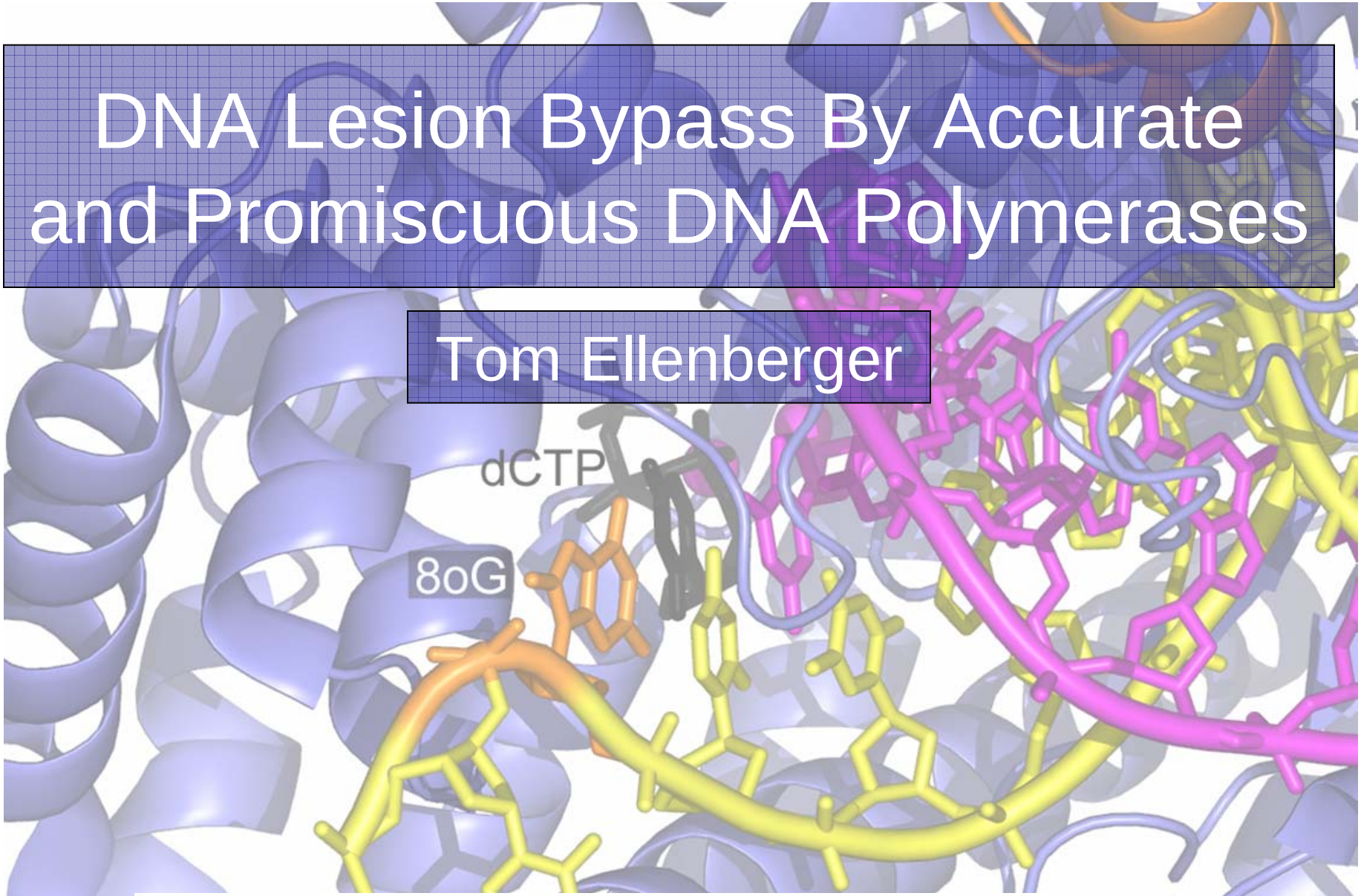
DNA Lesion Bypass By Accurate and Promiscuous DNA Polymerases

Tom Ellenberger



Department of Biochemistry and Molecular Biophysics

Washington University in St.Louis
SCHOOL OF MEDICINE

“Geometric Selection” of dNTPs

equipment, and to Dr. G. E. R. Deacon and the captain and officers of R.R.S. *Discovery II* for their part in making the observations.

[1] Young, F. B., Gerrard, H., and Jevons, W., *Phil. Mag.*, **40**, 149 (1920).
[2] Longuet-Higgins, M. S., *Mon. Not. Roy. Astro. Soc., Geophys. Supp.*, **5**, 285 (1949).
[3] Von Arx, W. S., Woods Hole Papers in Phys. Oceanog. Meteor., **11** (3) (1950).
[4] Ekman, V. W., *Arkiv. Mat. Astron. Fysik. (Stockholm)*, **2** (11) (1905).

MOLECULAR STRUCTURE OF NUCLEIC ACIDS

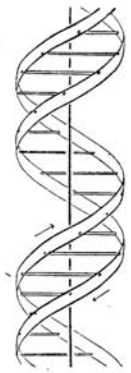
A Structure for Deoxyribose Nucleic Acid

WE wish to suggest a structure for the salt of deoxyribose nucleic acid (D.N.A.). This structure has novel features which are of considerable biological interest.

A structure for nucleic acid has already been proposed by Pauling and Corey[1]. They kindly made their manuscript available to us in advance of publication. Their model consists of three intertwined chains, with the phosphates near the fibre axis and the bases on the outside. In our opinion

> It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.

gested by Fraser (in the press). In his model the phosphates are on the outside and the bases on the inside, linked together by hydrogen bonds. This structure as described is rather ill-defined, and for this reason we shall not comment on it.



This figure is purely diagrammatic. The two ribbons symbolize the two phosphate—sugar chains, and the horizontal rods the pairs of bases holding the chains together. The vertical line marks the fibre axis

We wish to put forward a radically different structure for the salt of deoxyribose nucleic acid. This structure has two helical chains each coiled round the same axis (see diagram). We have made the usual chemical assumptions, namely, that each chain consists of phosphate diester groups joining β-D-deoxyribofuranose residues with 3′,5′ linkages. The two chains (but not their bases) are related by a dyad perpendicular to the fibre axis. Both chains follow right-handed helices, but owing to the dyad the sequences of the atoms in the two chains run in opposite directions. Each chain loosely resembles Furberg's[2] model No. 1; that is, the bases are on the inside of the helix and the phosphates on the outside. The configuration of the sugar and the atoms near it is close to Furberg's 'standard configuration', the sugar being roughly perpendicular to the attached base. There is a residue on each chain every 3·4 A. in the z-direction. We have assumed an angle of 36° between adjacent residues in the same chain, so that the structure repeats after 10 residues on each chain, that is, after 34 A. The distance of a phosphorus atom from the fibre axis is 10 A. As the phosphates are on the outside, cations have easy access to them.

The structure is an open one, and its water content is rather high. At lower water contents we would expect the bases to tilt so that the structure could become more compact.

The novel feature of the structure is the manner in which the two chains are held together by the purine and pyrimidine bases. The planes of the bases are perpendicular to the fibre axis. They are joined together in pairs, a single base from one chain being hydrogen-bonded to a single base from the other chain, so that the two lie side by side with identical z-co-ordinates. One of the pair must be a purine and the other a pyrimidine for bonding to occur. The hydrogen bonds are made as follows: purine position 1 to pyrimidine position 1; purine position 6 to pyrimidine position 6.

If it is assumed that the bases only occur in the structure in the most plausible tautomeric forms (that is, with the keto rather than the enol configurations) it is found that only specific pairs of

formed, it follows that if the sequence of bases on one chain is given, then the sequence on the other chain is automatically determined.

It has been found experimentally[3,4] that the ratio of the amounts of adenine to thymine, and the ratio of guanine to cytosine, are always very close to unity for deoxyribose nucleic acid.

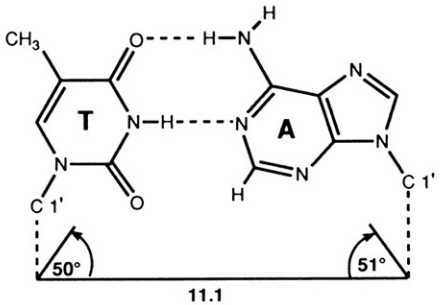
It is probably impossible to build this structure with a ribose sugar in place of the deoxyribose, as the extra oxygen atom would make too close a van der Waals contact.

The previously published X-ray data[5,6] on deoxyribose nucleic acid are insufficient for a rigorous test of our structure. So far as we can tell, it is roughly compatible with the experimental data, but it must be regarded as unproved until it has been checked against more exact results. Some of these are given in the following communications. We were not aware of the details of the results presented there when we devised our structure, which rests mainly though not entirely on published experimental data and stereochemical arguments.

It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.

Full details of the structure, including the conditions assumed in building it, together with a set of co-ordinates for the atoms, will be published elsewhere.

We are much indebted to Dr. Jerry Donohue for constant advice and criticism, especially on interatomic distances. We have also been stimulated by a knowledge of the general nature of the unpublished experimental results and ideas of Dr. M. H. F. Wilkins, Dr. R. E. Franklin and their co-workers at



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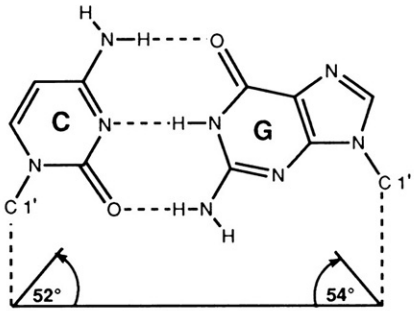

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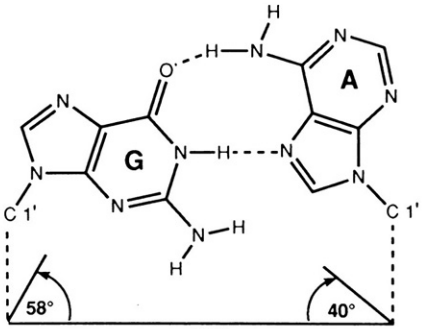

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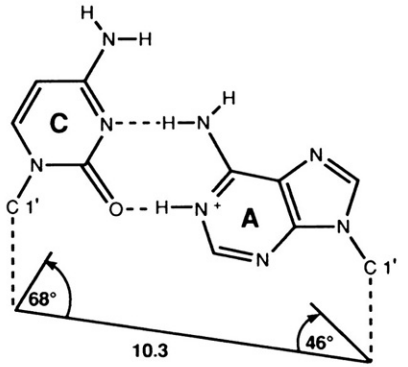

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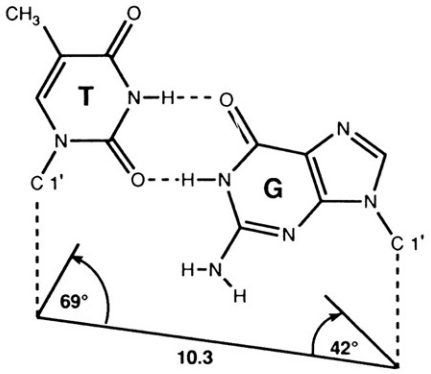
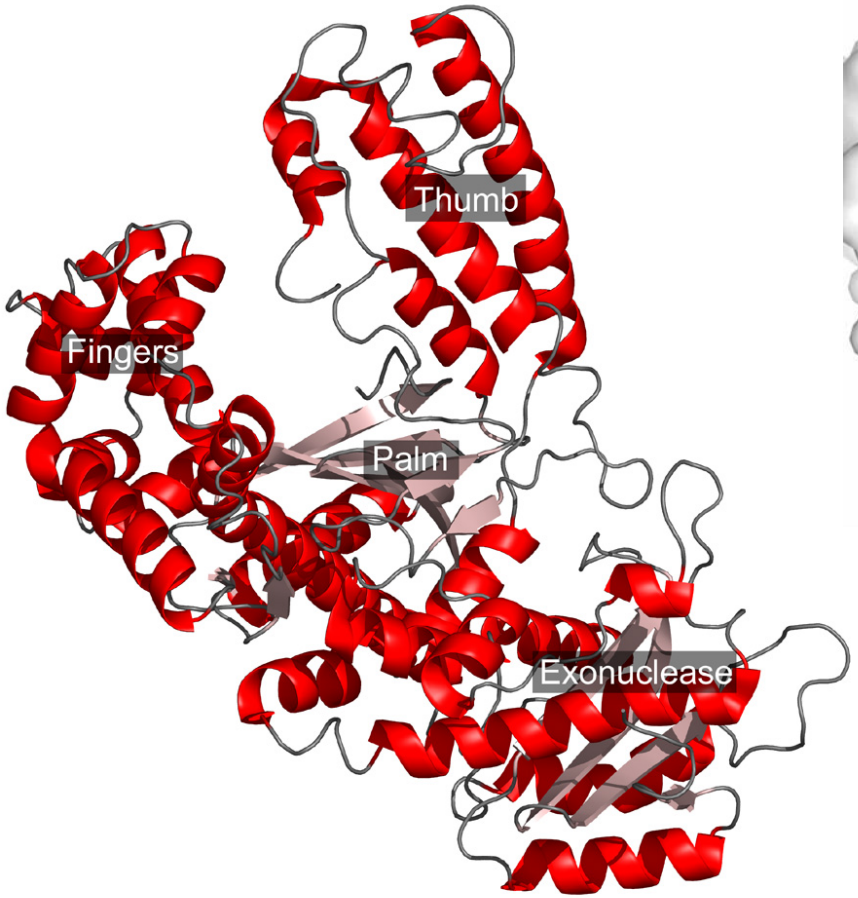
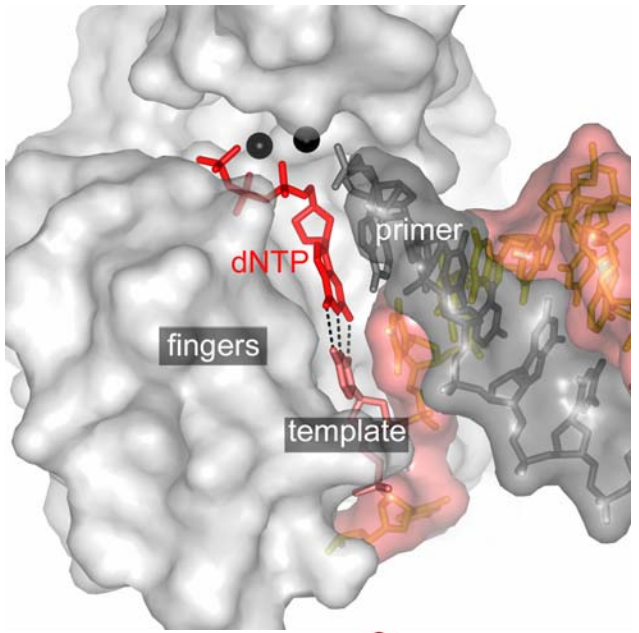


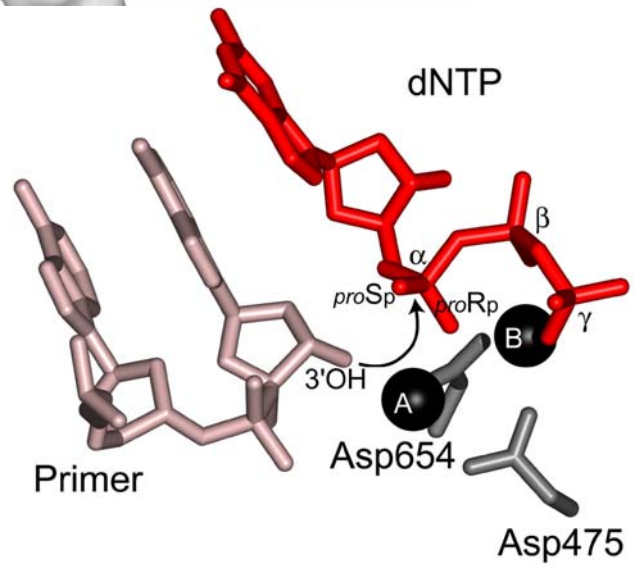
10.3

M. Goodman 1997.
PNAS 94, 10493.

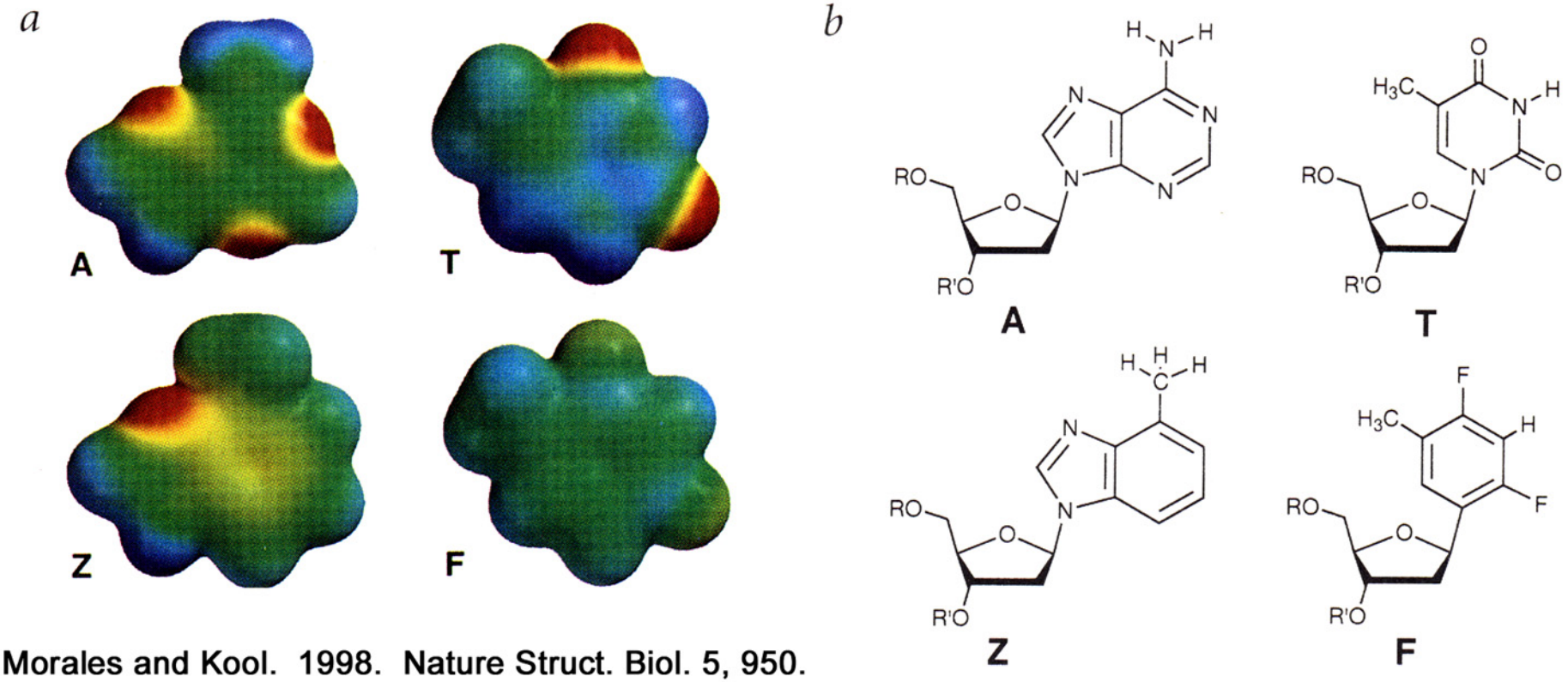
Templated DNA Synthesis







Selective Incorporation of Nonpolar Nucleobase Isosteres into DNA

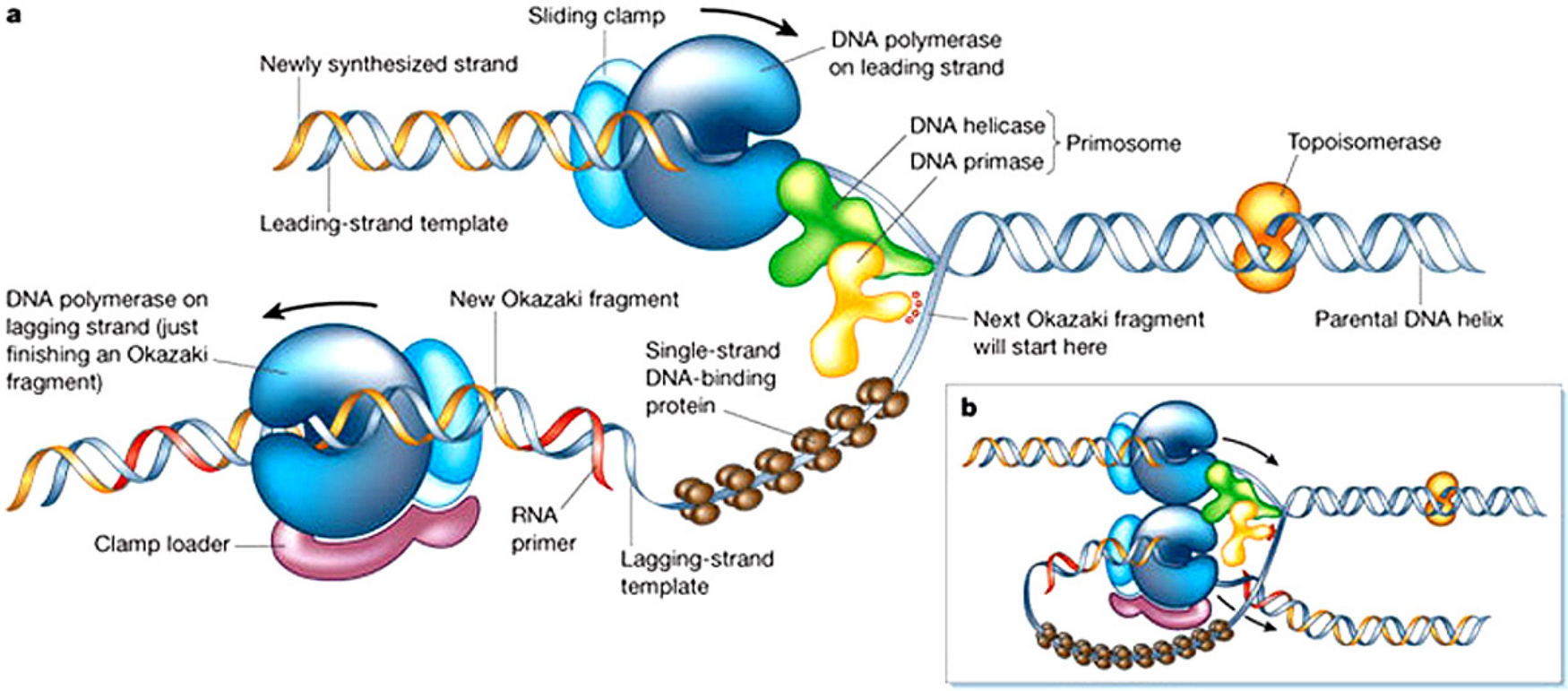


Morales and Kool. 1998. Nature Struct. Biol. 5, 950.

Bypass of Chemically Modified Bases

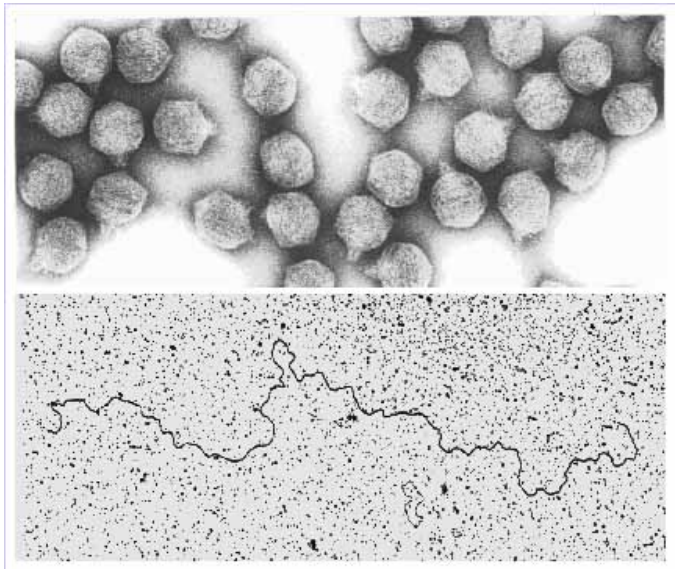
- Replication Arrest – block of replication fork.
- Mutagenesis – miscoding potential, template slippage.
- Y Family Polymerases – error-prone enzymes that bypass damage.

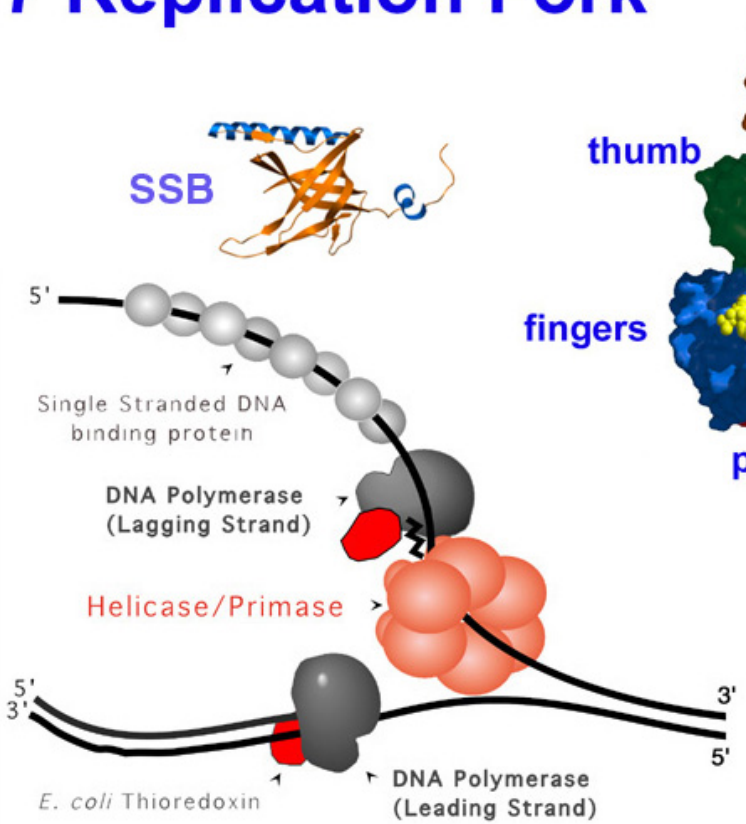
Physical Coupling of Enzymatic Activities at the Replication Fork

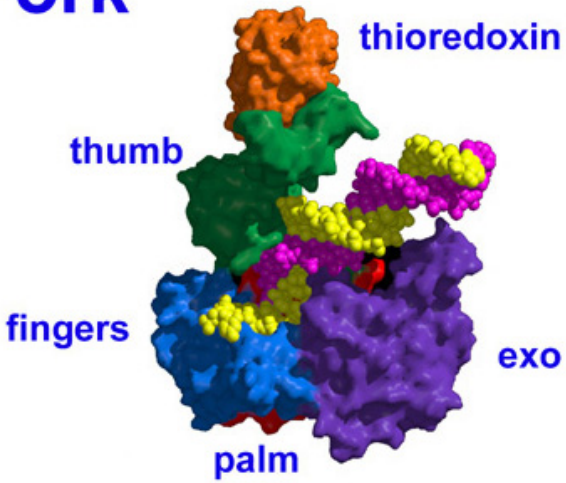


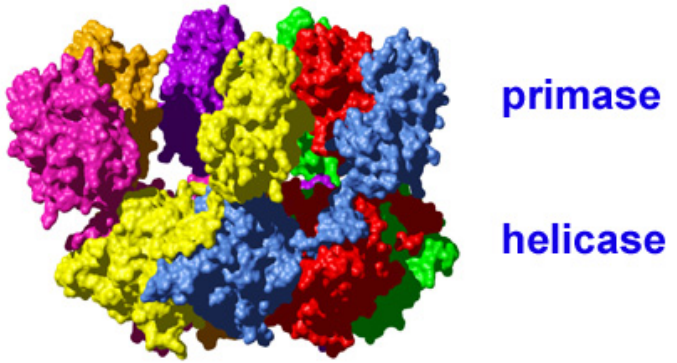
from Alberts, B. 2003. Nature 421, 431-435.

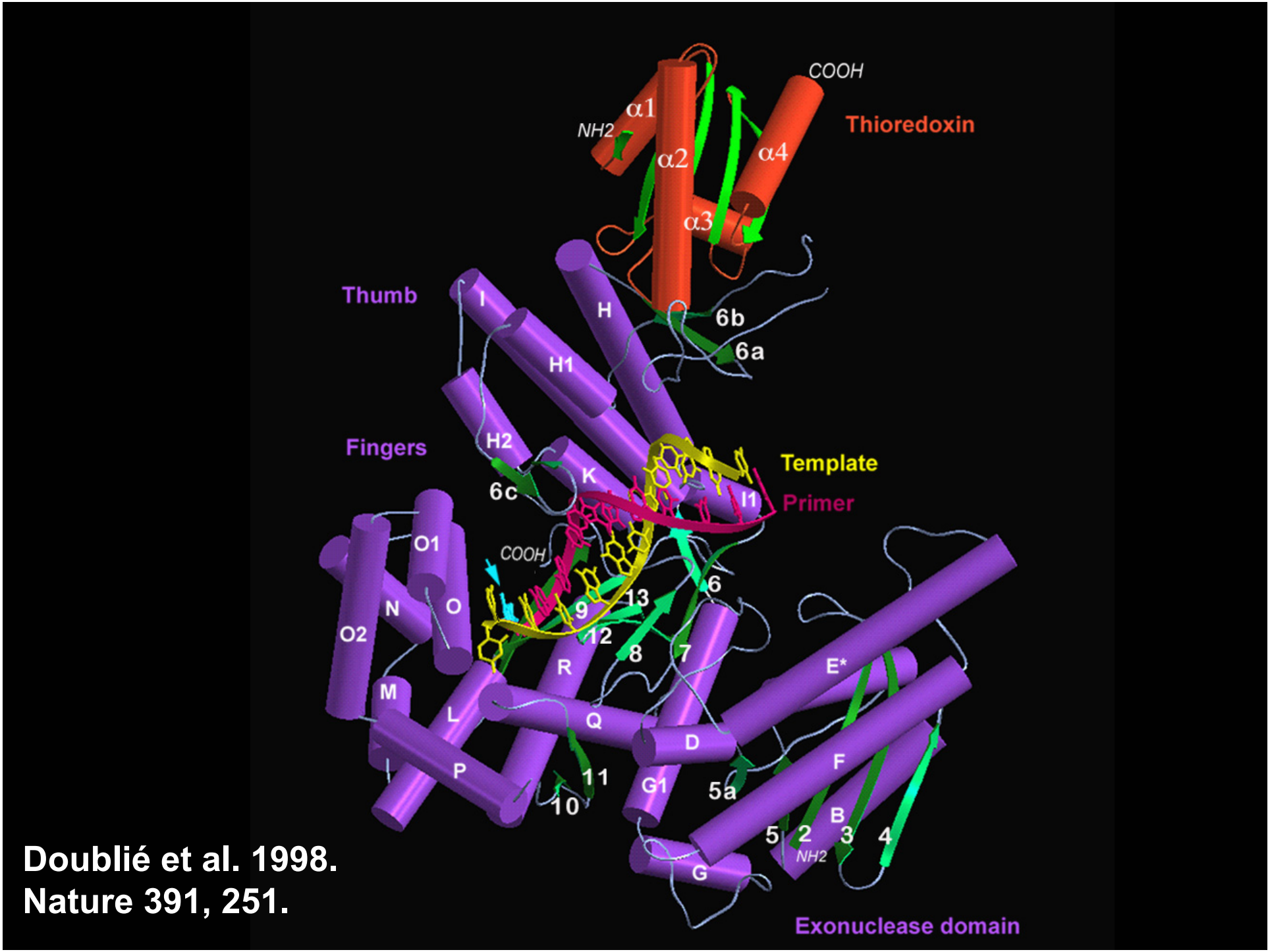
T7 Replication Fork





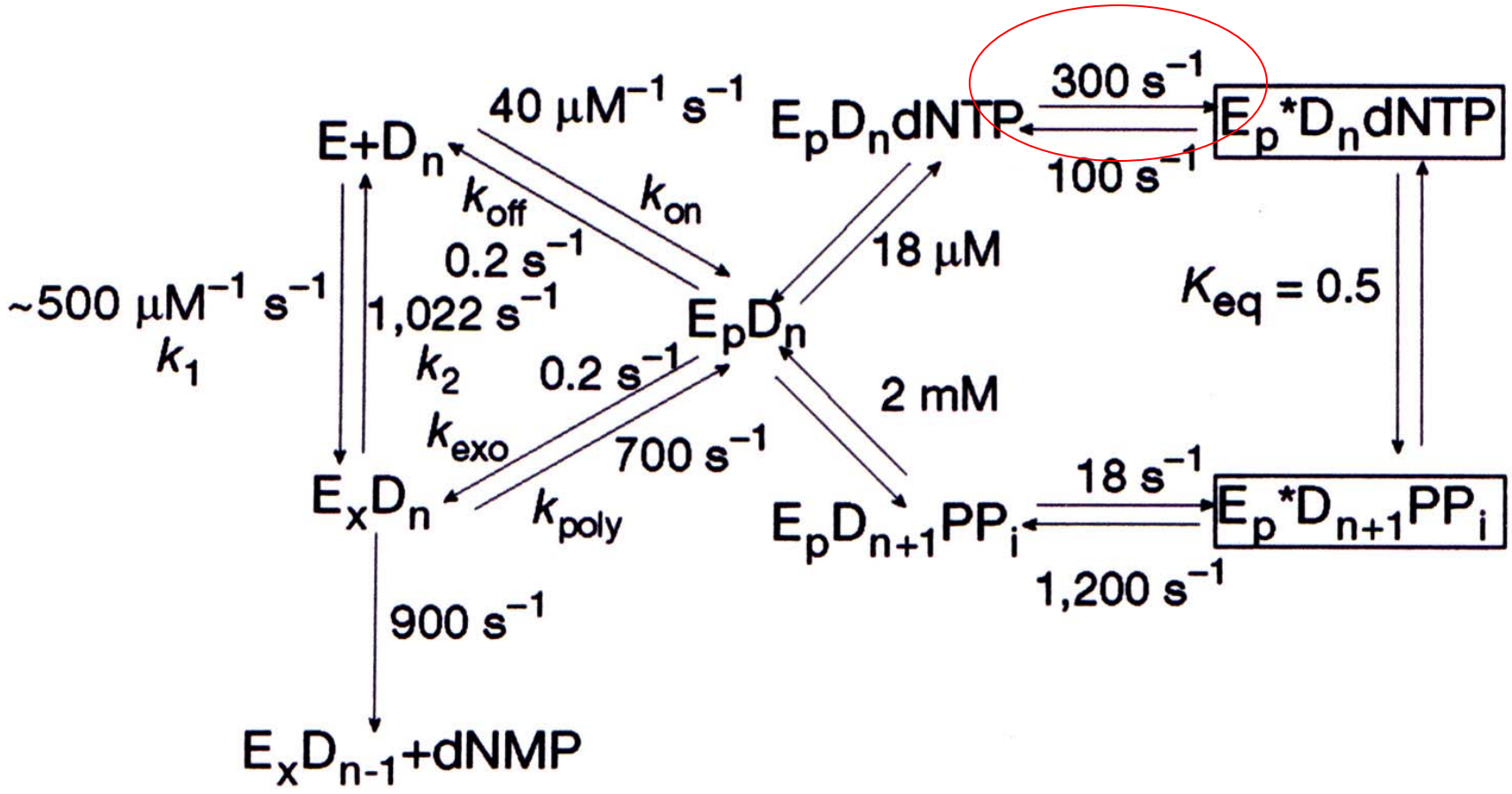






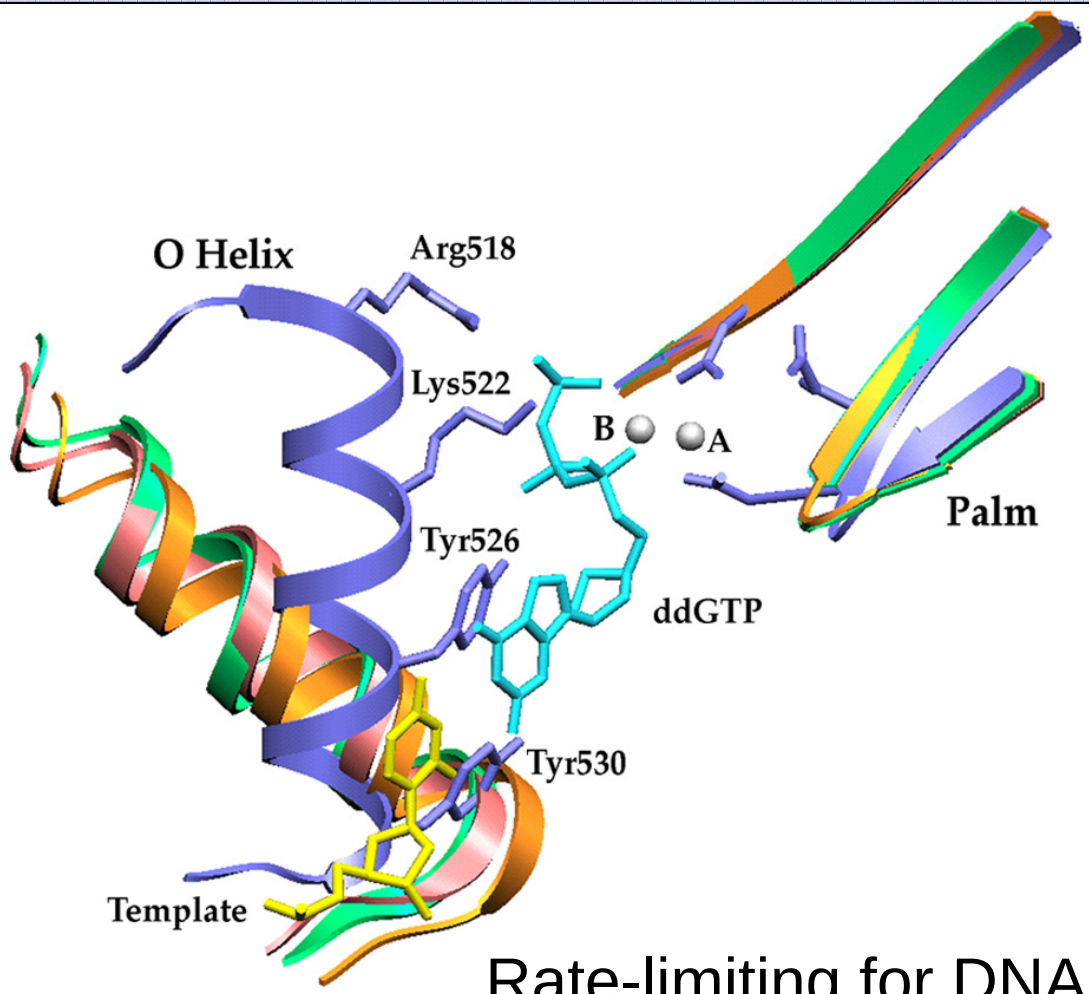
Doublié et al. 1998.
Nature 391, 251.

Kinetic Studies of T7 DNA Pol

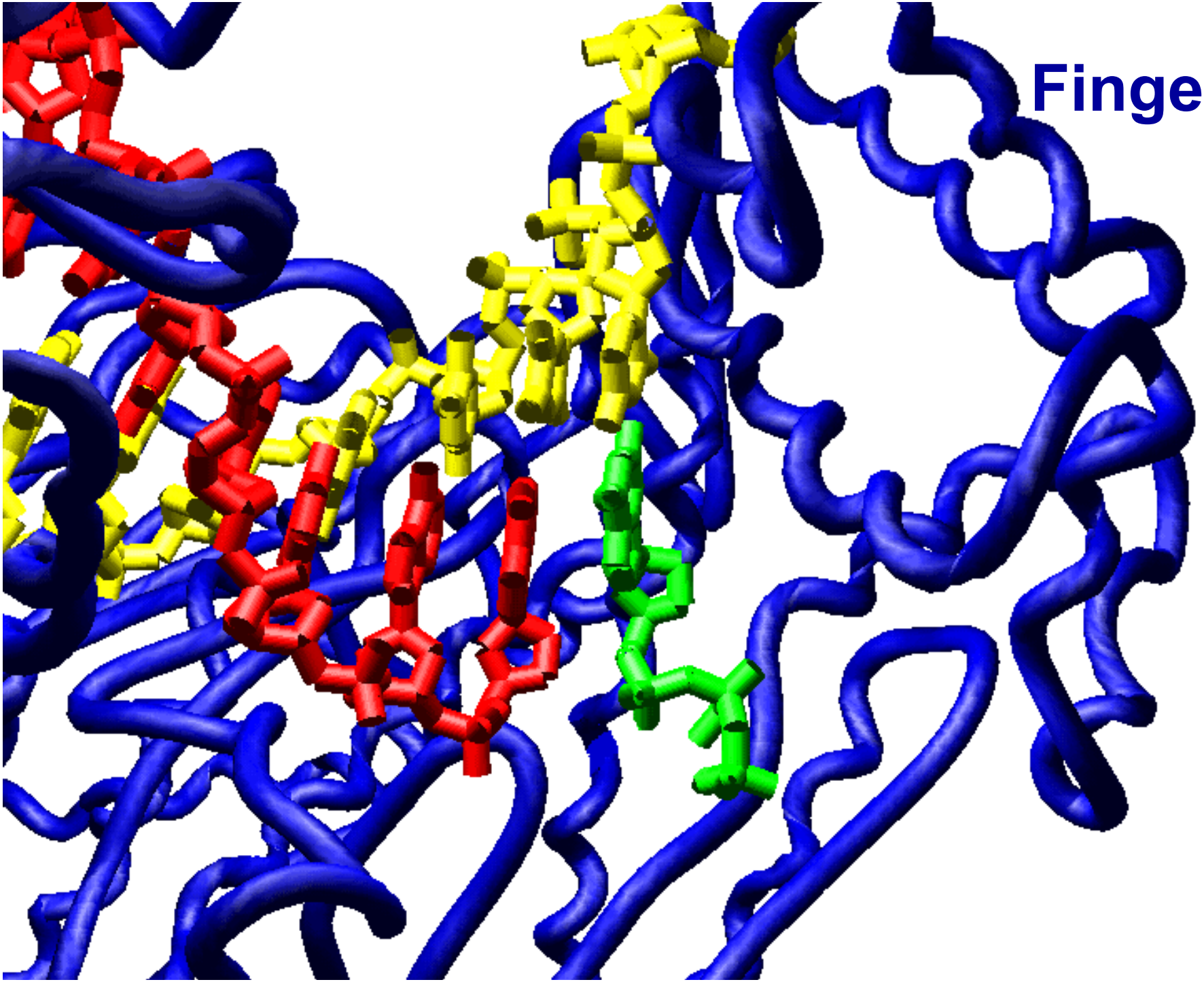


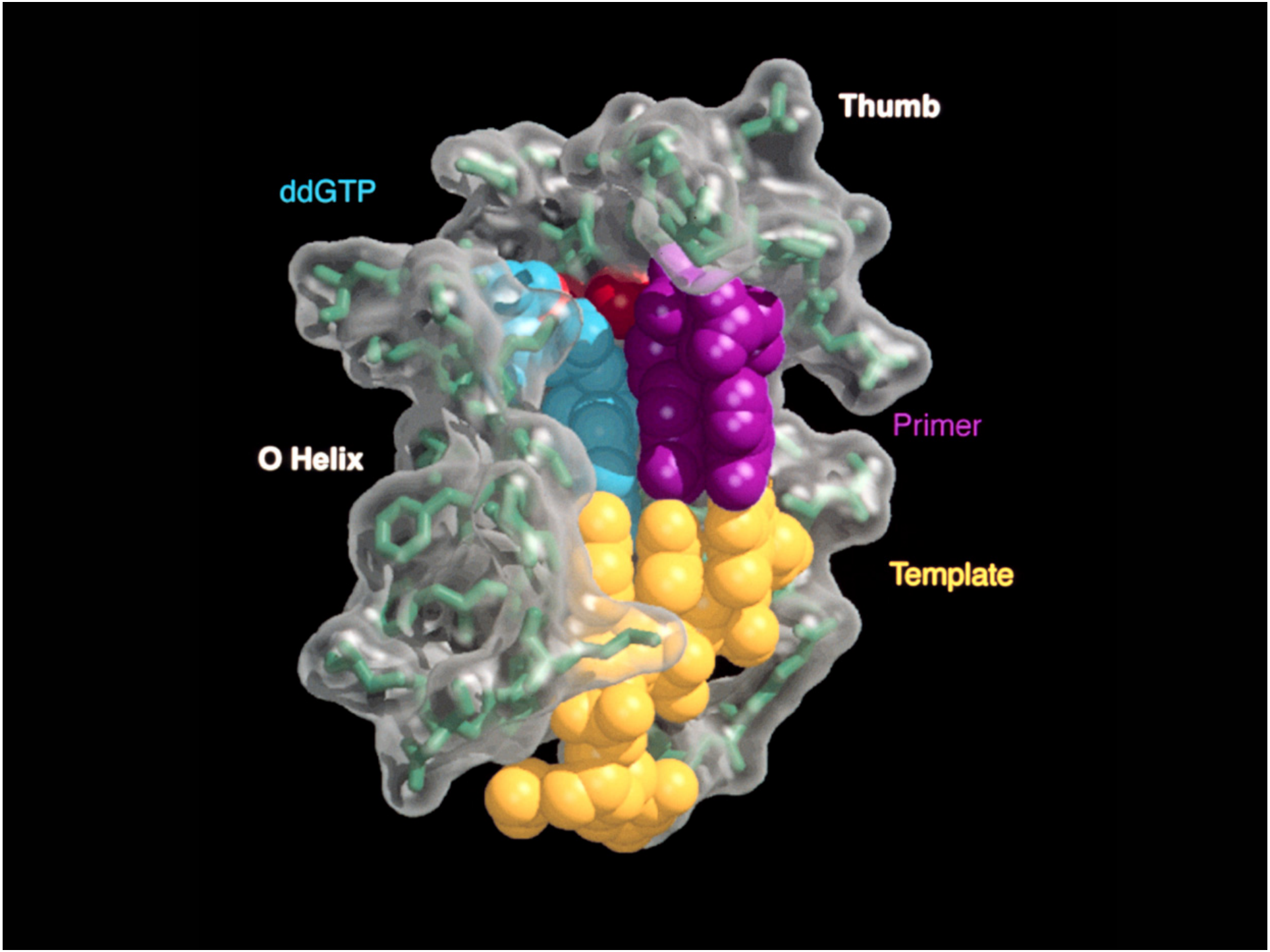
Patel, S.S., Wong, I., and Johnson, K.A. 1991. Biochemistry 30, 511-525.

Substrate-induced Fit of Enzyme Active Site

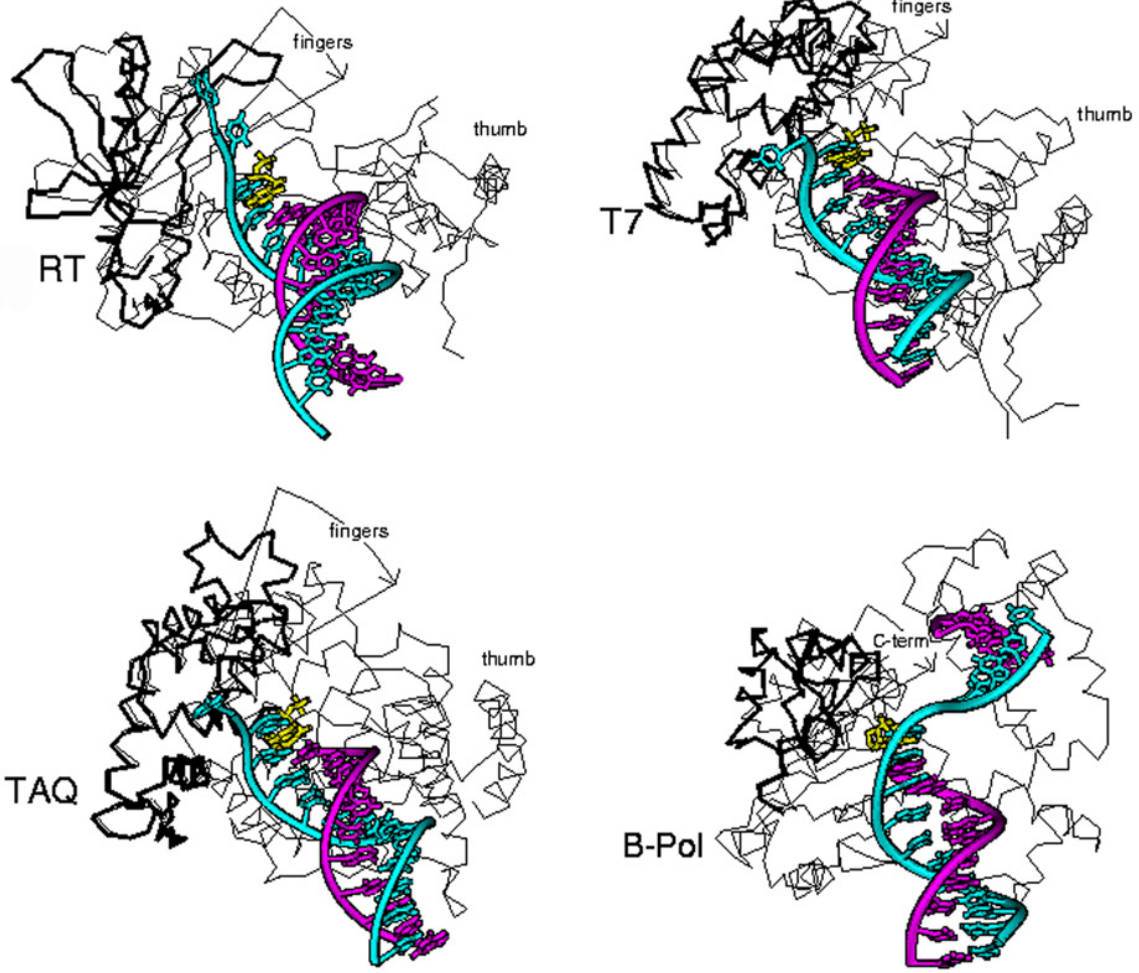


Rate-limiting for DNA synthesis?

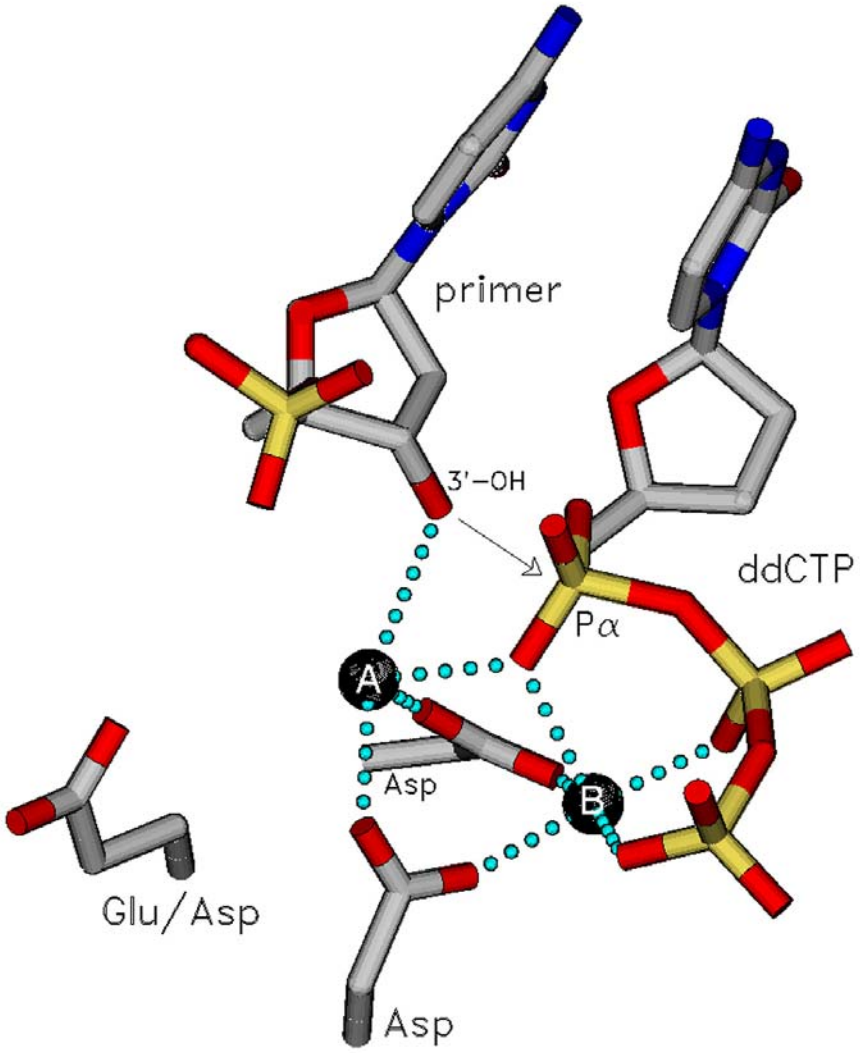

Fingers


Thumb
ddGTP
Primer
O Helix
Template

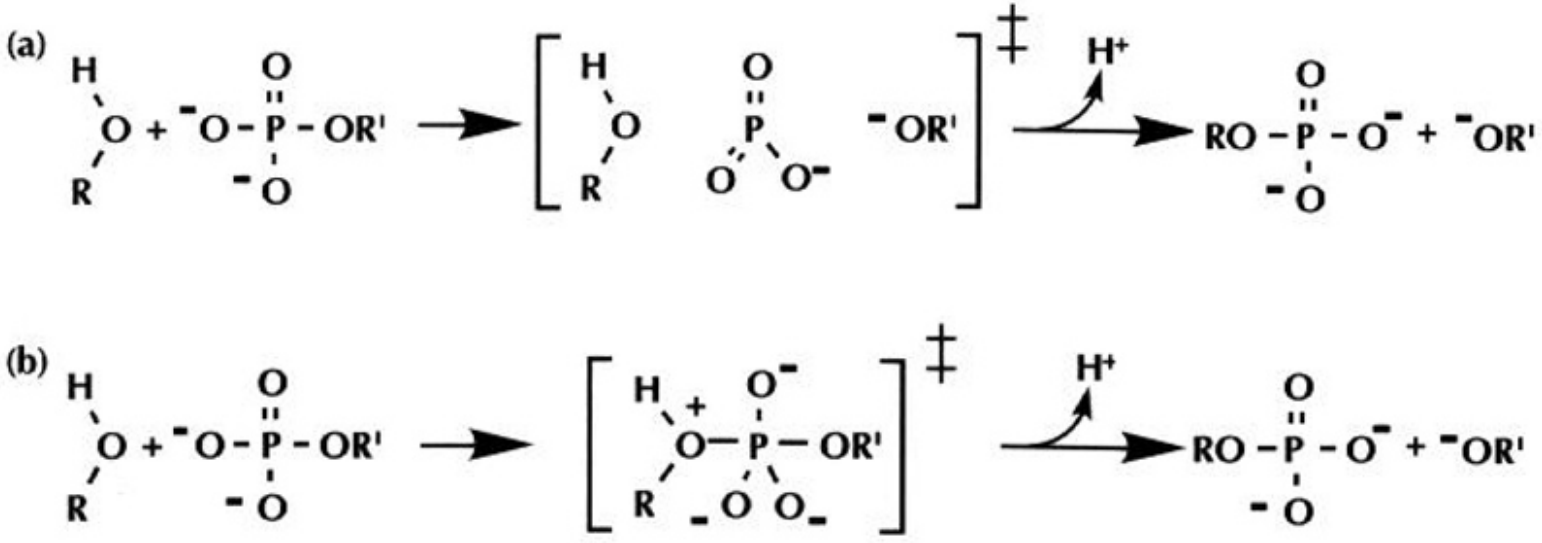
An Open & Closed Case for Templated DNA Synthesis



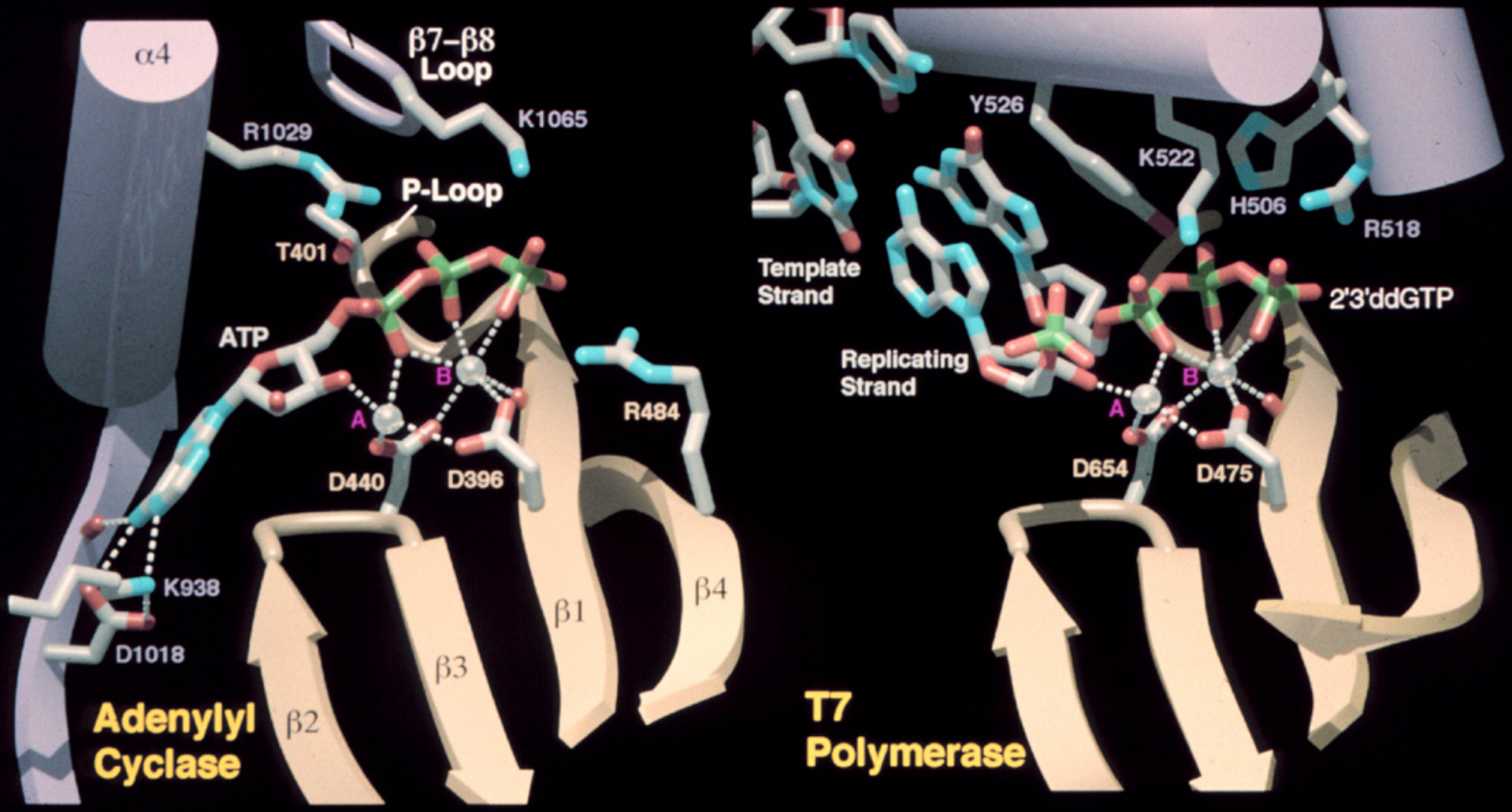
Metal-assisted Catalysis



Metal Stabilization of Transition State for Phosphoryl Transfer Reaction



A Common Strategy for Phosphoryl Transfer

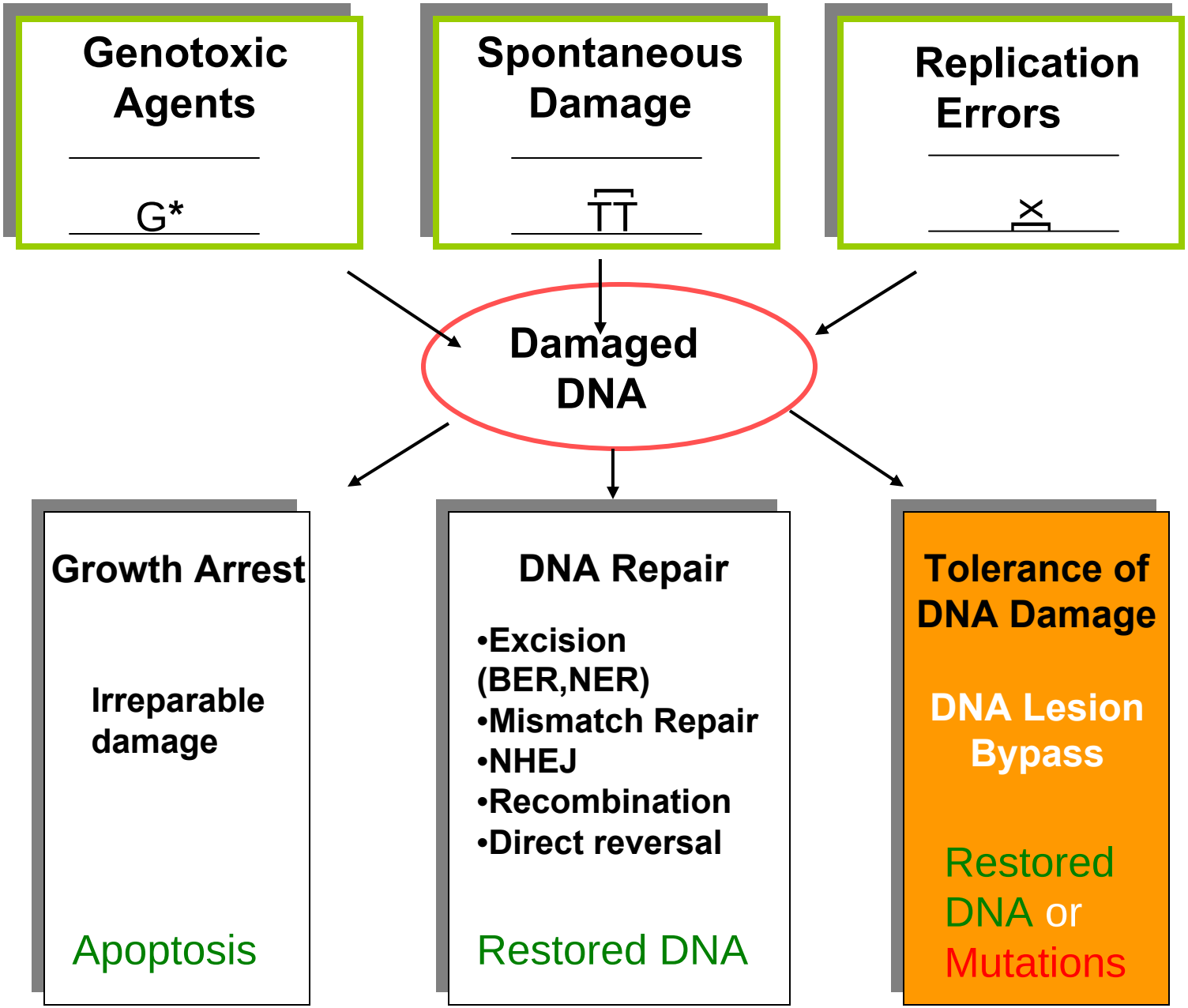


Tesmer et al. 1999. Science *285*, 756-60

Unanswered Questions

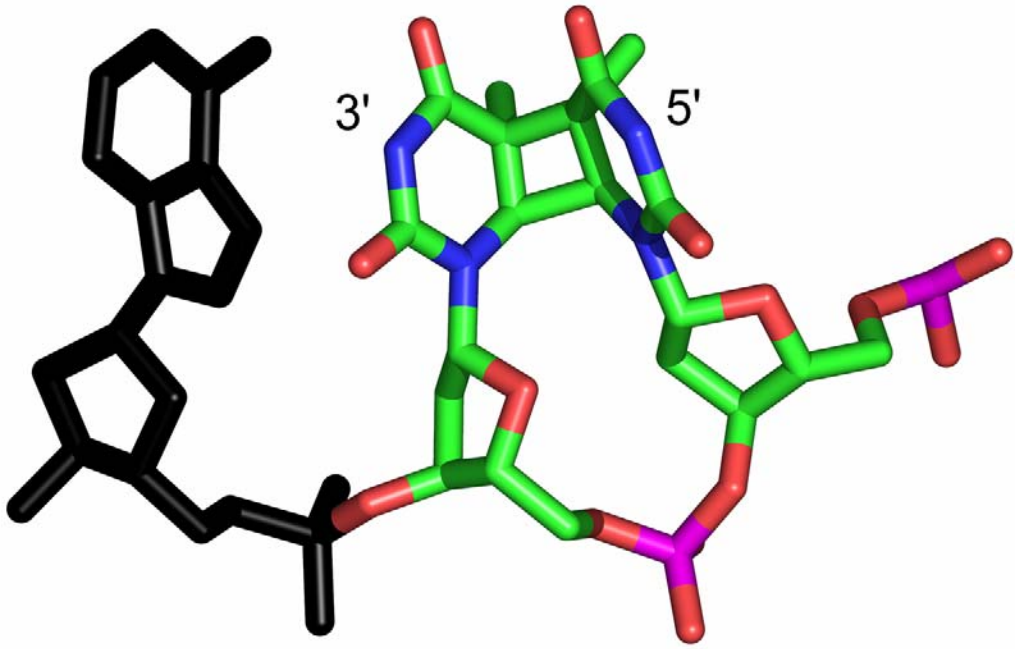
- Mechanism of DNA transport? Coupled to dNTP binding, hydrolysis?
- How does thioredoxin confer processivity?
- Switch from DNA synthesis to proofreading?
  - **Donlin, M.J., Patel, S.S., and Johnson, K.A. 1991. Kinetic partitioning between the exonuclease and polymerase sites in DNA error correction. Biochemistry. 30, 538-546.**
  - **Bailey, M.F., van der Schans, E.J. C., and Millar, D.P. 2004. Thermodynamic Dissection of the Polymerizing and Editing Modes of a DNA Polymerase J. Mol. Biol. 336, 673-693.**

- Bypass of chemical modifications that block synthesis or promote misinsertion of dNMPs.

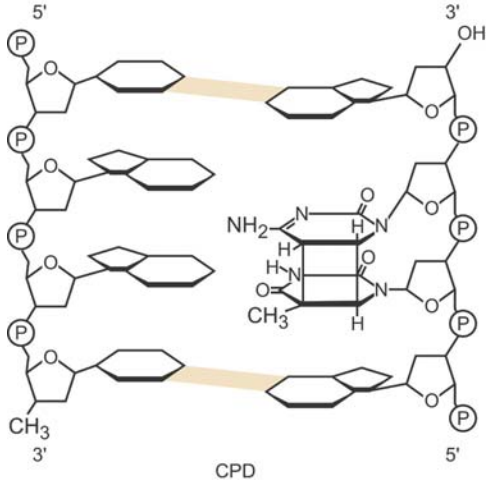


Adapted from Krokan et al. 1997 Biochem J. 325, 1-116.

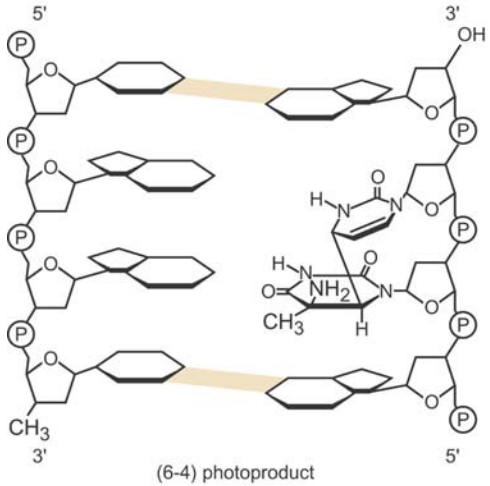
Pyrimidine Photodimers Generated by UV Irradiation of DNA



Cis-syn cyclobutane pyrimidine dimer

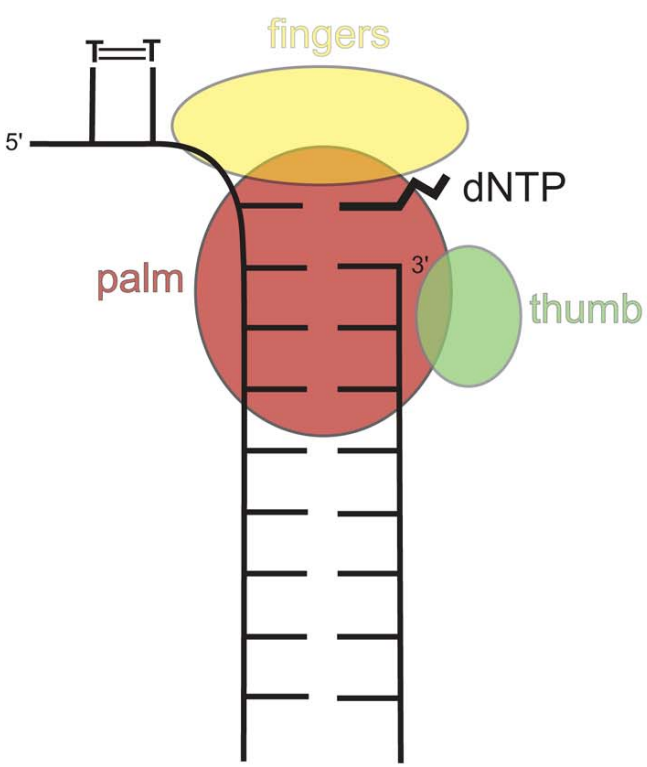


CPD

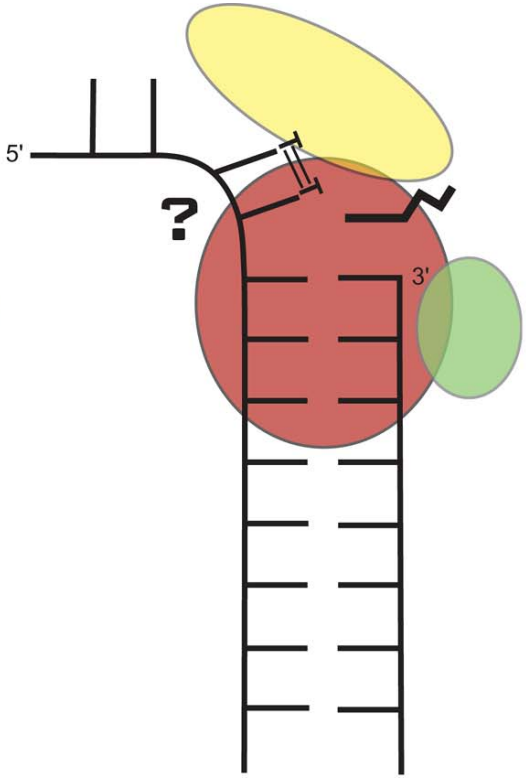


(6-4) photoproduct

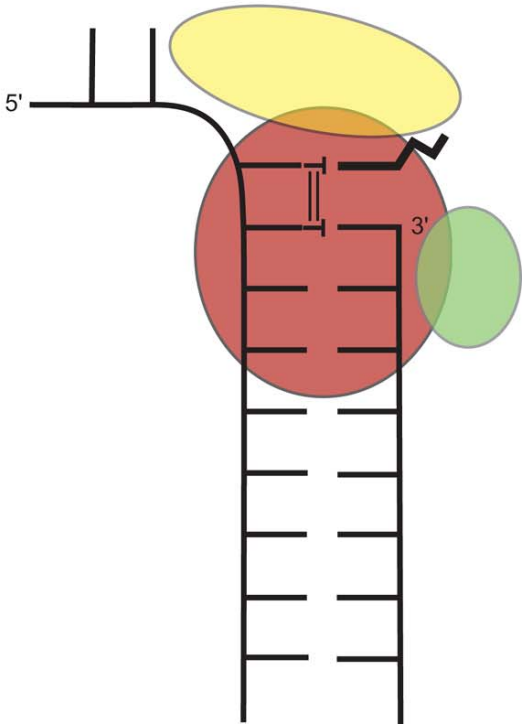
Bypass of UV Photodimers



Normal templated insertion

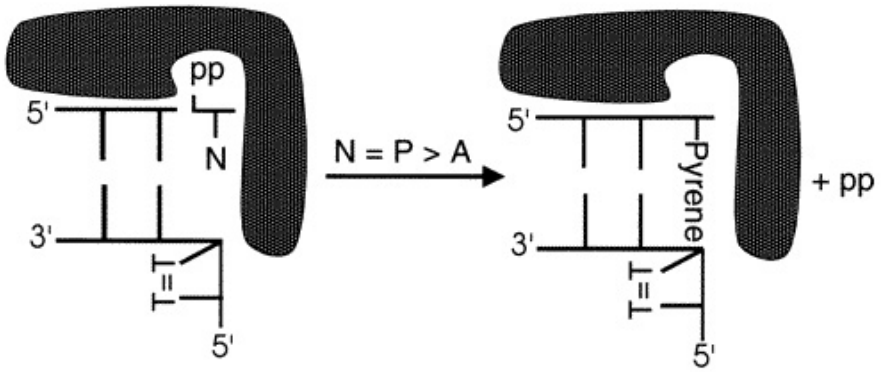


Insertion opposite 3’ T of CPD

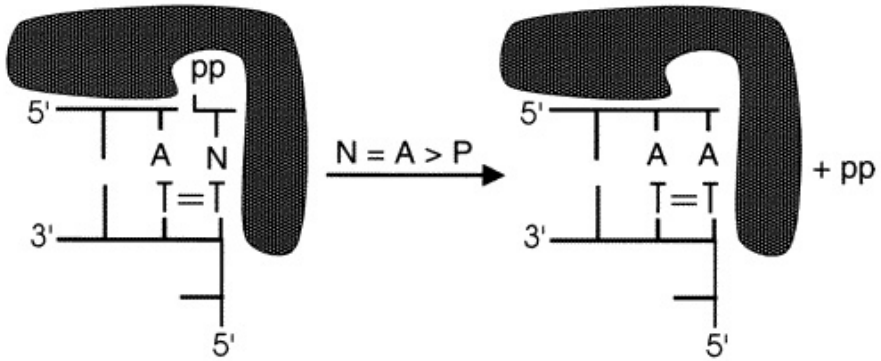


Insertion opposite 5’ T of CPD

A Model for CPD (TT Dimer) Bypass

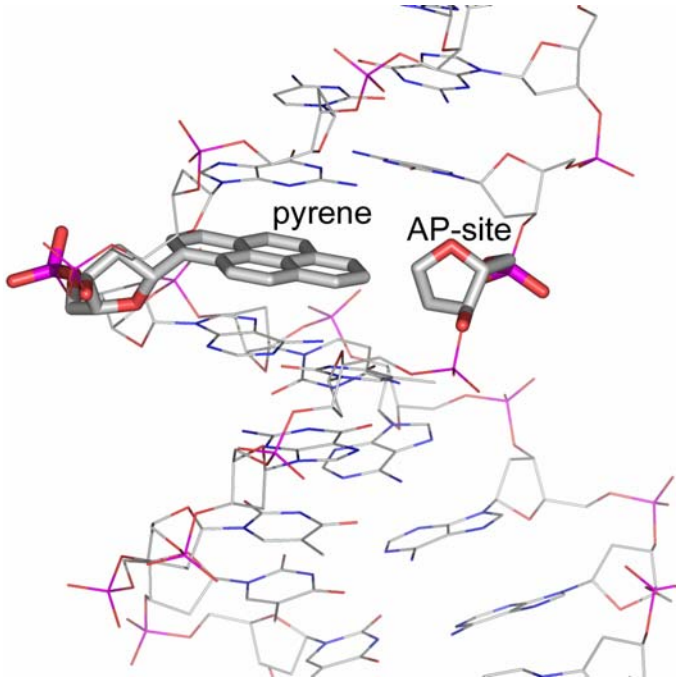


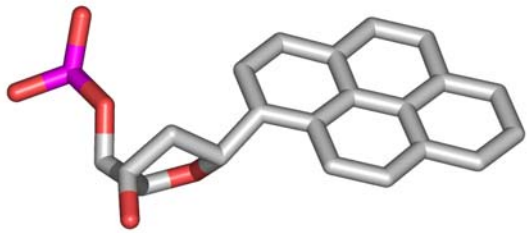
Insertion Opposite 3' T



Insertion Opposite 5' T

Sun, Taylor et al. 2000. Biochemistry 39, 14603.

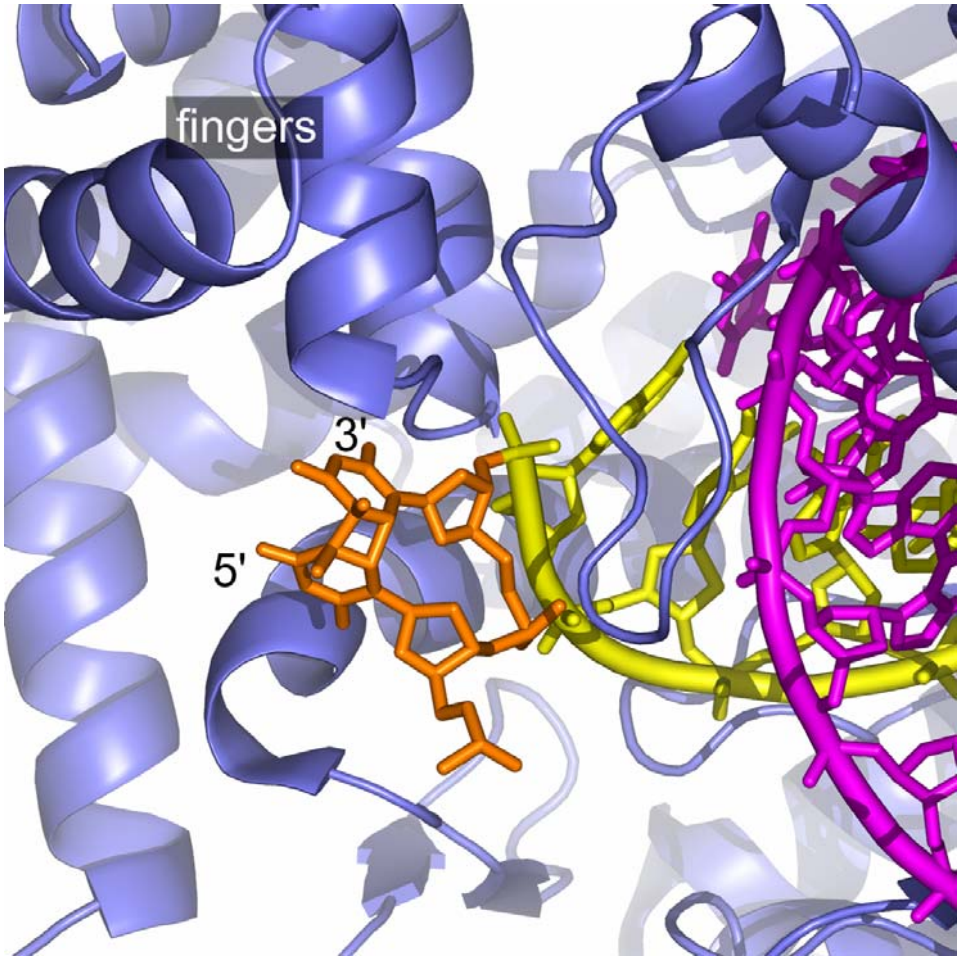




Pyrene Nucleoside

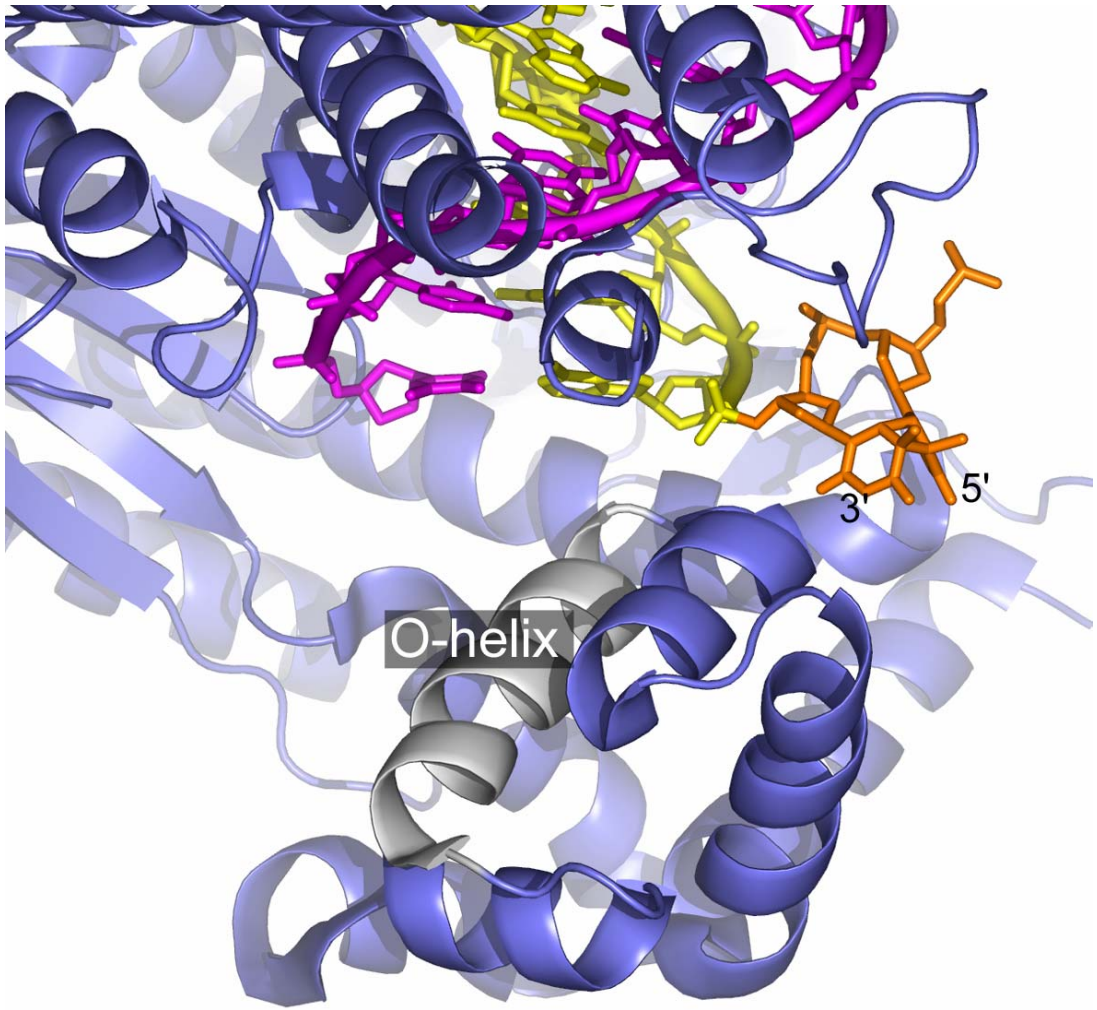
Smirnov, Kool, et al. 2002.
Nucleic Acids Res. 30, 5561.

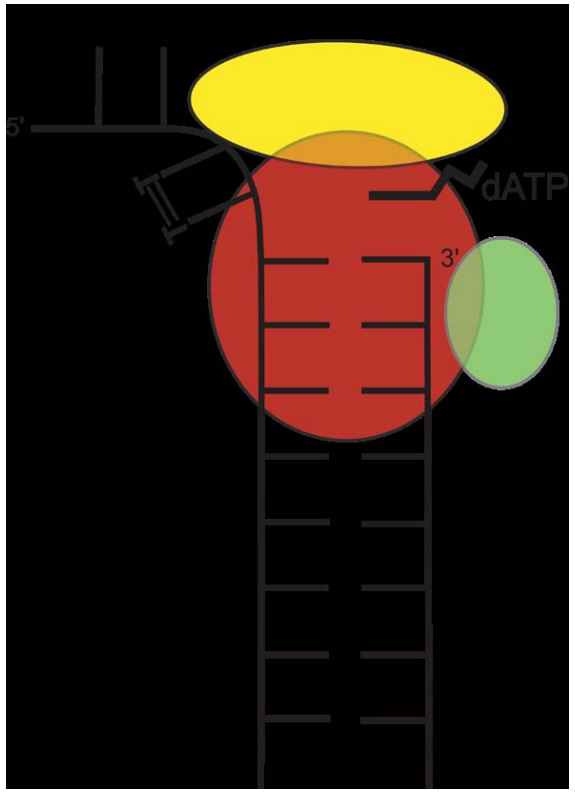
3’ T of a CPD Fails to Bind in the Active Site of T7 DNA Pol



Li et al. 2004. Nature Struct. Mol. Biol. 11, 784.

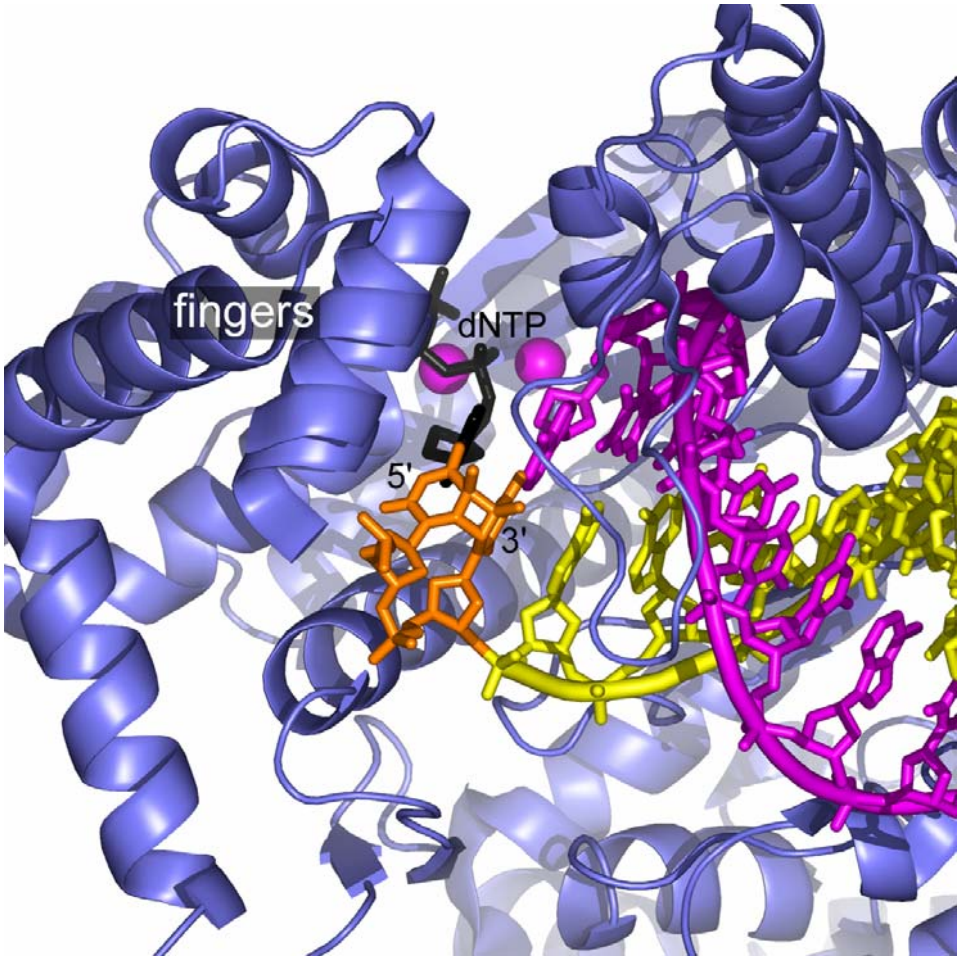
3’ T of CPD Is Non-instructional





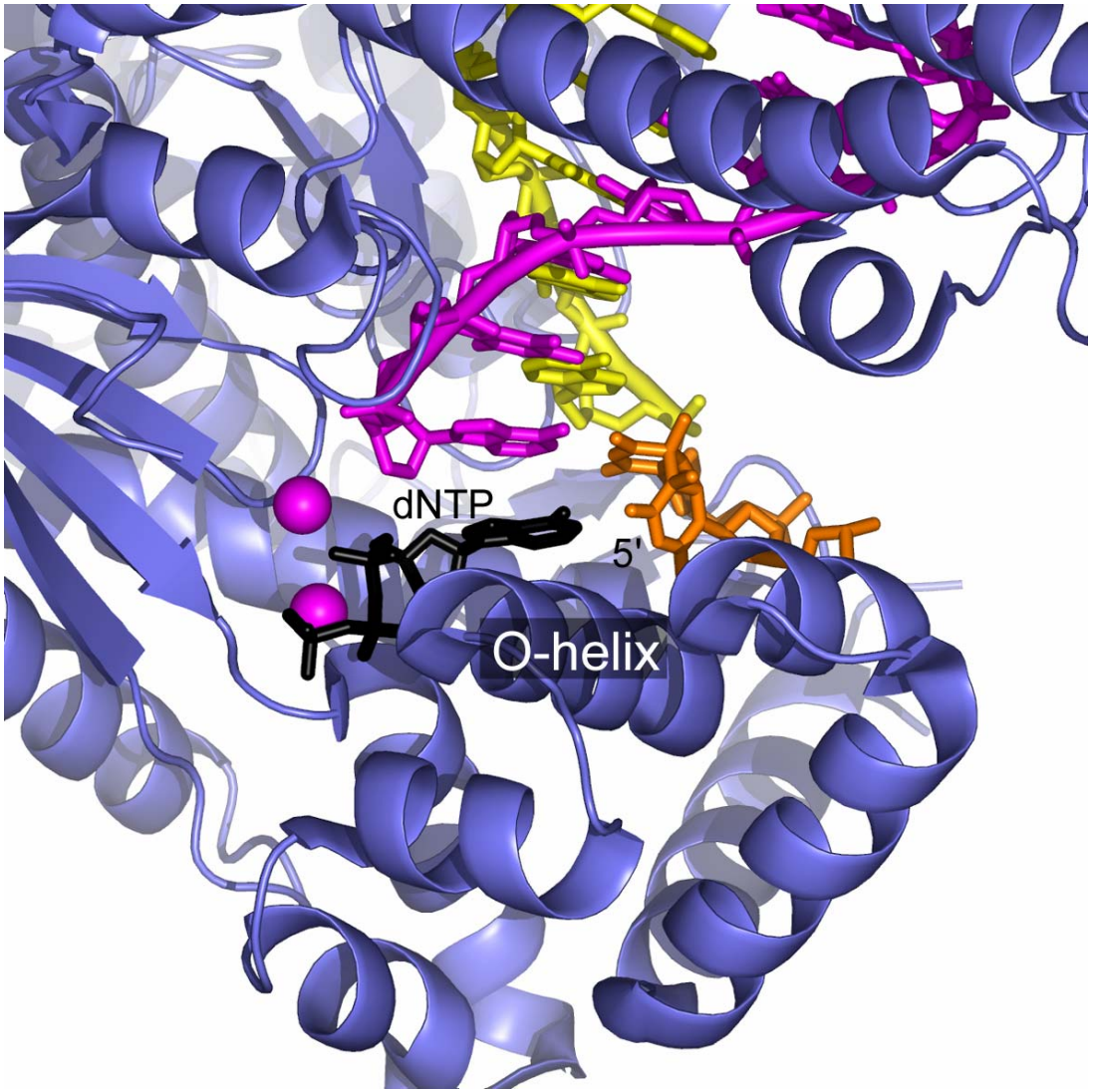
Ying Li, Shuchismita Dutta

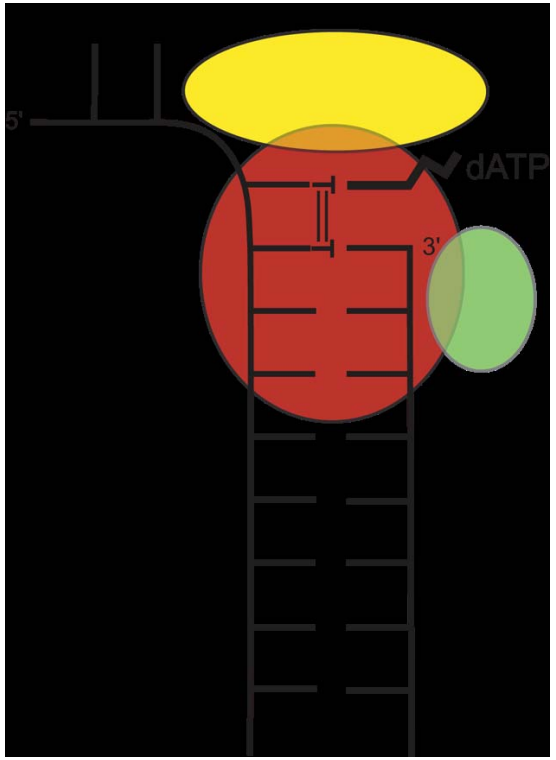
5’ T of CPD Base Pairs With dATP in the Active Site of T7 DNA Pol



Ying Li, Shuchismita Dutta

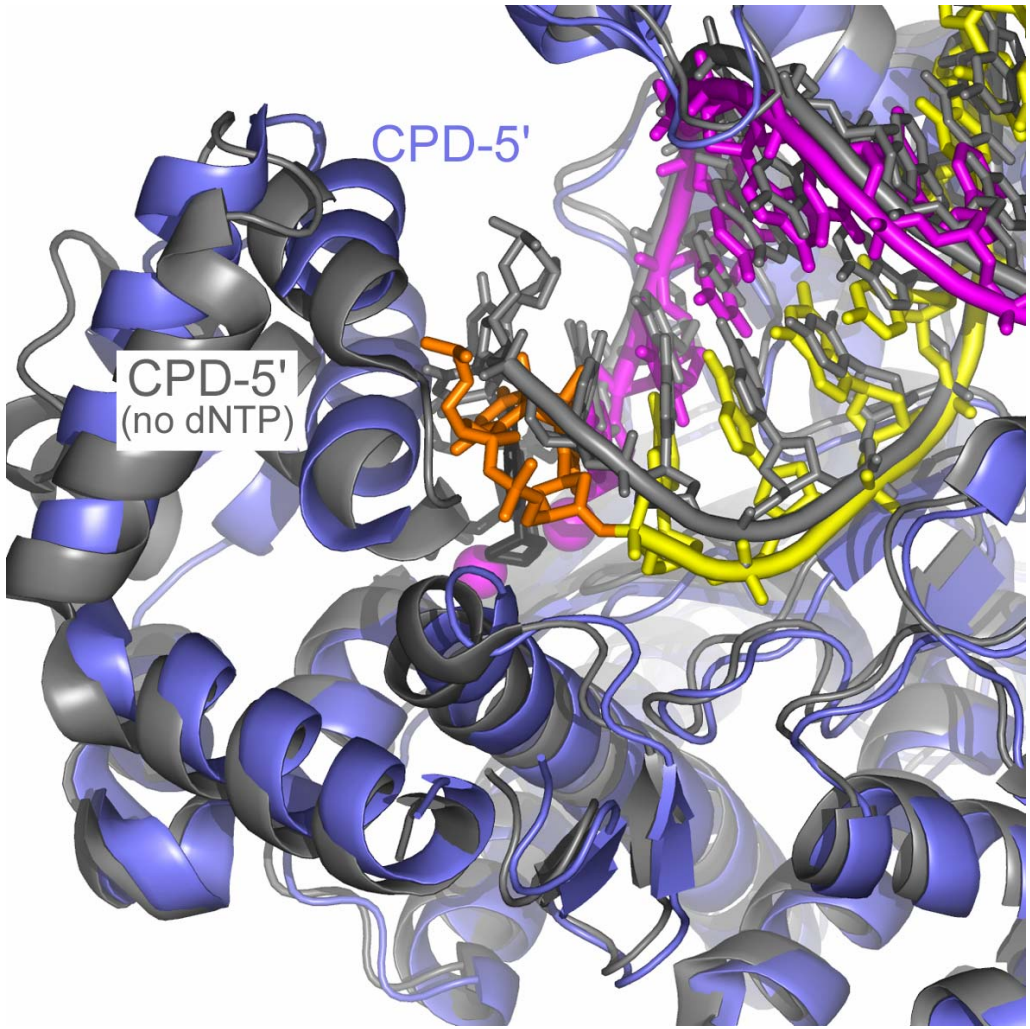
5’ T Templates Insertion of dAMP





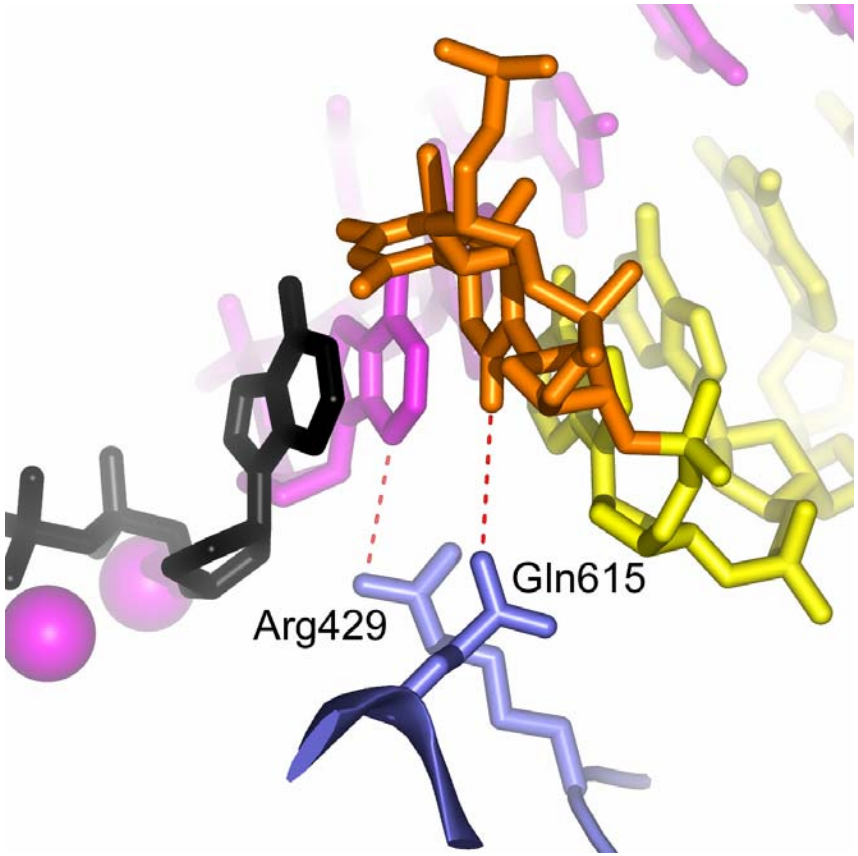
Ying Li, Shuchismita Dutta

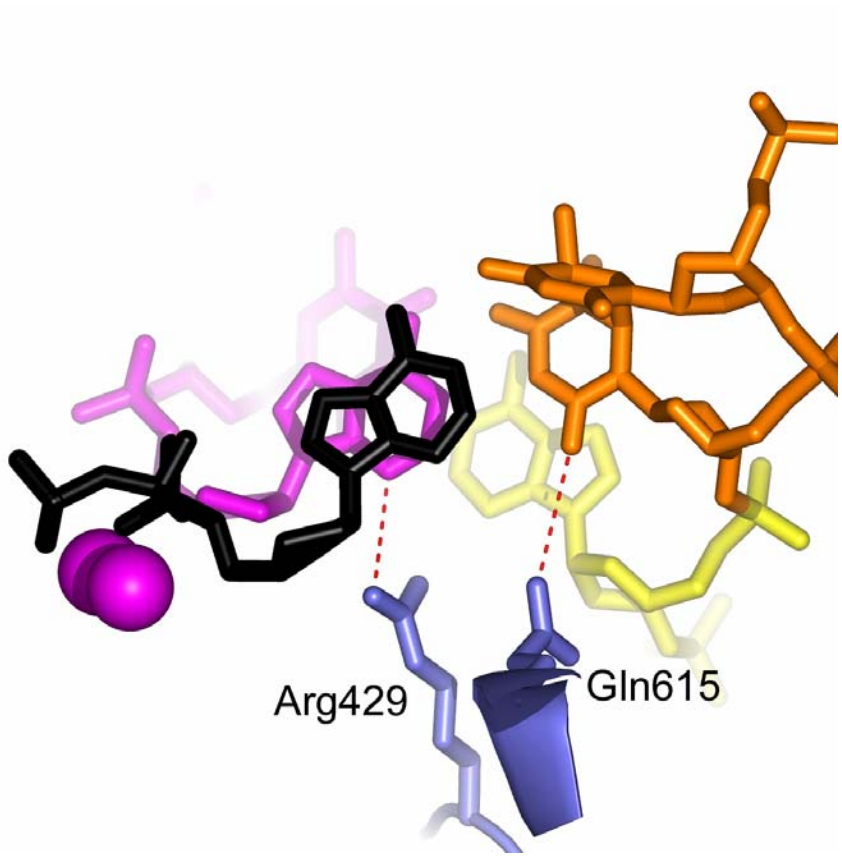
5’T(CPD):dATP Base Pair Is Destabilizing Compared to a Native T:dATP



Ying Li, Shuchismita Dutta

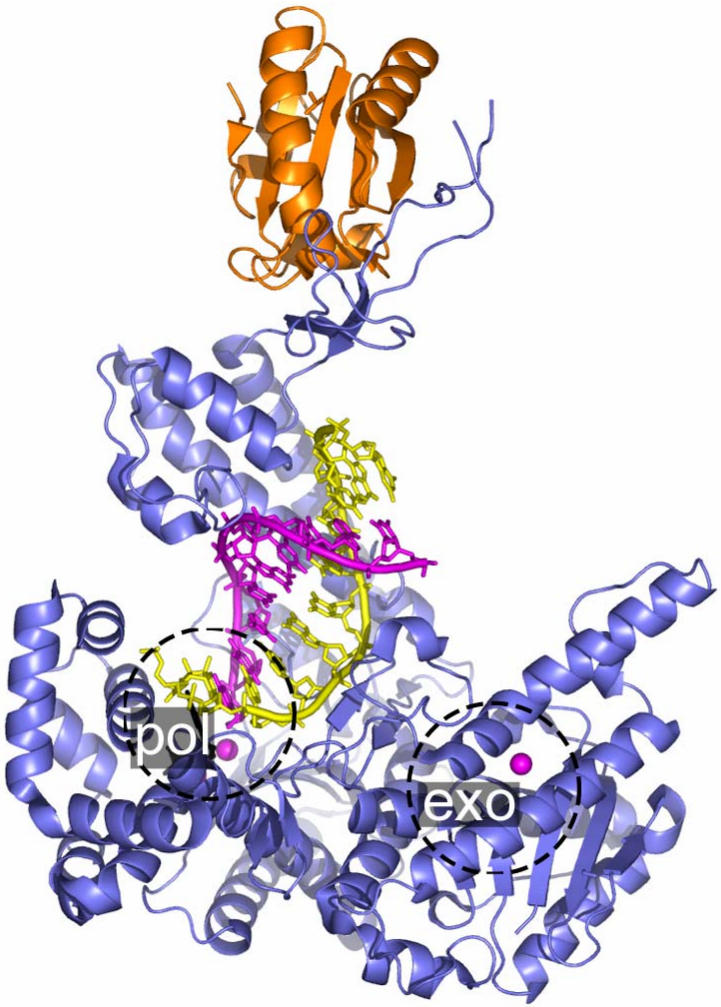
Minor Groove Interactions: A Trigger for Proofreading?





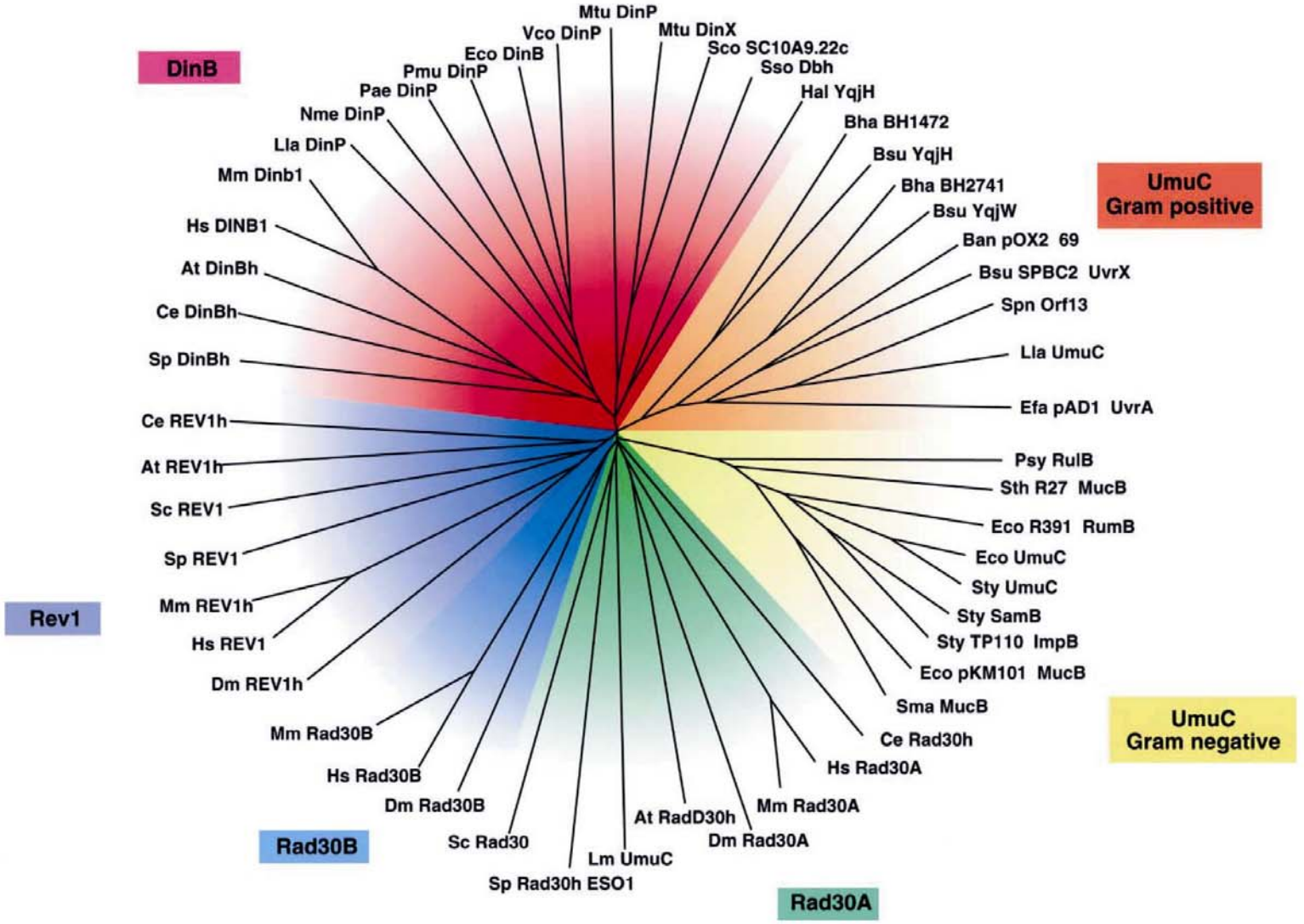
Doublié & Ellenberger. 1998. Curr. Opin. Struct. Biol. 8, 704.

Decreased Affinity for Pol Active Site Favors Proofreading Opposite a CPD



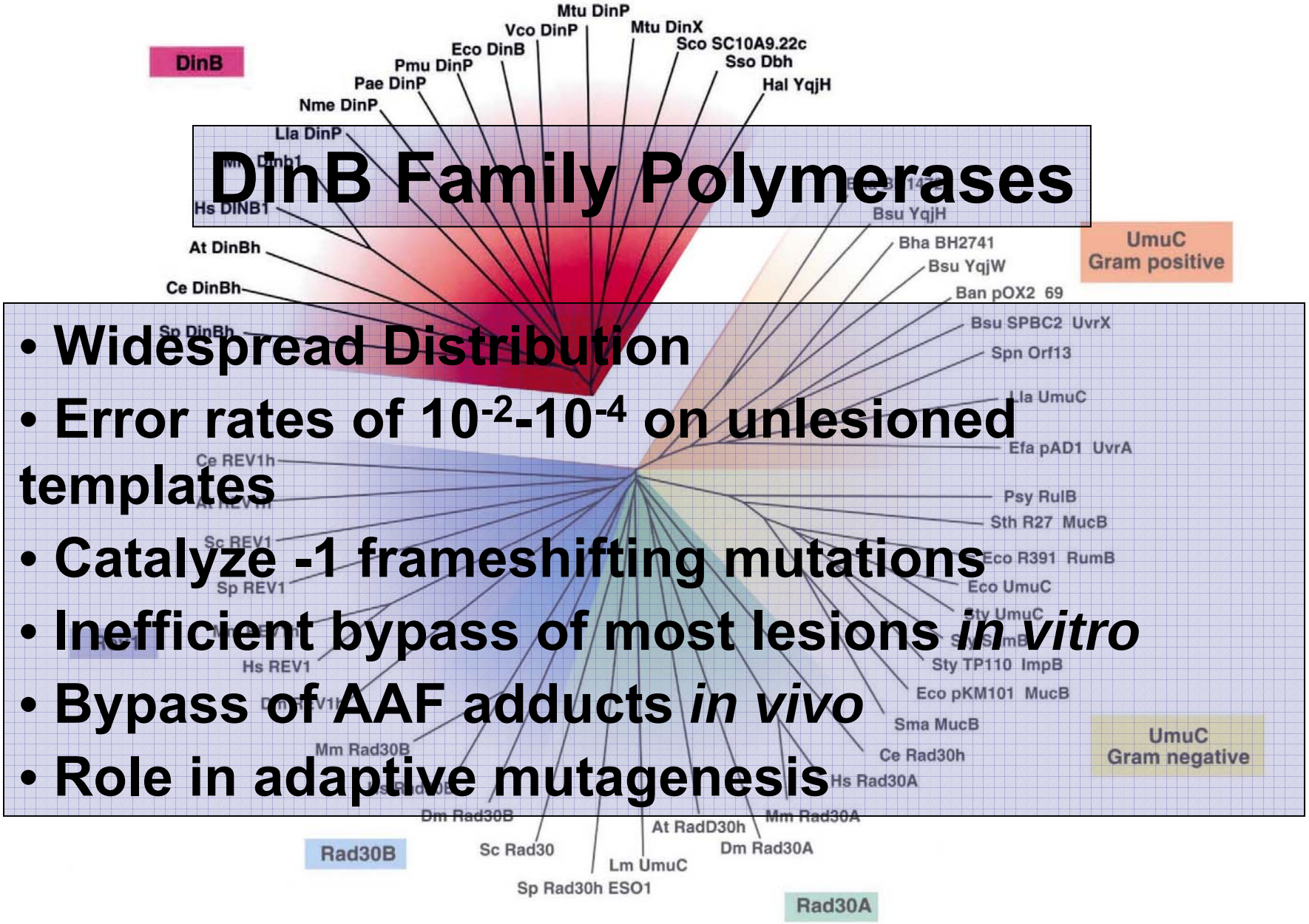
Doublié et al. 1998. Nature 391, 251.

Y Superfamily, Error-prone Polymerases



Ohmori et al. Molecular Cell, Vol 8, 7-8, July 2001

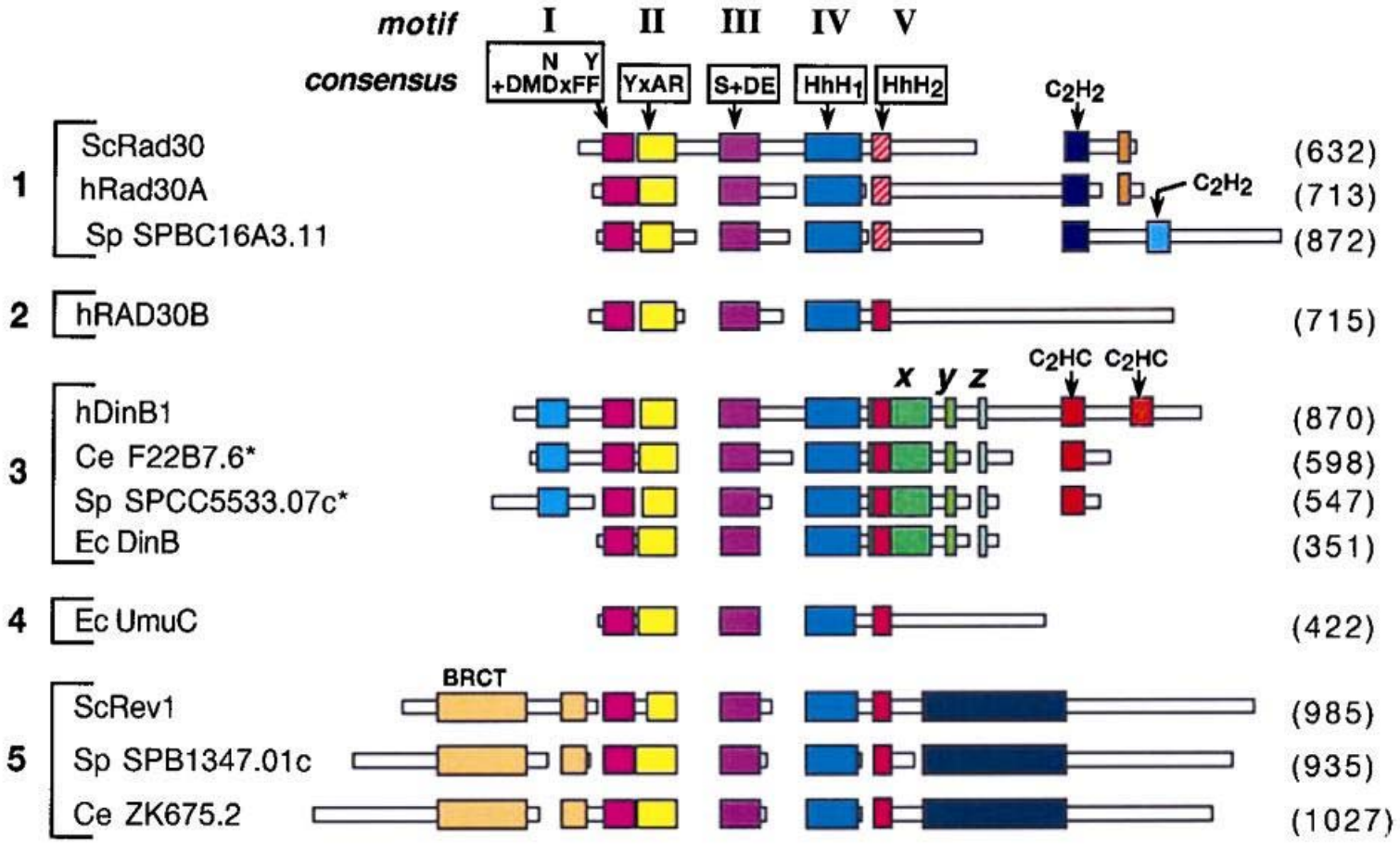
DinB Family Polymerases



- Widespread Distribution
- Error rates of 10^{-2}-10^{-4} on unlesioned templates
- Catalyze -1 frameshifting mutations
- Inefficient bypass of most lesions *in vitro*
- Bypass of AAF adducts *in vivo*
- Role in adaptive mutagenesis

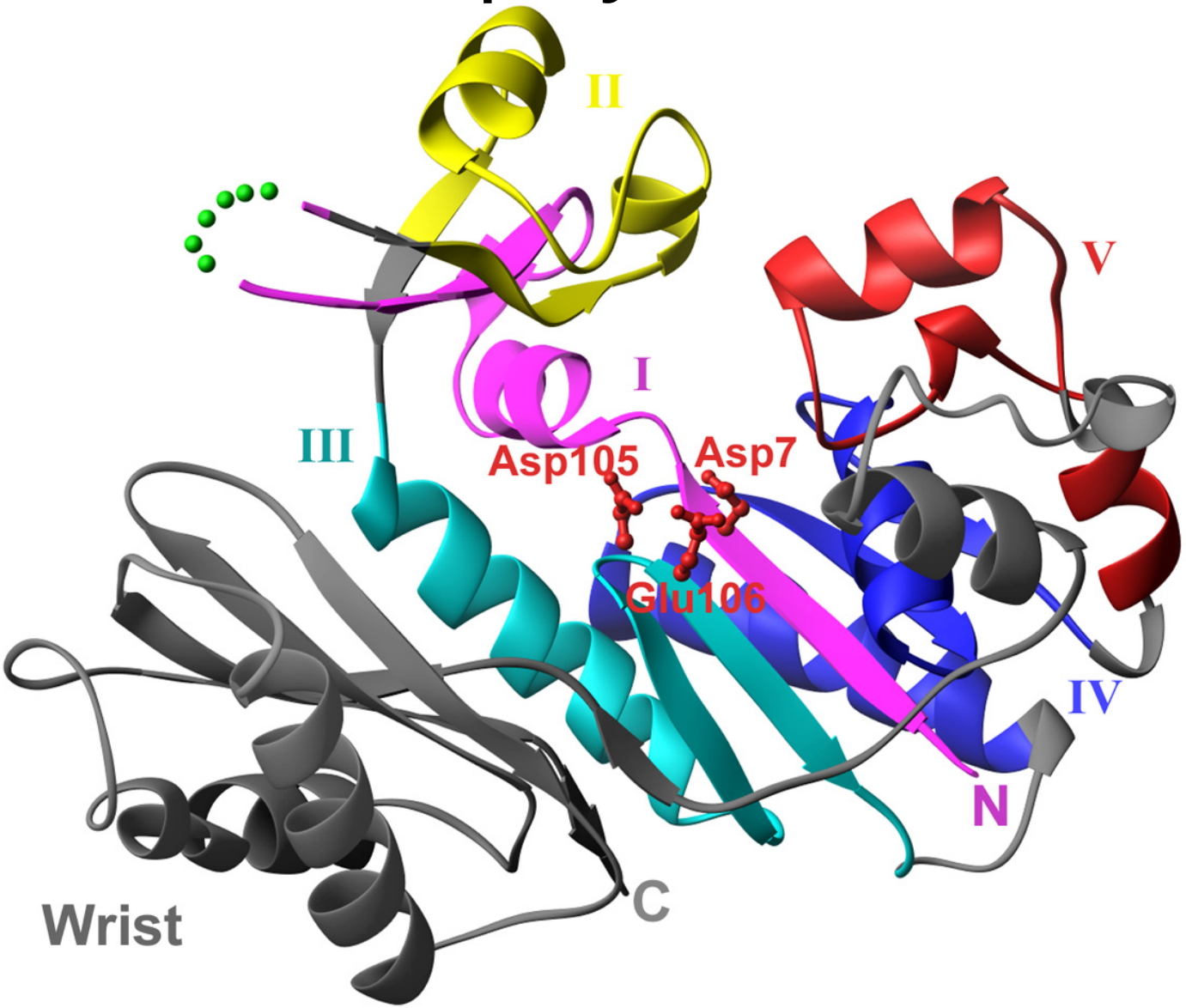
Ohmori et al. Molecular Cell, Vol 8, 7-8, July 2001

Motifs of Y Family Polymerases



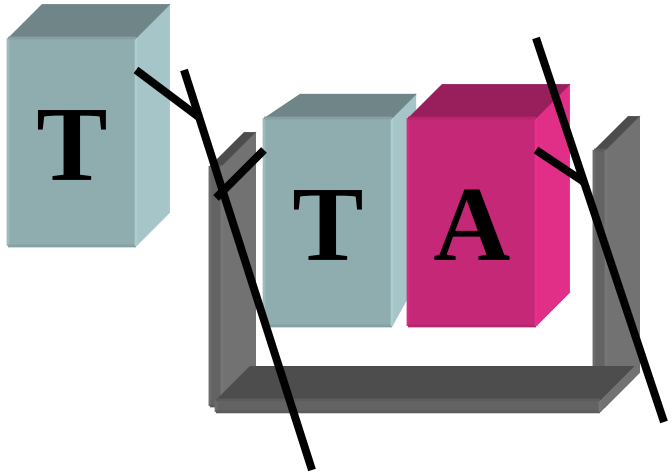
Johnson et al. 2000. Proc. Natl Acad. Sci. USA 96, 12224-12226.

Smaller Subdomains of Y Family DNA polymerases



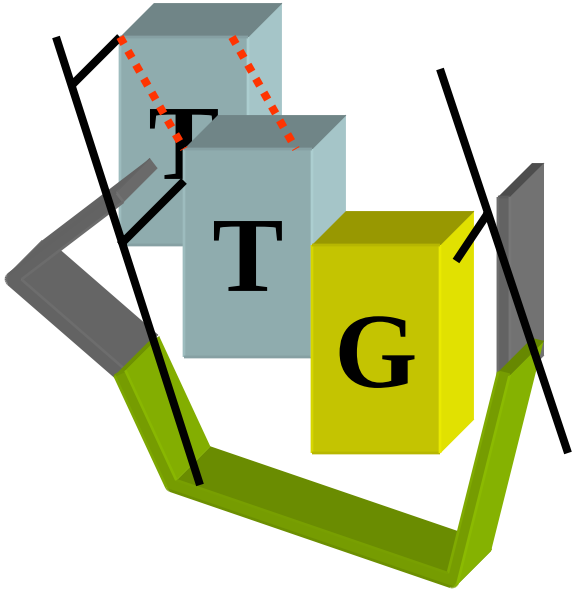
Loose, Flexible Active Site Proposal

Replicative/Repair Polymerases



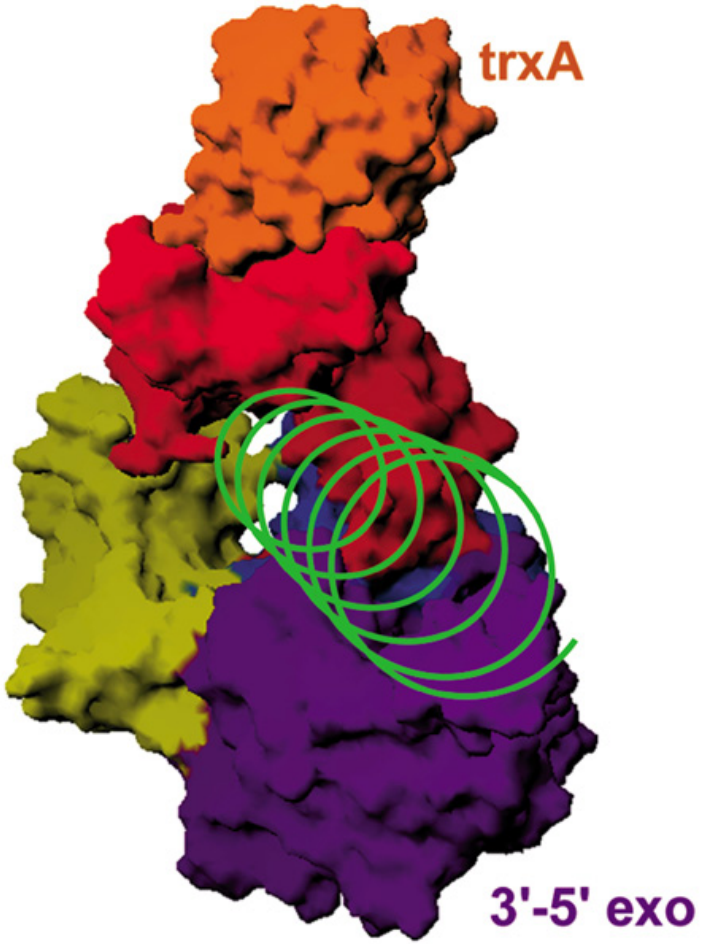
Geometric Selection for Watson-Crick pair

Lesion-bypass Polymerases

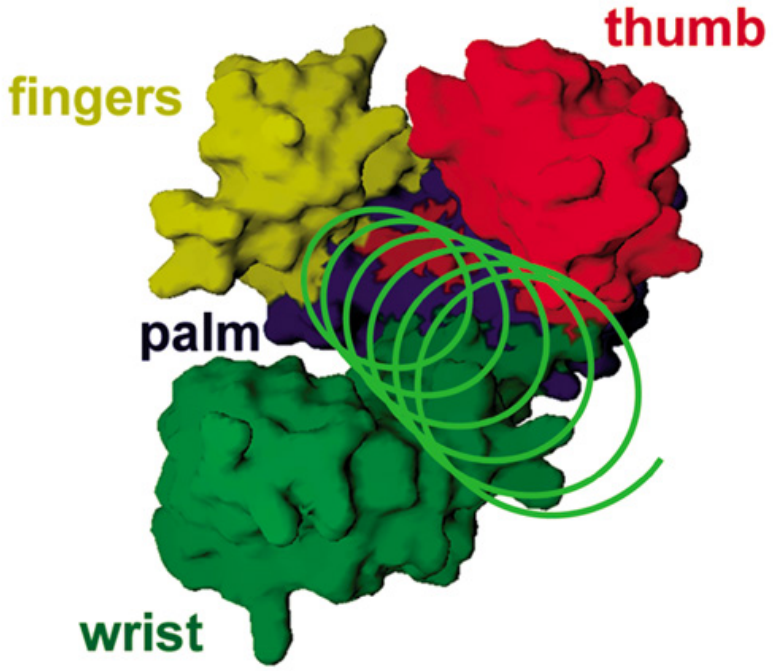


Active site with fewer constraints

Open Architecture Favors Distributive Synthesis

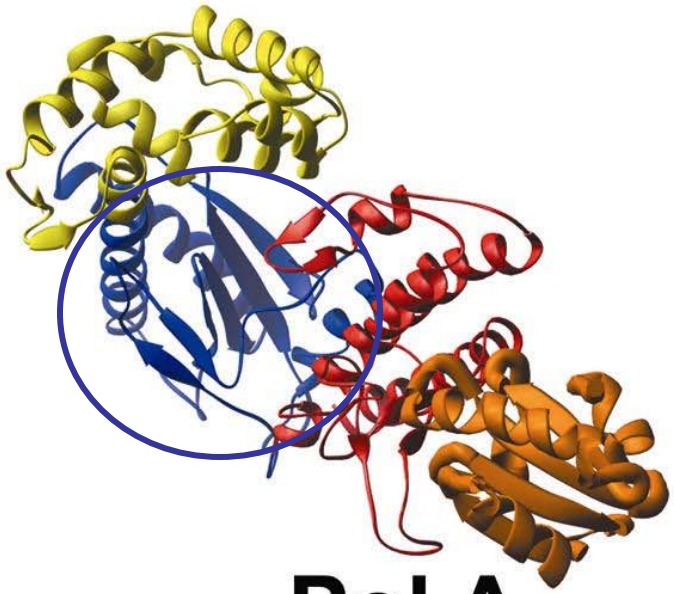


T7 DNA Pol

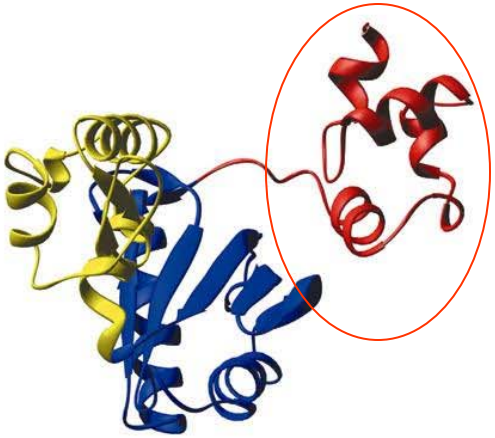


S. so Dbh

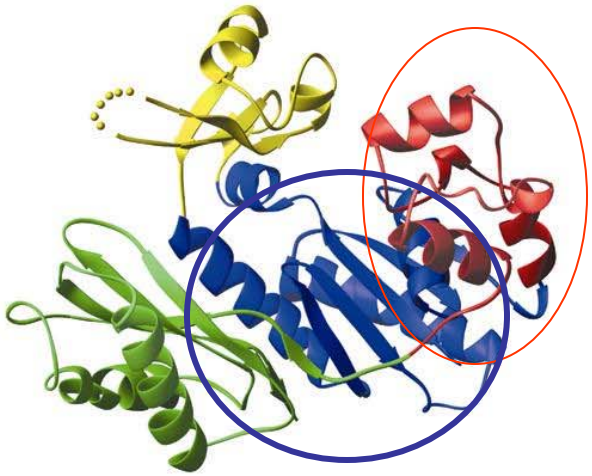
Dpo4/Dbh are Hybrid Polymerases



Pol A

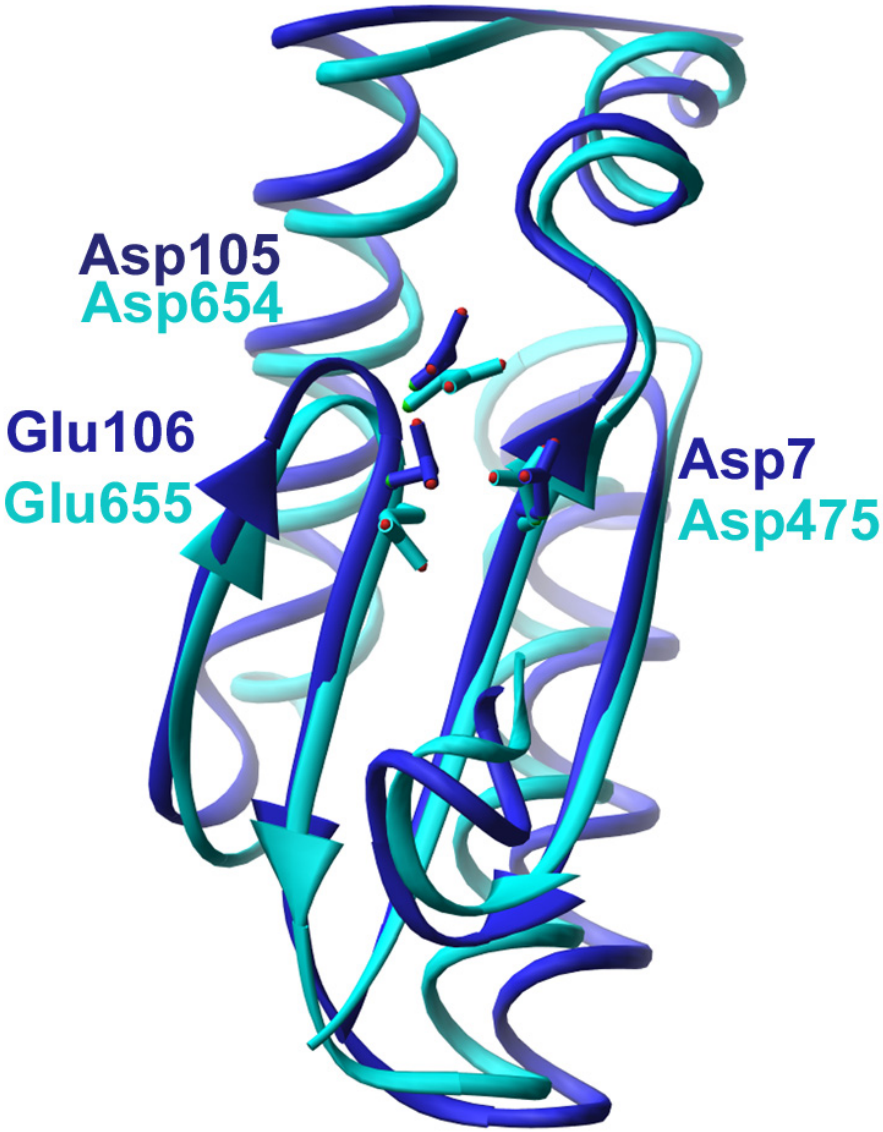


Pol X

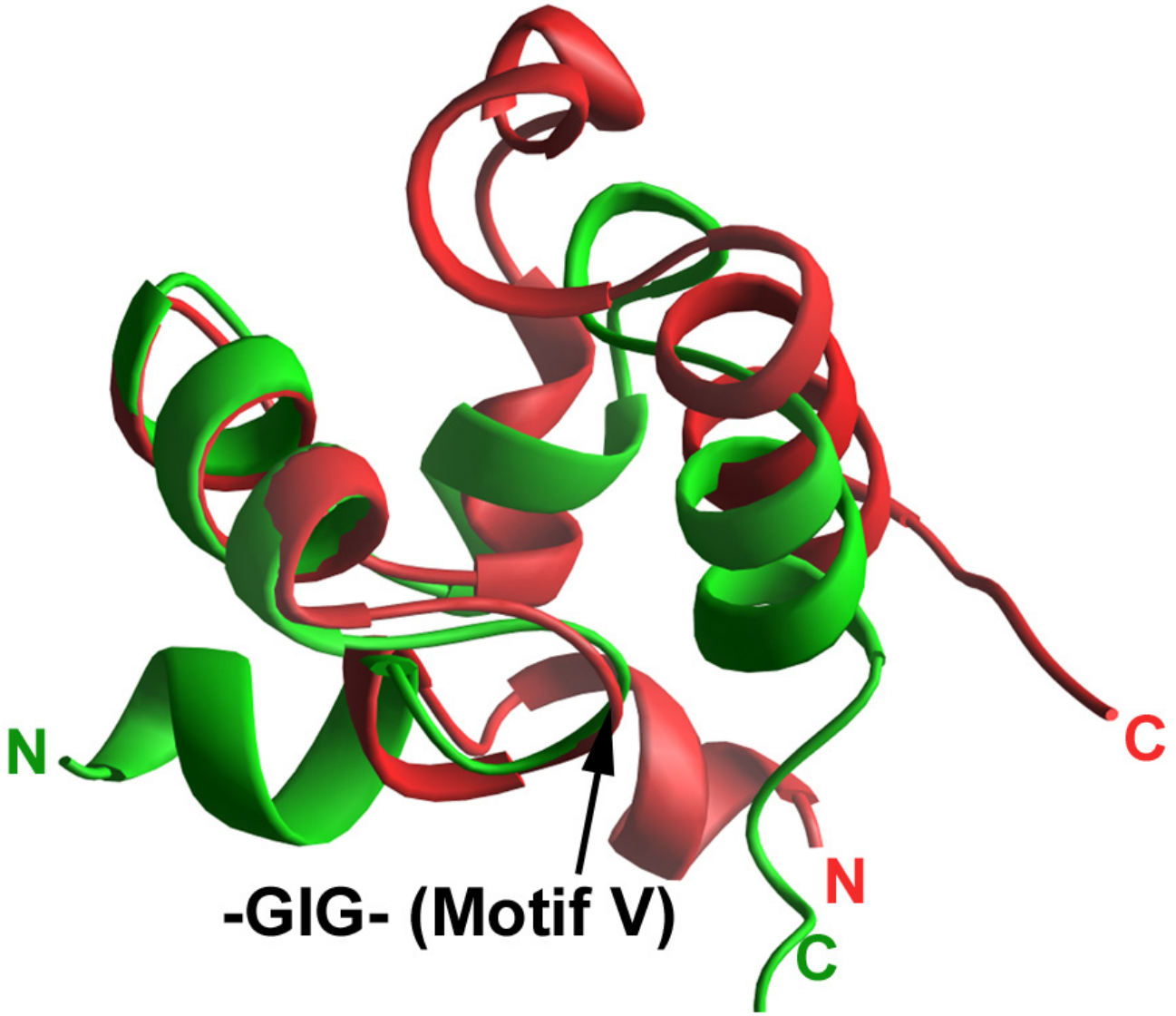


Pol Y

The Palm Subdomains of Dbh & T7DNAP Are Homologous



Thumbs of Dbh and Polβ Are Structurally Analogous



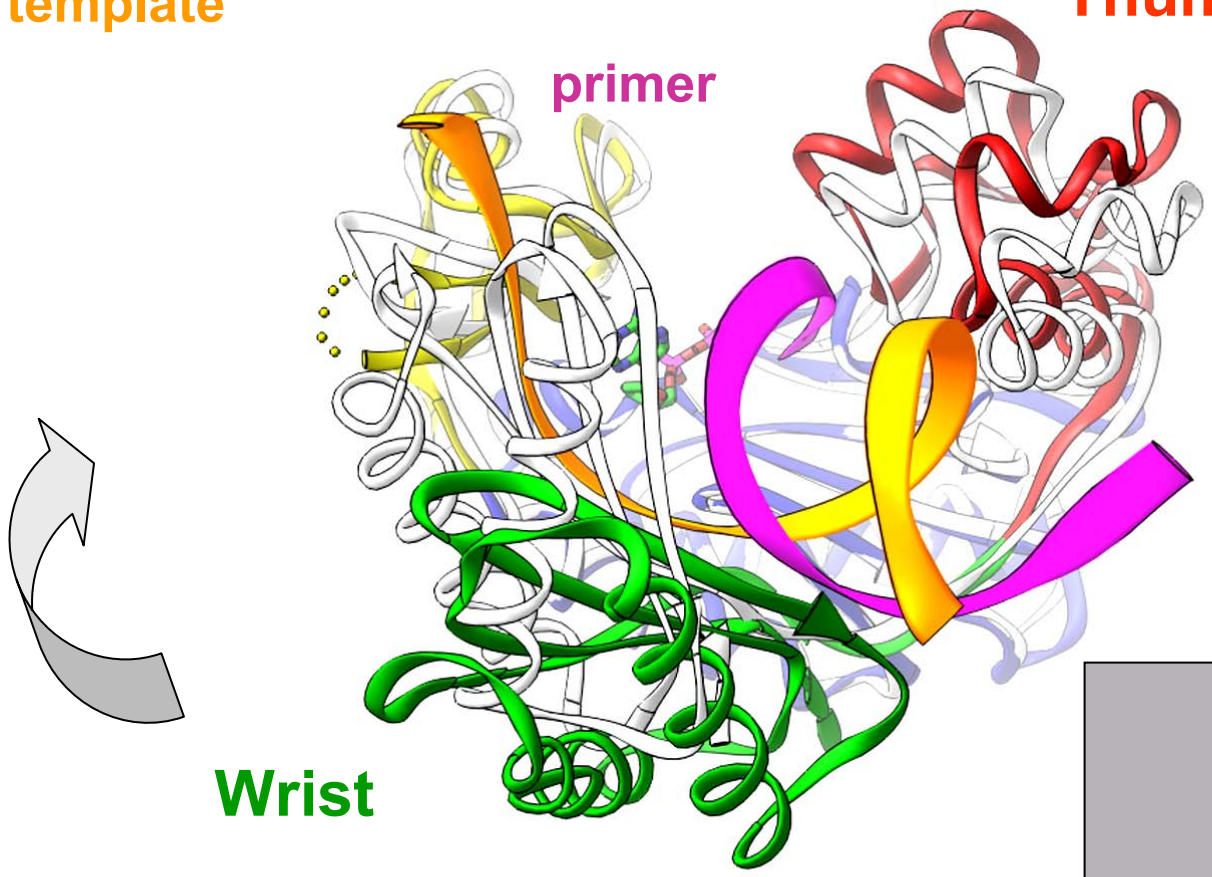
Wrist moves upon binding to DNA

Fingers

template

Thumb

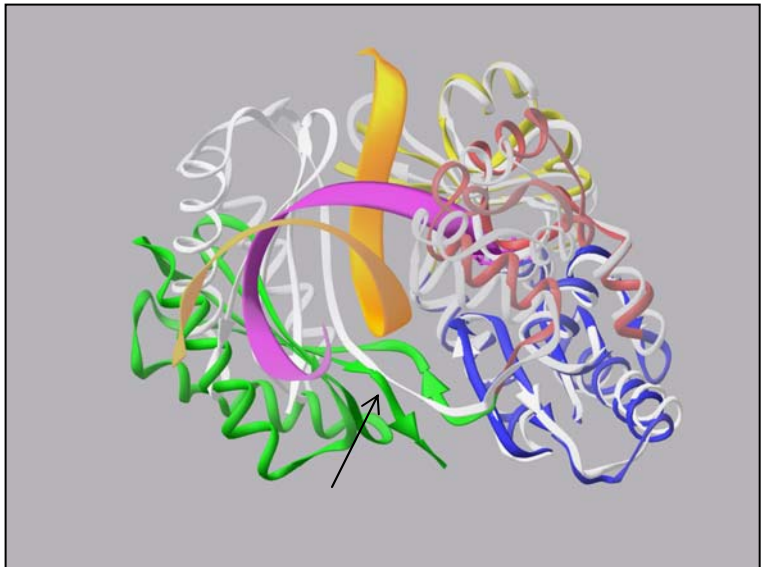
primer



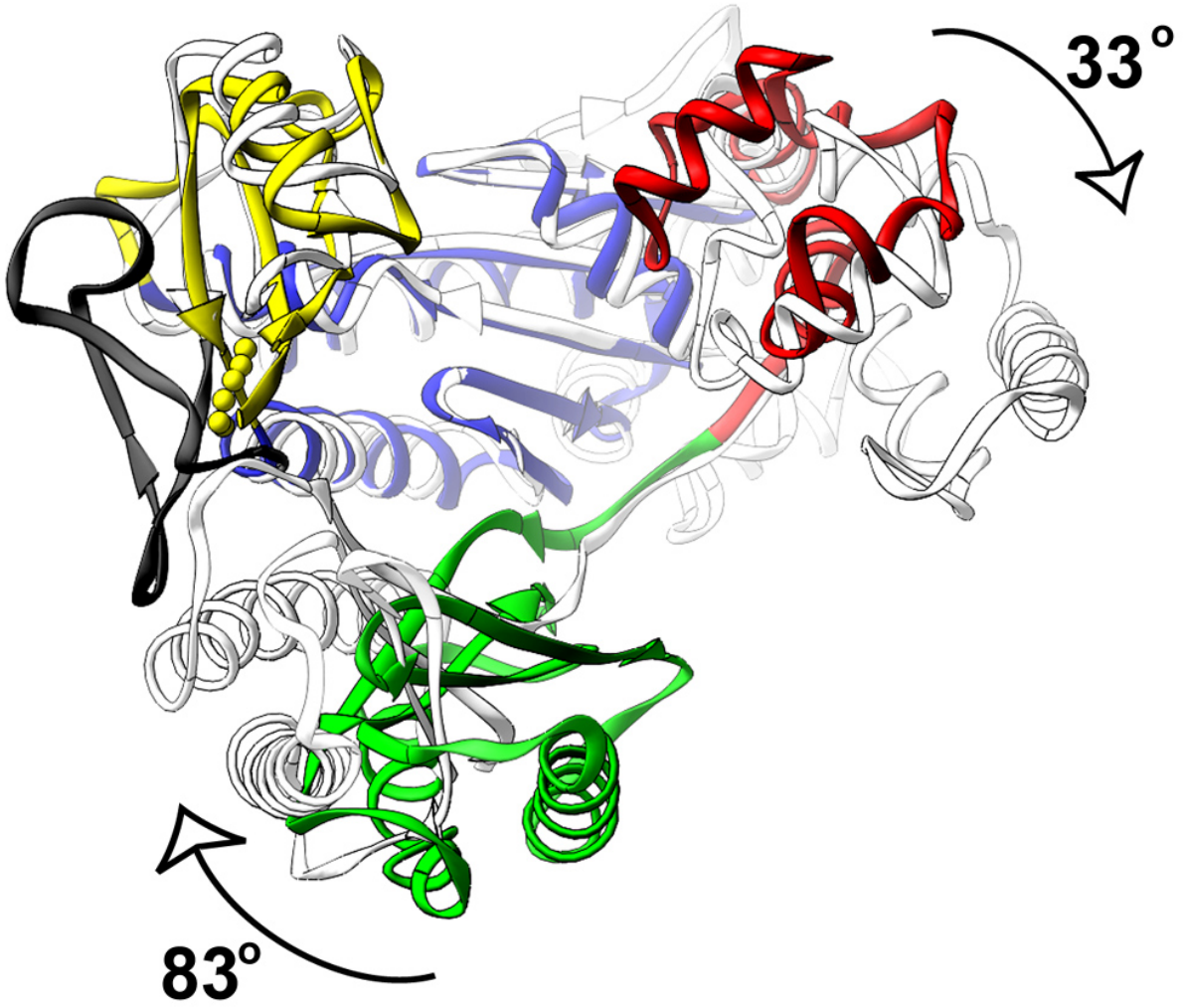
Wrist

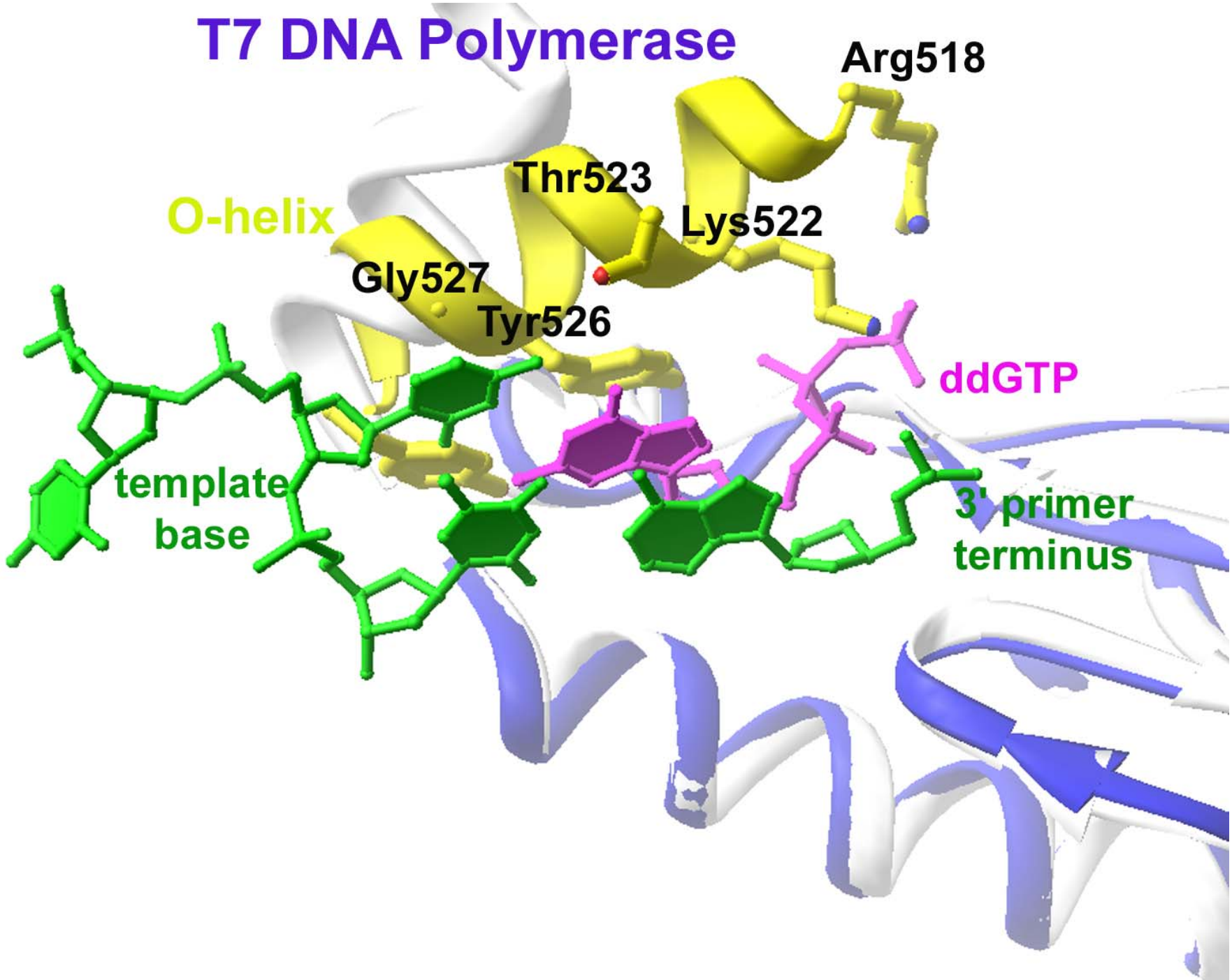
Dpo4-DNA-ddADP
ternary complex

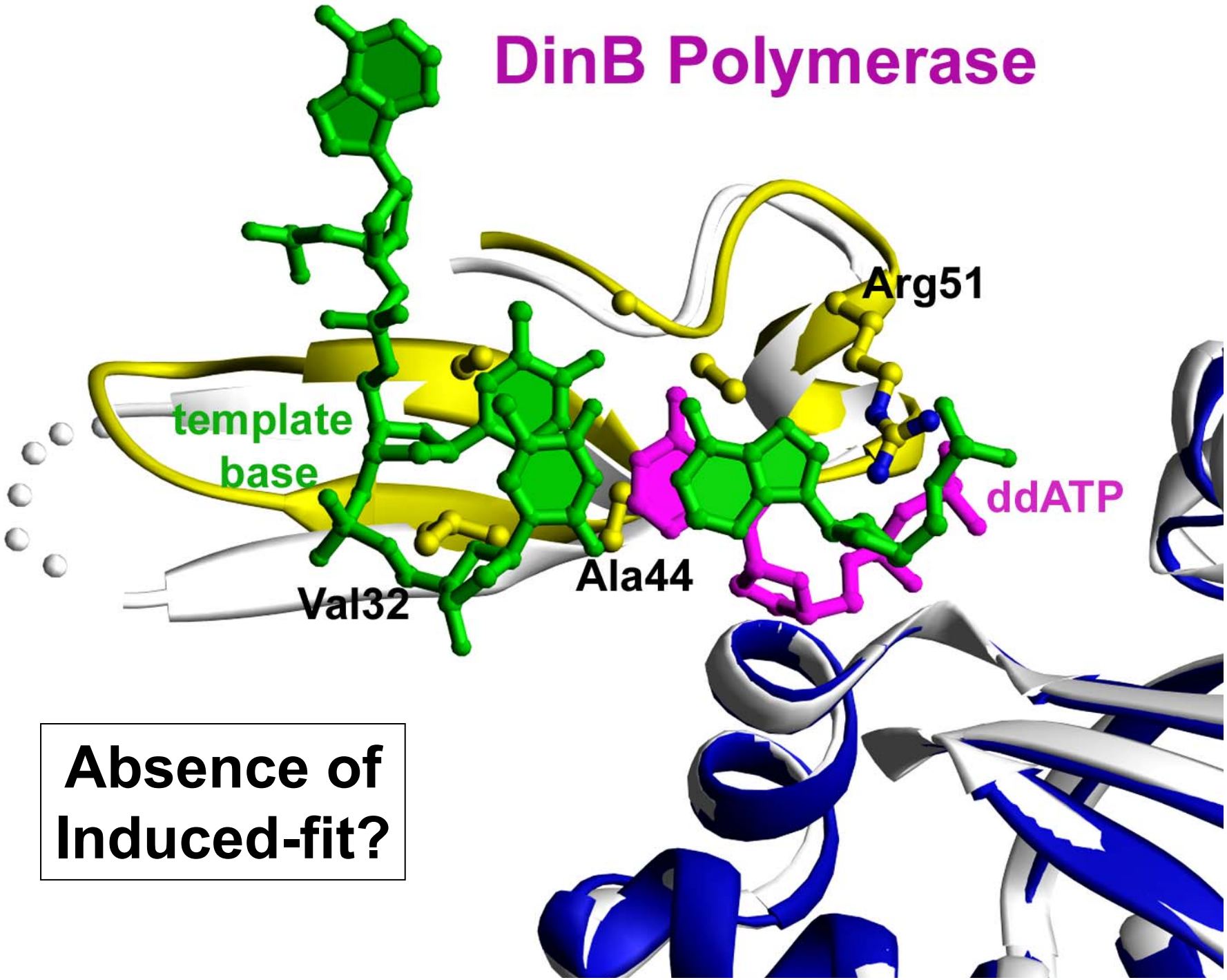
Dbh apo-enzyme



DNA-induced Movement of Domains

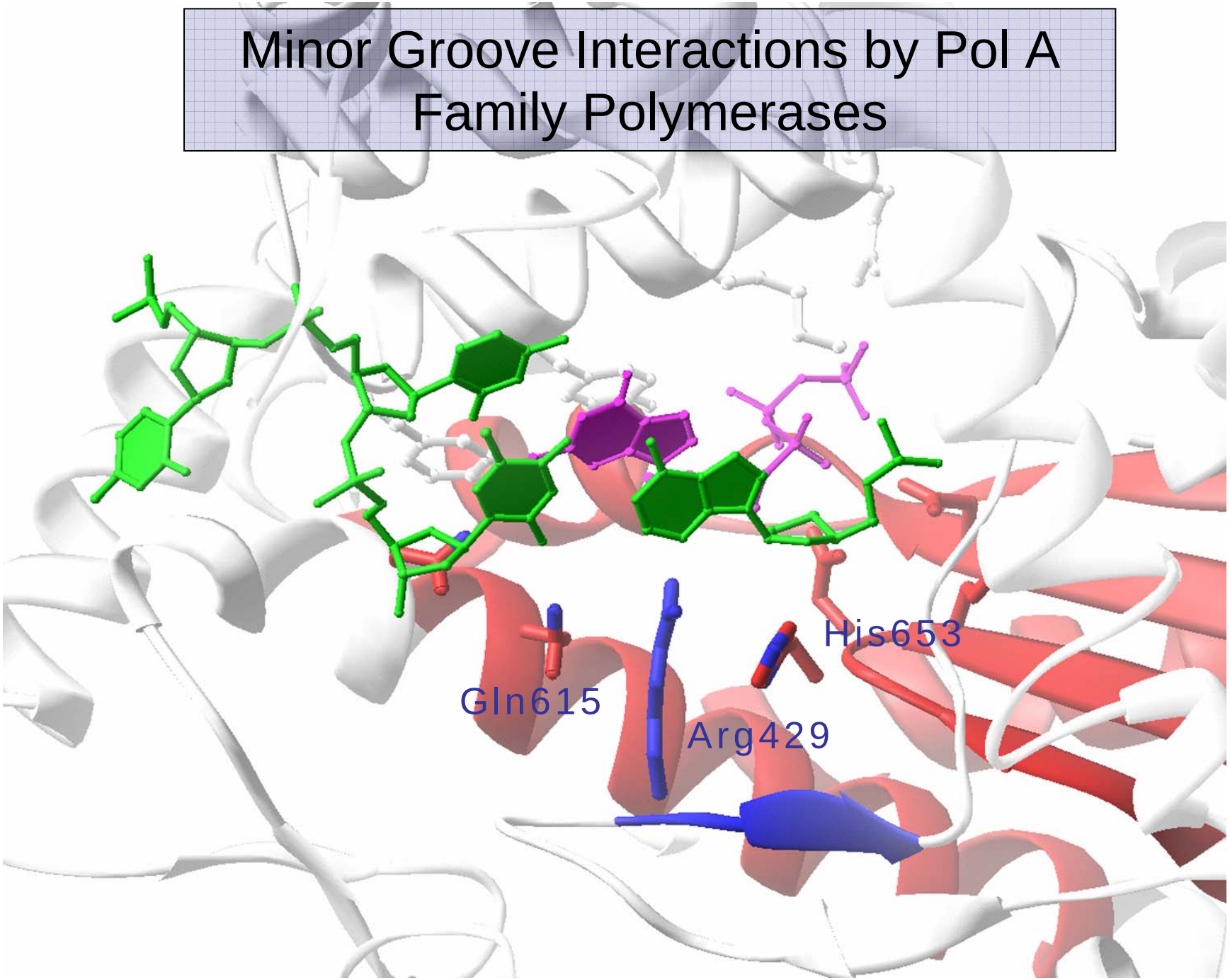



T7 DNA Polymerase
Arg518
Thr523
O-helix
Lys522
Gly527
Tyr526
ddGTP
template
base
3' primer
terminus

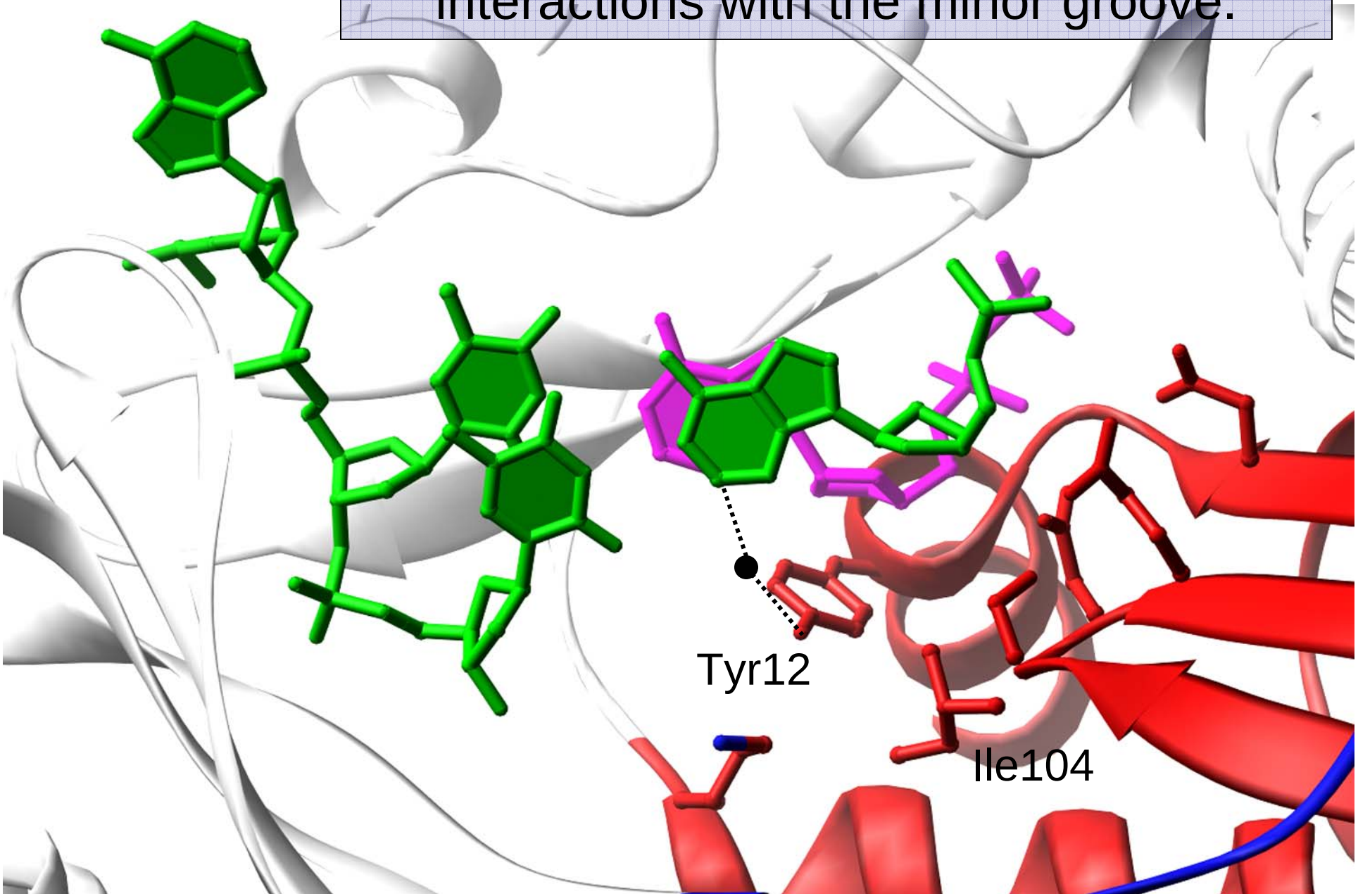


Absence of Induced-fit?

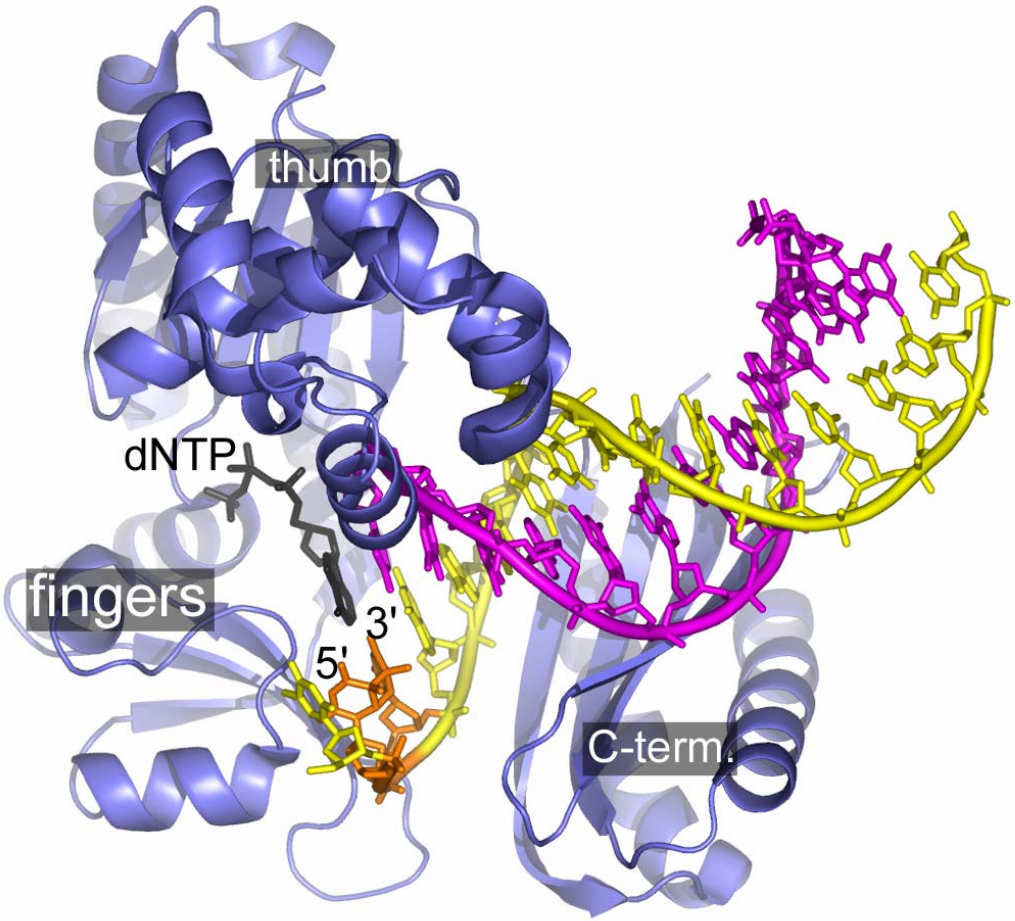
Minor Groove Interactions by Pol A Family Polymerases



DinB polymerase makes few interactions with the minor groove.

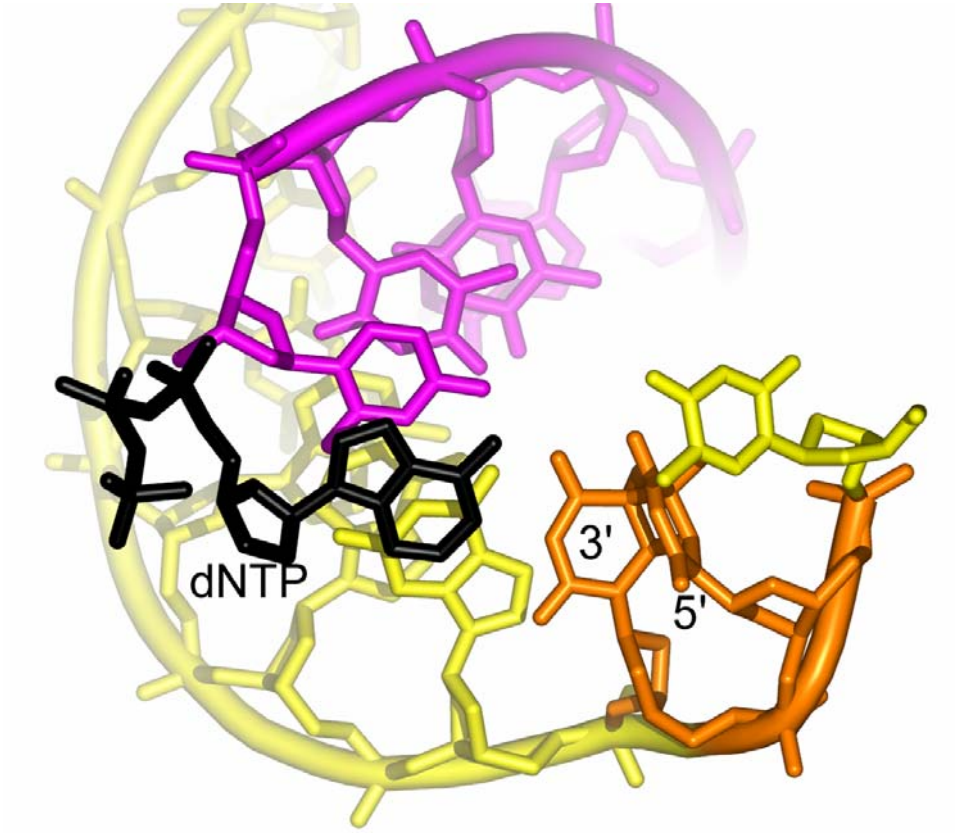


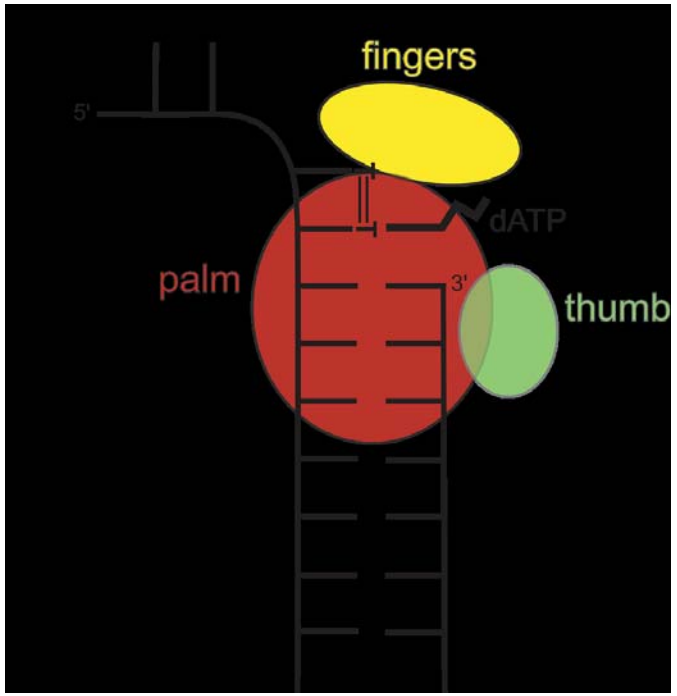
PolY Family Polymerase Dpo4 Accommodates Both T's of the CPD



H. Ling, W. Yang, et al. Nature. 2003 424, 1083.

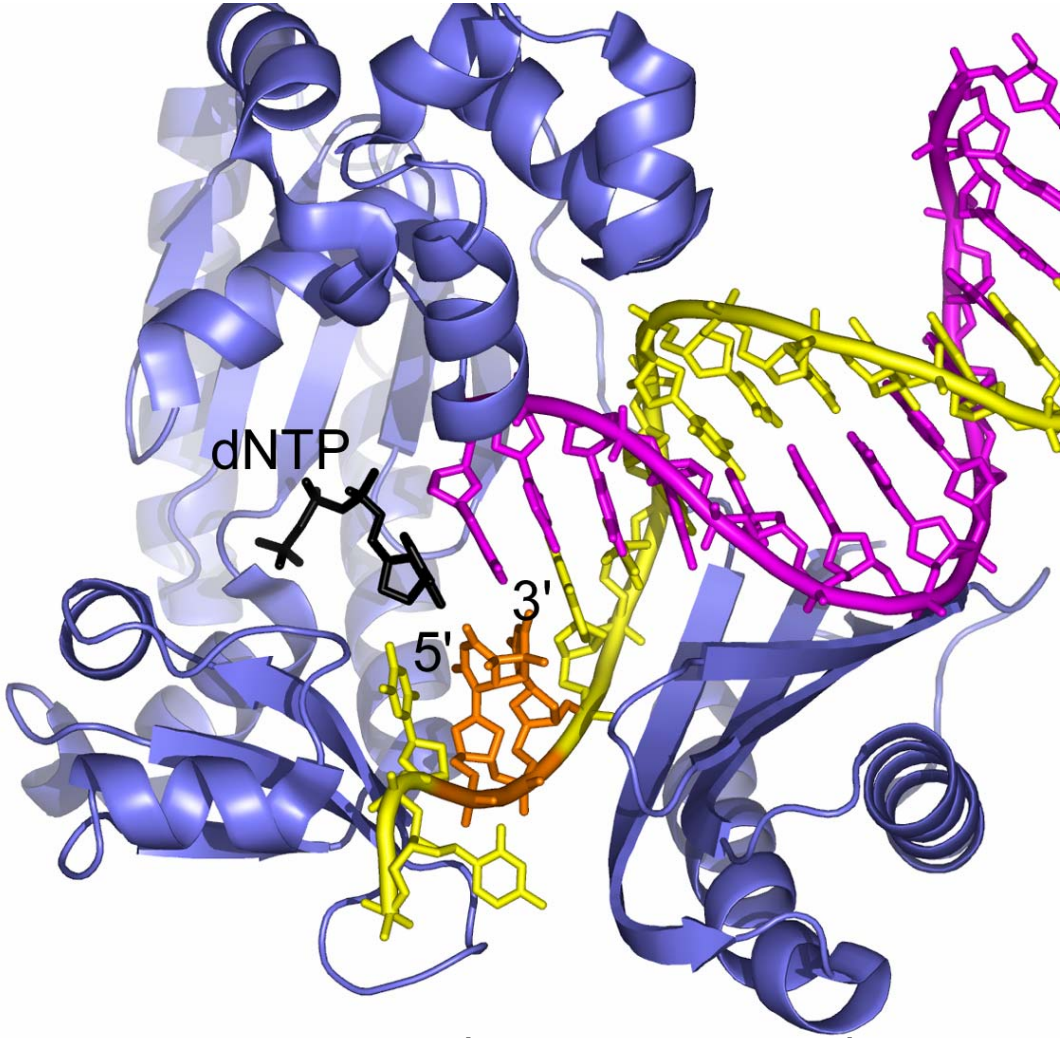
Dpo4's Spacious Active Site Allows Watson-Crick Pairing With the 3' T





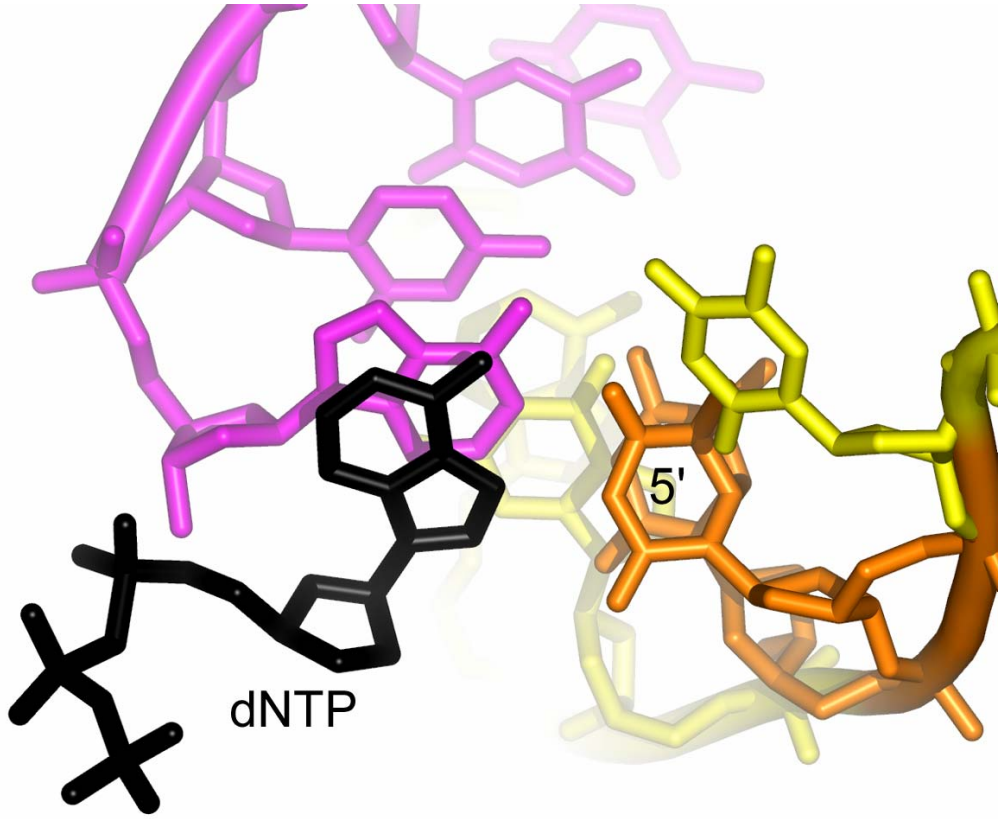
H. Ling, W. Yang, et al. Nature. 2003 424, 1083.

Templated Insertion of dAMP via Hoogsteen Pairing Opposite 5'T



H. Ling, W. Yang, et al. Nature. 2003 424, 1083.

Hoogsteen pairing with 5'T of *cis-syn* Cyclobutane Thymine Dimer



H. Ling, W. Yang, et al. Nature. 2003 424, 1083.

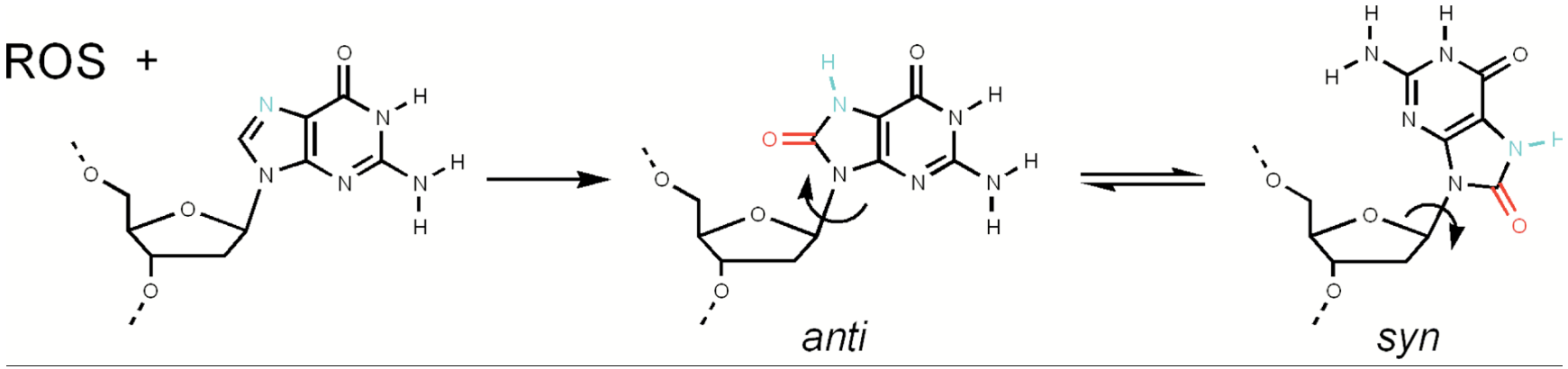
Lesion Bypass by Y Family Polymerases: Unanswered Questions

Mechanism of replication restart: Hand-off between error-free and lesion-bypass polymerases?

Other replication factors mediate polymerase switching? (SOS-response protein UmuD'$_2$C requires SSB, RecA*, Beta clamp).

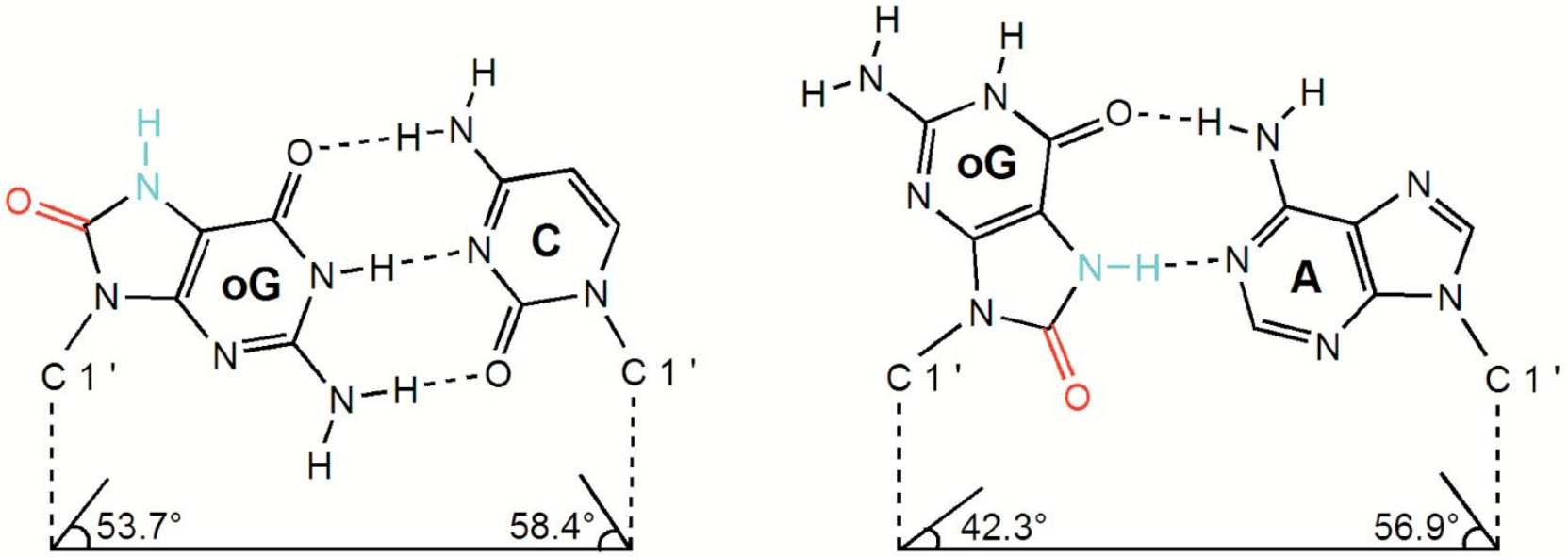
Specialized biological roles of large number of error-prone polymerases in higher eukaryotes?

Oxidative Damage of DNA Generates 8-oxoguanosine



Luis Brieba

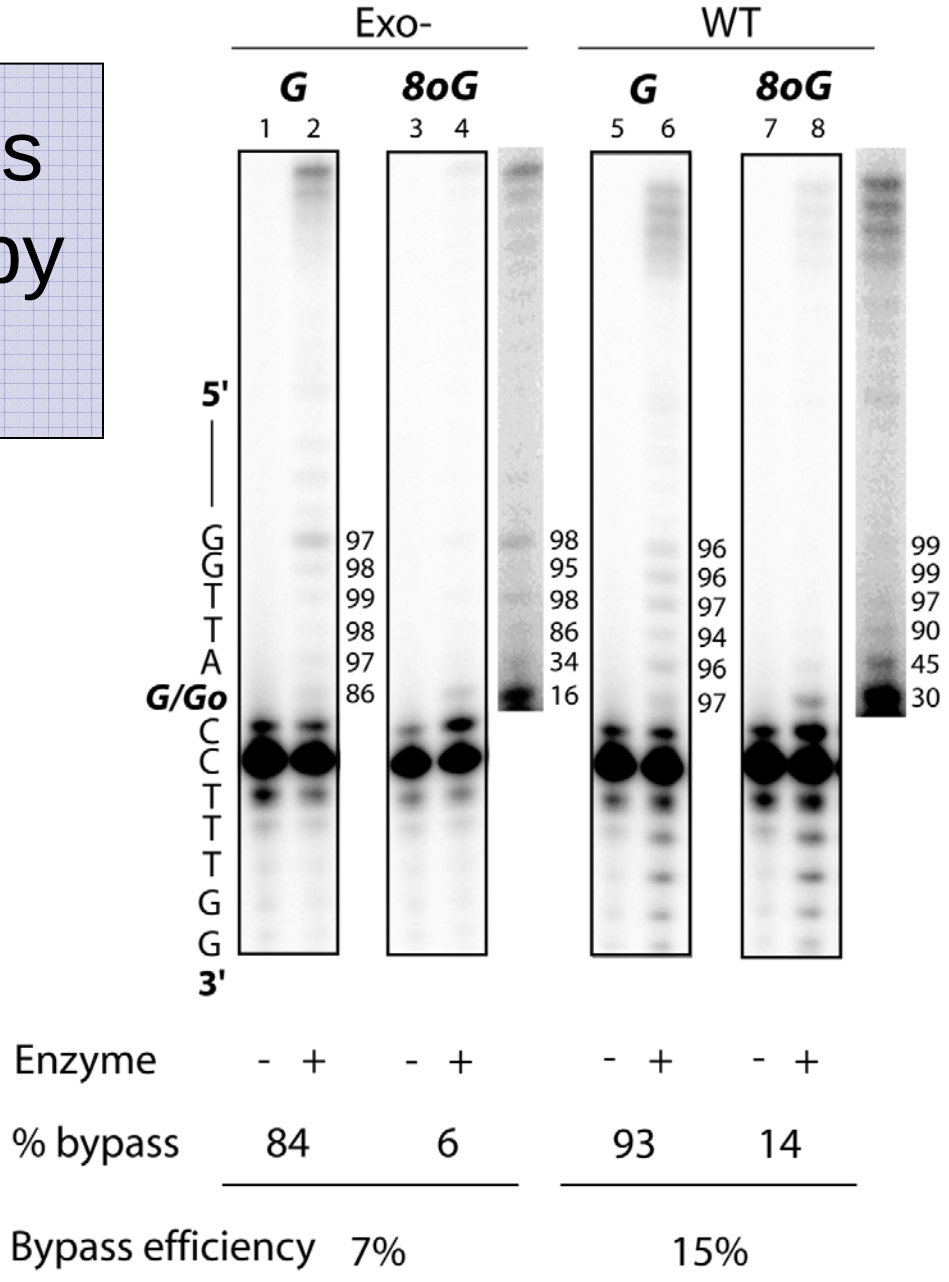
8oG Is Highly Mutagenic Because It Readily Pairs with C or A During Replication



- 8oG-induced mutagenesis implicated in cancer, aging, neurodegenerative disease.

Luis Brieba

Bypass of 8oG Is Not Diminished by Proofreading



Robert Kokoska & Tom Kunkel

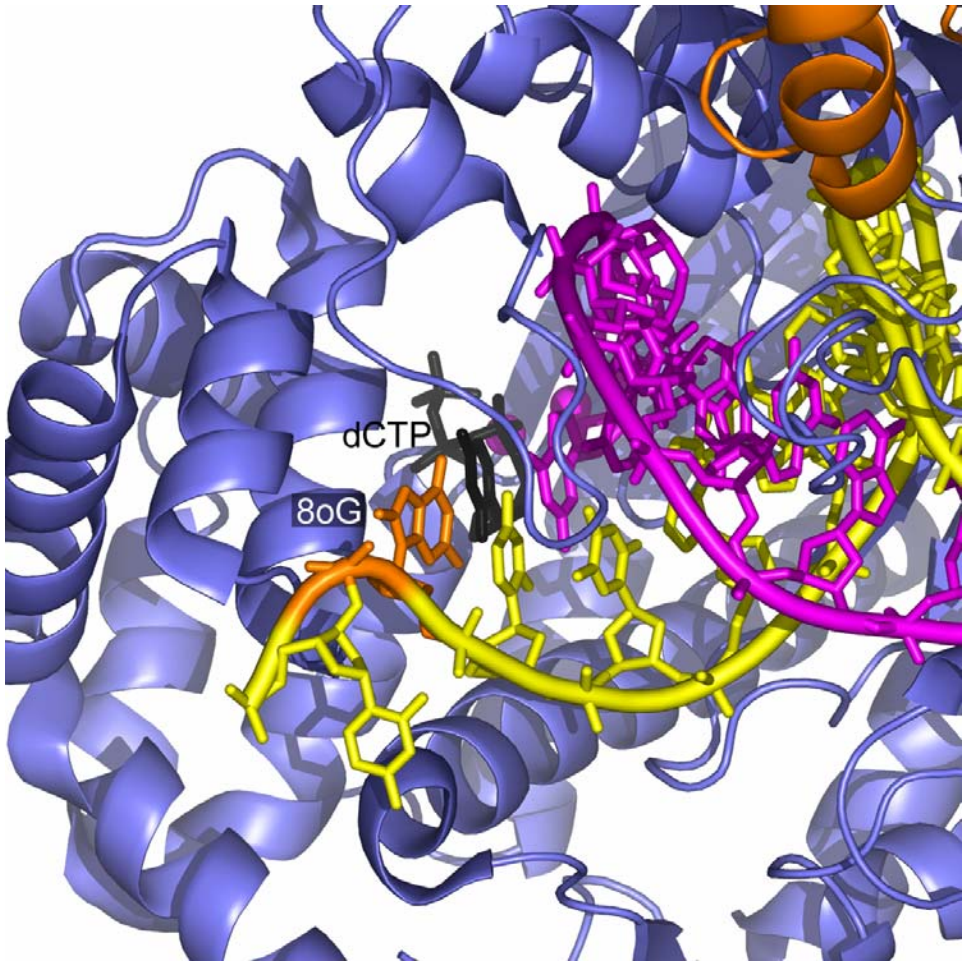
Low Fidelity of T7 DNA Polymerase When Copying 8oG *in vivo*

Polymerase	Template	Plaques		Dark Blue Frequency	dNMP incorporated	
		Dark Blue	Total		Dark Blue	Light Blue
Exo$^-$	8oG	187	876	0.21	31/31 dAMP	32/32 dCMP
		477	2059	0.24	24/24 dAMP	24/24 dCMP
	G	3	2137	0.0014		
		2	4071	0.00049		
WT	8oG	127	662	0.19	32/32 dAMP	29/29 dCMP
		469	1755	0.27	23/23 dAMP	24/24 dCMP
	G	0	5052	<0.00020		

*Our assay detects ~60% of polymerization errors. Est. **true error rate ~ 30%** of bypass events.

Robert Kokoska & Tom Kunkel

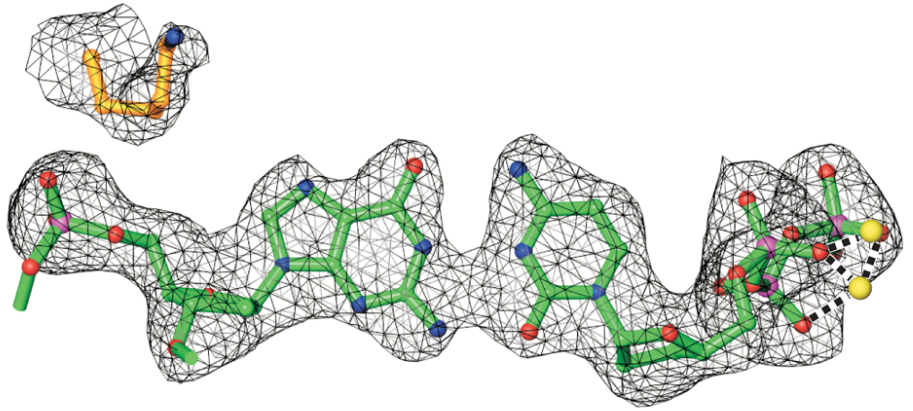
Mutagenic and Error-free Copying of the Oxidative Lesion 8oG in DNA



- 4 crystal structures
  - 8oG in templating position (8oG paired with dATP, dCTP in active site).
  - 8oG paired with 3' nucleotide of primer strand (i.e., extension following insertion opposite 8oG)

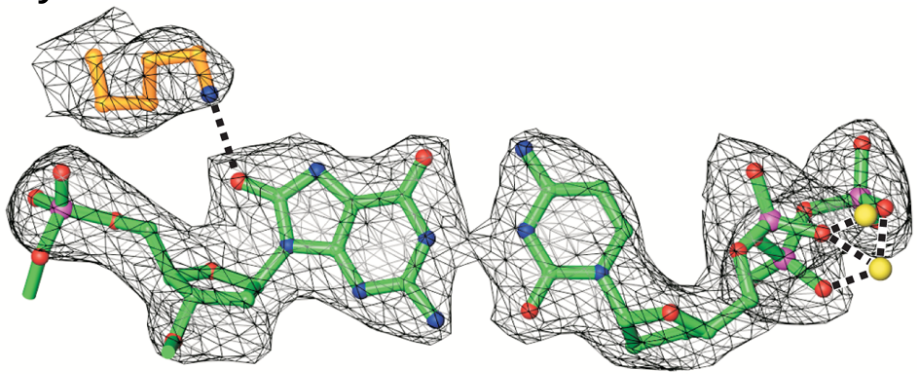
Brieba et al. 2004. EMBO J. 23, 3452

anti-8oG is accommodated by the sharp kink in the DNA template

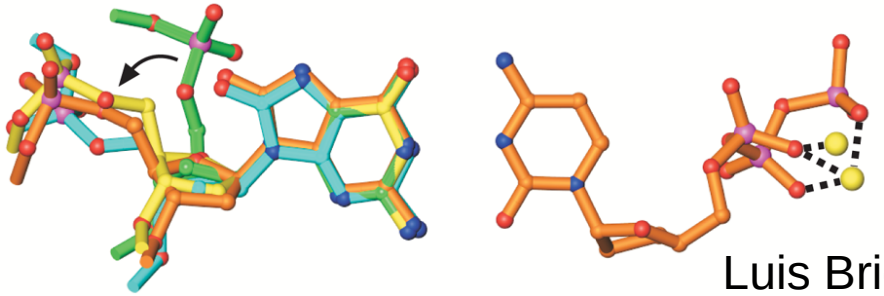


dG:dCTP

Lys536

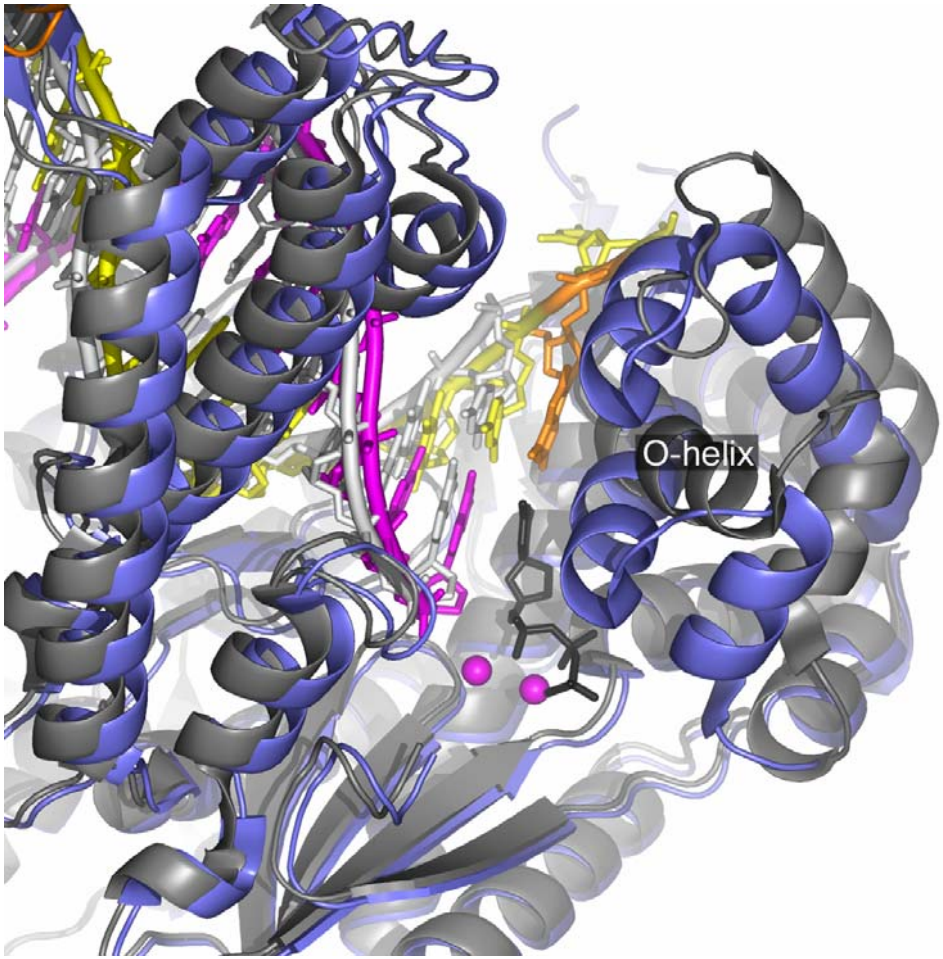


d8oG:dCTP



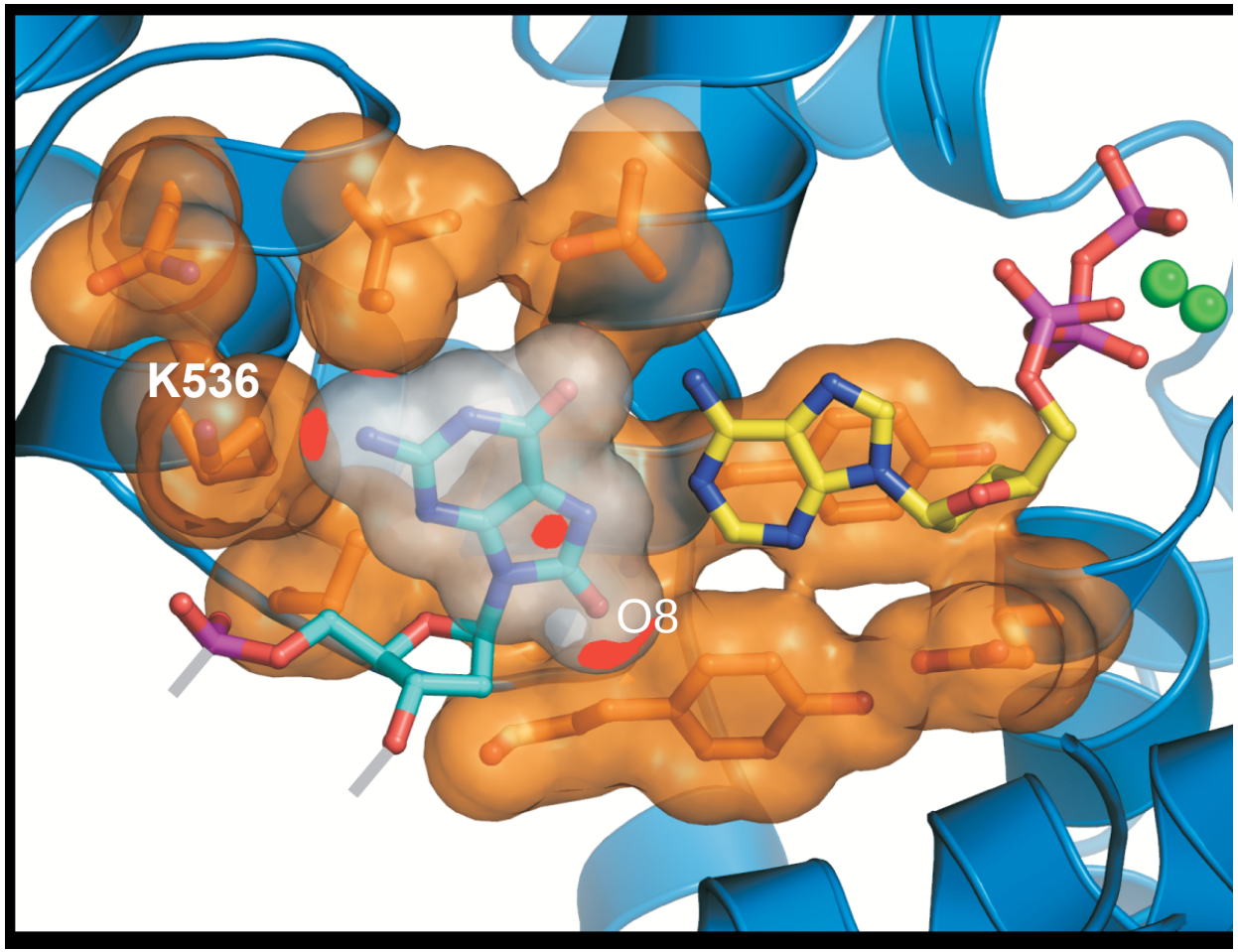
Luis Brieba

8oG:dATP Is Poorly Accommodated in the Polymerase Active Site



Luis Brieba

Modeling Insertion of dAMP Opposite 8oG



Luis Brieba

K536A Mutant Enhances Mutagenic Bypass of 8oG

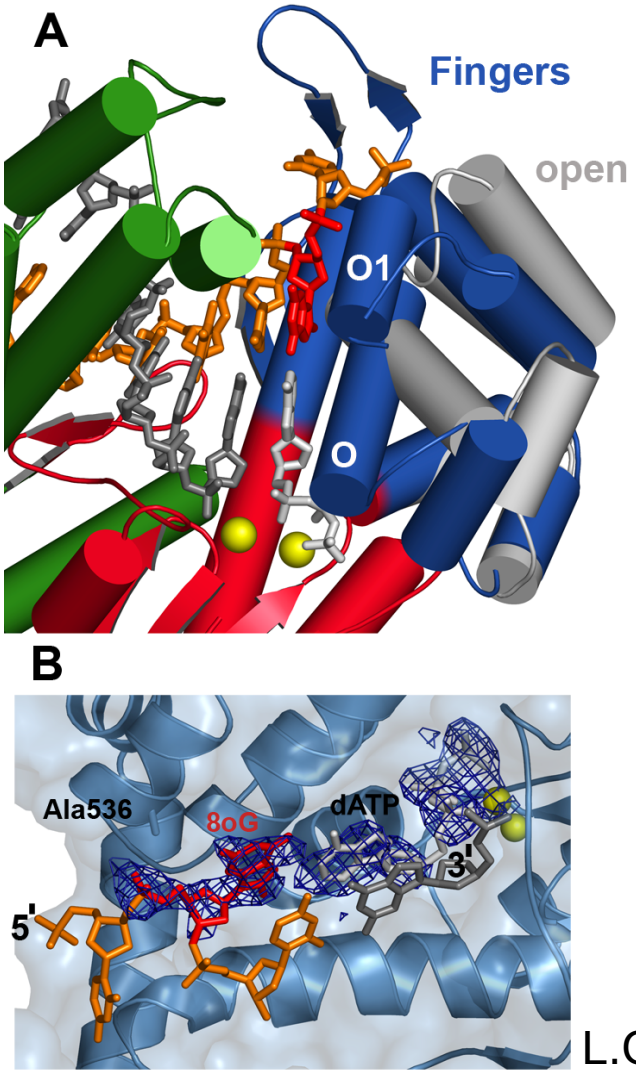
Nucleotide incorporation fidelity by Exo$^-$ and Lys536Ala T7 DNA polymerases

Polymerase	Template	Plaques Dark Blue	Plaques Total	Dark Blue frequency	dNMP incorporated (Dark Blues)
exo- Pol[a]	8oG	187	876	0.21	31/31 dAMP
		477	2059	0.24	24/24 dAMP
Lys536Ala		2214	5094	0.43	46/47 dAMP 1/47 dCMP
exo$^-$ Pol [a]	G	3	2137	0.0014	
		2	4071	0.00049	
Lys536Ala		5	11085	0.00045	

[a]From Brieba et al., EMBO J (2004) **23**:3452-3461.

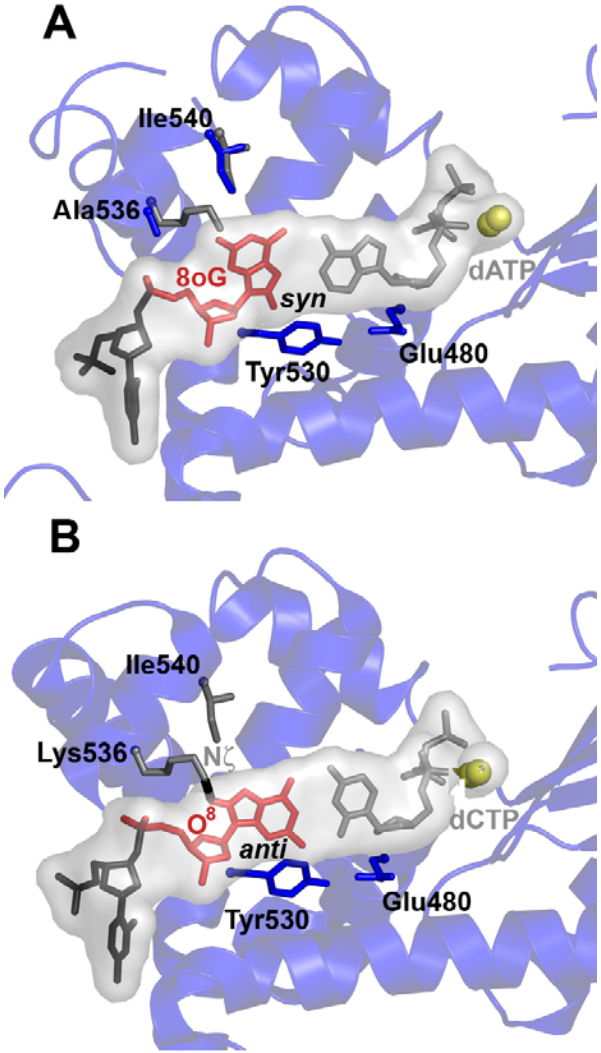
L.G. Brieba et al. 2005. Structure 13, 653-1659.

K536A Mutant Enhances Mutagenic Bypass of 8oG



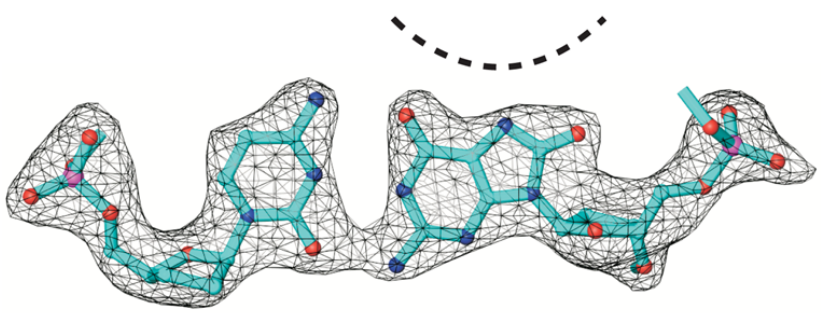
L.G. Brieba et al. 2005. Structure 13, 653-1659.

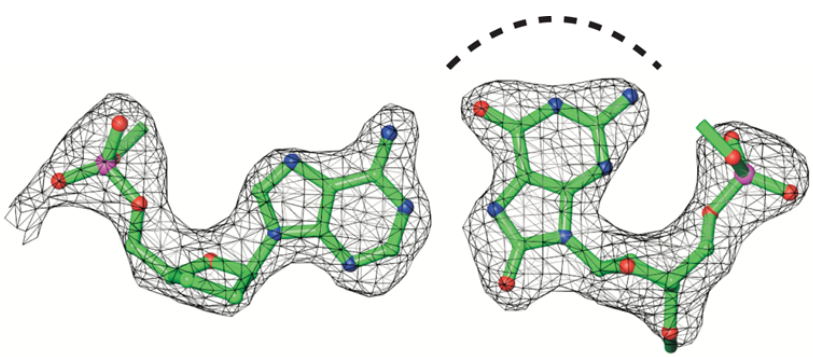
Faithful and Mutagenic Insertion of dNMP's Opposite 8oG

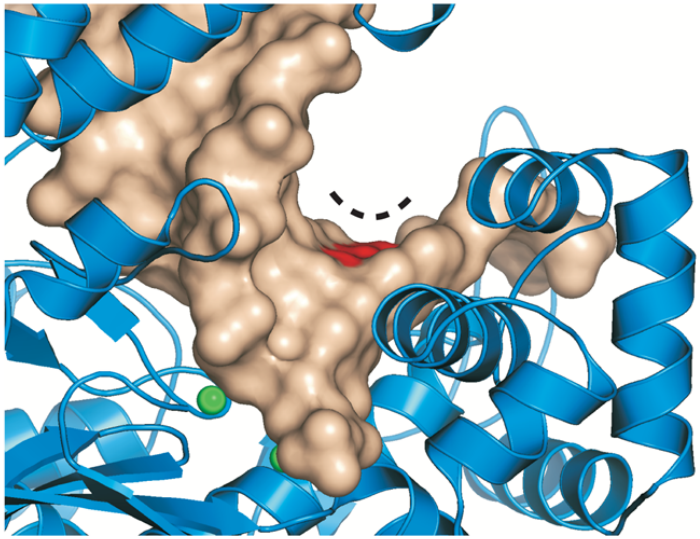


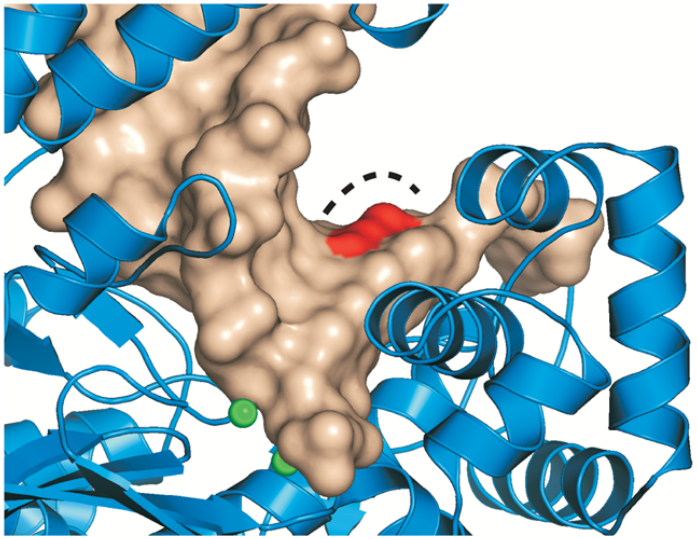
- K536A substitution stabilizes 8oG:dATP and promotes dAMP misinsertion.
- K536 (W-T) contributes to $Km_{(dCTP)}$ and suppresses dAMP insertion.

Primer Extension From a 8oG:dA Hoogsteen Pair

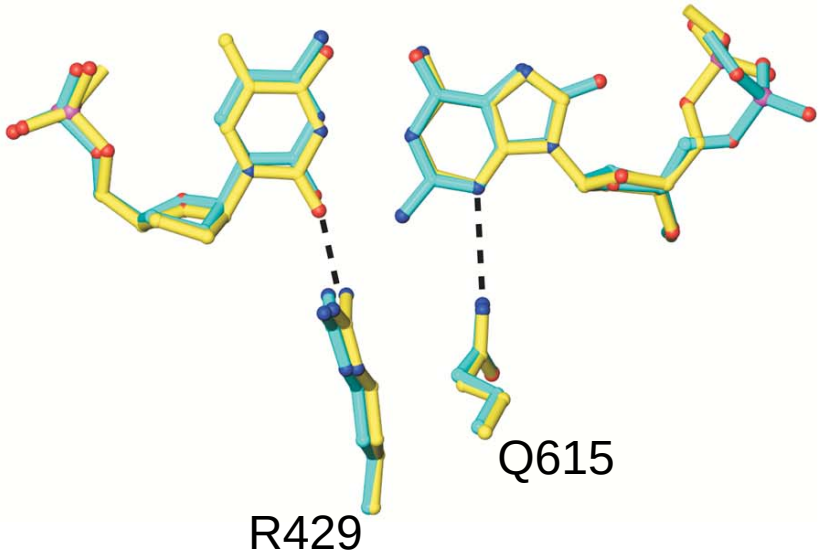




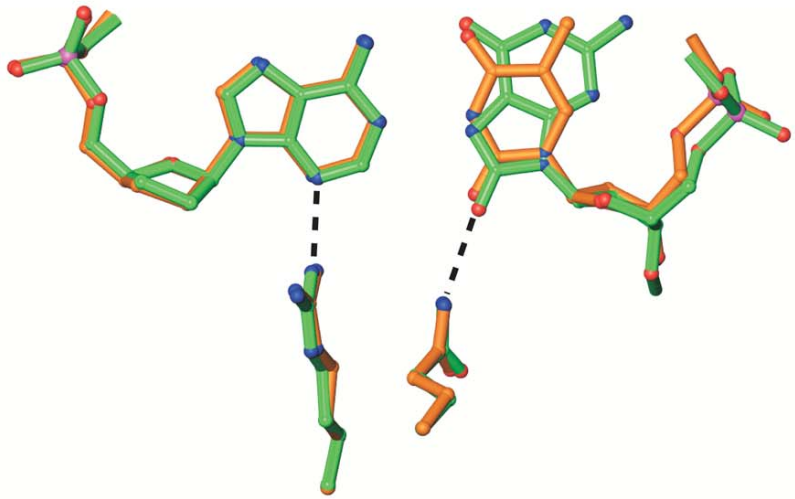




8oG:dA Mispairs Are Not Subject to Normal Mechanism of Proofreading

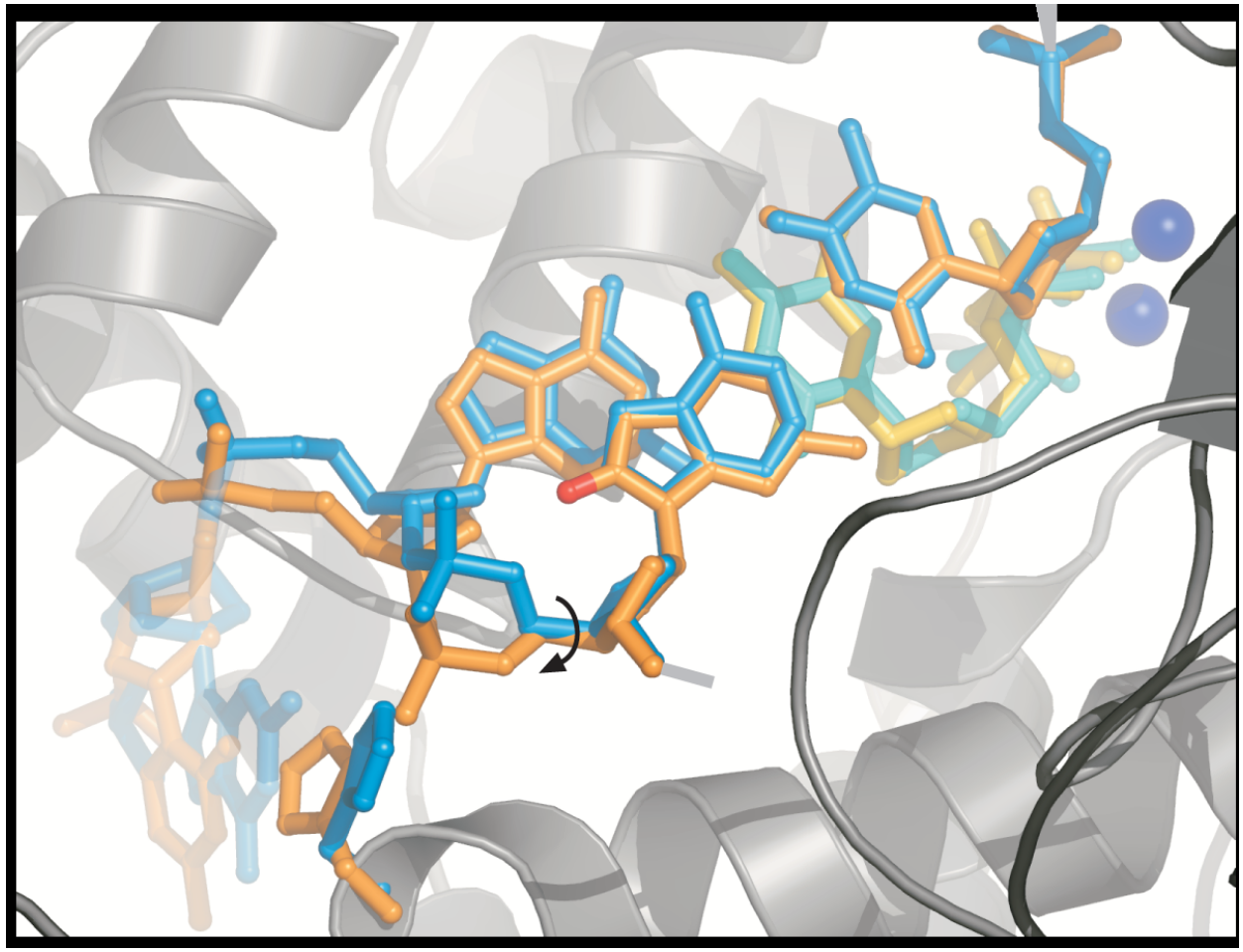


Minor Groove Readout of Watson:Crick Base Pairs



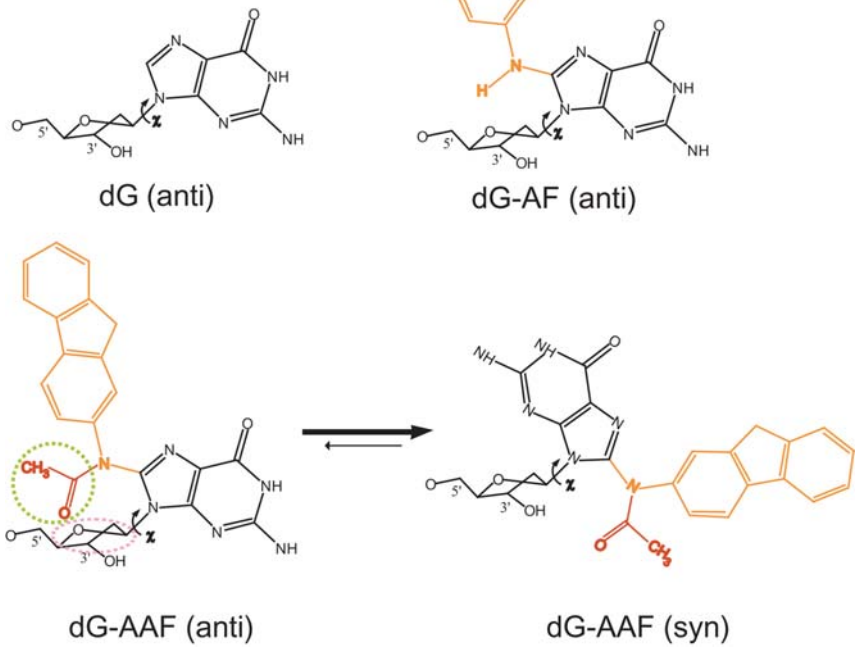
8-oxo Group Stands In As H-bond Donor In the Minor Groove

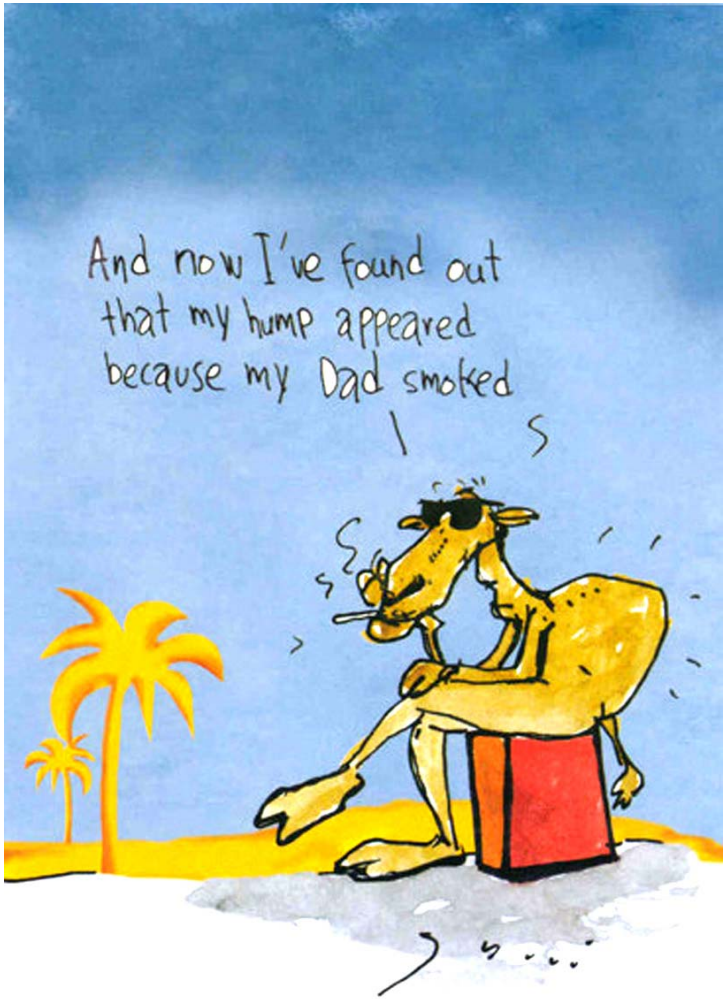
Local Adjustment of the DNA Backbone Accommodates *anti*-8oG



Luis Brieba

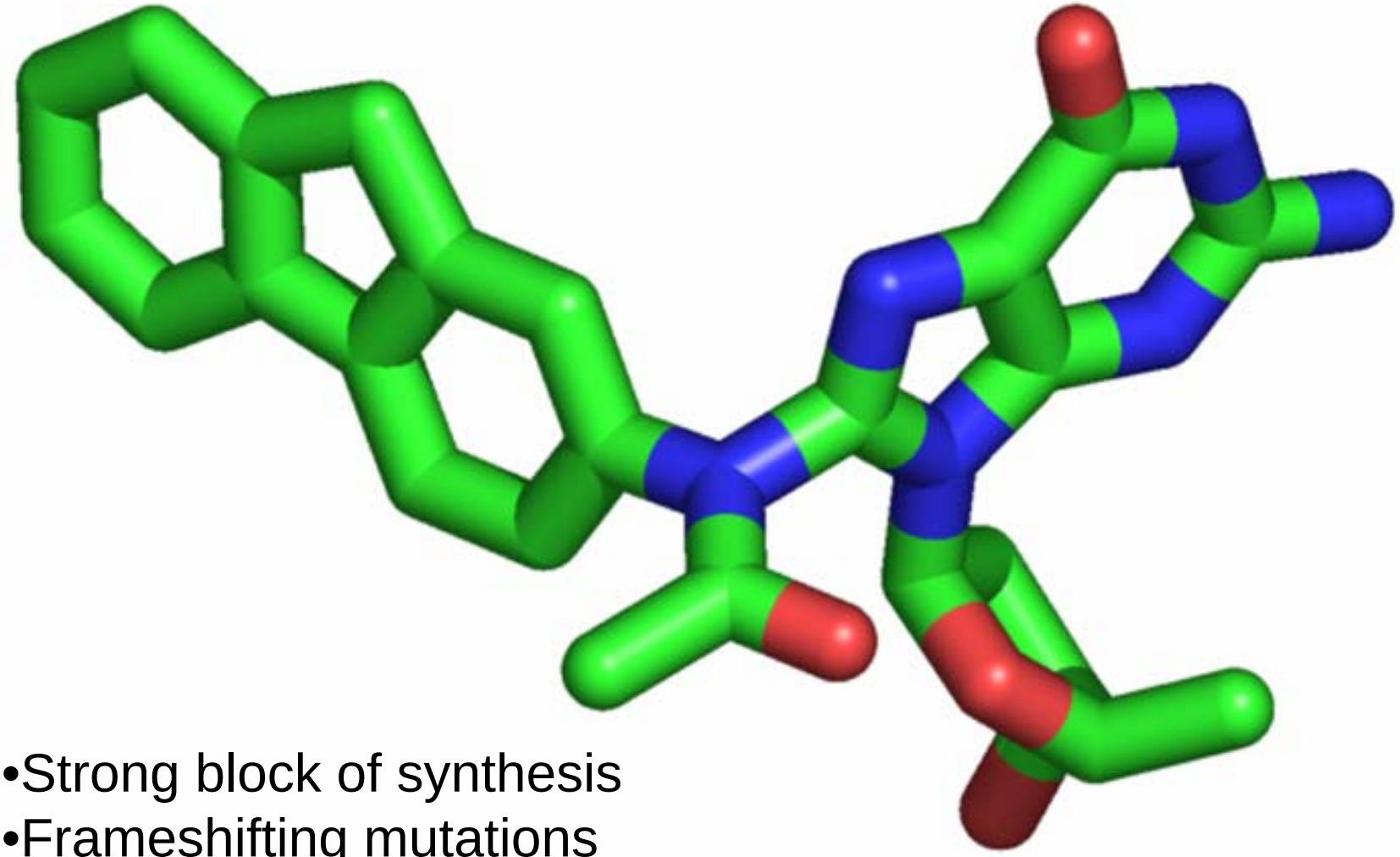
Bypass of Carcinogenic Adducts of 2-acetylaminofluorene





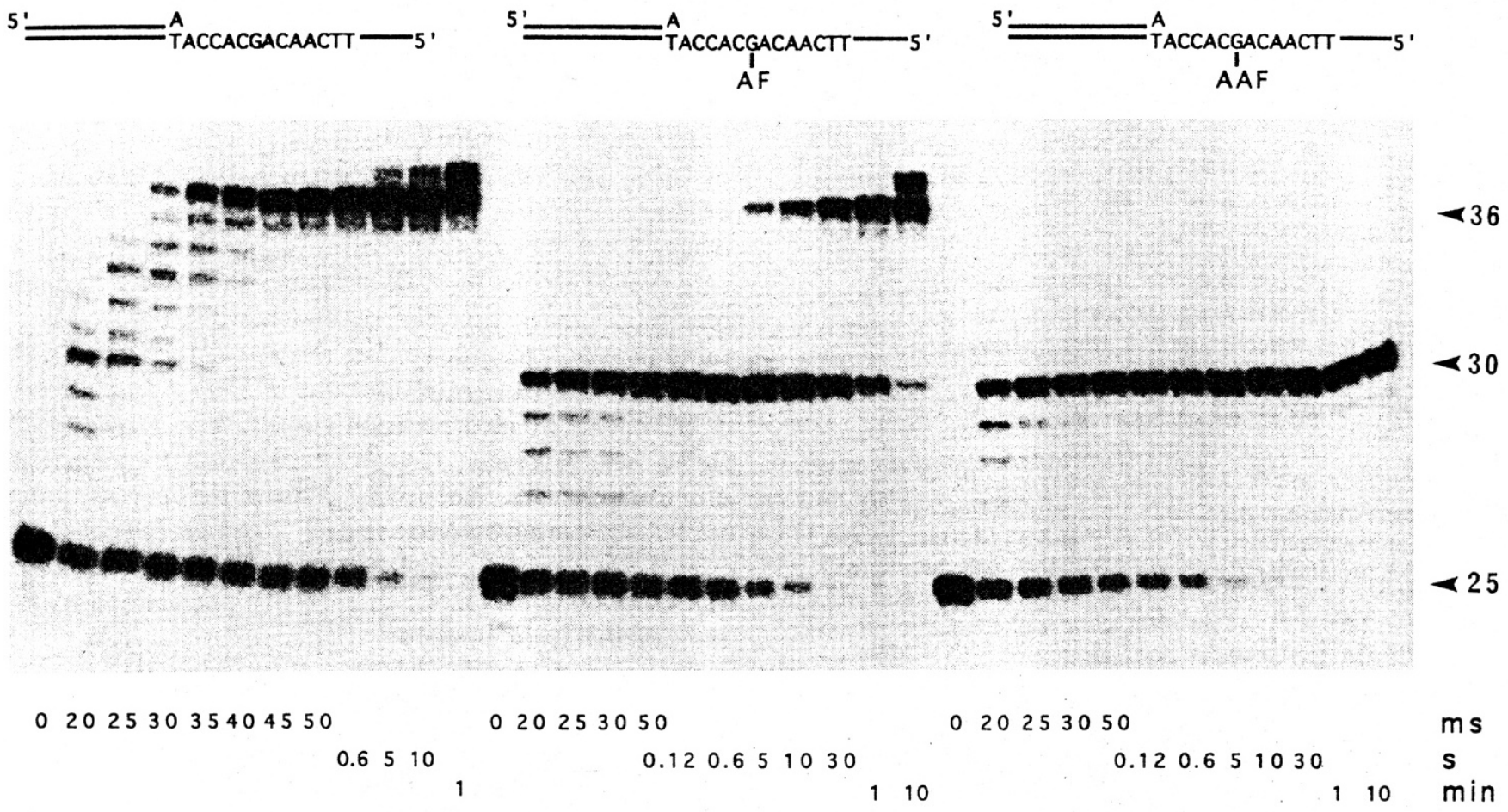
Shuchismita Dutta

Acetylaminofluorene (AAF) Adduct of dG



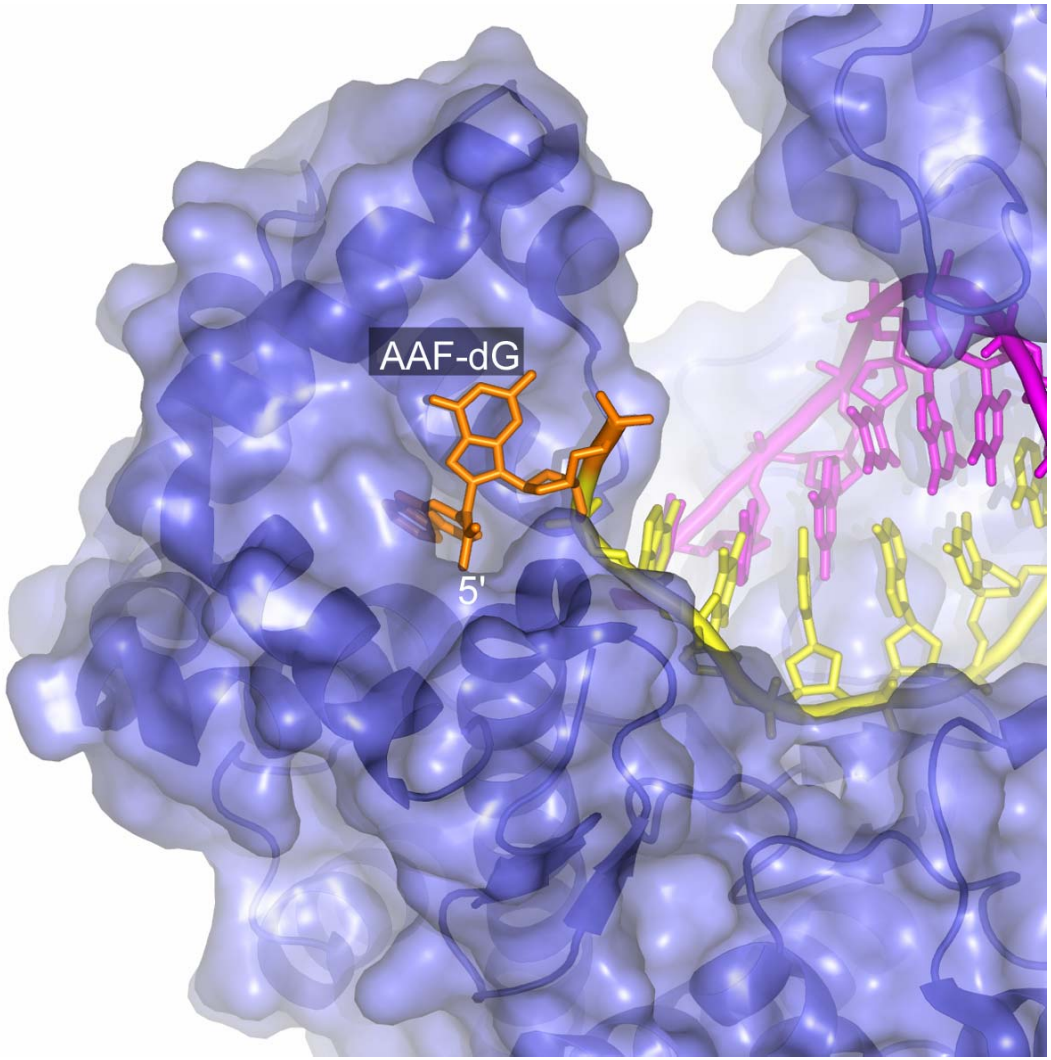
- Strong block of synthesis
- Frameshifting mutations

Bypass of Aminoflurene & Acetylaminoflurene Adducts of dG



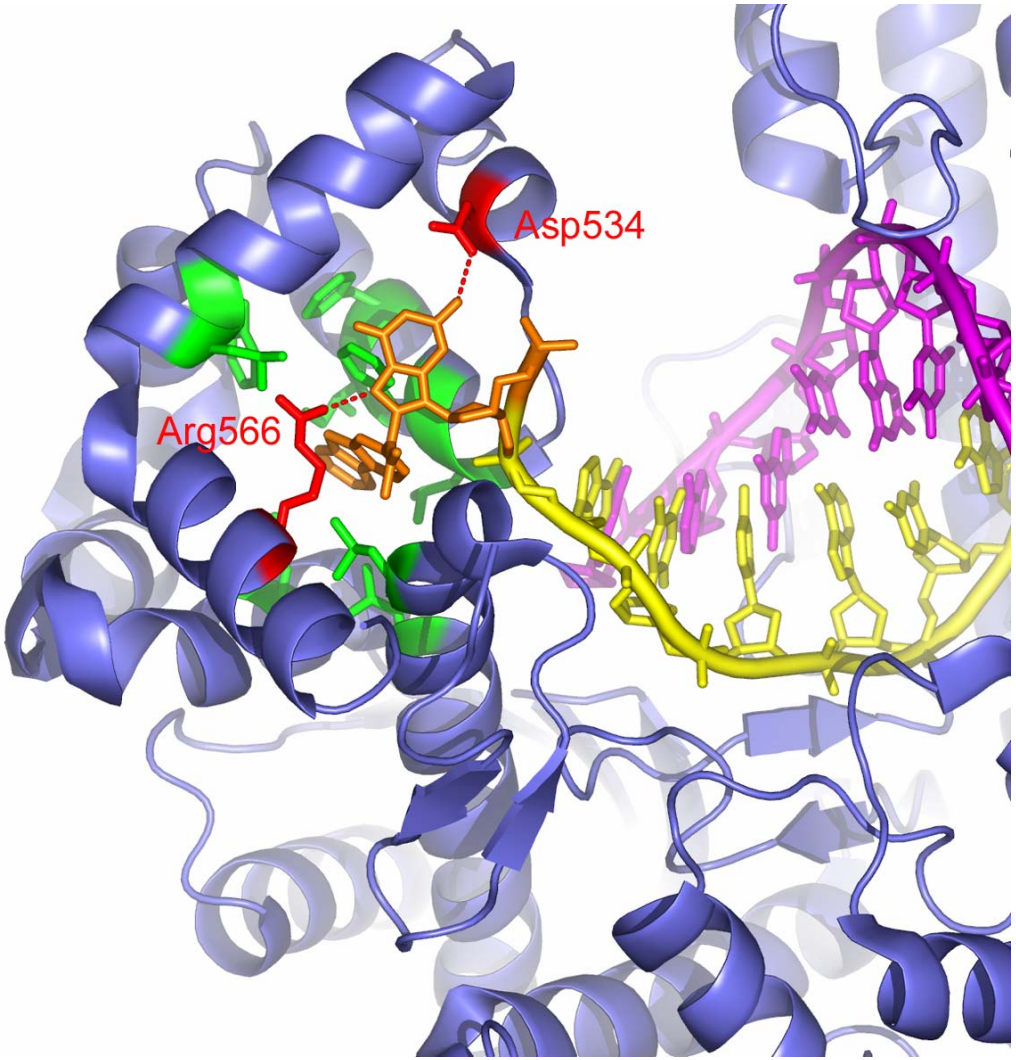
Lindsley & Fuchs 1994. Biochemistry 33, 764.

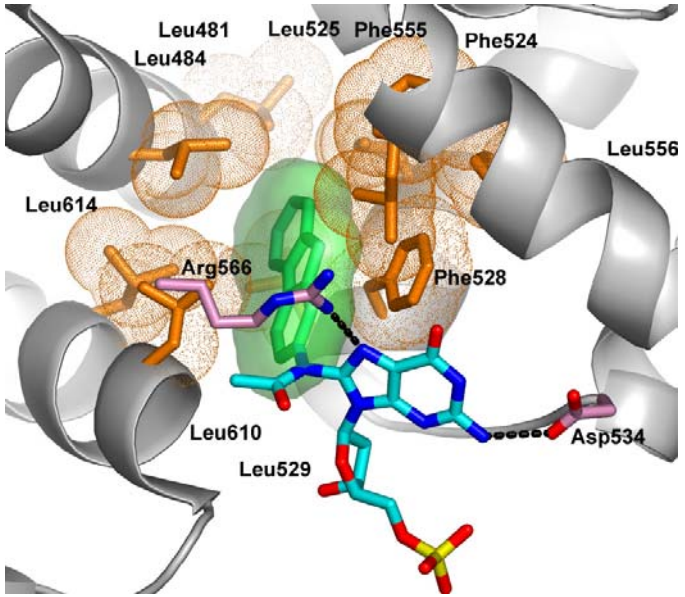
AAF-dG Intercalates into the Fingers of T7 DNA Polymerase



Dutta et al. 2004. PNAS 101, 16186.

Polar and Nonpolar Interactions Stabilize AAF-dG Binding at a Remote Site





Shuchismita Dutta, Ying Li

Conclusions

- Chemically modified template bases such as a CPD photoproduct interfere with geometric selection by hindering closure of the fingers and providing less stabilization of dNTPs bound in the active site.

- 8oG:dA is able to evade proofreading by mimicking the minor groove surface of T:dA.

- AAF-dG locks the fingers in an open conformation and tethers the templating G outside of the active site. The resulting strong pause in synthesis provides an opportunity for frameshifting mutagenesis.

Acknowledgements

Cis-syn thymine dimer

- Ying Li, Shuchismita Dutta, Hussam Moh'd Bdour, John-Stephen Taylor

8-oxoguanosine

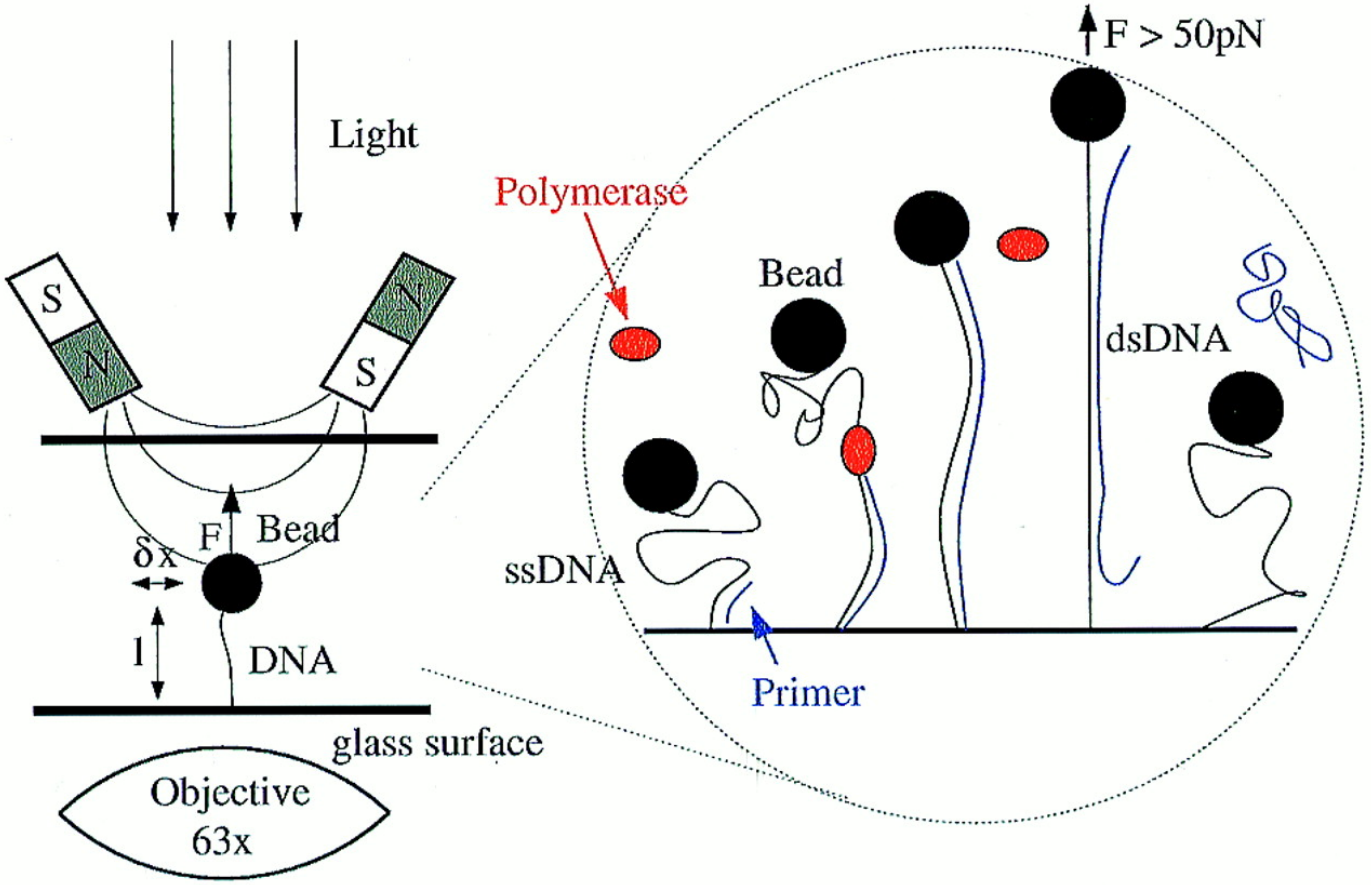
- Luis Brieba, Brandt Eichman, Sylvie Doublié, Robert Kokoska, Tom Kunkel

dG-AAF, dG-AF

- Shuchismita Dutta, Ying Li, Donald Johnson, Leonid Dzantiev, Charles Richardson, Louis J. Romano

FUNDING: R01 GM55390, F32 GM065746, Pew Latinoamerican Fellowship Program

DNA Synthesis by Single Polymerase Molecules



Maier, Berenike et al. (2000) Proc. Natl. Acad. Sci. USA 97, 12002-12007

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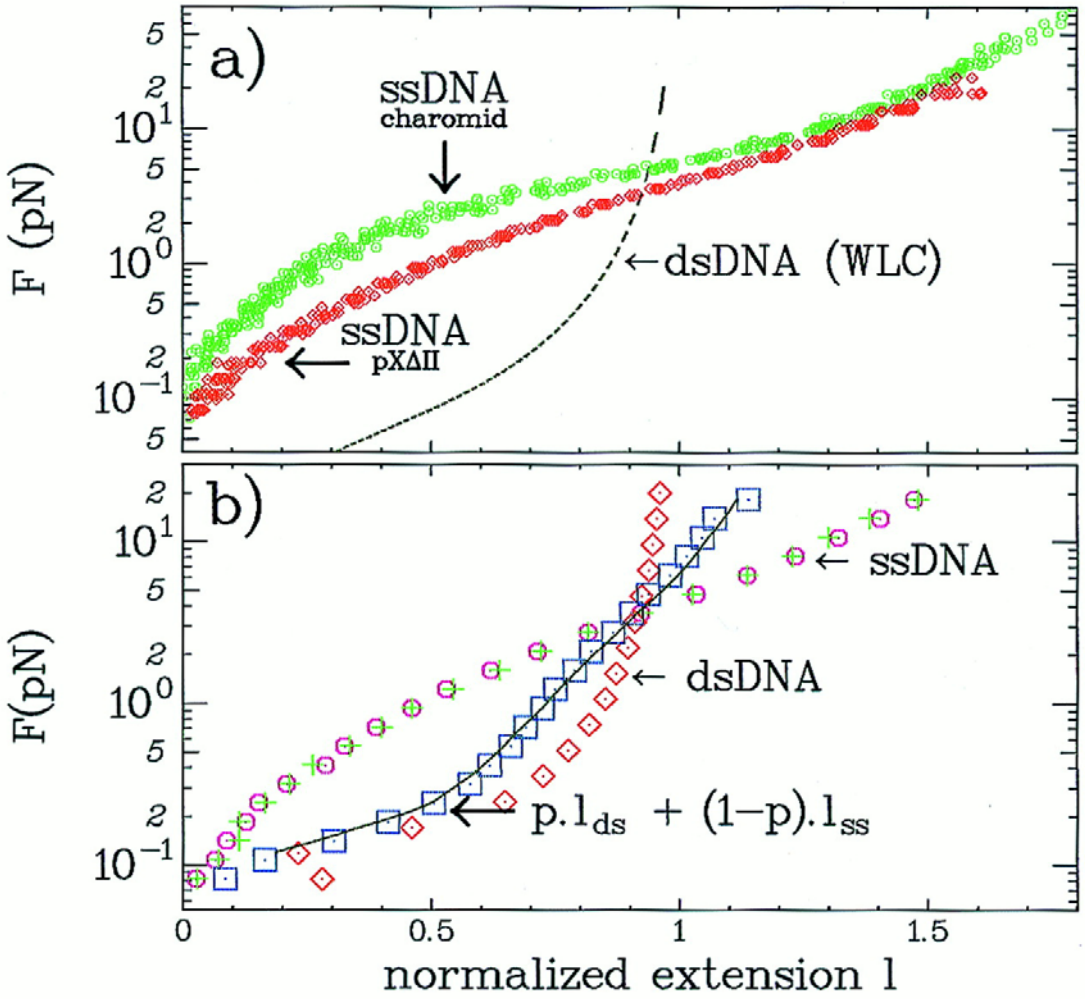
DNA Force-extension Curves



Maier, Berenike et al. (2000) Proc. Natl. Acad. Sci. USA 97, 12002-12007

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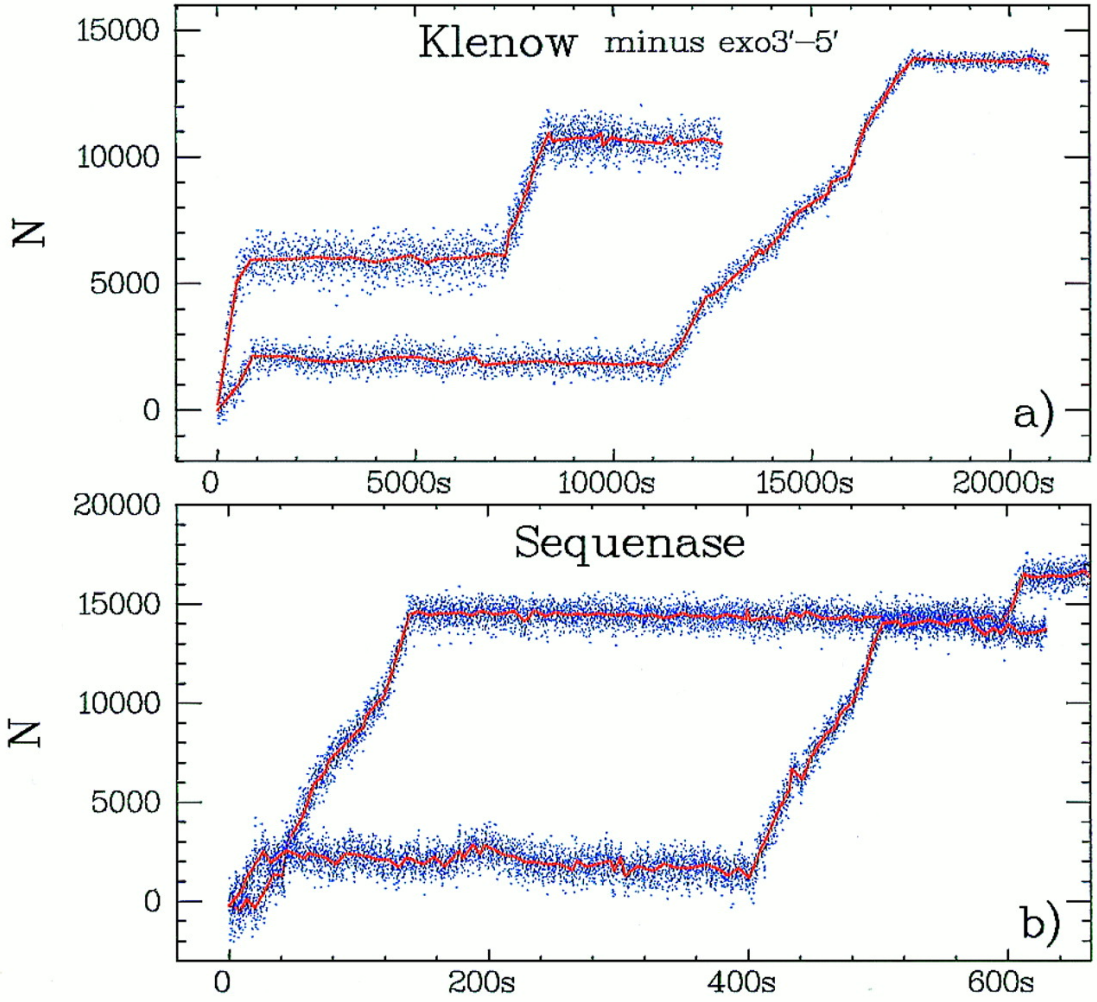
Pausing Events (1 pN load)



Maier, Berenike et al. (2000) Proc. Natl. Acad. Sci. USA 97, 12002-12007

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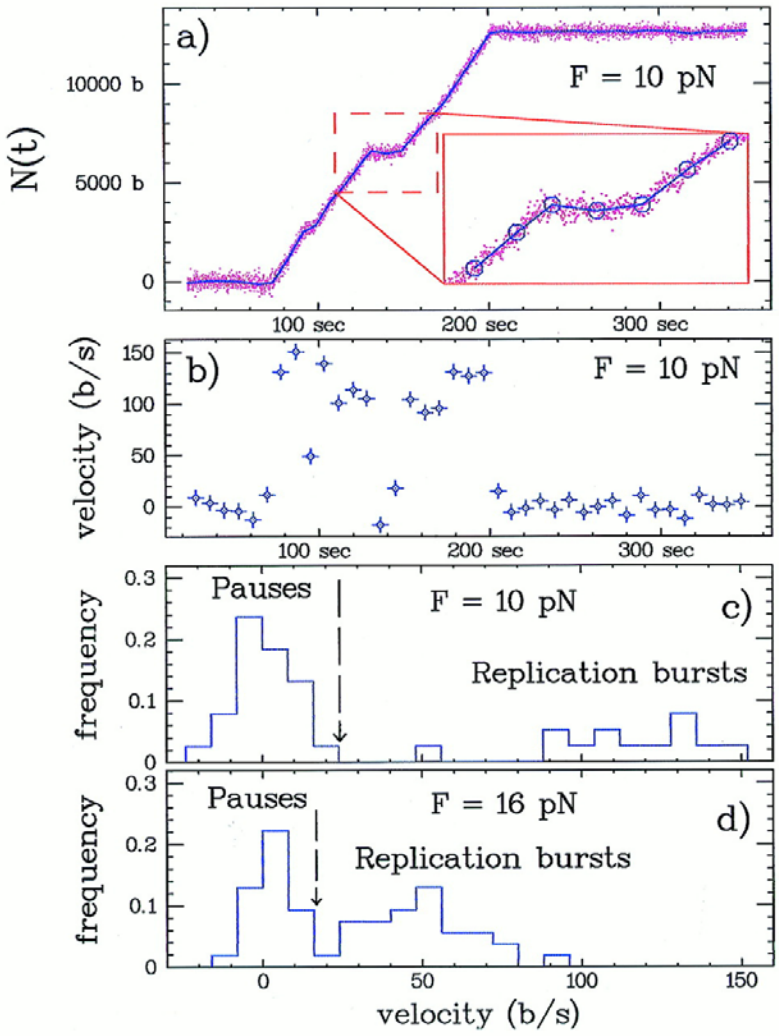
Replication & Bursting Behavior



Maier, Berenike et al. (2000) Proc. Natl. Acad. Sci. USA 97, 12002-12007

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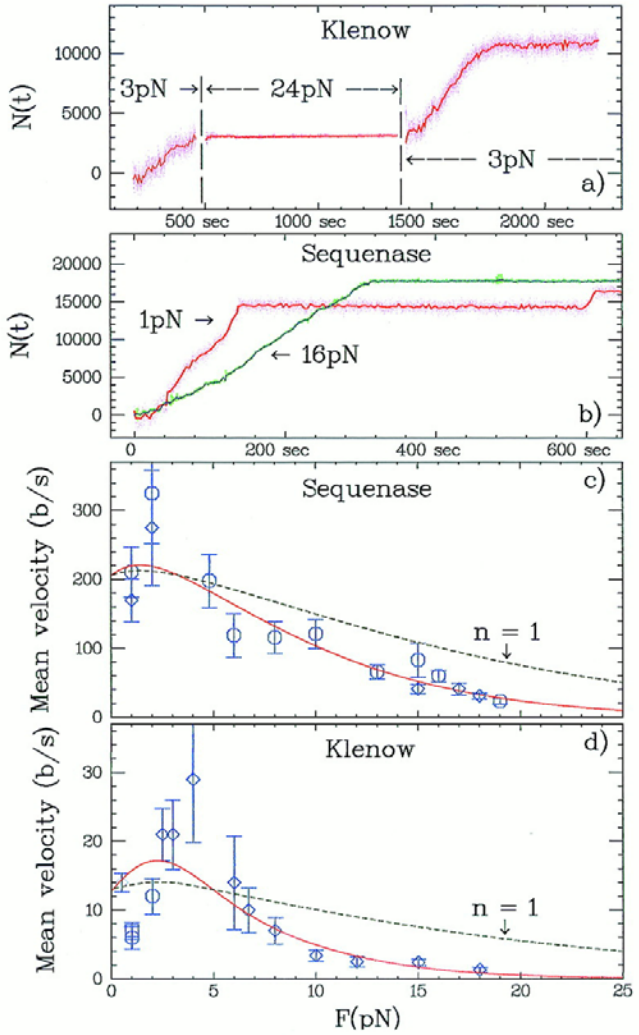
Force Dependence of Rate



$$\langle v(f) \rangle = v_0 \exp[-n\Delta h(f)/k_B T]$$

where $\Delta h(f) = h_{ds}(f) - h_{ss}(f)$

T7 DNA polymerase:
$n = 2.1$, $v_0 = 200$ bp s^{-1}

Klenow fragment:
$n = 4.0$, $v_0 = 13.5$ bp s^{-1}

Maier, Berenike et al. (2000) Proc. Natl. Acad. Sci. USA 97, 12002-12007

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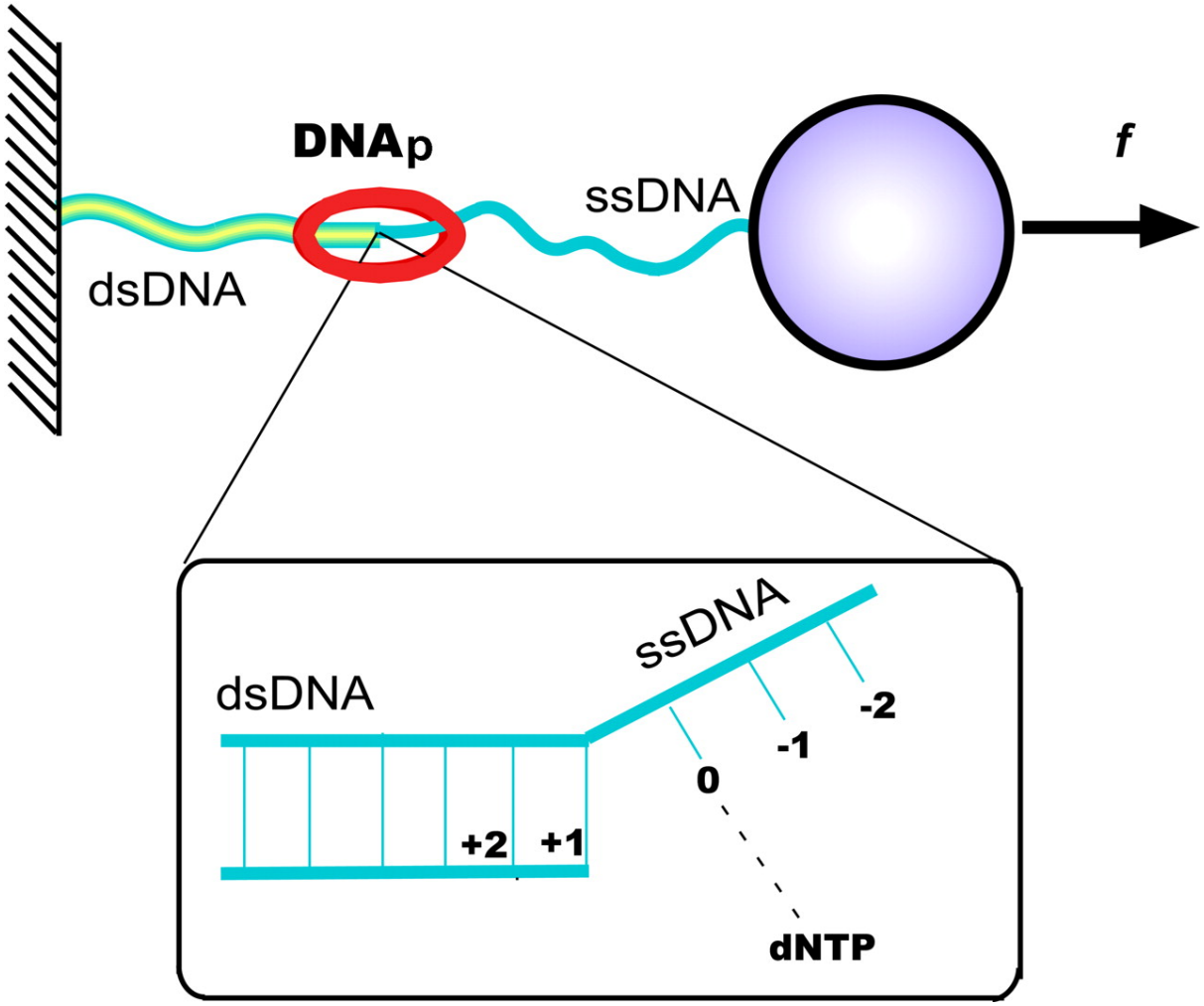
A Local, Enzyme-centric Model



Goel, Anita et al. (2001) Proc. Natl. Acad. Sci. USA 98, 8485-8489

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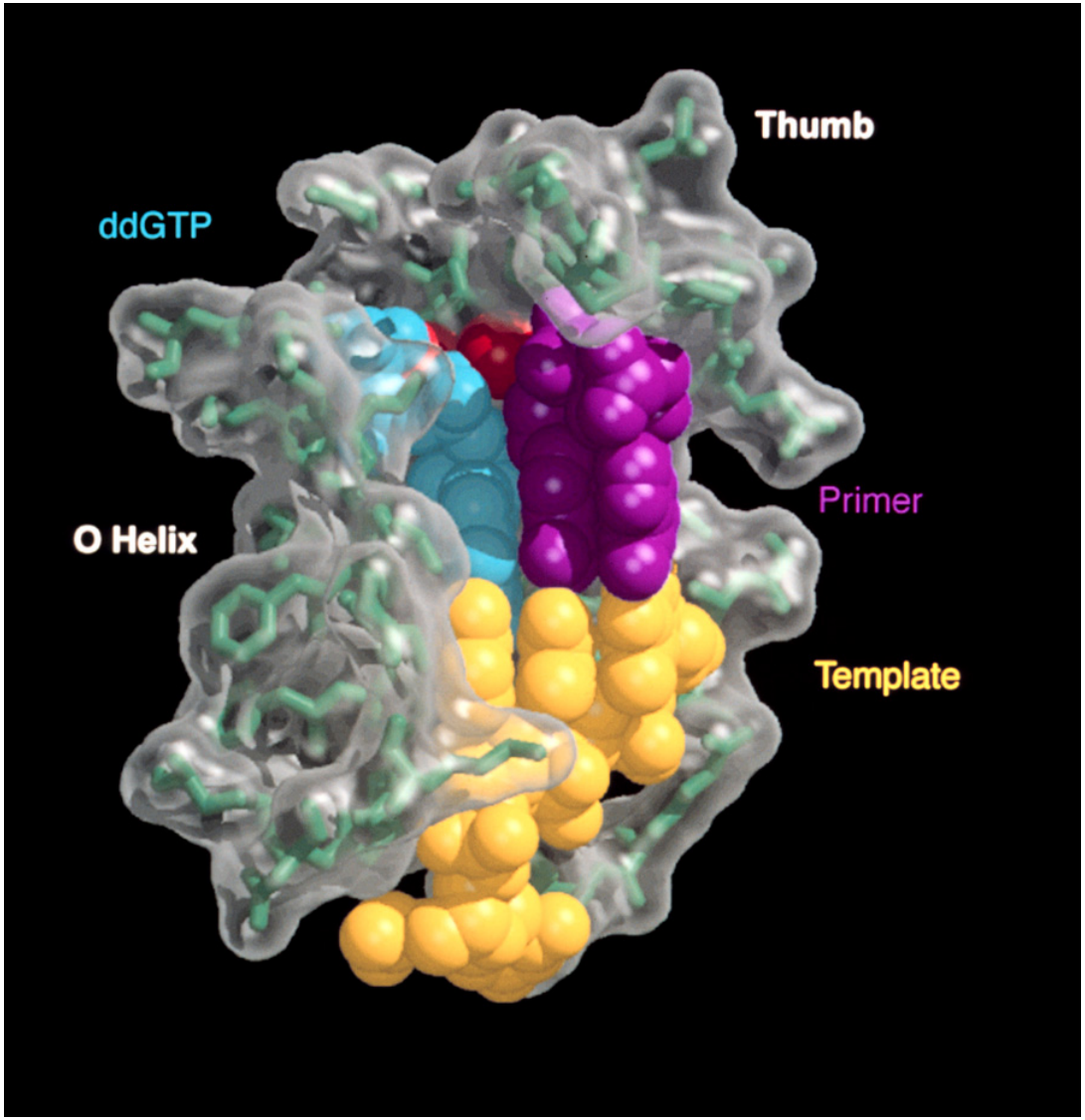
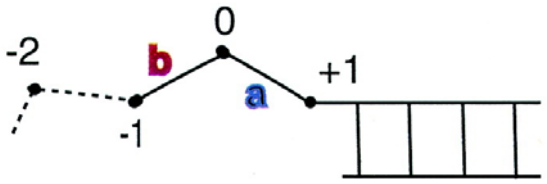
One Nascent Basepair in Active Site

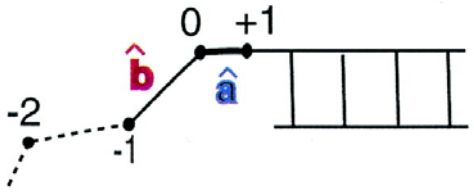


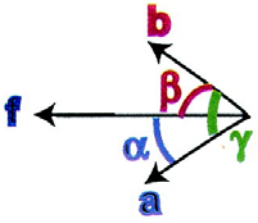
Local Geometry in Active Site

Open



Closed





$$w(f) = fL_{ss}(\cos\alpha + \cos\beta) - f(L_{ds}\cos\hat{\alpha} + L_{ss}\cos\hat{\beta})$$

Goel, Anita et al. (2001) Proc. Natl. Acad. Sci. USA 98, 8485-8489

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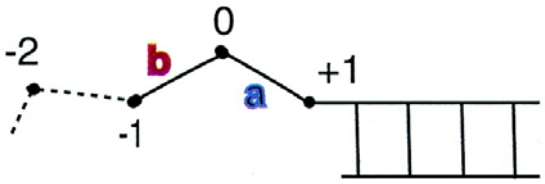
Local Geometry in Active Site

Open



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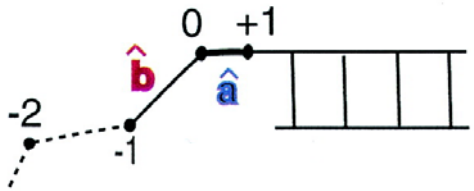
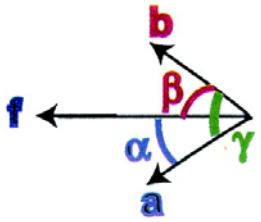


$$v(f) = v_0 \exp[- (\langle w_1(f) \rangle + \langle w2(f) \rangle)/k_B T]$$

$$\langle w_1(f) \rangle = f(L_{ss} \langle \cos\alpha \rangle - L_{ds} \langle \cos\hat{\alpha} \rangle)$$

$$\langle w_2(f) \rangle = f L_{ss} (\langle \cos\beta \rangle - \langle \cos\hat{\beta} \rangle)$$



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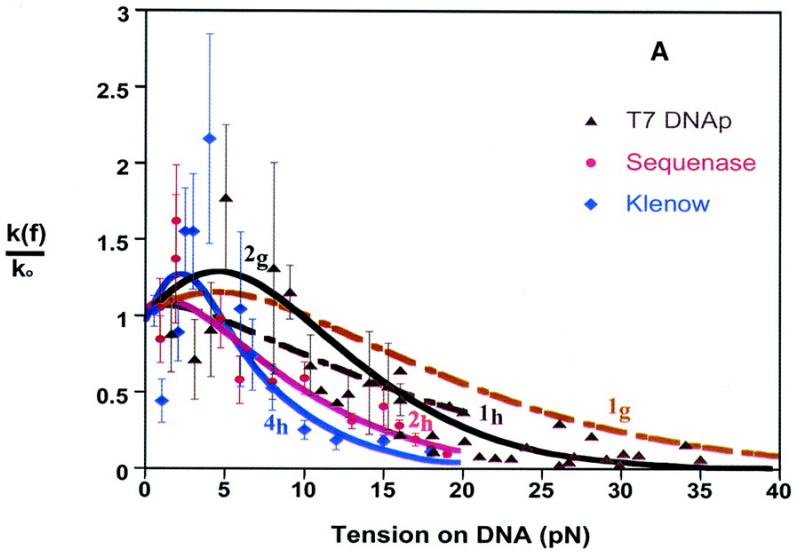
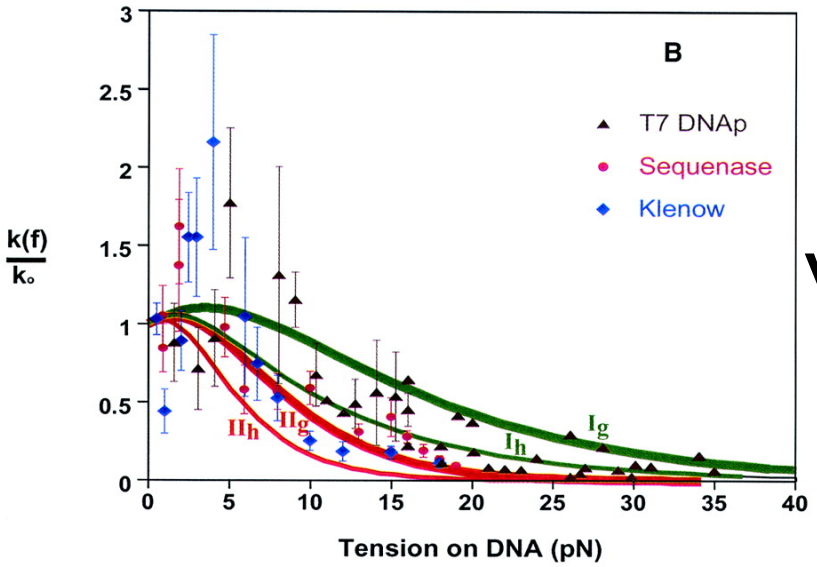
Fitting 2 Limiting Conditions



$$\langle v(f) \rangle = v_0 \exp[-n\Delta q(f)/k_B T]$$

where $\Delta q(f) = q_{ds}(f) - q_{ss}(f)$



$$v(f) = v_0 \exp[- (\langle w_1(f) \rangle + \langle w2(f) \rangle)/k_B T]$$

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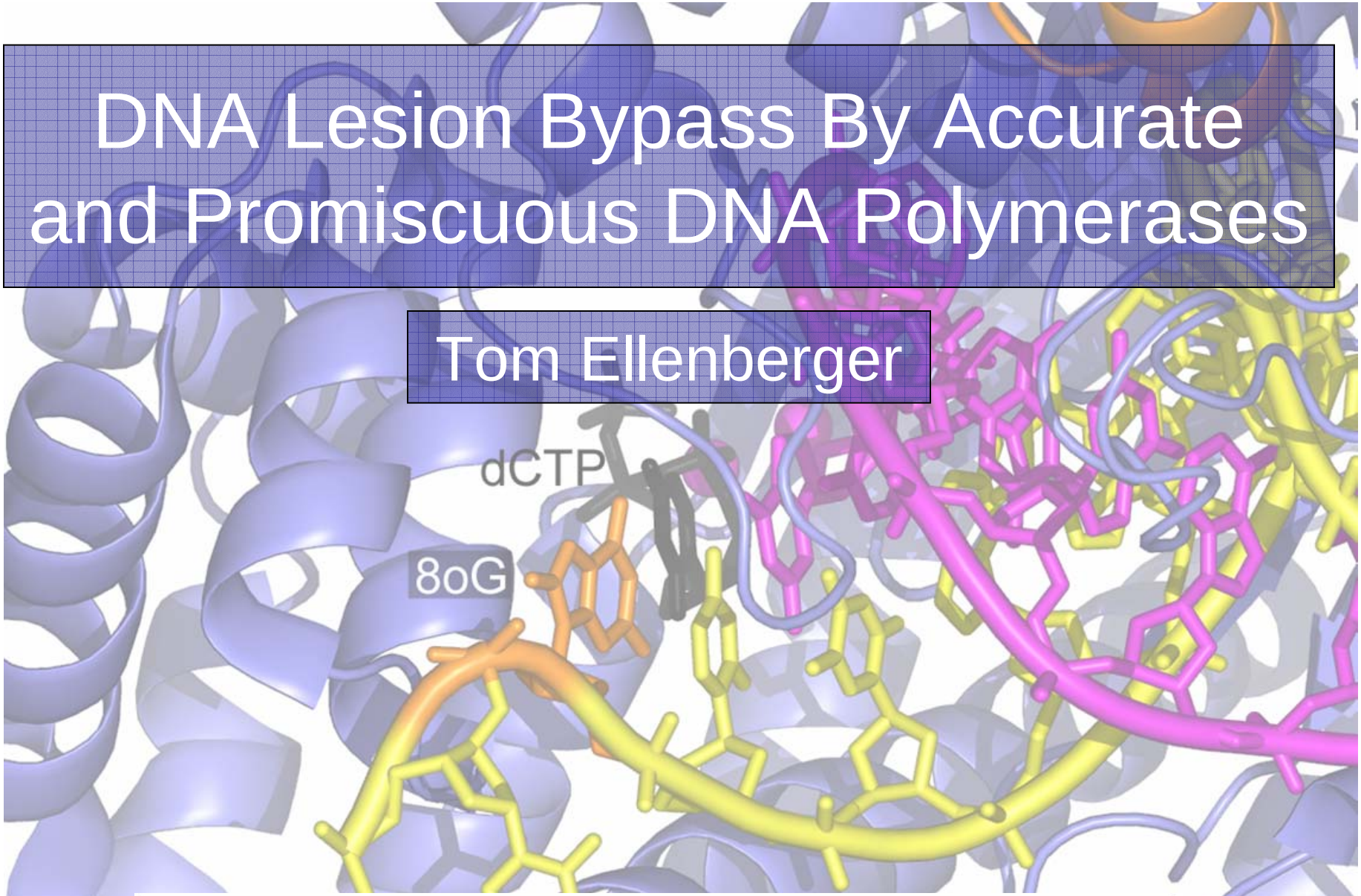
DNA Lesion Bypass By Accurate and Promiscuous DNA Polymerases

Tom Ellenberger



Department of Biochemistry and Molecular Biophysics

Washington University in St.Louis
SCHOOL OF MEDICINE