[P34] Free energy profiles of DNA Pol II interaction with matrix-complimentary/non-complimentary nucleotides – umbrella sampling approach

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The interaction of regular dNTPs with their natural cell targets DNA polymerases (DNA pols) is multistage process including, at least, primary inter recognition dNTP and DNA-bounded enzyme, nucleotide joining to active center and mutual structural adjustment nucleotide and active site. The next result of these processes is ether transition of enzyme complex from open to close form or NTP discrimination from active site. It's supposed the discrimination can be conditioned by structural inconsistency between non-complimentary nucleotide and pol active center, or by another reasons which basis is still unclear. The open-to-close form enzyme transition also has several vagueness in case of non-complimentary dNTP joining. The existent kinetic models aren't completely explanative because in case of their truth any non-complimentary dNTP is a DNA pol inhibitor that obviously doesn't observe in the nature.

It seems to be possible the preliminary dNTP discrimination can occur on stage primary inter recognition NTP and enzyme complex. To verify or refute this assumption, the investigation of free energy changes alone reaction coordinate during dNTP migration from cytoplasm/karyoplasm to active site is required. One of the best approach to such studies is umbrella sampling which lets to sufficiently describe the states of molecular system and accurately evaluate the free energy changes over association/dissociation of molecular complexes.

Free energy profiles were calculated for 4 ternary complexes of *Escherichia coli* DNA PolI II which represents the **high fidelity** DNA polymerase family B. The studied complexes have contained one of four regular deoxynucleotide triphosphates (dCTP, dATP, dTTP and dGTP). The umbrella sampling calculations were performed with GROMACS 5.1 software using Charmm27 force field. At whole, 132 trajectories in 30 ns each were computed. The weighted histogram analysis method was used for PMF extraction and free energy calculation.

It was revealed that interaction of *E.coli* Pol II complex with matrix-complimentary dGTP and non-complimentary dATP and dCTP is characterized by free energy minima which don't' correspond to natural position of incoming nucleotide in enzyme active site. In case of complimentary to matrix dGTP free energy barrier for nucleotide fitting into active center is 2.38 kcal/mol that can be overcome as a result of thermal fluctuations. In contrast, appropriate barriers for dATP and dCTP (7.64 and 6.48 kcal/mol, correspondently) cannot be got over by thermal fluctuations in physiological conditions and, thus, are one of **key factor** making for low level of them mismatched incorporation in grow DNA chain.

The dTTP binding in *E. coli* Pol II active center is barrier-free, but absolute free energy of enzyme complex interaction with dTTP is essentially more than with matrix-complimentary dGTP (-96.29 against - 293.97 kcal/mol), that testifies less probability of dTTP insertion into enzyme active site, but doesn't except this event.

One should mention the free energy of dATP interaction with Pol II complex is positive, and its complete issue from active site over 30 ns is observed. It can testify to the reduced level of G>A mutations in comparison with other ones and against an assumption about greater frequency of transitions in comparison with transversions.