

**Molecular basis of stepwise cyclic tetra-adenylate cleavage by the
type III CRISPR ring nuclease Crn1/Sso2081**

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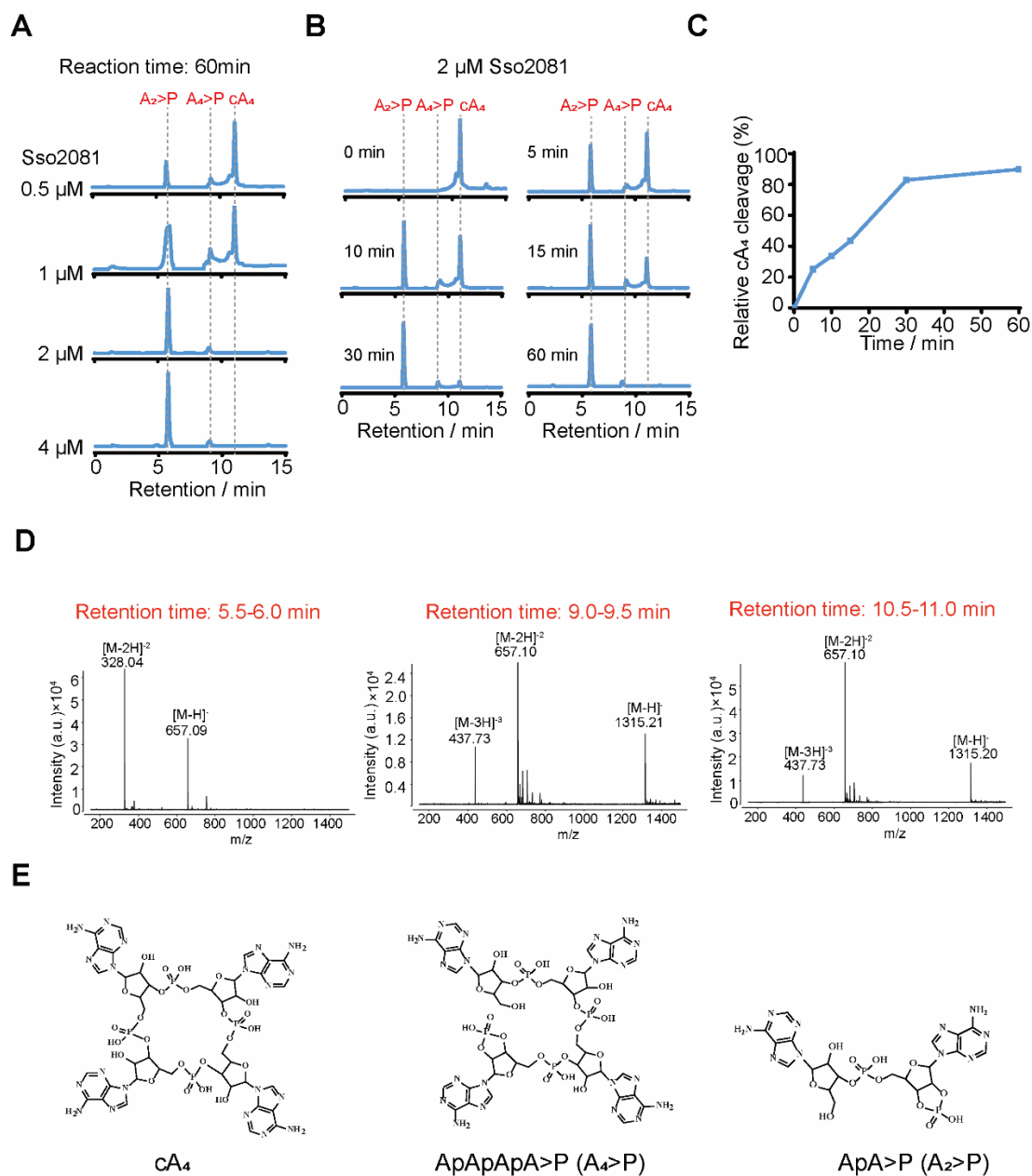
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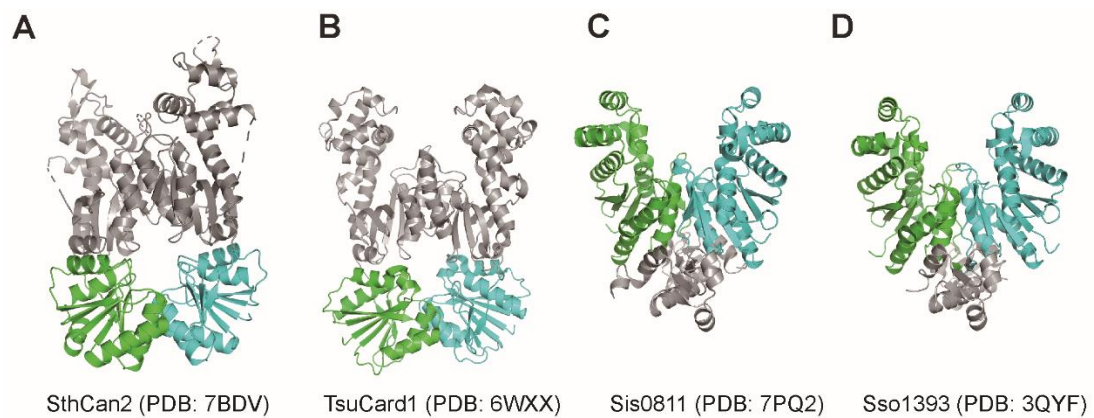
[\(Z. Lin\)](#)

SUPPLEMENTARY FIGURES

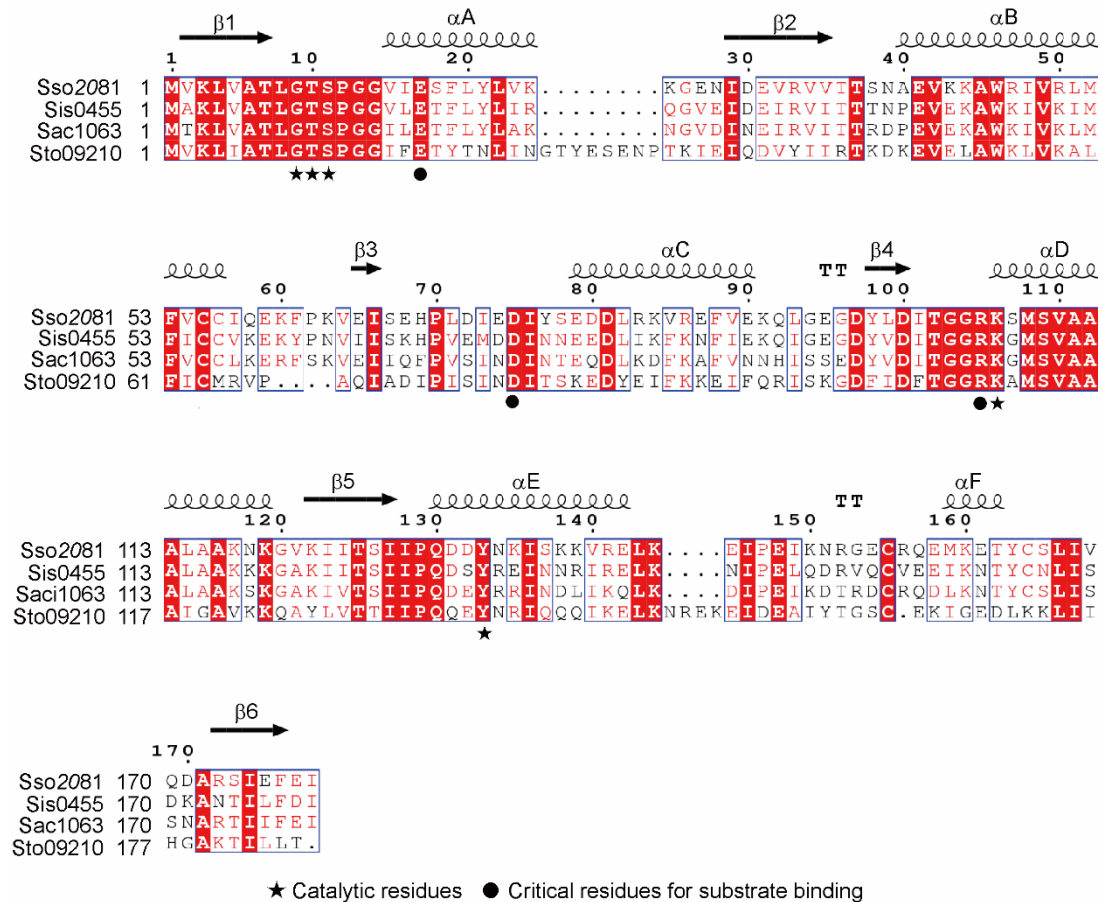


Supplementary Fig. S1 LC and MS analyses of cA₄ cleavage by Sso2081. (A) LC spectra of the reaction products of cA₄ with increasing concentrations of Sso2081. Reactions were conducted at 60 °C for 60 min. **(B)** LC spectra of the reaction products of cA₄ with 2 μM Sso2081 at 60 °C for 0 ~ 60 min. **(C)** The kinetic plot of cA₄ cleavage in (B). **(D)** Mass spectra of the samples eluted from HPLC column at indicated retention

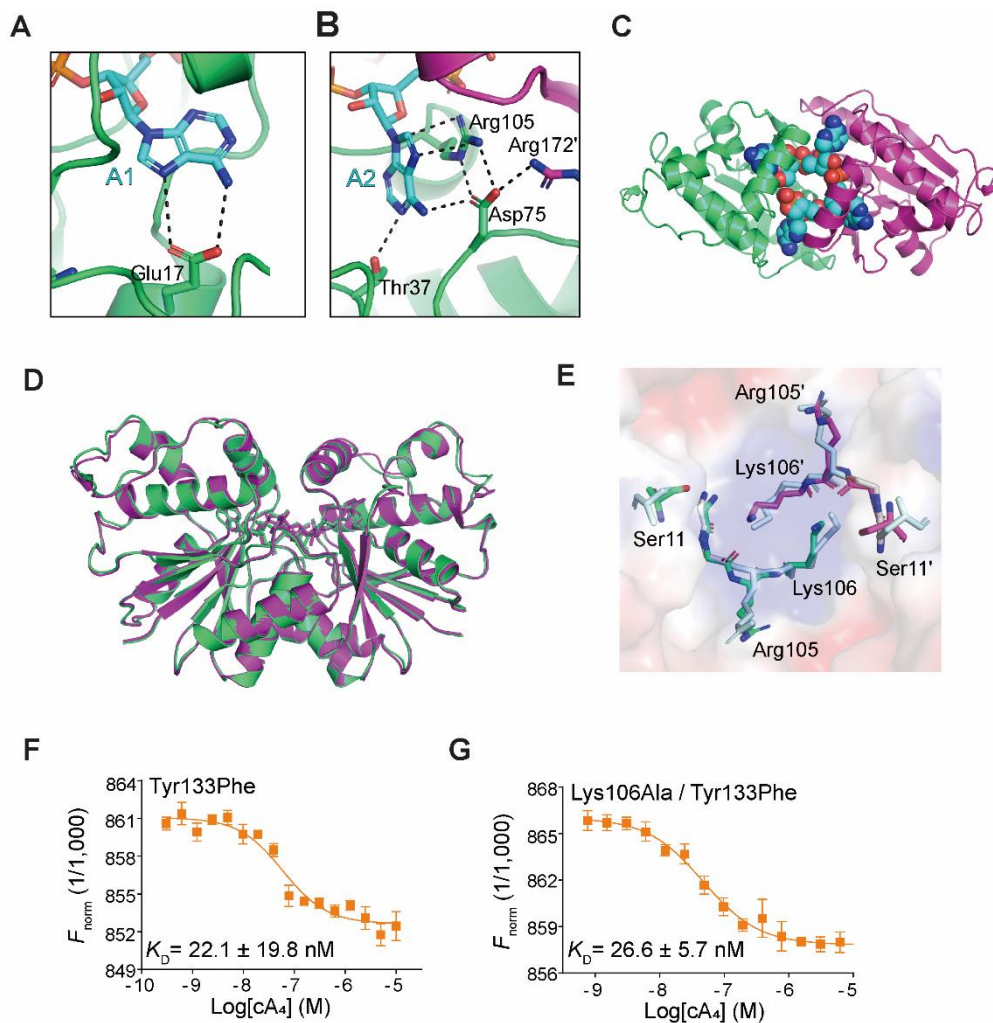
times. Retention time 5.5-6.0: m/z 657.09 for $A_2>P^{-1}$ ($ApA>P^{-1}$); m/z 328.04 for $A_2>P^{-2}$; Retention time 9.0-9.5 min: m/z 1315.21 for $A_4>P^{-1}$ ($ApApApA>P^{-1}$); 657.10 for $A_4>P^{-2}$; 437.73 for $A_4>P^{-3}$; Retention time 10.5-11.0: m/z 1315.20 for cA_4^{-1} ; m/z 657.10 for cA_4^{-2} ; m/z 437.73 for cA_4^{-3} . **(E)** The chemical structures of cA_4 and the cleavage products.



Supplementary Fig. S2 Comparison of the structures between various CARF domain-containing proteins. A-D, Structures SthCan2, TsuCard1, Sis0811 and Sso1393. The structures are shown in cartoon representation. CARF domains are highlighted in green / cyan colors. Sis, *Sulfolobus islandicus*; Sso, *Sulfolobus solfataricus*; Sth, *Sulfobacillus thermosulfidooxidans*; Tsu, *Treponema succinifaciens*.

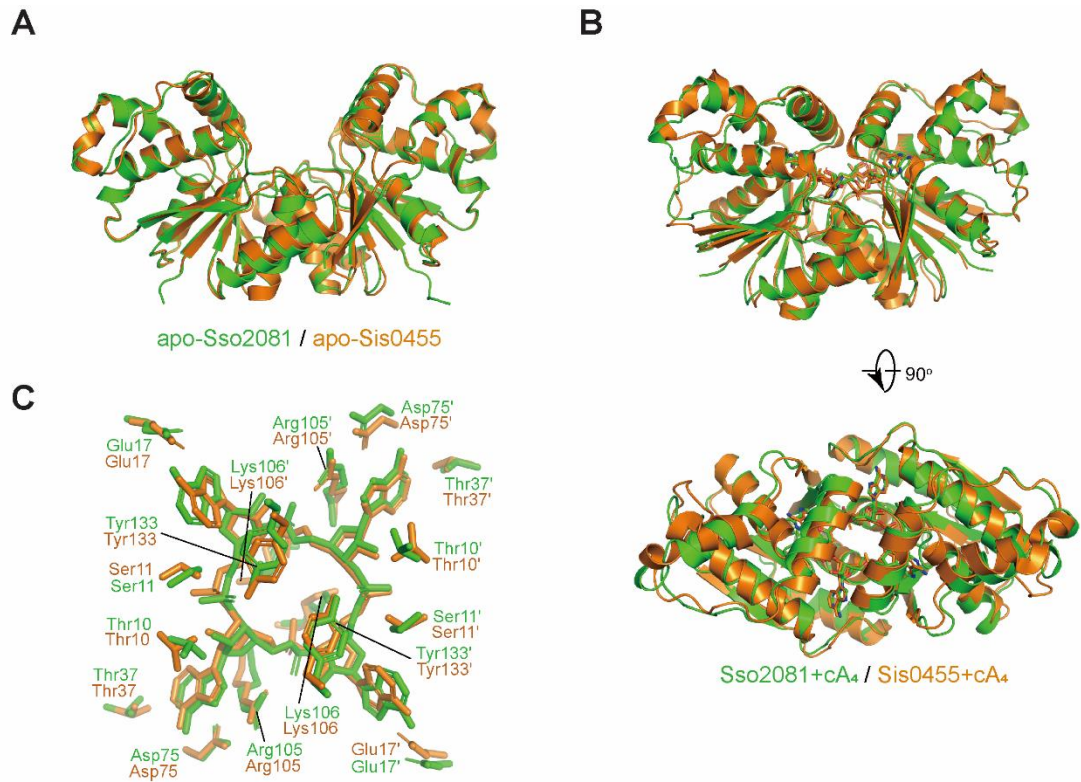


Supplementary Fig. S3 Sequence alignment of Sso2081 with its structural homologs. The alignment is generated using the online ESPript 3.0 server. Secondary structural elements of Sso2081 are indicated above the sequences. Abbreviations: Sso, *Saccharolobus solfataricus*; Sis, *Sulfolobus islandicus*; Sac, *Sulfolobus acidocaldarius*. Sto, *Sulfolobus tokodaii*;



Supplementary Fig. S4 Structural comparison between cA₄- and A₄>P-bound Sso2081. (A, B) The adenine binding sites for A1 (A) and A2 (B) of cA₄ in the complex of Sso2081/cA₄. (C) Top-view of the structure of Sso2081/A₄>P complex. Sso2081 is shown in cartoon representation and cA₄ is in sphere. (D) Superposition of the structures of cA₄- (green) and A₄>P- (magenta) bound Sso2081. (E) Local conformational changes in the active site of Sso2081 between phosphate- (light blue) and cA₄- (green and magenta) bound Sso2081. The structure is overlaid with 80% transparent surface of the active site of cA₄-bound Sso2081. (F, G) Binding isotherms

for cA₄ to Sso2081^{Tyr133Phe} (F) and Sso2081^{Lys106Ala/Tyr133Phe} (G) by the MST binding assay. Values are means \pm SD, n = 3.



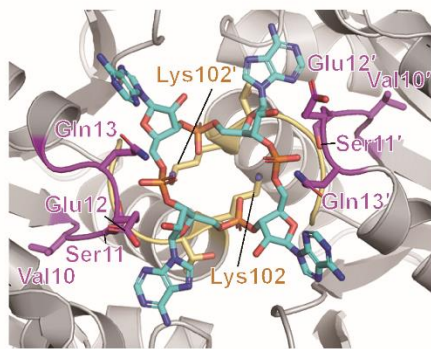
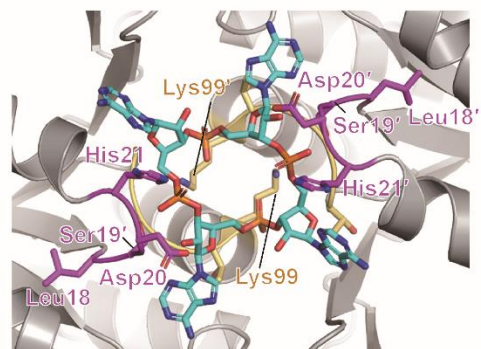
Supplementary Fig. S5 Structural comparison between Sso2081 and Sis0455. (A)

Structural comparison between apo-Sso2081 (green) and apo-Sis0455 (orange, PDB:

7Z56) from side view. **(B)** Structural comparison between cA₄-bound Sso2081 (green)

and Sis0455 (orange, PDB: 7Z55) from both side (top panel) and top (bottom panel)

views. **(C)** Comparison of the cA₄-binding residues between Sso2081 and Sis0455.

ACard1 CARF:cA₄ (PDB:6WXX)**B**Can2 CARF:cA₄ (PDB:7BDV)

Supplementary Fig. S6 The cA₄ binding site of Card1 and Can2. (A) The cA₄ binding site in the structure of TsuCard1 in complex with cA₄ (PDB: 6WXX). (B) The cA₄ binding site of Can2 in the structure of SthCan2 in complex with cA₄ (PDB: 7BDV). cA₄ is shown in cyan stick. Key residues in motif-I and motif-II are labeled and shown in magenta and yellow sticks, respectively.