Artificially regulated synthesis of nanocrystals in live cells

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ABSTRACT
Live cells, as reservoirs of biochemical reactions, can serve as amazing integrated chemical plants where precursor formation, nucleation and growth of nanocrystals, and functional assembly, can be carried out accurately following an artificial program. It is crucial but challenging to deliberately direct intracellular pathways to synthesize desired nanocrystals that cannot be produced naturally in cells, because the relevant reactions exist in different spatiotemporal dimensions and will never encounter each other spontaneously. This article summarizes the progress in the introduction of inorganic functional nanocrystals into live cells via the ‘artificially regulated space–time-coupled live-cell synthesis’ strategy. We also describe ingenious bio-applications of nanocrystal–cell systems, and quasi-biosynthesis strategies expanded from live-cell synthesis. Artificially regulated live-cell synthesis—which involves the interdisciplinary application of biology, chemistry, nanoscience and medicine—will enable researchers to better exploit the unanticipated potentialities of live cells and open up new directions in synthetic biology.

Keywords: cell, synthesis, nanocrystal, quantum dot, artificial, metabolism

INTRODUCTION
In the last decade, cells have been exploited as a powerful tool to accomplish unexpected tasks through artificial regulation. Given the numerous reactive intermediates generated in the sophisticated metabolic networks and the subtle redox balance that supports intracellular homeostasis, cells can function as chemical factories to produce various nanocrystals [1]. Some redox reactions endow the cell with the ability to change the valence of heavy metal ions, which is essential for its survival in stressful/toxic environments [2,3]. Under the pressure of natural selection, some microorganisms have evolved the ability to spontaneously synthesize nanoparticles, or even hierarchical structures. This is known as biomineralization, bioremediation or bioleaching. These processes have been incorporated into important strategies to produce minerals, mainly natural products, with marvelous functions [4].

However, it is still a great challenge to deliberately direct intracellular reactions and pathways to synthesize desired products that cannot be produced naturally in the cell, in cases where the required reactions exist in different spatiotemporal dimensions and would never coincide spontaneously.

To overcome this barrier, we proposed the concept of ‘artificially regulated space–time-coupled live-cell synthesis’ (ARLCS), which means purposefully and precisely coupling a series of intracellular metabolic pathways in an appropriate spatial and temporal sequence to synthesize nanocrystals, such as fluorescent semiconductor quantum dots (QDs), in live cells. Compared with conventional chemical synthesis approaches using toxic organic solvents at elevated temperature and pressure, ARLCS can be effectively controlled in mild (i.e. physiological) conditions, endowing the nanocrystals with inherent biostability and biocompatibility [5,6]. In addition to the specific desired physicochemical properties of the nanocrystals, they are protein encapsulated and water dispersible without needing to resort to additional biofunctionalization processes such as ligand exchange and encapsulation treatment. Thus, ARLCS offers a unique route to synthesizing nanocrystals that are suitable for specific bio-applications. The strategy has subsequently been expanded to synthesize nanocrystals in cell-free systems that mimic intracellular processes in a mild aqueous solution containing enzymes, electrolytes, peptides and coenzymes. The synthesis routes in these quasi-biosynthesis...
strategies can be elaborately designed based on an understanding of the principles underlying the live-cell synthesis of various nanocrystals (Fig. 1).

In this review, we focus on: (i) synthesis of inorganic nanocrystals with desired properties in live cells; (ii) promising and ingenious bio-applications of nanocrystal-synthesizing cells; and (iii) quasi-biosynthesis systems. Finally, we discuss future possibilities and challenges of the live-cell synthesis and cell-free quasi-biosynthesis strategies.

**HOW TO SYNTHESIZE DESIGNER NANOCRYSTALS IN LIVE CELLS**

Precursors resulting from cellular metabolism are a prerequisite for nanocrystal synthesis. The reactivity of the precursors, the fed amount of raw chemicals, the feeding ratio and the feeding order are all vitally important to the morphology, size and properties of the product nanocrystals. In conventional chemical synthesis, the precursors are usually prepared separately before the production of nanocrystals, whereas, in ARLCS, the precursors are produced by feeding the cell with suitable chemicals and triggering intended intracellular metabolic pathways. These reactive precursors are mostly complex, unstable and are present in the cell at trace levels. However, by regulating the amount and oxidation state of the chemicals, the time and order of addition, and the incubation time, we can adjust the production and reactivity of the cell-generated precursors.

In the case of live-cell synthesis of CdSe QDs, it is essential to produce both reactive Se- and Cd-containing precursors at the proper intracellular location and timepoint (Fig. 2). Selenium is a valence-variable element that can exist in multiple organic and inorganic forms. Among these, high-valence Na2SeO3 [Se(IV)] is generally selected as a selenium source; it can be reduced to selenodiglutathione (GSSeSG) in the intracellular redox environment, driven by reduced thiols (RSH) such as glutathione (GSH) (reaction 1). GSSeSG can be further reduced to unstable low-valence selenium, glutathioselenol (GSSeH), catalyzed by GSH-related enzymes such as glutathione reductase (GR) in the cytoplasm and mitochondria (reaction 2) [5]. GSSeH either spontaneously decomposes into GSH and elemental selenium (Se0) or is further reduced to volatile hydrogen selenide (H2Se/Se2−) by GSH (reactions 3 and 4) [7]. The downstream metabolites of hydrogen selenide are multiple organoselenium compounds, including selenocysteine (SeCys), L-selenocystine [(Cys-Se)2] and selenomethionine (SeMet) (Fig. 2); this has been confirmed in *Saccharomyces cerevisiae* cells by high-performance liquid chromatography coupled with inductively coupled plasma mass spectrometry analysis and the use of selective selenol probes [5,8,9]. The consumption of GSH induces the upregulation of the expression of cysteine-synthesis-related genes, which can promote the conversion of SeMet to SeCys [10]. Unstable low-valence selenium compounds are the reactive Se-containing precursors.
Figure 2. Metabolic pathways in ARLCS of fluorescent quantum dots in *Saccharomyces cerevisiae* and *Escherichia coli*.

As shown in reactions 3 and 4, the metabolite that is formed from GSSeH depends on the GSH concentration. When the level of GSH is moderate, Se⁰ is produced and accumulated, which is a detoxification mechanism in many microorganisms [11]; upon formation of Se⁰, the cells turn red (the color of Se⁰) [12]. A high level of both GSH and reduced nicotinamide adenine dinucleotide phosphate (NADPH) is essential to produce reactive Se-containing precursors (Fig. 3) [13]. In yeast cells, GSH and NADPH can be synthesized with high efficiency during the stationary phase (SP), which is hence usually selected as the period when the Se source is added to cells for ARLCS [5,12]. As well as GSH, other intracellularly generated RSH, including glutaredoxin (GRX) and thioredoxin (TRX), whose expression levels can be significantly elevated by addition of glucose in *Escherichia coli* (E. coli) cells, can also act as reducing agents to convert Na₂SeO₃ to reactive Se-containing precursors [14].

Particular pathways in certain bacteria can also affect the products of Se reduction. For instance, in *Shewanella oneidensis* MR-1, a widely distributed dissimilatory metal-reducing bacterium, fumarate reductase FccA reduces Na₂SeO₃ to Se⁰ in the periplasm [15]. The extracellular electron transfer (EET) ability of this bacterium enables the reduction, which is regulated by the key membrane-anchored protein CymA. Impairing EET by deleting *cymA* gene significantly enhanced the production of CdSe QDs in the cytoplasm, indicating that several intracellular Se reduction pathways can proceed in parallel, leading to distinct products [11]. In *E. coli*, Na₂SeO₃ uptake is inhibited by phosphate, and intracellular Se is transformed from Se⁰ to organoselenium compounds when the phosphate level is high. This is mainly attributed to the competitive uptake of phosphate and Na₂SeO₃ mediated by the low-affinity phosphate transporter PitA [16].

In addition, a high concentration of Na₂SeO₃ (≥10 mM) results in the generation of reactive oxygen species, bringing about oxidative stress, which

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**Figure 3.** Metabolic pathway of glutathione in *S. cerevisiae* yeast cells, which participates in the ARLCS of CdSe QDs. (A) The formation of γ-glutamylcysteine is the rate-limiting reaction of glutathione synthesis, catalyzed by the γ-glutamylcysteine ligase (GCL, *GSH1*-encoded). The γ-glutamylcysteine is further consumed to produce reduced glutathione (GSH) by reacting with glycine in the presence of catalyst glutathione synthetase (GS, *GSH2*-encoded). The oxidized glutathione (GSSG) is reduced to GSH under the catalysis of the glutathione reductase (GR, *GLR1*-encoded). (B) Fluorescence images (scale bar, 5 μm) and (C) fluorescence intensity of wild-type (WT) and engineered Δgsh1, Δgsh2, and Δgr1 *S. cerevisiae* cells. (D) Flow cytometry measurement and fluorescence images of WT (blue) and engineered (PGAL1::GSH1) *S. cerevisiae* (red) cells after ARLCS of CdSe QDs (scale bar, 10 μm) [13].

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**Reactions:***

\[
4\text{GSH} + \text{SeO}_3^{2−} + 2\text{H}^+ → \text{GSSeSG} + \text{GSSG} + 3\text{H}_2\text{O} \tag{1}
\]

\[
\text{GSSeSG} + \text{NADPH} + \text{H}^+ \xrightarrow{\text{GR}} \text{GSSeH} + \text{GSH} + \text{NADP}^+ \tag{2}
\]

\[
\text{GSSeH} → \text{Se}^0 + \text{GSH} \tag{3}
\]

\[
\text{GSSeH} + \text{GSH} → \text{H}_2\text{Se} + \text{GSSG} \tag{4}
\]

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**Figure 2.** Metabolic pathways in ARLCS of fluorescent quantum dots in *Saccharomyces cerevisiae* and *Escherichia coli*. **Figure 3.** Metabolic pathway of glutathione in *S. cerevisiae* yeast cells, which participates in the ARLCS of CdSe QDs.
inhibits the growth of yeast cells [5,17]. Therefore, once the concentration of Na$_2$SeO$_3$ is too high, a detoxification pathway will be triggered, and Se$^{0}$ becomes the predominant product. To maximize the yield of organoselenium products and hence improve the yield of QDs, the concentration of Na$_2$SeO$_3$ must be moderate and optimized. Although Na$_2$SeO$_3$ can also serve as a high-valence Se source, it is seldom employed because of its high toxicity and low transformation efficiency to reactive Se-containing precursors [9,18].

As well as Se, Cd is required for the synthesis of CdSe QDs. Because Cd(II) is very toxic to cells, organisms have evolved several mechanisms to counter Cd toxicity. Some intermediates generated in the detoxification process are suitable Cd-containing precursors for CdSe synthesis in live cells. For instance, in yeast cells, Cd(II) is sequestered by metallothioneins or chelated by GSH because of their high cysteine content [10,19–21]. The generated bis(glutathionato)cadmium [Cd(GS)$_2$], a reactive Cd-containing precursor, is subsequently transported to and isolated in vacuoles to achieve detoxification; this transport is regulated by adenosine triphosphate (ATP)-binding cassette transporters [22]. However, once Cd(GS)$_2$ is sequestered in the vacuole, there is little Cd-containing precursor left in the cytoplasm, which is unfavorable for the formation of CdSe. In E. coli, ingested Cd(II) binds predominantly with phosphate groups and is mainly transformed into Cd$_3$(PO$_4$)$_2$ precipitate to decrease the toxicity of cadmium [14,23]. These pathways for Cd(II) might outcompete the CdSe synthesis pathway [14]. However, glucose addition can enhance the synthesis of NADPH and RSH, favoring competitive binding of Cd with the RSH over the high cysteine content [10,19–21]. The generated bis(glutathionato)cadmium [Cd(GS)$_2$], a reactive Cd-containing precursor, might outcompete the CdSe synthesis pathway [14]. However, glucose addition can enhance the synthesis of NADPH and RSH, favoring competitive binding of Cd with the RSH over the high cysteine content [10,19–21]. The generated bis(glutathionato)cadmium [Cd(GS)$_2$], a reactive Cd-containing precursor, might outcompete the CdSe synthesis pathway [14]. However, glucose addition can enhance the synthesis of NADPH and RSH, favoring competitive binding of Cd with the RSH over the high cysteine content [10,19–21]. The generated bis(glutathionato)cadmium [Cd(GS)$_2$], a reactive Cd-containing precursor, might outcompete the CdSe synthesis pathway [14]. However, glucose addition can enhance the synthesis of NADPH and RSH, favoring competitive binding of Cd with the RSH over the high cysteine content [10,19–21]. The generated bis(glutathionato)cadmium [Cd(GS)$_2$], a reactive Cd-containing precursor, might outcompete the CdSe synthesis pathway [14]. However, glucose addition can enhance the synthesis of NADPH and RSH, favoring competitive binding of Cd with the RSH over the high cysteine content [10,19–21]. The generated bis(glutathionato)cadmium [Cd(GS)$_2$], a reactive Cd-containing precursor, might outcompete the CdSe synthesis pathway [14]. However, glucose addition can enhance the synthesis of NADPH and RSH, favoring competitive binding of Cd with the RSH over the high cysteine content [10,19–21]. The generated bis(glutathionato)cadmium [Cd(GS)$_2$], a reactive Cd-containing precursor, might outcompete the CdSe synthesis pathway [14]. However, glucose addition can enhance the synthesis of NADPH and RSH, favoring competitive binding of Cd with the RSH over the high cysteine content [10,19–21]. The generated bis(glutathionato)cadmium [Cd(GS)$_2$], a reactive Cd-containing precursor, might outcompete the CdSe synthesis pathway [14]. However, glucose addition can enhance the synthesis of NADPH and RSH, favoring competitive binding of Cd with the RSH over the high cysteine content [10,19–21]. The generated bis(glutathionato)cadmium [Cd(GS)$_2$], a reactive Cd-containing precursor, might outcompete the CdSe synthesis pathway [14]. However, glucose addition can enhance the synthesis of NADPH and RSH, favoring competitive binding of Cd with the RSH over the high cysteine content [10,19–21]. The generated bis(glutathionato)cadmium [Cd(GS)$_2$], a reactive Cd-containing precursor, might outcompete the CdSe synthesis pathway [14]. However, glucose addition can enhance the synthesis of NADPH and RSH, favoring competitive binding of Cd with the RSH over the high cysteine content [10,19–21]. The generated bis(glutathionato)cadmium [Cd(GS)$_2$], a reactive Cd-containing precursor, might outcompete the CdSe synthesis pathway [14]. However, glucose addition can enhance the synthesis of NADPH and RSH, favoring competitive binding of Cd with the RSH over the high cysteine content [10,19–21]. The generated bis(glutathionato)cadmium [Cd(GS)$_2$], a reactive Cd-containing precursor, might outcompete the CdSe synthesis pathway [14]. However, glucose addition can enhance the synthesis of NADPH and RSH, favoring competitive binding of Cd with the RSH over the high cysteine content [10,19–21]. The generated bis(glutathionato)cadmium [Cd(GS)$_2$], a reactive Cd-containing precursor, might outcompete the CdSe synthesis pathway [14]. However, glucose addition can enhance the synthesis of NADPH and RSH, favoring competitive binding of Cd with the RSH over the high cysteine content [10,19–21].
a broad range of transition metal chalcogenide semiconductor nanocrystals, such as CdS, PbS and ZnS, have been synthesized in different microorganisms [23,29–37]. These nanocrystals are formed following the typical intracellular biomineralization pathways triggered by metal–thiolate polynuclear clusters formed via the interaction between the transition metal and thiolate. This mechanism is different from the concept of ARLCS. On one hand, the biosynthesis of CdS and PbS in these reports generally makes use of thiolate-containing peptides in which sulfur naturally exists in a low oxidation state (S2−). The S2− ions can directly bind to metal ions, and generate polynuclear clusters that nanocrystals develop from. In contrast, the low-valence reactive Se in the ARLCS of CdSe is generated from the reduction of high-valence Se in raw chemicals via intracellular metabolic reactions. On the other hand, the CdS or PbS nanocrystals biosynthesized in the typical intracellular biomineralization pathways accumulate in vacuoles, in which the metal precursors are sequestered in metal–γ-glutamyl complexes [29]. However, in the ARLCS of CdSe, multiple metabolic pathways of Se and Cd are spatially and temporally coupled (reactions 1–4). As a result, the Cd-containing precursors generated in the cytoplasm and mitochondria are hijacked by reactive Se-containing precursors and transformed into the desired QDs before isolation in vacuoles can occur [5]. Because of this mechanism, the diameter and corresponding emission wavelength of the QDs can be tuned by the amount of the added raw chemicals and the incubation time, which has never been achieved using the biomineralization strategy. In addition, the growth of the nanocrystals can be controlled (made slow) in live cells, facilitating the crystallization and production of QDs with desired sizes and optical properties.

As well as fungal and bacterial cells, the ARLCS strategy has been extended to earthworms [38]. In 2013, Stürzenbaum et al. used the same strategy to expose Lumbricus rubellus earthworms to soil spiked with CdCl2 and Na2TeO3 for 11 days, achieving the synthesis of CdTe QDs in the earthworms. Although their proposed synthesis mechanism was similar to the above-mentioned reactions 1–4 (but replacing the Na2SeO3 with Na2TeO3), further experimental evidence is necessary to support such a mechanism [38]. Their as-prepared CdTe QDs are 2.3 nm in diameter with a fixed emission maximum at 520 nm. The fluorescence lifetime is only 4.54 ns, which is 20-fold lower than that of the CdSe synthesized in S. cerevisiae MR-1 cells (99.8 ns) [11,38]. The lifetime, defined by the average time the electron spends in the excited state prior to returning to the ground state, is one of the most important characteristics of a fluorescent nanocrystal. The fluorescent nanocrystals with long lifetimes are preferred in bio-applications. Based on the proposed mechanism of ARLCS, the optical properties of the QDs synthesized in L. rubellus earthworms could be enhanced by artificially regulating the amount of added raw chemicals and the incubation time.

The ‘space–time coupling ARLCS strategy’ was first proposed to account for the synthesis of CdSe QDs in S. cerevisiae in 2009 [5,8,26,28,39,40], and it has been expanded to the synthesis of various nanocrystals, such as CdTe, ZnSe, CuSe and Te nanorods, in other cell types including Staphylococcus aureus [41–43], Fusarium oxysporum [44], E. coli [45], S. oneidensis [11,15,46], Bacillus licheniformis [47], Bacillus amylopliticfaciens [48], Rhodotorula mucilaginosa [49], Candida utilis [50], mammalian cells [51], Tetrahymena pyriformis [52,53], Caenorhabditis elegans [54] and earthworms [38,55], by easily altering the raw chemicals fed to the cells (Fig. 1). As mentioned above, the redox reactions that participate in generating the reactive anion and cation precursors are dependent on the intracellular metabolic network. By elaborately coupling these reactions, supercells containing functional inorganic nanocrystals are created for some ingenious bio-applications.

**WHAT TO DO WITH THE NANOCRYSTAL-CONTAINING CELLS**

Despite terrific progress in the synthesis of nanocrystals in live cells, it is still challenging to conveniently and skillfully use the fluorescence properties of intracellular nanocrystals for further application. One straightforward idea is to isolate the synthesized fluorescent nanocrystals from cells by ultrasonication, ultrafiltration and centrifugation. For instance, extracted intracellular-synthesized core-shell QDs (CdS,Se1−x core with protein- and phosphate-rich capping synthesized in E. coli cells) can be directly used as a sensitive Hg(II) probe based on a Cd(II)–Hg(II) substitution; these QDs exhibit a linear fluorescent response to Hg(II) concentration in the range 1.5–100 nM. Strikingly, in the higher concentration range 0.1–10 μM, Hg(II) can be easily detected by the naked eye once the load of QDs has been raised. Therefore, this is a label-free method for Hg(II) detection, which implies the high potential of live-cell-synthesized QDs for environmental monitoring applications that advance the development of environmental analytical techniques toward higher sustainability [45].

In some cases, the intricate capping proteins of intracellular QDs make the extraction and
purification processes much more difficult. Even worse, the laborious and time-consuming extraction is just the first step, because the nanocrystals need to be further engineered with target molecules before application. Unfortunately, all these processes can induce aggregation of the nanocrystals and impair their fluorescence properties. One way to circumvent the above problems is to exert the fluorescence of intracellular nanocrystals in situ, without extraction and purification.

For instance, making use of the specific interaction between protein A expressed on the surface of S. aureus cells and the Fc fragment domain of antibodies, cells with in-situ-synthesized QDs can be readily transformed into nanobioprobes with strong, stable and uniform fluorescence (Fig. 4). This avoids the need for extraction, purification and cell surface modification procedures such as covalent conjugation or genetic and metabolic engineering. Hence, the fluorescence intensity can be maintained to the largest extent, which enhances the sensitivity of detection. Remarkably, this versatile nanobioprobe can be easily adapted to detect diverse pathogens, tumor cells and other biomolecules by simply changing the antibody conjugated to the cell surface. By combining with immunomagnetic beads, the detection limit reached 8.94 ng/mL in H9N2 influenza A virus detection [41].

Live-cell-synthesized QDs have also been employed to efficiently label microvesicles (MVs) in situ (Fig. 5). Cell-derived MVs can be secreted from almost all types of mammalian cells into the extracellular space, and they play crucial roles in intercellular signaling, communication and transporta-

![Figure 4](image-url)  
**Figure 4.** Schematic illustration of the generation and application of nanobioprobes. (A) Fabrication of bioprobes by ARLCS. (B) Using fluorescent-biotargeting bifunctional cells as bioprobes for pathogen detection [41].

![Figure 5](image-url)  
**Figure 5.** Designer cell-self-implemented labeling of microvesicles (MVs) in situ with intracellular-synthesized quantum dots. (A) Schematic illustration of an efficient and biofriendly strategy for one-step labeling of MVs by ARLCS of fluorescent QDs in live MCF-7 cells. (B) In situ high-resolution transmission electron microscopy (TEM) image of the QDs in an MV. (C) 90% of MVs can be labeled by the intracellular-synthesized QDs, as measured by flow cytometry analysis [51].
Moorella thermoacetica–CdS reaction schemes. (A) In the \textit{M. thermoacetica}–CdS hybrid system, the photogenerated electrons generated from CdS nanoparticles synthesized by \textit{M. thermoacetica} can drive the photosynthesis of acetic acid from CO$_2$. (B) Possible photosynthetic mechanism of the \textit{M. thermoacetica}–CdS hybrid system, the photogenerated electrons generated from CdS nanoparticles synthesized by \textit{M. thermoacetica}.}

**QUASI-BIOSYNTHESIS SYSTEMS EXPANDED FROM THE ARLCS**

As discussed in the last section, it is difficult to purify the QD products from the intricate intracellular environment. Therefore, it is necessary to develop methods that retain the green characteristics of live-cell synthesis whilst avoiding extraction and purification procedures. In the live-cell synthesis route of CdSe, the NADPH/GR system and GSH play important roles in maintaining the reducing environment required for production of the necessary Cd- and Se-containing precursors [5,7,10,13]. Inspired by the principles of ARLCS, we created a cell-free quasi-biological synthesis system containing GSH, NADPH and GR, which is simple compared with the intricate environment in live cells. These bioactive agents can reduce metal ions \textit{in vitro} and produce reactive precursors for QD synthesis, although they have seldom been used in the chemical synthesis of nanomaterials so far because of the lower reactivity of the produced precursors. Using this quasi-biological synthesis system, our group successfully synthesized ultrasmall (sub-3 nm) near-infrared (NIR) fluorescent and water dispersible Ag$_2$Se QDs at 90 °C [60].

As in ARLCS, the crucial point of quasi-biological synthesis is to obtain Ag- and Se-containing precursors in appropriate oxidation states. With the aid of GSH, NADPH and GR, SeO$_2$$^{2-}$ is reduced to low-valence GSSeH, as in the similar process that occurs in live cells in ARLCS. GSSeH, which can react with metal ions such as Ag(I), is predesigned as the Se-containing precursor (reactions 1 and 2). The intended processes of Na$_2$SeO$_3$ reduction have been evidenced, because all the intermediate products, including GSH, GSSG (oxidized GSH), GSSeSG, and GSSeH, were detected by high-performance liquid chromatography-mass spectrometry. To generate an appropriate Ag-containing precursor, alanine (Ala) is chosen as a stabilizer because it can form Ag(1)–Ala complex and also act as a ligand to stabilize the generated Ag$_2$Se QDs. The diameter of the synthesized Ag$_2$Se QDs can be precisely controlled by tuning the molar ratio of Ag-containing precursor to Se-containing precursor. Thereby, the photoluminescence (PL) emission peak can be tuned from 700 to 820 nm, corresponding to QD diameters from 1.5 to 2.4 nm [60]. These Ag$_2$Se QDs show a strong and efficient cathodic electrogenerated chemiluminescence (ECL) signal on a glassy carbon electrode with K$_2$S$_2$O$_8$ as co-reactant in aqueous solution. The ECL spectrum of the Ag$_2$Se QDs exhibited a peak at 695 nm, consistent with the peak in the PL spectrum of Ag$_2$Se QD solution, indicating that Ag$_2$Se QDs had no deep surface traps that usually impaired the fluorescent properties of QDs [61].

Furthermore, once the synthesized Ag$_2$Se QDs are functionalized by glucose (Glc–Ag$_2$Se QDs), they demonstrate high uptake in almost all types of cancer cell; this has been applied to \textit{in vivo} long-term tumor imaging. The fluorescence of Glc–Ag$_2$Se QDs from the targeted tumor can be observed for at least 7 days, indicating the outstanding \textit{in vivo} stability of the QDs [62]. More importantly, because the Glc–Ag$_2$Se QDs are ultrasmall, this probe can be excreted via the kidneys without significant long-term accumulation in organs, which is also favorable for \textit{in vivo} imaging applications [62,63]. The biosafety of the Ag$_2$Se QDs has been demonstrated by pathological analysis, blood biochemical analysis and body weights in which the QDs exhibit no appreciable \textit{in vivo} toxicity [63]. All these excellent features mean that the Ag$_2$Se QDs have great potential for future clinical use in tumor imaging.

The surface of these ultrasmall Ag$_2$Se QDs is Ag(I) rich, so it can be transformed into partial O$_2$−-terminated by NaOH treatment. This surface engineering facilitates Mn(II) doping via strong coordination with the surface O$_2$−, generating ultrasmall water dispersible Ag$_2$Se@Mn QDs. The resultant QDs possess high NIR fluorescence quantum yield (13.2%) and longitudinal relaxivity (12.87 mM$^{-1}$ s$^{-1}$). This longitudinal relaxivity is almost four times higher than that of the commercial magnetic resonance imaging contrast agent Gd-diethylenetriaminepentacetate (Gd-DTPA) (Fig. 7) [57]. The ultrasmall size of the Ag$_2$Se@Mn QDs enables them to be directly and efficiently loaded into MVs by electroporation, instantly and reliably conferring both NIR fluorescence and magnetic resonance traceability on MVs. The complementary imaging capabilities of the Ag$_2$Se@Mn QDs have enabled long-term dual-mode \textit{in vivo} tracking of MVs with high resolution,
Figure 7. Preparation and application of Ag₂Se@Mn QDs. (A) Fabrication of the Ag₂Se@Mn QDs by controlling the reaction of Mn²⁺ with Ag₂Se nanocrystals pre-treated in NaOH solution. (B) Labeling of cell-derived MVs with Ag₂Se@Mn QDs by electroporation, and high-resolution dual-mode in vivo imaging [57].

Similarly, by coupling reduction of Na₂SeO₃ with detoxification of Pb(II) in the quasi-biosystem, monodispersed PbSe nanocubes were synthesized in aqueous solution with controllable sizes (Fig. 8) [64]. The crystallization mechanism is that the amorphous precursors are transformed into mesocrystals as intermediates and finally to nanocubes, suggesting that a non-classical crystallization occurred during the formation of the PbSe nanocubes [64]. Using the similar reduction pathways of TeO₃²⁻, Te nanorods with uniform and tunable lengths (ranging from 10 to 200 nm) can be synthesized as we expected [46]. The molar extinction coefficients are \(1.54 \times 10^9\) (absorption at 549 nm) and \(8.06 \times 10^8\) M⁻¹ cm⁻¹ (absorption at 410 nm) for Te nanorods, respectively, which is comparable to gold nanorods with similar lengths, indicating that Te nanorods may serve as potential photothermal materials in tumor therapy [46].

The mild quasi-biosynthesis system can also be employed to synthesize noble-metal (i.e. gold and silver) clusters and nanoparticles. Compared with conventional methods, this system slows down the reduction of the Au-containing precursors by using a bioreducing agent, NADPH, instead of a strong reducing agent such as NaBH₄. NADPH molecules initially interact with Au(I) ions via electrostatic force and then reduce them to gold nanoclusters (AuNCs) [65]. Owing to the relatively slow rate of biomimetic reduction, the pH-dependent reduction potential of the reducing agent, and the favorable structure of the capping molecules, we have successfully realized kinetically controlled formation of gold clusters and nanoparticles in mild conditions. The resulting glutathione-capped gold clusters consist of \(~60\) Au atoms (Au₆₀NCs) with 1.3-nm diameter. Wheat germ agglutinin (WGA)-capped gold clusters consist of 25 Au atoms (Au₂₅NCs) with 1.2-nm diameter [66,67]. Generally, such small metal cores are energetically unstable in solution, and tend to aggregate to lower their specific surface energies. However, in the case of quasi-biosynthesis of Au₆₀NCs, the abundant GSH molecules capping the surface of the Au cores via strong Au–S bonds provide the product with high stability in aqueous solution. WGA was selected to stabilize the Au₂₅NCs via Au–S bonds as it is rich in cysteine residues. Matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry results indicate that each Au₂₅NC is stabilized by only one subunit of WGA [67]. Additionally, the slow production rate of the Au clusters led to separate clusters dispersing in solution, further diminishing the possibility of aggregation [66]. By adjusting the concentration of NADPH, which is easy,
the size of the gold nanoparticles (AuNPs) capped with glutathione can be tuned from 6.1 to 12.6 nm with good stability in aqueous solution even in the presence of a high salt concentration [65]. Furthermore, when Au(III) and Ag(I) ions are introduced into the system simultaneously, uniform sub-5 nm Au–Ag alloy NPs tightly capped by NADPH molecules are synthesized [68].

The quasi-biosynthesis strategy can be used to synthesize nanocrystals of interest in a cell-free system, based on an understanding of metabolic pathways. This strategy opens a new avenue for controllable, facile and efficient synthesis of designer nanocrystals for diverse industrial and biomedical applications.

**SUMMARY AND FUTURE PERSPECTIVES**

Live cells, as reservoirs of biochemical reactions, can serve as amazing integrated chemical plants for the synthesis of nanocrystals, where precursor formation, nucleation and growth of nanocrystals, as well as functional assembly, can be controlled accurately following an artificial program. Since 2009, by artificially coupling a series of intracellular redox reactions in an appropriate spatiotemporal sequence, various inorganic semiconductor QDs and other nanomaterials have been successfully synthesized in bacterial, fungal and mammalian cells. Inspired by these systems, a cell-free quasi-biosynthesis strategy that simplifies the regulation of intracellular reactions has been developed to produce a variety of nanocrystals in mild conditions, further verifying, strengthening and expanding the methodology for ARLCS.

Generally, the properties of elements in the same group of the periodic table are relatively similar; therefore, multiple elements in the same family may share common intracellular metabolic pathways, which can potentially be used to synthesize different nanocrystals in live cells as well as in cell-free quasi-biological systems. Unfortunately, because of the complexity of intracellular metabolic networks, the deduced practicable pathways have so far focused only on the reduction of chalcogenides (including Se and Te). Thus, the metabolic pathways that have been employed so far in ARLCS are merely the tip of the iceberg. In addition, the nanocrystals synthesized by ARLCS are generally distributed in the cytoplasm, and it is difficult to manipulate the synthesis location in the cell. With this review, we hope to intrigue more researchers to explore new strategies and mechanisms for producing diverse multifunctional crystals and even intricate heteronanostructures and hierarchical structures at desired locations and times.

Besides the metabolic networks, various intracellular biomolecules also play irreplaceable roles in ARLCS. Many biomolecules participate in metabolic reactions and regulate the nucleation and growth processes of nanocrystals. Some biomolecules act as ligands, stabilizing the nanoparticles, and may also provide unique features, endowing the inorganic–biological hybrid systems with potent properties. Therefore, it is necessary to explore the type and function of the biomolecule(s) on the surface of the nanocrystals and investigate the interface of the nanocrystals and biomolecules, but this is very challenging, and it has been largely ignored to date.

Generally, nanoparticles need to be isolated and purified before characterization. However, in the ARLCS system, the laborious and time-consuming extraction process can induce the aggregation of nanoparticles and impair their optical properties. Therefore, in situ measurements are required to characterize the size, shape, elemental composition and fluorescence properties of the nanocrystals. Although some powerful approaches, such as electron microscopy, fluorescence spectroscopy, Raman spectroscopy and X-ray absorption near-edge structure spectroscopy are useful for in situ studies, it is still difficult to obtain effective and well-defined results [5,11,69]. Studies of live-cell-synthesized nanocrystals, including the surface ligands for stabilizing them, the type and function of encapsulating proteins, and related underlying mechanisms, are still limited by current methodologies.

Compared with chemical synthesis, it is much easier to controllably produce nanocrystals on a large scale by ARLCS, as the number of cells (reactors) can be amplified exponentially and simply by cell culture.

The concept described here is a synthesis route complementary to chemical synthesis, meeting the need to produce materials that cannot be realized by chemical synthesis. It will enable researchers to better exploit the potentials of live cells and allow for new approaches in synthetic biology via the interdisciplinary application of biology, chemistry, medicine and nanoscience.

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