Aerogels are ultralight porous materials whose matrix structure can be formed by interlinking 880 nm long M13 phage particles. In theory, changing the phage properties would alter the aerogel matrix, but attempting this using the current production system leads to heterogeneous lengths. A phagemid system that yields a narrow length distribution that can be tuned in 0.3 nm increments from 50 to 2500 nm is designed and, independently, the persistence length varies from 14 to 68 nm by mutating the coat protein. A robotic workflow that automates each step from DNA construction to aerogel synthesis is used to build 1200 aerogels. This is applied to compare Ni–MnO\textsubscript{x} cathodes built using different matrixes, revealing a pareto-optimal relationship between performance metrics. This work demonstrates the application of genetic engineering to create “tuning knobs” to sweep through material parameter space; in this case, toward creating a physically strong and high-capacity battery.

1. Introduction

Aerogels are ultralight materials, sometimes referred to as “frozen smoke,” whose properties are determined by the matrix chemistry and pore structure.\textsuperscript{[1–7]} Their ultrahigh surface areas and ultralow densities make them ideal scaffolds to create nanomaterial composites with enhanced electrical conductivity and mechanical/ thermal stabilities (carbon nanotubes, graphene, metal oxides, silica, etc.).\textsuperscript{[3,6,8–14]} These properties are ideal for battery components, such as cathodes or anodes, and the hierarchical porous structures of aerogels effectively accommodate any volume changes within electrodes during the charge–discharge process, impossible with traditional electrodes.\textsuperscript{[11,15–21]} As such, aerogel-based batteries have been explored for many battery chemistries, including lithium ion/metal/oxygen/sulfur/Te and sodium ion/oxygen.\textsuperscript{[22–40]} Battery performance is dictated by the aerogel pore size and volume as well as the surface area, controlled by varying synthesis conditions; for example, acid concentration, solvents, and gas pressure.\textsuperscript{[24,26,28,41–51]}

Alternatively, aerogels can be templated by crosslinking M13 phage, which are linear 880 nm rods.\textsuperscript{[52]} M13-templated porous structures have been used for various applications, including batteries, solar cells, catalysts, and imaging.\textsuperscript{[53–59]} Aerogels built from M13 phage have densities between 2 and 5 mg cm\textsuperscript{-3} and porosities as high as 99%.\textsuperscript{[52]} The wild-type phage consists of a packed genome surrounded by an external shell of 2700 copies of the pVIII protein. pVIII has been mutated to alter charge, add binding sites for metals or nanomaterials, or enable protein–protein interactions to direct their self-assembly into defined structures.\textsuperscript{[54,60–70]} The addition of an N-terminal EEA\textsubscript{E} to pVIII increases the negative surface charge, which enhances the material nucleation and metal deposition onto the surface of the phage scaffolds.\textsuperscript{[71–73]} Phage flexibility can be changed by mutating pVIII (Y21M), which increases the persistence length and inverts the pVIII helix chirality from left- to right-handed.\textsuperscript{[74]}

So-called phagemid systems are used to produce the phage particles required for material synthesis.\textsuperscript{[75–78]} Phagemids are plasmids containing one or two f\textsubscript{1}-oris that replicate single-stranded DNA (ssDNA) in the presence of phage proteins, expressed by helper phages or plasmids in \textit{Escherichia coli}.\textsuperscript{[79–80]} The replication of ssDNA proceeds following a rolling circle mechanism in the presence of pII, a phage protein that binds to specific sequence sites in f\textsubscript{1}-ori (nicking region-TTTAATA, βTGGAC, γGTTCCA, and δTGGGAC) and induces a single strand break at the nicking site.\textsuperscript{[81]} The free 3′-hydroxyl end of the nick serves as a primer for ssDNA synthesis, which proceeds through displacement of the nicked strand as ssDNA by DNA polymerase III and the rep helicase in the presence of

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single stranded DNA binding protein (SSB).\textsuperscript{[82–83]} ssDNA replication terminates by pII cleavage of ssDNA at a second downstream nicking site.\textsuperscript{[84]} The ssDNA is circularized, bound by pV proteins for transport to the cell membrane, and wrapped by the major phage coat and capsid proteins during extrusion of the phage particle. The phage length is determined by the size of the packaged ssDNA.\textsuperscript{[85]} Fine control over the size of the packaged ssDNA was previously attempted by flanking the desired sequence length, containing a packaging signal (PS), between a full f1 ori and a truncated f1 ori\textsuperscript{A29}, which was designed to terminate replication.\textsuperscript{[86–90]} In this way, various groups have shown that the phage can be shortened to as little as 50 nm (\textapprox\ 95 pVIII copies) and lengthened to 1300 nm.\textsuperscript{[88,91]} The challenge is that the underlying populations are bimodal because unintended longer “genomes” are produced by the ssDNA starting at f1 ori\textsuperscript{A29} and ending at f1 ori. Phages produced from the f1 ori/f1 ori\textsuperscript{A29} system require an additional purification step to ensure uniform length phage batches, thus compromising the inherent scalability of the phage amplification process for downstream material applications.

Here, we present a phage production system that generates uniform phages with precisely controllable length and a high-throughput pipeline for their conversion to aerogels and the determination of material properties. This is achieved by modifying f1 ori and f1 ori\textsuperscript{A29} to block the readthrough of DNA polymerase. This yielded a unimodal distribution of phage lengths that can be varied up to five fold by making different amino acid substitutions to pVIII residue 21. Using automated liquid handling we can cotransform phagemid/helper plasmids into E. coli, produce phages, purify them, make phage-hydrogels, lyophilize hydrogels to make aerogels and characterize them, all in high-throughput (=1000 per week). Based on the measured aerogel properties, we selected five phage lengths to make Ni–MnO\textsubscript{2} cathodes, a promising material for lithium ion batteries due to its low cost and nontoxicity.\textsuperscript{[92–95]} The cathodes are produced by forming phage hydrogels that are converted to Ni nanofoams on which MnO\textsubscript{2} is deposited.\textsuperscript{[96–97]} By changing phage length and stiffness, we are able to control porosity, battery performance, and mechanical strength of the resulting cathode. The conditions for creating these materials are identical and the properties are tuned solely through the genetic manipulation of the phage.

2. Results

2.1. Phagemid Engineering to Generate Uniform Phage

The phage production system is based on E. coli K561 harboring the phagemid and helper plasmid R474.\textsuperscript{[91]} The phagemid contains the DNA sequence to be packaged, located between four pII sequences that are intended to initiate or terminate ssDNA production (Figure 1a). Internal to the ssDNA sequence, the hairpin PS structure is included to ensure recognition and encapsulation of the ssDNA by the assembly proteins encoded by the helper plasmid.\textsuperscript{[98]} With this design, we find that the wild-type f1-ori signal (146 bp) both initiated and terminated ssDNA production, whereas the f1-ori\textsuperscript{A29} (86 bp) terminated replication and also allowed some level of replication initiation, resulting in unwanted side products (Figure 1b,c).

Our starting phagemid (EP475) contained 561 bp of DNA from the natural phage genome M13KE.\textsuperscript{[99]} The construct was intended to generate 475 bp ssDNA (including the PS sequence) for the assembly of 100 nm phages. We quantified the population of phage sizes produced using this system. Transmission electron microscopy (TEM) images were taken, from which the phage lengths were measured (Figure 1c–f; Experimental Section). About one third of the phage were in the expected range of lengths (average \textapprox\ 97 nm), corresponding to the ssDNA produced from replication starting at f1 ori and ending at f1 ori\textsuperscript{A29} (475 bp). However, the remaining two thirds of the phage population was found to be much longer with an average length of 620 nm, which corresponds to the packaging of ssDNA initiated at f1 ori\textsuperscript{A29} and terminated at f1 ori (4422 bp). Note that longer phages are assembled despite the lack of a defined PS in this region. To confirm the origin of this population of longer phage, we designed another phagemid (EP1960) to package a larger sequence of 1960 bases. This again resulted in a mixed phage distribution centered at 310 and at 680 nm (Figure S1, Supporting Information). Therefore, we concluded that this was due to replication initiated at f1 ori\textsuperscript{A29} and not due to the formation of polyphages or phages produced from multiple, serial incorporation of ssDNA.\textsuperscript{[86]}

To correct this problem, we first designed a novel phagemid system by modifying the f1 ori to create versions that only initiate (f1-\textalpha) and only terminate (f1-\textdelta\textalpha\textsuperscript{A29}) ssDNA production. To make f1-\textalpha, the 10 bp \textalpha sequence (AGTCC ACGTT) required for termination is deleted.\textsuperscript{[90]} When this sequence is used for initiation (EP475\textalpha), a bimodal population remains, but the percent of phage that are the correct length improves from 31% to 86% (Figure S2, Supporting Information). This improvement is the result of incorrect replication of ssDNA from f1 ori\textsuperscript{A29} being unable to terminate at f1-\textalpha, thus decreasing production efficiency of incorrect phage. Still, 10% of the phage are the length of the incorrect sequence, indicating that the f1-\textalpha sequence still retains some ability to terminate.

Next, we created a version of f1 ori\textsuperscript{A29} that terminates but is not able to initiate. Viral genome replication occurs after the f1 ori sequence binds to pII proteins and is nicked.\textsuperscript{[100]} There are four pII binding sites (nicking site, \textbeta, \textgamma and \textdelta) and binding between four pII proteins and these sites induces conformation changes that promote nicking of the (+) strand (Figure 1b). We sequentially tested alternative f1 ori\textsuperscript{A29} sequences where each \textdelta site (f1-\textdelta\textalpha\textsuperscript{A29}) was deleted (Figure S2, Supporting Information). We found that the removal of the \textdelta site (f1-\textdelta\textalpha\textsuperscript{A29}), combined with f1-\textalpha to create EP475\textalpha\textdelta, yielded the largest percent of correct phage (95%) with no distinct subpopulation at a different phage length (Figure 1e,f).

2.2. Genetic Control of Phage Length and Stiffness

With the improved f1-\textalpha/f1-\textdelta\textalpha\textsuperscript{A29} system, phage length is controlled by changing the DNA length between the f1-\textalpha initiator and the f1-\textdelta\textalpha\textsuperscript{A29} terminator (Figure 2a). A set of 13 phagemids designed where this DNA ranges from 330 to 19 800 bp, all containing a PS.\textsuperscript{[98]} The non-PS “inactive”
The remainder of the ssDNA was filled in using a DNA sequence we had previously observed to be inactive in E. coli. The phagemids lead to uniform length distributions between 50 and 2500 nm (with an average standard deviation of ±5%) (Figure 2b; Figure S3, Supporting Information). There is a linear correlation of phage length L with the ssDNA size n that follows $L = 0.13n$ (Figure 2c). Note that phagemids with a single wild-type f1-ori can also lead to uniform distributions, but the length cannot be reduced to <200 nm because of additional required DNA in the plasmid backbone (ori and antibiotic resistance) (Figure S4, Supporting Information).

The pVIII Y21M mutation has been reported to stiffen fd phage from a persistence length of 2.8 to 9.9 μm (for 10 μm long multimers). We mutated this position on the M13 pVIII protein to all 20 amino acids and measured the impact on the persistence length for this smaller phage (Figure 2d; Experimental Section). The phage produced by each pVIII mutant with EP475αδ (100 nm phage) were purified and the hydrodynamic radiuses measured using dynamic light scattering (DLS). From this, the persistence lengths of the mutant 100 nm phages range from 14 to 68 nm, without affecting length (Figure 2e; Figure S5, Supporting Information). The Y21M mutation only showed a 50%
Figure 2. Independent control of phage length and stiffness. a) The set of phagemids are shown with different lengths of ssDNA (numbers are bp). The detailed plasmid map and DNA sequences are provided in Table S2 and Figure S14 in the Supporting Information. b) The length distributions are shown for each phagemid. The data were collected from three replicates, performed on different days, and compiled to create a single distribution (N = 99). The histogram was drawn using the bin width of 50. Representative TEM images are shown for four phagemids; yellow highlights the length of the largest phage. c) The relationship between mean phage length and ssDNA length. The mean is calculated from distributions obtained from three replicates performed on different days and the error bars are the standard deviations of these means. d) The helper plasmid is shown along with the location of pVIII and the Y21 mutation (the X in the name is the amino acid at position 21). The sequence and detailed plasmid map are provided in Table S1 and Figure S14 in the Supporting Information. e) The phage persistence lengths measured for pVIII mutants (using EP475αδ). WT indicates the amino acid at position 21 of wild-type pVIII. Data are the means of three replicates performed on different days and the error bars are the standard deviations.
increase in persistence length when introduced in M13 phage, in contrast to the larger effect observed for fd phage.

2.3. Automated Workflow for Aerogel Creation

Lab automation protocols were developed to encompass all steps from plasmid transformation into E. coli to aerogel formation (Figure S6, Supporting Information). The creation of phages of different lengths and stiffnesses was simplified by having each property encoded in separate phagemids and helper plasmids that can be cotransformed. To this end, 60 phagemids were constructed containing DNA sequences ranging from 330 to 19,800 bp in increments of 330 bp (Table S2, Supporting Information). These were cotransformed together with the 20 helper plasmids, each coding for a different pVIII mutant. The 1200 strains were constructed using Echo and Mantis liquid handlers to mix the plasmids/cells and transformations were performed in 96-well format using Bravo and Hamilton automated handling systems (the detailed workflow is described in the Experimental Section) (Figure 3a; Figure S6, Supporting Information). After transformation, cells were plated using the Hamilton, incubated and then picked into 96 deep-well plates (1.3 mL of 2xYT media instead of LB media to increase phage titer (Figure S7, Supporting Information)) using a colony picker (Figure S6, Supporting Information). The culture was grown, cells removed by centrifugation, and then PEG/NaCl was added using the Hamilton to precipitate the phage from the growth media. The precipitated phage pellets were then redispersed in water. Typically, $10^{13}$ phage particles were produced through

![Image](https://www.advancedsciencenews.com/)

Figure 3. High-throughput production and characterization of aerogels. a) Steps of the high-throughput production of aerogels. Details are provided in the Experimental Section and Figure S6 in the Supporting Information. The first step involves the mixing of different phagemids (P) and helper plasmids (H) to control length and stiffness, respectively. The high-throughput equipment used for each step are shown under the boxes. b) Representative SEM image of the phage–aerogel made from EP475αδ and pVIII Y21A using the high-throughput protocol. c) Force–displacement plot of raw AFM data are shown for an aerogel made from EP660αδ and pVIII Y21A. The Hertzian model is used to calculate $E$ by measuring force ($F$) and displacement ($\delta$). d) Three hundred samples are shown from the 1200 phagemid/helper plasmid combinations. Top to bottom (pVIII): A, D, M, Y, and G. Left to right: all 60 phagemids are shown. The data are representative of a single screen. e) The impact of length on Young’s modulus is shown. Each data point is the mean $E$ for the five pVIII mutants shown in panel (d). f) The impact of persistence length on Young’s modulus is shown. Each data point is the mean $E$ for the 60 lengths shown in panel (d).
this workflow and they follow the same length distribution as obtained from low-throughput techniques (Figure S8, Supporting Information).

Aerogels were synthesized from the phage variants using the Hamilton pump to dry them on hydrophobic glass slides that are then heated at 50 °C for 30 min to increase phase concentration past the critical point (0.08 wt%) where phage particles interlink through van der Waals forces.[104] The interlinked phage form a hydrogel that was then converted to an aerogel using the freeze drying method.[52] The resulting material had the highly porous structural characteristic of aerogels (Figure 3b).

The mechanical strengths (Young’s moduli, E) of the aerogels were measured using atomic force microscopy (AFM).[105–106] A force-displacement curve can be plotted from indentation at a single point on the phage-aerogel surface by the AFM tip in contact mode (Figure 3c). Using the Hertzian model,[107] the E of an aerogel can be calculated from the force-displacement curve assuming a Poisson ratio of 0.5, which is consistent with biomaterials (Figure 3c). Because AFM is difficult to automate, a subset of 300 variant samples was selected and their E measured (Figure 3d). The aerogels produced from smaller and more rigid phage tend to produce stiffer aerogels, with a sixfold range in E observed over the complete set (Figure 3e,f). These results are consistent with numerical simulations predicting particle size effects on the mechanical properties of silica aerogels[108–109] and has been experimentally observed for other porous nanomaterials.[110–122]

2.4. Genetic Optimization of Structural Battery Electrodes

High-performance structural batteries rely on simultaneous optimization of coupled electrochemical and mechanical properties, and we therefore sought to determine whether these properties could be controlled and co-optimized using phage genetics. Biotemplated nickel aerogels were produced from genetically modified phage and employed as current collectors by electrodeposition of an active material in order to produce robust Ni–MnO$_x$ biotemplated nanofoam electrodes. Different phage lengths were selected from our subset: 320, 430, 550, 750, 960, and 1300 nm (all pVIII-EEAE Y21). Biotemplated metal nanofoam current collectors were constructed following a previously reported protocol.[56,111–114] Briefly, the phages are chemically crosslinked with glutaraldehyde to form hydrogels onto which nickel is deposited first using an electrolotus deposition solution followed by the electrodeposition of a manganese oxide active material (Figure 4a; Experimental Section). The effect of heat treatment was assessed using thermogravimetric analysis (Figure S9, Supporting Information).

Materials produced using phage of different length and stiffness but identical processing conditions were evaluated (Figure 4b). Scanning electron microscope (SEM) images reveal that nanofoam-template phages exhibit distinct morphologies. This variation was quantified in order to assess the effect of phage genetics on foam porosity. Foam porosity was quantified from the SEM images, showing that an intermediate phage length (750 nm) yielded the maximum porosity (Figure 4c).

Batteries were constructed using the biotemplated Ni–MnO$_x$ electrodes as cathodes. The batteries were assembled using lithium foil as the negative electrode, a celgard separator, and an electrolyte consisting of 1 M LiPF$_6$ in EC:DMC (Experimental Section). The rate capability of the electrodes was determined by electrochemically cycling batteries using different currents, showing how rapidly a battery charge or discharge. The active material mass was calculated by weighing dry electrodes both before and after active material deposition, and nominal capacity upon discharge was calculated by dividing the electrochemical discharge capacity measured during cycling by these active material mass. Typical discharge curves are shown in Figure S10 in the Supporting Information. These exhibit a discharge capacity up to 135 mAh g$^{-1}$ similar to previous published manganese oxide data.[115–117] First cycle Coulombic efficiencies are given in Figure S11 in the Supporting Information, and are high due to organic residues. However, cycling data shown in Figure S12 in the Supporting Information show stability for 20 cycles. Electrodes made using higher porosity nanofoams show a shoulder near 2.8 V corresponding to manganese oxide intercalation. Electrodes based on lower porosity samples, by contrast, show a sloped capacitive behavior with no shoulder. This indicates that intercalation plays less of a role in electrochemical cycling when the porosity is low. This would be expected if lithium diffusion through the liquid electrolyte is blocked due to lower sample porosity. This effect of biologically controlled porosity on electrode rate capability was assessed by Ragone plots of batteries using phage of different lengths (Figure S13, Supporting Information). Low rates result in higher capacity for all electrodes. The strong effect of porosity on rate capability in these batteries indicates that these electrodes contain electrochemically inaccessible active material.

Figure 4e shows the measured nominal capacity as a function of the length of the phage used to make the biotemplated electrode. It follows the same trend as the porosity of the material, including an optimum at 750 nm. The peak value of 121 mAh g$^{-1}$ is similar to the theoretical maximum capacity of the manganese oxide active material of 147 mAh g$^{-1}$, indicating an 82% active material utilization. Because the active chemistry is the same for these materials, these data indicate that the morphology changes the lithium ion transport through the liquid electrolyte. Materials with lower porosity contain a lower volume fraction of liquid electrolyte, resulting in a corresponding rate limitation due to lithium diffusion constraints.

The mechanical strengths of the cathodes were measured using AFM (Figure 4f). There is a general trend of smaller phage leading to mechanically stronger materials, similar to the relationship between phage length and mechanical strength for the aerogels (Figure 3e). However, there is an optimum E for the cathode biotemplated by 430 nm phage (Figure 4f). This reveals the competing needs of a structural battery to have high mechanical strength, requiring robust scaffolds, and high capacity, which requires that the foam be highly porous. Using phage of different length, we can identify the optimum in balancing both of these constraints, which for our system is the biotemplated cathodes produced using 750 nm phage.

3. Discussion

Our system encodes the structural features of an aerogel or hydrogel in the genetics of the scaffolding phage. Mutations
Figure 4. Characterization of Ni–MnO\(_x\) nanofoam cathodes. a) An overview of the fabrication process is shown (Experimental Section). The additional peptides EEAE were attached on pVIII proteins. Engineered phages were crosslinked with the glutaraldehyde (red) to form hydrogels. The samples were sensitized with tetraamminepalladium chloride (green). The Ni\(^{2+}\) ions (orange) were integrated to synthesize phage-templated Ni nanofoams. After deposition of manganese oxide (purple), the phage-templated cathodes were fabricated. b) TEM images of cathodes synthesized using different lengths of engineered phage. c) Nanofoam porosity is shown as a function of the phage length used as a scaffold. For each data point, SEM images were taken at three randomly chosen positions in a sample. Porosity was calculated from the image as described in the Experimental Section. The error bars are the standard deviations of these measurements. d) Schematic of battery testing apparatus (Experimental Section) and the calculation of the nominal capacity. e) Capacity dependence on the phage length used to create the nanofoam. Samples were charged to 4.4 V and discharged at varying rates to 2 V (Figure S10, Supporting Information). Three cycles were performed at each rate and averaged to calculate the discharge capacity. The error bars are the standard deviations of these measurements. f) The Young’s moduli of the cathodes are shown as a function of phage length.
can then be made to sweep through material properties without changing the chemistry or processing conditions. As an 880 nm rod, the geometry of M13 phage has proven valuable to scaffold an enormous range of materials, including batteries, catalysis, photovoltaic cells, sensors, and optical tools.[118] Control over the rod length and stiffness paves the way for the exploration of new regions of the phage materials space, with potential new functional properties, but previous efforts have led to mixed populations that lead to heterogenous materials.

With the newly designed phagemid/helper plasmid system (f1/αf1-ΔA29) and automation of the material construction process, we are able to access new parameters for phage-templated material development. We can sweep through the gel porosities and scaffold morphologies to identify optimal materials for the target application. Porosity control is critical for many applications of phage-templated materials, including flexible electrodes, filtration membranes, and drug delivery systems.[119–122] Control over the metal nanowire morphology in the material is essential for photonic/electronic nanodevices and biomedical sensors.[123–124]

Here, phage genetic control used to create cathodes that balance the competing needs for high capacity (highly porous) with mechanical strength (robust scaffolding). The optimal phage-templated Ni-MnO₂ cathode using 750 nm phages has ~120 mAh g⁻¹ of nominal capacity as well as ~20 MPa of Young’s modulus. The capacity of the cathode is competitive compared to other cathodes of lithium ion batteries.[125] On the other hand, the Young’s modulus of cathode is lower than minimum value (~1 GPa) needed to structure panels of battery material.[126] However, using stiffer phages as a template could improve mechanical strengths of the materials as the cathode made by engineered phage with Y21A pVIII (exhibited ~2.4 times higher Young’s modulus that made by wild-type phage). Incorporation of strong metallic materials to the phage templates may additionally enhance the mechanical strength. Our ability to genetically manipulate the filament properties of phages can be used to optimally design for structural batteries that are able to carry a mechanical load as well as efficiently store electrical energy, with the ultimate objective of eliminating the need for a conventional battery by replacing it with multifunctional body panels.[127–128]

Our length-controlled phage have several advantages over abiological methods to create porous nanomaterials. They provide higher monodispersity and uniformity compared to the commercially available polymeric particles or carbon-based nanoparticles.[129–131] The crystal packing of M13 phage is 4 nm with evenly spaced nucleation events that gives small and uniform particle sizes. In addition, the proteins of phage can be easily engineered by adding specific peptides that allows us to attach various metal ions or organic/inorganic materials uniformly on the surfaces of M13 phage.[132–134] Furthermore, the phage is easily crosslinked with servers to form filaments, which allows us not to add any additives in the cathode synthesis. The disadvantage of using phage is the ability to scale-up for the production of bulk materials. Several companies have been able to scale phage production through fermentation to multikilogram scales. Another approach is to use what is learned from the phage materials to direct the synthesis of an abiological version that is easier to scale. An example of this was demonstrated by Cambrios, where silver nanowires for touch screens, phones and all-in-one computers were initially discovered using phage and then reconstructed for bulk production by Huawei. To this end, our ability to control phage properties allows for the systematic screening of thousands of materials with altered properties, beyond that which is easily accessible through chemical techniques.

4. Experimental Section

Strains, Plasmids, and Culture Media: Chemically competent E. coli XL1-Blue (recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 (F' proAB lacZΔM15 Tn10 (Tet')) (Agilent Technologies, 200249) was used for all molecular cloning and phage production experiments. Chemically competent cells were purchased or made using the Mix & Go E. coli Transformation Kit (Zymo Research, T3001). All E. coli strains were grown at 37 °C in LB-Miller media (BD Biosciences, 246620) and LB/agar plates (1.5% of Bacto Agar (BD Bioscience, 214010) unless otherwise indicated. The engineered phagemids were derived from the plasmid pL57,[16] and the helper plasmid M13-f1 was constructed based on M13mp18.[135] The sequence and detailed plasmid map are provided in Table S1 and Figure S14 in the Supporting Information. The following concentrations of antibiotics were added to maintain plasmids in liquid cultures and plates: 100 µg mL⁻¹ ampicillin (GoldBio, CAS#69-52-3), 40 µg mL⁻¹ tetracycline (GoldBio, CAS#64-75-5), and 50 µg mL⁻¹ kanamycin (GoldBio, CAS#25389-94-0).

Low-Throughput Phage Production and Purification: E. coli was first transformed with the phagemid and helper plasmid. Cells were then streaked on LB/agar plates with ampicillin and kanamycin and grown at 37 °C overnight. The single colonies were inoculated in 70 mL of LB media with ampicillin and kanamycin and grown in 250 mL Erlenmeyer flasks at 37 °C and 250 r.p.m. in a New Brunswick Innovia 44 shaker-incubator overnight. The cultures were transferred to a 250 mL Nalgene PPCO centrifuge bottle (Thermo Scientific, 3120-0250) and centrifuged at 10 000 × g for 30 min in a Sorvall RC 6+ centrifuge (Thermo Scientific) with the Fiberlite F14-6 × 250 fixed angle rotor (Thermo Scientific, 78500) and the supernatant containing phage was collected. To purify the phage, 20 mL 50% (w/v) PEG8000 (Sigma-Aldrich, 89510) and 10 mL 5 mL NaCl (Sigma-Aldrich, 53014) were added to the collected phage solution. The phage mixtures were incubated at 4 °C for 24 h and then centrifuged at 20 000 × g at 4 °C for 30 min. After gently removing the supernatant, the phage–PEG/NaCl precipitations were redispersed vigorously in 2 mL of 1× Tris-Buffered Saline (TBS) buffer (Sigma-Aldrich, T5912 diluted 10× into deionized (DI) water). To remove cell debris and extra PEG/NaCl, the solutions were transferred to prechilled 2.0 mL microcentrifuge tubes (VWR, 20170-170) and centrifuged at 15 000 r.p.m. at 4 °C for 30 min in a refrigerated benchtop Eppendorf Centrifuge 5424R (Eppendorf, 540400138) and the supernatants were collected and hereafter referred to as the “phage solution.” This protocol was used for the data shown in Figures 1 and 2.

Transmission Electron Microscopy: A 10 µL aliquot of phage solution was deposited on a 200-mesh formvar/carbon-coated copper TEM grid (Electron Microscopy Sciences, FCF200-Cu). The spot was allowed to rest for 3 min and then the excess liquid was removed using a Kimwipe. The phages were stained using 10 µL 1% uranyl acetate alternative (Gadolinium Triacetate, Ted Pella, 19485) that was deposited on the TEM grid and allowed to rest for 20 min. The excess stain solution was removed using a Kimwipe. High-resolution TEM (JEOL 2010) was performed at 200 kV accelerating voltage (Electron Microscopy lab at the Center for Materials Science and Engineering CMSE, MIT). The images were collected at 15 000× magnification for lengths EP330αoct to EP5280αoct and 10 000× magnification for lengths EP6600αoct to EP19800αoct. To quantify phage length, the TEM images were analyzed using ImageJ software. For each phage, the line was manually drawn along the phage, and then the “Measure” command was used to measure the line length. The histograms were drawn by randomly.
sampling 198 phage particles produced from phagemids EP475, EP1960, EP475sr, EP475srl, EP475srr, and EP475sm and the remaining histograms were drawn by randomly sampling 99 phage particles. The experiments were performed as three replicates where bacterial growth was initiated on different days and even numbers of phage particles were selected from each replicate (e.g., the 99 particles come from 3 replicates and 33 particles from each replicate).

**Dynamic Light Scattering:** Dynamic light scattering was performed using a cuvette-based DLS instrument (Wyatt Technology Corporation, DynaPro NanoStar). A 450 μL aliquot of phage solution was pipetted into a plastic cuvette (Eppendorf, 952010069) and the hydrodynamic radius ($R_h$) of phage was measured. The persistence length $P$ was calculated considering ($R_h$) and the phage length $L$, according to ($R_h^2 = 2P(1 - e^{-L/R_h})$).

**Phage-to-Aerogel Workflow:** To construct the 1200 strains, 60 phagemids and 20 helper plasmids were mixed in a 96-well PCR plate (Thermo Scientific, AB-800) using the Echo 550 acoustic liquid handler (Labcyte) and the Echo Cherry Pick software. A 10 μL aliquot of each lyophilized E. coli XLI-Blue (Agilent Technologies, 200249 or made in the laboratory) was added to 1.5 mL of LB media. A thermocycler (Bio-Rad C1000 Touch) was used to heat shock the cells at 42 °C for 35 s. Cell mixtures were recovered using 70 μL of SOC outbreak media (New England BioLabs, B90205) added using a Bravo automated liquid handling platform (Agilent).

After incubating at 37 °C for 60 min, the cells were transferred to 24 channel LB/agar trough plates (Analytical Sales and Services, Inc., 47025) with ampicillin and kanamycin using a Hamilton Microlab STAR liquid handling system (Hamilton). The plates were incubated at 37 °C for 2 days. The single colonies were inoculated in 96 deep-well plates (Southern Labware, 503501) (in 1.3 mL of 2×YT media (BD Biosciences, 244060)). Complete Colony Picker (Hudson Robotics) and incubated at 37 °C and 250 r.p.m. overnight in a Multitron Pro shaker-incubator (INFOR HT). To remove E. coli cells, the 96 deep-well plates were centrifuged at 4000 × g for 4 °C for 30 min in a Sorvall Legend XFR centrifuge. A 700 μL aliquot of supernatant was transferred to another centrifuge. A 700 μL aliquot of phage was pipetted onto a hydrophobic printed well slide (Fisher Scientific, 3120-0250) and centrifuged at 20 000 × g for 4 °C for 30 min. After removing the supernatant, the phage–PEG/Nal NaCl precipitations were redispersed vigorously into 7 mL of 1× TBS buffer (each bottle). A 35 mL redispersed phage mixtures were transferred to 100 mL round media storage bottles (Corning, 1395-100). As a second purification step, 12 mL of 50% (w/v) PEG8000 and 6 mL 5 m NaCl were added to the collected phage solution. The phage mixtures were incubated at 4 °C for 24 h and then divided into six samples, which were transferred to 250 mL Nalgene PPCO centrifuge bottles (Thermo Scientific, 3120-0250) and centrifuged at 20 000 × g for 4 °C for 30 min. After removing the supernatant, the phage–PEG/Nal NaCl precipitations were redispersed vigorously into 7 mL of 1× TBS buffer (each bottle) and 5 mL redispersed phage mixtures were transferred to 250 mL Nalgene PPCO centrifuge bottles (Thermo Scientific, 3119-0050PK) and centrifuged at 20 000 × g for 4 °C for 30 min in a Sorvall RC 6+ centrifuge with the Fibellite F252-8 × 50 fixed angle rotor (Thermo Scientific, 46923). After gently removing the supernatant, the resulting phage pellets were resuspended in 2 mL of 1× PBS buffer (Gibco, 10010023). To remove cell debris and excess PEG/Nal NaCl, the solutions were transferred to prechilled 2 mL microcentrifuge tubes and centrifuged at 15 000 r.p.m. at 4 °C for 30 min in a refrigerated benchtop Eppendorf Centrifuge 5424R. The supernatants were then collected, which contained 1013 phage mL−1.

**Measurement of Phage Concentration, Weight, and Titer:** The concentration of the phage solution was determined by measuring the OD at 269 nm and OD at 320 nm using an UV–vis spectrophotometer (Nanodrop, ND-1000). The difference between $OD_{269}$ nm and $OD_{320}$ nm reflected the absorbance of phage particles at 269 nm. The concentration $c$ was calculated using $c = \frac{A}{\varepsilon}$ where $A$ is the absorption ($OD_{269} - OD_{320}$), $\varepsilon$ is the extinction coefficient of phage at 269 nm (3.5 m L−1 cm−1). The molecular weight of M13 phage was 70 000dalton, and the OD269 nm concentration of the phage solution was determined by measuring the absorbance (OD269 nm) of the phage solution using a NanoDrop spectrophotometer (ND-1000). The difference between OD269 nm and OD320 nm reflected the absorbance of phage particles at 269 nm. The concentration $c$ was calculated using $c = \frac{A}{\varepsilon}$ where $A$ is the absorption ($OD_{269} - OD_{320}$), $\varepsilon$ is the extinction coefficient of phage at 269 nm (3.5 m L−1 cm−1). The molecular weight of M13 phage was 70 000dalton. As the molecular weight of M13 phage was 70 000dalton, the final concentration $c$ was calculated using $c = \frac{A}{\varepsilon}$ where $A$ is the absorption ($OD_{269} - OD_{320}$), $\varepsilon$ is the extinction coefficient of phage at 269 nm (3.5 m L−1 cm−1). The molecular weight of M13 phage was 70 000dalton. As the molecular weight of M13 phage was 70 000dalton, the final concentration $c$ was calculated using $c = \frac{A}{\varepsilon}$ where $A$ is the absorption ($OD_{269} - OD_{320}$), $\varepsilon$ is the extinction coefficient of phage at 269 nm (3.5 m L−1 cm−1). The molecular weight of M13 phage was 70 000dalton. As the molecular weight of M13 phage was 70 000dalton, the final concentration $c$ was calculated using $c = \frac{A}{\varepsilon}$ where $A$ is the absorption ($OD_{269} - OD_{320}$), $\varepsilon$ is the extinction coefficient of phage at 269 nm (3.5 m L−1 cm−1). The molecular weight of M13 phage was 70 000dalton. As the molecular weight of M13 phage was 70 000dalton, the final concentration $c$ was calculated using $c = \frac{A}{\varepsilon}$ where $A$ is the absorption ($OD_{269} - OD_{320}$), $\varepsilon$ is the extinction coefficient of phage at 269 nm (3.5 m L−1 cm−1). The molecular weight of M13 phage was proportional to ssDNA (6407 bases for the wild-type phage), the final equation for number of phage particles per volume was calculated based on the following equation: $OD_{269} - OD_{320} = 6 \times 10^{9}$ (number of DNA base). The phage titer was calculated by dividing the phage number by the culture media volume.

**Fabrication of Phage-Templated Cathode:** Metal nanofoam current collectors were synthesized following a procedure previously described, with the following modifications. The 1013 phage particles mL−1 solution in PBS buffer was prepared as described above. Substrates for electrode samples were cleaned by immersing electrode spacers (Pred Materials International, SUS316L) in 18 m sulfuric acid (VWR, 470302-872) for 30 min followed by thorough rinsing with DI water. Substrates for additional samples were prepared by cutting glass slides into 0.5 cm2 chips and treating with ozone (UV-O3) exposure for 10 min. To crosslink the phage, 10 μL of phage–PBS solution was pipetted onto the substrate and exposed to the polypropylene chamber (1 L volume) with 20 mL excess DI in order to maintain humidity for 4 h. Samples were then removed and allowed to sit in 0.2 mL 50%
glutaraldehyde (Sigma-Aldrich, 340855) under ambient conditions for 8 h in order to evaporate excess liquid. These samples were rinsed three times with DI water in order to remove glutaraldehyde. Samples were sensitized for 30 min using 1 × 10−2 M tetraamminepadlladium chloride solution (Sigma-Aldrich, 324348), and rinsed three times with DI water. The nickel electroleless deposition solution was made in a 2000 mL glass bottle (VWR, 10754-822) by first mixing 1000 mL of DI water with 7.17 g sodium δ-lactate (Sigma-Aldrich, L4263) and 20.93 g 3-(N-morpholino) propanesulfonic acid (MOPS, Sigma-Aldrich, 69947) and adjusting the pH to 7.0 using sodium hydroxide (Sigma-Aldrich, S8045). Nickel sulfate hexahydrate (Sigma-Aldrich, 227676) (8.417 g) was added and dissolved by stirring, followed by the addition of 3.948 g borane dimethylamine (Sigma-Aldrich, 180238). Samples were exposed to the electroleless deposition solution for 45 min, followed by rinsing three times with DI water. The sample was then dried under ambient conditions.

Deposition of Manganese Oxide Active Material: An electrodeposition solution of 0.1 M manganese acetate (Sigma-Aldrich, 221007) and 0.1 M sodium sulfate (Sigma-Aldrich, 239313) was prepared and kept in a glass bottle. Nickel current collectors were fabricated as described in the previous section and then heated in air in a small box furnace (MTI Corporation, KSL1200XF, 6 °C min−1 ramp followed by holding at 350 °C for 30 min). The nickel current collectors were weighed following heating. The back of the sample was covered with Kapton tape (Uline, S-11730) in order to block deposition. The sample, Pt counter electrode (Millipore Sigma 298093), and reference electrode (Ag/AgCl, BASI MF-2052) were placed in a three-electrode setup using 50 mL of deposition solution. Electrodeposition was performed using a potentiostat (BioLogic VMP3) by maintaining −1.8 V versus the counter electrode for 30 min (∼1.0 V vs reference electrode). Samples were then washed three times using DI water, allowed to dry, and heated in air in a box furnace (6 °C min−1 ramp followed by holding at 350 °C for 30 min). As-deposited manganese hydroxide films were golden brown and turned black upon conversion to manganese oxide during heating, forming the Ni–MnO2 electrode. The nickel current collector weight was subtracted from the final weight of the Ni–MnO2 electrode samples in order to determine the active material mass.

Calculation of Porosity: The porosities of nanofoams were calculated from SEM images. Grayscale images were loaded into FIJI(Image) and processed using the thresholding function (Image > Adjust > Threshold). The B&W setting was selected such that nanofoam struts would appear white and the background would appear black. The “Auto” setting was used to automatically choose a thresholding value that typically divided a bimodal brightness distribution between a bright foreground and a dark background. Following thresholding, the resulting average pixel value was measured (with Analyze > Set Measurements, select Limit to threshold setting enabled, the average pixel brightness was measured with Analyze > Measure). This pixel value was divided by the maximum brightness of 255 to achieve the fraction of the image occupied by the nanofoam sample. Following heating, the measured specific capacity at a given rate was the total discharge capacity over this voltage range divided by the active material mass. Three cycles were performed at each rate and averaged to calculate the discharge capacity.

Supporting Information
Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest
The authors declare no conflict of interest.

Data Availability Statement
Research data are not shared.

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