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ARTICLE *in* JOURNAL OF THE AMERICAN CHEMICAL SOCIETY · FEBRUARY 2004

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Reversible Modulation of Quantum Dot Photoluminescence Using a Protein-Bound Photochromic Fluorescence Resonance Energy Transfer Acceptor

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Nanotechnology explores the interface between biology and materials science in pursuit of functional nanodevices. Hybrid devices consisting of biomolecules coupled to inorganic opticoelectronic elements are attractive since the optoelectronic element can record and transduce the biological function.[1] Light-driven devices are of particular interest since the wavelength, intensity, and exposure to light can be readily controlled.[1,2] These hybrid technologies may function in nanosensing, computational devices, and as nanoprosthetic parts, and the toolbox for building these devices must be assembled. We report on a hybrid organic−inorganic light-driven modulator of quantum dot (QD) emission that may function as an element of this nanotoolbox. Colloidal luminescent semiconductor QDs have unique spectroscopic properties and several inherent functional advantages compared to organic dyes including size-dependent, tunable absorption, and photoemission properties with narrow photoemission spectra as well as high resistance to photobleaching.[3] Using engineered proteins, we have previously demonstrated their utility in numerous immunoassays and have utilized QDs as the fluorescence resonance energy transfer (FRET)-donating portion of a prototype hybrid inorganic−organic sensing nanoassembly that functions in sugar detection.[3,4a,5] We found that QDs not only function as efficient FRET donors, but also act as effective and integral scaffolds for these biosensing nanoassemblies.[4a,5]

Photochromic BIPS (1′,3-dihydro-1′-(2-carboxyethyl)-3,3-dimethyl-6-nitrospiro-[2H-1-benzopyran-2,2′-(2H)-indoline]) has functioned as a reversible FRET acceptor when conjugated to a Lucifer Yellow (LY) energy donor with a FRET efficiency approaching 100% in organic media.[6] Exposure to white light/UV light catalyzes the reversible photoconversion of BIPS from the colorless spiropyran (SP) to the colored merocyanine (MC) form which functions as the FRET acceptor.[6] Herein, we labeled maltose binding protein (MBP) with BIPS, coordinated multiple copies of this labeled MBP to individual QDs, and then used the photochromic BIPS properties to modulate QD emission, see Figure 1A. Sulfo-*N*-hydroxysuccinimide-activated BIPS (sulfo-*NHS*-BIPS) was synthesized as described (Figure 1).[6b] Mass spectral analysis confirmed the synthetic product. Maltose binding protein appended with a C-terminal pentahistidine sequence (5-HIS) was purified as described.[3d,4] Since the mechanism of MBP 5-HIS to QD surface Zn coordination is well understood, MBP can be exploited for protein−QD bioassembly, and it remains functional when surface-coordinated to QDs.[4a,b,5] MBP was prepared for labeling with sulfo-*NHS*-BIPS by dissolving 1−2 mg of protein in 0.1 M HEPES (pH 8) and adding 10% DMSO. The sulfo-*NHS*-BIPS was dissolved in DMSO, added to the protein/DMSO solution, and allowed to react for 1 h at room temperature in the dark. Labeled protein was separated from free label with a Bio-gel P6 (BioRad) or PD-10 (Amersham) column. Adjustment of protein concentration and reaction volumes allowed

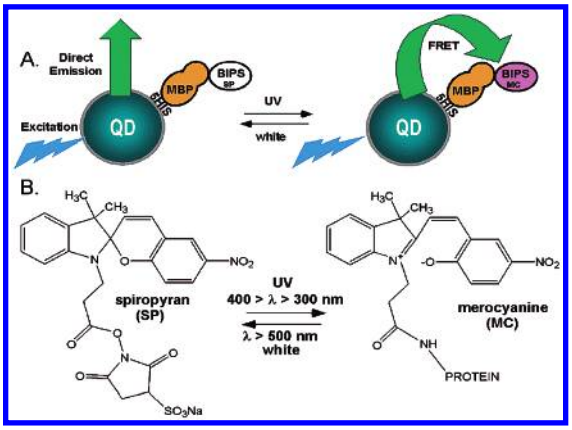

Figure 1. (A) Schematic representation of QD modulation by MBP−BIPS. When BIPS is converted to the MC form by UV light, the QD emission is reduced through FRET quenching. After exposure to white light and photoconversion to MBP−BIPS-SP, the QD direct emission is substantially increased. Although each QD in these nanoassemblies is coordinated to 20MBPs, a single QD−MBP−BIPS is presented for clarity. (B) Structure of sulfo-NHS-BIPS in the SP form preconjugation (left) and BIPS-MC shown post conjugation to protein, for brevity (right).

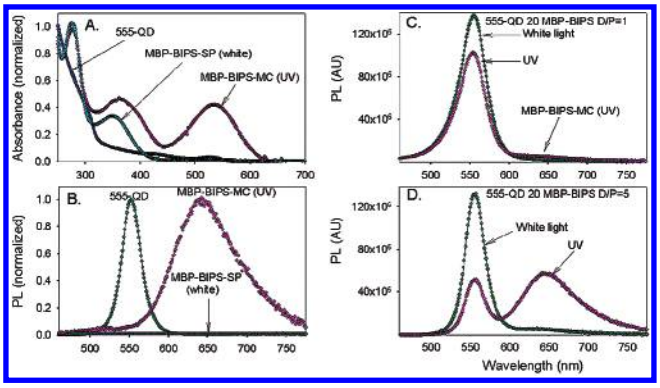

Figure 2. Spectral properties and modulation function of MBP−BIPS and the 555 nm emitting QDs. (A) Absorption spectra of MBP−BIPS after conversion to SP form followed by photoconversion to MC form. Absorbance spectra of 555 nm QD overlaid. White indicates exposure to visible light and the same for UV (B) Emission spectra of MBP−BIPS-MC, MBP−BIPS-SP, and 555 nm QD. Samples were excited at 440 nm and spectra collected from 460 to 775 nm. Photoluminescence (PL) in arbitrary units-AU (C) PL spectra of the QD−MBP−BIPS after BIPS SP−MC photoconversion. 555 QD−20MBP−BIPS/QD with MBP D/P = 1. (D) BIPS SP−MC photoconversion for QD−20MBP−BIPS/QD with MBP D/P = 5, (Optical density < 0.1).

labeling at two different dye-to-MBP ratios; D/P = 1 and D/P = 5. Ratios were determined using MBP (69 000 M^{-1} cm^{-1} at 280 nm) and BIPS-SP (40 000 $M^{-1}cm^{-1}$ at 350 nm) molar extinction coefficients.[4,6]

Figure 2A,B shows the absorption and emission spectra of the 555-QD and MBP−BIPS (SP and MC). CdSe−ZnS core−shell QDs were synthesized and made water soluble with dihydrolipoic

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10.1021/ja037970h CCC: $27.50

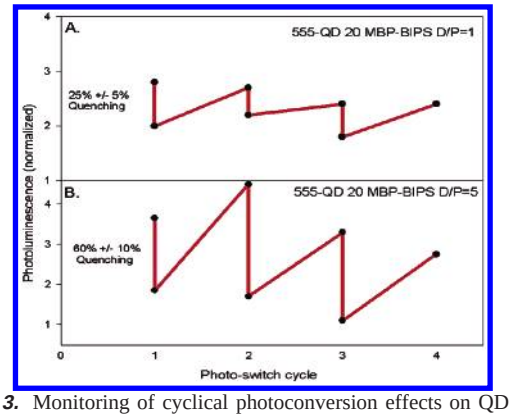

Figure 3. Monitoring of cyclical photoconversion effects on QD photoluminescence (initial white light to UV). (A) 555 QD−20MBP−BIPS/QD nanoassembly with MBP D/P = 1. (B) 555 QD−20MBP−BIPS/QD nanoassembly with MBP D/P = 5. PL monitored at 555 nm. Excitation at 440 nm, optical density < 0.1.

acid (DHLA) as described.[3] MBP protein appended with a C-terminal 5-HIS sequence is specifically coordinated to QDs in a controlled self-assembly process, through histidine−QD shell zinc coordination.[4a,b] Thirty pmol of 555 nm emitting QDs were mixed with the molar equivalent of 20MBP−BIPS per QD (essentially surface saturating), and complexes were allowed to self-assemble for 1 h in 250 μL of 10 mM Na tetraborate, 10 mM NaCl buffer (pH 9.55).[4] These solutions were stable for at least 8−12 h. Quantum dot photoemission in these nanoassemblies was modulated as presented schematically in Figure 1A. The QD−MBP−BIPS was exposed to 180 s of white light (>500 nm, Fiber-Lite High Intensity Illuminator, Dolan Jenner Ind. Inc., St. Lawrence, MA) to convert the BIPS into the SP form; the nanoassembly excited at 440 nm, and emission spectra were measured from 460 to 775 nm using a SPEX fluorometer.[4] The BIPS-SP in the QD−MBP−BIPS nanoassembly was then photoconverted to the MC form by exposure to 180 s of UV light using a Blak-Ray UV-365 nm lamp (UVP, Upland CA).

Figure 2C,D presents the QD emission profiles of these modulated nanoassemblies for both MBP-labeling ratios. Figure 3 shows QD emission spectra of both these modulated nanoassemblies subjected to repeated cyclical photoconversion. FRET efficiencies (E) derived from QD quenching, were estimated using:

$$E = (F_{DA}/F_D) \times 100$$

F_D is the relative fluorescence intensity of the donor in the absence of acceptor, and F_{DA}, in the presence of acceptor.[7] QD−MBP−BIPS with D/P = 5 averaged 60 ± 10% QD quenching while D/P = 1 nanoassembly averaged 25 ± 5%. These values represent an upper limit for FRET efficiency since non-FRET mechanisms may also contribute to QD PL loss.[5] FRET efficiencies of 20−95% have been reported for light-harvesting linear polymers and dye-labeled poly(aryl ether) dendrimers.[8] The data indicate that dynamic PL range decreases with increasing number of switching events. Assemblies were tested up to 10 switching events with a loss of ~10−20% PL over the cycles. This loss is under investigation and is believed to be caused by an increase in QD surface charge when exposed to intense prolonged light sources, which would alter FRET properties. BIPS dye−protein interactions are not understood and may contribute as well. Increasing the number of energy acceptors per QD donor increases overall FRET efficiency compared to that of complexes with a single QD donor−acceptor FRET pair. Binding of MBP−BIPS-MC/QD in the D/P = 5 nanoassembly substantially increases the effective acceptor extinction coefficient relative to the D/P = 1 case; therefore, multiple acceptors in each complex proportionally increase the effective overlap integral for single QD donor−multiple acceptor complexes.[4a,5] For the 20MBP/QD D/P = 5 nanoassembly, ~100 BIPS moieties surround each QD compared to 20BIPS/QD in the D/P = 1 case. Hybrid bionanoassemblies in which a single energy donor is surrounded by ~100 acceptors are unparalleled. The R_0 (Forster distance for 50% energy transfer[7]) for the 555 QD MBP−BIPS-MC (555 QD quantum yield 23.5% when coordinated to MBP) is 38.5 Å, nearly identical to the R_0 of 35−38 Å determined for LY−BIPS.[6,7] Separation distances are substantially larger here than the 20−22 Å of LY−BIPS, owing to overall 555 QD size (diameter = 70 Å), and thus LY−BIPS has better FRET efficiency.[4,5] Since MBP is randomly labeled on lysine residues by means of *NHS* chemistry, we estimate that BIPS/QD (center-to-center) distance ranging from 35 to 100 Å can occur, considering MBP molecular dimensions of ca. 30 Å × 40 Å × 65 Å.[4,5] More importantly, BIPS did function as a modulator even in the D/P = 1 nanoassemblies; therefore, it must be closer to the QD surface rather than further away. Evidence suggests that MBP is preferentially labeled by NHS esters on lysine 42 in the binding pocket and lysines 141, 143, and 145.[4b] This would place the BIPS at ~55 or ~45 Å from the QD core, respectively. Indeed the results seem to corroborate these less than optimal placements; again, the multiple acceptors in each complex help to overcome this.

In conclusion, we have demonstrated reversible modulation of QD emission using a photochromic dye attached to a QD-coordinated protein. Since QDs can be excited at almost any discrete wavelength short of their emission, excitation wavelengths could be adjusted without interfering with photochromic switching. As demonstrated, the level or efficiency of modulation can be adjusted by increasing the number of BIPS attached to each protein and therefore to each QD. Alternatively, different ratios of labeled to unlabeled protein could be controllably assembled on the QD,[4a,5] or specific cysteine mutants implanted within the protein could provide better control of labeling placement(s) and thus effectively control R_0 (requiring a thiol-reactive BIPS). We anticipate that photochromically switched devices or sensors, where QD emission modulation presets the device below some predetermined critical threshold, will be possible using these types of nanosensors.

Acknowledgment. We thank Aaron R. Clapp for discussion, and Michael E. Lassman for assistance with MS analysis. I.L.M. is supported by a National Research Council Fellowship through the Naval Research Laboratory. H.M. and J.M.M acknowledge K. Ward at the Office of Naval Research (ONR) for research support and Grant N001400WX20094 for financial support.

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JA037970H