Supporting Information

Phospholipid-Graphene Nanoassembly as A Novel Fluorescence Biosensor for Sensitive Detection of Phospholipase D Activity

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ADDITIONAL EXPERIMENTAL DETAILS

Characterization of Graphene. The transmission electron microscope (TEM) images were obtained on a field-emission high-resolution 2100F TEM (JEOL, Japan) at an acceleration voltage of 200 kV. The sample films for TEM analysis were formed by dropping the diluted solution of nanomaterial suspension on a holey carbon mesh grids (400 mesh) and air-dried at room temperature. The Raman scattering spectroscopic characterization was performed with graphene powders (reduced graphene oxide or graphene oxide) spreading on clean silicon wafers using a Confocal Raman System Laboram 010 (Jobin Yvon, France) with 632 nm HeNe laser. The infrared absorption spectroscopic measurements were taken with graphene powders in KBr pieces on a Nexus 870 FT-IR spectrophotometer (Thermo Electron, USA) under continuous N\(_2\) purge. X-ray diffraction (XRD) patterns of graphene samples were collected via a D8 Advance (Bruker, USA) X-ray diffractometer with Cu K\(\alpha\) radiation (\(\lambda = 1.5418\ \text{Å}\)). X-ray photoelectron spectroscopy (XPS) analysis were performed on a K-Alpha 1063 X-ray photoelectron spectrometer (Thermo Fisher, UK) with a monochromatic Al K\(\alpha\) X-rays source at an operation power of 72 W. Ultraviolet–visible spectroscopy (UV-VIS) measurements were obtained in a micro quartz cuvette on a UV 2450 spectrophotometer (Shimadzu, Japan) in the 400–650 nm range with a 1 nm interval. Dynamic light scattering (DLS) measurements were used to determine the hydrodynamic sizes of the liposome and the phospholipid-graphene nanoassembly using a Malvern Zetasizer 3000 HS particle size analyzer (Malvern Instruments, UK).

Optical microscopy characterization. Fluorescence and differential interference contrast (DIC) microscopy images was acquired using a Nikon Eclipse 80i microscope equipped with a 100× objective (NA 0.2~1.4) and a CoolSnap HQ2 CCD camera (Roper Scientific, USA). The samples were illuminated with a mercury lamp and the emitted fluorescence were passing through a FITC filter set (Chroma Technology Corp., USA) and the fluorescence images were taken at a exposure time of 20 ms. DIC images were taken with an oil immersion DIC condenser (NA 0.2~1.4) when illuminated by 100 W halogen lamp. The CCD camera was controlled by MetaVue (Universal Imaging Corp, USA) and the images were processed using Image J software (http://rsbweb.nih.gov/ij/). The samples were obtained by
dropping 10 µL phospholipid–graphene nanoassembly suspension before or after the enzymatic reaction with 1000 U/L PLD on a pre-cleaned glass slide and air-dried at room temperature.

**Fluorescence anisotropy measurements.** The reaction solution was prepared in fresh by mixing 6 µL phospholipid–graphene nanoassembly suspension with 14 µL reaction buffer containing 4 µL Tris-HCl buffer (50 mM, pH 8.0), 5 µL 30% DMF, 1.5 µL 100 mM CaCl₂, and 3.5 µL ultrapure water. In a typical assay, in 20 µL reaction solution, 10 µL PLD (dissolved in 10 mM Tris-HCl buffer, pH 8.0 with final concentrations ranging from 0 to 1000 U/L) was added followed by incubation at 37 °C for 2 h. The resulting mixture was diluted to a final volume of 100 µL with ultrapure water and subjected to fluorescence anisotropy measurements. Fluorescence anisotropy measurements were taken at room temperature in a 100 µL quartz cuvette on a PTI-QM-1 fluorescence spectrometer (PTI, USA). The excitation wavelength was 492 nm and the emission wavelengths were in the range from 505 to 600 nm with both excitation and emission slits of 2.5 nm.

**Surface Enhanced Laser Desorption/Ionization Time-of-Flight Mass Spectometry (SELDI-TOF MS) measurements.** The sample was formed by dropping 1 µL phospholipid–graphene nanoassembly suspension before or after the enzymatic reaction on the spot in a polished steel sample holder and air-dried at room temperature followed by direct detection using SELDI-TOF MS. SELDI-TOF MS was performed in deflection mode on a Proflex III mass spectrometer (Bruker, USA) equipped with a nitrogen laser of 337 nm with 3 ns duration pulse. The acceleration voltage was set to 20 KV. Laser power was set as 47%. The mass spectra were acquired as an average of 100 laser shots.
ADDITIONAL FIGURES

Figure S1. TEM images of freshly prepared reduced graphene oxide (a) and phospholipid–graphene nanoassembly (b).
Figure S2. Raman spectra obtained with reduced graphene oxide (red solid line) and graphene oxide (black solid line).

The Raman spectra show that the G band of reduced graphene oxide is shifted up to 1595 cm\(^{-1}\) compared with that of graphene oxide (1602 cm\(^{-1}\)), which corresponds to the recovery of the hexagonal network of carbon atoms. The intensity of the D band for reduced graphene oxide (1322 cm\(^{-1}\)) increases notably compared with that for graphene oxide, so the I\(_D\)/I\(_G\) ratio increase substantially for reduced graphene oxide. This indicates that the reduction process altered the structure of G-O and yields products with less structural defects.
**Figure S3.** FT-IR spectra obtained with reduced graphene oxide (red solid line) and graphene oxide (black solid line).

FT-IR spectra reveal that the oxygen peaks of reduced graphene oxide decreased dramatically compared with those of graphene oxide, indicating most of the epoxide and hydroxyl functional groups in graphene oxide had been successfully removed. This is attributed to the restoration of the electronic conjugation within hexagonal platelets after reduction.
**Figure S4.** XRD patterns obtained with graphene oxide (upper) and reduced graphene oxide (lower).

The XRD pattern (upper) shows a typical peak position near 11.54° (d-spacing ~7.58 Å) for graphene oxide. After reduction, the peak showed a dramatic shift to higher 2θ angles (24.07°; d-spacing ~3.69 Å). These data suggest that reduced graphene oxide is well ordered as two-dimensional sheets with a decreased interlayer spacing as compared with graphene oxide.
The XPS spectra show that the oxygen functionalities absorbance peak at 286 eV (C–O) sharply decreases and the peak at 287 eV (C=O) vanishes after the reduction, which gives clear evidence for the reduction of graphene oxide.
**Figure S6.** UV-VIS absorption spectra of liposome (blue solid line), graphene oxide (black solid line), phospholipid-coated graphene oxide (red solid line), reduced graphene oxide (green solid line), phospholipid–graphene nanoassembly (yellow solid line).
**Figure S7.** DLS analysis of the as-synthesized liposome (upper) and the phospholipid–graphene nanoassembly (lower). The average diameters are shown in the plot.

DLS analysis shows that the average hydrodynamic size of phospholipid-graphene nanoassembly (106 nm) was much smaller than that of the precursor liposome (285.5 nm). The decreased size indicates the precursor liposome collapsed in forming the phospholipid-coated graphene nanoassembly structure, since embedding of reduced graphene oxide in the liposome will not decrease the size of liposome.
Figure S8. Fluorescence anisotropy responses of phospholipid–graphene nanoassembly before (solid line) and after adding 1000 U/L PLD (dotted line).
Figure S9. Confocal fluorescence microscopy images of a casting film of phospholipid–graphene nanoassembly before (upper left) or after (upper right) reacting with 1000 U/L PLD. Differential interference contrast images of phospholipid–graphene nanoassembly before (Lower left) or after (Lower right) reacting with 1000 U/L PLD.
**Figure S10.** SELDI-TOF MS spectra obtained with phospholipid–graphene nanoassembly before (top) and after (bottom) hydrolysis by 1000 U/L PLD. The exact masses of the species involved are given: 

$M_{PC}$ is the molecular mass of DPPC, $M_{PA}$ is the molecular mass of phosphatidic acid.
**Figure S11.** The inhibition curve of halopemide obtained with phospholipid-graphene nanoassembly in hydrolysis by 10 U/L PLD in the presence of halopemide of varying concentrations (0, $10^{-6}$, $10^{-5}$, $10^{-4}$, $10^{-3}$, $10^{-2}$, 0.1, 1 mg/mL). The fluorescence intensity on Y-axis has been subtracted the fluorescence of background. According to the inhibition curve, the IC$_{50}$ is obtained to be 5.76 µM. Error bars are standard deviation across three repetitive experiments.