# Interactions of the SAP Domain of Human Ku70 with DNA Substrate: A Molecular Dynamics Study

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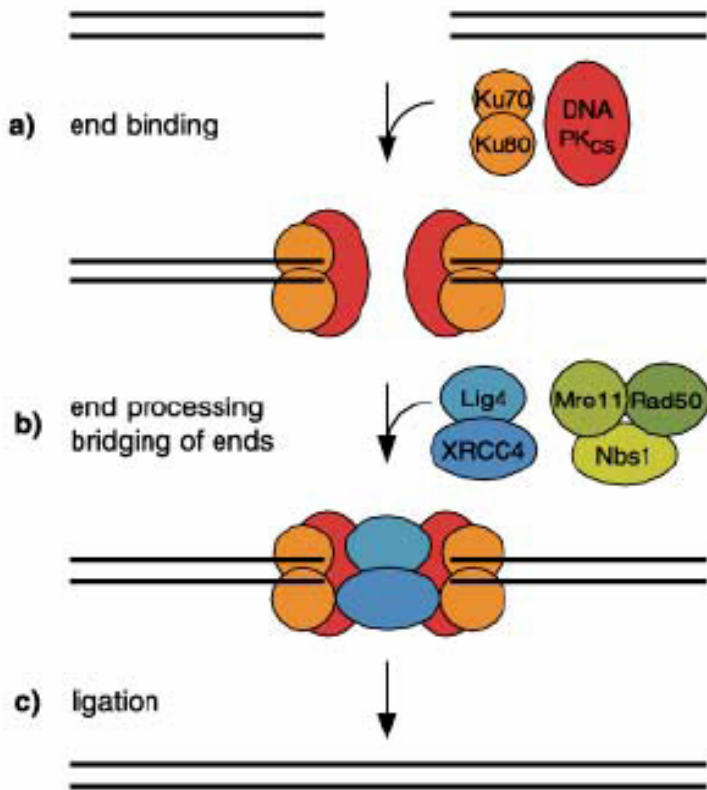
# Abstract

- NASA is developing a systems biology approach to improve the assessment of health risks associated with space radiation. The primary toxic and mutagenic lesion following radiation exposure is the DNA double strand break (DSB), thus a model incorporating proteins and pathways important in response and repair of this lesion is critical.  One key protein heterodimer for systems models of radiation effects is the Ku70/80 complex. The Ku70/80 complex is important in the initial binding of DSB ends following DNA damage, and is a component of nonhomologous end joining repair, the primary pathway for DSB repair in mammalian cells.  The SAP domain of Ku70 (residues 556-609), contains an a helix-extended strand-helix motif and similar motifs have been found in other nucleic acid-binding proteins critical for DNA repair. However, the exact mechanism of damage recognition and substrate specificity for the Ku heterodimer remains unclear in part due to the absence of a high-resolution structure of the SAP/DNA complex.

- We performed a series of molecular dynamics (MD) simulations on a system with the SAP domain of Ku70 and a 10 base pairs DNA duplex. Large-scale conformational changes were observed and some putative binding modes were suggested based on energetic analysis. These modes are consistent with previous experimental investigations.  In addition, the results indicate that cooperation of SAP with other domains of Ku70/80 is necessary to explain the high affinity of binding as observed in experiments.
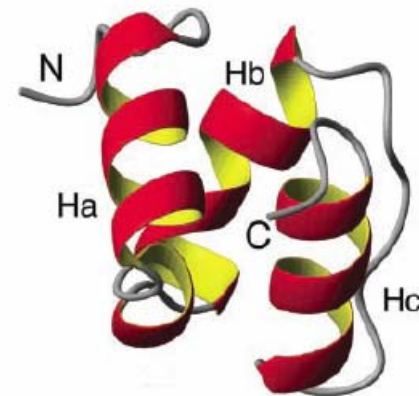
# Introduction



a) end binding

b) end processing
bridging of ends

c) ligation

DSB repair by nonhomologous
end joining (NHEJ)

About $5\times10^5$ Ku molecules per nucleus.

Ku70 is mainly responsible for DNA binding.

Two DNA-binding domains: N terminus before residue 440, C terminus (SAP domain)



Helix-extend strand-helix
structure of SAP domain

# Material and Methods

Resources:

- Computational programs package: AMBER 9.0
- Workhorse: Radiation Biology Laboratory Beowulf Cluster (16X4 AMD Opteron x86-64)
- SAP domain: NMR structure (PDB accession code 1JJR)
- DNA 10-mer: d(GCGTTAACGC)$_2$, generated by NUCGEN of AMBER

MD protocol:

1. Putative binding modes search: implicit solvent (GB2), multiple alternative constraint/restraint simulation annealing (SA) (250ps) and semi-flexible MD (250ps)

2. Binding affinity calculation: 5ns unconstraint explicit solvent (TIP3P water), counterions (Na$^+$), 300K, 1 atm, SHAKE, PME, MM_PBSA

# Force Fields and Solvent Models



Performance of three force fields on SAP
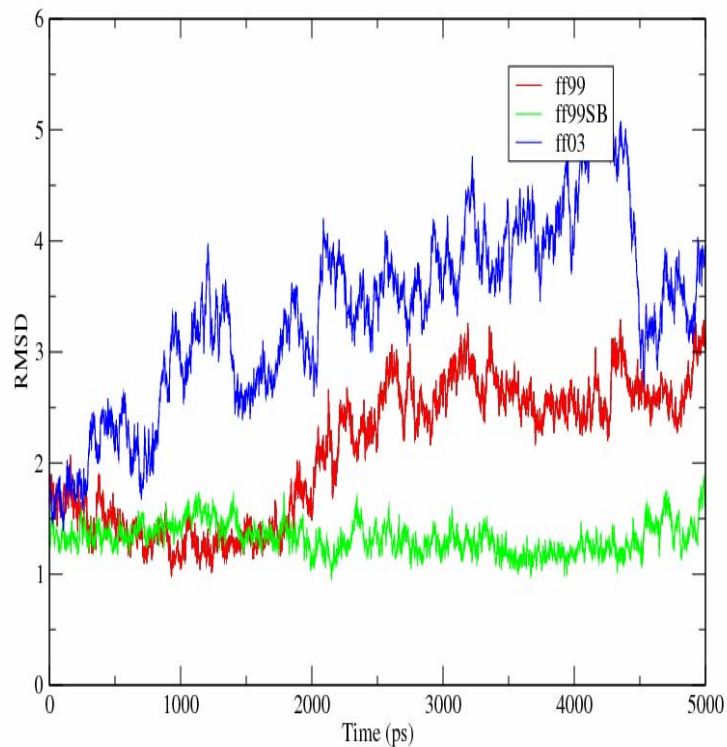
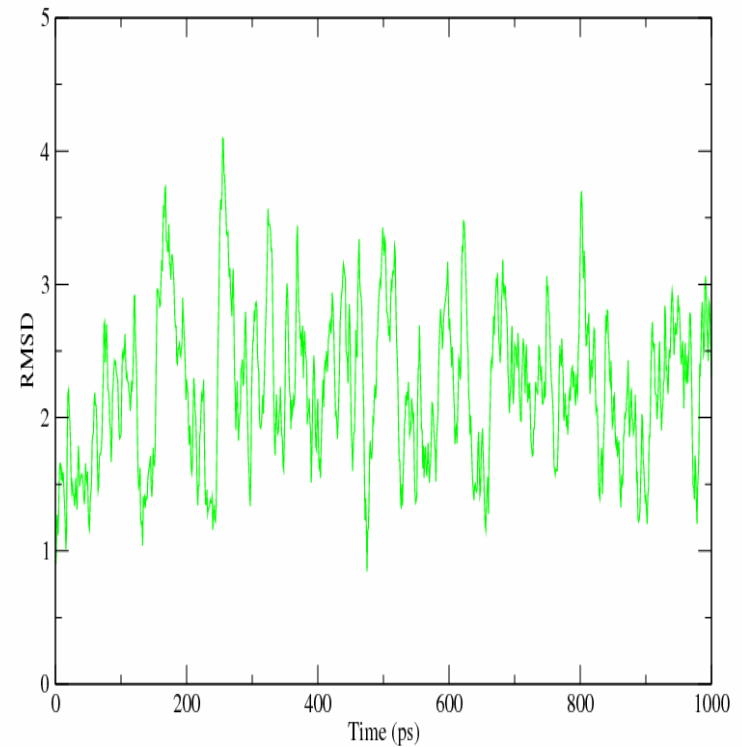Performance of three implicit solvent on SAP
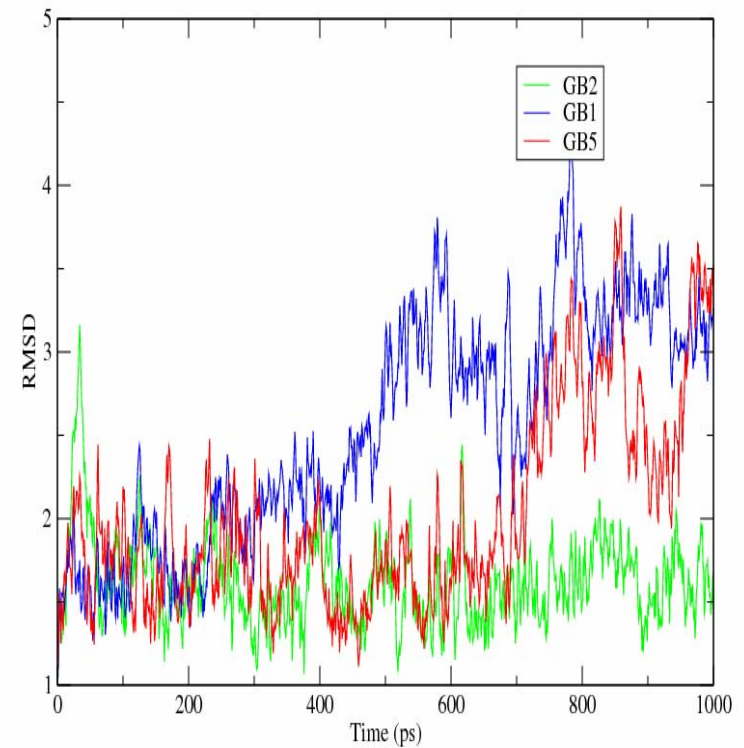
RMSD: root mean square deviation (Å) of heavy atoms of backbone.

# How about DNA?

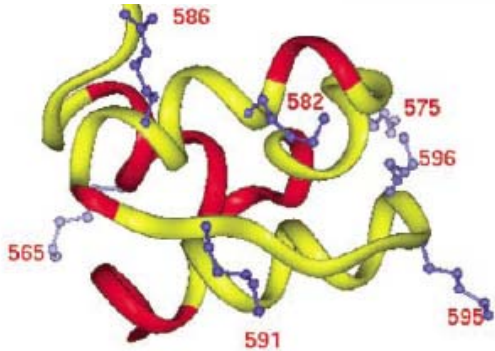Unconstraint simulation of SAP with ff99SB and TIP3P water
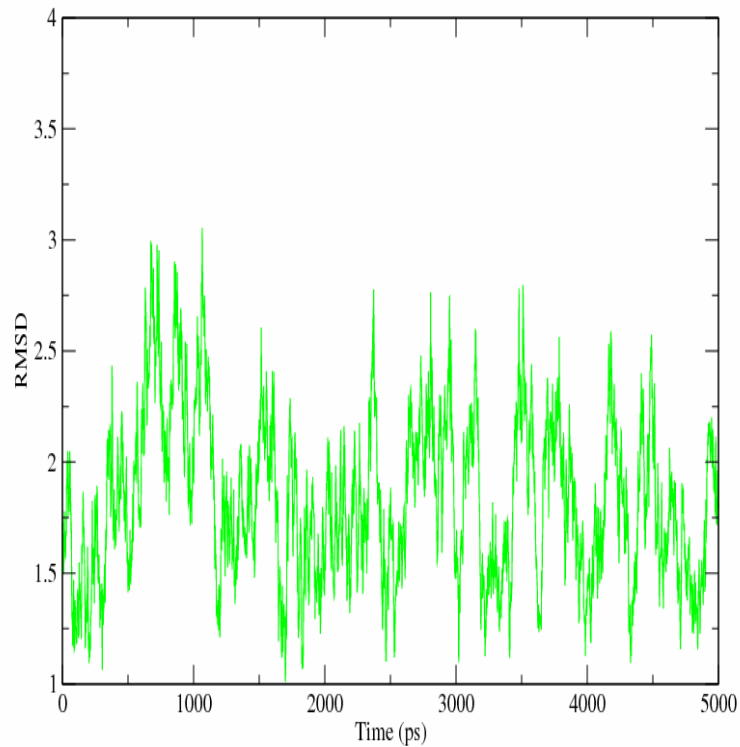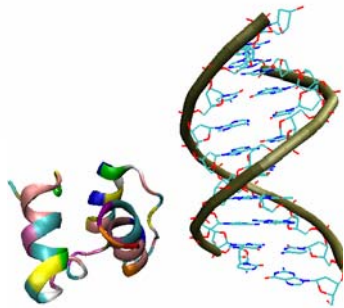
Unconstraint simulation of DNA with GB2 implicit solvent
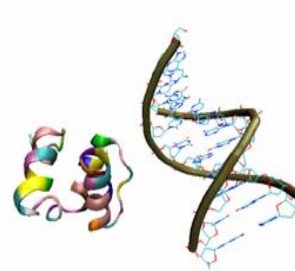
DNA is more flexible than SAP
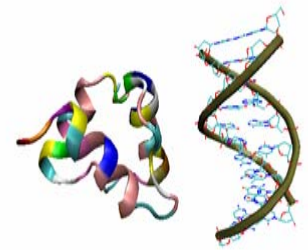
# Starting Structures (manually docked)



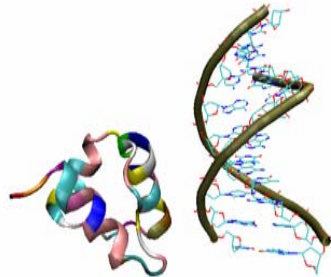Chemical shift changes upon complex formation ( in yellow) and conserved Arg or Lys residues.

kuc3

kuc4
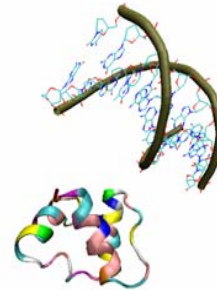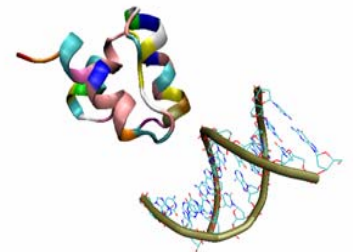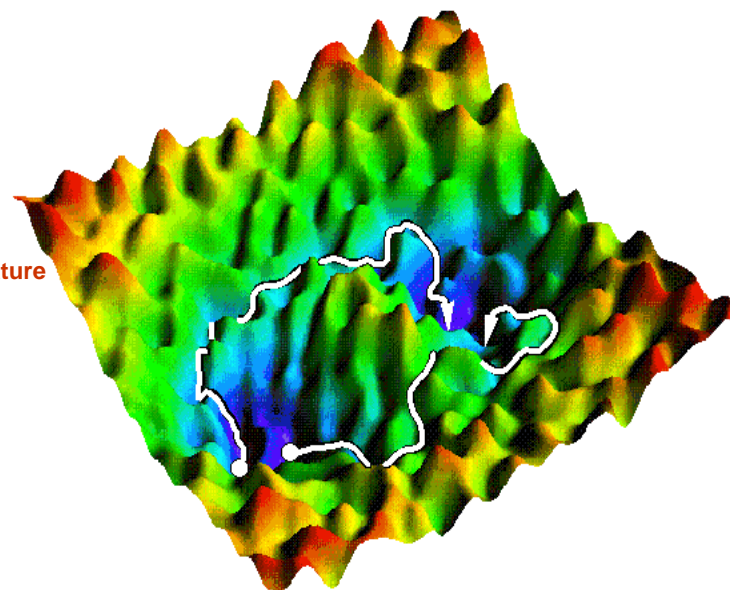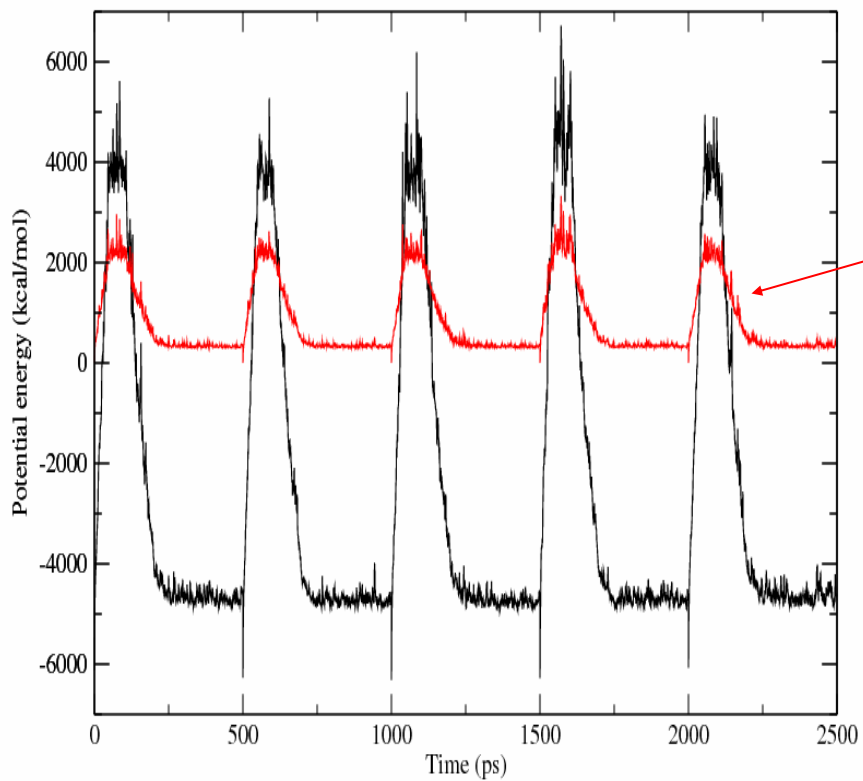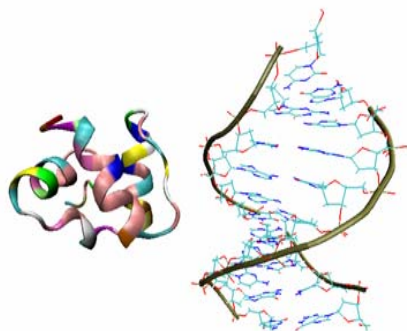
kuc5

kuc6

kuc7

kuc8

# Constraint/Restraint SA

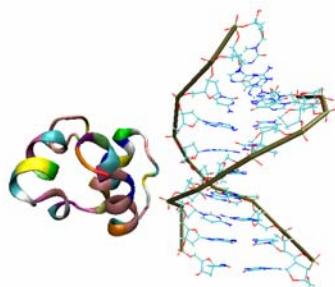**Potential energy variation along the path of SA**



Temperature
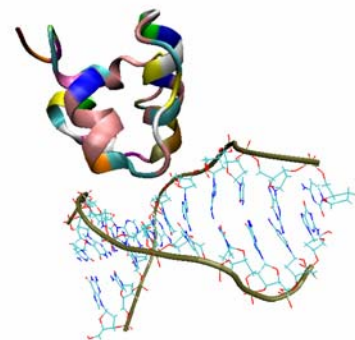


Overcome the barriers of potential energy surface

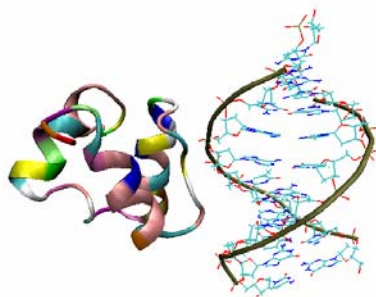# Annealed Structures (most visited conformations)

kuc3

kuc4

kuc5

kuc6
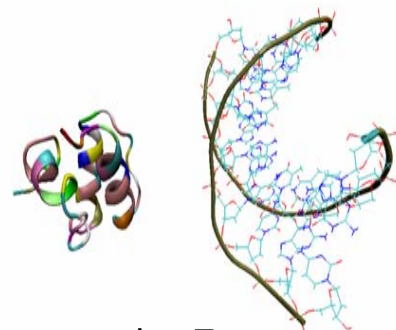
kuc7
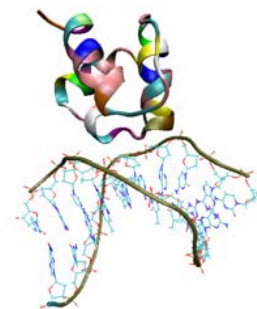
kuc8

➢Loops rather than helices directly interact with the minor groove of DNA.

➢DNA experiences apparent bending upon binding.

# Explicit Solvent Simulation

**RMSD (Å) of heavy atoms of the complex backbone**

**Separate RMSD (Å) of SAP and DNA**



➢Complex is stabilized after 2 ns equilibration.

➢There is apparent adjustment when the complex is immersed into explicit solvent, but the binding mode is well maintained.

# Binding Affinity Calculation

MM_PBSA approach:

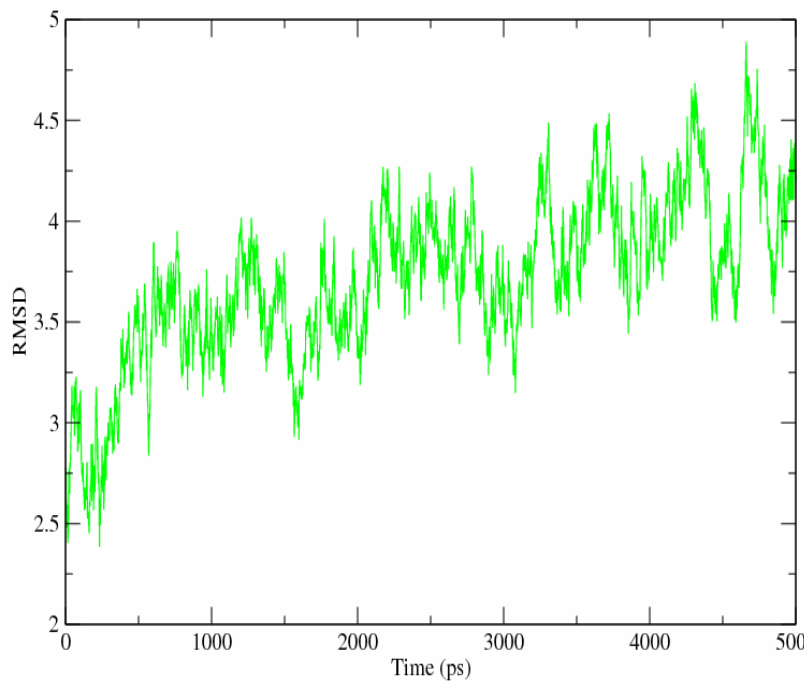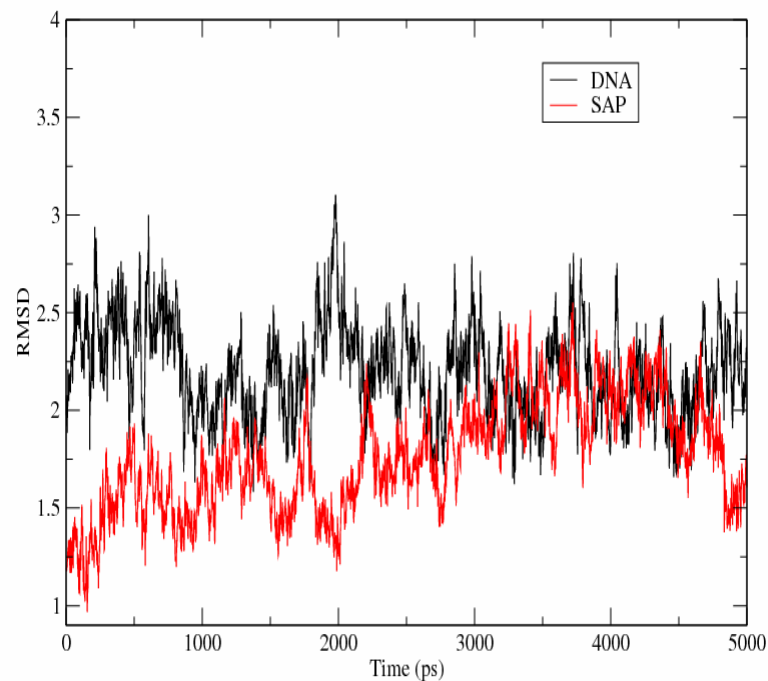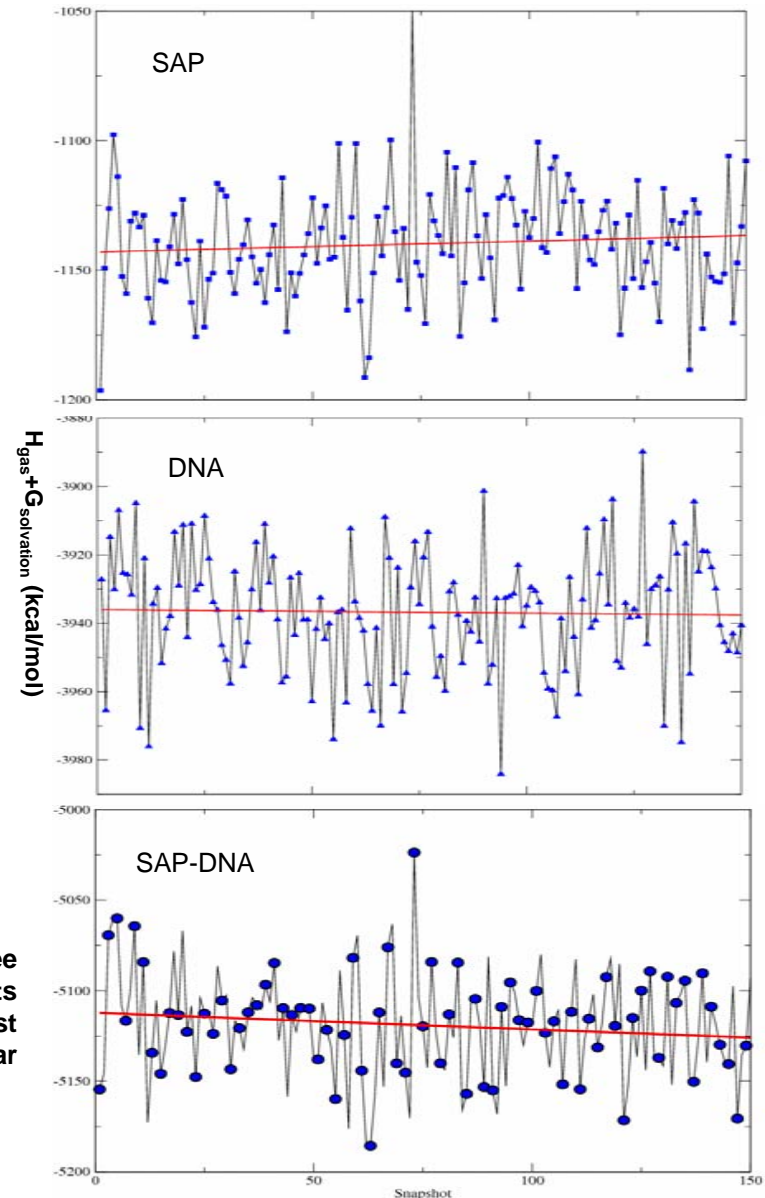$$\Delta G_{binding} = \left\langle G^{complex}(i) - G^{SAP}(i) - G^{DNA}(i) \right\rangle_i$$

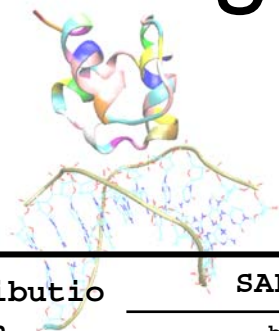$$G^x(i) = H^x_{gas} + G^x_{solvation}(i) - TS^x(i)$$

$$H_{gas} = H_{elec} + H_{vdw} + H_{int}$$

$$G_{solvation} = G_{PB} + G_{SA}$$

**Sum of gas-phase and solvation free energies calculated for 150 snapshots extracted at 20ps intervals from the last 3ns of MD simulations. Linear regression are indicated in red lines.**

# Binding Free Energy Components

| Contribution | SAP-DNA | | SAP | | DNA | | Delta[a] | |
|---|---|---|---|---|---|---|---|---|
| | Mean[b] | $\sigma^c$ | mean[b] | $\sigma^c$ | Mean[b] | $\sigma^c$ | Mean[b] | $\sigma^c$ |
| $H_{ele}$ | -2326.04 | 6.87 | -839.53 | 4.71 | -48.04 | 2.36 | -1438.47 | 8.06 |
| $H_{vdw}$ | -370.96 | 1.15 | -169.67 | 0.94 | -160.99 | 0.71 | -40.30 | 0.25 |
| $H_{gas}$ | -589.27 | 7.02 | 170.66 | 4.82 | 718.84 | 2.39 | -1478.77 | 7.73 |
| $G_{solvation}$ | -4529.80 | 6.27 | -1310.45 | 4.30 | -4655.62 | 2.13 | 1436.27 | 7.34 |
| $G_{gas+solvation}$ | -5119.08 | 2.31 | -1139.80 | 1.74 | -3936.78 | 1.43 | -42.50 | 0.64 |
| $TS$ | 1144.06 | 1.87 | 662.09 | 1.26 | 516.60 | 0.31 | -34.63 | 1.81 |
| $G_{total}$ | -6263.14 | 2.97 | -1801.89 | 2.15 | -4453.38 | 1.46 | **-7.87** | 1.92 |

[a] Contribution (SAP-DNA)-contribution (SAP)-contribution (DNA).

[b] kcal/mol. Average over 150 (15 in the case of entropy contributions) snapshots.

[c] Standard error of mean values.

# Conclusions and Future Work

- SAP domain of Ku70 is unlikely to be involved in DSBs end recognition, but shows apparent affinity to DNA minor groove.

- The loops rather than the helices of the SAP domain are more active in binding DNA, and in stable complex, the DNA substrate is bent while the structure of SAP is well preserved.

- The converged stable complex found by implicit solvent method are verified by explicit solvent computation, indicating a reasonably effective way to search the possible binding modes between macromolecules.

- Different sequences of DNA duplex will be investigated to understand the mechanism of pause sites when Ku translocating along DNA.