2015/2016 Summer Studentship Project Application Form

Send to: Research Office, University of Otago Christchurch, PO Box 4345, Christchurch, by 5pm on **3 July 2015**

Supervisor Information (First named supervisor will be the contact):	
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Research Category (Choose one category only – to be used for judging the students' presentations):		
Clinical	**Laboratory x**	**Community**

Project Title (20 words MAXIMUM):

Interplay between enterobactin & myeloperoxidase during infections with *E. coli.*

Project Description:

Introduction:

To establish an infection, gram-negative bacteria, such as *Escherichia coli* and *Salmonella typhimurium*, release enterobactin to capture iron, which is essential for their growth. Enterobactin is a small compound composed of three ortho-hydroquinones (Fig. 1) that bind iron with great affinity ($K = 10^{52}$ M^{-1}). Recently, it was proposed that enterobactin is an inhibitor of the human enzyme myeloperoxidase [1]. This enzyme acts within white blood cells to produce chlorine bleach which is targeted against pathogenic bacteria to kill them [2]. We have previously investigated the mechanisms by which several different types of compounds inhibit myeloperoxidase [3, 4]. Based on this work, we suggest that enterobactin is unlikely to be an effective inhibitor of myeloperoxidase. Rather, it is likely to be a good substrate for the enzyme.

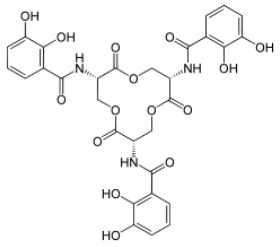

Fig. 1 Enterobactin

We hypothesize that during an infection, white blood cells will release myeloperoxidase to degrade enterobactin and thereby retard bacterial growth. Thus, by degrading enterobactin and preventing bacterial growth, myeloperoxidase may have an additional role in host defence besides directly killing bacteria.

Aim:

To establish how enterobactin and myeloperoxidase are likely to interact during an infection.

The aim will be achieved by addressing the following objectives:

1. Determine whether enterobactin is an inhibitor of hypochlorous acid production by myeloperoxidase.
2. Establish whether enterobactin is a substrate for myeloperoxidase.
3. Determine whether enterobactin can compete with other substrates of myeloperoxidase.
4. Identify the initial product of enterobactin oxidation and whether it forms adducts with cysteine residues on proteins and glutathione.
5. Establish whether oxidation of enterobactin by myeloperoxidase retards growth of *E coli*.

Method:

Enterobactin will be tested in our routine myeloperoxidase inhibition assays. The interactions of enterobactin with myeloperoxidase will be determined using spectrophotometric methods that are well established in our laboratory. Product analysis will be undertaken using mass spectrometry. The effect of myeloperoxidase on bacterial growth will be determined using standard plating assays. That is, *E. coli* will be incubated with myeloperoxidase under various conditions that either favour killing of the bacteria by chlorine bleach or favour oxidation of enterobactin.

References:

[1] Singh, V.; Yeoh, B. S.; Xiao, X.; Kumar, M.; Bachman, M.; Borregaard, N.; Joe, B.; Vijay-Kumar, M. Interplay between enterobactin, myeloperoxidase and lipocalin 2 regulates E. coli survival in the inflamed gut. *Nature communications* **6:**7113; 2015.
[2] Winterbourn, C. C.; Kettle, A. J. Redox reactions and microbial killing in the neutrophil phagosome. *Antioxid. Redox. Signal.* **18:**642-660; 2013.
[3] Forbes, L. V.; Furtmuller, P. G.; Khalilova, I.; Turner, R.; Obinger, C.; Kettle, A. J. Isoniazid as a substrate and inhibitor of myeloperoxidase: identification of amine adducts and the influence of superoxide dismutase on their formation. *Biochem. Pharmacol.* **84:**949-960; 2012.
[4] Forbes, L. V.; Sjogren, T.; Auchere, F.; Jenkins, D. W.; Thong, B.; Laughton, D.; Hemsley, P.; Pairaudeau, G.; Turner, R.; Eriksson, H.; Unitt, J. F.; Kettle, A. J. Potent reversible inhibition of myeloperoxidase by aromatic hydroxamates. *J. Biol. Chem.* **288:**36636-36647; 2013.

Student Prerequisites (eg. Medical Student) if applicable:
This project would be ideally suited to a student with a strong background in chemistry and or biochemistry.