Mutations in hSMUG1 and their effect in U/hmU excision: A computational study

Jorge Antonio Amador Balderas[1,2], Frank Beierlein[1,2], Celine Lorentsen[3], Trond Bærheim[3], Cyrell Ann Ruales[3], Svein Bjelland[3,4], Marina Alexeeva[3], Petra Imhof[1]

[1] Computer Chemistry Center, Department of Chemistry and Pharmacy, Friedrich-alexander Universität (FAU) Erlangen-Nürnberg, Nägelsbachstraße 25, 91054 Erlangen, Germany

[2] Erlangen National High Performance Computing Center (NHR@FAU), Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU), Martensstraße 1, 91058 Erlangen, Germany

[3]Department of Chemistry, Bioscience and Environmental Technology, University of Stavanger, 4021 Stavanger, Norway

[4]Department of Clinical Molecular Biology, Akershus University Hospital, 1478 Lørenskog, Norway

Mutagenic uracil (U) arises in DNA by hydrolytic deamination of cytosine (C) while non-mutagenic U results from erroneous incorporation of deoxyuridine monophosphate opposite adenine during replication. Human single-strand-selective mono-functional uracil-DNA glycosylase 1 (hSMUG1) was first described as one of several enzymes to initiate the base excision repair (BER) pathway by excising U from DNA. hSMUG1 was also found to excise bases damaged by oxidation like 5-hydroxymethyluracil (hmU) from DNA in addition to being involved in RNA metabolism, where it has been suggested that hmU is excised from RNA.

Connected to the latter functionality, is the interaction of several hSMUG1 residues including Ser26 and Glu35 with the pseudouridine synthase Dyskerin (DKC1) protein. Our experimental work shows that the S26R/E35D double mutant shows no excision activity compared to that of U, even when both residues are far away from the active site. We also observe that replacing Pro240 with Gly (P240G) abolishes hSMUG1 activity for hmU while U activity is retained.

Unfortunately, there is no hSMUG1 crystal structure available that can help explain our results. The *Xenopus laevis* SMUG1 (xSMUG1) crystal structure has been determined without association with substrate or product. Through *in silico* modelling and molecular dynamics (MD) simulations, we managed to produce a hSMUG1-DNA complex and study the interactions between the substrate base and the active site residues.

We observed that P240 stabilizes the interaction of H239 with the substrate, which explains the reduced stabilization in the P240G mutant. We also observed that E35 is part of a hydrogen bond chain that extends to an active site residue which discriminates between hmU and thymine as substrate. When E35 is replaced with a similar amino acid as in the E35D mutant, a slight conformational change misdirects the interaction; this prevents proper hmU binding while leaving U binding unaffected.

These findings, coupled with subtle differences between hmU and U inside the active site, suggest that hmU excision is more susceptible to be affected by active site residue rearrangements, even if small.