Direct conjugation of antibodies to the ZnS shell of quantum dots for FRET immunoassays with low picomolar detection limits

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The development of quantum dot-antibody (QD-AB) conjugates with compact surface functionalization and high-performance photophysical properties for efficient Förster resonance energy transfer (FRET) immunoassays in serum samples are of paramount importance for the integration of QDs into clinical *in vitro* diagnostics. They would allow overcoming the major limitations for developing QD-FRET immunoassays with limits of detection competitive to commercial kits which are: (i) insufficient colloidal stability of compact QDs in biological media, (ii) thick QD surface coatings (e.g., PEG, polymer, or lipid coatings that protect the inorganic QD from the biological environment and render them hydrosoluble), and (iii) insufficient AB-conjugation strategies for QDs with thinner organic capping. The latter two QD surface related aspects result in long FRET donor–acceptor distances and unfavorable binding conditions, respectively. This leads to lower sensitivities compared to conventional time-resolved FRET immunoassays that use lanthanide donor and dye acceptor AB conjugates.

Here, we present a simple strategy to directly conjugate antibodies via their endogenous disulfide groups directly to the inorganic ZnS shell of compact penicillamine-coated QDs. The functionality of the conjugates was demonstrated by Tb-to-QD FRET immunoassays against prostate specific antigen in serum samples. The major advantages of this novel method are i) generic applicability to IgG, $F(ab')_2$, and F(ab) antibodies; ii) functionality in serum-based immunoassays; and iii) superior diagnostic performance compared to maleimide-functionalized penicillamine-coated QDs or commercial polymer-coated QDs. Detection limits of 2.5 pM (0.080 ng/mL) were 10 and 25 times lower compared to conjugation via maleimide-terminated ligands and polymer chains, respectively. These more compact, simple, and sensitive QD-antibody conjugates will be highly advantageous for nanocrystal-based biosensing applications [1–3].

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