Supporting Information

Controlled Stoichiometric Synthesis of DNA-Quantum Dot Conjugates Using Ni-mediated Coordination Chemistry

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Experimental Section

1. Quantification of 6His binding on QDs. The polymer-coated QDs (25 pmol) were mixed with various equivalents of 2G6H containing an amidated C-terminal peptide (x100, 200, 500) in 10 mM borate buffer (pH 8.0) containing EDC (25 nmol). After 4 h at 25 ℃, the 2G6H-QD conjugates were washed five times with 10 mM borate buffer and characterized by agarose gel electrophoresis. Gel electrophoresis was run in a 0.5x TBE buffer on a 1.5 % agarose gel at 50 V for 1 h. The 6His on 2G6H-QD was quantified by collecting the unreacted peptide followed by a BCA peptide assay. The absorbance of the solution was measured using a UV-Vis spectrometer and the absorbance at 562 nm of each sample was plotted as a function of the amount of peptide that reacted with the QDs.

The BCA assay is a biochemical assay for determining the total level of protein in a solution. The total protein concentration is exhibited by a color change of the sample solution from green to purple in proportion to protein concentration, which can then be measured using colorimetric techniques.

Equivalent of 2G6H	2G6H / QD
100	60
200	150
500	270

Table S1. Quantification of 6xHis binding on QDs.

2. Synthesis of NTA ligand. NTA molecule was synthesized following the modified procedure reported in the literature.[S1]

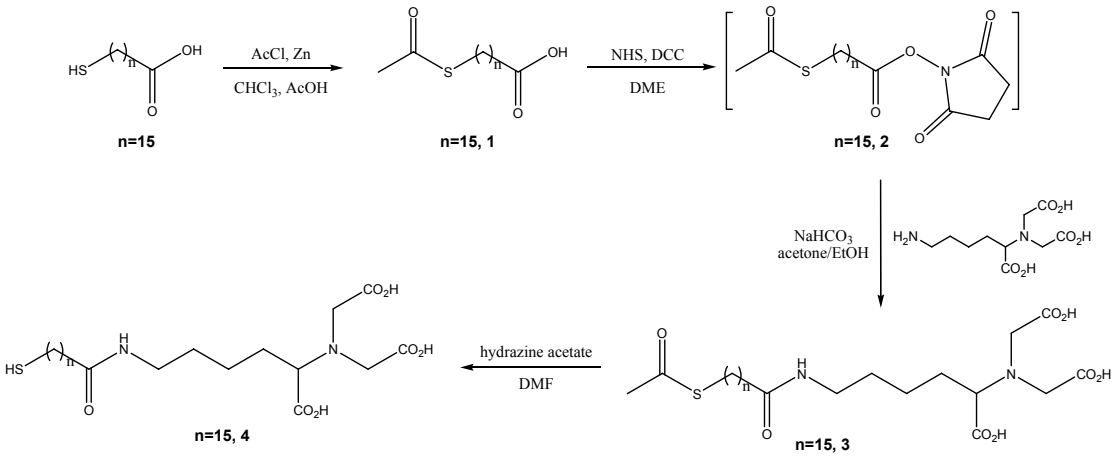


Figure S2. Synthetic procedures of the NTA ligand.

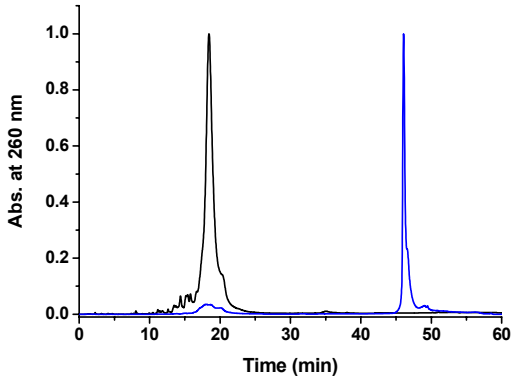
16-(Acetylthio)hexadecanoic Acid (Compound 1): The thiol group was protected by acetyl chloride according to the method reported by Svedhem et al.[S2] To a solution of MHDA (500 mg, 1.73 mmol, 30 mL $CHCl_3$, and 6 mL acetic acid) was added zinc powder (1.0 g, mmol). After stirring for 15 min, the solution was cooled to 0 °C and then treated with acetyl chloride (2.4 mL, 34 mmol). After stirring overnight, zinc was removed by filtration through a pad of Celite®, the solution was washed with 0.1 N HCl (2 × 30 mL) and water (30 mL). The organic layer was dried over Na_2SO_4, filtered, and concentrated in vacuum. The residue was purified via flash column chromatography to give compound 1 (436 mg, 76%) as a white powder; TLC Rf (chloroform:methanol 95:5) = 0.74.

N-[Nα,Nα-Bis(carboxymethyl)-L-Lysine]16-(Acetylthio)hexadecanamide (Compound 2): To a 0 °C solution of 16-(acetylthio) hexadecanoic acid 1 (338 mg, 1.02 mmol) and NHS (118 mg, 1.02 mmol) in anhydrous DME (15 mL) was added DCC (232 mg, 1.13 mmol). After 24 h at 0 °C, the white precipitate of DCU was removed by filtration and rinsed with dry 1,2-dimethoxyethane. The filtrate was concentrated in vacuum to give N-hydroxysuccinimide ester as a white powder and used in the following step without further purification. Then a solution of the resulted N-hydroxysuccinimide ester in acetone (1 mL) and EtOH (10 mL) was treated with a solution of Nα,Nα-

bis(carboxymethyl)-L-Lysine (322 mg, 1.23 mmol) and $NaHCO_3$ (340 mg, 4.05 mmol) in water (5 mL) at room temperature. After stirring for 43 h under Ar, the ethanol was removed under reduced pressure. The resulting residue was diluted with water (5 mL) and aqueous $NaHCO_3$ solution (0.25 M, 2 mL). The white precipitate of the remaining solution was filtered and discarded. The filtrate was acidified with 1.0 N HCl to pH = 3 and the resulting colloidal suspension was centrifuged (4000 rpm, 25 °C, 20 min) to give crude product 2. After removing supernant, the residue was washed with water by repeating resuspension/centrifugation twice. The resulting residue was dried under vacuum to give compound 2 (346 mg, 59%) as a pale yellow solid.

N-[Nα,Nα-Bis(carboxymethyl)-L-Lysine]16-Mercaptohexadecanamide (Compound 3): To a solution of N-[Nα,Nα-bis(carboxymethyl)-L-lysine]16-(acetylthio) hexadecane-amide 2 (120 mg, 0.203 mmol) in DMF (13 mL) was added hydrazine acetate (282 mg, 3.06 mmol). The resulting solution was bubbled with Ar for 20 min at room temperature. After degassing, the reaction mixture was stirred for 20 h under Ar. The solvent was removed under reduced pressure and then the residue was treated with 0.05 N HCl (20 mL). The resulting colloidal suspension was centrifuged (4000 rpm, 25 °C, 20 min) to give crude product 3, named NTA hereafter. After removing supernant, the residue was washed with water by repeating resuspension/centrifugation twice. The resulting residue was dried under vacuum to give compound 3 (102 mg, 91%) as a pale yellow solid.

3. Preparation and characterization of NiNTA-modified DNA. The retention time of starting oligonucleotides and NTA-modified oligonucleotide was 18 and 46 min, respectively.



	0	5	40	45	50	60	61
A	95	90	60	-	-	95	95
B	5	10	40	100	100	5	5

Eluent: (A) 50 mM Ammonium formate (in H_2O)
(B) 50 mM Ammonium formate (in H_2O/Acetonitrile: 50/50)

Figure S3. HPLC spectrum of starting DNA (black) and NTA-modified DNA (blue).

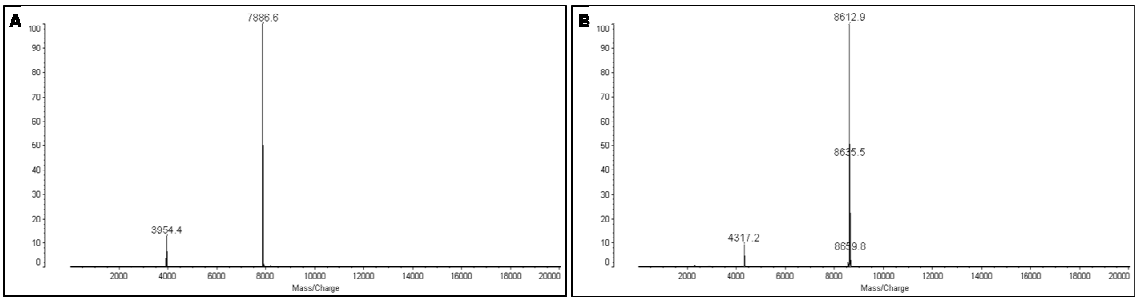


Figure S4. Maldi-TOF mass data of (A) starting oligonucleotide (MH^+=7886.5) and (B) NTA-modified oligonucleotide (MH^+=8612.5).

4. Preparation and characterization of 2G6H-QD conjugate. Gel electrophoresis was run in a 0.5x TBE buffer on a 2 % agarose gel at 50 V for 1 h.

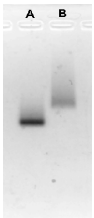


Figure S5. Agarose gel electrophoresis of (A) polymer-coated QD and (B) 2G6H-QD conjugate.

5. Quantification of NiNTA-DNA binding on 2G6H-QD. The 2G6H-QD conjugates were added to various equivalents of NiNTA-DNA (or NTA-DNA as a negative control) in 0.5x PBS (pH 7.4). The final QD solution was diluted to 70 nM. After 1 h incubation at 25 □, the characterization performed by gel electrophoresis. Gel electrophoresis was run in a 0.5x TBE buffer on a 1.5 % agarose gel at 50 V for 1 h.

To quantitative analysis, another set was performed. After 1 h incubation at 25 □, the QDs were washed several times with DI water. The DNA on 2G6H-QD was quantified by collecting the unreated DNA followed by an oligreen assay.[S3] The fluorescence of the solution was measured using a fluorescence microplate reader (Ex 485, Em 535).

QD : DNA	DNA / QD	Complex Eff. (%)
1 : 1	1	100
1 : 5	4	80
1 : 10	7	70
1 : 20	14	70

Table S6. Quantitation of NiNTA-DNA binding on 2G6H-QD.

6. Binding study of NiNTA-DNA with 6His-functionalized agarose bead. Two different agarose beads (AB) functionalized with different amino acid sequences were prepared in order to examine the potential of NiNTA-DNA as site-specific agent for the 6His-tagged QD. ABs have previously been modified by reductive amination of the glyoxal group with the N-terminal amino group 2G6H or 5GH2G. Both agarose beads were incubated with NiNTA-DNA at room temperature for 30 min. The unbound DNA were removed by washing with DI water and stained by SYBR Green. The fluorescence images of the beads were obtained using a fluorescence microscope.

7. Synthesis of $BHQ1_2$-tagged dsDNA. The 3 kinds of ssDNAs (DNA, NTA-DNA, NiNTA-DNA; 10 μM) were mixed with $BHQ1_2$-labeled target DNAs (10 μM) in 1x PBS (pH 7.4). The solution was incubated at 90 □ for 5 min, and then at 25 □ for 1 h. These dsDNAs were described as dsDNA($BHQ1_2$), NTA-dsDNA($BHQ1_2$) and NiNTA-

dsDNA(BHQ1$_2$).

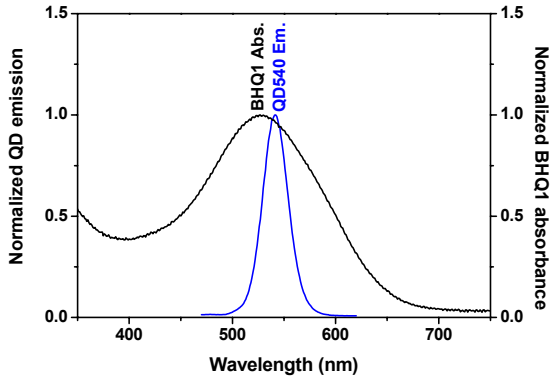


Figure S7. Normalized emission and absorbance spectra for QD donor and BHQ1 used in the experiment.

8. FRET efficiency. The FRET efficiency can be correlated with the number of acceptor dye molecules based on the following equation;

$$E = \frac{nR_0^{\,6}}{(nR_0^{\,6} + r^6)} \qquad \text{(Eq.1)}$$

where R_0 is the Förster radius at which 50 % donor fluorescence quenching occurs, *n* is the number of acceptor dye molecule, and *r* is the distance between the donor and acceptor. With the FRET pair we selected (QD with 540 nm and BHQ1 at of DNA), the R_0 value was calculated to be 5.1 nm based on the equation 2;

$$R_0 = 0.21(\kappa^2 n^{-4} Q_d J)^{1/6} \qquad \text{(Eq. 2)}$$

where *k* is the orientation factor (k_2 = 2/3 for random collisions), *n* is the refractive index in aq. solution (~ 1.33), Q_d is the quantum yield of donor (in this case, 0.32), and *J* is the spectral overlap integral between the acceptor and the QD (2.8 x 10^{-13}). A typical Förster distance $R_{0,}$ for the QDs (donor)-dye (acceptor) FRET system has been reported to be between 4 and 5 nm depending on the FRET pair.[S4] Our results are reasonably close to this range.

Reference

S1) 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**, 3392.

S2) S. Svedhem, C. -A. Hollander, J. Shi, P. Konradsson, B. Liedberg, and S. C. T. Svensson, *J. Org. Chem.* 2001, **66**, 4494.

S3) Molecular Probes® Handbook 11th Edition

S4) A. R. Clapp, I. L. Medintz, B. R. Fisher, G. P. Anderson and H. Mattoussi, *J. Am. Chem. Soc.* 2005, **127**, 1242.