Supporting Information for

Synthesis of Amphiphilic Reduced Graphene Oxide with Enhanced Charge Injection Capacity for Electrical Stimulation of Neural Cells

Qi Zhang[a,b], Jun Xu[c], Qin Song[c], Ning Li[c], Zhaolei Zhang[a], Kunyang Li[a], Yuyang Du[c], Liqiong Wu[c], Mingliang Tang[c], Liwei Liu[c], Guosheng Cheng*[c] and Jian Liu*[a]

[a]Jiangsu Key Laboratory for Carbon-Based Functional Materials and Devices, Institute of Functional Nano and Soft Materials (FUNSOM), Collaborative Innovation Center of Suzhou Nano Science and Technology,Soochow University, Suzhou, Jiangsu 215123, China. E-mail:jliu@suda.edu.cn

[b]State Key Laboratory of Molecular Engineering of Polymers, Fudan University, Shanghai 200433, China.

[c]Suzhou Institute of Nano-Tech and Nano-Bionics, Chinese Academy of Sciences, 398 Ruoshui Road, Suzhou Industrial Park, Jiangsu 215123, China. E-mail: gscheng2006@sinano.ac.cn

1. Activation of mPEG 5k

Materials Methoxy poly(ethylene glycol) 5,000 (mPEG 5k) and hexane-1,6-diisocyanate (HMDI, M_w 168.19 g/mol) were purchased from Sigma-Aldrich. All the reagents were used as received.

Preparation of A-mPEG 5k In a 50 mL flask equipped with a reflux condenser and a nitrogen gas bubbler, 5 g of mPEG 5k (1 mmol) was dissolved in 10 mL of $CHCl_3$, followed by addition of 20 mL (120 mmol, 120 times molar excess) of HMDI. The mixture was heated under reflux for 48 h. The HMDI-activated mPEG 5k (A-mPEG 5k) was precipitated in 200 mL of hexane. The precipitate was washed with a further 10 mL of hexane, redissolved in 20 mL of $CHCl_3$, and precipitated in 150 mL of hexane again. This reprecipitation and washing steps were repeated six times and dried at reduced pressure. 4.2 g of white solid was obtained with an 84.7% yield. The reaction routine was shown as Fig. S1.[1-3]

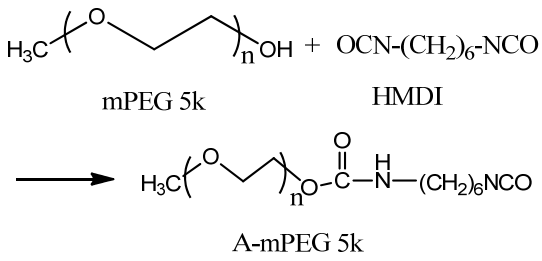

Fig. S1. Activation of mPEG 5k with HMDI

^{1}H NMR ($CDCl_3$) of A-mPEG 5k: δ = 1.32 (m, OCN(CH_2)$_3$CH_2-, 2H), 1.35 (m, OCN(CH_2)$_2$CH_2-, 2H), 1.48 (m, OCN(CH_2)$_4$CH_2-, 2H), 1.59 (m, OCNCH$_2$CH_2-, 2H), 3.14 (t, OCN(CH2)$_5$CH_2-, 2H), 3.28 (t, OCNCH_2-, 2H), 3.37 (s, -OCH_3, 3H), 3.65 (s, -OCH_2CH_2O-, 450H), 3.81 (m, -NHC(O)OCH$_2$CH_2O-, 2H), 4.19 (t, -NHC(O)OCH_2-, 2H) ppm.

2. Preparation of reduced graphene oxides

mPEG-rGO obtained at 60 °C The anhydrous graphene oxide (GO) foams (10 mg) were added into a 25-mL round-bottom flask equipped with a magnetic stir bar, followed by addition of 10 mL anhydrous DMF. The flask was then sonicated for 30 min under nitrogen. The A-mPEG 5k (0.5 g) was next loaded and the mixture was heated at 60 °C for 12-24 h. The product was centrifugated, washed with DMF and ethanol for six times. The as-prepared mPEG-rGO-60 °C can stably disperse in polar solvents such as water, *N,N*-dimethylformamide (DMF), ethanol, acetone and tetrahydrofuran (THF).

mPEG-rGO obtained at 80 °C The anhydrous GO foams (10 mg) were added into a 25-mL round-bottom flask, followed by addition of 10 mL anhydrous DMF. The flask was then sonicated for 30 min under nitrogen, followed by addition of A-mPEG 5k (0.5 g). The mixture was heated at 80 °C for 6-12 h. The product was centrifugated, washed with DMF and ethanol for six times. The as-prepared mPEG-rGO-80 °C can form stable dispersion in various solvents such as water, DMF, acetone and THF.

The control of mPEG-rGO The anhydrous GO foams (10 mg) were added into a 25-mL round-bottom flask, followed by addition of 10 mL anhydrous DMF. The flask was then sonicated for 30 min under nitrogen, followed by addition of mPEG 5k (0.5 g, Sigma-Aldrich). The mixture was heated at 160 °C for 1-5 h. The product was centrifugated, washed with DMF and ethanol for six times.

rGO_{N2H4} The homogeneous GO dispersion (1.2 mg/mL, 4 ml) was mixed with 10 ml of water, 10 μl of hydrazine solution (35 wt% in water, Aldrich) and 100 μl of ammonia solution (28 wt% in water) in a 25 mL round-bottom flask. The weight ratio of hydrazine to GO was around 1:1.4. After being sonicated for 30 minutes, the vial was put in a water

bath (~95 °C) for 1 h. The product was centrifugated and washed with water for six times.[4]

Graphene film transferring The rGO solutions were filtered to form films using a cellulose ester membrane with 0.25 μm pores. The as-prepared filtered membranes were wetted with ultrapure water and pressed against the clean solid substrate (e.g. Au-coated tissue culture polystyrene, TCPS). The filtered films were attached to the substrates under a 2 kg weight for 12 h. After that, the weight was removed and the rGO was adhered to the substrates. The substrates containing transferred films were rinsed with ethanol and dried by blowing with nitrogen.

3. Fourier transform infrared spectroscopy

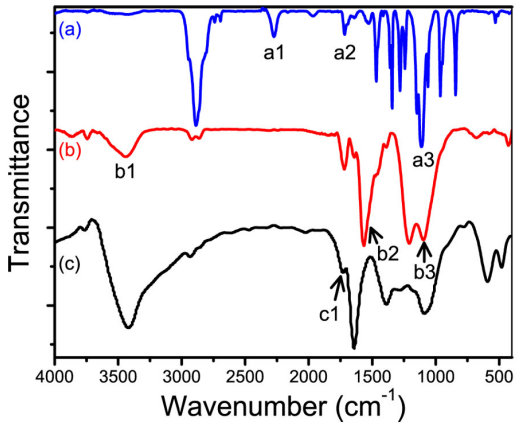

Fig. S2. Fourier transform infrared spectroscopy (FT-IR) spectra (from top to bottom) of A-mPEG 5k (a), mPEG-rGO (b), and GO (c). Characteristic peaks: (a1) O=C=N isocyanate stretching 2273 cm^{-1}, (a2) C=O carbamate stretching 1715 cm^{-1}, (a3) C–O ether stretching 1110 cm^{-1}; (b1) N–H amines stretching 3345 cm^{-1}, (b2) C=O carbamate or amide stretching 1564 cm^{-1}, (b3) C–O ether stretching 1096 cm^{-1}; (c1) C=O carbonyl or carboxyl stretching 1730 cm^{-1}.[1-3]

4. UV-Vis spectroscopy

The UV-Vis absorption spectra (Lambda 25, Perkin Elmer) of mPEG-rGO, mPEG 5k, HMDI and GO in ethanol were investigated to clarify the reduction of mPEG-rGO. As shown in Fig. S3, GO gave a typical absorption maximum at ~230 nm. After reduction, the absorbance of mPEG-rGO was tremendously higher than that of GO, mPEG 5k or HMDI, implying that the recovery of C–C bonding. According to the previous reported data[4], an absorption peak of rGO should appeared at ~ 270 nm . However, a new shoulder peak at 300 nm was observed. Compare with the spectrum of HMDI, this shoulder peak should be ascribed to the collaborative absorbent effect of rGO and amides/carbamate ester group originated from HMDI.

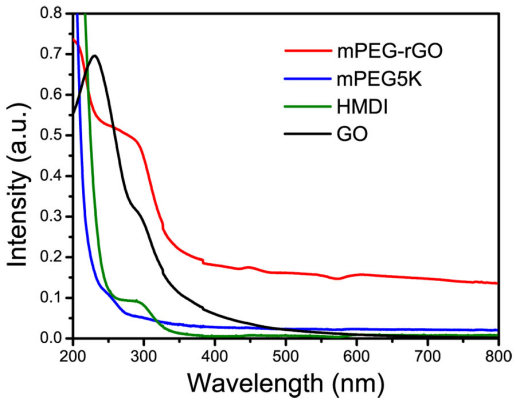

Fig. S3. UV-Vis spectra of GO, HMDI, mPEG 5k and mPEG-rGO at 160 °C in ethanol (~0.05 mg/mL). The HMDI was measured within 5 min after the addition of HMDI into ethanol solution.

5. X-ray photoelectron spectroscopy

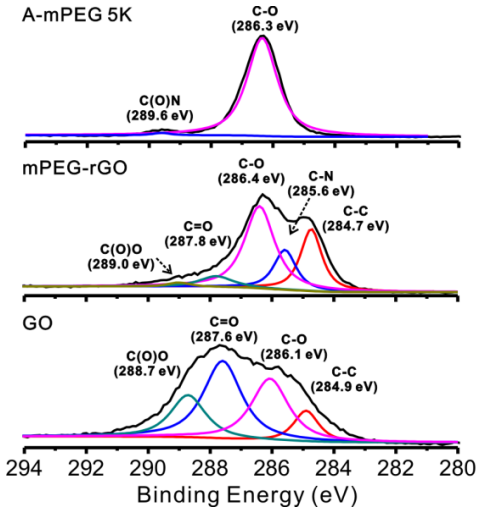

Fig. S4. High-resolution C (1s) XPS of A-mPEG 5k, mPEG-rGO and pure GO.

Detailed compositional analysis of mPEG-rGO was conducted and compared with GO and A-mPEG 5k by X-ray photoelectron spectroscopy (XPS, Axis Ultra DLD, Kratos, UK), which provided the information about the type and surface functionalization of the materials (Fig. S4). The XPS of GO showed four types of carbon bonds, i.e. C–C (284.9 eV) of sp^2 carbon in the plane of GO, the carbon in C–O (286.1 eV), the carbonyl carbon in C=O (287.6 eV) and the carboxylate carbon in C(O)=O (288.7 eV). After GO was reduced and functionalized to synthesize mPEG-rGO, the C–C peak at 284.9 eV was enhanced, while the peaks related to the oxidized carbon species become weak. Moreover, the peak at 285.6 eV assigned to C–N bonding. The strong peak at 288.4 eV, corresponded to C–O of mPEG, also appeared in the spectrum of mPEG-rGO. The appearance of C–N peak and the significant intensity enhancement in C–O peak for mPEG-rGO, as compared to GO and A-mPEG 5k, indicated the successful immobilization of mPEG chain onto rGO.

6. Raman spectroscopy

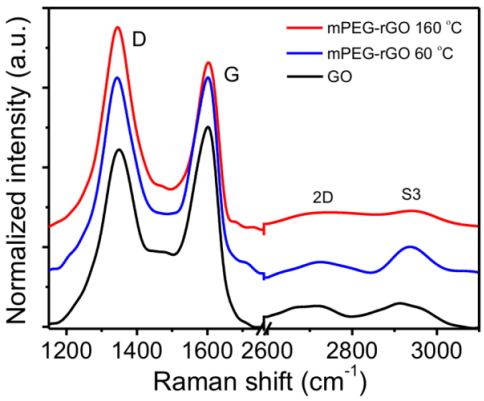

Fig. S5. Raman spectra of GO (black), mPEG-rGO obtained at 60 °C (bule) and mPEG-rGO obtained at 160 °C (red).

The structural change during reduction and functionalization was also investigated by Raman spectroscopy (lamRAM HR800, HORIBA, France). Fig. S5 presents the micro-Raman spectra of GO, mPEG-rGO obtained at 60 °C and 160 °C. Compared with GO and mPEG-rGO obtained at 60 °C, the I_D/I_G ratio of mPEG-rGO increased notably, indicating a decrease in the sp^2 cluster size perhaps caused by the defects, vacancies, or distortions of the sp^2 domains. Additionally, the relative intensity of the 2D (~2700 cm^{-1}) and S3 (~2930 cm cm^{-1}) peaks of mPEG-rGO obtained at 160 °C become obscure, showing the graphitization was distorted due to the bonding of mPEG chains.[5]

7. Thermogravimetric analysis

The reduction and functionalization of mPEG-rGO were further verified by thermogravimetric analysis (TGA, TG/DTA 6200, Seiko). In Fig. S6, GO had a major weight loss of ~40% in the temperature range from 100 to 500 ºC, which was mainly attributed to the loss of oxygen-containing functional groups such as COOH and OH groups. In contrast, A-mPEG 5k presented a totally different pyrolysis process. Near 97% of weight loss occurred at 250-450 °C. Almost no residue was observed in the pyrolysis

products. Compared with GO, the mPEG-rGO obtained at 80 °C started to decomposition at higher temperature, and the mPEG-rGO obtained at 160 °C exhibited a much higher decomposition temperature. The mPEG-rGO obtained at 80 °C loses ~70% weight in the temperature range of 250-450 °C, while mPEG-rGO obtained at 160 °C loses ~20% weight, which was higher than that of the control or rGO_{N2H4}. It's well known that the removal of oxygen-containing functional groups results in much increased thermal stability and reduction quality of carbon plane[6]. Hence, the change of weight loss was ascribed to the amount of mPEG chains on graphene surface. Since the functionalization and reduction were the competition reaction, a lower amount of mPEG was attached onto the mPEG-rGO at 160 °C for it contained lower amount of functional groups. Based on the TGA data and the yield of mPEG-rGO (~120%), it was estimated that mPEG-rGO comprises 20-50 wt% of mPEG chains.

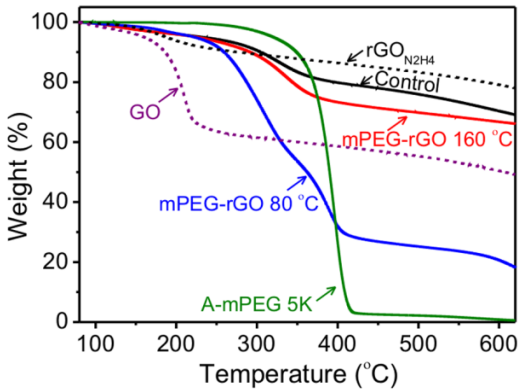

Fig. S6. Normalized weight of rGO_{N2H4}, the control, mPEG-rGO obtained at 160 °C & 80 °C, GO and A-mPEG 5K as a function of annealing temperature with a heating rate of 5 °C/min. The measurement was performed under a nitrogen flow (50 cm^3/min) on sample sizes from 2 to 3 mg, and the weight loss was detected in a Pt crucible from room temperature to 800 °C.

8. X-ray diffraction

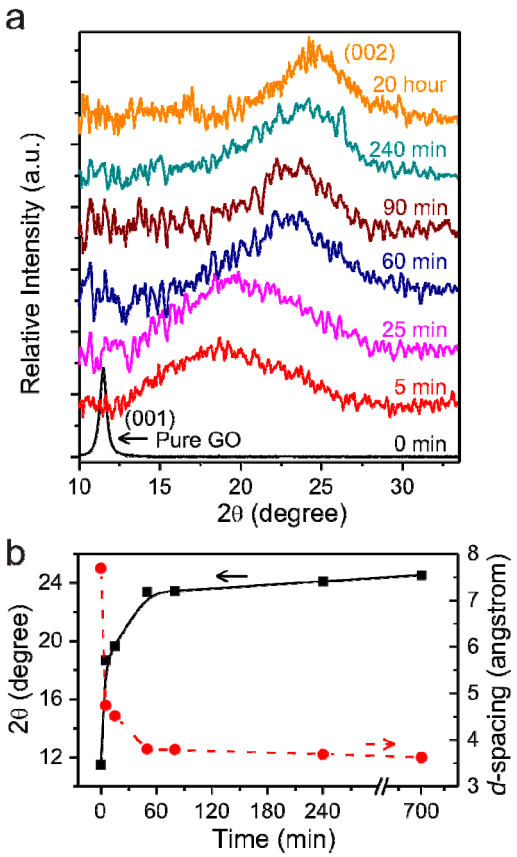

Fig. S7. (a) Time-dependent X-ray diffraction (XRD) patterns of mPEG-rGO obtained by reduction and functionalization at 160 °C for 5 min, 25 min, 60 min, 90 min, 240 min and 20 hour, respectively. (b) 2θ angles and *d*-spacing of mPEG-rGO (corresponding to panel a) as a function of reaction time.

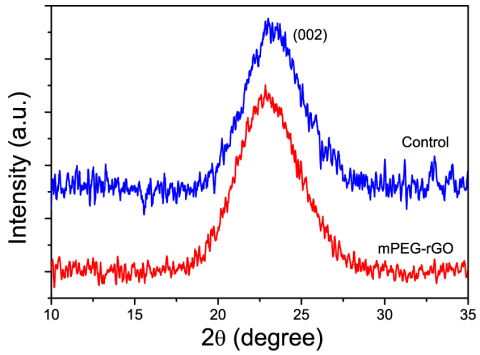

Fig. S8. XRD patterns of mPEG-rGO at 160 °C and control

9. Cell viability assay

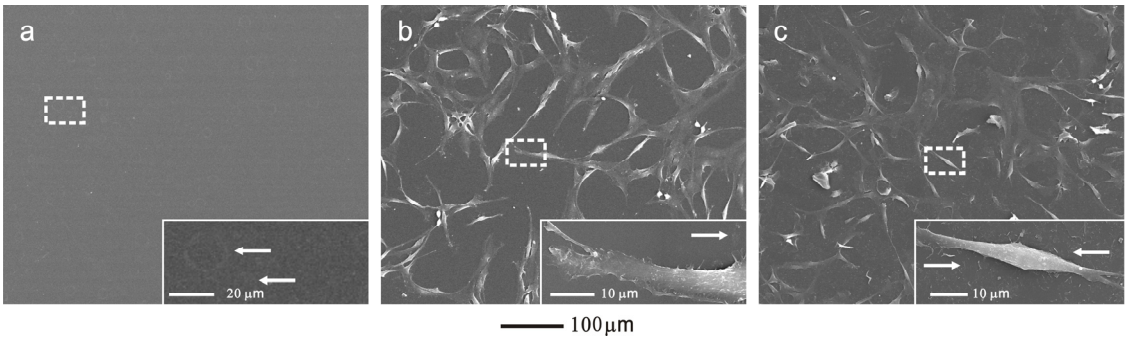

Fig. S9. Scanning electron microscopy (SEM, Quanta 400 FEG, FEI, USA) images of bare mPEG-rGO (a), and PC12 cells adhered on mPEG-rGO after 1 day culture (b&c). The insets illustrate the zoom-in view of white rectangular area. The white arrows point to the wrinkles of the mPEG-rGO film. For imaging, cells were fixed by 2.5% glutaraldehyde in pH 7.4 phosphate buffer, followed by post-fixation in 1% osmium tetroxide and by progressive dehydration in ethanol.

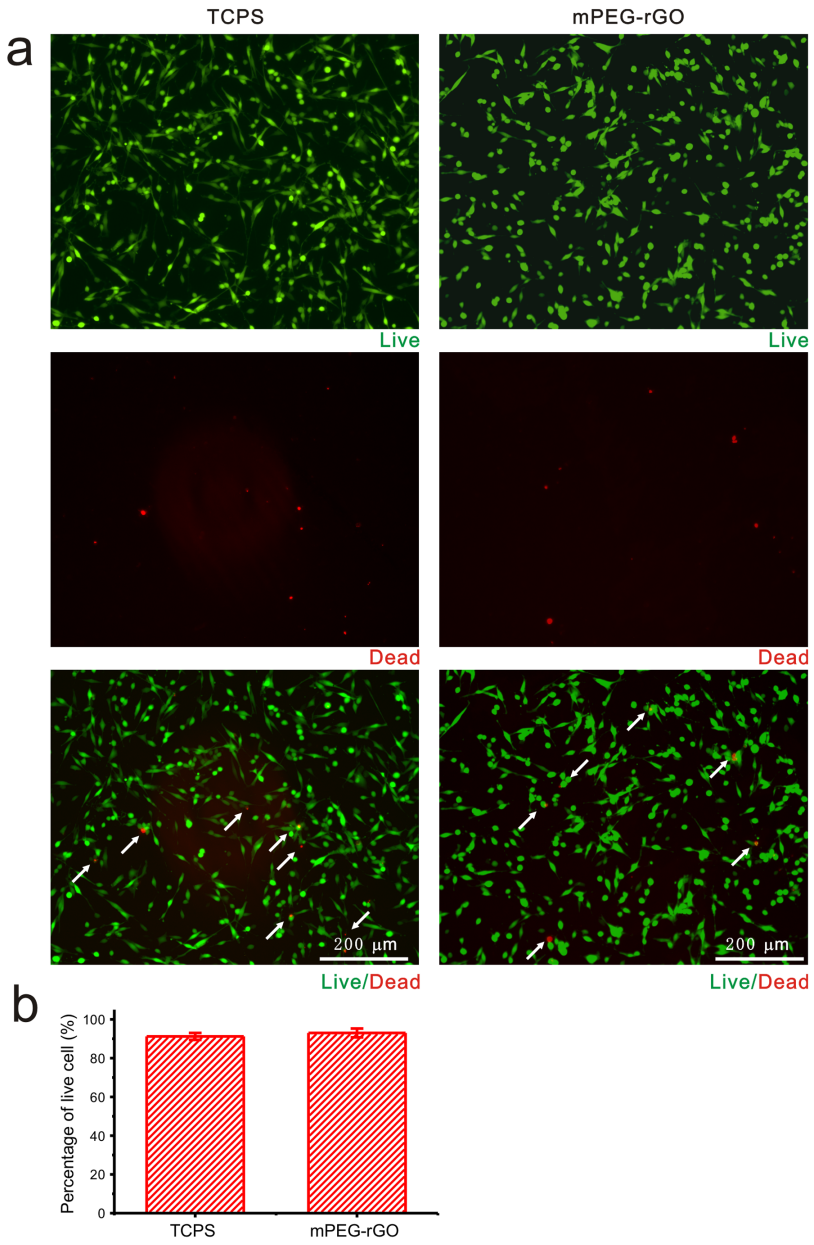

Fig. S10. Cell viability assay of PC12 cells on TCPS and mPEG-rGO after 2 days culture. In panel (a), the live cells are stained green and the dead cells are red, white arrows point to the dead cells. The histogram (b) depicts the percentage of live cell on TCPS and mPEG-rGO.

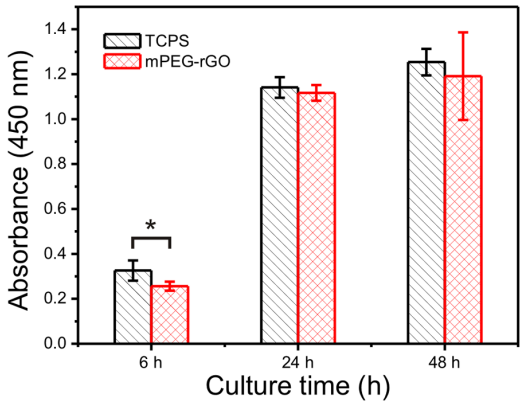

Fig. S11. Cell viability of PC12 cells on TCPS and mPEG-rGO was assessed at indicate time points. Results were shown as mean ± SEM. *$P < 0.05$. The cell viability assay was measured by using of the colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Cell Counting Kit-8, Dojindo) test. Briefly, following PC12 cell incubation, the media were aspirated and replaced with 100 μL of serum-free media. Each well was added 10 μL of an MTT stock solution (5 mg/mL), followed by incubation for 4 h at 37 °C. The supernatant was then removed, and cells were lysed with 100 μLof DMSO.[7] It was found that the cell number on mPEG-rGO was slightly lower than that on TCPS at 6 h, which was probably ascribed to the inhibition of cell adhesion on the amphiphilic molecule modified surface[8]. At 24 h and 48 h, no significant difference in the cell number of TCPS and mPEG-rGO could be observed.

10. Electrical stimulation of PC12 neural cells

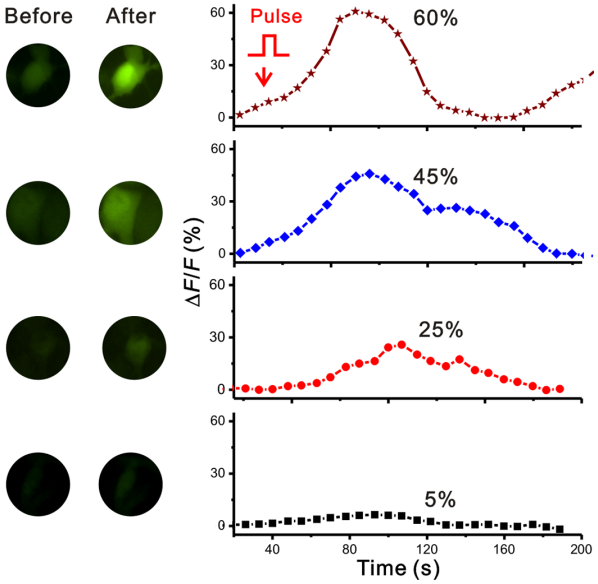

Fig. S12. Typical fluorescence changes of the PC12 cells pre-incubated with Fluo-4 AM dye on mPEG-rGO film before and after electrical stimulation. On the left panels, the circles contain the cells selected from the data set of Fig. 4 in the main manuscript. On the right panels, the diagrams plot the ΔF/F of corresponding cell at series of stimulation time. The maximum ΔF/F values were inserted into each diagram in percentage. The plots and diagrams illustrate that ΔF/F change of >5% can be obviously identified during electrical stimuli, whereas, the change of ≤5% is neglectable.

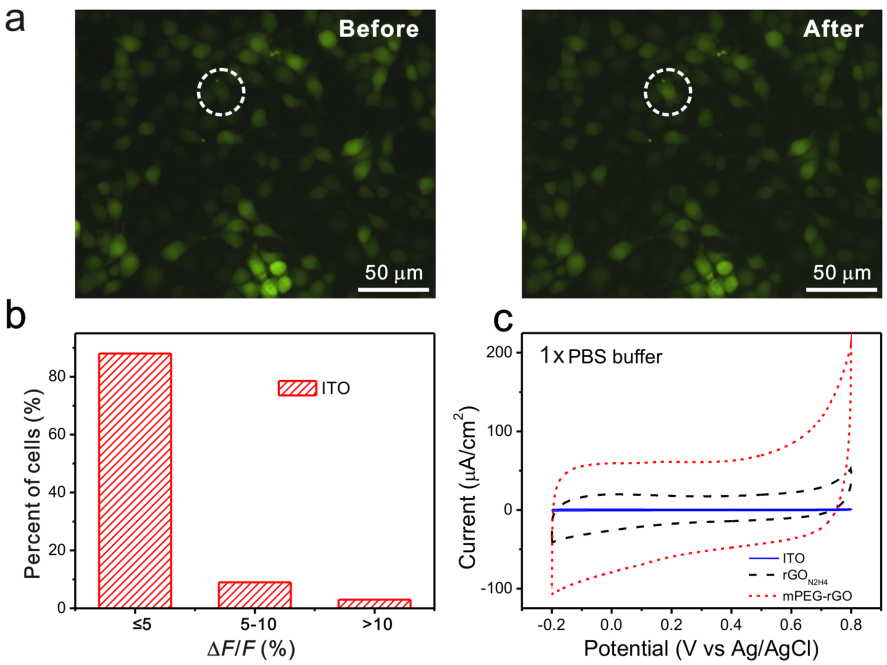

Fig. S13. Fluorescence imaging (a) of the PC12 cells pre-incubated with Fluo-4 AM dye on indium tin oxide (ITO) film before and after electrical stimulation. Histogram (b) depicts the percent of cells in the different range of *ΔF/F*. Panel (c) plots the CVs recorded at ITO, rGO_{N2H4}/GC or mPEG-rGO/GC electrode with 1× PBS buffer (b), scan rate: 50 mV/s.

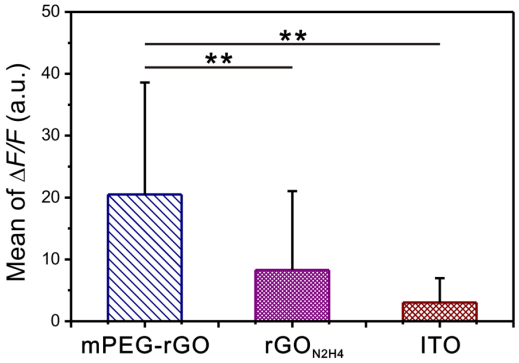

Fig. S14. The mean *ΔF/F* of PC12 cells pre-incubated with Fluo-4 AM dye on mPEG-rGO, rGO_{N2H4} and ITO during electrical stimulation. The data are expressed as mean ± standard deviation. $^{**}P < 0.01$.

Reference

1 M. Glodde, S. R. Sirsi and G. J. Lutz, *Biomacromolecules*, 2005, **7**, 347-356.
2 X. Zhang, S.-R. Pan, H.-M. Hu, G.-F. Wu, M. Feng, W. Zhang and X. Luo, *J. Biomed. Mater. Res.*, 2008, **84A**, 795-804.
3 H. Petersen, P. M. Fechner, D. Fischer and T. Kissel, *Macromolecules*, 2002, **35**, 6867-6874.
4 D. Li, M. B. Muller, S. Gilje, R. B. Kaner and G. G. Wallace, *Nat. Nanotechnol.*, 2008, **3**, 101-105.
5 I. K. Moon, J. Lee, R. S. Ruoff and H. Lee, *Nat. Commun.*, 2010, **1**, 73.
6 H.-K. Jeong, Y. P. Lee, M. H. Jin, E. S. Kim, J. J. Bae and Y. H. Lee, *Chem. Phys. Lett.*, 2009, **470**, 255-258.
7 Y. Zhang, S. F. Ali, E. Dervishi, Y. Xu, Z. Li, D. Casciano and A. S. Biris, *ACS Nano*, 2010, **4**, 3181-3186.
8 S. Park, N. Mohanty, J. W. Suk, A. Nagaraja, J. An, R. D. Piner, W. Cai, D. R. Dreyer, V. Berry and R. S. Ruoff, *Adv. Mater.*, 2010, **22**, 1736-1740.