A STUDY OF THE EFFECT OF MEMBRANE TARGETING ANTIMICROBIAL COMPOUNDS ON IONIC TRANSPORT ACROSS LIPID BILAYER MEMBRANES

A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

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CERTIFICATE OF ORIGINAL AUTHORSHIP

I, Thomas Berry declare that this thesis is submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the School of Life Sciences of the Faculty of Science at the University of Technology Sydney.

This thesis is wholly my own work unless otherwise reference or acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis. This document has not been submitted for qualifications at any other academic institution. This research is supported by an Australian Government Research Training Program.

Signature: Production Note: Signature removed prior to publication.

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Publications arising from this thesis

Berry, T., Dutta, D., Chen, R., Leong, A., Wang, H., Donald, W., Parviz, M., Cornell, B., Willcox, M., Kumar, N., Cranfield, C. G., *Lipid Membrane Interactions of the Cationic Antimicrobial Peptide Chimeras Melimine and Cys-Melimine.* Langmuir, 2018. 34(38): p. 11586-11592.

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Other publications

Nizalapur, S., Kimyon, O., Yee, E., Ho, K., **Berry, T.**, Manefield, M., Cranfield, C. G., Willcox, M., Black, D., Kumar, N., Amphipathic guanidine-embedded glyoxamide-based peptidomimetics as novel antibacterial agents and biofilm disruptors. Organic & Biomolecular Chemistry. 2017. 15(9): p. 2033-2051.

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Contents

List of Abbreviations

α-helices	alpha helices
β-sheet	beta sheets
AC	alternating current
AMP's	antimicrobial peptides
ANOVA	analysis of variance
ANSTO	Australian nuclear science and technology organisation
BLMs	black lipid membranes
CholPEG	cholesterol-pentaethyleneglycol
C_m	capacitance
CPP	critical packing parameter
C_s	counter electrode capacitor
C_{th}	tethering electrode capacitor
DLP	double-length reservoir half-membrane spanning diphytanyl ethylene glycol tethers
DLS	dynamic light scattering
DMPC	1,2-dimyristoyl-sn-glycero-3-phosphocholine
DOPC	1,2-dioleoyl-sn-glycero-3-phosphocholine
DPEPC	diphytanyldietherphosphatidylcholine
DPTL	2,3-di-O-phytanyl-sn-glycerol-1-tetraethylene glycol-DL-a-lipoic acid ester lipid

DSC	differential scanning calorimetry
EIS	electrochemical impedance spectroscopy
FminP	frequency of the minimum phase
GDPE	glyceroldiphytanylether
G_m	conductance
HPLC	high-performance liquid chromatography
KCl	potassium chloride
LPS	lipopolysaccharide
Lyso PC	1-oleoyl-2-hydroxy-sn-glycero-3-phosphocholine
MBC	minimum bactericidal concentration
MIC	minimum inhibitory concentration
NaCl	sodium chloride
NMR	nuclear magnetic resonance
OPEP	optimized potential for efficient structure prediction
PBS	phosphate-buffered saline
PC	phosphatidylcholine
PEG	poly(ethyleneglycol)
PminP	phase of the minimum phase
POPC	1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine
POPG	1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphoglycerol

Q	scattering vector
QCM-D	quartz crystal microbalance with dissipation
SA	structural alphabet
SEM	standard error of the mean
SLB	supported lipid bilayer
SLD	scattering length density
spacers	benzyl-disulfide-tetra-ethyleneglycol-OH
SPR	surface plasmon resonance
SUVs	small unilamellar vesicles
tBLMs	tethered bilayer lipid membranes
tethers	(tetra-ethyleneglycol)n=2 C20-phytanyl

Amino acids

Alanine (A)	Leucine (L)
Arginine (R)	Lysine (K)
Asparagine (N)	Proline (P)
Cysteine (C)	Serine (S)
Glutamine (Q)	Threonine (T)
Glycine (G)	Tryptophan (W)
Isoleucine (I)	Valine (V)

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Abstract

The novel synthetic cationic peptide *melimine* is a chimera of two natural peptides, *melittin* and *protamine*. This peptide has a broad spectrum of antibacterial activity against both gram-positive and gram-negative bacteria while having no toxicity in mammalian cells. Melimine was initially synthesised as a coating for contact lenses as a way of reducing keratitis, inflammation of the cornea. However, its antibacterial effects also have further potential use as an antimicrobial coating for other biomaterial surfaces.

This peptide has been studied along with four peptide derivatives to determine the effects that peptide hydrophobicity, charge and size have on the peptides' antibacterial properties through studying their peptide-membrane interactions. Melimine has previously been shown to reduce the integrity of membranes, observed through the leakage of dye from bacterial membranes. The membrane-peptide interactions were compared using advanced lipid membrane biophysical techniques, including in-silico structural modelling, fluorescent membrane dipole measures, differential scanning calorimetry, neutron reflectometry, dynamic light scattering and electrical impedance spectroscopy and Arrhenius measures of their interactions with tethered bilayer lipid membranes (tBLMs).

Through analysing the results obtained from these biophysical techniques, the peptide-membrane interactions of melimine and its derivatives were compared against known modes lipid membrane interactions of antimicrobial compounds. These interactions included the barrel-stave model, carpet model, interdigitated toroidal pore model, critical packing parameter model and other surfactant-like properties. The five peptides showed minimal peptide-membrane interactions and an inability to span a lipid bilayer, leading to a postulation that these five peptides do not conform to having one of these aforementioned modes of action in killing bacteria.

The limited peptide-membrane interactions of the five cationic peptides contrast with their known antibacterial activity against bacteria. This suggests that melimine and its derivatives may interact with other components of bacterial cell membranes; this could include extracellular components, such as porin channels. This research highlights that the assumption that cationic peptides adhere to established membrane disruption models for their antimicrobial

activity requires reconsideration. This study emphasises the need for an alternative model of the antibacterial effects of cationic peptides such as melimine and its derivatives.