This report features the work of Ho-Hsiu Chou and his co-workers published in Chem. Eng. J. ***438****, 135592 (2022).*

TPS 44A Quick-scanning X-ray Absorption Spectroscopy
TLS 23A1 Small/Wide Angle X-ray Scattering

- SAXS, XANES/EXAFS
- Soft Matter, Materials Science, Chemistry

Reference

1. T.-Y. Han, C.-H. Lin, Y.-S. Lin, C.-M. Yeh, Y.-A. Chen, H.-Y. Li, Y.-T. Xiao, J.-W. Chang, A.-C. Su, U.-S. Jeng, H.-H. Chou, Chem. Eng. J. **438**, 135592 (2022).

Polytherapy to Combat Antimicrobial-Resistant Bacteria

Polytherapy of antibiotic and lipid nanoparticles could be an alternative to fight against superbugs.

Gram-negative bacteria have caused significant diseases and have increasingly become one of the leading causes of high morbidity and mortality globally, which has led to the extensive development of antibiotics that are effectively against such bacteria. However, bacteria evolve new features and develop antibiotic resistance faster than the development of new antibiotics. Fortunately, nanosized particles can lower the treatment dose while maintaining high antimicrobial activities. This raises the hope that nanoparticles could reduce the probability of promoting resistant bacteria compared with conventional antibiotics. Accordingly, lipid nanoparticles, due to their high biocompatibility and ability to solubilize hydrophobic and hydrophilic molecules, have received considerable attention in the field of biological applications as drug carriers. These particles without exception simply function as nano vehicles that deliver the loaded cargo to the bacteria. Comparative studies have evidenced the superiority of polytherapy regimens of polymyxins with various drugs approved by the U.S. Food and Drug Administration over their corresponding monotherapies, either by producing an improved combination effect or, conversely, by achieving the same killing effect using lower doses of medicines, while diminishing or delaying the development of resistance. Therefore, superbugs can be effectively combatted *via* the polytherapy of antibiotics with lipid nanoparticles.

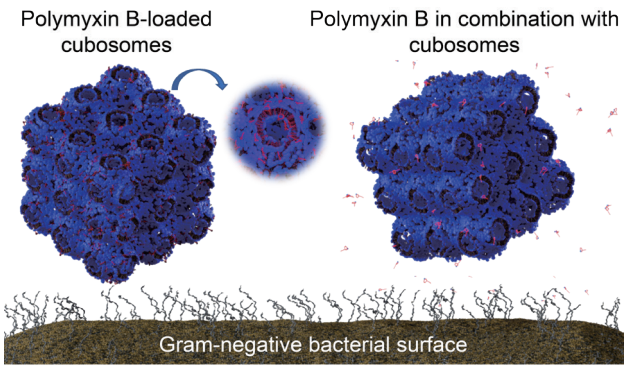

Fig. 1: Schematic diagram of polymyxin B-loaded cubosomes and interaction of the polytherapy treatment with the gram-negative bacterial outer membrane. [Reproduced from Ref. 1]

Hsin-Hui Shen's group (Monash University, Australia) recently conducted a study to investigate why the polytherapy of polymyxin B (PMB) with cubosomes exhibited better antibacterial activity than the traditional PMB-loaded cubosomes (**Fig. 1**).[1] Neutron reflectometry (NR) data was measured by the **PLATYPUS** time-of-flight neutron reflectometer at Australia's Nuclear Science and Technology Organisation (ANSTO). NR was used to investigate the interaction between the components of the polytherapy treatment and a model outer membrane (OM) of gram-negative bacteria (**Figs. 2(a) and 2(b)**). Shen's team found that the PMB-loaded cubosomes could simply attach to the bilayer surface without further penetrating (**Figs. 2(c) and 2(d)**), most likely due to the diffused cubosome layer preventing the released PMB from binding to the membrane. Therefore, the bacterial membrane remained relatively intact. Additionally, two distinct modes of action were found for the antibacterial activity of the polytherapy (**Figs. 2(e) and 2(f)**). Firstly, the interaction between the positively charged PMB and negatively charged phosphate groups on lipid A was driven by electrostatic force, which led to membrane stability. Secondly, the partially permeabilized membrane facilitated the entry of the cubosomes into the bilayer, which were

then expected to interact with the bilayer lipids *via* a lipid-exchange mechanism to further solvate the membrane bilayer.

In summary, the polytherapy of PMB with cubosomes achieved better antibacterial activity against a series of gram-negative bacteria than the traditional approach with PMB-loaded cubosomes. This novel approach paves the way for the future design of nanoparticle-based combination therapies. The use of state-of-the-art NR to non-destructively probe a model bacterial membrane offers a high-spatial-resolution technique for the study of antimicrobial–bacterium interaction. (Reported by Xiangfeng Lai, Monash University, Australia)

This report features the work of Jian Li, Hsin-Hui Shen and their collaborators, published in Nat. Commun. ***13****, 1 (2022).*

ANSTO PLATYPUS – Neutron Reflectometer

- NR
- Soft Matter, Biological Science, Polymer Chemistry, Interface and Thin-film Chemistry

Reference

1. X. Lai, M.-L. Han, Y. Ding, S. H. Chow, A. P. Le Brun, C.-M. Wu, P. J. Bergen, J.-h. Jiang, H.-Y. Hsu, B. W. Muir, Nat. Commun. **13**, 1 (2022).

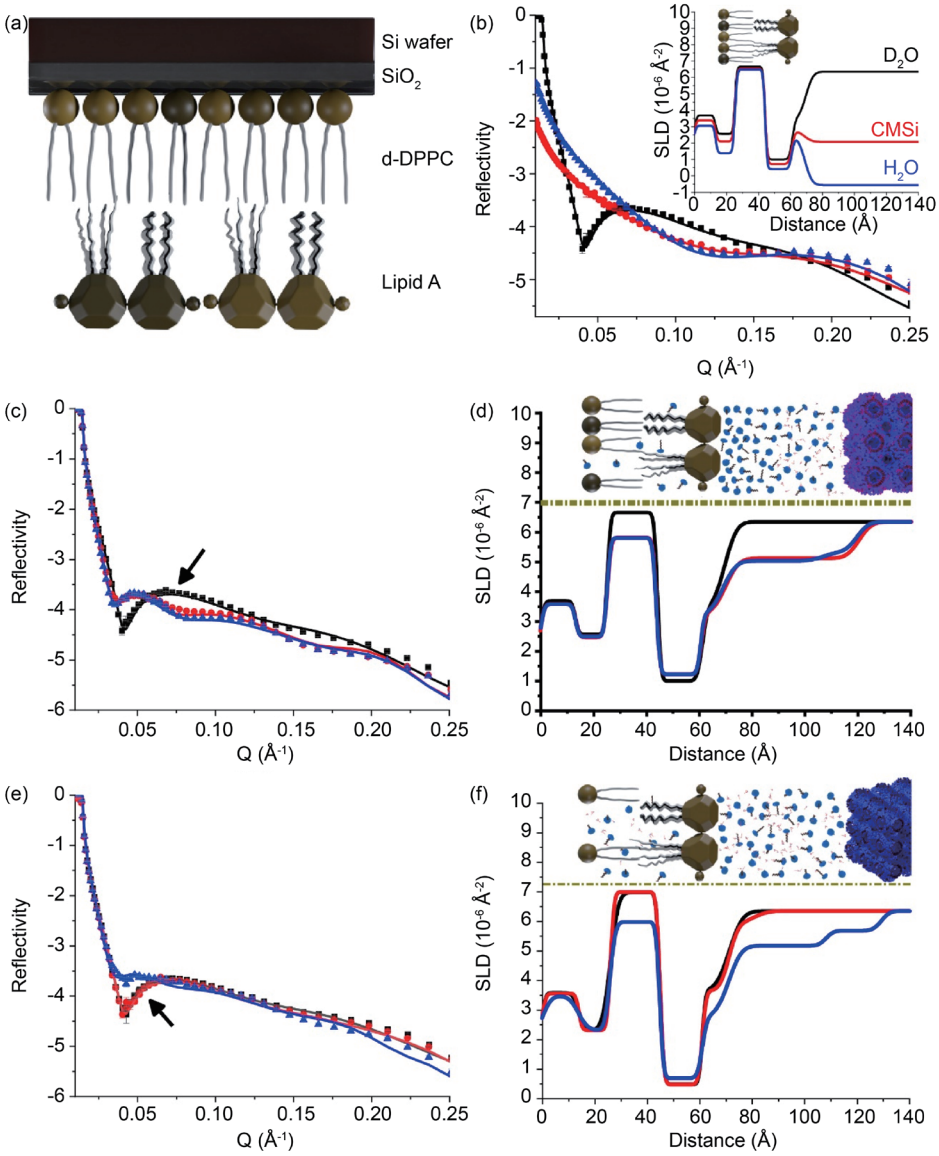

Fig. 2: Neutron reflectometry experiment. (a) Schematic representation of the model outer membrane bilayer. Lipid A (outer leaflet): the 1,2-dipalmitoyl-d_{62}-sn-glycero-3-phosphocholine (d-DPPC, inner leaflet) membrane bilayer is absorbed on the SiO_2 surface. During the experiments, all space inside the cell is always filled with aqueous solution, *i.e.*, D_2O, CMSi (contrast matched silicon), and H_2O buffer. (b) The NR profiles (symbols) and fits (lines) of the bilayer obtained from D_2O (black squares and line), CMSi (red circles and line), and H_2O (blue triangles and line). The insert shows the corresponding scattering length density (SLD) profiles of the bilayer and the diagram depicts the bilayer structure and distance relative to the SiO_2 surface. (c) Experimental (symbols) and fitted (curves) NR profiles of the bilayer (black squares and line), treated with 32 µg/mL cubosomes (red circles and line) followed by 4 µg/mL PMB (blue triangles and line) in D_2O; the black arrow indicates the fringe changing from the black to red curve after incubation with the cubosomes. (d) The corresponding SLD profiles of (c). The insert depicts the membrane bilayer after successive treatment with the cubosomes and PMB. (e) Experimental (symbols) and fitted (curves) NR profiles of the bilayer (black squares and line) treated with 4 µg/mL PMB (red circles and line) followed by 32 µg/mL cubosomes (blue triangles and line) in D_2O; the black arrow indicates that the fringe from the black to red curve remained the same after incubation with PMB. (f) The corresponding SLD profiles of (e). The insert depicts the bilayer after successive treatment with PMB and cubosomes. [Reproduced from Ref. 1]