Two-Photon Fluorescent Molybdenum Disulfide Dots for Targeted Prostate Cancer Imaging in the Biological II Window

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ABSTRACT: Molybdenum disulfide (MoS2) quantum dots (QDs) derived from this two-dimensional (2D) transition metal dichalcogenide are emerging zero-dimensional materials that possess very good optical properties. Bioimaging using light in the biological II window (950–1350 nm) is a next-generation approach that will allow clinicians to achieve deeper tissue imaging with better image contrast and reduced phototoxicity and photobleaching. This article reports the development of a water-soluble, zero-dimensional antibody-conjugated transition metal dichalcogenide MoS2 QD-based two-photon luminescence (TPL) probe for the targeted bioimaging of cancer cells in the biological II window. The data indicates that MoS2 QDs exhibit an extremely high two-photon absorption cross-section (σ = 58960 GM) and two-photon brightness (4.7 × 10^4 GM) because of the quantum confinement and edge effects. Experimental data show that anti-PSMA antibody-attached MoS2 QDs can be used for selective two-photon imaging of live prostate cancer cells using 1064 nm light because of the high two-photon brightness, very good photostability, and very good biocompatibility of these MoS2 QDs. The data demonstrate that the bioconjugated MoS2 QDs can distinguish targeted and nontargeted cells. This study illuminates the high two-photon brightness mechanism of MoS2 QDs and provides a zero-dimensional transition metal dichalcogenide-based selective TPL agent for high-efficiency live cell imaging.

1. INTRODUCTION

Two-dimensional (2D) transition metal dichalcogenides like MoS2, WS2, WSe2, and MoSe2 are promising building blocks for use in next-generation optoelectronics and photonics applications.1−3 The unsaturated d orbitals of Mo and W generate the band structures that help these materials to exhibit interesting properties.4−12 The 2D dichalcogenide MoS2, a structural analogue of 2D graphene, has received tremendous attention in the semiconductor industry because of its direct band gap of 1.8 eV in a monolayer and the layer dependence of its band structure.6−12 Monolayer MoS2 exhibits strong photoluminescence due to its indirect–direct band gap transition, and its luminescent enhancement factor can be 4 orders of magnitude higher than that of multilayer MoS2.5−12 However, the room-temperature photoluminescence quantum yield (QY) is extremely low (Φ ~ 0.01) for 2D MoS2, and as a result, its application in two-photon imaging applications is rare.10−18 To overcome this problem, we report the design of zero-dimensional transition metal dichalcogenide MoS2 quantum dots (QDs) using a bottom-up hydrothermal method. These MoS2 QDs exhibit extremely high two-photon absorption properties, with a two-photon absorption (TPA) cross-section (σ) of 58960 GM (Goeppert-Mayer units, where 1 GM = 10^{-40} cm^4 s photon^{-1}), which is several orders of magnitude higher than that of organic dyes and much higher than that of semiconductor QDs.18−32 The data indicate that their two-photon brightness (σ × Φ, where Φ is the two-photon fluorescence QY) is around 7.6 × 10^4 GM, which is very good for bright two-photon luminescence (TPL) imaging. In our design, by decreasing the size of MoS2 to between 3 and 5 nm, the quantum confinement and edge effects have been enhanced tremendously.

Bioimaging using two-photon near-infrared (NIR) light excitation is very popular method for in vitro and in vivo imaging, offering significant benefits over one-photon luminescence microscopy, including a very high penetration depth and less photobleaching.19−27 For efficient, noninvasive biological diagnostic use, NIR light in the second biological window (950–1350 nm) will provide a deeper penetration depth into biological tissues, better image contrast, and reduced phototoxicity and photobleaching.28−35 As a result, biological imaging using light in the NIR I and II windows is a very good option to avoid absorption by physiological fluids.30−35 Despite these advantages, due to the lack of biocompatible fluorescent probes with very good imaging capability in the biological I and II windows, fluorescence microscopy is not used routinely in the clinic.19−32 Two-photon fluorescence (TPF) imaging using NIR light in the biological I and II windows has opened up the possibility of new discoveries and breakthroughs in the biological sciences because it allows for the investigation of complex biological samples.19−36 The efficiency of bioimaging using a TPF microscope is highly dependent on the two.
Figure 1. (A) Schematic of the synthetic procedure for the development of transition metal dichalcogenide MoS$_2$ QDs. (B, C) Bright-field and fluorescence (under 385 nm UV light) images of the transition metal dichalcogenide MoS$_2$ QDs, clearly showing blue fluorescence under UV light excitation. (D) High-resolution TEM image of the morphology of the transition metal dichalcogenide MoS$_2$ QDs. The EDX data in the inset clearly show the presence of Mo and S. (E) HRTEM image indicating that the lattice fringe spacing is 0.27 nm, which is due to the (100) lattice of MoS$_2$. (F) Raman spectra showing two strong Raman bands. The first band is observed at 380.7 cm$^{-1}$, which is due to the in-plane (E$_{2g}$) vibration of the Mo$-$S bond in MoS$_2$ QDs. The second band is observed at 406.5 cm$^{-1}$, which is due to the vertical plane (A$_{1g}$) vibration of the Mo$-$S bond in MoS$_2$ QDs. (G) X-ray powder diffraction (XRD) patterns of MoS$_2$ QDs and bulk MoS$_2$. The XRD spectra show the (100) and (103) planes of the crystalline MoS$_2$ phase in MoS$_2$ QDs. (H) Histogram of the size distribution of MoS$_2$ QDs measured by DLS.
photon absorption cross-section and two-photon QY of the fluorescence probe.\textsuperscript{24−36} However, commonly used organic two-photon fluorescence probes are not photostable, and these dyes also exhibit a low two-photon absorption cross-section (∼1−100 GM), which hampers the use of TPF for real-life imaging.\textsuperscript{19−32}

To facilitate the use of TPF imaging tools in the bioimaging community, we report the development of a water-soluble antibody-conjugated transition metal dichalcogenide MoS\textsubscript{2} QD-based two-photon photoluminescence probe for targeted bioimaging of LnCaP prostate cancer cells using light in the biological I and II transparency windows, as shown in Figure 1A. For this purpose, MoS\textsubscript{2} QDs were modified with lipoi acid-terminated poly(ethylene glycol) (PEG) to increase their stability in physiological solutions, and an anti-PSMA antibody was attached to the MoS\textsubscript{2} QDs via PEG to allow their use in targeted imaging. The results show that due to their very high two-photon brightness, photostability, and lower cytotoxicity, antibody-conjugated MoS\textsubscript{2} QDs are a very good candidate for TPF imaging of live cells in the NIR biological I and II windows.

2. RESULTS AND DISCUSSIONS

2.1. Development and Characterization of Zero-Dimensional Bioconjugated MoS\textsubscript{2} QDs. Zero-dimensional transition metal dichalcogenide MoS\textsubscript{2} QDs were synthesized using a bottom-up hydrothermal method\textsuperscript{12−16} as shown in Figure 1A. Details of the synthesis route are discussed in Section 4.1. For this purpose, Na\textsubscript{3}MoO\textsubscript{4}·2H\textsubscript{2}O was used as the source of Mo and l-cysteine was used as a S precursor. At the end, the purified particles were characterized by high-resolution tunneling electron microscopy (TEM), energy-dispersive X-ray (EDX) spectroscopy, Raman spectroscopy, and dynamic light scattering (DLS), as reported in Figure 1 and Table 1.

Table 1. Size Distribution of MoS\textsubscript{2} QDs and PEG-LA-MoS\textsubscript{2} QDs

| nanoparticle description          | size measured by DLS |
|-----------------------------------|-----------------------|
| MoS\textsubscript{2} QDs           | 7 ± 2 nm              |
| PEG-LA-MoS\textsubscript{2}       | 16 ± 3 nm             |
| PSMA antibody-PEG-LA-MoS\textsubscript{2} | 25 ± 4 nm           |

Figure 1D shows a TEM image of freshly prepared transition metal dichalcogenide MoS\textsubscript{2} QDs, which indicates that the size of the MoS\textsubscript{2} QDs is 6 ± 2 nm. DLS data, reported in Figure 1H and Table 1, also indicate that the average size of transition metal dichalcogenide MoS\textsubscript{2} QDs is about 7 ± 2 nm, which matches the TEM data quite well. We also performed high-resolution TEM (HRTEM) to characterize the lattice structure of the transition metal dichalcogenide MoS\textsubscript{2} QDs developed using a bottom-up hydrothermal method. The HRTEM image in Figure 1E shows the crystalline lattice structure of the MoS\textsubscript{2} QDs with a fringe lattice spacing around 0.27 nm due to the (100) lattice plane. EDX elemental mapping, as shown in the inset of Figure 1D, clearly indicates the presence of Mo and S. The crystal structure of the MoS\textsubscript{2} QDs was investigated using X-ray powder diffraction (XRD) and compared with that of bulk MoS\textsubscript{2}, as shown in Figure 1G. For MoS\textsubscript{2} QDs, we observed intense diffraction peaks at 30.9 and 39°, which can be assigned to the (100) and (103) planes of the crystalline MoS\textsubscript{2} phase. As shown in Figure 1G, the XRD diffraction signals for the (002) and (105) planes totally disappeared for the MoS\textsubscript{2} QDs, which indicates the formation of few-layered MoS\textsubscript{2} QDs.

We also performed Raman spectroscopy, which shows two strong Raman bands for the transition metal dichalcogenide MoS\textsubscript{2} QDs. As shown in Figure 1F, the first band is at 380.9 cm\textsuperscript{-1}, which is due to the in-plane (E\textsubscript{1g}) vibration of the Mo=S bond in the MoS\textsubscript{2} QDs.\textsuperscript{12−16} The second band is observed at 405.4 cm\textsuperscript{-1}, which is due to the vertical plane (A\textsubscript{1g}) vibration of the Mo=S bond in the MoS\textsubscript{2} QDs.\textsuperscript{12−18} The observed E\textsubscript{1g} and A\textsubscript{1g} vibrational modes clearly indicate the presence of exfoliated MoS\textsubscript{2} nanostructures in the transition metal dichalcogenide QDs.\textsuperscript{12−18} The observed peak spacing for the MoS\textsubscript{2} QDs is much lower than that of bulk MoS\textsubscript{2} (25.7 cm\textsuperscript{-1})\textsuperscript{12−16} reported in the literature, which indicates that MoS\textsubscript{2} QDs contain a few-layered structure for MoS\textsubscript{2}.

Figure 1B,C shows that a solution of the water-soluble MoS\textsubscript{2} QDs emits blue fluorescence upon irradiation with UV light. The excitation wavelength-dependent single-photon photoluminescence spectra of the MoS\textsubscript{2} QDs at excitation wavelengths ranging from 300 to 532 nm, as reported in Figure 2A, clearly show strong blue photoluminescence under UV light excitation. To determine the photoluminescence QY of the transition metal dichalcogenide MoS\textsubscript{2} QDs, we used quinine sulfate as a standard, whose QY is well documented (Φ = 54%). The QY for the transition metal dichalcogenide MoS\textsubscript{2} QDs was determined using eq 1.\textsuperscript{8−20}

$$\Phi_{\text{TMD}} = \Phi_{\text{QS}}(I_{\text{TMD}}/A_{\text{TMD}})^2(I_{\text{QS}}/A_{\text{QD}})^2 \eta^2$$  (1)

where the transition metal dichalcogenide is denoted TMD, the quinine sulfate standard is denoted QS, Φ is the quantum yield under 375 nm excitation, A is the absorbance, I is the fluorescence intensity, and η is the refractive index.

By using eq 1 and counting the integrated luminescence intensity for the MoS\textsubscript{2} QDs and the standard, the photoluminescence QY for the transition metal dichalcogenide MoS\textsubscript{2} QDs was determined to be 13.2%, which is about 1 order of magnitude higher than the reported QY for a MOS\textsubscript{2} single-layer nanosheet.\textsuperscript{14}

As reported in Figure 2A, the photoluminescence of the transition metal dichalcogenide MoS\textsubscript{2} QDs can be changed by varying the excitation energy. Although the exact origin of the excitation-dependent single-photon luminescence of the transition metal dichalcogenide MoS\textsubscript{2} QDs is not known, there are several factors that might contribute to this, including (1) ground state heterogeneity of MoS\textsubscript{2} QDs due to their polydispersity, (2) the possibility of there being multiple discrete electronic states due to the presence of different types of aggregates, and (3) variation in the hot fluorescence from the K point of the Brillouin zone\textsuperscript{5−15} due to the polydispersity of the transition metal dichalcogenide MoS\textsubscript{2} QDs.

2.2. Two-Photon Absorption and Two-Photon Brightness of the MoS\textsubscript{2} QDs. As we discussed earlier, light in the second biological window (between 950 and 1350 nm) has to be used for biological imaging to avoid absorption by physiological fluids.\textsuperscript{19−32} As shown in Figure 2A, single-photon luminescence can be observed from the transition metal dichalcogenide MoS\textsubscript{2} QDs up to 532 nm excitation, which indicates that the transition metal dichalcogenide MoS\textsubscript{2} QDs are not suitable for biological imaging applications using light in the NIR biological II window. Thus, for biological imaging using transition metal dichalcogenide MoS\textsubscript{2} QDs, TPL imaging will be the best choice. Next, we explored the possible use of
MoS$_2$ QDs as a two-photon imaging material. For the two-photon absorption and imaging experiments, we used an 80 MHz Ti:sapphire laser as an excitation source with a 100 fs pulse width and 80 MHz repetition rate, as we have reported before. For measurements in the biological II window, tunable wavelengths between 700 and 1100 nm were generated using an optical parametric amplifier. For TPL imaging using MoS$_2$ QDs, we used a Nikon multiphoton microscope (FV1000MPE). Figure 2B shows the TPL spectra of the transition metal dichalcogenide MoS$_2$ QDs at an excitation of 1064 nm. The observed $\lambda_{\text{max}}$ for TPL emission is 660 nm, which matches quite well with the single-photon luminescence $\lambda_{\text{max}}$ (659 nm) at 532 nm excitation, as reported in Figure 2A. Figure 2C shows the excitation wavelength power-dependent plot for 1064 nm excitation light, which indicates that the photoluminescence intensity from the transition metal dichalcogenide MoS$_2$ QDs at 660 nm is proportional to the square of the 1064 nm excitation light intensity. The linear plots clearly confirm that the observed photoluminescence at 660 nm is indeed a two-photon process. Using fluorescein as the reference, whose QY has been determined to be 0.9, the TPL QY for the transition metal dichalcogenide MoS$_2$ QDs was determined to be 0.08. The observed two-photon QY for the MoS$_2$ QDs is slightly lower than the observed single-photon QY. This can be due to the charging process via Auger recombination. Since the charging process occurs on a subnanosecond time scale, it is much faster than what is measurable using conventional single-photon photoluminescence spectroscopy. Because we used a femtosecond laser for measuring the TPL QY, Auger recombination is more likely for this case than it is for the single-photon QY measurement using normal fluorescence spectroscopy.

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Figure 3. (A) Bright-field inverted microscope images of HaCaT normal skin cells after a 24 h incubation with transition metal dichalcogenide MoS$_2$ QDs. Cells were stained with trypan blue, which indicates no cell death after the 24 h incubation. (B) Bright-field inverted microscope images of LnCaP prostate cancer cells after a 24 h incubation with transition metal dichalcogenide MoS$_2$ QDs. Cells were stained with trypan blue, which indicates no cell death after the 24 h incubation. (C) Plot of the biocompatibility of the anti-PSMA antibody-attached transition metal dichalcogenide MoS$_2$ QDs against human prostate cancer LnCaP cells and human skin HaCaT keratinocyte cells. The data indicate that even after a 24 h incubation, about 98% of cells were viable for both the LnCaP cancer and HaCaT normal cells. (D) Bright-field inverted microscope images of LNCaP prostate cancer cells after a 24 h incubation with silver nitrate. Cells were stained with trypan blue, which indicates a significant amount of cell death after the 24 h incubation. (E) Intracellular reactive oxygen species formation upon exposure of HaCaT normal cells to PEG-LA-MOS$_2$ QDs during an 8 h incubation.
Figure 4. (A) TEM image showing that anti-PSMA antibody-attached transition metal dichalcogenide MoS$_2$ QDs are attached to the LnCaP prostate cancer cell surface after incubation for 30 min. (B) TEM image showing that anti-PSMA antibody-attached transition metal dichalcogenide MoS$_2$ QDs are not bound to the surface of HaCaT normal skin cells after incubation for 30 min followed by separating unconjugated QDs using...
centrifugation and washing with buffer three times. (C) Bright-field image of HaCaT normal skin cells after incubation with anti-PSMA antibody-attached MoS2 QDs followed by separation using centrifugation. (D) TPL image of the same HaCaT normal skin cells after incubation with anti-PSMA antibody-attached MoS2 QDs followed by separation using centrifugation. Our results clearly show that anti-PSMA antibody-attached MoS2 QDs do not bind the HaCaT normal skin cells. (E) Bright-field image of LnCaP human prostate cancer cells after incubation with anti-PSMA antibody-attached MoS2 QDs followed by separation by centrifugation. (F) TPL imaging of anti-PSMA antibody-attached MoS2 QDs incubated with LnCaP prostate cancer cells using 1064 nm excitation. For TPL imaging, we used a laser power density of 25 W/cm². The data shows that the anti-PSMA antibody-attached MoS2 QDs are also able to penetrate the cell membrane and localize inside the cells. (G) Percentage of cells attached to anti-PSMA antibody-conjugated MoS2 QDs when a mixture of (106 cells/mL each) HER2(+) SK-BR-3 breast cancer cells, PSMA(+) LnCaP human prostate cancer cells, and CD34(+) stem cells was incubated with anti-PSMA antibody-attached MoS2 QDs for 30 min followed by separating the MoS2 QD-attached cells by centrifugation.

The experimental data show that the photostability of the anti-PSMA antibody-attached MoS2 QDs is very good, thus indicating that they can be used as a TPL material. The observed TPL is found to be intense and does not blink, which could be due to the excellent crystalline quality of the MoS2 QDs, as reported in Figure 1E.

Because biocompatibility is one of the most important factors for live cell imaging, we performed biocompatibility experiments for anti-PSMA antibody-attached MoS2 QDs. We used silver nitrate as a standard to compare the cellular toxicity. For this purpose, 3.9 × 105 LnCaP cells/mL and 3.9 × 105 HaCaT cells/mL were incubated with anti-PSMA antibody-attached MoS2 QDs for different time intervals. In the next step, the cell viability was measured using trypan blue and MTT colorimetric tests to determine the amount of living and dead cells after incubation. As reported in Figure 3, the trypan blue and MTT tests clearly indicate that even after a 24 h incubation with LnCaP cancer cells, 98% cell viability was observed. On the other hand, more than 95% cells were dead after a 24 h incubation with silver nitrate, as shown in Figure 3D, which indicates the very good biocompatibility of the anti-PSMA antibody-attached MoS2 QDs.

To further explore the biocompatibility of the MoS2 QDs, we performed an intracellular reactive oxygen species (ROS) formation assay. For this purpose, we exposed HaCaT cells to MoS2 QDs for different time intervals and monitored the formation of ROS using a proprietary cell-permeable fluorogenic probe (Cell Biolabs, Inc., San Diego, CA). Control experiments were performed using cell-containing-only media. We performed fluorescence measurements using 480 nm excitation, and emission data at 530 nm was recorded using a multimode microplate reader (BioTek Instruments, Inc.). As reported in Figure 3E, we did not observe increased intracellular ROS production by MoS2 QDs even up to 7 h of incubation when compared with PBS as a control.

Next, for the two-photon imaging experiment, we incubated anti-PSMA antibody-attached transition metal dichalcogenide MoS2 QDs with different concentrations of LnCaP prostate cancer cells for 30 min. After that, unconjugated anti-PSMA antibody-attached MoS2 QDs were separated using centrifugation followed by washing with buffer three times to make sure that QDs not bound to prostate cancer cells were separated. To understand whether anti-PSMA antibody-attached transition metal dichalcogenide MoS2 QD-based TPL imaging is selective for targeted LnCaP prostate cancer cell imaging, we incubated anti-PSMA antibody-attached transition metal dichalcogenide MoS2 QDs with 9.2 × 10⁵ cells/mL of nontargeted HaCaT skin cells. After 30 min of incubation, anti-PSMA antibody-attached MoS2 QDs were separated using centrifugation followed by washing with buffer three times to make sure that QDs not bound to prostate...
cancer cells were separated. Figure 4A shows an HRTEM image, which clearly indicates that anti-PSMA antibody-attached transition metal dichalcogenide MoS2 QDs are attached to the LnCaP prostate cancer cell surface. On the other hand, as reported in Figure 4B, TEM clearly indicates that anti-PSMA antibody-attached MoS2 QDs are not bound to the surface of HaCaT normal skin cells after incubation with anti-PSMA antibody-attached MoS2 QDs for 30 min followed by separating unconjugated QDs using centrifugation and washing with buffer three times.

The data clearly show that anti-PSMA antibody-attached transition metal dichalcogenide MoS2 QDs are bound only to targeted cells. Figure 4F shows TPL imaging of LnCaP prostate cancer cells using a 1064 nm excitation wavelength. The TPL imaging data clearly demonstrate that the extremely bright TPL properties can be used for bioimaging using light in the biological II window. On the other hand, the TPL image of HaCaT cells in Figure 4D clearly demonstrates that non-targeted normal skin cells do not bind the anti-PSMA antibody-attached MoS2 QDs, and as a result, no TPL image was observed. All of these data clearly show that the anti-PSMA antibody-attached transition metal dichalcogenide MoS2 QD-based TPL imaging is highly selective for targeted LnCaP prostate cancer cells. The data in Figure 4F indicate that anti-PSMA antibody-attached transition metal dichalcogenide MoS2 QDs not only are localized in the proximity of the LnCAP prostate cancer cell membrane but also are also able to penetrate the cell membrane and localize inside the cell. As reported by us and others, small size nanoparticles can be internalized into cells either via the classic endocytic or nonendocytotic pathway.%2C13,29,32 Since the size of the anti-PSMA antibody-attached transition metal dichalcogenide MoS2 QDs is small, particles can enter the cells via both endocytic and nonendocytotic pathways.

To understand the real selectivity of the particles, we performed an experiment using a coculture with three cell lines: PSMS(+) LNCaP human prostate cancer cells, HER2(+) SK-Br-3 human breast cancer cells, and CD34(+) bone marrow stem cells. Using enzyme-linked immunosorbent assays, we found that no PSMA is present in SK-Br-3 human breast cancer cells and CD34(+) bone marrow stem cells. Using enzyme-linked immunosorbent assays, we found that no PSMA is present in SK-Br-3 human breast cancer cells and CD34(+) bone marrow stem cells. For demonstrating selectivity, we incubated anti-PSMA antibody-attached MoS2 QDs with 106 cells/mL PSAM(+) LNCaP prostate cancer cells, 105 cells/mL HER2(+) SK-Br-3 human breast cancer cells, and 105 cells/mL CD34(+) bone marrow stem cells. After incubating the anti-PSMA antibody-attached MoS2 QDs with the cell mixture for 30 min, cells that were not conjugated to the anti-PSMA antibody-attached MoS2 QDs were separated using centrifugation, followed by washing with buffer three times. Using enzyme-linked immunosorbent assays, we found that only HER2(+) and CD34(+) cells were present in the fractions of the cell suspension that did not bind to the MoS2 QDs. On the other hand, as shown in Figure 4G, we found only PSMA(+) cells bound to the MoS2 QDs. These data clearly show that anti-PSMA antibody-attached MoS2 QDs are highly selective for PSMA(+) LNCaP human prostate cancer cells.

3. CONCLUSIONS

In this article, we have reported the development of zero-dimensional transition metal dichalcogenide MoS2 QDs using a bottom-up hydrothermal method, which exhibits very strong two-photon photoluminescence. Our experimental data show that MoS2 QDs exhibit very high two-photon absorption properties with a TPA cross-section of 58 960 GM, which is several orders of magnitude higher than that of organic dyes and much higher than that of semiconductor QDs. We have demonstrated that due to their extremely high two-photon brightness of ∼4.7 × 105 GM, high photostability, and very good biocompatibility, anti-PSMA antibody-attached MoS2 QDs can be used for multiphoton imaging of live prostate cancer LnCaP cells. The data demonstrate that MoS2 QD-based two-photon imaging using light in the biological II window is capable of distinguishing targeted prostate cancer LnCaP cells from other nontargeted cells. The observed bright TPL imaging of selective cancer cells, photostability, and biocompatibility make the antibody-attached MoS2 QDs a good candidate for use as a TPL imaging material for cancer in clinical environments.

4. METHODS

Na2MoO4·2H2O, l-cysteine, LA-modified PEG, different solvents, and other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA). Human prostate cancer LnCaP cells and HaCaT normal skin cells were purchased from American Type Culture Collection (ATCC, Rockville, MD).

4.1. Synthesis of MoS2 QDs. The MoS2 QDs were synthesized using a bottom-up hydrothermal method.%2C12−16 as shown in Figure 1A. For this purpose, Na2MoO4·2H2O was used as the source of Mo and l-cysteine was used as the S precursor. Typically, 0.5 g of solid Na2MoO4·2H2O was dissolved in a beaker containing 50 mL of water and sonicated for 5 min. The pH of the solution was adjusted to 6.5 with diluted HCl. In another beaker, 1.0 g of l-cysteine was dissolved in 50 mL of water, followed by sonication for 10 min. Both solutions were transferred into a 100 mL Teflon-lined stainless steel autoclave and heated at 200 °C for 3 h. The resulting black suspensions were separated by centrifugation at 12 000 rpm for 30 min. Solid MoS2 QDs were obtained by evaporation of the solvent followed by lyophilization. At the end, the purified particles were characterized by HRTEM, EDX spectroscopy, Raman spectroscopy, and DLS measurements, as reported in Figure 1 and Table 1.

4.2. Development of Anti-PSMA Antibody-Conjugated MoS2 QDs. For the targeted capture and imaging of human prostate cancer LnCaP cells, an anti-PSMA antibody was attached to the fluorescent MoS2 QDs. To accomplish this, MoS2 QDs were modified with LA-terminated PEG to improve their stability in physiological solutions using a reported method.%2C11−14 During this process, LA covalently bonds to the edges of MoS2 to produce a PEG-LA-MoS2 conjugate.%2C11−14 In the next step, the anti-PSMA antibody was attached to the MoS2 QDs via PEG for their use in targeted imaging. For this purpose, the anti-PSMA antibody was attached to PEG-MoS2 via EDC/NHS chemistry, as we and others have reported.%2C11,13,27,29,31,34

4.3. Cell Culture and Incubation with Transition Metal Dichalcogenide MoS2 QDs. We purchased human prostate cancer LaCaP and normal skin HaCaT cells from ATCC and grew them according to ATCC’s procedure, as we have reported before.%2C37 Once the culture reached more than 106 cells/mL, different concentrations of anti-PSMA antibody-attached MoS2 QDs were mixed with the different cell lines for 30 min. After that, unconjugated anti-PSMA antibody-attached MoS2 QDs were separated using centrifugation followed by washing with buffer three times to make sure that QDs not bound to cells were separated.
4.4. Two-Photon Absorption Coefficient Measurement and Two-Photon Imaging. For two-photon absorption and imaging experiments, we used an 80 MHz Ti:sapphire laser as an excitation source with a 100 fs pulse width and 80 MHz repetition rate, as we have reported before.27,29,34–37 For measurements in the biological II window, light of tunable wavelengths between 700 and 1100 nm was generated using an optical parametric amplifier. For TPL imaging using MoS2 QDs, we used a Nikon multiphoton microscope (FV1000MPE), as we have reported before.27,29,31,34

4.5. Cell Viability Assay. To study the cytotoxicity of the transition metal dichalcogenide MoS2 QDs, different numbers of cancer and normal cells were treated with the MoS2 QDs for different time intervals. At the end, we measured cell viability using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. For the MTT assay, we measured the absorbance at 540 nm using a Multiskan Ascent plate reader with ASCENT software (Labsystems), as reported previously.36,37

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The authors declare no competing financial interest.

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