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Identification of Novel Short BaTiO₃-Binding/Nucleating Peptides for Phage-Templated in Situ Synthesis of BaTiO₃ Polycrystalline Nanowires at Room Temperature

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Abstract

Ferroelectric materials, such as tetragonal barium titanate (BaTiO₃), have been widely used in a variety of areas including bioimaging, biosensing, and high power switching devices. However, conventional methods for the synthesis of tetragonal phase BaTiO₃ usually require toxic organic reagents and high temperature treatments, and are thus not environment-friendly and energy-efficient. Here, we took advantage of the phage display technique to develop a novel strategy for the synthesis of BaTiO₃ nanowires. We identified a short BaTiO₃-binding/nucleating peptide, CRGATPMSC (named RS), from a phage-displayed random peptide library by biopanning technique and then genetically fused the peptide to the major coat protein (pVIII) of filamentous M13 phages to form the pVIII-RS phages. We found that the resultant phages could not only bind with the presynthesized BaTiO₃ crystals but also induce the nucleation of uniform tetragonal BaTiO₃ nanocrystals at room temperature and without the use of toxic reagents to form one-dimensional polycrystalline BaTiO₃ nanowires. This approach enables the green synthesis of BaTiO₃ polycrystalline nanowires with potential applications in bioimaging and biosensing fields.

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Author Contributions

Y.L. and B.C. contributed equally to this work.

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Supporting Information

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TEM image of as-purchased powders, HRTEM lattice image of the biotemplated nanocrystals, molecular model of the RS peptide, and sequences of peptides discovered by biopanning (PDF)

Graphical Abstract

Keywords

phage; bioassembly; polycrystalline nanowires; barium titanate; tetragonal structure

1. INTRODUCTION

Ferroelectric materials have widely been used in energy harvesting, energy storage, transducers, and actuators.^{1,2} Unlike the traditional $\text{Pb}(\text{Zr,Ti})\text{O}_3$ (PZT),^{3,4} which is toxic to the environment due to the presence of Pb, tetragonal Barium Titanate (BaTiO_3 or BT) is one of the most important green ferroelectric materials and has been extensively used in many areas such as biosensors,⁵ bioimaging,^{6,7} and thermistors⁸ due to its unique ferroelectric, optical, piezoelectric, dielectric, and electrochemical properties.^{9–14} BT crystals have five crystalline phases including rhombohedral, orthorhombic, tetragonal, cubic and hexagonal phases. Different phases of BT crystals have different applications based on their unique photo-refractive and electro-optical properties. Among them, the tetragonal BT crystals are more desired in practical applications because they possess the high ferroelectricity and highest stability at ambient condition.¹⁵ However, most of the conventional methods for the synthesis of tetragonal BT crystals involve high-temperature solid-state reactions ($> 1100^\circ\text{C}$).¹⁶ Even the recently low-temperature solution-based synthetic approaches still require the use of relatively high temperature ($> 100^\circ\text{C}$) and toxic organic reagents (such as diphenyl, $(\text{C}_5\text{H}_5)_2\text{O}$, and oleic acid).^{17–19}

Over the past decade, biotemplates,²⁰ such as bioengineered viruses, have been used for the synthesis of inorganic materials.²¹ For instance, viruses can be genetically modified to possess specific material-recognizing peptides, which are able to induce the nucleation of the materials (mostly crystals) from an aqueous precursor solution onto the virus surface at a mild condition. When viruses with various shapes and concentrations are used to synthesize or assemble inorganic materials, materials with controlled architectures can be produced,

including one-dimensional inorganic nanowires,^{22,23} two-dimensional inorganic nanofilms,²⁴ and three-dimensional inorganic scaffolds.^{25,26}

M13 bacteriophage (also termed phage) is a filamentous virus consisting of a circular single-stranded DNA (ssDNA, including 11 genes) of ~6400 nucleotides, ~2700 copies of major coat protein (pVIII) on the side wall surrounding the ssDNA,¹¹ and 5 copies each of four minor coat proteins (pIII, pVI, pVII, pIX) constituting either of the two ends.²⁷ All of the five coat proteins could be rationally modified as a display platform via accurate genetic engineering.^{28–35} From a materials science perspective, M13 phage is a naturally occurring bionanofiber (~900 nm in length and ~7 nm in diameter) with high monodispersity.^{26,36} Therefore, it is regarded as an ideal template to induce the synthesis and assembly of a variety of nanomaterials.^{21,22,37} We, along with others, have successfully employed the filamentous phages to synthesize and assemble semiconducting nanoparticles,^{38–40} metallic nanostructures,^{41,42} magnetic nanoparticles,^{25,43} silica,^{44,45} and bone minerals.^{46–49}

The nanowires of tetragonal BT crystal phase usually can be synthesized by a hydrothermal method without the use of templates at high temperature, such as 170 °C.^{9,50} To explore the probability of forming polycrystalline tetragonal BT nanowires at room temperature without the use of toxic reagents, we proposed to continue to exploit the power of filamentous phages in templating materials growth (Scheme 1). To achieve this goal, we first identified a BT-binding peptide via a phage display selection technique (also called biopanning, Figure 1) from a random peptide library where peptides are displayed on pIII of M13 phage.⁵¹ We then inserted the peptide-coding foreign gene into the gene of pVIII to display the peptide on the side wall of M13 phage. We found that the BT-binding peptide indeed nucleated tetragonal BT crystals on the side wall of phages, generating polycrystalline BT nanowires, at room temperature.

2. EXPERIMENTAL SECTION

2.1. Materials

Tetragonal BT nanocrystals (99.9% purity, 200 nm in diameter, Figure S1) were purchased from US Research Nanomaterials Inc. (Catalog Number: US3830), and was washed five times with 0.5% TBST solution (0.5% Tween 20 in Tris-buffered saline (TBS) solution) prior to use. A Ph.D.-C7C phage display peptide library from New England Biolabs, Inc., was used for the phage display selection against BT crystals.

2.2. Affinity-Selection of BT-Binding Peptides

Phage biopanning procedure (Figure 1) was performed by following the standard manual with minor modifications. Briefly, 5 mg of tetragonal BT nanocrystals were incubated with 10 μ L of the phage library solution (for the first round of selection) or the amplified phage sublibrary (from the previous round of selection for each subsequent round of selection) in 1 mL of 0.5% TBST solution on a rocking shaker for 2 h at room temperature. The mixture was centrifuged at 4000*g* for 20 min, then the supernatant containing nonbinding phages was discarded. The BT nanocrystals were washed with 0.5% TBST solution for five times to remove loosely bound phages. The tightly bound phages were eluted from BT nanocrystals

using 400 μL of elution buffer (0.2 M glycine-HCl, pH = 2.2) with incubation for 10 min on a shaker. The supernatant was transferred to a new microcentrifuge tube and neutralized with 100 μL of 1 M Tris-HCl (pH = 9.1) immediately. After the third round of selection, 50 random colonies were picked out separately for DNA sequencing with the primer of 5'-CCCTCATAGTTAGCGTAAC-3'.

2.3. Titer of a phage solution

The titer of phage solutions was calculated to evaluate each round of the biopanning process. Specifically, 10 μL of 10-fold serial dilutions of eluate phage solution was incubated with a total of 90 μL of mid log ER2738 culture ($\text{OD}_{600} = 0.5$) for 5 min, followed by adding 900 μL of LB medium with 0.2 $\mu\text{g}/\text{mL}$ of tetracycline to each tube and incubating for 15 min at 150 rpm, 37 °C. 100 μL of the suspensions from the tube was mixed with 3 mL prewarmed Top Agar and spread onto a LB/IPTG/Xgal culture plate (50 mg/L, 40 mg/L). The titer was calculated by this equation: $\text{titer} = 1000 \times N \times M \text{ pfu/mL}$, where N and M are the dilution factor and the number of the blue phage plaques on a plate, respectively.

2.4. Amplification and Purification of Selected Phages

After titering, the remaining eluate phages were incubated with 20 mL of early log ER2738 culture ($0.01 < \text{OD}_{600 \text{ nm}} < 0.05$) for 30 min at room temperature. The phage-infected cell culture was then incubated at a low speed of 100 rpm for 30 min at 37 °C and then at 200 rpm for an additional period of 4 h. After the amplification, the cell culture was transferred into fresh tubes and centrifuged at 7200g for 10 min. The supernatant was transferred into a new 250 mL flask, followed by adding 9 mL of a solution containing 16.7% PEG 8000 and 2.5 M NaCl (termed PEG-NaCl) and mixing thoroughly. The mixture was incubated at 4 °C overnight for precipitation. The mixture was centrifuged at 13000g for 30 min and the supernatant was discarded. The phage pellet was resuspended in 1 mL of TBS (100 mM NaCl, 25 mM Tris, pH = 7.4). The phage solution was transferred to a new microcentrifuge tube and centrifuged at 13000g for 30 min. Subsequently, the supernatant was transferred to a new microcentrifuge tube and mixed with 200 μL of the PEG-NaCl solution again, then incubated at 4 °C for 5 h. The resultant mixture was centrifuged at 13000g for 30 min and the pellet was dissolved in 200 μL of TBS.

2.5. Assay of Selected Phage Binding to BT Nanocrystals

The selected BT-binding phages were individually amplified and diluted to the same concentration of 5×10^9 pfu/mL in 0.5% TBST solution. One milligram of BT nanocrystals was suspended in 200 μL of each phage solution and incubated for 1 h with continuous rotation at room temperature. Then the solutions were centrifuged at 4000 g for 10 min and the BT crystal pellets were washed three times to remove unbound phages. The bound phages were transfected into the mid log ER2738 cells for titering.

2.6. Construction of Engineered Phages by Displaying BT-Binding Peptides on the Side Wall of Phages

Our identified BT-binding peptide, CRGATPMSC, was genetically displayed at the N-terminus of pVIII of M13 phage to allow BT crystals to be nucleated on the side wall of the

phages. The peptide display followed our previously published protocols.^{46,47} Briefly, both the forward primer (5'-ATCCATGGCGTGCCGCGGCGGACCCCGATGAGCTGC-GATCCCGCAAAAGCG-3'; the underlined nucleotide is *NcoI* recognition site, and the bold nucleotide is oligonucleotide sequence responsible for coding the RS peptide) and reverse primer (5'-GCAAGCTTTTATCAGCTTGCTTTCGAG-3'; the underlined nucleotide is *HindIII* recognition site) were used to insert the DNA sequence coding the peptide into the precise site of the gene of pVIII via polymerase chain reaction (PCR). The recombinant phagemid was transformed into *Escherichia coli* TG-1 competent cells and verified by both PCR and DNA sequencing. The resultant phage is termed as BT-binding phage.

2.7. Amplification and Purification of the Engineered Phages

The *E. coli* TG-1 strain containing the recombinant phagemid displaying the peptide CRGATPMSC was incubated in 100 mL of LB medium with shaking at 37 °C until mid log phase ($OD_{600\text{ nm}} = 0.5$) was reached. Then 3 μL of M13KO7 helper phages was added and the culture was incubated for 30 min with a speed of 90 rpm at 37 °C. Ten μL of 50 mg/mL of chloromycetin stock solution was added and incubated for 12 h at 37 °C. The purification of the engineered phages is described in the previous section 2.4.

2.8. Interaction of Synthetic BT Nanocrystals with the BT-Binding Phages

One hundred micrograms of tetragonal BT nanocrystals were incubated with 10 μL of purified BT-binding phage solution (10^{13} pfu/mL) for 2 h at room temperature. A total of 5 μL of the mixed solution was placed onto a carbon-coated copper grid for 2 min. The grid was then washed by water for 30 s. The excess water was subsequently wicked away by a filter paper. The grid was then stained with 1% uranyl acetate twice for 30 s and the excess uranyl acetate solution was wicked away by a filter paper. The grid was dried at room temperature and observed under a transmission electron microscope (TEM).

2.9. Nucleation Tests and Nanowire Formation

Ten micro-liters of phage solution (2×10^{10} virions) were incubated in a precursor salt solution containing 0.2 mM potassium bis(oxalato)-oxotitanate(IV) dihydrate ($\text{K}_2\text{TiO}(\text{C}_2\text{O}_4)_2 \cdot 2\text{H}_2\text{O}$) and 0.2 mM barium acetate ($\text{Ba}(\text{OOCCH}_3)_2$) overnight at room temperature. On the next day, the solution was centrifuged at $4000g$ for 10 min and the pellet was washed three times with water. The products were stained with 1% uranyl acetate and characterized under TEM to verify the nucleation of BT nanocrystals on the side wall of the phages to form polycrystalline nanowires.

3. RESULTS

3.1. Biopanning of the Phage Library against BT Nanocrystals

BT-binding peptides were identified from the Ph.D.-C7C phage library via phage biopanning (Figure 1). We chose the Ph.D.-C7C phage library instead of other commonly used phagemid libraries for the following considerations. First, in the Ph.D.-C7C phage library, the cyclic 9-mer peptide made of 7 random amino acids and 2 flanking cysteines is displayed on all copies of the pIII of phages. However, in the phagemid libraries (e.g.,

pSKAN Phagemid Display System, MoBiTec), peptides are only displayed on a subset of pIII. Second, the displayed peptides in the Ph.D.-C7C phage library are flanked by a pair of cysteine residues to form a loop structure, allowing the peptides to protrude for interactions. The affinity-selection process was performed for a total of four rounds. The titers of the selected phage increased from 4.9×10^4 pfu/mL (round two) to 1.94×10^6 pfu/mL (round three) and to 7.32×10^6 pfu/mL (round four), indicating that the biopanning was successful. In the third and fourth rounds of selection, 50 plaques were sent for DNA sequencing. The sequencing results show that the peptide CRGATPMS (termed RS) has 5 repeats, CTHLRHASC (termed TS) has 4 repeats, CMSTGLSSC (termed MS) has 3 repeats, CGGGPLYMC (termed GM) has 2 repeats and each of other 36 sequences only has 1 repeat (Table S1). Therefore, the four peptides with more than one repeat may have higher BT-binding affinity.

3.2. Binding-Affinity of the High-Frequency Phages

To test the binding-affinity of the four peptides (RS, TS, MS, and GM), we performed a binding assay using the four selected phages (Table 1) with each displaying one of the four peptides. The same amount of each phage (1×10^9 virions) was allowed to interact with 1 mg of BT nanocrystals (Figure S1) with 5 rounds of washing. Then the quantity of the surviving phages, those that still bound to the nanocrystals after washing but further were eluted using an elution buffer, was determined by phage titering. The results show that the phage displaying the peptide RS (termed pIII-RS phage) has the highest number of recovered phages ($3.3 \times 10^8 \pm 0.23 \times 10^8$) (Figure 2), suggesting RS has the highest affinity for BT.

3.3. Construction of pVIII-RS Phages and Their Interaction with Tetragonal BT Nanocrystals

To use M13 phages to template the formation of BT nanowires, RS peptide was genetically displayed on the side wall (assembled from pVIII) instead of at the end (pIII) of the phage body. The display was achieved using our previously validated protocol.³⁸ The resultant pVIII-RS phages (Figure 3) were then allowed to interact with BT nanocrystals at room temperature, washed with water, and imaged under TEM. The TEM images show that pVIII-RS phages could indeed bind the tetragonal BT nanocrystals efficiently (Figure 4a). However, the control phages displaying a control scrambled peptide (SMPTAGR) showed no interactions with BT crystals (Figure 4b). The results indicate that the identified RS peptide enables the engineered pVIII-RS phages to bind tetragonal BT nanocrystals.

3.4. Nucleation of BT Nanocrystals along pVIII-RS Phages to Form BT Nanowires

To test if the pVIII-RS phages could induce the direct nucleation of tetragonal BT nanocrystals, pVIII-RS phages were incubated in an aqueous precursor salt solution (0.2 mM $K_2(TiO(C_2O_4)_2) \cdot 2H_2O$ and 0.2 mM $Ba(OOCCH_3)_2$) at room temperature. The results show that uniform precipitates were formed on the surface of the pVIII-RS phages at room temperature, which was verified by transmission electron microscopy (TEM) observation (Figure 5a). The mineralization yield was found to be 91.2%, comparable to the reported yield (95.7%) of Au nanoparticles synthesized using M13 phage.⁵³ The electron diffraction pattern (Figure 5a), high resolution TEM image (Figure S2) and X-ray diffraction (XRD)

pattern (Figure 6a) obtained from the precipitates on the pVIII-RS phages can be indexed by the structure of tetragonal BT nanocrystals.⁵⁴ In contrast, when phages displaying the scrambled peptide were used, only nonuniform amorphous precipitates were found randomly distributed in the solution rather than along the side walls of phages (Figure 5b and 6b). This result is similar to that found in the nucleation experiments using wild-type phages (data not shown). Based on the above results, we believe that the pVIII-RS phages could efficiently induce the nucleation of tetragonal BT nanocrystals and assemble the crystalline nanoparticles into nanowires at room temperature.

4. DISCUSSIONS

Tetragonal BT is widely used for microelectronic devices in the technological ceramic industry due to its ferroelectric properties. Low-temperature routes, such as hydrothermal methods, sol–gel and phase transformation, have been explored to synthesize the tetragonal BT. For example, Wang et al. reported the hydrothermal synthesis of BT nanocrystals under strong alkaline condition at 180 °C.¹⁹ O'Brien et al. reported the synthesis of BT nanocrystals by sol–gel in the stabilizing organic agent (oleic acid) at 100–140 °C.¹⁷ Additionally, the tetragonal BT crystals could be transformed from the cubic phase around 132 °C. Among the five phases of BT crystals, tetragonal phase is the one that is the most stable at room temperature. To synthesize tetragonal BT nanocrystals, we took advantage of phage display technique in identifying BT-binding peptide and constructing the pVIII-RS phages as a catalytic biotemplate (Figure 1). Indeed, we found the pVIII-RS phages could successfully induce the nucleation of tetragonal BT crystals at room temperature under a neutral pH value (Figures 4 and 5). The phage-templated methods of synthesizing BT nanocrystals we developed here are environmentally benign, cost-efficient and energy-saving, compared to the reported methods.

One BT-binding peptide (NTISGLRYAPHM, pI = 8.8) has been previously identified using the Ph.D.-12 phage library, and used to induce the formation of tetragonal BT nanocrystals under neutral pH at room temperature.⁵⁵ In the published work, the authors did not test the formation of BT nanowires. In this work, we chose Ph.D.-C7C phage library (Figure 1) instead to identify BT-binding peptides because the displayed peptides in the Ph.D.-C7C phage library are flanked by a pair of cysteine residues to form a loop structure (a relatively complex structure compared with the linear peptide) that could increase the specificity of the biopanning.⁵⁶ Moreover, we displayed the identified peptide on the side wall of the filamentous phages and found that tetragonal BT nanocrystals could be nucleated on the side wall of the phages to form nanowires. It is known that BT nanowires have higher dielectric constant because of their higher aspect ratio than the individual BT nanocrystals.^{57,58}

BT nanowires have been previously synthesized by using phages displaying a nonspecific peptide, EEE (E₃), based on just the electrostatic interaction between the anionic phages and precursor salts.⁵⁹ Such phages could also nucleate the amorphous iron phosphate (a-FePO₄) because of the lack of specificity.⁶⁰ In addition, we previously found the E₈-displayed phages could attract calcium ions and then phosphate ions through electrostatic interactions, leading to the nucleation of calcium hydroxyapatite (HAP, Ca₁₀(PO₄)₆(OH)₂).⁴⁷ These results indicate that the phages displaying nonspecific peptides, such as E_n, could initiate the

nucleation of a variety of materials. This lack of specificity of the peptide displayed on the surface of phages could be disadvantageous because it may lower the nucleation efficiency of BT nanocrystals under an impure or complex reaction system. In our work, no materials other than BT were found to be formed on the phages according to the electron and X-ray diffraction (Figure 5a and 6a). In addition, we did not find that the BT-binding phages could nucleate other materials (data not shown). It should be noted that compared to the negative charge of E_n polypeptides involved in the production of the BT nanocrystals, the RS peptide identified in our biopanning contains positively charged amino acids (i.e., Arg2). We also analyzed the 3D peptide structure using the PEP-FOLD program of Mobyle (Figure S3). The results show that the disulfide bond formed between the two Cys residues (Cys1 and Cys9) results in a loop structure (Figure S3), in which the amine group from Arg2 and the hydroxyl groups from Thr5 and Ser8 are on the same side of the RS peptide and collectively constitute a plane facing the solution phase (before nucleation) or BaTiO₃ crystals (during and after nucleation). We hypothesize that the RS peptide nucleates and binds BaTiO₃ crystals through both the hydrogen bonding between the hydroxyl groups of the Thr5 and Ser8 residue and the TiO₃²⁻ ions of BaTiO₃ crystals,⁶¹ and the electrostatic interaction between the positively charged amine group from Arg2 and the negative charged TiO₃²⁻ ions from BaTiO₃ crystals. During the nucleation on the RS-phage, we believe that the RS-phages will catalyze the dissociation of barium titanyl oxalate (BaTi(O)(C₂O₄)₂, BTO),^{62,63} formed from a reaction of the two precursors we used in this work,^{63,64} into BaTiO₃ nanocrystals. Namely, the aforementioned hydrogen bonding and electrostatic interactions will enable the TiO₃²⁻ ions and the subsequently electrostatically attracted Ba²⁺ ions to be enriched on the surface of the RS-phages, favoring the nucleation of BaTiO₃ nanocrystals on the surface of the RS-phages at room temperature. Such site-specific nucleation will further promote the dissociation of BTO, leading to site-specific growth of the BaTiO₃ nanocrystals on the RS-phages at room temperature.

Pantazis et al. found two photons could interact with the BT nanoparticles to produce a single photon with 2-fold higher energy than the incident photon. This phenomenon lays the foundation for extending the applications of BT nanoparticles to whole animal imaging using 2-photon microscopy.⁶ In addition, Pattnaik et al. found that a BT thin film could detect glucose in blood better than piezoelectric materials.^{65,66} Hence, we fully anticipate that the polycrystalline BT nanowires synthesized by using our biotemplated nucleation method could be potentially used in bioimaging and biosensing.

5. CONCLUSIONS

In conclusion, we successfully identified a BT-binding peptide, CRGATPMS_C, via phage biopanning and verified its interaction with tetragonal BT nanocrystals. We also found that when the RS peptide was displayed on the side wall of phages, the resultant phages could efficiently not only bind to BT nanocrystals but also induce the nucleation of tetragonal BT nanocrystals on the surface of phages to form polycrystal-line nanowires under neutral pH at room temperature. Since other material-binding peptides could be identified by biopanning and displayed on the side walls of phages in the same manner, we believe such an approach could also be extended to the synthesis of other inorganic perovskite nanoparticles and nanowires.

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Figure 1.

Schematic of affinity-selection of BaTiO₃-binding peptides utilizing the Ph.D.-C7C phage library. (a) BaTiO₃ nanocrystals are dispersed in the buffer. (b) The phage library is allowed to incubate with the BaTiO₃ nanocrystals. (c) BaTiO₃-bound phages precipitate with nanocrystals during the centrifugation and the nonbound phages are washed away. (d) The BaTiO₃-binding phages are eluted from the surface of the nanocrystals. (e) The eluted phages are allowed to infect the ER2738 *Escherichia coli* host cells for amplification and used as a sublibrary for the next round selection (b → c → d → e). (f) The eluted phages are titered. (g) Starting from 3rd round, plaques are sequenced to determine the consensus sequence. After 4th round of selection, 50 plaques are picked out for DNA sequencing. (h) The structure of an M13 phage. A cyclic ssDNA is enclosed in a capsid made of five coat proteins (2700 copies pVIII constituting the side wall and five copies each of pIII, pVI, pVII, and pIX capping one of the two ends).

Figure 2.

Affinity-binding assay of the selected BaTiO₃-binding phage. For each phage type, 1×10^9 of the input phage particles are allowed to interact with BaTiO₃ nanocrystals, washed with washing buffer, and titred to determine the number of remaining phage. The results show that the pIII-RS phage has the highest affinity to BaTiO₃ nanocrystals. Each value represents the mean of three repeated independent experiment $\pm$ standard deviation. The statistical test method is student *t* test, and the RS has a statistically significant increase compared with the TS, MS and GM peptides (*, $P < 0.05$). pIII-RS, pIII-TS, pIII-MS, pIII-GM: phages displaying RS, TS, MS, GM peptide on the 5 copies of their pIII, respectively.

Figure 3. TEM (a) and AFM (b) image of the RS peptide displaying phages (pVIII-RS phages), showing that the phages are about 900 nm long.

Figure 4.

TEM images showing the interaction between phages displaying the selected BT-binding peptide and BaTiO₃ nanocrystals. (a) pVIII-RS phages retained on BaTiO₃ crystals after washing. (b) Phages displaying a scrambled peptide (SMPTAGR) could not be retained on the crystals after washing. Both samples were negatively stained with 1% uranyl acetate.

Figure 5.

TEM images showing the nucleation of BT nanocrystals on the side walls of phages. (a) BaTiO₃ nanocrystals nucleated on the surface of pVIII-RS phages at room temperature to form polycrystal-line BaTiO₃ nanowires. The electron diffraction pattern obtained from the nanocrystals on the phages matches the typical pattern of tetragonal BaTiO₃ crystals. (b) No crystals were formed on phages displaying the scrambled peptide sequence. Instead, only some nonuniform amorphous precipitates were formed.

Figure 6.

XRD pattern showing that the BaTiO₃ nanocrystals were formed on the side walls of the phages displaying the BT-binding peptides. (a) XRD pattern of polycrystalline BaTiO₃ nanowires formed by site-specific nucleation of BaTiO₃ nanocrystals on the surface of pVIII-RS phages at room temperature. The XRD pattern is in agreement with the typical pattern of tetragonal BaTiO₃ crystals where the (002) and (200) peak are split. (b) XRD pattern of the nonuniform amorphous precipitates formed in the mineralizing solution containing phages displaying the scrambled peptide sequence.

Scheme 1.

Schematic of Using Engineered Phages for the Synthesis of BaTiO₃ Polycrystalline Nanowires at Room Temperature: (a) Selection of BT-Binding Peptides via Biopanning and (b) Construction of the pVIII-RS Phage via Displaying of BT-Binding Peptides on the Side Wall of Phage, Which Guides the Nucleation of BT on Phage to Form Polycrystalline Nanowires

Table 1**BaTiO₃-Binding Peptides**

phage	amino acid sequence	pI ^a
pIII-RS	CRGATPMSC	8.07
pIII-TS	CTHLRHASC	8.09
pIII-MS	CMSTGLSSC	5.51
pIII-GM	CGGGPLYMC	5.51

^aThe theoretical isoelectric point (PI) calculated using the ProtParam tool.⁵²