ACES Asian Chemical Editorial Society

DOI: 10.1002/asia.202100546

CHEMISTRY AN ASIAN JOURNAL Communication

# Vancomycin-Functionalized Porphyrinic Metal-Organic Framework PCN-224 with Enhanced Antibacterial Activity against *Staphylococcus Aureus*

Li-Jian Chen,[a, b] Yao-Yao Liu,[a, b] Xu Zhao,[a, b] and Xiu-Ping Yan*[a, b, c]

**Abstract:** A vancomycin (Van) modification strategy on a porphyrinic metal-organic framework (MOF) PCN-224 is presented. The obtained Van-PCN-224 gives the combined advantages of porphyrinic MOF and Van with high photosensitive activity and excellent targeted antibacterial activity against *Staphylococcus aureus*. The features make Van-PCN-224 promising for antimicrobial therapy.

Bacterial infection has drawn widespread concern because of its serious threat to human health.[1] In particular, *Staphylococcus aureus* (*S. aureus*), one of the widely distributed Gram-positive bacteria, can cause various dangerous diseases including toxic shock syndrome septicemia, pneumonia, endocarditis, and osteomyelitis.[2] Antibiotics have proved highly effective in eradicating infectious diseases, but the abuse of antibiotics leads to the emergence and increase of antibiotic-resistant bacteria.[3] Vancomycin (Van), as a glycopeptide antibiotic, binds to a specific C-terminal sequence, D-Ala–D-Ala, in the peptidoglycan pentapeptide in the bacterial cell wall.[4] It has been widely used to combat infection arising from *S. aureus*, especially the strains which are resistant to penicillin. Van group has once become the last resort to fight against these bacteria. However, the appearance of Van-resistant enterococcus in recent years turns the problem into new concern.[1] Thus, the development of alternative effective therapeutic means available for *S. aureus* infection is of great importance.

Photodynamic therapy (PDT) uses photosensitizers that are activated by absorbing light in the presence of oxygen to generate reactive oxygen species, especially singlet oxygen ($^1O_2$) to kill microbes or cells via oxidative burst.[5] Photosensitizers with excellent photosensitivity play a critical role in PDT. Protoporphyrin is a natural molecule in all living cells. Protoporphyrin and its derivatives have been used as photosensitizers for a long time, but their poor solubility and self-aggregation in physiological environment limits their direct application for PDT.[3]

Porphyrinic metal-organic frameworks (MOFs) are an important subclass of MOFs, assembled from porphyrin or metalloporphyrin as an organic bridging ligand and metal ion/clusters as nodes through coordination bonds.[6] The stable crystalline structure, periodically arranged porphyrin molecules and high porosity beneficial to the diffusion of $^1O_2$ are advantages of porphyrinic MOFs to enhance $^1O_2$ generation. As such, nanoscale porphyrinic MOFs have been reported as photosensitizer in effective anticancer PDT.[7]

Porphyrinic MOF PCN-224 is favorable for anticancer PDT. PCN-224 has good biocompatibility and easy cellular internalization which benefits from the easy-controlled size and spherical morphology.[8] PCN-224 with good water stability as well as other base stable porphyrinic MOFs are quite suitable for applications, particularly for bio-applications.[9] PCN-224 has received great attention either as photosensitizer alone for antibacterial PDT or combined with other materials for synergistic therapy. Zhang et al fabricated a nanoplatform based on silver-infused PCN-224 for synergistic bacteria killing and wound disinfection.[10] Chen et al incorporated titanium into PCN-224 and reported the enhanced antibacterial activity against multidrug-resistant pathogens.[11] Han and coworkers reported $Cu^{2+}$-doped PCN-224 for rapid treatment of bacteria-infected wounds.[12] Nevertheless, to our best knowledge, no studies on functionalized PCN-224 for targeted antibacterial application have been reported so far.

Herein, we report the preparation of Van functionalized PCN-224 (Van-PCN-224) for targeted antibacterial application. Van molecule is used as targeting ligand as it not only enables selective targeting of Gram-positive bacteria, but also exhibits antibacterial activity against Gram-positive bacteria.[4,13] Thus, the prepared Van-functionalized PCN-224 gives the integrated merits of the bacteria-targeting ability of Van molecule and the PDT feature of PCN-224 for enhanced targeted antibacteria.

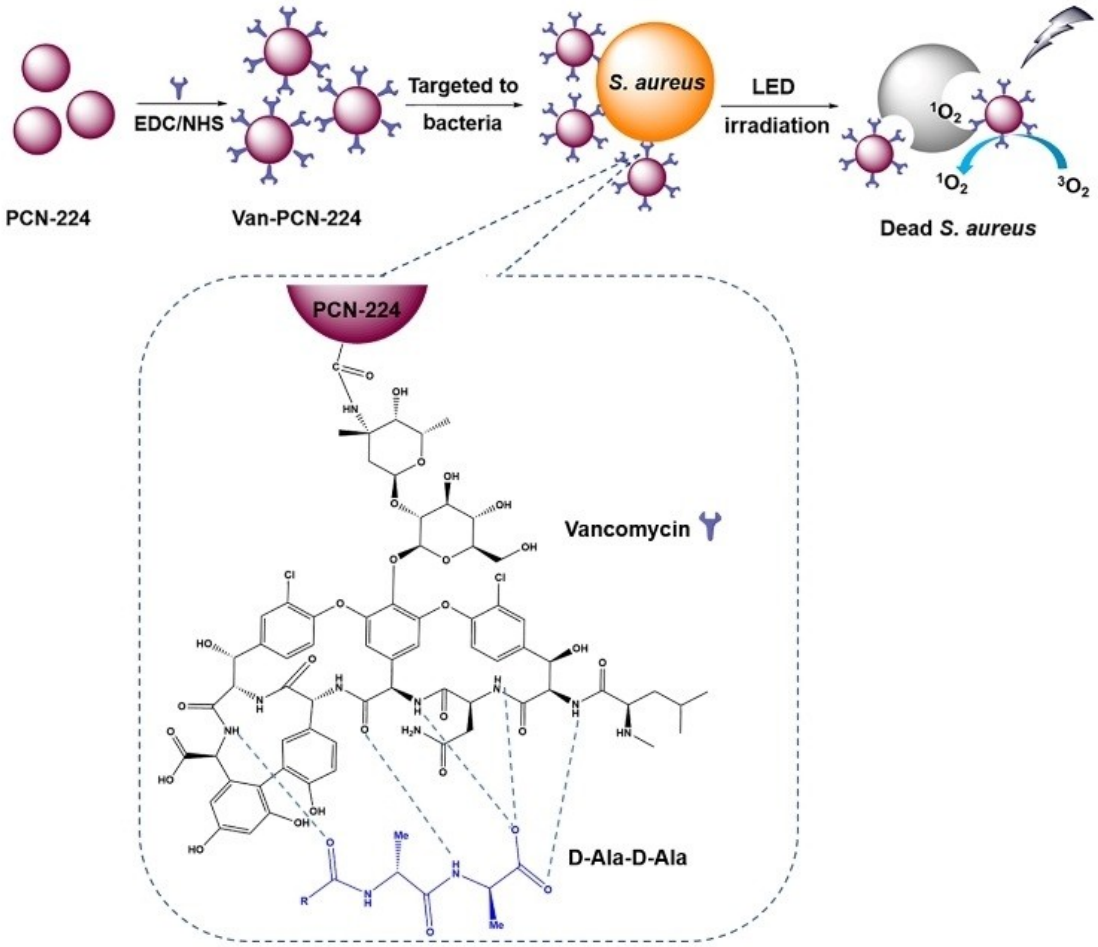
To obtain the ability to target gram-positive bacteria and enhanced antibacterial efficiency, surface functionalization of Van molecule on PCN-224 is necessary. Scheme 1 shows the functionalization of PCN-224 with Van molecule via carbodiimide method. Van-PCN-224 possesses the improved ability to kill the gram-positive bacterium under light-emitting diode (LED) irradiation. The carboxyl groups on the free ligands in PCN-224 frameworks reacted with the amino groups of Van molecules via ester-amide condensation with the aid of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride

[a] Dr. L.-J. Chen, Y.-Y. Liu, Dr. X. Zhao, Prof. X.-P. Yan
State Key Laboratory of Food Science and Technology
Jiangnan University
Wuxi 214122 (P. R. China)
E-mail: xpyan@jiangnan.edu.cn

[b] Dr. L.-J. Chen, Y.-Y. Liu, Dr. X. Zhao, Prof. X.-P. Yan
Institute of Analytical Food Safety
School of Food Science and Technology
Jiangnan University
Wuxi 214122 (P. R. China)

[c] Prof. X.-P. Yan
International Joint Laboratory on Food Safety
Jiangnan University
Wuxi 214122 (P. R. China)

Supporting information for this article is available on the WWW under https://doi.org/10.1002/asia.202100546




**Scheme 1.** Schematic for the functionalization of PCN-224 with Van and its application for targeted and synergistic killing of *S. aureus*.

(EDC·HCl) and N-hydroxysuccinimide (NHS) to obtain Van-PCN-224.

PCN-224 nanoparticles were prepared according to Park et al with modification.[8a] The prepared PCN-224 nanoparticles show a nearly spherical shape with an average size of 79.9 ± 7.2 nm (based on the 50 randomly selected nanoparticles) (Figure 1a). The Van functionalization did not have significant effect on the morphology of PCN-224 (Figure 1b). The crystal structures of PCN-224 and Van-PCN-224 display similar sharp diffraction peaks matching well with the simulated pattern of PCN-224 (Figure 1c). However, the zeta-potential decreased from 6.8 mV (PCN-224) to −14.2 mV (Van-PCN-224) due to the electronegativity of Van (−17.5 mV) (Figure 1d).

The change in Fourier transform infrared (FT-IR) spectra and hydrodynamic size shows the successful Van functionalization. Both Van and PCN-224 gave the absorption band of N=H vibration at 1659 $cm^{-1}$ from amido and imimo groups, while Van-PCN-224 offered the reduced absorption band of N=H vibration along with a new absorption band at 1611 $cm^{-1}$ for the stretching vibration of –CO–NH– formed in the amide condensation (Figure 2a). The hydrodynamic size increased from 91.3 nm (PCN-224) to 164 nm (Van-PCN-224) after Van functionalization (Figure 2b). The Van modification also made the Brunauer–Emmett–Teller (BET) surface area decrease from 1550 $m^2 g^{-1}$ (PCN-224) to 340 $m^2 g^{-1}$ (Van-PCN-224) (Figure 2c), and the major pores reduce from 2.0 nm (PCN-224) to 1.9 nm (Van-PCN-224) (Figure 2d). The loaded vancomycin on the PCN-224 was determined to be 0.18 mg/mg using UV spectrophotometry (Figures S1–S3). These data further prove the successful modification of Van on PCN-224. The stability of Van-PCN-224 was further evaluated by XRD spectrometry. The Van-PCN-224 was stable at least for 12 months for the diffraction peaks matching well with the original pattern (Figure S4).

To test the effect of Van functionalization on photodynamic generating $^1O_2$ of PCN-224, 1,3-diphenylisobenzofuran (DPBF) was used as the indicator. PCN-224 can be a photosensitizer to generate $^1O_2$ in the presence of $O_2$ under white light irradiation.[8a,10] The generated $^1O_2$ led to effective decrease of DPBF absorbance owing to redox reaction. The absorbance of DPBF obviously decreased in the presence of PCN-224 and Van-PCN-224 under white LED illumination for 8 min (Figure 3a and 3b), indicating PCN-224 and Van-PCN-224 had good ability to

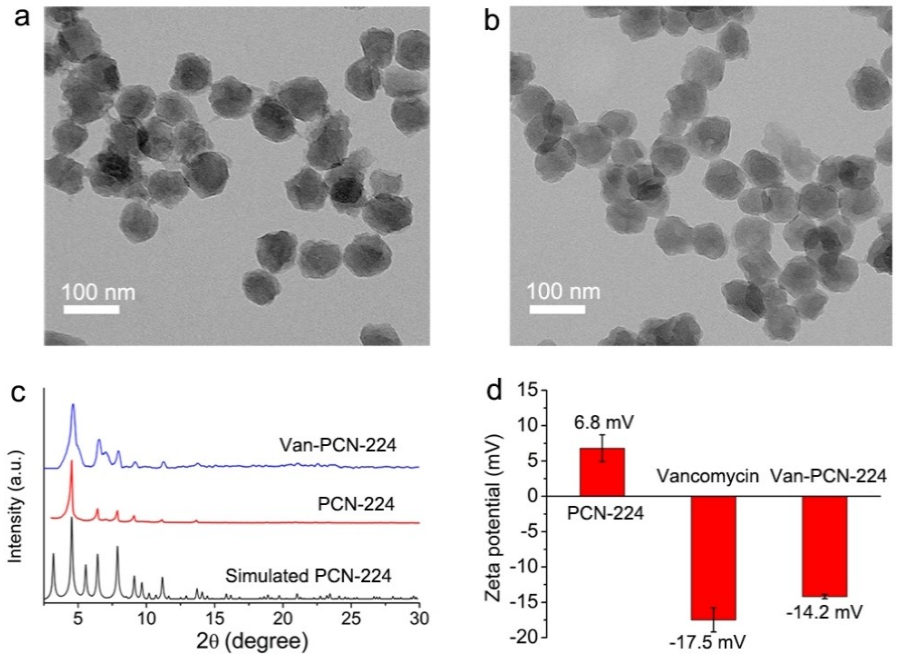



**Figure 1.** TEM images: (a) the prepared PCN-224; (b) the prepared Van-PCN-224. (c) XRD patterns of the prepared PCN-224 and Van-PCN-224. (d) Zeta potential of the prepared PCN-224, Van, and Van-PCN-224.

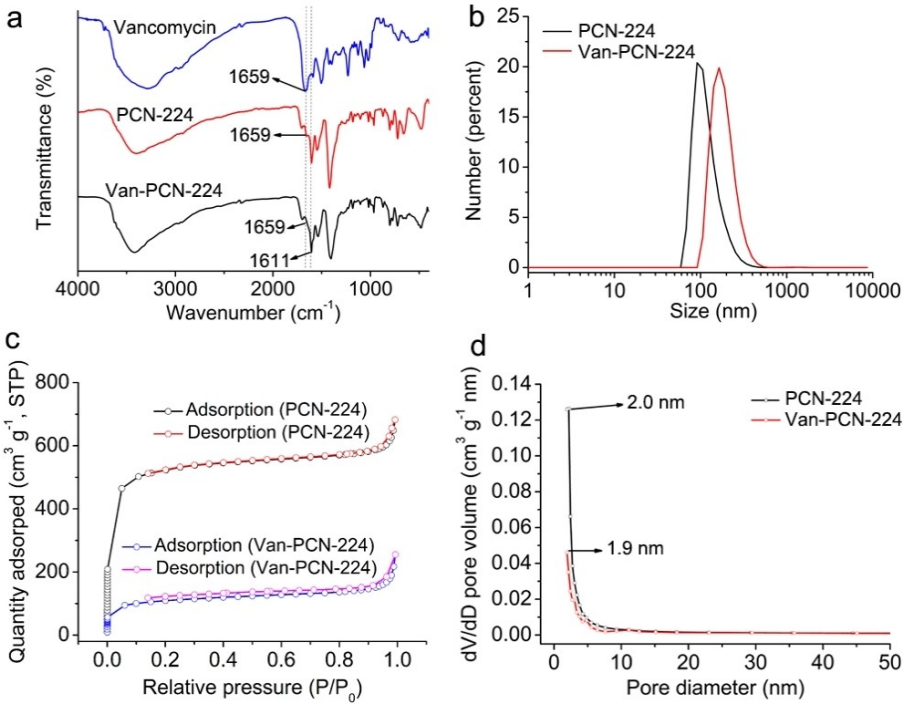



**Figure 2.** Characterization of the prepared PCN-224 and Van-PCN-224: (a) FT-IR spectra; (b) Hydrodynamic size distribution; (c) $N_2$ adsorption–desorption isotherms; (d) pore size distribution.

generate $^1O_2$ under light irradiation. Although the decrease of DPBF absorbance slightly slowed down in the presence of Van-PCN-224 (Figure 3c), the Van-PCN-224 could still be a good photosensitizer as the DPBF was halfly degraded in less than 8 min.

*S. aureus* was selected as representative bacterium to investigate the enhanced antibacterial activity of Van-PCN-224 against gram-positive bacteria. For comparison, bacteria incubating with unmodified PCN-224 was used as the control group. The bacterial viabilities of *S. aureus* in plate counting test were only 56.9%, 16.6% and 6.0% under dark conditions after

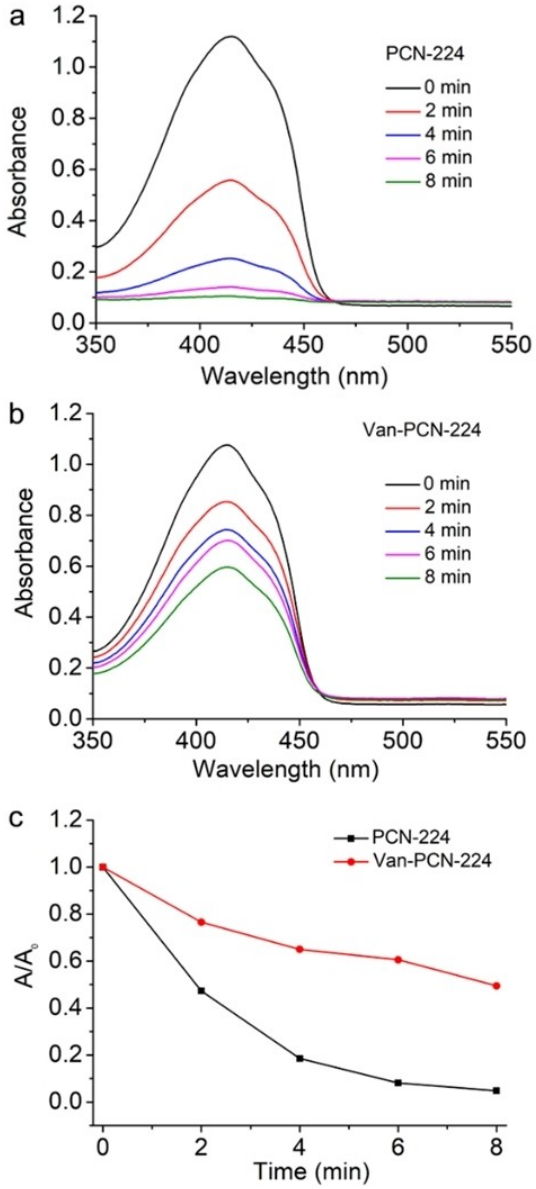



**Figure 3.** Time-dependent UV-vis absorption spectra of DPBF under white LED irradiation (4 mW/cm$^2$) in the presence of (a) PCN-224; (b) Van-PCN-224. (c) Time-dependent absorption change of DPBF in the presence of PCN-224 and Van-PCN-224 under white LED irradiation. $A_0$ and $A$ are the absorbance of DPBF at 359 nm before and after illumination, respectively.

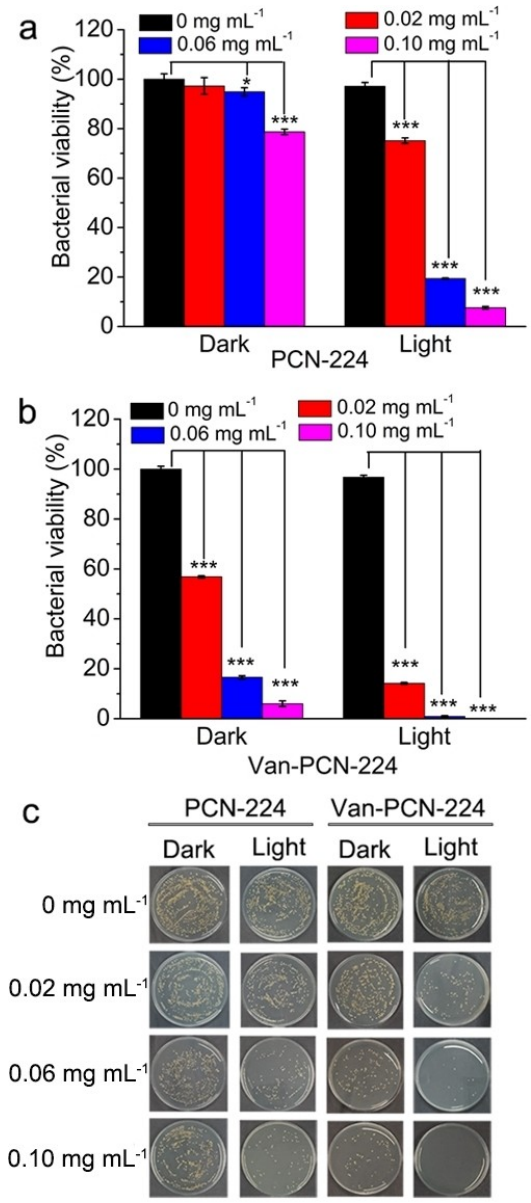



**Figure 4.** Bacterial viabilities of *S. aureus* incubated with different concentrations of PCN-224 (a) and Van-PCN-224 (b) under LED irradiation (4 mW/cm$^2$) for 20 min. *P* values obtained by Students' *t*-Test: * $<0.05$, *** $<0.001$. (c) The corresponding photographs of bacterial colonies of *S. aureus* on plates.

incubating with Van-PCN-224 of 0.02, 0.06 and 0.10 mg mL$^{-1}$, respectively. Because Van can endow Van-PCN-224 with targeting ability and native antimicrobial activity, the antibacterial ability mainly originated from the additional antimicrobial activity of Van in the dark (Figure 4b). LED irradiation for 20 min made the bacterial viabilities after incubating with Van-PCN-224 decreased to 14.2% and 0.9% even at the low concentrations of 0.02 mg mL$^{-1}$ and 0.06 mg mL$^{-1}$, respectively (Figure 4b) showing dramatically antibacterial ability of Van-PCN-224 against *S. aureus*. However, after incubating with PCN-224, the bacterial viabilities were all above 78.7% in the dark, and gradually decreased to 75.1%, 19.4% and 7.6% with different concentrations of 0.02, 0.06 and 0.10 mg mL$^{-1}$, respectively, under white LED irradiation for 20 min (Figure 4a). The decrease of bacterial viability derived from the photosensitive activity of PCN-224. The related photographs of *S. aureus* bacterial colonies on plates were also shown in Figure 4c. The above results indicate that Van-PCN-224 possess synergistic antibacterial ability against *S. aureus* which combined the targeted antimicrobial activity of Van and the photodynamic antibacterial ability of PCN-224. Thus, the Van-PCN-224 can be a good candidate agent for further antibacterial PDT.

In summary, we have reported vancomycin-functionalized PCN-224 as an effective antibacterial agent for targeted and enhanced killing of *S. aureus*, one of the Gram-positive bacteria.

The functionalization of Van endows the Van-PCN-224 with excellent antibacterial efficiency against *S. aureus*, which benefits from the targeting ability and antibacterial activity of Van for Gram-positive bacteria and the high photosensitive activity of PCN-224. The designed Van-PCN-224 is promising for further antimicrobial PDT application.

## Acknowledgements

*The authors are thankful for the financial support from National Natural Science Foundation of China (No. 21934002, 21804057, and 21804056), and Natural Science Foundation of Jiangsu Province, China (No. BK20180584 and BK20180581), the Fundamental Research Funds for the Central Universities (No. JUSRP121005) and Collaborative Innovation Center of Food Safety and Quality Control in Jiangsu Province.*

## Conflict of Interest

*The authors declare no conflict of interest.*

**Keywords:** Porphyrinic Metal-Organic Framework · Vancomycin functionalization · Antibacterial ability

[1] D. H. Williams, B. Bardsley, *Angew. Chem. Int. Ed.* **1999**, *38*, 1172–1193; *Angew. Chem.* **1999**, *111*, 1264–1286.
[2] a) H. F. L. Wertheim, D. C. Melles, M. C. Vos, W. van Leeuwen, A. van Belkum, H. A. Verbrugh, J. L. Nouwen, *Lancet Infect. Dis.* **2005**, *5*, 751–762; b) S. Yang, H. Ouyang, X. Su, H. Gao, W. Kong, M. Wang, Q. Shu, Z. Fu, *Biosens. Bioelectron.* **2016**, *78*, 174–180.
[3] L. G. Ning, P. Liu, B. Wang, C. M. Li, E. T. Kang, Z. S. Lu, X. F. Hu, L. Q. Xu, *J. Colloid Interface Sci.* **2019**, *549*, 72–79.
[4] Y. E. Hur, Y. Park, *J. Nanosci. Nanotechnol.* **2016**, *16*, 6393–6399.
[5] a) F. Cieplik, D. Deng, W. Crielaard, W. Buchalla, E. Hellwig, A. Al-Ahmad, T. Maisch, *Crit. Rev. Microbiol.* **2018**, *44*, 571–589; b) M. R. Hamblin, *Curr. Opin. Microbiol.* **2016**, *33*, 67–73.
[6] a) O. K. Farha, A. M. Shultz, A. A. Sarjeant, S. T. Nguyen, J. T. Hupp, *J. Am. Chem. Soc.* **2011**, *133*, 5652–5655; b) D. Feng, Z. Y. Gu, J. R. Li, H. L. Jiang, Z. Wei, H. C. Zhou, *Angew. Chem. Int. Ed.* **2012**, *51*, 10307–10310; *Angew. Chem.* **2012**, *124*, 10453–10456.
[7] a) K. Lu, C. He, W. Lin, *J. Am. Chem. Soc.* **2014**, *136*, 16712–16715; b) K. Lu, C. He, W. Lin, *J. Am. Chem. Soc.* **2015**, *137*, 7600–7603; c) J. Liu, Y. Yang, W. Zhu, X. Yi, Z. Dong, X. Xu, M. Chen, K. Yang, G. Lu, L. Jiang, Z. Liu, *Biomaterials* **2016**, *97*, 1–9.
[8] a) J. Park, Q. Jiang, D. Feng, L. Mao, H. C. Zhou, *J. Am. Chem. Soc.* **2016**, *138*, 3518–3525; b) L. He, M. Brasino, C. Mao, S. Cho, W. Park, A. P. Goodwin, J. N. Cha, *Small* **2017**, *13*; c) G. Lan, K. Ni, W. Lin, *Coord. Chem. Rev.* **2019**, *379*, 65–81.
[9] a) K. Wang, X. L. Lv, D. Feng, J. Li, S. Chen, J. Sun, L. Song, Y. Xie, J. R. Li, H. C. Zhou, *J. Am. Chem. Soc.* **2016**, *138*, 914–919; b) X. L. Lv, K. Wang, B. Wang, J. Su, X. Zou, Y. Xie, J. R. Li, H. C. Zhou, *J. Am. Chem. Soc.* **2017**, *139*, 211–217.
[10] Y. Zhang, P. Sun, L. Zhang, Z. Wang, F. Wang, K. Dong, Z. Liu, J. Ren, X. Qu, *Adv. Funct. Mater.* **2019**, *29*.
[11] M. Chen, Z. Long, R. Dong, L. Wang, J. Zhang, S. Li, X. Zhao, X. Hou, H. Shao, X. Jiang, *Small* **2020**, *16*, e1906240.
[12] D. Han, Y. Han, J. Li, X. Liu, K. W. K. Yeung, Y. Zheng, Z. Cui, X. Yang, Y. Liang, Z. Li, S. Zhu, X. Yuan, X. Feng, C. Yang, S. Wu, *Appl. Catal. B* **2020**, *261*, 118248.
[13] a) X. Su, M. Wang, H. Ouyang, S. Yang, W. Wang, Y. He, Z. Fu, *Sens. Actuators B* **2017**, *241*, 255–261; b) S. Singh, A. Moudgil, N. Mishra, S. Das, P. Mishra, *Biosens. Bioelectron.* **2019**, *136*, 23–30.

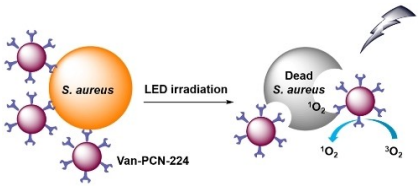
Manuscript received: May 20, 2021
Revised manuscript received: June 3, 2021
Accepted manuscript online: June 6, 2021
Version of record online: ■■, ■■■

ACES Asian Chemical Editorial Society

CHEMISTRY
AN ASIAN JOURNAL
Communication

# COMMUNICATION

**Vancomycin-functionalized porphyrinic metal-organic framework PCN-224** displays the integrated merits of the bacteria-targeting ability of vancomycin and the photodynamic effect of PCN-224 for enhanced targeted sterilization.




*Dr. L.-J. Chen, Y.-Y. Liu, Dr. X. Zhao, Prof. X.-P. Yan**