Mechanistic Enzymology of DNA Polymerases in Base-Excision Repair (1996-present). The objective of this proposal is to use enzymological and structural approaches to enhance understanding of the mechanisms of DNA polymerases in the base excision repair (BER) pathway.

DNA Polymerase β (Pol β): Early structural and kinetic studies by many prominent groups suggested that the major factor contributing to the high fidelity of DNA polymerases is a rate-limiting conformational change, induced by binding of correct dNTP, and involving closing of subdomains. We first set out to test this theory by studying mammalian DNA polymerase β (Pol β), which functions in base excision repair (BER). Our goal is to measure all "physical steps" of the enzyme – binding of DNA, dNTP, two Mg^{2+} ions, and associated changes in conformation – and examine how each specific step is affected by mutation of a specific residue. We obtained results that could have broad impact to the entire field of DNA polymerases. For example, we have, for the first time, dissected the catalytic roles of the two metal ions during the catalytic cycle, by trapping the intermediates and characterizing their structures by stopped-flow and X-ray crystallography (collaboration with M. Chan). Furthermore, our results indicate that, in contrast to the "established theory" noted above, there is no rate-limiting conformational change in the catalysis by Pol β. This is a major development in the field and our theory is being tested for other polymerases by many other labs. The work has been featured in a news article in *Chemical & Engineering News* (May 13, 2002). Our further kinetic analyses led us to propose a unified kinetic mechanism applicable to multiple DNA polymerases, which sets a platform for comparative analyses of various mutants and substrate analogs including mismatch incorporations.

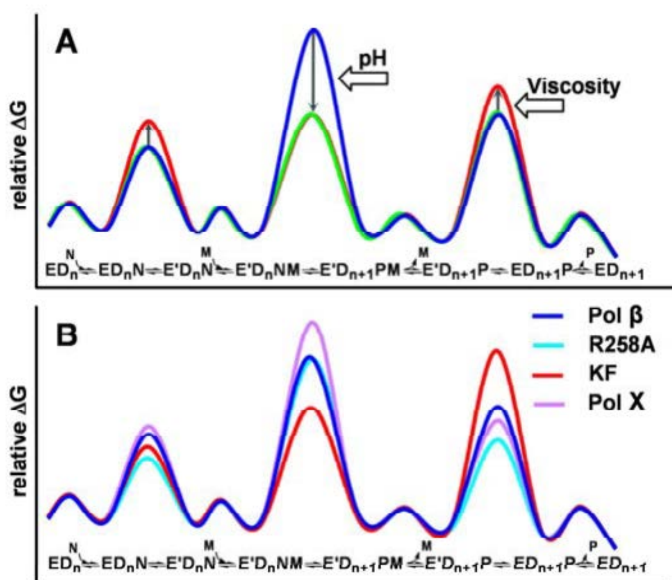

Figure. Qualitative free energy diagram for correct single-nucleotide incorporation. *A*, The blue curve represents neutral pH, where chemistry is the rate-determining step. The green curve represents high pH, where chemistry is selectively facilitated. The red curve represents high pH and high viscosity, where chemistry is selectively facilitated and conformational steps are selectively retarded. *B*, Comparison of Pol β WT, R258A mutant, KF, and Pol X. **E** = DNA polymerase in open conformation; **E'** = closed conformation; $\mathbf{D_n}$ = DNA; **N** = MgdNTP; **M** = catalytic Mg^{2+}; **P** = $MgPP_i$. (Taken from Bakhtina et al., 200

DNA Polymerase X (Pol X): Pol X from African swine fever virus (ASFV) was first studied due to its small size – it lacks the thumb subdomain required for DNA binding in all other polymerases. To our total surprise, our kinetic results indicate that it catalyzes G:G mispair formation with comparable efficiency to that of all four Watson-Crick base pairs. On the basis of such properties, we postulated that Pol X as a strategic DNA mutase, promoting viral hypervariability via low-fidelity synthesis. This work, published in *JACS* in 2001 (entitled "A DNA Polymerase with Specificity to Five Base Pairs"), has been featured prominently by *Chemical & Engineering News* (March 12, 2001). We then solved the first structure of Pol X by NMR. The structure is unique within the realm of nucleotide polymerases, consisting of only palm and fingers subdomains. Despite the absence of a thumb subdomain that is important for DNA binding in other polymerases, we showed that Pol X binds DNA with very high affinity. Further structural analyses suggest a novel mode of DNA binding that may contribute to low-fidelity synthesis. This work has been published in *Nature Structural Biology* and featured in *News and Views*. Subsequently we have solved the structure of the Pol X complexed with DNA in the presence of incorrect dNTP, which reveal the structural basis of the G:G specificity for Pol X. In another front, we recently showed that Pol X also possesses the properties of legion-bypass.

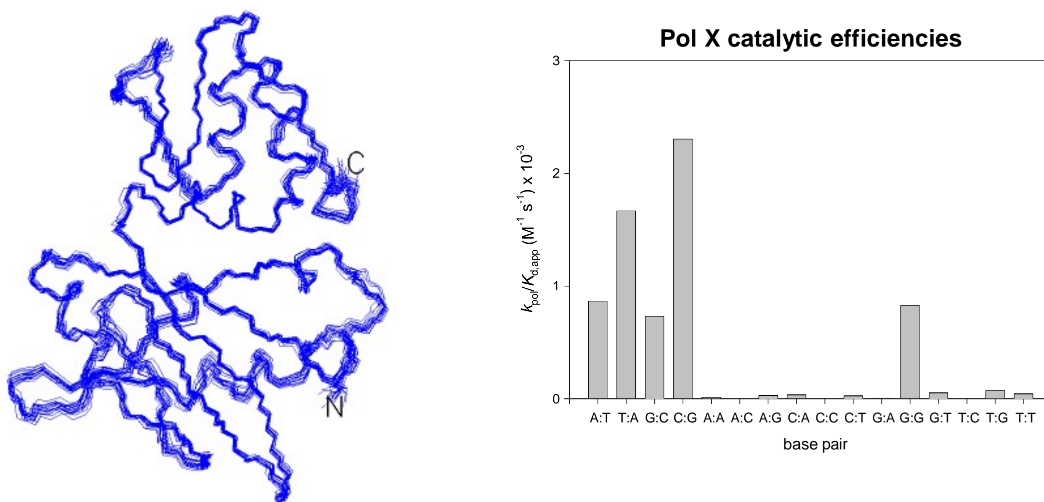

Figure: Structure of DNA polymerase X from African Swine Fever virus [*Nature Struc. Biol. 8,* 942-946 (2001)], which are specific to G:G mismatch in addition to the four Watson-Crick base pairs [*J. Am. Chem. Soc. 123*, 1776-1777 (2001)].

In a related paper, we used gene shuffling to create new hybrid polymerases between Pol β and Pol X, featured in the cover of JMB (publication #160). This work was features on the cover of JMB.

In another related direction, since the incorporation of dNTP into DNA by DNA polymerases requires sealing of the nick by DNA ligase, we asked whether there may exist high-fidelity and error-prone DNA ligases. We performed detailed pre-steady-state and steady-state kinetic and mechanistic analyses of ASFV DNA lingase and

human DNA ligases I and IV (also in the presence of its partner protein XRCC4). The results indicate that the fidelities of ligases fall into a very narrow range. The enzymes examined show different specificity toward different base pairs, but there is no clear evidence for two types of ligases – low and high fidelity. Publication: #188, 202.

In the most recent development, we have employed small angle X-ray scattering (SAXS) to study the solution conformational states of Pol β and Pol X. The work led to identification and structural modeling of a 2:1 enzyme:DNA complex, and a hypothesis that it represents the functional form of Pol β in solution. In addition, our results indicate that the mismatched ternary complex lies in-between the open and the closed forms, but more closely resembles the open form.

Current and Future Directions: Having established the kinetic mechanism of correct dNTP incorporation, the main thrust is to elucidate that of incorrect dNTP incorporation – since the fidelity is based on the comparison between correct dNTP and incorrect dNTP incorporations. We will continue to use kinetic and structural approaches to move toward this goal, and significant progresses have already been made.

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