DATE: Day____Month____Year 2024

SUMMARY of
FY2023 RESEARCH RESULTS REPORT
For International Collaborative Research with IPR, Osaka University

Research Title		Using an Enzymatic Method to Synthesize Heterochromatin Protein 1 (HP1a)
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Summary

Peptidyl asparaginyl ligases (PALs) are powerful enzyme tools for peptide/protein conjugations [1]. Commonly PALs are used for peptide cyclization and protein labelling. Since we have increase the ligation yield of PAL by coupling PAL with other enzyme (QC) [2]. Now we want to broaden the application scape of PAL for the synthesis of Heterochromatin Protein 1 (HP1a) [3]. Heterochromatin Protein 1 (HP1a) is a eukaryotic chromosomal protein that is prominently associated with pericentric heterochromatin, therefore HP1a plays many important roles in chromatin architecture and impacts both gene expression and gene silencing, utilizing a variety of mechanisms. HP1a consist of N-terminal chromo domain (CD), flexible hinge region (H), and C-terminalchromo shadow domain (CSD). HP1a dimerized at its chromo shadow domain while its CD recognizes and directly binds to key histone modification sites (primarily H3K9me2/3) to form a repressive chromatin environment. Understanding the conformational change and the dynamics of HP1 dimerization become crucial in epigenetic study.

In this study, we aim to synthesis HP1a with a chemically modified TOAC at N terminal of HP1a. We are going to ligate the chemically modified CD part with recombinantly expressed CSD part using PAL-QC coupled ligation which has been established in our lab, in order to get a high yield of the ligation product. The TOAC labelled [Ser(PO3H2)]4-HP1a will enable us to study the conformational change of HP1a during dimerization, furthermore, during binding to chromatin. Additionally, we aim to investigate the effect of post-modification on N terminal Ser residues to the binding of HP1a towards chromatin.

During the study, we have found out the best suited ligation site and the best suited PAL for ligating CD and CSD of HP1a. However, the efficiency and reaction yield still require further optimization.

Reference:
[1] G. K. Nguyen, S. Wang, Y. Qiu, X. Hemu, Y. Lian, J. P. Tam, *Nat. Chem. Biol.* **2014**, 10, 732 – 738
[2] Y.Xia et al*., J. Am. Chem. Soc.* **2023**, 145, 6838-6844.
[3] C. D. Allis, T. W. Muir, *ChemBioChem* **2011**, 12, 264.

***Deadline: May 10, 2024**
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