Interfacial sensing: surface assembled molecular receptors

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The controlled surface assembly of biological or appropriately designed synthetic host systems on optically or electrochemically-active surfaces has advanced considerably during the past decade. Recent activities, from the authors laboratory and elsewhere, and possible future directions are discussed herein.

Introduction

Interest in the reliable detection and quantification of potentially harmful or physiologically-active chemical agents at robust and re-usable interfaces has grown sharply in recent years. Key to many drug screening, environmental, military, medical or food safety assays is the requirement of high selectivity and, therefore, the absence of "false positives". This is achievable by appropriate design of "recognition surfaces" based either on the immobilisation of natural bio-recognition entities (biosensors) or the surface assembly of designed and synthesised receptors.

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Biosensors

Biosensors, broadly definable as comprising "biologically-derived" molecules, organelles, or whole cells, in contact with a transducing element, have advanced considerably during the past decade. Of these, those based on enzymes are, by some margin, the most heavily developed. Enzymes exhibit a characteristic highly-specific, and, from an applied perspective, important substrate selectivity. By coupling this substrate recognition/turnover to a signal generation mechanism, derived devices capable of high limits of detection are feasible. When enzymes have a redox-addressable cofactor electroanalytical methodologies (primarily amperometric and potentiometric) have proved to be of considerable value. The direct electrochemical analysis of an enzyme is, however, generally non-trivial. In many cases, the redox-active site is buried deeply within an insulating polypeptide matrix and is, consequently, difficult to access electronically. This problem can often be solved through the use of electron-transfer mediators which shuttle electrons between electrode and (solution phase or surface-bound) enzyme. These effectively act as a substitute for the natural *in vivo* electron mediator and work effectively enough for robust sensory devices to be constructed and sold on an enormous commercial scale.[1–3]

From an applications perspective it is preferable to construct sensors without the need to add mediators to the analyte solution, that is, to construct "reagentless sensors". By appropriate interfacial design it is possible to establish reliable means of electrical communication between man-made electrodes and the redox sites of small proteins. Though the protein surface itself can be chemically or genetically modified in order to facilitate electron exchange at bare electrodes, it is, in general, the electrode surface which is modified.[4,5] This electrical coupling can, subsequently, be extended to the enzymic partner of the protein and the current measured then becomes a calibratable function of substrate concentration (Fig. 1).[6] In some cases, it has been possible to achieve (otherwise difficult) direct electrical coupling between an enzyme active site and an electrode by genetically engineering the former so as to facilitate a favourable surface-bound orientation (Fig. 2).[7] In this case, calibratable analyte detection proceeds when direct electron transfer between electrode and active site becomes catalytic.

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Jason Davis (b. 1971) studied Chemistry at King's College, London, where he was awarded The Victor Gold Prize for Chemistry in 1991, The Ivor John Prize for Organic Chemistry in 1992, and The Robert Wakeford Memorial Prize in Chemistry and a first class degree in 1993. He moved to the Inorganic Chemistry Laboratory at the University of Oxford in 1994. After obtaining a PhD and undertaking postdoctoral research on carbon nanotubes, electroanalysis and scanning probe microscopy he was elected to an Extraordinary Junior Research Fellowship at The Queen's College in 1998, a Royal Society University Research Fellowship in 1999 and a Lectureship in Chemistry at Jesus College, Oxford, in 2001. He was made a University Lecturer and Official Student and Tutor in Chemistry at Christ Church in 2003. His work during the past 5 years has focused on the molecular and nanometre-scale construction and analysis of bioinorganic, sensory, electronic and optical systems. He has published over 45 papers in leading peer-reviewed journals on carbon nanotube chemistry, nanotube biosensing, molecular sensing, electrochemistry, biomolecular electronics and nanotechnology.

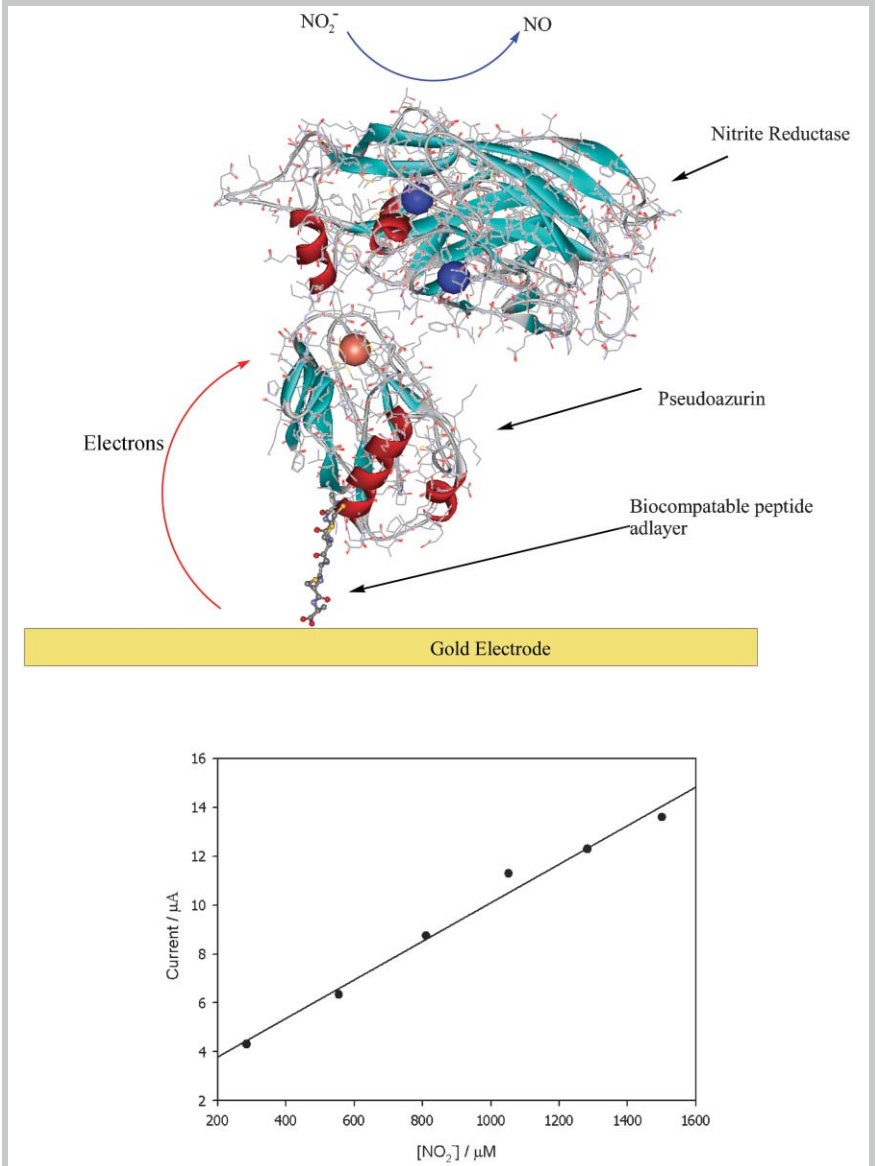

Fig. 1 The pseudoazurin metalloprotein is in direct electrochemical communication with the underlying peptide-modified gold electrode surface and is able to transfer electrons to the copper-based active site of its solution-phase enzyme partner, nitrite reductase. The current generated by direct electron transfer with the metalloprotein becomes, in the presence of the enzyme partner, a sensitive and calibratable function of substrate (in this case nitrite) concentration.

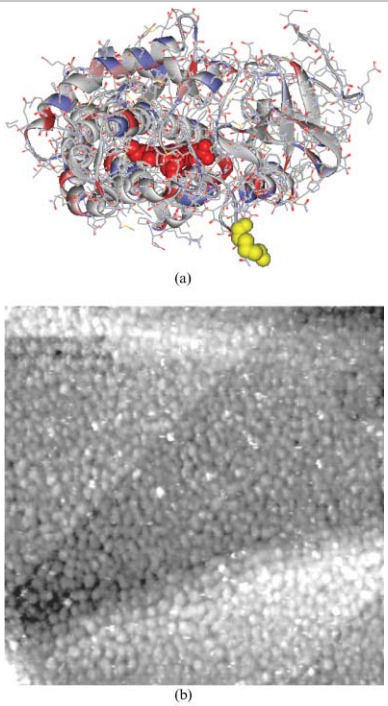

Fig. 2 a) Enzymes can be engineered such that surface assembly, direct electron transfer and substrate turnover can occur. Surface cysteine residues (yellow) can, for example, be utilised in the assembly of cytochrome P450cam molecules on gold electrodes in an orientation which brings the haem moiety close to the surface. The closely packed adlayers can be scrutinised by AFM or STM and utilised in the electrochemical detection of halogenated hydrocarbons. b) Fluid tunnelling image (250 × 250 nm) of a self-assembled electroactive P450cam adlayer on Au(III).[7]

Supramolecular receptors: synthetic host–guest sensing

Though of unquestionable value, sensory systems based on the immobilisation of biological molecules intrinsically suffer from complications associated with surface-induced structural change, an inability to control homogeneity and surface-bound orientation, and difficulties associated with (electrically) accessing the active/recognition site. Inspired by the selective host–guest recognition afforded by nature's receptors, chemists have attempted to generate laboratory-synthesised cavity-containing molecular receptors of comparable affinity and selectivity. From a sensing perspective, supramolecular systems may be loosely described as comprising a 'host' sensor and a 'guest' analyte species, the latter being neutral, cationic, or anionic. Individually the component host–guest interactions are relatively weak but in combination they lead to thermodynamically stable complexes, the formation of which can be utilised in the generation of robust sensors.

Through the incorporation of redox or photo-active moieties into a receptor moiety, host–guest association may be communicated to an observer (Fig. 3). A number of electrochemically-active sensory systems responsive to, for example, lithium, sodium, and potassium, as well as larger cations (some of considerable environmental concern), such as rubidium and caesium, have been developed.[8] Selective anion recognition, more demanding than cation detection, has become an active area of interest during

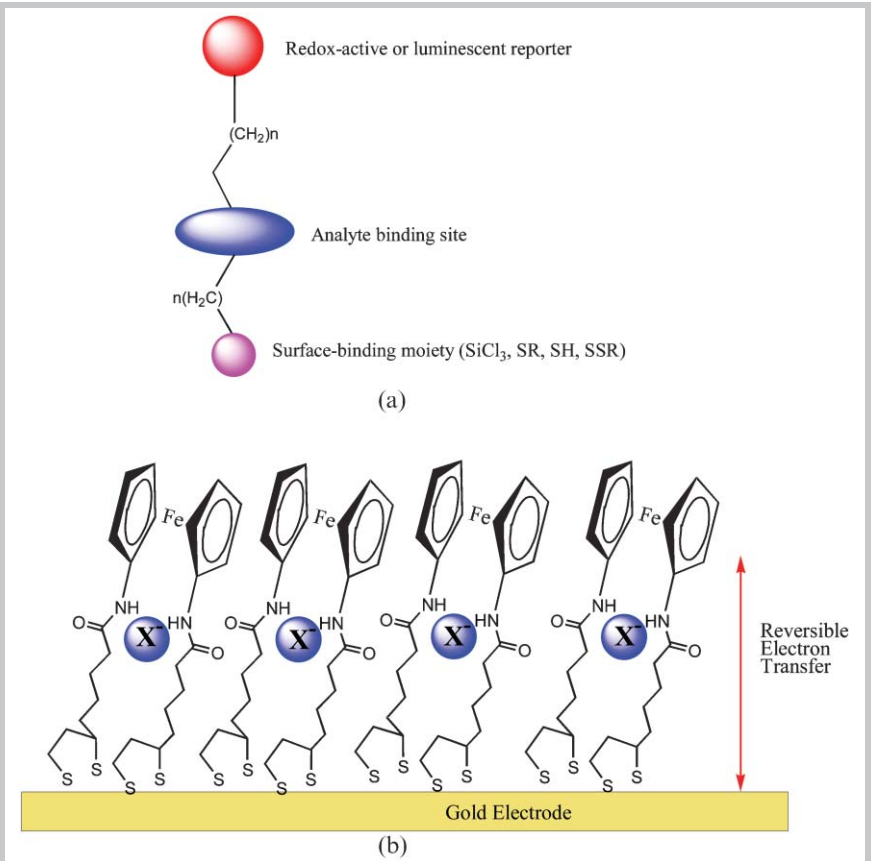

Fig. 3 a) The construction of a surface-confined (ideally robust, washable and re-usable) sensor can be achieved by the design and synthesis of appropriate "three-component molecules" which spontaneously chemisorb, possess a high-affinity host site and a redox or optically-active moiety. The properties of the latter should be detectably perturbed by host–guest association. With electrochemical sensors, perturbation of a voltammetrically monitored electron transfer process, achieved by localisation of a redox-active group (*e.g.*, ferrocene) in the vicinity of the receptor, may be achieved by one or a combination of through-space and through-bond interactions (optical or colorimetric sensing can be achieved through perturbation of an optical signal, typically by equivalent mechanisms). Analyte binding efficiency and selectivity are synthetically tunable. b) Schematic of a self-assembled, anion-coordinating ferrocene monolayer. Hydrogen bonding of specific anions results in predictable cathodic perturbation of the SAM voltammetry. Strikingly, the anion binding affinities associated with such SAMs are, in some cases, orders of magnitude greater than those associated with the same receptors free in solution.

the past decade or so.[9] A number of anions, such as perchlorate (known to interfere with thyroid production and fetal brain development) for example, have become, through accidental release or inappropriate disposal, significant contaminants of surface and ground water.

Surface assembled supramolecular receptors

Self-assembled monolayers (SAMs) can be formed from the exposure of a pristine metallic surface to suitably-functionalised molecules which subsequently form ordered two-dimensional crystalline arrays; the degree of ordering being dependent on molecular structure and size, substrate morphology, coverage, temperature and solvent. Through the introduction of a surface-binding moiety into the "bifunctional" recognition/signalling molecules mentioned above, it is possible to combine molecular-scale recognition with such assembly and to, subsequently, develop a powerful means of generating interfaces capable of analyte detection in a variety of media.

The "surface tethering" of receptors yields a number of specific additional advantages; efficiency, in terms of quantity of material required, is high. The sensor can also be portable, robust, and capable of use in a variety of (polar and non-polar) environments. In addition to this, the preorganisation of molecular receptors at a surface enhances the thermodynamic driving force associated with receptor/analyte binding and, therefore, both binding affinity and detection limits. This "surface amplification", which can lead to the development of highly effective sensors, is broadly attributable to two effects. (i) The "macrocyclic effect" is the entropy-driven increased thermodynamic stability of a complex formed between an ion (usually cationic) and a multi-dentate macrocyclic ligand in comparison to the equivalent complex formed with open chain (non-cyclic) ligands. By "pre-organising" the host pseudo macrocycle on a surface, and thereby restricting its vibrational and rotational degrees of freedom, the entropic driving force accompanying complex formation is further increased. (ii) The comparatively low dielectric constant of the SAM receptor binding site is also likely to significantly enhance ionic binding efficacy.[10,11]

The ability to tune both geometry and binding affinity of the host molecular receptor allows one to achieve highly-selective sensing. Even the structurally-simple SAMs shown in Fig. 3b, for example, are able to sense dihydrogen phosphate in the presence of more than a ten thousand fold molar excess of halide in solvent. The advantages associated with detection at an interfacial assembly are highlighted by the fact that functional (but *uncharged*) surfaces of this type are able to detect low levels of the perrhenate anion (a model for pertechnetate, an environmentally-important radioactive waste product of the nuclear and radiopharmaceutical industries) in *aqueous* solution.[11] By synthetically increasing both receptor binding affinity and electroanalytical detection sensitivity, it should be possible to extend this aqueous phase sensing, challenging due to the commonly associated low binding constants, to a range of anions.

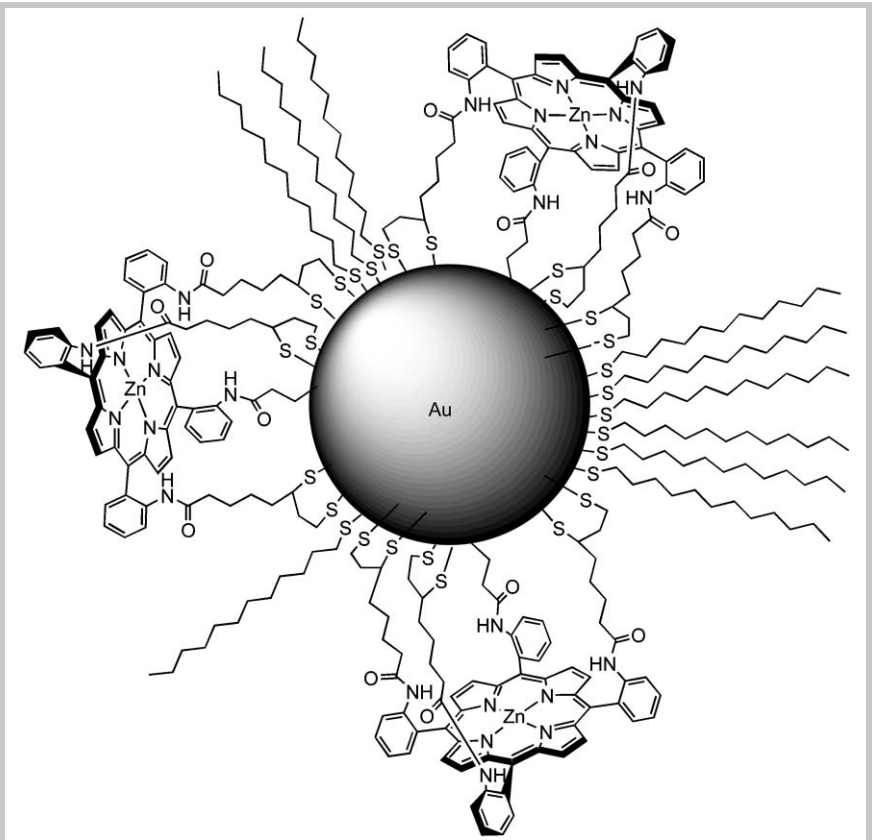

Fig. 4 Diffusing zinc porphyrin-modified gold nanoparticles. The sensitivity of porphyrin adsorption to anion binding at the metal facilitates the formation of nanoparticles able to detect micromolar levels of chloride and dihydrogen phosphate in polar or aqueous solution. The association constants, are, in some cases, an order of magnitude greater on the particle than with the free porphyrin.[22]

Sensing at noble metal nanoparticle surfaces

During the past decade, as it becomes possible to controllably generate homogeneous samples and subsequently scrutinise them at useful levels of resolution, there has been an explosion of interest in metallic nanoparticles and a corresponding exponential growth in associated publications. The optical properties of these are, in particular, of considerable theoretical, experimental and applied interest.[12,13] Specifically, particles of suitable size exhibit a strong adsorption in the visible due to the coupling of incident wavevectors to the localised plasmons at the particle surface and are associated with enormous (up to 10–14 orders of magnitude) local electromagnetic field enhancements. In recent years, much progress has been made in the controllable surface-modification of nanoclusters; specifically, both gold and silver nanometre-sized clusters have been decorated with self-assembled adlayers which can be used to confer not only analyte binding but also desired solubility.[14,15] Through the decoration of nanoparticles with optically-active ion binding molecules, it is possible to make use of the advantages associated with receptor surface packing and organisation whilst keeping the sensory particle as a whole mobile in solution (Fig. 4).

One can additionally seek to combine receptor surface organisation with the intrinsic optical properties of the nanoparticle. Specifically, the high sensitivity of the ''plasmonic'' visible adsorption to changes in local refractive index can be utilised in the detection of surface binding events at particles which are either freely diffusing or surface-confined.[16–23] (Fig. 5) This mechanism of sensing does not require the analyte in question to be labelled or the binding site to be active in anything but recognition and binding. Though propagating surface plasmon resonance (SPR) has been a demonstrably powerful means of assaying recognition events at a surface,[24] the excitation of plasmon modes in evaporated metal film requires the use of a rather elaborate and experimentally inflexible configuration. By contrast, the localised plasmon modes of nanometre-sized gold and silver particles can be readily excited with extremely high extinction.[25,26] The fact that these sensitive particles can be loaded with numerous binding sites, dispersed on a surface, and readily optically excited should enable not only the direct detection of the binding events at a single nanoparticle but also the construction of arrays of particles singularly responsive to different moieties (enabling high-sensitivity multi-analyte detection).

In summary, our ability to non-destructively interrogate the activities of redox-active enzymes and host–guest receptors has increased dramatically during the past 20 years or so and has led to the development of a range of highly-specific amperometric and potentiometric assays. The highly-specific molecular recognition characteristics inherent in biomolecules (or appropriate synthetic hosts) more generally, can be coupled to the high environmental sensitivity of, for example, nanometre scale optically- or electrically-active surfaces. An area likely to develop considerably during the next five years is, for example, the generation of selective and sensitive protein detection methods, something which would constitute a highly significant development in medical diagnostics. The recent marriage of protein aptamers to gold nanoparticles in establishing an attomolar detection limit has already highlighted the value of such ''nano-bio'' integration[27] and this is likely to increase exponentially as our abilities to synthesise, modify and analyse at the molecular-scale develop.

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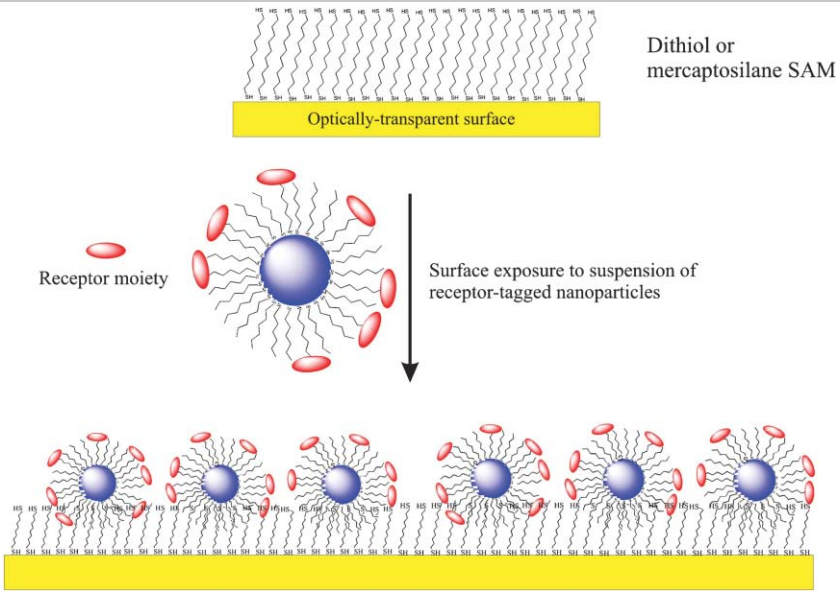

Fig. 5 Receptor-functionalised metallic nanoparticles can be prepared by place exchange at the surface of an alkyl thiol stabilised particle.[10,28] Depending on respective particle and receptor sizes, each nanoparticle may be loaded with up to several hundred receptor sites. Through appropriate surface chemistry, analyte-responsive nanoparticles can be controllably assembled on optically-transparent substrates. The loading of these particles with the surface-assembling amide receptors such as those shown in Fig. 3b, for example, facilitates the formation of a surface capable of hydrogen-bond recognition based detection of anions at the nanomolar level.

Wong, Professor Paul Beer (all of the Department of Chemistry, University of Oxford), Professor Gerard Canters (Leiden Institute of Chemistry) and both past and present members of his research group.

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