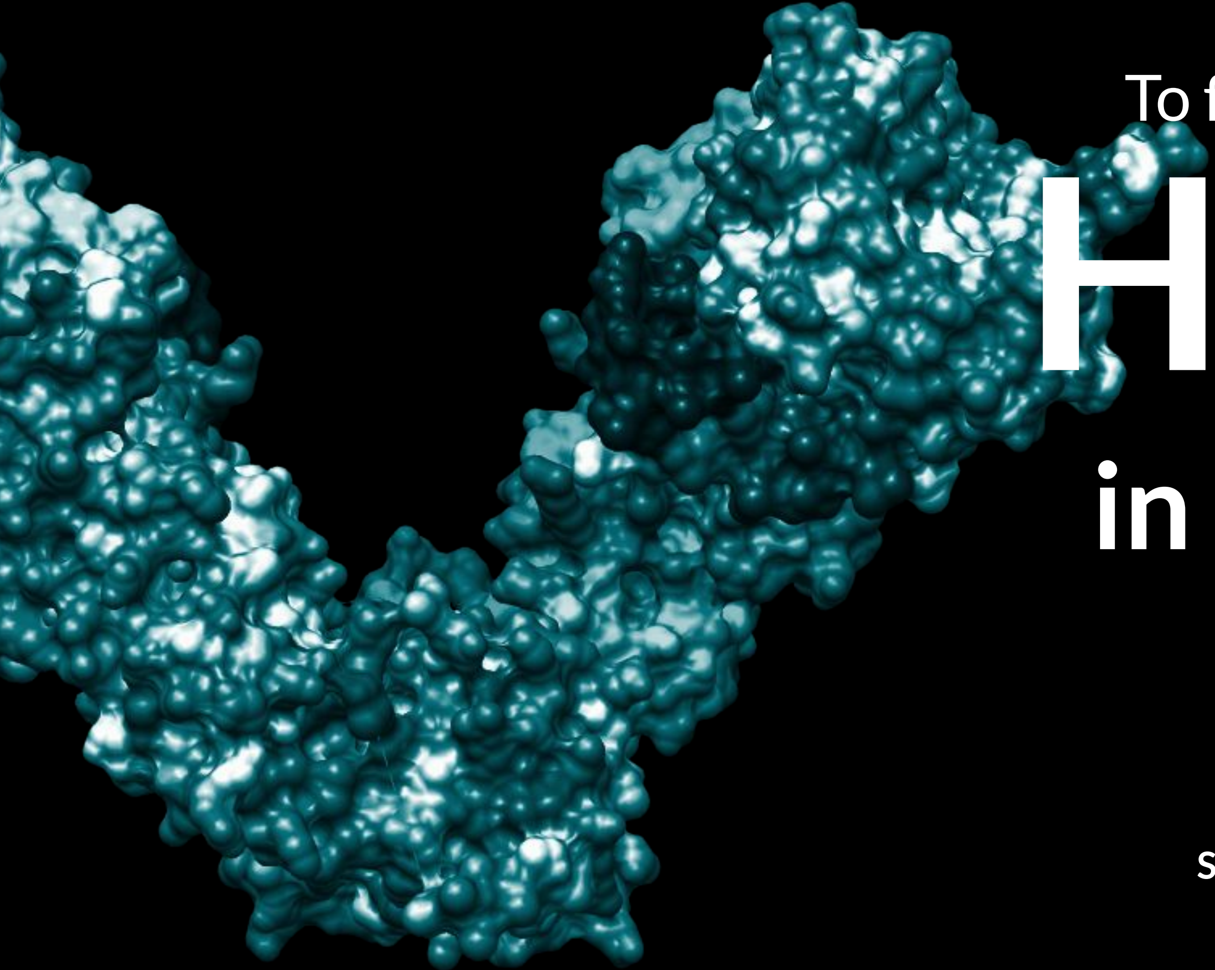


To fold or not to fold?

Hsp90
in a nutshell

Carla García
Pedro Morata
Ainoa Tejedera
Mariona Vidal

Structural Biology Project
Human Biology
UPF 19/20

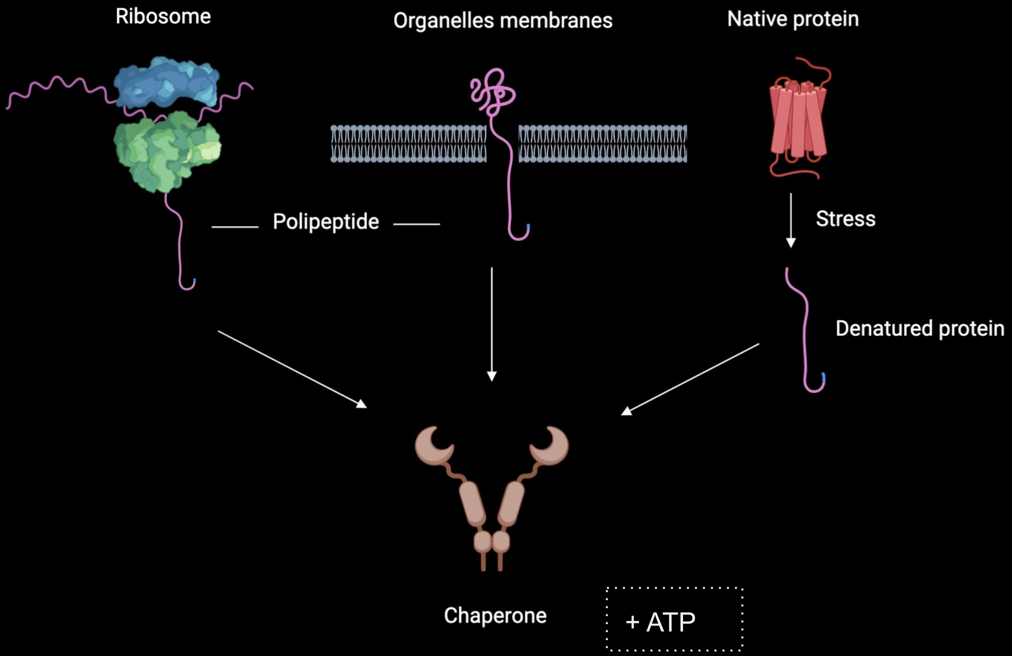
Index

INTRODUCTION

Molecular chaperons

Proteins that assist the conformational **folding** and the assembly of **other** macromolecular structures. **Functions**:

- Folding of *de novo* synthesized proteins.
- Folding of proteins after denaturation.
- Cell transport.

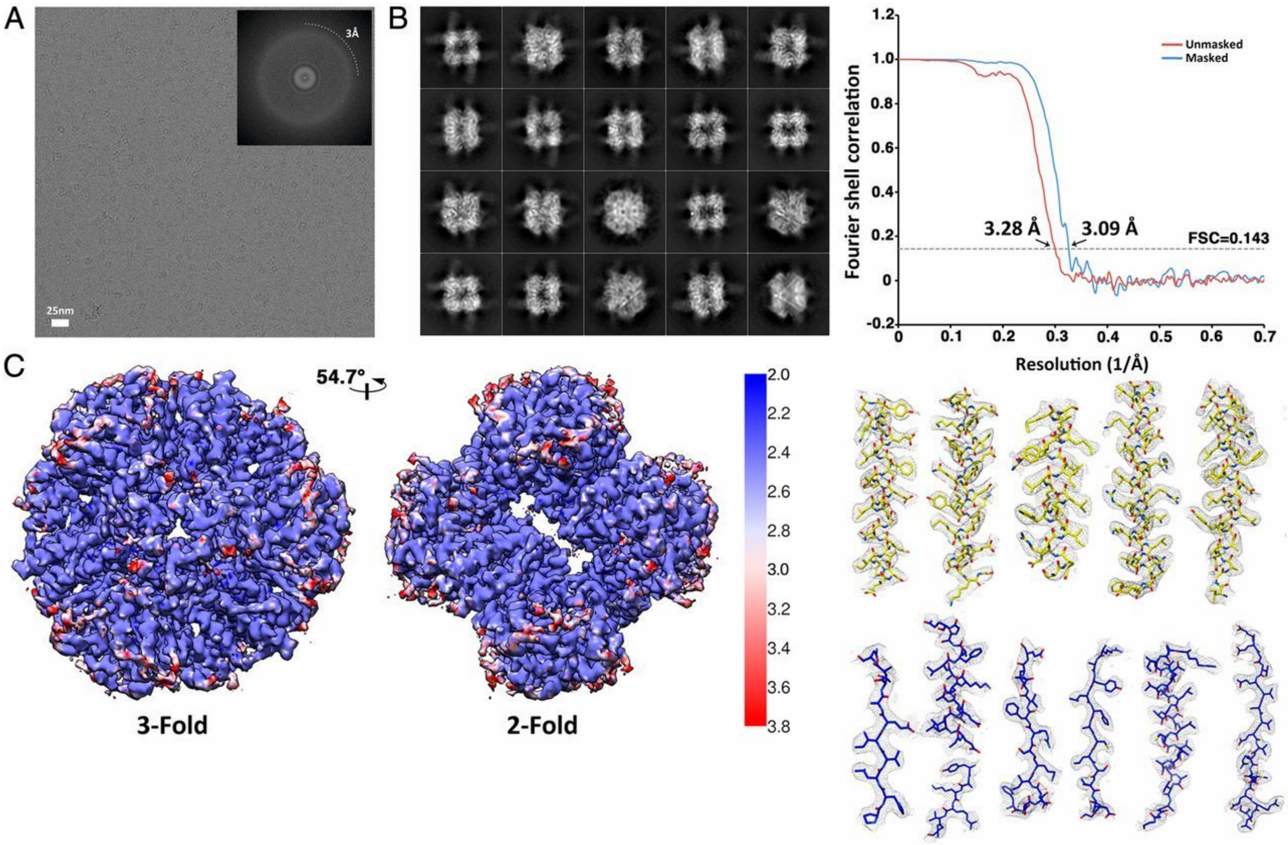


Molecular chaperons

Main types of chaperons
Nucleoplasmins
Hsp60 or chaperonins
Hsp70 chaperons
Hsp90 chaperons

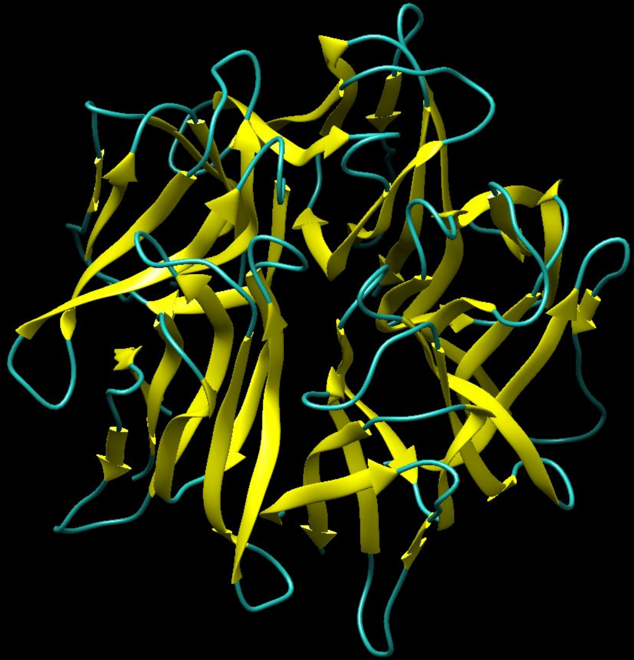
Cryo-EM

Additional information

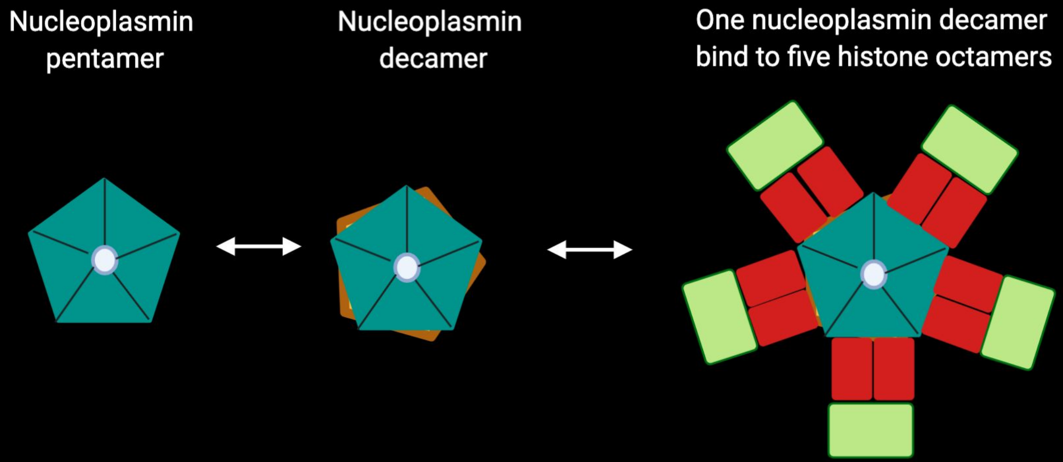


Near-atomic cryo-EM imaging of a small protein displayed on a designed scaffolding system. Liu Y, Gonen S, Gonen T, Yeates TO.

Classification: Nucleoplasmin

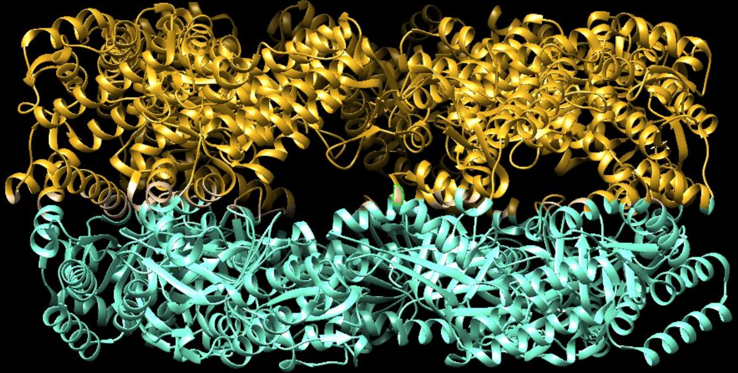


Human nucleophosmin-core in complex with cytochrome c

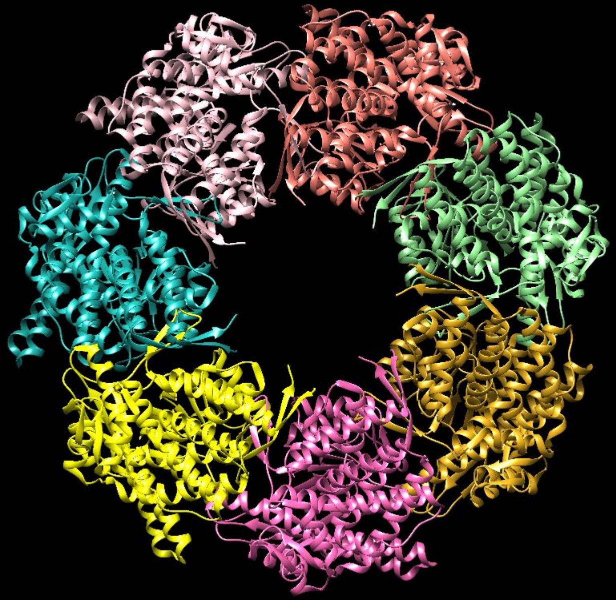


Adapted from: Molecular chaperones: assisting assembly in addition to folding. Ellis RJ. Trends Biochem Sci.

Classification: Hsp60 (chaperonins)



GroEL side view



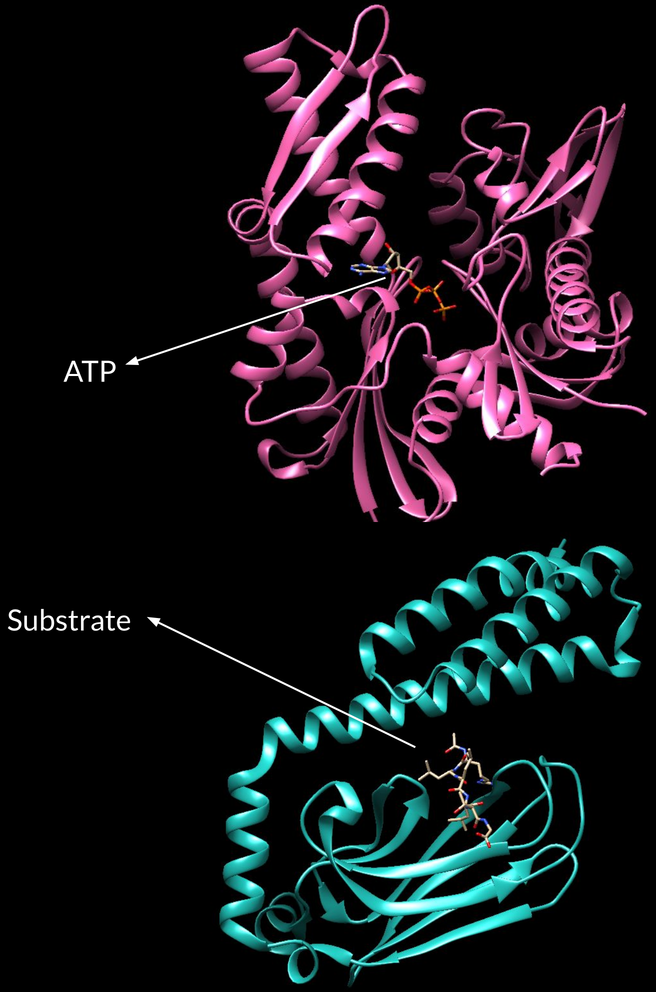
GroEL top view

Classification: Hsp70

Two domains:

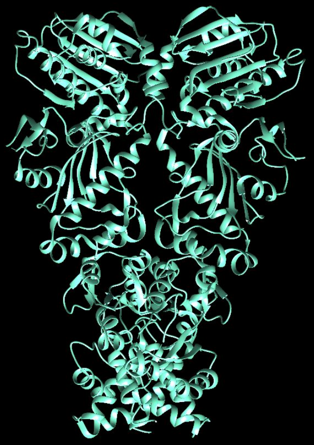
N-terminal domain or **nucleotide binding domain**.

C-terminal domain or **substrate binding domain** .



INTRODUCTION
to HSP90

HSP90: ISOFORMS



GRP94

TRAP1

HtpG

HSP90: ISOFORMS

RMSD: 0.31

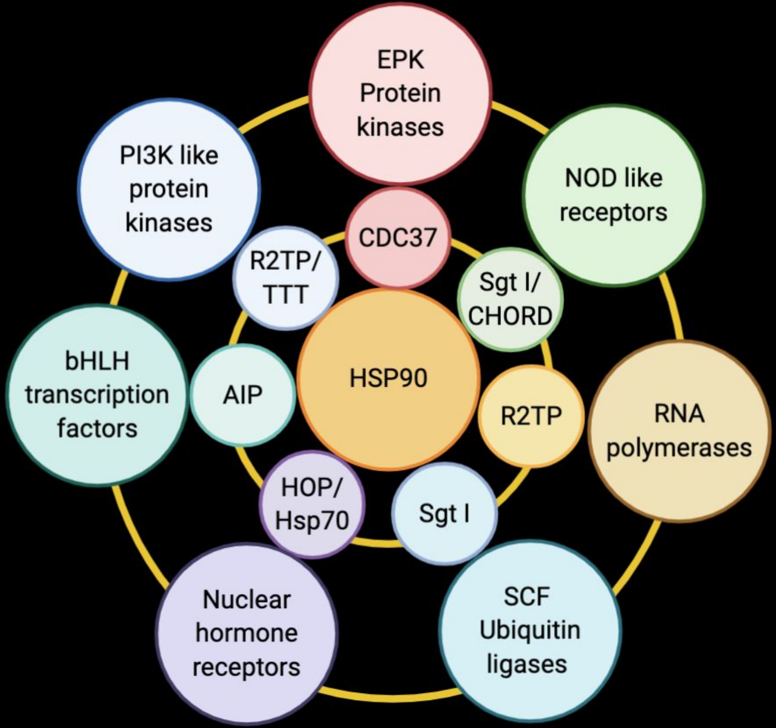
N-terminal Hsp90α
and Hsp90β

HtpG

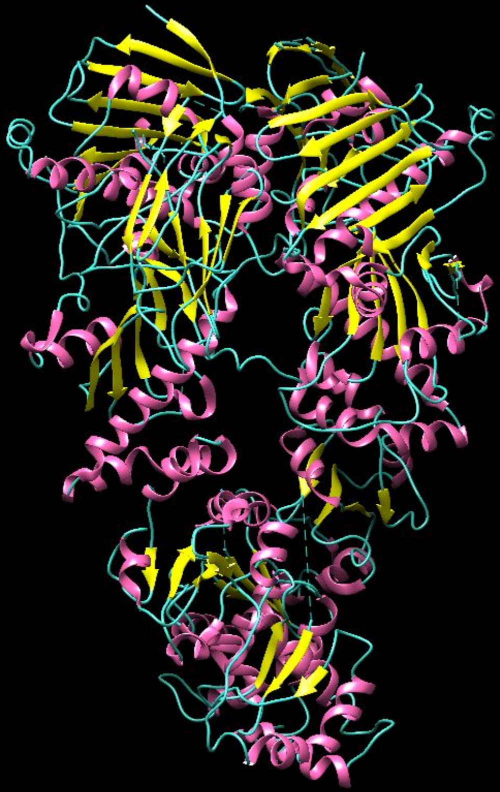
HSP90: CO-CHAPERONS AND CLIENTS

Clients:

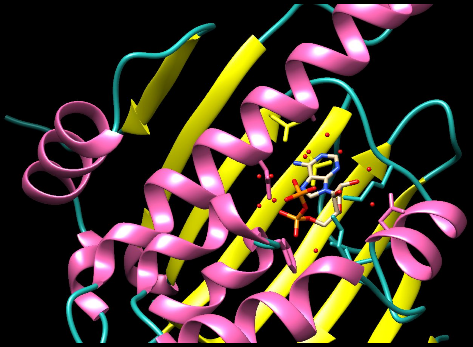
- Receptors.
- Transcription factors.
- Kinases.
- Other unrelated proteins



Hsp90: Cancer



Geldanamycin



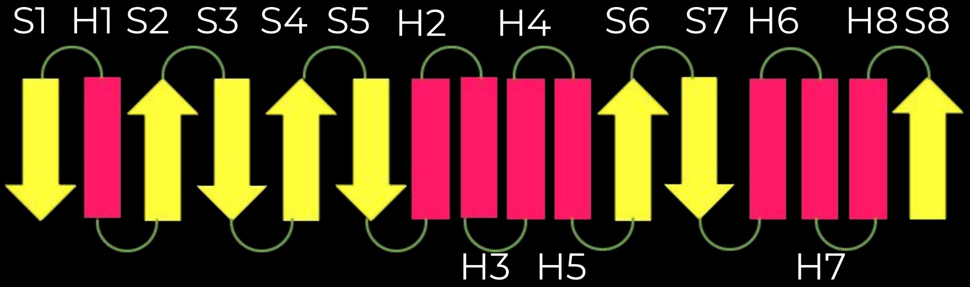
ATP

STRUCTURAL ANALYSIS

Class	Alpha and Beta proteins (α+β)		
Fold	ATPase domain of Hsp90 chaperone / DNA topoisomerase II / histidine kinase	Ribosomal S5 domain 2-like	Hsp90 C-terminal domain
Superfamily	ATPase domain of Hsp90 chaperone / DNA topoisomerase II / histidine kinase	Ribosomal S5 domain 2-like	Hsp90 C-terminal domain
Family	Heat shock protein 90, Hsp90, N-terminal domain	HSp90 middle domain	Hsp90 C-terminal domain

N-TERMINAL DOMAIN

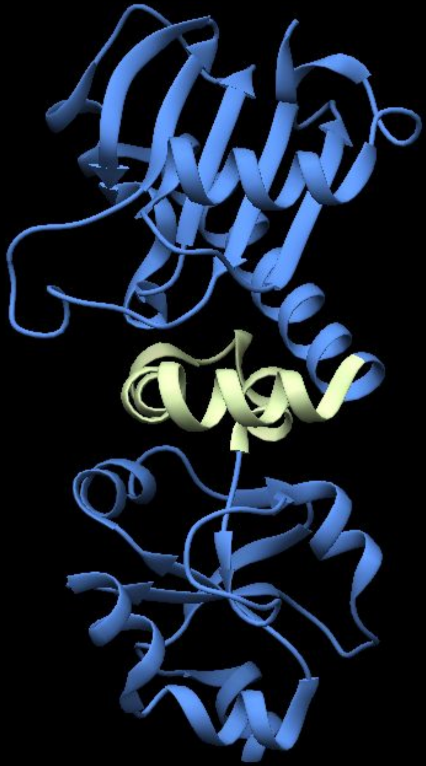
α-ß sandwich: Eight stranded ß sheet (strands S2–S9) flanked by eight **α** helices (H1–H8)



MIDDLE DOMAIN

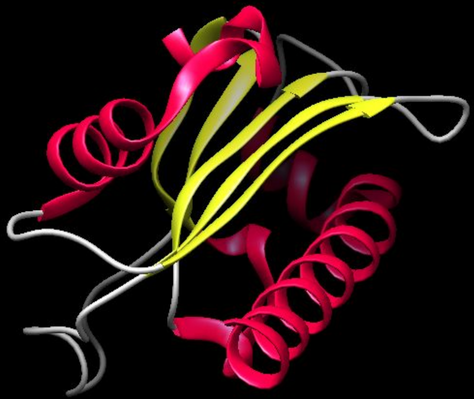
Three subdomains:

- **α**-ß-**α** sandwich
- Spiral of three helices
- **α**-ß-**α** sandwich

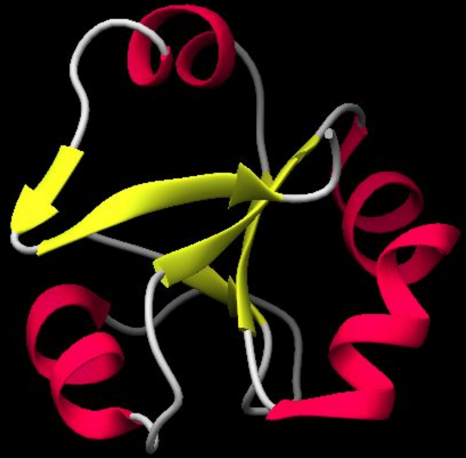


MIDDLE DOMAIN

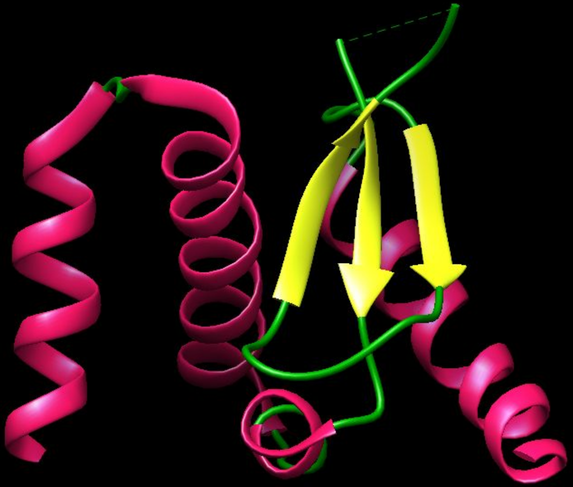
TOP **α**-ß-**α** sandwich



BOTTOM **α**-ß-**α** sandwich



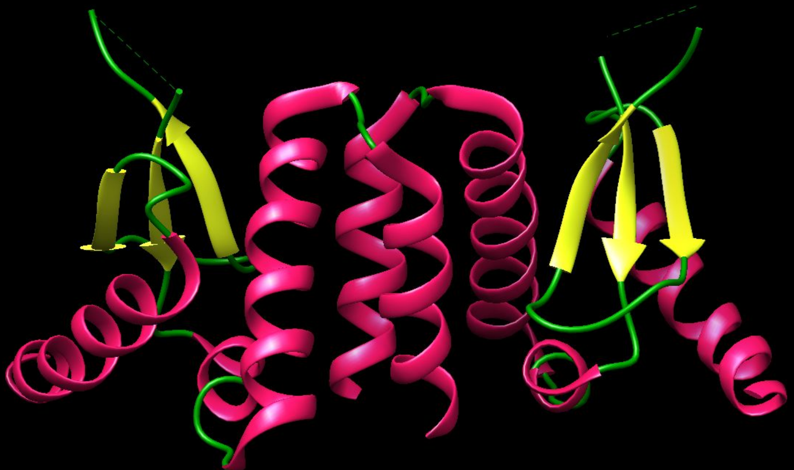
C-TERMINAL DOMAIN



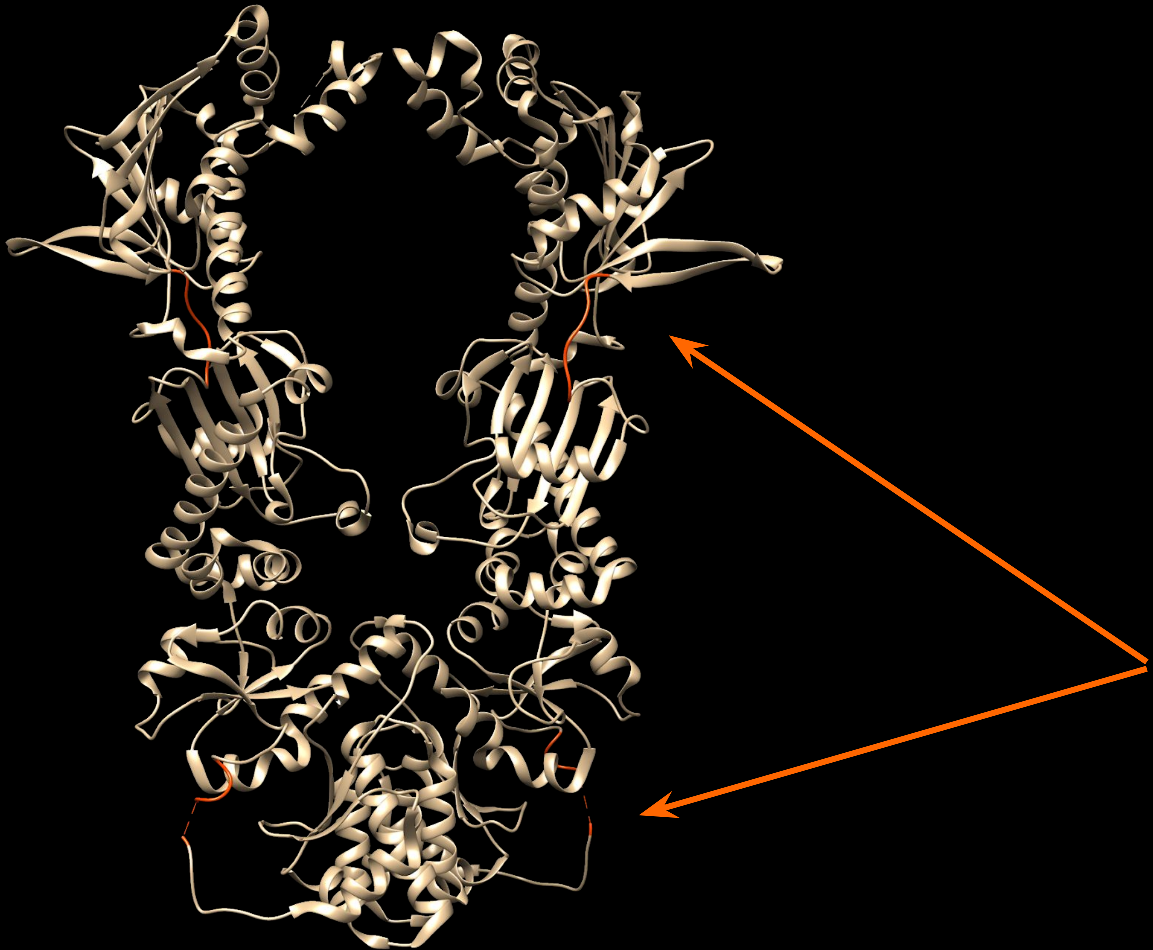
Unique mixed **α**-ß fold

Responsible of dimerization

Homodimer: Four helix bundle



DOMAIN LINKERS



NTD-MD: 7 residues

MD-CTD: Highly mobile tether

Accomodation for client proteins

RELEVANT REGIONS

N-TERMINAL DOMAIN

ACTIVE SITE LID

Two **helices** and an intervening **loop**

Adjacent to **ATP binding site**

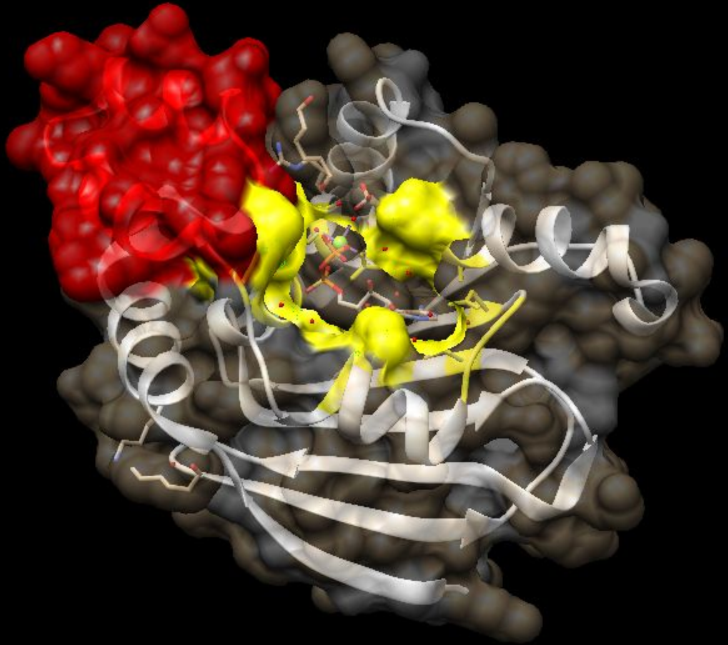
Helices are parallel to NTD **ß sheet**

Conserved motif: IGQFGVG

N-TERMINAL DOMAIN

ACTIVE SITE LID AND ATP BINDING

Function: link between ATP binding / hydrolysis and client-protein binding / release

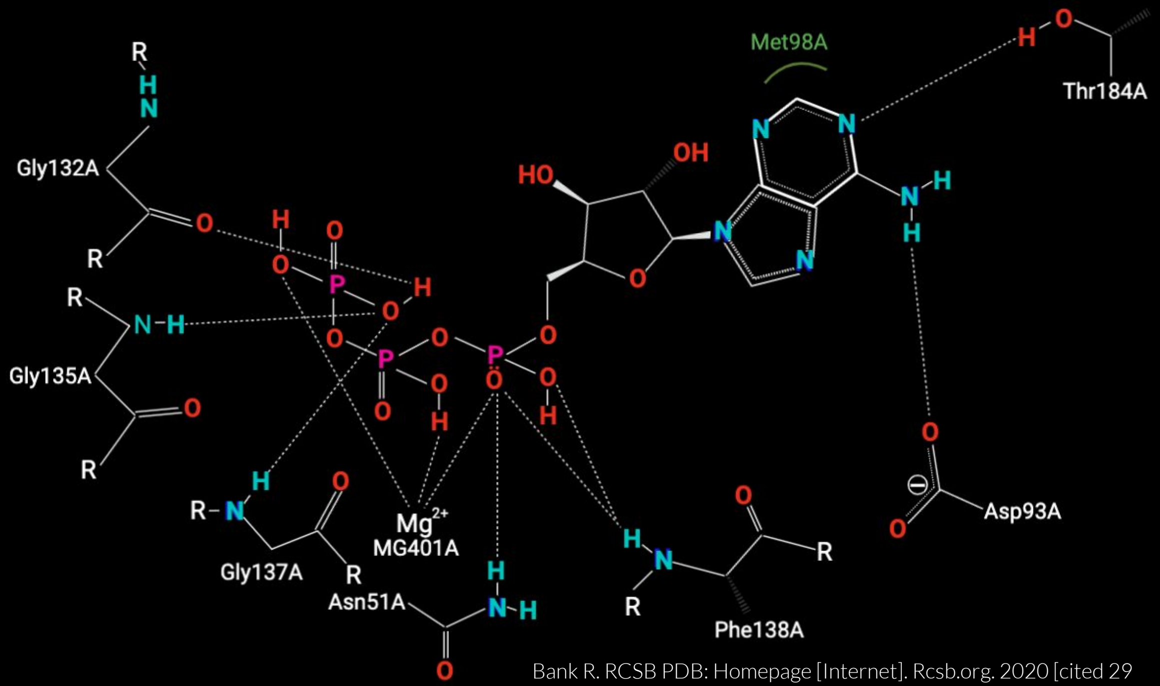


N-TERMINAL DOMAIN
ATP BINDING AND HYDROLYSIS

Main elements: GLU, ARG, GLN, PHE

MD (ARG) + dimerization are required for full activity

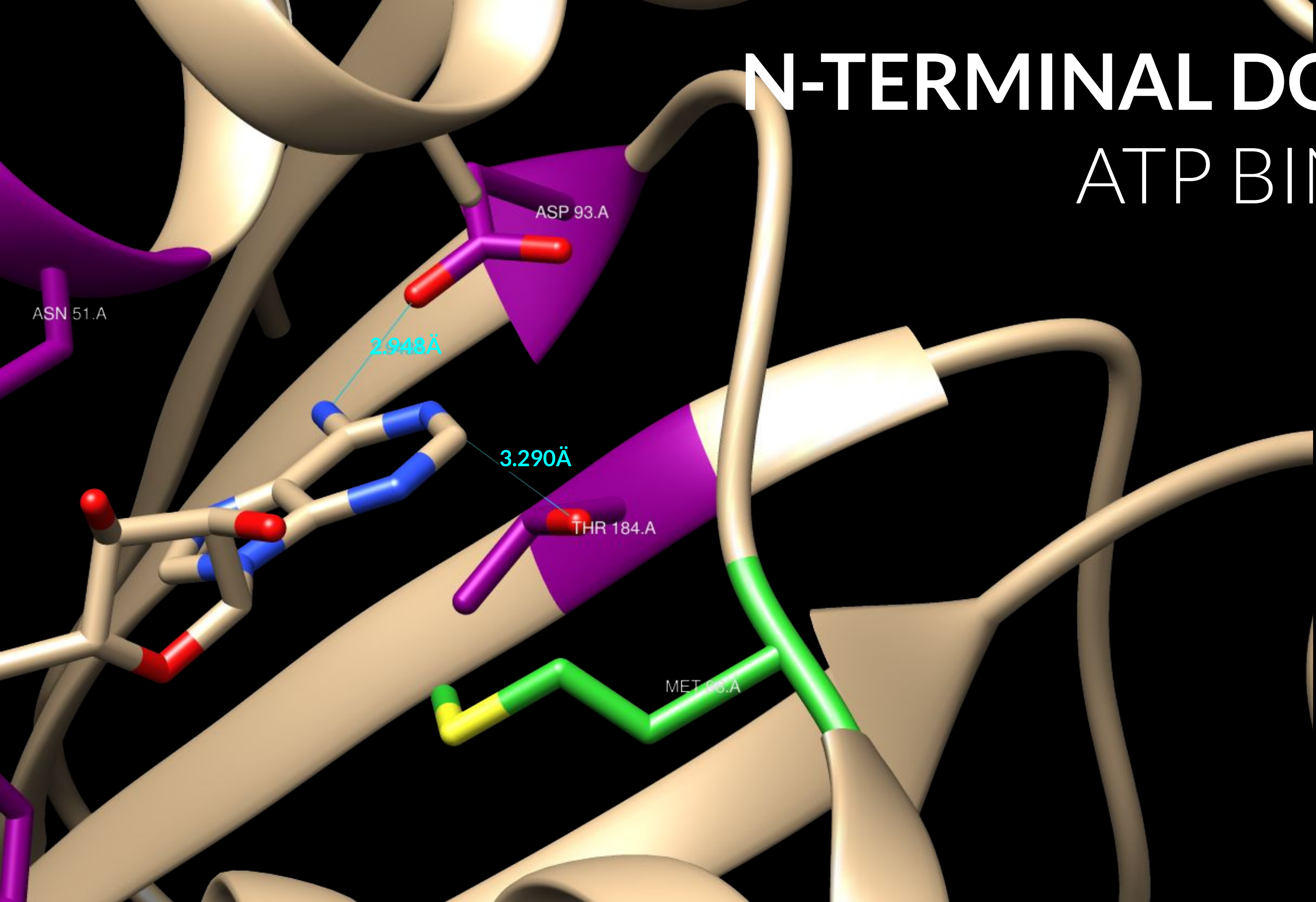
Pre-existing equilibrium of states



Bank R. RCSB PDB: Homepage [Internet]. Rcsb.org. 2020 [cited 29 February 2020]. Available from: https://www.rcsb.org/

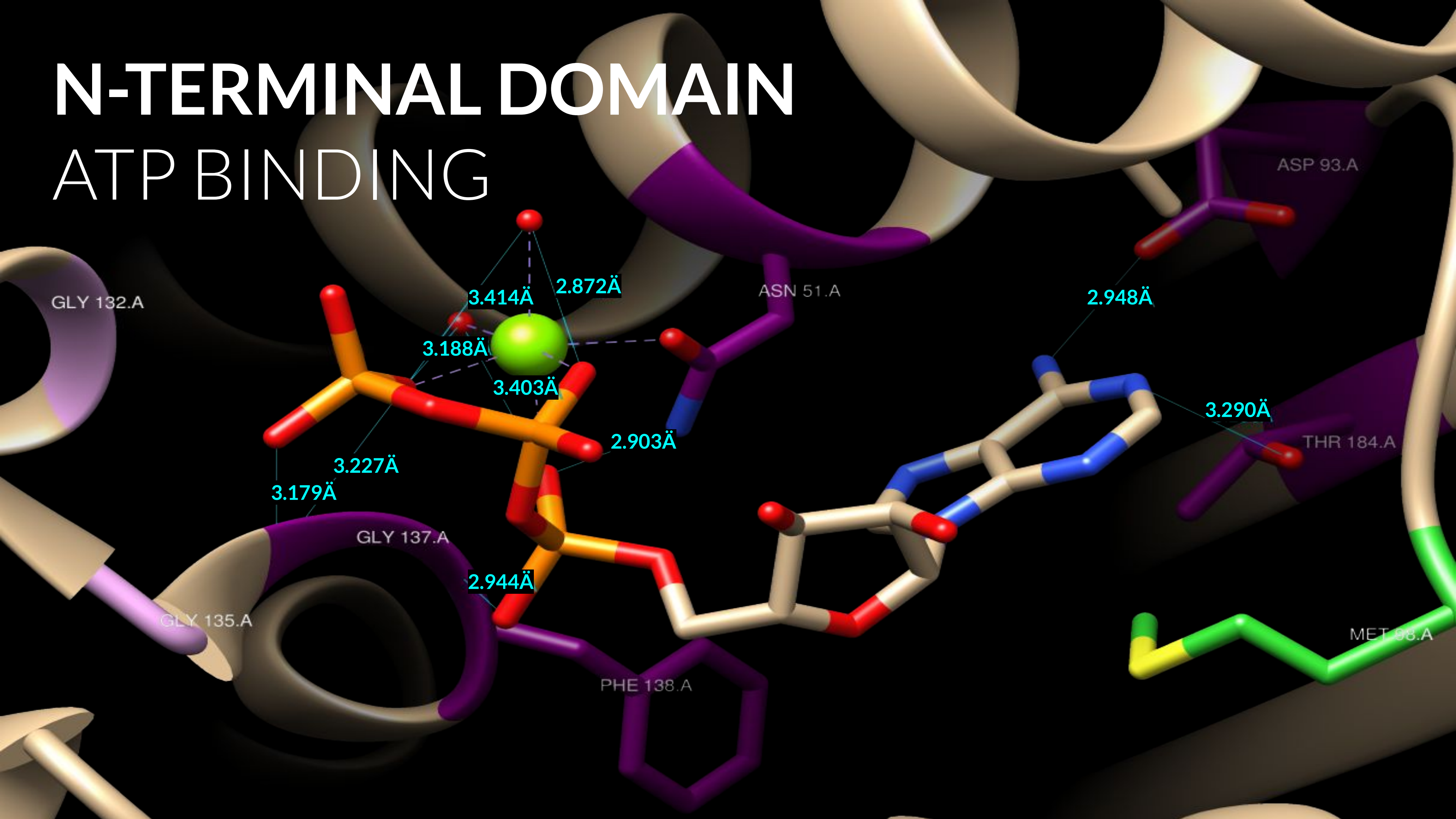
N-TERMINAL DOMAIN

ATP BINDING



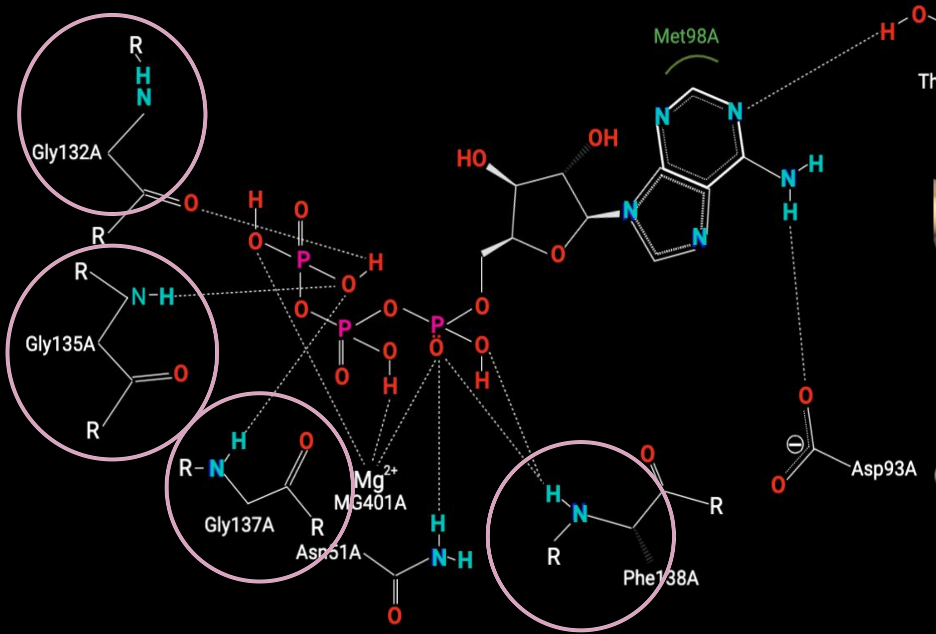
N-TERMINAL DOMAIN

ATP BINDING



N-TERMINAL DOMAIN

ATP BINDING



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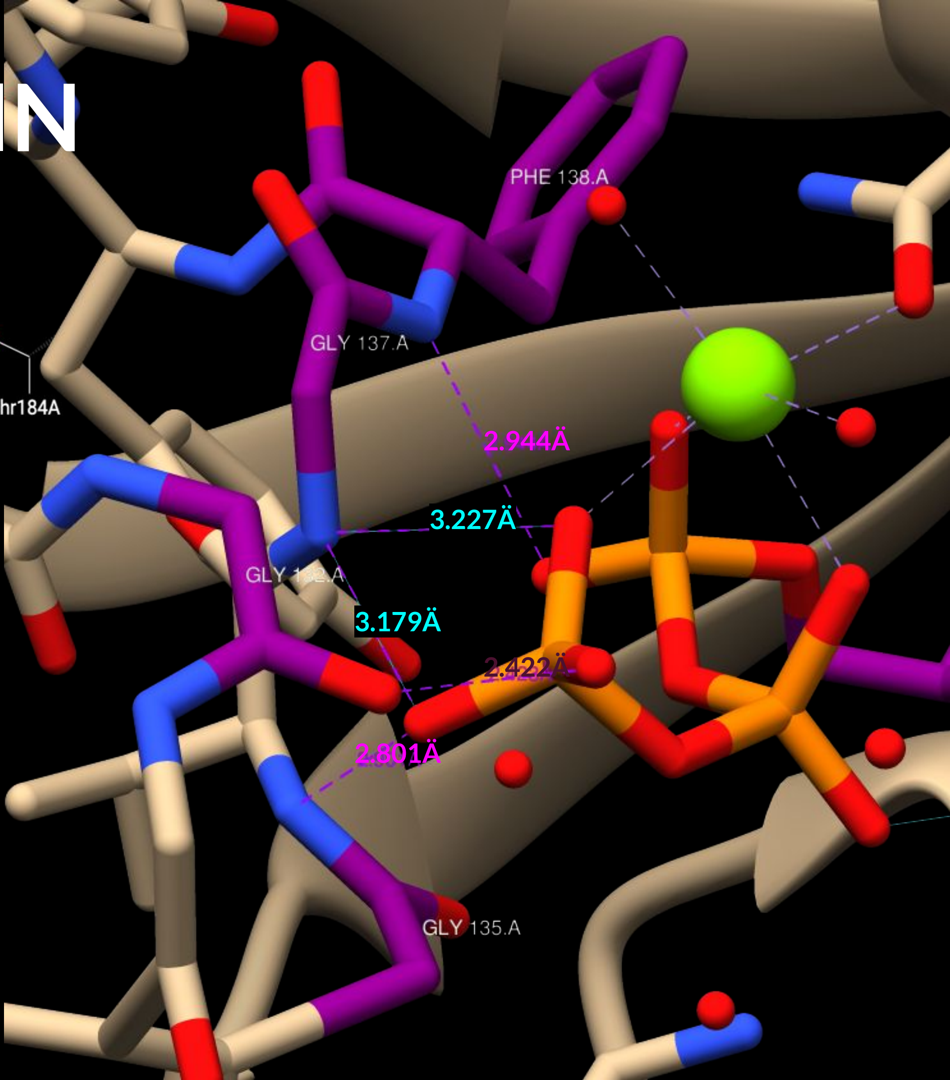
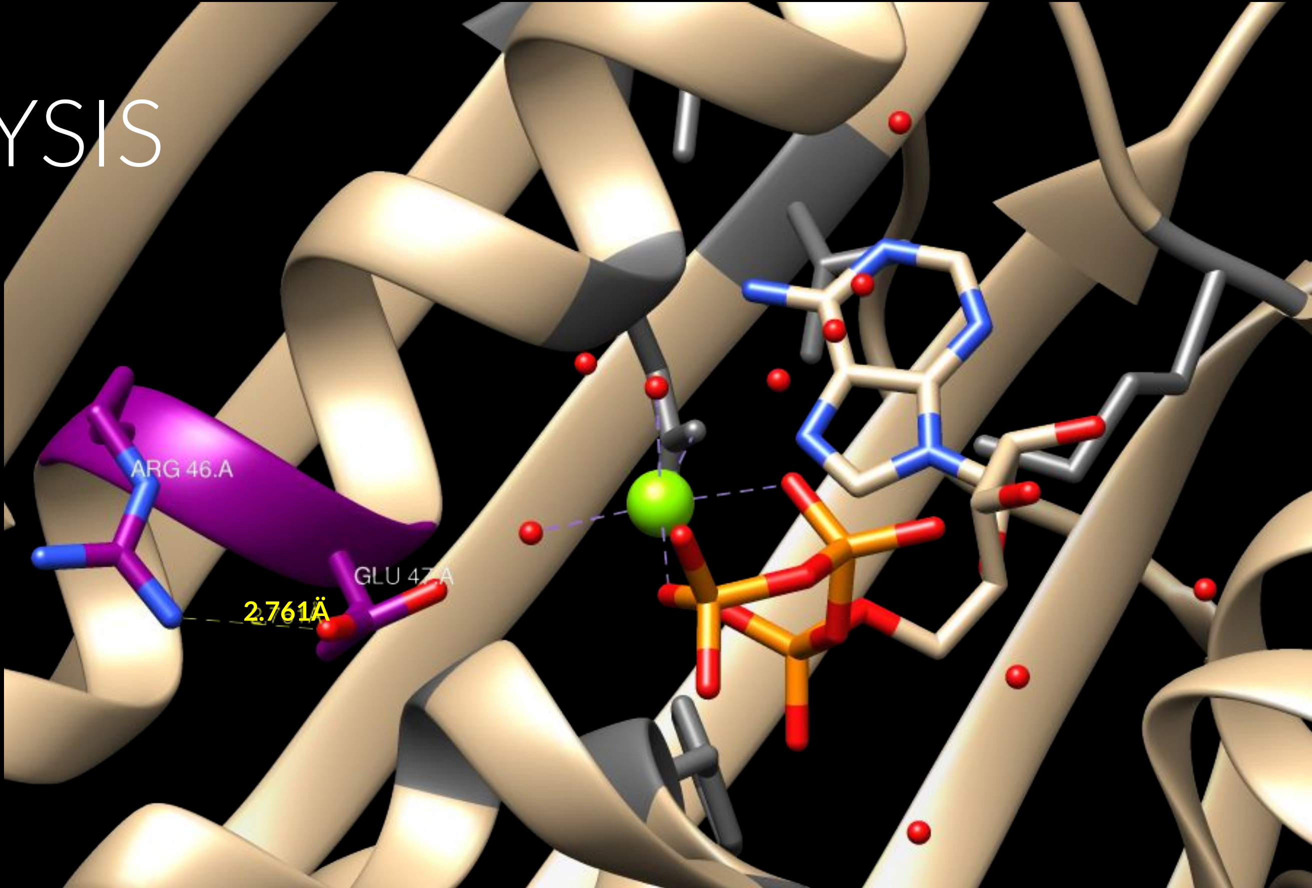


N-TERMINAL DOMAIN

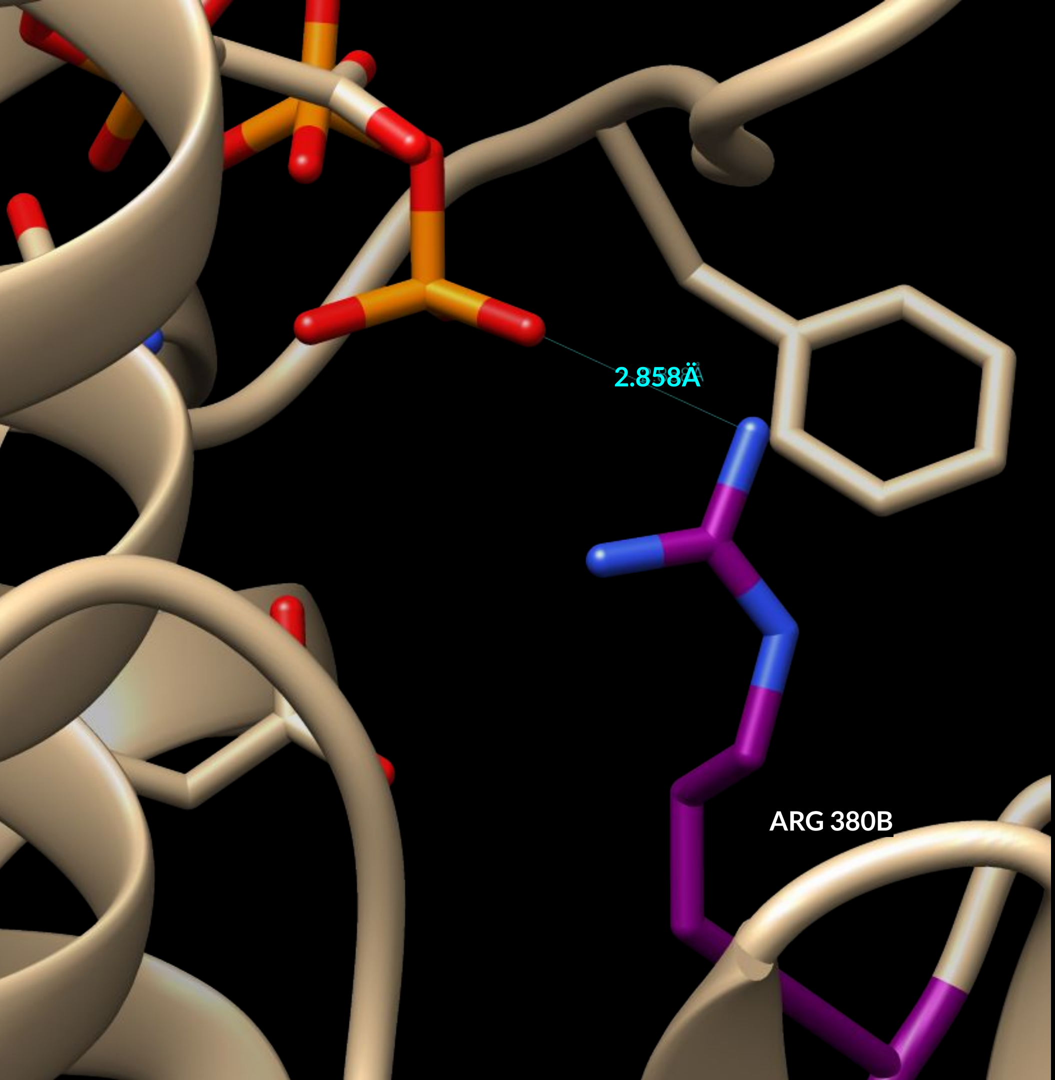
ATP HYDROLYSIS

1. ARG forms salt bridge with carboxyl group of GLU
2. GLU gets polarized
3. Nucleophilic attack between GLU and H_2O
4. H_2O becomes negatively charged
5. H_2O attacks P atom of γ-phosphate
6. Covalent bond between γ and β phosphate is broken
7. Products: hydrogen phosphate and ADP



N-TERMINAL DOMAIN

ATP HYDROLYSIS



Salt bridge between ARG-MD and nucleotide gets broken

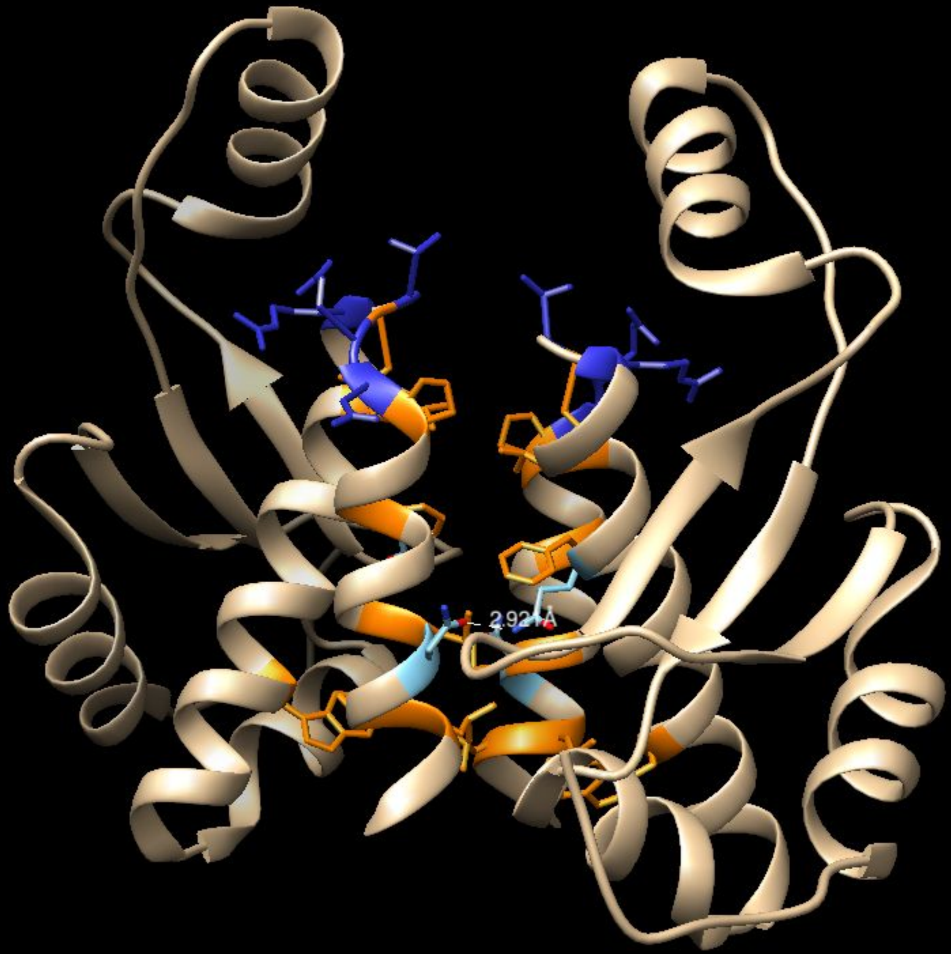
Breaking of this interaction + Release of **γ**-phosphate after hydrolysis ⟶ Release of the client protein

C-TERMINAL DOMAIN DIMERIZATION

Four helix bundle (not purely)

Surface: Predominantly **hydrophobic** with few charged residues

Intermonomeric hydrogen bonds:
ASN-GLN

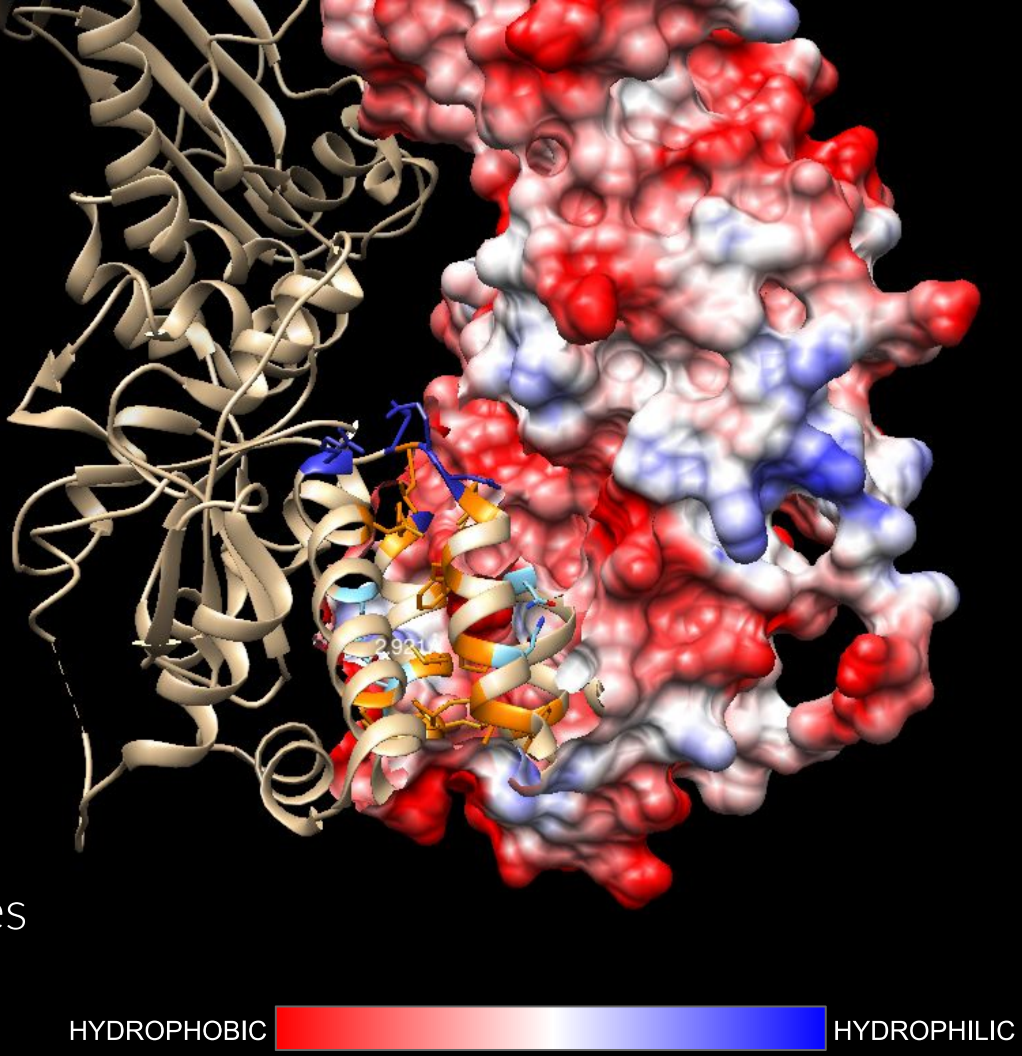


C-TERMINAL DOMAIN DIMERIZATION

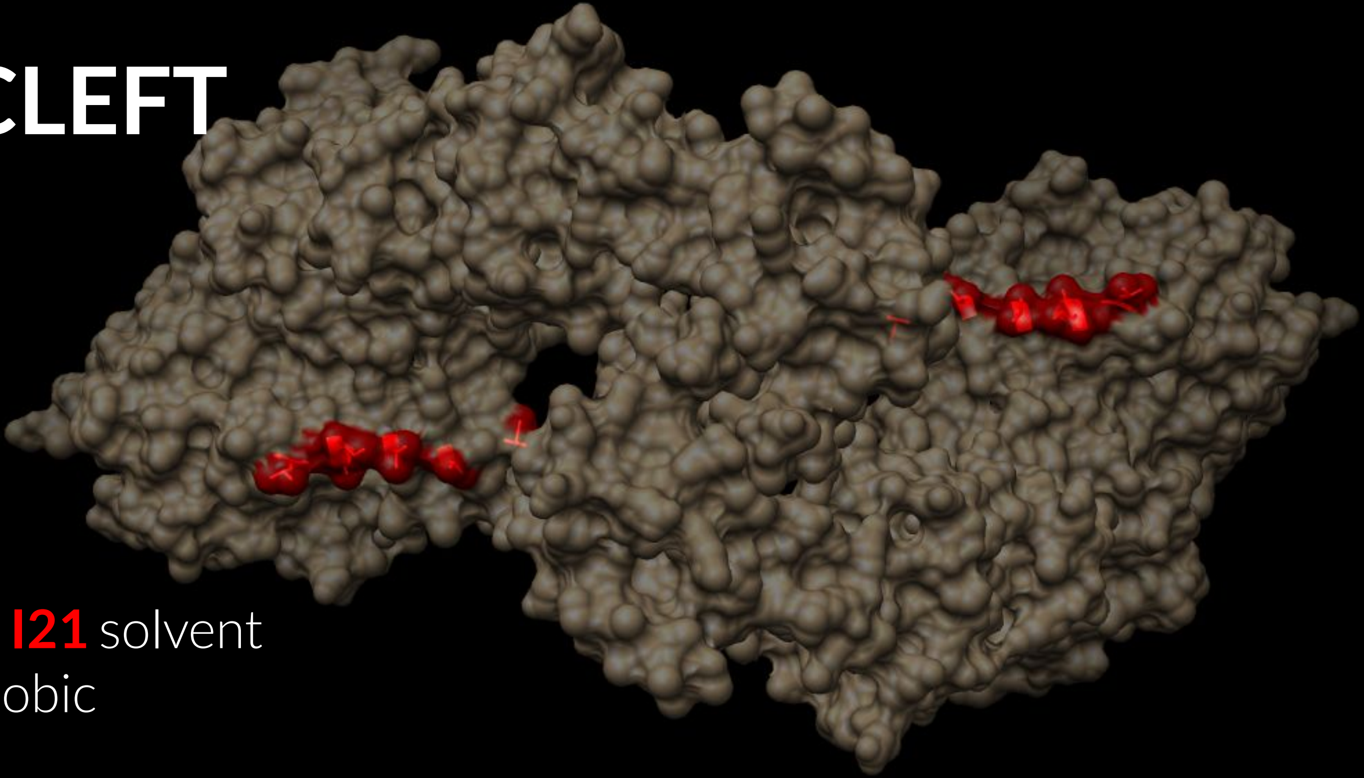
Long **hydrophobic** groove with conserved hydrophobicity

Conserved motif: MEEVD for co-chaperone binding

H21: conserved hydrophobic residues → role in client - protein interaction



CENTRAL CLEFT
CLIENT BINDING



NTD **V13, L17, M20, I21** solvent exposed and hydrophobic

Cluster with **F9, L24**

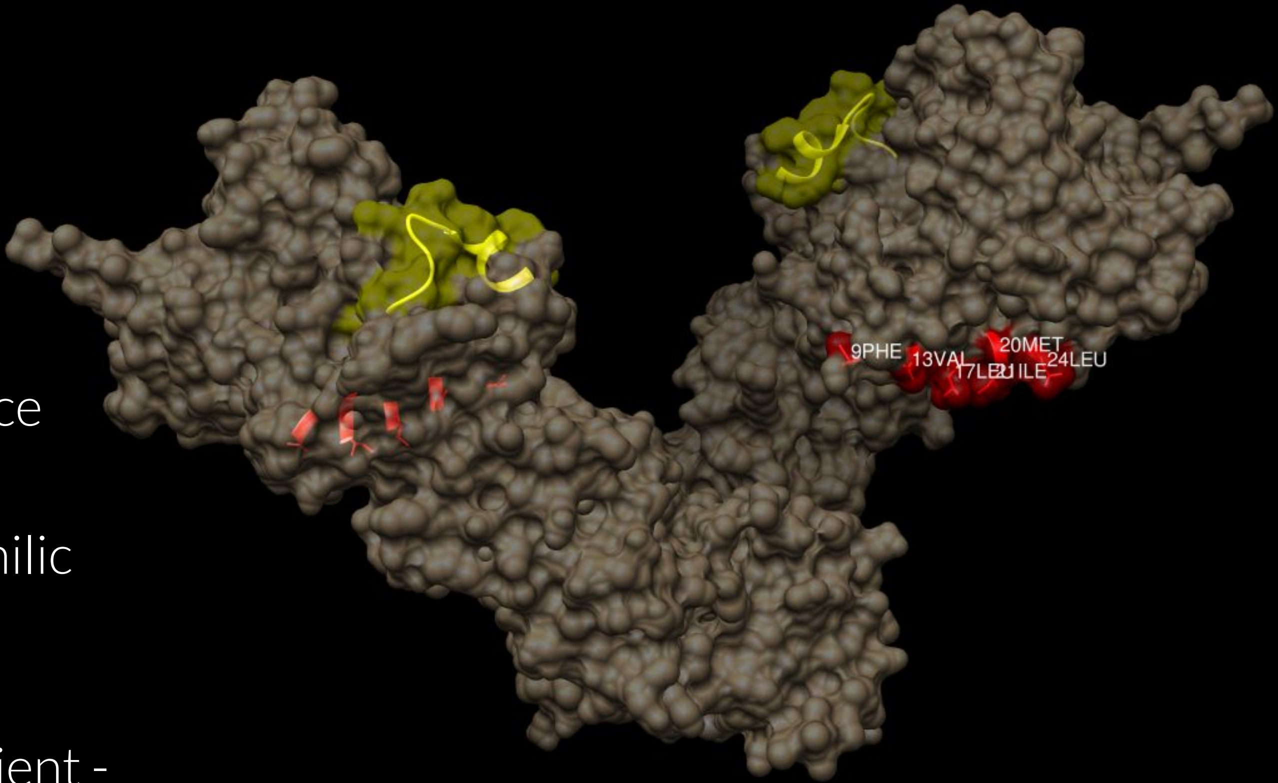
Hydrophobic patch: Protein / protein interaction

CENTRAL CLEFT CLIENT BINDING

MD **src loop**

Region with poor sequence conservation but similar hydrophobic and hydrophilic residues

Conservation suggests client - protein interaction region

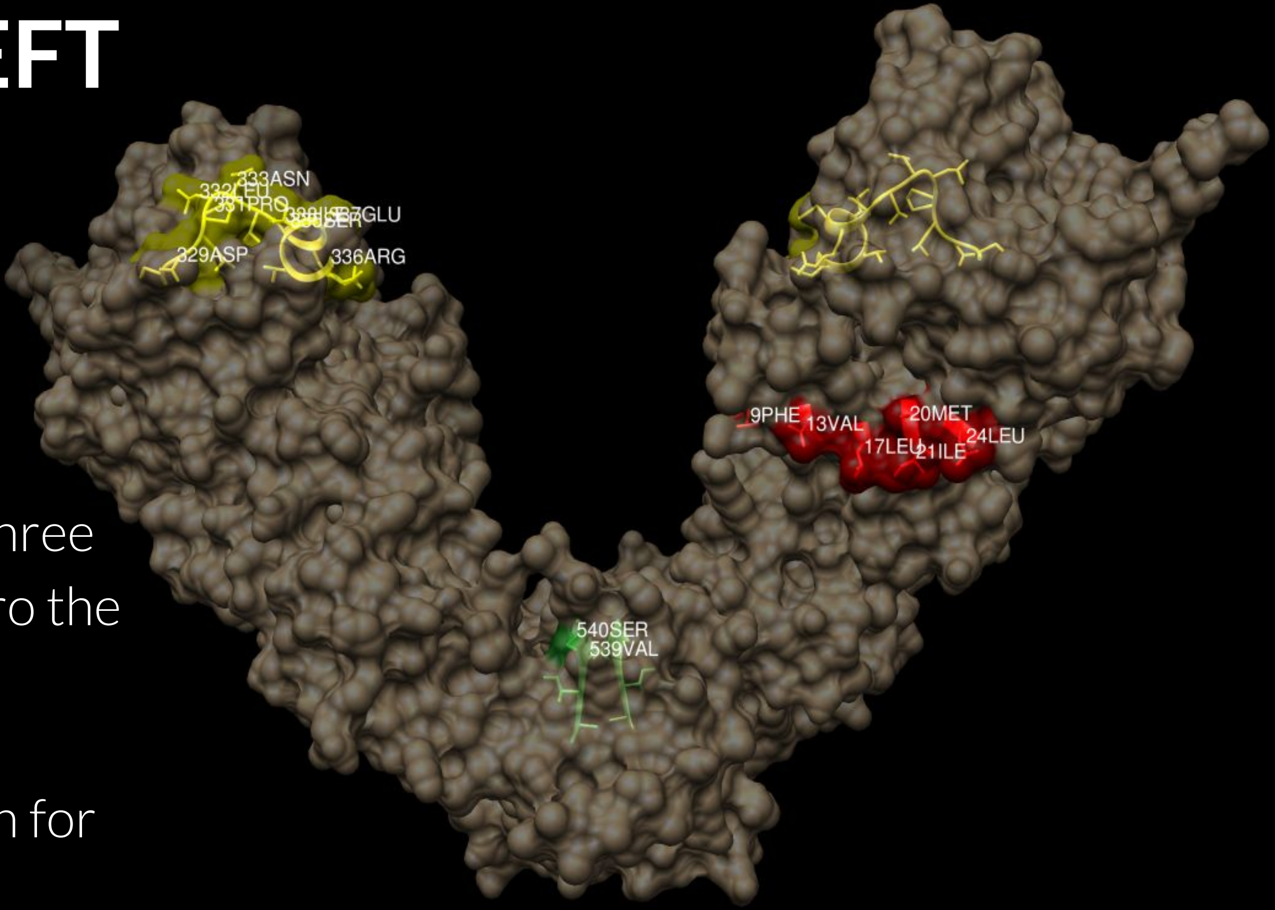


CENTRAL CLEFT
CLIENT BINDING

CTD **H21**

Each monomer presents three hydrophobic elements intro the inner concave surface

Central cleft: Ideal location for client - protein binding



VERTICAL ANALYSIS

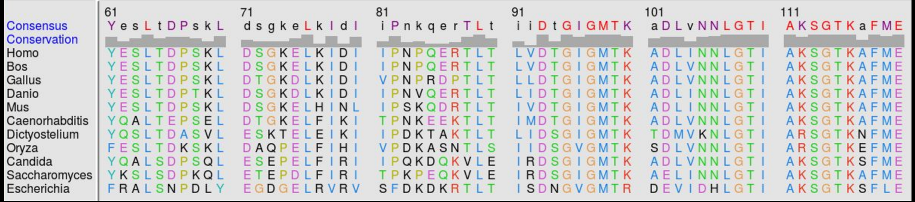
N-terminal Domain

Well-conserved N-terminal in general

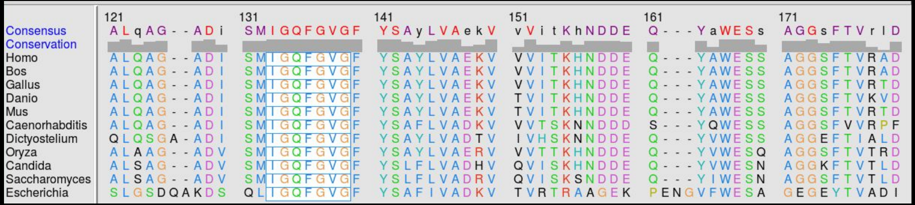
Active site lid presents shares little sequence homology beyond a well conserved IGQFGVG motif

Hydrophobic residues involved in client binding site conserved

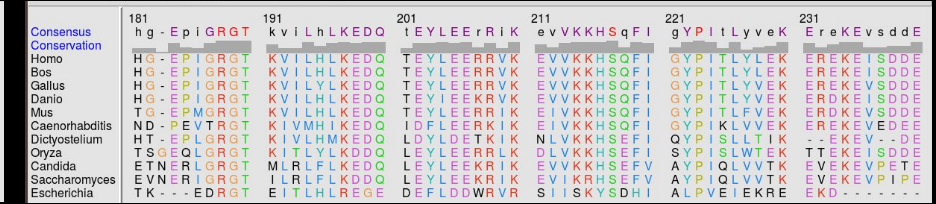
	1	11	21	31	41	51
Consensus	----------	---eeevETF	aFQAEIaQLM	SLIINTfYSN	KEIFLRELIS	NASDALDKIR
Conservation						
Homo	MPEEVHHG--	---EEEVETF	AFQAEIAQLM	SLIINTFYSN	KEIFLRELIS	NASDALDKIR
Bos	MPEEVHHG--	---EEEVETF	AFQAEIAQLM	SLIINTFYSN	KEIFLRELIS	NASDALDKIR
Gallus	MPEQVQHG--	---EDEVETF	AFQAEIAQLM	SLIINTFYSN	KEIFLRELIS	NASDALDKIR
Danio	MPEEMRQ---	---EEEAETF	AFQAEIAQLM	SLIINTFYSN	KEIFLRELVS	NASDALDKIR
Mus	MPEETQTQDQ	PMEEEEVETF	AFQAEIAQLM	SLIINTFYSN	KEIFLRELIS	NSSDALDKIR
Caenorhabditis	----------	--MSENAETF	AFQAEIAQLM	SLIINTFYSN	KEIYLRELIS	NASDALDKIR
Dictyostelium	----------	-MAESQVERF	TFQAEINQLM	SLIINTFYSN	KEVFLRELIS	NASDALDKIR
Oryza	----------	--MASETETF	AFQAEINQLL	SLIINTFYSN	KEIFLRELIS	NSSDALDKIR
Candida	----------	-MADAKVETH	EFTAEISQLM	SLIINTVYSN	KEIFLRELIS	NASDALDKIR
Saccharomyces	----------	-MAS---ETF	EFQAEITQLM	SLIINTVYSN	KEIFLRELIS	NASDALDKIR
Escherichia	----------	---MKGQETR	GFQSEVKQLL	HLMIHSLYSN	KEIFLRELIS	NASDAADKLR



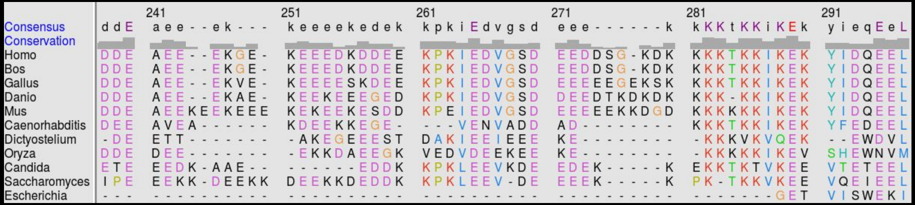
	61	71	81	91	101	111
Consensus	YesLtDPskL	dsgkeLkIdI	iPnkqerTLt	iiDtGIGMTK	aDLvNNLGTI	AKSGTKaFME
Conservation						
Homo	YESLTDPSKL	DSGKELKIDI	IPNPQERTLT	LVDTGIGMTK	ADLINNLGTI	AKSGTKAFME
Bos	YESLTDPSKL	DSGKELKIDI	IPNPQERTLT	LVDTGIGMTK	ADLVNNLGTI	AKSGTKAFME
Gallus	YESLTDPSKL	DTGKDLKIDI	VPNPRDPTLT	LLDTGIGMTK	ADLVNNLGTI	AKSGTKAFME
Danio	YESLTDPTKL	DSGKDLKIDI	IPNVQERTLT	LIDTGIGMTK	ADLINNLGTI	AKSGTKAFME
Mus	YESLTDPSKL	DSGKELHINL	IPSKQDRTLT	IVDTGIGMTK	ADLINNLGTI	AKSGTKAFME
Caenorhabditis	YQALTEPSEL	DTGKELFIKI	TPNKEEKTLT	IMDTGIGMTK	ADLVNNLGTI	AKSGTKAFME
Dictyostelium	YQSLTDASVL	ESKTELEIKI	IPDKTAKTLT	LIDSGIGMTK	TDMVKNLGTI	ARSGTKNFME
Oryza	FESLTDKSKL	DAQPELFIHI	VPDKASNTLS	IIDSGVGMTK	SDLVNNLGTI	ARSGTKEFME
Candida	YQALSDPSQL	ESEPELFIRI	IPQKDQKVLE	IRDSGIGMTK	ADLVNNLGTI	AKSGTKSFME
Saccharomyces	YKSLSDPKQL	ETEPDLFIRI	TPKPEQKVLE	IRDSGIGMTK	AELINNLGTI	AKSGTKAFME
Escherichia	FRALSNPDLY	EGDGELRVRV	SFDKDKRTLT	ISDNGVGMTR	DEVIDHLGTI	AKSGTKSFLE



	121	131	141	151	161	171
Consensus	ALqAG--ADi	SMIGQFGVGF	YSAyLVAekV	vVitKhNDDE	Q---YaWESs	AGGsFTVrID
Conservation						
Homo	ALQAG--ADI	SMIGQFGVGF	YSAYLVAEKV	VVITKHNDDE	Q---YAWESS	AGGSFTVRAD
Bos	ALQAG--ADI	SMIGQFGVGF	YSAYLVAEKV	VVITKHNDDE	Q---YAWESS	AGGSFTVRAD
Gallus	ALQAG--ADI	SMIGQFGVGF	YSAYLVAEKV	VVITKHNDDE	Q---YAWESS	AGGSFTVRTD
Danio	ALQAG--ADI	SMIGQFGVGF	YSAYLVAEKV	TVITKHNDDE	Q---YAWESS	AGGSFTVKVD
Mus	ALQAG--ADI	SMIGQFGVGF	YSAYLVAEKV	TVITKHNDDE	Q---YAWESS	AGGSFTVRTD
Caenorhabditis	ALQAG--ADI	SMIGQFGVGF	YSAFLVADKV	VVTSKNNDDD	S---YQWESS	AGGSFVVRPF
Dictyostelium	QLQSGA-ADI	SMIGQFGVGF	YSAYLVADTV	IVHSKNNDDE	Q---YVWESS	AGGEFTIALD
Oryza	ALAAG--ADV	SMIGQFGVGF	YSAYLVAERV	VVTTKHNDDE	Q---YVWESQ	AGGSFTVTRD
Candida	ALSAG--ADV	SMIGQFGVGF	YSLFLVADHV	QVISKHNDDE	Q---YVWESN	AGGKFTVTLD
Saccharomyces	ALSAG--ADV	SMIGQFGVGF	YSLFLVADRV	QVISKSNDDE	Q---YIWESN	AGGSFTVTLD
Escherichia	SLGSDQAKDS	QLIGQFGVGF	YSAFIVADKV	TVRTRAAGEK	PENGVFWESA	GEGEYTVADI



	181	191	201	211	221	231
Consensus	hg-EpiGRGT	kviLhLKEDQ	tEYLEErRiK	evVKKHSqFI	gYPItLyveK	EreKEvsddE
Conservation						
Homo	HG-EPIGRGT	KVILHLKEDQ	TEYLEERRVK	EVVKKHSQFI	GYPITLYLEK	EREKEISDDE
Bos	HG-EPIGRGT	KVILHLKEDQ	TEYLEERRVK	EVVKKHSQFI	GYPITLYLEK	EREKEISDDE
Gallus	HG-EPIGRGT	KVILYLKEDQ	TEYLEERRVK	EVVKKHSQFI	GYPITLYVEK	EREKEVSDDE
Danio	HG-EPIGRGT	KVILHLKEDQ	TEYIEEKRVK	EVVKKHSQFI	GYPITLYVEK	ERDKEISDDE
Mus	TG-EPMGRGT	KVILHLKEDQ	TEYLEERRIK	EIVKKHSQFI	GYPITLFVEK	ERDKEVSDDE
Caenorhabditis	ND-PEVTRGT	KIVMHIKEDQ	IDFLEERKIK	EIVKKHSQFI	GYPIKLVVEK	EREKEVEDEE
Dictyostelium	HT-EPLGRGT	KIVLHMKEDQ	LDYLDETKIK	NLVKKHSEFI	QYPISLLTIK	--EKEV--DE
Oryza	TSGEQLGRGT	KITLYLKDDQ	LEYLEERRLK	DLVKKHSEFI	SYPISLWTEK	TTEKEISDDE
Candida	ETNERLGRGT	MLRLFLKEDQ	LEYLEEKRIK	EVVKKHSEFV	AYPIQLVVTK	EVEKEVPETE
Saccharomyces	EVNERIGRGT	ILRLFLKDDQ	LEYLEEKRIK	EVIKRHSEFV	AYPIQLVVTK	EVEKEVPIPE
Escherichia	TK---EDRGT	EITLHLREGE	DEFLDDWRVR	SIISKYSDHI	ALPVEIEKRE	EKD-------



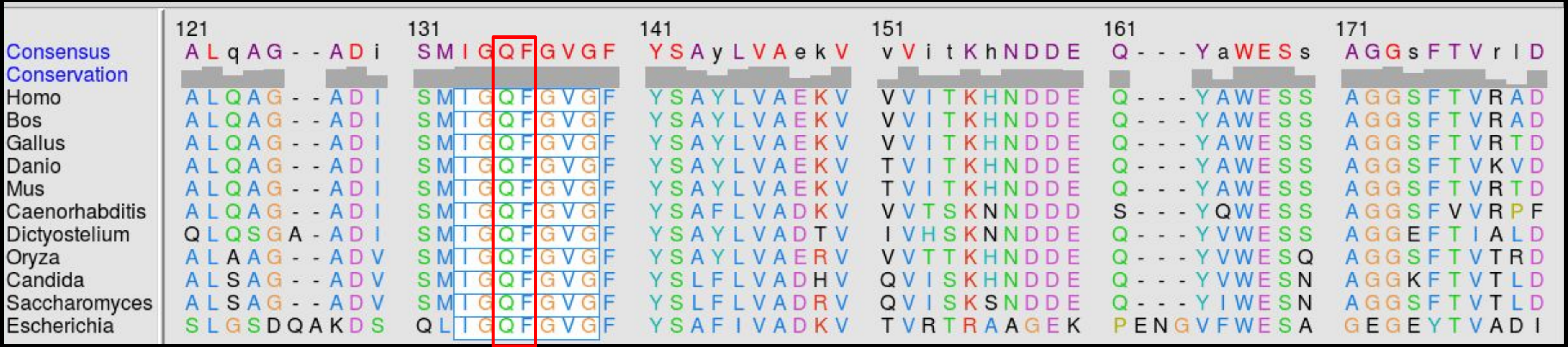
		241	251	261	271	281	291
Consensus	ddE	aee--ek---	keeeekedek	kpkiEdvgsd	eee------k	kKKtKKiKEk	yieqEeL
Conservation							
Homo	DDE	AEE--EKGE-	KEEEDKDDEE	KPKIEDVGSD	EEDDSG-KDK	KKKTKKIKEK	YIDQEEL
Bos	DDE	AEE--EKGE-	KEEEDKDDEE	KPKIEDVGSD	EEDDSG-KDK	KKKTKKIKEK	YIDQEEL
Gallus	DDE	AEE--EKVE-	KEEEESKDEE	KPKIEDVGSD	EEEEEGEKSK	KKKTKKIKEK	YIDQEEL
Danio	DDE	AEE--EKAE-	KEEKEEEGED	KPKIEDVGSD	DEEDTKDKDK	KKK-KKIKEK	YIDQEEL
Mus	DDE	AEEKEEKEEE	KEKEEKESDD	KPEIEDVGSD	EEEEEKKDGD	KKKKKKIKEK	YIDQEEL
Caenorhabditis	DEE	AVEA------	KDEEKKEGE-	---VENVADD	AD--------	KKKTKKIKEK	YFEDEEL
Dictyostelium	-DE	ETT-------	-AKEGEEEST	DAKIEEIEEE	KE--------	-KKKVKVQEK	--EWDVL
Oryza	DDE	DEE-------	-EKKDAEEGK	VEDVDEEKEE	KE--------	-KKKKKIKEV	SHEWNVM
Candida	ETE	EEDK-AAE--	------EDDK	KPKLEEVKDE	EDEK-----K	EKKTKTVKEE	VTETEEL
Saccharomyces	IPE	EEKK-DEEKK	DEEKKDEDDK	KPKLEEV-DE	EEEK-----K	PK-TKKVKEE	VQEIEEL
Escherichia	---	----------	----------	----------	----------	-------GET	VISWEKI

N-terminal Domain

Well-conserved N-terminal in general

Active site lid presents shares little sequence homology beyond a well conserved IGQFGVG motif

Hydrophobic residues involved in client binding site conserved



	121	131	141	151	161	171
Consensus	ALqAG--ADi	SMIGQFGVGF	YSAyLVAekV	vVitKhNDDE	Q---YaWESs	AGGsFTVrID
Conservation						
Homo	ALQAG--ADI	SMIGQFGVGF	YSAYLVAEKV	VVITKHNDDE	Q---YAWESS	AGGSFTVRAD
Bos	ALQAG--ADI	SMIGQFGVGF	YSAYLVAEKV	VVITKHNDDE	Q---YAWESS	AGGSFTVRAD
Gallus	ALQAG--ADI	SMIGQFGVGF	YSAYLVAEKV	VVITKHNDDE	Q---YAWESS	AGGSFTVRTD
Danio	ALQAG--ADI	SMIGQFGVGF	YSAYLVAEKV	TVITKHNDDE	Q---YAWESS	AGGSFTVKVD
Mus	ALQAG--ADI	SMIGQFGVGF	YSAYLVAEKV	TVITKHNDDE	Q---YAWESS	AGGSFTVRTD
Caenorhabditis	ALQAG--ADI	SMIGQFGVGF	YSAFLVADKV	VVTSKNNDDD	S---YQWESS	AGGSFVVRPF
Dictyostelium	QLQSGA-ADI	SMIGQFGVGF	YSAYLVADTV	IVHSKNNDDE	Q---YVWESS	AGGEFTIALD
Oryza	ALAAG--ADV	SMIGQFGVGF	YSAYLVAERV	VVTTKHNDDE	Q---YVWESQ	AGGSFTVTRD
Candida	ALSAG--ADV	SMIGQFGVGF	YSLFLVADHV	QVISKHNDDE	Q---YVWESN	AGGKFTVTLD
Saccharomyces	ALSAG--ADV	SMIGQFGVGF	YSLFLVADRV	QVISKSNDDE	Q---YIWESN	AGGSFTVTLD
Escherichia	SLGSDQAKDS	QLIGQFGVGF	YSAFIVADKV	TVRTRAAGEK	PENGVFWESA	GEGEYTVADI

N-terminal Domain

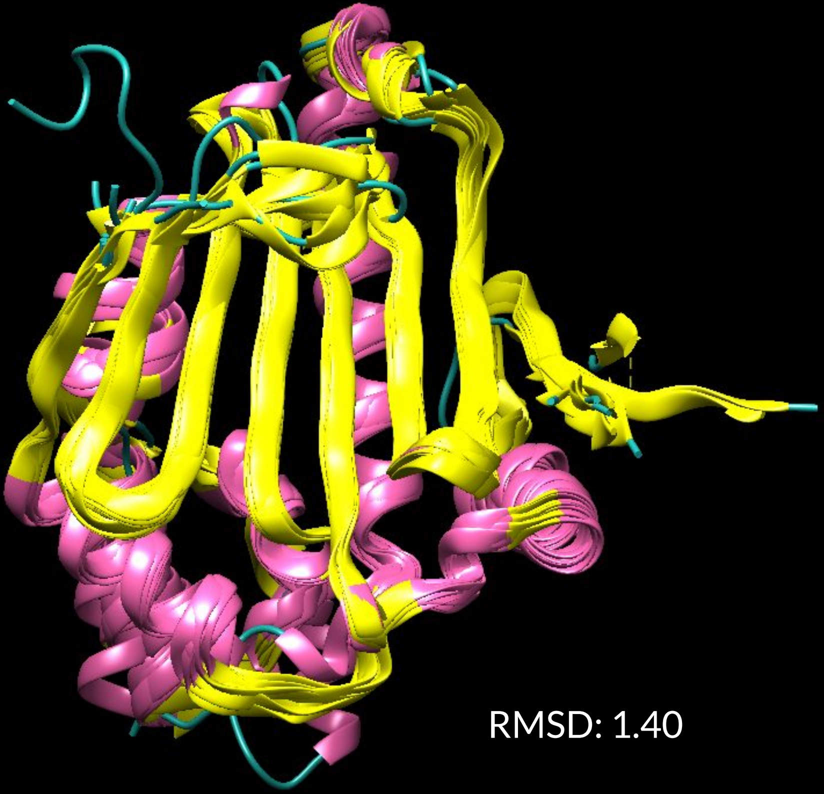
Well-conserved N-terminal in general

Active site lid presents shares little sequence homology beyond a well conserved IGQFGVG motif

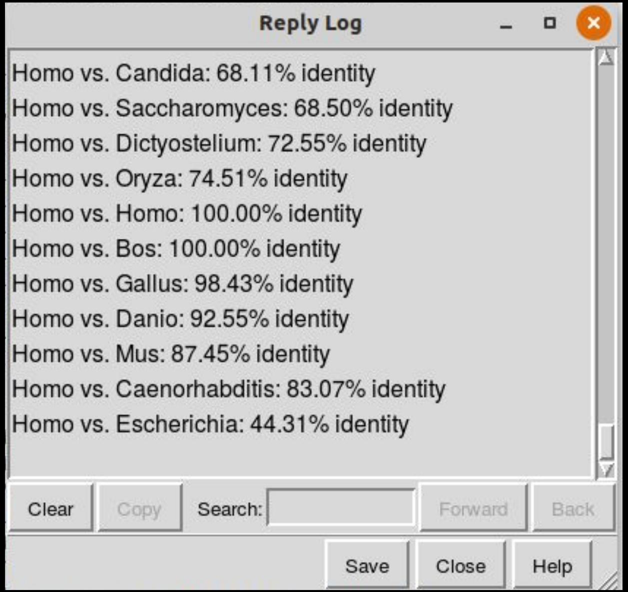
Hydrophobic residues involved in client binding site conserved

	1	11	21	31	41	51
Consensus	----------	---eeevETF	aFQAEIaQLM	SLIINTfYSN	KEIFLRELIS	NASDALDKIR
Conservation						
Homo	MPEEVHHG--	---EEEVETF	AFQAEIAQLM	SLIINTFYSN	KEIFLRELIS	NASDALDKIR
Bos	MPEEVHHG--	---EEEVETF	AFQAEIAQLM	SLIINTFYSN	KEIFLRELIS	NASDALDKIR
Gallus	MPEQVQHG--	---EDEVETF	AFQAEIAQLM	SLIINTFYSN	KEIFLRELIS	NASDALDKIR
Danio	MPEEMRQ---	---EEEAETF	AFQAEIAQLM	SLIINTFYSN	KEIFLRELVS	NASDALDKIR
Mus	MPEETQTQDQ	PMEEEEVETF	AFQAEIAQLM	SLIINTFYSN	KEIFLRELIS	NSSDALDKIR
Caenorhabditis	----------	--MSENAETF	AFQAEIAQLM	SLIINTFYSN	KEIYLRELIS	NASDALDKIR
Dictyostelium	----------	-MAESQVERF	TFQAEINQLM	SLIINTFYSN	KEVFLRELIS	NASDALDKIR
Oryza	----------	--MASETETF	AFQAEINQLL	SLIINTFYSN	KEIFLRELIS	NSSDALDKIR
Candida	----------	-MADAKVETH	EFTAEISQLM	SLIINTVYSN	KEIFLRELIS	NASDALDKIR
Saccharomyces	----------	-MAS---ETF	EFQAEITQLM	SLIINTVYSN	KEIFLRELIS	NASDALDKIR
Escherichia	----------	---MKGQETR	GFQSEVKQLL	HLMIHSLYSN	KEIFLRELIS	NASDAADKLR

N-terminal Domain

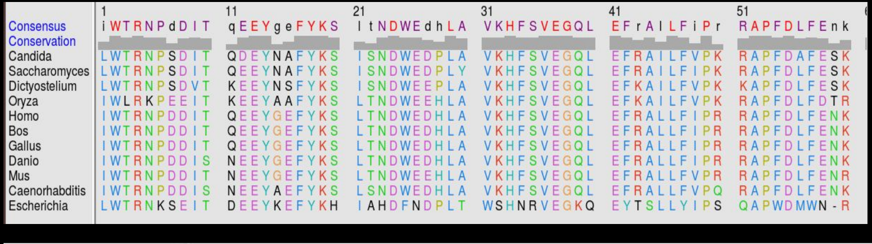


RMSD: 1.40



```
Homo vs. Candida: 68.11% identity
Homo vs. Saccharomyces: 68.50% identity
Homo vs. Dictyostelium: 72.55% identity
Homo vs. Oryza: 74.51% identity
Homo vs. Homo: 100.00% identity
Homo vs. Bos: 100.00% identity
Homo vs. Gallus: 98.43% identity
Homo vs. Danio: 92.55% identity
Homo vs. Mus: 87.45% identity
Homo vs. Caenorhabditis: 83.07% identity
Homo vs. Escherichia: 44.31% identity
```

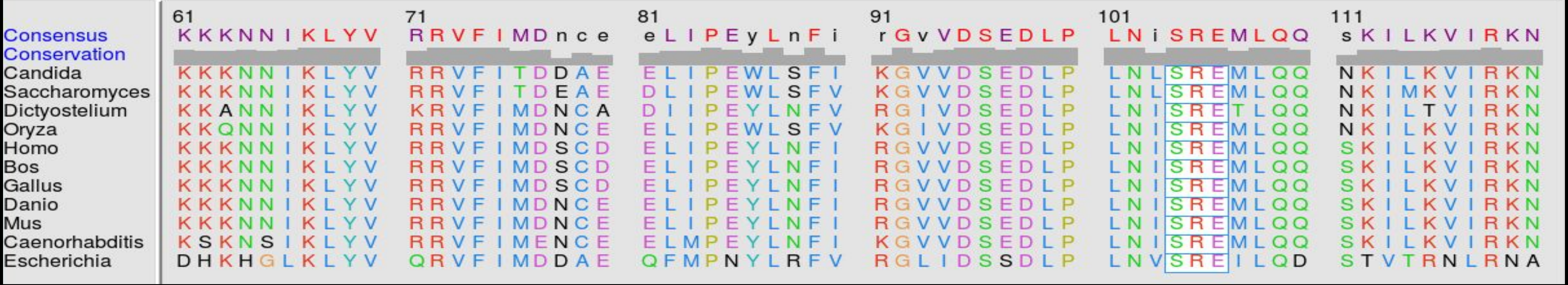
Middle Domain



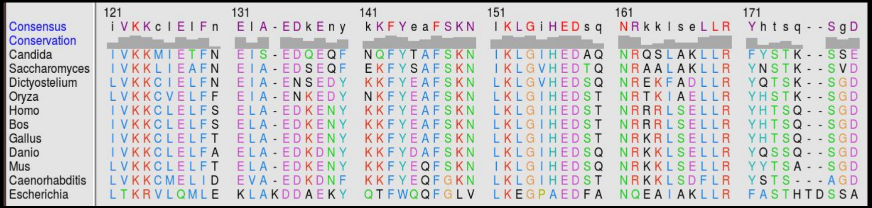
	1	11	21	31	41	51
Consensus	iWTRNPdDIT	qEEYgeFYKS	ltNDWEdhLA	VKHFSVEGQL	EFrAILFiPr	RAPFDLFEnk
Conservation						
Candida	LWTRNPSDIT	QDEYNAFYKS	ISNDWEDPLA	VKHFSVEGQL	EFRAILFVPK	RAPFDAFESK
Saccharomyces	LWTRNPSDIT	QEEYNAFYKS	ISNDWEDPLY	VKHFSVEGQL	EFRAILFIPK	RAPFDLFESK
Dictyostelium	LWTRNPSDVT	KEEYNSFYKS	ISNDWEEPLA	VKHFSVEGQL	EFKAILFVPK	KAPFDLFESK
Oryza	IWLRKPEEIT	KEEYAAFYKS	LTNDWEEHLA	VKHFSVEGQL	EFKAILFVPK	RAPFDLFDTR
Homo	IWTRNPDDIT	QEEYGEFYKS	LTNDWEDHLA	VKHFSVEGQL	EFRALLFIPR	RAPFDLFENK
Bos	IWTRNPDDIT	QEEYGEFYKS	LTNDWEDHLA	VKHFSVEGQL	EFRALLFIPR	RAPFDLFENK
Gallus	IWTRNPDDIT	QEEYGEFYKS	LTNDWEDHLA	VKHFSVEGQL	EFRALLFIPR	RAPFDLFENK
Danio	IWTRNPDDIS	NEEYGEFYKS	LTNDWEDHLA	VKHFSVEGQL	EFRALLFIPR	RAPFDLFENK
Mus	IWTRNPDDIT	NEEYGEFYKS	LTNDWEEHLA	VKHFSVEGQL	EFRALLFVPR	RAPFDLFENR
Caenorhabditis	IWTRNPDDIS	NEEYAEFYKS	LSNDWEDHLA	VKHFSVEGQL	EFRALLFVPQ	RAPFDLFENK
Escherichia	LWTRNKSEIT	DEEYKEFYKH	IAHDFNDPLT	WSHNRVEGKQ	EYTSLLYIPS	QAPWDMWN-R

Well-conserved middle domain

Conserved Arginine (ArgMD) with significant role in ATP hydrolysis

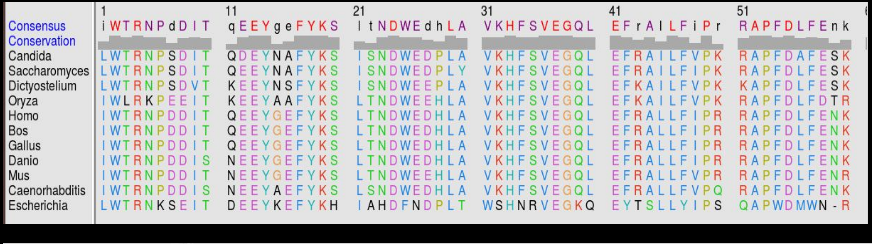


	61	71	81	91	101	111
Consensus	KKKNNIKLYV	RRVFIMDnce	eLIPEyLnFi	rGvVDSEDLP	LNiSREMLQQ	sKILKVIRKN
Conservation						
Candida	KKKNNIKLYV	RRVFITDDAE	ELIPEWLSFI	KGVVDSEDLP	LNLSREMLQQ	NKILKVIRKN
Saccharomyces	KKKNNIKLYV	RRVFITDEAE	DLIPEWLSFV	KGVVDSEDLP	LNLSREMLQQ	NKIMKVIRKN
Dictyostelium	KKANNIKLYV	KRVFIMDNCA	DIIPEYLNFV	RGIVDSEDLP	LNISRETLQQ	NKILTVIRKN
Oryza	KKQNNIKLYV	RRVFIMDNCE	ELIPEWLSFV	KGIVDSEDLP	LNISREMLQQ	NKILKVIRKN
Homo	KKKNNIKLYV	RRVFIMDSCD	ELIPEYLNFI	RGVVDSEDLP	LNISREMLQQ	SKILKVIRKN
Bos	KKKNNIKLYV	RRVFIMDSCD	ELIPEYLNFI	RGVVDSEDLP	LNISREMLQQ	SKILKVIRKN
Gallus	KKKNNIKLYV	RRVFIMDSCD	ELIPEYLNFI	RGVVDSEDLP	LNISREMLQQ	SKILKVIRKN
Danio	KKKNNIKLYV	RRVFIMDNCE	ELIPEYLNFI	RGVVDSEDLP	LNISREMLQQ	SKILKVIRKN
Mus	KKKNNIKLYV	RRVFIMDNCE	ELIPEYLNFI	RGVVDSEDLP	LNISREMLQQ	SKILKVIRKN
Caenorhabditis	KSKNSIKLYV	RRVFIMENCE	ELMPEYLNFI	KGVVDSEDLP	LNISREMLQQ	SKILKVIRKN
Escherichia	DHKHGLKLYV	QRVFIMDDAE	QFMPNYLRFV	RGLIDSSDLP	LNVSREILQD	STVTRNLRNA



	121	131	141	151	161	171
Consensus	iVKKcIEIFn	EIA-EDkEny	kKFYeaFSKN	IKLGiHEDsq	NRkkIseLLR	Yhtsq--SgD
Conservation						
Candida	IVKKMIETFN	EIS-EDQEQF	NQFYTAFSKN	IKLGIHEDAQ	NRQSLAKLLR	FYSTK--SSE
Saccharomyces	IVKKLIEAFN	EIA-EDSEQF	EKFYSAFSKN	IKLGVHEDTQ	NRAALAKLLR	YNSTK--SVD
Dictyostelium	LVKKCIELFN	EIA-ENSEDY	KKFYEAFSKN	LKLGVHEDSQ	NREKFADLLR	YQTSK--SGD
Oryza	LVKKCVELFF	EIA-ENKEDY	NKFYEAFSKN	LKLGIHEDST	NRTKIAELLR	YHSTK--SGD
Homo	IVKKCLELFS	ELA-EDKENY	KKFYEAFSKN	LKLGIHEDST	NRRRLSELLR	YHTSQ--SGD
Bos	IVKKCLELFS	ELA-EDKENY	KKFYEAFSKN	LKLGIHEDST	NRRRLSELLR	YHTSQ--SGD
Gallus	IVKKCLELFT	ELA-EDKENY	KKFYEAFSKN	LKLGIHEDST	NRKRLSELLR	YHTSQ--SGD
Danio	IVKKCLELFA	ELA-EDKDNY	KKFYDAFSKN	LKLGIHEDSQ	NRKKLSELLR	YQSSQ--SGD
Mus	LVKKCLELFT	ELA-EDKENY	KKFYEQFSKN	IKLGIHEDSQ	NRKKLSELLR	YYTSA--SGD
Caenorhabditis	LVKKCMELID	EVA-EDKDNF	KKFYEQFGKN	LKLGIHEDST	NRKKLSDFLR	YSTS---AGD
Escherichia	LTKRVLQMLE	KLAKDDAEKY	QTFWQQFGLV	LKEGPAEDFA	NQEAIAKLLR	FASTHTDSSA

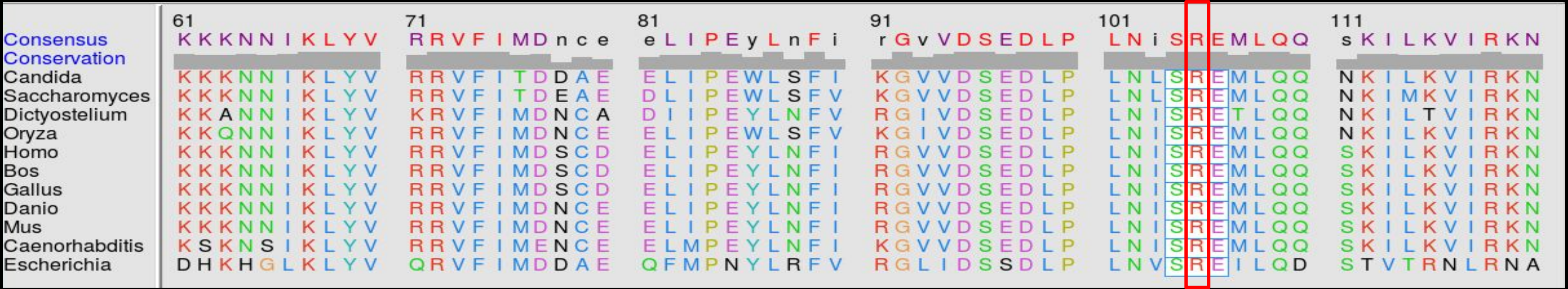
Middle Domain



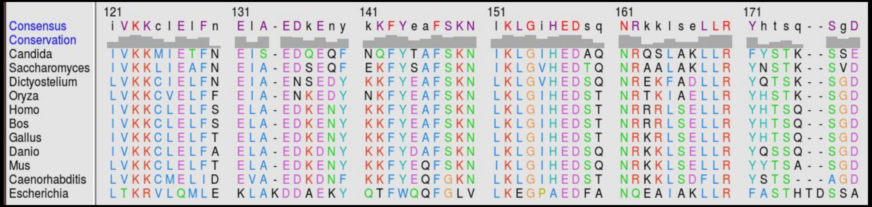
	1	11	21	31	41	51
Consensus	iWTRNPdDIT	qEEYgeFYKS	ItNDWEdhLA	VKHFSVEGQL	EFrAILFiPr	RAPFDLFEnk
Conservation						
Candida	LWTRNPSDIT	QDEYNAFYKS	ISNDWEDPLA	VKHFSVEGQL	EFRAILFVPK	RAPFDAFESK
Saccharomyces	LWTRNPSDIT	QEEYNAFYKS	ISNDWEDPLY	VKHFSVEGQL	EFRAILFIPK	RAPFDLFESK
Dictyostelium	LWTRNPSDVT	KEEYNSFYKS	ISNDWEEPLA	VKHFSVEGQL	EFKAILFVPK	KAPFDLFESK
Oryza	IWLRKPEEIT	KEEYAAFYKS	LTNDWEEHLA	VKHFSVEGQL	EFKAILFVPK	RAPFDLFDTR
Homo	IWTRNPDDIT	QEEYGEFYKS	LTNDWEDHLA	VKHFSVEGQL	EFRALLFIPR	RAPFDLFENK
Bos	IWTRNPDDIT	QEEYGEFYKS	LTNDWEDHLA	VKHFSVEGQL	EFRALLFIPR	RAPFDLFENK
Gallus	IWTRNPDDIT	QEEYGEFYKS	LTNDWEDHLA	VKHFSVEGQL	EFRALLFIPR	RAPFDLFENK
Danio	IWTRNPDDIS	NEEYGEFYKS	LTNDWEDHLA	VKHFSVEGQL	EFRALLFIPR	RAPFDLFENK
Mus	IWTRNPDDIT	NEEYGEFYKS	LTNDWEEHLA	VKHFSVEGQL	EFRALLFVPR	RAPFDLFENR
Caenorhabditis	IWTRNPDDIS	NEEYAEFYKS	LSNDWEDHLA	VKHFSVEGQL	EFRALLFVPQ	RAPFDLFENK
Escherichia	LWTRNKSEIT	DEEYKEFYKH	IAHDFNDPLT	WSHNRVEGKQ	EYTSLLYIPS	QAPWDMWN-R

Well-conserved middle domain

Conserved Arginine (ArgMD) with significant role in ATP hydrolysis



	61	71	81	91	101	111
Consensus	KKKNNIKLYV	RRVFIMDnce	eLIPEyLnFi	rGvVDSEDLP	LNiSREMLQQ	sKILKVIRKN
Conservation						
Candida	KKKNNIKLYV	RRVFITDDAE	ELIPEWLSFI	KGVVDSEDLP	LNLSREMLQQ	NKILKVIRKN
Saccharomyces	KKKNNIKLYV	RRVFITDEAE	DLIPEWLSFV	KGVVDSEDLP	LNLSREMLQQ	NKIMKVIRKN
Dictyostelium	KKANNIKLYV	KRVFIMDNCA	DIIPEYLNFV	RGIVDSEDLP	LNISRETLQQ	NKILTVIRKN
Oryza	KKQNNIKLYV	RRVFIMDNCE	ELIPEWLSFV	KGIVDSEDLP	LNISREMLQQ	NKILKVIRKN
Homo	KKKNNIKLYV	RRVFIMDSCD	ELIPEYLNFI	RGVVDSEDLP	LNISREMLQQ	SKILKVIRKN
Bos	KKKNNIKLYV	RRVFIMDSCD	ELIPEYLNFI	RGVVDSEDLP	LNISREMLQQ	SKILKVIRKN
Gallus	KKKNNIKLYV	RRVFIMDSCD	ELIPEYLNFI	RGVVDSEDLP	LNISREMLQQ	SKILKVIRKN
Danio	KKKNNIKLYV	RRVFIMDNCE	ELIPEYLNFI	RGVVDSEDLP	LNISREMLQQ	SKILKVIRKN
Mus	KKKNNIKLYV	RRVFIMDNCE	ELIPEYLNFI	RGVVDSEDLP	LNISREMLQQ	SKILKVIRKN
Caenorhabditis	KSKNSIKLYV	RRVFIMENCE	ELMPEYLNFI	KGVVDSEDLP	LNISREMLQQ	SKILKVIRKN
Escherichia	DHKHGLKLYV	QRVFIMDDAE	QFMPNYLRFV	RGLIDSSDLP	LNVSREILQD	STVTRNLRNA

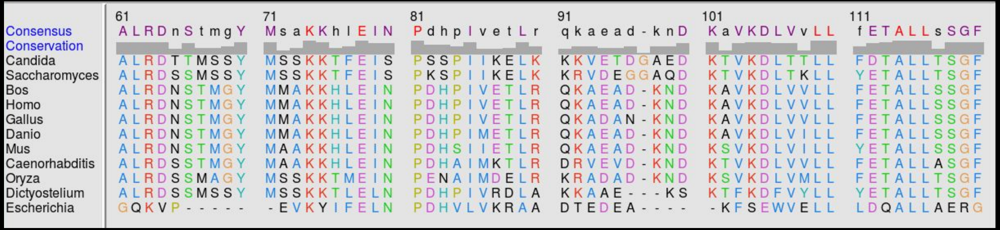


	121	131	141	151	161	171
Consensus	iVKKcIEIFn	EIA-EDkEny	kKFYeaFSKN	IKLGiHEDsq	NRkkIseLLR	Yhtsq--SgD
Conservation						
Candida	IVKKMIETFN	EIS-EDQEQF	NQFYTAFSKN	IKLGIHEDAQ	NRQSLAKLLR	FYSTK--SSE
Saccharomyces	IVKKLIEAFN	EIA-EDSEQF	EKFYSAFSKN	IKLGVHEDTQ	NRAALAKLLR	YNSTK--SVD
Dictyostelium	LVKKCIELFN	EIA-ENSEDY	KKFYEAFSKN	LKLGVHEDSQ	NREKFADLLR	YQTSK--SGD
Oryza	LVKKCVELFF	EIA-ENKEDY	NKFYEAFSKN	LKLGIHEDST	NRTKIAELLR	YHSTK--SGD
Homo	IVKKCLELFS	ELA-EDKENY	KKFYEAFSKN	LKLGIHEDST	NRRRLSELLR	YHTSQ--SGD
Bos	IVKKCLELFS	ELA-EDKENY	KKFYEAFSKN	LKLGIHEDST	NRRRLSELLR	YHTSQ--SGD
Gallus	IVKKCLELFT	ELA-EDKENY	KKFYEAFSKN	LKLGIHEDST	NRKRLSELLR	YHTSQ--SGD
Danio	IVKKCLELFA	ELA-EDKDNY	KKFYDAFSKN	LKLGIHEDSQ	NRKKLSELLR	YQSSQ--SGD
Mus	LVKKCLELFT	ELA-EDKENY	KKFYEQFSKN	IKLGIHEDSQ	NRKKLSELLR	YYTSA--SGD
Caenorhabditis	LVKKCMELID	EVA-EDKDNF	KKFYEQFGKN	LKLGIHEDST	NRKKLSDFLR	YSTS---AGD
Escherichia	LTKRVLQMLE	KLAKDDAEKY	QTFWQQFGLV	LKEGPAEDFA	NQEAIAKLLR	FASTHTDSSA

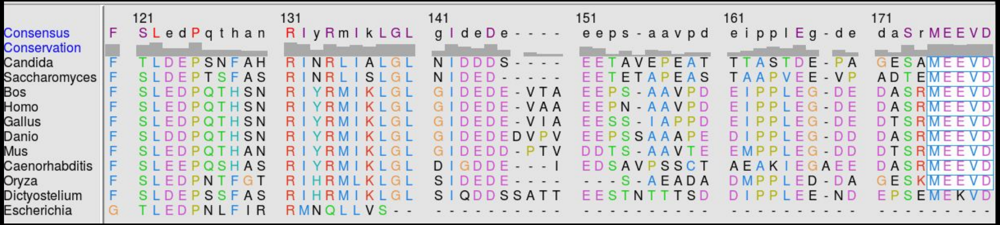
Middle Domain

RMSD: 1.60

C-terminal Domain



	61	71	81	91	101	111
Consensus	ALRDnStmgY	MsaKKhlEIN	PdhpIvetLr	qkaead-knD	KaVKDLVvLL	fETALLsSGF
Conservation						
Candida	ALRDTTMSSY	MSSKKTFEIS	PSSPIIKELK	KKVETDGAED	KTVKDLTTLL	FDTALLTSGF
Saccharomyces	ALRDSSMSSY	MSSKKTFEIS	PKSPIIKELK	KRVDEGGAQD	KTVKDLTKLL	YETALLTSGF
Bos	ALRDNSTMGY	MMAKKHLEIN	PDHPIVETLR	QKAEAD-KND	KAVKDLVVLL	FETALLSSGF
Homo	ALRDNSTMGY	MMAKKHLEIN	PDHPIVETLR	QKAEAD-KND	KAVKDLVVLL	FETALLSSGF
Gallus	ALRDNSTMGY	MMAKKHLEIN	PDHPIVETLR	QKADAN-KND	KAVKDLVVLL	FETALLSSGF
Danio	ALRDNSTMGY	MMAKKHLEIN	PDHPIMETLR	QKAEAD-KND	KAVKDLVILL	FETALLSSGF
Mus	ALRDNSTMGY	MAAKKHLEIN	PDHSIIETLR	QKAEAD-KND	KSVKDLVILL	YETALLSSGF
Caenorhabditis	ALRDSSTMGY	MAAKKHLEIN	PDHAIMKTLR	DRVEVD-KND	KTVKDLVVLL	FETALLASGF
Oryza	ALRDSSMAGY	MSSKKTMEIN	PENAIMDELR	KRADAD-KND	KSVKDLVMLL	FETALLTSGF
Dictyostelium	ALRDSSMSSY	MSSKKTLELN	PDHPIVRDLA	KKAAE---KS	KTFKDFVYLL	YETALLTSGF
Escherichia	GQKVP-----	-EVKYIFELN	PDHVLVKRAA	DTEDEA----	-KFSEWVELL	LDQALLAERG



		121	131	141	151	161	171
Consensus	F	SLedPqthan	RIyRmIkLGL	gIdeDe----	eeps-aavpd	eippIEg-de	daSrMEEVD
Conservation							
Candida	F	TLDEPSNFAH	RINRLIALGL	NIDDDS----	EETAVEPEAT	TTASTDE-PA	GESAMEEVD
Saccharomyces	F	SLDEPTSFAS	RINRLISLGL	NIDED-----	EETETAPEAS	TAAPVEE-VP	ADTEMEEVD
Bos	F	SLEDPQTHSN	RIYRMIKLGL	GIDEDE-VTA	EEPS-AAVPD	EIPPLEG-DE	DASRMEEVD
Homo	F	SLEDPQTHSN	RIYRMIKLGL	GIDEDE-VAA	EEPN-AAVPD	EIPPLEG-DE	DASRMEEVD
Gallus	F	SLEDPQTHSN	RIYRMIKLGL	GIDEDE-VIA	EESS-IAPPD	EIPPLEG-DE	DTSRMEEVD
Danio	F	SLDDPQTHSN	RIYRMIKLGL	GIDEDEDVPV	EEPSSAAAPE	DIPPLEG-DD	DASRMEEVD
Mus	F	SLEDPQTHAN	RIYRMIKLGL	GIDEDD-PTV	DDTS-AAVTE	EMPPLEG-DD	DTSRMEEVD
Caenorhabditis	F	SLEEPQSHAS	RIYRMIKLGL	DIGDDE---I	EDSAVPSSCT	AEAKIEGAEE	DASRMEEVD
Oryza	F	SLEDPNTFGT	RIHRMLKLGL	SIDEDE----	---S-AEADA	DMPPLED-DA	GESKMEEVD
Dictyostelium	F	SLDEPSSFAS	RIHRMIKLGL	SIQDDSSATT	EESTNTTTSD	DIPPLEE-ND	EPSEMEKVD
Escherichia	G	TLEDPNLFIR	RMNQLLVS--	----------	----------	----------	---------

Well-conserved C domain

Conserved hydrophobic (Leu/Ile, Pro, Phe/His) and hydrophilic residues (Asn/Tyr/His and Gln/Thr) involved in dimerization.

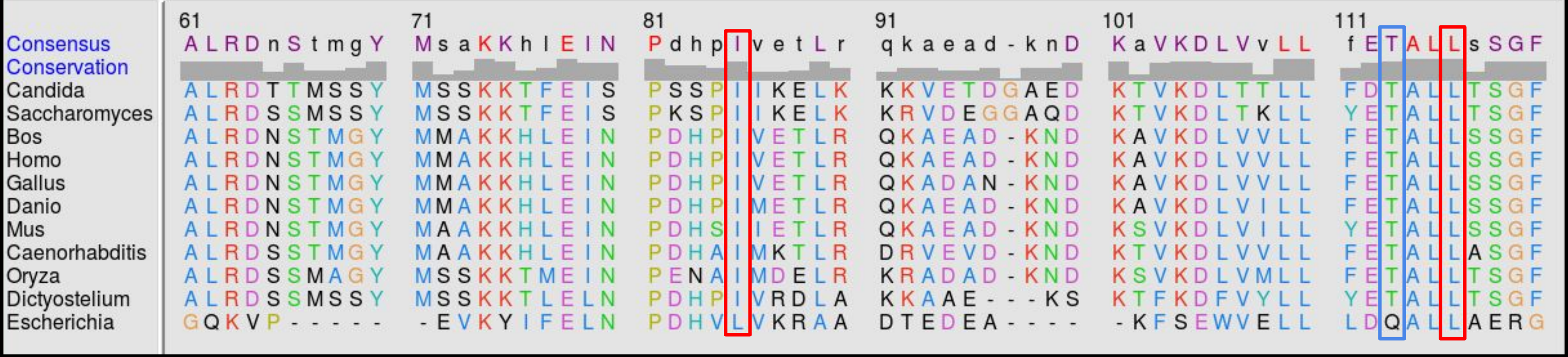
Conserved MEEVD for cochaperones binding (absent in *E. coli* since Htpg doesn't use cochaperones)

C-terminal Domain

Well-conserved C domain

Conserved hydrophobic (Leu/Ile, Pro, Phe/His) and hydrophilic residues (Asn/Tyr/His and Gln/Thr) involved in dimerization

Conserved MEEVD for cochaperones binding (absent in *E. coli* since Htpg doesn't use cochaperones)



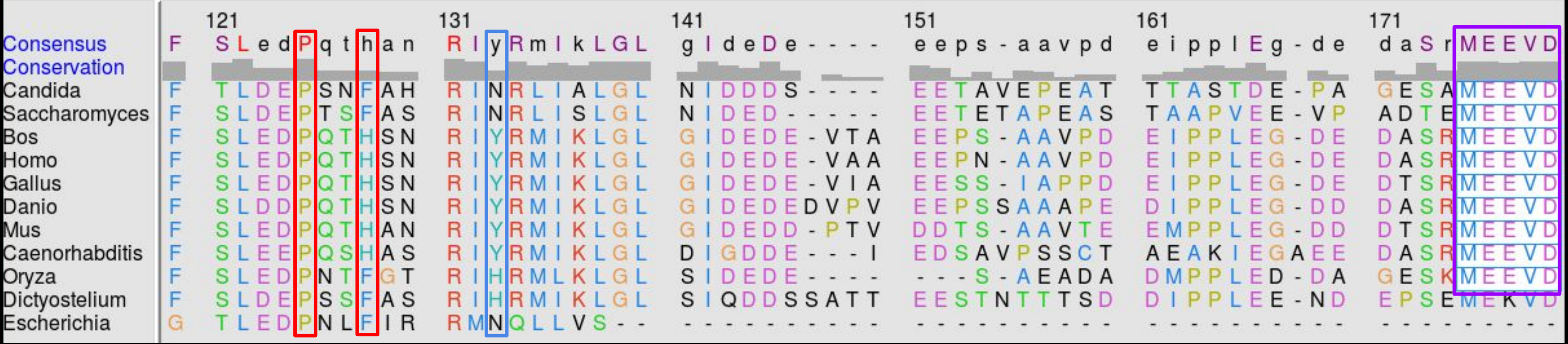
	61	71	81	91	101	111
Consensus	ALRDnStmgY	MsaKKhlEIN	PdhpIvetLr	qkaead-knD	KaVKDLVvLL	fETALLsSGF
Conservation						
Candida	ALRDTTMSSY	MSSKKTFEIS	PSSPIIKELK	KKVETDGAED	KTVKDLTTLL	FDTALLTSGF
Saccharomyces	ALRDSSMSSY	MSSKKTFEIS	PKSPIIKELK	KRVDEGGAQD	KTVKDLTKLL	YETALLTSGF
Bos	ALRDNSTMGY	MMAKKHLEIN	PDHPIVETLR	QKAEAD-KND	KAVKDLVVLL	FETALLSSGF
Homo	ALRDNSTMGY	MMAKKHLEIN	PDHPIVETLR	QKAEAD-KND	KAVKDLVVLL	FETALLSSGF
Gallus	ALRDNSTMGY	MMAKKHLEIN	PDHPIVETLR	QKADAN-KND	KAVKDLVVLL	FETALLSSGF
Danio	ALRDNSTMGY	MMAKKHLEIN	PDHPIMETLR	QKAEAD-KND	KAVKDLVILL	FETALLSSGF
Mus	ALRDNSTMGY	MAAKKHLEIN	PDHSIIETLR	QKAEAD-KND	KSVKDLVILL	YETALLSSGF
Caenorhabditis	ALRDSSTMGY	MAAKKHLEIN	PDHAIMKTLR	DRVEVD-KND	KTVKDLVVLL	FETALLASGF
Oryza	ALRDSSMAGY	MSSKKTMEIN	PENAIMDELR	KRADAD-KND	KSVKDLVMLL	FETALLTSGF
Dictyostelium	ALRDSSMSSY	MSSKKTLELN	PDHPIVRDLA	KKAAE---KS	KTFKDFVYLL	YETALLTSGF
Escherichia	GQKVP-----	-EVKYIFELN	PDHVLVKRAA	DTEDEA----	-KFSEWVELL	LDQALLAERG

C-terminal Domain

Well-conserved C domain

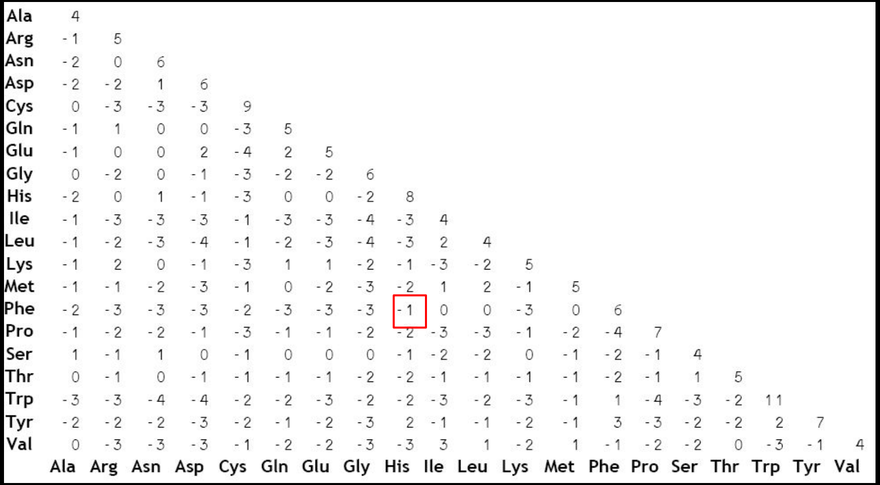
Conserved hydrophobic (Leu/Ile, Pro, Phe/His) and hydrophilic residues (Asn/Tyr/His and Gln/Thr) involved in dimerization

Conserved MEEVD for cochaperones binding (absent in *E. coli* since Htpg doesn't use cochaperones)



```
                   121         131         141         151         161         171
Consensus       F  SLedPqthan  RIyRmIkLGL  gIdeDe----  eeps-aavpd  eipplEg-de  daSrMEEVD
Conservation
Candida         F  TLDEPSNFAH  RINRLIALGL  NIDDDS----  EETAVEPEAT  TTASTDE-PA  GESAMEEVD
Saccharomyces   F  SLDEPTSFAS  RINRLISLGL  NIDED-----  EETETAPEAS  TAAPVEE-VP  ADTEMEEVD
Bos             F  SLEDPQTHSN  RIYRMIKLGL  GIDEDE-VTA  EEPS-AAVPD  EIPPLEG-DE  DASRMEEVD
Homo            F  SLEDPQTHSN  RIYRMIKLGL  GIDEDE-VAA  EEPN-AAVPD  EIPPLEG-DE  DASRMEEVD
Gallus          F  SLEDPQTHSN  RIYRMIKLGL  GIDEDE-VIA  EESS-IAPPD  EIPPLEG-DE  DTSRMEEVD
Danio           F  SLDDPQTHSN  RIYRMIKLGL  GIDEDEDVPV  EEPSSAAAPE  DIPPLEG-DD  DASRMEEVD
Mus             F  SLEDPQTHAN  RIYRMIKLGL  GIDEDD-PTV  DDTS-AAVTE  EMPPLEG-DD  DTSRMEEVD
Caenorhabditis  F  SLEEPQSHAS  RIYRMIKLGL  DIGDDE---I  EDSAVPSSCT  AEAKIEGAEE  DASRMEEVD
Oryza           F  SLEDPNTFGT  RIHRMLKLGL  SIDEDE----  ---S-AEADA  DMPPLED-DA  GESKMEEVD
Dictyostelium   F  SLDEPSSFAS  RIHRMIKLGL  SIQDDSSATT  EESTNTTTSD  DIPPLEE-ND  EPSEMEKVD
Escherichia     G  TLEDPNLFIR  RMNQLLVS--  ----------  ----------  ----------  ---------
```

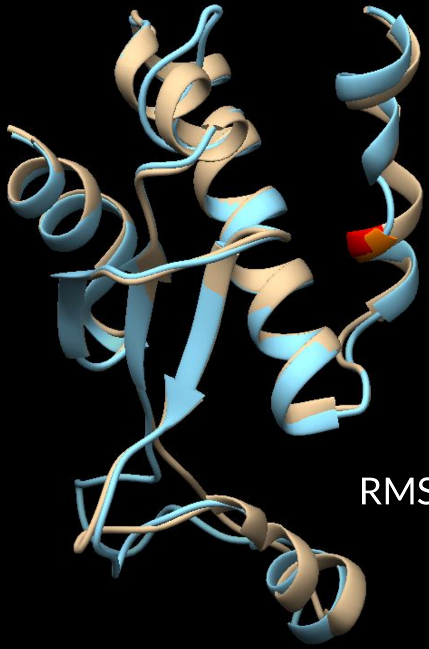
C-terminal Domain



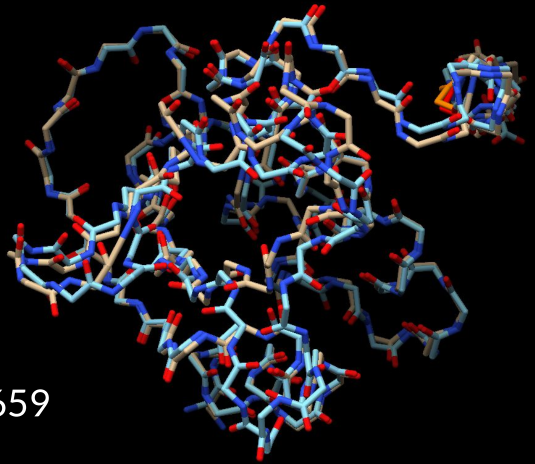
Ala	4																			
Arg	-1	5																		
Asn	-2	0	6																	
Asp	-2	-2	1	6																
Cys	0	-3	-3	-3	9															
Gln	-1	1	0	0	-3	5														
Glu	-1	0	0	2	-4	2	5													
Gly	0	-2	0	-1	-3	-2	-2	6												
His	-2	0	1	-1	-3	0	0	-2	8											
Ile	-1	-3	-3	-3	-1	-3	-3	-4	-3	4										
Leu	-1	-2	-3	-4	-1	-2	-3	-4	-3	2	4									
Lys	-1	2	0	-1	-3	1	1	-2	-1	-3	-2	5								
Met	-1	-1	-2	-3	-1	0	-2	-3	-2	1	2	-1	5							
Phe	-2	-3	-3	-3	-2	-3	-3	-3	-1	0	0	-3	0	6						
Pro	-1	-2	-2	-1	-3	-1	-1	-2	-2	-3	-3	-1	-2	-4	7					
Ser	1	-1	1	0	-1	0	0	0	-1	-2	-2	0	-1	-2	-1	4				
Thr	0	-1	0	-1	-1	-1	-1	-2	-2	-1	-1	-1	-1	-2	-1	1	5			
Trp	-3	-3	-4	-4	-2	-2	-3	-2	-2	-3	-2	-3	-1	1	-4	-3	-2	11		
Tyr	-2	-2	-2	-3	-2	-1	-2	-3	2	-1	-1	-2	-1	3	-3	-2	-2	2	7	
Val	0	-3	-3	-3	-1	-2	-2	-3	-3	3	1	-2	1	-1	-2	-2	0	-3	-1	4
	Ala	**Arg**	**Asn**	**Asp**	**Cys**	**Gln**	**Glu**	**Gly**	**His**	**Ile**	**Leu**	**Lys**	**Met**	**Phe**	**Pro**	**Ser**	**Thr**	**Trp**	**Tyr**	**Val**

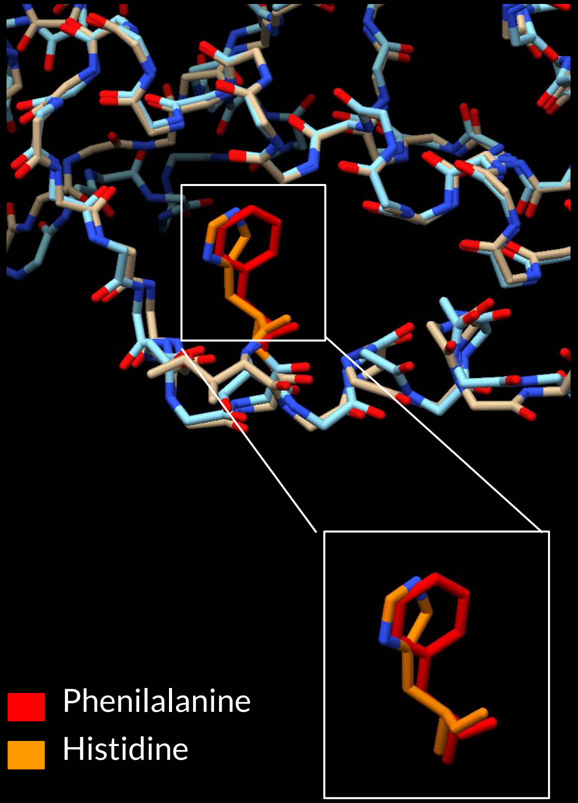
HtpG E.coli

C-terminal Domain



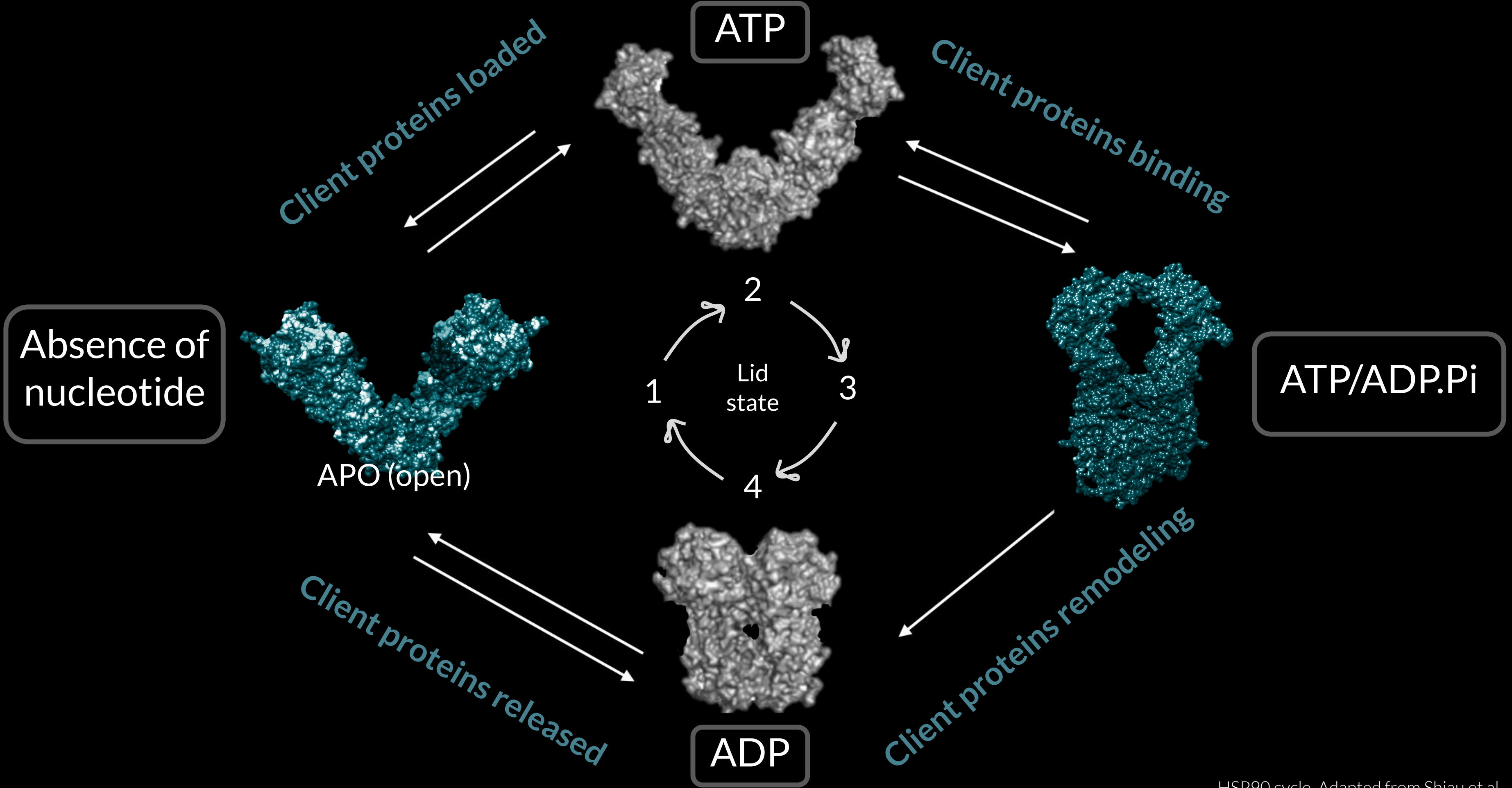
RMSD: 0.659





E.coli HtpG + human Hsp90 model

HSP90
CYCLE



ATP

Client proteins loaded

Client proteins binding

Absence of nucleotide

APO (open)

2

1

Lid state

3

4

ATP/ADP.Pi

Client proteins released

Client proteins remodeling

ADP

HSP90 cycle. Adapted from Shiau et al.

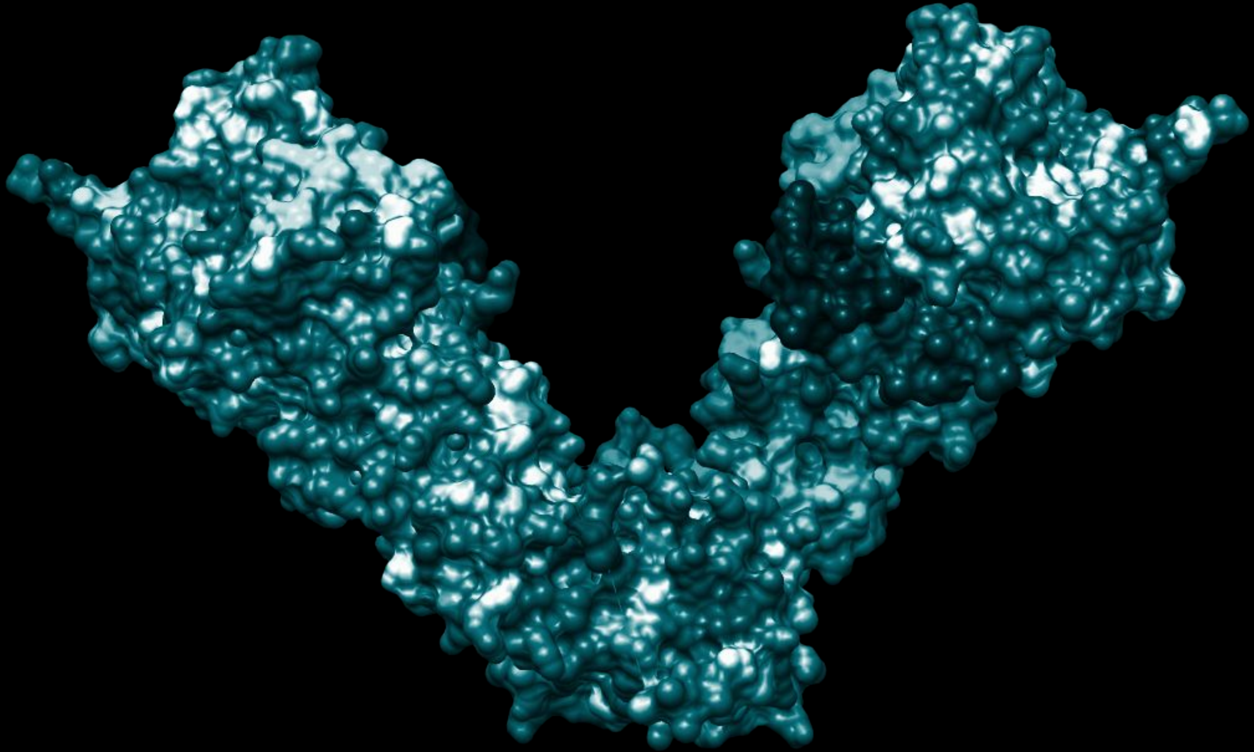
1. APO (open) STATE

Absence of nucleotide

Lid state 1

Hydrophobic central cleft

Client proteins loading



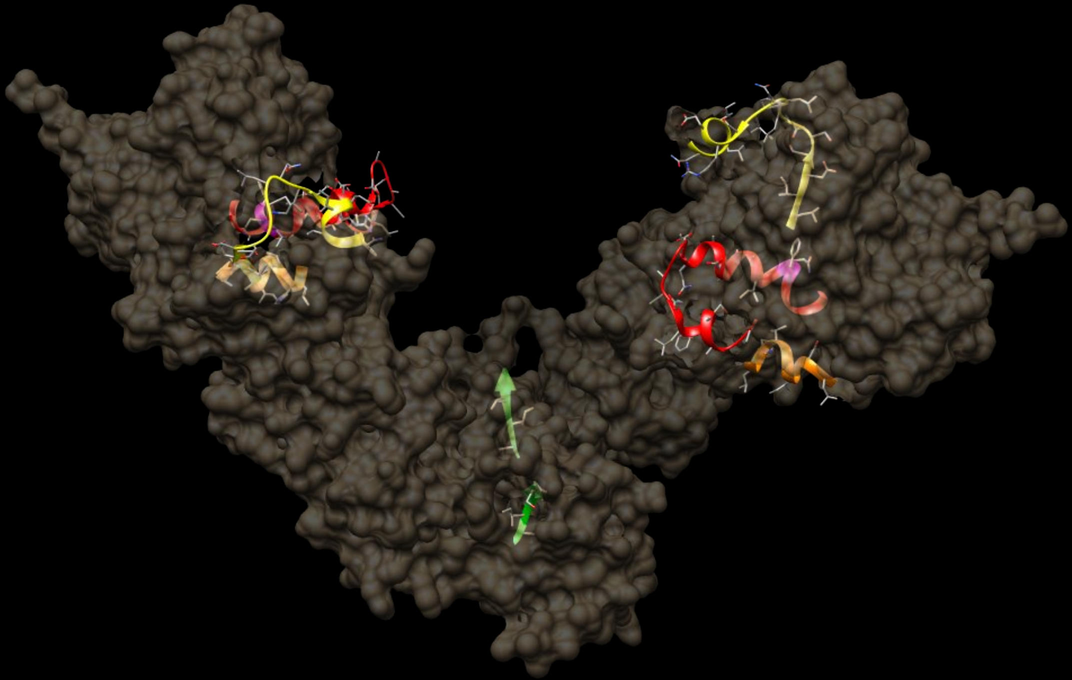
1. **APO STATE:** central cleft

Client proteins loading-binding

Large central cleft lined by **hydrophobic** elements from each of the **three domains**:

Client-protein binding
- **active-site lid**
- **helix H1**

Minimal MD/CTD interface:
- **MD src loop**
- **CTD helix 21**



side view

1. **APO STATE:** central cleft

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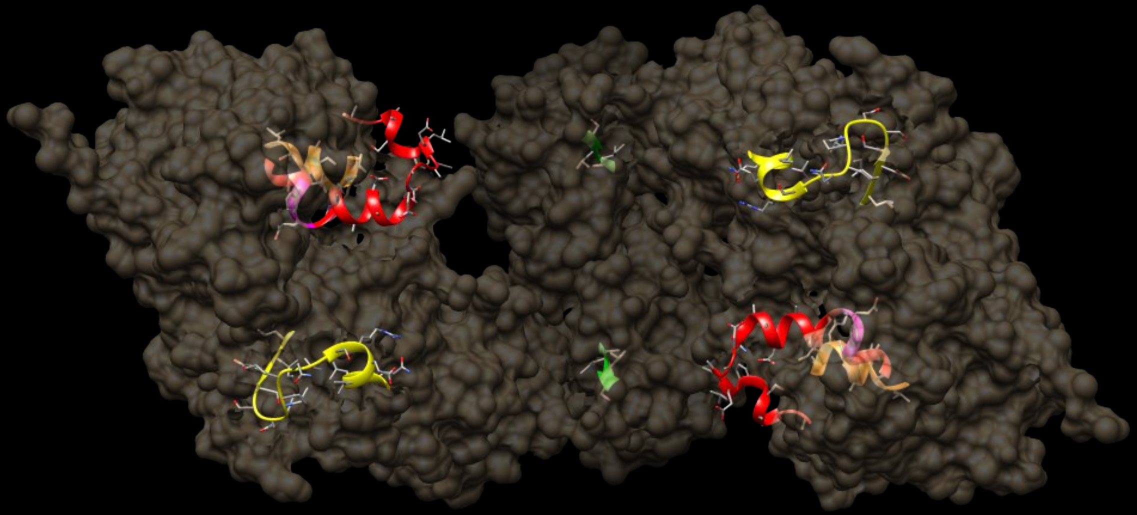
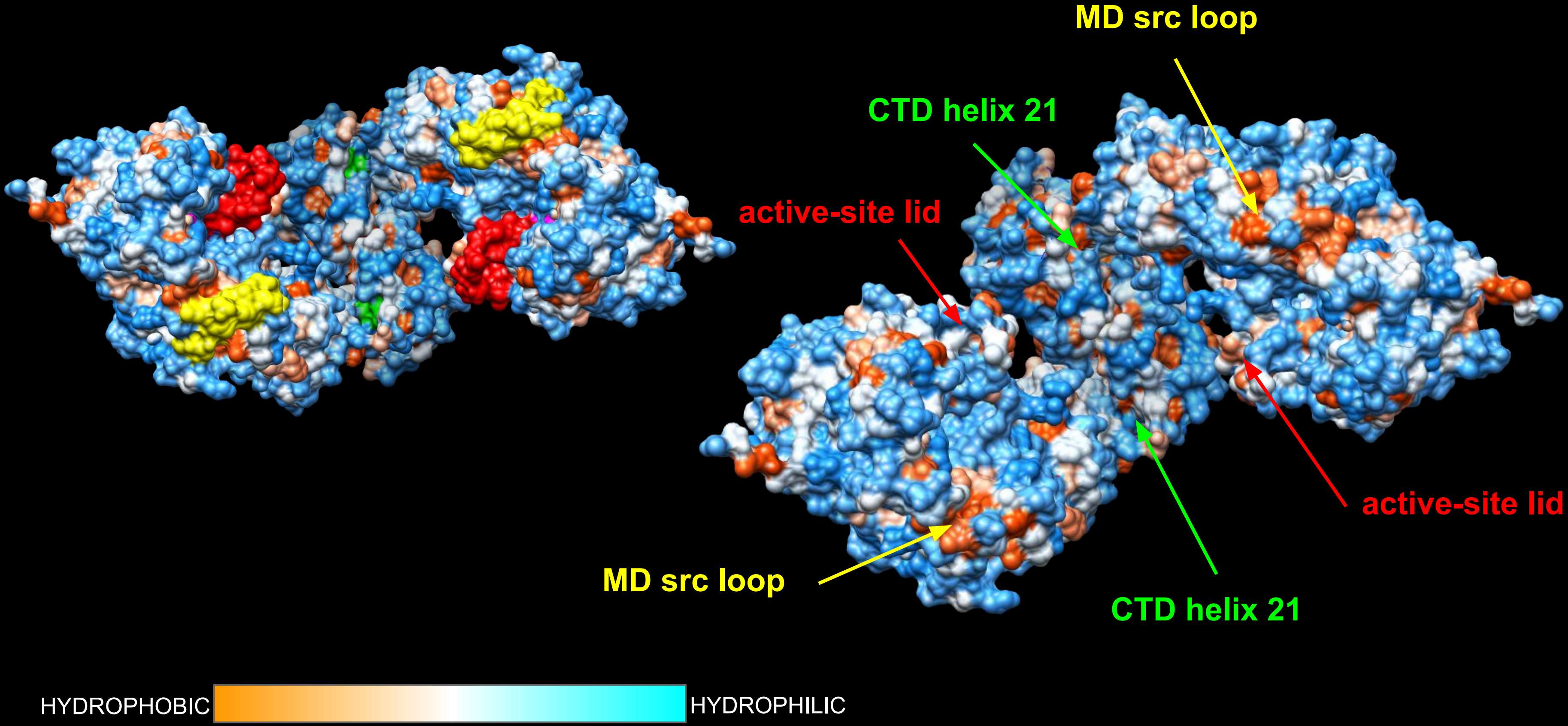
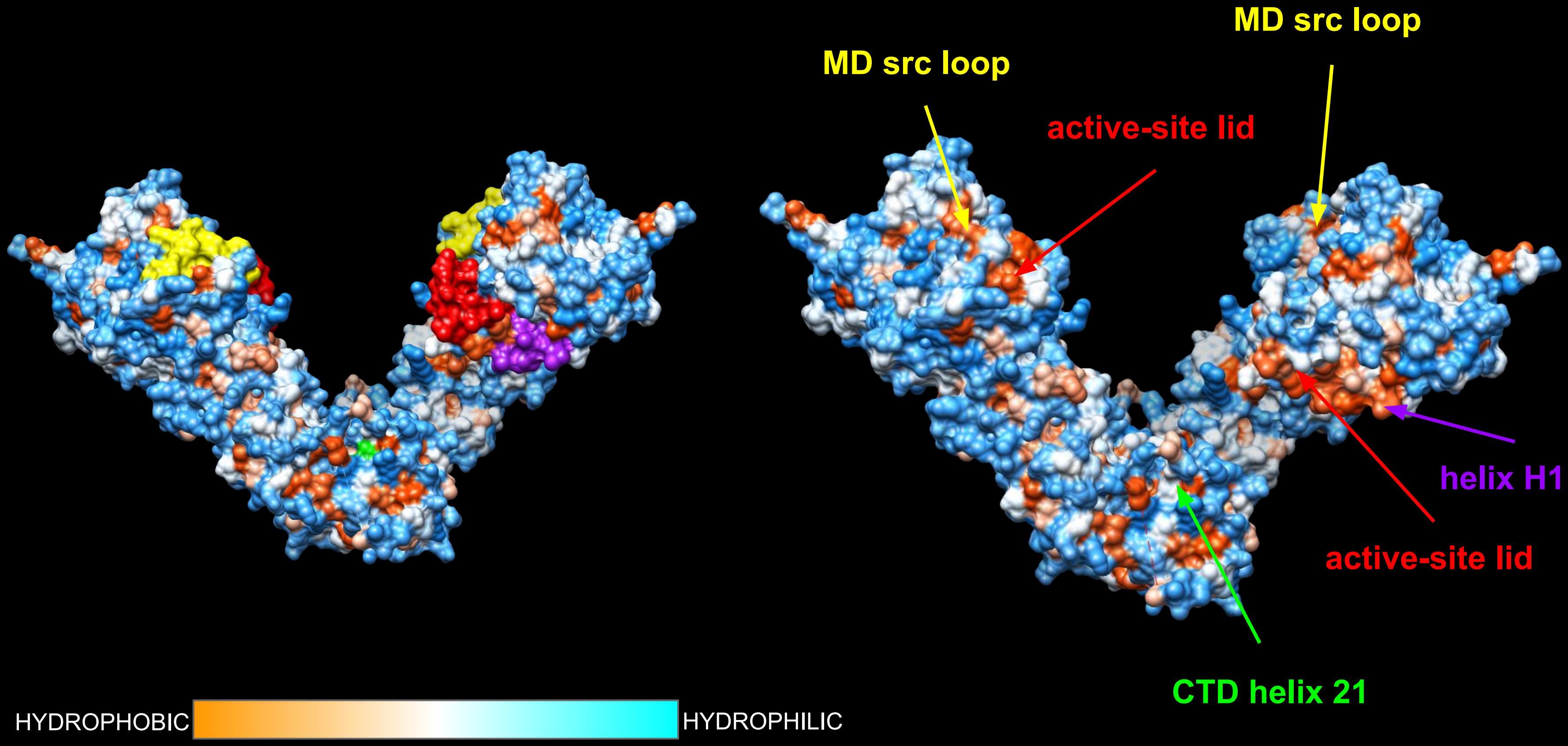


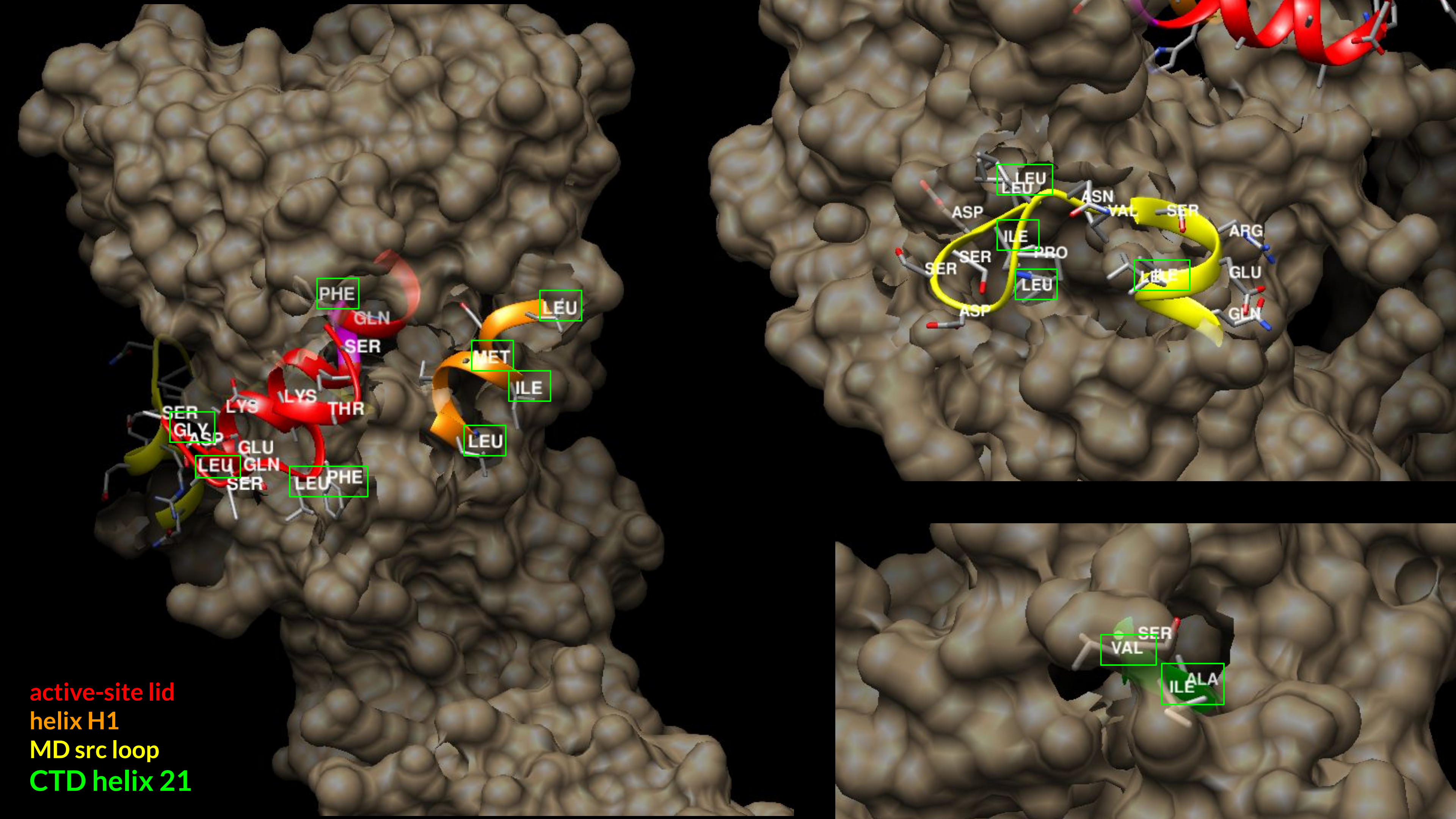
top view

1. **APO STATE:** central cleft



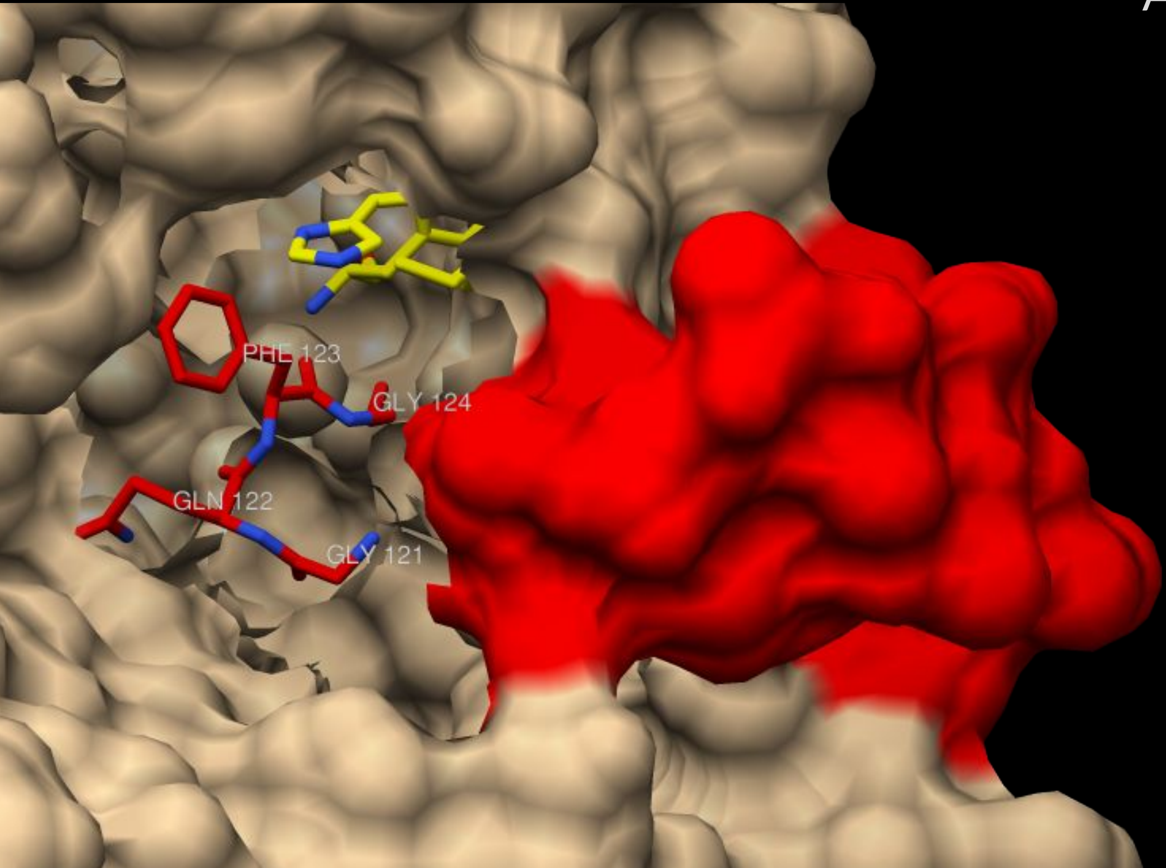
1. **APO STATE:** central cleft





active-site lid
helix H1
MD src loop
CTD helix 21

1. **APO STATE:** active lid



Active-site lid is **disordered** and not **visible**

↓

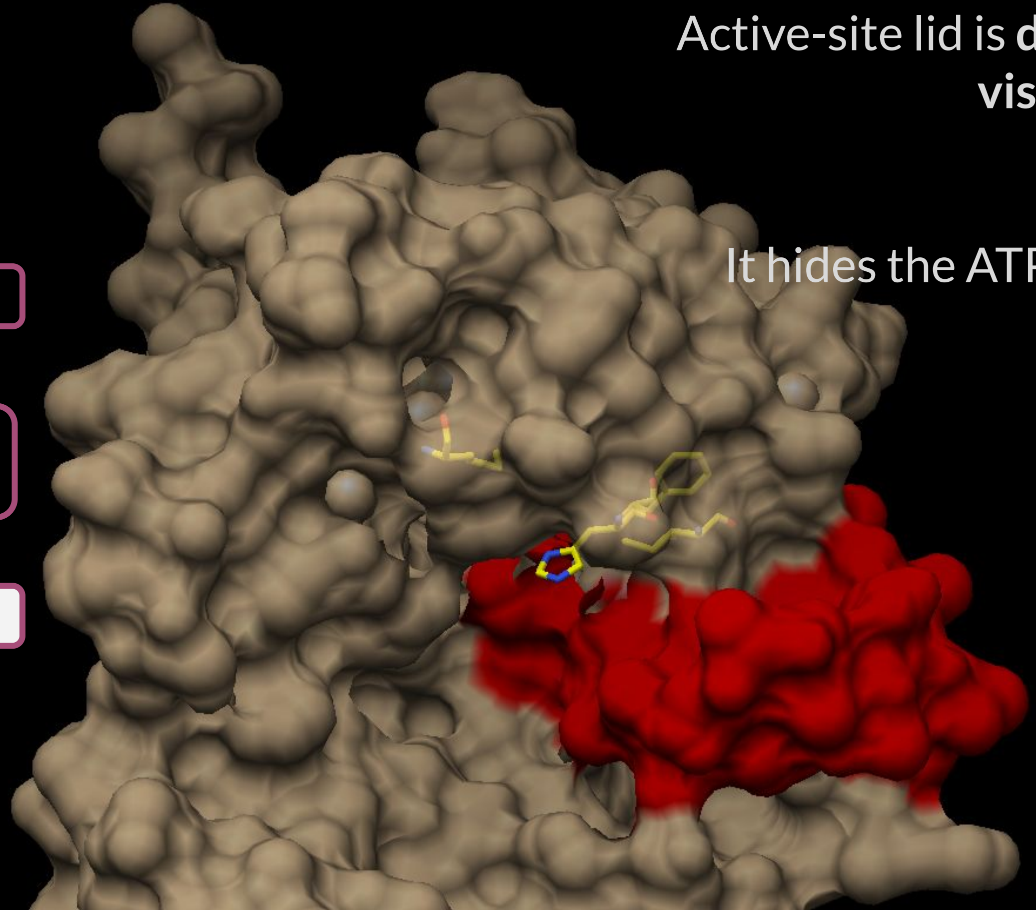
It hides the ATP binding pocket

Residues 121–124 from the lid cross the region occupied by the a and ß phosphates of the nucleotide

Active -site lid

ATP binding pocket

1. **APO STATE:** active lid



Active-site lid is **disordered** and not **visible**

↓

It hides the ATP binding pocket

Client binding

↓

Lid disordering
NTD/MD reorientation

↓

Nucleotide binding

Active -site lid

ATP binding pocket

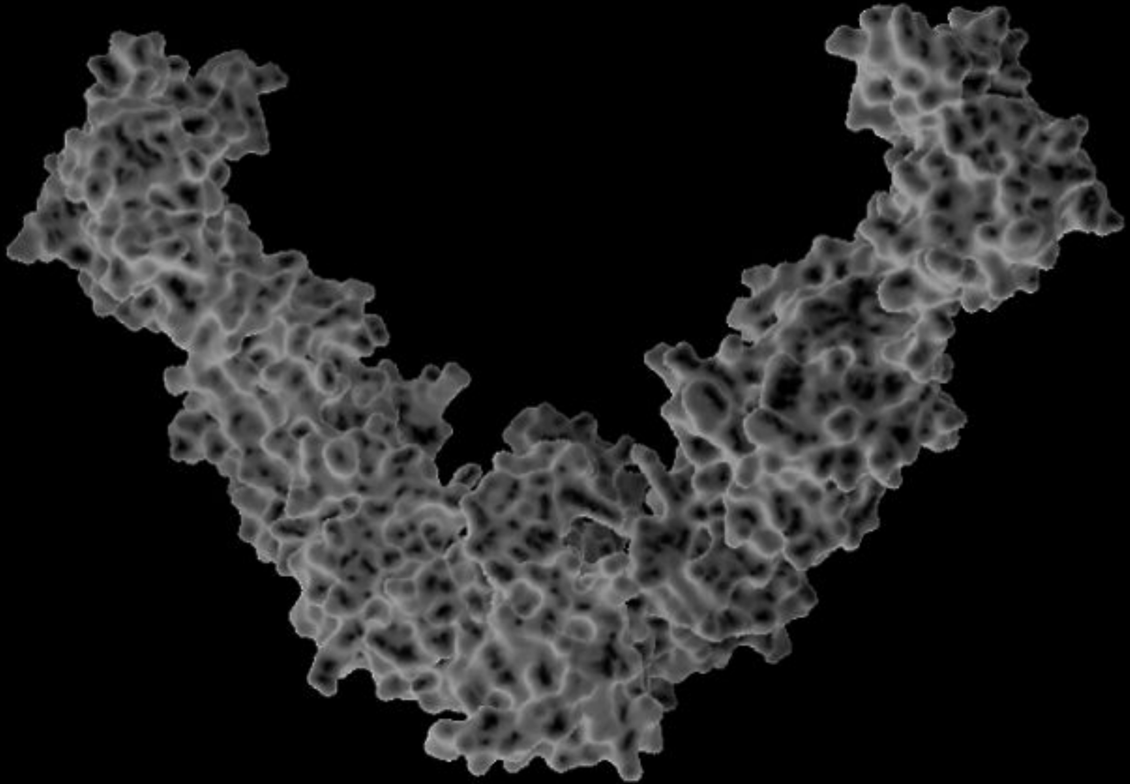
2. ATP STATE

Client proteins binding

Lid state 2

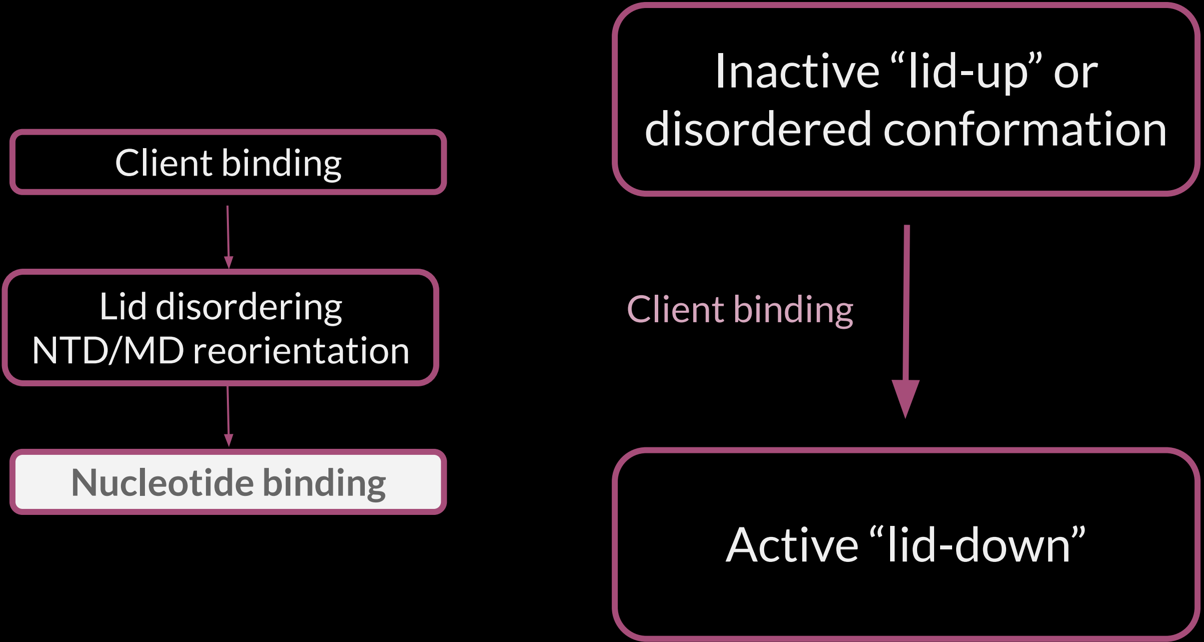
Lid rearrangement

ATP binding



Adapted from Shiau et al. 2006

2. ATP STATE: lid rearrangement



Client binding

Lid disordering
NTD/MD reorientation

Nucleotide binding

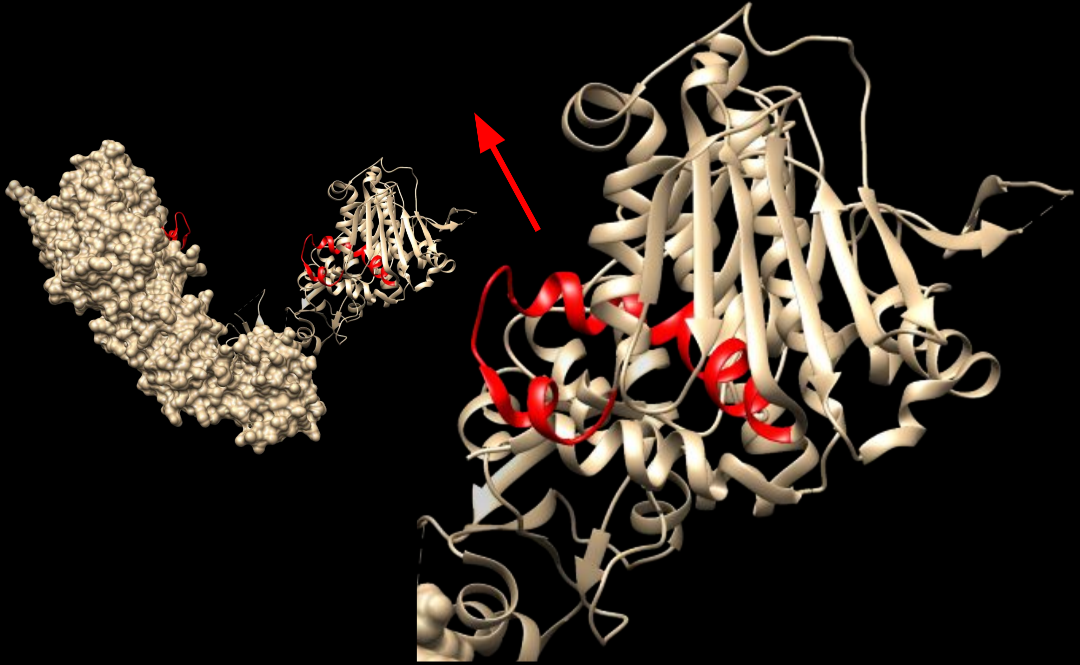
Inactive “lid-up” or disordered conformation

Client binding

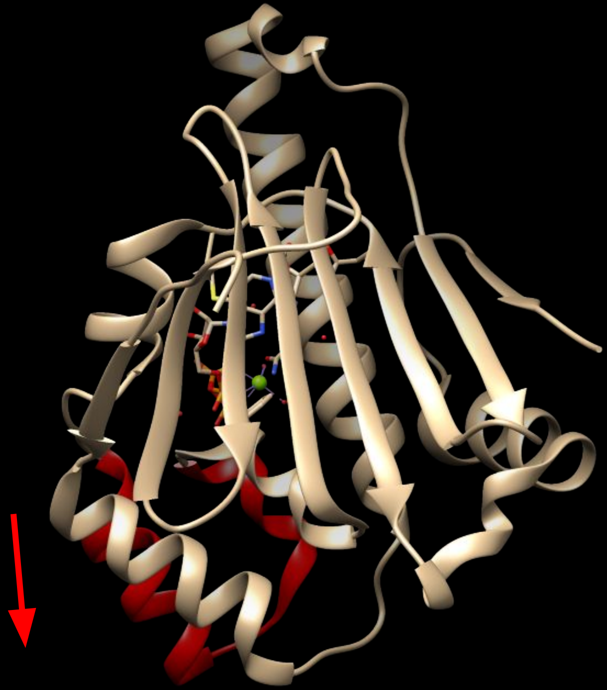
Active “lid-down”

2. ATP STATE: lid rearrangement

Lid-up → Lid-down



Apo full length



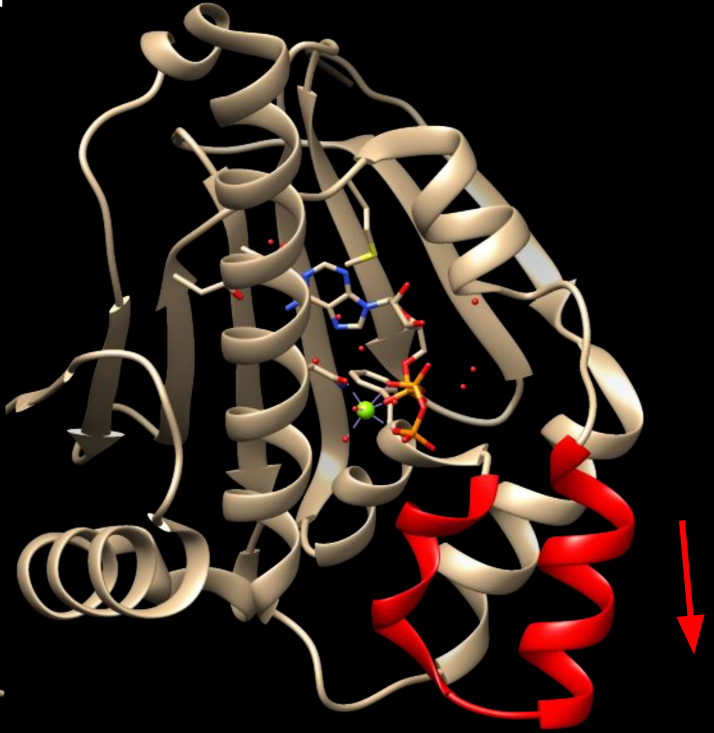
NTD isolated with ATP

Lid

2. ATP STATE: lid rearrangement

Lid-up → Lid-down

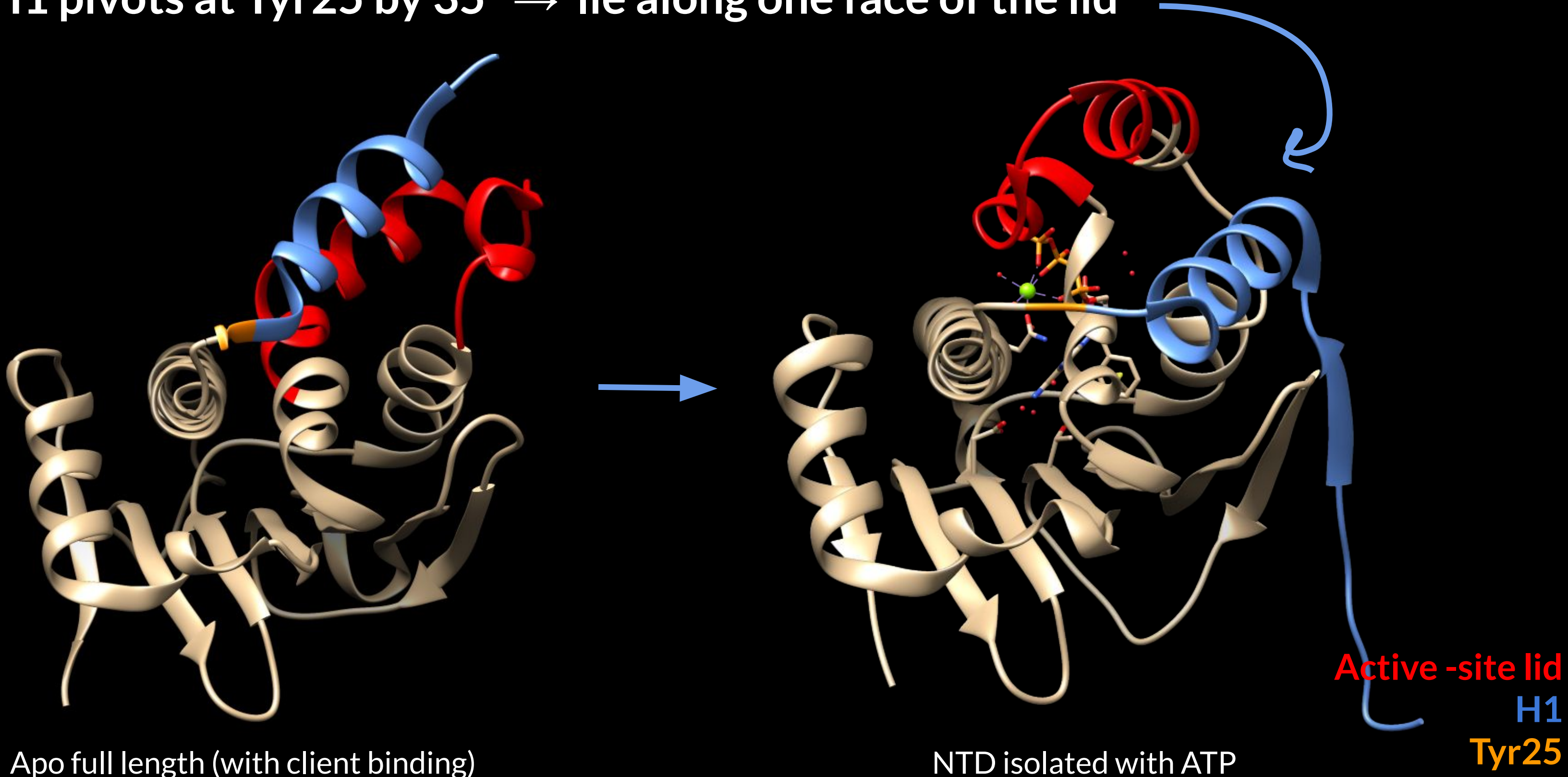
Apo full length



NTD isolated with ATP

2. ATP STATE: lid rearrangement

1. Helix H1 pivots at Tyr25 by 35° → lie along one face of the lid



Apo full length (with client binding)

NTD isolated with ATP

2. ATP STATE: lid rearrangement

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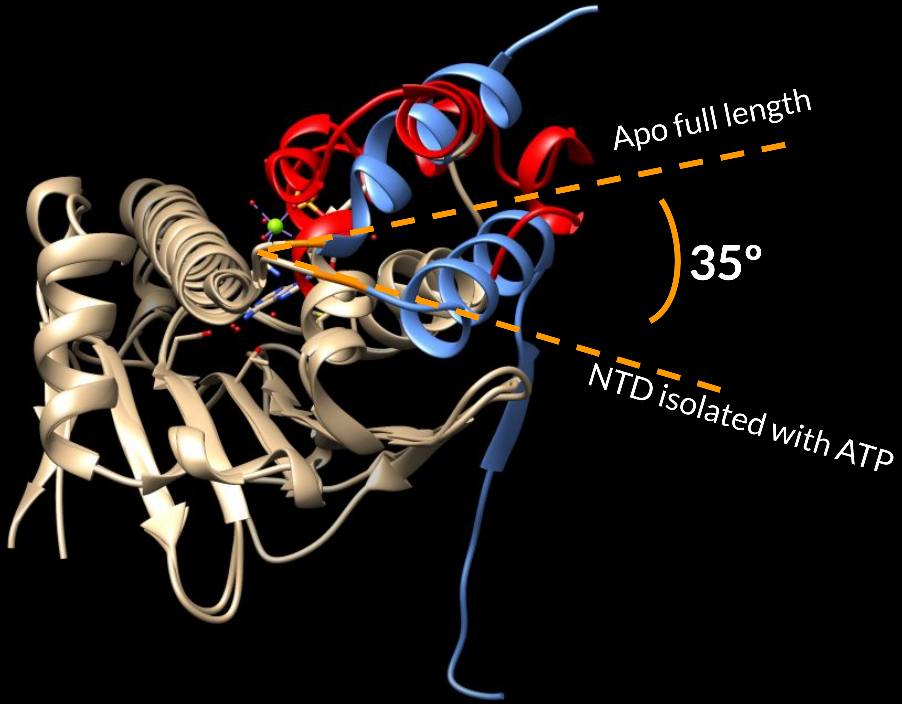
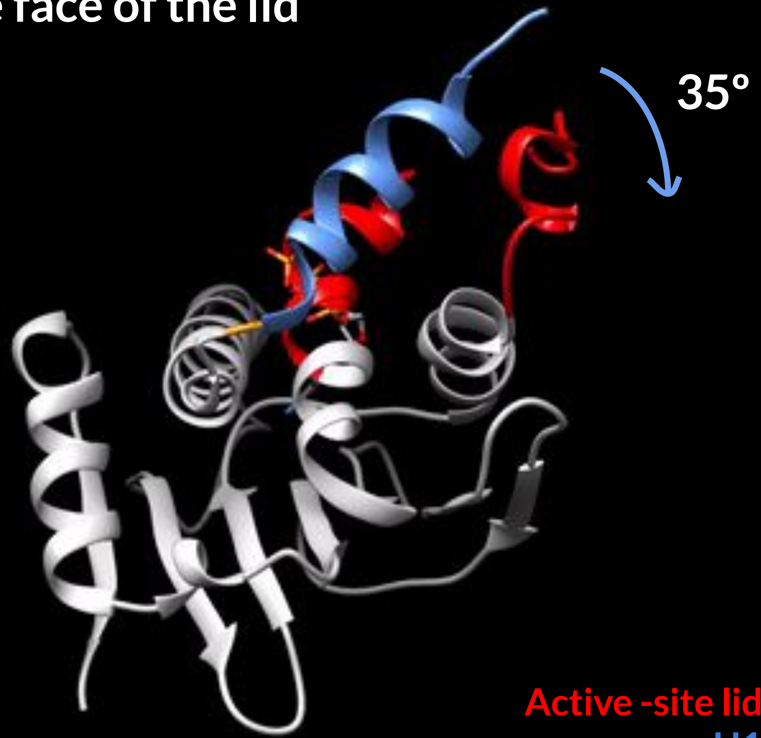


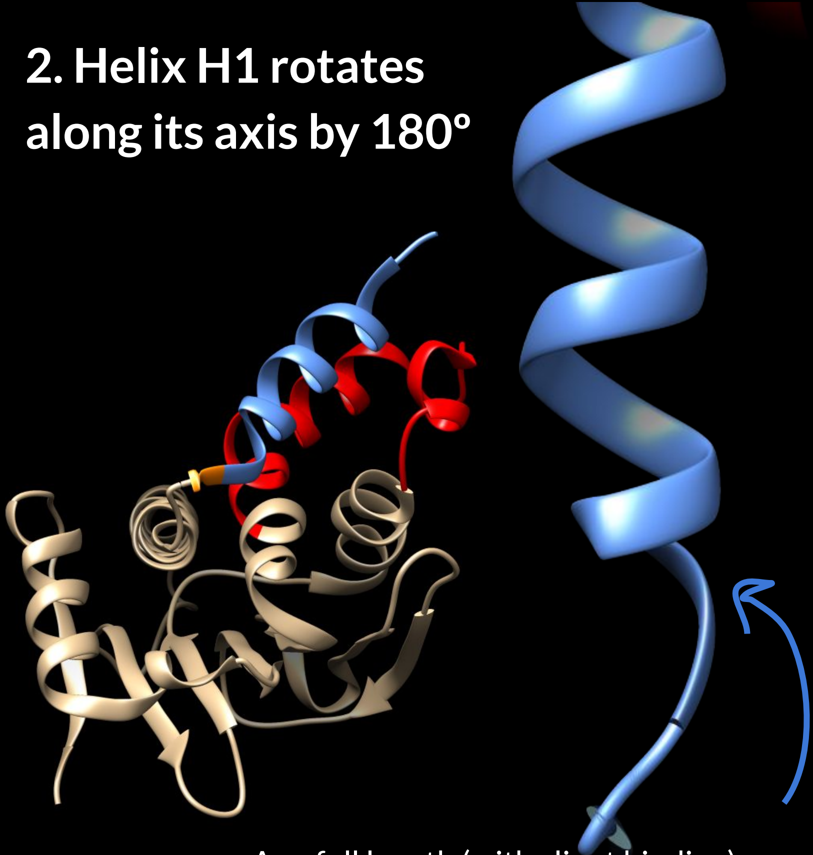


Morphological changes modelled with Chimera

Active -site lid
H1
Tyr25

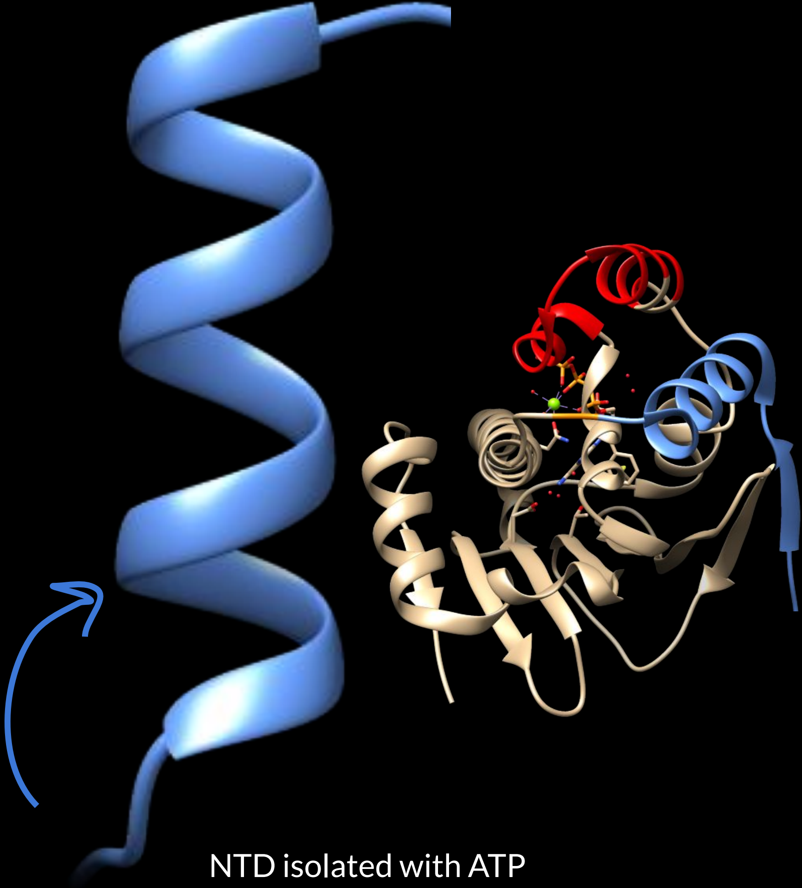
2. ATP STATE: lid rearrangement

2. Helix H1 rotates along its axis by 180°



Apo full length (with client binding)

180° rotation →

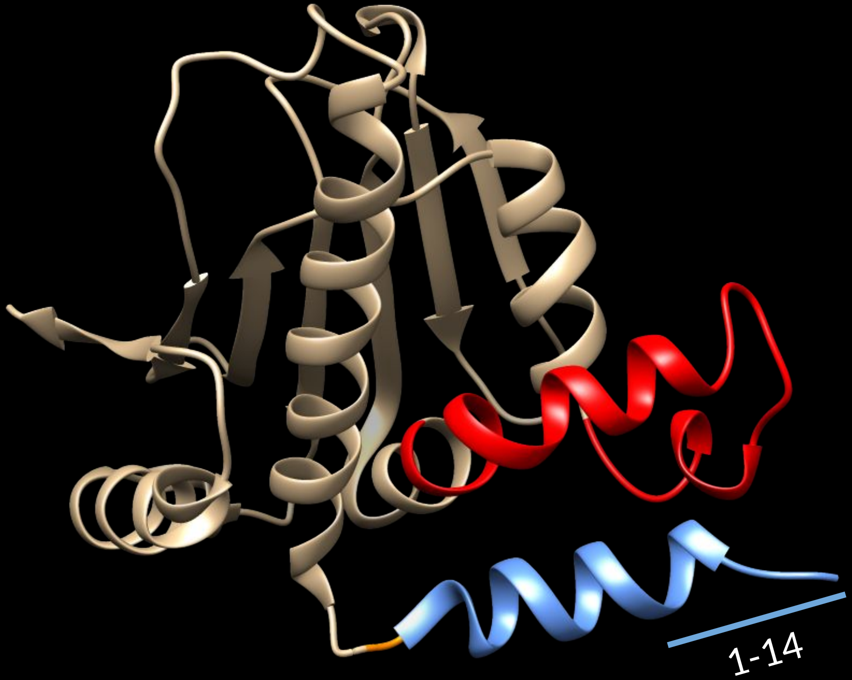


NTD isolated with ATP

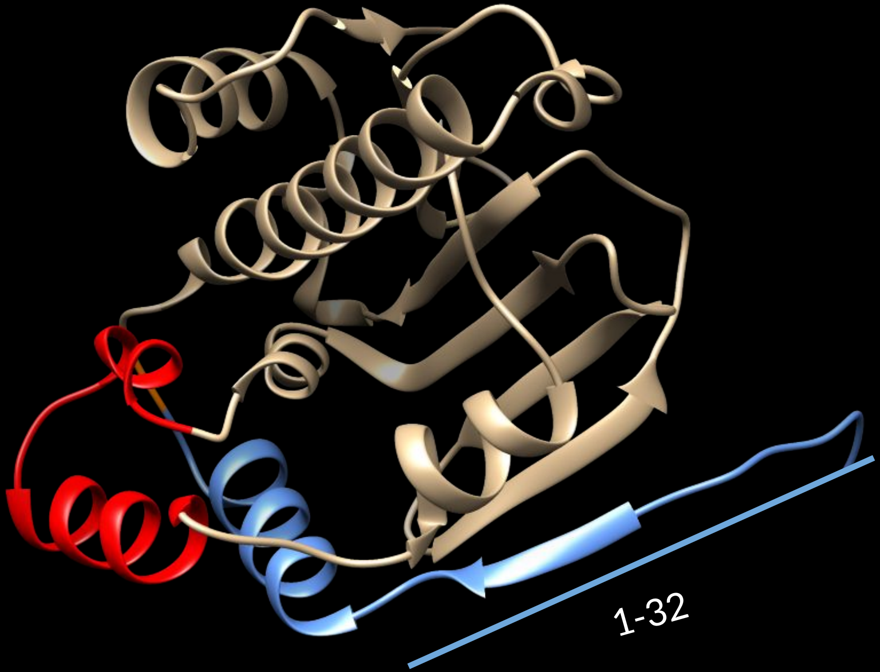
2. ATP STATE: lid rearrangement

3. Residues 1-14 (1-32 in *H. sapiens*) are disordered

Active -site lid
H1
Tyr25



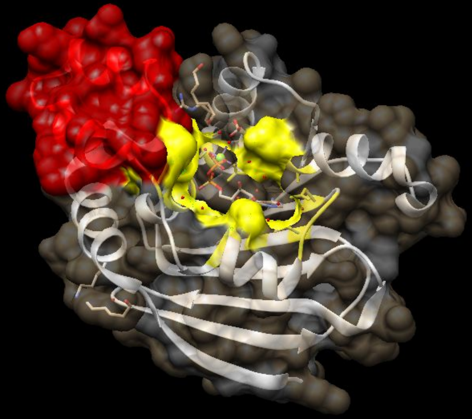
Apo full length (with client binding)

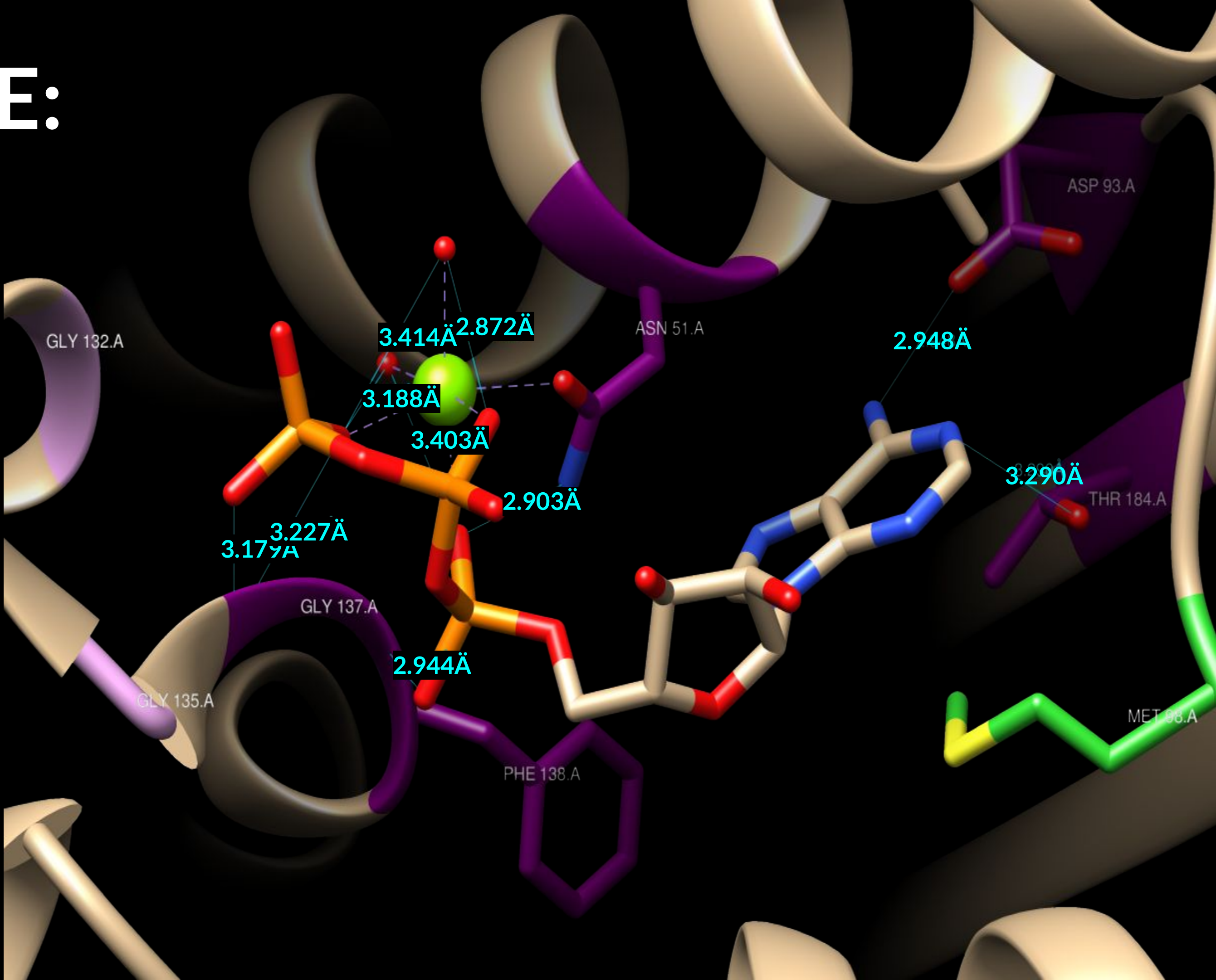


NTD isolated with ATP

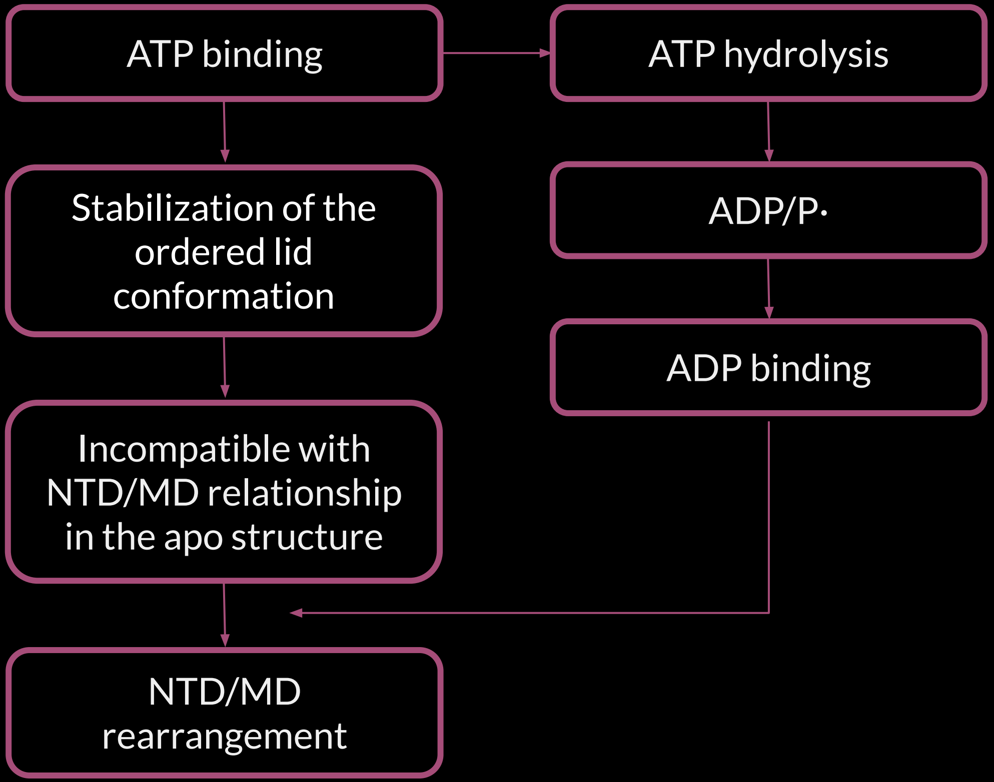
2. ATP STATE:

ATP binding





2. **ATP STATE:** ATP binding



ATP binding

ATP hydrolysis

Stabilization of the ordered lid conformation

ADP/P·

ADP binding

Incompatible with NTD/MD relationship in the apo structure

NTD/MD rearrangement

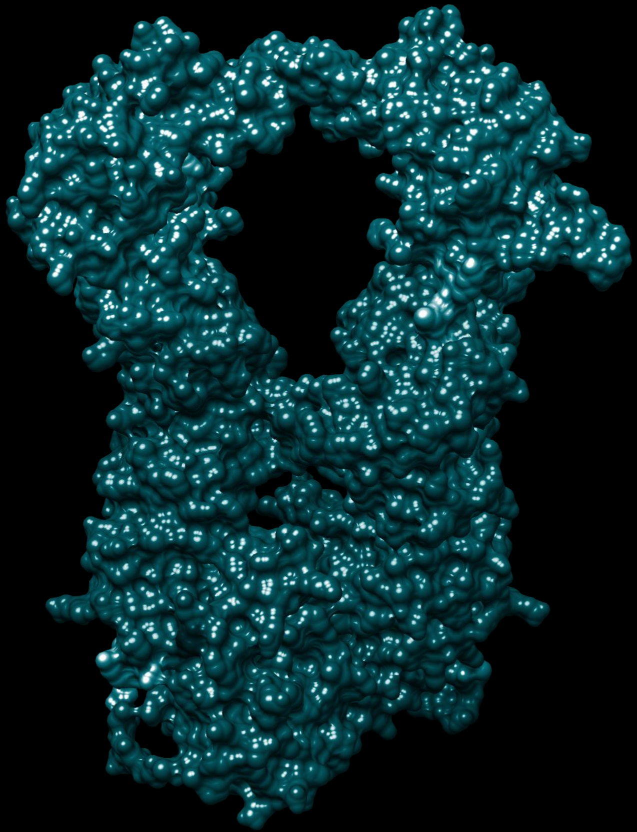
3. ATP/ADP·P_i STATE

Lid state 3

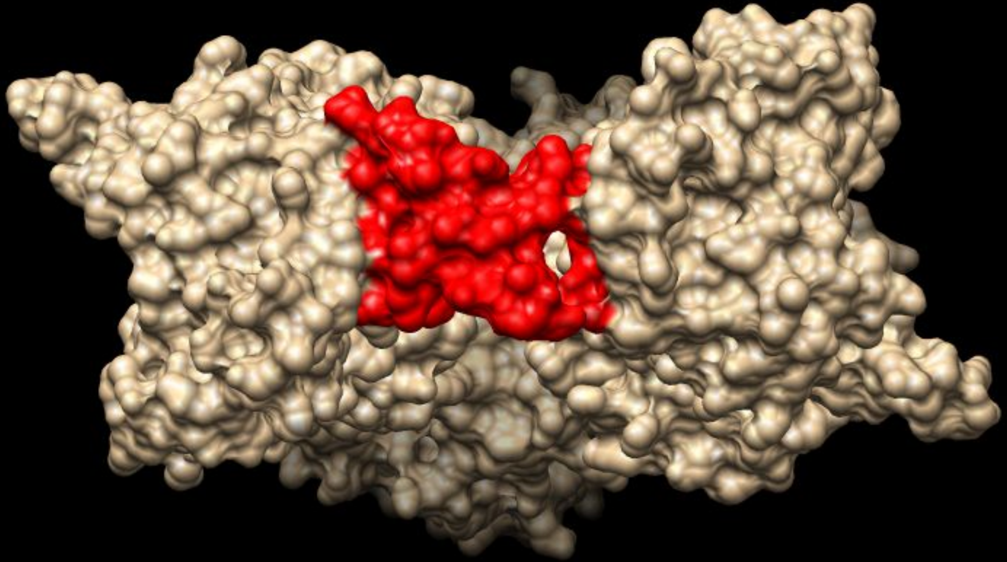
NTD dimerization

ATP hydrolysis and ADP binding

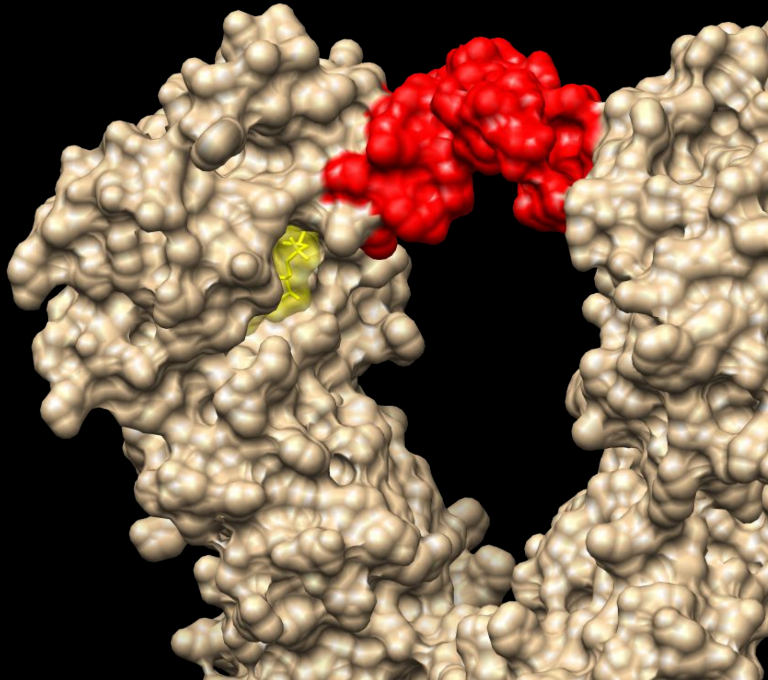
Client protein remodeling



3. ATP/ADP·P_i STATE



The lid covers the bound nucleotide

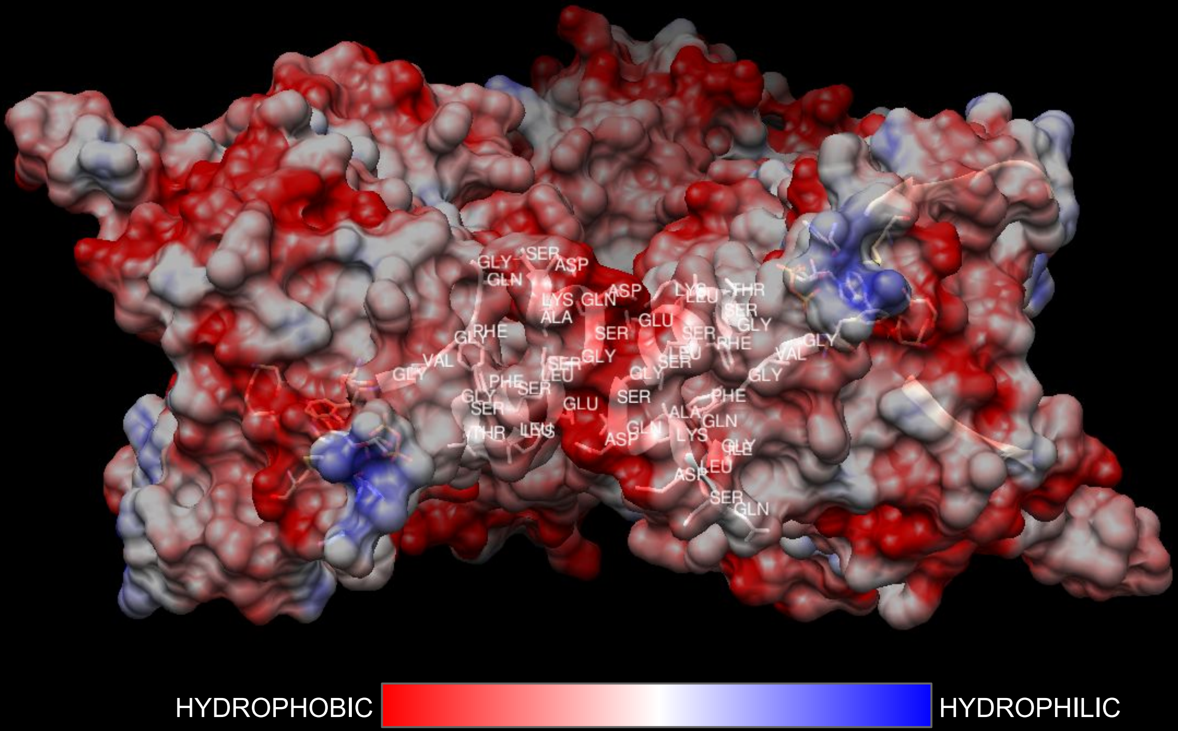


3. ATP/ADP·P_i STATE: NTD dimer

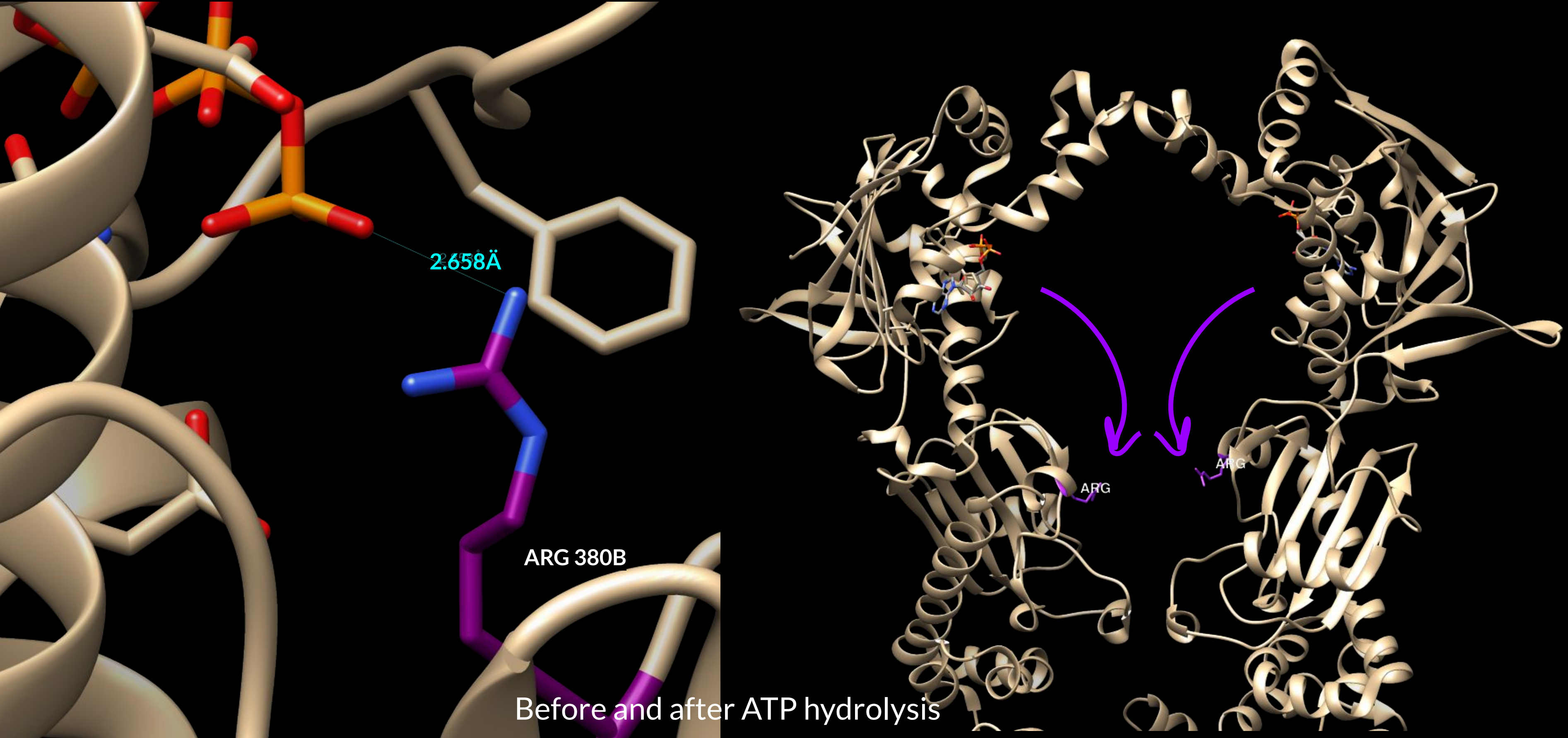
ATP-mediated interprotomer closing → well drive client-protein remodeling

Exposition of the hydrophobic face of the lid

Stabilization of the **NTD dimerization** by **N-ter residues**



3. ATP/ADP·P_i STATE: ATP hydrolysis



Before and after ATP hydrolysis

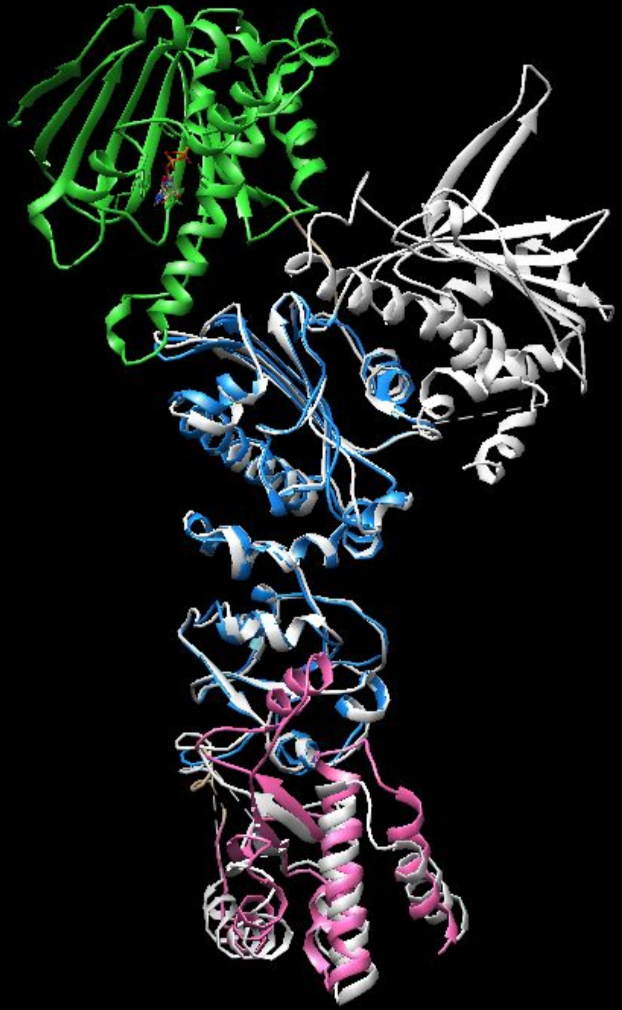
3. ATP/ADP·P_i STATE: Rearrangement

Hydrolysis occurs and ArgMD interaction is broken

Based on superimposition:

- **CTD is unaffected**
- **MD pivotes 15° away**
- **NTD rotates 127° → nucleotide binding pocket exposition**

NTD and MD of each monomer are closer to each other



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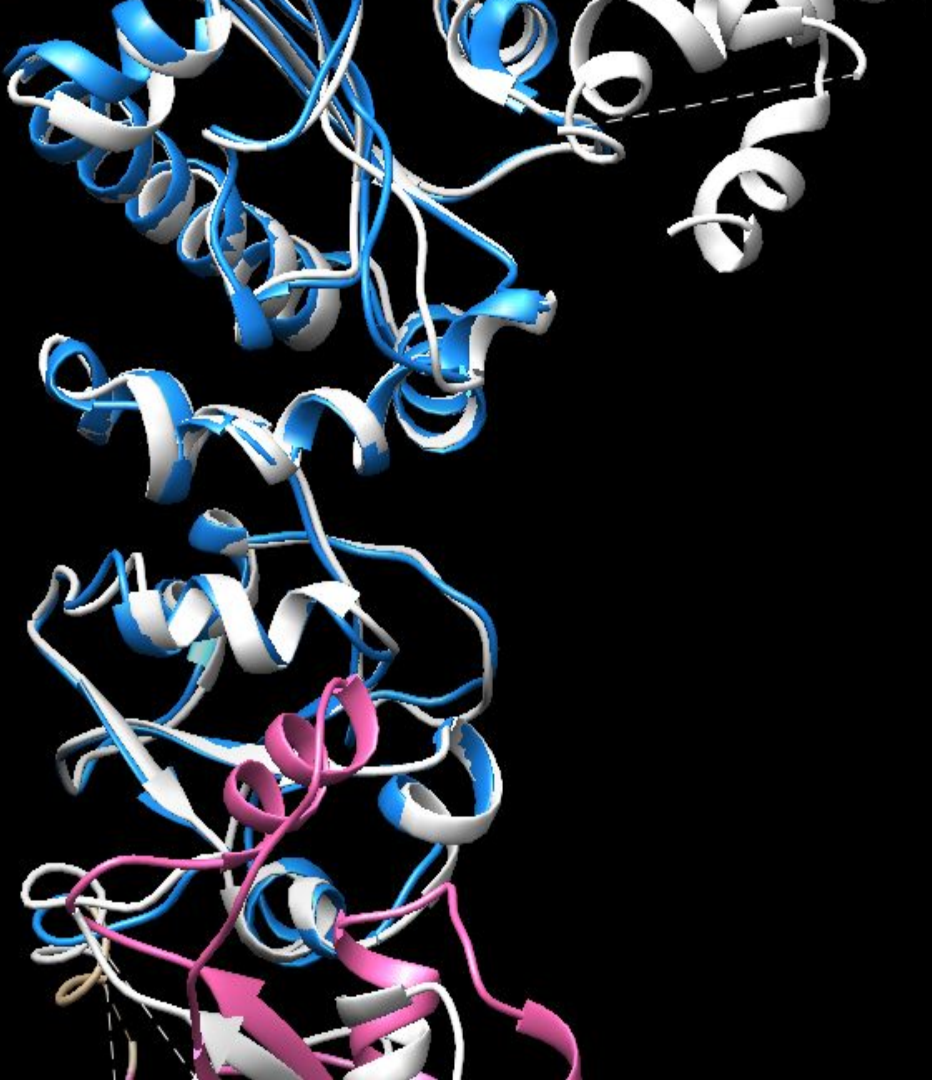

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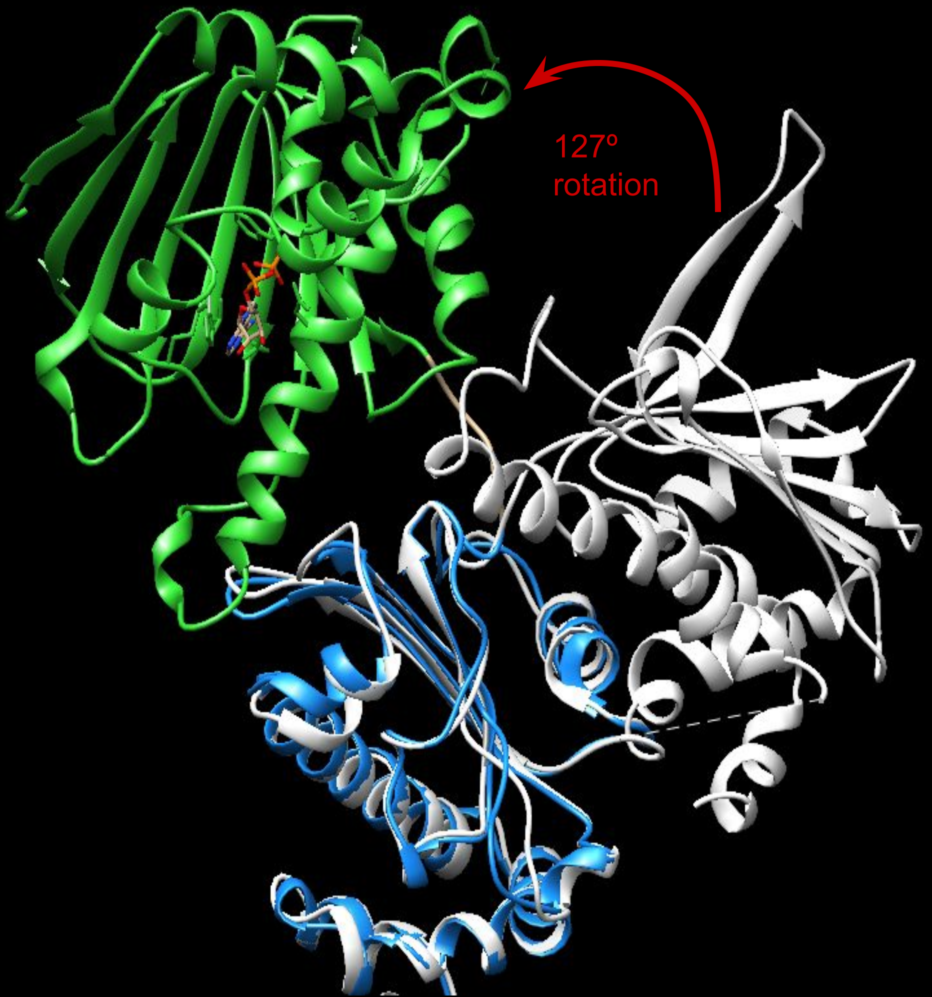

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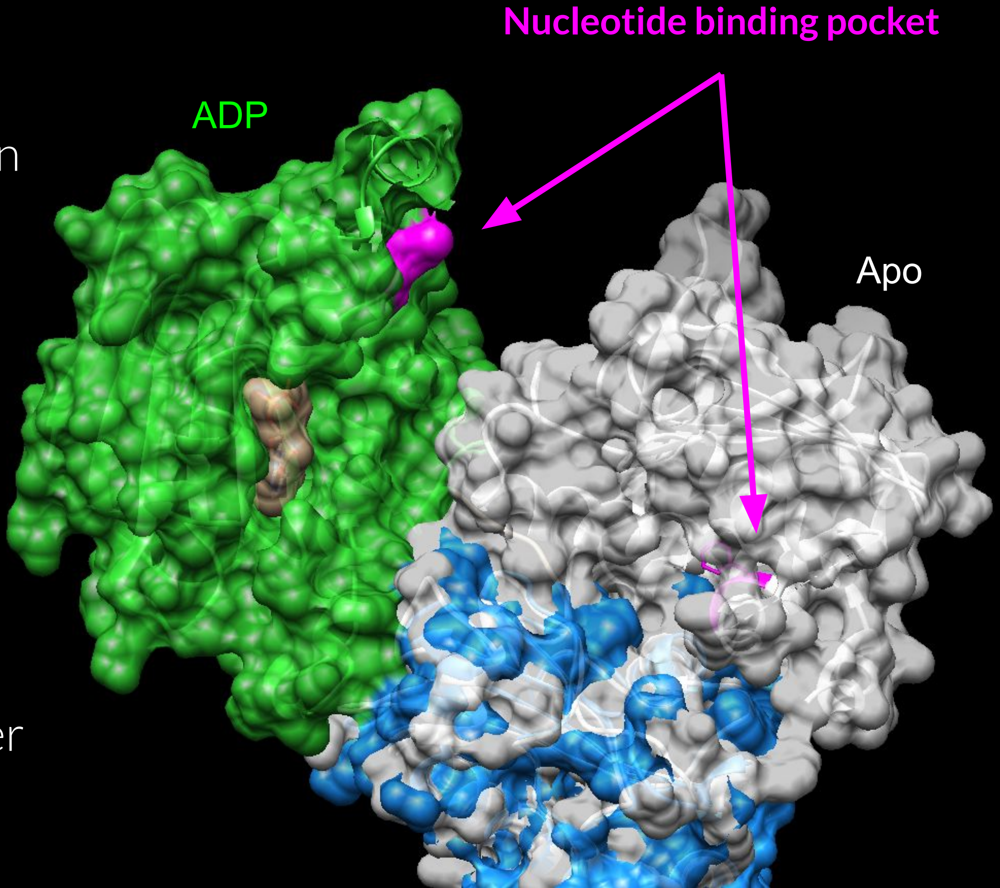
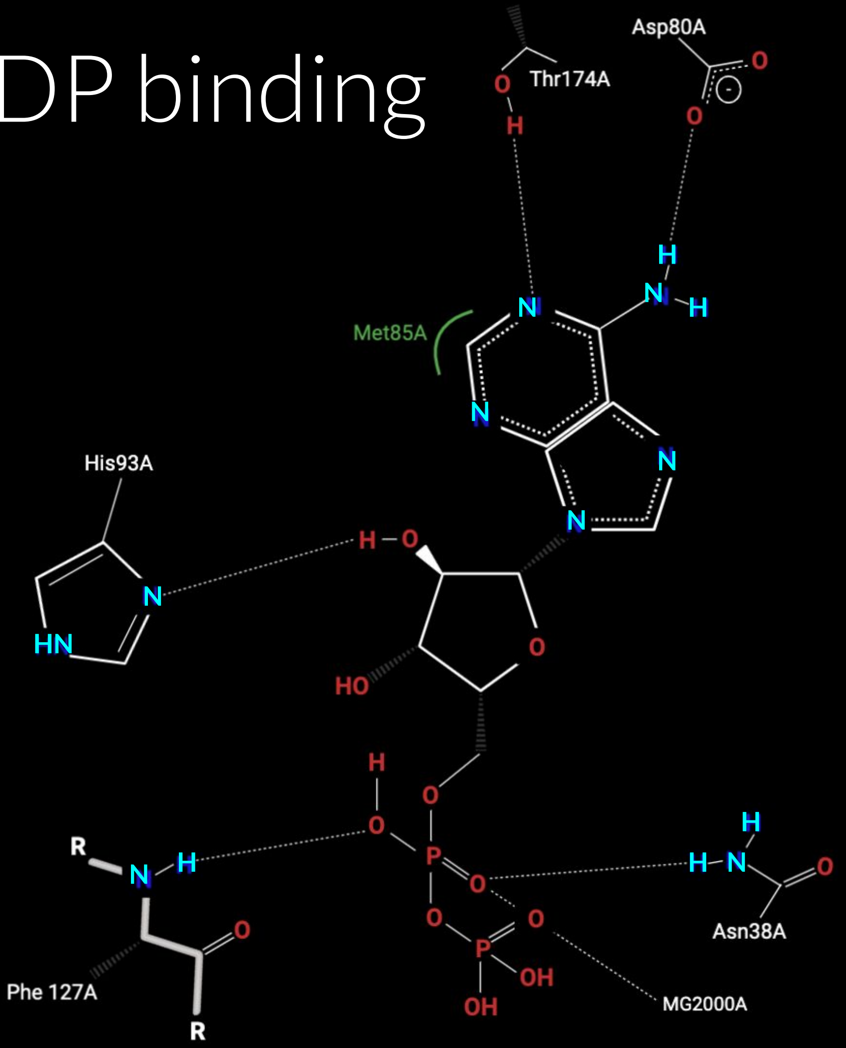
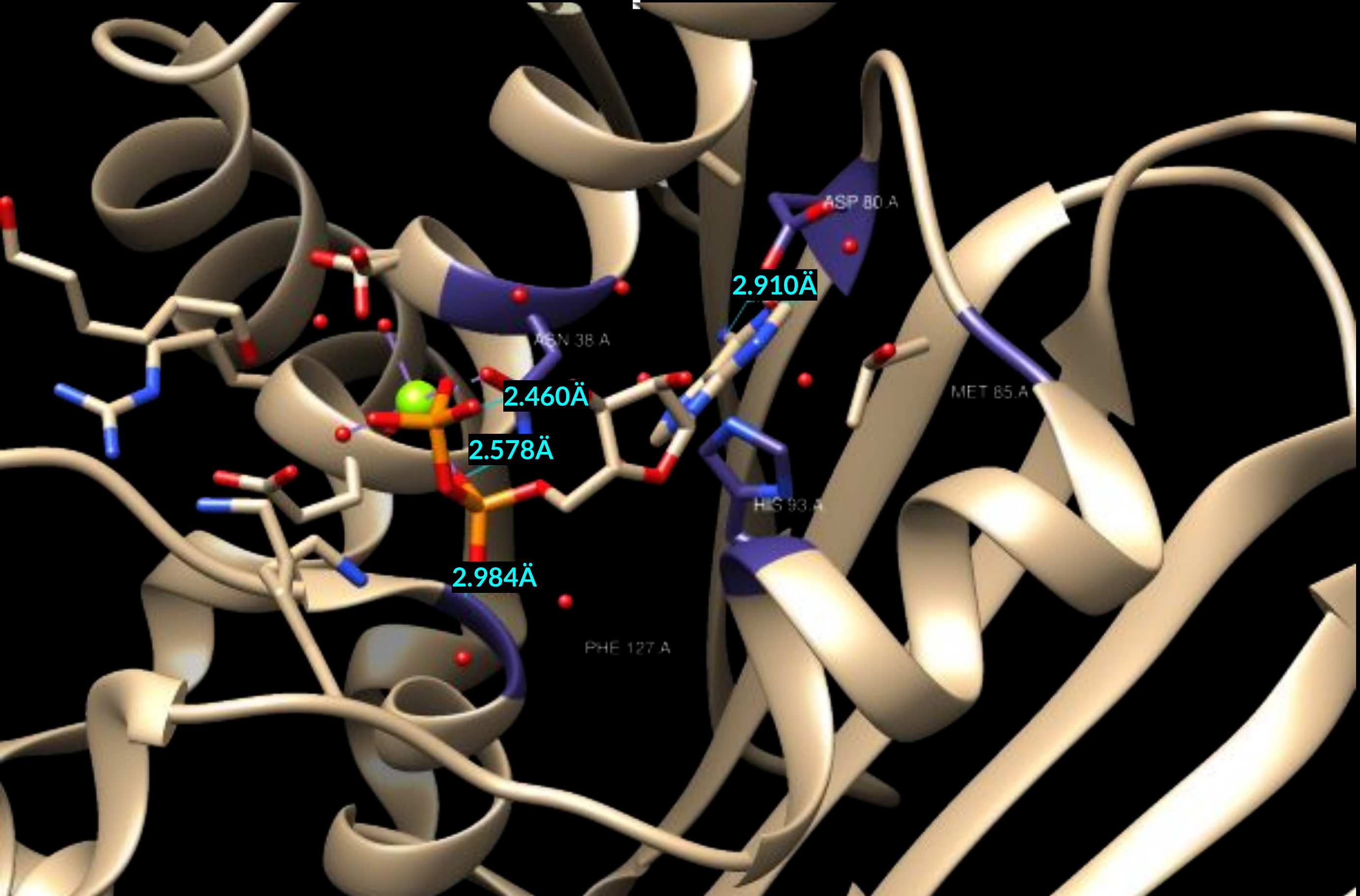


3. **ATP/ADP·P_i STATE:** ADP binding



Bank R. RCSB PDB: Homepage [Internet]. Rcsb.org. 2020 [cited 29 February 2020]. Available from: https://www.rcsb.org/

3. ATP/ADP·P$_i$ STATE: ADP binding

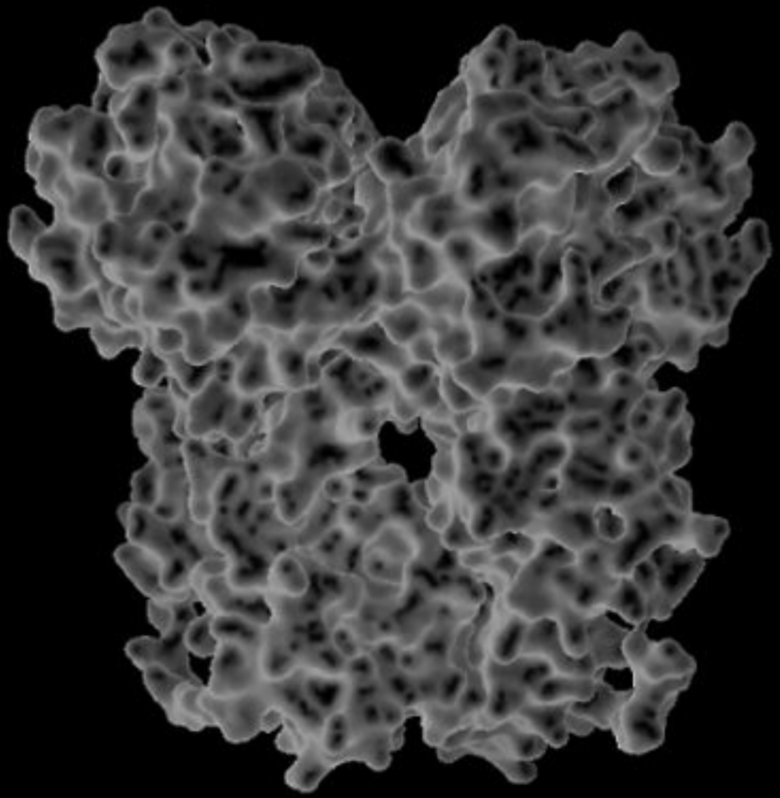


4. ADP STATE

Lid rearrangement

Lid state 4

Client proteins release



Adapted from Shiau et al. 2006

4. ADP STATE: lid rearrangement

Lid state 4:

- "Gasket" to help seal the **NTD** against the **MD,** stabilizing the very compact configuration
- Collapsed hydrophobic core, formed by the interdigitation of the lid, the src loop and the **CTD** H21, mutually masking their otherwise exposed hydrophobic surfaces

→ **Client proteins release**

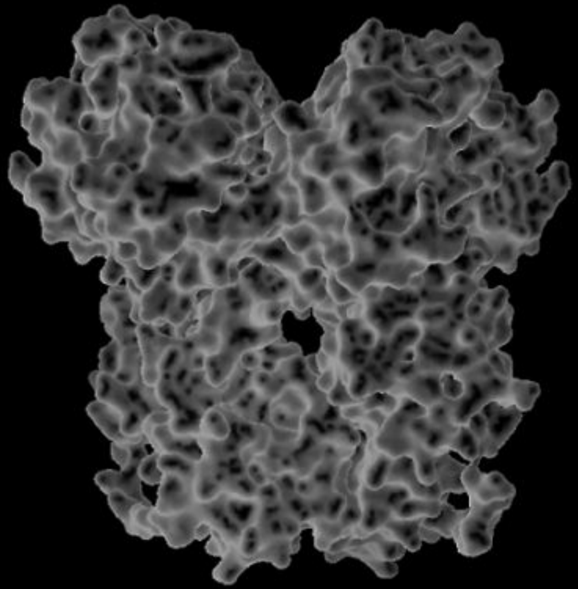
Adapted from Shiau et al. 2006

4. ADP STATE

The client proteins released from hsp90

The nucleotides dissociate

Cleft open → new round of client-protein binding



Adapted from Shiau et al. 2006

Conformational Changes in Hsp90

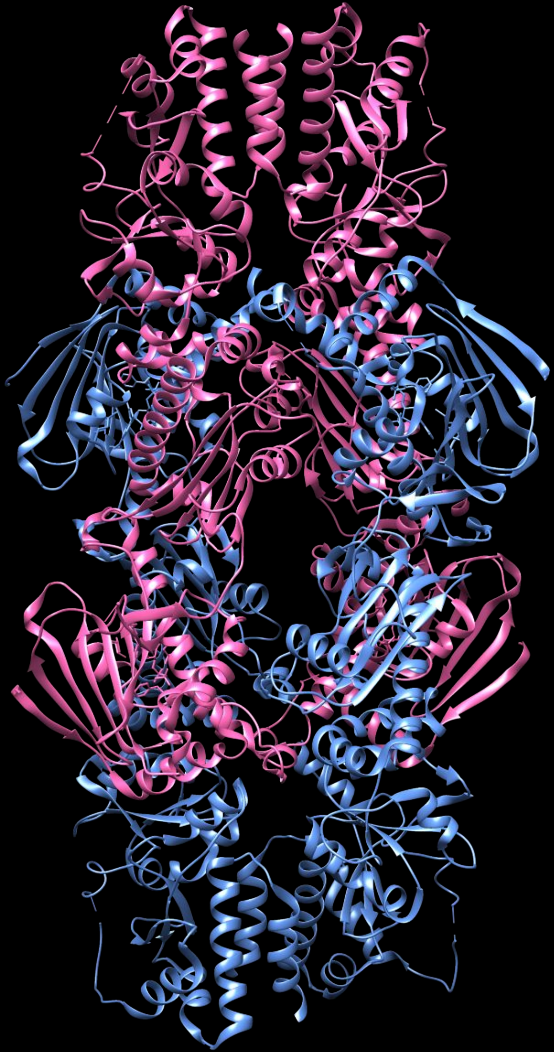
Shiau et al. 2006

DIMER OF DIMERS

DIMER OF DIMERS

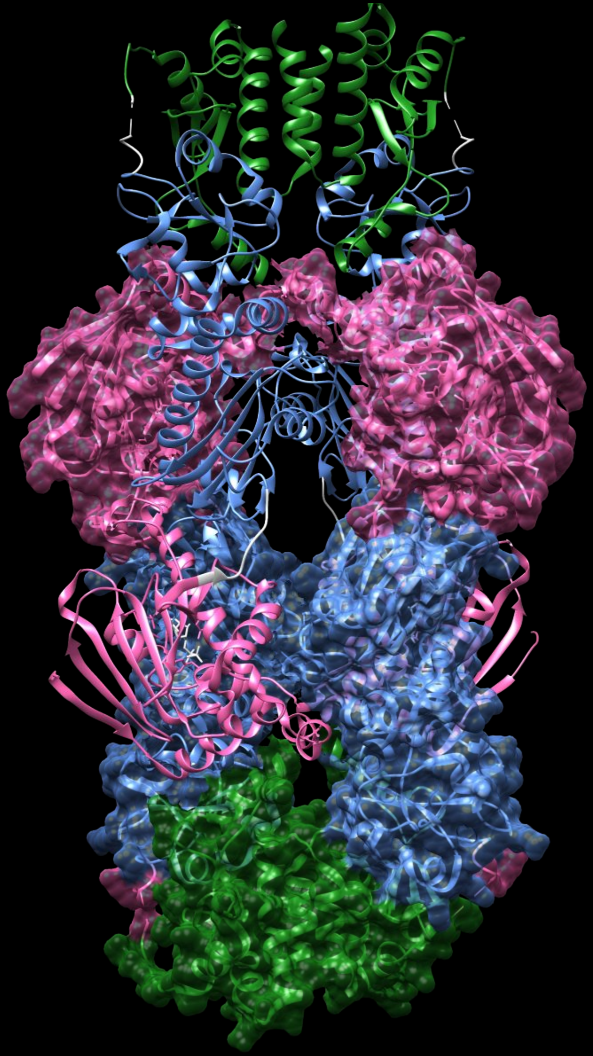
Two inverted interlocking dimers

Stabilized: Interaction of NTDs from one dimer with MDs and CTDs of the other



DIMER OF DIMERS

NTD cradled against the 1st subdomain of **MD** and lies on top of the **CTD** of the other protomer

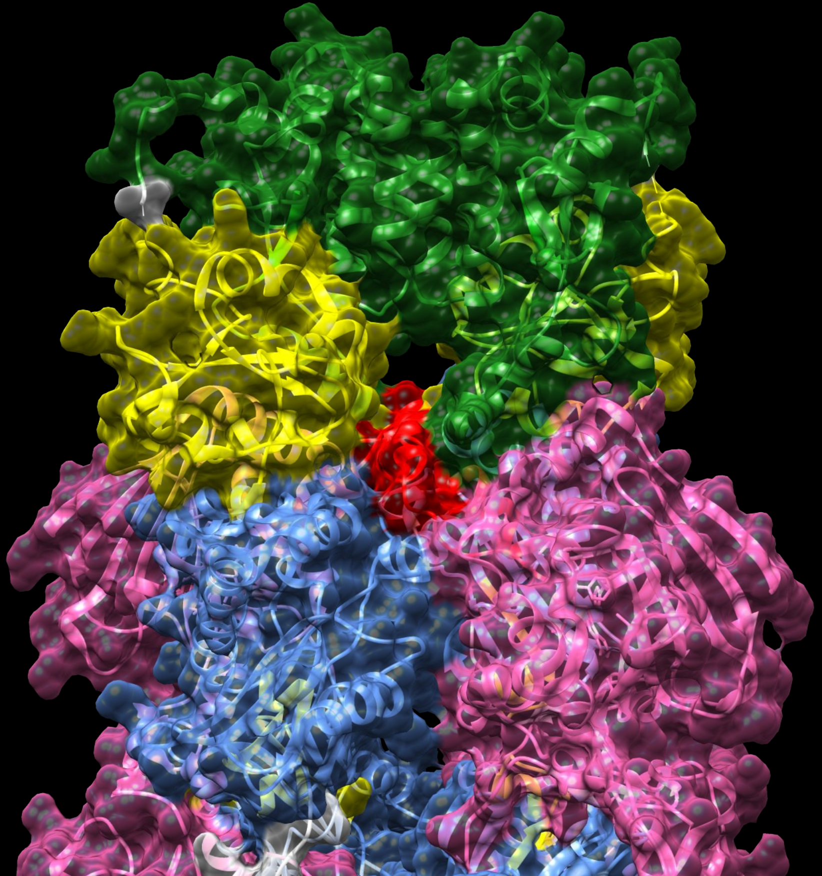


DIMER OF DIMERS

The **NTD** lids lie at the heart of the rearrangement

Each of the lids is inserted into a channel bound by the **MD src loops**, the **last domain of the MD** and the **N-ter end of H21** of the other dimer

The hydrophobic surfaces come together to form a well-packed hydrophobic core



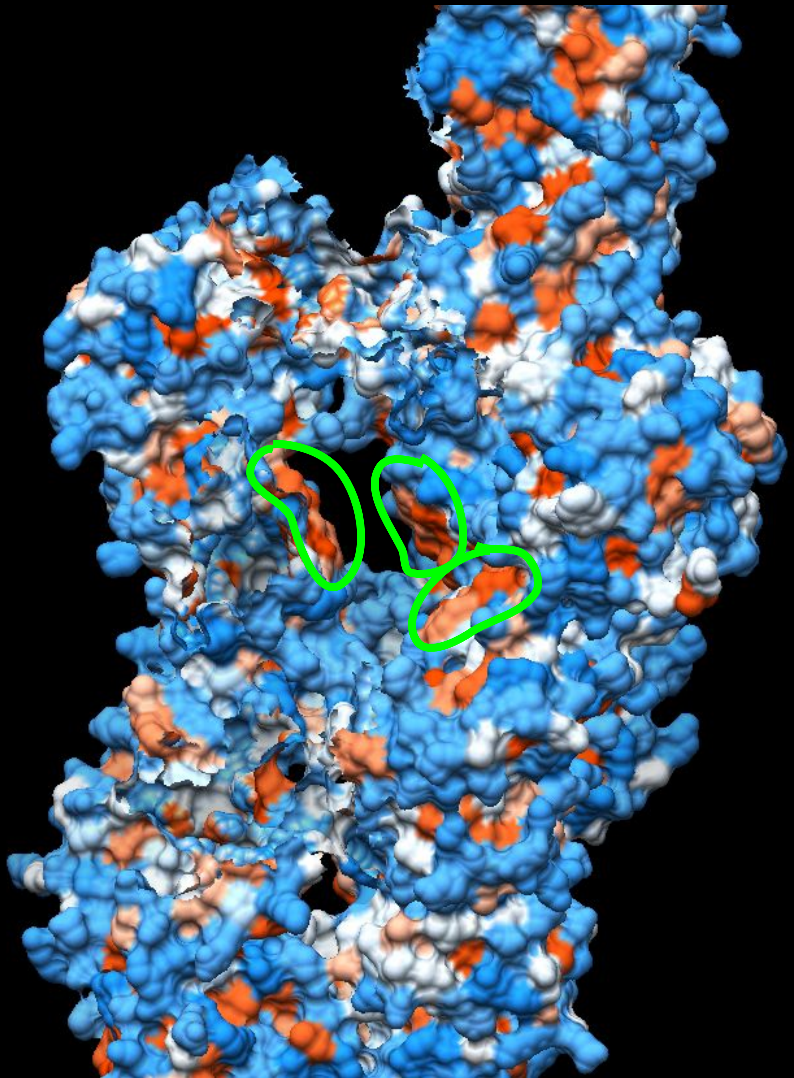
DIMER OF DIMERS

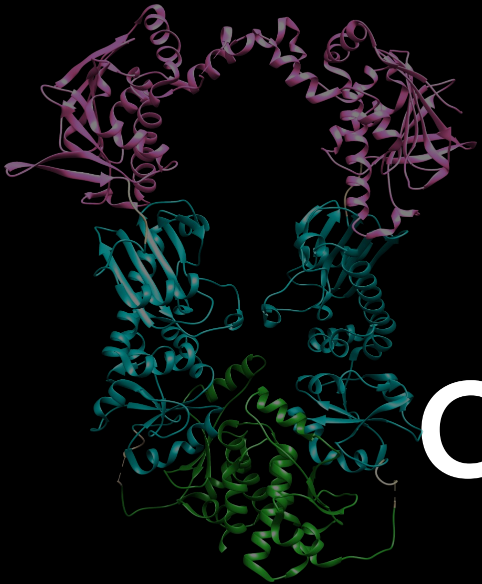
The NTD lids lie at the heart of the rearrangement

Each of the lids is inserted into a channel bound by the MD src loops, the last domain of the MD and the N-ter end of H21 of the other dimer

The **hydrophobic surfaces come together** to form a well-packed hydrophobic core

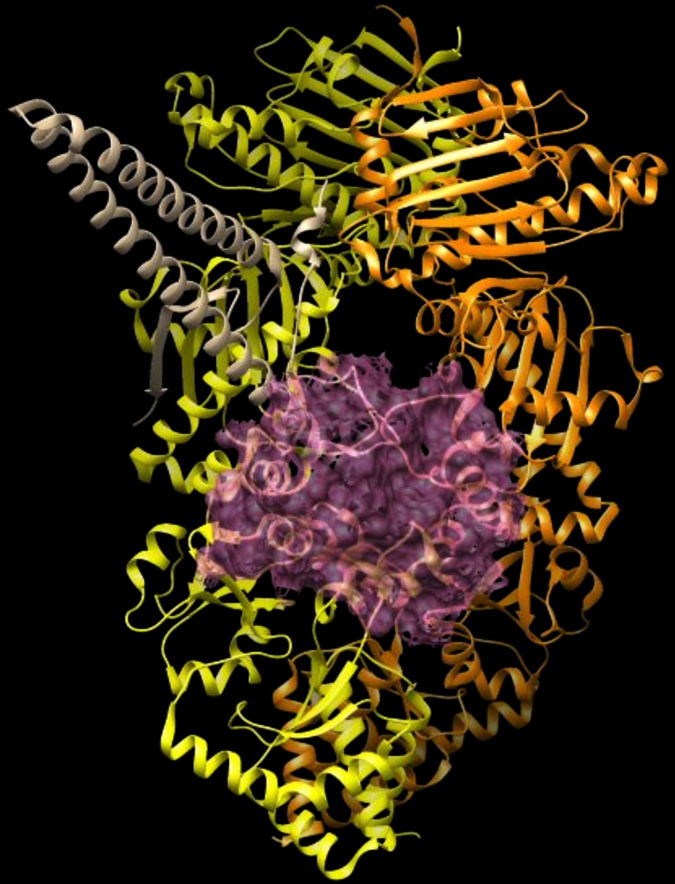
HYDROPHOBIC HYDROPHILIC





CLIENT BINDING AND FOLDING

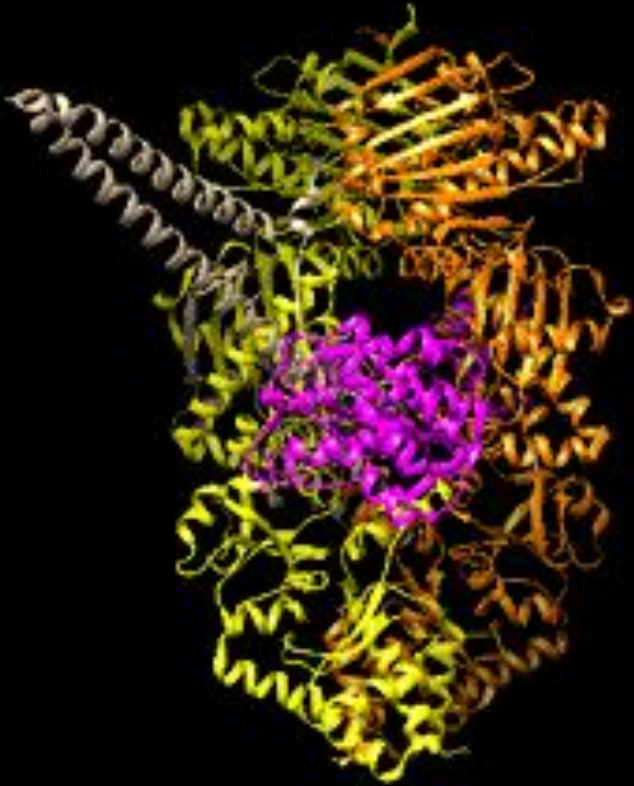
CLIENT BINDING



Coexpression in Sf9 cells of **HSP90** +
CDC37 + **CDK4**

Symmetrical closed conformation

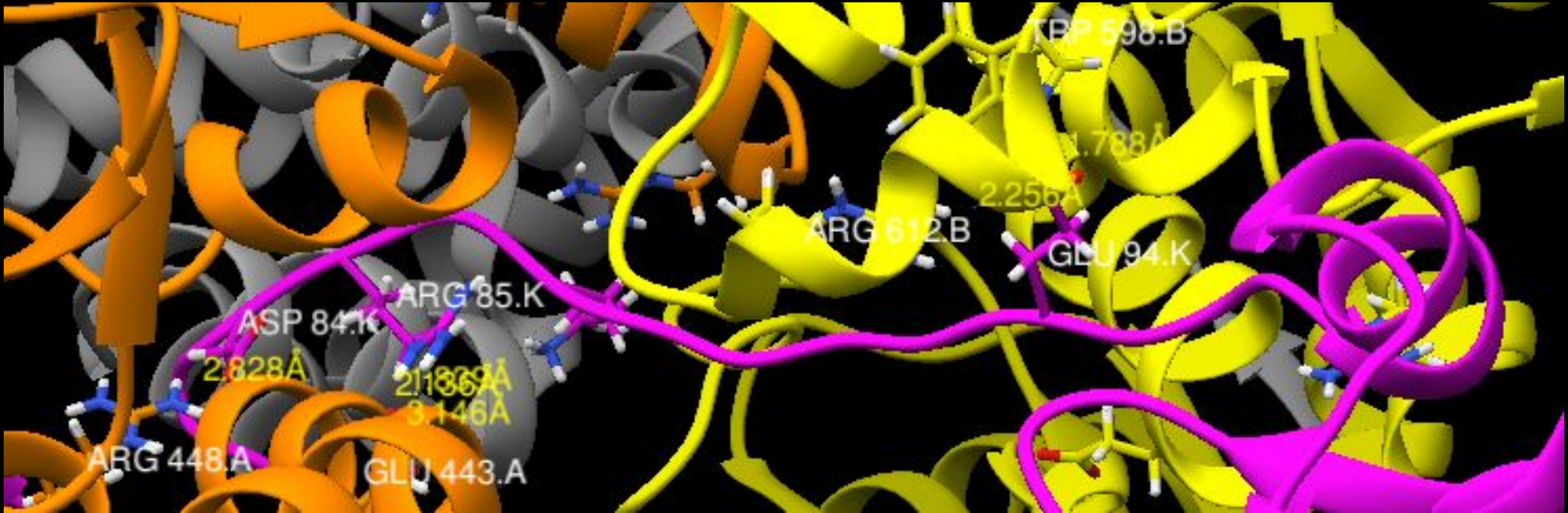
CLIENT BINDING



CLIENT BINDING INTERACTIONS

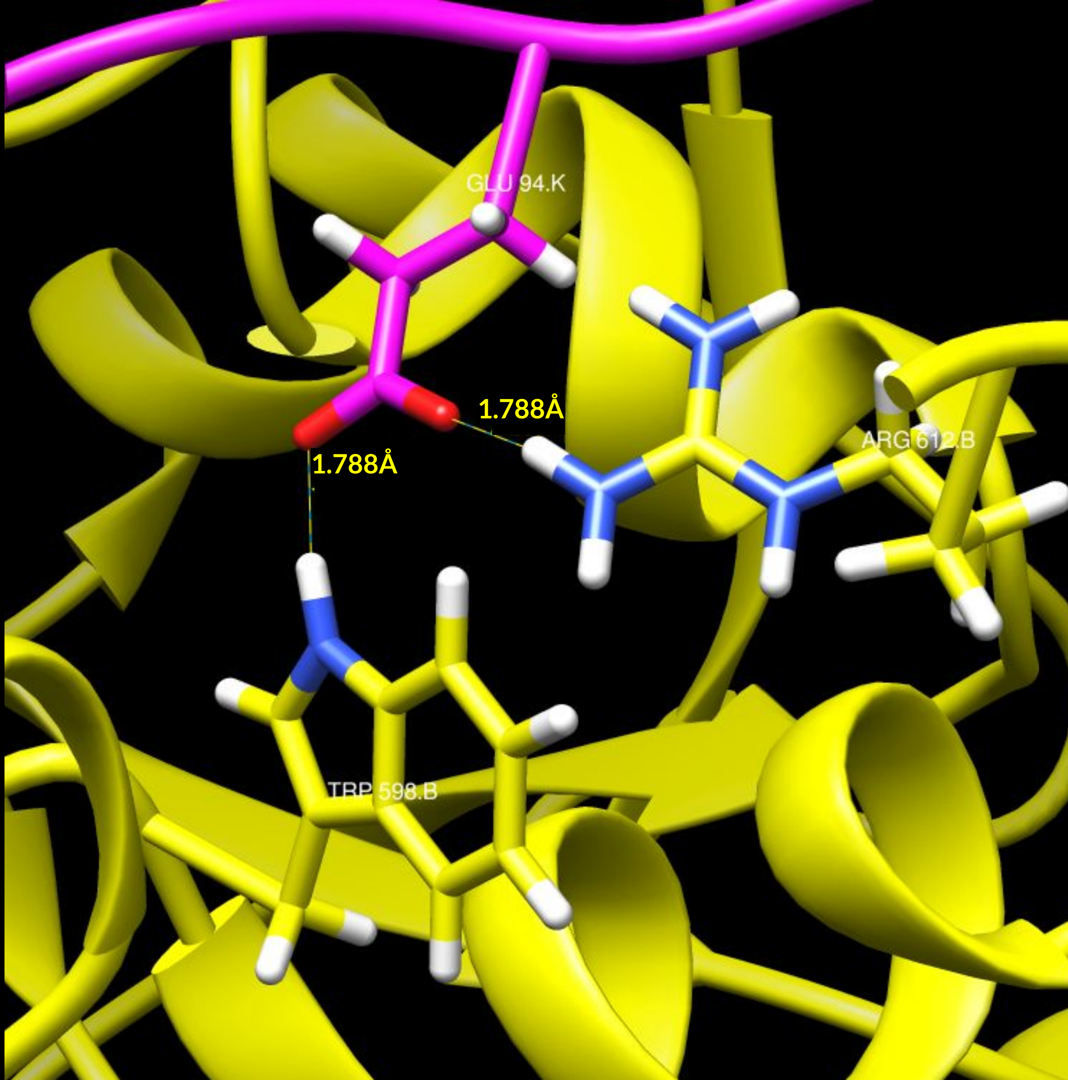
HSP90 - Tubular Domain

HSP90 interacts with the tubular domain of Cdk4 preventing the folding of the protein



CLIENT BINDING INTERACTIONS

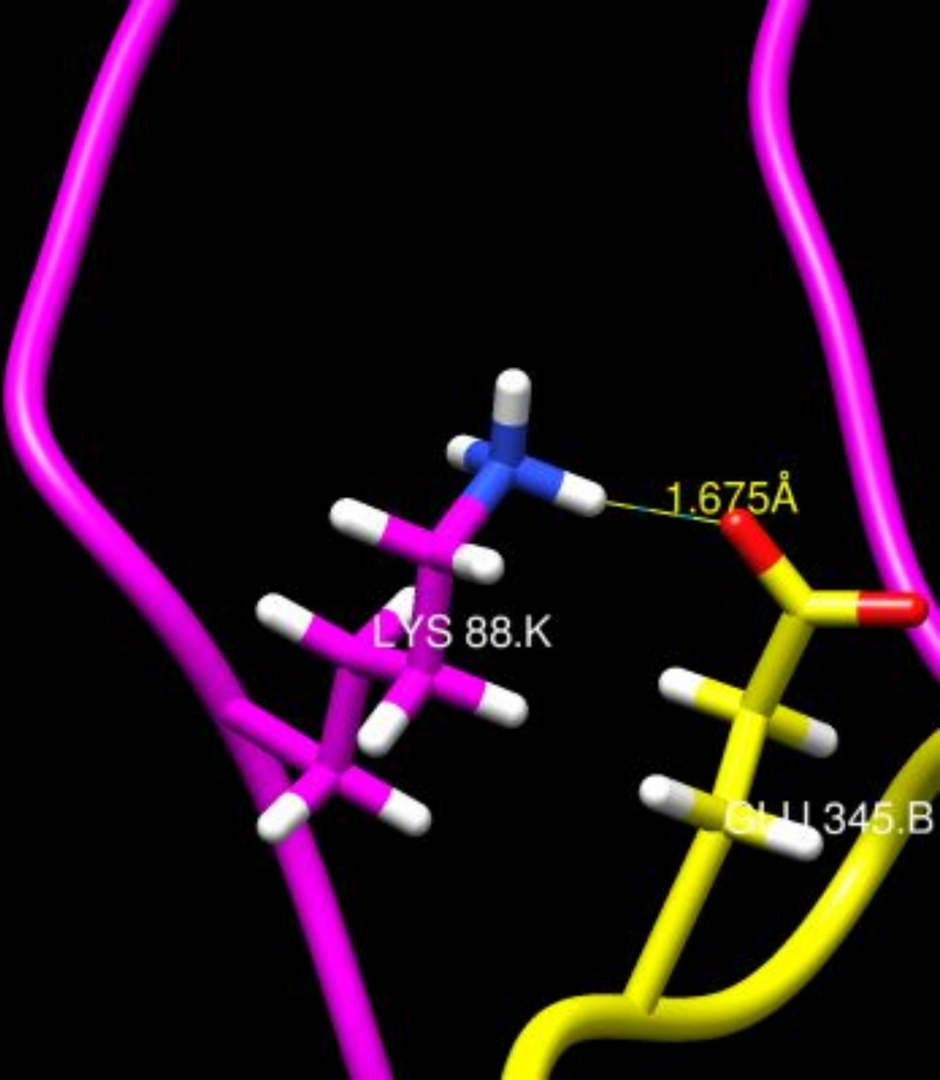
Salt bridge:
GLU (CDK4) - ARG (HSP90)
Hydrogen bond:
GLU (CDK4) - TRP (HSP90)



CLIENT BINDING INTERACTIONS

Salt bridge:

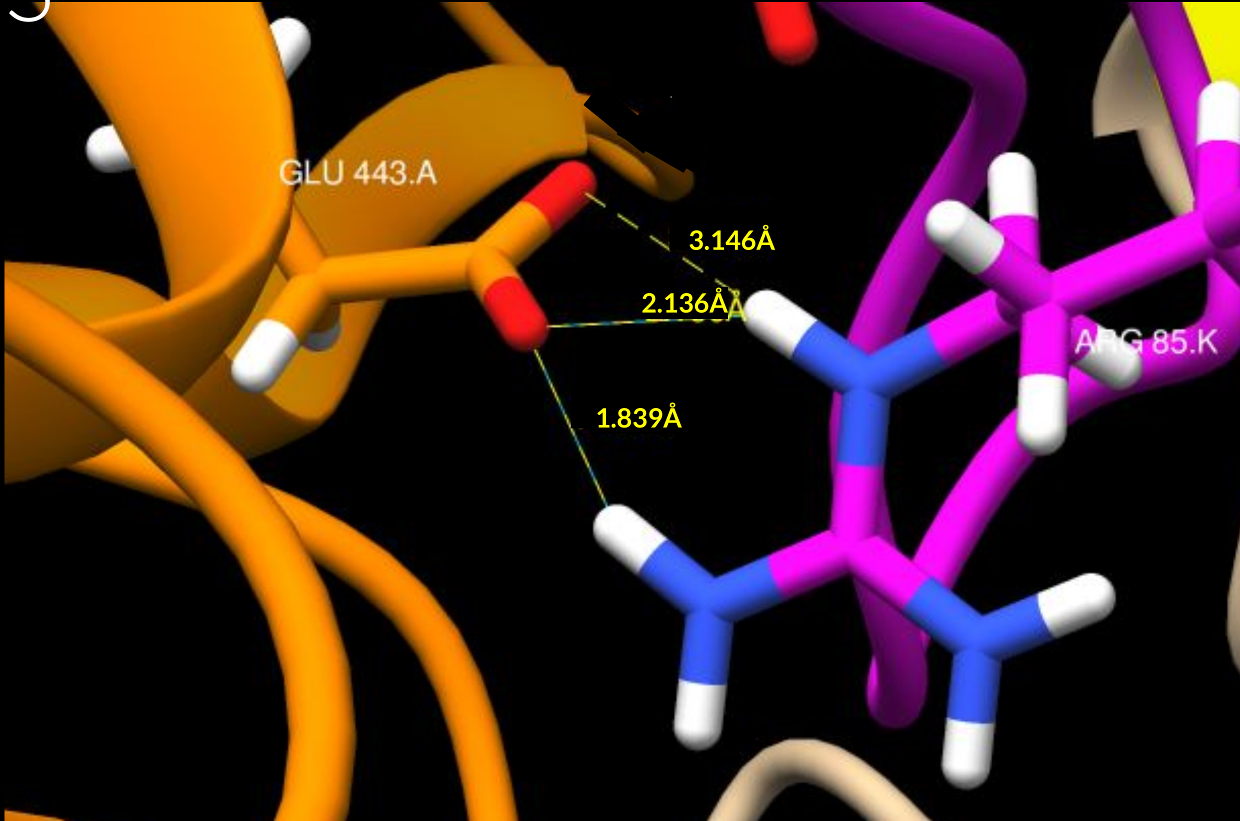
LYS (CDK4) - GLU (HSP90)



CLIENT BINDING INTERACTIONS

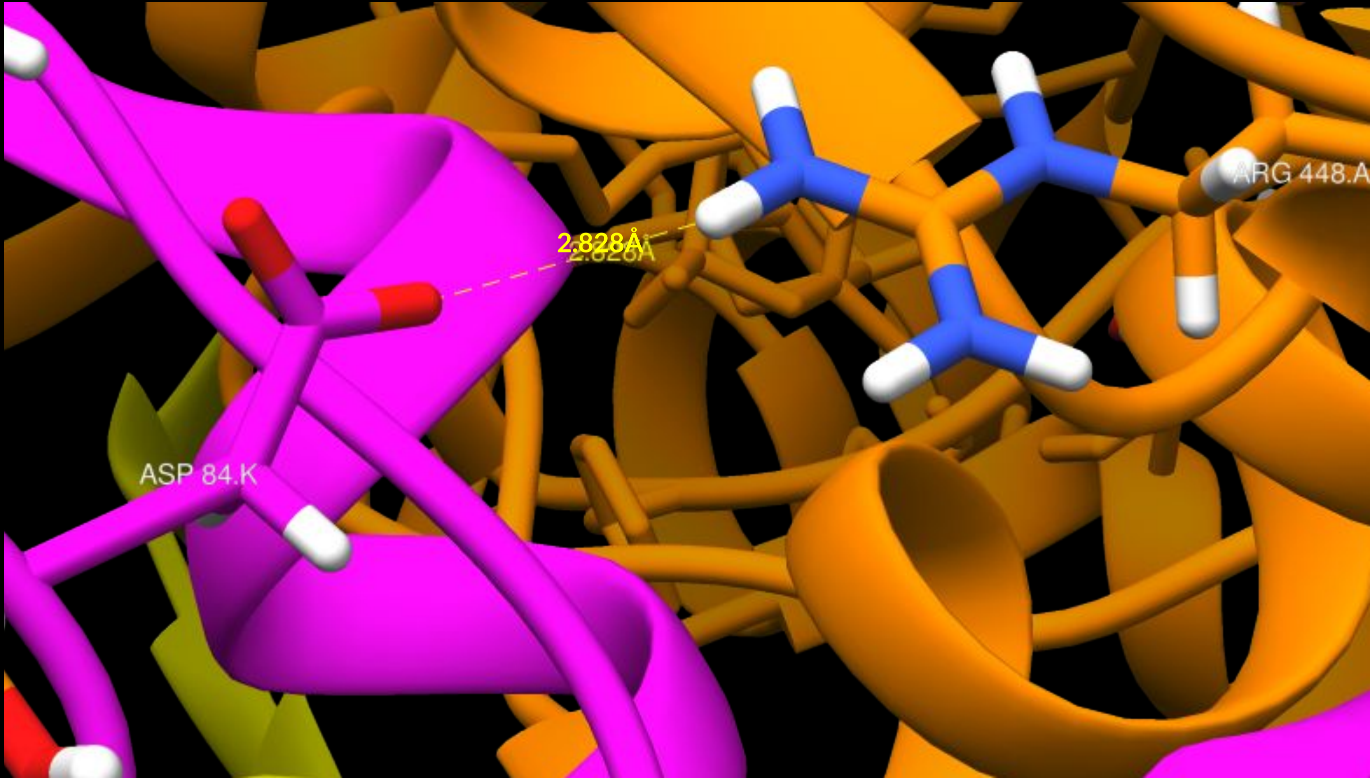
Hydrogen bond:

ARG (CDK4) - GLU (HSP90)
ARG (CDK4) - GLU (HSP90)

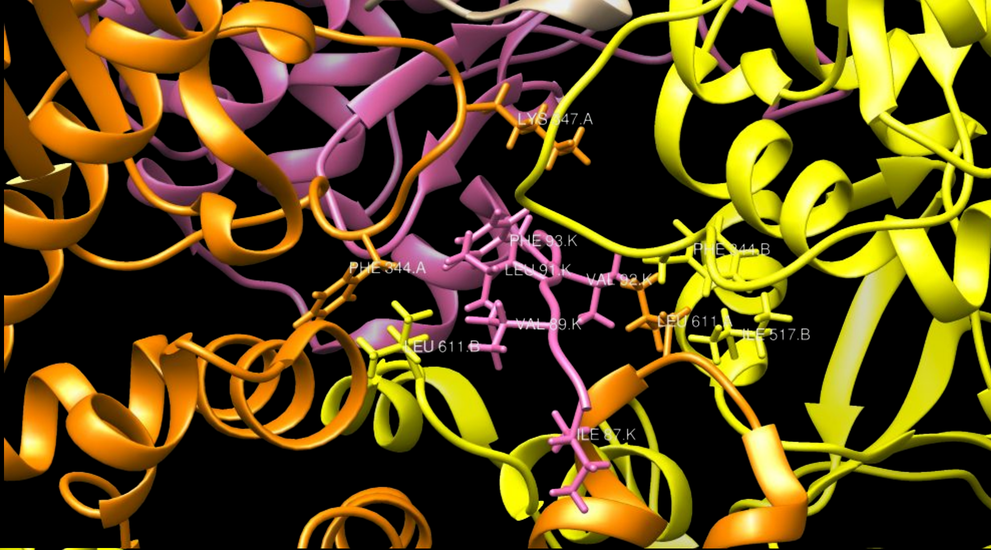


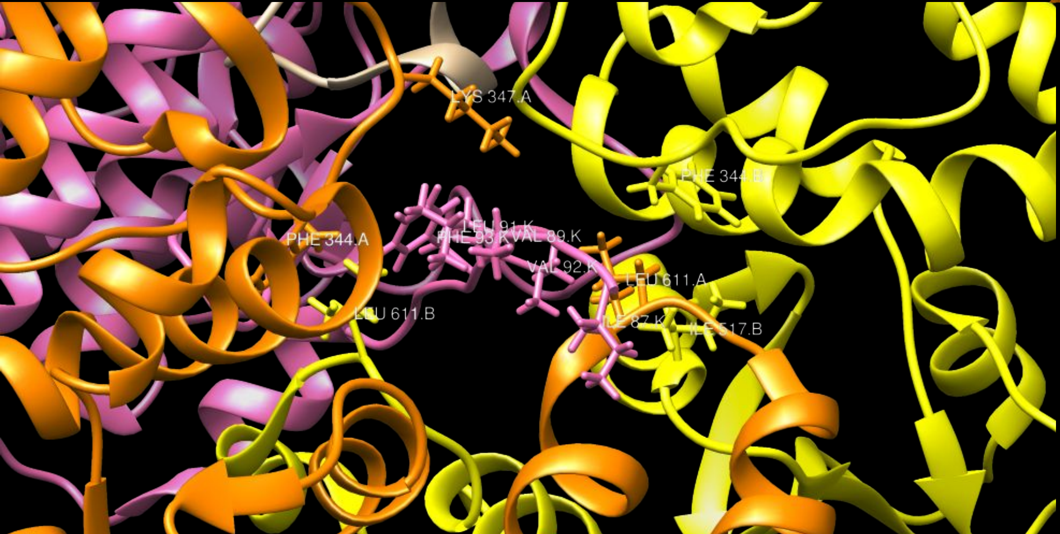
CLIENT BINDING
INTERACTIONS

Salt Bridge:
ASP (CDK4) -
ARG (HSP90)



CLIENT BINDING
INTERACTIONS

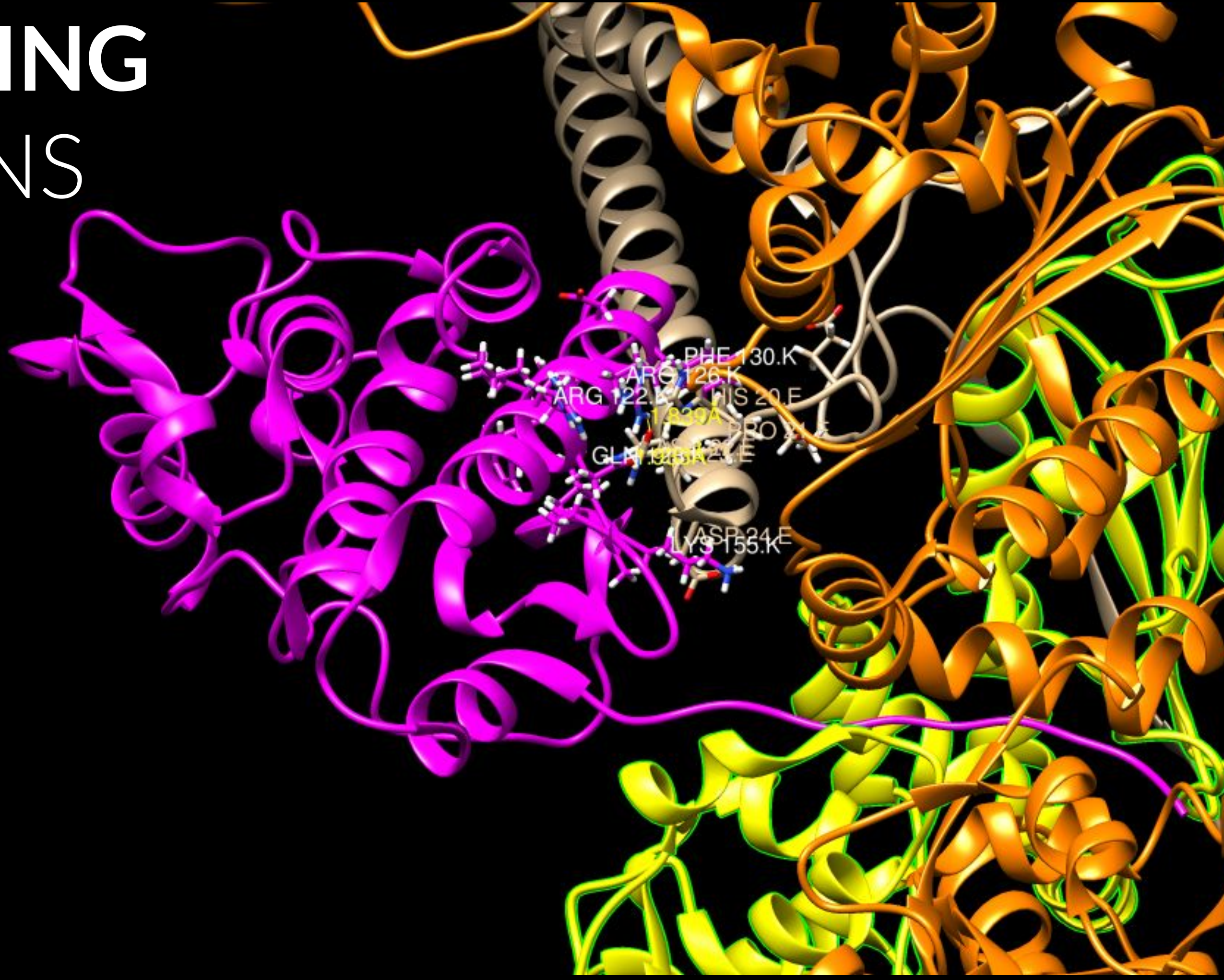




Hydrophobic interactions that stabilize the complex

CLIENT BINDING INTERACTIONS

CLIENT BINDING INTERACTIONS



NTD - NTD Interactions

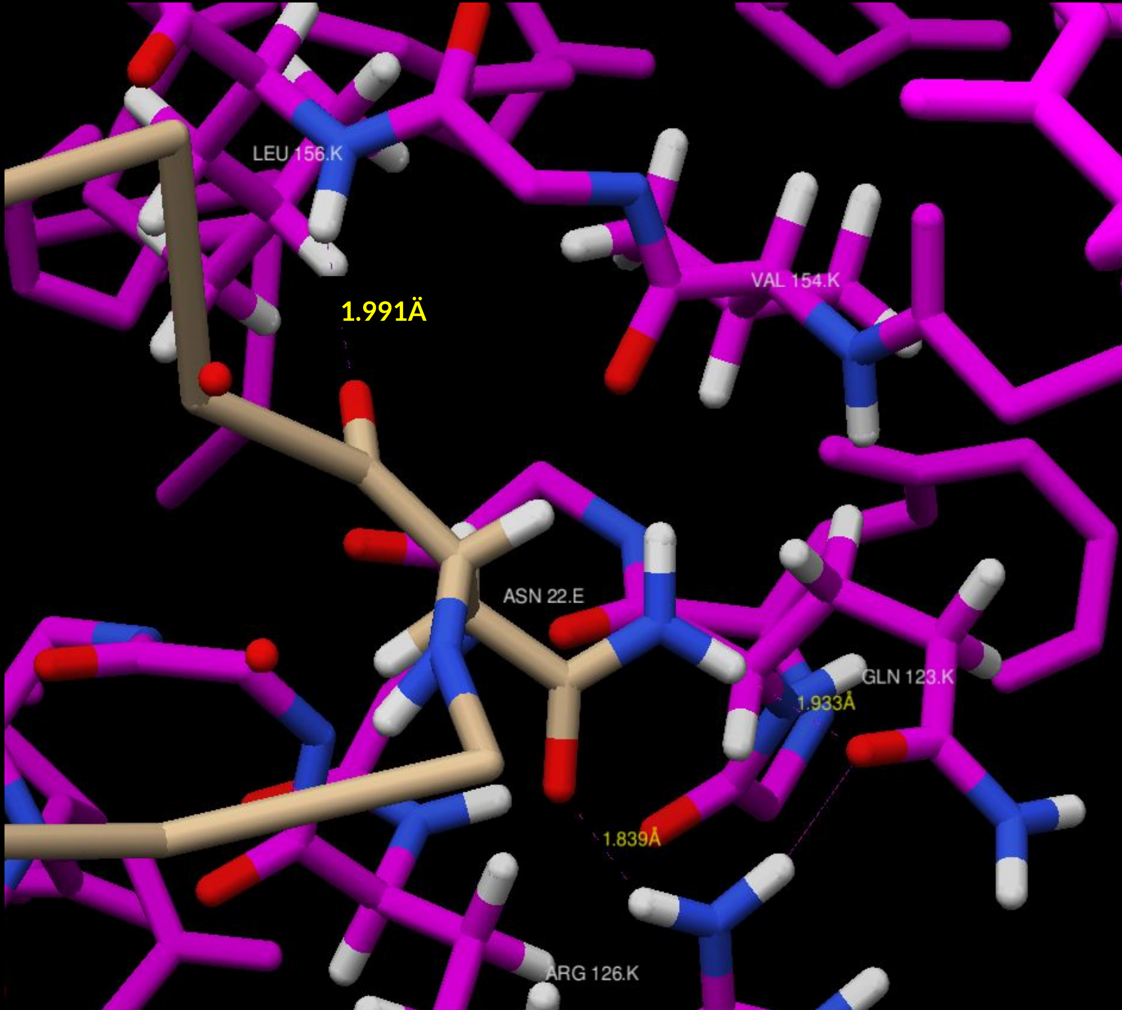
CDK4 interacts with the **co-chaperone** through extensive hydrophobic and hydrogen bonding interactions

Mimicking of the N-C interaction

CLIENT BINDING INTERACTIONS

Hydrogen bonds:

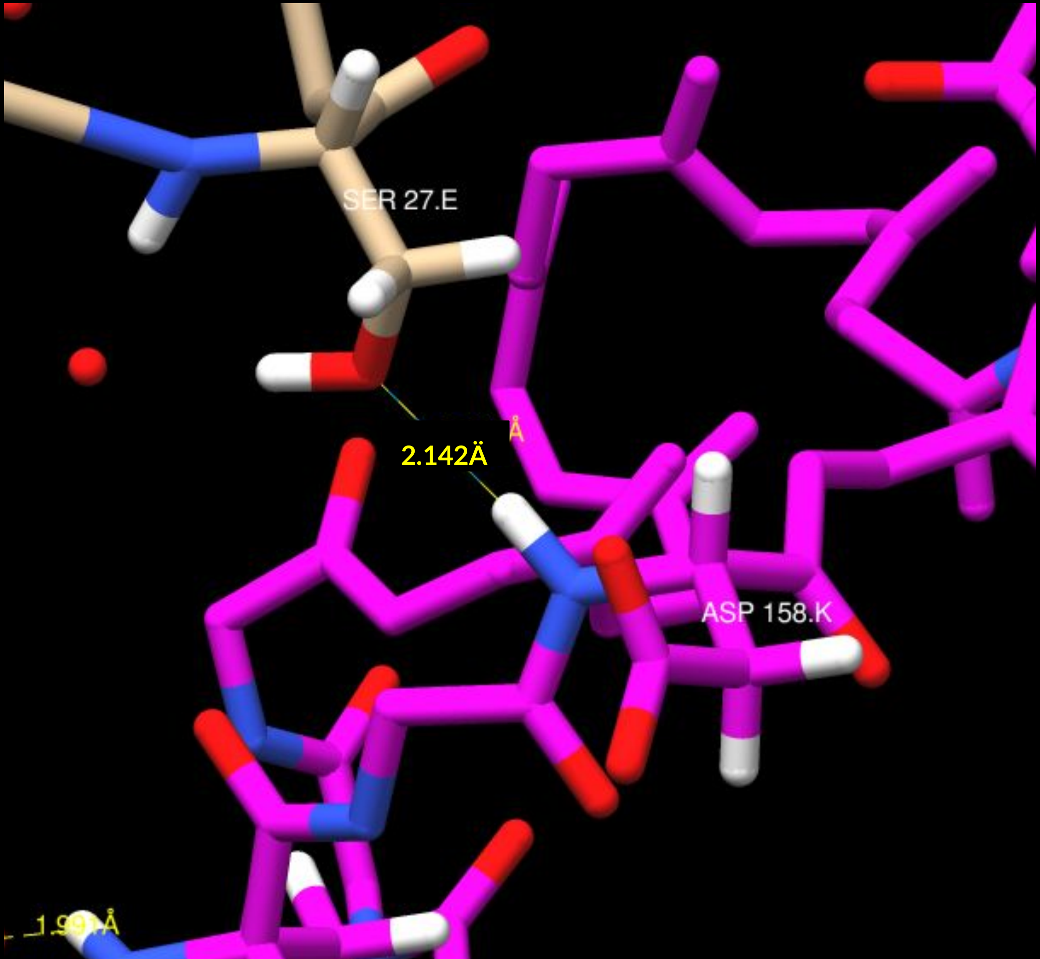
- **GLN (CDK4)** - **ASN (CDC37)**
- **ARG (CDK4)** - **ASN (CDC37)**
- **LEU (CDK4)** - **ASN (CDC37)**



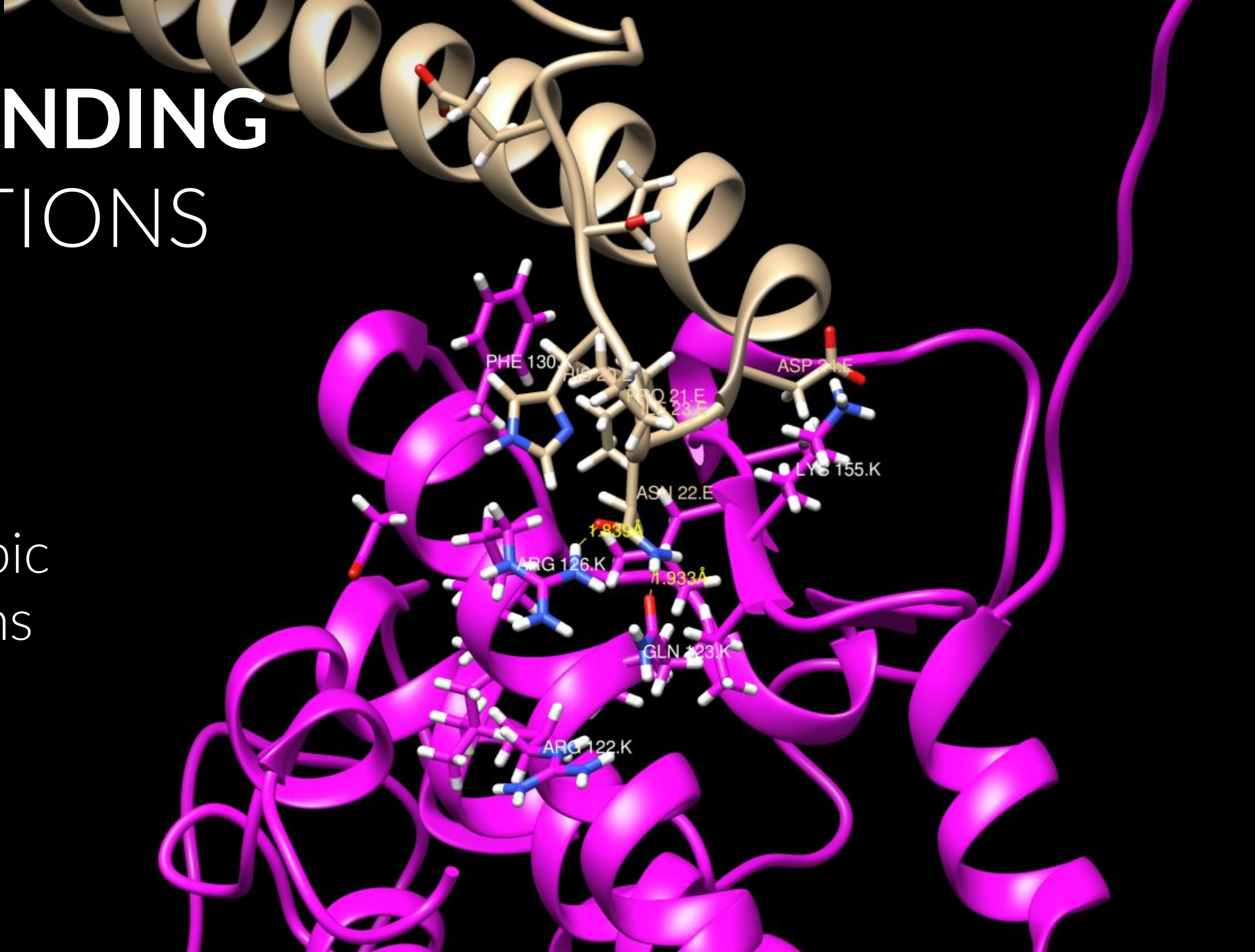
CLIENT BINDING INTERACTIONS

Hydrogen bonds:

ASP (CDK4) - SER (CDC37)



CLIENT BINDING INTERACTIONS

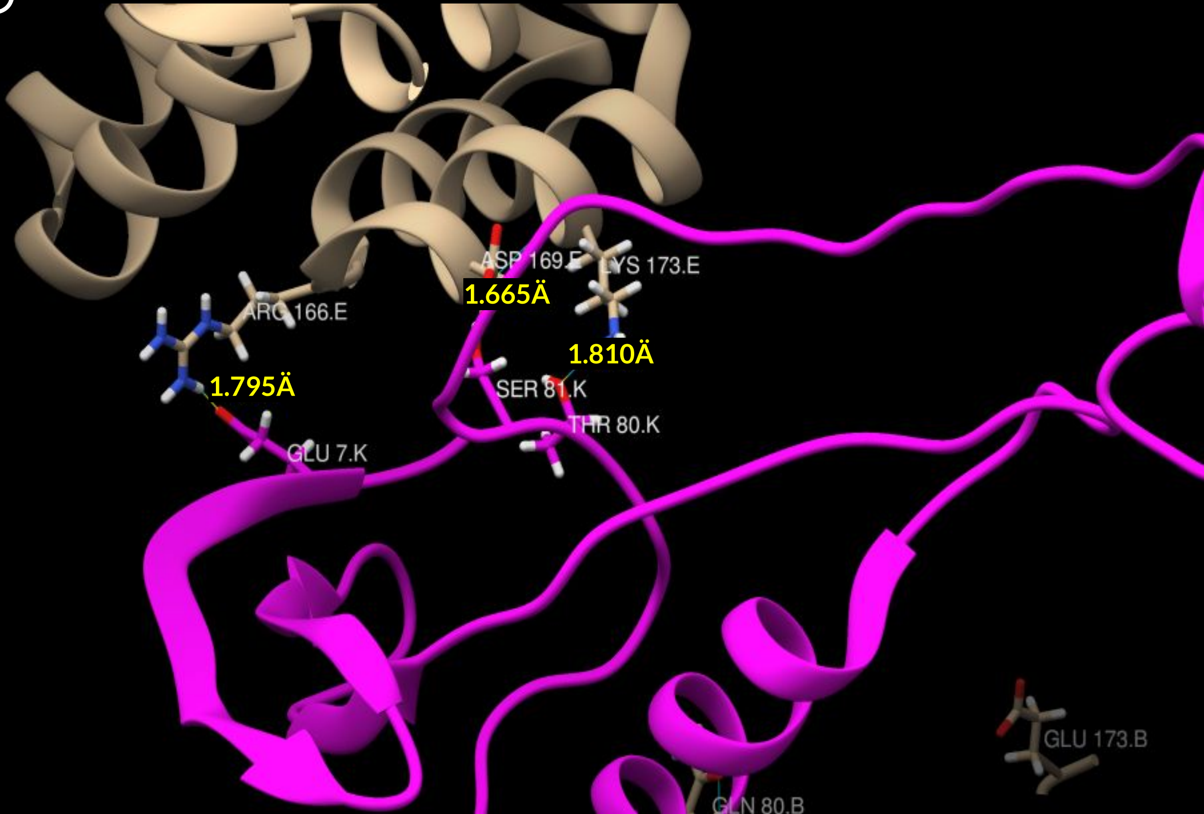


Stabilizing hydrophobic interactions

CLIENT BINDING
INTERACTIONS

CTD - CTD Interaction

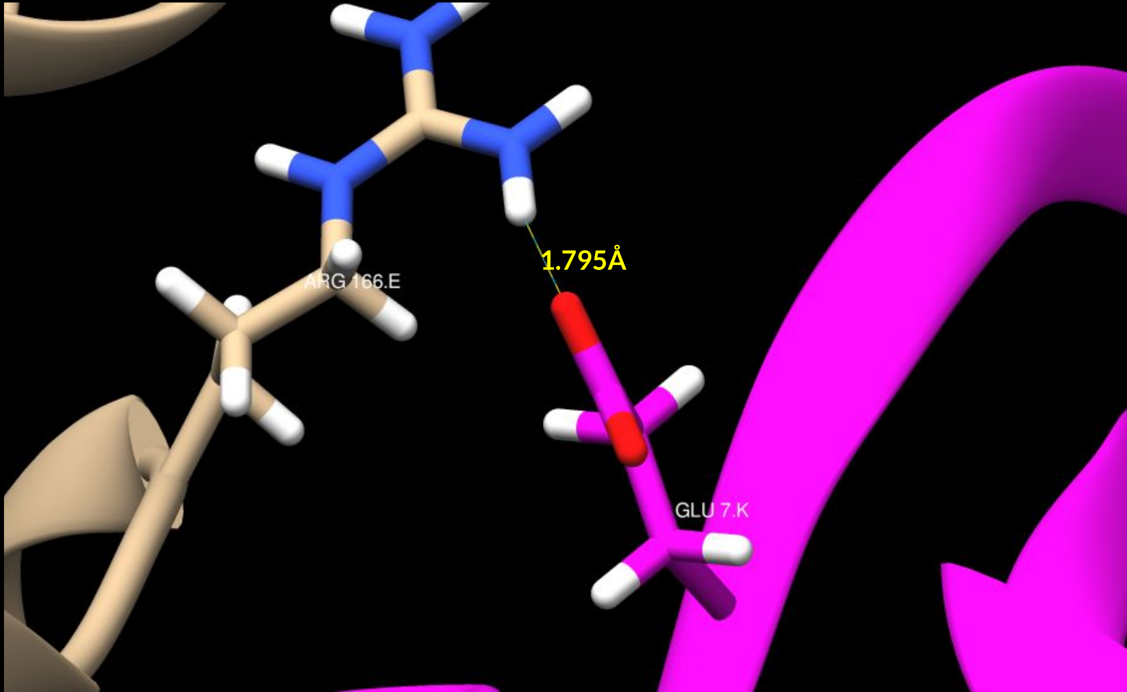
Helps covering the other side of the CDK NTD - CTD interaction



CLIENT BINDING
INTERACTIONS

Hydrogen bonds:

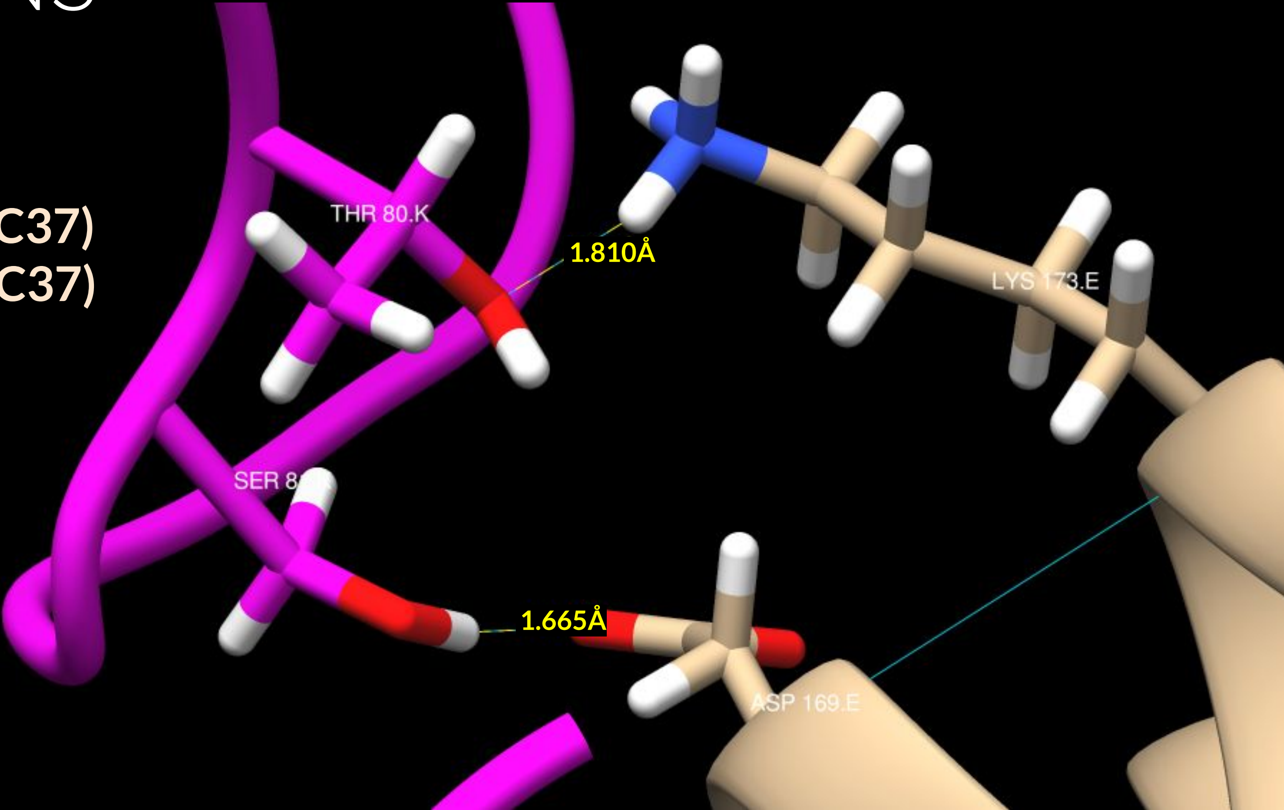
LYS (CDK4) - ARG (CDC37)



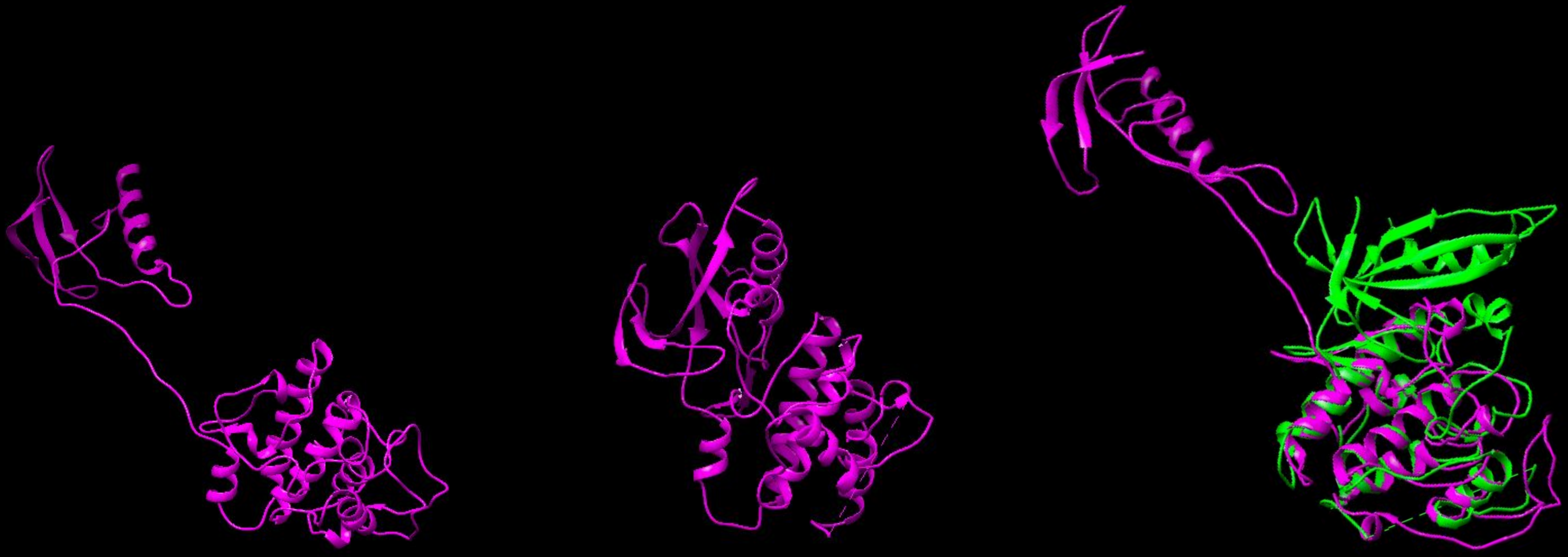
CLIENT BINDING INTERACTIONS

Hydrogen bonds:

- **THR (CDK4)** - **LYS (CDC37)**
- **SER (CDK4)** - **ASP (CDC37)**



CLIENT BINDING INTERACTIONS



BULLET POINTS

- Hsp90 is a highly conserved chaperon among all its representatives. Even though sequence homology is not always observed in HSP90, the structure is highly conserved through species following similar hydrophobic and hydrophilic patterns.

- It works in an ATP dependent manner. This nucleotide helps to switch between different equilibrium states that compose the HSP90 cycle. The key factor of the Hsp90 function lies in its cycle of conformational changes.

- HSP90 is a constitutive dimer. Nonetheless, it is usually found as a dimer of dimers to maintain its stability by hiding the hydrophobic exposed residues.

- Each domain has conserved hydrophobic regions responsible for the client-protein interactions. Overall, this pattern is known as the central cleft and it’s composed by: the hydrophobic stripe of the NTD, the src loop of the MD, and the H21 helix of CTD.

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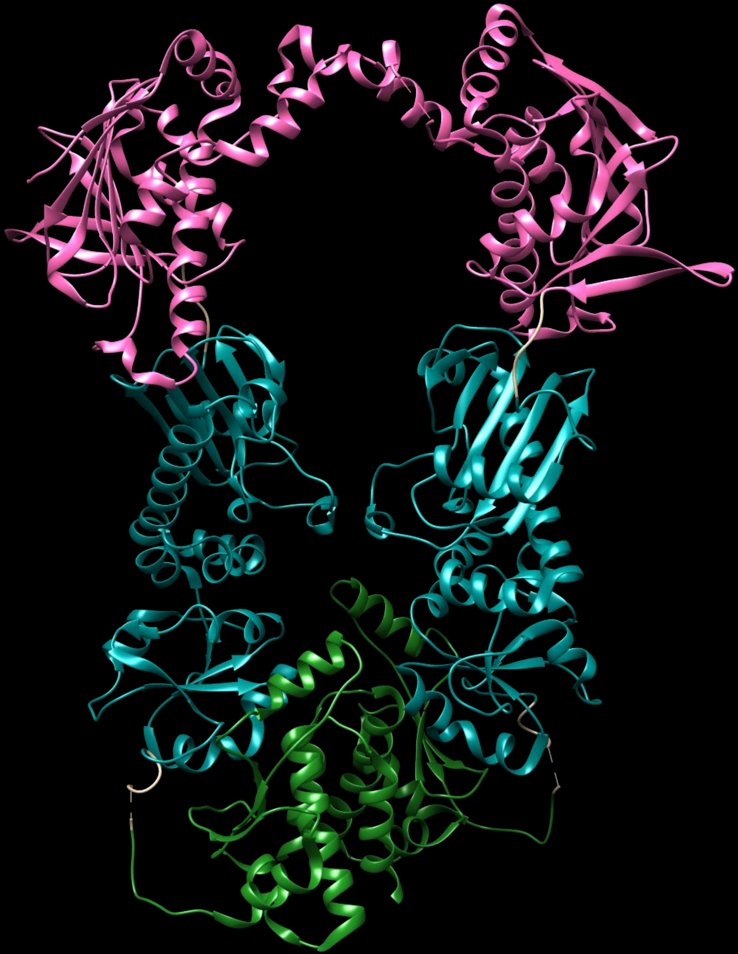
THANK YOU FOR YOUR ATTENTION!



ANY QUESTION?

PEM

1. Which are the main types of chaperones?
    a. Nucleoplasmins.
    b. Chaperonins.
    c. Both are correct..
    d. Hsp70 and Hsp90.
    e. **All of the above**.

2. Hsp90 is responsible for:
    a. The folding of *de novo* synthesised proteins.
    b. The folding of unfolded proteins after a denaturation process.
    c. Glycosylation of proteins.
    d. Cargo export.
    e. DNA folding.

3. How many domains does the HSP90 have?
    a. Only one.
    b. Two: the NTD and the CTD
    c. Three: NTD, MD, CTD
    d. It depends on the species.
    e. None of the above are true.

PEM

4. Which is the region responsible for the ATP binding?

   a. Only the NTD.
   b. Only the MD
   c. The NTD together with an ARG from the MD.
   d. The CTD.
   e. HSP90 does not bind to ATP.

5. In which species is the MEEVD motif not conserved?

   a. Homo sapiens.
   b. Mus musculus.
   c. Bos taurus.
   d. E. coli.
   e. S. cerevisiae.

6. About the cycle of the HSP90:

   1. The cycle consists of multiple states of equilibrium directionally determined by ATP binding and hydrolysis.
   2. It regulates the protein binding and release.
   3. Client proteins are loaded during the apo state.
   4. There is only one equilibrium state (when the client protein is bound).

      a) 1, 2 and 3
      b) 1 and 3
      c) 2 and 4
      d) 4
      e) 1, 2, 3 and 4

PEM

7. About the cycle of the HSP90:
    1. The lid remains with the same conformation during all the cycle
    2. The hydrophobic central cleft is exposed at the end of the cycle
    3. The client binding needs a previous ATP binding
    4. The ATP binding needs a previous client binding

    a) 1, 2 and 3
    b) 1 and 3
    c) 2 and 4
    d) 4
    e) 1, 2, 3 and 4

8. In which state does the hydrophobic core collapse to allow the release of the client proteins?
    a) Apo state
    b) ATP state
    c) ATP/ADP state
    d) ADP state
    e) The hydrophobic core never collapses because the client proteins don't release from the Hsp90

PEM

9. What kind of bonds are involved in the Hsp90 - Cdk4 interaction?
   1. Hydrogen Bonds
   2. Salt Bridges
   3. Hydrophobic interactions
   4. Disulphide bonds
      - **a. 1, 2 and 3**
      - b. 1 and 3
      - c. 2 and 4
      - d. 4
      - e. 1, 2, 3 and 4

10. About the Hsp90 - Cdc37 - Cdk4 interactions (choose the right one):
    - a. Three Hsp90 are present in the complex
    - b. Only one of the Hsp90s is interacting with the client protein
    - c. Cdc37 is the client protein
    - d. Cdk4 is the cochaperone
    - **e. Cdc37 NTD domain interacts with Cdk4 NTD**