

## Near-Infrared Light Emitting Luciferase via Biomineralization

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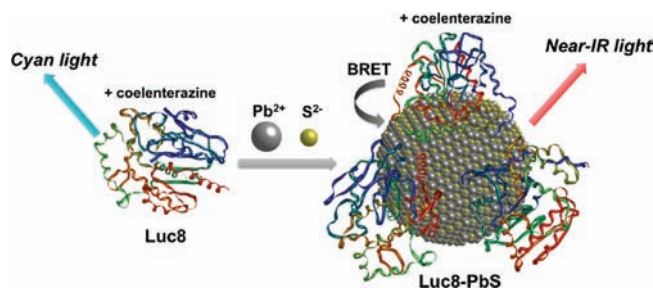
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The biomineralization processes use biomolecules to mediate the growth of inorganic structures in living organisms with distinct functions such as mollusks protection, tissue scaffolding and hardening, iron storage, and metal detoxification.<sup>1</sup> The mild condition and high efficiency of the biomineralization process have inspired research to mimic it *in vitro* using a variety of biomolecules including proteins, nucleic acids, and polysaccharides to build functional inorganic materials.<sup>2</sup> Biomolecules used in most studies merely serve as stabilizers or scaffolds to achieve bottom-up control of the properties of inorganic materials. Synthesis of dual functional bioinorganic hybrid nanostructures directed by active enzymes remains little explored.

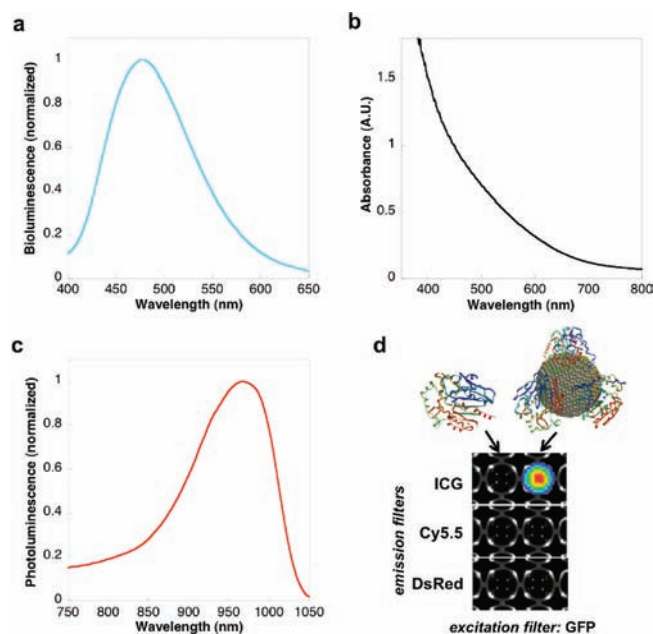
Luciferase is a class of bioluminescent proteins that convert chemical energy into light by oxidizing its substrate luciferin. The emissions of most natural and recombinant luciferases are normally in the visible region from blue to yellow and can be further tuned via bioluminescence resonance energy transfer (BRET) in living organisms or biomimetic systems.<sup>3</sup> Bioluminescence beyond the natural range especially in the near-infrared (NIR) region is highly desired for deep-tissue imaging yet difficult for protein or substrate engineering.<sup>4</sup> Inspired by the biomolecule-templated nanomaterial synthesis, we describe here a new strategy based on biomineralization to generate NIR light emitting luciferase.

Biomolecules are effective ligands for inorganic nanomaterials with functional groups such as amine, thiol, and phosphate chelating metal ions and passivating nanoparticle surfaces postsynthesis.<sup>5,6</sup> In this work we use luciferase enzyme as the template to grow PbS quantum dots (QDs) as a Luc–PbS hybrid nanostructure (Scheme 1). We expect that luciferase can act with dual functions for both QD growth and energy production. The addition of substrate (coelenterazine) initiates BRET and enables the Luc–PbS complex to emit NIR light. In the current study we select an eight-mutation variant of *Renilla reniformis* luciferase (Luc8) that possesses enhanced stability versus the wild-type luciferase.<sup>7</sup> The synthesis was initiated by incubating luciferase with lead acetate (Pb(Ac)<sub>2</sub>) at ambient conditions to allow the binding of Pb<sup>2+</sup> to Luc8 and facilitate the heterogeneous nucleation. Next, a sodium sulfide (Na<sub>2</sub>S) solution was quickly injected into the Luc8–Pb<sup>2+</sup> mixture followed by intense agitation to promote nanocrystal growth. A light brown solution was produced immediately after the introduction of Na<sub>2</sub>S, and the resulting materials were stably dispersed in the solution with high solubility. In contrast, the reaction that proceeded in the absence of Luc8 only produced large insoluble aggregates, which indicates that Luc8 is required for stabilizing PbS nanocrystals. We chose a Pb<sup>2+</sup> to S<sup>2-</sup> ratio of 2:1 for the synthesis given that excess metal ions are usually required for QD surface passivation and preservation of high quantum yield (QY). Different buffers including 1X PBS (pH 7.4), 50 mM Tris

**Scheme 1.** Schematic Illustration of NIR Light Emitting Luciferase via Biomineralization



(pH 7.5), and pure H<sub>2</sub>O were tested for the synthesis. The PbS QDs with the highest luminescence intensity were produced in 50 mM Tris buffer. Those synthesized in pure H<sub>2</sub>O exhibited moderate luminescence. Interestingly, no luminescent materials were generated in 1X PBS which could interfere with the nanocrystal formation and quench their photoluminescence.<sup>8</sup> The luminescence of QDs is also dependent on the protein concentration, and as high as 0.5 mg/mL of Luc8 is required to synthesize luminescent product. The as-synthesized PbS QDs exhibit NIR luminescence with an emission peak in an approximate range of 800–1050 nm (Figure 1c) and with the QY of 3.6% that is comparable to other NIR QDs prepared

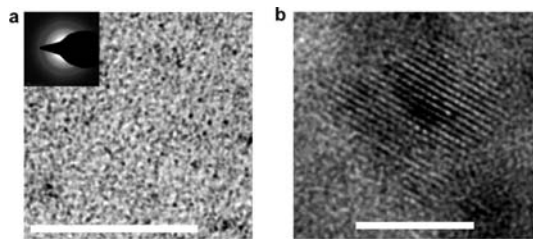


**Figure 1.** Characterization of Luc8 and Luc8–PbS complex. (a) Bioluminescence spectrum of Luc8 in the presence of coelenterazine. (b) Absorption spectrum of PbS QDs. (c) Fluorescence spectrum of Luc8–PbS QDs (excitation: 480 nm). (d) Imaging Luc8–PbS complex by IVIS Imaging System (with light excitation).

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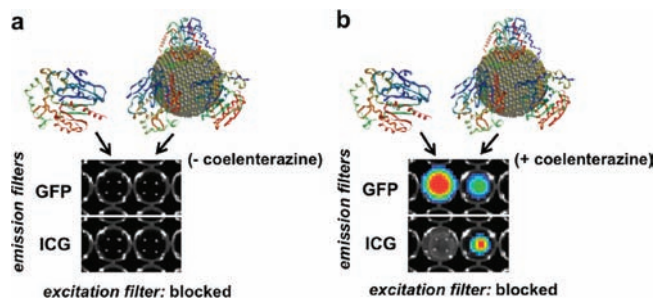
**Figure 2.** Low (a) and high (b) magnification TEM images of Luc8–PbS QDs; scale bars: 200 nm (a) and 5 nm (b). Inset: SAD of QDs.

in water phase.<sup>9</sup> The absorption spectrum of PbS QDs overlaps well with the emission spectrum of Luc8 (Figure 1), which is the prerequisite for BRET. Synthesis of PbS QDs with a different protein–bovine serum albumin as the template under the same condition produced the QDs with same emission spectrum and similar QY.

We further analyzed the luminescence of Luc8–PbS in a 96-well plate with an IVIS imager. A GFP filter (445–490 nm) was used for excitation, and three different band-pass emission filters were used: ICG (810–875 nm), Cy5.5 (695–770 nm), and DsRed (575–650 nm). As shown in Figure 1d, the luminescence of PbS QDs was detected only via an ICG filter, which is consistent with the emission spectrum of PbS QDs. No signal was detected from the control Luc8 alone. The stability of Luc8–PbS was tested at 37 °C over a time course of 72 h (Supporting Information (SI)). No aggregates formed during the incubation but a slow decrease in luminescence intensity was observed, which is probably due to the QD surface oxidation.<sup>10</sup>

The size and morphology of as-synthesized Luc–PbS QDs were characterized using transmission electron microscopy (TEM). Monodisperse spherical nanoparticles can be easily identified with a mean diameter of ~4 nm (Figure 2). The diffraction rings obtained by selected area diffraction (SAD) further confirm that the nanoparticles are crystalline materials (Figure 2a inset). The lattice fringes of a single PbS QD can be clearly visualized in the high resolution TEM image (Figure 2b). The mean hydrodynamic diameter of the Luc8–PbS complex is 19.9 nm as measured by dynamic light scattering (SI).

Next, we proceeded to explore the BRET within the Luc8–PbS complex. The excess Luc8 in the Luc8–PbS solution was removed by filtering through a Microcon YM-100 centrifugal device. The luminescence signals were monitored using an IVIS Imager. The excitation was blocked to monitor the bioluminescence signals. Two emission filters, the GFP filter (515–575 nm) and ICG filter (810–875 nm), were used to detect the Luc8 bioluminescence and PbS luminescence, respectively. As expected, no signal was detected in the absence of coelenterazine (Figure 3a); upon the addition of coelenterazine, bioluminescence signals were detected from Luc8–PbS under the GFP emission filter, confirming that the Luc8 remains active while passivating on the PbS QD surface. The bioluminescence signal of Luc8 alone (left well in Figure 3b) is stronger than that of Luc8–PbS (right well in Figure 3b) because the energy is partially transferred to the PbS QD nonradiatively. The NIR luminescence signal was detected only from Luc8–PbS QDs under the ICG emission filter, confirming that BRET occurred. The BRET ratio of the complex is estimated to be 0.118 (see SI for details). The stability of Luc8 within the Luc8–PbS complex was further monitored (SI Figure S3): 86% bioluminescence



**Figure 3.** Luminescence of Luc8 and Luc8–PbS complex in the absence (a) and presence (b) of coelenterazine (without light excitation).

retained after 1 h. Further decreases after prolonged incubation may be caused by the interactions between the nanoparticle and the protein which could promote protein conformation change and impair its catalytic activity.<sup>11</sup>

In summary, we report a new strategy to construct NIR light emitting luciferase via a biomineralization process. To the best of our knowledge, it is the first example of enzyme-templated synthesis of NIR QDs. The finding that luciferase remains active and enables BRET within the Luc8–PbS complex represents an important advance over previous templated nanomaterial synthesis in which the biomolecules are merely used as stabilizers whereas their biological functions are neglected. This study can be extended to other biomolecules and nanomaterials to generate dual functional nanostructures by integrating the functions of biomolecules and nanomaterials via a simple one-pot synthesis.

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**Supporting Information Available:** Experimental details. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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