

30 July 2019

Dear ACFRT Board Trustees,

I am writing to provide a final report for my ACFRT funded project entitled: “*Subcellular-Specific Characterisation of the Aberrant Structure and Function of the Glycoproteome of CF and Healthy Neutrophils - Using Glycobiology to Decode Contributing Disease Mechanisms in CF*”. In short, the focus of this scholarship project spanning the period 2017-19 was to decode the glycobiology of neutrophils our critical first-line immune cells found in abundance in the lungs of individuals affected by cystic fibrosis (CF).

Over the first part of the ACFRT project period, I focused on investigating the asparagine (N-) linked glycosylation of proteins residing in the four major neutrophil compartments (hereafter granules) i.e. primary granules, secondary granules, tertiary granules and secretory vesicles using liquid chromatography tandem mass spectrometry-driven glycomics and glycoproteomics technologies. In collaboration with leading neutrophil biologists i.e. Prof Anna Karlsson and Prof Johan Bylund at Gothenburg University, Sweden, I discovered that each of these four granule compartments harbours proteins decorated with distinct glycosylation signatures. This fascinating granule-specific N-glycosylation has not been reported before. Interestingly, these studies also showed that the primary granule contained proteins that were abundantly glycosylated with a previously overlooked type of truncated N-glycosylation termed “paucimannosylation” (*pauci*, Latin for few). I found this discovery particularly interesting since the primary granule is known to contain hydrolytic enzymes that are responsible for the antibacterial/antimicrobial function of neutrophils as well as degranulation and phagocytosis of pathogens in the CF lung. These discoveries are currently being written up as two original research manuscripts for publication (one paper for the glycomics and one for the glycoproteomics experiments) with me as a data contributing co-author on both manuscripts (Venkatakrishnan et al., *in preparation* and Loke et al., *in preparation*, see full details below). These two papers, which are scheduled for publication later this year, will form an integral part of my PhD thesis.

To further explore my discovery of paucimannosidic proteins in neutrophils and its role in various inflammatory processes including the processes that pertain to the CF pathophysiology, I have recently dedicated a significant amount of time to survey and compile the entire glycobiological literature for previous reports of paucimannosylation not only in human and mammalian glycobiology but across the entire eukaryotic domain. This resulted in an extensive “synthetic” review covering a total of 423 citations that previously reported the presence of paucimannosidic proteins across 77 eukaryotic species. This review article featuring me as a first-author contributor was recently accepted

for publication in a very high impact journal (Tjondro et al., Human Protein Paucimannosylation: Cues from the Eukaryotic Kingdoms, *Biol Rev Camb Philos Soc*, impact factor 10.3) (DOI: 10.1111/brv.12548). In this synthetic review, I found evidence for the notion that paucimannosidic proteins are involved in diverse biological processes such as development, fertility and in the immune system in particular within the innate immune system of relevance to the CF aetiology. Taking cues from the “lower” species, I suggested that protein paucimannosylation is important in human neutrophil maturation and function and that aberrant glycosylation processes may lead to an impaired innate immune system. Following on from this review, it is therefore now of particular interest to investigate whether neutrophils isolated from CF patients display an impaired response to inflammation and infection.

Together with my collaborators at Gothenburg University, I have over the past year (2018-19) conducted a large *ex vivo* study in which I infected neutrophils isolated from healthy individuals with bacteria common to CF including *S. aureus* in order to study their longitudinal response to infection and degranulation of antimicrobial paucimannosidic proteins. This involved a visit to the Swedish research lab for almost two weeks where I learned new techniques and biology valuable to the completion of my PhD candidature. My preliminary data have shown an interesting time-dependent increase of human myeloperoxidase (MPO) being released from *S. aureus* stimulated neutrophils. MPO is a well-studied glycoprotein with antibacterial properties residing in the primary granule of neutrophils. This abundant and neutrophil-specific glycoprotein has previously been associated with important processes in the CF pathogenesis, but limited is still known of the site-specific N-glycosylation of MPO despite reports demonstrating the importance of glycans for the antimicrobial function of MPO. I therefore have dedicated much time and effort to perform a detailed glycoprofiling of MPO over the past year. To this end, I am currently writing a structural characterisation paper on MPO that is expected to be published later this year in a high impact journal (Tjondro et al., *in preparation*). In short, this study showed that MPO is decorated by both oligomannosidic and paucimannosidic N-glycans at specific positions highly relevant to the 3D structure of the MPO dimer and its inhibition by ceruloplasmin. Interestingly, a glycoproteomic analysis of granule-separated MPO showed that only two of the five sites are glycosylated in a granule-specific manner, the functional relevance of which I am currently investigating. These findings are important to better understand the trafficking, processing and maturation of MPO in neutrophils, knowledge that contributes to an improved understanding of the innate immune system.

A relevant opportunity for a research project arising from my findings involves studying the effect of neutrophils upon challenge with *P. aeruginosa* also typically found in the CF lung. This is particularly interesting given our access to unique *P. aeruginosa* CF isolates (PASS1-4) that we previously have characterised as part of a larger ARC sponsored program. In such experiments neutrophils isolated

from both healthy individuals and CF patients should be compared. Other avenues for future research include studying the reduced capacity of neutrophils deficient in CFTR (CF-neutrophils) to eliminate pathogens relevant to the CF lung environment.

In conclusion, the studies and experiments described herein, which were enabled by the generous ACFRT funding, have provided support for the notion that novel paucimannosidic glycoproteins carried by neutrophils play critical functions during infection and inflammation (patho)physiological processes highly relevant to the CF disease mechanisms. Although this scholarship has produced new exciting knowledge mostly of fundamental character many aspects relating to neutrophil glycobiology remain to be explored in the context of CF and related disorders. This fundamental knowledge contributes to the essential knowledge base required to develop novel therapeutics and treatment options to assist lowering the disease burden of CF patients.

I am very much honoured and privileged to be selected as the recipient for this scholarship. If given the opportunity to do so, I would have a great interest in pursuing a career in CF research in the coming years.

Thank you.

Sincerely,

Mr Harry Tjondro, M.Sc
Ph.D Candidate
Department of Molecular Science
Macquarie University
Australia

List of outcomes:

Papers (*published and in preparation*):

1. **Tjondro, H.**, Loke, I., Chatterjee, S., Thaysen-Andersen, M. (2019). Human Protein Paucimannosylation: Cues from the Eukaryotic Kingdoms. *Biol Rev Camb Philos Soc*. DOI: 10.1111/brv.12548 (accepted on the 17th July, 2019)
2. **Tjondro, H.**, Ugonotti, J., Chatterjee, S., Loke, I., Chen, S.Y., Hinneburg, H., Parker, B.L., Venkatakrishnan, V., Dieckmann, R., Struwe, W., Karlsson, A., Thaysen-Andersen, M.

Neutrophil Granule-Specific Glycosylation and Functions of Human Myeloperoxidase (*in preparation*).

3. Venkatakrishnan, V., Dieckmann, R., Loke, I., **Tjondro, H.**, Chatterjee, S., Bylund, J., Thaysen-Andersen, M., Karlsson, N.G., Karlsson, A. Revealing The Neutrophil Granule-specific Glycosylation Signature. (*in preparation*).
4. Loke, I., Venkatakrishnan, V., Dieckmann, R., **Tjondro, H.**, Chatterjee, S., Bylund, J., Karlsson, N.G., Karlsson, A., Thaysen-Andersen, M. Mapping the Granule-specific N-Glycoproteome of Human Neutrophils (*in preparation*).

Talks:

1. **Tjondro, H.**, Venkatakrishnan, V., Loke, I., Chatterjee, S., Dieckmann, R., Horn, C., Parker, B.L., Packer, N.H., Borregaard, N., Karlsson, N.G., Bylund, J., Karlsson, A., Thaysen-Andersen, M. Glycosylation Features of Neutrophilic Granules. Talk presented at: 23rd Lorne Proteomics Symposium; 1st-4th Feb 2018; Lorne, VIC, Australia
2. **Tjondro, H.**, Venkatakrishnan, V., Loke, I., Chatterjee, S., Dieckmann, R., Horn, C., Parker, B.L., Packer, N.H., Borregaard, N., Karlsson, N.G., Bylund, J., Karlsson, A., Nenne, M., Torpy, D., Thaysen-Andersen, M. Probing Glyco-signatures of the Neutrophil Granules in Bacteria-infected Human Sera. Talk at the 2nd GlycoCom and 1st Human Glycome Project Meeting 2018; 3rd-6th Oct 2018; Dubrovnik, Croatia.

Posters:

1. **Tjondro, H.**, Venkatakrishnan, V., Loke, I., Chatterjee, S., Dieckmann, R., Horn, C., Parker, B.L., Packer, N.H., Borregaard, N., Karlsson, N.G., Bylund, J., Karlsson, A., Thaysen-Andersen, M. Glycosylation Features of Neutrophilic Granules. Poster at the 23rd Lorne Proteomics Symposium; 1st-4th Feb 2018; Lorne, VIC, Australia
2. **Tjondro, H.**, Venkatakrishnan, V., Loke, I., Chatterjee, S., Dieckmann, R., Horn, C., Parker, B.L., Packer, N.H., Borregaard, N., Karlsson, N.G., Bylund, J., Karlsson, A., Nenne, M., Torpy, D., Thaysen-Andersen, M. Probing Glyco-signatures of the Neutrophil Granules in Bacteria-infected Human Sera. Poster at the 2nd GlycoCom and 1st Human Glycome Project Meeting 2018; 3rd-6th Oct 2018; Dubrovnik, Croatia.
3. **Tjondro, H.**, Ugonotti, J., Chatterjee, S., Loke, I., Chen, S.Y., Hinneburg, H., Parker, B.L., Venkatakrishnan, V., Dieckmann, R., Struwe, W., Karlsson, A., Thaysen-Andersen, M. Unusual Site- and Granule-Specific N-Glycosylation of Human Myeloperoxidase from Resting Neutrophils. Poster at the 18th Human Proteome Organization World Congress; 14th-18th Sept 2019; Adelaide, SA, Australia.