Electronic Supplementary Material (ESI) for Chemical Communications.

Supramolecular fluorogenic peptide sensor array based on graphene oxide for the differential sensing of Ebola virus

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Contents list

S1. Additional figures

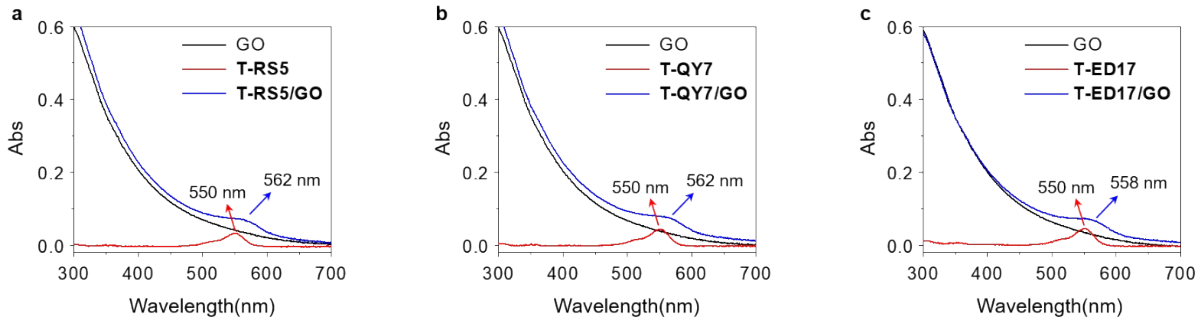

Fig. S1. UV-vis spectra of (a) GO (40 μg mL^{-1}), **T-RS5** (10 μM) and **T-RS5/GO** (peptide/GO = 10 μM/40 μg mL^{-1}), (b) GO (40 μg mL^{-1}), **T-QY7** (10 μM) and **T-QY7/GO** (peptide/GO = 10 μM/40 μg mL^{-1}), and (c) GO (40 μg mL^{-1}), **T-ED17** (10 μM) and **T-ED17/GO** (peptide/GO = 10 μM/40 μg mL^{-1}). All measurements were carried out in PBS (0.01 M, pH 7.4).

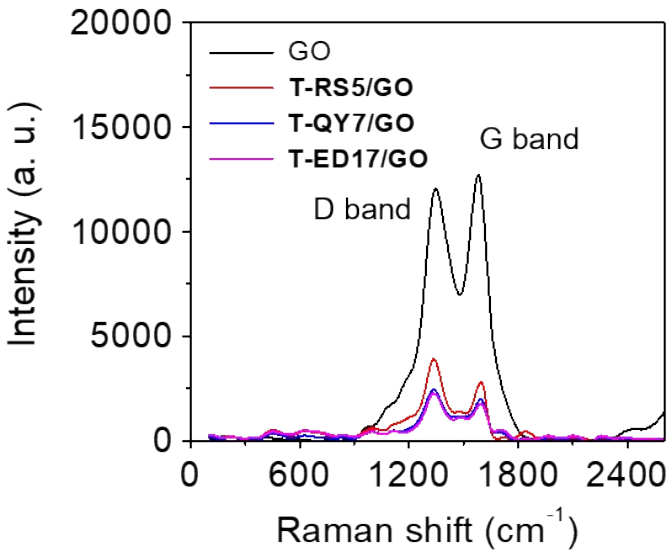

Fig. S2. Raman spectra of GO (40 μg mL^{-1}), **T-RS5/GO** (peptide/GO = 10 μM/40 μg mL^{-1}), **T-QY7/GO** (peptide/GO = 10 μM/40 μg mL^{-1}) and **T-ED17/GO** (peptide/GO = 10 μM/40 μg mL^{-1}). All measurements were carried out in PBS (0.01 M, pH 7.4).

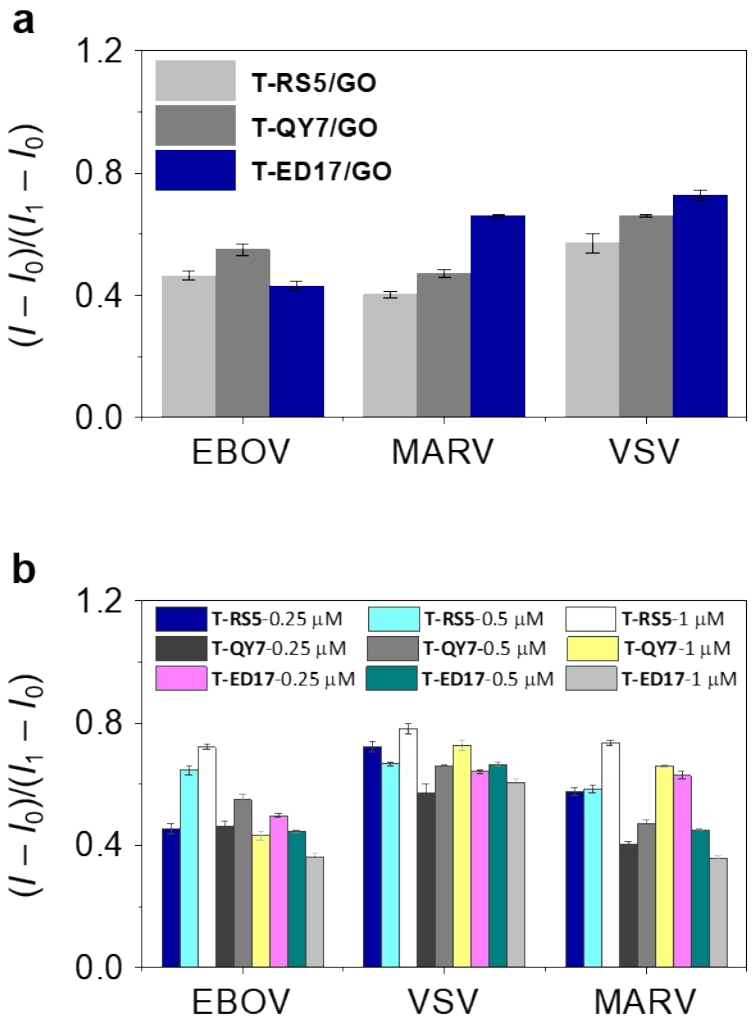

Fig. S3. (a) Normalized fluorescence intensity of **T-RS5/GO** (peptide/GO = 0.5 μM/10 μg mL^{-1}), **T-QY7/GO** (peptide/GO = 0.5 μM/40 μg mL^{-1}) and **T-ED17/GO** (peptide/GO = 0.5 μM/24 μg mL^{-1}) in the presence of EBOV, MARV and VSV (0-15 ng mL^{-1}). (b) Normalized fluorescence intensity of **T-RS5/GO** (peptide/GO = 0.25 μM/10 μg mL^{-1}, 0.5 μM/20 μg mL^{-1} and 1 μM/40 μg mL^{-1}), **T-QY7/GO** (peptide/GO = 0.25 μM/20 μg mL^{-1}, 0.5 μM/40 μg mL^{-1} and 1 μM/80 μg mL^{-1}) and **T-ED17/GO** (peptide/GO = 0.25 μM/12 μg mL^{-1}, 0.5 μM/24 μg mL^{-1} and 1 μM/36 μg mL^{-1}) in the presence of EBOV, MARV and VSV (0-15 ng mL^{-1}). All measurements were carried out in PBS (0.01 M, pH 7.4) with an excitation of 525 nm on a M5 microplate reader. I_1, I_0 and I are the fluorescence intensity of peptide probe alone, peptide probe with GO and peptide/GO in the presence of a virus.

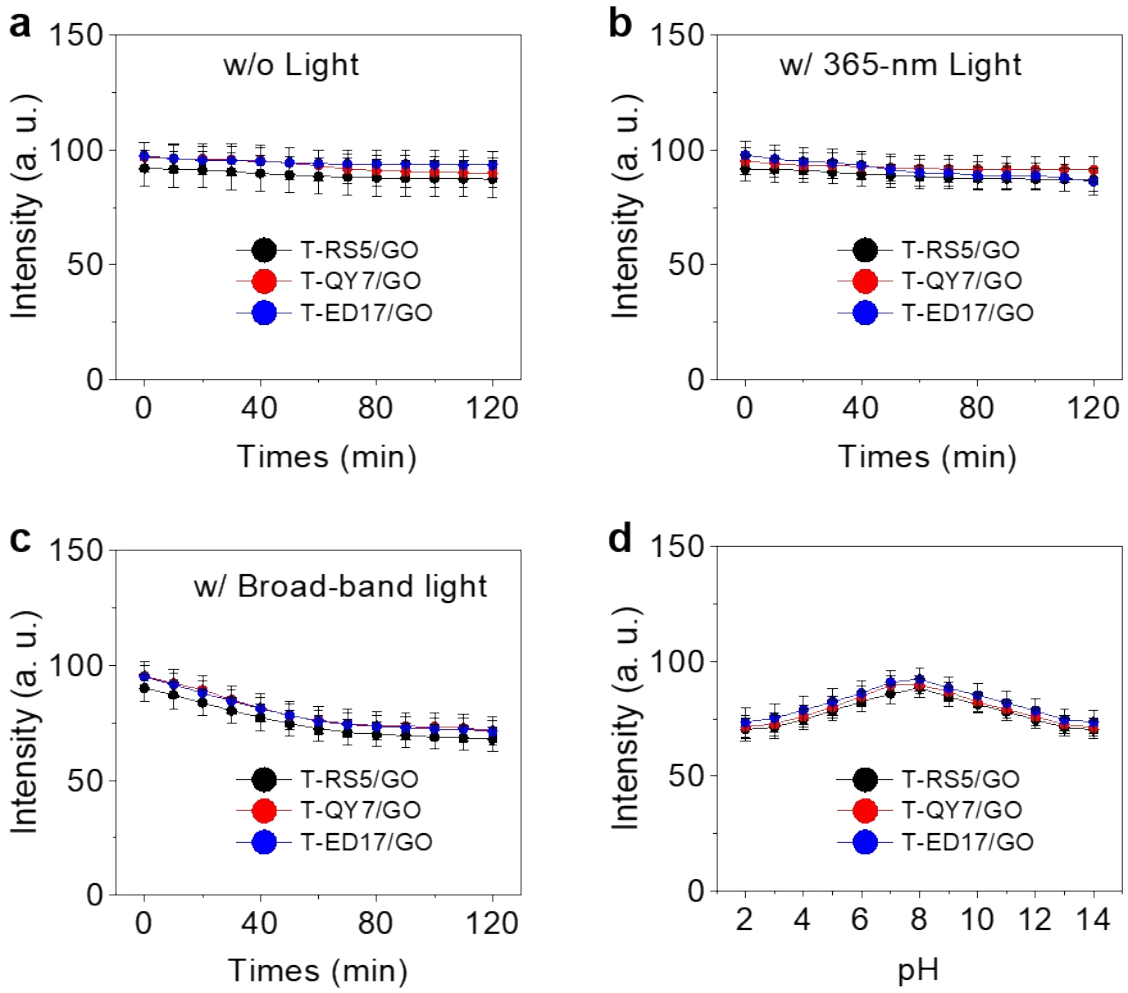

Fig. S4. Plotting the fluorescence intensity of **T-RS5/GO** (peptide/GO = 0.5 μM/10 μg mL^{-1}), **T-QY7/GO** (peptide/GO = 0.5 μM/40 μg mL^{-1}) and **T-ED17/GO** (peptide/GO = 0.5 μM/24 μg mL^{-1}) (a) in the dark for 120 min, (b) with 365-nm light irradiation for 120 min, (c) with broad-band light irradiation (400-700 nm) for 120 min and (d) at different pH. All measurements were carried out in PBS (0.01 M, pH 7.4, 1% DMSO, v/v) with an excitation of 525 nm.

S2. Experimental section

General remarks.

All purchased chemicals and reagents are of analytical grade. High-resolution transmission electron microscope (HRTEM) images were obtained with JEOL 2100 equipped with a Gatan Orius charged-coupled device camera and Tridiem energy filter operating at 200 kV. Raman spectra were obtained using a Renishaw InVia Reflex Raman system (Renishaw, UK). Fluorescence measurements were recorded on a Varian Cary Eclipse fluorescence spectrophotometer or a Microplate reader (Synergy H4 from Botten, USA). Zeta potential was measured with a Horiba LB-550 DLS Nano-Analyzer. Ultraviolet-visible spectra were recorded on a Varian Cary 100 Spectrophotometer. Graphene Oxide (GO) dispersion (2 mg mL^{-1}, 50-200 nm) was purchased from Xianfeng Nano Materials Technology Co., Ltd. Fluorescent peptide probes, **T-RS5** (TAMRA-RYWMS), **T-QY7**(TAMRA-QGYGYNY) and **T-ED17** (TAMRA-EINPDSSTINYTPSLKD), were customized from Shanghai Mujin Biotechnology Co., Ltd.

Preparation of pseudoviruses. The plasmid used for transfection was extracted by using an endotoxin-free plasmid extraction reagent kit (NucleoBond Xtra Midi EF; Macherey-Nagel). Twenty-four hours before transfection, 293T cells were inoculated onto 10 cm plates at a density of 4–5 $\times 10^6$ cells/mL in 10 mL of Dulbecco's modified Eagle's medium (DMEM; Hyclone) containing 10% fetal bovine serum (FBS; Gibco) and 1% Penicillin/Streptomycin (Gibco). The cells were then cultured at 37 °C with 5% CO_2. When cell growth reached 80% to 90% confluence, the culture medium was replaced with fresh complete medium before transfection. For EBOV pseudovirus ,10 μg of the plasmid HIV-luc, 10 μg of the Ebola-Z plasmid, and 1.25 μg of Δ8.9 plasmid was transfected into the 293T cells by using PEI transfection reagent. For MARV pseudovirus, 20 μg of the plasmid HIV-luc and 2 μg of the pCDNA3.1-MARV-G plasmid were transfected into the 293T cells by using PEI transfection reagent. For VSV pseudovirus, 24 μg of the plasmid PNL4-3 and 6 μg pVSVG plasmid were transfected into the 293T cells by using PEI transfection reagent. After the cells were cultured at 37 °C with 5% CO_2 for 10 to12 h, the culture medium was replaced with 10 mL of fresh 293T culture medium.

After 48 h of transfection, the cell culture supernatant was collected, placed into a 50 mL centrifuge tube, and centrifuged at 4 °C and 3500 rpm for 10 min. The supernatant was then collected, filtered through a 0.45 μm filter, and transferred into a new 50 mL centrifuge tube. Finally, the filtrate was transferred into a centrifuge tube and add proper volume of 20-30% sucrose solution to the bottom with a syringe, centrifuge at 4 °C and 25000 rpm for 3 to 4 hours. The supernatant was discarded and the precipitate in the bottom was resuspended with the proper volume of medium. The viruses were aliquoted and stored at −80 °C.

Preparation of the graphene oxide (GO)-based fluorogenic peptide sensors. Fluorescent peptide probes (**T-RS5**, **T-QY7** and **T-ED17**) were dissolved in N,N-dimethylformamide (DMF) to prepare a 250 μmol L^{-1} stock solution. Then, 40 μL of the stock solution (250 μmol L^{-1}) and 20 μL of the GO dispersion (2 mg mL^{-1}) were mixed with a 940 μL PBS (phosphate buffered saline) solution (0.01 mol L^{-1}, pH 7.4), followed by gently shaking for 15 min, producing the peptide sensors (**T-RS5/GO**, **T-QY7/GO** and **T-ED17/GO**), which were stored at -4 °C.

Transmission electron microscopy. A droplet of the fluorogenic sensors (peptide/GO = 10 μM/40 μg mL^{-1}) was dropped onto a 300-mesh holey carbon copper grid. After 30 min, filter paper was used to carefully dry the copper net from the edge, followed by a further drying with infrared light for 10 min. JEOL 2100 equipped with a Gatan Orius charged-coupled device camera and Tridiem energy filter operating at 200 kV was used for TEM imaging; data were processed using Image J software.

Fluorescence quenching assays. T-RS5, **T-QY7** and **T-ED17** were dissolved in DMF to prepare a 250 μmol L^{-1} stock solution. Then, 1 μL of the stock solution (250 μmol L^{-1}) was mixed with 500 μL of a PBS solution (0.01 mol L^{-1}, pH 7.4), followed by an addition of GO dispersion at different concentrations. After a gentle shake for 15 min, the fluorescence spectra were recorded with an excitation of 525 nm.

Fluorescence recovery assays. EBOV, VSV and MARV pseudovirus stock solutions (500 ng mL^{-1}) were prepared in PBS (0.01 mol L^{-1}, pH 7.4). Then, 0-15 ng mL^{-1} of the pseudovirus solution was added to **T-RS5/GO** (0.5 μmol L^{-1}/10 μg mL^{-1}), **T-QY7/GO** (0.5 μmol L^{-1}/40 μg mL^{-1}) or **T-ED17/GO** (0.5 μmol L^{-1}/24 μg mL^{-1}) system, followed by a gentle shake for 15 min. The fluorescence spectra were recorded with an excitation of 525 nm.

Differential sensing of the viruses using PCA. EBOV, VSV and MARV pseudovirus stock solutions (200 ng mL^{-1}) were prepared in PBS (0.01 mol L^{-1}, pH 7.4). **T-RS5/GO** (0.5 μmol L^{-1}/10 μg mL^{-1}), **T-QY7/GO** (0.5 μmol L^{-1}/40 μg mL^{-1}) and **T-ED17/GO** (0.5 μmol L^{-1}/24 μg mL^{-1}) were added a 96-well plate, followed by addition of 10 ng mL^{-1} of a pseudoviral solution (200 ng mL^{-1}). The mixture was incubated for 5 min, and then the fluorescence intensity was monitored with a Microplate reader (Synergy H4 from Botten, USA) with an excitation of 525 nm. Each group of experiments was repeated six times, and the obtained fluorescence responses were processed by PCA according to the literature.[1,2]

S3. Additional refs

1. D. Svechkarev, M. R. Sadykov, K. W. Bayles and A. M. Mohs. *ACS Sens.*, **2018**, *3*, 700-708.
2. C. Zhou, W. Xu, P. Zhang, M. Jiang, Y. Chen, R. T. K. Kwok, M. M. S. Lee, G. Shan, R. Qi, X. Zhou, J. W. Y. Lam, S. Wang and B. Z. Tang. *Adv. Funct. Mater.*, **2019**, *29*, 1805986.