

Zebrafish imaging and two-photon fluorescence imaging using ZnSe quantum dots

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Zebrafish imaging and two-photon fluorescence imaging using ZnSe quantum dots*

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This study is to report a ZnSe quantum dot with a large two-photon absorption cross section and good biocompatibility, which can be used in bioimaging. Fluorescence emission at 410 nm is observed in the quantum dot under 760-nm laser excitation. These biocompatible quantum dots exhibit a two-photon cross-section of 9.1×10^5 GM (1 GM = 10^{-50} cm^4·s/photon). Two-photon excited laser scanning microscopic images show that cells co-cultured with ZnSe quantum dots are found in the blue channel at a fluorescence intensity that is 14.5 times that of control cells not co-cultured with quantum dots. After incubating zebrafish larvae with ZnSe quantum dots for 24 h, the fluorescence intensity of the yolk sac stimulated by ultraviolet light is 2.9 times that of the control group. The proposed material shows a great potential application in biological imaging.

Keywords: ZnSe quantum dots, two-photon absorption, biological imaging, zebrafish

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1. Introduction

Semiconductor quantum dots (QDs) have attracted much attention in recent years due to their unique optical properties.[1–3] The QDs have good chemical stability and can adapt to environments with large variations in pH range.[4] By adjusting their composition and size, QDs can emit the lights with various colors to supplement the green autofluorescence of living organisms.[5,6] Therefore, high-quality imaging can be performed using the fluorescence of quantum dots for biological imaging. The use of two-photon absorption (TPA) imaging is an important direction for quantum dot biological imaging. The TPA process is a third-order nonlinear optical process, which refers to the process in which two photons are simultaneously absorbed by an electron from the ground state to the excited state under the excitation of strong light.[7] The TPA fluorescence materials used in biological imaging have the following advantages: significantly improving the depth and spatial resolution of imaging the fluorescence of biological samples,[8–10] reducing the degree of damage to biological tissues,[11,12] and improving the image signal-to-noise ratio.[13] The effect of TPA fluorescence bioimaging depends on the TPA absorption cross section and the biocompatibility of the fluorescent probe.[14–16] Quantum dots have better biocompatibility and photochemical stability than other TPA materials, such as organic materials.[17,18] These characteristics indicate that QDs have a great potentional application in biological imaging. However, reports on TPA luminescence of QDs, showing that QDs suitable for biological imaging have a large TPA cross section, are lacking. The QDs with a large TPA cross section can help obtain clear images by low-power laser excitation and reduce the cell damage caused by the laser.

In this study, ZnSe QDs with upconversion blue fluorescence emission properties and good biocompatibility which can be used in vitro bioimaging are explored. These ZnSe QDs are used for TPA biological microscopic imaging to obtain images with high brightness. The water-soluble ZnSe QDs are applied to cell and zebrafish imaging. The uptake of ZnSe QDs by HepG2 cells is high, 7.19×10^7 for each cell. Under these conditions, the viability of HepG2 cells is more than 90%. The viability of zebrafish larva after being exposured to 0.8-μM ZnSe QDs for 24 h is 90%. These results indicate that ZnSe QDs have good biocompatibility and low biotoxicity. Under the excitation of a 760-nm femtosecond pulsed laser, the ZnSe QDs exhibit a strong TPA effect, and the TPA cross section is 9.1×10^5 GM. The TPA fluorescence is 410 nm in the visible range. These characteristics indicate that ZnSe QDs are suitable for biological imaging. Laser scanning microscopy images of TPA excitation show that HepG2

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cells incubated with ZnSe QDs produce bright blue TPA fluorescence, and the brightness is 14.5 times that of the control group. The images of zebrafish larvae incubated with ZnSe QDs for 24 h are also studied. Under the stimulation of ultraviolet light, the bright blue fluorescence of the experimental zebrafish larva incubated with ZnSe QDs is observed. The fluorescence intensity of yolk sac is 2.9 times that in the control group. This result opens a new avenue for developing highly effective multiphoton absorbing materials for biological imaging.

2. Materials and methods

The details of the synthesis, characterization, and biocompatibility of the ZnSe QDs and the experiment setup can be seen in supplementary materials.[19–21]

3. Results and discussion

3.1. Morphology and structure of ZnSe QDs

The morphology and structure of ZnSe QDs are characterized by using TEM (Fig. S1 in supplementary materials). The average diameter is about 4.7 nm. The corresponding crystal face indices of the polycrystalline diffraction ring in the selected area electron diffraction SAED pattern are (111), (220), and (311), which are consistent with the crystal face indices of the cubic blende structure of the bulk ZnSe materials.[22] This finding indicates that water-soluble ZnSe QDs has a cubic blende structure.

3.2. Optical properties of ZnSe QDs

Figure 1(a) shows the ultraviolet–visible absorption spectrum and fluorescence spectrum of ZnSe QDs. The absorption range of ZnSe QDs could be as long as 400 nm. The ZnSe QDs produce 410-nm blue fluorescence under the excitation of 380 nm, which shifts about 50 nm compared with the intrinsic fluorescence (460 nm) of the bulk ZnSe material.[23] The blue shift is caused by the quantum confinement effect of QDs.[24] The fluorescence peak at 410 nm is attributed to the band edge radiation or the excite radiation.[25] Besides, a relatively weak fluorescence emission peak is observed at 500 nm due to the presence of various defect states on the surface or inside of ZnSe QDs.[26]

Based on the result of absorption spectrum, afemtosecond laser of 760-nm is used to excite the ZnSe QDs and the upconversion fluorescence is observed. The fluorescence peak is 410 nm, which is consistent with the corresponding single-photon fluorescence emission peak (Fig. 1(b)). Fluorescence spectra with different laser power values are measured to study the mechanism of the upconversion fluorescence. The results are plotted in logarithms further for understanding the mechanism of fluorescence in ZnSe QDs. The logarithm of the intensity and the laser power exhibit a linear relationship as shown in Fig. 1(c). The fitted k slope is 1.91, indicating that the upconversion fluorescence of ZnSe QDs is the TPA excitation fluorescence.[27] The TPA fluorescence lifetime of ZnSe QDs is 1.83 ns.

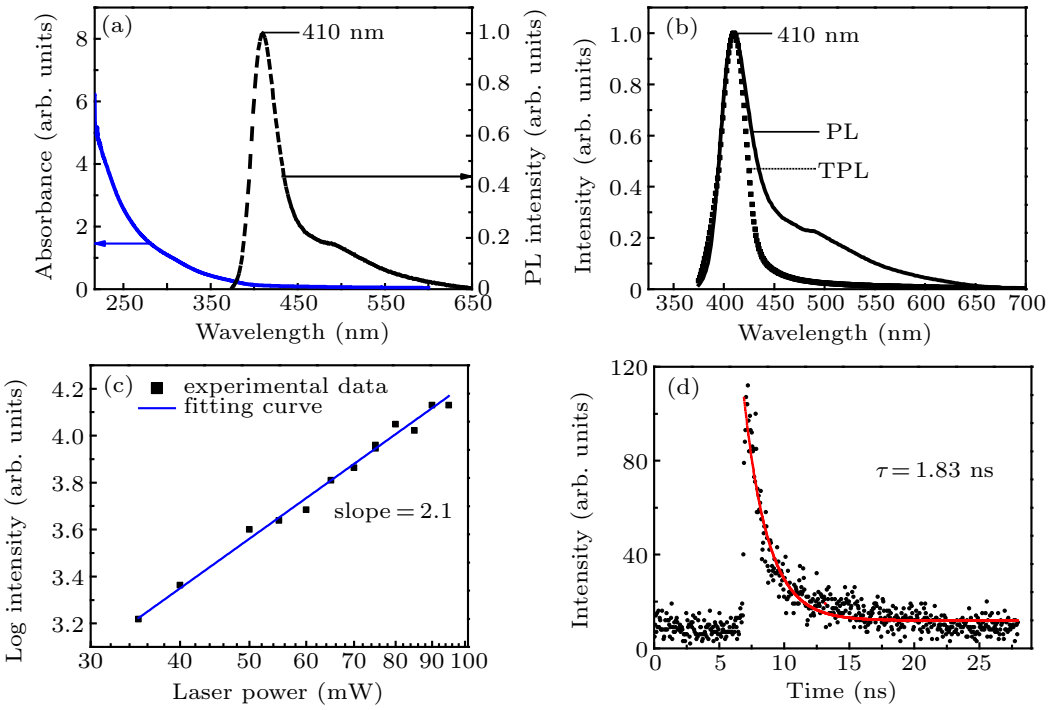


Fig. 1. (a) Absorption and fluorescence spectrum of the ZnSe quantum dots, with solid line and dash line representing absorption spectrum and fluorescence spectrum, respectively. (b) Fluorescence and TPA excited fluorescence spectrum of ZnSe quantum dots, with the solid line and dash dotted linedenoting fluorescence spectrum and TPA excited fluorescence spectrum, respectively. (c) Power dependence of TPA excited fluorescence intensity of ZnSe quantum dots on 760-nm excitation power. (d) TPA excited luminescence decay curves (dots) of the ZnSe quantum dots solution and the best fitting curves (red line).

Table 1. Optical property of ZnSe quantum dots.

Sample	λ_{ex}^{a} (nm)	λ_{ex}^{b} (nm)	λ_{em}^{c} (nm)	λ_{em}^{d} (nm)	Φ (%)	σ_2 (GM)
DAPI	320	–	460	–	4.5	–
R6G	–	760	–	553	95	31.5
ZnSe	320	760	410	430	0.22	757[e], 9.1×10^5 [f]

[a]One-photon excitation wavelength, [b]two-photon excitation wavelength, [c]emission wavelength of DAPI and ZnSe QDs under excitation of 320 nm, [d]emission wavelength of R6G and ZnSe QDs under excitation of 760 nm, [e]results corresponding to molecular concentration, [f]results corresponding to particle concentration.

The excitation peak and emission peak of DAPI are 345 nm and 475 nm, respectively, which are close to those of ZnSe QDs. Therefore, the The photoluminescence quantum yields (PL QYs) of ZnSe QDs are measured by using DAPI as the reference solution. The PL QY of ZnSe QDs is around 4.9% of that of the fluorescent dye DAPI, or an absolute QY of 0.22%. Using R6G as a reference dye, the TPA cross section of ZnSe QDs is measured by a TPA-induced fluorescence method. Comparing with other reported quantum dots,[28] the absorption cross section of this quantum dot is relatively high, reaching 9.1×10^5 GM per QD (757 GM for molecule, the detail can be seen in part 2 of Supplementary material). Although the PL QY of this kind of quantum dot is very low, the strong TPA characteristics make it potentially useful in biological imaging. The PL QYs and the TPA cross section of ZnSe QDs are shown in Table 1.

3.3. Biocompatibility of ZnSe QDs in HepG2 Cells

Toxicity is considered when ZnSe QDs are applied to cellular imaging. Different concentrations of ZnSe QDs are incubated with HepG2 cells for 24 h, and the viability is measured by using the MTT assay. As shown in Fig. 2. When the concentration of ZnSe QDs is 0.8 μM, the cell viability is still 92%, indicating that this concentration is not toxic to HePG2 cells.[28–31] The morphology of HepG2 cells changes little within 24 h when the particle concentration is less than 0.8 μM (Fig. S2).

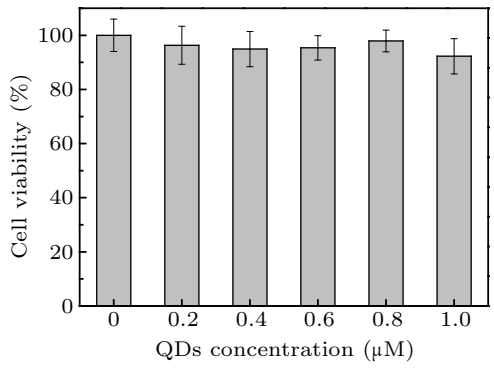


Fig. 2. Viability of HepG2 cells with different concentrations of ZnSe quantum dots, measured by MTT assay.

The TEM images are taken after incubating the cells with ZnSe QDs for 24 h to determine the cellular uptake and the distribution of ZnSe QDs in HepG2 cells. Figures 3(a) and 3(b) show that the ZnSe QDs enter into the organelle and the nanoparticle clusters form naturally. Figure 3(c) shows the energy-dispersive x-ray spectrum (EDS) of QD clusters inside the cell. The map shows that the nanoparticles in the cells are ZnSe QDs. The O, Pb, and other elements, apart from Zn and Se, are found in the EDS map due to additional chemicals and nickel meshes used for preparing cell slices.

The cellular internalization process is crucial in the bioimaging of nanoparticles. The cellular uptake in a solution with a particle concentration of 0.8 μM is measured by using ICP-MS. The molar mass for 4.7-nm ZnSe QDs is 1.774×10^5 g/mol. The uptake of a single HepG2 cell for 4.7-nm ZnSe QDs is 2.12×10^{-11} g. Therefore, the intake for each cell is about 7.19×10^7 ZnSe QDs.

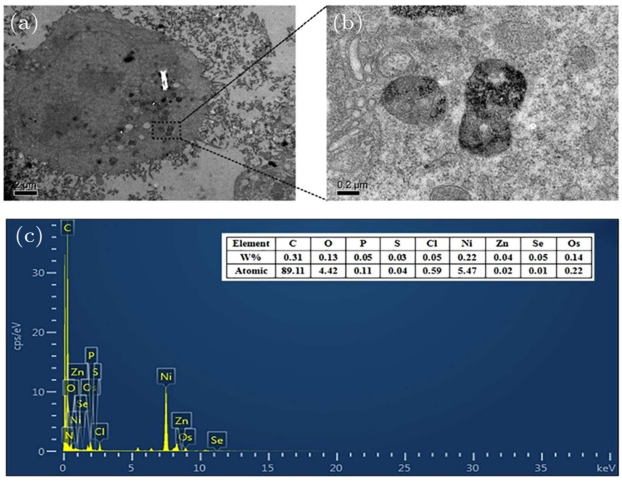


Fig. 3. (a) TEM image of HepG2 cell incubated with ZnSe quantum dots for 24 h. (b) Enlarged TEM image of ZnSe quantum dots naturally created in lysosome of HepG2 cell. (c) EDS spectrum of ZnSe quantum dots inside HepG2 cell.

3.4. TPA excitation cellular imaging of ZnSe QDs

In this paper, the application of ZnSe quantum dots in TPA fluorescence bioimaging ios also discussed. After incubating HepG2 cells with 0.8-μM ZnSe QDs in a culture dish for 24 h, the cells are washed three times with PBS. The experimental group is cells incubated with ZnSe QDs, and the control group is cells incubated without ZnSe QDs. Subsequently, The TPA fluorescence images are obtained by scanning them with a two-photon scanning microscope excited by a 760-nm femtosecond pulse laser. The structure of the two-photon microscope system is shown in Fig. S3. Figures 4(a)–4(d) show the bright-field image, images of the green and blue

channels, and the combination image of green and blue channels of the control group, respectively. Figures 4(e)–4(h) show the bright-field image, images of the green and blue channels, and the combination image of the experimental group, respectively. When cells are excited by a 760-nm femtosecond pulsed laser, the TPA fluorescence intensity of the cells is recorded with the photomultiplier tube of each channel in the microscope. Therefore, the TPA excited cellular imaging is obtained. The bright blue fluorescence is observed in the experimental group, indicating that the ZnSe QDs enter into the cells, while the blue fluorescence intensity is very weak in the control group.[32] The fluorescence brightness of blue channel of the labelled cells in Fig. 4 is listed in Table 2. The fluorescence brightness in the blue channel of cell 5 is 14.5 times higher than that of cell 3. These results indicate that the water-soluble ZnSe QDs are suitable for the TPA fluorescence cellular imaging.

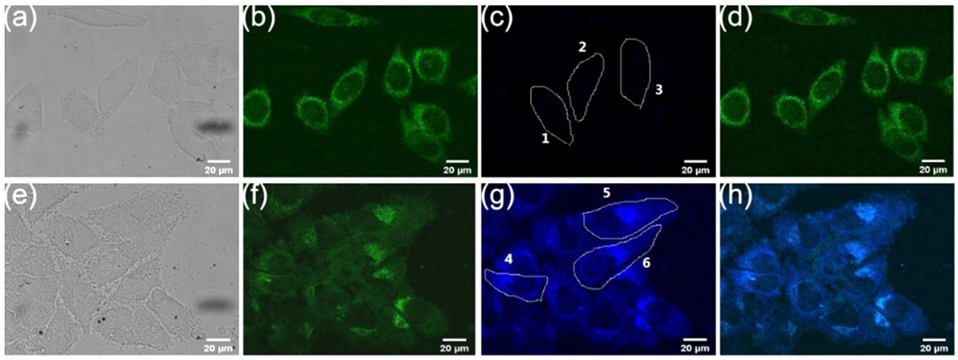


Fig. 4. Images of control group HepG2 cells without culturing with ZnSe quantum dots: (a) bright field image, (b) TPA fluorescence image with green channel, (c) blue channel, (d) the combination image of geen and blue channels. Images of HepG2 cells incubated with ZnSe quantum dots for 24 h: (e) bright field image, (f) TPA fluorescence images with green channel, (g) blue channel, and (h) combination image of geen and blue channel. Scale bars correspond to 20 μm.

Table 2. Fluorescence brightness in blue channel of selected cells.

Sample	Area (cell)	Intensity (min)	Intensity (max)	Intensity (mean)
1	1	0	3	0.69
2	1	0	3	1.06
3	1	0	3	1.01
4	1	4	34	14.05
5	1	4	34	14.51
6	1	4	33	14.35

3.5. Biological imaging of ZnSe QDs in zebrafish larva

After exploring that ZnSe QDs significantly enhance the TPA fluorescence of cells, a series of experiments are performed to investigate the application of ZnSe QDs in zebrafish imaging. The zebrafish larvae with rapid embryonic development, short growth time, simple incubation, and transparent body is chosen as model organism[33–35] to study the zebrafish imaging of ZnSe QDs. Figure 5 shows a fluorescence image of zebrafish larvae exposed to ZnSe QDs. Figures 5(a) and 5(b) show the bright-field image and fluorescence image of zebrafish larvae in the control group incubated without ZnSe QDs, respectively. Figures 5(c) and 5(d) show the bright-field image and fluorescence image of zebrafish larvae in the experimental group incubated with 0.8-μM ZnSe QDs for 24 h. The morphology of zebrafish larvae in the bright-field imaging of the experimental group as shown in Fig. S4 does not significantly change compared with that in the control group. The viability of zebrafish larvae after being exposured to ZnSe QDs for 24 h is 90% (Fig. S5). These two results indicate that 0.8-μM ZnSe QDs are safe for zebrafish larvae. The fluorescence images of zebrafish larvae are obtained by selecting the light range of 300–410 nm as an excitation source for the fluorescence stereomicroscope. Comparing Fig. 5(b) with Fig. 5(d), it is found that the zebrafish larvae incubated with ZnSe QDs emits the bright blue fluorescence, while the zebrafish larvae incubated without QDs (control group) produces the weak blue fluorescence. As shown in Table 3, the average brightness of blue fluorescence produced in the control group is 15.4 and the maximum brightness is 33. The zebrafish larvae incubated with ZnSe QDs produces the blue fluorescence

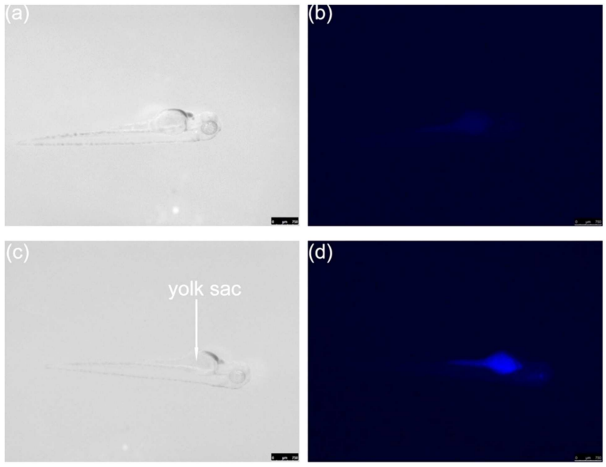


Fig. 5. (a) Bright field image of zebrafish larva incubated without QDs, (b) fluorescent image of zebrafish larva incubated without QDs, (c) bright field image of zebrafish larva incubated with ZnSe QDs, and (d) fluorescent image of zebrafish larva incubated with ZnSe QDs. Scale bar is 750 μm in size.

with an average brightness of 25.6 and a maximum brightness of 86. This finding indicates that the ZnSe QDs enter into the zebrafish larvae exposed to the ZnSe QDs. The average brightness of the yolk sac of zebrafish larvae incubated with ZnSe QDs is 58.1, which is 2.9 times of the blue fluorescence brightness of yolk sacs of zebrafish larvae incubated without QDs (19.9). This observation indicates that the ZnSe QDs mainly assemble in the yolk sac of zebrafish larvae after ingestion.

The thioglycolic acid (TGA) is used as the stabilizer in the synthesis of quantum dots. For TGA, the mercapto group binds to the Zn atom on the surface of QDs, and the polar carboxylic acid group renders the QDs water-soluble. The free carboxyl group is also available for covalent coupling to various biomolecules (such as proteins). After these ZeSe QDs enter into the zebrafish larvae, they are likely to bind to yolk proteins in the yolk sac. So the yolk sac appears bright blue.

Table 3. Fuorescence brightness of zebrafish larva incubated without QDs.

Sample	Intensity (min)	Intensity (max)	Intensity (mean)
Zebrafish larva in Fig. 5(b)	5	33	14.5
Zebrafish larva in Fig. 5(d)	6	86	25.5
Yolk sac of zebrafish larva in Fig. 5(b)	8	33	19.9
Yolk sac of zebrafish larva in Fig. 5(d)	19	86	58.1

4. Conclusions

In this study, ZnSe QDs with good biocompatibility and strong TPA properties are synthesized by the hydrothermal method. The ZnSe QDs have a TPA cross section of about 9.1×10^5 GM. After 24-h-incubation with 0.8-μM ZnSe QDs, the viability of HepG2 cells is still more than 92%. The uptake of ZnSe QDs by a single cell is 7.17×10^7 particles. When the cells are excited by the 760-nm femtosecond laser, the TPA fluorescence images of cells are obtained by using a laser scanning microscope. The experimental group cells co-cultured with ZnSe QDs produce bright blue TPA fluorescence, and the brightness of fluorescence is 14.5 times that of the control cells incubated without QDs. The morphology of zebrafish larvae does not change significantly after being incubated with 0.8-μM ZnSe QDs for 24 h. Under the stimulation of ultraviolet light, bright blue fluorescence is observed in the zebrafish larvae incubated with ZnSe QDs. These results opened up a new way for developing new biological imaging materials.

References

[1] Guo H M, Wen J W, Xue X G and Ma H L 2008 *Chin. Phys. B* **17** 1280
[2] Li shuo, Shi L and Yan Z W 2020 *Chin. Phys. B* **29** 097802
[3] Qin Y D, Cai W H, Zeng L X, Feng Q J and Wei F 2010 *Chin. Phys. B* **19** 017304
[4] Kaur G and Tripathi S K 2014 *Mater. Chem. Phys.* **143** 514
[5] Liu Q, Guo B, Rao Z, Zhang B and Gong J R 2013 *Nano Lett.* **13** 2436
[6] Han M Y, Gao X H, Su J Z and Nie S 2001 *Nat. Biotechnol.* **19** 631
[7] Dyer J, Jockusch S, Balsanek V, Sames D and Turro N J 2005 *J. Org. Chem.* **70** 2143
[8] Maestro L, Ramirez-Hernandez J, Bogdan N, Capobianco J, Vetrone F, Solé J G and Jaque D 2012 *Nanoscale* **4** 298
[9] Miller D R, Hassan A M, Jarrett J W, Medina F A, Perillo E P, Hagan K, Shams Kazmi S M, Clark T A, Sullender C T, Jones T A, Zemelman B V and Dunn A K 2017 *Biomed. Opt. Express* **8** 3470
[10] Perillo E P, McCracken J E, Fernée D C, Goldak J R, Medina F A, Miller D R, Yeh H C and Dunn A K 2016 *Biomed. Opt. Express* **7** 324
[11] Denk W, Strickler J H and Webb W W 1990 *Science* **248** 73
[12] Helmchen F and Denk W 2005 *Nat. Methods* **2** 932
[13] Piston D W 1999 *Trends Cell Biol.* **9** 66
[14] Zhao W, Ribeiro R M and Eda G 2014 *Acc. Chem. Res.* **48** 91
[15] Ray P C 2010 *Chem. Rev.* **110** 5332
[16] Chinen A B, Guan C M, Ferrer J R, Barnaby S N, Merkel T J and Mirkin C A 2015 *Chem. Rev.* **115** 10530
[17] Xu G, Zeng S, Zhang B, Swihart M T, Yong K T and Prasad P N 2016 *Chem. Rev.* **116** 12234
[18] Sharma A, Gadly T, Gupta A, Ballal A, Ghosh S K and Kumbhakar M 2016 *J. Phys. Chem. Lett.* **7** 3695
[19] Sureshkumar S, Jothimani B, Sridhar T M and Venkatachalapathy B 2016 *RSC Adv.* **6** 16081
[20] Zhou C, Song J, Zhou L, Zhong L, Liu J and Qi Y 2015 *J. Lumin.* **158** 176
[21] Zhang N N, Liu X, Wei Z C, Liu H Y, Peng J, Zhou L Y, Li H M and Fan H H 2019 *Nanomaterials* **9** 369
[22] Yang C, Feng G, Dai S, Wang T, Li G, Zhang H and Zhou S 2017 *Appl. Surf. Sci.* **414** 205
[23] Zhao B, Yao Y, Yang K, Rong P, Huang P, Sun K and Li W 2014 *Nanoscale* **6** 12345
[24] Sharma A, Gadly T, Gupta A, Ballal A, Ghosh S K and Kumbhakar M 2016 *J. Phys. Chem. Lett.* **7** 3695
[25] Xing G, Ji W, Zheng Y and Ying J Y 2008 *Opt. Express* **16** 5715
[26] Yang C C and Li S 2008 *J. Phys. Chem. C.* **112** 2851
[27] Lu J F, Xu C X, Dai J, Li J T, Wang Y Y, Lin Y and Li P L 2015 *Nanoscale* **7** 3396
[28] Subha R, Nalla V, Yu J H, Jun W J, Shin K S, Hyeon T W, Vijayan C and Ji W 2013 *J. Phys. Chem. C* **117** 20905
[29] Mirnajafizadeh F, Ramsey D, Mcalpine S, Wang F, Reece P and Stride J A 2016 *Mater. Sci. Eng. C* **64** 167
[30] Derfus A M, Chan W C W and Bhatia S N 2004 *Nano Lett.* **4** 1118
[31] Xu Z, Wu Z, Sun J and Gui R 2015 *Mater. Chem. Phys.* **162** 286
[32] Ohulchanskyy T Y, Roy I, Yong K T, Pudavar H E and Prasad P N 2010 *Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol.* **2** 162
[33] Drummen G P C 2010 *Int. J. Mol. Sci.* **11** 154
[34] Weber T and Köster R 2013 *Methods* **62** 279
[35] Lee K J, Nallathamby P D, Browning L M, Osgood C J and Xu X H N 2007 *ACS Nano* **1** 133