Atomistic Simulations of Complex DNA DSBs and the Interactions with Ku70/80 Heterodimer

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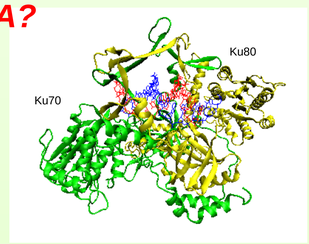
Introduction

What is complex DNA damage?

- Two or more lesions within one or two helical turns of the DNA double-helix by a single radiation track.
- Modified bases (8-oxoG and thymine glycol), AP sites, 2-deoxyribonolactone, SSBs or DSBs.
- For low LET radiation, 30% of DSBs formed are complex, where at least one lesion is in close proximity of DSB.
- For high LET radiation, over 90% of DSBs contain clustered DNA damages nearby.

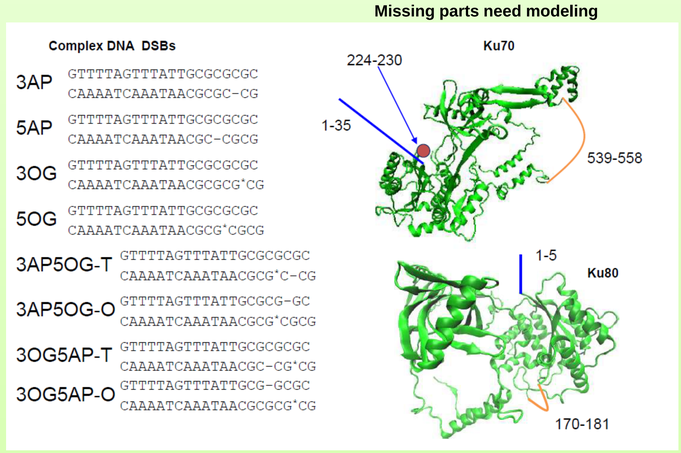
How does Ku bind DNA?

- The channel structure of Ku heterodimer
- Two steps:
  1. recognize the DNA ends
  2. translocate to internal sites



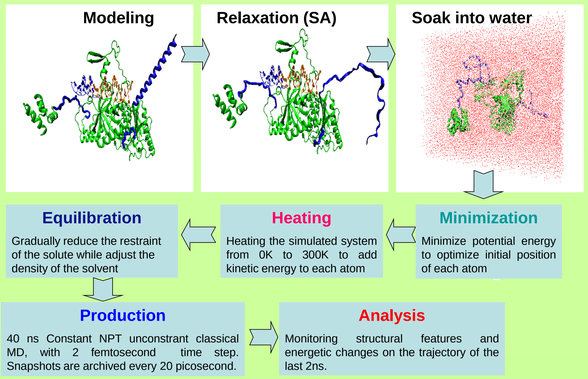
Modeling

Missing parts need modeling



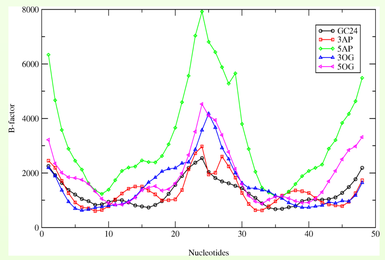
Methods and Procedure

- **Modeling:** with DeepView Swiss-PdbViewer
- **Simulated annealing (SA):** using AMBER 9, ff99SB force field, GB5 implicit solvent, 5X100ps, with crystal structure and DNA atoms constraint, and missing residues flexible
- **MD simulation:** each system 40 ns, with TIP3P water & counter-ions & Periodic boundary & PME &SHAKE
- **Structural and energetic analysis:** VMD, ptraj and MM-PBSA of AMBER

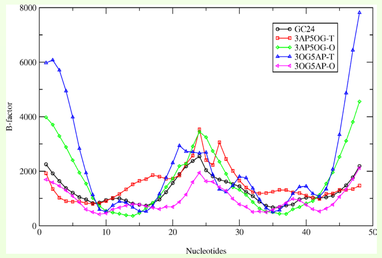


Results

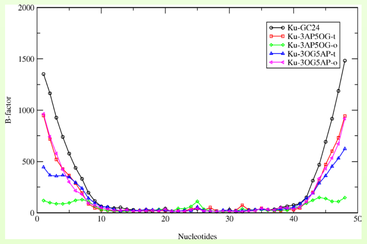
I. Flexibility of nucleotides



B-factors of I site lesions compared with simple DSB



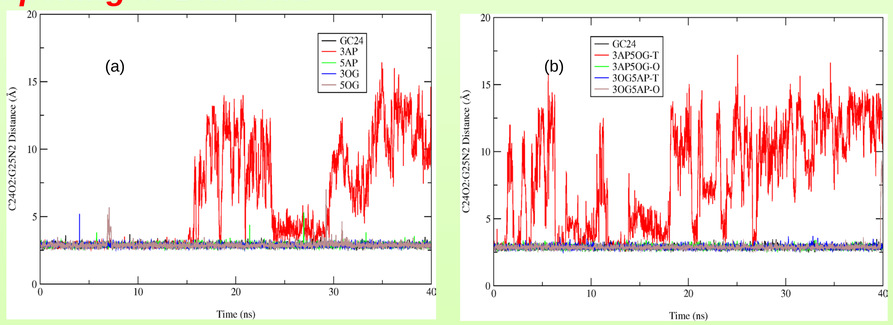
B-factors of 2 sites lesions compared with simple DSB



B-factors of the nucleotides in Ku-DNA systems

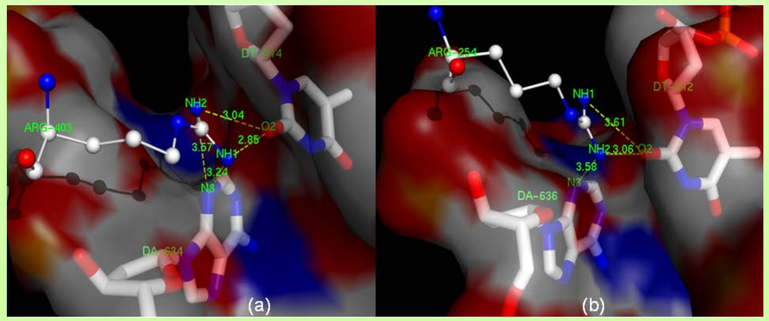
- Complex DSBs are more flexible than simple DSBs.
- Binding with Ku drastically stabilizes the DNA duplexes.

II. Opening of DNA end



Base opening rates at DNA ends for 1 site lesions (a) and 2 sites lesions (b)

III. Base interactions



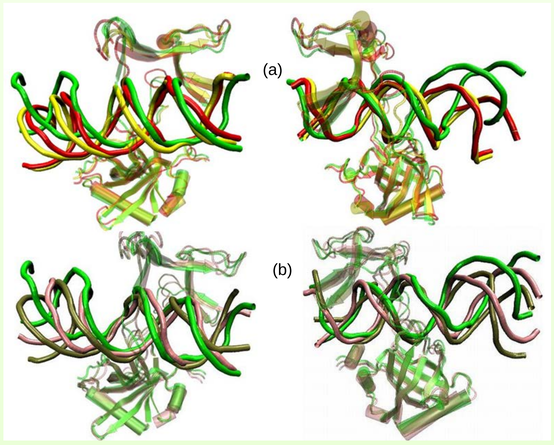
H-bond network of R403 (a) and R254 (b) of Ku70

IV. Energetic analysis

System	Ku-DNA	Ku70	Ku80	Ku70 Ring	Ku80 Ring	DNA	DNA End (6bps)	DNA Center (9bps)
Ku-GC24	-238.3	-105.6	-63.9	-88.9	-40.7	-68.8	-38.0	-26.3
Ku-3AP5OG-T	-229.4	-84.4	-82.8	-71.0	-70.6	-62.2	-27.7	-27.1
Ku-3AP5OG-O	-238.0	-97.6	-84.4	-75.2	-65.8	-56.0	-23.4	-23.9
Ku-3OG5AP-T	-266.3	-90.4	-101.0	-63.3	-73.9	-74.9	-20.8	-35.1
Ku-3OG5AP-O	-274.7	-112.0	-82.0	-79.2	-63.2	-80.7	-37.7	-35.8

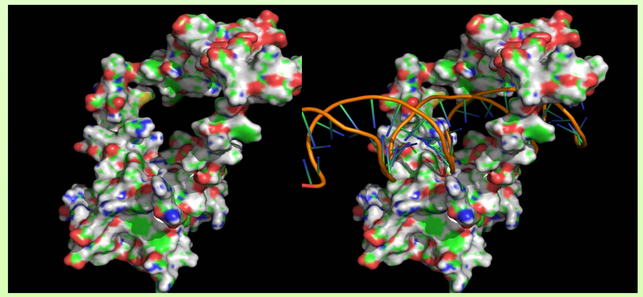
Binding free energy decomposition. Unit: kcal/mol. Calculated with GB1SA from the last 2ns. Entropies not included.

V. Binding mode changed

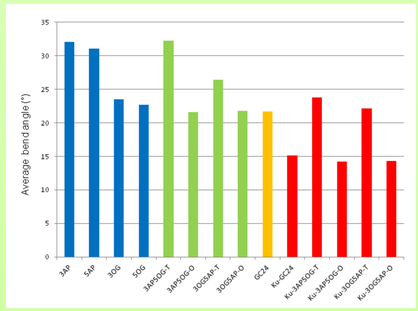


The Ku80 ring tends to bind more tightly with complex DSBs than with simple DSB. Note the angles of DNA duplexes of complexes damages (colored in red, yellow, brown, and tan) and simple DSB (colored in green), with respect to the superposed Ku80 ring.

VI. Clamp structure of the inner surface of the ring



VII. Bend of helix



Conclusions

- Compared to DNA with simple DSBs, the complex lesions can enhance the hydrogen bonds opening rate at the DNA terminus, and increase the mobility of the whole duplex.
- Binding of Ku drastically reduces the structural disruption and flexibility caused by the complex lesions.
- In all complex DSBs systems, the binding of DSB terminus with Ku70 is softened while the binding of the middle duplex with Ku80 is tightened.
- Binding of Ku promotes the rigidity of DNA duplexes, due to the clamp structure of the inner surface of the rings of Ku70/80.

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