DATE: Day 9 Month May Year 2025

SUMMARY of
FY2024 RESEARCH RESULTS REPORT
For International Collaborative Research with IPR, Osaka University

Research Title		Functional dynamics of a human ubiquitin C-terminal hydrolase studied by SAIL NMR
Applicant	**Name**	Hsu Shang-Te Danny
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	Present Title	Research fellow and Deputy director
Research Collaborator (Host PI)		**Yohei Miyanoiri**

Summary

BRCA1-associated protein 1 (BAP1) is a tumor-suppressive deubiquitinase, with many cancer-associated mutations localized in its ubiquitin C-terminal hydrolase (UCH) domain. Individuals with germline mutations have an approximately 85% risk of developing BAP1-related cancers. The applicants have investigated the structural and functional impacts of these mutations, identifying an allosteric communication network centered on conserved residues. Mutations in Phe residues led to loss of DUB activity, suggesting that aromatic interactions are critical for functional regulation.

To elucidate the mechanism, stereo-array isotope labeling (SAIL) technology is employed to optimize NMR relaxation properties and enable site-specific assignment and dynamic analysis of aromatic residues. To overcome the poor spectral resolution from uniformly ^{13}C-labeled BAP1-UCH, the project aims to use SAIL Phe-specific labeling, combined with selective methyl labeling of Ile, Leu, and Val residues.

Results

Preliminary experiments revealed that BAP1-UCH has low thermal stability, necessitating rapid sample handling. A large-scale expression and purification system was established at the IPR.
Optimization allowed successful soluble expression and partial purification of BAP1-UCH.
SAIL-labeled BAP1-UCH samples (~20 μM) were prepared using 200 mL cultures.
^{1}H–^{13}C aromatic CH-TROSY spectra acquired on a 950 MHz NMR spectrometer successfully detected all Phe-derived signals, enabling further structural and dynamic analysis.

***Deadline: May 9, 2025**
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