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Preparation of bioconjugates of CdTe nanocrystals for cancer marker detection

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Abstract
Highly fluorescent CdTe quantum dots (Q-dots) stabilized by 3-mercaptopropionic acid (MPA) were prepared by an aqueous solution approach and used as fluorescent labels in detecting a cancer marker, carcinoembryonic antigen (CEA), expressed on human colon carcinoma cell line LS 180. Nonspecific adsorptions of CdTe Q-dots on carcinoma cells were observed and effectively eliminated by replacing MPA with a thiolated PEG (poly(ethylene glycol), $Mn = 750$) synthesized according to literature. It was unexpectedly found out that the PEG-coated CdTe Q-dots exhibited very strong and specific affinity to anti-CEA monoclonal antibody rch 24 (rch 24 mAb). The resultant CdTe–(rch 24 mAb) conjugates were successfully used in detections of CEA expressed on the surface of cell line LS 180. Further experiments demonstrated that the fluorescent CdTe Q-dots exhibited much better photostability and a brighter fluorescence than FITC, which consequently led to a higher efficiency in the cancer marker detection.

1. Introduction

Quantum dots (Q-dots) have been demonstrated to be a new generation of fluorescent markers in various bioapplications [1–10]. In comparison with conventional dyes, the fluorescence of Q-dots is generally characterized by narrow, symmetrical and particle size-dependent features, as well as very broad excitation wavelength range, which make them very useful in high throughput biodetections and multicolour imaging. Furthermore, their excellent photostability is also practically welcome in many sophisticated bioapplications [1, 2, 11].

The research on the uses of Q-dots as biolabels was originally pioneered by Alivisatos and Nie independently in 1998. In the investigations of Alivisatos *et al*, two different size CdSe@CdS particles covered by a thin layer of silica were prepared to fluorescently image fixed mouse fibroblast cells. In their experiments, the red emission Q-dots were modified to selectively stain F-actin filaments, while the green emission ones were developed to link to the cell nucleus. In this way, the fibroblast cell together with its nuclear can clearly pictured under UV-irradiation [1]. In the independent work of Nie *et al*, the mercapto-solubilized CdSe@ZnS core–shell Q-dots were adopted to label transferrin via an (ethyl-3-(dimethylaminopropyl)carbodiimide)-mediated coupling reaction, and the resultant conjugates were successfully used to reveal the transportation of transferrin into cultured HeLa cells by means of receptor-mediated endocytosis [2].

Following these investigations, Wu *et al* recently used Q-dots linked with IgG (immunoglobulin G) and streptavidin to label different types of targets such as cell surface receptors, cytoskeleton components, and nuclear antigens at different subcellular locations, i.e., surface, intracellular, and intranuclear in cultured live cells, fixed cells, as well as tissue sections, and principally demonstrated the practicality of Q-dots as an attractive class of fluorescence labels for biological and biomedical cellular imaging [3]. Simon's research on a living cell level also reveals that Q-dots have almost no influence on normal cellular growth as well as

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development during quite a long period of time [5]. Nie's investigations further demonstrated that Q-dots as fluorescent markers can even be used directly in living animals [4]. In addition, it was demonstrated the cytotoxicity of Q-dots was significantly reduced by coating the surface of Q-dots with ZnS or protein [12, 13]. These exciting results indeed provide new possibilities by using Q-dots to investigate quite a range of phenomena in cells, developmental biology, cancer targeting and imaging that have not been explored due to the lack of suitable fluorescent labels.

Although the excellent optical properties of Q-dots have already promised a great number of bioapplications, to use Q-dots as fluorescent markers, the preparation of durable and highly fluorescent Q-dots is the very first step. So far, there are mainly two types of synthetic route that have maturely developed for synthesizing highly fluorescent Q-dots. The first is based on the TOP/TOPO (tri-*n*-octylphosphine/tri-*n*-octylphosphine oxide) method invented by Bawendi [14]. It is a high-temperature approach and the direct products, in most cases CdSe Q-dots, are only soluble in nonpolar solvents. The second adopts water-soluble thiol molecules as the particle-stabilizing agents and the preparations can be conducted directly in water. The excellent aqueous solubility of the direct products, typically CdTe nanocrystals, should make them superior to CdSe Q-dots prepared by the TOP/TOPO method as biolabels.

The second step towards the bioapplications of Q-dots is to effectively couple Q-dots with biomolecules meanwhile maintaining their optical properties and biological functions. In general, this step requires the water solubility of Q-dots and the availability of functional moieties on the Q-dot surface to interact with biomolecules. To achieve water solubility and obtain surface reactive moieties are sophisticated for CdSe Q-dots prepared by the TOP/TOPO method. Nonetheless, they remain the most successfully used Q-dots in various bioapplications so far [1–6, 15]. Typically the bioconjugates of Q-dots can be obtained mainly by two types of method. The first relies on covalent bonds formed by chemical reactions [1–4, 16], while the second is dependent on weaker interactions between Q-dot probes and biomolecules, such as electrostatic interactions [5, 17–19], hydrophobic attractions [17], and coordination of histidine residues to metal ions [20].

In comparison with the preparations of Q-dot bioconjugates, nonspecific labelling is much less investigated or reported, although the nonspecific adsorptions of Q-dots on nuclear membrane [1], liver, and spleen [21, 22] have already been observed. Therefore, to eliminate nonspecific adsorption has become an equally important issue to be taken into consideration in the applications of Q-dot bioconjugates.

In this paper, we report our investigations on the detection of carcinoembryonic antigen (CEA), a cancer marker, by using CdTe nanocrystals as fluorescent labels. The CdTe Q-dots stabilized by 3-mercaptopropionic acid were directly synthesized in water. A nonspecific adsorption of CdTe Q-dots on carcinoma cells was observed and effectively eliminated by modifying the Q-dots with a thiolated PEG. Unexpectedly, it was observed that the anti-CEA monoclonal antibody rch 24 (rch 24 mAb) presents a very special and strong affinity to the PEG-modified CdTe Q-dots. The conjugates obtained just by mixing the PEG-modified CdTe Q-dots with rch 24 mAb exhibited promising potentials in detecting cancer marker CEA expressed on the surface of the human colon carcinoma cell line LS 180.

2. Experimental section

2.1. Chemicals and materials

3-mercaptopropionic acid (MPA) (Aldrich, 99+%, product M5801), Al_2Te_3 (CERAC Inc., 99.5%), $CdCl_2{\cdot}2.5H_2O$ (99+%), methoxypolyethylene glycol amine 750 (NH_2-PEG 750) (Fluka, product No. 07964), 2-iminothiolane hydrochloride (Sigma, 98%, product No. I6256), paraformaldehyde (Sigma, product No. P6148), coomassie brilliant blue R-250 (Sigma, 99+%, product No. B-0149) are commercially available products and used as obtained. The chimeric anti-CEA monoclonal antibody rch 24 was gifted from Cancer Institute, Chinese Academy of Medical Sciences. FITC-conjugated sheep anti-human IgG was purchased from Vector. Dulbecco's Modified Eagle Media (DMEM) and Dulbecco's Modified Eagle Media/Ham's F12 media (DMEM/F12) were obtained from Invitrogen.

2.2. Synthesis of fluorescent CdTe Q-dots

The CdTe Q-dots were synthesized according to a synthetic route reported earlier [23]. Briefly, 1.039 g (4.55 mmol) of $CdCl_2{\cdot}2.5H_2O$ was dissolved in 350 ml of water, and then 0.96 ml (10.92 mmol) MPA was added under stirring. After the pH of the mixture was adjusted to 11.2 by using 1 M NaOH, the solution was bubbled for 30 min by N_2. Under stirring, H_2Te gas (generated by the reaction of 0.389 g (0.89 mmol) of Al_2Te_3 lumps with 30–40 ml of 0.5 M H_2SO_4 under N_2 atmosphere) carried by nitrogen flow was introduced. The reaction between Te^{2-} and Cd^{2+} was allowed for approximately 20 min at room temperature. The fluorescent CdTe Q-dots were finally obtained by refluxing the reaction mixture for a certain period of time. The CdTe Q-dots with a luminescence peak centred at 632 nm were purified by a G25-Superfine column before further experiments.

2.3. Preparation of PEG-modified CdTe Q-dots

First, thiolated PEG was prepared by a reaction between NH_2-PEG 750 and 2-iminothiolane hydrochloride in a PBS (137 mM NaCl, 2.7 mM KCl, 10 mM phosphate buffer) solution of pH 7.4 [21]. Typically, the molar ratio between NH_2-PEG 750 and 2-iminothiolane hydrochloride was set to 1:1 and the reaction time was 1 h. Then MPA-capped CdTe Q-dots were mixed with the thiolated PEG in PBS to exchange the MPA molecules on the Q-dot surface. This exchange reaction typically lasted for more than 12 h at room temperature. In order to tune the surface coverage of PEG, three molar ratios, i.e., 2:6, 3:6, and 4:6, between thiolated PEG and CdTe Q-dots were chosen to generate three different PEG-modified CdTe Q-dot samples. Finally, the three samples were purified through a G25-Superfine column and used for further experiments.

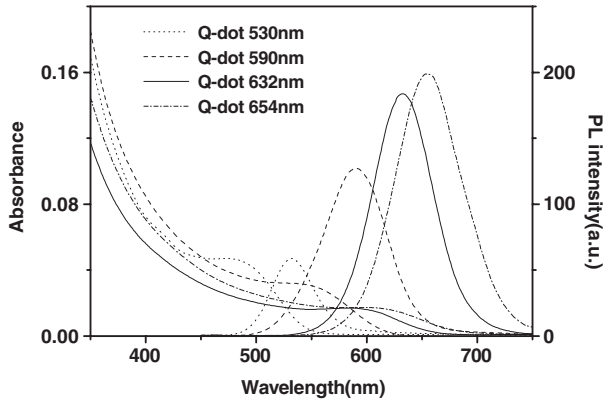

Figure 1. Absorption and fluorescence spectra of a series of CdTe Q-dots prepared by an aqueous solution approach. The spectra in solid lines were recorded from the CdTe Q-dots used in the current investigations.

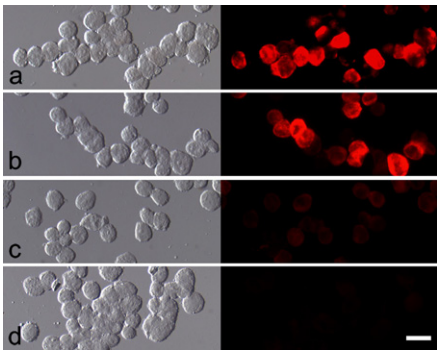

Figure 2. Bright field images (left row) and dark field images (right row, taken under UV light) of LS 180 cells after incubations with PEG-coated CdTe Q-dots obtained by thiolated PEG to CdTe feed ratios of 0:6 (a), 2:6 (b), 3:6 (c), and 4:6 (d). The scale bar corresponds to 20 μm.

2.4. Preparation of Q-dot bioconjugates

The Q-dot bioconjugates were prepared by incubating the mixture of the PEG-modified CdTe Q-dots and rch 24 mAb in PBS buffer for more than 12 h at room temperature under gentle stirring. The typical concentration of the PEG-modified CdTe Q-dots was 0.002 M and the concentration of rch 24 mAb was 0.4 mg ml^{-1}.

2.5. Immunofluorescence detection of carcinoma cells

The procedure for detecting LS 180 cells which were CEA-positive ran as follows. Typically, LS 180 cells were first washed three times with PBS, then approximately 2×10^6 cells were incubated with 120 μl CdTe-(rch 24 mAb) conjugates under gently stirring. The incubation temperature was set to 37 °C. After approximately 1 h, the mixture was subject to a centrifugation to collect cells that were subsequently washed by PBS buffer three times. The finally obtained cells were suspended in 50% (vol/vol) glycerol–PBS for further measurements. For the immunofluorescence experiment, one drop of the cell suspension was placed on a glass slide and examined and imaged under a fluorescence microscope. Normally each experiment was repeated three times.

2.6. Cell culture and fixation

Human colon carcinoma cell line LS 180 and human lung carcinoma cell line GLC-82 were chosen in the detection experiments. Cells were cultured on glass chamber slides in DMEM/F12 (for LS 180) or DMEM (for GLC-82) supplemented with 10% fetal bovine serum and penicillin/streptomycin. All cells were grown at 37 °C under an atmosphere containing 5% CO_2. When cell lines were grown to 80% confluency, they were detached by using 0.25% typsin/0.03% EDTA and fixed with 4% paraformaldehyde for 30 min at room temperature.

2.7. Gel electrophoresis

5% polyacrylamide separating gel was used. The gel was run at 10 V cm^{-1} with a running buffer composed of 25 mM Tris, 0.192 M glycine, 0.1% SDS. After the electrophoresis was finished, the gel was stained overnight in a mixture of 0.1% coomassie brilliant blue R-250, 40% methanol and 7% glacial acetic acid and then destained in the same solution without coomassie brilliant blue R-250.

2.8. Characterizations

Gel electrophoresis was assayed using a Tanon GIS-2008 analytical system (Tanon Technical Co. Ltd, China). The cancer marker detections were performed using an Olympus IX71 fluorescence microscope equipped with a cooled charge-coupled device (CCD) camera. Images capturing and processing were done by using DVCView software (version 2.2.8, DVC company).

3. Results and discussion

Figure 1 shows the absorption and fluorescence spectra of a series of differently sized CdTe Q-dots prepared by the aqueous synthetic approach. The particle sample used in the current investigations exhibits a symmetrical emission centred around 632 nm, which gives rise to a red fluorescence colour under an irradiation below 450 nm. Its fluorescence quantum yield was 31% using Rhodamin 6G as fluorescence standard. Since MPA was used as the surface capping molecule, the Q-dot surface was rich in free carboxylic groups, making the Q-dots perfect candidates as biomarkers.

Since nonspecific adsorptions of Q-dots on nuclear membrane [1], liver, and spleen [21, 22] have already been observed, the nonspecific interactions between CdTe Q-dots and carcinoma cells were examined first before further experiments. In a typical test, MPA-capped CdTe Q-dots were incubated with LS 180 cells for 1 h. It was found out that the LS 180 cells were heavily stained by CdTe Q-dots (figure 2(a)), indicating that MPA-capped CdTe Q-dots have strong nonspecific interactions with LS 180 cells. Therefore, to eliminate the nonspecific adsorptions becomes the first problem to resolve.

PEG is a widely used biocompatible material known to have good resistance to nonspecific bindings with biological molecules [7, 21, 22, 24–28]. By encapsulating nanocrystals

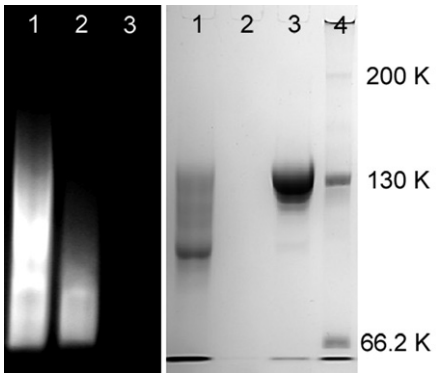

Figure 3. 5% SDS-PAGE (sodium dodecyl sulphate-polyacrylamide gel electrophoresis) of CdTe–(rch 24 mAb) bioconjugates (lane 1), CdTe Q-dots (lane 2), rch 24 mAb (lane 3) and a standard protein ladder (lane 4). The left panel is a fluorescence image and the right panel is bright field image obtained after staining the gel with coomassie blue. Molecular weights marked on right-hand side image are in kiloDaltons.

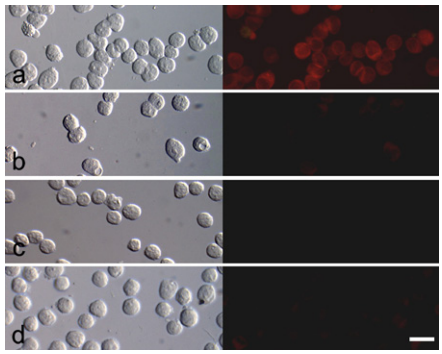

Figure 4. Bright field images (left row) and dark field images (right row, taken under UV light) of CEA-positive LS 180 cells obtained after incubations with the CdTe–(rch 24 mAb) conjugates (a), PEG-modified CdTe Q-dots (b) and CdTe–(irrelevant antibody) conjugates (CdTe–IgG) (c), respectively. Images of CEA-negative GLC-82 cells obtained after incubation with the CdTe–(rch 24 mAb) conjugates are given at the bottom (d). The scale bar corresponds to 20 μm.

in phospholipid block-copolymer micelles which possess a dense layer of PEG locating at the outmost surface, Dubertret *et al* have successfully obtained composite particles which can be used in both *in vivo* and *in vitro* imaging due to a great reduction in nonspecific adsorption [7]. In investigations of Akerman *et al*, PEG coating was also demonstrated to be effective in eliminating the nonspecific uptake of Q-dots into the liver and spleen [21]. According to these investigations, PEG was also chosen in the current investigations to modify the MPA-coated Q-dots in order to eliminate their nonspecific adsorptions. Thiolated PEG 750 was first prepared according to reference via a reaction between NH_2-PEG 750 and 2-iminothiolane hydrochloride [21], and then used to replace the MPA on the Q-dot surface. The surface coverage of PEG was controlled by varying the feed molar ratio between the thiolated PEG and Q-dots. The results shown in figures 2(b)–(d) clearly demonstrate that nonspecific interactions between CdTe Q-dots and carcinoma cells can dramatically be weakened by increasing the ratio of thiolated PEG to CdTe Q-dots and finally be eliminated (figure 2(d)) when the ratio reaches 4:6.

The next step towards the detection of the carcinoma cells is to couple the Q-dots with an antibody which can specifically target the marker expressed on the carcinoma cells. In the current experiments, anti-CEA monoclonal antibody rch 24 (rch 24 mAb) was chosen for detecting human colon carcinoma cell line LS 180 with CEA being expressed on the cell surface. Although the CdTe Q-dots were coated by the thiolated PEG, there is no reason to believe that MPA was completely replaced. Therefore the carboxylic residues may still be utilizable for covalently linking antibody. Unexpectedly, it was found out during the amidation reaction that rch 24 mAb presented very special and strong affinity to PEG-modified CdTe Q-dots, which therefore provided a more convenient way to couple CdTe Q-dots with rch 24 mAb. The feasibility of this approach was confirmed by gel electrophoresis. Two types of image were taken from the same gel after electrophoresis to identify the effectiveness of the coupling reaction. A comparison of a fluorescence image and an image obtained by staining the gel with coomassie blue is shown in figure 3. Lanes 1, 2, 3 and 4 were filled with conjugates, CdTe Q-dots, rch 24 mAb and a standard protein ladder, respectively. Quite obviously, the fluorescence of CdTe Q-dots survived the electrophoresis, which allows an evaluation on the feasibility of the aforementioned conjugation process. In comparison with lane 2, lane 1 presents a very broad band with the nonoverlapping part being stainable by coomassie blue, which strongly suggests that the conjugation between CdTe Q-dots and antibody was successfully achieved even though a certain percentage of free CdTe Q-dots remained in the mixture. But this will not affect the following detection of carcinoma cells since the PEG-coated Q-dots which are not conjugated with antibody will not interact with carcinoma cells, as demonstrated above. In addition, the stained part of lane 1 presents a higher electrophoretic mobility than that of lane 3 (pure antibody), indicating that the antibody in lane 1 has an increased charge density and further supporting the successful conjugation between rch 24 mAb and CdTe Q-dots. In addition, it was also demonstrated that CdTe–IgG (normal human immunoglobulin G) conjugates, obtained in the same way, also presented a quite different electrophoretic behaviour in comparison with pure normal human IgG, suggesting that the current conjugation strategy was applicable not only for rch 24 mAb. It is reasonable to deduce that the coupling reactions were mainly driven by hydrogen bonding as there are an imine and a secondary amine groups in the thiolated PEG. This speculation was further confirmed by the experimental fact that the CdTe–(rch 24 mAb) conjugates were completely decoupled upon introduction of urea, a known hydrogen bonding breaker [29], into the dispersion of the conjugates.

The CdTe–(rch 24 mAb) conjugates obtained as mentioned above were used in detections of LS 180 cells with CEA being expressed on the surface (figure 4(a)). In parallel, three control experiments were performed to show the specificity of CdTe–(rch 24 mAb) conjugates, i.e., CEA-positive LS 180 cells incubated with PEG-modified CdTe Q-dots (figure 4(b)) or CdTe–(irrelevant antibody) conjugates (CdTe–IgG) (figure 4(c)), CEA-negative GLC-82 cells incubated with CdTe–(rch 24 mAb) conjugates (figure 4(d)). It is quite obvious that only CdTe–(rch 24 mAb) conjugates can specifically detect the

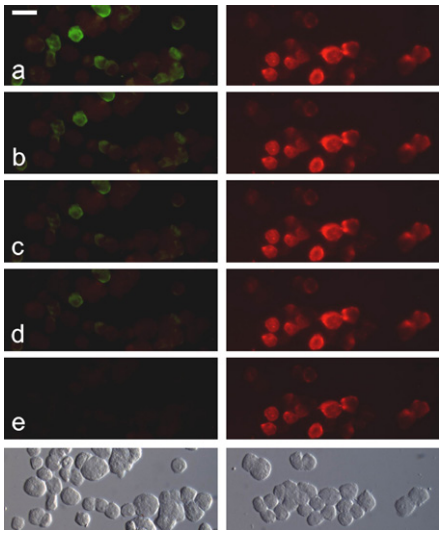

Figure 5. Comparison of photostabilities of CdTe Q-dots (right row) and FITC (left row) labelled on the carcinoma cells. The images were taken under a continual irradiation (460–490 nm) from a 100 W mercury lamp for irradiation times of 0 (a); 1 (b); 2 (c); 3 (d) and 30 min (e), respectively. The scale bar corresponds to 20 μm.

carcinoma cells. Furthermore, nearly one-to-one correspondence between cells shown in both bright field and dark field under fluorescence mode not only demonstrates that the CdTe–(rch 24 mAb) conjugates are still optically and biologically active, but also manifests the feasibility of using the current conjugates in cancer marker detection.

One of the most important reasons to use Q-dots as biomarkers is because they possess excellent photostability in comparison with conventional dyes. However, the photostability of Q-dots is usually very sensitive to their surface structure as well as their surrounding environments. Therefore, it is necessary to evaluate the photostability of CdTe Q-dots in the CdTe–(rch 24 mAb) conjugates. FITC was chosen for a comparison since it has widely been used for biological detections as a standard fluorescence marker. In detail, CEA-positive LS 180 cells were incubated first with rch 24 mAb and then with FITC-conjugated sheep anti-human IgG. Both of these two procedures lasted for 1 h. Finally the FITC-labelled LS 180 cells together with those labelled with Q-dots were subject to a continual UV irradiation (460–490 nm) from a 100 W mercury lamp equipped on an Olympus fluorescence microscope. Fluorescence images were taken at 1 min intervals for the same exposure time. Five groups of representative images captured at different time points during illumination, i.e., 0, 1, 2, 3 and 30 min, are shown in figure 5. It is quite obvious that the fluorescence of FITC fades much faster than that from Q-dots and is almost gone after 3 min illumination. In huge contrast, the Q-dots remain luminescing throughout the test with only 10% loss in intensity. This dramatic difference will be beneficial to CdTe Q-dots prepared by the aqueous solution approach in applications of long-term tracking of biological processes. Moreover, statistical results demonstrated that CdTe Q-dots were 1.2 times more effective than FITC in detections of the CEA-positive LS 180 cells. This makes CdTe Q-dots potentially useful in immunofluorescence detection of cancer cells.

4. Conclusions

Aqueous colloidal CdTe nanocrystals were synthesized by using 3-mercaptopropionic acid as surface-stabilizing agent and used as fluorescent markers in the immunofluorescence detection of carcinoma cells. Detailed experimental results reveal that the nonspecific adsorption of CdTe Q-dots on carcinoma cells can effectively be eliminated after MPA molecules capped on CdTe Q-dots are replaced by thiolated PEG synthesized according to the literature. Unexpectedly, the resultant PEG-capped Q-dots presented specific and strong affinity to the anti-CEA monoclonal antibody rch 24. The bioconjugates obtained simply by mixing PEG-covered CdTe and rch 24 mAb have been demonstrated to be able to specifically stain human colon carcinoma cell line LS 180 with CEA being expressed on surface, which therefore opens up a facile way for CEA detection. Due to the excellent photostability of the CdTe–(rch 24 mAb) conjugates, CdTe Q-dots prepared by the aqueous solution approach also exhibit great potential for further biological and biomedical applications, such as multicolour imaging and long-term tracking of biological processes.

Acknowledgments

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References

[1] Bruchez M J, Moronne M, Gin P, Weiss S and Alivisatos A P 1998 *Science* **281** 2013–6
[2] Chan W C W and Nie S 1998 *Science* **281** 2016–8
[3] Wu X, Liu H, Liu J, Haley K N, Treadway J A, Larson J P, Ge N, Peale F and Bruchez M P 2003 *Nat. Biotechnol.* **21** 41–6
[4] Gao X, Cui Y, Levenson R M, Chung L W K and Nie S 2004 *Nat. Biotechnol.* **22** 969–76
[5] Jaiswal J K, Mattoussi H, Mauro J M and Simon S M 2003 *Nat. Biotechnol.* **21** 47–51
[6] Larson D R, Zipfel W R, Williams R M, Clark S W, Bruchez M P, Wise F W and Webb W W 2003 *Science* **300** 1434–6
[7] Dubertret B, Skourides P, Norris D J, Noireaux V, Brivanlou A H and Libchaber A 2002 *Science* **298** 1759–62
[8] Medintz I L, Uyeda H T, Goldman E R and Mattoussi H 2005 *Nat. Mater.* **4** 435–46
[9] Zhang C Y, Yeh H C, Kuroki M T and Wang T H 2005 *Nat. Mater.* **4** 826–31
[10] Dubertret B 2005 *Nat. Mater.* **4** 797–8
[11] Niemeyer C M 2001 *Angew. Chem. Int. Edn* **40** 4128–58
[12] Sinani V A, Koktysh D S, Yun B G, Matts R L, Pappas T C, Motamedi M, Thomas S N and Kotov N A 2003 *Nano Lett.* **3** 1177–82
[13] Derfus A M, Chan W C W and Bhatia S N 2004 *Nano Lett.* **4** 11–8
[14] Murray C B, Norris D J and Bawendi M G 1993 *J. Am. Chem. Soc.* **115** 8706–15
[15] Bakalova R, Ohba H, Zhelev Z, Nagase T, Jose R, Ishikawa M and Baba Y 2004 *Nano Lett.* **4** 1567–73
[16] Pathak S, Choi S K, Arnheim N and Thompson M E 2001 *J. Am. Chem. Soc.* **123** 4103–4
[17] Mattoussi H, Mauro J M, Goldman E R, Anderson G P, Sundar V C, Mikulec F V and Bawendi M G 2000 *J. Am. Chem. Soc.* **122** 12142–50
[18] Goldman E R, Anderson G P, Tran P T, Mattoussi H, Charles P T and Mauro J M 2002 *Anal. Chem.* **74** 841–7

[19] Goldman E R, Balighian E D, Mattoussi H, Kuno M K, Mauro J M, Tran P T and Anderson G P 2002 *J. Am. Chem. Soc.* **124** 6378–82
[20] Goldman E R, Medintz I L, Hayhurst A, Anderson G P, Mauro J M, Iverson B L, Georgiou G and Mattoussi H 2005 *Anal. Chim. Acta* **534** 63–7
[21] Akerman M E, Chan W C W, Laakkonen P, Bhatia S N and Ruoslahti E 2002 *Proc. Natl Acad. Sci.* **99** 12617–21
[22] Ballou B, Lagerholm B C, Ernst L A, Bruchez M P and Waggoner A S 2004 *Bioconjug. Chem.* **15** 79–86
[23] Gaponik N, Talapin D V, Rogach A L, Hoppe K, Shevchenko E V, Kornowski A, Eychmuller A and Weller H 2002 *J. Phys. Chem.* B **106** 7177–85
[24] Harris J M and Zalipsky S 1997 *Poly(ethylene glycol): Chemistry and Biological Applications* (Washington, DC: American Chemical Society) chapter 2
[25] Zheng M, Davidson F and Huang X 2003 *J. Am. Chem. Soc.* **125** 7790–1
[26] Zheng M, Li Z and Huang X 2004 *Langmuir* **20** 4226–35
[27] Gerion D, Parak W J, Williams S C, Zanchet D, Micheel C M and Alivisatos A P 2002 *J. Am. Chem. Soc.* **124** 7070–4
[28] Bentzen E L, Tomlinson I D, Mason J, Gresch P, Warnement M R, Wright D, Sanders-Bush E, Blakely R and Rosenthal S J 2005 *Bioconjug. Chem.* **16** 1488–94
[29] Ilmain F, Tanaka T and Kokufuta E 1991 *Nature* **349** 400–1