Hollow graphitic nanocapsules as efficient electrode materials for sensitive Hydrogen peroxide detection

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ABSTRACT

Carbon nanomaterials are typically used in electrochemical biosensing applications for their unique properties. We report a hollow graphitic nanocapsule (HGN) utilized as an efficient electrode material for sensitive hydrogen peroxide detection. Methylene blue (MB) molecules could be efficiently adsorbed on the HGN surfaces, and this adsorption capability remained very stable under different pH regimes. HGNs were used as three-dimensional matrices for coimmobilization of MB electron mediators and horse-radish peroxidase (HRP) to build an HGN–HRP–MB reagentless amperometric sensing platform to detect hydrogen peroxide. This simple HGN–HRP–MB complex demonstrated very sensitive and selective hydrogen peroxide detection capability, as well as high reproducibility and stability. The HGNs could also be utilized as matrices for immobilization of other enzymes, proteins or small molecules and for different biomedical applications.

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1. Introduction

Carbon nanomaterials have attracted wide attention based on their unique physical and chemical properties. Different kinds of carbon-based nanomaterials have been developed, such as fullerene, carbon nanotubes (CNTs), graphene, carbon dots, and carbon cages (Smalley, 1992; Tans et al., 1998; Chen et al., 2008; Novoselov et al., 2004; Chen et al., 2010; Sun et al., 2006; Sheng and Wang, 2008; Yang et al., 2010). Among these materials, graphic nanocapsules, as a type of carbon cage, have recently been explored for such applications as lithium ion batteries (Wang et al., 2005), catalysts (Wu et al., 2007; Wu et al., 2008; Schaefer et al., 2010), supercapacitors (Bushueva et al., 2008; Xie et al., 2012), immunoassays (Cui et al., 2008; Ho et al., 2009), and drug delivery (Uo et al., 2005). Unlike other amorphous carbon nanocapsules, graphic nanocapsules have properties similar to those of graphene and carbon nanotubes, such as unique structures, very high conductivity, and good mechanical and biocompatible properties (Hwang, 2010). Their high surface area, good conductivity and three-dimensional structure could make graphitic nanocapsules a good electrode material for electrochemical biosensing. Graphitic nanocapsules also have high chemical stability and excellent dispersion characteristics which are important both for optimizing synergistic nanoparticle-support interactions and maximizing the mass activity of expensive precious enzymes. However, many applications and properties unique to graphic nanocapsules remain to be explored. For example, increasing interest has been shown in controllable synthesis of graphitic nanocapsules and constructing bio-hybrid nanocomposites and structures for broader biomedical applications.

In particular, hydrogen peroxide is a universal molecule and has significant functions as a signaling molecule in the regulation of a variety of biological processes, including aging and carcinogenesis (Veal et al., 2007; Giorgio et al., 2007; López-Lázaro, 2007). As such, the accurate determination of hydrogen peroxide is essential in the biological, environmental and clinical fields. Accordingly, many methods have been developed for the highly sensitive detection of hydrogen peroxide. The electrochemical method is a widely used approach by its simplicity and high sensitivity, different strategies and electrode systems have been explored, such as HRP-peptide on gold electrode (Zhao et al., 2013), HRP–Au–chitosan–clay (Zhao et al., 2008), HRP–gold nanowire (Xu et al., 2010), HRP–attapulgitc on glassy carbon (GC) (Wu et al., 2011), HRP–CNT–chitosan–sol–gel (Kang et al., 2009), HRP–CNT–methylene blue (Xu et al., 2003; Zhang et al., 2010), Pt on glassy carbon (O’Neil et al., 2004), Pt–MnO–graphene (Xiao et al., 2012), TiO–cytochrome C (Luo et al., 2009), and PtPd–Fe3O4 (Sun et al., 2012). While these methods have all demonstrated their utility, it is worthwhile exploring new electrode materials and developing a simpler and more effective approach to fabricate reagentless amperometric biosensors for hydrogen peroxide. One such candidate is the graphic nanocapsule.
Herein, we developed a method of synthesizing hollow graphitic nanocapsules (HGN) and using them as an electrode material for hydrogen peroxide detection. These HGNs were used as three-dimensional matrices to effectively immobilize enzymes, proteins, and small molecules. Specifically, a reagentless amperometric biosensor was created by coimmobilization of methylene blue (MB) electron mediators and horseradish peroxidase (HRP) enzyme on the HGN-coated electrode. The positively charged HRP and MB molecules were anchored on the HGN surface through electrostatic adsorption. As a consequence of the extra strong π–π interaction, HGNs exhibited a high loading capacity for the MB and electrochemical mediator-molecules. The constructed HGN–HRP–MB platform ably facilitated electron shuttling between the active center of HRP enzyme and the surface of the electrode. Thus, by using HGN as the electrode matrix, together with a simple MB and HRP assembly strategy, the sensing platform demonstrated the ability to detect hydrogen peroxide with high sensitivity, selectivity, reproducibility, and stability. Indeed, hydrogen peroxide molecules could be detected less than 1 μM.

2. Experimental

2.1. Chemicals

Methylene blue and horseradish peroxidase (300 U/mg) were obtained from Shanghai Reagents Company (Shanghai, China). All other chemicals of analytical reagent grade or higher were obtained from Changsha Chemical Reagents Company (Changsha, China) and used as received without further purification. All aqueous solutions were prepared using ultrapure water (Milli-Q, Millipore). The buffer solutions were used as received without further purification. Other chemicals of analytical reagent grade or higher were obtained from Shanghai Reagent Company (Shanghai, China). All aqueous solutions were prepared using ultrapure water (Milli-Q, Millipore). The buffer solutions were used as received without further purification. Other chemicals of analytical reagent grade or higher were obtained from Shanghai Reagent Company (Shanghai, China).

2.2. Preparation of HGN

Hollow graphitic nanocapsules were obtained from a core–shell magnetic graphitic nanomaterial (MG). MG was synthesized with a chemical vapor deposition (CVD) system as reported previously (Chen et al., 2012; Song et al., 2013). Briefly, we first impregnated fumed silica (1.00 g, Aladdin) with Co(NO₃)₂·6H₂O (2.06 g) in methanol. Then the methanol was removed, the mixture dried, and the powder ground. Typically, 0.50 g of the powder was used for methane CVD growth in a tube furnace at 800°C for 5 min. After growth, the sample was etched with 10% HF in H₂O (80%) and ethanol (10%) to dissolve the silica. We collected the MG solid product through centrifugation and washed thoroughly. To obtain the HGN, a solution of sulfuric and nitric acid was utilized to polish the as-received MG for 4 h and solubilize it in water. The excess MGs were removed by an external magnet. The HGNs were collected through centrifugation and washed thoroughly with ultrapure water.

2.3. Adsorption measurement of methylene blue on HGN

Adsorption measurements were performed with simple mixing of the MB and HGN solutions. Typically, the MB solution at a concentration of 2 mg/L was used for the preparation of MB–HGN nanocomposites through a premixing procedure with different amounts of HGNs. The mixture was shaken for 30 min at room temperature and then centrifuged at 7000 rpm for 5 min. The resulting supernatant was collected, and UV–vis spectra were recorded using a Shimadzu UV-2550 spectrophotometer (Shimadzu International Trading Co., Ltd., Shanghai, China).

2.4. Preparation of HGN–HRP–MB-modified electrode

Prior to modification, the bare glassy carbon electrode was thoroughly polished with emery paper and alumina slurry in the order of 1.0, 0.5, and 0.03 μm, followed by ultrasonication in water. Next, the electrode was immersed in a freshly prepared piranha solution (30% H₂O₂ and 98% H₂SO₄, 1/3, v/v) for 40 min. After this, the electrode was rinsed with ultrapure water and electrochemically pretreated with cyclic potential scanning to obtain a clean glassy carbon electrode. Then, 5 μL, 1.75 mg/mL HGN was cast on the electrode surface and air-dried at room temperature. Following this step, the electrode was soaked in 10 mg/mL HRP solution and incubated overnight. Meanwhile, a homogeneous solution (I) composed of 1 mg/mL HRP and 0.33 mg/mL MB was prepared in PBS (pH 7.4). The HGN–HRP–MB electrode was obtained by dipping the HRP-coated HGN glassy carbon electrode into solution I (PBS, pH 7.4) for 12 h at 4°C, followed by careful rinsing with ultrapure water. Finally, 5 μL of 1% Nafion ethanolic solution was cast on the electrode surface and air-dried at room temperature to ready the instrument for electrochemical measurements.

2.5. Characterization and electrochemical measurements

Raman spectroscopy was performed on a Horiba Joyn Yvon LabRAM-010 Raman microscope with 632 nm He–Ne laser excitation. The hydrodynamic diameters of the HGNs under investigation were measured using a Zetasizer Nano ZS90 Dynamic Light Scattering (DLS) system equipped with a red (633 nm) laser and an Avalanche photodiode detector (APD) (quantum efficiency > 50% at 633 nm) (Malvern Instruments Ltd., Worcestershire, England). Zeta potential measurements were performed in water. The measurements were carried out at room temperature on the ZetaSizer Nano ZS90 equipped with MPT-2 Autotitrator and 4 mL He–Ne laser (λ₀=633 nm) using the Laser Doppler Electrophoresis technique. Electrochemical measurements were carried out with a CHI 760 electrochemical analyzer (CH Instrument Company, Shanghai, China). A conventional three-electrode cell was used. A bare or modified glassy carbon electrode (GCE) was used as a working electrode, and a platinum disk was used as an auxiliary electrode, with a saturated calomel electrode (SCE) as reference.

3. Results and discussion

3.1. Characterization of hollow graphitic nanocapsules

The prepared HGNs were analyzed by scanning electron microscopy (SEM), as shown in the images pictured in Fig. 1A and B. These HGNs showed uniform size distribution. Transmission electron microscopy (TEM), which was utilized for the structural characterization of HGNs, showed a hollow structure with some wrinkles on the surface (Fig. 1C). In the high-resolution TEM image of the HGN (Fig. 1D), the graphitic shell structure was clearly observed, and the intralayer distance of the shell was around 0.34 nm, which is consistent with that of graphite. The digital camera image shows the graphitic nanocapsule in water solution before and after the hollow structure was prepared (Fig. 1E). After the acid treatment, the HGNs form a black suspension which could remain stable for months.

The HGNs were further characterized with selected area electron diffraction (SAED) and Raman spectroscopy, and both techniques indicated the graphitic structure of the nanocapsules. As demonstrated in Fig. 2A, the SAED diffraction patterns can be assigned to the (002), (100), (004) and (112) facets of the hexagonal crystalline graphite, respectively. The graphitic shell was also proved through Raman
spectroscopy (Fig. 2B, Fig. S1, Supporting information), showing a graphitic carbon (G) peak at ~1590 cm⁻¹ and a disordered (D) peak at ~1300 cm⁻¹. The high Raman D peak reflects the high strain of the graphite shells caused by the small size of the MG and defects presented during the acid treatments. Analyses of the hydrodynamic diameters and surface ζ-potential of the suspended HGNs was investigated with dynamic light scattering (Fig. 2C). The average size was around 60 nm, which agreed with the size measured from TEM. Fig. 2D shows the ζ-potential curves of the HGN water solution. HGNs were negatively charged, and the ζ-potential was around 36 mV. The mixed-acid treatments produced massive suspended hydroxyl and carboxyl groups at the HGN surface, which were believed to be the origin of the negative charge observed in the ζ-potential curves.

3.2. Isothermal adsorption of hollow graphitic nanocapsules

HGNs have high surface areas resulting from their hollow and three-dimensional graphitic structure. Methylene blue was used as the model molecule to explore the isothermal adsorption properties of HGNs (Fig. 3). HGNs exhibited high loading capacity for MB molecules, and the positively charged MB molecules were anchored on the HGN surface through strong electrostatic adsorption and π–π interactions. Fig. 3A shows the UV–vis spectra of the 2 mg/L MB molecules (a) mixed with 0.175 mg/mL HGNs after 0 min (b), 30 min (c), 1 h (d) and 5 h (e) incubation and separation, respectively. Two absorption peaks around 613 and 664 nm, respectively, can be observed in the UV–vis spectra. These peaks belong to the MB molecules whose intensities are proportional to the concentration of MB in solution. As determined from the spectra, most MB molecules were adsorbed by the HGNs and reached isothermal equilibrium after 30 min. The inset of Fig. 3A shows the digital camera images of the original MB solution (a) and following the addition of HGNs (b). The HGNs demonstrated good MB adsorbing capability, which remained very stable under different pH regimes (Fig. 3B). More specifically, HGNs can stably adsorb the MB dyes, ranging from very acidic pH 3 to alkaline pH 11 conditions, suggesting that HGNs could be a potentially efficient adsorbent for the removal of organic environmental contaminants. We further investigated MB adsorption as a function of HGN concentration. Accordingly, UV–vis spectra were collected from MB solution after different concentrations of HGN were incubated and separated (Fig. 3C). By adding certain amounts of HGNs into the MB solution, the peaks around 613 and 664 nm nearly disappeared. The inset shows the digital camera images of the MB solution with different concentrations of HGN added. The curve of adsorption efficiency as a function of the added HGN concentration is shown in Fig. 3D, which could be well fit into an equilibrium isotherm adsorption equation. With around 0.08 mg/mL HGN added into the 2 mg/L MB solution, the adsorption reached saturation, and most of the MB molecules were adsorbed on the HGN surfaces.

3.3. Hydrogen peroxide detection strategies

Having determined the efficient surface adsorption capability and excellent conductivity of the HGNs, we designed an HGN electrochemical sensing platform for hydrogen peroxide detection. Hydrogen peroxide, the simplest perioxide, is a strong oxidizer and considered a highly reactive oxygen species. Therefore, its accurate determination is essential in biological, environmental and clinical fields. Fig. 4 shows the assembly of the HGN–HRP–MB electrochemical sensing platform. Briefly, HGNs were used as three-dimensional matrices for coimmobilization of methylene blue (MB) electron mediators and horseradish peroxidase (HRP) (Fig. S2, Supporting information). First, HGNs were coated on the glassy carbon electrode (GCE) surface through physical
absorption (Fig. S3 for electrochemical impedance characterization, Supporting information). Next, the electrode was soaked in the HRP solution for the preassembly of HRP enzymes. Following that, the HRP-coated electrode was immersed in the HRP–MB solution for further assembly (Fig. 4A). Fig. 4B displays the interface view of the active HGN–HRP–MB electrode structure and the hydrogen peroxide detection processes. The high conductivity of the HGN graphitic shells clearly enhances the electrochemical performance of the HGN electrode. The HGNs were also a high surface area supporter, and both the HRP and MB molecules could be easily adsorbed, while maintaining their activity as a direct result of the three-dimensional structure of the HGN. The adsorbed MB molecules on the HGN–HRP particle surface could act as an efficient electrochemical mediator to improve electron transport. While the HRP-coated electrode loses its enzymatic bioactivity and shows poor electron transport, the HGN platform has higher hydrogen peroxide sensing activity and better electron transport efficiency.

3.4. Electrochemical properties of HGN–HRP–MB-modified electrode

HGNs, as an efficient electrode material, have unique and stable electrochemical properties, as characterized in the HGN–HRP–MB detection platform shown in Fig. 5. Typical cyclic voltammograms of HGN–HRP–MB in PBS solution (Fig. 5A, Supporting information) at different scanning rates in the potential range from 0.7 V to −0.7 V demonstrate the symmetrical and good redox behavior of MB in contributing to the efficiency of HGNs (Fig. 5B). As the scanning rate increased, the redox current correspondingly increased. The peak currents of HGN–HRP–MB complexes are proportional to the scanning rate up to 700 mV/s, indicating the redox reaction of HGN–HRP–MB complexes on the electrode surfaces. The redox peak currents increased linearly (linear correlation coefficient $R^2 > 0.999$) with the scanning rate between 50 and 700 mV/s, as expected, further indicating a surface-confined process of the HGN–HRP–MB redox reaction (Fig. 5B).

3.5. Electrochemical detection of H$_2$O$_2$ by HGN–HRP–MB electrode

We next investigated the hydrogen peroxide detection capability of the HGN–HRP–MB electrochemical sensing platform (Fig. 6). In particular, electrochemical activity is compared among the bare glassy carbon (a), HRP only (b) and HGN–HRP–MB (c) electrode in 100 μM of hydrogen peroxide, respectively (Fig. 6A). Similar to the peak current of the bare GCE, the HRP-only electrode also shows a relatively low peak current, possibly caused by the denaturation of the HRP enzyme molecules and the low electron transport efficiency between the electrode and HRP molecules. However, for the HGN–HRP–MB matrix-modified electrode, the redox peak current is significantly increased. The high conductivity of HGN was believed to improve the electron transport, and the 3D hollow structure of the HGN also helps to maintain HRP enzyme activity during the electrochemical measurements. MB, as an electrochemical reaction mediator, displayed excellent mediating ability in the electrocatalytic reactions and also contributed to the increase of peak current. The HGN–HRP–MB matrix-modified electrode, the redox peak current is significantly increased. The high conductivity of HGN was believed to improve the electron transport, and the 3D hollow structure of the HGN also helps to maintain HRP enzyme activity during the electrochemical measurements. MB, as an electrochemical reaction mediator, displayed excellent mediating ability in the electrocatalytic reactions and also contributed to the increase of peak current. The HGN–HRP–MB matrix-modified electrode, the redox peak current is significantly increased. The high conductivity of HGN was believed to improve the electron transport, and the 3D hollow structure of the HGN also helps to maintain HRP enzyme activity during the electrochemical measurements. MB, as an electrochemical reaction mediator, displayed excellent mediating ability in the electrocatalytic reactions and also contributed to the increase of peak current. The HGN–HRP–MB matrix-modified electrode, the redox peak current is significantly increased. The high conductivity of HGN was believed to improve the electron transport, and the 3D hollow structure of the HGN also helps to maintain HRP enzyme activity during the electrochemical measurements. MB, as an electrochemical reaction mediator, displayed excellent mediating ability in the electrocatalytic reactions and also contributed to the increase of peak current. The HGN–HRP–MB matrix-modified electrode, the redox peak current is significantly increased. The high conductivity of HGN was believed to improve the electron transport, and the 3D hollow structure of the HGN also helps to maintain HRP enzyme activity during the electrochemical measurements. MB, as an electrochemical reaction mediator, displayed excellent mediating ability in the electrocatalytic reactions and also contributed to the increase of peak current. The HGN–HRP–MB matrix-modified electrode, the redox peak current is significantly increased. The high conductivity of HGN was believed to improve the electron transport, and the 3D hollow structure of the HGN also helps to maintain HRP enzyme activity during the electrochemical measurements. MB, as an electrochemical reaction mediator, displayed excellent mediating ability in the electrocatalytic reactions and also contributed to the increase of peak current. The HGN–HRP–MB matrix-modified electrode, the redox peak current is significantly increased. The high conductivity of HGN was believed to improve the electron transport, and the 3D hollow structure of the HGN also helps to maintain HRP enzyme activity during the electrochemical measurements. MB, as an electrochemical reaction mediator, displayed excellent mediating ability in the electrocatalytic reactions and also contributed to the increase of peak current. The HGN–HRP–MB matrix-modified electrode, the redox peak current is significantly increased. The high conductivity of HGN was believed to improve the electron transport, and the 3D hollow structure of the HGN also helps to maintain HRP enzyme activity during the electrochemical measurements. MB, as an electrochemical reaction mediator, displayed excellent mediating ability in the electrocatalytic reactions and also contributed to the increase of peak current. The HGN–HRP–MB matrix-modified electrode, the redox peak current is significantly increased. The high conductivity of HGN was believed to improve the electron transport, and the 3D hollow structure of the HGN also helps to maintain HRP enzyme activity during the electrochemical measurements. MB, as an electrochemical reaction mediator, displayed excellent mediating ability in the electrocatalytic reactions and also contributed to the increase of peak current.

Fig. 2. Advanced structural analysis of HGNs. (A) Based on selected area electron diffraction measurement of the HGNs, the diffraction patterns can be assigned to the (002), (100), (004) and (112) facets of the hexagonal crystalline graphitic structure. (B) A Raman spectrum (excitation 632 nm) of the HGNs with the G and D bands of graphitic carbon. (C) and (D) were the size and \( \zeta \)-potential distribution of the HGNs, respectively, as measured by the DLS system equipped with a red (633 nm) laser at room temperature. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
The HGN–HRP–MB platform also demonstrated high detection sensitivity and good reproducibility for the determination of hydrogen peroxide in its linear range. Hydrogen peroxide could be detected down to 1 μM. The cyclic voltammograms of PBS solution without (a) and with (b) the addition of 1 μM hydrogen peroxide clearly demonstrate the sensing capability of the HGN–HRP–MB platform.

Fig. 3. The isothermal adsorption properties of the HGNs. (A) UV–vis spectra of 2 mg/L MB molecules (a) mixed with 0.175 mg/ml HGNs after 0 min (b), 30 min (c), 1 h (d) and 5 h (e) incubation and separation, respectively (see inset for digital camera images of solution a and b before separation). (B) UV–vis absorption of MB molecules adsorbed on HGNs under different pH regimes (A, A0 were the intensity of the solution with and without HGN adsorption, respectively). (C) UV–vis spectra of 2 mg/L MB solution after different amounts of HGNs were added (see inset for the digital camera images of the different solutions). (D) Adsorption efficiency of 2 mg/L MB as a function of the added HGN concentration.

Fig. 4. The strategy of using HGNs for hydrogen peroxide detection. (A) Procedure for preparing the HGN–HRP–MB matrix on the glassy carbon electrode (GCE). (B) Interface view of the active electrode structure and the hydrogen peroxide detection process.
Sensing response of five different platforms was also investigated with fixing the hydrogen peroxide concentration under optimum conditions, and the HGN–HRP–MB platform demonstrated good fabrication reproducibility. Moreover, HGN as electrode material can also be utilized for different electrochemical detection and it demonstrated good generality and reproducibility (Supporting information, Figs. S5, S6 and S7).

### 3.6. Amperometric and selectivity measurements of hydrogen peroxide detection

The amperometric response of the HGN–HRP–MB platform was attempted under the optimized operating electrode potential of −0.4 V with successive injections of hydrogen peroxide in PBS solution. Fig. 7A shows a typical amperometric response by varying
H$_2$O$_2$ concentration from 500 nM to 500 μM, respectively. Amperometric response down to 500 nM H$_2$O$_2$ indicates the good sensitivity of the HGN–HRP–MB sensing platform. Selectivity of the HGN–HRP–MB platform was also investigated. Hydrogen peroxide, l-Cys, NO$_3^-$ and glycine were compared with the platform, as demonstrated under voltammetry tests (Fig. 7B). The 50 μM H$_2$O$_2$ solution showed around three times higher positive signal than the 50 μM l-Cys, NO$_3^-$ or glycine solution, indicating good selectivity of the HGN–HRP–MB sensing platform. For maximum efficiency and selectivity, more effort should be directed toward optimizing HGN size and graphitic shell thickness, designing multilayer HRP and MB coating structures, or further amplifying the sensing with other strategies.

## 4. Conclusion

In summary, we prepared and utilized hollow graphitic nanocapsules as efficient electrode materials for the sensitive detection of hydrogen peroxide. The HGNs were synthesized through a CVD system and then with mixed-acid treatments. HGNs have large surface areas, high conductivity, as well as good mechanical and biocompatible properties, based on their hollow and three-dimensional graphitic structure. Methylene blue molecules can be efficiently adsorbed on the HGN surfaces, and this adsorption capability remained very stable under different pH regimes. The positively charged MB molecules were anchored on the HGN surface through strong electrostatic adsorption and π–π interactions. Using HGNs as three-dimensional matrices for coimmobilization of methylene blue electron mediators and horseradish peroxidase, an HGN reagentless amperometric sensing platform was designed to detect hydrogen peroxide. The simple HGN–HRP–MB electrochemical sensing platform was able to facilitate electron shuttling between the active center of HRP enzyme and the surface of the electrode. Benefiting from efficient surface adsorption capability and excellent conductivity of the HGN, the sensing platform demonstrated very sensitive hydrogen peroxide detection capability, and less than 1 μM hydrogen peroxide could be detected. The HGN–HRP–MB electrochemical sensing platform also demonstrated good hydrogen peroxide detection selectivity and high reproducibility and stability. Considering that no noble metal electrode is used, this detection capability of HGN platform is excellent. The HGNs could also be utilized as matrices for immobilization of other enzymes, proteins or small molecules and for different biomedical applications.

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [http://dx.doi.org/10.1016/j.bios.2013.08.023](http://dx.doi.org/10.1016/j.bios.2013.08.023).

## References


