A Graphene–Conjugated Oligomer Hybrid Probe for Light-Up Sensing of Lectin and *Escherichia Coli*

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With the rapid development of nanotechnology, many more nanomaterials, including quantum dots,[1] carbon nanotubes,[2] and gold nanoparticles,[3] are being integrated with biomolecules and subsequently applied for advanced biological applications in biosensing, bioimaging, and gene delivery. Graphene oxide (GO), a 2D carbon nanosheet generated from oxidation of graphene, has recently emerged as a unique nanomaterial that is competent for all these vital applications.[4] The broad biological usability of GO results from its heterogeneous plane structure featuring ionic and aromatic components that facilitate chemical bonding and non-covalent interacting with a variety of biomolecules.[5] Particularly, the sp^2 aromatic domains within GO induce efficient fluorescence quenching of nearby fluorescent substances via fluorescence resonance energy transfer (FRET) or non-radiative dipole–dipole coupling,[6] ultimately enabling GO to serve as an optical basis for fluorescent light-up biosensing.[7]

Despite the existence of several GO-based bioassays, they largely rely on commercially available dye-labeled DNA, peptide, and protein as the probe.[8–10] Due to the limited biorecognition capability of these probes, the targeting species are constrained to a few simple biomolecules such as DNA,[9] helicase,[10] adenosine-5′-triphosphate (ATP),[11] and thrombin.[12] In addition, because of the charged nature of these probes, non-specific electrostatic interaction between the probes and charged biomolecules inevitably occurs during assay operation, which is likely to decrease selectivity. To further advance GO-based bioassays, customized neutral probes with fluorescence that can be efficiently quenched by GO, while simultaneously having high specificity toward complicated biomolecules are in high demand.

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DOI: 10.1002/adma.201102227

Conjugated polymers (CPs) are fluorescent macromolecules with advantages over traditional chromophores including large absorption extinction coefficient, high photostability, and efficient energy and charge transfer.[13] Due to their highly electron-delocalized backbones, CPs were found to show very strong π–π interaction with GO similar to that for other molecules such as DNA, peptides, and small organic chromophores,[12,14] potentially leading to high quenching efficiency.[15] In addition, the side chains of CPs can be facilely functionalized with neutral and hydrophilic groups to endow biorecognition capability and water-solubility, respectively.[16] With these desirable features, functionalized CPs are good candidates for constituting GO-based bioassays.

In this study, we report a GO-based bioassay using a conjugated oligomer as the water-soluble neutral probe for light-up detection of Concanavalin A (ConA) and *Escherichia coli* (*E. coli*). *E. coli* is one of the most dangerous pathogens that can cause serious food-borne diseases, therefore requiring quick and accurate detection.[17] As *E. coli* is covered with tiny finger-like projections containing an amino acid–sugar complex called lectin, the sugar groups with strong affinity toward lectin, such as α-mannose, are useful for *E. coli* detection.[18] Although sugar-substituted fluorescent CPs have been designed to study carbohydrate–protein interactions and perform lectin assays, these glycopolymers are of low water-solubility and the assay sensitivity is often limited by the high background fluorescence arising from non-specific interactions.[19] As such, we develop a conjugated oligomer (4,7-bis(9,9-bis(2-(2-(2-(2,3,4,5,6-pentahydroxyhexanal)-ethoxy)ethyl)fluorenyl)benzothiadiazole (FBT), **Figure 1**a) having a high density of α-mannose side chains and a relatively short backbone to increase water-solubility. By virtue of the fluorescence quenching capability of GO, the background fluorescence is low, which ultimately leads to efficient visual detection of ConA and *E. coli* with high sensitivity and selectivity. We believe that the new sensor design and the fundamental mechanisms demonstrated here should provide valuable information for further development of GO-based biological applications.

The conjugated oligomer FBT was synthesized using a post-functionalization strategy based on click chemistry.[20] The UV-vis and photoluminescence (PL) spectra of FBT in 25 mM phosphate buffer containing 0.1 mM Ca^{2+} and Mn^{2+} (PB buffer) are shown in Figure 1b. FBT exhibits two character absorption peaks at 314 nm and 412 nm, which have been assigned to fluorene and benzothiadiazole, respectively.[20] The yellow aqueous solution of FBT has excellent stability at room temperature. Upon excitation at 350 nm, FBT exhibits green fluorescence with an emission maximum at 595 nm. The quantum yield of FBT in PB buffer is ≈0.35, measured using quinine sulfate in 0.1 M H_2SO_4 (quantum yield = 0.55) as the standard.

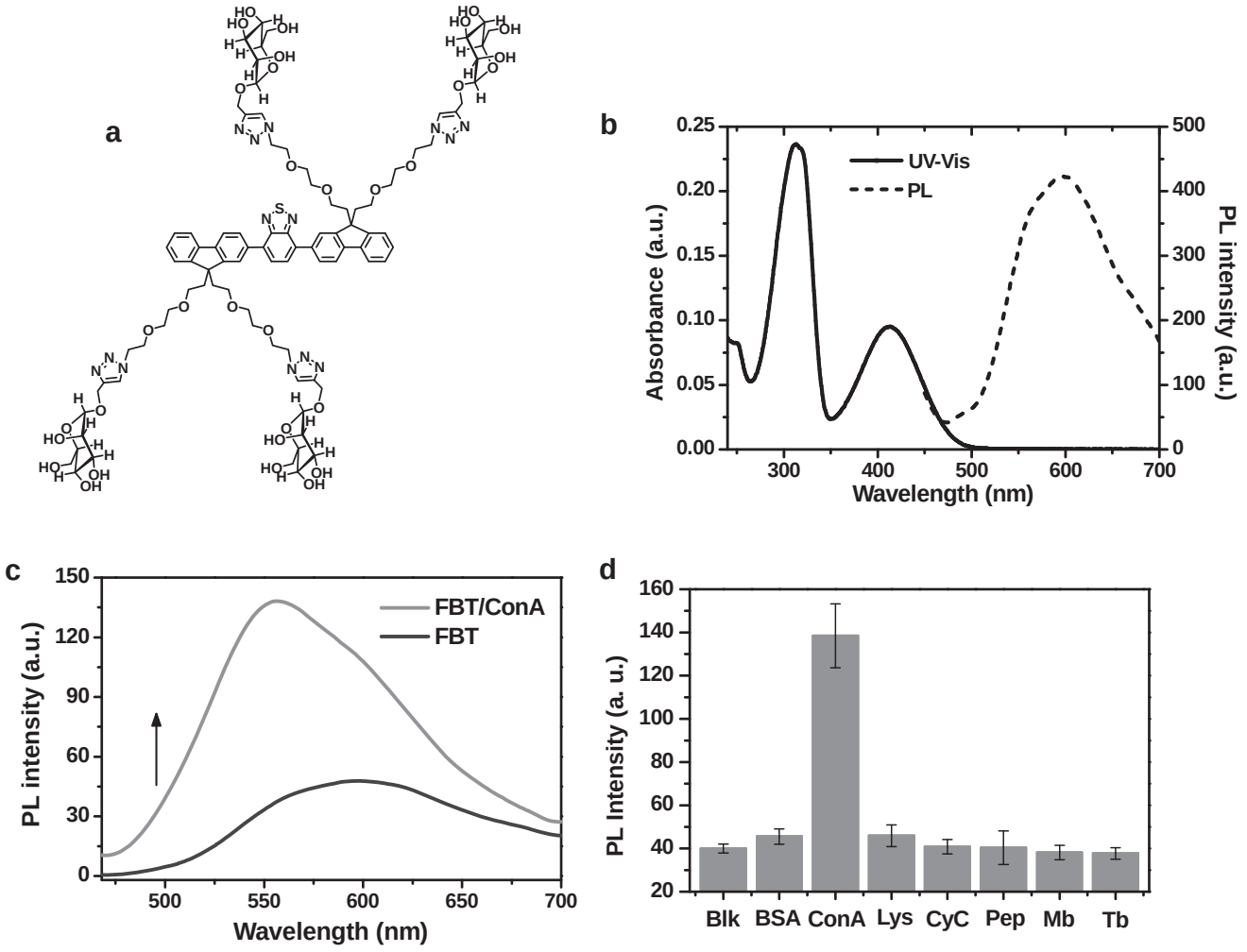


Figure 1. a) Chemical structure of FBT. b) UV-vis and PL spectra of FBT in PB buffer. FBT concentration, [FBT],= 3.0 μM for UV-vis and 0.1 μM for PL measurement. Exitation at 420 nm. c) PL spectra of FBT in the absence and presence of ConA in PB buffer. d) Selectivity analysis with various proteins. [FBT] = 20 nM, [protein] = 20 nM for (c) and (d).

The fluorescent response of FBT toward ConA was studied first. ConA is a mannose-specific lectin with four identical subunits that each contain a single small mannose binding site.[21] As illustrated in Figure 1c, the emission intensity of FBT (20 nM) at 595 nm increases by approximately threefold upon addition of ConA (20 nM), which is concomitant with the blue-shift of emission maximum from 595 nm to 555 nm. Previous reports have revealed that 2,1,3-benzothiadiazole (BT) emission is sensitive to environmental polarity due to its charge-transfer electronic states, and the BT emission intensity increases as the environment becomes more hydrophobic.[22] Since mannose binds to ConA through hydrogen bonds, hydrophobic interactions, and coordination with metal ions,[23] FBT molecules are brought to the proximity of the hydrophobic pocket of ConA. Under such circumstances, the environment of FBT becomes more hydrophobic, which leads to blue-shifted and enhanced fluorescence of FBT in the presence of ConA.

Figure 1d displays the PL intensity of FBT at 555 nm in the presence of various proteins, including ConA, bovine serum albumin (BSA), lysozyme (Lys), cytochrome *c* (CyC), pepsin (Pep), myoglobin (Mb), trypsin (Try), and thrombin (Tb). PB buffer was used as blank (Blk). The selectivity of FBT was evaluated by measuring the PL intensity ratio of target to nontarget (F_T/F_{NT}), where F_T, F_{NT} are the PL intensities of ConA and noncognate proteins, respectively. The selectivity ratio is typically in the range of approximately 2.4 to 3. 6. This study indicates that the fluorescence change of FBT is only specific for ConA, while other noncognate proteins do not significantly alter its fluorescence. Despite the effectiveness of FBT in ConA sensing, the relatively high background emission of FBT limits the assay selectivity.

GO was synthesized from microwave-expanded graphite by a modified Hummers method.[15,24] Before integration of GO with FBT to form a hybrid probe, the quenching of FBT by GO is examined. **Figure 2**a shows the PL spectra of FBT (20 nM) upon addition of GO. The PL intensity of FBT gradually decreases with increasing GO concentration. The Stern–Volmer constant (K_{SV}) of GO to FBT was calculated according to the equation, $F_0/F = 1 + K_{SV}$ [quencher], where F_0 and F are the PL intensities at 555 nm for FBT in the absence and

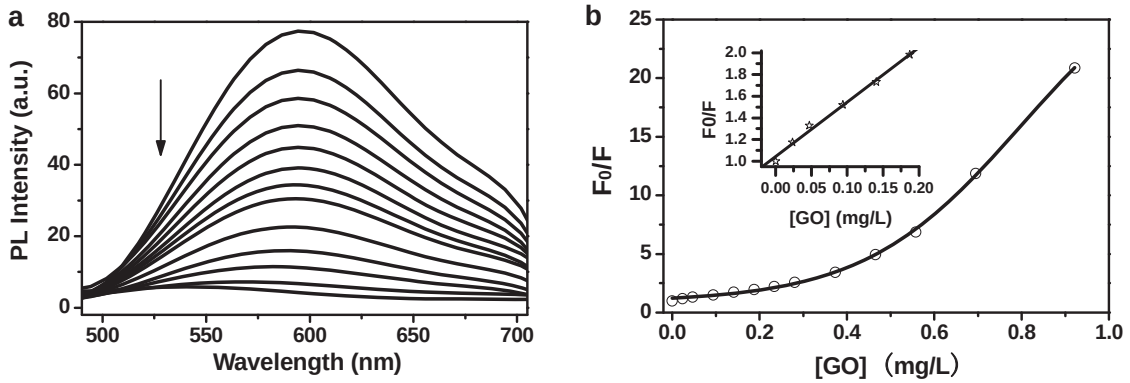
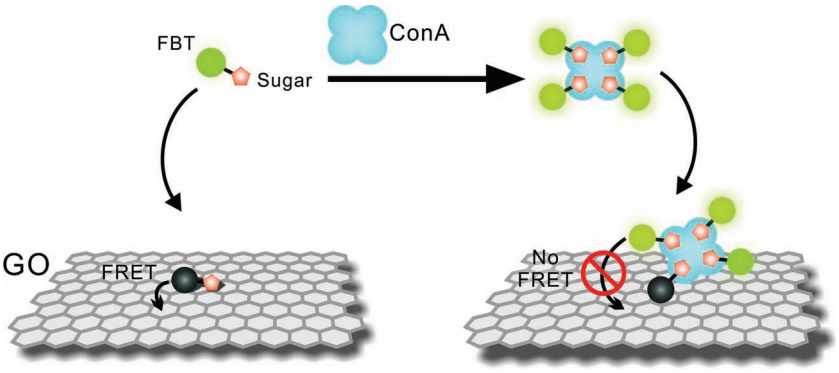


Figure 2. a) PL spectra of FBT in the presence of various concentrations of GO. [FBT] = 20 nM, [GO] = 0, 0.0235, 0.047, 0.094, 0.141, 0.188, 0.235, 0.282, 0.376, 0.47, 0.564, 0.705, and 0.94 mg L^{-1} (from top to bottom). b) The Stern–Volmer plots of FBT quenched by GO deriving from (a).

presence of GO, respectively. As shown in Figure 2b, the Stern–Volmer plot exhibits upward curvature as the concentration of GO increases. The upward Stern–Volmer plots are widely observed for water-soluble CPs, which is ascribed to the quencher-induced aggregation at high quencher concentration, which contributes to the self-quenching of CPs.[16c] To estimate the intrinsic quenching ability of GO toward FBT, the linear range of the Stern–Volmer plot (inset of Figure 2b) is used to calculate K_{SV}. Since the molar concentration of GO cannot be estimated due to its polydispersity, we only provide a K_{SV} value of 5.024×10^4 L g^{-1} in mass concentration. Furthermore, at GO concentration, [GO], = 0.94 mg L^{-1}, the fluorescence of the GO and FBT solution is as low as the background, which can be considered as almost non-fluorescent. Similar to the previous theoretical and experimental studies,[7a,24] the quenching ability of GO to FBT should be mainly attributed to strong π–π interaction between FBT and GO, although the upward Stern–Volmer plot suggests that GO-induced aggregation of FBT is also contributive. The strong interaction between GO and FBT is also verified by atomic force microscopy (AFM) measurements (Figure S1, Supporting Information). The average single-layer thickness of GO is increased by 0.232 nm in the presence of FBT.

Given that the background emission of FBT could be greatly suppressed by GO, we then integrated GO with FBT to form a hybrid probe for ConA sensing. A post-mixing strategy was employed in the sensing process, i.e., FBT was incubated with ConA prior to GO addition under optimized conditions. We reason that specific binding between bulky ConA to FBT should significantly prevent GO/FBT contact (**Scheme 1**). Since GO-based quenching follows long-range nanoscale surface resonance energy transfer that exhibits a (distance)$^{-4}$-dependent quenching efficiency,[25] the binding process may switch from the high-quenching (almost non-fluorescent) state to the low-quenching (highly fluorescent) state in the presence of ConA rather than other non-specific proteins. As such, ConA is expected to be easily distinguished from other proteins.



Scheme 1. The principle of ConA detection with the GO/FBT hybrid probe.

As demonstrated in **Figure 3**a, the PL intensity of FBT (20 nM) in the absence of ConA was greatly quenched (>95%) by 0.94 mg L^{-1} of GO. In contrast, the fluorescence quenching of FBT in the presence of ConA was only 33% of its original intensity. More importantly, as a result of the GO-suppressed background, the signal-to-noise (F_{ConA}/F_{Blank}) ratio increased from approximately 2.4 to 3.6 for FBT to 20 for the GO/FBT hybrid probe (Figure 3b). Therefore, improved selectivity and high discrimination of target molecule from other interference proteins were easily obtained by the GO/FBT hybrid probe.

To gain insight into the interactions among FBT, GO, and ConA, real-time monitoring of the fluorescence quenching of FBT by GO at 555 nm was conducted in the absence and presence of ConA. As shown in Figure 3c, the fluorescence of FBT is almost completely quenched by GO within 10 s in the absence of ConA, while the quenching kinetics for the FBT/ConA complex by GO is slow and nearly half of the fluorescence remains after 10 min. The kinetic difference clearly proves that the specific protein–carbohydrate interaction between FBT and ConA screens the π–π interaction between FBT and GO. As such, the contact between GO and FBT is substantially inhibited in the presence of ConA, leading to less quenched fluorescence.

The remarkable quenching efficiency of GO provides the possibility to visually identify ConA with GO/FBT probe. Figure 3d displays the photographs of both FBT (top row) and GO/FBT (bottom row) solutions under UV radiation at 365 nm in the

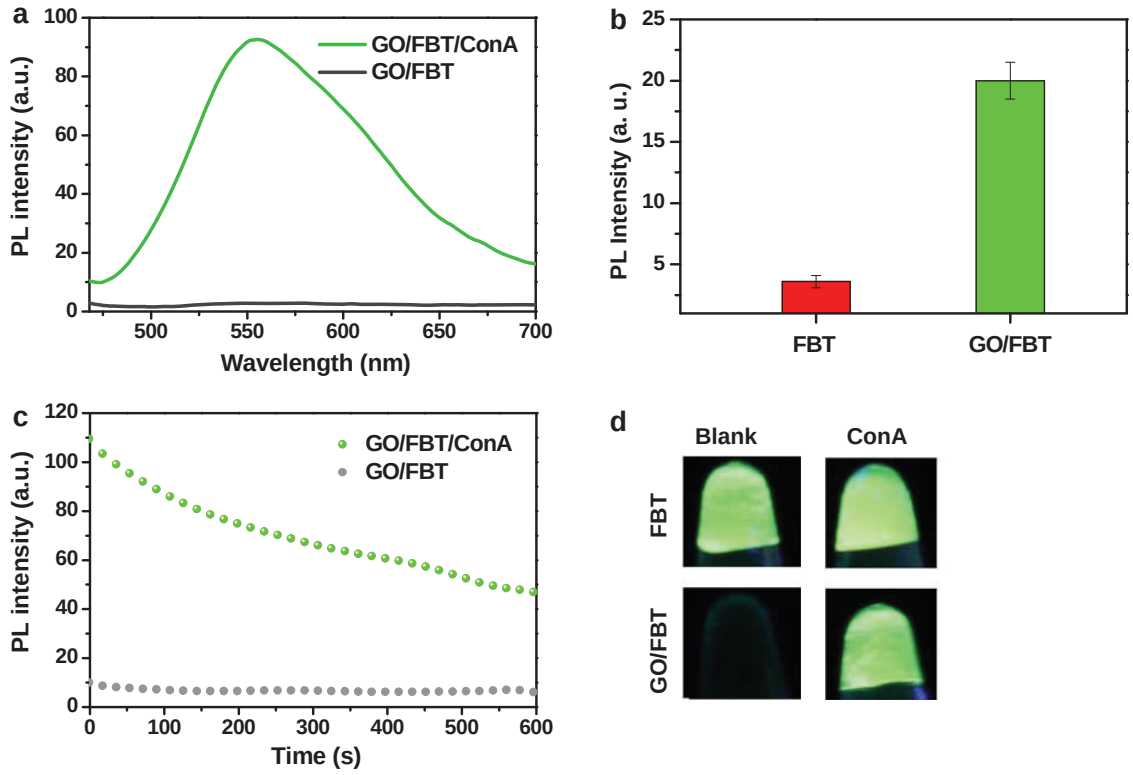


Figure 3. a) In the presence of GO, the PL spectra for FBT in the presence and absence of ConA. b) The comparison of signal-to-noise ratio of FBT versus GO/FBT probe. c) Kinetic studies for the fluorescence change of GO/FBT ([FBT] = 20 nM, [GO] = 0.94 mg L^{-1}) in the presence and absence of ConA (20 nM). d) Photographs for ConA detection based on FBT and GO/FBT ([ConA] = 2.63 μM, [FBT] = 10.52 μM, and [GO] = 124 mg L^{-1}).

absence and presence of ConA, respectively. The solution of FBT (10.52 μM) emits bright green fluorescence itself, and addition of ConA (2.63 μM) leads to slightly intensified fluorescence, which is not possible for visual detection. In contrast, the dark fluorescence of GO/FBT solution ([FBT] = 10.52 μM and [GO] = 124 mg L^{-1}) significantly lights up upon addition of ConA (2.63 μM). Therefore, this hybrid probe forms the basis for mix-and-detect of ConA with visual readout.

The performance of GO/FBT probe is also examined in terms of ConA quantification and assay selectivity. **Figure 4**a provides a calibration curve for ConA detection with the GO/FBT probe. The PL intensity at 555 nm intensified with increasing ConA concentrations. This assay has a linear range of 0–50 nM for ConA. As shown in Figure S2 (Supporting Information), the deteciton limits for GO/FBT and FBT are measured to be ≈0.5 nM and ≈1.0 nM, using a standard fluorometer. This reflects that the sensitivity for ConA detection is also enhanced by intergration of GO. The specificity of this assay was also evaluated with various proteins. As illustrated in Figure 4b, there is only minimal fluorescence increase in the presence of the interference proteins, while significant fluorescence enhancement is observed for ConA. The fluorescence ratio of F_T/F_{NT} is calculated to be in the range of approximately 16 to 28, representing an order of magnitude improvement in selectivity over FBT itself (≈2.4–3.6). Therefore, the GO enhanced assay is of excellent sensitivity and selectivity for ConA detection, which has potential applications in pathogen diagnosis and cell biology.

With the established assay for mannose-binding ConA, we further study and compare the performance of FBT and GO/FBT probe for bacterial detection using *E. coli* strains (MG1655 and Top 10). MG1655 produces Type 1 fimbriae containing the fimH protein that possesses carbohydrate recognition sites (CRS) with high affinity for mannose (dissociation constant K_d = 2.3 μM),[26] while the other *E. coli* strain, Top10, does not produce Type 1 fimbriae. As shown in Figure 4c, d, MG1655 could bind to FBT to result in high PL intensity. However, the Top 10 strain exhibits nearly the same PL intensity as that induced by MG1655, which leads to poor selectivity for target determination. Using the GO enhanced strategy, the F_T/F_{NT} ratio increased from 1.1 (without GO) to 2.2 (with GO), making discrimination between the two *E. coli* strains feasible.

In conclusion, we have customized a neutral probe (FBT) based on a conjugated oligomer that is hybridized with GO to constitute a novel light-up probe for detection of ConA and *E. coli*. Although FBT binds specifically to a lectin protein, ConA, with high selectivity over noncognate proteins, it is not ideal for visual sensing due to the high background signals. Integration of GO can almost completely quench the fluorescence of FBT as a result of π–π interaction between GO and FBT. Such interactions can be inhibited by specific protein–carbohydrate interaction between FBT and ConA, and the quenched florescence of GO/FBT is greatly recovered in the presence of ConA. As a result, this hybrid probe has the significant advantage of light-up visual discrimination of ConA from other proteins, which

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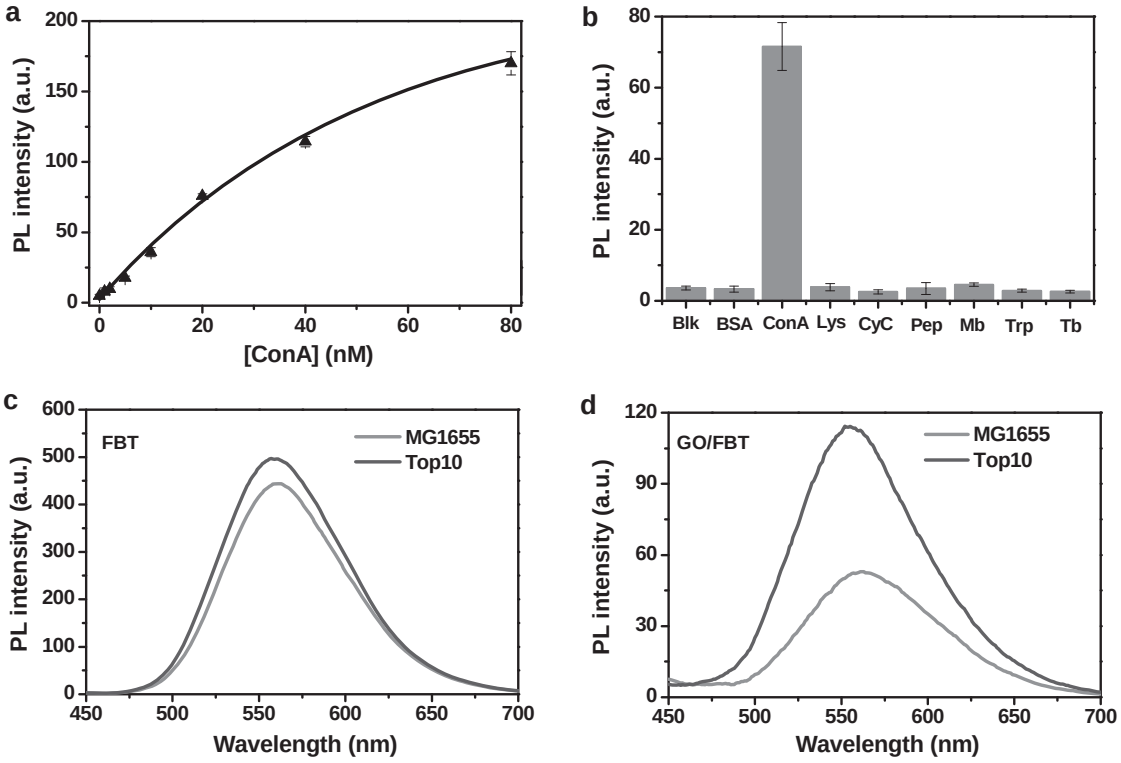

Figure 4. Fluorescence of GO/FBT hybrid probe in the presence of a) different concentrations of ConA (0, 1, 2, 5, 10, 20, 40, and 80 nM) and b) ConA and noncognate proteins 20 nM each. The PL spectra of c) FBT and d) GO/FBT hybrid probe upon binding with mannose-positive MG1655 and mannose-negative Top 10 strains.

also allows quantification of ConA in the range of 0–50 nM with a detection limit of ≈0.5 nM. This specific protein–carbohydrate interaction induced a light-up response of GO/FBT probe also makes it useful for distinguishing different *E. coli* strains. This study highlights that integration of π-conjugated system with specific affinity groups to GO is an effective strategy to develop novel GO-based assays. Given the advantages arising from the combination of probe design and novel nanomaterials, we expect this assay could form a generic platform for advanced molecular sensing with a high promise in practical applications.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements

L.H.W. and K.Y.P. contributed equally to this work. The authors are grateful for the support from the Defense Research Innovative Program of Singapore, Singapore Ministry of Defense (R279-000-301-232), the National University of Singapore (R279-000-301-646, R-279-000-340-232), the Science Foundation of China (21073221, 90913014 and 20725516), Ministry of Science and Technology (2007CB936000), CAS (KJCX2-EW-N03), the K. C. Wong education foundation in Hong Kong, and AcRF 2010-T2-1-060 from MOE, NRF-CRP2-2007-01 from NRF, No. 092 101 0064 from A*STAR, CREATE program from NRF, New Initiative Fund FY 2010 (M58120031) from NTU in Singapore. The authors also thank Dr. J. B. Shi for monomer synthesis.

Received: June 14, 2011
Revised: July 13, 2011
Published online: August 23, 2011

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