Electronic Supplementary Material (ESI) for Chemical Communications

Supporting information

Homogeneous electrochemical monitoring of exonuclease III activity and its application to nucleic acid testing by target recycling.

Rebeca Miranda-Castro, Damien Marchal, Benoît Limoges* and François Mavré*

Laboratoire d'Electrochimie Moléculaire, UMR 7591 CNRS, Université Paris Diderot, Sorbonne Paris Cité, 15 rue Jean-Antoine de Baïf, F-75205 Paris Cedex 13, France.

Experimental section

Apparatus

Cyclic voltammetric experiments were carried out with a computer-controlled AutoLab PGSTAT 12 potentiostat interfaced to a GPES 4.9007 software (EcoChemie B.V. Utrecht, The Netherlands) in a micro-electrochemical cell equipped with a working carbon-based screen-printed electrode (sensing area of 0.071cm^2), an auxiliary carbon-based screen-printed electrode, and a reference saturated calomel electrode. The screen-printed electrodes were prepared with a DEK model-65 semiautomatic screen-printer (Presco, USA) on a planar polyethylenterephtalate film, using carbon ink (Acheson Colloids Co).

Real-time electrochemical experiments for monitoring the enzyme kinetic reaction were performed with custom designed single-use 48-well electrochemical microplates, whereby the flat bottom of each well was integrated with 3 screen-printed electrodes (a carbon working electrode of 4.8 mm^2, a carbon counter electrode, and a silver pseudo-reference electrode) interfaced with a multiplexed potentiostat (LEO, Easy Life Science, www.elice.fr) previously developed in the group.[1] The overall 48 electrochemical wells can be scanned by square wave voltammetry (SWV) in less than 5 seconds. The SWV parameters were as follow: square-wave frequency of f = 100 Hz, pulse amplitude of ΔE_p = 40 mV, and potential step increment of E_{SW} = 5 mV. The SWV peak current integrations were automatically extracted from the raw signals after baseline correction and then normalized to the value recorded just before the injection of the target to account for well-to-well variations. For the kinetic plots, the response was also corrected from the blank response (i.e., zero target concentration) and normalized by the maximal recoverable signal, leading thus to the percentage signal recovery S_r.

Reagents

Exonuclease III (Exo III) was purchased from EUR_X (Poland) and used as received. The complex $Os[(bpy)_2(dppz)](PF_6)_2$ was synthesized according to published protocols.[2] Dithiothreitol (DTT) and polyvinylpyrrolidone (PVP) were obtained from Sigma-Aldrich. Salts for buffer solutions (KCl, $MgCl_2$, $(NH_4)_2SO_4$, and Trizma base) were also purchased from Sigma-Aldrich. HPLC-purified synthetic oligonucleotides were obtained from Eurogentec

(France). Their sequences are listed in Table S1. All other reagents were of analytical grade. Milli-Q water (18.2 MΩ cm, TKA MicroPure UV) was used to prepare all aqueous solutions.

Table S1: DNA sequences used for the assay development

Name	Function	Sequence (5'-3')	
T	Target	CGGGTGGATCGGCGTTTTATTCTTGTTCAGATATTCA	37-mer
P1	Capture probe	AACAAGAATAAAACG***CCGATCCACCCG***	27-mer
P2	Auxiliary probe	CGTTTTATTCTTGTT***CCCTA***	20-mer
P2-b	Auxiliary probe	CGTTTTATTCTTGTT***CCCTsA***	20-mer
P2-c	Auxiliary probe	TTTTATTCTTGTT***ATATA***	18-mer
P2-d	Auxiliary probe	TTTTATTCTTGTT***ATATATAT***	21-mer
M_3	Interference	CGGGTGGAACCCCGTTTTATTCTTGTTCAGATATTCA	37-mer
M_2	Interference	CGTGAGGATCGGCGTTTTATTCTTGTTCAGATATTCA	37-mer
R	Interference (random sequence)	ACTTATAGACTTCTGTTATTTTTGCGGCTAGGTGGTG	37-mer

S = phosphorothioate linkage
Within the probe sequences, the bold and italicized bases constitute the Exo-III-resistant 3' protruding terminus. The mismatches of the interfering sequences are underlined.

Buffers

Digestion buffer containing 10 mM Tris-HCl (pH 7.5), 10 mM KCl, 1.5 mM $MgCl_2$, 1 mM $(NH_4)_2SO_4$, and 100 µM DTT was used in all reported experiments. DDT is essential for the enzyme activity.

The presence DTT in the buffer was observed to be catalytically oxidized by the electrogenerated osmium(III) complex according to a redox mediated catalytic reaction. This can be clearly seen on the cyclic voltammograms in Figure S1, showing an amplified catalytic signal in the presence of DTT. In SWV, the peak current responses are thus more complex than that expected for the reversible one-electron transfer of the osmium compound. Another observation that needs to be mentioned was a systematic shift of the potential of the silver pseudo-reference electrode after the addition of DTT, probably due to the strong adsorption of the thiol groups of DTT on the silver electrode surface. Therefore, the concentration of DTT has to be carefully controlled.

Table S2. Description of the successive additions involved in the kinetic experiments described in Fig. 1B. The symbol (+) means the addition of the component and the symbol (-) the addition of Milli-Q water.

Injection step	(a)		(b)	(c)
Added component	**P1**	**P2**	**Exo III**	**T**
Blue curve	+	+	+	+
Red curve	+	+	+	-
Black curve	+	+	-	-
Green curve	-	-	+	-
Violet curve	-	+	+	-

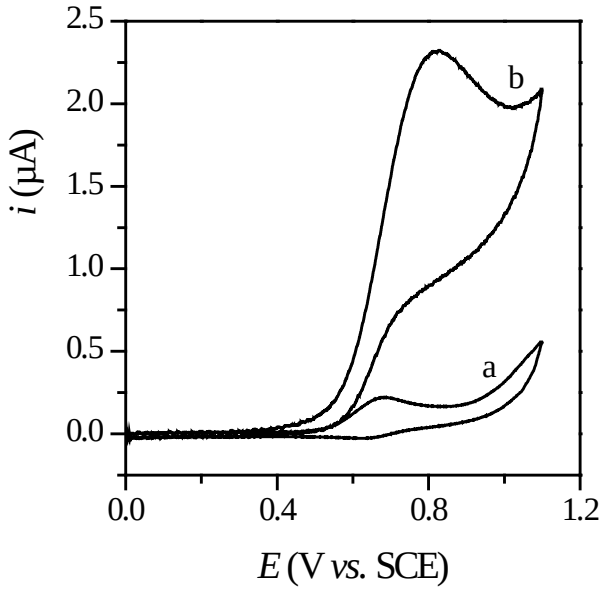

Fig. S1. Cyclic voltammograms of 2 µM $Os[(bpy)_2(dppz)]^{2+}$ (E^0 = +0.65 V *vs.* SCE) in (a) the DTT-free digestion buffer and (b) after addition of 100 µM DTT. Scan rate: 0.1 V s^{-1}.

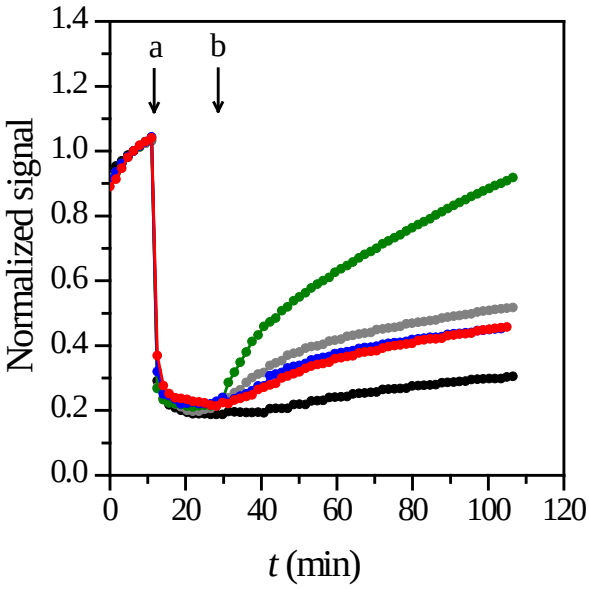

Fig. S2. Integrated SWV peak current response of 2 µM $[Os(bpy)_2(dppz)]^{2+}$ as a function of time. Injections of (a) 0.8 µM duplex reservoir (red: **P1-P2**; blue: **P1-P2-b**; grey: **P1-P2-c**, and green: **P1-P2-d**), (b) 1 U/ µL Exo III. Control experiment: 0.8µM duplex reservoir in the absence of enzyme (black curve).

The effect of the 3'-protruding ends at the duplex probe reservoir on Exo III activity was investigated. With this aim, different length overhangs at 3'-end to the auxiliary probe were tested: 5-nt (**P2**, **P2-b**, **P2-c**) and 8-nt (**P2-d**). In contrast to the available information about Exo III activity (≥ 4-base protruding 3' termini and phosphorothioate linkages are protected from Exo III digestion) a monotonic signal increase for the longest tail, **P2-d**, was observed after enzyme addition (Fig. S2, green curve) suggesting that DNA enzymatic hydrolysis takes place. It might be due to the formation of auxiliary probe dimers which could be exonuclease substrates. Therefore, for suitable duplex probe protection against Exo III activity, not only the tail length but also its base sequence should be considered. Also, the efficiency of the 5-nt tail was checked by comparing with a tail that has a phosphorothioate link that cannot be hydrolyzed by the action of Exo III. No differences in signal recovery are observed whether the phosphorothioate link is present or not, meaning that the chosen 5-nt tail is completely protective.

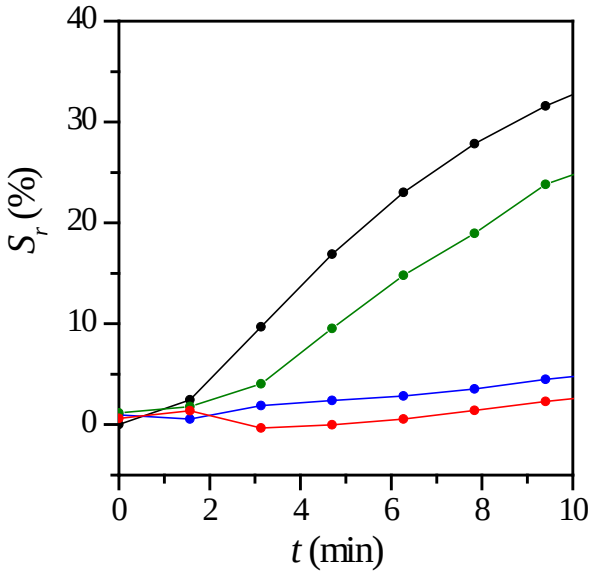

Fig. S3. Time-dependent signal recovery (S_r) after the addition of 50 nM of different DNA sequences: (red) **R**, (blue) $\mathbf{M_3}$, (green) $\mathbf{M_2}$, and (black) **T**. Experiments were performed in the presence of 1 U/μL Exo III, 2 μM $[Os(bpy)_2(dppz)]^{2+}$, and 0.8 μM of **P1**-**P2**.

References:

(1) T. Deféver, M. Druet, D. Evrard, B. Limoges and D. Marchal, *Anal. Chem.*, 2011, **83**, 1815.

(2) E.M. Kober, J.V. Caspar, B.P. Sullivan and T. Meyer, *Inorg. Chem.*, 1988, **27**, 4587.