A Proposed Inhibition Mechanism for Ethacridine with Topoisomerase IA

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Abstract

Topoisomerase IA (Topo1A) is essential for regulation of DNA supercoiling in E. coli and related prokaryotes. The compound Ethacridine (ETC) has been shown to specifically inhibit Topo1A. This is hypothesized to occur due to ETC binding to the interface between Topo1A and DNA. We are currently investigating the interactions between ETC and Topo1A , and the role of DNA specificity using VMD software to model and observe the inhibition mechanism. After creating a model of Topo1A we inserted various DNA sequences to create a model of the cleaved state with and without ETC. The NAMD software package is used to simulate and observe the motion and events associated with the Topo1A-DNA complex and inhibitor. It has been shown that DNA specificity at the cleavage site contributes to the reaction by enhancing the inhibition. Early data suggests that this enhancement is linked to the presence of pyrimidines at the cleavage site, and that ETC may prefer to bind at cleavage sites containing pyrimidines rather than purines. Our investigation will continue to explore the role of DNA sequence specificity in the enhancement of Topo1A inhibition by ETC.

Objectives

During our exploration of Topo1A inhibition by ETC we hope to discover both the mechanism by which ETC binds to Topo1A and the role of DNA specificity in the enhancement of this binding. We also hope to discover if the structural effects of DNA sequence variations such as motion or specific interactions contributes to this binding. As Topo1A is essential in both E. Coli and other prokaryotes, we hope that this will allow for better understanding of how DNA supercoiling in prokaryotes may be inhibited. Long term, this would allow for the possible design of antibiotics that are capable of treating a wide range of diseases caused by prokaryotic bacteria by damaging prokaryotic DNA through inhibition of the DNA supercoiling mechanism

Materials and Methods

During this experiment we performed our simulations using the VMD and NAMD software to model and simulate the inhibition mechanism of ETC. Several DNA sequences were inserted to create a model of the enzyme-DNA complex based on homology modeling with related enzyme-DNA complexes. After the DNA insertion we energy minimized the models. A model for the cleaved covalently bound DNA substrate was incorporated into the model to simulate the proposed mechanism step inhibited by ETC. Using this cleaved state, we ran simulations to determine the behavior of the enzyme without the presence of ETC. This solvated enzyme with ions was heated to 300K, and simulated for 2 ns in the NPT ensemble to observe the reaction and the changes in configuration of the enzyme. Simulations with ETC were started from the endpoint of the prior simulations and also simulated for 2 ns. ETC parameters were derived from the CGenFF force field implemented through the PARAMCHEM website (www.paramchem.org)

Results
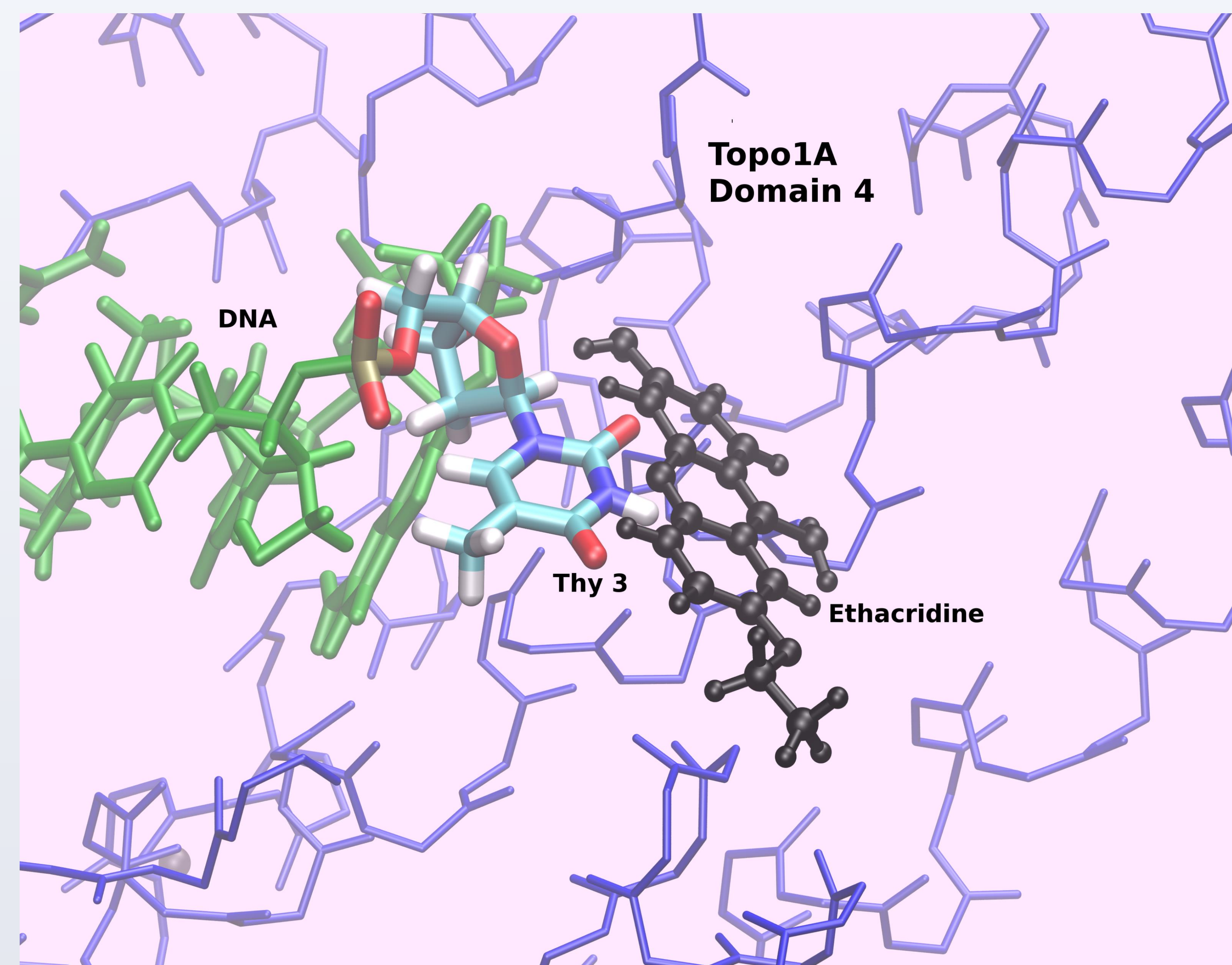
This image shows an interaction between ETC and the third residue of the "Strong" DNA variant. ETC came within 4 angstroms of the base pair and appeared to form a stacking interaction with it. Given the interaction of ETC with thymine and the presence of thymine at the cleavage site, this suggests that ETC may intercalate into DNA . Low RMSD values (table on right), show this interaction stabilized the DNA.
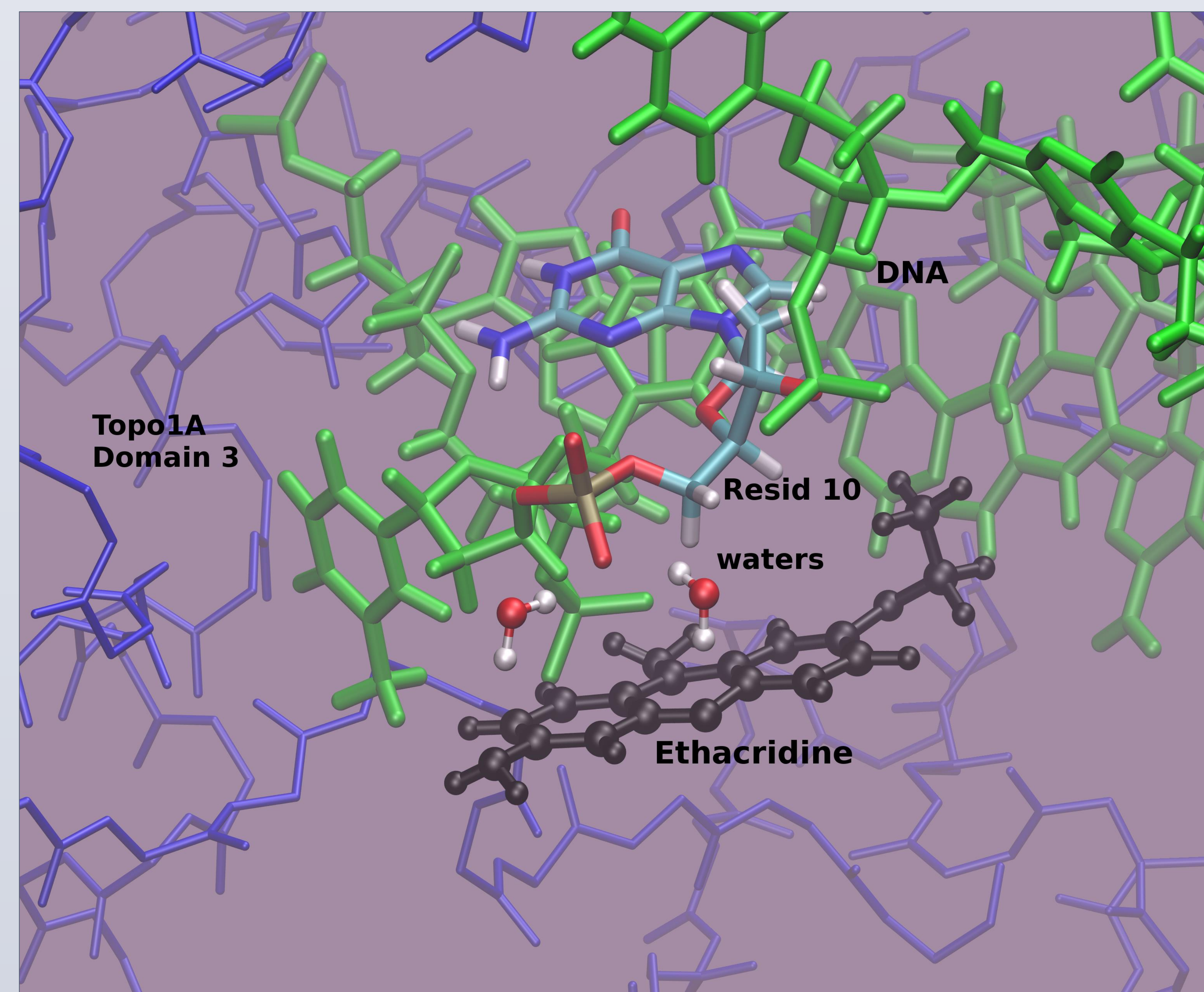
This image shows an interaction between ETC and the tenth residue of the "Moderate" DNA variant via a water molecule bridge. ETC was not able to interact directly with the backbone of the Guanine. This interaction may be significant because it supports our hypothesis that ETC prefers to bind to pyrimidines . The raised RMSD values (table on right) suggests this interaction destabilizes the DNA.

DNA Sequences

	13	12	11	10	9	8	7	6	5	4	3	2
Strong	G	G	C	G	A	T	C	T	C	G	T	C
Moderate	C	T	C	G	T	C	G	A	G	C	T	C
No Enhancement 2	T	T	C	C	A	T	C	G	C	C	G	C

The cleavage site lies between the 7TH and 8th bases

Range of RMSD values during simulation

	Strong	Moderate	No Enhancement 2
DNA	2.61±.36	2.54± .39	2.76±. 82
DNA + ETC	3.00 ±.39	3.09± .18	**1.62± .26**

The flexibility of each variant strand was estimated using the variance in the RMSD . In calculating the RMSD values, an average structure for the nucleic acid was utilized. ETC showed a preference for the DNA that was most flexible in its presence, supporting our hypothesis that the relative flexibility of the DNA plays a role in the binding interface.

Strong RMSD vs time	0.1 ns	0.2 ns	0.5 ns
Nucleic 2-7 RMSD with ETC	1.68	2.09	4.46
Nucleic 8-13 RMSD with ETC	1.53	1.56	2.74
Nucleic RMSD with ETC	1.61	1.84	3.69
Nucleic RMSD without ETC	4.61	6.48	6.18

In the figure at left, ETC interacts with the "Strong" DNA at the 0.1 ns time; this interaction ends at approx. 0.2 ns.   As is demonstrated by the lower RMSD values, ETC has a stabilizing effect on the structure of the DNA. During the interaction with ETC, the DNA has a more stable confirmation than during the simulation without ETC.

Moderate RMSD vs time	1.3 ns	1.4 ns	1.5 ns
Nucleic 2-7 RMSD with ETC	1.82	2.01	1.60
Nucleic 8-13 RMSD with ETC	5.13	4.92	4.60
Nucleic RMSD with ETC	3.85	3.76	3.44
Nucleic RMSD without ETC	3.66	3.62	3.11

In the figure at left, ETC interacts with the "Moderate" DNA through a water bridge at 1.3 ns; this interaction ends at approx. 1.4 ns. As is demonstrated by the higher RMSD values, ETC has a destabilizing effect on the structure of the DNA when interacting via a water bridge.

Conclusion

Our early data strongly indicates that DNA specificity plays a role in the binding of ETC to Topo1A as is evidenced through ETC's interaction with thymine. The direct interaction with thymine may confirm a preference for pyrimidines. It also indicates that both the presence of pyrimidines at the cleavage site and the flexibility of the DNA strand influence the binding preferences of ETC.