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Srikanth Banda

Department of Chemistry and Biochemistry, Florida International University, sbanda@fiu.edu

Purushottam Babu Tiwari

Georgetown University

Yesim Darici

Department of Physics, Florida International University, dariciy@fiu.edu

Yuk-Ching Tse-Dinh

Biomolecular Sciences Institute and Department of Chemistry and Biochemistry, Florida International University, ytsedinh@fiu.edu

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Investigating direct interaction between *Escherichia coli* topoisomerase I and RecA

Srikanth Banda^{a,#}, Purushottam Babu Tiwari^{b,#}, Yesim Darici^{c,*}, and Yuk-Ching Tse-Dinh^{a,d,*}

^aDepartment of Chemistry and Biochemistry, Florida International University, Miami, Florida, USA

^bDepartment of Oncology, Georgetown University, Washington, District of Columbia, USA

^cDepartment of Physics, Florida International University, Miami, Florida, USA

^dBiomolecular Sciences Institute, Florida International University, Miami, Florida, USA

*Corresponding Authors:

Yesim Darici E-mail: dariciy@fiu.edu

Phone: +1(305) 348-2606 Fax: +1 (305) 348-6700

Yuk-Ching Tse-Dinh E-mail: ytsedinh@fiu.edu

Phone: +1(305) 348-4956 Fax: +1 (305) 348-3772

#Authors with equal contribution

Highlights

- *E. coli* topoisomerase I (EcTOP1) physically interacts with RecA.
- EcTOP1 can interact with RecA directly in the absence of DNA.
- RecA interacts with the N-terminal domain of EcTOP1 that forms the active site region.
- RecA interaction with EcTOP1 is stimulated by ATP.

ABSTRACT

Protein-protein interactions are of special importance in cellular processes, including replication, transcription, recombination, and repair. *Escherichia coli* topoisomerase I (EcTOP1) is primarily involved in the relaxation of negative DNA supercoiling. *E. coli* RecA, the key protein for homologous recombination and SOS DNA-damage response, has been shown to stimulate the relaxation activity of EcTOP1. The evidence for their direct protein-protein interaction has not been previously established. We report here the direct physical interaction between *E. coli* RecA and topoisomerase I. We demonstrated the RecA-topoisomerase I interaction via pull-down assays, and surface plasmon resonance measurements. Molecular docking supports the observation that the interaction involves the topoisomerase I N-terminal domain that forms the active site. Our results from pull-down assays showed that ATP, although not required, enhances the RecA-EcTOP1 interaction. We propose that *E. coli* RecA physically interacts with topoisomerase I to modulate the chromosomal DNA supercoiling.

Keywords

Protein-protein interactions, RecA, DNA topoisomerase I, SPR, Molecular docking, Pull-down assay

1. Introduction

Protein-protein interactions (PPIs) are essential features of almost every cellular process (Coulombe et al., 2004; Perkins et al., 2010). Genomic processes including DNA replication, transcription, translation, recombination, and repair require an ensemble of proteins (Coulombe et al., 2004). PPIs, especially transient protein interactions, are vital in the regulation of the above-mentioned genomic processes (Perkins et al., 2010; Ngounou Wetie et al., 2013). Proteins involved in transient interactions can function as independent units in the cells, and certain post-translational modifications on these proteins or binding of ligands can trigger the protein interactions. A protein's function is defined and controlled through interaction with other proteins, or biomolecules (Ngounou Wetie et al., 2013).

Understanding protein-protein interaction network in *Escherichia coli* would be essential in broadening current insight on the fundamental cellular processes. PPIs involved in DNA damage response would be important for the development of antibiotic resistance (Marceau et al., 2013). We are reporting here a direct physical interaction of RecA, the key player of homologous recombination and SOS DNA-damage response in *E. coli*, with DNA topoisomerase I. RecA family of recombinases, conserved in most of the bacteria, are ATP-dependent proteins mediating homologous recombination, DNA repair and genome integrity (Karlin and Brocchieri, 1996; Lin et al., 2006; Cox, 2007). Homolog searches have provided evidence for conservation of RecA in bacteria, archaea, and eukaryotes, although, the functions of the homologs have diversified with evolution. Most of the archaeal species have two RecA homologs (RadA and RadB), whereas the eukaryotes have multiple representatives of the RecA family (Rad51, Rad51B, Rad51C Rad51D, Dmc1, XRCC2, XRCC3, and RecA) (Lin et al., 2006). RecA monomers bind to single-stranded DNA (ssDNA) in an ATP-dependent manner forming an active nucleoprotein filament (McGrew

and Knight, 2003; Bell, 2005). *E. coli* RecA, a prototype of RecA family of proteins, has multiple roles in the cell. RecA catalyzes the DNA strand exchange mechanism by coupling with ATP hydrolysis, promoting the recombination process (Cox, 1999; Cox, 2002; Lusetti and Cox, 2002; Cox, 2003; Renzette and Sandler, 2008). RecA can also function as a coprotease of LexA, and UmuD proteins. RecA facilitates the autocatalytic cleavage of LexA repressor, which is required for inducing the SOS response (Little, 1991; Harmon et al., 1996). It can also facilitate the autocatalytic cleavage of UmuD to an active UmuD', which is a component of a low fidelity DNA polymerase V that is involved in the translesion DNA synthesis (Patel et al., 2010).

The topology of DNA is maintained by an important group of evolutionarily conserved enzymes called topoisomerases (Wang, 2002). The essential genomic processes such as replication, transcription, recombination, and repair can create topological strain or entanglement on the double helix of DNA (Vos et al., 2011). Topoisomerases transiently cleave and rejoin DNA (Wang, 1971) to resolve the topological strain or entanglement, and maintain the genomic stability (reviewed in (Wang, 1971; Berger, 1998; Champoux, 2001; Chen et al., 2013)).

E. coli DNA topoisomerase I is primarily involved in the relaxation of negatively supercoiled DNA by the strand passage mechanism (Brown and Cozzarelli, 1981; Tse-Dinh, 1986; Champoux, 2002). It has an important function in preventing excess negative supercoiling of DNA (Drlica, 1992) which can affect global transcription and result in growth inhibition. According to a previous report, the relaxation activity of *E. coli* topoisomerase I is stimulated by RecA; suggesting a functional interaction between RecA and topoisomerase I (Reckinger et al., 2007). It remains unclear whether this stimulatory effect is due to direct protein-protein interaction between *E. coli* RecA and topoisomerase I, or is only due to the effect of *E. coli* RecA on DNA conformation. More recent results showed that mutations in *E. coli topA* gene coding for

topoisomerase I can diminish the *E. coli* SOS response to DNA damage and antibiotics treatment (Liu et al., 2011; Yang et al., 2015). The interaction between RecA and topoisomerase I may influence the increase in antibiotic resistance (Hastings et al., 2004; Beaber et al., 2004; Thi et al., 2011) and persistence shown to be associated with the SOS response (Dörr et al., 2009). In this study, we tested the hypothesis that *E. coli* RecA might physically interact with topoisomerase I to modulate the topoisomerase I catalytic activity and DNA supercoiling.

Herein, we present evidence for a direct physical interaction between *E. coli* RecA, and topoisomerase I in solution by pull-down assays (Yang et al., 2015) as well assess the influence of ATP, and the domains of topoisomerase I involved in the protein-protein interaction with RecA. We further investigated the inter-protein interaction between *E. coli* RecA and topoisomerase I by using surface plasmon resonance (SPR) and molecular docking. SPR is a widely accepted label-free biophysical tool in order to investigate biomolecular interactions (Wilson, 2002; Willander and Al-Hilli, 2009; Tiwari et al., 2014), including PPIs (Berggård et al., 2007; Tiwari et al., 2015), whereas molecular docking can be used to provide structural insights for PPIs (Smith and Sternberg, 2002; Gray et al., 2003). The structural basis for the protein-protein interaction was predicted by molecular docking that shows the N-terminal domain (NTD) of topoisomerase I is involved in the interaction with RecA. The NTD (amino acids 1-597) contain the active site for DNA cleavage-religation (Lima et al., 1994). Experimental evidence supporting this prediction was provided from pull-down assays.

2. Material and methods

2.1. Bacterial strains and plasmids

E. coli strain BW25113 ($\Delta(araD-araB)567$, $\Delta lacZ4787(::rrnB-3)$, λ^- , *rph-1*, $\Delta(rhaD-rhaB)568$, *hsdR514*), obtained from Yale CGSC (Datsenko and Wanner, 2000), was used for preparing the cell lysate used in the pull-down of RecA from total cellular proteins. Plasmid, pLIC-ETOP was used for the expression and purification of recombinant *E. coli* topoisomerase I with 6x-His tag (Sorokin et al., 2008). A plasmid, pLIC-NTD-ETOP was made similarly as pLIC-ETOP by introducing the coding sequence of the NTD of *E. coli* topoisomerase I (amino acids 1-597) into a pLIC-*HK* cloning vector that allows T7 RNA polymerase-dependent expression of His-tagged NTD of topoisomerase I for purification (Sorokin et al., 2008). A pET His₆-Mocr TEV cloning vector (2O-T) (gift of Scott Gradia, Addgene #29710) was used for expression and purification of a recombinant viral protein, His-Mocr (DelProposto et al., 2009) that was used as negative control in the pull-down assays.

2.2. Purified Proteins

E. coli topoisomerase I with a N-terminus 6x-His tag (His-EcTOP1) was expressed from pLIC-ETOP in *E. coli* BL21AI by induction with 1mM IPTG, 0.02% L-Arabinose as described previously (Sorokin et al., 2008). N-terminal domain of the *E. coli* topoisomerase I with a N-terminus 6x-His tag (His-NTD-EcTOP1) was expressed from pLIC-NTD-ETOP in BL21 Star (DE3) by induction with 1mM IPTG. Expression of recombinant His-tagged Mocr was induced in BL21 star (DE3) with 1mM IPTG. Ni Sepharose 6 Fast Flow beads (GE Healthcare Life Sciences) were used to purify these proteins by affinity chromatography (Cheung et al., 2012) to near homogeneity as described previously (Sorokin et al., 2008) with some modifications (Supplementary Material, section S1, Fig. S1). Purified *E. coli* RecA was purchased from New

England BioLabs for use in assays involving verification of direct protein-protein interactions with topoisomerase I.

2.3. Pull-down assays to study direct physical interactions between purified proteins

Pull-down assays were carried out to establish the physical interactions of proteins in solution (Yang et al., 2015). An assay involving the incubation of purified RecA and topoisomerase I was carried out to study the direct physical interactions between these proteins. Purified His-EcTOP1 serves as bait in these assays. Individual pull-down reactions were set up by incubating constant amount of bait (10nM) with varying concentrations (0-80nM) of RecA (prey) for 2 hours at 4°C. The bait-prey interactions were set up in pull-down buffer with 10mM HEPES, pH 7.4, 100mM NaCl, 0.5mM MgCl₂, 0.005% v/v Tween-20. The HisPur Cobalt Agarose resin (Thermofisher), previously equilibrated in the above-mentioned pull-down buffer, was mixed with the bait-prey reaction. Following an overnight incubation at 4°C, the reactions were centrifuged and the supernatant was discarded. The resin pellet was then washed three times in HEPES buffer, and the proteins bound to the resin were eluted with pull-down buffer containing 400mM imidazole. The eluates were electrophoresed in a polyacrylamide SDS gel, and RecA was detected by western blotting (Burnette, 1981) with Anti-RecA monoclonal antibody (MBL International Corp.). A C-DiGit blot scanner (LI-COR) was used to detect the chemiluminescent western blot signal, and the signal intensity was quantified (Image Studio Digits version 4.0).

A comparative study was performed to compare the RecA-topoisomerase I binding efficiency in the presence, and absence of 5 mM ATP. The assay was carried out with a constant amount (10nM) of His-EcTOP1 as bait, and varying concentrations (0-80nM) of RecA as prey. An independent similar assay was carried out with a constant amount (10nM) of NTD-EcTOP1 as bait, and varying RecA concentrations (0-60nM) as prey in the presence of ATP.

2.4. Pull-down assays on *E. coli* soluble cell lysate

In this assay, *E. coli* strain (BW25113) was allowed to grow in LB medium for 16 hrs to stationary phase ($OD_{600}=2.5$), and the culture was pelleted. The cell pellet was suspended in pull-down buffer with 1mg/ml lysozyme. The suspended cells were subjected to lysis by four freeze-thaw cycles. The lysate was centrifuged at 13000xg for 2 hrs at 4°C. The soluble fraction was precleared with HisPur Cobalt Agarose resin before incubation with the bait. Either full length purified EcTOP1 or NTD-EcTOP1 was used as bait. A Bacteriophage T7 protein, Mocr, with a N-terminus 6x-His tag was used as bait in the negative control for the pull-down assay (DelProposto et al., 2009).

Bait (40nM), and total cellular proteins in lysate (150µg) were incubated at 4°C for 2 hrs. HisPur Cobalt Agarose resin (Thermofisher) was mixed with the reaction, and incubated overnight at 4°C. On the following day, the resin-reaction mixture was spun, and the supernatant was discarded. The bead pellet was washed three times in pull-down buffer with 10mM imidazole to minimize non-specific binding of histidine rich proteins to the resin. The proteins bound to the resin were finally eluted in 400mM imidazole, and the eluates were subjected to SDS-PAGE analysis. A western blot was performed to probe for RNA polymerase, and RecA in the eluates using a monoclonal antibody against RNA polymerase beta (BioLegend), and RecA respectively.

2.5. SPR

Biacore T200 SPR instrument was used to record SPR sensorgrams. EcTOP1 was immobilized onto CM5 sensor surface using standard amine coupling chemistry. Buffered

solutions with various concentrations of RecA were flown over EcTOP1 immobilized sensor surface. A detailed explanation of SPR experimental procedures, including data analysis, is included in the Supplementary Material (section S2).

2.6. Molecular docking

The formation of inter-protein complex between EcTOP1 and RecA was optimized using pyDockWEB (Jiménez-García et al., 2013). Protein coordinates from pdb entries 4RUL (full length EcTOP1, (Tan et al., 2015)) and pdb entry 2REB (*E. coli* RecA, (Story et al., 1992)) were used in the docking study as receptor (EcTOP1) and ligand (RecA), respectively. The top ten docked complexes from the pyDockWEB outputs, based on energy scoring, were used to predict the RecA interaction site on EcTOP1. The output pdb file of the top scored complex was analyzed using PDBsum database (Laskowski et al., 1997; Laskowski, 2001). Chimera molecular graphics software (Pettersen et al., 2004) was used to visualize the structure and to generate images of the docked complexes.

3. Results

3.1. Pull-down assay demonstrates a direct physical interaction between *E. coli* RecA and topoisomerase I

A functional association between *E. coli* RecA and topoisomerase I have been reported previously (Cunningham et al. 1981; Reckinger et al., 2007). More recently, a role of topoisomerase I was observed in *E. coli* SOS response (Liu et al., 2011; Yang et al., 2015), which prompted us to verify the possibility of a direct physical interaction between these proteins. Purified His-EcTOP1 and RecA were incubated together in the presence of ATP, and pulled-down with Cobalt agarose resin. The amount of RecA bound to EcTOP1 was determined by western blot analysis of the eluates from the reaction with monoclonal antibodies against RecA. The results

(Fig. 1) confirmed the possibility of a direct interaction between these proteins. Pull-down of RecA by the resin required the presence of His-EcTOP1. Both *E. coli* RecA, and topoisomerase I bind strongly to single-stranded DNA. However, according to this pull-down result with purified RecA and topoisomerase I, the association between these proteins does not require the presence of DNA.

3.2. Influence of ATP on the binding efficiency of RecA with EctopI

The functional interactions between *E. coli* RecA and topoisomerase I were observed in the presence of ATP. According to a previous report (Konola et al., 1994), ATP binds to the P-loop of RecA. *E. coli* RecA undergoes ATP dependent conformational change (Cox, 2003) that could affect its interaction with topoisomerase I (Cunningham et al. 1981; Reckinger et al., 2007). We, therefore tested the influence of ATP on the physical interaction between *E. coli* RecA and topoisomerase I with pull-down reactions in the absence or presence of ATP.

While the results from the pull-down assay suggested that the protein-protein interaction between *E. coli* RecA and topoisomerase I may not require ATP, the presence of ATP was found in pull-down assay to enhance the protein-protein interaction significantly (Fig. 2). Experimental data from one trial of pull-down experiment is shown here. Similar enhancement of the interaction by the presence of ATP were seen in two additional trials of the experiment (Supplementary Material, section S3, Fig. S3). However, ATP did not appear to be absolutely required for the interaction. Direct protein interaction between *E. coli* RecA and topoisomerase I in the absence of ATP has been confirmed by surface plasmon resonance (SPR) measurements (Supplementary Material, section S2). We could not obtain meaningful SPR sensorgrams for RecA-EcTOP1 interactions in the presence of ATP due to technical difficulty (Supplementary Material, section S2).

3.3. Molecular docking results for the complex formation between RecA and EcTOP1

Fig. 3 depicts the binding complex, as predicted by pyDockWEB, between EcTOP1 (receptor) and RecA (ligand). Fig. 3A shows the surface representation for the binding of EcTOP1 (green) and with RecA (10 different colors, except green, representing the RecA conformations upon binding with EcTOP1). Fig. 3B shows the cartoon representation for the top-scored EcTOP1-RecA docked complex as well as the interacting amino acid residues, as predicted by PDBsum, across the binding interface. The amino acid residues predicted to be responsible for the formation of hydrogen bonds and salt bridges are listed in the Supplementary Material (section S4).

3.4. Pull-down assay for complex formation between NTD-Ectopoi and RecA

Molecular docking results (section 3.3) have suggested that the NTD of Ectopoi can interact with RecA in *E. coli*. The possibility of the direct interaction of RecA with the NTD-Ectopoi was verified by pull-down assays, involving the direct incubation of purified recombinant NTD-Ectopoi and RecA, in the presence of ATP. In these assays, NTD-Ectopoi (bait) and RecA (prey) were incubated with HisPur Cobalt agarose resin, in the presence of ATP. The eluates from the pull-down reactions were analyzed by western blotting with monoclonal RecA antibodies. The results from the assay suggest that the N-terminal domain of topoisomerase I and RecA can interact physically (Fig. 4).

3.5. Pull-down of RecA from *E. coli* soluble cell lysate by recombinant EcTOP1 and NTD-ECTOP1

E. coli RecA has a stimulatory effect on the topoisomerase I relaxation activity, suggesting a possible protein-protein interaction between RecA and topoisomerase I (Reckinger et al., 2007). We were able to verify a direct physical interaction between RecA and EcTOP1 with purified proteins in solution (Fig 1A). A pull-down assay using the *E. coli* cell lysate was performed to

further confirm the interaction of *E. coli* topoisomerase I NTD with *E. coli* RecA. The cell lysate, and EcTOP1 or NTD-EcTOP1 were incubated together with HisPur Cobalt agarose beads that have high affinity for the 6x-Histidine tag. The complexes recovered from the beads after the pull-down protocol were resolved by SDS-PAGE, and analyzed by western blot. The nitrocellulose membrane was probed for RecA and RNA polymerase with monoclonal antibodies against RecA and RNA polymerase beta subunit respectively. EcTOP1 is known to interact with *E. coli* RNA polymerase via its CTD (Cheng et al., 2003).

The results showed that both RecA, and RNA polymerase were pulled down by full-length topoisomerase I as expected (Fig. 6, lane 2). The data also confirmed that the NTD of topoisomerase I can interact with RecA (Fig. 6, lane 3). Interaction between NTD-EcTOP1 and RNA polymerase was not observed, demonstrating the domain specific interactions between EcTOP1 and its partners (Cheng et al., 2003).

4. Discussion

In a previous study (Reckinger et al., 2007), the stimulation of topoisomerase I relaxation activity by *E. coli* RecA was seen only for topoisomerase I protein from *E. coli* and not for the topoisomerase I proteins from other species. This indicated that the stimulation of relaxation activity by RecA was not entirely due to the effect of RecA on DNA conformation. Even though this previous results suggested that *E. coli* RecA may stimulate topoisomerase I relaxation activity via direct protein-protein interaction, data to support such interaction was not available (Reckinger et al., 2007). We have presented evidence here for the first time to confirm the direct physical interaction between *E. coli* RecA and topoisomerase I. The presence of DNA was not required for this interaction. ATP, although not absolutely required, can enhance the protein-protein interaction between *E. coli* RecA and topoisomerase I. *E. coli* topoisomerase I plays an important role in the

regulation of local and global DNA supercoiling (Liu and Wang, 1987; Drlica, 1992). The stimulation of topoisomerase I relaxation activity by RecA via direct protein-protein interaction allows RecA to add modulation of DNA supercoiling to its multiple roles. This stimulation of topoisomerase I relaxation activity by RecA may enable relaxation-dependent *E. coli* promoters to have higher transcription activities following DNA damage, as observed following norfloxacin treatment (Jeong et al., 2006; Reckinger et al., 2007).

As a type IA DNA topoisomerase, EcTOP1 binds to the single-stranded region of negatively supercoiled DNA to initiate its relaxation activity (Champoux, 2001). The active site region with the Tyr-319 nucleophile for single-stranded DNA cleavage and religation by EcTOP1 is located in its NTD, formed at the interface between the subdomains that enclose the toroid hole in its structure (Lima et al., 1994; Berger, 1998). Molecular docking and pull-down results reported here showed that RecA interacts with the NTD of topoisomerase I. The protein-protein interactions may either facilitate the loading of negatively supercoiled DNA onto topoisomerase I, or increase the catalytic rate of DNA relaxation by inducing conformational change in topoisomerase I. It is notable that EcTOP1 interacts with RNA polymerase via its CTD (Cheng et al., 2003) so that the transcription-driven negative supercoiling can be relaxed efficiently to prevent hypernegative supercoiling of DNA and suppress R-loop stabilization (Tan et al., 2015). Interaction with RecA takes place via a different domain in topoisomerase I and may also have functional significance for the physiological response of *E. coli* to DNA damage and antibiotics to improve survival. Future studies will investigate further the mechanism of the RecA-topoisomerase I interaction, and the physiological consequence of perturbation of this specific protein-protein interaction.

Conflict of interests

The authors declare no competing interests.

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Figure captions:

Fig. 1. Direct physical interaction between purified *E. coli* RecA and topoisomerase I

(A) pull-down scheme (B) Pull-down of *E. coli* RecA by topoisomerase I at an increasing RecA:EcTOP1 molar ratios, as measured by western blot using antibodies against RecA. Lanes 1-4: Eluates from pull-down reactions with increasing RecA:EcTOP1 molar ratios. Lane 5: Negative control in the absence of EcTOP1. (C) Graph showing average values (symbols) of RecA band intensities, from three independent experiments, relative to the maximal intensity of RecA in the pull-down reactions. The error bars represent standard deviations of three measurements.

Fig. 2. ATP promotes binding of *E. coli* RecA to topoisomerase I.

(A) Comparative analysis of ATP's influence on the direct protein interactions between RecA, and EcTOP1. Lanes 1-4: Eluates from pull-down reactions in the presence of 5mM ATP. Lanes 5-8: Eluates of pull down reactions devoid of ATP. Lane 9: negative control with no EcTOP1 present. (B) The quantified RecA band intensities.

Fig. 3. RecA-EcTOP1 complex predicted by molecular docking:

(A) Green colored surface represents EcTOP1 with light green as its C-terminal domain (CTD) and dark green as N-terminal domain (NTD). The surfaces in the other colors represent ten different predicted RecA conformations when it binds to EcTOP1, all with NTD of EcTOP1 as the binding domain interacting with RecA. (B) Cartoon representation of the top scored docked RecA-EcTOP1 complex. EcTOP1 is shown in green color and RecA in blue color. The amino acid residues across the EcTOP1-RecA binding interface that form hydrogen bonds

and salt bridges are shown in sticks representation (orange colored sticks for EcTOP1 and magenta colored sticks for RecA).

Fig. 4. NTD of EcTOP1 can interact with RecA as efficiently as full length EcTOP1.

(A) Pull-down of RecA, at an increasing RecA: NTD-EcTOP1 molar ratios, as measured by western blot using antibodies against RecA. Lane 1-4: Eluates from the pull-down reactions of increasing RecA (prey) to NTD-Ectop1 (bait) in the presence of ATP. Lane 5: negative control for the assay with RecA only. Lane 6, 7: Eluates from reaction carried out with full-length EcTOP1, as bait. (B) Quantified RecA band intensities relative to the band intensity observed with pull-down reaction corresponding to 1: 6 molar ratios of EcTOP1 RecA in lane 7 of Fig. 4A. The average values of three experiments (symbols) are shown here with the error bars representing the standard deviations.

Fig. 5. Pull-down of RecA from *E. coli* cell lysates by EcTOP1 and NTD-EcTOP1.

Lane 2 and 3 represent the eluates from the pull-down reactions containing EcTOP1, NTD-EcTOP1 as bait respectively. Lane 1, representing the eluate from the pull-down reaction with Mocr as bait, serves as a negative control.

Fig. 1.

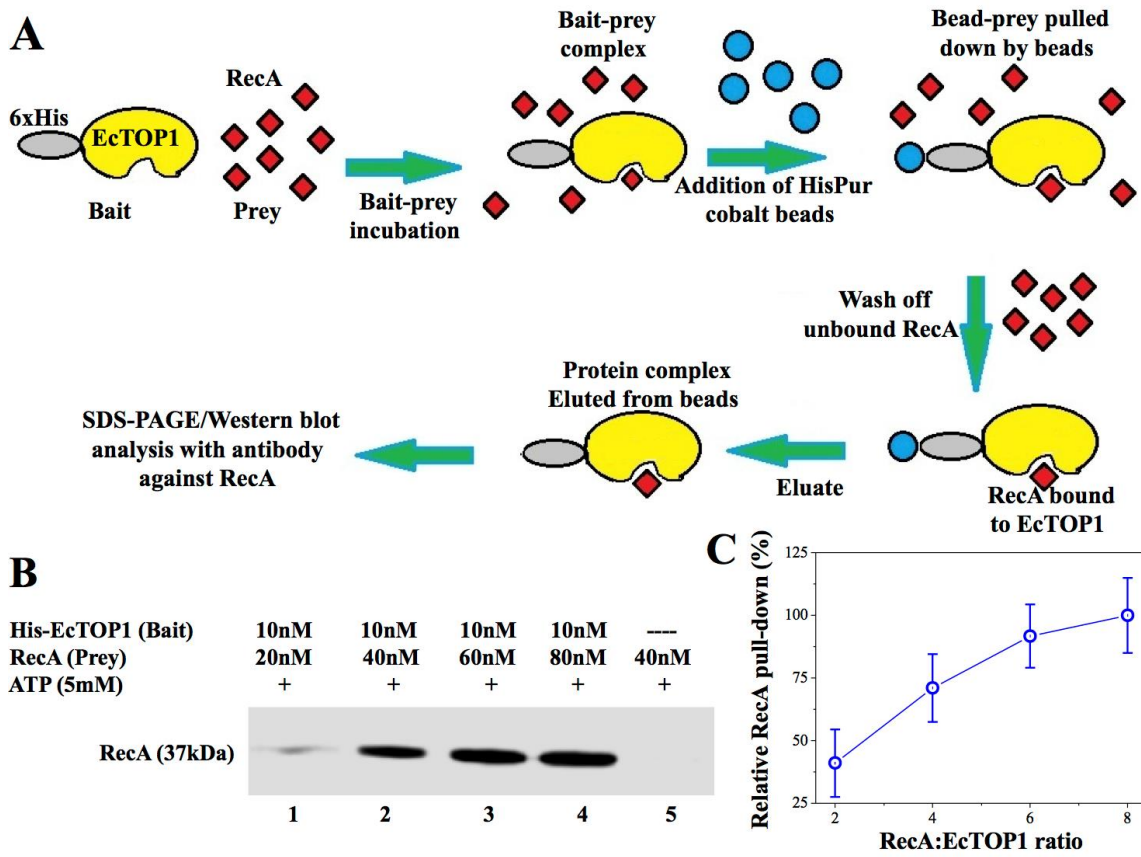


Fig. 2.

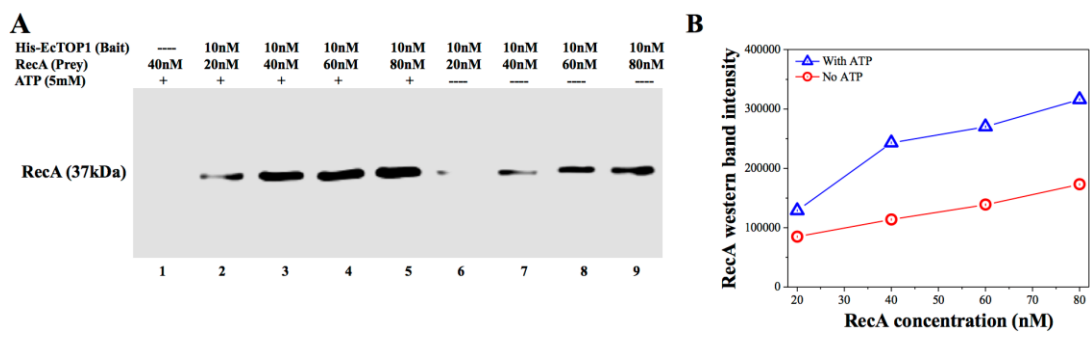


Fig. 3.

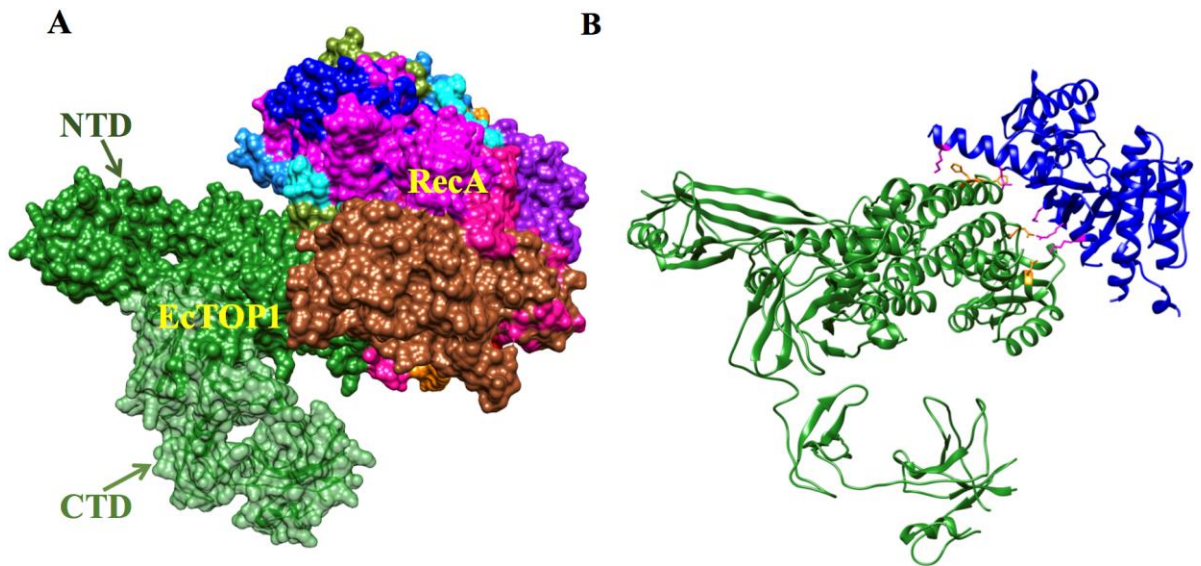


Fig. 4:

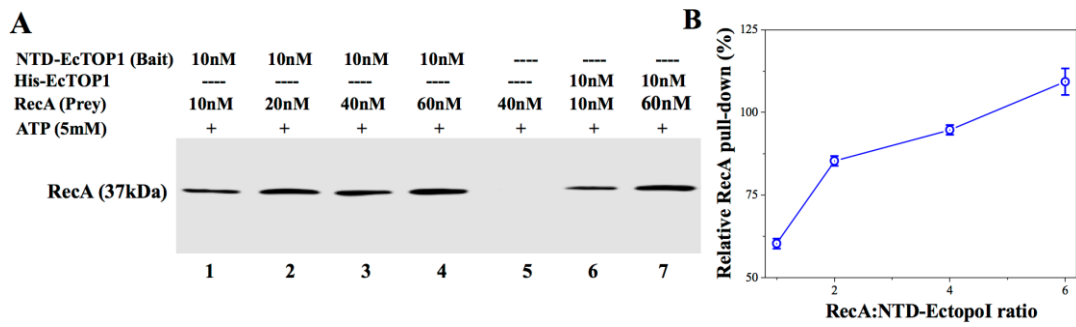
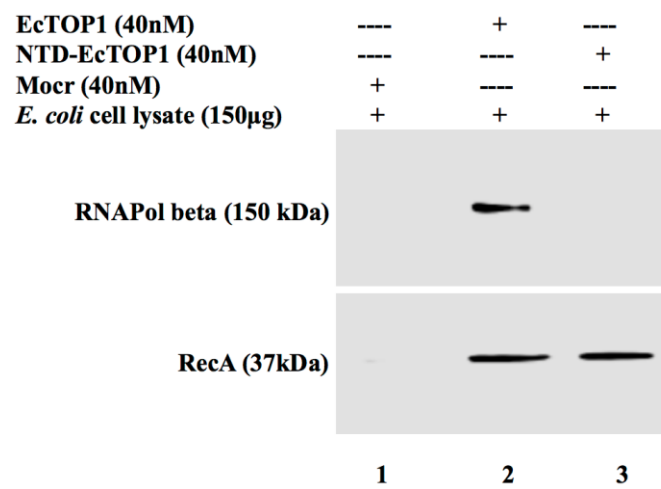


Fig. 5.



*Abbreviations list

Abbreviations:

CTD – C-terminal domain

EcTOP1 – *Escherichia coli* topoisomerase I

NTD – N-terminal domain

PPI – protein-protein interactions

SPR – surface plasmon resonance