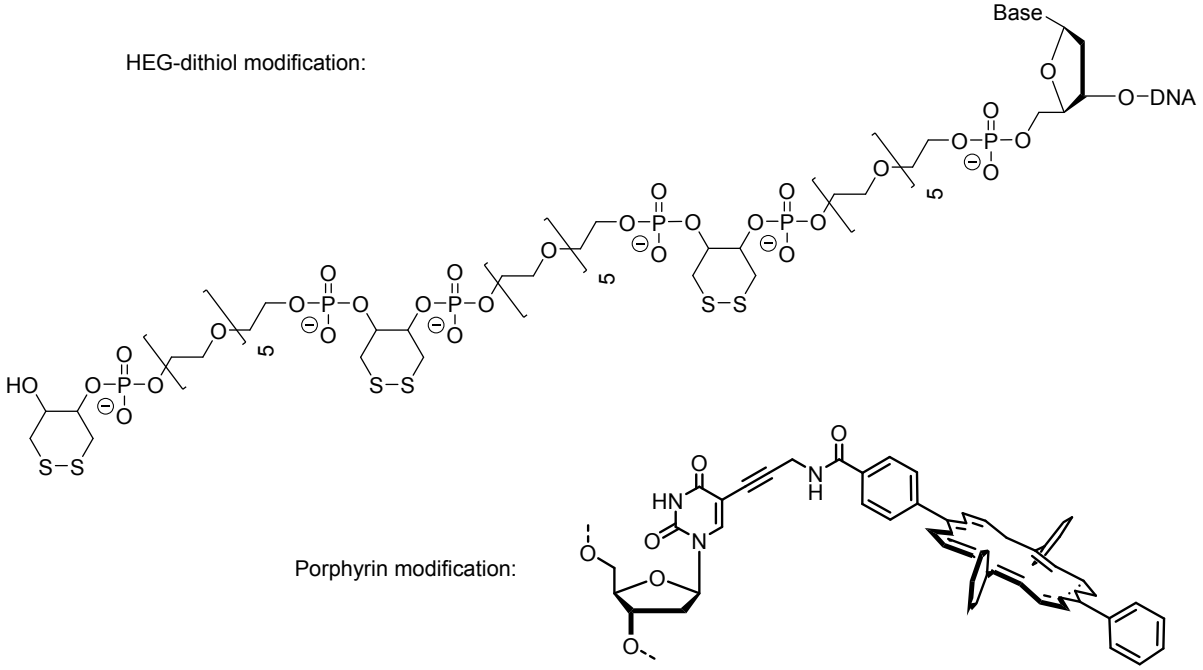
Experimental:

Reagents and Materials. 6-mercaptohexanol ($HS(CH_2)_6OH$), sodium perchlorate ($NaClO_4$), sodium nitrate ($NaNO_3$), sodium chloride (NaCl), sodium tetrafluoroborate ($NaBF_4$), sodium citrate ($HOC(COONa)(CH_2COONa)_2 \cdot 2H_2O$), potassium chloride (KCl), cesium chloride (CsCl) were purchased from Sigma-Aldrich (Poznan, Poland). KOH, H_2SO_4, ethanol, methanol were obtained from ABChem, Gliwice, Poland. All aqueous solutions were prepared with deionized and charcoal-treated water (resistivity of 18.2 MΩcm) purified with a Milli-Q reagent grade water system (Millipore, Bedford, MA).

The modified oligonucleotide: CoP-ssDNA (5'-$(SH\text{-}HEG)_3$-ATT-**P** TGG AGC TAT AGC AGG TT-3'; where **P** is the cobalt metallated porphyrin modified thymidine) was synthesized according to established protocols (A. Brewer, G. Siligardi, C. Neylon and E. Stulz, Org. Biomol. Chem., 2011, 9, 777-782; L. A. Fendt, I. Bouamaied, S. Thöni, N. Amiot and E. Stulz, J. Am. Chem. Soc., 2007, 129, 15319-15329) and was used as probe for immobilization on a surface of gold electrodes.

Solid phase synthesis (SPS) of the DNA followed standard phosphoramidite chemistry for the unmodified parts of the ODN using an Applied Biosystems Expedite DNA synthesiser (1000 Å CPG) with reagents obtained from Tides Service, Haar, Germany (standard phosphoramidites, reagents, solvents), from Link Technologies Ltd, Bellshill, Scotland (HEG modifier) and from GlenResearch, Sterlind, US (dithiol modifier). The complementary oligonucleotides were obtained from biomers.net GmbH, Germany. For insertion of the dU-TPP building block, a freshly made solution of the building block in DCM-MeCN 1:1 (50 mM) was used with an extended coupling time (600 s), otherwise using standard SPS protocols; the modifiers were incorporated using the suppliers' protocols. The Co-P-ssDNA probe contains the DNA sequence characteristic for gene encoding hemagglutinin of H5N1

AIV. The modified DNA was cleaved from the CPG and deprotected using aqueous ammonia solution at ambient temperature overnight, followed by purification using NAP-25 column, RP-HPLC and denaturing PAGE (see below). HPLC was run using HFIP-TEA buffer at pH 7.0. The porphyrin-DNA is isolated as free base porphyrin due to loss of zinc during acid detritylation in the SPS and remetallated using $Co(OAc)_2$ according to published procedures (OBC 2011).



Electrode Preparation. Gold disk electrodes of 2 mm^2 area (Bioanalytical Systems (BAS), West Lafayette, IN) were used for the experiments. The electrodes were polished with wet 0.3 and 0.05 μm alumina slurry (Alpha and Gamma Micropolish, Buehler, Lake Bluff, IL) on a flat pad for at least 10 min and rinsed repeatedly with water and finally in a sonicator (30 s). The polished electrodes were then dipped in 0.5 M KOH solution deoxygenated by purging with argon for 15 min, and the potential was cycled between -400 and -1200 mV (versus Ag/AgCl reference electrode) with a scan rate of 100 mVs^{-1} until the CV no longer changed. On the polished electrodes, 10 µl of the following solution was dropped: 100µM CoP-ssDNA and 10 µM 6-mercaptohexanol in buffer: 0.9 M NaCl, 0.09 M sodium citrate, pH 7.0 at room temperature for 3 hours. Then, the electrodes were rinsed with solution of 0.9 M NaCl, 0.09 M sodium citrate, pH 7.0, water and again buffer solution. In the next step, on the gold electrodes 10 µl of 1 mM 6-mercaptohexanol in 0.9 M NaCl, 0.09 M sodium citrate, pH 7.0 for 0.5 hour was dropped. Next, after rinsing with the same buffer, the electrodes were left in

refrigerator for 24 hours and stored in buffer solution (0.9 M NaCl, 0.09 M sodium citrate, pH 7.0) at 4° until used.

Immobilization on the electrode surface of CoP-ssDNA probes complexed with ssDNA targets. The appropriate mixtures were prepared in the tube in the following way. 100 μM CoP-ssDNA was mixed with corresponding complementary ssDNA in buffer: 0.9 M NaCl, 0.09 M sodium citrate, pH 7.0 and left at room temperature for at least 24 hours. After this time, the mixtures was mixed with 10 μM 6-mercaptohexanol and were dropped on the surface of gold electrodes and left at room temperature for 3 hours. And then, in the next step, on the gold electrodes 1 mM 6-mercaptohexanol solution in 0.9 M NaCl, 0.09 M sodium citrate was dropped for 0.5 hour and stored as described above.

Hybridization of the CoP-ssDNA probe and the target sequences. The CoP-ssDNA modified gold electrode was exposed to 10 μl of the solution containing targets: 20-mer complementary or no-complementary in buffer solution as described above, for 0.5 h at room temperature. After the hybridization with the particular concentration of targets, the electrodes were rinsed thoroughly with the following buffer: 0.3 M NaCl, 0.03 M sodium citrate at 42 °C and transferred to the electrochemical cell for electrochemical measurements.

Electrochemical Measurements. All electrochemical measurements were performed with a potentiostat-galvanostat AutoLab (Eco Chemie, Utrecht, Netherlands) with a three-electrode configuration. Potentials were measured versus the Ag/AgCl electrode, and a platinum wire was used as an auxiliary electrode.

Cyclic voltammetry (CV) was performed in the potential window: -200 mV to 700 mV with different scan rates: 10, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 mVs^{-1}. Osteryoung square-wave voltammetry (OSWV) was performed with a potential scanned in the potential window: -200 mV to 600 mV, and with a step potential of 1 mV, a square-wave frequency of 50 Hz, and an amplitude of 25 mV. Differential pulse voltammetry was performed with a potential scanned in the potential window: -200 mV to 600 mV or 600 mV to – 200 mV, with a step potential of 1 mV, and an amplitude of 25 mV.

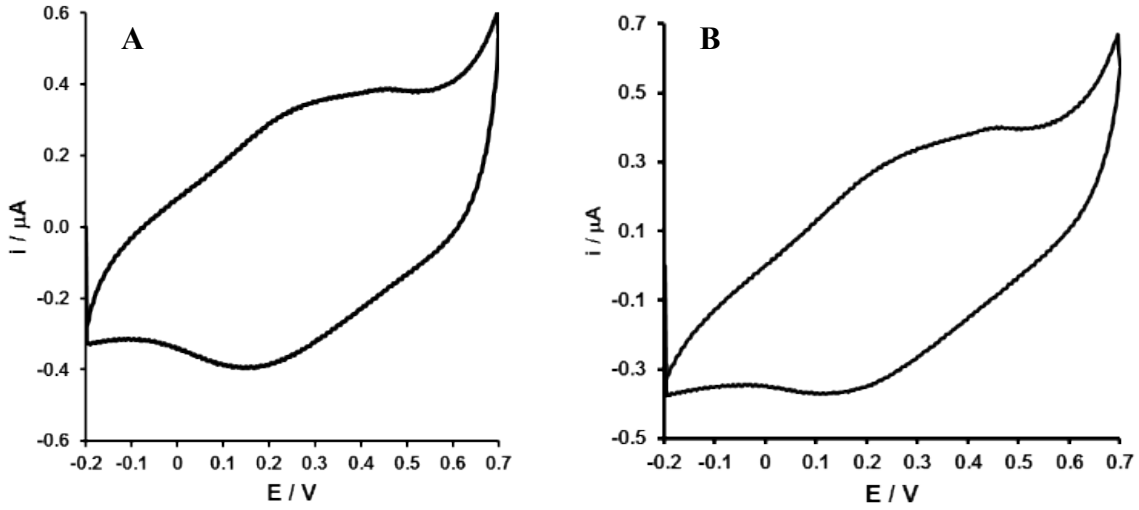


Fig. S1

Representative cyclic voltammograms of gold electrode modified with **CoP-DNA** in (**A**) ssDNA form and (**B**) dsDNA form. Buffer conditions: 1 M NaCl, 0.01 M sodium citrate, pH 7.0, scan rate 100 mV s-1.

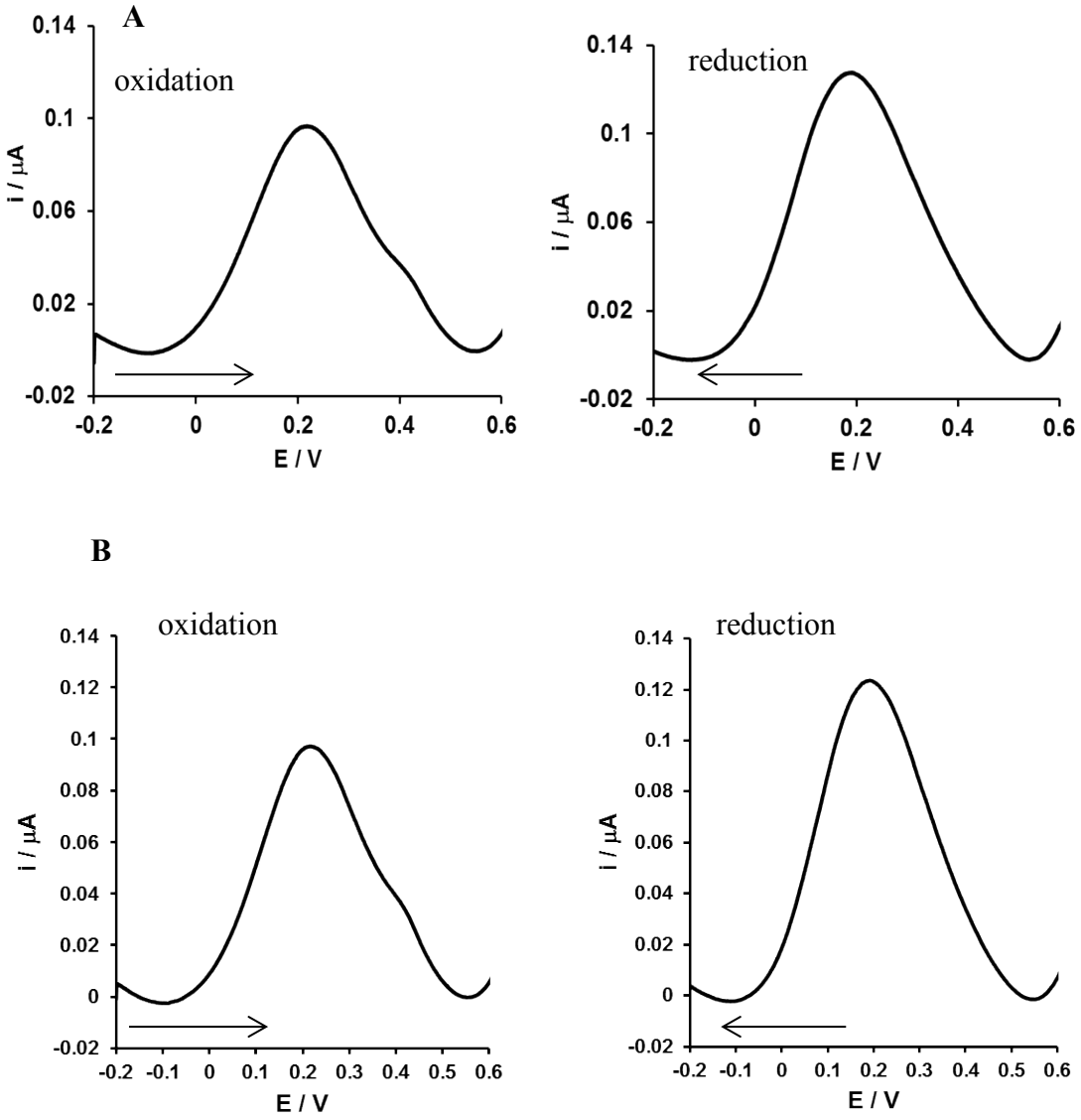


Fig. S2

Representative differential pulse voltammograms of gold electrode modified with : (**A**) **CoP-ssDNA** and (**B**) **CoP-dsDNA** . Buffer conditions: 1 M NaCl, 0.01 M sodium citrate, pH 7.0, step potential of 1 mV, and an amplitude of 25 mV.

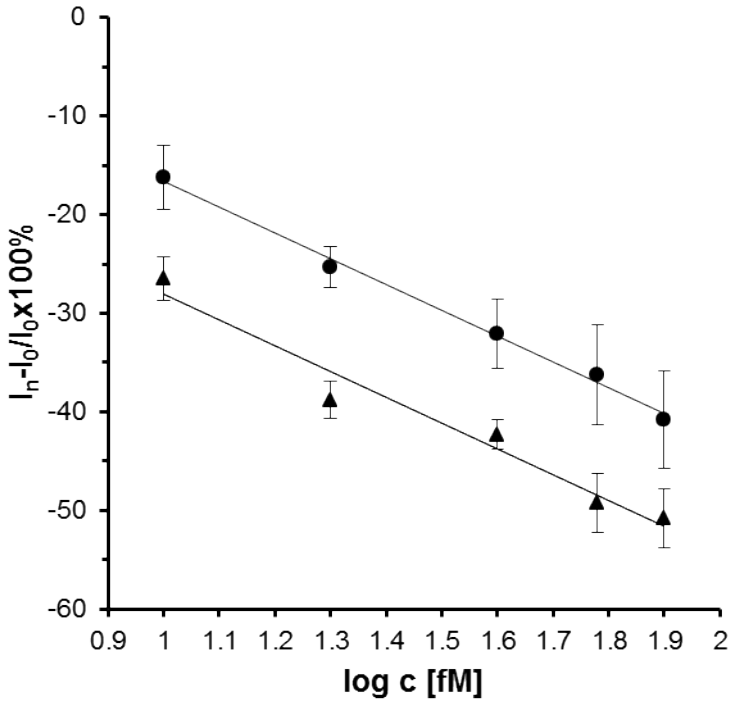


Fig. S3

Relative intensity of redox Co(II)/Co(III) current *vs* log of concentration of 20-mer complementary ssDNA after one day (●) (I_n-I_0/I_nx100% = -26.244logc (fM) + 9.6665; R^2 = 0.9946) and three days (▲) (I_n-I_0/I_nx100% = -26.104logc (fM) - 1.9668; R^2 = 0.9625) of genosensor storing during 3 days in buffer 1 M NaCl, 0.01 M sodium citrate, pH 7.0 in addition of 0.05% sodium azide, in 4°C.

I_n is the value of peak current measured in the presence of particular concentration of ssDNA, and I_0 is the value of peak current measured in the presence of buffer before hybridization reaction.

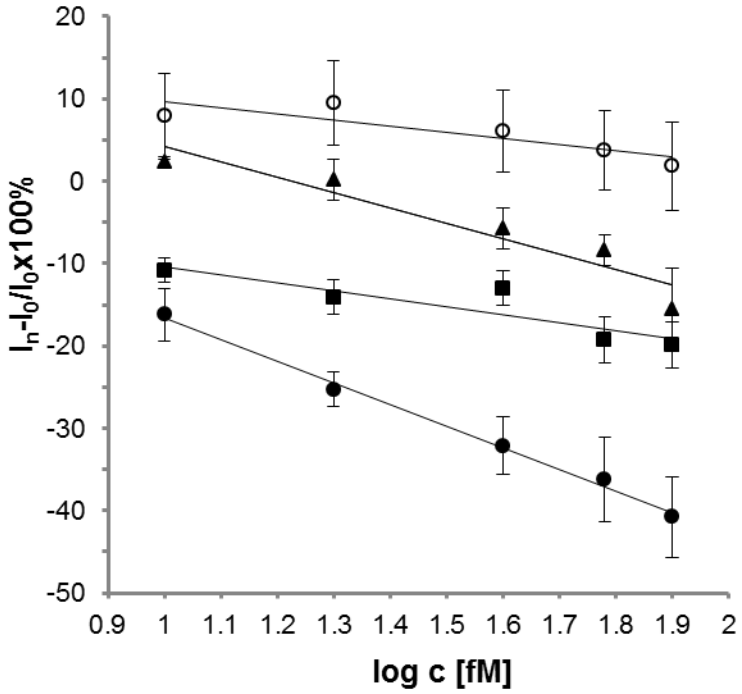


Fig. S4
Measurement with complementary and non-complementary DNA probes. DNA probe: CoP-ssDNA: 5`-AT**P** TGG AGC TAT AGC AGG TT-3`;

1. (●) complementary sequence.: 5`- **AAC CTG CTA TAG CTC CAA AT**-3`
I_n-I_0/I_nx100% = -26.244logc (fM) + 9.6665; R^2 = 0.9946

2. (■) sequence with three complementary bases.: 5`- GGA G**T**T **C**CT CTC TCA TC**A** TC-3`
I_n-I_0/I_nx100% = -9.6515logc (fM) - 0.7684; R^2 = 0.7904

3. (▲) sequence with one complementary base.: 5`-G**A**A GAA GAG AGA GGA ACT CC-3`
I_n-I_0/I_nx100% = -18.559logc (fM) + 22.715; R^2 = 0.901

4. (○) totally non-complementary sequence.: 5`-TTG GAC GAT ATC GAG GTT TA-3`
I_n-I_0/I_nx100% = -7.3503logc (fM) + 16.943; R^2 = 0.7518

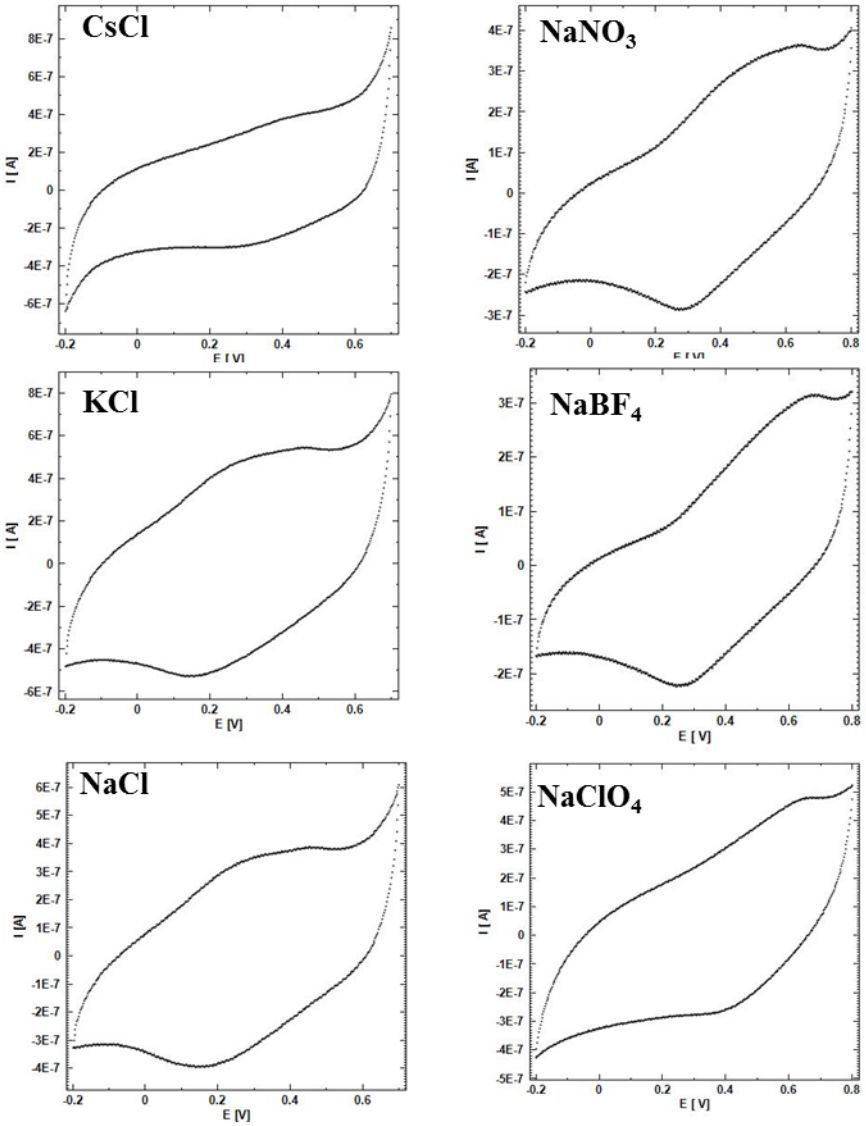


Fig. S5
Representative cyclic voltammograms of gold electrode modified with **CoP-ssDNA** measured in 1.0 M solutions of selected supporting electrolytes (with addition of 0.01 M sodium citrate). Scan rate 100 mVs^{-1}.

Table S1
Electrochemical parameters of gold electrodes modified with **CoP-ssDNA** and **CoP-dsDNA** estimated in different electrolytes together with hydration energy ($-\Delta G_s$) and size of ions (a) used in supporting electrolytes. Calculations were done for 100 mV/s.

electrolyte 1.0 M + 0.01 M sodium citrate	$-\Delta G_s$ kJ/mol	a Å	**CoP-ssDNA**				**CoP-dsDNA**			
			$E_{ox.}$ [V]	$Q_{ox.}$ x10^{-8} [C]	$E_{red.}$ [V]	$Q_{red.}$ x10^{-8} [C]	$E_{ox.}$ [V]	$Q_{ox.}$ x10^{-8} [C]	$E_{red.}$ [V]	$Q_{red.}$ x10^{-8} [C]
CsCl	276	1.62[b]	0.379±0.028	nd	0.304±0.023	1.6±0.2	0.388±0.009	nd	0.307±0.015	1.6±0.6
KCl	322	1.06[b]	0.310±0.025	2.8±0.6	0.180±0.010	4.6±0.4	0.290±0.009	3.5±0.7	0.189±0.016	4.7±1.1
NaCl	406	0.67 (Na^+)[b] 1.86 (Cl^-)[b]	0.285±0.022	2.9±1.0	0.190±0.014	4.1±0.5	0.275±0.005	2.3±0.2	0.182±0.001	3.6±1.0
$NaNO_3$	306[a]		0.525±0.012	2.1±0.4	0.292±0.012	4.4±0.7	0.531±0.040	1.9±0.1	0.288±0.011	4.1±0.3
$NaBF_4$	243		0.632±0.019	0.6±0.1	0.254±0.026	2.3±0.3	0.596±0.003	1.0±0.1	0.274±0.015	2.8±0.6
$NaClO_4$	214[a]	2.35[b]	0.633±0.026	nd	0.385±0.034	2.3±0.8	0.624±0.019	nd	0.361±0.032	2.3±0.2

[a] from G. Valincius, G. Niaura, B. Kazakeviciene, Z. Talaikyte, M. Kazemekaite, E. Butkus, V. Razumas, *Langmuir*, 2004, **20**, 6631; [b] from L. Medda, A. Salis, E. Magner, *Phys.Chem. Chem.Phys*, 2012, **14**, 2875.

nd - not determined

Table S2
Electron transfer coefficient (α) and electron rate constant (k_s , s^{-1}) for **CoP-ssDNA** and **CoP-dsDNA** modified gold electrodes estimated in different supporting electrolytes.

electrolyte 1.0 M + 0.01 M sodium citrate	**CoP-ssDNA**		**CoP-dsDNA**	
	α	k_s [s^{-1}]	α	k_s [s^{-1}]
CsCl	0.27 ± 0.06	0.74 ± 0.13	0.24 ± 0.06	0.71 ± 0.09
KCl	0.77 ± 0.12	0.44 ± 0.10	0.56 ± 0.02	0.73 ± 0.17
NaCl	0.78 ± 0.05	0.65 ± 0.26	0.72 ± 0.04	0.69 ± 0.05
$NaNO_3$	0.64 ± 0.05	0.19 ± 0.09	0.58 ± 0.11	0.18 ± 0.11
$NaBF_4$	0.46 ± 0.02	0.04 ± 0.02	0.48 ± 0.07	0.07 ± 0.01
$NaClO_4$	0.61 ± 0.04	0.19 ± 0.06	0.56 ± 0.04	0.15 ± 0.09