

DATE: Day ____ Month ____ Year 2026

SUMMARY of
FY2025 RESEARCH RESULTS REPORT
For International Collaborative Research with IPR, The University of Osaka

Research Title		Structural analysis of a plant glucosyl ceramidase (GBA2)-like enzyme from <i>Arabidopsis thaliana</i>
Applicant	Name	James Ketudat Cairns
	Affiliation	Suranaree University of Technology
	Present Title	Professor Emeritus
Research Collaborator (Host PI)		Prof. Genji Kurisu
Summary The project focuses on the structural analysis of <i>At</i>4GH116 (<i>At</i>GCD3), a plant glycoside hydrolase family 116 (GH116) enzyme from <i>Arabidopsis thaliana</i> that functions as a glucosylceramidase-like enzyme. Glucosylceramides are important glycosphingolipids involved in stress responses and cellular functions, but the structural and biological roles of plant GH116 enzymes remain poorly understood. Although the cryo-EM structure of <i>At</i>GCD3 has recently been reported, the molecular basis of <i>At</i>GCD3–glucosylceramide recognition and substrate binding remain unclear. The study aimed to determine the structure of <i>At</i>GCD3 and clarify its substrate recognition and catalytic mechanism. Previous crystallization and cryo-EM studies produced only low-resolution structural information, making atomic-level interpretation difficult. Therefore, the current work focused on producing improved protein samples and generating mutants for future structural studies. Two catalytic mutants, D647N and E499A, were expressed in <i>Escherichia coli</i> BL21(DE3) using a pET30 expression system. The proteins were purified through Ni²⁺-IMAC, TEV protease cleavage, and size-exclusion chromatography. Both mutants were successfully purified to homogeneity and showed good stability suitable for structural analysis. Future work will focus on cryo-electron microscopy (cryo-EM) sample preparation, data collection, and determination of the <i>At</i>GCD3–glucosylceramide complex structure to better understand substrate binding and enzyme catalysis.		

*Deadline: May 11, 2026

*Please submit it to E-mail: tanpakuken-kyoten@office.osaka-u.ac.jp.

*Please describe this summary within 1 sheet. Please DON'T add some sheets.

*This summary will be published on the web.