No.	K25-2153	
Project Title	Structural basis of the DNA methylation maintenance	
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(Form 2)

Principal Investigator
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IMSUT International Joint Usage/Research Center Project <International>

Joint Research Report (Annual/Project Completion)

Annual Report

Report

We are conducting research aimed at high-resolution structural analysis of methylated DNA–associated factor complexes present in Xenopus egg extracts using cryo-electron microscopy (cryo-EM).
In the last fiscal year, we focused on developing the technical foundation required to achieve this goal. Traditionally, DNA-conjugated magnetic beads have been incubated in Xenopus egg extracts to assemble DNA-binding protein complexes. However, this approach does not allow efficient release of the assembled DNA–protein complexes from the magnetic beads, making the samples unsuitable for cryo-EM analysis. To overcome this limitation, we applied the well-established FLAG-tag–based immunopurification strategy to develop elutable DNA beads (Fig. A). In this method, a FLAG-M2 antibody is first immobilized on protein G magnetic beads, followed by assembly of mono-FLAG–tagged monovalent streptavidin, which is used to attach DNA.

As a proof of concept, we used these beads to assemble the DNA-dependent protein kinase (DNA-PK) complex, a DNA damage repair protein complex that recognizes damaged DNA, in Xenopus egg extracts. We then systematically evaluated elution conditions. Multiple parameters—including DNA length, FLAG peptide concentration, pH, temperature, and reducing agent concentration—were titrated. Through this analysis, we identified conditions that enable efficient elution of the DNA–protein complexes, as shown in Fig. B.

In the next phase of the project, we will further purify the eluted DNA–protein complexes and perform pilot cryo-EM experiments. In parallel, we will optimize gentle purification protocols and vitrification conditions suitable for cryo-EM analysis. Additionally, we will generate beads conjugated with DNA substrates relevant to the collaborative research goals, such as methylated DNA and hemimethylated DNA, and quantitatively assess the extent to which the target complexes of interest can be purified under these conditions.