

## Supporting Information

### **Cu Single-Atom Embedded g-C<sub>3</sub>N<sub>4</sub> nanosheets Rehabilitate Multidrug-Resistant Bacteria Infected Diabetic Wounds via Photoswitchable Cascade Reaction**

*Xichen Sun<sup>1,2†</sup>, Pengqi Zhu<sup>3,4†</sup>, Liuyan Tang<sup>1,2</sup>, Pengfei Wang<sup>1,2</sup>, Ningning Li<sup>1,2</sup>, Qing Wang<sup>1,2</sup>, Yan-Ru Lou<sup>5</sup>, Yuezhou Zhang<sup>1,2\*</sup>, and Peng Li<sup>1,2,6\*</sup>*

<sup>1</sup>State Key Laboratory of Flexible Electronics (LOFE) & Institute of Flexible Electronics (IFE), Northwestern Polytechnical University, 127 West Youyi Road, Xi'an, 710072, China

<sup>2</sup>Ningbo Institute of Northwestern Polytechnical University, Frontiers Science Center for Flexible Electronics (FSCFE), Key laboratory of Flexible Electronics of Zhejiang Province, Ningbo Institute of Northwestern Polytechnical University, 218 Qingyi Road, Ningbo, 315103, China

<sup>3</sup> Shanxi Bethune Hospital, Shanxi Academy of Medical Sciences, Tongji Shanxi Hospital, Third Hospital of Shanxi Medical University, 99 Longcheng Street, Taiyuan, 030032, China

<sup>4</sup>Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, 430030, China

<sup>5</sup>Faculty of Pharmacy, University of Helsinki, P.O. Box 56, FI-00014 Helsinki, Finland

<sup>6</sup>School of Flexible Electronics (SoFE) and Henan Institute of Flexible Electronics (HIFE), Henan University, 379 Mingli Road, Zhengzhou, 450046, China

† These authors contributed equally to this work.

E-mail: [iamyzzhang@nwpu.edu.cn](mailto:iamyzzhang@nwpu.edu.cn); [iamppli@nwpu.edu.cn](mailto:iamppli@nwpu.edu.cn)

## ***Materials***

Melamine, glucose oxidase (GOx), cupric nitrate trihydrate and glucose were purchased from Aladdin Industrial Co., Ltd. (Shanghai, China). The rest reagents were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

## ***Preparation of CN***

3 g of melamine was put into porcelain cup with a cap, then calcined at 550 °C for 4 h with a ramping rate of 2.2 °C min<sup>-1</sup> in a muffle furnace in air. After heating, the resulting yellow agglomerates were gently ground and treated under ultrasonication for 3 h as an aqueous solution (1 g L<sup>-1</sup>). Then the powder was filtered, washed, and dried at 80 °C for further tests (the sample was named as CN<sub>550</sub>).

## ***Preparation of nitrogen vacancy-rich CN***

For the quick thermal treatment, a horizontal tube furnace was heated to target temperatures (600 °C, 650 °C, 700 °C and 750 °C) and 250 mg of CN<sub>550</sub> was put in an alumina crucible. A stopwatch was started as soon as the alumina crucible was placed at the targeted heating zone. When the watch ran for 5 min, the crucible was fished out as soon as possible and cooled down in air. The whole process is carried out in well-ventilated space. The resulting products were collected and named as CN<sub>600</sub>, CN<sub>650</sub>, CN<sub>700</sub>, CN<sub>750</sub>, according to the target temperature. When the treatment temperature was up to 800 °C, the CN<sub>550</sub> was completely decomposed.

## ***Synthesis of Cu/CN***

1 g CN<sub>700</sub> was dispersed into 10 mL 5wt% cupric nitrate trihydrate solution, stirred at room temperature for 1 hour, and evaporated at 80°C. The yellow-green powder was washed with deionized water and dried at 80 °C overnight. Through ICP analysis, the actual loading amounts were 1wt%, abbreviated as Cu/CN.

## ***Characterization***

The morphology and size of CN were measured by TEM (HT7800, Hitachi, and JEM-200F). X-ray diffraction (XRD) patterns were recorded on a D8 Advance diffractometer (Germany Brock AXS Company, Cu K $\alpha$ ,  $\lambda$  = 1.54056Å) operated at 40 kV and 200mA at room temperature. The X-ray photoelectron spectroscopy (XPS) was measured on a Thermo Scientific K $\alpha$  photoelectron spectroscopy with Al K $\alpha$  radiation. The surface

morphology and element mapping of CN and Cu/CN were using a field emission scanning electron microscope (FE-SEM, ZEISS Gemini SEM 300) after lyophilization. The diffuse reflectance UV-vis (DR UV-vis) spectra were recorded on the UV-vis spectrophotometer (UV-3600 Plus, Shimadzu). The electron paramagnetic resonance (EPR) spectra were tested via Bruker EMXPLUS10/12 EPR electron paramagnetic resonance spectrometer.

### ***Hypoglycemic ability of CN and Cu/CN***

CN with different nitrogen vacancies were dispersed in the glucose solution (20 mmol/L) and irradiated using an Xe lamp cutoff filter 420 ( $\lambda > 420$ , 0.5 W/cm<sup>2</sup>, 10 min), and the consumption of glucose is measured by the amount of H<sub>2</sub>O<sub>2</sub> produced. Cu/CN was dispersed in glucose solution (20 mmol/L), irradiated with Xe lamp cut-420 ( $\lambda > 420$  nm, 0.5 W/cm<sup>2</sup>, 10 min), and measure glucose consumption using a glucose assay kit.

### ***Calculate the charge carrier density ( $N_d$ )***

As shown in Figure 2e, the positive slope of the M-S curve indicates that CN<sub>700</sub> are n-type semiconductors and thus, their  $N_d$  can be calculated by the following equation

$$N_d = 2 / (e \times \epsilon_0 \times \epsilon) \times (d(1/C^2) / dV_s))^{-1} \quad (1)$$

where C is the capacitance of the space charge layer;  $\epsilon_0$  denotes the vacuum dielectric constant ( $8.85 \times 10^{-14}$  F/cm);  $\epsilon$  is the dielectric constant of the sample (15.6 for g-C<sub>3</sub>N<sub>4</sub>); e is the electron charge ( $1.602 \times 10^{-19}$  C).

### ***Calculate the standard hydrogen electrode***

The potential of the catalyst, relative to the standard hydrogen electrode, can be calculated from the Nernst equation

$$E_{RHE} = E_{Ag/AgCl} + 0.059 \times pH + E^\circ_{Ag/AgCl} \quad (2)$$

### ***In vitro antibacterial performance of CN and Cu/CN***

The methicillin-resistant *Staphylococcus aureus* (MRSA, Gram-positive) and extended-spectrum  $\beta$ -lactamases producing *Escherichia coli* (ESBL *E. coli*, Gram-negative) were used to evaluate the antibacterial performance of all samples. Briefly, stock bacteria were added into 5 mL of sterilized fresh LB liquid medium and cultivated at 37 °C for 4-6 h.

Then, the concentration of bacterial suspensions was measured by utilizing a multifunctional microplate reader (OD600) at 600 nm. The contact-active antimicrobial efficacy of CN<sub>700</sub> and Cu/CN was determined using a reported protocol. Typically, 20  $\mu\text{L}$  of  $1 \times 10^6$  colony-forming units  $\text{mL}^{-1}$  (CFU/ $\text{mL}$ ) bacterial solution was mixed with 20  $\mu\text{L}$  Cu/CN solution, and 20  $\mu\text{L}$  of the bacterial solution alone was used as control. 180  $\mu\text{L}$  of aseptic PBS was added to suspend residual bacteria after being incubated with or without light ( $\lambda > 420 \text{ nm}$ ) for 15 min. 50  $\mu\text{L}$  resuspension was dropped onto a standard agar plate and then cultured for 12 h at 37 °C to observe the surviving bacteria colony which. To count the surviving bacteria colony, various 10-fold gradient dilutions in aseptic PBS were spread onto agar plates and the survivors were obtained through images of plates after cultured. Each group was repeated three times, and the killing rate was calculated by the following Equation

$$\log \text{ reduction} = \log (\text{cell count of control} / \text{survivor count treated with materials}) \quad (3)$$

#### ***In vitro hemolysis assay***

The hemolysis test was performed using fresh whole blood from KM mice. First, 1 mL of fresh mice blood was centrifuged for 10 minutes at 1000 RPM using a centrifuge to collect red blood cells (RBC) and washed with Tris-HCl 3 times. Then 500  $\mu\text{L}$  of RBC Tris-HCl solution (5%) was added to 500  $\mu\text{L}$  of Tris-HCl solution containing different materials, in which Tris-HCl and Triton X-100 was used as negative and positive controls, respectively. The mixture was centrifuged at 1000 RPM for 10 minutes after being incubated at 37 °C for 1 h centrifuge it at to test the absorbance of the supernatant at 540 nm. The formula for calculating hemolysis rate (%) is: hemolysis rate (%) =  $(A_M - A_N) / (A_W - A_N) \times 100\%$ , where  $A_M$ ,  $A_N$  and  $A_W$  represent absorbance after co-incubation of red blood cells with the materials, Tris-HCl and Triton X-100, respectively.

#### ***In vitro cytotoxicity assay***

The CN<sub>700</sub> and Cu/CN were mixed with 3T3 fibroblasts was added to a 96-well microtiter plate and incubated at 37 °C for 24 h. The growth and proliferation of 3T3 fibroblasts with the CN<sub>700</sub> and Cu/CN were observed using an optical microscope. Then the cells were stained with Almar Blue and incubated at 37 °C for 4 hours. The

fluorescence intensity was calculated with a microplate reader to quantify cell activity. Each test was repeated 3 times.

### ***Diabetic chronic wound healing experiment***

KM mice (30-40 g) were provided by Beijing Vital River Laboratory Animal Technology Co., Ltd. All animal-based experimental studies were conducted in accordance with the guidelines of the Administration of Laboratory Animals of China and approved by the animal ethics committee of Northwestern Polytechnical University (Number: NPU201906). The wound healing performance was studied using a murine diabetic wound healing model. STZ was used to establish the diabetic mouse model, and then these mice were divided into five different treatment groups, randomly, three in each group: control, CN<sub>700</sub>+vis, and Cu/CN+vis. The diabetic wound in the whole group was fixed with medical tape and then all the groups were irradiated with Xe lamp cut-420 for 10 min. The mice were killed by anesthesia on days 3, 7 and 14 days, and the wound tissue was collected for later tests. Hematoxylin and eosin (H&E), Masson, and CD31 staining were used for the histological analysis. The unrepaired area ratio of the wound was calculated by equation:

$$\text{Wound area ratio (\%)} = ((A_x - A_y) / A_y) \times 100\% \quad (4)$$

where  $A_x$  and  $A_y$  are the wound area of the infected and primary area.

### ***Density functional theory calculation***

First-principles calculations were performed by using periodic DFT with the Perdew-Burke-Ernzerhof (PBE) exchange-correction functional under the generalized gradient approximation. The plane waves forming the wave functions were expanded with an energy cutoff set to 500 eV. A gamma-centered k-point mesh of  $3 \times 3 \times 1$  was applied for the geometry optimization. The convergence criteria for the geometry optimization were established at  $1.0 \times 10^{-6}$  eV/atom for the total energy and 0.02 eV/Å for the force. To minimize the interaction between surfaces in the periodically repeated slabs, a substantial vacuum gap of 15 Å was introduced. The energy of adsorption was determined by using a standard formula, as follows:

$$E_{\text{ads}} = E_{\text{Total}} - E_{\text{Cat}} - E_{\text{molecular}} \quad (5)$$

In the calculations of free energies, adjustments for entropic corrections and zero-point energy (ZPE) were incorporated. The free energy of various species was determined based on the following standard formula:

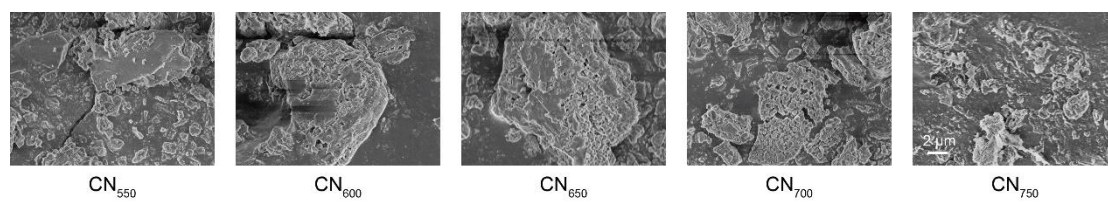
$$\Delta G = E + \Delta ZPE + \Delta H - \Delta TS \quad (6)$$

where ZPE represents the zero-point energy,  $\Delta H$  is the change in enthalpy or the integrated heat capacity,  $T$  denotes the temperature at which the system or product is analyzed, and  $S$  signifies the entropy of the system.

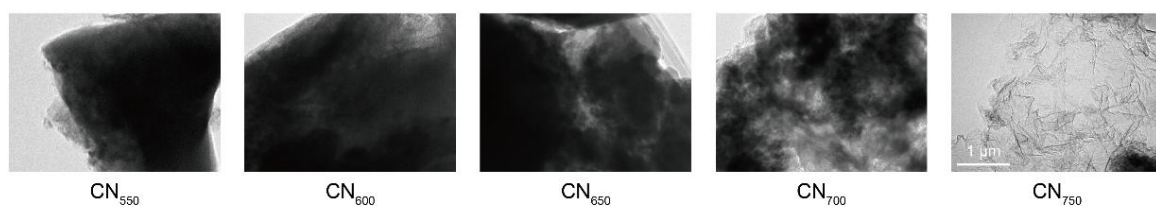
#### ***In vitro ROS scavenging activities of Cu/CN***

Initially, HUVECs were seeded into cell culture plates and stimulated with H<sub>2</sub>O<sub>2</sub> (0.5 mmol/L) to produce ROS. The cells were then co-incubated with PBS, CN<sub>700</sub>, and Cu/CN. The intracellular ROS levels were subsequently measured by fluorescence microscopy, following staining with the ROS-specific probe, 2,7-dichlorodihydrofluorescein diacetate (DCFH-DA).

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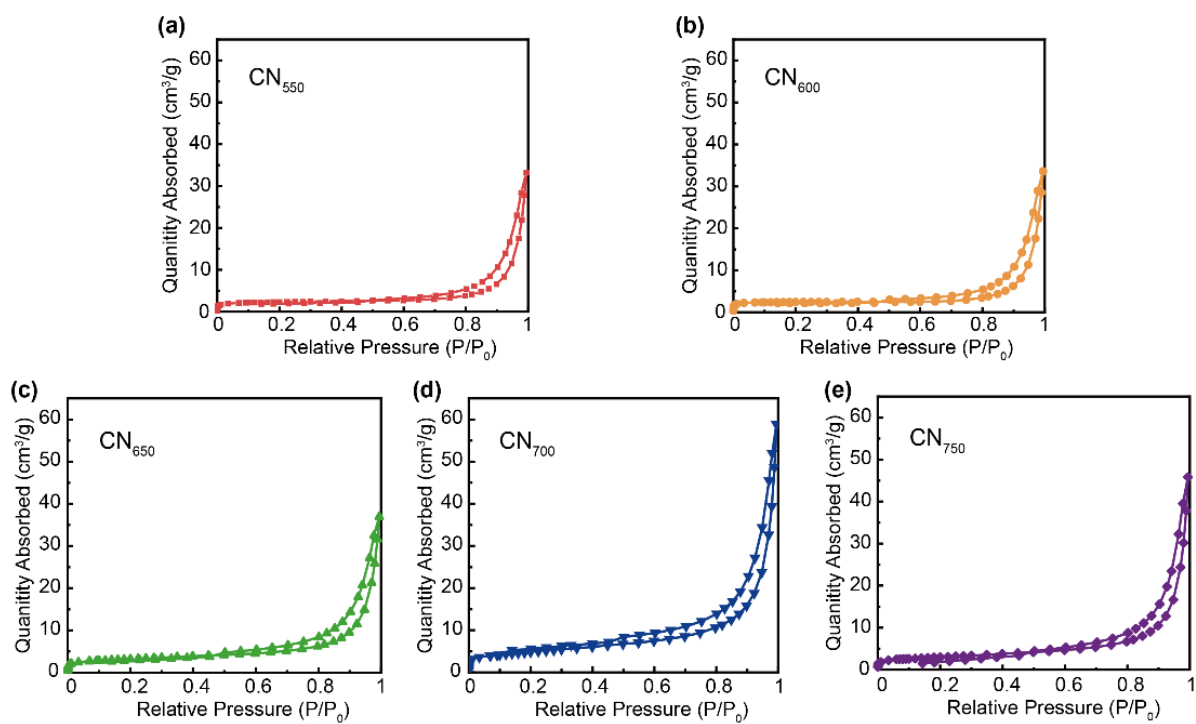


**Figure S1.** SEM of CN treated at 550 °C, 600 °C, 650 °C, 700 °C and 750 °C.

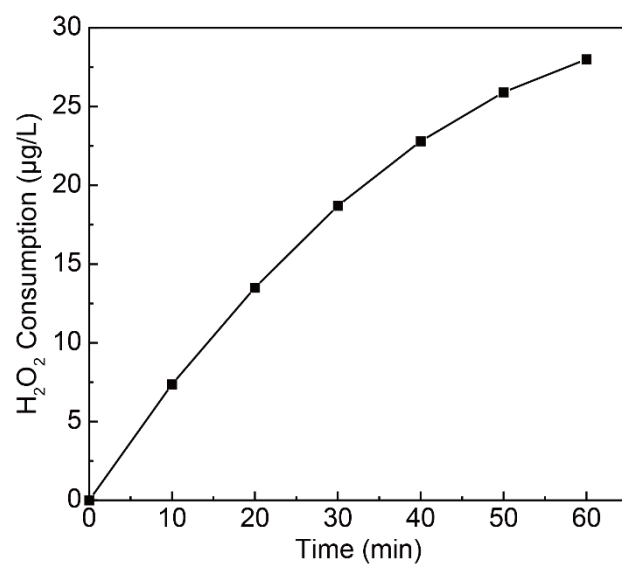


**Figure S2.** TEM of CN treated at 550 °C, 600 °C, 650 °C, 700 °C and 750 °C.

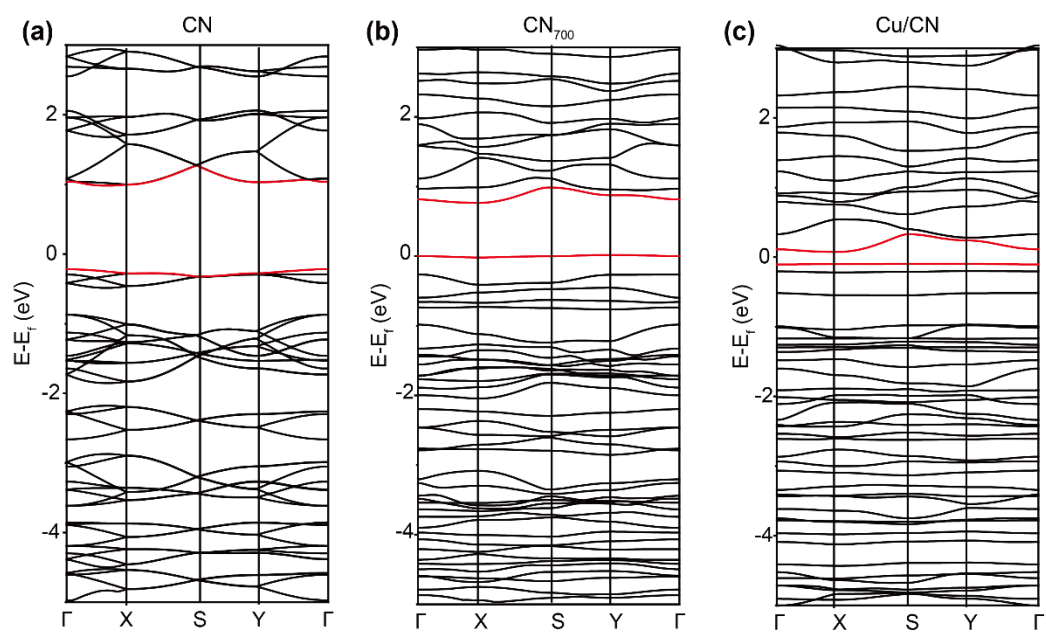




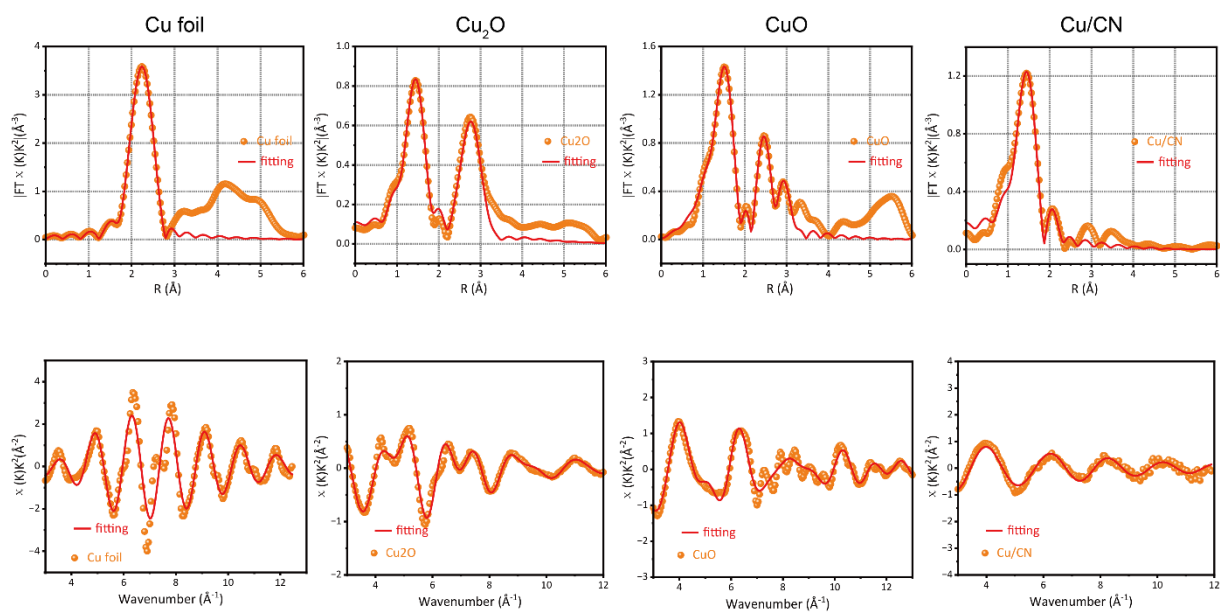
**Figure S3.** BET of CN treated at 550 °C, 600 °C, 650 °C, 700 °C and 750 °C.



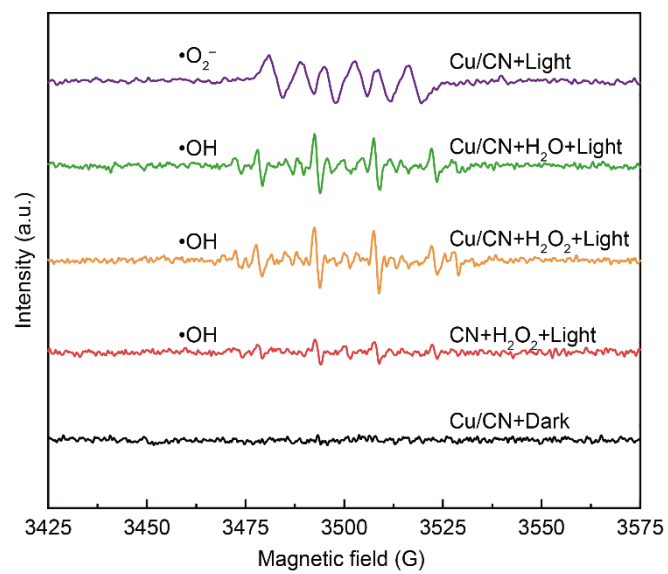
**Figure S4.** Kinetics of  $\text{H}_2\text{O}_2$  consumption with Cu/CN.



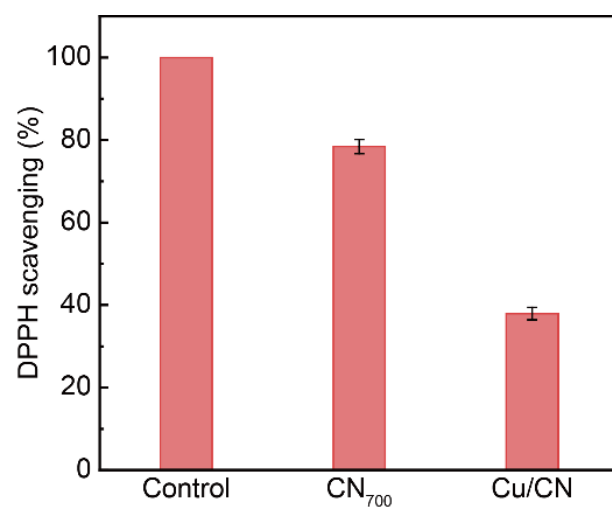
**Figure S5.** PBE of (a) CN, (b) CN<sub>700</sub>, and (c) Cu/CN.



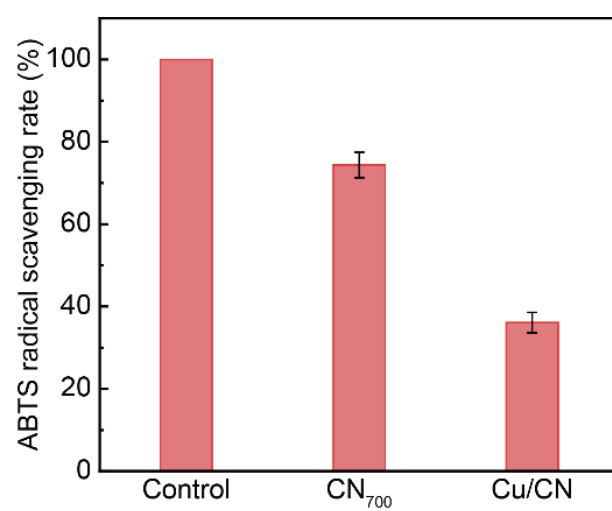
**Figure S6.** EXAFS R-space and K-space fitting curve of Cu foil, Cu<sub>2</sub>O, CuO, and Cu/CN.



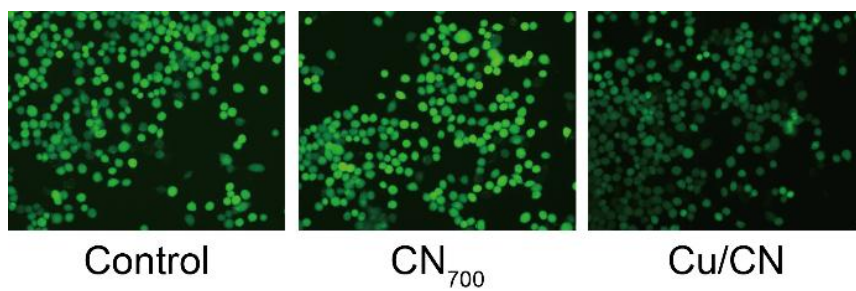
**Figure S7.**  $\bullet\text{OH}$ , and  $\bullet\text{O}_2^-$  EPR spectra of CN<sub>700</sub> and Cu/CN.



**Figure S8.** ROS-scavenging efficiency of CN<sub>700</sub> and Cu/CN determined by DPPH method.

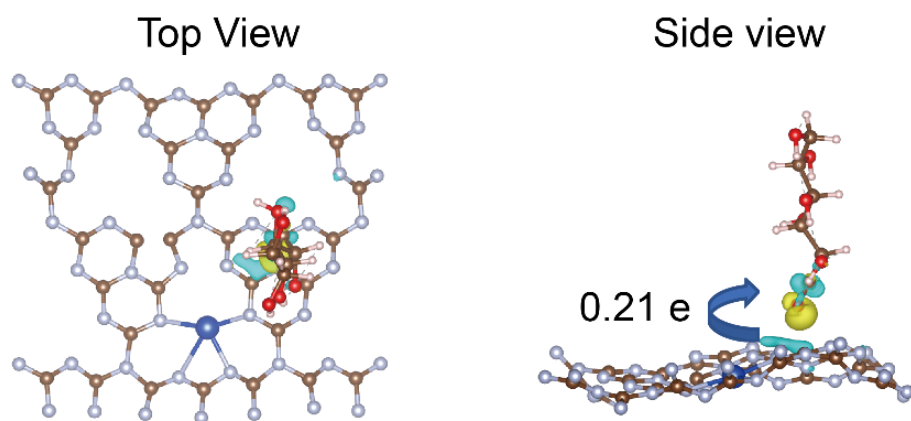


**Figure S9.** ROS-scavenging efficiency of CN<sub>700</sub> and Cu/CN determined by ABTS method.



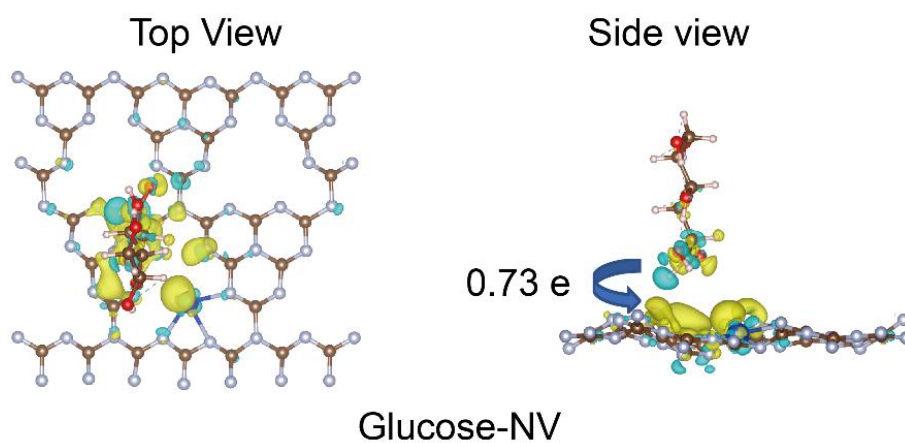
**Figure S10.** Intracellular ROS-scavenging performance of  $\text{CN}_{700}$  and Cu/CN.



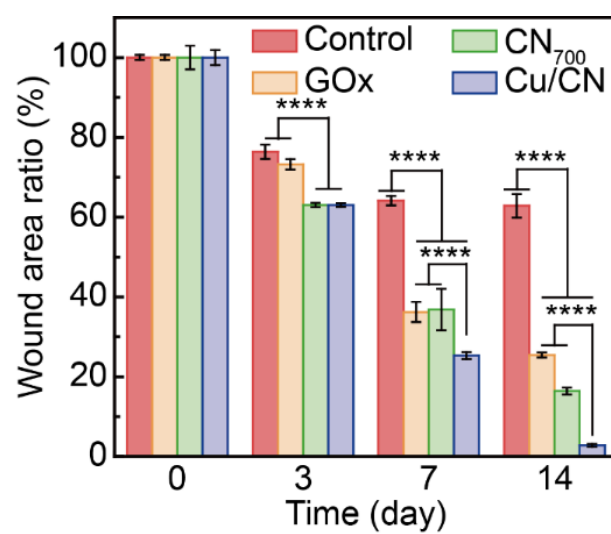


### Glucose-N

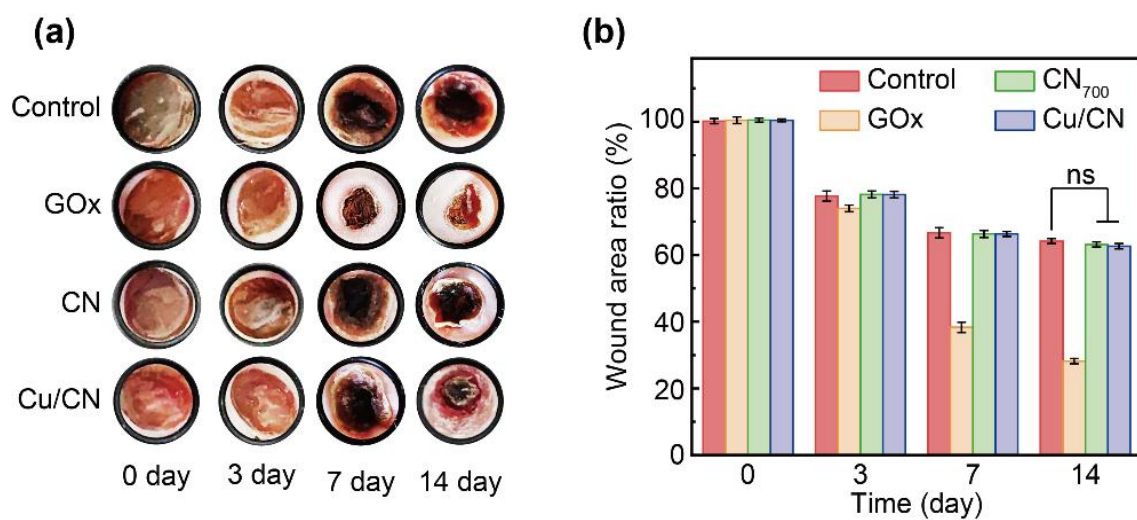
**Figure S11.** Top and side views of the Charge Density Difference Analysis for the glucose-N. The yellow and the cyan areas represent charge accumulation and depletion, respectively.



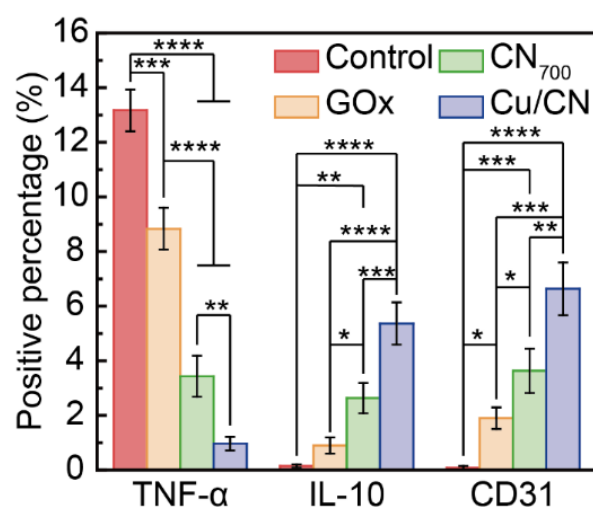
**Figure S12.** Top and side views of the Charge Density Difference Analysis for the glucose-NV. The yellow and the cyan areas represent charge accumulation and depletion, respectively.



**Figure S13.** Quantitative analysis of wound area in different groups treated with light ( $\lambda > 420$  nm).



**Figure S14.** (a) Photographs of the wound closure procedure treated without light. (b) Assessment of the wound area ratios.



**Figure S15.** Fluorescence quantitative analysis of TNF- $\alpha$ , IL-10, and CD31 from different treatment groups on day 14.

**Table S1.** The specific surface area and pore size of CN

| Entry | sample            | SBET (m <sup>2</sup> g <sup>-1</sup> ) | Pore Volume (cm <sup>3</sup> g <sup>-1</sup> ) |
|-------|-------------------|--|--|
| 1     | CN <sub>550</sub> | 7.2909                                 | 0.007877                                       |
| 2     | CN <sub>600</sub> | 7.3677                                 | 0.007493                                       |
| 3     | CN <sub>650</sub> | 10.9141                                | 0.012754                                       |
| 4     | CN <sub>700</sub> | 16.7939                                | 0.022491                                       |
| 5     | CN <sub>750</sub> | 10.3465                                | 0.015052                                       |

**Table S2.** Charge carrier density ( $N_d$ ) of CN<sub>550</sub>, CN<sub>600</sub>, CN<sub>650</sub>, CN<sub>700</sub>, and CN<sub>750</sub>.

| Entry | Photocatalyst     | $d(1/C^2/dV_s)$      | $N_d$ (cm <sup>-3</sup> ) |
|-------|-------------------|----------------------|---------------------------|
| 1     | CN <sub>550</sub> | $4.8821 \times 10^7$ | $1.8522 \times 10^{21}$   |
| 2     | CN <sub>600</sub> | $3.9056 \times 10^7$ | $2.3153 \times 10^{21}$   |
| 3     | CN <sub>650</sub> | $3.8556 \times 10^7$ | $2.3453 \times 10^{21}$   |
| 4     | CN <sub>700</sub> | $2.8251 \times 10^7$ | $3.2008 \times 10^{21}$   |
| 5     | CN <sub>750</sub> | $4.4798 \times 10^7$ | $2.0185 \times 10^{21}$   |

**Table S3.** Potentials of CN<sub>550</sub>, CN<sub>600</sub>, CN<sub>650</sub>, CN<sub>700</sub>, and CN<sub>750</sub>.

| Entry | Photocatalyst     | E <sub>Ag/AgCl</sub> (V) | E <sub>RHE</sub> (V) | E <sub>NHE</sub> (V) |
|-------|-------------------|--------------------------|----------------------|----------------------|
| 1     | CN <sub>550</sub> | -0.5698                  | 0.0284               | -0.1716              |
| 2     | CN <sub>600</sub> | -0.5869                  | 0.0113               | -0.1887              |
| 3     | CN <sub>650</sub> | -0.6615                  | -0.0633              | -0.2633              |
| 4     | CN <sub>700</sub> | -0.7696                  | -0.1714              | -0.3714              |
| 5     | CN <sub>750</sub> | -0.5874                  | 0.0108               | -0.1892              |



**TableS4.** EXAFS fitting parameters at the Cu K-edge for various samples

| Sample            | Shell | $CN^a$  | $R(\text{\AA})^b$ | $\sigma^2(\text{\AA}^2)^c$ | $\Delta E_0(\text{eV})^d$ | $R$ factor |
|-------------------|-------|---------|-------------------|----------------------------|---------------------------|------------|
| Cu foil           | Cu-Cu | 12.0*   | 2.53±0.01         | 0.0082                     | 5.5                       | 0.0005     |
| Cu <sub>2</sub> O | Cu-O  | 1.5±0.2 | 1.84±0.01         | 0.0025                     | 6.6                       |            |
|                   | Cu-Cu | 12.0*   | 3.04±0.01         | 0.0225                     | 8.8                       | 0.0167     |
| CuO               | Cu-O  | 4.0*    | 1.95±0.01         | 0.0044                     | -1.3                      |            |
|                   | Cu-Cu | 4.0*    | 2.91±0.01         | 0.0059                     |                           | 0.0083     |
|                   | Cu-Cu | 6.0*    | 3.10±0.01         | 0.0090                     | 3.7                       |            |
| Cu/CN             | Cu-N  | 4.3±0.3 | 1.98±0.01         | 0.0037                     | -2.8                      | 0.0103     |

<sup>a</sup> $CN$ , coordination number; <sup>b</sup> $R$ , distance between absorber and backscatter atoms; <sup>c</sup> $\sigma^2$ , Debye-Waller factor to account for both thermal and structural disorders; <sup>d</sup> $\Delta E_0$ , inner potential correction;  $R$  factor indicates the goodness of the fit.  $S_0^2$  was fixed to 0.91. A reasonable range of EXAFS fitting parameters:  $0.700 < S_0^2 < 1.000$ ;  $CN > 0$ ;  $\sigma^2 > 0 \text{ \AA}^2$ ;  $|\Delta E_0| < 15 \text{ eV}$ ;  $R$  factor  $< 0.02$ .