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# Towards intrinsic graphene biosensor: A label-free, suspended single crystalline graphene sensor for multiplex lung cancer tumor markers detection

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ABSTRACT

Graphene biosensors reported so far are based on polycrystalline graphene flakes which are anchored on supporting substrates. The influence of grain boundary and the scattering from substrate drastically degrade the properties of graphene and conceal the performance of intrinsic graphene as a sensor. Here we report a label-free biosensor based on suspended single crystalline graphene (SCG), which can get rid of grain boundary and substrate scattering, revealing the biosensing mechanism of intrinsic graphene for the first time. Monolayer SCG flakes were derived from low pressure chemical vapor deposition (LPCVD) method. Multiplex detection of three different lung cancer tumor markers was realized. The suspended structure can largely improve the sensitivity and detection limit (0.1 pg/ml) of the sensor, and the single crystalline nature of SCG enable the biosensor to have superior uniformity compared to polycrystalline ones. The SCG sensors exhibit superb specificity and large linear detection range from 1 pg/ml to 1 μg/ml, showing the prominent advantages of graphene as a sensing material.



## 1. Introduction

Tumor markers are small biological molecules in human's blood and tissue, and their high level of concentrations indicate certain cancers are very likely present inside the human body (Zhong et al., 2010). Therefore, tumor marker detection is very critical in disease prediction, diagnosis, and cancer treatment monitoring. The clinical utility of tumor marker to discriminate health and disease requires the capability to measure extremely low concentration protein biomarkers, which is also important to understand cellular processes and to search for new protein biomarkers (Mohammed and Desmulliez, 2014). Conventional techniques, such as enzyme linked immunosorbent assay (ELISA) (Sato et al., 2014), surface plasmon resonance (SPR) (Liang et al., 2012), and magnetic beads detection (Chang et al., 2006), have been widely used for detecting tumor markers. However, the detection limits of these methods have lagged behind the requirements for clinical utility and research. Additionally, these techniques require time-consuming target-labeling process, such as fluorescent dyes and quantum dots labeling (Petryayeva et al., 2013), or sophisticated optical detection system. These drawbacks limit the clinical application of tumor marker detection.

Graphene is an attractive material for label-free detecting tumor markers in clinical applications and research, which can overcome the hurdles of the previous detection methods. Graphene, a two-dimensional honeycomb crystal of $sp^2$-bonded carbon atoms (Novoselov et al., 2004), has drawn significant attention in both fundamental and applied research fields, owing to its extraordinary electrical, mechanical, and chemical properties. Graphene's extremely high carrier mobility (Du et al., 2008), thermal conductivity (Balandin et al., 2008), Young's modulus (Lee et al., 2008, 2012), breaking strength (Lee et al., 2013; Rasool et al., 2013), and extremely low noise (Robinson et al., 2008) make it an excellent candidate for nanoelectromechanical systems (NEMS) sensors and actuators (Bunch et al., 2007; Chen et al., 2009; Gomez-Navarro et al., 2008; Chen C. et al., 2013). Graphene based label-free biosensors hold the great potential to have superb performances with low cost (suitable for long term dynamic monitoring) (Zhang et al., 2012). They are able to detect many types of molecules and ions in very low concentration situations by

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monitoring the resistance shift caused by the adsorption of target molecules.

The performances of the sensors largely rely on the quality of graphene film. Several techniques have been developed for producing high-quality graphene films, including mechanical cleavage from graphite (Liang et al., 2007; Song et al., 2009), decomposition of SiC (Shivaraman et al., 2009), and chemical vapor deposition (CVD) growth on metallic surfaces such as copper (Li et al., 2009; van der Zande et al., 2010; Yu et al., 2011), nickel (Kim et al., 2009;), and platinum (Gao et al., 2012). CVD with copper substrate has the ability for the growth of large-area monolayer graphene with relatively low cost, attracting increasing attention recently. During growth, graphene grains initially nucleate from random locations. Then the growth of such grains proceeds, and these grains eventually form a polycrystalline film. Therefore, sensors based on large area graphene film reported so far are polycrystalline (Jin et al., 2014; Her et al., 2013). The grain boundaries degrade graphene material properties drastically, thus lead to deterioration on device's performance (Milaninia et al., 2009). The performances of polycrystalline graphene biosensors show large deviation from device to device (Yue et al., 2014), which reason has not been investigated so far. It has become the hurdle of their practical application. Additionally, the research has been focused on graphene sensors anchored on supporting substrates (Yue et al., 2014; Zheng et al., 2005; Xu et al., 2014). Carrier scattering from the substrate largely reduces the carrier mobility of the film, drastically affecting the performance of the sensor. Therefore, the influence of grain boundary and the scattering from substrate deteriorate the properties of graphene and the performance of biosensor based on it.

Here we report a label-free biosensor based on suspended single crystalline graphene, which can get rid of grain boundary and substrate scattering, revealing the sensing mechanism of intrinsic graphene. The SCGs were derived from LPCVD by controlling the growth duration. Multiplex detection of lung cancer biomarkers with easy operation was realized. The lung cancer sensors are ultra-sensitive due to free-standing structure, and they show improved uniformity because of the single-crystalline nature of the film.

## 2. Experiments

### 2.1. Materials

0.1% Poly-L-lysine (PLL) was received from Sigma-Aldrich Inc. without further treatments. 3% Bovine serum albumin (BSA) was purchased from Sigma-Aldrich Inc. Three types of lung cancer bio markers, ANXA2, ENO1, and VEGF (both antibody and antigen), were purchased from Sigma-Aldrich Inc. The copper foil (25 μm thick, 99.8%, Product no. 046365) was received from Alfa Aesar.

### 2.2. LPCVD SCG synthesis

The LPCVD graphene growth was carried out in a 2 inch quartz tube furnace on polycrystalline copper substrates with a mixture of research grade methane (as the carbon source) and hydrogen. Before growth, the as-received copper foil was pre-treated by 1:10 diluted nitric acid to remove the native copper oxide for a better growth of graphene, since the oxide could degrade the catalytic ability of copper. Copper foil was quickly loaded into CVD furnace and pumped down to base pressure ( $< 5$ mTorr). Then the furnace was heated up to 1050 °C under 10 SCCM hydrogen. After reaching 1050 °C, the sample was annealed for 30 min or longer, which can

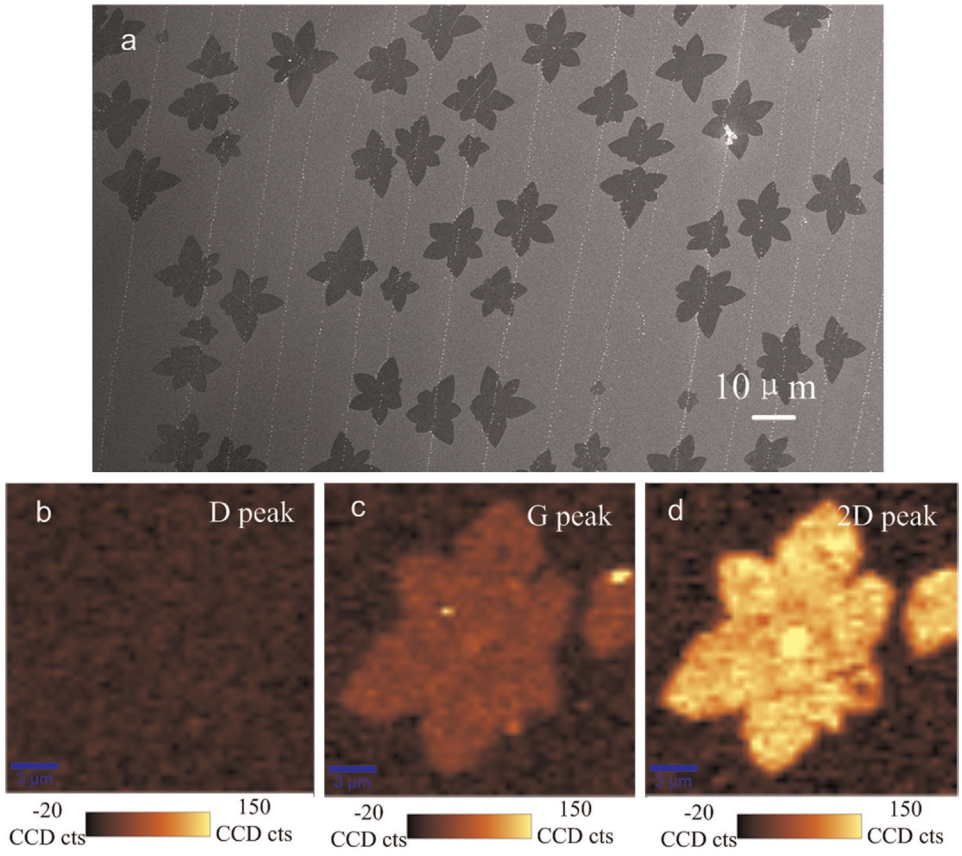


**Fig. 1.** CVD growth of SCG. (a) SEM image of LPCVD growth of SCGs on copper foil. (b) Raman D peak intensity image of a SCG which shows negligibly small D peak intensity within SCG grain, indicating low defect of the film. (c) Raman G peak intensity image of the same SCG. (d) Raman 2D peak intensity image of the same SCG.