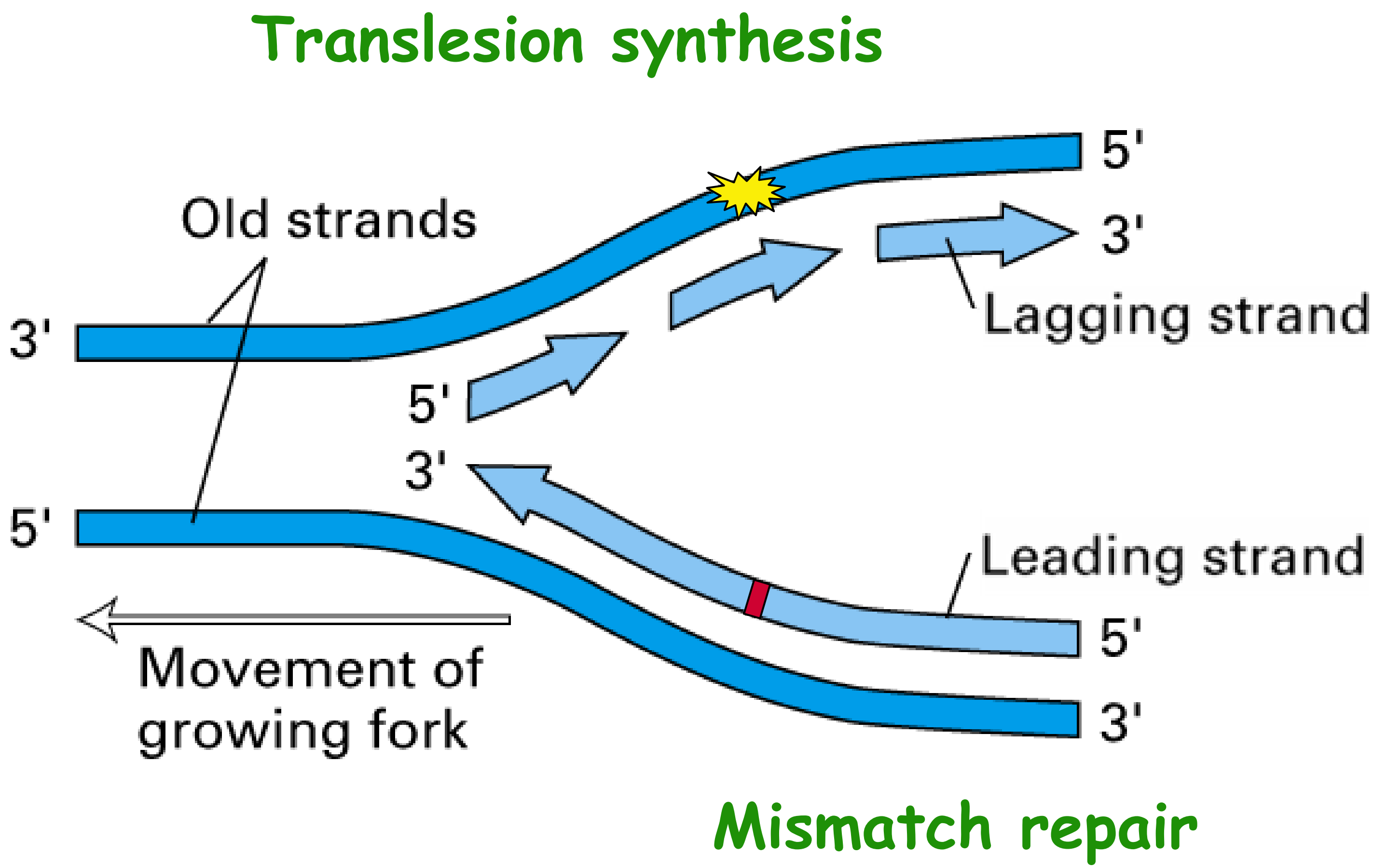
DNA Repair in 3D



Wei Yang, NIDDK, NIH

DNA Polymerases

family	examples	error rate	function
A	Pol I, T7, Taq	10^{-5} to 10^{-6}	replication
B	Pol II, RB69, PolB, Pol α, δ, ε (cat. subunit)	10^{-5} to 10^{-6}	replication
C	Pol III α subunit	10^{-5} to 10^{-6}	replication
D	Archaeal PolD	10^{-5} to 10^{-6}	replication ?
X	Pol β, Pol μ, TdT	10^{-4} to 10^{-5}	repair, Ig, TCR
Y	DinB, UmuCD', Dpo4, Dbh, Pol η, ι, κ	10^{-2} to 10^{-4}	mutagenic, lesion bypass

Bacteria,* *Archaea,* *Eukarya

An Updated Phylogeny of Bacterial and Archaeal Y-Family Polymerases

	UmuC	DinB	Outlyers
# of proteins	81	97	2
Mobile?	22	3	–
Gram Positive	40	52	–
Gram Negative	41	36	1
Archaea	–	9	1
UmuD present?	41	–	–

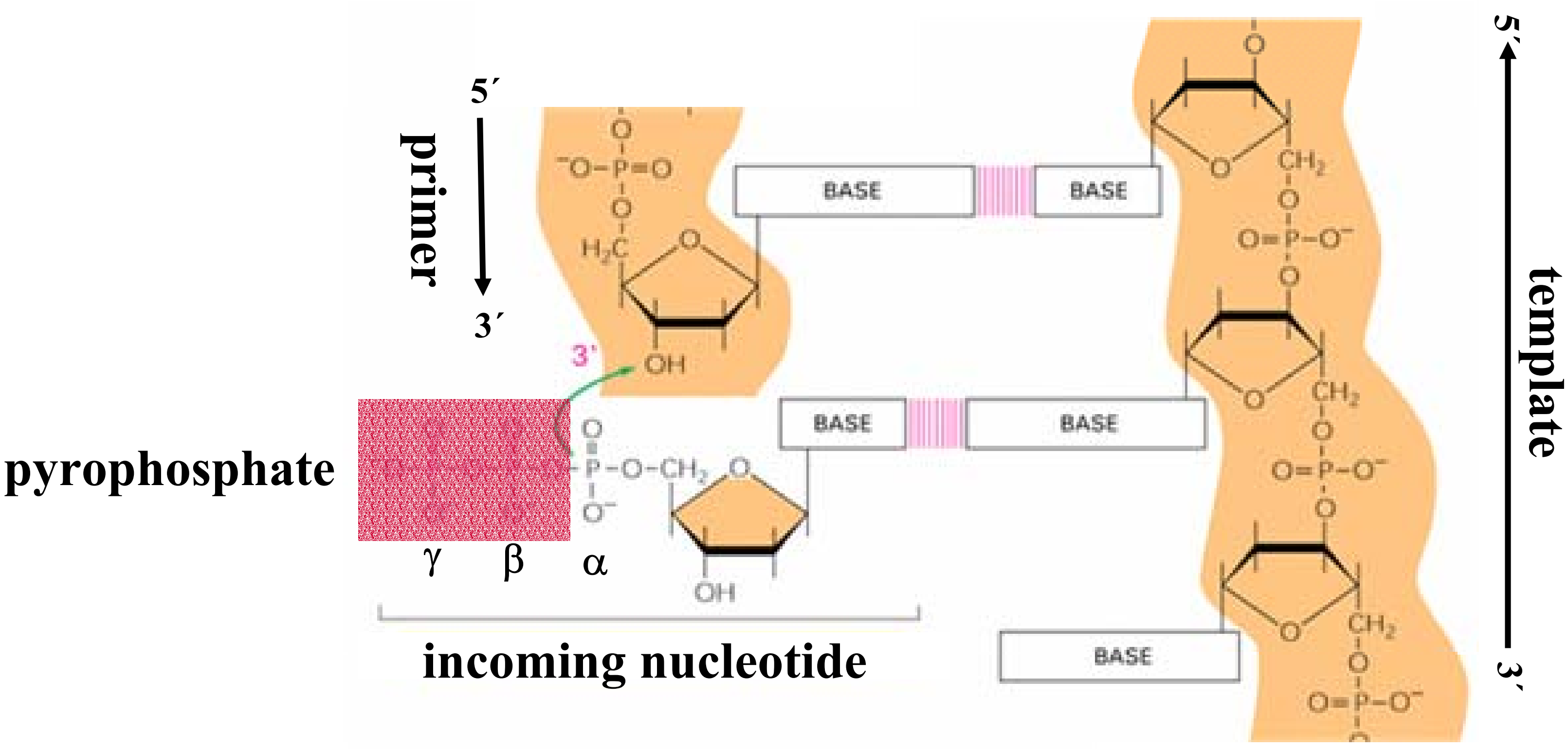
Species	Polη	Polι	Polκ	Rev1
Homo sapiens	+	+	+	+
Rattus norvegicus	+	+	+	+
Mus musculus	+	+	+	+
Bos taurus	+	+	?	+
Gallus gallus	+	–	+	+
Fugu rubripes	+	+	+	+
Drosophila melanogaster	+	+	+	+
Anopheles gambiae	+	+	+	+
Arabidopsis thaliana	+	–	+	+
Oryza sativa	+	–	+	+
Caenorhabditis elegans	+	–	+	+
Caenorhabditis briggsae	+	–	+	+
Neurospora crassa	+	+	+	+
Magnaporthe grisea	+	+	+	+
Aspergillus nidulans	+	+	+	+
Gibberella zeae	+	+	+	+
Saccharomyces cerevisiae	+	–	–	+
Schizosaccharomyces pombe	+	–	+	+

Defective Pol η
Leads to Xeroderma Pigmentosum

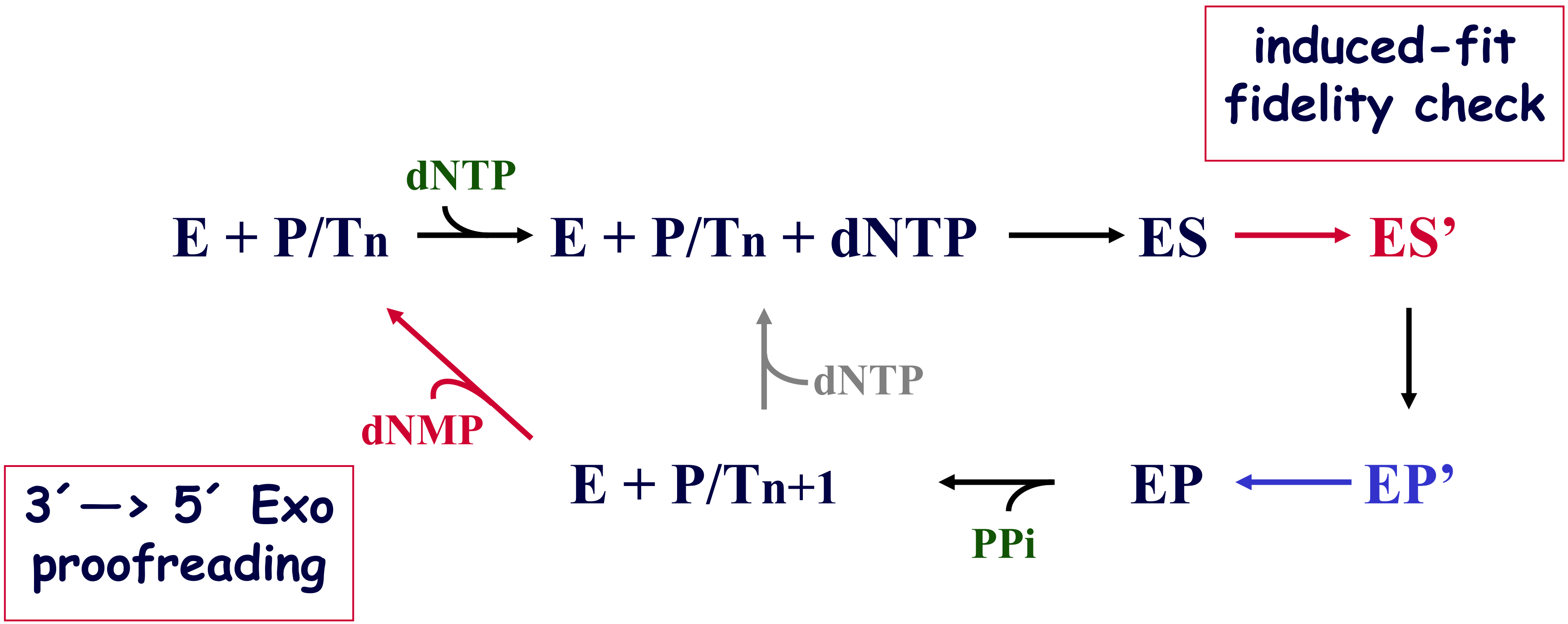
Question

What is the structural basis for low fidelity DNA synthesis by the Y-family polymerases?

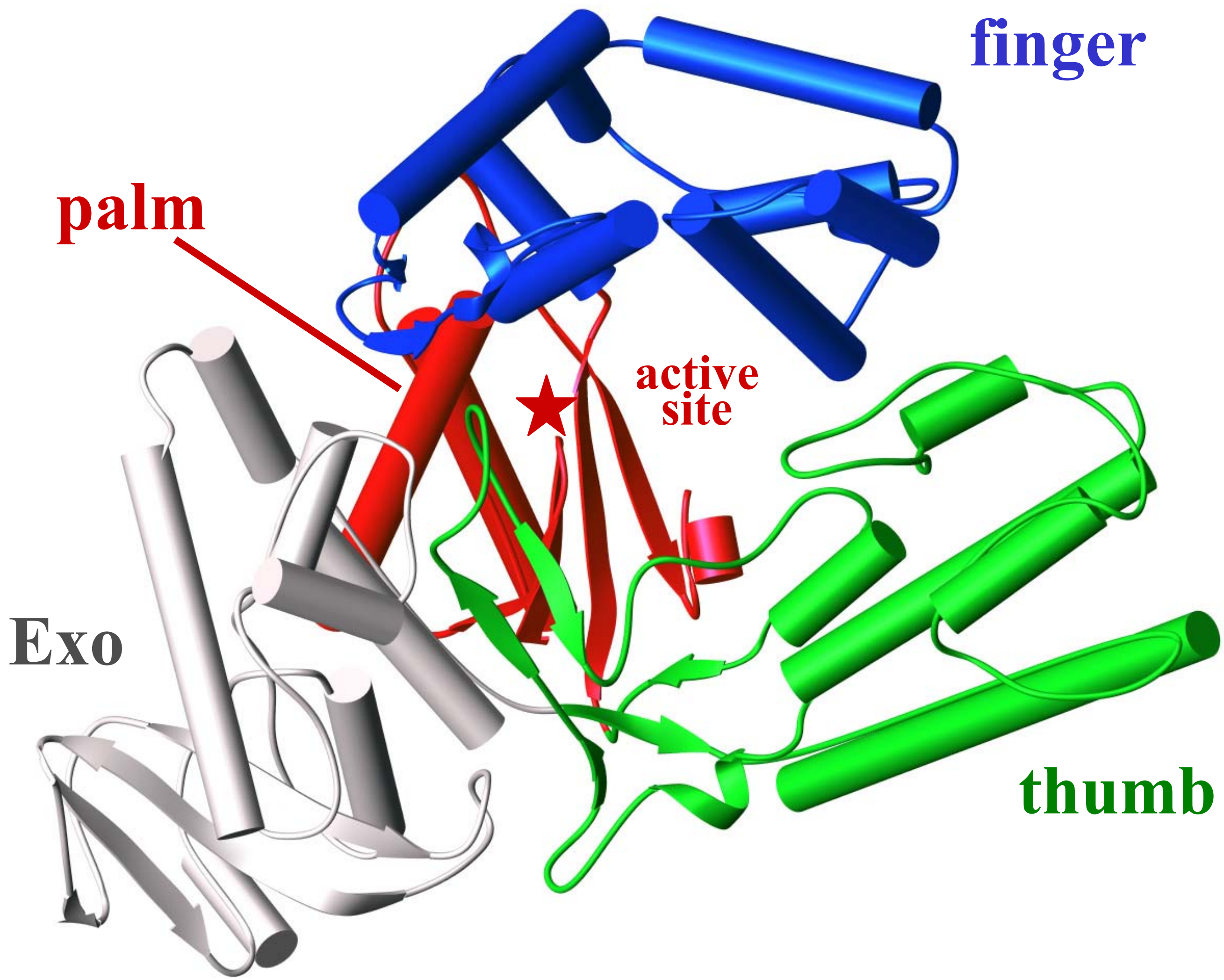
Chemistry of DNA Replication



Fidelity of DNA Replication

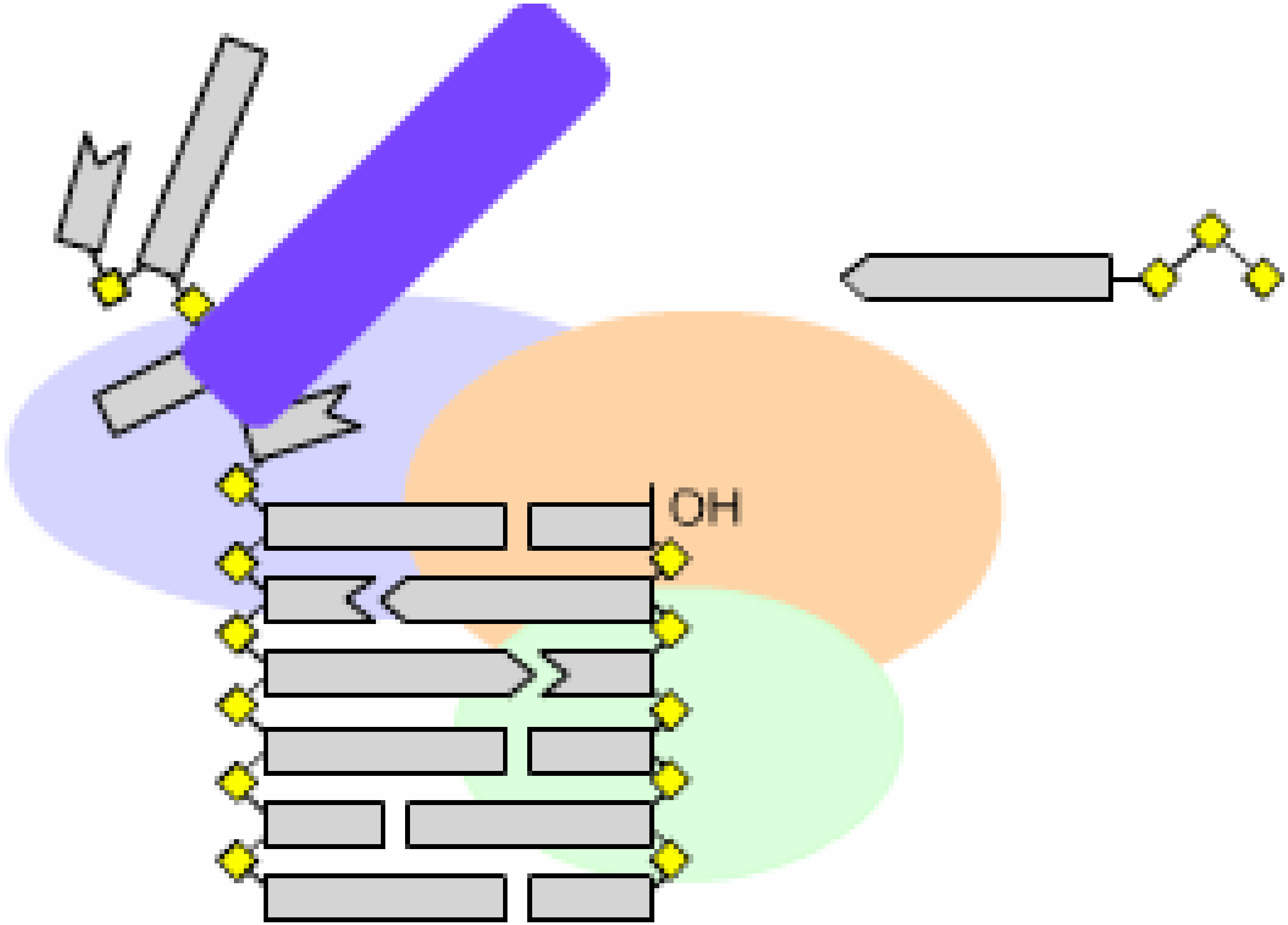


A Classic DNA Polymerase



Klenow, Taq, T7, RT, RB69, etc

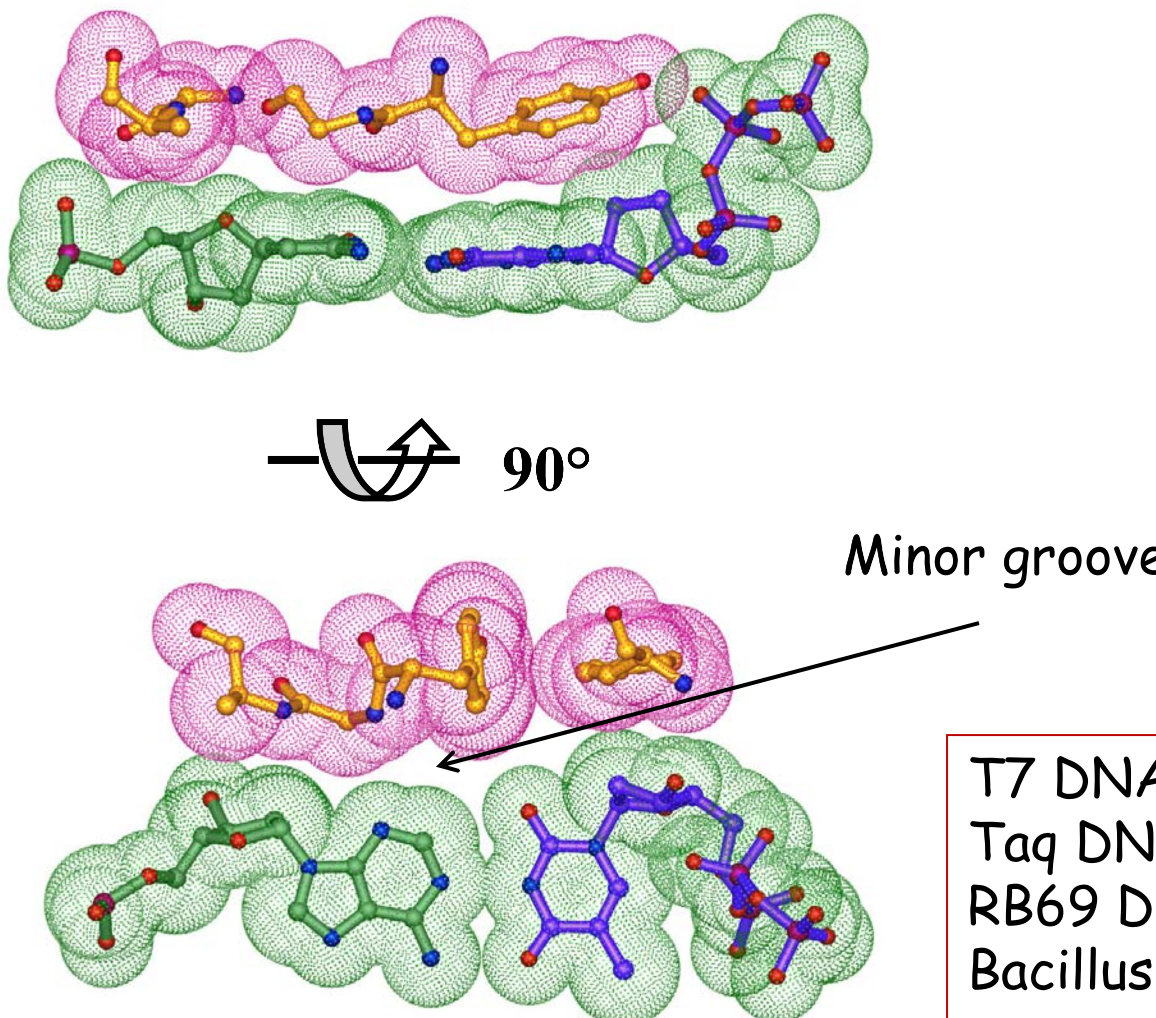
Fidelity Check by Replicative Polymerases



Structural Evidence for Fidelity Check

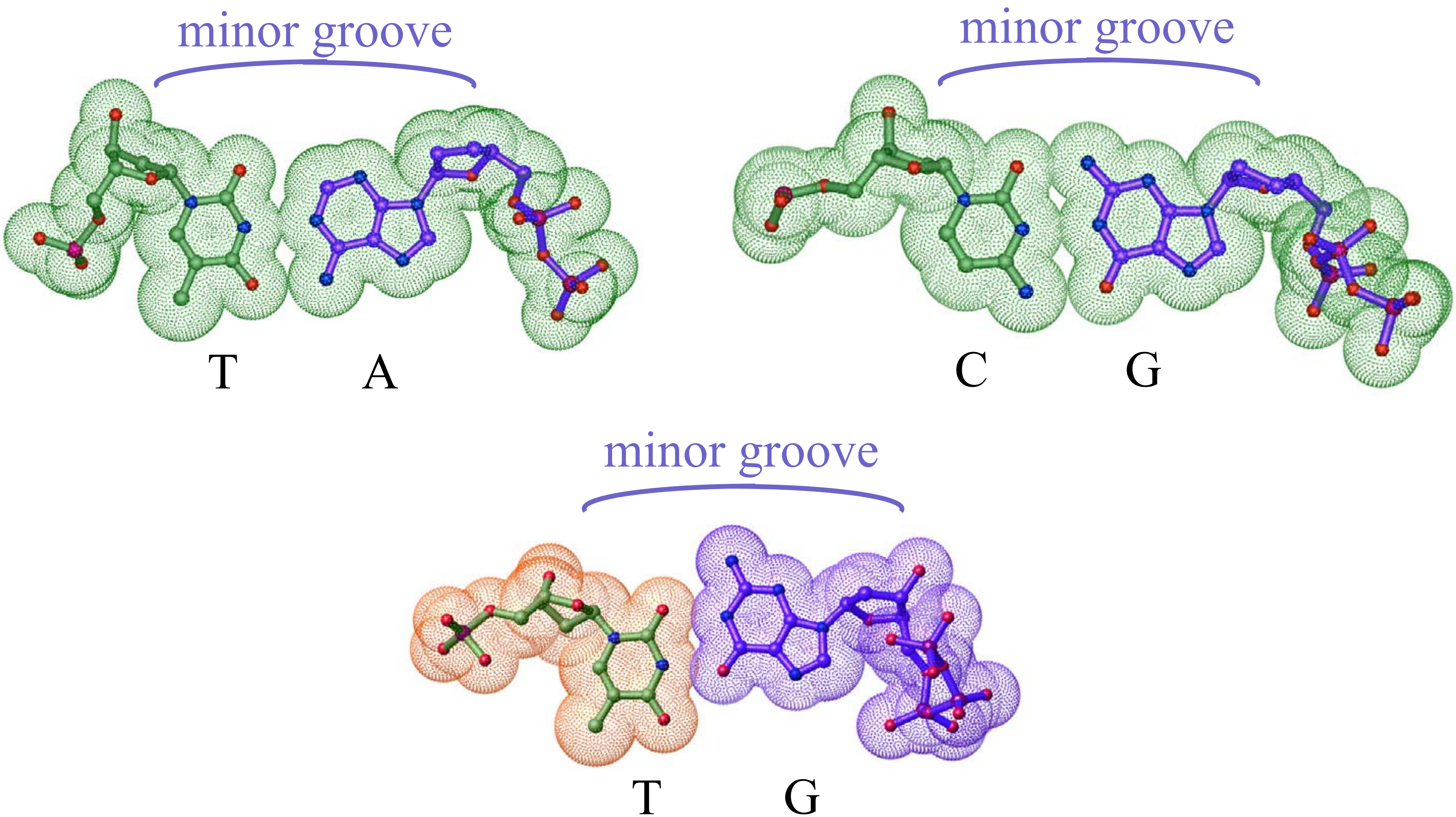
Li et al. Waksman. 1998

Complementary Interface Between A Replicating Base Pair And Polymerase



T7 DNA Pol
Taq DNA Pol
RB69 DNA Pol
Bacillus DNA Pol

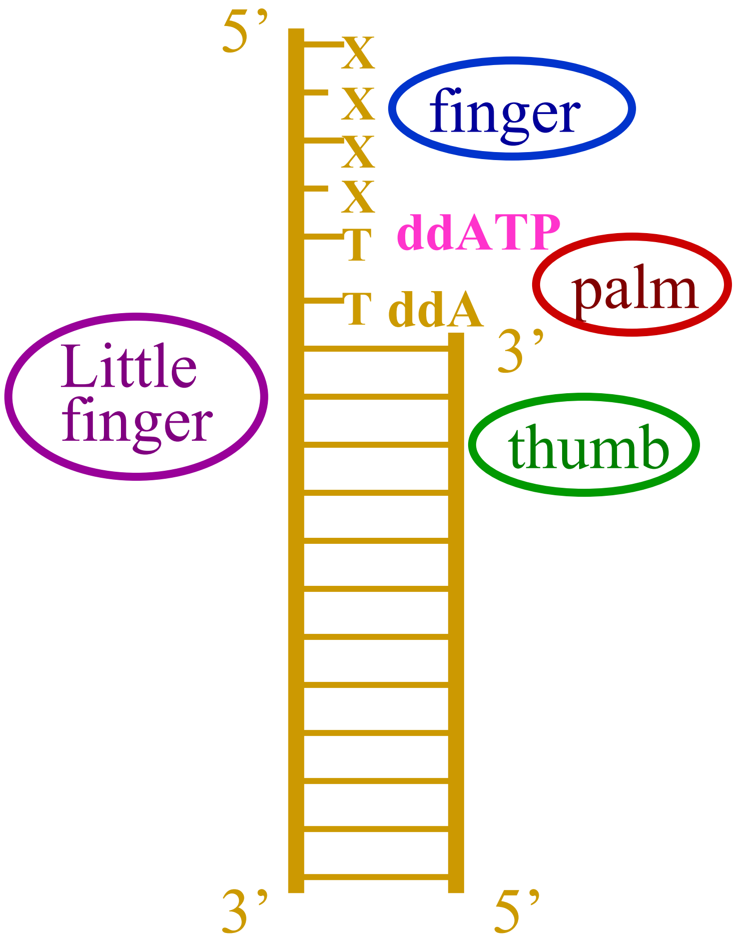
Watson-Crick and Mismatched Base-pairs Differ in The Minor Groove

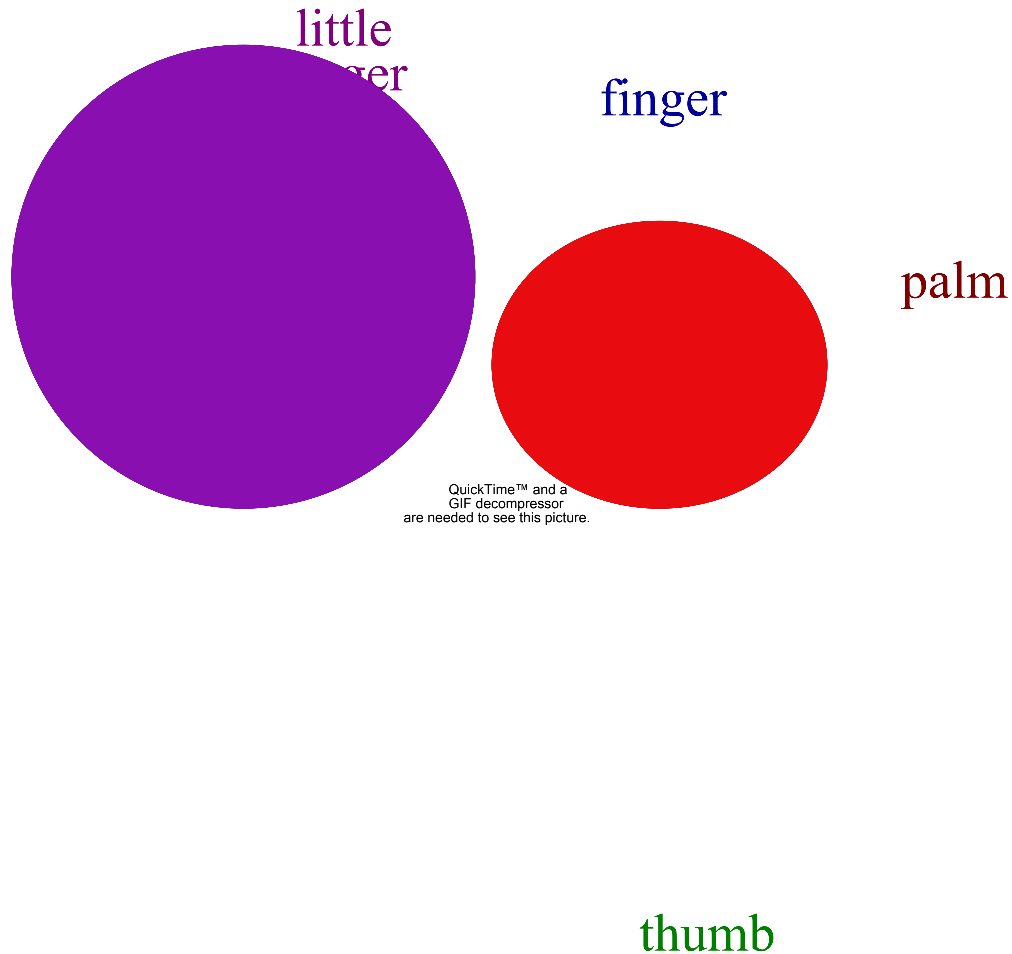


Dpo4 from *Sulfolobus Solfataricus* Is a DinB Homolog

P1 is *Sulfolobus Acidocaldarius*
P2 is *Sulfolobus Solfataricus*

Crystal Structure of Dpo4-Substrate Complex



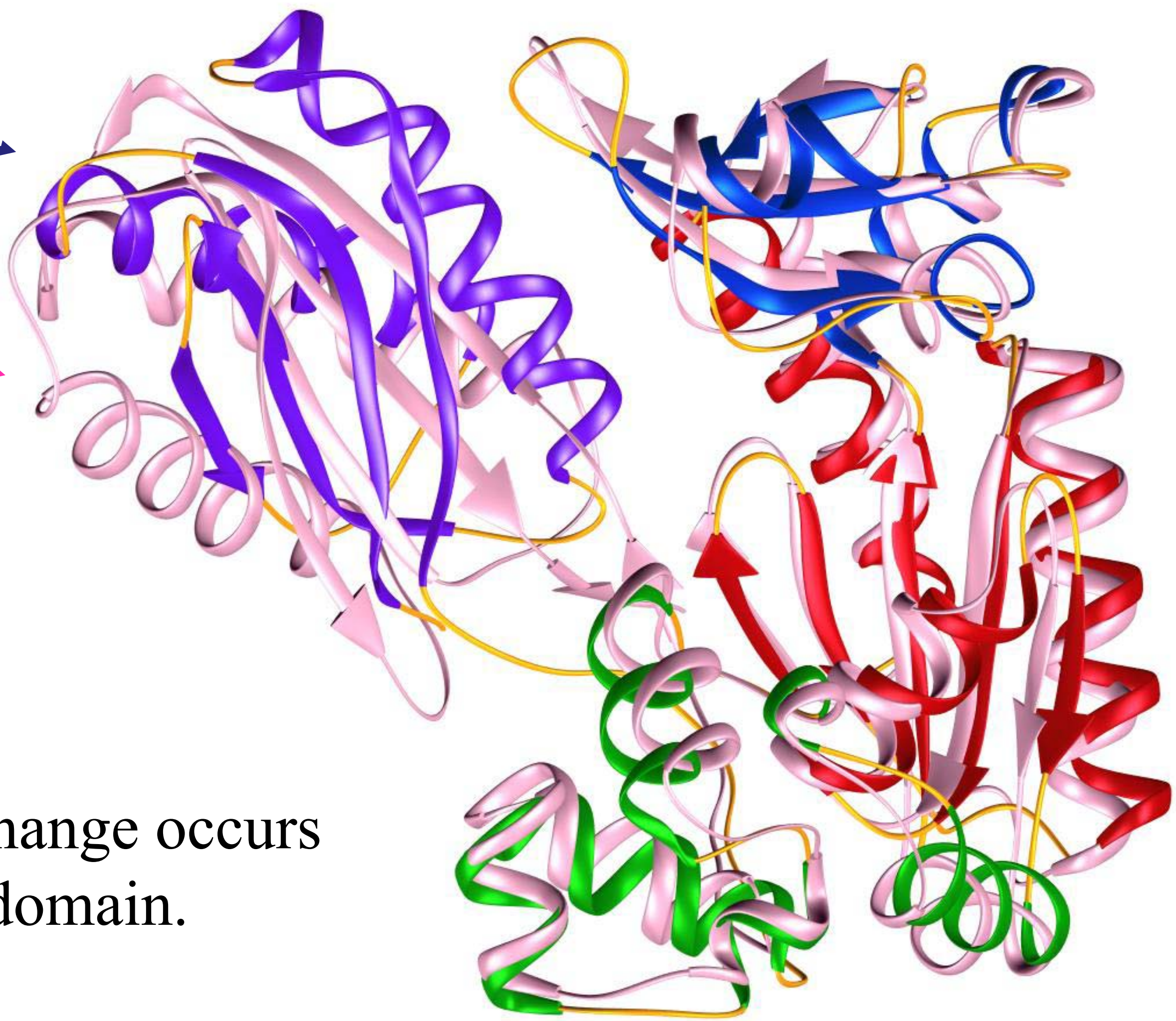


Ling et al., Yang (2001) Cell

The Dpo4 Active Site Is Preformed Without Substrates

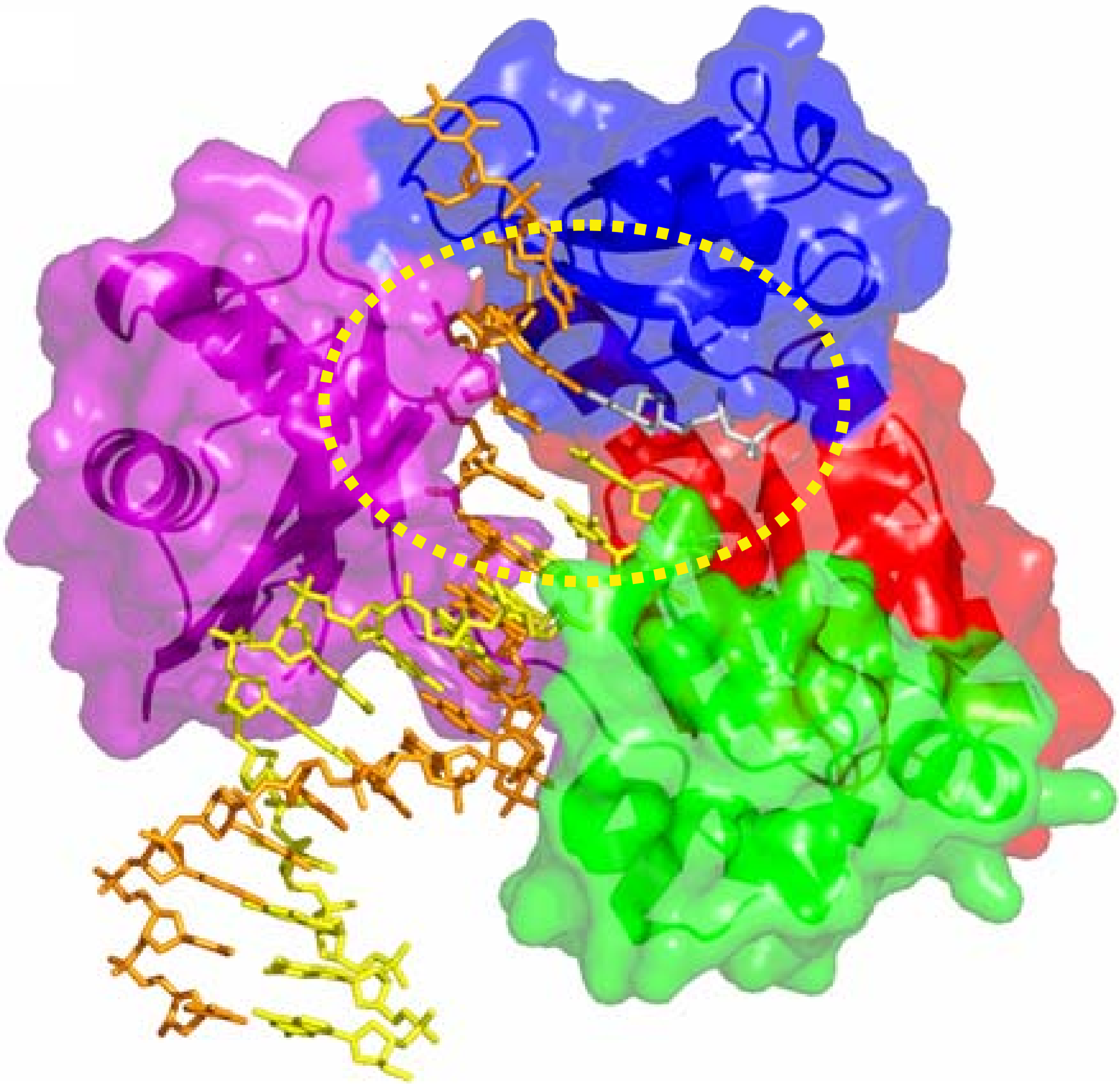
Ternary complex

Binary complex

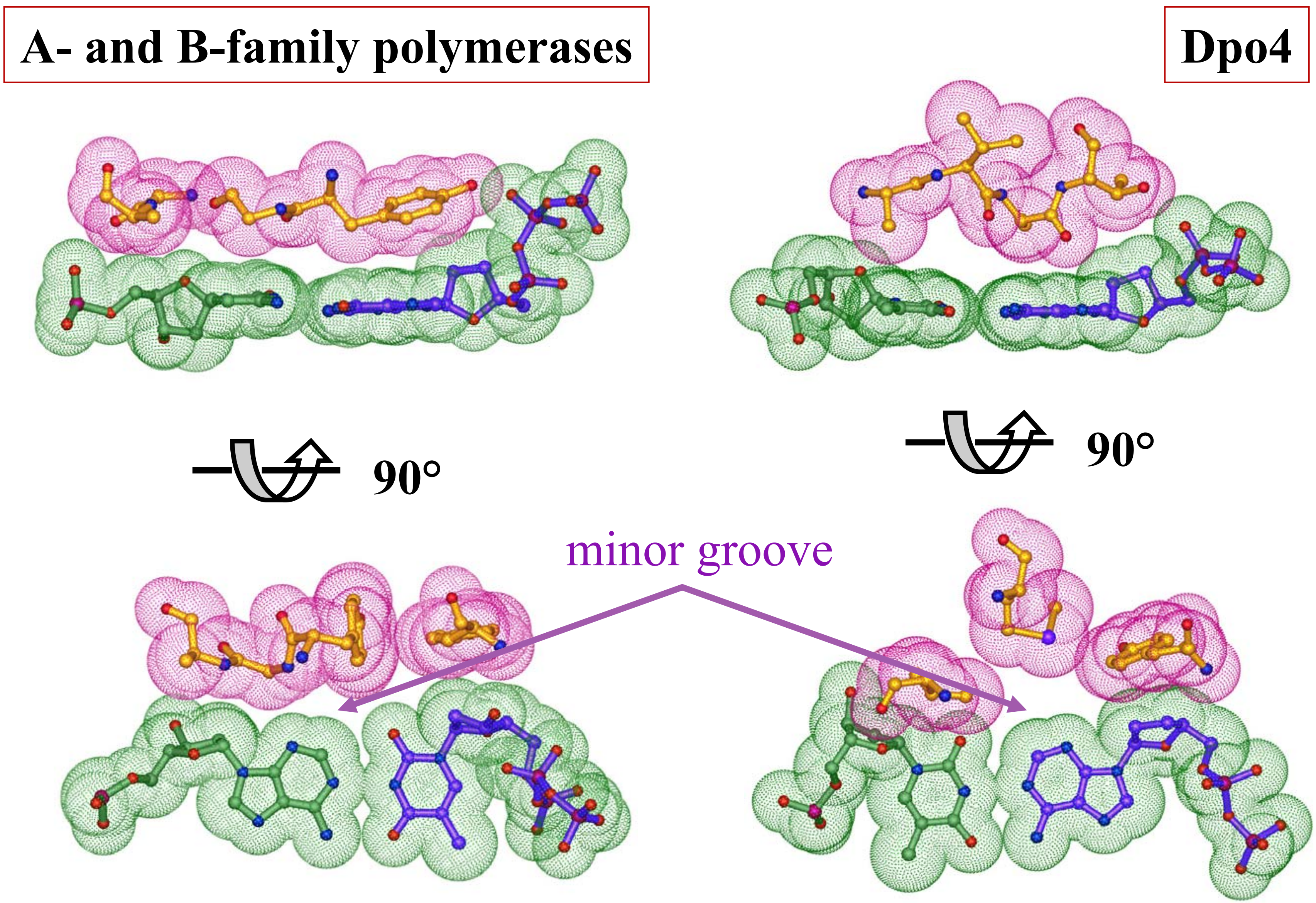


Conformational change occurs mainly in the LF domain.

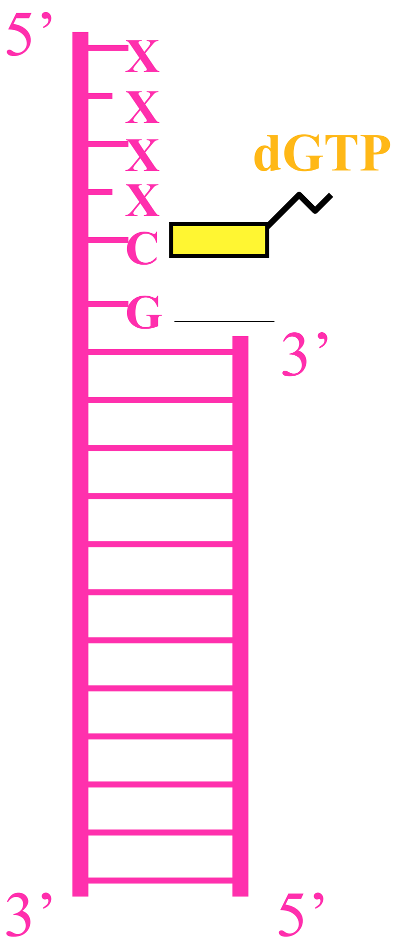
The Dpo4 Active Site Is Open

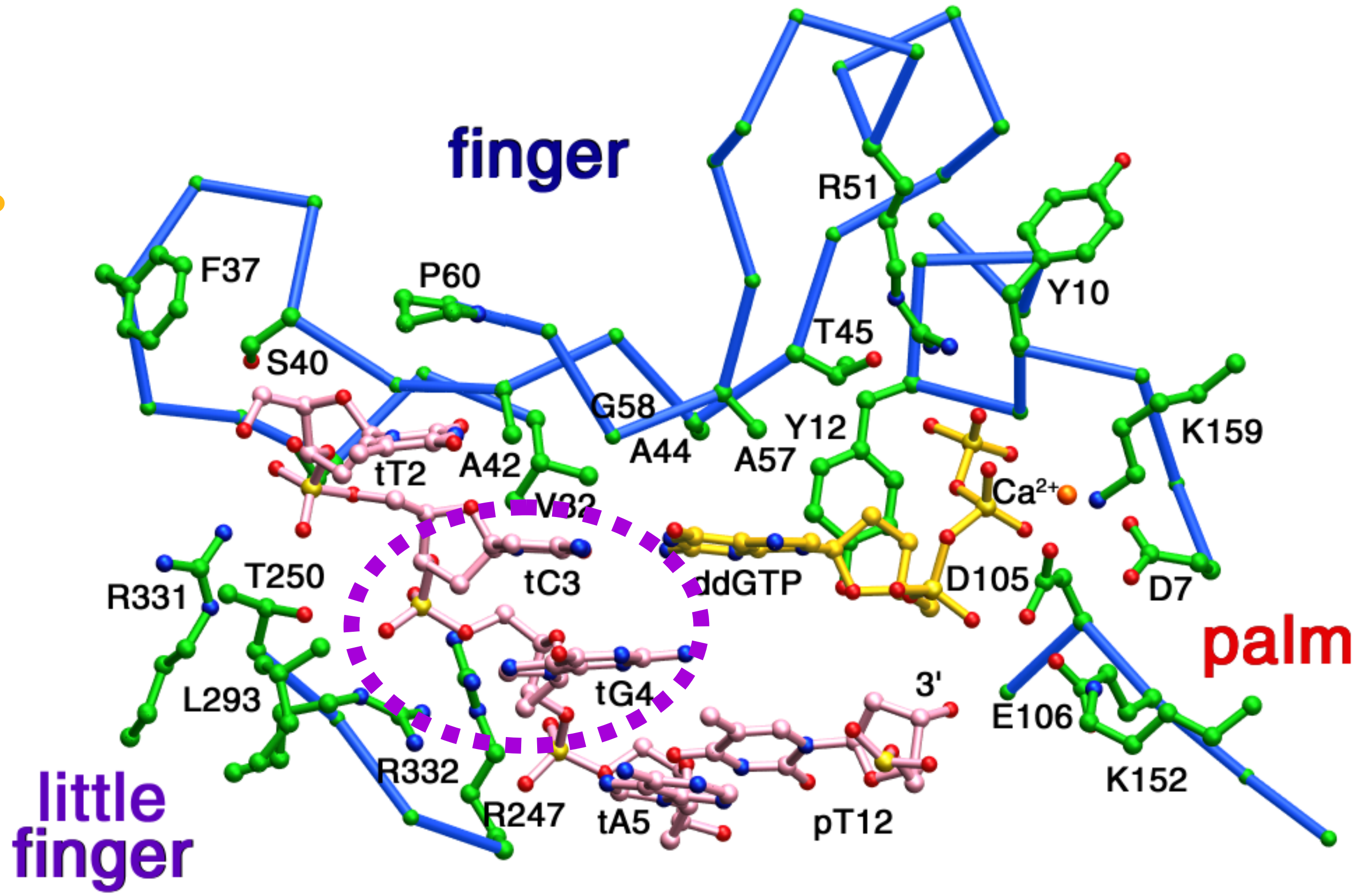


Reduced Stringency of Base Selection by Dpo4



A Frameshift Resulting from Misalignment





Type II Dpo4-DNA crystal

Incorporation of A Mismatched Base Pair By Dpo4

Question

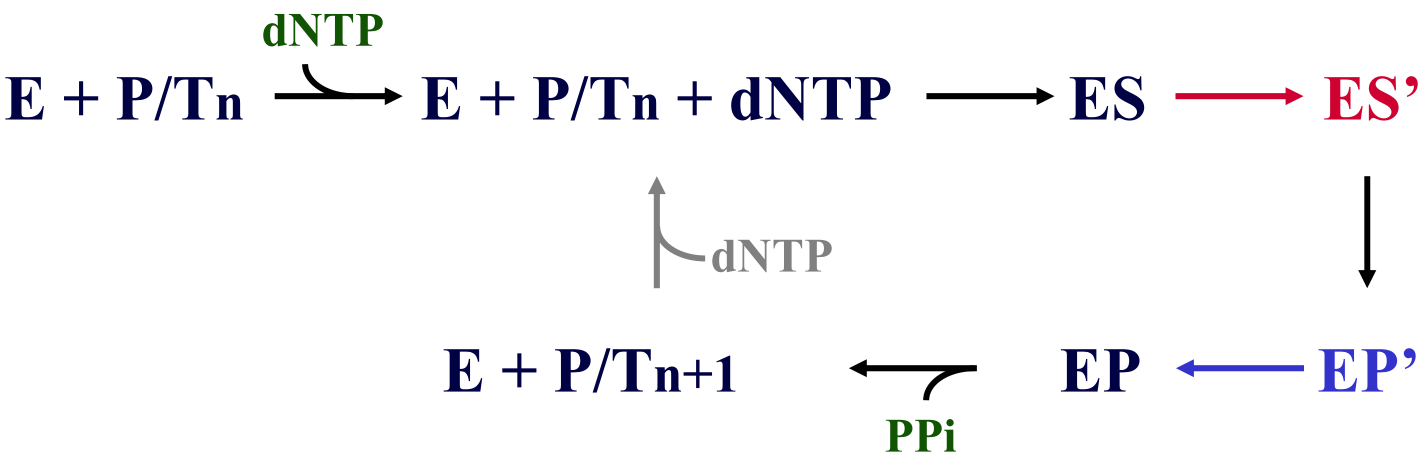
The expected error rate is 10^{-2} if fidelity is solely dependent of base pairing, but the actual error rate of Dpo4 is ~ 10^{-4}. How does Dpo4 discriminate again mismatched base pairs ?

The triphosphate of the incoming nucleotide is misplaced in the mismatch complex

The metal ions are mobile in the Dpo4 ternary complexes

Could Metal Ions Perform Fidelity Check ?

Metal-dependent fidelity check ?



Mg^{2+} Favors Incorporation of Correct dNTP

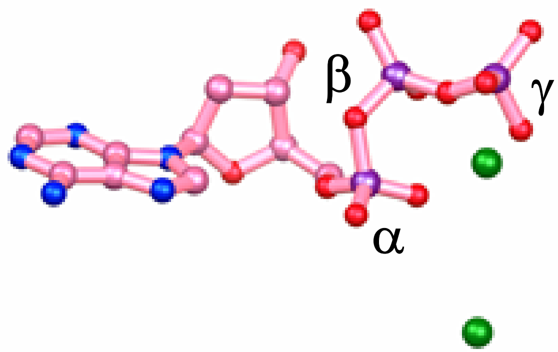
Mn^{2+} Ions Increases Translesion Synthesis by Dpo4

Mg and Mn influence the fidelity of Dpo4 more than T7 as the active site of T7 is better defined than Dpo4

Side Reactions by Dpo4 : dNTP Degradation

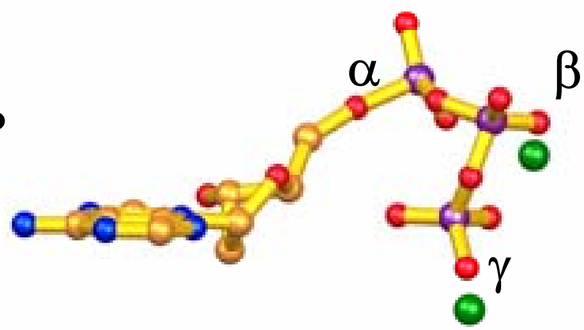
standard

dATP

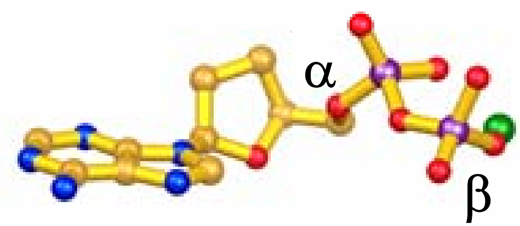


distorted

dATP



distorted & hydrolyzed



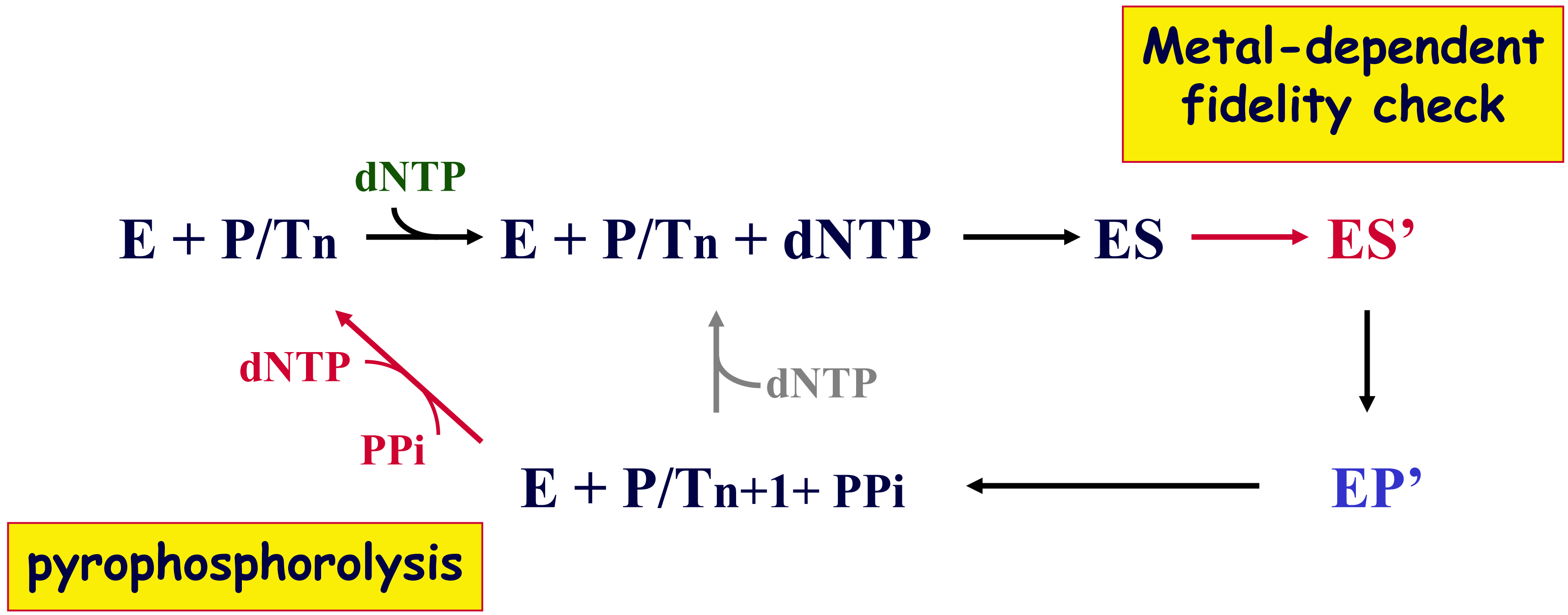
dADP

ddATP —> ddADP + Pi

When misincorporation occurs, Pyrophosphate remains bound to Dpo4

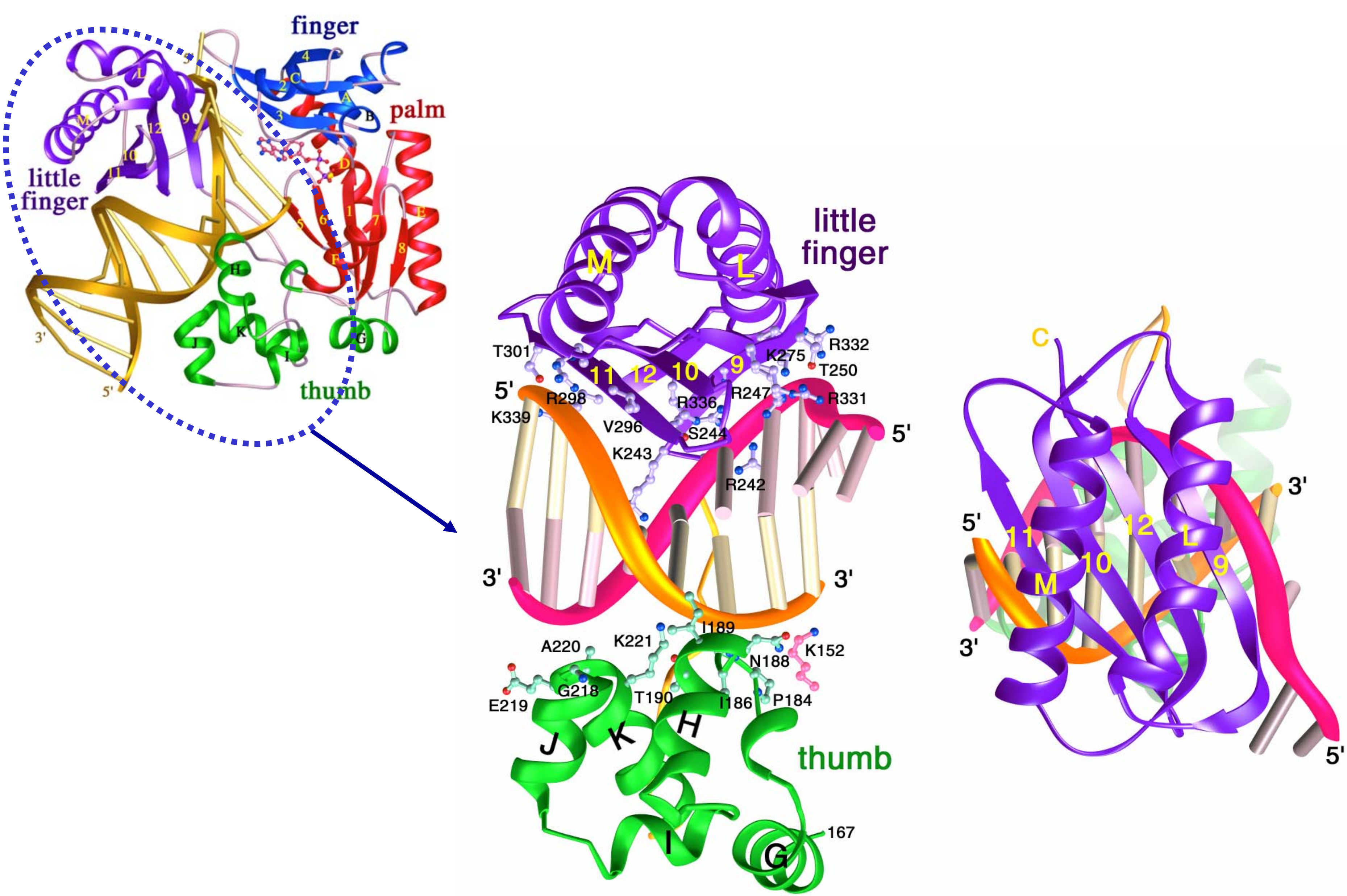
$$\mathrm{d(NMP)n + dNTP} \underset{?}{\rightleftarrows} \mathrm{d(NMP)n{+}_1 + PPi}$$

Could Dpo4 Perform Pyrophosphorolysis ?



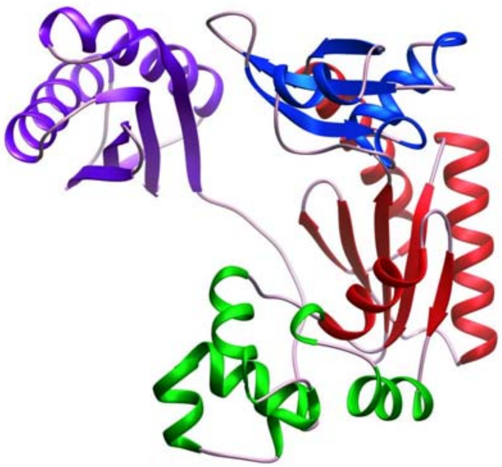
Pyrophosphorolysis Catalyzed by Dpo4

The Little Finger Domain Is Unique in the Y-family

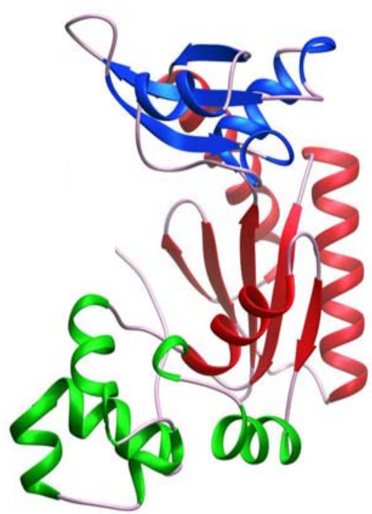


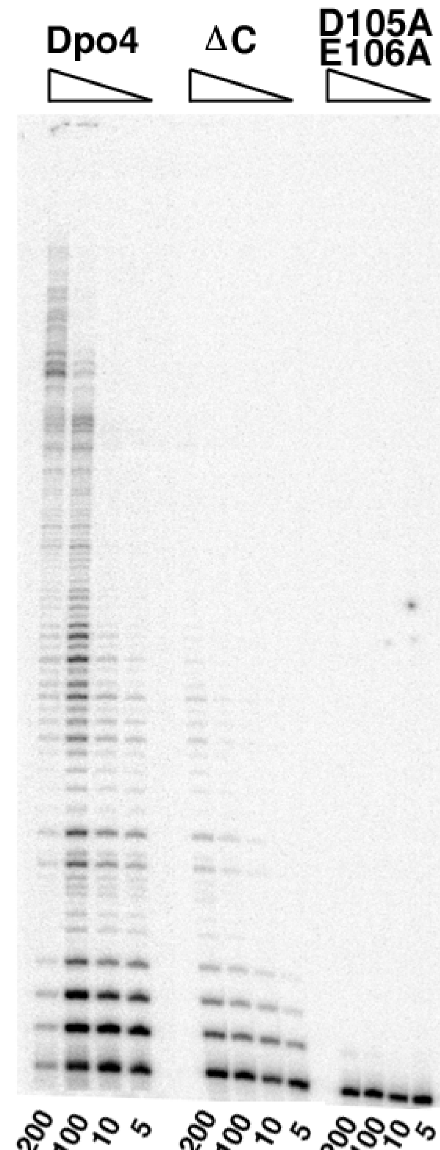
The Little Finger Enhances The Polymerase Activity

Dpo4



ΔC

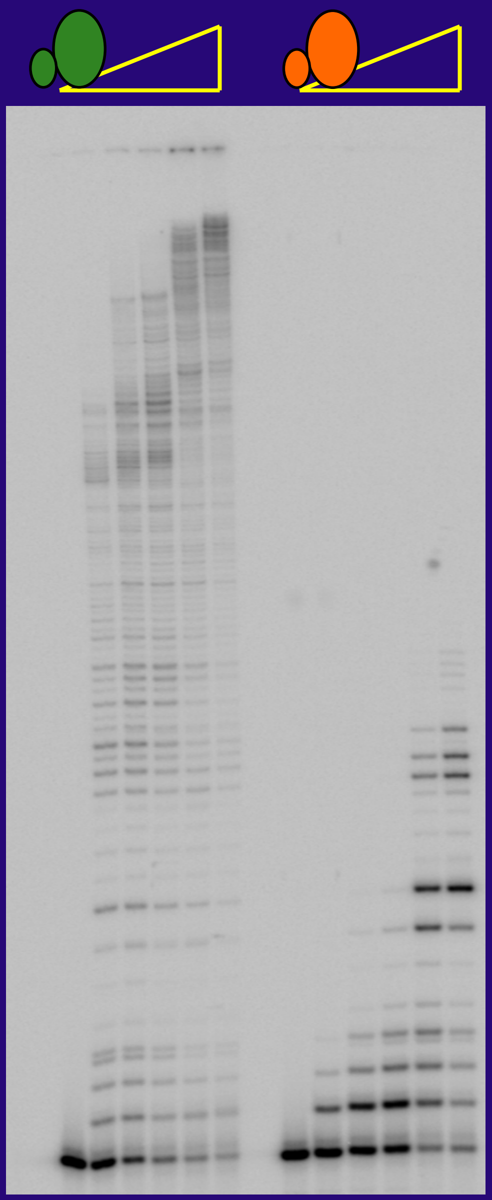




LF Domain Determines the Catalytic Efficiency

Dpo4
(*S. sof*)

Dbh
(*S. aci*)



10 nM M13 DNA,
100 μM dNTPs
0.01-2 μM P1, P2
37°C, 5 min

LF Domain Also Determines Pyrophosphorolysis Efficiency

Variations of Pyrophosphorolysis

1. <u>Pyrophosphate exchange</u> (Klenow fragment by A. Kornberg)

   dNTP + dXMP —> dNMP + dXTP

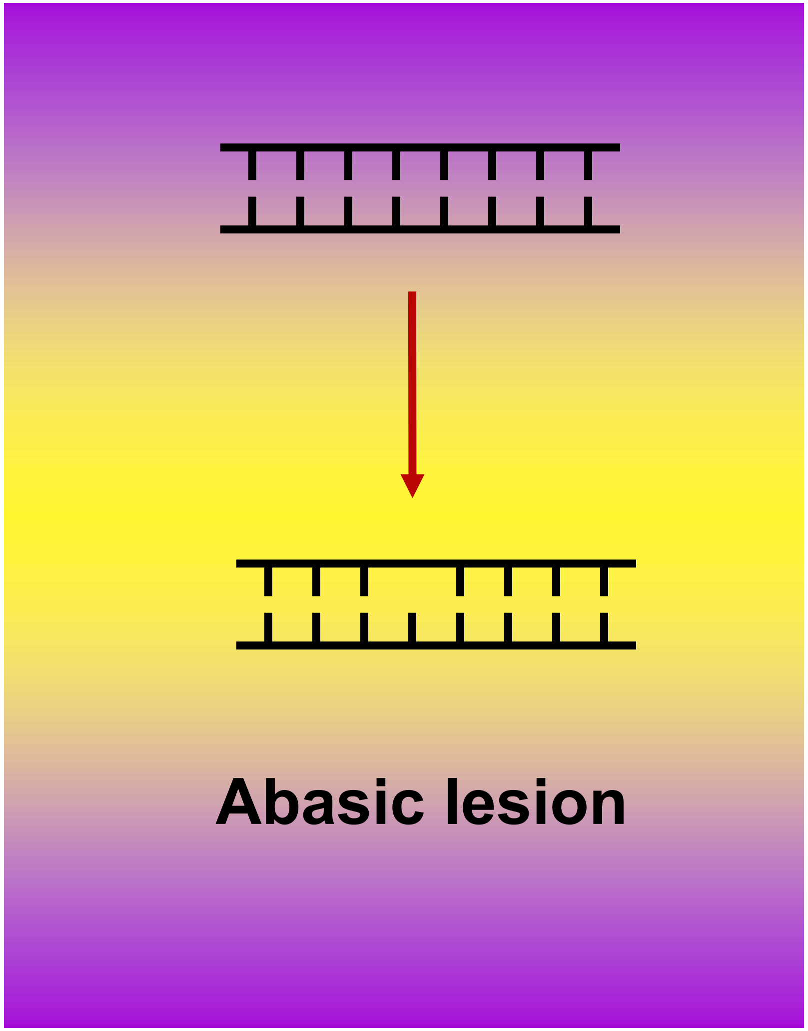
2. <u>NTP-dependent Pyrophosphorlysis by RT</u> (M. Parniak & W. Scott)

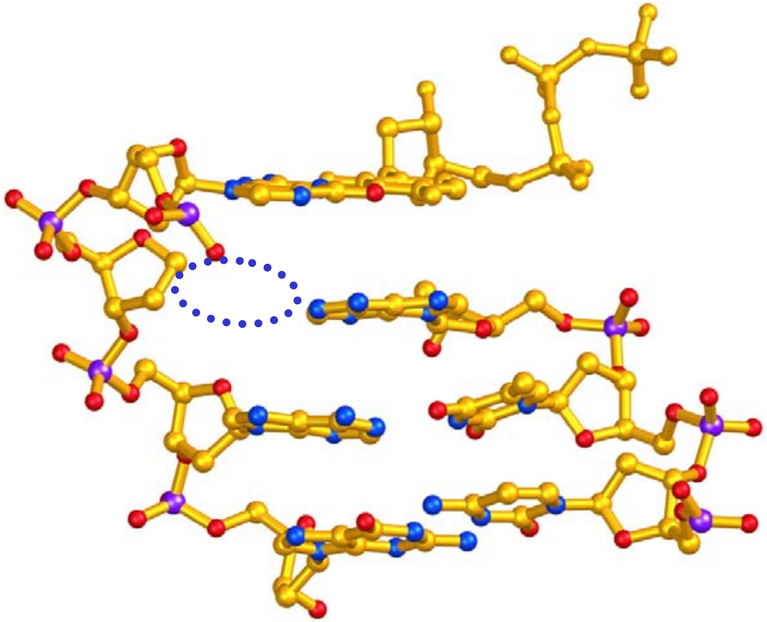
   T/P-**AZT** + NTP (or PPi) —> T/P + NTP-**AZT**

Question

What is the structural basis for translesion DNA synthesis by the Y-family polymerases?

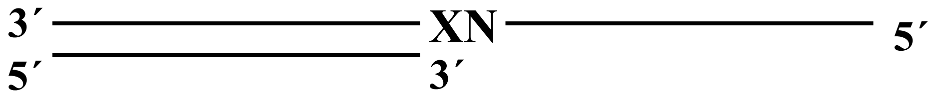
Spontaneous Loss of Bases



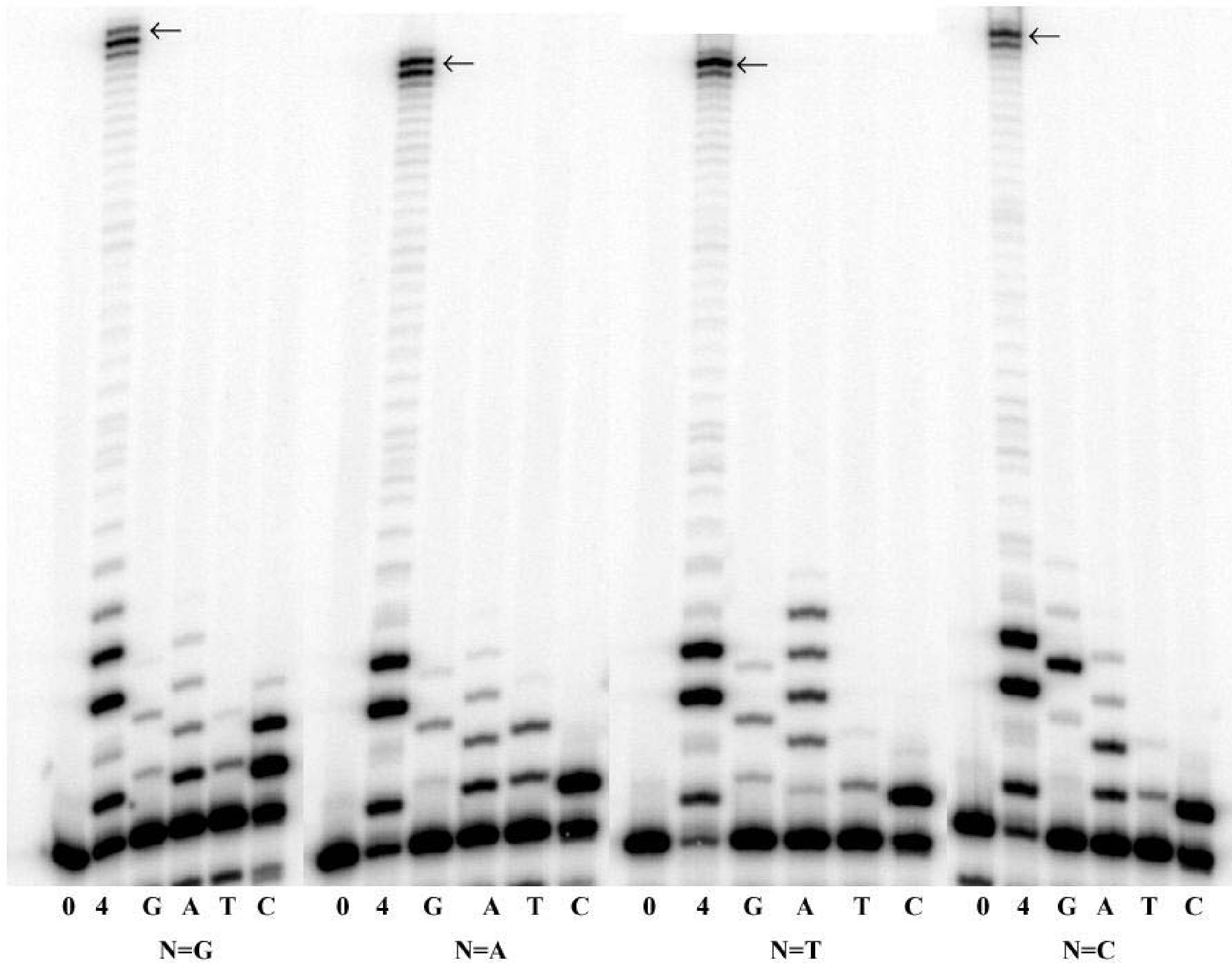


9000 per cell per day !

Dpo4 Efficiently Bypasses Abasic Lesions



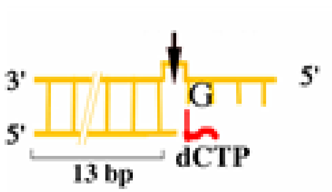
X = abasic site
N = G, A, T or C

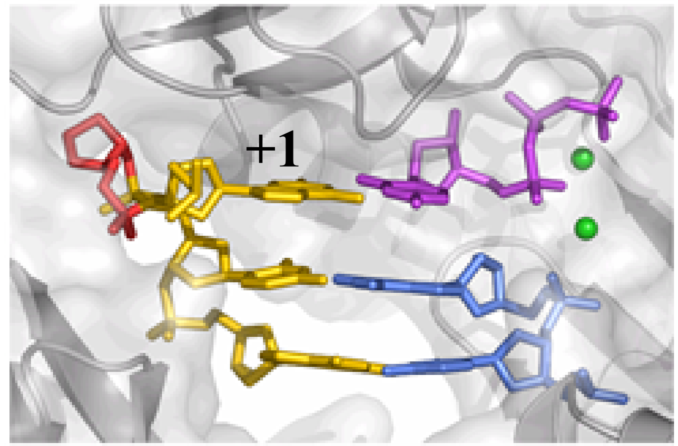


5' ATGATTAAGACTCCTTATT
3'-TTCTTGACCGTACTAATTCTGAGGAATAAXNCTTCATACAATCGTTTGCATCTTTTATGT

Dpo4 Loops out Abasic Lesions and Realigns Template

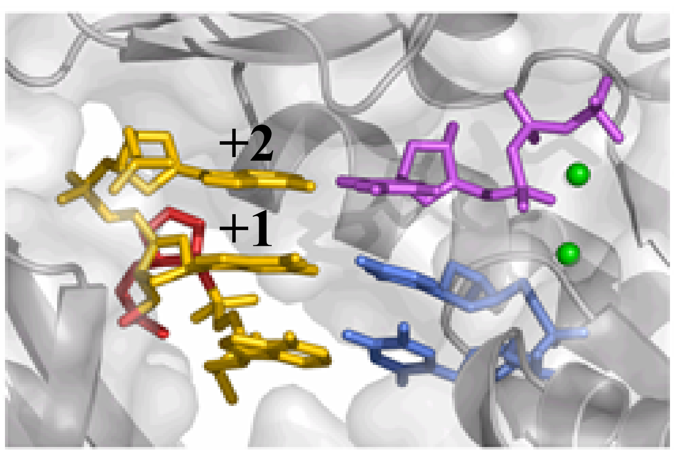
Step 1:
looping out the abasic lesion



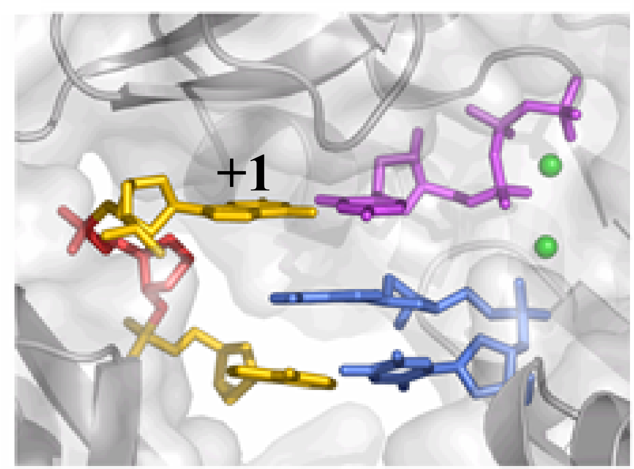


Step 2: **keeping the abasic site out**

realigning the template



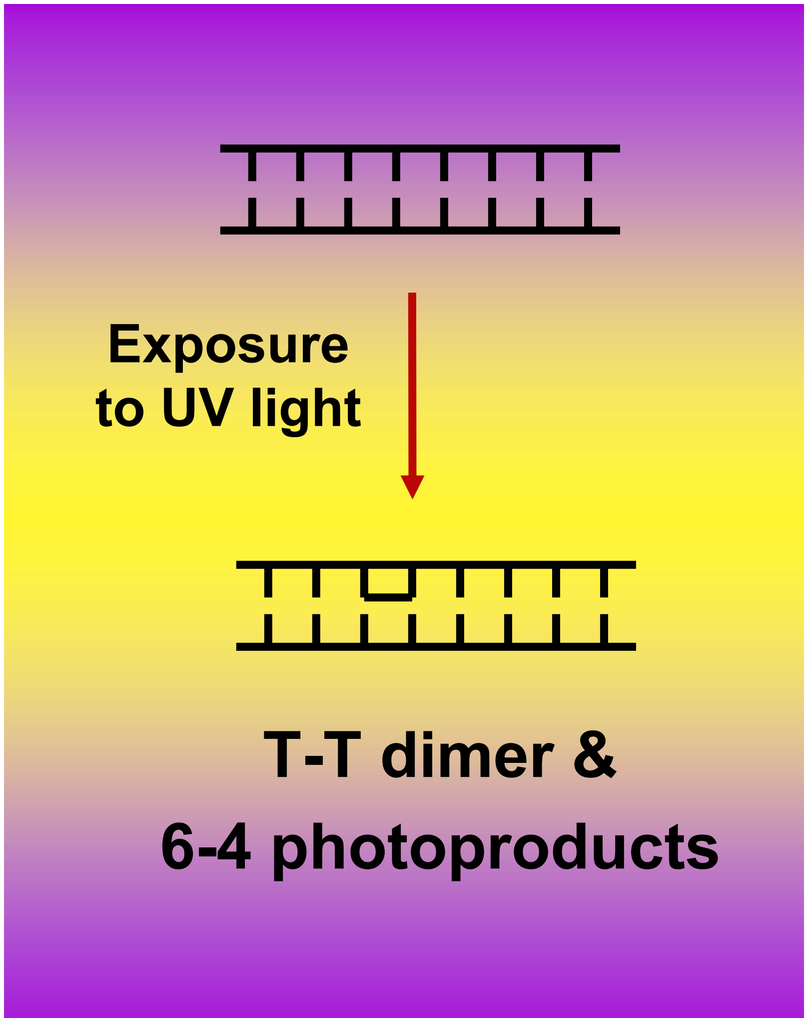
or



-1 frameshift

base substitution

UV-Induced Damage

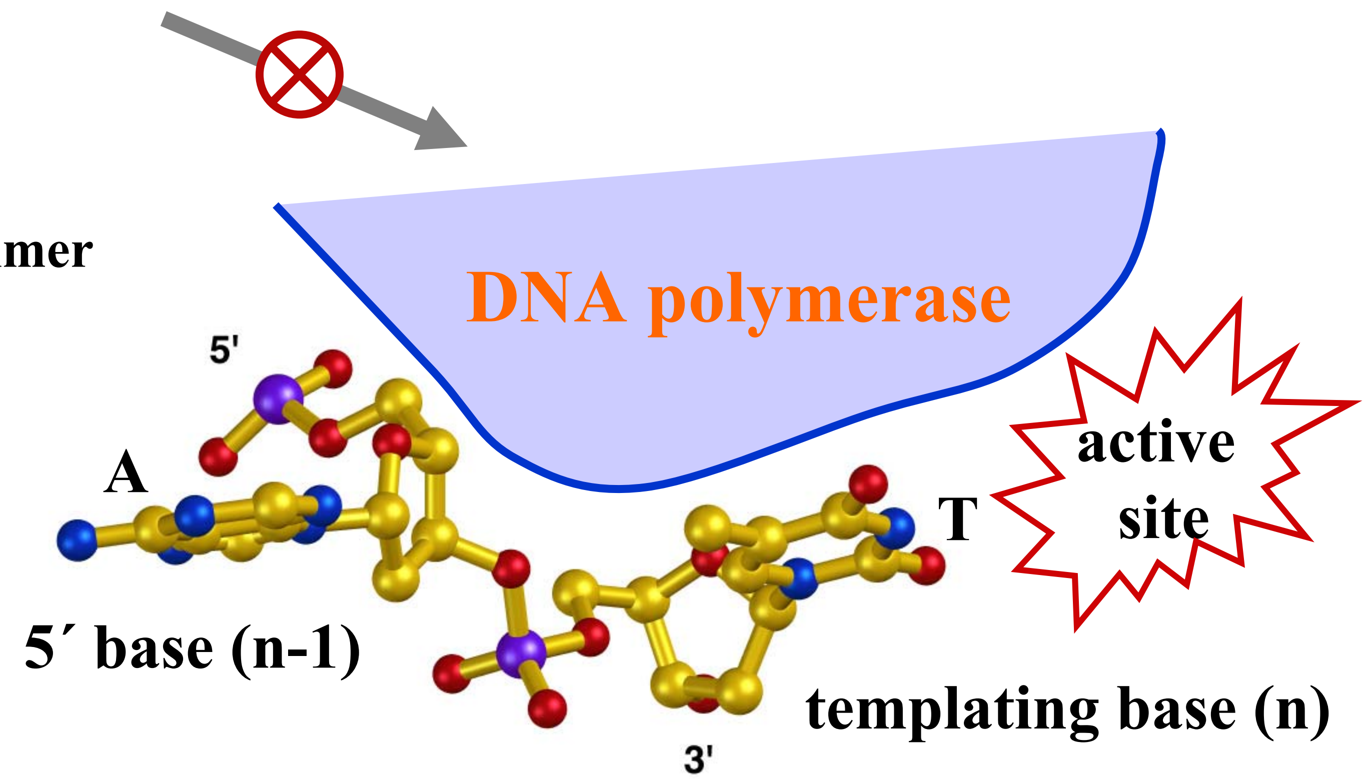


QuickTime™ and a TIFF (Uncompressed) decompressor are needed to see this picture.

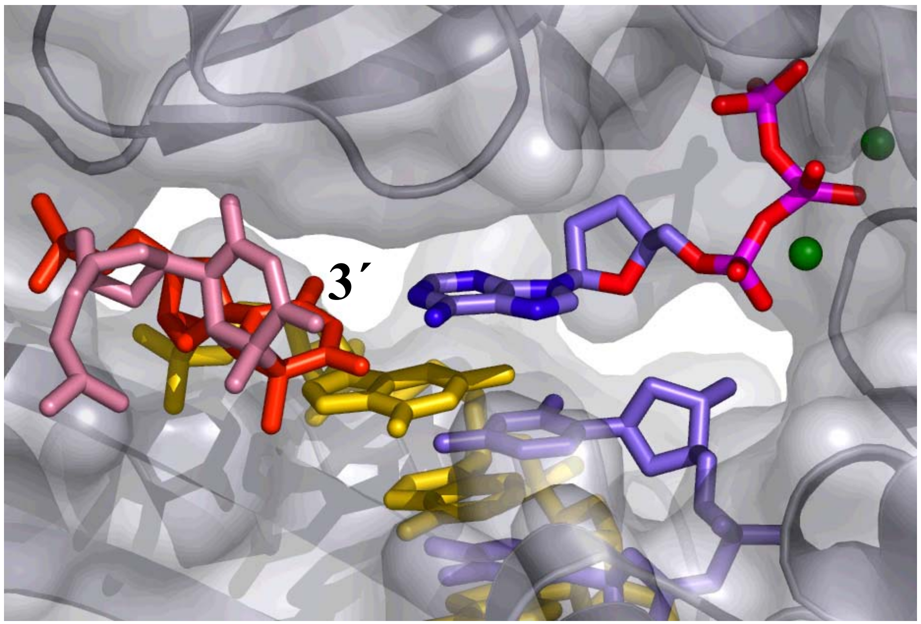
Thymine Dimer Is Incompatible With Replicative DNA Polymerases

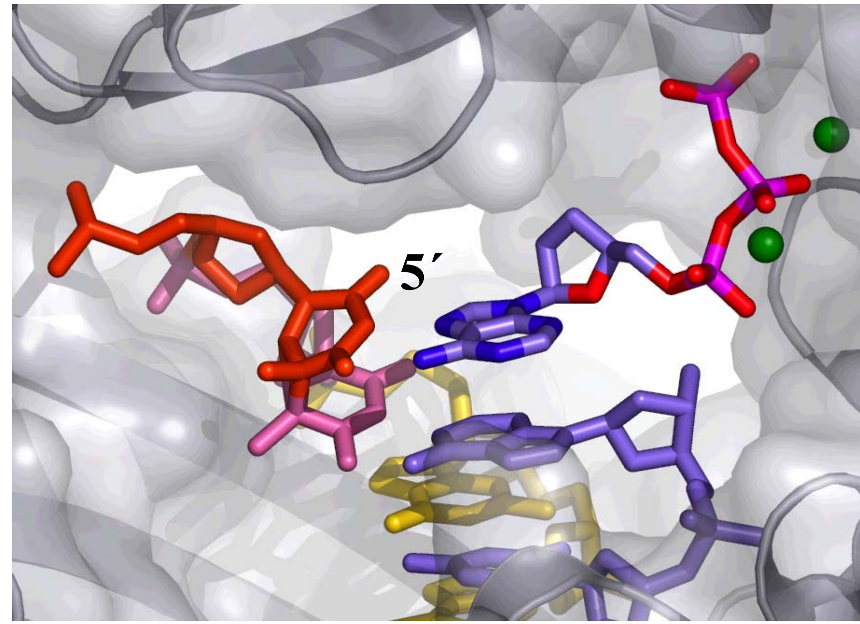
QuickTime™ and a TIFF (Uncompressed) decompressor are needed to see this picture.

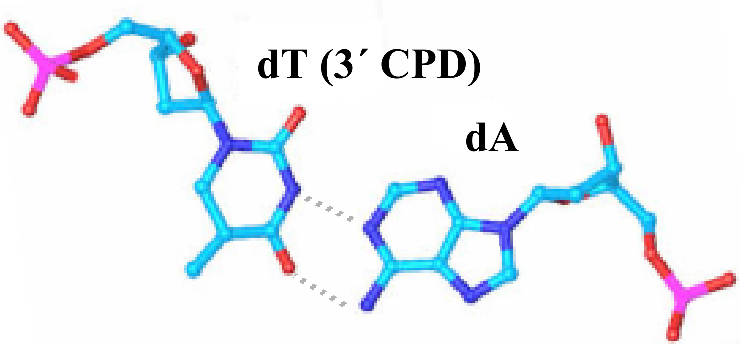
cis-syn thymine dimer
(CPD)



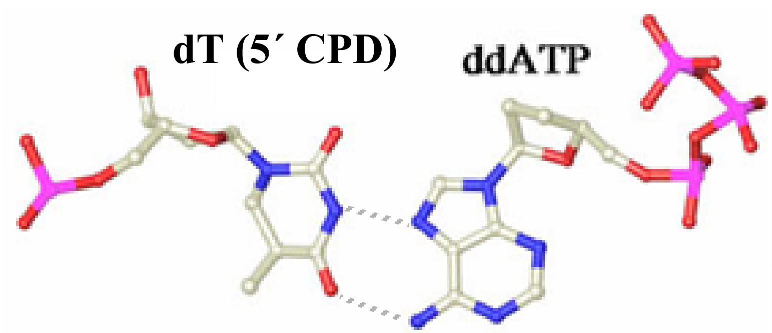
Dpo4 Inserts dATP opposite a CPD in Two Ways







Watson-Crick base pair



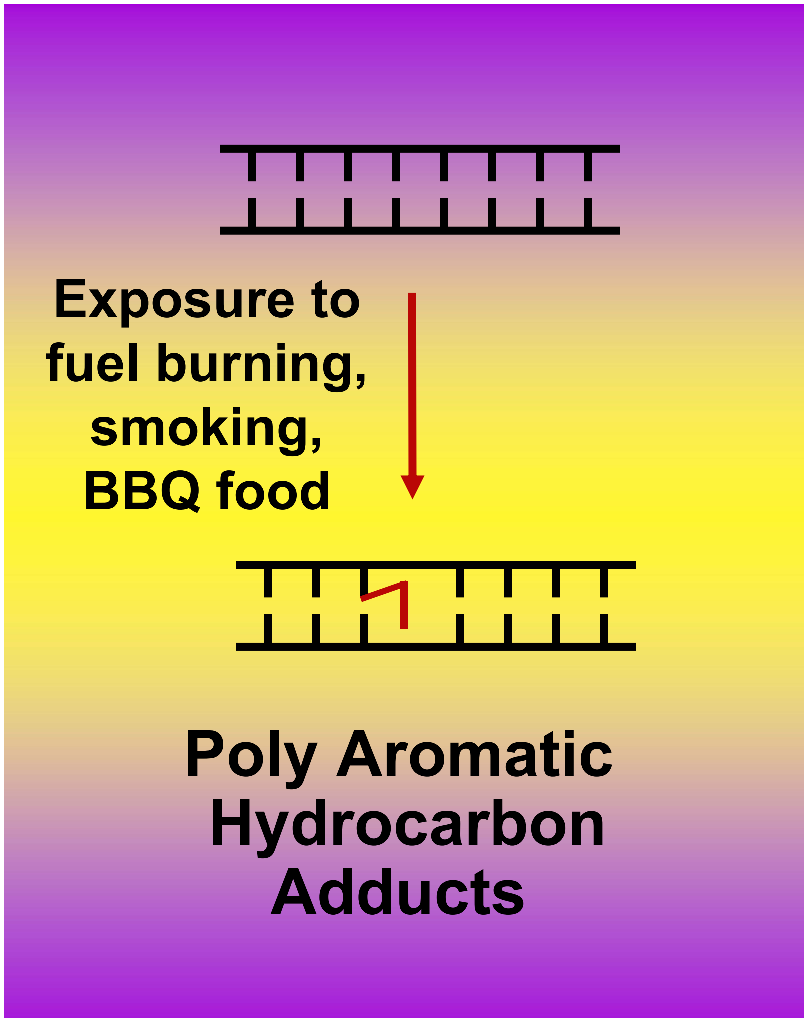
Hoogsteen base pair

Modeling Polη and Thymine Dimer Complex

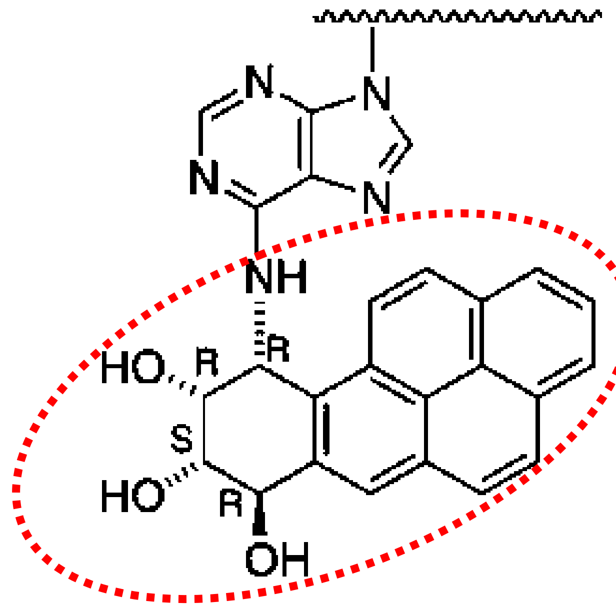
QuickTime™ and a
GIF decompressor
are needed to see this picture.

Thymine dimer appears to be more protected from solvent by Polη than Dpo4

Poly Aromatic Hydrocarbon (PAH) Adducts

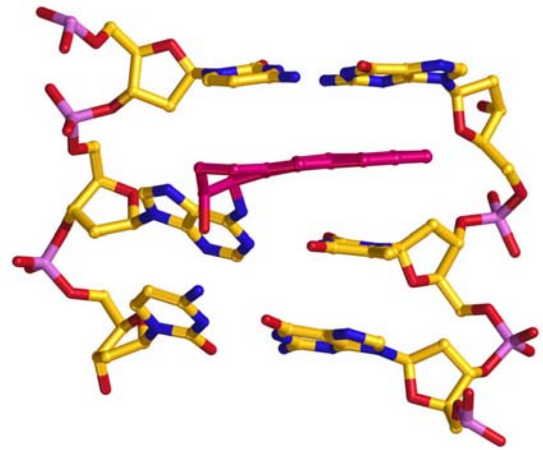


BPDE-dA

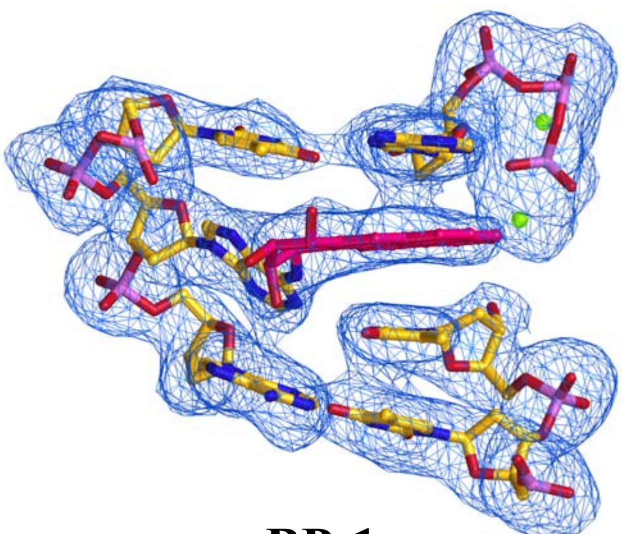


DNA Adducts

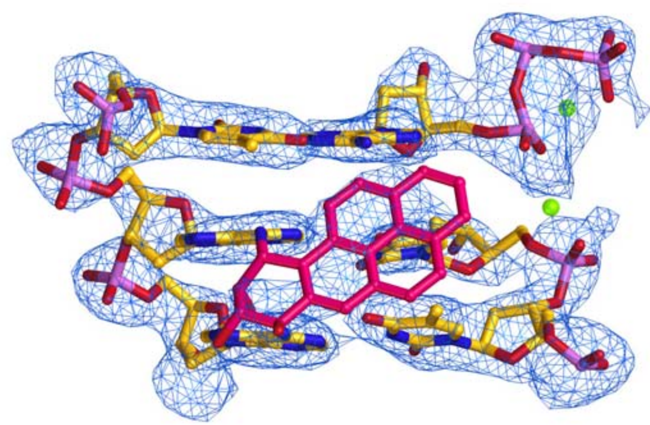
Dpo4 Changes BPDE Conformation



Solution Structure
NMR (1N8C)
(BPDE is intercalated)



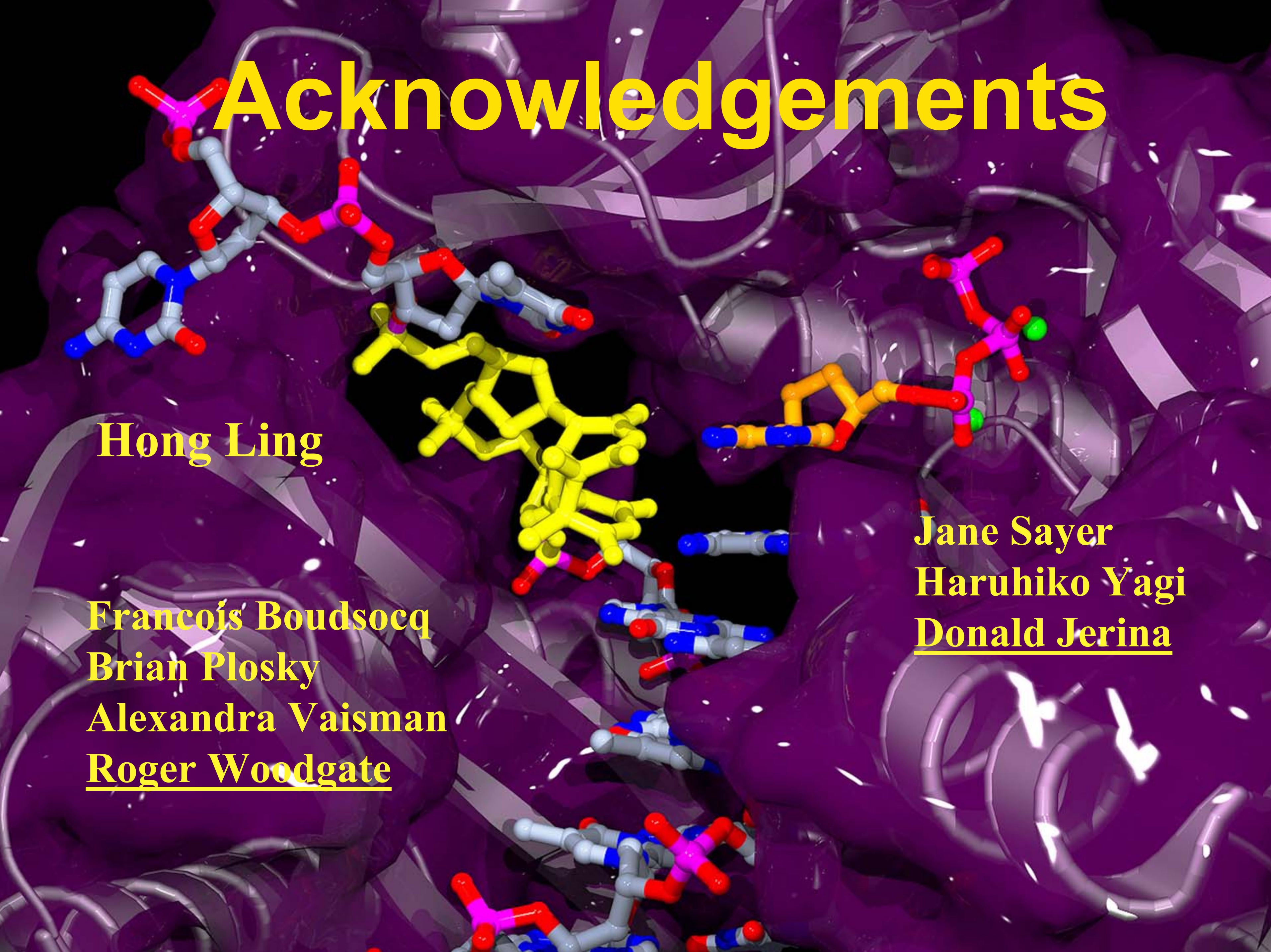
BP-1



BP-2

Co-crystal structure of Dpo4 and DNA adduct

Ling et al. & Yang (2004) PNAS



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