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Supporting Information

N-doped Porous Graphitic Carbon with Multi-flaky Shell Hollow Structure by Green and 'Useful' Template $CaCO_3$ for VOCs Fast Adsorption and Small Peptides Enrichment

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Experimental Section

Dopamine hydrochloride (> 98%), Bovine serum albumin (BSA) and ammonia aqueous solution (25~28 wt%) and trypsin (1:250) were purchased from Sigma-Aldrich Chemical Company. Acetonitrile (ACN) and trifluoroacetic acid (TFA) were obtained from Merck (Darmstadt, Germany). Ethanol (99.7%) was purchased from Kelong, China. Calcium chloride ($CaCl_2$, ≥ 96%), sodium carbonate (Na_2CO_3, ≥ 99.8%), polyvinylpyrrolidone (PVP-40) and sodium dodecyl sulfate (SDS, ≥ 98.5%) were purchased from Sinopharm, China. All the reagents were used without further purification.

Synthesis of HS-$CaCO_3$ particles

The hollow spherical $CaCO_3$ particles (HS-$CaCO_3$) with a diameter of 4.5 μm were prepared according to previously described in the literature[1]. First, 10.0 $g \cdot L^{-1}$ of PVP and SDS were dissolved in 0.1 M of Na_2CO_3 solution. Then, an equal volume of $CaCl_2$ solution (0.1 M) with SDS was completely rapidly added into the beaker at 20 °C under magnetic agitation,

and $CaCO_3$ crystals were generated and precipitated subsequently. After being stirred for 2 h, the deposit was centrifuged, and washed three times with homemade deionized water and dried at 80 °C overnight.

Synthesis of N-doped porous graphitic carbon with hollow structure (S1-NPGC and S2-NPGC)

A synthesis procedure was conducted to prepare S1-NPGC and S2-NPGC, as shown in Scheme 1. Firstly, 50 mL $CaCO_3$ suspension (8.0 $g \cdot L^{-1}$) was added dropwise in a stirred 100 mL aqueous solution of dopamine hydrochloride (DA, 5.0 $g \cdot L^{-1}$) for 30 min at room temperature. Then, the mixture solution with $CaCO_3$/DA was added into the ammonia (0.75 mL) / ethanol (40 mL) / water (90 mL) solution, and reacted for 20 h at room temperature (see Scheme 1, step I). Secondly, the $CaCO_3$@PDA black particles were washed with absolute ethanol and dried at 80 °C for 24 h. Thirdly, the dried $CaCO_3$@PDA samples were carbonized under N_2 atmosphere at 750 °C for about 1.0 h with a heating rate of 5.0 $°C \cdot min^{-1}$. The obtained grey black powders were named as CaO@S1-NPGC and S1-NPGC before and after being washed with deionized water till pH ~7. The S1-NPGC samples were filtered and dried under vacuum at 150 °C overnight (see Scheme 1, step II and III-1). While other part of CaO@S1-NPGC were further activated by $Ca(OH)_2$ formed by the introduction of H_2O vapors reacted with template remained CaO (S2-NPGC, Scheme 1III-2 and IV). Then, the $Ca(OH)_2$@S1-NPGC samples were continuously carbonized under N_2 atmosphere at 750 °C for about 1 h with a heating rate of 5.0 $°C \cdot min^{-1}$. The final product was washed by deionized water until pH ~7, which was denoted as S2-NPGC (see Scheme 1, step IV).

Materials Characterization

The morphology and structure of the HS-$CaCO_3$, $CaCO_3$@PDA, $Ca(OH)_2$@S1-NPGC, S1-NPGC and S2-NPGC samples were analysed by scanning electron microscopy (SEM, S-3400N, Hitachi), transmission electron microscopy (TEM, FEI TECNAI G2 F30, USA), powder X-ray diffraction (XRD, Rigaku, Smart Lab diffractometer using Cu *K*α radiation at 30 kV, Japan), respectively. The size distributions and Zeta potential of the HS-$CaCO_3$ and $CaCO_3$@PDA were measured from dynamic light scattering (DLS) using a Zetasizer (Nano ZS, Malvern). The element components of the surface of the synthesized samples were analysed by X-ray photoelectron spectroscopy (XPS) on a Kratos Axis Ultra spectrometer using a focused monochromatized Al Kα radiation ($h\upsilon$=1486.6 eV). The carbon structures of the S-NPGCs in powder form were studied using a Microscopes Raman Spectrometer (HORIBA Jobin Yvon, France) with a 532 nmlaser radiation source at room temperature. The thermogravimetric analysis (TG) was carried out on a NETZSCH F3 at a heating rating of 5 $K \cdot min^{-1}$ under N_2. Nitrogen sorption isotherms were measurements with an ASAP 2420 (Micro-meritics) at 77 K. Before these measurements, all samples were degassed overnight at 493 K in a vacuum line. The surface area, pore volume and the pore size distribution of samples were determined based on the Brunauer-Emmett-Teller (BET) method and Horvath-Kawazoe (HK) model.

Adsorption and Diffusion Measurements

Adsorption and diffusion of toluene on several porous materials were carried out gravimetrically in a Dynamic Vapor Sorption (3H-2000PW). In 3H-2000PW, an ultrasensitive microbalance of resolution 0.1 μg is mounted in the thermostated heatsink with

high precision temperature control. The sample weighed about 30-40 mg for each run. Before the measurements, the samples were degassed at 423 K for 8 h. The measurements were carried out at 298 K and 1.32 kPa. The transient adsorption uptakes of toluene on the samples were calculated as follows:

$$Q_t = \frac{W_t - W_a}{W_a} \quad \text{Eq. (S1)}$$

where W_t ($g \cdot g^{-1}$) are the weight of adsorbed adsorbents at time t(s), and W_a ($g \cdot g^{-1}$) is the initial weight of adsorbents; Q_t is the adsorbed amount per gram at time t(s).

Tryptic digestion of BSA

BSA was hydrolyzed as described in previous reports[2, 3]. 30 mg of BSA was dissolved in 10 mL of ultrapure water, and reduced by dithiothreitol (DTT) at 65 °C for 1.0 h. After that, the proteins were alkylated by iodoacetamide (IAA) at room temperature for 45 min in dark, followed by dilution with 100 mM $(NH_4)_2CO_3$ (pH 8.1-8.3). Then, trypsin was added with a weight ratio of trypsin/BSA of 1:50 and incubated at 37 °C overnight (16 h).

Adsorption and Enrichment of Peptides from BSA Digestion

S2-NPGC material was added into the BSA tryptic peptides (< 3 kDa, 2.84 $mg \cdot mL^{-1}$) solution with the ratio of peptides/S2-NPGC at 1:1 (W/W). The mixture was then incubated at 30 °C for only 1.0 h with gentle vibration at ~1000 rpm. Then, 0.1 mL emulsion was taken from the bulk solution and centrifuged at 20,000 rmp for 3 min. The amount of peptides adsorbed on the S2-NPGC was measured by UV spectrometry.

The adsorbed peptides were eluted from S2-NPGC by 120 μL of ACN/TFA/H_2O (80/0.1/19.9, V/V/V) three times and dried by using vacuum-freezing dryer, then re-dissolved in 200 μL of TFA/H_2O (0.1/99.9, V/V) for analysis by the Time of Flight Mass Spectrometry (TOF-MS).

Assay of inhibitory activity of ACE

Angiotensin converting enzyme (ACE) is known as one of important components to cause hypertension[4]. In this work, we used our synthesized S2-NPGC to enrich small peptides and test their inhibitory activities towards ACE. ACE activity is usually evaluated by determining the amount of hippuric acid (HA) generated. All assays for the ACE inhibitory activities of the BSA digested peptides before being adsorbed and after enriched from the S2-NPGC were conducted in triplicate, respectively. Here, ACE activity was measured using the improved method as described previously[5]: (1) ACE (40 μL) and substrate Hippuryl-L-histidyl-L-leucine (HHL, 10 μL) were separately dissolved in 0.1 M of BBS (pH 8.3, 200 μL); (2) 130 μL of borate buffer (BBS) and 30 μL of ACE solution were mixed and pre-incubated at 37 °C for 10 min; (3) 40 μL of HHL was added to start the reaction, and subsequently terminated by adding 150 μL of 1.0 M HCl after the incubation at 37 °C for 30 min; (4) 40 μL of HHL were added to start the reaction. The reaction was terminated by adding 50 μL of 1.0 M HCl after 30 min of incubation at 37 °C.

The HA content of the mixture was determined through RP-HPLC (Agilent 1260) with Zorbax SB C18 column (4.6×150 mm, 5 μm, Agilent, USA). The mobile phase was water-0.1% trifluoroacetic acid (TFA): methanol with a volume ratio of 85:15 at a flow rate of 1.0 mL/min, and the effluent was monitored at 228 nm with the diode array detector (DAD).

ACE in vitro inhibitory activity was measured as described as following equation:

$$\text{ACE inhibition rate (\%)} = (A_1 - A_2)/A_1 \times 100\% \quad \text{Eq. (S2)}$$

where A_1 is the peak area of HA in the control group, and A_2 is the peak area of HA with inhibitor. The IC_{50} value refers to the concentration of inhibitor producing 50% of inhibition of ACE.

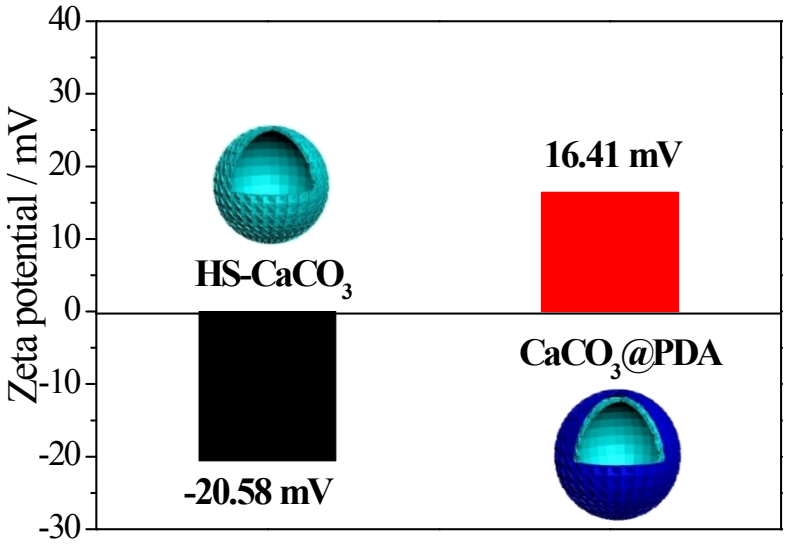

Figure S1. Zeta potentials of the $CaCO_3$ hollow spheres and the $CaCO_3$@PDA.

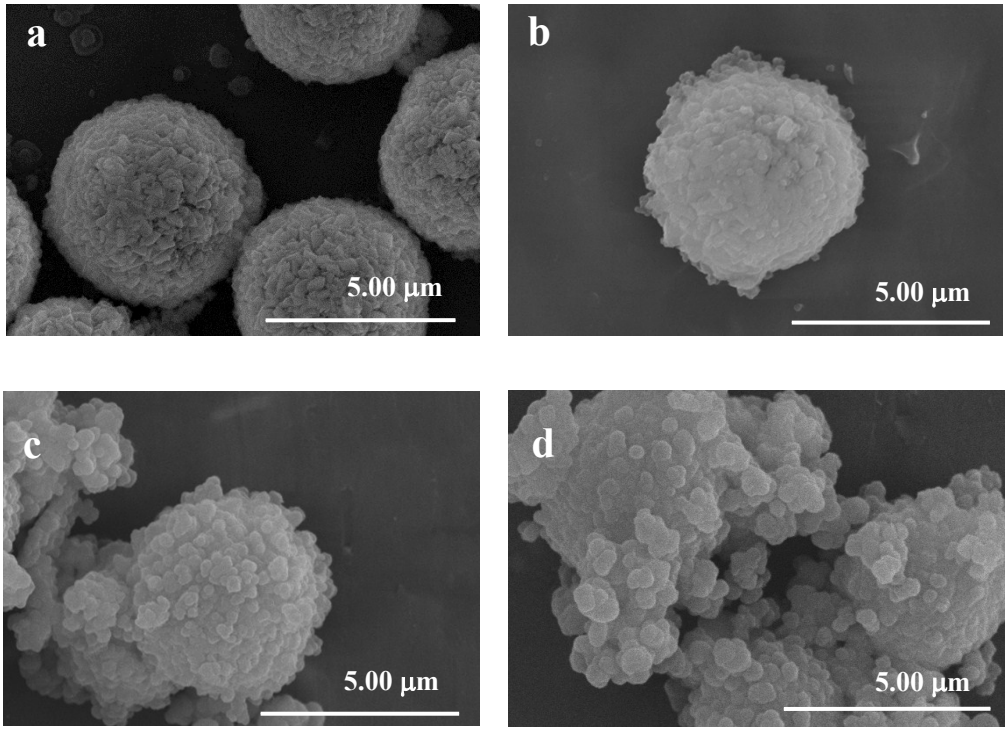

Figure S2. SEM images of $CaCO_3$@PDA obtained with different concentrations of dopamine hydrochloride solution: (a) 3 $g \cdot L^{-1}$, (b) 5 $g \cdot L^{-1}$, (c) 6 $g \cdot L^{-1}$, (d) 7 $g \cdot L^{-1}$.

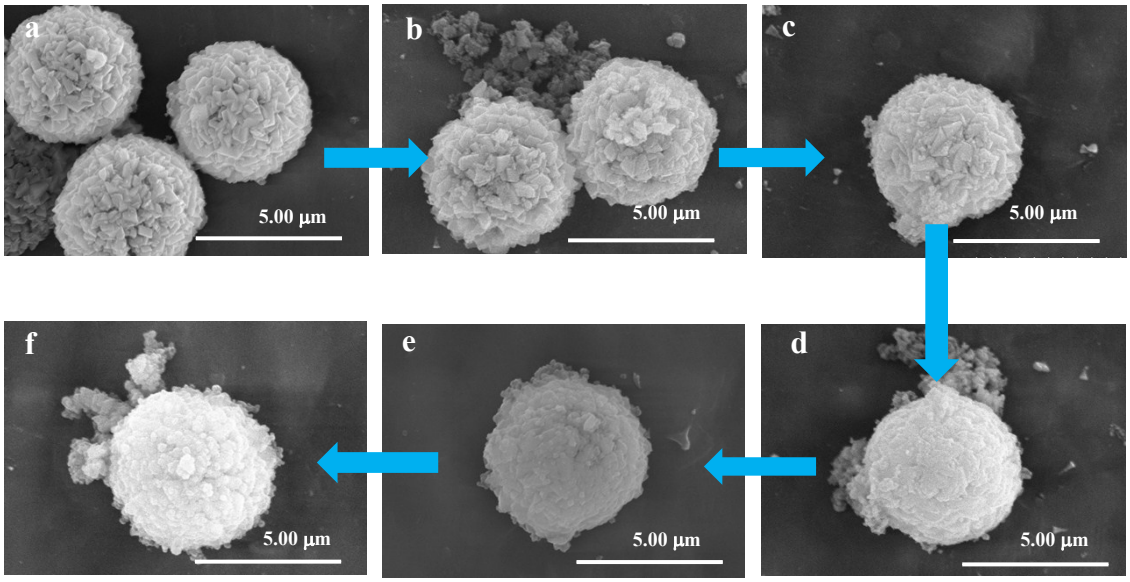

Figure S3. SEM images of $CaCO_3$ hollow spheres and $CaCO_3$@PDA synthesized at different reaction times under 5 $g \cdot L^{-1}$ of dopamine hydrochloride solution: (a) 0 h; (b) 2 h; (c) 6 h; (d) 10 h; (e) 20 h; (f) 30 h.

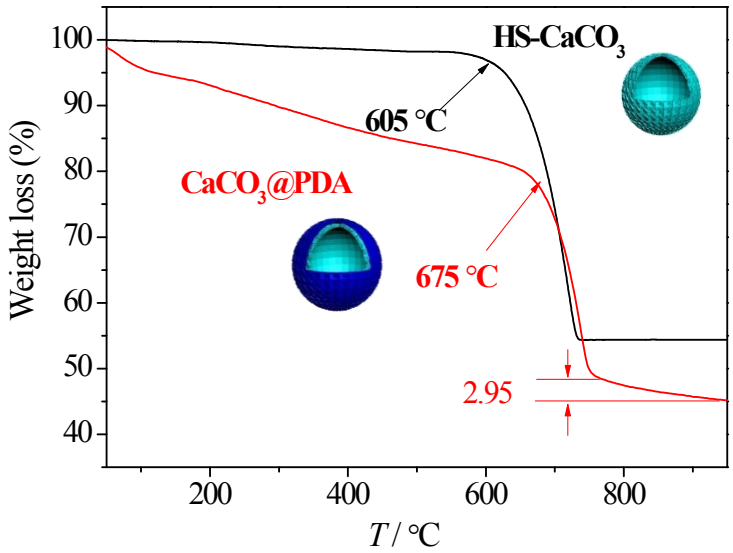

Figure S4. TGA curves of the HS-$CaCO_3$ and $CaCO_3$@PDA.

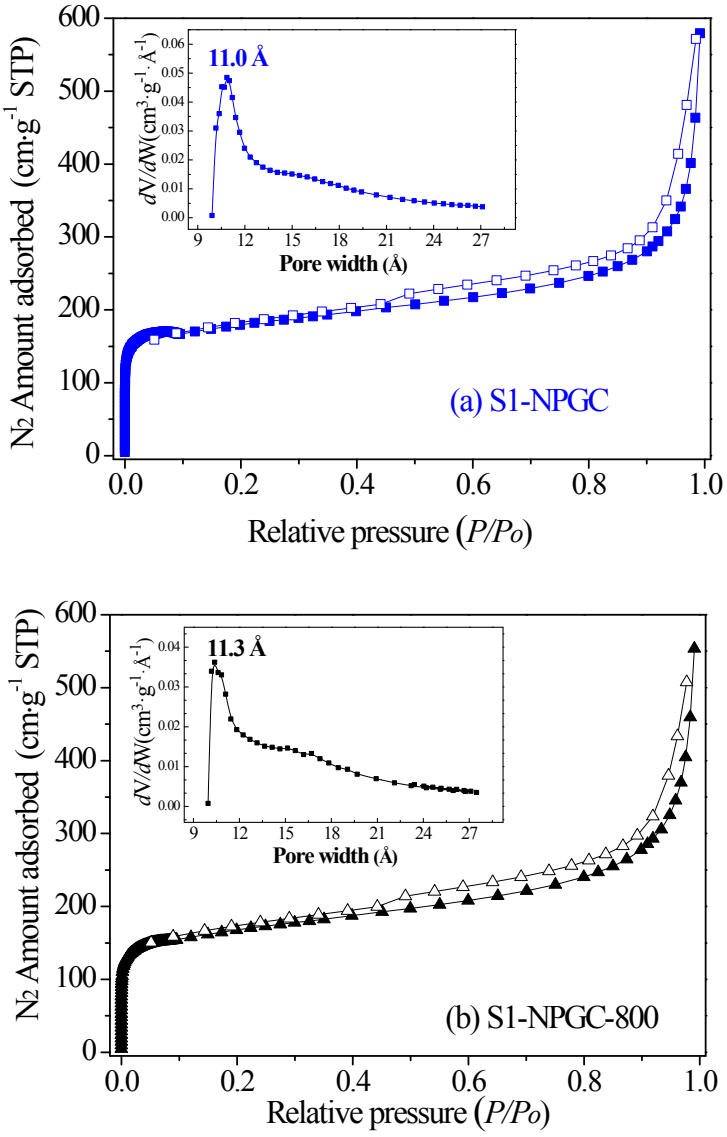

Figure S5. N_2 adsorption-desorption isotherms and pore size distribution (HK) of the S1-NPGC and S1-NPGC-800.

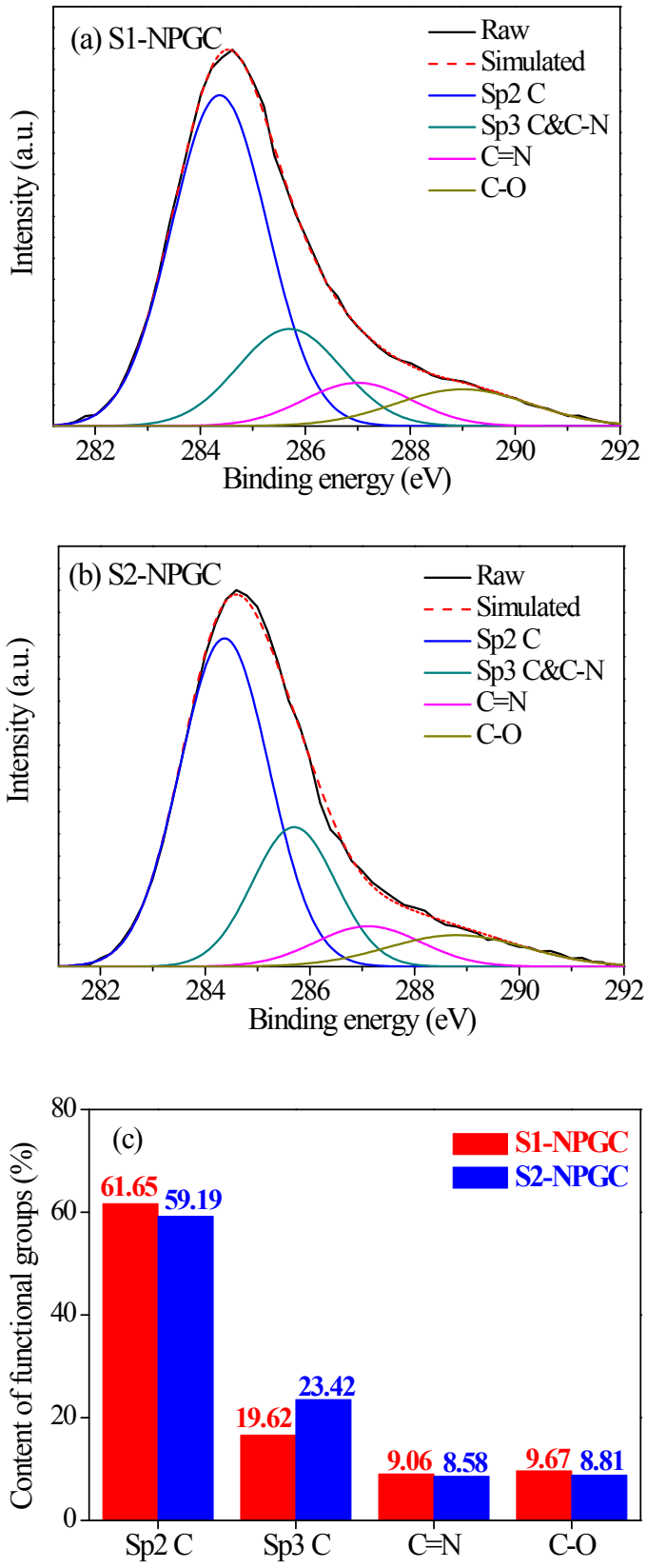

Figure S6. C 1s XPS spectra of (a) S1-NPGC and (b) S2-NPGC samples, and (c) their contents of functional groups.

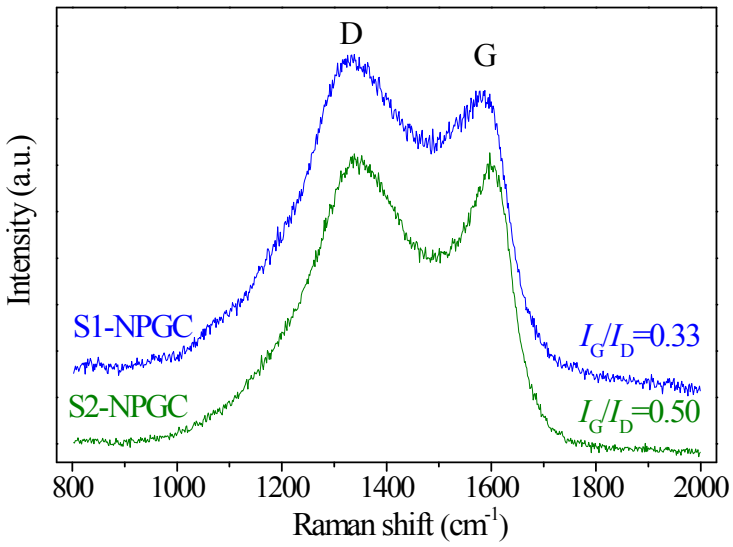

Figure S7. Raman spectrum of the S-NPGCs sample.

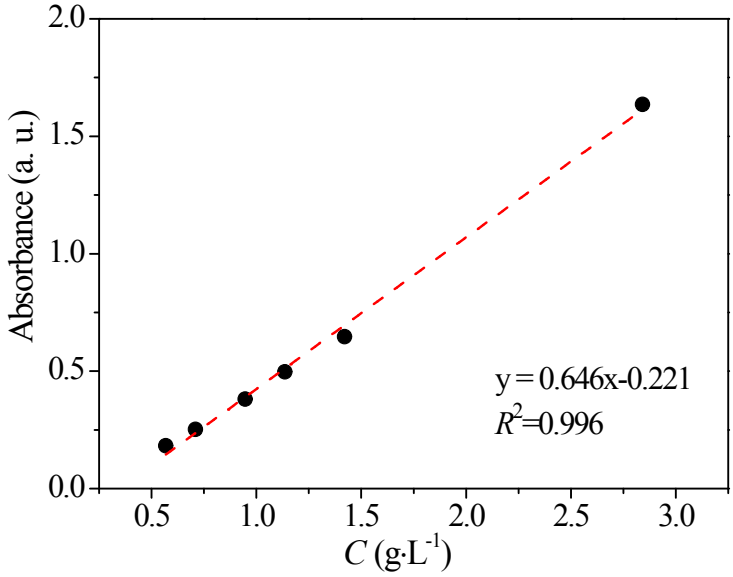

Figure S8. The standard curve of peptides adopted UV absorption spectrum.

Table S1. Pore structure parameters of S1-NPGC and S2-NPGC materials**.**

Sample	Langmuir ($m^2 \cdot g^{-1}$)	BET ($m^2 \cdot g^{-1}$)	V_t ($cm^3 \cdot g^{-1}$)	V_m ($cm^3 \cdot g^{-1}$)	S_m ($m^2 \cdot g^{-1}$)	S_m/S_t (%)	*Yield* (%)
S1-NPGC	955.6	719.9	0.836	0.218	560.5	77.86	50.48
S2-NPGC	938.7	736.1	0.642	0.261	634.5	86.20	32.18
S1-NPGC-800	804.1	638.9	0.829	0.186	457.3	71.57	41.21
S1-NPGC-900	509.4	361.3	0.513	0.057	112.0	31.00	36.64

Table S2. Comparison of experimental toluene diffusion data for the S2-NPGC with MSPDA, MILs and HKUST-1.

Sample	S2-NPGC	MSPDA	HKUST-1	MIL-101	MIL-Z1
Surface areas (BET, $m^2 \cdot g^{-1}$)	736.1	1836.6	1568.5[6]	3054[7]	2086[8]
Particle size (μm)	2.0	0.40	2.5	0.15	0.2
D_M/r_c^2 ($\times 10^{-3}$, s^{-1})	8.221	0.971	0.259	0.956	0.997
D_M ($\times 10^{-12}$, $cm^2 \cdot s^{-1}$)	331.2	1.553	16.192	0.215	0.399

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