

Fig. S1. Negative staining EM of RecA filaments formed with or without DinI, full-length LexA, and NTD truncated LexA.

(A) A representative EM photo of RecA filaments in the absence of DinI. The scale bar is 100 nm. Fifteen random fields were imaged with similar results.

(B) A representative EM photo of RecA filaments in the presence of DinI. The scale bar is 100 nm. Fifteen random fields were imaged with similar results.

(C) A representative EM photo of RecA filaments in the presence of full-length LexA (K156A). The scale bar is 100 nm. Fifteen random fields were imaged with similar results.

(D) A representative EM photo of RecA filaments in the presence of NTD truncated LexA (K156A). The scale bar is 100 nm. Fifteen random fields were imaged with similar results.

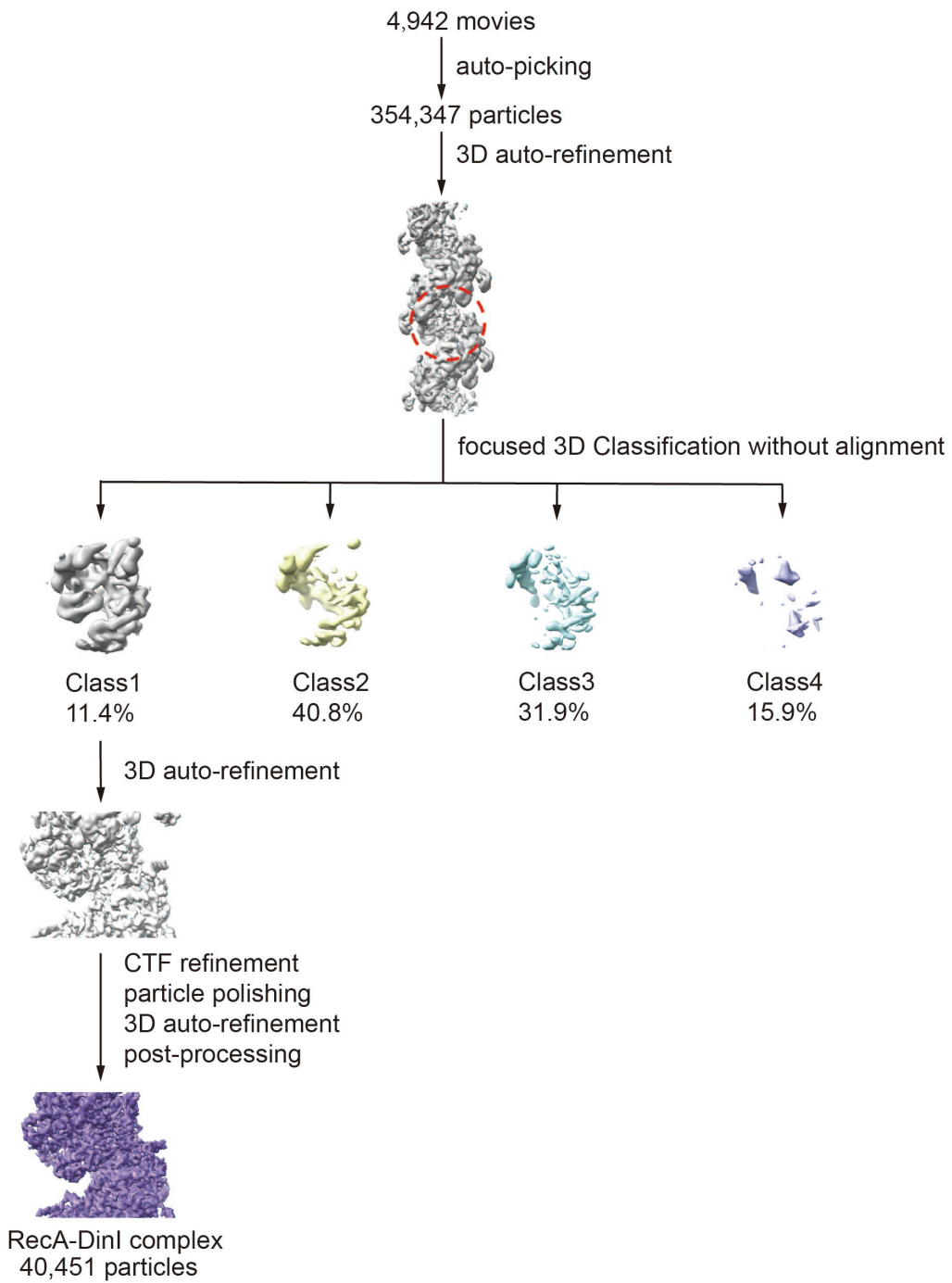


Fig. S2. Data processing pipeline for the dataset of RecA-DinI.

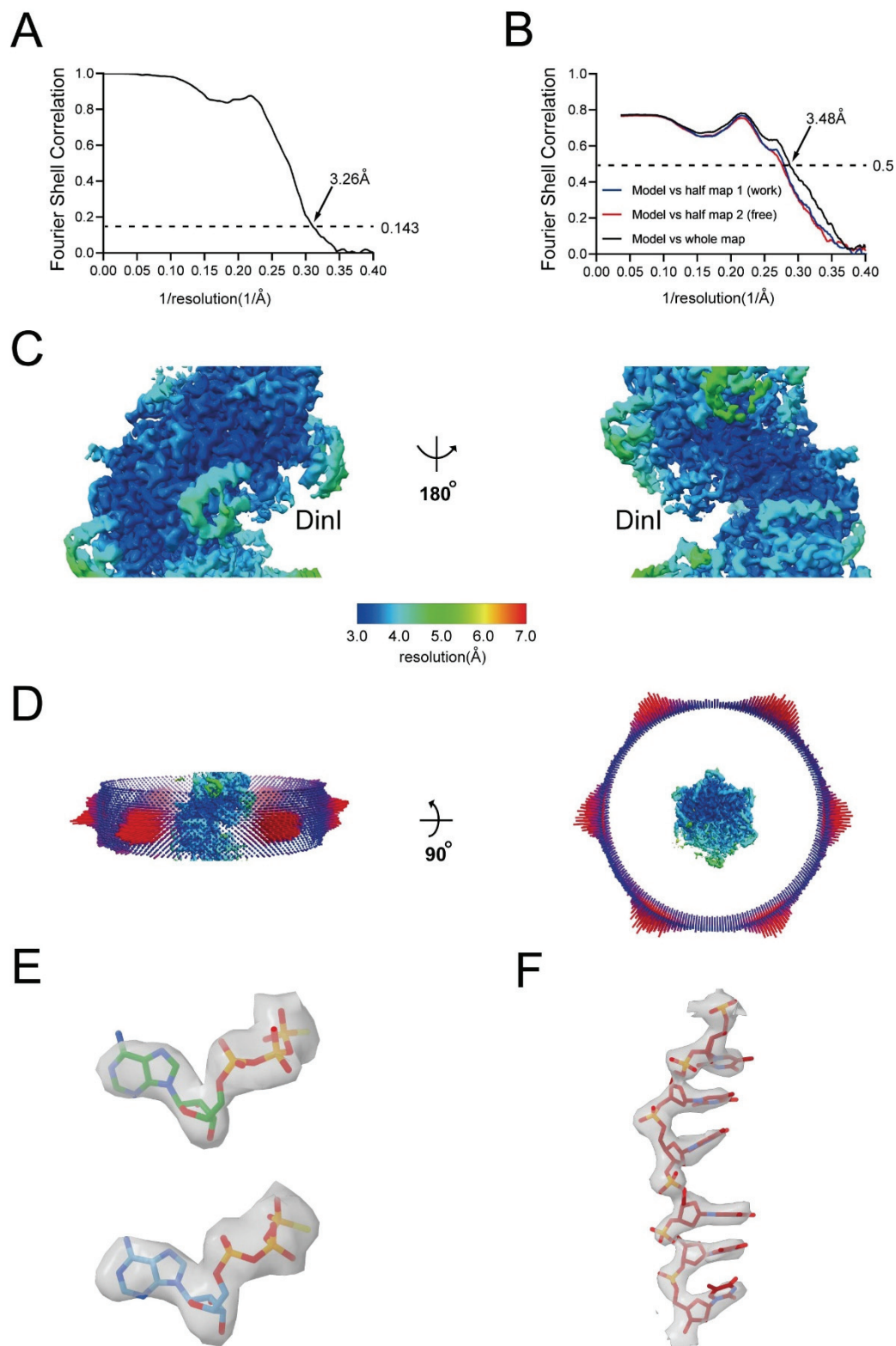


Fig. S3. Data validation for RecA-DinI.

(A) The gold-standard FSC of RecA-DinI. The gold-standard FSC is calculated by comparing the two independently determined half-maps from RELION. The dashed line represents the 0.143 FSC cutoff.

(B) FSC calculated between the model and the half map used for refinement (work), the other half map (free), and the full map.

(C) Cryo-EM density map colored by local resolution.

(D) Angular distribution of particle projections.

(E) The cryo-EM density map of two ATP γ S molecules in each asymmetrical unit.

(F) The cryo-EM density of 6-nt oligo (dT) ssDNA in each asymmetrical unit.

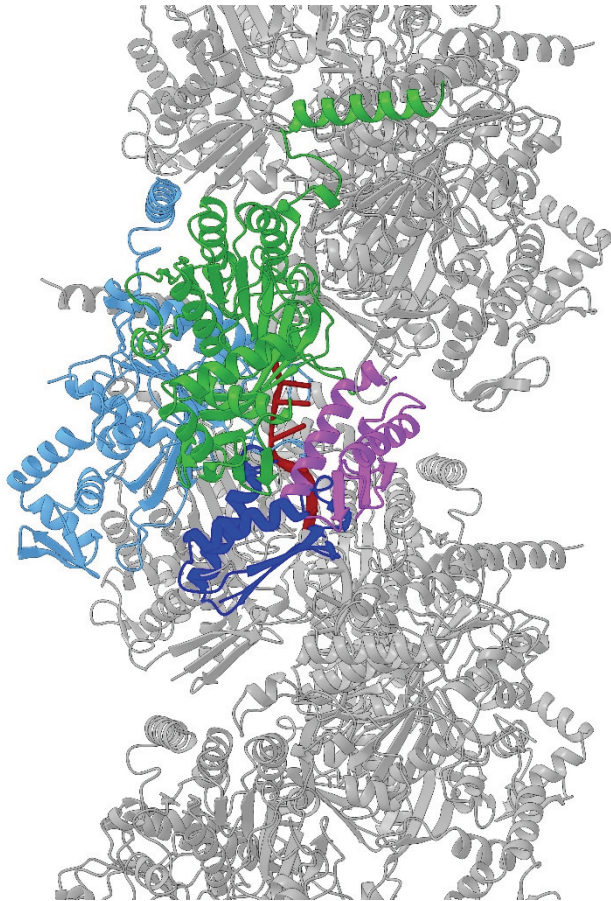


Fig. S4. The asymmetrical unit of RecA-DinI is composed of two RecA protomers and one DinI molecule.

The asymmetrical unit of RecA-DinI is colored as in Fig. 1A. If a second DinI molecule (dark blue) was modeled into the asymmetrical unit, there would be a steric clash between DinI molecules.

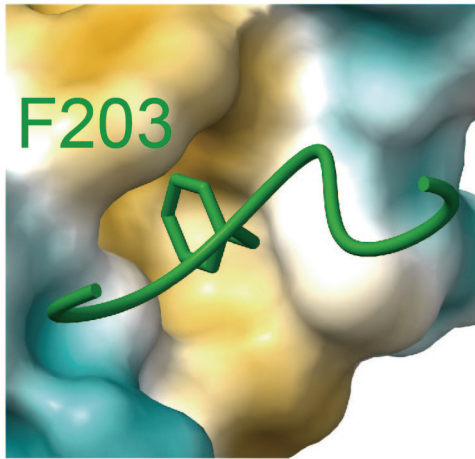


Fig. S5. F203 of RecA binds into a hydrophobic pocket in DinI.

DinI surface is colored according to hydrophobic potential. The surface coloring ranges from dark goldenrod for the most hydrophobic potentials to dark cyan for the most hydrophilic potentials.

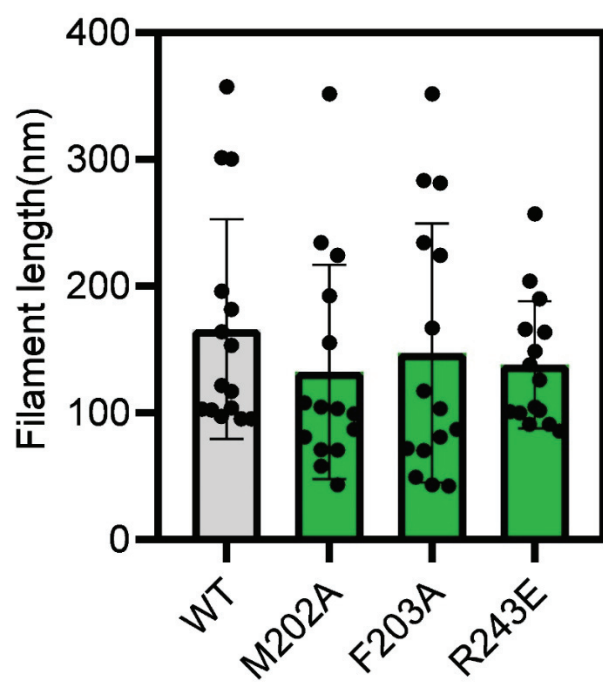


Fig. S6. Substitution of RecA residues doesn't affect RecA filament formation in the absence of DinI.

Fifteen random fields were imaged and the lengths of filaments longer than 40 nm were summed for each field.

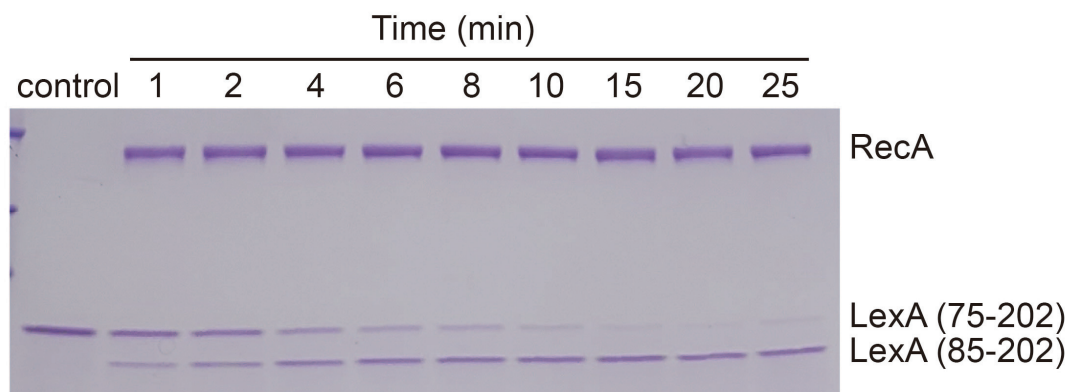


Fig. S7. LexA (residues 75 - 202) undergoes RecA mediated cleavage.

Experiments were repeated independently three times with similar results.

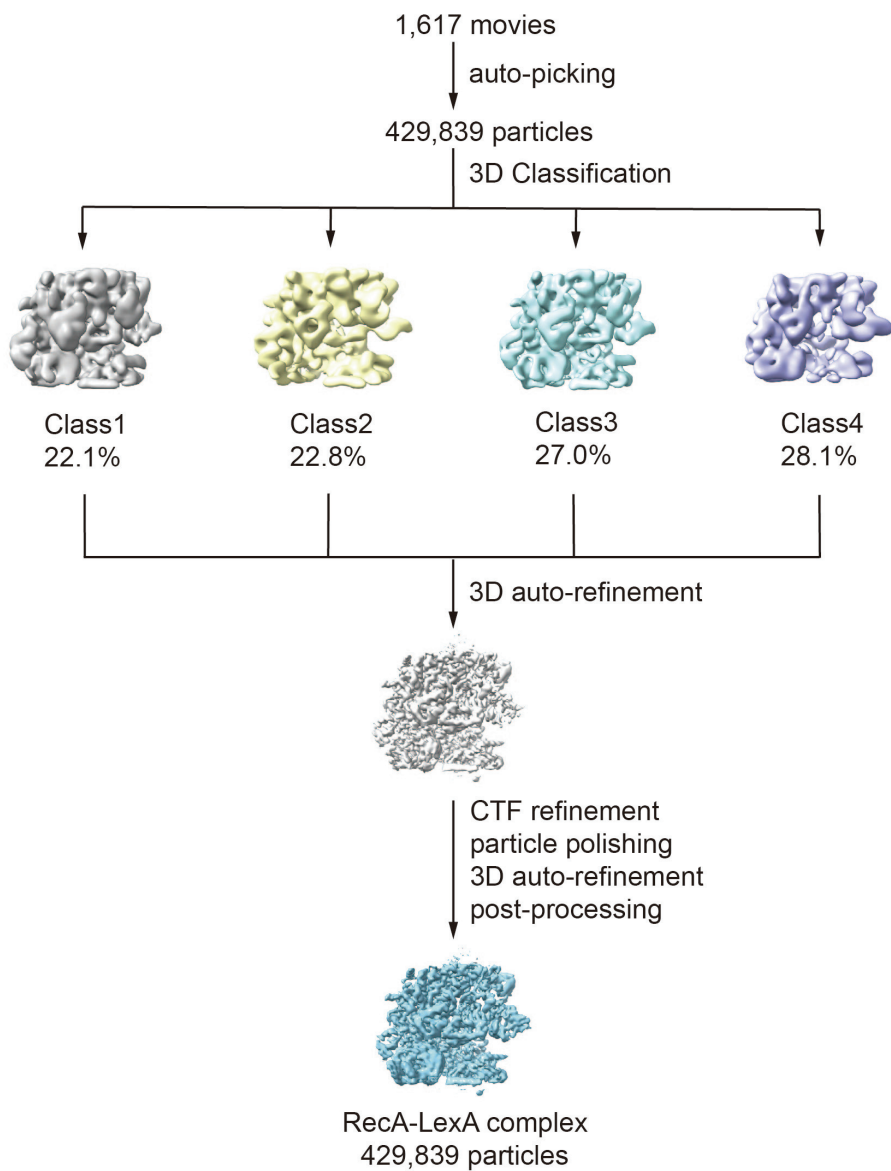


Fig. S8. Data processing pipeline for the dataset of RecA-LexA.

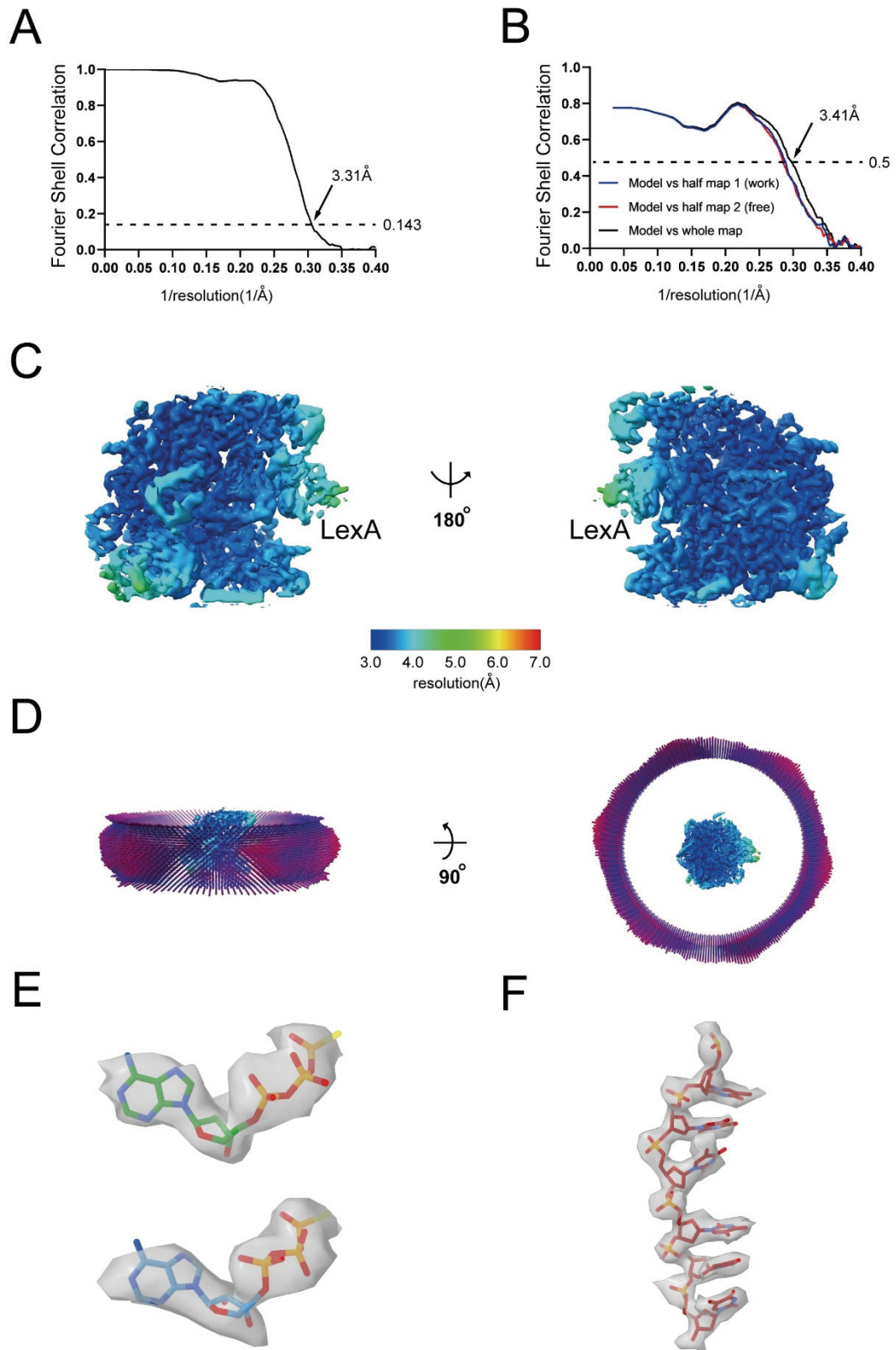


Fig. S9. Data validation for RecA-LexA.

(A) The gold-standard FSC of RecA-LexA. The gold-standard FSC is calculated by comparing the two independently determined half-maps from RELION. The dashed line represents the 0.143 FSC cutoff.

(B) FSC calculated between the model and the half map used for refinement (work), the other half map (free), and the full map.

(C) Cryo-EM density map colored by local resolution.

(D) Angular distribution of particle projections.

(E) The cryo-EM density map of two ATP γ S molecules in each asymmetrical unit.

(F) The cryo-EM density of 6-nt oligo (dT) ssDNA in each asymmetrical unit.

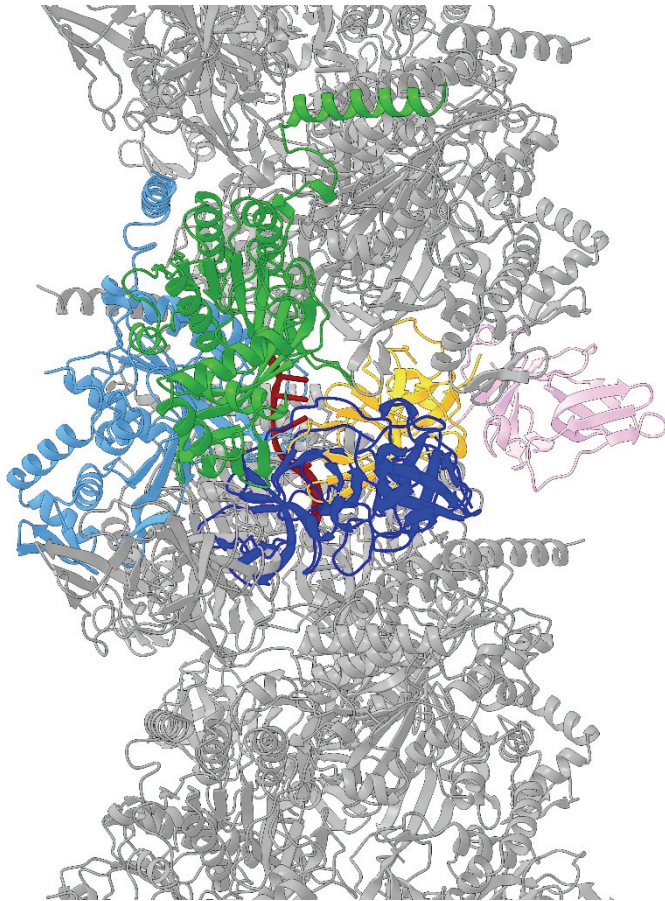


Fig. S10. The asymmetrical unit of RecA-LexA is composed of two RecA protomers and one LexA dimer.

The asymmetrical unit of RecA-LexA is colored as in Fig. 2A. If a second LexA dimer (dark blue) was modeled into the asymmetrical unit, there would be a steric clash between LexA dimers.

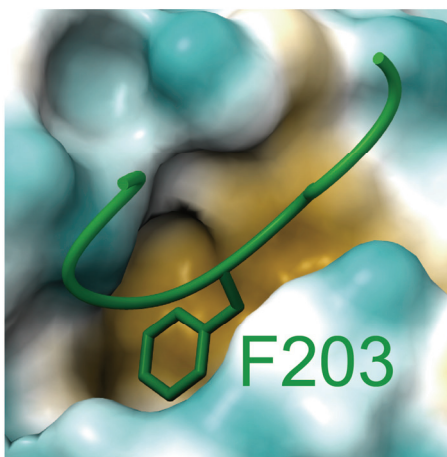


Fig. S11. F203 of RecA binds into a hydrophobic pocket in LexAⁱ.

LexA surface is colored according to hydrophobic potential. The surface coloring ranges from dark goldenrod for the most hydrophobic potentials to dark cyan for the most hydrophilic potentials.

▼▼

RECA_ECOLI	171	MSQAMRKL	LAGN	LKQ	SNTLLI	FINQI	IRMKI	GVMFGN	PETT	GGN	ALKFY	ASVRLD	IRRI	GA
RECA_SHISO	171	MSQAMRKL	LAGN	LKQ	SNTLLI	FINQI	IRMKI	GVMFGN	PETT	GGN	ALKFY	ASVRLD	IRRI	GA
RECA_SALTY	171	MSQAMRKL	LAGN	LKQ	SNTLLI	FINQI	IRMKI	GVMFGN	PETT	GGN	ALKFY	ASVRLD	IRRI	GA
RECA_SALPK	171	MSQAMRKL	LAGN	LKQ	SNTLLI	FINQI	IRMKI	GVMFGN	PETT	GGN	ALKFY	ASVRLD	IRRI	GA
RECA_KLEP7	171	MSQAMRKL	LAGN	LKQ	SNTLLI	FINQI	IRMKI	GVMFGN	PETT	GGN	ALKFY	ASVRLD	IRRI	GA
ReCa_ENTCC	171	MSQAMRKL	LAGN	LKQ	SNTLLI	FINQI	IRMKI	GVMFGN	PETT	GGN	ALKFY	ASVRLD	IRRI	GA
RECA_VIBCH	169	LSQAMRKL	LTGN	LKQ	SNCMCI	FINQI	IRMKI	GVMFGN	PETT	GGN	ALKFY	ASVRLD	IRRT	GA
RECA_HAEIN	171	MSQAMRKL	LTGQ	IKNAN	CLVVF	FINQI	IRMKI	GVMFGN	PETT	GGN	ALKFY	ASVRLD	IRRT	GS
RECA_PSEAE	169	MSQAMRKL	ITCNI	IKNAN	CLVVF	FINQI	IRMKI	GVMFGN	PETT	GGN	ALKFY	ASVRLD	IRRT	GA
RECA_ACIBC	169	MSQALRKL	ITGN	AKRSN	CMVIF	FINQI	IRMKI	GVMFGS	PETT	GGN	ALKFY	ASVRLD	IRRI	GQ
RECA_MYCTU	171	MSQALRKM	TGAL	NNSG	TTAIF	INQI	LRDKI	GVMFGS	PETT	GGK	ALKFY	ASVRMD	VRRV	ET
RECA_MYCA1	171	MSQALRKM	TGAL	NNSG	TTAIF	INQI	LRDKI	GVMFGS	PETT	GGK	ALKFY	ASVRMD	VRRI	ET
RECA_MYCS2	171	MSQALRKM	TGAL	NNSG	TTAIF	INQI	LRDKI	GVMFGS	PETT	GGK	ALKFY	ASVRMD	VRRI	ET
RECA_MYCA9	171	MSQALRKM	TGAL	NNSG	TTAIF	INQI	LRDKI	GVMFGS	PETT	GGK	ALKFY	ASVRMD	VRRI	ET
RECA_STAAC	169	MSQALRKL	LSGAI	ISK	SNTTAI	FINQI	IREKVI	GVMFGN	PETT	GGR	ALKFY	SSVRLE	VRRAE	EQ
RECA_STAEQ	169	MSQALRKL	LSGAI	ISK	SNTTAI	FINQI	IREKVI	GVMFGN	PETT	GGR	ALKFY	SSVRLE	VRRAE	EQ
RECA_BACSU	168	MSQALRKL	LSGAI	INK	SKTIAI	FINQI	IREKVI	GVMFGN	PETT	GGR	ALKFY	ATVRLE	VRRAE	EQ
RECA_ENTFA	169	MSQALRKL	LSGSI	INK	SKTIAI	FINQI	IREKVI	GVMFGN	PETT	GGR	ALKFY	ATVRLE	VRRAE	EQ
RECA_CLOD6	171	MSQALRKL	LTGSI	IKNS	NCVAI	FINQI	LRDKI	GVMFGN	PETT	GGR	ALKFY	SSVRLD	VRRK	IDT
RECA_RHIL3	181	MSQALRKL	LTASIS	IKS	ENTMVI	FINQI	IRMKI	GVMFGS	PETT	GGN	ALKFY	ASVRLD	IRRI	GA

L2 Loop

▼ ▼▼

RECA_ECOLI	231	VKEGEN	VVGS	ETRVK	VVKNKI	APFKQAEF	QILYGE	GINFY	C	ELVDL	LGV	KEKLI	EKA	GAW							
RECA_SHISO	231	VKEGEN	VVGS	ETRVK	VVKNKI	APFKQAEF	QILYGE	GINFY	C	ELVDL	LGV	KEKLI	EKA	GAW							
RECA_SALTY	231	VKEGDN	VVGS	ETRVK	VVKNKI	APFKQAEF	QILYGE	GINFY	C	ELVDL	LGV	KEKLI	EKA	GAW							
RECA_SALPK	231	VKEGDN	VVGS	ETRVK	VVKNKI	APFKQAEF	QILYGE	GINFY	C	ELVDL	LGV	KEKLI	EKA	GAW							
RECA_KLEP7	231	VKEGDN	VVGS	ETRVK	VVKNKI	APFKQAEF	QILYGE	GINFY	C	ELVDL	LGV	KEKLI	EKA	GAW							
ReCa_ENTCC	231	VKEGEN	VVGS	ETRVK	VVKNKI	APFKQAEF	QILYGE	GINFY	C	ELVDL	LGV	KEKLI	EKA	GAW							
RECA_VIBCH	229	IKEGEE	VVGN	ETRIK	VVKNKI	APFKQAEF	QIMYG	QGFN	RE	C	ELIDL	LGV	KHKM	VEK	GSW						
RECA_HAEIN	231	VKDG	ENI	IGNET	RVK	VVKNK	LAPFR	QVDF	Q	ILYGE	GISKA	GELLE	LGV	KHK	LVE	K	GSW				
RECA_PSEAE	229	VKEG	DEI	VVGS	ETRVK	VVKNK	VSPFF	RQAEF	Q	ILYK	KG	IYRT	GEI	IDL	LGV	QLG	LVE	K	GSW		
RECA_ACIBC	229	VKEG	DEI	VVGS	ETRVK	VVKNK	MAPP	FKQAEF	Q	ILYK	KG	TNQL	GEL	VDL	AV	QDD	I	V	K	GSW	
RECA_MYCTU	231	LKDG	TNA	VGNR	TRVK	VVKNK	CSE	PFKQAEF	D	ILYK	KG	ISRE	GSL	IDM	GV	DQGL	I	R	K	GSW	
RECA_MYCA1	231	LKDG	TNA	VGNR	TRVK	VVKNK	VSPFF	RQAEF	D	ILYGR	GISRE	GSL	IDM	GV	DQGF	I	R	K	GSW		
RECA_MYCS2	232	LKDG	TDA	VGNR	TRVK	VVKNK	VSPFF	RQAEF	D	ILYQ	GISRE	GSL	IDM	GV	EHGF	I	R	K	GSW		
RECA_MYCA9	231	LKDG	TDA	VGNR	TRVK	VVKNK	VSPFF	RQAEF	D	ILYK	KG	ISKE	GSL	IDM	GV	EQGF	I	R	K	GSW	
RECA_STAAC	229	LKQG	QEI	VGNR	TKIK	VVKNK	VAPP	FRVAE	VD	IMYG	QGIS	KEG	ELI	DL	GV	END	I	V	D	K	GSW
RECA_STAEQ	229	LKQG	QEI	VGNR	TKIK	VVKNK	VAPP	FRVAE	VD	IMYG	QGIS	KEG	ELI	DL	GV	END	I	V	D	K	GSW
RECA_BACSU	228	LKQG	NDI	VGNK	TKIK	VVKNK	VAPP	FRVAE	VD	IMYG	EGIS	KEG	ELI	DL	GV	TELD	I	V	D	K	GSW
RECA_BACSU	228	LKQG	NDI	VGNK	TKIK	VVKNK	VAPP	FRVAE	VD	IMYG	EGIS	KEG	ELI	DL	GV	TELD	I	V	D	K	GSW
RECA_ENTFA	229	LKQG	TDI	VGNR	TKIK	VVKNK	VAPP	FRVAE	VD	IMYG	QGIS	QEG	GEL	LD	MA	EKDL	I	S	K	GSW	
RECA_CLOD6	231	IKQG	DKI	VIGSR	TRVK	VVKNK	VAPP	FKQAEF	D	IMYGE	GISK	I	GD	LD	LA	DVD	I	V	K	GSW	
RECA_RHIL3	241	VKER	E	VIGN	QTRVK	VVKNK	MAPP	FKQVE	F	IMYGE	GVS	KT	GEL	VDL	LGV	KAG	I	V	K	GSW	

Fig. S12. Sequence alignment of RecA homologs.

The sequences were aligned using Clustal Omega and the figure was prepared using ESPrict 3.0. Black and cyan triangles indicate residues making hydrophobic interactions and electrostatic interactions, respectively. Species are as follows: *Escherichia coli* (ECOLI), *Shigella sonnei* (SHISO), *Salmonella typhimurium* (SALTY), *Salmonella paratyphi A* (SALPK), *Klebsiella pneumoniae* (KLEP7), *Enterococcus cloacae* (ENTCC), *Vibrio cholerae* (VIBCH), *Haemophilus influenzae* (HAEIN), *Pseudomonas aeruginosa* (PSEAE), *Acinetobacter baumannii* (ACIBC), *Mycobacterium tuberculosis* (MYCTU), *Mycobacterium avium* (MYCA1), *Mycolicibacterium smegmatis* (MYCS2), *Mycobacterium abscessus* (MYCA9), *Staphylococcus aureus* (STAAC), *Staphylococcus epidermidis* (STAEQ), *Bacillus subtilis* (BACSU), *Enterococcus faecalis* (ENTFA), *Clostridioides difficile* (CLOD6), *Rhizobium leguminosarum* (RHIL3).

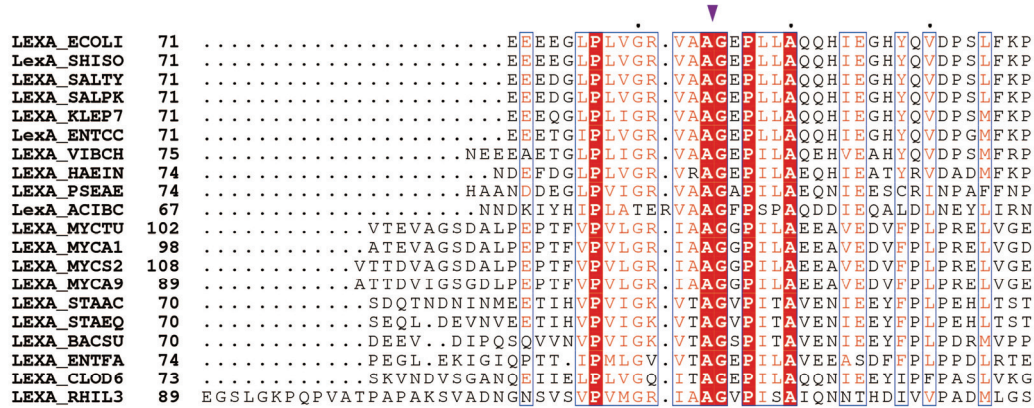


Fig. S13. Sequence alignment of LexA homologs.

The sequences were aligned using Clustal Omega and the figure was prepared using ESPrIPT 3.0. Black and red triangles indicate residues making hydrophobic interactions and electrostatic interactions, respectively. Purple triangle, the cleavage peptide bond; orange triangles, the Ser-Lys catalytic dyad. Species are as follows: *Escherichia coli* (ECOLI), *Shigella sonnei* (SHISO), *Salmonella typhimurium* (SALTY), *Salmonella paratyphi A* (SALPK), *Klebsiella pneumoniae* (KLEP7), *Enterococcus cloacae* (ENTCC), *Vibrio cholerae* (VIBCH), *Haemophilus influenzae* (HAEIN), *Pseudomonas aeruginosa* (PSEAE), *Acinetobacter baumannii* (ACIBC), *Mycobacterium tuberculosis* (MYCTU), *Mycobacterium avium* (MYCA1), *Mycolicibacterium smegmatis* (MYCS2), *Mycobacterium abscessus* (MYCA9), *Staphylococcus aureus* (STAAC), *Staphylococcus epidermidis* (STAEQ), *Bacillus subtilis* (BACSU), *Enterococcus faecalis* (ENTFA), *Clostridioides difficile* (CLOD6), *Rhizobium leguminosarum* (RHIL3).

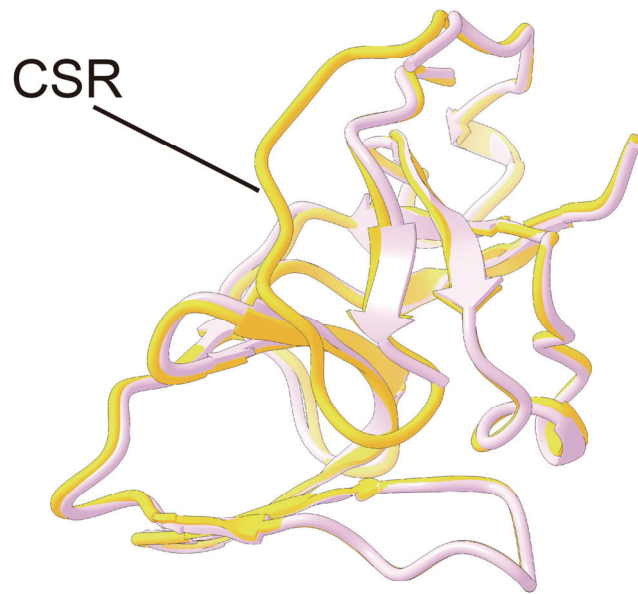


Fig. S14. Structural comparison of LexAⁱ and LexA^o shows that the CSR of LexA^o is disordered.

Yellow, LexAⁱ; pink, LexA^o.

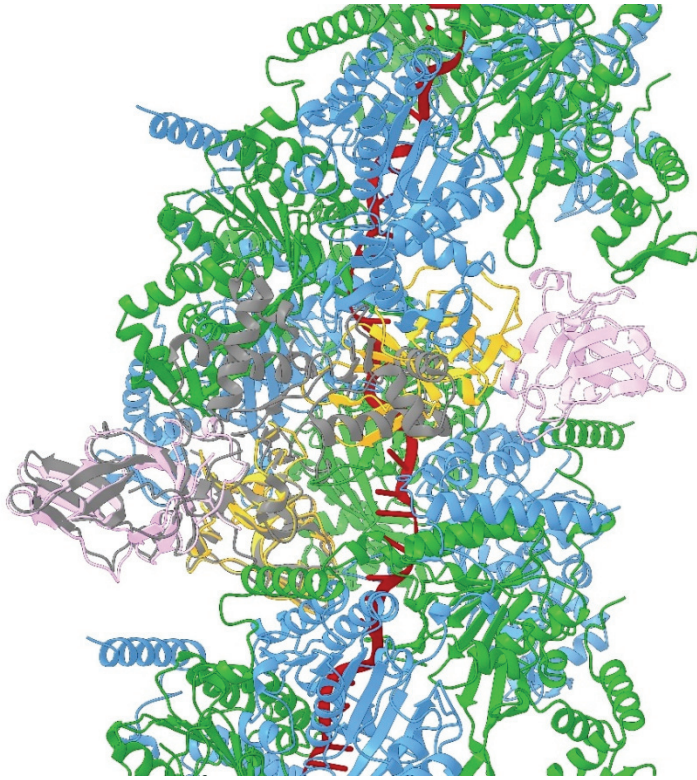


Fig. S15. Superposition of RecA-LexA and full-length LexA structures shows steric clashes between the NTD and CTD of neighboring LexA molecules.

Yellow and pink, LexA in RecA-LexA; gray, crystal structure of full-length LexA (PDB 3JSP).

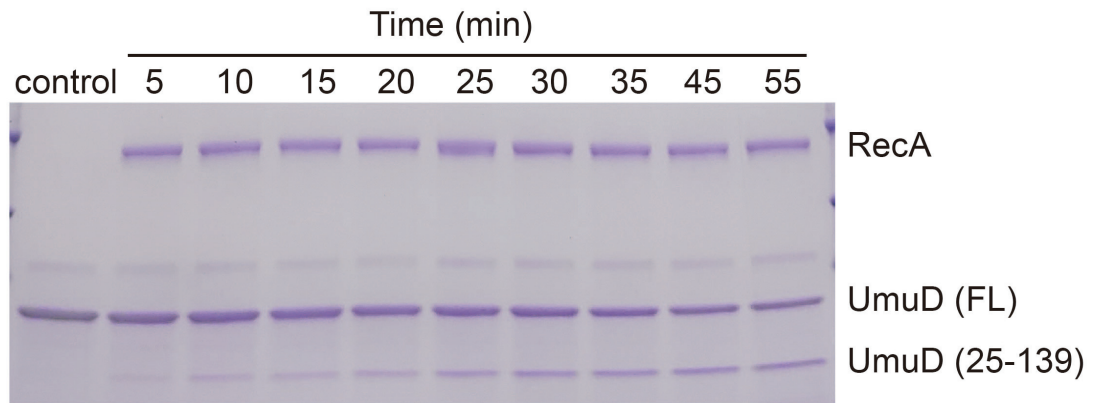


Fig. S16. UmuD undergoes RecA mediated cleavage.

Experiments were repeated independently three times with similar results.

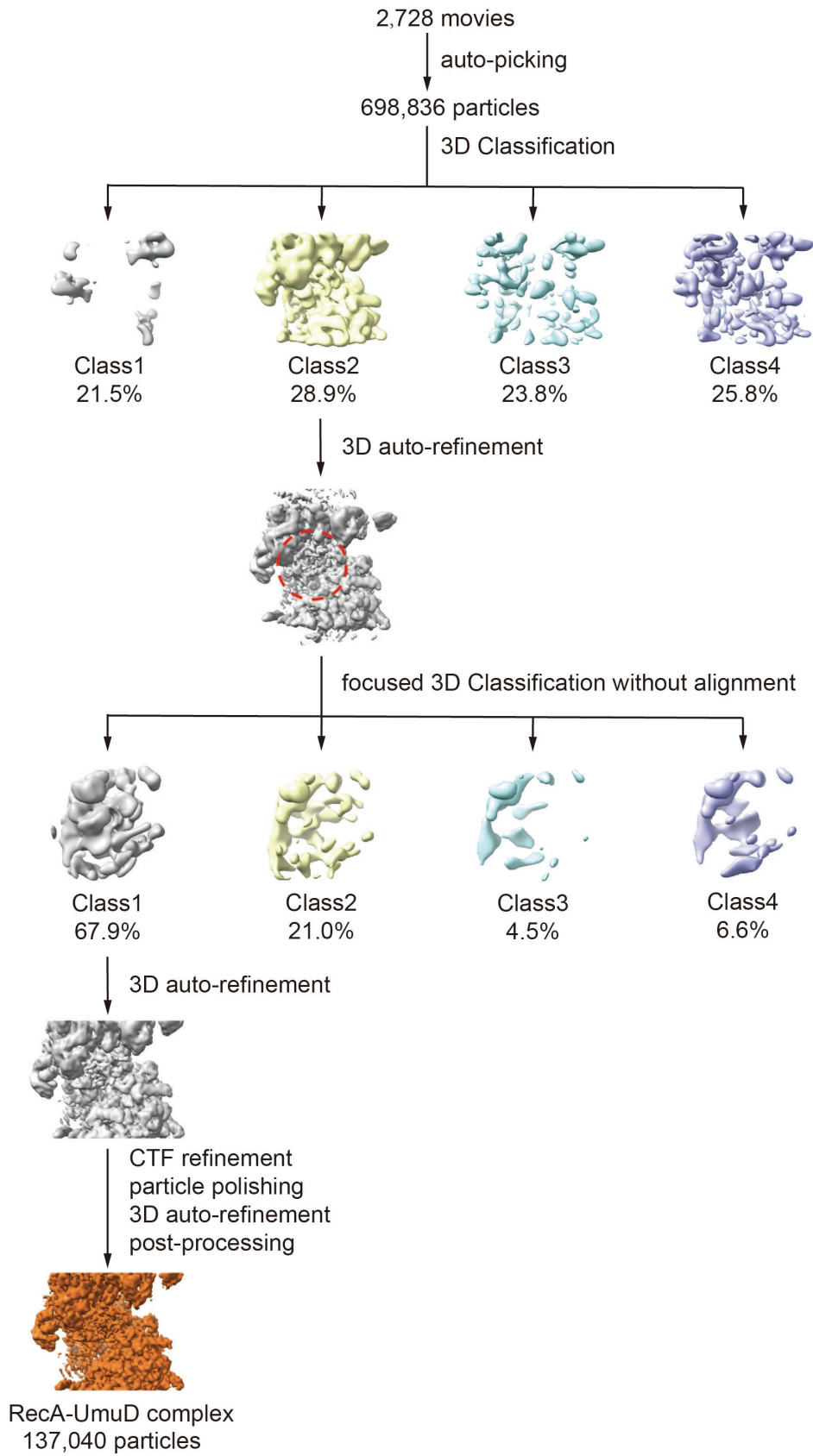


Fig. S17. Data processing pipeline for the dataset of RecA-UmuD.

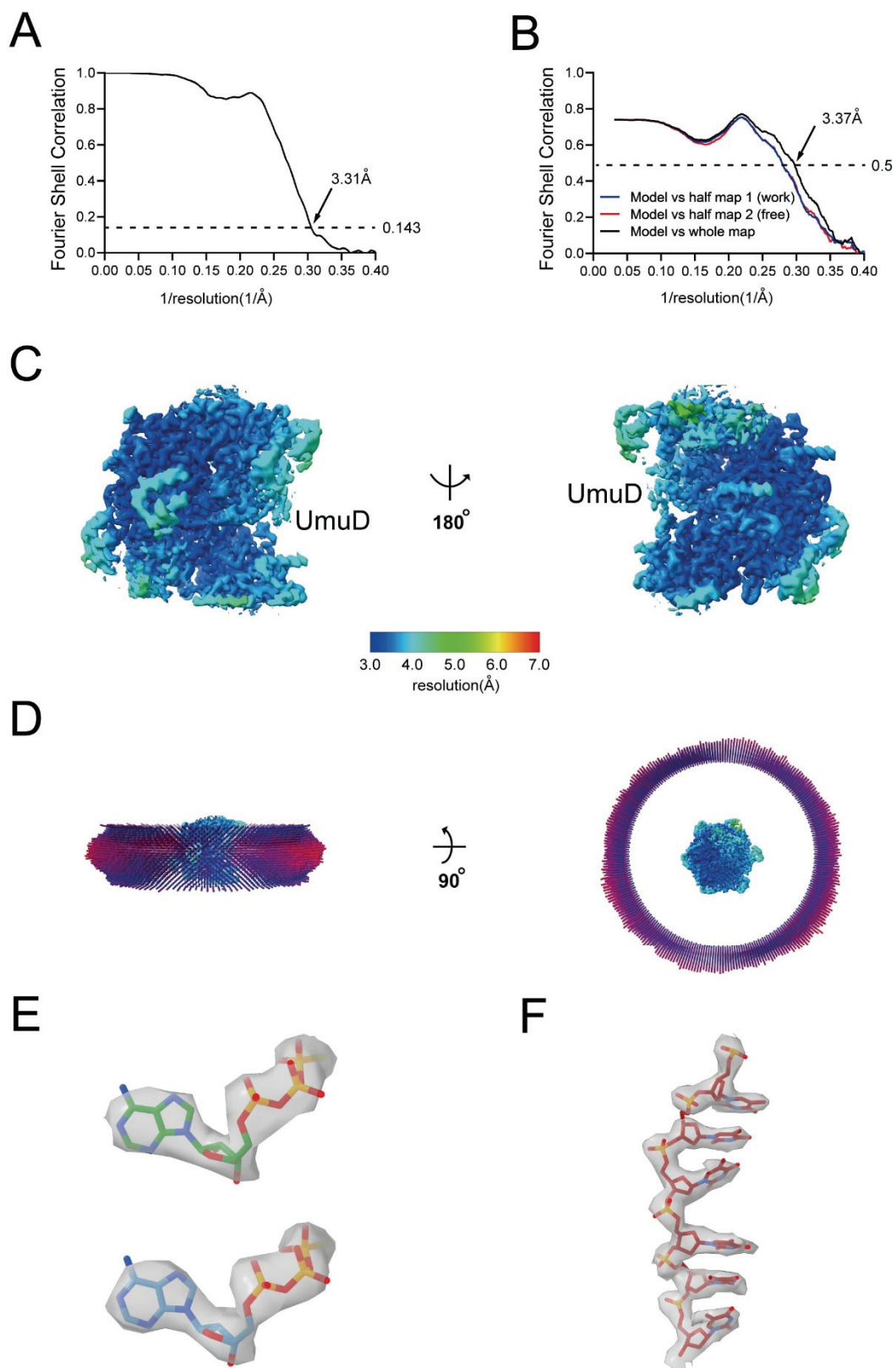


Fig. S18. Data validation for RecA-UmuD.

(A) The gold-standard FSC of RecA-UmuD. The gold-standard FSC is calculated by comparing the two independently determined half-maps from RELION. The dashed line represents the 0.143 FSC cutoff.

(B) FSC calculated between the model and the half map used for refinement (work), the other half map (free), and the full map.

(C) Cryo-EM density map colored by local resolution.

(D) Angular distribution of particle projections.

(E) The cryo-EM density map of two ATP γ S molecules in each asymmetrical unit.

(F) The cryo-EM density of 6-nt oligo (dT) ssDNA in each asymmetrical unit.

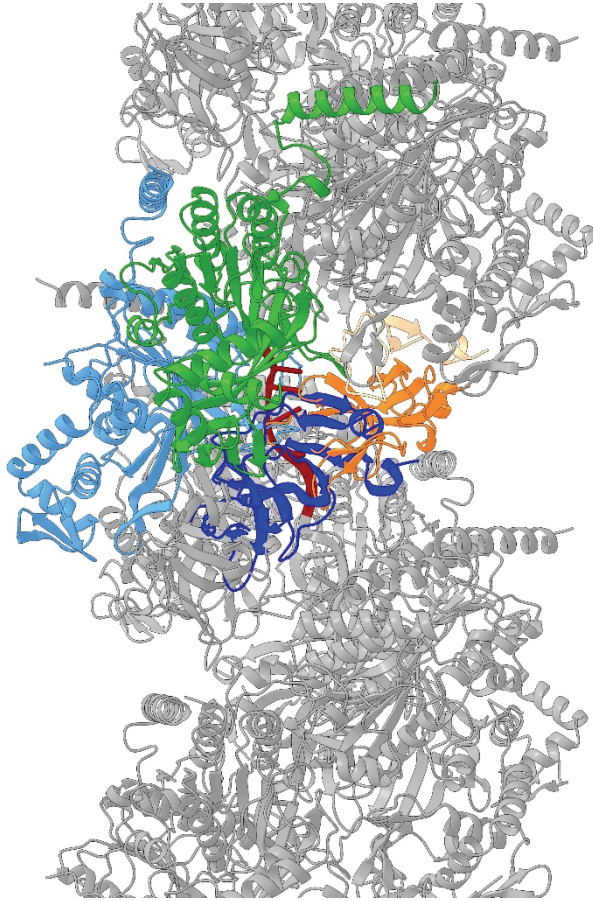


Fig. S19. The asymmetrical unit of RecA-UmuD is composed of two RecA protomers and one UmuD dimer.

The asymmetrical unit of RecA-UmuD is colored as in Fig. 3A. If a second UmuD dimer (dark blue) was modeled into the asymmetrical unit, there would be a steric clash between UmuD dimers.

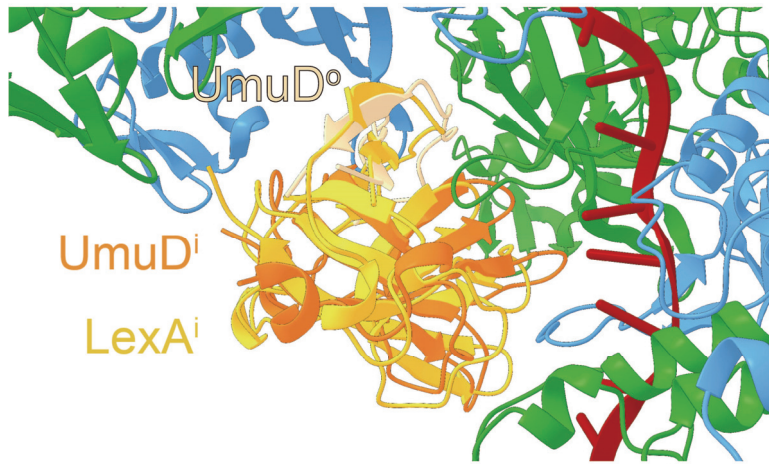


Fig. S20. Structural comparison of RecA-LexA and RecA-UmuD.

LexA^o and part of UmuD^o is omitted for clarity.



Fig. S21. F203 of RecA binds into a hydrophobic pocket in UmuD.

UmuD surface is colored according to hydrophobic potential. The surface coloring ranges from dark goldenrod for the most hydrophobic potentials to dark cyan for the most hydrophilic potentials.

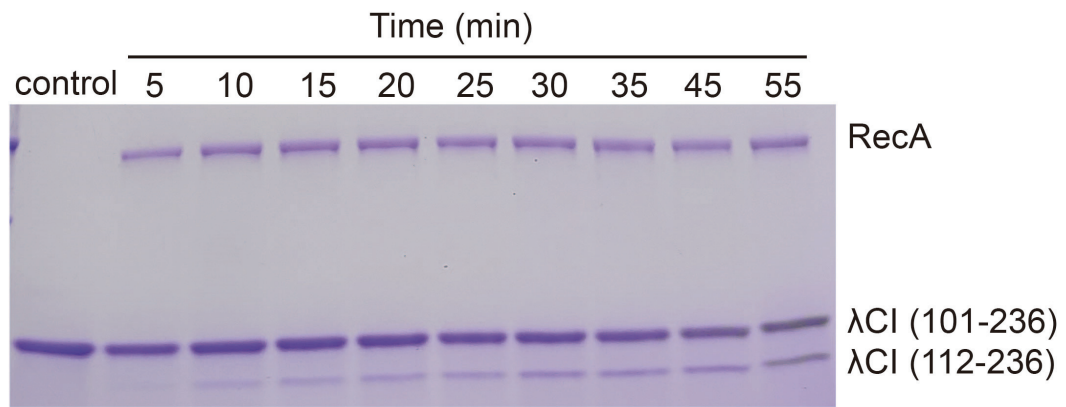


Fig. S22. λCI (residues 101 - 236) undergoes RecA mediated cleavage.

Experiments were repeated independently three times with similar results.

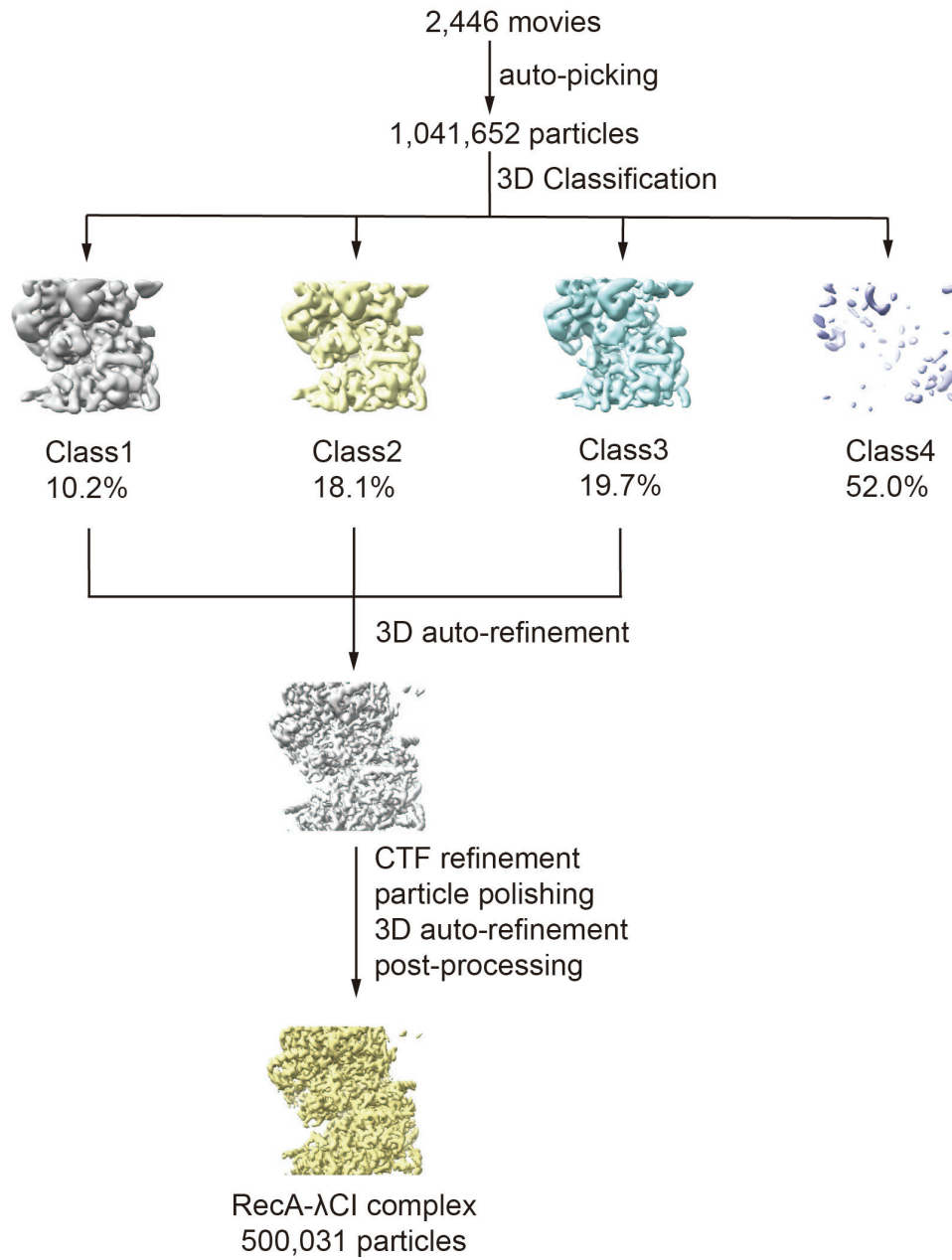


Fig. S23. Data processing pipeline for the dataset of RecA-λCI.

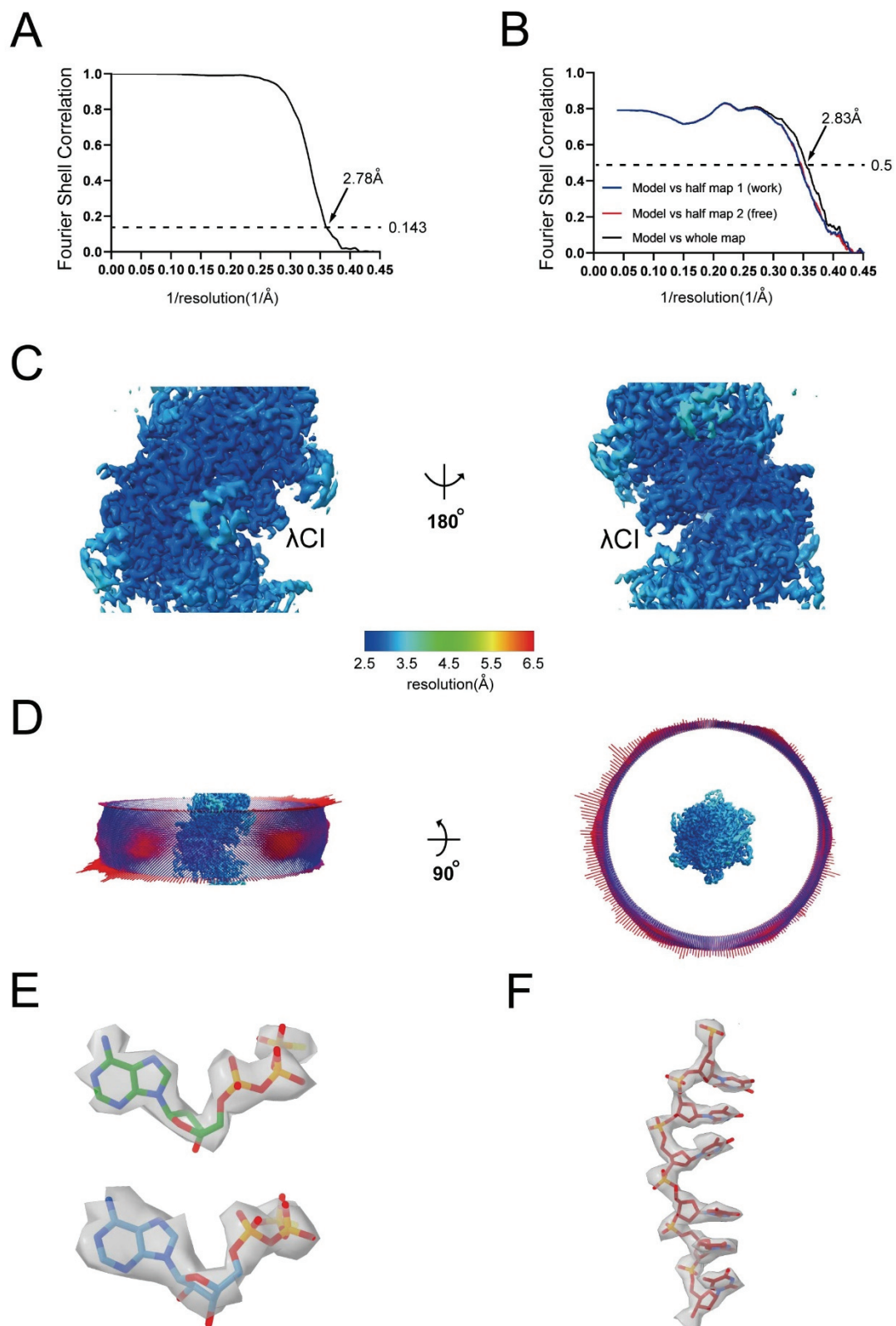


Fig. S24. Data validation for RecA-λCI.

(A) The gold-standard FSC of RecA- λ CI. The gold-standard FSC is calculated by comparing the two independently determined half-maps from RELION. The dashed line represents the 0.143 FSC cutoff.

(B) FSC calculated between the model and the half map used for refinement (work), the other half map (free), and the full map.

(C) Cryo-EM density map colored by local resolution.

(D) Angular distribution of particle projections.

(E) The cryo-EM density map of two ATP γ S molecules in each asymmetrical unit.

(F) The cryo-EM density of 6-nt oligo (dT) ssDNA in each asymmetrical unit.

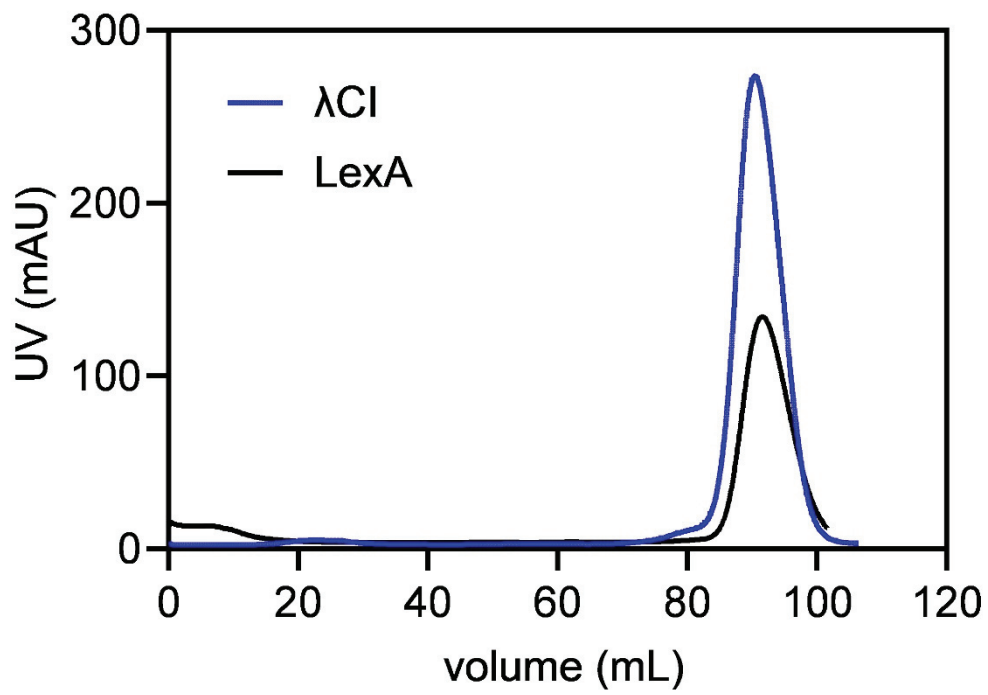


Fig. S25. The elution volume of NTD truncated λ CI is the same as NTD truncated LexA in gel filtration experiments.

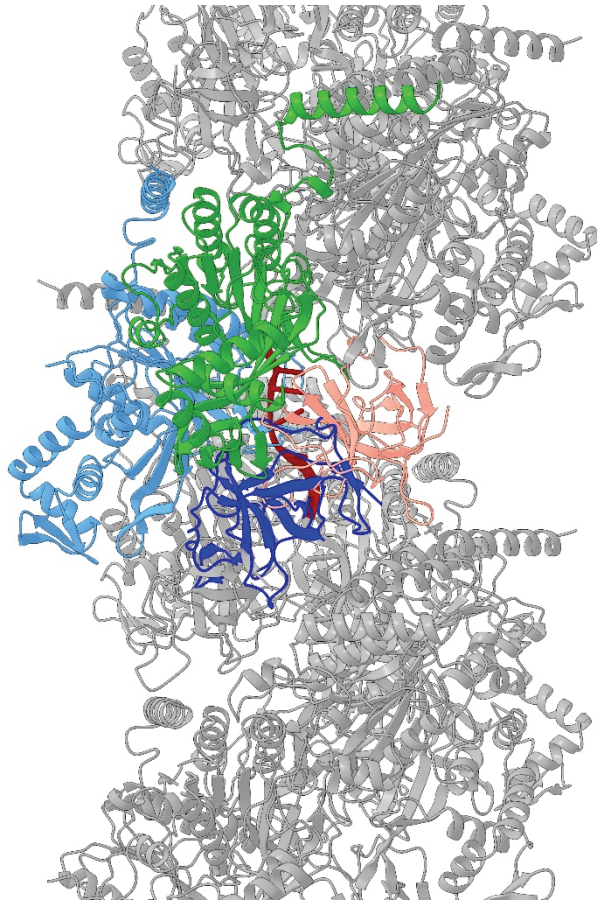


Fig. S26. The asymmetrical unit of RecA- λ CI is composed of two RecA protomers and one λ CI molecule.

The asymmetrical unit of RecA- λ CI is colored as in Fig. 4A. If a second λ CI molecule (dark blue) was modeled into the asymmetrical unit, there would be a steric clash between λ CI molecules.

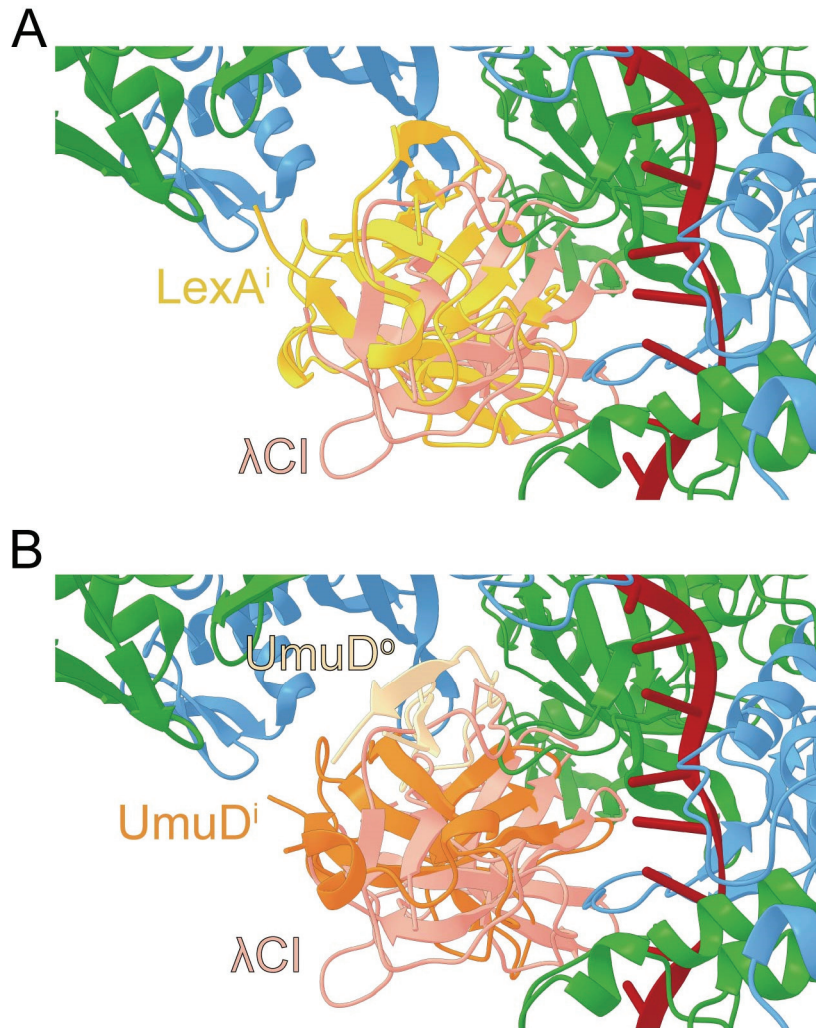


Fig. S27. Structural comparison of RecA-λCI, RecA-LexA, and RecA-UmuD shows that the orientation of λCI is different from LexA and UmuD.

(A) Structural comparison of RecA-λCI and RecA-LexA. LexA^o is omitted for clarity.

(B) Structural comparison of RecA-λCI and RecA-UmuD. Part of UmuD^o is omitted for clarity.

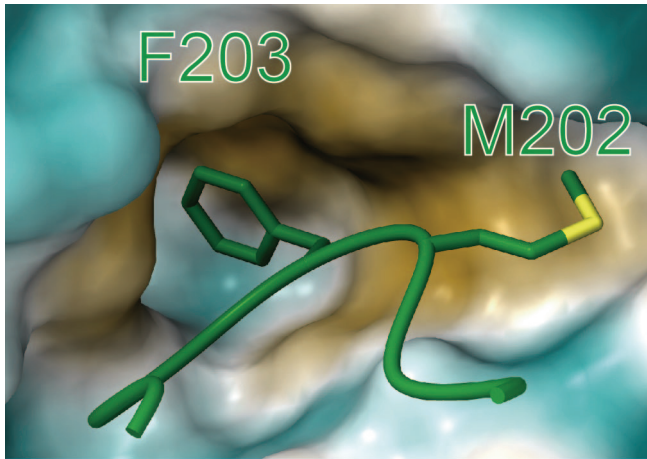


Fig. S28. M202 and F203 of RecA bind into a hydrophobic pocket in λ CI.

λ CI surface is colored according to hydrophobic potential. The surface coloring ranges from dark goldenrod for the most hydrophobic potentials to dark cyan for the most hydrophilic potentials.

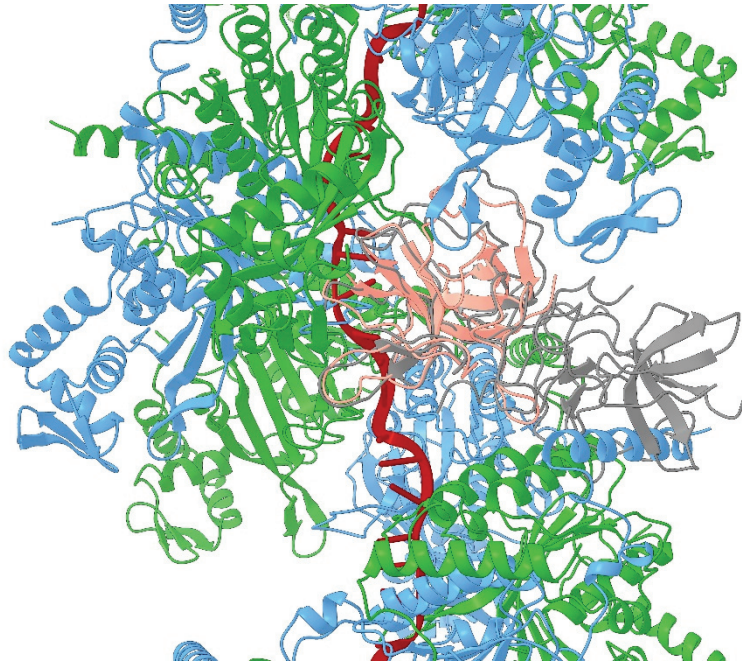


Fig. S29. Superposition of RecA-λCI and λCI dimer structures shows steric clashes between RecA and the outside λCI protomer.

Salmon, λCI in RecA-λCI; gray, crystal structure of λCI dimer (PDB 3BDN, NTD is omitted for clarity).

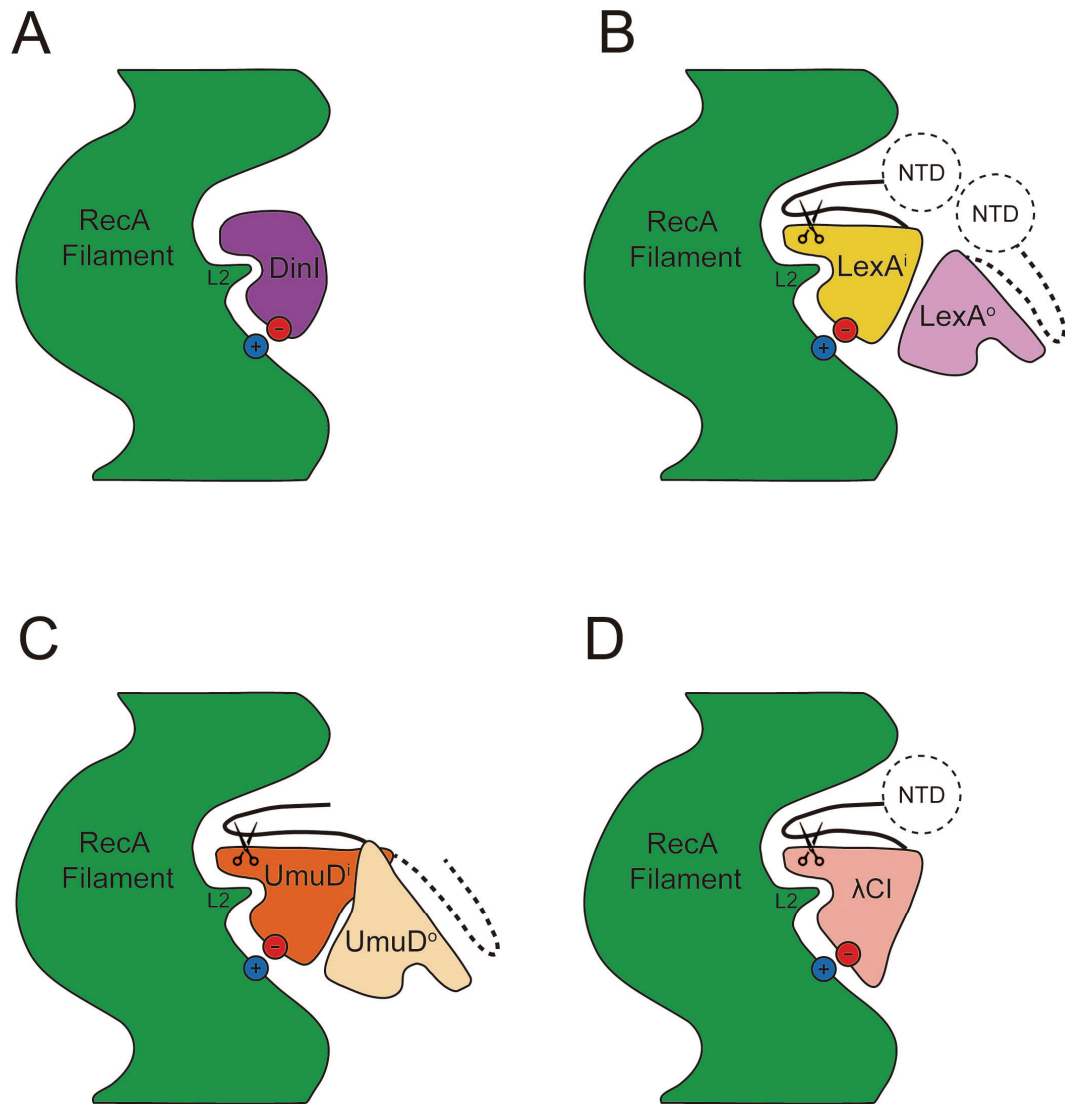


Fig. S30. The schematic diagrams of RecA-DinI (*A*), RecA-LexA (*B*), RecA-UmuD (*C*), and RecA-λCI (*D*).

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

	RecA-DinI	RecA-LexA	RecA-UmuD	RecA-λCI
Data collection and processing				
Microscope	Titan Krios	Titan Krios	Titan Krios	Titan Krios
Voltage (kv)	300	300	300	300
Detector	Falcon 4	Falcon 4	Falcon 4	Falcon 4
Electron exposure (e/Å ²)	62	52	52	50
Defocus range (μm)	1.0 - 2.0	1.0 - 2.0	0.8 - 1.7	0.8 - 1.7
Data collection mode	Counting	Counting	Counting	Counting
Physical pixel size (Å/pixel)	0.93	1.19	1.19	1.19
Symmetry imposed	Helical	Helical	Helical	Helical
Twist (°)	118.0	118.6	118.3	118.1
Rise (Å)	31.5	31.5	31.6	31.5
Initial particle images	354,347	429,839	698,836	1,041,652
Final particle images	40,451	429,839	137,040	500,031
Map resolution (Å) ^a	3.3	3.3	3.3	2.8
Refinement				
Root-mean-square deviation				
Bond lengths (Å)	0.003	0.003	0.003	0.003
Bond angles (°)	0.631	0.564	0.794	0.497
Molprobrity statistics				
Clashscore	11.67	9.68	8.99	8.36
Rotamer outliers (%)	0.34	0.41	0.31	1.89
Cβ outliers (%)	0	0	0	0
Ramachandran plot				
Favored (%)	97	97	98	98
Outliers (%)	0	0	0	0.25

^aGold-standard FSC 0.143 cutoff criteria.

Table S2. RecA-DinI interface surface area (\AA^2) statistics.

	DinI
RecA²	238
RecA³	370
RecA⁴	231
RecA⁵	125
RecA⁶	19
Sum	983

Table S3. RecA-LexA interface surface area (\AA^2) statistics.

	LexAⁱ	LexA^o
RecA¹	58	246
RecA²	339	0
RecA³	480	0
RecA⁴	251	0
RecA⁵	89	0
RecA⁶	77	0
Sum	1294	246

Table S4. RecA-UmuD interface surface area (\AA^2) statistics.

	UmuDⁱ	UmuD^o
RecA¹	0	51
RecA²	21	502
RecA³	439	302
RecA⁴	216	0
RecA⁵	28	0
RecA⁶	8	0
Sum	712	855

Table S5. RecA- λ CI interface surface area (\AA^2) statistics.

	λ CI
RecA²	274
RecA³	846
RecA⁴	360
RecA⁵	192
RecA⁶	86
RecA⁸	45
Sum	1803