Figures and Preliminary results

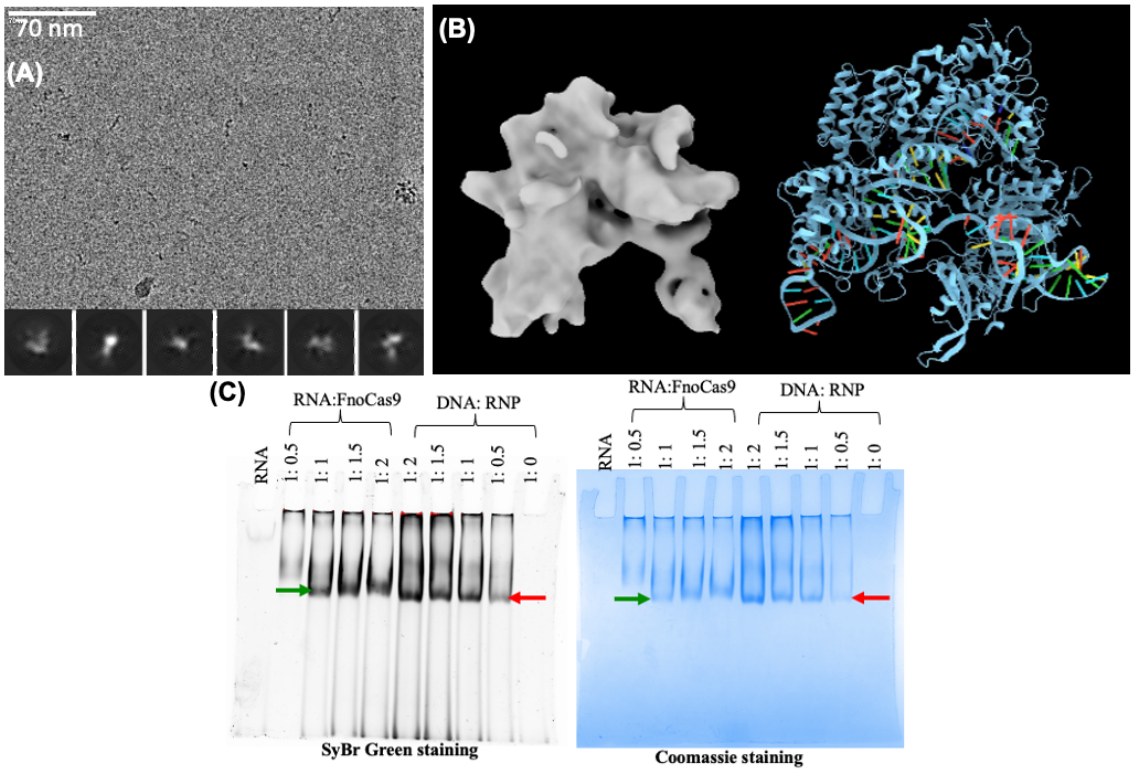
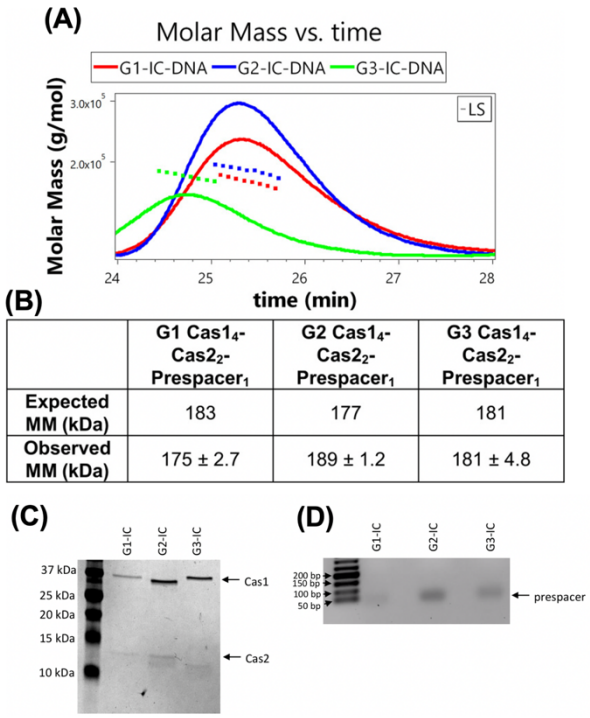


Figure 1. Preliminary results for Cas9. (A) Micrograph of SpyCas9-2Pro-gRNA-DNA complex collected at the MSU cryo-EM facility. Blob picking was performed using 571 micrographs which gave the 2D classes shown in the inset. **(B)** Volume created by *ab-initio* modeling and further refinement using 35,000 particles yielded a low resolution map (~7.4 Å), which has similar overall features as the SpyCas9-sgRNA-DNA complex (PDB ID: 4OO8).[1] The proposed data collection with SpyCas9-2Pro [2,3] and FnoCas12a-KD2P [4] will help advance our research goals on creating high-fidelity Cas proteins for genome applications. **(C)** Electrophoretic mobility shift assay (EMSA) using FnoCas9-RNA-DNA complex. The gels show bands with RNA/DNA and protein stains indicating the presence of a homogenous complex. FnoCas9 gets more compact when it binds to RNA (green arrow), which is further compacted after binding to DNA (red arrow) as indicated by lower bands on the gel.



(B)

	G1 $Cas1_4$-$Cas2_2$-$Prespacer_1$	G2 $Cas1_4$-$Cas2_2$-$Prespacer_1$	G3 $Cas1_4$-$Cas2_2$-$Prespacer_1$
Expected MM (kDa)	183	177	181
Observed MM (kDa)	175 ± 2.7	189 ± 1.2	181 ± 4.8

Figure 2. Preliminary results for Aim 2. (A) SEC-Multi Angle Light Scattering (SEC-MALS) analysis showing integration complex (IC) formation of Cas1-Cas2-DNA (termed prespacer) belonging to three different type II-A groups (G1, G2, and G3). The graph shows the light scattering (LS, colored lines) signal and the molar mass (MM, triangles) calculations for the complexes. **(B)** Table shows the expected and observed MM values of a 4:2:1 Cas1:Cas2: prespacer DNA. SDS PAGE **(C)** and an agarose gel **(D)** of fractions from SEC-MALS. The gels show that all three complexes (G1, G2, and G3) contain Cas1, Cas2 and prespacer DNA. The proposed data collection will help us decipher the protein and DNA sequence determinants of site-specific DNA insertion by different type II-A CRISPR adaptation complex.[5]

References Cited:

1. Nishimasu, H., Ran, F.A., Hsu, P.D., Konermann, S., Shehata, S.I., Dohmae, N., Ishitani, R., Zhang, F. & Nureki, O. Crystal structure of Cas9 in complex with guide RNA and target DNA. *Cell* **156**, 935-49 (2014).
2. Babu, K., Amrani, N., Jiang, W., Yogesha, S.D., Nguyen, R., Qin, P.Z. & Rajan, R. Bridge Helix of Cas9 Modulates Target DNA Cleavage and Mismatch Tolerance. *Biochemistry* **58**, 1905-1917 (2019).
3. Babu, K., Kathiresan, V., Kumari, P., Newsom, S., Parameshwaran, H.P., Chen, X., Liu, J., Qin, P.Z. & Rajan, R. Coordinated Actions of Cas9 HNH and RuvC Nuclease Domains Are Regulated by the Bridge Helix and the Target DNA Sequence. *Biochemistry* **60**, 3783-3800 (2021).
4. Parameshwaran, H.P., Babu, K., Tran, C., Guan, K., Allen, A., Kathiresan, V., Qin, P.Z. & Rajan, R. The bridge helix of Cas12a imparts selectivity in cis-DNA cleavage and regulates trans-DNA cleavage. *FEBS Lett* (2021).
5. Van Orden, M.J., Newsom, S. & Rajan, R. CRISPR type II-A subgroups exhibit phylogenetically distinct mechanisms for prespacer insertion. *J Biol Chem* **295**, 10956-10968 (2020).