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Graphene-Based SELDI Probe with Ultrahigh Extraction and Sensitivity for DNA Oligomer

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Abstract: We demonstrate that graphene can be used as an ultrahigh efficiency preconcentration and detection platform for ssDNA. DNA-adsorbed graphene can be used directly for Surface Enhanced Laser Desorption Ionization-Time of Flight-Mass Spectrometry (SELDI-TOF-MS). The rapid enrichment of biomolecules and direct, label-free detection are potentially useful for analysis in proteomics and genomics.

Graphene (G), a two-dimensional sheet of sp^2-conjugated atomic carbon, has stimulated intense research interest because of its unique band structure, massless fermions, and ultrahigh carrier mobility.[1] Besides its use as an electronic material, G is emerging as a new tool for bioapplications.[2] G is a double-sided aromatic scaffold with a high specific surface area of 2630 m^2/g and potentially can afford an ultrahigh loading capacity for biomolecules and drugs.[2] Solution-phase G synthesis, unlike carbon nanotubes, does not involve the use of metal catalysts; thus there is no associated problems of biotoxicity. Graphene oxide (GO), the oxidized counterpart of G, contains functional groups such as epoxide, carboxyl, and hydroxyl groups which can undergo covalent, electrostatic, or hydrogen bonding with proteins.

Laser Desorption/Ionization-Mass Spectrometry (LDI-MS) has emerged as an important tool for the rapid and sensitive analysis of biomolecules.[3] A major advancement of this technology is the incorporation of photoabsorbing media for the indirect transfer of laser energy to analyte molecules and the suppression of molecular fragmentation.[3] One of the most popular LDI-MS methods is Matrix-assisted Laser Desorption/Ionization (MALDI). However, matrix interference in the low-mass regions poses a serious problem for small molecule analysis. Furthermore, in proteomics and genetics research, it is often necessary to extract the target molecule from unfractionated biological mixtures. This requires various chromatography and affinity purification methods. As an alternative to MALDI, Surface-Enhanced Laser Desorption/Ionization (SELDI) eliminates the use of the acidic organic matrix and utilizes a template for the direct extraction, amplification, desorption, and ionization of the target analyte, thereby cutting the time and cost for analysis.[4] Herein, we report the ultrahigh efficiency extraction and analysis of DNA oligomers (ssDNA) using G as a combined extraction and analysis platform for SELDI-TOF-MS.

Briefly, G flakes (Figure 1a) were synthesized by lithium ion intercalation followed by high power sonication. Details of synthesis and characterization can be found in the Supporting Information. The performance of our synthesized G as a SELDI substrate was compared with GO flakes synthesized by Hummer's method.[5] Due to the presence of the oxidized functional groups, GO is soluble in water and forms a homogeneous dispersion. In contrast, hydrophobic G sheets are not dispersible in water and floats (Figure 1b). However, after interaction with single stranded DNA (ssDNA), the

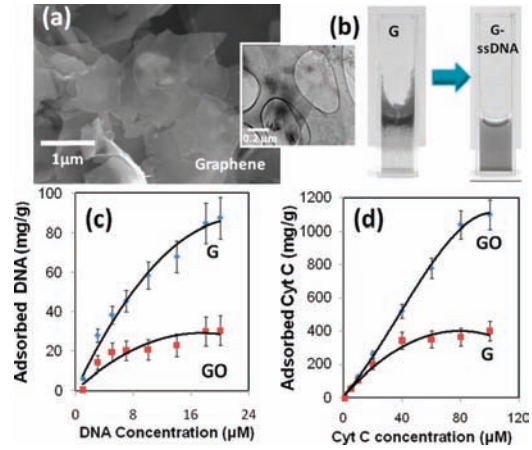

Figure 1. (a) SEM image of Graphene (G); inset: TEM image of G. (b) Photographs of pristine G sheets floating on water (left) and G-ssDNA after interaction with single-stranded DNA (right). Adsorption isotherm of ssDNA (c) and Cyt C (d) on G and GO.

solubility of G improves remarkably. This attests to the high loading capacity of G for DNA *via* $\pi-\pi$ interactions. The effect of these noncovalent binding interactions results in quenching of the fluorescence from the dye-labeled ssDNA (Figure S2).

The adsorption isotherms of ssDNA and Cytochrome C (Cyt C) protein on GO and G were recorded and compared (Figure 1c−d).

The adsorption capacity is judged from the saturation point of the adsorption isotherm. G shows a higher adsorption capability for ssDNA compared to GO (Figure 1c). At a concentration of 20 μM of ssDNA, the adsorption capacity corresponds to 87 mg/g of G and 30 mg/g of GO. The amount of ssDNA adsorbed on G is four times higher than in the case of the polylysine-coated nanodiamond platform (22 mg/g at neutral pH).[6] The adsorption capability of functionalized nanodiamond particles relies on the electrostatic binding capacity of polylysine and is pH dependent. In contrast, the as-prepared G platform provides effective $\pi-\pi$ cooperative interactions with ssDNA under physiological conditions. Evidence of ssDNA binding can be seen from the circular dichroism (CD) spectra (Figure 2a) of free ssDNA and DNA-bound G. In the CD spectrum of DNA-bound G, the bands that are characteristic of the G-quadruplex and B-DNA configurations of free-DNA[7] vanish upon $\pi-\pi$ binding interactions with G, due to unfolding of the intrinsic structures. The binding interactions of GO with ssDNA are aided mainly by H-bonding or electrostatic interactions. At pH 7, the zeta potential of GO is determined to be −20 mV, which arises from its weakly acidic COOH and OH groups. The electrostatic repulsion between anionic GO and negatively charged ssDNA decreases the adsorption capacity of GO for ssDNA compared to G.

The situation is reversed with regards to protein molecules like Cyt C where electrostatic interactions play a more important role

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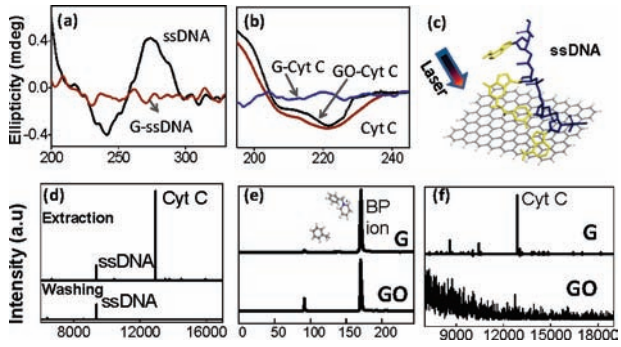

Figure 2. CD spectra of (a) free ssDNA and G-ssDNA and (b) native Cyt C as well as Cyt C adsorbed on G and GO. (c) G-ssDNA SELDI probe. (d) SELDI-TOF MS spectra acquired directly after extraction of 1:5 ssDNA and protein mixture (top) and after washing with DI water (bottom). (e) MS spectra of BP parent ion and fragmented ion using G (top) and GO (bottom) as SELDI probe; the laser fluence was set at 40 mJ cm^{-2}. (f) MS spectra of Cyt C using G (top) and GO (bottom) SELDI probe.

than $\pi-\pi$ bonding. In this case, due to H-bonding and electrostatic interactions, the binding capacity of GO for Cyt C is three times that of G (Figure 1d). The reorientation of Cyt C in order to expose its hydrophobic moieties to bind to G resulted in its denaturization, as judged from CD measurements (Figure 2b). The broad negative band (200−240 nm) corresponding to the α-helix structure[8] of the protein vanishes for Cyt C-adsorbed G, whereas, for Cyt C-adsorbed GO, the α-helix negative band is clearly seen and similar to the native Cyt C band (Figure 2b). The loading capacity of GO for Cyt C, i.e. 1100 mg of Cyt C per g of GO, is very high compared to any form of extraction media that has been reported so far (1 order of magnitude higher than that for the nanodiamond-based extraction platform).[9] Our studies show that the lowest detection limit for Cyt C using GO for MALDI-TOF MS is 1 fM (Figure S3); this is 5 orders of magnitude lower than that for the nanodiamond-based MALDI platform.[9] GO is thus useful for the high efficiency extraction of proteins and can be used as a detection probe in MALDI-TOF.

The ability of G to act as a SELDI probe for DNA and proteins was investigated. The unique aspect of G is that it can combine high loading capacity with high selectivity in the case of DNA extraction, by virtue of the $\pi-\pi$ cooperative interactions between DNA and G. To examine the selective extraction of DNA by G, G was applied as the extraction platform in a mixture of Cyt C protein and DNA (5:1 ratio). A simple extraction procedure involves the vortexing of G flakes in the mixture, followed by high speed centrifugation (14 000 rpm, 5 min) to recover the biomolecule-loaded G. This is then drop-casted onto a metal plate and used directly for SELDI analysis (Figure 2c) with a Bruker Daltonics Autoflex II ion extraction linear time-of-flight mass spectrometer. Positive and negative ion spectra were recorded with a nitrogen laser ($\lambda = 337$ nm) to ionize the biomolecules with a typical energy of 20 μJ/pulse. Figure 2d (top) shows the SELDI mass spectrum of G following extraction of DNA and protein in the mixture without washing. It can be seen that the peak due to Cyt C is significantly higher than that of ssDNA due to its 5× higher concentration. After the mixture is rinsed with deionized water, the Cyt C peak disappears completely, although the signal of ssDNA remains unattenuated [Figure 2d (bottom)]. Using G as the SELDI probe, the lowest concentration detected for Cyt C is 1 pM (Figure S4a). The detection limit for ssDNA is 100fM (Figure S4d), which is 1 to 3 orders of magnitude lower than that for the MALDI method using the polymeric or nanodiamond platform.[6,10] In addition, a range of other ssDNA and proteins of different lengths and surface charges have also been tested using the G platform, achieving the pM and fM detection limit (Figure S4).

The desorption−ionization processes of SELDI matrices involve complex optical and mechanical phenomena as well as thermodynamic and physicochemical processes of phase transition and ionization. Our studies show that G has distinct advantages compared to GO in terms of optical absorption and suppression of fragmentation in SELDI. First, the absorbance of G is much higher than that of GO at the excitation laser wavelength of 337 nm used in the SELDI (Figure S5) study. In fact G shows universal absorbance that is independent of frequency over a wide range, meaning broad spectral excitation is possible. Next, we observed a significantly lower degree of fragmentation of the analyte molecule on G compared to GO. Benzylpyridinium (BP) ion, which is a standard thermometer chemical used to probe the desorption properties of the matrix,[3] was used to compare the desorption process of the G- and GO-based SELDI probe (Figure 2e). The much lower fragmentation and higher survival yield of BP ion for the G SELDI probe compared to GO is attributed to the efficient electron−phonon coupling in G and its extraordinary high thermal conductivity (4840−5300 W m^{1-} K^{-1}),[11] which allows it to act as a thermal sink during the rapid thermalization of laser-excited electrons. In G, collective phonon modes that are IR-active can be efficiently coupled to a continuum of electron−hole excitations through electron−phonon interactions. In addition, the weaker binding interactions of G with proteins favor a more efficient desorption/ionization process for Cyt C compared to GO. For example, although GO shows a greater binding affinity and loading capacity for Cyt C compared to G, only a noisy spectrum is obtained in SELDI, compared to a sharp signal for G (Figure 2f).

In conclusion, we have demonstrated that G can be used as a high efficiency preconcentration and direct SELDI analysis platform in bioanalytics. In addition, the binding interactions of G with biomolecules is governed by hydrophobic and $\pi-\pi$ interactions; these binding forces favor the desorption/soft ionization processes specific to SELDI. The electrostatic bonding interactions of GO with proteins render it more suitable as a MALDI probe. The low noise level/interference of the G-based SELDI probe can open up a new level of sensitivity for biomarker recovery in proteomics and genomics study.

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Supporting Information Available: Detailed experimental methods and additional figures. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Geim, A. K. *Science* **2009**, *324*, 1530.
(2) Liu, Z.; Robinson, J.; Sun, X.; Dai, H. *J. Am. Chem. Soc.* **2008**, *130*, 10876.
(3) Tang, H. W.; Ng, K. M.; Lu, W.; Che, C. M. *Anal. Chem.* **2009**, *81*, 4720.
(4) Tang, N.; Tornatore, P.; Weinberger, S. *Mass Spectrom. Rev.* **2004**, *23*, 34.
(5) Hummers, W. S., Jr.; Offeman, R. E. *J. Am. Chem. Soc.* **1958**, *80*, 1339.
(6) Kong, X.; Huang, L. C. L.; Liau, S. C. V.; Han, C. C.; Chang, H. C. *Anal. Chem.* **2005**, *77*, 4273.
(7) Patil, S. D.; Rhodes, D. G. *Nucleic Acids Res.* **2000**, *28*, 4125.
(8) Shang, W.; Nuffer, J. H.; Muñiz-Papandrea, V. A.; Colón, W.; Siegel, R. W.; Dordick, J. S. *Small* **2009**, *5*, 470.
(9) Kong, X. L.; Huang, L. C. L.; Hsu, C. M.; Chen, W. H.; Han, C. C.; Chang, H. C. *Anal. Chem.* **2005**, *77*, 259.
(10) Ibanez, A. J.; Schueler, T.; Moeller, R.; Fritzsche, W.; Saluz, H. P.; Svatos, A. *Anal. Chem.* **2008**, *80*, 5892.
(11) Balandin, A. A. *Nano Lett.* **2008**, *8*, 902.

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