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Virus-Enabled Synthesis and Assembly of Nanowires for Lithium Ion Battery Electrodes

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The selection and assembly of materials are central issues in the development of smaller, more flexible batteries. Cobalt oxide has shown excellent electrochemical cycling properties and is thus under consideration as an electrode for advanced lithium batteries. We used viruses to synthesize and assemble nanowires of cobalt oxide at room temperature. By incorporating gold-binding peptides into the filament coat, we formed hybrid gold–cobalt oxide wires that improved battery capacity. Combining virus-templated synthesis at the peptide level and methods for controlling two-dimensional assembly of viruses on polyelectrolyte multilayers provides a systematic platform for integrating these nanomaterials to form thin, flexible lithium ion batteries.

There is an increasing need for smaller and more flexible Li ion batteries and for methods to assemble battery materials. Nanoparticles, nanotubes (1, 2), and nanowires (3), as well as several assembly methods based on lithography, block copolymer (4), or layer-by-layer deposition (5), have been introduced for constructing dimensionally small batteries. In addition to their utility in nanoelectronics, there is also growing evidence that nanostructured materials can improve the electrochemical properties of Li ion batteries compared to their bulk counterparts (6). However, to maximize this potential, monodisperse, homogeneous nanomaterials and hierarchical organization control are needed. Biosystems have the inherent capabilities of molecular recognition and self-assembly and thus are an attractive template for constructing and organizing the nanostructure (7–13). We have previously used viruses to assemble semiconductor and magnetic nanowires (14, 15) and consider whether they can be used for device fabrication. Using batteries as our example device, we also explore whether the viruses can be modified to improve the electrode materials. Because the viruses can assemble on multiple length scales, there may be scope for designing hierarchical self-assembling batteries. For this biological approach, once the genes are programmed for a functional device, very little postassembly processing is necessary. Additionally, this biological route uses room-temperature, aqueous synthesis conditions.

The M13 virus consists of ~2700 major coat proteins (p8) helically wrapped around its single-stranded DNA, with minor coat proteins (p3, p6, p7, and p9) at each end of the virus. The functionality of these subunit proteins can be modified specifically through additions in the M13 genome. Modification of the major coat proteins, as well as minor coat proteins at the virus ends, has been used successfully to form functional heterostructured templates for precisely positioned nanomaterials (16, 17). Furthermore, the intrinsic anisotropic virus structures are well suited for the growth of monodisperse, highly crystalline nanowires (14, 15). In addition, the anisotropic virus structures are promising as elements of well-ordered nanostucture, as demonstrated in three-dimensional (3D) liquid crystal film (18, 19).

Predictive-based design was used for engineering the virus to satisfy the multifunctional purpose of electrode formation and assembly with a polymer electrolyte for the Li ion battery (Fig. 1). Tetruglutamate (EEEE-) was fused to the N terminus of a copy of the major coat p8 protein with 100% expression. This clone, named E4, was designed with three objectives. (i) It can serve as a general template for growing nanowires through the interaction of the glutamate with various metal ions (Virus Biotemplating in Fig. 1). Carboxylic acid, the side chain of glutamate, binds positive metal ions via ion exchange, as demonstrated in polymeric templates (20). Glutamate is also believed to be important in biomineeralization, as evident in its role in specific proteins that regulate the nucleation of biominerals in nature. (ii) Tetruglutamate acts as a blocking motif for gold nanoparticles (21), due to the electrostatic repulsion. Therefore, tetruglutamate reduces nonspecific gold nanoparticle binding to phase, thereby increasing the specificity of a gold-specific peptide to bind gold in low concentration. (iii) The E4 clone is ideally suited for electrostatically driven assembly with a charged polymer (Assembly Engineering in Fig. 1). E4 is more negatively charged than wild-type virus, which enables it to interact favorably with the positively charged electrolyte polymer. Zeta potential measurements of the E4 clone reveal a dramatic change in the potential between pH 4.5 and 5.5, thus enabling a certain degree of charge control.

To design the cobalt oxide (Co3O4) nanowires electrodes, we incubated the E4 virus templates in aqueous cobalt chloride solution (1 mM) for 30 min at room temperature to promote cobalt ion binding (22). Co3O4 was chosen as one of a family of new lithium-active compounds with an extremely large reversible storage capacity arising from displacement reactions (23), approximately three times as large as the capacity of carbon-based anodes currently used in commercial batteries. After reduction with NaBH4 and spontaneous oxidation in water, monodisperse, crystalline Co3O4 nanowires were produced (24). Figure 2, A and B, show some examples of these nanowires grown on substrates.

**Fig. 1.** Schematic diagram of the virus-enabled synthesis and assembly of nanowires as negative electrode materials for Li ion batteries. Rationally designed peptide and/or materials-specific peptides identified by biopanning were expressed on the major coat p8 proteins of M13 viruses to grow Co3O4 and Au-Co3O4 nanowires. Macroscopic ordering of the engineered viruses was used to fabricate an assembled monolayer of Co3O4 nanowires for flexible, lightweight Li ion batteries.

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185
shows transmission electron microscope (TEM) images of the virus-templated Co$_3$O$_4$ nanowires, where Co$_3$O$_4$ nanocrystals of ~2 to 3 nm in diameter were uniformly mineralized along the length of the virus. For TEM observation, a dilute suspension was dropped on a carbon-coated TEM grid, washed with distilled water, and dried. The high-resolution TEM electron diffraction pattern and lattice spacing (Fig. 2), together with x-ray diffraction, confirm that the crystal structure is Co$_3$O$_4$. The inset in Fig. 2B shows that the measured lattice spacing corresponds to the planes of Co$_3$O$_4$. Because p8 proteins were genetically engineered with 100% expression and cobalt ions have a strong binding affinity to the carboxyl groups of glutamate, homogeneous and high-crystalline nanowires were synthesized. Furthermore, viral Co$_3$O$_4$ nanowires had a large surface area of 141.7 m$^2$/g, as measured by the Brunauer-Emmett-Teller method. The mass ratio of Co$_3$O$_4$ and virus is 0.837:0.163. Unlike E4 viruses, Co$_3$O$_4$ nucleating viruses, solutions of wild-type virus expressing no peptide insert, or solutions without viruses formed irregular and large precipitates of Co/Cu$_3$O$_4$ mixtures.

For electrochemical evaluation of the Co$_3$O$_4$ nanowires, positive electrodes were prepared by mixing together 3.29 mg of the virus-based nanowires, Super P (MMM Carbon, Brussels, Belgium) carbon black, and poly(vinylidene fluoride)-hexafluoropropylene binder in a mass ratio of 74:15:11. Swagelok design cells using Li fluoride)-hexafluoropropylene binder in a mass ratio of 74:15:11. Swagelok design cells using Li$_2$CO$_3$ as the negative electrode and a metal foil used as the negative electrode and a separator film of Celgard 2400 were assembled and saturated with the liquid electrolyte, 1 M LiPF$_6$ in ethylene carbonate and dimethyl carbonate (1:1 by volume). The assembled cells were galvanostatically cycled between 3.0 and 0.01 V using a Maccor automated tester. The behavior of voltage/capacity curves (Fig. 2C) for the Co$_3$O$_4$/Li half cell was similar to that of Co$_3$O$_4$ nanoparticles produced by other methods (25). The larger first-insertion capacity compared to that in subsequent discharge is characteristic of this material and is due to irreversible reactions occurring upon initial lithiation. Any biphasic nature (25) of Co$_3$O$_4$ and Li$_{x}$Co$_{3}$O$_{4}$ ($x$ = Li$_{1.45}$Co$_{0.5}$) at the early stages of discharge was not clearly evident from the voltage traces (Fig. 2C). We observed reversible capacity (Fig. 2D) ranging from 600 to 750 mA/hour/g, which is about twice that of current carbon-based negative electrodes. The charge and discharge capacities stabilized at 600 mA/hour/g over 20 cycles. The reversible formation of Li$_2$O accompanying the redox of cobalt nanoparticles and the reversible growth of a gel-like polymeric layer (26), resulting from electrolyte degradation, are believed to contribute to this reversible capacity. The existence of higher than theoretical specific capacity in Co$_3$O$_4$ has been observed before (27), and it plausibly attributed to the reversible formation of a Li-bearing solid-electrolyte interface.

At the nanometer scale, both the reversible formation of Li$_2$O, which is known to be electrochemically inactive in bulk, and the reversible formation of the gel-like layer catalyzed by cobalt nanoparticles can occur (23). A control experiment revealed that the virus is electrochemically inactive and stable under the electrochemical conditions of our experiments, as indicated by the absence of decomposition in cyclic voltammograms (fig. S1). The virus capsid-mediated growth of uniform-sized Co$_3$O$_4$ nanomaterials, in addition to the structural integrity and dense packing (Fig. 2B) imparted by the virus, provides electrochemical advantages. For instance, when all other experimental conditions were held constant, the capacity of samples fabricated without the virus templates faded rapidly (Fig. 2D). This phenomenon, which is most likely attributable to incomplete oxidation of cobalt, inhomogeneous composition, and large particle size, has also been observed for Co$_3$O$_4$ prepared at low temperatures, in which high polarization is a contributing factor (25). However, the properties of Co$_3$O$_4$ nanowires, templated by M13 virus and oxidized spontaneously at room temperature, were comparable to those of particles fabricated at temperatures above 500°C.

An added advantage of this system is that the nanotexture of viral Co$_3$O$_4$ nanowires can be manipulated by controlling the interactions between the peptides and cobalt ions. Higher cobalt chloride concentration (5 mM) with 10 mM NaBH$_4$ produced branchlike nanowire structures (Fig. 2E); in contrast, nucleation and growth of Co$_3$O$_4$ nanowires at 4°C with 1 mM
cobalt ion and 5 mM NaBH₄ resulted in the assembly of discrete nanoparticles (Fig. 2F).

To design new hybrid material electrodes with higher capacity, we engineered additional material-specific peptide motifs into the major coat p8 protein. This provides a general method for the systematic and controlled arrangement of two distinct nanomaterials, which can enhance the electrochemical properties through the cooperative contribution of each material. Increasingly, efforts to improve battery properties have focused on composite material design (28, 29). However, notable challenges, such as the achievement of uniform distributions of multiple phases, are encountered when components are combined at the nanoscale. Gold nanoparticles were chosen on the basis of their ability to provide high electronic conductivity where needed, the ability to maintain a thermodynamically stable interface with Co₃O₄, and the potential to catalyze electrochemical reactions at the nanoscale. We designed a bifunctional virus template that simultaneously expressed two different peptide motifs. To accomplish this, we isolated a gold-binding peptide motif (LKHALPPSRPSLPS) by screening against a gold substrate with a phage display library (30), which contains random 12–amino acid peptide sequences. Then, we assembled bifunctional viruses constructed to express both Au- and Co₃O₄-specific peptides with the virus coat. Phagemid constructs (15) were inserted into host bacterial cells encoding the gold-binding peptide motif (31). Thus, upon infection of the plasmid-incorporating host cells with the E4 virus, a small percentage of the resulting E4 p8 proteins also displayed the gold-specific peptide. Therefore, two types of p8 proteins were produced: intact p8 proteins of E4 viruses and engineered p8 proteins containing the gold-binding peptide motif, randomly packaged onto the virus progeny (Fig. 3A). This hybrid clone was named AuE4 virus. Incubation of the amplified AuE4 clones with a 5-nm gold colloid suspension (5 × 10¹³ particles/ml; Ted Pella) resulted in 1D arrays of Au nanoparticles bound to the gold-binding peptides distributed among p8 proteins (Fig. 3B). In contrast, wild-type viruses and the E4 virus, which do not have gold-binding motifs, did not bind gold nanoparticles along the length of the virus. After removal of excess unbound gold nanoparticles by centrifugation, Co₃O₄ was nucleated and grown via the tetraglutamate functionality, resulting in hybrid nanostructures of 5-nm Au nanoparticles spatially interspersed within the Co₃O₄ wires (Fig. 3C). The crystal structure of the Co₃O₄ was confirmed by electron diffraction. Inductively coupled plasma mass spectrometry (ICPMS) analysis indicated that Au nanoparticles were associated with Co₃O₄ in a mass ratio of 0.024:0.976.

We evaluated the electrochemical properties of the hybrid Au-Co₃O₄ nanowires by using galvanostatic cycling and cyclic voltammetry. The mass of Au-Co₃O₄ nanowires deposited on the Cu substrate for one electrochemical cell was 3.41 mg. The virus-mediated hybrid composite generated higher initial and reversible lithium storage capacity than the pure Co₃O₄ nanowires when tested at the same current rate (Fig. 3D). The higher lithium storage capacity may result from the formation of Au-Li intermetallic compound or the conductive or catalytic effects of Au nanoparticles on the reaction of Li with Co₃O₄. Au is known to be electrochemically active, which leads to the formation of Li₂Au in the alkali metals (32). However, based on the Au:Co₃O₄ ratio, the contribution of Li₂Au alloys to the lithium storage capacity is likely negligible. Cyclic voltammetry (Fig. 3E) shows no notable new redox peaks that could be associated with the lithiation of Au (4). Given the unique charging/discharging mechanism of Co₃O₄ wherein cobalt nanoparticles promote the reversible reaction of an organic layer that then contributes to the Li capacity, it is more likely that Au nanoparticles play a role in this displacement reaction. This role may be one of improving electronic conductivity to the Co₃O₄ nanoparticles, or it may be catalytic in nature. Indeed, decreased cell polarization was observed in the galvanostatic voltage-capacity curves, which could result from either of these mechanisms. Furthermore, incorporation of Au clearly increases the reaction rate upon reduction of Co₃O₄, as indicated by the enhanced reduction peak seen by cyclic voltammetry in Fig. 3E (measured on samples of similar mass at the same voltage sweep rate). Although the exact electrochemical mechanism is under investigation, our results show that a small amount of Au nanoparticles dispersed within Co₃O₄ to produce a hybrid material markedly improves electrochemical performance. The specific capacity of the hybrid is estimated to be at least 30% greater than that of our Co₃O₄ nanowires.

Fig. 3. Characterization of the hybrid nanostructure of Au nanoparticles incorporated into Co₃O₄ nanowires. (A) Visualization of the genetically engineered M13 bacteriophage viruses. P8 proteins containing a gold-binding motif (yellow) were doped by the phagemid method in E4 clones, which can grow Co₃O₄. (B) TEM images of the assembled gold nanoparticles on the virus. Control experiments showed that gold nanoparticles were bound by the gold-specific peptides. (C) TEM image of hybrid nanowires of Au nanoparticles/Co₃O₄. (D) Specific capacity of hybrid Au-Co₃O₄ nanowires. Half cell with Li electrode was cycled at a rate of 0.265. Virus mass was subtracted and the mass of active materials such as Co₃O₄ and Au was counted. The capacity of virus-directing Co₃O₄ nanowires without Au nanoparticles was also compared. (E) Cyclic voltammograms of hybrid Au-Co₃O₄ and Co₃O₄ nanowires at a scanning rate of 0.3 mV/s.
The principles of self-assembly and biotemplating can be further extended to control virus-virus interactions for organizing nanoscale components. The ease of genetic modification of the virus allows for the growth and assembly of other virus-based materials for the generation of anodic as well as cathodic materials for the further oxidation of nanowires. The control in the viral synthesis of monodisperse oxide nanowires and the nanoscale structure of hybrid nanowires can be advanced through further modification of other proteins. Heterostructured nanowires, composed of anode and solid electrolyte, and bioenergy-transducing nanowires, coupled with biomolecules, are currently being investigated. Moreover, we anticipate that self-organized virus nanolayers for the formation of anodic as well as cathodic materials on ionically conducting polyelectrolyte films may present potential architectures for interdigitated batteries. The ease of genetic modification allows for the growth and assembly of other functional nanomaterials for applications such as photovoltaic devices, high-surface area catalysts, and supercapacitors.

References and Notes

17. The mass spectrum acquired by matrix-assisted laser desorption/ionization confirmed that cobalt was bound with β8 proteins of E4 clones.
19. A 100-nl sample of E4 viruses (10^12 phage/ml) was incubated with 1 ml of 1 m MCoCl2 6H2O for 1 hour. Then, 1 ml of 5 m NaBH4 was added, and the solution was kept at room temperature for the further oxidation of nanowires.
21. The mass spectrum acquired by matrix-assisted laser desorption/ionization confirmed that cobalt was bound with β8 proteins of E4 clones.
22. The ease of genetic modification of the virus allows for the growth and assembly of other virus-based materials for the generation of anodic as well as cathodic materials on ionically conducting polyelectrolyte films may present potential architectures for interdigitated batteries. The ease of genetic modification allows for the growth and assembly of other functional nanomaterials for applications such as photovoltaic devices, high-surface area catalysts, and supercapacitors.

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Fig. S1

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Fig. 51

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