Electrochemical Studies of Redox-Functionalized Monolayer-Protected Gold Clusters

A Summary Report to The Electrochemical Society for the 1999 Edward G. Weston Summer Research Fellowship

by Allen C. Templeton

Nanometer-sized metallic and semiconducting particles have been the subject of considerable research attention over the last two decades.[1] This interest stems in part from their intermediate station between bulk materials and small molecular systems, giving rise for potentially unusual chemical, electronic, and physical properties. Numerous routes exist for the production of colloidal gold particles; however, the description by Brust *et al.* of nanometer-sized alkanethiolate monolayer-protected gold clusters (MPCs)[2] was one of the first examples of truly chemically robust metallic clusters. Thus, MPCs can be repeatedly isolated from and re-dissolved in common organic solvents without irreversible aggregation or decomposition, exhibit substantial air-stability, and can be characterized using standard analytical approaches.

Two main approaches have been developed for producing functionalized MPCs: (1) place-exchange reactions of MPCs with functionalized thiolates,[3] and (2) using simple chemical reactions of MPC pendant groups to affix target substituents (redox groups, flourophores, chromophores, etc.) to the nanoparticle surface.[4] The latter is often the preferred route since the synthesis of thiolates bearing redox groups or other groups of interest can present a more significant challenge. S_N2,[4a] amide,[4b] and ester[4b] coupling have all been explored as pathways to produce MPCs bearing multiple copies of a diverse selection of structural groups. Moreover, we have recently successfully extended this chemistry to functionalize alkanethiolate-MPCs to tiopronin, water-soluble MPCs.[5] The demonstration of cluster reactivity and functionalization is a prerequisite to probing important applications of these materials, including exploring the electrochemistry of redox-functionalized MPCs.

MPCs bearing multiple redox groups were first formed using place-exchange reactions with redox-alkanethiols,[3] and later using amide and ester coupling reactions.[4b] For example, we coupled multiple copies of a phenothiazine derivative to an MPC (see lower inset, Fig. 1).[4b] A solution of this cluster exhibits voltammetry (Fig. 1, solid line) nearly identical to that of the phenothiazine monomer (Fig. 1, dashed line). Currents for the MPC are smaller owing to its slower diffusion. Coulometry in thin-layer cells as a function of cell thickness, L, (Fig. 1, upper inset) showed that the average number of electroactive phenothiazines per cluster was 7.6, a result consistent with ^{1}H NMR data which gave an average of 7.4 phenothiazines per cluster. The coulometry provides both an independent measure of cluster loading and a confirmation of that all attached phenothiazines are electroactive.

As this example shows, electrode reactions of poly-redox-functionalized clusters are unusual in that many equivalents of redox charge per MPC can be delivered, under diffusion-control, to the electrode/solution interface. The few known precedents of such reactions include soluble redox polymers and redox-labeled dendrimers. Other examples of poly-redox-functionalized alkanethiolate-MPCs include ferrocene (≤ 25 per MPC by place-exchange),[3] and anthraquinone (≤ 25 per MPC by place-exchange).[6] When detectable, the electrochemical observations include currents for charging of the electrical double layers of the MPC Au cores, a characteristic reflecting the equivalence of MPCs to diffusing nanoelectrodes.[3, 6] Alkanethiolate-MPCs have also been prepared which bear mixtures of different redox groups (poly-hetero-functionalized MPCs), such as a mix of ferrocene and anthraquinone sites,[7] the voltammetry of each of the electron donor and acceptor groups was found to occur independently.

We recently prepared an example of poly-redox-functionalized water-soluble MPCs bearing multiple copies of a viologen derivative.[5] The [viologen]$^{1+/0}$ couple could be observed in aqueous solutions of monomer, by suppressing background currents at the Au electrode with a hexadecanethiol monolayer, but this second couple was not observed for cluster-bound viologen over the same range of potentials. Adsorption of reduced products may interfere with this second reaction.

Adsorption was detected and quantified using an electrochemical quartz crystal microbalance (EQCM). A frequency loss, Δf, of 37 Hz was observed, upon scanning through the potential

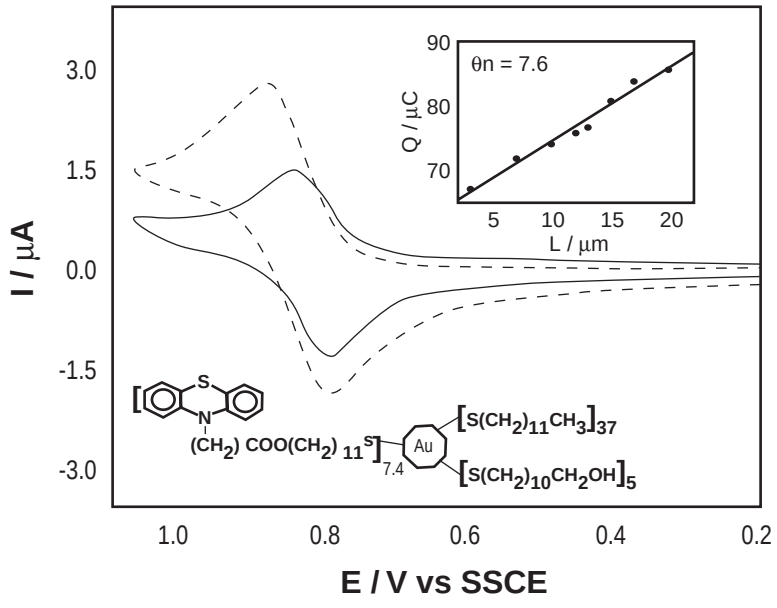

FIG. 1. *Electrochemical characterization of 10H-(phenothiazine-10)propionic acid-functionalized MPC (lower inset). Cyclic voltammetry of 0.8 mM 10H-(phenothiazine-10)propionic acid-functionalized MPC (-) and of 1 mM 10-H-(phenothiazine-10)propionic acid (--) in 2:1 toluene/Ch_3CN (v/v) at 100 mV/s. Upper inset: Thin layer coulometry charge Q vs. cell length L (r^2 = 0.98, slope = 11.47 X 10^{-3} C/cm).*

interval for the [viologen]$^{2+/1+}$ reaction, that was constant at potential scan rates between 10 and 100 mV/s. Assuming that the 37 Hz frequency decrease results from mass deposition on the Au surface, this frequency change corresponds roughly to a single, loosely packed monolayer of cluster on the electrode surface. Other interesting features of these experiments are noted in detail elsewhere.[5]

Multiple MPC electron donor and acceptor groups invite applications to mediated electrocatalysis. The basic prospects include concerted poly-electron exchanges with substrates, intramolecular electron transfer reactions during binding of MPCs to substrates (perhaps transiently), and (co-factor) MPCs that are multi-purpose by allowing both electron and proton transfers with a substrate in electron-proton coupled reactions. A significant step toward truly concerted poly-electron transfers will be shortening (or altogether removing) the connecting spacers between the redox groups and the metal-like Au so as to promote strong electronic coupling between multiple MPC electron donor-acceptor sites. Experiments are underway aimed at probing this effect.

In conclusion, electrochemical studies of poly-redox-functionalized MPCs are still at an early stage. Prospects for electrocatalysis and inducing electronic coupling between cluster-bound redox sites or the underlying gold core represent the frontiers of this newly emerging field of study. ■

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Electrochemical Characterization of Viologen-Functionalized PAMAM Dendrimers at a Pt Disk Microelectrode

A Summary Report to The Electrochemical Society for the 1999 Colin Garfield Fink Summer Research Fellowship

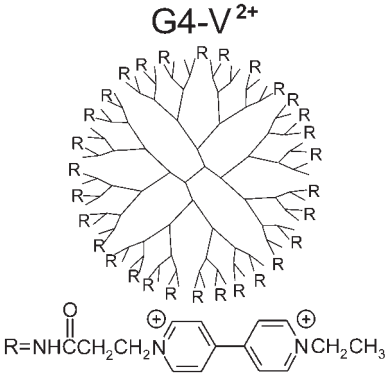
by Wendy S. Baker

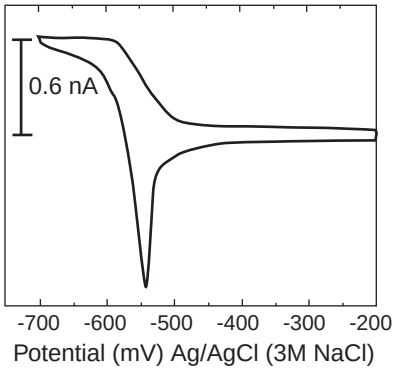
In previous studies, researchers have observed size-based molecular recognition effects at the very smallest microelectrodes.[1] For example, White *et al.* noted deviations from classical microelectrode behavior for ferrocene and decamethylferrocene at band electrodes having widths smaller than 10 nm.[2] Unfortunately, difficulties in the preparation of nanoscale electrodes of well-defined geometries have impeded this line of research. In a previous study we demonstrated that arrays of nanoscale microelectrodes could be fabricated by cyanide-etching of a hexadecanethiol monolayer.[3] The electrodes, whose size and shape were independently characterized using electrochemical and scanning probe techniques, had diameters ranging from 5 to 200 nm. A principal objective of our current project is to study size-based molecular recognition behavior at such nanoscale electrodes using redox-probe molecules of variable sizes (closely matched to the electrode dimensions), but which have similar electrochemical properties. To address this objective, a series of water-soluble, viologen-functionalized poly (amidoamine) (PAMAM) dendrimers has been synthesized and characterized.

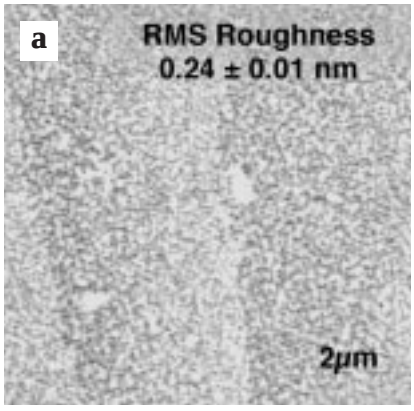
Figure 1 shows an idealized two-dimensional projection of an amine-terminated, fourth-generation PAMAM dendrimer ($G4\text{-}NH_2$) functionalized with 1-ethyl-1'-(3-propionic acid)-4-(4'-pyridyl)pyridinium dibromide ($G4\text{-}V^{2+}$). $G4\text{-}NH_2$ has a diameter of 4.5 nm, however, dendrimers having smaller ($G0\text{-}NH_2$/1.5 nm) and larger ($G6\text{-}NH_2$/6.7 nm) diameters have also been functionalized.[4] A combination of 1-[3-(dimethylamino)propyl-3-ethyl carbodiimide hydrochloride(EDC) and N-hydroxysuccinimide(NHS) coupling reagents was employed to form an amide linkage between the dendrimer and the viologen precursor.[5] Ratioing the integrated ^1H NMR intensities of the viologen aromatic protons with respect to the aliphatic dendrimer protons indicates that 20% to 30% of the dendrimer primary amine groups are functionalized using this approach.

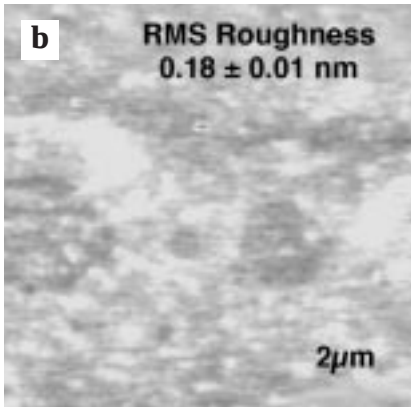
Figure 2 shows a cyclic voltammogram obtained at a 5 µm-radius Pt microelectrode in a deoxygenated 0.2 mM solution of a $G4\text{-}V^{2+}$. On the negative scan, a steady-state, limiting current response ($i_{l,c}$) of 0.6 nA was obtained, while on the reverse scan, a -0.75 nA peak due to oxidation of a precipitate is present. This peak suggests that the radical cation of $G4\text{-}V^{2+}$ ($G4\text{-}V^{+\bullet}$) electroprecipitates onto the electrode surface.

Figure 3 shows tapping-mode AFM data for adsorbed $G4\text{-}V^{2+}$ films obtained on Au(111) surfaces. Figure 3a was obtained after soaking the substrate for 1 min in a 0.2 mM $G4\text{-}V^{2+}$ solution. The image indicates that the oxidized dendrimer adsorbs on Au(111). Electroprecipitation at -0.8 V (vs. Ag/AgCl, 3M NaCl) yields the film shown in Figure 3b. The key point is that AFM shows adsorption of both the oxidized and reduced species, while the electrochemical data suggests that only the reduced species is adsorbed.









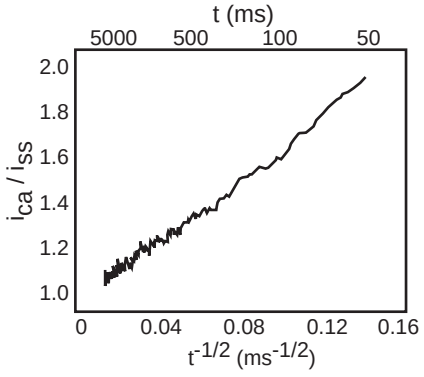


FIG. 1. (top left) Two-dimensional projection of a fourth-generation PAMAM dendrimer and the viologen functional group used in this study.

FIG. 2. (bottom left) Cyclic voltammogram of an aqueous, deoxygenated 0.2 mM $G4\text{-}V^{2+}$ solution. Electrolyte: 0.1 M KNO_3; Scan Rate: 100 mV/s.

FIG. 3. (above and above right) Tapping-mode AFM of a Au(111) surface after (a) soaking in an aqueous 0.2 mM $G4\text{-}V^{2+}$ solution for 1 min and then rinsing in deionized water and (b) reduction of a 0.2 mM $G4\text{-}V^{2+}$ electrolyte solution and emersion at -0.8 V vs. Ag/AgCl (3 M NaCl) followed by rinsing. Electrolyte: 0.1 M KNO_3.

FIG. 4. (bottom right) Normalized chronoamperometric response in aqueous, deoxygenated 0.2 mM $G4\text{-}V^{2+}$ solution. Electrolyte: 0.1 M KNO_3; Initial Potential: -0.3 V; Final Potential: -0.7 V.

The objective of this study is to use viologen-terminated dendrimers as probes of nanoscopic electrode size-exclusion effects, and therefore it is necessary to know both the diffusion coefficient (D_o) and the number of electrons transferred (n) during reduction of G4-V^{2+}. Accordingly, we used chronoamperometry at a Pt disk microelectrode to determine both D_o and n for G4-V^{2+}. Data from such experiments are treated by employing the equation for the chronoamperometric current (i_{ca})at a microdisk electrode.[6]

Figure 4 depicts the chronoamperometric response in 0.2 mM G4-V^{2+} normalized with respect to the steady-state current (i_{ss}) at a 5 µm-radius Pt electrode. The calculated diffusion coefficient is 1.2 x 10^{-6} cm ^{2}/s and n is 13. NMR and calibrated UV-vis data (obtained using the viologen precursor as the calibration standard) have shown that each dendrimer is functionalized with an average of 19 viologen moieties. The difference between the electrochemically determined value and that calculated from the spectroscopic data may be due to the low signal-to-noise ratio of the chronoamperometry data, or complications in the data interpretation arising from precipitation of G4-$V^{+\bullet}$ (Fig. 2). Analysis of the smaller G2-V^{2+} dendrimer indicates n=3 regardless of the analysis method.

In conclusion, the synthesis and electrochemical characterization of a series of viologen-functionalized PAMAM dendrimers have been described. Chemisorption of the oxidized species to Au(111) has been shown by tapping-mode AFM, while electroprecipitation of the reduced species at a Pt microdisk electrode is apparent from both cyclic voltammetry and tapping-mode AFM. Values for D_o and n were calculated for both G4-V^{2+} and G2-V^{2+} by chronoamperometry, and the values for n were in reasonable agreement with the degree of functionalization measured by NMR. Efforts are currently underway to employ the molecules as probes for characterizing nanoscale electrodes. ■

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An Electrochemical Approach for Developing a Chip-Based Immunosensor

A Summary Report to The Electrochemical Society for the 1999 Joseph W. Richards Summer Research Fellowship

by C. Ajith Wijayawardhana

Electrochemical immunoassay, like all other immunoassay methods, is an analytical technique based on the binding reactions between antibodies and antigenic sites on the analyte molecule.[1] The principles of the electrochemical immunoassay protocol currently used in our labs is illustrated in Fig. 1. In this method, the analyte is first captured by antibodies attached to the surface of paramagnetic beads (2.8 µm). The analyte is then sandwiched by a conjugate antibody carrying the enzyme label alkaline phosphatase (ALP). The enzyme substrate para-aminophenyl phosphate (PAPP) is then added. Quantitative measurement of the analyte is accomplished by electrochemical detection of the enzymatic product 4-aminophenol (PAP), the concentration of which relates directly to the number of analyte molecules.[2]

Over the last two years, our group, in collaboration with the Center for Microelectronic Sensors and MEMS, has been involved in developing a fully automated electrochemical immunosensor on a chip. The final goal is to use the sensor in detecting biological warfare agents. However, with the proper choice of antibodies, the sensor can be readily adapted to clinical and environmental immunoassays.

In reaching this goal, we have adopted a scale-down approach in which the initial focus was to develop a model bead-based electrochemical immunoassay in larger volumes, a task that has been successfully accomplished.[3, 4] Following this, we developed an automated meso version of the ultimate chip sensor that is partly fitted with microfabricated components used in the chip. Recently, a complete model assay for the analyte mouse-IgG was accomplished in the meso system. The assay procedure was very similar to that proposed for the chip system. The reagents were contained in reservoirs that open through a combination of valves to a central microfluidic path. Magnets placed at different positions along the path were used to capture beads for the various incubations and rinses necessary in an assay. A microfabricated interdigitated array type electrode (IDA) with 7 µm band widths and spacings, placed just before the path outlet, was used for a detector. Briefly, the assay involved 5 minute antigen and conjugate incubation times and the collection of beads slightly upstream of the electrode for PAPP incubation. The result of a 2500 ng/mL mouse IgG assay is shown in Fig. 2. As expected, the signal grows with the length of PAPP incubation. Although detectable signal change is attained in 1-2 minutes, longer incubation times were necessary for smaller antigen concentrations. For a 5 minute incubation cut-off time, a detection limit of 5 ng/mL was obtained. These results hold great promise for the chip detector because two key factors that determine the assay performance, the incubation volumes (~300 nL in this case) and the choice of electrochemical detector, will remain the same if not improved in the chip.

In order to better mimic larger biological warfare agents, an artificial microorganism or bug-bead model is being developed for testing. The bug-beads consist of 0.33 µm beads with surface attached dendrimers carrying the two epitopes, mouse IgG and guinea pig IgG. Mouse IgG is recognized by anti-mouse IgG coated paramagnetic beads for the capturing step

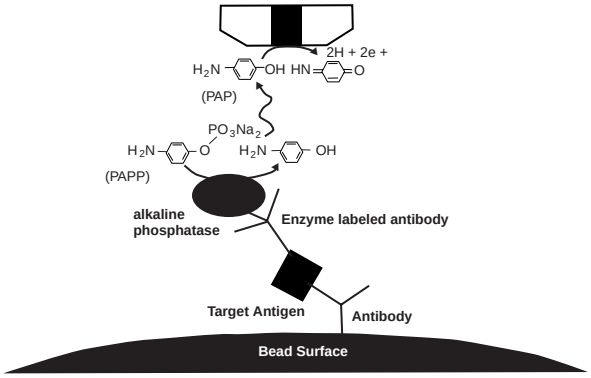

FIG 1. A schematic representation of the principles of an electrochemical immunoassay.

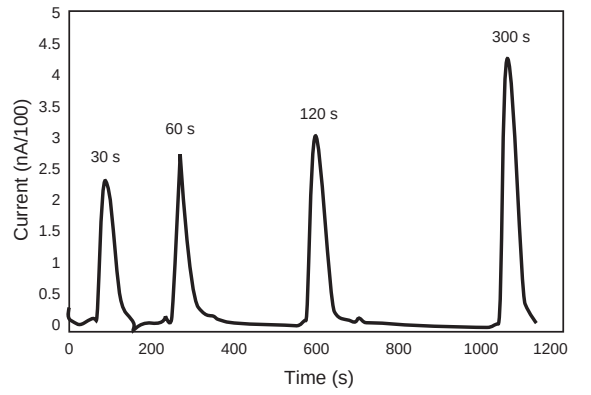

FIG 2. Immunoassay result for a 2500 ng/mL mouse-IgG assay in the meso immunosensor. The detection was done with an IDA type electrode with 7 µm band widths and spacing. The signals from the two band electrodes were added to give one enhanced signal. The different peaks correspond to 30, 60, 120, and 300 second enzyme substrate incubation prior to detection.

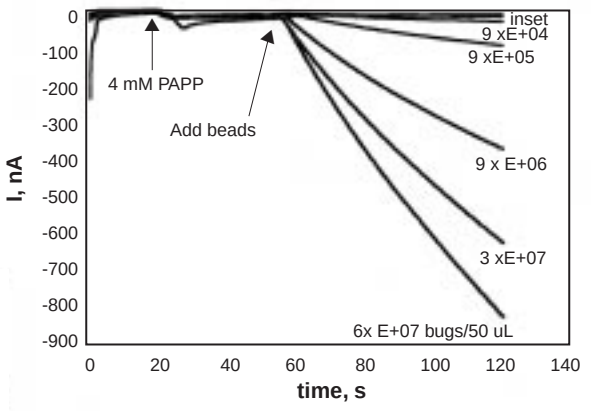

FIG 3. RDE current-time plots for a "bug-bead" sandwich immunoassay. The RDE electrode was held at 290 mV versus Ag/AgCl reference electrode and rotated at 3000 rpm. Two µL of bead mixtures (specified in the figure) were added to 40 µL of 4 mM PAPP in a micro-volume RDE.[4]

and the guinea pig IgG by AP labeled conjugate molecules for the labeling step. The immunoassay result from the bug-bead assay using micro volume amperometric detection with a rotating disk electrode (RDE) is shown in Fig. 3. The steadily growing anodic current following the addition of beads is due to the increasing concentration of PAP from the enzymatic reaction. The rate of current change is proportional the number of bug-beads in an assay as expected from a successful immunoassay. A detection limit of 360 bug-beads was obtained in the study (data not shown). ■

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