Supporting Information

Graphene Quantum Dots as Universal Fluorophores and Their Use in Revealing Regulated Trafficking of Insulin Receptors in Adipocytes

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Quantum yield (QY) measurement

Fluorescein in water (QY = 0.79) was chosen as the standard. The quantum yield of GQDs (in water) was calculated according to: $\phi_x = \phi_{st}(I_x / I_{st})(\eta_x^2 / \eta_{st}^2)(A_{st} / A_x)$, where ϕ is the quantum yield, I is the measured integrated emission intensity, η is the refractive index of the solvent, and A is the optical density. The subscript "st" refers to the standard and "x" for the sample.

Cytotoxicity test

The viability of cells were evaluated using 3-[4,5-dimethylthialzol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay (Figure S1). Briefly, PC12 cells were seeded into 96-well plates at a density of 1×10^4 per well in 200 μL of media for 24 h. The cells were then incubated with various concentrations of GQDs (0, 10, 20, 40, 60, 80, 100, 200, 400 μg/mL) for 24 h. Then, MTT solution (20 μL, 5 mg/mL) was added to each well for 4 h. Thereafter, the MTT solution was removed and the precipitated

violet crystals were dissolved in 200 μL of DMSO. The absorbance at 570 nm was measured using a BioTek microplate reader.

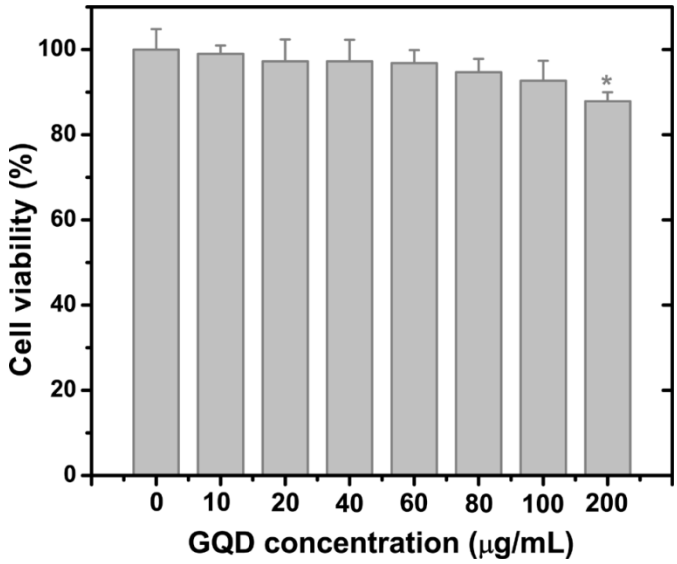

Figure S1. Cell viability measurements of PC12 cells (absorbance ratio normalized to that from control cells), without (control) or with treatment of GQDs at various concentrations (n = 4). The error bars indicate the standard deviations. Student's *t*-test: *p<0.05 *vs.* control.

Cell culture

PC 12 cells were incubated in RPMI 1640 medium (Invitrogen) with 10% fetal bovine serum, 5% horse serum, and 1% penicillin-streptomycin (37 ^{o}C, 5% CO_2). Mouse 3T3-L1 pre-adipocytes purchased from American Type Culture Collection (Rockville, MD, USA) were cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco) containing 10% (v/v) heat-inactivated fetal bovine serum (FBS; Gibco) and 1% penicillin-streptomycin (37 ^{o}C, 5% CO_2).

Adipocyte differentiation

3T3-L1 pre-adipocytes were differentiated into adipocytes as described previously.[1] After 3T3-L1 pre-adipocytes reaching confluence (defined as day 0), the cells were cultured in DMEM containing 10% FBS, 10 μg/mL insulin, 0.5 mM isobutyl-1-methyl xanthine and 1 μM dexamethasone for the first 2 days, and changed to DMEM with 10% FBS and 10 μg/mL insulin for another 2 days. Cells were then maintained in DMEM with 10% FBS for 4-5 days. Most of the cells were differentiated on day 8 as confirmed by appearance of intracellular lipid droplets.

Gel electrophoresis of GQD-protein conjugates

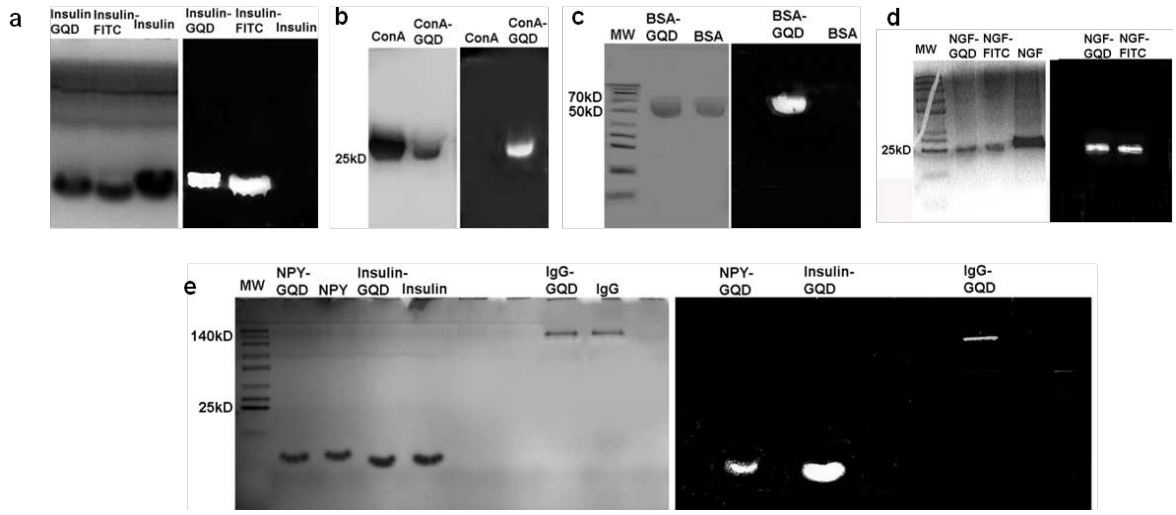

Figure S2. Full gel images of (a) insulin-GQD, insulin-FITC and insulin; (b) concanavalin A (conA) and conA-GQD; (c) bovine serum albumin (BSA)-GQD and BSA; (d) nerve growth factor (NGF)-GQD, NGF-FITC and NGF; (e) neuropeptide Y (NPY)-GQD, NPY, insulin-GQD, insulin, immunoglobulin G (IgG)-GQD and IgG.

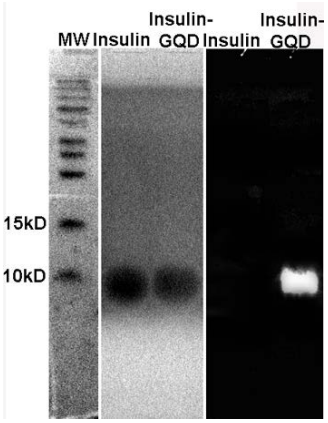

Figure S3. Gel electrophoresis of insulin and insulin-GQD conjugates purified by gel filtration.

Insulin-GQD complexes cannot form without the linker

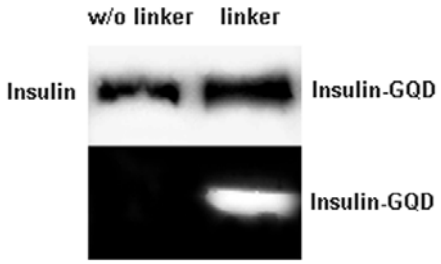

Figure S4. Gel electrophoresis of insulin and GQD mixture in the absence or presence of EDC/NHS linkers. Fluorescent insulin-GQD conjugates are not formed by simple mixing of insulin and GQD for 4h without linkers.

Diffusion of GQD labeled insulin receptor clusters

Mean square displacement (MSD) is a common measure of particle random motion and is calculated as described previously.[2] A Brownian random walk gives a linear MSD over time (Figure S5, dashed line) whereas a directional movement produces an upward-bent MSD (Figure S5, red line) and the MSD of a confined random movement quickly approaches a plateau (Figure S5, blue line).

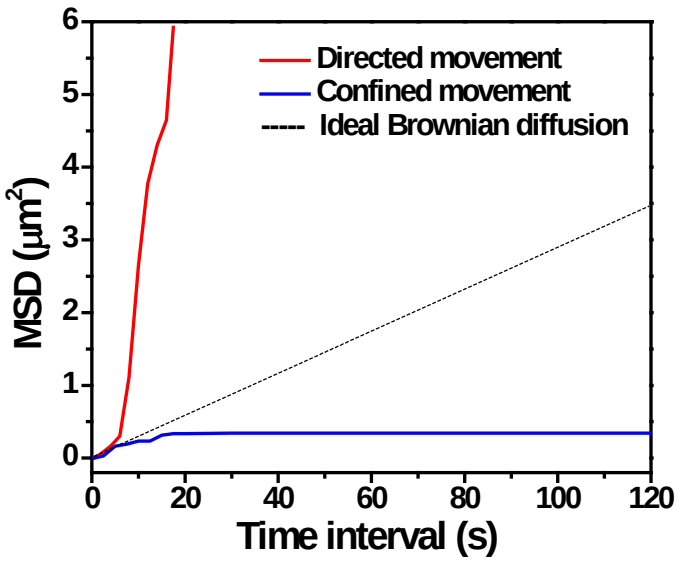

Figure S5. Time-evolved mean-square-displacement (MSD) of a GQD-labeled type-I cluster (blue) and a GQD-labeled type-II cluster (red).

TIRFM video

A TIRFM video (video S1) obtained from a control adipocyte pre-incubated with insulin-GQDs for 1 h is provided as supplementary data. It is noted, however, that the video is largely compressed from the original version and the 2-min recording is displayed in an accelerated speed (7 s).

Reference

1. Than, A.; Cheng, Y. Q.; Foh, L. C.; Leow, M. K. S.; Lim, S. C.; Chuah, Y. L.; Kang, Y. J.; Chen, P., Apelin Inhibits Adipogenesis and Lipolysis through Distinct Molecular Pathways. *Mol. Cell. Endocrinol.* **2012**, 362, 227-241.

2. Levi, S.; Schweizer, C.; Bannai, H.; Pascual, O.; Charrier, C.; Triller, A., Homeostatic Regulation of Synaptic GlyR Numbers Driven by Lateral Diffusion. *Neuron* **2008**, 59, 261-273.