Supporting Information

Zhang et al. 10.1073/pnas.1100300108

Fig. S1. *Front view (looking into the ssDNA binding groove) of the structural alignment of topoisomerase I covalent complex and the apo enzyme*. The alignment is optimized for the overlapping of domains II and III.

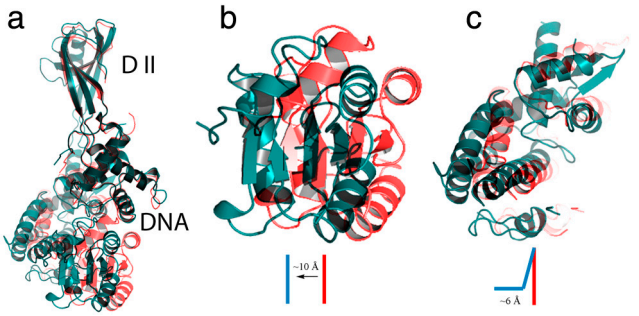

Fig. S2. *Domain rearrangement upon the formation of the covalent complex*. Domains II and III of the covalent complex and the apo enzyme were aligned with rmsd of 0.64 Å for the C^{α}s. (*a*) Overview of the relative domain rearrangement from the side view. The apo structure is in red and the covalent complex is in blue. (*b*) Relative to domains II and III, domain I is shifted back about 6 to 10 Å when viewed from the side. (*c*) Domain IV rotates backward approximately 5° in addition to its internal rearrangement.

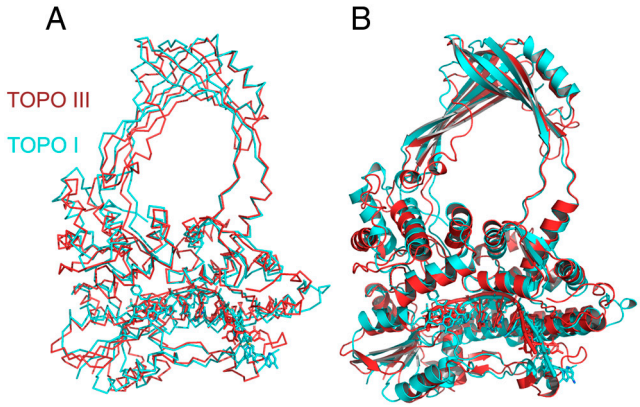

Fig. S3. *Structural alignment of covalent complex of topoisomerase I (cyan) with noncovalent complex of topoisomerase III (red) with DNA substrate [Protein Data Bank (PDB) ID code 1I7D]*. The bound DNA substrates adopt similar orientation in both complexes.

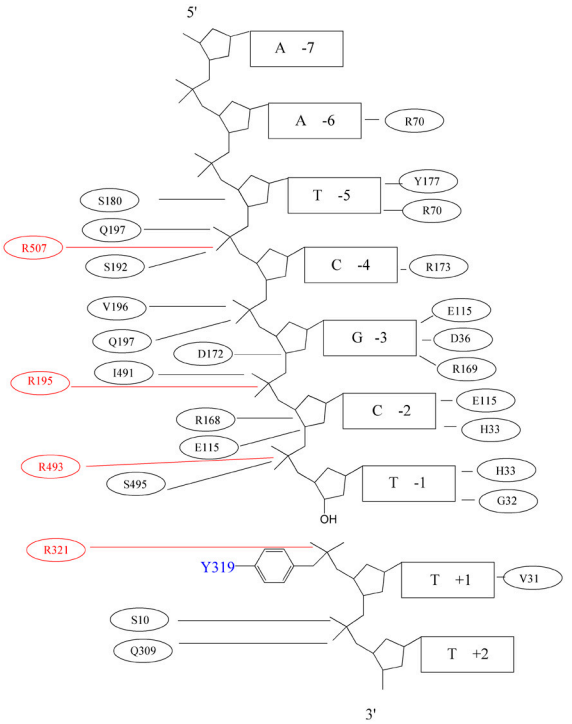

Fig. S4. *Schematic representation of the protein–DNA interactions in the covalent complex*. The bases (rectangles) and deoxyriboses form hydrogen bonds with topoisomerase I. The residues in red form salt bridges with DNA.

				NCBI ID	Organism
*EcTop1	161	RVNAQQARRF	MDRVVGYMVS	415338	*Escherichia coli*
HiTop1	167	RVNAQQTRRF	LDRVVGFMVS	687790	*Haemophilus influenzae*
SaTop1	134	LVDAQQARRI	LDRLVGYNIS	82750851	*Staphylococcus aureus*
*BcTop1	132	LVDAQQARRI	LDRLVGYNIS	52141655	*Bacillus cereus*
BsTop1	132	LVDAQQARRI	LDRLVGYKIS	520753	*Bacillus subtilis*
BaTop1	132	LVDAQQARRI	LDRLVGYNIS	30258481	*Bacillus anthracis*
*MlTop1	160	LVDAGETRRI	LDRLFGYELS	289557429	*Micrococcus luteus*
*MtTop1	160	LVDAGETRRI	LDRLYGYEVS	15610782	*Mycobacterium tuberculosis*
*TmTop1	133	KVRAQLARRI	LDRIVGYSLS	881494	*Thermotoga maritima*
MgTop1	132	WVESQFARQI	LDRMIGFRLS	12044974	*Mycoplasma genitalium*
*TmRG	718	LVKAQIVRRV	QDRWIGFELS	15642947	*Thermotoga maritima*
*SbRG	762	LVMSQIVRRI	EDRWIGFTLS	1679872	*Sulfolobus shibatae*
AfRG	680	LVKAQVVRRI	EDRWIGFVLS	11498629	*Archaeoglobus fulgidus*
SaRG	808	LVKSQLVRRI	EDRWIGFKLS	152943	*Sulfolobus acidocaldarius*
MkRGB	955	RVSAQILRRV	ADRWIGFSLS	1173903	*Methanopyrus kandleri*
*EcTop3	158	LCVSALARAR	ADWLYGINMT	148026	*Escherichia coli*
HiTop3	158	LATSALARAR	ADWLYGINMT	1174743	*Haemophilus influenzae*
*SfTop3	160	MINAGIARHK	IDWLWGINVS	15897795	*Sulfolobus solfataricus*
SaTop3	155	LYYAALARSE	ADWIVQINAT	81704216	*Staphylococcus aureus*
*BcTop3	156	LYASAVARSE	ADWYIGLNAT	52144873	*Bacillus cereus*
BsTop3	156	LYHSAVARAE	ADWIVGINAT	81345907	*Bacillus subtilis*
*ScTop3	175	SVHAVGTRIE	IDLRAGLRAG	173002	*Sacharomyces cerevisiae*
*HsTop3a	200	VSDAVDVRQE	LDLRIGAAFT	2501242	*Homo sapiens*
DmTop3a	192	QSDAVDVRTE	LDLRTGAAIT	33860232	*Drosophila melanogasta*
HsTop3b	176	EALSVDARQE	LDLRIGCAFT	6686033	*Homo sapiens*
*DmTop3b	173	EAKSVDARQE	LDLRIGCAFT	14286175	*Drosophila melanogasta*

Fig. S5. *Alignment of type IA topoisomerase sequences in region important for DNA substrate binding and recognition*. The residues highlighted in red are conserved in all type IA topoisomerases. The residues highlighted in blue are conserved for topoisomerase I and reverse gyrase enzymes that cleave DNA with the specificity of C nucleotide at the −4 position. *Topoisomerases with cleavage sequence selectivity determined experimentally (1–11).

1. Tse YC, Kirkegaard K, Wang JC (1980) Covalent bonds between protein and DNA. Formation of phosphotyrosine linkage between certain DNA topoisomerases and DNA. *J Biol Chem* 255:5560–5565.
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3. Bouthier de la Tour C, et al. (2008) Mutational analysis of the helicase-like domain of *Thermotoga maritima* reverse gyrase. *J Biol Chem* 283:27395–27402.
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7. Kim RA, Wang JC (1992) Identification of the yeast TOP3 gene product as a single strand-specific DNA topoisomerase. *J Biol Chem* 267:17178–17185.
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9. Wilson-Sali T, Hsieh TS (2002) Preferential cleavage of plasmid-based R-loops and D-loops by Drosophila topoisomerase IIIbeta *Proc Natl Acad Sci USA* 99:7974–7979.
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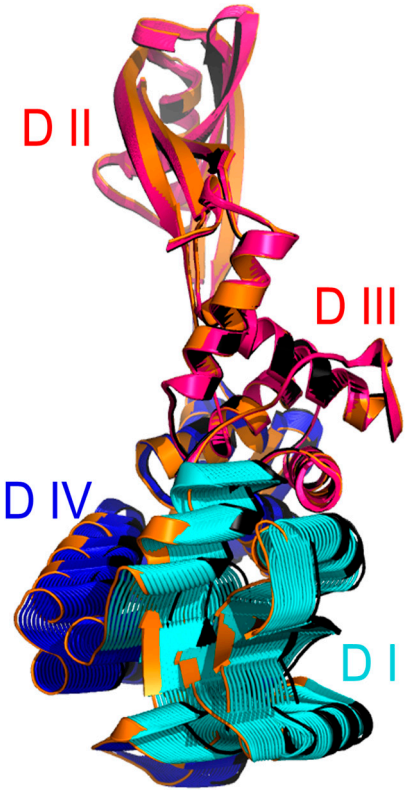
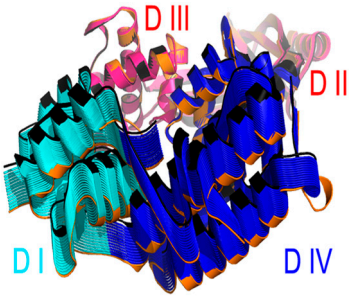
11. DiGate RJ, Marians KJ (1988) Identification of a potent decatenating enzyme from *Escherichia coli*. *J Biol Chem* 263:13366–13373.



Movie S1. Side view (in reference to Fig. 1*A*) domain mobility animation of an interpolation between the topoisomerase I apo enzyme (PDB ID code 1ECL, black) and the topoisomerase I-substrate covalent complex (orange). Domains I and II are in red, domain III in cyan, and domain IV in blue. The coordinate interpolation was performed by the Database of Molecular Movements Morph Server at Yale University (1), and the movie was rendered with PyMol.

Movie S1 (MOV)

1. Flores S, et al. (2006) The database of macromolecular motions: New features added at the decade mark. *Nucleic Acids Res* 34:D296–301.



Movie S2. View from the bottom (in reference to Fig. 1*A*) domain mobility animation of an interpolation between the topoisomerase I apo enzyme (PDB ID code 1ECL, black) and the topoisomerase I-substrate covalent complex (orange). Domains I and II are in red, domain 3 in cyan, and domain IV in blue. The coordinate interpolation was performed by the Database of Molecular Movements Morph Server at Yale University (1), and the movie was rendered with PyMol.

Movie S2 (MOV)

1. Flores S, et al. (2006) The database of macromolecular motions: New features added at the decade mark. *Nucleic Acids Res* 34:D296–301.

Table S1. Data collection and refinement statistics (molecular replacement)

Data collection	D111N mutant	Covalent complex
Space group	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions		
a, *b*, *c* (Å)	63.5, 79.3, 141.0	61.6, 91.76, 141.98
α, β, γ (°)	90, 90, 90	90, 90, 90
Resolution (Å)	1.9 (1.93–1.90)	2.3 (2.34–2.30)
R_{sym} or R_{merge}	6.4% (64%)	9% (76%)
$I/\sigma I$	39 (3)	33 (3)
Completeness (%)	99.8 (100)	99.6(100)
Redundancy	9.7 (9.8)	4.9 (5.1)
Refinement		
Resolution (Å)	500–1.9	500–2.3
No. reflections	56,450	58,558
R_{work}/R_{free}	21.7%/24.9%	23.0%/26.9%
No. atoms		
Protein	4,483	4,408
DNA ion	15	1,763
Water	421	140
B factors		
Protein	39.5	50.2
DNA		59.0
Water Hg	44.2	41.846.1
rms deviations		
Bond lengths (Å)	0.007	0.007
Bond angles (°)	1.2	1.7