

Supplementary Table 1. Data collection, processing, model refinement, and validation for IscB.m13.

Sample	IscB.m13 pre-R-loop	IscB.m13 early R-loop	IscB.m13 late R-loop
EMDB ID	EMD-81326	EMD-81327	EMD-81328
PDB ID	27PP	27PQ	27PR
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)	0.83		
Symmetry imposed	C ₁		
Number of movies	4,452		5,413
Initial particle images (no.)	3,772,093		5,044,029
Final particle images (no.)	71,392	80,465	117,593
Map resolution (Å)	2.80	2.60	2.99
FSC threshold	0.143		
3DFSC analysis			
Global resolution (Å)	3.41	3.31	3.61
Sphericity	0.980	0.980	0.857
Model building and refinement			
Map sharpening <i>B</i> factor (Å ²)	−67.6	−73.5	−71.5
Protein atoms	481	481	384
Model composition			
Nucleic acid atoms	215	226	234
Other atoms	2	2	0
Resolution (Å) by model to map FSC, threshold 0.50	2.78	2.68	2.97
Model refinement			
Average <i>B</i> factor (Å ²) (protein/RNA/ DNA/Other atoms)	77.3/142.6/187.6/133.9	88.1/137.6/143.6/167.5	93.4/151.4/131.7/NaN
R.m.s. deviations			

Bond lengths (Å)	0.0077	0.0072	0.006
Bond angles (°)	1.46	1.52	1.36
Validation			
MolProbity score	1.18	1.26	1.41
Clashscore	2.18	2.6	3.27
Rotamer outliers (%)	0.99	0.74	0
C _β outliers (%)	1.36	1.13	1.12
Ramachandran plot			
Favored (%)	96.87	96.66	95.79
Allowed (%)	3.13	3.34	4.21
Outliers (%)	0	0	0

Bond lengths (Å)	0.0276	0.0284	0.0254	0.03	0.0203	0.0218	0.0241
Bond angles (°)	1.79	1.75	1.7	1.46	1.39	1.53	1.46
Validation							
MolProbity score	2.27	1.89	1.7	1.18	0.98	1.04	0.82
Clashscore	9.61	5.65	4.07	0.97	0.37	0.64	0.47
Rotamer outliers (%)	3.7	2.81	2.34	1.25	1.53	0.73	0.46
C _β outliers (%)	0.32	0.44	0.15	0	0	0	0
Ramachandran plot							
Favored (%)	95.2	96.37	96.51	95.45	96.73	95.27	97.17
Allowed (%)	4.8	3.49	3.49	4.55	3.27	4.73	2.83
Outliers (%)	0.00	0.14	0.00	0.00	0.00	0.00	0.00

Supplementary Table 3. Data collection, processing, model refinement, and validation for CjCas9.

Sample	CjCas9 early R-loop	CjCas9 middle R-loop	CjCas9 late R-loop-I	CjCas9 late R-loop-II
EMDB ID	EMD-81321	EMD-81322	EMD-81323	EMD-81324
PDB ID	27PJ	27PK	27PL	27PM
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)	0.83			
Symmetry imposed	C ₁			
Number of movies	8,428	4,543	5,091	
Initial particle images (no.)	7,918,902	3,956,469	6,499,577	
Final particle images (no.)	72,494	54,963	171,614	260,322
Map resolution (Å)	2.94	2.71	2.89	2.61
FSC threshold	0.143			
3DFSC analysis				
Global resolution (Å)	2.96	2.93	2.96	2.69
Sphericity	0.900	0.916	0.872	0.971
Model building and refinement				
Map sharpening <i>B</i> factor (Å ²)	−78.1	−70.1	−82.7	−86.2
Protein atoms	5,105	7,865	6,406	7,981
Model composition				
Nucleic acid atoms	2,564	2,838	3,190	3,437
Other atoms	2	2	0	8
Resolution (Å) by model to map FSC, threshold 0.50	2.97	2.89	2.86	2.68
Model refinement				
Average <i>B</i> factor (Å ²) (protein/RNA/ DNA/Other atoms)	78.4/76.7/110.7 /65.7	101.4/70.5/79 /64.8	96.6/87.1/124.8 /NaN	76.1/67.5/103.9 /64.7
R.m.s. deviations				

Bond lengths (Å)	0.0075	0.0061	0.0058	0.009
Bond angles (°)	1.53	1.52	1.42	1.62
Validation				
MolProbity score	1.00	1.29	1.10	1.12
Clashscore	0.49	1.83	0.90	0.89
Rotamer outliers (%)	0.55	0.47	0.58	0.57
C _β outliers (%)	0.51	0.33	0	0.43
Ramachandran plot				
Favored (%)	95.21	94.94	95.34	95.02
Allowed (%)	4.79	5.06	4.66	4.98
Outliers (%)	0	0	0	0

Supplementary Table 4. Data collection, processing, model refinement, and validation for PsCas9.

Sample	PsCas9 early R-loop	PsCas9 middle R-loop	PsCas9 late R-loop-I	PsCas9 late R-loop-II
EMDB ID	EMD-81331	EMD-81332	EMD-81333	EMD-81334
PDB ID	27PU	27PV	27PW	27PX
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)	0.83			
Symmetry imposed	C ₁			
Number of movies	5,535			
Initial particle images (no.)	3,028,412			
Final particle images (no.)	48,542	90,162	44,741	62,378
Map resolution (Å)	3.29	3.12	3.04	2.69
FSC threshold	0.143			
3DFSC analysis				
Global resolution (Å)	3.52	3.21	3.15	2.87
Sphericity	0.797	0.900	0.865	0.974
Model building and refinement				
Map sharpening <i>B</i> factor (Å ²)	−79.4	−88.4	−58.9	−72.8
Protein atoms	11,425	11,425	9,911	11,314
Model composition				
Nucleic acid atoms	3,071	3,259	3,957	4,060
Other atoms	2	4	4	7
Resolution (Å) by model to map FSC, threshold 0.50	3.54	3.30	3.06	2.86
Model refinement				
Average <i>B</i> factor (Å ²) (protein/RNA/ DNA/Other atoms)	85.1/89.0/85.5 /29.7	139.0/115.8/141.5 /59.6	77.8/75.8/132.6 /53.2	64.4/59.5/89.3 /63.9
R.m.s. deviations				

Bond lengths (Å)	0.0063	0.0059	0.0054	0.0075
Bond angles (°)	1.39	1.3	1.32	1.48
Validation				
MolProbity score	1.45	1.06	1.09	1.05
Clashscore	5.71	0.79	2.97	2.00
Rotamer outliers (%)	1.27	0.32	0.18	1.04
C _β outliers (%)	0.37	0	0.09	1.13
Ramachandran plot				
Favored (%)	97.73	95.52	98.1	97.7
Allowed (%)	2.27	4.48	1.9	2.3
Outliers (%)	0	0	0	0