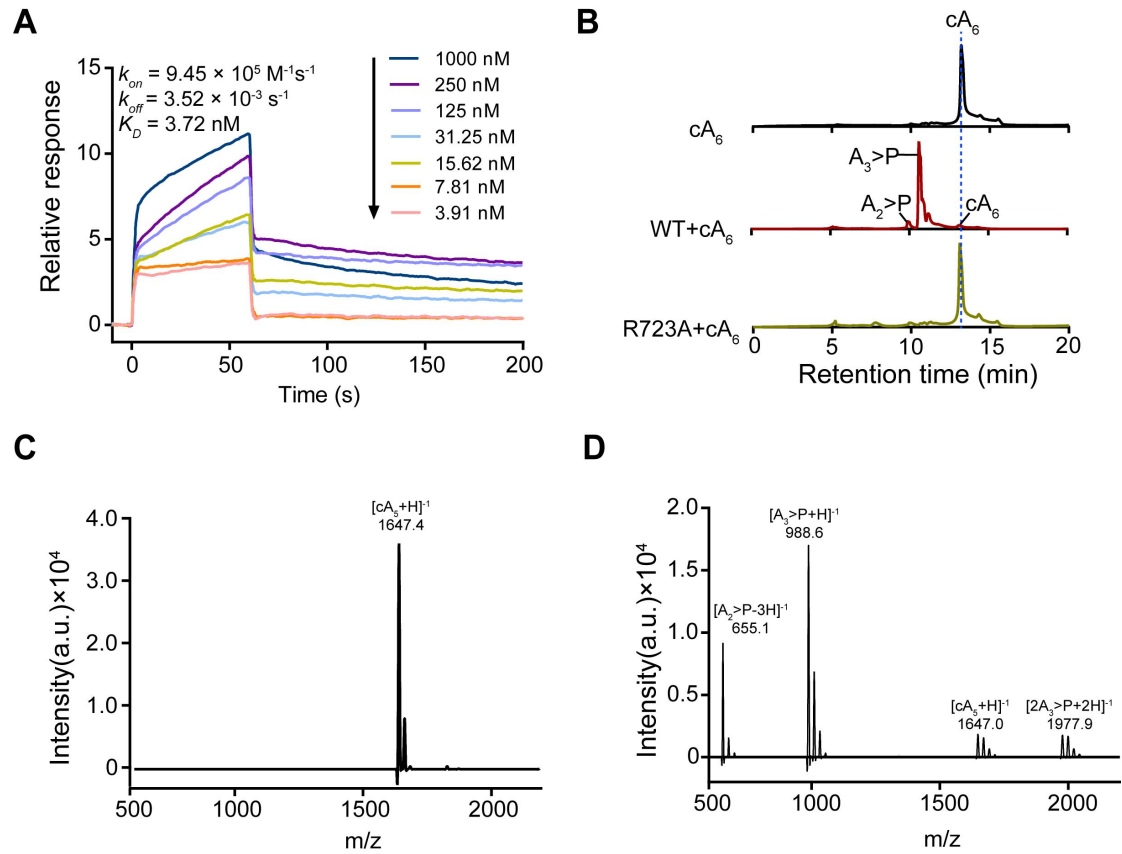


Appendix for

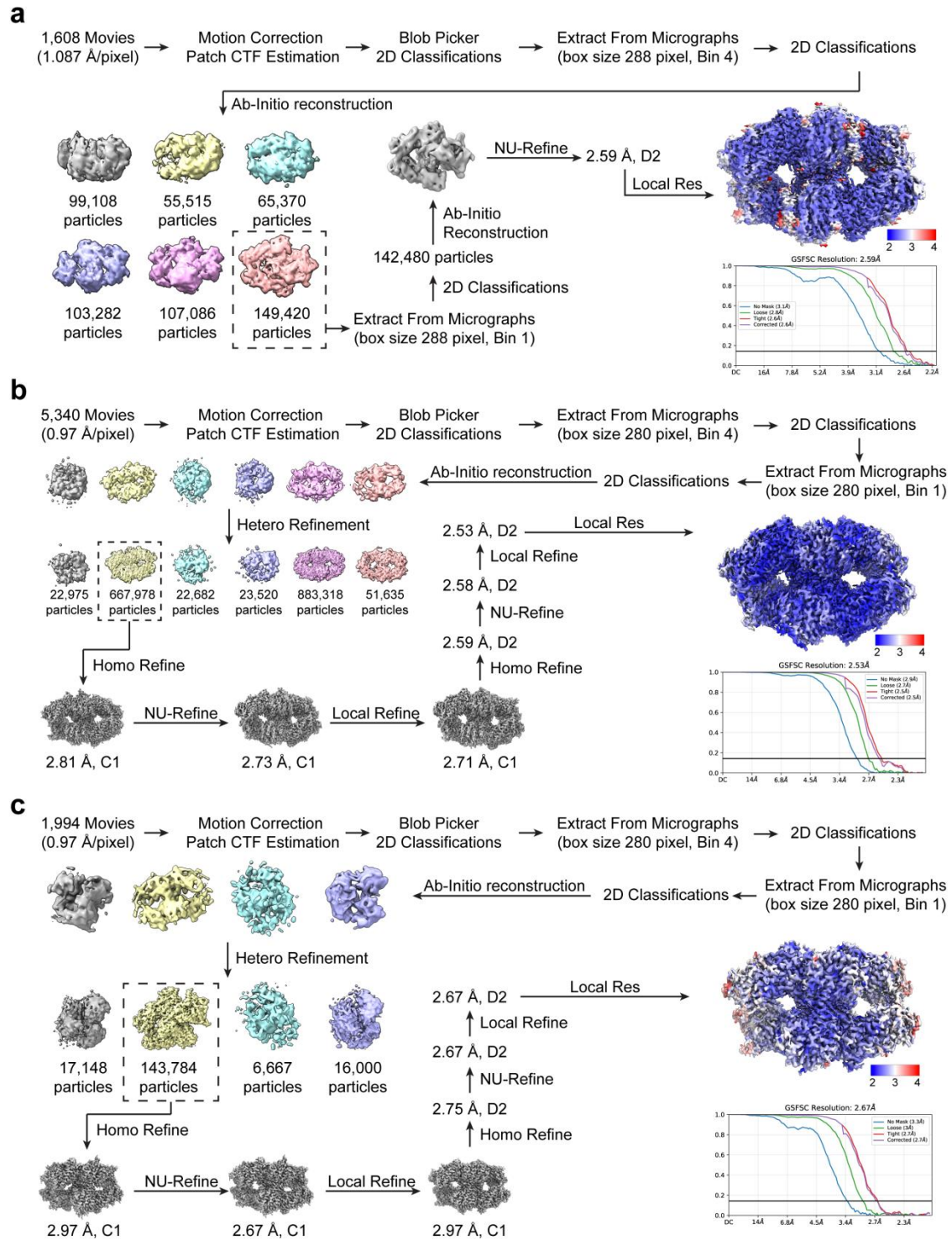
Mechanistic Basis for Selective Csm6-2 Activation by Cyclic Penta-Adenylate in a Type III CRISPR-Cas system

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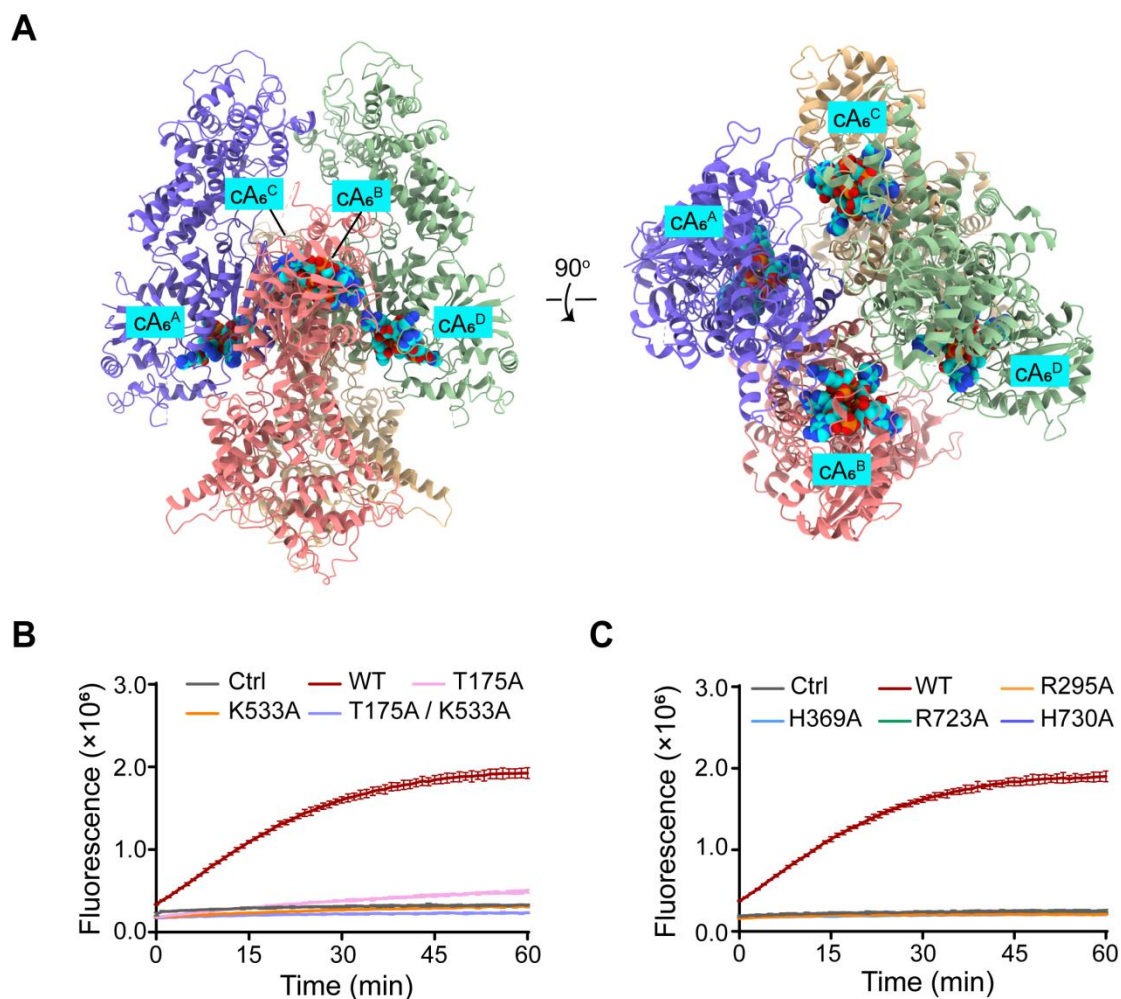
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Appendix Fig S1. Biochemical characterization of Csm6-2-cOA interactions and degradation activity. **A**, Dose-response curves from SPR measurements at indicated concentrations of cA₆ against immobilized Csm6-2 protein. **B**, HPLC analysis of cA₆ degradation by WT and mutant Csm6-2. **C**, **D**, Mass spectra of cA₅ (**C**) and its reaction products after incubation with Csm6-2 (**D**).



Appendix Fig S2. Flowchart of cryo-EM data processing. A, Cryo-EM analysis of Csm6-2. B, Cryo-EM analysis of Csm6-2 in complex with cA₆. C, Cryo-EM analysis of Csm6-2 in complex with cA₅.



Appendix Fig S3. Structure of Csm6-2 in complex with cA₆. **A**, Cartoon representation of Csm6-2-cA₆ complex. cA₆ molecules are depicted as cyan sphere. Each individual Csm6-2 monomer is shown in a distinct color. **B**, **C**, Effect of various mutations on cA₆-dependent activation of Csm6-2 ribonuclease activity. 200 nM Csm6-2 and 200 nM cA₆ were incubated with 200 nM FAM/BHQ1-labeled ssRNA at 37°C. Fluorescence intensities were recorded at 1-min intervals. Values are means $\pm$ SD, n = 3 replicates.