Living Yeast Cells as a Controllable Biosynthesizer for Fluorescent Quantum Dots

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There are currently some problems in the field of chemical synthesis, such as environmental impact, energy loss, and safety, that need to be tackled urgently. An interdisciplinary approach, based on different backgrounds, may succeed in solving these problems. Organisms can be chosen as potential platforms for materials fabrication, since biosystems are natural and highly efficient. Here, an example of how to solve some of these chemical problems through biology, namely, through a novel biological strategy of coupling intracellular irrelated biochemical reactions for controllable synthesis of multicolor CdSe quantum dots (QDs) using living yeast cells as a biosynthesizer, is demonstrated. The unique fluorescence properties of CdSe QDs can be utilized to directly and visually judge the biosynthesis phase to fully demonstrate this strategy. By such a method, CdSe QDs, emitting at a variety of single fluorescence wavelengths, can be intracellularly, controllably synthesized at just 30°C instead of at 300°C with combustible, explosive, and toxic organic reagents. This green biosynthetic route is a novel strategy of coupling, with biochemical reactions taking place irrelatedly, both in time and space. It involves a remarkable decrease in reaction temperature, from around 300 °C to 30 °C and excellent color controllability of CdSe photoluminescence. It is well known that to control the size of nanocrystals is a mojor challenge in the biosynthesis of high-quality nanomaterials. The present work demonstrates clearly that biological systems can be creatively utilized to realize controllable unnatural biosynthesis that normally does not exist, offering new insights for sustainable chemistry.

1. Introduction

In the current climate, the need for sustainable use of resources and for environmentally safe processes and products is becoming more and more pressing.[1] These problems exist in conventional synthesis of nanomaterials such as CdSe quantum dots (QDs), which usually requires high temperatures (around 300 °C) and the use of combustible, explosive, and toxic organic reagents, such as trioctylphosphane (TOP) or trioctylphosphane oxide (TOPO), under an oxygen- and water-free atmosphere.[2–4] Such synthesis methods are environmentally unfriendly, inefficient, and can be unsafe. In recent years, natural and highly efficient biosystems have exhibited their potential to establish a platform for fabrication of materials to address these problems. However, the controllable synthesis of high-quality nanomaterials, for example, controlling the size of nanocrystals, remains a challenge for such biosynthesis.[5]

Herein, we demonstrate an example of how to solve seemingly intractable chemical synthesis problems through biology, namely, through a novel biological strategy of coupling intracellular irrelated biochemical reactions to controllably synthesize multicolor CdSe QDs using living yeast cells as a biosynthesizer. CdSe QDs were chosen as the target product to validate the strategy because their unique fluorescence properties, which can be used to directly and visually judge the completion of the biosynthesis phase. Such properties include broad excitation, size-dependent photoluminescence, unusual photochemical stability, single-excitation/multiple-emission and relatively high photoluminescence quantum yields,[6–8]

Yeast, which is widely used as a model organism,[9,10] is an ideal candidate biosynthesizer for fluorescent semiconductor CdSe QDs. The eukaryotic *Saccharomyces cerevisiae* cell is a sophisticated mini-machine, in which thousands of biochemical reactions precisely govern cellular behavior.[11–13] Yeast cells can quickly and exactly respond to various changing environments through their regulation networks.[12] Such cells have provided not only a consolidated basis and platform but also a powerful tool for the bioengineering and life sciences.[9,14] It is expected that

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intracellular reactions that are unrelated in the normal physiological state of cells can be skillfully utilized to produce the desired product.

The synthesis of CdSe requires that both cadmium and selenium are in the appropriate valence states and react with each other in the right place and at an appropriate time. It is known that yeast cells can produce valence-fit cadmium and selenium via natural reactions concerning the intracellular metabolism of Na_2SeO_3[15,16] and detoxification of $CdCl_2$.[17,18] However, because these two reactions are unrelated in yeast cells, they must be coupled collaboratively, in an appropriate time and space sequence, in order to obtain the final product.

2. Results and Discussion

2.1. Reduced Selenium Species in Yeast Cells

Selenite can be reduced into the selenotrisulfide derivative of glutathione (GSSeSG) by reduced glutathione (GSH), and further reduced into low-valence selenium by GSH-related enzymes, such as NADPH (reduced β-nicotinamide adenine dinucleotide 2′-phosphate tetrasodium salt) and glutathione reductase (GR), in the cytoplasm and mitochondria.[19,20] Only during the stationary phase (SP) can yeast cells synthesize the GSH and GSH-related enzymes with a high efficiency, which is required for the reduction of selenium; this is a physiological response to intracellular excess free radicals, oxidative stress and lack of nutrition.[12,21,22]

High-performance liquid chromatography coupled with inductively coupled plasma mass spectrometry (HPLC-ICP-MS) analysis verified that the yeast cells in the SP could reduce Na_2SeO_3 into organoselenium compounds, such as selenocystine [$(Cys\text{-}Se)_2$] and selenomethionine (Se-Met) after 24 h of co-incubation with $CdCl_2$ (Fig. 1). Moreover, the relative activity of GSH-related enzymes in the yeast cells was decreased by 1 mM $CdCl_2$ (data not shown), as found in previous studies.[18,23] Since the activity of GSH-related enzymes is the key to the reduction of selenium,[19,20] $CdCl_2$ can inhibit the reduction of selenium. Therefore, coupling the intracellular reactions in the correct space and time sequence is crucial for the successful synthesis of CdSe QDs. A biosynthesis route was designed involving the co-incubation of Na_2SeO_3 and the yeast cells at SP to reduce Na_2SeO_3 into organoselenium compounds and then the addition of $CdCl_2$ into the seleniumized yeast cell culture at the appropriate moment (time coupling), such that $Cd(SG)_2$ can react exactly with the reduced selenium to create CdSe QDs just before being transported into the vacuole (space coupling) (Figs. 2b and 3, and movie in the Supporting Information). The different *Z*-axis focal plane confocal images of yeast cells (Fig. 3 and movie in the Supporting Information) show that the fluorescence of yeast cells appeared in the cytoplasm outside vacuole, as designed. And the experiments in vitro suggested that selenocysteine (se-cys, one of the reduced selenium compounds) was necessary for the synthesis of CdSe QDs (Supporting Information, Figs. S1 and S2).

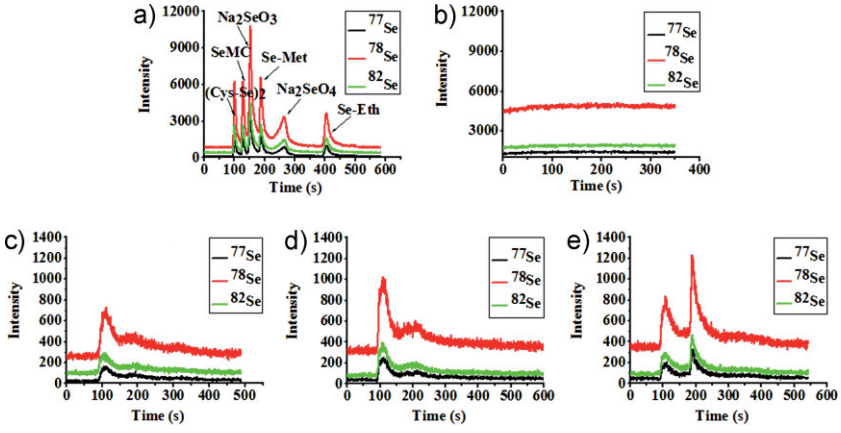

Figure 1. HPLC-ICP-MS chromatograms of intracellularly reduced selenium species. a) Standards of selenium speices: D,L-Selenomethionine (Se-Met), Se-methylseleno-L-cysteine (SeMC), L-selenocystine $(Cys\text{-}Se)_2$, seleno-D,L-ethionine (Se-Eth), Na_2SeO_3 and Na_2SeO_4; b) Yeast cells untreated with Na_2SeO_3 (no peak of any selenium species was found in the chromatogram); c) Intracellular components of the seleniumized yeast cells. Chromatogram pattern of intracellular components was the same with those of $(Cys\text{-}Se)_2$ and Se-Met-mixed standards. d) Addition of $(Cys\text{-}Se)_2$ standard into (c); e) Addition of Se-Met standard into (c). The introduction of standards of $(Cys\text{-}Se)_2$ for (d) and Se-Met for (e) would increase the corresponding peaks in the HPLC-ICP-MS chromatogram. All chromatograms of (c), (d) and (e) indicate that intracellular components of the seleniumized yeast cells contained $(Cys\text{-}Se)_2$ and Se-Met. Three isotopes of ^{77}Se, ^{78}Se and ^{82}Se which were free from the spectral interferences were simultaneously monitored.

2.2. Well-dispersed CdSe QDs in Fluorescing Yeast Cells

The seleniumized yeast cells surprisingly began to fluoresce after 6–8 h of co-incubation with $CdCl_2$. Such fluorescence came from inside the cells and there was no fluorescence for the control cells (Fig. 4a–d). In situ transmission electron microscopy (TEM) images for fluorescing cells displayed well-dispersed nanocrystals, with the size of (2.69 ± 0.07) nm (mean $\pm$ s.d., $n = 79$). The unit cell parameters (JCPDS card, no. 77-2307) of these nanocrystals are consistent with those previously reported.[24] Energy dispersive X-ray spectroscopy (EDX) characterization of the isolated particles (Supporting Information, Fig. S3) proved that the elements of the product were Cd and Se, and the molar ratio of Cd to Se in the EDX spectrum is 1:1.28. On the contrary, no nanocrystals were found in the control cells. These data indicate that the biochemical reactions in yeast cells were successfully coupled to allow the synthesis of fluorescing CdSe QDs (Fig. 2a): upon being added into the yeast cell suspension, Na_2SeO_3 immediately reacted with GSH to form GSSeSG,[15] which is a substrate for GSH-related enzymes. Reduction of GSSeSG catalyzed by GSH-related enzymes (thioredoxin reductase and glutathione reductase)[16,19,20] resulted in low-valenced selenocompounds (Fig. 1). Among the selenocomponds, only Se-Cys is suggested to react with $Cd(SG)_2$ (Supporting Information, Figs. S1 and S2), which, added at the proper time, produced CdSe QDs.

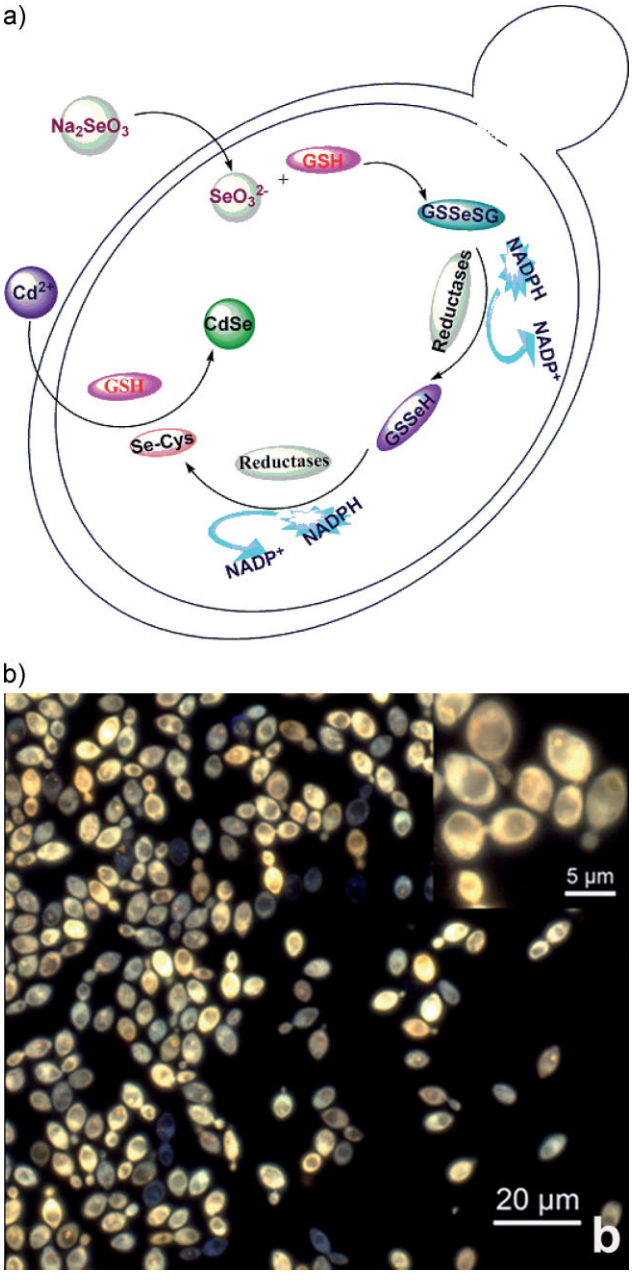

Figure 2. a) Route for unnatural biosynthesis of fluorescent CdSe quantum dots and b) images for subcellular location of intracellular fluorescence. Yeast cells treated according to the designed protocol (exposure time: 50 ms).

2.3. Controllability of the CdSe QDs Diameter

It is exciting that CdSe QDs with different sizes and fluorescence wavelengths could purposely be obtained simply by controlling the incubation duration of the yeast cells with $CdCl_2$. In situ laser confocal scanning microscopy results showed that, when the duration of incubation was extended from 10 to 40 h, the color of fluorescence from in cells shifted from green first to yellow, and then to red (Fig. 5a–c), with corresponding increases in emission wavelengths from ca. 520 to ca. 560 and then to ca. 670 nm (Fig. 5d–f). In situ fluorescence spectra directly proved that the fluorophores had the same emission peaks as those of CdSe QDs synthesized by conventional methods reported previously. The spectra of QDs isolated from the cells are given in Figure 5h–j. TEM images confirmed the size of corresponding CdSe QDs increased from 2.69 to 6.34 nm (Fig. 5g). These showed that the CdSe QDs in yeast cells grew more slowly than those synthesized by conventional chemical routes, which will facilitate the exact and purposeful production of QDs with desired sizes, at least for the case studied here.

The most important element of our strategy is the rational coupling, in an appropriate space and time sequence, of multiple indispensable but separate reactions that are unrelated, so that Cd(II) can collide with low-valenced selenium at the proper time and in the proper place to yield the desired CdSe QDs; this strategy is totally different from previous reports on the syntheses of CdS and PbS nanocrystals using yeast cells.[25,26] Firstly, the previous work followed the normal pathways of the cellular metabolism to synthesize CdS and PbS nanocrystals using yeast cells and the size of the products could not be tuned.[25,26] Secondly, the CdS or PbS nanocrystals accumulated in the vacuoles;[26] this is rather different to the present work, in which the fluorescence appeared in the cytoplasm and the mitochondria rather than the vacuole (see Figs. 2b and 3, and movie in the Supporting Information), indicating that CdSe QDs had been synthesized outside of the vacuoles and, therefore, via a different pathway than in previous reports on the syntheses of CdS and PbS nanocrystals using yeast cells.

2.4. The Influence of the Concentration of Selenite or $CdCl_2$

The concentration of selenite or $CdCl_2$ added to the cell culture is key to the synthesis. It has been found that high concentration of selenite (10 mM or above) or $CdCl_2$ (1 mM or above) completely inhibit the growth of normal yeast cells (see the Supporting Information, Fig. S4a and b). If cellular metabolism had been so restrained, intracellular reactions could not have been utilized; hence, 5 mM selenite was used to seleniumize yeast cells. 1 mM $CdCl_2$ was still used to synthesize CdSe QDs because, despite inhibiting the growth of normal yeast cells, such a $CdCl_2$ concentration less affected the growth of seleniumized cells (Supporting Information, Fig. S4c).

3. Conclusions

A novel route for the controllable biosythesis of fluorescent CdSe QDs has been demonstrated by aboratively coupling unrelated intracellular biochemical reactions in an appropriate space and time sequence.

Selenocompounds of selenocystine and selenomethionine required for the biosynthesis of CdSe QDs in the living yeast cells were confirmed by HPLC-ICP-MS. Adding $CdCl_2$ into the seleniumized yeast cells could make them fluorescent after an appropriate duration of incubation, yielding CdSe nanocrystals. In situ TEM confirmed that the fluorescence resulted from intracellular CdSe nanocrystals. In vitro biomimetic synthesis of CdSe suggested that selenocysteine had participated in this

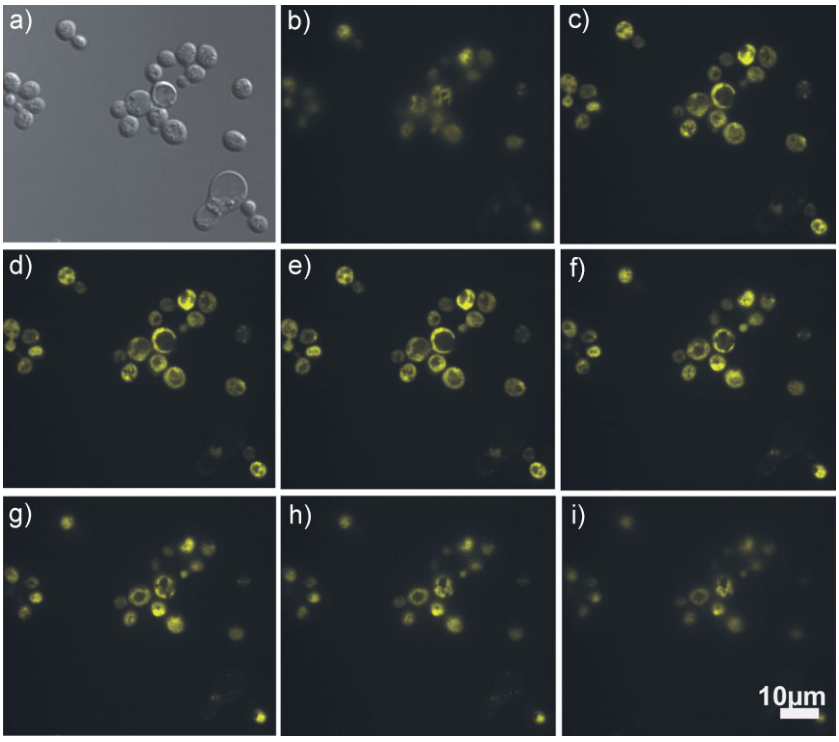

Figure 3. Laser confocal scanning images of fluorescing yeast cells. a) Differential interference contrast (DIC) bright field; b–i) Different *Z*-axis focal planes excited by 488 nm laser beam. The images were obtained on a laser confocal scanning microscope (Olympus I × 81, 100 × objective, *N.A.* = 1.40, Andar Revolution XD)

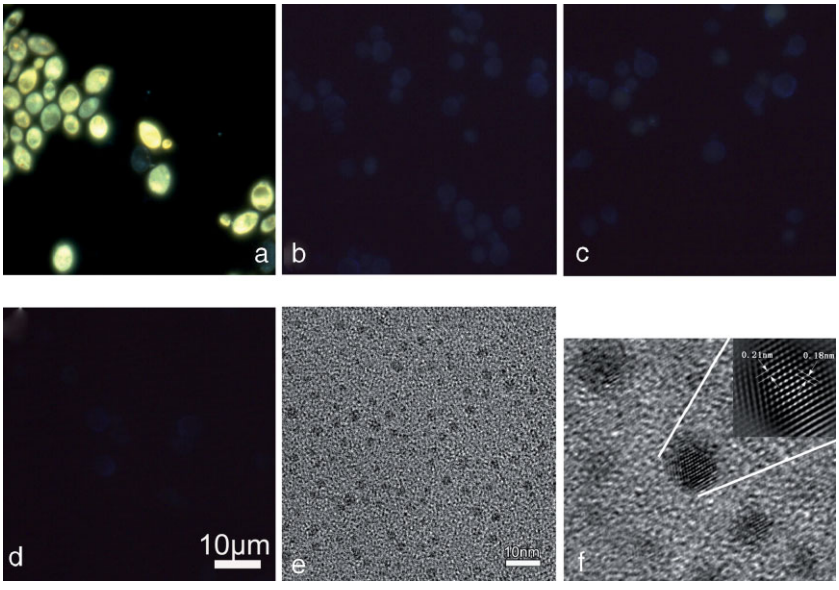

Figure 4. Fluorescence images for the yeast cells and in situ TEM for intracellular CdSe quantum dots. a) The seleniumized yeast cells cultured with $CdCl_2$ for 20 h according to the designed route; b) Control of yeast cells seleniumized for 24 h at the stationary phase; c) Control of yeast cells co-incubated with $CdCl_2$ for 20 h; d) Control of yeast cells cultured neither with Na_2SeO_3 nor $CdCl_2$. The exposure time of (a–d) was 50 ms. e) In situ TEM image of intracellular CdSe QDs; f) In situ high-resolution TEM (HRTEM) image of several individual intracellular CdSe QDs. The crystal data obtained from (f) indicates that the CdSe QDs had the structure of wurtzite with the unit cell parameters of $a = 0.4299$ nm and $c = 0.7010$ nm, and with planar faces of $(1\bar{1}1)$ ($d = 0.21$ nm) and (201) ($d = 0.17$ nm) at an interplane angle of 58°.

process (Supporting Information, Figs. S1 and S2). By simply modulating the duration of co-incubation of the seleniumized yeast cells and $CdCl_2$, CdSe QDs with a variety of sizes and fluorescence emission wavelengths can be controllably synthesized.

This strategy is a good example of how the use of biological systems can address some of the problems associated with traditional methods of chemical synthesis, opening up a window for making use of cells, both natural and unnatural. Such a green biosynthesis is simple, low-cost, mild, less toxic and highly repeatable, offering new insights for sustainable chemistry.

4. Experimental

HPLC-ICP-MS: Measurements were performed using an HPLC system (Shimadzu, Japan) for chromatographic separations and Aligent 7500a ICP-MS (Aligent, USA) for online element-specific detection. The chromatographic system consisted of a high pressure liquid chromatographic pump (LC-10AD, Japan) and a 5 μm ODS stationary phase column (4.6 mm × 150 mm shim-pack CLC, Japan). The mobile phase compositions used were as follows: 99.2% Milli-Q ultra-pure water (18.2 $M\Omega \cdot cm^{-1}$, Millipore, Molsheim, France), 0.4% 1-butyl-3-methylimidazolium chloride and 0.4% 1-butyl-2,3-dimethylimidazolium tetrafluroborate (Hangzhou Chemer Chemical Co., Ltd., Hangzhou, China). Selenium standards, including D,L-selenomethionine (>99%), Se-methylseleno-L-cysteine (98%) and L-selenocystine (98%), were purchased from Acros Organics, USA. Seleno-D,L-ethionine was obtained from Toronto Research Chemicals, Inc., Canada, and both Na_2SeO_3 and Na_2SeO_4 from Wako, Japan. Single-standard stock solutions (100 $\mu g \cdot mL^{-1}$) were prepared by dissolving each of them in ultra-pure water, except for L-selenocystine with 0.1 M hydrochloric acid. These stock solutions were kept at 4 °C in dark. The intracellular components of the seleniumized yeast cells were extracted as previously reported.[27]

Method for Synthesizing CdSe QDs in Live Yeast Cells: The strain *Saccharomyces cerevisiae* BY4742 *MATα his3-Δ1, leu2-Δ0, lys2-Δ0, ura3-Δ0* was used. The yeast cells were cultured for 24 h with YPD broth (yeast extract 10 $g \cdot L^{-1}$, tryptone 20 $g \cdot L^{-1}$, glucose 20 $g \cdot L^{-1}$) to make sure the yeast cells were in the SP, and then co-incubated with 5 mM Na_2SeO_3 (see the Supporting Information, Fig. S4a) for 24 h at 30 °C, followed by centrifuging, harvesting, transporting into fresh YPD culture, and mixing with $CdCl_2$ (final concentration: 1 mM) (see the Supporting Information, Fig. S4c). The mixture was finally cultivated with shaking at 30 °C in the dark for 10–40 h to obtain desired CdSe QDs with different colors of fluorescence.

Imaging: The fluorescence microscopic images were captured by a CCD camera (MicroPublisher 5.0 RTV, QIMAGING) mounted on an inverted fluorescence microscope (Olympus I × 51, 100× objective, N.A. = 1.35, (U-MWU 330–385/400/420 nm) excitation filter). The imaging software was Image-Pro Plus

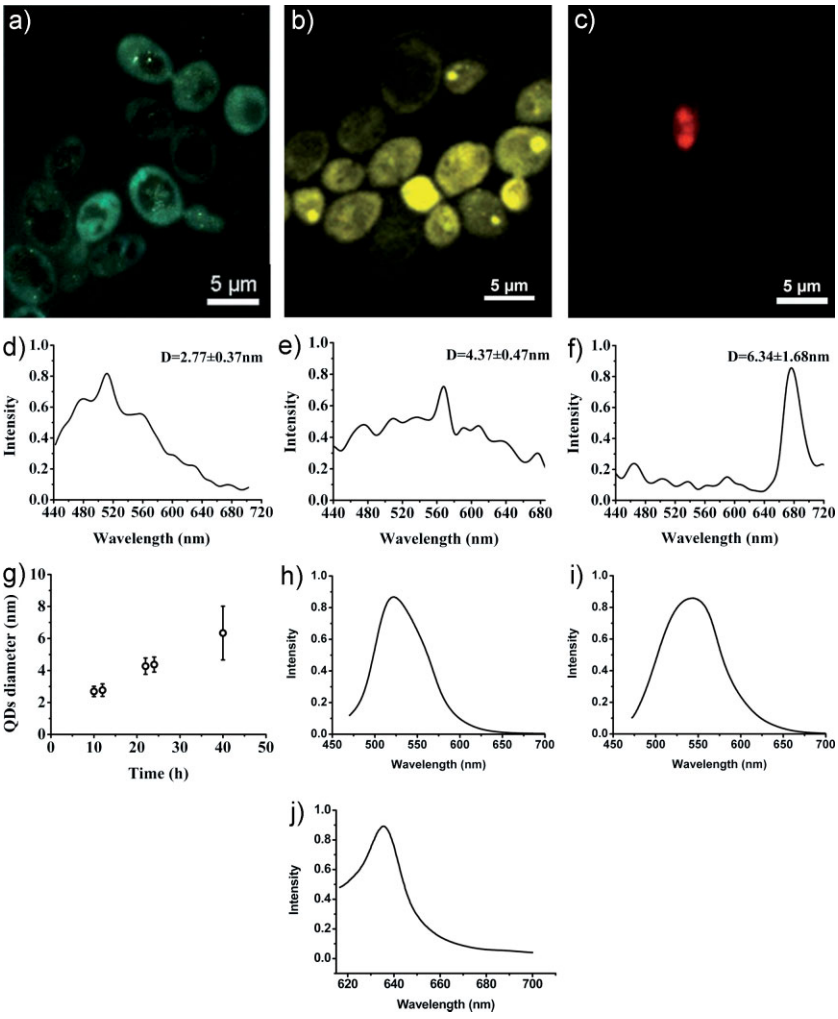

Figure 5. a–c) Confocal images of yeast cells cultured according to the designed route; d–f) In situ fluorescence spectra of intracellular CdSe QDs corresponding to a–c, respectively; g) the dependence of the CdSe QDs diameter on the incubation time; and, h–j) and the fluorescence spectra of CdSe QDs isolated from the different colours of fluorescing yeast cells. The fluorescing color, i.e. the size, of CdSe quantum dots could be simply controlled by adjusting the duration of the co-incubation of the seleniumized yeast cells with $CdCl_2$. Different durations of $CdCl_2$ treatment led to various fluorescence colors of the yeast cells, for example, 12 h for green (a), 24 h for yellow (b) and 40 h for red (c). The results in (g) show the sizes of CdSe QDs increasing gradually from 2.69 ± 0.07 nm ($n = 79$) to 3.04 ± 0.20 nm ($n = 20$), 4.15 ± 0.18 nm ($n = 20$), 4.37 ± 0.17 nm ($n = 31$) and 6.34 ± 0.79 nm ($n = 18$) with increasing duration of $CdCl_2$ treatment. The alpha level was 0.05 for all the QD diameter statistical tests and n is the number of QDs. The corresponding fluorescence spectra of isolated CdSe QDs from the fluorescing yeast cells of different duration of the co-incubation of the seleniumized yeast cells with $CdCl_2$: 12, 14 and 40 h for (h–j), respectively. Fluorescence spectra were recorded at room temperature on a spectrofluorophotometer using PMT R3788-02 and PMT R212-14 as the detectors (RF-530 LPC, Shimadzu, Japan).

6.0. In situ laser confocal scanning microscopic images were obtained on a laser confocal scanning microscope (Nikon TE2000-E, C1SI), which was also used for recording the in situ fluorescence spectra. The spectra were analyzed with the software EZ-C1. In Figure 5a–c, the fluorescence of the images was excited by a 405 nm laser beam. The fluorescing cells were harvested by centrifuging at 2 000 rpm for 5 min, washing three times with Tris-HCl (pH 8.0), and then resuspending in the same buffer.

In situ TEM images for fluorescing cells were acquired with a CCD camera (Dual vision 300 W CCD, Gatan Corporation) equipped on an HRTEM (JEOL-JEM 2010EF). The cells were harvested by centrifuging at 2 000 rpm for 5 min, washing three times with ultra-pure water, resuspending in ultra-pure water and then drying on a piece of ultra-thin carbon coated copper grid.

Isolation of CdSe QDs from the Fluorescing Yeast Cells: The fluorescing yeast cells were harvested by centrifuging at 2 000 rpm for 5 min, washing and resuspending with 0.02 M sodium phosphate buffer (pH 8.0). The cells were crushed with glass beads (acid-washed, 425–600 μm, Sigma, U.S.A.) for 20 min on a vortex (Barnstead International, IOWA, U.S.A.) and then ultrasonicated for 30 min with a sonicator (XinZi Tech, Ningbo, China). The suspension was centrifuged at 12 000 rpm for 20 min to precipitate out the fragments of yeast cells. Then the CdSe QDs were purified by filtrating with centrifugal filter devices (amicon ultra–4, 100k, Millipore Corporation, U.S.A) to remove the compounds smaller than 100 kDa.

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