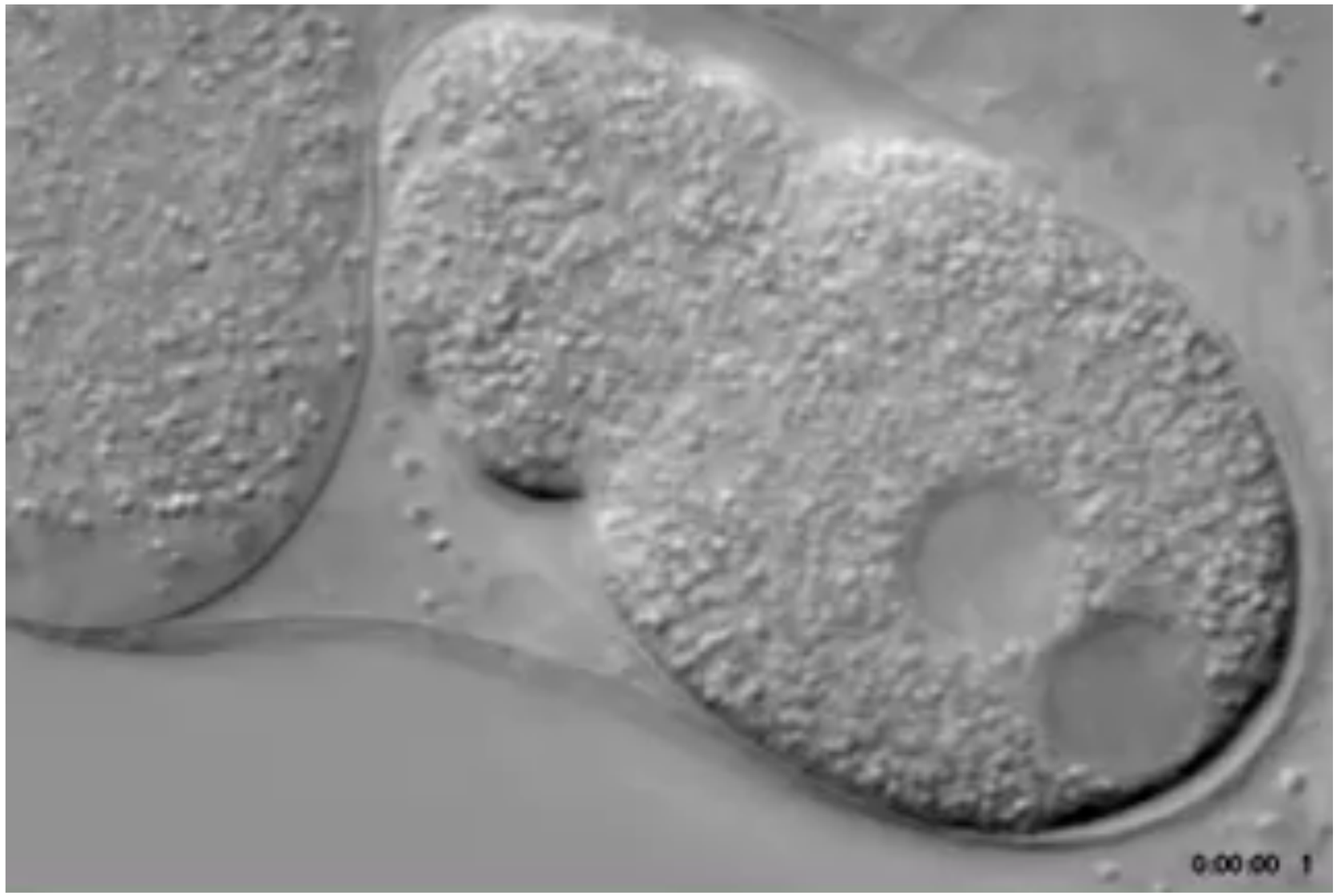
Molecular Acrobat of DNA Translesion Synthesis

Wei Yang, NIH

DNA Replication is Essential



Williams et al. (2004) Human Mol Gen

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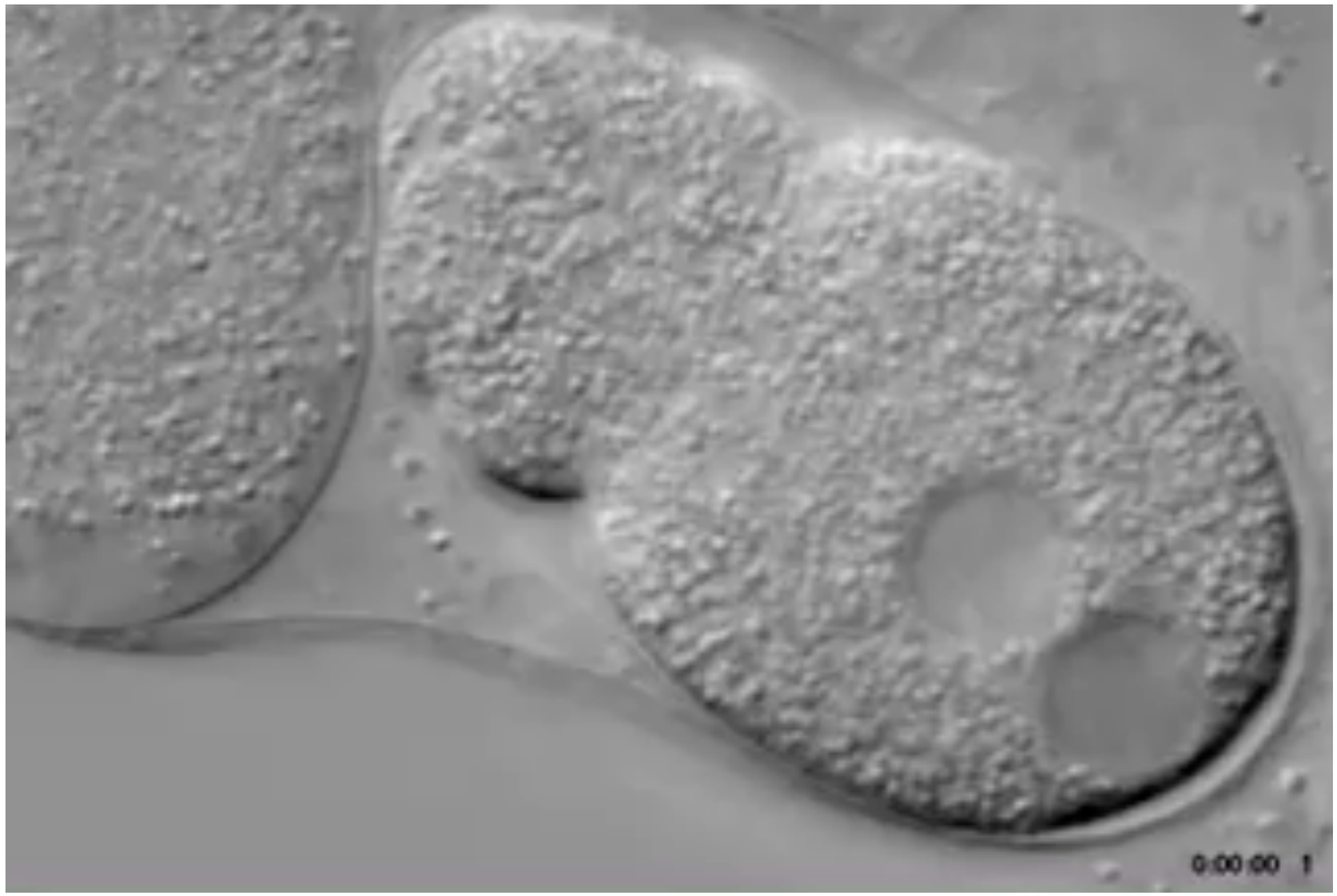

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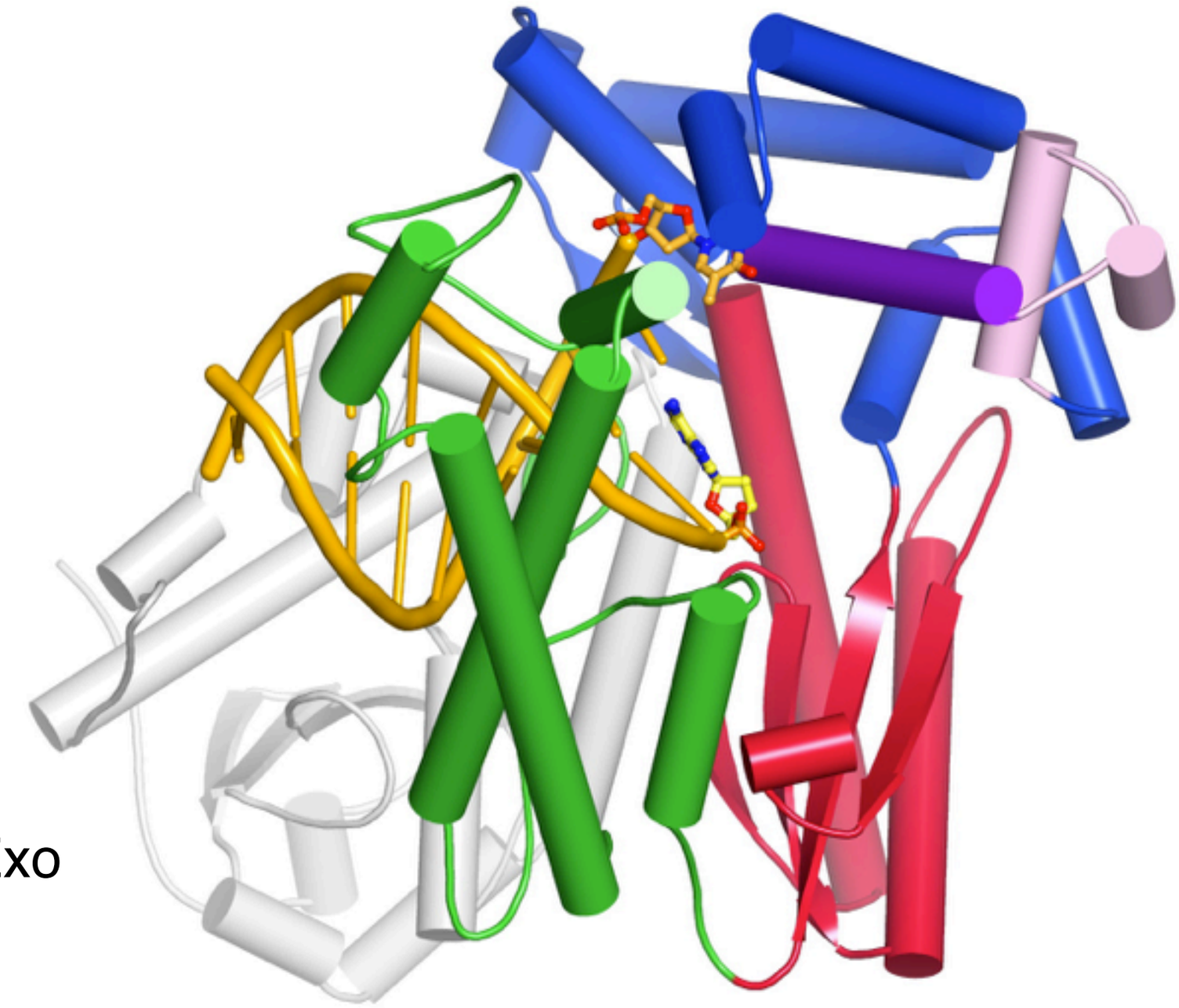
Conserved DNA Replicase, Proofreading & Conformational Changes



Pelletier et al. Kraut (1994) Science
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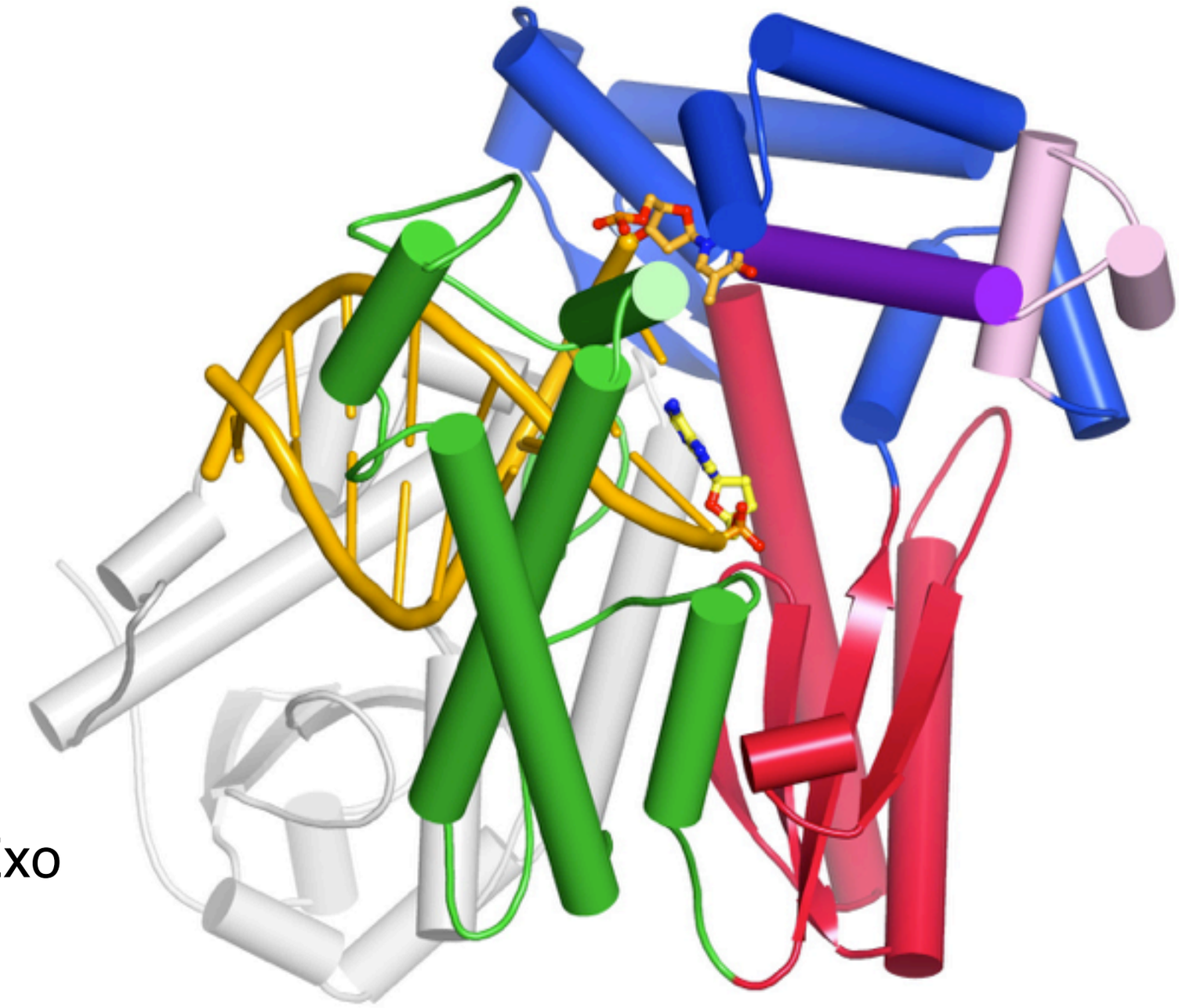

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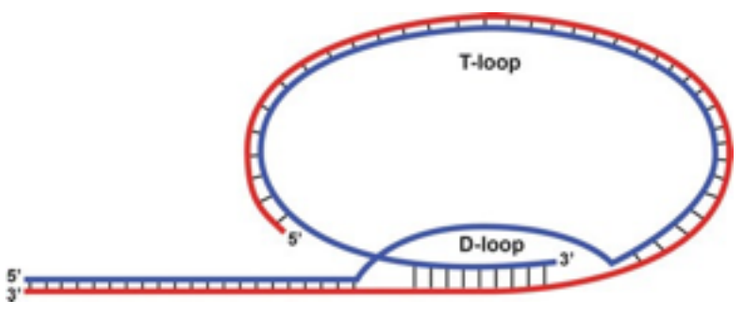
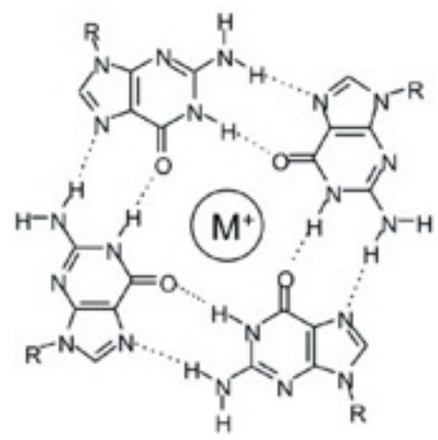
Naturally Occurring Roadblocks in DNA Replication
Fragile Sites, Centromere and Telomere

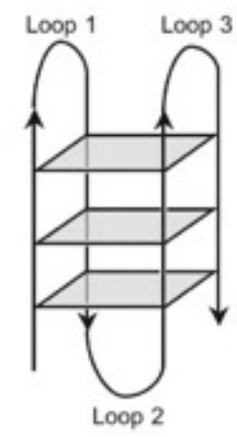
Telomere — (TTAGGG)n

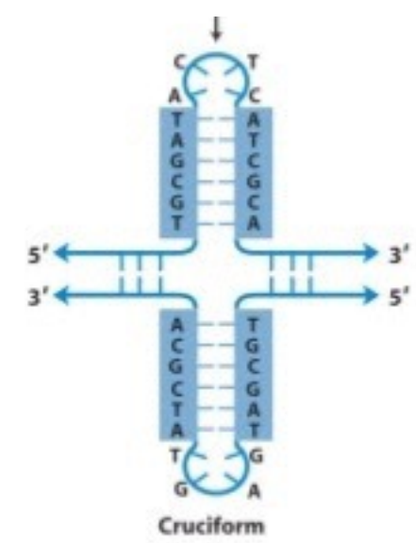
Centromere — a-satellite repeats
AATAT, TTCTC

Fragile sites — > 120 breakage sites,
palindromic AT-rich &
simple 2-3 nt repeats

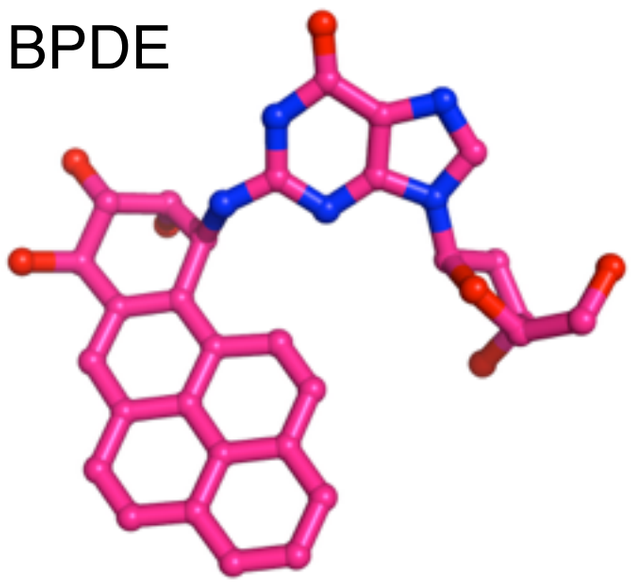




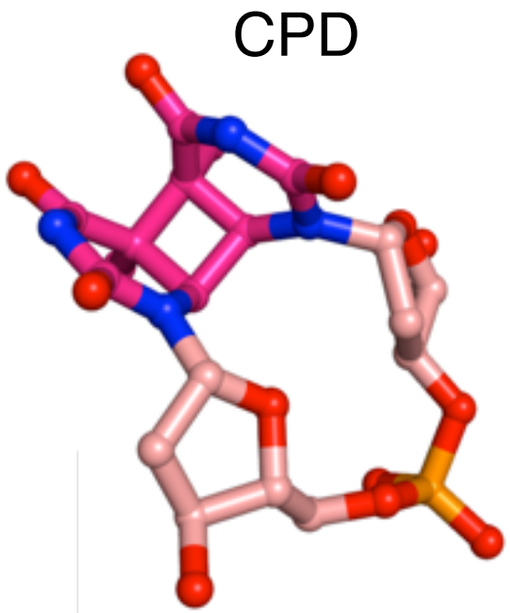


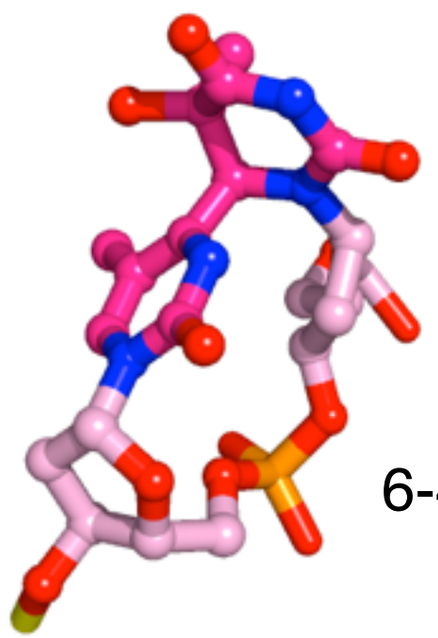


DNA Lesions are Unavoidable and Varied



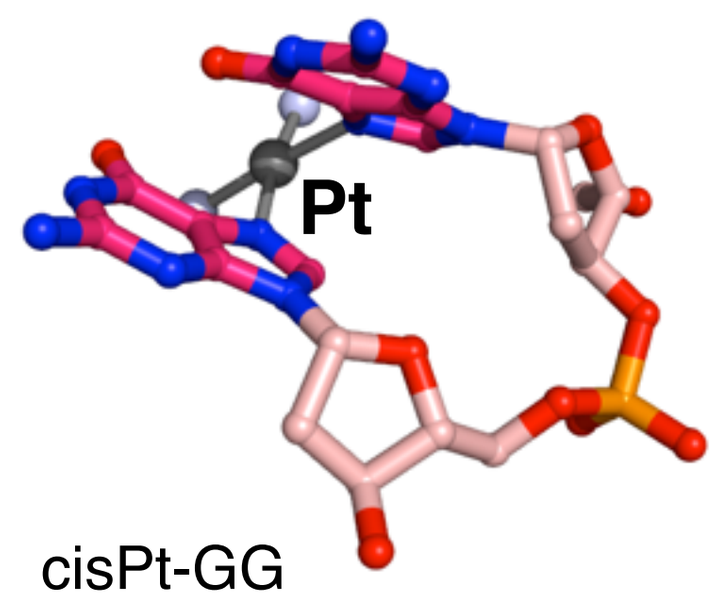
Smoke, BBQ





UV-induced

Chemotherapy



cisPt-GG

Seventeen Human DNA Polymerases

Family	Name	Error rate	Function
A	Pol γ	10^{-5} to 10^{-6}	Mitochondrial replication
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Replication, Repair, Translesion

Lange, Takata & Wood (2010) Nat Rev; Garcia-Gomez et al. & Blanco (2013) Mole Cell

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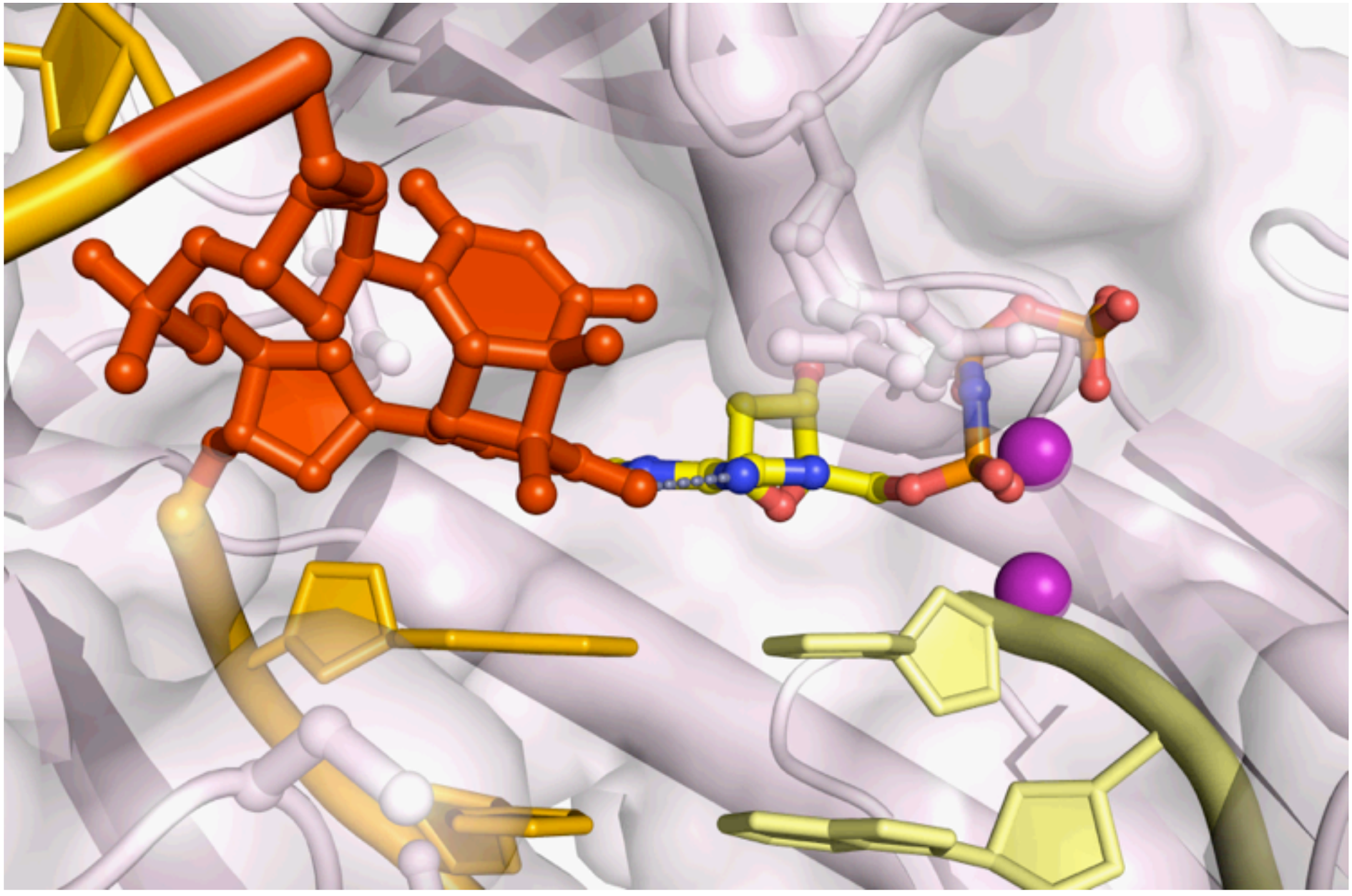
The Active Site of hPol η is Unusually Large & Snugly Accommodates a CPD



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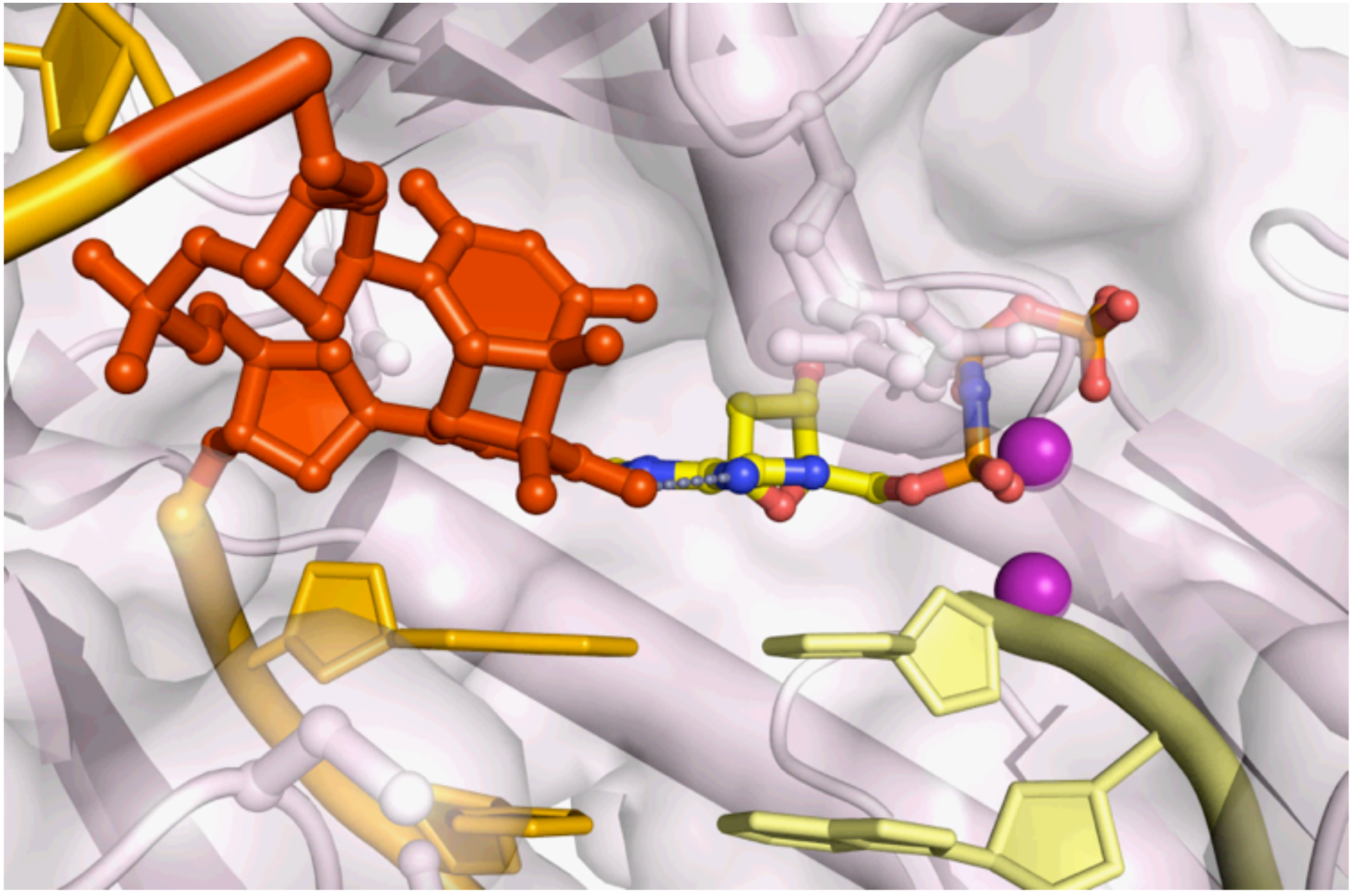

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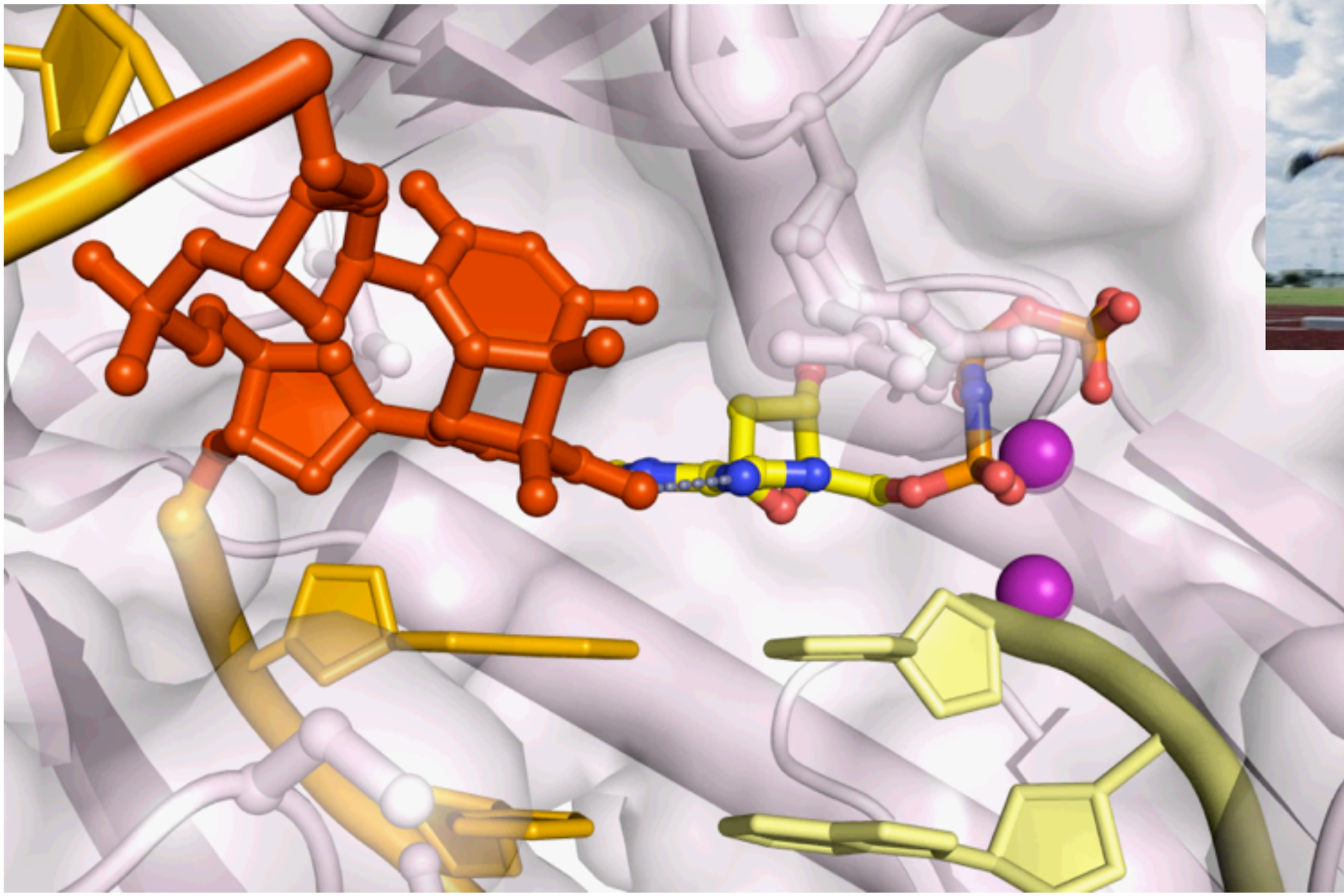

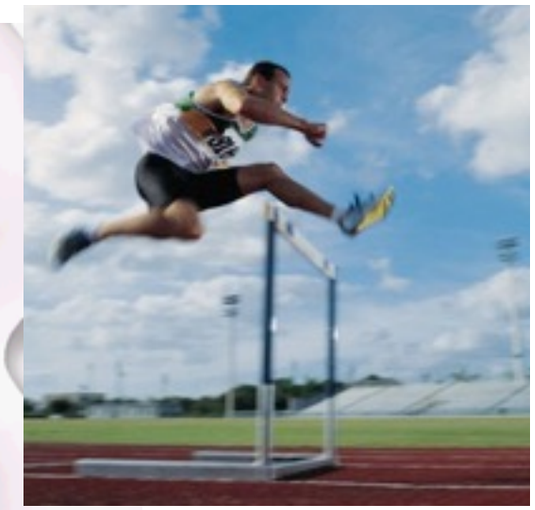

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Mechanisms for Translesion Synthesis by the Y-family DNA Polymerases

Y-family TLS polymerases: lesion accommodation and bypass

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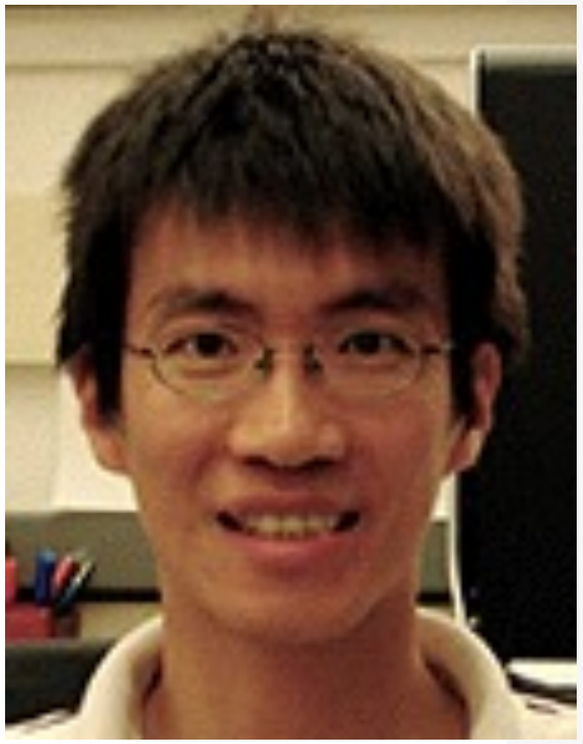
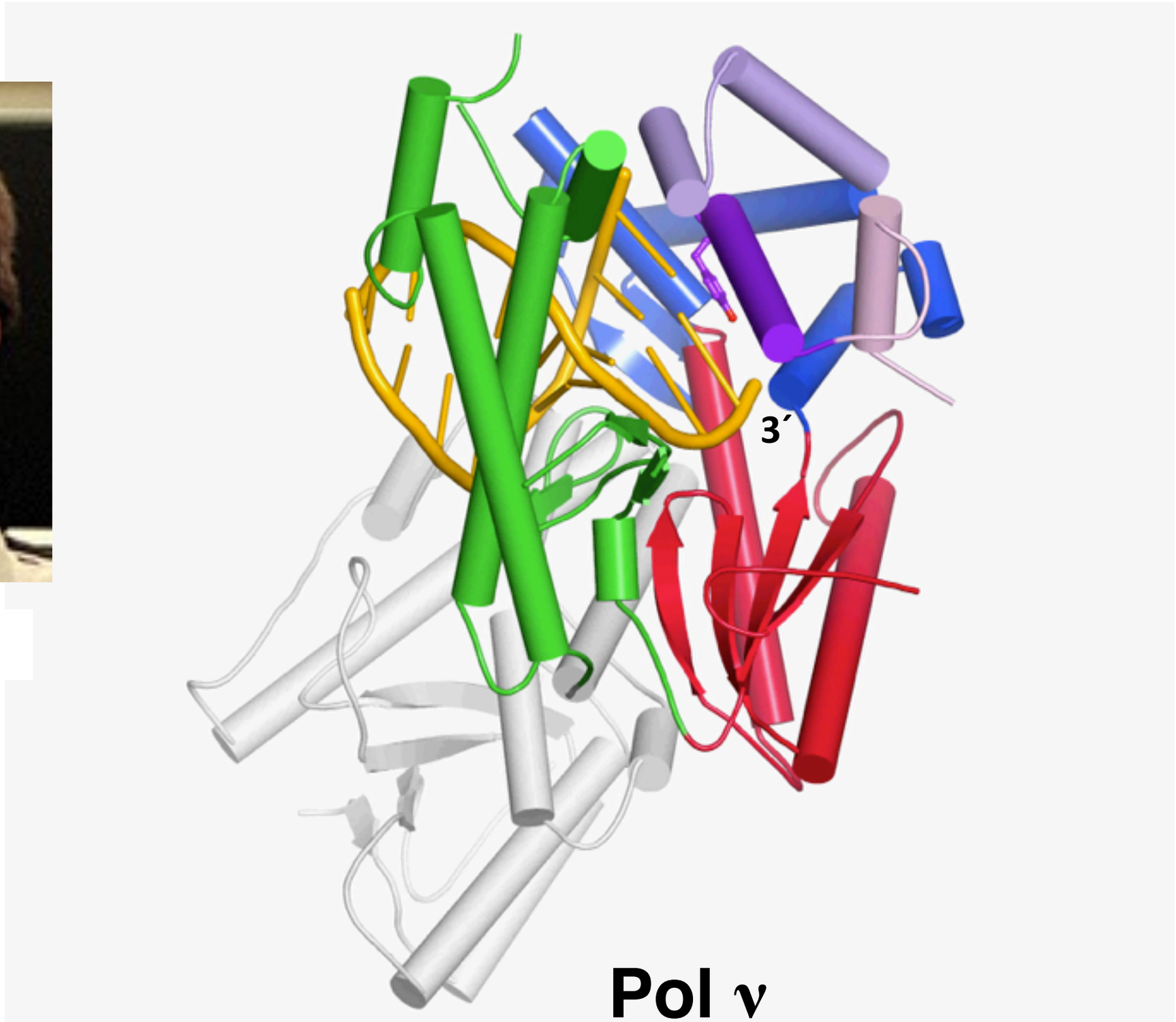
How A Homolog of High-fidelity DNA polymerases Carries out Mutagenic DNA Synthesis



Young-Sam Lee



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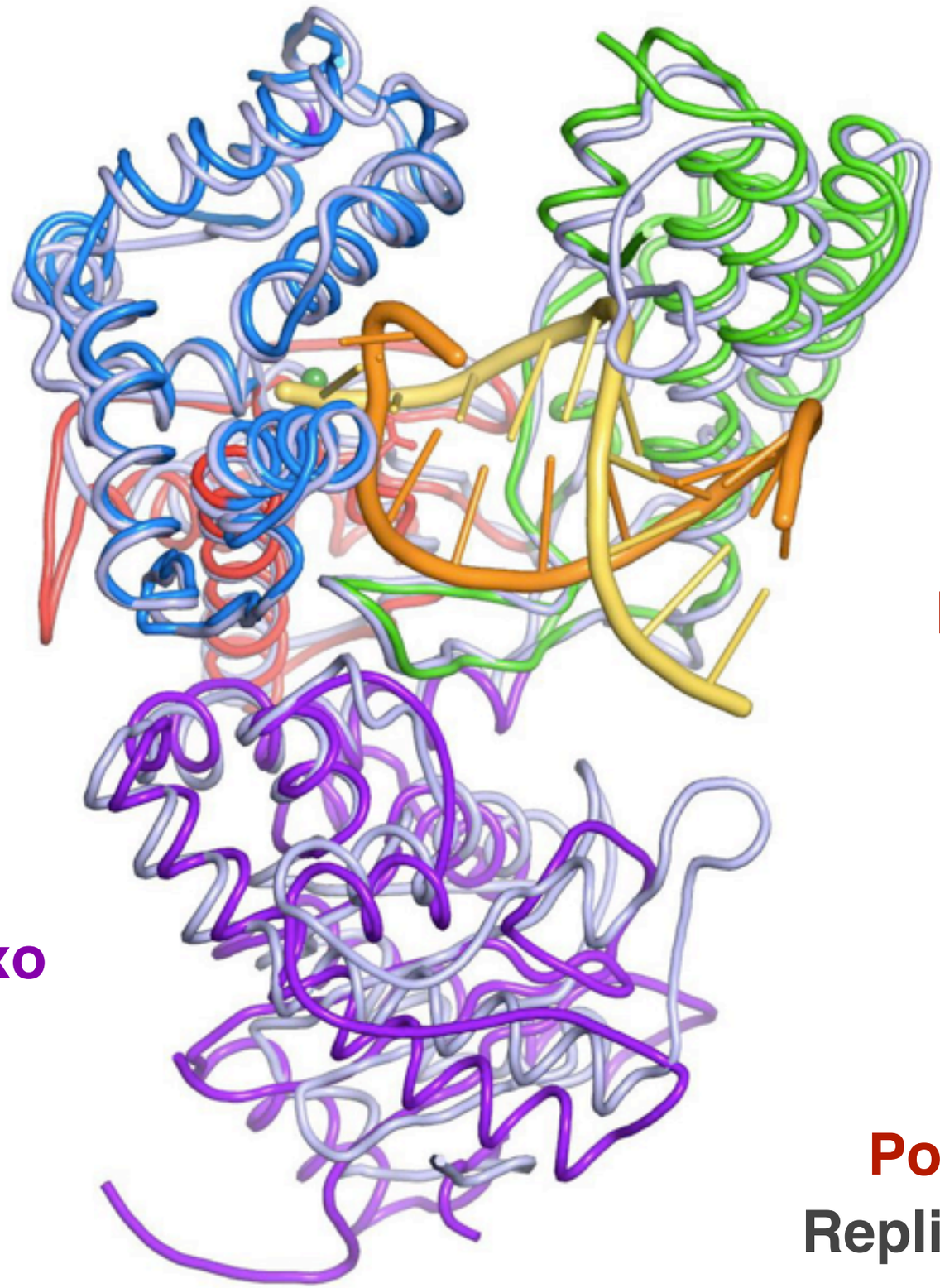
Pol ν is Mostly Homologous to Replicases Except for a few Loops and a Lack of Proofreading



Lee, Gao & Yang (2015) NSMB

Pol ν in four colors

Replication Pol I in grey

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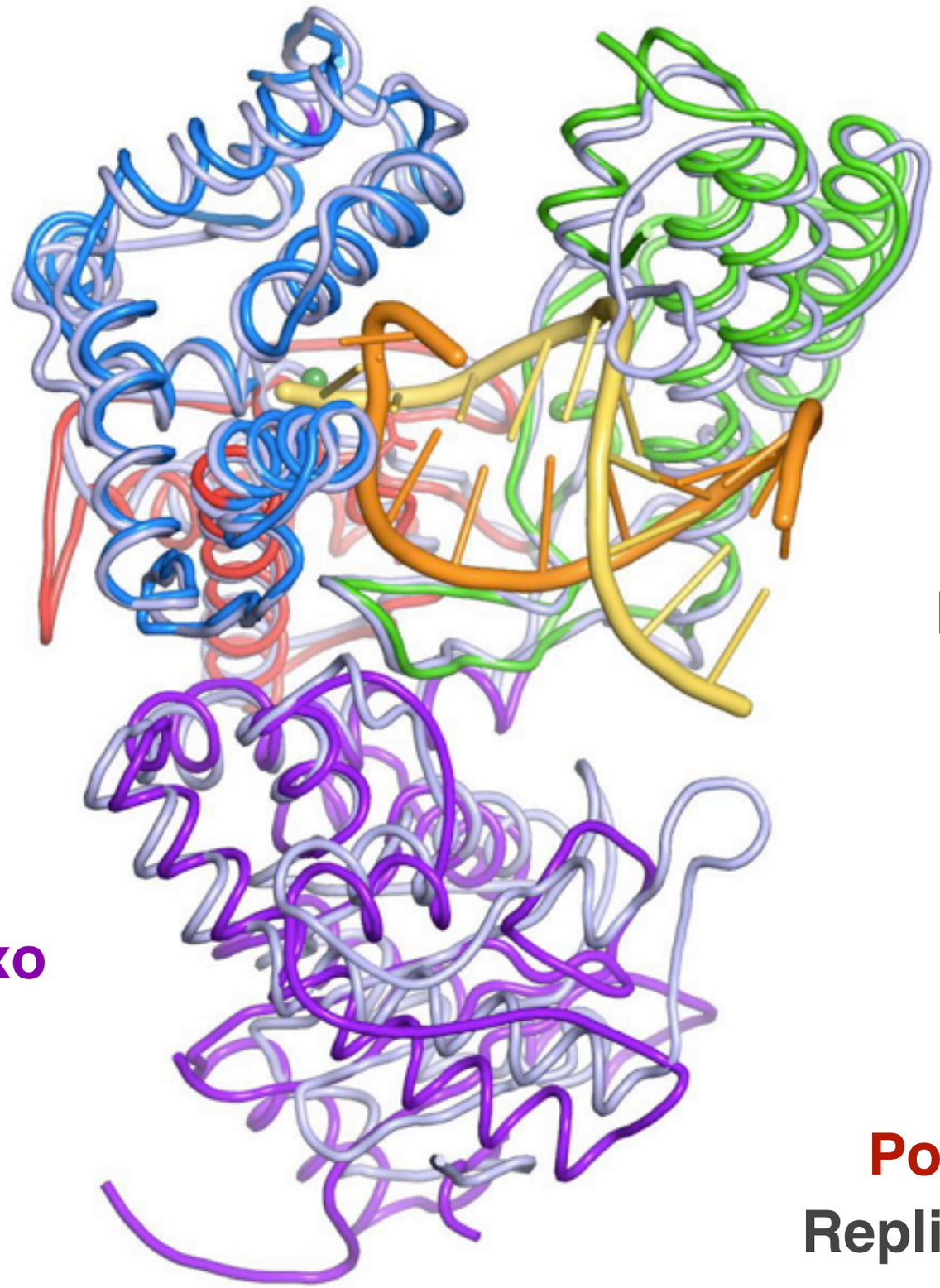

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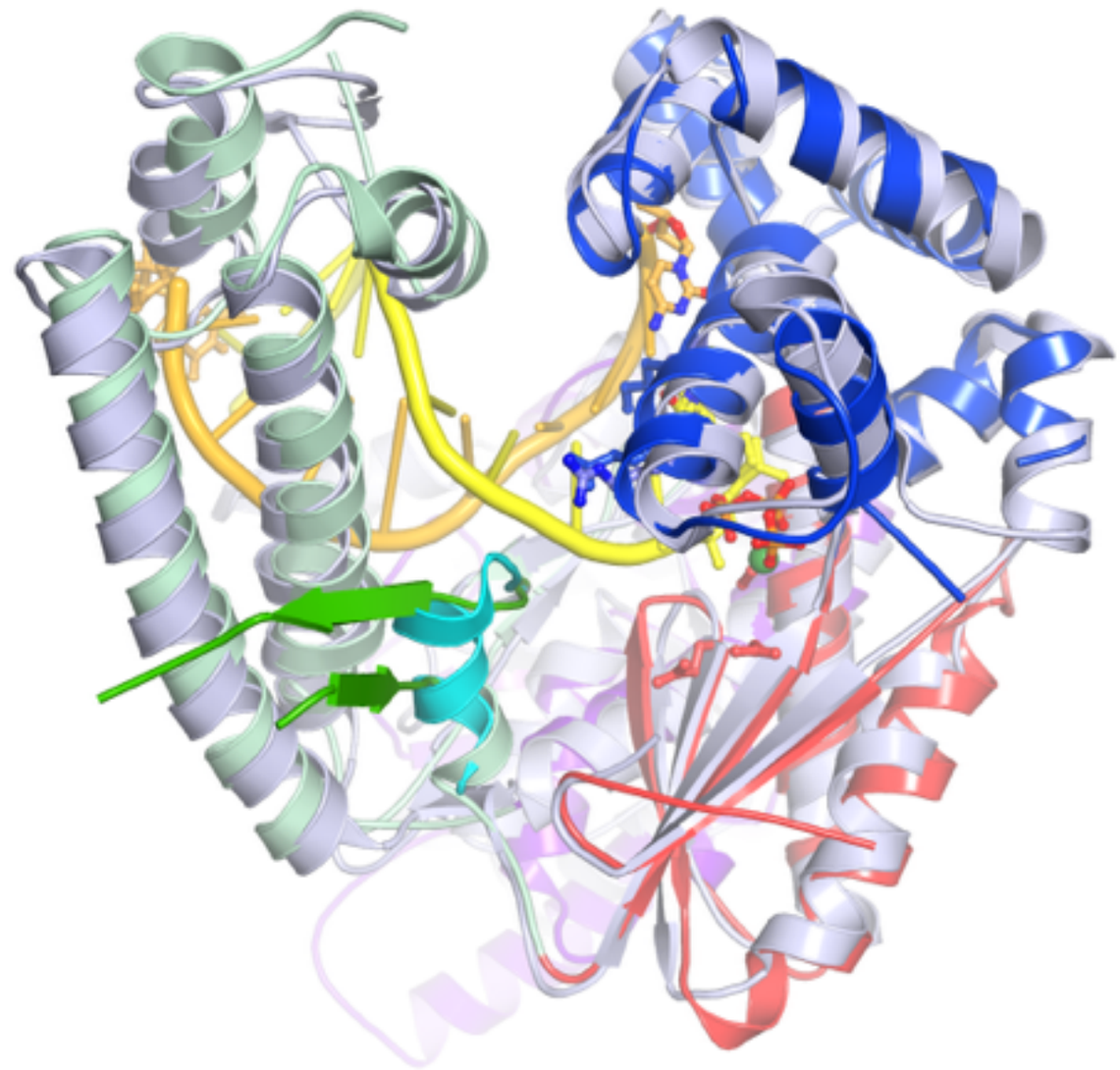
The Insertion in Pol ν's Thumb Leads to Solvent Exposure of the Primer Strand

Pol ν

TLS

Pol I



Replicative

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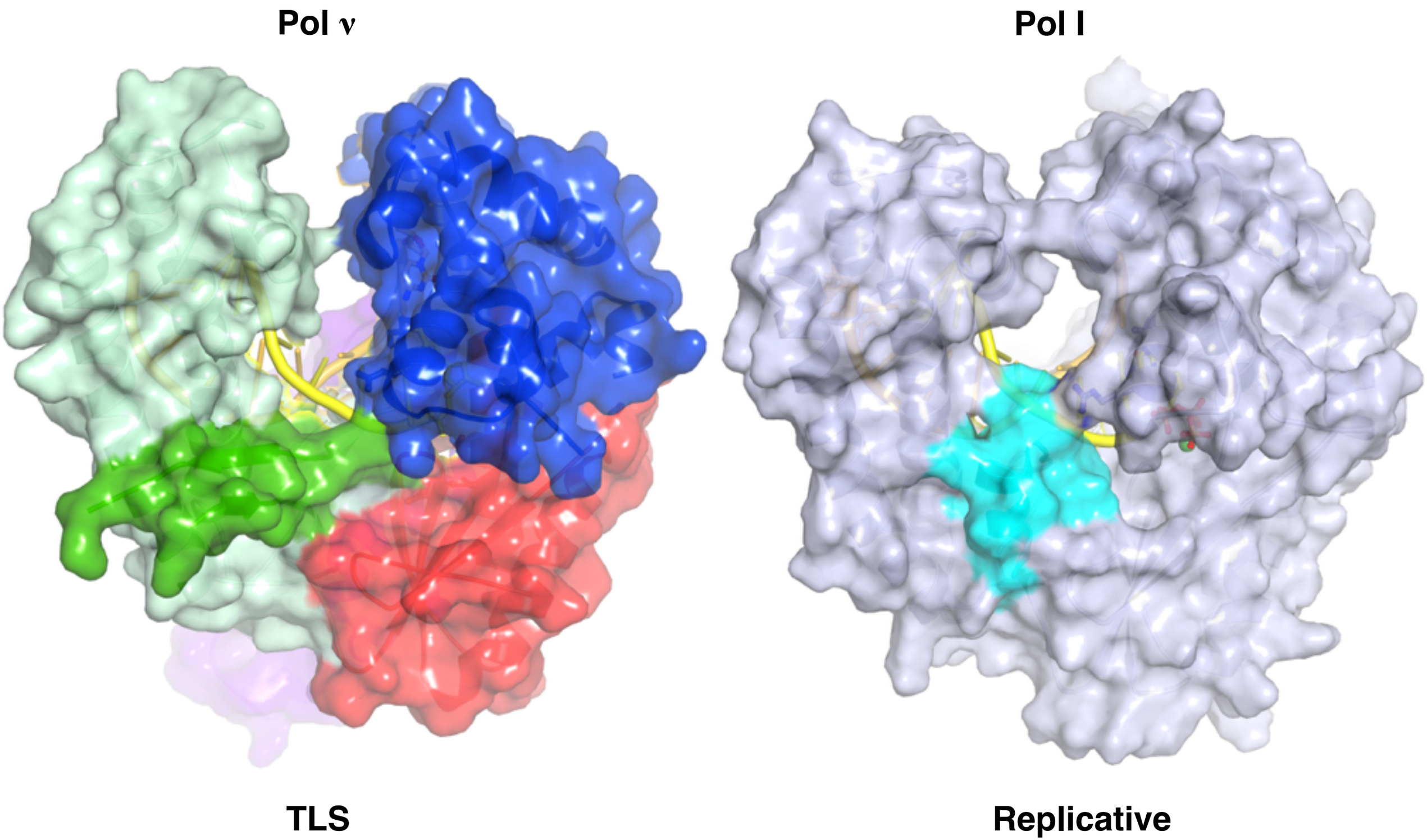

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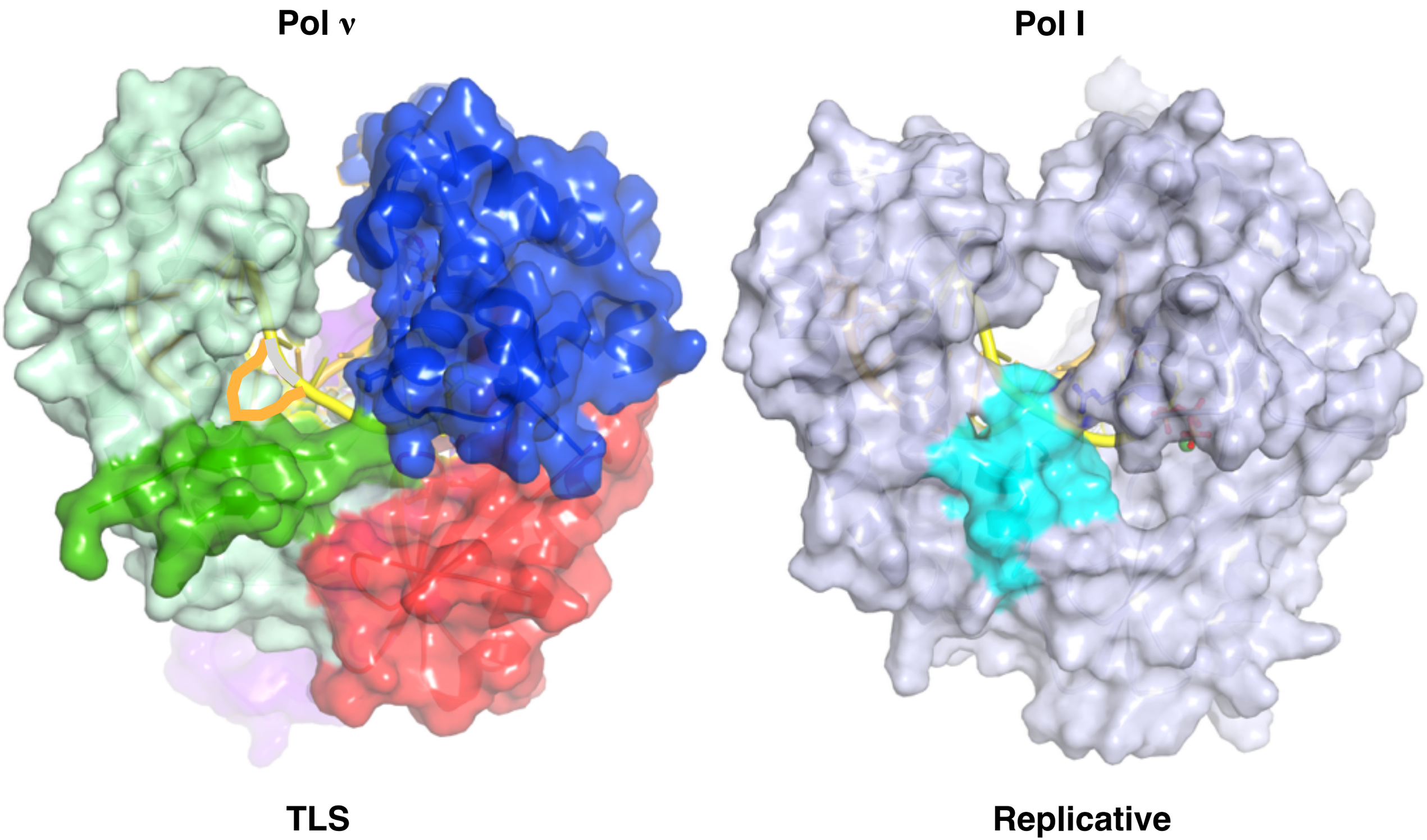
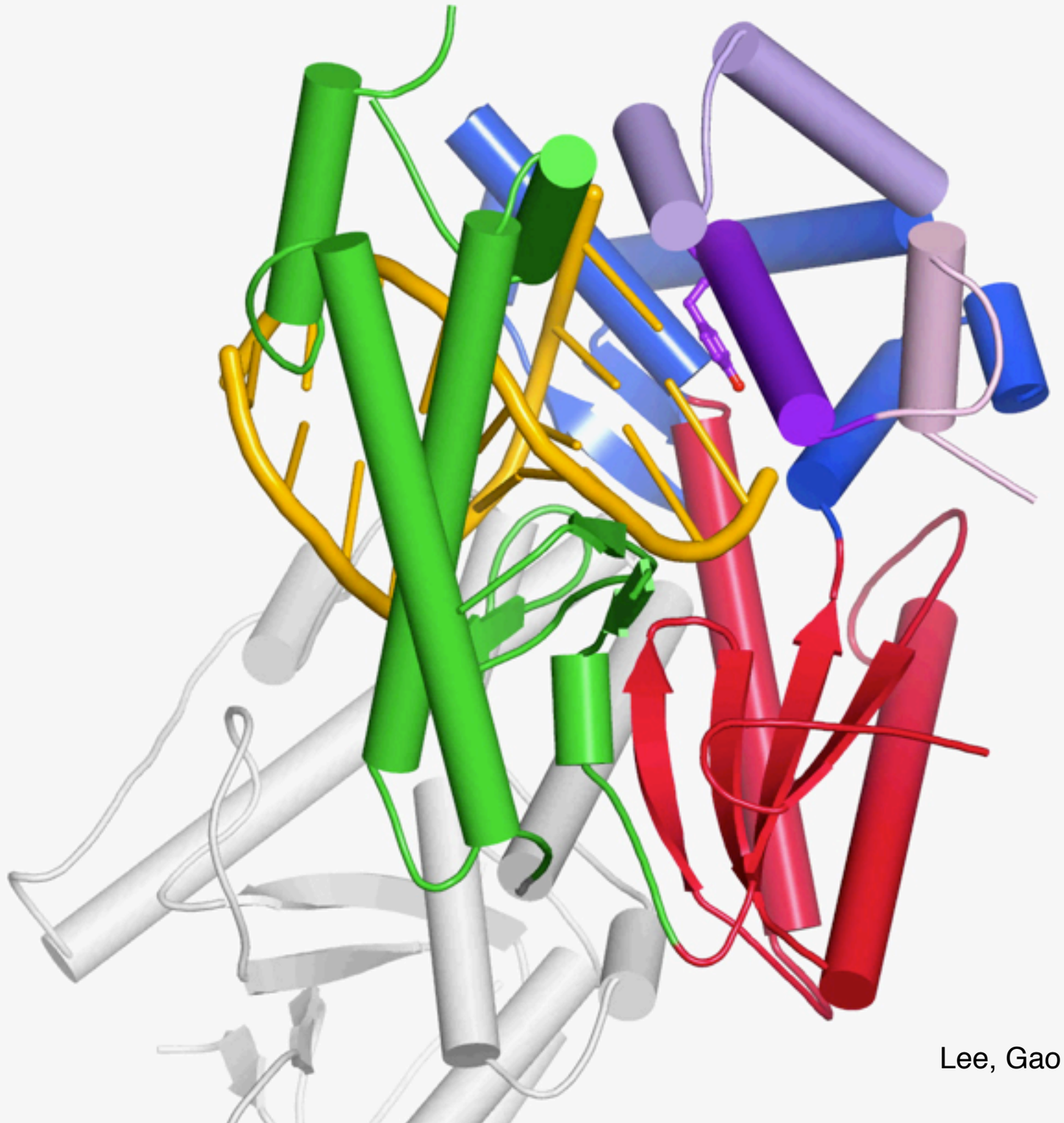


A Moving Thumb of Pol ν Potentially Allows Loopout and Realignment of Primer Strand



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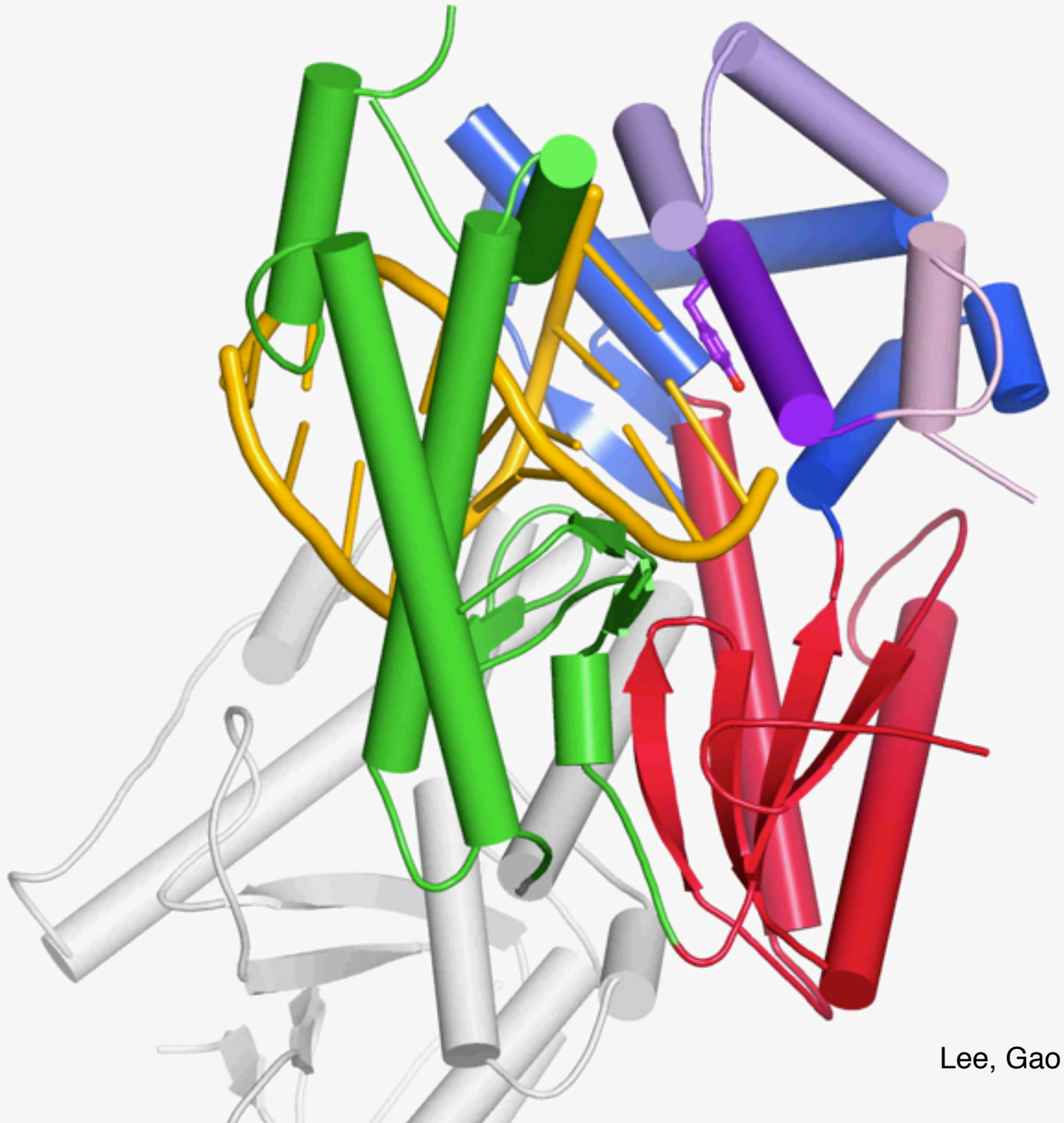

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Translesion DNA Synthesis & Primer Loopout Leads to DNA Expansion

A-family TLS polymerases: stalled DNA synthesis

Lee, Gao & Yang (2015) NSMB

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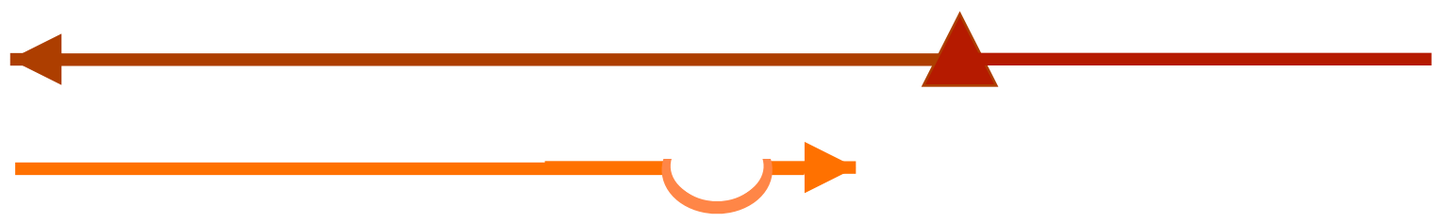
A-family TLS polymerases: DNA primer loopout



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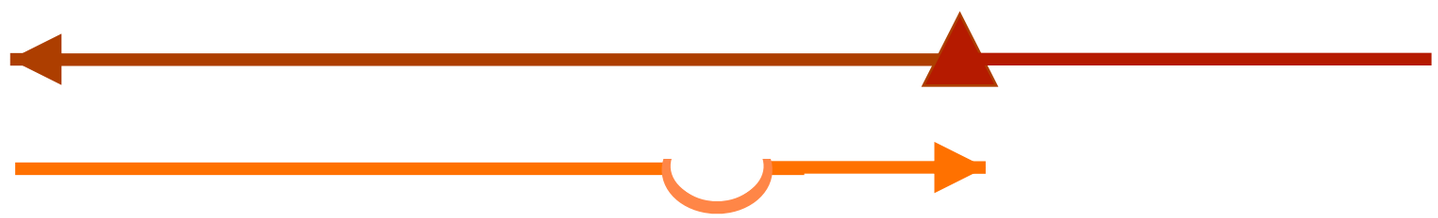
A-family TLS polymerases: TLS DNA synthesis



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A-family TLS polymerases: DNA primer realignment

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A-family TLS polymerases: resumed replicative DNA synthesis

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Repeat Expansion: stalled DNA synthesis

Lee, Gao & Yang (2015) NSMB

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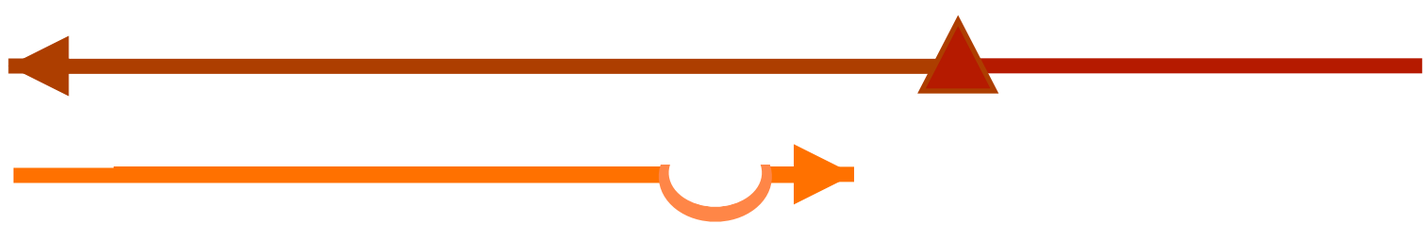
Repeat Expansion: DNA primer loopout



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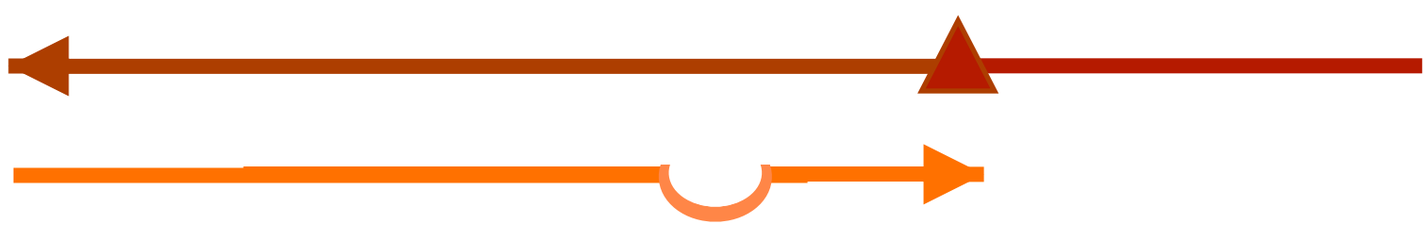
A-family TLS polymerases: resumed replicative DNA synthesis

Repeat Expansion: Repeat synthesis



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A-family TLS polymerases: resumed replicative DNA synthesis

Repeat Expansion: DNA primer realignment

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Repeat Expansion: Repeat loopout again

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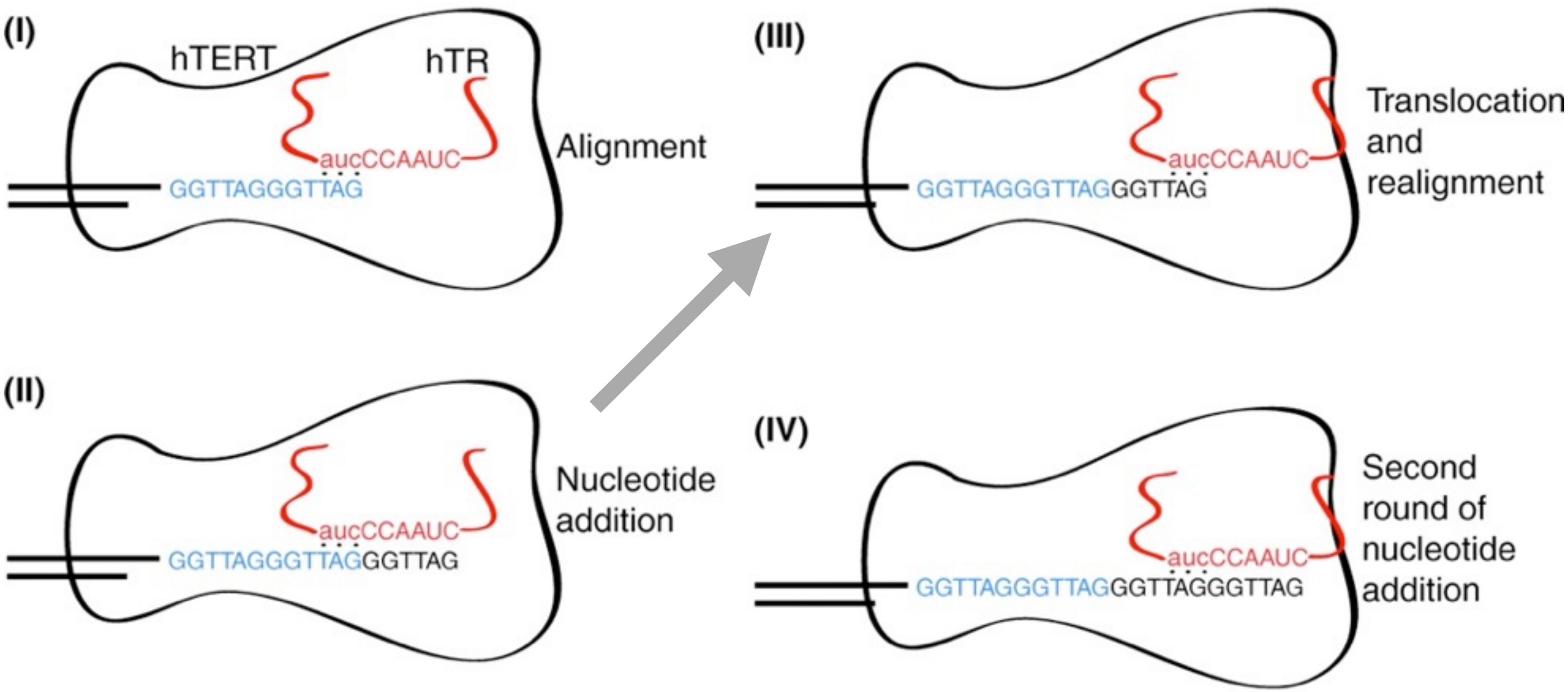
The Mystery of Processive Telomere Synthesis and Repeat Addition

Telomerase = telomere reverse transcriptase (**TERT**) + telomere template RNA (**TR**)



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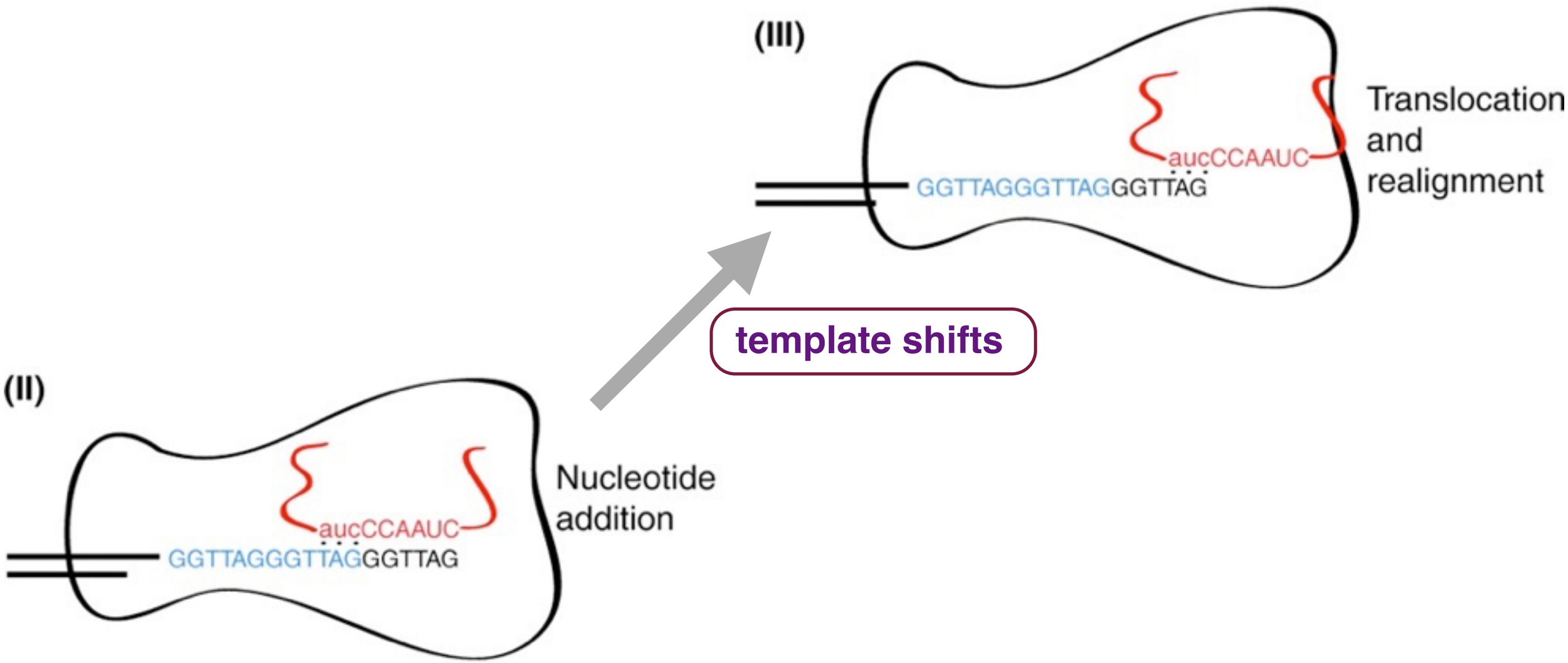

Telomere Repeat can Form a Hairpin Loop

Vertebrates:

Plants:

Tetrahymena:

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Telomere Repeat can Form a Hairpin Loop

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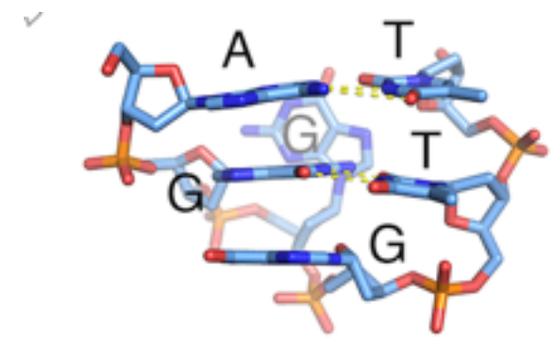
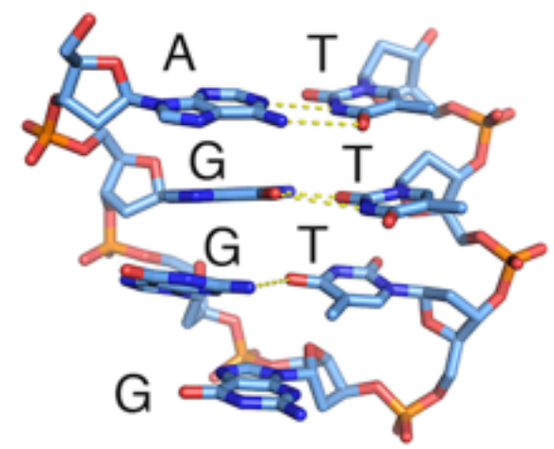
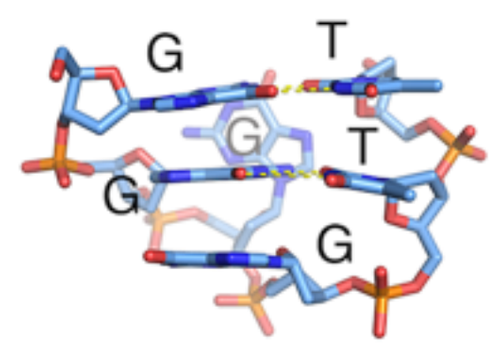


Plants:



Tetrahymena:



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Telomere Synthesis & Mechanism for Repeat Addition Processivity

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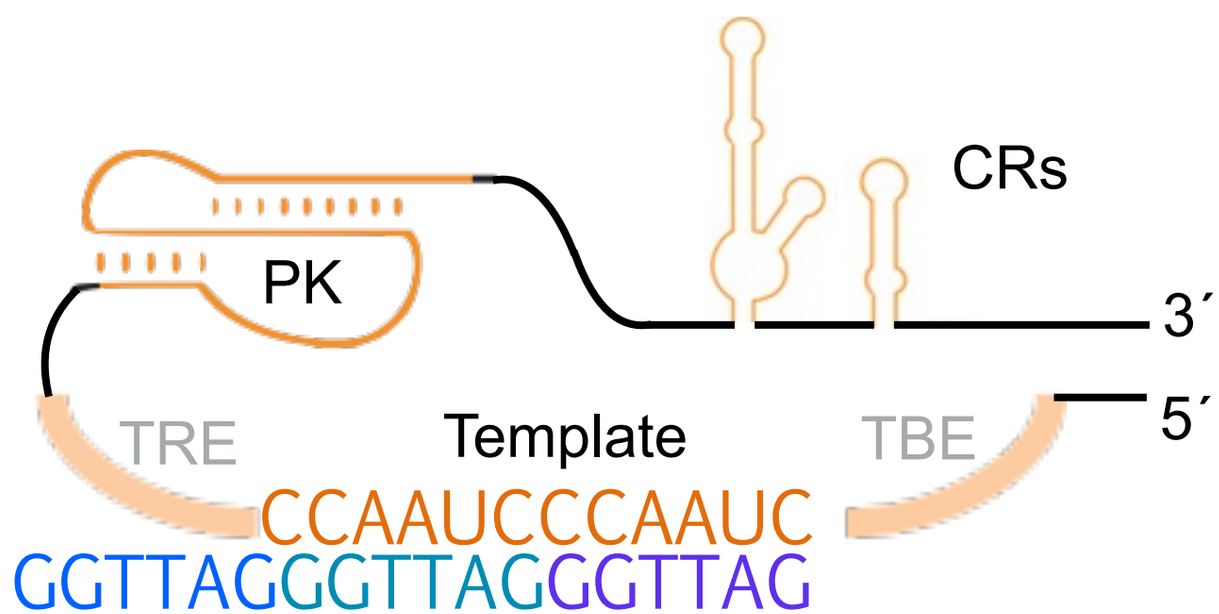
TERT

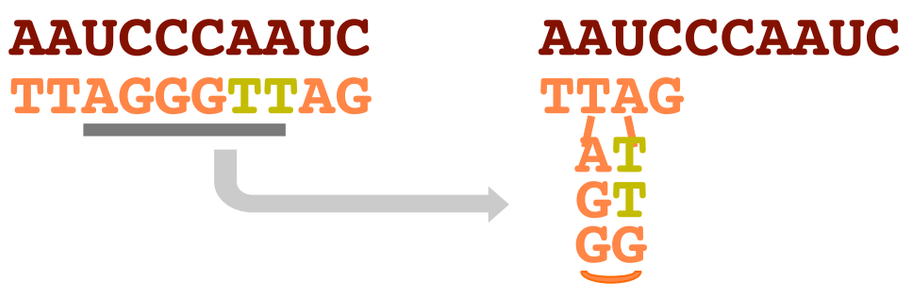
TR



A Hairpin Loop Model (HLM) for Telomere Synthesis

DNA primer looping out, RNA template translocation

Vertebrates:



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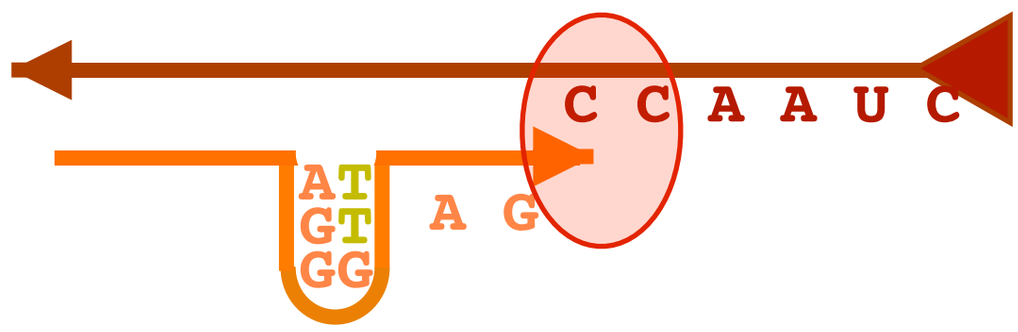
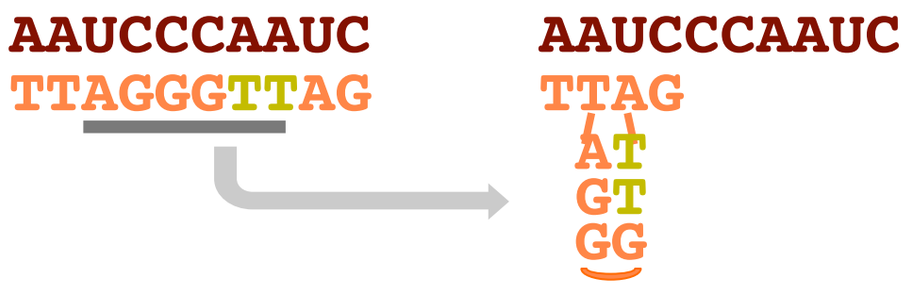
Incoming dGTP stabilizes the looped out primer



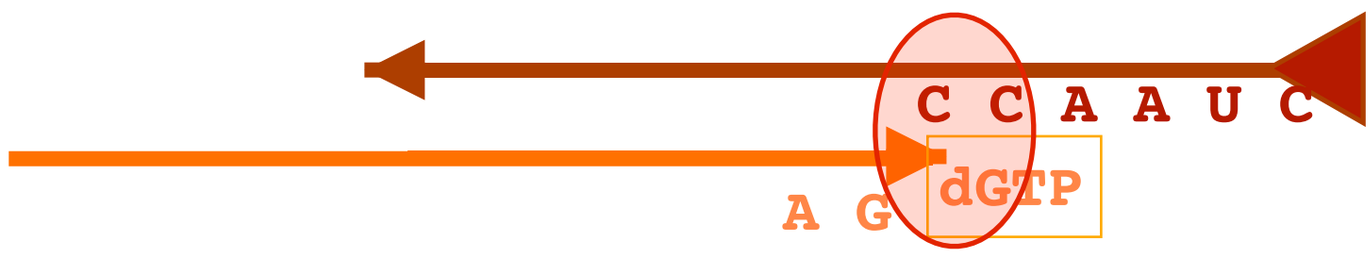
Vertebrates:



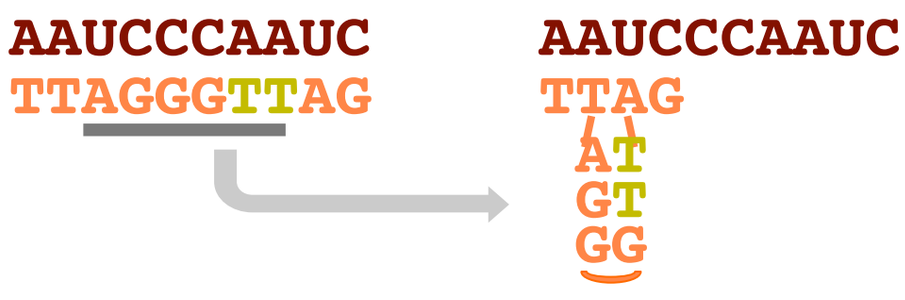
Yang & Lee (2015) NSMB

A Hairpin Loop Model (HLM) for Telomere Synthesis

DNA primer realignment



Vertebrates:

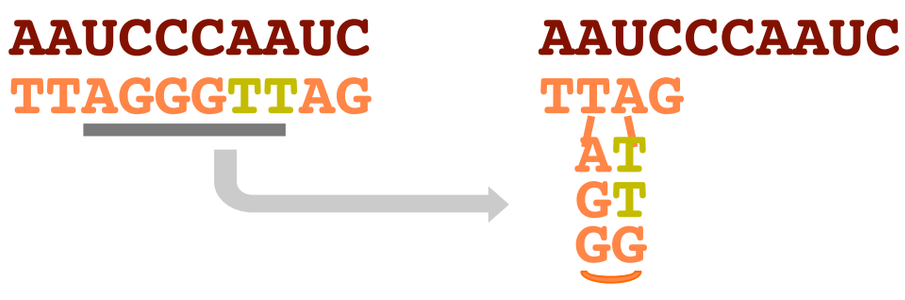


Yang & Lee (2015) NSMB

A Hairpin Loop Model (HLM) for Telomere Synthesis

DNA synthesis

Vertebrates:



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A Hairpin Loop Model (HLM) for Telomere Synthesis

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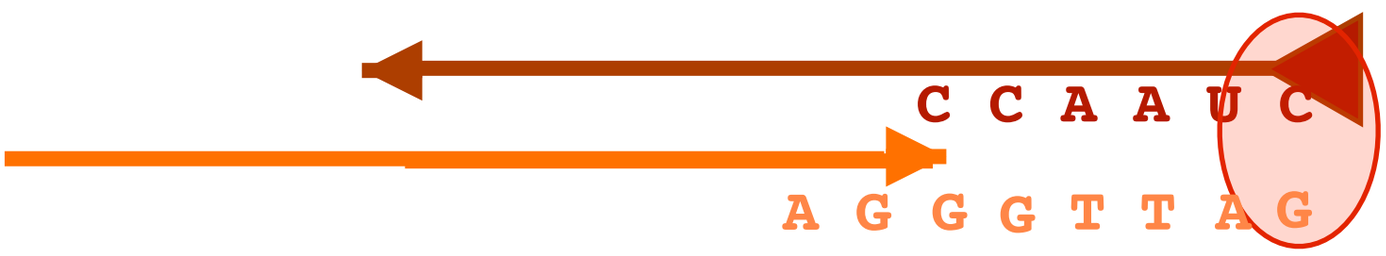
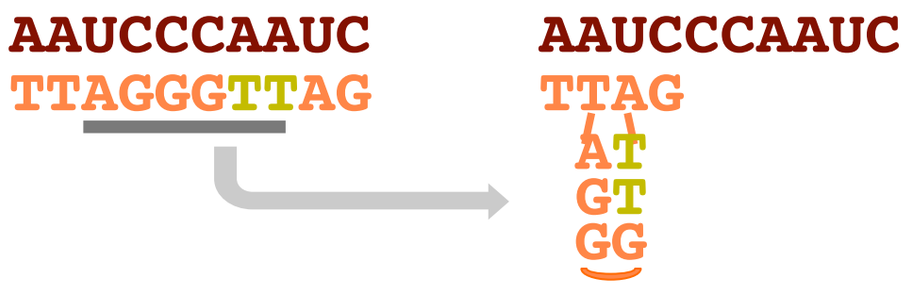


Vertebrates:



Yang & Lee (2015) NSMB

Behind Every (E)Motion is Chemistry

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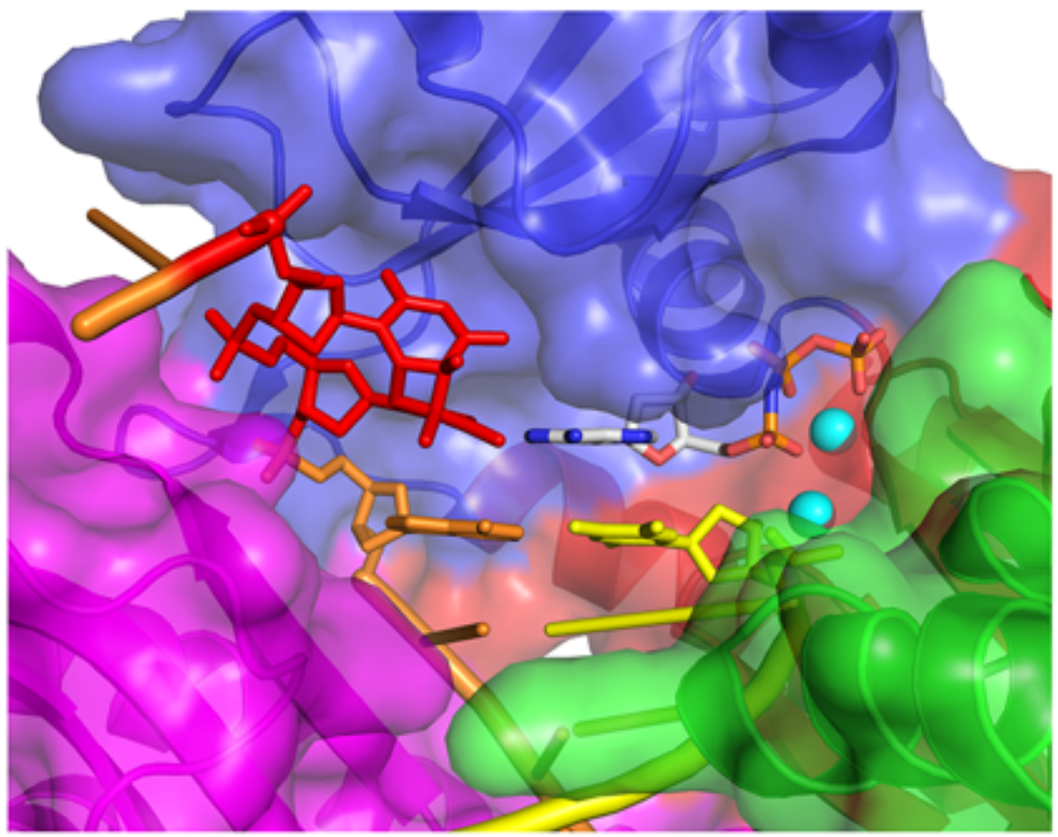
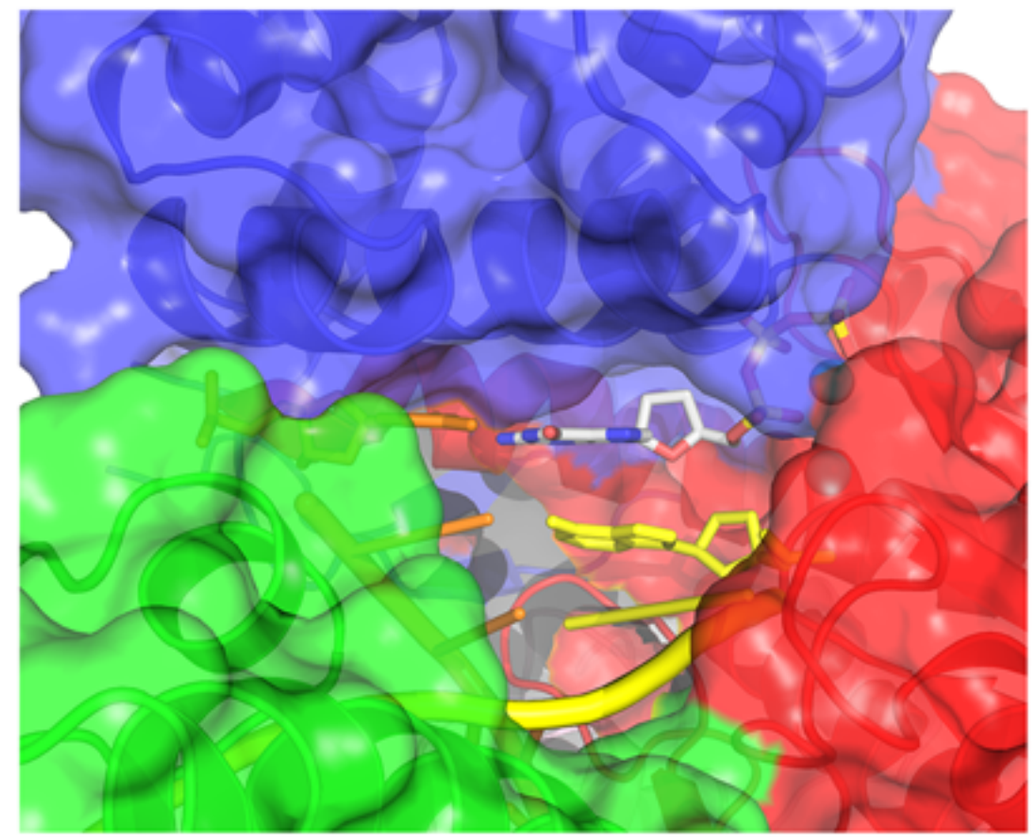
Chemistry of DNA Synthesis

DNA pol η



Biertümpfel et al., 2010 Nature

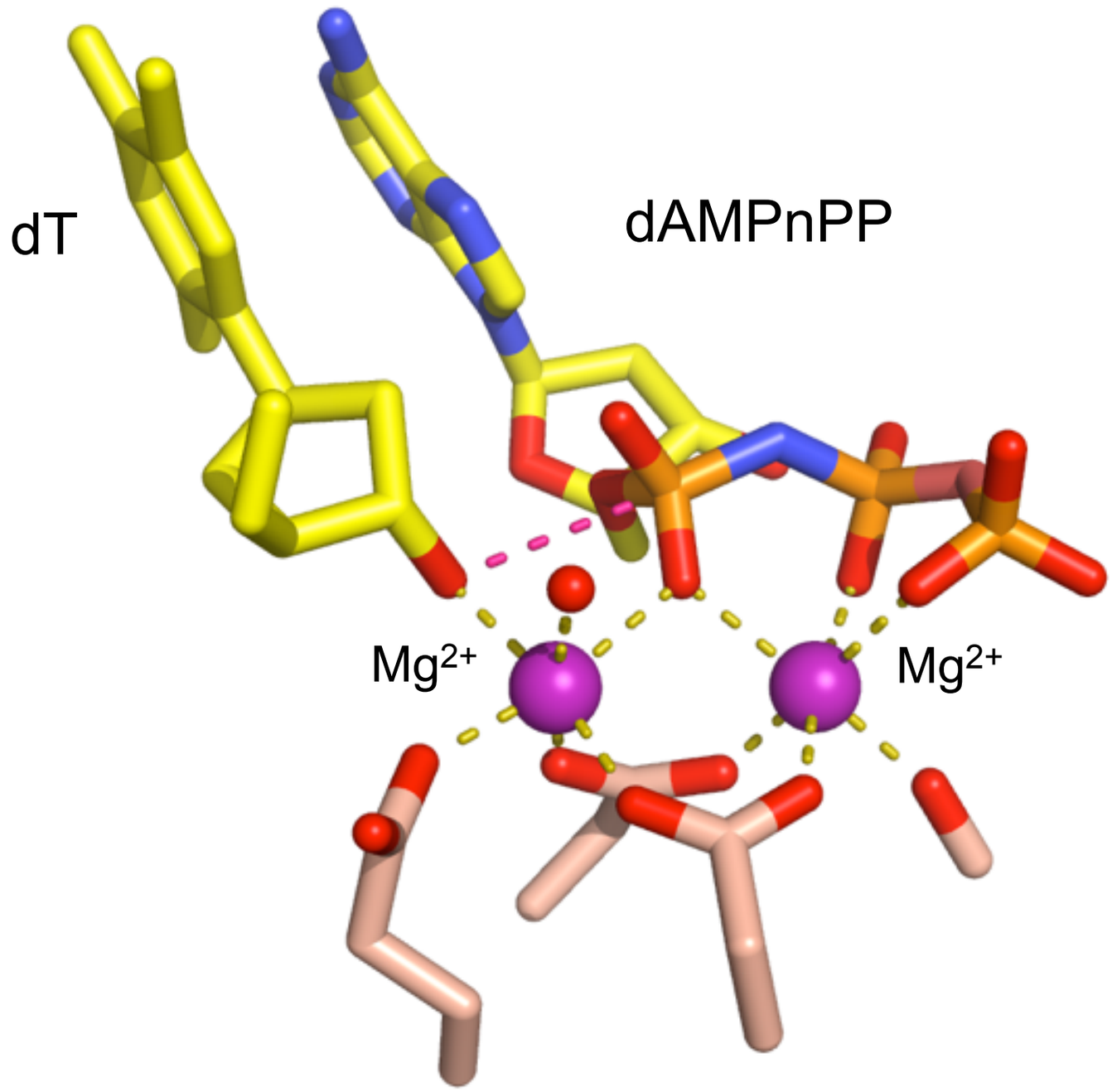
T7 DNA pol



Doublié et al. (1998) Nature

No proofreading
No conformational change

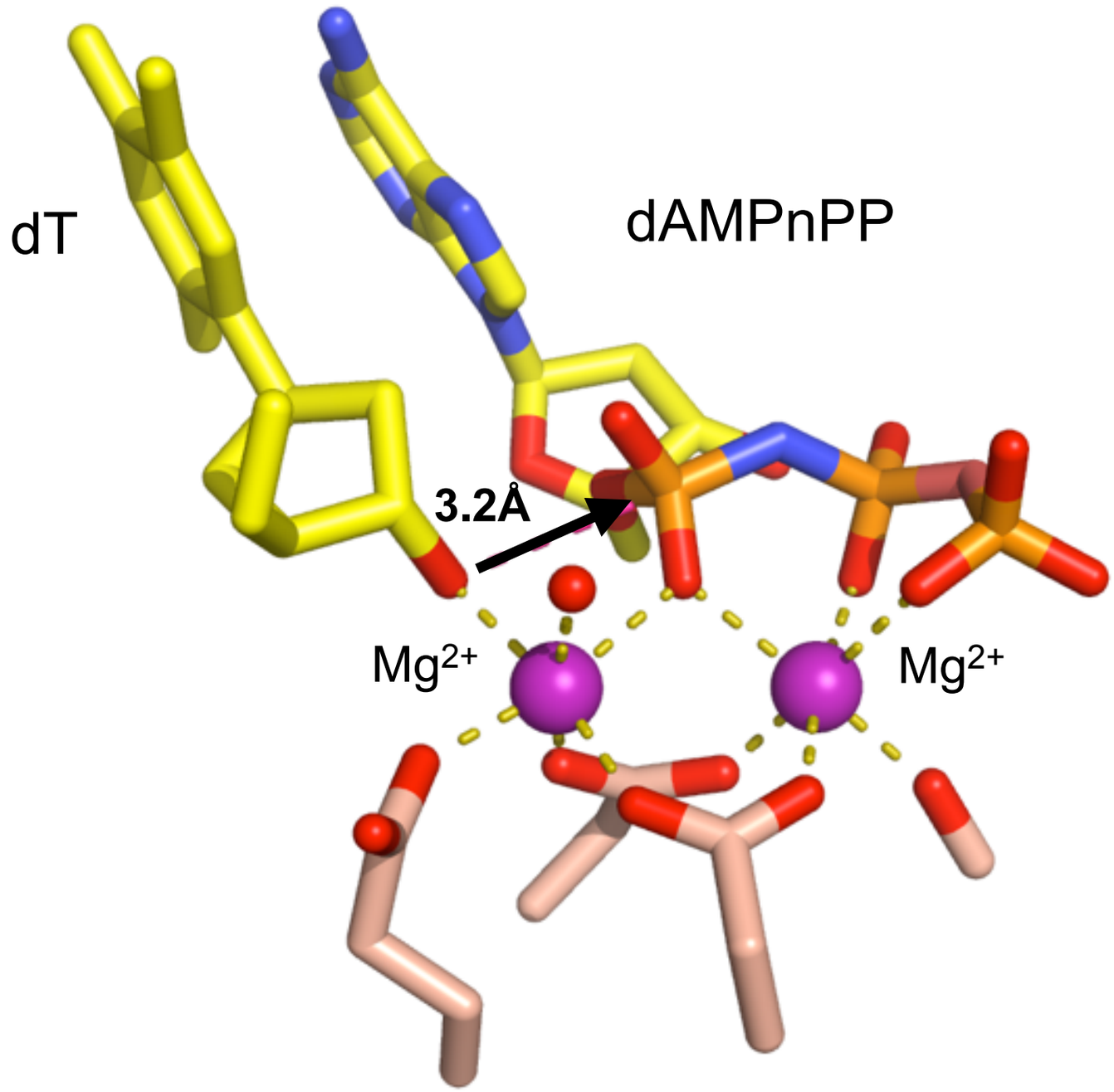
Mg^{2+}-dependent Substrate Alignment & Acid-Base Catalysis of all DNA Polymerases



1.7 Å

Biertuempfel, et al. & Yang (2010) Nature

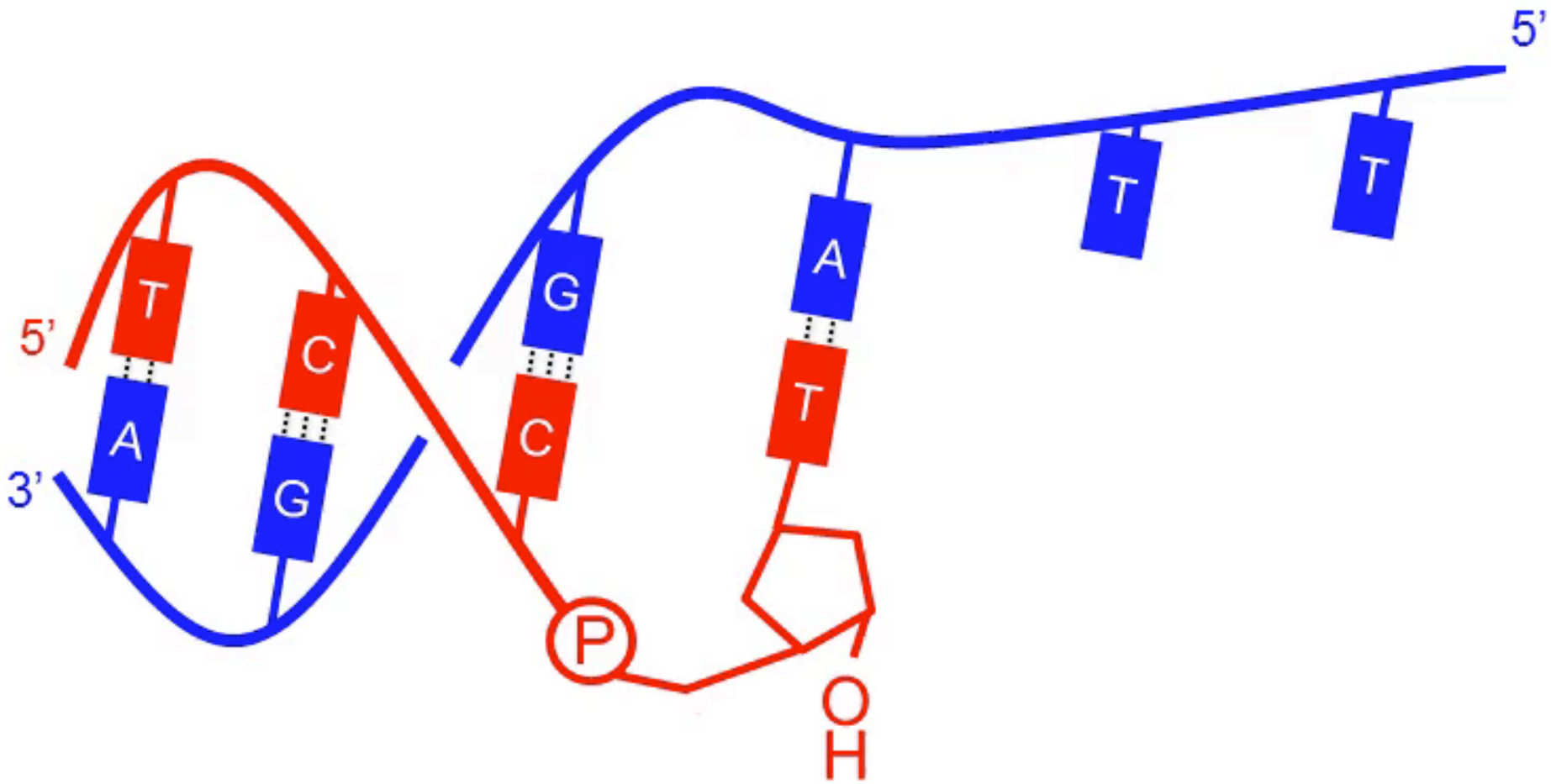
Mg^{2+}-dependent Substrate Alignment & Acid-Base Catalysis of all DNA Polymerases



1.7 Å

Biertuempfel, et al. & Yang (2010) Nature

How Does Catalysis of a Phosphoryl-transfer Reaction Occur ?



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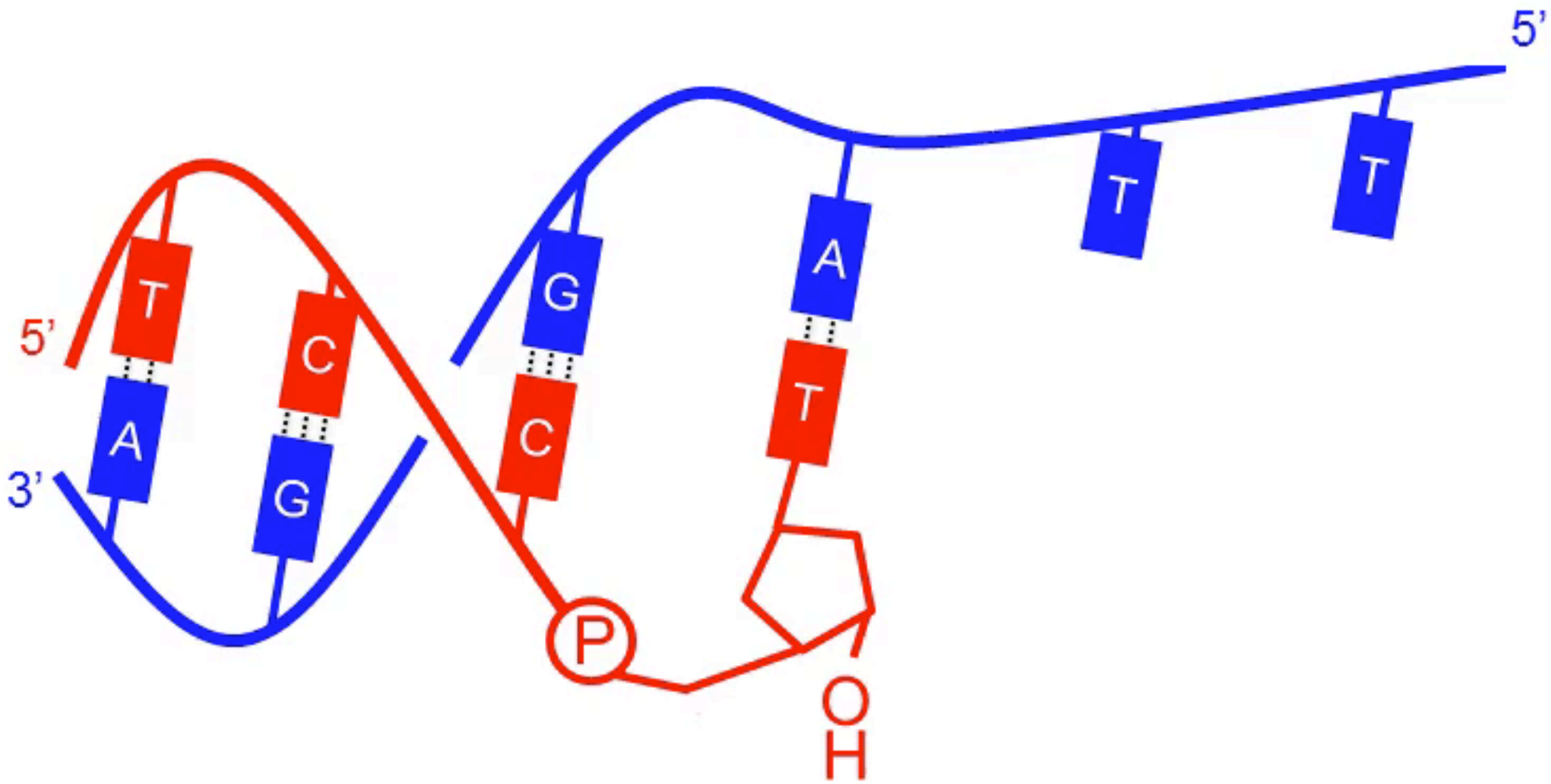

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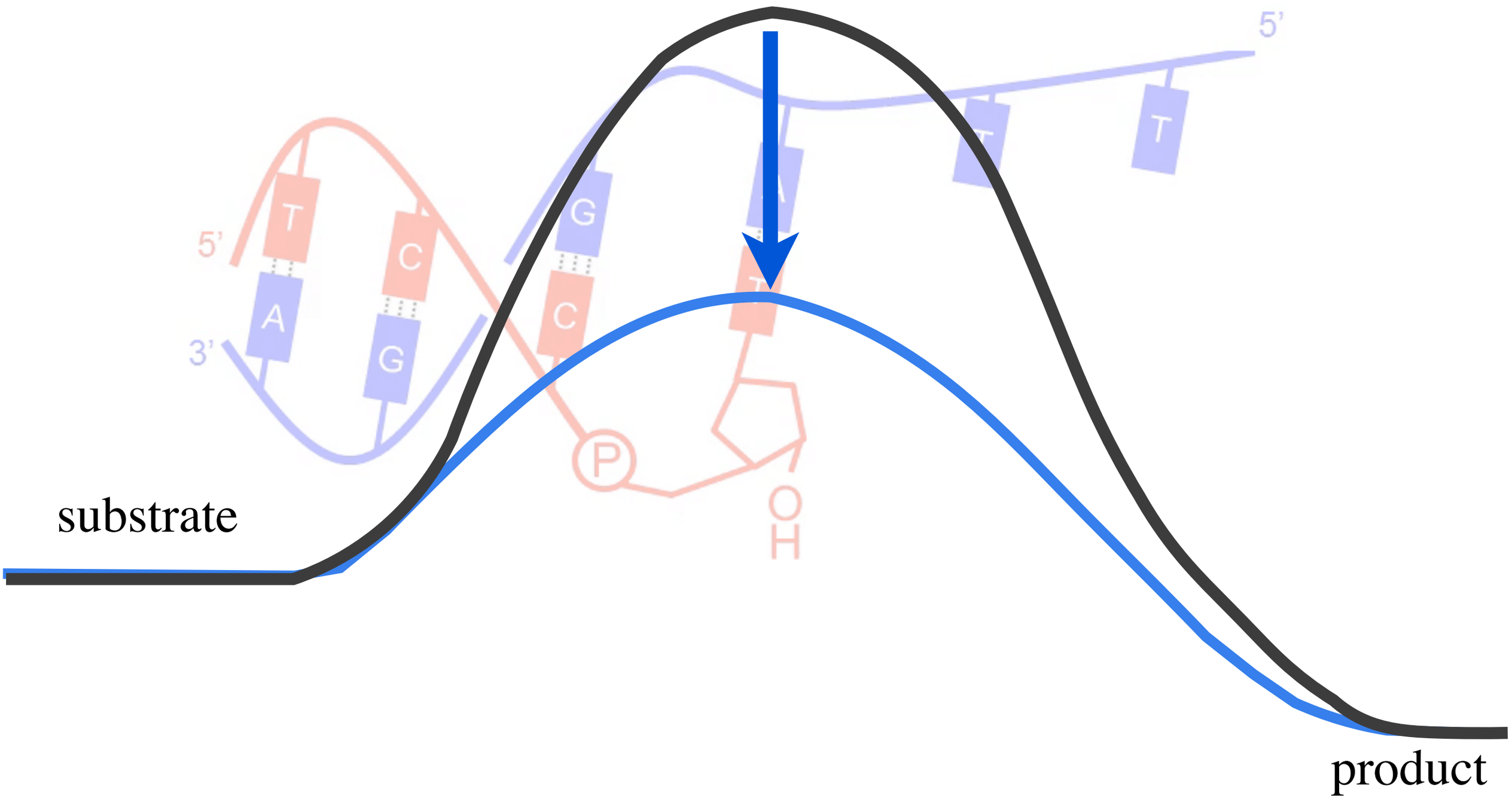
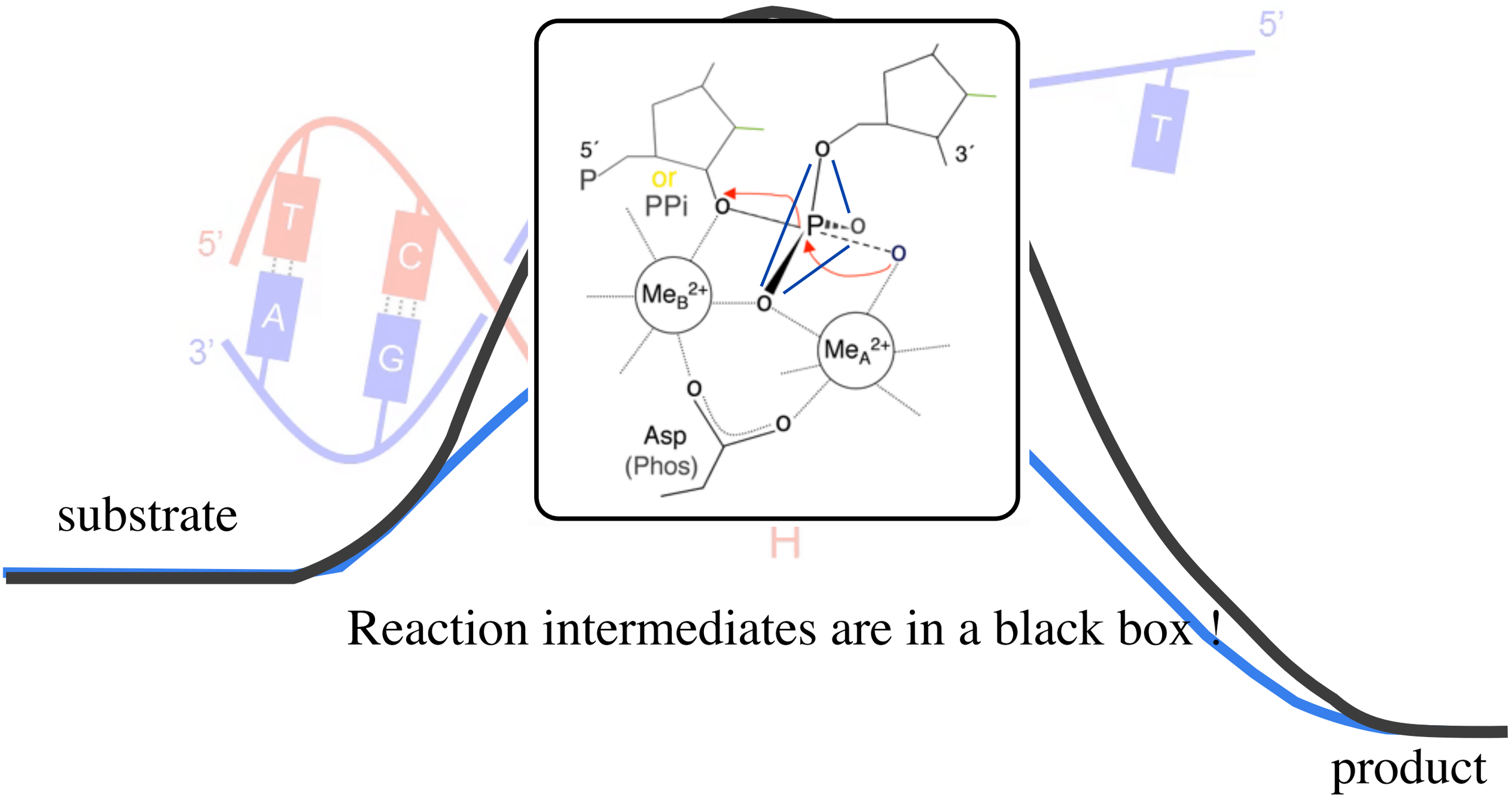
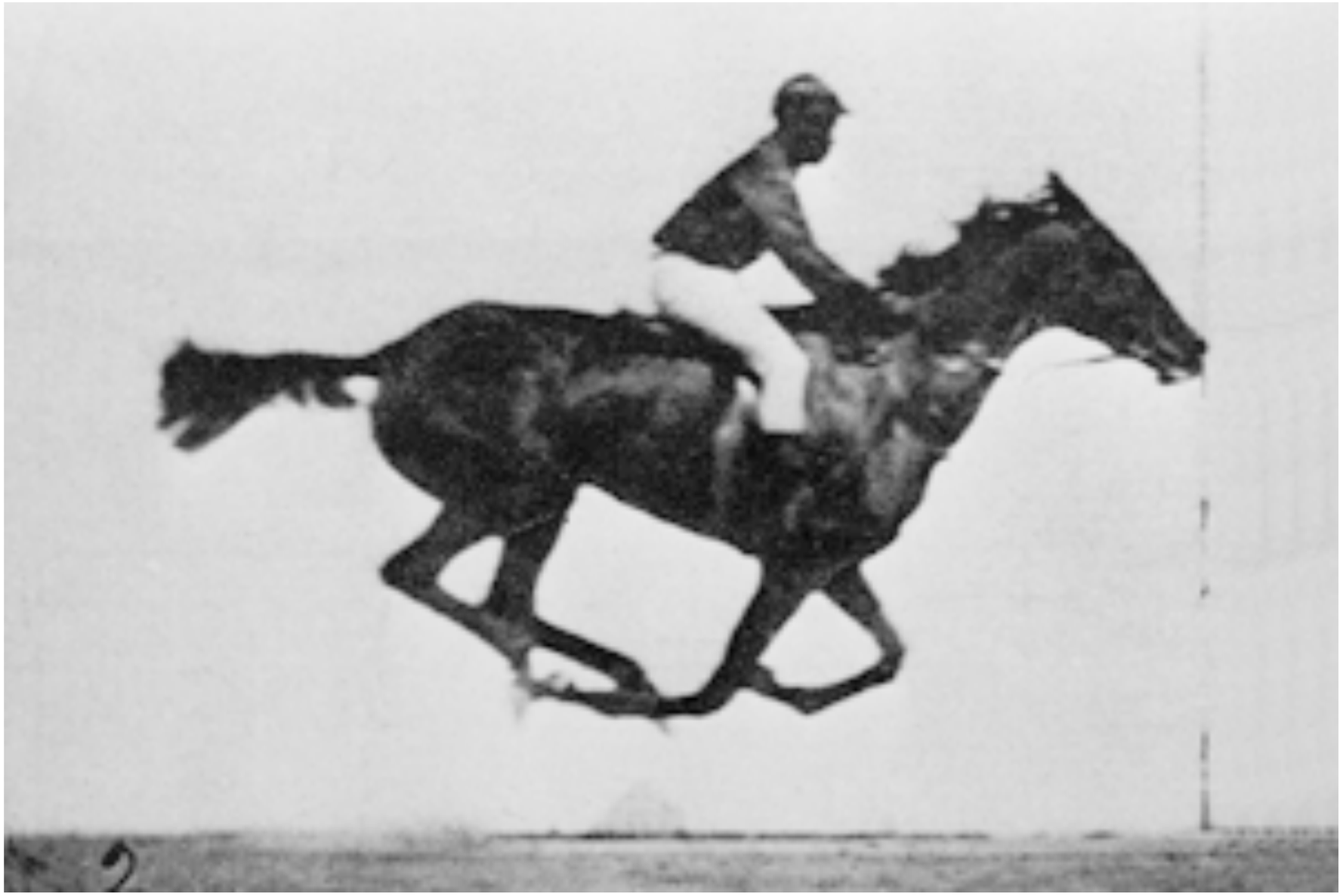


How Does Catalysis of a Phosphoryl-transfer Reaction Occur ?



Reaction intermediates are in a black box !

Static Photographs Recapitulate Movement



Eadweard Muybridge, 1878

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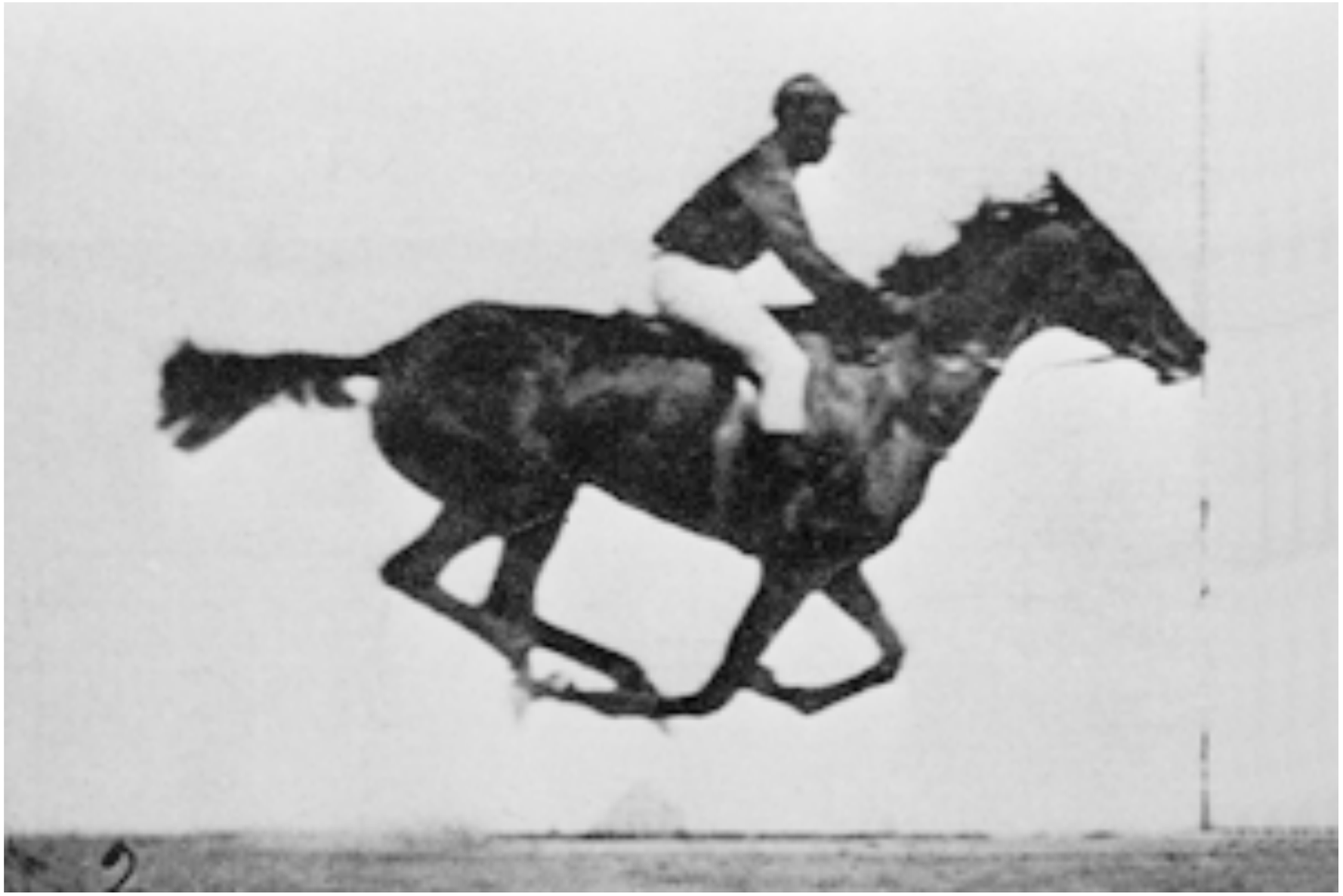
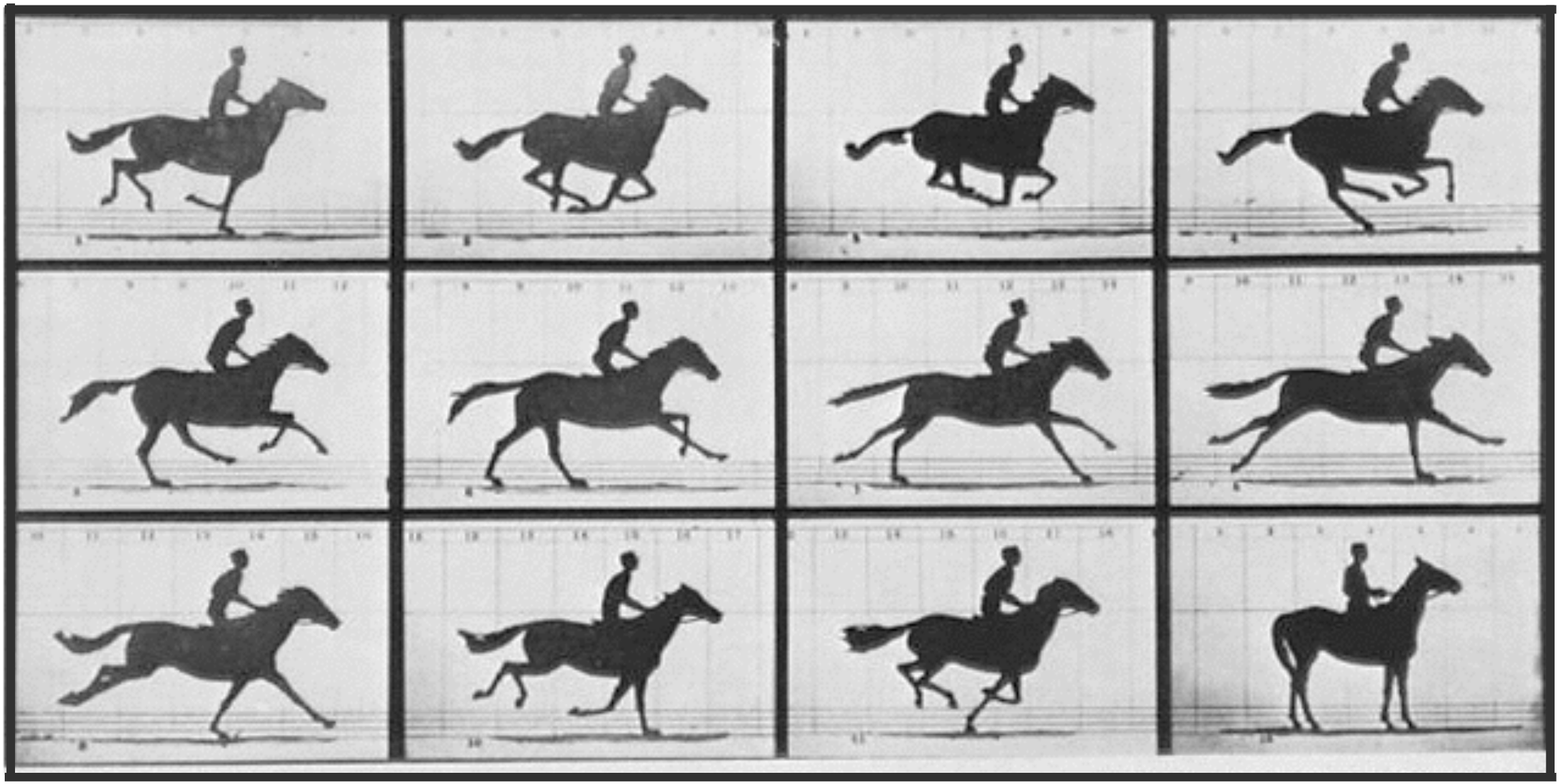


Eadweard Muybridge, 1878

To Capture Transient Intermediates of a Dynamic Process by Still Photography



Eadweard Muybridge, 1878

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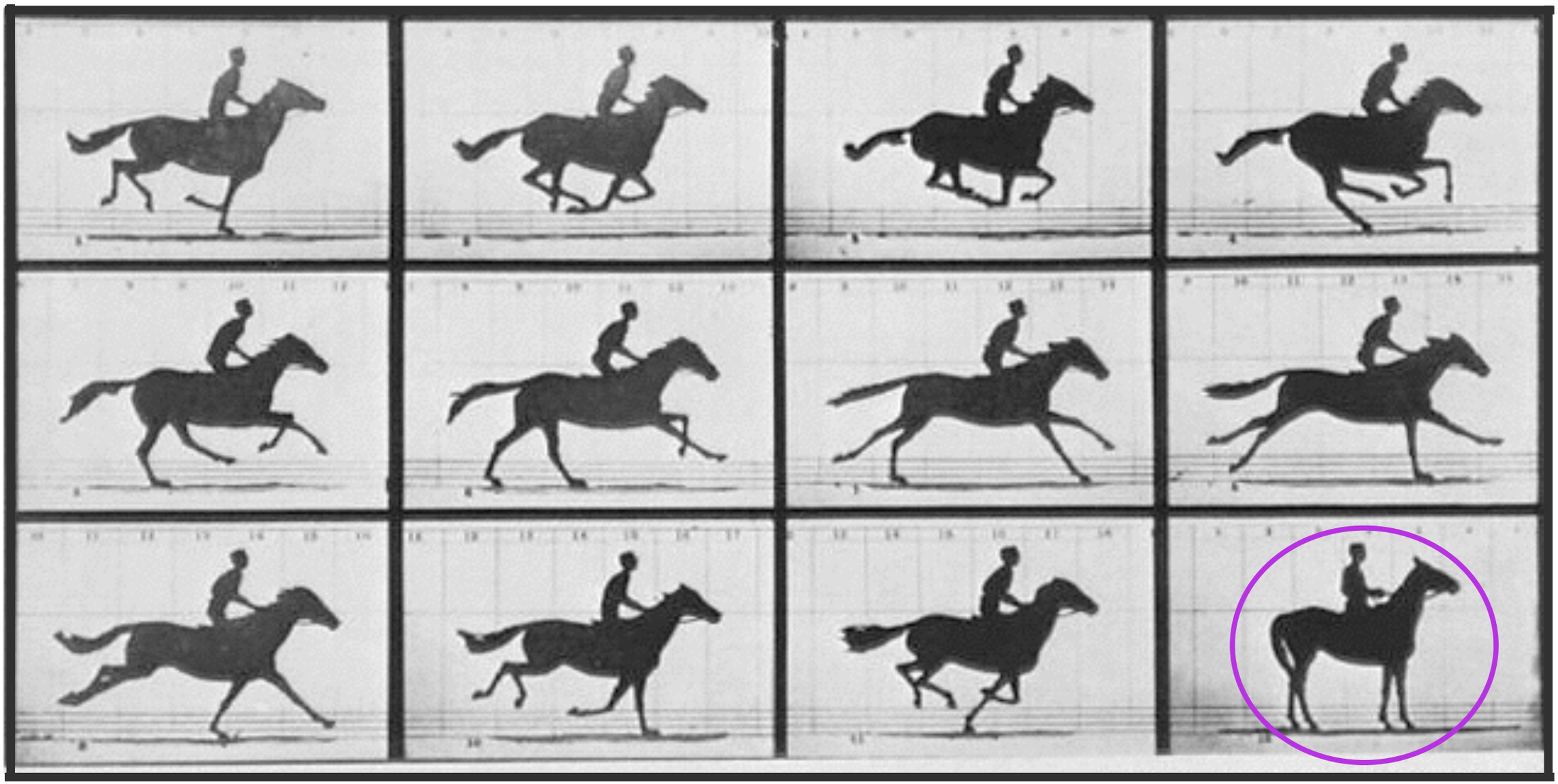

Eadweard Muybridge, 1878

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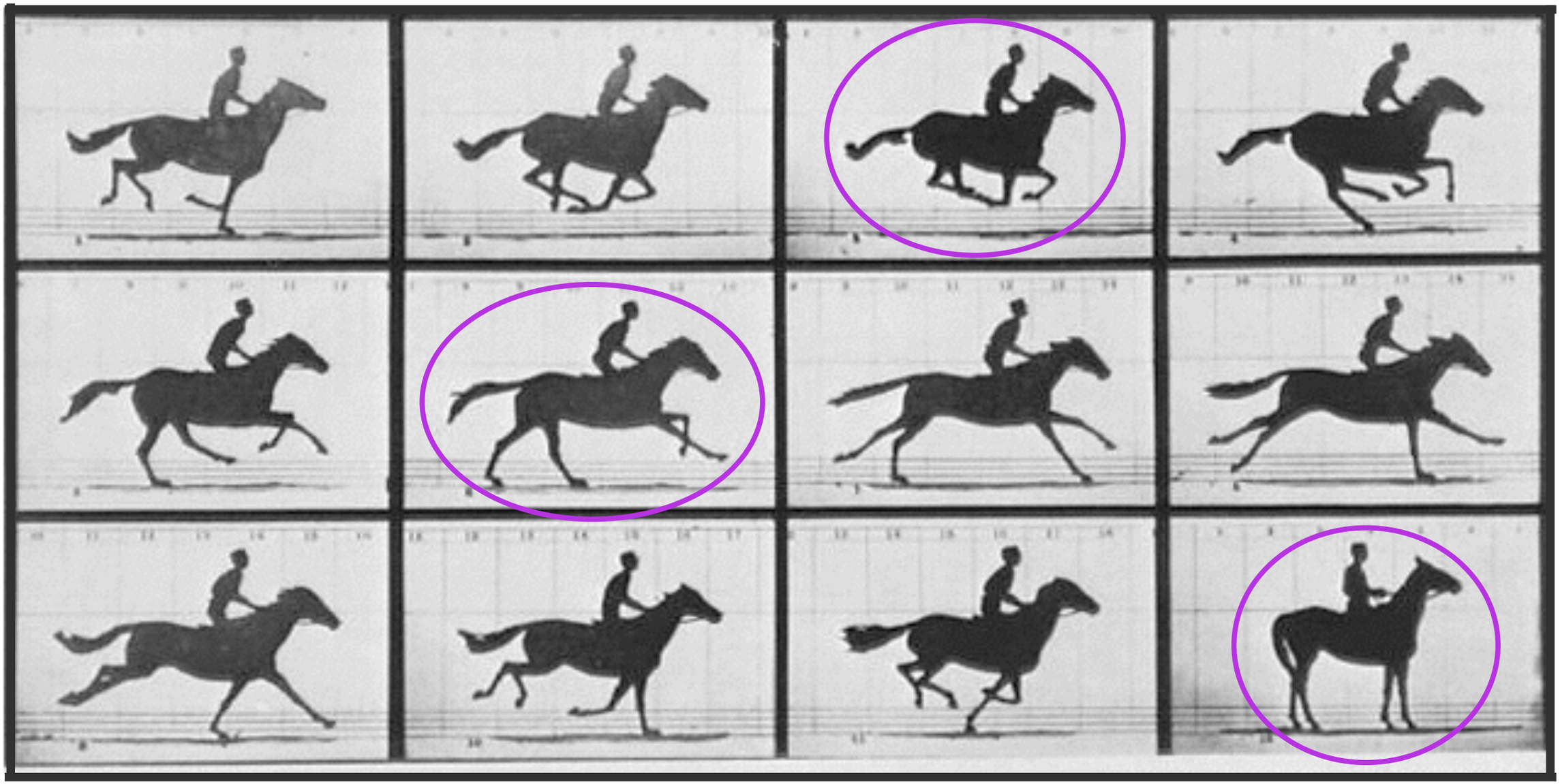


Eadweard Muybridge, 1878

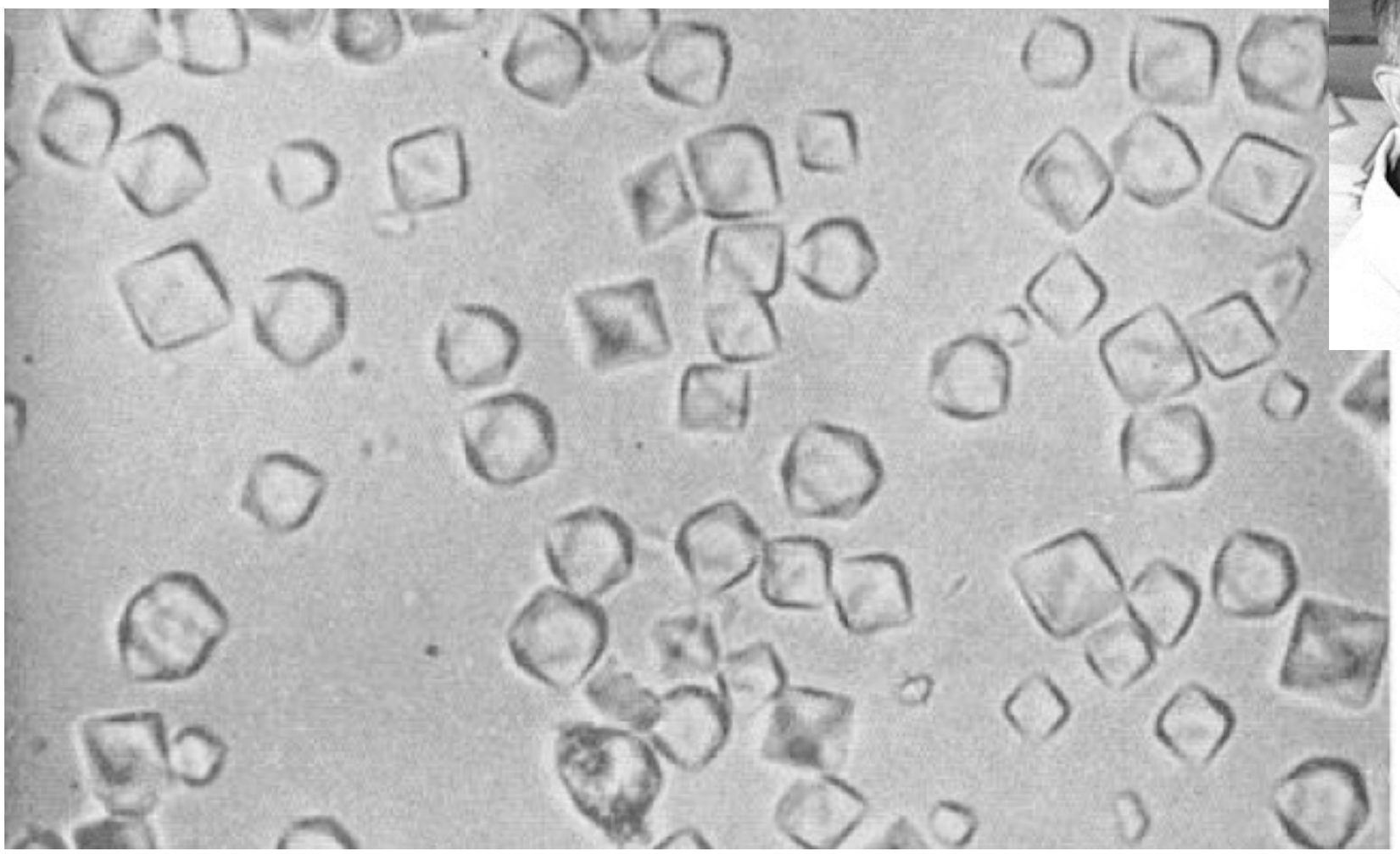
THE ISOLATION AND CRYSTALLIZATION OF THE ENZYME UREASE.

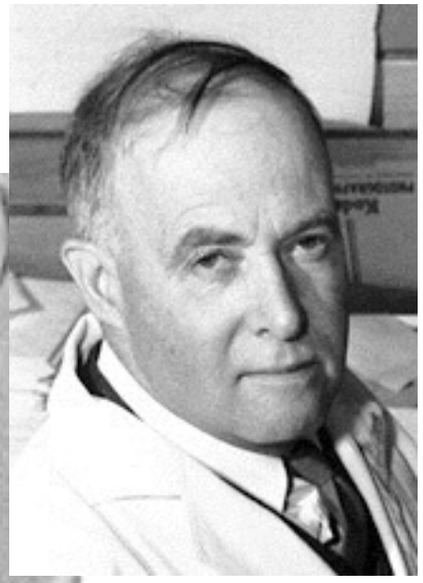
PRELIMINARY PAPER.

By JAMES B. SUMNER.

(*From the Department of Physiology and Biochemistry, Cornell University Medical College, Ithaca.*)

(Received for publication, June 2, 1926.)





Catalysis *in Crystallo* by Controlled Mg^{2+} Exposure

GS

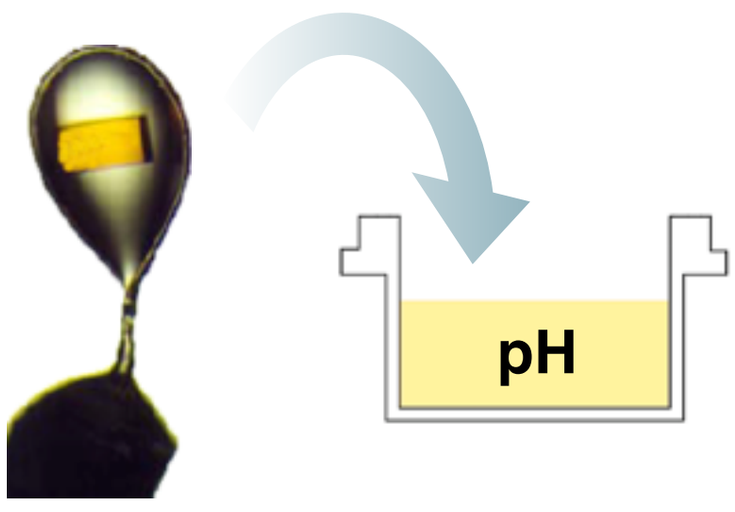
pH 6.0,
1Ca^{2+} /
complex

Nakamura, et al., & Yang (2012) Nature

Catalysis *in Crystallo* by Controlled Mg^{2+} Exposure

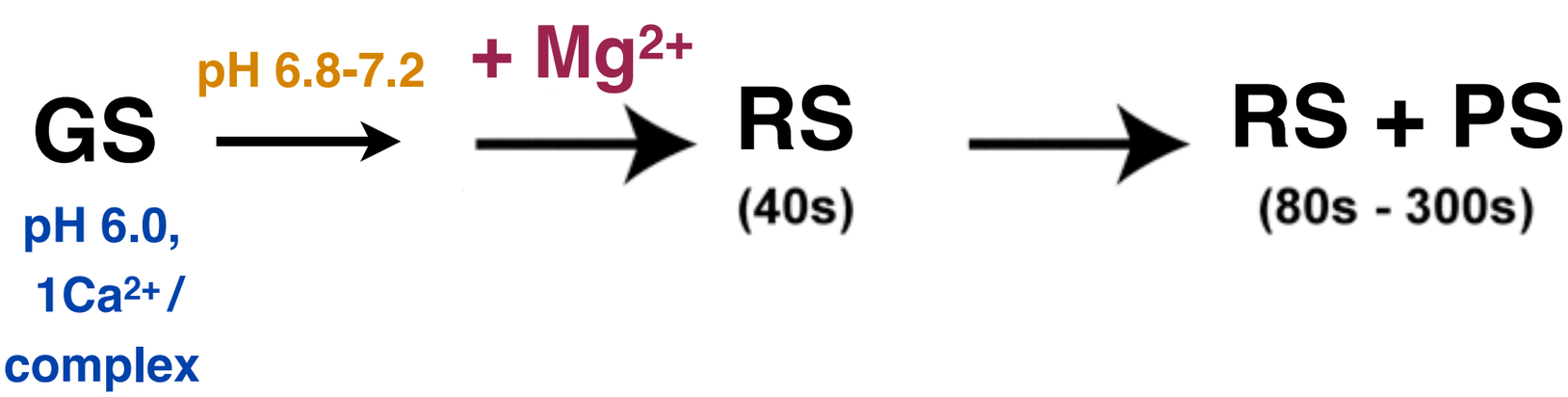
GS $\xrightarrow{\text{pH 6.8-7.2}}$

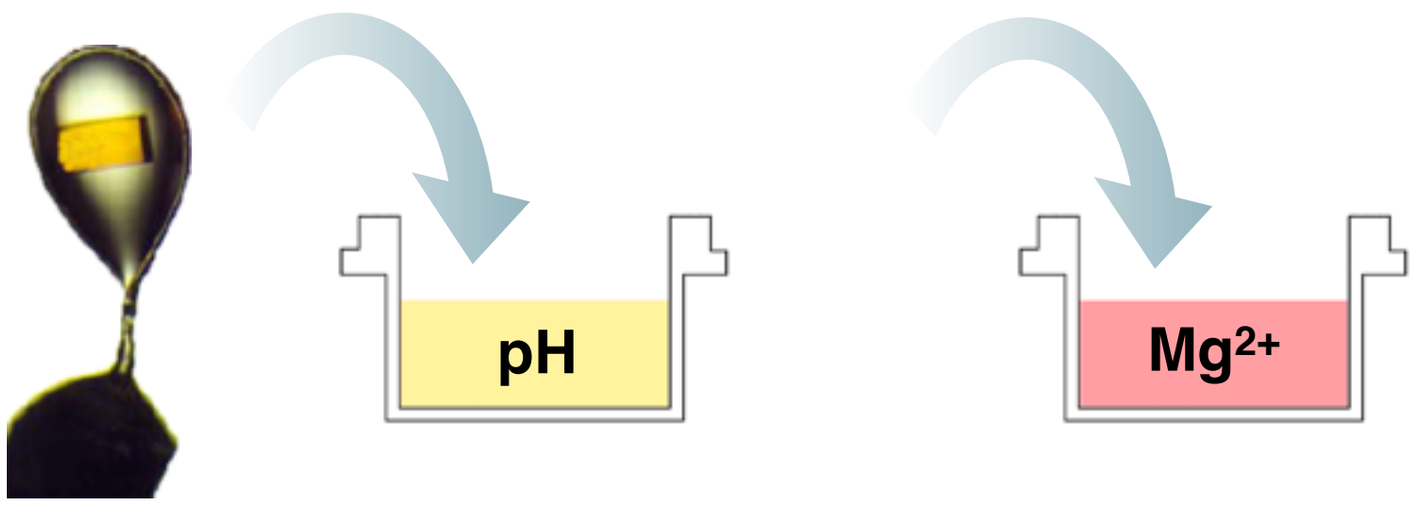
pH 6.0, 1Ca^{2+} / complex



Nakamura, et al., & Yang (2012) Nature

Catalysis *in Crystallo* by Controlled Mg^{2+} Exposure





Nakamura, et al., & Yang (2012) Nature

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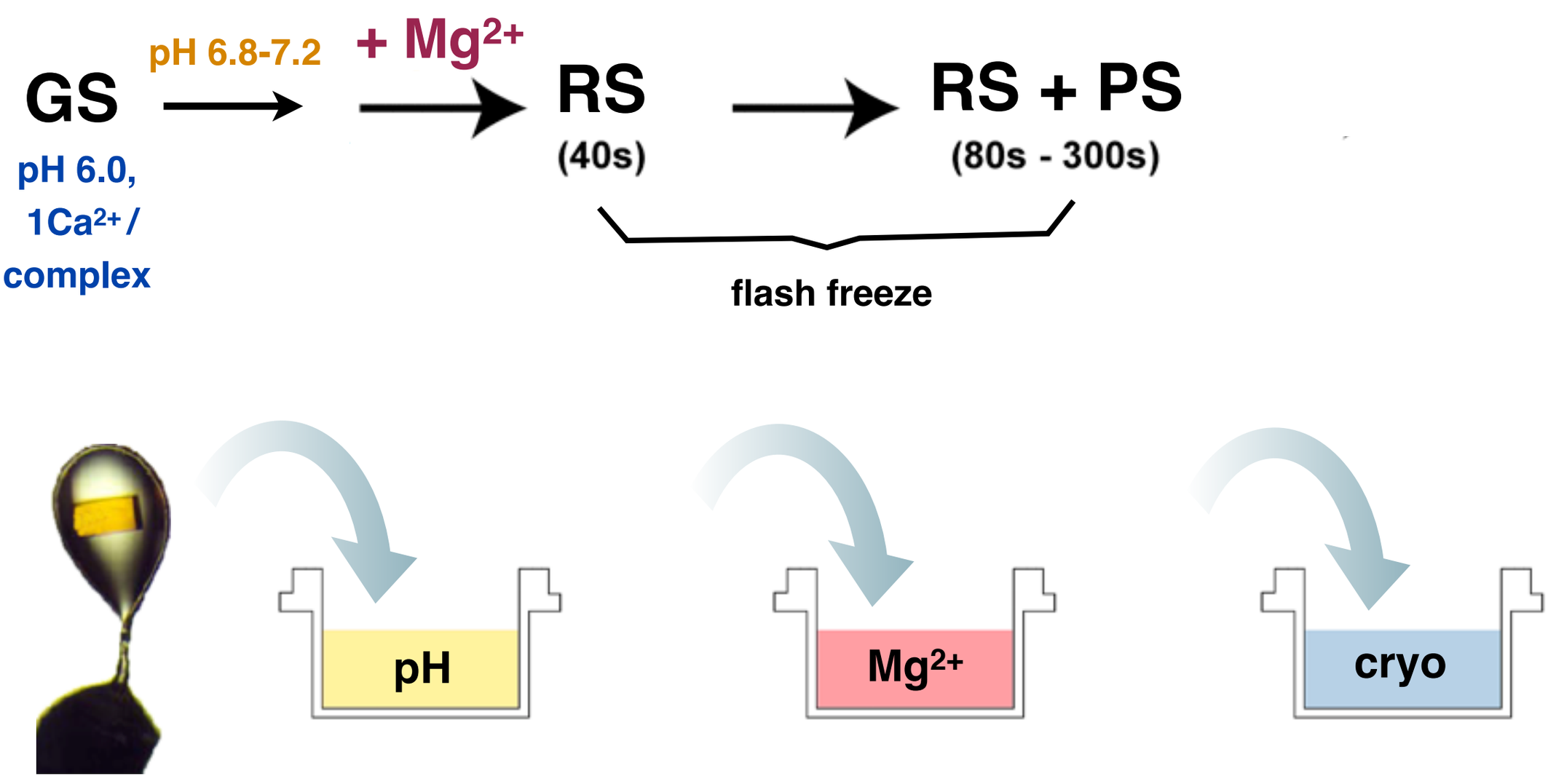

Nakamura, et al., & Yang (2012) Nature

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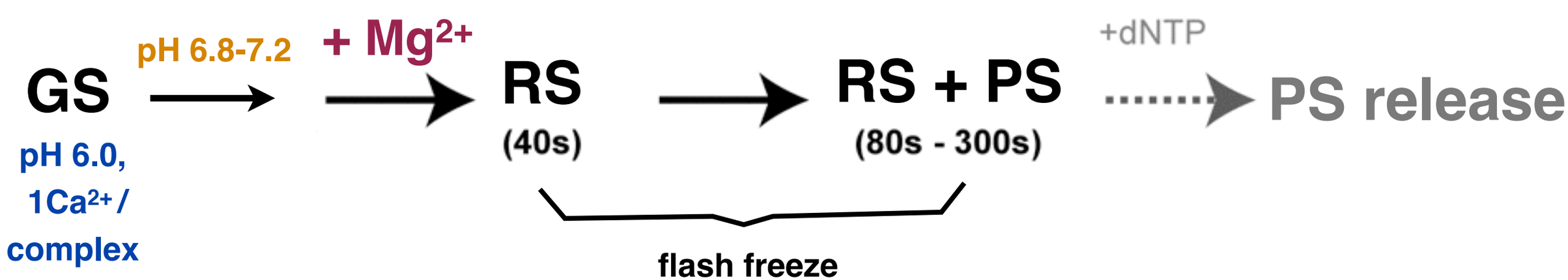
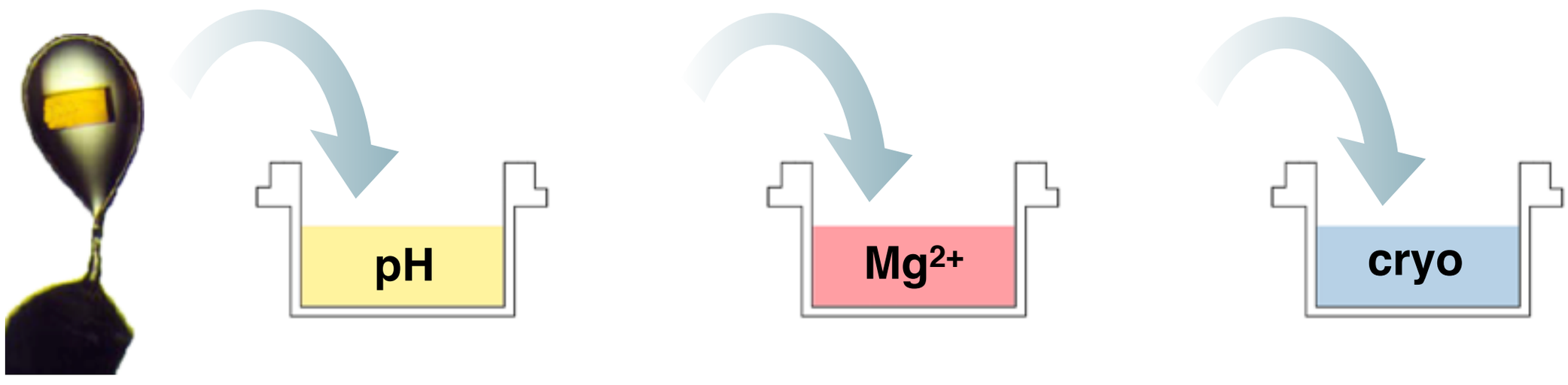
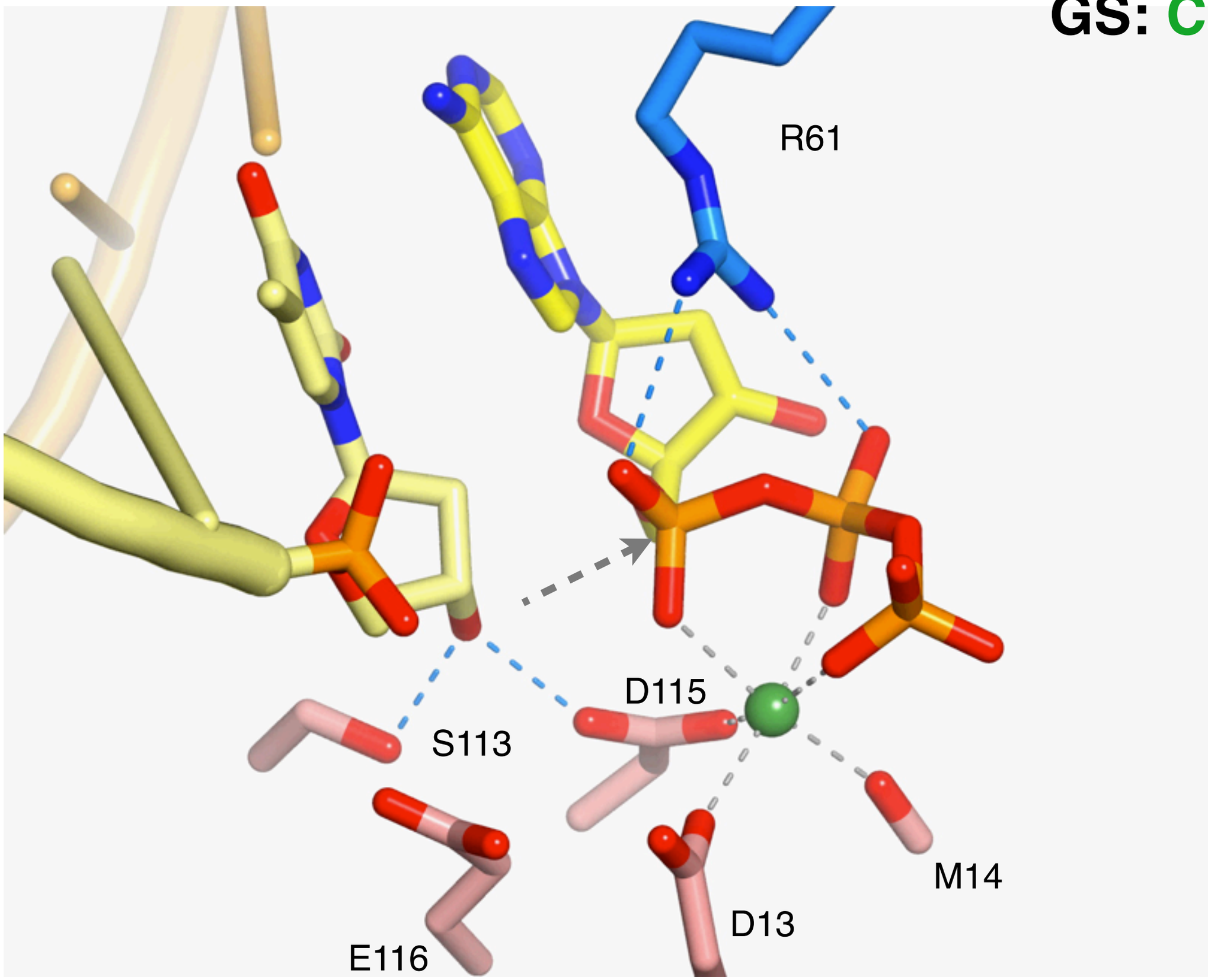




Nakamura, et al., & Yang (2012) Nature

Ground State: Misaligned Reactants



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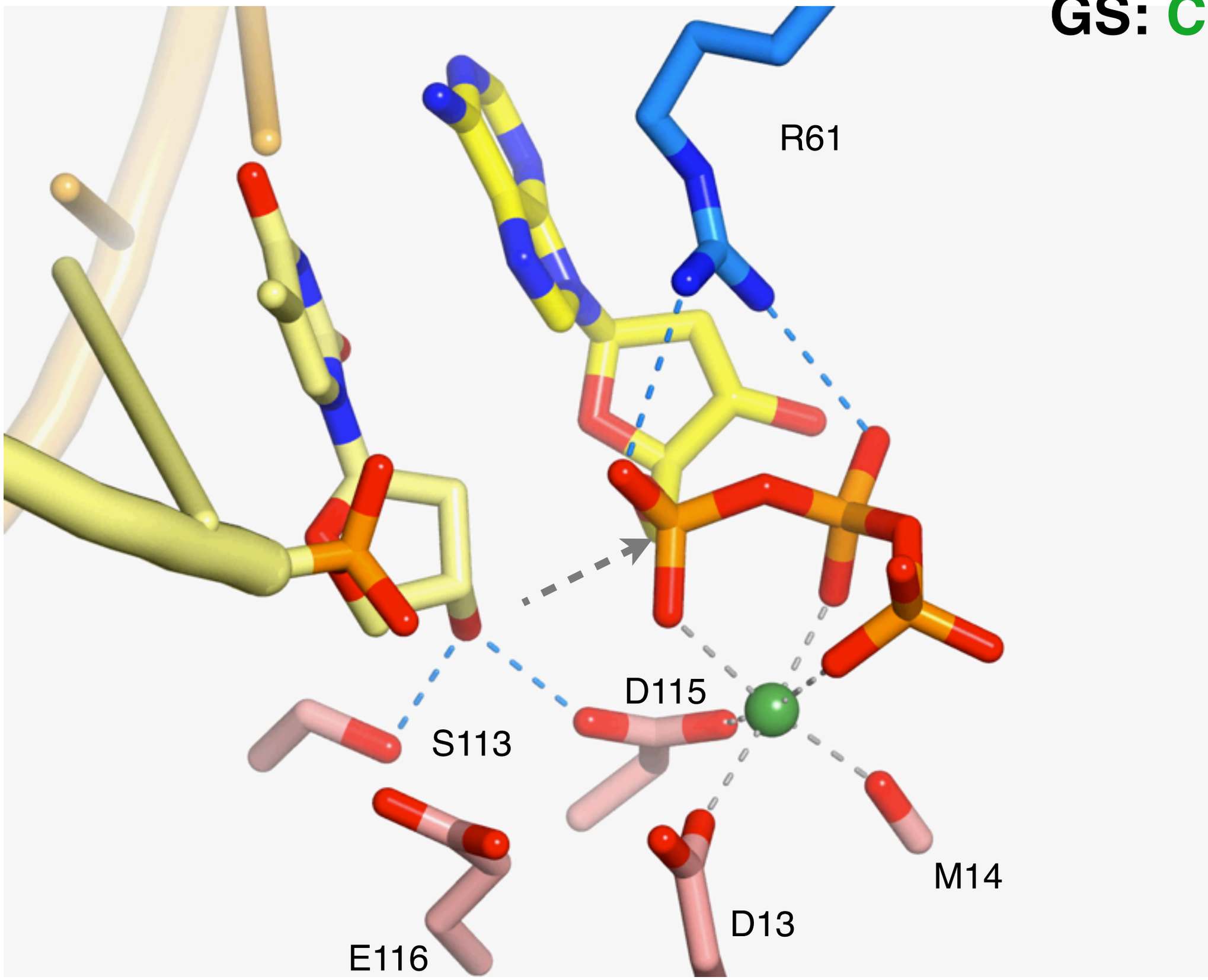
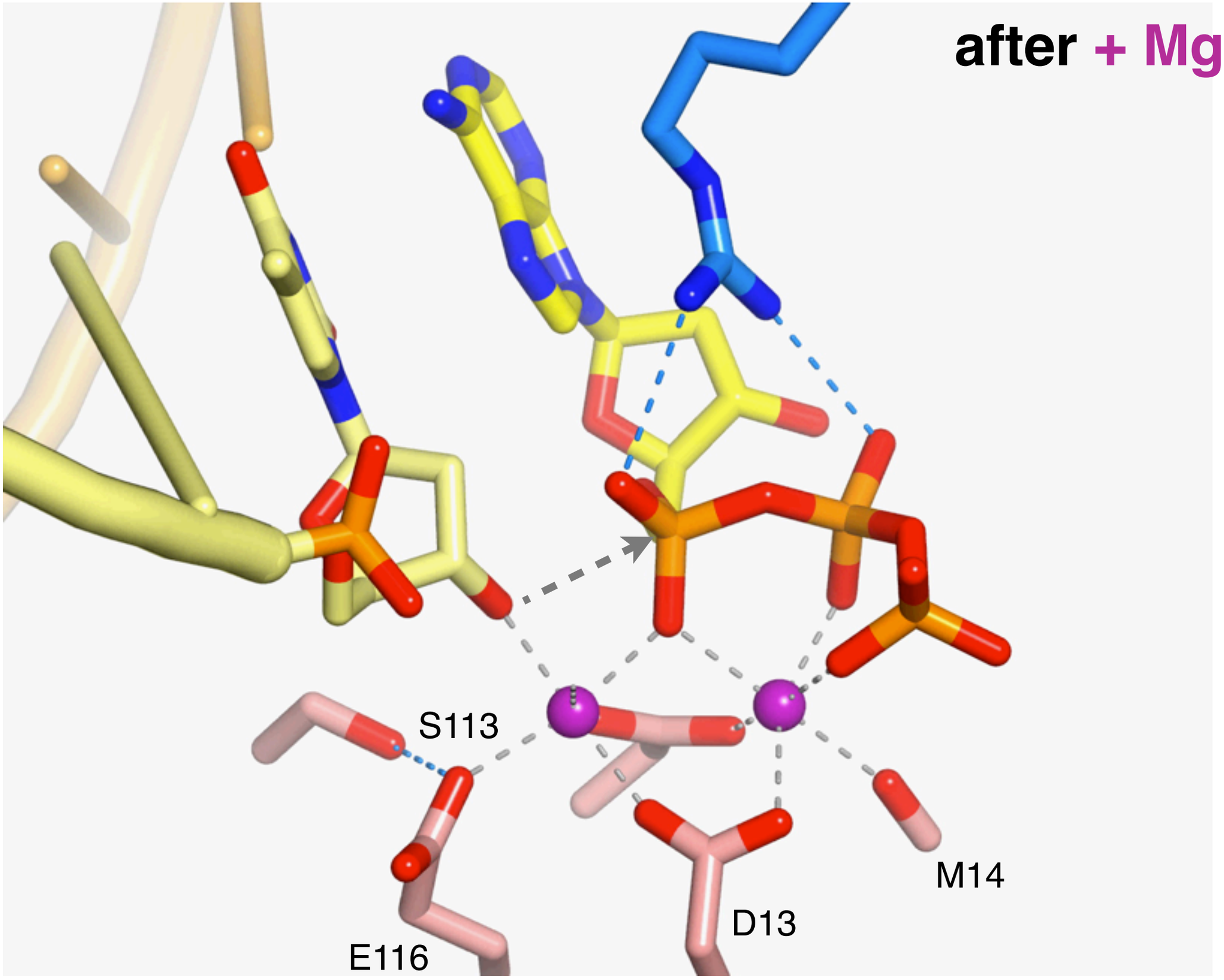


Two Mg^{2+} Align the Reactants



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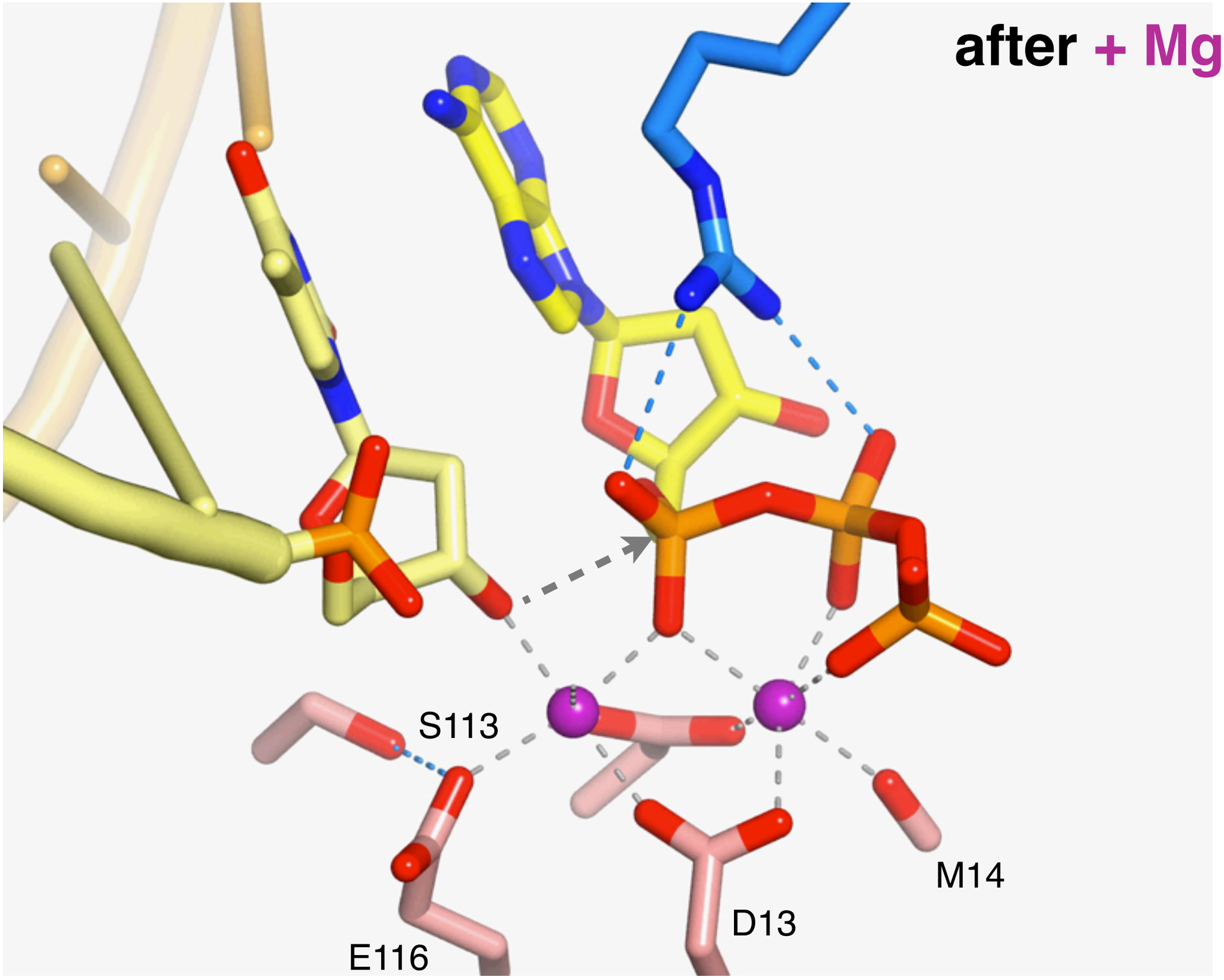
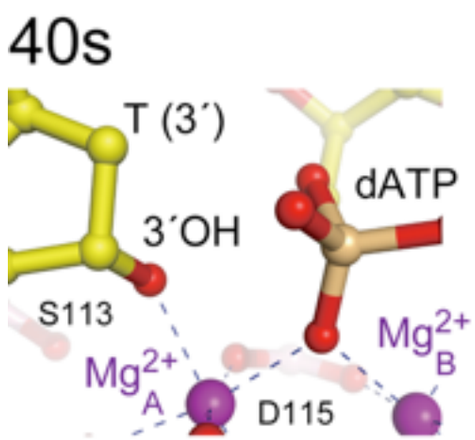


Reaction Time Course:
Monitor the new Bond Formation



Nakamura, et al., & Yang (2012) Nature

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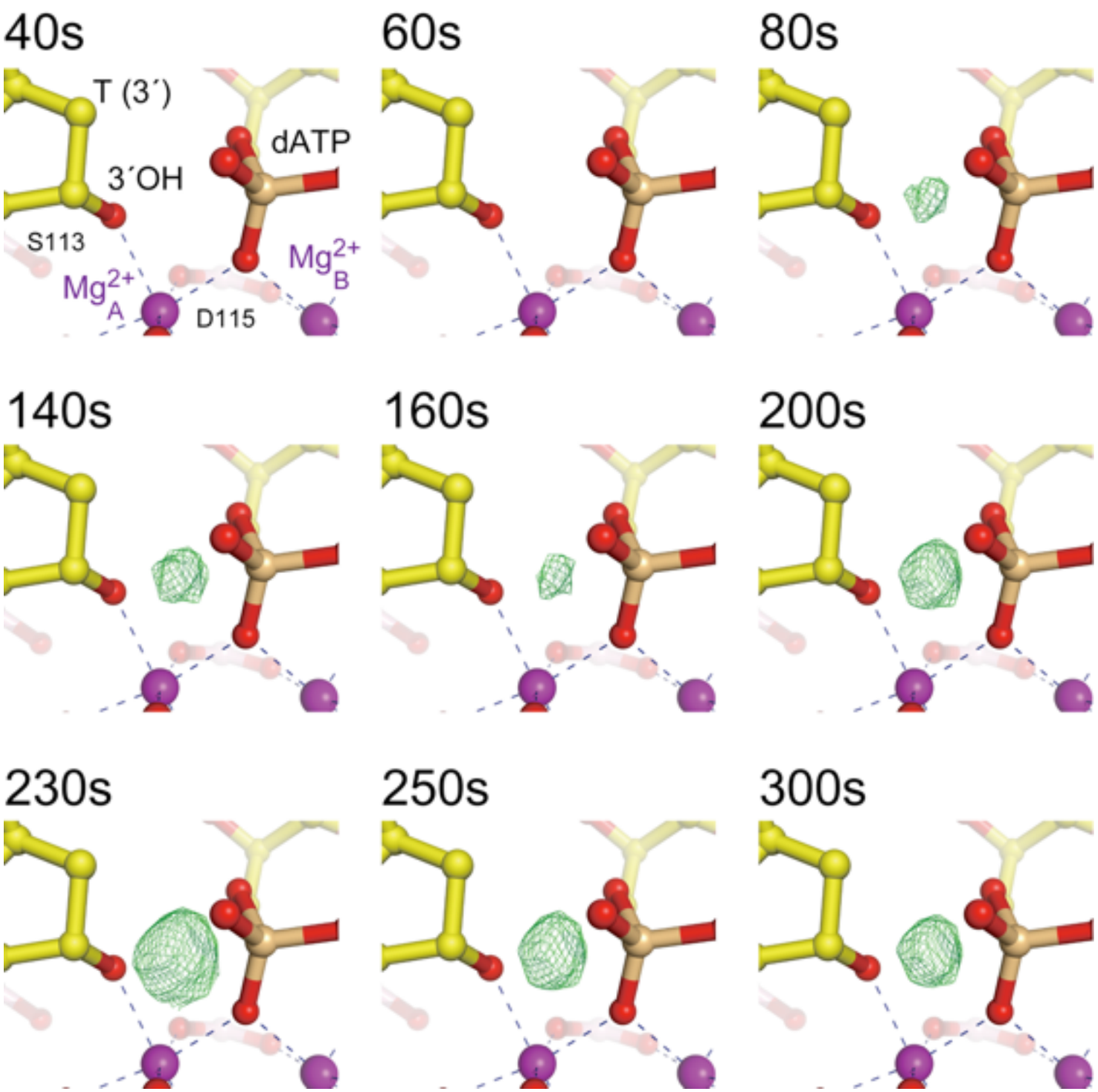

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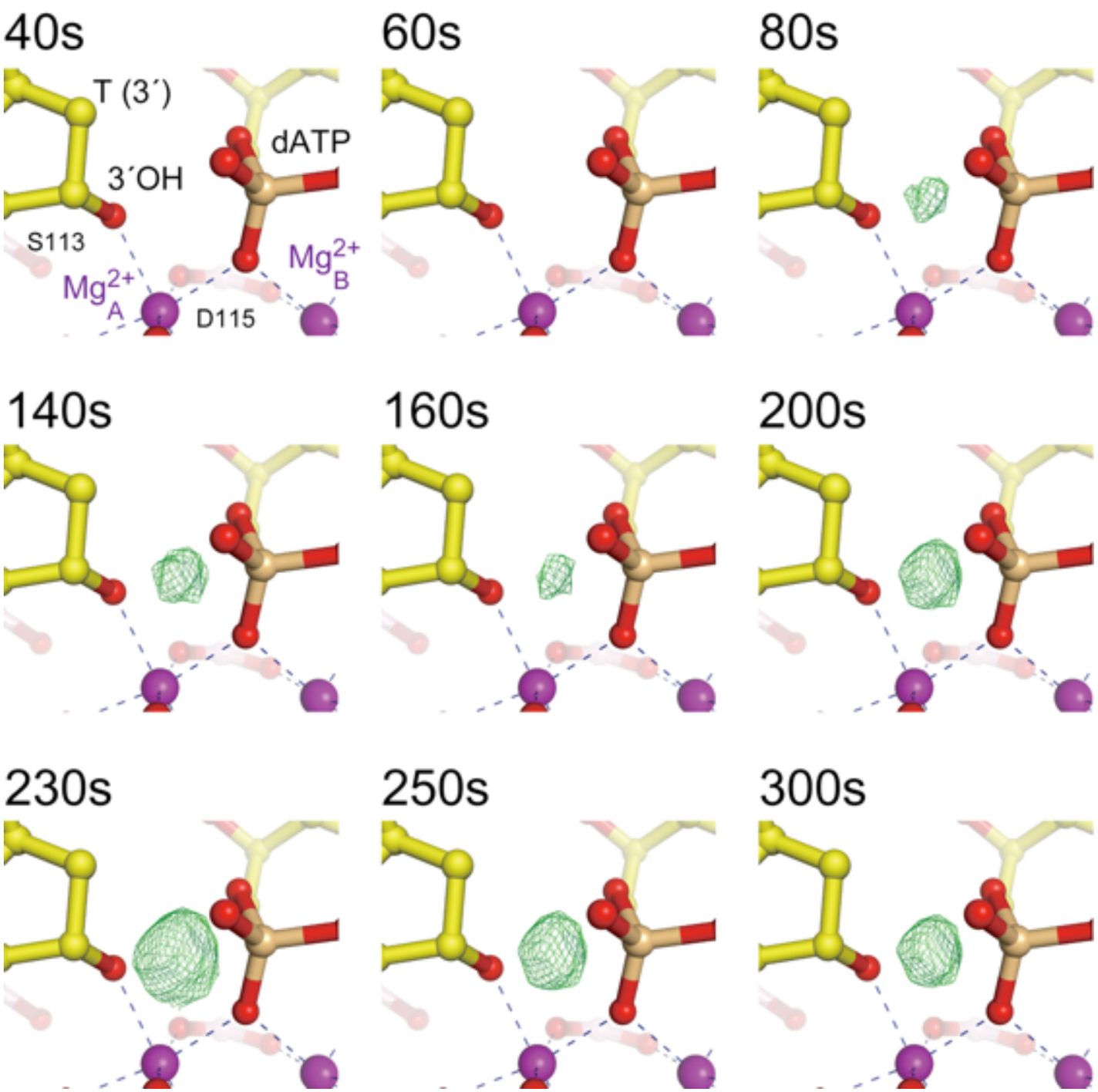
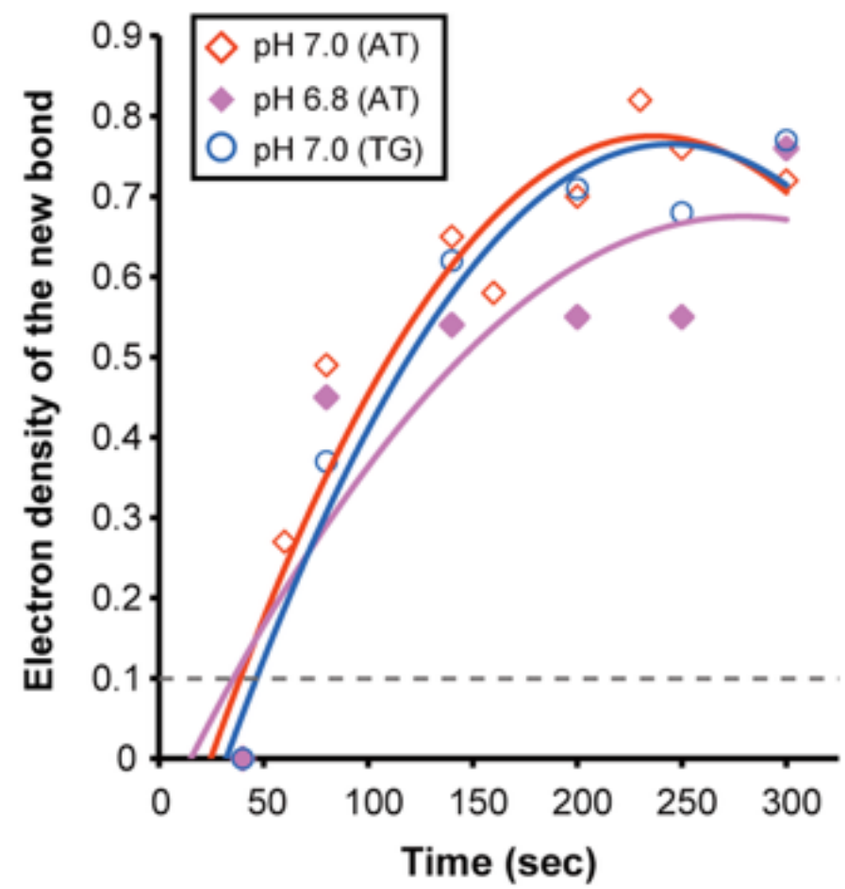
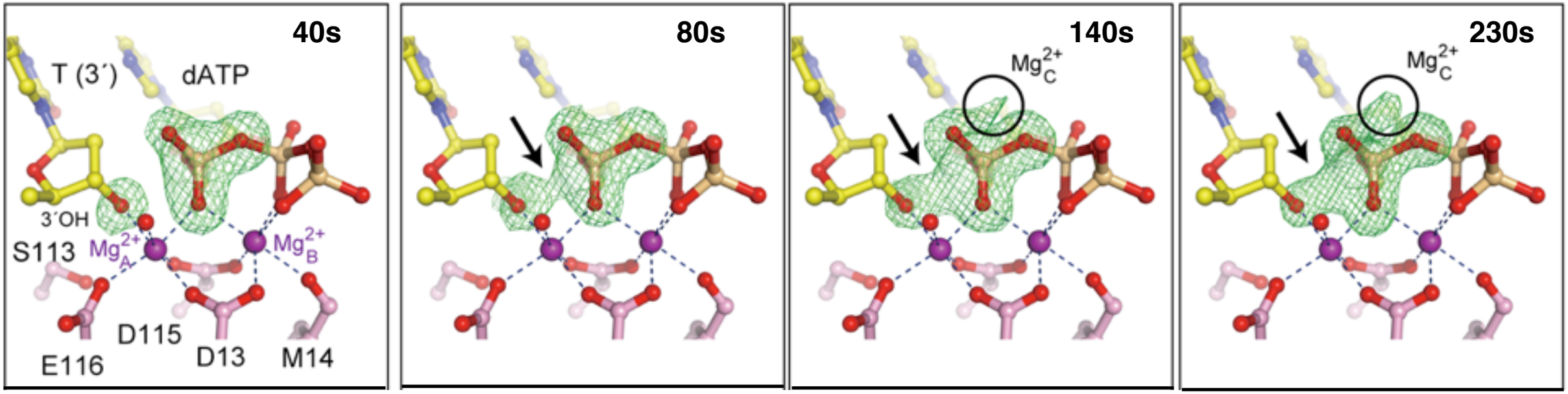




Nakamura, et al., & Yang (2012) Nature

Revelations: A Transiently Bound 3rd Metal ion

Mg^{2+} (1 mM)



Gao & Yang, (2016) Science

Nakamura, et al., & Yang (2012) Nature

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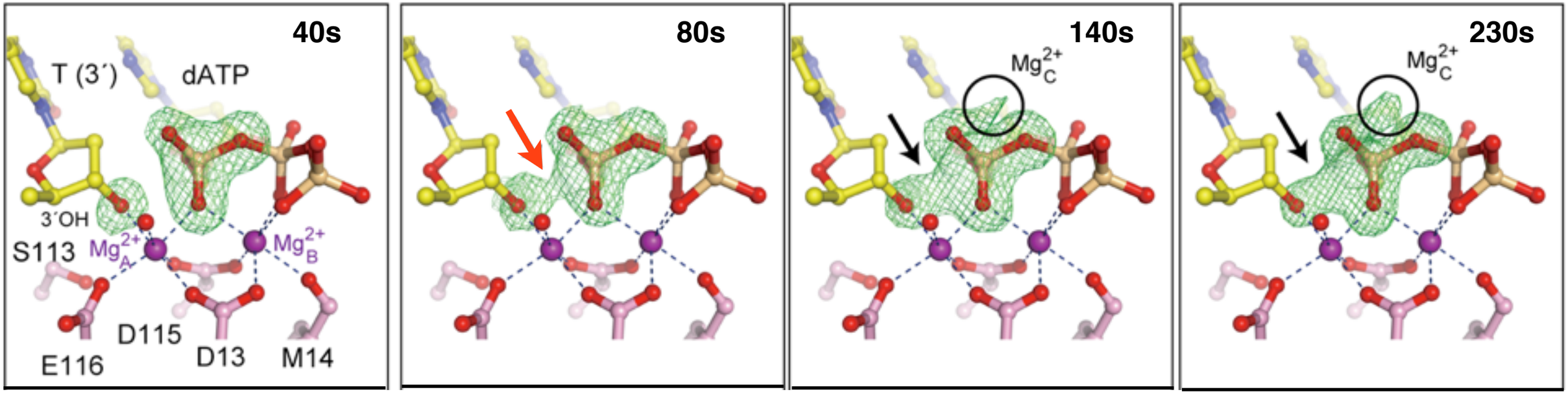

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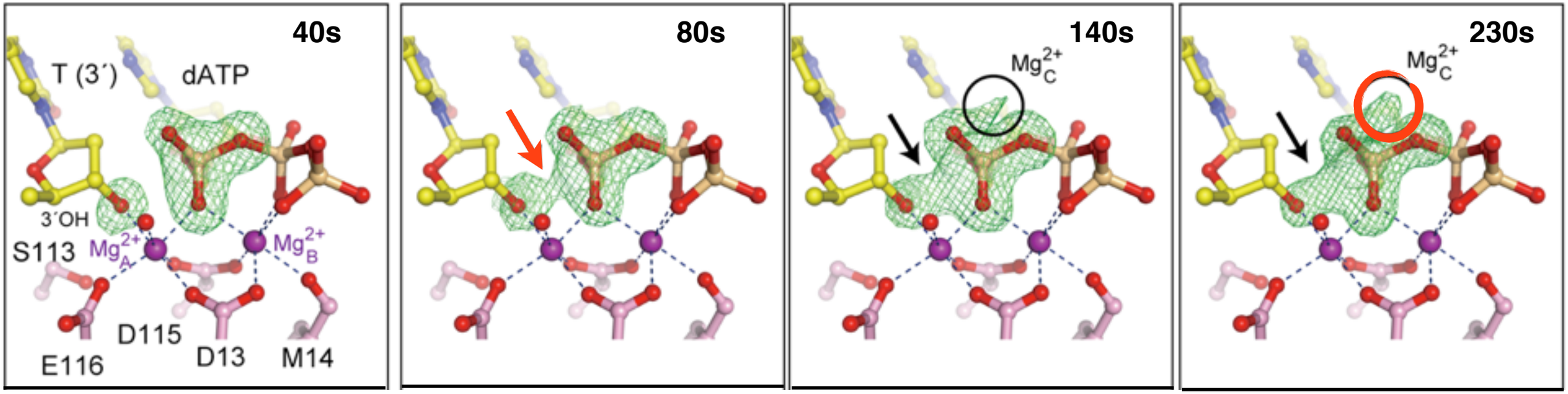

Gao & Yang, (2016) Science

Nakamura, et al., & Yang (2012) Nature

Revelations: A Transiently Bound 3rd Metal ion

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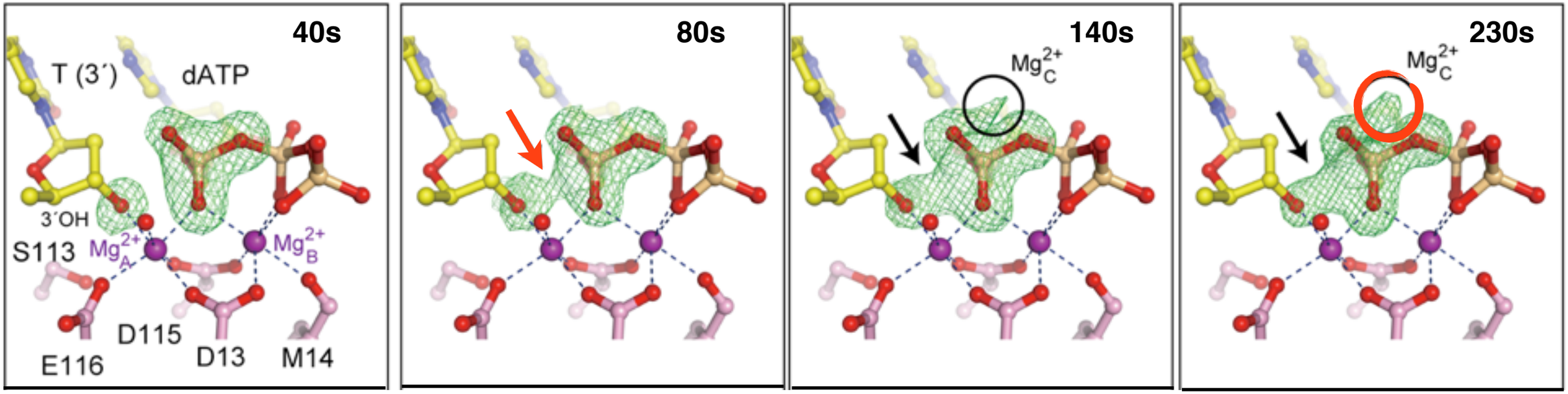
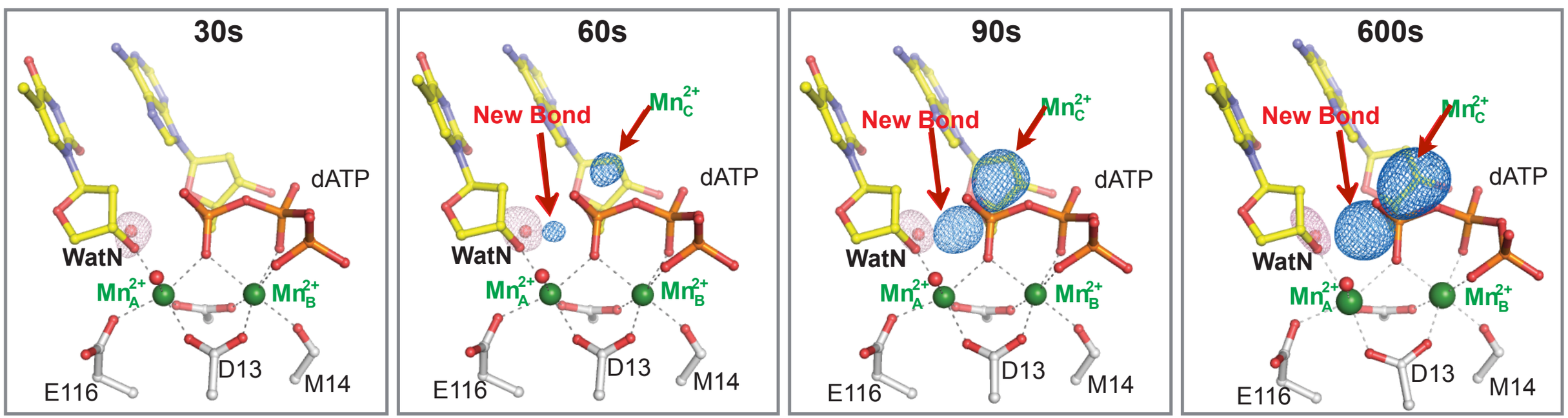


Mn^{2+} (10 mM)



Gao & Yang, (2016) Science

Nakamura, et al., & Yang (2012) Nature

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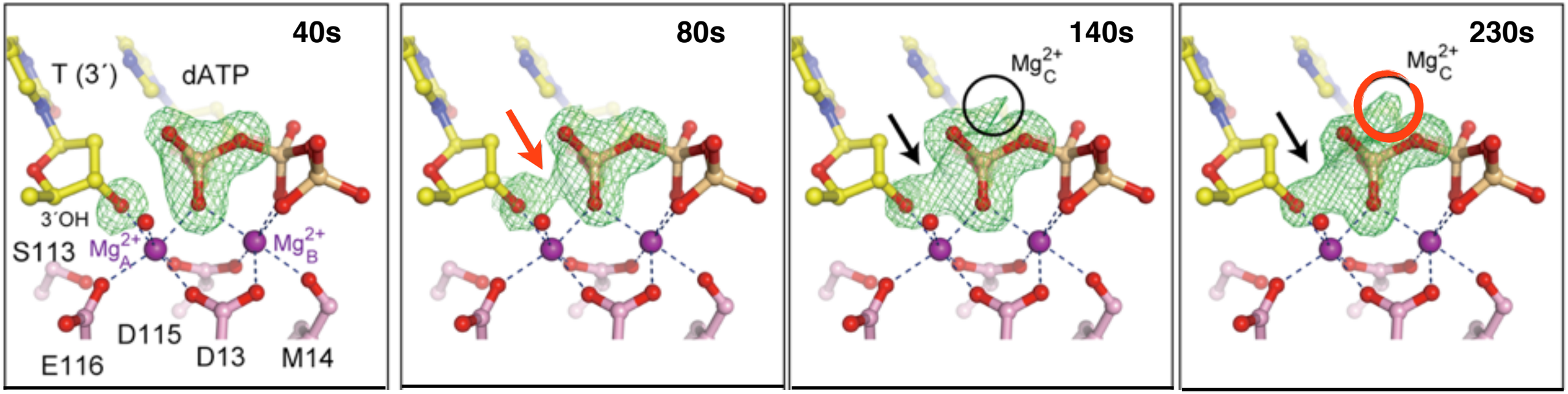

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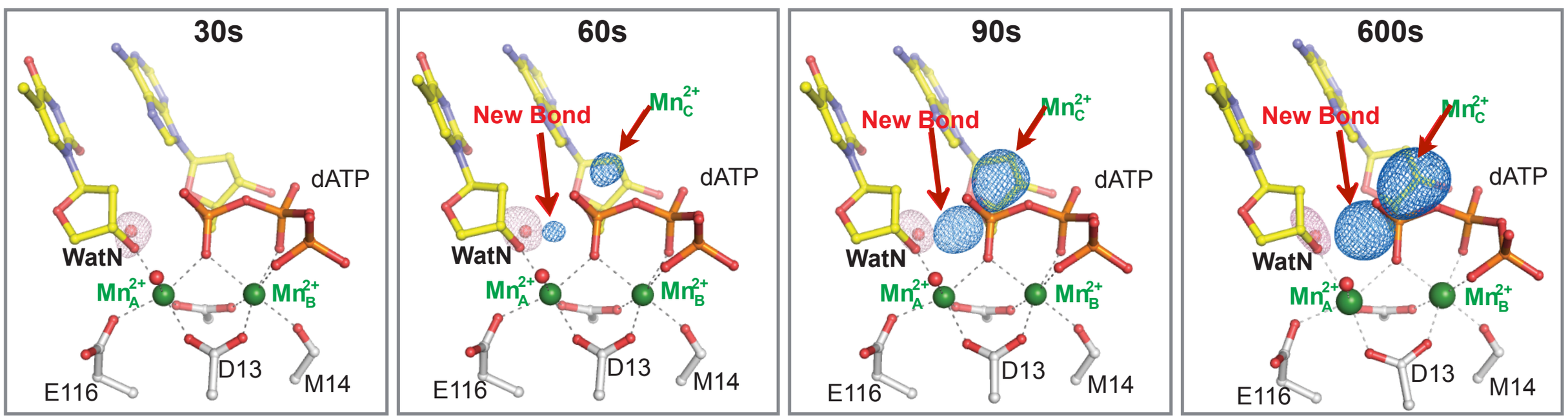
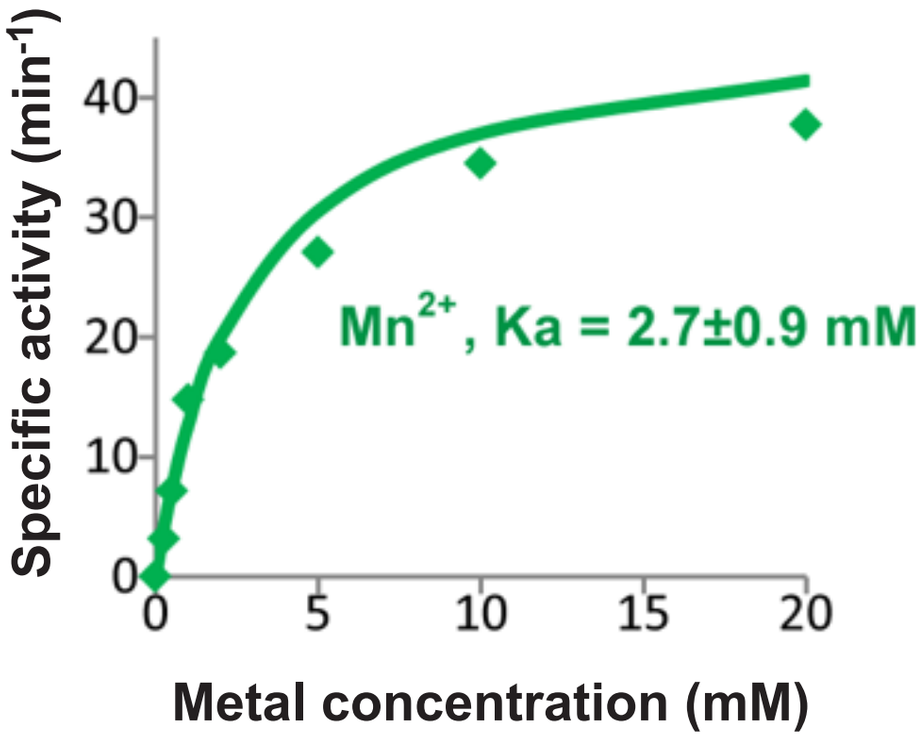


Gao & Yang, (2016) Science

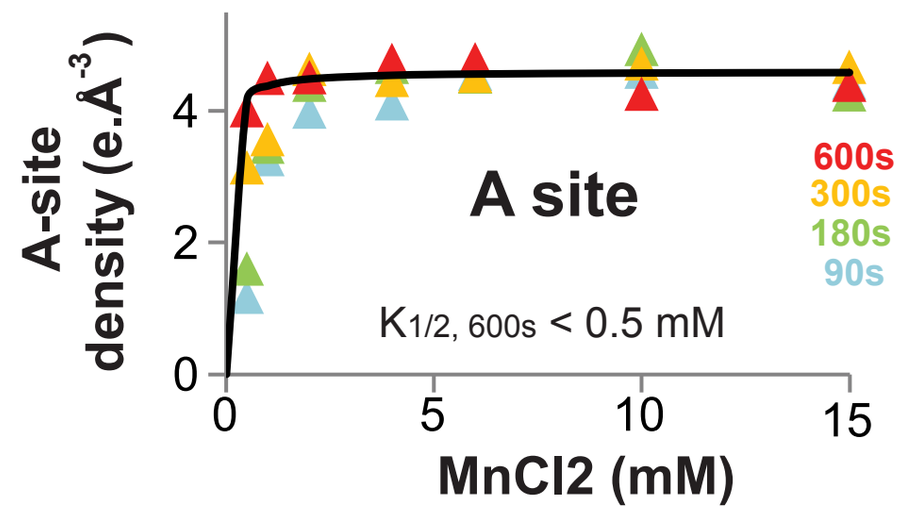
Nakamura, et al., & Yang (2012) Nature

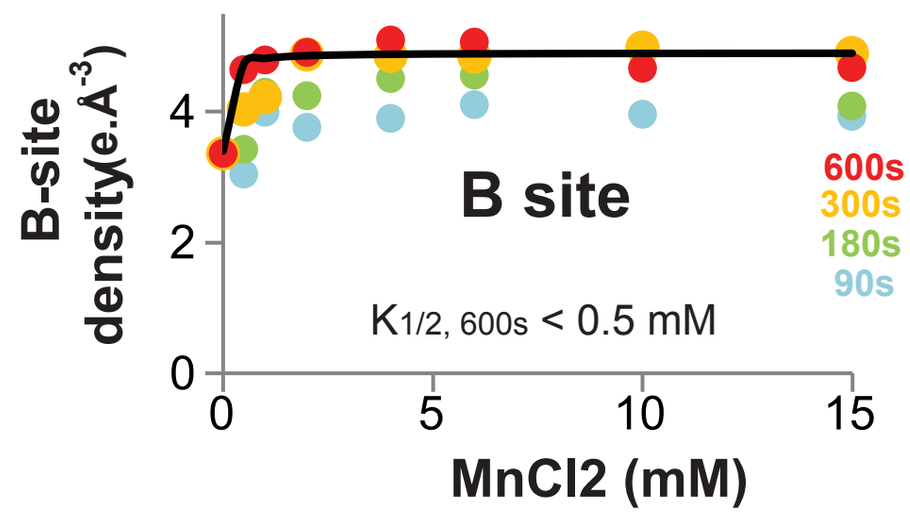
Affinity of the 3rd Metal ion is the Determinant

Solution Titration



***in crytallo* Measurement**





Gao & Yang, (2016) Science

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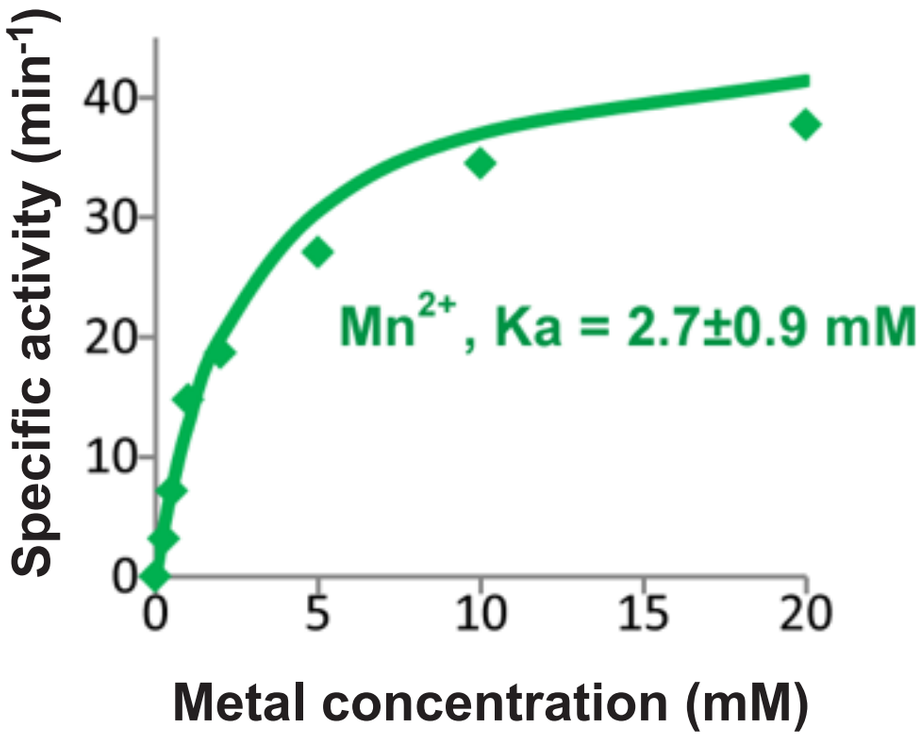

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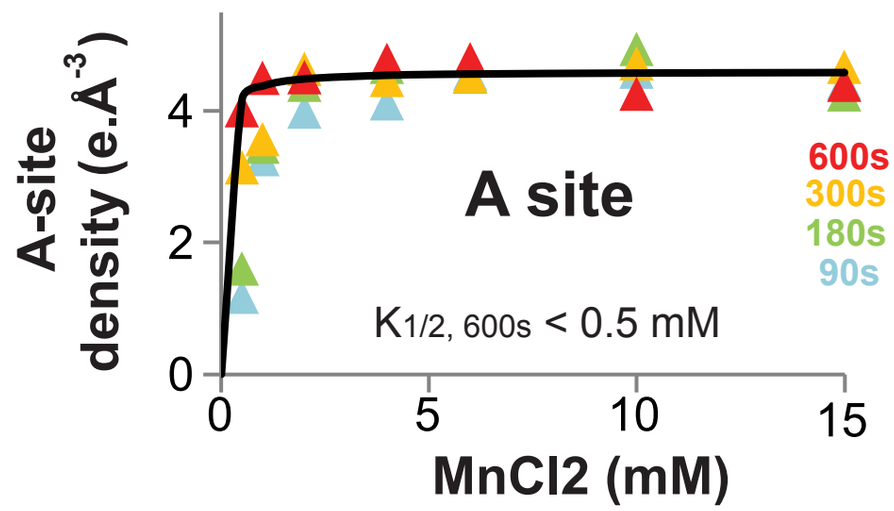
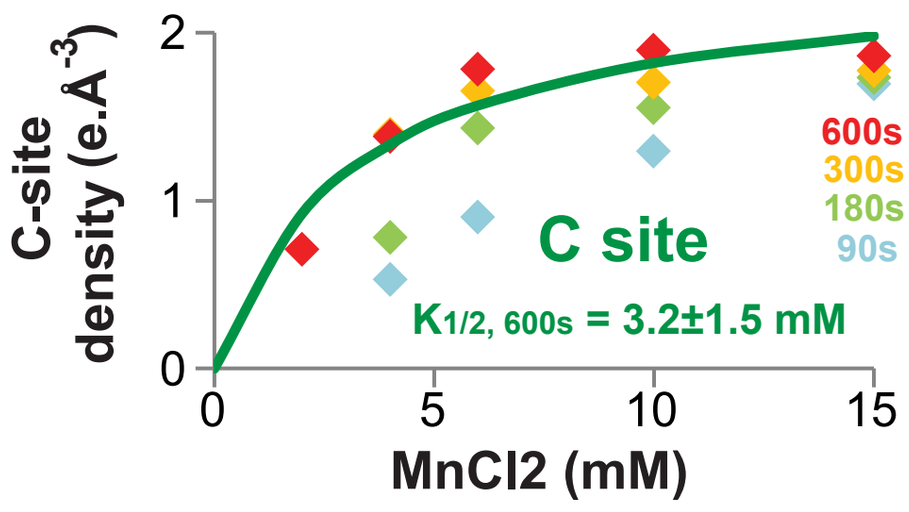
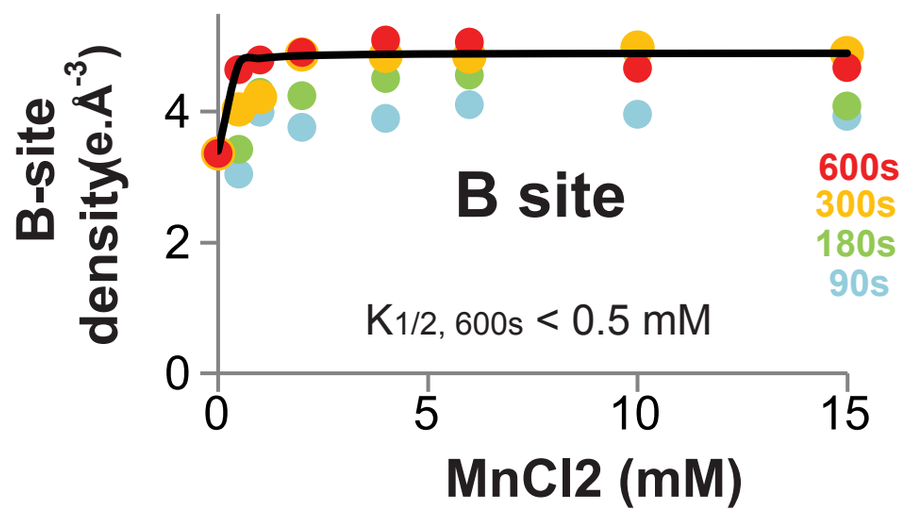
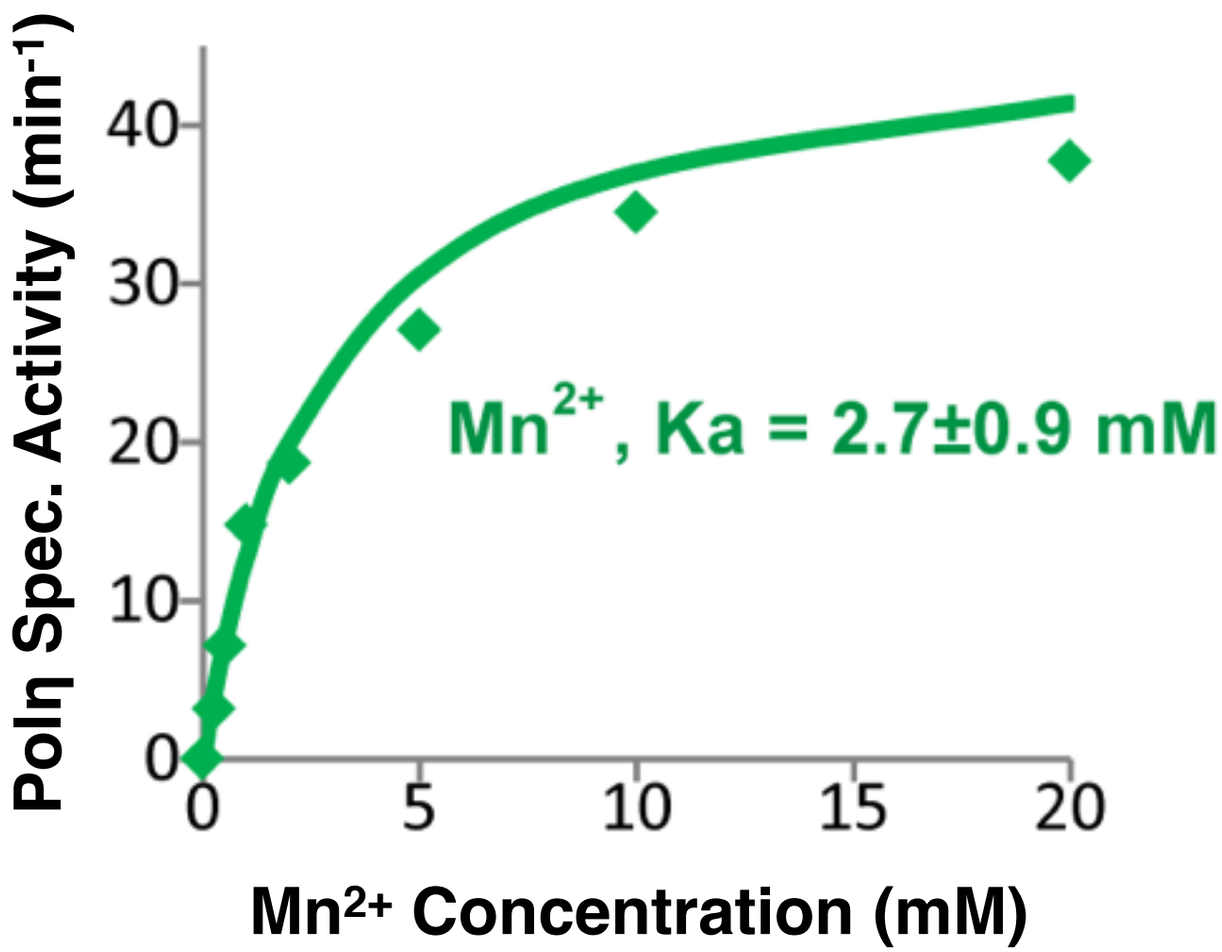






Gao & Yang, (2016) Science

The 3rd Metal ion is Required for DNA Synthesis Reaction !!!



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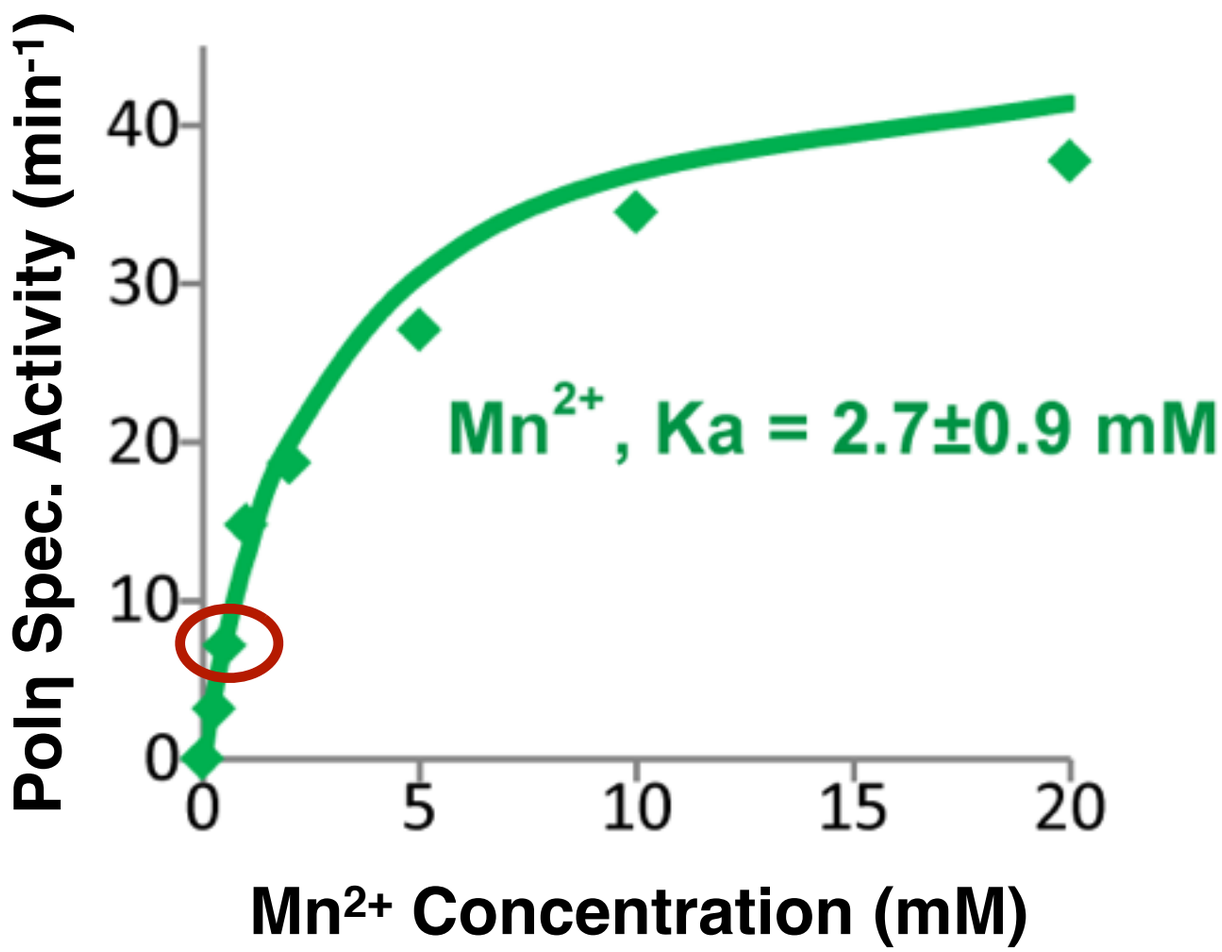
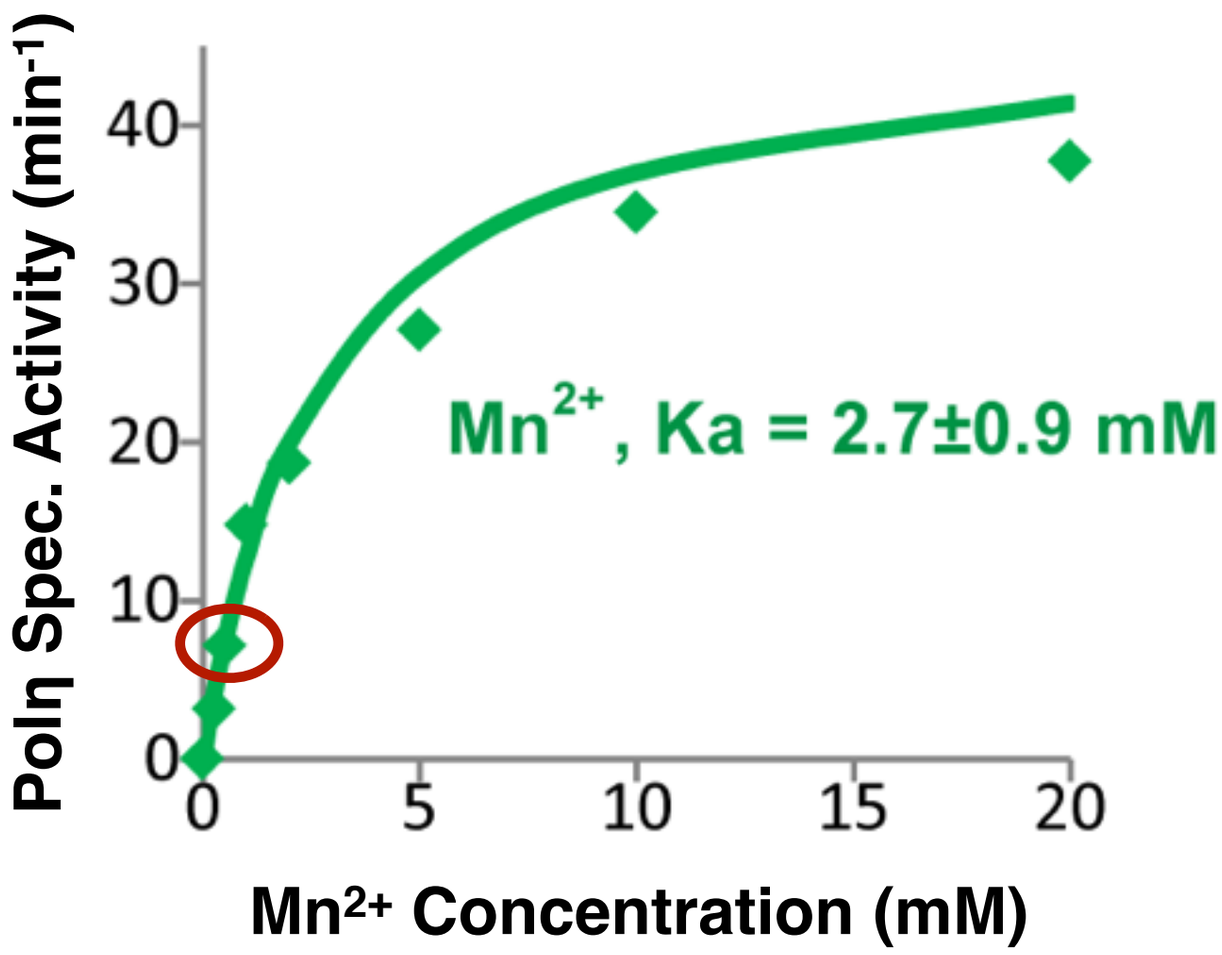
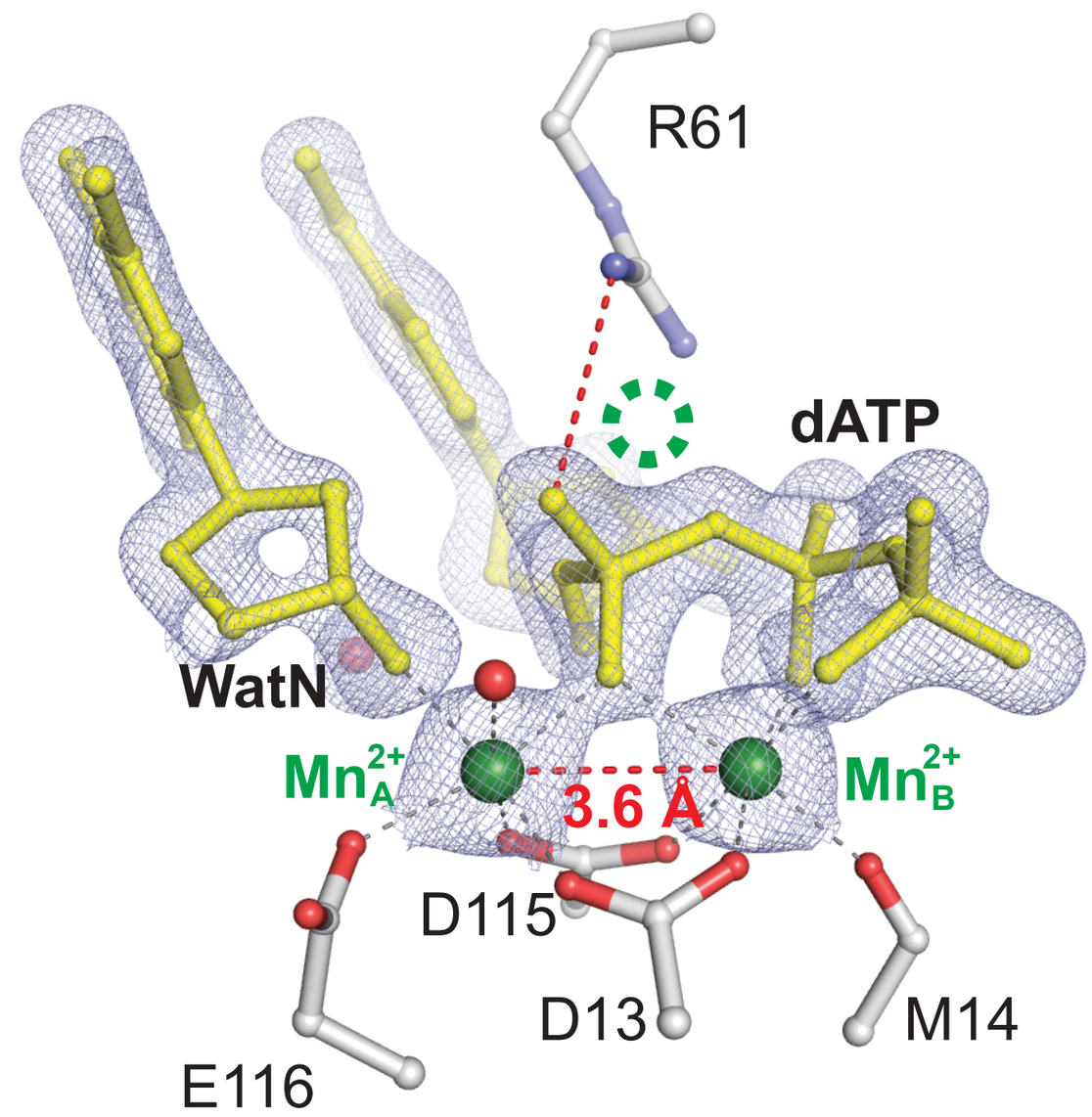


Gao & Yang, (2016) Science

The 3rd Metal ion is Required for DNA Synthesis Reaction !!!





1 mM Mn^{2+}, 1800s
No reaction

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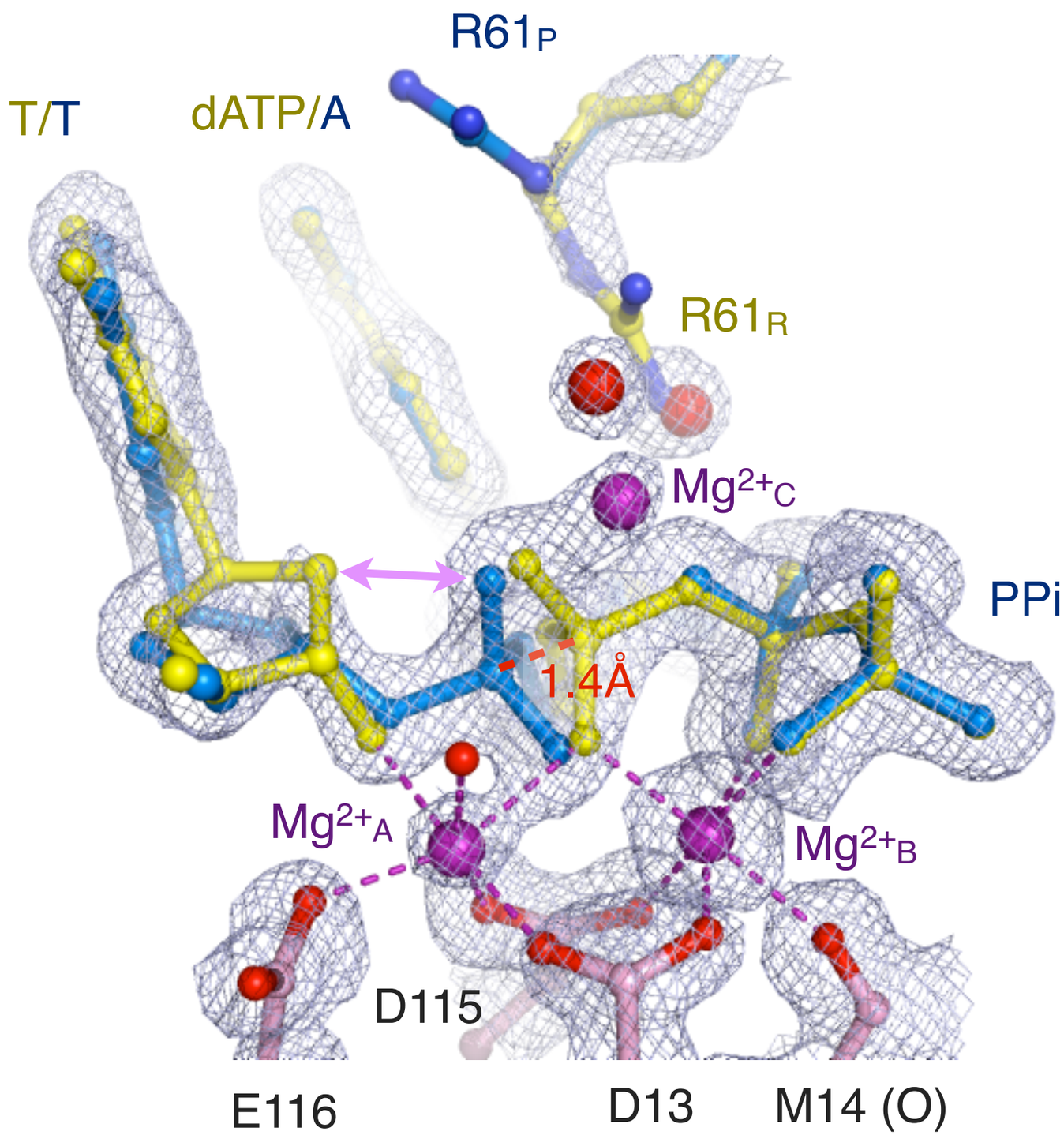
Puzzle: When Does the 3rd Metal Ion Bind ?



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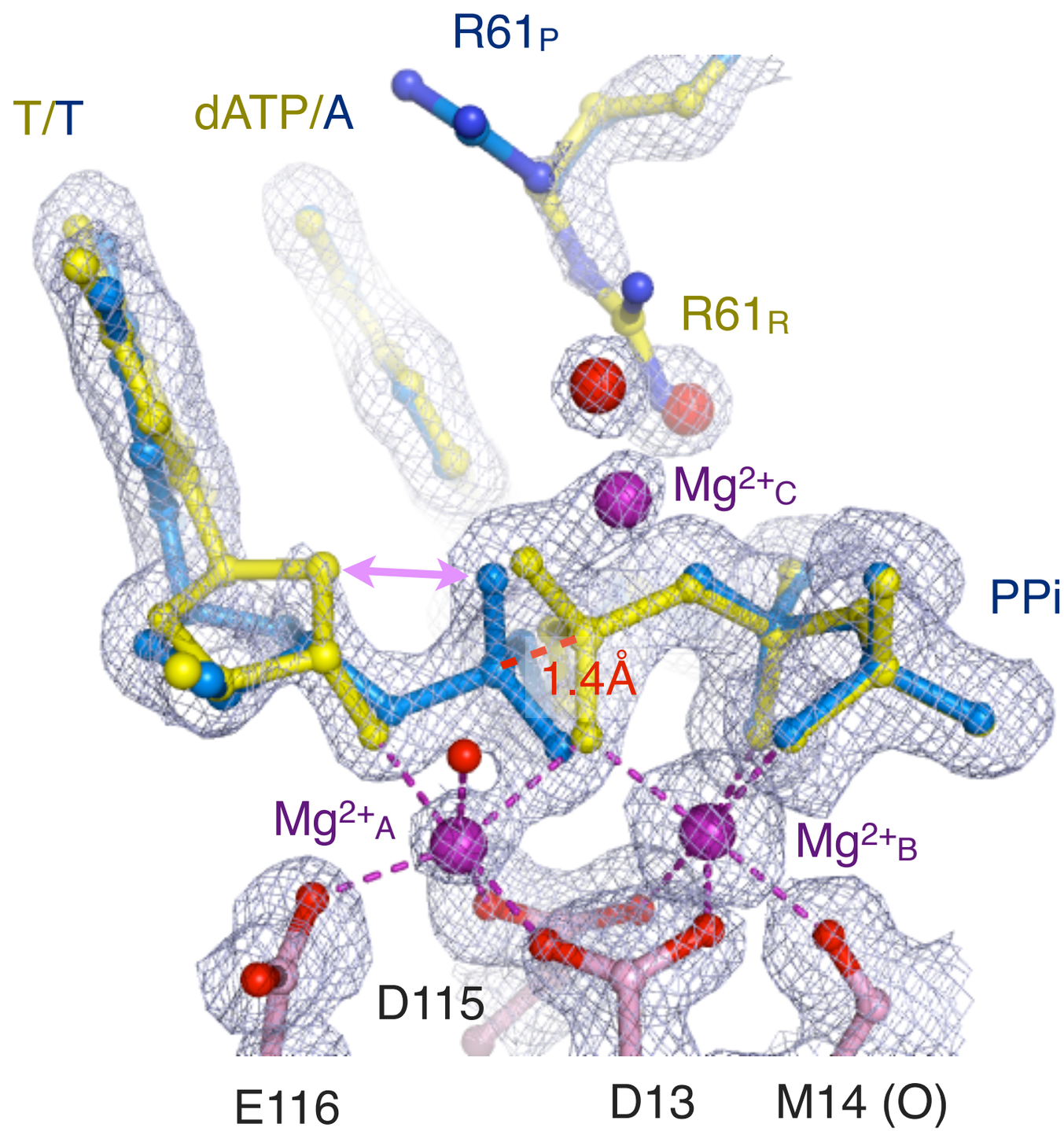
Reactant State (RS)
Product State (PS)



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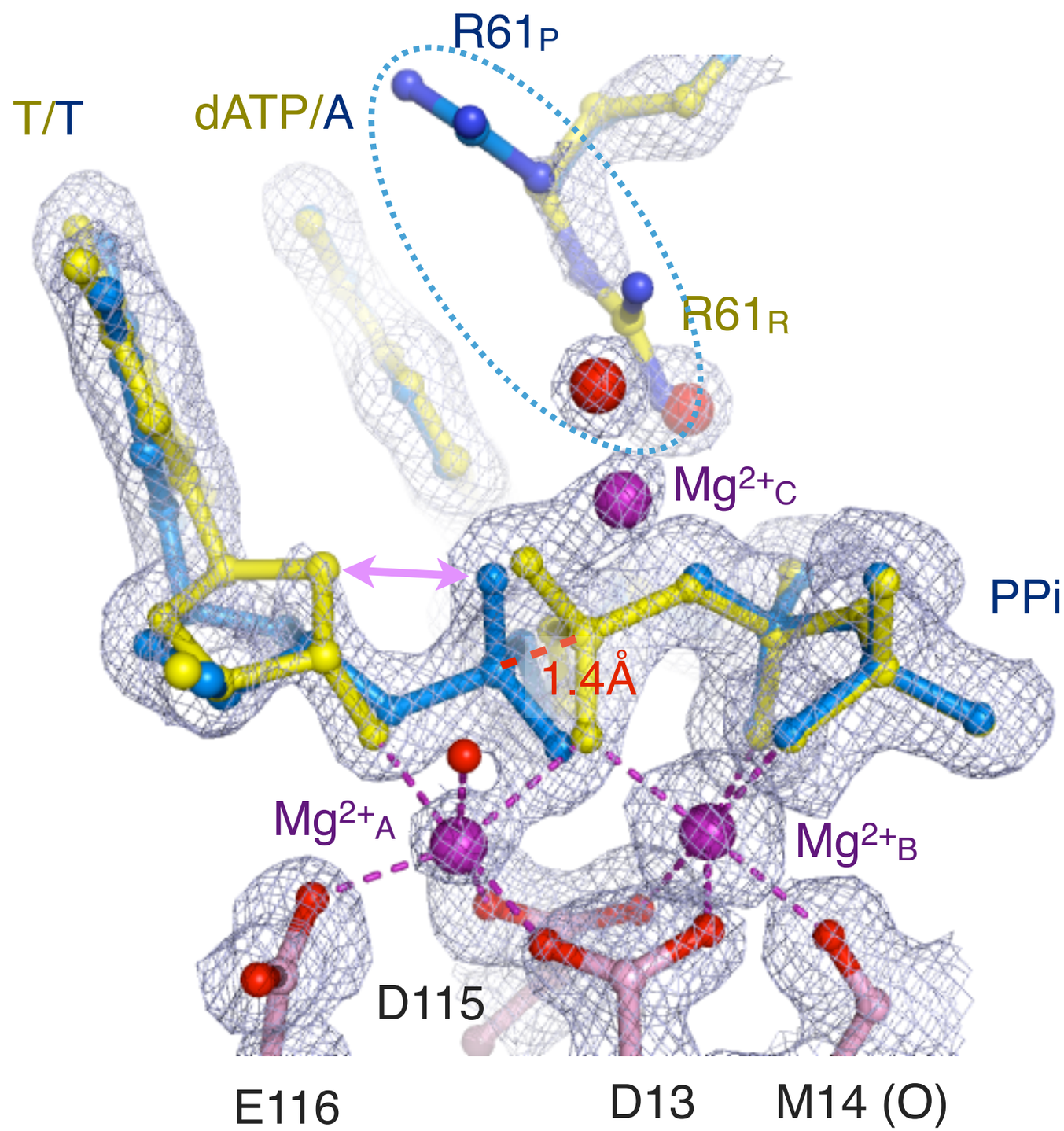

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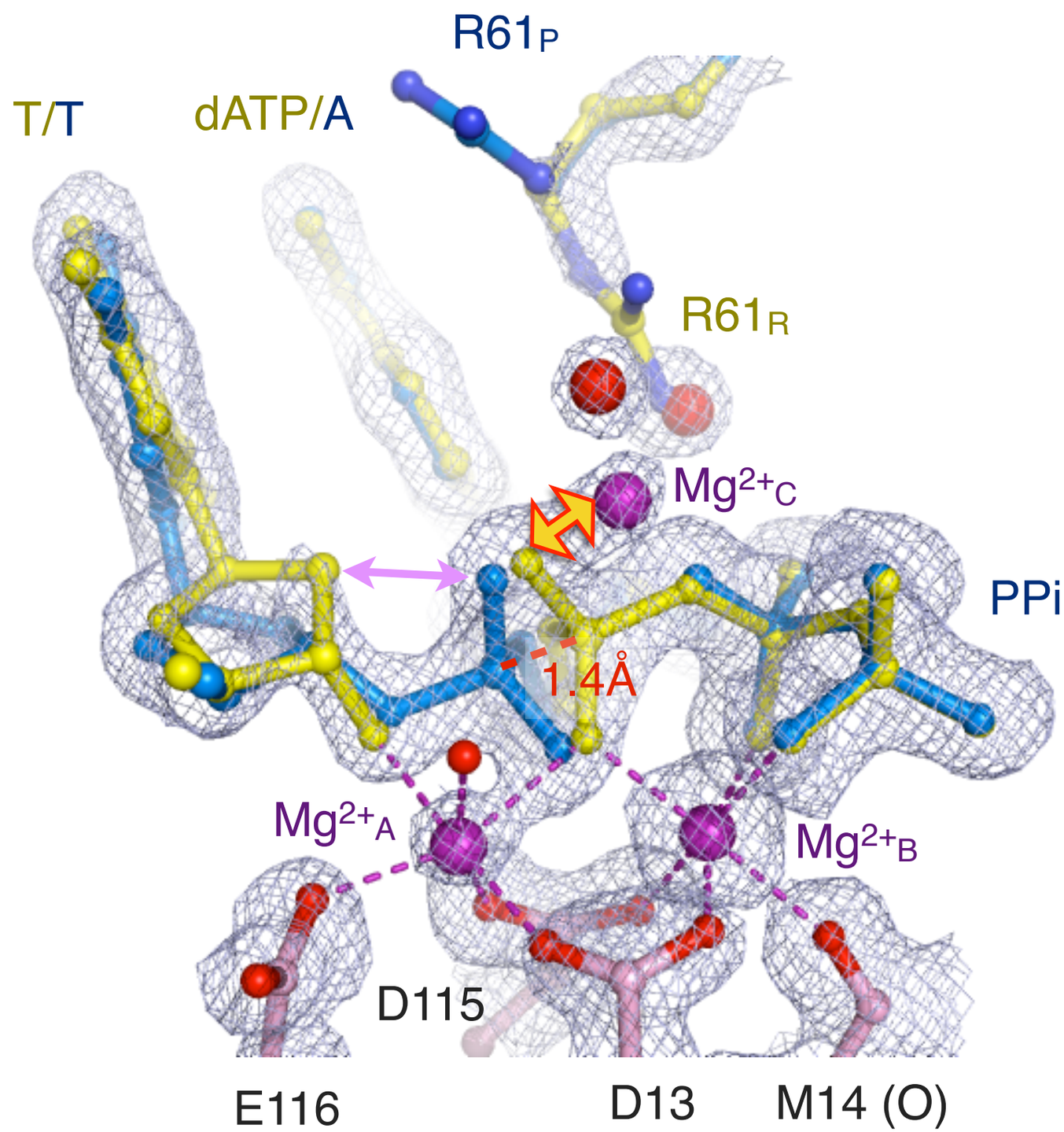
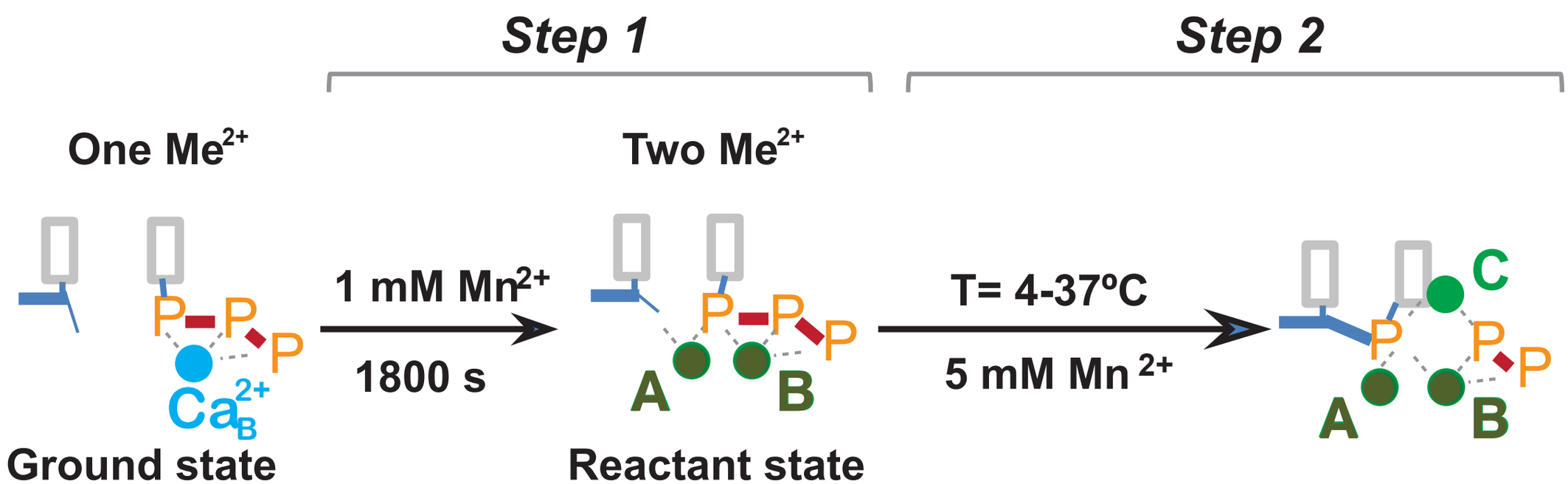


Binding of the 3rd Metal Ion Occurs in Transition State & Requires Thermal Energy



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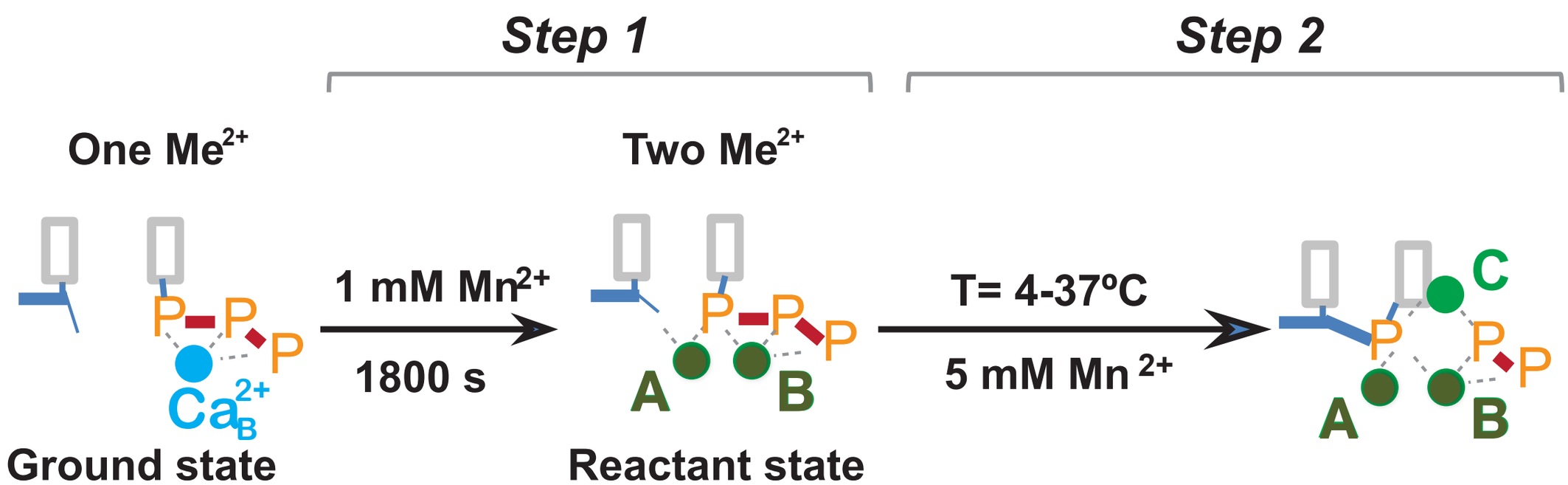

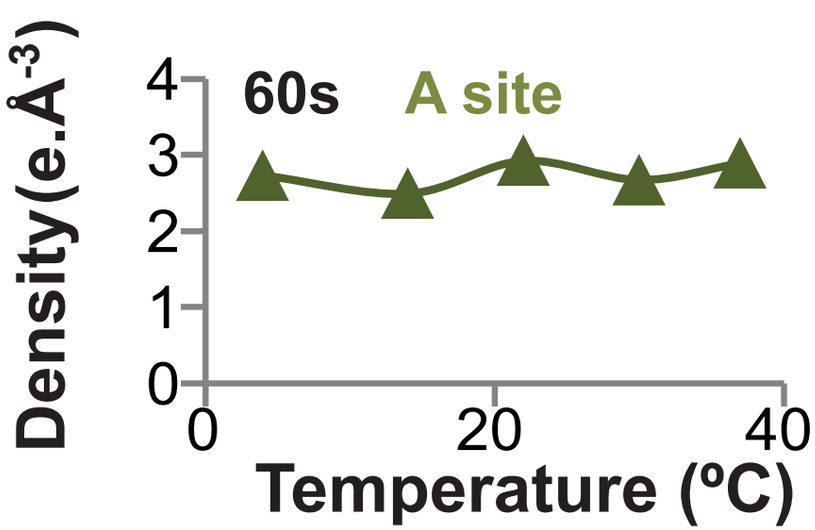

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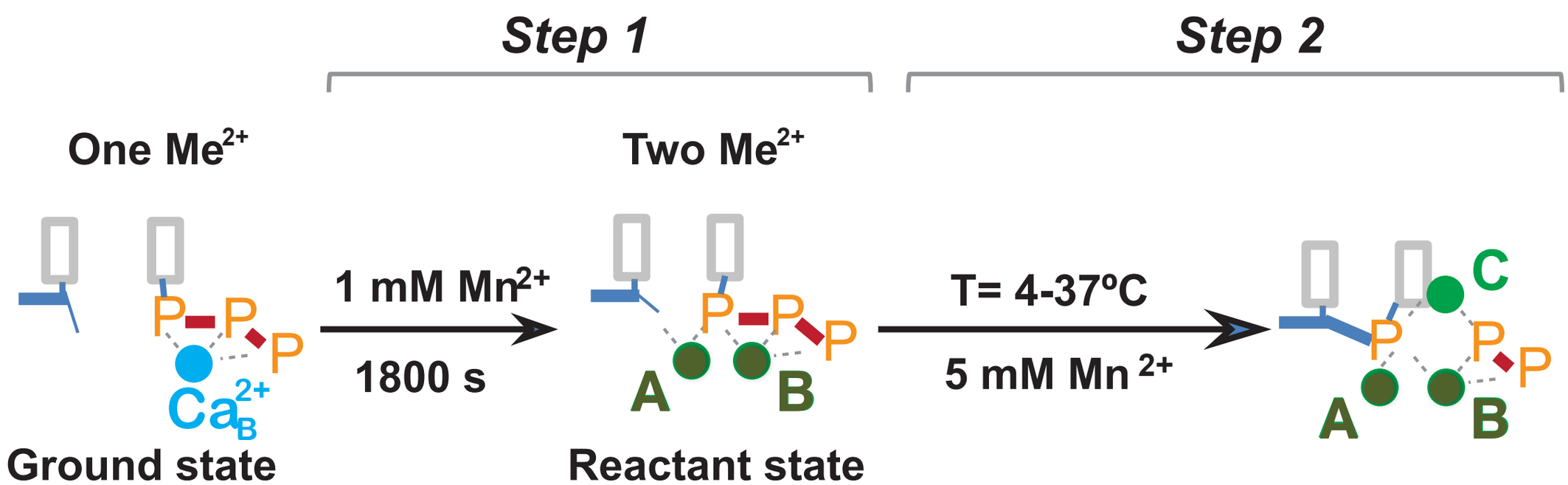

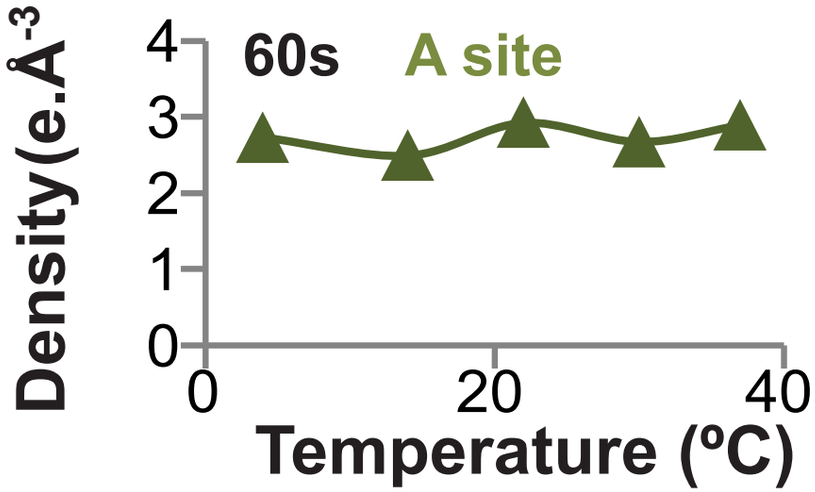

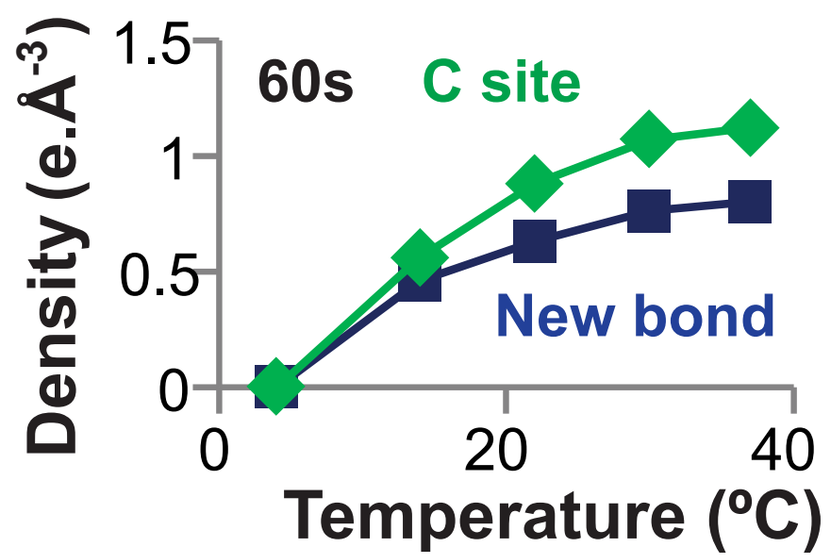

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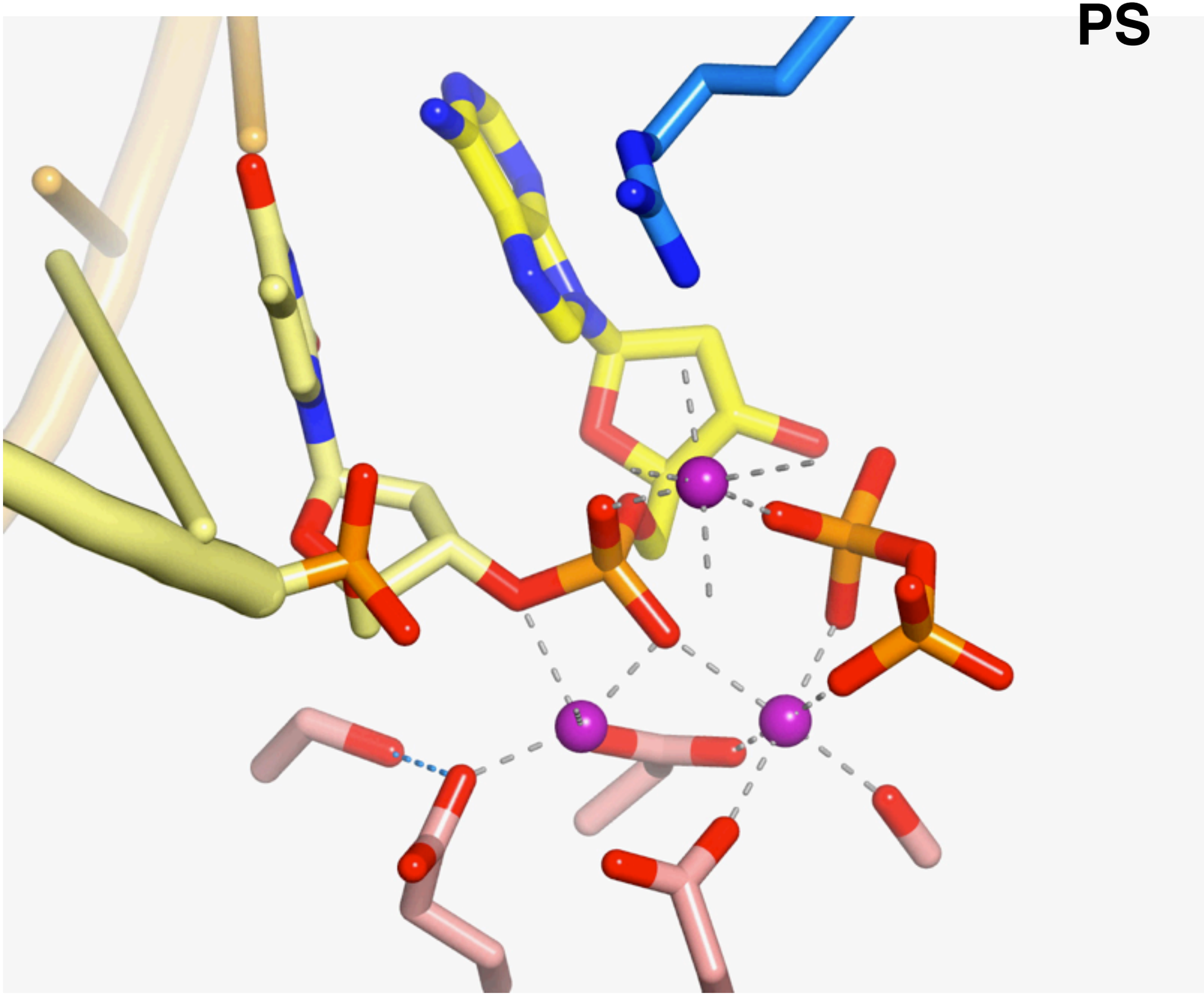
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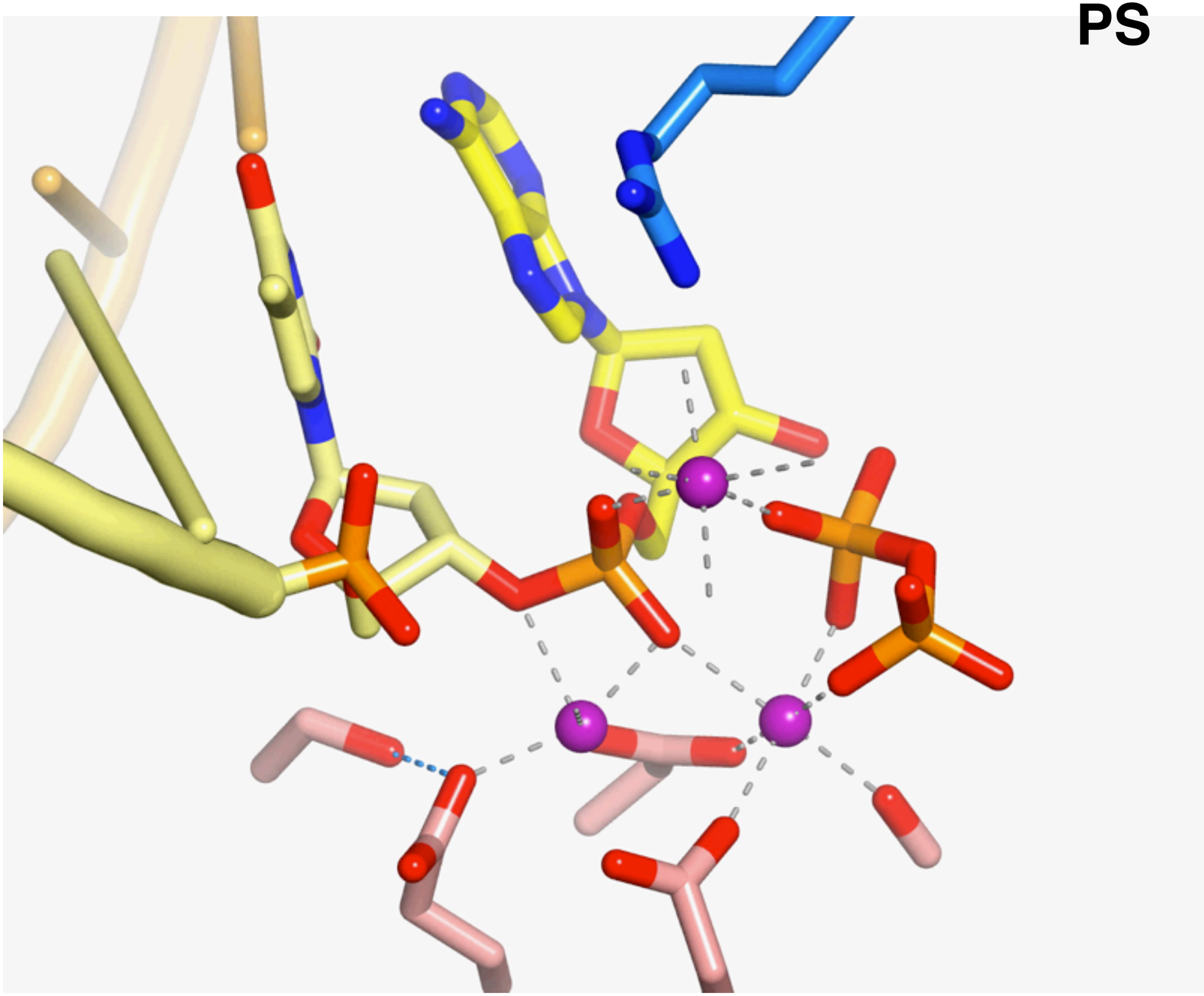
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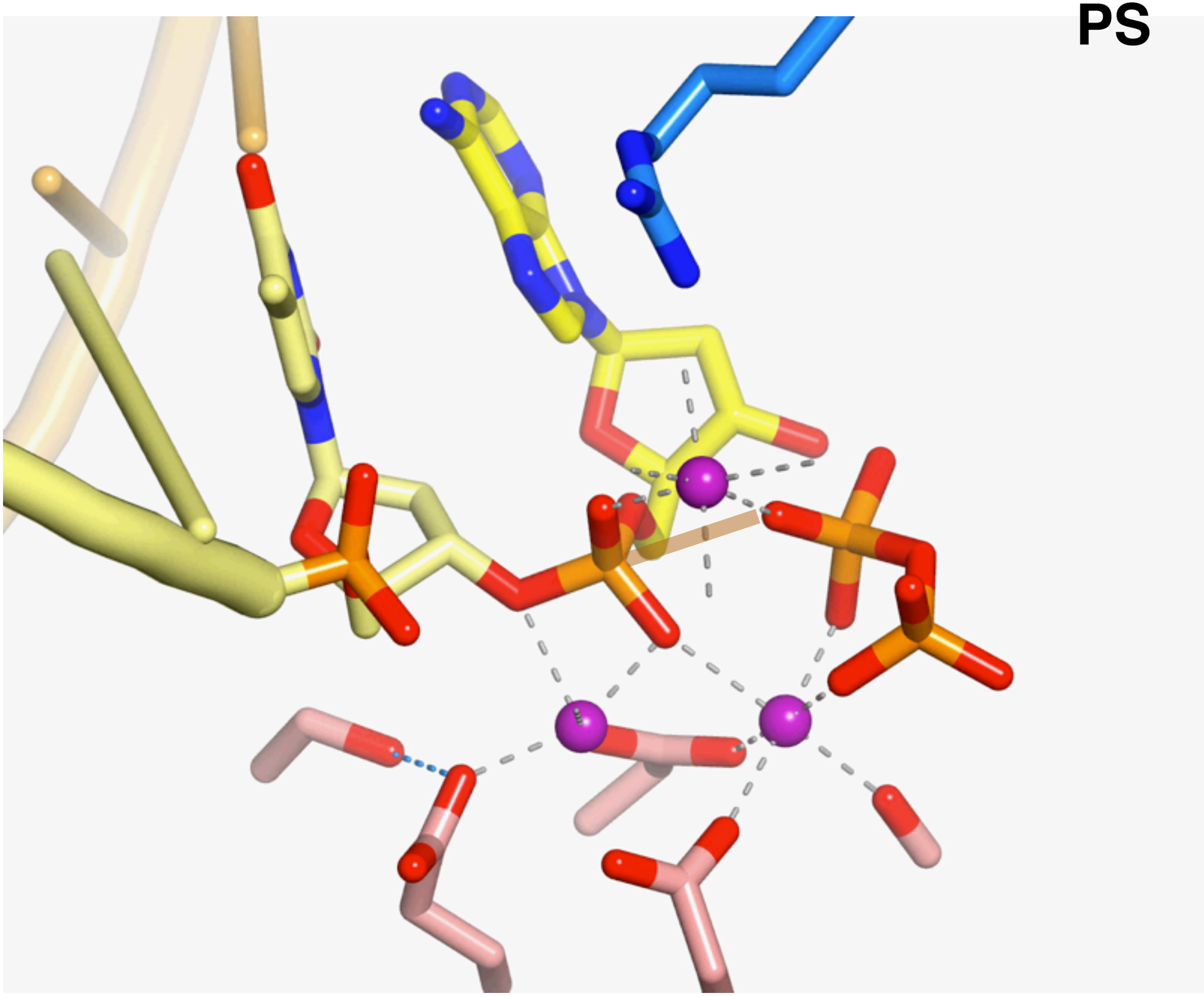
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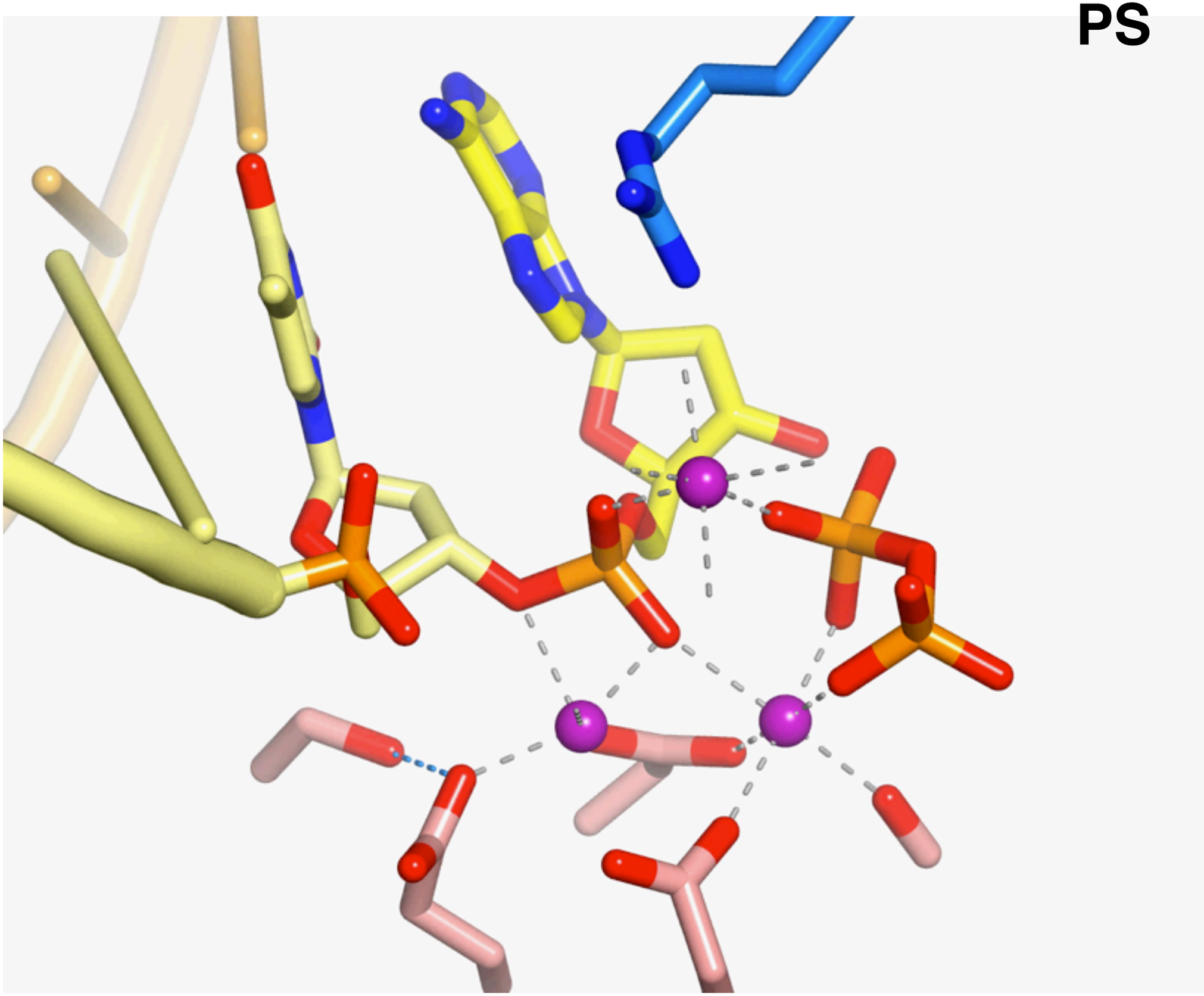
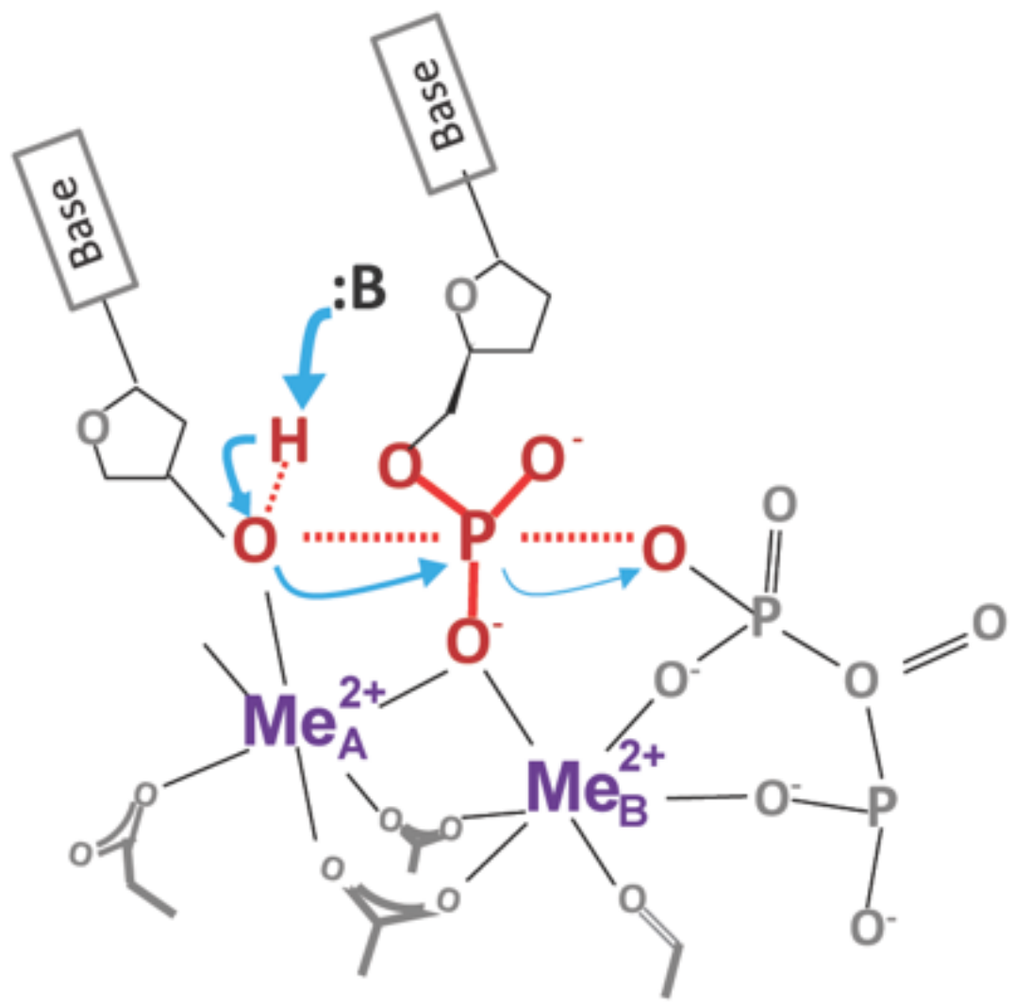

PS

DNA Synthesis Reaction is Likely Initiated by the 3rd Mg^{2+} and not by a General Base



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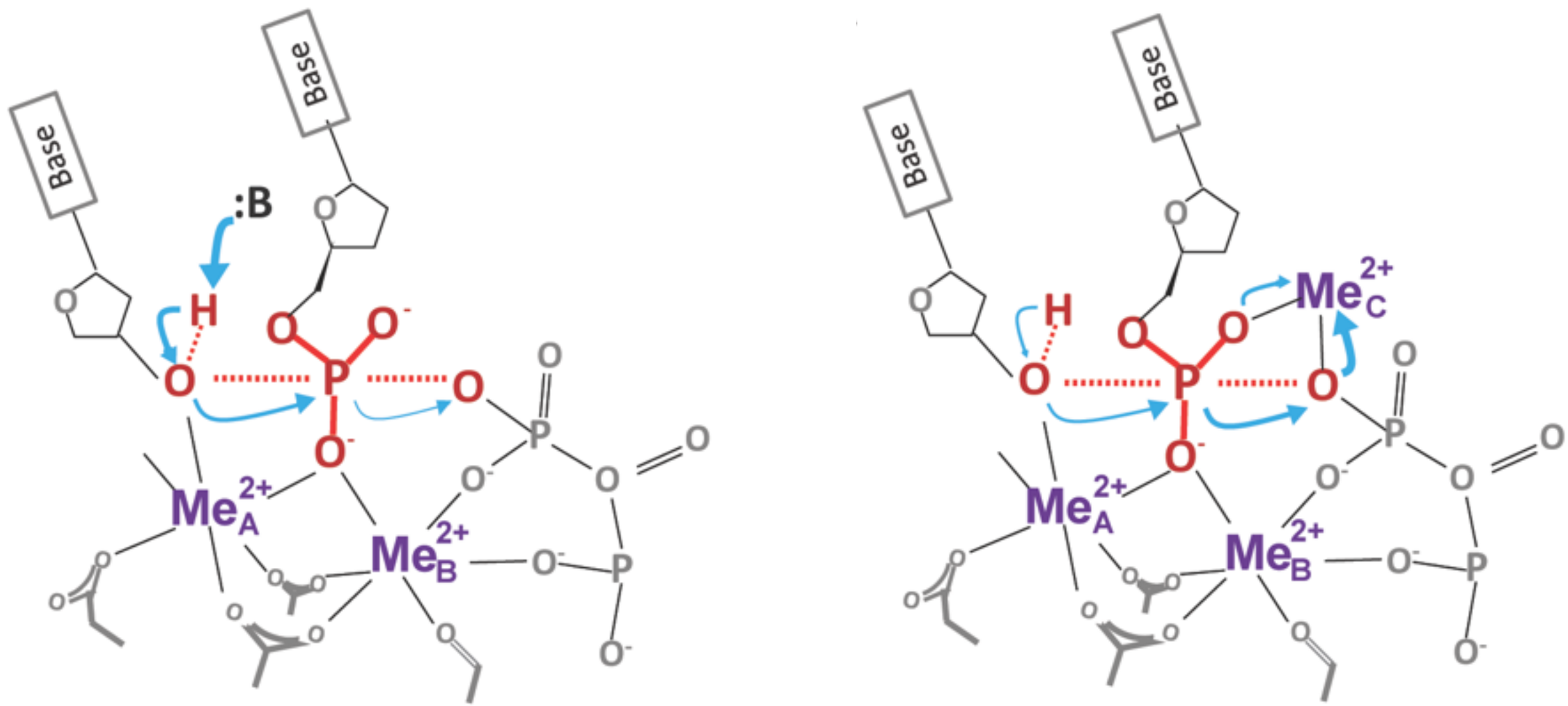
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Base
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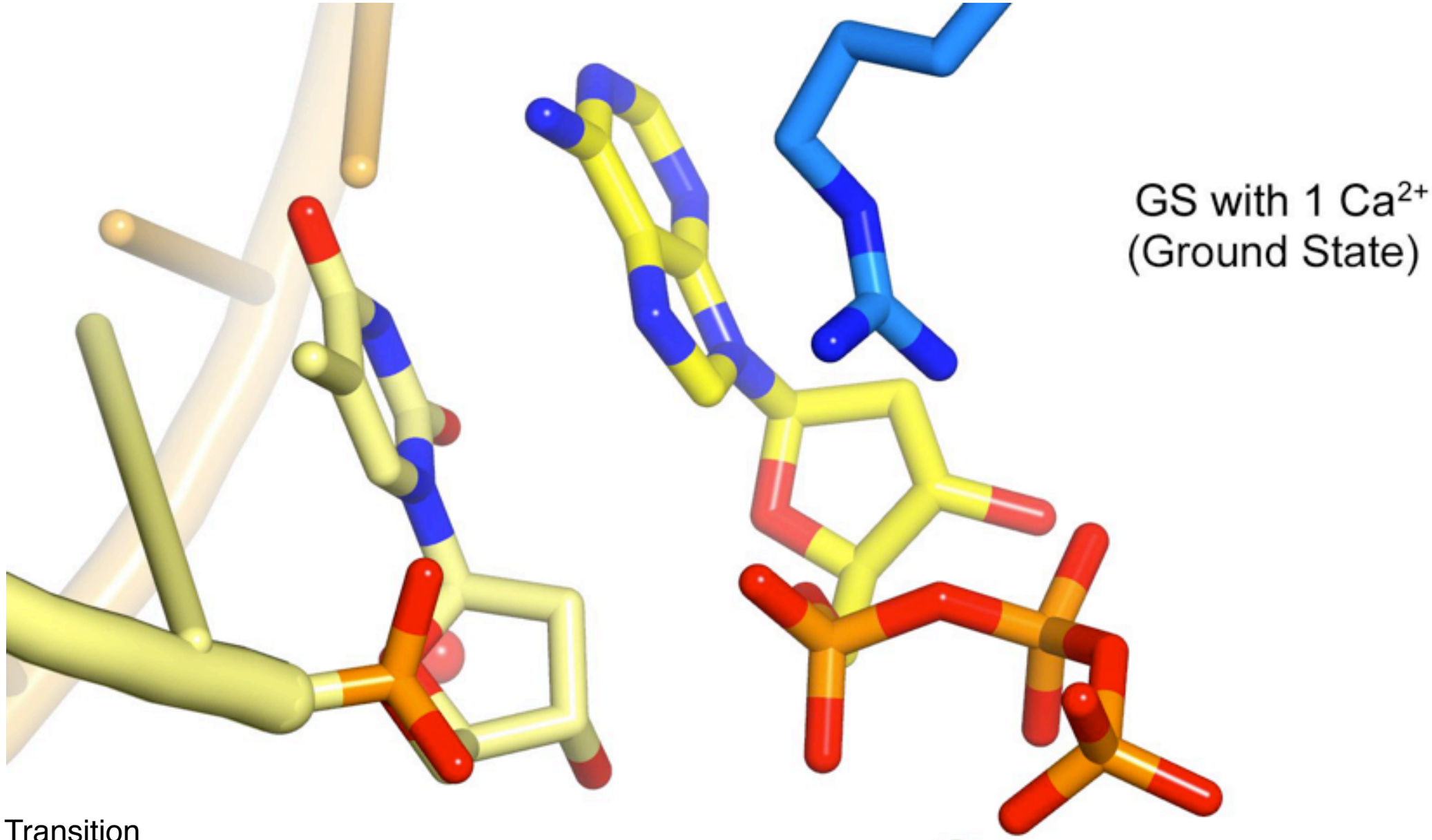

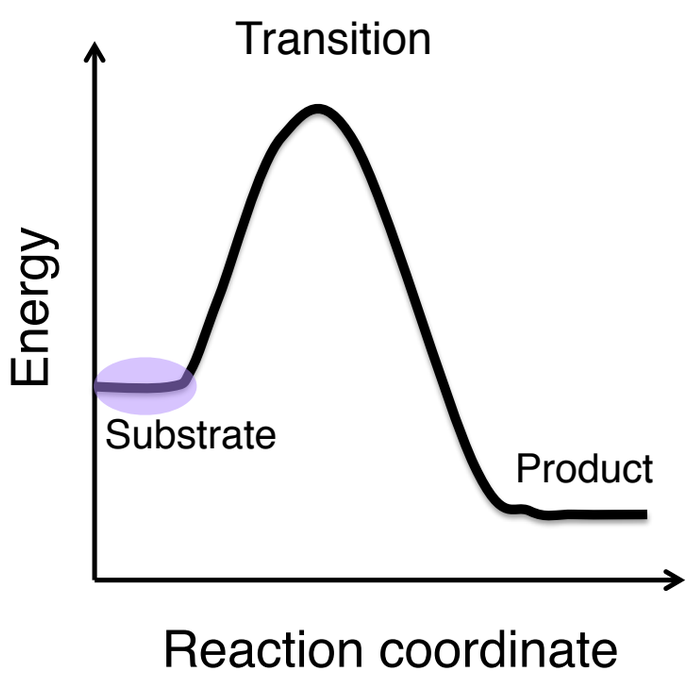

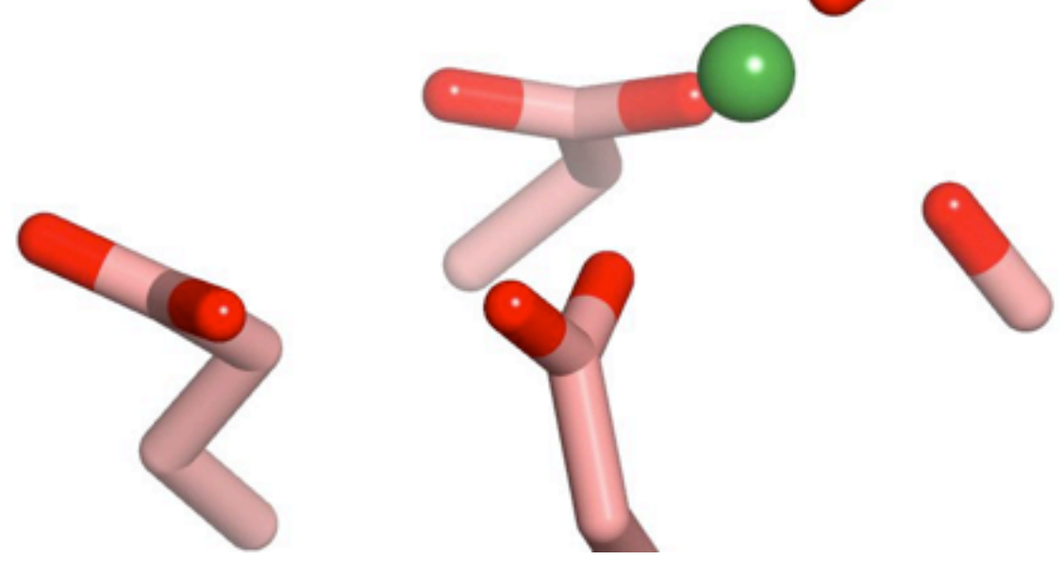

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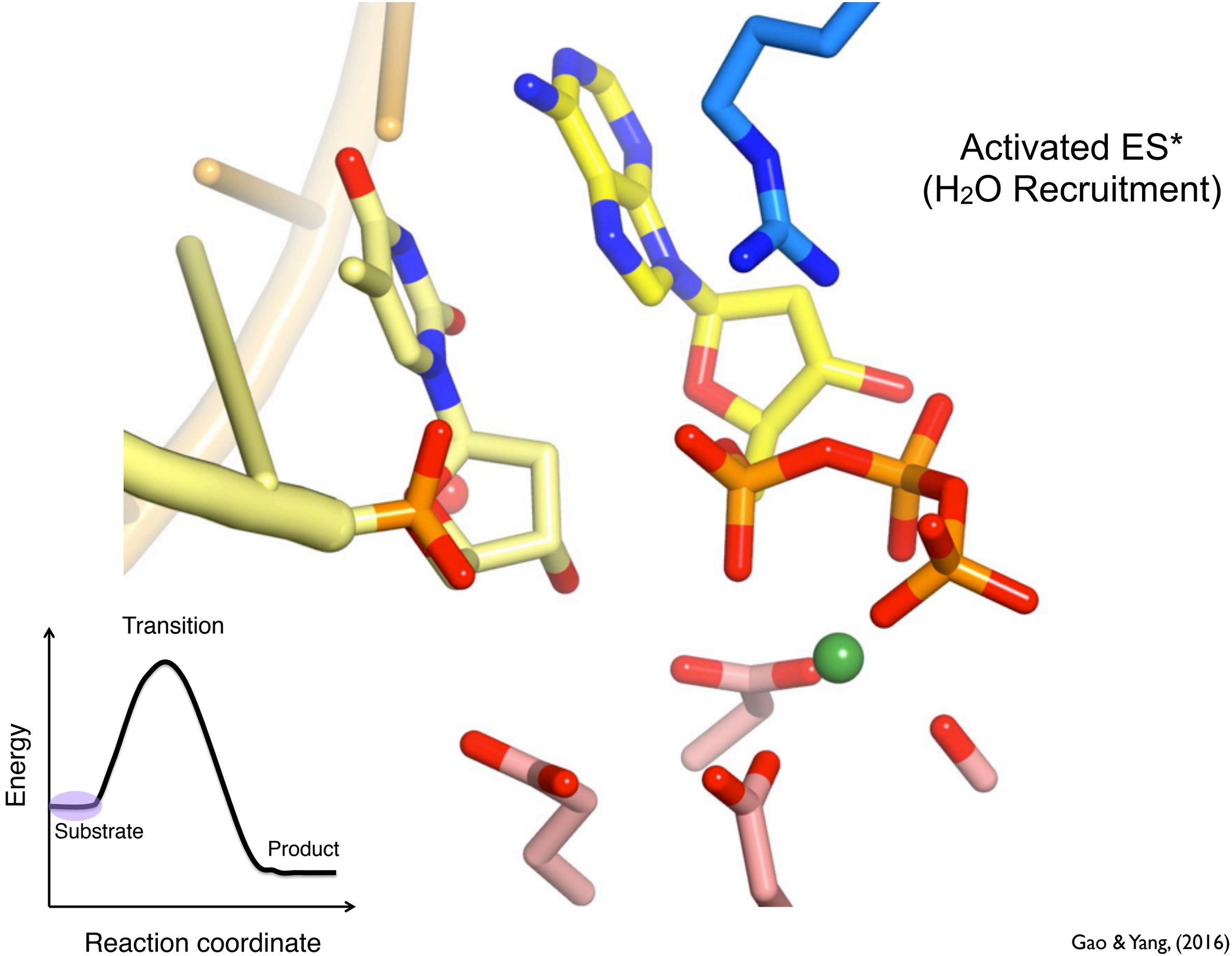

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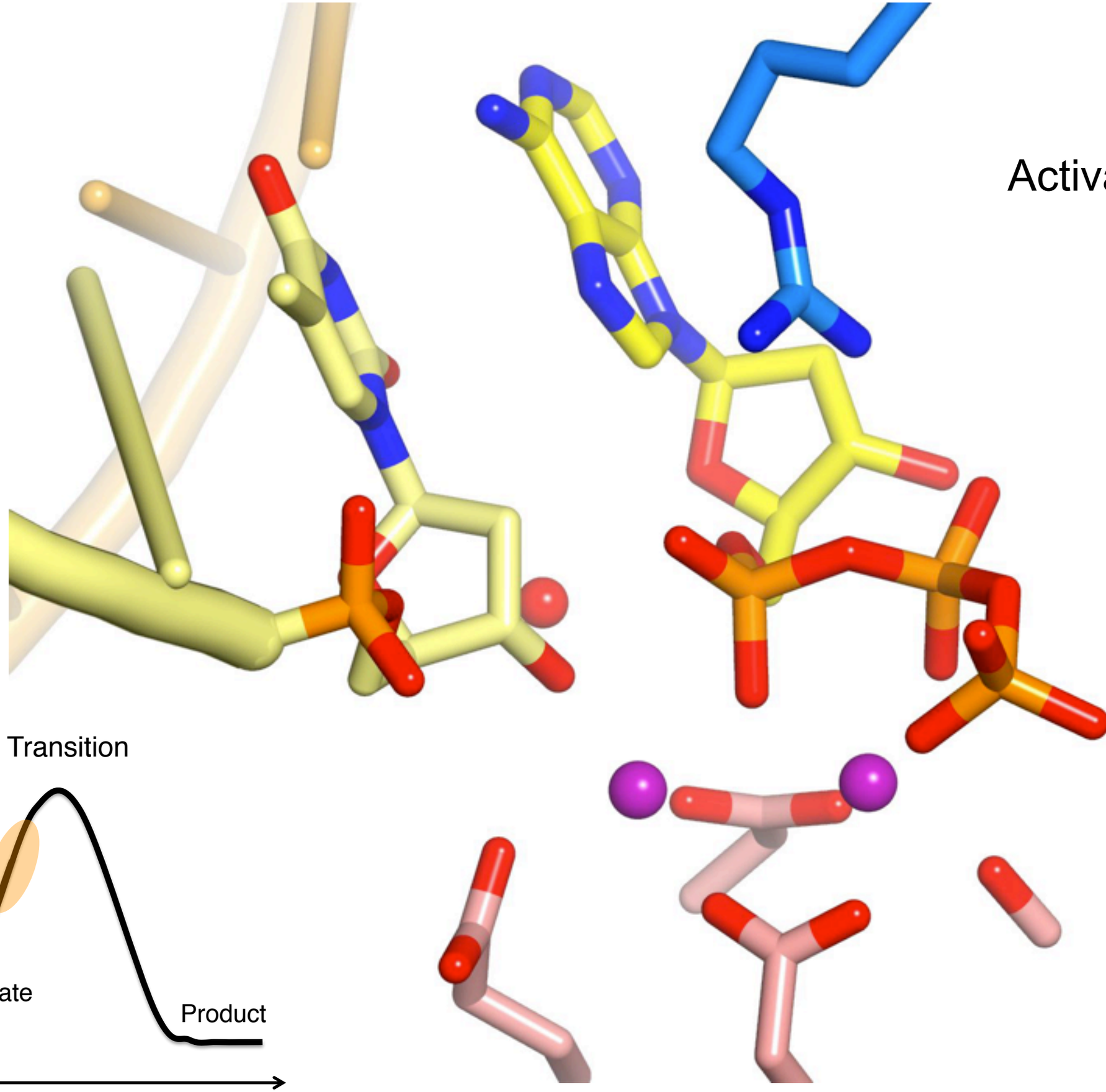

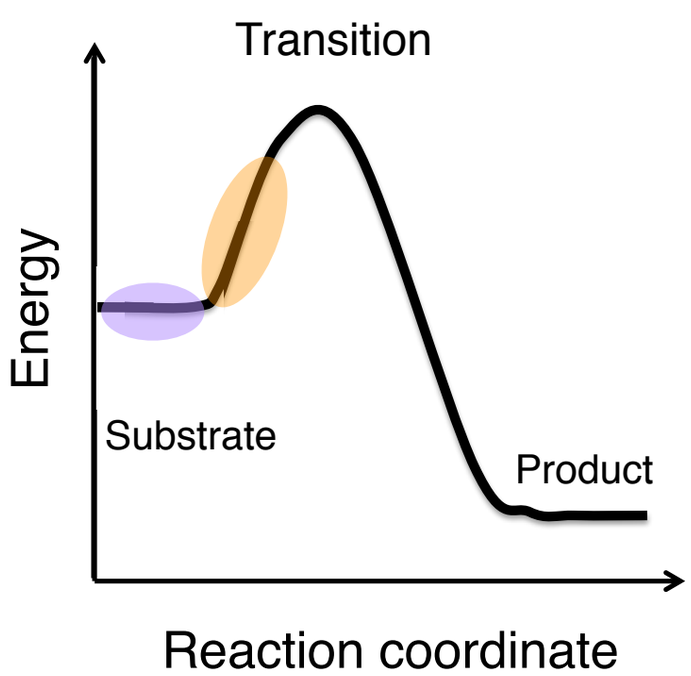

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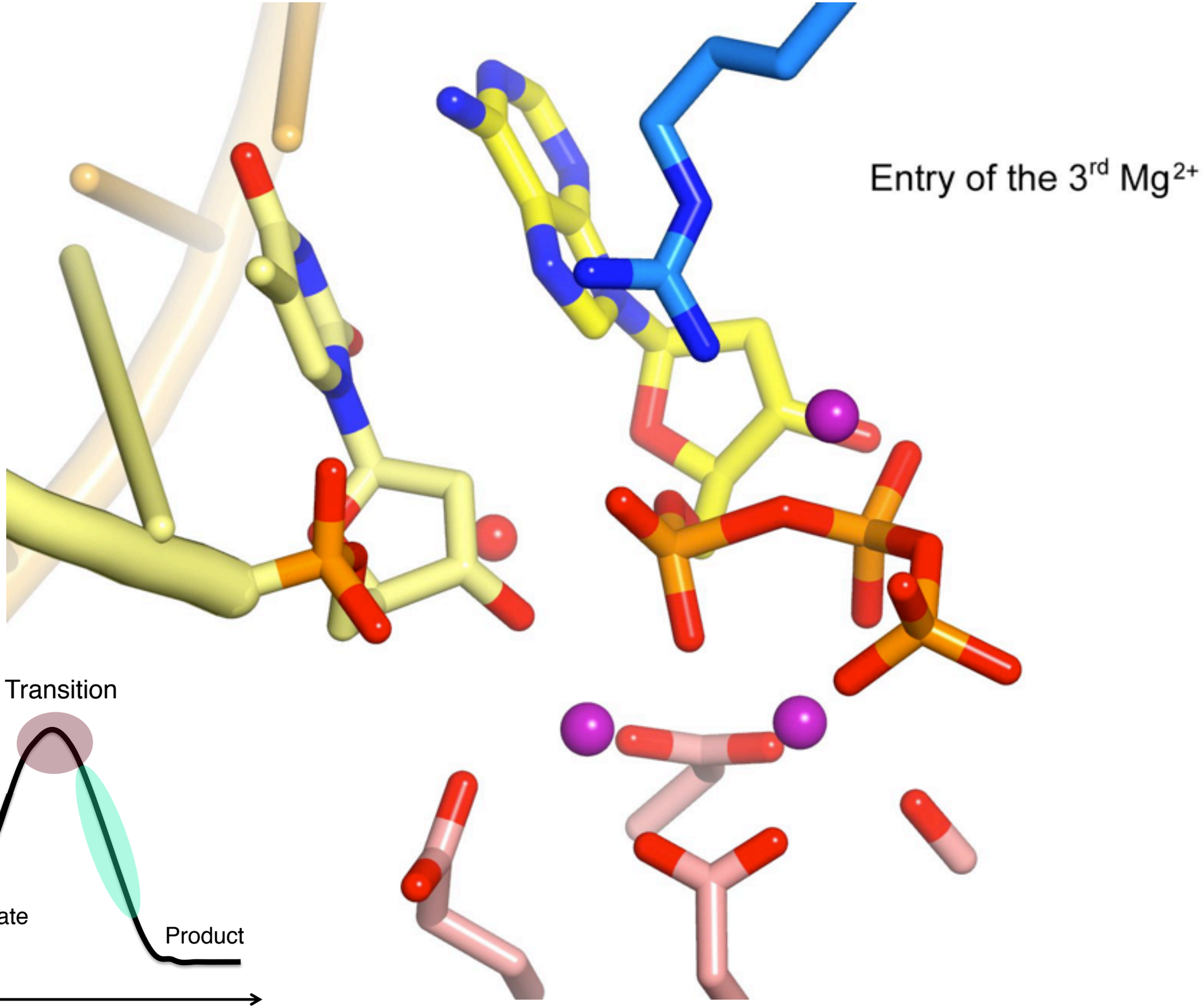

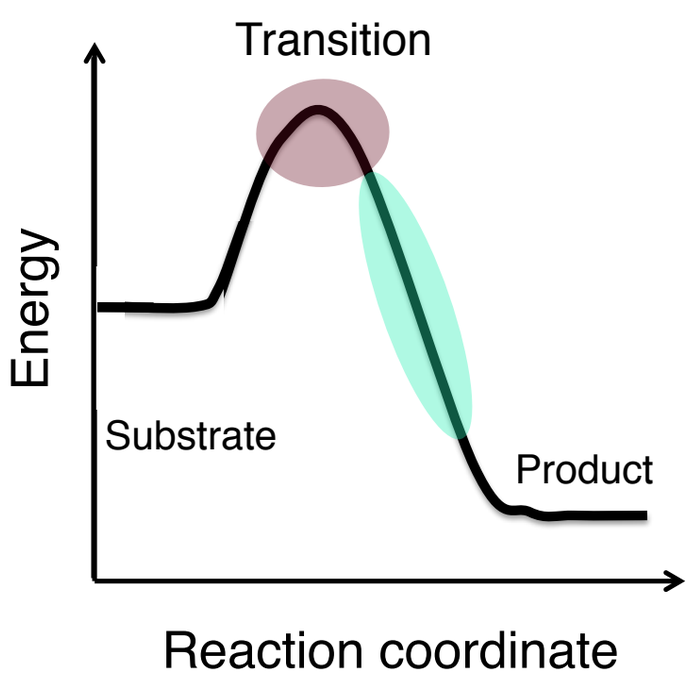

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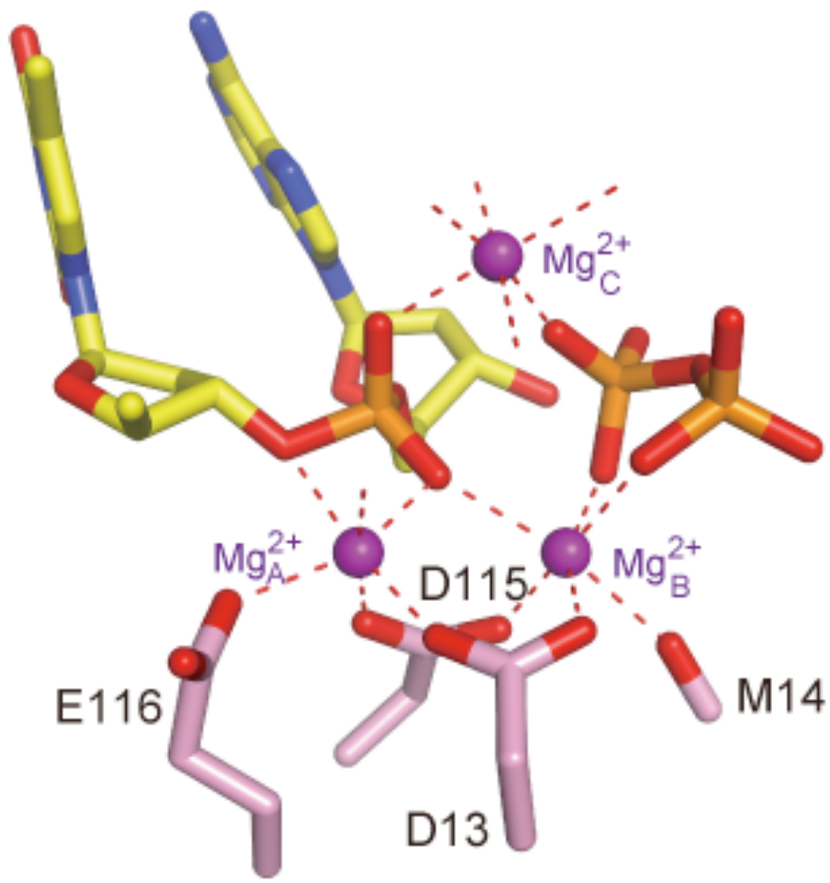
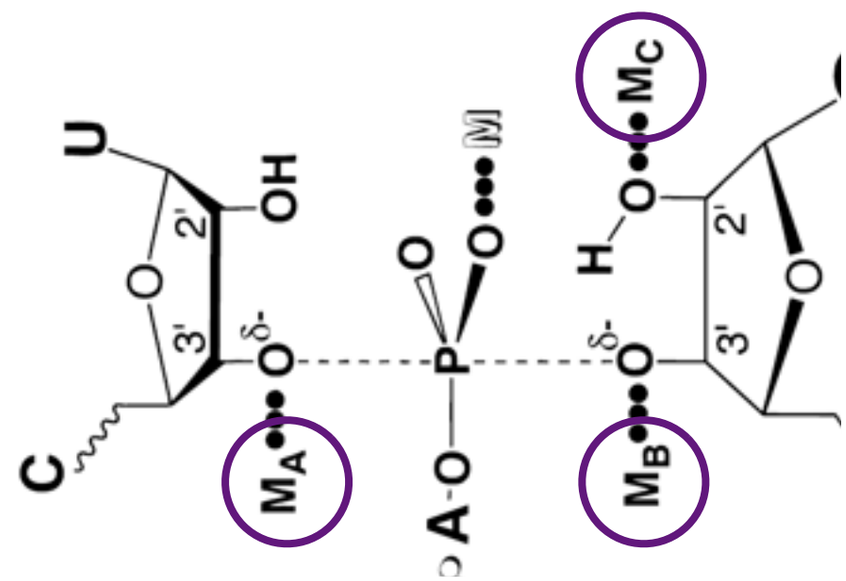
A Third Metal Ion in Two-Metal-Ion Catalysis

hPol η



Gao & Yang, (2016) Science
Nakamura, et al., & Yang (2012) Nature

Group I Intron



Shan & Hershlag., *Biochem,* (1999)

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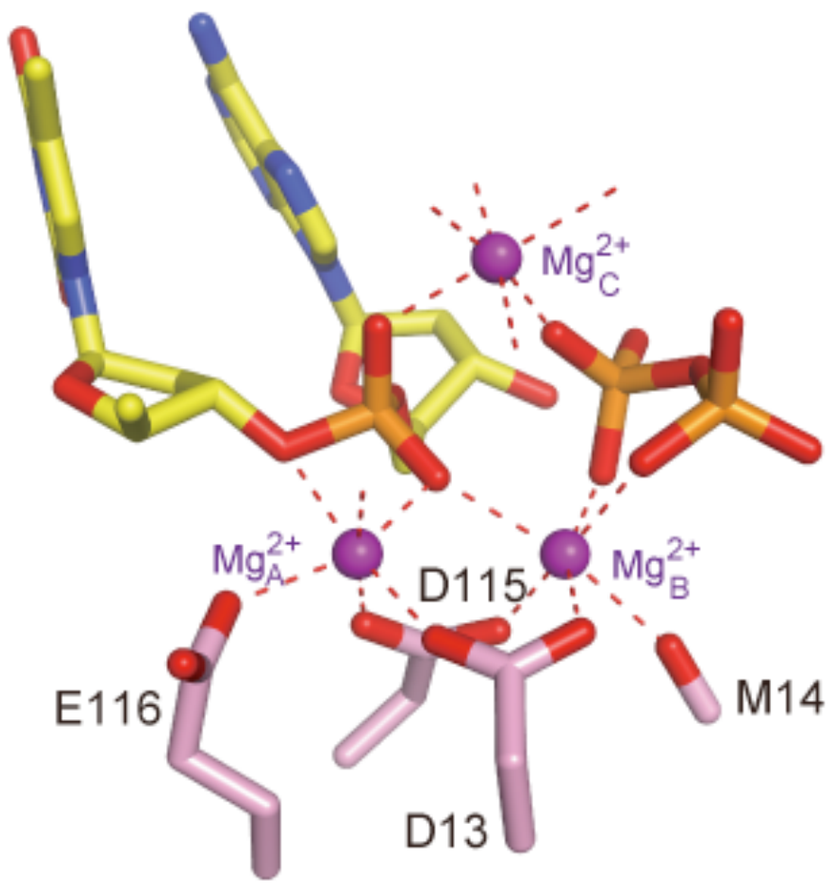

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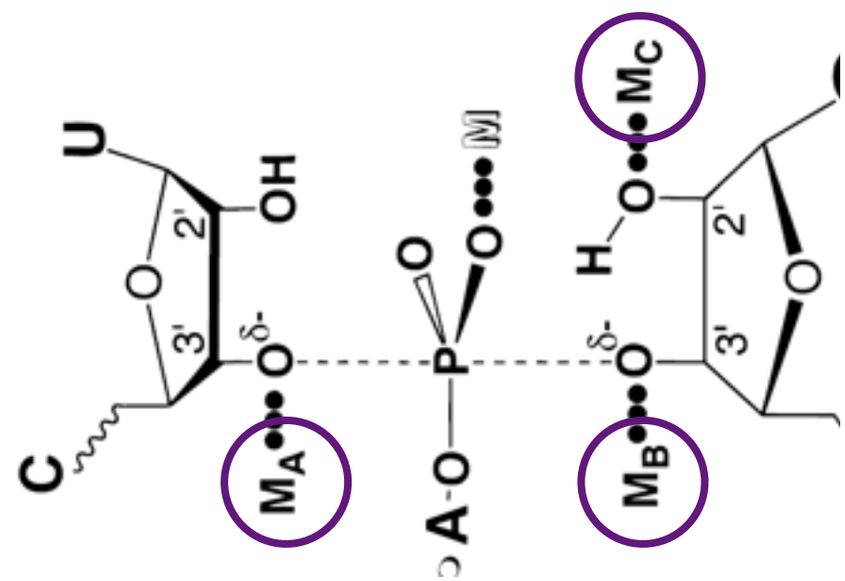

$$E + S \rightleftharpoons ES \longrightarrow ES^* \longrightarrow E + P$$

Gao & Yang, (2016) Science
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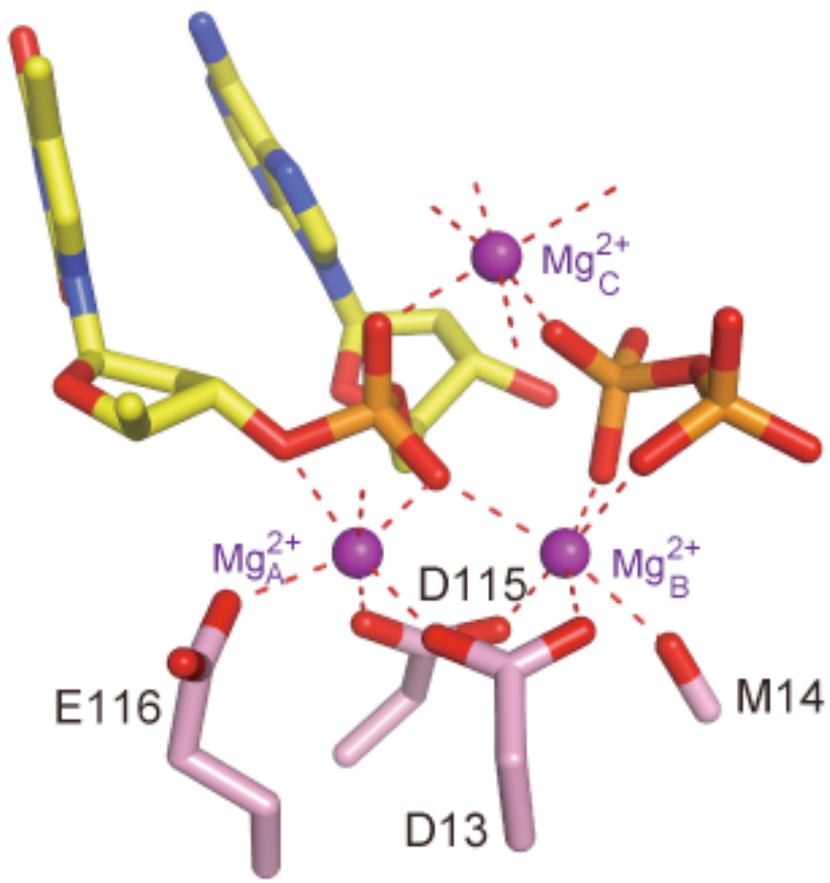

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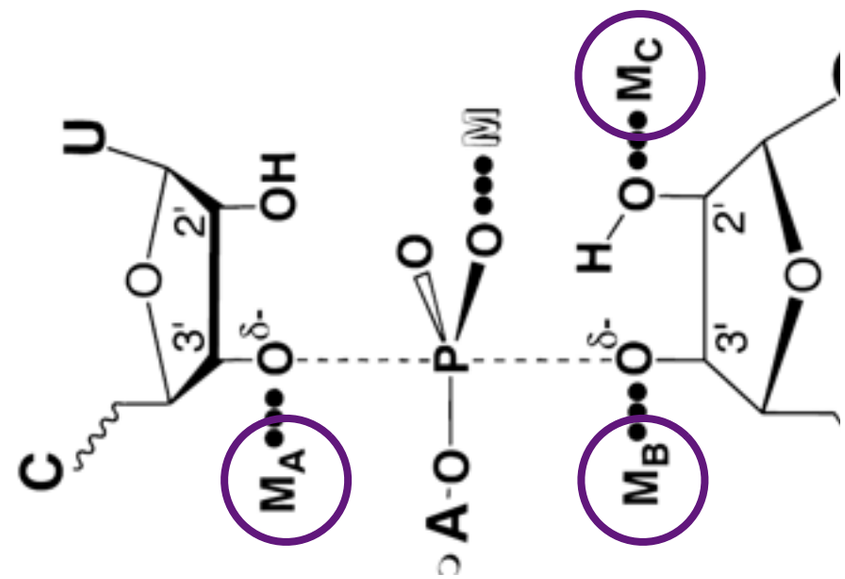

$$E + S \rightleftharpoons ES \longrightarrow ES^* \xrightarrow{Me^{2+}} E + P$$

Gao & Yang, (2016) Science
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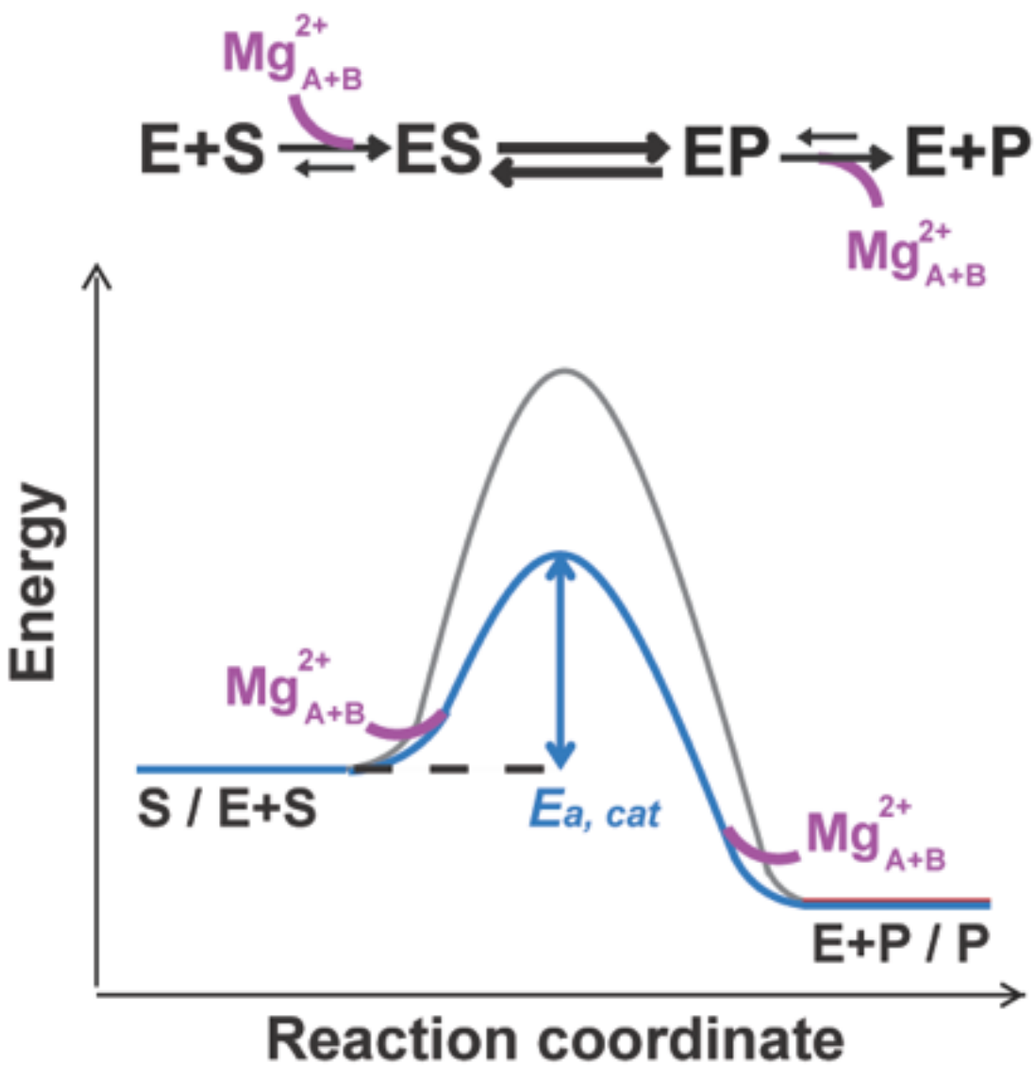
Shan & Hershlag., *Biochem,* (1999)

A New Paradigm for Enzyme Catalysis

Classic two-metal-ion catalysis



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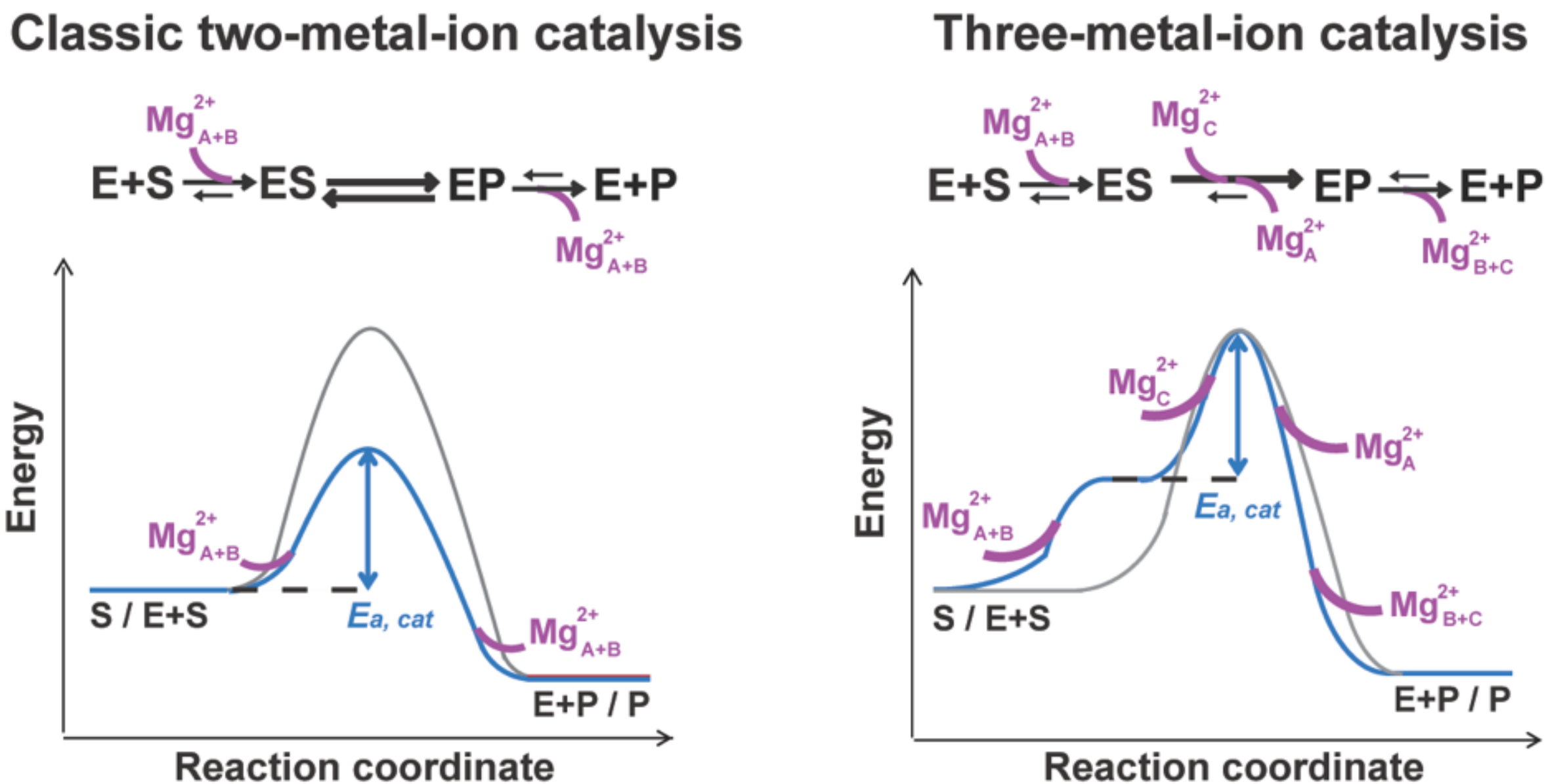
A New Paradigm for Enzyme Catalysis



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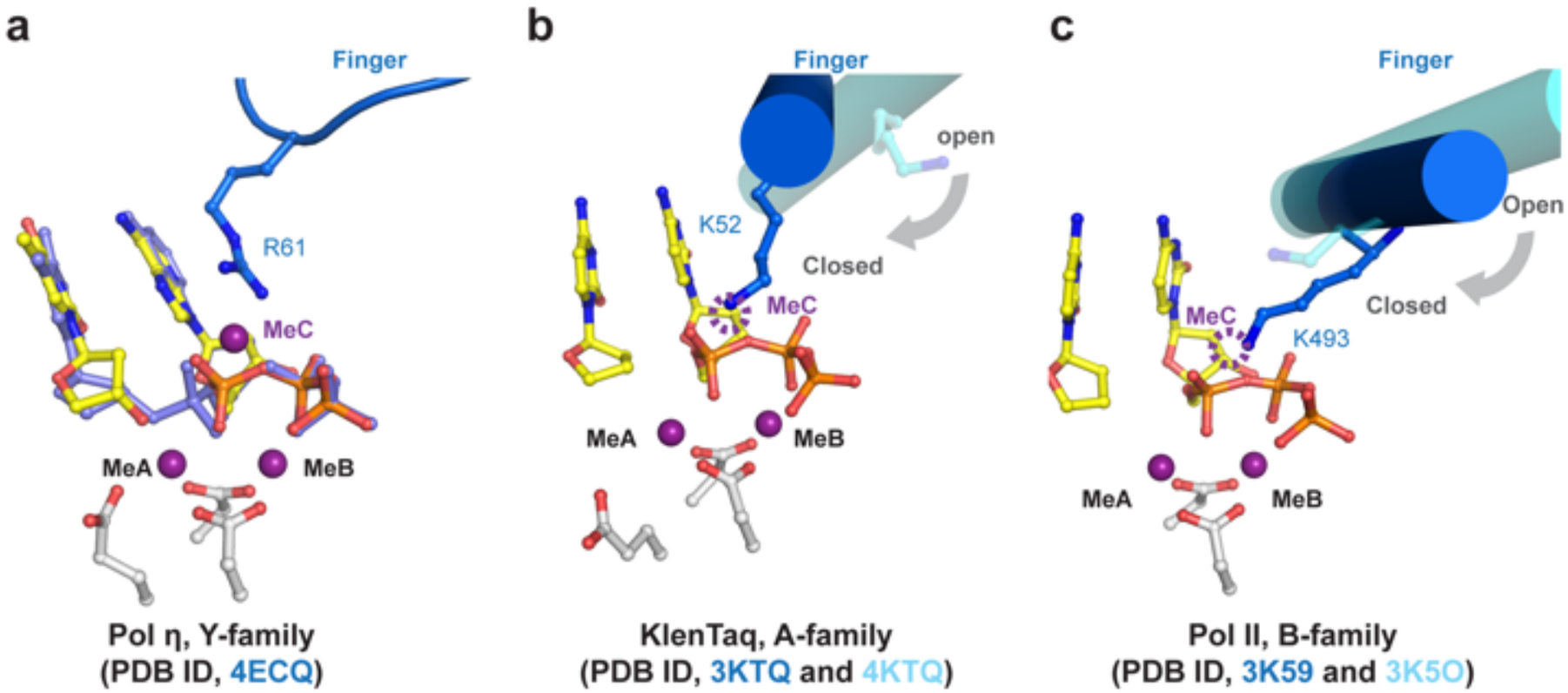
Three Me^{2+} may be Required for Catalysis by all Polymerases



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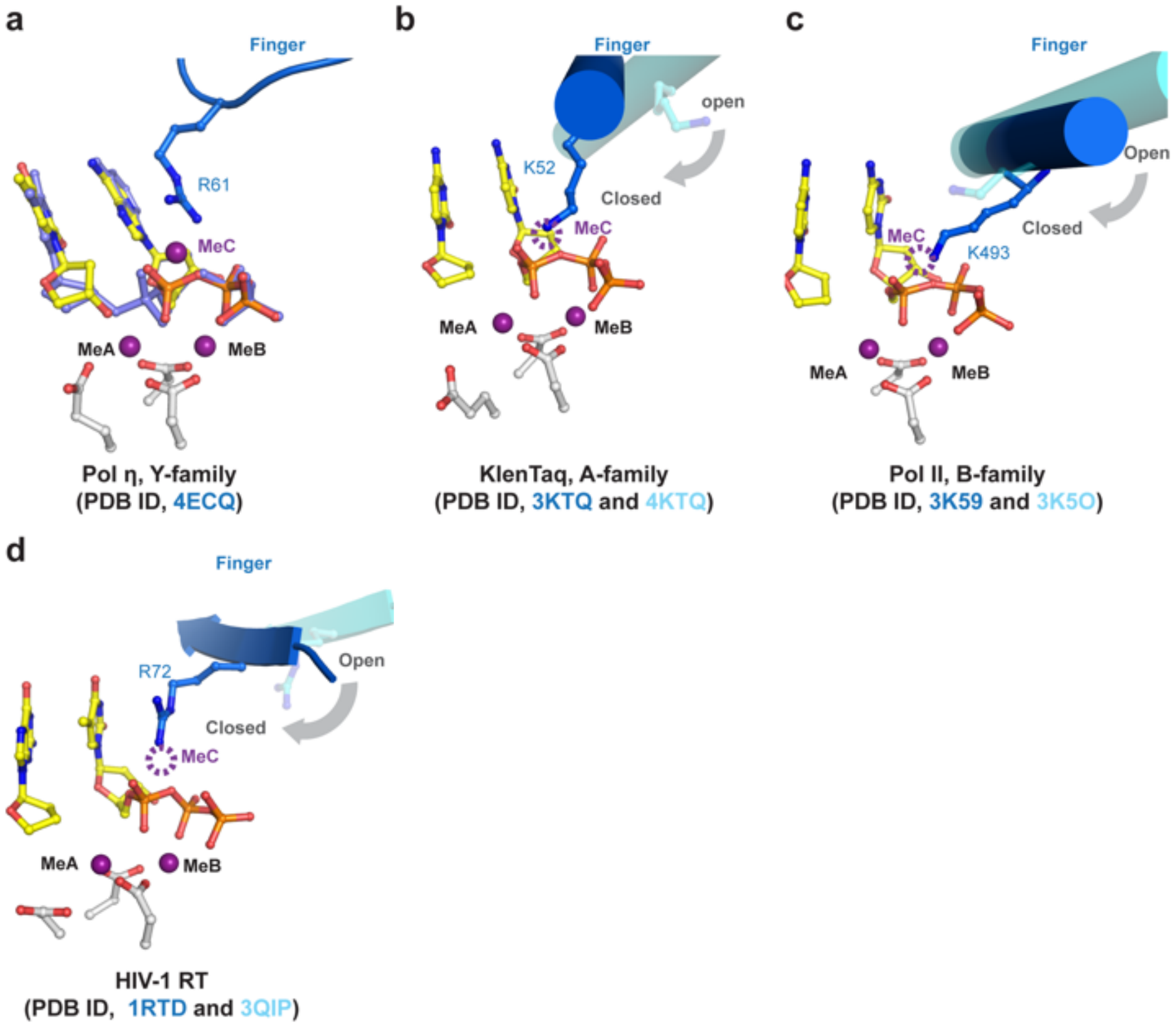

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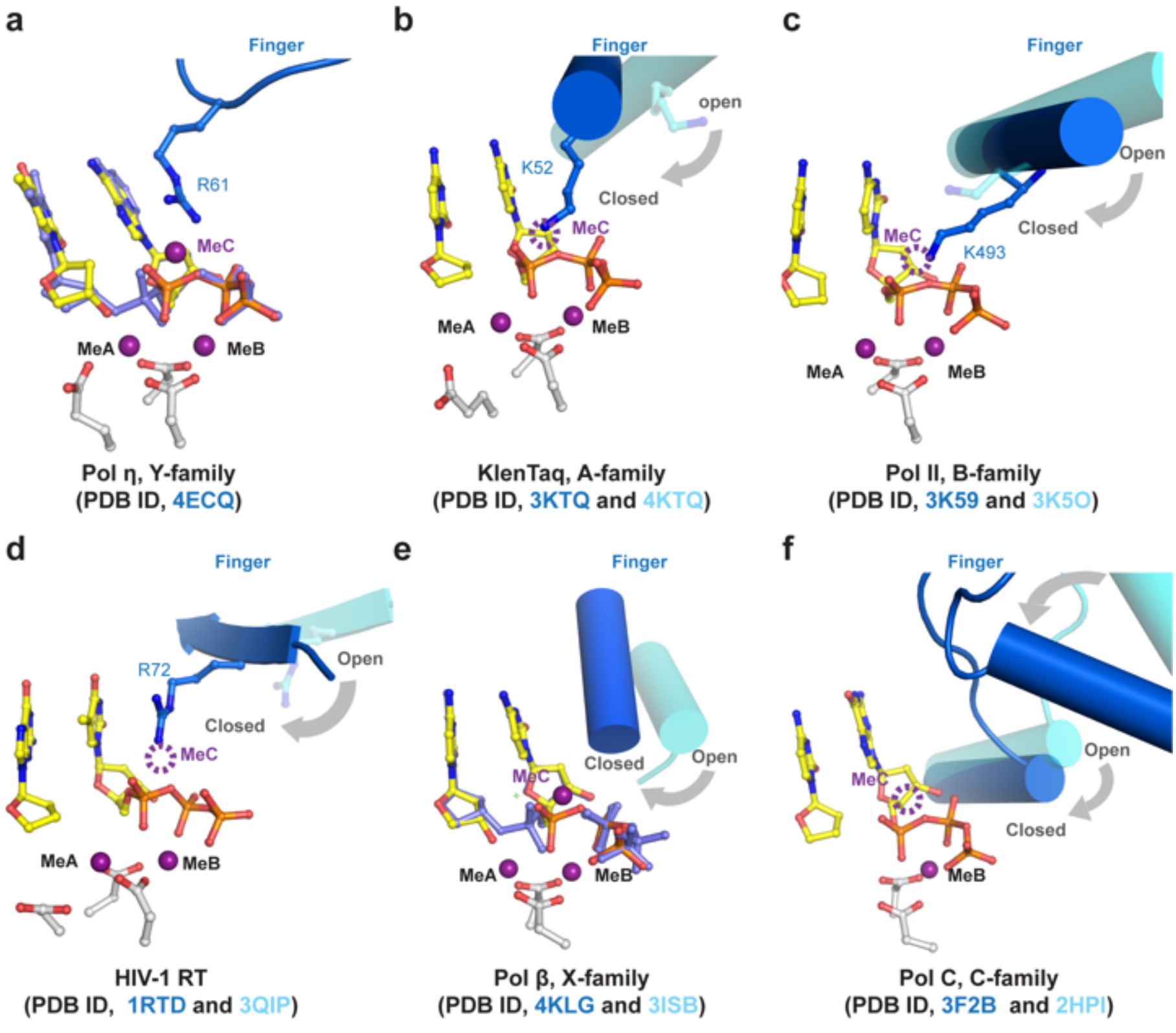

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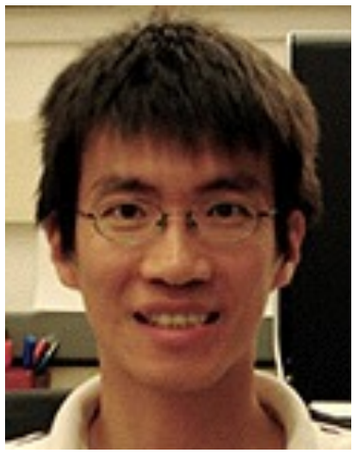

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(Max Planck)

Peter Weng
(NIH)

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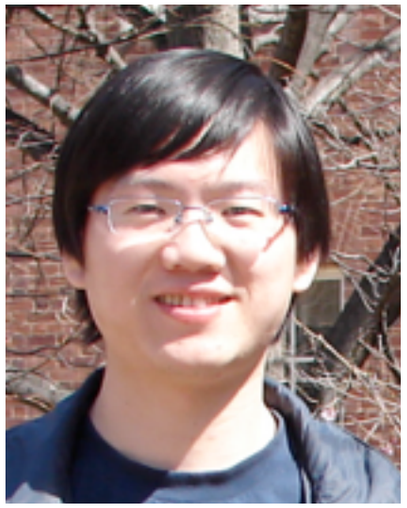
(Kumamoto Univ.)



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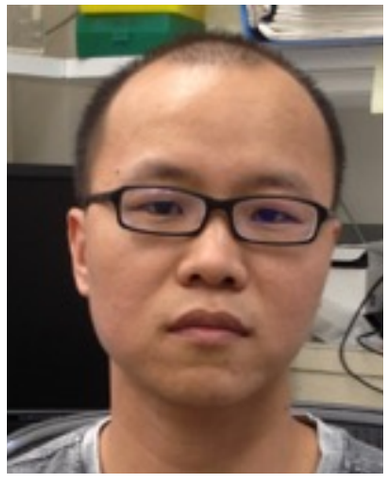

Yang Gao
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NIDDK, NIH, China Scholarship Council,
$$ HFSP, Kumamoto University

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