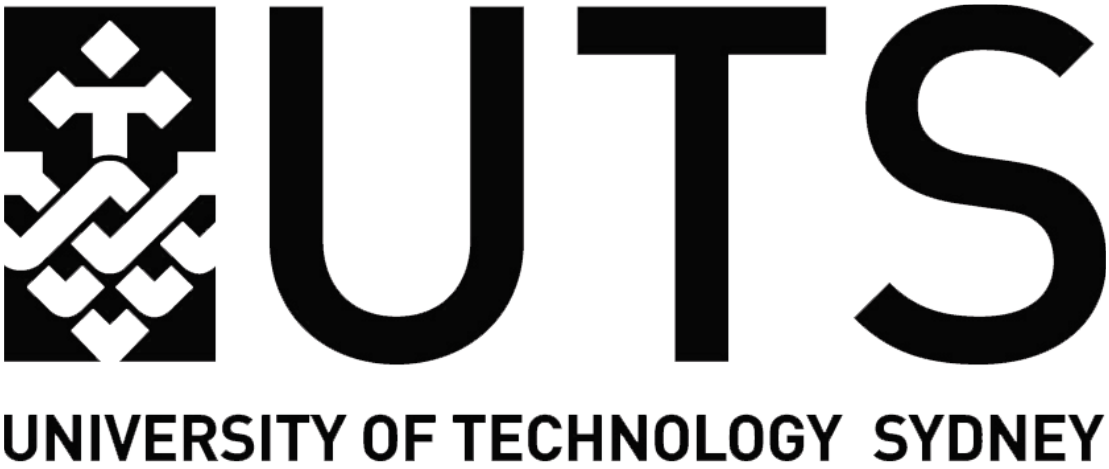

BACTERIAL CELL DIVISION; A NOVEL TARGET FOR NEW ANTIBACTERIALS

Kennardy Dewanto Kusuma

January 2019

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

Certificate of originality

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

I also verify that the research conducted for this thesis is supported by the Australian Government Research Training Program.

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Kennardy D. Kusuma

August 2018

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Table of Contents

List of Figures and Tables

Abbreviations

°C	Degrees Celcius
µ	Micro (10^{-6})
^{15}N	Nitrogen 15
Å	Angstrom
ADP	Adenosine 5'-diphosphate
Amp^r	Ampicillin resistance
APS	Ammonium per sulfate
ATP	Adenosine 5'-triphosphate
bp	Base pair(s)
cm	centimeter
Cα	alpha carbon
Da	Dalton(s)
DNA	Deoxyribose nucleic acid
dNTPs	deoxyribonucleotide triphosphate
DTT	Dithiothreitol
FD	Faraday constant
fts	Filamentous temperature sensitive
g	Gram(s)
GDP	Guanosine 5'-diphosphate
GTP	Guanosine 5'-triphosphate
h	Hour(s)
HEPES	(4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid)
IPTG	Isopropyl-1-thio-β-D-galactopyranoside
K	Kelvin

Kan^r	Kanamycin resistance
kb	Kilobase(s)
K_d	Dissociation constant
kDa	Kilo (10^3)
L	Liter
LB both	Luria-Bertani broth
m	Milli (10^{-3})
M	Moles per Liter (Molar)
min	Minute(s)
MTS	Membrane targeting sequence
MWCO	Molecular weight cut off
n	Nano (10^{-9})
NBD	nucleotide-binding domain
$OD_{(x)}$	Optical density measured at x nanometer wavelength
p	Probability
PCR	Polymerase chain reaction
PEG	Poly-ethylene glycol
PMSF	Phenylmethylsulfonyl fluoride
ps	Picosecond
R	resolution
RMSD	root mean square deviation
RNA	Ribonucleic acid
rpm	Revolution per minute
rRNA	Ribosomal ribonucleic acid
SDS	Sodium dodecyl sulphate

SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
sec	Second(s)
STD-NMR	Saturated transfer difference nuclear magnetic resonance
TEMED	Tetramethylethylenediamine
Tet^r	Tetracycline resistance
Tris	Tris(hydroxymethyl)methylamine
TROSY-HSQC	Transverse relaxation optimized spectroscopy-heteronuclear single quantum coherence
U	Units (enzyme activity)
V	Volts
v/v	Volume per volume
w/v	Weight per volume
Xg	Times gravity
Ω	Ohms

Publications

Journal articles

Bottomley AL, Liew ATF, **Kusuma KD**, Peterson E, Seidel L, Foster SJ, et al. Coordination of Chromosome Segregation and Cell Division in *Staphylococcus aureus*. Frontiers in Microbiology. 2017;8(1575).

Kusuma KD, Griffith R, Harry EJ, Bottomley AL, Ung AT. *In silico* Analysis of FtsZ Crystal Structures Towards a New Target for Antibiotics. Australian Journal of Chemistry. 2018

Conference proceedings

Kusuma KD, Amy Bottomley, Alison Ung, Aaron Oakley, Nick Dixon, Nan Li, Elizabeth Harry **(2015)** Cell division as a new target for the development of antibiotics against *Acinetobacter*, **East Coast Protein Meeting, Brisbane, Poster presentation.**

Kusuma KD, Amy Bottomley, Elizabeth Harry, Renate Griffith, Alison Ung **(2017)** Understanding FtsZ as an antibacterial target, **Solution for Drug-Resistant Infections Conference, Brisbane, Poster presentation.**

Abstract

The problem of antibiotic resistance is a complex issue and one that urgently needs addressing from multiple sectors, including agriculture, medicine, science/research, government, social science, businesses and the community. Although many strategies are being implemented around the world to address these different aspects that contribute to the rise and spread of antibiotic resistance, one further possibility to help alleviate this problem is through the design of novel antibiotics. The essential process of bacterial cell division, is yet to be targeted by any of the FDA-approved antibiotics and represents an untapped area of potential drug targets. In this thesis, the overall strategy of inhibiting the bacterial cell division process is to target the essential and conserved protein FtsZ in two ways: Firstly, to understand the essential interaction of FtsZ with another division protein, FtsA as a starting point to design inhibitors of division complex formation and, secondly, to develop compounds that inhibit FtsZ function.

Characterizing the protein-protein interaction of FtsZ and its partner FtsA used the proteins from the organism *Acinetobacter* spp.. This is because *A. baumannii* is now classified by the World Health Organization as a priority 1 pathogen that urgently needs an antibiotic against due to its high multidrug resistance profile, as well as causing high mortality rates. Two of the most highly conserved bacterial cell division proteins, FtsZ and FtsA, have been recognised as promising drug targets in *Acinetobacter* spp. and other bacteria. The interaction of these two proteins has been known for over a decade with mutational studies indicating that the conserved aspartate and proline at the extreme C-terminal peptide of FtsZ being the important amino acid residues for the interaction of FtsZ and FtsA in *Escherichia coli* and other bacterial species. Co-crystallography of *Thermotoga maritima* FtsZ C-terminal peptide and FtsA identified

an additional amino acid; arginine, to be important in the interaction of FtsZ and FtsA. In the *Acinetobacter* spp. the aspartate, proline and arginine have been changed to a serine, glutamine and lysine, respectively. Understanding this could potentially be used to develop new narrow-spectrum antimicrobials to specifically treat *Acinetobacter* infections. The work in this thesis attempted to understand the implication of these amino acid differences by initially conducting an *in silico* study. The data obtained suggests that the serine, glutamine and lysine are important for the FtsZ/FtsA interaction of *Acinetobacter* spp. Further follow up co-crystallographic studies were planned using full-length *Acinetobacter* FtsZ and FtsA. Both of these full-length *Acinetobacter* proteins were successfully purified in this study but, the purified FtsZ protein was unable to form crystals of acceptable size for structure determination, while the purified FtsA were found to be aggregated. Therefore, in the interest of time and for gaining positive results, the focus of the project was shifted towards solely understanding and targeting FtsZ.

Thus far, many published FtsZ inhibitors have been shown to target FtsZ in one of the three druggable regions on the protein: nucleotide-binding domain, interdomain cleft and T7-loop. A missing piece of information is an in-depth understanding of FtsZ structure at the molecular level across diverse bacterial species to ensure inhibitors have high affinity for the FtsZ target in a variety of clinically relevant pathogens. To address this, an *in silico* investigation was conducted by analysing multiple FtsZ structures, which revealed that FtsZ groups into two distinct classes based on structural differences. The outcome of this analysis lead to the suggestion of several binding pockets on FtsZ which can potentially be used as a broad- and narrow-spectrum target. The use of fragment-based drug discovery approach, allowed the confirmation of one of the suggested pockets, which is located towards the front of the nucleotide-binding domain.

This pocket is yet to be reported in the literature, therefore, allowing the possibility of novel drug design to contribute in tackling the global issue of antimicrobial resistance.