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Simple Conjugation and Purification of Quantum Dot−Antibody Complexes Using a Thermally Responsive Elastin-Protein L Scaffold As Immunofluorescent Agents

U Loi Lao, Ashok Mulchandani, and Wilfred Chen*

Department of Chemical and Environmental Engineering, University of California, Riverside, California 92521

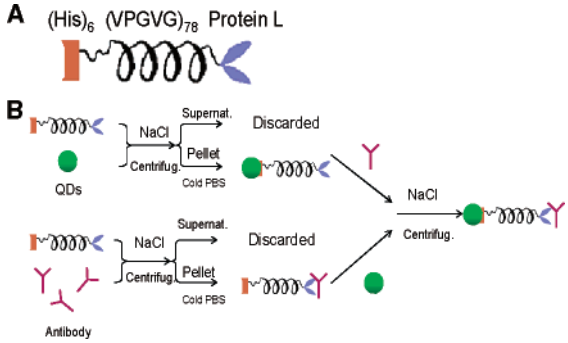
Received July 25, 2006; E-mail: wilfred@engr.ucr.edu

The exploitation of colloidal semiconductor nanocrystals, often referred to as quantum dots (QDs), in biological applications has increased dramatically since the first benchmark utility in 1998[1] because of their size-tunable spectral properties, high quantum yield, narrow photoemission spectra, and high resistance to photobleaching.[2] Simultaneous multiplex labeling and detection are also possible on the basis of the broad excitation spectra, enabling excitation of distinctive QDs at a single wavelength.[3] This unique property offers significant advantages over conventional organic fluorophores that have closely overlapping spectra and is ideal for detecting multiple analytes in high-throughput antibody arrays.[4] Methods based on covalent attachment of antibodies to QDs have been developed.[2] However, the relatively tedious preparation in combination with the resulting low quantum yield and low quantity[2] severely hinder their utility. Non-covalent bioconjugation of antibodies to QDs has been made possible on the basis of the electrostatic interaction between an engineered adaptor protein employing the immunoglobulin G (IgG)-binding domain of streptococcal protein G modified by a positively charged leucine zipper tag and the negatively charged capped QDs.[5] However, final purification of the conjugates requires affinity chromatography based on interaction between the maltose-binding protein (MBP) and amylose and involves multiple binding, washing, and elution steps. Additionally, the inability to be configured in a high-throughput setting on top of the cost-effectiveness concern of current chromatographic techniques provide a strong motivation for the development of an alternative bioconjugation and purification method.

Affinity precipitation based on thermally responsive polymers is an effective alternative to affinity chromatography,[6] affording one-step purification by a simple environmental trigger in combination with the specificity and affinity of the binding proteins. Although tunable polymers such as poly(*N*-isopropylacrylamide) (poly-NIPAM) have been used for this purpose, the ability to tune for network formation and to provide IgG- and QD-binding functionality is far from straightforward. Elastin-like polypeptide (ELP), consisting of the repeating pentapeptide VPGVG, can undergo a reversible phase transition from water-soluble forms into aggregates similar to PNIPAM polymer within a wide range of conditions.[7] ELPs can be easily tailored genetically with active fusion partners for diverse applications.[8,9] Herein, we propose a simple approach of bioconjugation and purification of QDs with IgGs by combining this chromatography-free purification method with QD conjugation moiety as well as antibody recognition domain into a tripartite ELP fusion protein.

The tripartite fusion protein (His-ELP-PL) (Scheme 1A) consists of (1) an N-terminus histidine tag (His) used for QD conjugation via strong metal-affinity coordination with Zn^{2+} on the core−shell,[5] (2) an ELP midblock comprising 78 VPGVG repeating units for stimuli-responsive purification, and (3) a C-terminus protein L (PL), a cell-wall component of *Peptostreptococcus magnus* that has a high affinity toward immunoglobulin (Ig) κ-light chains of a wider range of species[10] and was used to prepare aggregate-free solutions of QD−IgG conjugates. Detection of tumor markers in an antibody array was demonstrated using these QD-conjugated IgGs.

Scheme 1. Schematic Representation of Tripartite Protein HIS-ELP-PL and Procedures to Conjugate and Purify Quantum Dots as Fluorescent Probes for Antibody Microarray Detection[a]



[a](A) Fusion structure. The elastin-like protein was flanked by an N-terminus hexahistidine tag and a C-terminus protein L. (B) Conjugation and separation scheme.

The direct conjugation of QD with His-ELP-PL was examined by observing the photoluminescence at 520 nm. The protein was incubated with carboxyl-functionalized CdSe−ZnS QDs (Evident Tech. Inc.) of 520 nm emission spectra in phosphate buffered saline (PBS) solution. Consistent with previous reports,[11] enhanced photoluminescence was observed upon the binding of His-ELP-PL; a saturation ratio of 20 proteins per QD was determined by the functional assay and used in subsequent experiments. The ability to achieve a thermally triggered separation of QD−His-ELP-PL conjugates was accomplished according to Scheme 1B. NaCl was added to the solution to induce ELP phase transition at room temperature and the aggregates of the QD−His-ELP-PL complex were separated from the solution by centrifugation. The resulting pellets were resolubilized in cold PBS. The efficiency of separation was visualized qualitatively using an UV illuminator and monitored quantitatively using a fluorescence microplate reader (BMG laboratory) (Figure 1). Nearly no fluorescence loss was observed in the precipitation step as demonstrated by the fluorescence measurements (Figure 1A). Conversely, no QDs were found to associate with ELP alone upon precipitation.

The feasibility of His-ELP-PL mediated formation of QD-antibody complexes was further investigated. Formation of the three-component complex was accomplished by conjugating with either QD or IgG as shown in Scheme 1B. An excess amount of IgG was added to ensure a saturating level of conjugation, and the resulting QD−IgG complex was precipitated and separated from the free IgGs by centrifugation. Independent of the conjugation scheme, the resulting QD−His-ELP-PL−IgG complexes were

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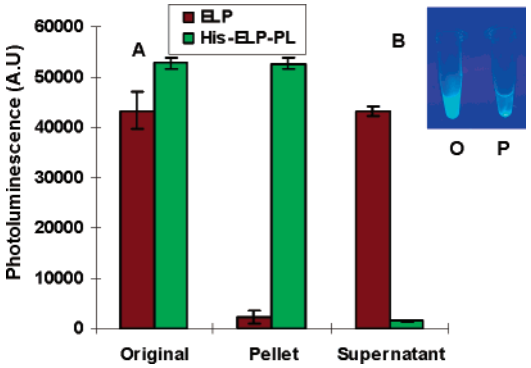

Figure 1. Separation of QD−His-ELP-PL by thermally triggered precipitation: (A) fluorescence measurements for each step of separation; (B) highly fluorescent QD−His-ELP-PL pellets after centrifugation was visualized under UV illumination (P) and compared with the original QD solution (O).

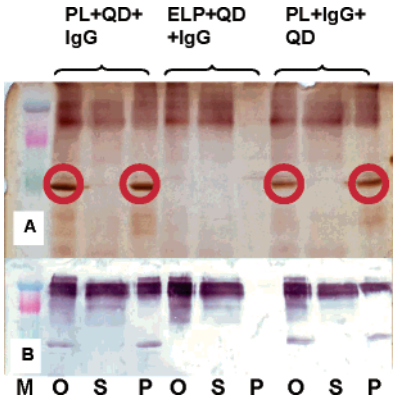

Figure 2. Formation of QD−His-ELP-PL−IgG complexes: PL, His-ELP-PL; M, marker; O, original; P, pellet; and S, supernatant. Total proteins from each step were analyzed by (A) SDS-PAGE (PL is marked by a red circle) or (B) Western blot analysis.

highly fluorescent, retaining more than 80% of the original fluorescence intensity after the final conjugation. The presence of the PL domain also enabled the efficient conjugation of IgG (Figure 2). In contrast, ELP by itself lacked the ability to conjugate either QD or IgG.

The utility of QD−His-ELP-PL−IgG conjugates as a sensitive immuno-fluorescent agent for antibody array analysis was demonstrated. Carcinoembryonic antigen (CEA) was chosen as a representative tumor marker, which is used frequently for colorectal cancer screening. A sandwich immunoassay configuration was constructed. Briefly, a capture antibody (Ab) for CEA was suspended in sodium bicarbonate buffer pH 8.6 and immobilized onto the OTS-modified glass slide[12] via hydrophobic interaction. The glass surface was blocked with bovine serum albumin to reduce the nonspecific absorption. After samples with different concentrations of CEA were loaded, a CEA-specific detection antibody decorated with QD via conjugation with His-ELP-PL was used to probe the antigen. The amount of CEA in the sample was quantified by relating the fluorescence signal detected using a microarray

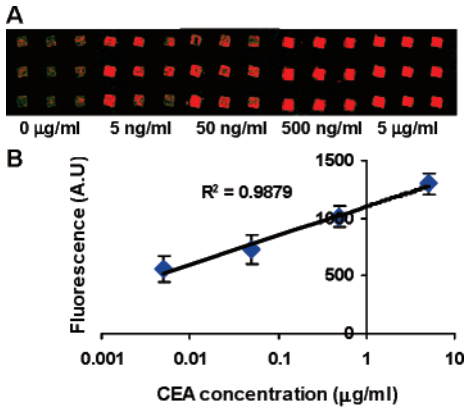

Figure 3. The utility of QD−IgG conjugates as highly fluorescent probes for antibody array detection: (A) fluorescence images of the array when different CEA concentrations are probed by QD−IgG conjugates; (B) fluorescence intensity as a function of the CEA concentration.

scanner. Figure 3 shows the corresponding calibration plot of the fluorescence intensity as a function of the CEA concentration.

In summary, we demonstrated a simple platform for the direct conjugation and separation of CdSe−ZnS QD−IgG complexes using a genetically engineered His-ELP-PL trifusion protein. The stimuli-triggered separation eliminates the need of chromatography enabling the easy preparation of sensitive immuno-fluorescent agents. An antibody array for the detection of CEA was fabricated to demonstrate utility of the biofunctionalized QD probes. Because of the inherent characteristics of QD and the flexibility of engineering ELP fusion, we anticipate that this methodology will be useful for the detection of a wide range of analytes.

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Supporting Information Available: Experimental procedure. This material is available free of charge via the Internet at http://pubs.acs.org.

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