

SUPPORTING INFORMATION

CAPACITANCE SPECTROSCOPY: A VERSITILE APPROACH TO RESOLVING THE REDOX DENSITY OF STATES AND KINETICS IN REDOX-ACTIVE SELF-ASSEMBLED MONOLAYERS

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SI.1 Experimental Procedures and Methods

SI.1.1. Materials

1-hexanethiol, 1-octanethiol, 1-decanethiol and 1-dodecanethiol were purchased from Sigma-Aldrich. 11-ferrocenyl-1-undecanethiol was purchased from Dojindo Molecular Technologies, Japan. All were used without further modification. Tetrabutylammonium salts (perchlorate, hexafluorophosphate) HPLC pure ethanol, dichloromethane and acetonitrile were used as received (Sigma). *Pseudomonas aeruginosa* azurin was kindly donated by Prof. Canters group (Leiden University, NL). All solutions were prepared in MilliQ water (18.2 MΩ).

SI.1.2. Sample preparation

Polycrystalline Gold Disk Electrodes (GDE) (Cypress Gold, diameter 1mm) were cleaned following a published procedure¹ and immersed in 1mM thiol solutions in HPLC-pure ethanol (overnight, room temperature). The electrodes were then rinsed with ethanol and water and dried under nitrogen. Azurin physisorption on hydrophobic SAM surfaces of (hexanethiol, octanethiol, decanethiol, dodecanethiol) was obtained by depositing for 30 min. an aliquote of 5μL of 0.5mM protein in 20mM MES buffer at pH7.0. The so-modified electrodes were then rinsed copiously with buffer and immersed in the electrochemical cell for analysis.

SI.1.3. Instrumentation and procedure

Cyclic Voltammetry (CV) and impedance measurements were carried out using an Autolab potentiostat PGSTAT20 (Ecochemie NL) equipped with an ADC750 and a FRA (frequency response analyses) module and FRA software. The Cyclic voltammograms were acquired only with the purpose of comparisons between the two methodologies. Single CV sweeps were also performed to pre-define faradaic windows prior to EIS analysis (required only if the redox potentials are not known prior). The AC frequencies for impedance experiments ranged from 1 MHz to 10 mHz, with an amplitude of 10 mV. All the obtained impedance data were checked regarding to compliance with the constraints of linear systems theory by Kramers-Kronig using the appropriate routine of the FRA AUTOLAB software. The complex $Z^*(\omega)$ (impedance) function was converted into $C^*(\omega)$ (capacitance) through the physical definition $Z^*(\omega) = 1/j\omega C^*(\omega)$ in which ω is the angular frequency (for detailed information see SI. 3). CVs and EIS scans were measured in a 5 mL, one compartment cell, containing the GDE, a saturated calomel reference (SCE) and a platinum gauze as

counter electrode. As a supporting electrolyte, 200 mM NaClO₄ and 5 mM MES, buffered at pH 5.0 with NaOH, was used. All the solutions used for electrochemistry were deoxygenated by bubbling with ultrapure argon and purging the surface of the electrolyte for the duration of the experiment. After protein physisorption, thermodynamic and kinetic parameters were determined, by CV for comparative purposes only, by fitting to Butler-Volmer theory (SI. 2). Impedance data were acquired at fixed potentials of -200 mV and 90 mV vs. SCE, with modulation frequencies were varied in 80 steps from 0.1 mHz to 10 MHz, and analyzed as described below.

SI.2Azurin film electron transfer kinetics: CV determination

CVs were acquired in the potential window between -0.30 and 0.40 V vs. SCE after protein physisorption, generating typical responses as shown in Figure S1. The only purpose of CV taken here was to obtain the apparent electron transfer rate to be compared with EMCS methodology described in the main text for the same systems.

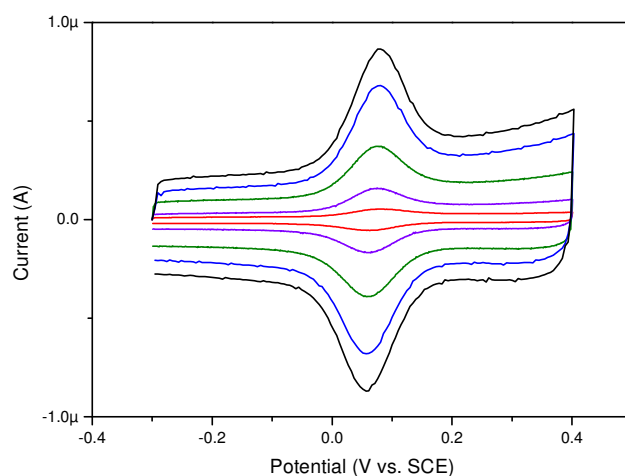


Figure S1. Cyclic voltammograms measured for azurin immobilized on an octanethiol SAM in pH5.0 MES buffer with 200mM NaClO₄ supporting electrolyte. Starting from the central line, data were acquired at a scan rate of 0.5 V/s (red), 3 V/s (purple), 5 V/s (green), 10 V/s (blue) and 12 V/s (black).

The anodic and cathodic peaks positions were determined by using the peaks fitting function provided in the software GPES (Ecochemie, NL); the baseline of the peaks was determined by polynomial interpolation of the capacitive currents at the extremes of the potential window. As expected for surface confined electroactive molecules², the peaks height scaled linearly with the scan rate applied as in Figure S2.

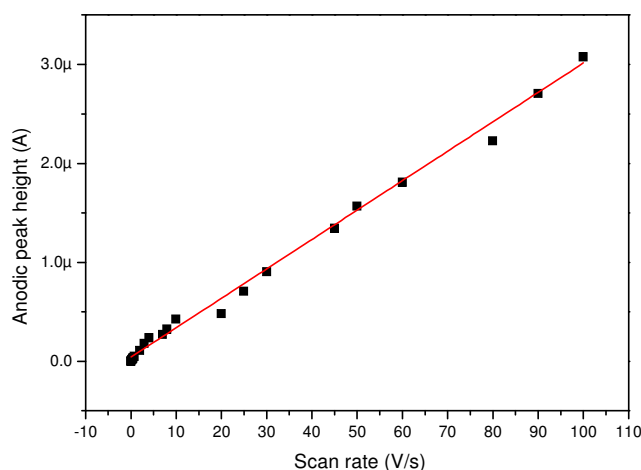


Figure S2. Current peaks height increases linearly with the applied scan rate for a film of azurin at an octanethiol-modified electrode.

Plotting the experimentally obtained peak potentials for the cathodic and anodic processes against the logarithm of the scan rate for surface immobilized redox species gives a ‘trumpet plot’, so called because of its characteristic shape. A finite differential procedure³ was used to fit the peak to peak separation to the Butler-Volmer equations, after subtracting contributions from the constant peak splitting at low scan-rates.³ Trumpet plots of azurin immobilized on hexanethiol, octanethiol, decanethiol and dodecanethiol are displayed in Figure S3, where the continuous lines represent the best fit to the data and the corresponding electron transfer rates are listed in the first row in Table S1. As the thickness of the SAM increases the electron transfer rate diminishes and the peaks start to separate at higher scan rates applied, at thinner SAMs the electron transfer reaches an upper rate limit of $\sim 3000\text{s}^{-1}$.

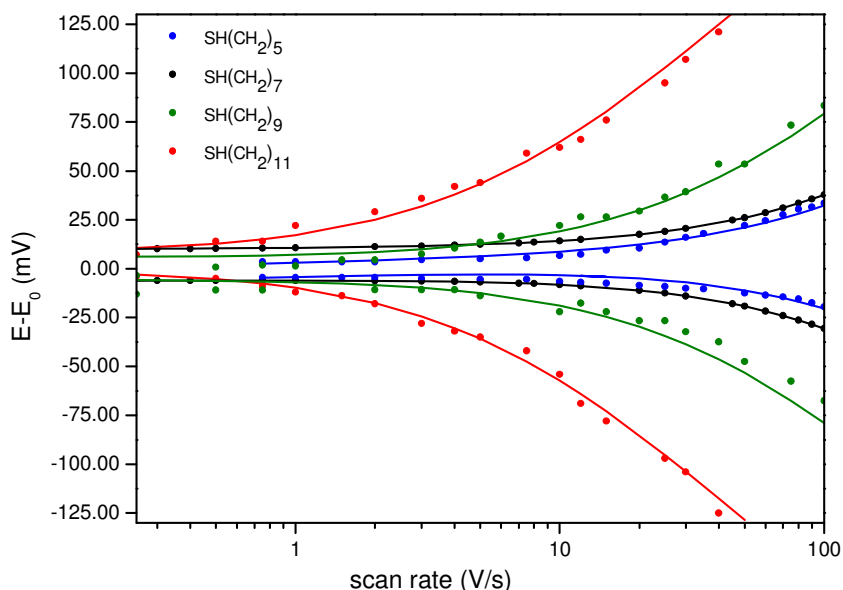


Figure S3. Trumpet plot for data acquired on several alkanethiol SAMs, with continuous lines representing fits to Butler-Volmer theory for the values reported in Table S1.

SI.3 Electroactive Monolayer Capacitive Spectroscopy

EMCS data were acquired at fixed potentials of -200 mV and 90 mV vs. SCE, potentials at which the faradaic currents are at their minimum and at a minimum and maximum respectively. Modulation frequencies were varied in 80 steps from 0.1 mHz to 10 MHz. The resulting FR data were processed and treated to obtain the imaginary part of the capacitance as mentioned previously, i.e. $Z^* = 1/j\omega C^*$. From this operation, note that $C'' = \phi Z'$ and $C' = \phi Z''$, where $\phi = (\omega |Z|^2)^{-1}$ and $|Z|$ is the modulus of Z^* . The advantages in using the outlined EMCS technique is that the end user eliminates not only the uncompensated resistance but also all the capacitive and resistive “parasitic” terms related to the non-electroactive components of any given film (see main text).

SI.4 Comparison of EMCS and CV determined electron transfer kinetics

The relative electron transfer rates evaluated with the two different methods are reported in Table S1 for four examined supporting SAM thicknesses.

	(CH ₂) ₅	(CH ₂) ₇	(CH ₂) ₉	(CH ₂) ₁₁
<i>k</i> determined by CV	3050 s ⁻¹	3390 s ⁻¹	520 s ⁻¹	80 s ⁻¹
<i>k</i> determined by EMCS	740 Hz (s ⁻¹)	1195 Hz (s ⁻¹)	141 Hz (s ⁻¹)	30 Hz (s ⁻¹)

Table S1. Electron transfer rates determined by fitting the Trumpet plot at different SAMs. Note that *k* obtained by CV is the apparent electron transfer rate from a Butler-Volmer analysis of trumpet plots, without uncompensated resistance considerations, i.e. where the monolayer polarization cannot be avoided. We believe that the differences regarding to the values found in these methodologies comes mainly from this fact.

SI.5 Electroactive Film Capacitance Spectroscopy applied to 11-ferrocenyl-1-undecanethiol films

As discussed in the main text, with the electrode potential poised outside of the faradaic window, such plots resolve only the high (kHz) frequency contributions from the alkanethiol bridge. On moving the surface potential to appropriate values, strong contributions from C_r are observed (and maximized at

the electrochemical reversible potential) at frequencies related to those resolved in frequency domain analyses. A subtraction of these contributions enables a clean quantification of C_r , meaning only the contribution of redox process.

The electron transfer rate obtained from EMCS technique is $\sim 13 \text{ s}^{-1}$ in this solvent/electrolyte system (Figure S4) in fair agreement with that resolved for the same film from chronoamperometry $40 \pm 7 \text{ s}^{-1}$ or a Butler Volmer analysis of CV data ($67 \pm 7 \text{ s}^{-1}$). We note that the $\sim 13 \text{ s}^{-1}$ determination is “pure” in the sense it does not have potential iR drop or polarization contributions, as discussed in the main text.

The uncompensated contributions of R_e , R_t and C_t to CV analyses will potentially distort the dependence of peak current or peak potential magnitude/position on voltage sweep rate or overpotential, leading to errors in the determination of electron transfer rate. An accurate determination of the manner of this kinetic distortion is the subject of ongoing work and lies beyond the remit of this current work.

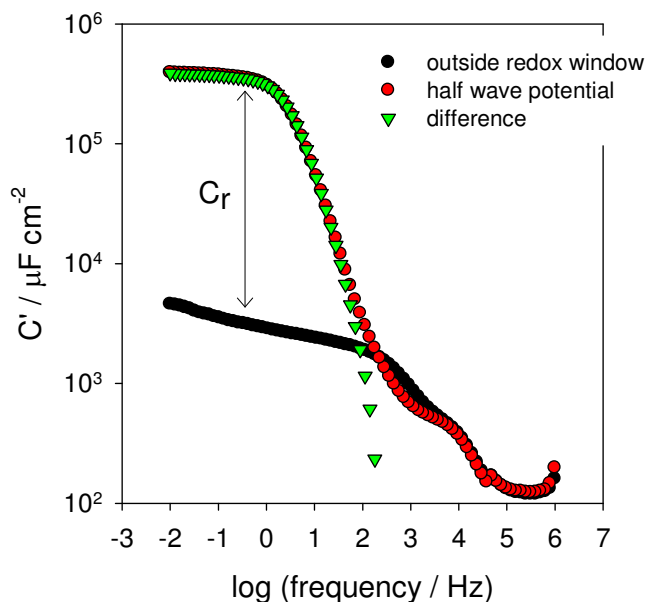


Figure S4. The real part of complex capacitance as a function of frequency mapping the capacitive contributions associated with an 11-ferrocenyl-1-undecanethiol monolayer. By using the methodology previously detailed to eliminate parasitic contributions (mainly affecting regions higher than 1 kHz, as shown), the redox capacitance is resolved and quantified.

SI.6 Electroactive Monolayer Capacitance Spectroscopy, EMCS: fundamentals

A full theoretical paper about EMCS is underway and will give more details of both the theoretical background of this methodology and further examples of its broad application. The theory is largely based on the microscopic Gerisher-Marcus model and on fundamental theories of dielectric relaxation in solids and polymers (applied for the first time herein to electroactive monolayer films).

To define the faradaic window in EMCS only a single sweep at frequencies where only faradaic processes are likely to contribute significantly (from 1 to 0.01 Hz) is required— a peak in imaginary part of complex capacitance (after background corrections as described in the main text), visualized from Bode diagrams, allows the EMCS user to define electron transfer rate of the process, and this is a simple way to obtain the electron transfer rate, without need of a model fitting or any other additional correction procedure, since the background correction eliminates all the spurious

contribution, for instance, double layer or monolayer polarization contribution, the latter responsible for uncompensated resistance.

SI.7. Mapping the redox states DOS

Diagrammatic representations of the Gaussian DOS associated with a surface confined azurin-on-decanethiol film (occupied/reduced states shaded) is shown in Figure S5 for three different steady-state potentials. It is important to note that the distributions shown on the right were extracted experimentally from capacitive analyses at the half wave potential within the EMCS experiments discussed herein. The oxidized/non-occupied $[1 - f(E_0 - E_F)]$, and the reduced/occupied $f(E_0 - E_F)$ distributions are, of course, interlinked and of relative magnitudes as dictated by the electrode potential (integrating to the same at the electrochemical half wave potential). The product of these two functions, has a maximum at the reversible potential, where $(E_0) = 1/2$ and consequently $1 - f(E_0) = 1/2$. Note that the energy states in the electrode close to E_F are considered constant and to overlap with the distributed redox states in the protein layer (DOS shown in grey).

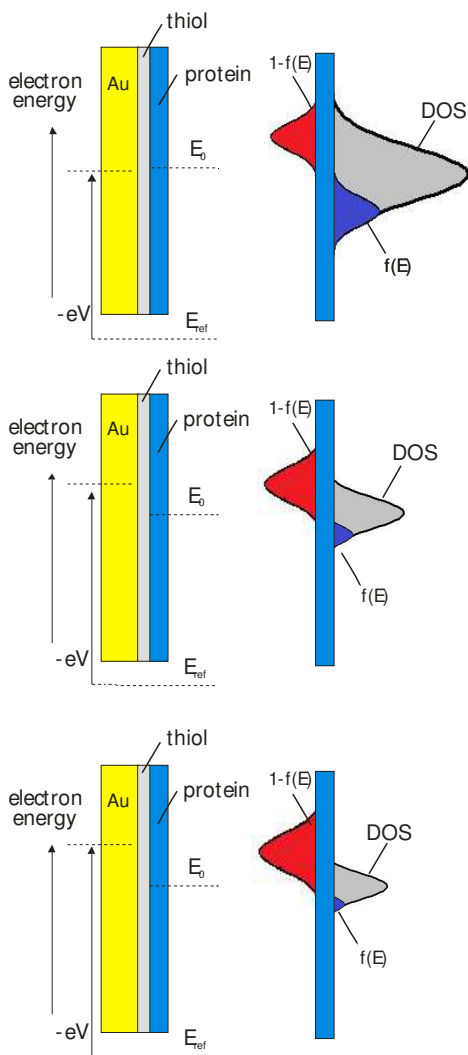


Figure S5. Diagrammatic representation of the distribution of oxidized ($1-f$) and reduced (f) redox centres in an azurin-on-decanethiol film at three different electrode potentials, showing progressive changes in sub-populations as the surface potential moves away from the half wave potential (a) to 0.25 V and 0.35 V positive of this (b) and (c) respectively.

SI.8. Visualisation and correction of iR drop contributions

As the cell and interface geometry is the same inside and outside faradaic region the iR drop contributions are likely to be equivalent and removeable by the same procedure. The validity of assuming polarization contributions are largely potential independent is testable (see SI. 11). To additionally confirm an ability to cleanly resolve the effects of solution resistance we have carried out analyses across different cell configurations. In Figures S6 and S7 below impedance spectra are shown at two different solution resistances (R_e) (tuned by changing the Working Electrode (WE) and Reference Electrode (RE) distances) for electroactive azurin-on-hexanethiol film with all other conditions unchanged. Figures S6 and S7 show data acquired outside of and then inside the redox window respectively. As can be seen in the high frequency region of the impedance spectra (insets) where faradaic processes do not contribute regardless of the redox window shown in Figure S7, the R_e value obtained are 181 Ω and 566 Ω , respectively, i.e. almost 3 times higher for the latter. In Figure S7 the R_e values obtained in the two electrode distance situations are 186 Ω and 570 Ω , respectively. The methodologies applied herein cleanly resolve iR drop.

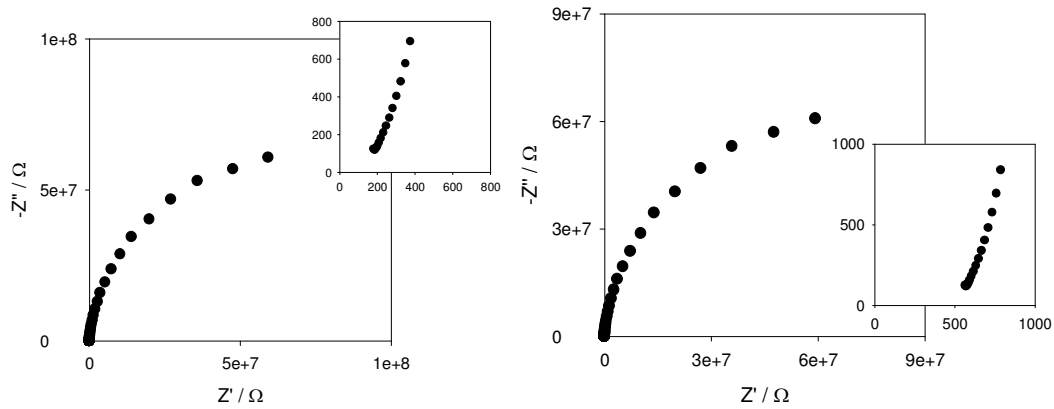


Figure S6. Impedance Nyquist diagrams of electroactive azurin-on-hexanethiol film at -200 mV vs. SCE (outside the redox window where the impedance response is predominantly due to the SAM parasitic terms) at two different solution resistances. The solution resistance can be evaluated from the high frequency region of the spectra, (the insets here as 181 Ω and 566 Ω).

R_e is eliminated from subsequent spectral analysis by means of $Z'(\omega) - R_e$ and some practical examples were shown in the previous paragraphs. At the end of this procedure, then, the $C^*(\omega)$ function is obtained from $Z^*(\omega)$ without unwanted iR drop contributions.

The effectiveness of this procedure is shown in Figure S8 in which Bode plots at two different cell resistances are presented after correction – they are clearly almost perfectly superimposed. It is clear, then, that the data we present is unaffected by solution resistance (which, as we have stated, is utterly removed from the raw data prior to further corrections as detailed below).

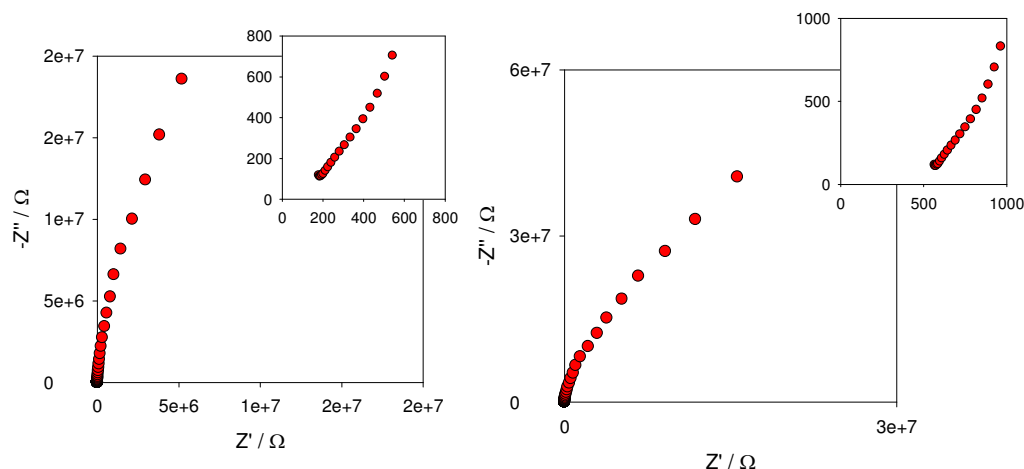


Figure S7. Impedance Nyquist diagrams of electroactive azurin-on-hexanethiol film at -90 mV vs. SCE (meaning around reversible potential where the impedance response has both component, i.e. SAM parasitic non-faradaic relaxation and redox relaxation due to faradaic effects) for the same systems at two different solution resistance. The solution resistance can be readily evaluated from the high frequency region of the spectra (insets here 186 Ω and 570 Ω).

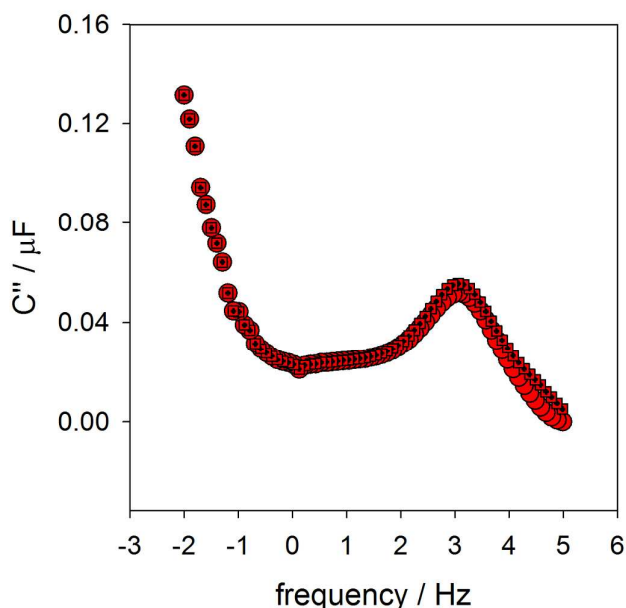


Figure S8. Comparative plots of the imaginary part of complex capacitance, as used to determine faradaic electron transfer kinetics, after iR drop correction in the case of two different solution resistances. The film here is azurin-on-hexanethiol.

SI.9. Visualisation and removal of SAM polarization contributions

The previous section dealt with solution iR drop effects. We deal separately here with SAM relaxation and the associated parallel double layer contributions to acquired spectra. Figure S9 shows the contribution of the parasitic relaxation (obtained when measurement is conducted outside faradaic windows, *black curve*) and the capacitive response of the electroactive azurin-on-hexanethiol film conducted at the reversible potential. The subtraction of these two responses gives a capacitive spectrum in which the influence of SAM dielectric polarization (and double layer) is accounted for and then subtracted. Note that, as mentioned above, this system is in the adiabatic regime and the subtraction works well. The vast majority of interfacial electrochemical analysis that will be relevant

to readers will be nonadiabatic where the native frequencies of the faradaic and polarization contributions are far apart, but here the data illustrate the ability of the methodology in doing the necessary corrections on the data.

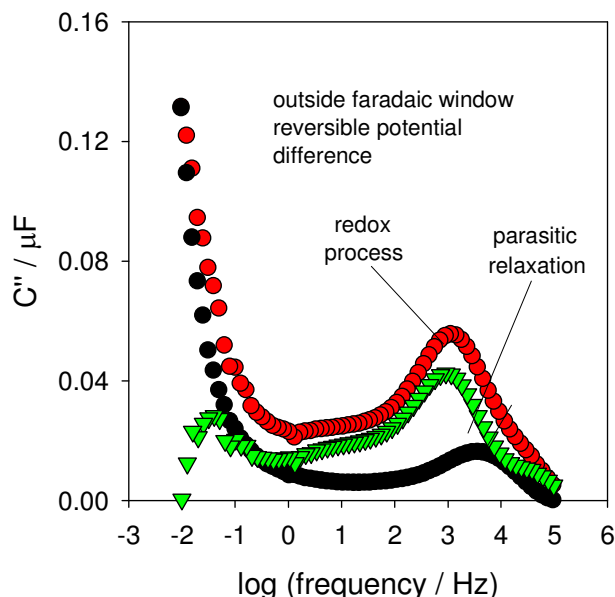


Figure S9. Comparison of the imaginary part of complex capacitance of a redox active film obtained for different solution resistance, after iR drop correction (see above). The film is composed of electroactive azurin-on-hexanethiol. Observe that the redox relaxation is essentially superposed on the SAM parasitic relaxation (red curve). At this stage of the methodology, the SAM and double layer parasitic polarization contributions are coupled to the observed kinetics despite the solution resistance compensation (red curve). After subtraction of the black curve (the response of the film outside redox window where only the parasitic terms contribute) from the red curve, the green curve is obtained. The green curve is the response of the redox process corrected in terms of SAM and double layer parasitic relaxation, after the iR drop corrections in the terms discussed previously.

SI.10. Further clarification of nonfaradaicmonolayer dielectric parasitic polarization

The double layer in series with monolayer capacitance generates parallel parasitic contributions. These terms can be obtained when the system is polarized outside redox windows and then subtracted accordingly in the capacitive domain of FRA data when the electrode has the additional redox contribution, i.e. when subsequently the electrode is poised inside redox window. Here we will provide an additional explanation using capacitive Nyquist diagrams for slow (azurin-on-dodecanethiol film) and fast (azurin-on-hexanethiol film) faradaic processes, complementary to the Bode analysis conducted in Figure 3b and 3c of the manuscript.

After the iR drop correction conducted by $Z'(\omega) - R_e$, where R_e is the electrolyte resistance, it is possible to observe the faradaic response in the capacitive domain affected only by double layer and monolayer capacitance in parallel to the monolayer dielectric polarization process, as can be observed in FigureS10 alternatively in Nyquist capacitive diagrams for azurin-on-dodecanethiol film, complementary to what is shown in Figure 3 of the manuscript. The combined double layer capacitance is around $0.8 \mu\text{F}.\text{cm}^{-2}$ as shown from the diameter of the semicircle in Figure S10a. When the electrode poised in the reversible potential inside redox windows, an additional faradaic process contribute with an additional semicircle and capacitance, precisely the redox capacitance, identified as $3 \mu\text{F}.\text{cm}^{-2}$. After the subtract procedure i.e. the subtraction of black curve in Figure S10a from that shown in red in Figure S10b the redox faradaic process is corrected for nonfaradaic contributions and the resultant curve is shown in Figure 7b in the main manuscript.

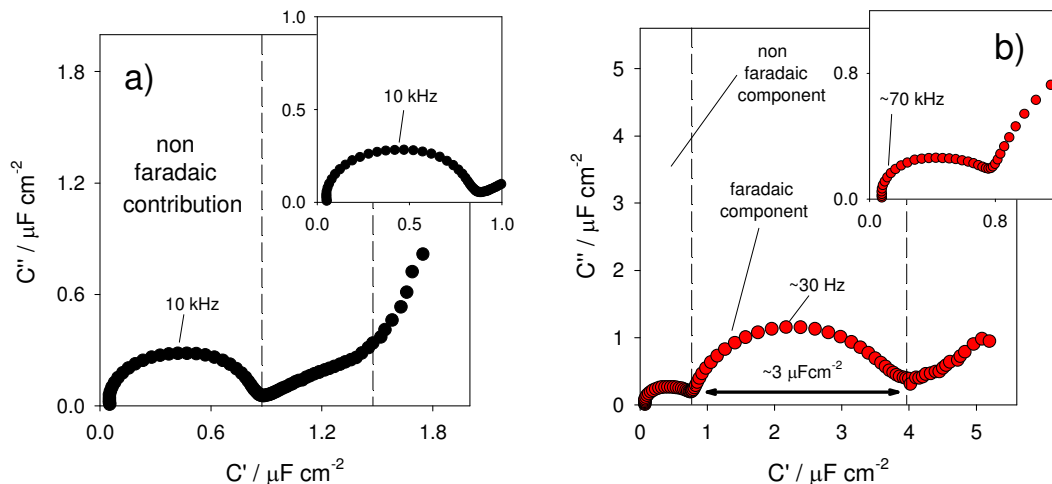


Figure S10. Nyquist capacitive plots of EMCS analysis with the electrode potential poised (a) outside of the redox window where the response is dominated by the nonfaradaic process (here of dodecanethiol) and (b) at the electrochemical reversible potential, i.e. with the electrode poised inside redox windows. The resultant EMCS spectrum (a subtraction of (a) from (b)) is shown in Figure 7b of the manuscript, where the nonfaradaic contribution is corrected and only one semicircle remains. The value of C_r can be clearly obtained from the diameter of the semicircle (b) or Figure 7b. Insets correspond to magnifications of the respective high frequency spectral regions shows the successful subtraction of thiol dielectric contribution (in that the faradaic contributions are corrected from nonfaradaic, with the faradaic onset commencing at zero in Figure 7b).

In Figure S11 the same process is highlighted within Nyquist capacitive diagrams for azurin-on-hexanethiol film, where the nonfaradaic and faradaic processes are, this time, highly superimposed. As illustrated (and complementary to the analysis conducted in Figure 3b of the manuscript), the nonfaradaic contributions can still be subtracted.

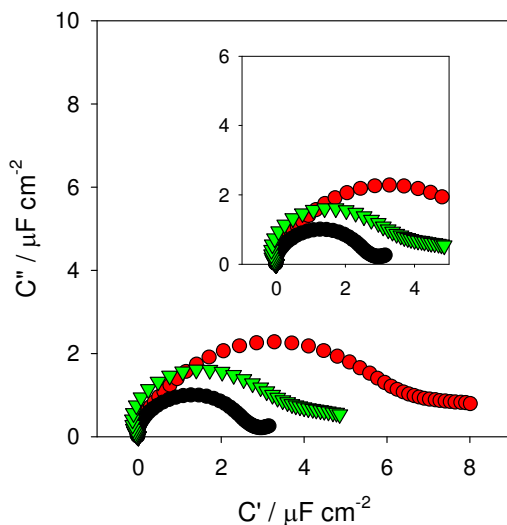


Figure S11. Nyquist capacitive plots of EMCS analysis with the electrode potential poised (black curve) outside of the redox window where the response is dominated by the nonfaradaic process (here hexanethiol) and (red curve) at the electrochemical reversible potential, i.e. with the electrode poised inside redox windows. The resultant EMCS spectrum (green curve), where the nonfaradaic contribution is corrected. Observe that in such a case the processes are highly convoluted (see also Figure 3b of the main text). The value of C_r can be obtained from the diameter of the remained semicircle (green curve). Insets correspond to magnifications of the respective high frequency spectral regions.

SI.11. Polarization test

EMCS is a steady-state technique as noted in the introduction section of the manuscript. To investigate the potential limitations of assuming that polarisation contributions (“parasitic contributions”) are equivalent inside and outside of the redox window, we include here an analysis of across a broad range of potentials (Figure S12) at a fixed frequency of 100 Hz. As can be seen the capacitive response at -200 mV (outside redox window) is essentially the same as that at 90 mV (inside the redox window). The capacitive response after systematic solution resistance and SAM relaxation subtractions is, thus, likely to be very close to a “pure” redox response.

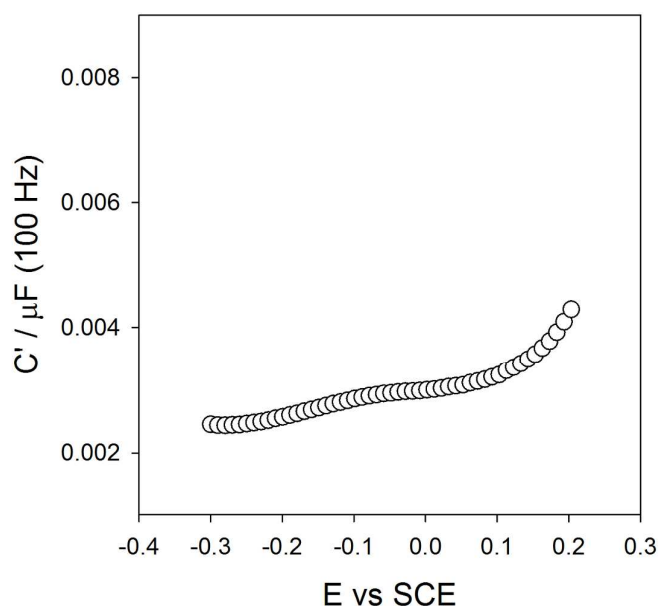


Figure S12. Real component of complex capacitance for SAM scanned at 100 Hz, demonstrating that the capacitance response of the hexanethiolSAM outside redox windows is essentially the same as that found inside redox window.

References

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