



Structural analysis of strand misalignment during DNA synthesis

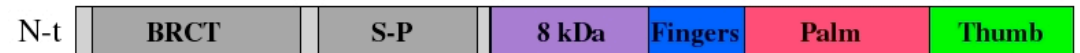
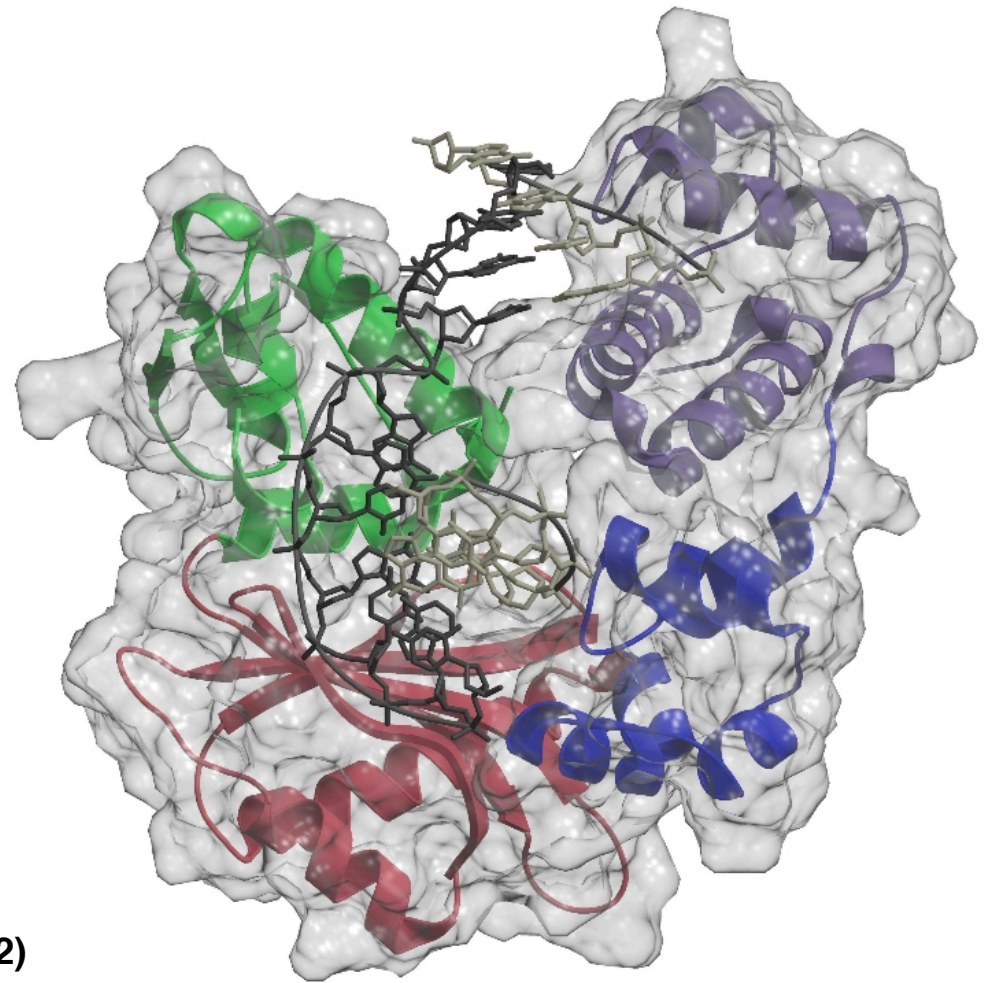
Miguel Garcia-Diaz



DNA Polymerase lambda

- Family X
- Distributive
- Processivity in small gaps
- NHEJ

- Pol λ (Lee *et al.*, 2004)
- Pol λ (Mahajan *et al.*, 2002)
- Pol IV (Wilson & Lieber, 1999)
(Tseng & Tomkinson, 2002)



Gap-filling on a normal substrate

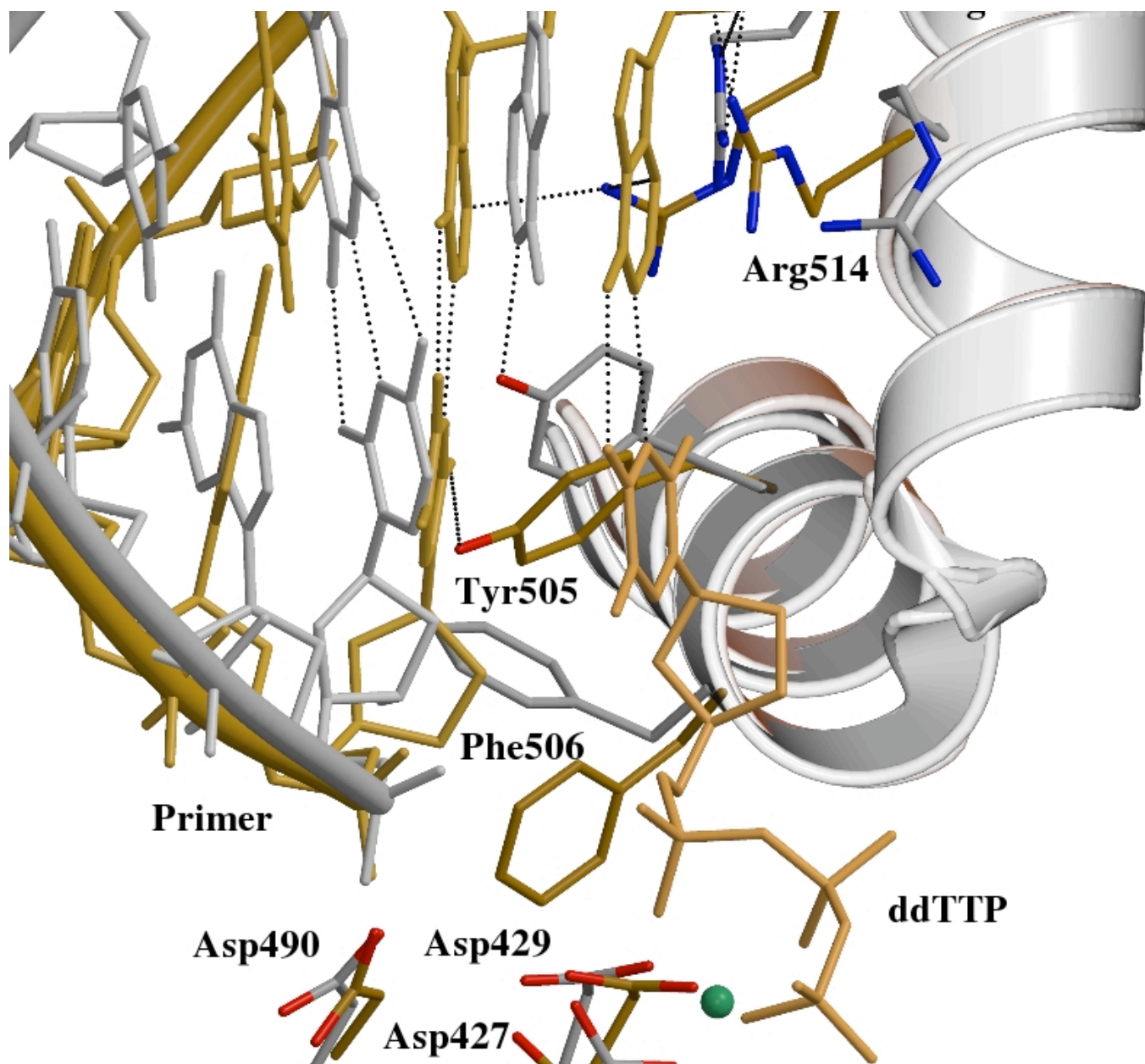
1nt gap



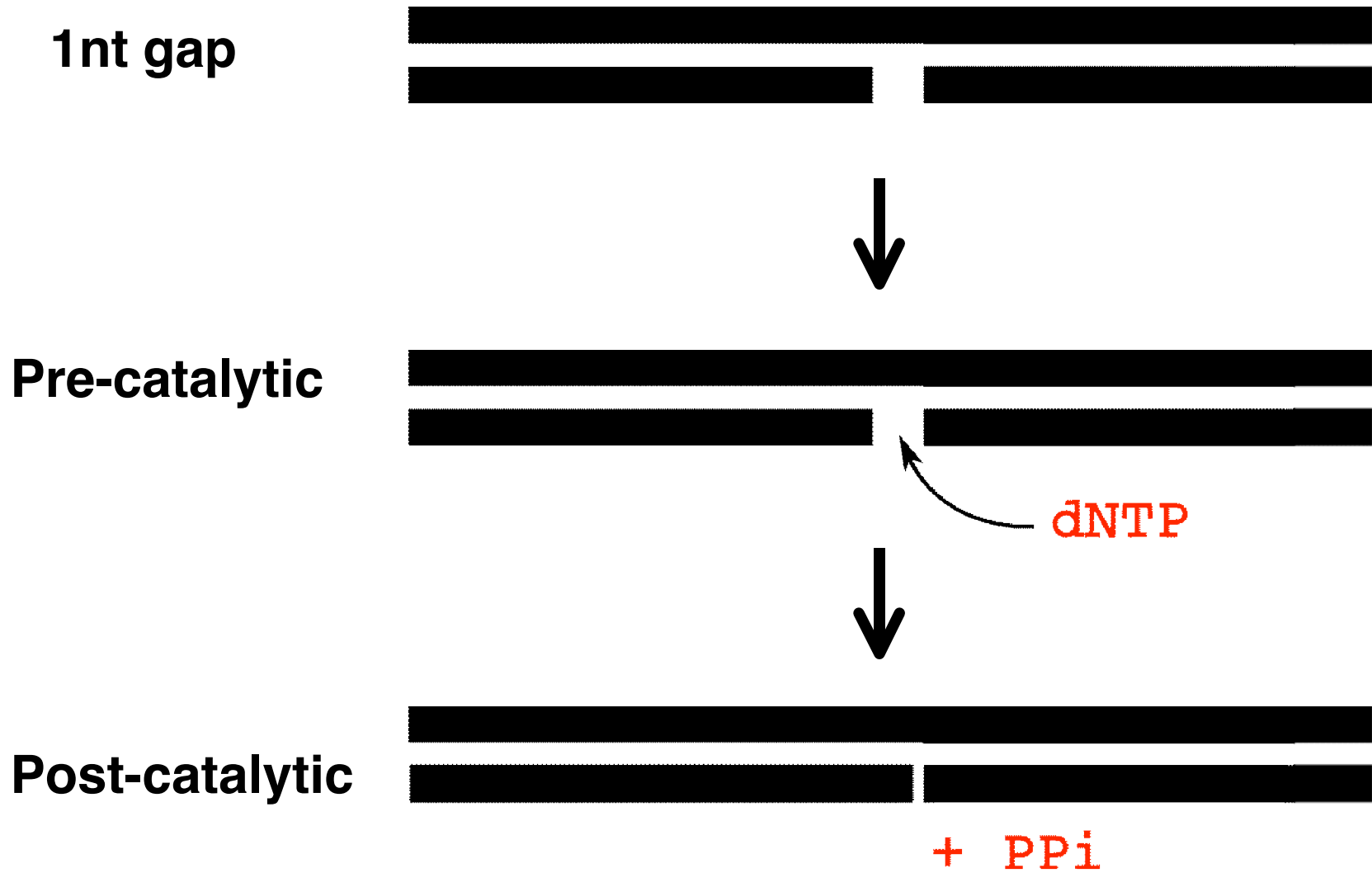
Pre-catalytic

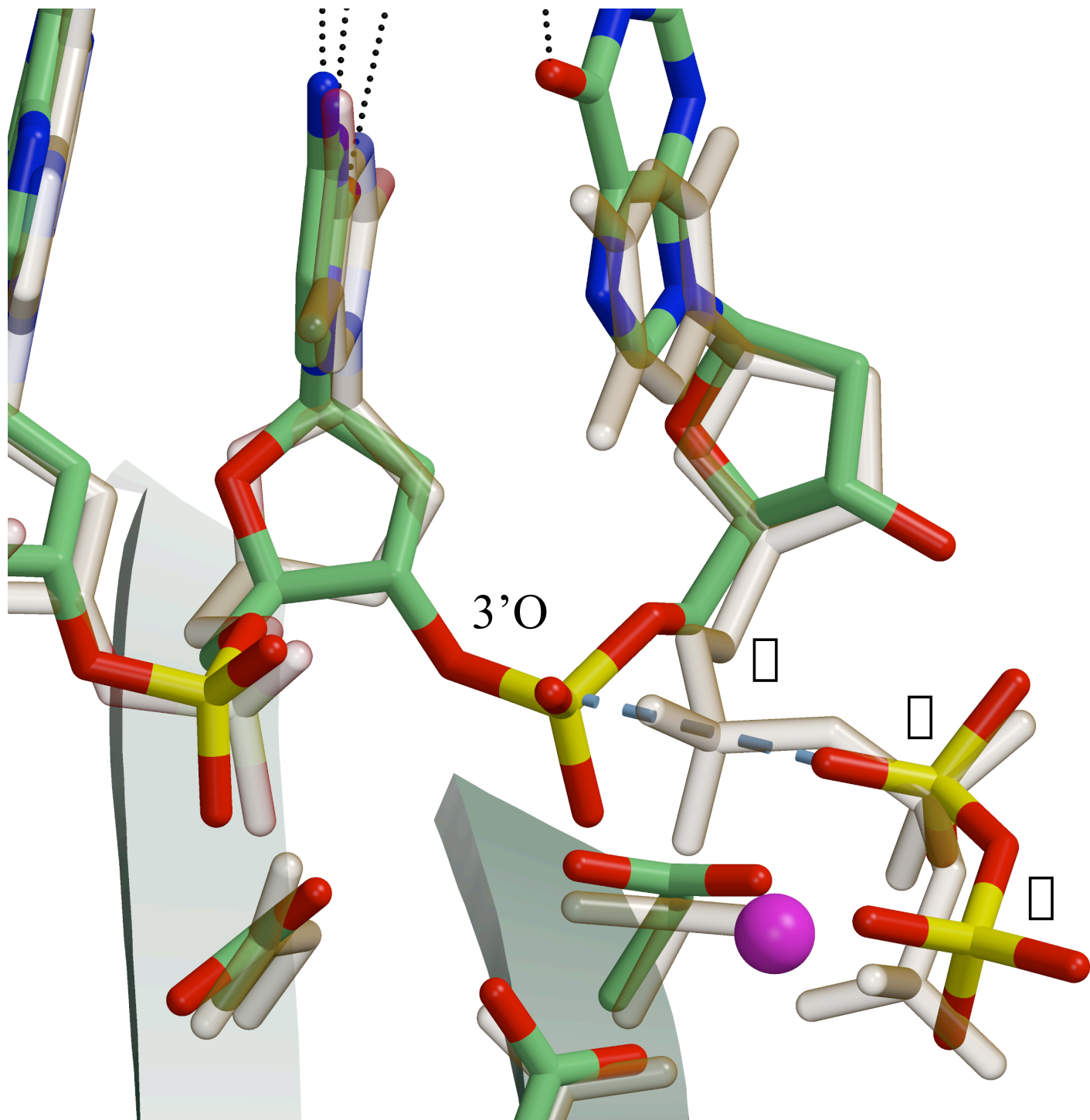


dNTP



Gap-filling on a normal substrate





Pol ϵ error rates

Compared to other eukaryotic DNA polymerases

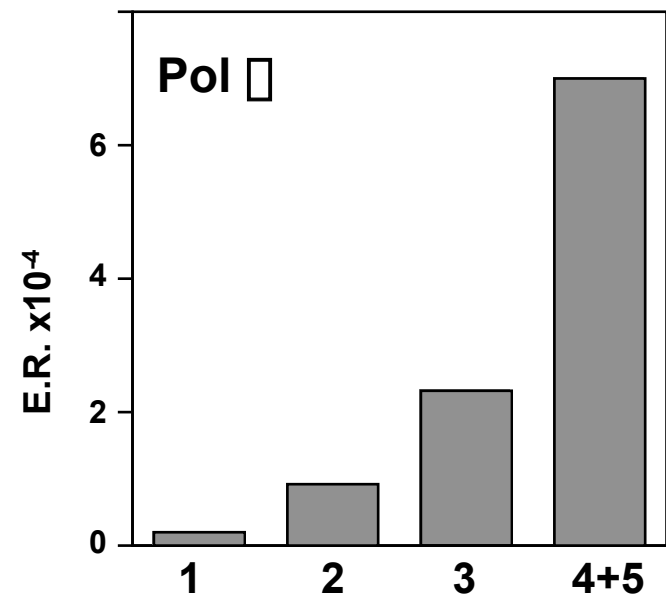
Enzyme	Family	Error rate ($\times 10^{-4}$)	
		Base substitutions	Deletions
Pol ϵ	Y	350	24
Pol δ	Y	58	18
Pol ϵ	X	9	45
Pol γ	X	7.4	1.3
Pol β	B	1.6	0.5
Pol δ	B	~ 0.1	0.2
Pol α	B	≤ 0.1	≤ 0.1
Pol θ	A	≤ 0.1	≤ 0.1

What are the Sequence Dependent Variations in One-Base Deletion Rates of Pol δ ?

Run length	One Base Deletion Error Rate ($\times 10^{-4}$)
	Pol δ
1	0.2
2	0.9
3	2.3
4 +5	7.0

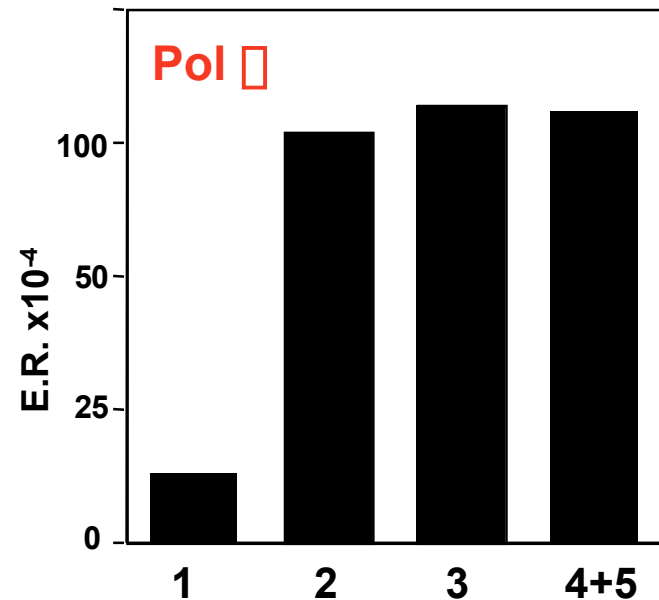
ACTGGATCGT^CCAGGTT
TGACCTAGCAG^B

ACTGG^CCCCCCAGGTT
TGACC^BGGGGG

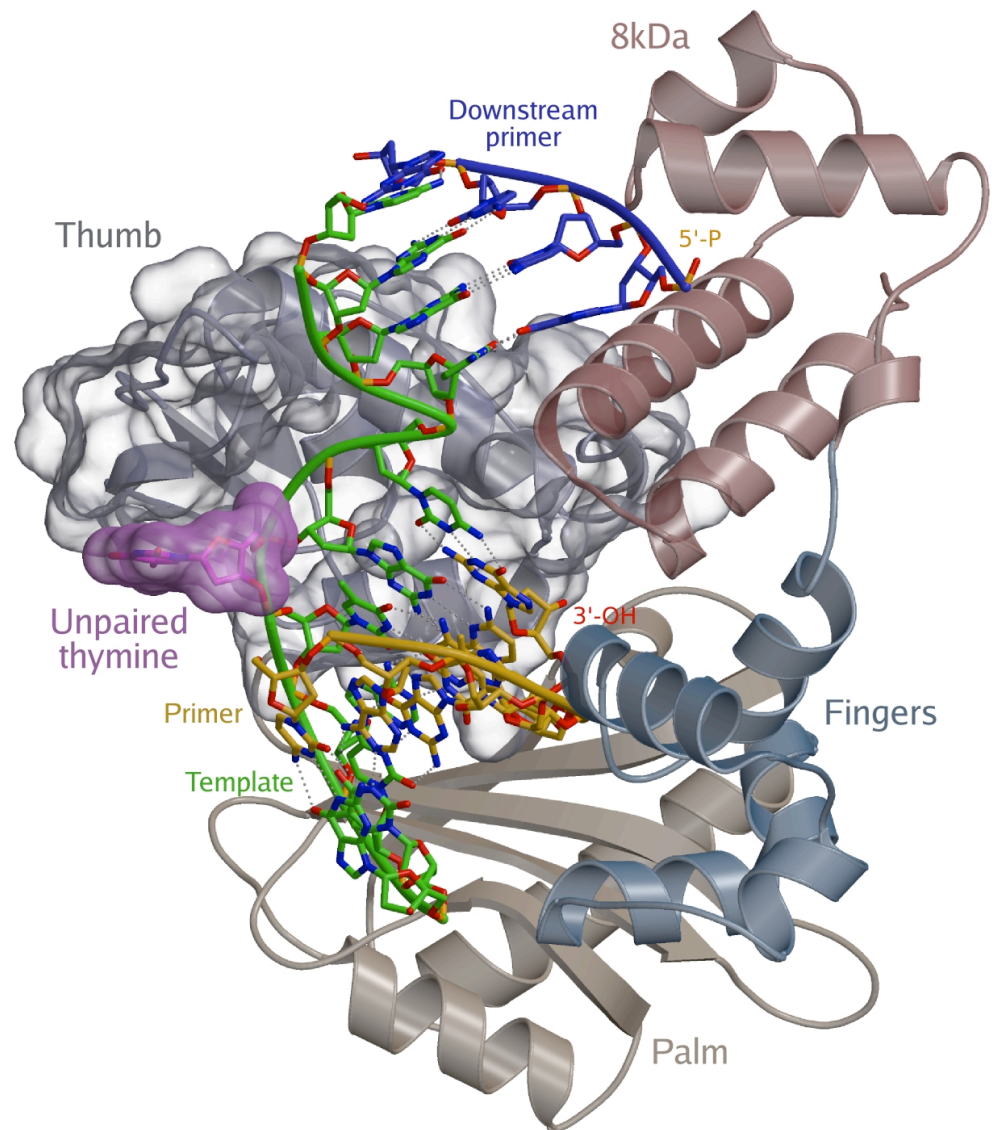
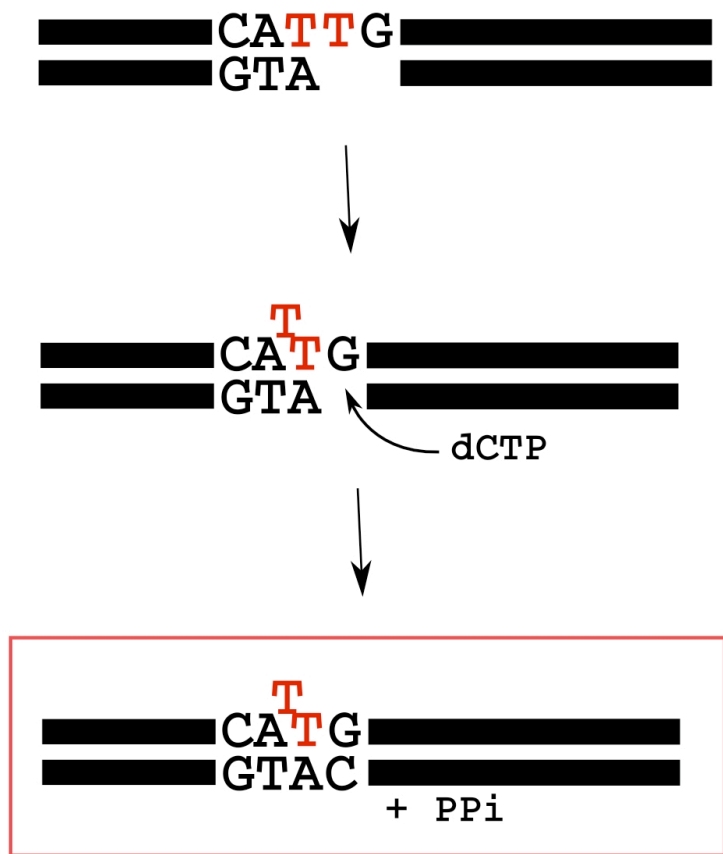


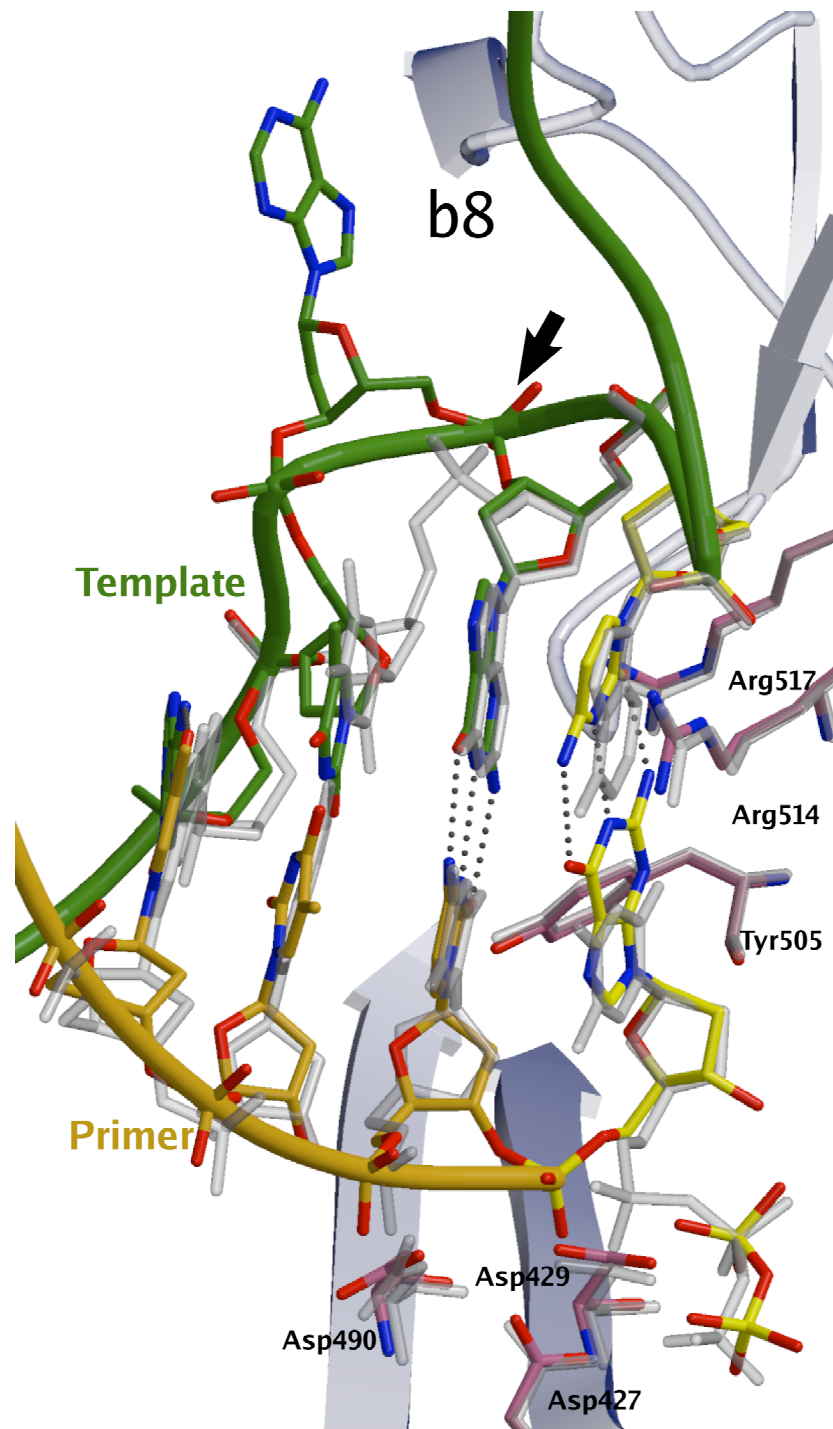
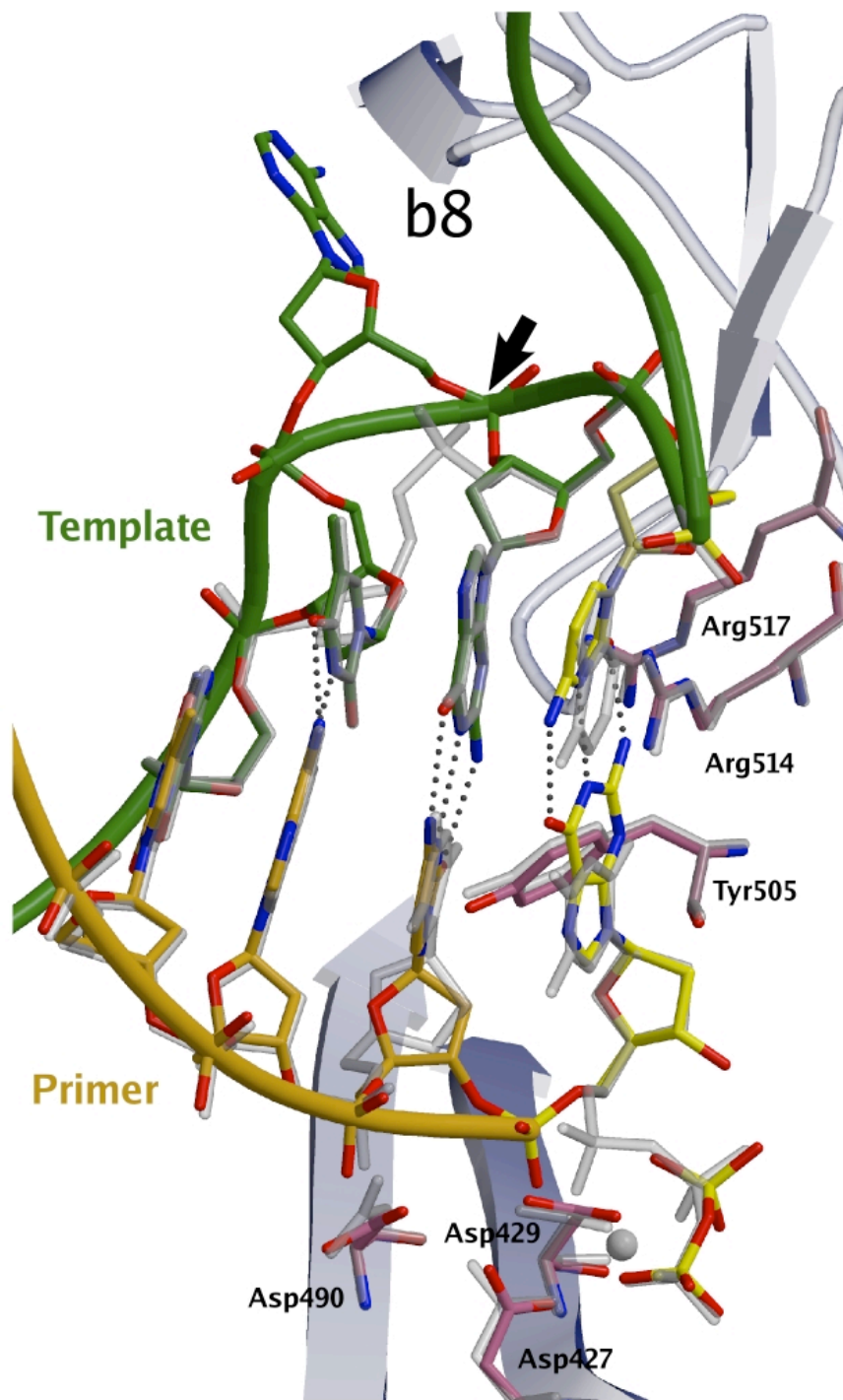
What are the Sequence Dependent Variations in One-Base Deletion Rates of Pol ϵ ?

Run length	One Base Deletion Error Rate ($\times 10^{-4}$)	
	Pol ϵ	Pol δ
1	0.2	13
2	0.9	77
3	2.3	82
4 +5	7.0	81



ACTGGATTAT^CCAGGTT
TGACCTAATAG^G





_____TACAG_____

_____ATG_____



_____TACAG_____

_____ATG_____

↖ dCTP

_____TACCG_____

_____ATG_____



_____TACCG_____

_____ATG_____

↖ dCTP

_____CGATGG_____

_____ATACTC_____



_____CGATGG_____

_____ATACTC_____

↖ dCTP

Summary

- Structural characterization of gap-filling by Pol η on normal substrate
- Structure of Pol η on a Streisinger misalignment reveals the unpaired nucleotide in an extrahelical position.
- Pol η seems to be adapted to utilize minimal base-pairing, a characteristic that is consistent with its role in NHEJ.

Acknowledgments

Kasia Bebenek

Joe Krahn

Lars Pedersen

Tom Kunkel

NIEHS

Luis Blanco

CBMSO (Madrid)