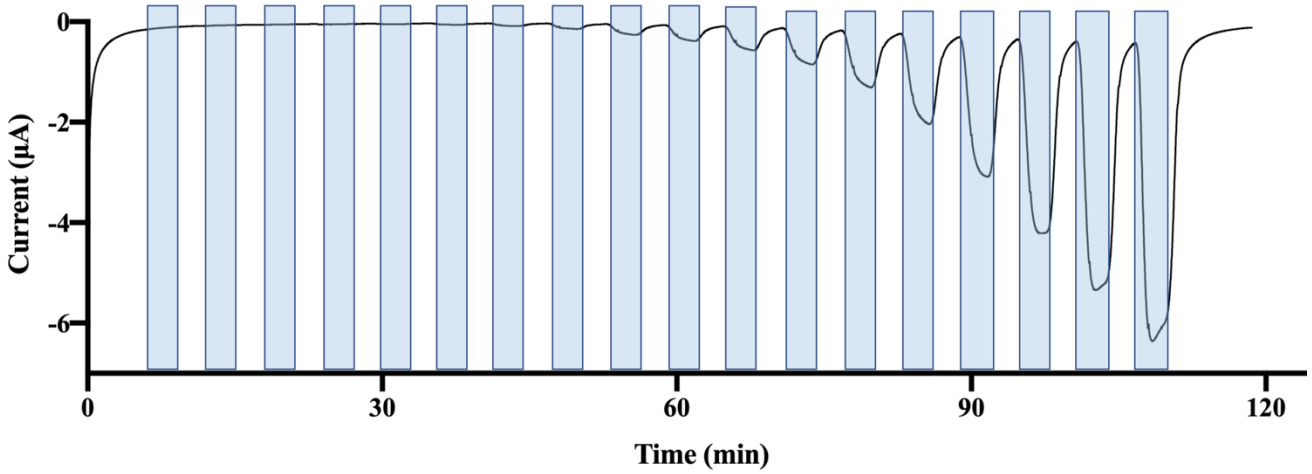
Electronic Supplementary Material (ESI) for Analytical Methods.

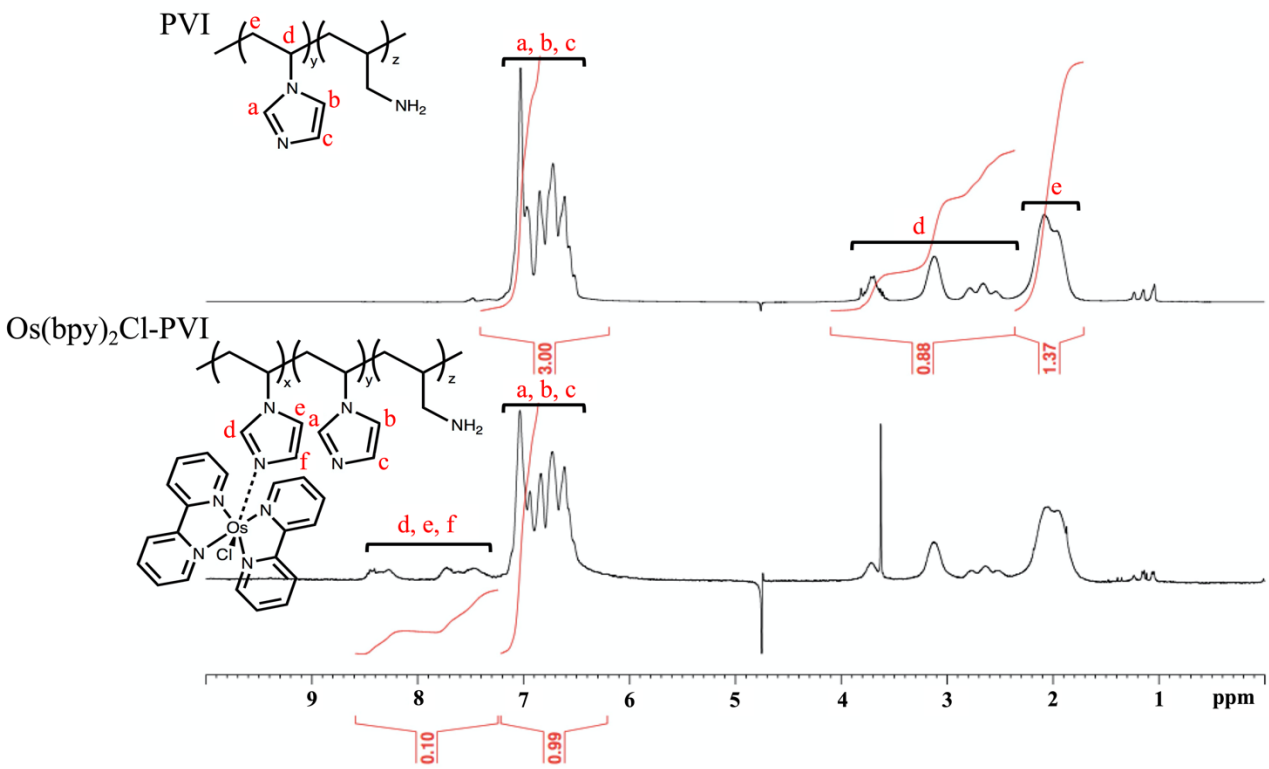
A Low-Interference, High-Resolution Multianalyte Electrochemical Biosensor

Sara L. Melow[1], Dusty R. Miller[1], Evan A. Gizzie[1], and David E. Cliffel[1-2*]

Supplementary Information.



Supplemental Figure 1 (Continuous Measurement of Os-based GOx Sensor). Graphical representation of the current (µA) vs. time (min) using an Os-based GOx sensor for glucose detection. One example for a continuous measurement using this sensor for glucose with 18 calibrants ranging from 0 to 23 mM where the calibrants are highlighted in blue. There is buffered saline solution in between calibrants to obtain a steady state in current before measuring the next calibrant. Experiment was performed in buffered saline solution (ambient conditions) at 0.2 V.



Supplemental Figure 2 (^{1}H-NMR of Poly(N-vinylimidazole-allylamine) and Os(bpy)$_2$Cl-PVI). The ^{1}H-NMR spectra of PVI and Os-based PVI (20 mg mL^{-1} in D_2O) were collected from at least 128 scans using a 400 MHz Bruker spectrometer with water suppression. There is a multiplet at δ 6.5-7 ppm that corresponds to the protons a, b, and c on the structure for both polymers (I and II). The PVI spectrum (top) shows a single proton (d) that produced three groups of peaks. At δ 2 ppm, there are two protons (e) that produced a doublet. The Os-polymer spectrum (bottom) had imidazole protons (d, e, and f) that produced signals at δ 7.5-8.5 ppm, resulting from the Os-complex substitution on the polymer backbone.

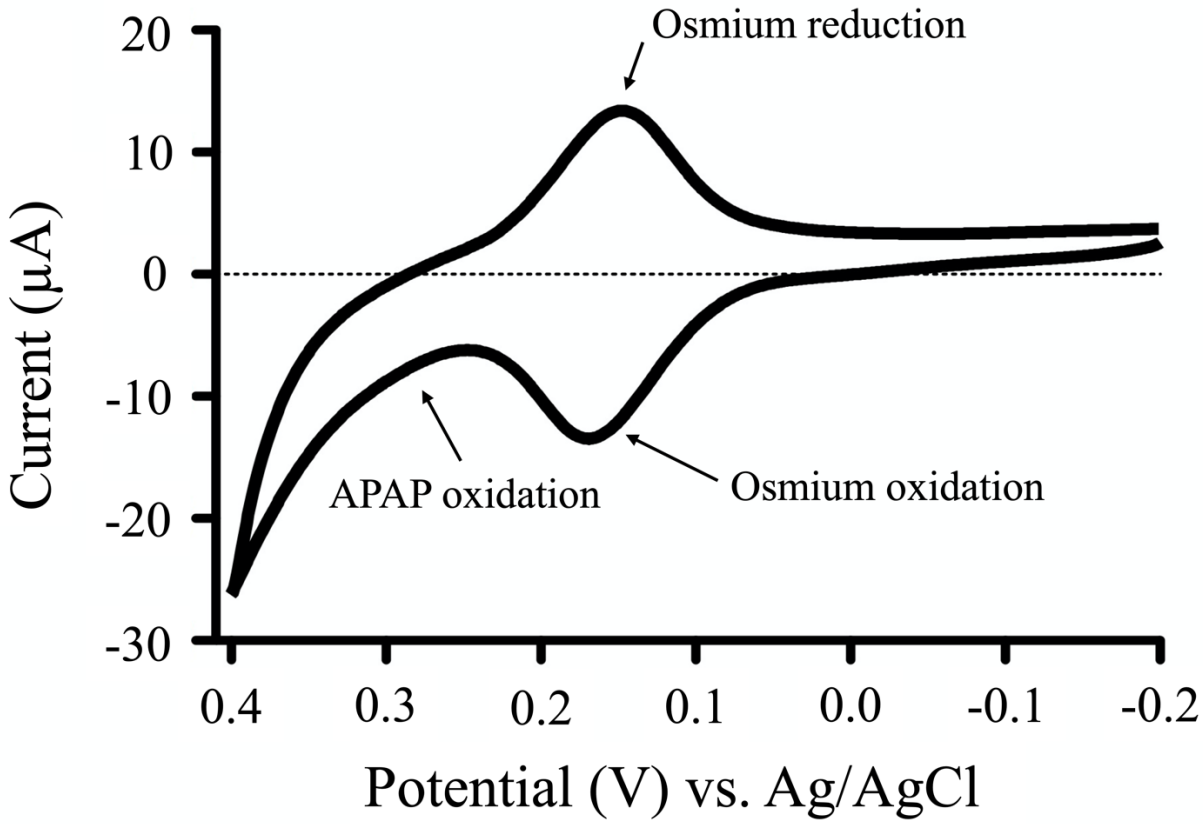
The unloaded polymer, PVI, (**Supplemental Figure 2 PVI**) was analyzed by ^{1}H-NMR. Peak integrations were normalized to three protons for the multiplet of peaks in the downfield region (δ 6.5-7 ppm) corresponding to the aromatic protons of the imidazole sidechain (PVI – a, b, and c). Due to the mixed tacticity of this polymer, the proton signal attributed to the single proton (PVI – d) is observed as three different groups of peaks (δ 2.4-3.6 ppm). Finally, the two

protons found on the unsubstituted carbon in the polymer backbone (PVI – e) produced a doublet (δ 2 ppm).

After covalently attaching the Os-coordination compound to the polymer backbone, ^{1}H-NMR was used to calculate the amount of Os loading percentage on the PVI. Due to the sensitivity of the imidazole protons in their chemical environment, a large downfield shift was observed when the Os-complex substituted onto the imidazole side chain the (**Supplemental Figure 2 Os(bpy)$_2$Cl-PVI**). Imidazole protons (Os(bpy)$_2$Cl-PVI – d, e, and f) produced signals further downfield than the unsubstituted polymer (δ 7.5-8.5 ppm). By comparing the integration of protons on the unmodified imidazole (PVI – a, b, and c) to the total integration of protons for both the downfield, Os-bound imidazole (Os(bpy)$_2$Cl-PVI – d, e, and f) and the unmodified (PVI – a, b, and c), an approximation for the metal-loading percentage was calculated. This relationship is summarized below in Equation 2.

$$metal - loading\ percentage = \frac{\text{unmodified protons}}{\text{unmodified protons + bound protons}}\ X\ 100\% \quad (2)$$

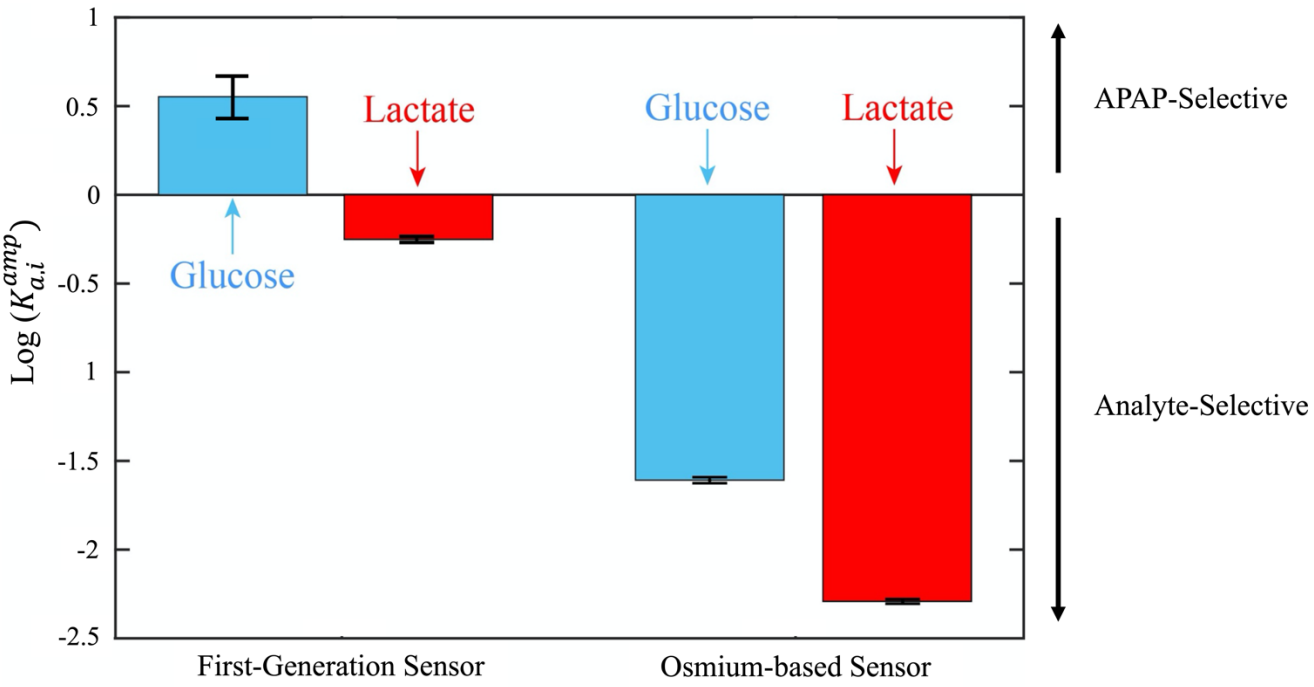
For the Os-polymer used in these studies, there was an osmium metal-loading of approximately 9%.



Supplemental Figure 3 (Cyclic Voltammogram of $Os(bpy)_2Cl$-PVI). The cyclic voltammogram was performed using an SPE modified with Os-polymer in a 26-μL flow chamber under a flow rate of 100 μL min^{-1}, showing the oxidation of the osmium at 0.177 V and a reduction of the osmium at 0.135 V (1 mM phosphate buffer, 120 mM KCl, 10 mM APAP, pH = 7.00). The current vs. potential is shown in the CV (-0.2 to 0.4 V, 0.5 V s^{-1}) of the Os-polymer. Oxidation of APAP began around 0.250 V (vs. Ag/AgCl). An internal reference, Ag/AgCl, and counter electrode, Pt, were used.

Based on the cyclic voltammogram, a redox potential of 0.156 V (vs. Ag/AgCl) was determined. The electrochemical reversibility of this redox couple was evident in the ΔE_p of 21 mV. To identify the onset potential of bulk APAP relative to the $Os^{2+/3+}$ redox couple, APAP was included in this voltammogram. The onset of APAP oxidation occurred around 0.250 V (vs.

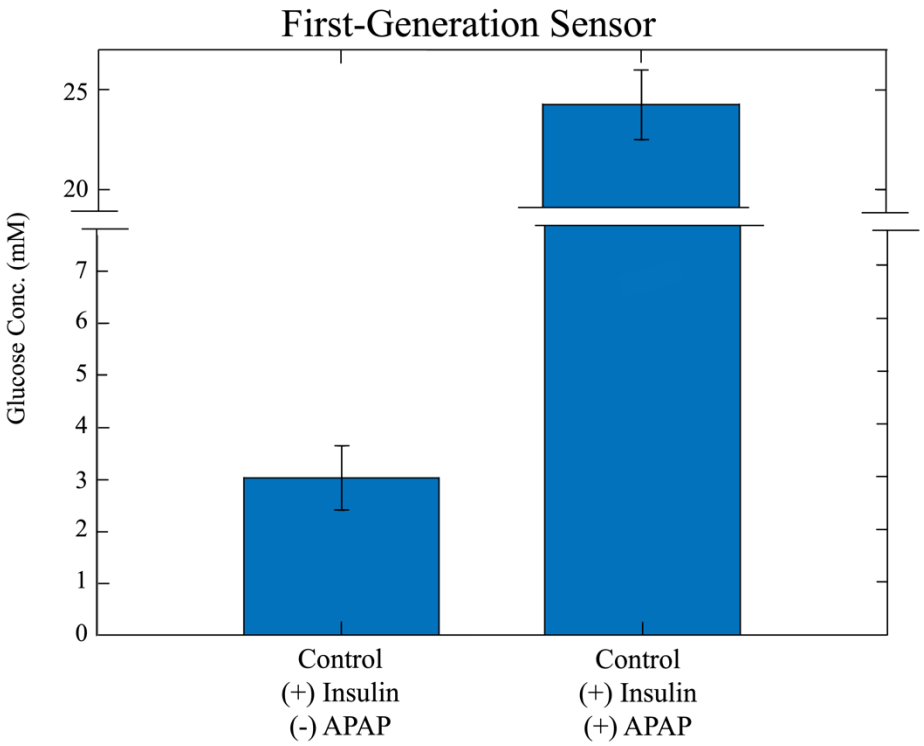
Ag/AgCl). To oxidize the Os couple to the 3+ state, a potential that was slightly more positive than the peak in the oxidation portion of the voltammogram was selected. In addition, to avoid APAP interference, this potential must be less than 0.250 V (vs. Ag/AgCl), thus 0.2 V (vs. Ag/AgCl) was ideal for this sensor operation and was used in all studies and experiments.



Supplemental Figure 4 (Selectivity Assay Graph Comparing Synthesized Os-Polymer Sensors and First-Generation Sensors). Logarithmic representation of the selectivity coefficients for APAP with glucose (**blue**) and lactate (**red**) sensors. The first-generation sensors (**left**)—oxidase enzyme with BSA—had log ($K_{a,i}^{amp}$) values of 0.5 ± 0.1 for glucose and -0.25 ± 0.01 for lactate, indicating a preference of APAP over glucose and a slight preference for lactate over APAP, respectively. After coupling one of the oxidase enzymes with the Os-polymer, the Os-based sensors (**right**) had selectivity coefficients for glucose at -1.61 ± 0.03 and lactate at -2.29 ± 0.02, indicating a strong preference for the analyte in both cases. Potentials were held at 0.6 V (vs. Ag/AgCl) for the first-generation sensors and 0.2 V (vs. Ag/AgCl) for the Os-polymer sensors.

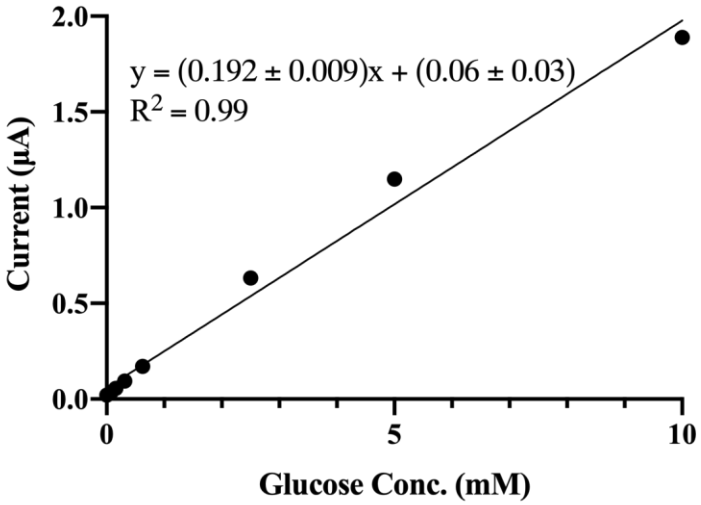
Experiments were performed in buffered saline solution at ambient conditions. Data represented as the average with standard deviation, n = 3.

The amperometric selectivity coefficients are presented as $\log(K_{a,i}^{amp})$ values. A positive selectivity value indicated the sensor was APAP-selective, and a negative selectivity value was analyte-selective. The first-generation sensors had a positive value for the glucose selectivity coefficient and a slightly negative coefficient for lactate. Therefore, the first-generation sensor was more selective for APAP with the glucose sensor, while the lactate sensor was only slightly more selective for the analyte. However, the Os-polymer sensors were significantly more negative for both analytes compared to the first-generation ($p < 0.01$), meaning they were more selective for the analytes. Direct comparison of these sensor types revealed the increased selectivity of Os-polymer sensors to the analyte of interest over a model interferent, APAP.

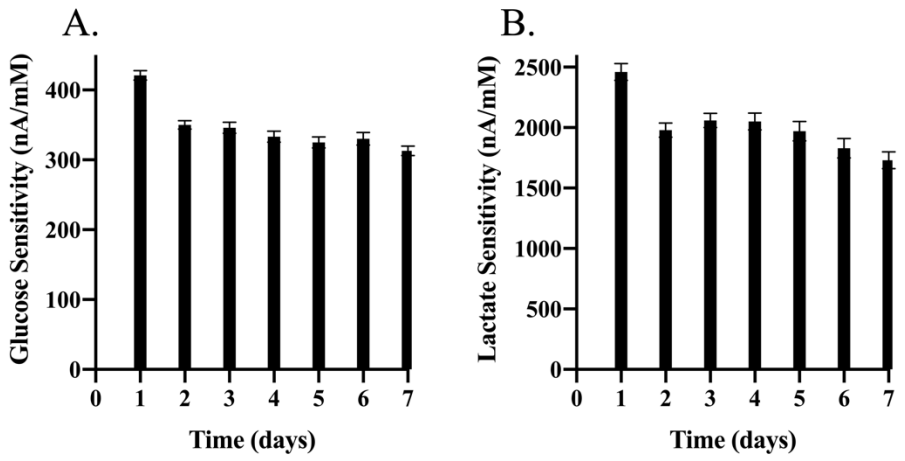


Supplemental Figure 5 (Comparison Graph of Control Media with First-Generation Sensors). Bar graph displaying the glucose concentration vs. controls (no cells) using a first-

generation sensor for analysis. The glucose concentrations of the control without APAP (reduced glucose media with 10 μg mL^{-1} insulin) was 3.0 ± 0.6 mM glucose, while the same control with added APAP (1.4 mM) had 24 ± 2 mM glucose. Experiments were performed in reduced glucose DMEM/F12 media (ambient conditions). Data represented as the average and the standard error, n = 3 biological replicates.



Supplemental Figure 6 (First-Generation Glucose Sensor Calibration Curve). Representative calibration curve of anodic current vs. analyte concentration showing the linear range of a first-generation GOx sensor. This shows the calibration from 0 to 10 mM. The slope is represented by a solid black line [y = (0.192 ± 0.009)x – (0.06 ± 0.03), R^2 = 0.99] for the first-generation GOx sensor. Experiments were performed in buffered saline solution (ambient conditions).



Supplemental Figure 7 (Comparison Graphs for Glucose and Lactate Sensitivities for Operational Longevity). Operational longevity of the Os-based glucose oxidase (A) and lactate oxidase (B) sensors was tested by calibrating daily for seven days. Sensitivity (nA/mM) vs. calibration day is reported above. The sensitivity of the GOx sensor decreased from 420 ± 7 nA mM^{-1} to 313 ± 7 nA mM^{-1}, and the LOx sensor sensitivity decreased from 2460 ± 70 nA mM^{-1} to 1730 ± 70 nA mM^{-1} in the course of the week. Experiments were performed in buffered saline solution (ambient conditions) at 0.2 V. Data represented as the average, n = 3 replicates.