ADVANCED MATERIALS

Supporting Information

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Label-Free Polypeptide-Based Enzyme Detection Using a Graphene-Nanoparticle Hybrid Sensor

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1. Synthesis of the polypeptide polymer

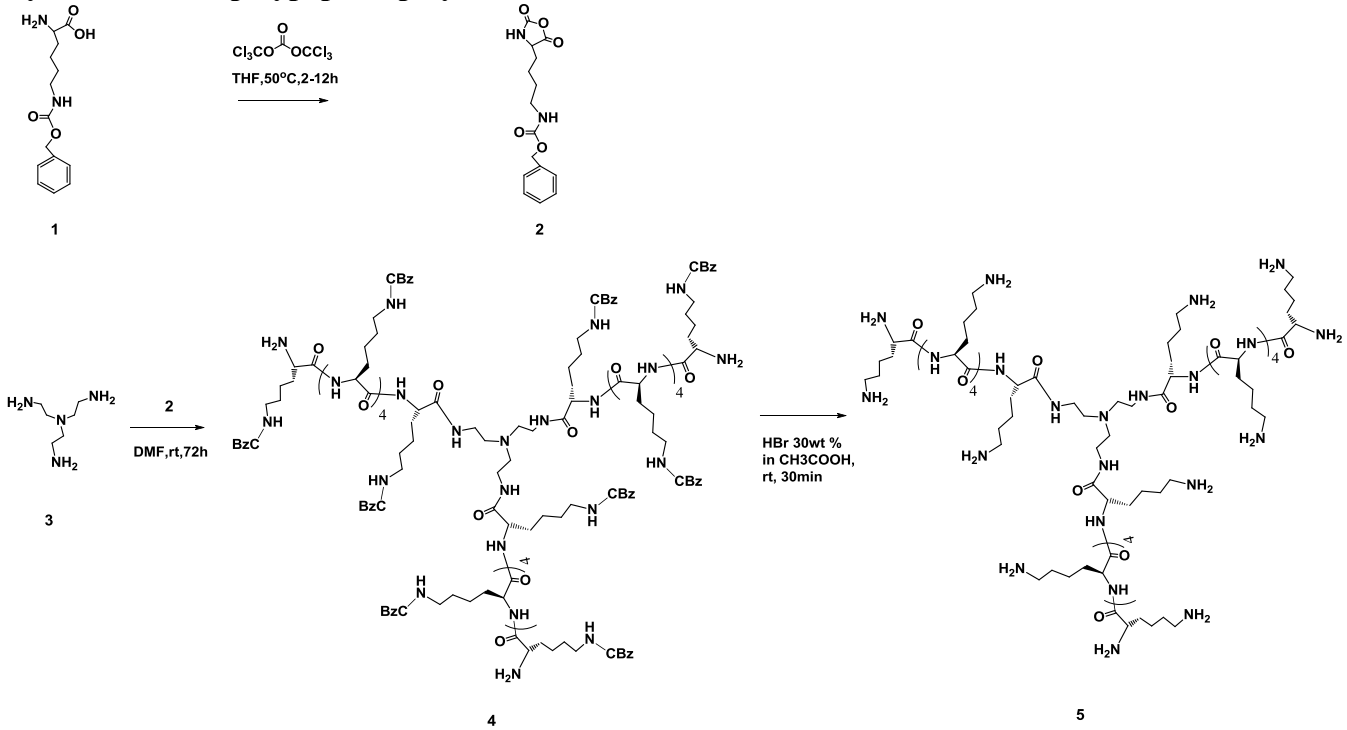

Figure S1. Synthetic Scheme of polypeptide linkers that is specific for our target enzyme, Carboxypeptidase B.

1.1 *Synthesis of the amino acid N-carboxy anhydrides (2, NCAs)*

To synthesize the amino acid N-carboxy anhydrides, *N*-CBZ-l-lysine was converted to the corresponding *N*-carboxy anhydrides (NCAs). Specifically, *N*-CBZ-l-lysine was suspended in anhydrous THF (10 wt. %), and the reaction temperature was increased to 55 °C. A calculated amount of triphosgene, dissolved in anhydrous THF, was added dropwise into the reaction mixture. Due to the addition of triphosgene, the suspended mixture became a clear solution as NCA was formed after a certain reaction time. To ensure ring formation, the reaction mixture was vigorously stirred for an additional 1 h. The reaction mixture was then condensed and poured into a 10-fold excess amount of anhydrous *n*-hexane to precipitate the amino acid NCAs. Finally, the amino acid NCAs were recovered by filtration and dried for 48 hours using a vacuum. Characterization of the amino acid NCAs was completed using ^{1}H NMR.

1.2 ***Synthesis of 3-armed polypeptides*** **(5)**

The lysine-based polymer was synthesized by ring opening polymerization via the amino acid NCA using amino acids and triphosgene (Van Dijk-Wolthuis et al., 1997; Daly and Poche, 1988). Calculated amounts of amino acid NCAs were placed in an 100 ml two-neck round bottom flask and dissolved in anhydrous DMF (10 wt.%) in a nitrogen atmosphere (Van Dijk- Wolthuis et al., 1997). Freshly distilled *n*-trisamine, previously diluted in anhydrous DMF, was added into the solution to initiate the ring-opening polymerization. The polymerization was continued for 72 h at room temperature to ensure consumption of all the NCA monomers. The resulting slightly viscous solution was then precipitated in 20-fold excess water. The precipitate was filtered and dried in vacuum overnight. Then, the protection groups of the polymer (CBZ groups in lysine side chains) were removed using HBr (Deming, 2000). Specifically, the polymer with protection groups was dissolved in 30% HBr/glacial acetic acid solution (20 ml/g). As the deprotection reaction progressed, carbon dioxide evolved out and the polymer rapidly precipitated from the solution. After 30 mins, a 10-fold excess amount of anhydrous diethyl ether was added to the reaction mixture. The precipitate was filtered and washed with diethyl ether. After drying, the deprotected polymer was dissolved again in deionized water, transferred to a pre-swollen dialysis membrane (MWCO= 500) and dialyzed against deionized water for 2 days. Polylysine was obtained by freeze drying for 3 days. Polylysine was characterized by ^{1}H NMR.

2. Deposition and Patterning of Graphene

The same method was used as was previously described in reference 2. In brief, graphene was grown on nickel layers using chemical vapor deposition. To transfer the graphene, an aqueous $FeCl_3$ solution was used as an oxidizing etchant. This solution slowly etched the nickel layers, separating the graphene film from the substrate. The resulting graphene film was then transferred to our device by simple contact with our SiO_2 substrate.

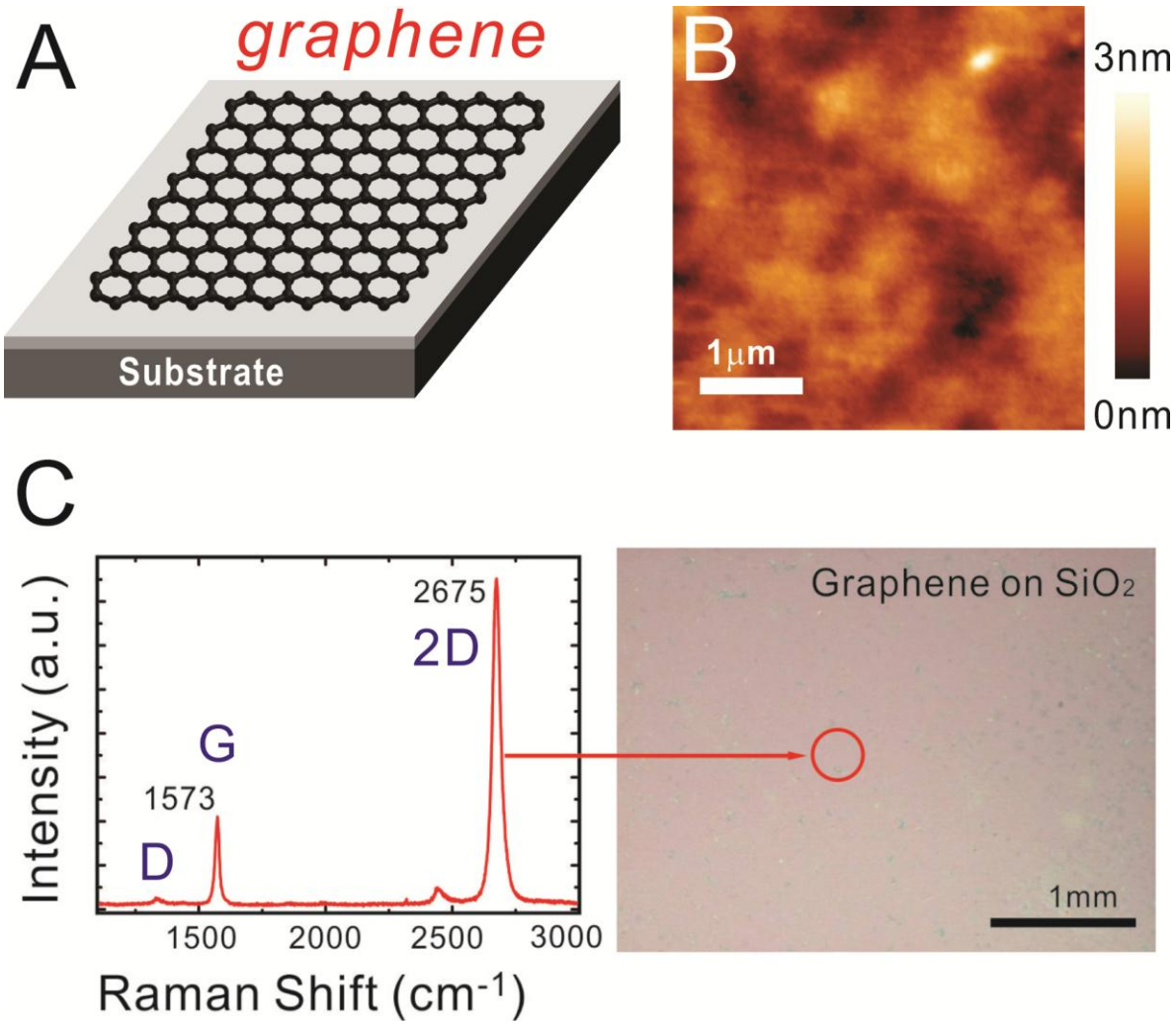

Figure S2. Characterization of graphene deposited (A) on the substrate. (B)AFM topographical image of the graphene surface deposited on the substrate. (C) Raman spectroscopy of a single graphene layer on a SiO_2 substrate (left) and an optical microscope image of a single graphene layer (right).