SUPPLEMENTARY DATA for:

An 'open' structure of the RecOR complex supports ssDNA binding within the core of the complex

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Figure S1: SAXS curves of various RecR constructs. RecRdC (black) comprises residues 1-197, RecR (red) corresponds to full-length RecR with no His-Tag, RecRCH (blue) to full-length RecR with a C-terminal His-Tag and RecRNH (yellow) to full-length RecR with an N-terminal His-Tag.



Figure S2: Comparison of the RecOR-'closed' structure (A) with that of the RecOR-'open' structure (B). For clarity, only two RecR molecules are illustrated (in red and yellow) and the two RecO molecules are colored in light and dark blue. In (A), the two RecO molecules interact tightly, whereas in (B) a wide groove is formed between the two RecO molecules and through the RecR tetramer, which could accommodate ssDNA (illustrated as a space-filling model, colored red and green).



Figure S3: Time evolution of the atom-positional rmsd over the backbone atoms of RecOR in *i*) RecOR wild-type -*apo*, *ii*) RecOR wild-type -ssDNA, *iii*) RecOR^{K23A/R27A} -*apo* and *iv*) RecOR^{K23A/R27A} ssDNA with respect to the RecOR-'open' crystal structure.



Figure S4: Time evolution of the atom-positional rmsd over the backbone atoms of each RecOR individual protein (chain A,B,C,D of RecR and E,F of RecO) in *i*) RecOR wild-type - *apo*, *ii*) RecOR wild-type- ssDNA, *iii*) RecOR^{K23A/R27A} -*apo* and *iv*) RecOR^{K23A/R27A} -ssDNA with respect to the RecOR- 'open' crystal structure.



Figure S5: Probability distribution and time evolution (inset) of the distance between the center of mass of the two RecO subunits monitored along an independent 100-ns trajectory of RecOR wild-type-*apo*. Dotted and dashed lines (inset) depict the corresponding distances observed in RecOR- 'closed' (61.4 Å) and RecOR- 'open' (71.6 Å), respectively.



Figure S6: Probability distribution and time evolution (inset) of the tilt angle between the normal to the RecR tetrameric ring and the axis connecting the center of mass of the two RecO subunits in *i*) RecOR wild-type *-apo*, *ii*) RecOR wild-type *-ssDNA*, *iii*) RecOR^{K23A/R27A} *-apo* and *iv*) RecOR^{K23A/R27A} ssDNA. Dotted and dashed lines (inset) depict the corresponding angles observed in RecOR-'closed' (16°) and RecOR-'open' (36°), respectively.



Figure S7: Probability distribution of R_g obtained from the fit of SANS curves against 1.500 complex structures taken all along the 150 ns production runs for *i*) RecOR wild-type - *apo*, *ii*) RecOR wild-type - ssDNA, *iii*) RecOR^{K23A/R27A} - *apo* and *iv*) RecOR^{K23A/R27A} - ssDNA.



Figure S8: Probability distribution and time evolution (inset) of the tilt angle between the normal to the RecR tetrameric ring and the axis connecting the center of mass of the two RecO subunits monitored along an independent 100-ns trajectory of RecOR wild-type - *apo*.



Figure S9: Sequence alignment of RecR proteins from *D. radiodurans, Thermus thermophilus, Thermoanaerobacter tengcongensis, Escherichia coli, Bacillus subtilis, Helicobacter pylori, Mycobacterium tuberculosis and Staphylococcus aureus.* The secondary structure elements of drRecR are shown above the sequences. The alignment was prepared with ClustalW (65) and rendered with ESPript (66).

DECO DETRA		β1		β2	• mm	β3		β4
RECU_DEIRA	i i	10	20		30	40	50	
RECO_DEIRA RECO_THET RECO_ECOLI RECO_ECOLI RECO_BSUB RECO_HPYL RECO_MTUB RECO_SAUR	MRSRTANR MAARMRTG MRFLKT MEGWQI MLTKCI MLTKCI MRLYRDI MLMRQI	SGIVIRRRV TAYLRRRRI EAIVLKSNLJ RAFVLHSRPV EGIVLRTND 2GFLLQTQSJ RAVVLRQHKJ KGIIIKAVD	IPAGDI. SERARRI ISEKDK. VSETSL. (GETNK. IRDEDL. GEADR. (GESDK.	IVTLI LPRKVKI IATLI MLDVI IVTLI IVRVI IVTLI	LTPQ.GKL IHPPSQKA TRDYGKL TEESGRV LTREHGKI LTKNQLKT LTRDHGLV LNEHGAKV	KAIARGG RSTPTTTK QAVAKGAR RLVAKGAR GVMARGAK LYRFYGKF RAVAKGVF PLMARRAK	.VKGPLSSSLN SPSTTHSRMSI RSKSRFVNAVR SKRSTLKGALG KPNSRLSAVSG HSVLNVGRKID RTRSKFGARLE KVKTGLQAQTG	LFHHVGVQ SVRSMAPA PFIVANYV PFTPLLLR PFLYGSFL PFAHIEVQ LFVYGLFI
RECO_DEIRA		β5 70	→ η1 80 80	00000 90	αl 20000000 1	000	110	α2 00000000 120
RECO_DEIRA RECO_THET RECO_THETN RECO_ECOLI RECO_BSUB RECO_HPYL RECO_MTUB RECO_SAUR	VYQGPHND YTGKVERY IFEGQNYY FGGRGEVK MQKTSGLG FEEENDDK LHPGRNLD YNQWRGMG	LASVKQAVLI RLEEGIVVGI YIDQWELVKI TLRSAEAVSI TLQQGEMILS FLPKLRNILI IVTQVVSVDJ TLNSVDVIS(CGALPTLA RKPLPQGD VFEN.IEK LALPL MRG.IRE HLGYIWER AFATDIVA 2HYK.LQM	PERYAI LLRLV GITLY MERLFI YGRYTC LYVSS	FAHLMAEF IPKGSLEA LASYISET SGLYINEL YAAYVAEL FWQRFCTL CGCAILET YAALAAET	ADALF.QE VVRKGQRE ISRVL.EE LSRVL.EY VDRGT.EE LFKHLEGV AERLAGEE IERSM.DE	GEFSEQAFDLF TGRTGRLSLFH KQKNTKLYFFT ETRFSELFFDY KKPNPYLFEFI HSLDSVYFDTI RAPAPALHRLT GDIAPYNYQLI	AASIRGVA HVRFQLYA KHSLEAVE LHCIQSLA LESLKQLN DDGASKLS VGALRAVA QFVLEKIE
RECO DETRA	ò 000	α3	0.0		ጥጥጥ		<u>ተ</u> ጥ ጥ	
NECO_DEINA	130	140		150	p	160	170	180
RECO_DEIRA RECO_THET RECO_THETN RECO_ECOLI RECO_BSUB RECO_HPYL RECO_MTUB RECO_SAUR	HQ.PDPEW KGEGLPTL SLNVETSI GVTGTPEP EG.TDPDV KQ.HPLR DGQRPRDL SG.TSAQL	VALVMSYKLI IQAELLGRLE FLFSYTLKLI ALRRFELALI ITFIVQMKMI VVLEMYATLI LLDAYLLRAN MSVVVMLKCN	GLAG IGLEAPRRI SLLG GHLG GVMG NFEG GIAG KRFG	VIPQ FLLAAFI .YMPVJ .YGVNI .LYPEJ .RLQS .WAPAJ .FTASI	TARCARCG LAELAYRL LDSCAVCG FTHCAGSG LNHCVHCK YNSCFLCD LTECARCA FNRCAVSG	APDPE ASPEAAPE KKENLS EPVDDTMT SQDGTE AKLE TPGPHE NDTQADLI	HPDPLGGOLL YPLLVSGLRG YFSSSCGGAVC 'YRYREEKGFIA 'HFSVRDNGFIC RSVALAQGFII AFHIATGGSVC	SKCAALPP IAKHEDPL KDCNETCK SVVIDN HRCFEKDP AHPSCLKA AHCRPAGS RQEASKDV
RECO_DEIRA	α4 202020 19	000	20	α5 20000 00	210	α6 220 220	230	η2 222
RECO_DEIRA RECO_THET RECO_THETN RECO_ECOLI RECO_BSUB RECO_HPYL RECO_MTUB RECO_SAUR	YPPAVLDF LPLVWAGWI DAKFLNKK KTF YRIPIKPQ KSLDLERI TTPPLG HAVILSNK	LRHAVRRT. RVAKAGGIGH VLKFLLYL. TGRQLKAL. TARLLRLF. QAFFRTQS. VVDLMSAL. TLYLLDVL.	SLEGEGLI LI 	RASFEQI RLKGGRI KAKYEKI AREFPDI YFDLSRI OGDWEAJ KLPIDKI	VPSADRP GEEGVYL LERISVPG DTLR GNVSLKE EAAPQ NSLNIHQ	ALWRATEK GREGVEAI VIKEEADK AAKF ETKAELKÇ SARSHVSG EIIDEMSD	KFVTVQVGGVHS KATLRLPGAQA IITEYVRTHLE FTRMALKPYLG VIDLYEEYSG JULETEEVEE LVAAHLQWHLE JIILMLYREYAG	WRQLVPSG LPHLEGAP .MDFKSKD GKPLKSRE .LVRTLNLG .RQLKTLNLG .RQLKTLP .MFFKSQK
RECO_DEIRA 240								
RECO_DEIRA RECO_THET RECO_ECOLI RECO_BSUB RECO_BSUB RECO_HPYL RECO_MTUB RECO_SAUR	VPVLS LNRLFLAL FAMKLSD. LFRQFMPKI FLDQMESMI F LVERFYQAI LINQLKRL	KAHAEEALGI RTVKTHYE. KHLMGENKS DRSVAERRAA	LRSAEAIC	GV GV GG				

Figure S10: Sequence alignment of RecO proteins from *D. radiodurans, Thermus thermophilus, Thermoanaerobacter tengcongensis, Escherichia coli, Bacillus subtilis, Helicobacter pylori, Mycobacterium tuberculosis and Staphylococcus aureus.* The secondary structure elements of drRecO are shown above the sequences. The alignment was prepared with ClustalW (65) and rendered with ESPript (66).



Figure S11: Non-specific recognition of ssDNA by RecOR. Close-up view of the binding of ssDNA to RecO predicted by molecular dynamics, involving electrostatic interactions between lysine residues from the two RecO subunits and phosphate groups from the DNA backbone.