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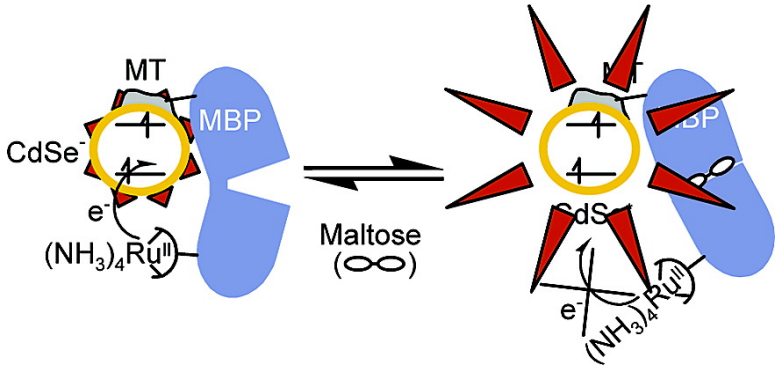

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A Modular Nanoparticle-Based System for Reagentless Small Molecule Biosensing

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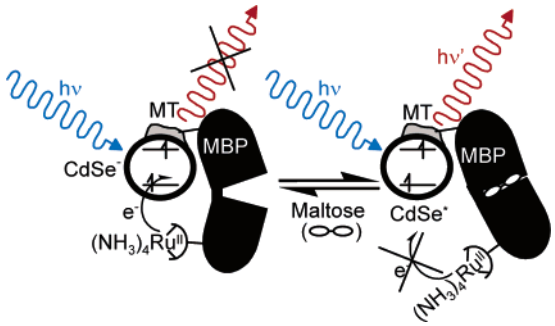
Semiconducting nanoparticles are a highly luminescent, photostable class of fluorophores that have recently been used for sensing applications.[1] Biopolymer-tethered, semiconducting nanoparticles have been used as in vivo localization probes[2] and ex vivo oligonucleotide detectors.[3] These semiconducting nanoparticle biosensors take advantage of the molecular recognition properties of biomolecules while retaining the detection limits and photostability of semiconducting nanoparticles. Small molecule sensing with biopolymer-attached semiconducting nanoparticles has remained a difficult goal. Recently, maltose and trinitrotoluene sensing by protein-bound CdSe/ZnS nanoparticles overcome this difficulty.[4] This method displaces a ligand-tethered fluorescence emission acceptor molecule to confer fluorescent signaling.[4] Unfortunately, displacement and other multimolecular sensing strategies are susceptible to diffusion- and dilution-based interferences. Unimolecular, or reagentless, biosensors avoid such interferences.[5a] Here we present a facile, reagentless method for generating protein-based semiconducting nanoparticle sensors for small molecules.

Maltose binding protein (MBP) is typically used for biosensor method development.[5] MBP undergos a reversible, ligand-dependent conformational change, similar to all members of the bacterial periplasmic binding protein superfamily.[6] These movements have been harnessed for maltose sensing either by differentially opening/closing crevices on the protein surface[5a−c] or by using lever-action molecular displacement.[5d,e] The approach reported here employs the lever-action strategy to alter the interaction between an MBP-attached [(tetraamine)(5-maleimido-phenanthroline)ruthenium]-$[PF_6]_2$ (**1**) and the surface of a water-soluble CdSe nanoparticle. This system provides reagentless, selective detection of maltose by changing the interaction between **1** and the CdSe nanoparticle surface in a distance-dependent fashion (Scheme 1). Since CdSe nanoparticle anions are nonfluorescent,[7] electron transfer from **1** to the valence-band hole of the CdSe nanoparticle excited-state is suggested as the sensing mechanism, similar to photoconductivity biosensors.[8]

Chimeric MBP-metallothionein proteins (MBP-MTs) with surface cysteine residues allow for orthogonal, site-specific attachment of MBP to both **1** and thiolhexadecanoate (THDA) capped CdSe nanoparticles. This site-selective derivatization has been reported for N282C MBP-MT attachment to a Bodipy 577/618 maleimide fluorophore and CdSe, CdSe/ZnS core−shell, or Au nanoparticles.[9] The K25C, K46C, Q72C, and N282C surface mutant MBP-MTs were used in this system and generated by standard recombinant DNA methods. All surface cysteine MBP-MTs were bacterially expressed, purified by affinity chromatography, and characterized by MALDI mass spectrometry. Complex **1** was synthesized by a route similar to that of a previous method.[5c] This approach[5c,d,6] allows for rapid **1**−MBP-MT attachment by surface cysteine nucleophilic attack of the maleimide functional group. Thus, **1** is tethered to MBP-MT by the maleimide, leaving the Ru^{II} ion coordination sphere to freely interact with the CdSe nanoparticle surface. Attachment of complex **1** to the surface cysteine of each mutant MBP-MT was performed in a site-specific fashion using Cd^{2+} ion protection of the eleven metallothionein-domain cysteines, reported previously.[9] After Cd^{2+} ion chelation, the metallothionein-domain cysteines were available to coordinate the water-soluble CdSe nanoparticles and displace the THDA capping groups, which was monitored by fluorescence.[9] Kinetic analysis of **1** modified MBP-MTs binding to 3.0−3.5 nm diameter THDA capped CdSe nanoparticles saturated after 1 h with a 5-fold decrease in the fluorescence emission intensity. By contrast a 2-fold increase in fluorescence emission intensity was observed upon MBP-MT binding to 3.0−3.5 nm diameter THDA capped CdSe nanoparticles.[9] The affinity of complex **1** modified MBP-MTs was found ($K_A = 1.9 \times 10^7\ M^{-1}$, $K_D = 53$ nM) to be similar to the affinity of MBP-MTs for THDA capped CdSe nanoparticles.[9]

Scheme 1. Maltose-Dependent Change in CdSe Emission



The significantly different emission intensities observed for MBP-MTs binding to THDA capped CdSe nanoparticles suggested different MBP−CdSe interactions. Since the affinities of **1** modified and unmodified MBP-MTs for THDA capped CdSe nanoparticles are similar, no change in Cd−Cys coordination is presumed. The different emission intensities are explained by strongly coupled **1**−CdSe interactions. While it is possible complex **1** alters the electrostatic interactions, the addition of 500 mM NaCl showed no change in CdSe emission intensity when **1** modified MBP-MT was attached. In the absence of **1** and the presence of 500 mM NaCl the fluorescence emission intensity decreased 1.3-fold, yielding less than a 1.5-fold increase with MBP-MT binding. These results argue against surface-charge mediated effects in the **1** modified MBP-MT case. Alternatively, electron transfer from **1** to the valence band of the CdSe excited state (Scheme 1), forming a nonfluorescent CdSe anion,[7] is consistent with the decrease in CdSe emission intensity.

Maltose-dependent changes in CdSe emission intensity were observed for **1** modified K46C MBP-MT bound to 3.0−3.5 nm diameter THDA capped CdSe nanoparticles (K46C system, Figure 1). A 1.4-fold increase in fluorescence intensity was observed upon maltose addition (Figure 1A). This change indicated a decrease in the **1**−CdSe electron transfer quenching (Scheme 1). The addition

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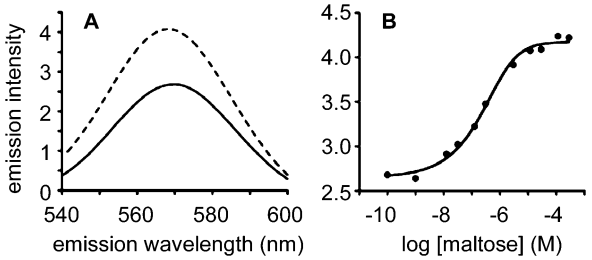

Figure 1. Maltose-dependent fluorescence of complex **1** modified-K46C MBP-MT attached to 3.0−3.5 nm diameter THDA capped CdSe nanoparticles ($\lambda_{ex} = 363$ nm) at pH 7.5 (20 mM 3-(*N*-morpholino)propanesulfonic acid, MOPS): (A) fluorescence emission spectra of a solution (5 nM biosensor) without (solid line) and with 1 mM maltose (dashed line); (B) fluorescence emission intensity (filled circles) versus maltose concentration, fit to a maltose binding isotherm[10] ($K_A = 1.9 \times 10^6$ M^{-1}, $F_o = 2.65$, $F_{max} = 4.17$, $R = 0.997$).

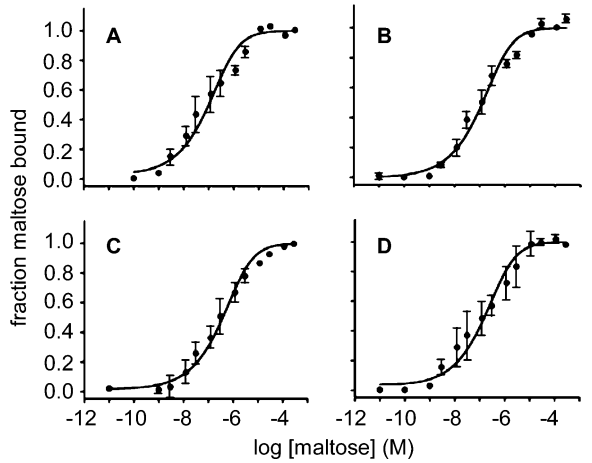

Figure 2. Reproducibility of maltose-dependent emission intensities (●) for four complex **1** modified MBP-MTs attached to 3.0−3.5 nm diameter THDA capped CdSe nanoparticles. Error bars denote standard deviations from three independent determinations, and lines denote fits of the average values to a maltose binding isotherm:[10] (A) K25C, $K_A = 4.8 \times 10^6$ M^{-1}, $K_D = 200 \pm 50$ nM, $R = 0.985$; (B) K46C, $K_A = 3.9 \times 10^6$ M^{-1}, $K_D = 250 \pm 53$ nM, $R = 0.991$; (C) Q72C, $K_A = 1.3 \times 10^6$ M^{-1}, $K_D = 780 \pm 120$ nM, $R = 0.994$; (D) N282C, $K_A = 3.0 \times 10^6$ M^{-1}, $K_D = 340 \pm 70$ nM, $R = 0.989$.

of 10 mM glucose or lactose to the K46C system showed no change in the fluorescence emission spectra. When maltose was added incrementally (Figure 1B), the change in fluorescence emission intensity fit[10] a profile consistent with a single maltose binding site isotherm. An association constant was determined from the average of three maltose titrations to be 3.9×10^6 M^{-1} (Figure 2B). This association constant is similar to the maltose−maltose binding protein affinity.[11] Therefore, the K46C system provides a selective, reagentless, protein-based nanobiosensor for maltose.

All four surface cysteine MBP-MT mutants were screened for maltose biosensing. Surprisingly, all mutant MBP-MT systems exhibited similar fluorescence enhancements (K25C, 1.4-fold; K46C, 1.4-fold; Q72C, 1.3-fold; N282C, 1.3-fold) upon maltose addition. Additionally, maltose affinities for each system were similar (K25C, 4.8×10^6 M^{-1}; Q72C, 1.3×10^6 M^{-1}; N282C, 2.9×10^6 M^{-1}, Figure 2). The only significant difference between each of these systems is the degree of sample-to-sample variation (Figure 2, error bars). K46C MBP-MT represents the most reproducible system. The universal fluorescent response of these systems is an important finding since the attachment sites are on different faces of the amino-terminal MBP domain. By contrast, similar sensors that use organic fluorophores require multiple surface cysteine mutation/fluorophore combinations to be screened before a viable sensor is identified.[12] Figure 2 clearly demonstrates that when using the lever-action strategy every surface cysteine MBP-MT mutant screened has a maltose-dependent response.

Each surface Cys attachment site in this MBP-MT system provided a ligand-dependent readout with CdSe fluorescence emission intensity. The general response of the four MBP-MT systems suggest this method will easily provide biosensors for other analytes (glucose, phosphate, histidine, etc.) by taking advantage of the ligand diversity of the bacterial periplasmic binding protein superfamily. The mechanism by which **1** modified MBP-MT provides maltose-dependent changes in CdSe emission intensity is, presumably, due to electron transfer from **1** to the valence band of photoexcited CdSe nanoparticles. Therefore, site-specific attachment of a Ru(II) complex and water soluble CdSe nanoparticles to MBP-MT provides a reagentless, selective, and reproducible protein-based nanobiosensor method for small molecule detection.

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Supporting Information Available: Sample preparations, maltose titrations, equation fitting, and control experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

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