http://dx.doi.org/10.5012/bkcs.2013.34.3.997

RNA Aptamer-Functionalized Quantum Dots for Detection of His-Tagged Proteins in *Escherichia coli*

Hye Yeon Nam,[a] Yea Seul Cho,[a] Woong Jung,[†,a] Ho-Jung Kang, and Sang Soo Hah[*]

Department of Chemistry and Research Institute for Basic Sciences, Kyung Hee University, Seoul 130-701, Korea
[*]E-mail: sshah@khu.ac.kr
[†]Department of Emergency Medicine, Kyung Hee University Hospital at Gangdong, Seoul 134-727, Korea
Received November 29, 2012, Accepted December 26, 2012

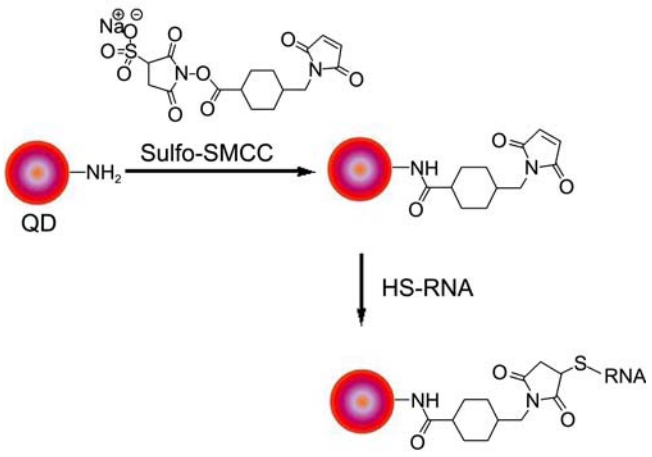
Key Words : Aptamer, Quantum dot, His-tagged protein

One of the fundamental formats in biological research is to monitor and understand the spatio-temporal activity of biomolecules from the cellular to the integrative level. To study these interactions, researchers commonly use selective fluorescent labeling both *in vivo* cellular imaging and *in vitro* assay detection.[1] Recombinant strategies, for example, fuse fluorescent proteins directly to the target of interest[2] or engineer chimeric enzyme target fusions that bind exogenously added fluorescent substrates.[3] And, chemical or affinity interactions specifically targeting small peptidyl residues appended onto proteins are also available as exemplified by Tsien's FlAsH/ReAsH biarsenical fluorophores,[4] which react with vicinal tetracysteine motifs and nitrilotriacetic acid (NTA) functionalized dye to be coordinated to oligohistidine sequences. However, the intrinsic photophysical properties of organic and genetically encoded fluorophores, which generally have several intrinsic photophysical properties of the fluorophores themselves, such as broad absorption/emission profiles and low photobleaching thresholds,[1] have limited their effectiveness in long-term imaging and 'multiplexing' (simultaneous detection of multiple signals) without complex instrumentation and processing. Taken together, these issues can significantly complicate long-term *in vivo* monitoring of labeled proteins.

In these regards, the studies on the biological uses of semiconductor quantum dots (QDs) demonstrated that the unique properties of QDs could overcome the issues and that the optical properties of QDs would be ideally suited for long-term monitoring of intracellular protein dynamics.[5,6] The optical properties of QDs of interest to biologists include high quantum yields, high molar extinction coefficients (~10-100-fold higher than those of organic dyes), resistance to chemical degradation, photostability, large and effective Stokes shifts, broad absorption with narrow-symmetric photoluminescence (PL) spectra (full-width at half-maximum ~25-40 nm) spanning the UV to near-infrared, and choice of size-tunable photoluminescence.[5,6] These properties also make them useful for multiplexing applications as well as single-molecule tracking assays.[5] Clearly, it is of great importance to uniquely conjugate QDs to a target protein *in vivo* to expand the intracellular fluorescent labeling "toolset" to include these unique nanomaterials, which may provide far more versatile research formats.

We have previously reported the use of QD-conjugated RNA aptamers targeting histidine tags (His-tags) as an alternative to the conventional Western blot analysis.[6] Aptamers are a special class of nucleic acids that can specifically bind, with high affinity, to a target molecule.[6,7] Emerging as alternatives to antibodies, a wide range of aptamers have been found to bind specifically to targets, thus they have been used in many bioanalytical applications, such as for specific detection of proteins,[6,7] metal ions,[8] and small molecules,[9] and for target-specific delivery.[11] By virtue of the highly selective interaction of an RNA aptamer complex with oligohistidine (K_d ~3.78 pM),[6] which is comparable or superior to that of protein-antibody (K_d ~10^{-5}-10^{-12} M),[6] we could successfully develop a simple, time-saving, selective and sensitive method to detect the His-tagged proteins. In the present study, we expand the scope of the applicability of the RNA aptamer-functionalized QDs to intracellular target detection for live cell imaging.

To accomplish this task, we generated RNA aptamer conjugated QDs (Scheme 1). Amino-modified QDs were conjugated to thiol-containing RNA aptamers using sulfo-SMCC cross-linker according to the literature.[6] In brief, QDs were resuspended in 50 mM sodium phosphate, 150 mM



Scheme 1. RNA aptamer conjugation on quantum dots (QDs).

[a]These authors contributed equally to this work.

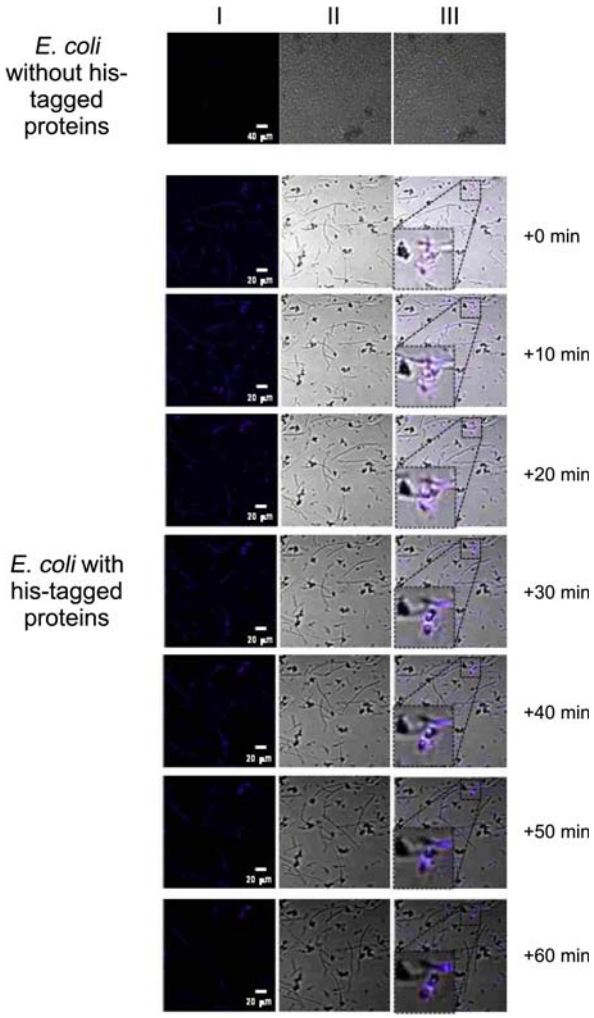


Figure 1. Fluorescence images of His-tagged proteins in *E. coli* using *anti*-His-tag aptamer-conjugated QDs. Heat-treated *E. coli* cells containing His-tagged proteins were mixed with the aptamer-conjugated QDs and 0.5 μL of the mixed solution was dropped on a glass slide. After additional 0-, 10-, 20-, 30-, 40-, 50-, and 60-min incubation, fluorescence microscopy was performed. Panel I was obtained with band-pass filters for green fluorescence (excitation: 365 ± 10 nm and emission: 565 ± 30 nm). The DIC images are shown in panel II, and panel III represents the merged images.

sodium chloride, pH 7.2. Cross-linker (100-fold excess) was added to QDs and allowed to react for 1 h. Samples were filtered on a NAP-5 gravity column (to remove excess cross-linker) using similar buffer supplemented with 10 mM EDTA. 5'-Thiol-containing RNA aptamer[6,10] was added to filtered QDs and allowed to react overnight at 4 °C. Using three consecutive Amicon filters, product was filtered twice with Dulbecco's phosphate-buffered saline (PBS), twice with a high salt buffer (1.0 M sodium chloride, 100 mM sodium citrate, pH 7.2), and twice again with PBS.

After His-tagged proteins (His-tagged β-galactosidase in this study) were expressed in *E. coli* according to the literature,[11] and *anti*-His-tag aptamer-conjugated QDs were introduced to the live *E. coli* cells containing His-tagged proteins, we monitored the interactions between QD-functionalized aptamers and His-tagged proteins within cells as they grew (Fig. 1), since the interactions as a functions of time could be observed under a fluorescence microscope. For comparison, the DIC images of *E. coli* are also provided. As shown in Figure 1, only the QDs that were conjugated with *anti*-His-tag aptamers could bind to the His-tagged proteins within *E. coli* cells and show green fluorescence under the fluorescence microscope. To prove that the fluorescence signals really came from *E. coli*, the bright field (DIC) image of *E. coli* was put next to the fluorescence image. A control experiment was also performed by using the cells without the His-tagged proteins. As illustrated in Figure 1, no green fluorescence was observed under the fluorescence microscope. From these results, we can conclude that the *anti*-His-tag aptamers have been successfully conjugated to QDs and still retains its specific biorecognition ability in live cells. More interestingly, the data indicate that the location of the proteins of interest can be tracked by the fluorescence signals from the aptamer-functionalized QDs as a function of time.

To summarize, we report a generic method for specific and efficient targeting of nanoparticles to fusion proteins containing His-tag in the cytoplasm of *E. coli* cells. Our results indicate that we succeeded to track the diffusion of individual proteins as a function of time within the membrane of an organelle with a localization precision of a few nanometers. This approach can be adapted to other types of aptamer-functionalized nanoparticles and will considerably extend the application of nanoparticles towards probing protein functions and dynamics in the cytoplasm and on the surface of intracellular membranes.

Experimental Section

General Methods. Unless otherwise noted, reagents were obtained from commercial suppliers and were used without further purification, and depc-treated deionized water was used for all experiments. RNA aptamer-functionalized QDs were prepared as previously described. In brief, QDs (emission maxima at 655 nm) modified with PEG and amino groups were obtained from Invitrogen (Carlsbad, CA). QD concentrations were measured by optical absorbance, using extinction coefficients provided by the supplier. Sulfosuccinimidyl 4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate (sulfo-SMCC, Sigma) was used as cross-linker. RNA-based aptamers for specific binding to His-tagged proteins were synthesized, using the antisense oligonucleotide containing the T7 promoter sequence at the 5'-end (5'-GCCAG CTCCC GGGGC CAATC CCAAC CAGAC CACCC ATAGC CCCCC CTATA GTGAG TCGTA TTAGT CC-3'),[6] and the resulting RNA was modified to contain a 5'-thiol group, *via* an enzymatic method for the introduction of 5'-terminal sulfhydryl group at the 5'-termini of RNA molecules according to the literature.[6,10] Prior to the transcription, the 5'-deoxy-5'-thioguanosine-5'-monophosphorothioate (GSMP) was synthesized,[10] as substrate for T7 RNA polymerase that requires guanosine to efficiently initiate tran-

scription. The *in vitro* transcription followed by treatment of alkaline phosphatase was used to incorporate a sulfhydryl moiety to 5'-end of RNA molecule.

Preparation of *E. coli* containing His-tagged Proteins. His-tagged β-galactosidase (β-Gal-His) encoding plasmids (pRSET/*lac*Z) was purchased from Invitrogen. Transformation of *E. coli* BL21/DE3 harboring the pLysS vector was carried out utilizing the transformation and storage solution method.[11] Briefly, competent *E. coli* were prepared in LB broth containing 15% polyethylene glycol. Afterwards, 0.2 ng of the plasmid were added to 200 μL of competent cells and incubation was performed for 1 h at 0 °C. After incubation consecutively at 42 °C for 1.5 min and at 0 °C for 2 min, 8 mL of LB medium were added followed by incubation at 37 °C for 1 h. The cells were harvested by incubating the cells in LB broth containing 50 mg/mL ampicilin and 35 mg/mL chloramphenicol. The cultivations were performed in shaking flasks with 100 mL of LB medium (10 g/L rtyptone, 10 g/L NaCl, 5 g/L yeast extract) supplemented with 50 mg/mL ampicilin and 35 mg/mL chloramphenicol. At an optical density at 600 nm of 0.5-0.6, IPTG was added to induce the expression of β-Gal-His, followed by 6-h incubation at 37 °C. The resulting cells were harvested by centrifugation at 2,300 × *g* for 10 min at 4 °C, and the cell pellets were resuspended in 10 mM NaH_2PO_4, 140 mM NaCl, 2.7 mM KCl, pH 7.4, and disrupted at 4 °C with ultrasound. After centrifugation and filtration the *E. coli* cell lysates were used for further confirmation of the cellular His-tagged protein expression. Confirmation of β-Gal-His from *E. coli* cell lysate was performed utilizing Ni^{2+}-loaded affinity chromatography column (His SpinTrap, GE Healthcare) as recommended by the manufacturer.

Transfection and Fluorescence Imaging. Heat-treated *E. coli* cells were mixed with the aptamer-conjugated QDs and 0.5 μL of the mixed solution was dropped on a glass slide. After additional 0-, 10-, 20-, 30-, 40-, 50-, and 60-min incubation, fluorescence microscopy was performed with an LSM510 confocal laser scanning microscope (Carl Zeiss Inc., USA). Instrument and measurement details can be found elsewhere.[12]

Acknowledgments. This work was supported by a grant from the Kyung Hee University in 2012 (KHU-20120480).

References

1. (a) Emptage, N. J. *Curr. Opin. Pharmacol.* **2001**, *1*, 521. (b) Stephens, D. J.; Allan, V. J. *Science* **2003**, *300*, 82. (c) Haugland, R. P. *Handbook of Fluorescent Probes and Research Products*; Molecular Probes: Eugene, 2002.
2. Shaner, N. C.; Steinbach, P. A.; Tsien, R. Y. *Nat. Methods* **2005**, *2*, 905. (b) Giepmans, B. N. G.; Adams, S. R.; Ellisman, M. H.; Tsien, R. Y. *Science* **2006**, *312*, 217.
3. (a) Los, G. V.; *et al. ACS Chem. Biol.* **2008**, *3*, 373. (b) Berrade, L.; Camarero, J. A. *Cell Mol. Life Sci.* **2009**, *66*, 3909. (c) George, N.; Pick, H.; Vogel, H.; Johnsson, N.; Johnsson, K. *J. Am. Chem. Soc.* **2004**, *126*, 8896.
4. Adams, S. R.; Tsien, R. Y. *Nat. Protoc.* **2008**, *3*, 1527.
5. (a) Alivisatos, A. P.; Gu, W.; Larabell, C. *Annu. Rev. Biomed. Eng.* **2005**, *7*, 55. (b) Alivisatos, P. *Nat. Biotechnol.* **2004**, *22*, 47. (c) 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. (d) Shin, S.; Nam, H. Y.; Lee, E. J.; Jung, W.; Hah, S. S. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 6081. (e) Kim, Y. S.; Kim, M. Y.; Song, J. K.; Kim, T. J.; Kim, Y. D.; Hah, S. S. *Chem. Commun.* **2012**, *48*, 723. (f) Kim, M. Y.; Kim, Y. S.; Kim, J.; Hah, S. S.; Kim, T. J.; Kim, Y. D. *Biotechnol. Lett.* **2011**, *33*, 623.
6. Shin, S.; Kim, I.-H.; Kang, W.; Yang, J. K.; Hah, S. S. *Bioorg. Med. Chem. Lett. 20*, 3322.
7. (a) Ferreira, C. S. M.; Matthews, C. S.; Missailidis, S. *Tumor Biol.* **2006**, *27*, 289. (b) Lee, G.-H.; Hah, S. S. *Bioorg. Med. Chem. Lett.* **2011**, *22*, 1520. (c) Nimjee, S. M.; Rusconi, C. P.; Sullenger, B. A. *Annu. Rev. Med.* **2005**, *56*, 555. (d) Pavlov, V.; Xiao, Y.; Shlyahovsky, B.; Willner, I. *J. Am. Chem. Soc.* **2004**, *126*, 11768.
8. (a) Kim, J.; Kim, M. Y.; Kim, H. S.; Hah, S. S. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 4020. (b) Wang, L.; Liu, X.; Hu, X.; Song, S.; Fan, C. *Chem. Commun.* **2006**, 3780. (c) He, F.; Tang, Y.; Wang, S.; Li, Y.; Zhu, D. *J. Am. Chem. Soc.* **2005**, *127*, 12343. (d) Ueyama, H.; Takagi, M.; Takenaka, S. *J. Am. Chem. Soc.* **2002**, *124*, 14286.
9. (a) Sankaran, N. B.; Nishizawa, S.; Seino, T.; Yoshimoto, K.; Teramae, N. *Angew. Chem. Int. Ed.* **2006**, *45*, 1563. (b) Liu, J.; Lu, Y. *Angew. Chem. Int. Ed.* **2006**, *45*, 90.
10. Kim, I.-H.; Shin, S.; Jeong, Y.-J.; Hah, S. S. *Tetrahedron Lett.* **2010**, *51*, 3446.
11. Chung, C. T.; Niemela, S. L.; Miller, R. H. *Proc. Natl. Acad. Sci. USA* **1989**, *86*, 2172.
12. Shin, S.; Kwon, H.-M.; Yoon, K.-S.; Kim, D.-E.; Hah, S. S. *Mol. BioSyst.* **2011**, *7*, 2110.