The self-assembled monolayer modification of electrodes

Some recent advances in biological application

Justin Gooding and Wenrong Yang

Résumé **Les électrodes modifiées par des monocouches de molécules auto-assemblées. Quelques avancées récentes d'application dans le domaine de la biologie**

La modification d'une surface d'électrode au niveau moléculaire en utilisant la technique du dépôt de monocouches auto-assemblées (SAM) constitue un exemple typique d'une des techniques utilisées dans les nanotechnologies, découlant du procédé « bottom up », qui consiste à créer une nanostructure par additions successives d'entités moléculaires ou atomiques sur une surface. Cet article présente quelques avancées récentes dans le domaine, avec pour exemples : le développement de systèmes SAM hybridés par des biomolécules, des nanoparticules ou des nanotubes en bioélectronique ; l'utilisation d'électrodes commutables pour étudier l'adhésion et la migration de cellules biologiques ; et l'intégration de fils moléculaires dans les SAM pour reconnaître et permettre la transduction d'une réaction biologique autorisant la pratique de l'électrochimie dans un milieu biologique complexe.

Mots-clés **Monocouches auto-assemblées, reconnaissance moléculaire, nanomatériaux, surfaces commutables, fils moléculaires, biocapteurs.**

Abstract The modification of an electrode on a molecular level using self-assembled monolayers (SAMs) is essentially an example of the "bottom-up" fabrication principle of nanotechnology. This review outlines some recent advances and applications in this field, showing some examples: the development of hybrid systems of SAMs, biomolecules and nanoparticles or nanotubes for bioelectronics; switchable surfaces for integrating electrodes with cells to allow the investigation of biological cell migration and adhesion; integrating molecular wires into SAMs to transduce biorecognition reactions for bioelectronics allowing electrochemistry to be performed in complex media.

Keywords **Self-assembled monolayers, molecular recognition, nanomaterials, switchable surfaces, molecular wires, biosensors.**

Self-assembled monolayers (SAMs) for the modification of electrodes have been used in electrochemistry for almost 25 years with considerable advances having been made on how to use this incredibly flexible chemistry to give electrode surfaces a whole host of desired functionalities [1-4]. SAMs are monomolecular layers which form spontaneously when a solid substrate is immersed in a solution containing amphifunctional molecules. Two examples of SAM forming molecules are silanes which are used to modify silica surfaces and alkanethiols which have an affinity for coinage metals [5]. Being compatible with metal surfaces, it is the alkanethiol system that is more applicable to electrochemistry. The head groups of these amphifunctional molecules provide the driving force for adsorption onto the solid substrate while van der Waals interactions between their alkyl chains determine the organization of the monolayers. One of the important advantages of SAMs over many other methods of electrode modification is molecular level control over the architecture of the interface. As multiple molecular components can be incorporated within a monolayer, the potential exists for functional molecular systems to be prepared using self-assembly [6].

Alkanethiols adsorb spontaneously onto the surface of gold, silver, platinum and copper. In the case of gold, the most frequently used surface, the thiol groups chemisorb onto the gold to form a gold-thiolate bond [7-8]. The resultant monolayer has the alkyl chains in the all *trans*-conformation, tilted ~ 20-30° from normal to the metal surface. With solution assembly of alkanethiols, a monolayer forms within a few minutes of contact of the metal with the alkanethiol solutions whereupon there is a slow reorganization over a period of several hours [9]. The slow reorganization indicates that even after the alkanethiol has adsorbed onto a gold surface, the monolayer is still relatively dynamic with the adsorbate retaining some mobility. The dynamic behaviour of SAMs is further demonstrated by the observation that when a SAM modified gold surface is placed in a different alkanethiol solution, exchange occurs at the grain boundaries of the underlying metal surface [10].

The simplest functional molecular systems formed on electrodes using alkanethiol SAMs are where a recognition species at the distal end of the molecule is attached to a SAM modified electrode [1-2, 11-12]. Increased complexity is achieved by controlling the spacing of the recognition molecule on the surface by forming a mixed SAM where only one component can be coupled to the biomolecules [13-14] or using mixed monolayers to control the microenvironment of

the recognition molecule [13]. Control over the microenvironment of a biomolecule attached to the SAM can be used to orientate the recognition molecule [15-16], resist adsorption of electrode fouling species [17-19] or prevent interferences interacting with the electrode *via* charge exclusion [20]. Still greater complexity can be introduced into the functional molecular system on the electrode surface by building up multilayers with more than one type of recognition molecule which operate cooperatively [6, 21], combining an anchor for the recognition molecule with a method of transducing the biorecognition event [22-24] and isolating the electrode from the sample solution all in the one system [19, 22].

One of the main emphases in SAM research is electrode integration with biological systems, such as proteins, DNA and whole cells [25]. Two of the interesting challenges for this field are:

- how to electrically communicate with biomolecules on electrode surfaces (biomolecules do not naturally allow the easy flow of electrons to and from them);
- how to design electrodes so they can interact with the biological media in a well understood way while resisting fouling of the electrode surface.

The purpose of this paper is to highlight just a few of the major advances in using SAMs to integrate with biological systems which exploit the design of new SAM forming molecules and/or incorporate nanomaterials into the surface modification layer. The examples that are highlighted all provide electrochemistry with a means of integrating with biological systems, or transducing biorecognition reactions, in a way that was previously not possible [26-29].

Incorporation of nanomaterials with SAMs for interfacing with proteins

The integration of nanoparticles with electrodes for biosensing and bioelectronics applications has most frequently been used in one of two ways. The first is as electrical contacts between the electrode and a redox protein. Perhaps the most interesting example of this application is using nanoparticles to wire into glucose oxidase by Willner and co-workers [30]. In this work the redox active centre of glucose oxidase, flavin adenine dinucleotide (FAD), was attached to a SAM modified nanoparticle which was connected to an electrode surface. Reconstitution of the apo-enzyme around the nanoparticle immobilized FAD produced an enzyme electrode which responded to glucose in the absence of oxygen at a rate seven times the maximum rate observed when oxygen recycles the enzyme. Although the exact mechanism of this enzyme "wiring" is not well understood what the study highlights is the enormous possibilities of connecting electronic elements to proteins using nanoparticles.

The second major use of nanoparticles in electrochemical biosensing is as labels. This is a strategy pioneered by Wang and co-workers [31]. The majority of these labeling approaches have been similar to the sandwich assay principle of immunoassays where the target analyte binds to a recognition molecule immobilized onto the electrode surface. Subsequently a nanoparticle modified recognition molecule binds to another site on the analyte. The nanoparticle now bound onto the electrode surface is then used for transduction of the original biorecognition event. Wang *et al.* has recently reported a simplification of this approach based on a competition assay for the detection of specific glycosylation markers, indicative of disease, on cell surfaces [26]. The new bioassay relies on the competition between a nanocrystal (CdS)-tagged sugar and the target sugar for the binding sites of surface-confined lectin (*figure 1*). The extent of competition, and hence the amount of the specific glycan in the sample is determined *via* the electrochemical stripping of cadmium from the captured nanocrystal. Unlike the earlier two-step sandwich bioassays [32-34], the present protocol only relies on a single step (a competitive assay in using nanocrystal-tagged sugar), which is more suitable for monitoring small sugar molecules and lectin-sugar interactions. The advantages of the strategy for detecting lectins are short assay time, sensitivity, low cost and reliability. Importantly, the strategy is generic for the detection of small analytes such as sugars, drugs and signaling molecules.

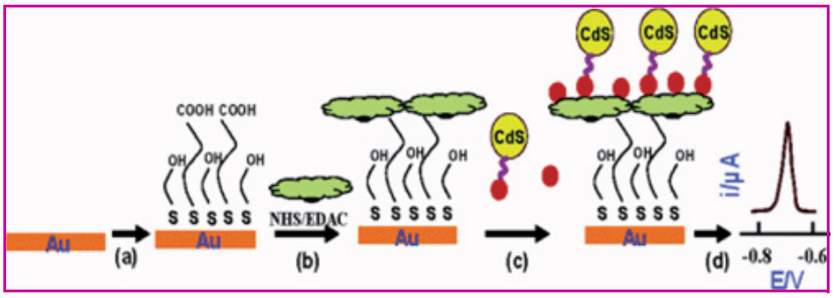

Figure 1 - Schematic of the steps in performing a bioassay using nanoparticle-based bioelectronic sensor for the detection of glycans.
The bioassay involves competition between a nanoparticle tagged sugar and the target analytes for the binding sites on a lectin ligand immobilized onto an electrode (a) mixed self-assembled monolayer on the gold substrate; (b) covalent immobilization of the lectin; (c) addition of the tagged and untagged sugars; (d) dissolution of the captured nanocrystals, followed by their stripping-voltammetric detection at a mercury-coated glassy carbon electrode.
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Carbon nanotubes (CNTs) revealing metallic or semiconducting properties represent a novel class of nanoparticles with anisotropic properties. The walls of CNTs are relatively poor electrode materials whilst the ends are very electrochemically active [35-36]. This anisotropy in electrochemical properties means that if a CNT is connected to a macroelectrode, it can be considered the smallest possible electrode (in the case of a single walled carbon nanotube (SWCNT) only 1 nm in diameter) [35]. CNTs have been used to integrate biomolecules with electrodes. The integration of biomaterials with CNTs enables the use of the hybrid systems as active field-effect transistors or biosensor devices (enzymes electrodes, immunosensors or DNA sensors). The specific advantage of CNTs for integration with biomolecules is the small size, allowing these active electrodes to be plugged into locations where electrochemistry would otherwise be unable to be performed, such as inside proteins [28, 37-40].

A major advance in the direct electrical contacting of redox enzymes and electrodes using SWCNTs was recently accomplished (*figure 2*) [28]. The enzyme microperoxidase (MP) was attached to the ends of SWCNTs, which were aligned normal to the electrode surface using self-assembly to give a nanoelectrode array [28]. An array of perpendicularly oriented SWCNTs on a gold electrode was fabricated by covalently attaching carboxylic acid functionalized SWCNTs, generated by the oxidative scission of the carbon nanotubes,

to a cysteamine monolayer-functionalized gold electrode. The efficiency of the nanotubes acting as molecular wires was determined by calculating the rate constant of heterogeneous electron transfer between the electrode and microperoxidase MP-11 attached to the ends of the SWCNTs. At the same time, using a similar strategy of CNTs aligned by self-assembly, Yu *et al.* [41] reported that quasi-reversible Fe^{III}/Fe^{II} voltammetry was observed for the iron heme enzymes, myoglobin and horseradish peroxidase.

An exciting extension of this idea of assembling enzymes onto the ends of carbon nanotube electrodes was to plug the electrodes inside proteins. This has been achieved by both Willner and co-workers [38] and by us [39] with surprisingly similar results considering the complexity of the systems. In both cases plugging the nanotubes inside glucose oxidase was achieved by first covalently attaching the FAD (flavin Adenine dinucleotide) cofactor carboxylic groups at the free ends of the aligned SWCNTs. Apo-glucose oxidase was then reconstituted around the FAD units linked to the ends of the standing SWCNTs to give active enzyme [38-39]. Willner and co-workers measured the interfacial electron-transfer rate constants to be 83 s^{-1}, 42 s^{-1}, 19 s^{-1} and 12 s^{-1}, for assemblies that include standing SWCNTs of mean length 25, 50, 100 and 150 nm average length, respectively.

What these examples of integrating nanoparticles and nanotubes with biological molecules on electrode surfaces shows us is that new opportunities exist for electrically connecting to biological molecules and that these nanomaterials provide unique strategies for labeling in bioassays with potentially greater sensitivity than most existing strategies. Thus the combination of nanomaterials and biomolecules provides unprecedented control over the interaction of electrode surfaces with individual biomolecules but they do not solve the challenge of allowing electrodes to be used in complex biological media or to follow biological processes in these media. Recent advances in self-assembled monolayer modified electrodes however have opened up new opportunities for these applications as well.

Switchable surfaces for integrating with cells

SAMs containing two or more constituent molecules provide a practical experimental system with which to generate model systems to study fundamental aspects of the interactions of surfaces with biological systems. The primary advantage of SAMs (especially mixed SAMs) over other methods of creating organic surfaces (polymer films, adsorbed proteins) is that the chemical composition of the surface can be modified in a deliberate manner. A reversibly switching surface was reported recently [42] which used electrical potential to control the orientation of SAM molecules on a gold electrode surface. This change in orientation altered the wettability of the surface almost instantaneously. This concept of designing switchable surfaces on electrodes such that the exquisite control over electrode potential can be used to control what the surface presents to its environment is ideal for conducting mechanistic studies of cell attachment and the dynamics of how changes to a surface influence cell structural organization and intracellular signaling. Such studies have important implications for biomaterial developments.

A good example is the demonstration that switchable surfaces based on SAMs can be used in controlling cell migration [43]. This was achieved using SAMs, patterned by micro-contact printing [44].

More advanced electrochemical manipulation of cell on surfaces can be achieved with custom synthesized switchable molecules. Mrksich and co-workers recently [45] demonstrated a SAM on gold with a 4-*H*-benzo[*d*][1,3] dioxinol terminal group. Subjecting the monolayer to a potential of 900 mV versus Ag/AgCl caused the oxidation of the aromatic ring of the 4-*H*-benzo[*d*][1,3] dioxinol with hydrolysis of the acetal to yield the aldehyde. The resulting aldehyde can be used for ligand immobilization, with corresponding protein capture or alternatively, the surface can be used for studies of cell migration.

The examples of switchable surfaces for controlling cell adhesion demonstrate the application of SAM modified surfaces with specifically designed molecules to enhance our understanding of cell adhesion. However, in these examples, although the cells interact with the surfaces in a highly defined manner, the electrode is only a vehicle for switching the surface and there is no electrochemical communication between the electrode and the cell activity. Recent advances in forming mixed SAMs composed of molecular wires and oligo (ethylene glycol) groups may allow controlled interaction of electrode with biological media and still permit electrochemistry to be performed.

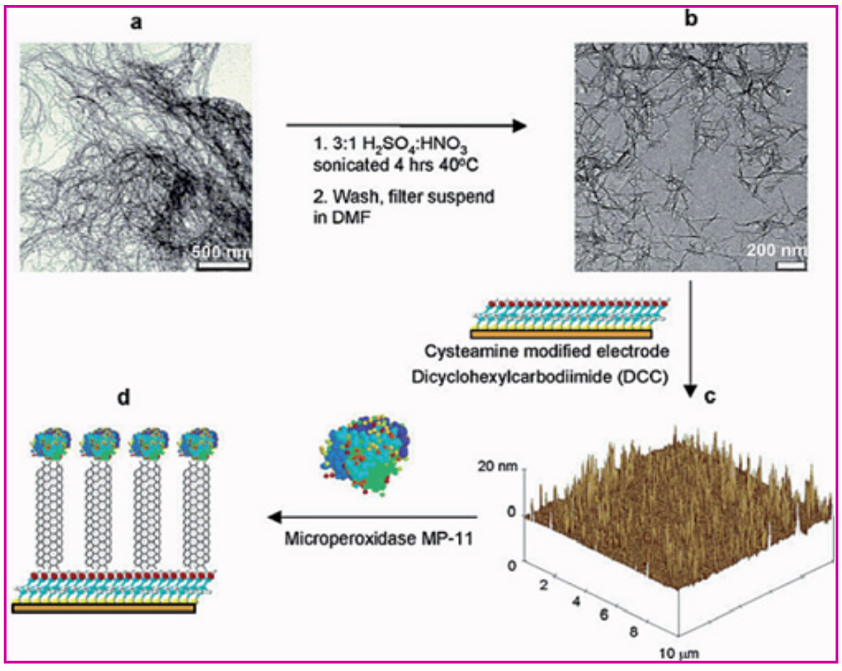

Figure 2 - A schematic illustrating the steps involved in the fabrication of aligned shortened SWNT arrays for direct electron transfer with enzymes such as microperoxidase MP-11.
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Integrating molecular wires into SAMs to transduce biorecognition reactions

The attraction of molecular wires in bioelectronics is that good electronic coupling exists between the electron donor at one end and the acceptor at the other over long distances [29, 46]. For biological systems there is general agreement that the electronic coupling between electron donors and acceptors plays a critical role in controlling long-range electron-transfer (ET) processes. An important issue for electrode interface constructs where a "molecular wire" (MW) penetrates into the biology is to ensure the biological molecules interact specifically with the MW, rather than the rest of the electrode surface. However, nonspecific adsorption of proteins is a problem that exists with most surfaces when exposed to biological samples. Thus, to achieve a generic surface which ensures specific interactions between a protein and an electrode requires two important things: molecular wires that can interact directly with the protein and exhibit efficient electron transfer behavior, and diluent molecules that are able to resist nonspecific adsorption of proteins.

The electrode interface used in the electrochemical DNA biosensor developed by Clinical Microsensors System (CMS) [19] was the first example of an electrode construct that met the two criteria above.

We have adapted this type of interface for protein electrochemistry and to allow electrochemistry to be performed in biological media without electrode fouling (see *figure 3*) [29]. The self-assembled monolayer depicted in *figure 3* is not an alkanethiol monolayer on gold but is derived from aryl diazonium salts reductively desorbed onto a glassy carbon electrode, and form much more stable monolayers on carbon and metal surfaces than the alkanethiol system [47-48]. The two components are an oligophenylethynyl molecular wire and a polyethylene glycol terminate antifouling component. As with the CMS system, the molecular wire allows electrochemical communication with the electrode and the PEG (polyethylene glycol) molecules resist nonspecific adsorption of proteins to the surface whilst preventing electroactive interferences reaching the electrode surface. The ability of the interface to ensure proteins are selectively attached to the molecular wires was shown using horseradish peroxidase (HRP), a protein to which direct electron transfer can easily be achieved. The electrochemistry of the heme centre shows a close to idea full width half maximum for the oxidation peak that indicates that the proteins are all in a similar environment.

The monolayer construct in *figure 3* has already been shown to have advantages for protein electrochemistry [29] and DNA biosensors [19] but its potential has yet to be touched for performing electrochemistry in complex biological media such as found in cell culture media. The important features of this system for performing electrochemistry in complex media are the highly stable aryl diazonium salt SAMs, the protein resistance and restriction of electroactive interferences from accessing the electrode surface. The challenge is to configure the interface with a biorecognition molecule that can be attached to the molecular wire that will allow detection of an analyte of interest. This is the challenge we are now pursuing.

Conclusions

This article highlights some recent advances in the rapidly developing area of bioelectronic systems using SAMs as a platform. SAMs are useful as model surfaces for studying biological and biochemical processes. SAMs can assemble onto surfaces of any geometry or size, which provide a general and highly flexible method to tailor the interfaces between biological systems and electrodes. The combination of the unique surface properties of SAMs and the recognition and catalytic features of biomolecules provides a unique opportunity for physicists, chemists, biologists and material scientists to mold a new area of nanobiotechnology. Based on recent advances in the field, exciting new science and novel systems can be anticipated for the interfacing of electrodes with biological systems.

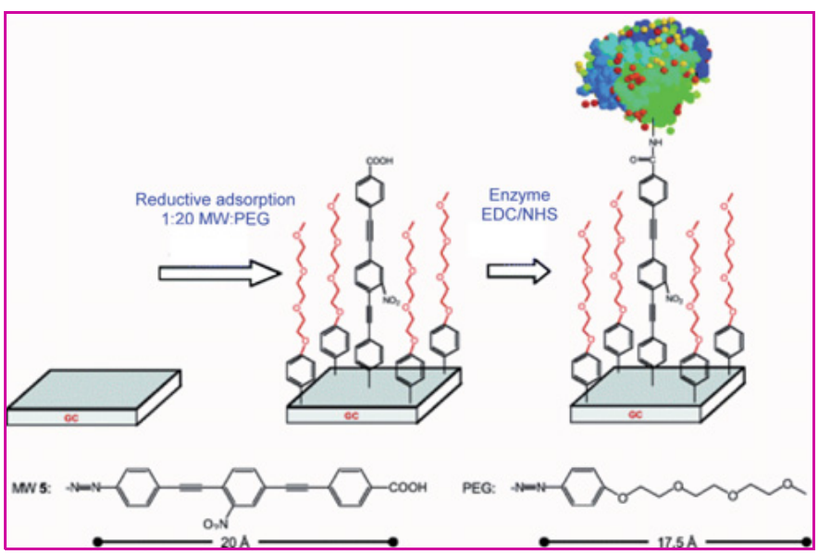

Figure 3 - Schematic of the modification of a glassy carbon electrode with aryl diazonium salts composed of a molecular wire and a polyethylene glycol diluent for the purpose of protein electrochemistry.
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J. Gooding

W. Yang

Justin Gooding is Professor at the School of Chemistry, University of New South Wales (Australia)[1].

Wenrong Yang is Postdoctoral research associate at the Electron Microscope Unit (EMU), University of Sydney (Australia)[2].

1 School of Chemistry, University of New South Wales, Sydney, NSW 2052 (Australia).
E-mail: justin.gooding@unsw.edu.au

2 Madsen Building F09, University of Sydney, NSW 2006 (Australia).
E-mail: wenrong.yang@emu.usyd.edu.au