Exploring uracil base opening dynamics in dsDNA through enhanced molecular dynamics simulations

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Introduction - DNA damage

- DNA damage refers to alterations in the chemical structure of DNA, which can affect its normal function. These changes can occur in various forms, including

S. No	Types of damage	
1	Single-Strand Breaks (SSBs)	Breaks in one of the two strands of the DNA helix
2	Double-Strand Breaks (DSBs)	Breaks in both strands of the DNA helix, which are particularly harmful and can lead to cell death or cancer if not properly repaired
3	Base Modifications	Changes to the individual bases in DNA, which can result from oxidation, alkylation, or deamination.
4	Cross-Linking	Covalent bonding between two DNA strands or between DNA and proteins, which can interfere with DNA replication and transcription.
5	Bulky Adducts	Large chemical groups that attach to DNA bases, often caused by exposure to certain chemicals or environmental agents.

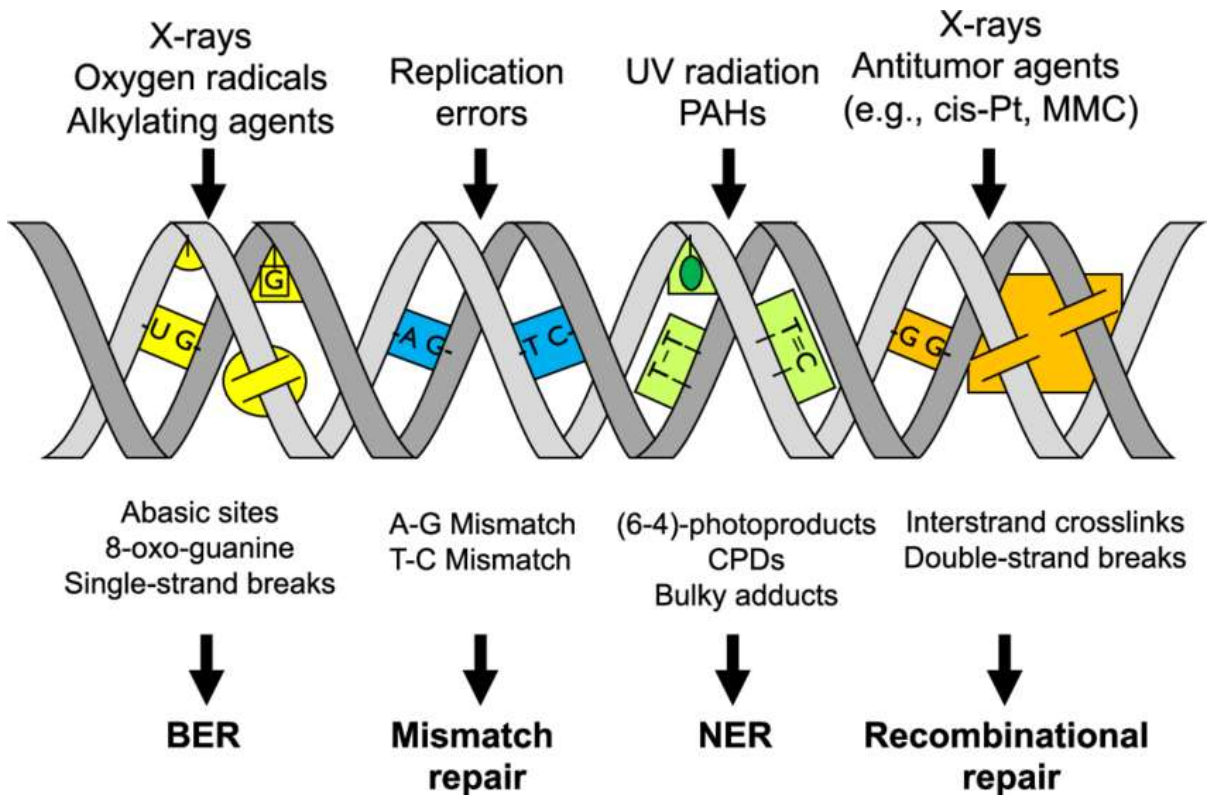
Introduction – Causes and repair mechanism

- DNA damage can be caused by a variety of internal and external factors
  - **Internal factors** - Reactive Oxygen Species (ROS), Replication Errors, Endogenous Alkylating Agents, etc.
  - **External factors** - Ultraviolet (UV) Radiation, Ionizing Radiation, Chemical Exposure, etc.

- **Repair mechanism**

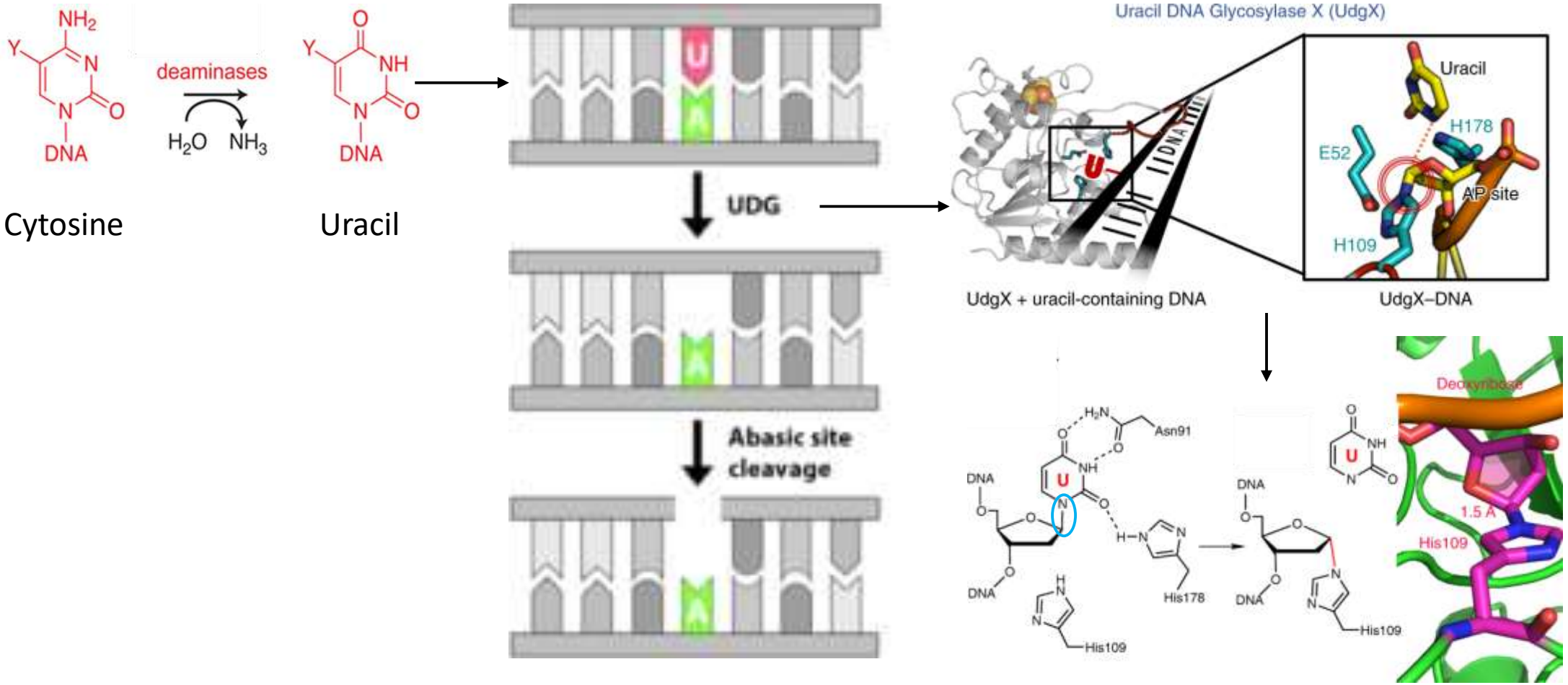
  It is essential for preventing mutations, maintaining genomic stability, and ensuring proper cellular function. There are several primary DNA repair pathways

  i. **BER**
  ii. Mismatch
  iii. NER
  iv. Recombinational repair



Introduction – BER (Base-excision repair)

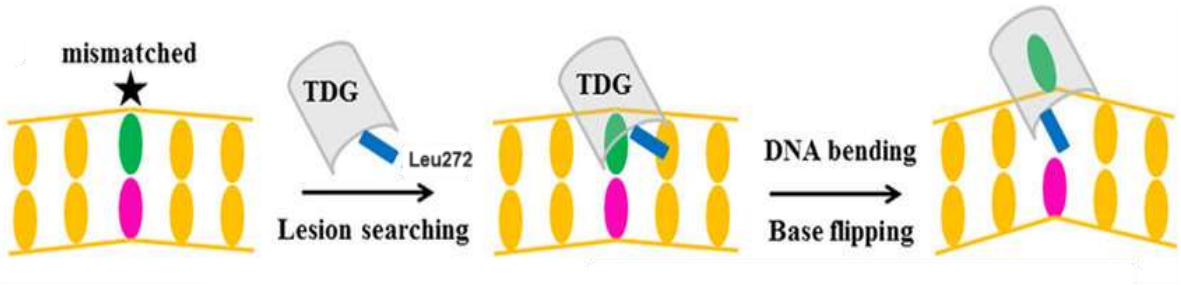
- Base excision repairs DNA when a base of a nucleotide is damaged; for example cytosine deamination

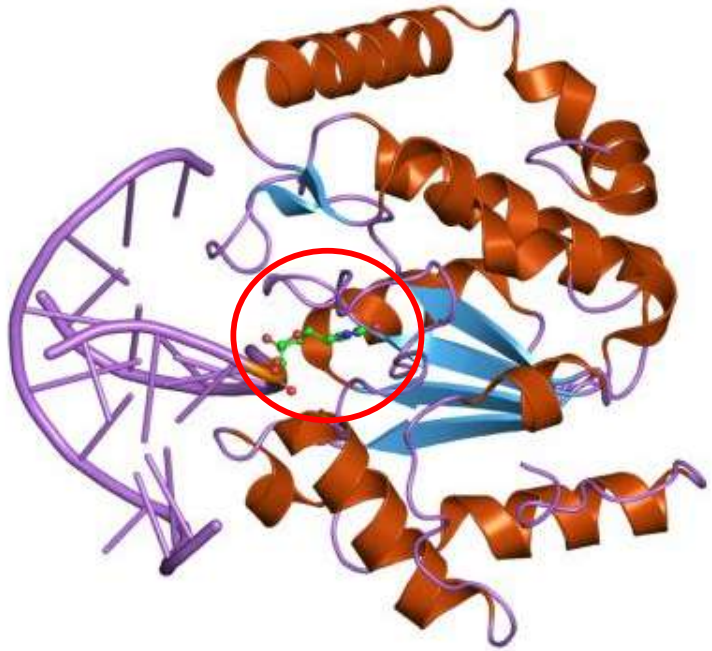


UDG catalyzes the cleavage of the glycosidic bond between the uracil base and the sugar phosphate backbone of the DNA

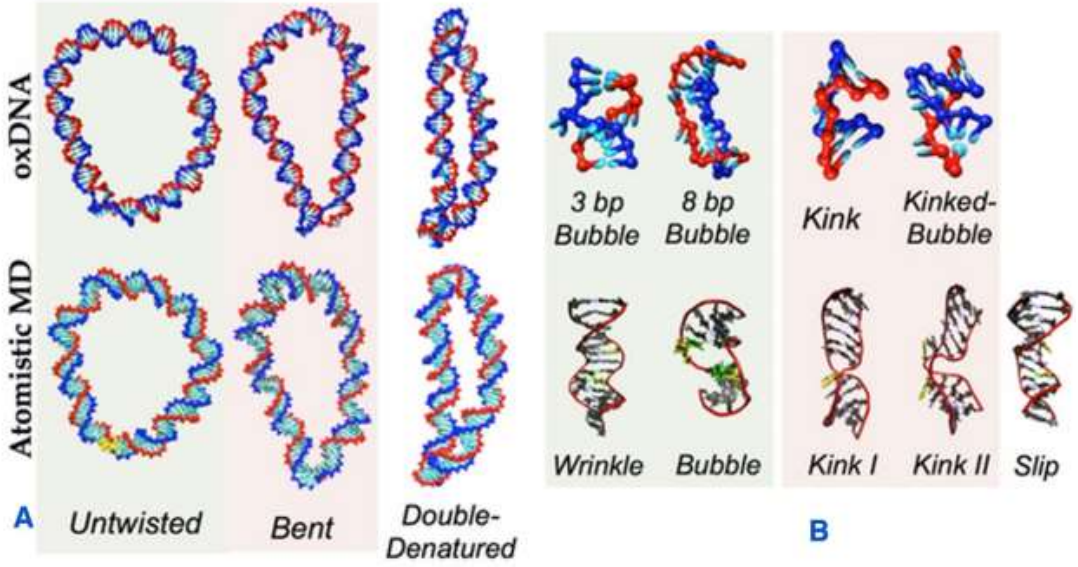
How does UDG recognize the uracil base?

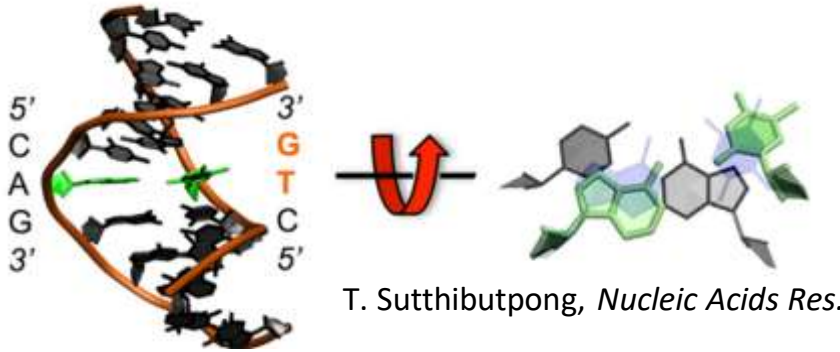
i. Active mechanism - **pinch**-push-**pull**



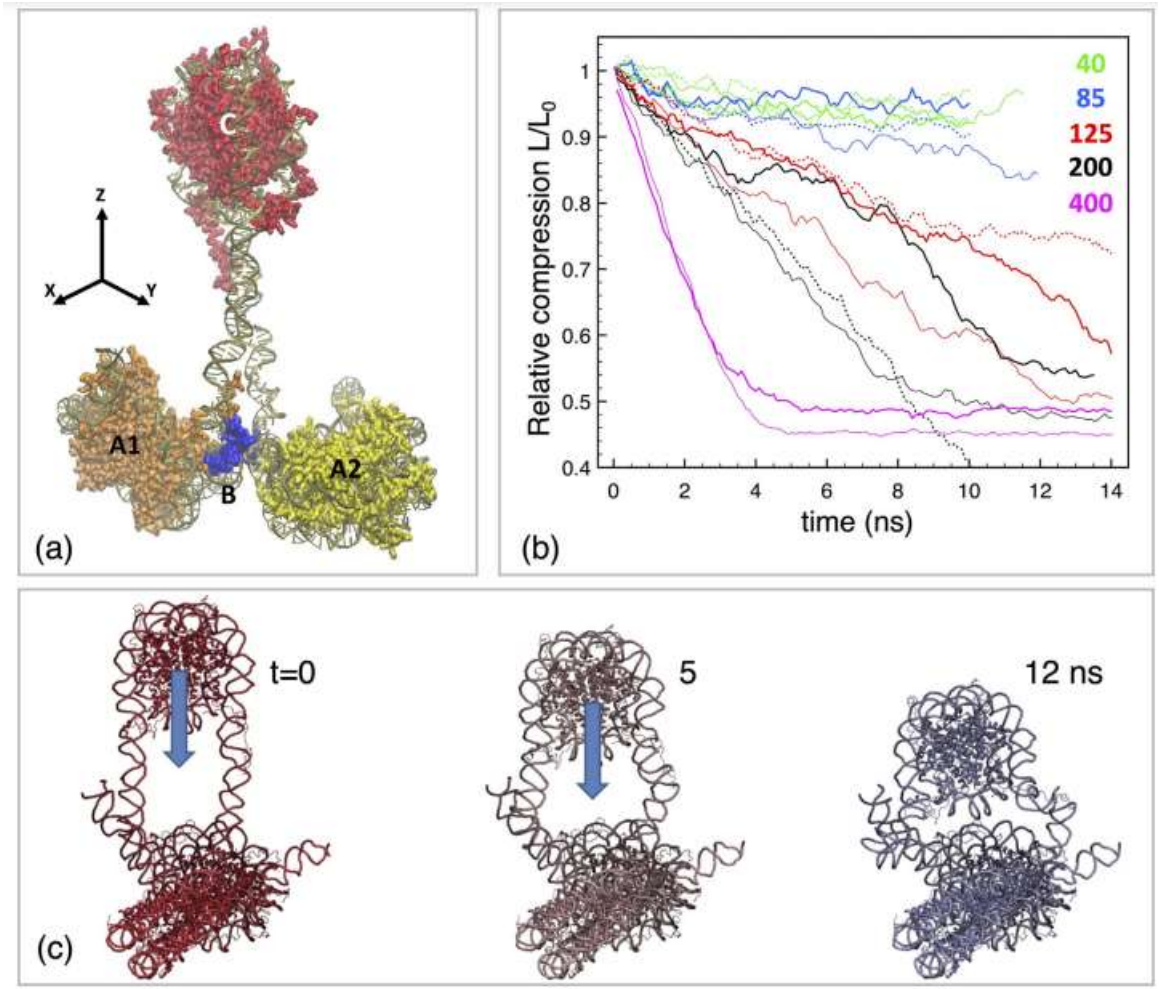


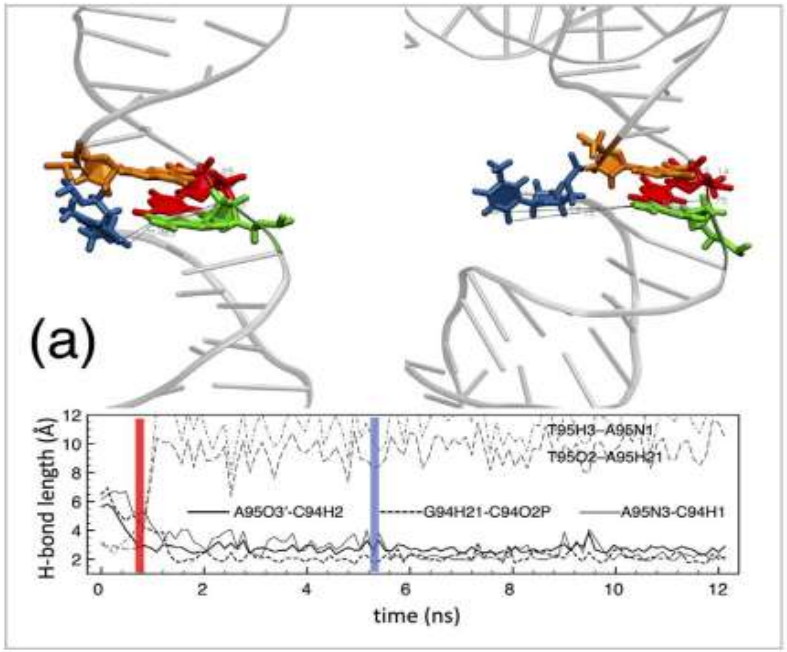
ii. Passive mechanism

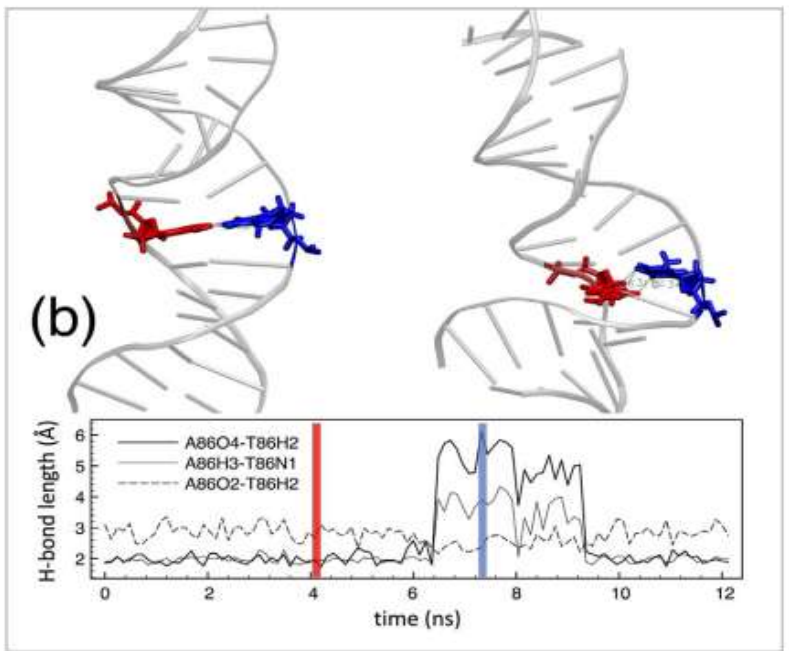




T. Sutthibutpong, *Nucleic Acids Res.*, 2016, gkw815







F. Cleri, S. Giordano, R. Blossey, J. Mol. Biology (2023)

Results section

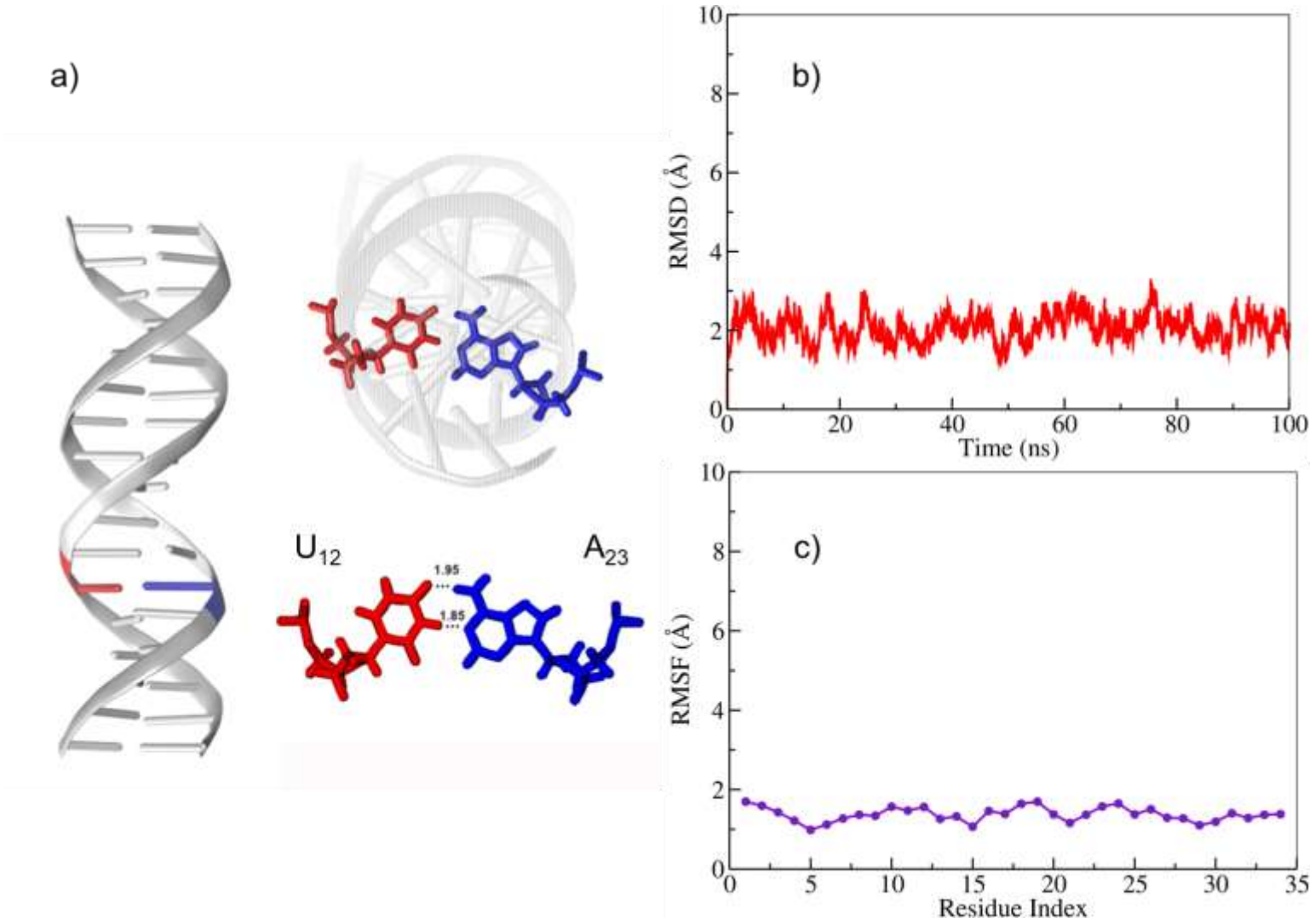
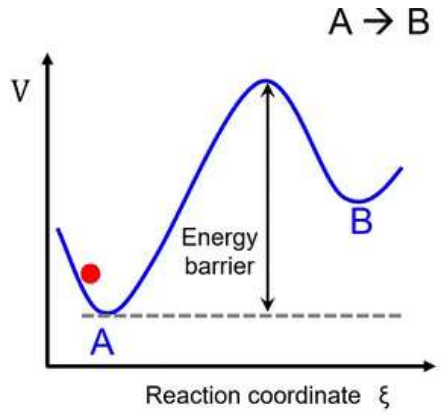


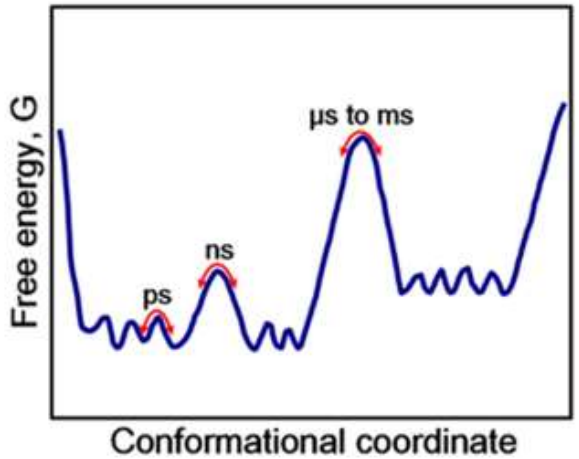
Figure 1: a) 17-mer uracil-mutated dsDNA system with red and blue colours representing uracil and adenine residues, respectively. b) RMSD and c) RMSF of the uracil-mutated dsDNA system during the first 100 ns of the MD trajectory.

Computational Methods- Enhanced Sampling technique

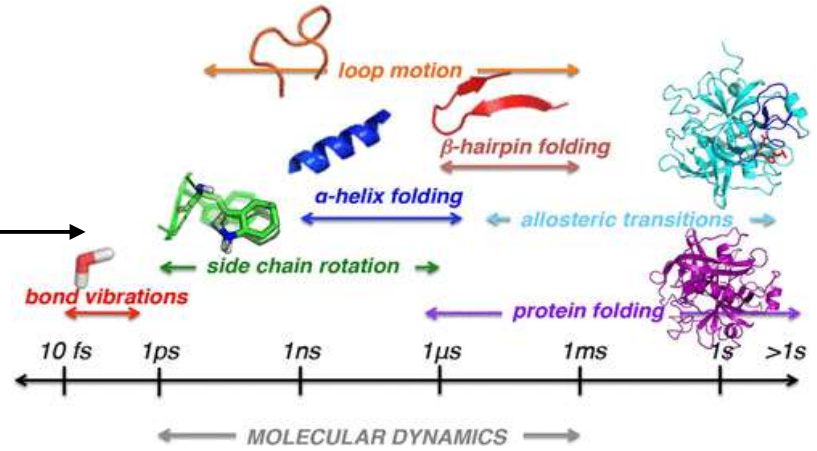
Challenges of MD simulations

- Molecular dynamics may be trapped at the local minima

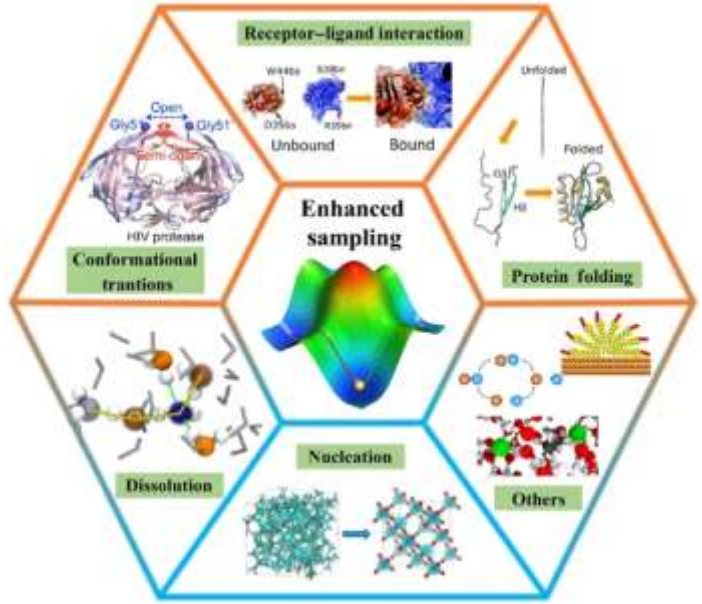




- Time scale challenges



- Enhanced sampling: apply biases
  - i. Umbrella Sampling
  - **ii. Metadynamics**
  - iii. Steered MD
  - iv. Replica Exchange MD
  - v. Gaussian accelerated

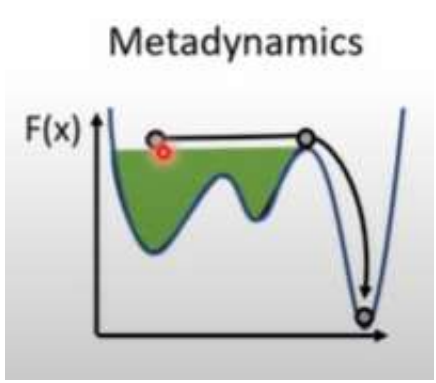


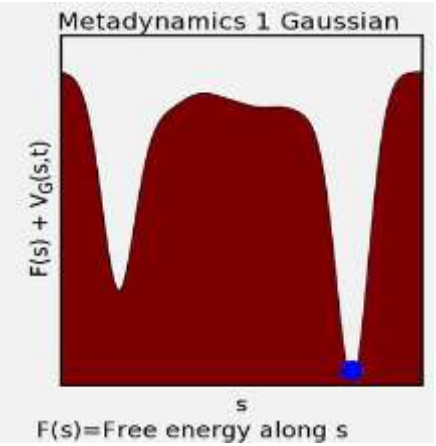
Metadynamics simulation

- A general idea of MtD is to enhance the system sampling by discouraging revisiting of sampled states.

- It is achieved by History-dependent bias potential acting on selected **collective variables** (CVs) or **reaction coordinates**.

$$V(s,t) = \sum_{k\tau < t} W(k\tau) \exp - \sum_{i=1}^{N_{CV}} \frac{(s_i - s_i(q(k\tau)))^2}{2\sigma_i^2}$$





Laio, A., & Parrinello, M. (2002)

Here, V(s, t) – biasing potential

σ_i - width of the Gaussian function

W(kτ) - height of the Gaussian at the simulation time t = kτ

Reaction coordinates schemes

- Collective variables (CVs) in metadynamics simulations are low-dimensional descriptors that represent the essential degrees of freedom of a molecular system.

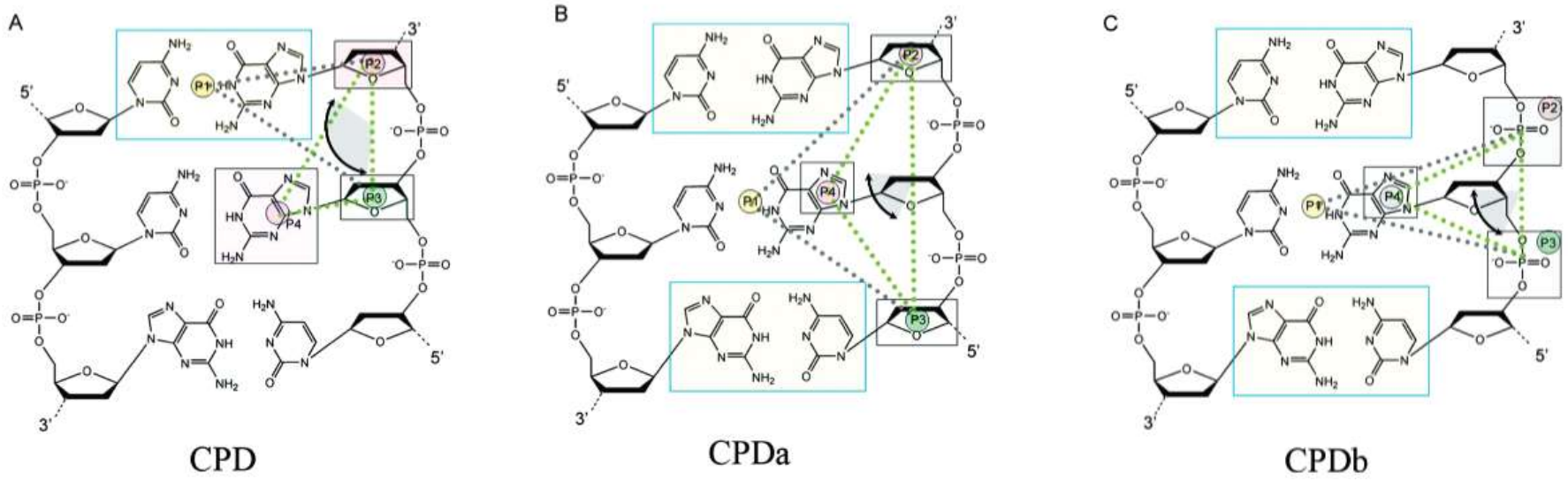
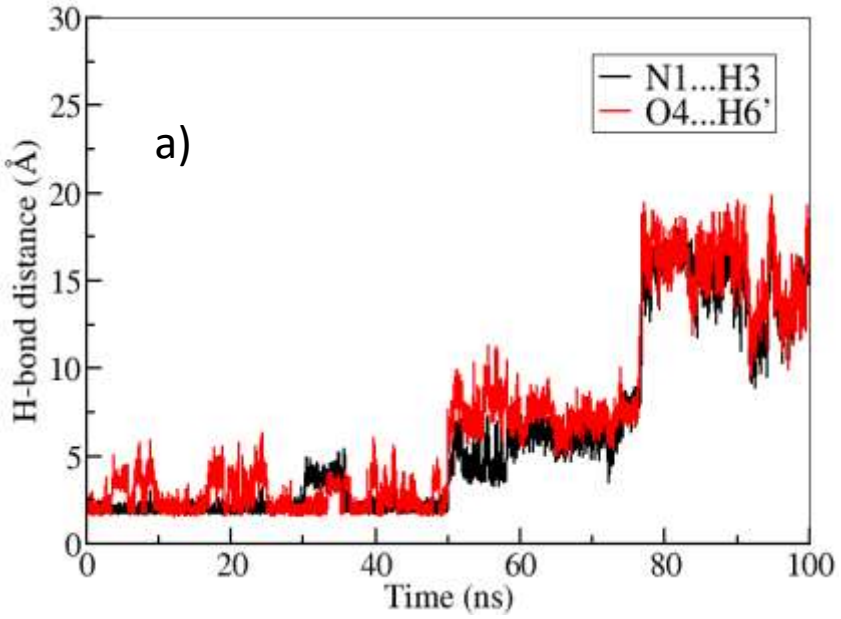


Figure 2: Definitions of various reaction coordinates for base flipping. (A) CPD: MacKerell et al.'s original COM pseudo-dihedral definition. (B) CPDa: the modified COM pseudo-dihedral (CPD) angle definition, in which p1 is defined by the mass center of the two flanking base pairs, p2 and p3 are defined by the flanking sugar groups, and p4 is defined by the five-member ring of the flipping purine (or the entire six-membered ring for a flipping pyrimidine). (C) CPDb: a similar definition to that of CPDa, but using the phosphate groups for p2 and p3. The dotted lines show the two planes which define the pseudo dihedral angles. (*J. Chem. Theory Comput. 2009, 5, 11, 3105–3113*)

Hydrogen bond analysis



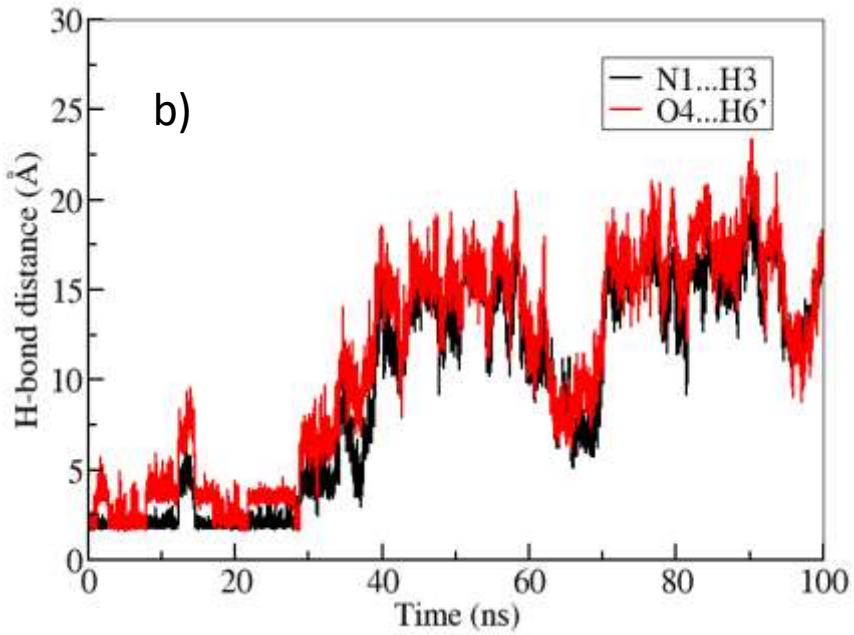


Figure 3: Hydrogen bond distance between N1⋯H3 and O4⋯H6' a) CPD and b) CPDb

Free energy landscape for base opening

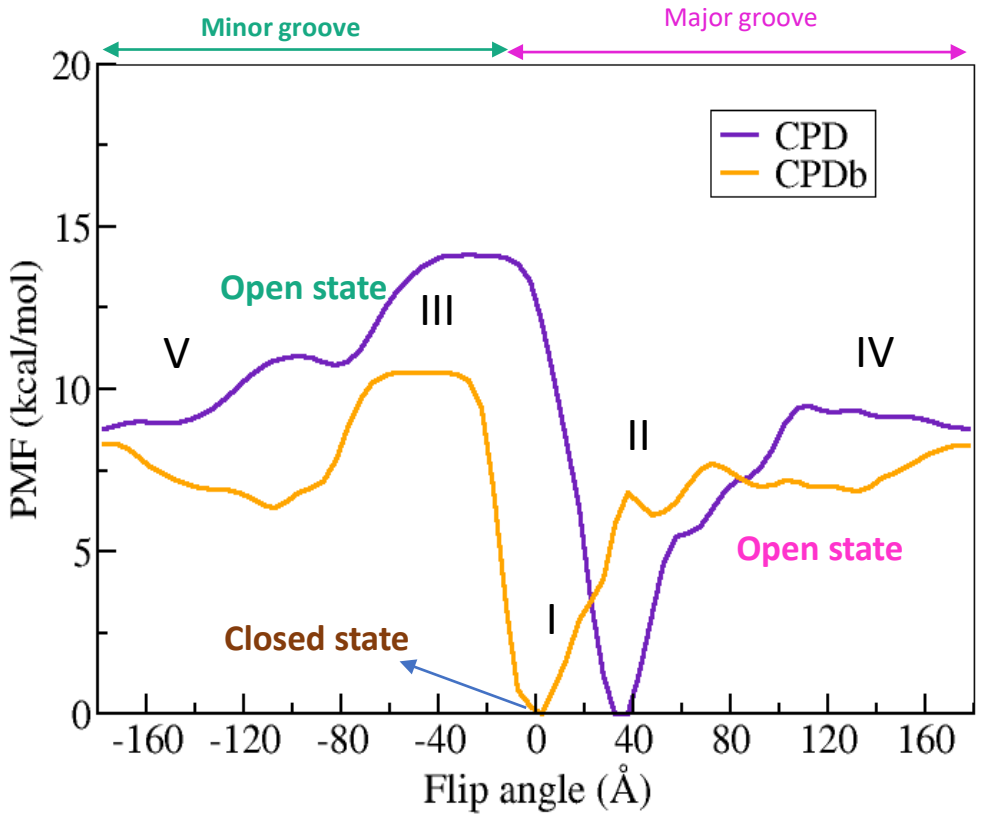


Figure 4: PMF profiles of uracil flipping out of the dsDNA

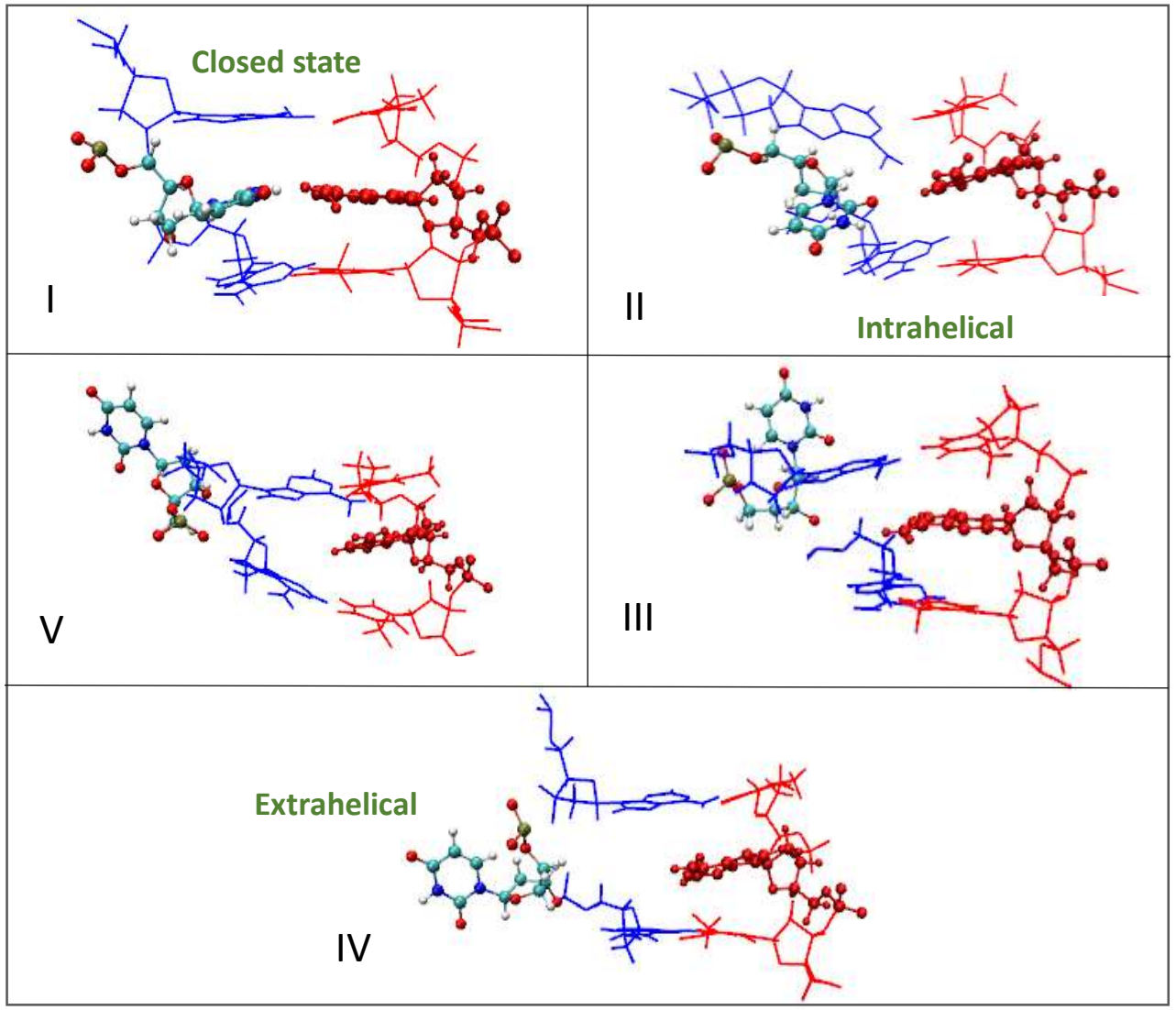


Figure 5: Snapshots of closed, semi flipped, and fully flipped Uracil nucleotide

Rigid base-pair parameters for dsDNA

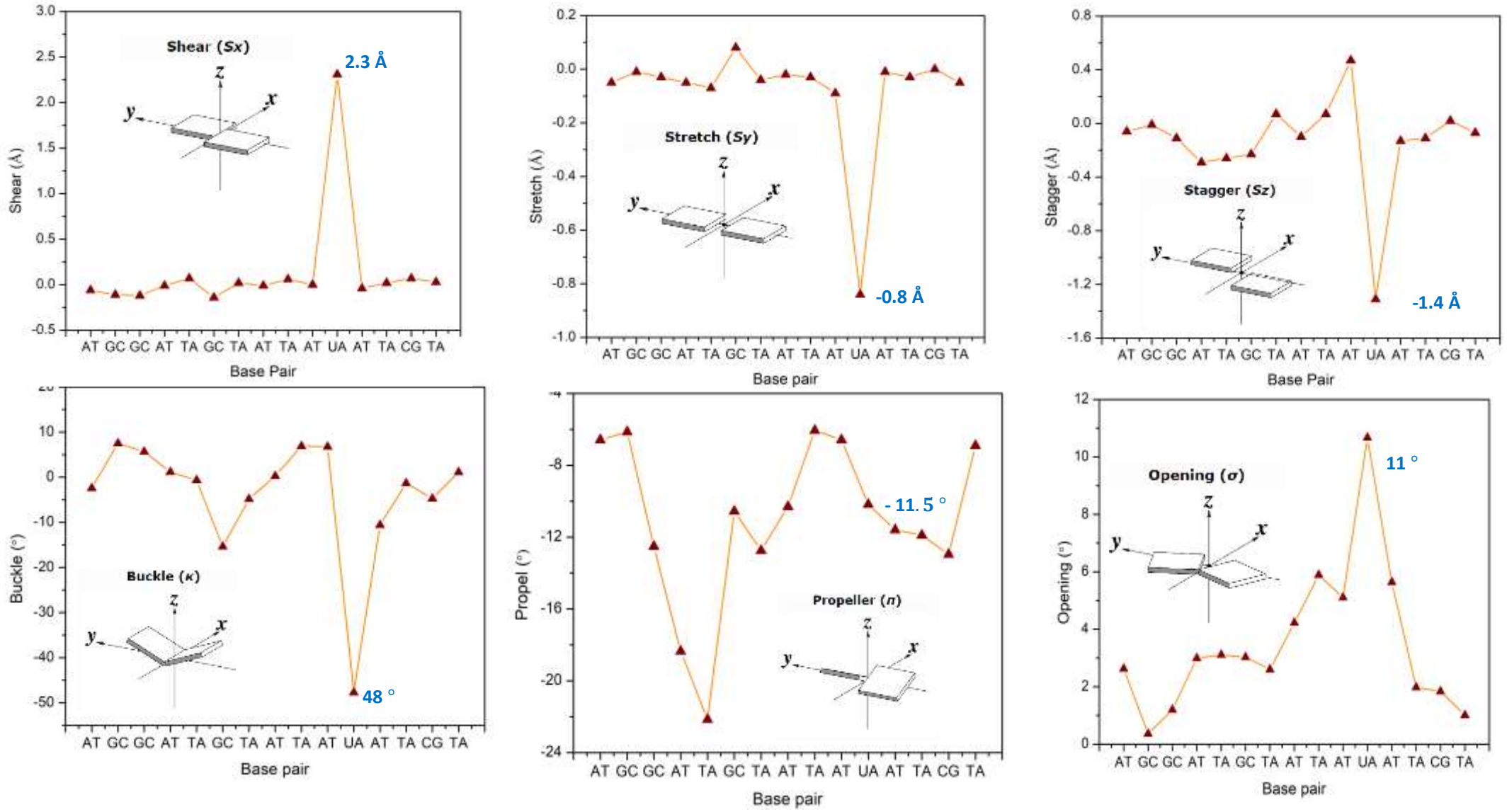
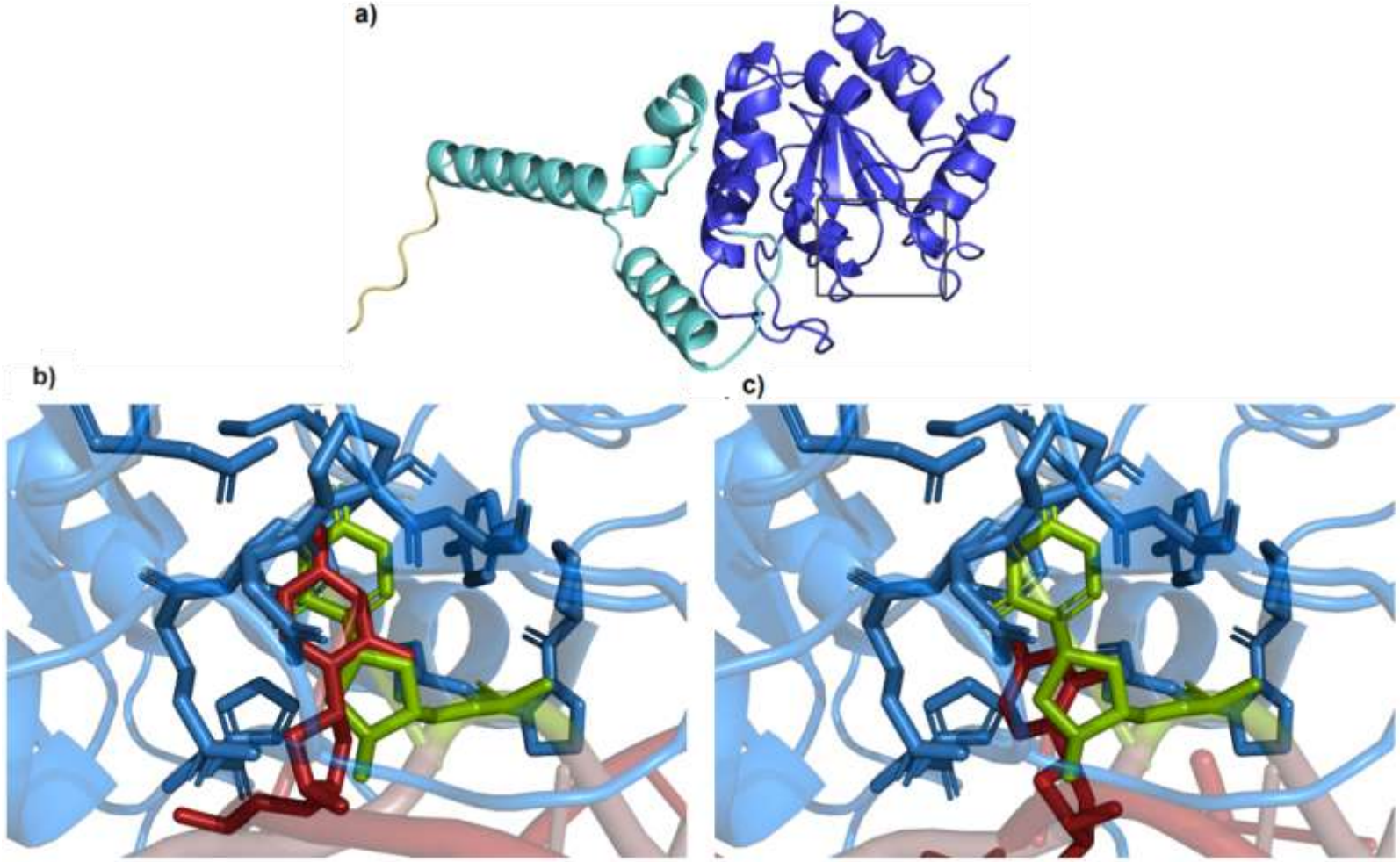


Figure 6: DNA helical parameters analysis (intra-base pair) by curves+ program

Molecular Docking results



PydockDNA

- Predicted several conformations
- Rank them with scoring function
- Selected the best complex structure that is closest to the experimental prediction

Figure 7: a) Cartoon representation of UDG tertiary structure. Its residues are colored according to the backbone qualitative flexibility evaluation. The three flexibility levels are colored as follows: yellow for flexible, blue for rigid and cyan for intermediate level between flexible and rigid. The black box designates UDG's catalytic pocket which is enlarged in b) and c), b) 'C6' DNA structures (red) docked to UDG (blue; cartoon) with a focus on its catalytic pocket (blue; sticks). The uracil (sticks; red) from the docked DNA is compared to the one from 1EMH's (cartoon; green) as a reference, and c) Same system as c) with T11 as the docked DNA structure.

Conclusion

Damaged DNA lesions spontaneously flip out from the dsDNA helix, providing enzymes with access to faulty genetic information, or else remain hidden within the dsDNA helix

i. In this study, we employed computational methods to investigate the Uracil base flipping mechanism in dsDNA. The initial structural stability of dsDNA was calculated using all-atom molecular dynamics simulations.

ii. Free energy calculations were performed via metadynamics simulations to explore spontaneous uracil base flipping in dsDNA. In the case of CPD, the energy barrier values for the major groove and minor groove are 6.9 kcal/mol and 13.0 kcal/mol, respectively.

iii. For CPDb, the corresponding energy barrier values are 7.8 kcal/mol for the major groove and 10.0 kcal/mol for the minor groove.

iv. Subsequently, partially and fully flipped uracil-mutated dsDNA structures generated by MD simulations were docked into the UDG enzyme using molecular docking tools.

v. Based on the molecular docking results, we confirmed that the fully flipped state of dsDNA fits well into the catalytic site of the UDG enzyme, which correlates with experimental predictions.