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2013

Subha, R., Nalla, V., Yu, J. H., Jun, S. W., Shin, K., Hyeon, T., Vijayan, C., & Ji, W. (2013). Efficient Photoluminescence of Mn²⁺-Doped ZnS Quantum Dots Excited by Two-Photon Absorption in Near-Infrared Window II. *The Journal of Physical Chemistry C*, 117(40), 20905-20911.

<https://hdl.handle.net/10356/107466>

<https://doi.org/10.1021/jp404124c>

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[DOI:<http://dx.doi.org/10.1021/jp404124c>].

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Efficient Photoluminescence of Mn^{2+} -Doped ZnS Quantum Dots Excited by Two-Photon Absorption in Near-IR Window II

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ABSTRACT.

Highly fluorescing biological labels with excitation in near-infrared window II have attracted the interest of scientific community as they are capable of increasing both penetration depth and imaging quality. However, studies on the utilization of quantum dots (QDs) in biological imaging appear to be rather limited to the near-infrared window I (NIR-I: 650 to 950 nm). We herein report on the observation of efficient photoluminescence (PL) in Mn^{2+} -doped ZnS QDs excited by two-photon absorption (2PA) in near-infrared window II (NIR-II: 1000 to 1350 nm). Multi-photon-absorption-induced PL measurements indicate that these biocompatible QDs exhibit a two-photon action cross-section of 265 GM at 1180 nm, the highest value reported to date among conventional fluorescent probes on excitation in NIR-II. This value is one to two orders of magnitude higher than that for organic dye molecules excited by NIR-I photons and three to four times greater than that of fluorescent proteins excited in the NIR-II. The underlying NIR-II excitation mechanism for the Mn^{2+} emission at 586 nm on account of the $^4\text{T}_1\text{-}^6\text{A}_1$ transition is attributed to the transitions from the valance subband of ZnS QDs (or ground states of Mn^{2+} ions) to the excited states of Mn^{2+} ions by direct two-photon absorption. Transient PL measurements reveal single exponential decay with a PL lifetime of 0.35 ± 0.03 ms irrespective of excitation wavelength, which are four to five orders longer than that of conventional fluorescent probes. With the excitation in NIR-II window and the unique combination of photo-physical properties such as a greater two-photon action cross-section, longer PL lifetime and larger anti-Stokes shift (450 nm or more), Mn^{2+} -doped ZnS QDs appear to be a promising candidate for deep tissue imaging applications.

Keywords: Mn²⁺-doped ZnS QDs, Multi-photon excited photoluminescence, Two-photon fluorescent probes, Fluorescence life time, Near-IR excitation

INTRODUCTION

Two-photon microscopy has emerged as a powerful tool for “deep tissue imaging” applications in recent years.^{1, 2} In this context, performance of two-photon microscopy depends greatly on fluorescent probes that penetrate deep into tissues and are capable of making efficient fluorescence through two-photon absorption (2PA). Various types of chromophores have been widely investigated as biological probes, ranging from organic dyes, metal-ligand complexes, fluorescent proteins, to semiconductor quantum dots (QDs).³ Among these, semiconductor QDs exhibit outstanding features such as tunable excitation wavelength, narrow and symmetric emission, high fluorescence quantum yield, considerably large 2PA cross-section and good photostability, making it an excellent candidate for two-photon imaging.⁴⁻⁶ Despite their interesting properties, cadmium-based semiconductor QDs (CdS, CdSe, CdS/ZnS, etc.) are not suitable for bio-imaging since they release cadmium and reactive oxygen species on photo oxidation, leading to cytotoxicity.^{7, 8}

In addition, most of the two-photon microscopic imaging was carried out with laser excitation in the spectral range from 700 to 1000 nm. Recently, investigations show that in addition to the

light absorption, scattering of incident photons has a strong influence on the penetration depth.⁹ This is due to the high probability of light scattering in biological tissues, mainly because of the difference in refractive index offered by the heterogeneous mixture of molecules and cell organelles, making even a coherent laser beam incoherent within an imaging length. Only ballistic (non-scattered) photons can cause signal generation in the laser focused volume. The ballistic power varies with the imaging depth z as e^{-z/l_s} where l_s is the scattering length. Since the fluorescence signal due to two-photon absorption has quadratic dependence on intensity, the fluorescence signal varies as $(e^{-z/l_s})^2$ which is equal to $(e^{-2z/l_s})^9$. Light scattering in biological tissues has been found to scale with the excitation wavelength (λ_{exc}) as λ_{exc}^{-2} approximately, indicating that a longer wavelength is more desirable for minimizing light scattering in bio-imaging.^{10, 11} Consequently, a new transparency window called Near-Infrared window II (NIR-II) in the range from 1000 nm to 1350 nm has been proposed for bio-imaging applications with the aim of reducing scattering and minimizing autofluorescence in biological tissues.^{12, 13} Beyond 1350 nm, light absorption of water prevents from decent penetration lengths.¹⁴ Enhanced penetration depth has been successfully demonstrated in liver tissues, lymph nodes and brain cells at 1070 nm, 1110 nm and 1280 nm.^{11, 15, 16} Recent studies on carbon nanotubes report that NIR-II imaging can also improve the spatial resolution of the image down to 30 μ m, making it possible to image blood vessels, taking advantage of the reduced scattering of NIR-II photons.¹⁷ Also, the greater difference between excitation wavelength and PL emission wavelength should readily result in a higher signal-to-noise ratio, leading to quality imaging.³

Fluorescent proteins are promising candidate materials in this regard as they exhibit TPA in NIR-II and emission in the orange and red region of the visible spectrum. Drobizhev *et al.*

suggested tdTomato as the best alternative among the fluorescent proteins with a two-photon action cross-section of 60 GM (or 120 GM per protein chain) in the range between 1000 and 1100 nm.^{18, 19} However, both photostability and photobleaching of these probes limit its usage in two-photon microscopy with excitation of high-repetition-rate laser pulses and long data acquisition time.¹⁹

To address the above-discussed issues, we believe that 2PA-induced fluorescent ZnS QDs doped with Mn^{2+} ions having excitation in the wavelength range from 1050 nm to 1300 nm (NIR-II) and emission at 586 nm would be an ideal candidate. ZnS is a low cytotoxic material with an extremely low absorption cross-section in NIR-II. Doping of Mn^{2+} ions is known to enhance the PL quantum yield of ZnS QDs to 65% with emission in the range of 585-600 nm, depending on the parameters such as QD size, synthesis methods, concentrations of Mn^{2+} ions, etc.^{20, 21} Very recently, Mn^{2+} -doped ZnS QDs were found to be suitable for high-resolution cellular imaging and *in vivo* tumour-targeting imaging under non-invasive conditions by utilizing their three-photon-absorption-induced PL characteristics at 920~950 nm.²² In view of its interesting properties of biocompatibility, photostability and high-resolution *in vivo* imaging capability in NIR-I window reported elsewhere²², we are extending our studies in Mn^{2+} -doped ZnS QDs to NIR-II window where the excitation wavelength is capable of achieving larger imaging depth than NIR-I photons. In this work, the luminescence characteristics of the emission at 586 nm owing to $^4\text{T}_1\text{-}^6\text{A}_1$ transition in Mn^{2+} -doped ZnS QDs were investigated using multi-photon absorption induced PL emission measurements and transient PL lifetime measurements on NIR-II excitation. We have observed a PL lifetime of the order of 0.35 ± 0.03 ms in these Mn^{2+} -doped ZnS QDs, which is four to five orders of magnitude longer than that of the fluorescent proteins and other chromophores. Longer lifetimes enhance the temporal

discrimination of the signal from the auto-fluorescence background.³ Apart from this, these QDs are found to exhibit a large two-photon action cross-section in NIR-II window with a magnitude of 265 GM, which is one to two orders higher than that for organic dye molecules (NIR-I excitation) and three to four times higher than the above-mentioned fluorescent proteins (37 to 63 GM in the range from 1000 to 1100 nm). Our findings indicate that Mn²⁺-doped ZnS QDs are better alternative materials for bio-imaging in view of their higher two-photon action cross-section on NIR-II excitation, longer PL lifetime, and greater anti-Stokes shift (> 450 nm, difference between excitation and PL wavelength).

RESULTS AND DISCUSSIONS

In this work, ZnS QDs doped with 1.1% Mn²⁺ ions were investigated and the dopant concentration was determined using inductively coupled plasma atomic emission spectroscopy. The TEM measurements (Figure 1a) reveal that the QDs are nearly spherical and monodisperse with an average particle size of 5.5 nm and a size dispersion of 0.5 nm. The measured UV-Visible absorption spectrum shows resolvable one-photon absorption induced optical transitions from valence subbands to conduction subbands of ZnS QDs, depicted in Figure 1b, where we fit with three Gaussian profiles, with the lowest peak at 318 nm and second lowest peak at 293 nm corresponding to the excitonic transitions of $1S(e)-1S_{3/2}(h)$ and $1S(e)-2S_{3/2}(h)$, respectively. These transitions are illustrated in the energy level diagram of Mn²⁺-doped ZnS QDs documented in Figure 2 (transitions (a) and (b)) where the continuous energy bands of the bulk counterpart are replaced by discrete energy levels arising from quantum confinement effect in QDs. With the measured excitonic transitions, we carried out theoretical calculations based on the effective mass approximation and realistic tight binding method,²³ and the average size of the

QDs was found to be 5.5 nm if the theory fit to the above measured spectrum. Furthermore, the size dispersion was found to be around 18% by Gaussian fitting of the excitonic peak of width of 210 meV, consistent with the HRTEM results.

Doping introduces new levels within the bandgap, modifying the linear photo-physical properties of ZnS QDs, particularly the photoluminescence of ZnS QDs. One-photon-induced PL spectra in Mn^{2+} -doped ZnS QDs (Figure 1c) shows emission peaked around 586 nm, attributed to the transition from Mn^{2+} excited state ($^4\text{T}_1$) to Mn^{2+} ground state ($^6\text{A}_1$).^{24, 25} The emission wavelength implies that Mn^{2+} ions are doped inside the nanocrystal volume and not on the surface.²⁶ Intrinsic PL emission in undoped ZnS QDs peaked around 438 nm (due to the vacancy of S^{2-} ions in undoped ZnS QDs) was quenched in Mn^{2+} -doped ZnS QDs. This shows that there is a strong interaction between the d -electron states of Mn^{2+} ions and the s - p states of ZnS host lattice, providing an effective channel for the transfer of electrons and holes leading to the subsequent emission in Mn^{2+} -doped ZnS QDs. The photoluminescence excitation (PLE) spectrum depicted in Figure 1c indicates that the one-photon absorption is dominated by the transition across the valence and conduction subbands of ZnS QDs.

PL lifetime is a major factor impeding the signal detection and hence we have performed transient PL measurements for excitation in the near-IR wavelength region. The time-resolved PL emission observed in Mn^{2+} -doped ZnS QDs on excitation at 900-1100 nm is depicted in Figure 3a. The results suggest that the lifetime for PL emission at 586 nm in Mn^{2+} -doped ZnS QDs is independent of excitation wavelength. This is expected since the emission is always due to the transition from $^4\text{T}_1$ state to $^6\text{A}_1$ state of Mn^{2+} ions. As shown in Figure 3b, the decay curves are fitted with the exponential function $Ae^{-t/\tau}$ and $\tau = 0.35 \pm 0.03$ ms. Such a long lifetime is owing to the fact that the transition between Mn^{2+} states is a partially allowed transition, due to

spin-orbit coupling and *sp-d* mixing of the electronic states in Mn^{2+} -doped ZnS QDs.^{24, 25} Our measurement indicates a shortened PL lifetime compared to the decay lifetime of 1.8 ms reported in Mn^{2+} single ion pairs,²⁷ whereas it is comparable to the PL lifetime in ZnS with higher Mn^{2+} ion concentration, similar to previous reports.²⁷ Lifetime of 0.4 ms and 0.09 ms was observed in Mn^{2+} pairs with less effective coupling and Mn^{2+} nearest neighbor coupled pairs with strong coupling respectively, when the concentration is greater than 1%.²⁷ The decrease in lifetime is a result of the increase in the oscillator strength when another Mn^{2+} ion is incorporated even on a distant site. The PL lifetime and the PL emission wavelength observed in our samples indicate effective doping of Mn^{2+} ions inside the QD volume. The observed PL emission lifetime in Mn^{2+} -doped ZnS QDs is four to five orders higher than other fluorescent probes such as dyes (1-10 ns), fluorescent proteins (1-3 ns) and three to four orders higher compared to other semiconductor QDs (10-100 ns).^{3, 18} The longer lifetime of PL and single exponential decay should facilitate data acquisition in bio-imaging, particularly, in the temporal discrimination of the PL emission from the scattered excitation light and auto fluorescence background in the surrounding tissues, thereby enhancing the signal-to-noise ratio.³

To investigate the utilization of these QDs as nanoprobe for deep-tissue imaging, multi-photon-absorption-induced PL emission of Mn^{2+} -doped ZnS QDs in NIR-II was recorded on femtosecond laser excitation in the wavelength range from 1050 nm to 1310 nm. Figure 1d shows the emission spectrum of Mn^{2+} -doped ZnS QDs normalized with the Rhodamine 6G emission in the same experimental set-up under laser excitation at 1060 nm. The emission spectrum on excitation in NIR-II is observed to be similar to one-photon case with peak at 586 nm. The PL emission on multi-photon absorption depends non-linearly on the excitation fluence and hence, to ascertain two-photon-induced or three-photon-induced PL processes, the measured

PL signal was plotted as a function of excitation fluence. The fluence dependence of PL observed in Mn^{2+} -doped ZnS QDs at different wavelengths is illustrated in Figure 4a. It is clear that the PL emission peaked around 586 nm in Mn^{2+} -doped ZnS QDs is higher on excitation at 1180 nm and 1100 nm, compared to that at 900 and 1000 nm, indicating higher PL efficiency on two-photon excitation in NIR-II wavelength photons when compared to three-photon excitation in NIR-I window. Figure 4a also indicates that the emission at 586 nm observed in Mn^{2+} -doped ZnS QDs when excited at NIR-II wavelengths is stronger than the emission from Rhodamine 6G molecules excited at 900 nm. From the fluence dependence of PL, we obtained the slopes, and the observed slope at different wavelengths is displayed in Figure 4b, whereby the error bar results from the standard deviation from several measurements of PL signal for a given excitation fluence in Figure 4a.

Between 900 nm and 1040 nm, the slopes of PL from Mn^{2+} -doped ZnS QDs are close to 3, clearly indicating three-photon absorption (3PA). In this excitation range, corresponding to process “c” in Figure 2, $3\hbar\omega$ energy matches with the difference between the valence and conduction subbands of ZnS QDs, and hence, 3PA is allowed to excite electrons to the conduction subbands of ZnS QDs. Subsequently, these excited electrons relax to $^4\text{T}_1$ states of Mn^{2+} ions due to non-radiative relaxation (see process “f” in Figure 2), leading to PL (see process “e” in Figure 2). Furthermore, because of the same selection rule for 1PA and 3PA, this range also matches with the 1PA spectrum of 300-347 nm.

As the excitation wavelength is increased to the range between 1050 nm and 1310 nm, the slope is reduced to be around 2, which corresponds to the switching of excitation mechanism from 3PA to 2PA. In this case, however, the excitation mechanism is no longer involving the conduction subbands of ZnS QDs. $2\hbar\omega$ energy directly matches with the difference between

Mn^{2+} excited state $^4\text{T}_1$ and valance subband of ZnS QDs (or ground states of Mn^{2+}), as indicated by process “ d ” in Figure 2. It should be pointed out that the selection rule of 2PA is different from that of 1PA, that explains why no 1PA is observed in the range of 525 – 640 nm. So, our studies indicate that in addition to the change in the linear optical properties, doping of Mn^{2+} ions can effectively modify the nonlinear absorption behavior of ZnS QDs as well, thereby enhancing the PL emission in NIR-II window.

A direct measurement of PL brightness is the two-photon action cross-section, which is the product of two-photon absorption cross-section and PL quantum yield. The two-photon-excited PL strength F_2 can be found out by integrating Δf_2 given by $\Delta f_2 = \eta_2 \phi \sigma_2 \rho \cdot ds \cdot dz \cdot I_r^2$, over the entire laser focused volume and time where η_2 is the PL quantum yield, ϕ is the fluorescence collection efficiency of the experimental setup, σ_2 is the two-photon absorption cross-section, ρ is the sample concentration, $ds \cdot dz$ is the small volume of the focused laser beam considered, and I_r is the nearly constant laser intensity at this small volume.²⁸ By assuming Gaussian functions for temporal and spatial profiles of input laser pulses, the total-collected PL signal is given by $F_2 = \frac{\pi^{5/2}}{4} \tau \phi \eta_2 \sigma_2 \rho I_{00}^2 \omega_0^2 z_0$ where τ is the half width of the Gaussian laser pulse, I_{00} is the peak intensity on the beam propagation axis, ω_0 is the beam waist and z_0 is the diffraction length. To calibrate our measurements, we used Rhodamine 6G molecules in methanol (0.1 mM). Two-photon absorption cross-section of Rhodamine 6G molecule (quantum yield 0.95) is reported to be 10 GM ($10^{-49} \text{ cm}^4 \text{ photon}^{-1}$) at 1060 nm.²⁹ Then, the two-photon action cross-section of Mn^{2+} -doped ZnS QDs can be obtained from the ratio of the measured PL from

Rhodamine 6G to Mn^{2+} -doped ZnS QDs ($\frac{F_{2(Rh)}}{F_{2(QD)}} = \frac{(\eta_2 \sigma_2)_{Rh} \cdot \rho_{Rh} \cdot I_{00}^2}{(\eta_2 \sigma_2)_{QD} \cdot \rho_{QD} \cdot I_{00}^2}$) where $(\eta_2 \sigma_2)_{Rh}$ is known

and $F_{2(Rh)}$, the PL intensity is measured at 1060 nm. Figure 5a depicts the measured two-photon action cross-sections for Mn^{2+} -doped ZnS QDs, showing the values of 100 ~ 265 GM with a maximum of 265 GM at 1180 nm.

Even though higher two-photon action cross-section on NIR-I wavelength excitation (650 - 1000 nm) has been reported in Cd-based semiconductor QDs, such as CdTe QDs (150 GM - 8 GM at 840 nm)⁴, CdSe QDs (10^3 - 10^2 GM at 840 nm)⁴, CdSe/ZnS QDs (10^4 GM at 700-1000 nm)³⁰, their nonlinear absorption properties in NIR-II are unknown. Also, high cytotoxicity prevents its use as nanoprobe in biological tissues. The observed two-photon action cross-sections in Mn^{2+} -doped ZnS QDs compare favorably with other chromophores such as organic dye molecules and fluorescent proteins, as illustrated in Figure 5b. It also shows the optical transparency window for the biological tissues, NIR-I and NIR-II constrained by the absorption of melanin and hemoglobin in the shorter wavelength and water absorption in the longer wavelength. It is clear from Figure 5b that the two-photon action cross-section observed in Mn^{2+} -doped ZnS QDs is one to two orders greater than the organic dye molecules and three to four times greater than the fluorescent proteins excited in NIR-II. In addition to the higher two-photon absorption cross-section in the NIR-II window, these QDs also exhibit high anti-Stokes shift. The anti-Stokes shift is defined as the difference between the excitation maximum and the PL emission wavelength. The excitation in the near IR wavelengths from 1050 to 1300 nm with emission near 586 nm indicates an anti-Stokes shift greater than 450 nm, which is highly desirable in two-photon microscopy, as it considerably enhances the signal to noise ratio³.

As discussed previously, one of the major challenges in two-photon microscopy is the prerequisite to keep sufficiently high intensity at the focal plane, since the biological tissues cause scattering of incident photons¹¹. The tactics to solve the problem is shifting the excitation

to NIR-II window and using two-photon excited highly fluorescing biological labels. Our studies indicate that Mn^{2+} -doped ZnS QDs is promising in this regard. In addition to this, NIR-II excitation can also increase the imaging depth and henceforth Mn^{2+} -doped ZnS QDs are excellent candidate materials for “deep tissue” imaging.

CONCLUSION

In summary, we have investigated various photo-physical properties of Mn^{2+} -doped ZnS QDs such as QD brightness (action cross-section) and PL lifetime on excitation in the NIR-II window (1050 nm to 1310 nm). Transient PL measurements of Mn^{2+} -doped ZnS QDs show that emission at 586 nm owing to ${}^4\text{T}_1\text{-}{}^6\text{A}_1$ transition of Mn^{2+} ions has a lifetime of 0.35 ± 0.03 ms, which is almost five to six orders longer than that in other chromophores like organic dyes and fluorescent proteins and four to five orders higher than other semiconductor QDs. Apart from a high anti-Stokes shift of 450 nm between the excitation and emission, these QDs possess high two-photon action cross-section (265 GM per QD) on excitation in the window of NIR-II, the excitation mechanism is attributed to the direct transitions from ZnS QD valence subbands (or Mn^{2+} ground state) to the Mn^{2+} excited state. This action cross-section is observed to be one to two orders greater than organic dye molecules and three-four times higher than fluorescent proteins in the NIR-II window. A higher two-photon action cross-section and PL emission efficiency on NIR-II window excitation, longer emission lifetime and larger anti-Stokes shift observed in these biocompatible Mn^{2+} -doped ZnS QDs can open new perspectives in the field of multi-photon microscopy especially in “deep tissue” imaging.

EXPERIMENTAL METHODS

Synthesis and Characterization of Mn^{2+} -doped ZnS QDs: 5.5 nm-ZnS QDs doped with 1.1% Mn^{2+} ions (Mn^{2+} -doped ZnS QDs) were synthesized from a previously reported colloidal synthesis technique using Zinc chloride, Manganese chloride and elemental Sulphur in dibenzylamine²². These QDs were post-treated with oleyl amine and were dissolved in octane. The size of the as-synthesized QDs was determined from transmission electron microscope (TEM, JEOL EM-2010) images. Inductively coupled plasma-atomic emission spectra (ICP-AES) were measured using Shimadzu ICPS-1000 IV ICP-AES spectrometer. The nanocrystal concentration was determined by calibrating the zinc concentration obtained from ICP-AES with the nanocrystal size obtained from TEM analysis. These QDs dissolved in octane with a QD molar concentration of 13 μM (7.8×10^{15} QDs per cm^3) were used for optical characterizations described as follows. All the optical characterizations described below were conducted at room temperature.

Spectroscopy measurements: One-photon absorption spectra of Mn^{2+} -doped ZnS QDs were acquired using a UV-visible-near IR spectrophotometer (Shimadzu, UV-1700) and the one-photon-absorption-induced PL spectra were collected with a Jasco FP-6300 spectrofluorometer.

Multi-photon-absorption induced PL emission in Mn^{2+} -doped ZnS QDs was studied in the range from 1050 nm to 1350 nm. The sample was optically excited using laser pulses with tunable wavelength (300-1600 nm, 150 fs, 1 kHz) produced by a mode-locked Ti: Sapphire laser seeded Ti: Sapphire regenerative amplifier (COHERENT, Vitesse) pumped OPA (TOPAS). The incident laser pulses (860-1300 nm, 150 fs, 1 kHz) were focused by a 10-cm focal length lens onto a 1-cm-thick quartz cell containing QD solution. The laser beam was focused into the QD solution by means of a lens of focal length 10 cm and the beam spot size at the focal plane was

26-28 μm . The multi-photon-excited PL signal was collected in the perpendicular direction of the incident light using a collection system of two 10-cm focal length lenses; and then coupled into a spectrometer (Avaspec- 2048-SPU, Resolution 0.5 nm). As a calibration, a standard sample, Rhodamine 6G which shows quadratic dependence to excitation fluence at 900 nm, was employed in the same experimental set-up²⁹.

PL lifetime measurements: Transient PL measurements were carried out in the same experimental set-up. PL signal due to multi-photon excitation from Mn^{2+} -doped ZnS QDs were collected with a Silicon fast photo-diode (EOT, Silicon PIN detector ET-2040, 30 ns) which was input to an oscilloscope (CRO, Tektronix TDS 380, 50-ohm-terminated, 400-MHz bandwidth). The measurements were carried out in the range from 900 nm to 1310 nm with an excitation fluence of 0.25 J/cm^2 at all excitation wavelengths. The resolution of the set-up is nearly 30 ns.

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FIGURES

Figure 1. (a) Transmission Electron Microscopy (TEM) images of Mn²⁺-doped ZnS QDs showing an average size of 5.5 nm. Inset shows the lattice fringes demonstrating the crystalline quality of QDs. (b) UV-Visible absorption spectrum for Mn²⁺-doped ZnS QDs (blue) fitted with Gaussian curves (red), showing the first excitonic transition from 1S_{3/2}(h) to 1S(e) and the second excitonic transition from 2S_{3/2}(h) to 1S(e) at 318 nm (3.9 eV) and 293 nm (4.24 eV), respectively. (c) One-photon PLE(blue) and PL(red) spectra of Mn²⁺-doped ZnS QDs. (d) Multi-

photon-excited PL emission spectra of Mn^{2+} -doped ZnS QDs and Rhodamine 6G with PL signal being normalized to the PL peak intensity of Rhodamine 6G.

Figure 2. Energy level diagram explaining the one-photon- and multi-photon-absorption-induced photodynamics in Mn^{2+} -doped ZnS QDs. a) First excitonic transition from $1S_{3/2}(h)$ to $1S(e)$ by one-photon absorption. b) Second excitonic transition from $2S_{3/2}(h)$ to $1S(e)$ by one-photon absorption. c) Excitation from $1S_{3/2}(h)$ to $1S(e)$ by three-photon absorption. d) Excitation from valence subbands (or Mn^{2+} ground state (GS)) to Mn^{2+} excited states (ES) (4T_2 , 4T_1) by two-photon absorption. e) Emission peaked at 586 nm due to transitions from 4T_1 to 6A_1 states of Mn^{2+} ions. f) Wiggled arrows showing non-radiative relaxation.

Figure 3. (a) Multi-photon-excited PL lifetime measurements in Mn²⁺-doped ZnS QDs (emission at 586 nm) at different excitation wavelengths from 900 to 1100 nm (fluence = 0.25 J/cm²). The decay kinetics remains the same for all excitation wavelengths. (b) Normalized two-photon excited PL lifetime curve for excitation at 1100 nm (fluence = 0.25 J/cm²) fitted with the function of $Ae^{-(t/\tau)}$.

Figure 4. (a) Excitation fluence dependence of multi-photon excited PL peak intensity for Mn^{2+} -doped ZnS QDs at different excitation wavelengths. As a calibration, excitation fluence dependence of Rhodamine 6G is measured at 900 nm. Solid lines are linear fits, and S is the slope observed at each wavelength. The efficiency of PL emission in Mn^{2+} -doped ZnS QDs is observed to be the highest when excited at 1180 nm compared to other wavelengths. Also the PL emission efficiency of Mn^{2+} -doped ZnS QDs in NIR-II is observed to be higher than Rhodamine 6G molecules at 900 nm. (b) Slope obtained from the log-log plot of PL peak intensity (586 nm) with excitation fluence for Mn^{2+} -doped ZnS QDs at different excitation wavelengths. A change

in slope from 3 to 2 is observed at ~ 1050 nm, indicating switching of mechanism responsible for PL emission from three-photon absorption to two-photon absorption.

Figure 5. (a) Two-photon action cross-section estimated for Mn^{2+} -doped ZnS QDs in the range from 1050 nm to 1300 nm. (b) Comparison of two-photon action cross-section in Mn^{2+} -doped ZnS QDs in NIR-II (g) with other chromophores such as organic dye molecules (a-f) and fluorescent proteins (h-m). ((a) Rhodamine B, (b) Fluorescein, (c) Coumarin 307, (d) Cascade

blue, (e) Dansyl and (f) Lucifer Yellow)³¹ and ((h) tdTomato, (i) mBanana, (j) mRFP, (k) mCherry, (l) mStrawberry, (m) mTangerine)¹⁸