**Libor Janu**[1]
**Maja Stanisavljevic**[1]
**Sona Krizkova**[1,2]
**Pavlina Sobrova**[1,2]
**Marketa Vaculovicova**[1,2]
**Rene Kizek**[1,2]
**Vojtech Adam**[1,2]

[1]Department of Chemistry and Biochemistry, Faculty of Agronomy, Mendel University in Brno, Brno, Czech Republic
[2]Central European Institute of Technology, Brno University of Technology, Brno, Czech Republic

Received February 14, 2013
Revised March 26, 2013
Accepted March 27, 2013

Research Article

# Electrophoretic study of peptide-mediated quantum dot-human immunoglobulin bioconjugation

The bioconjugation of quantum dots (QDs) is a key process in their application for bioanalysis as well as imaging. The coupling of QDs with biologically active molecules such as peptides, nucleic acids, and/or antibodies enables their fluorescent labeling, and therefore, selective and sensitive tracking during the bioanalytical process, however, the efficiency of the labeling and preservation of the biological activity of the bioconjugate have to be considered. In this study, a new approach of the bioconjugation of CdTe-QDs and human immunoglobulin employing a small peptide is described. The heptapeptide (HWRGWVC) was synthesized and characterized by mass spectrometry, liquid chromatography, and capillary electrophoresis. Moreover, the peptide was used as a capping agent for QDs synthesis. The CdTe-QDs were synthesized by microwave synthesis (600 W, 20 min) using 3.2 mM $CdCl_2$ and 0.8 mM $Na_2TeO_3$. The bioconjugation of QDs capped by this peptide with immunoglobulin was investigated by capillary electrophoresis and magnetic immunoextraction coupled with electrochemical detection by differential pulse voltammetry. Furthermore, the applicability of prepared bioconjugates for fluorescent immunodetection was verified using immobilized goat antihuman IgG antibody.

**Keywords:**
Antibody / Bioconjugation / Gel electrophoresis / Immunoglobulin / Quantum dot
DOI 10.1002/elps.201300088

## 1 Introduction

The development of biocompatible, highly fluorescent nanoparticles including quantum dots (QDs) for chemical and biochemical labeling, immunoanalysis, molecular imaging, and/or targeted therapy is a field attracting an extensive attention [1–15]. The main reason is that nanometer-sized particles have functional and structural properties that are not available from either discrete molecules or bulk materials [16–19]. When conjugated with biomolecular affinity ligands, such as antibodies, peptides, or small molecules, these nanoparticles can be used to target specific molecules such as DNA, proteins, and/or even cells [20–23]. Conjugation of QDs and other biomolecules can be done by covalent coupling, physical adsorption, and hydrophobic adsorption. One of the most frequently used methods is the cross-linking through an *N*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride (EDC)/*N*-hydroxysuccinimide reaction [24–28]. In the EDC coupling, there is a possibility that the antigen binding sites of antibodies are blocked by the nonselective formation of amide bonds at the vicinity of Fab (fragment antigen-binding) region of the antibody.

Another conjugation method is based on avidin and/or streptavidin-biotin linkage [29], which provides high specificity and stability. However, due to the sizes of all components (QD, streptavidin, antibody) the result of this method is relatively large nanoparticle (more than 40 nm). Moreover, the biotinylated antibody usually contains multiple biotinylated sites, which leads to the multiple labeling and also the orientation of antibody relative to the QD surface cannot be controlled and may lead to the production of nanoprobes without required functionality. To address these issues, numerous other methods have been developed including high-resolution hybrid gel system specially designed for fractionation of nanoparticle bioconjugates [30]. The conjugation strategy employing an engineered molecular adaptor protein, attached to the QDs via electrostatic/hydrophobic self-assembly [31, 32] and/or protein A as an adaptor protein for binding of antibody to QDs [33]. Protein A is a surface protein found in the cell wall of the bacteria *Staphylococcus aureus*. This protein has an ability to bind immunoglobulins through interaction with their Fc region [34]. Protein-A-mediated antibody conjugation has an advantage that the orientation of antibody can be controlled to face the antigen

**Correspondence:** Dr. Vojtech Adam, Department of Chemistry and Biochemistry, Mendel University, Brno, Zemedelska 1, CZ-613 00 Brno, Czech Republic
**E-mail:** vojtech.adam@mendelu.cz
**Fax:** +420-5-4521-2044

**Abbreviations: HIgG**, human Ig; **HWR**, synthetic heptapeptide HWRGWVC; **QD**, quantum dot

**Colour Online:** See the article online to view Figs. 1–5 in colour.

 

binding site outward. Moreover, it was found that a family of linear hexapeptides composed of histidine on the N-terminus followed by aromatic amino acids and positively charged amino acids are able to recognize human Ig (HIgG) through its Fc region, and their selectivity to Fc is comparable to protein A [35]. One of such peptides, HWRGWV, binds all HIgG subclasses and IgGs from bovine, mouse, goat, and rabbit. Capillary electrophoresis provides a high separation power, and therefore, it has a high application potential for analysis and characterization of peptides, QDs, and their bioconjugates [36–39].

In this study, a novel self-assembling bioconjugation method of QDs and antibodies was developed employing the synthetic heptapeptide (HWRGWVC, abbreviated as HWR) and its selective affinity to Fc fragment of IgG.

## 2 Materials and methods

### 2.1 Chemicals, pH measurements, and MiliQ water preparation

Cadmium chloride, water, and other chemicals were purchased from Sigma-Aldrich (USA) in ACS purity (chemicals meet the specifications of the American Chemical Society) unless noted otherwise. The pH value and conductivity was measured using inoLab Level 3 (Wissenschaftlich-Technische Werkstatten, Weilheim, Germany). Deionized water underwent demineralization by reverse osmosis using the instruments Aqua Osmotic 02 (Aqua Osmotic, Tisnov, Czech Republic) and then it was subsequently purified using Millipore RG (Millipore, USA, 18 MΩ)—MilliQ water.

### 2.2 Synthesis of HWR peptide

Peptide with the amino acid sequence HWR was prepared on Prelude peptide synthesizer (Protein Technologies, USA) by standard Fmoc solid-phase peptide synthesis. We used fourfold excess of amino Fmoc-acid with respect to the resin. Deblock of Fmoc protecting group was performed with 20% piperidine v/v in DMF. Coupling was achieved using 1:1:0.5:2 amino acids/*O*-benzotriazole-*N*,*N*,*N*′,*N*′-tetramethyl-uronium-hexafluoro-phosphate/*N*-hydroxybenzotriazole/*N*,*N*-diisopropylethylamine. Cleavage of side chain protecting groups was performed by treating the peptidyl resin with 91.5% TFA v/v, 1.5% phenol v/v, 5% $H_2O$ v/v, and 2% triisopropylsilane v/v for 2 h.

Purity of the crude peptide was analyzed using HPLC (Shimadzu, Japan) with standard mobile phases 0.1% TFA (in water, v/v, A); 80% ACN (in water, v/v), and 0.08% TFA (in water, v/v) (B). MALDI-TOF-MS (Ultraflex III instrument, Bruker Daltonik, Germany) was used to verify the identity of the final product.

### 2.3 Synthesis of QD-HWR

Cadmium chloride solution (0.04 M, 2 mL) was diluted to 21 mL with ACS water. Then, 50 mg trisodium citrate dihydrate and 2 mL $Na_2TeO_3$ (0.01 mol/L) were added successively under magnetic stirring. After complete dissolution of the precursors, 0.5 mL of the reaction mixture was transferred to the glass reaction vessel. Two milligrams of peptide HWR was dissolved in 50 μL DMF and then added to the 0.5 mL of reaction mixture. After that excess of $NaBH_4$ was added immediately. Reaction vessels were loaded into Microwave digestion system (Anton Paar, Germany) and irradiated with 600 W for 20 min. Temperature limit was 160°C. After microwave irradiation, the mixture was cooled down to 50°C and removed from digestion system.

### 2.4 Conjugation of QDs with IgG

One hundred microliters of QDs was mixed with 13 μL IgG from human serum (concentration of IgG was 10 mg/mL) in a sealed vial and 100 μL of QDs was mixed with 5 μL IgY (chicken immunoglobulins) from chicken yolk (concentration was 26.2 mg/mL) in a sealed vial. The mixture was vortex-mixed for 2 h at 20°C.

### 2.5 CE

Electrophoretic measurements were carried out using CE system Beckman P/ACE MDQ with absorbance detection at 214 nm (CE-UV) and Beckman PACE/5500 with LIF detection with excitation at 488 nm (CE-LIF). Uncoated fused silica capillary was used with total length of 60 cm and effective length of 50 cm for CE-UV and 47 cm of total length and effective length of 40 cm for CE-LIF. In both cases, the internal diameter of the capillary was 75 μm and the outer diameter was 375 μm. A total of 20 mM sodium borate buffer prepared from sodium tetraborate (the pH 9 was adjusted by 1 M NaOH solution) was used as a background electrolyte and the separation was carried out using 20 kV with hydrodynamic injection for 20 s at 3.4 kPa.

### 2.6 Fluorimetric measurement

Fluorescence spectra were acquired by multifunctional microplate reader Tecan Infinite 200 PRO (TECAN, Switzerland). An excitation wavelength of 480 nm was used and the fluorescence scan was measured within the range from 510 to 850 nm per 5 nm steps. Each intensity value is an average of five measurements. The detector gain was set to 100. The sample (50 μL) was placed in transparent 96 well microplate with flat bottom by Nunc.

 

### 2.7 SDS-PAGE

Electrophoresis was performed using a Mini Protean Tetra apparatus with gel dimensions of 8.3 × 7.3 × 0.1 cm (Bio-Rad, USA). First 7% w/v running, then 5% w/v stacking gel was poured. The gels were prepared from 30% w/v acrylamide stock solution with 1% w/v bisacrylamide. The polymerization of the running or stacking gels was carried out at room temperature for 45 min. Prior to analysis, the samples were mixed with reducing (3.3% β-mercaptoethanol, v/v) or nonreducing sample buffer in a 1:1 ratio. "Precision plus protein standards" protein ladder from Bio-Rad was used to determine molecular mass. The electrophoresis was run at 150 V for 45 min at laboratory temperature (Power Basic, Bio-Rad) in Tris-glycine buffer (0.025 M Trizma-base, 0.19 M glycine and 3.5 mM SDS, pH 8.3). In order to confirm the conjugation of QDs and IgG, the pH of the running buffer was adjusted to 9.0 with NaOH. After separation the gels were stained with Coomassie-blue according to Wong et al. [40].

### 2.8 Agarose gel electrophoresis

One-centimeter-thick gels were prepared from 1.5% agarose v/v in 0.5 × TAE buffer (20 mM Tris, 10 mM acetic acid, 0.5 mM EDTA, pH 8). The samples were loaded 5:1 in tris-tricine sample buffer (Bio-Rad). The electrophoresis was run at 100 V for 30 min at laboratory temperature (Biometra, Germany) in 0.5 × TAE buffer.

### 2.9 Paramagnetic beads modification

IgG from human serum (#I4506) were purchased from Sigma-Aldrich. Chicken antibodies were prepared by HENA, (Prague, Czech Republic) according to the following protocol. Two hens were immunized with Zn-KLH (keyhole limpet hemocyanin) complex. IgY fraction reactive to Zn-KLH was obtained from egg yolk. The antibodies were stabilized with 0.1% $NaN_3$ v/v in PBS and protein concentration was 39.6 mg/mL in immunoglobulin fraction.

For covalent antibody immobilization, *p*-toluenesulphonyl chloride – activated superparamagnetic polystyrene beads coated with polyurethane layer were used (Dynabeads® MyOne™ Tosylactivated, #655.01). Antibody preparation and immobilization protocol was adapted from the supplier's manual (Invitrogen, Norway). For immobilization, 1000 μg of the antibodies per 25 mg of beads was used. Prior to immobilization, $NaN_3$ was removed and antibodies were acidified to pH 2.5 by addition of HCl. After 15 min, the antibodies were brought into physiological pH 7.4. For all buffer exchanges, Amicon Ultra 0.5 columns with membrane cutoff 50 K (Millipore) were used. Covalent immobilization was carried out in total volume of 625 μL in the presence of 0.1 M borate buffer of pH 9.5 with 0.1 M $(NH_4)_2SO_4$ for 24 h under mild rotation. Particle-free surfaces were then blocked with 0.5% BSA in PBS w/v and 0.05% Tween-20 v/v for 10 h. After blocking, the beads were washed three times with 1 mL of 0.1% BSA in PBS w/v with 0.05% Tween-20 v/v and resuspended in 625 μL of storage buffer (washing buffer with 0.02% $NaN_3$ w/v).

### 2.10 Immunomagnetic isolation of CdTe-QDs

A 15 μL of Dynabeads® MyOne™ Tosylactivated with human antibodies (Invitrogen) was washed three times with 15 μL of 0.1% BSA in PBS w/v with 0.05% Tween-20 v/v in 1.5 mL tube (Eppendorf, Germany). The washed beads were dispensing to 15 μL of borate buffer (0.1 M NaOH + $Na_2B_4O_7 \cdot 10\ H_2O$; adjusted by HCl on pH 9.24). Finally, 15 μL CdTe-QDs were added. Immunoextraction was performed for 2 h at room temperature on a rotating programmable rotator-mixer (Biosan, Latvia). The beads were then separated from the solution and washed with 15 μL of borate buffer. Beads were used for the mineralization. The same procedure was carried out for bioconjugation with chicken antibodies.

### 2.11 Method for detection of cadmium in QDs

Prior to Cd determination, the samples were digested using microwave heating. The mineralization of samples was carried out using a microwave system Multiwave3000 (Anton-Paar). The beads conjugated with QDs (15 μL) was placed into MG5 glass vials and 150 μL of hydrogen peroxide (30%, w/w) and 350 μL of nitric acid (65%, w/w) were added. Prepared samples were sealed and placed into a 64MG5 rotor (Anton-Paar). The rotor with the samples was inserted into the microwave system and the microwave digestion was carried out under the following conditions: power 50 W for 10 min, power 100 W for 30 min, cooling (power 0 W) for 10 min, maximum temperature 80°C. After mineralization, 10 μL mineralized sample was pipetted into Eppendorf tubes with 990 μL of 0.2 M sodium acetate buffer (pH 5.00 adjusted by mixing 0.2 M sodium acetate and 0.2 M acetic acid) and electrochemically analyzed.

Electrochemical measurements were performed at 663 VA Stand, 800 Dosing and 846 Dosing Interface (Metrohm, Switzerland) using a standard cell with three electrodes. A hanging mercury drop electrode with a drop area of 0.4 $mm^2$ was employed as the working electrode. An Ag/AgCl/3 M KCl electrode served as the reference electrode and auxiliary electrode was a glassy carbon electrode. All measurements were performed in the presence of 0.2 M sodium acetate buffer (0.2 M $CH_3COOH$ + 0.2 M $CH_3COONa$, pH 5.0) at 25°C. Samples were deoxygenated with argon (99.99%, 60 s). For smoothing and baseline correction, the software GPES 4.9 supplied by EcoChemie was employed. For electrochemical detection of cadmium, differential pulse voltammetry was used. The parameters of electrochemical determination were as follows: initial potential −0.9 V; end potential −0.1 V; deposition potential −0.9 V; duration 600 s; equilibration time 5 s; modulation time 0.057; time

 

interval 0.2 s; potential step 0.00195 V; modulation amplitude 0.02505.

### 2.12 ELISA

Microtitration plate was coated either with of 1 μg/mL of goat antihuman IgG antibody (Greiner Diagnostics, Germany) or chicken IgY (HENA) diluted in 0.05 M carbonate buffer (0.032 M $Na_2CO_3$ and 0.068 M $NaHCO_3$, pH 9.6) in amount of 50 μL per well overnight at 4°C. Then, the wells were washed five times with 350 μL of 0.005% PBS-T v/v (Hydroflex, TECAN) and blocked for 30 min at 37°C with 50 μL of 1% BSA w/v diluted in PBS. After washing with PBS-T, 50 μL of QDs was pipetted in and the plate was incubated in 37°C for 60 min. After the removing of the solution and washing with PBS-T, the fluorescence scan (510–850 nm) was measured by multifunctional microplate reader Tecan Infinite 200 PRO (TECAN) at excitation wavelength of 480 nm. The fluorescence measuring step was 5 nm. Each intensity value is an average of five measurements and the detector gain was set to 100.

### 2.13 Statistics

Data were processed using MICROSOFT EXCEL® (USA) and STATISTICA.CZ Version 8.0 (Czech Republic). Results are expressed as mean ± SD unless noted otherwise (EXCEL®).

## 3 Results and discussion

### 3.1 Characterization of the peptide

A short amino acid sequence composed of aromatic and positively charged amino acids is mostly responsible for the specific interaction of HIgG and protein A. Based on this knowledge, an artificial hexapeptide HWRGWV was synthesized with additional cysteine at the C end (HWR) ensuring the ability to work as a capping agent for stabilization of colloidal CdTe quantum dot solution. The synthetically prepared heptapeptide was characterized by mass spectrometry (Fig. 1A), HPLC (Fig. 1B) and capillary electrophoresis (Fig. 1C). According to the mass spectrometry, the molecular mass of the heptapeptide is 942.452 Da. The other found in the spectrum is caused by the presence of adduct of the heptapeptide with tert-butyl, which serves as a protective agent during the peptide synthesis. The characterization by liquid chromatography confirmed the presence of the majority of product by the peak with retention time of 22.5 min (and the contamination with retention time of 23.7 min). The purity of the product was 70%. Finally, one major peak with migration time of 3.8 min and number of un-resolved peaks of possible contaminants were obtained by capillary electrophoresis with photometric detection. Based on the characterization by above-mentioned methods, the quality of the synthesized peptide was found sufficient for further experiments.

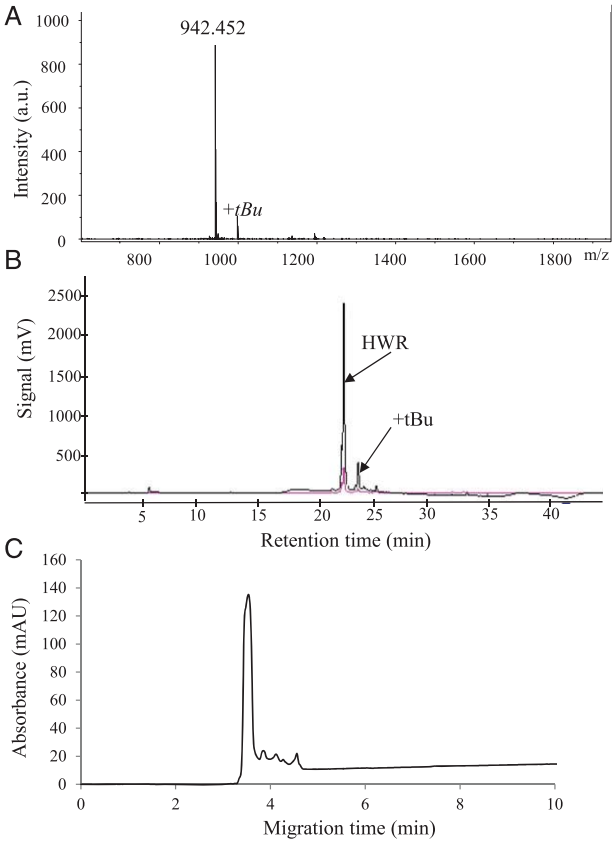


**Figure 1.** Characterization of synthetic HWR peptide. (A) Mass spectrum of the peptide. (B) HPLC characterization of the peptide. (C) CE characterization of the peptide. CE-UV, conditions—detection: 214 nm; capillary: 75 μm id, 60 cm/50 cm; BGE: 20 mM sodium borate, pH 9.2; voltage: +20 kV; injection: 3.4 kPa, 20 s. MS and HPLC conditions are described in Section 2.

### 3.2 CdTe-QDs covered with the synthesized peptide

This peptide was subsequently employed as a capping agent for CdTe-QDs. These QDs were characterized by fluorescence spectrometry (Fig. 2A) and capillary electrophoresis (Fig. 2B). Scheme of ideal HWR-QDs structure is shown in the inset of Fig. 2A. The excitation maximum of the HWR-QDs was found to be 480 nm and the emission maximum was 525 nm. These properties are suitable for CE-LIF with excitation wavelength of 488 nm. Prepared QDs were conjugated with HIgGs and because the heptapeptide HWR binds all HIgG subclasses and IgGs from bovine, mouse, goat, and rabbit [35], IgY were used as a nonreactive control, as IgY lack Fc region in their structure [41].

The conjugates were separated by capillary electrophoresis with both laser-induced fluorescence (Fig. 3A) and UV absorbance detection (Fig. 3B). The signals obtained were well developed and separated. QDs incubated with IgY exhibited the same electromigration properties as nonconjugated QDs (migration time of 4.1 min, Fig. 3A and B); however, the migration time of IgG-conjugated QDs (scheme in the

 

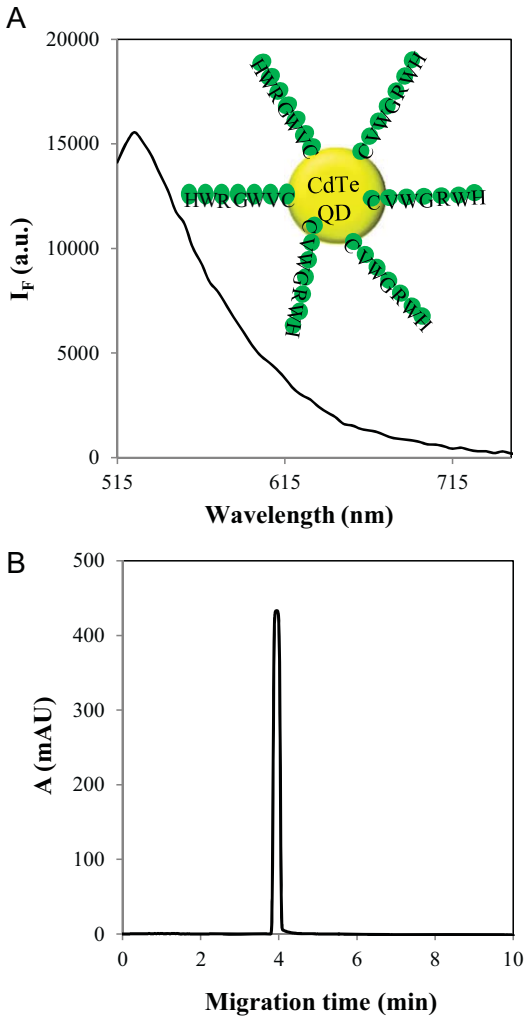


**Figure 2.** Characterization of HWR-QDs. (A) Emission spectrum of HWR-QDs (excitation 480 nm); inset: scheme of ideal structure of HWR-QDs. (B) CE-UV of HWR-QDs. Experimental conditions—detection wavelength: 214 nm; capillary: 75 μm id, 60 cm/50 cm; BGE: 20 mM sodium borate, pH 9.2; voltage: +20 kV; injection: 3.4 kPa, 20 s.

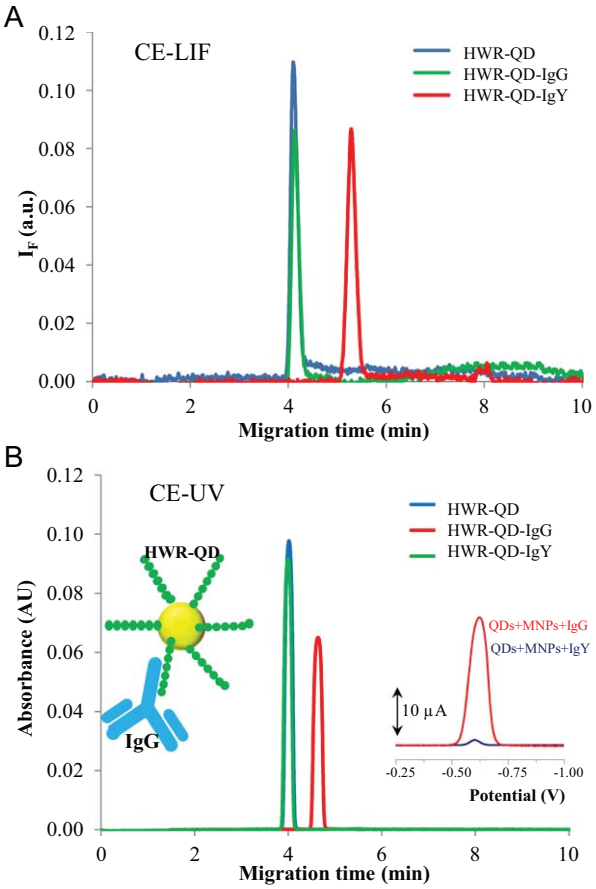


**Figure 3.** CE of HWR-QD and their bioconjugates with immunoglobulins. (A) CE-LIF, conditions—excitation: 488 nm; emission: 520 nm; capillary: 75 μm id, 47.5 cm/40 cm; BGE: 20 mM sodium borate, pH 9.2; voltage: +20kV; injection: 3.4 kPa, 20 s. (B) CE-UV, conditions—detection: 214 nm; capillary: 75 μm id, 60 cm/50 cm; BGE: 20 mM sodium borate, pH 9.2; voltage: +20 kV; injection: 3.4 kPa, 20 s; left inset: scheme of HWR-QD-IgG; right inset: differential pulse voltammograms of Cd determination extracted by magnetic particles coated with IgG and IgY. Experimental conditions—dilution: 1:1000; electrolyte: 0.2 M acetate buffer (0.2 M $CH_3COOH$ + 0.2 M $CH_3COONa$, pH 5.0) at 25°C; initial potential: −0.9 V; end potential: −0.1 V; deposition potential: −0.9 V; duration: 600 s; equilibration time: 5 s; modulation time: 0.057; time interval: 0.2 s; potential step: 0.00195 V; modulation amplitude: 0.02505.

inset of Fig. 3B) was increased due to the conjugation. After binding of IgG, migration time enhanced to 5 min.

It is known that pI of HIgG is within the range from 6.4 to 9.0 and therefore under the CE conditions used. The IgG molecule is negatively charged so as HWR-QDs. The electrophoretic mobility of HWR-QDs of $-8.13 \pm 0.41 \times 10^{-9}$ $m^2$ $V^{-1}$ $s^{-1}$ and $-7.81 \pm 0.38 \times 10^{-9}$ $m^2$ $V^{-1}$ $s^{-1}$ was determined by CE-UV and CE-LIF, respectively. After the conjugation with IgG, the electrophoretic mobility changed to $-14.91 \pm 0.73 \times 10^{-9}$ $m^2$ $V^{-1}$ $s^{-1}$ and $-14.4 \pm 1.1 \times 10^{-9}$ $m^2$ $V^{-1}$ $s^{-1}$ determined by CE-UV and CE-LIF. Moreover, we analyzed the conjugate using differential pulse voltammetry after purification, because the dots contain cadmium, which is highly electroactive. Using this, we verified the presence of the HWR-QDs because of detection of Cd(II) peak (inset of Fig. 3B).

### 3.3 Gel electrophoretic analysis

To verify the conjugation of HWR-QD and IgG the gel electrophoresis was employed. At first, the SDS-PAGE analysis of HWR-QDs, IgG and IgY standards as well as HWR-QD-IgG or HWR-QD-IgY conjugates under reducing and nonreducing conditions was carried out (Fig. 4A). After fluorescence imaging it was not possible to detect any fluorescence (not shown). IgG and IgY standards were resolved as expected. Under nonreducing conditions at IgG, three bands in size of approximately 150–170, 140, and 100 kDa corresponding to whole and partially fragmented IgG molecules were present and three bands in size of approximately 180, 170,

 

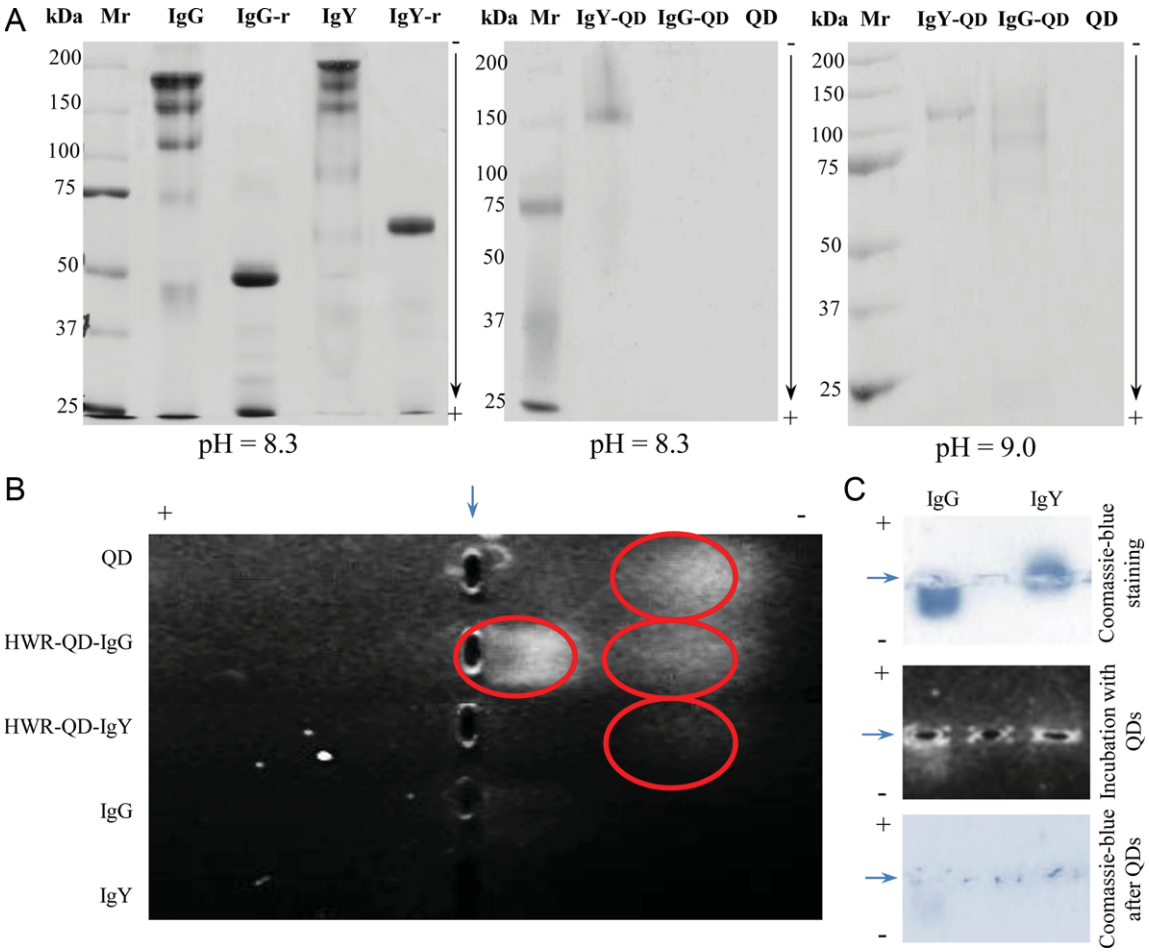


**Figure 4.** (A) SDS-PAGE electropherogram of 1000 ng of IgG and IgY standards under reducing (3.3% β-mercaptoethanol v/v, labeled as -r) and nonreducing conditions in running electrolyte pH 8.3 (left), SDS-PAGE electropherogram of QD-IgG and IgY conjugates in running electrolyte pH 8.3 (in the middle), SDS-PAGE electropherogram of QD-IgG and IgY conjugates in running electrolyte pH 9.0 (right). (B) Agarose gel electropherogram of QD-IgG and IgY conjugates, and pH of the running buffer is 8.0. Arrow indicates wells position, and (+) and (−) indicate poles orientation. (C) Agarose gel electropherogram of IgG and IgY stained either with Coomassie-blue (above) or incubated with QDs (in the middle) and consequently with Coomassie-blue (below), and pH of the running buffer is 8.0. Arrows indicate wells positions, and (+) and (−) indicate poles orientation.

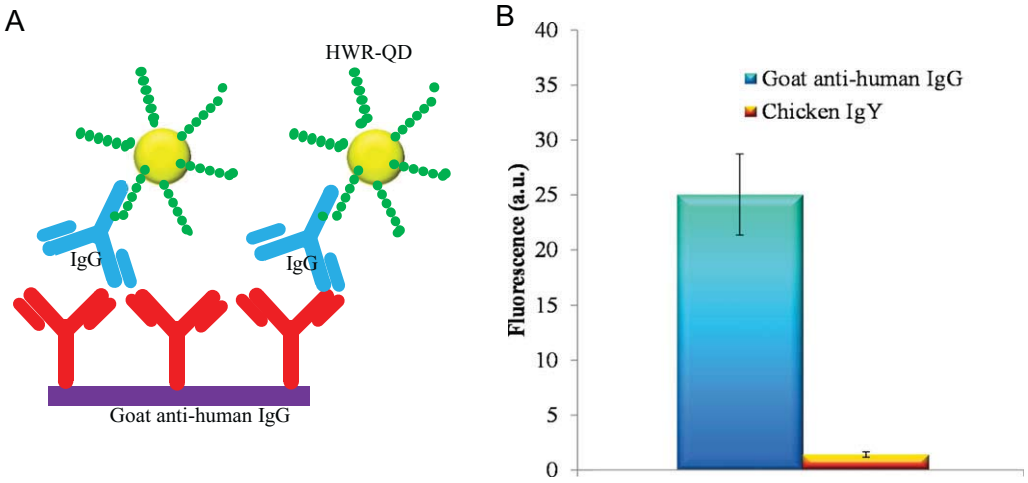


**Figure 5.** Fluorescent immunodetection. (A) Scheme of the possible interaction between HWR-QD-IgG and goat antihuman IgG. (B) Fluorescence intensity determined in the wells coated with goat antihuman IgG and chicken IgY (excitation: 480 nm and emission: 525 nm).

 

and 130 kDa corresponding whole and partially fragmented IgY molecules were present at IgY. Under reducing conditions at IgG two bands in size of approximately 50 and 25 kDa corresponding to large and small IgG subunits were present and two bands in size of approximately 65 and 20 kDa corresponding to large and small IgY subunits were present at IgY. For HWR-QD-IgG conjugate or HWR-QDs alone, no bands were detected, only a weak band of molecular size corresponding to partially reduced IgY molecule was observed for HWR-QD-IgY.

Binding of QDs modified with HWR peptide to IgG might affect migration of IgG conjugate, even though SDS binding to the proteins surface unifies their charge. Reduced SDS binding to HWR-QD-IgG conjugate is also possible. Therefore, the samples were run under the same conditions, but with switched poles. However, still no bands were observed (not shown). After changing of running buffer pH to 9.0, bands of sizes corresponding to partially reduced whole immunoglobulin molecules were observed after Coomassie-blue staining for both HWR-QD-IgG and HWR-QD-IgY conjugates, but no fluorescence was detected even for HWR-QDs alone. Different behavior of IgG and IgY mixtures dependent on running buffer pH indicates that HWR-QDs bound to IgG molecules, and did not bind to IgY molecules, but the fluorescence of HWR-QDs might be quenched during the electrophoresis. Fluorescence of both HWR-QDs and HWR-QDs-IgG conjugates was observed after agarose gel electrophoresis (Fig. 4B). In running electrolyte of pH 8.0, both HWR-QDs and HWR-QD-IgG conjugates migrated to negative pole and at HWR-QD-IgY mixture only the band corresponding to unconjugated HWR-QDs was recorded. Only very weak fluorescence was detected for IgG and IgY standards. As it is shown in Fig. 4C, after staining the agarose gel of immunoglobulin standards with Coomassie-blue, the weak fluorescence signals might be caused by IgG themselves. To confirm it, we incubated IgG and IgY resolved in agarose gel with HWR-QDs in running electrolyte for 16 h. After incubation, the fluorescence was observed in IgG only. The same gel was stained with Coomassie-blue and very weak band disproportional to original signal was observed in IgG only. This indicates that IgG was stabilized in the gel by HWR-QDs binding, while IgY was completely washed out.

The results of SDS-PAGE and agarose electrophoresis are consistent and confirm binding of QDs modified with HWR to IgG, but not to IgY. In addition, after HWR-QDs binding to IgG, their charge is modified. Moreover, HWR-QDs are applicable for IgG detection in agarose gels.

### 3.4 ELISA detection of HWR-QDs

To demonstrate the applicability of developed conjugates for immunodetection, ELISA experiment was carried out. The microtitration plate was coated with goat anti-human IgG (or chicken IgY—as a nonbinding control) in concentration of 1 μg/mL and subsequently incubated with HWR-QD-IgG according to scheme shown in Fig. 5A. It was found out that the intensity of fluorescence in the wells coated with goat antihuman IgG exhibited significantly higher fluorescence at 525 nm than the wells coated with chicken IgY (Fig. 5B). The suggested procedure could be used for sensitive immunobased techniques [42], for imaging approaches [6] and for microarrays technologies to determine Alzheimer's disease biomarkers [43], tumor markers [44], and C-reactive peptides [45].

## 4 Concluding remarks

The surface modification and functionalization of QDs is extensively studied due to the possibility of fine-tuning of the properties according to the requirements. Their functionalization by antibodies enables the fluorescent visualization of interactions between the antigen and antibody as well as biodistribution. However, the bond between the antibody and QD has to exhibit correct sterical orientation to preserve the biological activity of the antibody and to provide required efficiency. It was demonstrated that small synthetic heptapeptide (HWR) can serve as a QD capping agent providing suitable surface properties for interaction with Fc fragment of human IgG.

*Financial support from the following projects PGS07_2012, NanoBioTECell GA CR P102/11/1068, CEITEC CZ.1.05/1.1.00/02.0068 and CZ.1.07/2.3.00/30.039 is highly acknowledged. The authors wish to express their thanks to Eva Jilkova for perfect technical assistance.*

*The authors have declared no conflict of interest.*

## 5 References

[1] Medintz, I. L., Uyeda, H. T., Goldman, E. R., Mattoussi, H., *Nat. Mater.* 2005, *4*, 435–446.

[2] Michalet, X., Pinaud, F. F., Bentolila, L. A., Tsay, J. M., Doose, S., Li, J. J., Sundaresan, G., Wu, A. M., Gambhir, S. S., Weiss, S., *Science* 2005, *307*, 538–544.

[3] Drbohlavova, J., Chomoucka, J., Hrdy, R., Prasek, J., Janu, L., Ryvolova, M., Adam, V., Kizek, R., Halasova, T., Hubalek, J., *Int. J. Electrochem. Sci.* 2012, *7*, 1424–1432.

[4] Drbohlavova, J., Vorozhtsova, M., Hrdy, R., Kizek, R., Salyk, O., Hubalek, J., *Nanoscale Res. Lett.* 2012, *7*, 1–4.

[5] Krejcova, L., Huska, D., Hynek, D., Kopel, P., Adam, V., Hubalek, J., Trnkova, L., Kizek, R., *Int. J. Electrochem. Sci.* 2013, *8*, 689–702.

[6] Ryvolova, M., Chomoucka, J., Drbohlavova, J., Kopel, P., Babula, P., Hynek, D., Adam, V., Eckschlager, T., Hubalek, J., Stiborova, M., Kaiser, J., Kizek, R., *Sensors* 2012, *12*, 14792–14820.

[7] Krejcova, L., Dospivova, D., Ryvolova, M., Kopel, P., Hynek, D., Krizkova, S., Hubalek, J., Adam, V., Kizek, R., *Electrophoresis* 2012, *33*, 3195–3204.

[8] Chen, G. H., Sun, J., Dai, Y. J., Dong, M., *Electrophoresis* 2012, *33*, 2192–2196.

 

[9] Hlavacek, A., Skladal, P., *Electrophoresis* 2012, *33*, 1427–1430.

[10] Chen, Q. D., Fung, Y. S., *Electrophoresis* 2010, *31*, 3107–3114.

[11] Kleparnik, K., Voracova, I., Liskova, M., Prikryl, J., Hezinova, V., Foret, F., *Electrophoresis* 2011, *32*, 1217–1223.

[12] Ryvolova, M., Chomoucka, J., Janu, L., Drbohlavova, J., Adam, V., Hubalek, J., Kizek, R., *Electrophoresis* 2011, *32*, 1619–1622.

[13] Wang, J. J., Huang, X. Y., Zan, F., Guo, C. G., Cao, C. X., Ren, J. C., *Electrophoresis* 2012, *33*, 1987–1995.

[14] Frigerio, C., Ribeiro, D. S. M., Rodrigues, S. S. M., Abreu, V., Barbosa, J. A. C., Prior, J. A. V., Marques, K. L., Santos, J. L. M., *Anal. Chim. Acta* 2012, *735*, 9–22.

[15] Kuang, H., Zhao, Y., Ma, W., Xu, L. G., Wang, L. B., Xu, C. L., *Trac-Trends Anal. Chem.* 2011, *30*, 1620–1636.

[16] Biju, V., Itoh, T., Anas, A., Sujith, A., Ishikawa, M., *Anal. Bioanal. Chem.* 2008, *391*, 2469–2495.

[17] Ledentsov, N. N., Ustinov, V. M., Shchukin, V. A., Kop'ev, P. S., Alferov, Z. I., Bimberg, D., *Semiconductors* 1998, *32*, 343–365.

[18] Yoffe, A. D., *Adv. Phys.* 2001, *50*, 1–208.

[19] Chomoucka, J., Drbohlavova, J., Masarik, M., Ryvolova, M., Huska, D., Prasek, J., Horna, A., Trnkova, L., Provaznik, I., Adam, V., Hubalek, J., Kizek, R., *Int. J. Nanotechnol.* 2012, *9*, 746–783.

[20] Auyeung, E., Cutler, J. I., Macfarlane, R. J., Jones, M. R., Wu, J. S., Liu, G., Zhang, K., Osberg, K. D., Mirkin, C. A., *Nat. Nanotechnol.* 2012, *7*, 24–28.

[21] Boukany, P. E., Morss, A., Liao, W. C., Henslee, B., Jung, H. C., Zhang, X. L., Yu, B., Wang, X. M., Wu, Y., Li, L., Gao, K. L., Hu, X., Zhao, X., Hemminger, O., Lu, W., Lafyatis, G. P., Lee, L. J., *Nat. Nanotechnol.* 2011, *6*, 747–754.

[22] Chivers, C. E., Crozat, E., Chu, C., Moy, V. T., Sherratt, D. J., Howarth, M., *Nat. Methods* 2010, *7*, 391-U376.

[23] Tikhomirov, G., Hoogland, S., Lee, P. E., Fischer, A., Sargent, E. H., Kelley, S. O., *Nat. Nanotechnol.* 2011, *6*, 485–490.

[24] Wang, Y., Bai, Y., Wei, X., *IET Nanobiotechnol.* 2011, *5*, 14–19.

[25] Li, J., Zhou, X. P., Ni, S. Y., Wang, X. Q., *Colloid J.* 2010, *72*, 710–715.

[26] Hua, X. F., Liu, T. C., Cao, Y. C., Liu, B., Wang, H. Q., Wang, J. H., Huang, Z. L., Zhao, Y. D., *Anal. Bioanal. Chem.* 2006, *386*, 1665–1671.

[27] Kuo, Y. C., Wang, Q., Ruengruglikit, C., Yu, H. L., Huang, Q. R., *J. Phys. Chem. C* 2008, *112*, 4818–4824.

[28] Llopis, X., Pumera, M., Alegret, S., Merkoci, A., *Lab. Chip.* 2009, *9*, 213–218.

[29] Goldman, E. R., Balighian, E. D., Mattoussi, H., Kuno, M. K., Mauro, J. M., Tran, P. T., Anderson, G. P., *J. Am. Chem. Soc.* 2002, *124*, 6378–6382.

[30] Liu, H. Y., Gao, X. H., *Bioconjugate Chem.* 2011, *22*, 510–517.

[31] Goldman, E. R., Anderson, G. P., Tran, P. T., Mattoussi, H., Charles, P. T., Mauro, J. M., *Anal. Chem.* 2002, *74*, 841–847.

[32] Goldman, E. R., Balighian, E. D., Kuno, M. K., Labrenz, S., Tran, P. T., Anderson, G. P., Mauro, J. M., Mattoussi, H., *Phys. Status Solidi B-Basic Res.* 2002, *229*, 407–414.

[33] Jin, T., Tiwari, D. K., Tanaka, S., Inouye, Y., Yoshizawa, K., Watanabe, T. M., *Mol. Biosyst.* 2010, *6*, 2325–2331.

[34] Krizkova, S., Ryvolova, M., Hynek, D., Eckschlager, T., Hodek, P., Masarik, M., Adam, V., Kizek, R., *Electrophoresis* 2012, *33*, 1824–1832.

[35] Yang, H., Gurgel, P. V., Carbonell, R. G., *J. Pept. Res.* 2005, *66*, 120–137.

[36] Carrillo-Carrion, C., Moliner-Martinez, Y., Simonet, B. M., Valcarcel, M., *Anal. Chem.* 2011, *83*, 2807–2813.

[37] Huang, X. Y., Weng, J. F., Sang, F. M., Song, X. T., Cao, C. X., Ren, J. C., *J. Chromatogr. A* 2006, *1113*, 251–254.

[38] Kasicka, V., *Electrophoresis* 2012, *33*, 48–73.

[39] Song, X. T., Li, L., Chan, H. F., Fang, N. H., Ren, J. C., *Electrophoresis* 2006, *27*, 1341–1346.

[40] Wong, P., Barbeau, A., Roses, A. D., *Anal. Biochem.* 1985, *150*, 288–293.

[41] Mizutani, N., Sugita-Konishi, Y., Omoe, K., Shinagawa, K., Kawakami, H., Kanno, S., Sugiyama, K., Kamata, Y., *Int. J. Food Sci. Technol.* 2012, *47*, 155–159.

[42] Liu, J. M., Lin, L. P., Liu, Z. B., Yang, M. L., Wang, X. X., Zhang, L. H., Cui, M. L., Jiao, L., *J. Fluoresc.* 2012, *22*, 419–429.

[43] Morales-Narvaez, E., Monton, H., Fomicheva, A., Merkoci, A., *Anal. Chem.* 2012, *84*, 6821–6827.

[44] Tian, J. N., Zhou, L. J., Zhao, Y. C., Wang, Y., Peng, Y., Zhao, S. L., *Talanta* 2012, *92*, 72–77.

[45] Luo, Y., Zhang, B., Chen, M., Jiang, T. L., Zhou, D. Y., Huang, J. F., Fu, W. L., *J. Transl. Med.* 2012, *10*, 24.

 