Supporting information

Biofriendly molecular and protein release substrates with integrated piezoelectric motivation and anti-oxidative stress capabilities

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Section 1. The β-phase fractions calculated of the samples.

The β-phase fractions of the films are calculated by the portion of the vibrational bands of the α and β-phases in ATR-FTIR spectra according to equation 1.

$$F(\beta) = \frac{X(\beta)}{X(\alpha) + X(\beta)} = \frac{A_\beta}{1.26A_\alpha + A_\beta} \quad (1)$$

Where X_α and X_β were the α and β phases' crystalline mass fractions, and A_α and A_β were their absorption at 764 and 840 cm^{-1}. The 1.26 factor accounts for the ratio in absorption coefficients at 764 and 840 cm^{-1}. The calculated consequence of the piezoelectric composite film was 58.52% and the pure PVDF film was 39.82%, which increased 46.96%.

Section 2. Figures.

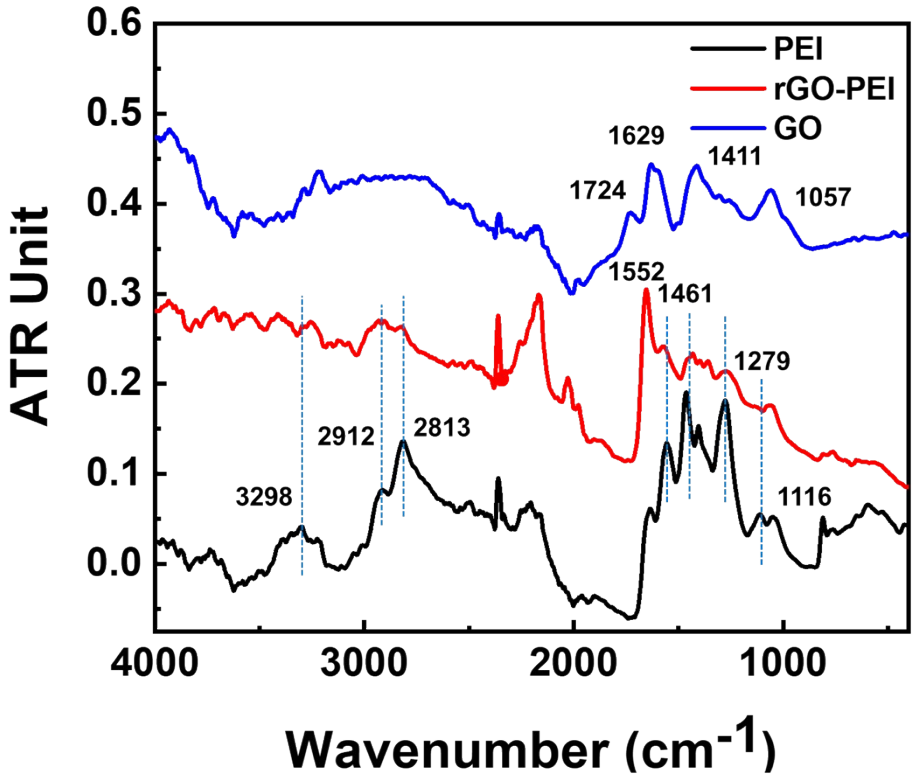

Figure S1. ATR-FTIR spectrums of PEI, GO and rGO-PEI.

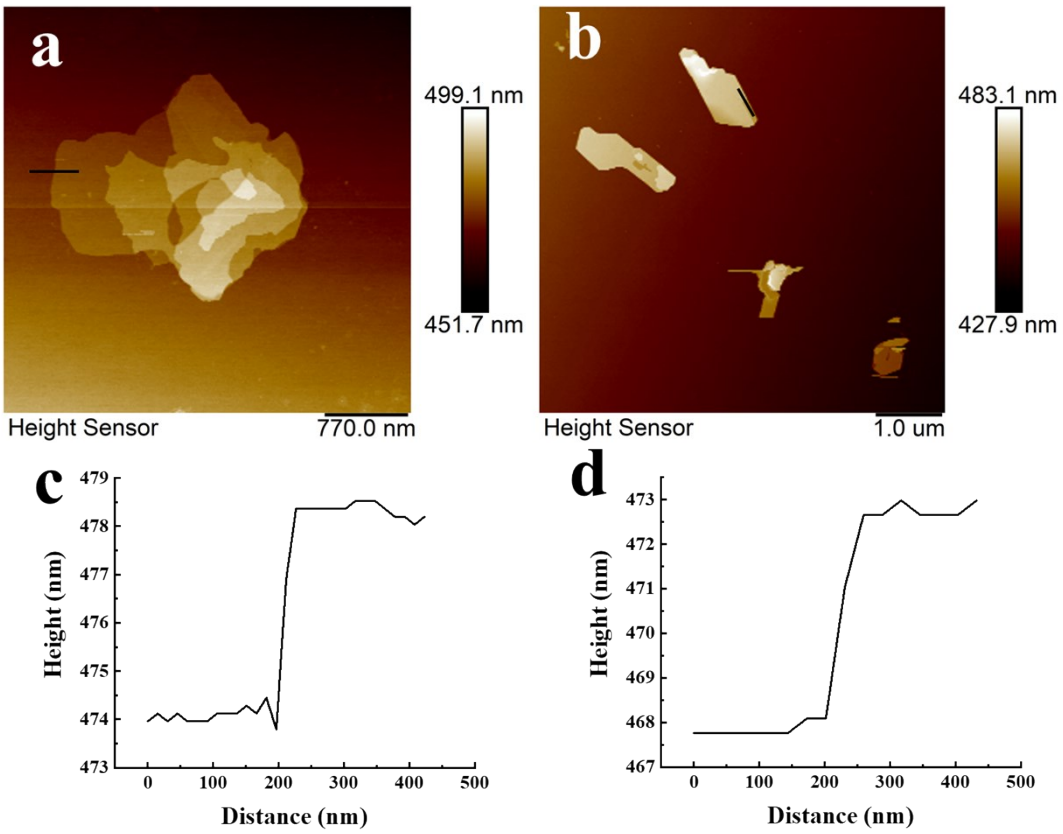

Figure S2. The AFM images and thickness of GO and rGO-PEI. The AFM images of (a) GO and (b) rGO-PEI. The thickness of (c) GO and (d) rGO-PEI

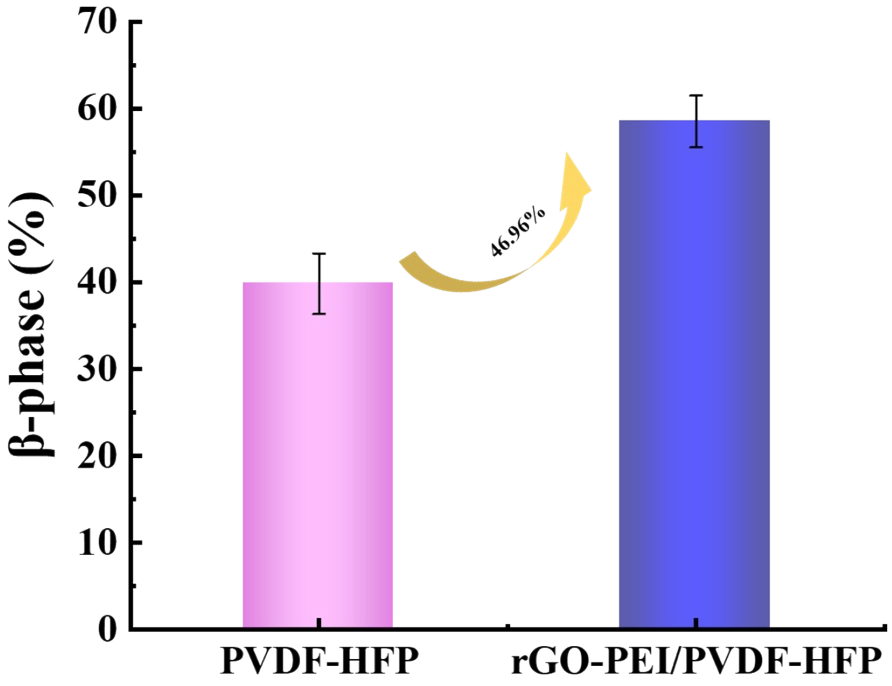

Figure S3. The β-phase of PVDF-HFP and rGO-PEI/PVDF-HFP.

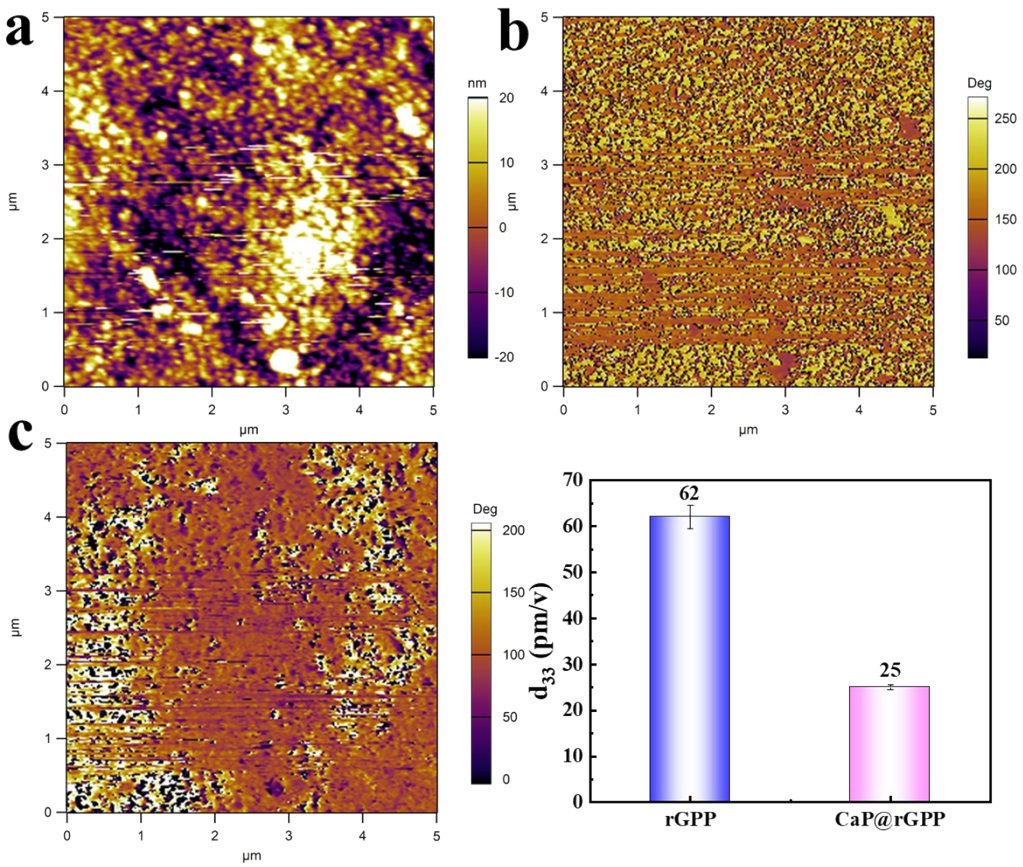

Figure S4. The PFM images of rGPP. (a) Surface morphology image. (b) In-plane and (c) out-of-plane piezoelectric response images. (d) The d_{33} of rGPP.

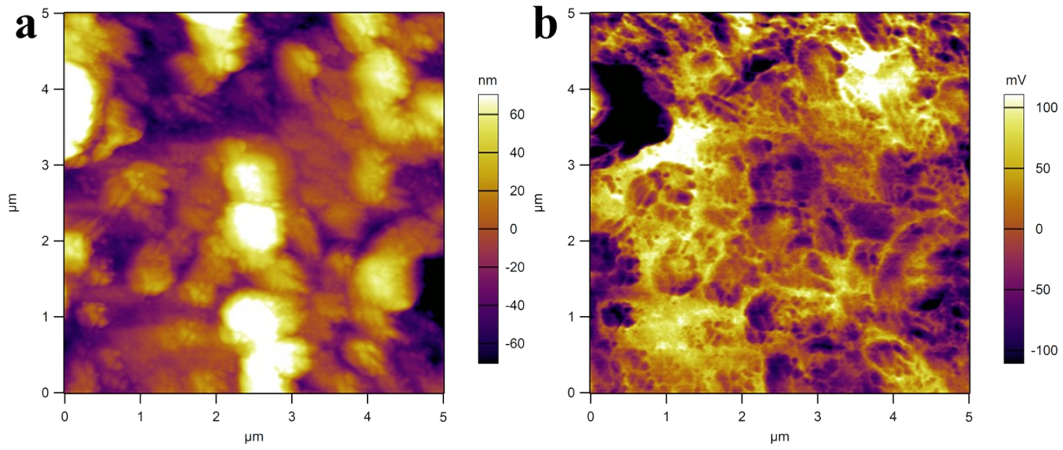

Figure S5. The KPFM image of rGPP. (a) Surface morphology image. (b) Surface potential image.

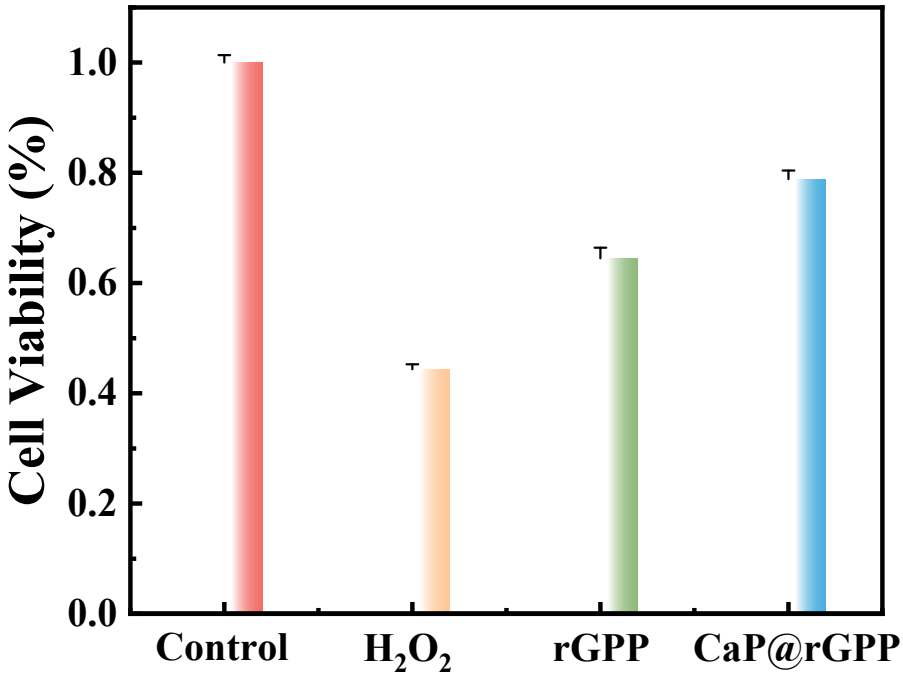

Figure S6. The cell viability treated by H_2O_2 with or without rGPP and CaP@rGPP.

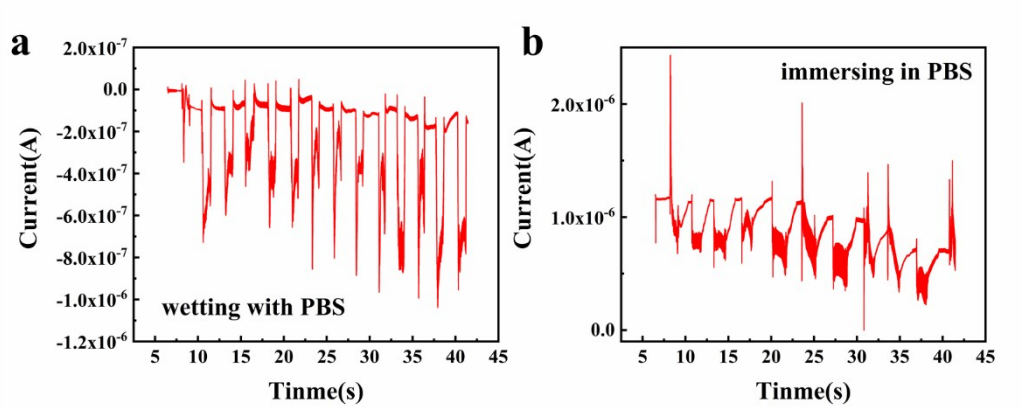

Figure S7. The short circuit current of CaP@rGPP in aqueous environment.

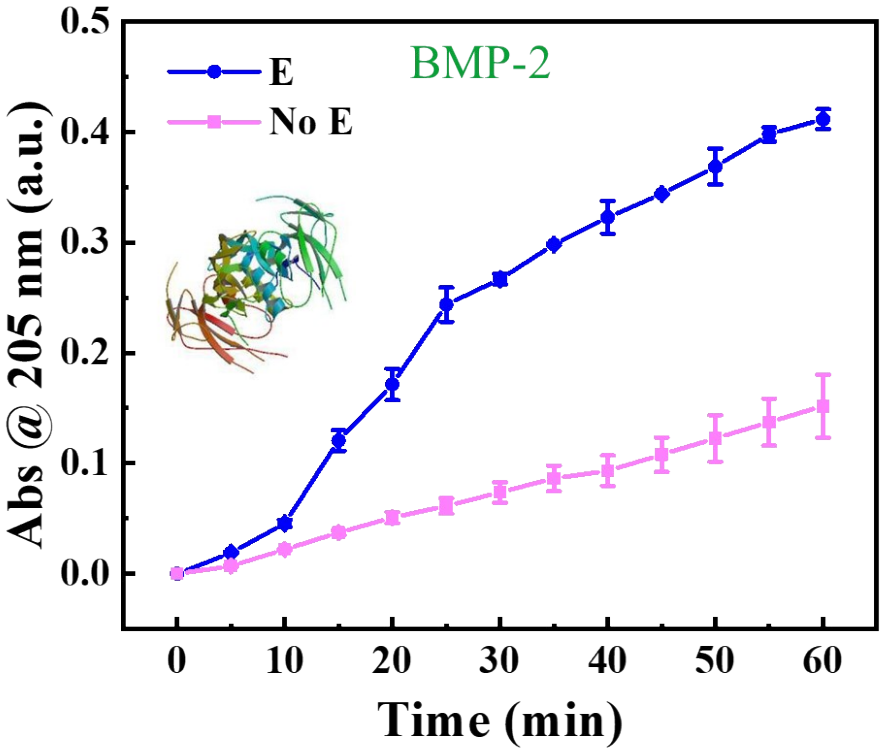

Figure S8. The release profile of BMP-2.

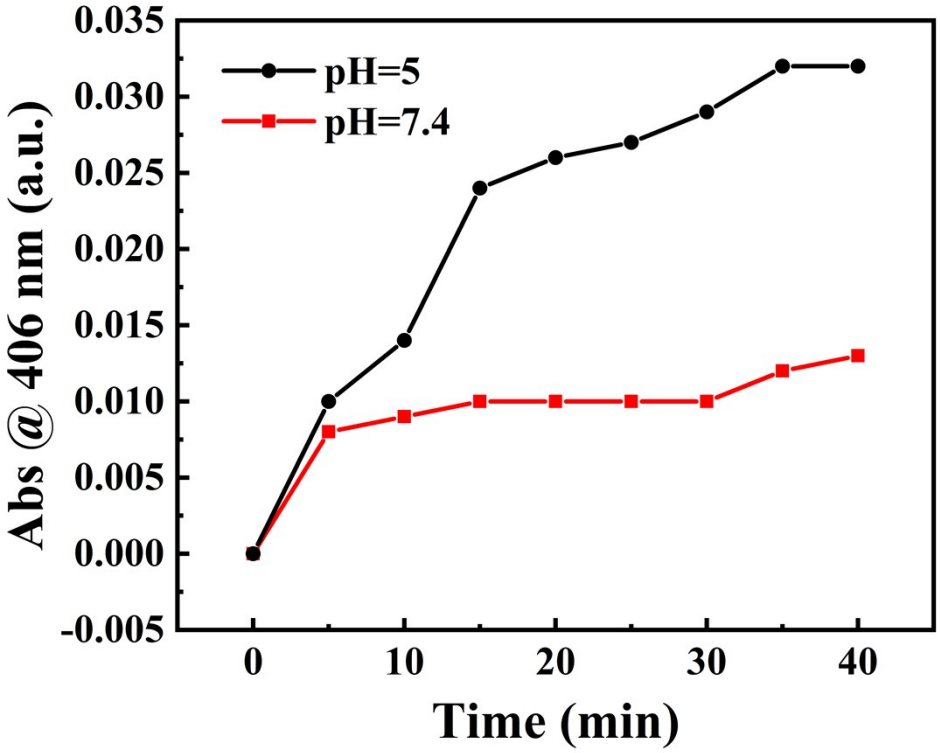

Figure S9. The pH-responsive release of CaP@rGPP.

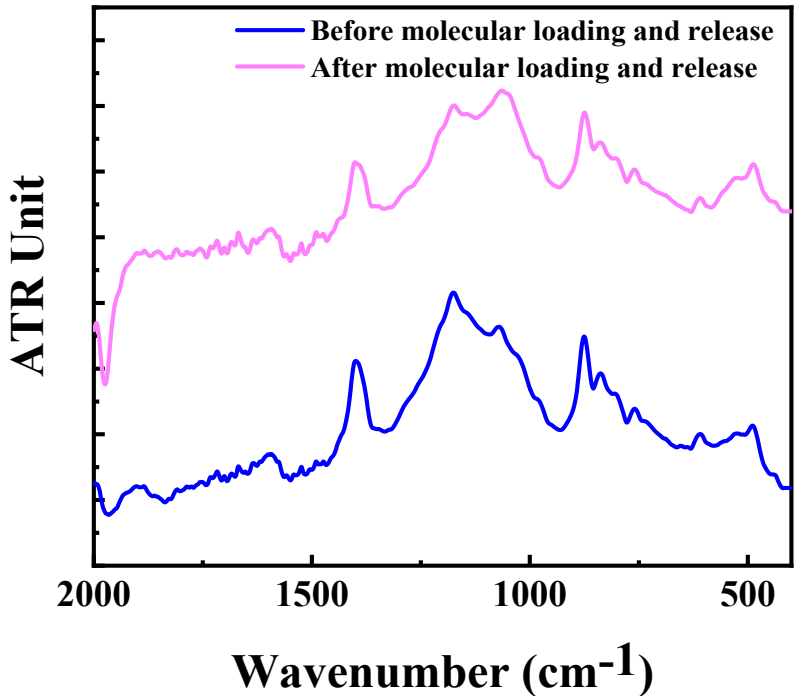

Figure S10. ATR-FTIR spectrum of CaP@rGPP before and after molecular loading and release.

Section 3. Experimental section.

Materials: Poly(vinylidene fluoride-co-hexafluoropropylene) (PVDF–HFP) (density 1.78 g cm^{-3}, 5–20% molar of hexafluoropropene), was purchased from Sigma–Aldrich. Polyethyleneimine (PEI) (M.W. 70,000, 50% aqueous solution) and polyethylene glycol (PEG) was purchased from Aladdin. Collagen (COL) was obtained from Beijing Pashionbio. Co. Hydrogen peroxide (H_2O_2, 30%), N,N-dimethyl formamide (DMF, P99.5%) and all other reagents were obtained from Sinopharm Chemical Reagent Beijing Co., Ltd (Beijing, China). The reagents were all analytical grade and used without further purification.

Preparation of rGO-PEI: The improved Hummers method was used to prepare GO. Surface modification of GO, and then reduction to obtain rGO-PEI, as shown below. 0.1g GO was dispersed in 200mL distilled water, and ultrasonicated in a 250ml round-bottomed flask for 10 min, 0.2g KOH and 4g PEI were added. The mixture was heated to 80°C for 10 hours, and PEI was used as both a surface modifier and a reducing agent. The mixture was washed with distilled water to remove excess PEI. Finally, the rGO-PEI was freeze-dried.

Preparation of rGO-PEI/PVDF-HFP piezoelectric composite film: Dissolve 1 g of PVDF-HFP particles in 4 mL dimethylformamide and stir at 80°C for 30 min to obtain a PVDF-HFP solution. 8% rGO-PEI is added to the PVDF-HFP solution to obtain a mixture with a specific amount of

rGO-PEI components. Disperse uniformly under the action of ultrasound, and stir for 2 h at 80°C. Finally, the mixture was cast into a thin film and kept in an oven at 80°C for 3 h to evaporate the solvent, and then kept in an oven at 220°C for 1 h to further reduce GO. Thus, a composite film with higher piezoelectric properties (higher β-phase content) is obtained.

Preparation of COL/PEG multilayer film on piezoelectric film: First, the piezoelectric film is used as a substrate and treated with a plasma cleaner for 10 minutes to obtain hydrophilicity. The assembly of the LbL COL/PEG multilayer film is as follows: Dissolve COL in acetic acid to form a uniform solution with a concentration of 1 mg mL^{-1}, and adjust the pH to 3 with 1 M sodium hydroxide. Dissolve PEG in deionized water to make a 1 mg mL^{-1} solution. The treated substrate was immersed in COL for 10 minutes, washed with deionized water, and then dried under a stream of nitrogen. Then, the substrate was immersed in the COL solution for 10 minutes, washed with deionized water, and then dried under a stream of nitrogen. Repeat the soaking cycle until the desired number of double layers is obtained.

Preparation of CaP@rGPP piezoelectric film: Dissolve $CaCl_2$ and K_2HPO_4 in deionized water to make a 300 mM and 500 mM solution respectively. The COL/PEG multilayer film on the piezoelectric film is immersed in the $CaCl_2$ solution for 3 minutes, dried under a stream of nitrogen. And then immersed in the K_2HPO_4 solution for 3 minutes, dried

under a stream of nitrogen, and finally CaP@rGPP is obtained.

Superoxide Radical Scavenging Activity: The superoxide radical ($O_2^{\cdot -}$) scavenging efficiency of CaP@rGPP was achieved by measuring the inhibition ratio of the photoreduction of NBT. The solutions containing riboflavin (20 μM), methionine (12.5 mM), NBT (75 μM) were prepared in PBS. The mixtures were illuminated upon ultraviolet (UV) radiation with a constant light intensity for 15 min at 25℃. After illumination, removed CaP@rGPP, the absorbance of the supernatant was measured at 560 nm immediately. The entire reaction assembly was enclosed in a constant temperature incubator lined with aluminum foil. The sample containing riboflavin, methionine, and NBT but placed in the dark was defined as the negative control. The sample containing riboflavin, methionine, and NBT after UV illumination for certain time was defined as the positive control. All the experiments were carried out in dark without illumination. Inhibition percentage was calculated as the following formula:

$$O2.^{-} \text{ scavenging effect } (\%) = 1-(Ao-An)/(Ap-An)\times 100\% \quad (1)$$

where, Ao, An, and Ap are the absorbance of the treated samples, negative control, and positive control, respectively.

Hydroxyl Radical Scavenging Activity: By measuring the presence of the specific probe salicylic acid (SA), the scavenging efficiency of CaP@rGPP on hydroxyl radicals (·OH) was determined. In the Fe^{2+}/H_2O_2

system, the Fenton reaction produces ·OH, and SA can detect ·OH. After SA is mixed with Fe^{2+}/H_2O_2 system, there is an obvious absorption peak at 510 nm. The solutions containing different concentrations of $FeSO_4$ (2 mM), H_2O_2 (5 mM), SA (1.5 mM) were prepared in PBS. As a compound, SA can capture ·OH and generate 2-hydroxysalicylic acid. The mixture was incubated at 37°C for 30 min, and then the absorbance of the mixture was measured. The percentage of ·OH. The removal efficiency of the scavenger was calculated using the given formula:

·OH scavenging effect (%) =(Ac-As)/Ac×100% (2)

where Ac is the absorbance of the control, and As is the absorbance in the presence of Fe3O4@TaN NFS. The control contains only $FeSO_4$, H_2O_2 and SA, which can produce ·OH through the Fenton reaction.

H2O2 Scavenging Activity: The H_2O_2 removal experiment was carried out according to the procedure of Ruch et al. [1]. In order to evaluate its ability to remove H_2O_2, a 15 mM H_2O_2 solution was prepared in PBS. Then, rGPP and CaP@rGPP were added to the H_2O_2 solution. Then, the absorbance of the reaction mixture was measured at 240 nm. Take the PBS and H_2O_2 solution without samples as the control. The percentage of H_2O_2 scavenging was calculated using the following equation:

H_2O_2 scavenging effect (%)=(Ac-As)/Ac×100% (3)

where, Ac is the absorbance of the control and As is the absorbance in the presence of samples.

Peroxides Activity: Hydrogen peroxide (POD) can catalyze the oxidation of the substrate TMB to blue in the presence of H_2O_2, with a maximum absorption wavelength of 652 nm. The POD activity of samples can be evaluated by monitoring the absorbance. In this study, TMB and H_2O_2 were mixed with different samples. One group containing Fe_3O_4 nanoparticles was a positive control, and the other group containing TMB and H_2O_2 was a negative control. Take TMB, H_2O_2 and samples as the experimental groups. Then measure the absorbance of the mixed solution at 652 nm.

Cell Culture: The L929 cells were incubated in Dulbecco's modified eagle medium (DMEM) containing 1% Penicillin-Streptomycin and 10 % fetal bovine serum (FBS) under 37℃ in a humidified atmosphere of 5 % CO_2.

Cytotoxicity Study: For the cytotoxicity of samples, L929 cells were seeded in a 48-well tissue culture polystyrene plate (TCPS) at a density of 10000 cells per well for 24 hours. After that, samples were added into the DMEM. The L929 and samples were further incubated at 37℃ for 5 days. To determine the cytotoxicity of samples, 100 μL of CCK-8 (final dilution: 1:10), which can react with dehydrogenase in mitochondria to form a water-soluble formazan, was added onto the medium to evaluate the viability. Afterwards, 100 μL of the supernatant was transferred into a 96-well plate to measure the absorbance at 490 nm.

Intracellular ROS Scavenging: 2',7'-dichlorofluorescein diacetate (DCFH-DA) is a non-fluorescent compound which reacts with intracellular RONS and generates the fluorescent product dichloro-fluorescein (DCF). In this study, it was used to monitor the production of RONS, and the fluorescence intensity depends on the amount of RONS in cellular environment. L929 cells were seeded in a 48-well plate with a density of 10000 per well for 24 hours. Then, cells were incubated with Rosup reagent and rGPP and CaP@rGPP for another 1 h. Subsequently, DCFH-DA was added for another 0.5 h at 37 ℃ in dark environment. Rosup reagent is a kind of compound mixture with the concentration of 50 mg/mL, and was purchased from Beyotime (S0033S). Fluorescence microscopy images were collected on the Leica fluorescence microscope (DMI8, Leica, Germany). The excitation wavelength was 488 nm and signals were collected at FITC channel 500–560 nm.

Live/Dead staining of L929 cells after H_2O_2 treatment: L929 cells were seeded in a 48-well plate at a density of 10000 cells per well for 24 hours. Then cells were incubated with 100 μM H_2O_2, 100 μM H_2O_2 and rGPP, 100 μM H_2O_2 and CaP@rGPP for another 1 h. Then, cells were stained by Live/Dead staining kit and observe through a fluorescence microscope.

Molecular adsorption and release: 1 mg mL^{-1} of molecular MB, SF, RhB, HSA, Hb and CAT were used as the feed liquid. The CaP@rGPP

film is immersed in the solution for 24 hours to achieve equilibrium absorption. All multilayer films are rinsed briefly in deionized water to remove surface-adsorbed molecules after the loading step. The film was then soaked in PBS to release the loaded molecules. During the release process, piezoelectricity is generated by pressing a multilayer film based on a piezoelectric film. Take out the 3 mL solution for measurement, monitor the absorbance of the released solution at a specific time, and return the solution after the measurement is over. An external mechanical disturbance is applied by tapping a point of the substrate with a finger to apply force to generate a piezoelectric signal. The magnitude of the force is about 10 N, and the frequency is about 0.5 Hz. Unless otherwise stated, the force is applied continuously.