

Maltose-Responsive Protein Nanoparticles: Using Ligand Gated Electron Transfer

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Biosensors are analytical devices that use the molecular recognition properties of biological macromolecules to report the concentration of molecules by rapid and sensitive analytical methods. Fluorescent tags, such as semiconducting nanoparticles, overcome limitations encountered by organic fluorophores by providing high quantum efficiency and high photobleaching threshold. A number of detection strategies have been reported using nanoparticle assemblies for ligand dependent signaling by energy transfer between a nanoparticle and an analyte attached to an organic fluorophore, but none provide a reagentless method for sensing. We will demonstrate that ligand-controlled electron transfer quenching is a viable and modular method for developing reagentless protein based nanobiosensors. This strategy will apply information gleaned from photo-induced electron transfer studies of metalloproteins. Metallothionein (MT), a cysteine rich peptide has been genetically fused to the carboxy-terminal domain of maltose binding protein (MBP), which enables the attachment of CdSe nanoparticles. On the amino terminal domain of MBP, four different surface cysteine point mutations have been generated (Q72C, N282C, K25C and K46C). A redox-active, thiol reactive metal complex, $[\text{Ru}^{\text{II}}(1,10\text{-phenanthroline-5-maleimide})(\text{NH}_3)_4][\text{PF}_6]_2$, was attached to the amino-terminal domain surface cysteines. Protection of the MT cysteines during this reaction was performed with Cd^{2+} ions, allowing for ruthenation at the surface cysteine of these mutant MBP-MTs, which are then attached to water soluble CdSe nanoparticles. Maltose dependent fluorescence enhancement in Ru-MBP-MT-CdSe nanoparticle assemblies (N282C, K46C, Q72C, and K25C MBP-MT) was observed and maltose affinities were determined for all four assemblies. We will report the progress to date and the potential for this method as a modular approach to detect a variety of organic compounds in aqueous environments.