Supporting Information:

Strongly Enhanced Antibacterial Action of Copper Oxide Nanoparticles with Boronic Acid Surface Functionality

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The Schematic of the Synthesis Method of CuONPs.

**Figure S1.** The schematic of the synthesis method of CuONPs.
Fourier Transform Infrared Spectroscopy (FTIR) Analysis of CuONPs Calcined at Various Temperatures.

Figure S2 presented the FTIR spectra of CuONPs annealed at 100°C, 200°C, 300°C, 400°C, 500°C and 600°C. The broad absorption peak at about 3445.89 cm$^{-1}$ was caused by the adsorbed water molecules. Because of the nano crystalline materials possess a high surface to volume ratio, they can absorb moisture. Similar peak at 3434 cm$^{-1}$ in the FTIR spectra of CuONPs are described.$^{34,35}$ The peaks at 1632.77 might be for the Cu-O symmetrical stretching.$^{34,36}$ The two infrared absorption peaks observed the vibrational modes of CuONPs in the range of 500 - 700 cm$^{-1}$. These peaks were detected at 533.33 cm$^{-1}$ and 585.41 cm$^{-1}$, respectively. The peak at 533.33 cm$^{-1}$ could be because of stretching of Cu-O.$^{37}$ The two peaks at 533.33 cm$^{-1}$ and 585.41 cm$^{-1}$ showed the creation of the CuONPs. These two peaks provision the existence of monoclinic phase. No other IR active modes are detected in the range of 500- 700 cm$^{-1}$, which completely rules out the presence of Cu$_2$O. Two peaks at 525 cm$^{-1}$ and 580 cm$^{-1}$ in the FTIR spectra described for CuONPs which closely matches with our results.$^{38}$ Thus, the metal-oxygen frequencies observed for CuONPs are in near agreement with that of literature values.$^{34}$

![Figure S2. FTIR spectra of prepared CuONPs at different calcination temperatures (A) Cu(OH)$_2$ without calcinated, (B) 100°C, (C) 200°C, (D) 300°C, (E) 400°C, (F) 500°C and (G) 600°C in the range of 500–4000 cm$^{-1}$.](image-url)
XRD Pattern of CuONPs Annealed at Different Temperatures

**Figure S3.** XRD pattern of CuONPs annealed at (A) 100 °C, (B) 200 °C, (C) 300 °C, (D) 400 °C, (E) 500 °C and (F) 600 °C with different crystallite size. The largest peak in the XRD results was applied to measure the crystallite size.
Effect of the Annealing Temperature on the Particles Size and Zeta Potential of the CuONPs

The particle size and zeta potential of CuONPs were examined at different calcination temperature as appeared in Figure S4 and Figure S5. From Figure S4, it is clear that the hydrodynamic diameter is increasing with increasing of the annealing temperature. Therefore, it was found that CuONPs with same crystal type but various particle size could be obtained by changing the calcination temperature and also these results were in agreement with the previous studies. These results may be explained that at higher calcination temperatures, the agglomeration of CuONPs begin to occur and hence the particle size increased. In addition to that, the zeta potential was tested for every calcined sample of CuONPs, and it can be seen from the Figure S5 that at 100°C, the zeta potential was +37 mV which means it was a highly stable while, at 600°C, the zeta potential was -4 mV.

Figure S4. The hydrodynamic diameter of CuONPs annealed at various temperatures.
Figure S5. The zeta potential of CuONPs annealed at different temperatures.
Figure S6. EDX diagram of *E.coli* cells with CuONPs at 20 µg mL\(^{-1}\); (A) *E.coli* inside membrane and (B) *E.coli* edge membrane areas and (C) *E.coli* outside membrane areas. The result shows the existence of CuONPs on the inner and outer part of the cell membrane.
Figure S7. The zeta potential of (A) bare CuONPs and (B) CuONPs functionalized with GLYMO and 4-HPBA at different concentrations (5, 15 and 25 µg mL\(^{-1}\)) after exposure to UV light for 0 day, 1 day, 2 days and 3 days.
Colony Forming Units assessment of the viability of *E. coli* and *R. rhodochrous* after treatment with bare and functionalized CuONPs

**Figure S8.** Colony forming unit (CFU) count of *E. coli* following up to 6 hours of exposure to increasing concentrations of the bare CuONPs (A), and surface functionalized of CuONPs with GLYMO (B) and (C) 4-HPBA/GLYMO, values are shown as mean ±SD.

**Figure S9.** Colony forming unit (CFU) count of *R. rhodochrous* following up to 6 hours of exposure to increasing concentrations of the bare CuONPs (A), and surface functionalized of CuONPs with GLYMO (B) and (C) 4-HPBA/GLYMO, values are shown as mean ±SD.

We present the numeric data for CFU/mL in Figures S8 and S9 in table format in Tables S1 and S2 below.
Table S1. Experimental data for the CFU/mL for *E.coli* after treatment with various concentrations of CuONPs, CuONPs/GLYMO and CuONPs/GLYMO/HPBA (0, 5, 10, 15, 20, 25 mg L\(^{-1}\)) in dark conditions for 10 min, 1 h and 6 h, respectively. CFU mL\(^{-1}\) were calculated as (no. of colonies per plate × dilution factor) / volume of culture plate (mL). The dilution factor is 10000 and the volume of culture plate 0.100 mL.

| time          | NPs conc. (µg mL\(^{-1}\)) | CuONPs | Log(Average (CFU mL\(^{-1}\))) | CuONPs/GLYMO | Log(Average (CFU mL\(^{-1}\))) | CuONPs/GLYMO/4-HPBA | Log(Average (CFU mL\(^{-1}\))) |
|---------------|-----------------------------|--------|--------------------------------|---------------|--------------------------------|---------------------|--------------------------------|
|               |                             | Average (CFU mL\(^{-1}\)) |                  | Average (CFU mL\(^{-1}\)) |                  | Average (CFU mL\(^{-1}\)) |                  |
| 10 min        | 0                           | 66 ± 5 \times 10^5         | 6.823            | 66 ± 5 \times 10^5         | 6.823            | 66 ± 5 \times 10^5         | 6.823            |
|               | 5                           | 58 ± 2 \times 10^5         | 6.765            | 66 ± 3 \times 10^5         | 6.821            | 40 ± 4 \times 10^5         | 6.605            |
|               | 10                          | 53 ± 3 \times 10^5         | 6.729            | 64 ± 2 \times 10^5         | 6.806            | 28 ± 2 \times 10^5         | 6.452            |
|               | 15                          | 52 ± 4 \times 10^5         | 6.721            | 64 ± 5 \times 10^5         | 6.808            | 23 ± 4 \times 10^5         | 6.367            |
|               | 20                          | 43 ± 3 \times 10^5         | 6.640            | 66 ± 8 \times 10^5         | 6.819            | 22 ± 2 \times 10^5         | 6.348            |
|               | 25                          | 43 ± 2 \times 10^5         | 6.633            | 58 ± 4 \times 10^5         | 6.768            | 15 ± 2 \times 10^5         | 6.176            |
| 1 hour        | 0                           | 72 ± 2 \times 10^5         | 6.857            | 72 ± 2 \times 10^5         | 6.857            | 72 ± 2 \times 10^4         | 6.857            |
|               | 5                           | 49 ± 6 \times 10^5         | 6.693            | 59 ± 3 \times 10^5         | 6.773            | 15 ± 3 \times 10^5         | 6.185            |
|               | 10                          | 35 ± 2 \times 10^5         | 6.544            | 56 ± 2 \times 10^5         | 6.753            | 8 ± 2 \times 10^5          | 5.937            |
|               | 15                          | 27 ± 2 \times 10^5         | 6.431            | 55 ± 7 \times 10^5         | 6.740            | 6 ± 1 \times 10^5          | 5.801            |
|               | 20                          | 23 ± 2 \times 10^5         | 6.361            | 50 ± 4 \times 10^5         | 6.698            | 5 ± 3 \times 10^5          | 5.753            |
|               | 25                          | 19 ± 3 \times 10^5         | 6.293            | 44 ± 3 \times 10^5         | 6.646            | 3 ± 2 \times 10^5          | 5.477            |
| 6 hours       | 0                           | 80 ± 2 \times 10^5         | 6.903            | 80 ± 2 \times 10^5         | 6.903            | 80 ± 2 \times 10^5         | 6.903            |
|               | 5                           | 21 ± 3 \times 10^5         | 6.329            | 47 ± 7 \times 10^5         | 6.672            | 7 ± 5 \times 10^5          | 5.845            |
|               | 10                          | 14 ± 2 \times 10^5         | 6.146            | 47 ± 3 \times 10^5         | 6.675            | 3 ± 2 \times 10^5          | 5.477            |
|               | 15                          | 11 ± 3 \times 10^5         | 6.054            | 40 ± 5 \times 10^5         | 6.602            | 0                         | 0                 |
|               | 20                          | 2 ± 2 \times 10^5          | 5.301            | 37 ± 5 \times 10^5         | 6.575            | 0                         | 0                 |
|               | 25                          | 0                         | 0                | 37 ± 2 \times 10^5         | 0                | 0                         | 0                 |
Table S2. Experimental data for the CFU/mL for *R.*rhodochrous after treatment with various concentrations of CuONPs, CuONPs/GLYMO and CuONPs/GLYMO/HPBA (0, 5, 10, 15, 20, 25 mg L^-1) in dark conditions for 10 min, 1 h and 6 h, respectively. CFU mL^-1 were calculated as (no. of colonies per plate × dilution factor) / volume of culture plate (mL). The dilution factor is 10000 and the volume of culture plate 0.1 mL.

| R. rhodochrous | CuONPs | CuONPs/GLYMO | CuONPs/GLYMO/4-HPBA |
|----------------|--------|--------------|---------------------|
| time (min)     | Average (CFU mL^{-1}) | Log (CFU mL^{-1}) | Average (CFU mL^{-1}) | Log (CFU mL^{-1}) | Average (CFU mL^{-1}) | Log (CFU mL^{-1}) |
| 10 min         | 0      | 51 ± 3 × 10^5 | 6.713               | 51 ± 3 × 10^5 | 6.713               | 51 ± 3 × 10^5 | 6.713               |
|                | 5      | 50 ± 5 × 10^5 | 6.701               | 49 ± 3 × 10^5 | 6.693               | 47 ± 4 × 10^5 | 6.675               |
|                | 10     | 50 ± 2 × 10^5 | 6.701               | 49 ± 6 × 10^5 | 6.693               | 44 ± 2 × 10^5 | 6.643               |
|                | 15     | 50 ± 5 × 10^5 | 6.698               | 50 ± 2 × 10^5 | 6.698               | 47 ± 2 × 10^5 | 6.678               |
|                | 20     | 48 ± 4 × 10^5 | 6.687               | 45 ± 3 × 10^5 | 6.659               | 45 ± 3 × 10^5 | 6.653               |
|                | 25     | 44 ± 2 × 10^5 | 6.649               | 50 ± 2 × 10^5 | 6.698               | 44 ± 3 × 10^5 | 6.646               |
|                | 250    | 39 ± 3 × 10^5 | 6.598               | 48 ± 2 × 10^5 | 6.681               | 30 ± 5 × 10^5 | 6.486               |
| 1 hour         | 0      | 52 ± 2 × 10^5 | 6.716               | 52 ± 2 × 10^5 | 6.716               | 52 ± 2 × 10^5 | 6.716               |
|                | 5      | 44 ± 3 × 10^5 | 6.649               | 50 ± 2 × 10^5 | 6.698               | 25 ± 2 × 10^5 | 6.397               |
|                | 10     | 39 ± 8 × 10^5 | 6.591               | 48 ± 2 × 10^5 | 6.681               | 17 ± 3 × 10^5 | 6.247               |
|                | 15     | 34 ± 4 × 10^5 | 6.539               | 46 ± 4 × 10^5 | 6.662               | 11 ± 2 × 10^5 | 6.041               |
|                | 20     | 31 ± 2 × 10^5 | 6.491               | 45 ± 2 × 10^5 | 6.656               | 7 ± 5 × 10^5  | 5.845               |
|                | 25     | 26 ± 5 × 10^5 | 6.420               | 47 ± 2 × 10^5 | 6.675               | 0              | 0                   |
|                | 250    | 16 ± 6 × 10^5 | 6.213               | 37 ± 5 × 10^5 | 6.572               | 0              | 0                   |
| 6 hours        | 0      | 53 ± 5 × 10^5 | 6.724               | 53 ± 5 × 10^5 | 6.724               | 53 ± 5 × 10^5 | 6.724               |
|                | 5      | 30 ± 1 × 10^5 | 6.486               | 45 ± 2 × 10^5 | 6.653               | 14 ± 2 × 10^5 | 6.156               |
|                | 10     | 29 ± 4 × 10^5 | 6.467               | 43 ± 3 × 10^5 | 6.640               | 3 ± 2 × 10^5  | 5.522               |
|                | 15     | 23 ± 3 × 10^5 | 6.374               | 36 ± 4 × 10^5 | 6.564               | 0              | 0                   |
|                | 20     | 19 ± 5 × 10^5 | 6.278               | 31 ± 4 × 10^5 | 6.491               | 0              | 0                   |
|                | 25     | 10 ± 3 × 10^5 | 6.028               | 27 ± 1 × 10^5 | 6.436               | 0              | 0                   |
|                | 250    | 0.3±5 × 10^5  | 4.522               | 27 ± 3 × 10^5 | 6.441               | 0              | 0                   |
Antimicrobial efficiency of the bare and HPBA-functionalized CuONPs

We have calculated the percentage reduction in the required CuONPs particle concentration after functionalization with HPBA to achieve the same antibacterial action as with the bare CuONPs (Figure S10 and Figure S11). We have done this by fitting the experimental data for the bacterial cell viability upon treatment with CuONPs/GLYMO/HPBA with a logarithmic law (see below) which allows to evaluate what particle concentration would produce the same CFU/mL as the bare CuONPs of fixed concentration. The calculation steps are presented as follows:

For bare CuONPs

\[ y_B = a_1 \ln(x_B) + b_1, \quad (y_B = \text{CFU/mL with bare CuONPs at particle concentration } x_B) \]

For CuONPs/GLYMO/4-HPBA

\[ y_F = a_2 \ln(x_F) + b_2, \quad (y_F = \text{CFU/mL with CuONPs/GLYMO/4-HPBA at particle concentration } x_F) \]

The coefficients \( a_2 \) and \( b_2 \) are determined from the fit with the experimental data for CFU/mL with CuONPs/GLYMO/4-HPBA. Hence for any value \( y_B \) at \( x_B \) from the bare CuONPs for the CFU/mL, \( y_2 = a_2 \exp \left( \frac{y_B - b_2}{a_2} \right) \), i.e.

\[ x_F = \exp \left( \frac{y_B - b_2}{a_2} \right) \]

Finally, the reduction in the functionalised CuONPs concentration needed to achieve the same antibacterial activity as the bare CuONPs is determined as follows:

\[ \% \text{ reduction} = \left( \frac{x_F}{x_B} \right) \times 100 \]  
(Percentage reduction of CuONPs which achieves the same antibacterial action).

![Graph showing % reduction in the required CuONPs/GLYMO/HPBA nanoparticle concentration after functionalization to achieve the same antibacterial action on E.coli as with the bare CuONPs. The data are calculated by using direct CFU/ml measurements.](image)

**Figure S10.** The % reduction in the required CuONPs/GLYMO/HPBA nanoparticle concentration after functionalization to achieve the same antibacterial action on *E.coli* as with the bare CuONPs. The data are calculated by using direct CFU/ml measurements.
Figure S11. The % reduction in the required CuONPs nanoparticle concentration after functionalization to achieve the same antibacterial action on *R. rhodochrous* as with the bare CuONPs. The data illustrate the increased antimicrobial efficiency of the HPBA-functionalised CuONPs compared with the bare CuONPs.
Figure S12. SEM images of HaCaT cells after being incubated for 36 hours with bare CuONPs and CuONPs functionalized with 4-HPBA: (A) HaCaT cell before treatment, (B) HaCaT cells incubated with 25 µg mL\(^{-1}\) CuONPs and (C) HaCaT cells incubated with 25 µg mL\(^{-1}\) CuONPs/GLYMO/4-HPBA and the scale bars are 30 µm.
EDX diagrams of the outer surface of *E. coli* treated with bare- and HPBA-functionalized CuONPs

Figure S13. EDX diagram of *E.coli* outside membrane areas with (A) bare CuONPs and surface functionalized of CuONPs with GLYMO and (B) 4-HPBA at 20 µg mL⁻¹. The result shows the existence of CuONPs on the outer part of the cell membrane.