DNA POLYMERASE I

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INDEX

DNA polymerase

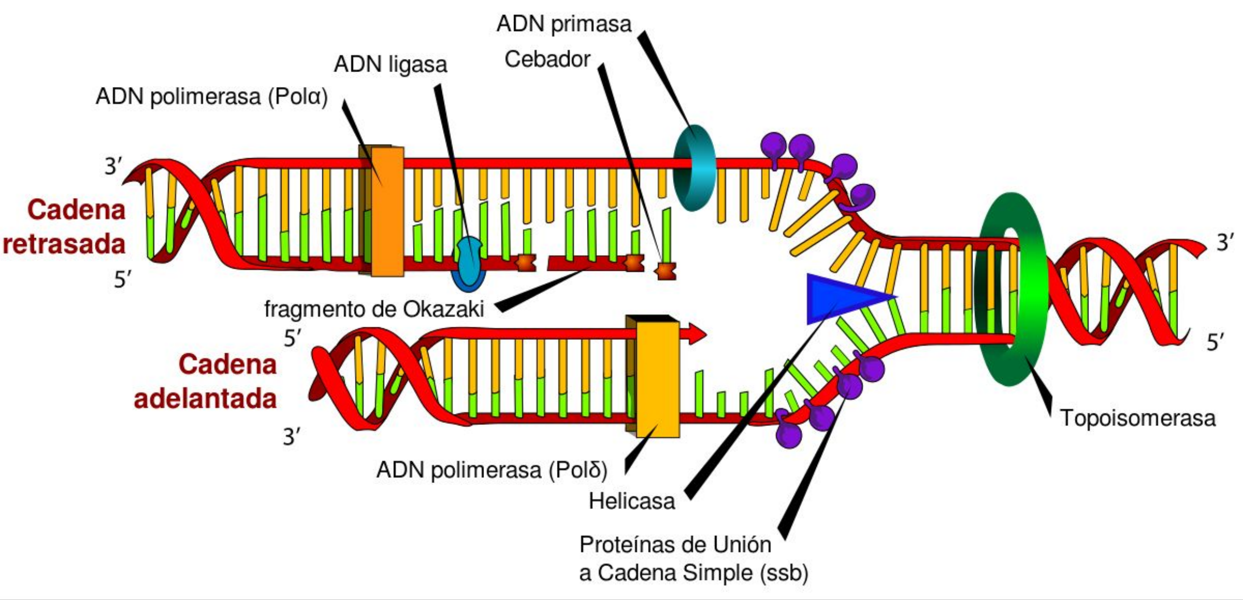
DNA pol I: RNA → DNA

DNA pol III: nucleotide insertion

Error rate: 10^{-6}

Exonuclease activity 3’ → 5’ and 5’ → 3’

Essential survival



Families

A

DNA pol I
Phage T7 and γ polymerase

DNA reparation, Okazaki fragments, exonuclease activity

Mammals : ν, θ

B

Replicative enzymes eukaryotes: δ, ε, α, ζ

Exonuclease 3’ → 5’ and primase activity
Bacteriophage

C

DNA pol III

Bacteria polymerase

D

Euryarchaeota

Mainly replicative

Not clearly defined

X

Small monomeric polymerases → filling small gaps in replication

Polymerase β: BER process

λ, μ pol: V(D)J recombination

σ: sister chromatid cohesion

Y

Injury tolerance pathways → translesion synthesis

DNA distorted structure active site

Polymerase η: UV lesions
Pols κ, Rev 1

DNA polymerase I

One polypeptide chain **928 aa** with molecular weight **109 kDa**

Codifying gene: **polA**

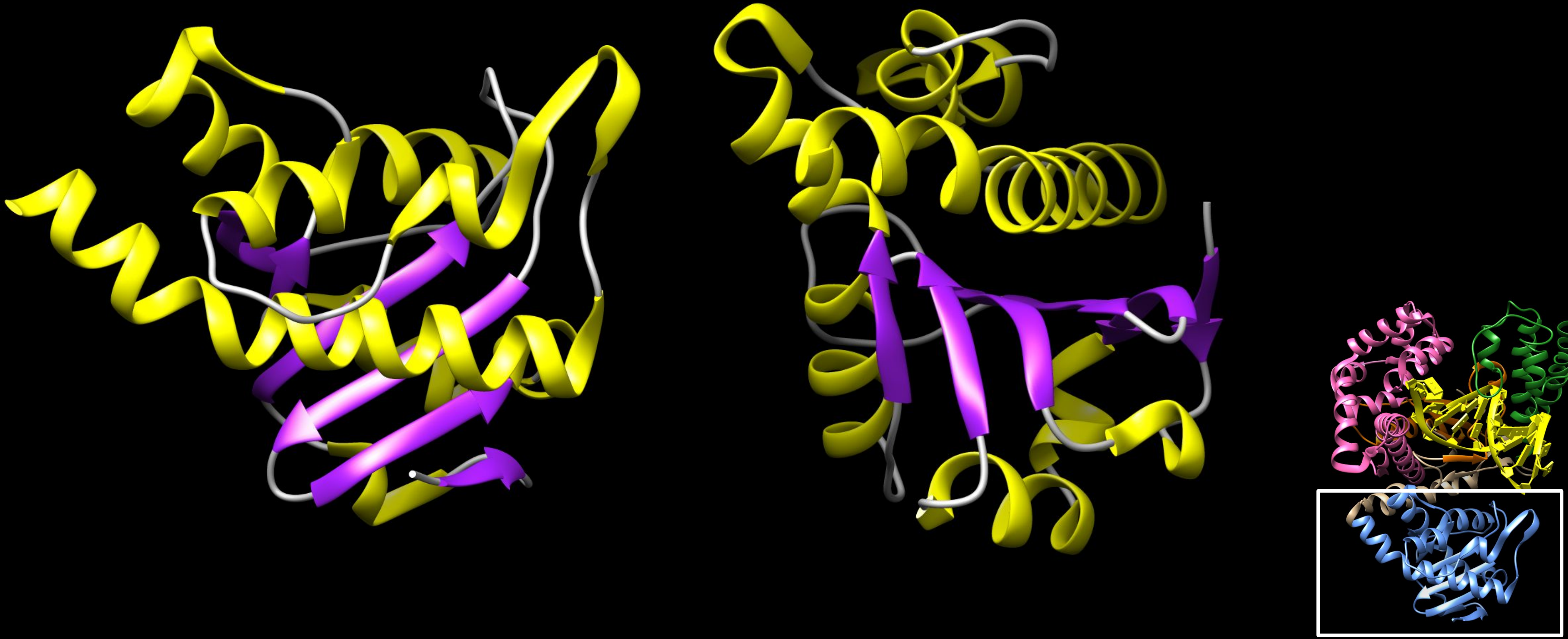
1st polymerase → E.coli (1956)

- 2 domains
  - Large fragment (Klenow)
    - Polymerase and exonuclease 3’ - 5’
    - ↑ fidelity
  - Small fragment
    - Exonuclease 5’ - 3’
    - RNA primer → DNA

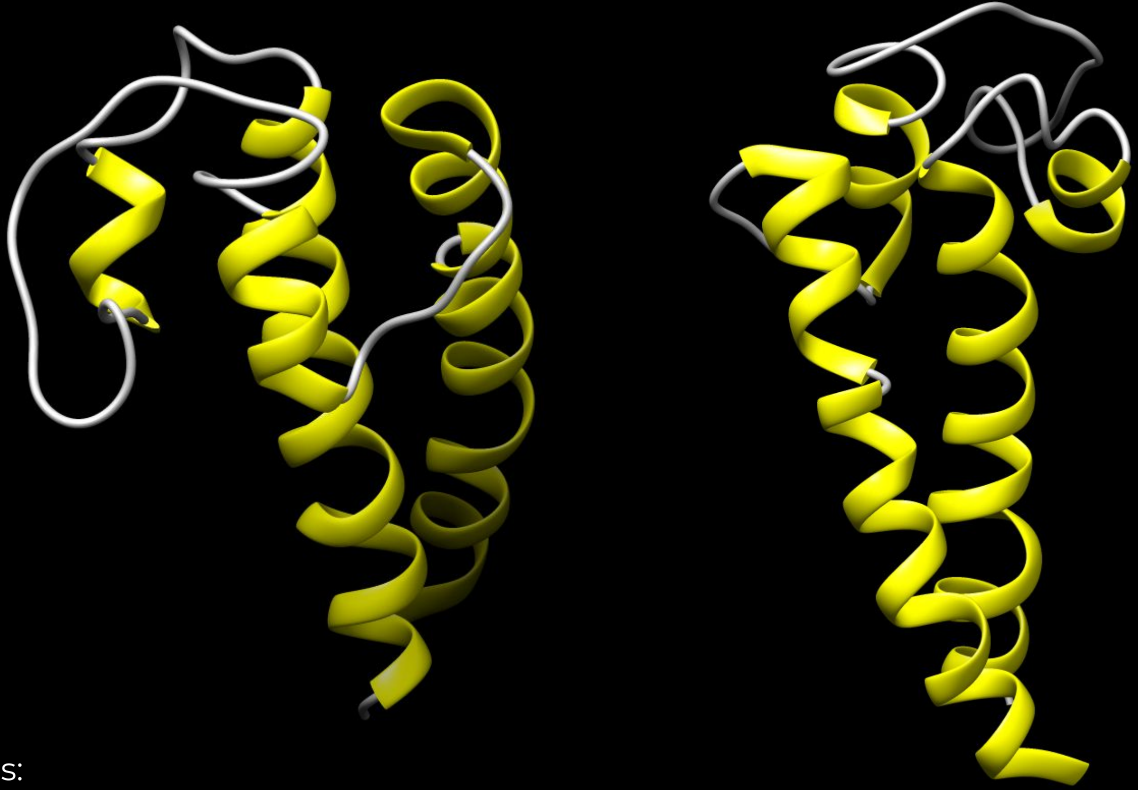
Repair and recombination DNA functions → double stranded DNA

DOMAINS

Exonuclease 3’ → 5’

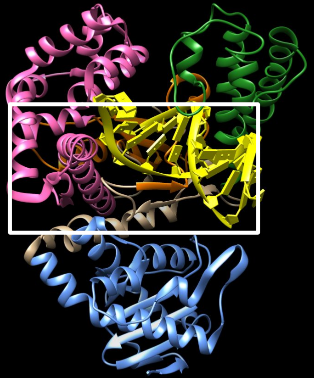


Thumb



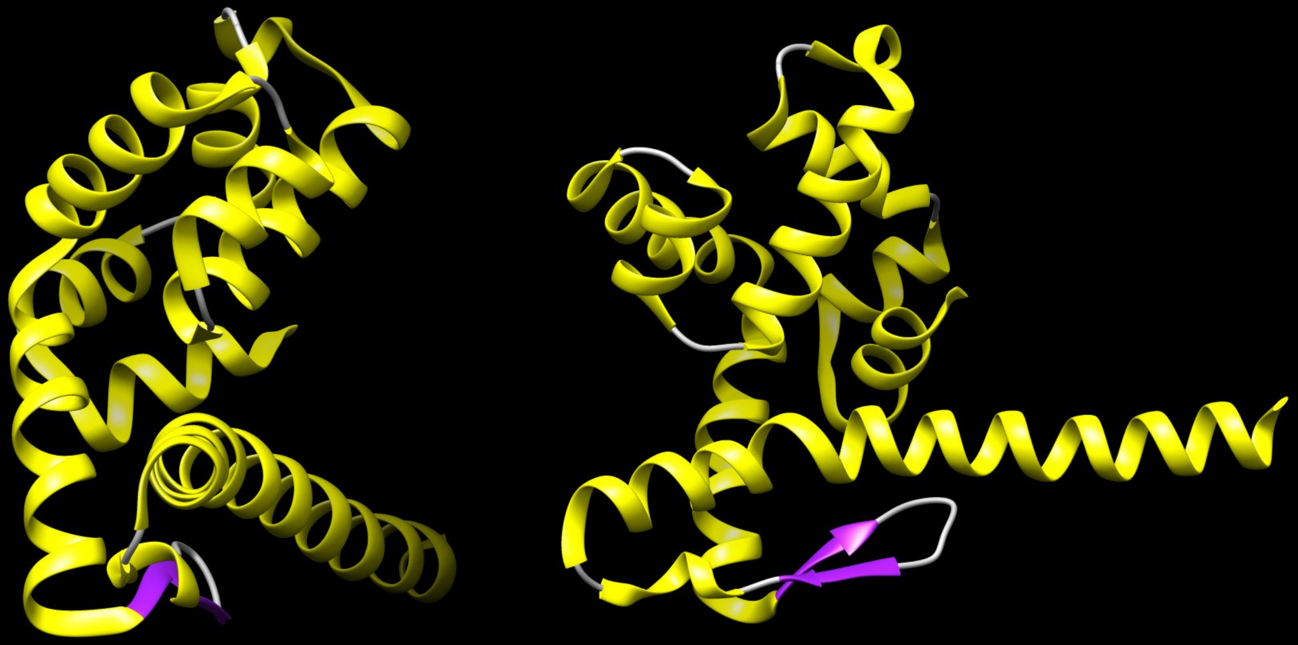
Residues:
496 - 595

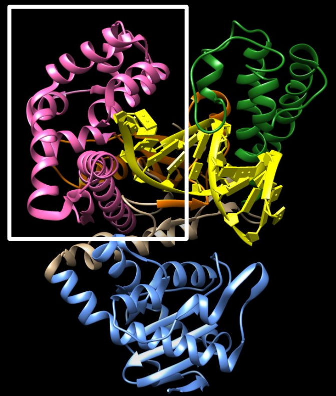
Palm



Residues:
617- 655
830-869

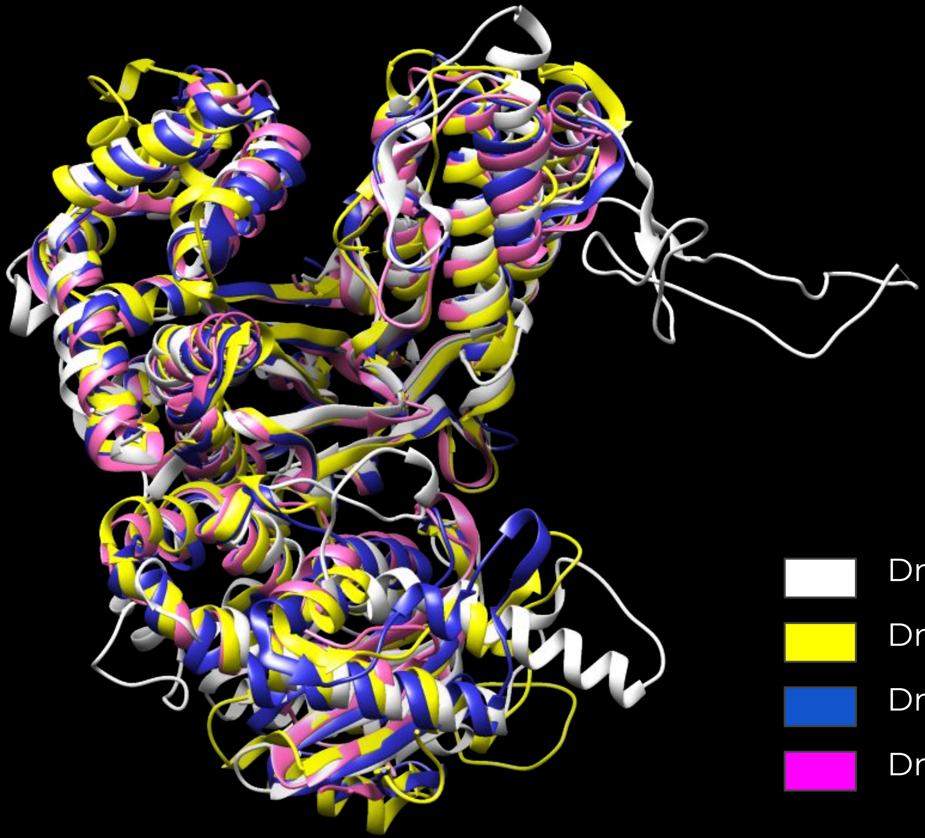
Finger





Residues:
656 - 818

Structural alignment



Sc = 7.092114
RMSD = 2.212980

⬜ Dna Polymerase Phage T7
🟨 Dna Polymerase I *Escherichia coli*
🟦 Dna Polymerase I *Geobacillus streatotermophilus*
🟪 Dna Polymerase I *Thermus aquaticus*

Structural alignment

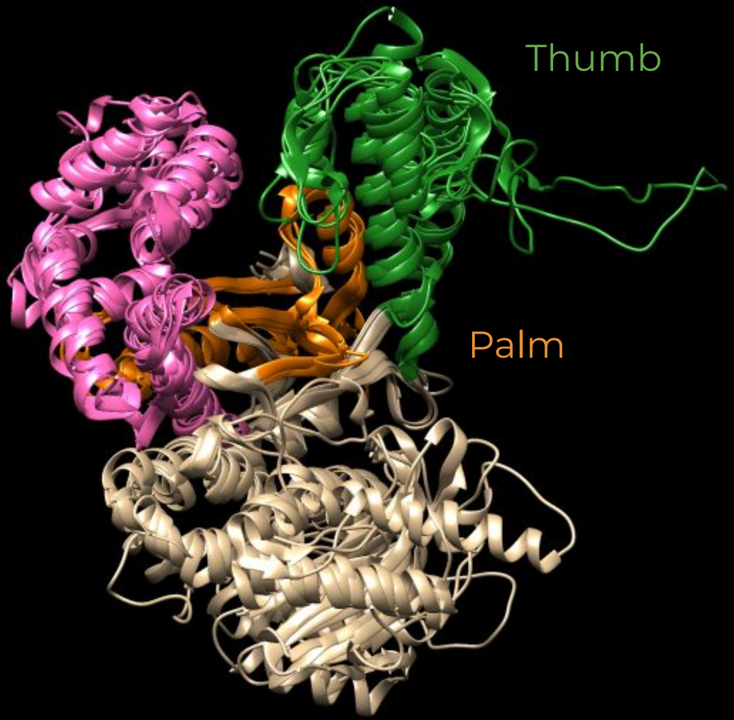
Sc = 8.235916
RMSD = 1.921819

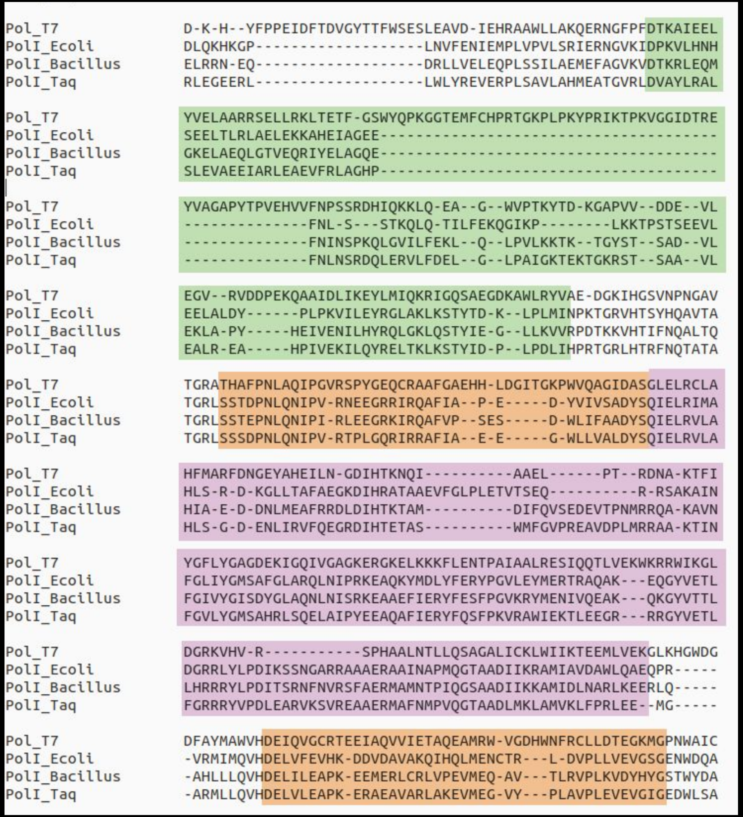
🟨 Dna Polymerase I *Escherichia coli*

🟦 Dna Polymerase I *Geobacillus streatotermophilus*

🟪 Dna Polymerase I *Thermus aquaticus*

Structural alignment: domains





```
Pol_T7          D-K-H--YFPPEIDFTDVGYTTFWSESLEAVD-IEHRAAWLLAKQERNGFPFDTKAIEEL
PolI_Ecoli      DLQKHKGP-------------------LNVFENIEMPLVPVLSRIERNGVKIDPKVLHNH
PolI_Bacillus   ELRRN-EQ-------------------DRLLVELEQPLSSILAEMEFAGVKVDTKRLEQM
PolI_Taq        RLEGEERL-------------------LWLYREVERPLSAVLAHMEATGVRLDVAYLRAL

Pol_T7          YVELAARRSELLRKLTETF-GSWYQPKGGTEMFCHPRTGKPLPKYPRIKTPKVGGIDTRE
PolI_Ecoli      SEELTLRLAELEKKAHEIAGEE--------------------------------------
PolI_Bacillus   GKELAEQLGTVEQRIYELAGQE--------------------------------------
PolI_Taq        SLEVAEEIARLEAEVFRLAGHP--------------------------------------

Pol_T7          YVAGAPYTPVEHVVFNPSSRDHIQKKLQ-EA--G--WVPTKYTD-KGAPVV--DDE--VL
PolI_Ecoli      --------------FNL-S---STKQLQ-TILFEKQGIKP--------LKKTPSTSEEVL
PolI_Bacillus   --------------FNINSPKQLGVILFEKL--Q--LPVLKKTK--TGYST--SAD--VL
PolI_Taq        --------------FNLNSRDQLERVLFDEL--G--LPAIGKTEKTGKRST--SAA--VL

Pol_T7          EGV--RVDDPEKQAAIDLIKEYLMIQKRIGQSAEGDKAWLRYVAE-DGKIHGSVNPNGAV
PolI_Ecoli      EELALDY------PLPKVILEYRGLAKLKSTYTD-K--LPLMINPKTGRVHTSYHQAVTA
PolI_Bacillus   EKLA-PY-----HEIVENILHYRQLGKLQSTYIE-G--LLKVVRPDTKKVHTIFNQALTQ
PolI_Taq        EALR-EA-----HPIVEKILQYRELTKLKSTYID-P--LPDLIHPRTGRLHTRFNQTATA

Pol_T7          TGRATHAFPNLAQIPGVRSPYGEQCRAAFGAEHH-LDGITGKPWVQAGIDASGLELRCLA
PolI_Ecoli      TGRLSSTDPNLQNIPV-RNEEGRRIRQAFIA--P-E-----D-YVIVSADYSQIELRIMA
PolI_Bacillus   TGRLSSTEPNLQNIPI-RLEEGRKIRQAFVP--SES-----D-WLIFAADYSQIELRVLA
PolI_Taq        TGRLSSSDPNLQNIPV-RTPLGQRIRRAFIA--E-E-----G-WLLVALDYSQIELRVLA

Pol_T7          HFMARFDNGEYAHEILN-GDIHTKNQI----------AAEL------PT--RDNA-KTFI
PolI_Ecoli      HLS-R-D-KGLLTAFAEGKDIHRATAAEVFGLPLETVTSEQ----------R-RSAKAIN
PolI_Bacillus   HIA-E-D-DNLMEAFRRDLDIHTKTAM----------DIFQVSEDEVTPNMRRQA-KAVN
PolI_Taq        HLS-G-D-ENLIRVFQEGRDIHTETAS----------WMFGVPREAVDPLMRRAA-KTIN

Pol_T7          YGFLYGAGDEKIGQIVGAGKERGKELKKKFLENTPAIAALRESIQQTLVEKWKRRWIKGL
PolI_Ecoli      FGLIYGMSAFGLARQLNIPRKEAQKYMDLYFERYPGVLEYMERTRAQAK---EQGYVETL
PolI_Bacillus   FGIVYGISDYGLAQNLNISRKEAAEFIERYFESFPGVKRYMENIVQEAK---QKGYVTTL
PolI_Taq        FGVLYGMSAHRLSQELAIPYEEAQAFIERYFQSFPKVRAWIEKTLEEGR---RRGYVETL

Pol_T7          DGRKVHV-R-----------SPHAALNTLLQSAGALICKLWIIKTEEMLVEKGLKHGWDG
PolI_Ecoli      DGRRLYLPDIKSSNGARRAAAERAAINAPMQGTAADIIKRAMIAVDAWLQAEQPR-----
PolI_Bacillus   LHRRRYLPDITSRNFNVRSFAERMAMNTPIQGSAADIIKKAMIDLNARLKEERLQ-----
PolI_Taq        FGRRRYVPDLEARVKSVREAAERMAFNMPVQGTAADLMKLAMVKLFPRLEE--MG-----

Pol_T7          DFAYMAWVHDEIQVGCRTEEIAQVVIETAQEAMRW-VGDHWNFRCLLDTEGKMGPNWAIC
PolI_Ecoli      -VRMIMQVHDELVFEVHK-DDVDAVAKQIHQLMENCTR---L-DVPLLVEVGSGENWDQA
PolI_Bacillus   -AHLLLQVHDELILEAPK-EEMERLCRLVPEVMEQ-AV---TLRVPLKVDYHYGSTWYDA
PolI_Taq        -ARMLLQVHDELVLEAPK-ERAEAVARLAKEVMEG-VY---PLAVPLEVEVGIGEDWLSA
```

Function of the DNA Pol I

Bacillus stearothermophilus

Steps of the Process

$$\begin{tikzcd}
E_o + \text{DNA} \arrow[d, leftrightarrow] \\
E_o \cdot \text{DNA} \arrow[d, leftrightarrow, "\text{dNTP-Mg}^{2+}"'] \\
E_{PC} \cdot \text{DNA} \cdot \text{dNTP-Mg}^{2+} \arrow[d, leftrightarrow] \\
E_{PC} \cdot \text{DNA}^{*} \cdot \text{dNTP-Mg}^{2+} \arrow[d, leftrightarrow, "\text{Fingers-closing}"'] \\
E_C \cdot \text{DNA}^{*} \cdot \text{dNTP-Mg}^{2+} \arrow[d, leftrightarrow, "\text{Mg}^{2+}"'] \\
E_C \cdot \text{DNA}^{*}\text{dNTP-}(\text{Mg}^{2+})_2
\end{tikzcd}$$

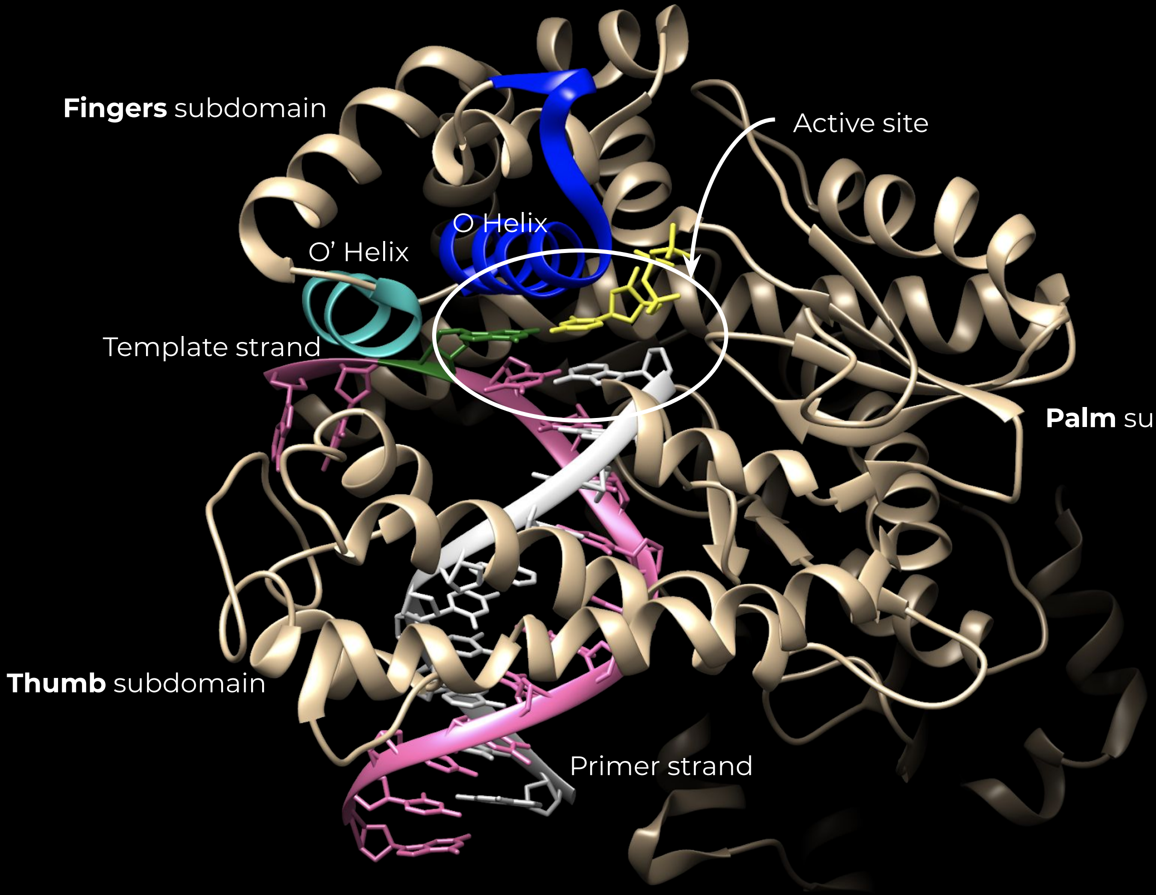
Conformational change of the DNA

Binary state

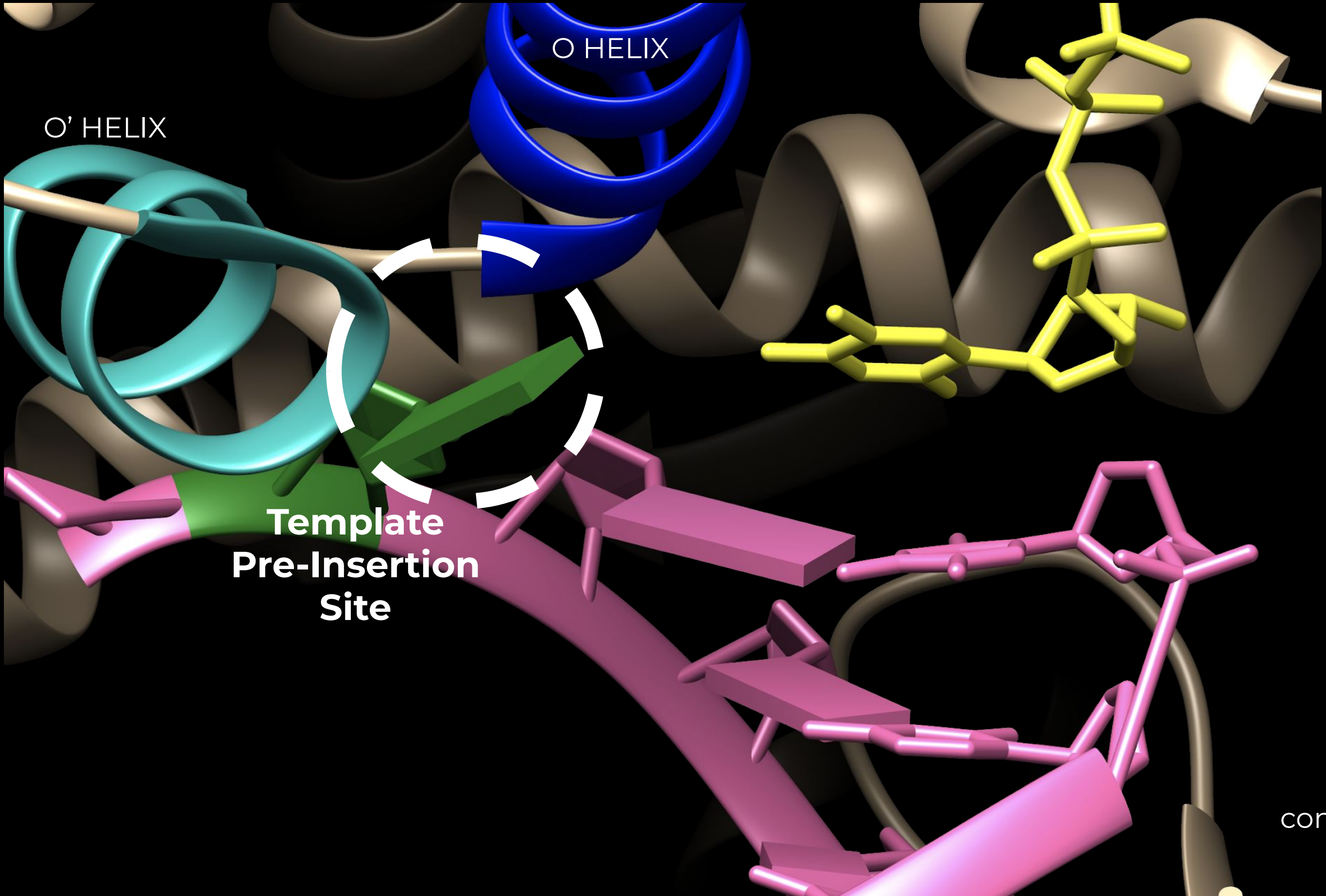
Ternary state

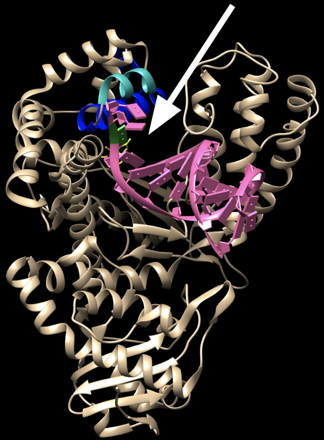
Miller B, Beese L, Parish C, Wu E. (2015)
Structure 23, 1609-1620. Elsevier.

General View



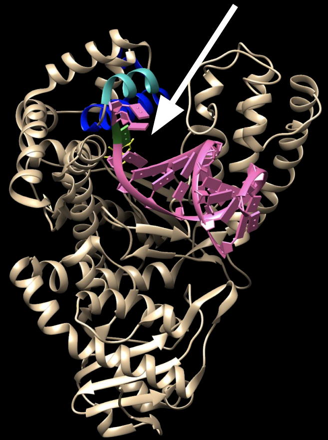
SITES : Protein/DNA interaction

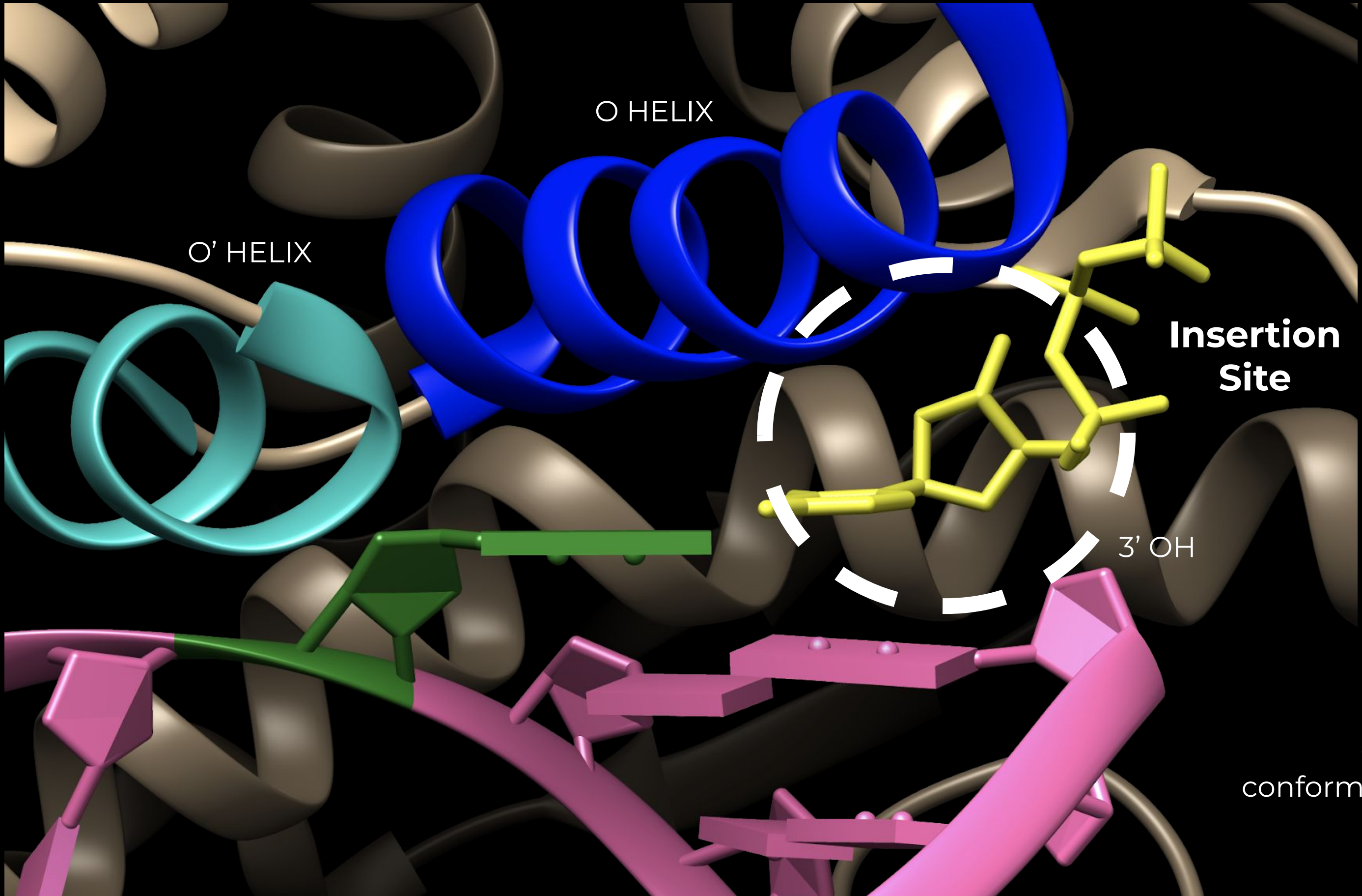




Open
conformation

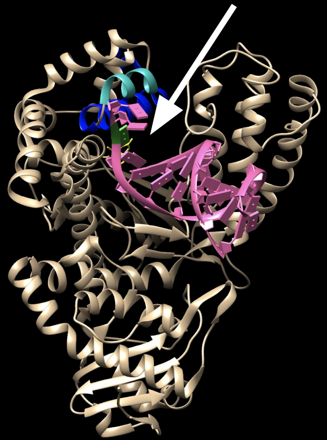
SITES : Protein/DNA interaction

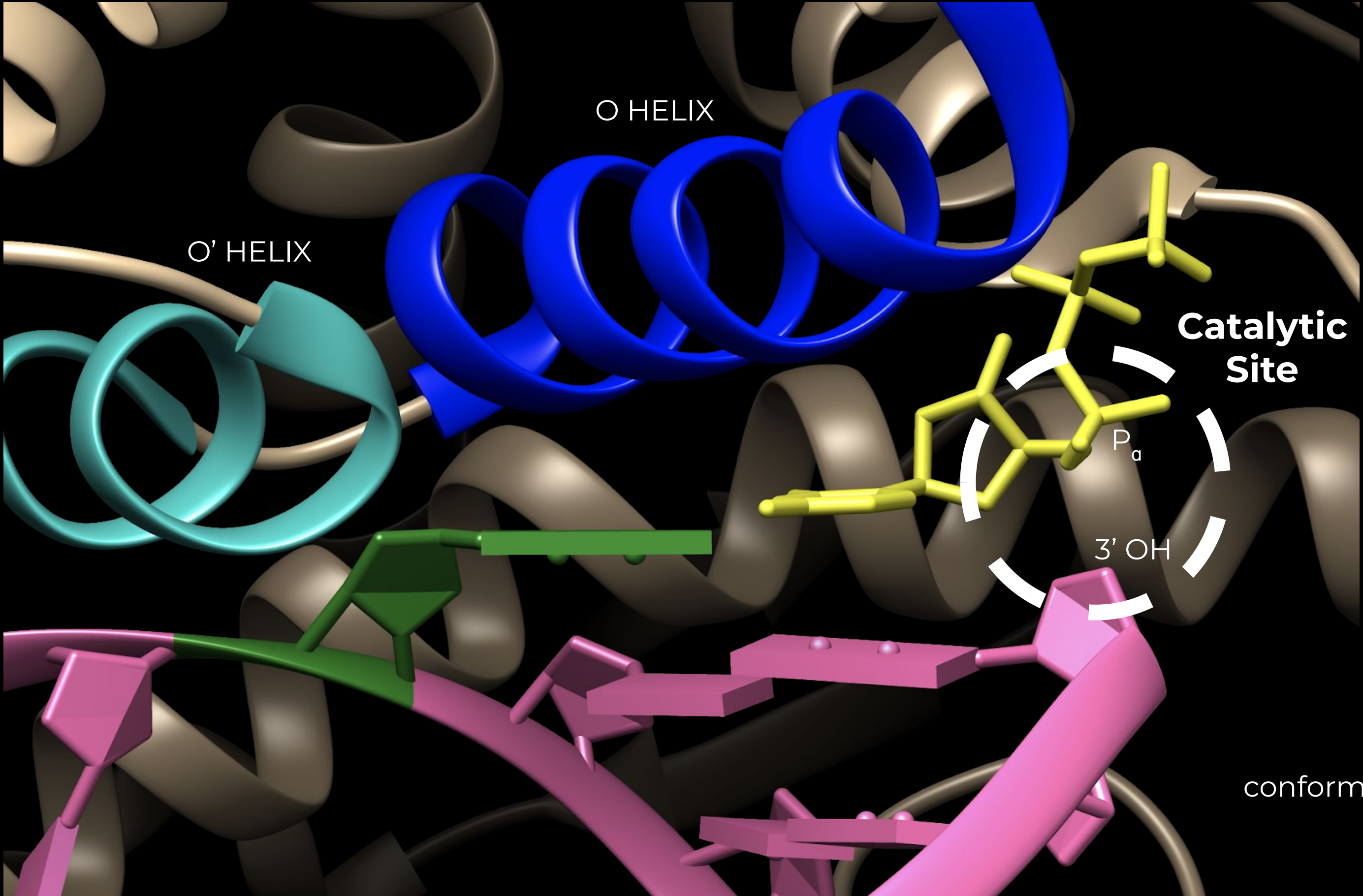




Close conformation

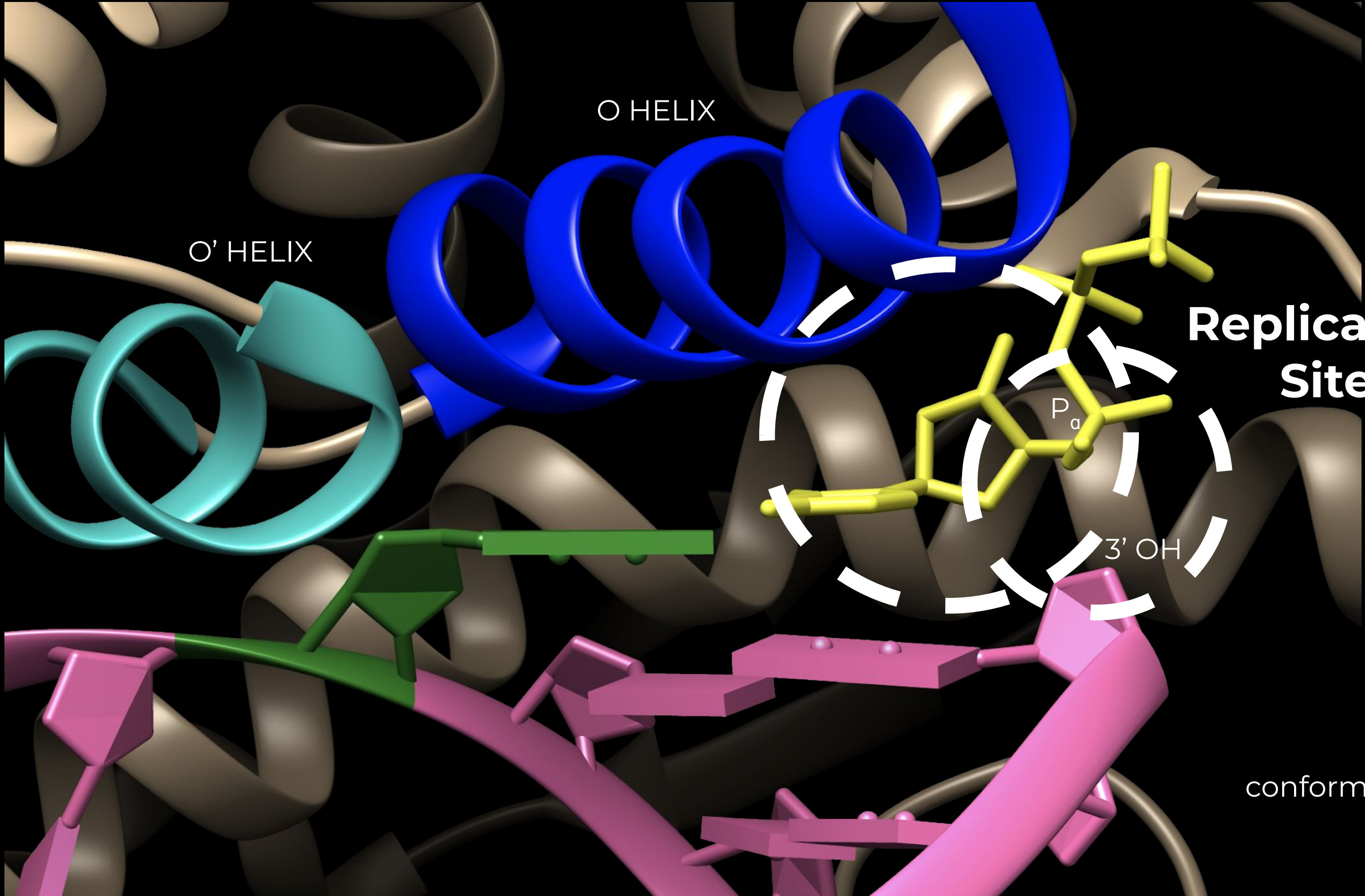
SITES : Protein/DNA interaction





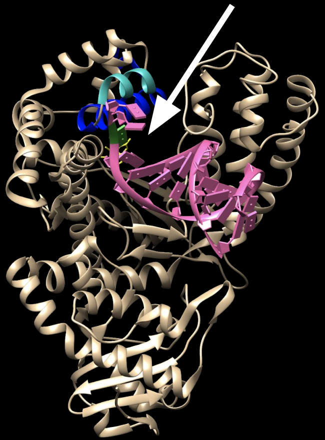
Close conformation

SITES : Protein/DNA interaction

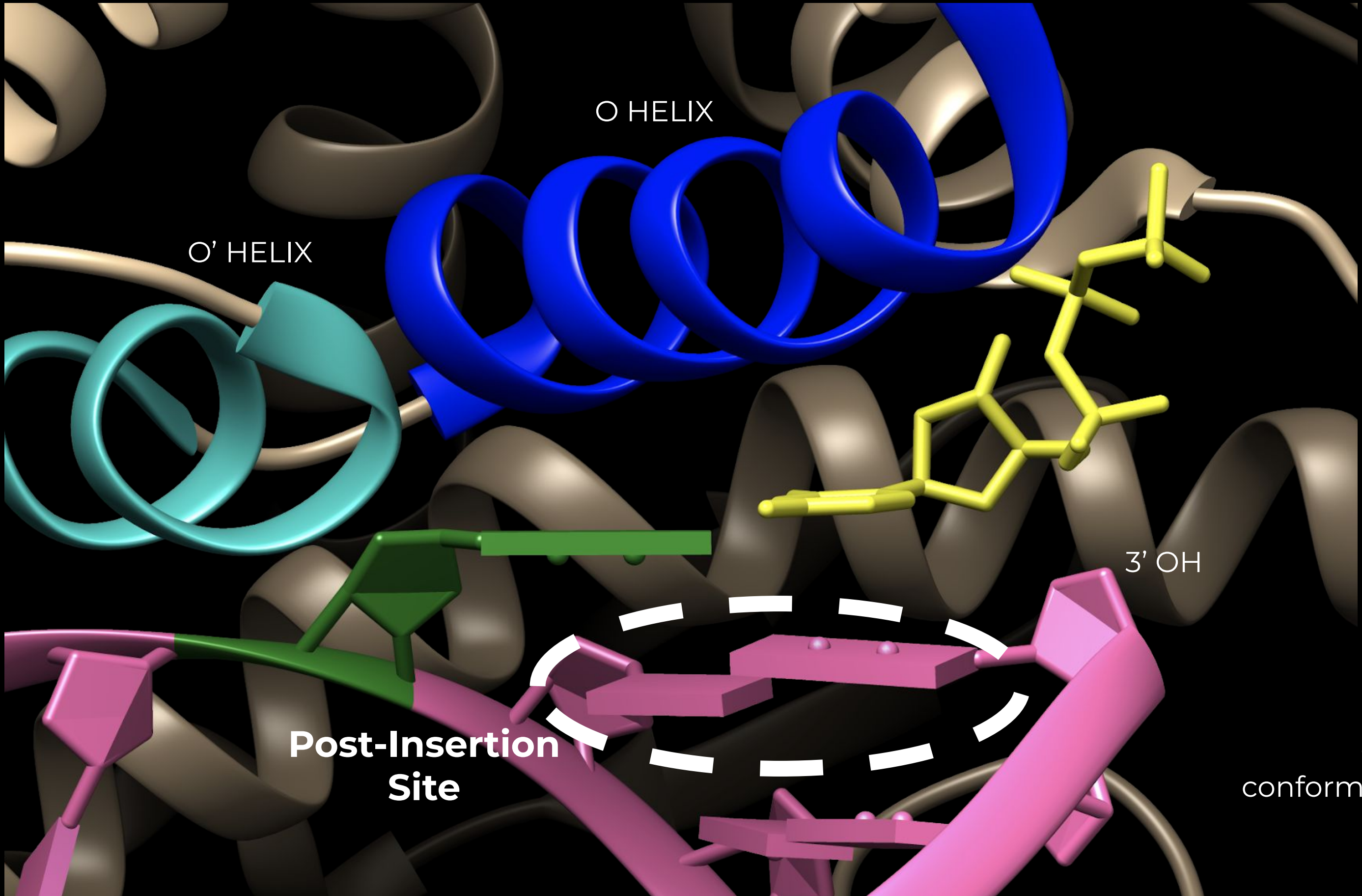


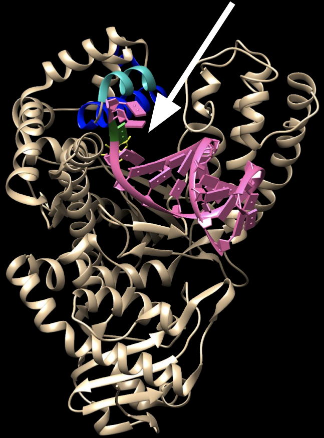
Replication Site

Close conformation



SITES : Protein/DNA interaction

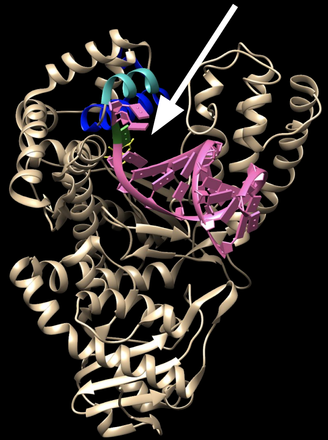


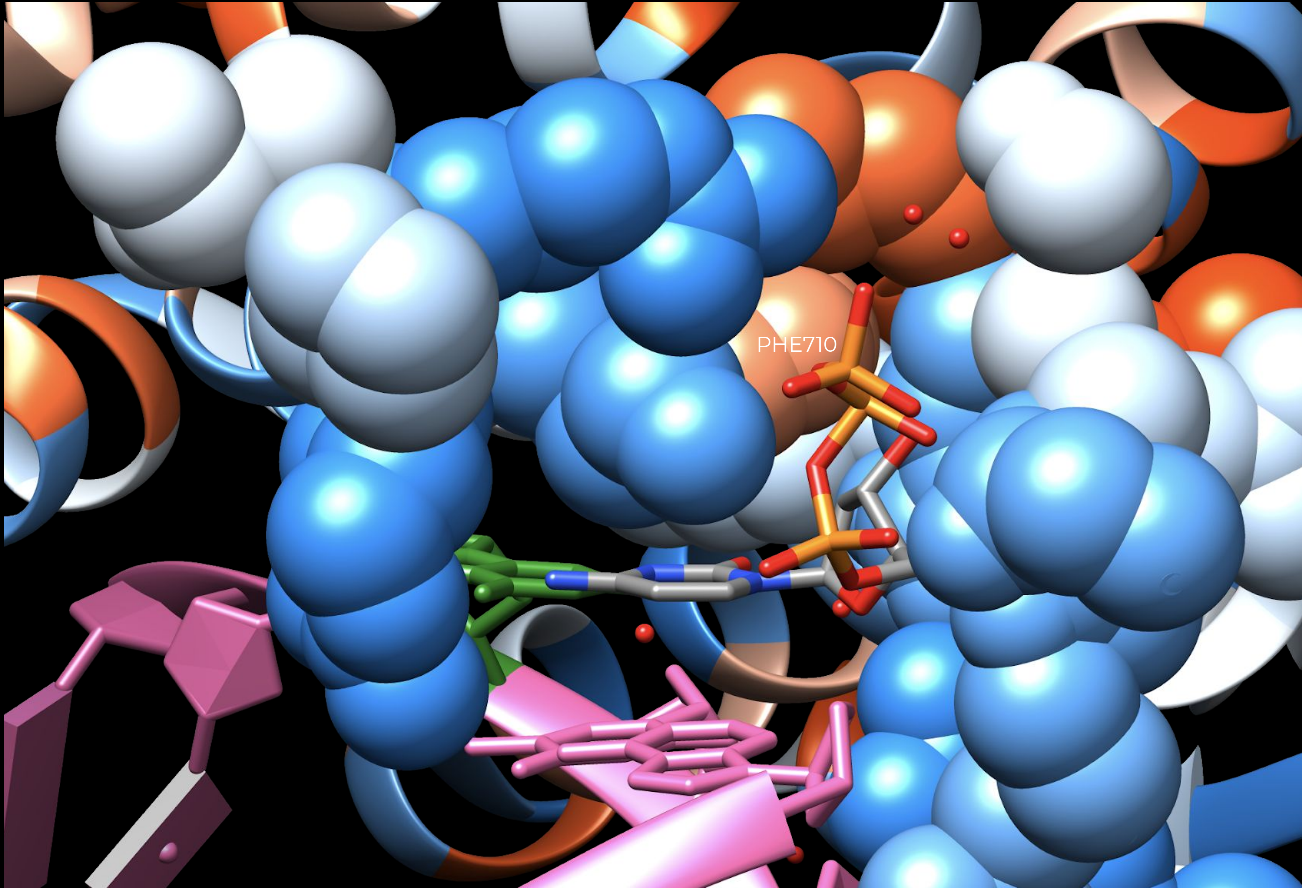


Close conformation

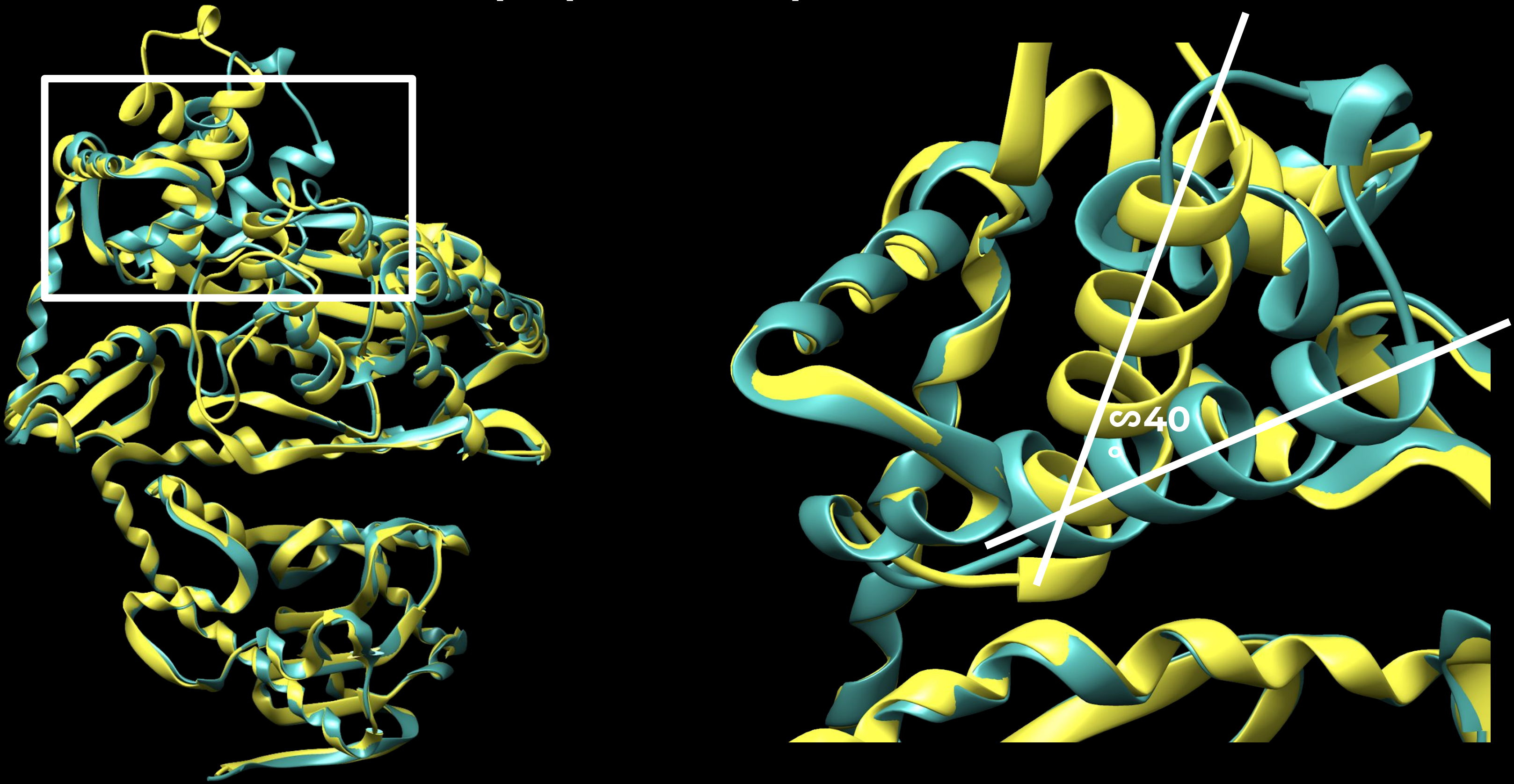
Hydrophilic Active Site

- The active site presents a mostly hydrophilic center





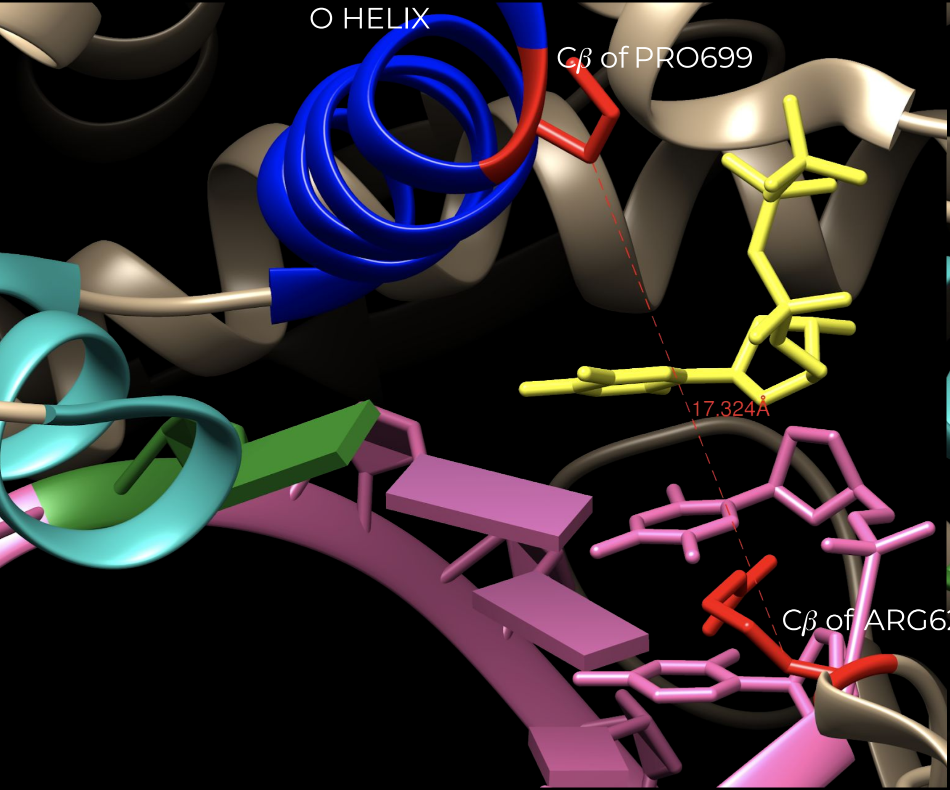
Superposition: Open - Close



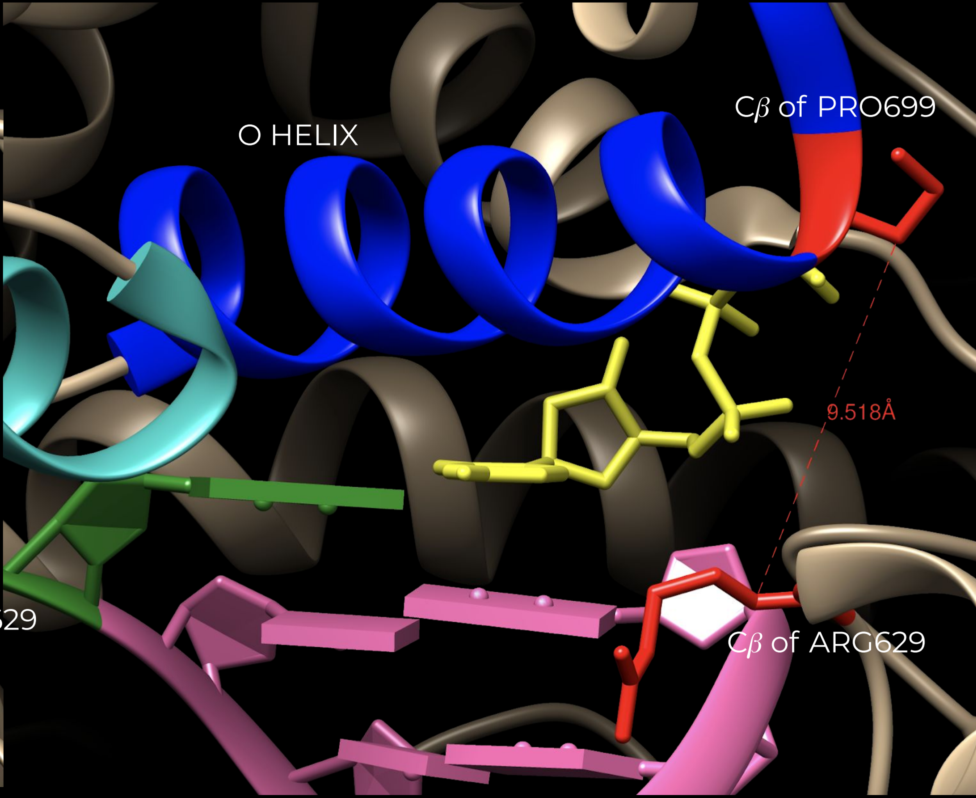
Close conformation: blue
Open conformation: yellow

Differences between Open and Close

Open conformation

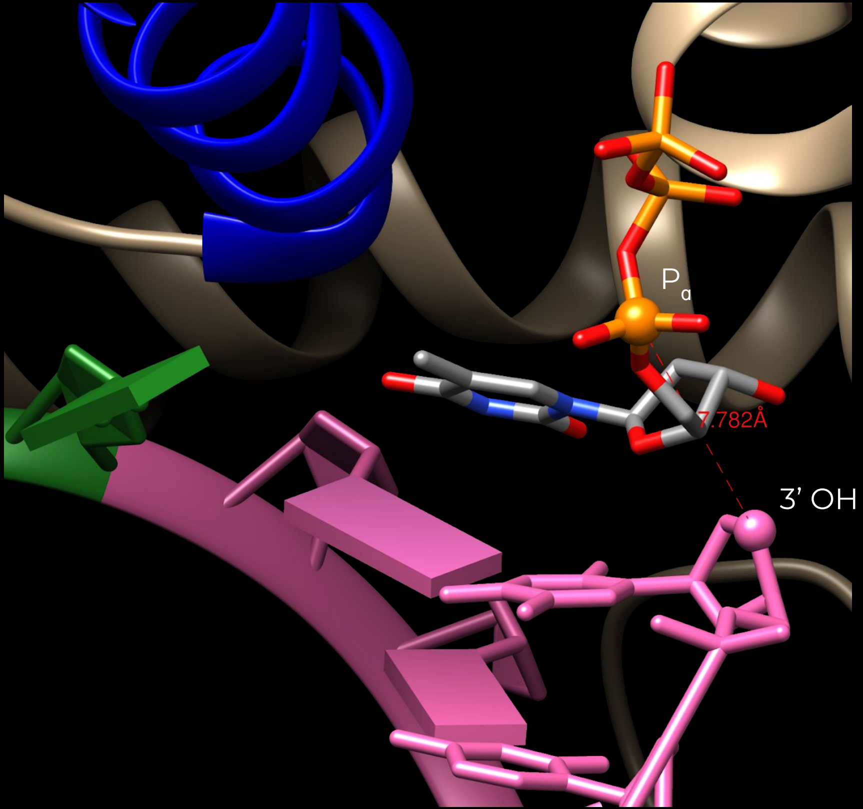


Close conformation

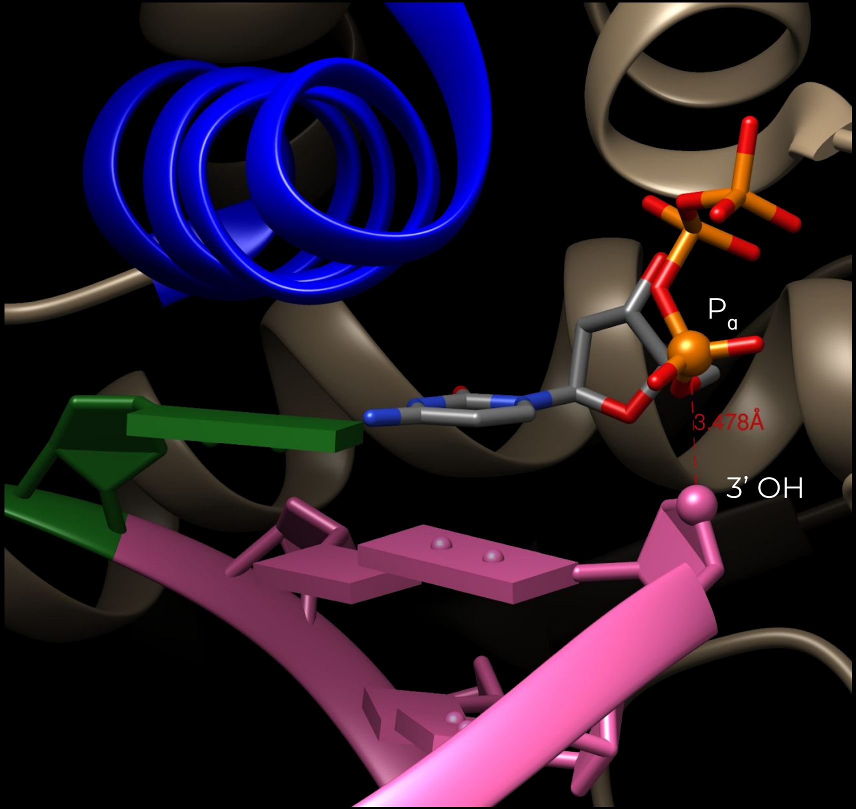


Differences between Open and Close

Open conformation

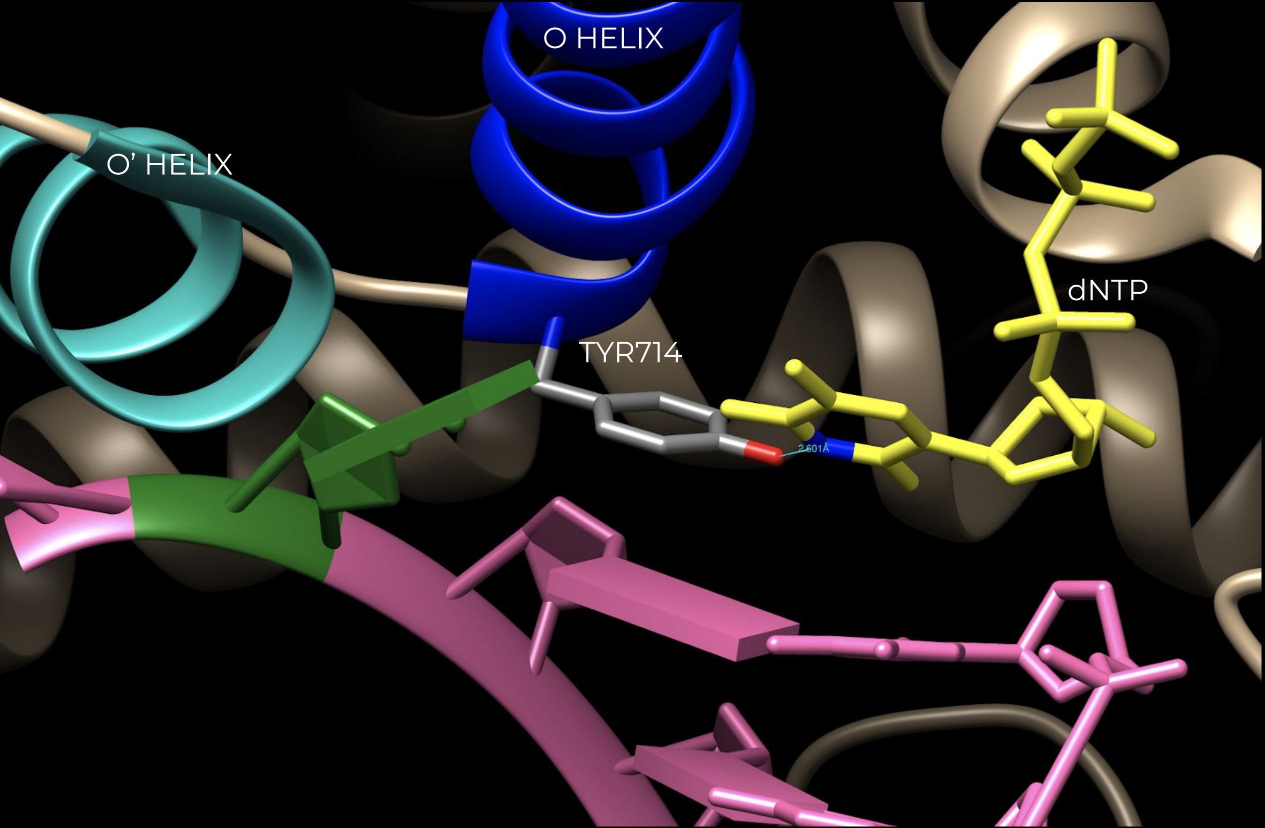


Close conformation



OPEN Conformation

Residue Tyr714



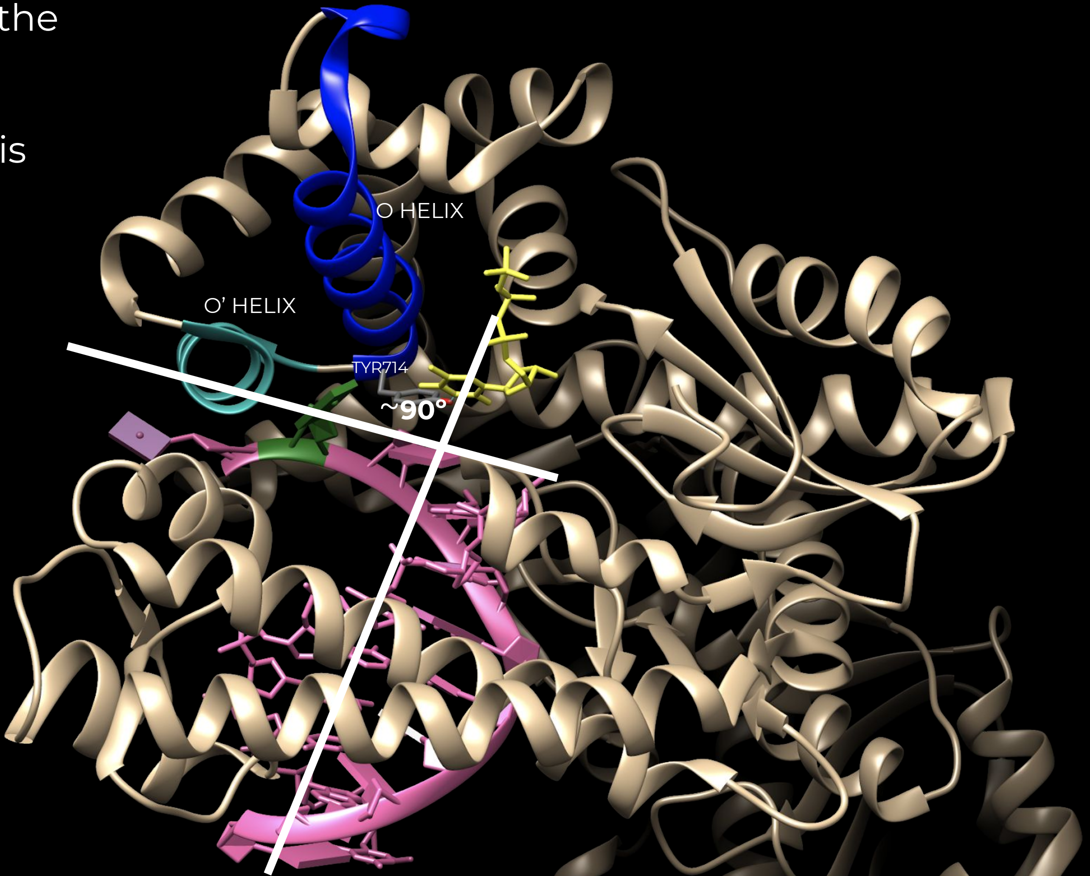
- Tyr714 is at the same position predicted for the acceptor base (T)
- Hydrogen Bond with the N of the base (P)

Residues of the loop

```
sp|Q9RAA9|DPO1_RICFE      ACQIFNLQK-HELTSEHRRKAKAINFGIIYGISAFGLAKQLNVS------
sp|P56105|DPO1_HELPY      SKALFG----EYLAKEKRSIAKSINFGLVYGMGSKKLSETLNIS------
sp|P74933|DPO1_TREPA      AAYIFNVSI-DDVQPAMRRIAKTINFGIVYGMSAFRLSDELKIS------
sp|O51498|DPO1_BORBU      ASKLFKIEE-KEITPNLRRIAKSINFGIIYRMSDFRLAKELGIT------
sp|P52026|DPO1_GEOSE      AMDIFHVSE-EDVTANMRRQAKAVNFGIVYGISDYGLAQNLNIT------
sp|Q04957|DPO1_BACCA      AMDIFQVSE-DEVTPNMRRQAKAVNFGIVYGISDYGLAQNLNIS------
sp|O32801|DPO1_LACLM      AMRVFGIEKAEDVTANDRRNAKAVNFGVVYGISDFGLARNLGIT------
sp|P59200|DPO1_STRR6      AMRVFGIERPDNVTANDRRNAKAVNFGVVYGISDFGLSNNLGIS------
sp|Q59156|DPO1_ANATD      AAEVFGVDI-ADVTPEMRSQAKAVNFGIVYGISDYGLARDIKIS------
sp|P46835|DPO1_MYCLE      ASRAFGIPI-EDITPELRRRVKAMSYGLAYGLSAYGLATQLKIS------
sp|P00582|DPO1_ECOLI      AAEVFGLPL-ETVTSEQRRSAKAINFGLIYGMSAFGLARQLNIP------
sp|Q9F173|DPO1_SALTY      AAEVFGLPL-DSVTGEQRRSAKAINFGLIYGMSAFGLSRQLNIP------
sp|P43741|DPO1_HAEIN      AAEIFGVSL-DEVTSEQRRNAKAINFGLIYGMSAFGLSRQLGIS------
sp|Q9HT80|DPO1_PSEAE      AAEVFGVPL-EDVSGDQRRSAKAINFGLIYGMSAFGLAKQIGVE------
sp|Q9S1G2|DPO1_RHILE      ASEMFGVPV-EGMPGEVRRRAKAINFGIIYGISAFGLANQLSIE------
sp|P19821|DPO1_THEAQ      ASWMFGVPR-EAVDPLMRRAAKTINFGVLYGMSAHRLSQELAIP------
sp|P52027|DPO1_DEIRA      AAQVLGLDE-ATVDANQRRAAKTVNFGVLYGMSAHRLSNDLGIP------
sp|O08307|DPO1_CHLAA      ASRLFGVEP-TAVDKNQRRVAKTVVFGVIYGISAFGLAQRLGIE------
sp|Q55971|DPO1_SYNY3      AKLLFGKED---ITPAERNLGKTINFGVIYGMGAQRFARETGIS------
sp|Q7TQ07|DPOLN_MOUSE     LTSQWKDIPIERVTHMDREQTKKVVYSVVYGAGKERLAACLGVT------
sp|Q7Z5Q5|DPOLN_HUMAN     LTSQWKDVPVEQVTHADREQTKKVVYAVVYGAGKERLAACLGVP------
sp|O75417|DPOLQ_HUMAN     AAEWKMIEP-ESVGDDLRQQAKQICYGIIYGMGAKSLGEQMGIK------
sp|P00581|DPOL_BPT7       LNGDIHTKNQIAAELPTRDNAKTFIYGFLYGAGDEKIGQIVGAG------
```

Residue Tyr714

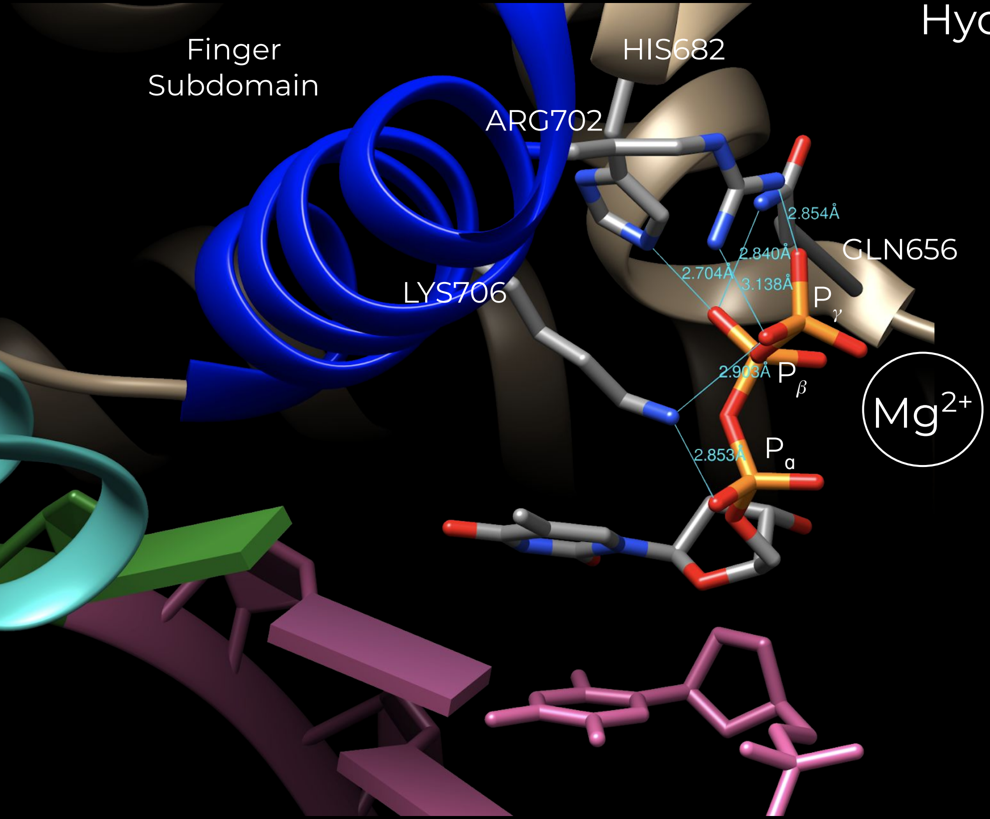
The overhang of the template strand turns ~90° in relation to the axis of the duplex



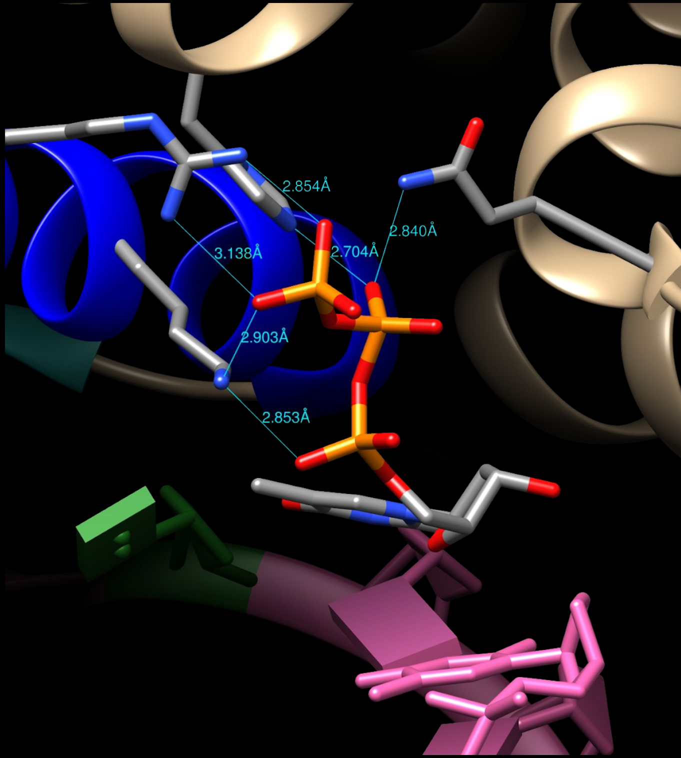
Residue Tyr714

```
sp|Q9RAA9|DPO1_RICFE       ACQIFNLQK-HELTSEHRRKAKAINFGIIYGISAFGLAKQLNVS------
sp|P56105|DPO1_HELPY       SKALFG----EYLAKEKRSIAKSINFGLVYGMGSKKLSETLNIS------
sp|P74933|DPO1_TREPA       AAYIFNVSI-DDVQPAMRRIAKTINFGIVYGMSAFRLSDELKIS------
sp|O51498|DPO1_BORBU       ASKLFKIEE-KEITPNLRRIAKSINFGIIYRMSDFRLAKELGIT------
sp|P52026|DPO1_GEOSE       AMDIFHVSE-EDVTANMRRQAKAVNFGIVYGISDYGLAQNLNIT------
sp|Q04957|DPO1_BACCA       AMDIFQVSE-DEVTPNMRRQAKAVNFGIVYGISDYGLAQNLNIS------
sp|O32801|DPO1_LACLM       AMRVFGIEKAEDVTANDRRNAKAVNFGVVYGISDFGLARNLGIT------
sp|P59200|DPO1_STRR6       AMRVFGIERPDNVTANDRRNAKAVNFGVVYGISDFGLSNNLGIS------
sp|Q59156|DPO1_ANATD       AAEVFGVDI-ADVTPEMRSQAKAVNFGIVYGISDYGLARDIKIS------
sp|P46835|DPO1_MYCLE       ASRAFGIPI-EDITPELRRRVKAMSYGLAYGLSAYGLATQLKIS------
sp|P00582|DPO1_ECOLI       AAEVFGLPL-ETVTSEQRRSAKAINFGLIYGMSAFGLARQLNIP------
sp|Q9F173|DPO1_SALTY       AAEVFGLPL-DSVTGEQRRSAKAINFGLIYGMSAFGLSRQLNIP------
sp|P43741|DPO1_HAEIN       AAEIFGVSL-DEVTSEQRRNAKAINFGLIYGMSAFGLSRQLGIS------
sp|Q9HT80|DPO1_PSEAE       AAEVFGVPL-EDVSGDQRRSAKAINFGLIYGMSAFGLAKQIGVE------
sp|Q9S1G2|DPO1_RHILE       ASEMFGVPV-EGMPGEVRRRAKAINFGIIYGISAFGLANQLSIE------
sp|P19821|DPO1_THEAQ       ASWMFGVPR-EAVDPLMRRAAKTINFGVLYGMSAHRLSQELAIP------
sp|P52027|DPO1_DEIRA       AAQVLGLDE-ATVDANQRRAAKTVNFGVLYGMSAHRLSNDLGIP------
sp|O08307|DPO1_CHLAA       ASRLFGVEP-TAVDKNQRRVAKTVVFGVIYGISAFGLAQRLGIE------
sp|Q55971|DPO1_SYNY3       AKLLFGKED---ITPAERNLGKTINFGVIYGMGAQRFARETGIS------
sp|Q7TQ07|DPOLN_MOUSE      LTSQWKDIPIERVTHMDREQTKKVVYSVVYGAGKERLAACLGVT------
sp|Q7Z5Q5|DPOLN_HUMAN      LTSQWKDVPVEQVTHADREQTKKVVYAVVYGAGKERLAACLGVP------
sp|O75417|DPOLQ_HUMAN      AAEWKMIEP-ESVGDDLRQQAKQICYGIIYGMGAKSLGEQMGIK------
sp|P00581|DPOL_BPT7        LNGDIHTKNQIAAELPTRDNAKTFIYGFLYGAGDEKIGQIVGAG------
```

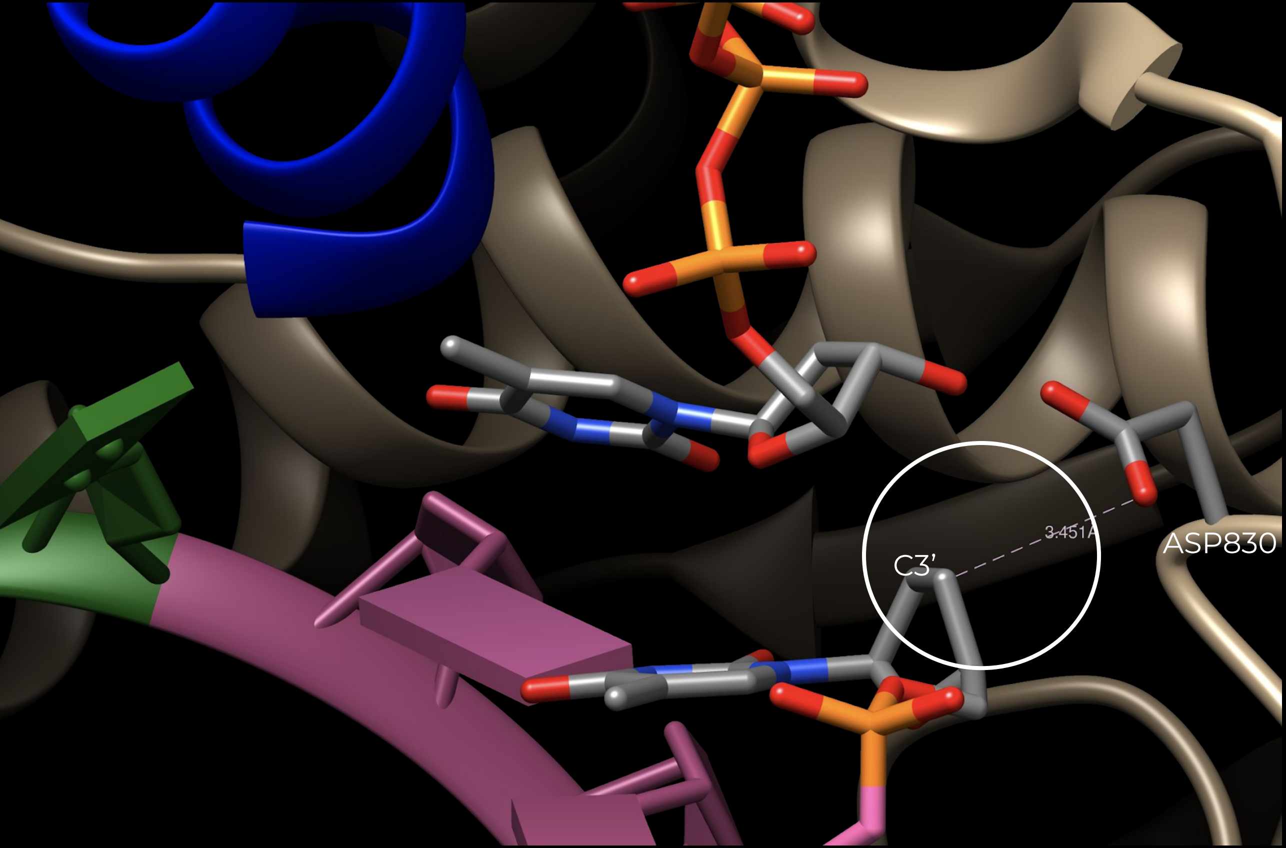
Phosphates of dNTP and O Helix



Hydrogen bonds

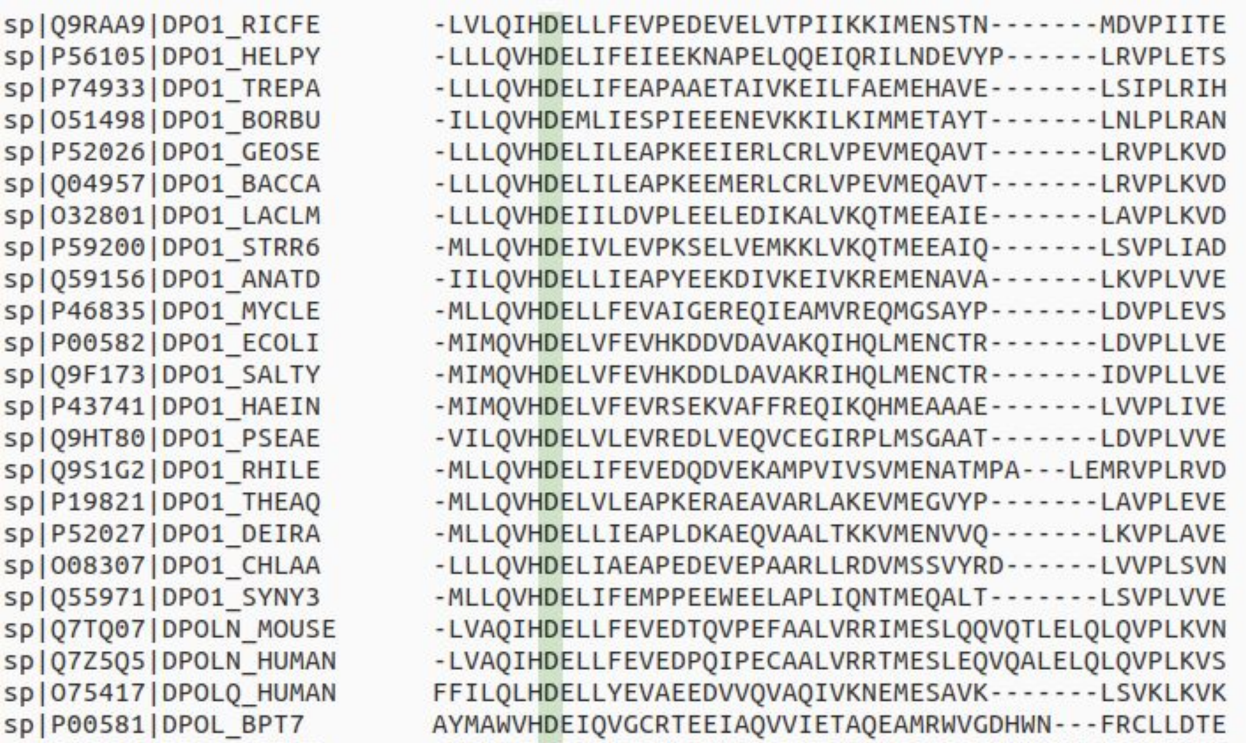


3’ OH “hidden” by Asp830



- A ddNTP was used at the end of the primer strand
- Asp830 avoids phosphodiester bonds in open conformation

Residue Asp830



```
sp|Q9RAA9|DPO1_RICFE       -LVLQIHDELLFEVPEDEVELVTPIIKKIMENSTN-------MDVPIITE
sp|P56105|DPO1_HELPY       -LLLQVHDELIFEIEEKNAPELQQEIQRILNDEVYP------LRVPLETS
sp|P74933|DPO1_TREPA       -LLLQVHDELIFEAPAAETAIVKEILFAEMEHAVE-------LSIPLRIH
sp|O51498|DPO1_BORBU       -ILLQVHDEMLIESPIEEENEVKKILKIMMETAYT-------LNLPLRAN
sp|P52026|DPO1_GEOSE       -LLLQVHDELILEAPKEEIERLCRLVPEVMEQAVT-------LRVPLKVD
sp|Q04957|DPO1_BACCA       -LLLQVHDELILEAPKEEMERLCRLVPEVMEQAVT-------LRVPLKVD
sp|O32801|DPO1_LACLM       -LLLQVHDEIILDVPLEELEDIKALVKQTMEEAIE-------LAVPLKVD
sp|P59200|DPO1_STRR6       -MLLQVHDEIVLEVPKSELVEMKKLVKQTMEEAIQ-------LSVPLIAD
sp|Q59156|DPO1_ANATD       -IILQVHDELLIEAPYEEKDIVKEIVKREMENAVA-------LKVPLVVE
sp|P46835|DPO1_MYCLE       -MLLQVHDELLFEVAIGEREQIEAMVREQMGSAYP-------LDVPLEVS
sp|P00582|DPO1_ECOLI       -MIMQVHDELVFEVHKDDVDAVAKQIHQLMENCTR-------LDVPLLVE
sp|Q9F173|DPO1_SALTY       -MIMQVHDELVFEVHKDDLDAVAKRIHQLMENCTR-------IDVPLLVE
sp|P43741|DPO1_HAEIN       -MIMQVHDELVFEVRSEKVAFFREQIKQHMEAAAE-------LVVPLIVE
sp|Q9HT80|DPO1_PSEAE       -VILQVHDELVLEVREDLVEQVCEGIRPLMSGAAT-------LDVPLVVE
sp|Q9S1G2|DPO1_RHILE       -MLLQVHDELIFEVEDQDVEKAMPVIVSVMENATMPA---LEMRVPLRVD
sp|P19821|DPO1_THEAQ       -MLLQVHDELVLEAPKERAEAVARLAKEVMEGVYP-------LAVPLEVE
sp|P52027|DPO1_DEIRA       -MLLQVHDELLIEAPLDKAEQVAALTKKVMENVVQ-------LKVPLAVE
sp|O08307|DPO1_CHLAA       -LLLQVHDELIAEAPEDEVEPAARLLRDVMSSVYRD------LVVPLSVN
sp|Q55971|DPO1_SYNY3       -MLLQVHDELIFEMPPEEWEELAPLIQNTMEQALT-------LSVPLVVE
sp|Q7TQ07|DPOLN_MOUSE      -LVAQIHDELLFEVEDTQVPEFAALVRRIMESLQQVQTLELQLQVPLKVN
sp|Q7Z5Q5|DPOLN_HUMAN      -LVAQIHDELLFEVEDPQIPECAALVRRTMESLEQVQALELQLQVPLKVS
sp|O75417|DPOLQ_HUMAN      FFILQLHDELLYEVAEEDVVQVAQIVKNEMESAVK-------LSVKLKVK
sp|P00581|DPOL_BPT7        AYMAWVHDEIQVGCRTEEIAQVVIETAQEAMRWVGDHWN---FRCLLDTE
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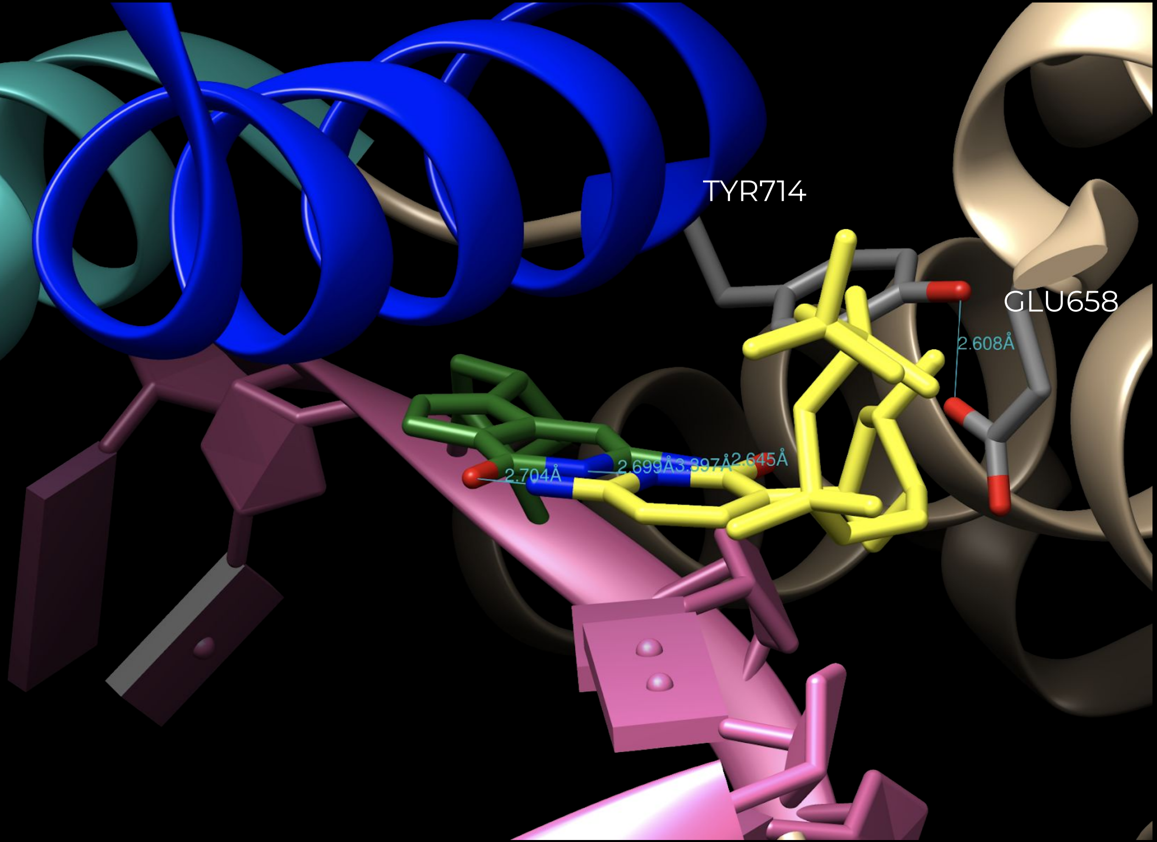
CLOSE Conformation

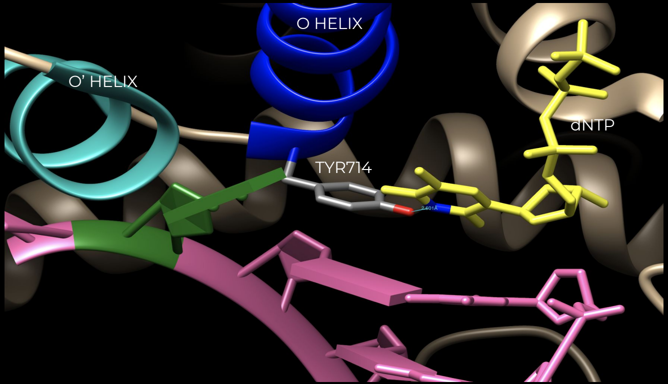
Residue Tyr714



- The acceptor base of the template is now in the **insertion site**

- Tyr714: water salt bridge with Glu658
- HBond between bases

Open Conf.



Residue Glu658

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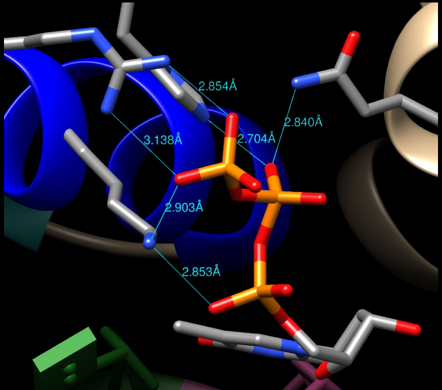
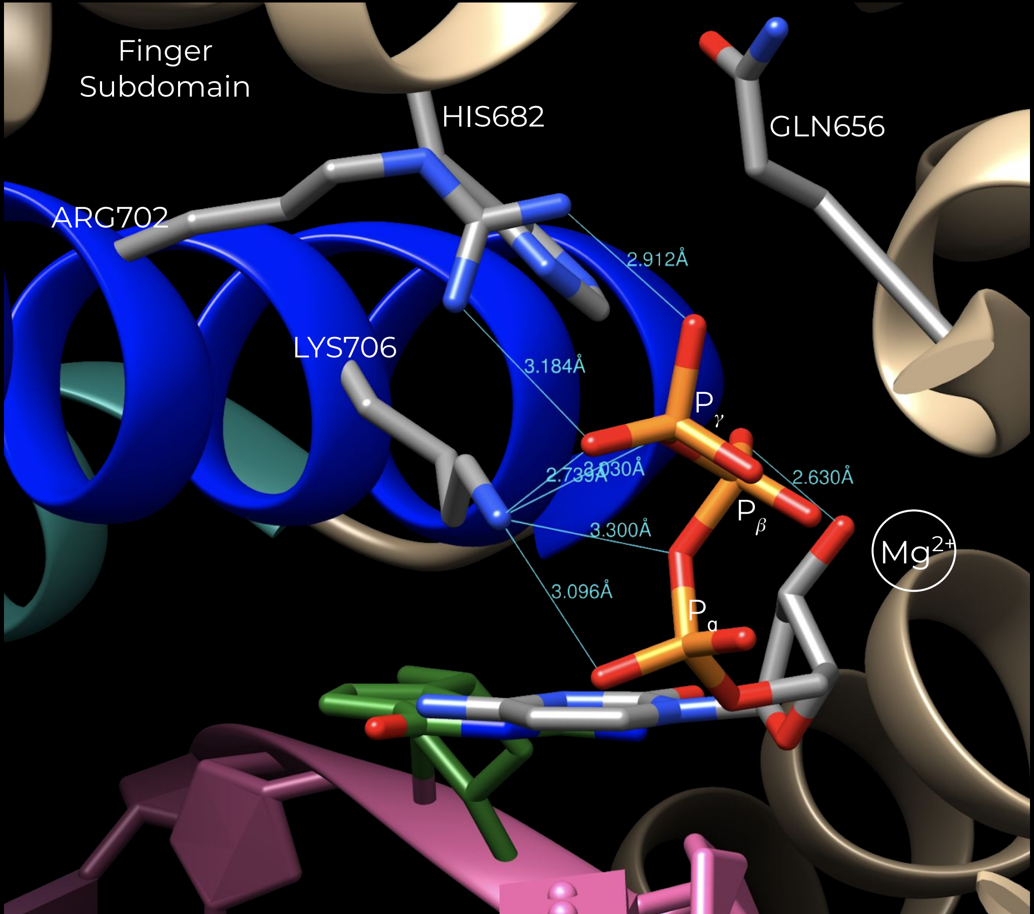
sp|P56105|DPO1_HELPY      -------YCLLGVDYSQIELRLLAHFSQDKDLMEAFLKG----RDIHLET
sp|P74933|DPO1_TREPA      ------GHELISADYTQIELVVLAHLSQDRNLLNAFRQH----IDIHALT
sp|O51498|DPO1_BORBU      ------GNIFISADYSQIELAILAHLSQDEVLIKAFENN----KDIHTET
sp|P52026|DPO1_GEOSE      -----PDWLIFAADYSQIELRVLAHIAEDDNLIEAFRRG----LDIHTKT
sp|Q04957|DPO1_BACCA      -----SDWLIFAADYSQIELRVLAHIAEDDNLMEAFRRD----LDIHTKT
sp|O32801|DPO1_LACLM      -----QDSLLLSSDYSQIELRVLAHISADEHLIDAFKHG----ADIHTST
sp|P59200|DPO1_STRR6      -----EDSVLLSSDYSQIELRVLAHISKDEHLIKAFQEG----ADIHTST
sp|Q59156|DPO1_ANATD      -----GGHVLIDADYSQIELRILAHISEDERLISAFKNN----VDIHSQT
sp|P46835|DPO1_MYCLE      N----GYTELMTADYSQIEMRIMAHLSRDEGLIEAFHTG----EDLHSFV
sp|P00582|DPO1_ECOLI      ------DYVIVSADYSQIELRIMAHLSRDKGLLTAFAEG----KDIHRAT
sp|Q9F173|DPO1_SALTY      ------DYLIVSADYSQIELRIMAHLSRDKGLLTAFAEG----KDIHRAT
sp|P43741|DPO1_HAEIN      ------GYSIVAADYSQIELRIMAHLSGDQGLINAFSQG----KDIHRST
sp|Q9HT80|DPO1_PSEAE      ------GYKLLAADYSQIELRIMAHLAKDDGLLDAFRHD----LDVHRAT
sp|Q9S1G2|DPO1_RHILE      ------GHKLISADYSQIELRVLAHVAEIPQLTKAFEDG----VDIHAMT
sp|P19821|DPO1_THEAQ      ------GWLLVALDYSQIELRVLAHLSGDENLIRVFQEG----RDIHTET
sp|P52027|DPO1_DEIRA      ------GFTLIAADYSQIELRLLAHIADDPLMQQAFVEG----ADIHRRT
sp|O08307|DPO1_CHLAA      ------GWRFVAADYSQIELRVLAHMSGDENLIAAFQQG----LDIHAAT
sp|Q55971|DPO1_SYNY3      ------DWLLVSADYSQIELRILAHLSQEPVLLQAYGDR----QDVHGVT
sp|Q7TQ07|DPOLN_MOUSE     ------GHTFLAADFSQIELRILAHLSGDPELLKLFQESE---RDDVFST
sp|Q7Z5Q5|DPOLN_HUMAN     ------GHTFLAADFSQIELRILTHLSGDPELLKLFQESE---RDDVFST
sp|O75417|DPOLQ_HUMAN     ------GGSILAADYSQLELRILAHLSHDRRLIQVLNTG----ADVFRSI
sp|P00581|DPOL_BPT7       DGITGKPWVQAGIDASGLELRCLAHFMAR------FDNG-----EYAHEI
```

Phosphates of dNTP and O Helix

- Change in the HBonds interactions between the O helix and the dNTP, compared to open conformation.

Open conformation



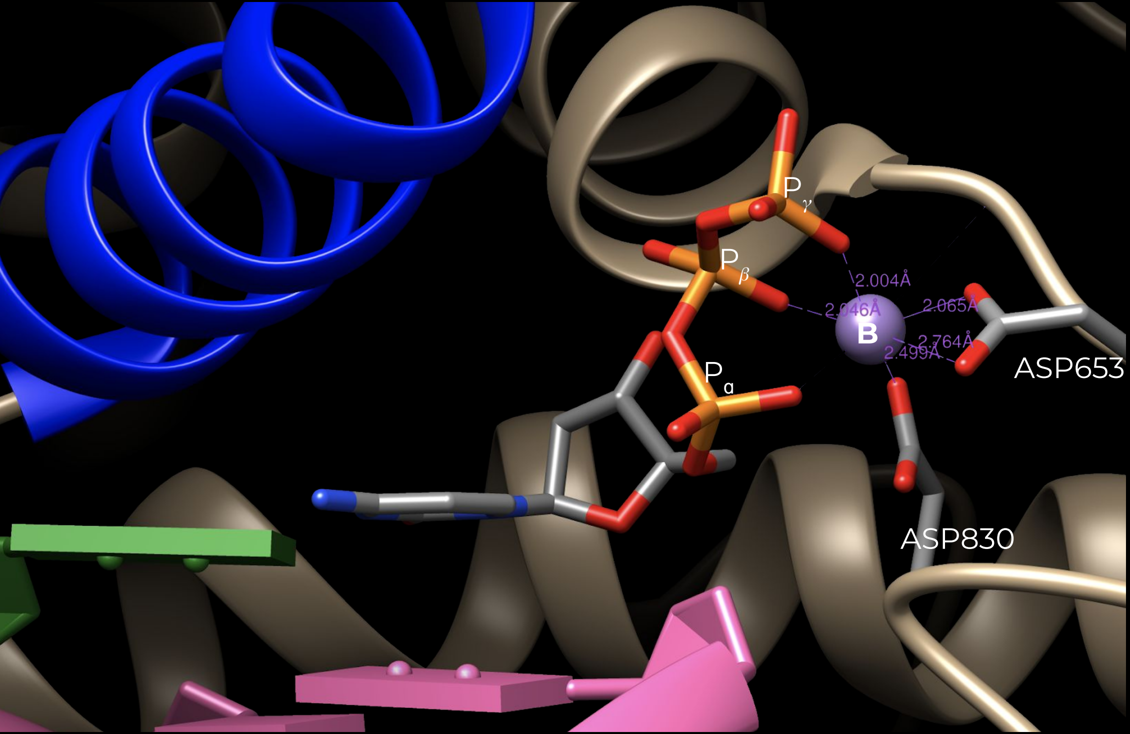


Residues of the O Helix

```
sp|Q9RAA9|DPO1_RICFE      ACQIFNLQK-HELTSEHRRKAKAINFGIIYGISAFGLAKQLNVS------
sp|P56105|DPO1_HELPY      SKALFG----EYLAKEKRSIAKSINFGLVYGMGSKKLSETLNIS------
sp|P74933|DPO1_TREPA      AAYIFNVSI-DDVQPAMRRIAKTINFGIVYGMSAFRLSDELKIS------
sp|O51498|DPO1_BORBU      ASKLFKIEE-KEITPNLRRIAKSINFGIIYRMSDFRLAKELGIT------
sp|P52026|DPO1_GEOSE      AMDIFHVSE-EDVTANMRRQAKAVNFGIVYGISDYGLAQNLNIT------
sp|Q04957|DPO1_BACCA      AMDIFQVSE-DEVTPNMRRQAKAVNFGIVYGISDYGLAQNLNIS------
sp|O32801|DPO1_LACLM      AMRVFGIEKAEDVTANDRRNAKAVNFGVVYGISDFGLARNLGIT------
sp|P59200|DPO1_STRR6      AMRVFGIERPDNVTANDRRNAKAVNFGVVYGISDFGLSNNLGIS------
sp|Q59156|DPO1_ANATD      AAEVFGVDI-ADVTPEMRSQAKAVNFGIVYGISDYGLARDIKIS------
sp|P46835|DPO1_MYCLE      ASRAFGIPI-EDITPELRRRVKAMSYGLAYGLSAYGLATQLKIS------
sp|P00582|DPO1_ECOLI      AAEVFGLPL-ETVTSEQRRSAKAINFGLIYGMSAFGLARQLNIP------
sp|Q9F173|DPO1_SALTY      AAEVFGLPL-DSVTGEQRRSAKAINFGLIYGMSAFGLSRQLNIP------
sp|P43741|DPO1_HAEIN      AAEIFGVSL-DEVTSEQRRNAKAINFGLIYGMSAFGLSRQLGIS------
sp|Q9HT80|DPO1_PSEAE      AAEVFGVPL-EDVSGDQRRSAKAINFGLIYGMSAFGLAKQIGVE------
sp|Q9S1G2|DPO1_RHILE      ASEMFGVPV-EGMPGEVRRRAKAINFGIIYGISAFGLANQLSIE------
sp|P19821|DPO1_THEAQ      ASWMFGVPR-EAVDPLMRRAAKTINFGVLYGMSAHRLSQELAIP------
sp|P52027|DPO1_DEIRA      AAQVLGLDE-ATVDANQRRAAKTVNFGVLYGMSAHRLSNDLGIP------
sp|O08307|DPO1_CHLAA      ASRLFGVEP-TAVDKNQRRVAKTVVFGVIYGISAFGLAQRLGIE------
sp|Q55971|DPO1_SYNY3      AKLLFGKED---ITPAERNLGKTINFGVIYGMGAQRFARETGIS------
sp|Q7TQ07|DPOLN_MOUSE     LTSQWKDIPIERVTHMDREQTKKVVYSVVYGAGKERLAACLGVT------
sp|Q7Z5Q5|DPOLN_HUMAN     LTSQWKDVPVEQVTHADREQTKKVVYAVVYGAGKERLAACLGVP------
sp|O75417|DPOLQ_HUMAN     AAEWKMIEP-ESVGDDLRQQAKQICYGIIYGMGAKSLGEQMGIK------
sp|P00581|DPOL_BPT7       LNGDIHTKNQIAAELPTRDNAKTFIYGFLYGAGDEKIGQIVGAG------
```

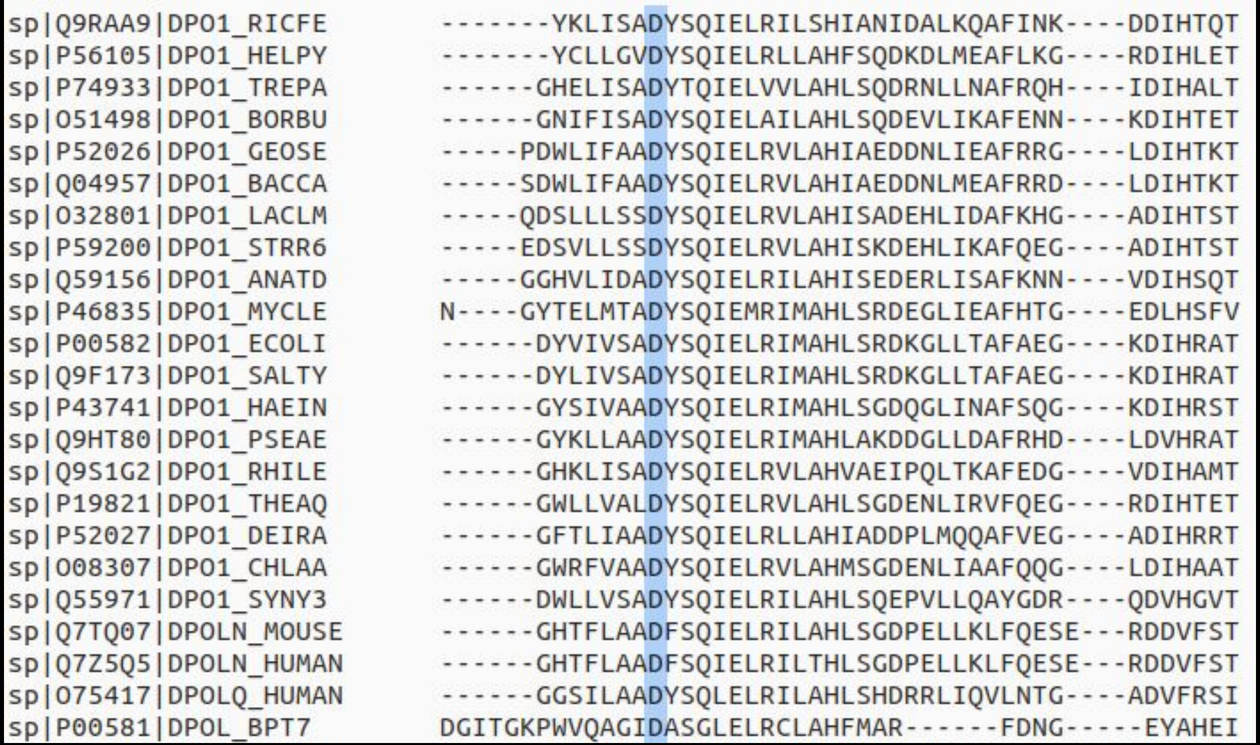
Mg^{2+}: Metal Sites A and B

Metal Site B



- dNTP-Mg^{2+}
- The β and γ phosphates interact with the Mg^{2+} through metallic bonding

Residue Asp653



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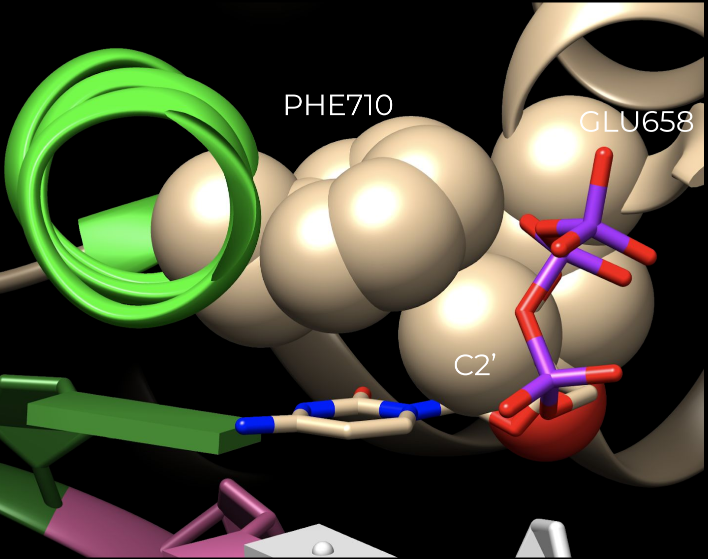
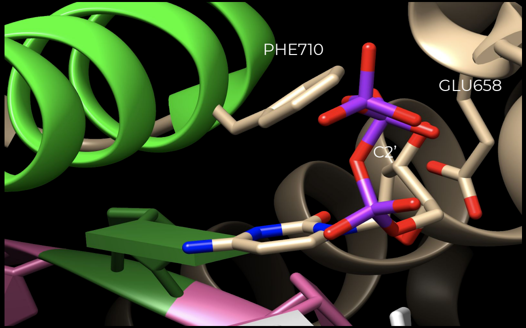
sp|Q9RAA9|DPO1_RICFE       -------YKLISADYSQIELRILSHIANIDALKQAFINK----DDIHTQT
sp|P56105|DPO1_HELPY       -------YCLLGVDYSQIELRLLAHFSQDKDLMEAFLKG----RDIHLET
sp|P74933|DPO1_TREPA       ------GHELISADYTQIELVVLAHLSQDRNLLNAFRQH----IDIHALT
sp|O51498|DPO1_BORBU       ------GNIFISADYSQIELAILAHLSQDEVLIKAFENN----KDIHTET
sp|P52026|DPO1_GEOSE       -----PDWLIFAADYSQIELRVLAHIAEDDNLIEAFRRG----LDIHTKT
sp|Q04957|DPO1_BACCA       -----SDWLIFAADYSQIELRVLAHIAEDDNLMEAFRRD----LDIHTKT
sp|O32801|DPO1_LACLM       -----QDSLLLSSDYSQIELRVLAHISADEHLIDAFKHG----ADIHTST
sp|P59200|DPO1_STRR6       -----EDSVLLSSDYSQIELRVLAHISKDEHLIKAFQEG----ADIHTST
sp|Q59156|DPO1_ANATD       -----GGHVLIDADYSQIELRILAHISEDERLISAFKNN----VDIHSQT
sp|P46835|DPO1_MYCLE       N----GYTELMTADYSQIEMRIMAHLSRDEGLIEAFHTG----EDLHSFV
sp|P00582|DPO1_ECOLI       ------DYVIVSADYSQIELRIMAHLSRDKGLLTAFAEG----KDIHRAT
sp|Q9F173|DPO1_SALTY       ------DYLIVSADYSQIELRIMAHLSRDKGLLTAFAEG----KDIHRAT
sp|P43741|DPO1_HAEIN       ------GYSIVAADYSQIELRIMAHLSGDQGLINAFSQG----KDIHRST
sp|Q9HT80|DPO1_PSEAE       ------GYKLLAADYSQIELRIMAHLAKDDGLLDAFRHD----LDVHRAT
sp|Q9S1G2|DPO1_RHILE       ------GHKLISADYSQIELRVLAHVAEIPQLTKAFEDG----VDIHAMT
sp|P19821|DPO1_THEAQ       ------GWLLVALDYSQIELRVLAHLSGDENLIRVFQEG----RDIHTET
sp|P52027|DPO1_DEIRA       ------GFTLIAADYSQIELRLLAHIADDPLMQQAFVEG----ADIHRRT
sp|O08307|DPO1_CHLAA       ------GWRFVAADYSQIELRVLAHMSGDENLIAAFQQG----LDIHAAT
sp|Q55971|DPO1_SYNY3       ------DWLLVSADYSQIELRILAHLSQEPVLLQAYGDR----QDVHGVT
sp|Q7TQ07|DPOLN_MOUSE      ------GHTFLAADFSQIELRILAHLSGDPELLKLFQESE---RDDVFST
sp|Q7Z5Q5|DPOLN_HUMAN      ------GHTFLAADFSQIELRILTHLSGDPELLKLFQESE---RDDVFST
sp|O75417|DPOLQ_HUMAN      ------GGSILAADYSQLELRILAHLSHDRRLIQVLNTG----ADVFRSI
sp|P00581|DPOL_BPT7        DGITGKPWVQAGIDASGLELRCLAHFMAR------FDNG-----EYAHEI
```

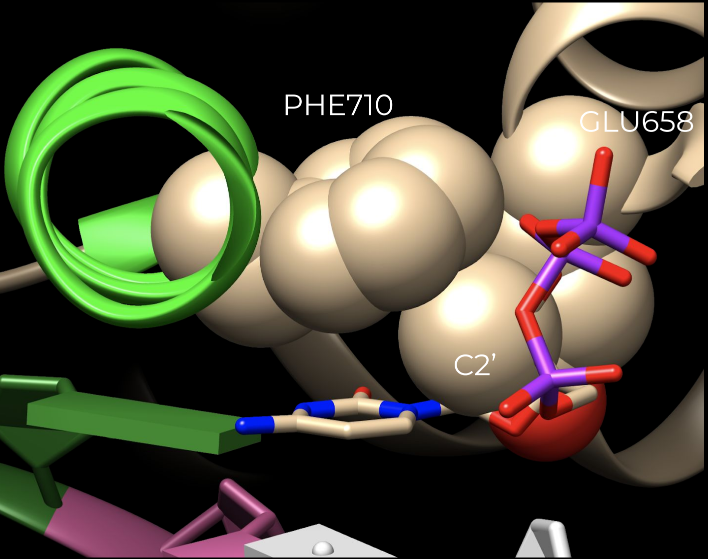
Sugar specificity of the dNTP



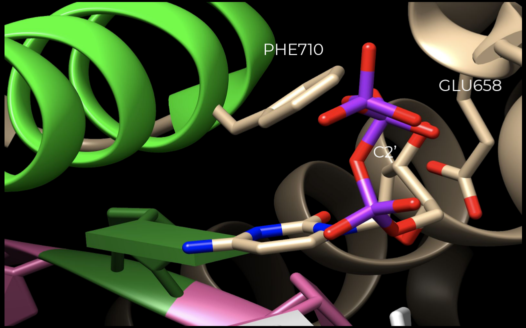
← ·Specificity of a deoxyribose over a ribose

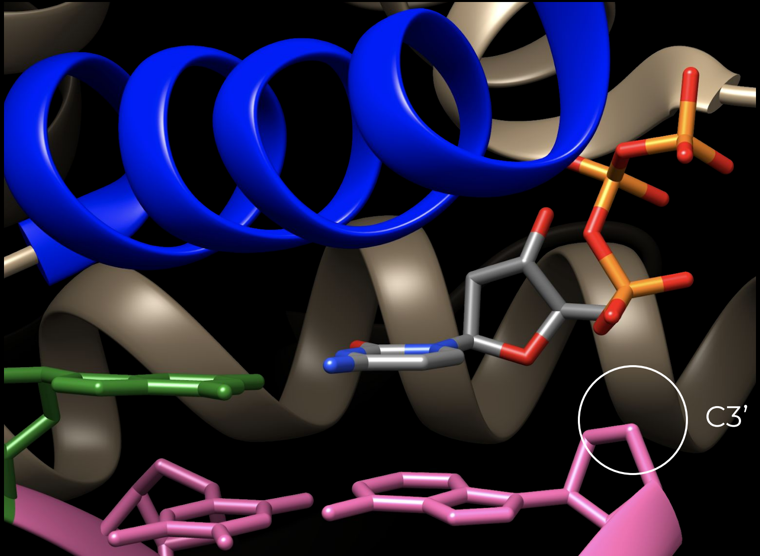


Sugar specificity of the dNTP

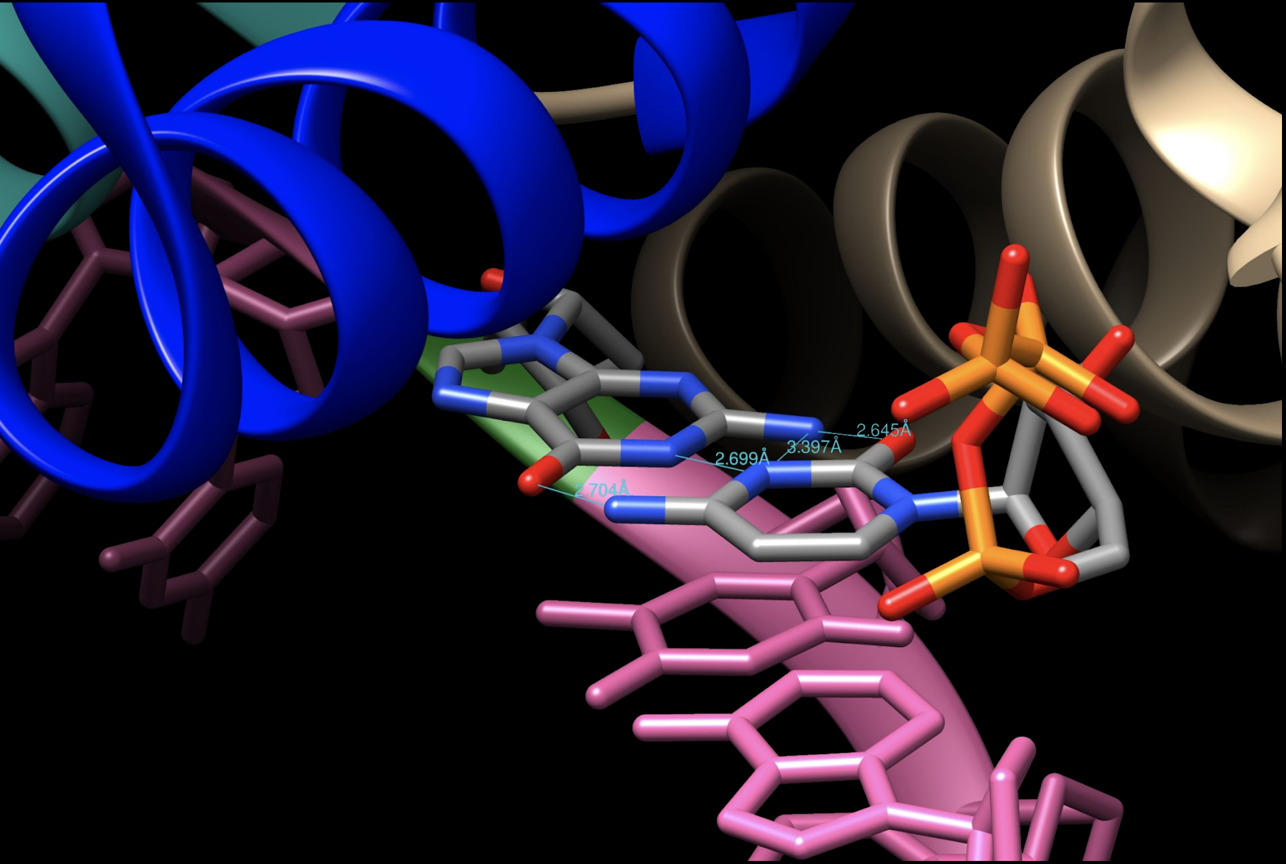


- Specificity of a deoxyribose over a ribose
- Specificity of a deoxynucleotide over a dideoxynucleotide





Hydrogen Bonds between base pairs

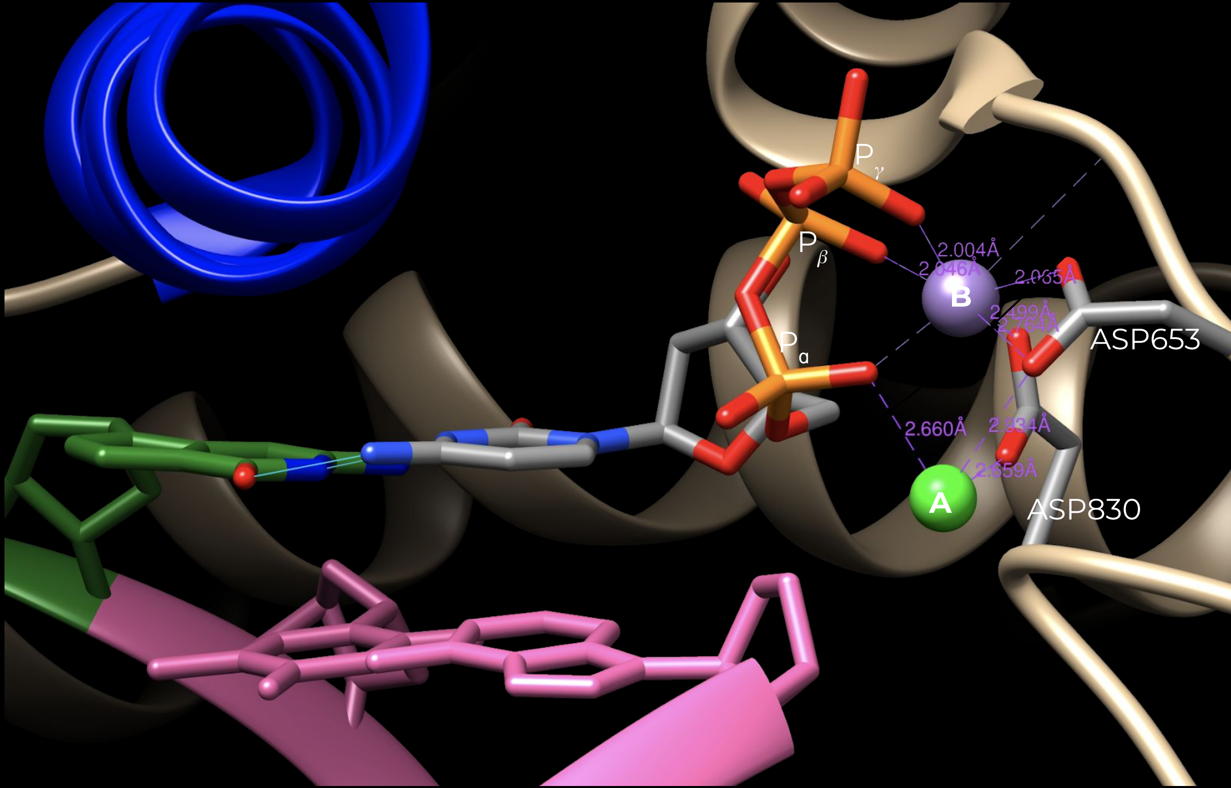


- The insertion site must be geometrically and energetically favourable in order to establish HBonds between bases

Mg^{2+}: Metal Sites A and B

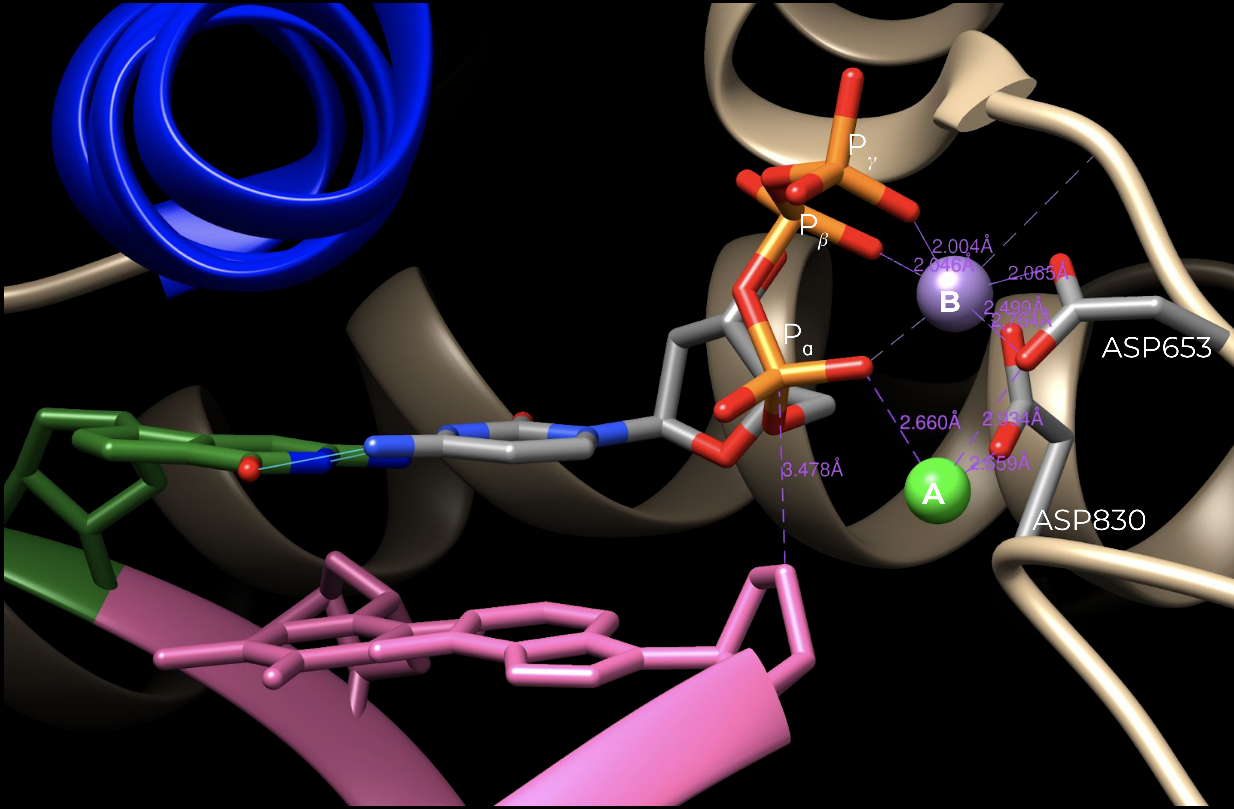
- Second Mg^{2+} ion
- The α phosphate interacts with the Mg^{2+} through metallic bonding

Metal Site A



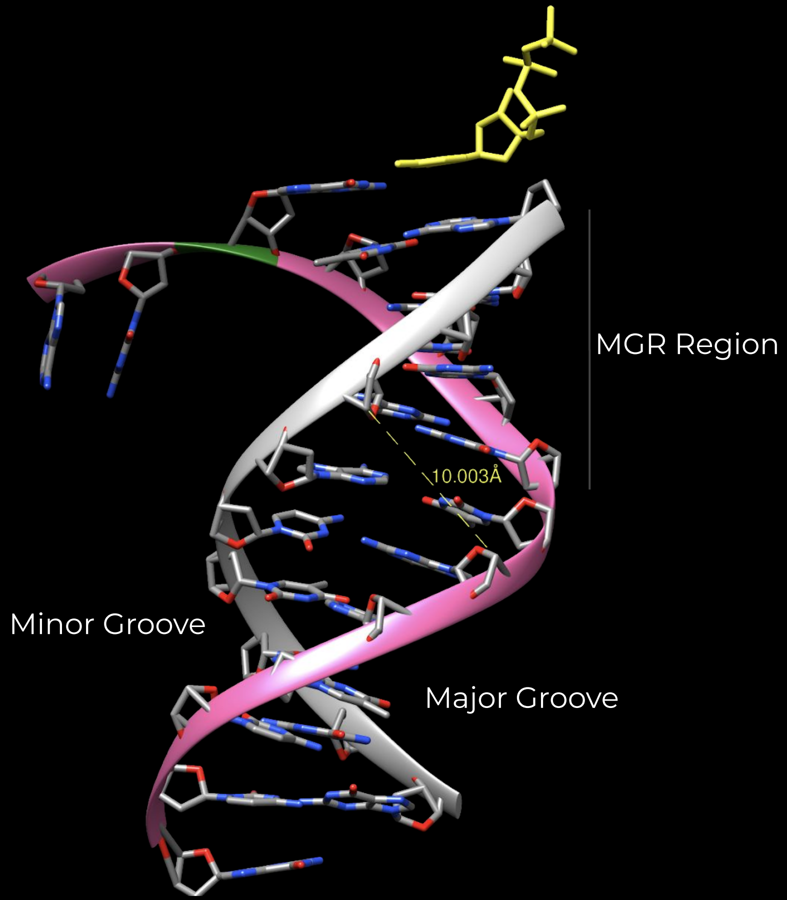
Mg^{2+}: Metal Sites A and B

- The distance (P_α and C3’) is lower than ~4Å
- The second Mg^{2+} enables catalytic reaction
- The C3’ of the deoxyribose must switch to C3’-endo sugar pucker (A-form)



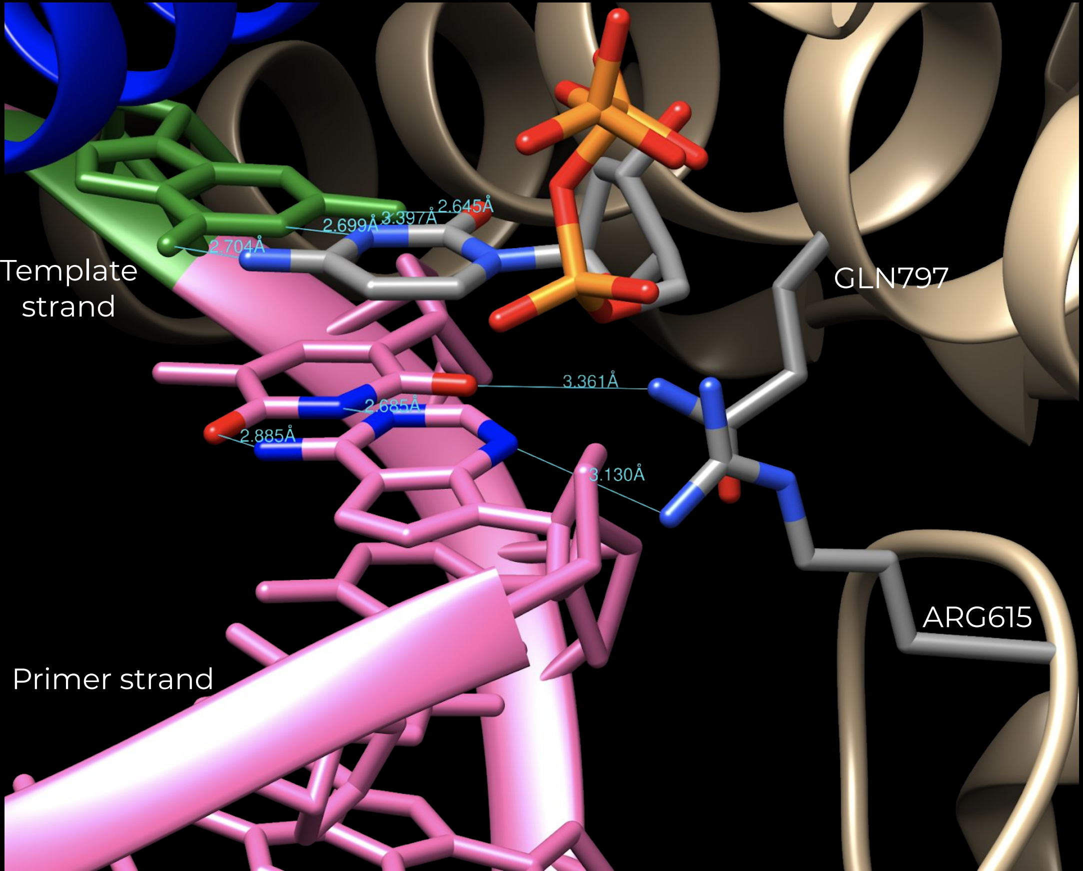
DNA Duplex Binding Region

Change in the DNA conformation



- The first three base pairs of the DNA duplex adopt a A-form DNA conformation
- The Minor Groove becomes more shallow and wider
- The Minor Groove is sequence-independent

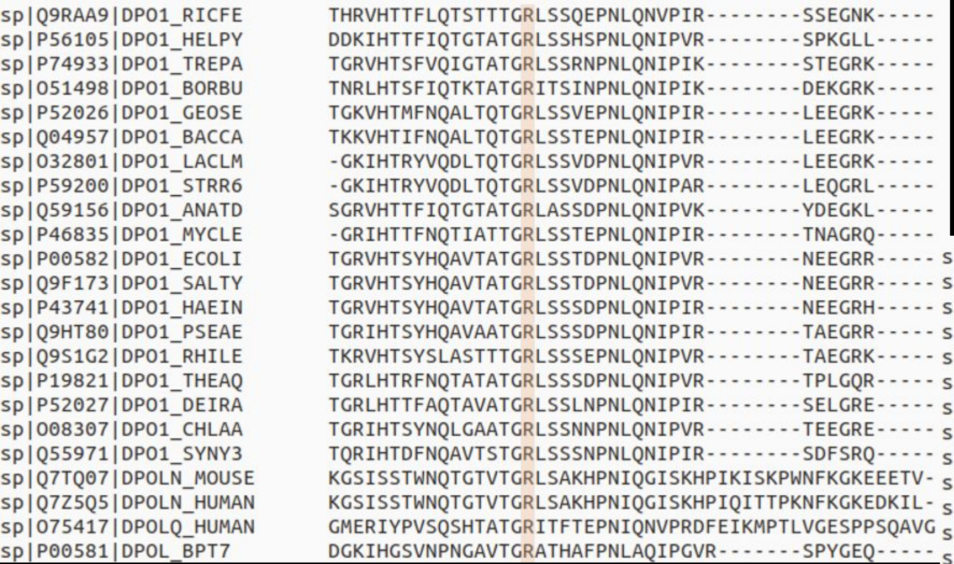
DNA duplex binding region



- **Post-Insertion site: 1st base pair**
- HBonds with the Minor Groove N bases
- Steric complementarity

Residues Arg615 and Gln797

ARG615



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sp|P56105|DPO1_HELPY      DDKIHTTFIQTGTATGRLSSHSPNLQNIPVR--------SPKGLL-----
sp|P74933|DPO1_TREPA      TGRVHTSFVQIGTATGRLSSRNPNLQNIPIK--------STEGRK-----
sp|O51498|DPO1_BORBU      TNRLHTSFIQTKTATGRITSINPNLQNIPIK--------DEKGRK-----
sp|P52026|DPO1_GEOSE      TGKVHTMFNQALTQTGRLSSVEPNLQNIPIR--------LEEGRK-----
sp|Q04957|DPO1_BACCA      TKKVHTIFNQALTQTGRLSSTEPNLQNIPIR--------LEEGRK-----
sp|O32801|DPO1_LACLM      -GKIHTRYVQDLTQTGRLSSVDPNLQNIPVR--------LEEGRK-----
sp|P59200|DPO1_STRR6      -GKIHTRYVQDLTQTGRLSSVDPNLQNIPAR--------LEQGRL-----
sp|Q59156|DPO1_ANATD      SGRVHTTFIQTGTATGRLASSDPNLQNIPVK--------YDEGKL-----
sp|P46835|DPO1_MYCLE      -GRIHTTFNQTIATTGRLSSTEPNLQNIPIR--------TNAGRQ-----
sp|P00582|DPO1_ECOLI      TGRVHTSYHQAVTATGRLSSTDPNLQNIPVR--------NEEGRR-----
sp|Q9F173|DPO1_SALTY      TGRVHTSYHQAVTATGRLSSTDPNLQNIPVR--------NEEGRR-----
sp|P43741|DPO1_HAEIN      TGRVHTSYHQAVTATGRLSSSDPNLQNIPIR--------NEEGRH-----
sp|Q9HT80|DPO1_PSEAE      TGRIHTSYHQAVAATGRLSSSDPNLQNIPIR--------TAEGRR-----
sp|Q9S1G2|DPO1_RHILE      TKRVHTSYSLASTTTGRLSSSEPNLQNIPVR--------TAEGRK-----
sp|P19821|DPO1_THEAQ      TGRLHTRFNQTATATGRLSSSDPNLQNIPVR--------TPLGQR-----
sp|P52027|DPO1_DEIRA      TGRLHTTFAQTAVATGRLSSLNPNLQNIPIR--------SELGRE-----
sp|O08307|DPO1_CHLAA      TGRIHTSYNQLGAATGRLSSNNPNLQNIPVR--------TEEGRE-----
sp|Q55971|DPO1_SYNY3      TQRIHTDFNQAVTSTGRLSSSNPNLQNIPIR--------SDFSRQ-----
sp|Q7TQ07|DPOLN_MOUSE     KGSISSTWNQTGTVTGRLSAKHPNIQGISKHPIKISKPWNFKGKEEETV-
sp|Q7Z5Q5|DPOLN_HUMAN     KGSISSTWNQTGTVTGRLSAKHPNIQGISKHPIQITTPKNFKGKEDKIL-
sp|O75417|DPOLQ_HUMAN     GMERIYPVSQSHTATGRITFTEPNIQNVPRDFEIKMPTLVGESPPSQAVG
sp|P00581|DPOL_BPT7       DGKIHGSVNPNGAVTGRATHAFPNLAQIPGVR-------SPYGEQ-----
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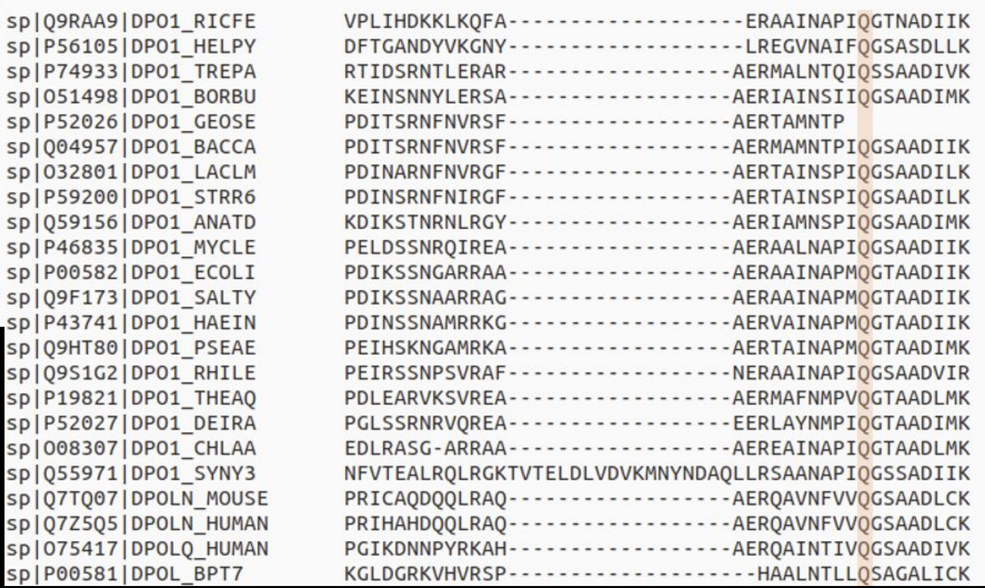
GLN797



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sp|Q9RAA9|DPO1_RICFE      VPLIHDKKLKQFA-------------------ERAAINAPIQGTNADIIK
sp|P56105|DPO1_HELPY      DFTGANDYVKGNY-------------------LREGVNAIFQGSASDLLK
sp|P74933|DPO1_TREPA      RTIDSRNTLERAR------------------AERMALNTQIQSSAADIVK
sp|O51498|DPO1_BORBU      KEINSNNYLERSA------------------AERIAINSIIQGSAADIMK
sp|P52026|DPO1_GEOSE      PDITSRNFNVRSF------------------AERTAMNTP
sp|Q04957|DPO1_BACCA      PDITSRNFNVRSF------------------AERMAMNTPIQGSAADIIK
sp|O32801|DPO1_LACLM      PDINARNFNVRGF------------------AERTAINSPIQGSAADILK
sp|P59200|DPO1_STRR6      PDINSRNFNIRGF------------------AERTAINSPIQGSAADILK
sp|Q59156|DPO1_ANATD      KDIKSTNRNLRGY------------------AERIAMNSPIQGSAADIMK
sp|P46835|DPO1_MYCLE      PELDSSNRQIREA------------------AERAALNAPIQGSAADIIK
sp|P00582|DPO1_ECOLI      PDIKSSNGARRAA------------------AERAAINAPMQGTAADIIK
sp|Q9F173|DPO1_SALTY      PDIKSSNAARRAG------------------AERAAINAPMQGTAADIIK
sp|P43741|DPO1_HAEIN      PDINSSNAMRRKG------------------AERVAINAPMQGTAADIIK
sp|Q9HT80|DPO1_PSEAE      PEIHSKNGAMRKA------------------AERTAINAPMQGTAADIMK
sp|Q9S1G2|DPO1_RHILE      PEIRSSNPSVRAF------------------NERAAINAPIQGSAADVIR
sp|P19821|DPO1_THEAQ      PDLEARVKSVREA------------------AERMAFNMPVQGTAADLMK
sp|P52027|DPO1_DEIRA      PGLSSRNRVQREA------------------EERLAYNMPIQGTAADIMK
sp|O08307|DPO1_CHLAA      EDLRASG-ARRAA------------------AEREAINAPIQGTAADLMK
sp|Q55971|DPO1_SYNY3      NFVTEALRQLRGKTVTELDLVDVKMNYNDAQLLRSAANAPIQGSSADIIK
sp|Q7TQ07|DPOLN_MOUSE     PRICAQDQQLRAQ------------------AERQAVNFVVQGSAADLCK
sp|Q7Z5Q5|DPOLN_HUMAN     PRIHAHDQQLRAQ------------------AERQAVNFVVQGSAADLCK
sp|O75417|DPOLQ_HUMAN     PGIKDNNPYRKAH------------------AERQAINTIVQGSAADIVK
sp|P00581|DPOL_BPT7       KGLDGRKVHVRSP--------------------HAALNTLLQSAGALICK
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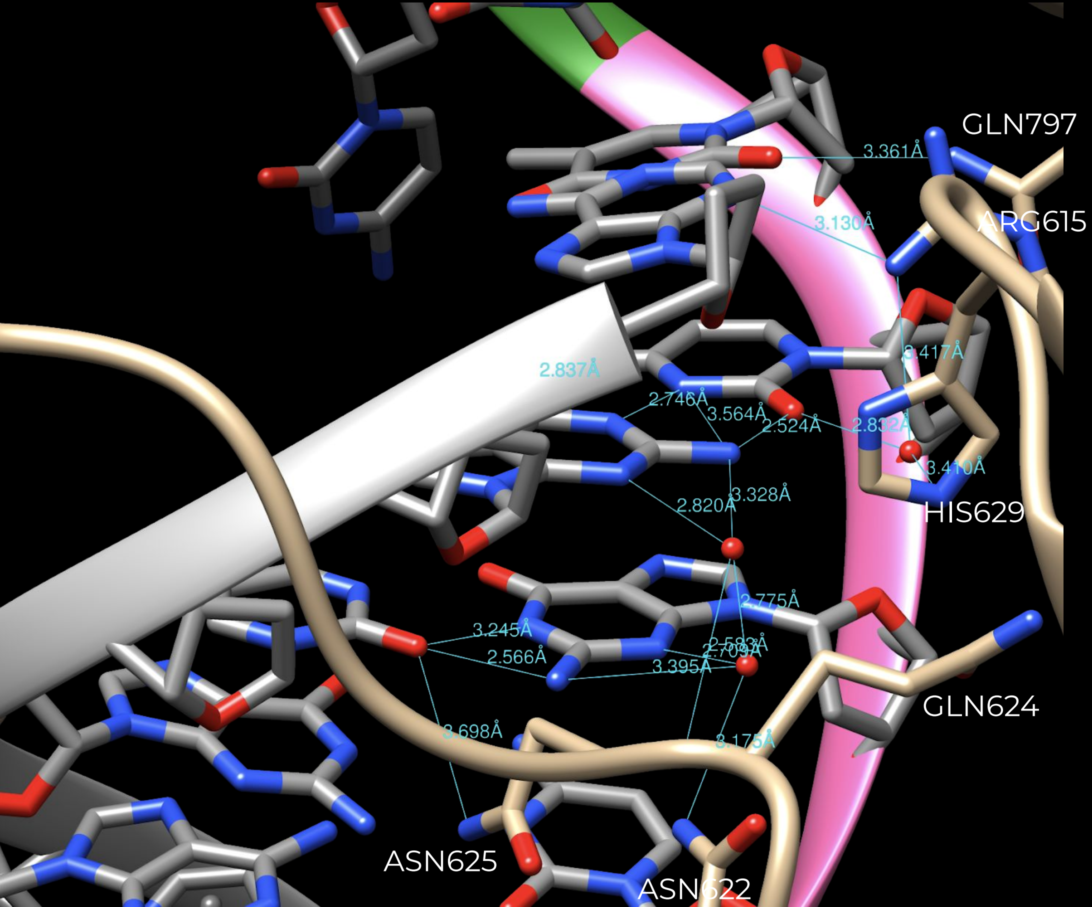
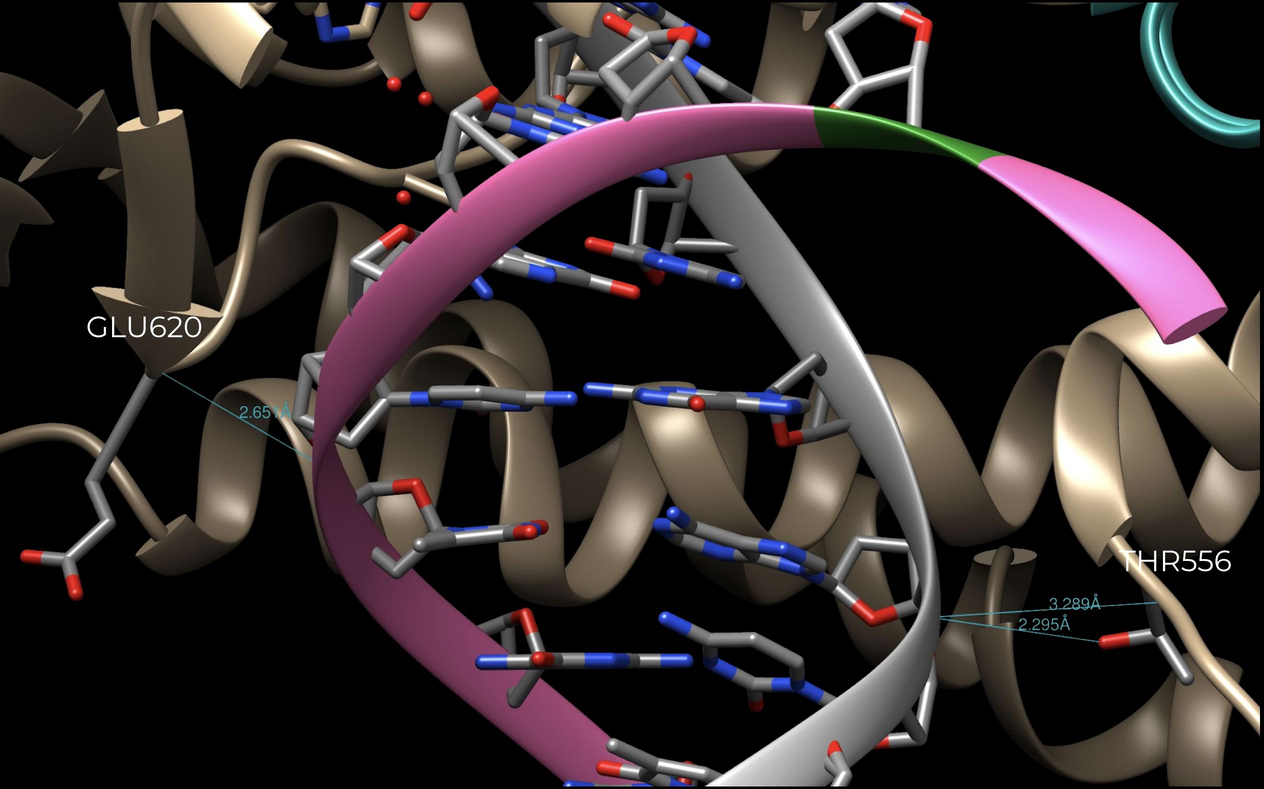
DNA duplex binding region



- **Minor-Groove Recognition (MGR) region**
- A-form DNA: C3’ -endo conformation
- Interaction between **the first 3 base pairs** of the duplex with side-chains or water molecules anchored to the side chains of the **palm** subdomain (HBonds)

DNA duplex binding region



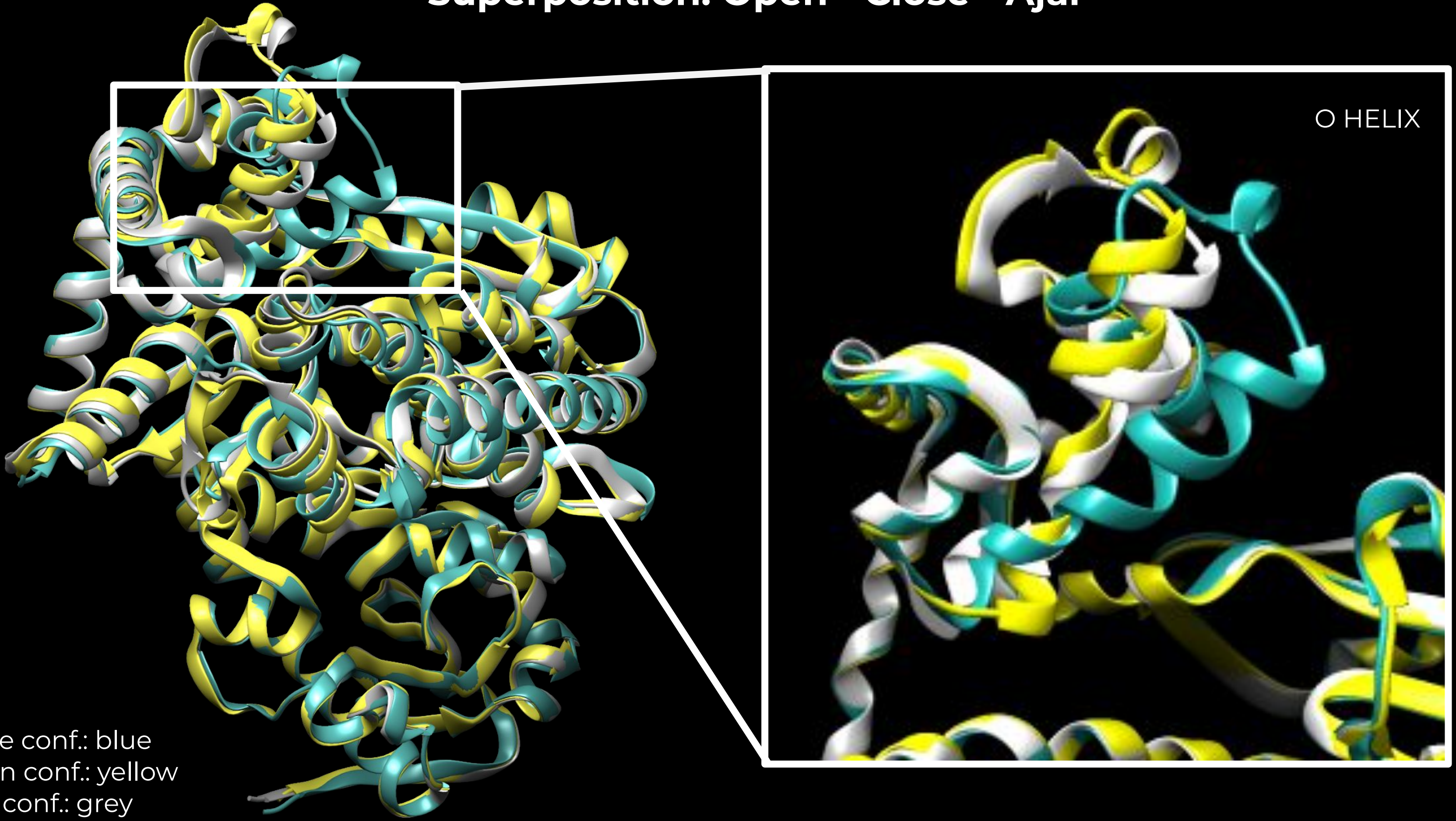
- **Distal part of the DNA duplex binding region**
- Sequence-independent binding region

  ↓

  Sugar-phosphate bb
- B-form DNA, a more hydrated conformation

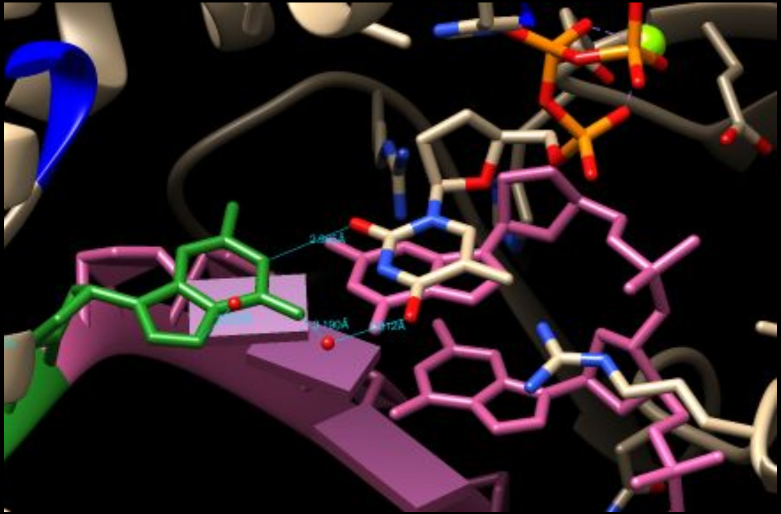
Ajar conformation

Superposition: Open - Close - Ajar

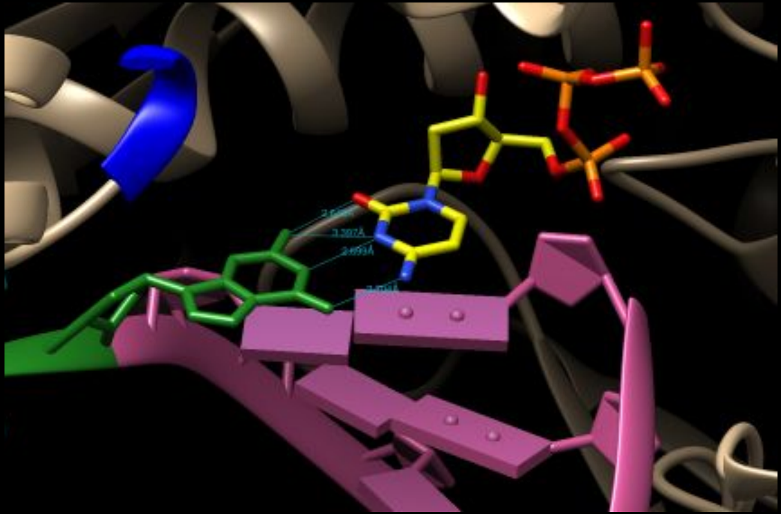


Close conf.: blue
Open conf.: yellow
Ajar conf.: grey

Ajar: base pair mismatch

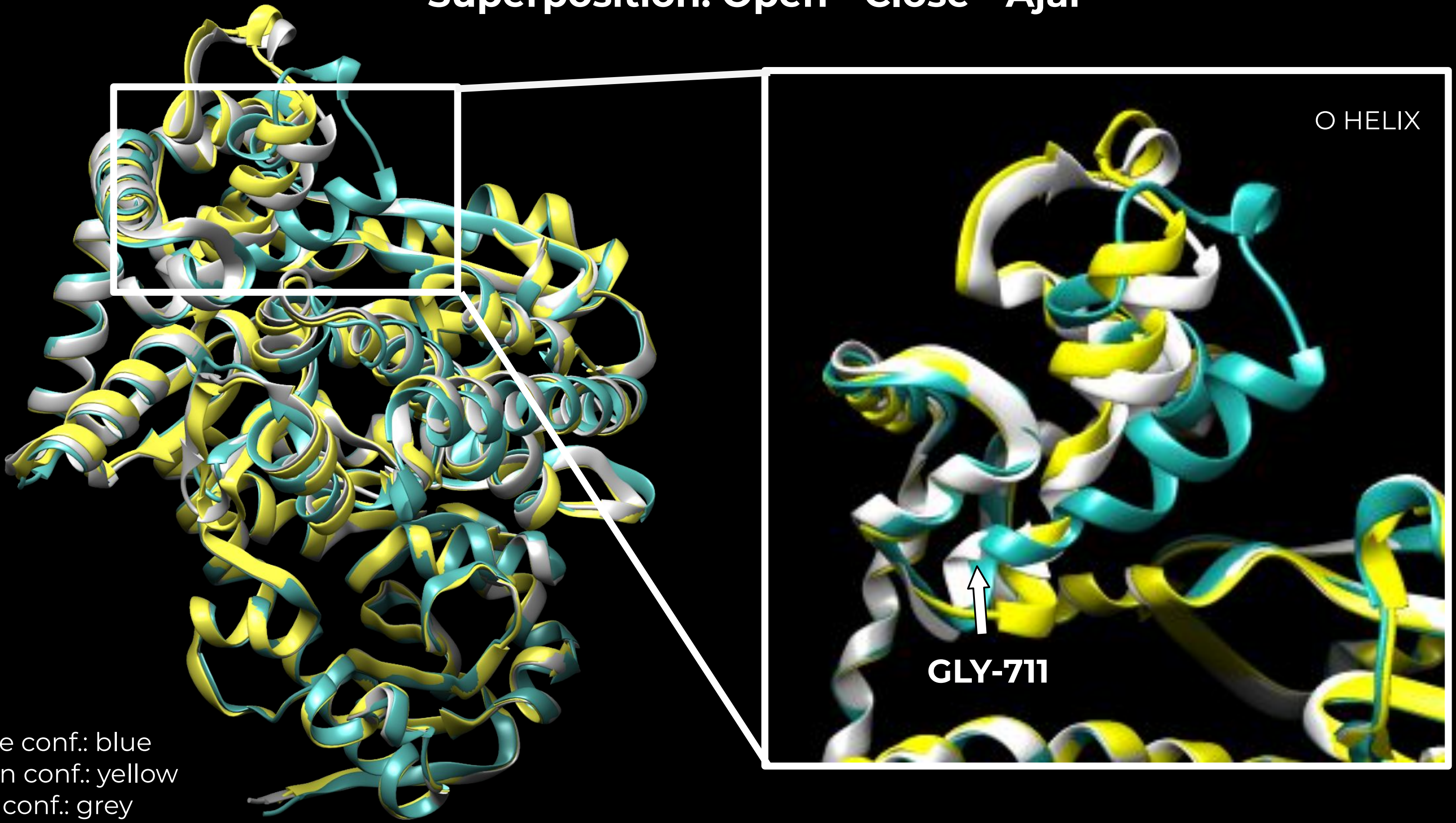


Mismatch



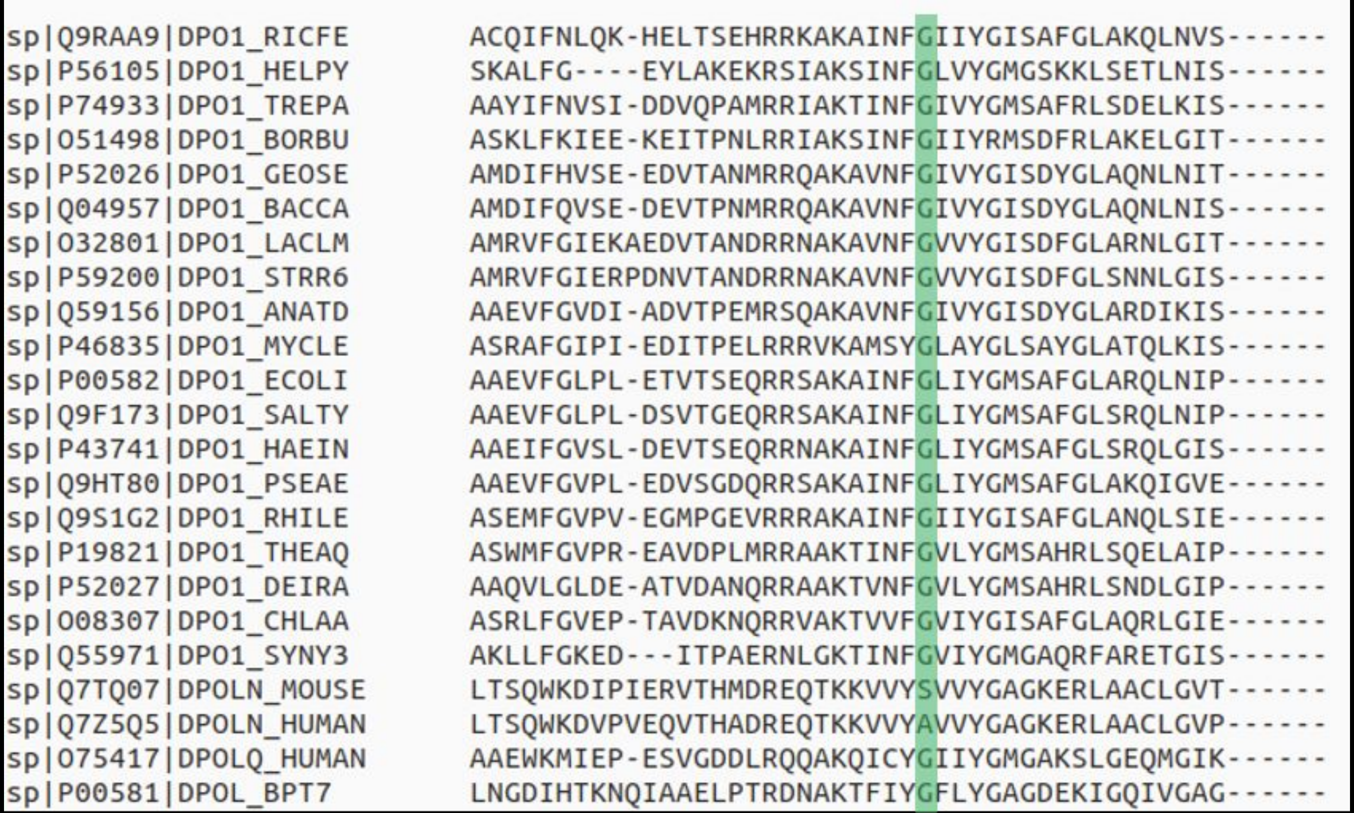
Correct pairing

Superposition: Open - Close - Ajar



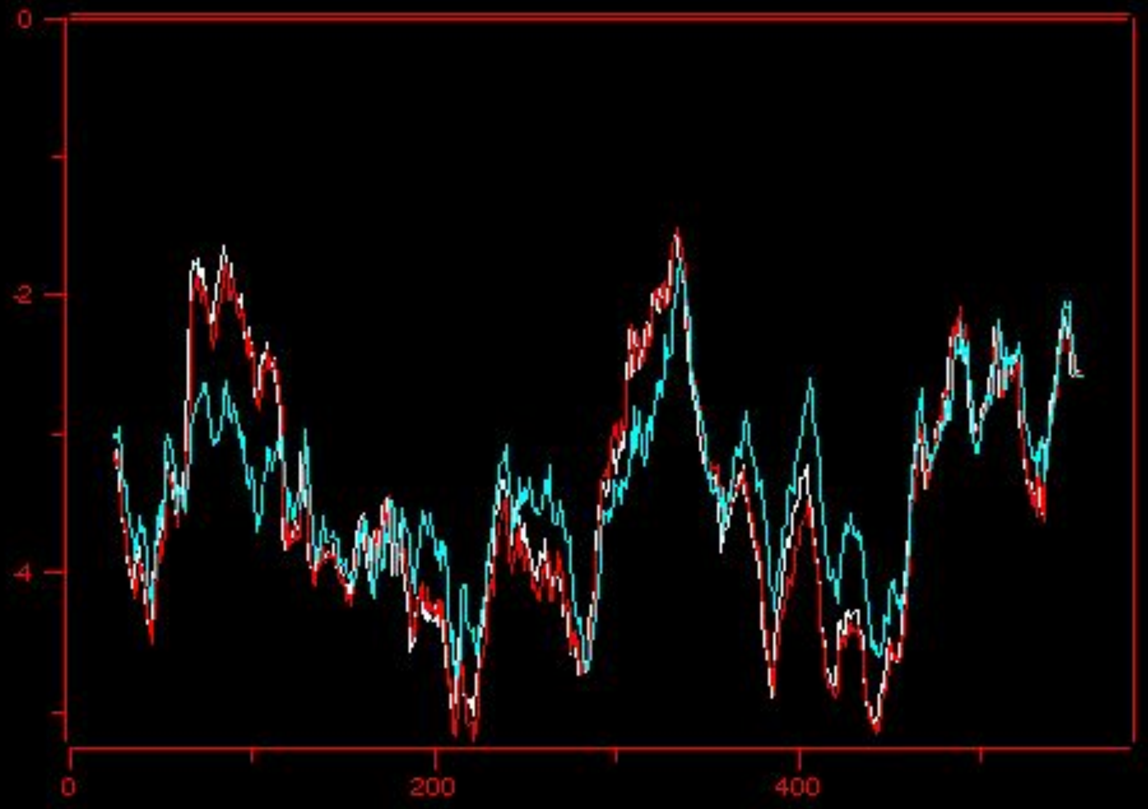
Close conf.: blue
Open conf.: yellow
Ajar conf.: grey

Residue Gly711



```
sp|Q9RAA9|DPO1_RICFE      ACQIFNLQK-HELTSEHRRKAKAINFGIIYGISAFGLAKQLNVS------
sp|P56105|DPO1_HELPY      SKALFG----EYLAKEKRSIAKSINFGLVYGMGSKKLSETLNIS------
sp|P74933|DPO1_TREPA      AAYIFNVSI-DDVQPAMRRIAKTINFGIVYGMSAFRLSDELKIS------
sp|O51498|DPO1_BORBU      ASKLFKIEE-KEITPNLRRIAKSINFGIIYRMSDFRLAKELGIT------
sp|P52026|DPO1_GEOSE      AMDIFHVSE-EDVTANMRRQAKAVNFGIVYGISDYGLAQNLNIT------
sp|Q04957|DPO1_BACCA      AMDIFQVSE-DEVTPNMRRQAKAVNFGIVYGISDYGLAQNLNIS------
sp|O32801|DPO1_LACLM      AMRVFGIEKAEDVTANDRRNAKAVNFGVVYGISDFGLARNLGIT------
sp|P59200|DPO1_STRR6      AMRVFGIERPDNVTANDRRNAKAVNFGVVYGISDFGLSNNLGIS------
sp|Q59156|DPO1_ANATD      AAEVFGVDI-ADVTPEMRSQAKAVNFGIVYGISDYGLARDIKIS------
sp|P46835|DPO1_MYCLE      ASRAFGIPI-EDITPELRRRVKAMSYGLAYGLSAYGLATQLKIS------
sp|P00582|DPO1_ECOLI      AAEVFGLPL-ETVTSEQRRSAKAINFGLIYGMSAFGLARQLNIP------
sp|Q9F173|DPO1_SALTY      AAEVFGLPL-DSVTGEQRRSAKAINFGLIYGMSAFGLSRQLNIP------
sp|P43741|DPO1_HAEIN      AAEIFGVSL-DEVTSEQRRNAKAINFGLIYGMSAFGLSRQLGIS------
sp|Q9HT80|DPO1_PSEAE      AAEVFGVPL-EDVSGDQRRSAKAINFGLIYGMSAFGLAKQIGVE------
sp|Q9S1G2|DPO1_RHILE      ASEMFGVPV-EGMPGEVRRRAKAINFGIIYGISAFGLANQLSIE------
sp|P19821|DPO1_THEAQ      ASWMFGVPR-EAVDPLMRRAAKTINFGVLYGMSAHRLSQELAIP------
sp|P52027|DPO1_DEIRA      AAQVLGLDE-ATVDANQRRAAKTVNFGVLYGMSAHRLSNDLGIP------
sp|O08307|DPO1_CHLAA      ASRLFGVEP-TAVDKNQRRVAKTVVFGVIYGISAFGLAQRLGIE------
sp|Q55971|DPO1_SYNY3      AKLLFGKED---ITPAERNLGKTINFGVIYGMGAQRFARETGIS------
sp|Q7TQ07|DPOLN_MOUSE     LTSQWKDIPIERVTHMDREQTKKVVYSVVYGAGKERLAACLGVT------
sp|Q7Z5Q5|DPOLN_HUMAN     LTSQWKDVPVEQVTHADREQTKKVVYAVVYGAGKERLAACLGVP------
sp|O75417|DPOLQ_HUMAN     AAEWKMIEP-ESVGDDLRQQAKQICYGIIYGMGAKSLGEQMGIK------
sp|P00581|DPOL_BPT7       LNGDIHTKNQIAAELPTRDNAKTFIYGFLYGAGDEKIGQIVGAG------
```

Conformations Energies



⬜ Open

🟦 Close

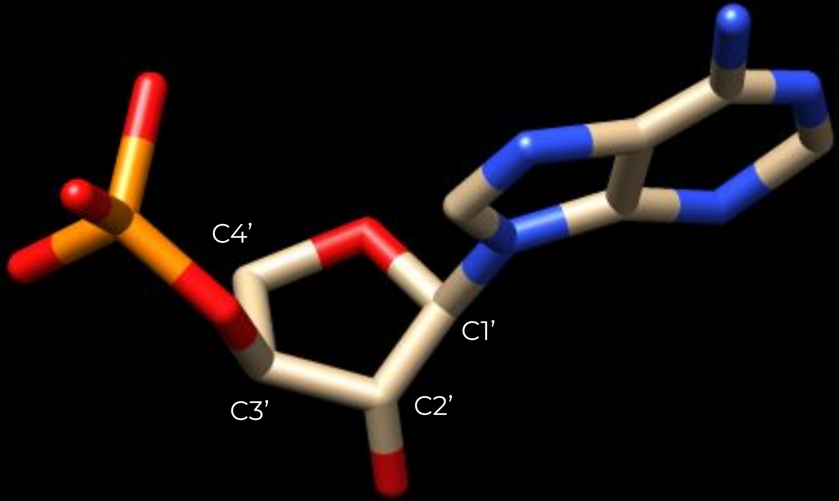
🟥 Ajar

- The Close conformation is the most stable one
- Open and Ajar conformations are more energetically unfavourable

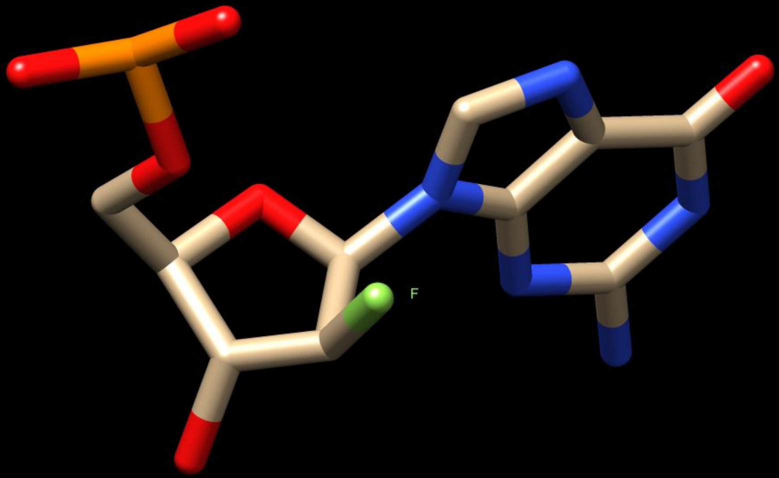
XNA reverse transcriptase

Jackson LN, Chim N, Shi C, Chaput JC. Crystal structures of a natural DNA polymerase that functions as an XNA reverse transcriptase. *Nucleic Acids Research*. 2019 Jun;47(13):6973–83.

Sugar differences

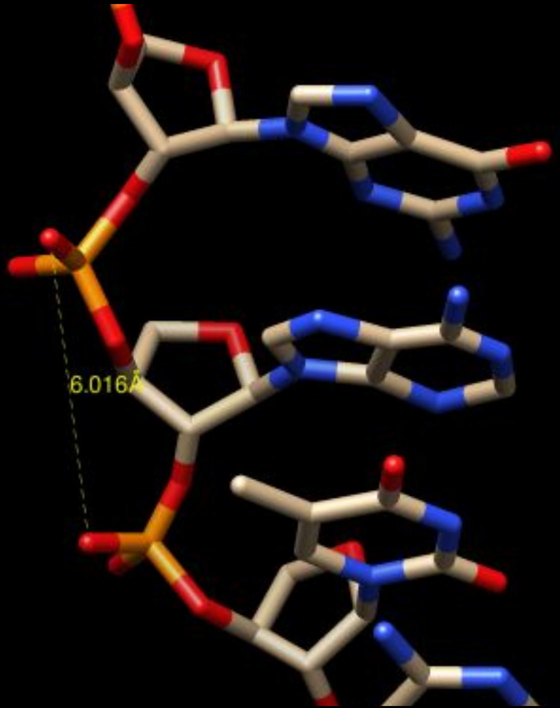


TNA

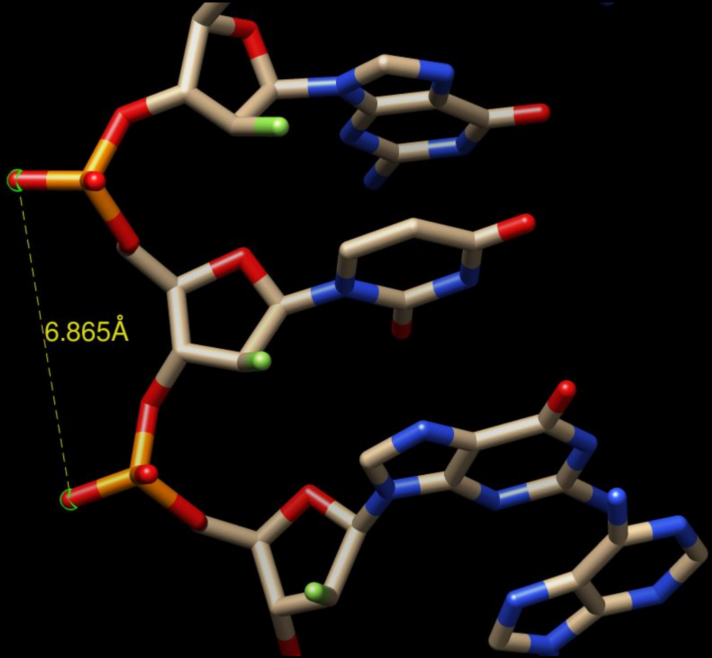


FANA

Intranucleotide distances

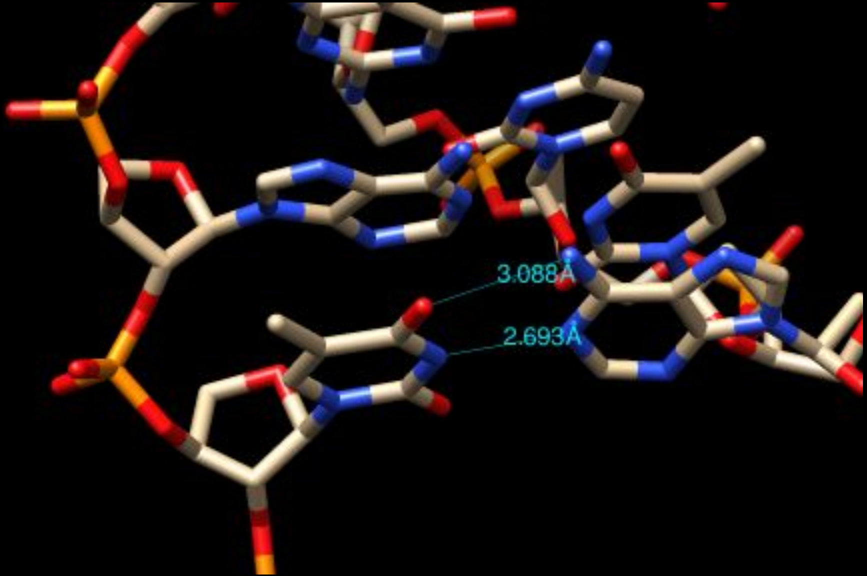


TNA

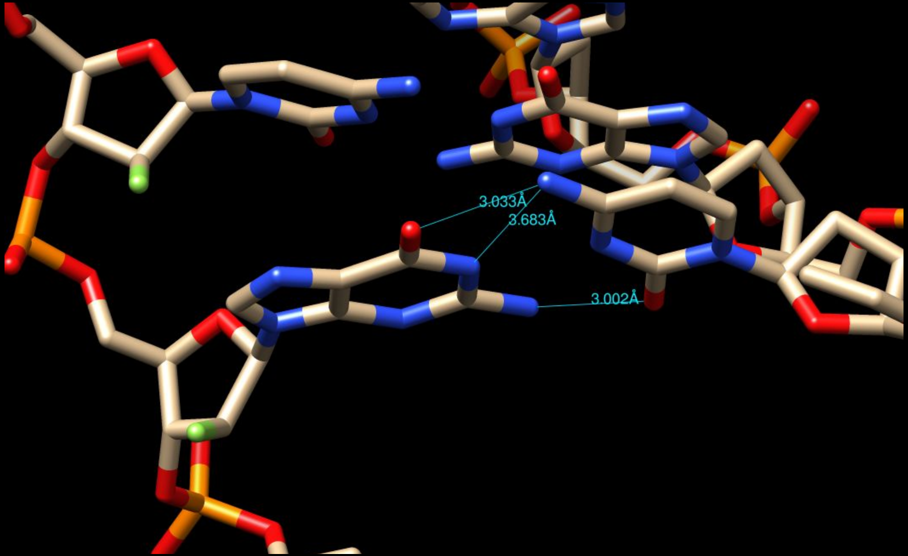


FANA

HBonds base pairs



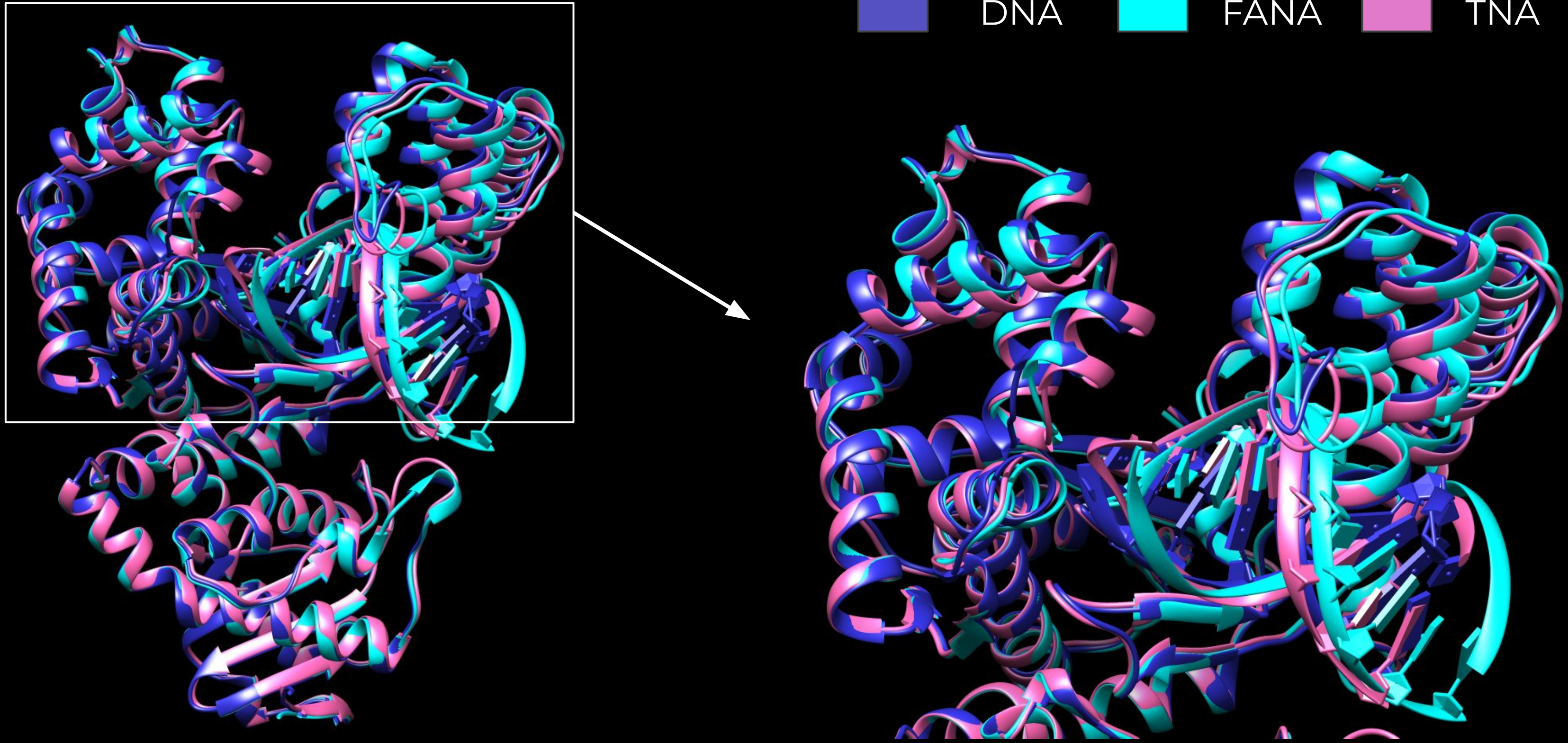
TNA



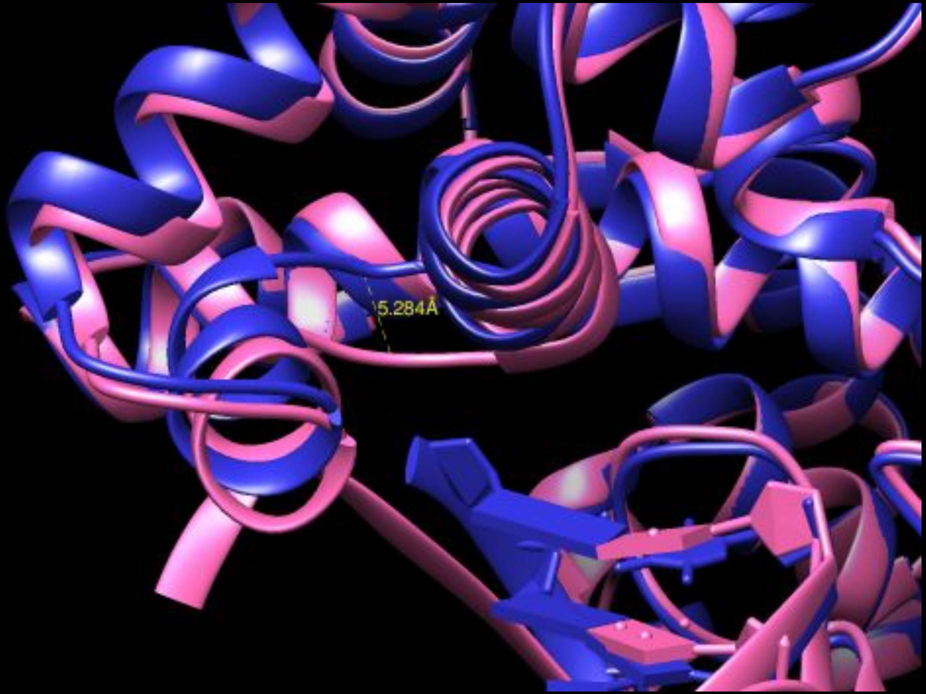
FANA

Superimposition

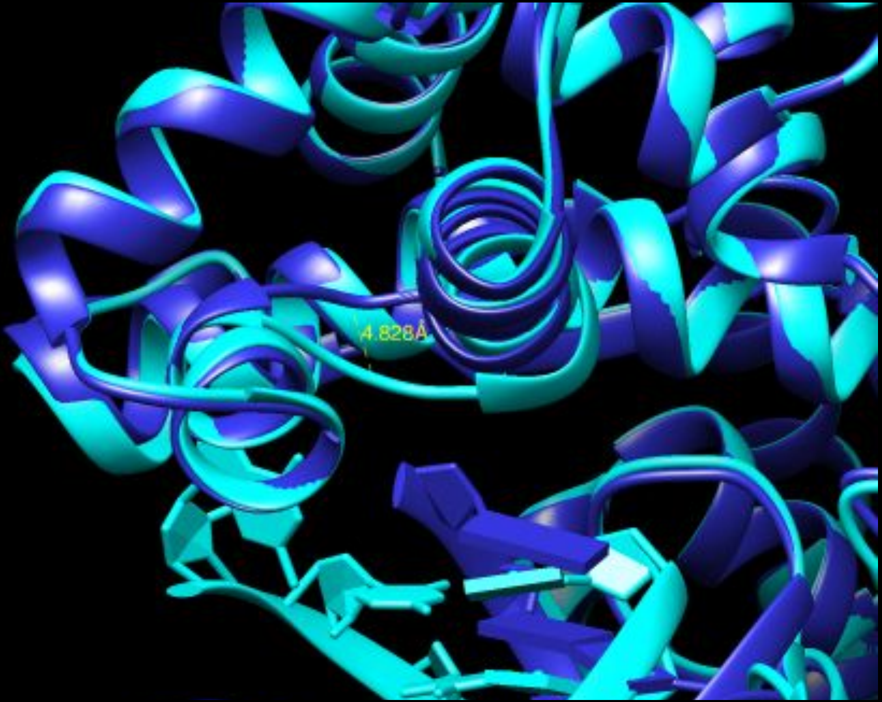
🟦 DNA 🟦 FANA 🟪 TNA



O helix



DNA/TNA



DNA/FANA

Conformations and Interactions

- Movement → finger domain and helix O/O1
- Interactions palm → recognition template
- Tyr 714 similar orientation
- B-form helix duplex

Final View

- DNA Polymerases is a **huge protein superfamily** present in all domains of life
- There are different **families**, adopting new additional functions
- Its **conformation** is essential for a correct nucleotide incorporation: Ajar, Open and Close are the only ones crystallized
- DNA Pol I presents a high **fidelity** rate thanks to the Pre-Insertion Site
- The **Pre-Insertion Site** is highly conserved in A-family DNA Polymerases

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Thanks for your attention

PEM Questions

1. Which is the main change observed between open and closed conformations?
    a. A new interaction between residues of the palm
    b. A 40° rotation of the O Helix
    c. A shorter distance between the alpha-phosphate of the dNTP and 3’OH of the primer strand
    d. The incorporation of two $Mg^{2`}$ ions
    e. B and C are correct

2. In relation to the polymerization process, the <u>incorrect</u> answer is:
    f. The Pre-Insertion Site avoids an early contact between the two bases
    g. The phosphodiester bond occurs after the hydrogen bonds between the base pair
    h. Two metal ions (Mg^{2+}) are needed in order to catalyze the reaction
    i. The base pair formed don’t need to be align with the other base pairs
    j. The active site is mostly hydrophilic

3. At the DNA duplex binding region:
    k. There are Hydrogen bonds between the bases and side-chains with water molecules as intermediaries
    l. The DNA is adopting a A-from conformation in the first three base pairs
    m. The interactions in this region are in the minor groove and the phosphate-sugar backbone
    n. All the answers are correct
    o. All the answers are wrong

4. Which domains are involved in the nucleotide incorporation?
    p. Thumb, palm, exonuclease
    q. Thumb, palm, finger
    r. Finger
    s. Palm, exonuclease
    t. Finger, exonuclease, thumb

PEM Questions

5. To which DNA polymerase family does the DNA polymerase I belong?
    a. Family X
    b. Family B
    c. Family A
    d. Family C
    e. Family D
6. Which is the most stable conformation that participates in the polymerase activity?
    a. Open
    b. Closed
    c. Ajar
    d. A and C are correct
    e. All the conformations are unstable
7. Relative to DNA polymerase general functions, which is the <u>incorrect</u> answer:
    a. It has proofreading activity (exonuclease 3’ - 5’)
    b. Okazaki fragments belong to the lagging strand
    c. Single stranded binding proteins are necessary to stabilize DNA
    d. Exonuclease 5’ - 3’ activity remove RNA primer
    e. All the answers are incorrect
8. The correct answer is:
    a. FANA contains a fluorine in the sugar
    b. DNA pol conformation that adopts with TNA is not exactly the same as with DNA
    c. A and B are correct
    d. Geobacillus stearothermophilus polymerase can naturally catalyze DNA from XNA templates
    e. **All the answers are correct**

PEM Questions

9. Ajar conformation is normally done when:

   a. Mismatch
   b. Before open conformation
   c. Between open and close conformation
   d. Only at high temperatures
   e. All the answers are correct

10. Which of these reasons does <u>not</u> happen during the polymerization?

   a. The alignment between the two nitrogenous bases
   b. The closing of the conformation thanks to the O Helix
   c. A conformational change of the DNA
   d. An hydratation of the DNA inside the active site
   e. Two Mg^{2+} ions binding inside the insertion site