DNA-Repair Mechanisms: Molecular Simulations and Computational Alchemy

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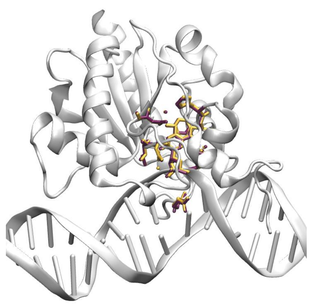
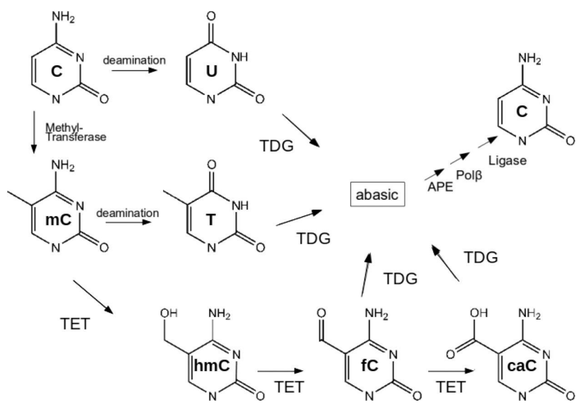
Thymine DNA Glycosylase (TDG)



TDG in complex with DNA (PDB codes 6U17 and 5T2W): substrate base (5caC^F, 5fC^F) flipped into active site

How does TDG achieve its specificity?

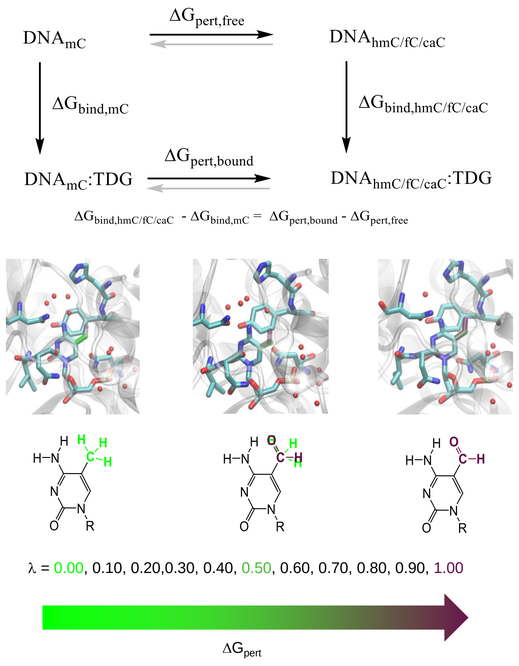
- removes mispaired thymine (T), uracil (U) and modified cytosine bases (oxC)
- recognizes and excises formyl-cytosine (fC) and carboxyl-cytosine (caC), but not methyl-cytosine (mC) or hydroxymethyl-cytosine (hmC)



Methods

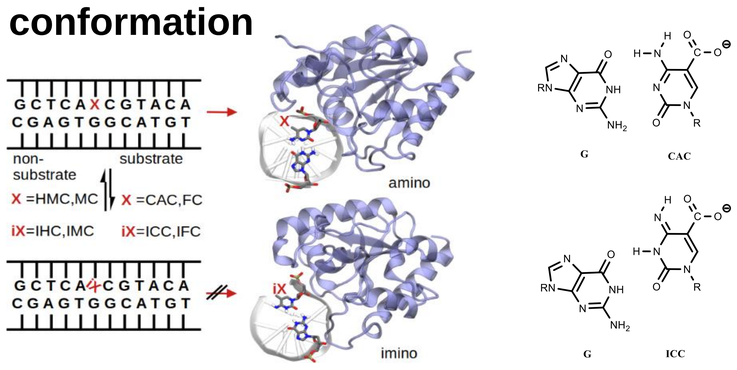
Atomistic molecular dynamics (MD) simulations of TDG-DNA complexes, amino-/imino tautomers of bases, intra-/extrahelical bases, His151 as HIP/HIE

Thermodynamic integration (TI): “alchemistic” perturbations

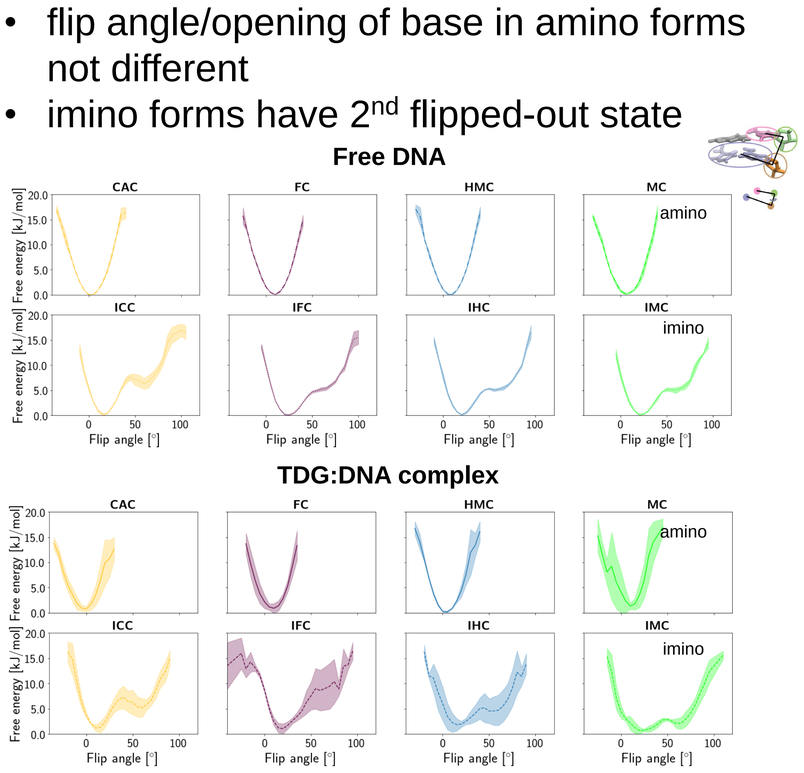


Unbiased MD: Amber 18/20 on GPUs, 3 x 600 ns, 298 K, 1 bar, SHAKE, 2 fs time step, WC restraints on DNA termini, BSC1 for DNA, BSC1/Gaff (RESP) for modified residues, ff14SB for protein, TIP3P water, 150 mM NaCl (Joung/Cheatham)
TI: Amber 20 on GPUs, 21 λ windows, 30 ns per λ window, 1 fs time step, 3 runs, dual-topology, vdW/electrostatics soft-core, no SHAKE on base

No oxC discrimination in intrahelical conformation



- flip angle/opening of base in amino forms not different
- imino forms have 2nd flipped-out state



- flipped-out state stabilized in complex of *non*-substrate bases
- imino tautomers not relevant in intra-helical (flipped-in) conformation

Rel. binding affinity [kcal/mol]

CAC→ICC	0.17 ± 1.10
FC→IFC	2.26 ± 0.23
HMC→IHC	-0.65 ± 0.43
MC→IMC	2.37 ± 0.26

Conclusions

- substrate specificity for fC and caC at least partially achieved by favorable binding to TDG in extrahelical complex
- damaged bases in suitable conformation for subsequent base excision

References

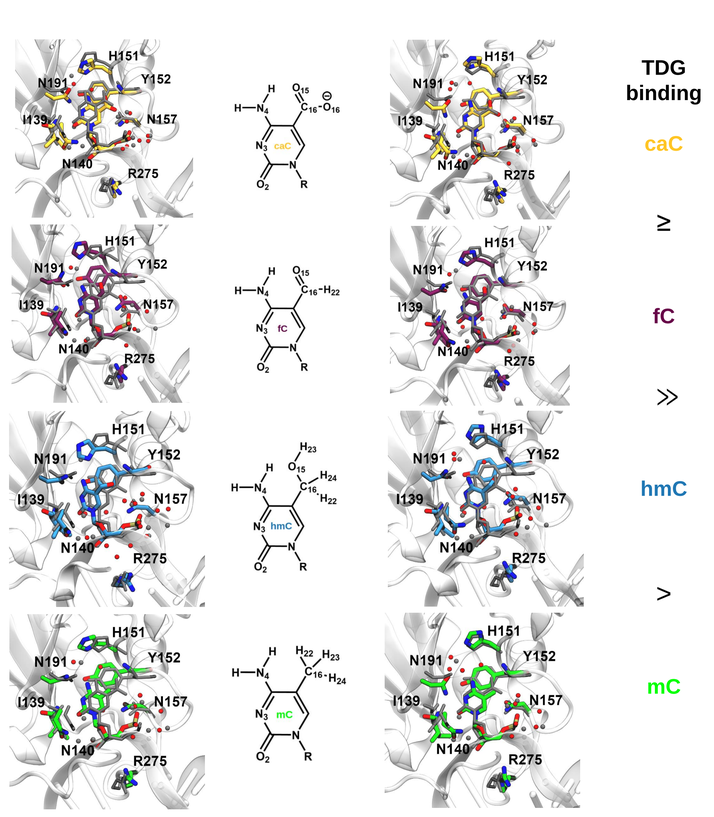
- F. Beierlein, S. Volkenandt, P. Imhof, *J. Phys. Chem. B* **2022**, *126*, 1188. (DOI: 10.1021/acs.jpcb.1c09896)
- S. Volkenandt, F. Beierlein, P. Imhof, *Molecules* **2021**, *26*, 5728. (DOI: 10.3390/molecules26195728)

https://chemistry.nat.fau.eu/ccc

Discrimination of fC and caC in extra-helical conformation by better binding

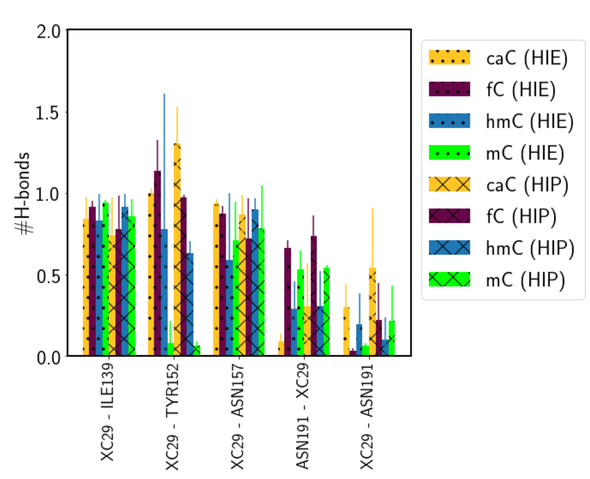
Rel. binding affinity [kcal/mol]	HIE	HIP
mC → caC	6.35 ± 0.62	-6.05 ± 0.80
caC → mC	-2.40 ± 0.41	7.52 ± 0.39
mC → fC	-2.92 ± 0.09	-6.11 ± 0.05
fC → mC	3.70 ± 0.23	6.43 ± 0.46
mC → hmC	-0.24 ± 0.26	-0.98 ± 0.36
hmC → mC	0.23 ± 0.10	3.62 ± 0.18

- caC and fC prefer HIP151 over HIE



Active site of TDG with extrahelical XC bases (colored) superimposed on crystal structure (gray).

- important interactions (with backbone NH of Y152) only observed for caC and fC



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