DATE: Day 11 Month 05 Year 2026

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

Research Title		To study the role of ^{15}N-dynamics on cognate DNA recognition of two transcription factors with diverse modes of DNA interaction.
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	Present Title	**Associate Professor**
Research Collaborator (Host PI)		**Dr. Yohei Miyanoiri**

Summary

IRF and NF-κB are two major transcription factor (TF) families that play crucial roles in the eukaryotic immune response against pathogens. IRF3 and IRF7 activate transcription by binding to Interferon Regulatory Elements (IREs; consensus: 5'-AANNGAAA-3'), while p50 and RelA of the NF-κB family recognize pseudosymmetric κB motifs (consensus: 5'-GGGRNWYYCC-3') on target gene promoters and enhancers. Both families function as dimers and engage DNA through their N-terminal DNA-binding domains (DBDs); however, their interaction modes differ — IRF3 DBD contacts the IRE via a helix inserted into the major groove, whereas p50 DBD engages κB DNA through flexible loops. Mechanistic insights into these contrasting DNA-binding modes will aid in the rational design of inhibitors with therapeutic potential against diseases involving aberrant activity of these TFs.

Preliminary ^{15}N backbone dynamics experiments on IRF3 DBD, conducted on the 950 MHz and 700 MHz NMR spectrometers at the University of Osaka and IISER Berhampur, have revealed an excited-state conformer comprising approximately 2% of the total population. ^{15}N-CEST experiments identify three residues involved in β-strand capping, while ^{15}N-CPMG relaxation dispersion reveals additional residues undergoing slow conformational exchange. Several CPMG-detected residues are contiguous with those identified by CEST, suggesting a structurally connected network of exchanging residues. Additional ^{13}C-CEST and ^{13}C-methyl CPMG experiments will be required to fully characterize the structural details of the excited-state conformer. To probe the functional relevance of this minor conformer, single-point mutants of the exchanging residues have been generated; DNA binding affinity measurements and ^{15}N relaxation dynamics using these mutants are expected to yield mechanistic insights into IRF3 DNA binding. In parallel, analogous experiments are underway on the NF-κB p50 DBD, providing a comparative framework for understanding how structurally distinct DBDs recognize their cognate DNA sequences.

***Deadline: May 11, 2026**
***Please submit it to E-mail: tanpakuken-kyoten@office.osaka-u.ac.jp.**
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