In Section S.I of this Supplemental Information we describe cyclic voltammetry (CV) scans that were collected to further confirm the behavior of the optical baseline signal of the single-mode EA-IOW under potential modulation. The results show a regular and persistent change in the baseline signal. These results prompted us to develop the mathematical formalism described in the companion article to properly analyze data under AC potential modulation.

In Section S.II, we show optical absorbance data of the electro-active cytochrome c protein layer collected under CV scans. The results clearly demonstrate that the surface-density of redox species is constant during potential modulation.
In Section S.III, we display the electrical impedance data, $Z(\omega)$, of the electrochemical cell as measured by the potentiostat during the AC potential modulation simultaneously to the acquisition of the optical data described in the article. The electrical impedance data is used in the article for calculations of the reaction rate constant.

In Section S.IV we show our measured data for the capacitance, $C_{dl}$, of the electric double layer, which is also used in the article for the characterization of the RC constants of the electro-active layer.

In Section S. V we experimentally determined the sensitivity factor of the single-mode EA-IOW and confirm our modelling results for such parameter.

S.I. EA-IOW Baseline Signal under Electric Potential Modulation

We describe here the impact of the electrical potential modulation in the optical signal propagating through the single-mode EA-IOW device when no redox species were present inside the electrochemical cell. Cyclic voltammetry (CV) scans were applied to the working electrode and the optical signal was simultaneously monitored. The CV scans were set to a speed of 0.02 V/s from -0.4 V to 0.8 V. For this experiment, a broadband light source from a tungsten-halogen lamp was in-coupled to the single-mode EA-IOW device. A monochromator connected to an intensified CCD (PI-MAX3) were used to collect the optical spectra out-coupled from the single-mode EA-IOW device. The ICCD was set to a 2-Hz acquisition rate and a 200-ms exposure time for each frame. The transverse electric (TE) polarization was selected for all experiments described in this work. As an illustration of the data acquired, Figure S.1.a shows the intensities of the optical signal at two wavelengths (550 and 600 nm) and the associated trace of the CV
potential scan against time. We observe in Figure S.1.a that when the potential moves towards low values (-0.4 V) the optical intensity at both wavelengths goes down, however as the potential approaches high values (+0.8 V) then the optical intensities for those two wavelengths go in opposite directions. In Figure S.1.b the multiple (about 5) CV cycles are folded into a single cycle and displayed for 10 different wavelengths. For better comparison between the different wavelengths, Figure S.1.b shows the normalized throughput where (for each wavelength) the lowest and highest optical intensities are assigned values of 0 and 1 respectively, and the other signals during the multiple cycles are then linearly scaled between those two extreme values. The normalized throughput is plotted against the electric potential, and the traces for each wavelength are shifted in increments of 1 in the y-axis to facilitate visualization. First, we observe in Figure S.1.b that the optical signals during the CV scans are remarkably reversible and repeatable. Next, we note that the optical throughput is clearly dependent on the applied potential. Finally, we remark that the potential for maximum optical intensity shifts from a lower value (-0.2 V) at 510 nm to a higher value (+0.8 V) at 600 nm with an almost linear relationship.
Figure S.1: (a) Waveform of the out-coupled optical intensity from the single-mode EA-IOW device for the 550-nm and 600-nm wavelengths and the CV scanning potential against time. (b) Normalized optical throughput (see text for its definition) under CV potential scan at several wavelengths. Each color includes traces of many cycles obtained under the CV scan to demonstrate the reversibility and repeatability of the optical signal with respect to the potential modulation.

The optical signal above corresponds to the baseline in an absorbance measurement, thus we can conclude that the optical baseline changes under an electric potential modulation. Most likely those changes, which have been reported earlier \(^1\), are related to the electro-optical characteristics of the ITO working electrode. In addition, our experimental data here indicate that this behavior is different for each wavelength. Therefore, to obtain accurate results of absorbance measurements in the single-mode EA-IOW platform under potential modulation (either CV or AC) those effects must be considered. For CV scans at relatively slow scan speeds, one can acquire data in the absence and in the presence of the sample of interest, and then determine the absorbance at each electric potential on a point-by-point basis. That is the approach described in the following Section S.II for obtaining the absorbance under CV scan modulation. However, for AC potential modulation described in the companion article, where the optical response has a time delay that varies with the modulation frequency, the previous approach would be at least cumbersome. To overcome those difficulties we have developed in Section III of the companion article a mathematical formalism that provides an accurate protocol to retrieve information from optical measurements under AC potential modulation. Such
analysis is certainly required to reach accurate optical absorbance results, and therefore crucial to
the electro-chemical information that are derived from those optical results.

S.II. Absorbance Results with Cyclic Voltammetry in the Single-Mode EA-IOW

For the measurements of optical absorbance under CV scans, we used as the probing
wavelengths two narrow bands (about 3 nm of full-width at half maxima (FWHM) centered at
550 and 556.5 nm) from a super-continuum laser source (FemtoPower 1060, Fianium Ultrafast
Fiber Lasers) connected to an acousto-optical tunable filter. At the 550-nm wavelength, cytochrome c protein undergoes a large change in molar absorptivity between the two redox states \( \epsilon_{\text{red}} = 27.7 \times 10^3 \text{ M}^{-1}\text{cm}^{-1}, \epsilon_{\text{ox}} = 9.0 \times 10^3 \text{ M}^{-1}\text{cm}^{-1} \) which is quite helpful to follow the redox process. On the other hand, the 556.5-nm wavelength corresponds to an isosbestic point for the cytochrome c redox couple which can be useful to monitor possible desorption/adsorption from the analytical surface. A CV potential scan with a speed of 0.02 V/s operating in the potential range from -0.4 V to 0.4 V was used for these experiments. The ICCD simultaneously collected data for those two wavelengths under the same setting conditions as described in the companion article. Initially, a baseline signal for the single-mode EA-IOW under CV scan was acquired by the ICCD with just the buffer solution inside the flowcell. After a relaxation period of 10 minutes, about 2 mL of oxidized cytochrome c dissolved in the buffer solution was injected into the flowcell. Another 30 minutes was used for stabilization of cytochrome c adsorption inside the electro-chemical cell. Then a similar CV scan was applied and another set of ICCD data was collected. The electrical current signal measured by the potentiostat was recorded as well during both acquisition steps. Finally, at the end of the
experimental section, and in order to account for effects of stray light and dark current, black ink was injected into the flowcell and a dark signal was collected by the ICCD.

A standard expression for optical absorbance, $A$, accounting for the presence of dark signal is described by Equation (S.1), where $I$ is the optical intensity under the presence of the cytochrome $c$ adlayer, $I_0$ is the baseline signal, and $I_d$ is the dark signal.

$$A(\lambda, E) = -\log_{10} \left[ \frac{I(\lambda,E) - I_d(\lambda,E)}{I_0(\lambda,E) - I_d(\lambda,E)} \right]$$  (S.1)

![Figure S.2](image)

**Figure S.2:** (a) Absorbance versus potential from multiple CV scans of 0.02 V/s with a sub-monolayer of cytochrome $c$ adsorbed onto the single-mode EA-IOW surface. (b) Faradaic current density as determined from Equation (S.4) using the CV scan data at the 550-nm wavelength.

The experimental results for optical absorbance of the cytochrome $c$ layer as measured by the single-mode EA-IOW under the CV scan are summarized in Figure S.2.a, where the traces correspond to about 5-folded cycles. We first notice in Figure S.2.a that the optical absorbance
at the 556.5-nm wavelength (the isosbestic point) is almost constant under potential modulation, which indicates that the total surface-density of cytochrome c molecules, $\Gamma_{tot}$, is unchanged during the CV scans. Although the fractions in each redox state, $\Gamma_{ox}(E)$ and $\Gamma_{red}(E)$ depend on the specific potential, this experimental result gives us the following relation: $\Gamma_{tot} = \Gamma_{ox}(E) + \Gamma_{red}(E)$. Such result allows us to write $\Gamma_{red}(E) = f(E) \Gamma_{tot}$ and $\Gamma_{ox}(E) = [1 - f(E)] \Gamma_{tot}$, where $f(E)$ is a function of the applied electric potential, $E$, and $f(E)$ varies from 0 (fully oxidized) to 1 (fully reduced) and then accounts for the fraction in each redox state.

Next, we focus on the experimental results from the 550-nm wavelength. Figure S.2.a shows the measured absorbance from 5-folded cycles of CV scans at 550 nm calculated using Equation (S.1). The potential-dependent absorbance as measured by the single-mode EA-IOW can be described by the sensitivity factor $S^3$, the surface-density of species in each oxidation state, and the corresponding molar absorptivities, $\epsilon_{ox}$ and $\epsilon_{red}$ using the following relation:

$$A(E) = S \left[ \epsilon_{ox} \Gamma_{ox}(E) + \epsilon_{red} \Gamma_{red}(E) \right] \quad (S.2)$$

Because the total surface-density was previously determined to be constant, we can then write Equation (S.2) as:

$$A(E) = S \Gamma_{tot} \left[ [1 - f(E)] \epsilon_{ox} + f(E) \epsilon_{red} \right] \quad (S.3)$$

As we have initially injected oxidized cytochrome c into the flowcell and observed the absorbance to stabilize at a value of about 0.42, we can conclude that at positive values of the potential all cytochrome molecules are fully oxidized and we have $f = 0$ in this potential range. Then we use Equation (S.3) to determine the total surface coverage, $\Gamma_{tot} = 3.2 \text{ pmol/cm}^2$, which corresponds to about 15% of a full monolayer (22 pmol/cm$^2$). Towards negative values
of the potential, cytochrome c molecules start to reduce, and the redox process is observed to reach a plateau for potential values smaller than -0.20 V. Again, by using the measured absorbance values (ca. 0.85) into Equation (S.3) we found $f = 0.49$, which is equivalent to an amount of 1.59 pmol/cm$^2$ species that were electrically reduced. In other words, we observe that, from the total amount of cytochrome c adsorbed on the ITO electrode surface, only a fraction of about 49% was effectively driven by the redox process. This fraction is actually consistent with results from previous works $^4$.

Another important piece of information that can be retrieved from the absorbance versus potential plot is the faradaic current density by using the following equation $^{3b}$:

$$i_F = \frac{nFv}{2F\Delta E} \frac{dA}{dE}$$

(S.4)

where $i_F$ is the faradaic current density from the redox reaction, $n$ is the number of electron transferred in the redox process, $F$ is the Faraday constant, $E$ is the applied potential, and $v$ is the potential scanning speed. Optically-reconstructed faradaic current density against potential is shown in Figure S.2.b. The data shows that the faradaic current density is repeatable over several cycles, and the peaks of the anodic and cathodic current density are located at about the same potential point. Reversible redox reactions with a formal potential of approximately –0.14 V was measured. And the intensity of those two peaks is also about the same. In addition, information on the apparent number of electrons (related to intermolecular interaction) involved in the redox reaction can be obtained by using Equation (S.5) $^5$, where $\Delta E_{1/2}$ is the FWHM of the faradaic current density peak. From Figure S.2.b we get $\Delta E_{1/2} = 0.1 V$, so $n_a$ is approximately 0.9, and very close to 1 indicating that the interaction between cytochrome c
molecules was weak, as one would expect due to the sub-monolayer coverage. A similar result was also obtained under AC potential modulation.

\[ n_a = \frac{90.6 \text{ mV}}{\Delta E_{1/2}} \]  

(S.5)

S.III. Electrical Impedance of the Electro-Chemical Cell

In Figure S.3 we summarized the data of the electrical impedance as measured by the potentiostat for the electrochemical cell during the AC potential modulation measurements described above. The data displayed in Figure S.3 corresponds to a DC bias potential of -0.08 V, and it shows both the amplitude and phase of the total electrical impedance across the electrochemical cell. From the plot below we can estimate the solution resistance \( R_s \) and the magnitude of the total current \( I_t \) inside the electrochemical cell. At higher frequencies, we have \( |Z_t| \cong R_s \cong 100 \ \Omega \), which allows us to estimate the magnitude of the total current to be \( I_t \cong 100 \ \mu\text{A} \) (10 mV / 100 \( \Omega \)). As the magnitude of the faradaic current \( I_F \) is smaller than 1.8 \( \mu\text{A} \) (250 nA/cm\(^2\) \( \times \) 7.1 cm\(^2\)) we confirm that our assumption \( I_F \ll I_t \) is satisfied.
Figure S.3: Amplitude (blue trace with legend on the left) and phase (red trace with legend on the right) of the total electrical impedance, \(Z_t\) of the electrochemical cell as measured by the potentiostat for different modulation frequencies of potential at the DC bias potential of -0.08 V (closest data to the formal potential).

S.IV. Electric Double-Layer Capacitance

The double-layer capacitance was measured by collecting current, \(i\), and potential data using a potentiostat under CV potential modulation with the flowcell filled with buffer solution (i.e. in the absence of cytochrome \(c\) species inside the cell). Such data is displayed for different scanning speeds, \(v\), in Figure S.4.a. Then, we apply Equation (S.6) at a particular potential to determine the double-layer capacitance. The results are shown in Figure S.4.b, where the slope gives us the following result for the double-layer capacitance, \(C_{dl} = 111.3 \, \mu F\). As the solution resistance is about 100 \(\Omega\), we find an \(R_s \, C_{dl}\) time-constant of approximately 11 ms.

\[
i = C_{dl} \, v \quad \text{(S.6)}
\]
Figure S.4: (a) Electric current versus potential at different scanning speeds, \( v \). (b) Electric current versus scanning speed at a fixed electric potential (-0.20 V).

S.V. Experimental Data on the Sensitivity Factor of the Single-Mode EA-IOW

We have used blue dextran in aqueous solutions of several concentrations and performed absorbance measurements using both i) a conventional spectrophotometer with a cuvette of 1-cm pathlength and ii) the single-mode EA-IOW. The experimental results of the IOW absorbance versus solution absorbance are plotted in the Figure S.5 below and, as described elsewhere\(^6\), the slope of the curve can be used to determine the sensitivity factor (\( S = \text{slope} \times \frac{4 \pi \sqrt{N^2-n_c^2}}{\lambda(cm)} \)). Our results for the sensitivity factor of the single-mode EA-IOW gave a value of \( S = (1.5 \pm 0.2) \times 10^4 \) averaged over the two wavelengths used in the measurements; such measurement of the sensitivity factor agrees reasonably well with the modelling results calculated using the optical constants and thickness of each layer in the single-mode EA-IOW structure.
**Figure S.5:** Absorbance of blue dextran at several concentrations in aqueous solution measured by a regular spectrophotometer (x-axis) with a 1-cm pathlength and by the single-mode EA-IOW device (y-axis). The slope of the curve is used to determine the sensitivity factor, as described elsewhere. The blue curve corresponds to a wavelength of 560 nm and the red curve corresponds to a wavelength of 570 nm.

**S.V. References**

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