SUPPLEMENTARY INFORMATION

Os(II/III) complex supports pH-insensitive electrochemical DNA-based sensing with superior operational stability than the benchmark methylene blue reporter

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SUPPLEMENTARY FIGURES



Figure S1. The two-step synthesis of $[Os(dmebpy)_2Cl(ampy)]^+$. (**A**) In the first step, 4 chlorides from the starting Os (IV) salt are substituted by 2 dmebpy. (**B**) In the second step, one more chloride is substituted by one ampy and the complex is precipitated as a PF_6^- salt.



Figure S2. Covalent attachment of redox reporters to the surface of gold electrodes. (A) Freshly polished gold electrodes were modified with MUA for 2 h. Next, either [Os(dmebpy)₂Cl(ampy)]⁺ or NMB were covalently attached via the free carboxylic groups of MUA using EDC chemistry. After the coupling reaction, electrodes were thoroughly washed with acetonitrile/sulfuric acid and guanidinium chloride. Last, electrodes were incubated overnight with MCH to backfill. (B-**C**) Cyclic voltammograms show that using this protocol we can successfully attach each redox reporter to MUA monolayers, observing a clean difference between electrodes that were incubated with the reporter in the presence or absence of EDC. The broad peaks observed in the case of $[Os(dmebpy)_2Cl(ampy)]^+$ have been reported before for analogous Os-based redox active monolayers.^{1, 2} Peak broadness in these systems may arise because the osmium complex has a positive charge in both oxidation states; thus, repulsive interactions between the positively charged redox centers can increase the overvoltage needed for electron transfer. Additionally, the electrostatic shielding of the positive charges by solvated ions and packing density can both affect the observed peak broadness.² Cyclic voltammograms were collected in 1X PBS at 5 V/s. Solid traces and shaded areas represent the average and standard deviation of 4 electrodes.



Figure S3. Effect of solvent washes on the voltammetric response of redox reporter-modified alkanethiols monolayers. (A) When we interrogate NMB-modified electrodes with no washing steps other than with deionized water, cyclic voltammograms of the resulting surfaces show voltammetric peaks for NMB molecules non-specifically adsorbed on the surface (for reporter incubations in the absence of EDC reagent). (B) However, washing the electrodes completely non-specifically adsorbed NMB. (**C**) This effect is removes more clear on [Os(dmebpy)₂Cl(ampy)]⁺-modified electrodes, as the complex is highly hydrophobic, observing similar voltammetric responses on electrodes modified with the complex in the presence or absence of EDC. (D) Washing is critical to remove the fraction of complex non-specifically adsorbed on the surface. Cyclic voltammograms were collected in 1X PBS at 1 V/s. Solid traces and shaded areas represent the average and standard deviation of 4 electrodes.



Figure S4. Evaluation of 3' vs 5' MB-modified tobramycin-binding E-AB sensors. The E-AB sensors were fabricated by incubating activated electrodes in 500 nM aptamer solutions for 2 h and then backfilled with MCH overnight. Titration curves were obtained via square wave voltammetry at a frequency of 300 Hz, amplitude of 75 mV, and voltage step of 1 mV.



Figure S5. Frequency maps for [Os(dmebpy)₂Cl(ampy)]⁺-based E-AB sensors. (A) For the tobramycin sensors we chose 1,000 Hz and 14,500 Hz as SWV frequencies to build the signal ON and OFF titration curves, respectively. (B) In the case of procaine sensors, we did not observe signal OFF behavior, so we selected 1,500 Hz for the signal ON titration curves.

Figure S6. Using EDC coupling reactions, we covalently attached (**A**) NMB and (**B**) $[Os(dmebpy)_2Cl(ampy)]^+$ to the terminal end of COOH-modified tobramycin aptamers. By integrating the area under the anodic peak of the voltammograms, we could estimate the surface coverage, obtaining values of 0.4 ± 0.1 pmol cm⁻² and 0.6 ± 0.1 pmol cm⁻² for NMB and Os-based sensors, respectively.

Figure S7. One of the main features of E-AB sensors is their ability to continuously monitor molecular target concentrations in real time. (**A**) We evaluated this ability using $[Os(dmebpy)_2Cl(ampy)]^+$ -modified tobramycin sensors in their signal-OFF mode, as these sensors displayed a larger signal change in their OFF mode. (**B**) Conversely, with procaine sensors we monitored their ON mode, observing in both cases that the signal is recovered after adding/removing target from the sample. Frequencies of 1000 Hz and 1500 Hz were used for the tobramycin and procaine sensors, respectively. Solid lines and shaded areas represent the average and standard deviation of 4 electrodes.

Figure S8. Predicted secondary structures for the two DNA sequences used in this work via the *Nupack*³ website. Colors represent the probability of nucleotide position.

Figure S9. We used a free carboxylic group at the terminal end of procaine aptamers to chemically attach them to the sensor surface via EDC chemistry. (A) [Os(dmebpy)₂Cl(ampy)]⁺ and (B) NMB-modified srufaces. Black traces show control experiments done in the absence of EDC, showing that even after washing the electrodes both reporters remain non-specifically bound to the surface-immobilized oligos. Solid lines and shaded areas represent the average and standard deviation of 4 electrodes.

Figure S10. $[Os(dmebpy)_2Cl(ampy)]^+$ -based sensors can be used to detect hybridization events (Fig. 5). To assess the specificity of these sensors to detect the hybridization of the immobilized strand with its complementary strand, we challenged the fabricated tobramycin sensors to a non-complementary strand (15-nt Poly T) at a concentration of 10 µM. After an initial decay of ~ 5%, likely due to the non-specific interaction of the negatively charged molecules of the Poly T strand, the sensors signal remained constant after 45 min of incubation, indicating that no hybridization events took place.

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