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Bioinspired Piezoelectric Nanogenerators Based on Vertically Aligned Phage Nanopillars

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Experimental Methods

Genetic modification of M13 Phages

We genetically engineered the major coat proteins (pVIII) of the M13 phage (New England Biolabs) using a recombinant DNA engineering technique[1]. An inverse polymerase chain reaction (PCR) cloning method was used to replace the desired peptide sequences positioned between the first and fifth amino acids of the N-terminus of the wild-type pVIII with residues 2-4 (Ala-**Glu-Gly-Asp**-Asp to Ala-**Glu-Glu-Glu-Glu**-Asp). We used the M13KE vector with the engineered *PstI* site as the template and 5'-

ATATATCTGCAGGAAGAAGAGGAACCCGCAAAAGCGGCCTTTAACTCCC-3’ peptides as insertion primers. The sequences of the products were verified *via* DNA sequencing at Cosmo Gentech (Daejeon, Republic of Korea).

Phage Mass Amplification

To begin the phage amplification, a wild or 4E phage plaque was chosen from a lawn in a cultivation dish and immersed in 1mL lysogeny broth (LB) containing *Escherichia coli* (*E. coli*., New England Biolabs). We incubated the mixture at 37°C for 6 h, resulting in the phages naturally infecting the *E. coli*. and then self-replicating. One millilitre of preamplified M13 phage in LB was mixed with 100 mL LB and 1 mL *E. coli* mixture that had been growing for 6 h and then incubated under vigorous shaking at 37°C for approximately 7 h in a 250-mL flask. After centrifugation of the amplified mixture, 10 mL of the resulting supernatant was transferred to 1.6 L fresh LB containing 10 mL *E. coli* mixture. The mixture was vigorously shaken at 37°C for approximately 7 h in a 4-L flask. The M13 phages were easily refined by consecutive centrifugation. The incubated mixture was rapidly centrifuged to precipitate the *E. coli.*, and the resulting supernatant was added to a solution of 20% (w/v) poly(ethylene glycol) (PEG) and 2.5 M NaCl. After storage overnight at 4 °C, the phages could be precipitated by centrifugation and suspended again in a fresh PEG/NaCl solution. This precipitation was repeated three times to further purify the phages. Then, the phages were suspended in a TAE (Tris/acetate/EDTA) buffer after finishing the last centrifugation and filtered through membranes with a 0.45-μm pore size. To verify phage stability, DNA sequences were confirmed at each step of the amplification.

Phage Quantification

We used an ultraviolet (UV)-visible spectrometer (Evolution™ 300, Thermo Fisher Scientific) to determine the concentration of the M13 phage suspension. From the absorbance spectrum, the concentration of the phage was calculated with the following equation, as previously reported[2]:

$$\textbf{mg of phages/mL} = (A_{269} - A_{320})/3.84$$

where A_{269} and A_{320} are the UV absorbances at 269 and 320 nm, respectively.

Phage Nanopillar Fabrication

A custom-made phage infiltration apparatus was constructed using a syringe pump (kd Scientific, Legato 111, Holliston, MA 01756, USA) and acrylic membrane holder (Supplementary Fig. S17). The porous template was placed between two ~2-mm-thick PMDS gaskets that have a hole with a diameter of 17 mm to prevent leakage of the phage suspension; then, two acrylic plates were tightly combined with the matrix of the porous template and PDMS. After connecting the membrane holder to the syringe pump, the phage suspension was extruded into the porous template at a precisely controlled speed of 70 μL min^{-1}. Varying phage concentrations (0.3, 0.1 and 0.05 mg mL^{-1}i.e.; 9.6X 10^{12}, 3.2X 10^{12} and1.6X 10^{12}phages mL^{-1}) and infiltration cycles (1, 2, 4, 7, 10) were used to fabricate the phage nanopillars. The porous

templates were carefully washed with deionized (DI) water and fully dried under ambient conditions. The extruded phage solution was collected to measure the phage concentration. A commercial anodic aluminium oxide (Whatman, anodisc 25) with a thickness of 60 μm and a pore diameter of 100 nm was used as a porous template.

Characterization of the Phage Nanopillar Morphologies

The morphologies of the phage nanopillar-embedded porous template were observed using a field-emission scanning electron microscope (FE-SEM, Hitachi S-4700). Atomic force microscopy (AFM) images were collected using an NX10 AFM (Park Systems) that operated by the data acquisition program XEP 3.0.4 (Park Systems) and analysed by the image processing program XEI 1.8.2 (Park Systems).

Characterization of the Piezoelectric Response

We used an AFM (NX10 AFM, Park Systems) equipped with an internal lock-in amplifier to characterize the piezoelectric response. We used a platinum-coated tip (Multi-75E, Budgetsensors) with a spring constant of 3 nN nm^{-1}, a resonance frequency of 75 kHz and a tip radius of < 25 nm. The force applied to the sample during PFM measurements was ~56 nN, which corresponds to a ~2 nm indentation, as determined by the force-distance curve. An AC peak-to-peak voltage of 1 V_{pp} at 552 Hz was applied to the AFM tip. The piezoresponse amplitude was monitored while probing the AFM tip on the sample surface. The internal lock-in

amplifier in the AFM can separate the electrostatic component from the output signal of the quadrant photodetector.

Fabrication of the Phage Nanopillar-based Nanogenerators

Piezoelectric nanogenerators were prepared by depositing 5 nm of chromium and 150 nm of gold on both surfaces of the infiltrated porous templates. After they were completely dried, two copper wires were attached to both gold electrodes using silver paint. Then, the nanogenerators were encapsulated with polydimethylsiloxane (PDMS) elastomer to enhance their robustness and durability.

Fabrication of the Phage Film-based Nanogenerators

The substrates were prepared by depositing 5 nm of chromium and 150 nm of gold on a silicon wafer. We used the PDMS mould to precisely define the phage-coated area. PDMS elastomer was poured in a Petri dish and fully cured at 70 °C for 30 min. The desired area of 342.3 mm^2 was removed from the PDMS mould using a razor blade. We gently peeled the PDMS mould out from the Petri dish and placed it on the gold-coated Si wafer. Then, 500 μL of 1.6 mg mL^{-1} (0.8 mg) phage solution was dropped onto the defined region of the substrate and fully dried. We obtained the phage films with a thickness of up to ~ 1.5 μm. Another phage-dropped substrate was overlaid on the film, and then, two PDMS elastomers were placed over the film-based nanogenerator. Copper wires were connected to gold electrodes using silver paint.

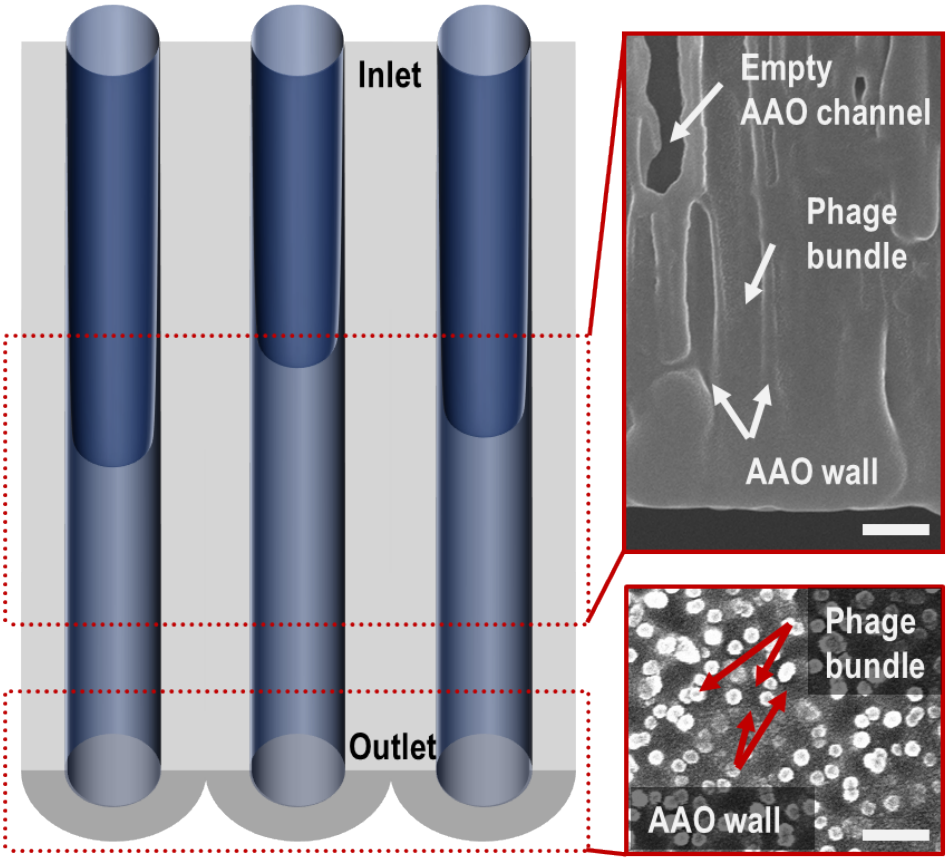
Characterization of the Piezoelectric Output Performance

A custom-built mechanical test system was constructed with a linear motor (LS Mechapion APM-SB02ADK) and a sensitive scale (Supplementary Fig. S18). An oscilloscope (Agilent DSO-X-2014A) was used for low-noise voltage and current measurements while monitoring the force applied to the nanogenerator. The load resistance for the current measurements was 1 MΩ. The piezoelectric output voltage and current were measured under periodic compression and release conditions with a constant strain rate of 150 mm s^{-1}. Load resistance dependency was performed using a variable resistor on the circuit board.

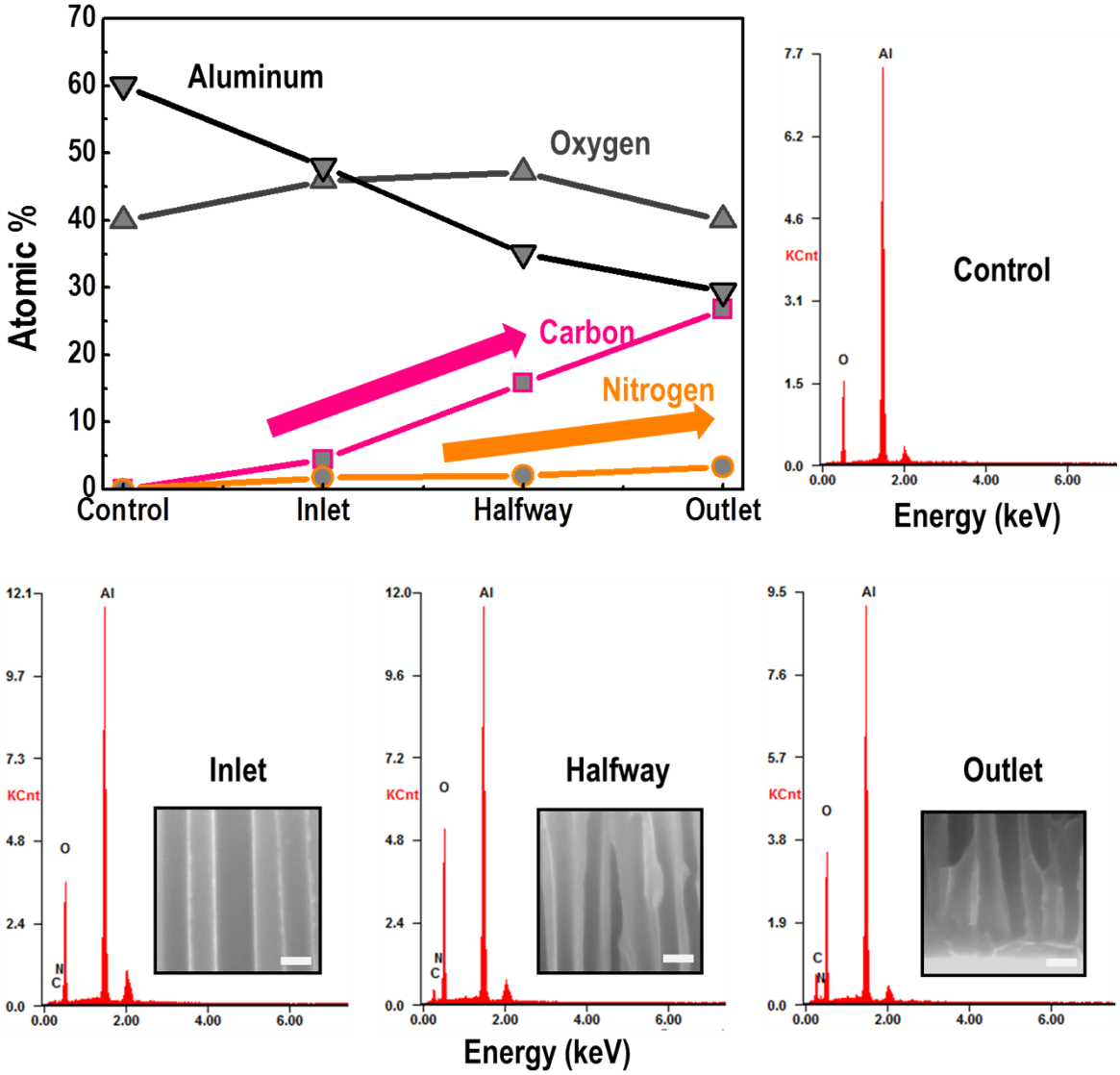
Powering the Liquid Crystal Display

The LCD screen was taken from a commercial digitalized table clock, and a proper connection was selected to display the number "1" on the front panel. We connected the LCD screen to the nanogenerator through the combination of copper clip and wire. Every junction was coated by silver paint to minimize the electrical loss. The LCD screen was directly connected to two serially combined nanogenerators without any external electrical devices. When we applied a compressive force using fingers, we could light up the LCD (Supplementary Video S1). The external voltages were applied to the LCD for observing the required voltage to light up the LCD, and then we could light up the LCD when we applied the voltage more than ~ 0.3 V.

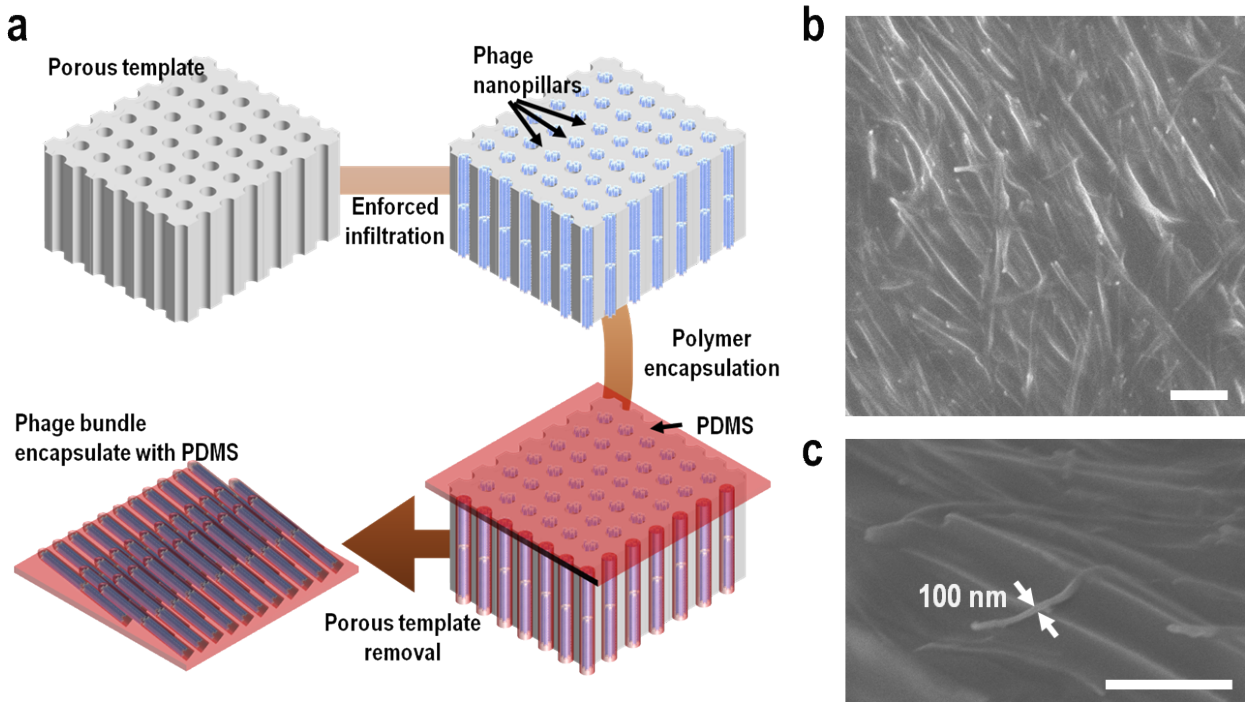
Supplementary Figures



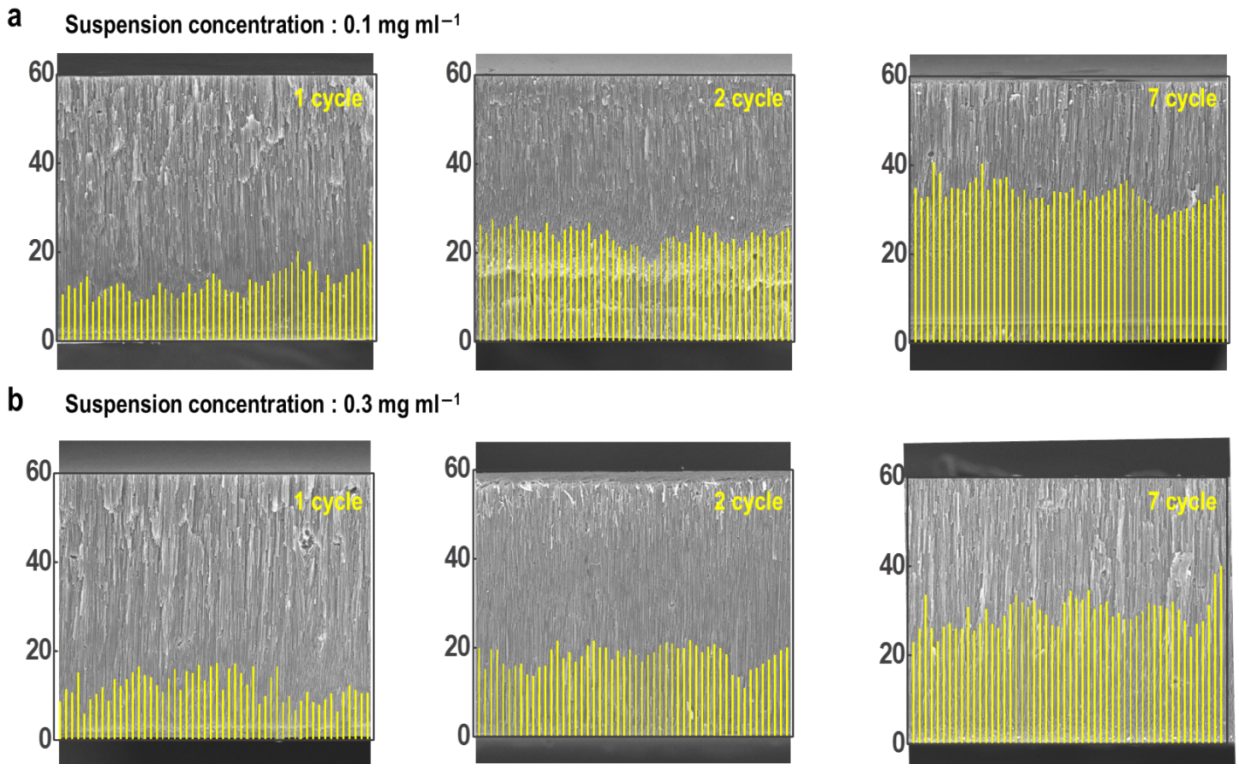
Supplementary Figure S1. Phage nanopillars after a single enforced infiltration. Cross-sectional SEM image showing that porous channels were densely packed with phage bundles at the near outlet. In addition, nominal pores at the outlet were fully filled with phage bundles. Scale bar=200 nm.



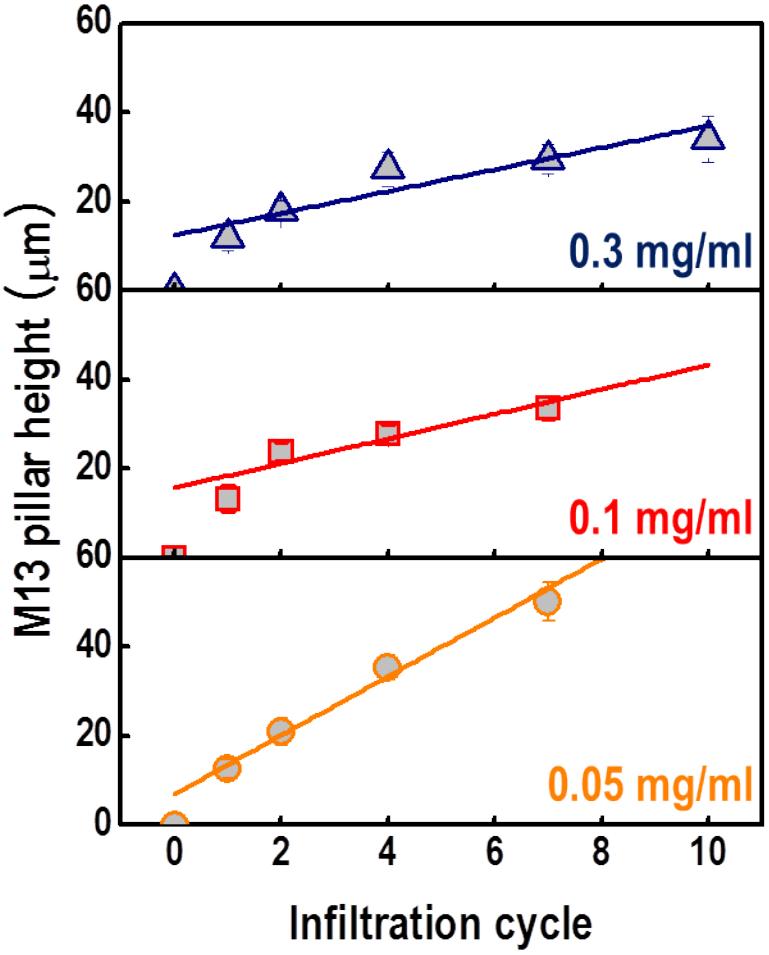
Supplementary Figure S2. Energy-dispersive X-ray spectroscopy analysis along the porous channel after a single enforced infiltration. Atomic percentages of carbon and nitrogen from an M13 phage were significantly increased when it progressed from the inlet to the outlet, indicating that phages were successfully adsorbed after a single infiltration. Scale bar=200 nm.



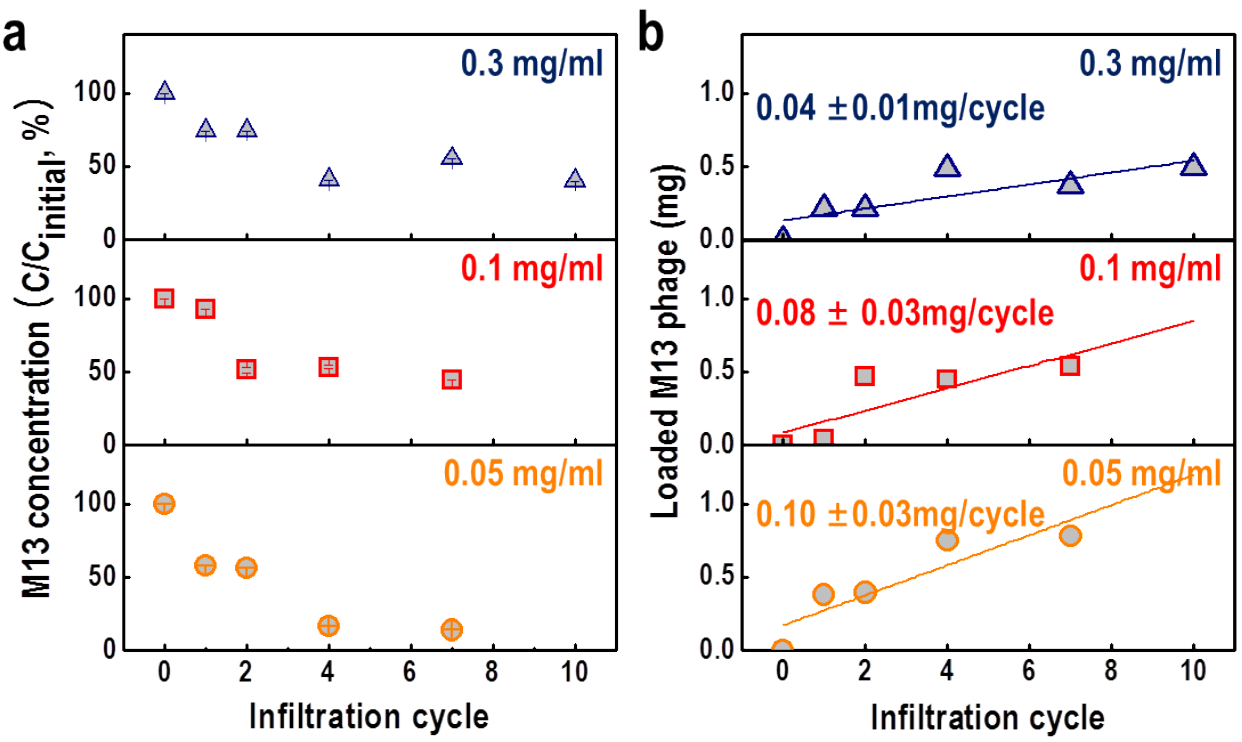
Supplementary Figure S3. Freestanding phage nanopillars. a) Schematic of the fabrication of freestanding phage nanopillars. M13 phages were enforcedly infiltrated into the porous template. Phage nanopillars then were encapsulated by PDMS elastomer as a supporting material to maintain the shape of the phage nanopillars. We fully dissolved the porous template in 0.5 M H_3PO_4 aqueous solution, resulting in phage bundles that were encapsulated with PDMS. **b**,**c**) Large-scale (**b**) and magnified (**c**) SEM images of freestanding phage nanopillars. Remarkable phage nanopillars were observed even though phage bundles were lying on a substrate. Scale bar = 1 μm.



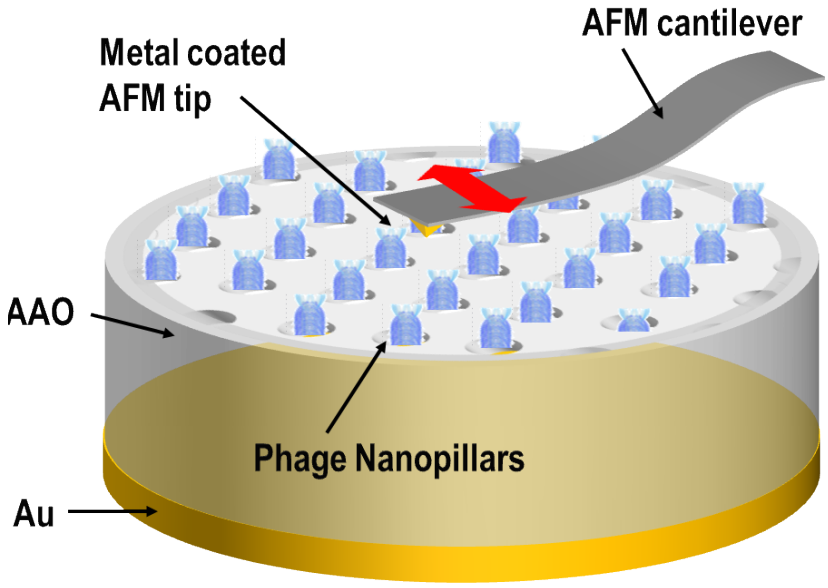
Supplementary Figure S4. Height modulation of phage nanopillars by controlling either the phage suspension concentration or infiltration cycle. a,**b**)The height of phage nanopillars increased with an increasing number of infiltration cycles, whereas they decreased with an increasing phage suspension concentration.



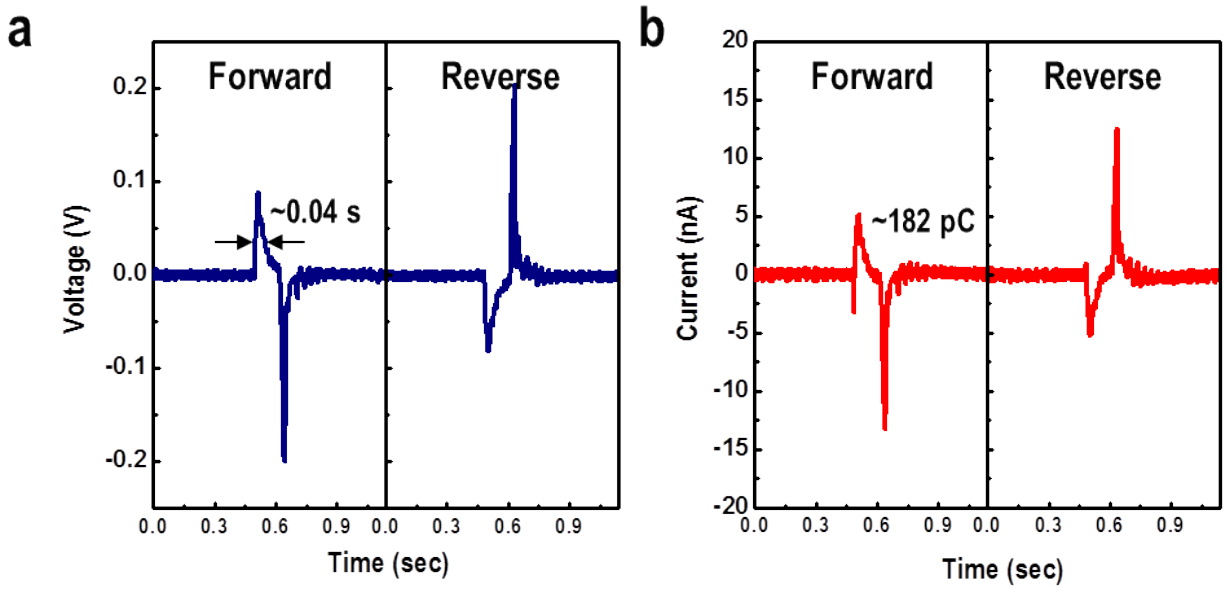
Supplementary Figure S5. Infiltration cycle dependent on the height of the M13 phage pillars. The heights of the phage nanopillars are plotted as a function of the infiltration cycle at different initial phage concentrations. Phage nanopillars using initial concentrations of 0.3, 0.1 and 0.05 mg mL^{-1} exhibited the filling rate values of 2.5±0.5, 2.8±0.6 and 6.6±0.7 μm $cycle^{-1}$, respectively.



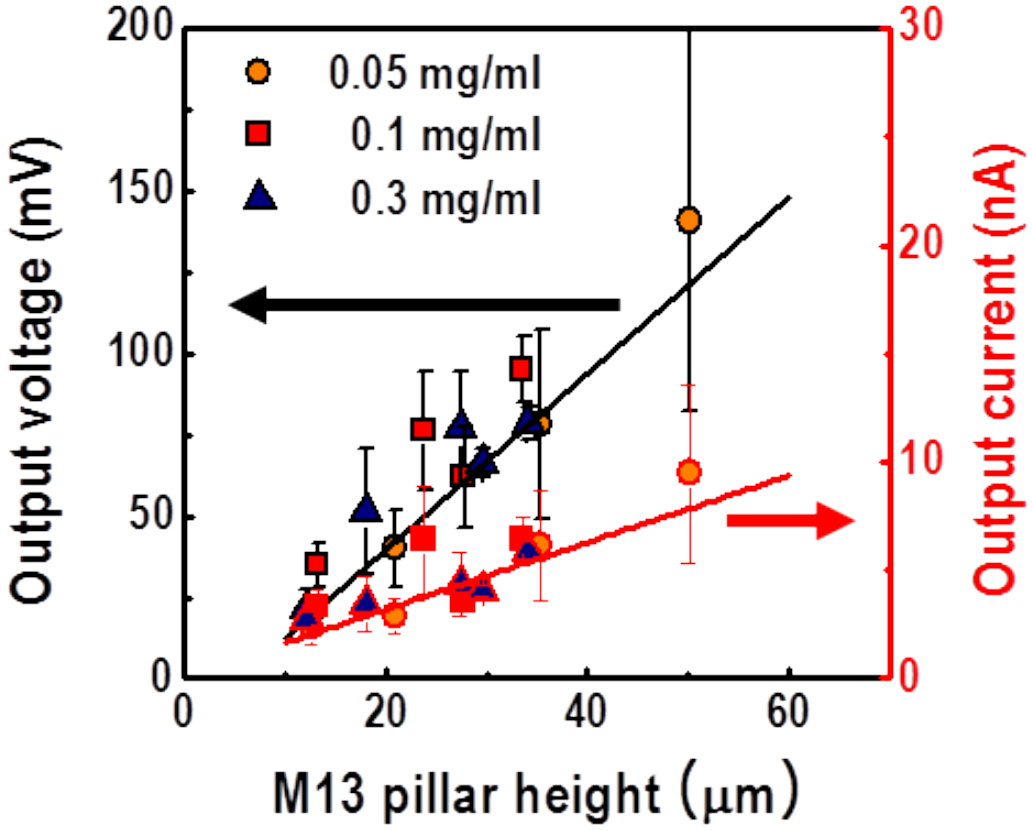
Supplementary Figure S6. Phage suspension concentration and loaded phages in the porous template after cycled infiltration. a) Concentration of the M13 phage suspension after infiltration. **b**) Calculated amount of M13 phages in the porous template. When we assumed that the disappeared phages in the phage suspension fully moved to the porous template, a loaded M13 phage, m_{phage}, could be approximated by $m_{phage}=(C_{initial}-C_{extruded})$ x V, where $C_{initial}$ and $C_{extruded}$ are the initial concentration and extruded concentration of the phage suspension, respectively, and V is the volume of phage suspension. This relationship can be used to estimate the loaded M13 phage value after infiltration. The adsorbed rates for different concentrations from 0.3 to 0.05 mg mL^{-1} increased from 0.04±0.01 to 0.1±0.03 mg $cycle^{-1}$.



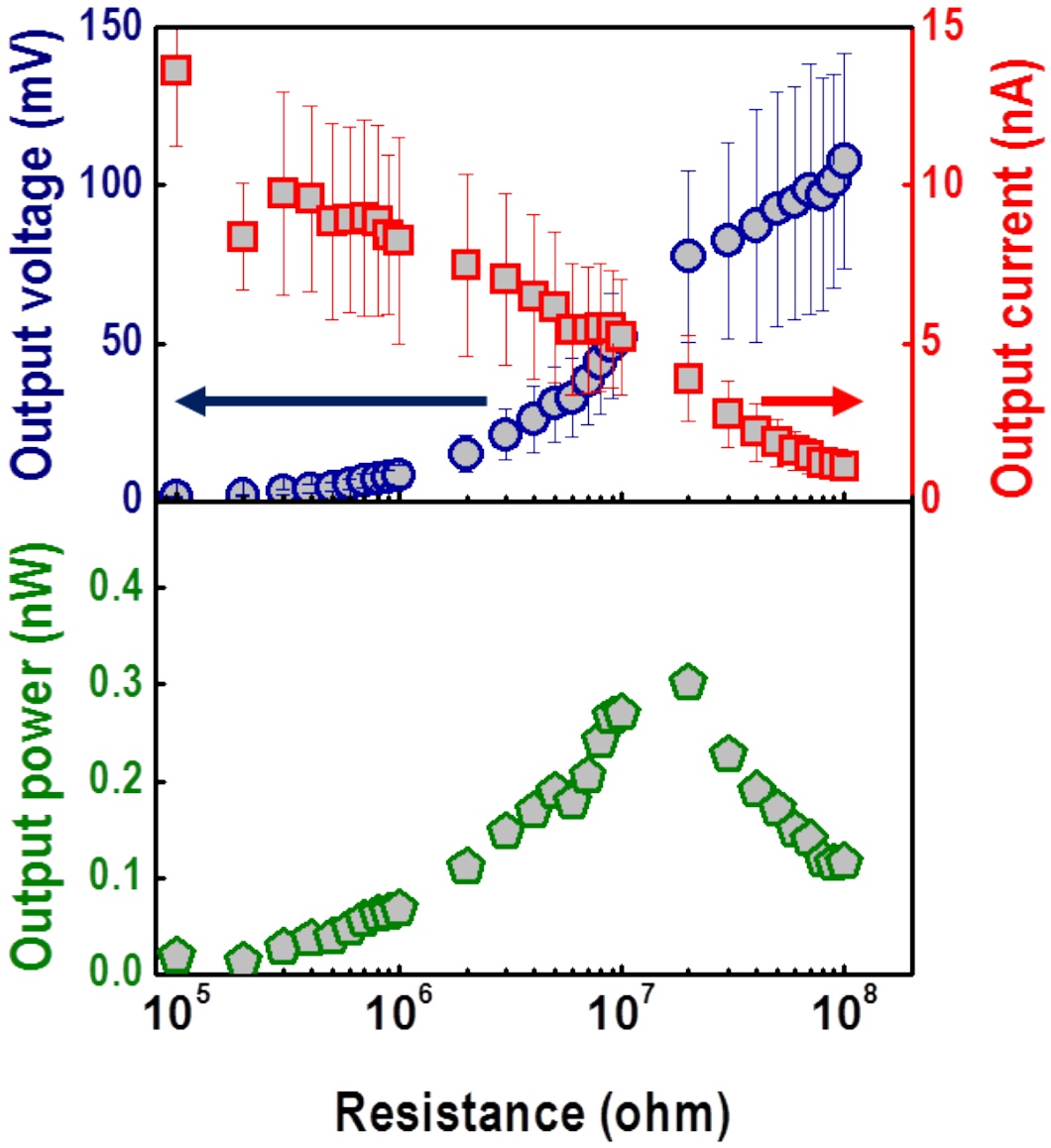
Supplementary Figure S7. Schematic of the PFM measurement. Gold electrode was thermally coated on the single surface of phage nanopillar-embedded porous template. PFM responses were measured using a later PFM mode.



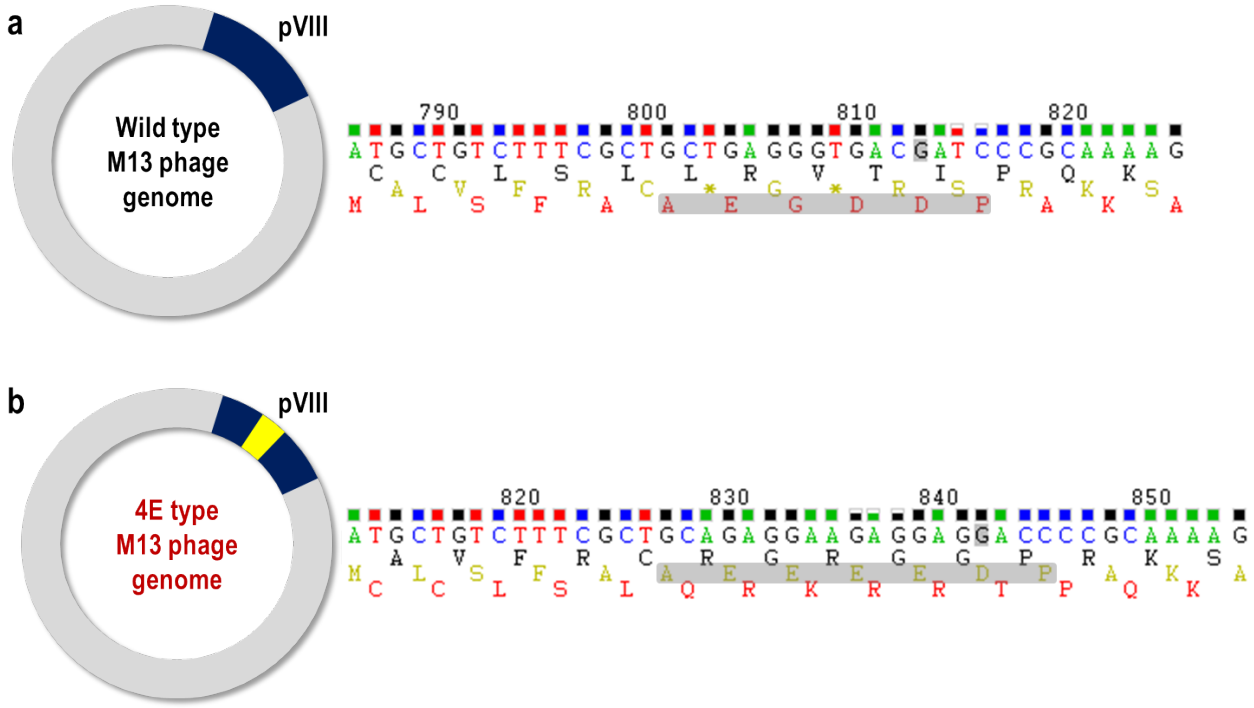
Supplementary Figure S8. Polarity switching test of wild phage nanopillar-based nanogenerators. a,**b**) Output voltage (**a**) and current (**b**) exhibited by the opposite signals when we reversely connected the nanogenerator to the measurement system.



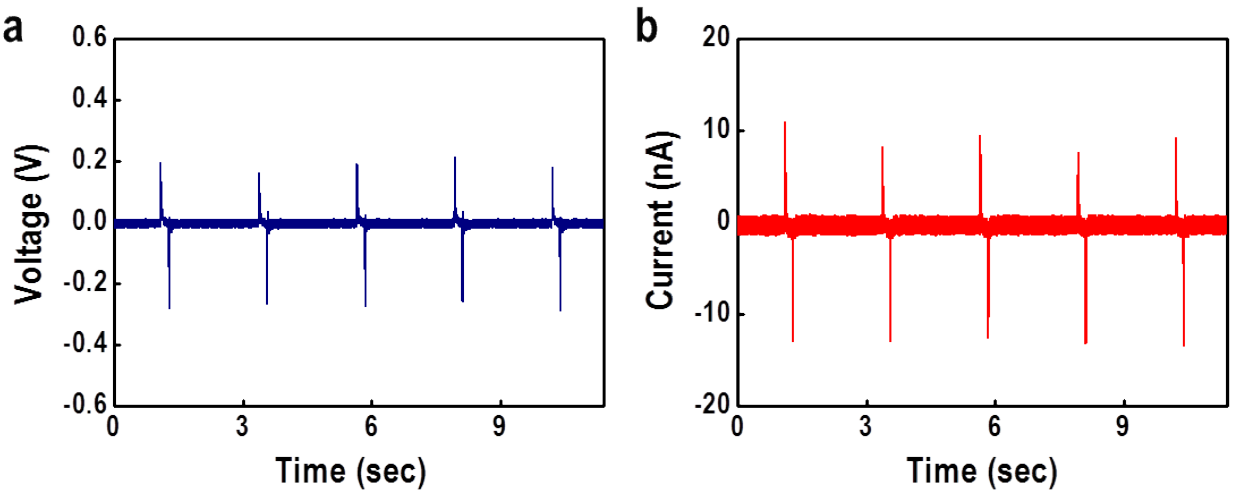
Supplementary Figure S9. Phage nanopillar height-dependent electrical outputs. The output voltage and current linearly increased while increasing the height of the phage nanopillars when applying a constant external force of F=30 N



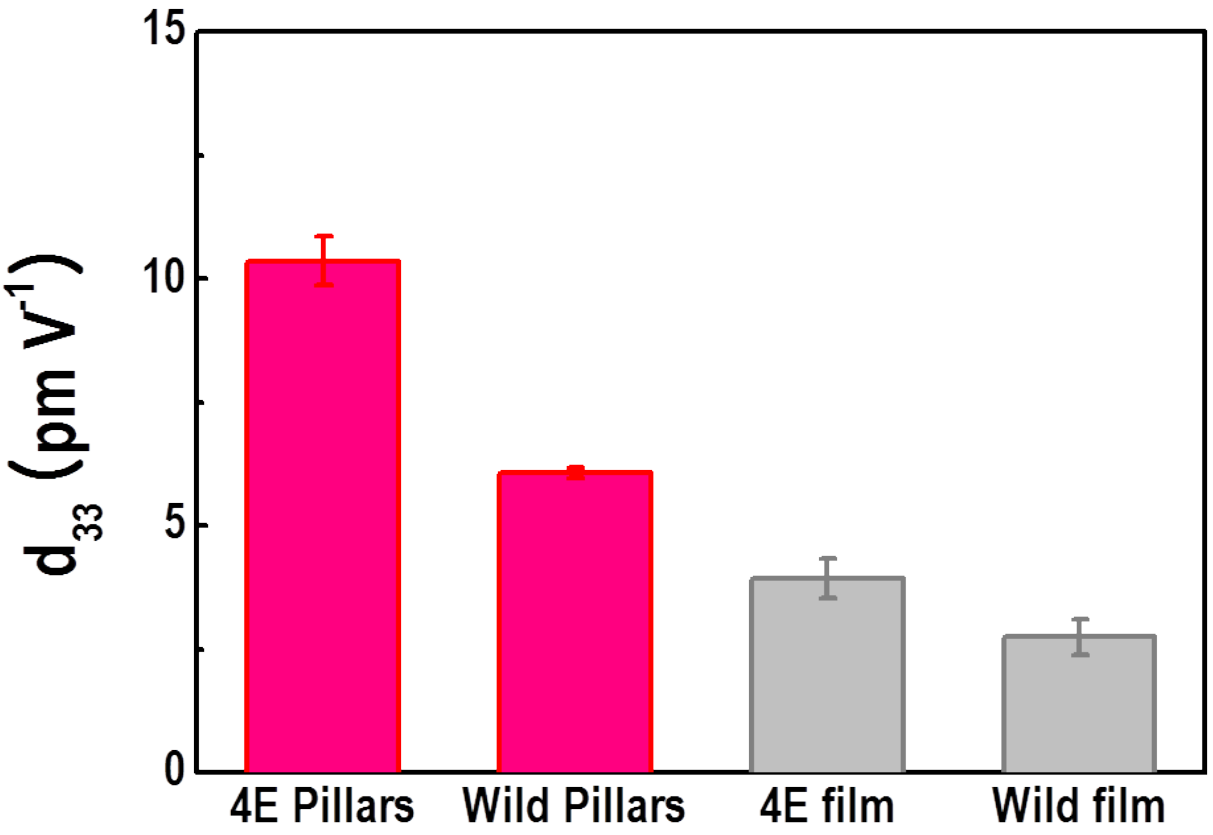
Supplementary Figure S10. Resistance-dependent electrical outputs of the wild phage nanopillar-based nanogenerator. The output voltage increased with increasing external resistance, whereas the output current decreased with increasing load. The corresponding power curve was calculated, and the maximum value was ~0.3 nW at ~10 MΩ(applied force =30N).



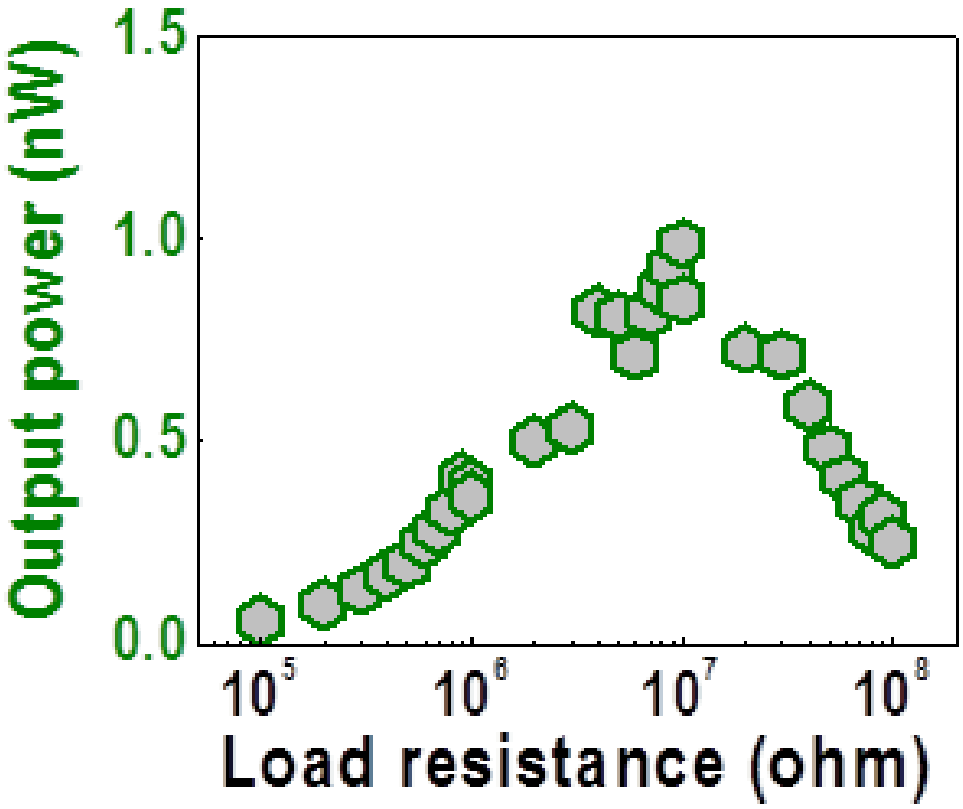
Supplementary Figure S11. pVIII protein sequences of wild- and 4E-type phages. Four glutamates (E) were positioned between the first and fifth amino acids of the pVIII protein in the wild phage. We highlight the modified protein sequence in grey.



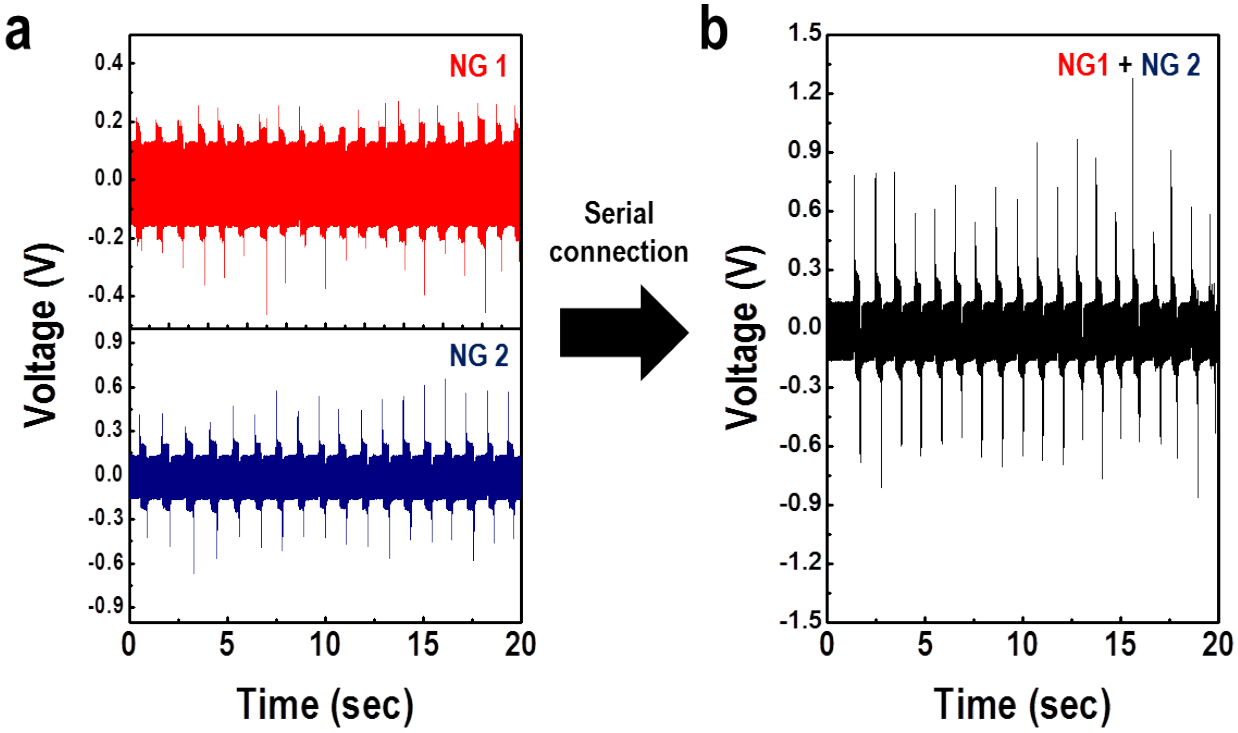
Supplementary Figure S12. Electrical outputs of the 4E phage nanopillar-based nanogenerator when applying a compressive force of 30 N.



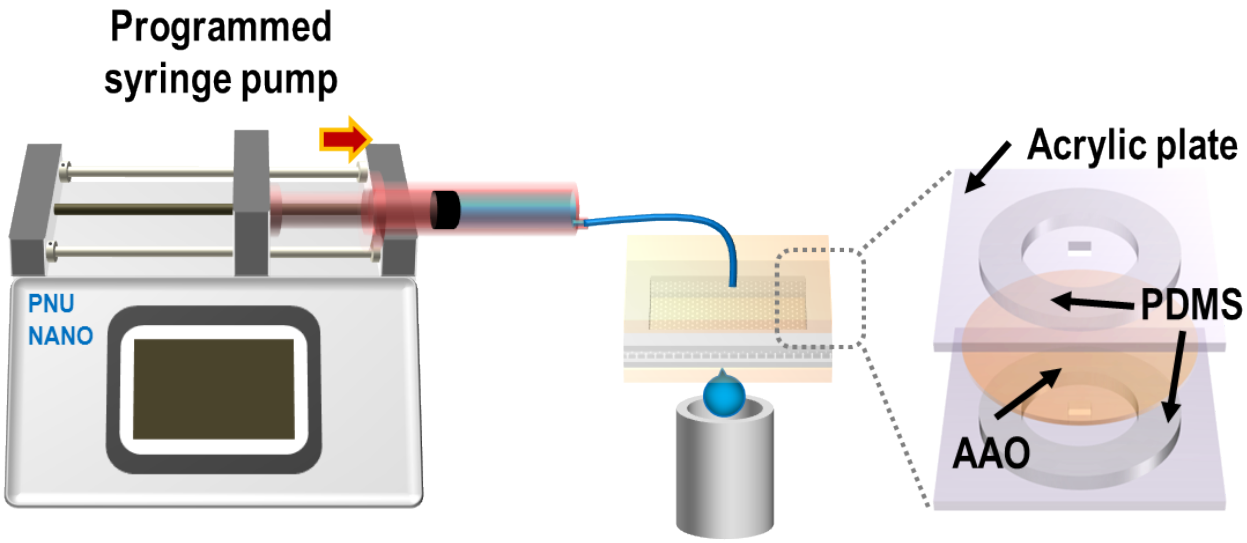
Supplementary Figure S13. Estimated piezoelectric coefficient for phage nanopillars and films based nanogenerator. Quasi-static piezoelectric coefficient can be approximated by $d_{33}=Q/F$, where Q is the generated charge and F is the applied force. The generated charge is calculated from integral of single current peak at the applied force of F=30N. The phage nanopillars exhibited higher d_{33} values than those of the phage films.



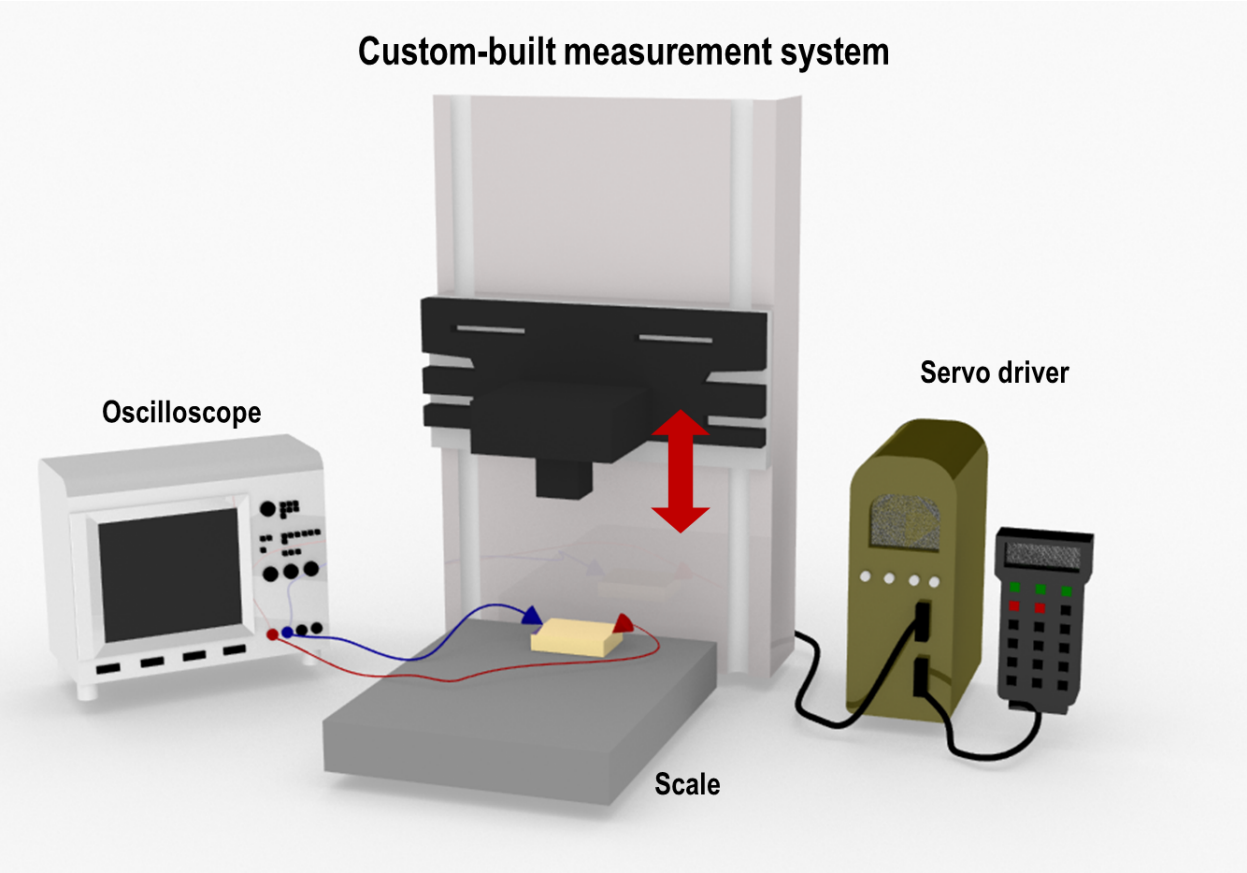
Supplementary Figure S14. Power spectrum of 4E phage nanopillars based nanogenerator. Power spectrum was calculated from the external load dependent electrical outputs, and maximum value was ~ 1nW at ~ 10MΩ. (applied force=30N).



Supplementary Figure S15. Output voltage summation of two different nanogenerators for powering the liquid crystal display. Each nanogenerator exhibited the output voltages of 0.3±0.1 and 0.5±0.1 V. Electrical signals were successfully added up when the nanogenerators were serially connected, showing output voltage of 0.7±0.2 V.



Supplementary Figure S16. Schematic illustration of custom-made enforced infiltration system. The system is composed of a syringe pump and an acrylic template holder. After placing the syringe that included the phage suspension, the solution was extruded into porous template at precisely controlled speed. Extruded phage solution was collected for concentration measurement.



Supplementary Figure S17. Schematic of custom-built mechanical test system. The system is composed of a linear motor, a sensitive scale and an oscilloscope. Electrical signals were recorded by oscilloscope while applying the periodic compressive load.

Supplementary Movie Legend

Supplementary Move 1.Movie of powering the LCD using phage nanopillars based nanogenerator. A movie was taken using two 4E phage nanopillars based nanogenerators. A smartphone (SHV-E250K (Galaxy Note 2), Samsung Electronics) was used to take video.

Supplementary References

1. A. Merzlyak and S.-W. Lee, Bioconjugate chemistry, 2009, 20, 2300-2310.
2. Sambrook, J. Rissell, D. W., *Molecular Cloning: A Laboratory Manual*, CSHL Press, New York, 2001.