Supporting Information for

Light-Controlled Graphene-Elastin Composite Hydrogel Actuators

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Table S1. ELP names and sequences used in this study.

ELP Name	N-terminal	Backbone	C-terminal
V50	MSGVG	$(VPGVG)_{50}$	VPG
V50GB	MSGVG	$(VPGVG)_{50}$	VPG*HNWYHWWPH*[a]
V50CK1	MSGVG	$(VPGVG)_{50}$	VPG*K*G [b]

[a] Graphene-binding sequence in italics [b] Lysine used for crosslinking in italics

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Table S2. Maximum ionic strength and the lowest pH before aggregation was observed.

	rGO	rGO+V50	rGO+V50GB
Maximum [NaCl] before aggregation	< 12.5 mM	At least 50mM, less than 100 mM	At least 1.0 M
Lowest pH before aggregation	~7	~6	Not observed

Table S3. Densities and swelling ratios of the hydrogel actuators.

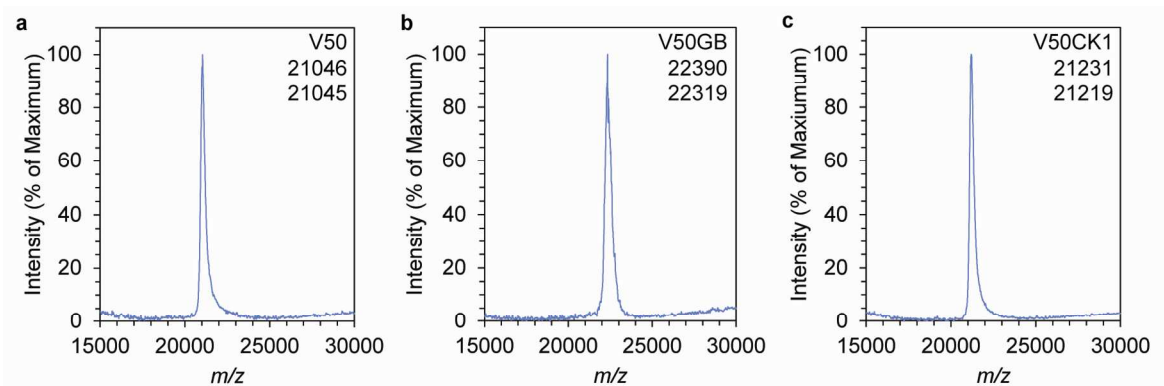|	Dry Density (mg/mm^3)	Swelling Ratio
Whole gel	0.050	23.8
Non-porous area	0.061	19.8
Porous area	0.014	88.3

Figure S1.MALDI-TOF MS of ELPs.Protein name, theoretical molecular weight (assuming N-terminal methionine cleavage), and experimentally measured molecular weights in kDa. (a)V50, (b)V50GB, and(c)V50CK1.
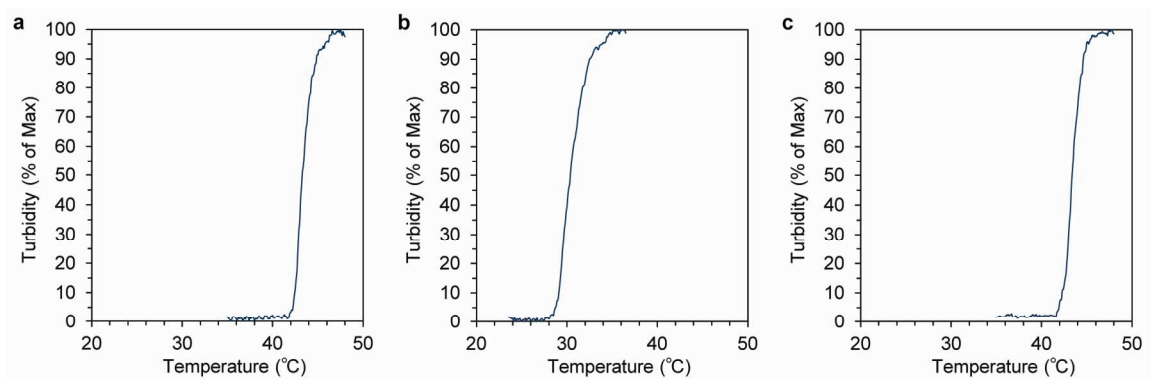
Figure S2.ELP transition temperature measurements.The T_t for (a) V50; (b) V50GB; and (c)V50CK1 were 43.3 °C, 30.4 °C and 43.4 °C, respectively in 10 mM Tris-Cl, 25 mM NaCl, pH 7.4.
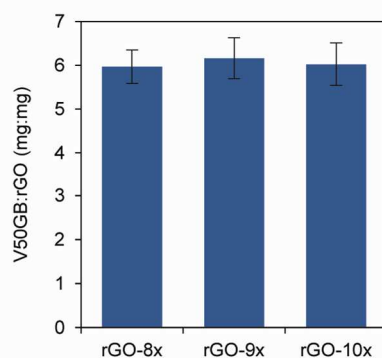

Figure S3. Characterization of V50GB binding to rGO.V50GB was added at eight, nine, or ten times the mass of rGO in solution. The mass of V50GB bound relative to the mass of rGO was determined by measuring the concentration of free V50GB. The ratios were not significantly different under the conditions tested (ANOVA P-value = 0.54) suggesting that V50GB binding was saturated in all conditions. (Error bars represent one standard deviation from the mean of three measurements).
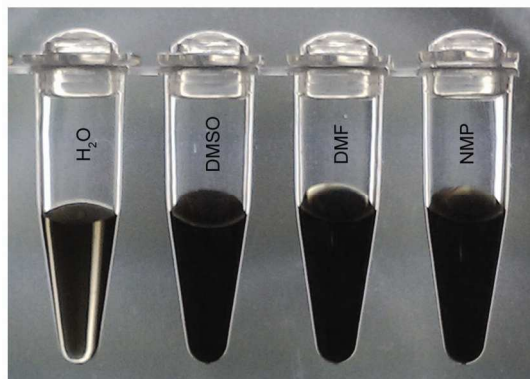
Figure S4. Dispersion of V50GB-rGO hybrid nanoparticles in organic solvents. V50GB-rGO at 0.1 mg mL^{-1} rGO in H_2O, and at 1.0 mgmL^{-1} rGO in DMSO, DMF, and NMP.
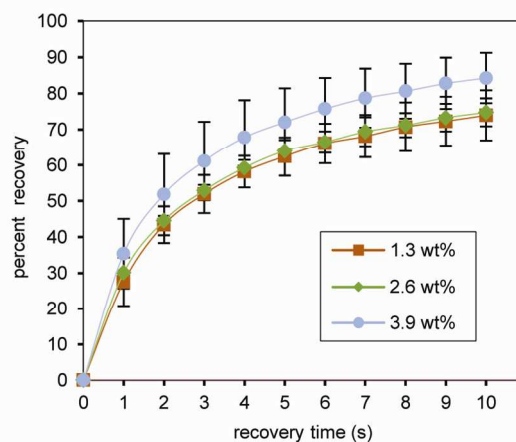

Figure S5. Recoveries of hydrogels from figure 2b after the nIR stimuli were removed. At all wt% conditions approximately 74-84% recovery occurs within 10 seconds.
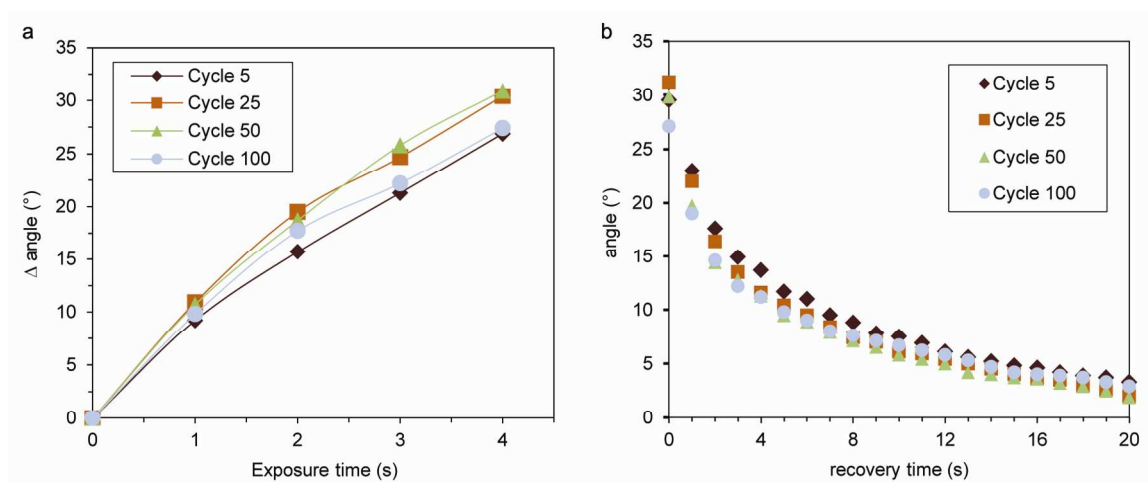
Figure S6.Repeatable bending response of composite hydrogels (2.6 wt%).The bending and recovery trajectories of a hydrogel were analyzed from the 5th, 25th, 50th, and 100th cycle. (a) Change in bending angle in response to repeated nIR laser exposure (3.0 W/cm^2). (b) Recovery of gels after the nIR stimulus was removed. No significant change in response over the course of 100 cycles was observed.
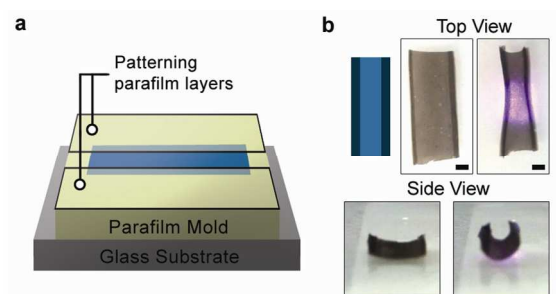
Figure S7. Surface patterning of composite hydrogels.(a)Schematic of patterning method. Parafilm was used to block water adsorption at specific regions of the gel surface.(b)Schematic of patterned hydrogel (top left). Darker regions represent stiffer regions that were formed under the parafilm. Top view (top right two images) and side view (bottom images) of a patterned hydrogel forced to bend along its long axis due to the presence of the more rigid patterned regions. Scale bars: 1 mm.

Supporting Movie 1. Composite hydrogel curling in one direction due to cooling from room temperature then curling in the other direction after heating above room temperature.

Supporting Movie 2. Upward bending of a composite hydrogel with its porous side facing up due to nIR laser exposure, then downward bending of the same hydrogel with its porous side facing down.

Supporting Movie 3. Response of an isotropic gel to nIR laser exposure. Accelerated 2x.

Supporting Movie 4. Rapid, site-specific finger-like flexing motions of a hand-shaped composite hydrogel.

Supporting Movie 5. Laser-path determined bending of a circular hydrogel.

Supporting Movie 6. Long-axis bending caused by composite hydrogel surface patterning. Accelerated 1.5x

Supporting Movie 7.nIR-driven motion of a crawling hydrogel atop a glass slide.Accelerated 1.5x.

Experimental:
Engineering of ELP genes: A gene encoding 50 VPGVG repeats was engineered into the pJ54 vector (DNA 2.0) as described previously.[1]The pET28b plasmid was digested with NcoI and XhoI restriction endonucleases and two annealed, oligonucleotides were inserted (Table S3) depending on the desired N- and C-terminal sequences. The VPGVG repeats were separated from the pJ54 plasmid by digestion with BpiI and Eco31I and then ligated with the modified pET28b vectors that were prepared by digestion with Eco31I.

Table S3. List of oligonucleotides used to synthesize the terminal sequence vectors in this study.

Oligo	Oligo Sequence (5′ to 3′)	Terminal sequence
N-F	CATGAGCGGCGTTGGCGTCCTGAGACC	MSGVG
N-R	GTGACCAGTGGGTCTCAGGACGCCAACGCCGCT	
C-GB-F	CACTGGTCACGGTCTCGGTCCCGGGTCACAACTGGTACCACTGGTGGCCGCACTAATAA	VPGHNWYHWWPH
C-GB-R	GATCTTATTAGTGCGGCCACCAGTGGTACCAGTTGTGACCCGGGACCGAGACC	
C-CK1-F	CACTGGTCACGGTCTCGGTCCCGGGTAAAGGCTAATAA	VPGKG
C-CK1-R	GATCTTATTAGCCTTTACCCGGGACCGAGACC	

Protein expression and purification: The ELP genes were transformed into BLR(DE3) *E. coli* for expression. Expression and purification of each ELP was performed as described previously.[1] The purified proteins were dialyzed into deionized water then lyophilized prior to further use.

Protein characterization: ELP molecular weights were verified by MALDI-TOF MS (Applied Biosystems Voyager DE). The matrix solution used was sinapinic acid (10 mg mL^{-1}) in 30% acetonitrile, 0.1% TFA. Transition temperatures were measured using ELP solutions (1 mg mL^{-1} of ELP in 10 mM Tris-Cl, 25 mM NaCl, pH 7.4). The ELP solution was placed in a glass cuvette with 5mm path length. The bottom half of the cuvette was placed in a stirred water bath. A light source was passed through the cuvette and the absorbance at 600 nm was measured using a spectrometer (Ocean Optics USB4000) as the temperature of the water bath was increased. The inflection point of a sigmoidal curve fit to the absorbance data was used to determine the T_t.

rGO synthesis: GO was synthesized from SP1 graphite (Bay Carbon) using a modified Hummer's method.[2] Dry, oxidized graphite was resuspended in water to ~2.0 wt%. The slurry was subjected to extensive dialysis for one week then freeze dried to form a spongy brown mass. The graphite oxide was exfoliated to single GO sheets by suspending the freeze-dried material to the desired concentration in water and then subjecting it to bath sonication. Reduction of GO to rGO was performed in batches using ascorbic acid as the reducing agent.[3] GO solutions (0.1 mg mL^{-1}) were adjusted to pH ~ 9-10 using 35% ammonium hydroxide then sonicated for an additional hour. Unexfoliated material was pelleted by spinning the solution for 20 min at 1950 x g and placed in a round bottom flask. Ascorbic acid was then added to a concentration of 1 mM. The flask was closed with a rubber stopper then sealed tightly with parafilm and Teflon tape. The flask was held in a silicone oil bath at 95 °C for 1 hour with stirring then cooled to room temperature with continued stirring. After reduction, the mass of rGO was considered equal to the mass of the GO precursor.

AFM binding analysis: Silicon wafer sections were treated with piranha solution (3:1 Sulfuric Acid: 30% hydrogen peroxide) for 10 minutes, rinsed with water and ethanol, and finally blown dry in a stream of nitrogen. The sections were then immersed in 3-Aminopropyltriethoxysilane (APTES) (1% in ethanol) for 30 minutes, rinsed with ethanol, blown dry in a stream of nitrogen, then baked in a 70 °C oven for 30 minutes. APTES treated wafer fragments were immersed in

solutions of rGO (5 µgmL^{-1} pH ~ 9) for 5 minutes, rinsed with water, and blown dry in a stream of nitrogen. Binding was performed by dipping the rGO treated fragments in solutions of V50 or V50GB (0.5 mg mL^{-1}) for 20 minutes, rinsing with water, and then blowing them dry in a stream of nitrogen. Imaging was performed using an MFP3D AFM (Asylum Research, Santa Barbara, CA) in tapping mode and analyzed using Igor Pro 6.0 software.

ELP binding to rGO: To remove byproducts from the reduction process, rGO solutions (0.1 mg mL^{-1}) were dialyzed against water that had been adjusted to a pH of ~9. The initial volume of rGO was divided by the final volume after dialysis to determine the final concentration of the dialyzed rGO solution (86.6 µg mL^{-1}). V50GB (100 µL)was added to rGO solutions (300 µL)at an 8:1, 9:1, or 10:1 mass ratio. The solutions were rotated end-over-end for 16 hours at 4 °C then placed in centrifugal filters (0.5 mL capacity, 100 kDa cutoff) (Millipore). The filters were centrifuged at 5000 x g for 30 minutes at 4 °C. The concentration of V50GB in the flow through was determined by comparison to a standard curve made with V50GB using a bicinchoninic acid assay (Thermo-Scientific).

Colloidal stability: The colloidal stability of rGO was measured by adding 25 µL of V50, V50GB (2.4 mg mL^{-1}), or water to rGO (100 µL of 0.1 mg mL^{-1}) on ice. Cold NaCl or HCl (75 µL) of varying concentration was then added to adjust the ionic strength or pH to the desired values, respectively. The solutions were kept at 4 °C and visibly monitored for signs of aggregation for one day.

*Colloidal dispersion in organic solvents:*Solutions of rGO (0.1 mg mL^{-1}) were mixed with V50GB to a 6:1 ELP to rGO mass ratio. The solutions were then heated to induce aggregation and pelleted by centrifugation. The supernatant was removed and replaced with ethanol. The solution was vortexed, pelleted again, and the supernatant was removed. The ethanol wash was repeated and then the pellet was allowed to dry in a vacuum chamber. The pellet was then resuspended in DMF, DMSO, or NMP and bath sonicated (rGO concentration of 1.0 mg mL^{-1}).

Thermoresponsive Characterization: Solutions of rGO (0.1 mg mL^{-1}) were mixed with V50GB to a 6:1 ELP to rGO mass ratio. NaCl was added to reach a concentration of 25 mM. The solutions were heated to ~45 °C and vortexed to test for aggregation. The solutions were then cooled on an ice bath and vortexed again to test for resdispersion. The same solutions were placed in 10 mm path length cuvettes and irradiated by an 808 nm nIR laser (Lazerer Electronics). The laser power was set at ~0.4 W using a laser power meter (Molectron PowerMax 5100) and a DC power supply.

Gel Synthesis: V50GB functionalized rGO was prepared by mixing the two components at a 6:1 mass ratio. The solution was then heated to 37 °C to induce aggregation and then pelleted by centrifugation at 1950 x g in a swinging arm centrifuge. The pellet was further centrifuged at 5000 x g in a fixed angle rotor (Beckman JA-20). The supernatant was removed and replaced with half the initial volume of deionized water. NaCl was added to a concentration of 5 mM. The mixture was pelleted by heating and centrifugation again and resuspended in the same volume of deionized water. This solution was frozen and lyophilized. The lyophilized product was weighed and resuspended in a 75% DMSO/25% DMF solution to a concentration of 35 mg mL^{-1}. V50CK1, triethylamine, and 4-arm PEG-NHS (Succinimidyl Carboxy Methyl ester) crosslinker

(CreativePEGWorks) were prepared in the same solvent. The four components were mixed such that the final percent weight by volume of V50CK1 would be 13% (assuming each mg of V50-CK1 added 1 μL to the volume). The TEA concentration to add was determined by the formula, [primary amines from V50GB] + [primary amines from V50-CK1] + 20·(mg of rGO). The 4-Arm PEG-NHS was added so that the NHS concentration would equal the concentration of primary amines under the assumption that each crosslinker had 3.3 arms. The value of 3.3 was determined empirically based on testing with various crosslinker concentrations. The crosslinker was always added last and mixed on ice to prevent rapid crosslinking before thorough mixing could occur.

To create anisotropically porous gels, molds were cut out of parafilm layers that were adhered onto glass microscope slides to a thickness of ~0.45 mm. The parafilm molds were adhered to the concave side of curved glass substrates (12.5 mm radius of curvature) for the preparation of the crawling gels. The pre-gel mixtures were then pipetted into the molds. The gel was allowed to stand at room temperature for 10 minutes before being put in a box containing a saturated aqueous slurry of NaCl. The box was sealed and placed in a 37 °C incubator for 20 hours. The gels were removed from their molds then soaked in water to remove any organic solvents and uncrosslinked materials.

SEM Analysis: The hydrogels were solvent-exchanged into ethanol by progressively increasing the ethanol concentration from 10% to 100% over the course of two days and then critical point dried. The dried gels were then visualized by SEM (Hitachi TM-1000).

Density and Swelling Measurements: The wet mass and dimensions of anisotropic gels and isotropic gels (not exposed to vapor) were measured. They were then critical point dried and their dry mass was measured. The dry densities of the porous and non-porous region were calculated under the assumption that the non-porous region was equivalent to an isotropic gel and with the following equations

$$(\rho_p)(X_p) + (\rho_b)(1-X_p) = \rho_t \qquad \text{and} \qquad \rho_b = \rho_n$$

where ρ_p = density of porous layer, ρ_b = density of bottom layer, ρ_t = density of the whole gel, X_p = volume fraction of porous layer, and ρ_n = density of an isotropic gel. Volume fractions were estimated based on the relative thicknesses of the porous and non-porous regions from SEM images. The swelling ratios (wet mass/dry mass) were then calculated using the following equations:

$$Q_t\rho_t = (Q_p)(\rho_p)(X_p) + (Q_b)(\rho_b)(1-X_p) \qquad \text{and} \qquad Q_b = Q_n$$

where the Q_t is the swelling ratio of the whole gel, Q_p is the swelling ratio of the porous layer, and Q_b is the swelling ratio of the bottom layer which we again consider equivalent to that of an isotropic gel.

Thermoresponsive Curling: Gels were clamped by a binder clip and suspended vertically in a ~50 mL solution of room temperature water. To cool the solution, ~40 mL of ice cold water was then manually injected into the solution by syringe while simultaneously removing ~40 mL of solution using a second syringe. The final temperature of the solution was ~10 °C. To heat the solution, ~40 mL of 55 °C water was then injected by syringe while simultaneously removing ~40 mL of water. The final temperature of the solution was ~ 35 °C.

Laser Actuation: Gel bending was characterized by pinning one end of rectangular gels (~2 mm wide) between glass slides. The gel was then irradiated by the 808 nm near-IR laser near the pinning point. Bending rates were determined by measuring the angle between the laser spot and the end of the gel using individual video frames. Bending fatigue was tested by cycling the laser on for 5 seconds and off for 25 seconds, 100 times.

Supporting References:
    (1)   Wang, E.; Lee, S.-H.; Lee, S.-W. *Biomacromolecules* **2011**, *12*, 672-680.
    (2)   Li, D.; Muller, M. B.; Gilje, S.; Kaner, R. B.; Wallace, G. G. *Nature Nanotech.* **2008**, *3*, 101-105.
    (3)   Fernández-Merino, M. J.; Guardia, L.; Paredes, J. I.; Villar-Rodil, S.; Solís-Fernández, P.; Martínez-Alonso, A.; Tascón, J. M. D. *J. Phys. Chem. C* **2010**, *114*, 6426-6432.