

**Probing self-association of CetZ1
cytoskeletal protein from *Haloferax
volcanii***

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Thesis submitted in fulfilment of the
requirements for the degree of
Master of Science by Research

under the supervision of A. Prof. Iain Duggin

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Faculty of Science

March 2021

CERTIFICATE OF ORIGINAL AUTHORSHIP

I, Vinaya Devidas Shinde, declare that this thesis, is submitted in fulfilment of the requirement for the award of Master of Science by Research degree, in the School of Life Sciences/Faculty of Science at the University of Technology Sydney.

This thesis is wholly my own work unless otherwise referenced or acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

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This research is supported by an Australian Government Research Training Program.

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ACKNOWLEDGMENT

There are many people who helped me along the way on this journey. I want to thank all of them. First and foremost, special thanks to my supervisor, A/Prof. Iain Duggin for providing me with the opportunity to undertake a Master of Science by Research under his guidance and supervision. Without your support, significant advice, I would not be able to complete this project. I am very grateful for your time and patience throughout my journey.

To my co-supervisor and dear friend Yan Liao, she always provided constant support, availability and constructive suggestions which helped in completing the research. Thank you Yan for everything, I have learned a lot on how to be a good scientist.

A big thank you to my past and present lab mates Dora, Hannah, Tamika, Solenne, Tayla and Carly for your friendship and for creating a wonderful vibe to work in. Special thanks to Hannah for reading my thesis and her constant support throughout the thesis writing process. I am so grateful to have met you.

I am thankful to Mark Lockrey from MAU for training me on the electron microscope. Thank you to Rodrigues lab for attending our lab meetings and for the insightful discussions about my project. Thank you to Chris from the MIF for training me on the microscopes, and finally, thank you to everyone at itthree institute.

Finally, I must express my profound gratitude to my husband, Vishal for providing me with unfailing support and continuous encouragement throughout the degree. Also, to my family, even though they are not in Sydney, I still appreciated all the support they have shown, which gave me strength to be better each day. This accomplishment would not have been possible without them. Thank you.

CONFERENCE PROCEEDINGS

2020

Lorne Conference on Protein Structure and Function, Cumberland, Lorne, Australia. Presented poster titled, **“Structure function studies of CetZ tubulin like cytoskeletal proteins from haloarchaea”**. **Shinde V**, De Silva R.T. Liao Y, Duggin I.

2019

Foster, Sydney, Australia. Presented poster titled, **“Study of a divergent cytoskeletal protein from haloarchaea”**. **Vinaya Shinde**, Roshali De Silva, Yan Liao, Iain Duggin

ABBREVIATIONS

AMP	Ampicillin
BSA	Bovine Serum Albumin
CetZ	Cell-structure-related Euryarchaeota tubulin/FtsZ
DNA	deoxyribonucleic acid
g	Gram
GTP	Guanosine triphosphate
GDP	Guanosine diphosphate
h	Hour(s)
IPTG	Isopropyl β -D-1-thiogalactopyranoside
kDa	Kilo base pair(s)
min	Minute(s)
mL	Millilitre(s)
MQW	Milli-Q-water
OD	Optical Density
PBS	Phosphate Buffered Saline
PIPES	1,4-Piperazinediethanesulfonic acid
PIPES Buffer	800 mM PIPES, 3M KCl, 10 mM MgCl ₂ , pH 7.3
M	Molar concentration
mM	Millimolar concentration
MW	Molecular Weight
MWCO	Molecular weight cut-off
n	Nano
ROW	Reverse osmosis water
RT	Room Temperature
rpm	Revolution per minute
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
sec	Seconds
TEM	Transmission electron microscope

TEMED	N,N,N',N'-Tetramethylethane-1,3 diamine
Tris	Tris (hydroxymethyl) methylamine
v	Volume
w	Weight
W	Wild-type
μ	Micro
Δ	Delta (change in)

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ABSTRACT

The cytoskeleton is a dynamic network of proteins, which are required by all cells for cell division, growth, and maintenance of cell shape. A major group of cytoskeleton proteins present in nearly all cells are the tubulin superfamily proteins. Archaea, the third domain of life, encode a great diversity of tubulin superfamily proteins, including FtsZ and the tubulins that are more similar to those in eukaryotes. Recently a new group of cytoskeletal proteins was found in archaea, named “CetZ”, which show characteristics in common with both tubulin and FtsZ and are involved in cell shape regulation. They form dynamic cytoplasmic filaments at or near the cell envelope, which are required for cell shape determination (Duggin et al., 2015). However, the mechanisms by which CetZ proteins lead to remodelling of the cell envelope to modulate cell shape remain unknown.

Based on crystal structures of CetZ proteins and their likely manner of self-association, we have initiated a structure-function analysis of CetZ interactions *in vivo* (De Silva, 2019 PhD Thesis) and, in the present study, *in vitro*. Point mutations were introduced into the *Haloferax volcanii* CetZ1 protein, designed to target putative functional interactions in self-association and putative membrane association. In the present study, mutations that disrupt the longitudinal and lateral interactions were selected for the *in vitro* analysis. Light scattering and TEM were used as an approach to analyse the polymerization cycle and structural features of CetZ polymers, correlating these to the *in vivo* structures observed by high- and super-resolution fluorescence microscopy.

The *in vitro* studies demonstrated that CetZ1 forms GTP-dependent single-stranded filaments and polymer stability was clearly altered in predicted self-association mutants. The longitudinal interface mutants demonstrated that the GTP binding site and GTPase activity controls the longitudinal interaction of CetZ1 polymer formation by GTP hydrolysis. Lateral interaction mutants showed decreased polymerization ability. The mutation in the M-loop region, revealed that it is crucial for polymer formation. Preliminary investigation into possible CetZ1 lipid membrane binding suggests that CetZ1 can bind to the lipid membrane and modify shape changes in them, dependent on the polymerization ability of CetZ1.

These findings have contributed to the understanding of tubulin-like cytoskeleton proteins in archaea, which, by comparison to the cytoskeletons of bacteria and eukaryotes, are

expected to provide future insights into cytoskeleton evolution and help reveal fundamental principles of cytoskeletal function across the three domains of life.