Near infrared fluorescence quenching properties of copper (II) ions for potential applications in biological imaging

Dolonchampa Maji, Mingzhou Zhou, Pinaki Sarder, Samuel Achilefu

Dolonchampa Maji, Mingzhou Zhou, Pinaki Sarder, Samuel Achilefu, "Near infrared fluorescence quenching properties of copper (II) ions for potential applications in biological imaging," Proc. SPIE 8956, Reporters, Markers, Dyes, Nanoparticles, and Molecular Probes for Biomedical Applications VI, 89560K (7 March 2014); doi: 10.1117/12.2053746

SPIE.

Event: SPIE BiOS, 2014, San Francisco, California, United States

Near infrared fluorescence quenching properties of copper (II) ions for potential applications in biological imaging

Dolonchampa Maji[1, 2], Mingzhou Zhou[2], Pinaki Sarder[2], Samuel Achilefu[1, 2, 3,*]

[1]Department of Biomedical Engineering, Washington University in St. Louis, Missouri, USA;
[2]Department of Radiology, Washington University School of Medicine, St. Louis, Missouri, USA.
[3]Department of Biochemistry and Molecular Biophysics, Washington University School of Medicine, St. Louis, MO, USA.

*Correspondence: achilefus@mir.wustl.edu

ABSTRACT

Fluorescence quenching properties of copper(II) ions have been used for designing Cu(II) sensitive fluorescent molecular probes. In this paper, we demonstrate that static quenching plays a key role in free Cu(II)-mediated fluorescence quenching of a near infrared (NIR) fluorescent dye cypate. The Stern-Volmer quenching constant was calculated to be $K_{SV} = 970{,}000\ M^{-1}$ in 25 mM MES buffer, pH 6.5 at room temperature. We synthesized LS835, a compound containing cypate attached covalently to chelated Cu(II) to study fluorescence quenching by chelated Cu(II). The fluorescence quenching mechanism of chelated Cu(II) is predominantly dynamic or collisional quenching. The quenching efficiency of chelated Cu(II) was calculated to be 58% ± 6% in dimethylsulfoxide at room temperature. Future work will involve further characterization of the mechanism of NIR fluorescence quenching by Cu(II) and testing its reversibility for potential applications in designing fluorophore-quencher based molecular probes for biological imaging.

Keywords: Copper, cypate, fluorescence quenching, near infrared fluorescence, Stern-Volmer quenching constant.

1. INTRODUCTION

Metal ions have been shown to interact with and alter the spectral properties of fluorescent molecules. This feature is widely used to design metal sensitive fluorescent molecular probes to detect the presence of metal ions[1,2]. Such interactions result in effects ranging from quenching of fluorescence by metal ions such as iron (Fe (III))[3] and copper (Cu(II))[4] to fluorescence enhancement by zinc (Zn(II))[5]. Cu(II) ions have been shown to quench fluorescence of organic dyes, fluorescent proteins and quantum dots[6-10]. Fluorescence quenching properties of Cu(II) are very prominent in the near infrared (NIR) fluorescence range (700-900 nm). In this study we studied and quantified the fluorescence quenching properties Cu(II) ions for a NIR dye cypate. We used both free Cu(II) as well as chelated Cu(II) attached to the dye molecule for this study. The ultimate goal is to demonstrate the feasibility of exploiting this NIR fluorescence quenching properties of Cu(II) to design fluorophore-quencher pair based molecular probes for potential applications in biological imaging.

2. MATERIALS AND METHODS

2.1 Materials

Dimethylsulfoxide (DMSO), dimethylformamide (DMF), ethylenediamine, N,N-Diisopropylethylamine (DIEA), MES hydrate, hydrion (sodium carbonate and sodium bicarbonate) buffer powder, ammonium acetate, and copper (II) chloride

Reporters, Markers, Dyes, Nanoparticles, and Molecular Probes for Biomedical Applications VI, edited by Samuel Achilefu, Ramesh Raghavachari, Proc. of SPIE Vol. 8956, 89560K · © 2014 SPIE · CCC code: 1605-7422/14/$18 · doi: 10.1117/12.2053746

dihydrate were purchased from Sigma Aldrich. p-SCN-Bn-PCTA was purchased from Macrocyclics. 2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) and N-Hydroxybenzotriazole (HOBt) were purchased from AAPPTec. All commercially purchased materials were used without any further purification. NIR dye cypate was synthesized in the lab using previously described procedure as reported earlier[11]. MES buffer (25mM), pH 6.5 was prepared by dissolving required quantity of MES hydrate in milliQ water and adjusting pH using sodium hydroxide. Hydrion buffer (pH 10) was prepared following vendor's protocol.

2.2 Spectroscopic studies of titration of cypate with Cu(II)

A solution of cypate (0.2 μM) was prepared in MES buffer. Small amount of DMSO (<2% v/v final concentration) was added to enhance the solubility of the dye in aqueous buffer. MES buffer was chosen for this study as it has been shown not to interact with Cu(II) ions in solution[12]. Stock solution of copper (II) chloride (100 μM), prepared in the same buffer and 1mL of the dye solution in a cuvette were used for the analysis. Copper(II) chloride solution was added to the dye solution and mixed for approximately 10 s before recording the absorption and emission spectra. Absorption spectra (600-900 nm) were recorded on a DU 640 spectrophotometer (Beckman Coulter). Fluorescence spectra were recorded on a FluoroLog 3 spectrofluorometer (Horiba Scientific) using 720 nm/735-900 nm as excitation/emission wavelength. Measurements were made for increasing final concentrations of Cu(II) at 0, 0.1, 0.2, 0.5 and 1 μM in the same dye solution. These concentrations correspond to 0, 0.5, 1, 2.5 and 5 molar ratios of Cu(II) with respect to cypate in the solution.

Stern-Volmer plot was generated for the above spectral measurements. For each emission spectrum, total fluorescence signal was quantified by integrating the area under the emission curve. This value was normalized to absorbance at the excitation wavelength 720 nm. The ratios of fluorescence in absence of copper to the presence of copper were plotted versus the final concentration of copper in the solution, and the data were fitted to a straight line representing the Stern-Volmer relation for fluorescence quenching as follows:

$$F_0/F = 1 + K_{SV}[Q] \quad \text{.....(1)}$$

where, F_0 = Fluorescence signal in absence of copper; F = Fluorescence signal in presence of copper; [Q] = concentration of the quencher Cu(II), and K_{SV} = Stern Volmer quenching constant. Goodness of fit was judged by regression value ($R^2 > 0.96$).

2.3 Spectral properties of LS835

Absorption and emission spectra of cypate and LS835 were recorded in DMSO. For all measurements, absorbance at the excitation wavelength was kept below 0.05. Quenching efficiency (QE) of Cu(II) was calculated by quantifying the number of photons emitted (measured by the area under fluorescence emission curve) of molecules normalized to absorbance value at excitation wavelength 720 nm. The following formula was used,

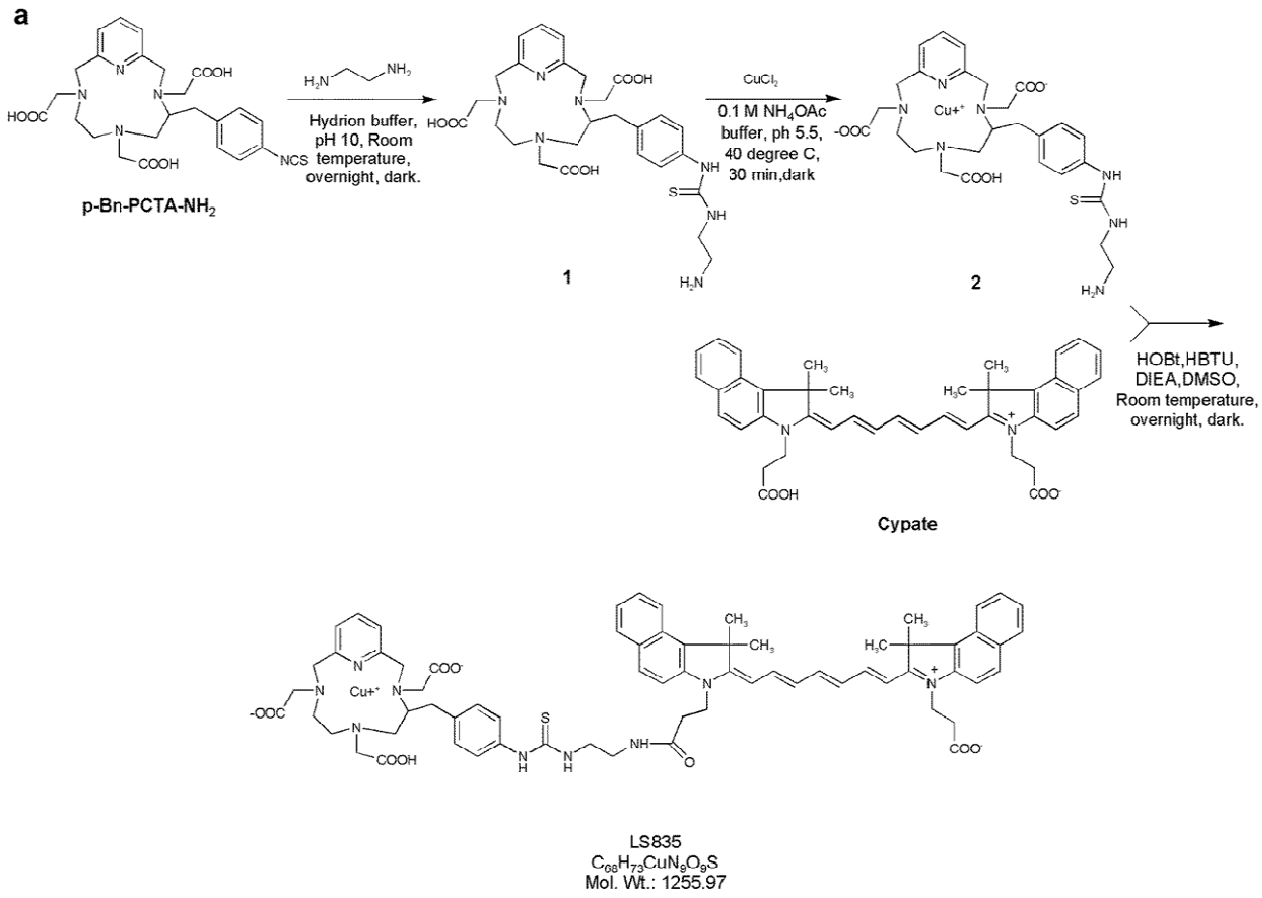
$$\mathrm{QE} = \left\{1 - \frac{\text{Normalized area under emission curve for LS835}}{\text{Normalized area under emission curve for cypate}}\right\} * 100 \quad \text{..... (2)}$$

3. RESULTS AND DISCUSSION

3.1 Synthesis and characterization of LS835

The synthesis scheme for LS835 is shown in Fig. 1a. The final molecules at each step of the synthesis were characterized by LC/MS(ESI) analysis in the positive ion mode conducted in a Shimadzu LCMS 2010A machine.

1: A solution of p-SCN-Bn-PCTA (5 mg, 7.8 µmoles) was prepared in 2 mL of hydrion buffer (sodium carbonate – sodium bicarbonate), pH 10. Within one minute, ethylenediamine (4.7 mg, 78 µmoles) was added to this solution. The mixture was stirred at room temperature overnight in dark. The crude product was further purified by HPLC equipped with a reverse-phase C-18 column to afford **1** as white solid. MS (ESI) m/z obtained: 588($M+H^+$).



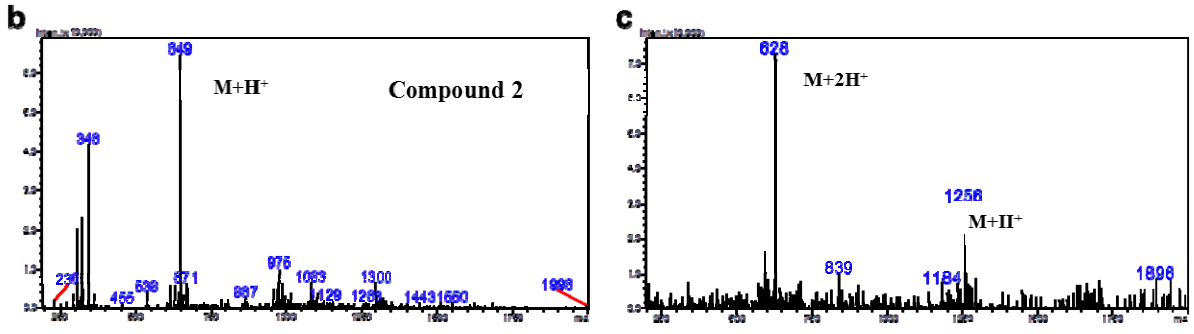


Figure 1: Synthesis and characterization of LS835: (a) Synthesis scheme for LS835. (b) MS (ESI) spectra of compound **2** showing ($M+2H^+$) peak. (c) MS (ESI) spectra of LS835 showing ($M+H^+$) and ($M+2H^+$) peaks.

2: To a solution of **1** (1 mM) in 0.1 M ammonium acetate buffer (pH 5.5), copper(II) chloride (5 mM) was added. The mixture was stirred at 40˚C for 30 min in dark. The crude product was further purified by HPLC equipped with a reverse-phase C-18 column to afford **2** as greenish white solid. MS(ESI) m/z obtained: 649($M+H^+$) (Fig. 1b).

LS835: A mixture of cypate (10 mg, 14 μmoles), HOBt (1.2 mg, 8 μmoles) and HBTU (5.3 mg, 14 μmoles) was prepared in 1mL DMSO. This mixture was shaken at room temperature for 20 min in dark. A mixture of **2** (2.6 mg, 4 μmoles) and DIEA (7.2mg, 56 μmoles) was prepared in 1 mL DMSO and shaken at room temperature for 5 min. Both were mixed together and the resulting mixture was stirred at room temperature overnight in dark. The crude product was further purified by HPLC equipped with a reverse-phase C-18 column to afford **LS835** as green solid. MS (ESI) m/z obtained: 628($M+2H^+$), 1256($M+H^+$) (Fig. 1c).

3.2 Fluorescence quenching of cypate by free Cu(II) ions

The changes in absorption and emission spectra of cypate upon addition of Cu(II) are shown in Fig. 2a and Fig 2b respectively. The absorption spectra show gradual decrease in the absorption peak with a bathochromic shift of 3 nm from 779 nm to 782 nm with increasing concentration of Cu(II). These changes were associated with the existence of one isosbestic point approximately at 812 nm. These results indicate the existence of two molecular species in the solution at varying concentrations upon addition of copper. Our result suggests that one of the species is cypate only and the other is a complex of cypate and Cu(II). As concentration of Cu(II) is increased, increasing amounts of the free dye is converted to the complex.

The corresponding fluorescence emission intensities also showed a gradual decreasing trend. The changes in absorbance value at the excitation wavelength 720 nm are shown in Fig. 2c. Decrease in emission intensity can be attributed to the combined effect of decrease in absorbance at the excitation wavelength and due to complex formation between copper and cypate.

These results demonstrate the NIR fluorescence quenching properties of free Cu(II). Spectral studies suggest complex formation between cypate and copper and thus the quenching mechanism is majorly by static quenching, where the quencher Cu(II) interacts with the dye in its ground state and the spectral properties of the complex is different from the dye only. In this case, the cypate-Cu(II) complex is either non fluorescent or has decreased fluorescence emission compared to cypate alone.

We used the Stern-Volmer plot to further quantify the quenching properties of free Cu(II) (Fig. 2d). From this plot, the Stern-Volmer quenching constant for free Cu(II) was calculated to be $K_{SV} = 970{,}000\ M^{-1}$.

3.3 Fluorescence quenching of cypate by chelated Cu(II) ion

To demonstrate the quenching properties of chelated Cu(II) ion, the compound LS835 was synthesized. It contains the dye cypate covalently attached to Cu(II) ion chelated to a metal chelator (PCTA). The absorption and emission spectra of cypate and LS835 were measured and compared as shown in Fig. 3a and Fig. 3b respectively. No significant difference was observed in the absorption spectra of both molecules. Fluorescence emission intensity from LS835 was decreased compared to that for cypate at approximately equal absorbance at the excitation wavelength 720 nm.

These results demonstrate the NIR fluorescence quenching properties of chelated Cu(II) when present at 1:1 molar ratio with the dye cypate. The similarity of the absorption spectra in the presence and absence of Cu(II) indicates dynamic quenching mechanism where the quencher Cu(II) interacts with the dye only in the excited state. The quenching efficiency of chelated copper in LS835 was calculated to be 58% ± 6% (mean ± SD, n=3) in DMSO at room temperature.

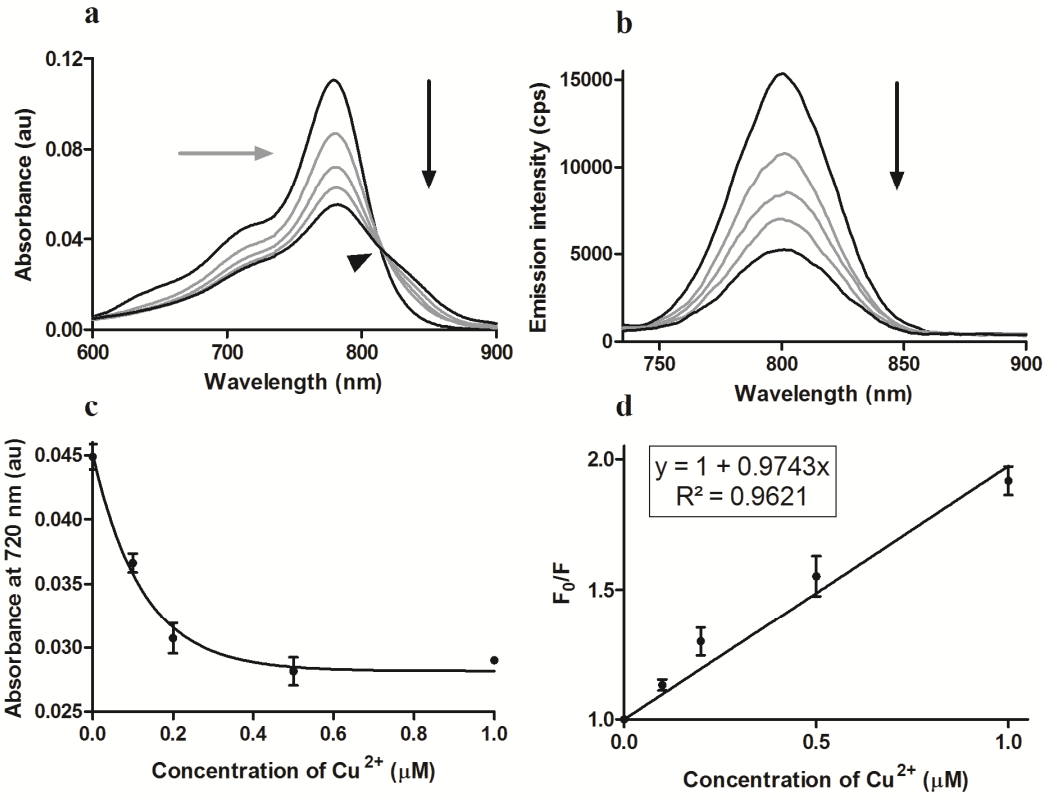


Figure 2: Spectroscopic studies of the titration of NIR fluorescent dye cypate (0.2 μM) with copper (II) ions (0 μM to 1 μM). (a) Representative figure for the changes in absorption spectra of cypate upon addition of Cu(II) . Black arrow indicates decreasing absorption with increasing concentration of Cu(II). Grey arrow indicates the associated bathochromic shift of absorption peak. (b) Corresponding changes in emission spectra of cypate. Black arrow indicates decreasing fluorescence emission intensity with increasing concentration of Cu(II). Arrowhead indicates isosbestic point approximately at 812 nm. (c) Changes in absorbance value at 720 nm with addition of Cu(II). (d) Stern-Volmer plot for Cu(II) induced fluorescence quenching of cypate. Inset shows the fitted equation with corresponding regression value. Error bar represent standard errors of mean for three independent experiments.

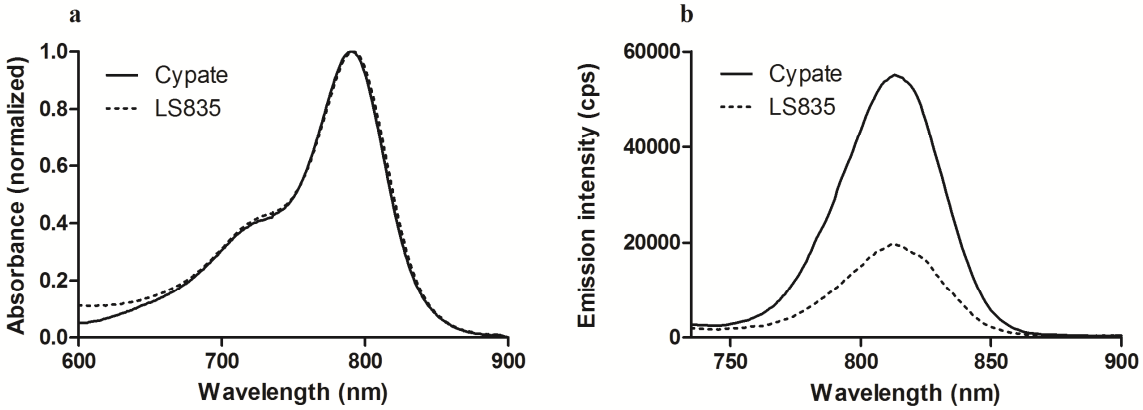


Figure 3: Absorption and emission spectra of cypate and LS835. (a) Normalized absorption spectra show no change in spectra of cypate on attachment with chelated Cu(II) in **LS835**. (b) Emission spectra at approximately equal absorbance at excitation wavelength 720 nm shows fluorescence quenching of cypate due to the presence of copper in **LS835**.

4. CONCLUSION

We have shown that the fluorescence quenching of cypate by free Cu(II) is predominantly by static quenching, whereas attachment of chelated Cu(II) to the dye in 1:1 molar ratio resulted in dynamic quenching. The Stern-Volmer quenching constant for free Cu(II) was calculated to be $K_{SV} = 970{,}000\ M^{-1}$. The quenching efficiency of chelated Cu(II) when attached to cypate in **LS835** was calculated to be 58 ± 6% in this study. Fluorescence quenching by excited state energy transfer and charge transfer has been used to explain such dynamic quenching by Cu(II) ion[1, 6-10]. Since such interactions are reversible, we postulate it is possible that the quenched fluorescence of cypate can be recovered after removal of the chelated Cu(II) ion from the dye molecule. In future work, we will study the reversible nature of Cu(II) induced quenching of NIR fluorescence of organic dyes such as cypate. We also plan to evaluate the effect of copper quenching on fluorescence lifetime of the dye. If successful, this strategy could be used to design fluorophore–quencher pair based molecular probes for applications in biological imaging using both fluorescence and fluorescence lifetime imaging.

5. ACKNOWLEDGMENT

This study was supported by grants from the National Institutes of Health (NIBIB R01 EB008111 and NCI R01CA171651). D.M. is supported by the NIH 21st Century Imaging Training Grant (T32 EB014855).

6. REFERENCES

[1] Domaille D.W., Que, E.L., Chang, C,J., "Synthetic fluorescence sensors for studying the cell biology of metals," Nat. Chem. Biol., 4(3), 168–75 (2008).
[2] Rurack, K., "Flipping the light switch 'on' –the design of sensor molecules that show cation-induced fluorescence enhancement with heavy and transition metal ions," Spectrochim. Acta A Mol. Biomol. Spectrosc., 57(11), 2161–95 (2001).
[3] Peng, R., Wang, F., Sha, Y., "Synthesis of 5-dialkyl(aryl)aminomethyl-8-hydroxyquinoline dansylates as selective fluorescent sensors for Fe^{3+}," Molecules., 12(5), 1191-201 (2007).
[4] Liu, Z.C., Yang, Z.Y., Li, T.R., Wang, B.D., Li, Y., Qin, D.D., Wang, M.F., Yan, M.H., "An effective Cu(II) quenching fluorescence sensor in aqueous solution and 1D chain coordination polymer framework," Dalton Trans. 40(37), 9370-3 (2011).
[5] Mikata, Y., Sato, Y., Takeuchi, S., Kuroda, Y., Konno, H., Iwatsuki, S.,"Quinoline-based fluorescent zinc sensors with enhanced fluorescence intensity, Zn/Cd selectivity and metal binding affinity by conformational restriction," Dalton Trans., 42(26), 9688-98 (2013).
[6] Berezin, M. Y., Guo, K., Teng, B., Edwards, W. B., Anderson, C. J., Vasalatiy, O., Achilefu, S., "Radioactivity-synchronized fluorescence enhancement using a radionucleitide fluorescence-quenched dye," J Am Chem Soc. 131(26), 9198–200 (2009).
[7] Brunner, J., Kraemer, R., "Copper(II) quenched oligonucleotide probes for fluorescence DNA sensing," J Am Chem Soc. 126(42), 13626-7 (2004).
[8] Rahimi, Y., Goulding, A., Shrestha, S., Mirpuri, S., Deo, S. K. "Mechanism of copper induced fluorescence quenching of red fluorescent protein, DsRed," Biochem Biophys Res Commun., 370(1), 57-61 (2008).
[9] Chen, Y., Rosenzweig, Z., "Luminescent CdS quantum dots as selective ion probes," Anal Chem. 74(19), 5132–8 (2002).
[10] Kotagiri, N., Niedzwiedzki, D. M., Ohara, K., & Achilefu, S., "Activatable probes based on distance-dependent luminescence associated with Cerenkov radiation," Angew. Chem. Int. Ed. Engl. 52(30), 7756–60 (2013).
[11] Achilefu, S., Dorshow, R.B., Bugaj, J.E., Rajagopalan, R., "Novel receptor-targeted fluorescent contrast agents for in vivo tumor imaging," Invest Radiol., 35(8), 479-85 (2000).
[12] Mash, H.E., Chin, Y.P., Sigg, L., Hari, R., Xue, H., "Complexation of copper by zwitterionic (good) buffers," Anal Chem. 75(3), 671-7 (2003).