Ni–nitrilotriacetic acid-modified quantum dots as a site-specific labeling agent of histidine-tagged proteins in live cells†

Junwon Kim,‡[a] Hye-Young Park,[a] Jaeseung Kim,‡[a] Jiyoung Ryu,[a] Do Yoon Kwon,[b] Regis Grailhe[b] and Rita Song*[a]

Received (in Cambridge, UK) 17th December 2007, Accepted 24th January 2008
First published as an Advance Article on the web 19th February 2008
DOI: 10.1039/b719434j

Ni–nitrilotriacetic acid (NTA) functionalized CdSe/ZnS quantum dots (QDs) were exploited as a site-specific labeling agent of histidine-tagged biomolecules in live cells; the QDs were found to be water-soluble, aggregation free and stable for several months.

Labeling of proteins with various chromophores is fundamental for the elucidation of the structure and localization of proteins *in vitro* and in live cells. In this context, the development of a selective and sensitive method using simple chemical interactions to overcome the limitation caused by conventional antibody-based protein labeling has been an important subject for related areas. In addition, the need for more efficient fluorophores has been continuously raised to minimize photobleaching and overlapping problems of organic dyes.

Semiconductor quantum dots (QDs) have emerged as useful and alternative materials for labeling biomolecules because of their unique optical advantages including photostability, narrow emission bands, broad excitation spectra, and accessibility to versatile functionalization allowing for site-specific targeting.[1,2]

In this study, we used the highly selective and simple chemical interaction of Ni–nitrilotriacetate (Ni–NTA) complex with oligohistidine ($K_d' \approx 10^{-13}$). The Ni–NTA moiety has been used extensively for purification, surface immobilization, and *in vitro* detection of recombinant histidine-tagged proteins.[3,4] In particular, organic fluorescent dyes or gold nanoparticles linked to the Ni–NTA complex have shown potential for tracking biological events by specific binding to His-tagged proteins.[5] Although there are a variety of strategies for labeling proteins with conventional organic fluorescent dyes, from practical point of view, such approaches to visualize specific proteins have been limited by rapid photobleaching[3a] or the lack of availability of a detection device.[5a,b]

Here, we report for the first time a simple and efficient method using Ni–NTA conjugated CdSe/ZnS QDs (referred to as "QDs" hereafter) for site-specific and stable non-covalent fluorescence labeling of histidine-tagged proteins in live cells (Fig. 1).

Scheme 1 illustrates the synthetic route to the Ni–NTA QDs. First, thiolated NTA ligand **2** was synthesized using a previously reported method with slight modifications.[4a] Then, amino-PEG coated QDs (545 nm λ_{max}, See ESI† for TEM image) **1** were treated with sulfo-SMCC, a hetero-bifunctional crosslinker, at room temperature for 1 h followed by the removal of the excess reagent by membrane filtration. The resulting retentate was re-dissolved in phosphate buffered saline (PBS, pH 7.4, 50 mM PB, 150 mM NaCl) and reacted with ligand **2** in DMSO. After vigorous stirring at ambient temperature for 2 h, the reaction mixture was purified using membrane filtration to obtain NTA-modified QDs **3**, which were then treated to form the Ni complex by reacting with an excess of $NiCl_2{\cdot}6H_2O$. Finally, unreacted Ni^{2+} was removed by extensive washing with water yielding pure Ni–NTA modified QDs **4**. Each QD nanoparticle contains about 15 Ni–NTA complexes on the surface as determined by inductively coupled plasma mass spectrometry (ICP-MS) analysis. Non-specific binding of Ni^{2+} ions on the surface of QDs **1** was negligible even after prolonged incubation with $NiCl_2{\cdot}6H_2O$. Non-specific electrostatic interaction of ligand **2** with the amine groups on QDs **1** can be minimized by maintaining the basic reaction solution. The quantum yield (QY) of the initial QDs remained unchanged after the conjugation and Ni complexation steps, although some reports have described that the QY is significantly reduced due to non-radiative energy transfer following metal ion complexation.[3d] Alternatively, another type of NTA modified-QD was also successfully prepared *via* a ligand exchange reaction of trioctylphosphine oxide (TOPO) capped CdSe/ZnS nanoparticles with ligand **2** (see ESI†).[6]

To explore the site-specific interaction of Ni–NTA modified QDs with oligohistidine-tagged proteins, a cell-mimic system

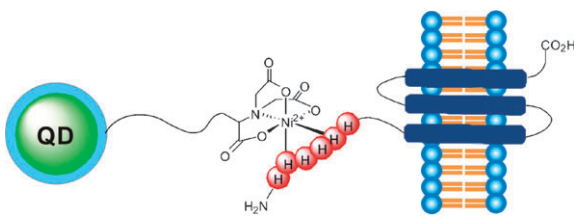
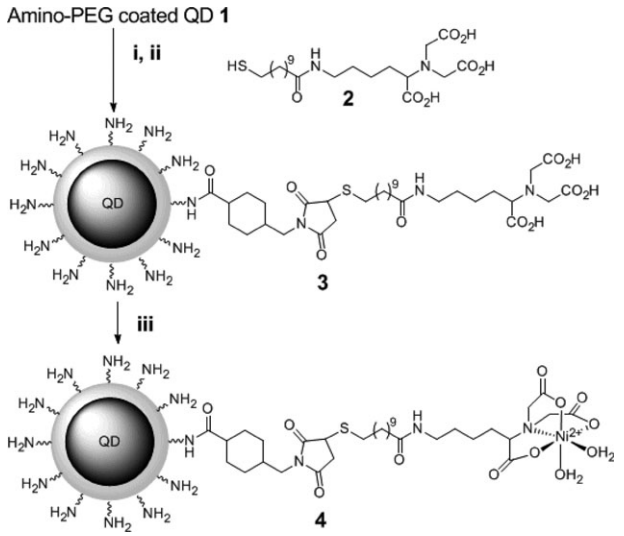


Fig. 1 Ni–NTA modified quantum dots interact selectively with histidine-tagged membrane proteins in live cells.

[a] *Nano/Bio Chemistry Laboratory, Institut Pasteur Korea (IP-K), 39-1 Hawolgok-dong, Seongbuk-gu, 136-791 Seoul, Korea. E-mail: rsong@ip-korea.org; Fax: +82-31-546-6710; Tel: +82-31-546-6701*
[b] *Dynamic Imaging Platform Group, Institut Pasteur Korea (IP-K), 39-1 Hawolgok-dong, Seongbuk-gu, 136-791 Seoul, Korea*
† Electronic supplementary information (ESI) available: Data including detailed Ni–NTA conjugation procedures, stability test, and fluorescence microscope images, IR spectra and TEM images. See DOI: 10.1039/b719434j
‡ Present address: Medicinal Chemistry Laboratory, IP-K.



Scheme 1 Ni–NTA conjugation to amino-PEG coated QDs. Conditions: (i) sulfo-SMCC in borate buffer (pH 8.4), rt, 1 h; (ii) ligand **2** in DMSO, PBS buffer (pH 7.4), rt, 2 h; (iii) $NiCl_2 \cdot 6H_2O$, rt, 30 min; sulfo-SMCC = sulfosuccinimidyl 4-*N*-maleimidomethyl cyclohexane-1-carboxylate.

was constructed using agarose beads (AB, 45–180 μm in diameter).[7] The peptide-functionalized beads, with two glycines (G) and six histidines (H) (AB-G2H6), were used to mimic cells expressing 6 × His on the membrane. AB-G5HG2, representing cells with a natural amino acid composition on the membrane, was used as a negative control. Both ABs were incubated with Ni–NTA QDs at room temperature for 15 min and the unbound QDs were washed out with 10 mM PBS. The fluorescence images were obtained using a fluorescence microscope.

Ni–NTA modified QDs were found to be specifically bound to 6 × His-tagged agarose beads, as evidenced by the strong fluorescence of AB-G2H6 (Fig. 2a) whereas practically no fluorescence was detected for AB-G5HG2. (See ESI†) The affinity of Ni–NTA QDs for 6 × His was further investigated by measuring the photoluminescence (PL) of the AB-G2H6 incubated with various concentrations of QDs **4**. Fig. 2c shows the specific interaction between 6 × His on the agarose beads and Ni–NTA modified QDs **4**. Binding of an increasing amount of Ni–NTA QDs **4** to AB-G2H6 resulted in a saturable sigmoidal increase of the fluorescence intensity with an EC_{50} value of 71 nM, where EC_{50} is the concentration of Ni–NTA QD that elicits the half-maximal response. In the other control experiments the PL increases linearly with the increase of QD concentration, indicating absence of any specific interaction. Labeling was also fully reversed upon the addition of 1 M imidazole solution, indicating the specific interaction of the Ni–NTA probes with 6 × His.[7] This is confirmed by the absence of fluorescence upon addition of NTA probes without Ni.

The site-specific labeling of Ni–NTA QDs **4** was subsequently investigated in live cells to visualize the 5HT2C serotonin receptor into which hexahistidine sequences were genetically engineered at the extracellular NH_2 terminal as shown in Fig. 1. After incubation of HEK-293 cells expressing His-5HT2C with 90 nM QDs **4** for 30 min, the images were

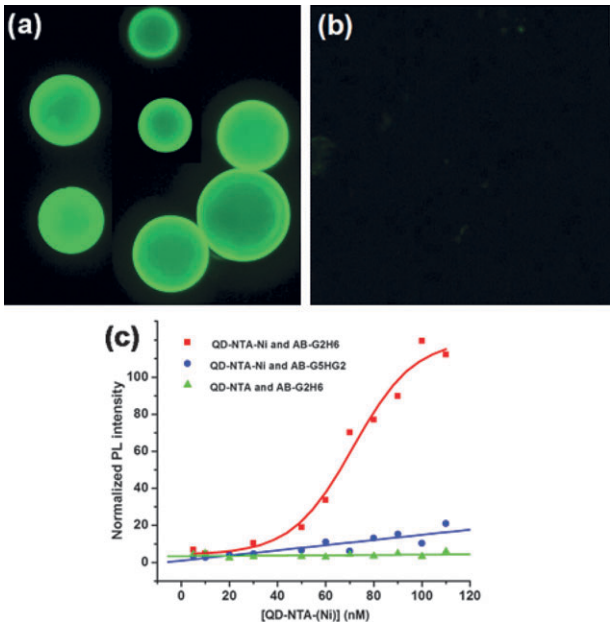


Fig. 2 Fluorescence microscope image of Ni–NTA modified QDs (545 nm) incubated with (a) G2H6-modified AB and (b) G5HG2-modified AB (control). (c) PL intensity of G2H6-modified AB after incubation with Ni–NTA modified QDs (red) and without Ni (green) and 5GH2G-modified AB with Ni–NTA modified QDs (blue).

obtained using a Zeiss LSM-5 live confocal microscope (Fig. 3). Fig. 3 shows that the Ni–NTA QDs **4** can selectively label His-5HT2C transfected HEK-293 cells (Fig. 3a). The control reaction of Ni–NTA QDs **4** with wild-type HEK-293 cells showed a significantly low fluorescence signal (Fig. 3b).

The binding affinity of the Ni–NTA QDs was found to be dependent on the QD probe concentration (Fig. 3c). Quantitative image processing (ImageJ software) revealed that the binding of QDs **4** to His-5HT2C transfected HEK-293 cells resulted in a similar sigmoidal increase of the fluorescence intensity, which confirmed a specific binding between the QD probe and His-tagged protein. The EC_{50} of QDs **4** in live cells was determined to be 41.7 nM, which is quite similar to that obtained in the cell-mimic bead system (71 nM). The addition of QDs **4** to untransfected HEK-293 cells exhibited a negligible effect and was found to follow a linear function which is

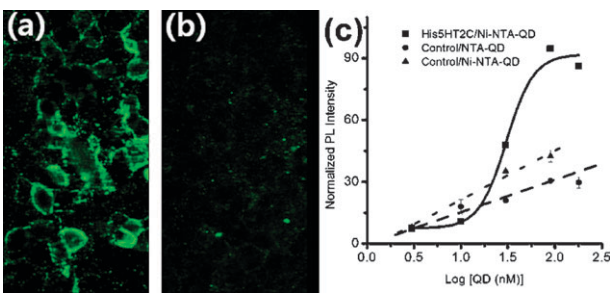


Fig. 3 Confocal microscope images of (a) His-5HT2C transfected HEK-293 cells incubated with Ni–NTA QDs and (b) untransfected HEK-293 cells incubated with Ni–NTA QDs. (c) Normalized PL intensity of His-5HT2C transfected HEK-293 cells (■), untransfected HEK-293 cells (▲) and transfected HEK-293 cells labelled with NTA QDs (●).

indicative of non-specific interaction. These results clearly show that Ni–NTA QDs can be used to determine the topology and localization of specific proteins in living cells without the need for fixation and/or permeabilization of the cells. These Ni–NTA QDs exhibit unique advantages over the conventional protein labeling: (i) quantitative binding and stability, (ii) site-specificity, (iii) controllable linkage length, (iv) reversible binding, (v) cost effectiveness, (vi) universality because a type of Ni–NTA QDs can be used for any kind of specific protein of interest, and (vii) non-toxicity as all the cells studied in our experiment remained alive even after 24 h incubation. More detailed cytotoxicity studies are under investigation for further *in vivo* imaging. We are currently developing strategies to label intracellular proteins by using cell membrane penetrating peptides and to switch on and off the fluorescence signal of Ni–NTA QDs utilizing their reversible labeling ability.

In conclusion, we have demonstrated the synthesis of Ni–NTA modified QDs as a site-specific protein labeling agent. The interesting property of such an interaction is that it is virtually unaffected by high salt, non-ionic detergent or highly denaturing conditions. These Ni–NTA modified QDs are water-soluble, aggregation free, stable for several months, and were found to be promising nanoprobes for histidine-tagged proteins in live cells. Because of the extensive usage of $6 \times$ His-tagged proteins in biological research, this system will be a useful alternative for labeling histidine-tagged proteins.

This work was supported by the Korean Ministry of Science and Technology (MOST).

Notes and references

1 (*a*) Y. A. Yang, H. M. Wu, K. R. Williams and Y. C. Cao, *Angew. Chem., Int. Ed.*, 2005, **44**(41), 6712; (*b*) D. V. Talapin, A. L. Rogach, A. Kornowski, M. Haase and H. Weller, *Nano Lett.*, 2001, **1**(4), 207; (*c*) P. Reiss, J. Bleuse and A. Pron, *Nano Lett.*, 2002, **2**(7), 781; (*d*) Z. A. Peng and X. G. Peng, *J. Am. Chem. Soc.*, 2001, **123**(7), 1389; (*e*) S. Kim, B. Fisher, H. J. Eisler and M. Bawendi, *J. Am. Chem. Soc.*, 2003, **125**(38), 11466; (*f*) Z. Zhelev, H. Ohba, R. Bakalova, R. Jose, S. Fukuoka, T. Nagase, M. Ishikawa and Y. Baba, *Chem. Commun.*, 2005, 1980; (*g*) M. Laferriere, R. E. Galian, V. Maurel and J. C. Scaiano, *Chem. Commun.*, 2006, 257.

2 (*a*) X. H. Gao, Y. Y. Cui, R. M. Levenson, L. W. K. Chung and S. M. Nie, *Nat. Biotechnol.*, 2004, **22**(8), 969; (*b*) S. Kim, Y. T. Lim, E. G. Soltesz, A. M. De Grand, J. Lee, A. Nakayama, J. A. Parker, T. Mihaljevic, R. G. Laurence, D. M. Dor, L. H. Cohn, M. G. Bawendi and J. V. Frangioni, *Nat. Biotechnol.*, 2004, **22**(1), 93; (*c*) I. L. Medintz, H. T. Uyeda, E. R. Goldman and H. Mattoussi, *Nat. Mater.*, 2005, **4**(6), 435; (*d*) X. Michalet, F. F. Pinaud, L. A. Bentolila, J. M. Tsay, S. Doose, J. J. Li, G. Sundaresan, A. M. Wu, S. S. Gambhir and S. Weiss, *Science*, 2005, **307**(5709), 538; (*e*) H. T. Uyeda, I. L. Medintz, J. K. Jaiswal, S. M. Simon and H. J. Mattoussi, *J. Am. Chem. Soc.*, 2005, **127**(11), 3870; (*f*) J. H. Warner, A. Hoshino, K. Yamamoto and R. D. Tilley, *Angew. Chem., Int. Ed.*, 2005, **44**(29), 4550.

3 (*a*) S. Lata, M. Gavutis, R. Tampe and J. Piehler, *J. Am. Chem. Soc.*, 2006, **128**(7), 2365; (*b*) C. Buchel, E. Morris, E. Orlova and J. Barber, *J. Mol. Biol.*, 2001, **312**(2), 371; (*c*) E. G. Guignet, R. Hovius and H. Vogel, *Nat. Biotechnol.*, 2004, **22**(4), 440; (*d*) A. N. Kapanidis, Y. W. Ebright and R. H. Ebright, *J. Am. Chem. Soc.*, 2001, **123**(48), 12123.

4 (*a*) C. J. Xu, K. M. Xu, H. W. Gu, X. F. Zhong, Z. H. Guo, R. K. Zheng, X. X. Zhang and B. Xu, *J. Am. Chem. Soc.*, 2004, **126**(11), 3392; (*b*) H. G. Bagaria, E. T. Ada, M. Shamsuzzoha, D. E. Nikles and D. T. Johnson, *Langmuir*, 2006, **22**(18), 7732; (*c*) C. Xu, K. Xu, H. Gu, R. Zheng, H. Liu, X. Zhang, Z. Guo and B. Xu, *J. Am. Chem. Soc.*, 2004, **126**(32), 9938.

5 (*a*) A. N. Kapanidis, Y. W. Ebright and R. H. Ebright, *J. Am. Chem. Soc.*, 2001, **123**(48), 12123; (*b*) S. Lata, A. Reichel, R. Brock, R. Tampe and J. Piehler, *J. Am. Chem. Soc.*, 2005, **127**(29), 10205.

6 (*a*) S. F. Wuister, I. Swart, F. van Driel, S. G. Hickey and C. D. Donega, *Nano Lett.*, 2003, **3**(4), 503; (*b*) Y. Li, M. Afzaal and P. O'Brien, *J. Mater. Chem.*, 2006, **16**(22), 2175.

7 K. E. Sapsford, T. Pons, I. L. Medintz, S. Higashiya, F. M. Brunel, P. E. Dawson and H. Mattoussi, *J. Phys. Chem. C*, 2007, **111**(31), 11528.

