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Catalytic Peptides for Inorganic Nanocrystal Synthesis Discovered by New Combinatorial Phage Display Approach

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Nature tends to find the easiest way to grow materials with high efficiency and selectivity at room temperature, and thus biomimetic approach is a potential pathway to break through novel room-temperature inorganic nanocrystal synthesis because enzymes can catalyze the growth of materials in desired structures at low temperature. Recently, several promising peptides and proteins were demonstrated to catalyze the growth of semiconductors[1], however a successful discovery of the catalytic peptide sequences needed to go through trial-and-error processes[2], and the development of the systematic methodology with combinatorial selection is desirable for future materials chemistry. Here, we report a novel evolutionary approach to identify catalytic peptides for the room-temperature growth of target semiconductor materials. The conventional phage display technique is limited to find peptide sequences that bind specific target surfaces, however our combinatorial phage display approach directly screens peptides that catalyze the target material growth. The unique feature in this technique is that the panning process of peptides undergoes in precursor solutions where no reactions are expected to occur without catalysts. Thus, the product is observed only when there are phages in the solution which display peptides to catalyze the target reactions. Our methodology provides a simple and convenient route to discover a catalytic peptide for ZnO nanocrystal growth at room temperature and the ZP-1 peptide induces non-classical crystallization process which conventional ZnO synthetic methods cannot match. The broad impact is highly expected from this outcome since this novel screening technology can be applied to generate a wide range of new catalyses.

Previously, biomineralizing peptides that catalyze the growth of metal nanocrystals have been isolated from tissues and cells of animals and microorganisms[3]. However, a broad range of materials cannot be generated by peptides since there is no combinatorial method to determine specific sequences of catalytic peptides.

Recently, the *in vivo* combinatorial biological protocol is utilized to screen peptides exhibiting selectivity for binding particular inorganic surfaces[4]. Since these peptides cap specific crystalline faces of target materials, some of these peptides happen to mediate the formation of specific inorganic nanoparticles. However, a critical drawback still exists that the selected peptides do not necessarily catalyze the nucleation of nanocrystals at low temperature, and in many cases the selected peptides only influence the shape and the

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structure of resulting nanocrystals[4b, 5]. Therefore, it is strongly desirable to establish a simple method for the combinatorial selection of catalytic peptides to grow semiconductor nanocrystals at room temperature for future materials engineering. By undergoing the biopanning process in the material growth medium, for the first time we developed an evolutionary methodology that can isolate the catalytic peptides directly from the semiconductor growth solution. Our method could identify the peptide that nucleated zinc oxide (ZnO) nanoparticles at room temperature via the non-classical crystallization path[6]. The majority of important oxide semiconductor nanocrystals are grown at high temperature for solar cells, microelectronics, medical imaging, data storage, and sensors, and the low temperature synthesis will be effective to reduce energy consumption in manufacturing processes due to the reduction of the production cost, the facility size (such as cooling systems), and the manpower[1b]. Our methodology provides a simple and convenient route for the discovery of biomineralizing peptides for a variety of inorganic nanocrystals at room temperature.

In the proof-of-concept study, ZnO nanoparticle growth with the proposed combinatorial approach is investigated. ZnO is a useful large band gap semiconductor material, which has widely been applied in solar cells, gas sensors, ultraviolet nanolasers, and blue light-emitting diodes (LEDs)[7]. As illustrated in Fig. 1, first phages were incubated with zinc precursor (10 mg/mL zinc nitrate solution) at room temperature (steps a-b). Among random peptide sequences of phage viruses on the order of 10^{11}, if some of them catalytically grow ZnO particles (step c), theses phages growing the ZnO nanocrystals by their displayed peptides were recovered from the solution by a simple centrifuge method (step d). In this step, the nanocrystals grown on the viruses were confirmed as crystalline ZnO by transmission electron microscopy (TEM) and selected area electron diffraction (SAED) (also see Supplementary Information). After unbound phages were removed by extensive washing with a 0.5% Tween 20 aqueous solution, the residual phage viruses were released from ZnO with a 0.2 M glycine/HCl solution at pH = 2.2, and then the eluted phage viruses were amplified (step e). After three rounds of selection, the peptide sequences displayed on the phage viruses were analyzed for the identification of catalytic peptides (step f) and one peptide, ZP-1 (GAMHLPWHMGTL), was identified. In step b, careful selection of Zn precursors is important to succeed this evolutionary selection method. If conventional Zn precursors that form zinc hydroxide solids in aqueous solution are used, amorphous $Zn(OH)_x$ might grow in addition to ZnO nanoparticles in the phage solution and then the panning process could also contain the sequences of peptides that bind amorphous $Zn(OH)_x$ nanoparticles. It should also be noted that our method has an advantage to reduce the number of non-targeted phages accidently contained in the final selection because inorganic NP-free viruses are less likely to be collected with NP-grown viruses by the centrifugation due to the significant mass difference[8].

Next, to confirm whether the selected peptide can catalyze the crystallization without virus templates, we mixed ZP-1 peptide (50 mg/mL) with the same zinc precursors (10 mg/mL). When ZP-1 peptide was incubated in the growth solution for 4 days, polydispersed particles with diameter of 20-100 nm were observed in the TEM image (Fig. 2a) after purifying with centrifugation three times. A control experiment without using peptides fails to grow any nanoparticles, demonstrating that the ZP-1 peptide plays a critical role in the nucleation and the catalytic crystal growth. High-resolution TEM (HR-TEM) image reveals that the white-circled area in the inset of Fig. 2a possesses multiple crystalline domains with diameters less than 5 nm (Fig. 2b). The *d* spacing of this single crystal domain is measured in 2.82 ± 0.05 Å, corresponding to the (100) lattice fringe of wurtzite ZnO. SAED patterns further confirm the single crystalline nature of this domain (Fig. 2f and Fig. S3b) and the *d* spacings in a = 0.324 982 nm and c = 0.520 661 nm agree with the wurtzite structure of ZnO (JCPDS card no. 36-1451). The energy dispersive X-ray (EDX) spectrum revealing nearly pure

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compositions of zinc and oxygen also supports the ZnO nanocrystal formation from the catalytic ZP-1 peptide (Fig. S2). Among 100 samples 86% of nanoparticles were crystalline with the wurtzite structure.

To study the crystallization process of ZnO on the catalytic ZP-1 peptide, TEM and SAED of ZnO particles were monitored with time. As the mineralization time increased, the evolution of crystallinity is detected in SAED pattern changes with the development of (100) and (110) faces after mixing the ZP-1 peptide with the zinc precursor (Figs. 2e to g), indicating that the growth directions are along <01-10> and <11-20>. Notably, the growth of ZnO crystals along these directions are rarely reported, especially at low temperature because the growth along [0001] axis is usually much faster than other directions[9]. Previously, it was reported that under the condition of insufficient growth medium, ZnO tends to grow along unusual directions corresponding to the nonpolar crystalline faces[10]. Because both (100) and (110) are nonpolar faces[11], we attribute the growth of ZnO along the directions of <01-10> and <11-20> to a slow diffusion of zinc ions towards the peptide surfaces[3], which would create the insufficient growth medium condition. In addition, charged groups of the ZP-1 peptide could preferentially attach polar crystalline faces such as (001) and (00-1)[11] and as a result, the anisotropic growth along these directions could be quenched.

Previously, the peptide-assisted aggregation-driven crystal fusion via the non-classical crystallization path was observed in other nanocrystal growths[12], and the similar fusing process also undergoes in the peptide-catalyzed ZnO nanocrystal growth. When morphologies of ZnO nanocrystals between 4 days and 3 weeks are compared with HR-TEM (Figs. 2b and d), these images indicate that the primitive crystalline domains of ZnO are fused in the anisotropic direction to form larger single crystalline domains as the incubation time is increased. In Fig. 2b, the crystalline domains in the diameter of 5 nm are randomly oriented in the 4 day incubation. However, after 3 weeks these domains are aligned and fused on {100} to form the larger single crystalline domain as shown in Fig. 2d. This image also shows the subtle short-range order of domains on {110}. These observations indicate that the fusion of primitive nanoparticles mainly occurs on {100} interface. After 3 weeks, diffraction spots in the SAED pattern (Fig. 2g) are attenuated except (100) and (110) as compared to the one with the 4 day-incubation (Fig. 2f), consistent with the HR-TEM result. When the structure of the ZnO single nanoparticle after 3 weeks is studied with the nano-beam electron diffraction (NBED) (the inset of Fig. 2c), the diffraction pattern reveals the single crystallinity induced by the anisotropic fusion of nano-crystalline domains.

Previously, metal nanoparticles were observed to coagulate in larger particles by photo-mediation[13]. The driving force can be the induced electromagnetic field around the excited particles[13b], while the redistribution of ligands and ions on the particle surfaces could also allow an anisotropic growth[13a]. Extended structures from oriented nanoparticles can also be formed by dipole moments that drives the fusion of nanoparticles on high-energy faces[14]. However, in this case the fusion of semiconductor nanoparticles occurs on nonpolar crystalline faces that are rarely observed[12]. Our outcomes suggest that the ZP-1 peptide can not only nucleate the semiconductor nanoparticle growth catalytically but also induce the anisotropic coagulation of primitive crystalline domains at room temperature to form single crystalline nanoparticles without irritation or adding complex capping reagents[15].

In conclusion, a novel evolutionary protocol was established to discover catalytic peptides for new material synthesis at low temperature. We were succeeded in synthesizing single-crystalline ZnO nanocrystals with the catalytic peptide discovered by this combinatorial

technique at room temperature. This combinatorial phage display approach discovered the ZP-1 peptide, which displayed the strong catalytic activity for the growth of ZnO nanocrystals at room temperature.

Experimental Section

Combinatorial peptide display

To select ZnO-minerallizing peptides, Ph.D.-12 phage display peptide library kit (New England Bio Labs, Beverly, MA) was used. Phages library (10^{11} pfu) was mixed with $Zn(NO_3)_2$ solution (10 mg/mL, 300 μL) and incubated for 4-6 days at room temperature. Resulting nanocrystals were recovered by centrifuge (18,000 × g, 30 min), and then washed 3 times with 1 mL of deionized water containing 0.01 % tween 20. The phages were eluted from nanocrystals by the addition of 0.2 M glycine-HCl (pH = 2.2) for 20 min. The eluted phages were amplified through infection into *Escherichia coli* strain ER2738, followed by PEG-purification. The amplified and purified phages were tittered on LB plates containing X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactoside) and IPTG (isopropyl-β-D-thiogalactopyranoside), and this panning is repeated for 2 more rounds. Then, DNAs were isolated from 12 independent blue plaques and sequenced using ABI Prism™ 3730×l DNA sequencers (SeqWright, Houston, TX).

Growth and analysis of ZnO nanoparticles

The peptides discovered by the phage display (50 μL, 50 mg/mL) were mixed with 50 μL $Zn(NO_3)_2$ solution (10 mg/mL). After incubated for 4 days at room temperature, the nanoparticles were collected by centrifugation. After 3 rounds of washing and centrifugation, the nanoparticles were characterized by TEM (JEM 2100 (JEOL) with the acceleration voltage of 200 kV). Samples are prepared by drying one drop of the nanoparticle solution on carbon-coated copper grids.

Acknowledgments

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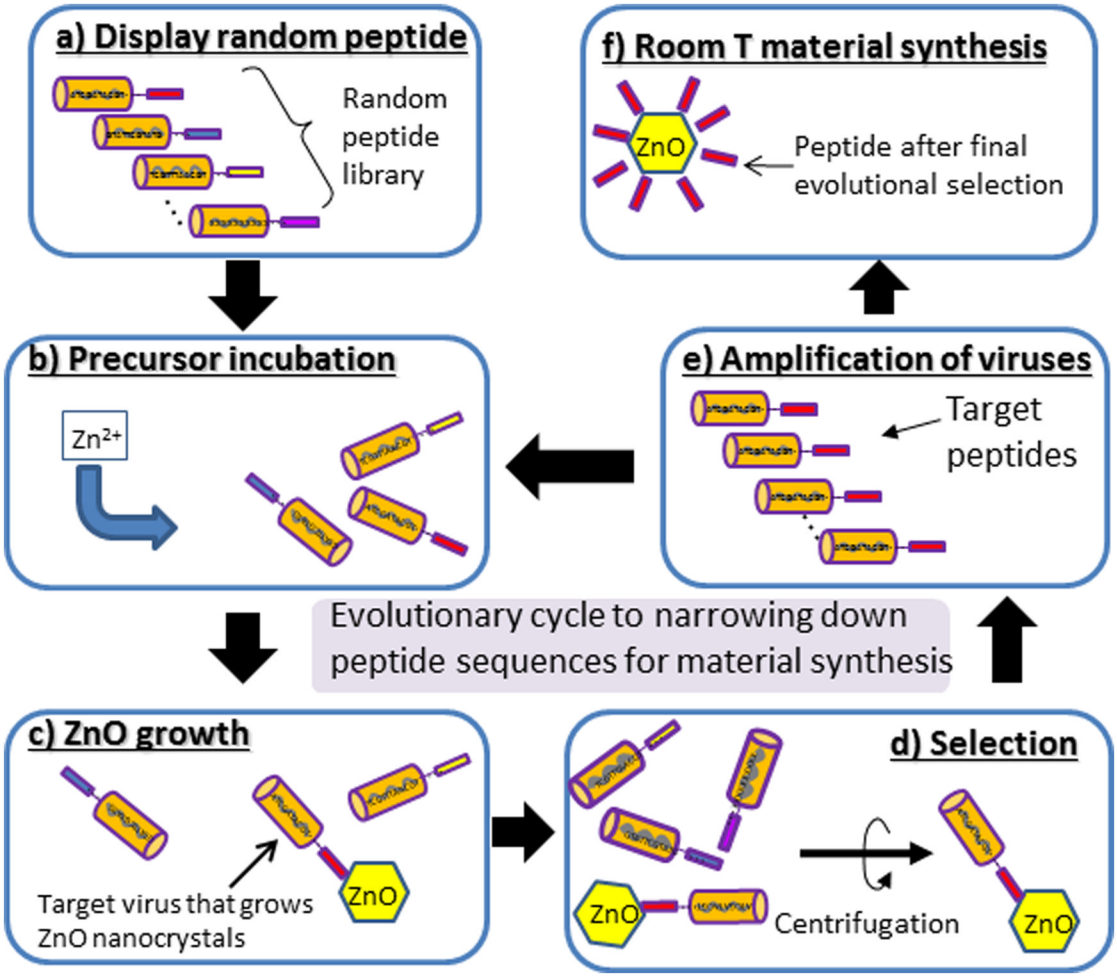

Figure 1.
Scheme of the proposed evolutionary material synthesis with discovering catalytic peptides that can grow ZnO at room temperature.

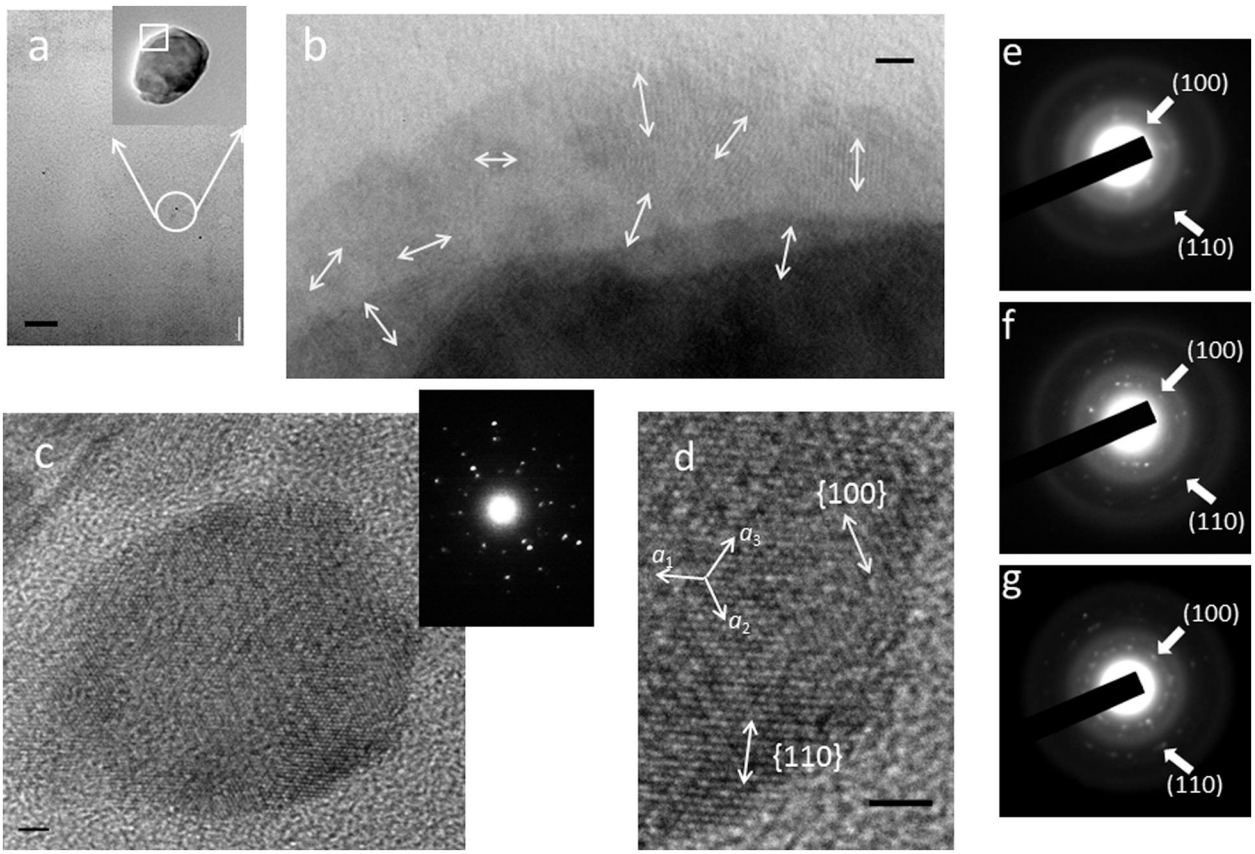

Figure 2.

Microscopic studies on the mineralization process. (a) TEM image of ZnO nanoparticles after 4 day incubation in the peptide-growth precursor solution, scale bar = 1 μm. (inset) Magnified image of the white-circled area. (b) HR-TEM image of the square region in (a), showing nanoparticle domains with (100) faces (white lines) oriented in random directions. The *d* spacing of nanocrystals = 2.82 ± 0.05 Å, corresponding to (100) faces. Scale bar = 2 nm. (c) TEM image of ZnO nanocrystals after 3 week incubation in the peptide-growth precursor solution. (inset) nano-beam electron diffraction (NBED) pattern of this nanocrystal showing the single crystallinity with [0001] transmission direction. See details of the diffraction pattern indexation in Fig. S3. Scale bar = 2 nm. (d) HR-TEM image of (c) resolving {100} and {110} faces (shown by arrows). Scale bar = 2 nm. (e) Selected area electron diffraction (SAED) pattern for ZnO nanoparticles after 12 hour incubation in the peptide-growth precursor solution. (f) SAED pattern for ZnO nanoparticles after 4 day incubation in the peptide-growth precursor solution. (g) SAED pattern for ZnO nanoparticles after 3 week incubation in the peptide-growth precursor solution. In (e) – (g), (100) and (110) faces are shown by arrows, respectively.

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