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## I. Conjugation of Quantum dots to DNA

Streptavidin coated QD655 was purchased from Life Technologies Inc. Biotin modified 99mer and Amino functionalized 10mers were purchased from IDT DNA Technologies with the following sequences:

| | |
|---|---|
| 99mer with 3` Biotin modifier | 5'-GATGATGGATTATACCAGTAACAGCTTCTATTTAGGTT TCATGTTTTATCGAAGTAAACTGGGAACCGAAGCAGTACA TGCCCCCATTAGCAACTCTGT-3' |
| 10mer at 1-10 base with 5` Amino modifier | 5`-ACAGAGTTGC-3 |
| 10mer at 7-17 base with 5` Amino modifier | 5`-TGCTAATGGG-3` |
| 10mer at 15-25 base with 5` Amino modifier | 5`-GGGGCATGTA-3` |
| 10mer at 30-40 base with 5` Amino modifier | 5`-CTTCGGTTCC-3` |
| 10mer at 44-54 base with 5` Amino modifier | 5`-TTACTTCGAT-3` |
| 10mer at 59-69 base with 5` Amino modifier | 5`-ATGAAACCTA-3` |
| 10mer at 88-98 base with 5` Amino modifier | 5`-ATAATCCATC-3` |
| 60mer target | 5`-ACAGAGTTGCTAATGGGGGCTGTTTCTATCTA GGCGTGATTTGTTAGAAAAGTTCGATTG-3` |
| 100mer Hairpin with 5` Amino and 3` Biotin modifiers | 5`GCCTTGGCAAACTTTCTTTTTTTATCATTATTACACTAT CGTATTAATTTCCATCTAAAAACTAGACCAAGAAATTTA GAAAAAGAAAGTTTGCCAAGGC-3` |
| 60mer Hairpin target | 5`-TCTAAATTTCTTGGTCTAGTTTTTAGATGGAAATTAAT ACGATAGTGTAATAATGATAAA-3` |
| 99mer Internal DBCO (31nm) | 5`-ACAGAGTTGCTAATGGGGGCATGTACTGCTTCGGTT CCCAGTTTACTTCGATAAAACATGAAACCTAAATAGA AGCTGTTACTGGT/**i5DBCO**/ ATAATCCATCATC- 3’ |

‘Click’ chemistry reagent DBCO-$PEG_4$-NHS was purchased from Clickchemistrytools. 0.2 μM of QD655-streptavidin were incubated with 2 μM of 99mer ssDNA in 50 mM borate buffer pH 8.5 for 1 h at room temperature (RT). The Amino modified 10mers and hairpin DNA (2 μM) were modified to include DBCO functionality by incubating with 60 mM of DBCO-$PEG_4$-NHS in borate buffer for 4 h at RT. Excess reagent was removed by dialyzing the ssDNA and QDs for 8 h in a 2000 Da MW Slide-A-Lyzer MINI Dialysis Device purchased from Thermo Scientific Inc. Characterization of successful bioconjugation and subsequent hybridization assays was performed using FITC labeled ssDNA in a fluorometer (data not shown). 100mer hairpin DNA (2 μM) was conjugated to QD-avi (0.2 μM) by incubating the mixture in borate buffer for 30 min at room temperature.

## II. Labeling DNA with $^{64}$Cu

All work involving the use of radioactive materials at Washington University is conducted under Radiation Safety Committee approved authorizations in accordance with the University's Nuclear Regulatory Commission license. $^{64}CuCl_2$ was produced in a biomedical cyclotron CS-15. Azido-mono-amide-DOTA (100 μM) purchased from Macrocyclics Inc. dissolved in 50 mM Ammonium acetate buffer pH 5.5 was incubated with $^{64}$Cu (1 mCi) for chelation at 45$^{o}$C for 1 h. Similarly, Biotin-DOTA (100 μM) was used to chelate $^{64}$Cu (1 mCi). A Waters HPLC system was used to purify Azide-DOTA using a reverse phase C8 column. The flow rate was set to 1 ml/min. The solvents were A-0.1%Trifluoracetic acid (TFA) in water and B-0.1% TFA in Acetylnitrile. After 5 min hold at 5% B the gradient was programed linearly to 100%B at 40 min. The sample was collected for 2 min at 6 min timepoint corresponding to the Azide-DOTA peak and at 11 min timepoint for the Biotin-DOTA peak in the radiometer and UV detector. The samples were then dried in a rotary shaker for 4 h before resuspending in borate buffer. 'Click' reaction was carried out by incubating 2 μM of 10mer-DBCO and hairpin-DBCO with Azide-DOTA-$^{64}$Cu for 4 h at 37$^{o}$C. Unreacted reagents were removed using a 3 kDa Amicon Ultra centrifugal membrane filtration device purchased from Millipore.

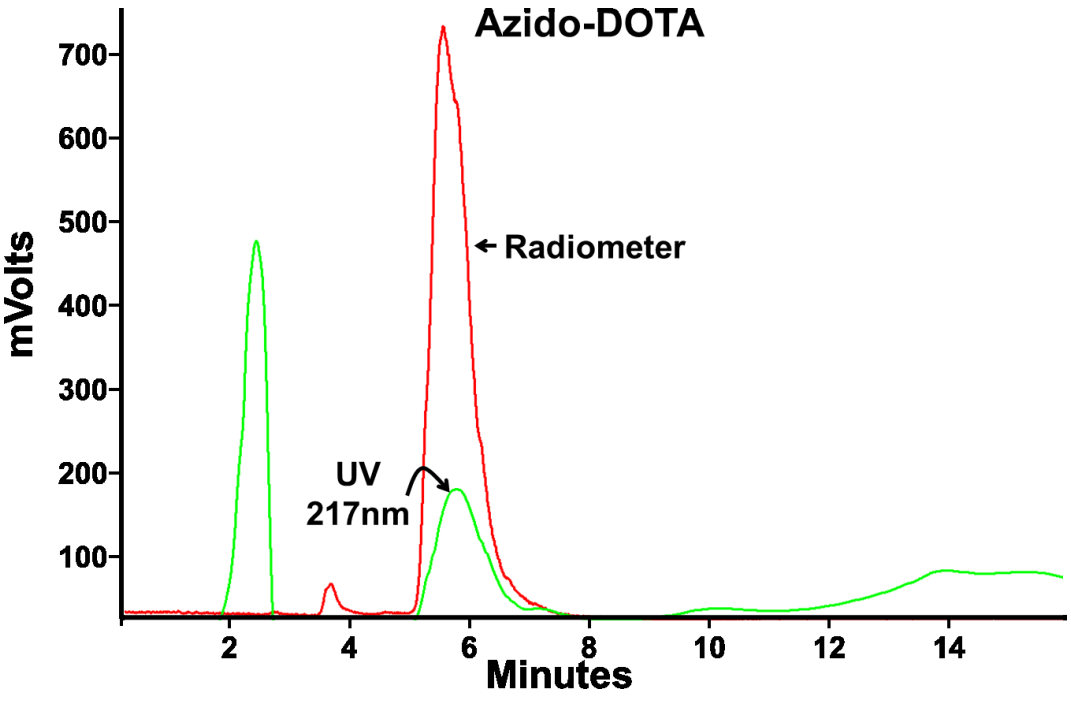


***Figure S1.*** HPLC chromatogram showing superimposed UV (green) and radiometer (red) peaks of Azido-DOTA eluting at 6 min.

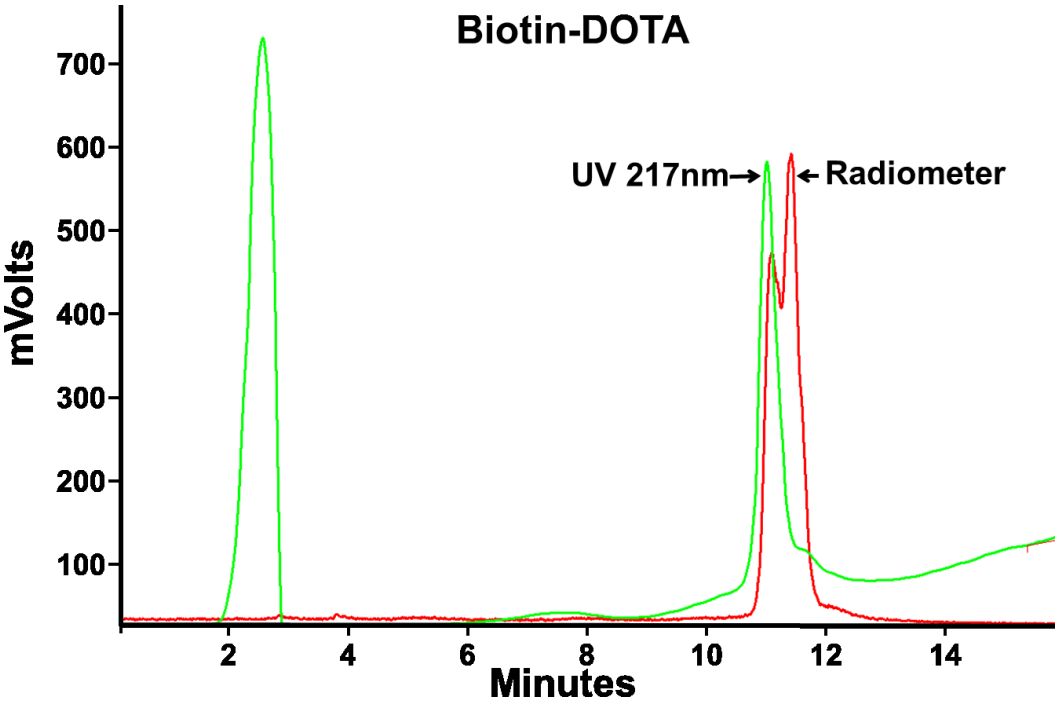


***Figure S2.*** HPLC chromatogram showing superimposed UV (green) and radiometer (red) peaks of Biotin-DOTA eluting at 11 min.

**III. Hybridization of QD-99mer and QD-hairpin with $^{64}$Cu-10mer**

Hybridization was performed in 50 mM borate buffer pH 8.5 by heating the sample to 95$^{o}$C for 2 min and allowing it to cool to room temperature for 20 min. The probes were kept on ice till measurements were taken. The same procedure was followed for the strand displacement and hairpin-target annealing. Excess and unreacted reagents were removed using a 50 kDa Amicon Ultra centrifugal membrane filtration device.

**IV. Luminescence imaging**

The samples were imaged in a 96 well plates by IVIS lumina XR multimodal imaging system (Caliper Life Sciences Inc.) with a minimum detectable radiance of 100 photons/s/sr/cm$^2$ and significant dark current reduction of $< 3 \times 10^{-4}$ e$^-$/pixel/s. The images were captured at a resolution of 1024 X 1024 using the Cy5.5 emission filter (690-770 nm) with Living Image software. Radiance readings were also recorded using Living Image software through the in-built analysis tool by drawing regions of interest

(ROI). Graphpad Prism statistical software was used to analyze and plot the data. Non-linear regression fitting analysis was applied after background correction from the well containing $^{64}Cu$ alone. Radiance was normalized to the maximum value.

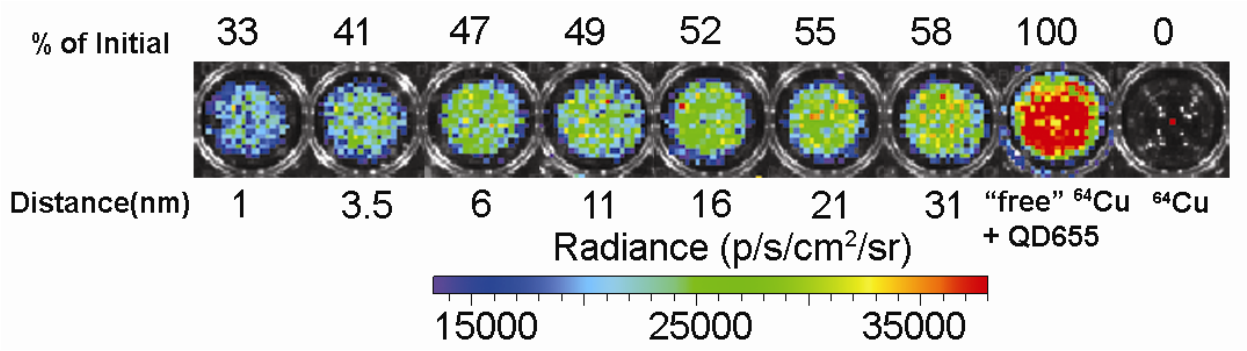


***Figure S3.*** Luminescence of QD655 recorded using IVIS Lumina XR using a Cy5.5 emission filter (690-770 nm) in a well plate. Sequential positioning of $^{64}Cu$ from the QD surface along the DNA ladder showed a direct relation between radiance and distance. Maximum radiance was observed in QD655 admixed with free $^{64}Cu$ and minimal radiance from $^{64}Cu$ alone.

**V. "Cold" Copper experiments and steady-state optical measurements**

Serial dilutions of Copper chloride were made with respect to a fixed QD655 concentration (0.2 μM). 1:1, 1:2, 1:5, 1:10 and 1:100 molar ratios were chosen. Similarly, serial dilutions for chelated Cu(II) were prepared using equimolar DOTA. Endpoint fluorescence readings were recorded using Synergy HT multimode plate reader (BioTek Instruments Inc.). For distance validation experiments, aforementioned method was followed where Azido-mono-amide-DOTA (100 μM) was incubated with 500 μM $CuCl_2$ instead. Fluorescence spectra of 20 nM QD655 were recorded on a Fluorolog-3 spectrofluorometer (Horiba Jobin Yvon Inc.) with an excitation of 460 nm and emission scan 550-800 nm. Absorbance spectra of 1.6 mM $CuCl_2$ were recorded on a Beckman Coulter DU 640 UV-visible spectrophotometer and analyzed using Graphpad Prism statistical software.

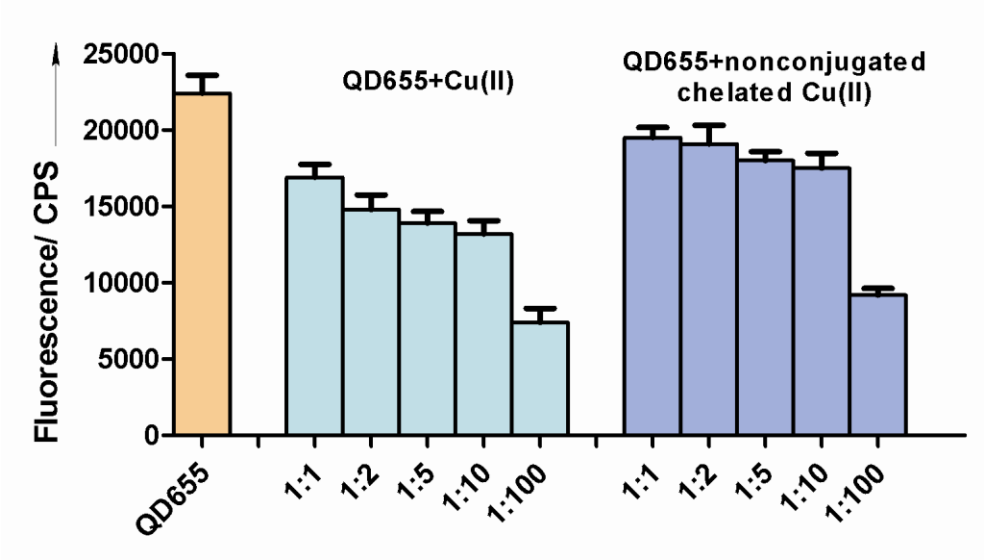


***Figure S4.*** Fluorometric titrations of unchelated (green) and chelated (blue) "cold" Cu(II) admixed with QD655 in comparison to QD655 alone (orange).

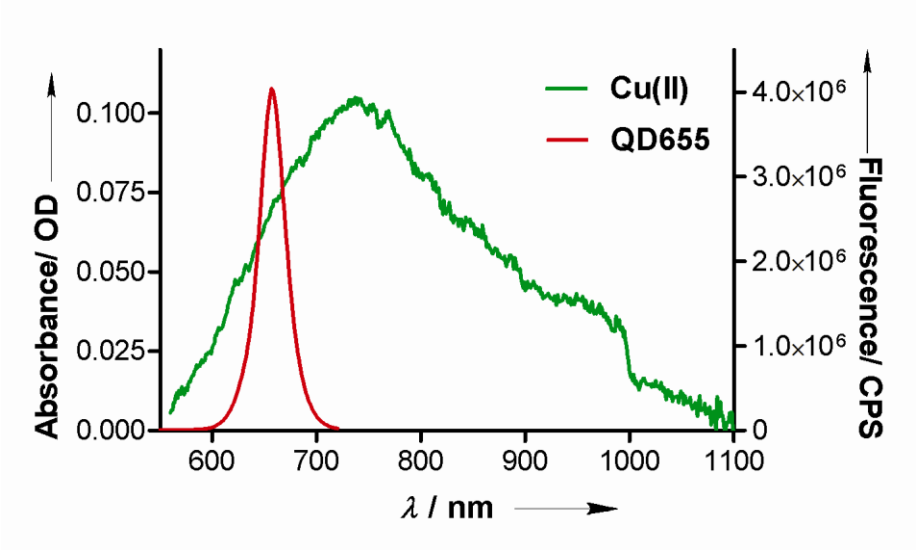


***Figure S5.*** Absorption spectrum of Cu(II) and fluorescence emission spectrum of QD655 showing overlap, suggesting possibility of energy transfer.

**VI. Picosecond time-resolved fluorescence spectroscopy**

Time-resolved fluorescence (TRF) experiments were carried out using Hamamatsu universal streak camera consisting of a cooled N51716-04 streak tube, C5680 blanking unit, digital CCD camera (Orca2), slow speed unit M5677, C10647 and C1097-05 delay generators and equipped with Bruker

A6365-01 spectrograph. Excitation pulses (at 520 nm) were produced by an ultrafast optical parametric oscillator (OPO) Inspire100 (Radiantis-Spectra-Physics) that was pumped with a Mai-Tai ultrafast Ti:Sapphire laser generating ~90 fs laser pulses at 820 nm with a frequency of 80 MHz. After the OPO, the repetition rate of the excitation beam was lowered to 8 MHz, consisting of a 125 ns time gap between subsequent excitations. Excitation beam with power of ~0.5 mW was depolarized to avoid polarization effects and focused on the sample in a circular spot of ~1 mm diameter, which corresponds to a photon intensity of ~$2\times10^{10}$ photons/cm$^2$pulse.

Upon photo excitation of QD nanoparticle, electrons from the valence band (VB) are promoted into the unoccupied conductive band (CB). Florescence emitted from QD nanoparticle is a result of emissive recombination of pairs of so-called deeply trapped electrons and holes. The deep traps lie lower in energy than exciton states thus fluorescence spectrum of QD is significantly shifted to longer wavelengths compare to its absorption.[1] In general an average number of electron-hole pairs $p$ present in a QD at time *t* after photogeneration can be described by following general function [2]:

$$p\ t\ =\ \sum_{n=1}^{\infty}(2\exp\ -\ p_{\ 0}\ (-1)n\ \ \sum_{j=1}^{\infty}\frac{p_{\ 0}i}{n+i\ !}\ \ \prod_{j=1}^{n}(n-i-j))\exp(-n^2kt) \quad (1)$$

Where $p_{\ 0}$ is an average number of electron-hole pairs at time $t = 0$, $n$ – number of QD particles per unit volume.

If $p_{\ 0}$ is small, the equation (1) can be simplified to [2]:

$$p\ \ t\ =\ p_{\ 0}\exp(-\mathrm{k}t) \quad (2)$$

If If $p_{\ 0}$ is large, the equation (1) can be simplified to [3]:

$$p\ \ t\ =\frac{p_{\ 0}}{1+\ p_{\ 0}kt} \quad (3)$$

Upon assumption of absense of non-radiative recombination of electron-hole pairs, $p$ will be proportional to number of photon counts recorded in TRF measurement (one photon origins from emissive recombination of one electron-hole pair) and the equations (2) and (3) can be described as follows:

$$\mathrm{F}\ t\ =\mathrm{F}_0\exp(-\mathrm{k}t) \quad \text{(small number of } p \text{ per QD nanoparticle)} \quad (4)$$

and

$$\mathrm{F}\ t\ =\frac{\mathrm{F}_0}{1+\mathrm{F}_0kt} \quad \text{(large number of } p \text{ per QD nanoparticle)} \quad (5)$$

where F is fluorescence intensity denoted in photon counts.

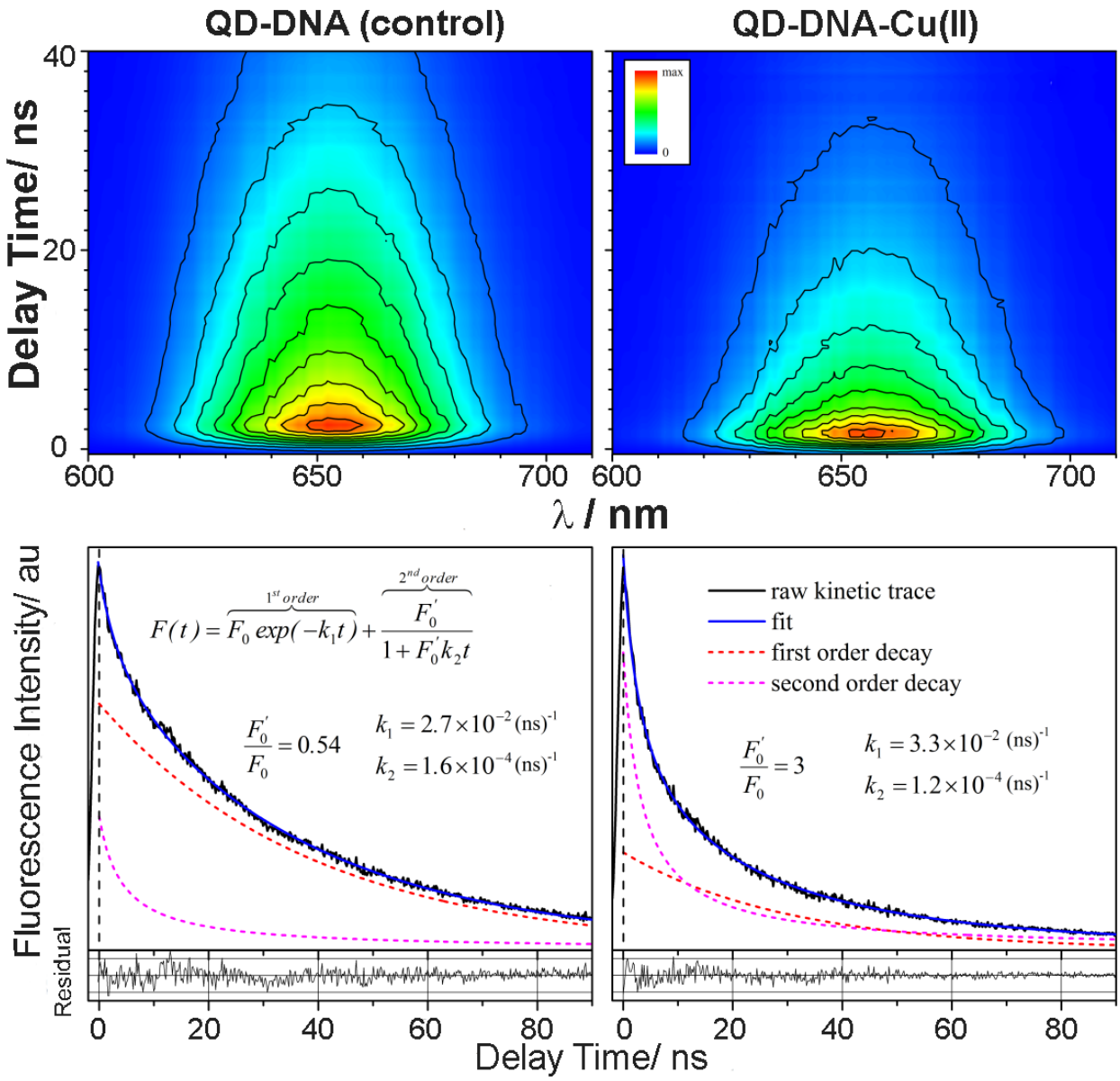


***Figure S6.*** TRF of the QD-DNA without Cu(II) (Top left). TRF of the QD-DNA attached to Cu(II) at a distance of 1nm from QD (Top right). Kinetic traces of fluorescence decay of QD-DNA without Cu(II) (Bottom left). Kinetic traces of fluorescence decay of QD-DNA with Cu(II) (Bottom right), accompanied with fits obtained according to a linear combination of equation 4 and 5. Decay constants and proportions of electron-hole pairs emitting according to 1st and 2nd order decay are also provided.

| Sample | Exponential component | | | Non-exponential component | | | $F_1\tau_{1/2}/ F_2\tau'_{1/2}$ | $(F_1\tau_{1/2}+ F_2\tau'_{1/2})/2$ (ns) |
|---|---|---|---|---|---|---|---|---|
| | $F_1^*$ | Decay rate $[(ns)^{-1}]$ (mean lifetime) | Half-life ($\tau_{1/2}$) (ns) | $F_2^*$ | Decay rate $[(ns)^{-1}] /10^{-6}$ | ($\tau'_{1/2}$) (ns) | | |
| QD-DNA Control | 0.65 | 0.026 (38.4 ns) | 26.6 | 0.35 | 160 | 4.2 | 11.8 | 9.4 |
| QD-DNA-$Cu^+$ (1 nm) | 0.25 | 0.034 (29.4 ns) | 20.4 | 0.75 | 120 | 3.2 | 2.1 | 3.8 |
| QD-DNA-$Cu^+$ (3.5 nm) | 0.34 | 0.035 (28.6 ns) | 19.8 | 0.76 | 94 | 3.0 | 3.4 | 4.5 |
| QD-DNA-$Cu^+$ (6 nm) | 0.16 | 0.036 (27.8 ns) | 19.3 | 0.84 | 150 | 2.4 | 1.5 | 2.6 |
| QD-DNA-$Cu^+$ (11 nm) | 0.34 | 0.036 (27.8 ns) | 19.3 | 0.66 | 190 | 2.2 | 4.5 | 4.0 |
| QD-DNA-$Cu^+$ (16 nm) | 0.36 | 0.034 (29.4 ns) | 20.4 | 0.64 | 350 | 2.9 | 3.9 | 4.6 |
| QD-DNA-$Cu^+$ (21 nm) | 0.27 | 0.033 (30.3 ns) | 21.0 | 0.73 | 170 | 2.7 | 2.5 | 3.8 |
| QD-DNA-$Cu^+$ (31 nm) | 0.18 | 0.03 (27.0 ns) | 18.7 | 0.82 | 116 | 2.3 | 1.8 | 2.6 |

***Table S1.*** Results of fitting of fluorescence decay kinetic traces of QD-DNA-Cu(II) systems with various distances between QD and Cu(II). (*) fraction of initial amplitude of raw fluorescence signal. The fractional contribution and average half-life weighted by component amplitude contribution are included in the last two columns.

## VII. Femtosecond time-resolved transient absorption spectroscopy

Time-resolved pump-probe absorption experiments were carried out using Helios, a femtosecond transient absorption spectrometer (Ultrafast Systems, LCC) coupled to a femtosecond laser system described in detail previously. [3] The QD samples were excited at 470 nm. Energy of the pump beam was set to ~200 nJ/pulse in a spot size of 1 mm diameter corresponding to an intensity of $6\times10^{13}$ photons/$cm^2$pulse.

Representative results obtained for the QD-DNA and QD-DNA-Cu(II) samples are shown in figure S5. Figure shows transient absorption (TA) contours and spectra recorded at various delay times after excitation. TA spectra of both samples consist of two negative spectral features. The band that is located at 520 nm is associated with so-called “bleaching” of steady-state absorption and origins from difference in population of QD nanoparticles being in ground state before and after excitation. The

second band that spans spectral range from 550 to 700 nm is associated with stimulated fluorescence (SE) induced by the incident white light from the probe beam. The shape and position of the SE band does not remain constant while time elapses and center of the band shifts 20 nm toward longer wavelengths. The part of the spectrum located between 550 and 620 nm decays much faster and decays completely within few hundred ps. Fast decaying fluorescence that is substantially shifted toward shorter wavelengths may be interpreted as light induced (stimulated) emissive recombination of shallow traps that normally do not contribute in steady-state (spontaneous) fluorescence.

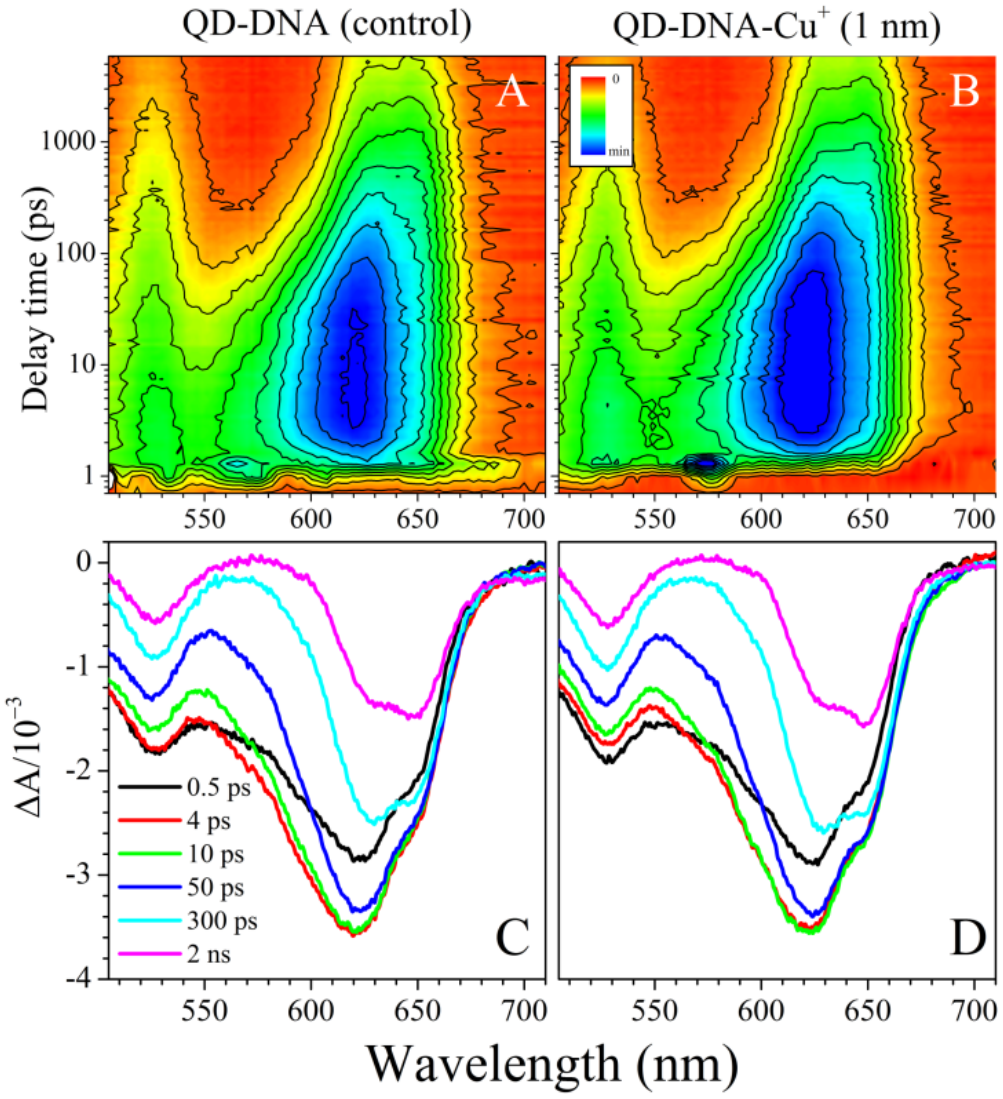


***Figure S7.*** Time-resolved absorption of the QD-DNA and QD-DNA-Cu(II) upon excitation at 490 nm: (A, B) transient absorption contours, (C, D) transient absorption spectra taken at various delay times after excitation. The band that is observed at ~520 nm is associated with bleaching of steady-state absorption, the band spanning range from 550 to 700 nm is associated with stimulated fluorescence.

**VIII. Direct attachment of Biotin-DOTA to streptavidin-QD655**

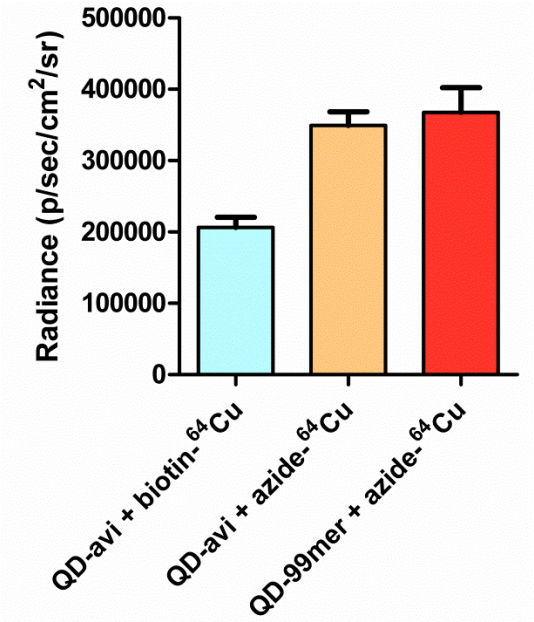


***Figure S8.*** Comparison of radiance from QD-streptavidin conjugated to biotin-$^{64}$Cu, QD-avi admixed with azide-$^{64}$Cu and QD-99mer admixed with azide-$^{64}$Cu.

**IX. Comparison of 10mer plus 'anchor' with 99mer design at 31 nm distance:**

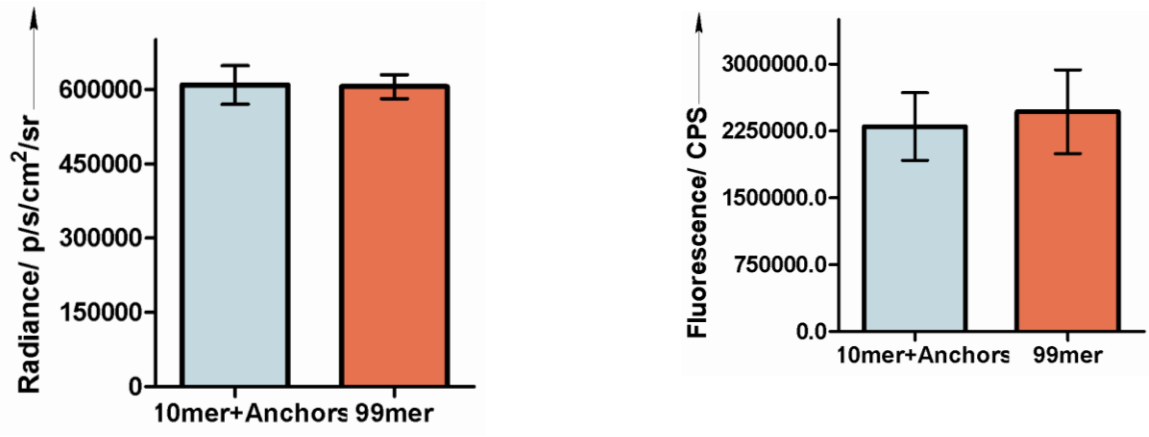


***Figure S9.*** Comparison of 10mer plus anchor ssDNA and 99mer ssDNA through luminescence (left), using $^{64}$Cu, and fluorescence (right), using Cu(II), measurements.

**References**

[1] N. Chestnoy, T.D. Harris, R. Hull, L.E. Brus, *J. Phys. Chem*. **1986**, *90*, 3393-3399.

[2] G. Rothenberger, J. Moser, M. Gratzel, N. Serpone, D.K. Sharma, *J. Am. Chem. Soc.* **1985**, *107*, 8054-8059.

[3] D.M. Niedzwiedzki, M. Fuciman, H.A. Frank, R.E. Blankenship, *Biochim. Biophys. Acta*., **2011**, *1807*, 518-528.