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Template-directed hierarchical self-assembly of graphene based hybrid structure for electrochemical biosensing

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ABSTRACT

A template-directed self-assembly approach, using functionalised graphene as a fundamental building block to obtain a hierarchically ordered graphene–enzyme–nanoparticle bioelectrode for electrochemical biosensing, is reported. An anionic surfactant was used to prepare a responsive, functional interface and direct the assembly on the surface of the graphene template. The surfactant molecules altered the electrostatic charges of graphene, thereby providing a convenient template-directed assembly approach to a free-standing planar sheet of sp^2 carbons. Cholesterol oxidase and cholesterol esterase were assembled on the surface of graphene by intermolecular attractive forces while gold nanoparticles are incorporated into the hetero-assembly to enhance the electro-bio-catalytic activity. Hydrogen peroxide and cholesterol were used as two representative analytes to demonstrate the electrochemical sensing performance of the graphene-based hybrid structure. The bioelectrode exhibited a linear response to H_2O_2 from 0.01 to 14 mM, with a detection limit of 25 nM ($S/N=3$). The amperometric response with cholesterol had a linear range from 0.05 to 0.35 mM, sensitivity of 3.14 μA/μM/cm^2 and a detection limit of 0.05 μM. The apparent Michaelis–Menten constant (K_m^{app}) was calculated to be 1.22 mM. This promising approach provides a novel methodology for template-directed bio-self-assembly over planar sp^2 carbons of a graphene sheet and furnishes the basis for fabrication of ultra-sensitive and efficient electrochemical biosensors.

1. Introduction

A range of nanoscale carbon-based building blocks, including nanotubes (Xia et al., 2003), graphite (Novoselov et al., 2004), fullerenes (Liu et al., 1998) and graphene (Stankovich et al., 2006), have attracted significant interest due to their extraordinary physical and chemical properties. Recently, research focused on graphene has advanced rapidly as this extraordinary material has become the preferred alternative to provide high surface area, high electrical conductivity, strong mechanical strength, biocompatibility and low manufacturing cost (Chen et al., 2008; Li et al., 2009). The extension of long range π-conjugation over the whole structure makes graphene a unique material for biosensors (Schedin et al., 2007; Shan et al., 2010), chemical sensors (Robinson et al., 2008), energy storage devices (Sofo et al., 2007), field effect transistor (Zhang et al., 2009), light emitting devices (Bonaccorso et al., 2010) and transparent, flexible, conductive polymer nanocomposite applications (Chen et al., 2011; Wang et al., 2008). Graphene-based materials are now being used widely in electroanalytical research for electrochemical sensing and immobilisation of biomolecules with high sensitivity and selectivity.

The biocompatibility and excellent electron transfer properties of graphene pave the way for its use in chemical and biological sensing (Lu et al., 2009). Covalent interactions are mostly obtained by doping or by chemical reaction with the functional groups which are formed during the synthesis of graphene (Veca et al., 2009). The former approach partly destroys the graphene conjugation system and results in suppression of its intrinsic properties. However, non-covalent interactions *via* van der Waals forces, electrostatic attraction, hydrogen bonding, coordination bonds or $\pi \rightarrow \pi$ stacking interactions mostly preserve the natural properties of graphene. In addition, although non-covalent bonds may be weak compared to covalent interactions, it is well-known, especially in biological systems, that multiple non-covalent bonds can provide quite enough stability for post-functionalisation (Liu et al., 2012). Recently, Li et al. showed that horseradish peroxidase can easily be assembled under physiological conditions on surfactant-modified graphene to yield high stability and sensitivity (Zeng et al., 2010). It is also known that assembly of gold nanoparticles into a graphene–enzyme structure increases electron transfer and the effective surface area of bioelectrodes (Lambert et al., 2009). The driving force for gold nanoparticle assembly is the interaction between the hydrophilic graphene surface and the gold

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nanoparticles. The functional and large surface area of graphene should be a promising building block for hybridisation with gold nanoparticles. To date, only a few studies on graphene/gold nanoparticle assembly have been reported *via* the self-assembly approach, based on π–π stacking interactions and electrochemical interaction for sensing applications (Fang et al., 2010). Uniform distribution and sufficiently high loading are still challenges to be overcome. Herein, we describe a self-assembled structure containing functionalised graphene–enzyme and gold nanoparticles to deliver high sensitivity for cholesterol sensing, accelerated electron transfer and good retention of enzyme activity.

The self-assembly approach has been widely used to produce more efficient and more functional materials (Mirkin et al., 1996; Sanchez et al., 2005). With an appropriate design, self-assembled hybrid structures can deliver synergistic effects, resulting in highly desirable materials with advanced performance. The forces behind the thermodynamic equilibrium state can be manipulated by conventional chemistry or more specifically directed by using templates or applying external fields. In order to achieve directed self-assembly, the base constituent should be carefully chosen and molecular interactions tailored by its functionalisation while the whole process can be modulated in terms of thermodynamic forces. In this way, a new ordered equilibrium may be established within the whole self-assembled structure. Directional interactions are achieved by designing stimuli–responsive interfaces or specific physical properties, provided by post-functionalisation under external directing fields or in the presence of directing surfaces.

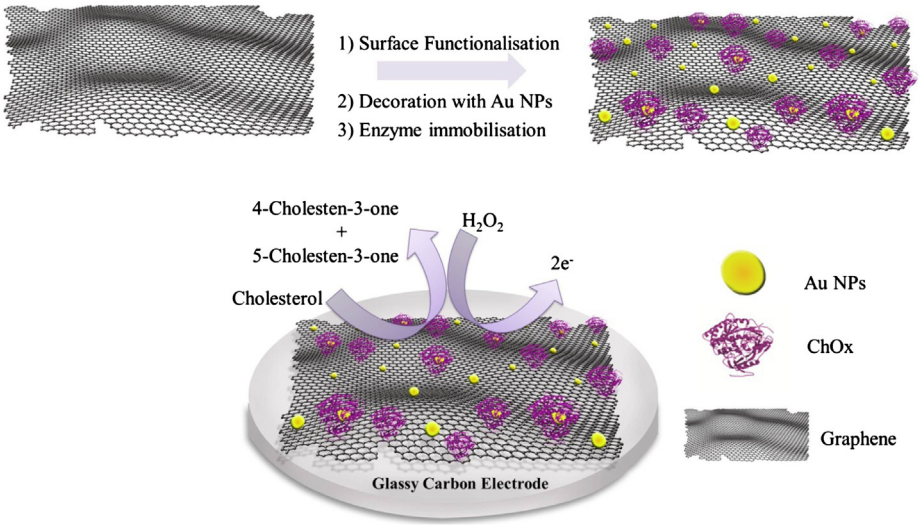
In this report, we focus on the template directed approach to construct a self-assembled structure. Template-directed assembly is defined as a process which modulates a self-assembled system using surface-modified one-dimensional, two-dimensional or three-dimensional materials having a functionalised surface suitable for additional assembly. Here, the surface-modified graphene was chosen as a model two-dimensional template. A straightforward method is described to assemble graphene with enzyme and gold nanoparticles by hierarchical structuring using electrostatic and hydrophilic interactions, respectively, for the fabrication of an enzyme electrode as illustrated in Scheme 1. The graphene plays a key role in the directed self-assembly by electrostatic and hydrophilic interactions. In the study, a negatively charged surfactant, sodium dodecyl benzene sulphonate (SDBS), has been employed to functionalise the graphene surface to not only assist the dispersion in aqueous media, but also to direct the self-assembly of both the enzyme and the metallic nanoparticles. Surfactant molecules on the graphene surface become the fundamental building blocks of the self-assembly structure that help to direct gold nanoparticles and enzyme molecules on the graphene template. As model enzymes, cholesterol oxidase and cholesterol esterase, which are positively charged in physiological media (pH 7.4), were chosen. It is shown that functionalised graphene and enzymes are spontaneously assembled into hierarchical structures due to electrostatic attraction. In other words, surface functionalisation directs enzyme molecules to assemble on the surface of the graphene, followed by gold nanoparticles as a third constituent of the assembled structure. Gold nanoparticle assembly on the surface of functionalised graphene was achieved via hydrophilic interactions. Thus, surfactant molecules create a hydrophilic environment on the highly hydrophobic graphene surface and direct hydrophilic gold particles on to the surface without any additional coupling chemistry.

For the proof of principle, we have chosen cholesterol oxidase and cholesterol esterase enzymes. Cholesterol and cholesteryl ester are two of the main constituents of all animal cells, and they are mostly present in the brain and nerve tissues (Dey and Raj, 2010; Kuila et al., 2011). The estimation of cholesterol in blood is a crucial step for controlling and early diagnosis of many life-threatening diseases, such as cerebral thrombosis, coronary heart disease, hypertension and Alzheimer. Various analytical techniques have been reported for monitoring cholesterol level in serum and food samples, including colorimetric (Li et al., 2011), spectrophotometric (Kumar et al., 2000) and non-enzymatic methods (Li et al., 2010). Electrochemical methods, however, offer simplicity, high sensitivity and low production costs (Gopalan et al., 2009; Tan et al., 2005). These may be enhanced by the use of nanoscaled materials such as this graphene–enzyme–nanoparticle hierarchical hybrid. Resulting novel self-assembled structures provide a larger electrochemically active surface for the adsorption of large amounts of enzymes and promote rapid electron transfer, resulting in ultra-sensitive and selective sensors.

2. Materials and methods

2.1. Chemicals

Cholesterol (≥99%), cholesteryl stearate (96%), cholesterol oxidase (ChEt) (≥50 units/mg, from *Brevibacterium* species),



Scheme 1. Schematic representation of the self-assembled graphene–gold–enzyme hierarchical hybrid structures on glassy carbon electrode and sensing process on the electrode surface .