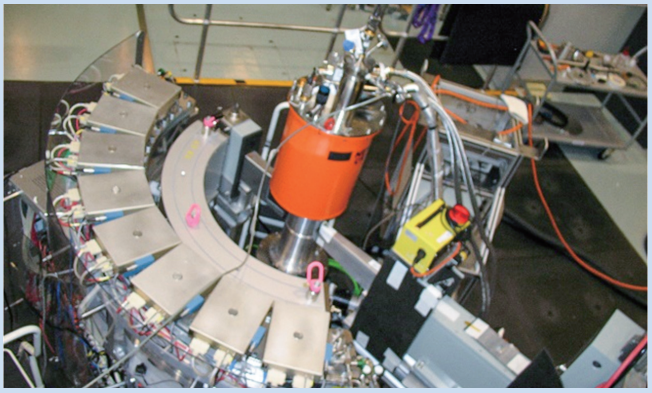
*This report features the work of J. G. A. Ramon and his collaborators published in Phys. Rev. B **99**, 214442 (2019).*

ANSTO WOMBAT—High-intensity Powder Diffractometer

- Powder diffraction, Single Crystal Diffraction
- Materials Science, Magnetism, Condensed-matter Physics

References

1. J. S. Gardner, M. J. P. Gingras, J. E. Greedan, Rev. Mod. Phys. **82**, 53 (2010).
2. C. R. Stanek, C. Jiang, B. P. Uberuage, K. E. Sickafus, Phys. Rev. B **80**, 174101 (2009).
3. J. G. A. Ramon, C. W. Wang, L. Ishida, P. L. Bernardo, M. M. Leite, F. M. Vichi, J. S. Gardner, R. S. Freitas, Phys. Rev. B **99**, 214442 (2019).
4. A. J. Studer, M. E. Hagen, T. J. Noakes, Physica B **385**, 1013 (2006).



Wombat high-intensity powder diffractometer.

How a Superbug Escape Antibiotic Attack?

The rise in antibiotic resistance represents one of the greatest threats to human health, with international organizations and governments calling for urgent action to tackle the crisis. Neutron diffraction has served in an investigation of how bacteria changes the membrane phospholipid composition to compromise antibiotic actions, which provides hints of innovative bacterial membrane-based therapies for treatment.

Dominating the list of 'red-alert' antibiotic resistant bacteria is methicillin-resistant *Staphylococcus aureus* (MRSA), which is well known as Golden staph. As one of the most notorious human pathogens, *S. aureus* has uncanny ability to adapt to antibiotic and host immune selection pressure, promoting bacterial survival, persistence and therapeutic failure. Treatment of severe MRSA infections increasingly relies on last-line antibiotics, including daptomycin (DAP). DAP targets bacterial cell membrane to execute its bactericidal effects and the emergence of resistance to daptomycin (DAP-R) in *S. aureus* is of serious concern. However, how *S. aureus* develop resistance is not entirely clear.

Anton Peleg's group at Monash University, Australia, discovered that clinical MRSA isolate was able to change its membrane phospholipid composition *via* single point mutation in the gene responsible for cardiolipin biosynthesis.[1] This compositional change of the membrane phospholipids led to daptomycin resistance. To further investigate the mechanism of resistance, membrane bilayers mimicking DAP-R MRSA membrane were reconstituted and Chun-Ming Wu (NSRRC) utilized the advantages of neutrons as a probe to analyze the structural changes on membrane bilayers caused by daptomycin.

Figure 1 shows the profiles of small-angle neutron scattering (SANS) of membrane bilayers. Synthetic phosphatidylglycerol, cardiolipin and lysyl-phosphatidylglycerol were dissolved in chloroform and mixed at the molar ratios of 69:12:19 and 23:60:17 for DAP-sensitive and DAP-R membranes respectively. The lipid mixtures were dried under nitrogen and resuspended in HEPES buffer (5mM $CaCl_2$, 150mM NaCl, 10mM HEPES, pH 7.4) with D_2O using a water bath sonicator. Daptomycin was added to the membrane suspension and the mixtures were transferred to Hellma cuvettes (Hellma Analytics, Germany). The samples were measured using the SANS instrument, **QUOKKA**, at ANSTO over a Q range of ~0.02–0.21 $Å^{-1}$ where $Q = 4\pi/\lambda \cdot sin\theta$, with λ = 5 Å, $\Delta\lambda/\lambda$ = 10% resolution and the scattering angle 2θ, providing a length scale of 3–31.4 nm. The distances of source-to-sample (L1) and sample-to-detector (L2) were 4 meters, with source and sample apertures of 50 mm and 5 mm in diameter respectively. The data were reduced using SANS reduction macros developed at

the National Institute of Standards and Technology Center for Neutron Research, USA within the Igor software package modified for the **QUOKKA** instrument, and transformed to absolute scale by the use of an attenuated direct beam transmission measurement.[2,3] At a clinically relevant daptomycin concentration of 4 µg/ml, daptomycin penetration and aggregation straddling the bilayer membrane for DAP-sensitive membrane was observed (**Fig. 1(a)**). However, DAP-R membrane resisted daptomycin actions on membrane (**Fig. 1(b)**).

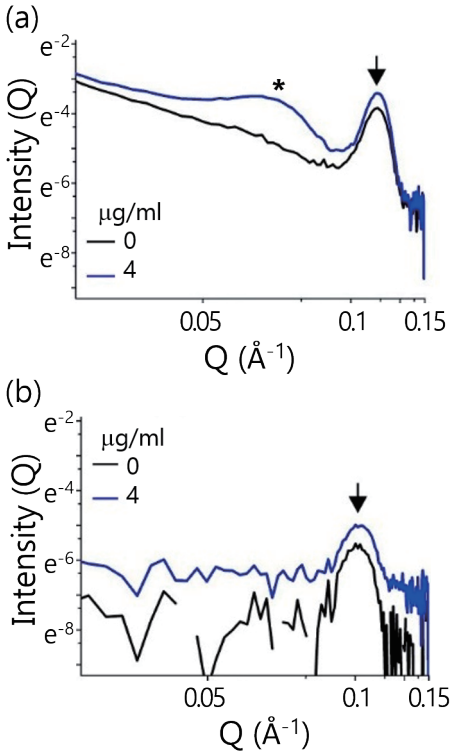


Fig. 1: Small-angle neutron scattering profiles measured for (a) DAP-sensitive and (b) DAP-resistant membranes treated with and without daptomycin (4 µg/mL). The arrow indicates the lipid bilayer, whereas the star indicates the Bragg peak as the sign of micelle formation. [Reproduced from Ref. 1]

Figure 2 shows the deduction of daptomycin-bound membrane structure. The data obtained from HEPES buffer in D_2O alone was used for background subtraction using the Igor pro package.[2] The spectra were analyzed using in-built algorithms within the SASview package 4.1 (http://www.sasview.org/). The standard core-shell and hollow-cylinder models were utilized for the fitting of the daptomycin micelles to calculate the radius of the core (Rc) and the thickness of the micelle (Rm).[4] It was estimated that DAP contains a 28 ± 0.86 Å radius spherical micelle structure within the DAP-sensitive membrane bilayer. This micelle structure was not observed in DAP-R membrane. Taken together, these results show that MRSA adapts during treatment with daptomycin in human infections by changing its membrane phospholipid content, which leads to a membrane that resists daptomycin membrane penetration and disruption. (Reported by Hsin-Hui Shen, Monash University)

This report features the work of Anton Peleg and his collaborators, published in Proc. Natl. Acad. Sci. USA ***116****, 3722 (2019).*

ANSTO QUOKKA—Small-angle Neutron Scattering

- Small-angle Neutron Scattering
- Antibiotic Resistance, DAP-R Membrane, Daptomycin, MRSA, Mutation

References

1. J.-H. Jiang, M. S. Bhuiyan, H.-H. Shen, D. R. Cameron, T. W. T. Rupasinghe, C.-M. Wu, A. P. Le Brun, X. Kostoulias, C. Domene, A. J. Fulcher, M. J. McConville, B. P. Howden, G. J. Lieschke, A. Y. Pelega, Proc. Natl. Acad. Sci. USA **116**, 3722 (2019).
2. S. R. Kline, J. Appl. Cryst. **39**, 895 (2006).
3. J. Ilavsky, P. R. Jemian, J. Appl. Cryst. **42**, 347 (2009).
4. A. Guinier, G. Fournet, Small-angle Scattering of X-rays. John Wiley & Sons, Inc., USA (1955).

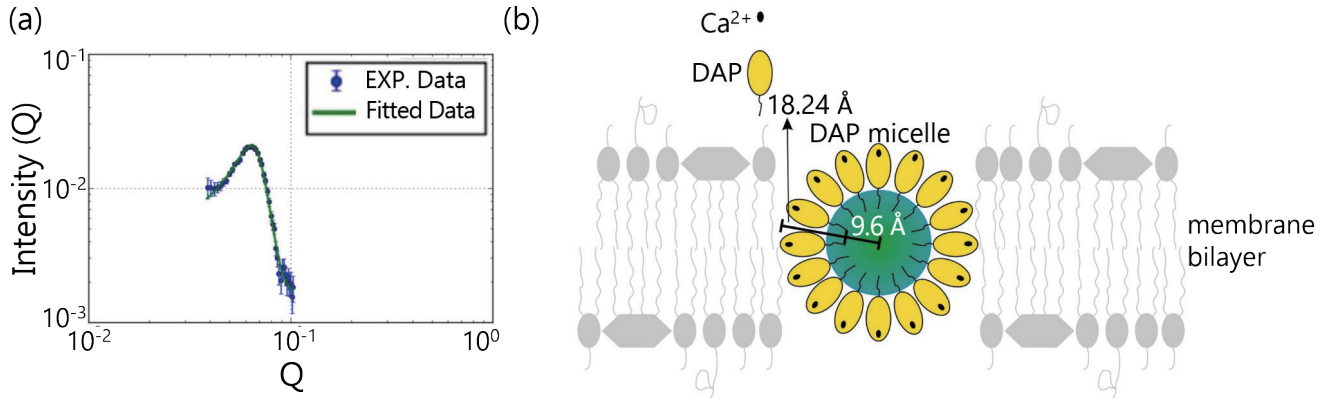


Fig. 2: Daptomycin micelle formation in the DAP-sensitive membrane as determined by small-angle neutron scattering. (a) The Bragg peak corresponding to the daptomycin micelle (as seen in **Fig. 1(a)**) was extracted for the calculation of the daptomycin micelle size and subunits using core-shell modelling. (b) Schematic illustration of the daptomycin micelle formed within the membrane. [Reproduced from Ref. 1]