

NUDIX superfamily



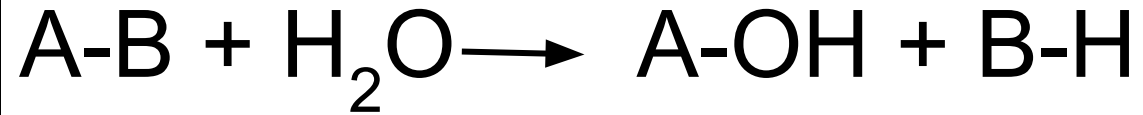
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Neus Font
Anna Llopart
Xavier Martí

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HYDROLASES

An hydrolase is an enzyme that catalyzes the hydrolysis of a chemical bond.



HYDROLASES CLASSIFICATION

HYDROLASES → EC 3

BREAK ACID ANHYDRIDES → EC 3.6

PHOSPHORUS-CONTAINING ANHYDRIDES → EC 3.6.1

NUDIX HYDROLASE SUPERFAMILY

- Found in all **3 domains**:
 - archaea
 - bacteria
 - eukarya
- This family catalyzes the hydrolysis of **Nucleoside Diphosphates** linked to other moieties, **X**.

Evolutionary related proteins → common mechanistic attribute

NUDIX FAMILIES

SCOP CLASSIFICATION

1. MutT-like family
2. MutY C-terminal domain-like family
3. NADH pyrophosphatase family
4. IPP isomerase-like family
5. GDP-mannose mannosyl hydrolase NudD family
6. BT0354 N-terminal domain-like family
7. mRNA decapping enzyme-like family

NUDIX MOTIF

X refers to any aminoacid

G₅X₇EXREUXEEEXGU

U refers to hydrophobic an
aminoacid

SEQUENCE ALIGNMENT

MUTT_D.melanogaster	GAIVANPTTgkniiQMVAIQSDN-----KLWAIP	GMVDPGENVSV-TLKREFTEEA-L
MUTY_H.sapiens	TCVLEQPGAl--gaQILLVQRPNSGL--LAGLWEPF	VTWEPSEQLQRkALLQELQRWAGP
NADH-pyrophosphatase_E.coli	IVAIRRDD-----SILLAQHTRHR---NGVHTVL	GFVEVGETLEQ-AVAREVMEESEGI
IPP-isomerase-like_D.radiodurans	NAFLRNSQG----QLWIPRRSPSKSLFPNALDVSV	GAVQSGETYEE-AFRREAREELNV
GDP-mannose-mannosyl-hydrolase_E.coli	DFIVENSRG----EFLLGKRTNRP---AQGYWVP	GRVQKDETLEA-AFERLTMAELGL
N-terminal-domain-like_B.thetha	-CIIFGFNEg--eiSLLLLKRNFEF---AMGEWSLM	GFVQKDESVDD-AAKRVLAELTGL
mRNA-decapping-complex-subunit2_S.pombe	-GAIMLDMSm---qQCVLVKGWKAS-----SGWGFP	GKIDKDESDVD-CAIREVYEETGF

GMVDPGENVSV-TLKREFTEEA-L
 VTWEPSEQLQRkALLQELQRWAGP
 GFVEVGETLEQ-AVAREVMEESEGI
 GAVQSGETYEE-AFRREAREELNV
 GRVQKDETLEA-AFERLTMAELGL
 GFVQKDESVDD-AAKRVLAELTGL
 GKIDKDESDVD-CAIREVYEETGF

G₅X₇REUXEE₇XGU

NUDIX STRUCTURE

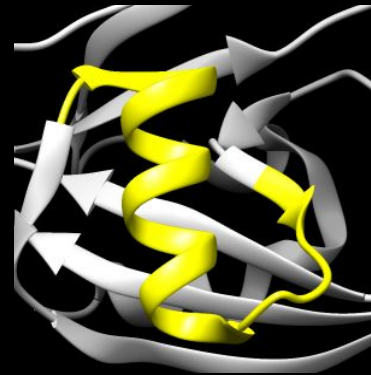
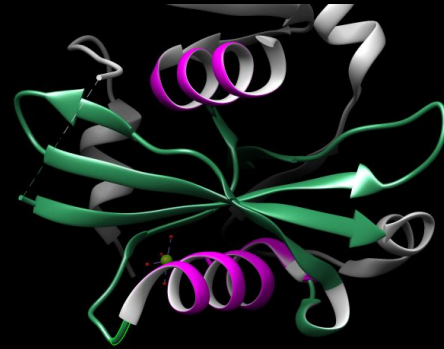
NUDIX FOLD
 $\alpha/\beta/\alpha$ sandwich



NUDIX MOTIF
loop- α helix-loop



GX₅EX₇REUXEEEXGU



NUDIX FAMILIES

1. MutT-like family

3.6.1.13 ADP-ribose diphosphatase

3.6.1.52 Diphosphoinositol-polyphosphate diphosphatase

3.6.1.55 8-oxo-dGTP diphosphatase

- COFACTOR UNION
- CATALYTIC MECHANISM
- SPECIFICITY

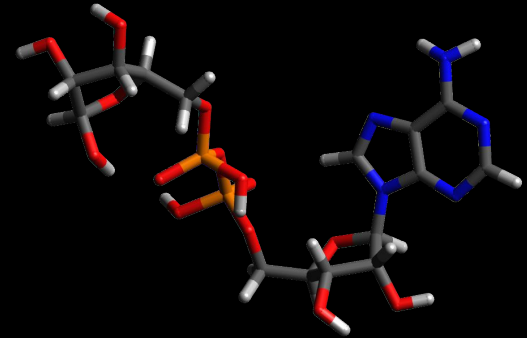
ADP-RIBOSE PYROPHOSPHATASES (*E. coli*)

- Subfamily → ADPR pyrophosphatases
- Gene: nudf
- Proline 15 residues downstream of Nudix Motif
- Avoid high levels of ADPR

└─→ nonenzymatic ADP-ribosylation of proteins

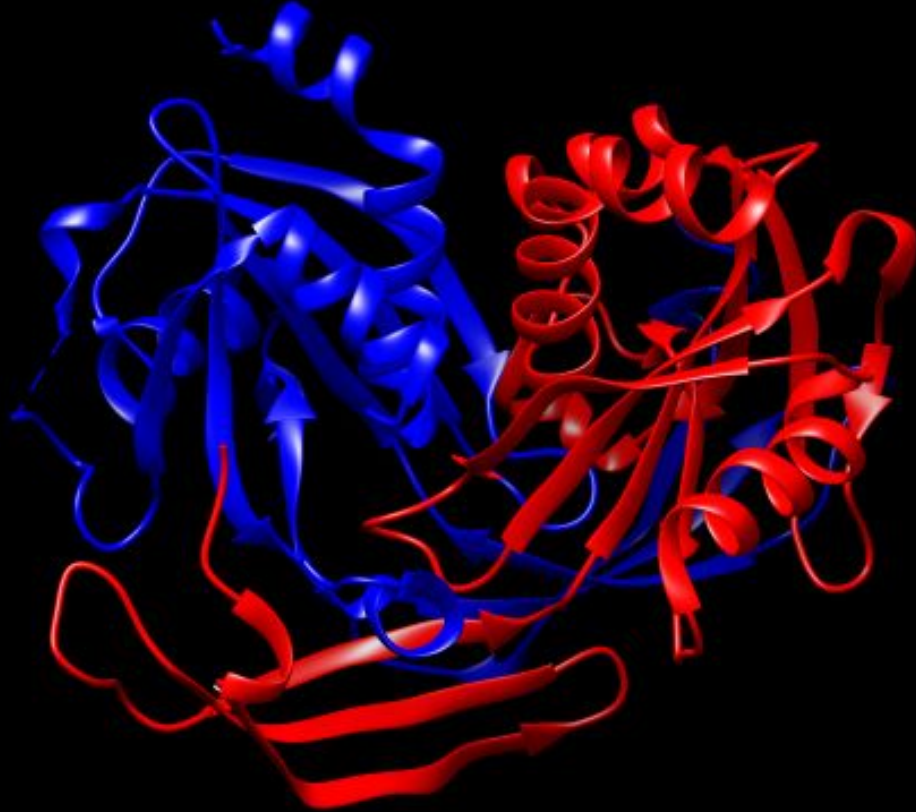


1. Enzyme inactivation
2. Apoptosis
3. Cytotoxicity



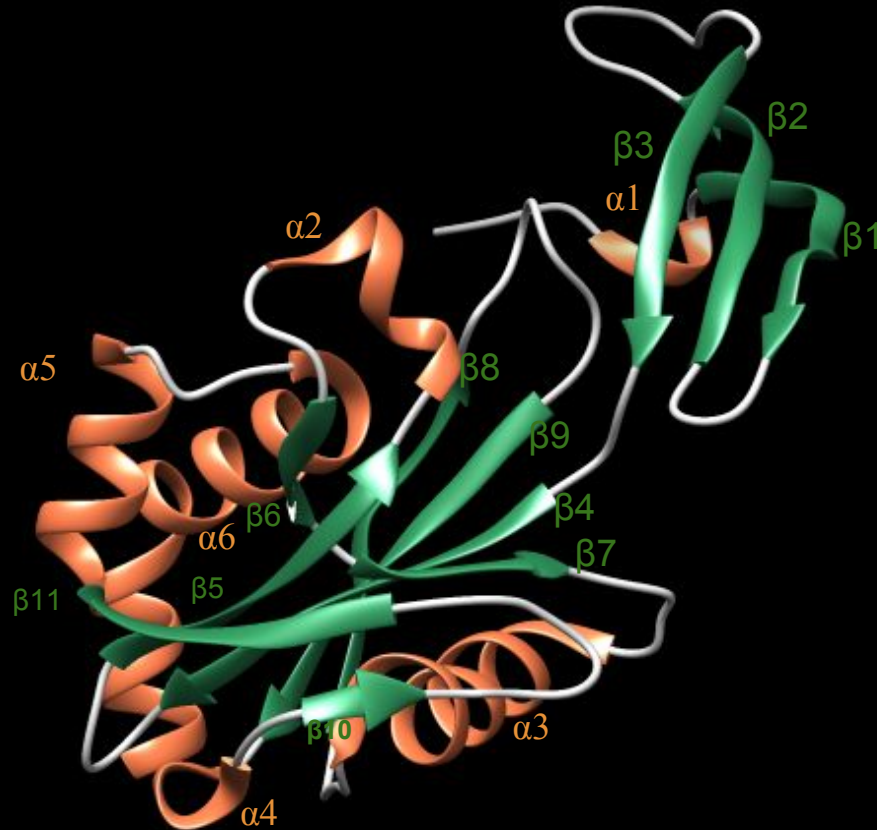
ADP-RIBOSE PYROPHOSPHATASE

Structure analysis



ADP-RIBOSE PYROPHOSPHATASE

Structure: Nudix domain

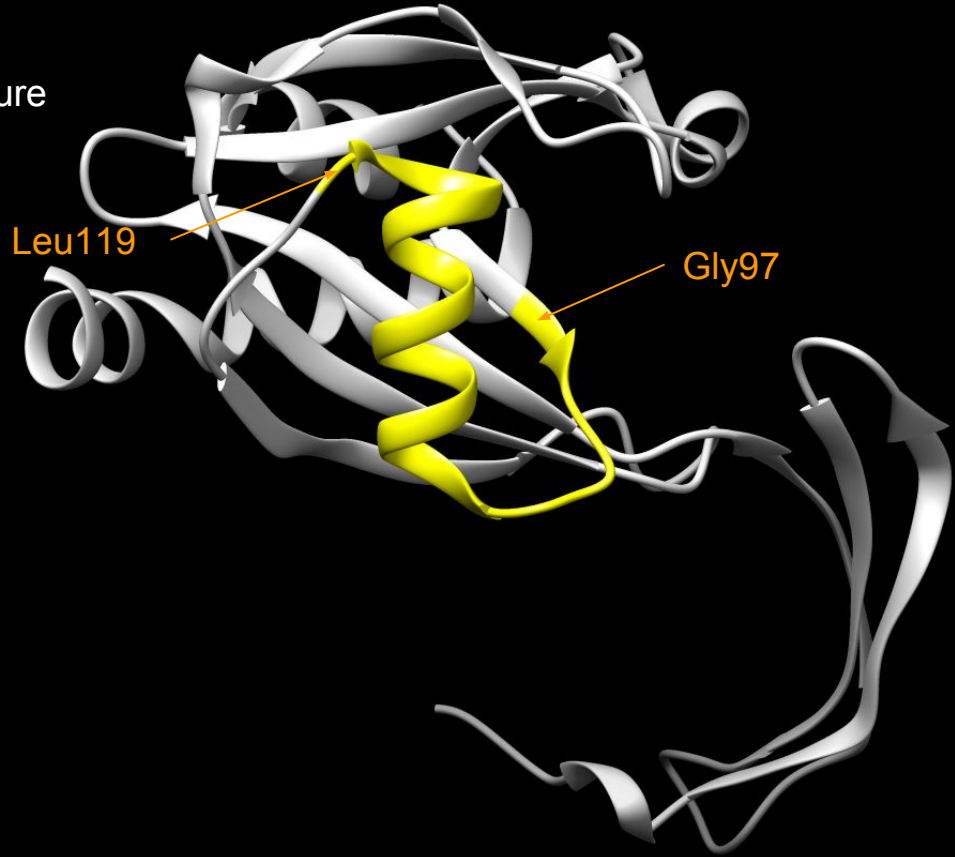


6 α -helices + 11 β -strands
alpha-beta-alpha sandwich

ADP-RIBOSE PYROPHOSPHATASE

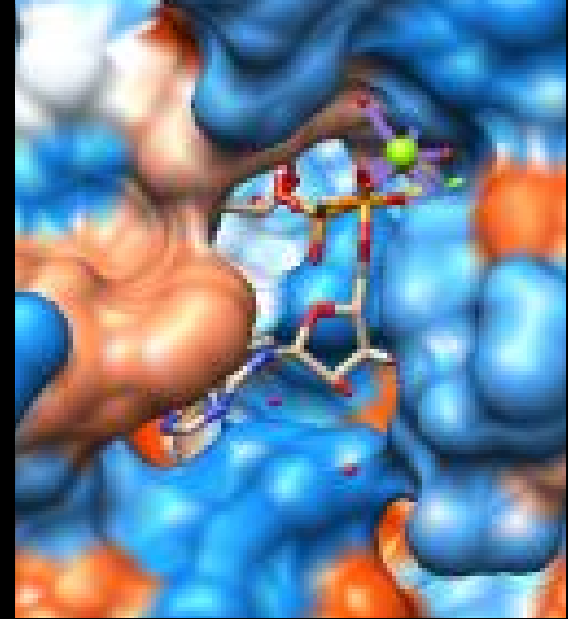
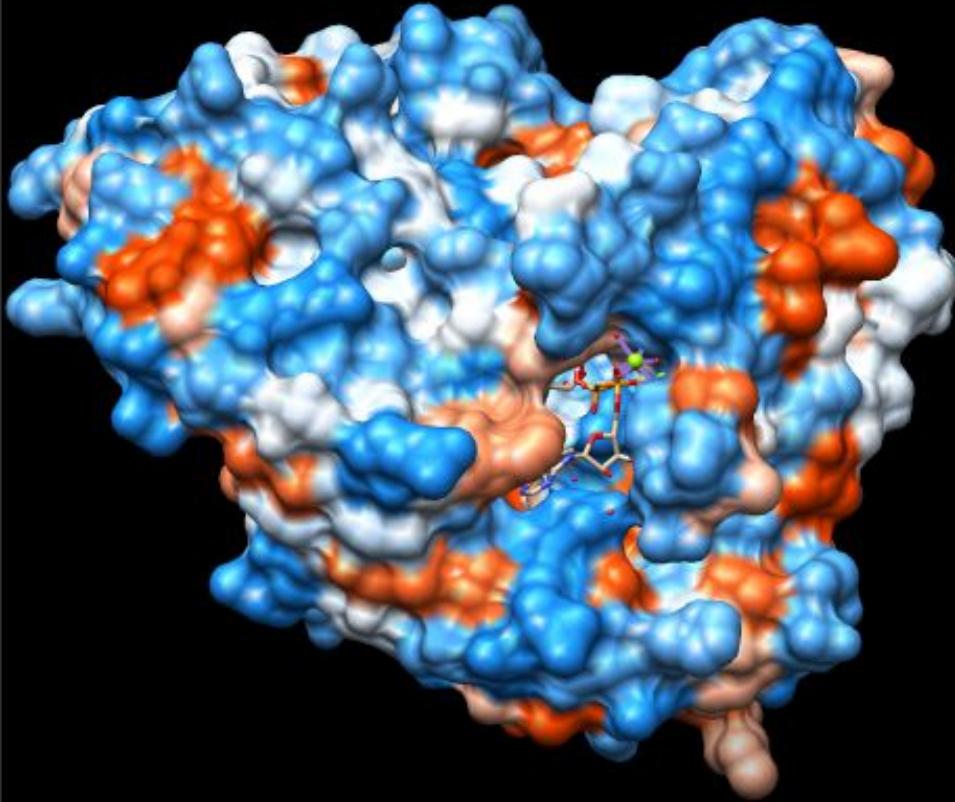
Structure: Nudix motif

Gly97 to Leu119
loop-helix-loop structure



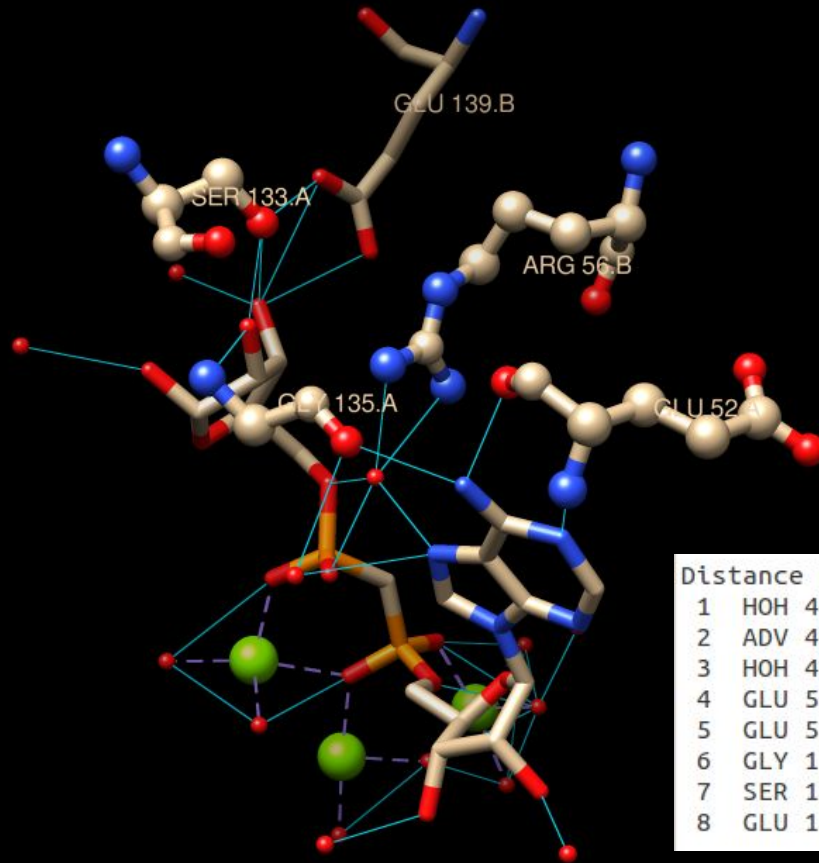
ADP-RIBOSE PYROPHOSPHATASES

Substrate Binding Site



ADP-RIBOSE PYROPHOSPHATASES

Substrate Binding Site

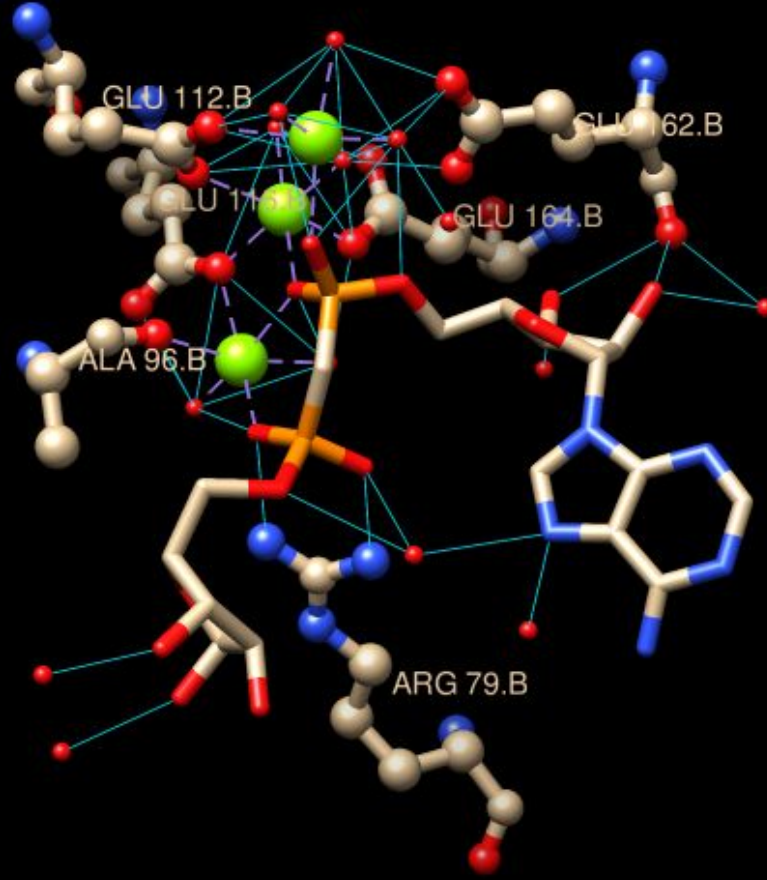


Distance information

1	HOH 408.B O <-> ARG 56.B NH2:	2.522
2	ADV 402.B OR5 <-> HOH 408.B O:	3.067
3	HOH 408.B O <-> ADV 402.B O1B:	2.789
4	GLU 52.A O <-> ADV 402.B N6:	2.958
5	GLU 52.A N <-> ADV 402.B N1:	3.040
6	GLY 135.A O <-> ADV 402.B N6:	3.099
7	SER 133.A OG <-> ADV 402.B OR3:	3.089
8	GLU 139.B OE2 <-> ADV 402.B OR3:	2.893

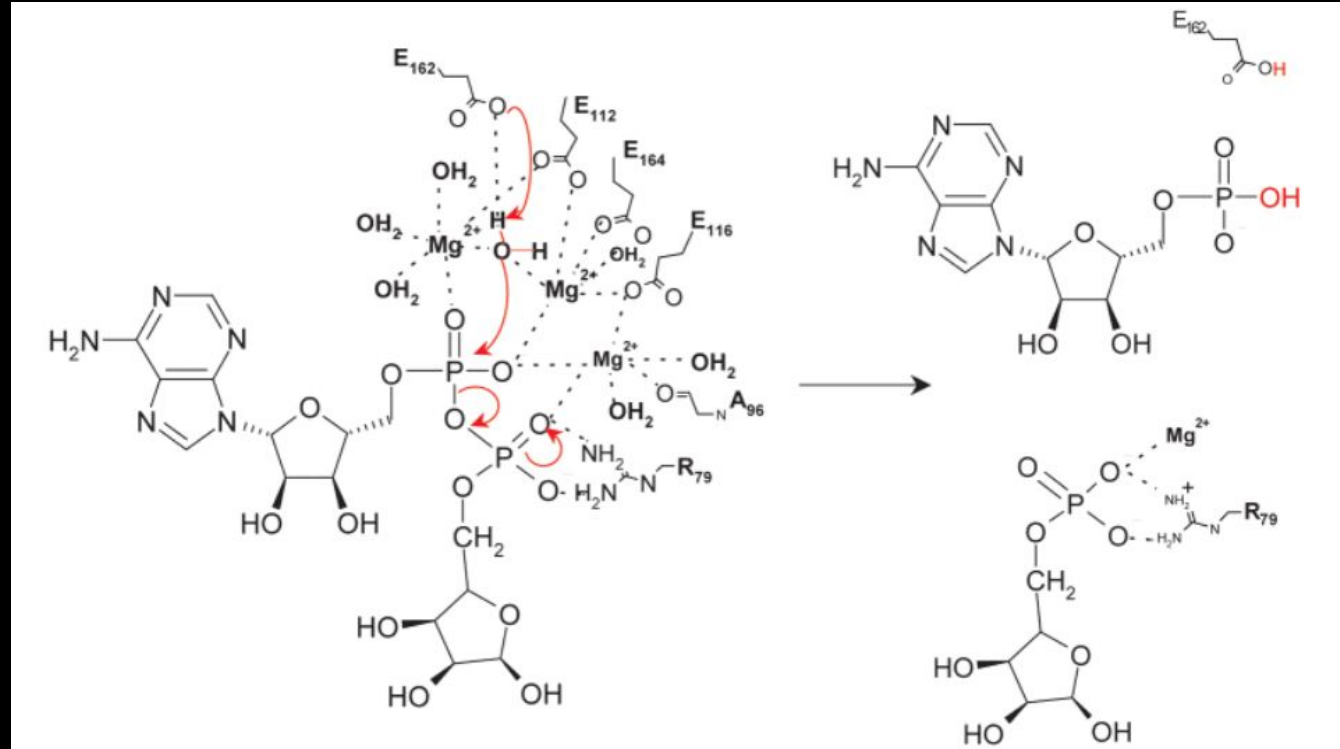
ADP-RIBOSE PYROPHOSPHATASES

Catalytic and Mg^{+} binding site



ADP-RIBOSE PYROPHOSPHATASES

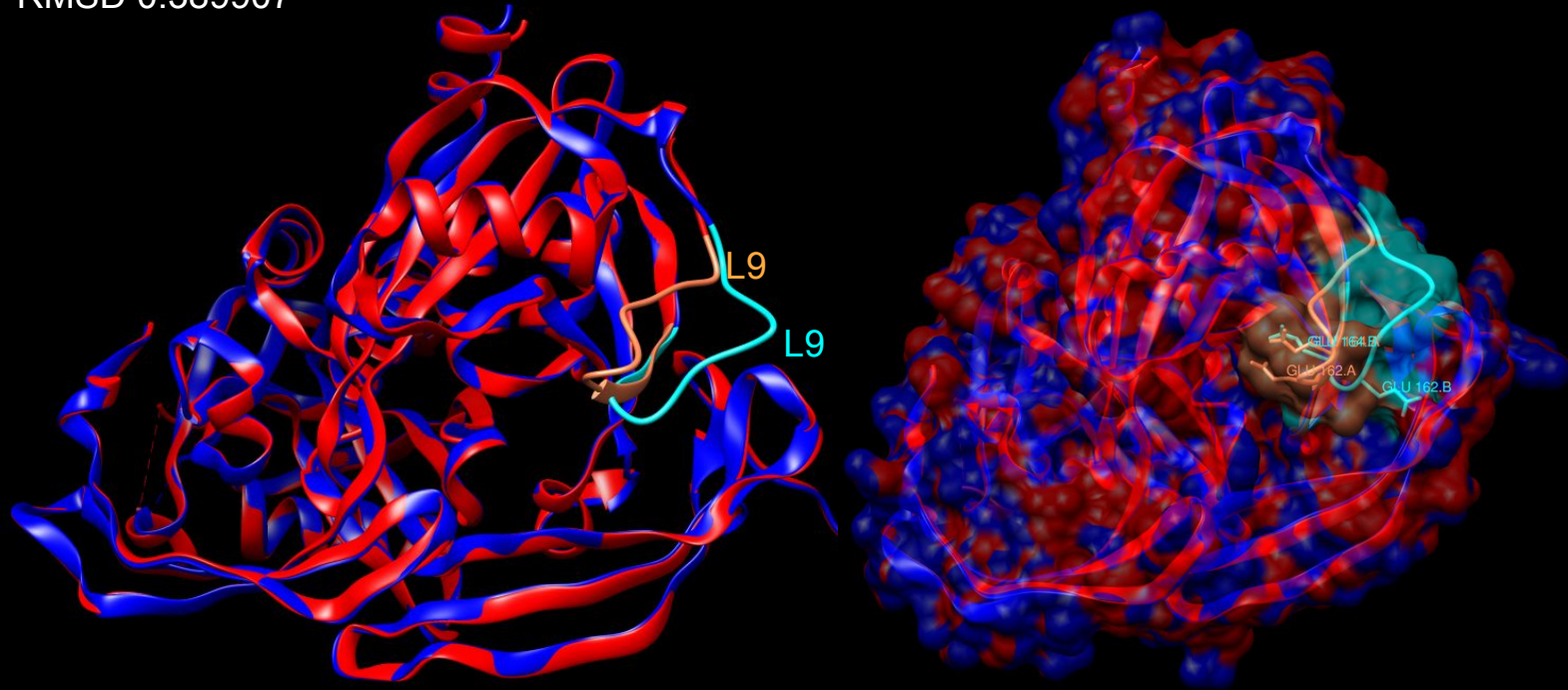
Catalytic Mechanism



ADP-RIBOSE PYROPHOSPHATASES

Superimposition

RMSD 0.389907



ADPR pyrophosphatase+substrate
ADPR pyrophosphatase

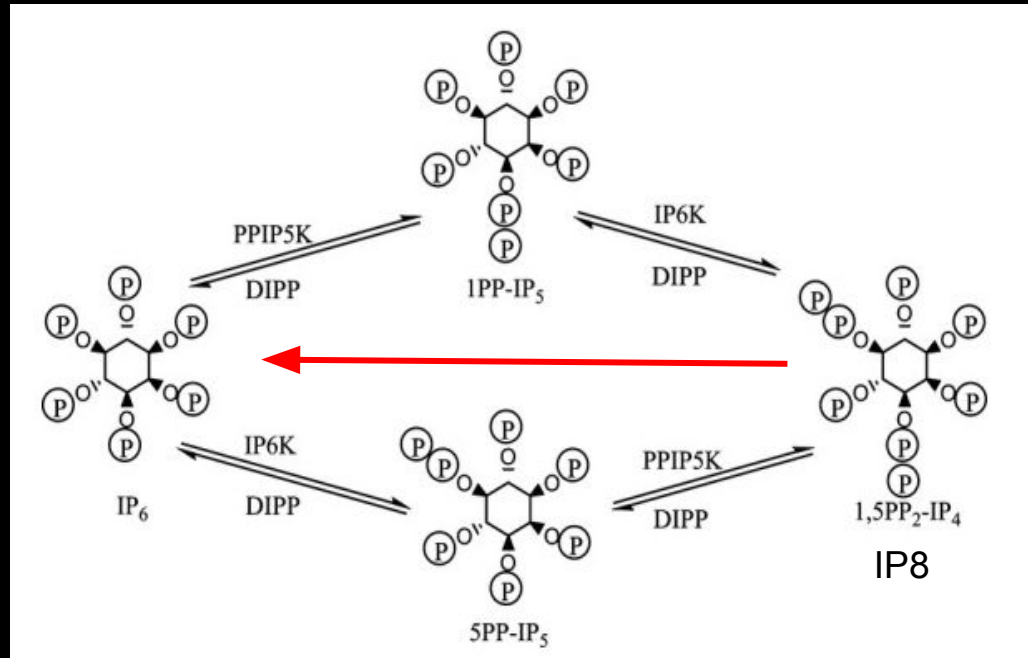
ADP-RIBOSE PYROPHOSPHATASES

Multiple Sequence Alignment

	10.	30.	50.
E. coli	-ml--KPDNLPVTFGK-NDVEIIARETLYRG-FFSLDLYRFRHRLFNGQMSHEVREIFE		
H. influenza	mqfWRKQMSEIQHFSQ-QDIEILGEQTLYEG-FFTLKRIQFKHKLFAGGQSGVVTTRELLI		
M. jannaschii	--m-----AKKCFICISGKIFALNLF GKRYSKKIIKKRDLKKYRLYL		
M. musculus	--m---ETRESTESSP-GKHLVTSEELISEGKWVKFEKTTYMDPTGKTRTWETVKLTTRK		
H. sapiens	-----MESQEPTESSQNGKQYIISEELISEGKWVKLEKTTYMDPTGKTRTWESVKRTTRK		
	70.	90.	
E. coli	-R-GHAAVLLPFDPV---RDEVVLIEQIRIAAYDTSE---TPWLLEMVAGMIEEGESVED		
H. influenza	-K-GAASAVIAYDPK---EDSVILVEQVRIGAAYHPeshrSPWLLELIAGMVEKGEKPED		
M. jannaschii	---HPAVAVDGIIEK---DNKILLIKRKNPPFKGC-----FALPGGFVECGETVEE		
M. musculus	gKSADAVSVIPVLQRtLhHECVILVKQFRPPMGSY-----CLEFFAGFIEDGESPEA		
H. sapiens	eQTADGVAVIPVLQRtLhYECIVLVKQFRPPMGY-----CIEFFAGLIDDGETPEA		
	110.	130.	150.
E. coli	VARREAIEEAGLIVKRTKPVLSFLASPGGTSSERSS---IMVGEVDATTAS--GIHGLADE		
H. influenza	VALRESEEEAGIQVKNLTHCLSVWDSPPGGIVERIH---LFAGEVDSAQAK--GIHGLAEE		
M. jannaschii	AVVREIKEETGLIPKVKs-LLGVYSSP-DRDPRGH---VISIVFILDVIG--GELKAGDD		
M. musculus	AALRELEEETGYKGEVAECSPAVCM DPGLSNCTTHvvtVTINGDDAGNVRpkPKPGDGEF		
H. sapiens	AALRELEEETGYKGDIAECSPAVCM DPGLSNCTIHivtVTINGDDAENARpkPKPGDGEF		
	170.	190.	209.
E. coli	NEDIRVH---VVSREQAYQWVEEGKIDNAASVIALQWLQLHHQALKNEWA-----		
H. influenza	NEDIKVH---VVKREQAYQWMCEGKIDNGIAVIGLQWLQLNYAQLQSQWKR-----		
M. jannaschii	AKAEFF---DLNNLPKLAFDHEKIIKD-----ymrwkng		
M. musculus	MEVISLPkndLLTRLDALGAEQHLTVDAKVYAYGLALKHANSKPFEVPFLKf-----		
H. sapiens	VEVISLPkndLLQRLDALVAEEHLTVDARVYSYALALKHANAKPFEVPFLKf-----		

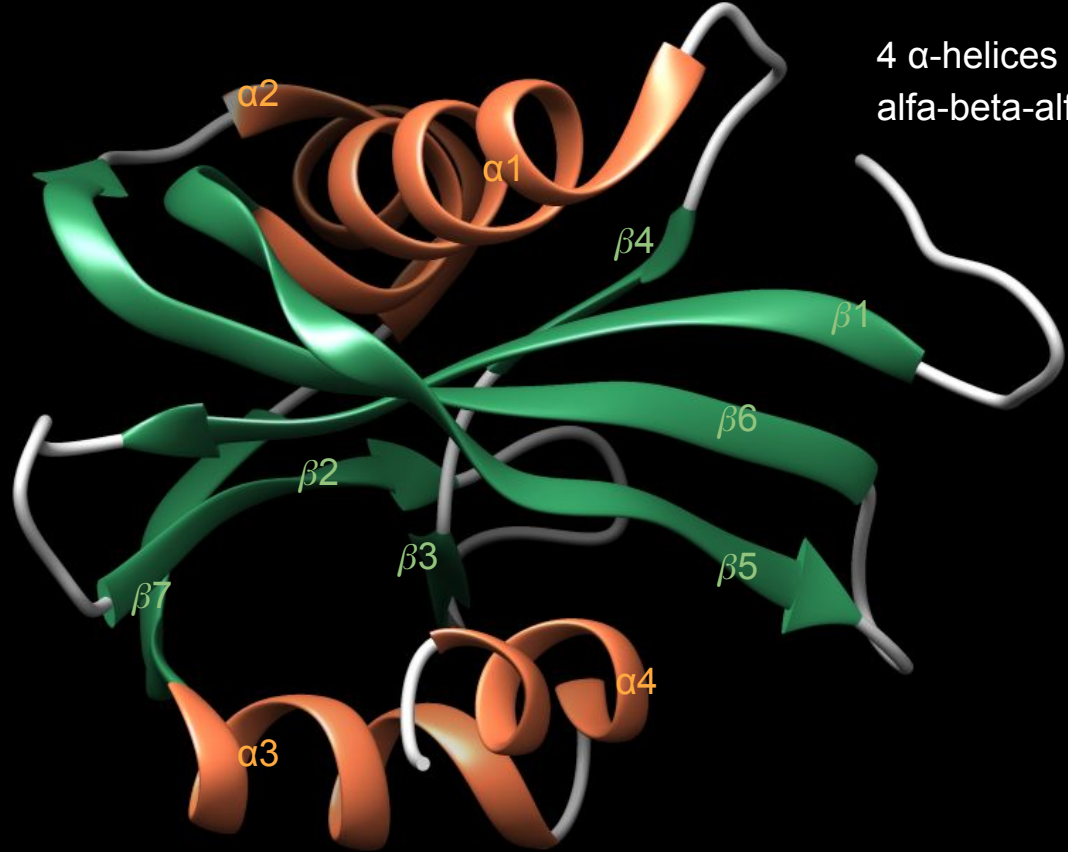
DIPHOSPHOINOSITOL POLYPHOSPHATE PHOSPHOHYDROLASE 1 (DIPP1)

H. sapiens



DIPP1

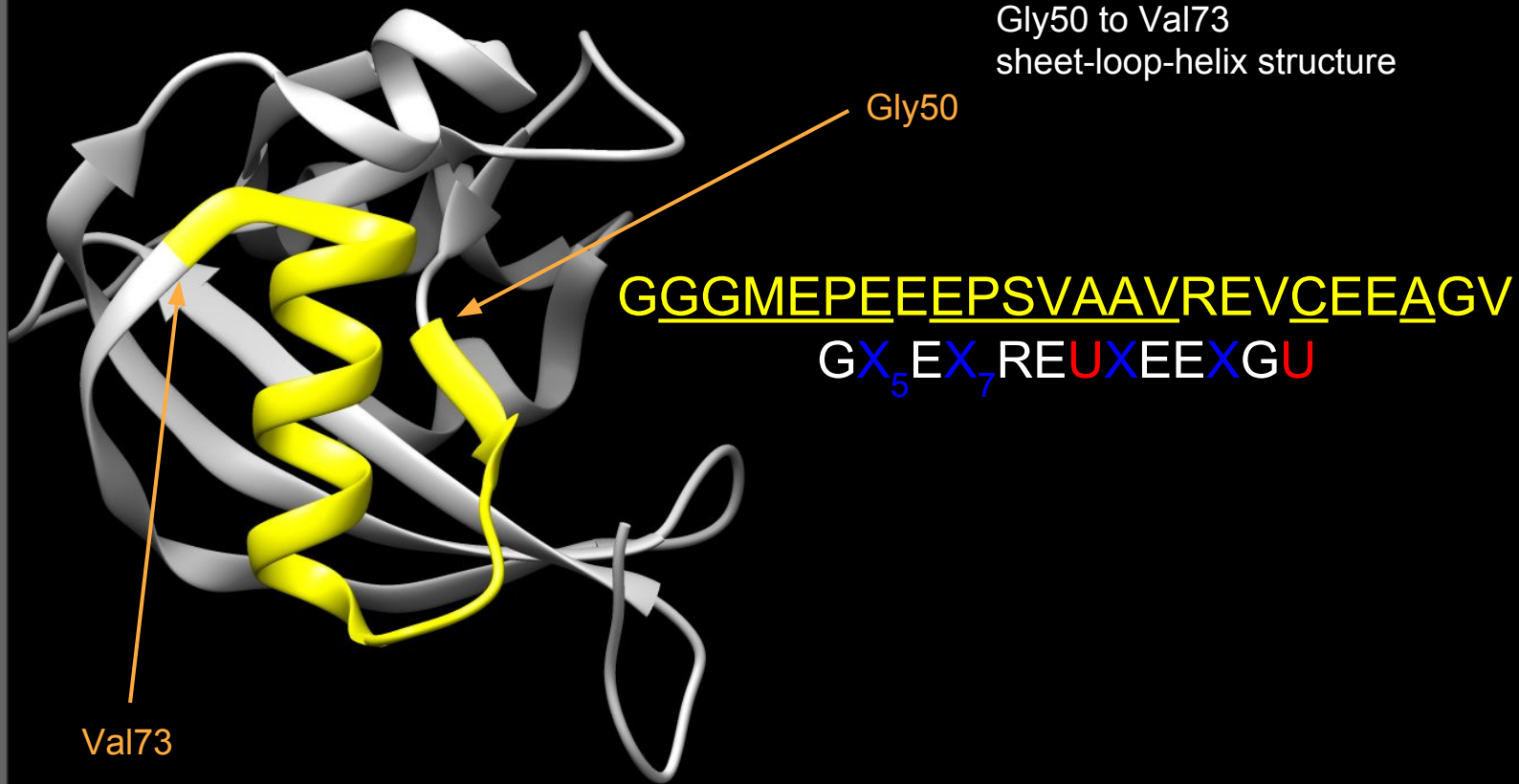
Structure: Nudix domain



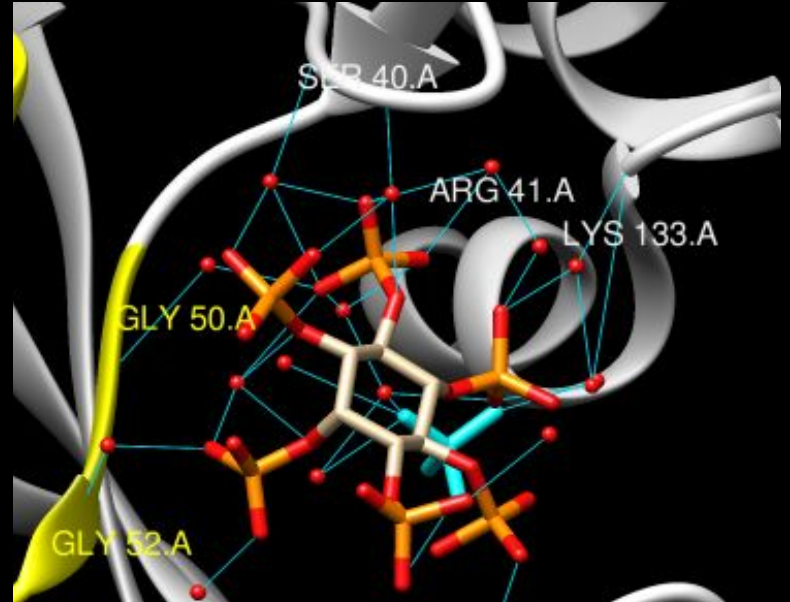
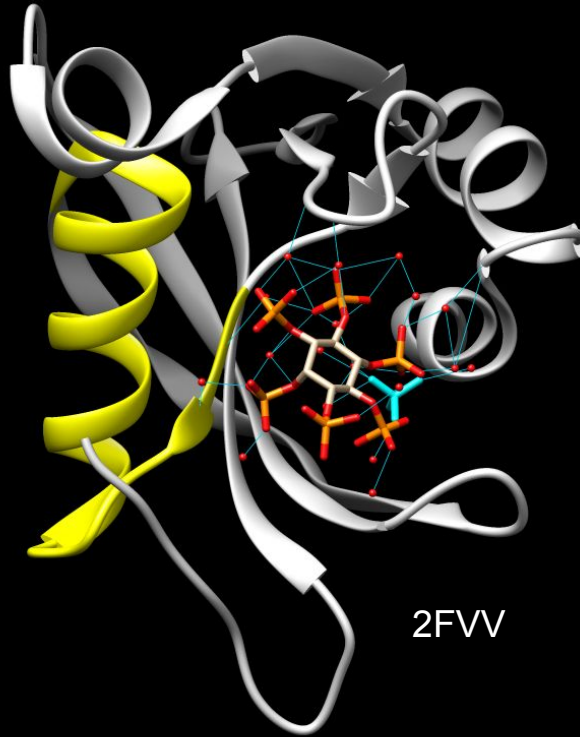
4 α -helices + 7 β -strands
alpha-beta-alpha sandwich

DIPP1

Structure: Nudix motif

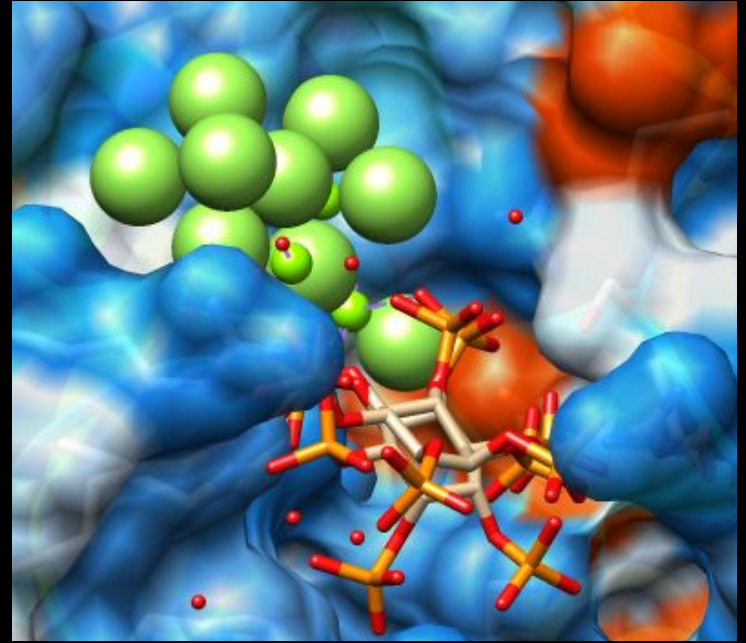
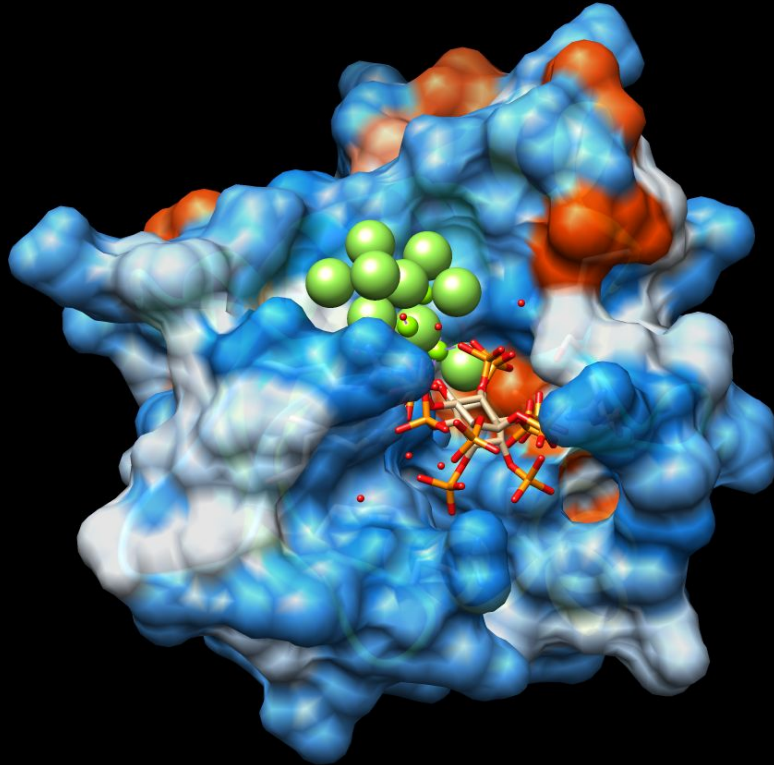


DIPP1



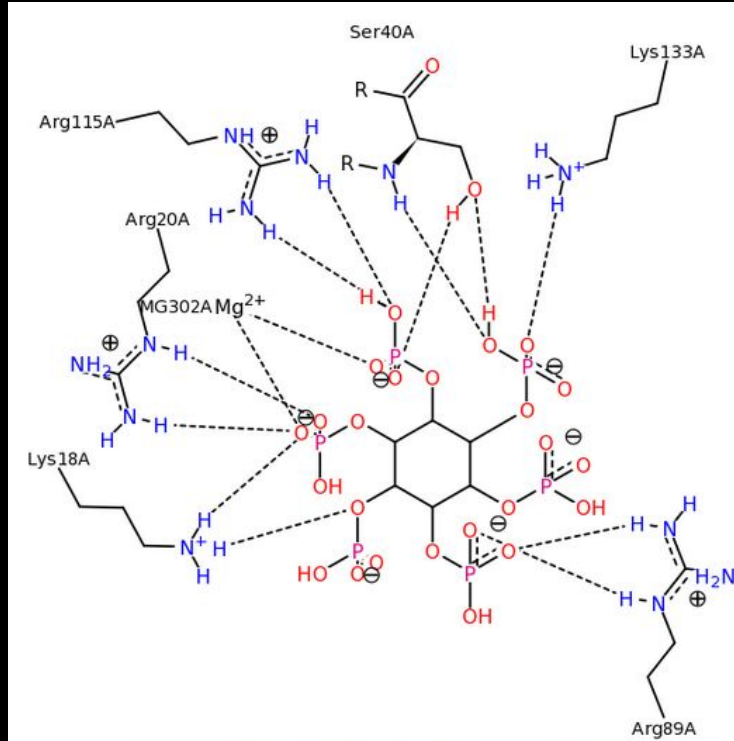
DIPP1

Substrate Binding Site



DIPP1

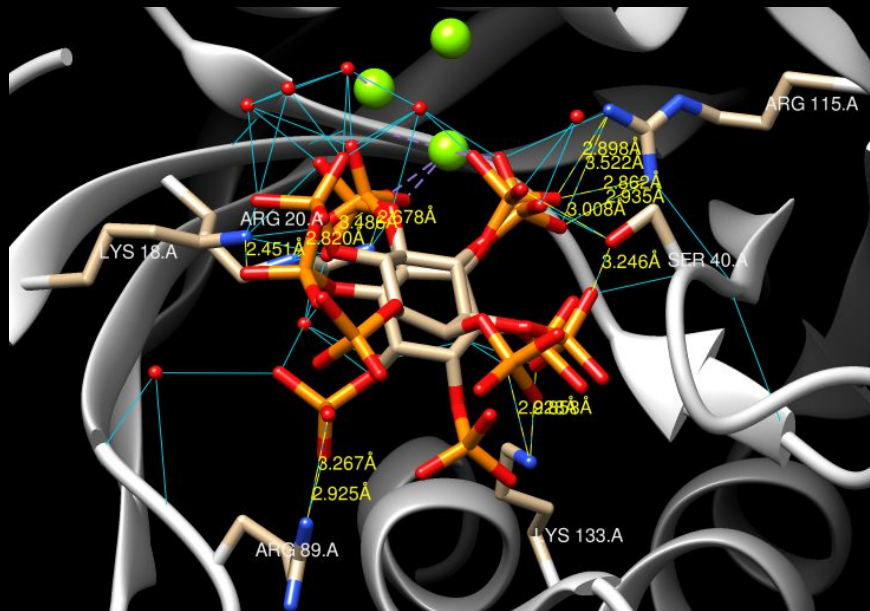
Substrate Binding Site



Thorsell, A. G. et. al.(2009)

DIPP1

Substrate Binding Site

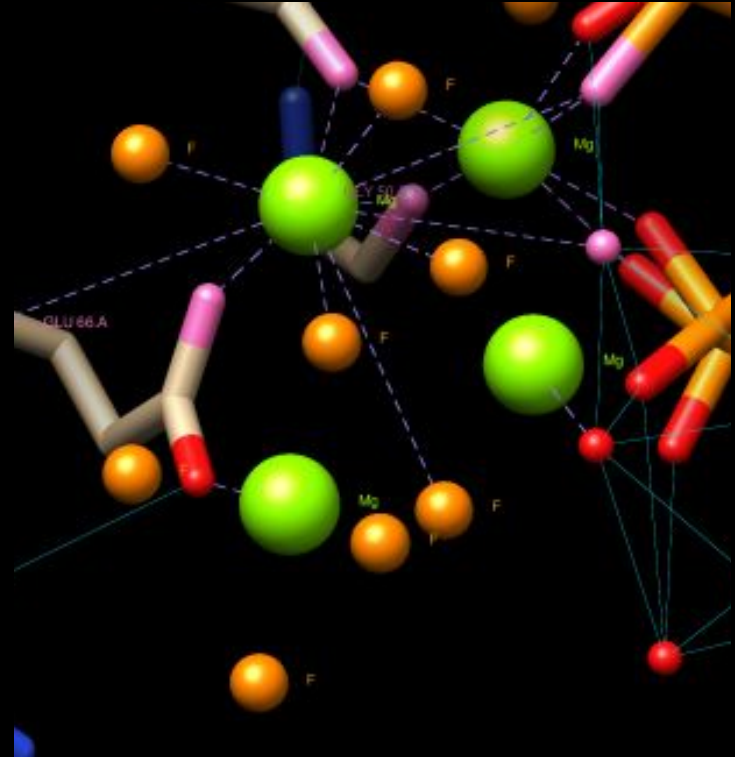
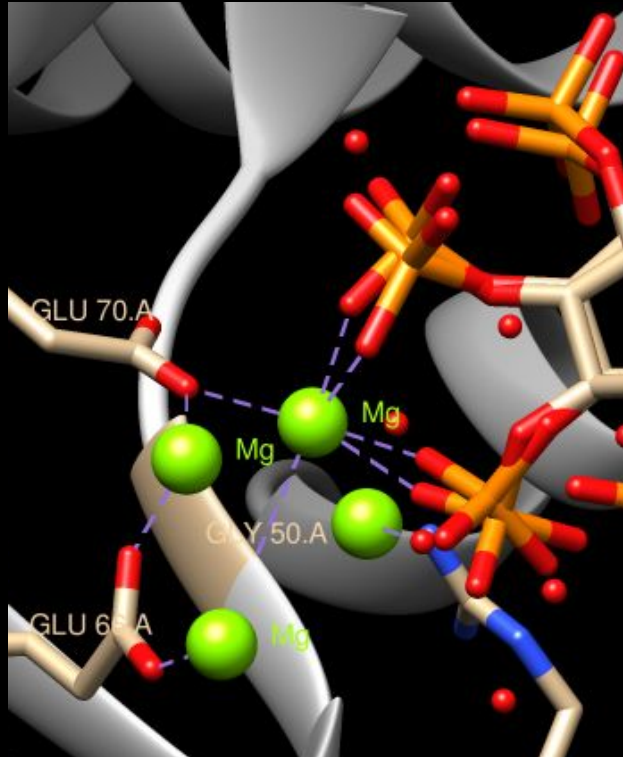


Distance information

1	IHP 201.A 025.A <-> SER 40.A OG:	3.008
2	IHP 201.A 046.A <-> SER 40.A OG:	3.246
3	IHP 201.A 045.A <-> ARG 115.A NH2:	2.898
4	IHP 201.A 044.B <-> ARG 115.A NH1:	2.935
5	IHP 201.A 045.A <-> ARG 115.A NH1:	2.862
6	ARG 115.A NH2 <-> IHP 201.A 044.B:	3.522
7	LYS 18.A NZ <-> IHP 201.A 023.A:	2.451
8	IHP 201.A 035.B <-> ARG 20.A NH2:	2.678
9	IHP 201.A 035.B <-> ARG 20.A NE:	3.486
10	IHP 201.A 034.A <-> ARG 20.A NE:	2.820
11	LYS 133.A NZ <-> IHP 201.A 043.B:	2.928
12	LYS 133.A NZ <-> IHP 201.A 026.A:	2.858
13	ARG 89.A NH2 <-> IHP 201.A 032.A:	3.267
14	ARG 89.A NE <-> IHP 201.A 022.A:	2.925

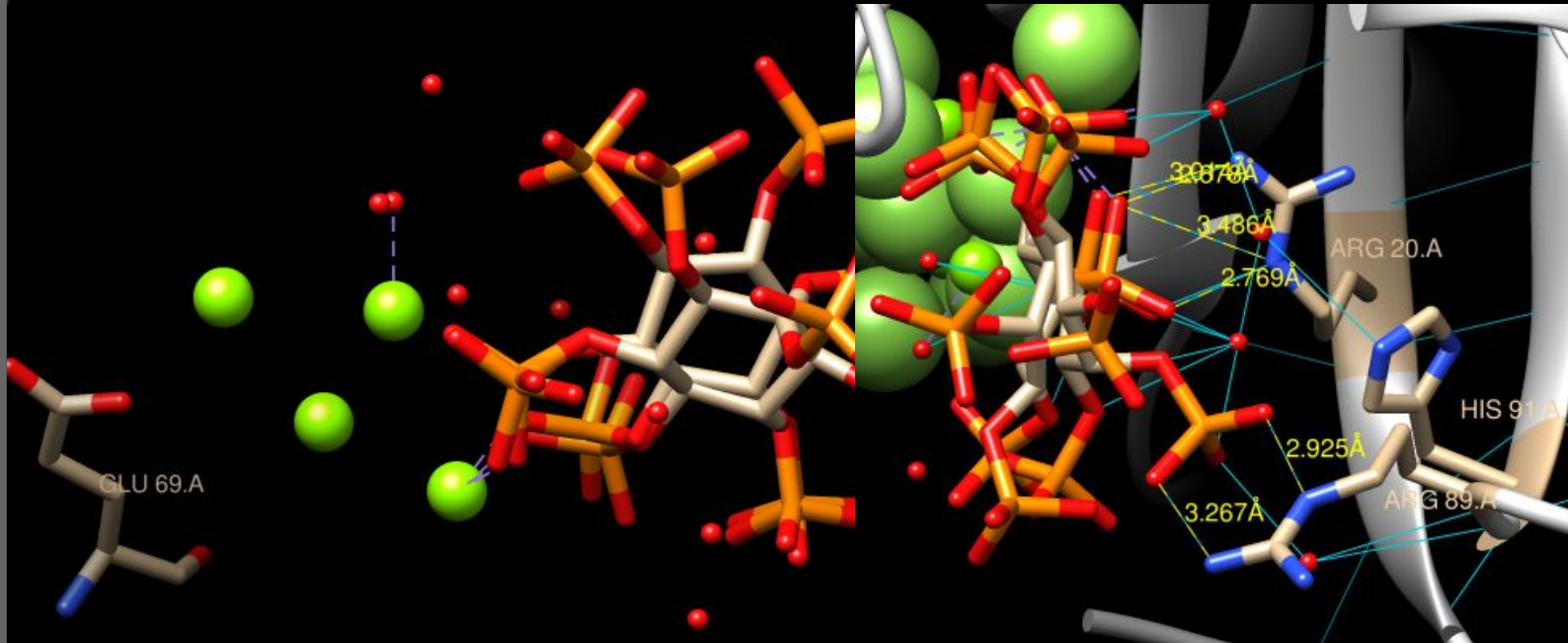
DIPP1

Mg⁺ Binding Site



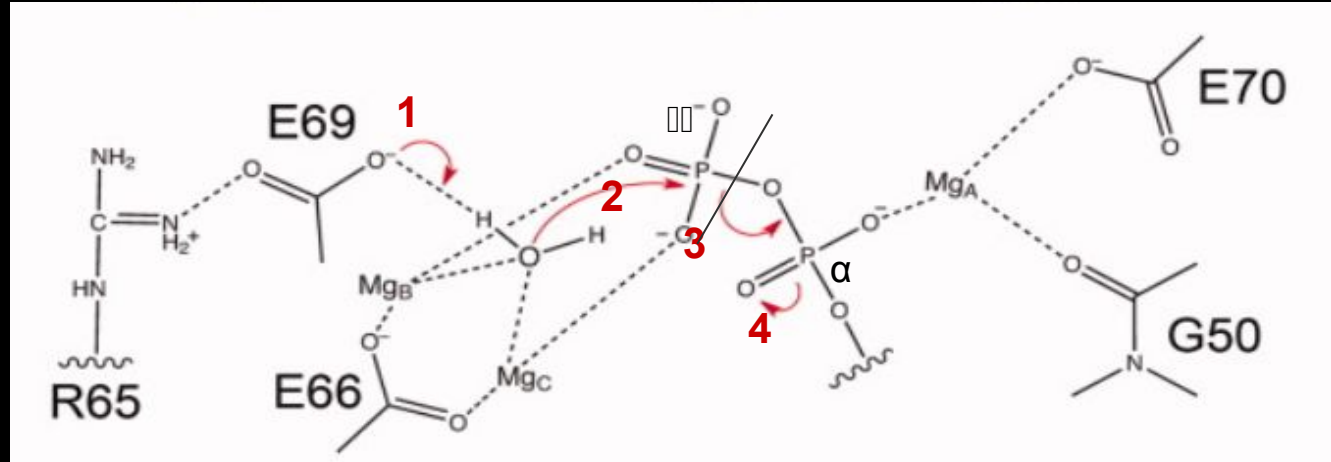
DIPP1

Catalytic Site



DIPP1

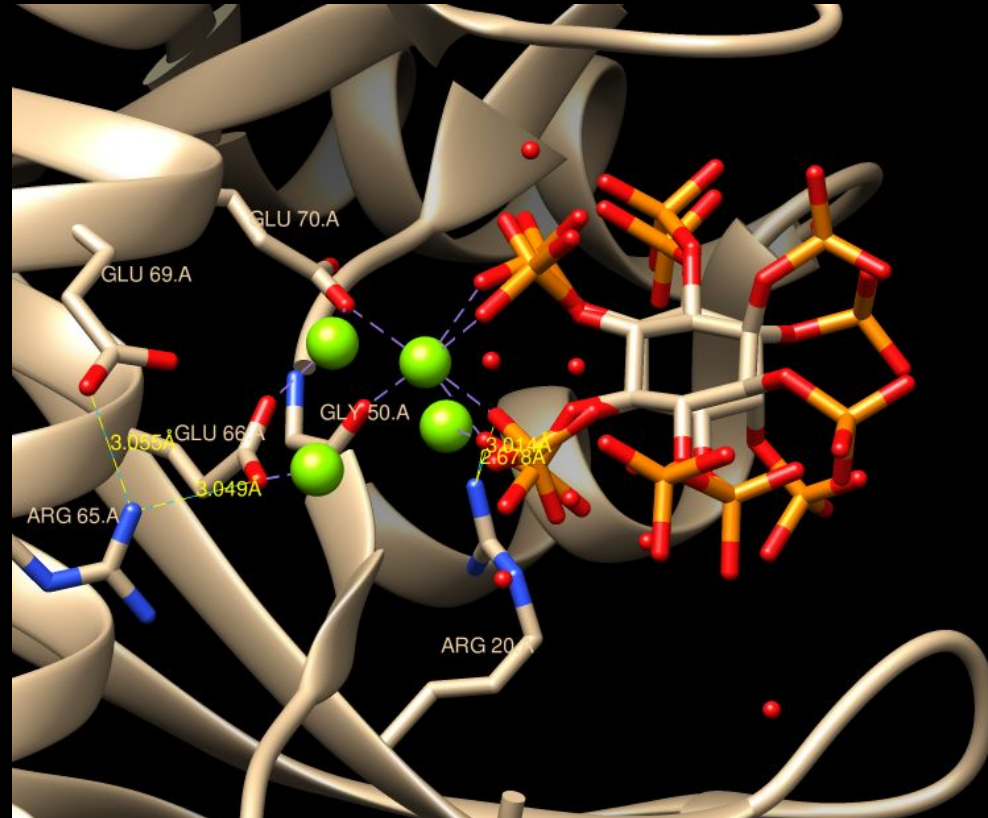
Catalytic mechanism



Modified from: Thorsell, A. G. et. al. (2009)

DIPP1

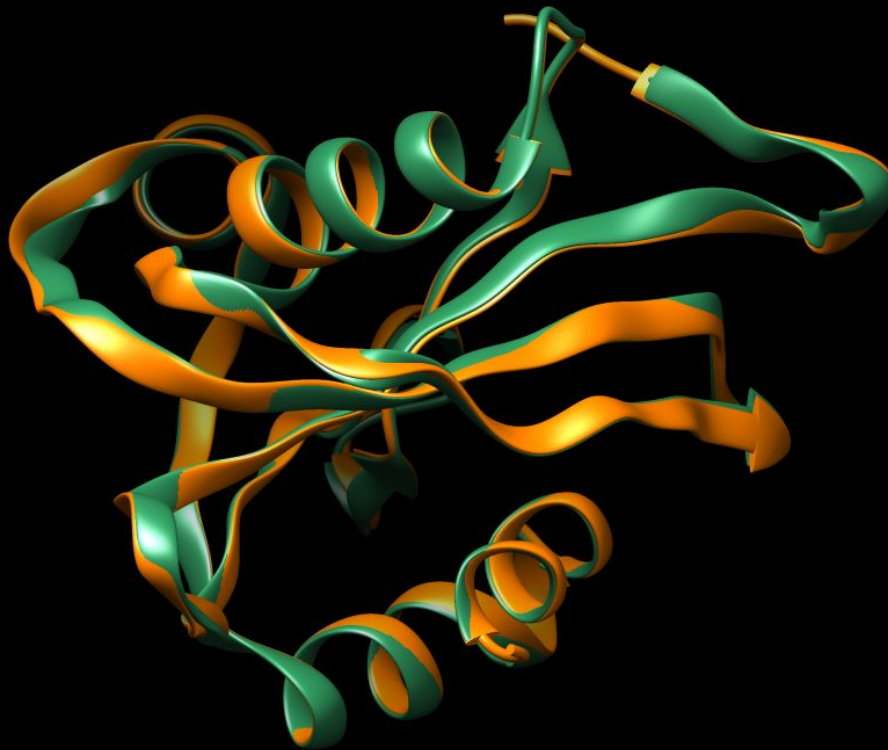
Catalytic mechanism



DIPP1

Superimposition

RMSD = 0.35



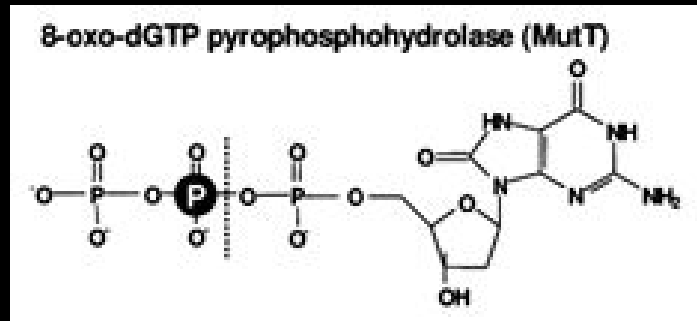
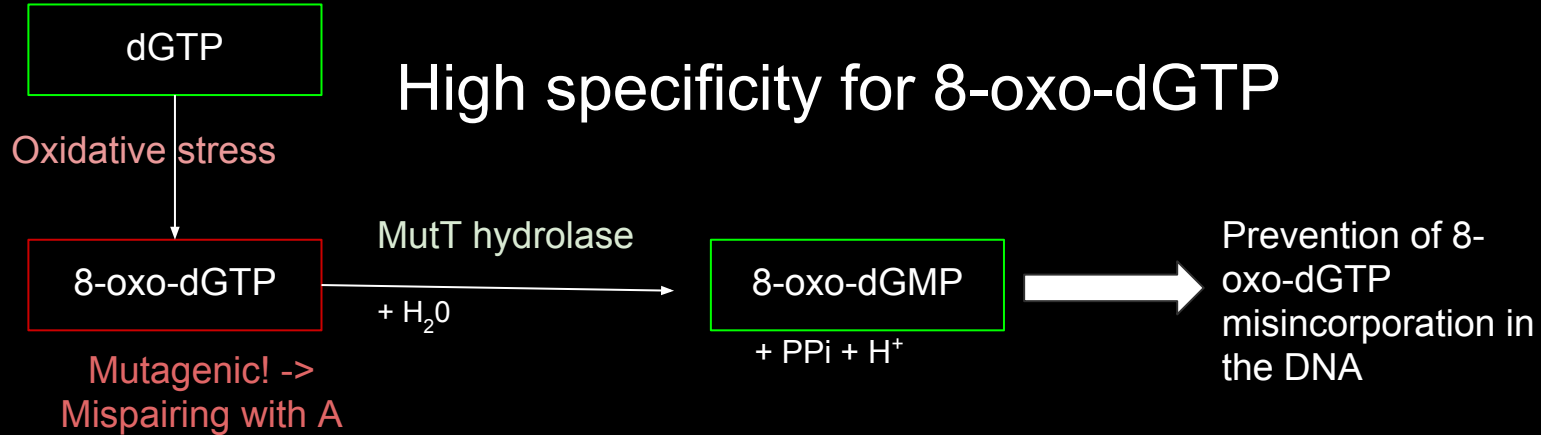
DIPP1-IP6
DIPP1-Inhibitor

DIPP1

Multiple Sequence Alignment

		20.
H.sapiens	-----MMKLKSNQTRTYDGDGYKKRAACLCFRSESEEEVLLVSSSRHPDRW	
M.musculus	-----MMKLKSNQTRTYDGDGYKKRAACLCFRSESEEEVLLVSSSRHPDRW	
M.nubicus	-----MMKLKSNQTRTYDGDGYKKRAACLCFRSESEEEVLLVSSSRHPDRW	
I.punctatus	-----MMKLKSNQTRTYDGDGYKKRAACLCFRSETEEEVLLVSSSRHPDKW	
H.saltator	-----MVKEKANSTRIYDSEGYRRRAACICVKNDLEDEVLLVTSSRKPD SW	
S.cerevisiae	mgktadNHGPVRSETAREGRENQVYSPVTGARLVAGCICLTP-DKKQVLMITSSAHKKRW	
	50 54 65 69	
H.sapiens	IVP GGGMEPEE- EPSVAAVRE VCEEAGV KGT LGR LVG IFEN- QER KHR TYV VV L IV TEVL	
M.musculus	IVP GGGMEPEE- EPSVAAVRE VCEEAGV KGT LGR LVG IFEN- QER KHR TYV VV L IV TEVL	
M.nubicus	IVP GGGMEPEE- EPSVAAARE VCEEAGV KGT LGR LVG IFEN- RDR KHR TYV VV L IV TEVL	
I.punctatus	IVP GGGMEPDE- EPNVAAVRE VCEEAGV KGT LGR LVG IFEN- QDR KHR TYV VV L IV KEVL	
H.saltator	IVP GGGVEPEE- EPAVTALRE VREEAGV LGQLGRCLGT FEN- VEH KHR TQV WVMQV TEEL	
S.cerevisiae	IVP KGGVEKDEpNYETTAQRETWEEAGCIGKIVANLGTVEDmRPPKDWNKDIKQFENS RK	
H.sapiens	EDWEDSVNIGRKREWFKIEDAIKVLQYHK- PVQASYFETLRQGYSANNGTPVVATTYSVS	
M.musculus	EDWEDSVNIGRKREWFKIEDAIKVLQCHK- PVQASYFETLRQGYPANNGTPVVPTTYS SS	
M.nubicus	EDWEDSVNIGRKREWFKIEDAIKVLQYHK- PVQASYFETLRQGCLANNGTPVMTTTTYSES	
I.punctatus	EDWEDSLNIGRKREWFKIDDAIQVLQCHK- PVQATYFEALQESCLTSNGTPLVATIAEDL	
H.saltator	PEWEDSRAIGRKRWFTIQEALLQLGQHK- PVQRSYLHSLHNTNPRHNSTTSPLSHHS HS	
S.cerevisiae	DSEVAKHPPRTEFH FYELEIENLLDKFPEcHKRHRKLYSYTEAKQNLIDAKRPELLEALN	

8-oxo-dGTP DIPHOSPHATASE (*E. coli*)

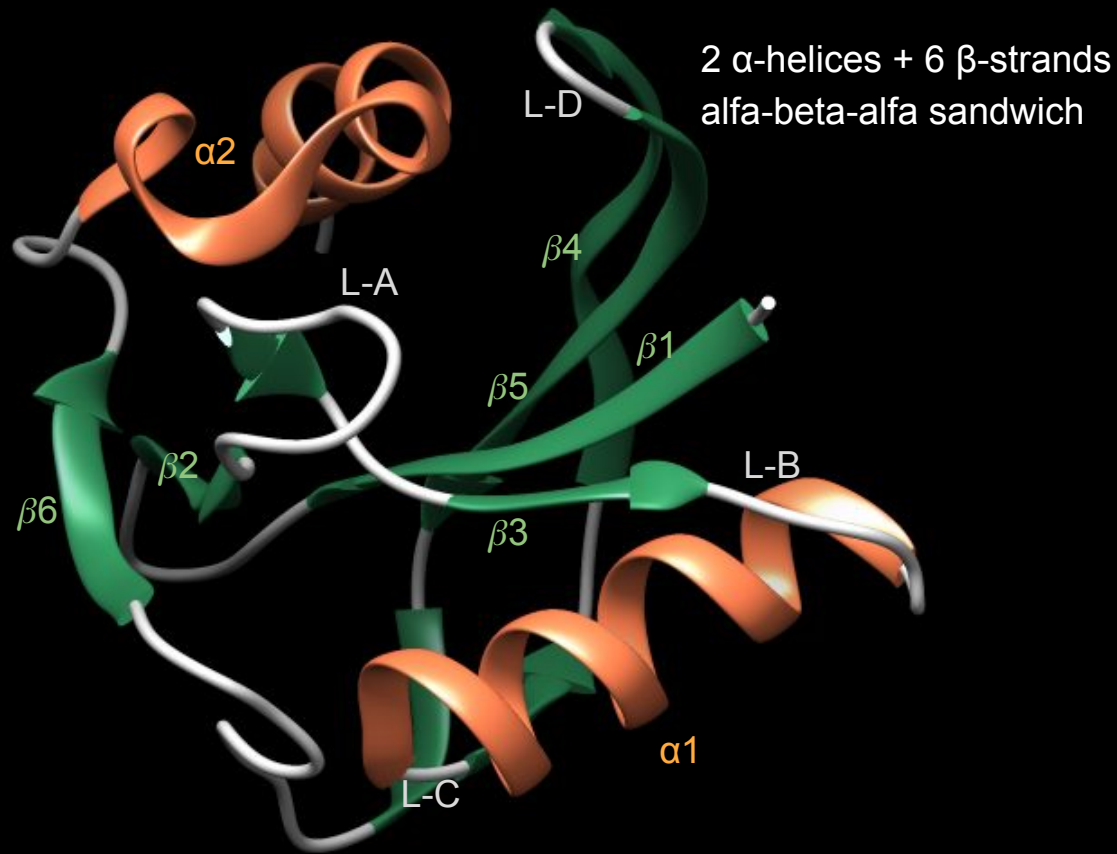


Mildvan AS et al (2005).

E. coli → Hydrolysis of damaged nucleotides by MutT can avoid replicational and transcriptional errors.

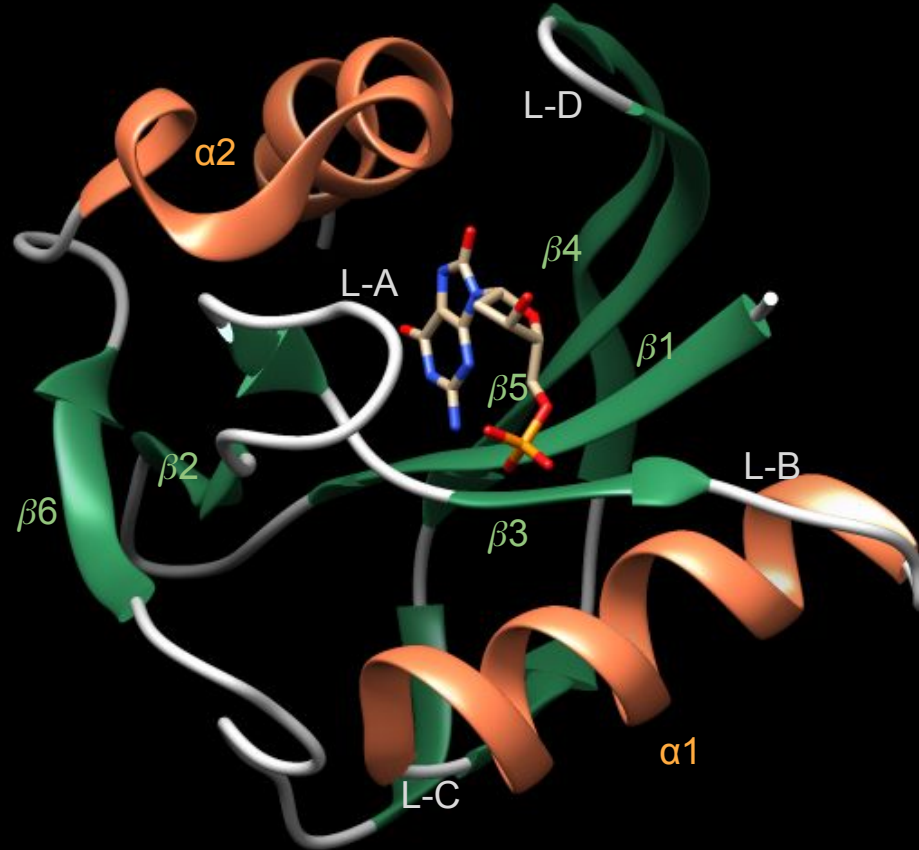
8-oxo-dGTP DIPHOSPHATASE (E. coli)

Structure: Nudix domain



8-oxo-dGTP DIPHOSPHATASE (*E. coli*)

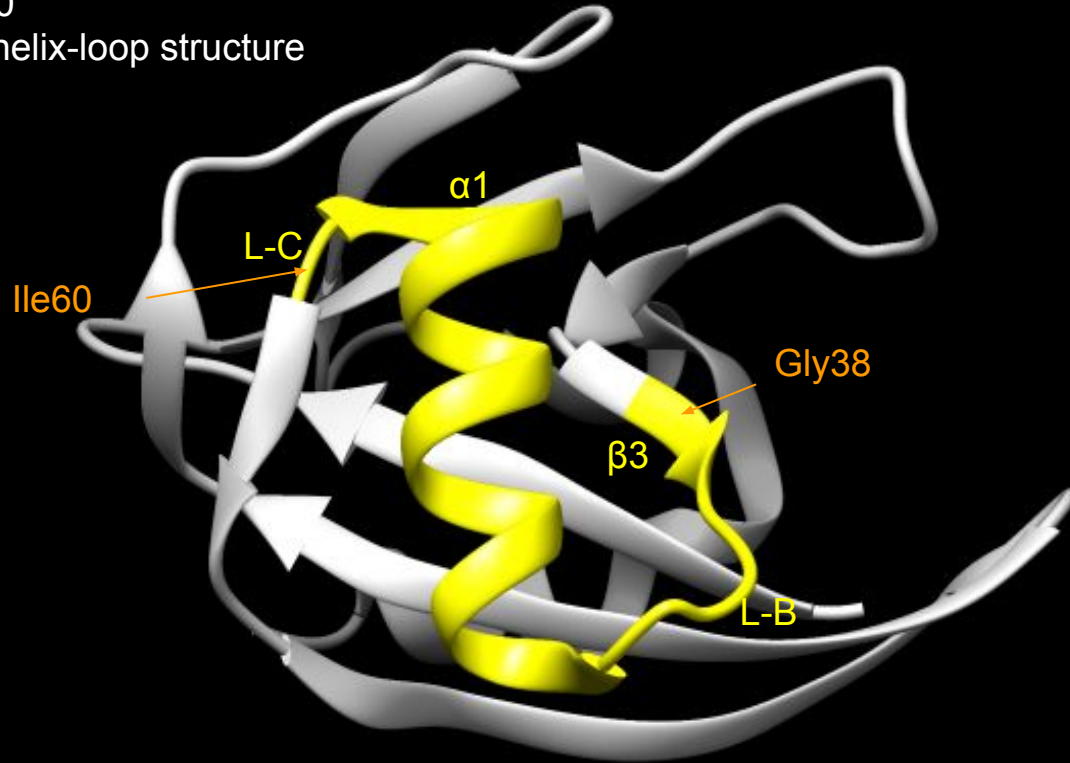
Structure: Nudix domain



8-oxo-dGTP DIPHOSPHATASE (E. coli)

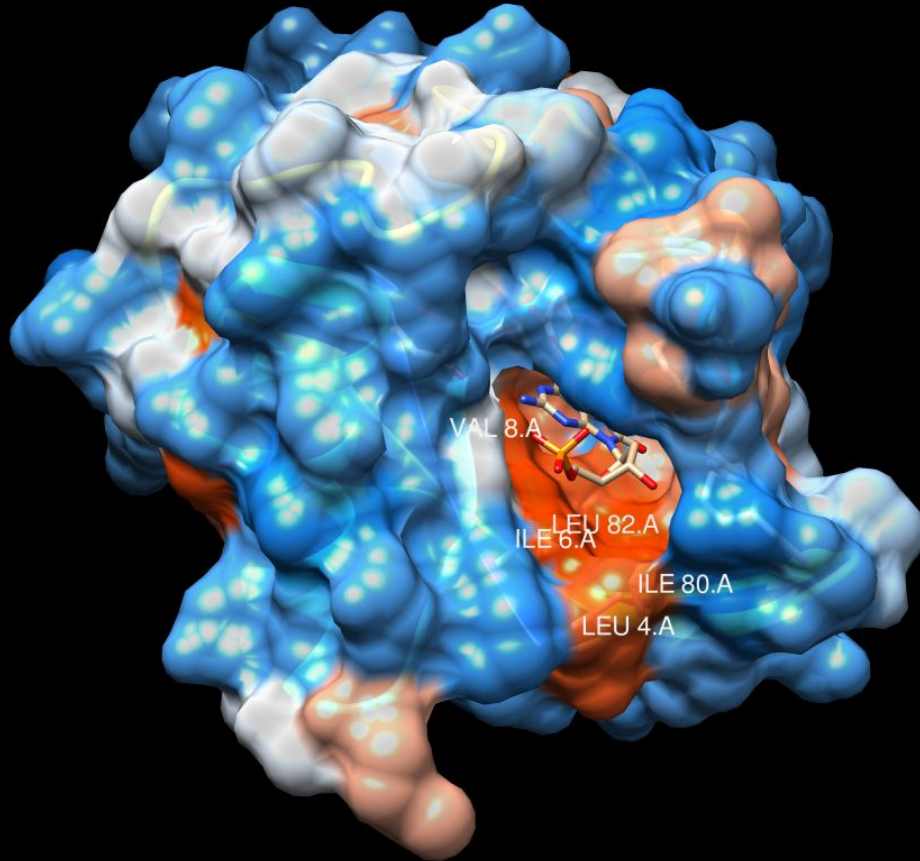
Structure: Nudix motif

Gly38 to Ile60
Strand-loop-helix-loop structure



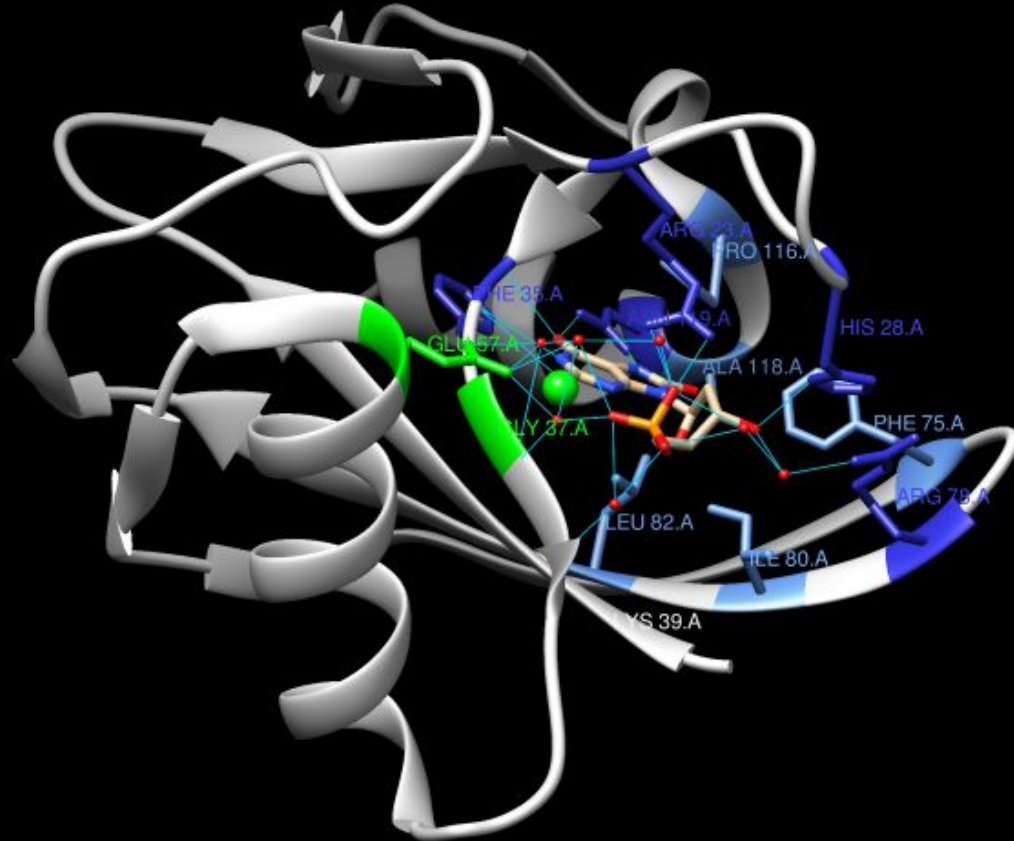
8-oxo-dGTP DIPHOSPHATASE (E. coli)

Substrate Binding Site



8-oxo-dGTP DIPHOSPHATASE (E. coli)

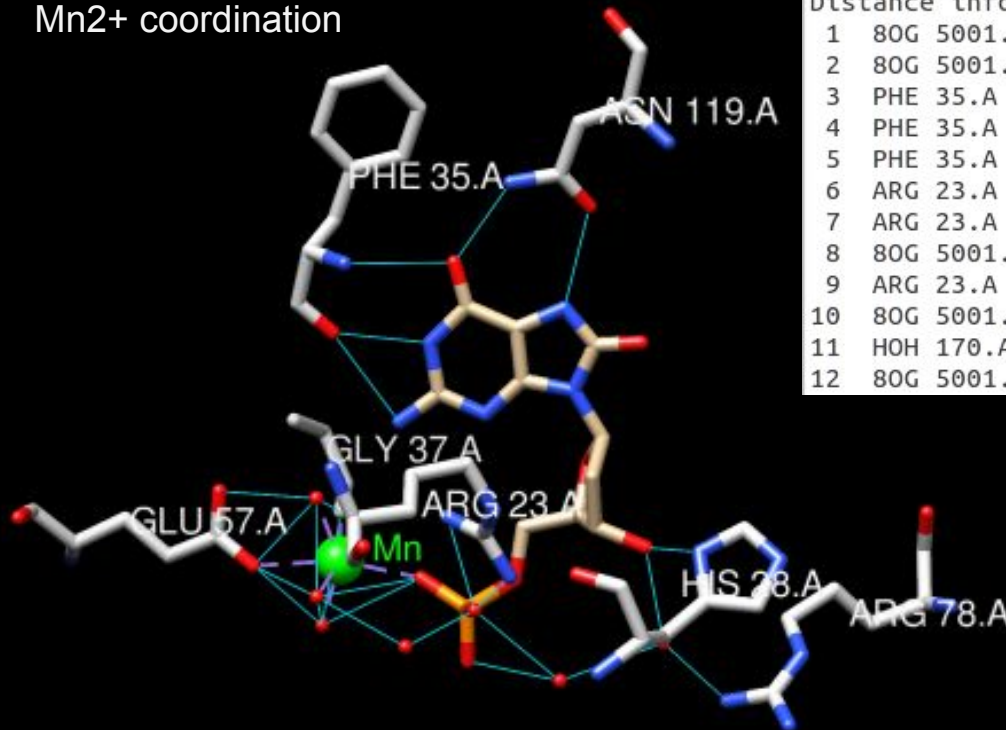
Substrate and Mn²⁺ Binding Site



8-oxo-dGTP DIPHOSPHATASE (E. coli)

Substrate and Mn²⁺ Binding Site

- Hydrogen bonds
- Mn²⁺ coordination

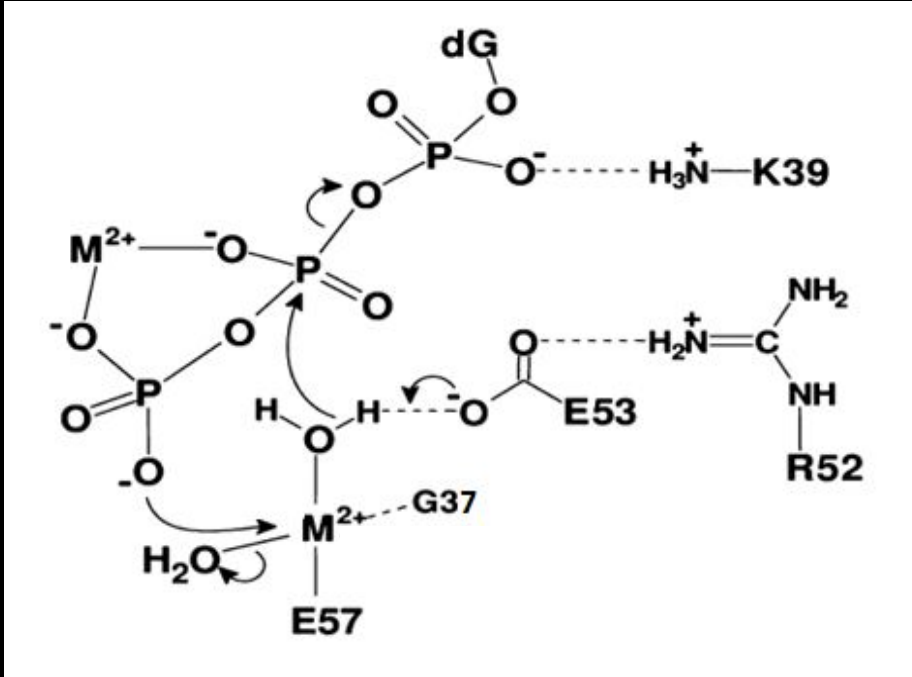


Distance information

1	8OG 5001.A N7 <-> ASN 119.A OD1:	2.774
2	8OG 5001.A O6 <-> ASN 119.A ND2:	2.782
3	PHE 35.A N <-> 8OG 5001.A O6:	3.032
4	PHE 35.A O <-> 8OG 5001.A N1:	2.635
5	PHE 35.A O <-> 8OG 5001.A N2:	2.963
6	ARG 23.A NE <-> HIS 28.A O:	2.800
7	ARG 23.A NH2 <-> HIS 28.A O:	2.775
8	8OG 5001.A OP3 <-> ARG 23.A NH2:	2.656
9	ARG 23.A NH1 <-> 8OG 5001.A OP3:	3.127
10	8OG 5001.A O3' <-> HIS 28.A ND1:	2.830
11	HOH 170.A O <-> ARG 78.A NH1:	2.683
12	8OG 5001.A O3' <-> HOH 170.A O:	2.786

8-oxo-dGTP DIPHOSPHATASE (E. coli)

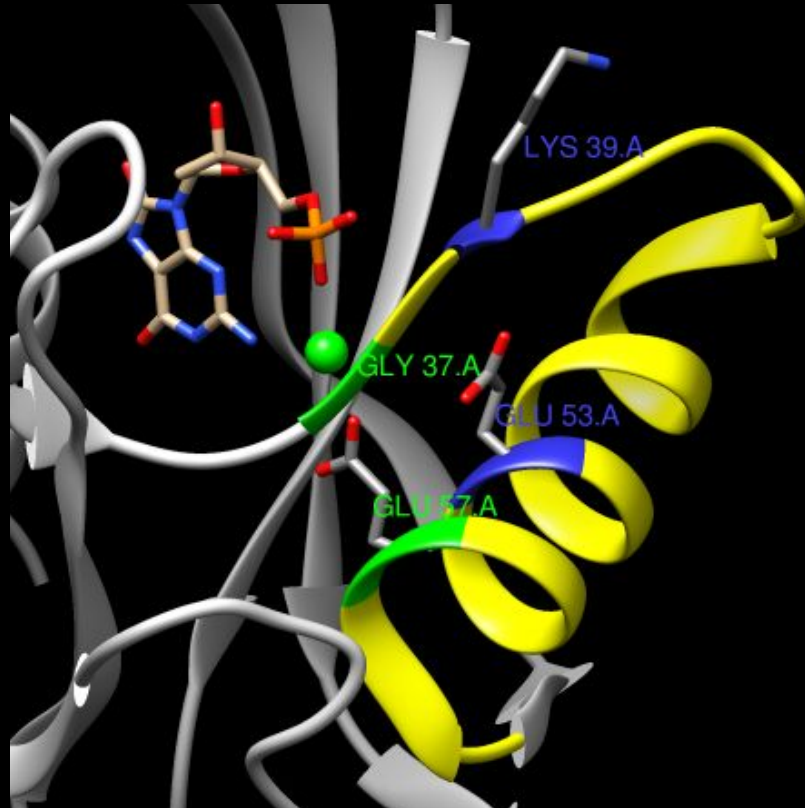
Catalytic mechanism



Modified from: Mildvan AS et al (2005).

8-oxo-dGTP DIPHOSPHATASE (E. coli)

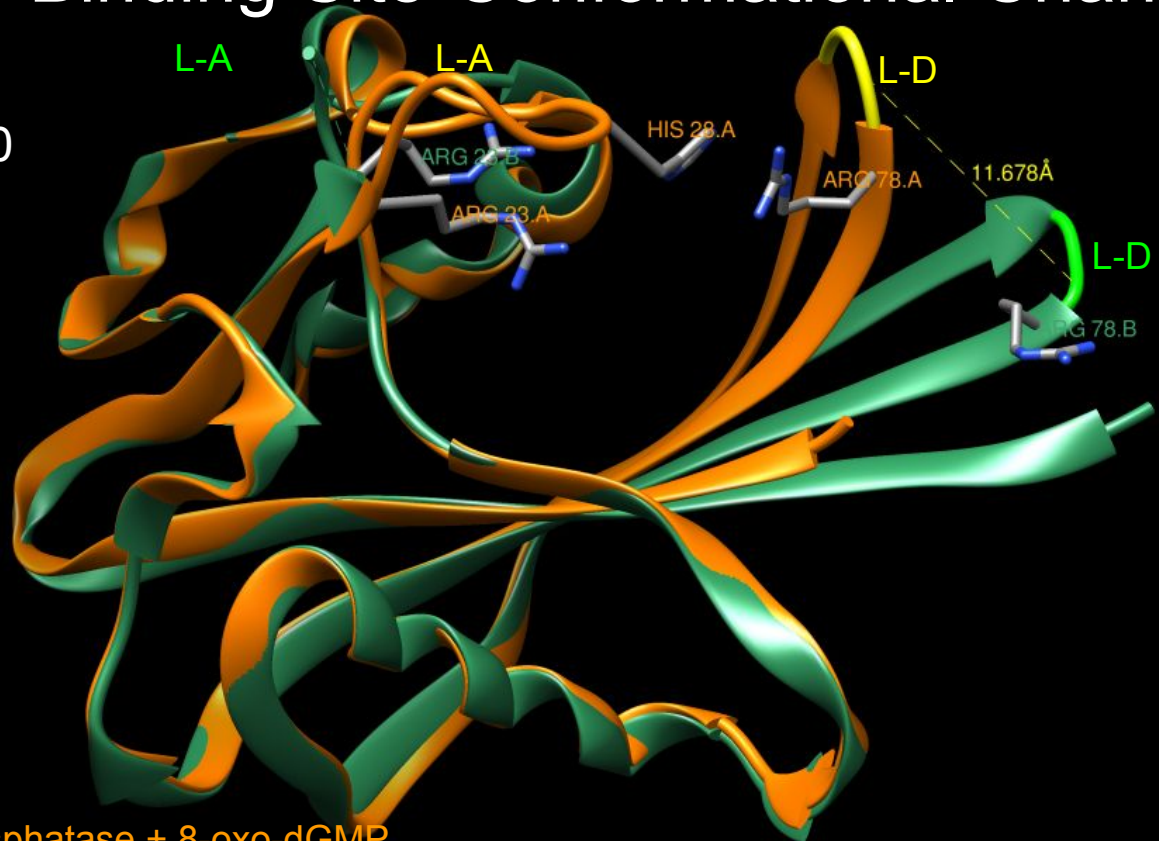
Catalytic mechanism



8-oxo-dGTP DIPHOSPHATASE (E. coli)

Substrate Binding Site-Conformational Change

RMSD = 0.80



8-oxo-dGTP diphosphatase + 8-oxo-dGMP

8-oxo-dGTP diphosphatase

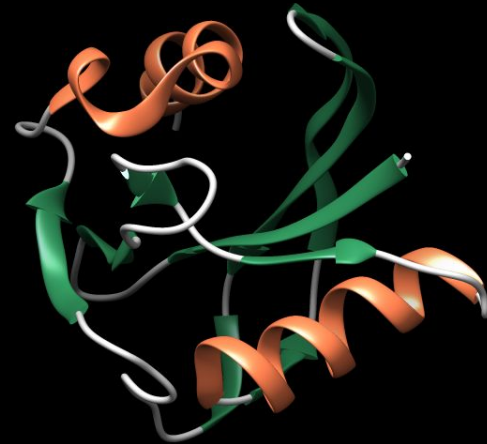
8-oxo-dGTP DIPHOSPHATASE (E. coli)

Multiple Sequence Alignment

A. thaliana	-----ms-TGEAIPRVAVVVFILNGNSILLGRRRSSIGNSTFALPGGHLEFGESFEECA
E. coli	-----MKKLQIAVGIIRNENNEIFITRRAADAHMANKLEFPGGKIEMGETPEQAV
H. sapiens	mtasaqprGRRPGVGVGVVVTSCKHPRCVLLGKRKGSVGAGSFQLPGGHLEFGETWEECA
M. musculus	maanaepr-RRPGVGVGVVLSCEHPRCVLLGKRKGSFGAGSFQLPGGHLEFGETWEECA
A. thaliana	AREVMEETGLKIEKMKLLTVTNNVFKEAPTFSHYVSVS ^{R23} iravlvdpsSQEPKNMEpEKCEG
E. coli	VRELQEEVGITPQHFSLFEEKLEYEFPDRHITLWFWLVE-----RWEGEPWG-KEGQP
H. sapiens	QRETWEEAALHLKNVHFASVNSFIEKENYHYVTILMKgev-dvthDSEPKNVEpEKNES
M. musculus	QRETWEEAGLHLKNVCFASVNSFVEKENYHYVTILMKgev-dmthDSEPRNMEpEKNES
A. thaliana	^{E53 E57} WDWYDWENLPKFLFWPLEKLF ^{D119} FGSGFNPF-----thgggd
E. coli	GEWMSLVGLNADDFPPANEPVIAKLK-----rl----
H. sapiens	WEWVPWEELPPLDQLFWGLRCLKEQGYDPFKEDLNHLVGKY-----gnhl--
M. musculus	WEWVPWEEFPPLDQLFWALRCLKEQGYDPFKEDLNHLEGYRGEHLERTTKTp----

CONCLUSION

1. Nudix motif is very conserved
2. Metal ions are needed as cofactors
3. Similar catalysis mechanisms but different substrates



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1. The Nudix family appears in :

- a) Only in archaea
- b) Only in bacteria
- c) Both domains
- d) Only in eukarya
- e) All 3 domains**

2. Nudix hydrolases are...:

- a) Pyrophosphatases that hydrolyze nucleoside diphosphates
- b) The nucleoside diphosphates that linked to another moiety (X)
- c) a and b are correct**
- d) There is only one family
- e) all options are correct.

3. The Nudix motif...

- a) is very conserved among different species.
- b) is barely conserved among different species.
- c) consists of 23 aminoacids.
- d) b and c are correct.
- e) a and c are correct.**

4. MutT...

- a) is a subfamily of Nudix.
- b) hydrolyzes 8-oxo-dGTP to 8-oxodGMP.
- c) a and b are correct.**
- d) doesn't present an alfa-beta-alfa sandwich (Nudix domain).
- e) All options are correct.

5. Indicate the correct answer:

- a) MutT is an heterodimer.
- b) 8-oxoguanine is not mutagenic at all.
- c) MutT hydrolyses the oxidized form of guanine.**
- d) MutT doesn't need any metal ion to be active.
- e) There isn't an homolog MutT in the *Homo sapiens* genome.

6. The ADP-ribose pyrophosphatase

- a) Degrades IPP.
- b) Contrarily to most Nudix proteins doesn't need Mg.
- c) its biological (functional) assembly is a dimer.**
- d) uses water as catalytic base.
- e) uses water as catalytic acid.

7. DIPPI1 needs for its interaction...

- a) Mg
- b) F
- c) a and b are correct**
- d) Cl
- e) All answers are correct

8. DIPPI1 function is...

- a) IPP hydrolysis**
- b) IPP synthesis
- c) Oxidized guanine hydrolysis
- d) Guanine hydrolysis
- e) ADP-R hydrolysis

9. About ADP ribose pyrophosphatase...

- a) Mg are coordinated in an octahedral conformation.
- b) It has a conserved Nudix motif among orthologs.
- c) a and b are correct.
- d) It hydrolyses ADPribose
- e) All are correct.**

10. About the ADP-ribose pyrophosphatase... Choose the wrong answer:

- a) **Magnesiums have a tetrahedral coordination**
- b) Uses hydroxide as nucleophile for the catalysis.
- c) a and b are wrong
- d) Can be found in humans
- e) All answers are wrong