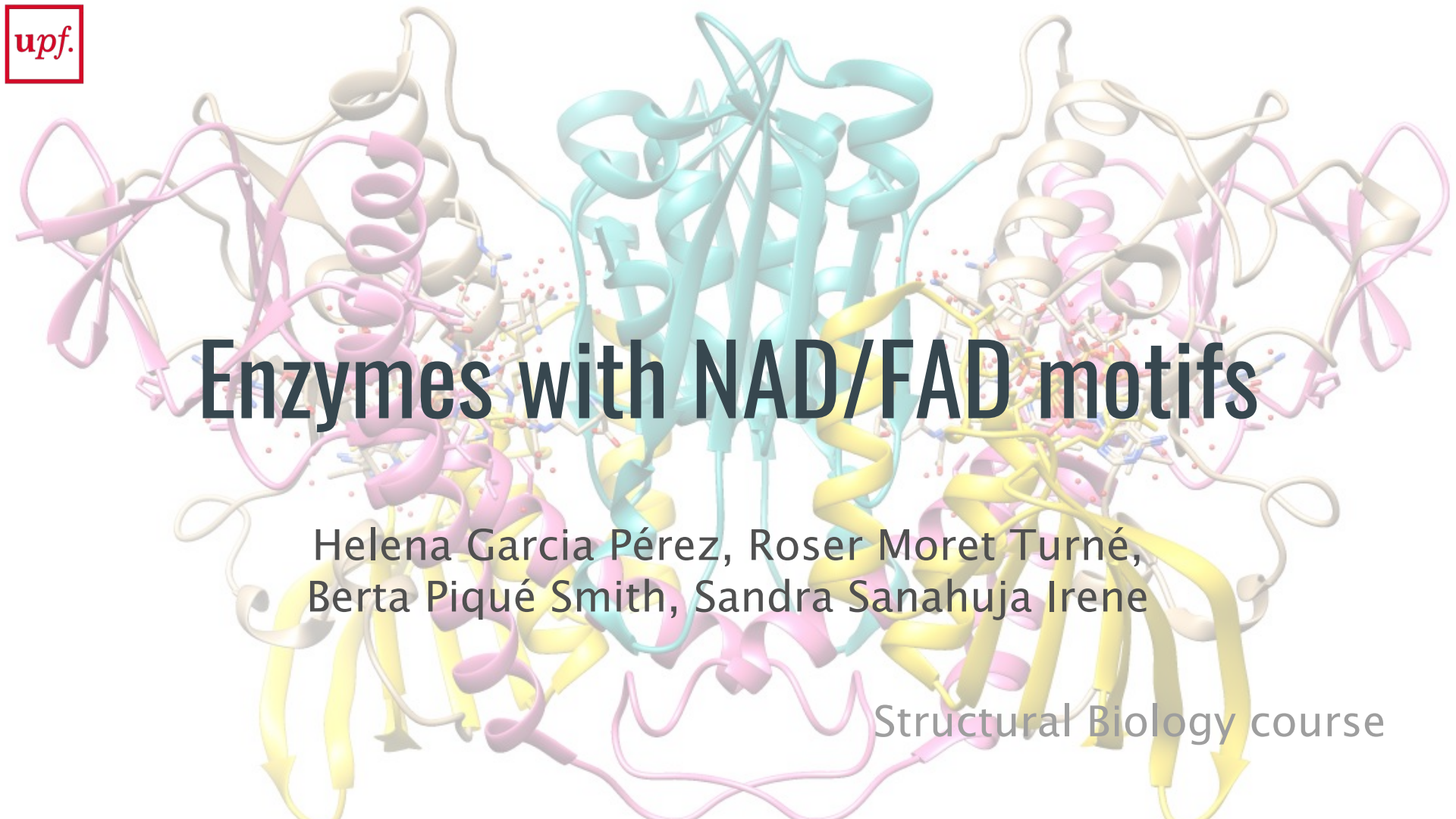




Enzymes with NAD/FAD motifs

Helena Garcia Pérez, Roser Moret Turné,
Berta Piqué Smith, Sandra Sanahuja Irene

Structural Biology course

Index

- Introduction

- FAD binding proteins
- Glutathion reductase
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- Catalytic cycle

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- FAD binding domain and Rossmann fold core
- NADPH binding domain and Rossmann fold core

- GR interactions

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- NADPH interactions
- Glutathione interactions

- Alignments

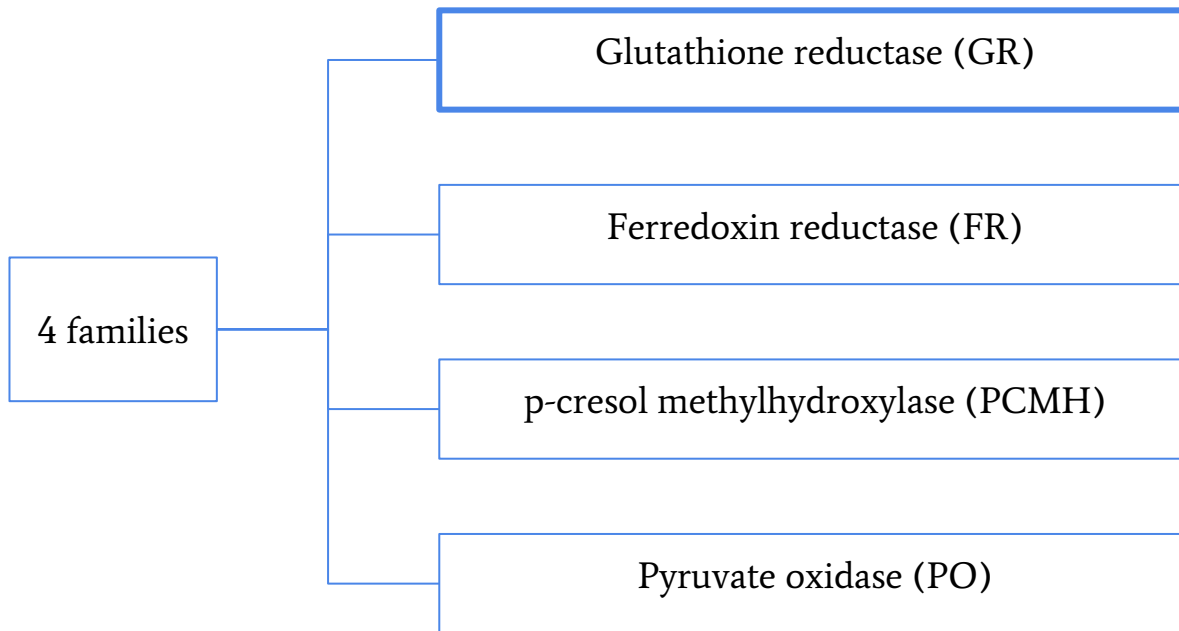
- Sequence alignment between species
- Structural alignment between species and structural superimpositions
- Catalytic cycle structural superimpositions

- PEM questions

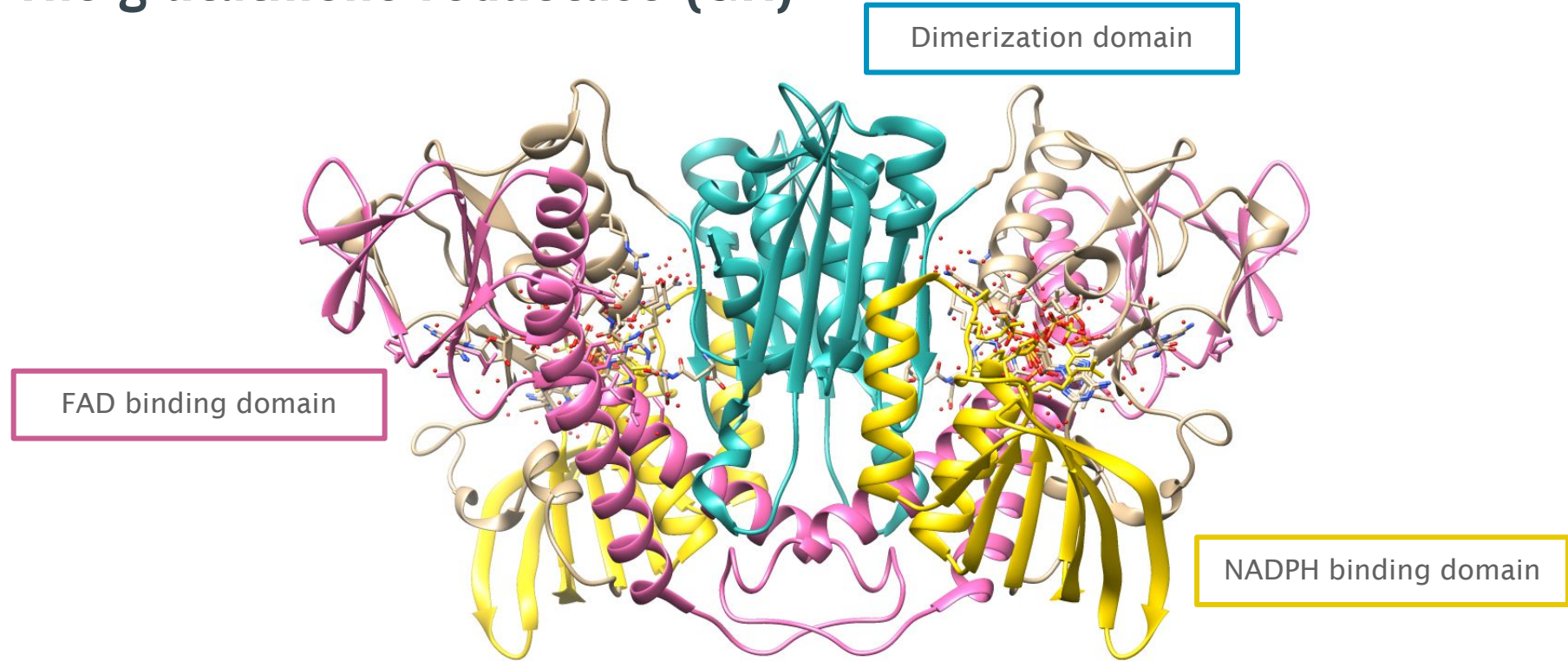
- Bibliography

FAD binding proteins

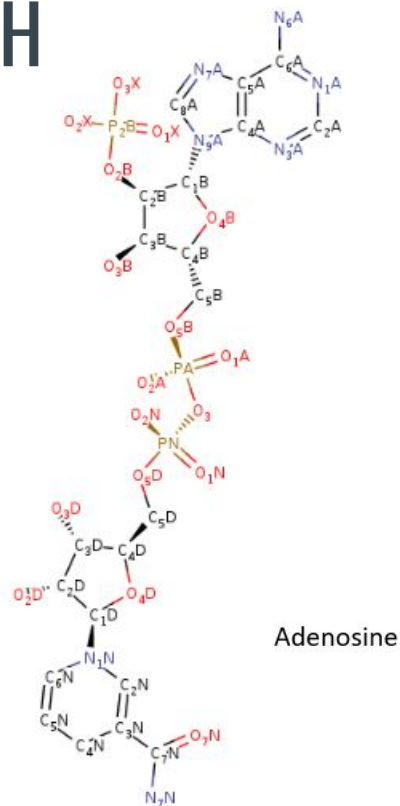
Proteins inside each family also
bind NADPH



The glutathione reductase (GR)



NADPH



Adenosine

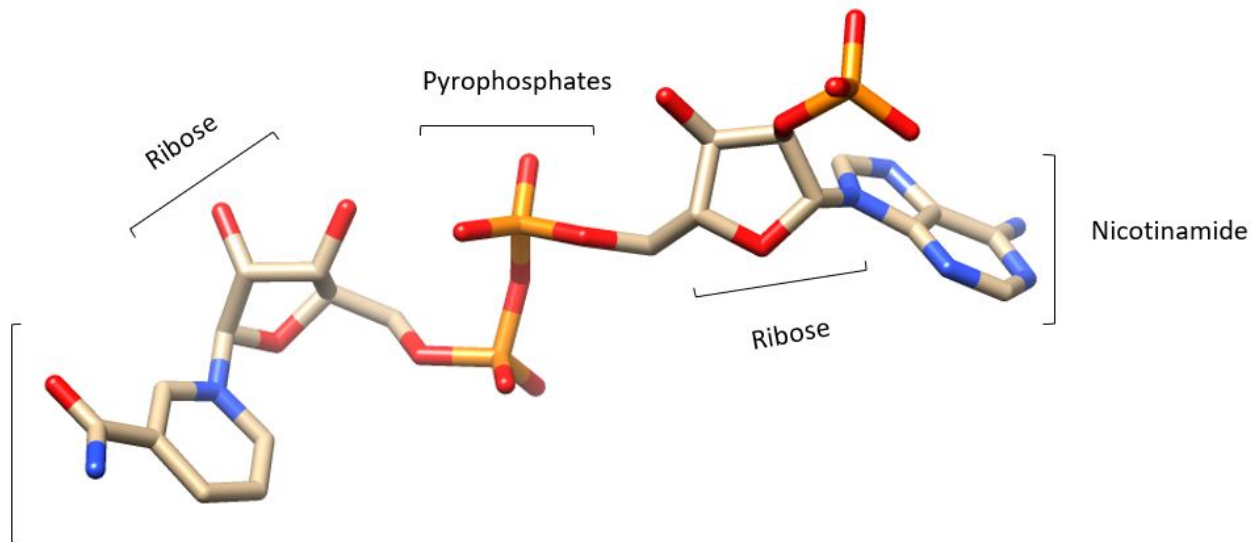
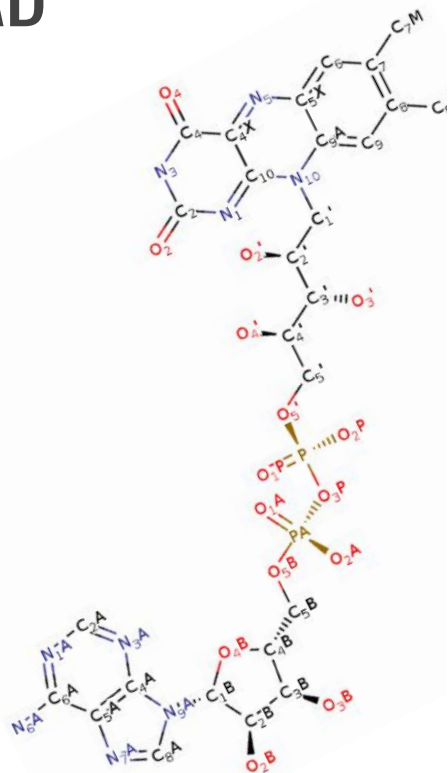


Image extracted from: Berkholz D, Faber H, Savvides S, Karplus P. 3DK4: Catalytic cycle of human glutathione reductase near 1 Å resolution [Internet]. RCSB PDB. 2008 [cited 16 February 2019]. Available from: <https://www.rcsb.org/structure/3dk4>

FAD



Adenosine monophosphate (AMP)

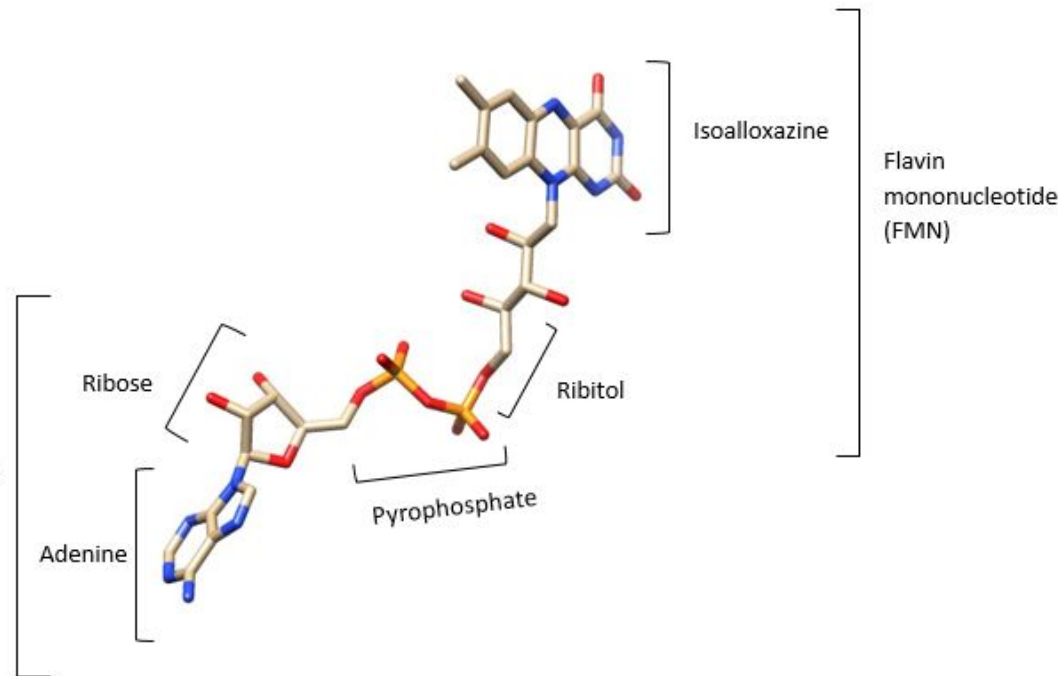


Image extracted from: Berkholz D, Faber H, Savvides S, Karplus P. 3DK4: Catalytic cycle of human glutathione reductase near 1 Å resolution [Internet]. RCSB PDB. 2008 [cited 16 February 2019]. Available from: <https://www.rcsb.org/structure/3dk4>

Catalytic cycle of the GR

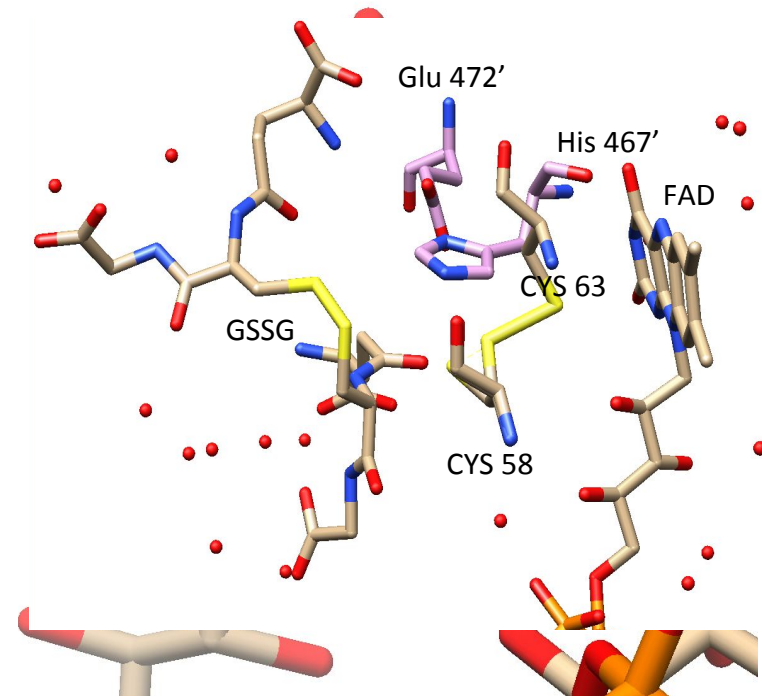
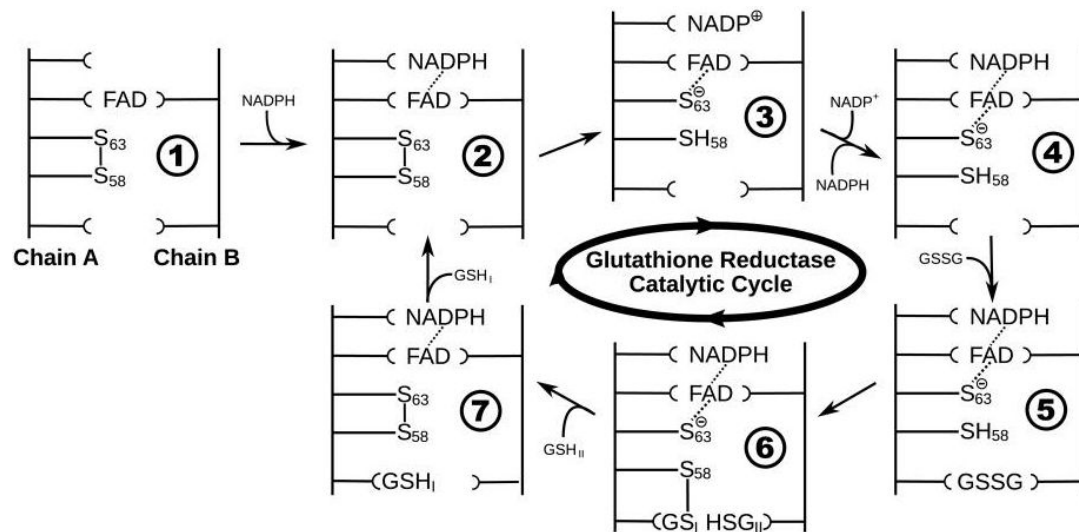
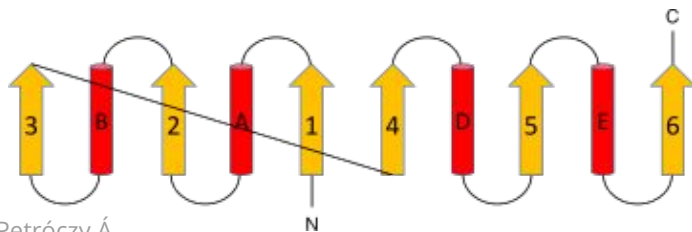
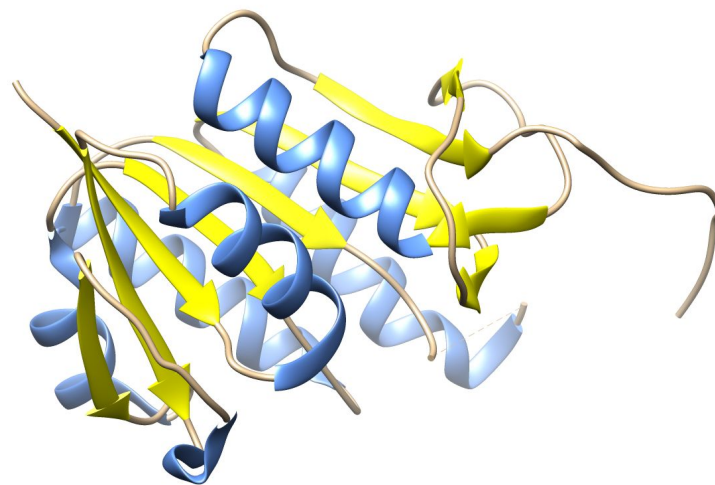
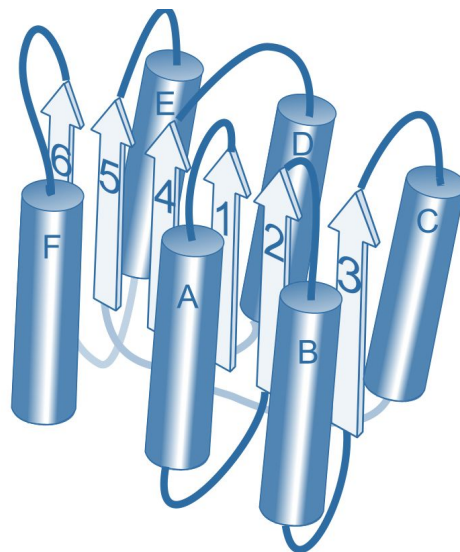


Image extracted from: Berkholz D, Faber H, Sawides S, Karplus P. Catalytic Cycle of Human Glutathione Reductase Near 1 Å Resolution. *J Mol Biol Mol.* 2008;382(2):371-384.

Canonical Rossmann Fold



Laurino P, Tóth-Petróczy Á, Meana-Pañeda et al. An Ancient Fingerprint Indicates the Common Ancestry of Rossmann-Fold Enzymes Utilizing Different Ribose-Based Cofactors. *PLOS Biology*. 2016;14(3):e1002396.



Structural motifs that bind nucleotides

6 β -strands connected by α -helices

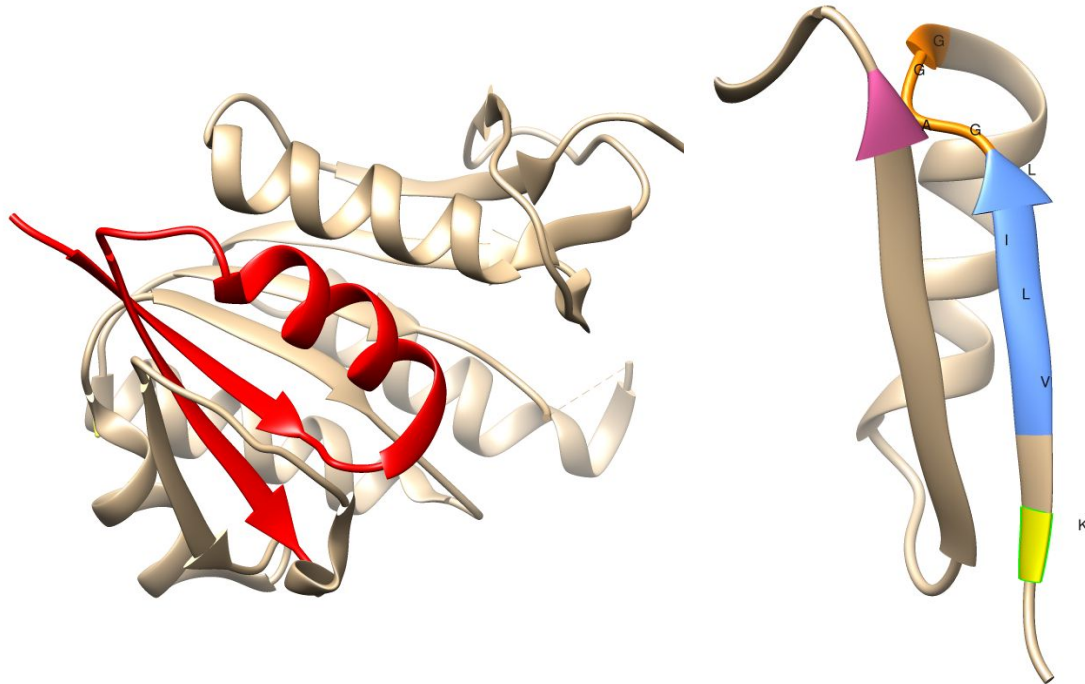
β -strands are hydrogen bonded and form a β -sheet

Europe P. Phaser - a "stunning" method for solving crystal structures < Quips < PDBe < EMBL-EBI [Internet]. Ebi.ac.uk. 2019 [cited 19 February 2019]. Available from: <http://www.ebi.ac.uk/pdbe/quips?story=P> haser

Rossmann fold core

Conserved

The minimum structure necessary to bind the cofactor



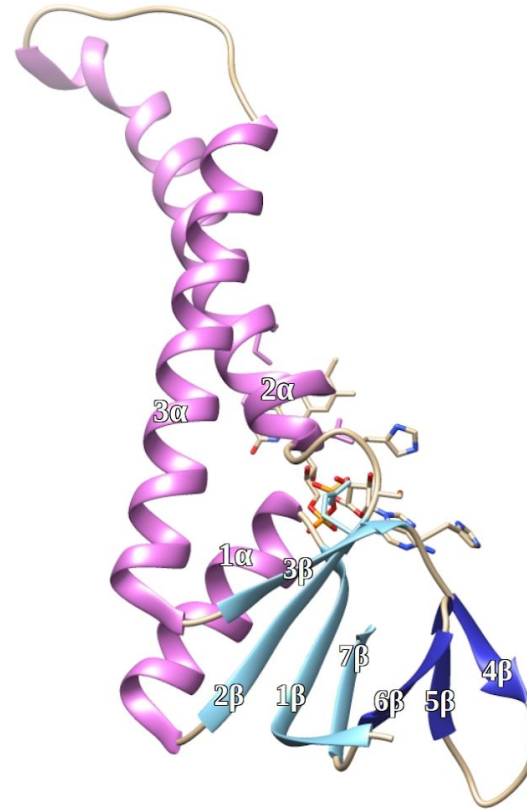
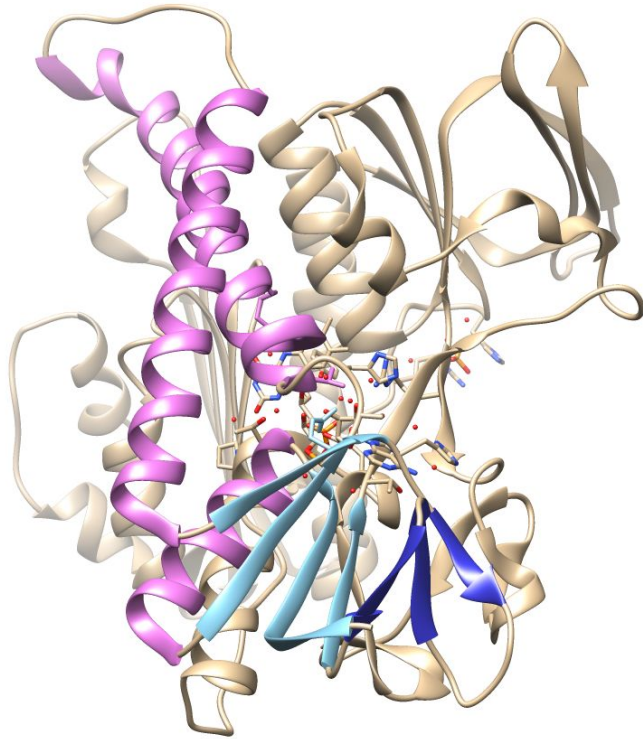
Phosphate binding region
GXGXXG/A or GXXXG/A

Core with small hydrophobic
amino acids

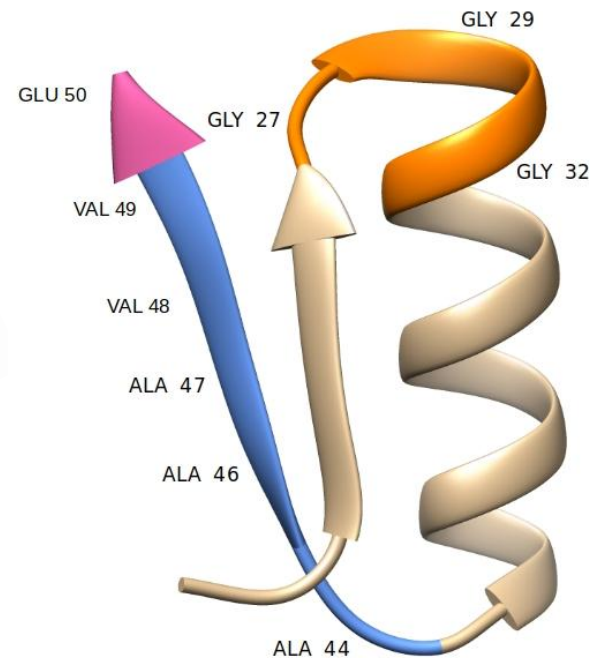
Negatively charged residue (Glu or
Asp) at the end of the second beta
strand

Positively charged residue (Arg or
Lys) at the beginning of the first beta
strand

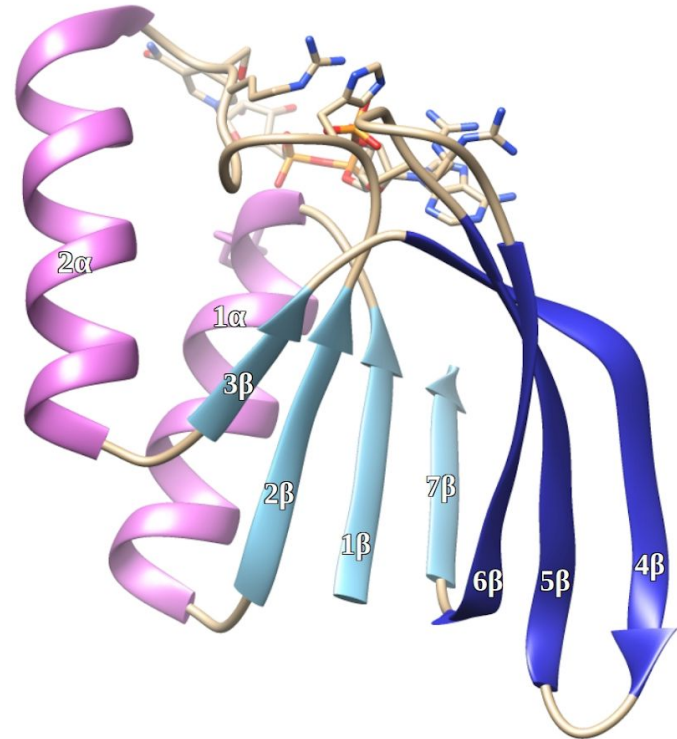
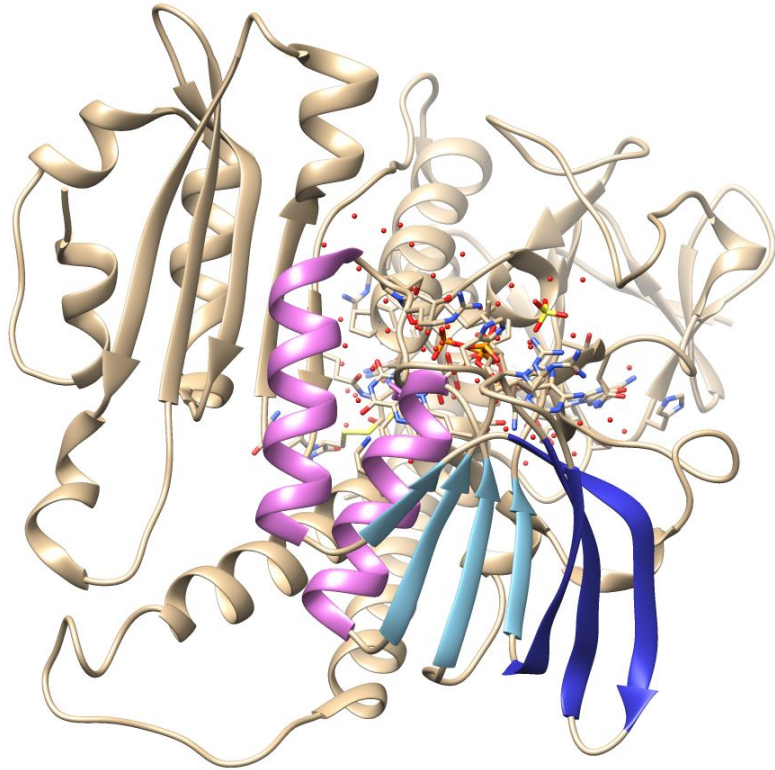
FAD binding domain



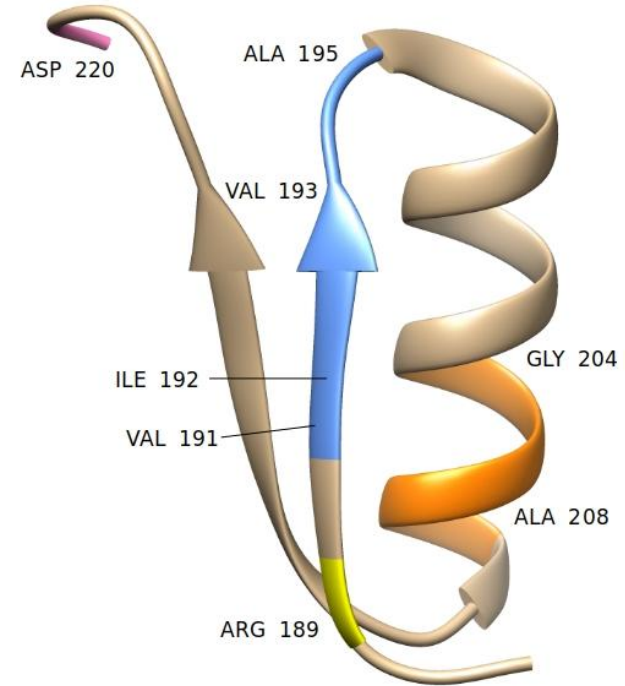
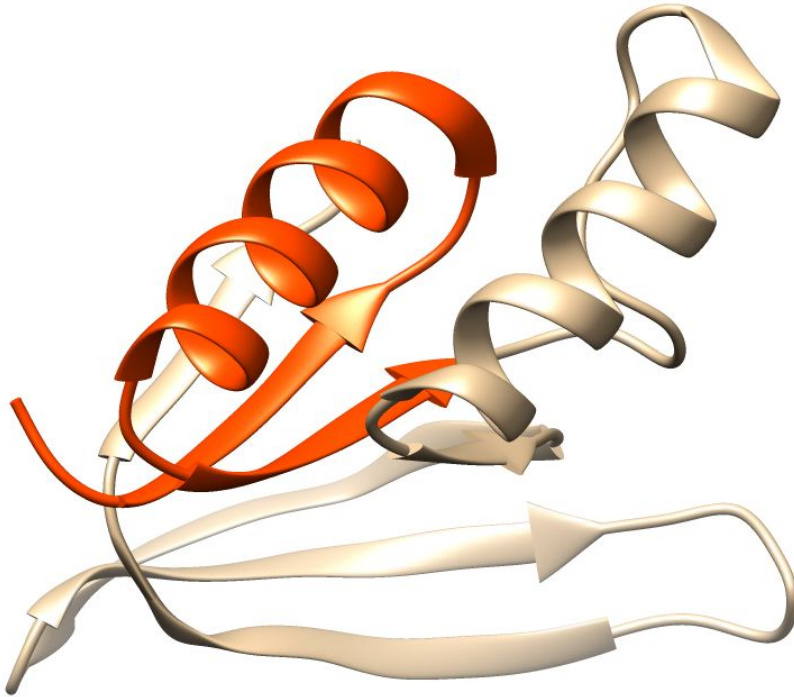
FAD's rossmann fold core



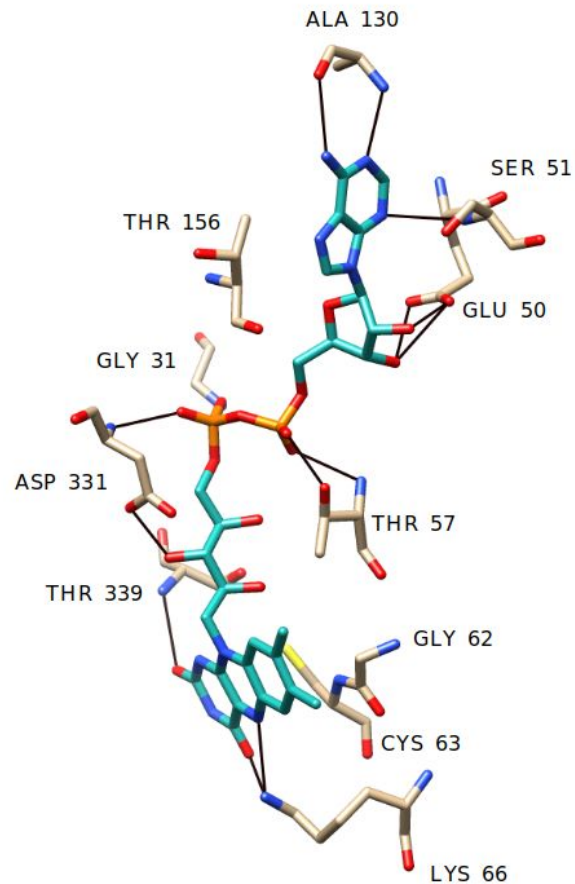
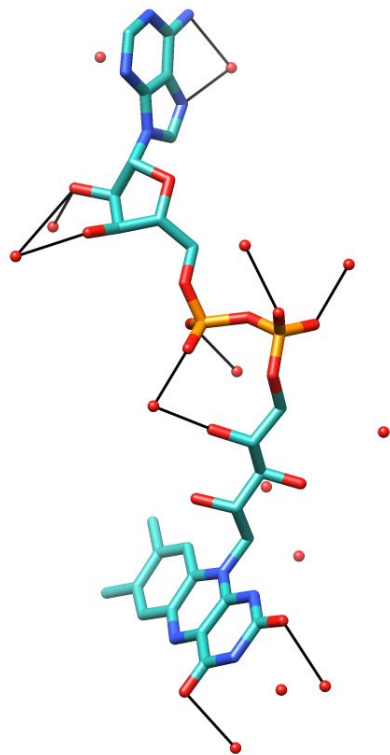
NADPH binding domain



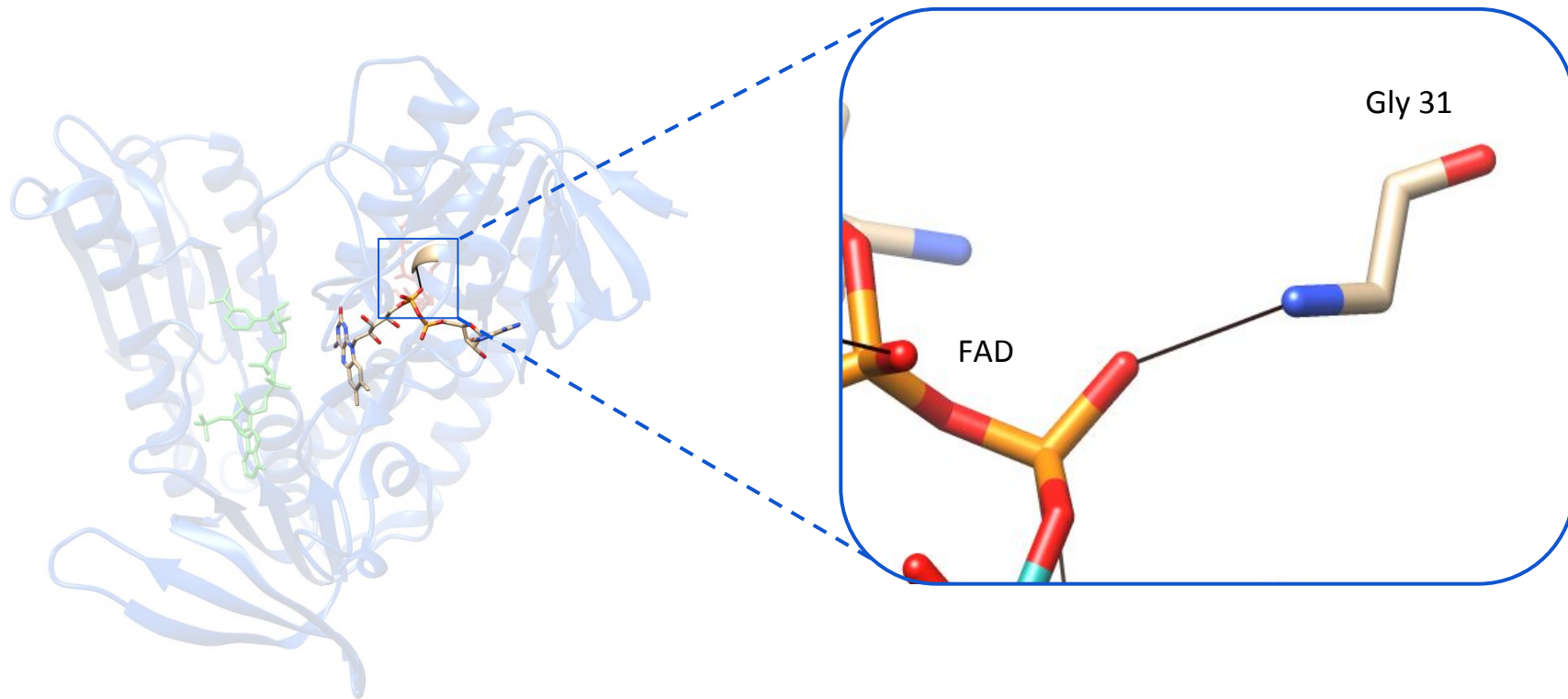
NADPH's rossmann fold core



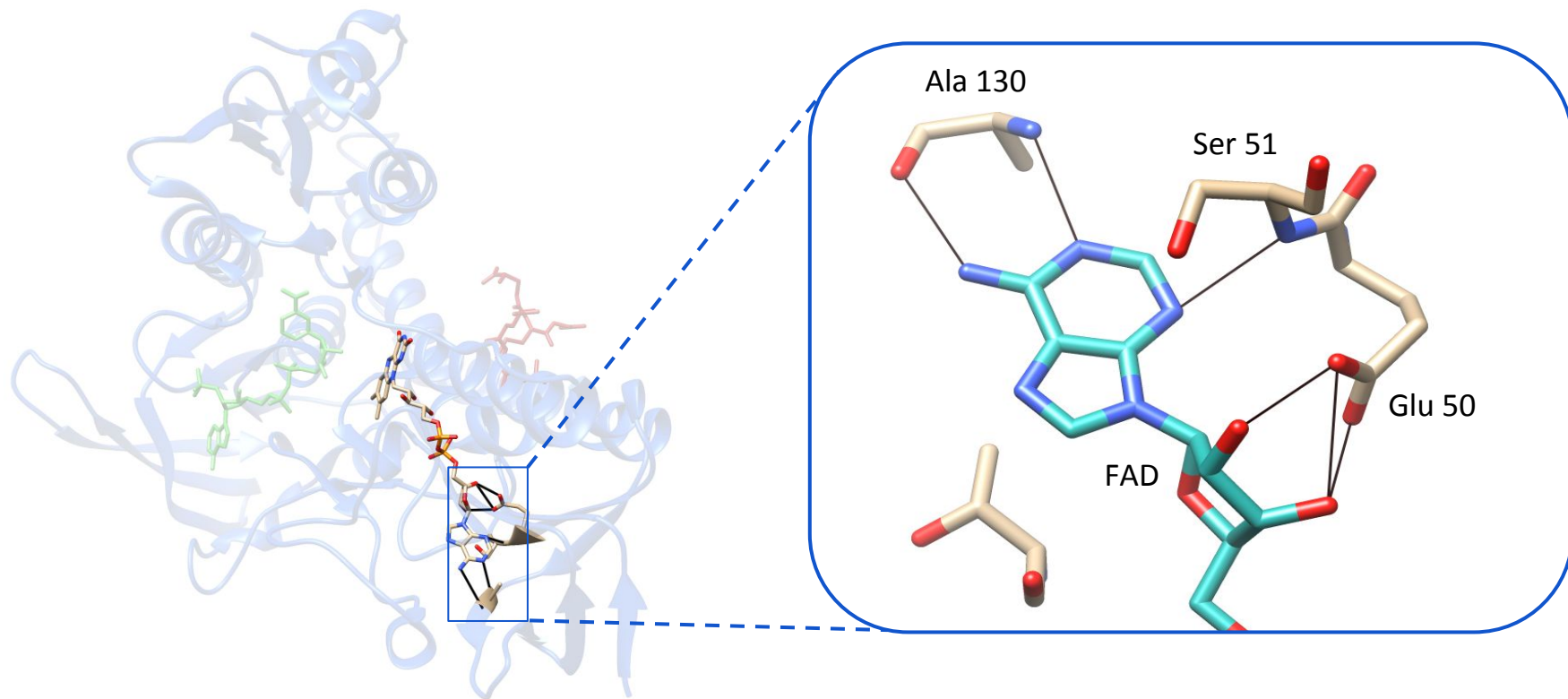
FAD Hbonds



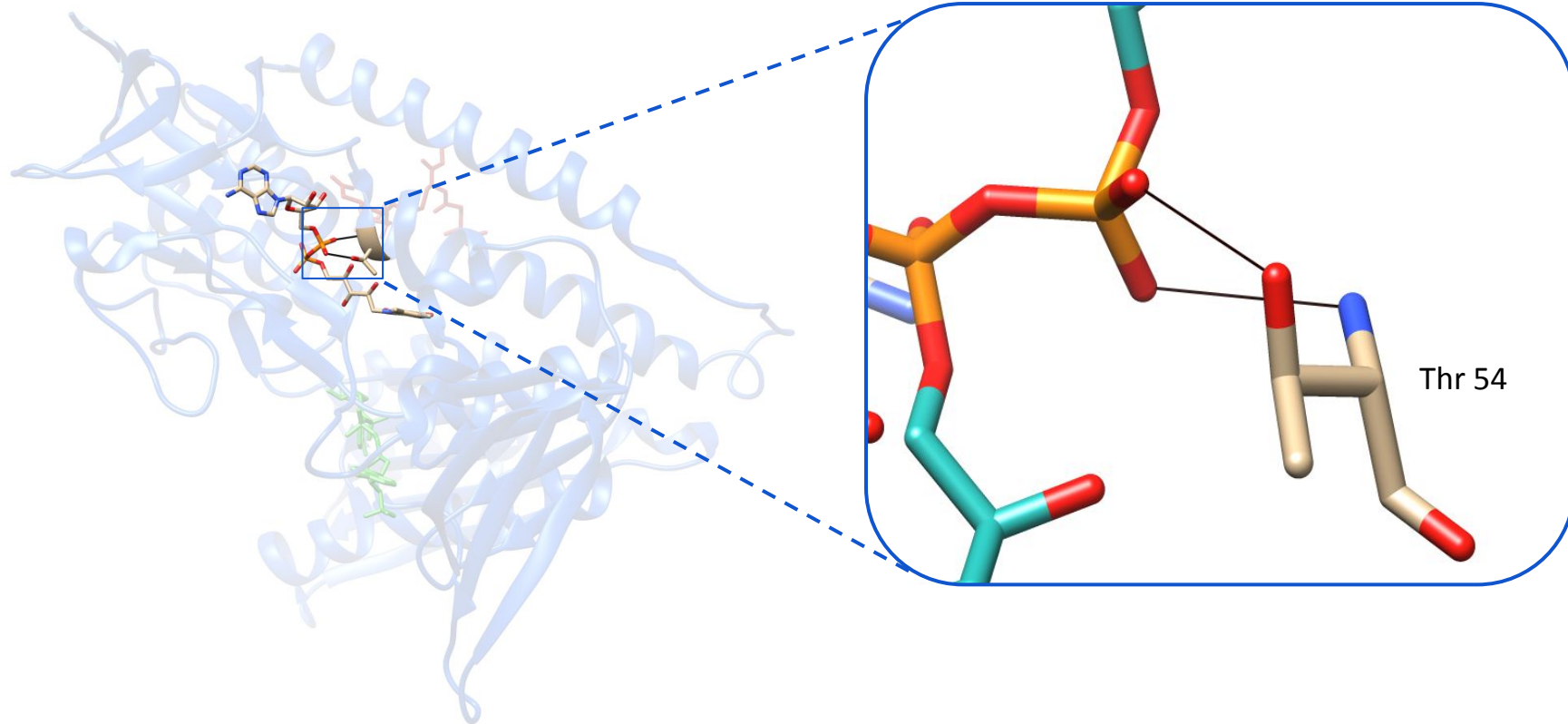
GLY 31



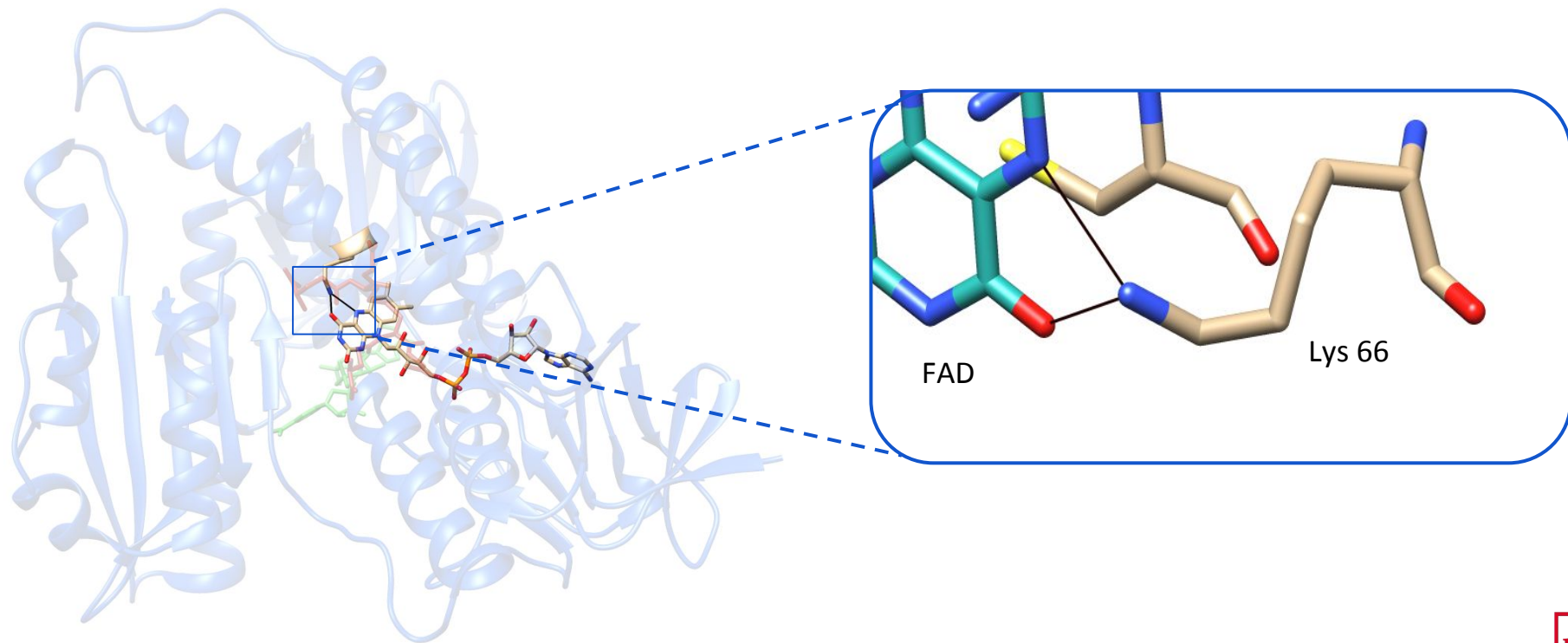
ALA 130 / SER 51 / GLU 50



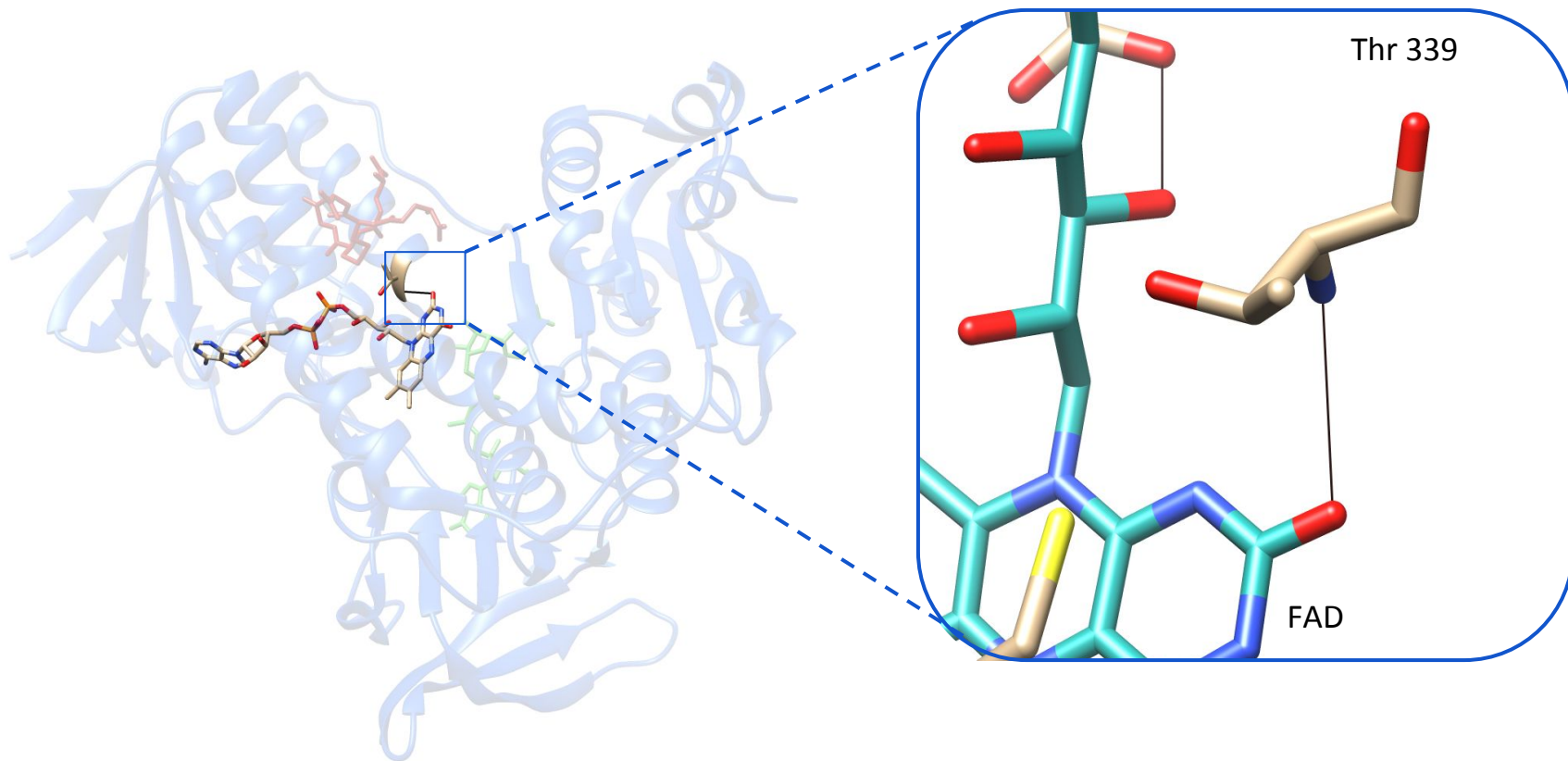
THR 57



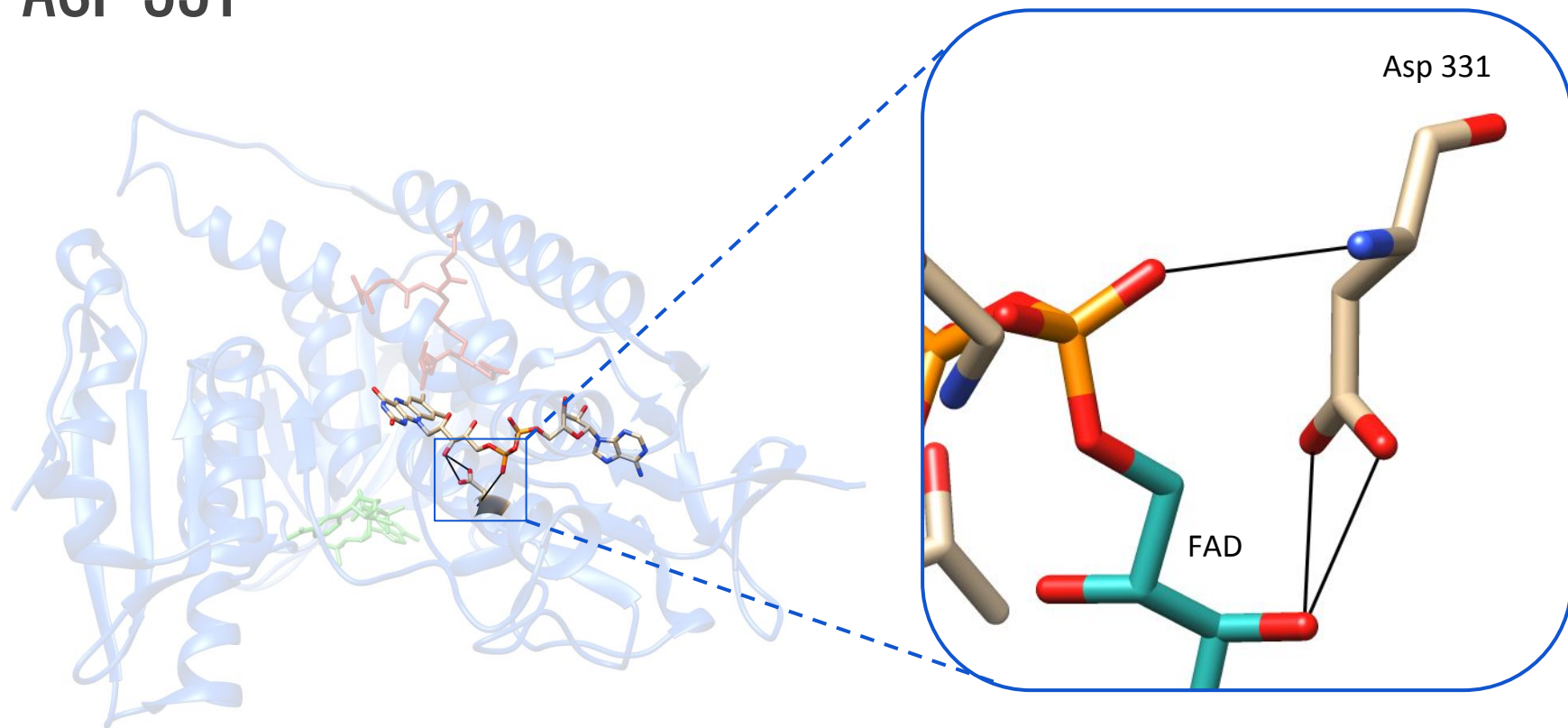
LYS 66



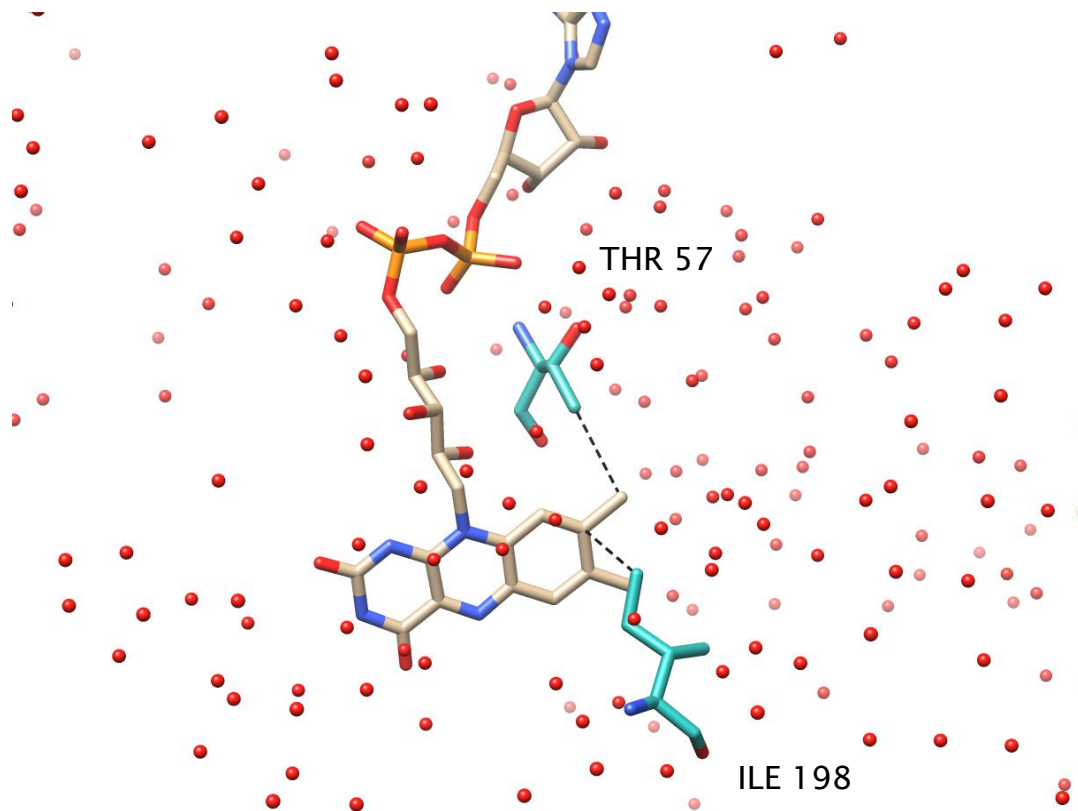
THR 339



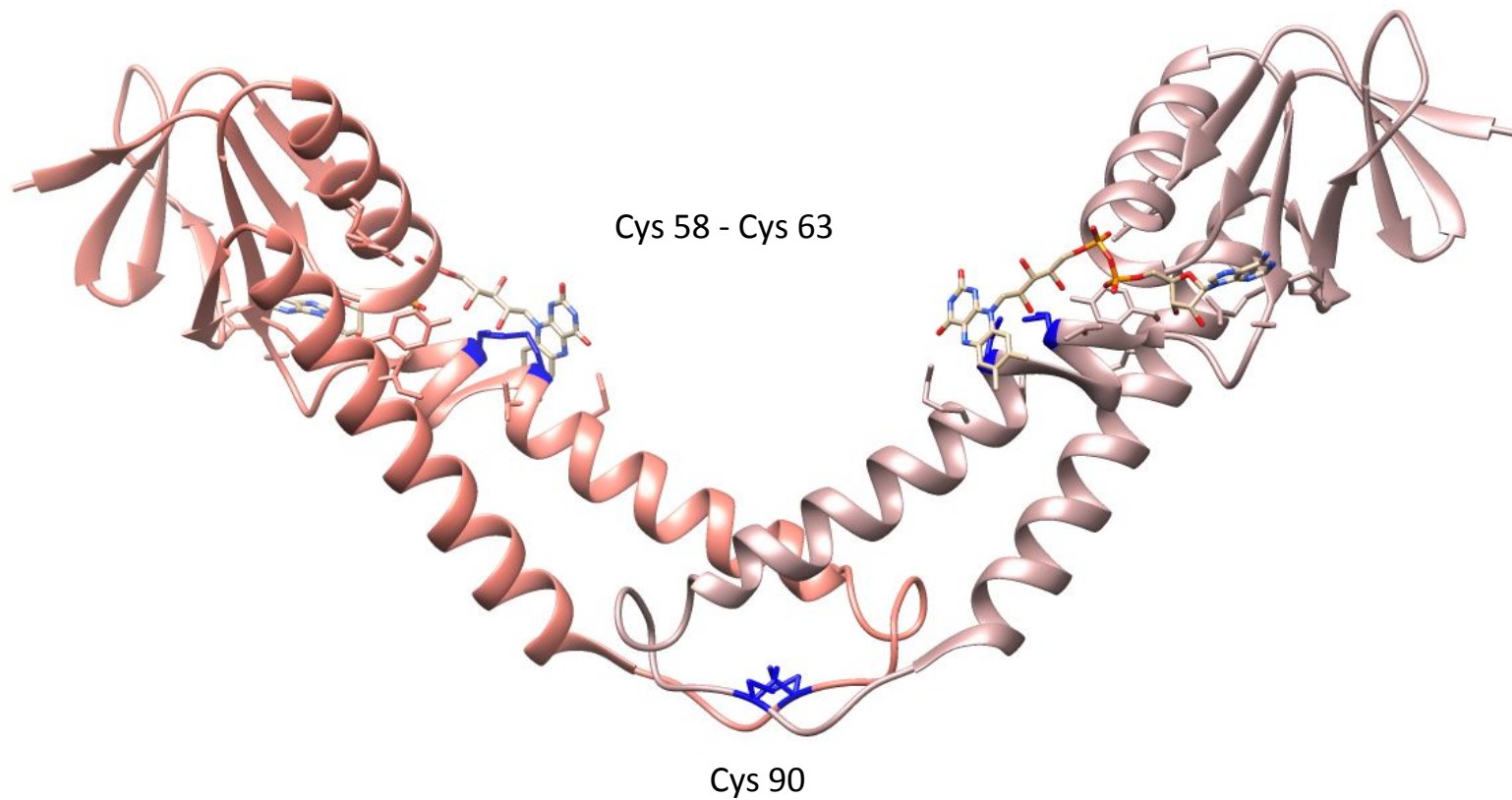
ASP 331



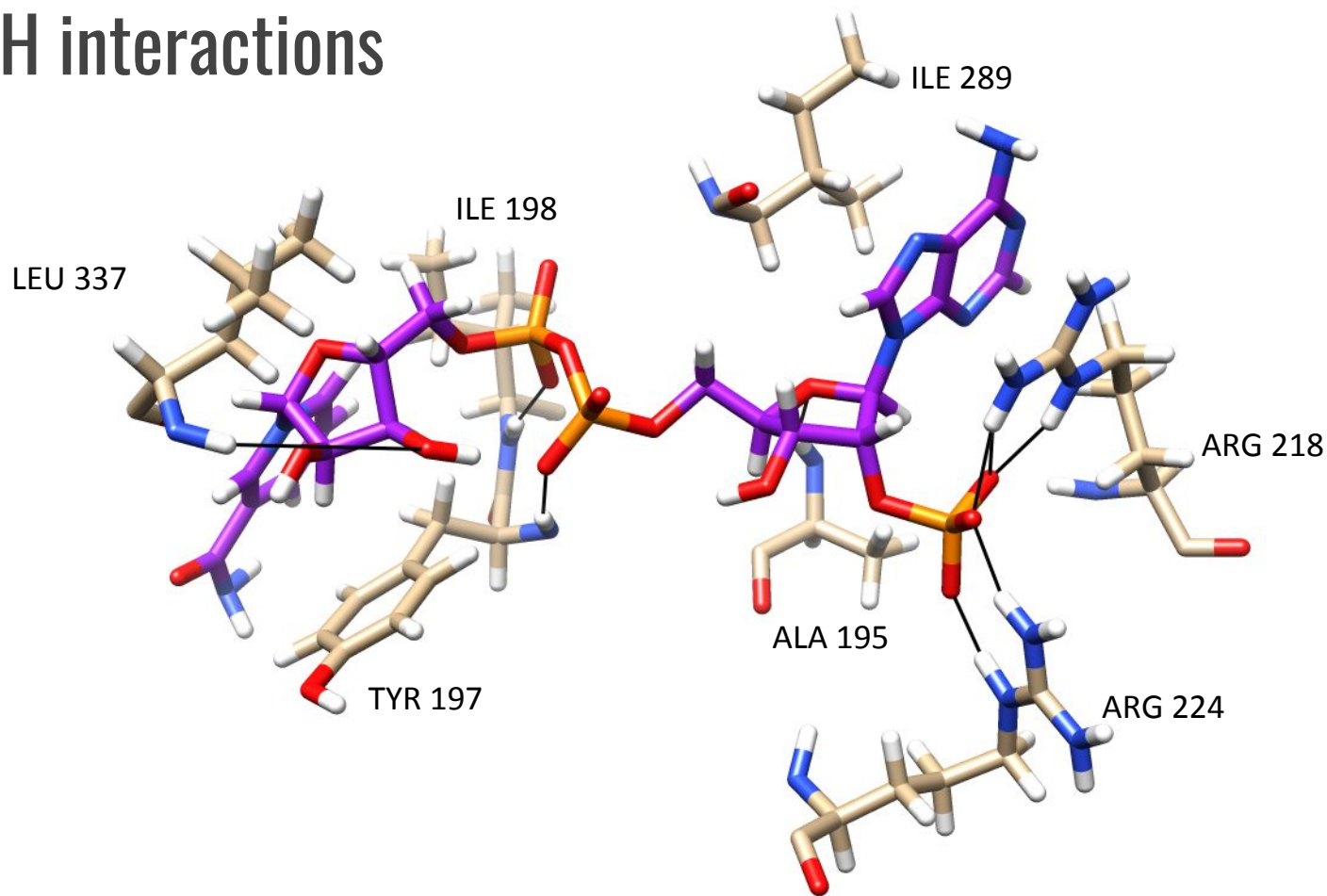
Hydrophobic interactions FAD



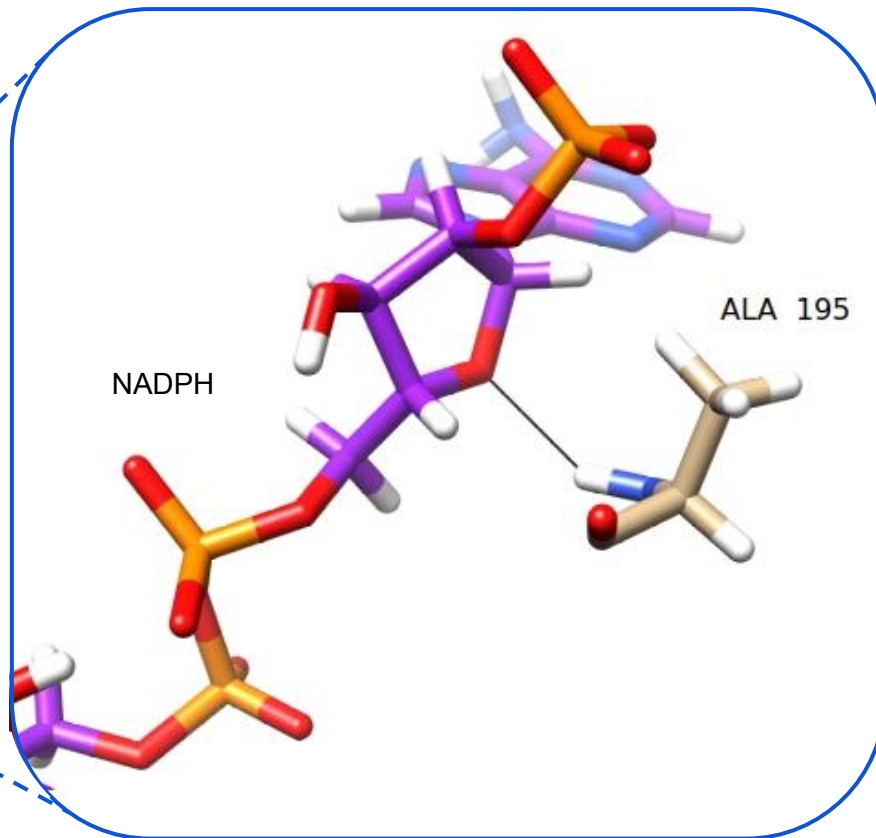
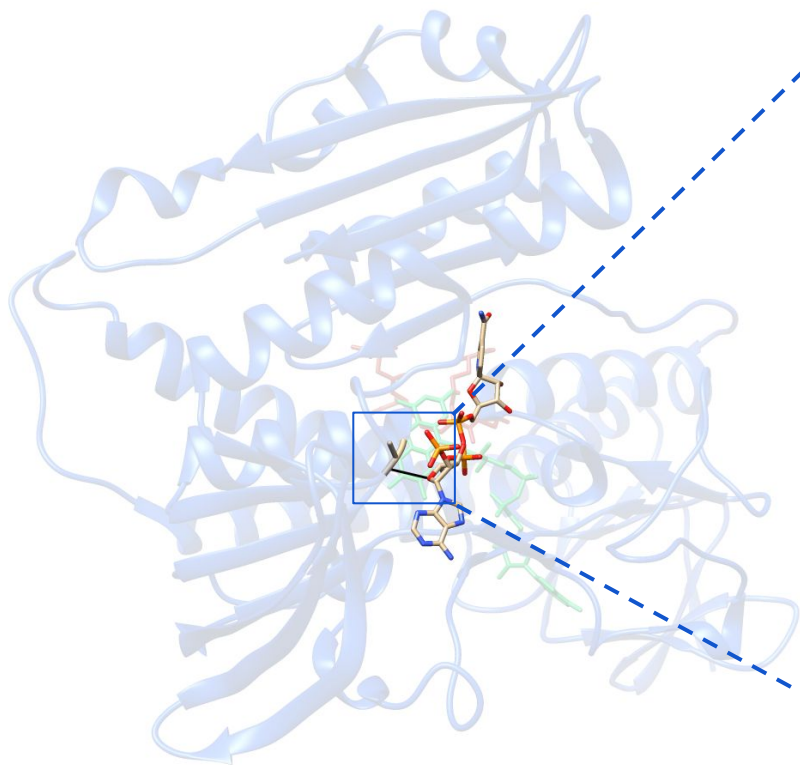
CYS interactions



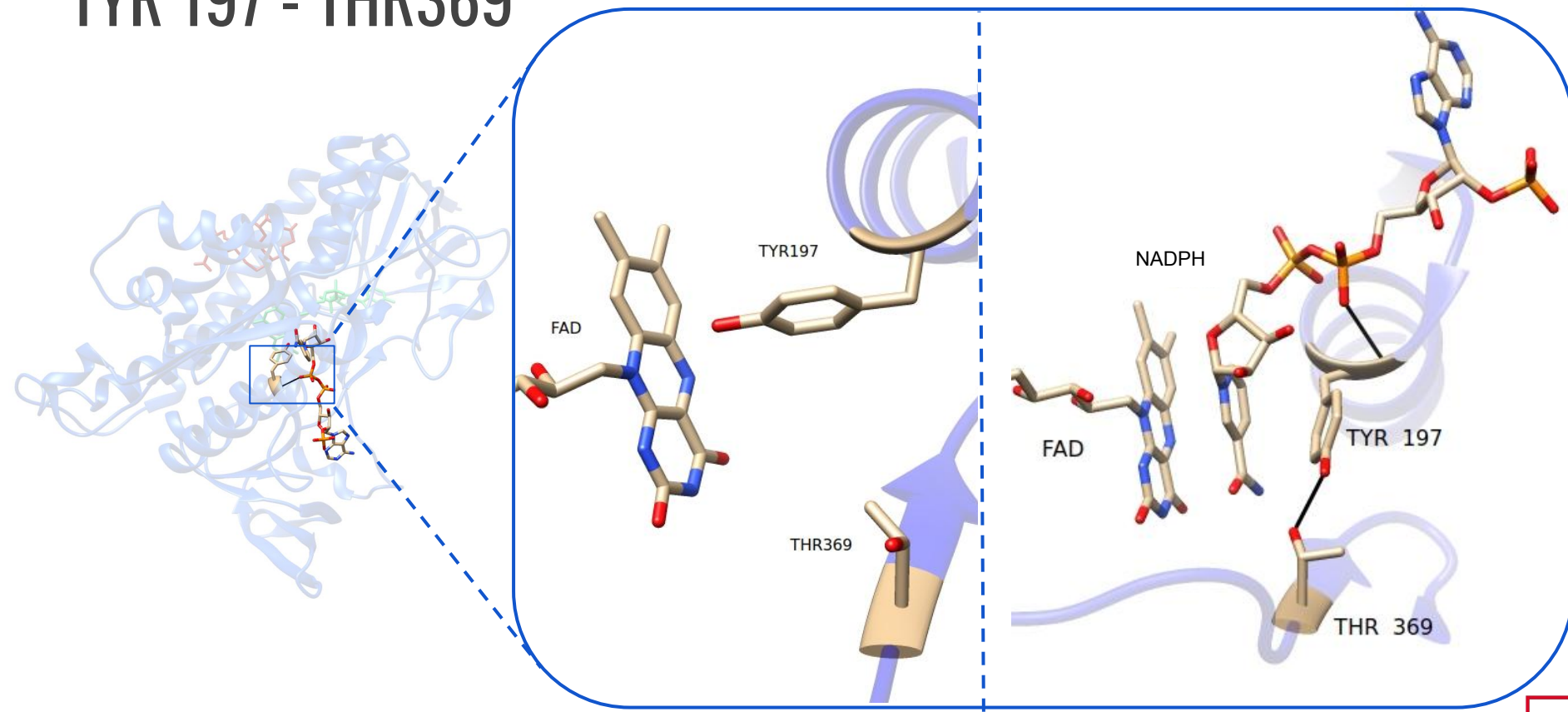
NADPH interactions



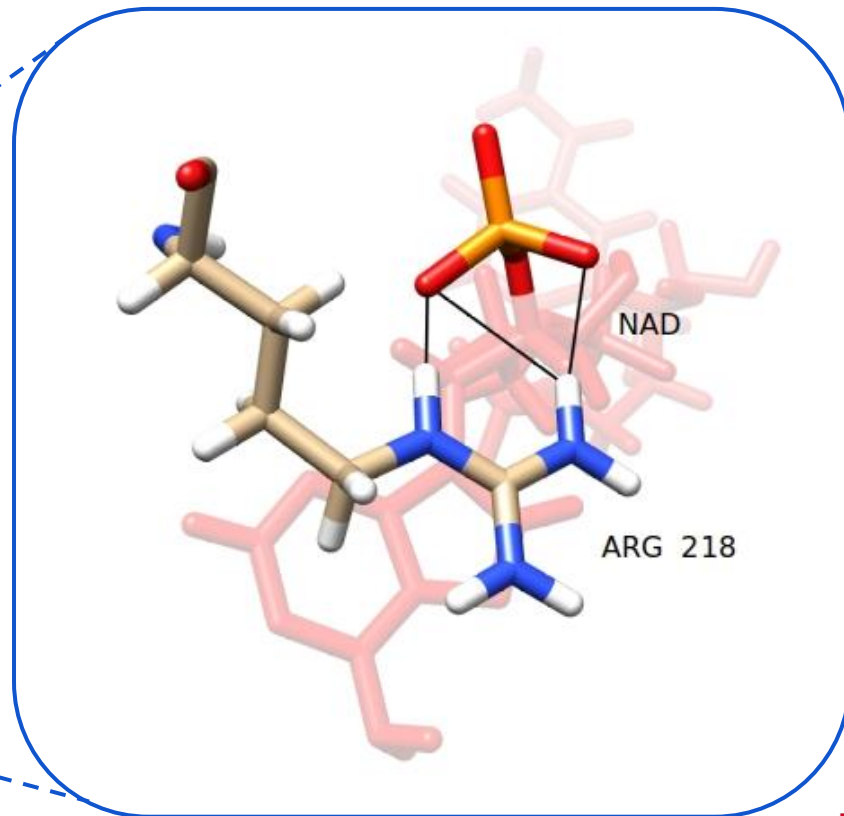
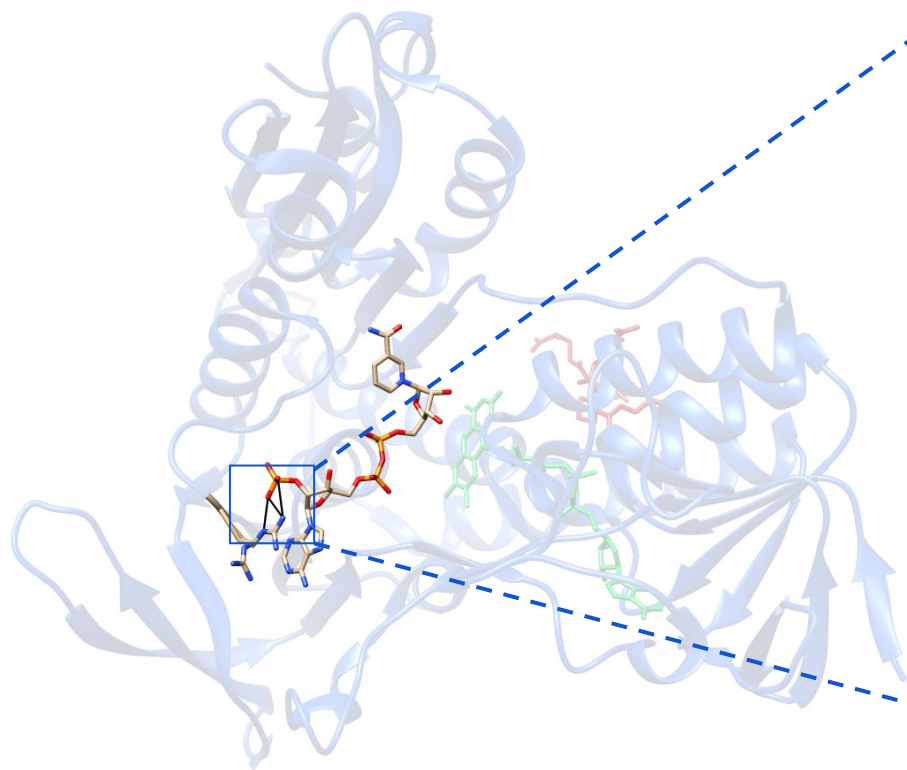
ALA 195



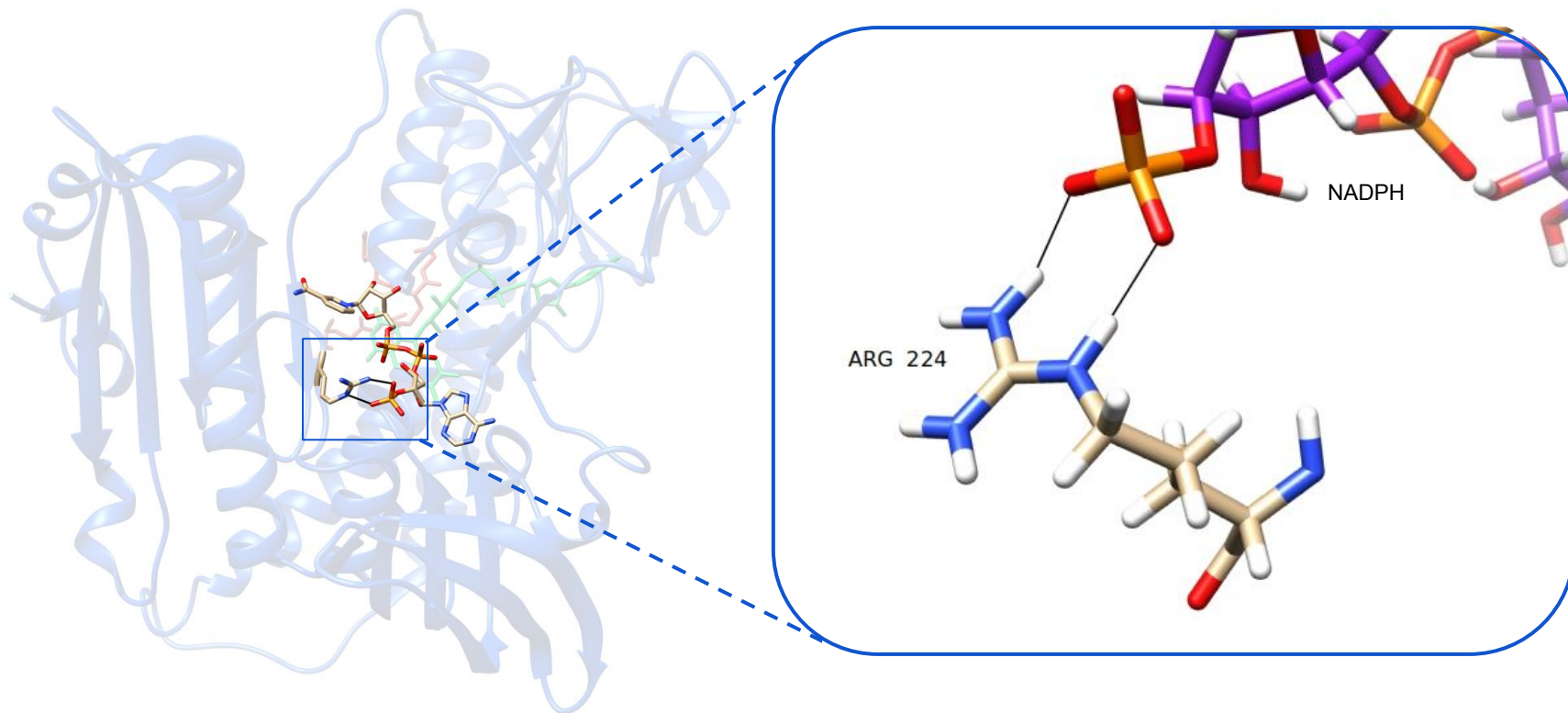
TYR 197 - THR369



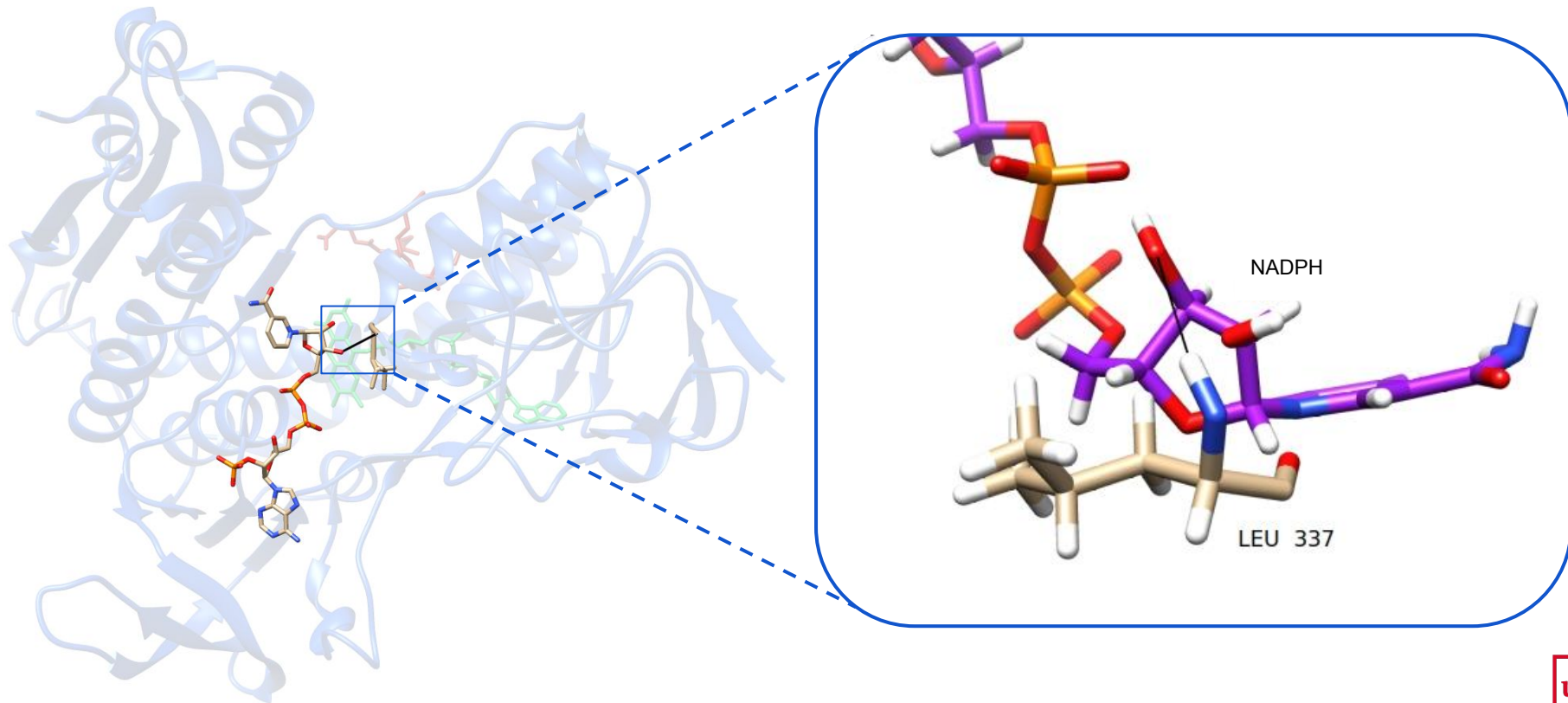
ARG 218



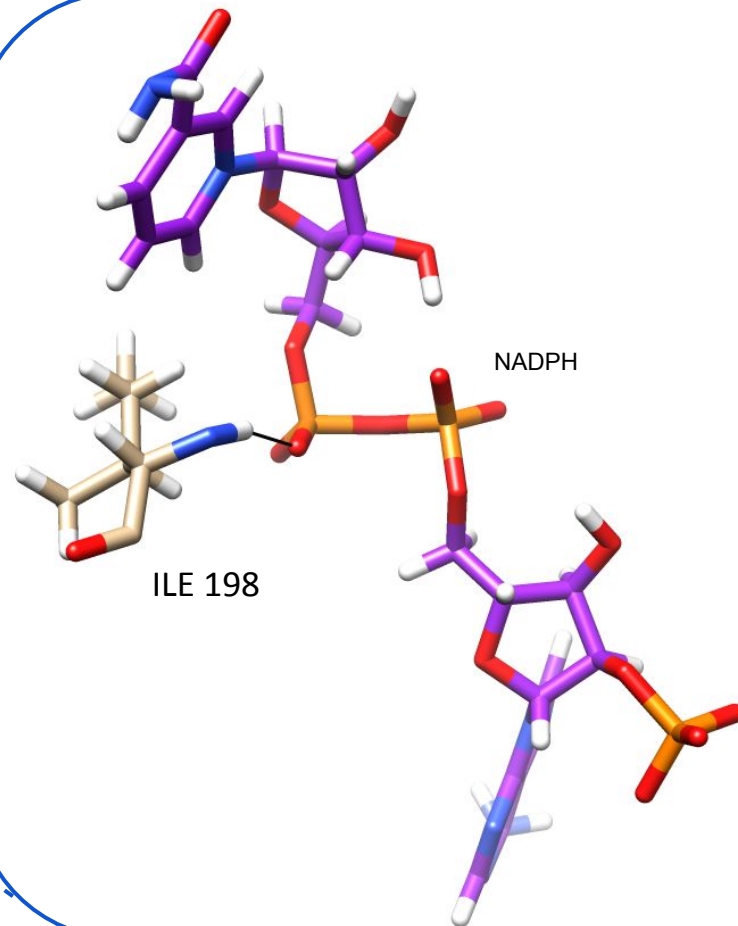
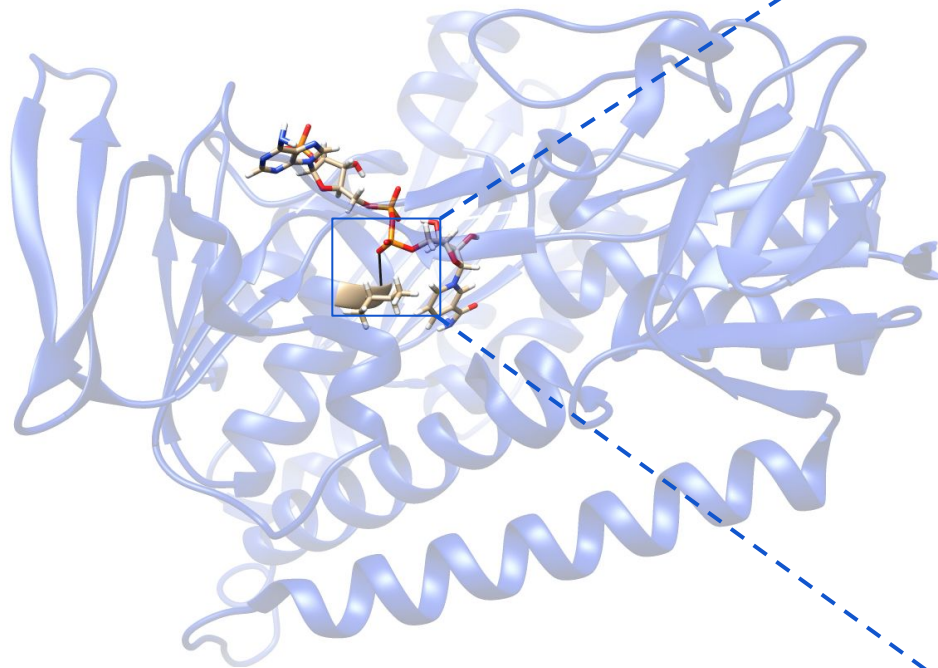
ARG 224



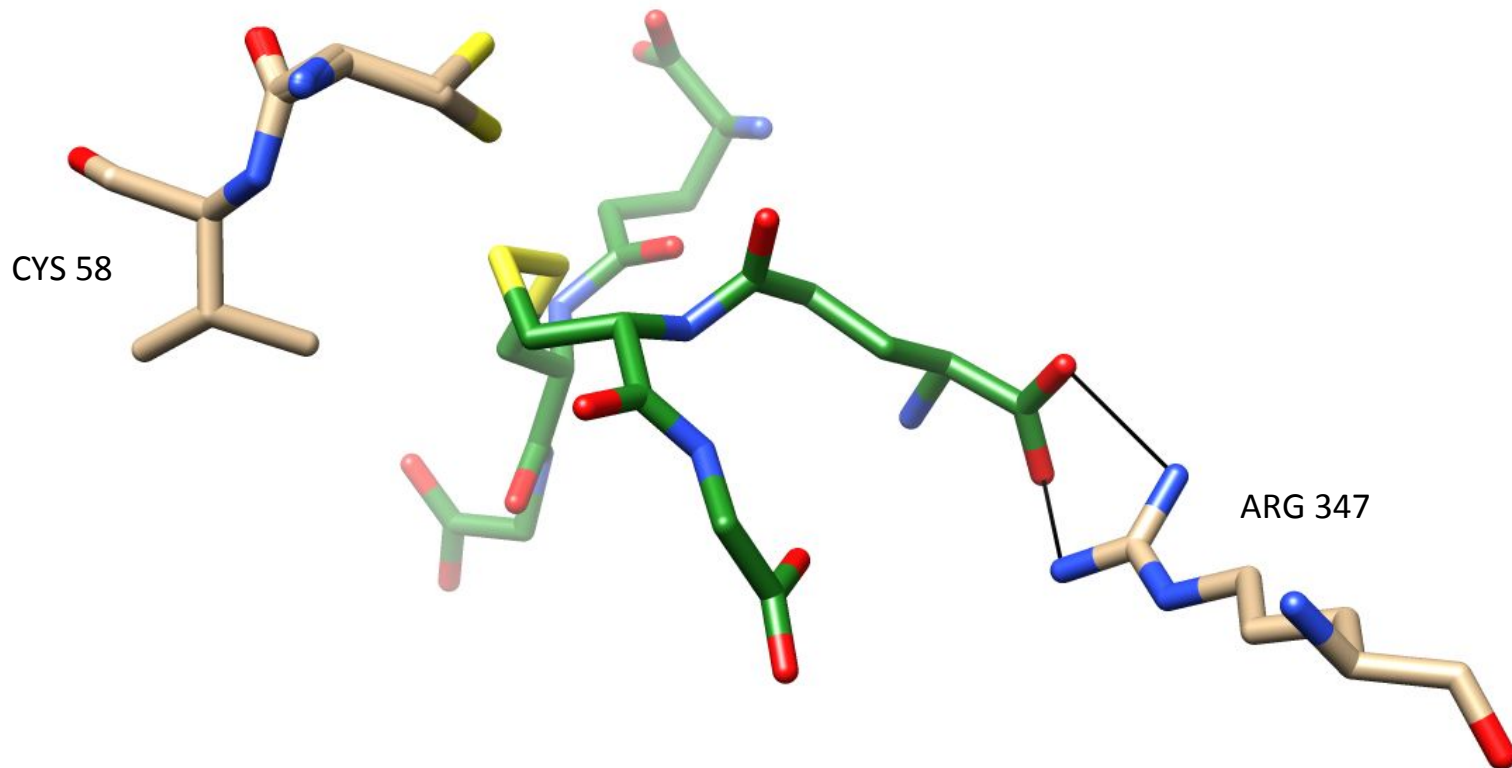
LEU 337



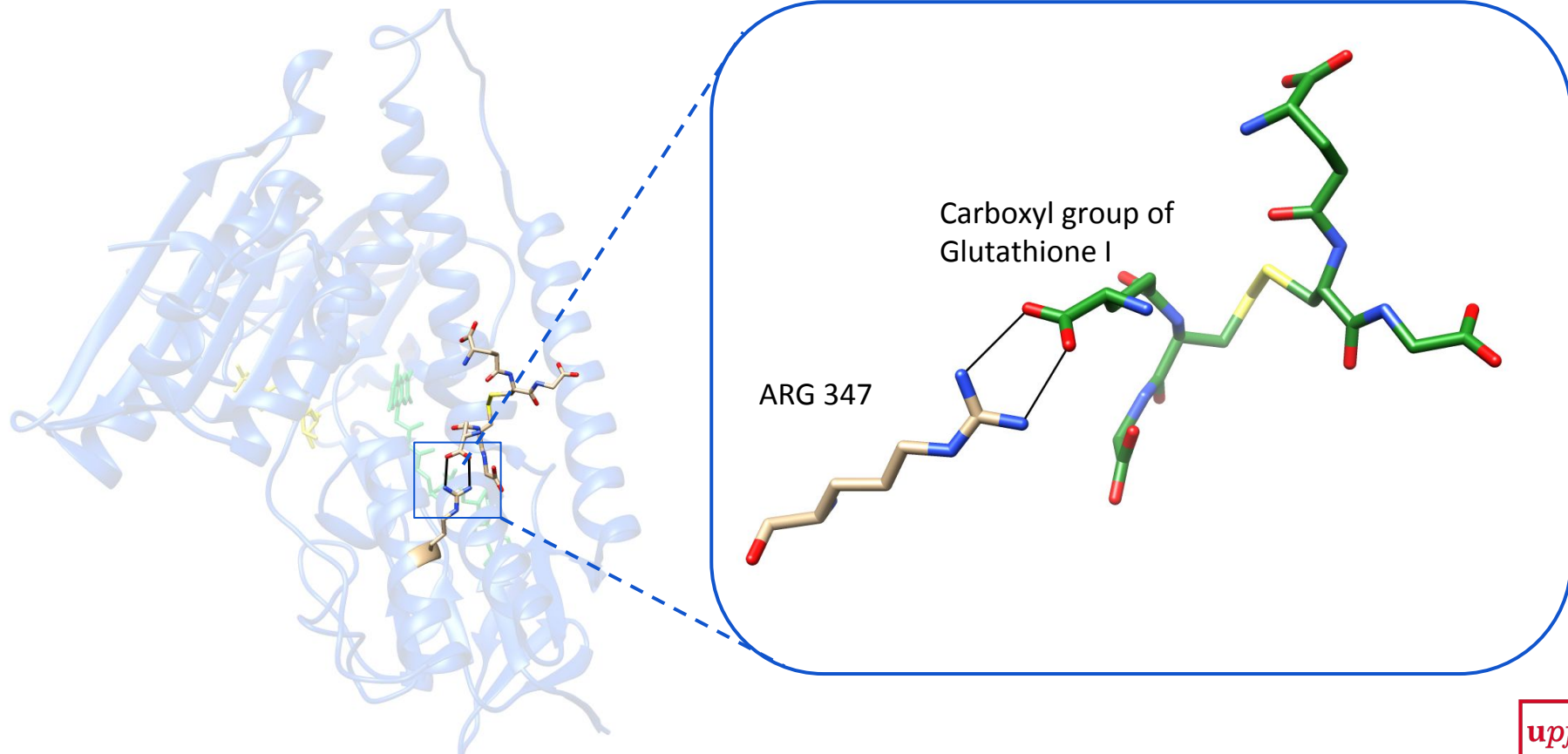
ILE 198



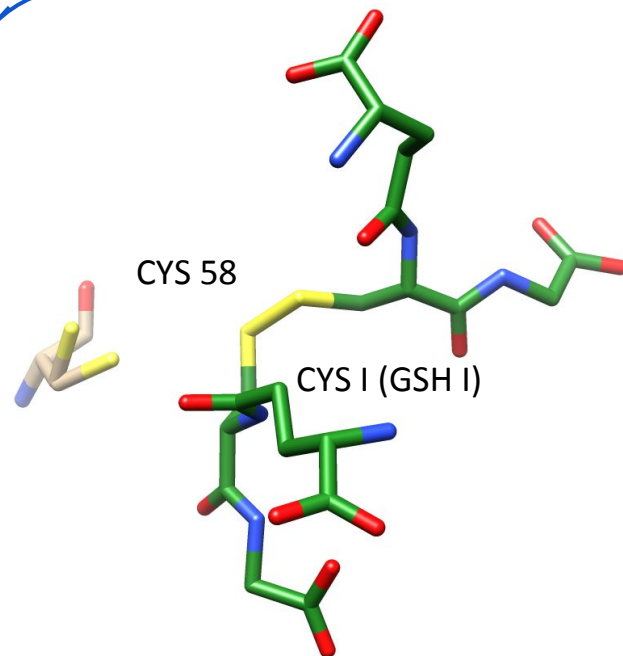
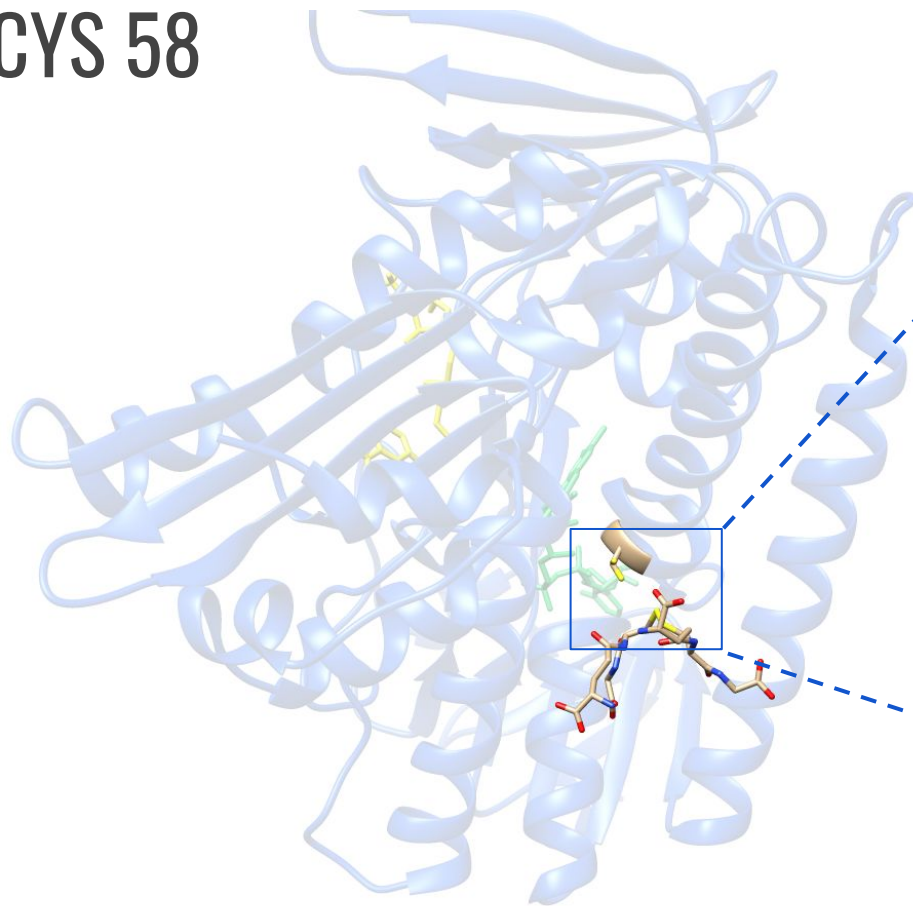
Glutathione interactions



ARG 347



CYS 58



Sequence alignment between species

Mitochondrial GR: 2, 4, 6, 9
Cytosolic GR: 1, 3, 5, 7, 8

Purple: conserved motifs
Red: FAD interactions
Blue: NADPH interactions
Green: GSSG interactions

		Gly 31	Glu 50 Ser 51	Thr 57 Cys 58	Gly 62 Cys 63	Lys 66	
1 Escherichia_coli	-MTKHYDYIAIGGGSGG	I	A	S	I	N	59
2 Caenorhabditis_elegans	SGVKEFDYLVIGGGSGG	I	A	S	A	R	75
3 Danio_rerio	GSVSRFDLVVGGGSGG	L	A	G	A	R	94
4 Danio_rerio	GSVSRFDLVVGGGSGG	L	A	G	A	R	63
5 Gallus_gallus	MAAAAYELLVLGGGSGG	L	A	G	A	R	60
6 Homo_sapiens	GAVASYDYLVIGGGSGG	L	A	S	A	R	119
7 Homo_sapiens	GAVASYDYLVIGGGSGG	L	A	S	A	R	119
8 Sus_scrofa	SVATSFDYLVIGGGSGG	L	A	S	A	R	116
9 Mus_musculus	PDTSSFYDYLVIGGGSGG	L	A	S	A	R	97
	. : : : ***** . . ** * : : *						

GXGXXG
FAD Rossmann fold
motif

Cys 58 Cys 63
Catalytic Cys motif

		Ala 130	
1 Escherichia_coli	REAIHMYGPDYGFDTTINKFNWETLIASRTAYIDRIHTSYENVLGKNNVDVIKGFARFVD		119
2 Caenorhabditis_elegans	AEFIR-DHADYGFDTVTLNKFDMKVIKKSDEYIKRLNGLYESGLKGSSVEYIRGRATFAE		134
3 Danio_rerio	AEYLH-DHEDYGFEGAKAHFSWQIIKHKRDAYVSRLNQIYRSNLEKGKIEFIHGYPARFTD		153
4 Danio_rerio	AEYLH-DHEDYGFEGAKAHFSWQIIKHKRDAYVSRLNQIYRSNLEKGKIEFIHGYPARFTD		122
5 Gallus_gallus	AEFIH-DHPDYGFEPGVRFNWRITKEKRDAYVRLNEIYENNVAKAHIDIIRGYGKFTA		119
6 Homo_sapiens	SEFMH-DHADYGFPSCEGKFNRVVIKEKRDAYVSRLNAIYQNNLTKSHIEIIRGHAAFTS		178
7 Homo_sapiens	SEFMH-DHADYGFPSCEGKFNRVVIKEKRDAYVSRLNAIYQNNLTKSHIEIIRGHAAFTS		178
8 Sus_scrofa	SEFMH-DHVDYGFQSCSKFNWRVVIKEKRDAYVSRLNTIYQNNLTKSHIEIIRGHAAFTS		175
9 Mus_musculus	SEFMH-DHVDYGFQSCGKFSWVVIKQKRDAYVSRLNTIYQNNLTKSHIEIIRGHAAFTAD		156
	* : : * * * * : * . . : . * * : * : : * . . : : : * : * . *		

Sequence alignment between species

Mitochondrial GR: 2, 4, 6, 9
Cytosolic GR: 1, 3, 5, 7, 8

Purple: conserved motifs
Red: FAD interactions
Blue: NADPH interactions
Green: GSSG interactions

Thr 156

1 Escherichia_coli	A--KTLEVNGETITADHILIA	TGGRP	SHPD---	IPGVEYGIDSDGFFALPALPERVA	WVG	174
2 Caenorhabditis_elegans	D--GTVEVNGAKYRGKNTLIA	VGGKPT	IPN---	IKGAEHGIDSDGFFDLEDLPSRT	WVG	189
3 Danio_rerio	DPEPTVEVNGKKYTATHILIS	TGGHPST	VSEDDVPGSSLGITSDGFFE	LESCPKRSV	IVG	213
4 Danio_rerio	DPEPTVEVNGKKYTATHILIS	TGGHPST	VSEDDVPGSSLGITSDGFFE	LESCPKRSV	IVG	182
5 Gallus_gallus	DPEPTIEVDGQKYTAPHILIA	TGGRP	WPPDCEVPGASLGITSDGFFD	LEELPRHS	WVG	179
6 Homo_sapiens	DPKPTIEVSGKKYTAPHILIA	TGGMPST	PHESQIPGASLGITSDGFFQ	LEELPGRS	VIVG	238
7 Homo_sapiens	DPKPTIEVSGKKYTAPHILIA	TGGMPST	PHESQIPGASLGITSDGFFQ	LEELPGRS	VIVG	238
8 Sus_scrofa	DPQPTVEVNGKKYTAPHILIA	TGGVPS	VPPESQIPGASLGITSDGFFQ	LEELPSRS	VIVG	235
9 Mus_musculus	GPRPTVEVNGKKFTAPHILIA	TGGVPT	VPHE	SQIPGASLGITSDGFFQ	LEDLPSRS	VIVG 216
	*:***. . . : **:	** *		: * . ** ***** *	* : . : **	

Ala 195

Tyr 197

Arg 218

Arg 224

G

1 Escherichia_coli	AGYIAVELAGVINGLGAKTHL	FVRKHAPLR	RSFDP	MISETLVEVMNA--	EGPQLHTNAIPK	232
2 Caenorhabditis_elegans	AGYIAVEIAGVLANLGS	DTHLIRYDKVLR	TFDKMLS	DELTA	MDDEETNPLHLHKNTQVT	249
3 Danio_rerio	AGYIAVEMAGILSTLGS	KTSSIIIRQGGVLR	NFDALISSNCT	KELQN--	NGIDL	RKNTQVK 271
4 Danio_rerio	AGYIAVEMAGILSTLGS	KTSSIIIRQGGVLR	NFDALISSNCT	KELQN--	NGIDL	RKNTQVK 240
5 Gallus_gallus	AGYIAVEIVGILSTLGS	KSSLLIRRD	KVLR	TFDSL	ISTNCTQELEN--	MGVDVWKHTQVQ 237
6 Homo_sapiens	AGYIAVEMAGILSALGS	KTSLMIRHDKVLR	RSFDS	MISTNCTE	ELEN--	AGVEVLKFSQVK 296
7 Homo_sapiens	AGYIAVEMAGILSALGS	KTSLMIRHDKVLR	RSFDS	MISTNCTE	ELEN--	AGVEVLKFSQVK 296
8 Sus_scrofa	AGYIAVEIAGILSALGS	KTSLMIRHDKVLR	RSFDS	IISNCTE	ELEN--	AGIEVLKYSQVK 293
9 Mus_musculus	AGYIAVEIAGILSALGS	KTSLMIRHDKVLR	NFDSLISSNCTE	ELEN--	AGVEVLKFTQVK	274
	*****:.*::	**::: :*: *	**.* :	* . ::	. : . :	

XGXXA

NADPH Rossmann fold motif

Mitochondrial GR: 2, 4, 6, 9
Cytosolic GR: 1, 3, 5, 7, 8

Red: FAD interactions

Blue: NADPH interactions

Green: GSSG interactions

Flavin ring contact motif

Species	Sequence	Score
1 Escherichia_coli	EKG YI V D K Y Q N T N I E G I Y A V G D V C G A L L T P V A V A A G R R L S E R L F N N K P D E H L D Y S N I P	340
2 Caenorhabditis_elegans	K S G H I I V D E Y Q N T S A P G I L S V G D D T G K F L L T P V A I A A G R R L S H R L F N G E T D N K L T Y E N I A	357
3 Danio_rerio	E R G H I V D E F Q N T S R P G V Y A V G D V C G R A L L T P V A I A A G R K L A H R L F E G K A D S K V D Y N N I P	390
4 Danio_rerio	E R G H I V D E F Q N T S R P G V Y A V G D V C G R A L L T P -----	331
5 Gallus_gallus	P H N H V V D E F Q N T T R K G I Y A I G D V C G K A L L T P V A I A A G R K L A L R L F G N Q Q H A R L D Y S N I P	354
6 Homo_sapiens	D K G H I I V D E F Q N T N V K G I Y A V G D V C G K A L L T P V A I A A G R K L A H R L F E Y K E D S K L D Y N N I P	412
7 Homo_sapiens	D K G H I I V D E F Q N T N V K G I Y A V G D V C G K A L L T P V A I A A G R K L A H R L F E Y K E D S K L D Y N N I P	412
8 Sus_scrofa	D K G H I I V D E F Q N T N V K G I Y A V G D V C G R A L L T P V A I A A G R K L A H R L F E C K E D S K L D Y D N I P	409
9 Mus_musculus	E K G H I L V D E F Q N T N V K G V Y A V G D V C G K A L L T P V A I A A G R K L A H R L F E C K Q D S K L D Y D N I P	390

Sequence alignment between species

Mitochondrial GR: 2, 4, 6, 9
Cytosolic GR: 1, 3, 5, 7, 8

Purple: conserved motifs
Red: FAD interactions
Blue: NADPH interactions
Green: GSSG interactions

1 Escherichia_coli	TWFSHPPIGTVGLTEPQAREQYGDDQVKVYKSSFTAMYTAVTTHRQPCRMKLVCVGSEE	400
2 Caenorhabditis_elegans	TWFSHPLIGTVGLTEAEAVEKYGKDEVTLYKSRFPMLFAVTKHKEKAAMKLVCVGKDE	417
3 Danio_rerio	TWFSHPPIGTVGLTEDEAVKTYGDKVKVYTTSTFTPMYYAITTRKSQCIMKLVCAGENE	450
4 Danio_rerio	-----DEAVKTYGDKVKVYTTSTFTPMYYAITTRKSQCIMKLVCAGENE	375
5 Gallus_gallus	TWFSHPPIGTVGLTEDEAISVYGKDNVKIYSTLFTPMYHAVTQRKVKCVMKLVCAGKEE	414
6 Homo_sapiens	TWFSHPPIGTVGLTEDEAIHKYGIENVKTYSTSTFTPMYHAVTKRKTCKVMKMCANKEE	472
7 Homo_sapiens	TWFSHPPIGTVGLTEDEAIHKYGIENVKTYSTSTFTPMYHAVTKRKTCKVMKMCANKEE	472
8 Sus_scrofa	TWFSHPPIGTVGLTEDEAICKYGENVKIYSTTFTPMYHAVTKRKTCKVMKMCANKEE	469
9 Mus_musculus	TWFSHPPIGTVGLTEDEAVHKYGKDNVKIYSTAFTPMYHAVTTRKTCKVMKMCANKEE	450
	:* ** :*. *.: *. * *: * :: . **: **...:*	

Interface domain: highly conserved

1 Escherichia_coli	KIVGIHGIGFGMDEMLQGFVAVALKMGATKKDFDNTVAIHPTAAEEFVTMR-----	450
2 Caenorhabditis_elegans	KVGVVHVFVGSGDEMLQGFVAVAVTMGATKKQFDQTVAIHPTSAAELVTMRGGVKPE	473
3 Danio_rerio	KVVG LHMQGFGCDEMLQGFVAVAVNMGATKADFDRITIAIHPTSSEELVTLR-----	500
4 Danio_rerio	KVVG LHMQGFGCDEMLQGFVAVAVNMGATKADFDRITIAIHPTSSEELVTLR-----	425
5 Gallus_gallus	KVVG LHMQGLGCDEMLQGFVAVAIKMGATKADFNTVAIHPTSAAELVTMR-----	464
6 Homo_sapiens	KVVG IHMQGLGCDEMLQGFVAVAVKMGATKADFNTVAIHPTSSEELVTLR-----	522
7 Homo_sapiens	KVVG IHMQGLGCDEMLQGFVAVAVKMGATKADFNTVAIHPTSSEELVTLR-----	522
8 Sus_scrofa	KVVG IHMQGIGCDEMLQGFVAVAVKMGATKSDFDNTVAIHPTSSEELVTLR-----	519
9 Mus_musculus	KVVG IHMQGIGCDEMLQGFVAVAVKMGATKADFNTVAIHPTSSEELVTLR-----	500
	*: ** :* *. * *****: . ***** : ** . *: *****: ** : ** : *	

Structural alignment between species

PDB code	Species
3GRS	<i>Homo sapiens</i>
1GER	<i>Escherichia coli</i>
1ONF	<i>Plasmodium falciparum</i>
6DU7	<i>Streptococcus pneumoniae</i>
3O0H	<i>Bartonella henselae</i> (strain ATCC 49882 / DSM 28221 / Houston 1)
6B4O	<i>Enterococcus faecalis</i>
5U1O	<i>Vibrio parahaemolyticus</i> serotype O3:K6 (strain RIMD 2210633)
4DNA	<i>Rhizobium meliloti</i>

GR + FAD

Chain A



Rough STAMP

Structural alignment between species

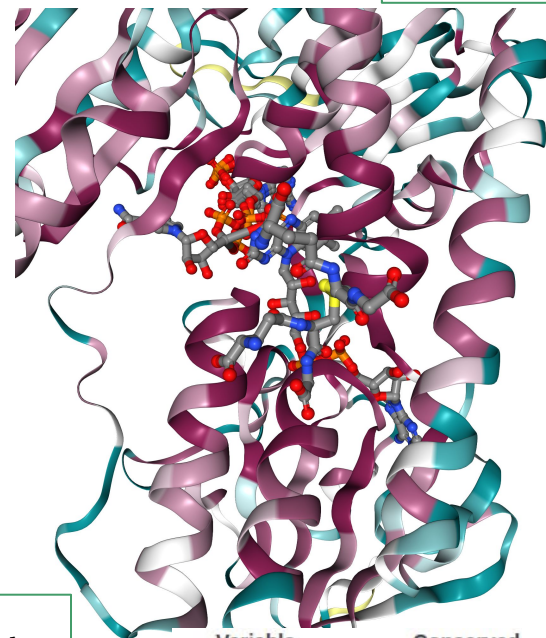
Pink: α -helix
Yellow: β -strand

1onf: *P. falciparum*
3grs: *H. sapiens*
6du7: *P. falciparum*
6b4o: *E. faecalis*
1ger: *E. coli*
5u1o: *V. parahaemolyticus*
3o0h: *B. henselae*
4dna: *R. meliloti*

	FAD Rossmann fold core			Molecular diad
	β -sheet		β -sheet	
1onf	---	VYDLIVIGGGSGGMAAARRAARHNAK	VALVEKSRLGGTCVNVGCVPKKIMFNAASV	
3grs	--	VASYDYLIVIGGGSGGLASARRAAELG	ARAAVVESHKLGGTCVNVGCVPKKVMWNTAVH	
6du7	--	MREYDIIAIGGGSGGIATMNRAGEHGA	QAAVIEEKKLGGTCVNVGCVPKKIMWYGAQI	
6b4o		NAMKTYDYIVIGGGSGGIASANRAGMHG	ANVLLIEGNEIGGTCVNVGCVPKKVMWQASSM	
1ger	--	KHYDYIAIGGGSGGIASINRAAMYGQK	CALIEAKELGGTCVNVGCVPKKVMWHAAQI	
5u1o	--	ATHFDYICIGGGSGGIASANRAAMYGA	KVALIEAQDLGGTCVNVGCVPKKVMWHGAQI	
3o0h	--	SFDFDLFVIGSGSGGVRAARLAGALGK	RVAIAEEYRIGGTCVIRGCVPKKLYFYASQY	
4dna	--	AFDYDLFVIGGGSGGVRSGRLLAALGK	VVAIAEEFRYGGTCVIRGCVPKKLYVYASQF	

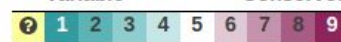
1onf	HDILE-NSRHYGFD-TKF-SFNLPLLVERRDKYIQRLNNIYRQNL	SKDKVDLYEGTASF-
3grs	SEFM-HDHADYGFPSC-E-GKFNWRVIKEKRDAYVSRLNAIYQNNL	TKSHIEIRGHAAFT
6du7	AETFHQFGEDYGFK-TTDLNDFATLRRNRESYIDRARSSYDGSF	KRNGVDLIEGHAEFV
6b4o	MEMMERDTAGYGFD-VEIKNFSFKQLVENREKYIDFLHGAYNRGL	DSNNIERIHGYATFT
1ger	REAIHMYGPDYGFD-TTINKFNWETLIASRTAYIDRIHTSYENVL	GKNNVDVIKGFARFV
5u1o	AEAMNLYAEDYGFD-VDVKGFDWSKLVESRQAYIGRIHQSYDRVL	GNKVMVIKGFAKFV
3o0h	AQEF-SKSIGFGWK-YADPIFNWEKLVAANKKEISRLEGLYREGL	QNSNVHIYESRAVFV
4dna	AEHF-EDAAGFGWT-VGESRFDWAKLVAAKEQETARLEGLYRKGL	ANAGAEILDTRAELA

Molecular diad

 β -sheet β
meandre

Variable

Conserved



Structural alignment between species

β meandre

```

1onf  -----L-----E-GRNIIAVGNKPVF-PP---VKGIENTISSDEFFNIKE-SKKI
3grs  SDPKPTIEV-S-GKKYTAPHILIAITGGMPST-PHESQIPGASLGITSDGFFQLEELPGRS
6du7  -D-SHTVSV-N-GELIRAKHIVIAITGAHPSI-PN---IPGAELGGSSDDVFAWEELPESV
6b4o  -G-EQTIEV-N-GTEYTAPHILIAITGGRPKK-LG---IPGEEYALDSNGFFALEEMPKRK
1ger  -D-AKTLEV-N-GETITADHILIAITGGRPSH-PD---IPGVEYGIDSDGFFALPALPERV
5u1o  -D-EKTVEV-N-GEHYTADHILIAVGGRTI-PN---IPGAEYGIDSNNGFFDLAEQPKRV
3o0h  -D-EHTLELSVTGERISA EKILIAITGAKIVS-NI----KGSDLCLTSNEIFDLEKLPKSI
4dna  -G-PNTVKLLASGKTVTAERIVIAVGGHPSPHDA---LPGHELCITSNEAFDLPALPESI
  
```

```

1onf  GIVGSGYIAVELINVIKRLGIDSYIFARGNRILRKFDSESVINVLENDMKKNINIVTFAD
3grs  VIVGAGYIAVEMAGILSALGSKTSLMRHDKVLRFSFSMISTNCTEELNAGVEVLKFSQ
6du7  AILGAGYIAVELAGVLHTFGVKTDLFVRRDRPLRGFDSYIVEGLVKEMERTNPLHHTKV
6b4o  VVVGAGYIAAELAGTLHGLGAETHWAFRHERPLRSFDDMLSEKVERYQEMGMQIHPNAT
1ger  AAVGAGYIAVELAGVINGLGAKTHLFVRKHAPLRSFDPMISETLVEVMNAEGPQLHTNAI
5u1o  AAVGAGYIAVEIAGVLHALGTETHLFVRKESPLRSFDPMIIDTLVEVMEAGPTLHTHSV
3o0h  VIVGGGYIGVEFANIFHGLGVKTTLLHRGDLILRNFDYDLRQLLNDAMVAKGISIIEAT
4dna  LIAGGGYIAVEFANIFHGLGVKTTLIYRGKEILSRFDQD-RRGLHAA-EEKGIRILCEDI
  
```

β-sheet

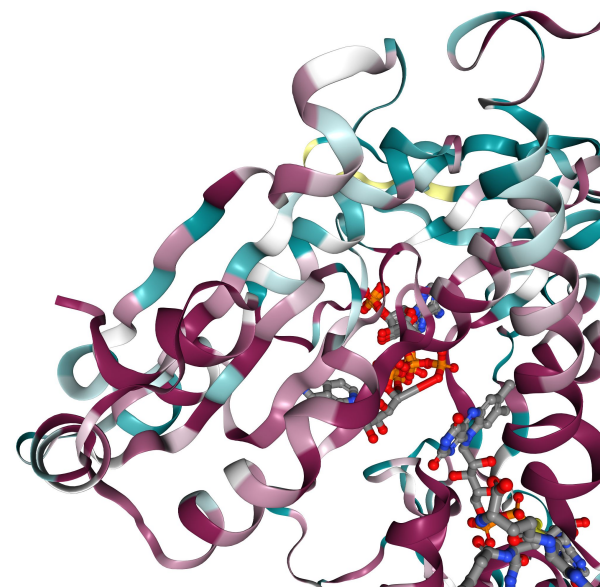
β-sheet

β-sheet

NADPH Rossmann fold core

Pink: α-helix
Yellow:
β-strand

1onf: P. falciparum
3grs: H. sapiens
6du7: P. falciparum
6b4o: E. faecalis
1ger: E. coli
5u1o: V. parahaemolyticus
3o0h: B. henselae
4dna: R. meliloti



Variable Conserved

Variable	1	2	3	4	5	6	7	8	9
Conserved	1	2	3	4	5	6	7	8	9

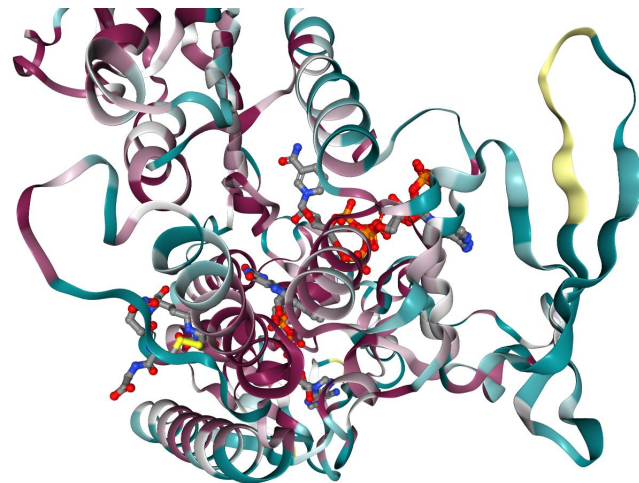
Structural alignment between species

Pink: α -helix
Yellow: β -strand

1onf: *P. falciparum*
3grs: *H. sapiens*
6du7: *P. falciparum*
6b4o: *E. faecalis*
1ger: *E. coli*
5u1o: *V. parahaemolyticus*
3o0h: *B. henselae*
4dna: *R. meliloti*

	β meandre	β -sheet
1onf	WEIKKVS-DKNSIHL-	-----DGR-IYEHFDHVIYCVGRSPD
3grs	VKEVKKTL--GLEVSMVT	AVPGRLPMT-MIPDVCLLWAIGRPNTKDL
6du7	PVKLEKTTD--GITIHFE-	-----DGT-SHT-ASQVIWATGRRPNV
6b4o	PAKIEKTAQN-EYVITFE-	-----NGE-SIT-TDAVIFGTGRQPNTD
1ger	PKAVVKNTDG-SLTLELE-	-----DGR-SET-VDCLIWAIGREPAND
5u1o	PKEVVKADG-SLTLHLE-	-----NGE-SQN-VDQLIWAIGRHPATD
3o0h	VSQVQSTEN--CYNVLT-	-----NGQ-TIC-ADRVMLATGRVPNTT
4dna	IQSVSADADG-RRVATT-	-----K-HGEIV-ADQV-LALGR-PNTNGL

1onf	N-NNYIVVDENQRTSVNNIYAVGDC	CMVKFYNVQLTPVAINAGRLLADRLFL-KKTRKTN
3grs	DDKGHIIVDEFQNTNVKGIYAVGDVCG---	KALLTPVAIAAGRKLALHRLFYKEDSKLD
6du7	NERGFIQVDEYQNTVVEGIYALGDVTG---	EKELTPVAIKAGRTLSERLFNGKTTAKMD
6b4o	DEKGYVKVDKFQNTTQNGIYAVGDVIG---	KIDLTPVAIAAGRRLSERLFNGQTDLYLD
1ger	NEKGYIVVDKYQNTNIEGIYAVGDNTG---	AVELTPVAVAAGRRLSERLFNNKPDEHLD
5u1o	NEKGYIKVDEYQETNVKGIYCVGDIME---	GGIELTPVAVKAGRQLSERLFNNKPNAMKD
3o0h	NEFGAVVDEKMTTNVSHIWA	VDVTG---HIQLTPVAIHDMCFVKNAF-E-T--TPD
4dna	NELGAIIVDAFSRTSTPGIYALGDVTD---	RVQLTPVAIHEA-CFIETEKNNP-TSPD



Structural alignment between species

Pink: α -helix
Yellow: β -strand

1onf: *P. falciparum*
3grs: *H. sapiens*
6du7: *P. falciparum*
6b4o: *E. faecalis*
1ger: *E. coli*
5u1o: *V. parahaemolyticus*
3o0h: *B. henselae*
4dna: *R. meliloti*

Interface domain

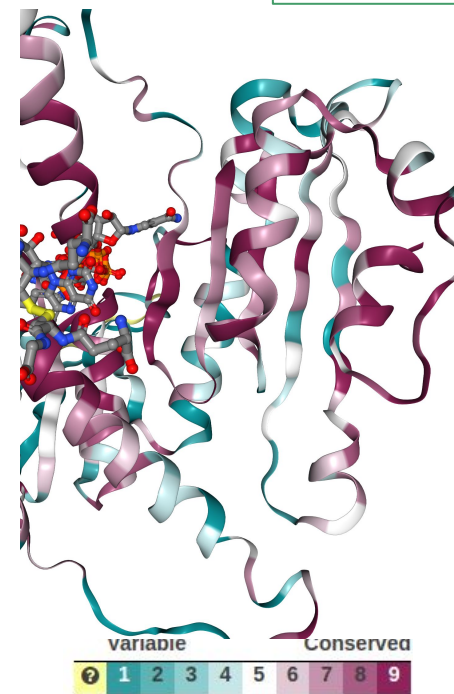
```

1onf  YKLIPTVIFSHPPIGTIGLSEEAIIQIYKENVKIIYESKFTNLFFSVYDIEPELKEKTYL
3grs  YNNIPTVWFSHPPIGTVGLTEDEAIHKYGIENVKTYSTSFTPMYHAVT-K---RKTCKVM
6du7  YSTIPTVWFSHPAIGTVGLTEEQAIIKEYGDDQIKVYKSSFASMYSACT-R---NRQESRF
6b4o  YNLVPTVWFTHPPVATIGLTEKAAL EYGEDQVKIYRSSFTPMYFALG-E---YRQKCDM
1ger  YSNIPTVWFSHPPIGTVGLTEPQAREQYGDDQVKVYKSSFAMYTAVT-T---HRQPCRM
5u1o  YDLVPTVWFSHPPIGTIGLTTQEAEKYGKDN IKVYTSGFTAMYTAVT-K---HRQPCKM
3o0h  YDLITTAVFSQPEIGTVGLSEEDALHRY--KRVEIYRTVFRPMRNVL-S---SPEKMFM
4dna  HDLIATAVFSQPEIGTVGIT EEEAARKF--QEIEVYRAEFRP-KATLS-G---RKEKTI-
  
```

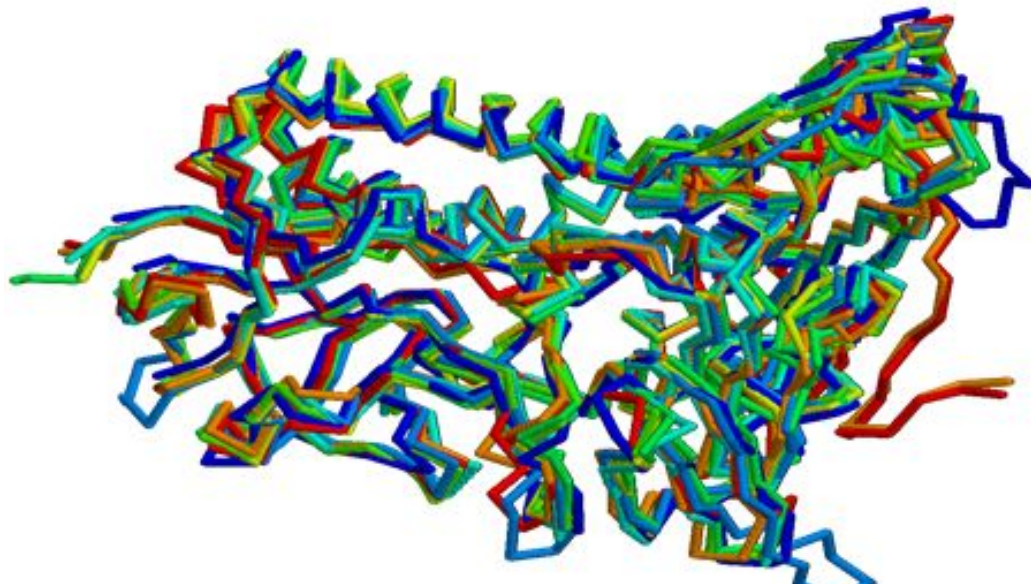
```

1onf  KLVCVGKDEL IKGLHII GLNADEIVQGF AVALKM NATKKDFDET IPIHPTAAEEFLTLQ-
3grs  K M V C A N K E E K V G I H M Q L G C D E M L Q G F A V A V K M G A T K A D F D N T V A I H P T S S E E L V T L R -
6du7  K L I T A G S E E K V V G L H G I G Y G V D E M I Q G F A V A I K M G A T K A D F D A T V A I H P T S S E E F V T M R -
6b4o  K L I C V G K E E K I V G L H G I G I G V D E M L Q G F A V A I K M G A T K A D F D N T V A I H P T G S E E F V T M R -
1ger  K L V C V G S E E K I V G I H G I G F G M D E M L Q G F A V A L K M G A T K K D F D N T V A I H P T A A E E F V T M R -
5u1o  K L V C A G E E E T V V G L H G I G F T V D E M I Q G F G V A M K M G A T K A D F D S V A I H P T G S E E F V T M R -
3o0h  K L V V D G E S R I V V G A H V L G E N A G E I A Q L I G I S L K G K L T K D I F D K T M A V H P T M S E E L V T M Y K
4dna  K L V V N A A D R K V V G A H I L G H D A G E - A Q L L G I S L R A G C T K D D F D R T - A V H P T A A E E L V T - Y Q
  
```

Interface domain

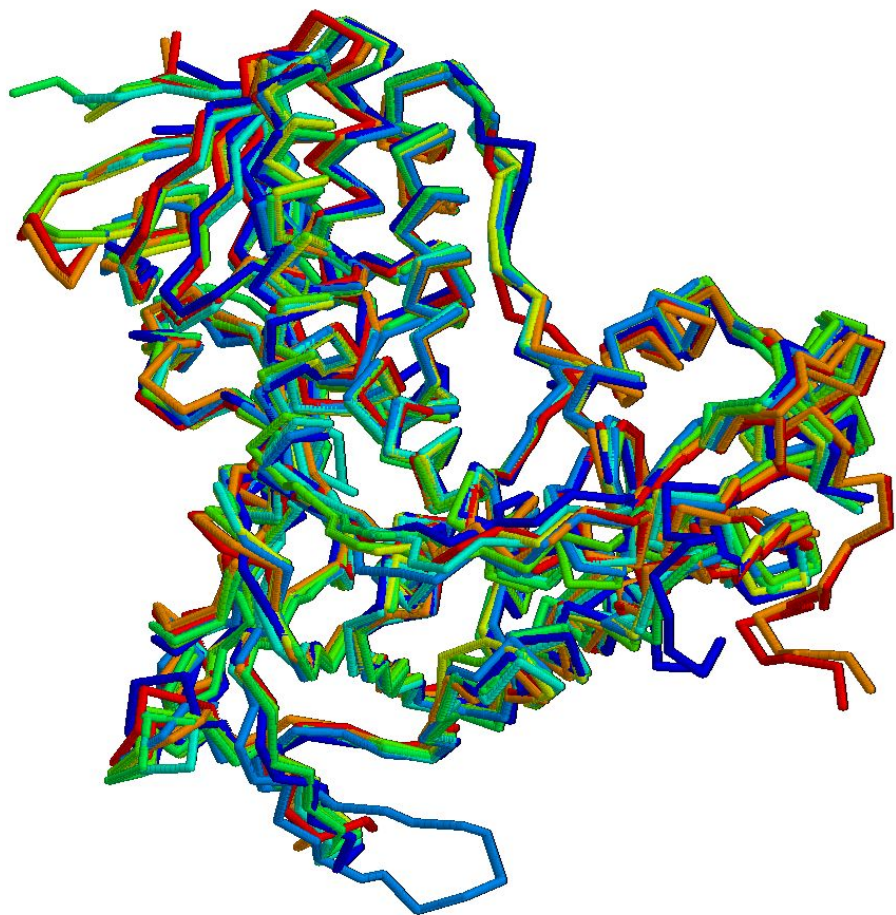


Structural superimposition between species



```
Doing cluster analysis...
Cluster: 1 ( 1ger & 5u1o ) Sc 9.45 RMS 0.75 Len 449 nfit 447
See file especes.1 for the alignment and transformations
Cluster: 2 ( 6b4o & 1ger 5u1o ) Sc 9.67 RMS 0.80 Len 450 nfit 447
See file especes.2 for the alignment and transformations
Cluster: 3 ( 6du7 & 6b4o 1ger 5u1o ) Sc 9.62 RMS 0.82 Len 450 nfit 443
See file especes.3 for the alignment and transformations
Cluster: 4 ( 3grs & 6du7 6b4o 1ger 5u1o ) Sc 9.37 RMS 0.90 Len 465 nfit 442
See file especes.4 for the alignment and transformations
Cluster: 5 ( 3o0h & 4dna ) Sc 8.80 RMS 0.81 Len 462 nfit 437
See file especes.5 for the alignment and transformations
Cluster: 6 ( 1onf & 3grs 6du7 6b4o 1ger 5u1o ) Sc 9.14 RMS 0.98 Len 471 nfit 420
See file especes.6 for the alignment and transformations
Cluster: 7 ( 1onf 3grs 6du7 6b4o 1ger 5u1o & 3o0h 4dna ) Sc 9.05 RMS 1.03 Len 477 nfit 405
See file especes.7 for the alignment and transformations
```

Chain	PDB code	Colour	Species
A	1onf	Dark blue	<i>Plasmodium falciparum</i>
B	3grs	Light blue	<i>Homo sapiens</i>
C	6du7	Turquoise	<i>Plasmodium falciparum</i>
D	6b4o	Light green	<i>Enterococcus faecalis</i>
E	1ger	Green	<i>Escherichia coli</i>
F	5u1o	Yellow	<i>Vibrio parahaemolyticus</i>
G	3o0h	Orange	<i>Bartonella henselae</i>
H	4dna	Red	<i>Rhizobium meliloti</i>

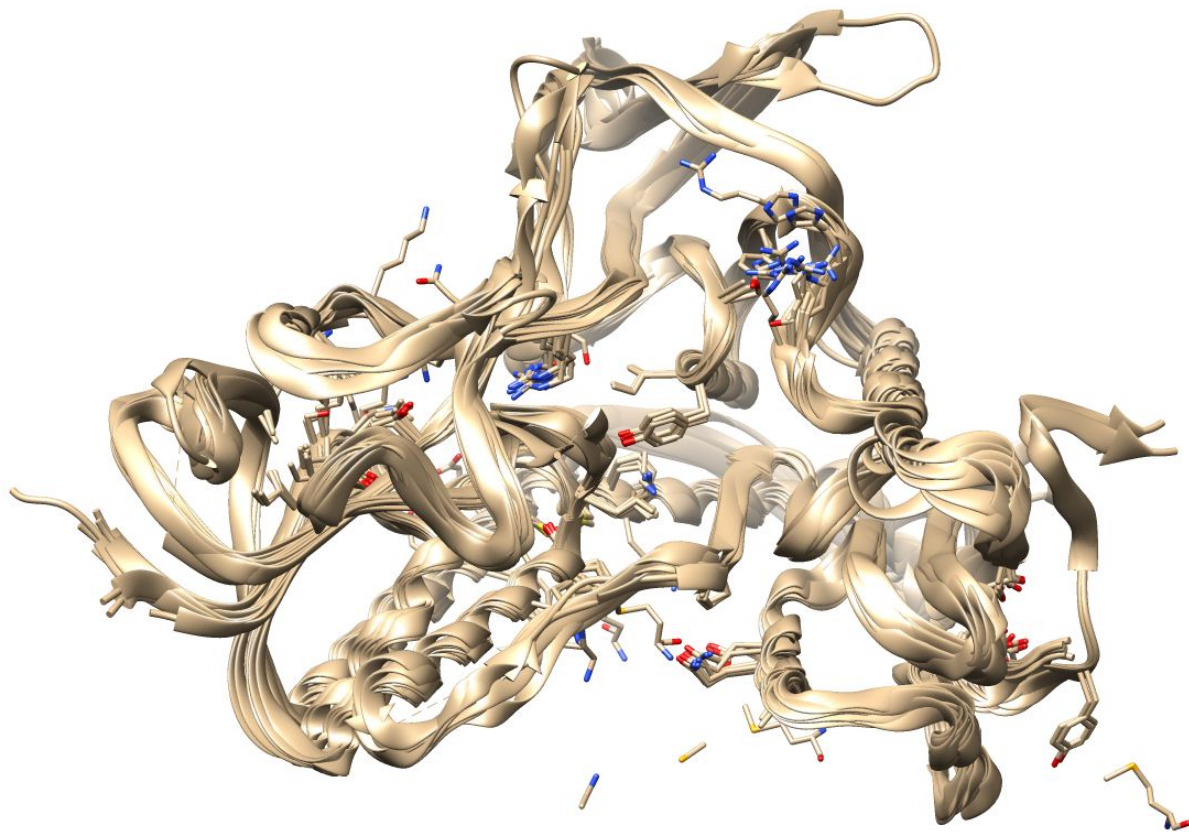


Overall similar
structure

More
changes in
the loops

Human structure
has bigger loops

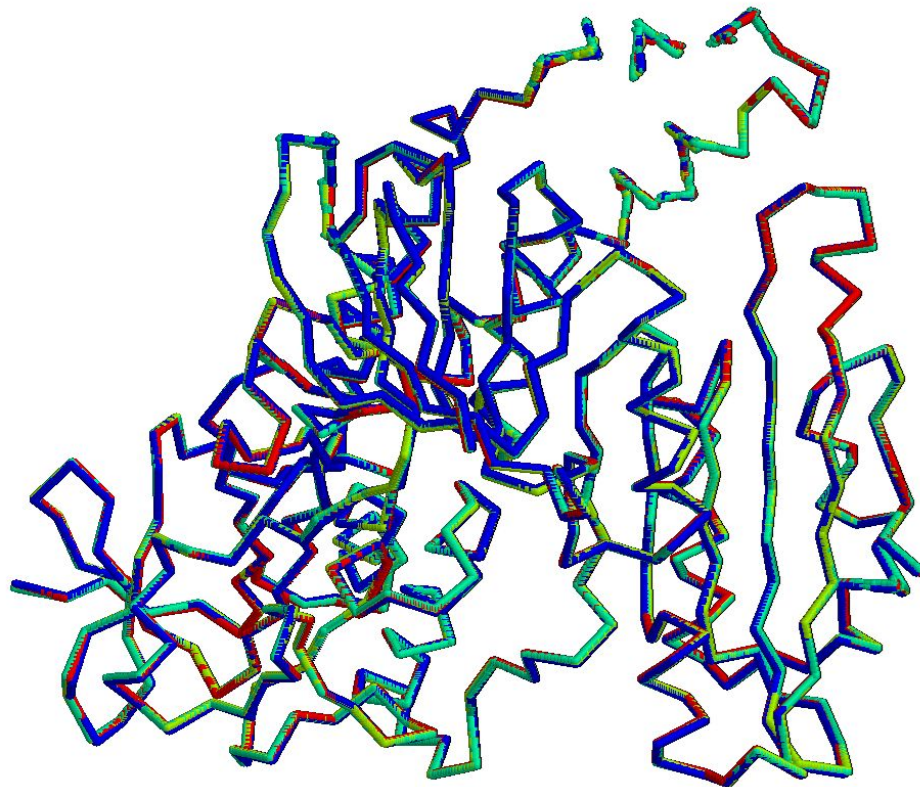
Changes in
the
Terminal
sites



Catalytic cycle structural superimposition on human GR

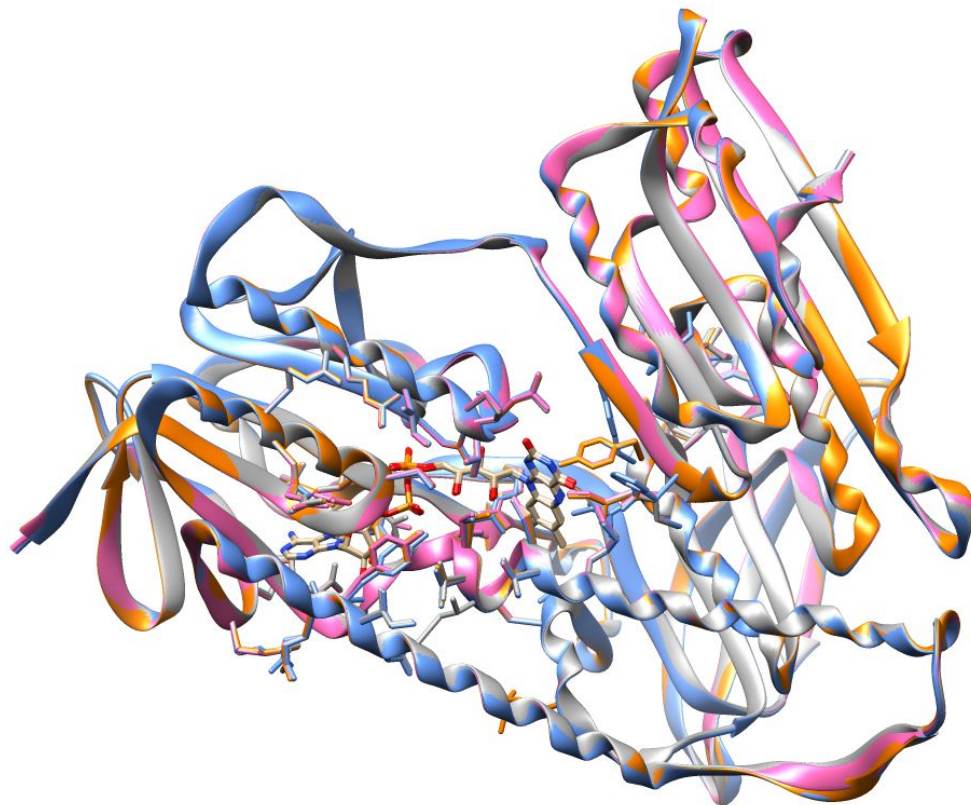
Homo sapiens

PDB code	Ligands/ cofactors
3DK9	FAD
3DK8	FAD GSH
3DK4	FAD NDP GSH
3DJJ	FAD NDP



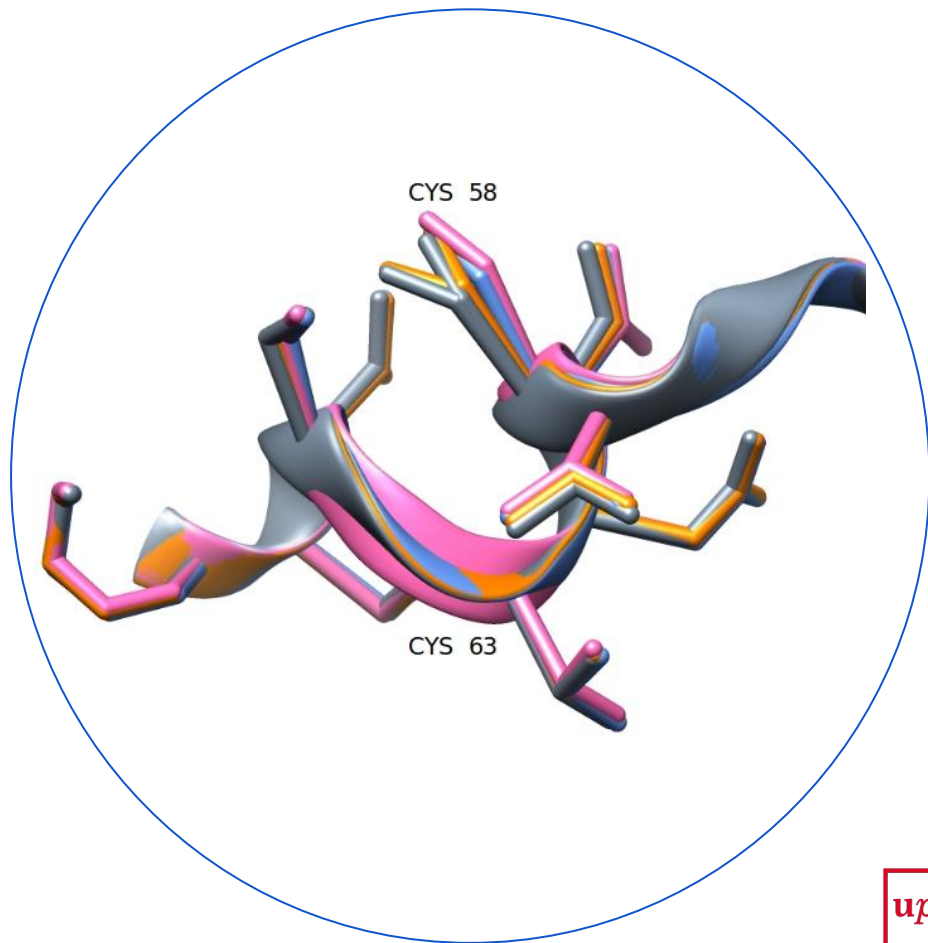
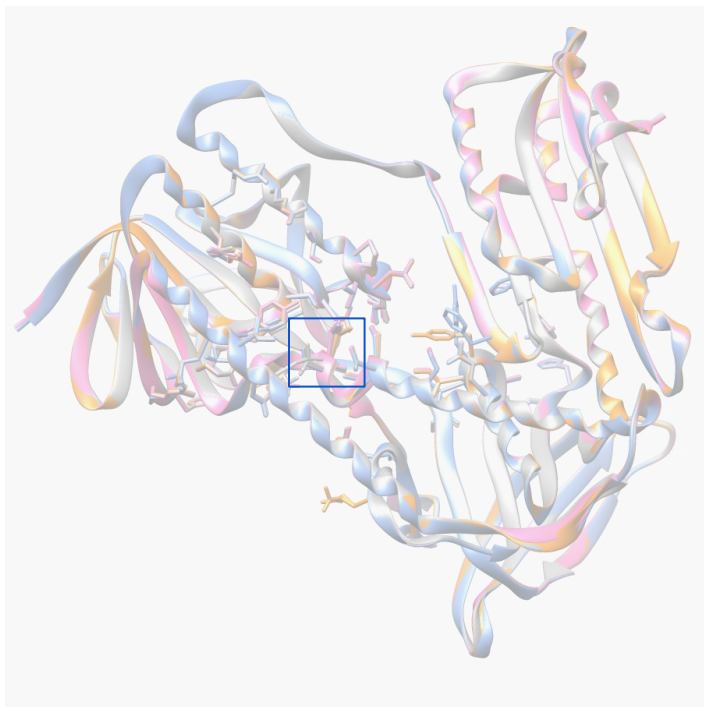
Changes in the
sidechain, not
on the
backbone

Catalytic cycle structural superimposition on human GR

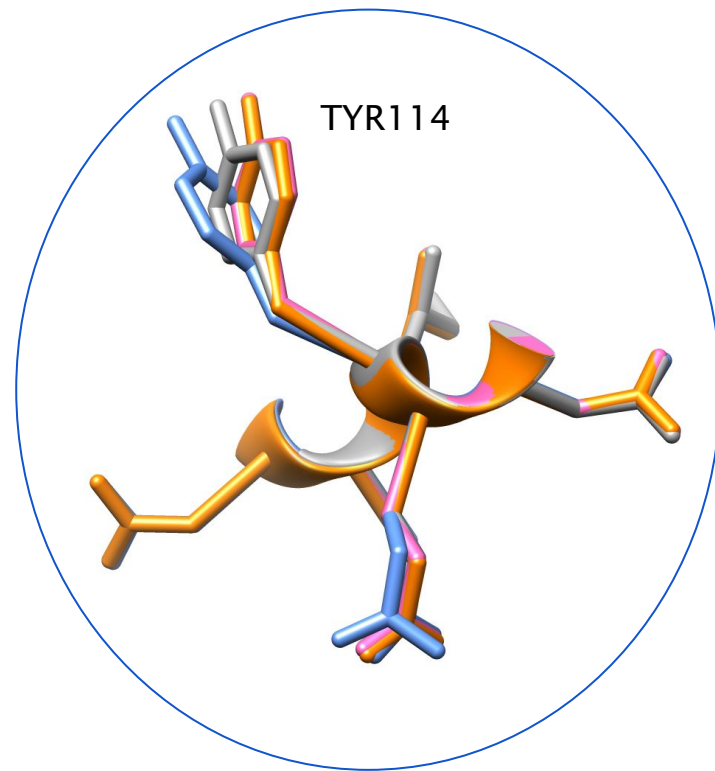
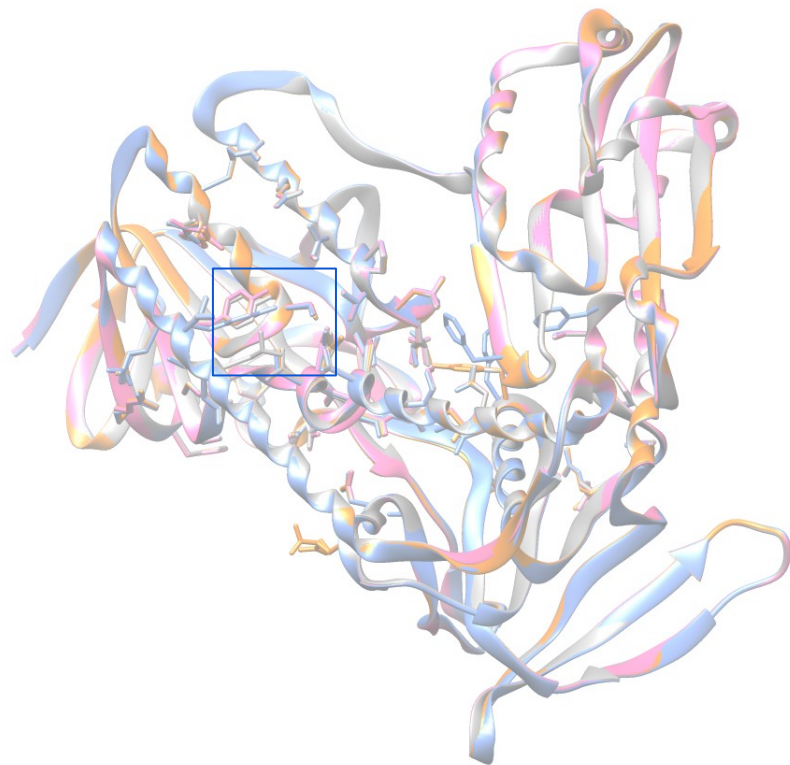


PDB code	Ligands/ cofactors	Color chain
3DK9	FAD	Grey
3DK8	FAD GSH	Pink
3DK4	FAD NDP GSH	Orange
3DJJ	FAD NDP	Blue

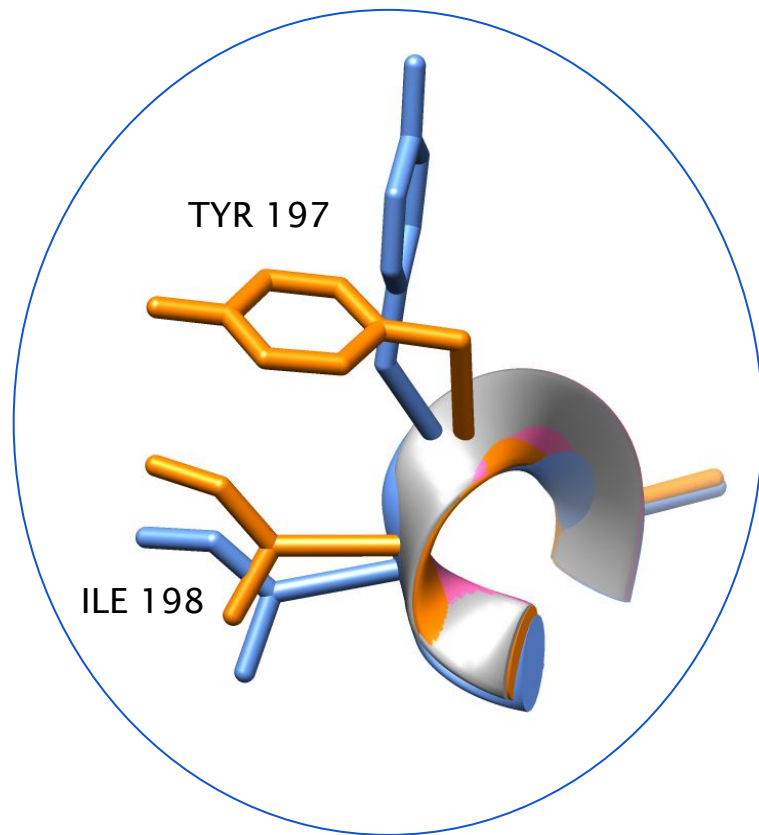
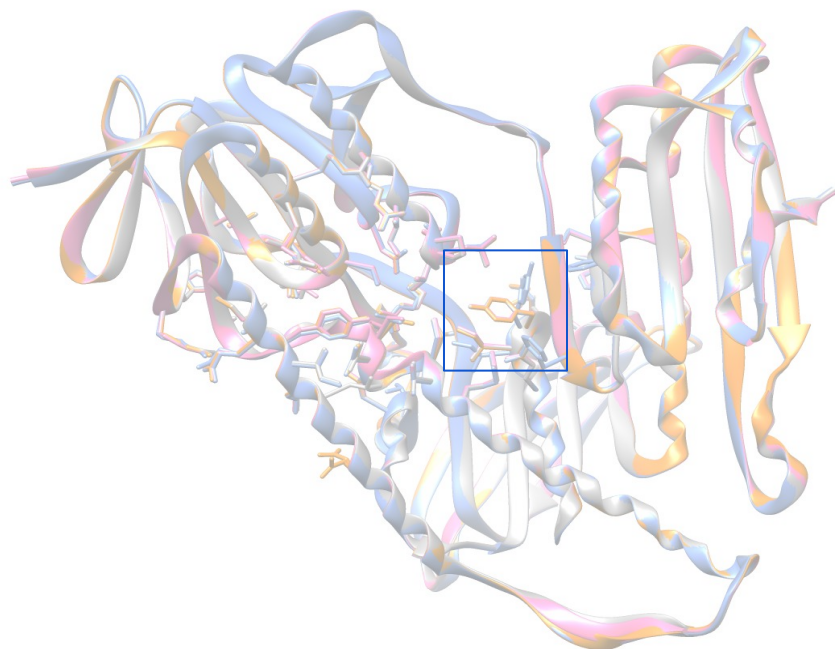
CYS 58 - CYS 63



TYR 114



TYR 197 - ILE 198



PEM questions

1. Which molecule binds to glutathione reductase?
 - a. NADPH
 - b. Glutathione
 - c. The two previous
 - d. FAD
 - e. All of the above**
2. The Rossmann fold:
 - a. Binds nucleotides**
 - b. Binds glutathione
 - c. Binds peptides
 - d. Binds steroids
 - e. All of the above
3. Which amino acid interacts with the NADPH, pushing it against the FAD?
 - a. Cys 63
 - b. Cys 58
 - c. Tyr 197**
 - d. Gly 31
 - e. Glu 50
4. The conserved motifs on the Rossmann fold are:
 1. AXXhXR
 2. HXXPXXC
 3. CVXTA
 4. GXGXXG
 - a. 1, 2, 3
 - b. 1,3
 - c. 2, 4
 - d. 1, 2, 3, 4
 - e. 4**
5. Which of these are FAD binding proteins?
 - a. glutathione reductase
 - b. Ferredoxin reductase
 - c. p-cresol methylhydroxylase
 - d. Pyruvate oxidase
 - e. all of the above**

PEM questions

6. Conserved characteristics of the Rossmann fold core are
1. Phosphate binding region GXGXXG or GXXXG/A
 2. Core with small hydrophobic amino acids
 3. Negatively charged residue (Glu or Asp) at the end of the second beta strand
 4. Positively charged residue (Arg or Lys) at the beginning of the first beta strand
- b. 1, 2, 3
c. 1,3
d. 2, 4
e. 1, 2, 3, 4
f. 4
7. Which of the following residues are conserved in the catalytic disulphide site of the Glutathione reductase?
- a. Cys 63
 - b. Cys 53
 - c. a and b are correct**
 - d. Arg 218
 - e. all of the above
8. Which of the following domains are present in the glutathione reductase:
- a. FAD domain
 - b. NAD domain
 - c. a and b are correct
 - d. Interface domain
 - e. All of the above**
9. Which aminoacid makes a Van der Waals contact with the glutathione?
- a. Cystein 63
 - b. Glycine
 - c. Threonine
 - d. Cystein 58**
 - e. None of the above
10. The Rossmann fold core is formed by:
- a. $\beta\alpha\beta$**
 - b. Two consecutive α helices
 - c. a and b are correct
 - d. β -meander
 - e. None of the above

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Thank you for your attention