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Supplementary Information

Tunable, Bacterio-Instructive Scaffolds Made from Functional Graphenic Materials

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1. Supplementary Materials and Methods

1.1 Materials

Table S1. Table of materials used in chemical synthesis.

Material(s)	Vendor
Graphite flakes (-325 mesh, 99.8% metal basis), Triethyl orthoacetate, Trifluoroacetic acid (TFA), Hydrobromic acid (HBr), Sodium hydroxide (NaOH)	Alfa Aesar, Ward Hill, MA, USA
Concentrated sulfuric acid (H_2SO_4), 30% Hydrogen peroxide (H_2O_2), Glacial acetic acid	Fisher Scientific, Waltham, MA, USA
Hexylamine, Thionyl chloride ($SOCl_2$), Potassium permanganate ($KMnO_4$), *p*-Toluene sulfonic acid Solvents (dioxane, acetone, dichloromethane, diethyl ether, dimethylformamide (DMF), tetrahydrofuran, ethanol)	Sigma–Aldrich, St. Louis, MO, USA
SnakeSkin™ dialysis tubing	Thermo Scientific, Waltham, MA, USA
N_6-carbobenzyloxy-L-lysine	Chem-Implex, Wood Dale, IL, USA
Triphosgene	TCI America, Portland, OR, USA

Dry tetrahydrofuran was obtained directly from a dry solvent still. Dioxane and dimethylformamide were dried by passing through a column of activated alumina. All other reagents and solvents were used without further drying or purification. N_6-carbobenzyloxy-L-lysine was used to synthesize Lysine(Z)-NCA according to a standard literature procedure.[1]

1.2 Graphene Oxide (GO) Synthesis

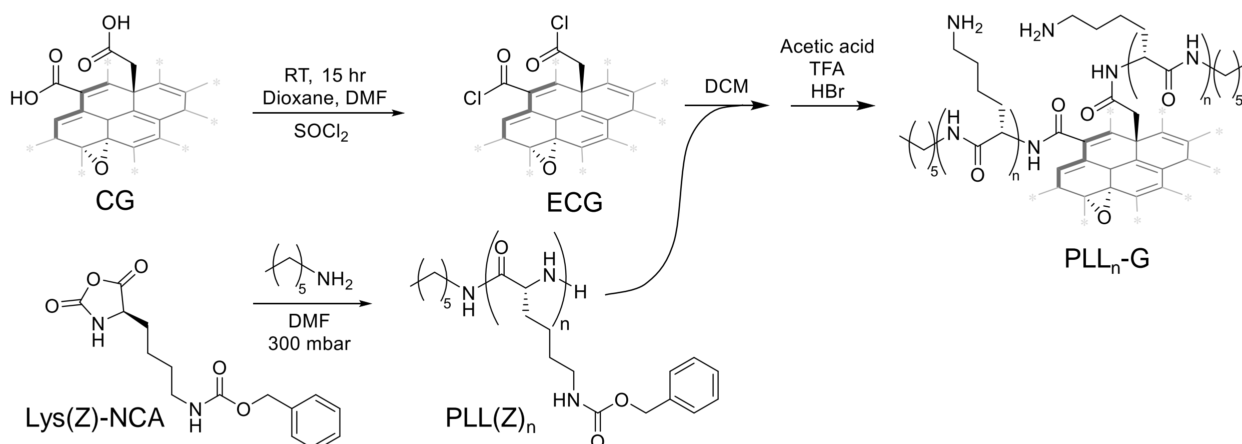
GO was synthesized using a modified Hummer's method.[2] Here, graphite (7 g) was dispersed in concentrated H_2SO_4 (175 mL) in a 1 L flask. While stirring over ice, $KMnO_4$ (14 g) was slowly added to the reaction mixture over 30 min. The ice bath was removed, and the reaction was stirred for 2 h while warming to room temperature. Then, the reaction was gently heated to 35°C and stirred for an additional 2 h. The heat was removed, and the reaction was quenched by quickly adding 980 mL of ice cold deionized (DI) water, 30% H_2O_2 (15 mL), and then 315 mL of DI water. The reaction was then stirred overnight. To purify the GO, the reaction solution was vacuum filtered through a Büchner funnel fitted with coarse filter paper (VWR grade 415). The resulting GO puck was carefully removed from the funnel without scraping the filter paper, loaded into dialysis tubing (3500 molecular weight cutoff), and dialyzed against DI water for 4 days. The DI water was changed twice the first day and then once per day for the next 3 days. Then, the dialyzed GO was frozen to –80°C and lyophilized for 3–5 days until dry.

1.3 Claisen Graphene (CG) Synthesis

CG was synthetized as previously described.[3] Briefly, GO (1.23 g) and triethyl orthoacetate (250 mL) were added to a flame dried round bottom flask under N_2. The reaction was bath sonicated

(240 W, 42 kHz ultrasonic cleaner, Kendal) for 10 min. Then, *p*-toluene sulfonic acid (21 mg) was added, and the reaction was refluxed (142°C) with stirring under N_2. After 36 h at reflux, the reaction was removed from heat and, when the reaction had cooled to approximately 85°C, 50 mL of 1.0 M NaOH (in ethanol) was added with rapid stirring. After stirring at room temperature for an additional 3 h, the reaction solution was centrifuged at 3600 × g for 10 min to pellet the CG (Z 366, HERMLE Labortechnik GmbH, Wehingen, Germany). The supernatant was discarded. The CG pellet was re–dispersed in DI water, centrifuged at 3600 × g for 10 min, and the supernatant discarded. The pellet was washed 3 additional times with DI water and 2 times with acetone. The CG pellet was then dried under vacuum overnight and stored in a desiccator.

1.4 PLL_n-G Synthesis



Scheme 1. Schematic representation of PLL_n-G synthesis. Bolded bonds indicate graphenic sheet edges, and asterisks indicate where the basal plane extends beyond the depicted structure.

PLL_n-G was synthesized using a previously described procedure.[4] Here, an oven–dried, round bottom flask was charged with CG (0.9 g) and dry dioxane (175 mL). The CG was dispersed *via* sonication (10 min, 240 W, 42 kHz, ultrasonic cleaner, Kendal), then dry DMF (0.9 mL) and $SOCl_2$ (5.3 mL) were added dropwise to the reaction flask while stirring vigorously under N_2. After stirring for 15 h at room temperature under N_2, the reaction solution was quickly vacuum filtered and rinsed with dichloromethane (under ambient conditions). The resulting filter cake of ECG (electrophilic Claisen graphene) was immediately used for PLL_n endcapping.

Poly(L-lysine-Z)$_n$ (PLL(Z)$_n$) was synthesized concurrently. Here, an oven–dried round bottom flask was charged with Lysine(Z)-NCA and vacuum backfilled thrice with N_2. Then, the monomer was dissolved in DMF (2.2 mL $mmol^{-1}$ monomer) and hexylamine initiator was added from a stock solution in DMF. After 10 min, the reaction was placed under light vacuum (approximately 300 mbar). After 2 days of stirring at room temperature under light vacuum, a 0.2 mL aliquot was withdrawn from the polymerization solution and precipitated into cold diethyl ether, filtered, and dried under vacuum to give a sample of free, unconjugated PLL(Z)$_n$ for analysis by GPC and ^{1}H-NMR.

The remaining polymerization solution was used to make PLL(Z)$_n$-G. The solution was cut with dichloromethane (2.2 mL $mmol^{-1}$ NCA monomer), the ECG filter cake was added, and the resulting solution was sonicated for 10 min. ECG was added in a ratio of 0.5 mmol of peptide per gram of CG. After stirring for 2 days, the endcapping reaction was vacuum filtered and rinsed

several times with DMF, deionized water, acetone, and dichloromethane to rinse away unconjugated peptide and reaction byproducts. The resulting product, $PLL(Z)_n$-G, was dried under vacuum overnight.

To remove the Z protecting group from the conjugate material, $PLL(Z)_n$-G (200 mg) was dispersed in glacial acetic acid (5 mL) *via* sonication (10 min). Trifluoroacetic acid (2 mL) and 48% aqueous hydrobromic acid (1 mL) were added to the dispersion, and the reaction was stirred at room temperature for 48 h. The resulting reaction solution was centrifuged at 2160 x *g* for 10 min and the supernatant discarded. The pellet was washed by resuspension in solvent, centrifugation at 2160 x *g* for 10 min, and decanting to discard the supernatant. Wash steps were performed twice with deionized water, once with acetone, and twice with diethyl ether. All supernatants from wash steps were discarded. The resulting deprotected PLL_n-G pellet was dried under vacuum overnight and stored in a desiccator.

1.5 Deprotection of free $PLL(Z)_{50}$

$PLL(Z)_{50}$ (73.6 mg) and trifluoroacetic acid (0.7 mL) were added to a scintillation vial and stirred until the peptide was completely dissolved. Then, HBr (1.2 mL, 48% v/v in H_2O) was added and the solution was stirred overnight. Next, the reaction solution was precipitated into ice cold tetrahydrofuran and filtered to give a PLL_{50} as a white solid. This material was used as the free (unconjugated) PLL control in the bacterial studies.

1.6 Fabrication of FGM scaffolds

A stainless steel die of 2.54 cm height, 6.350 cm outer diameter, and 3.749 mm inner diameter and punches of 3.749 mm diameter reference fit to die with 0.020 mm clearance per side were used to create pellets with a diameter of ~3.75 mm. 10–20 mg of powder material (GO, CG, PLL_6-G, or PLL_{50}-G) was added to the room temperature mold, pressed for 1 min with a Columbian D63 ½ bench vise, and removed. The resulting FGM pellets (Figure S1) were imaged via SEM and EDS Mapping (Figure S6). The pellets were also used to determine the contact angle of the materials and as scaffolds for the bacterial adhesion/repulsion study. The GO pellets immediately fell apart in water and re-dispersed as a powdered dispersion; thus, GO was not evaluated in the bacterial adhesion/repulsion study.

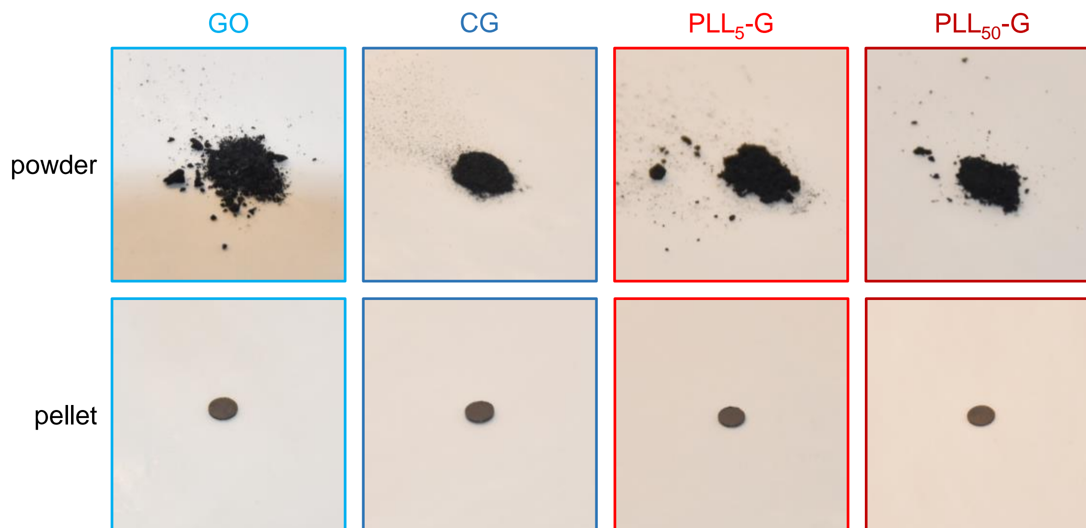


Figure S1. FGM powders (top row) were pressed into cylindrical pellets (bottom row) using a mold and benchtop vice. Images were captured using a Nikon D5600 camera with a AF-S NIKKOR 18-200 mm f/3.5-5.6G ED lens.

2. Supplementary Material Characterization

2.1 Instrumentation

Gel Permeation Chromatography (GPC)

Dispersity of the unconjugated PLL(Z) peptides was analyzed via size exclusion chromatography with dimethylformamide at 35˚C as eluent at a constant flow rate of 1.00 mL/min and a differential refractive index (RI) detector (Waters and Wyatt). The instrument was calibrated using polymethylmethacrylate standards.

Nuclear Magnetic Resonance (NMR) Spectroscopy

A 500 MHz NMR (Bruker Avance™ 500) was used to acquire ^{1}H NMR spectra of the unconjugated, PLL(Z) peptides in dimethyl sulfoxide-d_6. End group analysis was performed using Mestrenova (version 12.0.1) to determine the degree of polymerization (DP) of $PLL(Z)_n$, which allowed estimation of M_n. DP was calculated using the equation below, by comparing the absolute integral of the benzylic protons present in the repeated Z unit (δ4.94, singlet, 2H×n) to the absolute integral of the methyl protons from the hexylamine initiator (δ0.82, triplet, 3H), which are labelled in Figure S2A.

$$\mathrm{PLL(Z)_n\ DP} = \frac{\int \mathrm{Benzylic\ protons}/2}{\int \mathrm{Methyl\ protons}/3}$$

Fourier Transform Infrared (FTIR) Spectroscopy

FTIR attenuated total reflectance spectroscopy was performed using a PerkinElmer Frontier FTIR spectrometer with a germanium crystal. Spectra were acquired with a 0.25 cm^{-1} resolution over a range of 700-4000 cm^{-1}. Using the Spectrum software (PerkinElmer), spectra were corrected for attenuated total reflectance mode, baseline corrected, converted to absorbance, and normalized. The normalization of the GO spectrum was conducted using the epoxide peak (1000 cm^{-1}), due to the lack of prominent methylene peaks in the spectrum. The epoxide stretch for GO was normalized to an absorbance of 0.1. CG, $PLL(Z)_6$, and $PLL(Z)_{50}$ lacked epoxide stretches; thus, these spectra were normalized to an absorbance of 0.1 using the methylene peaks at 900 cm^{-1}.

X-Ray Photoelectron Spectroscopy (XPS)

Survey scans and high-resolution N1s and C1s scans were performed on each material using a Thermo Fisher ESCALAB 250 Xi XPS (Thermo Fisher, Waltham, MA). Samples were affixed to a piece of double-sided copper tape and irradiated with monochromatic aluminum Kα X-rays, charge compensation, and a 600-micron spot size. A minimum of 3 survey scans were performed for each spot with a 1.00 eV step size and 150.0 eV pass energy, with 3 spots per material. For N1s, a minimum of 10 scans were performed using a 0.1 eV step size and 50.0 eV pass energy, with 1 spot per material. N1s raw data was smoothed in Microsoft Excel using a moving average. The peak fitting procedure of high-resolution scans (N1s and C1s) for GO, CG, $PLL(Z)_6$, and $PLL(Z)_{50}$ has been described in more detail elsewhere.[4] Peak fitting was performed using Fityk

0.9.8.[13] Using this software, high resolution N1s and C1s data was Shirley baseline corrected and fit with a series of Lorentzian peaks using the Levenberg-Marquardt method.

Zeta Potential

Dispersions of graphenic materials with a concentration of 50 μg/mL in either LB Miller broth, Trypticase Soy broth, or water buffered to either pH 9 (GO and CG) or 5 (PLL_n-G) using NaOH and HCl, respectively. The FGM dispersions were then loaded into Malvern disposable folded capillary cells (DTS1070). Zeta potentials were measured using a Zetasizer Nano ZS (Malvern Instruments Ltd., Worcestershire, UK) with Zetasizer Software v7.12 (Malvern, Inc.). Three measurements were acquired using the optimal scanning parameters of the instrument (ranging from 10-100 scans per measurement). Data reported in Figure 3A (main text) and Figure S8 is expressed as the average ± the standard deviation of the three measurements. Zeta potential distributions of FGMs in buffered water are provided in Figure S7.

X-Ray Powder Diffraction (XRD)

XRD was conducted using an X'Pert powder diffractometer using Cu Kα radiation on a PIXcel detector (Malvern, Worcestershire, UK). The scan range was 5.005 - 27.77591 2θ with a step size of 0.0131303 2θ.

Contact Angle

Using a micropipette, 3 μL of deionized water was dispensed atop an FGM pellet. Then, the pellet with droplet was photographed straight on using a Nikon D5600 camera with an AF-S NIKKOR 18-200 mm f/3.5-5.6G ED lens. The images were analyzed using the Contact Angle plugin in ImageJ (Version 1.52a, Wayne Rasband, National Institute of Health, USA). Contact angle could not be determined with the GO pellet because the water droplet was instantly absorbed by the pellet.

Raman Spectroscopy

Raman samples were prepared by drop-casting FGM powder dispersions (approximately 1 mg/mL in acetone) onto silicon wafers. An XploRA ONE™ Raman microscope (HORIBA Scientific) with a 10× objective and a 532 nm laser line was used to acquire Raman spectra of FGMs. Data was acquired over a range of 58–3621 cm^{-1} with an average step size of 3.6 cm^{-1}, 20 accumulations, and accumulation time of 2 s.

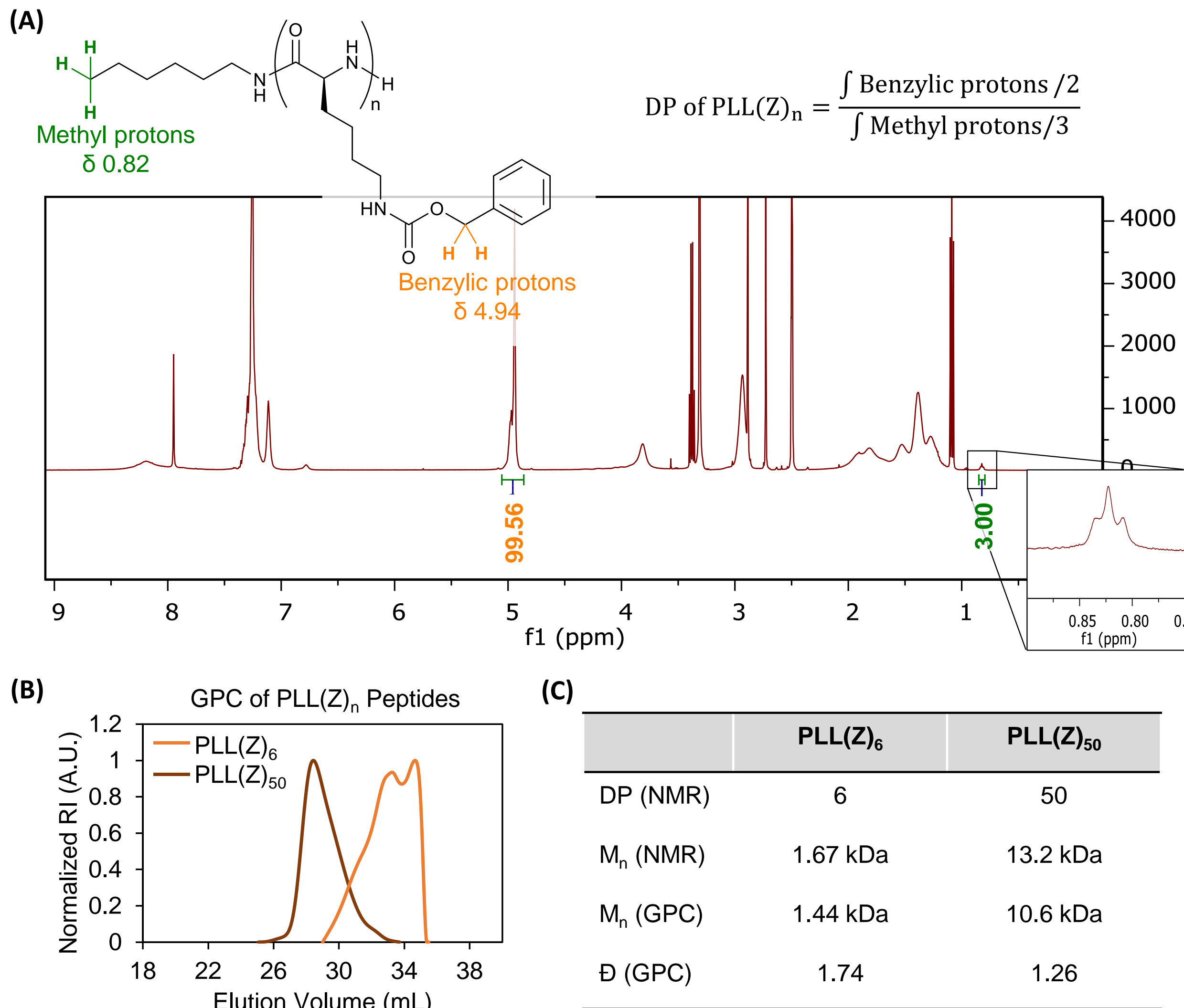
Scanning Electron Microscopy (SEM) and Energy-Dispersive X-Ray Spectroscopy (EDS)

SEM images of FGM pellets with and without bacteria were acquired using a FEI Quanta 600 FEG with an accelerated voltage of 20.00 kV and a magnification of up to 5000×. EDS elemental mapping of carbon, nitrogen, and oxygen on the FGM pellet surface (without bacteria) was obtained using an Oxford X-Max.

Pellets seeded with bacteria were prepared for SEM using previously established protocols.[6,7] Briefly, following 16 h incubation of bacteria with each FGM pellet (culture conditions described in section S3.3, "Bacterial Adhesion/Repulsion Study"), the pellets were rinsed with 250 μL of sterile 0.85% NaCl. Then, the bacteria were fixed by soaking the pellets for 2.5 h at room temperature in a solution of 2.5% glutaraldehyde (Electron Microscopy Sciences, Hatfield, PA, USA) in sterile water. Next, the pellets were rinsed with 250 μL of sterile 0.85% NaCl and

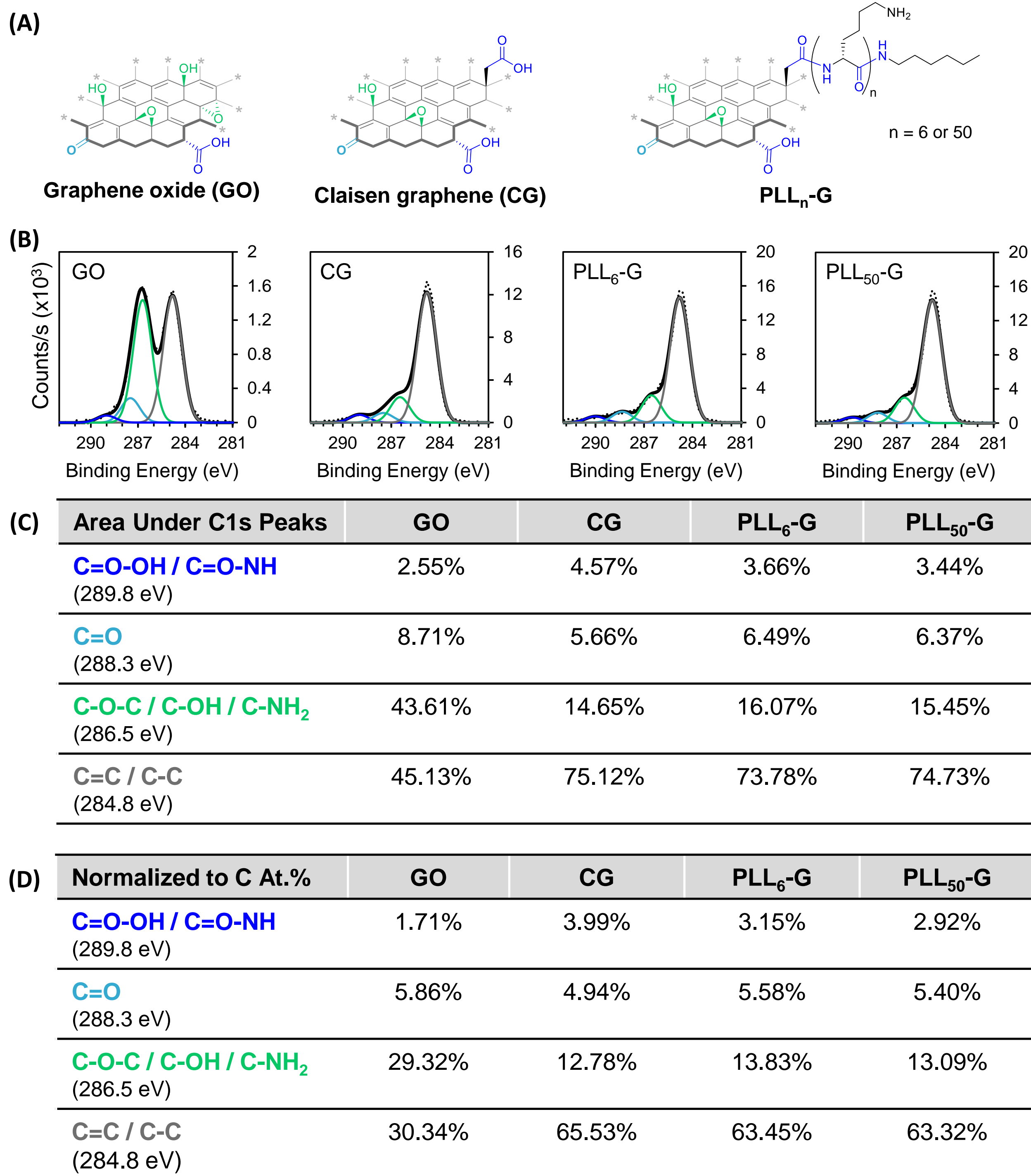
dehydrated by soaking for 10 minutes each in 500 μL of 30, 50, 70, 95, and 100% ethanol. The pellets were then flash frozen in liquid nitrogen and lyophilized overnight. Pellets were then imaged by SEM as described above.

2.2 Supplemental Results and Discussion



	PLL(Z)$_6$	PLL(Z)$_{50}$
DP (NMR)	6	50
M_n (NMR)	1.67 kDa	13.2 kDa
M_n (GPC)	1.44 kDa	10.6 kDa
Đ (GPC)	1.74	1.26

Figure S2. A) ^{1}H-NMR (500 MHz, DMSO-d$_6$) end-group analysis was used to determine the degree of polymerization (DP) of the PLL(Z)$_n$ peptides prior to conjugating to ECG. ^{1}H-NMR spectra of PLL(Z)$_{50}$ is shown as a representative sample. **B)** Gel permeation chromatography (GPC) traces of the PLL(Z)$_n$ peptides (unconjugated). The bimodal distribution of PLL(Z)$_6$ is likely due to the mix of α-helix and random coil secondary structures that possess different hydrodynamic volumes in DMF, as was previously observed.[8] **C)** The DP, number average molecular weight (M_n), and dispersity (Đ) of the PLL(Z)$_n$ peptides (unconjugated) were determined by GPC and ^{1}H-NMR. GPC predicts a lower M_n for PLL(Z)$_n$ when compared to M_n calculated from ^{1}H-NMR. This trend can be attributed to differences in the hydrodynamic volume of PLL(Z) compared to the standardization polymer, polymethylmethacrylate, in DMF.[8]



Area Under C1s Peaks	GO	CG	PLL$_6$-G	PLL$_{50}$-G
C=O-OH / C=O-NH (289.8 eV)	2.55%	4.57%	3.66%	3.44%
C=O (288.3 eV)	8.71%	5.66%	6.49%	6.37%
C-O-C / C-OH / C-NH$_2$ (286.5 eV)	43.61%	14.65%	16.07%	15.45%
C=C / C-C (284.8 eV)	45.13%	75.12%	73.78%	74.73%

Normalized to C At.%	GO	CG	PLL$_6$-G	PLL$_{50}$-G
C=O-OH / C=O-NH (289.8 eV)	1.71%	3.99%	3.15%	2.92%
C=O (288.3 eV)	5.86%	4.94%	5.58%	5.40%
C-O-C / C-OH / C-NH$_2$ (286.5 eV)	29.32%	12.78%	13.83%	13.09%
C=C / C-C (284.8 eV)	30.34%	65.53%	63.45%	63.32%

Figure S3. A) Chemical structures of functional graphenic materials (FGMs): GO, CG, PLL$_n$-G. Bolded bonds indicate graphenic sheet edges, and (*) indicate where the basal plane extends beyond the depicted structure. **B)** Deconvoluted C1s XPS spectra of FGMs, where functional groups are color coded to match the carbon-containing functional groups in panels A, C, and D. **C)** The atomic percent of the carbon functional groups in each FGM is calculated using the areas

under the corresponding C1s peaks in panel B. Note that these atomic percentages only represent carbon in the sample. **D)** The normalized atomic percent of each carbon functional group was calculated according to the equation below.

$$\%(C{=}O)_{normalized} = \%(C{=}O) \times \left(\frac{\%Carbon}{100}\right)$$

Where %Carbon is quantified from the XPS survey scans

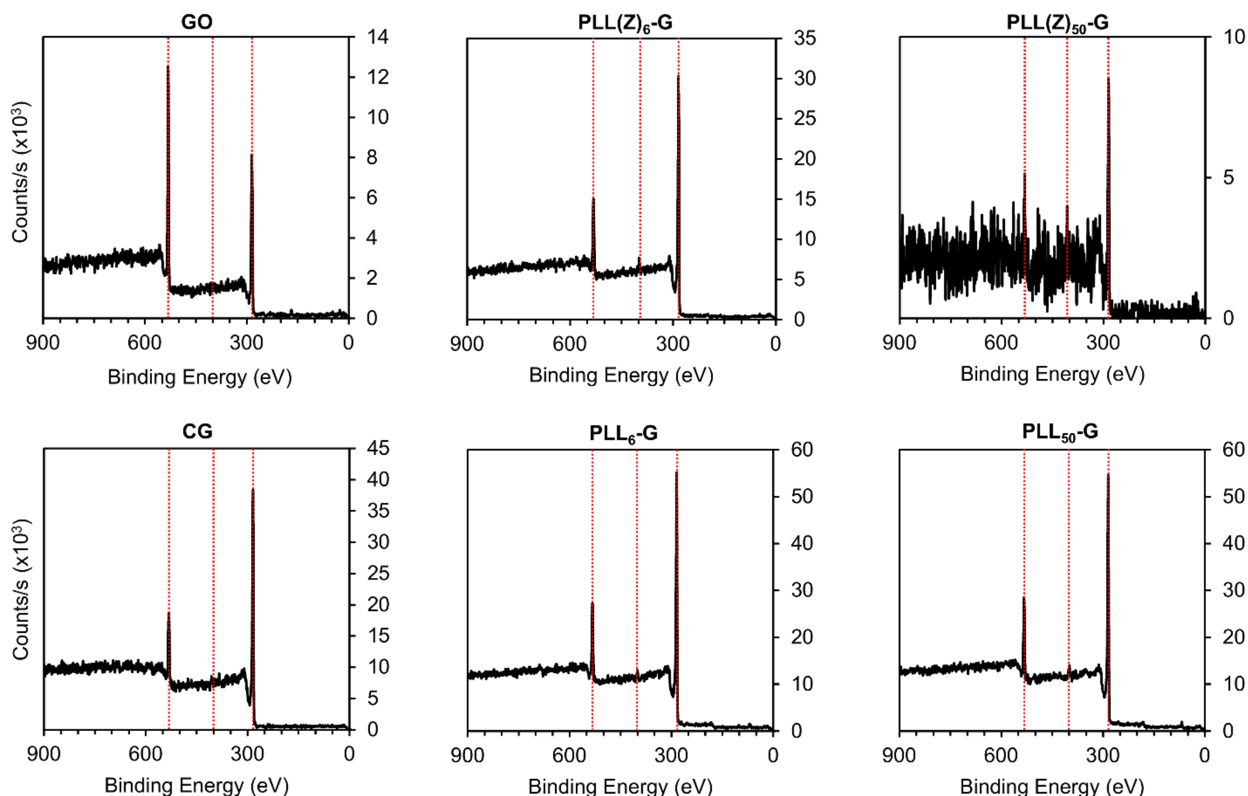
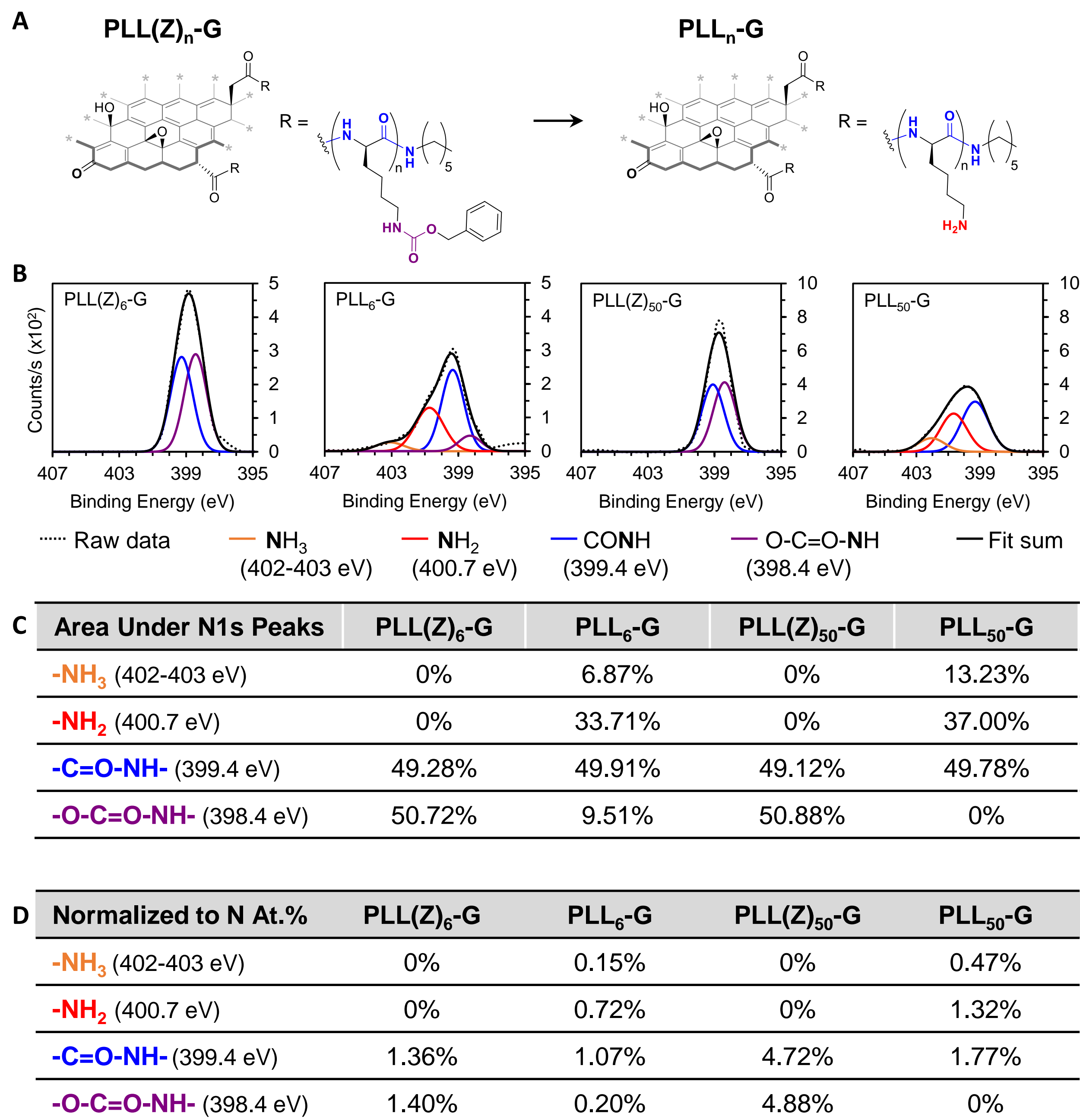


Figure S4. A representative XPS survey spectrum of functional graphenic materials (FGMs): GO, CG, PLL(Z)$_6$-G, and PLL(Z)$_{50}$-G, PLL$_6$-G, and PLL$_{50}$-G. The O1s, N1s, and C1s emission peaks that correspond to oxygen, nitrogen, and carbon are indicated by red lines from left to right in each spectrum. The intensity of each peak (O1s, N1s, and C1s) was used to calculate the quantitative elemental composition of each FGM displayed in Figure 2B. Elemental composition was calculated using CasaXPS software. Note that all three PLL(Z)$_{50}$-G spectra exhibited low signal count, resulting in more noise in the spectrum. The noise in the PLL(Z)$_{50}$-G spectra creates more error in the quantification of nitrogen.



Area Under N1s Peaks	PLL(Z)$_6$-G	PLL$_6$-G	PLL(Z)$_{50}$-G	PLL$_{50}$-G
-NH$_3$ (402-403 eV)	0%	6.87%	0%	13.23%
-NH$_2$ (400.7 eV)	0%	33.71%	0%	37.00%
-C=O-NH- (399.4 eV)	49.28%	49.91%	49.12%	49.78%
-O-C=O-NH- (398.4 eV)	50.72%	9.51%	50.88%	0%

Normalized to N At.%	PLL(Z)$_6$-G	PLL$_6$-G	PLL(Z)$_{50}$-G	PLL$_{50}$-G
-NH$_3$ (402-403 eV)	0%	0.15%	0%	0.47%
-NH$_2$ (400.7 eV)	0%	0.72%	0%	1.32%
-C=O-NH- (399.4 eV)	1.36%	1.07%	4.72%	1.77%
-O-C=O-NH- (398.4 eV)	1.40%	0.20%	4.88%	0%

Figure S5. A) Synthetic scheme shows deprotection of the PLL(Z)$_n$-G conjugate to give PLL$_n$-G. **B)** High resolution N1s XPS demonstrates the chemical changes that occur in the synthesis of the PLL$_n$-G conjugates. The protected PLL(Z)$_n$-G conjugates possess nitrogen in the form of carbamate and amide functional groups. Upon deprotection of the PLL(Z)$_n$-G conjugates to give PLL$_n$-Gs, carbamate functional groups are converted to amines or ammoniums. **C)** The atomic percent of the nitrogen functional groups in each FGM is calculated using the areas under the corresponding N1s peaks in panel B. Note that these atomic percentages only represent nitrogen in the sample. **D)** The normalized atomic percent of each nitrogen functional group was calculated according to the equation below.

$$\% \mathrm{NH_2}_{\mathrm{Normalized}} = \% \mathrm{NH_2} \times (\frac{\%\mathrm{Nitrogen}}{100})$$

Where %Nitrogen is quantified from the XPS survey scans

Upon deprotection of PLL(Z)$_n$-G to give PLL$_n$-G, the carbamate functional group in the peptide is removed to reveal either an amine or ammonium function group. Amide functional groups remain unchanged during the deprotection. This trend is observed in the conversion of PLL(Z)$_6$-G to PLL$_6$-G, where the decrease in carbamate functional groups (-O-C=O-NH-, 1.20%) in PLL(Z)$_6$-G is comparable to the increase in the sum of amine and ammonium functional groups in PLL$_6$-G (NH_2 + NH_3 = 0.87%). Meanwhile, the percent amides in these materials remains consistent (-C=O-NH-, 1.36% and 1.07%). While the remaining 0.20% carbamate functionality in PLL$_6$-G indicates incomplete deprotection, most of (86%) the peptide in this material was deprotected to give rise to amine and ammonium functionality.

However, this trend is not observed in the conversion of PLL(Z)$_{50}$-G to PLL$_{50}$-G. This is likely due to the noise in the survey scans of PLL(Z)$_{50}$-G (Figure S4), which introduces uncertainty in the percent nitrogen. This artificially inflates the atomic percent of amide and carbamate functional groups for PLL(Z)$_{50}$-G. Nevertheless, full deprotection of PLL$_{50}$-G is suggested by the complete disappearance of carbamate functionality (-O-C=O-NH-, 0%), giving rise to a combination of amine and ammonium functionality.

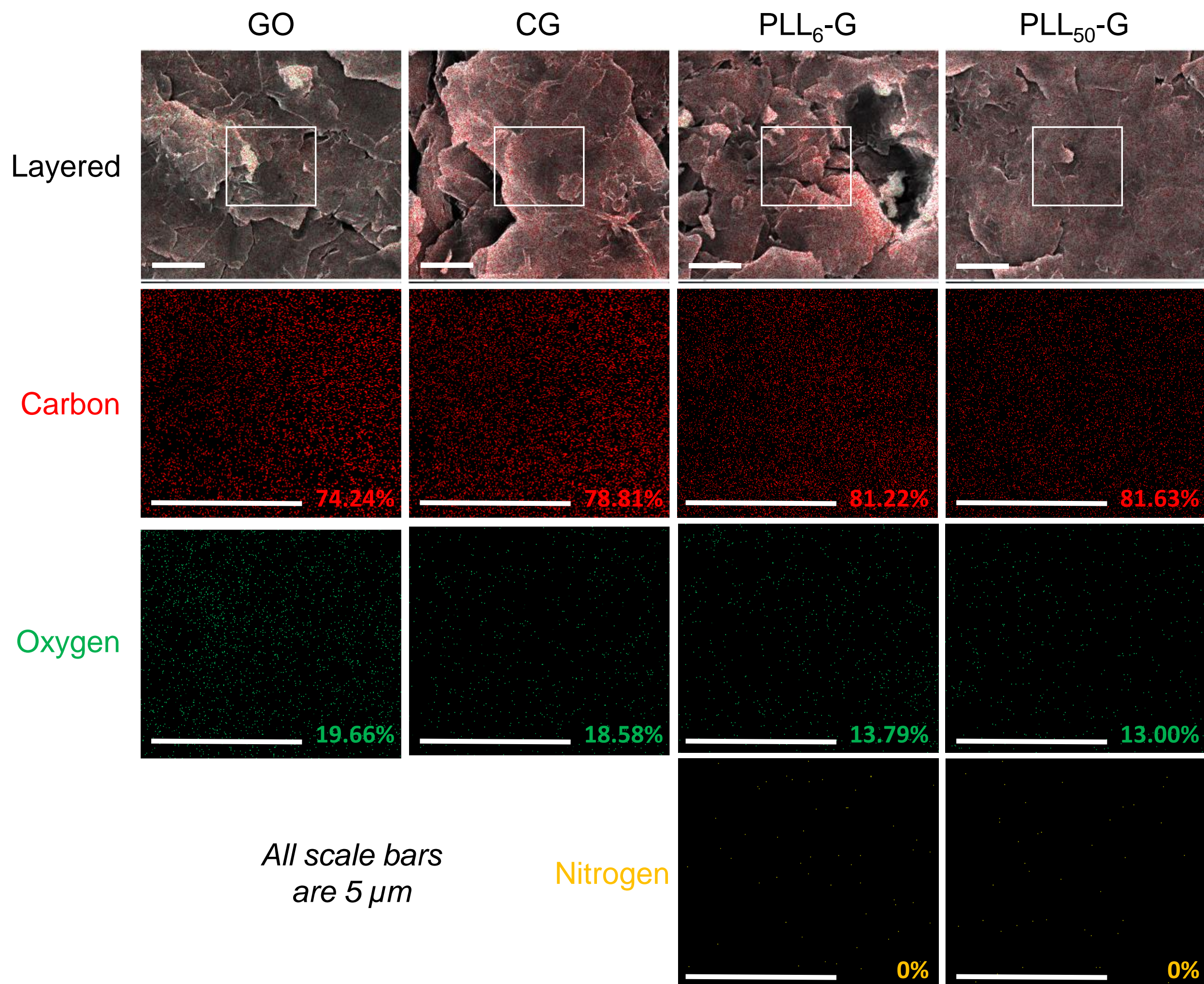


Figure S6. SEM images and EDS mapping of the surface of FGM pellets. EDS mapping is shown for an enlarged region of the layered SEM image (indicated by the white rectangle). EDS mapping demonstrates homogenous distribution of carbon and oxygen across the pellet surface. The atomic percent for carbon, oxygen, and nitrogen as determined by EDS mapping are displayed in the bottom right corner of each EDS image. Generally, the EDS signal for nitrogen is very weak and the low resolution of this technique can lead to inaccurate determination of atomic percent.[9] As such, the nitrogen content in the PLL_n-G conjugates (2 – 4% according to XPS survey scans) is expected to be below the detection limit for EDS mapping.[10]

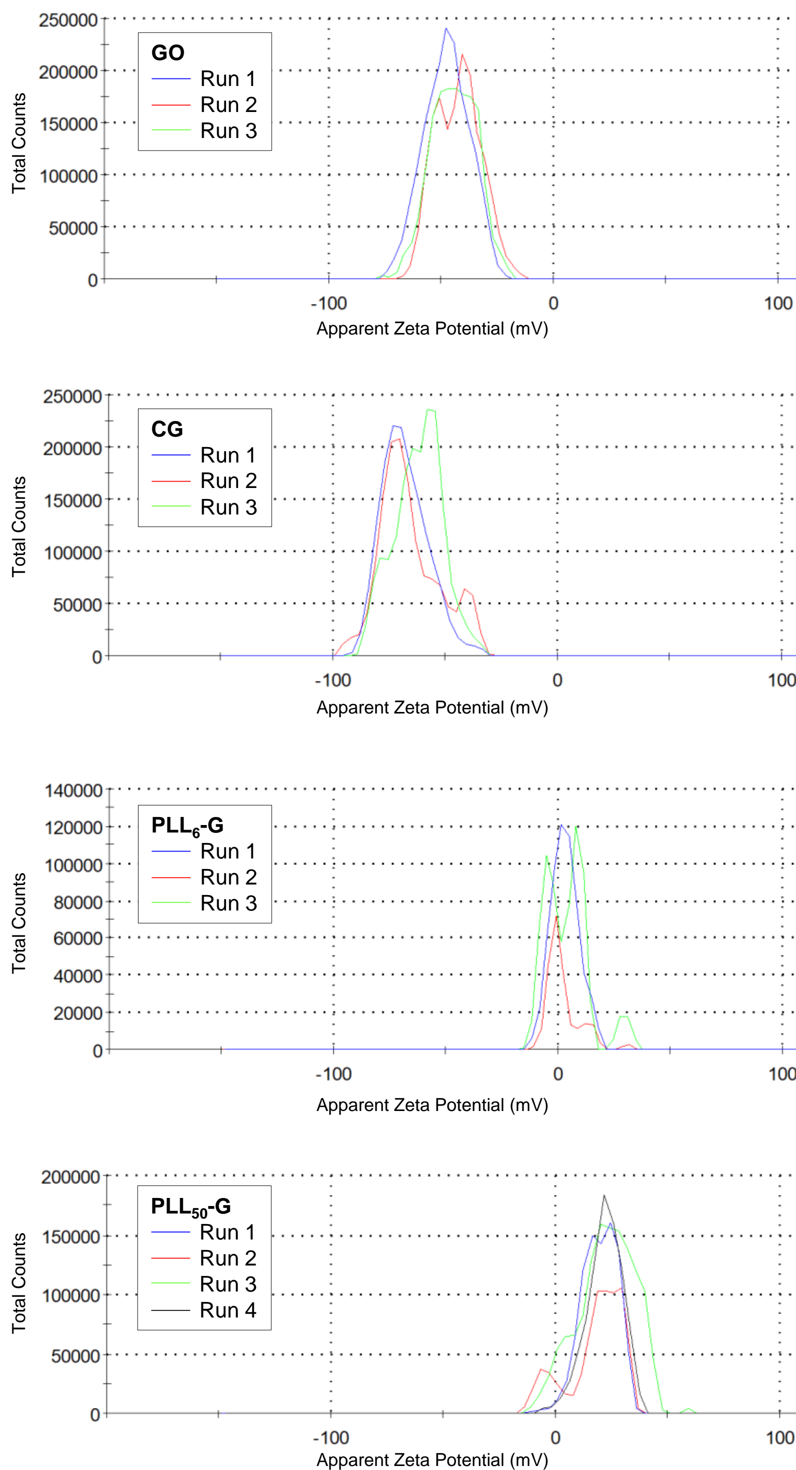


Figure S7. Zeta potential distributions of functional graphenic materials (FGMs): GO, CG, PLL_6-G, and PLL_{50}-G in buffered water.

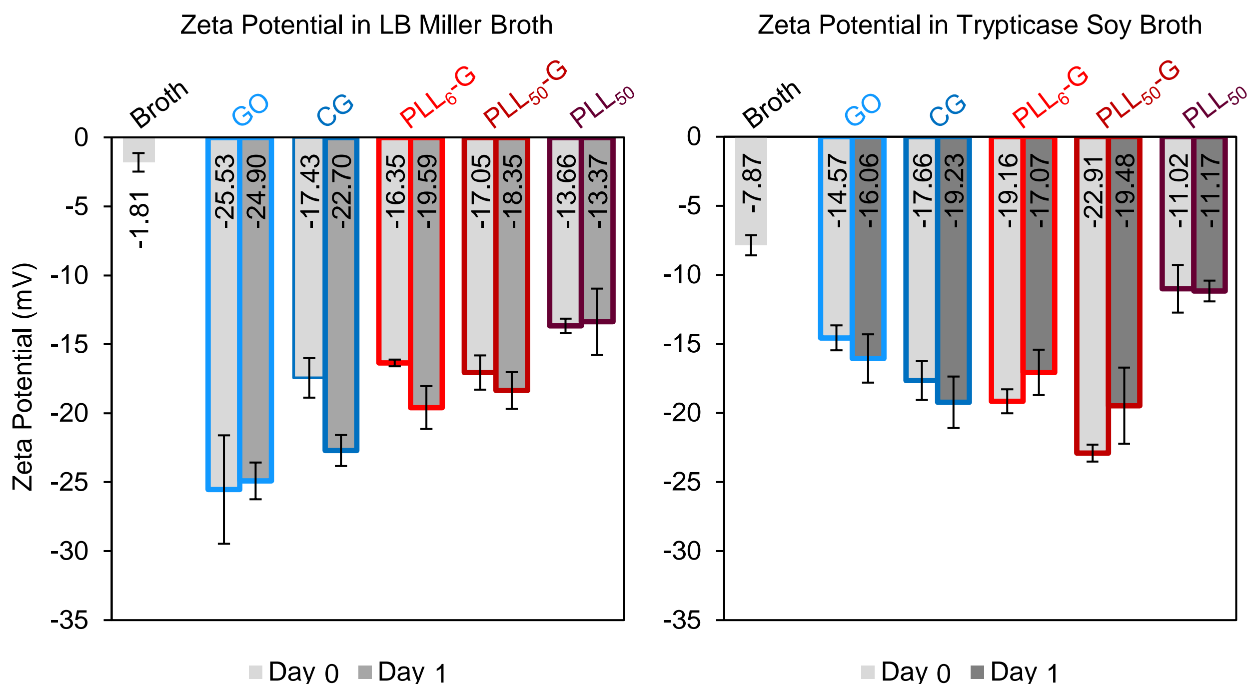


Figure S8. Zeta potential of FGMs (GO, CG, PLL_6-G, and PLL_{50}-G) and free PLL_{50} polypeptide in either LB Miller broth or Trypticase Soy broth. Day 0 data points were acquired immediately after the samples were prepared. Day 1 data points were acquired between 18-20 hours after the samples were prepared.

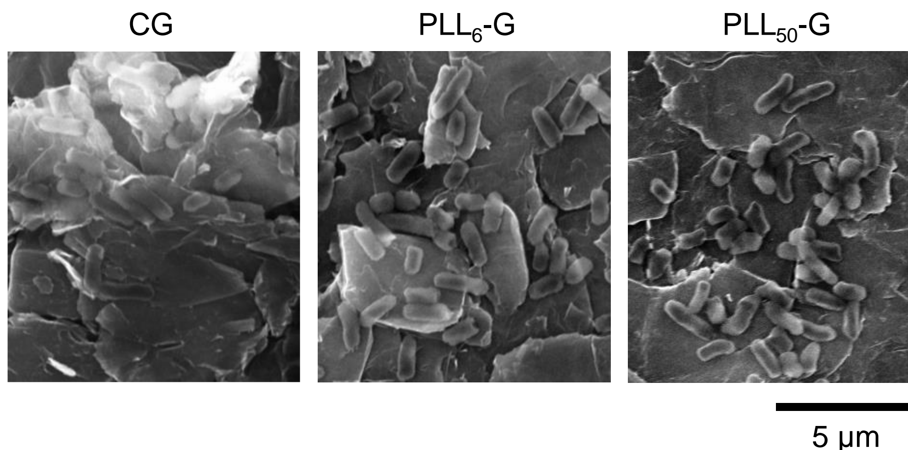


Figure S9. SEM images of *E. coli* on the FGM pellets show bacteria morphology.

3. Bacterial Culture

3.1 Bacterial Maintenance

Buffered LB Miller broth was prepared by dissolving 12.5 g of LB Miller dehydrate (Fisher BioReagents™, USA) and 0.75 g of Tris HCl (Promega, Madison, WI, USA) in 500 mL of DI H_2O to give a final media concentration of 10 g L^{-1} tryptone, 10 g L^{-1} NaCl, and 5 g L^{-1} yeast extract. Trypticase Soy broth was prepared by dissolving 15 g of Trypticase Soy dehydrate (BD™ BBL™) in 500 mL of DI H_2O to give a final media concentration of 17 g L^{-1} pancreatic digest of casein, 3 g L^{-1} papaic digest of soybean, 5 g L^{-1} sodium chloride, 2.5 g L^{-1} dipotassium phosphate, and 2.5 g L^{-1} dextrose. All media was autoclaved at 121 °C for 1 h and cooled to room temperature before use.

Escherichia coli (*E. coli*) strain K12 (ATCC® 25404™) was maintained as a frozen stock (-80 ˚C) in buffered LB Miller broth (media) with 30% glycerol. Liquid cultures were propagated in 5 mL of media with rotational shaking (MiniMixer™, Benchmark Scientific, Sayreville, NJ, USA) for 16 h at 37 ˚C (MyTemp Mini Digital Incubator, Benchmark Scientific). Then, the bacteria cultures were centrifuged at 10000 × *g* for 10 min to pellet the cells, followed by aspiration of the supernatant and resuspension of the pellet in 5 mL of fresh, media. Cultures were then used for experiments in a 1:5 split ratio (1 mL stock cell suspension + 4 mL fresh, media).

Bacillus subtilis (*B. subtilis*) Marburg strain (ATCC® 6051™) was maintained as a frozen stock (-80 ˚C) in Trypticase Soy broth (media) with 30% glycerol. Liquid cultures were propagated in 5 mL of media with rotational shaking for 16 h at 30 ˚C. Then, the bacteria cultures were centrifuged at 10000 × *g* for 10 min to pellet the cells, followed by aspiration of the supernatant and resuspension of the pellet in 5 mL of fresh, media. Cultures were then used for experiments in a 1:5 split ratio (1 mL stock cell suspension + 4 mL fresh, media).

3.2 Bacterial Vitality Study (in dispersion)

FGMs (GO, CG, PLL_6-G, and PLL_{50}-G) and the free PLL control (PLL_{50}) were weighed into 1.5 mL Eppendorf tubes and sterilized by irradiating with ultraviolet light (254 nm) for 5 minutes. Then, either buffered LB Miller broth (for *E. coli* experiments) or Trypticase Soy broth (for *B. subtilis* experiments) was added to each Eppendorf tube to give a concentration of 2 mg mL^{-1} (2X). FGM solutions were sonicated (240 W, 42 kHz, ultrasonic cleaner, Kendal) for 10 s to give a homogeneous dispersion; the free PLL control readily dissolved in media without sonication. The solutions were then serial diluted with media to give concentrations of 0.2 mg mL^{-1} (0.2X) and 0.02 mg mL^{-1} (0.02X).

The FGM dispersions and PLL solution were diluted with fresh media and the 1:5 split ratio to give FGM and PLL concentrations of 1, 0.1, and 0.01 mg mL^{-1} and a bacteria (either *E. coli* or *B. subtilis*) concentration of 4% v/v from the 1:5 split ratio. The positive control contained Penicillin/Streptomycin (ThermoFisher Scientific) diluted to 100 U mL^{-1} penicillin (approximately 60 mg/mL) and 100 mg/mL streptomycin with media and 4% v/v bacteria from the 1:5 split ratio. The "no treatment" (NT) control contained only 4% v/v bacteria from the 1:5 split ratio and media. To a sterile 96-well plate, 250 µL of each sample was dispensed into the

interior wells. All samples were run in triplicate. The 96-well plate (with cell culture plate lid) was incubated at 37 °C (for *E. coli*) or 30 °C (for *B. subtilis*) on a rotational shaker for 16 h.

After incubation, 125 µL was removed from the top of each well, being careful not to disturb the FGM particles settled at the bottom of the well. This aliquot was dispensed into a 1.5 mL snap-top Eppendorf tube and centrifuged (Eppendorf Microcentrifuge Model 5430) at 10,000 × *g* for 10 min to pellet bacteria. The supernatant was carefully aspirated, and the bacterial pellet was re-dispersed in 1.0 mL of sterile 0.85% NaCl buffer. Then, 100 µL of the bacterial dispersion in 0.85% NaCl was dispensed into a sterile 96-well plate. This plate was analyzed by absorbance and fluorescence.

3.3 Bacterial Adhesion/Repulsion Study

Each FGM pellet (n = 3 per FGM) was placed in an individual well of a sterile 48-well plate. To each well was added 750 µL of media – either buffered LB Miller broth (for *E. coli* experiments) or Trypticase Soy broth (for *B. subtilis* experiments) – with a bacteria concentration of 4% v/v from the 1:5 split ratio. A "no treatment" condition was prepared in the same way, except with no FGM pellet. The 48-well plate (with cell culture plate lid) was incubated at 37 °C (for *E. coli*) or 30 °C (for *B. subtilis*) on a rotational shaker for 16 h.

After incubation, 125 µL was removed from the top of each well. This aliquot was dispensed into a 1.5 mL snap-top Eppendorf tube and centrifuged (Eppendorf Microcentrifuge Model 5430) at 10000 × *g* for 10 min to pellet bacteria. The supernatant was carefully aspirated, and the bacterial pellet was re-dispersed in 1.0 mL of sterile 0.85% NaCl buffer. Then, 100 µL of the bacterial dispersion in 0.85% NaCl was dispensed into a sterile 96-well plate. This plate, containing non-adherent bacteria from the pellet co-culture, was analyzed by absorbance and fluorescence.

Pellets were rinsed three times with 0.85% NaCl (total volume of 750 µL). Two of each FGM pellet were placed in a new well of a sterile 96-well plate containing 250 uL solution of LIVE/DEAD® BacLight™ dual dye solution (3 µL of Syto® 9 and 3 µL of propidium iodide for every 2 mL of DI H_2O).[11] The plate was protected from light and incubated for 15 min at room temperature. One of each FGM pellet was not stained with dye – this unstained pellet was imaged as described below.

Fluorescence microscopy images were acquired for each FGM pellet (both stained and unstained) using an EVOS® FL Auto Cell Imaging System (ThermoFisher Scientific) with a 10×, 0.30 numerical aperture objective. Fluorescence images were acquired using the GFP (470/22 Ex; 510/42 Em) and RFP (531/40 Ex; 593/40 Em) light cubes, and phase contrast images were acquired at the same imaging parameters across all samples. Whole well automatically stitched together each component image to give an image of the whole pellet.

3.4 Bacterial Analysis by Absorbance

The 96-well culture plate containing bacterial dispersions in 0.85% NaCl was analyzed using a Spark® plate reader (Tecan) with SparkControlTM v2.2 software. Absorbance was measured from 200-1000 nm with a step size of 1 nm. Absorbance at 670 nm (OD670) was used as an indicator

of biomass, and thus, cell proliferation. Here, “Culture density (% of NT)” (Figures 5B, 5D, and 8) was calculated using the following equation:

$$(\text{Culture Density})_{\text{sample}} = 100 - \left(\frac{(\text{OD670}_{\text{sample}} - \text{OD670}_{\text{blank}})}{(\text{OD670}_{\text{NT}} - \text{OD670}_{\text{blank}})} * 100\right)$$

Where $\text{OD670}_{\text{sample}}$ = the absorbance at 670 nm of any given test sample,
$\text{OD670}_{\text{blank}}$ = the absorbance at 670 nm of the blank 0.85% NaCl with no cells,
and OD670_{NT} = the absorbance at 670 nm of the no treatment condition

3.5 Bacterial Analysis by Fluorescence

A modified procedure for the microplate LIVE/DEAD® *Bac*Light™ Bacterial Viability assay (Thermo Fisher) was used to evaluate bacterial vitality.[11] Briefly, the LIVE/DEAD® BacLight™ stain solution was prepared by dispersing 3 µL of Syto® 9 and 3 µL of propidium iodide for every 2 mL of DI H_2O. Then, 100 µL of the stain solution was added into each well of the 96-well culture plate containing 100 µL of sample (bacterial dispersions in 0.85% NaCl). The plate was protected from light and incubated for 15 min at room temperature.

Fluorescence data for all samples stained with the LIVE/DEAD® BacLight™ assay was collected on a Spark® plate reader with SparkControl™ v2.2 software. Emission spectra were acquired from 500–700 nm with a 5 nm step size using an excitation wavelength of 485 nm with a bandwidth of 10 nm and a manual gain of 60 from a z-position of 17,530 µm. Here, “Cell viability (% of NT)” (Figures 5C, 5E, and 8) was calculated using the following equation:

$$(\text{Cell Viability})_{\text{sample}} = 100 - \left(\frac{\left(\frac{\text{Em}_{530}}{\text{Em}_{630}}\right)_{\text{sample}}}{\left(\frac{\text{Em}_{530}}{\text{Em}_{630}}\right)_{\text{NT}}} * 100\right)$$

Where Em_{530} = the emission intensity at 530 nm (measuring living bacteria),
Em_{630} = the emission intensity at 630 nm (measuring dying bacteria),
$(\text{Em}_{530}/\text{Em}_{630})_{\text{sample}}$ = the living/dying ratio of any given test sample,
and $(\text{Em}_{530}/\text{Em}_{630})_{\text{NT}}$ = the living/dying ratio of the no treatment condition

3.6 Analysis of Pellet Images

ImageJ (Version 1.52a, Wayne Rasband, National Institute of Health, USA) was used to process whole pellet images. After cropping each image into a circular shape containing only the pellet surface (1068-pixel diameter), the red and green channels were split into separate images. Each channel (red and green) of each pellet (stained and unstained; CG, PLL_6-G, and PLL_{50}-G) was analyzed individually. The pellet image was split into 4 equal size quadrants and the integrated density was measured for each quadrant. The integrated density was used to calculate the absolute fluorescence intensity using the following equation:

$$\text{Fluorescence Intensity} = (\text{int den})_{\text{stained pellet}} - (\text{int den})_{\text{unstained pellet}}$$

Where “int den” is the integrated density

Values for fluorescence intensity in Figures 7A and 7C are reported as the average ± the standard deviation of the fluorescence intensity of the four quadrants of each pellet. The same imaging parameters (i.e. excitation intensity) were used for all samples; therefore, the absolute fluorescence intensity can be compared directly between samples.

3.7 Statistical Analysis

Bacterial absorbance and fluorescence assays were tested in three independent cultures (n = 3). Grubbs test was performed on each data set to identify outliers, if any. Data in main text Figures 5B–E, 7A, 7C, and 8 is presented as the sample mean ± standard deviation (SD). One-way analysis of variance was performed, and if significant differences were detected, a Bonferroni correction of post hoc t-tests was applied to identify which test conditions differed from the no treatment condition. $P < 0.05$ was deemed statistically significant.

4. Supplemental References

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