NTU-KU Joint Funding

Final Report

Section 1

NTU principle investigator	
Name (last name, first name)	**Miao-Hsia Lin**
Position	**Assistant professor**
Faculty/Department	**Medicine College/ Department of Microbiology**

KU principle investigator	
Name (last name, first name)	**Yasushi Ishihama**
Position	**Professor**
Faculty/Department	**Graduate School of Pharmaceutical Sciences/ Department of Molecular Systems BioAnalysis**
Visiting ECR*	
Name (last name, first name)	**I-Ying Lin**
Position	**Master student**
Faculty/Department	**Medicine College/ Department of Microbiology**

*Please complete this section if the KU principal investigators hosted ECRs from NTU.

Host researcher*	
Name (last name, first name)	
Position	
Faculty/Department	

*Please complete this section if the host researcher is different from the KU principal investigator.

Section 2

Project title
Long-term Tracing of Microbial Community Dynamics at the Gastrointestinal (GI) Tract in

post Menopause Model by Metaproteomics Analysis

Section 3

Period of project	
From dd/mm/yy to dd/mm/yy	**01/07/2022 to 31/08/2022**

Section 4

Summary of the project (approx. 100 words)
*KU PIs are required to submit a summary of the project in Japanese in addition to the English summary (approx. 200–300 characters).

(Please enter the summary of the project)

本プログラムの支援により、NTU側から大学院生I-Ying Linさんが、KU側の石濱研究室に2ヶ月間滞在し、メタプロテオミクス解析法を構築して技術移転することをプロジェクトの目的とした。I-Y. Linさんは計画通り来日し、石濱研究室でメタプロテオミクス技術を確立し、LCMSMSデータの処理方法を学んだ。これにより、微生物活性を属レベルで、場合によっては種レベルで区別し、定量することが可能となった。本メタプロテオミクスプラットフォームは、現在、NTUのM-H. Lin博士の研究室で順調に稼働しており、この共同研究は成功裏に完了した。

With the support of joint funding, one graduate student, Ms. I-Ying Lin, from Dr. Lin's lab (NTU side) has visited Dr. Ishihama's lab (KU side) for two months. Given the awareness of the important roles of microbiome in human health, we aimed to setup the metaproteomics approach and transfer it from KU side to NTU side and the animal experiment handling in return. During this period, I-Ying has optimized the metaproteomics methods in Dr. Ishihama's lab and learned how to processed the data generated from mass spectrometry. With her efforts and supervisions from Dr. Ishihama, we now are able to differentiate and quantify the microbial activity at genus level and in some cases, we can even reach the species level. Since we directly determined the bioactive molecules, metaproteomics provides more appropriate interpretation on biological issues. The information derived from metaproteomics is complement to metagenomics providing us the comprehensive view from genome to proteome. The metaproteomics platform has now been successfully transferred and setup in Dr. Lin's lab. Completion this collaboration, this metaproteomics analysis is now applicable for studying the role of microbiome in any mouse disease models which refers to the degree of involvement of each taxonomy at the molecular level.

Section 5 (Please complete this section if ECRs from NTU participated in collaborative research at KU)

Achievements and Outcomes of ECRs' Stay (approx. 100–250 words)
*This section should be filled by each of the ECR(s) (one paragraph per ECR) based on his/her experience of staying in Japan.

(Please enter the achievements and outcomes for each of the ECR(s).)

The microbial diversity and the host-microbial interaction of human microbiome both play crucial roles in human health. Proteins are the biomolecules to execute physiological regulations, therefore, metaproteomics displays as an appropriate mean to dissect the roles of microbiome by comprehensively protein identification and quantification. However, other than the sample complexity, the limited clinical sample amount makes the metaproteomics analysis difficult. To relieve these limitations, our group has collaborated with Prof. Yasushi Ishihama in Kyoto University to evaluate the protein extraction efficiency on six sample preparation workflows for metaproteomics analysis. Based on the previous published works (*PG Gavin, 2021*), we designed a serial modification to optimize protein extraction efficiency and sample cleanup by centrifugation, filtration, and liquid-liquid extraction approaches. In total, I evaluated the protein extraction and protein identification from six sample preparation workflows, in which I tried to either separate host proteins from bacterial proteins by differential centrifugation (DC) or filtration or extracted all protein without separation. Followed by liquid-liquid extraction, the protein extracts were subjected into trypsin digestion and LC-MS/MS analysis. In terms of peptide/protein identification, the centrifugation approach had better ability of extracting bacterial and total proteins in mouse feces samples. Furthermore, the protein compositions were similar among six approaches with up to 90 percent of bacterial proteins was co-identified in each sample. We have now established this analytic platform in our group (NTU side). Further applying this method on the mice with post menopause will help us to understand the roles of gut microbiome.

Section 6

Photographs with captions
*Please submit digital files (such as JPEG or GIF files) of the photographs used in your report as attachments. The size of each image should be at least 4MB, so that it can be used for printed materials. Please ensure that none of the photographs submitted will cause any issues relating to portrait rights.
URL at which project outcomes can be viewed (Optional)
*E.g. workshop notifications/programs/reports, evidence of academic papers published or otherwise made available, etc.
URL: