LANGMUIR

Subscriber access provided by CORNELL UNIVERSITY LIBRARY

Article

“Janus” calixarenes: double–sided molecular linkers for facile, multi-anchor point, multi-functional, surface modification

James P. Buttress, David P. Day, James M. Courtney, Elliot J. Lawrence, David L. Hughes, Robin J. Blagg, Alison Crossley, Susan E. Matthews, Carl Redshaw, Philip C. Bulman Page, and Gregory G. Wildgoose

Langmuir, **Just Accepted Manuscript** • DOI: 10.1021/acs.langmuir.6b02222 • Publication Date (Web): 15 Jul 2016

Downloaded from http://pubs.acs.org on July 19, 2016

Just Accepted

“Just Accepted” manuscripts have been peer-reviewed and accepted for publication. They are posted online prior to technical editing, formatting for publication and author proofing. The American Chemical Society provides “Just Accepted” as a free service to the research community to expedite the dissemination of scientific material as soon as possible after acceptance. “Just Accepted” manuscripts appear in full in PDF format accompanied by an HTML abstract. “Just Accepted” manuscripts have been fully peer reviewed, but should not be considered the official version of record. They are accessible to all readers and citable by the Digital Object Identifier (DOI®). “Just Accepted” is an optional service offered to authors. Therefore, the “Just Accepted” Web site may not include all articles that will be published in the journal. After a manuscript is technically edited and formatted, it will be removed from the “Just Accepted” Web site and published as an ASAP article. Note that technical editing may introduce minor changes to the manuscript text and/or graphics which could affect content, and all legal disclaimers and ethical guidelines that apply to the journal pertain. ACS cannot be held responsible for errors or consequences arising from the use of information contained in these “Just Accepted” manuscripts.

ACS Publications

Langmuir is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Published by American Chemical Society. Copyright © American Chemical Society. However, no copyright claim is made to original U.S. Government works, or works produced by employees of any Commonwealth realm Crown government in the course of their duties.

“Janus” calixarenes: double–sided molecular linkers for facile, multi-anchor point, multi-functional, surface modification

James P. Buttress,[a] David P. Day,[a] James M. Courtney,[a] Elliot J. Lawrence,[a] David L. Hughes,[a] Robin J. Blagg,[a] Alison Crossley,[b] Susan E. Matthews,[c] Carl Redshaw,[d] Philip C. Bulman Page,[a]* and Gregory G. Wildgoose.[a]*

[a] School of Chemistry, University of East Anglia, Norwich Research Park, Norwich, NR4 7TJ, United Kingdom.

[b] Department of Materials, Oxford University Begbroke Science Park, Sandy Lane, Yarnton, Oxford, OX5 1PF, United Kingdom

[c] School of Pharmacy, University of East Anglia, Norwich Research Park, Norwich, NR4 7TJ, United Kingdom

[d] Department of Chemistry, University of Hull, Cottingham Road, Hull, HU6 7RX, United Kingdom.

ABSTRACT: We herein report the synthesis of novel “Janus” calix[4]arenes bearing four “molecular tethering” functional groups on either the upper or lower rims of the calixarene. These enable facile multi-point covalent attachment to electrode surfaces with monolayer coverage. The other rim of the calixarenes bear either four azide or four ethynyl functional groups, which are easily modified by the copper(I)-catalyzed azide-alkyne cycloaddition reaction (CuAAC), either pre- or post- surface modification, enabling these conical, nanocavity reactor sites to be decorated with a wide range of substrates to impart desired chemical properties. Redox active species decorating the peripheral rim are shown to be electrically connected by the calixarene to the electrode surface in either “up” or “down” orientations of the calixarene.

Introduction

Heterogeneous support of surface-immobilized species can provide several advantages over homogenous systems, such as being reusable, non-leaching and non-intrusive to the systems they are employed within. When the heterogeneous support is an electrode, such systems have found myriad applications from medicinal devices[1] and flow reactors[2] to sensors.[3,4,5] To date, the vast majority of surface immobilization strategies employ an approach of “one attachment point per modifying molecular species”. While this approach is often synthetically convenient, the disadvantage is that if the molecular attachment to the surface is broken under the reaction and/or electrolysis conditions, then the modifying species may leach from the surface – a common problem encountered in this research area. Leaching of the modifier from heterogeneous supports risks reducing lifetime and catalytic efficiency of the system or contaminating the reaction solution/product mixture. Obviously devising multi-point attachment strategies and incorporating these into the synthesis of every individual molecule that one might wish to support on a surface would be both laborious and impractical. Instead, we have devised a generic macromolecular scaffold using modified calix[4]arenes that serve as double-sided, conductive, molecular “sticky tape”, which are facile to produce in gram quantities. Either the top or bottom of the calixarene may be used to form four-point attachments to a surface while the other side is decorated with up to four modifying species.[6] We have dubbed these “Janus” calixarenes after the two-faced Roman god.

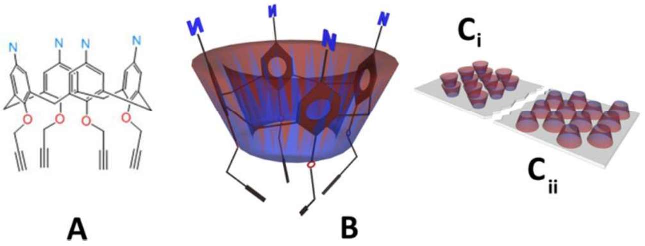


Figure 1. (A) Structure of "Janus" calix[4]arene ("N" groups are either azide or amine functionality). (B) Illustrates the cone structure of "Janus" calixarene. (C) The calixarene immobilized onto a surface by: (i) the lower rim; (ii) the upper rim.

Calix[4]arenes are multi-functionalizable macromolecules made up of four aromatic subunits connected by methylene linkers[7]. With suitable functional groups in place, calix[4]arenes can be locked into a cone-shaped geometry, which – like the Greek vase (“calix”) they are named after – have a wider upper rim and a narrower lower rim enclosing a hydrophobic, nanometer-sized, central cavity (Figure 1). Typically calixarenes are modified at two positions on the aromatic rings: the lower rim positions, which are commonly decorated with phenolic or ether groups, and the carbon atoms *para* to these positions which form the upper rim. The structural modification of calixarenes is an extensive area of research that has been reviewed by several authors in recent years,[8,9] with reports of upper-rim functionalization ranging from the use of biomolecular species such as peptides for the binding of small molecules and ligands,[10] urea-modified calix[4]arenes that participate in host-guest interactions with target substrates,[11] through to organometallic catalysts for polymerization reactions.[12] Modification of the lower rim of the calixarene structure, while less commonly reported, has been shown to enable calix[4]arenes to act as fluorescent reagents for sensing various anions and cations.[13] Furthermore, due to their unique 3D conical structure, calix[4]arenes have been shown to form densely packed monolayers on surfaces,[14] a property that we wish to exploit.

Previous strategies to attach large macrocyclic substrates such as calixarenes,[15] resorcinarenes,[16] and cyclodextrins[17] to surfaces have predominantly focused on the spontaneous adsorption of their thiol derivatives onto gold. This highly adaptable method is effective for a large array of substrates. However, this approach suffers one important limitation: the relatively weak gold-thiol bond is prone to cleavage resulting in a lack of thermal stability[18,19] and a relatively narrow electrochemical window in which to operate.[20] Furthermore this attachment strategy is only effective on "coinage" metals such as platinum, nickel and gold. In order to make our "Janus" calixarene approach universally applicable to the modification of both metallic and non-metallic (e.g. ubiquitous and inexpensive graphitic supports/electrode surfaces) we have chosen two alternative strategies: i) the reduction of aryldiazonium functionalities incorporated into the calix[4]arenes; ii) the oxidation of lithio-activated alkyne moieties on the calix[4]arene. Either strategy is applicable to the modification of a range of metallic and non-metallic surfaces[21,22,23] and can be selected to complement any redox sensitivity of the target modifying species when attached to the "Janus" calixarene prior to surface attachment.

The electrochemical one-electron reduction of aryl diazonium salts (generating a reactive aryl radical intermediate with loss of N_2) was first reported by Pinson *et al.*[24] in 1992 and has gained significant interest since then.[25,26] It is a highly versatile method of immobilizing compounds to solid supports, as aryl diazonium salts are easily generated from the corresponding aniline derivatives in either aqueous or non-aqueous solvents. Similarly, the electro-grafting process can take place in either aqueous[27] or organic media[28] and involves the formation of strong, robust C–C or C–metal bonds. Bulk surface modification is also readily achievable by chemical, rather than electrochemical, reduction of the diazonium salts, e.g. by using hypophosphorous acid.[29] The only minor drawback of this approach is that simple precautions must be taken to avoid the formation of disorganized, multilayer, films.[30,31]

The alternative surface attachment protocol we have selected, involving the oxidation of lithio-activated alkynes, was first described by Geiger *et al.* in 2013.[32] They initially demonstrated that several reactive organometallic complexes bearing pendent propargyl groups ($-CH_2C\equiv CH$) could be electrografted onto carbon and precious metal electrodes; in later work Geiger's group has extended this methodology to porphyrins.[33] The one-electron oxidation of either a terminal alkyne or lithio-alkyne results in the rapid loss of H^+ or Li^+ respectively, to form the desired propargyl radical intermediate for bond formation to the electrode surface.[33] Herein, we demonstrate that this approach can also be extended to covalently attach calixarenes to electrode surfaces.

Having selected two complementary and widely applicable surface attachment strategies, we were only left with the question of how to attach the target modifying molecules to the calixarene in a way that is applicable for a wide a range of different substrates. To this end, we report methods to functionalize either the upper or lower rim of calix[4]arenes, the rim not used for attachment to the surface, with either azide or ethynyl moieties. Simple incorporation of alkyne or azide groups onto the target substrate allows us to couple the substrate to the calixarene tethers using the extremely versatile copper(I) catalyzed alkyne-azide cycloaddition (CuAAC) reaction.[34] The substrate is then bound to the calixarene via a chemically and electrochemically robust triazole linkage.[35,36] Furthermore we show that full modification of each "Janus" calixarene with four modifying species can be accomplished using the CuAAC approach either before or, crucially, after the surface modification step.

Given the enormous scope of substrates that are amenable to this approach, in this report we are focused on presenting the synthesis, characterization and multi-point, robust, electrode surface modification of four different "Janus" calixarenes attached to the surface using two different strategies, and each bearing four identical functional groups on either upper or lower rims comprising of: i) organic nitro functionalities; ii) organometallic azide-functionalized ferrocenyl moieties; iii) ethynyl-functionalized ferrocenyl groups; iv) organic 1-ethynyl-3,5-bis(trifluoromethyl)benzene moieties. Substrates i)-iii) are used to demonstrate that the aromatic and unsaturated "Janus" calixarene linkers enable electron transfer to redox active centers attached at either the upper or lower rim of the calixarene. Substrate iv) is used as an XPS label to aid in surface characterization and to demonstrate that full functionalization of the "Janus" calixarenes can be readily achieved through CuAAC reactions *after* surface attachment of the calixarene. Aside from presenting synthetic routes to novel functionalized calix[4]arenes, including the first examples of tetraazido (upper rim) functionalized calix[4]arenes, this report seeks to demonstrate widespread applicability in providing a facile method of covalently attaching more than one species through more than one bond to a surface proffered by the use of "Janus" calixarenes.

Results and discussion

Suitable calix[4]arenes were synthesized for attachment to a glassy carbon electrode (GCE) surface at either the upper or lower rim. Once bound to the GCE surface, the exposed face of the calixarene was designed for facile modification using the copper-catalyzed azide alkyne cycloaddition (CuAAC) reaction.

Our starting material, *p-tert*-butylcalix[4]arene **1**, was *O*-alkylated with four propargyl groups following Chetcuti's procedure.[37] Attaching groups to the lower rim ensures that the calix[4]arenes maintain a cone confirmation (confirmed by the presence of two characteristic doublets in the ^{1}H-NMR spectra of every calix[4]arene studied, each corresponding to 4 methylene protons in the region of *ca.* 2.7–4.6 ppm)[38] throughout the synthesis. The *ipso*-nitration to functionalize the upper rim of the calixarene was conducted using fuming nitric acid and glacial acetic acid.[39] However, attempting the nitration reaction without first protecting the reactive terminal alkyne substituents failed to yield any of the desired product. Therefore, the propargyl groups were protected using *tert*-butyldimethylsilyl (TBDMS) groups, whereupon the nitration of the upper rim proceeded smoothly, leaving the alkyne groups intact. The nitro functionalities were then reduced using $SnCl_2.2H_2O$[40] in refluxing ethanol to afford the novel *cone*-5,11,17,23-tetra-amino-25,26,27,28-TBDMS-propargylcalix[4]arene **6**. Employing the Sandmeyer reaction, **6** was converted into compound **7**, through formation of the corresponding tetradiazonium salt by using sodium nitrite in dilute hydrochloric acid, and subsequently performing a nucleophilic aromatic substitution with sodium azide. Removal of the TBDMS protecting groups using a solution of 1 M tetra-*n*-butylammonium fluoride (TBAF) in THF afforded target molecule **8**. TBAF was also used to remove the TBDMS groups of molecule **4** to give the unprotected 5,11,17,23-tetra-nitro-25,26,27,28-propargylcalix[4]arene **5**. This novel calix[4]arene is highly versatile, with either rim being easily modifiable by the CuAAC reaction, affording us a very stable and functionalizable scaffold surrounding a hydrophobic cavity.

All novel compounds were fully characterized by mass spectrometry, nuclear magnetic resonance (NMR) and IR spectroscopy (see supporting information for more information). Only the final products require purification by flash chromatography before being attached to the electrode surface.

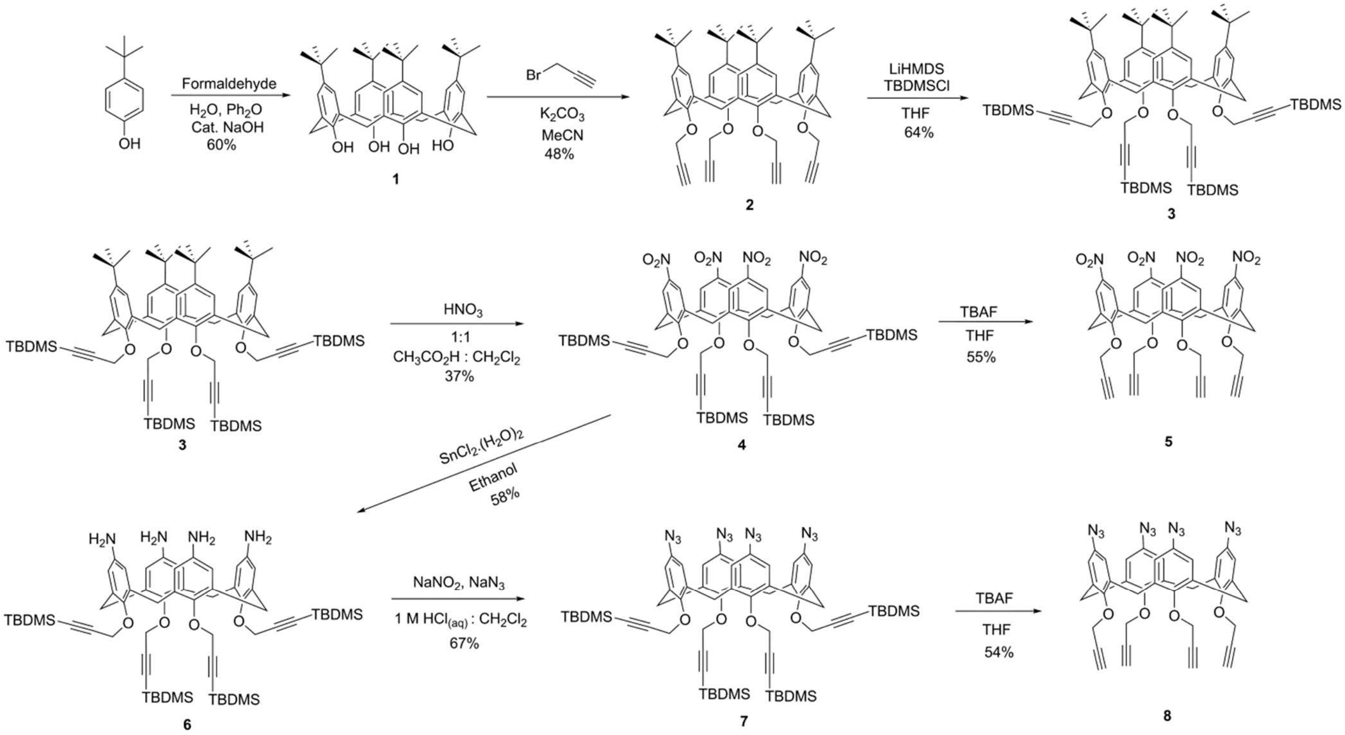


Figure 2. Synthesis of *cone*-5,11,17,23-tetra-azido-25,26,27,28-propargylcalix[4]arene **8**.

Attachment of "Janus" calixarenes by the upper rim

The surface of a GCE was modified with tetraaminocalix[4]arene **6** to form a robust, versatile platform that can be functionalized by a large array of substrates using the CuAAC reaction. The immobilization of calix[4]arene **6** was performed by the formation of its tetradiazonium salt, by reaction of **6** with stoichiometric sodium nitrite in 1 M hydrochloric acid. The diazonium salt was then reduced onto the electrode surface either *in situ*[41] or by isolating the salt and performing the reduction in anhydrous acetonitrile using $[^nBu_4N][PF_6]$ as electrolyte.[42] During the first reductive scan we observed a broad irreversible reduction at -0.35 V *versus* Ag/Ag^+, corresponding to the one-electron reduction of the diazonium salt to the phenyl radical, which then bonds to the electrode surface. Subsequent scans exhibited a large decrease in the background capacitance, suggesting that the exposed rim of the calixarene, modified with large silyl protecting groups, acts as a blocking layer to the electrode, passivating the surface (See Supporting Information Figure S45). The passivation observed is consistent with the electrografting of similar molecules in the literature. [30]

Having electro-reduced the diazonium salt onto the GCE surface, further functionalization was conducted using the CuAAC reaction.[43] The alkyne was deprotected using 1M TBAF, and ferrocenyl methyl azide was added to the immobilized calixarenes in a "click" reaction generating a triazole linker. This produced a ferrocene-modified calixarene on the surface of the GCE, as shown in figure 3A. In principle this method can be adapted for a large array of substrates. Models of all immobilized and post surface-modified calix[4]arenes were also separately synthesized in solution and fully characterized to show that they are indeed formed under these conditions and that they maintain their cone conformations (See Supporting Information, molecules **10** and **13**).

After attaching the ferrocene moieties by the CuAAC reaction, the electrode was thoroughly rinsed to remove any physisorbed material (see Supporting Information). The GCE was transferred to a fresh anhydrous electrochemical cell containing 0.1 M $[^nBu_4N][PF_6]$ in 10 mL dichloromethane. A well-defined reversible oxidation of the ferrocene moiety was observed, centered at 0.16 V *vs.* $Cp2Fe^{0/+}$, as illustrated in figure 3B. It was noted that the peak current corresponding to the reversible oxidation increases linearly upon increasing the voltage scan rate from 50 -750 mV s^{-1}, as shown in figure 3C. This result is clearly indicative of a surface–bound redox active species. In contrast, a redox active species in solution would exhibit a mass-transport limited diffusional response typified by a linear dependence of peak current upon the *square root* of the voltage scan rate. It is also important that over multiple scans the current corresponding to the ferrocene redox couple did not decrease, suggesting the surface adsorbed species is electrochemically stable.

The surface concentration, Γ (mol cm^{-2}), of ferrocene attached to the GCE surface was calculated from the peak areas. Assuming complete coupling of the ferrocenyl moieties to every available site on the calixarene, the surface coverage of ferrocene-modified calixarene was found to be Γ = 1.3 x10^{-10} mol cm^{-2}. Note that this assumption sets a lower limit on the calculated surface coverage of the calixarene linkers. Nevertheless, this value indicates a packing density of immobilized calixarenes on the surface of 0.78 calix nm^{-2}. This compares very favorably with the theoretical maximum value for a hexagonally packed monolayer of calixarenes (modelled as discs of diameter 11.6 Å derived from crystallographic dimensions of structurally similar calixarenes) of 0.85 calix nm^{-2},[44] strongly supporting the formation of a complete monolayer of surface immobilized calixarene linkers on the electrode surface. After formation of the monolayer, we were able to functionalize the adsorbed calixarenes with different organic and organometallic substrates using the CuAAC reaction. These calixarenes serve as excellent molecular tethers, connecting the redox active moieties to the electrode surface, while ensuring good electron conductivity between ferrocenyl units and the electrode, and effectively blocking the electrode surface to other redox active species that may be in solution.

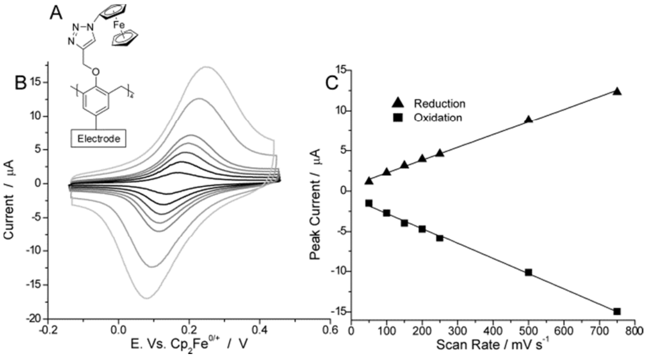


Figure 3. (A) Calix[4]arene **13** immobilized onto GCE surface. (B) Overlaid cyclic voltammograms recorded in CH_2Cl_2/0.1 M [$^nBu_4N$][PF_6], at scan rates of 50-750 mVs^{-1} for a GC electrode modified with calixarene **6** after further modification with ferrocenyl methyl azide moieties to form calix[4]arene **13** attached by the upper rim. (C) Corresponding plot of peak current versus scan rate.

Attachment of "Janus" calixarenes by the lower rim

Attachment of calix[4]arenes **5** and **8** at the lower rim to a GCE surface was achieved using the pendent propargyl ether groups. The formation of ethynyl-based radicals, generated by anodic oxidation of "lithio-activated" terminal ethynyl groups,[32] was used to electrochemically graft the substrates onto the surface. Lithiation of each calix[4]arene **5** or **8** was conducted in an anhydrous electrochemical cell containing a 0.1 M solution of [$^nBu_4N$][PF_6] and 4 mM calixarene dissolved in 10 mL THF cooled to -78 °C. Dilute (1.6 M) *n*-BuLi was added slowly to the reaction mixture and stirred for 20 minutes. Cyclic voltammetric characterization of the solutions revealed a large irreversible anodic oxidation wave present at 0.6 V vs Ag/Ag$^+$ for both calix[4]arenes **5** and **8** (see Supporting Information Figure S46), corresponding to the irreversible, one-electron oxidation of lithioethynyl groups on each calixarene.[32] Next, a chronoamperometric experiment was performed by holding the potential of the working electrode beyond the onset of oxidation, 0.9 V *vs.* Ag/Ag$^+$, for 300 s to electrograft substrates **5** and **8** separately onto different electrode surfaces. Note if the electrografting is attempted without the addition of *n*-BuLi, no oxidation is observed, suggesting that activation of the alkyne groups is essential to facilitate attachment onto the GCE.

With calixarene **5** bound to the GCE, the electrode was thoroughly rinsed to remove any physisorbed species and placed into an aqueous electrochemical cell containing 10 mL 1 M hydrochloric acid and 1.0 M KCl. The cyclic voltammetry obtained from this system showed a large irreversible reduction on the first scan at -0.52 V *vs.* SCE (peak 1 in Figure 4A), which corresponds to the chemically and electrochemically irreversible four-electron, four-proton reduction of the nitro functional groups on the calix[4]arene. After sweeping to potentials beyond this initial reduction and then reversing the scan direction a new oxidation wave was observed (peak 2). On further cycling, a new reduction peak (peak 3) was observed corresponding to peak 2; reduction peak 1 was no longer observed. This new, chemically and electrochemically reversible system (peaks 2 and 3), centered at 0.33 V *vs*. SCE, corresponds to the two-electron, two-proton arylhydroxylamine/arylnitroso redox couple. The observed voltammetric behavior is characteristic of the redox chemistry of arylnitro groups, and confirms the presence of calix[4]arene **5** on the electrode surface. Next, the voltage scan rate was varied between 50-750 mVs^{-1} over the *quasi*-reversible arylhydroxylamine/arylnitroso region (figure 4B). Once again, the oxidative and reductive peak currents were found to increase linearly with respect to the voltage scan rate (Figure 4C), confirming that the calix[4]arene **5** is indeed bound to the electrode surface.

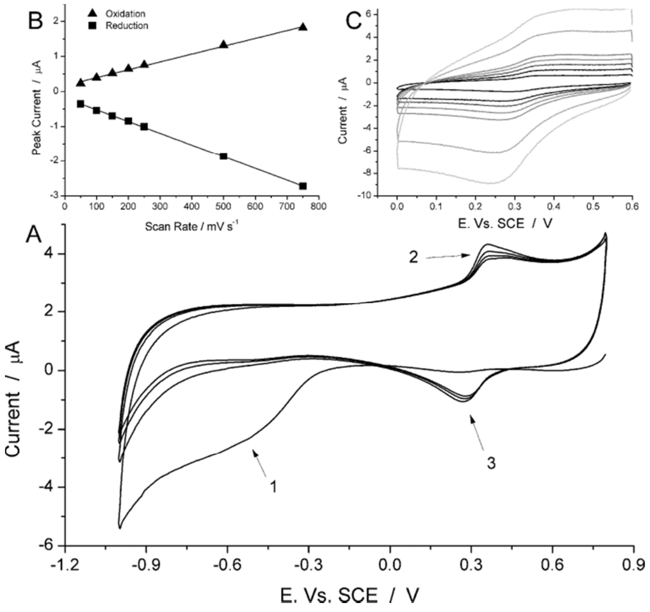


Figure 4. (A) Overlaid cyclic voltammograms of a GC electrode modified with **5** in 1 M HCl/1 M KCl, scan rate 0.1 V s^{-1}. (B) Corresponding plot of peak current *versus* scan rate. (C) Overlaid cyclic

voltammograms of the modified electrode recorded at 50-750 mVs^{-1} over the reversible arylhydroxylamine/arylnitroso redox couple.

The GCE modified with tetraazidocalix[4]arene **8** was treated with ethynyl ferrocene in the presence of Cu(I) to afford a ferrocenyl-modified calixarene immobilized onto the electrode surface shown in figure 5C. The electrode was thoroughly rinsed and transferred to an electrochemical cell containing 0.1M $[^nBu_4N][PF_6]$ in dry dichloromethane. Cyclic voltammetry was conducted at scan rates ranging from 50 mVs^{-1} to 750 mVs^{-1} (Figure 5A). We observed a non-ideal reversible oxidation of the ferrocenyl moiety centered at -0.27 *vs*. $Cp_2Fe^{0/+}$, where the peak current was again linearly dependent on the voltage scan rate, again indicative of a surface bound redox species (Figure 5B). Note, the waveshape of the redox couple of the ferrocene moieties is non-ideal, presumably, caused by the requirement for electrons to hop over the propargyl-ether linkers and/or the lateral interactions between charged centers around the upper rim. Whereas, the sharp reductive signal is attributed to the release of counter ions from the oxidized electrode surface, during the reduction process. Furthermore, it is important that the peak current remained constant over numerous scans suggesting that the chemisorbed material remains anchored to the electrode surface.

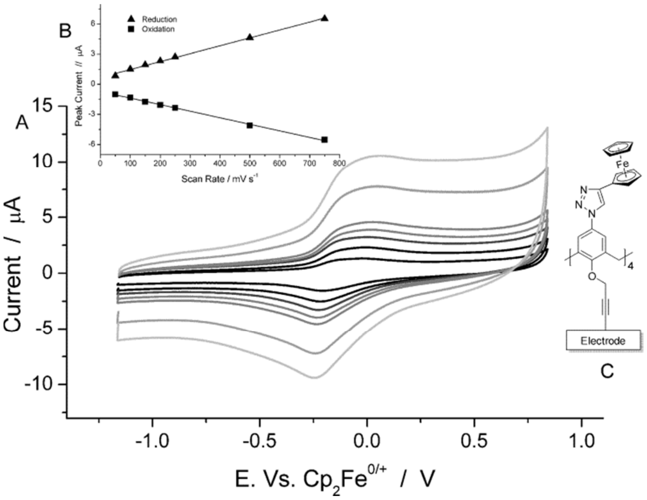


Figure 5. (A) Overlaid cyclic voltammograms of a GCE modified with, **8**, after post surface modification in CH_2Cl_2/0.1 M NBu_4PF_6, scan rate 0.05-0.75 mVs^{-1}. (B) Corresponding plot of peak current vs. scan rate. (C) Structure of modified electrode surface

The surface concentration Γ (mol cm^{-2}) of the ferrocene groups attached to the GCE surface was calculated from the oxidative and reductive peak areas to be $\Gamma = 5.6 \times 10^{-10}$ mol cm^{-2}. Assuming conversion of all the azide functional groups into the triazole-linked ferrocene moieties, this corresponds to a surface coverage of 0.84 calix nm^{-2}. Again, this value compares favorably with the theoretical value of 0.90 calix nm^{-2} for a hexagonally packed monolayer of calix[4]arenes (assumed to be discs of diameter 11.3 Å, derived from the crystallographically determined dimensions of **5**), indicating that we are able to attach a complete monolayer of calixarene linkers covalently to a GCE. Furthermore, this linker is shown to be easily functionalized *on the GCE surface* at all four sites on the upper rim using the CuAAC reaction.

XPS studies were also used to analyze the attachment and post-surface modifications of the calixarene linkers. This was conducted using a clean GCE that was modified with calixarene **8** using the lithiation and electrografting methodology described above. Instead of using a redox active probe we modified the immobilized calix[4]arene **8** molecular tether with 1-ethynyl-3,5-bis(trifluoromethyl)benzene, following the CuAAC reaction protocol as described in the Supporting Information. This introduces a fluorine-labelled probe onto the surface, whose presence is easily identified by X-ray photoelectron spectroscopy. The modified GCE was then thoroughly rinsed in a variety of solvents to remove any physisorbed species and analyzed using XPS. The full survey spectrum (See Supporting Information Figure S47) shows characteristic F_{1s} and N_{1s} signals from the probe molecules in addition to C_{1s} and O_{1s} signals arising from the GC surface. We also observed a small amount of Cl_{2p} and Si_{2p} impurities introduced by the polishing steps used to clean the electrode surface.

Strong evidence for the successful modification of the calixarenes after their surface immobilization onto the GCE is given by the presence of the peaks observed at binding energies of 399.7 eV and 688.3 eV (Figure 6). These correspond to the N_{1s} signal from the three nitrogen atoms in each of the triazole rings and the F_{1s} signal arising from the six fluorine atoms in the CF_3 groups of the 3,5-(trifluoromethyl)benzene moieties respectively. Note that had any unreacted azide groups been present on the GCE surface, these would have given rise to two characteristic nitrogen signals at 405 eV and 401 eV in a ratio of 1:2,[45] which are not observed. Figure 6A shows that we observe only one distinct triazole N_{1s} signal, suggesting that we have achieved complete modification of the calixarene molecular tether at almost all available reactive sites using the CuAAC reaction.

Further evidence in support of this claim is found in the ratio between the observed N_{1s} and F_{1s} signals measured in the XPS spectrum. After adjusting for the relative atomic sensitivity factors,[46,47] the relative abundance of the two elements on the surface of the GCE was found to be 1:1.9 (Nitrogen:Fluorine). This is very close to the 1:2 ratio of nitrogen:fluorine expected for complete modification of the surface–bound calixarene with 1-ethynyl-3,5-bis(trifluoromethyl)benzene moieties. Furthermore, the "click" reaction was performed on calix[4]arene **7** under similar conditions in solution to show that the molecule can be readily synthesized and maintains its cone confirmation (See Supporting Information Molecule **9**).

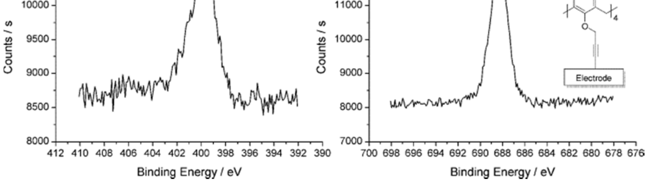


Figure 6. XPS spectra recorded over: (a) the N_{1s} region; (b) the F_{1s} region of the modified GCE. (c) Structure of the functionalized calixarene attached to the GCE surface.

Experimental

Commercially available reagents were purchased from Sigma-Aldrich (Gillingham, UK) and used without further purification unless stated otherwise. Anhydrous reactions were performed under a dry nitrogen atmosphere (BOC Gasses) using standard Schlenk-line techniques on a dual manifold vacuum/inert gas line. All glassware was dried in the oven overnight before use. Anhydrous solvents were dried via distillation over either sodium/benzophenone (tetrahydrofuran (THF) and diethyl ether) or calcium hydride (dichloromethane (DCM)) under an inert nitrogen atmosphere, and sparged with nitrogen gas to remove any trace amounts of dissolved oxygen. Water used in aqueous electrochemical reactions was also thoroughly sparged with nitrogen to remove dissolved oxygen prior to use. After any surface modification the electrode was sonicated for 10 minutes in each of the following solvents, water, acetone and dichloromethane before being allowed to dry.

Electrochemical measurements were performed under an inert atmosphere using computer-controlled potentiostat (Model PGSTAT 30, Autolab, Utrecht, The Netherlands) using a standard three–electrode configuration: a glassy carbon electrode (GCE) (BasiTechnicol, diameter 3 mm) served as the working-electrode with a platinum wire counter electrode (99.99%, GoodFellow, Cambridge, U.K.) and either a silver wire pseudo-reference electrode (99.99%, GoodFellow, Cambridge, U.K.) or a saturated calomel electrode (Radiometer, Copenhagen, Denmark) used as the reference electrode for non-aqueous and aqueous electrochemical measurements respectively. The working electrode surface was renewed by successive polishing with diamond paste slurries of decreasing particle size ranging from 3.0 μm to 0.1 μm (Kemmet, U.K.). The electrode was sonicated in deionized water and rinsed with ethanol between each polishing step to remove any adhered polishing materials.

Flash chromatography was performed on silica gel (SiO_2, 70-200 micron mesh 60 Å) purchased from Alfa Aesar. Thin layer chromatograph was performed using silica gel 60 Å F254 pre-coated plates.

Proton NMR (^1H NMR) spectra were recorded using a Bruker Avance DPX-500 MHz spectrometer. The data is reported as: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad; coupling constant(s) in Hz. Chemical shifts are reported in parts per million (ppm) relative to $CDCl_3$ (7.26 ppm). Carbon NMR (^{13}C NMR) spectra were recorded at 75 MHz and 125 MHz. Chemical shift is reported in ppm relative to the carbon resonance of $CDCl_3$ (77.00 ppm). IR spectra were recorded using a PerkinElmer *μ*-ATR Spectrum II spectrometer. X-ray photoelectron spectroscopy (XPS) was performed with a Thermo Scientific K-alpha instrument using monochromatic Al X-rays. All spectra were corrected relative to the graphitic C_{1s} peak position (284.97 eV). Calix[4]arenes **1**[48,49] and **2**[37] where synthesized following modified literature procedures full details in supporting information.

Synthesis of *cone*-5,11,17,23-tetra-*tert*-butyl-25,26,27,28-TBDMS-propargylcalix[4]arene 3. To a 3 necked 100 mL round bottom flask, *tert*-butyldimethylsilyl chloride (14.7 g, 97.5 mmol) was dissolved in anhydrous THF (50 mL). In a separate flask **2** (12 g, 16.2 mmol) was added and dissolved in anhydrous THF (100 mL), this was then cooled to -78 °C, and over a period of 15 minutes lithium bis(trimethylsilyl)amide solution (1.0 M in THF, 100 mL, 97.5 mmol) was added, resulting in a dark brown reaction mixture. After 30 mins the flask containing *tert*-butyldimethylsilyl chloride in THF was added dropwise, *via* cannula to the solution containing the deprotonated calixarene, **2**. After complete addition, the flask was allowed to warm to room temperature overnight. The reaction was then quenched by the addition of a saturated solution of ammonium chloride (100 mL). The organic layer was separated, and the aqueous layer washed with dichloromethane (100 mL). The organic extracts were combined, dried over $MgSO_4$, and remaining solvents removed *in vacuo*. The product **3** was isolated as a light brown solid (12.0 g, 64%). ^1H NMR (500 MHz, $CDCl_3$): 0.09 (24H, s), 0.90 (36H, s), 1.06 (36 H, s), 3.12 (4H, d, *J* = 13 Hz), 4.52 (4H, d, *J* = 13 Hz), 4.83 (8H, s), 6.75 (8H, s). ^{13}C NMR (125 MHz, $CDCl_3$): -4.5, 16.6, 26.3, 31.5, 32.7, 34.0, 61.4, 89.5, 103.6, 124.9, 134.8, 145.3, 152.1; IR (neat, ν / cm^{-1}): 2952, 2929, 2855, 2167 (alkyne), 1739, 1480, 1361, 1248, 1196, 1120, 1040; HRMS: *m/z*: $[M + NH_4]^+$ Calcd for $C_{80}H_{120}O_4Si_4$, 1274.8607, found 1274.8581

Synthesis of *cone*-5,11,17,23-tetra-nitro-25,26,27,28-TBDMS-propargylcalix[4]arene 4. To a round bottom flask, cooled to 0 °C, was added 3 (13 g, 10.4 mmol) dissolved in dichloromethane (130 mL) and glacial acetic acid (130 mL). Using a glass syringe, 100% nitric acid (54 mL, 1.29 mol) was added dropwise at 0 °C. After complete addition, the flask was allowed to warm to room temperature overnight. The reaction was then poured into iced water (200 mL), and the organic layer was separated by extraction. The aqueous layer was washed with dichloromethane (50 mL x3), and the remaining organic phases were combined, dried with $MgSO_4$, and remaining solvents were evaporated *in vacuo*. Re-dissolving the resulting residue in dichloromethane, followed by trituration with methanol yielded **4** as a cream powder (4.6 g, 37%). ^1H NMR (500 MHz, $CDCl_3$): 0.08 (24H, s), 0.86 (36H, s), 3.44 (4H, d, *J* = 14 Hz), 4.66 (4H, d, *J* = 14 Hz), 4.91 (8H, s), 7.71 (8H, s). ^{13}C NMR (125 MHz, $CDCl_3$):-4.7, 16.5, 26.0, 32.3, 62.6, 93.4, 99.9, 124.2, 136.3, 144.0, 159.7; IR (neat, ν / cm^{-1}): 2952, 2885, 2857, 2929, 2179 (alkyne), 1738, 1714, 1587, 1524 (nitro asymmetric), 1462, 1345 (nitro symmetric), 1251, 1205, 1096; HRMS: *m/z*: $[M + NH_4]^+$ Calcd for $C_{80}H_{120}O_4S_{i4}$, 1230.5501, found 1230.5497.

Synthesis of *cone*-5,11,17,23-tetra-nitro-25,26,27,28-propargylcalix[4]arene 5. Calixarene 4 (1 g, 0.82 mmol) was dissolved in a 1M solution of Tetra-n-butylammonium fluoride (TBAF) in THF (16.5 mL, 16.5 mmol) at room temperature, the reaction was monitored by TLC. Once complete the reaction was quenched by the addition of cold water (100 mL), and the product was extracted with dichloromethane (3x 50 mL) washings of the aqueous layer. The combined organic layers were then washed with water (2x 100 mL), dried using MgSO4. The filtrate was concentrated in vacuo, and the residue was purified using flash chromatography (acetone/petroleum ether 1:4) to afford **5** as a pale yellow-orange solid (0.34 g, 55%). 1H NMR (500 MHz, CDCl3): 2.60 (4H, t, J = 2 Hz), 3.49 (4H, d, J = 14 Hz), 4.72 (4H, d, J = 14 Hz), 4.86 (8H, d, J = 2 Hz), 7.71 (8H, s). 13C NMR (125 MHz, CDCl3): 26.0, 62.6, 93.4, 99.9, 124.2, 136.3, 144.0, 159.7; IR (neat, ν / cm-1): 3287, 3079, 2931, 2120 (alkyne), 1704, 1585, 1519 (nitro asymmetric), 1459, 1342 (nitro symmetric), 1202, 1093; HRMS: m/z: [M + NH4]+ Calcd for C40H32N5O12, 774.2042, found 774.2044.

Synthesis of cone-5,11,17,23-tetra-amino-25,26,27,28-TBDMS-propargylcalix[4]arene 6. Calix[4]arene 5 (4 g, 3.3 mmol) was dissolved in ethanol (200 mL), to which $SnCl_2.2H_2O$ (40 g, 177 mmol) was added and stirred at room temperature for 15 minutes. The reaction mixture was then submitted to reflux for 48 h. After allowing the reaction to cool to room temperature, 10% NaOH solution (200 mL) was added to basify the reaction mixture. Extraction of the aqueous layer with dichloromethane (3x 200 mL), followed by drying of the organic extracts over MgSO4 and solvent evaporation under reduced pressure afforded compound **6** as a light brown solid (2.05 g, 58%). 1H NMR (500 MHz, CDCl3): 0.08 (24H, s), 0.90 (36H, s), 2.93 (4H, d, J = 13 Hz), 4.45 (4H, d, J = 13 Hz), 4.69 (8H, s), 6.11 (8H, s). 13C NMR (125 MHz, CDCl3): -4.5, 16.6, 26.2, 31.1 (t, J = 167 Hz), 61.7, 89.4, 103.7, 115.6, 136.2, 141.2, 148.2; IR (neat, v / cm-1): 3325 (amine), 2954, 2928, 2855, 2173 (alkyne), 1606 (amine), 1471, 1361, 1249, 1207, 1097; HRMS: m/z: [M + H]+ Calcd for C64H93N4O4Si4, 1093.6267, found 1093.6269.

Synthesis of *cone*-5,11,17,23-tetra-azido-25,26,27,28-TBDMS-propargylcalix[4]arene 7. To a stirred solution of 6 (1.5 g, 1.37 mmol) in trifluoroacetic acid (TFA) (10 mL) at 0 °C a solution of NaNO2 (900 mg, 13 mmol) in cold water was added dropwise, the mixture was stirred at 0 °C for 10 mins. After this time a solution of NaN3 (1.8 g, 26 mmol) in cold water was added dropwise and the reaction mixture was stirred at 0 °C for 45 mins. Dichloromethane (10 mL) was then added and the reaction was allowed to warm up to room temperature overnight. The reaction was diluted with water and extracted with dichloromethane (3x 25 mL) washings of the aqueous layer. The combined organic layers were dried over MgSO4, filtered and concentrated in vacuo. The residue was purified using flash chromatography (ethyl acetate/petroleum ether 1:10) to afford **7** as pale yellow powder (1.0 g, 67 %). 1H NMR (500 MHz, CDCl3): 0.08 (s, 24H), 0.88 (s, 36H), 3.13 (d, J = 13.6 Hz, 4H), 4.53 (d, J = 13.6 Hz, 4H), 4.73 (s, 8H), 6.37 (s, 8H). 13C NMR (125 MHz, CDCl3): -4.58 16.53, 26.13, 61.96, 91.01, 101.97, 118.69, 134.78, 136.98, 152.12, IR (neat, v / cm-1): 2953, 2927, 2856, 2177 (alkyne), 2107 (azide), 1656, 1587, 1468, 1361, 1317, 1249, 1233, 1201, 1024; HRMS: m/z: [M + NH4]+ Calcd for C64H88N13O4Si4, 1214.6154, found 1214.6153.

Synthesis of *cone*-5,11,17,23-tetra-azido-25,26,27,28-propargylcalix[4]arene 8. Calixarene **7** (750 mg, 0.63 mmol) was dissolved in a 1M solution of TBAF in THF (13 mL mL, 13 mmol) at room temperature, the reaction was monitored by TLC. Once complete the reaction was quenched by the addition of cold water (100 mL), and the product was extracted with dichloromethane (3x 50 mL) washings of the aqueous layer. The combined organic layers were then washed with water (2x 100 mL), dried using $MgSO_4$. The filtrate was concentrated *in vacuo*, and the residue was purified using flash chromatography (acetone/petroleum 1:4) to afford **8** as a yellow solid (0.25 g, 54%). ^{1}H NMR (500 MHz, $CDCl_3$): 2.48 (t, *J* = 2.1 Hz, 1H), 3.17 (d, *J* = 13.8 Hz, 1H), 4.59 (d, *J* = 13.8 Hz, 1H), 4.70 (d, *J* = 2.1 Hz, 2H), 6.39 (s, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): 32.14, 61.63, 75.53, 79.88, 118.87, 135.10, 136.79, 152.41; IR (neat, v / cm^{-1}): 3295, 2921, 2195 (alkyne), 2104 (azide), 1587, 1529, 1467, 1366, 1314, 1232, 1201, 1001; HRMS: *m/z*: $[M + NH_4]^+$ Calcd for $C_{40}H_{32}N_{13}O_4$, 758.2695, found 758.2689.

Post surface modification of immobilized calyx[4]arene 6. Removal of the TBDMS protecting groups was conducted by submerging the GCE stub in a stirring solution of 1 M TBAF (10 mL) this reaction was ran for 14 hours. The electrode was removed from the solution and cleaned as described above. The CuAAC reaction of the unprotected alkyne with ferrocenylmethyl azide was achieved by immersing the stub in a rapidly stirred solution containing copper(II) sulfate (50 mg, 0.2 mmol) and sodium ascorbate (20 mg, 0.1 mmol) to this was added ethynyl ferrocene (10 mg, 0.05 mmol) dissolved in 2 mL dichloromethane, the reaction was ran for 12 hours. The GCE was then removed from the solution and cleaned before electrochemical analysis.

Post surface modification of immobilized calyx[4]arene 8. The post surface modification of molecule 8 was conducted by submerging the GCE stub in a rapidly stirring solution containing copper(II) sulfate (50 mg, 0.2 mmol) and sodium ascorbate (20 mg, 0.1 mmol) to this was added the desired alkynyl (0.05 mol) dissolved in 2 mL dichloromethane, the reaction was ran for 12 hours. The GCE was then removed from the solution and cleaned before electrochemical or XPS analysis.

Conclusion

In summary we describe two methods to form a monolayer of calix[4]arenes onto a GCE surface at either the upper or lower rims. These immobilized molecules have proven to be both chemically and electrochemically stable on an electrode surface, having up to four points of attachment to the surface per calixarene linker. These linkers can be easily functionalized by an array of substrates using the CuAAC reaction either before or after surface attachment, with four points of modification per calixarene. The use of bifunctional "Janus" calixarenes offers a simple, yet highly versatile route to robust, multi-attachment-point functionalization of surfaces for a wide range of potential chemical and biological species, with applications ranging from analytical sensors through to heterogeneous catalyst support.

ASSOCIATED CONTENT

Synthesis, electrode modification procedures and supplementary figures. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Authors

G.Wildgoose@uea.ac.uk
P.Page@uea.ac.uk

ACKNOWLEDGMENT

J.P.B. thanks the Leverhulme trust for financial support by PhD grant and the University of East Anglia for use of facilities. G.G.W. thanks the Royal Society for financial support through a University Research Fellowship. We thank the EPSRC UK National Crystallography Service at the University of Southampton for collection of the crystallographic data for compound 9. We acknowledge the use of the EPSRC funded National Chemical Database Service hosted by the Royal Society of Chemistry, and the EPSRC UK National Mass Spectrometry Facility (NMSF) at the University of Swansea. The research leading to these results has received funding from the European Research Council under the ERC Starting Grant Agreement No. 307061 (PiHOMER) and ERC PoC Grant Agreement No. 604988 (FLPower).

REFERENCES

(1) Zhang, Y.; Bai, Y.; Yan, B. Functionalized Carbon Nanotubes for Potential Medicinal Applications. *Drug Discov. Today* **2010**, *15* (11-12), 428–435.

(2) Tryba, B. Immobilization of TiO2 and Fe-C-TiO2 Photocatalysts on the Cotton Material for Application in a Flow Photocatalytic Reactor for Decomposition of Phenol in Water. *J. Hazard. Mater.* **2008**, *151* (2-3), 623–627.

(3) Sljukić, B.; Banks, C. E.; Compton, R. G. Iron Oxide Particles Are the Active Sites for Hydrogen Peroxide Sensing at Multiwalled Carbon Nanotube Modified Electrodes. *Nano Lett.* **2006**, *6* (7), 1556–1558.

(4) Boopathi, M.; Won, M. S.; Shim, Y. B. A Sensor for Acetaminophen in a Blood Medium Using a Cu(II)-Conducting Polymer Complex Modified Electrode. *Anal. Chim. Acta* **2004**, *512* (2), 191–197.

(5) Yemini, M.; Reches, M.; Gazit, E.; Rishpon, J. Peptide Nanotube-Modified Electrodes for Enzyme-Biosensor Applications. *Anal. Chem.* **2005**, *77* (16), 5155–5159.

(6) Fiore, M.; Chambery, A.; Marra, A.; Dondoni, A. Single and Dual Glycoside Clustering around calix[4]arene Scaffolds via Click Thiol-Ene Coupling and Azide-Alkyne Cycloaddition. *Org. Biomol. Chem.* **2009**, *7* (19), 3910–3913.

(7) Bohmer, V. Calixarenes , Macrocycles with (Almost) Unlimited Possibilities. *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 713–745.

(8) Nimse, S. B.; Kim, T. Biological Applications of Functionalized Calixarenes. *Chem. Soc. Rev.* **2013**, *42*, 366–386.

(9) Agrawal, Y. K.; Pancholi, J. P.; Vyas, J. M. Design and Synthesis of Calixarene. *J. Sci. Ind. Res.* **2009**, *68*, 745–768.

(10) Shuker, S. B.; Esterbrook, J.; Gonzalez, J. Solid-Phase Synthesis of a Novel Peptide Substituted Calix[4]arene. *Synlett* **2001**, *2*, 210–213.

(11) Julius, R. Host–guest Chemistry of Calixarene Capsules. *Chem. Commun.* **2000**, *8*, 637–643.

(12) Redshaw, C.; Walton, M.; Michiue, K.; Chao, Y.; Walton, A.; Elo, P.; Sumerin, V.; Jiang, C.; Elsegood, M. R. J. Vanadyl calix[6]arene Complexes: Synthesis, Structural Studies and Ethylene Homo-(Co-)Polymerization Capability. *Dalton Trans.* **2015**, *44* (27), 12292–12303.

(13) Kim, J. S.; Quang, D. T. Calixarene-Derived Fluorescent Probes. *Chem. Rev.* **2007**, *107* (9), 3780–3799.

(14) Lagrost, C.; Mattiuzzi, A.; Jabin, I.; Hapiot, P.; Reinaud, O. European Patent EP 12164038, 2012.

(15) Cormode, D. P.; Evans, A. J.; Davis, J. J.; Beer, P. D. Amplification of Anion Sensing by Disulfide Functionalized Ferrocene and Ferrocene-Calixarene Receptors Adsorbed onto Gold Surfaces. *Dalton Trans.* **2010**, *39* (28), 6532–6541.

(16) Collyer, S. D.; Davis, F.; Lucke, A.; Stirling, C. J. M.; Higson, S. P. J. The Electrochemistry of the Ferri/ferrocyanide Couple at a calix[4]resorcinarenetetrathiol-Modified Gold Electrode as a Study of Novel Electrode Modifying Coatings for Use within Electro-Analytical Sensors. *J. Electroanal. Chem.* **2003**, *549* (SUPPL.), 119–127.

(17) Nelles, G.; Weisser, M.; Back, R.; Wohlfart, P.; Wenz, G.; Mittler-Neher, S. Controlled Orientation of Cyclodextrin Derivatives Immobilized on Gold Surfaces. *J. Am. Chem. Soc.* **1996**, *118* (21), 5039–5046.

(18) Love, J. C.; Estroff, L. A.; Kriebel, J. K.; Nuzzo, R. G.; Whitesides, G. M. Self-Assembled Monolayers of Thiolates on Methals as a Form of Nanotechnology. *Chem. Rev.* **2005**, *105*, 1103–1169.

(19) Vericat, C.; Vela, M. E.; Benitez, G.; Carro, P.; Salvarezza, R. C. Self-Assembled Monolayers of Thiols and Dithiols on Gold: New Challenges for a Well-Known System. *Chem. Soc. Rev.* **2010**, *39* (5), 1805–1834.

(20) Wano, H.; Uosaki, K. In Situ Dynamic Monitoring of Electrochemical Oxidative Adsorption and Reductive Desorption Processes of a Self-Assembled Monolayer of Hexanethiol on a Au(111) Surface in KOH Ethanol Solution by Scanning Tunneling Microscopy. *Langmuir* **2005**, *21* (9), 4024–4033.

(21) Sheridan, M. V.; Lam, K.; Geiger, W. E. Spontaneous Attachment of Lithium-Activated Ferrocenylalkynes to Carbon and Gold. *Electrochem. Commun.* **2015**, *52*, 63–66.

(22) Kirkman, P. M.; Güell, A. G.; Cuharuc, A. S.; Unwin, P. R. Spatial and Temporal Control of the Diazonium Modification of sp2 Carbon Surfaces. *J. Am. Chem. Soc.* **2014**, *136* (1), 36–39.

(23) Celiktas, A.; Ghanem, M. A.; Bartlett, P. N. Modification of Nanostructured Gold Surfaces with Organic Functional Groups Using Electrochemical and Solid-Phase Synthesis Methodologies. *J. Electroanal. Chem.* **2012**, *670*, 42–49.

(24) Deiamar, 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* (114), 5884–5886.

(25) Lagrost, C.; Noël, J.-M.; Sjöberg, B.; Marsac, R.; Zigah, D.; Bergamini, J.-F.; Wang, A.; Rigaut, S.; Hapiot, P. Flexible Strategy for Immobilizing Redox-Active Compounds Using in Situ Generation of Diazonium Salts. Investigations of the Blocking and Catalytic Properties of the Layers. *Langmuir* **2009**, *25* (21), 12742–12749.

(26) Heald, C. G. R.; Wildgoose, G. G.; Jiang, L.; Jones, T. G. J.; Compton, R. G. Chemical Derivatisation of Multiwalled Carbon Nanotubes Using Diazonium Salts. *ChemPhysChem.* **2004**, *5* (11), 1794–1799.

(27) Corgier, B. P.; Marquette, C. A.; Blum, L. J. Diazonium-Protein Adducts for Graphite Electrode Microarrays Modification: Direct and Addressed Electrochemical Immobilization. *J. Am. Chem. Soc.* **2005**, *127* (51), 18328–18332.

(28) Kariuki, J. K.; McDermott, M. T. Formation of Multilayers on Glassy Carbon Electrodes via the Reduction of Diazonium Salts. *Langmuir* **2001**, *17* (19), 5947–5951.

(29) Pandurangappat, M.; Lawrence, N. S.; Compton, R. G. Homogeneous Chemical Derivatisation of Carbon Particles: A Novel Method for Funtionalising Carbon Surfaces. *Analyst* **2002**, *127* (12), 1568–1571.

(30) Leroux, Y. R.; Hapiot, P. Nanostructured Monolayers on Carbon Substrates Prepared by Electrografting of Protected Aryldiazonium Salts. *Chem. Mater.* **2013**, *25* (3), 489–495.

(31) Leroux, Y.; Fei, H.; Noël, J. Efficient Covalent Modification of a Carbon Surface: Use of a Silyl Protecting Group to Form an Active Monolayer. *J. Am. Chem. Soc.* **2010**, *132* (21), 14039–14041.

(32) Sheridan, M. V; Lam, K.; Geiger, W. E. An Anodic Method for Covalent Attachment of Molecules to Electrodes through an Ethynyl Linkage. *J. Am. Chem. Soc.* **2013**, *135*, 2939–2942.

(33) Sheridan, M. V; Lam, K.; Geiger, W. E. Covalent Attachment of Porphyrins and Ferrocenes to Electrode Surfaces through Direct Anodic Oxidation of Terminal Ethynyl Groups. *Angew. Chem. Int. Ed. Engl.* **2013**, *52* (49), 12897–12900.

(34) 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* (9), 3133–3139.

(35) Day, D. P.; Dann, T.; Hughes, D. L.; Oganesyan, V. S.; Steverding, D.; Wildgoose, G. G. Cymantrene−Triazole "Click" Products: Structural Characterization and Electrochemical Properties. *Organometallics* **2014**, *33*, 4687–4696.

(36) Day, D. P.; Dann, T.; Blagg, R. J.; Wildgoose, G. G. Synthesis and Characterization of Redox Active Cyrhetrene–triazole Click Products. *J. Organomet. Chem.* **2014**, *770*, 29–34.

(37) Chetcuti, M. J.; Devoille, A. M. J.; Othman, A. B.; Souane, R.; Thuéry, P.; Vicens, J. Synthesis of Mono-, Di- and Tetra-Alkyne Functionalized calix[4]arenes: Reactions of These Multipodal Ligands with Dicobalt Octacarbonyl to Give Complexes Which Contain up to Eight Cobalt Atoms. *Dalton Trans.* **2009**, *16* (16), 2999–3008.

(38) Gutsche, D. C.; Dhawan, B.; Levine, J. A.; Hyun No, K.; Bauer, L. J. Calixarenes 9: Conformational Isomers of the Ethers and Esters of calix[4]arenes. *Tetrahedron* **1983**, *39* (3), 409–426.

(39) Reinhoudt, D. N.; Verboom, W.; Durie, A.; Egberink, R. J. M.; Asfari, Z. Ipso Nitration of P-Tert-Butylcalix Arenes. *J. Org. Chem.* **1992**, *57*, 1313–1316.

(40) Bellamy, F. D.; Ou, K. Reduction of P-Nitrobenzoic Acid. *Tetrahedron Lett.* **1984**, *25*, 839–842.

(41) 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* (May), 1130.

(42) Laws, D. R.; Sheats, J.; Rheingold, A. L.; Geiger, W. E. Organometallic Electrodes: Modification of Electrode Surfaces through Cathodic Reduction of Cyclopentadienyldiazonium Complexes of Cobalt and Manganese. *Langmuir* **2010**, *26* (18), 15010–15021.

(43) Sayed, S. Y.; Bayat, A.; Kondratenko, M.; Leroux, Y.; Hapiot, P.; McCreery, R. L. Bilayer Molecular Electronics: All-Carbon Electronic Junctions Containing Molecular Bilayers Made With "click" chemistry. *J. Am. Chem. Soc.* **2013**, *135* (35), 12972–12975.

(44) Klimentová, J.; Vojtíšek, P. New Receptors for Anions in Water: Synthesis, Characterization, X-Ray Structures of New Derivatives of 5,11,17,23-Tetraamino-25,26,27,28-tetrapropyloxycalix[4]arene. *J. Mol. Struct.* **2007**, *826* (1), 48–63.

(45) Collman, J. P.; Devaraj, N. K.; Eberspacher, T. P. A.; Chidsey, C. E. D. Mixed Azide-Terminated Monolayers: A Platform for Modifying Electrode Surfaces. *Langmuir* **2006**, *22* (6), 2457–2464.

(46) National Institute of Standards and Technology (NIST) http://srdata.nist.gov/xps/.

(47) UK Surface Analysis Forum (UKSAF), http://www.uksaf.org/data.html.

(48) Gutsche, C. D.; Iqbal, M. P-Tert-BUTYLCALIX[4]ARENE. *Org. Synth.* **1990**, *68*, 234–235.

(49) Simaan, S.; Biali, S. E. Synthesis of P-Tert-Butylcalix[4]arene Derivatives with Trans-Alkyl Substituents on Opposite Methylene Bridges. *J. Org. Chem* **2003**, *68*, 3634–3639.

SYNOPSIS TOC (Word Style “SN_Synopsis_TOC”). If you are submitting your paper to a journal that requires a synopsis graphic and/or synopsis paragraph, see the Instructions for Authors on the journal’s homepage for a description of what needs to be provided and for the size requirements of the artwork.

To format double-column figures, schemes, charts, and tables, use the following instructions:

Place the insertion point where you want to change the number of columns
From the **Insert** menu, choose **Break**
Under **Sections**, choose **Continuous**
Make sure the insertion point is in the new section. From the **Format** menu, choose **Columns**
In the **Number of Columns** box, type **1**
Choose the **OK** button

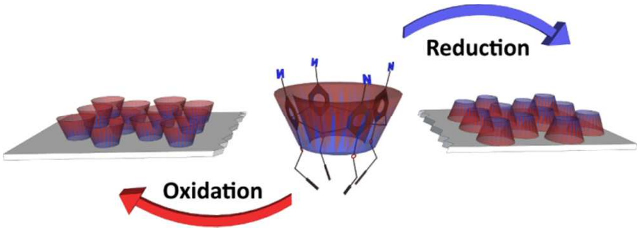
Now your page is set up so that figures, schemes, charts, and tables can span two columns. These must appear at the top of the page. Be sure to add another section break after the table and change it back to two columns with a spacing of 0.33 in.

Table 1. Example of a Double-Column Table

Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	Column 7	Column 8

Authors are required to submit a graphic entry for the Table of Contents (TOC) that, in conjunction with the manuscript title, should give the reader a representative idea of one of the following: A key structure, reaction, equation, concept, or theorem, etc., that is discussed in the manuscript. Consult the journal’s Instructions for Authors for TOC graphic specifications.

Insert Table of Contents artwork here



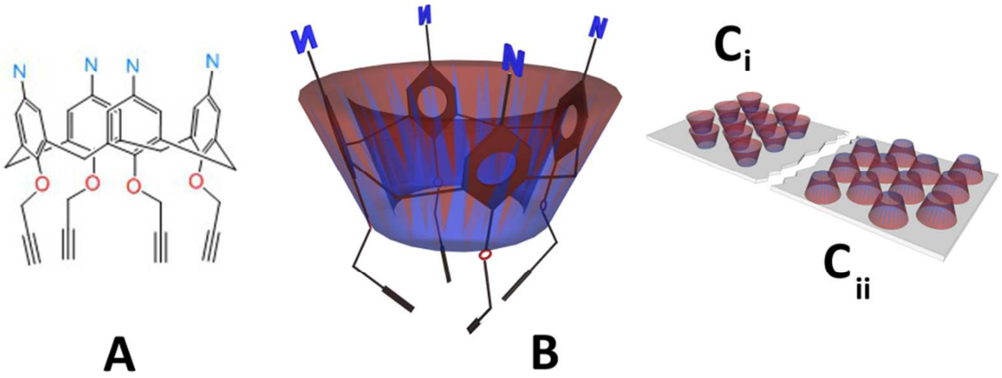


Figure 1. (A) Structure of "Janus" calix[4]arene ("N" groups are either azide or amine functionality). (B) Illustrates the cone structure of "Janus" calixarene. (C) The calixarene immobilized onto a surface by: (i) the lower rim; (ii) the upper rim.

84x34mm (300 x 300 DPI)

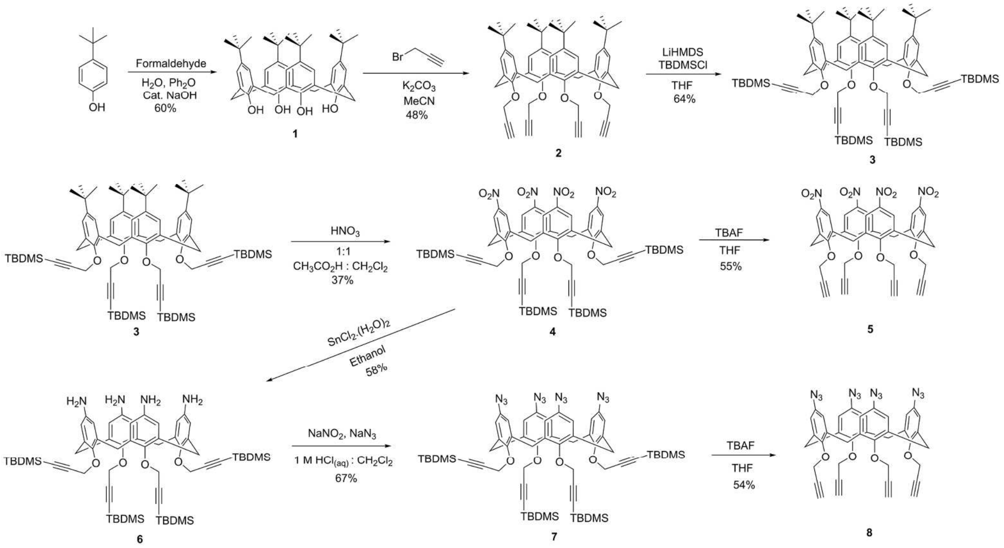


Figure 2. Synthesis of cone-5,11,17,23-tetra-azido-25,26,27,28-propargylcalix[4]arene 8.

97x53mm (600 x 600 DPI)

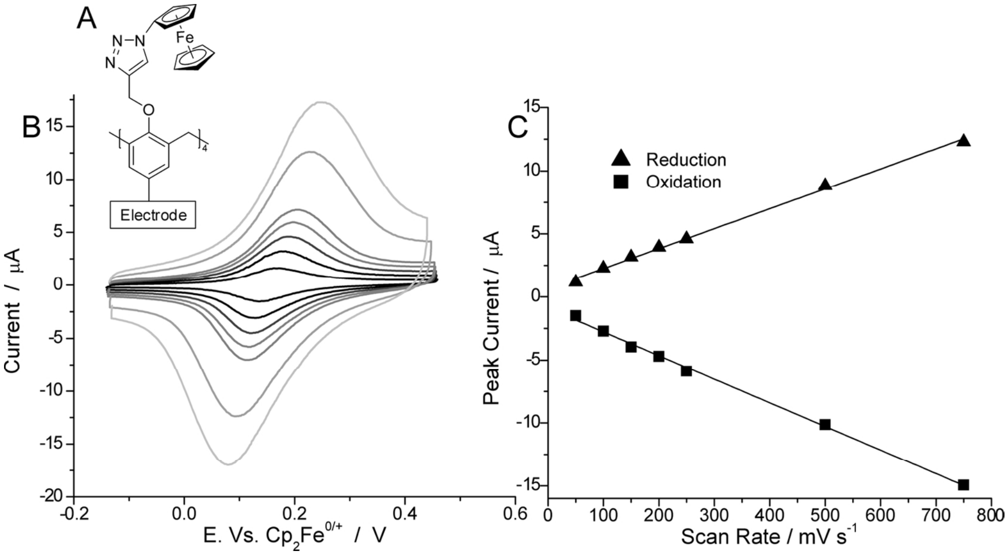


Figure 3. (A) Calix[4]arene 13 immobilized onto GCE surface. (B) Overlaid cyclic voltammograms recorded in CH2Cl2/0.1 M [nBu4N][PF6], at scan rates of 50-750 mVs-1 for a GC electrode modified with calixarene 6 after further modification with ferrocenyl methyl azide moieties to form calix[4]arene 13 attached by the upper rim. (C) Corresponding plot of peak current versus scan rate.

141x77mm (300 x 300 DPI)

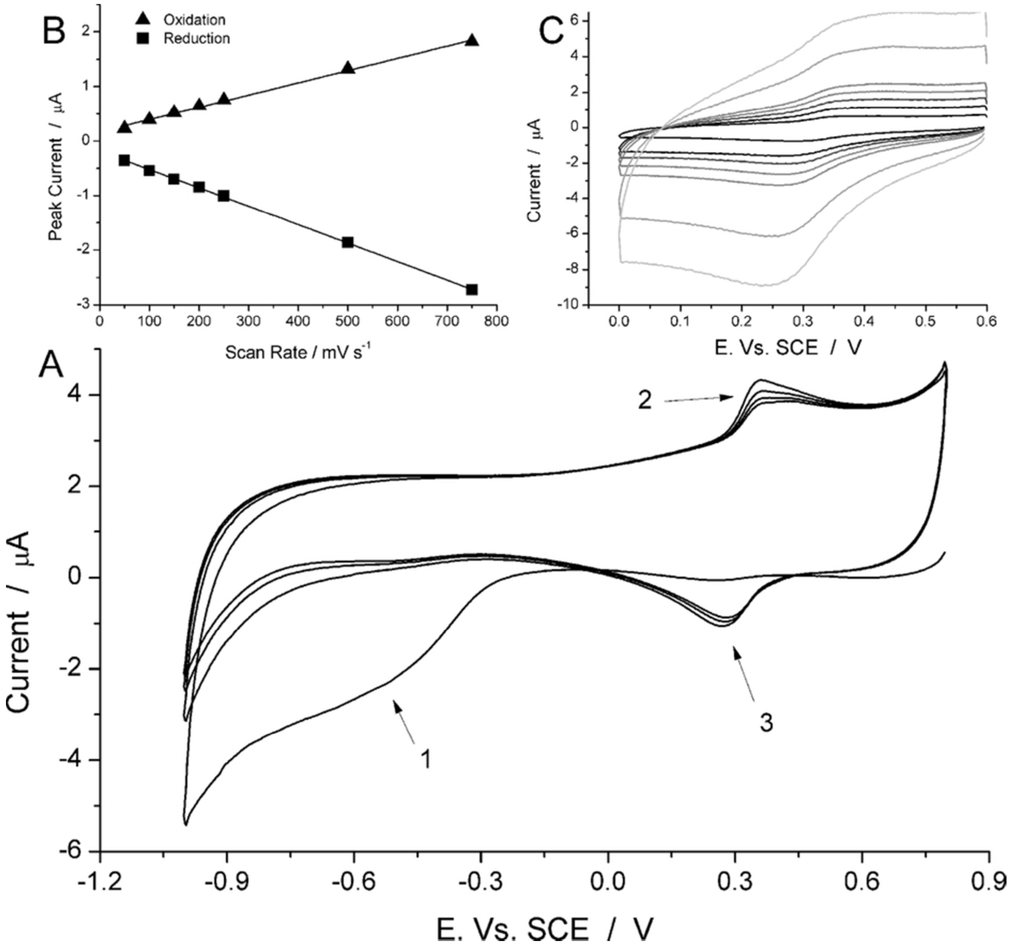


Figure 4. (A) Overlaid cyclic voltammograms of a GC electrode modified with 5 in 1 M HCl/1 M KCl, scan rate 0.1 V s-1. (B) Corresponding plot of peak current versus scan rate. (C) Overlaid cyclic voltammograms of the modified electrode recorded at 50-750 mVs-1 over the reversible arylhydroxylamine/arylnitroso redox couple.

79x74mm (300 x 300 DPI)

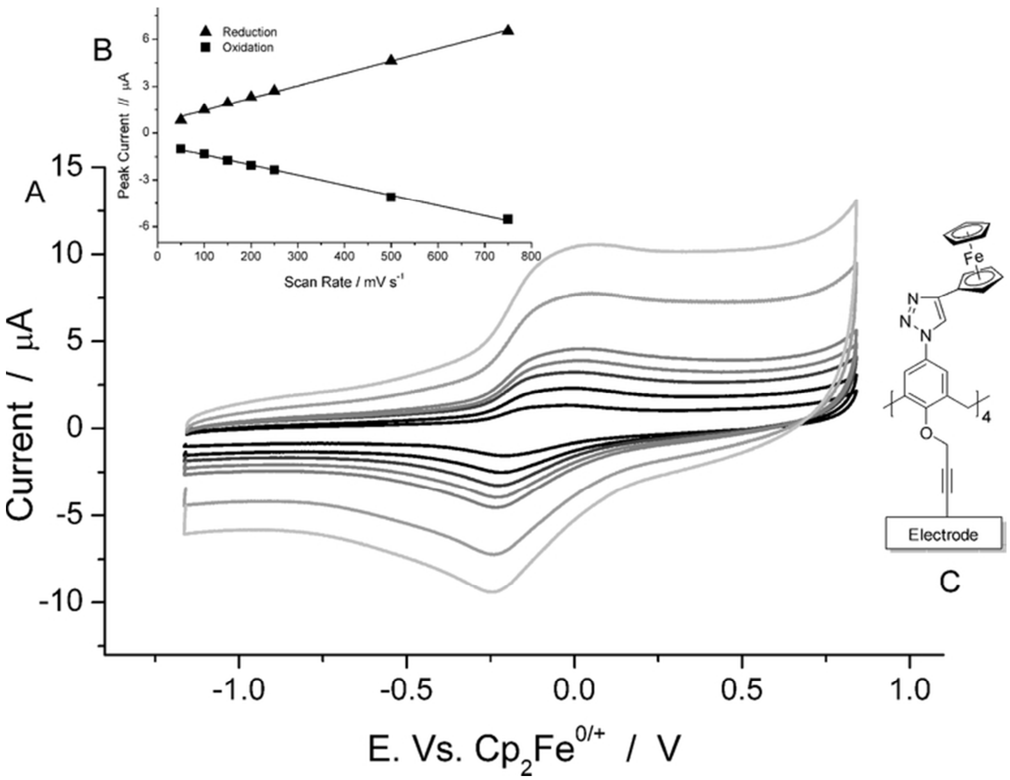


Figure 5. (A) Overlaid cyclic voltammograms of a GCE modified with, 8, after post surface modification in CH2Cl2/0.1 M NBu4PF6, scan rate 0.05-0.75 mVs-1. (B) Corresponding plot of peak current vs. scan rate. (C) Structure of modified electrode surface

65x50mm (300 x 300 DPI)

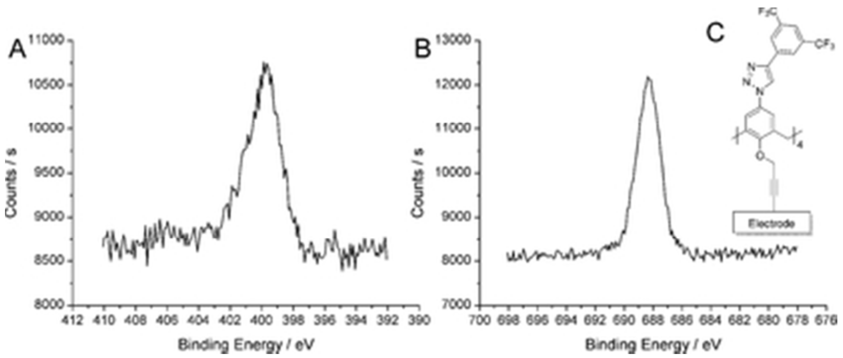
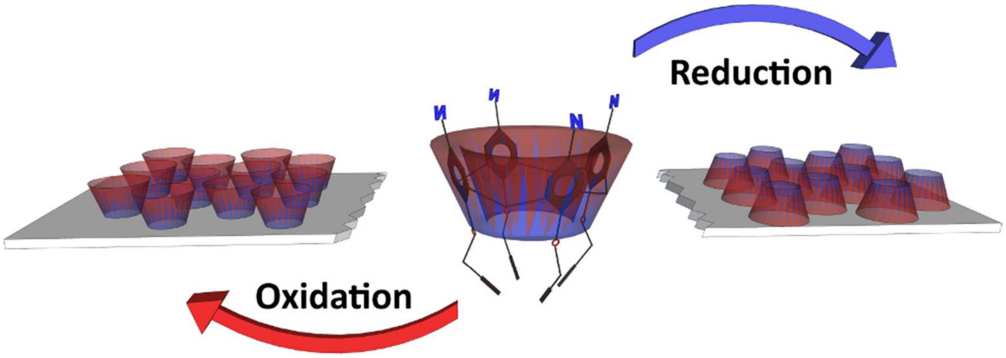


Figure 6. XPS spectra recorded over: (a) the N1s region; (b) the F1s region of the modified GCE. (c) Structure of the functionalized calixarene attached to the GCE surface.

35x14mm (300 x 300 DPI)



TOC image also found on last page of manuscript

83x29mm (300 x 300 DPI)