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Supplemental Information

Structure Studies of the CRISPR-Csm Complex Reveal Mechanism of Co-transcriptional Interference

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Table S1. Crystallographic Data Collection and Refinement Statistics. Related to Figure 1.

	Csm complex
Data collection*	
Space group	$P2_1$
Cell dimensions	
***a*, *b*, *c* (Å)**	123.54, 82.33, 161.36
α, β, γ (°)	90.00, 99.32, 90.00
Resolution (Å)	50.00-2.90 (3.00-2.90)
R_{merge}	0.069 (0.446)
$I/\sigma I$	8.0 (2.0)
Completeness (%)	97.2 (94.7)
Redundancy	4.5 (3.1)
Refinement	
Resolution (Å)	2.90
No. reflections	61,018
R_{work} / R_{free}	0.200/0.235
No. atoms	
protein	17,386
Nucleic Acid	670
***B*-factors (Å^2)**	
Protein	53.9
Nucleic Acid	46.9
R.m.s. deviations	
Bond length (Å)	0.010
Bond angles (°)	1.388
Ramachandran plot	
Favored region	95.3
Allowed region	4.2
Outlier region	0.5

* Highest resolution shell is shown in parentheses.

Table S2. Statistics of cryo-EM Data Processing and Model Refinement. Related to Figure 2 and Figure 3.

	Csm-CTR1	Csm-CTR2-ssDNA	Csm-CTR2-dsDNA	Csm-NTR
	EMD-9658 PDB-6IFY	EMD-9659 PDB-6IFZ	EMD-9657 PDB-6IFU	EMD-9654 PDB-6IFL
Data collection				
Electron microscope	Titan Krios			
Voltage (kV)	300			
Electron detector	K2 Summit			
Electron dose (e-/Å2)	60 (32 frames)			
Defocus range (μm)	1.0~3.0			
Pixel size (Å)	1.04			
3D Reconstruction				
Raw images	1,202	3,309	1,218	1,541
Initial particles autopicked	307,576	1,700,254	684,150	611,889
Particle for final refinement	46,360	109,805	75,887	133,127
Resolution of final map (Å)	3.80	3.58	3.05	3.16
FSC threshold	0.143	0.143	0.143	0.143
Map sharpening B-factor (Å2)	-139	-126	-115	-119
Model refinement				
R.m.s. deviations				
Bond length (Å)	0.008	0.013	0.008	0.006
Bond angles (°)	1.59	1.80	1.45	1.43
Ramachandran statistics (%)				
Favored regions	88.81	88.59	86.32	88.57
Allowed regions	11.19	11.32	12.86	10.92
Outlier regions	0.00	0.09	0.82	0.51
Rotamer outlier (%)	0.43	0.59	1.68	0.93
Clash score	9.56	7.71	8.13	6.81

Table S3. Statistics of cryo-EM Data Processing and Model Refinement. Related to Figure 4.

	Csm-CTR1-AMPPNP	Csm-CTR1-ATP	Csm-NTR-ATP
	EMD-9653	EMD-9660	EMD-9656
	PDB-6IFK	PDB-6IG0	PDB-6IFR
Data collection			
Electron microscope	Titan Krios		
Voltage (kV)	300		
Electron detector	K2 Summit		
Electron dose (e-/Å2)	60 (32 frames)		
Defocus range (µm)	1.0~3.0		
Pixel size (Å)	1.04		
3D Reconstruction			
Raw images	893	1,525	1,477
Initial particles autopicked	364,667	598,192	644,624
Particle for final refinement	61,538	58,587	54,012
Resolution of final map (Å)	3.20	3.37	3.40
FSC threshold	0.143	0.143	0.143
Map sharpening B-factor (Å2)	-128	-133	-133
Model refinement			
R.m.s. deviations			
Bond length (Å)	0.007	0.009	0.007
Bond angles (°)	1.30	1.35	1.33
Ramachandran statistics (%)			
Favored regions	89.50	88.31	87.07
Allowed regions	10.32	11.46	12.75
Outlier regions	0.18	0.22	0.18
Rotamer outlier (%)	0.85	0.64	0.80
Clash score	6.84	7.13	7.45

Table S4. Oligonucleotides Used in This Study. Related to Key Resources Table.

Type	Sequence 5′ to 3′	Source
CRISPR template	CATATGGATATAAACCTAATTACCTCGAGAGGGGACGGAAACGCTTTCTAGCTCGCTATAATTACCCATTCCTAGAAAGATATAAACCTAATTACCTCGAGAGGGGACGGAAACGCTTTCTAGCTCGCTATAATTACCCATTCCTAGAAAGATATAAACCTAATTACCTCGAGAGGGGACGGAAACGCTTTCTAGCTCGCTATAATTACCCATTCCTAGAAAGATATAAACCTAATTACCTCGAGAGGGGACGGAAACGCTTTCTAGCTCGCTATAATTACCCATTCCTAGAAAGATATAAACCTAATTACCTCGAGAGGGGACGGAAACGCTTTCTAGCTCGCTATAATTACCCATTCCTAGAAAGATATAAACCTAATTACCTCGAGAGGGGACGGAAACGCTTTCTAGCTCGCTATAATTACCCATTCCTAGAAAGATATAAACCTAATTACCTCGAGAGGGGACGGTACC	Sangon Biotech
RNA (NTR)	GGUAGGAAUGGGUAAUUAUAGCGAGCUAGAAAGCGUUUCCGUC	In vitro transcription
RNA (CTR1)	GGUAGGAAUGGGUAAUUAUAGCGAGCUAGAAAGCCAAAGGUC	In vitro transcription
RNA (CTR1 (-2)' mutation)	GGUAGGAAUGGGUAAUUAUAGCGAGCUAGAAAGCCUAAGGUC	In vitro transcription
RNA (CTR1 (-3)' mutation)	GGUAGGAAUGGGUAAUUAUAGCGAGCUAGAAAGCCAUAGGUC	In vitro transcription
RNA (CTR1 (-4)' mutation)	GGUAGGAAUGGGUAAUUAUAGCGAGCUAGAAAGCCAAUGGUC	In vitro transcription
RNA (CTR1 (-5)' mutation)	GGUAGGAAUGGGUAAUUAUAGCGAGCUAGAAAGCCAAACGUC	In vitro transcription
RNA (CTR1 (-2)'-(-3)' mutation)	GGUAGGAAUGGGUAAUUAUAGCGAGCUAGAAAGCCUUAGGUC	In vitro transcription
RNA (CTR1 (-4)'-(-5)' mutation)	GGUAGGAAUGGGUAAUUAUAGCGAGCUAGAAAGCCAAUCGUC	In vitro transcription
RNA (CTR1 (-2)'-(-4)' mutation)	GGUAGGAAUGGGUAAUUAUAGCGAGCUAGAAAGCCUUUGGUC	In vitro transcription
RNA (CTR1 (-3)'-(-5)' mutation)	GGUAGGAAUGGGUAAUUAUAGCGAGCUAGAAAGCCAUUCGUC	In vitro transcription
RNA (CTR1 (-2)'-(-5)' mutation)	GGUAGGAAUGGGUAAUUAUAGCGAGCUAGAAAGCCUUUCGUC	In vitro transcription
RNA (CTR2)	GGUAGGAAUGGGUAAUUAUAGCGAGCUAGAAAGCCAAAGGAAGUUUUGUC	In vitro transcription
RNA (for RNA cleavage assays)	GGUACCAUACCAAUCCGUAGACCCUAGCCCAGUAGGAAUGGGUAAUUAUAGCGAGCUAGAAAGCCAAAGGAAGUUUUGUC	In vitro transcription
ssDNA used in Csm-CTR2-ssDNA complex	TTTTTTTTAACTTCCTTATTTTTTT	Sangon Biotech
DNA used in Csm-CTR2-dsDNA complex	5'-TTTTTTTTACTTCCTTTCCTTTTTT-3' |||||||| |||||| 3'-AAAAAAAACCTTCAAAAGTAAAAAA-5'	Sangon Biotech

DNA used for in vitro cleavage assay	5'-Cy3-TTTTTTTTAACTTCCTTATTTTTTT-3'
	        |||||||| |||||||
	      3'-AAAAAAAATCCTTCAAATAAAAAAA-5'
		Sangon Biotech