

Supplementary Table S1. Cryo-EM data collection and refinement statistics

Data collection and processing					
Sample	Cas λ 2–crRNA–DNA Incompetent state	Cas λ 2–crRNA–DNA Intermediate state	Cas λ 2–crRNA–DNA NTS-cleaving state	Cas λ 2–crRNA–DNA TS-cleaving state	Cas λ 2–pre-crRNA Binary complex
EMDB ID	EMD-61038	EMD-61039	EMD-61040	EMD-61041	EMD-61037
PDB ID	9IZP	9IZQ	9IZR	9IZS	9IZM
Microscope			Titan Krios G3i		
Detector			Gatan K3 Camera		
Magnification			105,000		
Voltage (kV)			300		
Electron exposure (e ⁻ /Å ²)			50		
Defocus range (μm)			−0.8 to −1.6		
Pixel size (Å/px)			0.83		
Symmetry imposed			C ₁		
Number of movies	3,514	3,514	3,514	4,280	4,174
Initial particle images	4,084,635	4,084,635	4,084,635	5,253,669	4,767,330
Final particle images	149,163	65,575	97,017	128,740	85,316
Map resolution (Å)	2.89	3.06	2.93	2.84	3.02
FSC threshold			0.143		
Map-sharpening <i>B</i> factor (Å ²)	−80.0	−81.1	−69.4	−69.6	−68.4
3DFSC analysis					
Global resolution (Å)	2.96	3.15	3.02	2.90	3.08
Sphericity	0.879	0.939	0.847	0.808	0.820
Model building and refinement					
Model composition					
Protein atoms	5,246	6,140	6,133	6,133	6,137
Nucleic acid atoms	1,937	2,019	2,059	2,125	1,021
Metal ions	3	2	7	5	2
Model refinement					
Model-Map CC (CC _{mask} / CC _{box} / CC _{peaks} / CC _{volume})	0.83/ 0.60/ 0.62/ 0.79	0.81/ 0.79/ 0.77/ 0.80	0.81/ 0.59/ 0.61/ 0.78	0.78/ 0.58/ 0.59/ 0.75	0.76/ 0.77/ 0.73/ 0.76
Resolution (Å) by model to map FSC, threshold 0.50 (masked/ unmasked)	2.99/ 3.03	3.11/ 3.11	3.02/ 3.06	2.97/ 3.02	3.07/ 3.07
Average <i>B</i> factor (Å ²) (protein/ nucleotides/ ligands)	61.47/ 48.68/ 33.80	55.72/ 75.47/ 28.33	52.19/ 51.95/ 75.80	49.40/ 52.12/ 74.42	42.99/ 63.16/ 34.15
R.M.S. deviations from ideal					
Bond lengths (Å)	0.005	0.004	0.004	0.004	0.005
Bond angles (°)	0.552	0.566	0.535	0.556	0.603
Validation					
MolProbity score	1.62	1.62	1.57	1.88	1.86
CaBLAM outliers (%)	1.29	3.27	1.36	1.36	2.99
Clashscore	8.10	7.86	7.38	7.01	10.91
Rotamer outliers (%)	0.00	0.58	1.02	2.03	0.87
Cβ outliers (%)	0.00	0.00	0.00	0.00	0.00
EMRinger score	2.84	2.79	3.28	3.56	2.51
Ramachandran plot					
Favored (%)	96.96	96.88	97.15	96.20	95.66
Allowed (%)	3.04	3.12	2.85	3.80	4.34
Outlier (%)	0.00	0.00	0.00	0.00	0.00

Supplementary Table S1

Primers used in this study.

Oligo	Sequence
RNA-T7-f	GGATCCTAATACGACTCACTATAGG
crRNA-r	GCCACGCGCACCTCATCTCCGTCTTGGCCTTCGCCCCGCAAGCTGGGCTA TGACACCCTATAGTGAGTCGTATTAGGATCC

Target DNA sequences used for the structural determination

Name	Sequence
Target DNA strand	GATGGTGCCACGCGCACCTCATCTCCCAAATAGACA
Non-target DNA strand	TGTCTATTTGGGAGATGAGGTGCGCGTGGCACCATC

Supplementary Table S2