

Multifunctional and stable monolayers on carbon: a simple and reliable method for back-filling sparse layers grafted from protected aryldiazonium ions

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ABSTRACT

A new strategy for preparation of robust multifunctional low nanometer thickness monolayers on carbon substrates is presented. Beginning with protected aryldiazonium salts, sparse monolayers of ethynyl, amino and carboxy - terminated tethers are covalently anchored to the surface. The layers are then back-filled with a second modifier via the nucleophilic addition of an amine derivative to the surface. Through use of electroactive moieties coupled to the tethers, and an electroactive amine for back-filling, electrochemical measurements reveal that back-filling approximately doubles the surface concentration of the monolayer. Cyclic voltammetry of solution-based redox probes at the modified surfaces is consistent with the expected blocking properties at various stages of surface preparation. Fractional surface coverages of the layers are estimated using electrochemically-determined surface concentrations of modifiers and computationally-derived modifier footprints.

Assuming free rotation of the coupled ferrocenyl or nitrophenyl groups leads to physically unreasonable fractional surface coverages, indicating that these larger modifiers must be rotationally restricted. Using a conformationally constrained model produces lower bound estimates of the total fractional surface coverage close to 0.4, with tether-only coverages close to 0.2. The back-filled tether layers constitute practical platforms for controlled construction of complex interfaces with many potential applications including sensing, molecular electronics and catalysis.

Keywords: nucleophilic addition; amine; click reaction; amide coupling; DFT; fractional coverage

INTRODUCTION

Surface modification through electrografting of aryldiazonium salts has been subject of a major research effort since first reported by Pinson, Saveant and co-workers in 1992.¹ The wide substrate compatibility of the method and the possibility of strong covalent bonding to the substrate account for much of the interest in the method, with many potential (and under development) applications ranging from adhesion layers²⁻⁴ to antifouling coatings⁵⁻⁷ to sensors⁸⁻¹¹ to molecular electronic junctions.^{12,13}

Using aryldiazonium salts, the main pathway for electrografting proceeds via reduction concerted with loss of N₂ to generate aryl radicals. The radicals attack the substrate resulting in a covalent bond between the aryl groups and surface. However aryl radicals are unselective and can also attack already grafted groups, leading to a multilayer film.¹⁴ At least for substrates such as glassy carbon (GC), for which the bond between the substrate and modifying group is strong, (e.g. a C-C bond), the grafted groups are not labile on the surface and cannot self-organize into close-packed arrangements. Hence it is unsurprising that

detailed investigations of film thickness and surface concentration have revealed film densities well-below those expected for close-packed layers.^{15,16,17,18}

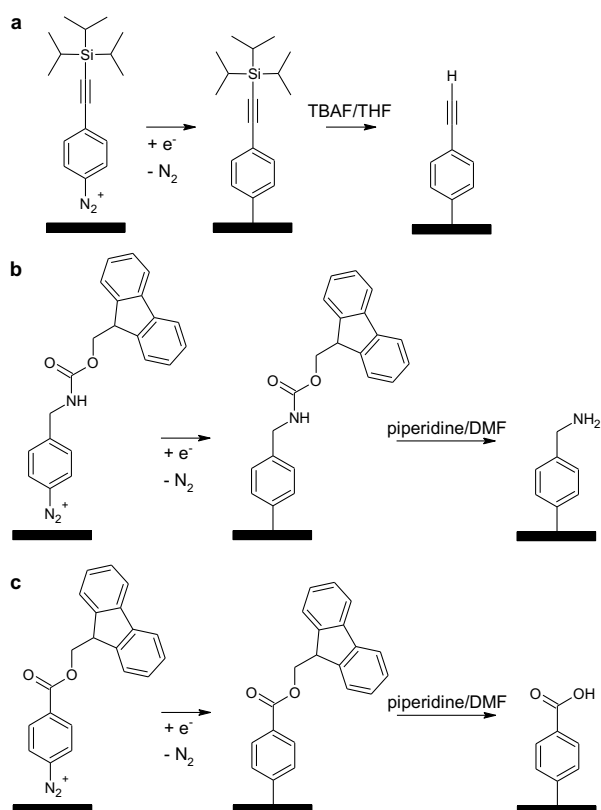
Multilayer films may be desirable for some applications of modified surfaces but, in other cases, monolayers are required. A single layer of modifiers can form the starting point for constructing a well-organized interface, such as required for sensing or for fundamental studies of electron transfer. A short distance between a redox center and the underlying electrode can also be achieved with monolayer modification and may be essential for electrochemical devices. The challenge of preparing monolayer films using aryldiazonium grafting has been addressed in two main ways. The first is to limit film growth to a monolayer. Examples of this approach are the use of aryldiazonium ions with bulky substituents that prevent attack at the aryl ring,^{19,20} or use of calix[4]arenediazonium ions in which the aryl groups are shielded by the macrocycle and its substituents on the upper rim.²¹ Alternatively, grafting the aryldiazonium ion in the presence of a radical scavenger has been shown to limit film growth to a monolayer.^{18,22} The second general strategy for monolayer preparation is to use aryldiazonium salts bearing a substituent that can be cleaved after grafting, removing multilayer growth. In the first examples, film thickness measurements after bond cleavage showed that most of the multilayer film had been removed leaving a near monolayer on the surface.^{23,24} Subsequently the concept was further developed into the ‘protection-deprotection’ strategy²⁵ which is based on aryldiazonium ions bearing bulky protecting groups.²⁵⁻³⁰ Use of sufficiently bulky groups in the *para* position prevents radical attack at already-grafted phenyl groups and ensures that after deprotection, only a monolayer film remains on the surface.

In the present work, we utilize three protected aryldiazonium ions: triisopropylsilyl (TIPS) - protected ethynylbenzenediazonium ion (TIPS-Eth-Ar-N₂⁺),^{25,26,30} fluorenylmethyloxycarbonyl (Fmoc)-protected aminomethylbenzenediazonium ion²⁷ (Fmoc-NH-CH₂-Ar-N₂⁺ and 9-fluorenylmethyl (Fm)-protected carboxybenzene diazonium ion²⁹

(Fm-COO-Ar-N₂⁺). The synthesis, electrografting, deprotection (Scheme 1a-c) and subsequent reactions of these modifiers have been characterized in detail. It has been unambiguously demonstrated that monolayers of H-Eth-Ar,^{25,26} NH₂-CH₂-Ar²⁷ and COOH-Ar²⁹ groups are generated by deprotection of the parent modifier. The monolayers function as reactive tethers for covalent immobilisation of target species via ‘click’ coupling (Cu(I)-catalysed Huisgen 1,3-dipolar cycloaddition^{31,32}) at the H-Eth-Ar monolayer,^{25,26,30} and amide coupling at NH₂-CH₂-Ar²⁷ and COOH-Ar²⁹ monolayers. Hence, these three monolayers constitute a versatile set of tethers for further on-surface reactions in which target species can be immobilized on the surface.

An inevitable outcome of the protection-deprotection approach is that after removing the bulky protecting groups, the tether layer remaining on the surface must have low fractional surface coverage because the footprint of the tether will be smaller than that of the protecting group. This provides the opportunity to introduce a second modifier to the layer by back-filling reactions at the bare surface. For maximum stability and fine control of the interfacial structure and properties, the second species should also be covalently anchored to the surface and attached only as individual molecule. Under these conditions, formation of highly stable complex multifunctional surfaces becomes a real possibility.

In earlier work, we investigated back-filling by electrografting aryldiazonium ion and arylhydrazine derivatives at deprotected H-Eth-Ar surfaces.³³ The radicals generated in the back-filling step were found to attack the already-grafted H-Eth groups and hence it was essential to carry out coupling reactions (through click chemistry) prior to back-filling. The conditions for the back-filling reaction also had to be strictly controlled to prevent formation of multilayer structures. Nevertheless, through trial and error, conditions were established in which back-filled, covalently attached monolayers were prepared.



Scheme 1. Grafting and deprotection of a) TIPS-Eth-Ar-N₂⁺; b) Fmoc-NH-CH₂-Ar-N₂⁺ and c) Fm-COO-Ar-N₂⁺.

We recently investigated a very different approach to monolayer formation using modifiers that can attach only to the surface and cannot form oligomers or polymers.³⁴ Several strategies were compared for reacting primary amines and carboxylic acids and their derivatives directly with GC. The experimentally simplest and highest yielding reaction involved immersing polished GC in an acetonitrile solution of the amine at room temperature for a few hours. In agreement with previous workers,^{35,36} our results were consistent with a nucleophilic addition reaction (Michael-like addition) at the surface, although the details of the mechanism remain unclear. More than a dozen primary, secondary and tertiary amines have been demonstrated to react directly with carbon substrates suggesting that this reaction can be used with a vast array of modifiers.³⁴⁻³⁶ All data were consistent with the attachment of only single molecules (i.e. not oligomers) at the surface.

Here we combine the use of protected aryldiazonium salts with back-filling via the nucleophilic addition of a primary amine, to prepare covalently attached mixed monolayers on GC. A key feature of the back-filling reaction at GC is its simplicity as no special conditions are required to prevent formation of oligomeric structures. The reactivity of tether groups in the back-filled layers is investigated and we explore the fractional coverages of the layers through the combination of electrochemically determined surface concentrations and computation of molecular footprints.

EXPERIMENTAL

Materials and reagents. Unless stated otherwise, all reagents were used as received.

Acetonitrile (ACN), from VWR BDH was HPLC grade; Millipore Milli-Q water (resistivity > 18 M Ω cm) was used for all aqueous solutions and washing. Tetrabutylammonium tetrafluoroborate (TBABF₄) was prepared as described previously.¹⁵

The protected aryldiazonium salts [TIPS-Eth- N₂]₂BF₄,³³ [Fmoc-NH-CH₂-Ar-N₂]₂BF₄,²⁷ and [Fm-COO-Ar-N₂]₂BF₄,²⁹ azidomethylferrocene (FcCH₂N₃),³⁷ and aminomethyl ferrocene (FcCH₂NH₂),³⁸ were prepared and characterized as described previously. All aryldiazonium salts were isolated as microcrystalline powders.

Electrochemistry and monolayer preparation. All monolayers were prepared on GC disk electrodes, area = 0.071 cm². Electrodes were polished with a slurry of 1 μ m alumina powder (Leco Corporation) on a piece of Lecloth (Leco Corporation) followed by sonicating in water for 5 min. A Pt mesh served as auxiliary electrode and the reference electrode was SCE for aqueous solutions and calomel with 1 M LiCl (here termed ‘CE’) for non-aqueous solutions. The ferrocene/ferrocenium couple appeared at $E_{1/2} = 0.36$ V vs CE (1 M LiCl) in 0.1 M TBABF₄-ACN solution.

Procedures for grafting and deprotection of aryldiazonium ions, and subsequent coupling reactions, are described in the Supporting Information. Nitrobenzylamine (NBA) groups were grafted to bare or modified GC electrodes by immersion of the electrodes for 16 h in a stirred

solution of 10 mM 4-nitrobenzylamine hydrochloride and 10 mM *N,N*-diisopropylethylamine (DIPEA) in ACN.

Electrochemistry at modified electrodes. Cyclic voltammograms (CVs) of immobilized Fc and NBA (or NP) groups were obtained in 0.1 M LiClO₄-EtOH at $v = 200$ mV s⁻¹ and 0.1 M H₂SO₄ at $v = 100$ mV s⁻¹, respectively. For mixed films, the CVs of Fc groups were recorded prior to CVs of NBA or NP groups. The surface concentration of immobilized Fc was determined by averaging the charge under the anodic and cathodic peaks from the second CV. The surface concentration of immobilized NBA and NP groups was estimated from the first cycle using the charge associated with the nitro reduction and the hydroxylamine oxidation peaks and the number of electrons involved in each redox process.³⁹

The nitro reduction peak corresponds to a mixed 6 and 4 electron process (equations 1 and 2) however groups reduced by 4 electrons to the hydroxylamine derivative, are subsequently oxidized to nitroso groups in a 2 electron oxidation (equation 3). Hence summing the charge associated with the nitro reduction and hydroxylamine oxidation gives the charge associated with a 6 electron redox process of each electroactive nitro group and allows the surface concentration to be established.



Charges arising from each redox process were determined from peak areas. Peak areas were calculated after correcting the baseline using a 3rd order polynomial. Uncertainties derived from the experimental data are indicated in the text. For sample sizes > 2, uncertainties are the standard deviation of the indicated number of samples (*n*). For sample size = 2, the uncertainty indicates the range of values obtained. There are estimated additional uncertainties of 20% and 10% for absolute surface concentrations of NBA (or NP) and Fc

respectively, reflecting the difficulty of defining appropriate peak baselines. The geometric working electrode area was assumed for all surface concentration calculations.

CVs of dopamine and ferrocyanide (both 1 mM) at polished and modified electrodes were obtained at $\nu = 100 \text{ mV s}^{-1}$ in 0.1 M H_2SO_4 and 0.1 M phosphate buffer, pH 7.1, respectively.

Computational methods and analysis. Trial conformers were generated using the Universal Conformer Generation and Analysis (UCONGA) algorithm,⁴⁰ and optimized at B3LYP/6-31G* using the Q-Chem 4.2 program package with the default parameters.⁴¹ For each lowest energy conformer, molecular radii were calculated as the in-plane distance from the surface-modifier bond to the outermost atom, plus the van der Waals radius of that atom. Heights were calculated as the C-C (or for NBA, C-N) bond length plus the distance from the atom bonded to the surface to the atom furthest from the surface, including van der Waals radii of the surface and furthestmost atoms. Ellipse parameters were calculated by aligning each molecule with its principal axes of inertia in the surface plane and finding the furthestmost atoms along each axis, including their van der Waals radii. Projected areas were calculated using a grid-based projection algorithm. All geometric and energetic parameters are reported, along with the xyz coordinates of each minimum energy conformer, in Tables S1-S9 (Supporting Information).

Theoretical 100% surface concentrations for each component of the grafted monolayers were calculated from molecular footprints, according to:

$$\Gamma_{\text{theo}} (\text{mol cm}^{-2}) = 1/[\text{footprint} (\text{cm}^2) \times N_{\text{A}} (\text{mol}^{-1})] \times f_{\text{SR}}$$

where $N_{\text{A}} = 6.022 \times 10^{23}$ and f_{SR} is the surface roughness factor. f_{SR} is defined as the ratio of the actual surface area to the geometric area of the electrode and is assumed to be 2 for the glassy carbon surfaces used here. No correction for packing efficiency was applied, to avoid complications associated with packing molecules with differently shaped and sized footprints.

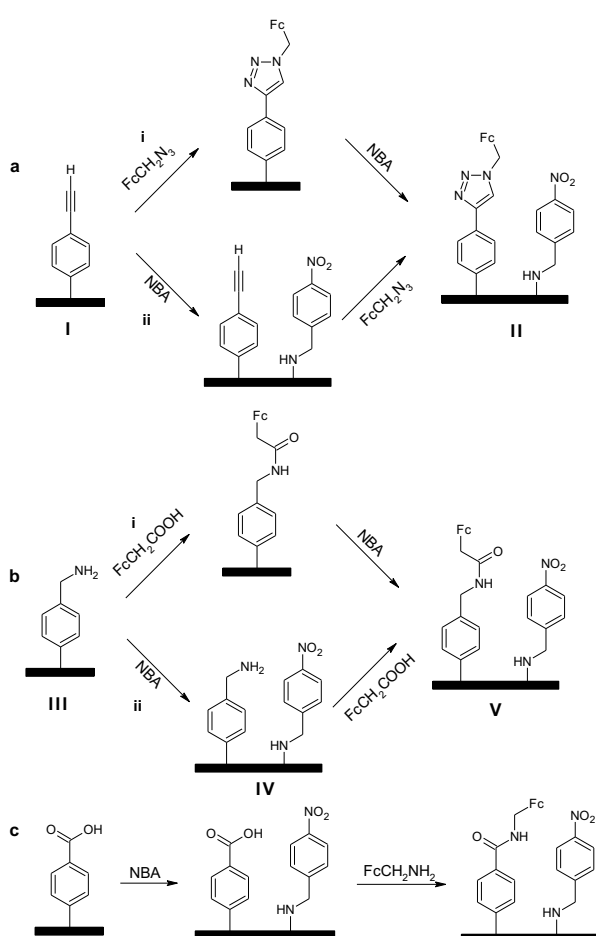
Fractional surface coverages for each modifier were then calculated as the ratio of experimentally determined surface concentration to the calculated maximum theoretical surface concentration assuming complete monolayer coverage:

$$\theta = \Gamma_{\text{expt}} / \Gamma_{\text{theo}}$$

Finally, the total fractional surface coverage was calculated as the sum of fractional surface coverages of each surface-bound species. We note that this value may be an underestimate, arising from the assumption of 100% packing efficiency when calculating Γ_{theo} .

RESULTS AND DISCUSSION

Back-filling strategy. The strategy for preparing back-filled monolayers is shown in cartoon-form in Schemes 1 and 2.



Scheme 2. Pathways for preparing mixed layers via back-filling, starting from deprotected tether layers a) H-Eth-Ar, b) NH₂-CH₂-Ar and c) COOH-Ar. Deprotected tether layers are

prepared as shown in Scheme 1. Nitrobenzylamine (NBA) is used for all back-filling reactions. Ferrocenyl groups are coupled to tethers to facilitate electrochemical characterization.

The procedure begins with electrografting a protected aryldiazonium ion followed by deprotection (Scheme 1). A second component is then added by back-filling with a primary amine derivative that reacts directly with GC (Scheme 2). If further on-surface chemistry is required to couple species to the deprotected aryl tethers, there are two possible orders of reaction steps: the deprotected layer can be back-filled followed by coupling to the tether groups, or the coupling can be carried out before back-filling. In this work, in order to demonstrate the reactivity and (minimum) surface concentration of grafted aryl tethers, electroactive ferrocene (Fc) moieties were coupled to the layer, using the derivatives shown in the Scheme. For convenience, nitrobenzylamine (NBA) was selected for all back-filling reactions because the immobilized groups can be detected and quantified electrochemically.

Although NBA has been reported to intercalate into graphite powder and multiwalled carbon nanotubes,⁴² there are no reports of its intercalation into GC, and its behavior is in line with that of other amine derivatives which undergo nucleophilic addition at GC.^{34,36} It is expected that numerous amines could be used for back-filling, including those with terminal functionalities that allow subsequent coupling reactions. For the H-Eth-Ar and NH₂-CH₂-Ar layers, backfilling with NBA groups either followed or preceded the coupling step (Schemes 2a, b). For the COOH-Ar layer, the back-filling step was always carried out first (Scheme 2c) to minimize nucleophilic addition of aminomethylferrocene to GC, or its reaction with carboxylic acid groups on the GC surface.³⁴

Single component layers. In initial experiments, single component layers were prepared by spontaneous reaction of NBA at GC and (separately) electrografting the three protected aryldiazonium salts, followed by deprotection and coupling Fc derivatives. Figure 1a shows two consecutive CVs of an NBA-modified surface obtained in 0.1 M H₂SO₄. The irreversible

reduction at $E_{pc} = -0.35$ V arises from a mixed six electron reduction of nitro to amino groups and a four electron reduction to hydroxyamino groups. The latter are oxidized to nitroso groups at ≈ 0.35 V and the chemically reversible hydroxyamino /nitrosobenzyl couple can be seen on the second scan scans.³⁹ From the charge associated with the CV peaks on the first scan, the surface concentration of NBA groups, Γ_{NBA} , was determined for two replicate samples, giving $\Gamma_{NBA} = (2.1 \pm 0.2) \times 10^{-10}$ mol cm⁻² (Table 1, entry 1). CVs of electrodes modified with TIPS-Eth-Ar, Fmoc-NH-CH₂-Ar and Fm-COO-Ar groups, followed by deprotection and coupling with Fc derivatives (these are termed Fc-T-Ar, Fc- NH-CH₂-Ar and Fc-COO-Ar, respectively) are shown in Figures 1b-d. Well-defined Fc responses are obtained at each surface with $E_{1/2} = 0.43$ V for Fc linked through the triazole moiety (Figure 1b) and $E_{1/2} = 0.33$ V for Fc coupled through amide linkages (Figures 1 c, d).

Table 1. Calculated surface concentrations of Fc and NP groups coupled to tether layers, and back-filled NBA^a

Table entry	Scheme	Aryldiazonium ion R- N ₂ ⁺ , R =	Coupled species	Back-fill species	Γ_{Fc} ($\times 10^{-10}$ mol cm ⁻²)	Γ_{NBA} ($\times 10^{-10}$ mol cm ⁻²)	Γ_{total} ($\times 10^{-10}$ mol cm ⁻²)
1		-	-	NBA	-	2.1 ± 0.2 (n=2)	
2		TIPS-Eth-Ar	FcCH ₂ N ₃	-	2.4 ± 0.3 (n=15) ^b	-	
3		Fmoc-NH-CH ₂ -Ar	FcCH ₂ COOH	-	1.4 ± 0.1 (n=2)	-	
4		Fm-COO-Ar	FcCH ₂ NH ₂	-	2.8 ± 0.7 (n=3)	-	
5	2ai	TIPS-Eth-Ar	FcCH ₂ N ₃	NBA	2.4 ± 0.2 (n=3)	1.8 ± 0.2 (n=3)	4.2 ± 0.4
6	2aii	TIPS-Eth-Ar	FcCH ₂ N ₃	NBA	1.9 ± 0.4 (n=4)	1.4 ± 0.6 (n=4)	3.3 ± 1.0
7	2bi	Fmoc-NH-CH ₂ -Ar	FcCH ₂ COOH	NBA	1.6 ± 0.2 (n=2)	1.8 ± 0.2 (n=2)	3.4 ± 0.4
8	2bii	Fmoc-NH-CH ₂ -Ar	FcCH ₂ COOH	NBA	1.6 ± 0.2 (n=3)	1.4 ± 0.3 (n=3)	3.0 ± 0.5
9	2c	Fm-COO-Ar	FcCH ₂ NH ₂	NBA	2.6 ± 0.1 (n=2)	1.0 ± 0.3 (n=2)	3.6 ± 0.3
10	3	1:1 TIPS-Eth-Ar: Fmoc-NH-CH ₂ -Ar	1.NPCOOH 2.FcCH ₂ N ₃	-	1.4 ± 0.2 (n=4)	1.1 ± 0.2 ^c (n=4)	2.5 ± 0.4
11	3	1:5 TIPS-Eth-Ar: Fmoc-NH-CH ₂ -Ar	1.NPCOOH 2.FcCH ₂ N ₃	-	0.5 ± 0.1 (n=2)	1.5 ± 0.1 ^c (n=2)	2.0 ± 0.2

^aCalculated based on geometric electrode area; ^bn is number of samples; ^c Γ_{NP}

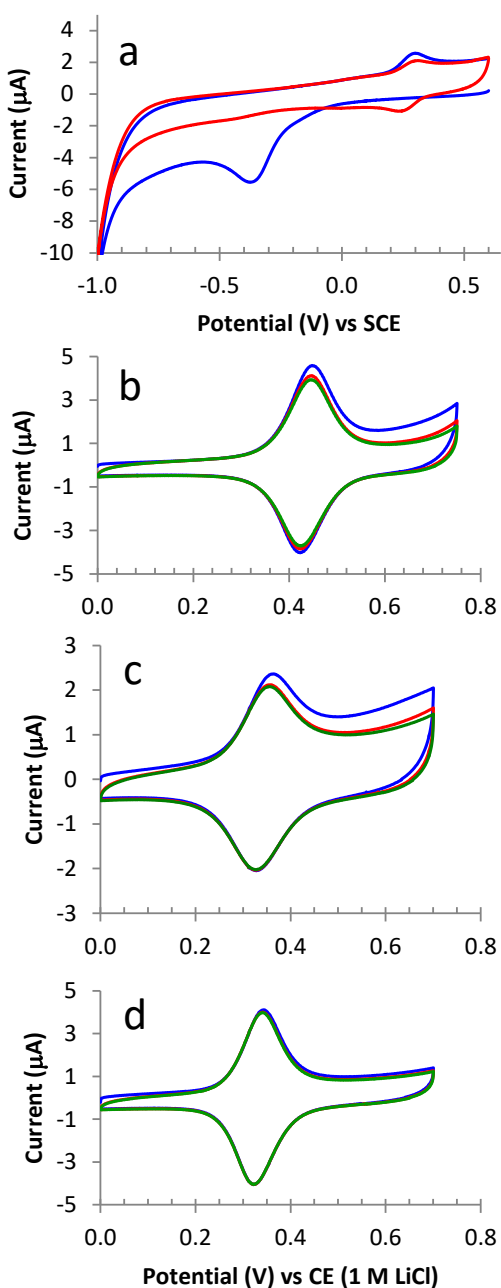


Figure 1. CVs obtained at single component electrodes modified with a) NBA; b) Fc-T-Ar; c) Fc- NH-CH₂-Ar and d) Fc-COO-Ar groups

Surface concentrations of Fc groups, Γ_{Fc} , were calculated from these CVs and are listed in Table 1, entries 2-4. Amounts of each grafted modifier in each layer may be estimated from these surface concentrations, assuming that all immobilized redox centres are electroactive; this is likely to be the case for a monolayer in which redox centres are tethered **at a** short distance from the surface. To equate the measured concentrations of coupled Fc groups (and

nitrophenyl (NP) groups, see below) to the concentration of tethers grafted to the surface also requires that all deprotection reactions and coupling reactions proceed quantitatively. In earlier work, we^{27,29,33} and others^{25,26} found no evidence that the deprotection reactions are not quantitative. Further, click reactions are typically close to quantitative and hence the concentration of Fc groups coupled to H-Eth-Ar tethers (for convenience, termed 'Fc-T-Ar' groups) can be assumed to closely correspond to the concentration of the grafted TIPS-Eth-Ar layer. On the other hand, yields for amide coupling reactions using HBTU (*N,N,N',N'*-tetramethyl-*O*-(1*H*-benzotriazol-1-yl)uronium hexafluorophosphate) are typically less than quantitative^{43,44} and hence Γ_{Fc} is likely to underestimate the concentration of grafted Fmoc-NH-CH₂-Ar groups. For the Fm-COOH-Ar layer, less than quantitative reaction between COOH-Ar tethers and FcCH₂NH₂ is expected, which would lead to an underestimation of the tether concentration. However FcCH₂NH₂ is also known to react directly with GC via nucleophilic addition and/or via amide bond formation with carboxylic acid groups on the surface.³⁴ That process will lead to immobilized Fc that is additional to that coupled to the tether groups. Hence depending on the magnitude of each effect, the surface concentration of grafted Fm-COOH-Ar may be greater or less than the measured Γ_{Fc} .

TIPS-Eth-Ar back-filled layers. Figures 2a, b show CVs of a modified electrode obtained by grafting TIPS-Eth-Ar-N₂⁺, deprotecting, coupling Fc to the layer by a click reaction with azidomethylferrocene and then back filling by immersing in NBA solution (Scheme 2a, pathway i). Well defined responses are obtained for immobilized Fc and NBA groups, similar to those described above for the single component layers. However, for NBA, several additional processes with low current are also seen in the CV (Figure 2b); their origin is unknown and was not investigated further. From the charge associated with each redox reaction, the surface concentrations of electroactive Fc and NBA groups were calculated and are listed in Table 1, entry 5.

The data in Table 1 reveal several interesting aspects of the modification procedure. First, Γ_{Fc} matches that for the single component layer, confirming that back-filling with NBA does not affect the electroactivity of Fc groups. Further, Γ_{NBA} is also not significantly different to that for a single component layer. This is surprising since grafting of NBA occurs at a surface pre-modified with Fc-T-Ar groups. When the aryl layer was back-filled prior to coupling Fc to the H-Eth-Ar groups (Scheme 2a_{ii}), the surface concentrations of immobilized Fc and NBA groups (Table 1, entry 6) were the same, within experimental uncertainty, as those described above. Evidently, the presence of the back-filling NBA groups does not inhibit the click reaction at the H-Eth-Ar groups and the amount of NBA that grafts to GC is approximately the same, whether the surface is pre-modified by H-Eth-Ar or Fc-Eth-Ar groups, or is bare GC. Models of surface coverage and the ability of NBA to access and graft to the surface pre- and post-modification will be discussed in more detail later, however in the context of using back-filling to add a second modifier to the surface and to increase the density of groups in a monolayer, the most important observation is that a significant amount of a second modifier can be added to the surface by back-filling, giving a concomitant increase in the total surface concentration of grafted groups.

In the context of the back-filled layers, we note that aryl radicals generated by reduction of aryldiazonium ions at GC bind to surface C atoms^{14,45} and it is assumed that nucleophilic addition of amines to GC also results in bonds to surface C atoms.^{34,36} However, the total surface concentration of modifiers does not appear to be limited by the concentration of surface C atoms on GC which has been estimated as $\sim 22 \times 10^{-10} \text{ mol cm}^{-2}$ for a flat surface and $\sim 44 \times 10^{-10} \text{ mol cm}^{-2}$ for GC with a roughness factor of 2.³⁴

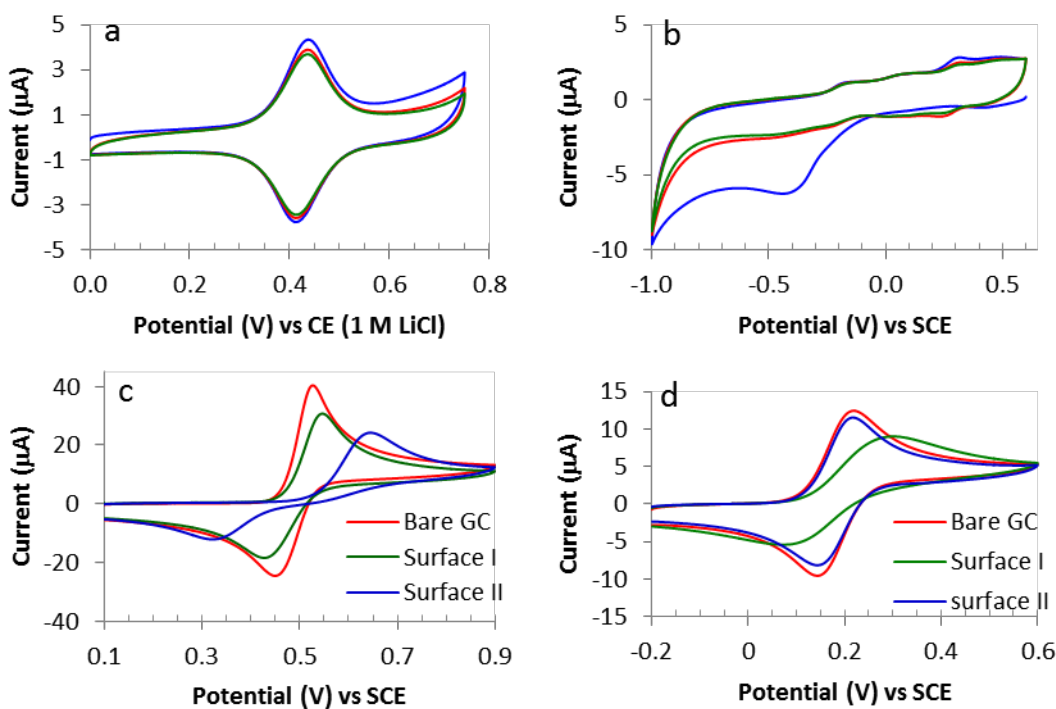


Figure 2. CVs of a), b) a Fc-T-Ar modified surface back-filled with NBA groups, prepared as shown in Scheme 2ai; and c) DA and d) ferrocyanide obtained at bare GC and at GC modified with H-Eth-Ar groups (surface I) and with Fc-T-Ar and NBA groups (surface II). The surface numbers refer to those shown in Scheme 2a.

The blocking properties of modified electrodes prepared by the route in Scheme 2ai were investigated using the redox probes dopamine (DA) and ferrocyanide. The voltammetry of DA was examined because it is an inner sphere redox probe for which the rate of electron transfer is catalysed by interaction directly at the GC surface.^{46,47} Ferrocyanide also has inner sphere character and its voltammetry is sensitive to structural differences even for very thin modified surfaces.⁴⁸ As shown in Figure 2c, at the H-Eth-Ar surface (structure I, Scheme 2ai), DA exhibits a well-defined CV although the peak currents are smaller and ΔE_p is larger (100 mV) than at the polished electrode ($\Delta E_p = 50$ mV). This response is consistent with DA interacting with accessible bare surface within the layer and undergoing fast electron transfer. After coupling Fc to the H-Eth-Ar groups and back-filling with NBA groups (structure II, Scheme 2ai), ΔE_p increases to 360 mV and the response is typical of DA undergoing

uncatalysed electron transfer. This change is the expected consequence of adding the large substituent to the H-Eth-Ar groups and back-filling, thereby blocking access of DA to the GC surface. In addition there will be electrostatic repulsion between protonated DA and the protonated secondary amine that links the NBA groups to the surface. For ferrocyanide, the CV at the H-Eth-Ar layer (Fig. 1d) has a larger ΔE_p and lower current than at the unmodified electrode, and approaches a sigmoidal shape. This response has previously been satisfactorily modelled²⁶ assuming electron transfer at interacting pinholes.⁴⁹ After coupling Fc and back-filling the CV is very similar to that at polished GC. The change in ferrocyanide response is attributed to electrostatic attraction between the anionic redox probe and the protonated secondary amine groups of NBA.

Fmoc-NH-CH₂-Ar back-filled layers. The procedures for preparation of mixed monolayers starting with the protected amine - terminated diazonium salt, Fmoc-NH₂-CH₂-Ar-N₂⁺ are shown in Schemes 1b and 2b. The tether layers were back-filled either before or after coupling Fc; Figures 3a,b show typical CVs of the modified electrodes obtained via pathway ii in Scheme 2b (back-filling prior to coupling). The responses of Fc and NBA groups in this monolayer are qualitatively similar to those shown in Figure 1a, c for the corresponding single component layers.

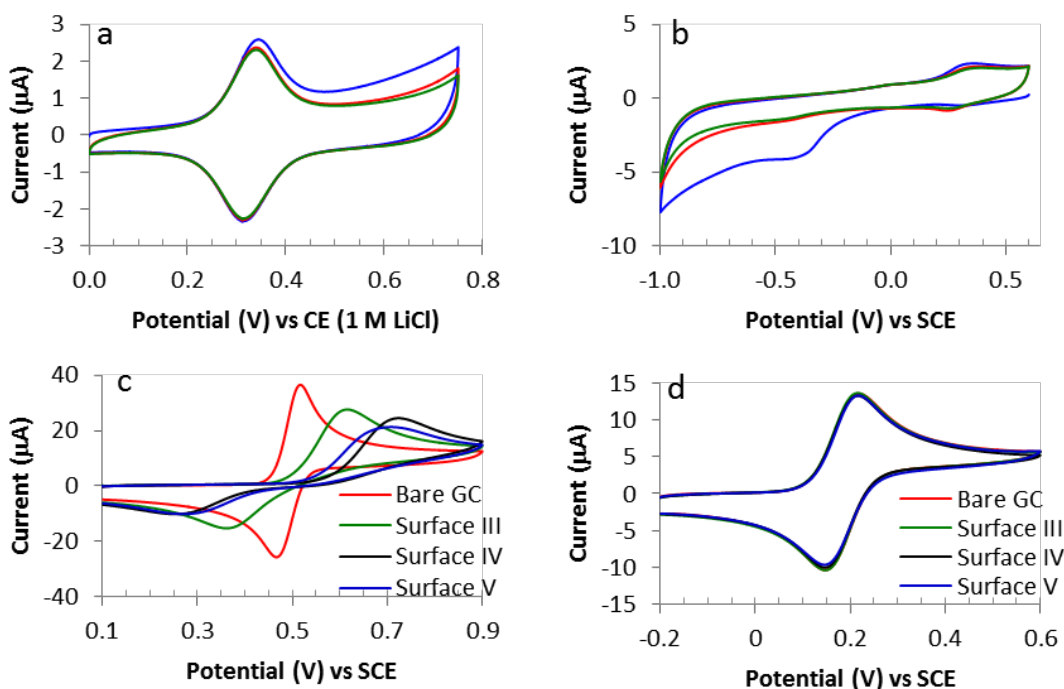


Figure 3. CVs of a), b) a Fc-NH-CH₂-Ar modified surface back-filled with NBA groups, prepared as shown in Scheme 2bii; and c) DA and d) ferrocyanide obtained at bare GC and at GC modified with NH₂-CH₂-Ar groups (surface III), a back-filled layer of NH₂-CH₂-Ar groups (surface IV) and with Fc-NH-CH₂-Ar and NBA groups (surface V). The surface numbers refer to those shown in Scheme 2b.

Table 1 entries 7 and 8 give the electrochemically measured surface concentrations of Fc and NBA groups in the layers. For these surfaces, as for the single component layer, it is likely that the surface concentrations of Fc groups are somewhat lower than the concentrations of grafted Fmoc-NH-CH₂-Ar groups as coupling reactions using HBTU are expected to be less than quantitative. Similarly to the H-Eth-Ar layers described above, Γ_{Fc} is the same for the layers prepared by coupling Fc to NH₂-CH₂-Ar groups before or after back-filling, and Γ_{Fc} is the same within experimental uncertainty as for the single component layer. Once again, it is also surprising that the order of preparing the mixed layer has an insignificant effect on the amount of NBA grafted to the surface, with the same amount

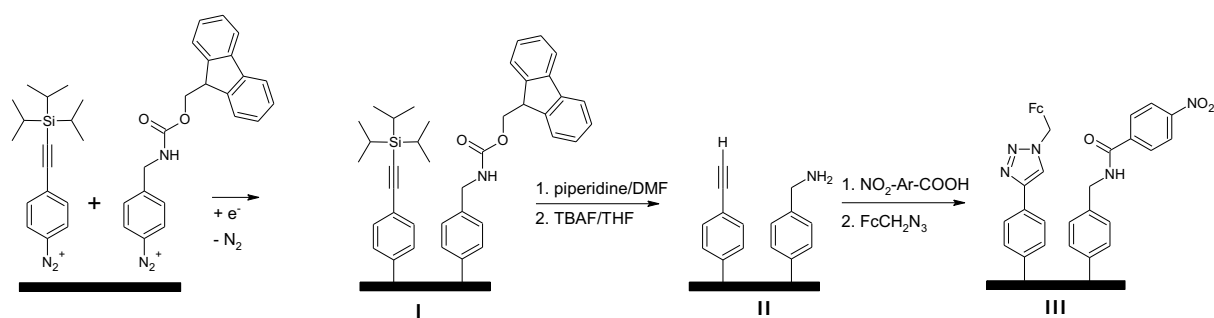
(within experimental uncertainty) of NBA grafted to the layers pre-modified with Fc- NH-CH₂-Ar groups and NH₂-CH₂-Ar groups.

Figures 3c, d show CVs of DA and ferrocyanide obtained at the electrodes at various stages of preparation. The blocking properties of the layers show some differences to those prepared from TIPS-Eth-Ar-N₂⁺ (Figures 2c,d) which can be attributed to the presence of protonated amines in all layers (structures III, IV and V, Scheme 2bii). The CV of DA at the NH₂-CH₂-Ar layer (Figure 3c, green) has a larger ΔE_p (240 mV) than that at the deprotected H-Eth-Ar layer ($\Delta E_p = 100$ mV, Figure 2c, green) showing a greater blocking effect of the NH₂-CH₂-Ar layer arising from electrostatic repulsions between protonated amine groups of DA and the modified surface. Back-filling with NBA groups gives a further increase in ΔE_p (Figure 3c, black, $\Delta E_p = 430$ mV) consistent with higher coverage of the surface and the introduction of additional protonated amine groups when NBA is linked to the surface. There is little change in ΔE_p after coupling Fc to the amine groups (Figure 3c, blue, $\Delta E_p = 410$ mV) even though the concentration of protonated amine groups must significantly decrease on reaction with FcCH₂COOH. However, Fc groups will be oxidized to cationic ferrocenium during scans in DA solution and this is assumed to result in little net change in electrostatic interactions at the surface. CVs of ferrocyanide obtained at the same surfaces (superimposed CVs in Figure 3d) are indistinguishable to those obtained at bare GC. Evidently the electrostatic attraction between ferrocyanide and the protonated amine groups of the NH₂-CH₂-Ar and NBA modifiers compensates for the blocking of the surface by grafted groups, resulting in no detectable change in response at the modified surfaces. With respect to the response at the final coupled and back-filled surface (Figure 3d blue line) an unperturbed response for ferrocyanide was also seen at the final surface prepared by from the TIPS-Eth-Ar-N₂⁺ (Figure 2d, blue line) and the same explanation applies here.

Fm-COOH-Ar back-filled layer. The protected carboxybenzene diazonium ion, Fm-COOH-Ar-N₂⁺ was also used for preparation of back-filled monolayers as shown in Scheme

2c. In this case, back filling with NBA always preceded coupling of aminomethylferrocene to the COOH-Ar groups because the presence of NBA should decrease reaction of aminomethylferrocene with the GC surface, via either nucleophilic addition or amide coupling to carboxylic acid groups naturally present on the GC surface. Surface concentrations of immobilized Fc and NBA groups were obtained from CVs (not shown) giving the data listed in Table 1, entry 9. As discussed above for the single component layers, the measured surface concentration of coupled Fc ($2.6 \pm 0.1 \times 10^{-10} \text{ mol cm}^{-2}$) is larger than expected based on the size of the initially grafted Fm-COO-Ar groups suggesting that despite back-filling with NBA, there is a contribution from aminomethylferrocene which has reacted directly with GC. For this layer, the amount of grafted NBA is significantly smaller than that grafted to a polished GC surface (Table 1, entry 1).

Mixed layers from grafting mixtures of protected aryldiazonium ions. The advantage of the back-filling strategy for preparing mixed monolayers can be illustrated by comparing with layers formed by grafting from mixtures of protected aryl diazonium salts. A major limitation of the mixed solution method, which applies to all aryldiazonium ions, not just those used here, is that to obtain layers in which the proportions of two modifiers can be varied over a wide range requires that the reduction peak potentials of both modifiers are very similar. Fortunately this is the case for TIPS-Eth-Ar-N₂⁺ and Fmoc-NH-CH₂-Ar- N₂⁺ which as 5 mM solutions and at scan rate, $\nu = 50 \text{ mV s}^{-1}$, show a reduction peak at 0.2 V and 0.15 and V (both vs calomel, 1 M LiCl), respectively.⁵⁰ (The corresponding potential is 0.35 V for COOH-Ar-N₂⁺.⁵⁰) Grafting from a mixed solution (1:1 molar ratio) of TIPS-Eth-Ar-N₂⁺ and Fmoc-NH-CH₂-Ar- N₂⁺, followed by deprotection and coupling of Fc to the H-Eth-Ar layer and nitrophenyl (NP) groups to the NH₂-CH₂-Ar layer (via amide coupling with 4-nitrobenzoic acid) as shown in Scheme 3, gave surfaces with similar concentrations of both redox groups (Table 1, entry 10).



Scheme 3. Strategy for preparation of mixed layers through grafting from a mixture of aryldiazonium salts.

It is immediately obvious that the total surface concentration of electroactive modifiers ($2.5 \times 10^{-10} \text{ mol cm}^{-2}$) is significantly smaller than for all back-filled layers (total surface concentrations range from $3.0 - 4.2 \times 10^{-10} \text{ mol cm}^{-2}$). The CVs of DA at the layers grafted from the mixture of aryldiazonium salts (Figure 4a) show smaller ΔE_p values than at the other modified surfaces indicating faster rates of electron transfer. For example at the final coupled layer (structure III, Scheme 3) $\Delta E_p = 270 \text{ mV}$ compared with $\Delta E_p = 360 \text{ mV}$ and 410 mV at the back-filled Fc-Eth-Ar and **Fc-NH-CH₂-Ar** layers, respectively (Figures 2c and 3c). While this is consistent with the lower surface coverage after grafting from a mixture of protected aryldiazonium salts, it should be noted that the final surface lacks the protonated amine groups that link the NBA groups to the surface in the other mixed layers and only ferrocenium groups can provide electrostatic repulsion. The ferrocyanide response shows no evidence of a blocking layer (Figure 4b) which is attributed to the low surface coverage combined with electrostatic attraction between ferrocyanide and protonated terminal amines at the deprotected layer (structure II, Scheme 3) and ferrocenium groups at the final coupled surface.

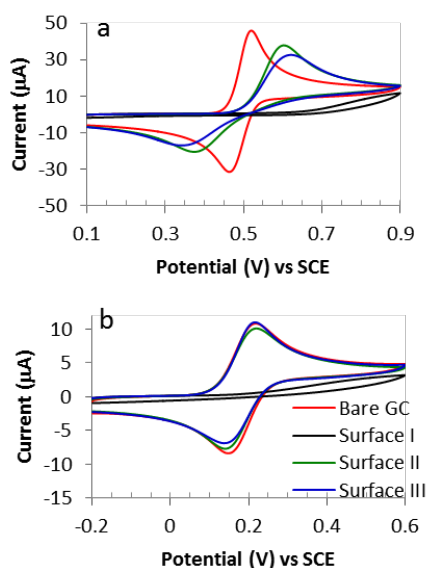


Figure 4. CVs of a) DA and b) ferrocyanide obtained at bare GC and at GC modified with a mixed layer of TIPS-Eth-Ar and Fmoc-NH-CH₂-Ar groups (surface I), a mixed layer of H-Eth-Ar and NH₂CH₂-Ar groups (surface II) and a mixed layer of Fc-NH-CH₂-Ar and Fc-T-Ar groups (surface III). The surface numbers refer to those shown in Scheme 3.

A mixed layer was also prepared using a 1:5 molar ratio of TIPS-Eth-Ar-N₂⁺ : Fmoc-NH-CH₂-Ar- N₂⁺. After coupling Fc and NP groups to the layer, the surface concentration of electroactive NP groups was found to have increased (Table 1, entry 11) and that of Fc decreased, compared to the layers grafted from an equimolar mixture of the two aryldiazonium salts. This is the consequence of the expected increase in the proportion of Fmoc-NH-CH₂-Ar groups on the surface when grafting from a solution with a higher relative concentration of Fmoc-NH-CH₂-Ar- N₂⁺. The data in the Table also show that the total surface concentration of the mixed layer of H-Eth-Ar and NH₂-CH₂-Ar groups is smaller (1×10^{-10} mol cm⁻²) than for the layer prepared from the equimolar solution. The larger footprint of Fmoc-NH-CH₂-Ar- N₂⁺ compared with TIPS-Eth-Ar-N₂⁺ (see below) accounts for the overall decrease in surface concentration.

Models of Fractional Surface Coverage. Having prepared monolayers with measurable surface concentrations it is interesting to examine the fractional surface coverages (θ values)

of the layers. For these calculations, a major question is the appropriate surface coverage (footprint) of a single modifier. As a starting point we consider the surface concentrations of the single component layers grafted from the protected aryldiazonium ions, Table 1, entries 2-4. In previous work, when calculating theoretical values for close-packed monolayers of the protected tether groups TIPS-Eth-Ar, Fmoc-NH-CH₂-Ar and Fm-COOH-Ar, we^{27,29} and others^{25,26,28} assumed free rotation around the phenyl-substituent bond of the lowest energy conformer, yielding circular footprints (Table 2). These footprints give theoretical surface concentrations of hexagonal close-packed layers of TIPS-Eth-Ar, Fmoc-NH-CH₂-Ar and Fm-COOH-Ar groups on a flat surface of 2.3, 1.2 and 1.7×10^{-10} mol cm⁻², respectively.^{26,27,29} The typical roughness factor of a GC electrode is 1.5-2.5,^{51,52} and hence assuming a value of 2 gives close-packed surface concentrations on GC approximately double those listed above. These values can be compared with the experimental surface concentrations for the single component layers prepared here (Table 1, entries 2-4), giving fractional surface coverages, θ , of approximately 0.5, 0.6 and 0.8 for the protected tether layers, TIPS-Eth-Ar, Fmoc-NH-CH₂-Ar and Fm-COO-Ar, respectively. Bearing in mind that these values are derived from measurement of the surface concentrations of Fc immobilized on the modified electrodes and the earlier discussion of the expected yields of the coupling reactions, Γ_{Fc} for the Fc-T-Ar layer should most closely correspond to the surface concentration of the grafted tether layer while Γ_{Fc} for Fc-NH-CH₂-Ar is expected to be an underestimate. This is inconsistent with the estimated experimental θ values, implying that the calculated footprint of Fmoc-NH-CH₂-Ar is too large. Note that the unexpectedly large calculated fractional surface coverage for the Fm-COO-Ar layer does not necessarily point to a problem with the assumed molecular footprints. In previous work³⁴ the reaction of aminomethylferrocene directly with GC under HBTU-coupling conditions gave $\Gamma_{Fc} = (1.3 \pm 0.2) \times 10^{-10}$ mol cm⁻². Hence it is likely that Γ_{Fc} for the Fm-COO-Ar layer includes a significant, but unknown, contribution from Fc not coupled to grafted COOH-Ar groups.

An alternative approach to calculating modifier footprints is to assume fixed orientation of the modifier relative to the surface and restricted internal conformational flexibility. This assumption was made by other workers to obtain the often cited values of 12×10^{-10} mol cm⁻²⁵² and (presumably) 12.5×10^{-10} mol cm⁻²⁵³ for a theoretical close-packed layer of NP groups on a flat surface.

Table 2. Calculated footprints and theoretical surface concentrations for modifiers based on lowest energy conformers and assuming vertical alignment on the surface, using a surface roughness factor of 2 and assuming 100% packing efficiency.

Modifier	Model A		Model B	
	Footprint, circular ^a (Å ²)	$\Gamma_{\text{theo}} (\times 10^{-10}$ mol cm ⁻²)	Footprint, ellipse ^b (Å ²)	$\Gamma_{\text{theo}} (\times 10^{-10}$ mol cm ⁻²)
TIPS-Eth-Ar	66.5 ^c	4.6		
Fmoc-NH-CH ₂ -Ar	138.9 ^d	2.4		
Fm-COO-Ar	90.6 ^e	3.4		
NBA	63.6	5.2	24.8	1.3
H-Eth-Ar	34.2	9.7	17.4	1.9
NH ₂ -CH ₂ -Ar	34.2	9.7	25.8	1.3
COOH-Ar	34.2	9.7	17.4	1.9
Fc-T-Ar	140.0	2.4	51.9	6.4
Fc-NH-CH ₂ -Ar	295.6	1.1	78.8	4.2
Fc-COO-Ar	128.7	2.6	48.3	6.9
NP-NH-CH ₂ -Ar	301.7	1.1	71.0	4.7

^a Calculated assuming free rotation around the surface-N bond for NBA, the surface-phenyl bond for H-Eth groups and the phenyl - substituent bond (equivalent to rotation around the surface-phenyl bond) for the remaining modifiers; ^b Calculated assuming no rotation and fitting an ellipse for which the main axes coincide with the principal axes of inertia; ^c ref 25; ^d ref 27; ^e ref 29

In the following discussion, we use each of these possible rotational freedom models and the experimentally determined surface concentrations of modifiers (Table 1) to estimate θ values for all grafted layers. For clarity, we refer to them as Models A and B, where Model A assumes circular footprints for all modifiers, arising from free rotation around the aryl-surface bond and/or the phenyl ring-substituent bond. Model B assumes completely restricted rotation, producing a 2D van der Waals projection on the surface that is well-fitted as an ellipse. The calculated areas of circular and elliptical footprints, and theoretical surface concentrations for 100% coverage are listed in Table 2; projected van der Waals areas are reported for completeness in the Supporting Information, Table S9.

Model A: Free Rotation. The data in Table 3 show that assuming that modifiers undergo free rotation produces a number of inconsistencies or seemingly unreasonable θ values. For example, $\theta_{\text{total (Fc-tether + NBA)}} > 1$ for all two-component back-filled layers (Table 3, entries 5-9). From the heights of NBA groups and the other modifiers and their lowest energy conformations (Tables S1-S9), freely rotating NBA groups cannot sit beneath the other component of the mixed layers, which are also freely rotating, and hence θ values significantly greater than 1 do not seem physically reasonable.

A similar conclusion applies to the mixed Fc-T-Ar and NP-NH-CH₂-Ar layers prepared from mixtures of protected aryldiazonium salts for which $\theta_{\text{total}} \approx 1.6$ once both coupling reactions have taken place (Table 3, entries 10 and 11). Further evidence indicating the limitations of assuming free rotation of the phenyl substituents comes from examining the preparation steps for this system. After deprotection of the grafted modifiers, NP groups were first coupled to NH₂-CH₂-Ar tethers followed by clicking Fc to the H-Eth-Ar groups. If free rotation of the phenyl substituents is assumed, after the first coupling step, $\theta_{\text{NP-NH-CH}_2\text{-Ar}} \approx 1.0$ and 1.4 for each of the two mixed layers. Therefore, the NP-NH-CH₂-Ar groups

would be expected to block access to the shorter H-Eth-Ar groups. However, based on the total fractional surface coverage values following both coupling steps, $\theta_{\text{total (coupled tethers)}}$, the click reaction appears to proceed with high yields.

Table 3. Calculated fractional surface coverages, θ , based on Model A (free rotation, circular footprint) for single component layers, two component back-filled layers, and two component layers prepared from mixtures of aryldiazonium salts. Calculated from mean surface concentrations (Table 1) and molecular footprints (Table 2), assuming a surface roughness factor of 2, 100% packing efficiency, and quantitative coupling of redox-active probes to tethers ($\Gamma_{\text{Fc-tether}} = \Gamma_{\text{tether}}$ and $\Gamma_{\text{NP-tether}} = \Gamma_{\text{tether}}$).

Single component layers, before and after coupling Fc to tethers						
Table 1 entry	Deprotected tether or NBA	θ_{NBA}	θ_{tether}	$\theta_{\text{Fc-tether}}$		
1	NBA	0.40	-	-		
2	H-Eth-Ar	-	0.25	1.02		
3	NH ₂ -CH ₂ -Ar	-	0.14	1.25		
4	COOH-Ar	-	0.29	1.09		
Pathway: (a) graft, deprotect, couple Fc to tether, (b) back-fill						
		$\theta_{\text{Fc-tether}}$	θ_{NBA}	θ_{total} (Fc-tether + NBA)		
5	H-Eth-Ar	1.02	0.34	1.36		
7	NH ₂ -CH ₂ -Ar	1.43	0.34	1.77		
Pathway: (a) graft, deprotect, (b) back-fill, (c) couple Fc to tether						
		θ_{tether}	θ_{NBA}	θ_{total} (tether + NBA)	$\theta_{\text{Fc-tether}}$	θ_{total} (Fc-tether + NBA)
6	H-Eth-Ar	0.20	0.27	0.47	0.81	1.08
8	NH ₂ -CH ₂ -Ar	0.16	0.27	0.43	1.43	1.70
9	COOH-Ar	0.27	0.19	0.45	1.01	1.20
Pathway: (a) graft from mixed solution, deprotect, (b) couple NP to NH ₂ -CH ₂ -Ar, couple Fc to H-Eth-Ar						
		$\theta_{\text{NH}_2\text{-CH}_2\text{-Ar}}$	$\theta_{\text{H-Eth-Ar}}$	$\theta_{\text{NP-NH-CH}_2\text{-Ar}}$	$\theta_{\text{Fc-T-Ar}}$	θ_{total} (coupled tethers)
10	NH ₂ -CH ₂ -Ar: H-Eth-Ar (1:1 [†])	0.11	0.14	1.00	0.59	1.59
11	NH ₂ -CH ₂ -Ar: H-Eth-Ar (5:1 [†])	0.15	0.06	1.36	0.21	1.57

[†] Ratio of protected aryldiazonium salts in grafting solution

The yields of the back-filling reactions are also inconsistent with θ values calculated assuming free rotation of modifiers and circular footprints. This is most easily seen by

comparing θ_{NBA} values in Table 3, entries 5 and 6. The values are the same, to within experimental uncertainty, even though surface 5 was prepared by back-filling NBA at surfaces pre-modified with a Fc-T-Ar layer, for which $\theta_{\text{Fc-tether}} \approx 1.0$, and surface 6, by back-filling at surfaces pre-modified with the deprotected H-Eth-Ar layer for which $\theta_{\text{tether}} \approx 0.2$. Similar observations can be made for the back-filled $\text{NH}_2\text{-CH}_2\text{-Ar}$ monolayers (Tables 3, entries 7 and 8). θ_{NBA} values are the same, within experimental uncertainty when NBA back-fills a Fc-NH- $\text{CH}_2\text{-Ar}$ layer ($\theta_{\text{Fc-tether}} \approx 1.4$) and a $\text{NH}_2\text{-CH}_2\text{-Ar}$ layer ($\theta_{\text{tether}} \approx 0.2$). Clearly the $\theta_{\text{Fc-tether}}$ values for the surfaces which are coupled with Fc prior to back-filling are not consistent with the observed yields of the back-filling reactions.

Model B: No Rotation. When modelled using elliptical footprints, Table 4 shows that calculated θ values are less than unity for all layers. Assuming no rotation and smaller footprints for the modifiers at least partially obviates the inconsistencies in grafting and back-filling reaction yields arising in Model A. For example, for NBA back-filling at pre-modified surfaces, the Fc-T-Ar and H-Eth-Ar layers have $\theta_{\text{B}} \approx 0.4$ and 0.1, respectively (Table 4, entries 5 and 6). Hence back-filling with NBA to give very similar surface concentrations and coverages at both layers is not unreasonable. As another example, for the two layers formed by grafting from mixed solutions of protected aryldiazonium salts, the NP-NH- $\text{CH}_2\text{-Ar}$ groups formed in the first coupling steps have $\theta \approx 0.2$ and 0.3 (Table 4, entries 10 and 11). With such low coverages, it is not surprising that Fc can be clicked with apparently high yield to the H-Eth-Ar groups in the layer.

With respect to blocking properties of the layers towards electron transfer reactions of solution redox probes, the CVs obtained in this study do not permit quantitative analysis of surface coverages. While trends in probe responses are qualitatively consistent with the modifying layers, it is apparent that electrostatic interactions between probes and the layers play a major role in the observed responses and prevent more detailed analysis.

Table 4. Calculated fractional surface coverages, θ , based on Model B (no rotation, elliptical footprint) for single component layers, two component back-filled layers, and two component layers prepared from mixtures of aryldiazonium salts. Calculated from mean surface concentrations (Table 1) and molecular footprints (Table 2), assuming a surface roughness factor of 2, 100% packing efficiency, and quantitative coupling of redox-active probes to tethers ($\Gamma_{\text{Fc-tether}} = \Gamma_{\text{tether}}$ and $\Gamma_{\text{NP-tether}} = \Gamma_{\text{tether}}$).

Single component layers, before and after coupling Fc to tethers						
Table 1 entry	Deprotected tether or NBA	θ_{NBA}	θ_{tether}	$\theta_{\text{Fc-tether}}$		
1	NBA	0.16	-	-		
2	H-Eth-Ar	-	0.13	0.38		
3	NH ₂ -CH ₂ -Ar	-	0.11	0.33		
4	COOH-Ar	-	0.15	0.41		
Pathway: (a) graft, deprotect, couple Fc to tether, (b) back-fill						
		$\theta_{\text{Fc-tether}}$	θ_{NBA}	θ_{total} (Fc-tether + NBA)		
5	H-Eth-Ar	0.38	0.13	0.51		
7	NH ₂ -CH ₂ -Ar	0.38	0.13	0.51		
Pathway: (a) graft, deprotect, (b) back-fill, (c) couple Fc to tether						
		θ_{tether}	θ_{NBA}	θ_{total} (tether + NBA)	$\theta_{\text{Fc-tether}}$	θ_{total} (Fc-tether + NBA)
6	H-Eth-Ar	0.10	0.10	0.20	0.30	0.40
8	NH ₂ -CH ₂ -Ar	0.13	0.10	0.23	0.38	0.48
9	COOH-Ar	0.14	0.07	0.21	0.38	0.45
Pathway: (a) graft from mixed solution, deprotect, (b) couple NP to NH ₂ -CH ₂ -Ar, couple Fc to H-Eth-Ar						
		$\theta_{\text{NH}_2\text{-CH}_2\text{-Ar}}$	$\theta_{\text{H-Eth-Ar}}$	$\theta_{\text{NP-NH-CH}_2\text{-Ar}}$	$\theta_{\text{Fc-T-Ar}}$	θ_{total} (coupled tethers)
10	NH ₂ -CH ₂ -Ar: H-Eth-Ar (1:1 [†])	0.09	0.07	0.23	0.22	0.45
11	NH ₂ -CH ₂ -Ar: H-Eth-Ar (5:1 [†])	0.12	0.03	0.32	0.08	0.40

[†] Ratio of protected aryldiazonium salts in grafting solution.

Other Models. Models A and B represent the extremes of possible modifier footprints and of θ values. Other models of the layers could be considered that would give θ values between these extremes. For example, smaller modifiers may undergo free rotation while larger modifiers are hindered by 2D steric packing. Additionally, larger modifiers have internal degrees of conformational flexibility that have not been accounted for here.

However, uncertainties in experimental measurement of surface concentrations and surface roughness, combined with neglect of surface packing effects, prevent quantitative analysis. Therefore, exploring more sophisticated models of rotational freedom is unlikely to provide any additional insight at this point.

Fractional Coverage and Extent of Back-filling. Regardless of which model of rotational freedom is assumed, the deprotected tethers are present at low surface concentrations (Tables 3 and 4, entries 2-4). This emphasizes the usefulness of back-filling as a strategy to incorporate additional functionality on the surface and increase the total coverage of the electrodes. Back-filling with NBA approximately doubles the coverage of the surface prior to coupling Fc (Tables 3 and 4, entries 6, 8, 9), but fractional coverage reaches a maximum value of only $\theta \approx 0.4 - 0.5$ if assuming rotating molecules or $\theta \approx 0.2$ if assuming no rotation. Considering that the grafted tethers are designed to be used for immobilizing target species, this incomplete coverage is not necessarily a drawback because as demonstrated here, it permits coupling reactions to proceed with high yields and in any case, after coupling target species, fractional surface coverage is higher.

These θ values for the back-filled tether layers can be compared with those for the mixed tether layers prepared by grafting from solutions of two protected aryldiazonium salts (Tables 3 and 4, entries 10 and 11). As expected, the latter monolayers have similar surface coverages to the single component layers grafted from protected aryldiazonium salts and correspondingly lower θ values than for the back-filled layers. However back-filling these layers via reaction with amines offers a straightforward method for obtaining monolayers with three components.

NP Monolayer. Finally, we note, as an aside, that for the very commonly studied NP film grafted from nitrobenzenediazonium salt, theoretical maximum surface concentrations for a monolayer on an ideally flat surface are 4.9×10^{-10} mol cm⁻² (Model A, circular footprint), 9.5×10^{-10} mol cm⁻² (Model B, elliptical footprint) and 9.1×10^{-10} mol cm⁻² (no

rotation, projected footprint). These values are the same as those for COOH-Ar reported in Tables 2 and S9. For hexagonally close-packed layers, the corresponding values are 4.4×10^{-10} mol cm⁻², 8.6×10^{-10} mol cm⁻² and 8.2×10^{-10} , respectively (assuming a packing density of 0.9 for all geometries). Irrespective of the rotational freedom model, these values are considerably smaller than $12 - 12.5 \times 10^{-10}$ mol cm⁻², the value frequently assigned to this monolayer.^{52,53}

CONCLUSION

Sparse monolayers prepared from protected aryldiazonium ions can be back-filled via reaction with primary amines, affording a very simple and reliable route to robust two component mixed monolayers. We anticipate that this methodology will find widespread use in constructing sensors and other smart surfaces that will be more physically stable than those based on the widely-used gold-thiol chemistry. The key to obtaining well-defined layers is that neither modification method can give oligomeric or polymeric surface groups. Back-filling significantly increases the total surface concentration of grafted groups giving concentrations that are up to double those obtained by grafting from a mixture of protected aryldiazonium salts. Modelling surface coverage by assuming free rotation of modifiers leads to physically unreasonable predictions such as monolayer fractional coverages > 1.0 , and yields for the back-filling reaction that are unaffected by a near-complete surface coverage by the first modifier.

ASSOCIATED CONTENT

Supporting Information

Procedures for grafting and deprotection of aryldiazonium ions, and subsequent coupling reactions. For each modifier, a table of all geometric and energetic parameters, along with the xyz coordinates of the minimum energy conformer. Table of calculated fractional surface coverage of each component for all modified surfaces for the mixed model (rotating tethers and NBA groups, and fixed orientation for couples tethers). Table of geometric parameters and

relative energies of flexible modifiers in different conformations, for the 5 lowest energy unique rotamers.

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REFERENCES

- (1) Delamar, M.; Hitmi, R.; Pinson, J.; Saveant, J. M. Covalent Modification of Carbon Surfaces by Grafting of Functionalized Aryl Radicals Produced from Electrochemical Reduction of Diazonium Salts. *J. Am. Chem. Soc.* **1992**, *114*, 5883-5884.
- (2) Hurley, B. L.; McCreery, R. L. Covalent Bonding of Organic Molecules to Cu and Al Alloy 2024 T3 Surfaces Via Diazonium Ion Reduction. *J. Electrochem. Soc.* **2004**, *151*, B252-B259.
- (3) Levy, Y.; Tal, N.; Tzemach, G.; Weinberger, J.; Domb, A. J.; Mandler, D. Drug-Eluting Stent with Improved Durability and Controllability Properties, Obtained Via Electrocoated Adhesive Promotion Layer. *J. Biomed. Mater. Res. Part B Appl. Biomater.* **2009**, *91B*, 819-830.
- (4) Mahouche-Chergui, S.; Gam-Derouich, S.; Mangeney, C.; Chehimi, M. M. Aryl Diazonium Salts: A New Class of Coupling Agents for Bonding Polymers, Biomacromolecules and Nanoparticles to Surfaces. *Chem. Soc. Rev.* **2011**, *40*, 4143-4166.
- (5) Zou, Q. J.; Kegel, L. L.; Booksh, K. S. Electrografted Diazonium Salt Layers for Antifouling on the Surface of Surface Plasmon Resonance Biosensors. *Anal. Chem.* **2015**, *87*, 2488-2494.
- (6) Fairman, C.; Ginges, J. Z.; Lowe, S. B.; Gooding, J. J. Protein Resistance of Surfaces Modified with Oligo(Ethylene Glycol) Aryl Diazonium Derivatives. *Chemphyschem* **2013**, *14*, 2183-2189.
- (7) Gam-Derouich, S.; Gosecka, M.; Lepinay, S.; Turmine, M.; Carbonnier, B.; Basinska, T.; Slomkowski, S.; Millot, M. C.; Othmane, A.; Ben Hassen-Chehimi, D.; Chehimi, M. M. Highly Hydrophilic Surfaces from Polyglycidol Grafts with Dual Antifouling and Specific Protein Recognition Properties. *Langmuir* **2011**, *27*, 9285-9294.

- (8) Bartolome, J. P.; Echegoyen, L.; Fragoso, A. Reactive Carbon Nano-Onion Modified Glassy Carbon Surfaces as DNA Sensors for Human Papillomavirus Oncogene Detection with Enhanced Sensitivity. *Anal. Chem.* **2015**, *87*, 6744-6751.
- (9) Gooding, J. J. Advances in Interfacial Design Sensors: Aryl Diazonium Salts for Electrochemical Biosensors and for Modifying Carbon and Metal Electrodes. *Electroanalysis* **2008**, *20*, 573-582.
- (10) Kong, L. T.; Wang, J.; Fu, X. C.; Zhong, Y.; Meng, F. L.; Luo, T.; Liu, J. H. P-Hexafluoroisopropanol Phenyl Covalently Functionalized Single-Walled Carbon Nanotubes for Detection of Nerve Agents. *Carbon* **2010**, *48*, 1262-1270.
- (11) Prieto-Simon, B.; Saint, C.; Voelcker, N. H. Electrochemical Biosensors Featuring Oriented Antibody Immobilization Via Electrografted and Self-Assembled Hydrazide Chemistry. *Anal. Chem.* **2014**, *86*, 1422-1429.
- (12) Ru, J.; Szeto, B.; Bonifas, A.; McCreery, R. L. Microfabrication and Integration of Diazonium-Based Aromatic Molecular Junctions. *ACS Appl. Mater. Interfaces* **2010**, *2*, 3693-3701.
- (13) McCreery, R. L.; Bergren, A. J. Progress with Molecular Electronic Junctions: Meeting Experimental Challenges in Design and Fabrication. *Adv. Mater.* **2009**, *21*, 4303-4322.
- (14) Belanger, D.; Pinson, J. Electrografting: A Powerful Method for Surface Modification. *Chem. Soc. Rev.* **2011**, *40*, 3995-4048.
- (15) Brooksby, P. A.; Downard, A. J. Electrochemical and Atomic Force Microscopy Study of Carbon Surface Modification Via Diazonium Reduction in Aqueous and Acetonitrile Solutions. *Langmuir* **2004**, *20*, 5038-5045.
- (16) Paulik, M. G.; Brooksby, P. A.; Abell, A. D.; Downard, A. J. Grafting Aryl Diazonium Cations to Polycrystalline Gold: Insights into Film Structure Using Gold Oxide Reduction, Redox Probe Electrochemistry, and Contact Angle Behavior. *J. Phys. Chem. C* **2007**, *111*, 7808-7815.
- (17) Garrett, D. J.; Lehr, J.; Miskelly, G. M.; Downard, A. J. Microcontact Printing Using the Spontaneous Reduction of Aryldiazonium Salts. *J. Am. Chem. Soc.* **2007**, *129*, 15456-15457.
- (18) Menanteau, T.; Levillain, E.; Downard, A. J.; Breton, T. Evidence of Monolayer Formation Via Diazonium Grafting with a Radical Scavenger: Electrochemical, Afm and Xps Monitoring. *PCCP* **2015**, *17*, 13137-13142.

- (19) Combellas, C.; Jiang, D. E.; Kanoufi, F.; Pinson, J.; Podvorica, F. I. Steric Effects in the Reaction of Aryl Radicals on Surfaces. *Langmuir* **2009**, *25*, 286-293.
- (20) Combellas, C.; Kanoufi, F.; Pinson, J.; Podvorica, F. I. Sterically Hindered Diazonium Salts for the Grafting of a Monolayer on Metals. *J. Am. Chem. Soc.* **2008**, *130*, 8576-8577.
- (21) Mattiuzzi, A.; Jabin, I.; Mangeney, C.; Roux, C.; Reinaud, O.; Santos, L.; Bergamini, J. F.; Hapiot, P.; Lagrost, C. Electrografting of Calix 4 Arenediazonium Salts to Form Versatile Robust Platforms for Spatially Controlled Surface Functionalization. *Nat. Commun.* **2012**, *3*.
- (22) Menanteau, T.; Levillain, E.; Breton, T. Electrografting Via Diazonium Chemistry: From Multilayer to Monolayer Using Radical Scavenger. *Chem. Mater.* **2013**, *25*, 2905-2909.
- (23) Malmos, K.; Iruthayaraj, J.; Pedersen, S. U.; Daasbjerg, K. General Approach for Monolayer Formation of Covalently Attached Aryl Groups through Electrografting of Arylhydrazines. *J. Am. Chem. Soc.* **2009**, *131*, 13926-+.
- (24) Nielsen, L. T.; Vase, K. H.; Dong, M. D.; Besenbacher, F.; Pedersen, S. U.; Daasbjerg, K. Electrochemical Approach for Constructing a Monolayer of Thiophenolates from Grafted Multilayers of Diaryl Disulfides. *J. Am. Chem. Soc.* **2007**, *129*, 1888-1889.
- (25) Leroux, Y. R.; Fei, H.; Noel, J. M.; Roux, C.; Hapiot, P. Efficient Covalent Modification of a Carbon Surface: Use of a Silyl Protecting Group to Form an Active Monolayer. *J. Am. Chem. Soc.* **2010**, *132*, 14039-14041.
- (26) Leroux, Y. R.; Hapiot, P. Nanostructured Monolayers on Carbon Substrates Prepared by Electrografting of Protected Aryldiazonium Salts. *Chem. Mater.* **2013**, *25*, 489-495.
- (27) Lee, L.; Leroux, Y. R.; Hapiot, P.; Downard, A. J. Amine-Terminated Mono Layers on Carbon: Preparation, Characterization, and Coupling Reactions. *Langmuir* **2015**, *31*, 5071-5077.
- (28) Liu, W. J.; Tilley, T. D. Sterically Controlled Functionalization of Carbon Surfaces with -C₆H₄CH₂X (X = Oso₂me or N-3) Groups for Surface Attachment of Redox-Active Molecules. *Langmuir* **2015**, *31*, 1189-1195.
- (29) Lee, L.; Ma, H. F.; Brooksby, P. A.; Brown, S. A.; Leroux, Y. R.; Hapiot, P.; Downard, A. J. Covalently Anchored Carboxyphenyl Monolayer Via Aryldiazonium Ion Grafting: A Well-Defined Reactive Tether Layer for on-Surface Chemistry. *Langmuir* **2014**, *30*, 7104-7111.

- (30) Leroux, Y. R.; Hui, F.; Hapiot, P. A Protecting-Deprotecting Strategy for Structuring Robust Functional Films Using Aryldiazonium Electroreduction. *J. Electroanal. Chem.* **2013**, *688*, 298-303.
- (31) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. A Stepwise Huisgen Cycloaddition Process: Copper(I)-Catalyzed Regioselective “Ligation” of Azides and Terminal Alkynes. *Angew. Chem. Int. Ed.* **2002**, *41*, 2596-2599.
- (32) Tornøe, C. W.; Christensen, C.; Meldal, M. Peptidotriazoles on Solid Phase: [1,2,3]-Triazoles by Regiospecific Copper(I)-Catalyzed 1,3-Dipolar Cycloadditions of Terminal Alkynes to Azides. *J. Org. Chem.* **2002**, *67*, 3057-3064.
- (33) Lee, L.; Brooksby, P. A.; Leroux, Y. R.; Hapiot, P.; Downard, A. J. Mixed Monolayer Organic Films Via Sequential Electrografting from Aryldiazonium Ion and Arylhydrazine Solutions. *Langmuir* **2013**, *29*, 3133-3139.
- (34) Lee, L.; Downard, A. J. Preparation of Ferrocene-Terminated Layers by Direct Reaction with Glassy Carbon: A Comparison of Methods. *J. Solid State Electrochem.* **2014**, *18*, 3369-3378.
- (35) Buttry, D. A.; Peng, J. C. M.; Donnet, J. B.; Rebouillat, S. Immobilization of Amines at Carbon Fiber Surfaces. *Carbon* **1999**, *37*, 1929-1940.
- (36) Gallardo, I.; Pinson, J.; Vilà, N. Spontaneous Attachment of Amines to Carbon and Metallic Surfaces. *J. Phys. Chem. B* **2006**, *110*, 19521-19529.
- (37) Casas-Solvas, J. M.; Ortiz-Salmeron, E.; Gimenez-Martinez, J. J.; Garcia-Fuentes, L.; Capitan-Vallvey, L. F.; Santoyo-Gonzalez, F.; Vargas-Berenguel, A. Ferrocene-Carbohydrate Conjugates as Electrochemical Probes for Molecular Recognition Studies. *Chem. Eur. J.* **2009**, *15*, 710-725.
- (38) Baramee, A.; Coppin, A.; Mortuaire, M.; Pelinski, L.; Tomavo, S.; Brocard, J. Synthesis and in Vitro Activities of Ferrocenic Aminohydroxynaphthoquinones against *Toxoplasma Gondii* and *Plasmodium Falciparum*. *Biorg. Med. Chem.* **2006**, *14*, 1294-1302.
- (39) Yu, S. S. C.; Tan, E. S. Q.; Jane, R. T.; Downard, A. J. An Electrochemical and Xps Study of Reduction of Nitrophenyl Films Covalently Grafted to Planar Carbon Surfaces. *Langmuir* **2007**, *23*, 11074-11082.
- (40) <https://Github.Com/Nrgunby/Uconga>. (accessed 10 December 2015).
- (41) Shao, Y.; Molnar, L. F.; Jung, Y.; Kussmann, J.; Ochsenfeld, C.; Brown, S. T.; Gilbert, A. T. B.; Slipchenko, L. V.; Levchenko, S. V.; O'Neill, D. P.; DiStasio Jr, R. A.; Lochan, R. C.; Wang, T.; Beran, G. J. O.; Besley, N. A.; Herbert, J. M.; Yeh Lin, C.; Van Voorhis, T.; Hung Chien, S.; Sodt, A.; Steele, R. P.; Rassolov, V. A.; Maslen, P. E.;

Korambath, P. P.; Adamson, R. D.; Austin, B.; Baker, J.; Byrd, E. F. C.; Dachsels, H.; Doerksen, R. J.; Dreuw, A.; Dunietz, B. D.; Dutoi, A. D.; Furlani, T. R.; Gwaltney, S. R.; Heyden, A.; Hirata, S.; Hsu, C.-P.; Kedziora, G.; Khalliulin, R. Z.; Klunzinger, P.; Lee, A. M.; Lee, M. S.; Liang, W.; Lotan, I.; Nair, N.; Peters, B.; Proynov, E. I.; Pieniazek, P. A.; Min Rhee, Y.; Ritchie, J.; Rosta, E.; David Sherrill, C.; Simmonett, A. C.; Subotnik, J. E.; Lee Woodcock Iii, H.; Zhang, W.; Bell, A. T.; Chakraborty, A. K.; Chipman, D. M.; Keil, F. J.; Warshel, A.; Hehre, W. J.; Schaefer Iii, H. F.; Kong, J.; Krylov, A. I.; Gill, P. M. W.; Head-Gordon, M. *Advances in Methods and Algorithms in a Modern Quantum Chemistry Program Package. PCCP* **2006**, *8*, 3172-3191.

(42) Wildgoose, G. G.; Hyde, M. E.; Lawrence, N. S.; Leventis, H. C.; Jiang, L.; Jones, T. G. J.; Compton, R. G. 4-Nitrobenzylamine Partially Intercalated into Graphite Powder and Multiwalled Carbon Nanotubes: Characterization Using X-Ray Photoelectron Spectroscopy and in Situ Atomic Force Microscopy. *Langmuir* **2005**, *21*, 4584-4591.

(43) Han, S. Y.; Kim, Y. A. Recent Development of Peptide Coupling Reagents in Organic Synthesis. *Tetrahedron* **2004**, *60*, 2447-2467.

(44) Montalbetti, C.; Falque, V. Amide Bond Formation and Peptide Coupling. *Tetrahedron* **2005**, *61*, 10827-10852.

(45) Combellas, C.; Kanoufi, F.; Pinson, J.; Podvorica, F. I. Time-of-Flight Secondary Ion Mass Spectroscopy Characterization of the Covalent Bonding between a Carbon Surface and Aryl Groups. *Langmuir* **2005**, *21*, 280-286.

(46) DuVall, S. H.; McCreery, R. L. Control of Catechol and Hydroquinone Electron-Transfer Kinetics on Native and Modified Glassy Carbon Electrodes. *Anal. Chem.* **1999**, *71*, 4594-4602.

(47) DuVall, S. H.; McCreery, R. L. Self-Catalysis by Catechols and Quinones During Heterogeneous Electron Transfer at Carbon Electrodes. *J. Am. Chem. Soc.* **2000**, *122*, 6759-6764.

(48) Chen, P. H.; McCreery, R. L. Control of Electron Transfer Kinetics at Glassy Carbon Electrodes by Specific Surface Modification. *Anal. Chem.* **1996**, *68*, 3958-3965.

(49) Amatore, C.; Saveant, J. M.; Tessier, D. Charge-Transfer at Partially Blocked Surfaces - a Model for the Case of Microscopic Active and Inactive Sites. *J. Electroanal. Chem.* **1983**, *147*, 39-51.

(50) Lee, L.; Brooksby, P. A.; Hapiot, P.; Downard, A. J. Electrografting of 4-Nitrobenzenediazonium Ion at Carbon Electrodes: Catalyzed and Uncatalyzed Reduction Processes. *Langmuir* **2016**, *32*, 468-476.

(51) McDermott, M. T.; Kneten, K.; McCreery, R. L. Anthraquinonedisulfonate Adsorption, Electron-Transfer Kinetics, and Capacitance on Ordered Graphite Electrodes - the Important Role of Surface-Defects *J. Phys. Chem.* **1992**, *96*, 3124-3130.

(52) Pontikos, N. M.; McCreery, R. L. Microstructural and Morphological Changes Induced in Glassy-Carbon Electrodes by Laser Irradiation. *J. Electroanal. Chem.* **1992**, *324*, 229-242.

(53) Allongue, P.; Delamar, M.; Desbat, B.; Fagebaume, O.; Hitmi, R.; Pinson, J.; Saveant, J. M. Covalent Modification of Carbon Surfaces by Aryl Radicals Generated from the Electrochemical Reduction of Diazonium Salts. *J. Am. Chem. Soc.* **1997**, *119*, 201-207.

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