Seeing Nanoparticles Combat Superbugs Through Neutrons

SANS is a powerful neutron technology to serve a new strategy to combat LPS-deficient Gram-negative pathogens with nano-technology which provides hints of innovative bacterial membrane-based therapies for treatment.

Antimicrobial resistance is becoming a severe threat to human health. Nanoparticles have increasingly emerged as new tools to combat fatal bacterial infections caused by resistant bacteria. Neutron scattering has been utilized to probe the changes in bacterial membrane structure upon nanoparticle actions, whereby the bactericidal process was seen, and the mechanism was deduced.

Hsin-Hui Shen's group in Monash University, Australia, found that lytropic liquid-crystalline lipid nanoparticles, *i.e.* cubosomes, were highly bactericidal against lipopolysaccharide (LPS)-deficient, *Acinetobacter baumannii* (*Ab*) strains.[1] The antibiotic-resistant strains of *Ab* strains are being reported increasingly in clinical settings. To unveil the killing mechanism, biomimetic membrane bilayers were reconstituted to replicate the composition of LPS-deficient strains, whereby neutrons can be employed to investigate the structure changes on bilayers upon cubosome treatment.

In their experiment, synthetic 1-palmitoyl-2-oleoyl-snglycero-3-phosphoethanolamine (16:0–18:1 POPE), 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-(1'-rac-glycerol) (sodium salt) (16:0–18:1 POPG) and 1',3'-bis-[1,2-dioleoyl-sn-glycero

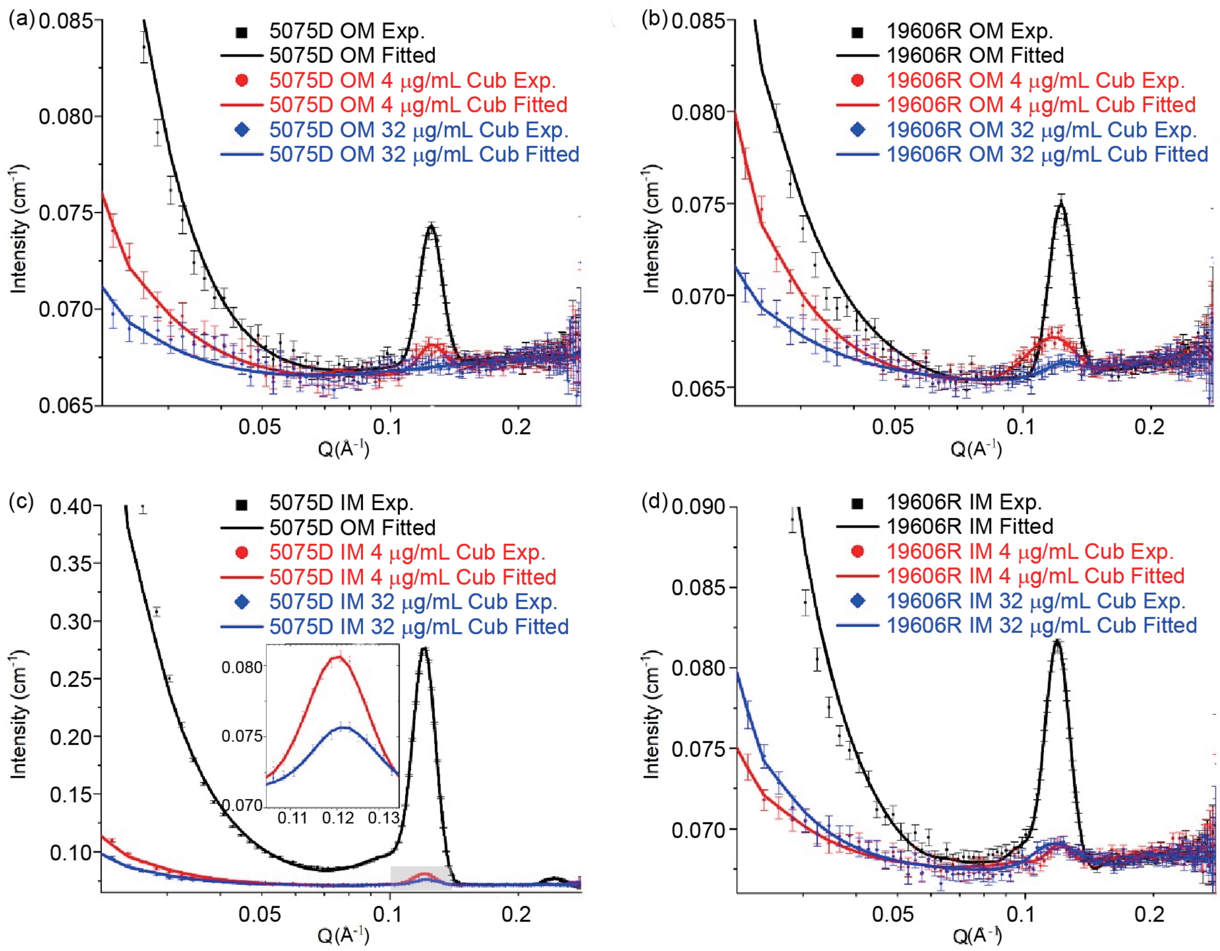

Fig. 1: SANS profiles (symbols) and best-fitted curves (lines) using a lamellar model for a biomimetic membrane before and after cubosome treatment in D_2O at 25 °C. The Gaussian signals indicate the lipid bilayer. (a) *Ab* 5075D OM; (b) *Ab* 19606R OM; (c) *Ab* 5075D IM, the inset magnifies the data around the scattering peak to unify the intensity scale; (d) *Ab* 19606R IM. [Reproduced from Ref. 1]

-3-phospho]-glycerol (sodium salt) (18:1 Cardiolipin, TOCL) were mixed thoroughly in trichloromethane at molar ratios 37:47:16, 64:26:10, 72:25:3 and 81:17:3 to represent an *Ab* 5075D outer membrane (OM), *Ab* 19606R OM, *Ab* 5075D inner membrane (IM) and *Ab* 19606R IM, respectively. The mixtures were then dried under N_2 and dispersed in D_2O with a HEPES buffer using bath sonication. The membrane dispersions were then transferred to quartz cuvettes and characterized at 25 °C to confirm the fabrication of the bilayer. Cubosomes at concentrations 4 and 32 µg/mL were consecutively added to the cuvettes and incubated for 30 min before being subjected to shows small-angle neutron scattering (SANS) measurements. The measurements were conducted on 40-m pinhole instrument **QUOKKA** at OPAL research reactor in Australian Nuclear Science and Technology Organisation (ANSTO), Sydney, Australia. Wave vector Q is calculated from θ (scattering angle) and λ (neutron wavelength): $Q = 4\pi \sin\theta/\lambda$. Two instrument configurations were applied to cover large Q and small Q. Distance L1 = 4 m from source to sample and distance L2 = 3 m at 5 Å and L1 = L2 = 12 m at 6 Å from sample to detector were used to give a Q range 0.0049–0.28 $Å^{-1}$.

Figure 1 shows SANS profiles of membrane bilayers before and after cubosome treatment. After the cubosome treatment, both OM and IM of *Ab* 5075D and *Ab* 19606R were evidently significantly disrupted, as indicated by the loss of the bilayer intensity. An analysis of SANS profiles was performed using SasView (version 4.2.2). The data were fitted using lamellar_hg and a Gaussian peak model. The calculated bilayer thicknesses are reported in **Table 1**. Notably, the thicknesses of OM and IM of *Ab* 5075D remained unchanged, while being altered for *Ab* 19606R after cubosome treatment. Taken together, these results indicate that cubosomes are bactericidal to *A. baumannii* on altering the membrane bilayer structure, whilst *Ab* 19606R is more susceptible to cubosome treatment, which is in accordance with results *in vitro* in which the minimum inhibitory concentration of cubosomes against *Ab* 19606R is 2 µg/mL compared with 4 µg/mL for *Ab* 5075D. (Reported by Xiangfeng Lai, Monash University, Australia)

Table 1: Bilayer thicknesses (δ) before and after cubosome treatment with a lamellar model for the OM and IM of *Ab* 19606R and *Ab* 5075D. [Reproduced from Ref. 1]

Membrane	$\delta_{bilayer}$ (Å)	$\delta_{Cub\ 4}$ (Å)	$\delta_{Cub\ 32}$ (Å)
Ab 5075D OM	47.2 ± 1.1	47.5 ± 1.2	-
Ab 19606R OM	47.6 ± 0.5	49.2 ± 0.4	-
Ab 5075D IM	51.7 ± 1.5	51.5 ± 0.9	50.9 ± 2.2
Ab 19606R IM	48.3 ± 0.4	45.0 ± 0.7	51.9 ± 2.5

*This report features the work of Mei-Ling Han, Jian Li, Hsin-Hui Shen and their collaborators, published in ACS Appl. Mater. Interfaces **12**, 44485 (2020).*

ANSTO Quokka – Small-angle Neutron Scattering

- SANS
- Antibiotic Resistance, Drag Delivery System, Polymers, Magnetism, Earth Science

Reference

1. X. Lai, Y. Ding, C. M. Wu, X. Chen, J. H. Jiang, H. Y. Hsu, Y. Wang, A. P. Le Brun, J. Song, M. L. Han, J. Li, H. H. Shen, ACS Appl. Mater. Interfaces **12**, 44485 (2020).

Neutron Experiments Exploring Advanced Metallurgy

AM and HEA are two emerging topics to advance metallurgy. Newly developed additive-manufacturing implants and a summary of how neutron experiments reveal the HEA deformation behaviours are reported.

A collaborative team, distinguished by an Innovation in Taiwan Award of the Institute for Biotechnology and Medicine Industry and Research Center for Biotechnology and Medicine Policy, has reported an investigation of the bone growth in additive-manufactured implants using Ti_6Al_4V and bioactive glass- powder composite.[1] E-Wen Huang (National Chiao Tung University & Industrial Technology Research Institute) led the multi-scale characterization team to investigate the underlying mechano-biological processes at varied scales due to the hierarchical nature of the bones.[2] With customized geometry made by one-step mixing of the bioactive ceramic in the metallic powders, additive-manufacturing implants demonstrate outstanding performance in bone growth. Shao-Ju Shih (National Taiwan University of Science and Technology University) designed the one-step ceramic-metal powder mixing; Nien-Ti Tsou (National Chiao Tung University) invented the healing pattern analysis for implant geometry, and Meng-Huang Wu (Taipei Medical University) performed the surgery.