Recipient Report

Recipient name: Jinlong Gao

Project title: Characterisation of novel haem binding proteins in the oral pathogen *Porphyromonas gingivalis*

Project summary: Periodontitis is one of the most prevalent bacterial-driven chronic inflammatory diseases of human. *Porphyromonas gingivalis* is the leading pathogen in chronic periodontitis, a disease process involving progressive destruction of the supporting tissues of the teeth. Accumulating evidence also implicate the involvement of this organism in the onset, development and progression of several systemic diseases. Haem is an essential nutrient for the organism, providing iron and porphyrin necessary for growth. Due to the absolute requirement for haem, *Porphyromonas gingivalis* has developed multiple uptake systems to scavenge haem from its environs. Over the past 30 years, several outer membrane haem receptors have been reported and characterised but most have relatively low haem binding affinities which may not be able to compete against host haem scavenging proteins or other haemophores secreted by other micro-organisms. The presence of high-affinity haem binding receptors has been detected in *P. gingivalis* but the identity of which is currently unknown.

An initial investigation identified several putative haem binding proteins from haem-rich outer membrane extracts of *P. gingivalis*. Utilizing bioinformatics analysis, the hypothetical protein PG2226 was predicted to be related to TonB-dependent haem binding receptors. Inactivation of this gene along with the gene immediately downstream, *PG2227*, provided evidence that they were important for the growth of the organism under low haem conditions. To characterise these two candidates, two main approaches were used in this study:

1. Recombinant proteins were expressed and purified from *Escherichia coli* to characterise the haem binding properties;

2. Gene inactivations were performed to evaluate the role of corresponding genes in the haem utilization and intracellular survival of *P. gingivalis*.

The results indicated that *PG2227* encodes for a haemophore-like protein, termed HusA, and *PG2226* encode for a TonB-dependent receptor, termed HusB. Together, they form core components of a novel haem uptake system: the Hus system. Unlike other haem binding proteins described in *P. gingivalis*, HusA can rapidly sequester free monomeric and dimeric haem in solution with high binding affinity at and 7×10^{-10} M, respectively. The *husA* and *husB* genes were revealed to be essential for growth under low haem conditions and both are expressed during infection by *P. gingivalis* and are critical for survival within cultured epithelial cells. The bound haem on HusA was proposed to be passed onto the specific outer membrane receptor

HusB, which subsequently transfer the substrate into the periplasm using the energy provided by the TonB complex. Knowledge of this new haem uptake pathway may offer a unique target for pharmaceutical intervention to control the growth of this organism.

The outputs have achieved (acknowledges have been made to the Bela Schwartz foundation):

Jinlong Gao, Ky-Anh Nguyen, Neil Hunter, Characterization of a hemophore-like protein from *Porphyromonas gingivalis*. The Journal of Biological Chemistry, 2010, Dec 17; 285(51):40028-38.

Jinlong Gao, The Missing Hemophore in *Porphyromonas gingivalis*. The 2nd Meeting of IADR Pan Asian Pacific Federation (PAPF) and the 1st Meeting of IADR Asia/ Pacific Region (APR). Wuhan, China, Sept. 22-24, 2009, oral presentation and poster presentation.

Jinlong Gao, Ky-Anh Nguyen, Neil Hunter, Characterization of Novel Heme Binding Outer Membrane Proteins by *Porphyromonas gingivalis*. The 87th General Session & Exhibition of the IADR and the 38th Annual Meeting of the AADR, Miami, U.S.A, oral presentation.

Jinlong Gao, Hongyu Xie, Ky-Anh Nguyen and Neil Hunter, Detection and Characterisation of Novel Outer Membrane Proteins Involved in Heme Binding by *Porphyromonas gingivalis.* The 13th International Biotechnology Symposium and Exhibition, Dalian, China, poster presentation.