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Mutual recognition of TNT using antibodies polymeric shell having CdS

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ABSTRACT

Click chemistry is the latest strategy called upon in the development of state of the art exponents of bioconjugation. In this study, we have proposed a covalent and photosensitive crosslinking conjugation of the antibody on nano-structures. For this purpose, quantum dots (QDs) without affecting conformation and function of proteins through the ruthenium-chelate based aminoacid monomer linkages have been applied. The aminoacid-monomer linkages called ANADOLUCA (AmiNoAcid Decorated and Light Underpining Conjugation Approach) give reusable oriented and cross-linked anti 2,4,6-trinitrotoluene (TNT) conjugated QD for TNT detection. In this work, a new and simple method has improved to design and prepare high sensitive nanoconjugates for TNT determination. We have demonstrated the use of luminescent QDs conjugated to antibody for the specific detection of the explosive TNT in aqueous environments. The binding affinity of each nanoconjugates for TNT detection by using Langmuir adsorption methods has also been investigated.

1. Introduction

2,4,6-Trinitrotoluene (2,4,6-TNT), 2,4-dinitrotoluene (2,4-DNT) and nitroglycerine are explosive materials most frequently used by terrorists. TNT is the most widely used explosive due to its low melting point, chemical and thermal stability, low sensitivity to impact and friction. Although it is basically a stable compound, it is highly photo reactive [1–3]. It is also the 'standard' explosive material by which the performance of all other explosive compounds are compared. For this reason, detection of TNT in aqueous environments has become a current priority. The detection of TNT can be carried out with gas chromatography–mass spectrometry (GC–MS), liquid chromatography–mass spectrometry (LC–MS), energy-dispersive X-ray diffraction and electrochemical sensors. These techniques are highly selective but expensive. In these days, nanoparticles can be used for the rapid detection of explosives. Quantum dots (QDs) [4,5], Au [6,7] and Si [8,9] nanoparticles can be effectively used for TNT detection.

Zero-dimensional semiconductor nanomaterials, known as QDs, which are composed of elements from the periodic groups II–VI, III–V or IV–VI. QDs are spherical particles and their sizes in the range of 1–12 nm in diameter [10–12]. When QDs size is small, their fluorescence shift to blue region [12,13]. QDs have many excellent optical properties such as high quantum yield, long fluorescence lifetime, wide excitation spectrum and narrow emission spectrum, tunable emission wavelength and so on, thus they have become a new popular type of fluorescence probes in these years. On the other hand, QDs typically exhibit higher fluorescence quantum yields than conventional organic fluorophores [14,15]. QDs can be made water-soluble and biocompatible by surface modification and bioconjugation. Attachment of molecules to surfaces is of great interest for the development of a large variety of applications. Tailorable physical properties and oriented surface modification are very important aspects of nanomaterials. Indeed, in this regard, nanomaterials and biology have a sustained history as nanoparticles have been used as bio-conjugation and cellular labeling agents for the past four decades [16]. At this point in time, the use of nanobioconjugates for life sciences and biotechnology applications is one of the fastest moving fields of nanobiotechnology. Bioconjugation encompasses a broad area of science at the interface between molecular biology and chemistry. It is essential to modulate the chemical nature of nanoparticle surfaces to alter their biocompatibility and add additional biochemical functionalities and stabilities in order to apply nanoparticles in biological systems or aqueous environment. By employing different conjugation technologies they cannot only be rendered biocompatible, but also, to fulfill tasks. These embody receptor are used for targeting, sensing, imaging, catalysis or preconcentrator. To achieve this goal, different monomeric or polymeric coatings are applied to provide biocompatibility and additional bioconjugation (also for multivalent interaction) for targeting those particular drugs which prevent the disease spreading and other applications [17].

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ANADOLUCA (AmiNoAcid Decorated and Light Underpining Conjugation Approach) is a novel protein conjugating approach which contains the novel synthetic materials and their applications in life sciences and biotechnology [18]. A "photosensitive ruthenium based aminoacid monomer chelate" is a hapten coordination compound that has at least one amino acid monomer as ligand and provides light induced covalent conjugation of biomolecules. A "photosensitive oligomer (polymer)" is a sequence of ruthenium chelate based amino acid monomers and dissolves in aqua and provides light induced covalent conjugation of biomolecules onto micro and nanoplatforms. The photosensitive, covalent and cross-linking conjugation methods based on aminoacid and ruthenium-chelate based monomers are used to provide accurately antibody orientation, preventing denaturation during bonding and after bonding. The photosensitive aminoacid monomer linkers can react via chemically and are biocompatible to a lot of different micro and nano-surface and then, to the protein when they act as a single-step cross-linking reaction using irradiation. To integrate proteins with inorganic nanostructures based on click chemistry that can be carried out at mild conditions, is independent of pH and temperature, without affecting conformation and function of protein. Recently, "click" chemistry has rapidly become a very popular method.

We present herein a novel approach to efficiently couple a molecule to nanoparticle surfaces. In this study, we have investigated and compared a covalent and photosensitive crosslinking conjugation of the anti TNT antibody on QDs without affecting conformation and function of proteins through the aminoacid-monomer and/or ruthenium-chelate based aminoacid monomer linkages called ANADOLUCA. We have prepared TNT sensors and the sensors consist of anti-TNT antibody specifically conjugated on the surface of QDs. We have synthesized photosensitive ruthenium based aminoacid monomer chelate and photosensitive oligomer (polymer) and characterized these monomers and oligomer with NMR and MALDI-TOF–MS. QDs have separately functionalized with methacryloyl cysteine (MACys), methacryloyl tyrosine (MATyr), methacryloyl tryptophan (MATrp), bis(2-2′-bipyridyl)bis(MATrp)-ruthenium(II) (MATrp-Ru(bpy)$_2$-MATrp), bis(2-2′-bipyridyl)-MATrp-MUABt ruthenium(II) (MUABt-Ru(bpy)$_2$-MATrp) monomers and polymerized in daylight. The interactions between nanoconjugate and TNT have been determined by fluorescence resonance energy transfer (FRET) at different TNT concentrations. TNT adsorption studies have performed in a batch system separately. We have demonstrated the use of luminescent QDs conjugated to antibody for the specific detection of the explosive TNT in aqueous environments. The binding affinity of each nanoconjugates for TNT detection by using Langmuir adsorption methods have also investigated.

2. Experimental procedures

2.1. Materials

Methacryloyl chloride was supplied by Aldrich and used as received. All chemicals were of reagent grade and were purchased from Aldrich and Sigma. All water used in the experiments was purified using a Barnstead NANOpure ultrapure water system. Elemental analyses were performed using Vario ELIII. ^{1}H NMR and ^{13}C NMR spectra were recorded at 500 MHz on a Bruker 500 MHz NMR spectrometer Ultra-shield FT-NMR spectrometer. All MALDI-TOF/MS were acquired on a Voyager Biospectrometry STR Workstation/the system utilizing a pulsed nitrogen laser, emitting 337 nm. The acceleration voltage was set to 20 kV and the delay time was 100 ns. Mass analysis was carried out in positive reflector mode and delayed extraction mode. α-Cyano-4-hydroxycinnamic acid (CHCA) was used as a matrix solution. 10 mg CHCA was solved in 1:1, 1 mL of 0.3% trifluoroacetic acid (TFA) solution and acetonitrile. 2 μL of sample solution was mixed with 23 μL of 10 mg mL^{-1} solution of CHCA in acetonitrile/0.3% TFA. This preparation (1 μL) was placed onto a MALDI-TOF/MS sample plate and allowed to dry. Photo-luminescence spectra were obtained using a Carry Eclipse Varian Model Fluorescence Spectrometer. All nanoparticules were excited at 310 nm, and emission was recorded at 621 nm. Fluorescence value was measured by 20 times dilution.

2.2. Preparation of aminoacid-monomer

3-(4-Hydroxyphenyl)-2-[(2-methylacryloyl)amino]-propanoic acid (methacryloyl tyrosine, MATyr), N-[1-(1H-Indol-3-methyl)-2-oxo-propyl]-2-methylacrylamide (methacryloyl tryptophan, MATrp), 2-[(2-methylacryloyl)amino]-3-sulfanylpropanoic acid (methacryloyl cysteine, MACys), and 11-(1H-benzotriazol-1-yl)-11-oxoundecane-1-thiol (MUABt) were prepared according to the previously published method by our group [19]. An appropriate amino acid (1 equiv.) was dissolved in 1 M aqueous solution of NaOH (1 equiv.) in a round bottom-flask. A solution of methacryloyl benzotriazole (MA-Bt) (1 equiv.) in 25 mL of 1,4-dioxane was slowly added into the amino acid solution. Reaction mixture was allowed to stir for 10–20 min at room temperature. Completion of reaction was monitored by TLC and after the reaction finished, 1,4-dioxane was evaporated under vacuum. The residue was diluted with water and extracted with ethyl acetate (3 × 50 mL) to remove 1H-benzotriazole. Collected water phases were neutralized to pH 6–7 using 10% water solution of HCl (pH should keep around 6–7 to prevent possible polymerization of methacryloyl group in acidic medium). Water was removed via rotary-evaporator to give MATyr in 90%, MATrp in 85%, and MACys in 83% yield.

2.2.1. Synthesis of 11-(1H-benzotriazol-1-yl)-11-oxoundecane-1-thiol (MUA-Bt) as ligand

MUA-Bt was prepared according to following literature method [20]. Thionylchloride ($SOCl_2$) (1 equiv.) was added to the solution of 1H-Benzotriazole (Bt-H) (3 equiv.) in dichloromethane CH_2Cl_2 (100 mL) under nitrogen atmosphere at room temperature. After 30 min stirring, 11-mercaptoundecanoic acid (MUA) was poured into the reaction mixture. Upon the addition, the white precipitate was formed. The reaction mixture was stirred for 3 h. After this period white solid was filtered and washed with CH_2Cl_2. The collected filtrate was extracted with 2 M NaOH (3 × 50 mL) to remove excess of Bt-H. The organic layer was dried using $MgSO_4$ and the solvent was evaporated under vacuum. The crude product was purified using ethyl acetate–hexane mixture over silica gel column to get MUA-Bt as white microcrystals in 87% yield.

2.2.2. Synthesis of photosensitive ruthenium based aminoacid monomers

1 equiv. $RuCl_2(MeSO)_4$ and 2 equiv. 2-2′-bipyridyl were refluxed in chloroform (30 mL) for ca. 1 h. The solvent was evaporated and the residue was dissolved in acetone. An orange complex was obtained after the addition of ether. This was filtered off, washed with ether and dried under vacuum. M.p.: 205–210 °C.

2.2.3. Synthesis of chlorobis(2-2′-bipyridyl) MATrp-ruthenium(II) (1) and bis(2-2′-bipyridyl)bis(MATrp)-ruthenium(II) (2)

1 equiv. $RuCl_2(bipyr)_2$ was dissolved in water. The solution cooled to 0 °C and N,N,N-triethylamine (NEt_3) was added. The aqueous solution of 1 equiv. and 2 equiv. MATrp were added, separately, dropwise into that solution and stirred at room temperature for 30 min. The mixture was heated to 80 °C for refluxing ca. 24 h. The

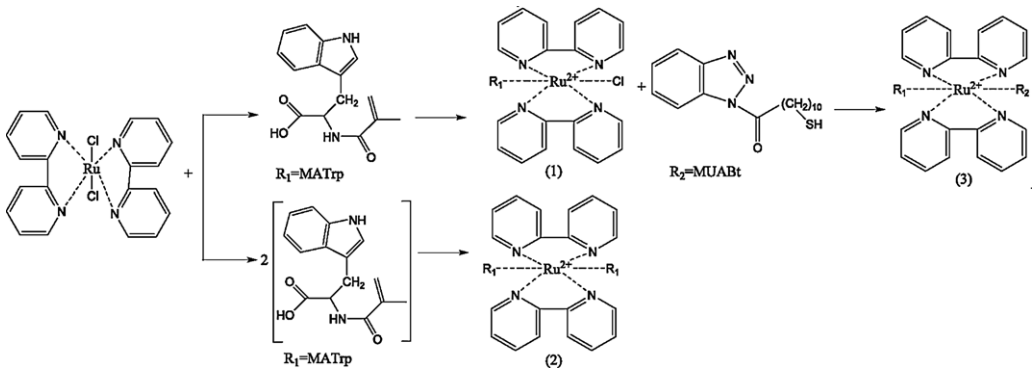

Fig. 1. Schematic representation of the photosensitive ruthenium based aminoacid-monomer preparation.

brown complex was filtered off, washed with ether and dried under vacuum (Fig. 1).

2.2.4. Synthesis of bis(2-2′-bipyridyl)-MATrp-MUABt ruthenium(II) **(3)**

1 equiv. **1** was dissolved in MetOH. Then, 1 equiv. MUABt was dissolved in DMSO and added to the first solution by dropwise at room temperature. The mixture was stirred at 110 °C for 6 h. Solvents were removed under reduced pressure and the residue was taken in CH_2Cl_2. Organic layer was washed with H_2O (3×10 mL), dried over $MgSO_4$, washed with ether and dried under vacuum. M.p.: >220 °C (Fig. 1).

2.2.5. Synthesis of quantum dots and functionalization with aminoacid monomer (MACys) and MUABt-Ru(bpy)$_2$-MATrp photosensitive ruthenium based aminoacid monomers

QDs were prepared according to the previously published method [15]. Firstly, 0.01 M $Cd(OAc)_2{\cdot}2H_2O$ solution (24 mL) was prepared with ethanol. The solution was stirred continuously for 30 min in nitrogen ambient. After that, Na_2S (0.01 M, 24 mL) was slowly added, stirred under nitrogen ambient for 30 min and then, centrifuged to collect precipitate. The precipitate was washed in double distilled water and dried in air. The entire synthesis was carried out at room temperature. QDs were modified with MACys aminoacid monomer and MUABt-Ru(bpy)$_2$-MATrp photosensitive ruthenium based aminoacid monomers, separately. Aminoacid monomer and photosensitive ruthenium based aminoacid-monomers were used for forming of reminiscent of self-assembled monolayer on the surface of QDs. For functionalization with MACys aminoacid monomers; 5 mL of QDs were immersed in ethanol containing 0.018 M, 10 mL of MACys in order to introduce methacryloyl groups onto the surface of QDs. The nanocrystals were then washed with ethanol and deionized water for 10 min to remove the excess of thiol groups. A stable self-assembled monolayer of MACys was formed onto the nanocrystal surfaces after all these steps. Fluorescence value was measured by 20 times dilution.

For modification with MUABt-Ru(bpy)$_2$-MATrp photosensitive ruthenium based aminoacid-monomers, 1 mL of QDs solution was added into 1 mL of MUABt-Ru(bpy)$_2$-MATrp solution (5 mg mL^{-1}), diluted with ethanol to 20 mL and mixed during a day. The nanocrystals were then washed with ethanol and deionized water for 10 min to remove the excess of ruthenium monomer groups. Consequently, a stable self-assembled monolayer of photosensitive ruthenium based aminoacid-monomer was formed onto the nanocrystal surfaces. Fluorescence value was measured by 20 times dilution.

After QDs were modified MACys aminoacid monomer, QDs were functionalized with MATyr and MATrp aminoacid monomer and MATrp-Ru(bpy)$_2$-MATrp and MUABt-Ru(bpy)$_2$-MATrp photosensitive ruthenium based aminoacid-monomers separately. 1 mL of MACys aminoacid monomer modified QDs was added into MATyr solution which was dissolved in (approximately 5 mg) 1 mL of water, diluted with ethanol to 20 mL and mixed during a day. Similarly, the other molecules (MATrp (aminoacid monomer), MATrp-Ru(bpy)$_2$-MATrp and MUABt-Ru(bpy)$_2$-MATrp photosensitive ruthenium based aminoacid monomers) were functionalized, separately. The nanocrystals were then washed with ethanol and deionized water for 10 min to remove the excess of monomer and photosensitive ruthenium based aminoacid monomer groups. Fluorescence value was measured by 20 times dilution.

2.3. Preparation of anti TNT cross-linked QDs and the evaluation of nanoshell QDs luminescence

For the synthesis of TNT recognized nanoshell/polymer nanocrystals; 10 ppm 1 mL of anti-TNT solution, 1 mg mL^{-1} 100 μL of photosensitive polymer solution and 100 mM 100 μL of ammonium persulfate (APS) solution in pH 7.4 buffer were added into 1 mL of aminoacid functionalized nanocrystal and polymerized in daylight during a day. All other QDs, which have functionalized with different photosensitive ruthenium based aminoacid monomers and interacted with anti-TNT solution were prepared with the same procedure, but photosensitive polymer solution did not use. APS was used through the aminoacid-monomer linkages to give covalent bonds with tyrosine, cysteine or a tryptophane of antibodies and other biomolecules. Nanocrystals were centrifuged and re-dispersed in pH 7.4 buffer.

2.4. Recognition of TNT by anti TNT cross-linked QDs

The sensing capability of the nanobioconjugate sensors were further explored by introducing TNT. The interaction with TNT was studied using fluorescence spectrophotometer. TNT adsorption studies were performed in a batch system. 4 mL of 10^{-2}, 0.2, 0.5, 0.8 and 1.0 ppm TNT solutions were interacted with 1 mL of nanocrystal solution, respectively. A big difference was observed between the excitation and emission wavelengths of QDs for the recorded fluorescence measurements.

3. Results and discussion

3.1. Characterization of aminoacid-monomer and photosensitive ruthenium based aminoacid-monomers

Aminoacid-monomers and photosensitive ruthenium based aminoacid-monomers were characterized by NMR and MALDI-TOF–MS. All data improved that aminoacid monomers and

photosensitive ruthenium based aminoacid monomers have been synthesized.

3.2. Data for MATrp

^{1}H NMR (500 MHz, DMSO-d6), δ: 10.7 (s, 1H), 7.45 (d, 1H, J = 7.89 Hz), 7.22–7.38 (m, 2H), 6.96–7.1 (m, 2H), 6.87 (t, 1H, J = 7.40, 14.86 Hz), 5.48 (s, 1H), 5.21 (s, 1H), 4.05 (dd, 1H, J = 4.80, 9.70 Hz), 3.28 (dd, 1H, J = 4.60, 14.16 Hz), 3.09 (dd, 1H, J = 4.60, 14.16 Hz), 1.78 (s, 3H) ppm. ^{13}C (125 MHz, DMSO-d6), δ: 19.0, 27.5, 55.5, 111.5, 112.0, 118.5, 119.0, 119.5, 121.0, 124.0, 129.0, 136.0, 141.0, 166.0, 173.5 ppm.

3.3. Data for MATy**r**

^{1}H (500 MHz, DMSO-d6), δ: 7.35 (d, J = 8.05 Hz, 2H), 7.05 (d, J = 8.40 Hz, 2H), 6.29 (s, 1H), 5.90 (s, 1H), 3.15 (dd, J = 4.49, 14.25 Hz, 1H), 3.02 (dd, J = 4.02, 14,12 Hz, 1H), 2.90 (dd, J = 7.75, 14.20 Hz, 1H), 2.00 (s, 3H) ppm. ^{13}C (125 MHz, DMSO-d6), δ: 170.9, 165.8, 149.6, 135.9, 130.9, 128.0, 121.8, 115.6, 55.9, 37.0, 18.5 ppm.

3.4. Data for MACys

^{1}H (500 MHz, DMSO-d6 and D_2O): Because of the solubility problem of MACys, NMR spectra for this compound was recorded both in DMSO-d6 and D_2O. In DMSO-d6 two single peaks between 7.67 and 7.36 ppm were recorded and since these peaks were disappeared in D_2O, it is assumed that these are —SH and —NH peaks. 5.67 (s, 1H) and 5.31 (s, 1H) peaks were assigned to the ethylene group of methacryloyl group. 4.16 (m, 1H) was assigned to the CH proton bonded to the NH group of amino acid. (—CH_2—**CH**—NH—). 3.10–3.02 (m, 2H) peak was assigned to the —CH_2— protons for amino acid. 1.88 (s, 3H) was assigned to the —CH_3 group on methacryloyl moiety.

^{13}C (125 MHz, D_2O), δ: 177.5, 172.9, 141.8, 122.6, 55.1, 30.1, 17.49 ppm.

3.5. Data for MUABt

^{1}H NMR (500 MHz, $CDCl_3$-d), δ: 8.32 (d, J = 8.27 Hz, 1H), 8.15 (d, J = 8.30 Hz, 1H), 7.68 (t, J = 7.15 Hz, 1H), 7.53 (t, J = 7.17 Hz, 1H), 3.44 (t, J = 7.50 Hz), 2.89 (t, J = 7.50 Hz, 1H), 2.70 (t, J = 7.50 Hz, 1H), 1.89–1.97 (m, 2H), 1.66–1.79 (m, 2H), 1.65–1.160 (m, 1H), 1.54–1.46 (m, 2H), 1.44–1.37 (m, 4H), 1.35–1.28 (m, 6H) ppm.^{13}C NMR (125 MHz, $CDCl_3$-d), δ: 24.5, 28.5, 28.8, 29.1, 29.2, 29.4, 29.4, 35.5, 38.9, 39.2, 114.5, 120.1, 126.1, 130.3, 131.14, 146.2, 172.7 ppm.

3.6. Data for $Ru(bpy)_2Cl_2$

Anal. for $C_{20}H_{15}Cl_2N_4Ru$: found: C 48.20%, H 3.01%, N 12.89%, calcd.: C 49.70%, H 3.13%, N 11.59%.

^{1}H NMR (500 MHz, $CDCl_3$), ppm: 10.25 (d, 2H, J = 4.35 Hz), 9.93 (d, 2H, J = 4.29 Hz), 9.73 (d, 2H, J = 5.86 Hz), 9.65 (d, 2H, J = 4.52 Hz), MALDI-TOF–MS spectrum of $Ru(bpy)_2Cl_2$ showed that the ion peaks at m/z 293, 327, 448 and 484 relating to RubpyCl, $RubpyCl_2$, $Ru(bpy)_2Cl$ and $Ru(bpy)_2Cl_2$, respectively. The MS and NMR spectrum data confirm that $Ru(bpy)_2Cl_2$ structure was produced exactly.

3.7. Data for chlorobis(2-2′-bipyridyl) MATrp-ruthenium(II) (1) and bis(2-2′-bipyridyl)bis(MATrp)-ruthenium(II) (2) photosensitive aminoacid monomers

Anal. for $C_{35}H_{31}ClN_6O_3Ru$ **(1)**: found: C 57.77%, H 4.22%, N 13.14%, calcd.: C 58.37%, H 4.34%, N 11.67%.

MALDI-TOF–MS spectrum of **1** showed that the ion peaks at m/z 272, 408, 529 and 564 relating to MATrp, RuMATrpCl, RubpyMATrp, RubpyMATrpCl, respectively. The peak at m/z 564 represents the binding of only one MATrp monomer to $Ru(bpy)_2Cl_2$.

Anal. for $C_{50}H_{46}N_8O_6Ru$ **(2):** found C 61.97%, H 4.3%, N 10.8, calcd.: C 62.82%, H 4.85%, N 11.72%.

^{1}H NMR (500 MHz, $CDCl_3$), ppm: 10.74 (d, 2H, J = 14.9 Hz), 9.76 (s, 1H), 7.99 (d, 1H, J = 7.68 Hz), 7.91 (d, 1H, J = 7.57 Hz), 7.54 (d, 2H, J = 7.94 Hz), 7.46 (d, 1H, J = 8.2 Hz), 7.31 (t, 4H, J = 6.76 Hz), 7.16 (s, 1H), 7.1 (d, 2H, J = 11.7 Hz), 7.07–7.00 (q, 5H, J = 8.0 Hz), 5.6 (d, 2H, J = 6.6 Hz), 5.3 (s, 2H), 4.57 (m, 1H, 4.39 (m, 2H), 1.24 (s, 3H).

MALDI-TOF–MS spectrum of **2** showed that the ion peaks at m/z 272, 373, 413, 529 and 685 relating to MATrp, Ru-MATrp, $Ru(bpy)_2$, Ru(bpy)-MATrp and $Ru(bpy)_2$-MATrp, respectively. MALDI-TOF–MS spectrum of **(2)** has not include any chloride complex. These data confirm that $(MATrp)_2Ru(bpy)_2$ structure was produced exactly.

3.8. Data for bis(2-2′-bipyridyl)-MATrp-MUABt ruthenium(II) (3) photosensitive aminoacid monomer

Anal. for $C_{52}H_{55}N_9O_4RuS$ **(3)**: found: C 61.53%, H 4.92%, N 12.99%, calcd.: C 62.26%, H 5.53%, N 12.57%.

^{1}H NMR (500 MHz, $CDCl_3$), ppm: 8.32 (d, 1H, J = 9.72 Hz), 8.14 (d, 1H, J = 8.26 Hz), 7.89 (s, 2H), 7.67 (t, 1H, J = 7.66 Hz), 7.52 (t, 1H, J = 7.65 Hz), 7.41 (d, 2H, J = 5.86 Hz), 3.68 (s, 10H), 3.44 (t, 2H, J = 7.48 Hz), 2.88 (t, 6H, J = 7.33 Hz), 2.33 (m, 16H), 1.92 (p, 3H), 1.74 (t, 8H, J = 7.35 Hz).

MALDI-TOF–MS spectrum of **3** showed that the ion peaks at m/z 419, 529 and 1003.1 peaks show Ru-MUABt, Ru(bpy)-MATrp and $Ru(bpy)_2$MATrp-MUABt, respectively. The peak at m/z 1003.1 proved both binding of MATrp and MUABt monomers.

3.9. Measurement of binding interactions of TNT

The binding ability of TNT with the QD nanoconjugate sensor was studied with fluorescence spectroscopy (Fig. 2). According to fluorescence measurements, a big difference was observed between the excitation and emission wavelengths of QDs. The wavelength difference between the light excitation and emission is called the Stokes shift. A large Stokes shift is desirable as it simplifies the technical aspects of achieving concurrent excitation with emission detection and also decreased interfering background. While QDs (blue line) were activated with hydrophilic thiol having aminoacid monomer with MACys (purple line) the fluorescence intensity increased. As expected, the fluorescence intensity of MACys modified QDs nanoparticle decreased when second functionalization carried out with MATyr and MATrp aminoacid monomers and MATrp-Ru(bpy)$_2$-MATrp and MUABt-Ru(bpy)$_2$-MATrp photosensitive ruthenium based aminoacid-monomers (green line).

A quenching was observed due to fluorescence resonance energy transfer (FRET). FRET is a chemical transduction method that occurs from the donor to the acceptor. When the fluorescence spectrum of the donor and the absorption spectrum of the acceptor overlap to an adequate degree, energy will be resonantly transferred from the donor to the acceptor. This can occur over distances of 10–100 Å, with the most efficient transfer occurring at 20–60 Å. FRET is extensively used in macromolecular interactions, such as protein–protein association, antibody-antigen binding and DNA hybridization [21–25]. In this study, FRET was used for supporting the accurate antibody orientation on the QD surface. MAC functionalized QD acts as donor group, second functionalization with MATyr and MATrp aminoacid monomers and MATrp-Ru(bpy)$_2$-MATrp and MUABt-Ru(bpy)$_2$-MATrp photosensitive ruthenium based aminoacid-monomers act as acceptor group

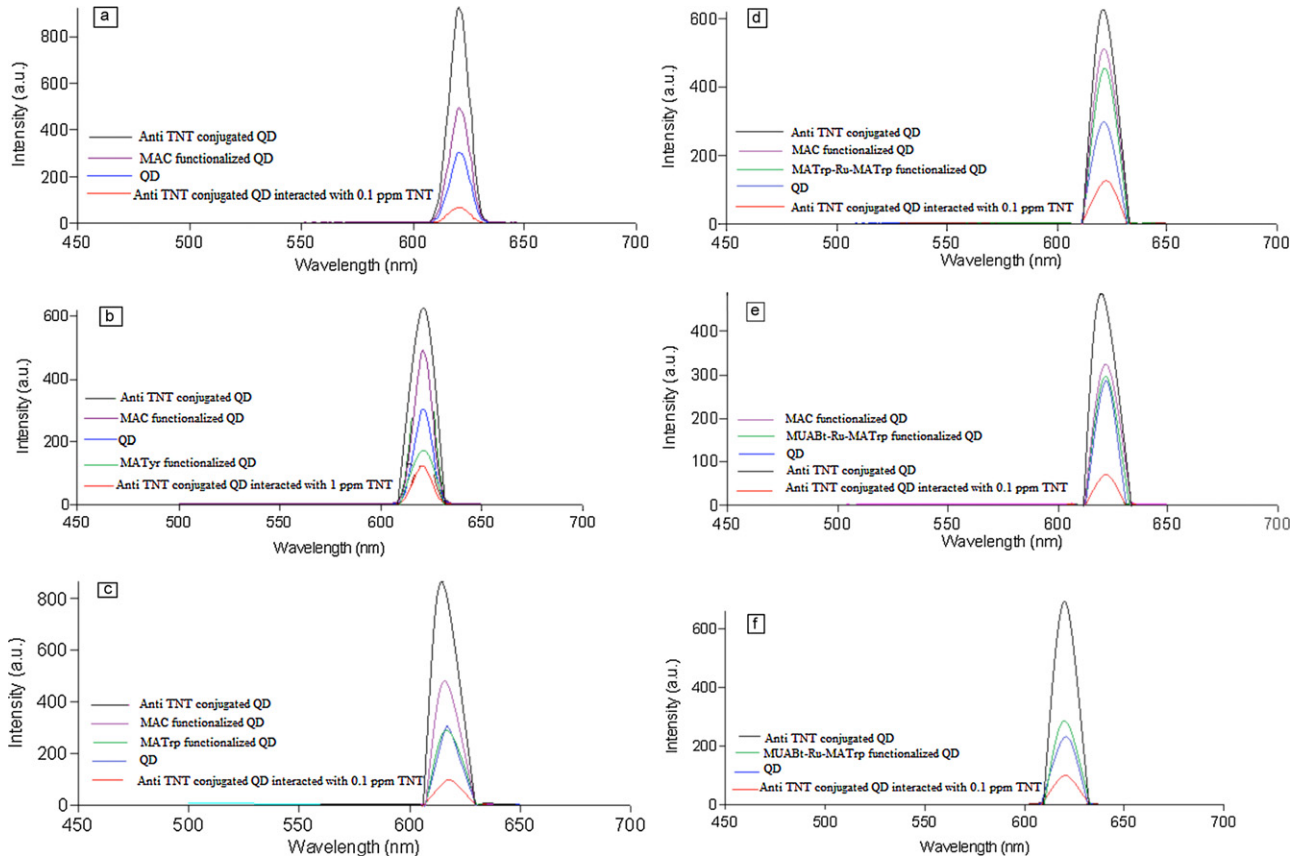

Fig. 2. Fluorescence intensities of (a) MAC functionalized, anti TNT conjugated QD's; (b) MATyr functionalized, anti TNT conjugated QD–MACys'; (c) MATrp functionalized, anti TNT conjugated QD–MACys'; (d) MATrp-Ru(bpy)$_2$-MATrp functionalized, anti-TNT conjugated QD–MACys'; (e) (MUABt-Ru(bpy)$_2$-MATrp) functionalized, anti-TNT conjugated QD–MACys'; and (f) MUABt-Ru(bpy)$_2$-MATrp functionalized, anti-TNT conjugated QD's. (For interpretation of the references to color in text, the reader is referred to the web version of this article.)

(Fig. 3). The fluorescence intensity of aminoacid monomer and photosensitive ruthenium based aminoacid-monomers functionalized QDs increased when they are polymerized with APS (black line in Fig. 2). The fluorescence intensities of the anti-TNT conjugated QDs can be enhanced by TNT concentration. The enhancement of fluorescence intensity is proportional to TNT concentration. The fluorescence intensity correlates with the amount of TNT bounded to the nanoshell having CdS nanocrystal in the cases of incubating the anti TNT attached nanoshell sensor with the TNT aqueous solution.

The affinity constants of TNT and its analogues to the nanoshell sensor can be estimated from the thermodynamic analysis of the fluorescence intensity as a function of the TNT concentration based on Scatchard analysis [26,27] and Langmuir isotherm [28].

If we consider a binding equilibrium scenario such as:

$$\mathrm{TNT} + \mathrm{QDs} \underset{k^{-1}}{\overset{k}{\rightleftarrows}} [\mathrm{TNT} - \mathrm{QDs}] \quad (1)$$

where QDs represent anti-TNT attached polymeric nanoshell having nanocrystal, and TNT – QDs is the TNT–nanocrystal bound complex.

The validity of the Langmuir isotherm can be tested by determining the affinity constant by measuring the fluorescence intensities at equilibrium with different bulk concentrations. Langmuir relationship can be obtained using the following equation:

$$\frac{C_0}{I} = \frac{1}{I_{\max} b} + \frac{C_0}{I_{\max}}$$

where C_0 is TNT concentration, b is Langmuir constant and $I_{\max}$ is the maximum fluorescence intensity. K_a and $I_{\max}$ values for the specific interaction between the ANADOLUCA functionalized QDs polymer and the TNT itself were determined by Langmuir isotherms, plotting C_0/I as a function of C_0. The Langmuir adsorption model assumes that the molecules are adsorbed at a fixed number of well-defined sites, each of which is capable of holding only one molecule. These sites are also assumed to be energetically equivalent, and distant from each other so that there are no interactions between molecules adsorbed on adjacent sites. According to the correlation coefficients of isotherms, Langmuir model was found to be applicable in interpreting TNT and ANADOLUCA functionalized QDs polymer. The comparison of the association constants, K_a, and the apparent maximum number of recognition sites, $I_{\max}$ values, are presented in Table 1.

As seen from Table 1, in general, the magnitude of K_a based on Langmuir analysis shows the accessibility of TNT molecules to all of the nanobioconjugates. Affinity constants for antibodies usually lie in the range of 10^6–10^9 M^{-1}. According to this data, the obtained K_a values suggest that the affinity of the binding sites are very strong.

In order to study the effect of selectivity of TNT on the affinity to the MACys aminoacid monomer modified QD, selectivities

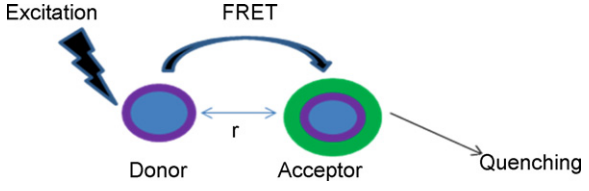

Fig. 3. Schematic representation of the FRET process.

Table 1
Comparision of Langmuir analysis for ANADOLUCA functionalized QDs polymer.

Process	K_a (M^{-1})	I_{max} (a.u.)	R^2
QD was modified MACys aminoacid monomer after that polymerized with APS and photosensitive polymer solution	4.41×10^7	73.529	0.9996
QD was modified MUABt-Ru(bpy)$_2$-MATrp photosensitive ruthenium based aminoacid monomer and polymerized with APS	3.48×10^7	108.696	0.9995
MACys aminoacid monomer modified QD was functionalized with MATyr amino acid monomer after that polymerized with APS and photosensitive polymer solution	6.3×10^6	120.482	0.9856
MACys aminoacid monomer modified QD was functionalized with MATrp amino acid monomer after that polymerized with APS and photosensitive polymer solution	1.18×10^7	96.154	0.9981
MACys aminoacid monomer modified QD was functionalized with photosensitive ruthenium based aminoacid monomer MATrp-Ru(bpy)$_2$-MATrp after that polymerized with APS	2.72×10^7	138.89	0.9991
MACys aminoacid monomer modified QD was functionalized with photosensitive ruthenium based aminoacid monomer MUABt-Ru(bpy)$_2$-MATrp after that polymerized with APS	1.22×10^7	92.593	0.9961

of the 2,4-dinitrotoluene and 2-nitrotoluene have been investigated. MACys aminoacid monomer modified QD were treated with 2,4-dinitrotoluene and 2-nitrotoluene to check whether the nanobioconjugate has any effect on recognition process. The results indicated that nanobioconjugate has 10 and 16 times greater selectivity for TNT than that of 2,4-dinitrotoluene and 2-nitrotoluene, respectively. The selectivity coefficient, k, of nanobioconjugate was calculated using the following equations:

$$k_{\text{nanobioconjugate}} = \frac{C_{\text{TNT}}}{C_{\text{2,4-dinitrotoluene (or 2-nitrotoluene)}}}$$

where C_{TNT} and $C_{\text{2,4-dinitrotoluene (2-nitrotoluene)}}$ are the concentrations of TNT, 2,4-dinitrotoluene and 2-nitrotoluene, respectively.

A 200 ppb TNT, 200 ppb 2,4-dinitrotoluene and 200 ppb 2-nitrotoluene solutions were prepared and nanobioconjugate was interacted using these solutions separately. Then, the fluorescence intensity of 2,4-dinitrotoluene and 2-nitrotoluene was recorded. The concentration of 2,4-dinitrotoluene and 2-nitrotoluene (considering the fluorescence intensity) was found using calibration graph. The results from the selectivity experiment clearly indicated that the change of fluorescence intensity is due to specific binding between TNT and anti TNT conjugated QD.

4. Conclusion

We have developed a novel protein conjugating method approach which contains the novel synthetic materials and their applications in TNT detection. The photosensitive, covalent and cross-linking conjugation methods based on aminoacid and photosensitive ruthenium based monomers were used to provide accurately antibody orientation, preventing denaturation during bonding and after bonding. The photosensitive aminoacid monomer linkers can react via chemically and are biocompatible to a lot of different micro and nano-surface and then to the protein when they act as a single-step cross-linking reaction using irradiation. The results showed that the fluorescence enhancement effect could be attributed to interaction between photosensitive ruthenium based aminoacid functionalized QDs and TNT. Langmuir adsorption isotherm showed that binding constants of nanobioconjugates are favorable for TNT detection. These nanosensors are potential candidate materials for detection of TNT in aqueous environments.

References

[1] P.T. Charles, L.C. Shrive-Lake, S.C. Francesconi, A.M. Churilla, J.G. Rangasammy, C.H. Patterson Jr., J.R. Deschamps, A.W. Kusterbeck, J. Immunol. Methods 284 (2004) 15–26.
[2] G. Bunte, J. Hürttlen, K. Hartlieb, H. Krause, Anal. Chim. Acta 591 (2007) 49–56.
[3] T. Alizadeh, M. Zare, M.R. Ganjali, P. Norouzi, B. Tavana, Biosens. Bioelectron. 25 (2010) 1166–1172.
[4] E.R. Goldman, I.L. Medintz, J.L. Whitley, A. Hayhurst, A.R. Clapp, H.T. Uyeda, J.R. Deschamps, M.E. Lassman, H. Mattoussi, J. Am. Chem. Soc. 127 (2005) 6744–6751.
[5] G.H. Shi, Z.B. Shang, Y. Wang, W.J. Jin, T.C. Zhang, Spectrochim. Acta A 70 (2008) 247–252.
[6] A.C.R. Pipino, V. Silin, Chem. Phys. Lett. 404 (2005) 361–364.
[7] T. Kawaguchia, D.R. Shankarana, S.J. Kima, K. Matsumoto, K. Toko, N. Miura, Sens. Actuators, B 133 (2008) 467–472.
[8] J. Wang, G. Liu, H. Wu, Y. Lin, Anal. Chim. Acta 610 (2008) 112–118.
[9] J. Feng, Y. Li, M. Yang, Sens. Actuators, B 145 (2010) 438–443.
[10] R.E. Galian, M. de la Guardia, Trends Anal. Chem. 28 (2009) 279–291.
[11] K. Kerman, T. Endo, M. Tsukamoto, M. Chikae, Y. Takamura, E. Tamiya, Talanta 71 (2007) 1494–1499.
[12] J.M. Costa-Fernaïndez, R. Pereiro, A. Sanz-Medel, Trends Anal. Chem. 25 (2006) 207–218.
[13] M.F. Frasco, N. Chaniotakis, Anal. Bioanal. Chem. 396 (2010) 229–240.
[14] N.C. Cady, A.D. Strickland, C.A. Batt, Mol. Cell. Probes 21 (2007) 116–124.
[15] S. Emir Diltemiz, R. Say, S. Buyuktiryaki, D. Hur, A. Denizli, A. Ersoz, Talanta 75 (2008) 890–896.
[16] N.L. Rosi, C.A. Mirkin, Chem. Rev. 105 (2005) 1547–1562.
[17] A.F.E. Hezinger, J. Tessmar, A. Göpferich, Eur. J. Pharm. Biopharm. 68 (2008) 138–152.
[18] R. Say, Photosensitive aminoacid-monomer linkage and bioconjugation applications in life sciences and biotechnology. PCT/IB2009/055707, Applied (2009).
[19] D. Hur, S.F. Ekti, R. Say, Lett. Org. Chem. 4 (2007) 585–587.
[20] A.R. Katritzky, Y. Zhang, S.K. Singh, Synthesis 18 (2003) 2795–2798.
[21] H. Mattoussi, I.L. Medintz, A.R. Clapp, J. Assoc. Lab. Auto. 9 (2004) 28–32.
[22] R.C. Stringer, S. Schommer, D. Hoehn, S.A. Grant, Sens. Actuators, B 134 (2008) 427–431.
[23] T. Jamiesona, R. Bakhshia, D. Petrovaa, R. Pococka, M. Imanib, A.M. Seifaliana, Biomaterials 28 (2007) 4717–4732.
[24] C. Xu, B. Xing, J. Rao, Biochem. Biophys. Res. Commun. 344 (2006) 931–935.
[25] A.M. Smith, H. Duan, A.M. Mohs, S. Nie, Adv. Drug Del. Rev. 60 (2008) 1226–1240.
[26] P.Y. Tsoi, J. Yang, Y.T. Sun, S.F. Sui, M.S. Yang, Langmuir 16 (2000) 6590–6596.
[27] M. Yang, P.Y. Tsoi, C.W. Li, J. Zhao, Sens. Actuators, B 115 (2006) 428–437.
[28] B. Persson, K. Stenhag, P. Nilsson, A. Larsson, M. Uhlen, P.A. Nygren, Anal. Biochem. 246 (1997) 34–44.