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DNA-Protein Binding Studies: Investigating an ATL Protein from T.thermophilus HB8

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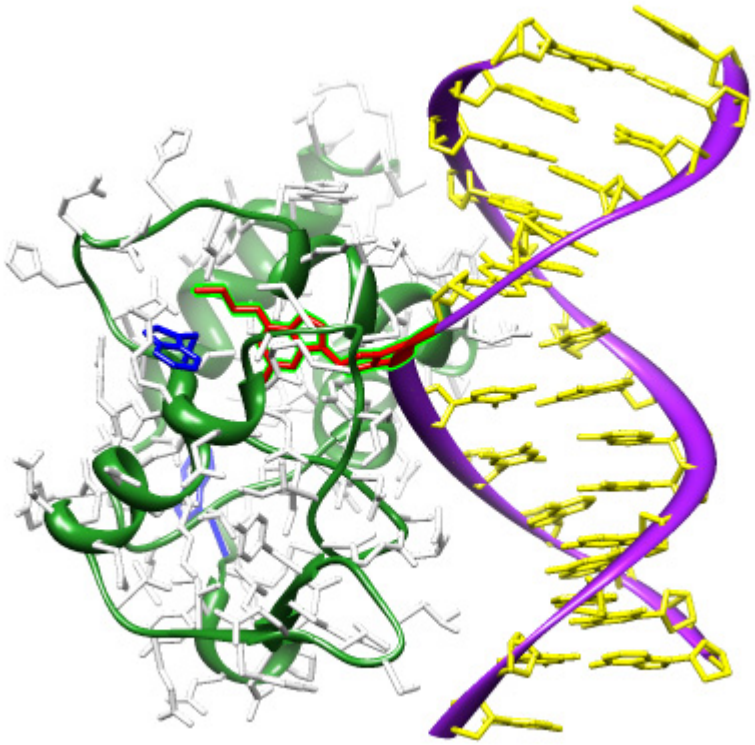
Maintaining the integrity of genetic information is essential in living systems. Certain proteins, such as alkylguanine transferases (AGTs), function to bind and repair a type of DNA damage (O^6-alkylation of guanine) which can prove both highly mutagenic and cytotoxic if left unrepaired. Alkyltransferase activity is achieved using an active-site cysteine residue to specifically remove an alkyl group from the O^6-position of guanine and return the DNA to its original, undamaged form. Many organisms, in all three kingdoms of life, have an AGT protein (the human form is known as MGMT). However, some organisms lack an AGT but instead have a homologue known as an alkyltransferase-like (ATL) protein.

AGT and ATL proteins are highly conserved, the significant difference being that in ATL proteins the active site cysteine is replaced with another residue (usually tryptophan or alanine). ATL proteins can still tightly bind O^6-alkylguanine-containing DNA but they are incapable of repairing the damage directly.(1) Interestingly, cells of organisms with no AGT (such as *S.pombe*) are still protected by ATL proteins from the harmful effects of alkylation damage, indicating that ATLs must have a role in an alternative repair mechanism (thought to be nucleoside-excision repair, NER). Indeed, TTHA1564, an ATL protein from *T.thermophilus*, has been shown to interact with UvrA, an NER protein.(2) It has been proposed that Atl1 from *S.pombe* forms a relatively stable and bulky complex with alkylated DNA, which is subsequently recognised and repaired by the NER machinery(3). In this context, it would seem advantageous for ATL proteins to have the ability to recognise and bind a broad range of O^6-alkylguanine adducts. Current research would suggest that Atl1 from *S.pombe* binds to DNA containing a wide variety of O^6-alkylguanine

residues, including many that are not repaired by MGMT. The purpose of this project is to investigate, using a fluorescence-based binding assay, whether TTHA1564 from *T.thermophilus* has similar binding preferences to Atl1, to further understand this unique family of proteins.



Structure of Atl1 from *S.pombe* bound to an O^6-propylguanine-containing oligomer

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