Figures and Preliminary Results

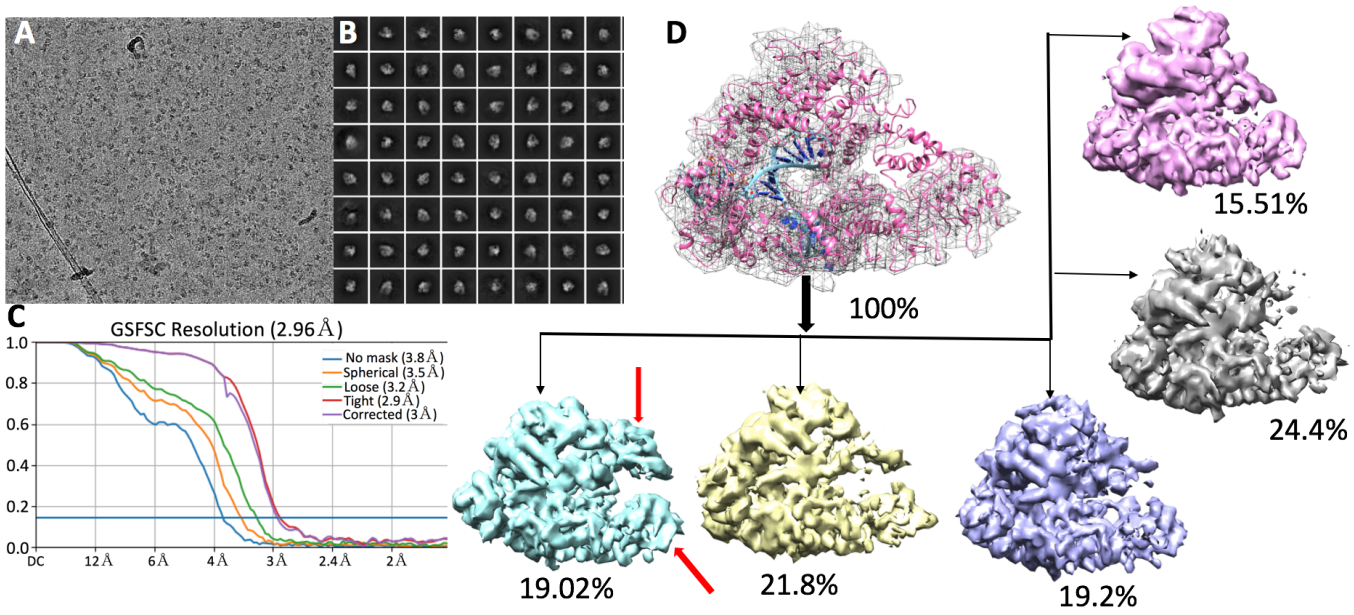

Figure 1. Cryo-EM structure determination of FnoCas12a-BH variant bound to crRNA and a matched DNA (i.e., complete complementarity between the base pairing regions of the gRNA and the target DNA)**. (A)** Particle distribution of a BH-variant of FnoCas12a (position BH3 in Figure 1D; K969P/L970P; FnoCas12a-KD2P) on a grid. **(B)** 2D class averages of FnoCas12a-KD2P. **(C)** Graph showing the gold-standard Fourier shell correlation curves for the dataset. The corrected map is at 3Å resolution. **(D)** An *ab-initio* map with a model derived from PDB ID 6GTG[1] fit onto the map. The map shows the general features of the crab-claw shaped FnoCas12a-gRNA-DNA ternary complex. A 3D variability analysis of the *ab-initio* map created five subpopulations (shown in black arrows) which vary in the orientations of the two domains shown in red arrows. The % of each population is also shown. Current work is to build models to fit the different maps that will create different intermediary conformations needed for DNA cleavage. We propose that the proline substitutions in the BH create distinct populations that arrest the conformational transitions at different stages that will help delineate the activation process for DNA cleavage and mismatch discrimination.

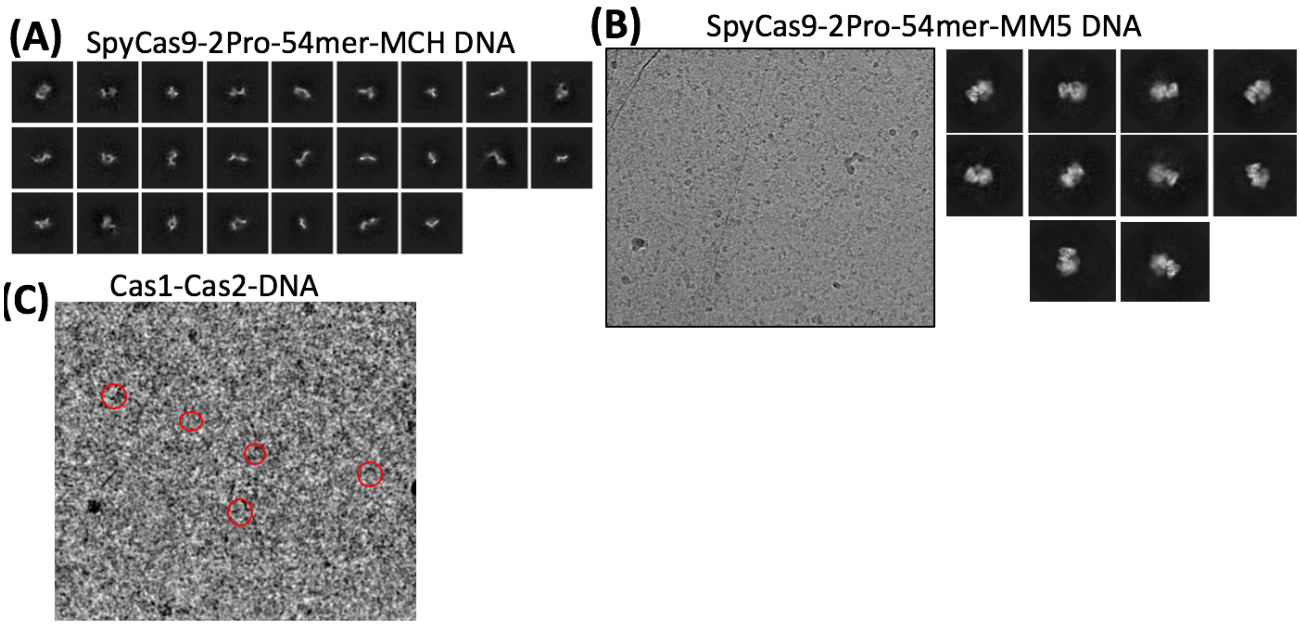

Figure 2. Cryo-EM structure determination of Cas9 and Cas1-Cas2 complexes. (A) Representative 2D classes for the SpyCas9-2Pro[2,3]-gRNA-54-mer matched DNA data collected during the NCCAT-TP1 June-2023 visit. **(B)** Representative micrograph and 2D classes for the SpyCas9-2Pro-gRNA-54-mer MM5 DNA data collected at SLAC. **(C)** Micrographs showing the distribution of Cas1-Cas2-DNA complex purified using our established methods[4] on a quantifoil grid. The particles possess elongated morphology similar to an available structure from an ortholog (e.g., PDB ID: 5XVN).[5]

References Cited:

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4. 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).
5. Xiao, Y., Ng, S., Nam, K.H. & Ke, A. How type II CRISPR-Cas establish immunity through Cas1-Cas2-mediated spacer integration. *Nature* **550**, 137-141 (2017).