Methods

Topo II expression and purification. Residues 409-1177 of *S. cerevisiae* topoisomerase II were expressed as a fusion with an N-terminal TEV protease-cleavable hexahistidine tag. Overexpression was carried out in *S. cerevisiae* strain BCY123 grown in URA^- dropout media supplemented with lactic acid and glycerol for a carbon source and galactose for induction (6 h at 30° C). Induced cells were harvested by centrifugation, flash frozen in liquid nitrogen, and lysed by grinding under liquid nitrogen. Ground cell powder was resuspended in lysis buffer (300 mM KCl, 20 mM Tris (pH 8.5), 10% glycerol, 20 mM imidazole (pH 8.0), 1 mM PMSF) and centrifuged to clarify the lysate. Protein was purified by tandem nickel-affinity and ion-exchange chromatography (nickel-chelating sepharose and HiTrap SP, GE), followed by removal of the His_6 tag with His-tagged TEV protease[31] and passage over a second nickel affinity column to remove uncleaved protein and the protease. The protein was then run over a gel-filtration column (S-300, GE) equilibrated in 10% glycerol, 500 mM KCl, and 20 mM Tris (pH 7.9). Peak fractions were pooled and concentrated to over 20 mg/mL.

Covalent complex formation and purification. Both standard and phosphorothiolate oligonucleotides were synthesized on an ABI synthesizer. Phosphorothioamidite for producing the 3'-bridging phosphorothiolate was synthesized as reported[30,32]. After synthesis, DNAs were purified by urea-formamide PAGE. For producing the cleavage complex, oligos were incubated for 60 min at room temperature at a 1:1 molar ratio with protein in 100 mM KCl, 10 mM Tris (pH 7.9), and 5 mM $MnCl_2$. Unreacted DNA and protein were separated from the complex by passing the sample over a gel-filtration column (S-300, GE) and cation exchange column (HiTrap SP, GE) connected in series and equilibrated with 100 mM KCl and 20 mM Tris (pH 7.9). The gel filtration column removed unbound DNA from the protein, while unbound protein and the DNA-cleavage complex were separated by the cation-exchange column. The protein/DNA complex was then concentrated to 3.5-6 mg/mL.

Crystallization and structure solution. Crystals were grown at 18˚ C in hanging drops by mixing 1 μL protein solution with 1 μL well solution containing 20% 1,4-butanediol and 100 mM sodium acetate (pH 4.5). For harvesting, crystals were looped and flash frozen in liquid nitrogen without additional cryoprotection. Data were collected at Beamline 8.3.1 at the Advanced Light Source (ALS) at Lawrence Berkeley National Laboratory[33] and reduced using HKL2000[34]. Initial phases were calculated by molecular replacement (MR), using the DNA binding and cleavage core without the coiled coil arms (residues 419-1004) of a noncovalent topoII/DNA complex (PDB ID code 2RGR)[13] as a search model for PHASER[35]. There is one monomer per asymmetric unit, with a canonical topo II homodimer recapitulated by a crystallographic dyad. All model building was carried out in COOT[36], followed by refinement using PHENIX[37]. Structure validation was assisted by MOLPROBITY[38]. Figures were rendered using PYMOL[39]. Ramachandran angles, as calculated by Procheck, are 90.6% most favored, 8.9% additionally allowed, 0.5% generously allowed, and 0% disallowed for the apo structure. For the Zn-soaked structure, these values are 89.0%, 10.0%, 0.9%, and 0%.

Notes on structure modeling. Because a non-palindromic DNA sequence was used for co-crystallization (Fig. 1b), and since the topoII-DNA dimer crystallized on a crystallographic dyad, electron density for the DNA corresponded to an average of the two half-sites. To account for this superposition, we modeled the DNA density as two alternate oligonucleotide segments, each present at half-occupancy; the covalent phosphotyrosine linkage was treated similarly. Refinement of this hybrid DNA model with a single protein chain produced significantly improved $2F_o$-F_c maps and far less F_o-F_c difference density, and substantially decreased both R_{work} and R_{free}. We note that such an approach has been used in similar circumstances with a STAT-1/DNA complex[40].

Interestingly, $2F_o$-F_c and composite simulated annealing omit maps not only showed features of both DNA sequences (Fig. S6), but also revealed electron density corresponding to only one catalytic tyrosine rotamer and a single 5’ DNA-end position (Fig. 1c). Thus, even

though only one active site can exist in a *bone fide* cleavage state with DNA (because the substrate contains only a single bridging phosphorothiolate modification), the nick is coordinated in essentially the same manner as the phosphotyrosyl bond. This state likely arises from the high cooperativity observed between the topoisomerase active sites during DNA cleavage *in vitro*[41].

Metal soaks. During building and refinement of our first model, we saw no evidence for metal ion factors within the active site. We suspected that the absence of metal ions might be due to the relatively acidic crystallization conditions (pH 4.5), which would favour protonation and hence charge neutralization of the acidic residue cluster within the TOPRIM domain that comprises the principal cation-binding site. Initial attempts to directly raise the pH of drop-grown crystals resulted in crystal dissolution, so we cross-linked the crystals by adding glutaraldehyde to the well solution (final concentration 0.09%), and allowing cross-linking to occur through the vapour phase for 16 hours. We then changed the crystal pH by moving the cover slip with the drop over a new well in which the acetate buffer was adjusted to pH 6.5; crystals were again allowed to equilibrate through the vapour phase overnight. Metal salt solutions were added either during this final step, or for 1-5 min prior to crystal harvesting.

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