Insertion of a flexible linker alters winged helix domain dynamics affecting substrate binding and unwinding activity of human RECQ1

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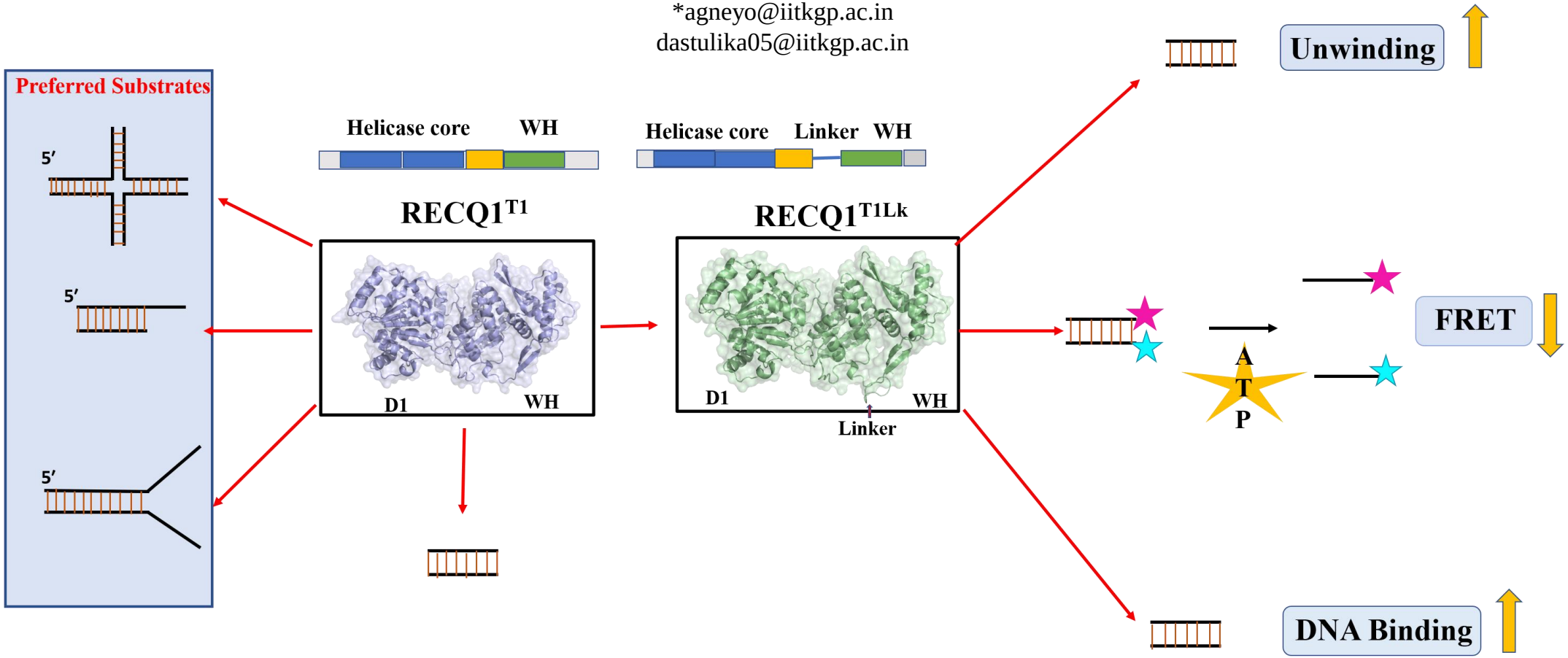


Introduction

RecQ helicases are ATP and Mg^{2+} dependent enzymes that unwind DNA with a 3′ to 5′ polarity and can recognize a wide spectrum of DNA substrates including duplex, triplex, quadruplex and Holliday junctions.

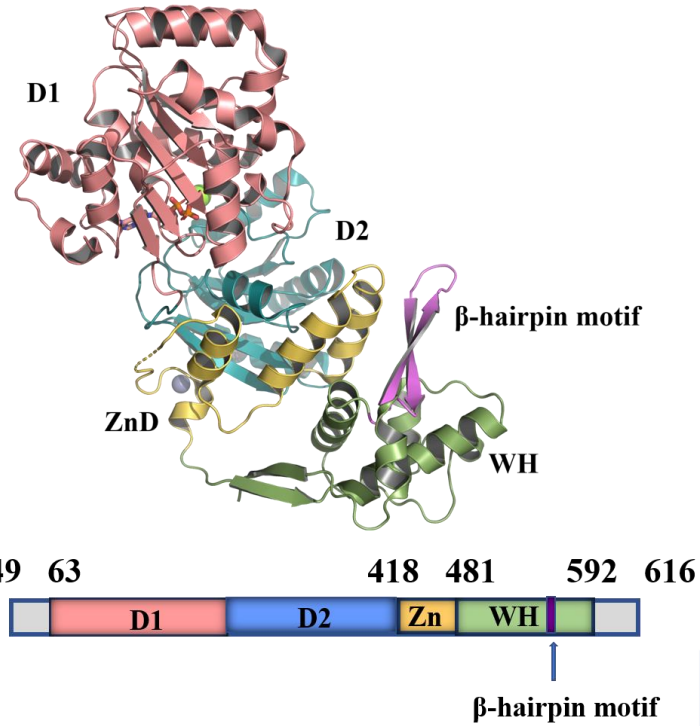


Figure 1. Crystal structure of a near full-length version of human RECQ1 ($RECQ1^{T1}$-49-616 of 649 amino acids) (PDB ID:2V1X)(Pike *et al.*, 2009). All domains are structurally conserved.

Structural alignment reveals that RECQ1 have a distinct WH domain conformation

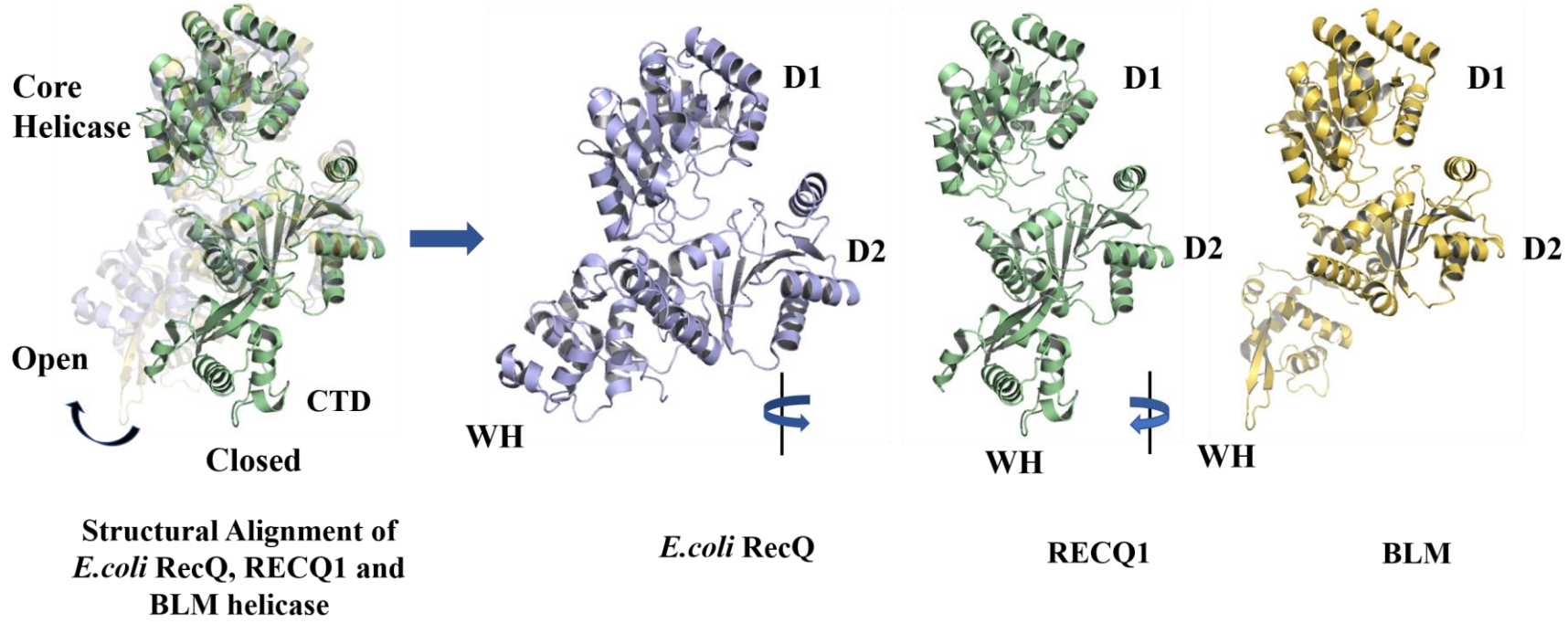
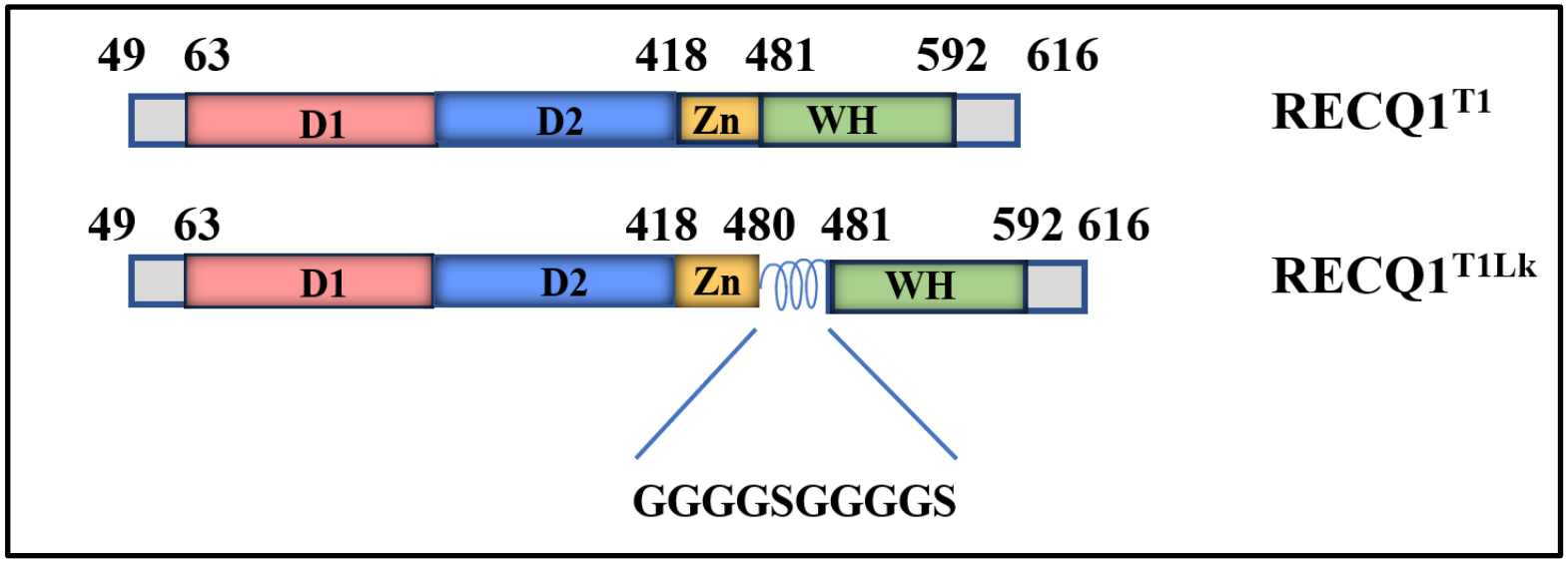


Figure 2. Structural alignment showing difference in orientation of C-terminal Domain in human RECQ1 (pale green) (PDB ID: 2V1X) (Pike *et al.*,2009), *E.coli* (pale blue) (PDB ID: 1OYY) (Bernstein *et al.*, 2003) and BLM (yellow orange) (PDB ID: 4CDG) (Newman *et al.*. 2015) RecQ Helicases.

***E.coli* RecQ**	+	+	+	**ND**	**ND**	+	+
RECQ1	-	++	++	+	~/-	-	+
BLM	-	+	~/-	+	+	+++	+++

Table 1. Comparison of helicase activity between E.coli RecQ, human RECQ1 and BLM helicase (Croteau *et al.*, 2014)

Research Approach



Purification of RECQ1^{T1} and RECQ1^{T1Lk}

Overexpression in Rosetta (DE3) cells

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Cell Lysis followed by 0.5% PEI precipitation

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Purification using Ni^{2+} -NTA affinity chromatography and size-exclusion chromatography

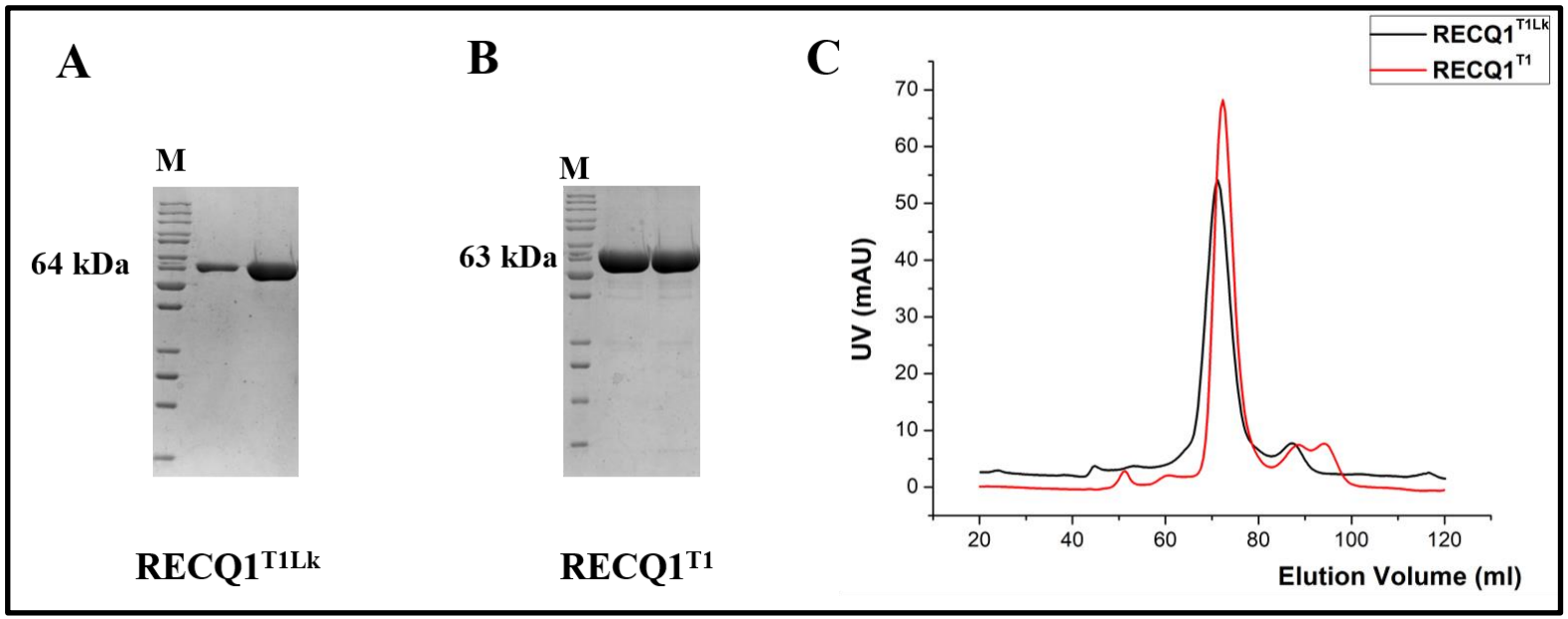


Figure 3. Purification profile of RECQ1^{T1} and RECQ1^{T1Lk}

A. 12% SDS PAGE analysis of purified RECQ1^{T1Lk} after size exclusion chromatography

B. 12% SDS PAGE analysis of purified RECQ1^{T1} after size exclusion chromatography

C. Chromatogram after size-exclusion chromatography (HiLoad 16/600 S200) of RECQ1^{T1} (red) and RECQ1^{T1Lk} (black) .

Insertion of linker improves unwinding of blunt duplex substrate

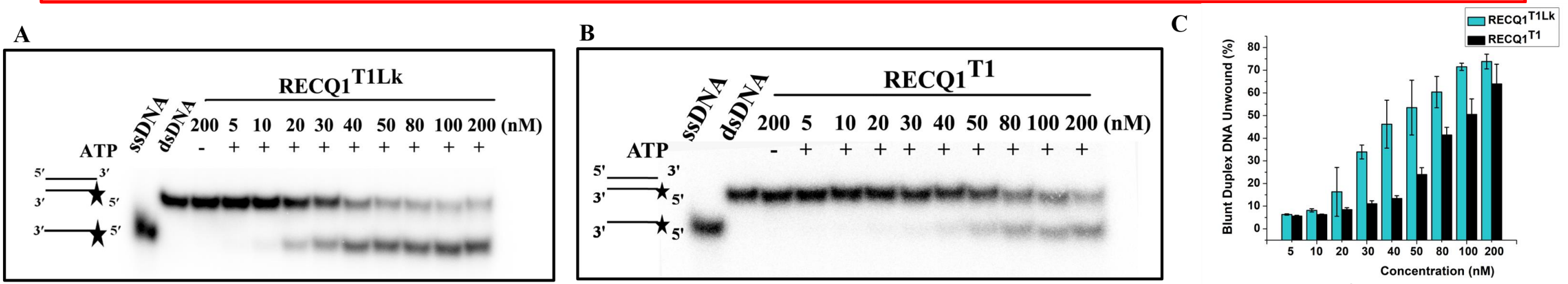
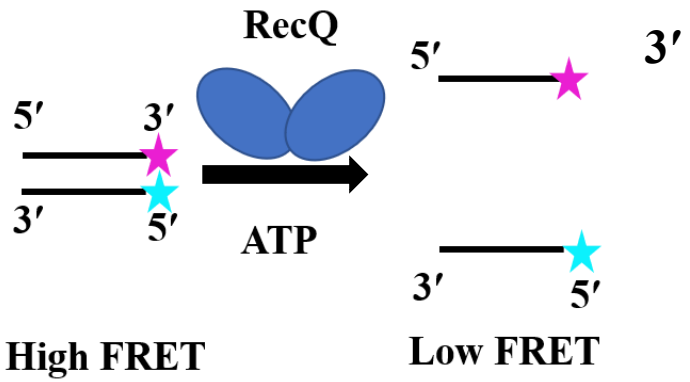


Figure 4. Unwinding of 20-mer blunt duplex substrate. Assays were performed in a reaction mixture containing 5 mM ATP, ^{32}P-labelled 20-mer blunt duplex substrate. Protein was added at increasing concentrations and the mixture was incubated at 37 °C for 30 minutes. The reaction was terminated by the addition of a stop solution. **A,B.** Concentration dependent unwinding of Blunt duplex DNA (20% Native PAGE) by RECQ1^{T1Lk} and RECQ1^{T1} respectively **C.** Comparison of the percentage of unwinding by RECQ1^{T1} (black) and RECQ1^{T1Lk} (deep cyan).



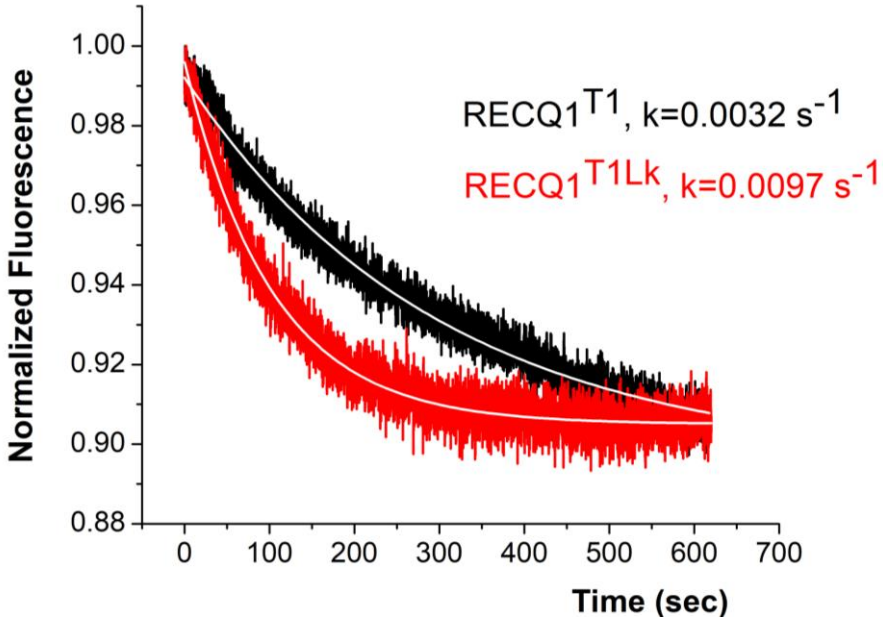


Figure 5. FRET based unwinding activity of blunt duplex DNA by RECQ1^{T1} (black, k=0.0032 s^{-1} and RECQ1^{T1Lk} (red, k=0.0097 s^{-1}). Assay was performed in a reaction mixture containing 5 mM ATP, fluorescent labelled blunt duplex DNA (100 nM) and 0.5 μM enzyme. Samples were excited at 550 nm and fluorescence emission was measured at 665 nm.

Linker containing construct has higher affinity for blunt and 3′ tailed duplex DNA in presence of non-hydrolysable ATP analogue

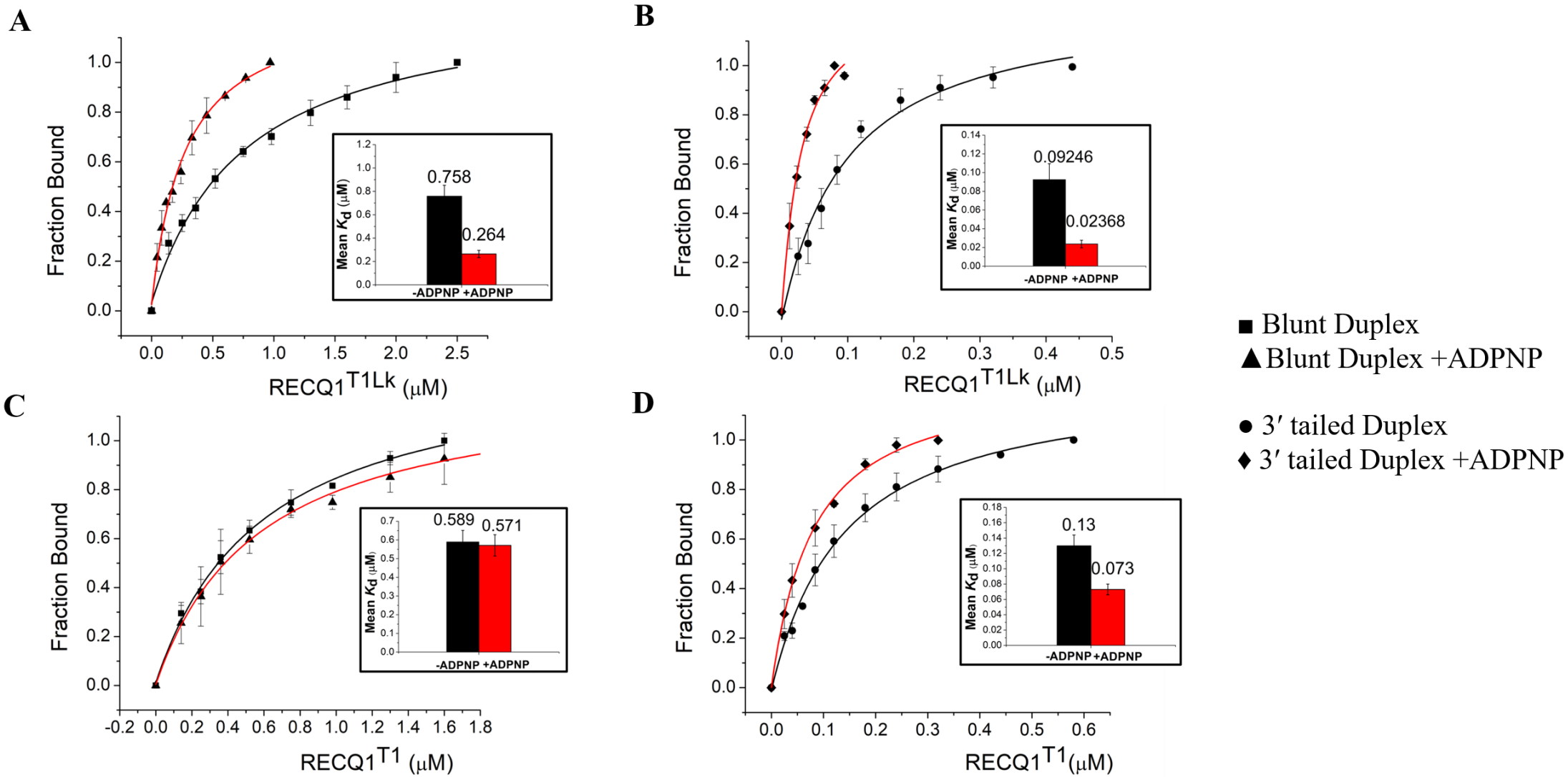


Figure 6. DNA Binding studies using Fluorescence Anisotropy for RECQ1^{T1Lk} (**A,B**) and RECQ1^{T1} (**C,D**) . Titrations were carried out with10 nM 5′6-FAM labelled blunt duplex and 3' tailed duplex substrate in presence and absence of 2 mM ADPNP. Inset shows a comparison of mean K_d values in presence and absence of ADPNP for different DNA substrates.

MD simulation and PCA analysis suggests altered domain dynamics in RECQ1^{T1Lk}

MD simulations were carried out for 100 ns under explicit solvent conditions for both RECQ1^{T1} and RECQ1^{T1Lk}. Simulations were implemented in GROMACS 5.0.7 software package using **Amber ff99SB** force field at **300K** and solvated using **TIP3P** water model. Equilibration was done for 100ps at constant temperature (100K) and pressure (1 bar). The time step employed was 2 fs, and coordinates were saved every 10 ps for analysis.

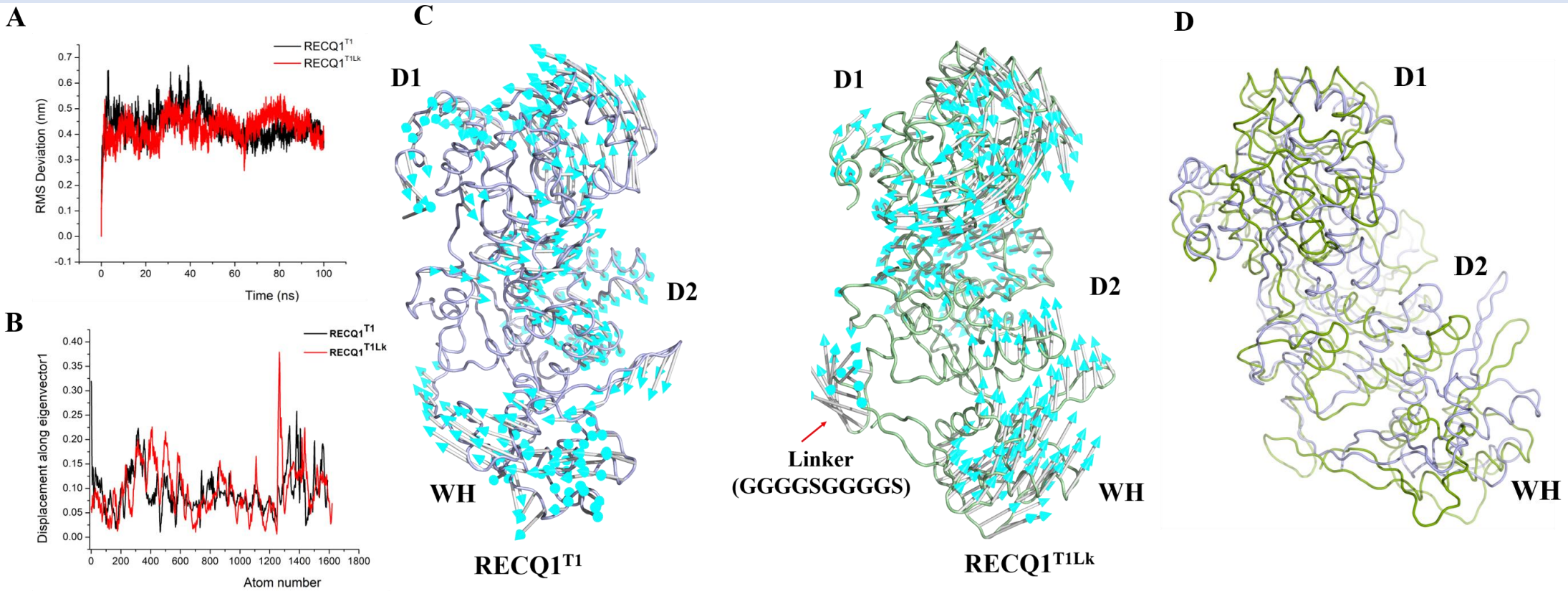


Figure 7. MD simulation and PCA analysis **A**. Backbone RMSD with respect to the equilibrated conformation as a function of time **B.** Backbone atom displacements in the subspace spanned by first eigenvector **C.** First eigenmode displacement visualization for RECQ1^{T1} (pale blue) and RECQ1^{T1Lk} (pale green). Cyan arrows indicate the direction of displacement. **D.** Superimposition of extreme structure extracted from projected trajectory along PC1 for RECQ1^{T1} (pale blue) and RECQ1^{T1Lk} (pale green).

Summary and Future Prospects

- We inserted a flexible glycine-serine rich linker between the Zn and WH domains of near full length human RECQ1 ($RECQ1^{T1}$) to assess its impact on WH domain conformation and enzyme functioning.
- Interestingly, linker incorporated construct ($RECQ1^{T1Lk}$) could unwind a 20-mer blunt duplex substrate more efficiently, which otherwise is not a preferred substrate for human RECQ1 helicase. FRET-based unwinding experiments confirmed that $RECQ1^{T1Lk}$ exhibited a faster rate of unwinding of blunt duplex substrate as compared to $RECQ1^{T1}$. Anisotropy experiments indicated $RECQ1^{T1Lk}$ exhibited 2-fold higher affinity for blunt and 3-fold higher affinity for 3′-tailed duplex in presence of ADPNP compared to $RECQ1^{T1}$.
- MD simulation and. PCA analysis indicated that $RECQ1^{T1Lk}$ had altered domain dynamics as compared to $RECQ1^{T1}$.
- Overall, our data suggests that insertion of linker affects conformational dynamics of WH domain in nucleotide bound form of RECQ1 exhibiting different substrate selectivity and unwinding activity.
- Further experiments using SAXS and single molecule fluorescence experiments can help to gain better insight regarding the conformational status of WH domain in $RECQ1^{T1Lk}$. Study of linker incorporated full length human RECQ1 is required for better understanding..

References

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