Supplementary Information

Gyrase containing a single C-terminal domain catalyzes negative supercoiling of DNA by decreasing the linking number in steps of two

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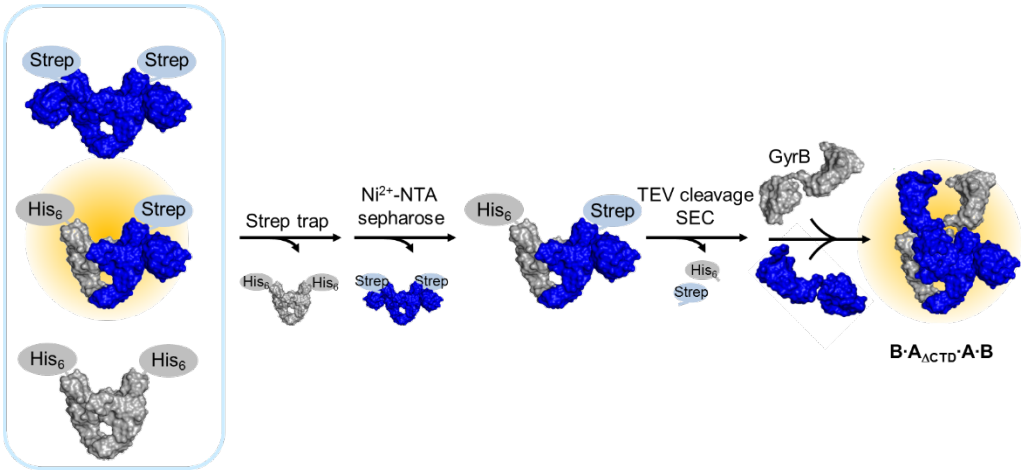
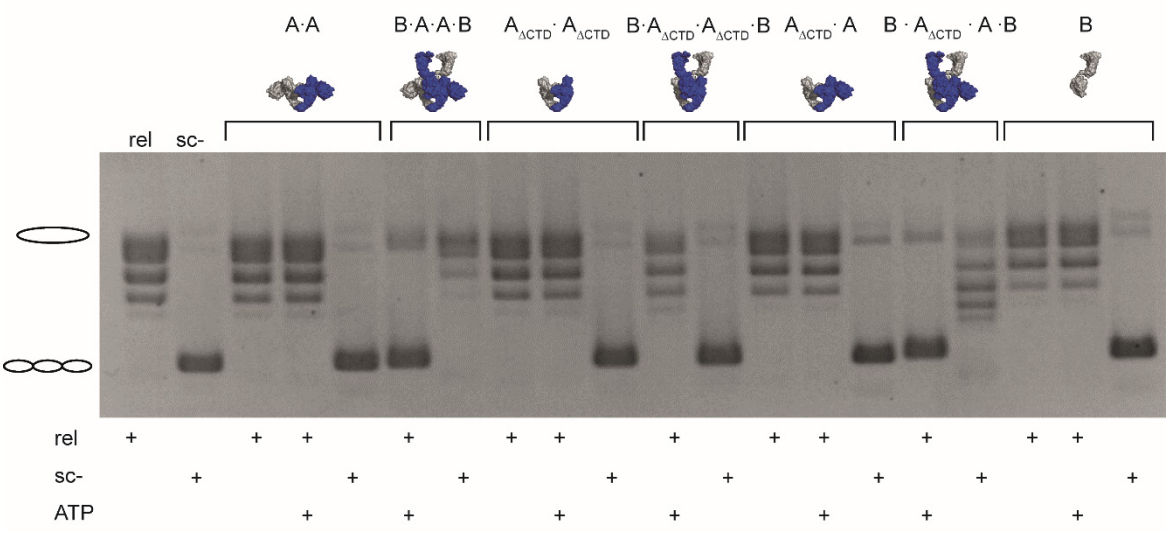
Supplementary Figures

Figure S1: **Tandem-affinity purification of gyrase containing only one CTD.**



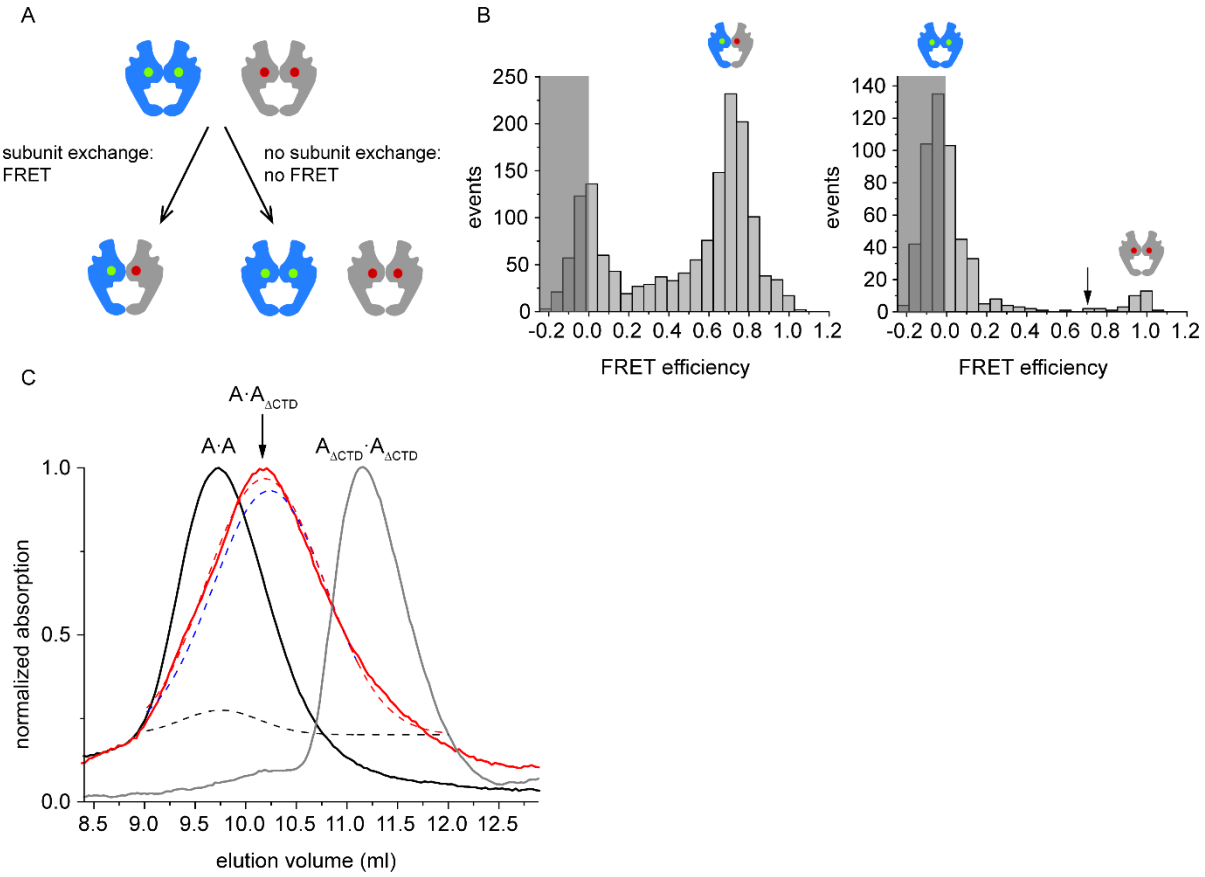
Strep-tagged wildtype GyrA (blue) and His_6-tagged GyrA lacking the CTD (gray) were coproduced in recombinant *E. coli*. Heterodimers were purified by tandem-affinity chromatography on StrepTactin sepharose and Ni^{2+}-NTA agarose (see main text and ref. (1) for details). Tags were removed by cleavage with TEV protease, and GyrB was added to generate gyrase with only a single CTD ($B{\cdot}A_{\Delta CTD}{\cdot}A{\cdot}B$).

Figure S2: **Effect of CTDs on DNA supercoiling.**



Supercoiling and relaxation activities of gyrase with two, one, or no CTD(s), and controls for isolated GyrA and GyrB subunits. Reactions were performed with 200 nM GyrA and/or 800 nM GyrB, and 20 nM relaxed pUC18 in 50 mM Tris/HCl pH 7.5, 100 mM KCl, 10 mM $MgCl_2$ at 37°C for 5 min (supercoiling) or 30 min (relaxation). For supercoiling reactions, 1.5 mM ATP was added. Gyrase with two CTDs supercoils DNA in the presence of ATP and relaxes DNA in the absence of ATP. Gyrase lacking the CTDs loses both activities. Gyrase with only one CTD shows ATP-dependent supercoiling activity and ATP-independent relaxation activity. No supercoiling or relaxation activities were observed for the individual subunits. A: GyrA, A·A: GyrA dimer, $A_{\Delta CTD}$: GyrA lacking the CTD, B: GyrB, B·A·A·B: gyrase heterotetramer, rel: relaxed pUC18 plasmid, sc-: negatively supercoiled pUC18 plasmid.

Figure S3: **No subunit exchange during storage or supercoiling experiments.**



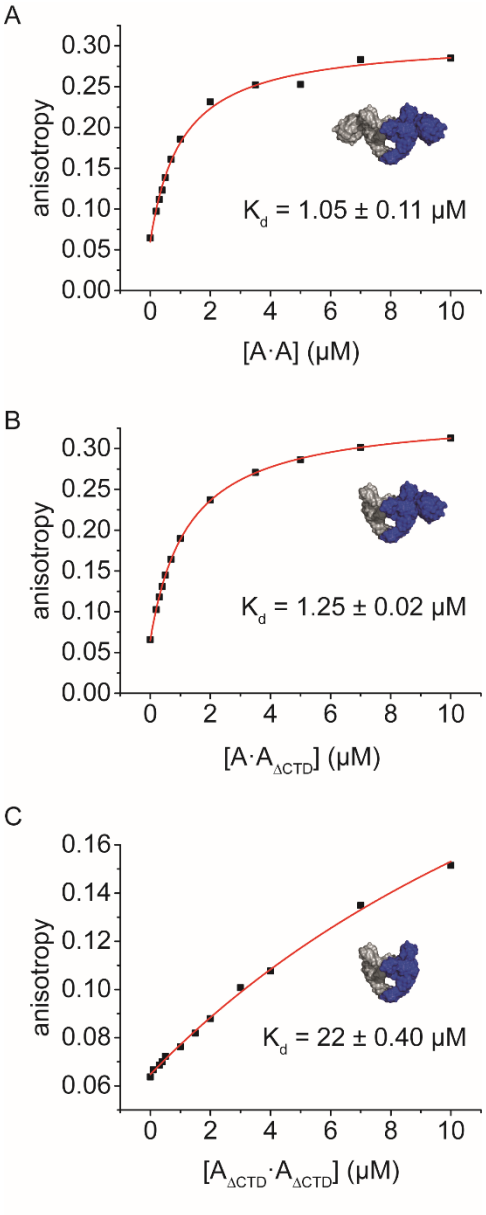
A: Schematic of a FRET measurement to test for subunit exchange. GyrA labeled with donor (green) or acceptor (red) in both subunits was mixed, and supplied with GyrB, relaxed pUC18 and ATP. In the absence of subunit exchange, no FRET is expected. If subunit exchange occurs, formation of donor/acceptor-labeled dimers can be detected by FRET.

B: Single-molecule FRET histograms of gyrase ($B{\cdot}A_{D145C}{\cdot}A_{D145C\_\Delta CTD}{\cdot}B$), labeled with donor and acceptor at position 145 in the A subunits (left), shows a FRET distribution centered at E_{FRET} = 0.7, and serves as a positive control. The peak at E_{FRET} = 0 represents molecules labeled with the donor only. FRET histograms of the mixture of donor-labeled and acceptor-labeled gyrase after 5 min of DNA supercoiling show peaks at E_{FRET} = 0 and E_{FRET} = 1, corresponding to donor/donor- and acceptor/acceptor-labeled molecules. No peak for donor/acceptor-labeled gyrase is observed, ruling out subunit exchange during the experiment.

C: Size-exclusion chromatography of GyrA with two, one, or no CTD(s). B·A·A·B: black; $B{\cdot}A_{\Delta CTD}{\cdot}A_{\Delta CTD}{\cdot}B$:·gray; $B{\cdot}A{\cdot}A_{\Delta CTD}{\cdot}B$: red. The chromatogram for gyrase with a single CTD ($B{\cdot}A{\cdot}A_{\Delta CTD}{\cdot}B$) can

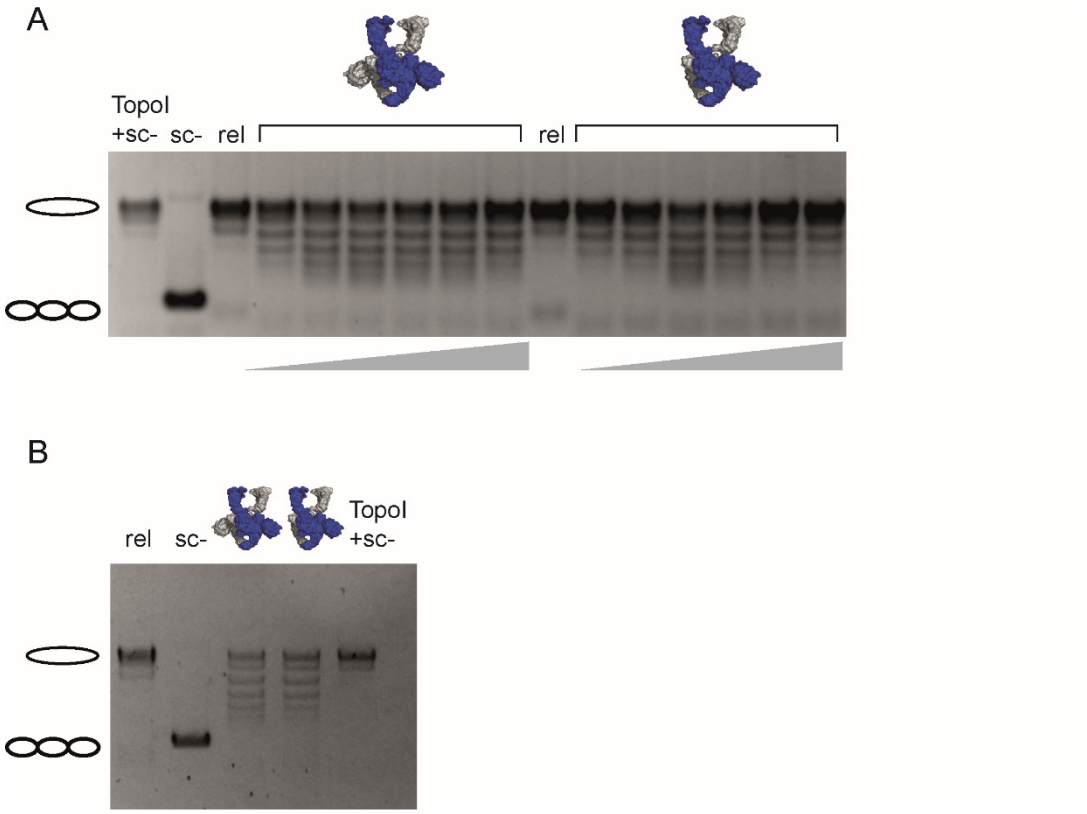
be described as the weighted sum (red broken line) of the chromatogram with two CTDs (B·A·A·B, black broken line, 5%) and a chromatogram with an elution maximum at 10.2 ml (blue broken line, 95%) corresponding to gyrase with one CTD (B·A·$A_{\Delta CTD}$·B).

Figure S4: **Effect of CTDs on DNA binding to GyrA**



Fluorescence anisotropy titration of 10 nM of an Alexa488/Alexa546-labeled 60 bp DNA with 0.2-2 μM GyrA (monomer concentration) containing two CTDs (A·A, panel A), one CTD (A·$A_{\Delta CTD}$, panel B) or no CTDs ($A_{\Delta CTD}$·$A_{\Delta CTD}$, panel C) at 37°C. Binding was followed using the fluorescence anisotropy of Alexa546 as a probe. The curves depicted are representatives of duplicate measurements; errors (±) denote the standard error of the mean. Deletion of one CTD does not affect the affinity of GyrA for the 60 bp DNA. Deletion of both CTDs leads to a 21-fold reduction in DNA affinity

Figure S5: **Trapping of positive supercoils by gyrase with one and two CTDs.**



Relaxation of gyrase-bound DNA by topoisomerase I. Gyrase and relaxed pUC18 (rel) were incubated in 50 mM Tris/HCl pH 7.5, 100 mM KCl, 10 mM $MgCl_2$ at 37°C for 15 min to enable binding. The DNA was then relaxed by topoisomerase I, gyrase was removed by incubation with proteinase K, and topoisomers were separated by electrophoresis on 1.3% agarose gels in TEP buffer (36 mM Tris, 36 mM NaH_2PO_4, 1 mM EDTA, pH 8.0; 2.6 V/cm, 3 h).

A: Gyrase with one and two CTDs trap positive supercoils in a concentration-dependent manner. Concentrations were 100, 200, 300, 400, 600, 800 nM GyrA and 200, 400, 600, 800, 1200, 1600 nM GyrB, 20 nM relaxed pUC18.

B: Side-by-side comparison of the topoisomer distribution after relaxation of DNA bound to gyrase with one and two CTDs at equal concentrations (300 nM GyrA, 600 nM GyrB, protein:DNA ratio 7.5:1)

sc-: negatively supercoiled pUC18 plasmid; rel: relaxed pUC18 plasmid, Topo I + sc-: relaxation of negatively supercoiled pUC18 by topoisomerase I (positive control).

Supplementary reference

1. Gubaev, A., Weidlich, D. and Klostermeier, D. (2016) DNA gyrase with a single catalytic tyrosine can catalyze DNA supercoiling by a nicking-closing mechanism. *Nucleic Acids Res.*, **44**, 10354-10366.