

New details of specific site recognition by human single-strand selective monofunctional uracil-DNA glycosylase SMUG1

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Introduction

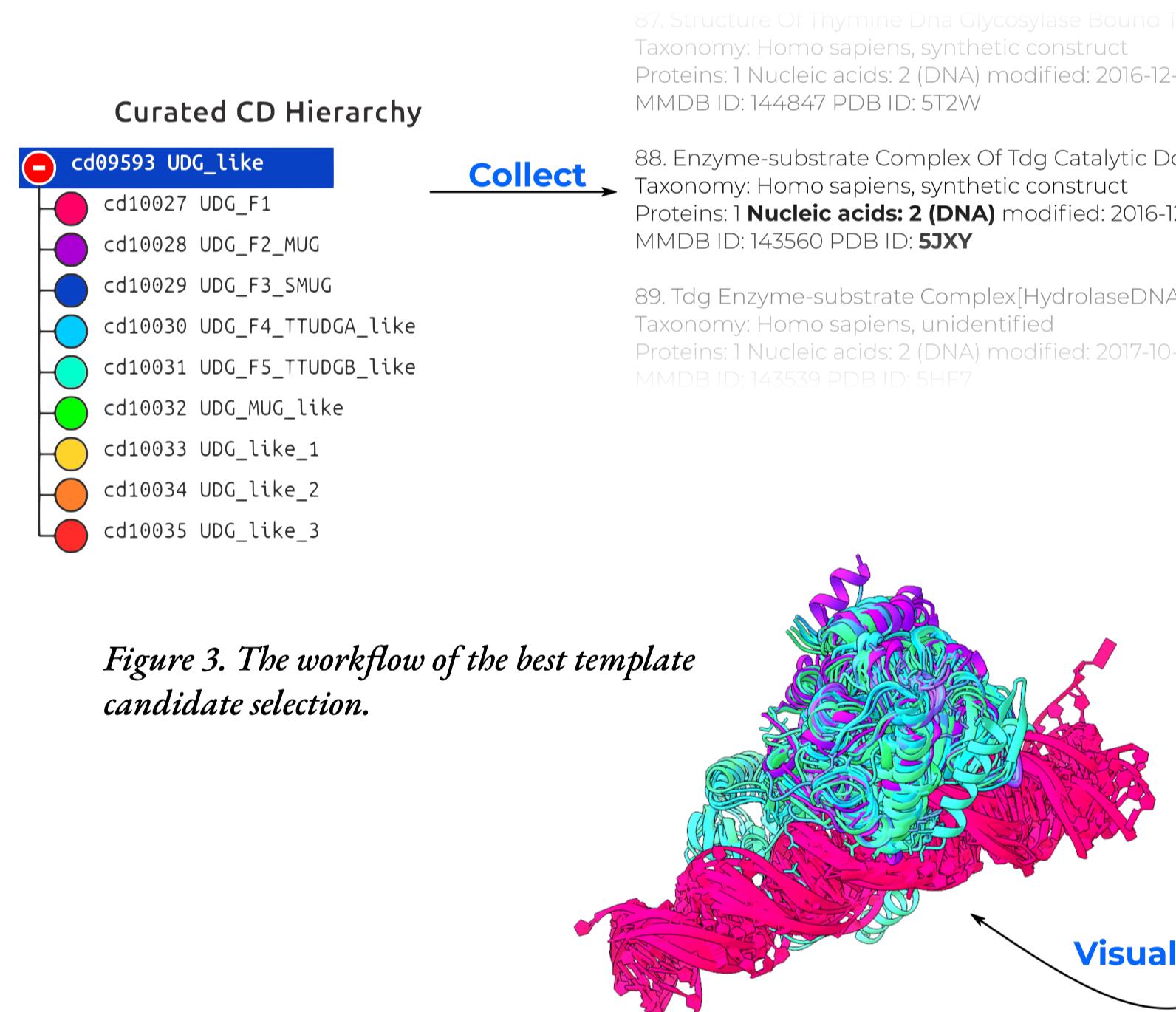
Human single-strand selective monofunctional uracil-DNA glycosylase SMUG1 is involved in base excision repair pathway. This enzyme recognizes and hydrolyses the N-glycosidic bond of uridine and uridine lesions bearing oxidized groups at C5: 5-hydroxymethyluracil, 5-formyluracil (5FU), and 5-hydroxyuracil. Uridine and its derivative lesions are the results of cytosine and 5-methylcytosine deamination and oxidation processes.

A comparison of pro- and eukaryotic DNA glycosylases, belonging to different structural families has revealed that the mechanism of catalytic complex formation includes initial DNA binding and formation of a nonspecific complex, DNA bending, damaged base flipping out from the double helix, and its placement into the enzyme's active site. Recently, we performed a pre-steady-state kinetic analysis of conformational transitions of hSMUG1 and DNA in the course of the catalytic cycle. The conformational dynamics were directly recorded by the stopped-flow technique combined with fluorescence detection.

Modelling of SMUG1-DNA Complex

As there is no structure of UDG F3 members in complex with DNA, we had to expand our research to whole UDG superfamily to predict hSMUG1-DNA complex.

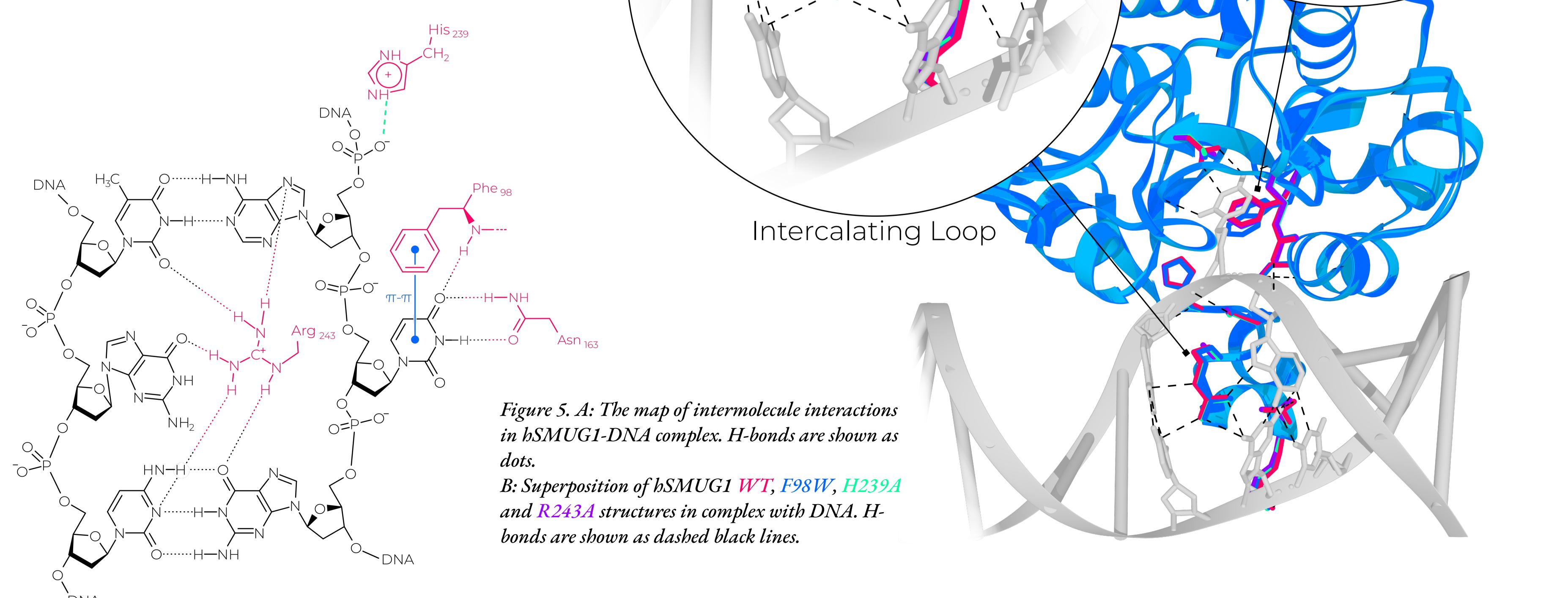
All PDB entries of protein-DNA structures in UDG structural superfamily were collected from NCBI CDD. Then all structures were aligned in two step manner: best DNA-DNA fitting, then best protein overlapping (figure 3). Following analysis of given structures showed, that DNA duplex bound by the member of UDG F1 hTDG (PDB 5T2W) best fitted both into the active site of hSMUG1 and into DNA binding domain. Therefore, combination of DNA from complex with hTDG and hSMUG1 model structure was optimized by molecular dynamics simulation for 15 ns, yielding the model structure of hSMUG1-DNA complex (figure 4).



Analysis of hSMUG1-DNA Complex Structure

Both active site and intercalating loop have an extensive network of DNA-protein intermolecular contacts, namely electrostatic and hydrogen bonds, π -stacking and hydrophobic interactions. Uridine is everted from the double helix and inserted into the protein active site. Extrahelical state of uridine is stabilized by the intercalating loop of protein (residues 239-249), which interacts with both DNA strands. R243 amino acid residue forms numerous hydrogen bonds with nucleobases.

Amino acids F98, H239 and R243 are highly involved in protein-DNA interactions, so they could play critical role in the DNA binding and catalysis. F98 is involved in stacking interaction with flipped U base, fixing it in the active site. H239 handles DNA backbone via electrostatic contact with phosphate group and R243 is inserted into DNA duplex replacing flipped U base and forms wide network of hydrogen bonds with nucleobases (figure 5).



SMUG1 Structure Prediction

Human SMUG1 has no crystal structure neither of free protein nor of protein-DNA complex. To gain deeper understanding of SMUG1 conformational dynamics we have created structural models of protein and protein/DNA complex. SMUG1 belongs to family 3 of UDG structural superfamily (figure 1). Structure of hSMUG1 was predicted by homology modelling using structures of homologous enzyme form *Xenopus laevis* (xSMUG1) as a template with following molecular dynamics for energy optimization for 5 ns (figure 2).

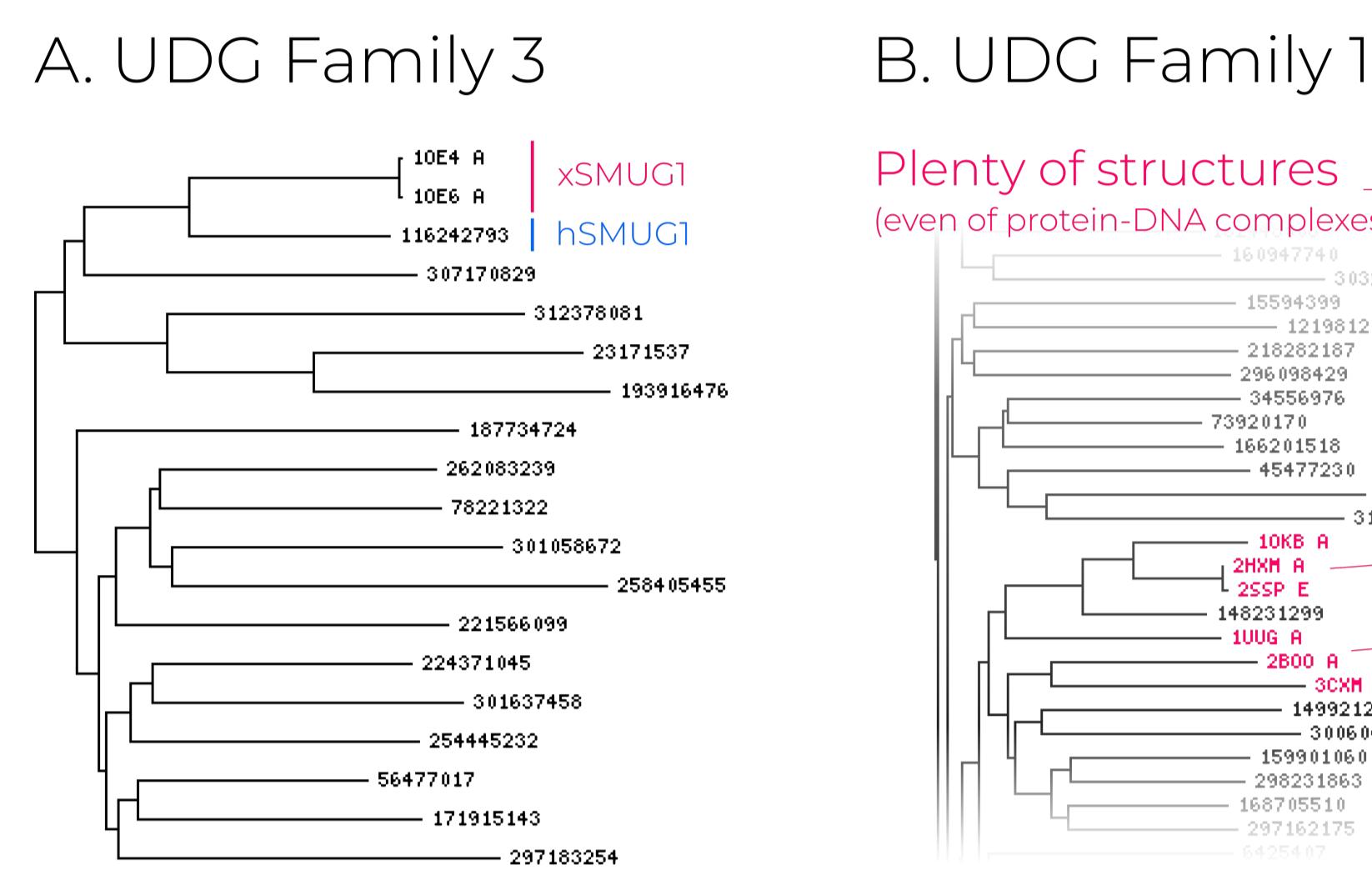


Figure 1. Phylogenetic trees of UDG subfamilies protein sequences from NCBI Conserved Domain Database (CDD).

- A: There are some structures of hSMUG1 near homologue, xSMUG1, in UDG F3.
- B: UDG F1 contains many structures suitable for further hSMUG1-DNA complex modelling.

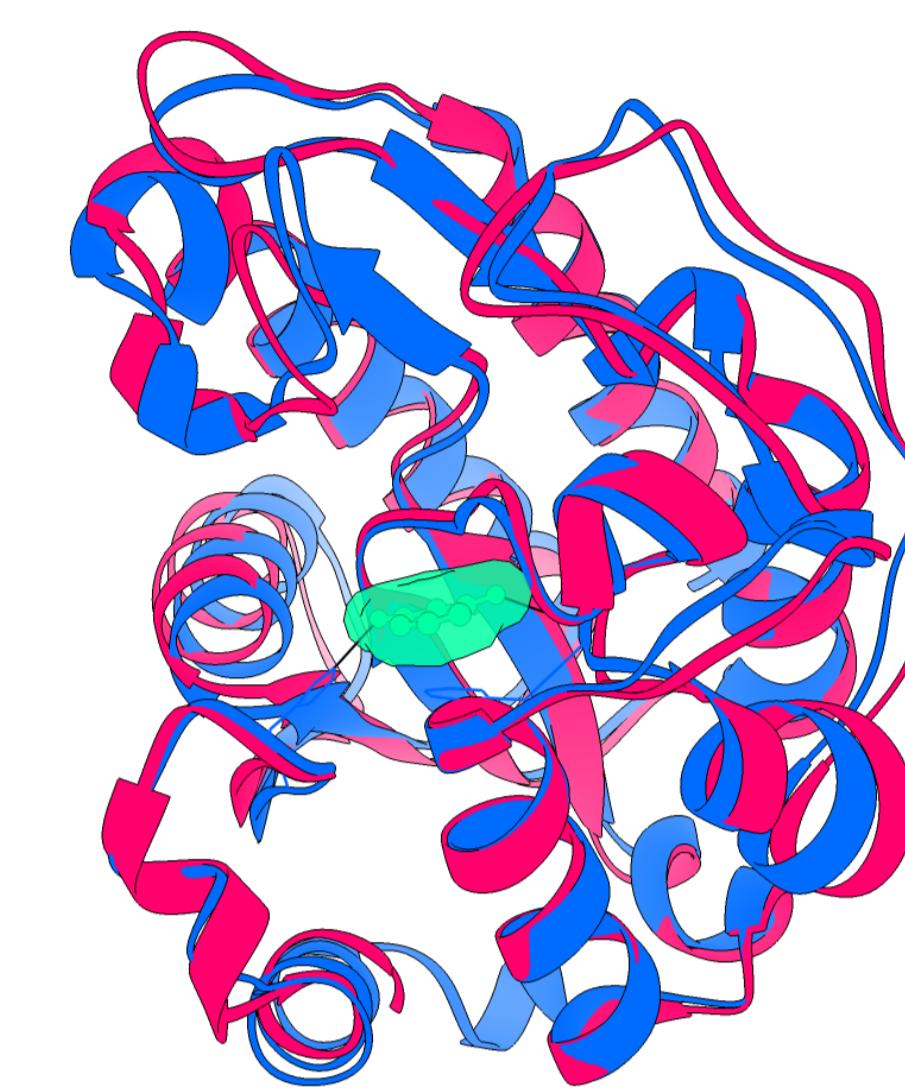


Figure 2. Superposition of hSMUG1 model structure (pink) and xSMUG1 structure (PDB 1OE5, blue).

These enzymes share very similar fold and active site structure. Uracil base from 1OE5 fits into the active site of predicted hSMUG1 structure.

Mutant Enzymatic Activity Assay

To determine the influence of mutations described, we tested catalytic activity of hSMUG WT and F98W, H239A and R243A mutant forms on model DNA duplex containing U:G pair. SMUG1 F98W and H239A are shown to be significantly less active than WT enzyme, R243A is shown to work faster than WT (figure 6).

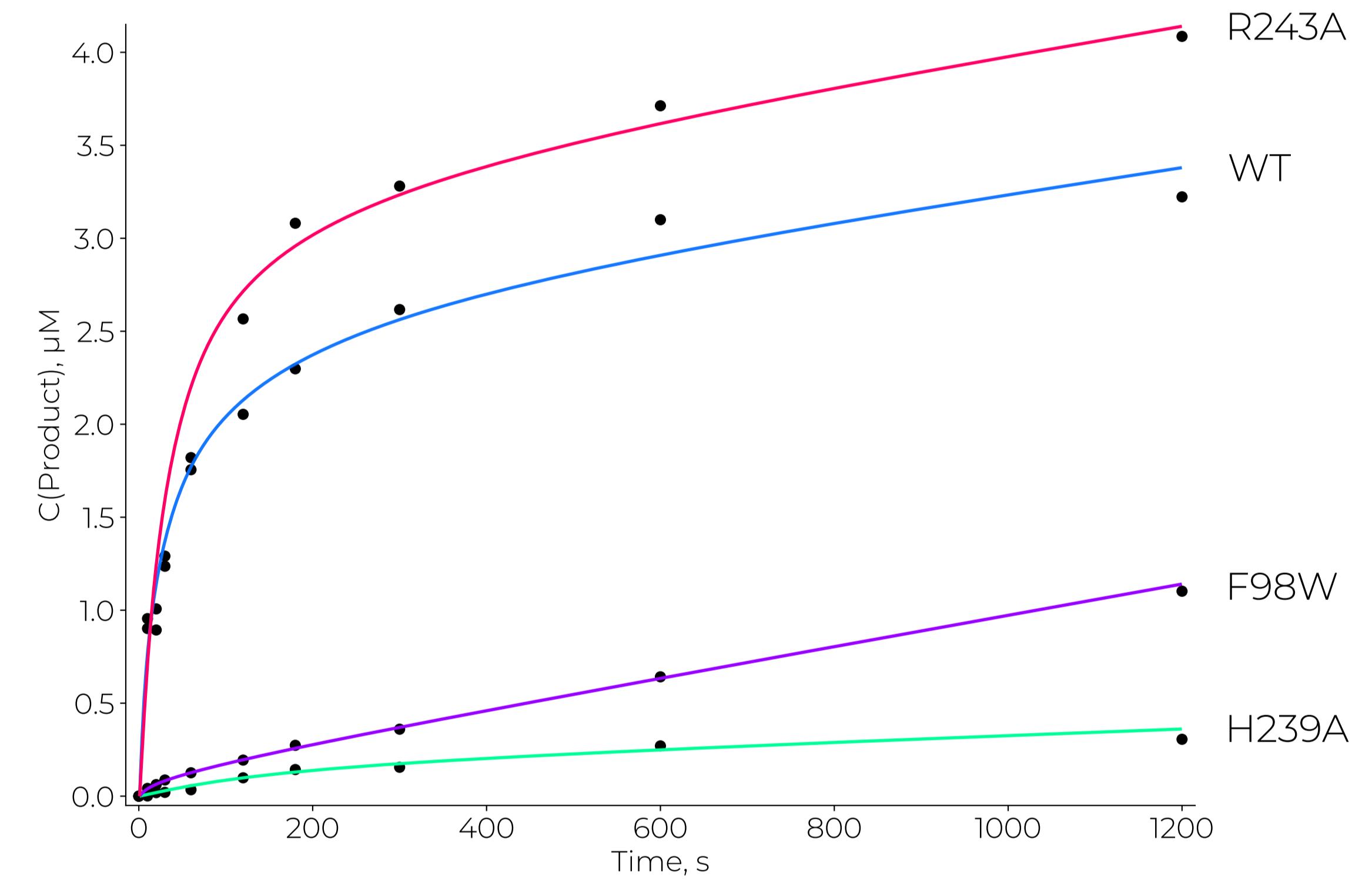


Figure 6. Accumulation of reaction product in the course of interaction hSMUG WT and F98W, H239A, R243A mutant forms with model DNA duplex containing U:G pair determined by PAGE. [Enzyme] = 1.0×10^{-6} M, [Substrate] = 5.0×10^{-6} M, T = 25°C, buffer: 50 mM Tris-HCl, pH 7.5, 50 mM KCl, 1.0 mM EDTA, 1.0 mM DDT and 7% glycerol (v/v). Product (DNA with apurinic/apirimidinic site) was treated with 10% piperidine solution for 30 min at 37°C, then reaction mixture was separated via denaturing PAGE and visualized with PhosphorImager.

Conclusion

The model structures of hSMUG1 wild type and F98W, H239A, R243A mutant forms were obtained. The network of contacts between F98, H239 and R243 residues and DNA was predicted. Steady-state kinetic assays revealed, that F98W and H239A mutant forms are significantly less active due to disruption of their contacts in the active site, indicating the critical role of these amino acids in the formation of enzyme catalytic state. It was shown that mutant form R243A is more active in comparison with wild type enzyme, probably, because of increased turnover rate and facilitation of product release from enzyme-product complex.

The obtained structures shed the light on the structural basis of hSMUG1 enzymatic activity and will allow us to better interpret pre-steady state kinetic analysis of conformational changes of enzyme and DNA substrates during their interaction.

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