analytical chemistry

Article

pubs.acs.org/ac

A Graphene Oxide-Based Fluorescent Biosensor for the Analysis of Peptide–Receptor Interactions and Imaging in Somatostatin Receptor Subtype 2 Overexpressed Tumor Cells

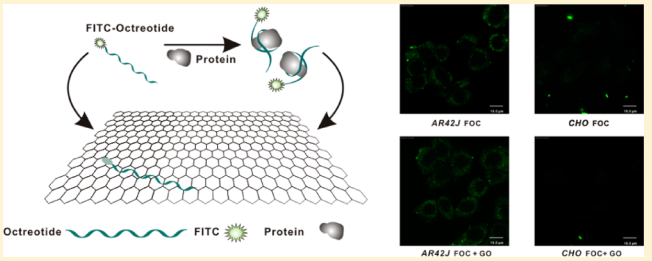
Feng Bianying,[†,‡,§] Guo Linjie,[‡,§] Wang Lihua,[‡] Li Fan,[‡] Lu Jianxin,[†] Gao Jimin,[†] Fan Chunhai,[‡] and Huang Qing*[,‡]

[†]Key Laboratory of Laboratory Medicine, Ministry of Education, Wenzhou Medical College, Wenzhou 325035, Zhejiang, China

[‡]Division of Physical Biology and Bioimaging Center, Shanghai Synchrotron Radiation Facility, Shanghai Institute of Applied Physics, Chinese Academy of Sciences, Shanghai 201800, China

Supporting Information

ABSTRACT: Analysis of peptide–receptor interactions provides insights for understanding functions of proteins in cells. In this work, we report the development of a fluorescent biosensor for the analysis of peptide–receptor interactions using graphene oxide (GO) and fluorescein isothiocyanate (FITC)-labeled octreotide (FOC). Octreotide is a synthesized cyclic peptide with somatostatin-like bioactivity that has been clinically employed. FOC exhibits high adsorption affinity for GO, and its binding results in efficient fluorescence quenching of FITC. Interestingly, the specific binding of the antibody anti-octreotide (AOC) with FOC competitively releases FOC from the GO surface, leading to the recovery of fluorescence. By using this GO-based fluorescent platform, we can detect AOC with a low detection limit of 2 ng/mL. As a step further, we employ this GO–FOC biosensor to image somatostatin receptor subtype 2 overexpressed AR42J tumor cells, which demonstrates high promise for molecular imaging in cancer diagnosis.



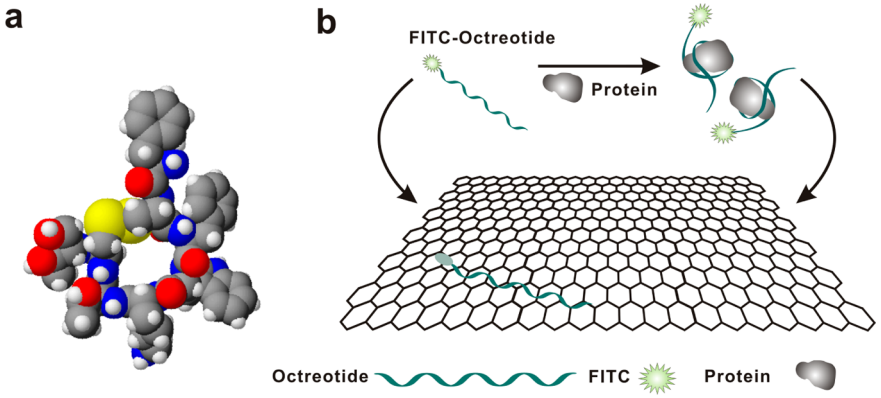
Peptides are ligands for a variety of receptors and have been extensively employed in clinical applications due to their high tissue penetration ability, low toxicity and immunogenicity, and high specificity.[1−5] Since peptides function in vivo through combination with their corresponding receptors, it is important to develop new tools to understand peptide–receptor interactions.[1] While there have been many reported methods for studying peptide–receptor interactions, most of them are limited by their complicated and time-consuming procedures and are lacking in the ability for real-time and multiplexed analysis.[3,6−8] Fluorescent biosensors employing either fluorescent dyes or proteins (e.g., green fluorescence protein, GFP) have been proven of high utility for real-time analysis of peptide–receptor interactions in living cells.[9−12] However, the limited availability of fluorescence pairs and the high cost of dually labeled peptide potentially restrict their widespread applications in medical studies. In this work, we report the development of a simple and sensitive fluorescent biosensor for the analysis of peptide–receptor interactions using graphene oxide (GO) and fluorescein isothiocyanate (FITC)-labeled octreotide (FOC).

GO is a single-atom-thick and two-dimensional carbon material that has received rapidly increasing attention in recent years because of its extraordinary electronic, optical, and thermal properties. Compared with other nanomaterials, those superior properties of GO, such as high quenching efficiency, large surface area, good water dispersibility and biocompatibility, facile surface modification, low manufacturing cost, and an amphiphile with hydrophilic ionizable edges (−COOH group) and a more hydrophobic basal plane, make it a promising candidate for biological applications, including biosensors.[13−20] In particular, by exploiting the superquenching property of GO, a range of fluorescent biosensors have been developed for the detection of DNA, adenosine-5′-triphosphate (ATP), reactive oxygen species (ROS), metal ions, and proteins.[14,21−25] In these GO-based sensing strategies, the sensitivity and selectivity have been improved due to the unique differentiation ability of GO for different structures of biomolecule probes and the ultrahigh fluorescent quenching property.

Octreotide is a synthesized cyclic peptide with eight amino acids, H-D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr (Scheme 1a), which is an excellent analogue for somatostatin owing to its high stability, good bioactivity, and pharmacokinetics behavior.[3] Octreotide has been widely used in clinical diagnosis and treatment after its FDA approval ([^{111}In]OctreoScan) in 1994. It has been employed to treat acromegaly, gigantism, thyrotropinoma, diarrhea, and flushing episodes associated

Received: March 30, 2013
Accepted: July 18, 2013
Published: July 18, 2013

Scheme 1. (a) Three Dimensional Structure of Octreotide and (b) Schematic Illustration of the Concept of Using FITC–Octreotide and GO to Detect Peptide–Receptor Interactions



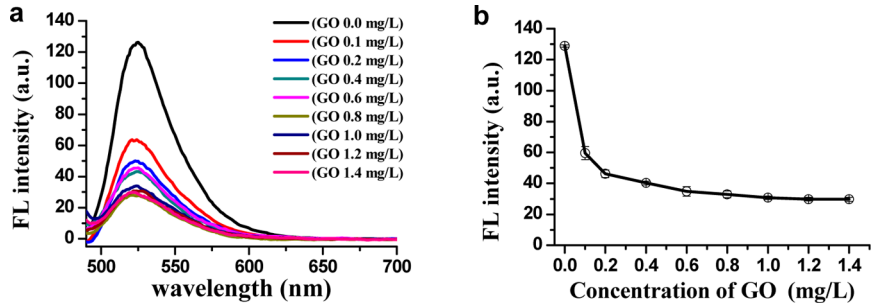


Figure 1. (a) Fluorescence spectra of 20 nM of FOC in the presence of various concentrations of GO. (b) Fluorescence intensity of FOC versus concentration of GO.

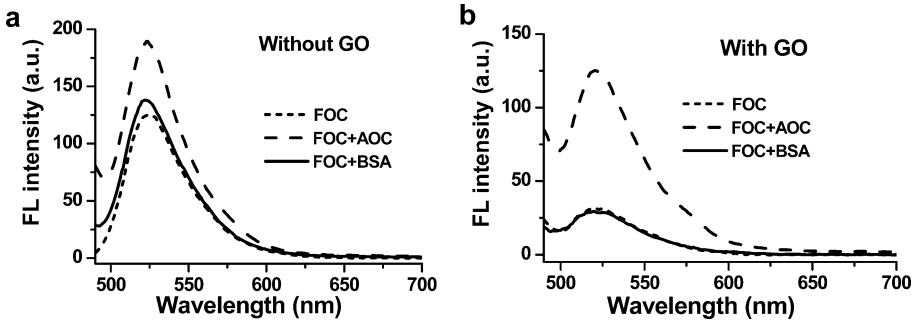


Figure 2. Fluorescence emission spectra of FOC, FOC–BSA, and FOC–AOC in the (a) absence and (b) presence of GO. [FOC] = 20 nM, [AOC] = [BSA] = 2 ng/mL.

with carcinoid syndrome, as well as diarrhea in patients with vasoactive intestinal peptide-secreting tumors via inhibiting secretion of growth hormone, glucagon, and insulin.[3] Moreover, octreotide has been used for receptor-targeted imaging of tumors by labeling with radioisotopes (e.g., indium-111, gallium-68, yttrium-90, or lutetium-177) or fluorescent tags, through which the noninvasive imaging of neuroendocrine and other tumors expressing somatostatin receptors has been well-performed.[26] In our design, we combine FITC–octreotide (FOC) with GO (Scheme 1b) to develop a biosensor for the analysis of interactions between octreotide and its receptor.

RESULTS AND DISCUSSION

We first studied the fluorescence change of FOC upon different concentrations of GO, and a titration experiment was performed for gaining an appropriate dose ratio of GO to FOC. The fluorescence spectra of FOC in the absence and presence of various doses of GO were recorded and are shown in Figure 1. The fluorescence intensity of FOC (20 nM) decreased with the increase of the GO concentration, implying that FOC had been efficiently adsorbed on the surface of GO. We found that ~78% of fluorescence was quenched by 0.8 mg/L of GO, and further increase of GO did not lead to higher fluorescence quenching.

As previously reported, the adsorption behavior of peptide on GO depended on the incorporated positively charged amino acids (Lys, His, and Arg) and aromatic-ring-containing hydrophobic ones (Trp, Tyr, and Phe), which were supposed to contribute ro the electrostatic force and $\pi-\pi$ interaction with negative-charged GO.[27] In this work, the cyclized octreotide was ready to adsorb on the surface of GO since it possessed two Phe, one Trp, and one Lys in a total 8 amimo acid sequence. We carefully studied the quenching efficiency, kinetics, and adsorption capacity in GO–FOC binding events

dx.doi.org/10.1021/ac4009463 | Anal. Chem. 2013, 85, 7732–7737

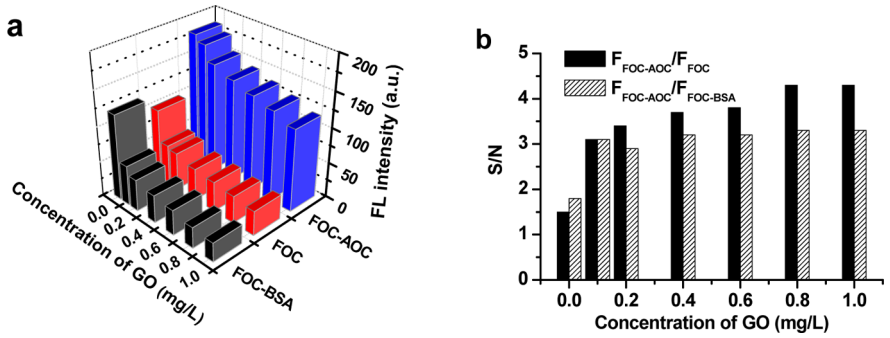


Figure 3. (a) FL intensity of FOC, FOC−BSA, and FOC−AOC and (b) the S/N ratio in the presence of different concentrations of GO. [FOC] = 20 nM, [AOC] = [BSA] = 2 ng/mL, [GO] = 0, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0 mg/L.

and made a further comparison with those of GO−DNA.[28,29] Figure S1 (Supporting Information demonstrated that both 20 nM of FAM−DNA (18 mer) and FOC were quenched to the lowest fluorescence by 0.8 mg/L of GO immediately; i.e., similar adsorption capacity was achieved for FAM−DNA and FOC (25 mmol/g GO). The kinetics study implied that both FAM−DNA and FOC adsorbed on GO with high kinetics, and the adsorption completed immediately after adding GO. However, the highest fluorescence quenching efficiency of GO for FOC (78%) was lower than that for single-standed (ss) DNA (100%)[13] and linear peptides (90%).[27] This is possibly because the intramolecular cyclization of two Cys enhanced the rigidity of peptide and then led to relatively longer GO−FOC distance and lower quenching efficiency.[30]

The interaction between FOC and its antibody, anti-octreotide (AOC), was investigated with the fluorescence emission spectra of FOC in the presence of AOC. Since most antibodies had K_d values in the low micromolar (10^{-6}) to nanomolar (10^{-7}−10^{-9}) range,[31] we considered that the specific binding of FOC−AOC would reduce the chance of GO−FOC interaction. As illustrated in Figure 2a, the fluorescence of FOC alone displayed strong fluorescence emission. Upon the addition of AOC, the fluorescence of FOC was enhanced, which might be explained by the high background arising from the autofluorescence of AOC (Figure S2, Supporting Information) and the variation of hydrophobic environment the dye molecule located after conjugation with protein.[32,33] As a contrast, the fluorescence of FOC increased very slightly after incubation with an interference protein, bovine serum albumin (BSA). While such fluorescence variations can differentiate proteins, the signal-to-noise (S/N) ratio, as calculated from $F_{FOC-AOC}/F_{FOC}$, was only of ∼1.5.

GO was then introduced to improve the S/N since it could quench the fluorescence of FOC molecules. As demonstrated in Figure 2b, the fluorescence of FOC was 122 au after the specific binding with AOC through antigen−antibody interaction in the presence of GO. The recovery of fluorescence implied the formation of FOC−AOC complex that had low binding affinity on the surface of GO, which competitively released FOC to the solution. The fluorescence quenching efficiency was calculated by $(F_{FOC-AOC} - F_{FOC-AOC/GO})/F_{FOC-AOC} \times 100\%$, and only 34% was achieved for FOC−AOC complex. Here, $F_{FOC-AOC}$ and $F_{FOC-AOC/GO}$ were the fluorescence intensity of FOC−AOC complex at 525 nm in the absence and presence of GO. However, the fluorescence of FOC itself and that with BSA were quenched efficiently, and 78% and 80% quenching efficiencies were obtained. Hence, the nonspecific binding with the interference proteins was not significant. By using GO to suppress the fluorescence background, the S/N ratio increased to 4.3.

We also studied the effect of GO concentration on the detection performance. As shown in Figure 3a, the fluorescence of FOC decreased dramatically in either buffer or BSA solution, while maintained in the solution of AOC. The S/N (Figure 3b) increased greatly after the addition of GO, and 3 times was achieved with 0.1 mg/L of GO while 4.3 times (the highest value) was reached with 0.8 mg/L of GO. This observation reveals that a small amount of GO could dramatically improved the performance of this peptide-based biosensor.

The performance of this GO/FOC-based sensor was also evaluated in terms of AOC quantification assay. Figure 4

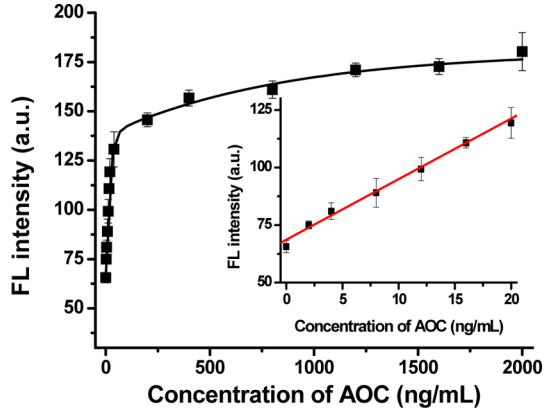


Figure 4. Fluorescence of GO/FOC hybrid probe in the presence of different concentrations of AOC (0, 2, 4, 8, 12, 16, 20, 40, 80, 1200, 1600, 2000 ng/mL). [GO] = 0.8 mg/L, [FOC] = 20 nM.

displayed a calibration curve for AOC detection, and a proportional relationship was observed between the concentration of AOC and relative fluorescent intensity. The fluorescence intensity of FOC at 525 nm gradually increased with the concentration of AOC. This assay had a linear range of 0−20 ng/mL, and then the fluorescence intensity reached a rough plateau with the increasing concentration of AOC, and as low as 2 ng/mL of AOC could be detected.

Cell imaging provides information on the motion and localization of biomolecules and their interactions with specific receptors.[33] Here, we employed the GO-based biosensor for analysis of peptide−receptor interactions inside the cell. The AR42J cell line is a type of rat pancreatic exocrine cell that overexpresses somatostatin receptor subtype 2 (SSTR2), which is the affinity receptor of octreotide (K_d = 0.4 pM).[34] CHO cells possessing low SSTR2 density were used as a negative

dx.doi.org/10.1021/ac4009463 | Anal. Chem. 2013, 85, 7732−7737

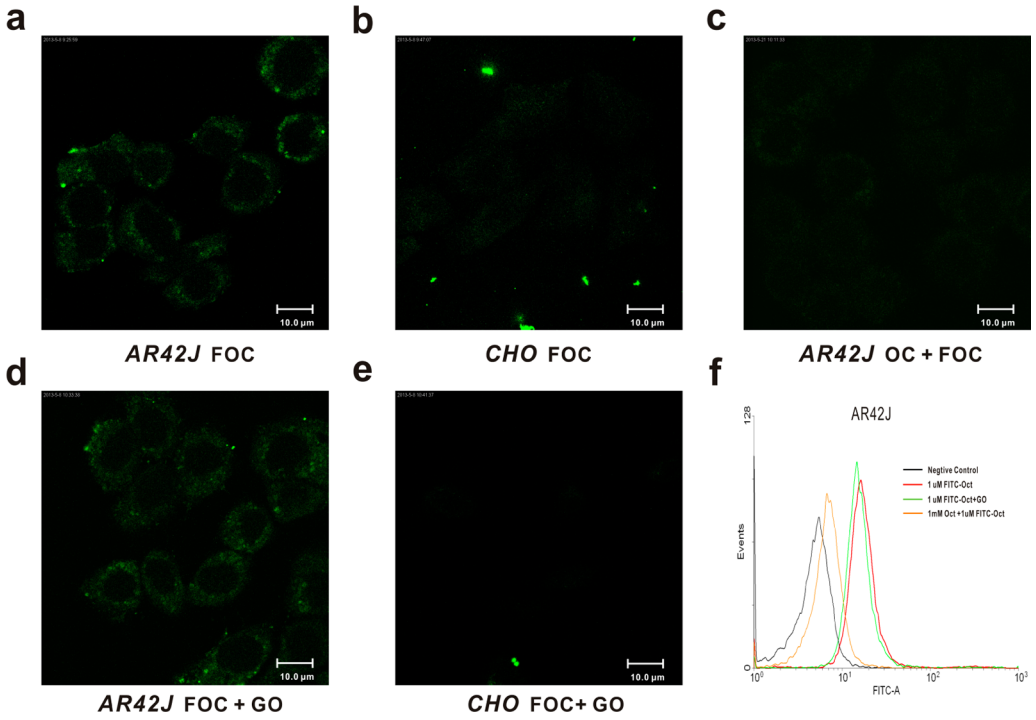


Figure 5. Confocal laser fluorescence images (a–e) and the flow cytometry data (f) of cell imaging experiments. (a) AR42J cells and (b) CHO cells were incubated with 1 μM of FOC for 1 h at 37 °C. (c) AR42J cells were pretreated with 1 mM of octreotide for 30 min and then incubated with 1 μM of FOC for another 1 h at 37 °C. (d) AR42J cells and (e) CHO cells were treated with 80 mg/L of GO for 5 min after incubation with 1 μM of FOC for 1 h at 37 °C.

control.[35] Both AR42J and CHO cells were grown in their corresponding cell growth medium for 12 h at 37 °C, and then 5×10^4 cells were put on the coverslip of each well. 1 μM of FOC was added into the medium solution and incubated with cells at 37 °C for 1 h. As shown in Figure 5a, AR42J cells incubated with FOC showed remarkable fluorescence on the cell surface owing to the strong binding of FOC with SSTR2. In order to confirm that the binding was specific for AR42J cells, both a control experiment with CHO cell line and a competitive binding test were performed here. Figure 5b exhibited the much weaker fluorescence for CHO cells in comparison with AR42J cells. For competitive tests, 1 mM octreotide (1000 times of FOC) was used to block the SSTR2 binding sites before addition of 1 μM of FOC. The ultralow fluorescence in Figure 5c disclaimed that the SSTR2-overexpressed cells were efficiently blocked by label-free octreotide, and only few sites were competitively bound by FOC. In addition, in order to achieve higher target/nontarget ratio, 80 mg/L of GO was used here to suppress the background fluorescence arising from nonspecific binding of surface proteins with FOC for CHO cells. After being treated with GO at room temperature for 5 min, the cells were imaged with confocal laser microscopy in a fixed status. As shown in Figure 5d,e, the fluorescence in AR42J cells maintained bright green color, while that in CHO cells was further quenched to negligible fluorescence upon the addition of GO. The flow cytometry data in Figure 5f were consistent with the results of confocal images. This observation proved that we could employ this GO-based biosensor to specifically image receptors in AR42J cells. Since most gastroenteropancreatic neuroendocrine tumors have been shown to overexpress SSTR2, this strategy has the potential in clinical receptor imaging of neuroendocrine gastroenteropancreatic tumors.[36−40]

CONCLUSIONS

In summary, we have proposed a new biosensing strategy for monitoring peptide−receptor interactions by employing GO and FOC. This platform has several excellent features for the analysis of peptide−receptor interactions. First, it is simple, fast, and cost-effective. It requires only one FITC label, and there is no need for modification of other expensive fluorophores. GO can also be synthesized in large quantities from graphite available at low cost. Second, the combination of GO and FOC can improve the specificity for the target and substantially suppress background fluorescence. Third, this assay can effectively identify gastroenteropancreatic neuroendocrine tumor cells, which shows high promise for screening antitumor drugs.

EXPERIMENTAL DETAILS

Materials and Apparatus. Graphene oxide (GO) was synthesized from natural graphite powder by the modified Hummers method.[41] FITC−octreotide was purchased from GL Biochem (Shanghai) Co. Ltd. Anti-octreotide was purchased from Shanghai Jin Ma Biological Technology Co. Ltd. Rat pancreatic (AR42J) cells were purchased from Shanghai Zhonghua Biological Technology Co. Ltd. Chinese hamster ovary cell line (CHO) was obtained from the cell bank of the Chinese Academy of Sciences. All other chemicals were of analytical grade and obtained from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Milli-Q water (18.2 MΩ) was used throughout all experiments; 1× PBS buffer (137 mM NaCl, 2.5 mM $MgCl_2$, 10 mM Na_2HPO_4, 2.0 mM KH_2PO_4, pH 7.4) was used for specific binding of anti-octreotide with FITC−octreotide, and F-12K medium (Gibco 21127022) was applied for specific binding between FITC−

dx.doi.org/10.1021/ac4009463 | Anal. Chem. 2013, 85, 7732−7737

octreotide and somatostain receptor-2, which have high densities on cell surface of rat pancreatic (AR42J) cells.

A Hitachi F-4500 spectrofluorometer was used for fluorescence spectra measurement, fluorescence images were acquired using confocal laser fluorescence microscope (Leica TCS SP5), and the flow cytometry data were achieved on a BD LSR II flow cytometer.

Quenching Studies. For the fluorescence quenching study of FOC, different volumes of GO (100 mg/L) were added into 20 nM of FOC solution, and 1× PBS buffer was used to make up 1 mL for further fluorescence detection. The final concentration of GO in the cuvettes was 0, 0.1, 0.2, 0.4, 0.8, 1.0, 1.2, and 1.4 mg/L, respectively, and the fluorescence spectra were recorded upon excitation at 490 nm and collection of emission from 500 to 700 nm. The quenching study for DNA was similar with that of FOC, and an equal concentration of DNA was used to replace FOC. The kinetics data were collected on 525 nm after the addition of GO, and the time gap between GO addition and spectra collection was unified as 15 s for gaining comparable results.

Detection of AOC. For specific binding of AOC with FOC, 20 μg/mL of AOC and 1 μM of FOC were mixed in 20 μL of 1× PBS buffer and incubated at 37 °C for 30 min first. After that, 980 μL of PBS buffer was added to make up 1 mL solution for fluorescence measurements. Finally, we introduced 8 μL of GO (100 mg/L) to the above mixture, and the fluorescence spectra were recorded immediately (the time gap between GO addition and spectra collection was less than 15 s). The excitation wavelength was 490 nm and the emission wavelengths were in the range from 500 to 700 nm with both excitation and emission slits of 5 nm.

For selectivity studies, an equal concentration of BSA was employed instead of AOC, and PBS buffer was used as a blank. The sensitivity was analyzed by investigating the fluorescence response from different concentrations of AOC, and the final concentrations in the cuvettes were 0, 2, 4, 8, 12, 16, 20, 40, 80, 1200, 1600, 2000 ng/mL, respectively.

Cell Imaging with FOC. Rat pancreatic (AR42J) cells, the cell surface of which have high densities of SSTR2, were used for binding studies, while CHO cells without SSTR2 were used as a control. AR42J cells were grown in RPMI 1640 medium (Gibco 11875) supplemented with 10% heat-inactivated fetal bovine serum (Invitrogen), 100 U/mL penicillin, and 100 U/mL gentamicin at 37 °C in a humidified atmosphere containing 5% CO_2. The corresponding cell growth medium for CHO cells was F-12K (Gibco 21127022). Both AR42J and CHO cells were incubated with the corresponding cell growth medium for 12 h at 37 °C with 5×10^4 cells/well, and each with the coverslip at the bottom. Besides, each cell had two groups accomplished with two wells for each group. After two washes with PBS, they were incubated with FOC at the same time in medium for 6 h at 37 °C in the dark place. After that, we treated one group of the two cells with GO (80 mg/L) at room temperature for 5 min. Then, all the cells were washed with PBS twice and fixed with 4% paraformaldehyde for 20 min at room temperature. Finally, we sealed coverslips with mounting medium after two washes with PBS and imaged with confocal laser fluorescence microscopy. For the flow cytometry analysis, 1×10^5 AR42J (or CHO) cells were cultured for 24 h and further incubated with 1 μM of FOC for 3 h at 37 °C. For groups with GO treatment, 80 μg/mL of GO was introduced to cells for 5 min.

■ ASSOCIATED CONTENT

Supporting Information

Additional information as noted in the text. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: huangqing@sinap.ac.cn. Tel: 8621-3919-4520. Fax: 8621-3919-4520.

Author Contributions

[§]These authors contributed to this work equally.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was financially supported by he National Basic Research Program of China (973 program, 2013CB933800, 2012CB825805), the National Natural Science foundation of China (Grant Nos. 31100716, 21077321), and Chinese Academy of Sciences (KJCX2-EW-N03).

■ REFERENCES

(1) Schottelius, M.; Wester, H. J. *Methods* **2009**, *48*, 161−177.
(2) Lee, I.; Date, S. V.; Adai, A. T.; Marcotte, E. M. *Science* **2004**, *306*, 1555−1558.
(3) Bessette, P. H.; Rice, J. J.; Daugherty, P. S. *Protein Eng., Des. Sel.* **2004**, *17*, 731−739.
(4) Sidhu, S. S. *Curr. Opin. Biotechnol.* **2000**, *11*, 610−616.
(5) Norman, T. C.; Smith, D. L.; Sorger, P. K.; Drees, B. L.; O'Rourke, S. M.; Hughes, T. R.; Roberts, C. J.; Friend, S. H.; Fields, S.; Murray, A. W. *Science* **1999**, *285*, 591−595.
(6) Schulze, W. X.; Mann, M. *J. Biol. Chem.* **2004**, *279*, 10756−10764.
(7) Ryan, D. P.; Matthews, J. M. *Curr. Opin. Struct. Biol.* **2005**, *15*, 441−446.
(8) Chen, K.; Liron, L. B.; Shahar, R. B.; Tiago, R.; Stefan, GD, R.; Assaf, F. *Chem. Soc. Rev.* **2011**, *40*, 2131−2145.
(9) Chen, X.; Conti, P. S.; Moats, R. A. *Cancer Res.* **2004**, *64*, 8009−8014.
(10) Shen, D.; Liang, K.; Ye, Y.; Tetteh, E.; Achilefu, S. *FEBS Lett.* **2007**, *581*, 1793−1799.
(11) Koyama, Y.; Barrett, T.; Hama, Y.; Ravizzini, G.; Choyke, P. L.; Kobayashi, H. *Neoplasia* **2007**, *9*, 1021−1029.
(12) Mankoff, D. A.; Link, J. M.; Linden, H. M.; Sundararajan, L.; Krohn, K. A. *J. Nucl. Med.* **2008**, *49*, 149S−163S.
(13) He, S. J.; Song, B.; Li, D.; Zhu, C. F.; Qi, W. P.; Wen, Y. Q.; Wang, L. H.; Song, S. P.; Fang, H. P.; Fan, C. H. *Adv. Funct. Mater.* **2010**, *20*, 453−459.
(14) Lu, C. H.; Li, J.; Qi, X. J.; Song, X. R.; Yang, H. H.; Chen, X.; Chen, G. N. *J. Mater. Chem.* **2011**, *21*, 10915−10919.
(15) Sun, X. M.; Liu, Z.; Welsher, K.; Robinson, J. T.; Goodwin, A.; Zaric, S.; Dai, H. J. *Nano Res.* **2008**, *1*, 203−212.
(16) Loh, K. P.; Bao, Q.; Ang, P. K.; Yang, J. *J. Mater. Chem.* **2010**, *20*, 2277−2289.
(17) Burress, J. W.; Gadipelli, S.; Ford, J.; Simmons, J. M.; Zhou, W.; Yildirim, T. *Angew. Chem., Int. Ed.* **2010**, *49*, 8902−8904.
(18) Mao, S.; Lu, G. H.; Yu, K. H.; Bo, Z.; Chen, J. H. *Adv. Mater.* **2010**, *22*, 3521−3526.
(19) Wu, W. H.; Hu, H. Y.; Li, F.; Wang, L. H.; Gao, J. M.; Lu, J. X.; Fan, C. H. *Chem. Commun.* **2011**, *47*, 1201−1203.
(20) Liu, Z.; Robinson, J. T.; Sun, X. M.; Dai, H. J. *J. Am. Chem. Soc.* **2008**, *130*, 10876−10877.
(21) Lu, C. H.; Li, J.; Lin, M. H.; Wang, Y. W.; Yang, H. H.; Chen, X.; Chen, G. N. *Angew. Chem., Int. Ed.* **2010**, *122*, 8632−8635.
(22) Liu, M.; Zhang, Q.; Zhao, H. M.; Chen, S.; Yu, H.; Zhang, Y. B.; Quan, X. *Chem. Commun.* **2011**, *47*, 4084−4086.

(23) Wen, Y. Q.; Xing, F. F.; He, S. J.; Song, S. P.; Wang, L. H.; Long, Y. T.; Li, D.; Fan, C. H. *Chem. Commun.* **2010**, *46*, 2596−2598.
(24) Chang, H. X.; Tang, L. H.; Wang, Y.; Jiang, J. H.; Li, J. H. *Anal. Chem.* **2010**, *82*, 2341−2346.
(25) Wang, L. H.; Pu, K. Y.; Li, J.; Qi, X. Y.; Li, H.; Zhang, H.; Fan, C. H.; Liu, B. *Adv. Mater.* **2011**, *23*, 4386−4391.
(26) Becker, A.; Hessenius, C.; Licha, K.; Ebert, B.; Sukowski, U.; Semmler, W.; Wiedenmann, B.; Grötzinger, C. *Nat. Biotechnol.* **2001**, *19*, 327−331.
(27) Zhang, M.; Yin, B. C.; Wang, X. F.; Ye, B. C. *Chem. Commun.* **2011**, *47*, 2399−2401.
(28) Wu, M.; Kempaiah, R.; Huang, P.-J. J.; Maheshwari, V.; Liu, J. W. *Langmuir* **2011**, *27*, 2731−2738.
(29) Huang, P.-J. J.; Liu, J. W. *Anal. Chem.* **2012**, *84*, 4192−4198.
(30) Swathi, R. S.; Sebastian, K. L. *J. Chem. Phys.* **2009**, *130*, 086101.
(31) http://www.abcam.com/index.html?pageconfig=resource&rid=15749.
(32) Toutchkine, A.; Kraynov, V.; Hahn, K. *J. Am. Chem. Soc.* **2003**, *125*, 4132−4145.
(33) Buschmann, V.; Weston, K. D.; Sauer, M. *Bioconjugate Chem.* **2003**, *14*, 195−204.
(34) Melmed, S. *J. Clin. Invest.* **2009**, *119*, 3189−3202.
(35) Zhang, J. L.; Jin, W.; Wang, X. Q.; Wang, J. C.; Zhang, X.; Zhang, Q. *Mol. Pharmaceutics* **2010**, *7*, 1159−1168.
(36) Reubi, J. C. *Endocr. Pathol.* **1997**, *8*, 11−20.
(37) Ehlers, R. A.; Kim, S. H.; Zhang, Y. J.; Ethridge, R. T.; Murrilo, C.; Hellmich, M. R.; Evans, D. B.; Townsend, C. M.; Evers, B. M. *Ann. Surg.* **2000**, *231*, 838−846.
(38) Reubi, J. C.; Lang, W.; Maurer, R.; Koper, J. W.; Lamberts, S. W. J. *Cancer Res.* **1987**, *47*, 5758−5764.
(39) John, M. W. M.; Richter, D.; Waser, B.; Schaer, J. C.; Scheruebl, H.; Boese-Landgraf, J.; Neuhaus, P.; Ziske, C.; Moelling, K.; Riecken, E. O.; Reubi, J. C.; Wiedenmann, B. *Gut* **1996**, *38*, 33−39.
(40) Schaer, J. C.; Waser, B.; Mengod, G.; Reubi, J. C. *Int. J. Cancer* **1997**, *70*, 530−537.
(41) Hummers, W. S.; Offeman, R. E. *J. Am. Chem. Soc.* **1958**, *80*, 1339−1339.