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Two-Component Mixed and Patterned Films on Carbon Surfaces through the Photografting of Arylazides

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Organic films have been grafted to glassy carbon surfaces by the photolysis of arylazides. Atomic force microscopy and electrochemical measurements reveal that the films are loosely packed. The methodology was expanded to prepare two-component thin films incorporating either a reactive tether species and a nonreactive background film or two different reactive tethers. Strategies were developed to generate both continuous mixed films and surfaces presenting patterns of two components. For patterning, the arylazide derivative was grafted onto previously modified glassy carbon surfaces. In this case, the first modification step is not limited to photografting, which increases the scope of the methods. For all grafted surfaces, the reactivity of tether species was confirmed by coupling electroactive targets to the tethers, followed by electrochemical monitoring. The ease of preparing surfaces with spatially controlled functionality offers promise for the design of sensing platforms on graphitic carbon substrates.

Introduction

Control of the composition of the sensing surface is an important aspect of chemical and biosensor design and implementation. Surface immobilization of the molecular recognition species can facilitate integration of the sensing element with the transducer and incorporation of these into a device. Manipulation of the chemical and physical properties of the surface can also address unwanted interactions at the interface such as nonspecific binding of the analyte and the adsorption of components of the sample matrix. The development of modification methods suitable for application to sensing surfaces is thus an active area of research.

Conductive carbon has attractive properties for sensor construction because it is an easy-to-handle and usually low-cost material with high mechanical stability.¹ Importantly, the surface of carbon can be readily modified by reactions involving the generation of solution-based organic radicals that attack the surface, resulting in the formation of C–C or C–N covalent bonds between the modifier and the surface.^{2–4} The best-known examples of such reactions are the reduction of aryldiazonium salts to generate the corresponding aryl radical^{5,6} and the oxidation of primary amines to yield N-centered radicals.⁷ In general, these radical-based reactions result in carbon surfaces modified

with disorganized multilayer organic coatings, less than 10 nm thick,^{8–11} that exhibit excellent thermal, mechanical, and chemical stability and are stable over a relatively wide potential range.^{5,12,13}

Although redox methods have been most widely investigated, there are some reports of photolytic approaches for the covalent attachment of organic modifiers to graphitic carbon surfaces. The photolysis of carbon substrates in solutions of alkenes and alkynes or after spin coating from the same solutions results in grafting of monolayer films to the substrate.^{14–16} Alkenes and alkynes do not absorb at the wavelength used for these reactions, and hence the activation of the substrate is implicated in the grafting reaction.¹⁷ In contrast, when irradiated with UV light, azide and diazine derivatives are well known to eliminate dinitrogen to generate reactive nitrenes and carbenes, respectively. Arylazides in particular have been widely used for photo-affinity labeling¹⁸ and for the preparation of protein chips.¹⁹ Nitrenes can insert into O–H, N–H, and C–H bonds and also undergo C=C addition reactions with neighboring molecules,²⁰ giving several pathways for covalent grafting to a carbon surface. These reactions have been developed by Kuhr and co-workers who used azide and diazine derivatives of biotin

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(photobiotin) to generate micrometer-scale patterns of biotin on glassy carbon (GC) surfaces.^{21–26}

Our goal is to develop simple, versatile, and patternable methods for the modification of planar graphitic carbon substrates. To our knowledge, the photolysis of azides, other than the biotin derivative (photobiotin),^{21–27} has not been used for the preparation of such surfaces. The thermally induced decomposition of azides to nitrenes has been successfully applied to the functionalization of carbon nanotube sidewalls^{28–31} and, most recently, graphene.^{32,33} However, photolytic methods are more easily adapted to surface patterning. This approach was recently demonstrated with the photopatterning of perfluoroazide derivatives onto carbon nanotube forests for the control of surface wettability.³⁴

In this work, we explore the photolytic modification of glassy carbon and glassy carbon-like thin films (pyrolyzed photoresist films, PPF)³⁵ with the arylazide derivatives shown in Figure 1. Mixed films were prepared by the photolysis of a spin-coated solution containing two arylazide derivatives, and we also prepared two-component films by photografting arylazide derivatives to preformed modified surfaces. By expanding the latter strategy, arylazide derivatives were patterned onto film-coated surfaces by irradiation through a mask.

Experimental Section

Materials and Reagents. 4-Azidophenyl isothiocyanate (ITC_A), 4-azidoaniline hydrochloride (AP_A), 4-nitrobenzoic tetrafluoroborate (TSTU) and *N,N*-diisopropyl ethylamine (DIPEA) (all from Sigma-Aldrich, purity ≥97%), 4-nitrobenzoyl chloride (>98%) and ethylenediamine (>99%) (both from Merck-Schuchardt), methoxy-poly(ethylene glycol)-amine (PEG) (>99%, Laysan Bio), triethylene glycolamine (TEG, >97%, Prime Organics), sodium nitrite (98%, Panreal), and sodium citrate and 4-nitrobenzoic acid (both from BDH, >99%) were used as received. Dichloromethane (DCM) and *N,N*-dimethylformamide (DMF) (both from Scharlau Chemie, 99.9%), acetone (99.5%, Mallinckrodt Baker), and ethanol (EtOH, 99.9%, Merck) were used as received. Acetonitrile (ACN, 99.9%, BDH) was dried over calcium hydride for >2 days and refluxed under N₂ for at least 2 h prior to distilling in a N₂ atmosphere. Aqueous solutions were prepared using Millipore Milli-Q water (>18 MΩ cm). The tetrafluoroborate salts of the 4-nitrobenzenediazonium cation (NP_D) and the 4-carboxybenzenediazonium cation (CB_D) were synthesized from their arylamine precursors (Aldrich) using standard procedures.³⁶ 4-Azidobenzene (NP_A) was synthesized by adding 1 equiv of sodium azide

Figure 1. Arylazides used for photografting and aryldiazonium salts and amines used for electrografting.

(99%, Fisons) to an aqueous solution of the 4-nitrobenzenediazonium salt with cooling in an ice bath. After being stirred for 30 min, the solution was filtered and the precipitated product was washed with cold water and acetonitrile (ACN), suction dried, and stored in the dark. The preparation and drying of [Bu₄N]BF₄ have been described previously.⁹ All synthesized salts and arylazides were stored under vacuum and in the dark. Citrate-capped gold nanoparticles with a diameter of approximately 13 nm were prepared from hydrogen tetrachloroaurate(III) hydrate (99.9%, Strem Chemicals) using the method of Natan and co-workers.³⁷

Carbon Surface Preparation. PPF surfaces were fabricated as described previously⁹ and sonicated in ACN for 5 min before modification. Glassy carbon (Tokai Carbon Co. Ltd.) was cut into plates measuring 15 × 15 × 4 mm³. Prior to use, GC surfaces were polished with alumina slurries (0.1 then a 0.05 μm particle size) on a polishing cloth (all Leco materials), with sonication in Milli-Q water for 5 min after each polishing step. Polished GC surfaces were sonicated in ACN for 5 min before use. An additional rinse in DCM or DMF was performed prior to photochemical modification.

Electrochemistry. All electrochemical measurements were performed at room temperature under N₂ using computer-controlled EG & G PAR model 362 and model 273A potentiostats or an Eco Chemie Autolab PGSTAT 302 potentiostat. Glassy carbon and PPF working electrodes were mounted horizontally on an insulated metal stage under a glass cell secured with four springs. A hole in the bottom of the cell was positioned on top of a Viton O-ring that sealed the solution above the sample. Electrical contact was maintained with a copper strip externally connected to the sample. The geometric area of the working electrodes was 0.22 cm² for electrografting and 0.11 cm² for subsequent electrochemical analysis of the modified surface. A standard three-electrode cell was used. The counter electrode was a Pt wire, and the reference electrode for aqueous solutions was either saturated calomel (SCE) or Ag/AgCl, as indicated in the text. For nonaqueous solutions, the reference electrode was Ag/Ag⁺ (10^{−2} M AgNO₃ in 0.1 M [Bu₄N]BF₄-ACN) and all potentials in ACN are given versus this reference. The hydroxymethylferrocene/ferrocenium (FcOH/FcOH⁺) couple appeared at *E*_{1/2} = 0.021 V versus Ag/10^{−2} M Ag⁺. Voltammetric peak areas were estimated by fitting Lorentzian, Gaussian, or a mixture of Lorentzian–Gaussian curves to the peaks via the Levenberg–Marquardt algorithm. Polynomial baseline corrections were made prior to curve fitting using Linkfit 4.1 software.³⁸

Electrografting was performed in 0.1 M [Bu₄N]BF₄-ACN solutions containing 1 mM NP_D or 5 mM CB_D or 3 mM solutions of TEG or PEG. NP_D was grafted using two scans from 0.3 to −0.65 V at 100 mV s^{−1}, followed by electrolysis at *E*_p − 150 mV for 300 s; CB_D was grafted using two scans from 0.3 to −0.80 V at 100 mV s^{−1}, followed by electrolysis at *E*_p − 150 mV for 30 s. (*E*_p is the diazonium cation reduction peak potential obtained during the first scan.) TEG and PEG were electrografted using two initial

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Scheme 1. Surface-Modification Strategies

scans from 0 to 1.35 V at 100 mV s⁻¹, followed by electrolysis at 1.35 V for 300 s. After modification, all surfaces were sonicated in ACN for 10 min and dried in a stream of N₂.

Photochemical Modification. For the standard photografting procedure, bare or film-modified carbon surfaces were spin coated with two coats of arylazide solution at 1000 rpm for 30 s. Solutions of NP_A (200 mM) and ITC_A (20 mM) were prepared in DCM, and AP_A (20 mM) was prepared in DMF. Nonpatterned surfaces were irradiated at 365 nm inside a Rayonet Srinivasan-Griffin photochemical reactor (equipped with six 365 nm reaction lamp tubes, ~1 mW cm⁻²) for 30 min. To prepare patterned surfaces, samples were irradiated through a photomask in an in-house-built photoreactor fitted with a 365 nm UV lamp (Philips HPR 125 W, 0.8 mW cm⁻²). The modified substrates were sonicated for 5 min in DCM or DMF and then for 5 min in acetone and dried in a stream of N₂.

Postmodification Coupling Reactions. Glassy carbon samples derivatized with isothiocyanate groups were immersed in an aqueous solution of 4-nitrobenzoic hydrazide (5 mM) for 30 min at room temperature with stirring. After reaction, the surfaces were sonicated for 2 min in DMF followed by 2 min in Milli-Q water. Amine-terminated surfaces were derivatized by immersion in a saturated DCM solution of 4-nitrobenzoyl chloride (~200 mM) for 15 h with vigorous stirring. Postmodification sonication for 20 min in, successively, DCM, DMF, and Milli-Q water was necessary to remove unreacted species. Surfaces photografted with NP_A were electrochemically reduced prior to coupling with an activated *N*-succinimidyl ester prepared by reacting 0.01 M 4-nitrobenzoic acid with 1.2 mol equiv of DIPEA and TSTU in dry DMF for 3 h under N₂. The reduction of NP_A films was carried out in H₂SO₄ (concentration specified in text) at -0.7 versus Ag/AgCl for 120 s. The resulting amine groups were reacted with the activated *N*-succinimidyl ester solution for at least 24 h under N₂. After coupling, the samples were sonicated for 5 min in DMF. Gold nanoparticles were assembled on bare and modified carbon surfaces by incubation in an as-prepared gold colloid solution for 20 min at room temperature in the dark. After immersion, the samples were rinsed with Milli-Q water and gently dried with N₂.

Surface Characterization. Atomic force microscopy (AFM, Digital Instruments Dimension 3100) film thickness measurements on modified PPF surfaces were made as described previously.⁹ In brief, an AFM tip was used to scratch a section of film from the surface. From the cross-sectional profile of the scratch, the film thickness was determined. Unless stated otherwise, all quoted thickness data have an associated uncertainty of ±0.2 nm. This value includes the uncertainty arising from instrumental

(AFM) limitations, the variation in the depth of PPF removed when the film is scratched off of the surface, and the variation in the measured film thickness for replicate samples.

Scanning electron microscopy (SEM) images were recorded using either a 7000 HRSEM (JEOL) or a Raith 150 e-beam lithography system operating with 8 and 10 keV accelerating voltages, respectively. Gold nanoparticles were counted by analyzing multiple SEM images obtained from different regions of a surface using ImageJ v1.37 software (Wayne Rasband, National Institutes of Health, Bethesda, MD).

Optical micrographs of condensation figures were obtained using an Olympus BX60 microscope equipped with a digital DP10 camera unit (Olympus). Condensation figures were obtained by delivering a small amount of water vapor to the carbon surface and immediately recording an image.

Results and Discussion

Figure 1 and Scheme 1 show, respectively, the surface modifiers and modification strategies investigated in this work. Arylazide derivatives ITC_A and AP_A were selected for study because their substituents give reactive groups for further coupling reactions. Nitrophenyl (NP) derivative NP_A is convenient for characterization studies because the electroactivity of the NP group enables electrochemical monitoring of its surface concentration. Furthermore, NP groups are reduced in protic media to (mainly) aminophenyl groups, giving a useful tether layer.³⁹ Table 1 lists surface concentration data for NP groups immobilized on the surface via different strategies, as described below. Both PPF and GC were used in initial characterization experiments. PPF has a very low surface roughness (≤0.6 nm)⁹ and hence is a useful substrate for film thickness measurements. However, each sample of PPF is used once only, and hence GC, which can be regenerated by polishing, is more convenient for other measurements.

Single-Component Surface Modification. The three arylazide derivatives, shown in Figure 1, were used for photografting directly onto PPF and GC (Scheme 1a). For convenience, as described above, a detailed examination of the grafted films was undertaken using NP_A. Solutions of NP_A in DCM were spin-coated onto GC and PPF surfaces and photolyzed at 365 nm for selected times. After sonication to remove weakly attached material, the samples were mounted in an electrochemical cell

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Table 1. Surface Concentration of NP Groups in Single and Two-Component Films at GC from Photografting, Electrografting, and Coupling Reactions^a

film	coupling reagent	surface concentration
		($\times 10^{-10}$ mol cm ⁻²) ^b
NP _A	none	21.0 ($n = 5$) ^c
NP _A ^d	none	5.8 ($n = 3$)
ITC _A	NO ₂ -benzoic hydrazide	4.1 ($n = 1$)
AP _A	NO ₂ -benzoyl chloride	2.7 ($n = 3$)
NP _A -AP _A	none	5.5 ($n = 1$)
TEG-NP _A	none	3.1 ($n = 3$)
TEG-NP _A ^e	NO ₂ -benzoyl chloride	1.7 ($n = 1$)
CB _D -AP _A	NO ₂ -benzoic acid	2.4 ($n = 3$)
NP _D	none	30.0 ($n = 3$)
NP _D -AP _A	none	26.6 ($n = 3$)

^a Preparation conditions are described in the text. ^b All surface concentrations have an estimated uncertainty of 20% based on the maximum observed RSD for repeated measurements. The error estimate includes sample-to-sample reproducibility and curve fitting. ^c n is the number of separate samples analyzed. ^d NP_A at PPF. ^e The TEG-NP_A film was electroreduced in 0.1 M H₂SO₄ prior to the coupling reaction.

Figure 2. Two consecutive cyclic voltammograms in 0.1 M H₂SO₄ of a GC surface spin-coated with two layers of 200 mM NP_A followed by photolysis for 30 min. The solid line represents the first scan; the dashed line represents the second scan.

for voltammetric analysis. Figure 2 shows two consecutive cyclic voltammograms in 0.1 M H₂SO₄ for a GC surface spin-coated with two layers of 200 mM NP_A, followed by photolysis for 30 min. The first scan shows the characteristic, irreversible reduction ($E_p = -0.40$ V vs Ag/AgCl) of the NP group to aminophenyl (eq 1) and hydroxyaminophenyl groups (eq 2) and, on the return scan, the oxidation ($E_p = 0.3$ V vs Ag/AgCl) of hydroxyaminophenyl to nitrosophenyl groups (eq 3).⁴⁰ In the second cycle, the only significant feature is the nitrosophenyl/hydroxyaminophenyl redox couple.

These cyclic voltammograms demonstrate that the GC surface has been modified with an NP_A film. When the procedure was

Figure 3. Surface concentrations of NP groups vs photolysis time at (a) GC after spin-coating solutions of NP_A [20 mM (●), 100 mM (■) and 200 mM (red ▲)] and (b) PPF after spin-coating a 200 mM solution of NP_A. (c) AFM line profiles of PPF (i) before and (ii) after photografting with NP_A using a photolysis time of 10 min and a 200 mM NP_A spin-coating solution.

repeated without photolysis (spin-coated surfaces were stored in the dark for 30 min), there was no evidence of the immobilization of NP groups.

The effects of photolysis time and concentration of the spin-coating solution on the surface concentration of NP groups grafted to GC were examined, giving the data shown in Figure 3a. Surface concentrations of electroactive NP groups were estimated from the integrated charges associated with the NP reduction and hydroxyaminophenyl oxidation waves in the cyclic voltammograms (eqs 1–3 and Figure 2).⁹ Figure 3a shows that at all photolysis times, lower surface concentrations are obtained from the 20 mM NP_A spin-coating solution than for the 100 and 200 mM solutions and that the surface concentrations are the same for the 100 and 200 mM solutions. Use of the latter spin-coating solutions gives a maximum average surface concentration of $\sim 21 \times 10^{-10}$ mol cm⁻², obtained for photolysis times of 5 min and longer (Table 1). When PPF is used as the substrate (Figure 3b), the maximum surface concentration of NP decreases to $\sim 5.8 \times 10^{-10}$ mol cm⁻² (200 mM NP_A spin-coating solution). The lower surface concentration is undoubtedly in part due to the lower surface roughness of PPF compared to that of GC; however, the grafting yield may also be lower at PPF than at GC.^{41,42}

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Figure 4. First scan cyclic voltammograms in (a) 0.1 M H₂SO₄ of GC photografted with ITC_A (—) and polished GC (---) after reaction with 4-nitrobenzoic hydrazide and (b) in 0.25 M H₂SO₄ of GC photografted with AP_A (—) and polished GC (---) after reaction with 4-nitrobenzoyl chloride.

A close-packed layer of NP groups on a flat surface has a calculated surface concentration⁴³ of $\sim 12 \times 10^{-10}$ mol cm⁻², and hence the NP_A film on PPF is assumed to be loosely packed.

The thickness of the NP_A films grafted to PPF surfaces after 10 min of photolysis (200 mM NP_A spin-coating solution) was measured by AFM depth profiling, giving an average thickness of 0.8 ± 0.2 nm. On the basis of the relevant molecular dimensions, a vertically aligned monolayer film of NP_A has an expected height of 0.8 nm.⁹ However, the AFM line profiles in Figure 3c show that, compared with bare PPF, the grafted PPF surface is very rough with peak-to-peak heights significantly greater than 0.8 nm. Hence, these measurements support the formation of a loosely packed film with multilayer domains.

Photografting of ITC_A and AP_A to PPF was examined using 30 min of photolysis time and 20 mM arylazide solutions for spin-coating. Evidence of surface grafting was obtained from AFM measurements, which gave average film thicknesses of 1.0 and 0.8 nm for ITC_A and AP_A, respectively. For both modifiers, AFM line profiles (not shown) were similar to that of NP_A, suggesting similar film structures. The use of higher-concentration arylazide solutions for spin-coating gave rougher layers, and hence the standard procedure used 20 mM solutions of these azide derivatives. Additional tests were also carried out to confirm the attachment of the expected groups. The presence of isothiocyanate groups after grafting ITC_A to GC was demonstrated indirectly by the reaction of a photografted surface with 4-nitrobenzoic hydrazide. After 30 min of reaction and cleaning by sonication, the electrode was transferred to an electrochemical cell. Figure 4a shows the first scans obtained in 0.1 M H₂SO₄ at the grafted electrode (—) and at a polished GC electrode (---) that had been incubated in 4-nitrobenzoic hydrazide under the same conditions. At both the grafted and polished GC electrodes, the irreversible reduction peak provides clear evidence of NP groups immobilized at the surface. Hydrazide reacts with surface

carbonyl groups invariably present on polished GC;⁴⁴ however, after photografting with ITC_A, the amount of NP coupled to the surface is significantly greater, consistent with the reaction of the hydrazide with grafted isothiocyanate groups.

The surface concentration of coupled NP groups at the ITC_A-grafted surface can be estimated from the voltammogram (—) of Figure 4a, giving a value of $\sim 4.1 \times 10^{-10}$ mol cm⁻². This value is significantly less than the concentration of NP groups directly photografted to GC (21×10^{-10} mol cm⁻²), which may indicate that ITC_A has a lower photografting yield on GC than does NP_A. However, the similar AFM line profiles and average thicknesses of ITC_A and NP_A films on PPF suggest that their surface concentrations are similar. We have previously observed examples where coupling reactions at film-coated GC surfaces appear to be limited to the outermost layer(s), presumably because of permeability and steric effects within the films.³⁹ In that work, the average (n (number of samples) = 4) surface concentration of electroactive coupled NP groups was $(4.6 \pm 0.2) \times 10^{-10}$ mol cm⁻², even though the underlying films had significantly higher concentrations of reactive groups. Hence we assume that the measured surface concentrations of coupled groups observed here do not accurately reflect the surface concentrations of reactive groups in the first layer. Nevertheless, the detection of coupled groups is a useful means of confirming the presence and reactivity of the first layer.

A similar approach was used to demonstrate the success of photografting AP_A to the GC surface. Polished and AP_A-modified GC surfaces were reacted with 4-nitrobenzoyl chloride, and the resulting surfaces were examined by cyclic voltammetry. The characteristic response of coupled NP groups is evident in the voltammogram (—) in Figure 4b obtained at the photografted AP_A surface in 0.25 M H₂SO₄. As found for the ITC_A surface discussed above, the NP reduction peak is broad and the estimated surface concentration of NP groups is relatively low (2.7×10^{-10} mol cm⁻²), presumably for the reasons outlined above. Figure 4b (---) shows the voltammogram of a polished GC electrode reacted with 4-nitrobenzoyl chloride under the same conditions as for the AP_A-modified electrode. Trace amounts of NP groups indicated by the voltammogram are attributed to a small amount of physisorbed acid chloride that persists despite extensive cleaning of the surface by sonication (Experimental Section).

Two-Component Films from the Photolysis of Mixtures of Arylazides. Photolyzing mixtures of arylazides spin-coated onto the GC surface is a simple method of preparing mixed films. Surfaces modified with mixed films are particularly useful for biosensing applications because they can incorporate reactive tethers for coupling biomolecules and additionally a diluent component that serves to separate the tethers spatially and at the same time reduces nonspecific interactions with the surface.^{45,46} Coupling more than one component through selective reactions with different tether groups is another possibility with mixed films.⁴⁷ To demonstrate the preparation of mixed films (Scheme 1b), a DCM/EtOH (50:50) solution of NP_A (100 mM) and AP_A (10 mM) was spin-coated and photolyzed as described in the Experimental Section. Cyclic voltammograms (not shown) of the photografted surfaces confirmed the presence of NP_A groups with a surface concentration of $\sim 5.5 \times 10^{-10}$ mol cm⁻².

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Table 2. Surface Concentration of Gold Nanoparticles at Single and Two-Component Films at GC from Photografting and Electrografting Procedures

	single-component films			two-component films	
	NP _A ^a	AP _A ^b	NP _D	NP _A ^a /AP _A ^b	NP _D /AP _A ^c
nanoparticle density (np/μm ²)	13 ± 2 ^d	297 ± 26	3 ± 1	87 ± 9	48 ± 5
	(n = 3) ^e	(n = 3)	(n = 3)	(n = 4)	(n = 3)

^a[NP_A] = 100 mM. ^b[AP_A] = 10 mM. ^c[AP_A] = 20 mM. ^dValues reported as the mean ± standard deviation. ^eNumber of individual SEM images sampled.

As expected, this value is significantly lower than that for single-component NP_A films, consistent with competition for surface sites during the photolysis of two-component mixtures. The successful grafting of AP_A in the mixed film was confirmed in a second set of experiments. Following photografting and sonication cleaning, the modified substrates were incubated in a solution of citrate-capped gold nanoparticles for 20 min. Single-component NP_A and AP_A films were also placed in the nanoparticle solution for the same length of time, and all samples were imaged by SEM. Nanoparticle counts at each type of modified surface are listed in Table 2; SEM images of the surfaces are shown in Figure S1 in Supporting Information. The data provide clear evidence for the presence of AP_A groups in the mixed film, but unsurprisingly, the surface concentration of nanoparticles appears to be less than (approximately 29%) that of single-component AP_A films.

Two-Component Films by the Photografting of Arylazides to Film-Modified Surfaces. The photolysis of mixtures of arylazides, as described above, is the most straightforward approach to mixed films but is limited by the requirement that all modifiers must be soluble in the same solvent or solvent mixture. Spin-coating an arylazide solution onto a preformed film of the first modifier, followed by photolysis, is an alternative route to two-component films and does not have this limitation. Furthermore, in the two-step strategy, the first modifier can be immobilized by the photografting of arylazides but also by a variety of other methods, including the photolytic or thermal grafting of alkenes and alkynes,^{14–16,48} by electrochemical grafting from aryldiazonium salts, aliphatic amines, and arylacetates,³ or by spontaneous grafting at open-circuit potential from diazonium salts and aliphatic amines.² This versatility greatly broadens the range of components that can be included on the surface. Another advantage of the two-step method is that it is easily adapted to surface patterning using standard photolithography techniques, as described in the next section.

To demonstrate the formation of mixed layers by sequential grafting, two-component films covering the whole surface were prepared and characterized (Scheme 1c). In the first example, NP_A was photografted to a GC surface modified with a triethylene glycol (TEG) layer. This layer was electrografted to GC by the oxidation of TEG as described in the Experimental Section. NP_A was spin-coated onto TEG-modified GC, and the surface was exposed to UV light for 30 min. After cleaning by sonication, the sample was transferred to an electrochemical cell and cyclic voltammograms were obtained in 0.1 M H₂SO₄. The voltammograms of Figure 5a demonstrate that NP_A groups (surface concentration $\sim 3.1 \times 10^{-10}$ mol cm⁻²) are immobilized on the surface, but it is not clear where the groups are located. The

Figure 5. Two consecutive cyclic voltammograms in 0.1 M H₂SO₄ of (a) TEG-modified GC after photografting NP_A and (b) the electrode in panel a after reduction and subsequent reaction with 4-nitrobenzoic acid. The solid line represents the first scan; the dashed line represents the second scan.

photogenerated nitrene is expected to be able to react with the TEG layer by insertion into C–H bonds,²⁰ and it seems likely that NP_A groups are grafted to both the TEG film and the GC surface. Considering this uncertainty, it is important to establish whether the aminophenyl and hydroxyaminophenyl groups generated by the reduction of NP_A are accessible for further coupling reactions or, alternatively, are buried in the TEG layer. To examine this question, the reactivity of the aminophenyl and hydroxyaminophenyl groups with 4-nitrobenzoic acid was examined under conditions that promote amide bond formation (Experimental Section). Figure 5b shows cyclic voltammograms obtained in 0.1 M H₂SO₄ after the coupling reaction. There is clear evidence of the presence of NP groups (surface concentration $\sim 1.7 \times 10^{-10}$ mol cm⁻²), demonstrating the success of the reaction and establishing that the photografted groups are accessible and reactive.

In a second example using a similar approach, benzoic acid groups were first electrografted to the GC surface by the reduction of the corresponding diazonium cation, CB_D. Amine groups were added to the surface by spin-coating a solution of AP_A onto the modified surface and photolyzing for 30 min. To demonstrate the presence of the amine groups, an amide coupling reaction between nitrobenzoyl chloride and the film-based amine moieties was used to introduce NP groups onto the surface. Subsequent voltammetric analysis in 0.25 M H₂SO₄ showed a response very similar to that obtained for NP groups coupled, by the same method, to an AP_A film directly grafted to GC (voltammogram not shown). For the two-component film, the estimated surface concentration of NP groups is 2.4×10^{-10} mol cm⁻², compared with 2.7×10^{-10} mol cm⁻² for NP coupled to the single-component AP_A film. This suggests that AP_A groups have comparable reactivity on the two surfaces.

To investigate photografting reactions at modified surfaces further, a multilayer film of NP groups was electrografted

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Figure 6. Cyclic voltammograms in 0.1 M H₂SO₄ of GC electrodes modified with (—) an electrografted NP_D film and (---) an electrografted NP_D film followed by a photografted AP_A film.

onto GC from the corresponding diazonium salt and AP_A was photografted to this surface from a spin-coated solution. The assembly of citrate-capped gold nanoparticles was examined at one set of two-component films and compared with the assembly at electrografted NP_D films and AP_A films directly photografted to GC using the same photolysis conditions as for the two-component films. The data in Table 2 show that after 20 min of immersion in the gold nanoparticle solution the surface coverage of nanoparticles at the two-component film is markedly greater than that at a single-component NP_D film, confirming that AP_A groups are immobilized at the surface by photografting. In a separate set of experiments, the electroactivity of the NP groups on the surface before and after the grafting of AP_A was assessed. Figure 6 shows that the NP reduction peak areas for the two films are similar; however, the peak potential is shifted ~80 mV in the positive direction in the two-component film. This shift is tentatively attributed to the addition of electron-withdrawing protonated AP substituents to the NP_D film. Importantly, the electroactivity of the NP_D film is not measurably decreased after grafting AP_A, confirming that the method affords two-component films in which each component retains its usual activity.

These examples of mixed-film preparation demonstrate that the photografting of arylazides is a simple approach both to generating surfaces in which reactive tethers are incorporated in a nonreactive background film (as exemplified by grafting a mixed NP_A and TEG layer) and to generating surfaces with two different reactive tethers for selective coupling to different targets (as demonstrated by the sequential grafting of a benzoic acid film and AP_A, and NP_D and AP_A). Control of the relative amounts of each component in the film and the extension of the methods to the preparation of surfaces with more than two components should be straightforward via control of the composition of the solutions used for grafting.

Patterned Two-Component Surface Modification. Having established the utility of preparing two-component films by sequential grafting, the method was applied to the preparation of patterned surfaces. We note that Kuhr's group and others have previously reported patterned grafting of the azide derivative, photobiotin, directly onto GC surfaces,^{21–27} but our goal here is to prepare film-coated surfaces on which different areas have different surface properties and reactivity. Modifying the whole surface enables a higher degree of control of surface properties than can be achieved by modifying only part of the surface. The general strategy for patterning is shown in Scheme 1d. In the first step, the first modifier is deposited as a continuous layer on the surface. In the second step, a solution of arylazide is spin-coated onto the modified surface and the surface is exposed to UV light

Figure 7. (a) Optical micrograph of a condensation figure obtained from an NP_A film photopatterned onto a TEG film. (b) SEM image of an ITC_A film photopatterned onto a PEG film. (c, d) SEM images of an NP_A film photopatterned onto a CB_D film. Arrows indicate the photografted component.

through a photomask. Activation of the arylazide occurs only in areas exposed to the light, and hence grafting occurs in these areas only. Figure 7 presents images of patterned surfaces prepared using three different film combinations. Figure 7a is an optical micrograph of a condensation figure⁴⁹ obtained from a surface on which NP_A was photopatterned onto an electrografted TEG film. The SEM image in Figure 7b shows an electrografted methoxy-poly(ethylene glycol)-amine (PEG) film photopatterned with ITC_A, and micrographs c and d show an electrografted CB_D film photopatterned with NP_A. These examples demonstrate the preparation of surfaces that have a reactive tether patterned onto a background film designed to resist nonspecific protein adsorption⁵⁰ (Figure 7a,b) and a surface bearing spatially separated tethers with different reactivities (Figure 7c,d). All images clearly show the expected patterned areas and confirm that the sequential grafting method is readily adapted to preparing two-component patterned surfaces. In fact, the method can be extended to pattern any number of arylazide derivatives onto the surface, with a resolution dependent on the mask and/or optical patterning method used.

Conclusions

Photografting of arylazides to GC and film-modified surfaces by the photolysis of spin-coated samples at 365 nm in air is a simple and versatile method of surface modification. Films incorporating more than one modifier can be prepared using mixtures of arylazides or by photografting an arylazide modifier to a preformed film. The latter strategy has the advantage that the first film component can be grafted to the GC surface by any convenient method, giving very wide possibilities for the incorporation of desired organic functionalities on the surface. This strategy can also be readily adapted to patterning modifiers onto film-coated surfaces. For two-component films, both components

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534 retain their reactivity for further coupling reactions, indicating
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