# Supporting Information

## Ultrasensitive QRS made by supramolecular assembly of functionalized cyclodextrins and graphene for the detection of lung cancer VOC biomarkers

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### Materials

1-pyrenebutyric acid N-hydroxysuccinimide ester (ALDRICH, 95 %), 1-adamantanemethylamine (Aldrich, 98%) triphenylphosphine (ALDRICH, 99 %), sodium azide (ALDRICH, 99.5 %), 2-propynyl-tetra-O-acetyl-β-D-glucopyranoside (ALDRICH, 97 %), ascorbic acid sodium salt (FLUKA, 99%), sodium methoxide (Fluka, 97 %), cupric sulfate pentahydrate (FLUKA, 99%), iodine (ALFA AESAR, 99%), anhydrous pyridine (ALDRICH, 99.8 %), acetic anhydride (ALDRICH, 99%) were used as received without further purification. $CH_2Cl_2$ (VWR, 100 %) was dried over $CaH_2$ and distilled just before use. The deuterated solvents ($D_2O$, DMSO-$d_6$) were purchased from EURISOTOP. The β-CD (ROQUETTE, 98 %) was purified before use. A saturated solution of β-CD was prepared at 80 °C, then slowly cooled to room temperature and left at 0 °C for one night. The crystallized β-CD was then filtered and dried under vacuum at 80 °C over one night. All solvents ethanol, methanol, DMF, formaldehyde, acetone, toluene, benzene, propanol, isopropanol, o-xylene, from Acros Organics (Belgium) were used as received.

### Synthesis of pyrene butyric acid adamantine methyl amide (PYAD)

0.77 g of 1-pyrenebutyric acid N-hydroxy succinimide ester (2 mmole) was dissolved in dried methylene chloride followed by the addition of 2 mmole of 1-adamantanemethylamine. The reaction mixture was stirred under nitrogen at room temperature for 91 h. After evaporation of

the solvent, the product was purified by silica flash chromatography. The used eluant was first $CH_2Cl_2$ then a mixture $CH_2Cl_2$/methanol (95/5). Pure pyrene butyric acid adamantanemethyl amide was obtained with a yield of 65%.

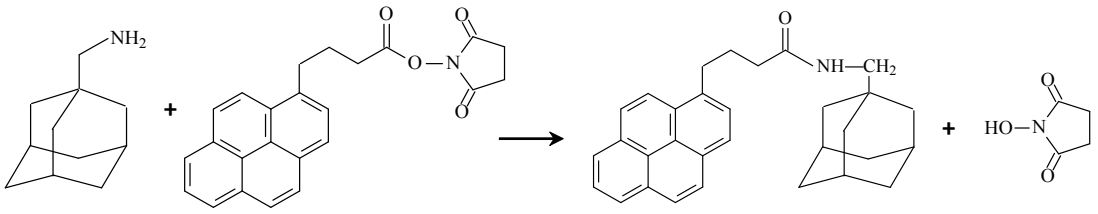
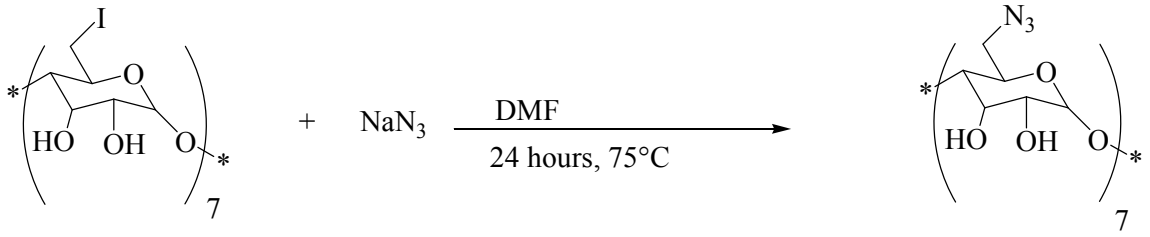



**Figure S5.** Synthesis of pyrene-adamantan.

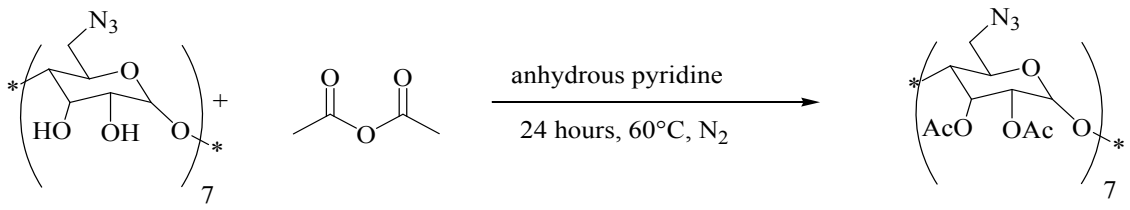
**Synthesis of RGO and PYAD linked RGO (RGO@PYAD)**

For preparation of PYAD linked RGO, 250 mg of PYAD was dispersed in ethanol and added to aqueous homogeneous dispersion of GO followed by stirring at room temperature for 6 hours. After addition of hydrazine hydrate, the in-situ reduction of GO was carried out at 100°C under reflux condition for 24 hours. The stable black dispersion was filtered with a nylon membrane (0.2 μm) to obtain PYAD linked RGO. Additionally, the preparation of pure graphene was similar except addition of PYAD.

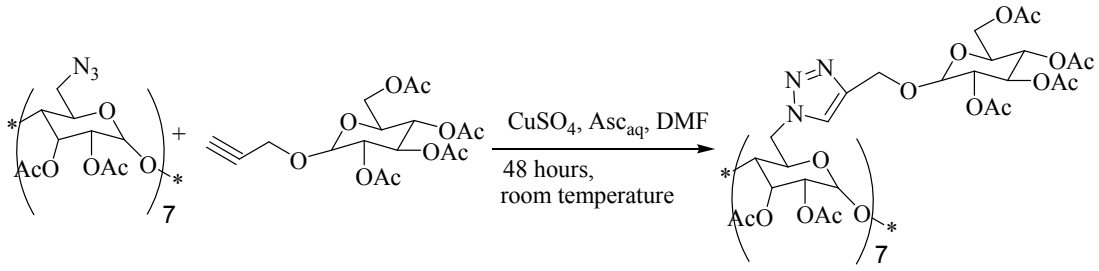
**Synthesis of heptakis(6-deoxy-6-(1,2,3-triazolyl)methylene-β-D-glucopyranoside)β-CD or mannose functionalized CD (MCD)**



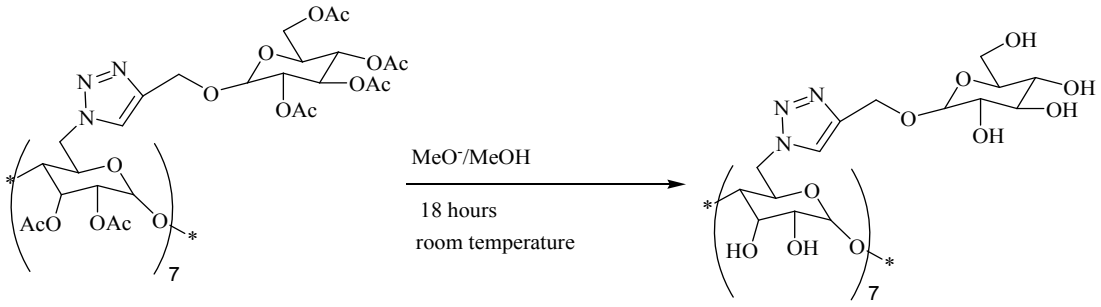

**Step 1** Synthesis of heptakis(6-deoxy-6-azido)β-CD




**Step 2** Synthesis of heptakis(6-deoxy-6-azido-2,3-di-O-acetyl)β-CD




**Step 3** Synthesis of heptakis(6-deoxy-6-(1,2,3-triazolyl)methylene-tetra-O-acetyl-β-D glucopyranoside -2,3-di-O-acetyl) β-CD




**Step 4** Synthesis of heptakis(6-deoxy-6-(1,2,3-triazolyl)methylene-β- glucopyranoside) β-CD.

**Figure S6.** Four steps synthesis of heptakis(6-deoxy-6-(1,2,3-triazolyl)methylene-β-glucopyranoside) β-CD (MCD)

A solution of β-cyclodextrin in anhydrous DMF (4.4 mmol in 90 $cm^3$), $I_2$ (92.5 mmol) and $Ph_3P$ (92.5 mmol) were added followed by stirring at 80 °C for 18 hours. A freshly prepared solution of NaOMe in MeOH (45 $cm^3$) was carefully introduced in the reaction vessel after the reaction mixture was cooled to room temperature and concentrated. The reaction mixture was precipitated

in methanol. The resulting brown solid was dried under high vacuum. 6.095 g of compound was recovered (yield of 72.6 %).

In the next step, $NaN_3$ (31.3 mmol,) was added to a solution of heptakis (6-deoxy-6-iodo) β-CD in DMF (3.2 mmol) and the resulting suspension was stirred at 75 °C for 24 h. The suspension was concentrated followed by treatment with a large excess of water. The precipitated compound was filtered, washed with water, and dried under high vacuum to yield a stable white powder (3.71 g, 88 %).

Then to a solution of heptakis(6-deoxy-6-azido)β-CD (2.7 mmol) in anhydrous pyridine (65.2 $cm^3$), acetic anhydride (215 mmol) was added. The mixture was stirred for 24 hours at 60°C under nitrogen. The solution was then concentrated and the residue was dissolved in dichloromethane and washed with 10 % HCl in water followed by water. The organic layer was dried over sodium sulfate and concentrated to yield white product (0.96 g, 66.8 %).

2-propynyl-tetra-O-acetyl-β-D-glucopyranoside (0.54 mmol) was added to a solution of heptakis(6-deoxy-6-azido-2,3-di-O-acetyl)β-CD (0.07 mmol) in DMF (2.4 mL). Cupric sulfate pentahydrate (0.54 mmol) and a freshly prepared solution of sodium ascorbate (1.08 mmol) in water (0.6 $cm^3$) were successively added to the mixture. The solution was stirred for 48hours at room temperature. After evaporation of solvents, the crude product was dissolved in $CH_2Cl_2$. The product was extracted three times with an ammonia solution (5N) and three times with water. The organic layer was retained, the solvent was removed and the product was dried under vacuum when the yield was 88.5% (290 mg).

Resulting,Heptakis(6-deoxy-6-(1,2,3-triazolyl)methylene-tetra-O-acetyl-β-Dglucopyranoside-2,3-di-O-acetyl) β-CD ($6.3.10^{-2}$ mmol) was added to the solution of NaOMe in MeOH (1M). The heterogeneous solution was stirred for 18 hours at room temperature. The mixture was filtered

and the precipitate was dried under vacuum. A white powder was recovered which was purified by silica chromatography with the mixture $H_2O$/ethanol as eluent (95/5 until 75/25). The expected product was collected with a yield of 40 %.

**Synthesis of heptakis(6-deoxy-6-amino)β-CD (NCD)**

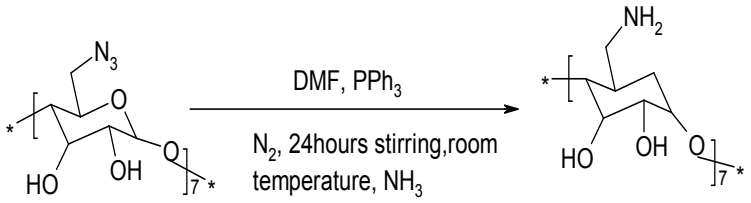



**Figure S7.** Synthesis of heptakis(6-deoxy-6-amino)β-CD(NCD).

The heptakis(6-deoxy-6-azido)β-CD (153 mmol) was dissolved in DMF (40 mL) and $PPh_3$ (24.2 mmol) was added. The solution was stirred under $N_2$ for 2h. Concentrated aqueous $NH_3$ (6 $cm^3$, ≈ 35 %) was then added dropwise to the solution followed by stirring. The suspension was then concentrated under reduced pressure to approximately 10 $cm^3$ and 150 $cm^3$ of ethanol was added in order to precipitate the product. The precipitate was washed with ethanol and dried under high vacuum to yield a white solid.

**Synthesis of perbenzylated β–cyclodextrin (PBCD)**

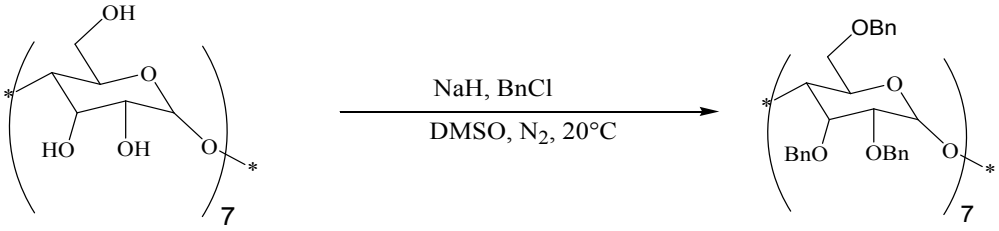



**Figure S8.** Synthesis of perbenzylated cyclodextrin.

7.1 g of β–cyclodextrin were added at room temperature under nitrogen to a solution of NaH (259 mmol) and benzyl chloride (259 mmol) was carefully added drop wise to the solution. The mixture was stirred vigorously at room temperature in anhydrous DMSO for 24 h. The reaction mixture was then neutralized with water followed by the extraction of the aqueous layer with $Et_2O$. The combined organic layers were dried, filtered and concentrated. Silica gel chromatography of the residue gave 17.9 g of perbenzylated β–cyclodextrin (yield: 94 %).

**Characterization Techniques**

The NMR spectra were recorded on a BRUKER AV300 spectrometer, operating at 300 MHz. Electrospray-ionization mass spectra (ESIMS) was recorded with an API 2000 spectrometer. The thermo-gravimetric analysis was done using METTLER TOLEDO Star instrument. Samples were heated from 25 ˚C to 700 ˚C with 5 ˚C /min heating rate in nitrogen atmosphere. UV-visible spectroscopy was done using CARY WinUV BIO-50 within the 250-750 nm range.

**Characterization of PYAD**

$^1H$ NMR ($CDCl_3$) shows the characteristic peaks as below:

ppm, δ: 8.33-7.86 (9H, Py), 5.39 (broad peak, 1H, NH), 3.43, 3.41, 3.38 (t, 2H, $H_a$), 2.97,2.95 (d, 2H, H1), 2.34-2.16 (m, 4H, Hc, Hb), 1.95 (broad peak, 3H, H5), 1.71, 1.67, 1.61,1.58 (q, 6H, H3), 1.46, 1.45 (d, 6H, H2)

$^{13}C$ NMR ($CDCl_3$) shows the following peaks:

ppm, δ: 172.83 (C=O), 136.08-123.57 (Py), 51.10 (C1), 40.40 (C2), 37.07 (C3), 36.42 (Cc), 33.76 (C4), 32.99 (Ca), 28.35 (C5), 27.71 (Cb)

For $C_{31}H_{33}NO$, ESIMS: 436.40 uma $[M+H]^+$, 458.40 uma $[M+Na]^+$, 474.40 uma $[M+K]^+$

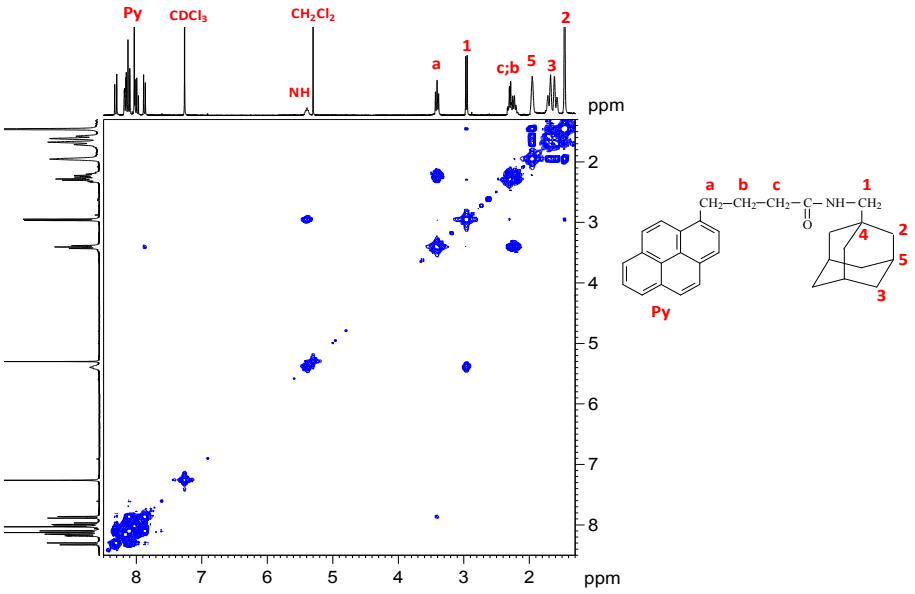



**Figure S9.** COSY NMR spectrum of pyrene butyric acid adamantanemethyl amide in $CDCl_3$.

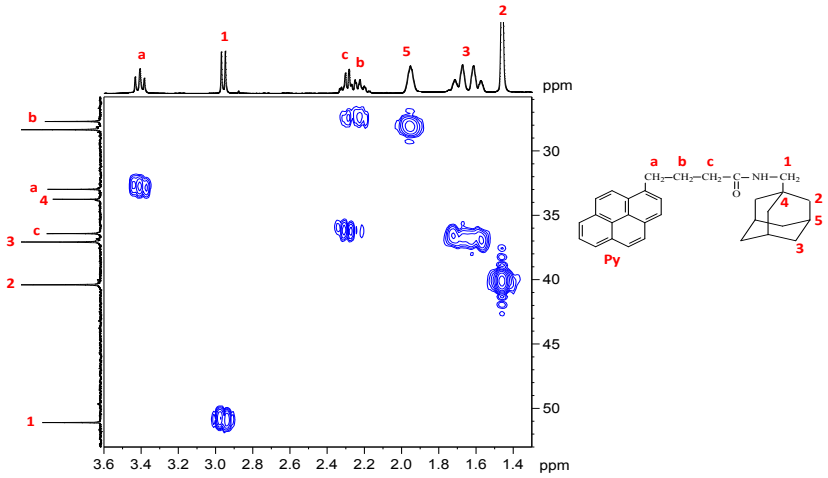



**Figure S10.** HMQC NMR spectrum of pyrene butyric acid adamantanemethyl amide in $CDCl_3$.

**Figure S11.** ESIMS of pyrene butyric acid adamantanemethyl amide.

**Characterization of the heptakis(6-deoxy-6-(1,2,3-triazolyl)methylene-β-D-glucopyranoside)β-CD (MCD)**

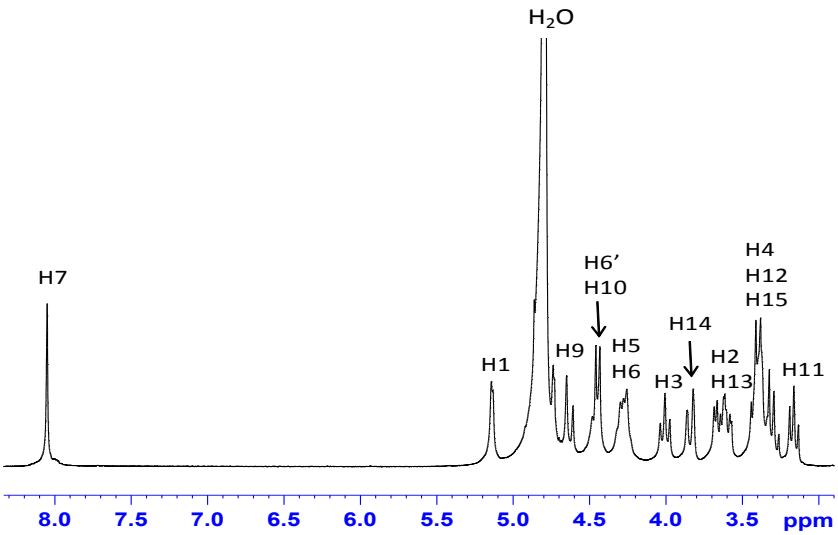



**Figure S12.** $^{1}H$ NMR spectrum of heptakis(6-deoxy-6-(1,2,3olyl)methylene-β-D-glucopyranoside)β-CD in $D_2O$ at 25 °C.

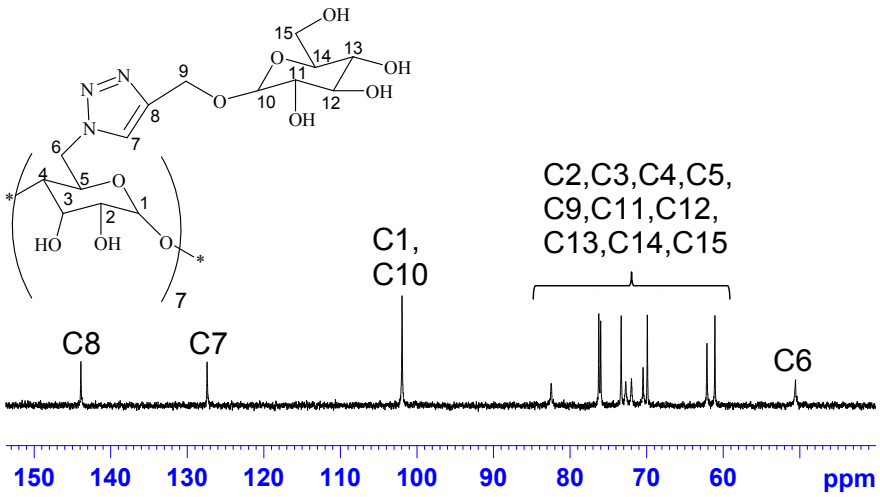



**Figure S13.** $^{13}C$ NMR spectrum of heptakis(6-deoxy-6-(1,2,3-triazolyl)methylene-β-D glucopyranoside)β-CD in $D_2O$ at 25 °C.

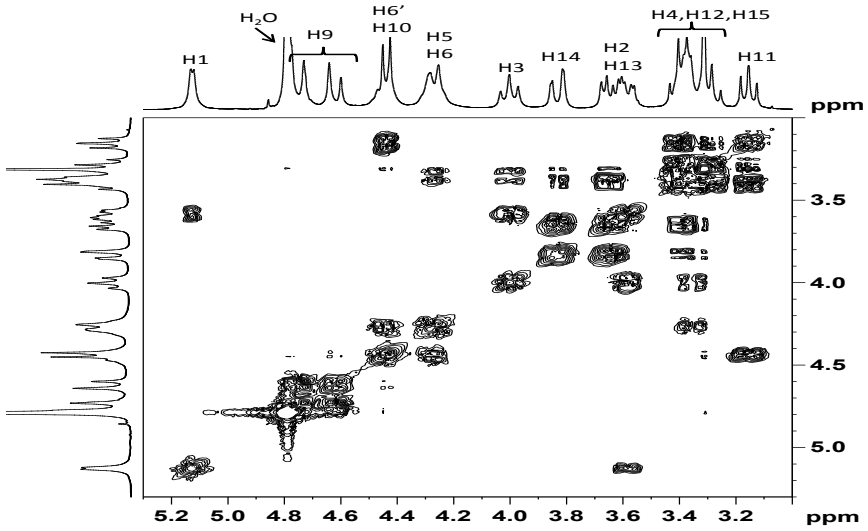



**Figure S14.** COSY NMR spectrum of heptakis (6-deoxy-6-(1,2,3-triazolyl)methylene-β-D-glucopyranoside)β-CD in $D_2O$ at 25 °C.

$^1H$ NMR ($D_2O$) ppm, δ: 8.04 (s, 7H, H7) 5.12 (d, 7H, H1), 4.68 (dd, 14H, H9), 4.44 (d,14H, H6, H10), 4.27 (d, 14H, H5, H6), 4.00 (t, 7H, H3), 3.84 (d, 7H, H14), 3.74-3.52 (m, 14H, H2, H13), 3.48-3.23 (m, 28H, H4,H12, H15), 3.15 (t, 7H, H11)

$^{13}$C NMR ($D_2O$) ppm, δ: 143.88 (7C, C8), 127.39 (7C, C7), 101.93 (14C, C1, C10), 83.27-60.52 (70C, C2 to C5, C9, C11 to C15), 50.55 (7C, C6).

For $C_{105}H_{161}N_{21}O_{70}$, ESIMS: 968.5 uma $[M+3Na]^{3+}$, 1440.6 uma $[M+2Na]^{2+}$

**Characterization of the heptakis(6-deoxy-6-amino)β-CD (NCD)**

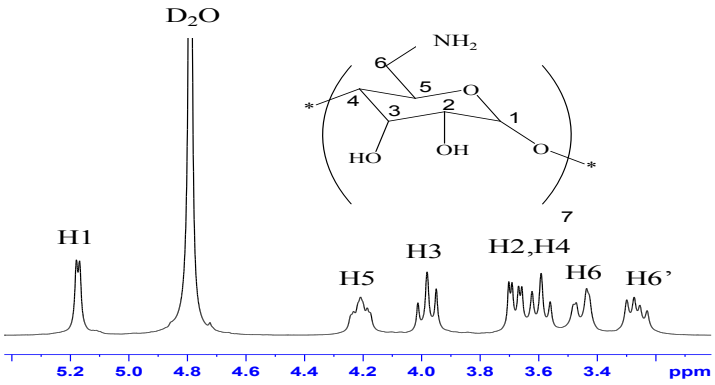



**Figure S15.** $^{1}$H NMR spectrum of heptakis(6-deoxy-6-amino)β-CD (NCD) in $D_2O$ at 25 °C.

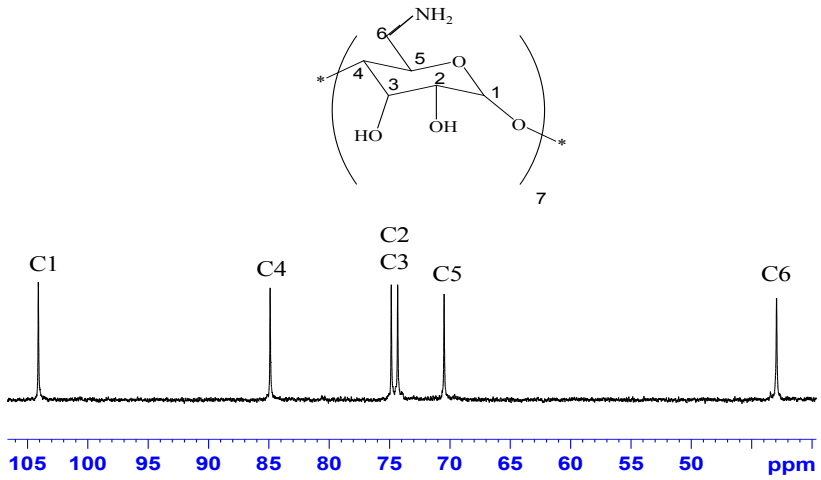



**Figure S16.** $^{13}$C NMR spectrum of heptakis(6-deoxy-6-amino)β-CD (NCD) in $D_2O$ at 25°C.

$^{1}$H NMR ($D_2O$) ppm, δ: 5.17 (d, 7H, H1), 4.21 (m, 7H, H5), 3.98 (dd, 7H, H3), 3.68 (dd, 7H, H2), 3.59 (t, 7H, H4), 3.46 (dd, 7H, H6), 3.26 (dd, 7H, H6') $^{13}$C NMR ($D_2O$) ppm, δ: 104.1 (7C, C1), 84.9 (7C, C4), 74.9, 74.3 (14C, C2, C3), 70.5 (7C, C5), 42.9 (7C, C6).

**Characterization of the perbenzylated β–cyclodextrin (PBCD)**

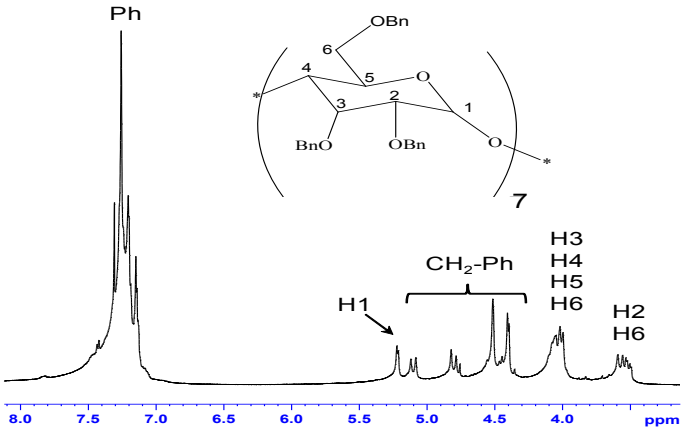



**Figure S17.** $^{1}H$ NMR of perbenzylated β–cyclodextrin in $CDCl_3$.

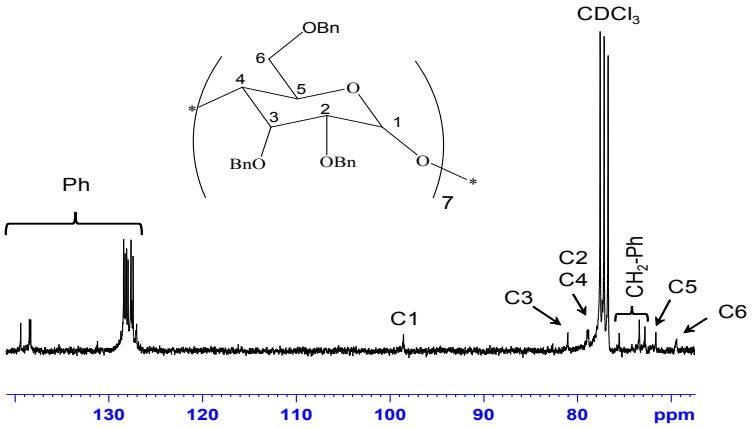



**Figure S18.** $^{13}C$ NMR of perbenzylated β–cyclodextrin in $CDCl_3$.

$^{1}H$ NMR ($CDCl_3$) ppm, δ: 8.40-6.84 (m, 105H, aromatic H), 5.22 (d, 7H, H1), 5.18-4.34 (m, 42H, $CH_2$-Ph), 4.24-3.92 (m, 28H, H3, H4, H5, H6), 3.69-3.45 (m, 14H, H2, H6)

$^{13}C$ NMR ($CDCl_3$) ppm, δ: 139.41, 138.48, 138.34 (3 × C aromatic quaternary), 130.0-126.2 (CH aromatic), 98.57 (C1), 81.04 (C3), 78.95 (C2), 78.82 (C4), 75.55, 73.41, 72.81 (3 × $CH_2$-Ph), 71.64 (C5), 69.44 (C6).

**Thermo gravimetric Analysis of modified graphene**

The comparison of thermo gravimetric analysis curves Figure 19**s** for RGO, RGO@PYAD and pyrene adamantan (PYAD) in Fig. S19 (a) confirms that about 60 weight % pyrene adamantan has been grafted onto RGO in RGO@PYAD. Hence the ratio of PYAD to RGO in RGO@PYAD is 1.5/1. Thermo Gravimetric Analysis (TGA) was done as a proof of successful non-covalent modification of RGO with perbenzylated β-cyclodextrin (PBCD) as shown in Figure S19 (b). Experiment proves that about 45% PBCD has been wrapped onto RGO in RGO@PBCD. In RGO@PBCD, the ratio of PBCD to RGO is 1/1.22.

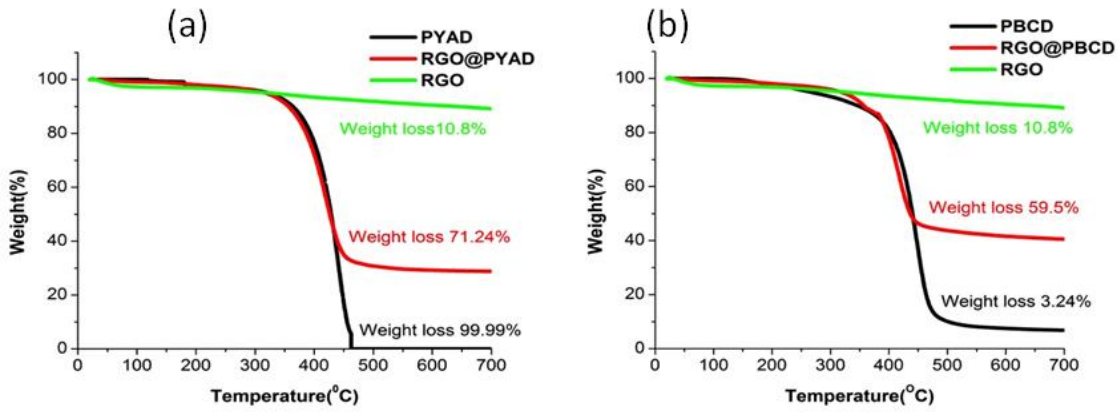



**Figure. S19.** a) TGA curves of RGO, RGO@PYAD and PYAD and b) Thermogravimetric analysis of RGO, RGO@PBCD and PBCD.

**UV-Visible Spectroscopy**

The determination of the amount of PYAD linked to RGO was also confirmed by UV-Vis spectroscopy as shown in Figure S20. A solution of PYAD in ethanol was made in 4 different concentrations as shown in Figure S20 (inset) and they were subjected to UV-visible spectroscopy. The absorbance was plotted as a function of concentration at constant wavelength to get the calibration curve. By using the calibration curve after doing UV-Vis spectroscopy for

the filtrate obtained after reaction of graphene and PYAD, it was found that the concentration of unreacted PYAD in filtrate was 0.43 mg.mL$^{-1}$ whereas the initial concentration of PYAD before reaction was 0.67 mg.cm$^{-3}$. Therefore the resulting amount of reacted PYAD was 0.24 mg.cm$^{-3}$, i.e. 54 mg. The total RGO@PYAD product obtained was 100 mg. So, the weight ratio of PYAD to graphene was found to be 1.1. Therefore the TGA and UV-Visible spectroscopy results are comparable and hence acceptable.

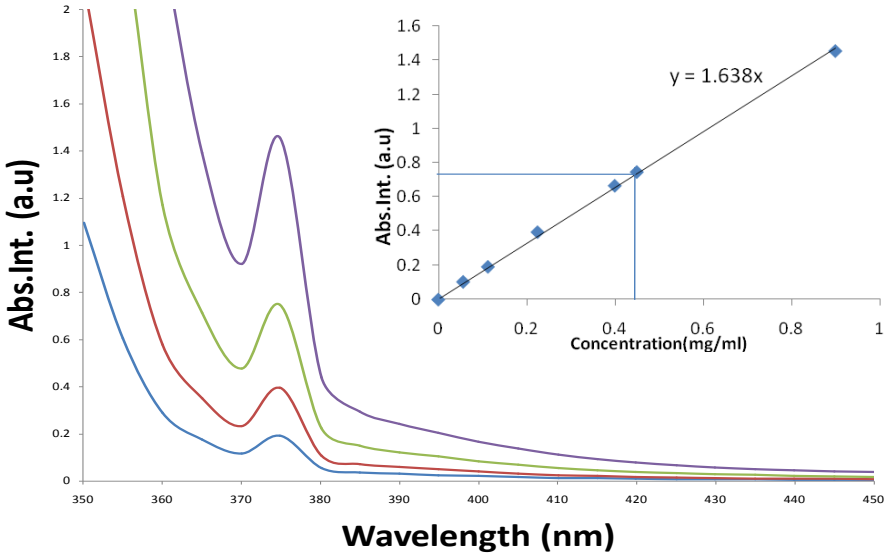



**Figure S20.** UV-vis spectra of PYAD in ethanol concentrations of 0.9 mg/mL (violet curve), 0.45 mg.cm$^{-3}$ (green curve), 0.225 mg.cm$^{-3}$ (red curve), 0.1156 mg.cm$^{-3}$ (blue curve) and Absorbance vs concentration at constant wavelength 286 nm curve following LAMBERT BEER's law (inset).

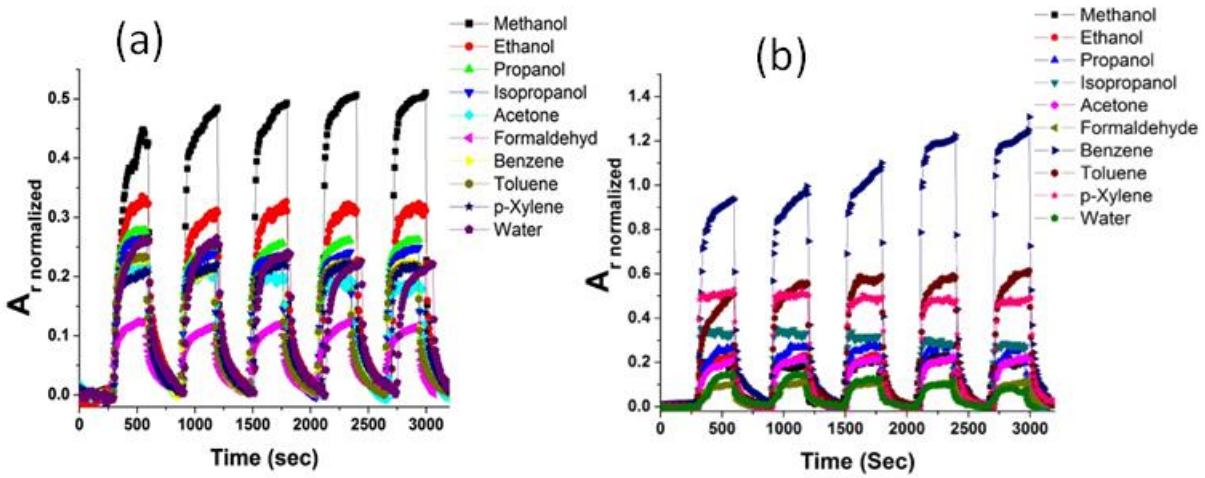



**Figure S21.** Chemo-electrical response of (a) RGO@PYAD-MCD and (b) RGO@PBCD sensor in a set of 10 VOC.

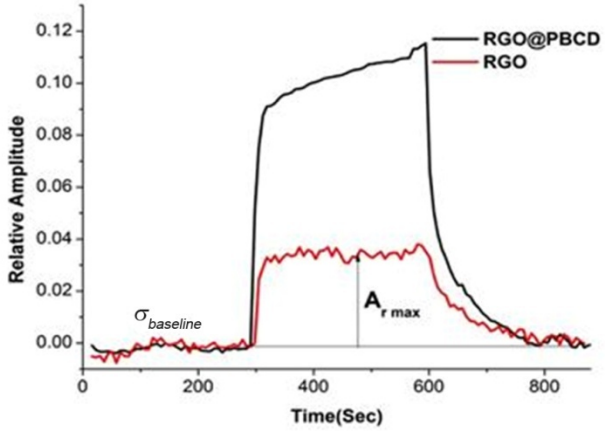



**Figure S22.** Signal to noise ratio of RGO and RGO@PBCD sensors at 400 ppb of benzene.

| VOC | $V_m$ ($cm^3.mol^{-1}$) | $\delta_{T\,sol}$ ($J^{½}.cm^{-3/2}$) | $(\chi_{12})_{CD}$ | $(\chi_{12})_{MCD}$ | $(\chi_{12})_{PBCD}$ | $(\chi_{12})_{NCD}$ |
|---|---|---|---|---|---|---|
| Methanol | 40.7 | 29.6 | 0.06 | 0.02 | 1.438 | 0.227 |
| Ethanol | 58.5 | 26.6 | 0.02 | 0.06 | 0.95 | 0.011 |
| Propanol | 74.84 | 23.8 | 0.44 | 0.62 | 0.37 | 0.13 |
| Isopropanol | 76.4 | 23.57 | 0.5 | 0.69 | 0.335 | 0.169 |
| Benzene | 89.7 | 18.5 | 3.44 | 3.51 | 0.117 | 2 |
| Toluene | 106.3 | 18.16 | 3.86 | 4.45 | 0.196 | 2.59 |
| p-Xylene | 122.8 | 18.2 | 4 | 5.1 | 0.218 | 2.968 |
| Water | 18.1 | 47.9 | 3 | 2.83 | 5.62 | 3.57 |
| Acetone | 74 | 19.7 | 1.88 | 2.23 | 0.01 | 1.16 |
| Formaldehyde | 36.8 | 24.6 | 1.195 | 0.205 | 0.278 | 0.025 |

**Table S2.** FLORY-HUGGINS interaction parameters of functionalized cyclodextrins.