Structure, Volume *28*

Supplemental Information

Mechanism of CcdA-Mediated Rejuvenation of DNA Gyrase

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Supplementary Information

Supporting tables:

Table S1: Interactions of CcdB residues with CcdA based on PDB structure 3G7Z which has two chains of CcdA bound to CcdB dimer. ΔASA is the difference between the solvent accessible surface area of the CcdB residues in free form (3VUB) and CcdA-bound form (3G7Z) (related to Figure 1).

CcdB Chain A	CcdA Interacting chain & residue no.	ΔASA - All atom (Å^2)	CcdB Chain B	CcdA Interacting chain & residue no.	ΔASA - All atom (Å^2)
8	D70, D,72	38.9	8	D65, D68, D69	27.1
			10	D68, D69	55.5
12	D72	31.5	12	D68	14.5
13	C58, D72	151.4	13	D62	101.8
14	C58, C59, D72	28.6	14	D58, D59, D65, D63	20.8
			17	D65	0.6
23	D45	20	23	C45	22.2
24	D41, D44, D45	85.7	24	C41, C44 C45	102.6
25	D44, D45, D52	22.8	25	C44, C45, C52	22.3
26	D45, D52	47.7	26	C52	69.9
27	D52	0.7	27	C52	4.1
28	D52, D53, D56	51.3	28	C52, C56	32
30	D64, D66, D67	42.6	30	D69, D71	54.3
35	D72	10.9	35	D65	10.9
37	C58	2.6	37	D58	1.3
41	C54, C58	63	41	D54, D58	56.8
42	C54, C55, C58	58	42	D54, D55, D58	64.3
43	C54	8.4	43	D54	5.5
45	C48, C50, C51	98	45	D48, D50, D51, D54	102.4
46	C51, C54, C55	57.5	46	D51, D54, D55	56.5
47	C44, C48	37.3	47	D44, D48	34.1
49	C44, C48	7			
50	C44, C52	15.8	50	D44, D52	15.8
51	C52, C55	30	51	D52, D55	29.5
64	C55, C58, C59	34.2	64	D55, D58, D59	33.9
66	C52, C55	1.1	66	D52, D55	0.6
67	C59, D71, D72	36.7	67	D59, D63, D65, D66	34.1
69	D70, D72	28.5	69	D65, D66, D69	26
70	D66, D70	12.7	70	D69, D71	16.7

71	D70, D72	16	71	D65, D69	16
			72	D69, D71	14.2
96	C44	41	96	D44	43
101	C40, C41, C44	92.5			

Table S2: Interactions of CcdA residues with CcdB based on PDB structure 3G7Z. ΔASA is the difference between solvent accessible surface area of the CcdA residues in the (fictitious) free form (removing CcdB from CcdBA complex) and CcdA-bound form (3G7Z) (related to Figure 1).

CcdA Chain C	**CcdB Interacting chain & residue no.**	**ΔASA - All atom (Å^2)**	**CcdA Chain D**	**CcdB Interacting chain & residue no.**	**ΔASA - All atom (Å^2)**
40	A101	17.6	40		
41	A101, B24	35.4	41	A24	37.6
44	A47, A49, A50, A96, A101, B24, B25	100.3	44	B47, B50, B96, A24, A25	133
45	B23, B24, B25	80.3	45	A23, A24, A25, A26	60
48	A47, A49	27.9	48	B47	34.8
50	A45	16	50	B45	33.3
51	A45, A46,	33.3	51	B45, B46	32.8
52	A50, A51, A66, B25, B26, B27, B28	105.4	52	B50, B51, B66, A26, A27, A28	106
53			53	A28	
54	A41, A42, A43, A46	71.6	54	B41, B42, B43, B45, B46	77.6
55	A42, A46, A51, A64, A66	80.7	55	B42, B46, B51, B64, B66	69.1
56	B28	32.1	56	A28	31.1
58	A13, A14, A37, A41, A42, A64,	76.9	58	B14, B37, B41, B42, B46, B64	97
59	A14, A64, A67	15.3	59	B14, B64, B67	39.9
			62	B13	10.1
			63	B14, B67	17.2
			64	A30	20.3
			65	B8, B17, B35, B67, B69, B71	162.6
			66	B67, B69, A70	64.8
			67	A30	27.9
			68	B8, B10, B12	48.6
			69	B8, B10, B30, B69, B70, B71, B72	71.1
			70	A8, A69, A70, A71	86.6
			71	B30, B70, B72	34.4

			72	A8, A12, A14, A35, A67, A69, A70, A71	211.6

Table S3: Interactions of CcdB residues with GyrA14 based on PDB structure 1X75. ΔASA is the difference between the solvent accessible surface area of the CcdB residues in free form (3VUB) and GyrA14-bound form (1X75) (related to Figure 1).

Chain C of CcdB	**GyrA interacting chain and residue no.**	**ΔASA – All atom (Å²)**	**Chain D of CcdB**	**GyrA interacting chain and residue no.**	**ΔASA - All atom (Å²)**
24	A375, A376, A379	76.4	24	B375, B376, B379	87.8
25	A376	3.4	25	B376	6
26	A368, A372	35.5	26	B368	30.9
87	A407, A456, A460	37.9	87	B407, B456, B460	34.9
88	A403, A456, A460, B464	53.2	88	B403, B456, B460, A464	53.7
91	B462	58.6	91	A462, B456, B460	54.6
92	B462, B464, B465	33.5	92	A462, A464, A465	25.5
95	A462, B462	36	95	A462, B462	34.1
96	B376	31.7	96	A376	32.4
99	A462, B460, B462	38.9	99	A460, A462, B462	35.5
100	B376	7.5	100	A376	7.5
101	B379, B380, B383, B457, B474	176.8	101	A379, A380, A383, A457, A461, A474,	170.5

Table S4: Interactions of GyrA14 residues with CcdB based on PDB structure 1X75. ΔASA is the difference between the solvent accessible surface area of the GyrA14 residues in the free form obtained after removing CcdB from 1X75 and GyrA14-bound form (1X75) (related to Figure 1).

Chain A of GyrA	**CcdB interacting chain and residue no**	**ΔASA – All atom (Å²)**	**Chain B of GyrA**	**CcdB interacting chain and residue no**	**ΔASA -All atom (Å²)**
368	C26	20.1	368	D26	16
372	C26				
375	C24	18.2	375	D24	15
376	C24, C25, D96, D100	98	376	D24, D25, C96, C100	97.5
379	C24, D101	29.7	379	D24, C101	34.8
380	D101	7.8	380	C101	9.2
383	D101	16.1	383	C101	16.4
403	C88	14	403	D88	15.5
407	C87	9.2	407	D87	8.6
456	C87, C88	53.5	456	D87, D88, D91	62.3
457	D101	7.2	457	C101	7
460	C87, C88, D91, D92, D95, D99	42.4	460	C99, D87, D88, D91	43.
461	D101	8.8			
462	C95, C99, D91, D92, D95 D99	99.2	462	C91, C92, C95, C99, D95, D99	77.9
464	D88, D92	30.9	464	C88, C92	21.5
465	D92	40	465	C92	26.9

Supporting figures:

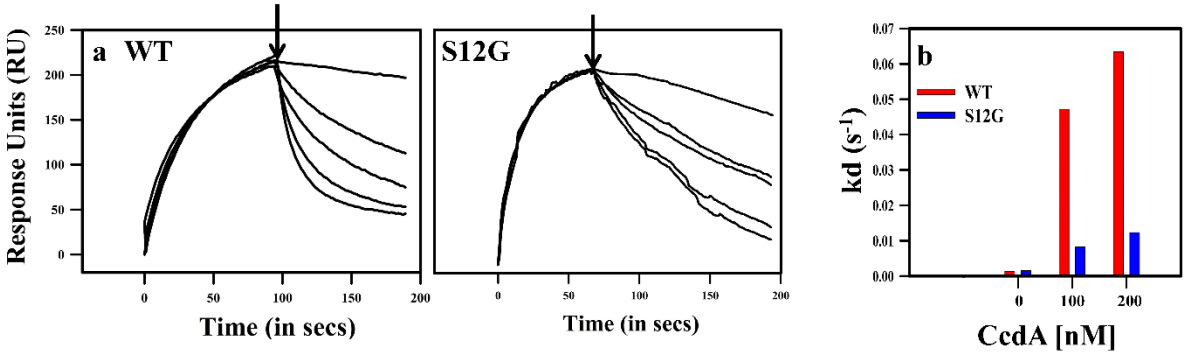

Figure S1: Dissociation of CcdB from GyrA14 in presence and absence of $CcdA_{45-72}$ (related to Figures 4 and 5). Overlays show the binding kinetics of 50 nM CcdB which is passed over Gyrase A immobilized on a CM5 chip for 100secs followed by dissociation mediated by different concentrations of $CcdA_{45-72}$. The arrow (↓) indicates the time of addition of CcdA. (a) Overlays show the dissociation of WT CcdB (left) bound to Gyrase with CcdA peptide, concentration increasing from the top to bottom (0 nM, 10 nM, 25 nM, 100 nM, 200 nM) and dissociation of S12G (right) bound to Gyrase with CcdA peptide, concentration increasing from the top to bottom (0 nM, 100 nM, 200 nM, 1000 nM, 5000 nM). The ligand GyrA14 was immobilized on the CM5 chip by standard amine coupling. (b) The apparent dissociation rate constants (kd) mediated by $CcdA_{45-72}$ are approximately five-fold lower for the S12G-CcdB-GyraseA14 complex than for WT-CcdB-GyraseA14.

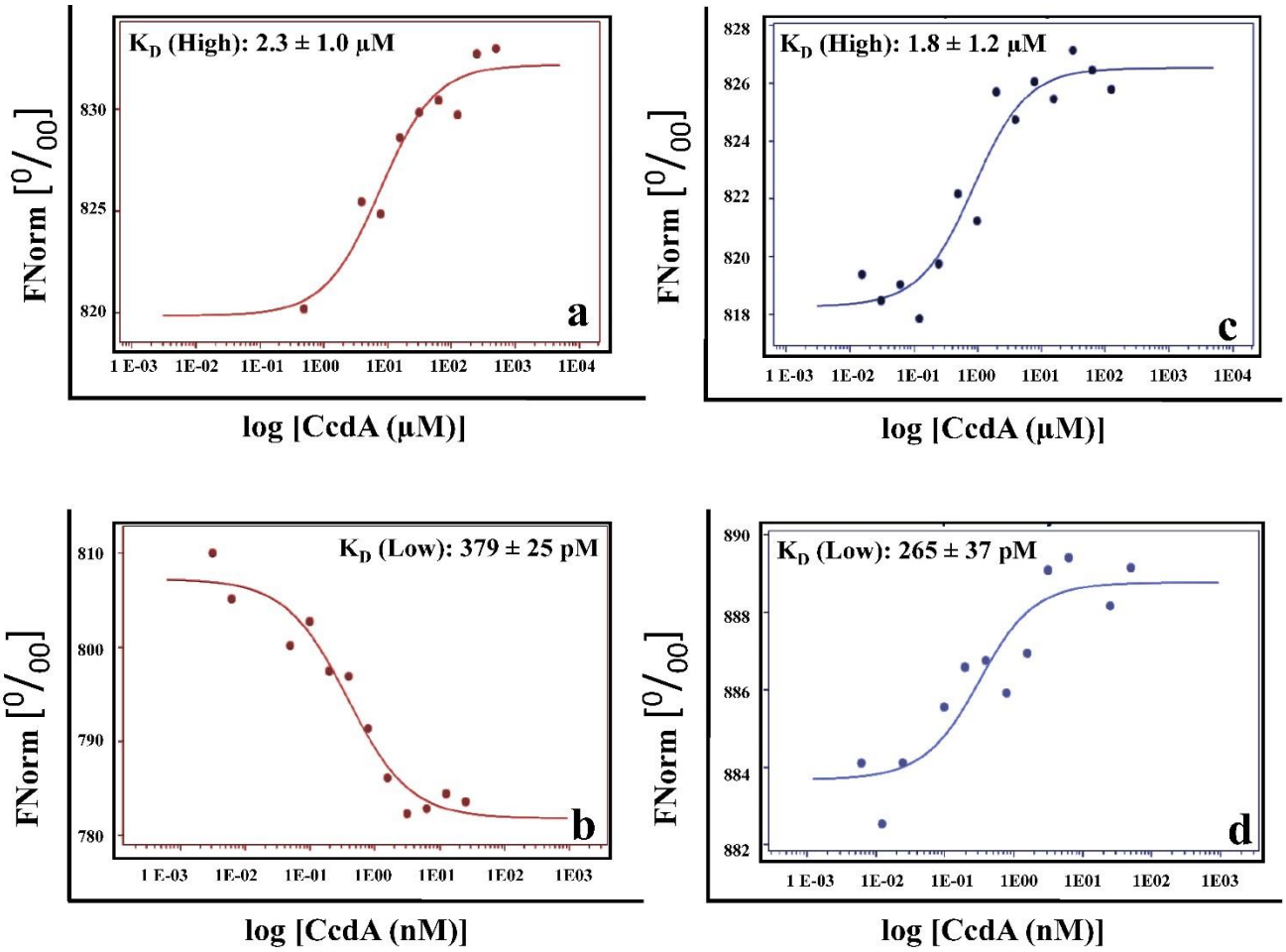

Figure S2: CcdB WT and S12G CcdB have similar affinity for CcdA$_{45\text{-}72}$ as determined by MicroScale Thermophoresis (related to Figures 4 and 5). CcdB WT (red) and S12G (blue) were labeled with Monolith™ Protein Labeling Kit NT-647-NHS dye (NanoTemper Technologies) according to the manufacturer's instructions, used at a concentration of 110 nM and titrated with two different concentration ranges of CcdA$_{45\text{-}72}$ to determine the K_D for both the high and low affinity binding sites for CcdB WT (a-b) and S12G (c-d). All studies were carried out in 200 mM HEPES, pH 8.4 and at 27 °C. The normalised fluorescence FNorm is plotted as parts per thousand [‰] as a function of [CcdB]. For each capillary (each measuring point), an MST trace is recorded. All traces are then normalised to start at 1000. For each trace, the FNorm value for the dose- response curve is calculated by dividing Fhot (MST laser on)/Fcold (MST laser off). The dissociation constants (K_D) were determined employing standard data analysis with MO.Affinity Analysis Software (Wienken *et al*, 2010).

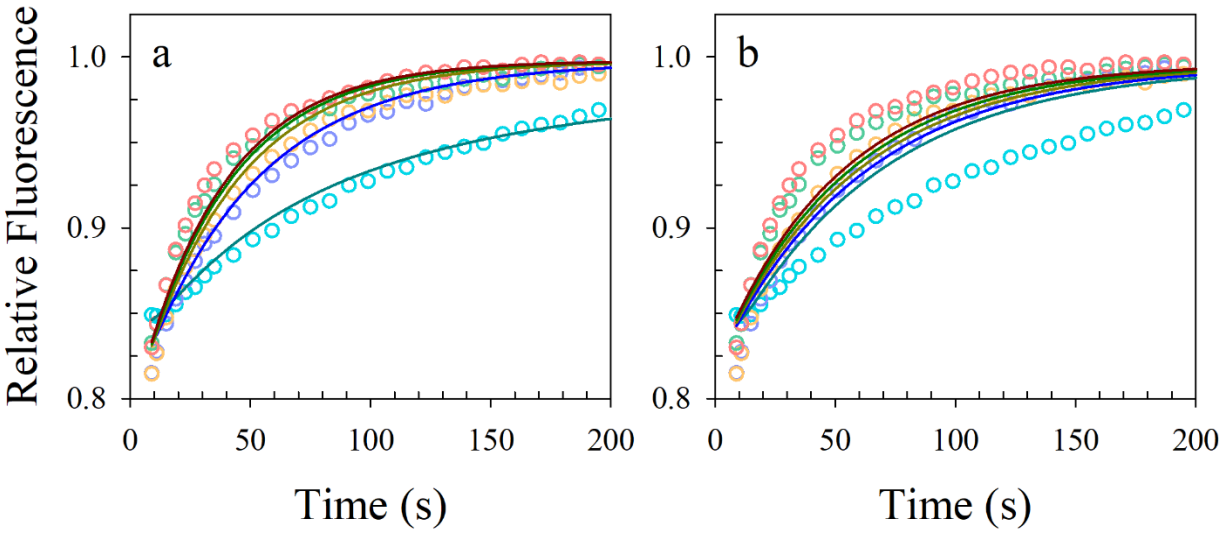

Figure S3: Global analysis of the rejuvenation data with single pathway (related to Figure 2). Panels a and b show global fitting to the concentration dependent rejuvenation traces using scheme 1 assuming $\lambda_4 << \lambda_3$ and $\lambda_2 << \lambda_4$, respectively. λ represents the kinetic flux through a given step, whereas k represents the corresponding microscopic rate constant.

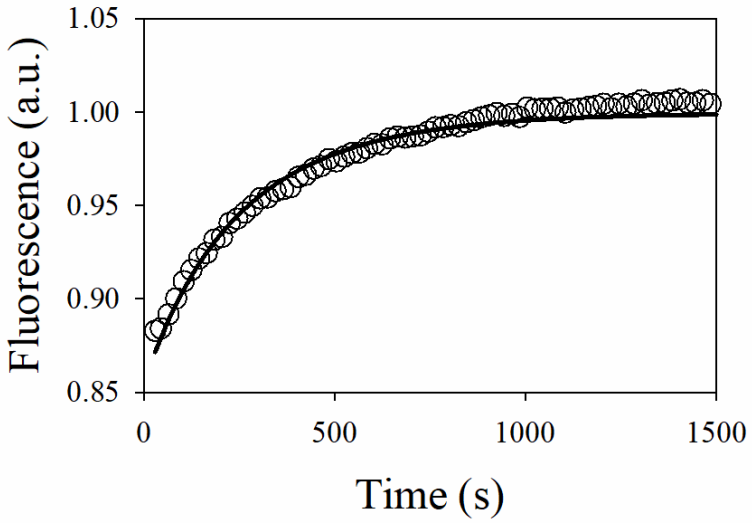

Figure S4: Rejuvenation using sub-stochiometric fraction of CcdA* (related to Figure 2). Rejuvenation trace acquired upon mixing CcdB-GyrA14* complex with CcdA* in 2:1 ratio. The rejuvenation process slows down by about fivefold with a twofold decrease in CcdA* concentration. This validates the requirement for two pathways in the rejuvenation process in scheme 1. The solid line represents fit to the data using scheme 1.

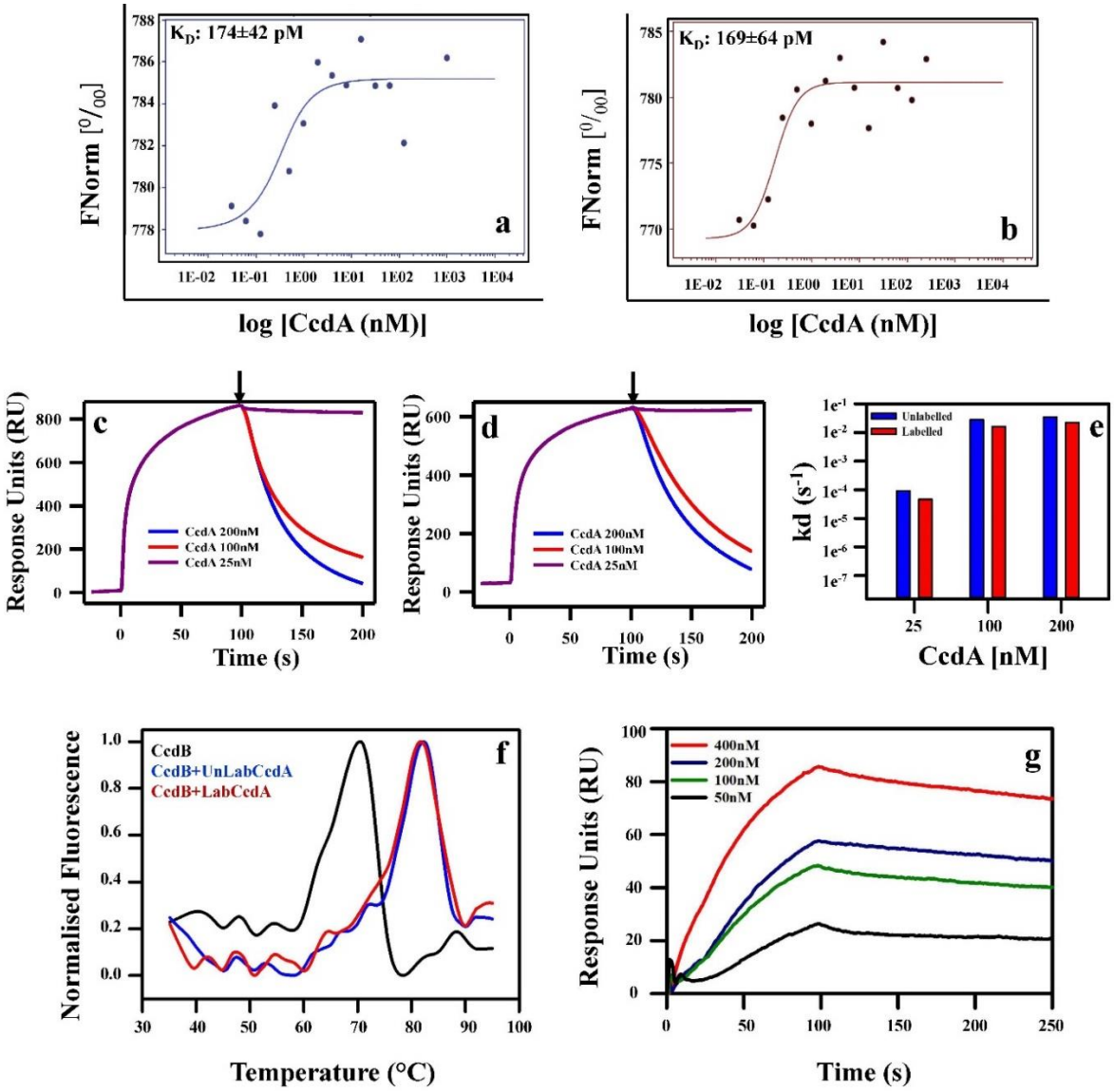

Figure S5: Both unlabelled and labelled $CcdA_{50\text{-}72}$ and Gyrase have similar affinity for CcdB WT as determined by MicroScale Thermophoresis, SPR and Tycho and SPR respectively (related to Figure 2). CcdB WT was labeled with Monolith™ Protein Labeling Kit NT-647-NHS dye (NanoTemper Technologies) according to the manufacturer's instructions, used at a concentration of 3 nM and titrated with different concentration ranges of (a) unlabelled $CcdA_{50\text{-}72}$ and (b) labelled $CcdA_{50\text{-}72}$ to determine the K_D for CcdB WT. All studies were carried out in 1XPBS, pH 7.4 and at 25 °C. The normalised fluorescence FNorm is plotted as parts per thousand [$^0/_{00}$] as a function of [CcdA]. For each capillary (each measuring point), an MST trace is recorded. All traces are then normalised to start at 1000. For each trace, the FNorm value for the dose-response curve is calculated by dividing Fhot (MST laser on)/Fcold (MST laser off). The dissociation constants (K_D) were determined employing standard data analysis with MO. Affinity Analysis Software (Wienken et al., 2010). (c-d) Overlays show the binding kinetics of 50 nM CcdB which is passed over Gyrase A immobilized on a CM5 chip for 100secs followed by dissociation

mediated by different concentrations of (c) unlabelled $CcdA_{50\text{-}72}$ and (d) labelled $CcdA_{50\text{-}72}$. The arrow (↓) indicates the time of addition of CcdA. Overlays show the dissociation of WT CcdB bound to Gyrase with unlabelled CcdA peptide (left), concentration increasing from the top to bottom (25 nM, 100 nM, 200 nM) and with labelled CcdA peptide (right), concentration increasing from the top to bottom (25 nM, 100 nM, 200 nM). The ligand GyrA14 was immobilized on the CM5 chip by standard amine coupling. (e) The apparent dissociation rate constants (kd) mediated by both unlabelled and labelled $CcdA_{50\text{-}72}$ are similar for the CcdB-GyraseA14 complex. (f) Thermal unfolding experiment of CcdB WT as well as complex with unlabelled $CcdA_{50\text{-}72}$ (blue) and labelled $CcdA_{50\text{-}72}$ (red) were carried out by nanoTemper Tycho (Tycho NT.6) by applying a thermal ramp of 30°C/min. 10µL of each CcdB protein (2 µM) with or without 5 µM of unlabelled and labelled $CcdA_{50\text{-}72}$ was subjected to thermal unfolding. (g) Binding sensogram of fluorescein labeled E487CGyrA14 (E487CGyrA14*) with CcdB, monitored by SPR on Biacore3000. Different concentrations of E487CGyrA14* mutant (50, 100, 200, and 400 nM from bottom to top), when passed as analytes over CcdB immobilized sensor channel surface, showed similar binding with CcdB. The E487CGyrA14* mutant binds with a K_D of 22 ± 10 nM with a k_{on} of 0.8 $\times 10^5$ ± 0.15 × 10^5 $M^{-1}s^{-1}$and a k_{off} of 1.46 × 10^{-3} ± 0.4 × 10^{-3} s^{-1}at 25 °C, similar to that previously reported for WT GyrA14 which binds with a k_{on} of 1.4×10^5 $M^{-1}s^{-1}$, a k_{off} of 1.3 × 10^{-3} s^{-1} and a K_D of 9.4 nM (Tripathi et al., 2019).