

An insight into Hsp70

Structural Biology Project 23/24

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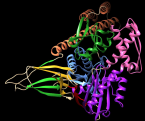


Introduction to chaperones

- Molecular Chaperones
- Classification of Chaperones



Introduction to Hsp70



Structural Analysis of Hsp70



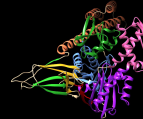
Hsp70 ATP/ADP

- Conformational changes and interactions
- Bonding cycle



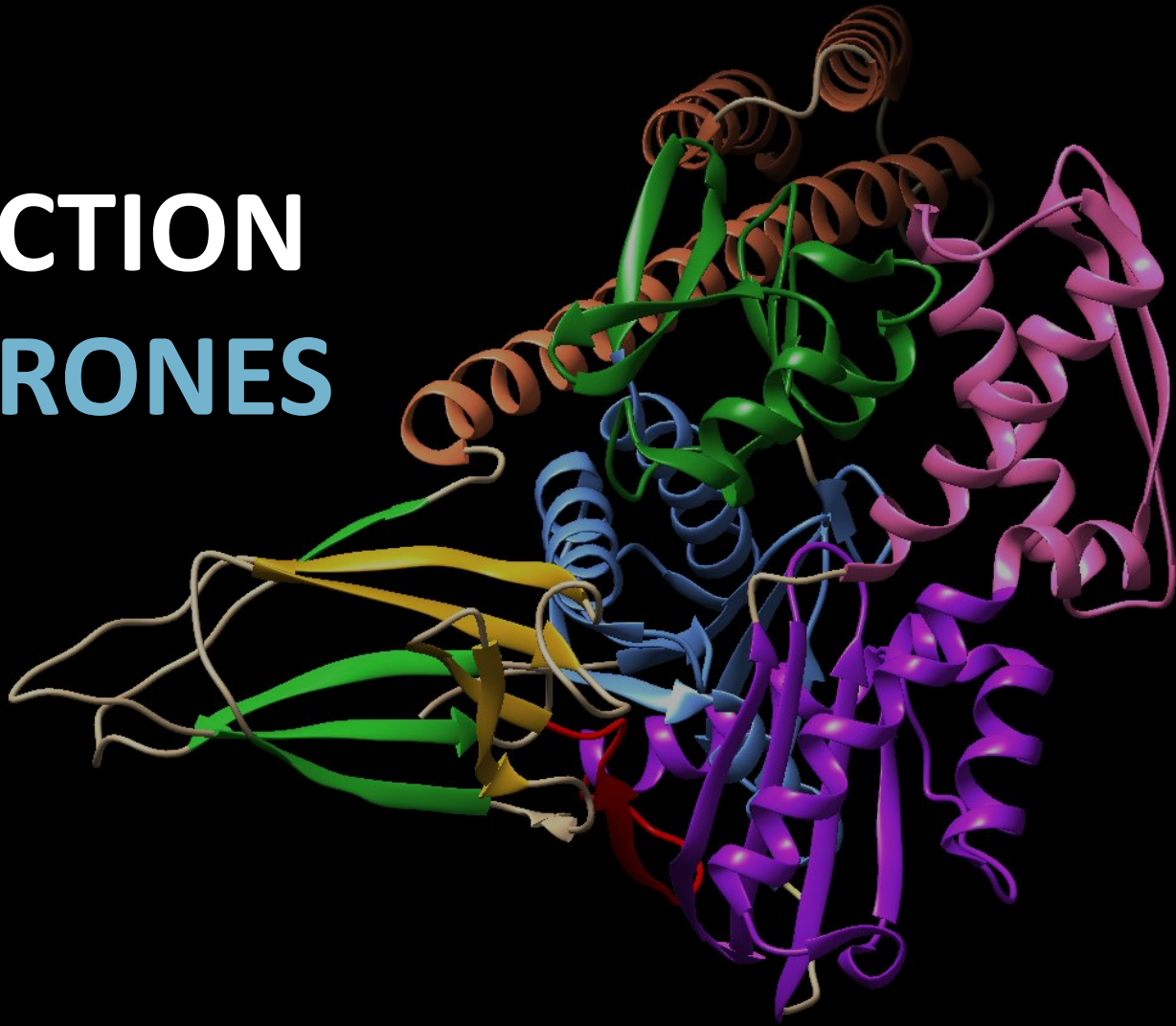
Hsp70 co-factors and co-chaperones

- Magnesium and Calcium
- J-domain Proteins
- Nucleotide Exchange Factors



Concluding remarks

INTRODUCTION TO CHAPERONES



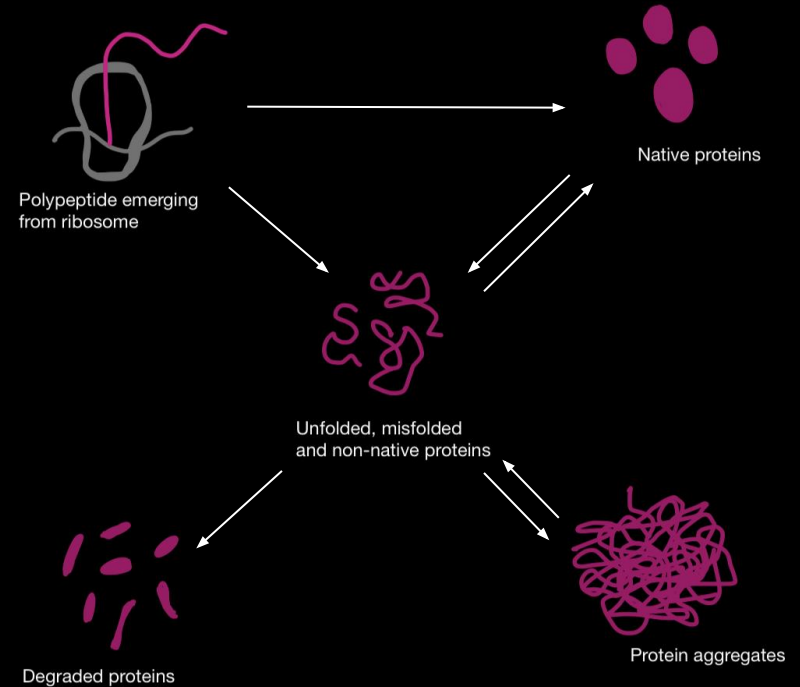
Molecular Chaperones

Chaperones are highly conserved molecular machines that control cellular protein homeostasis (proteostasis).

Chaperones are involved in

- De novo protein folding, maturation and translocation
- Protein disaggregation, refolding, and degradation

Heat shock proteins (Hsp) are chaperones that are produced by cells in response to stress.



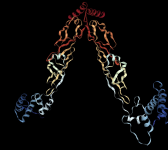
Classification

Classified based on **molecular weight (kDa)**.

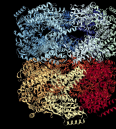
ATP-independent: passive holdases that can bind non-native proteins.

ATP-dependent: harness the energy from ATP binding and/or hydrolysis to assist proteostasis.

Hsp40



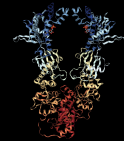
Hsp60



Hsp70



Hsp90



Others: sHsp and Hsp110.

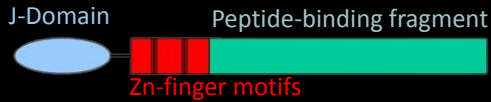
Hsp40

J-domain containing protein that functions as a co-chaperone to Hsp70.

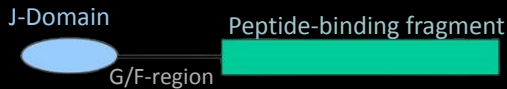
→ It can bind non-native proteins and direct them to Hsp70.

→ It accelerates ATP hydrolysis by Hsp70.

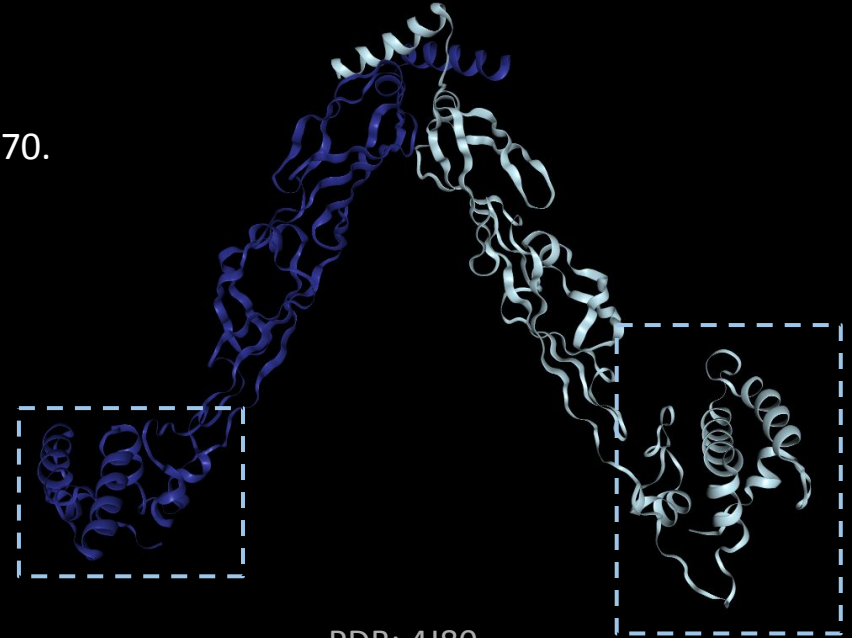
Type I



Type II



Type III



PDB: 4J80

E. coli

Hsp60

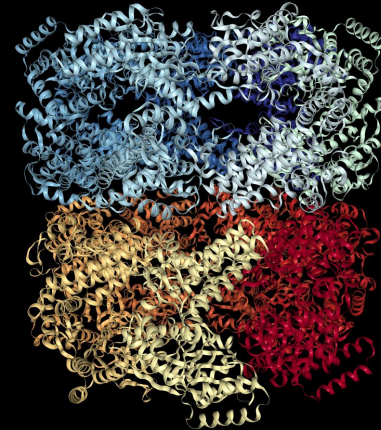
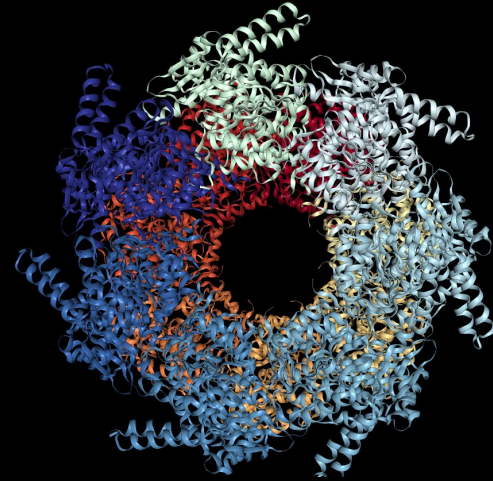
An **ATP-dependent** chaperone with a double ring structure, consisting of two heptameric rings.

Function:

Participate in assembly and disassembly of mitochondrial proteins.

Two groups:

1. GroEL of eubacteria, mitochondrial and chloroplast of eukaryotes
2. Chaperonins found in archaeobacteria and eukaryotic cytosol



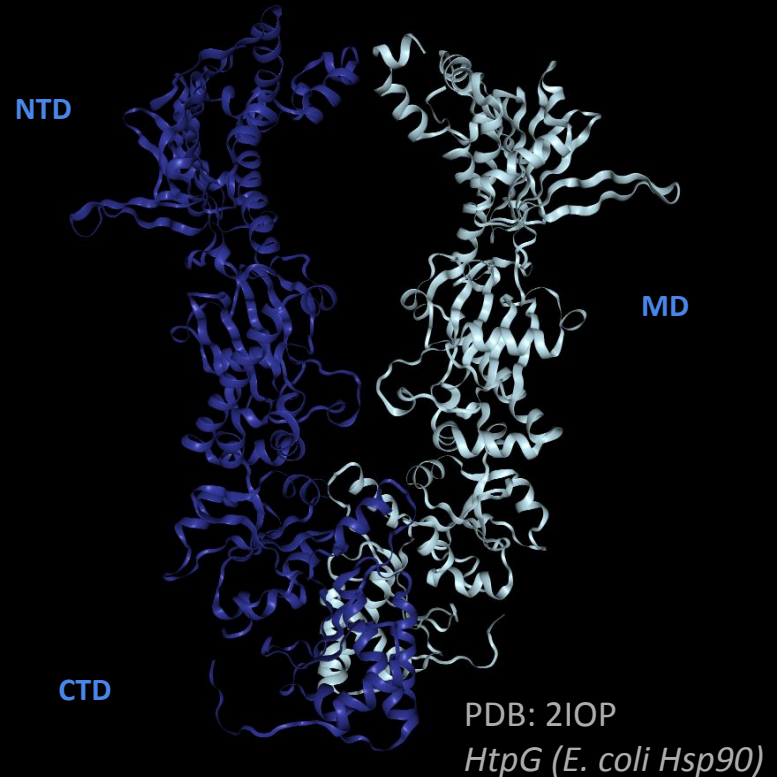
PDB: 2NWC
GroEL

Hsp90

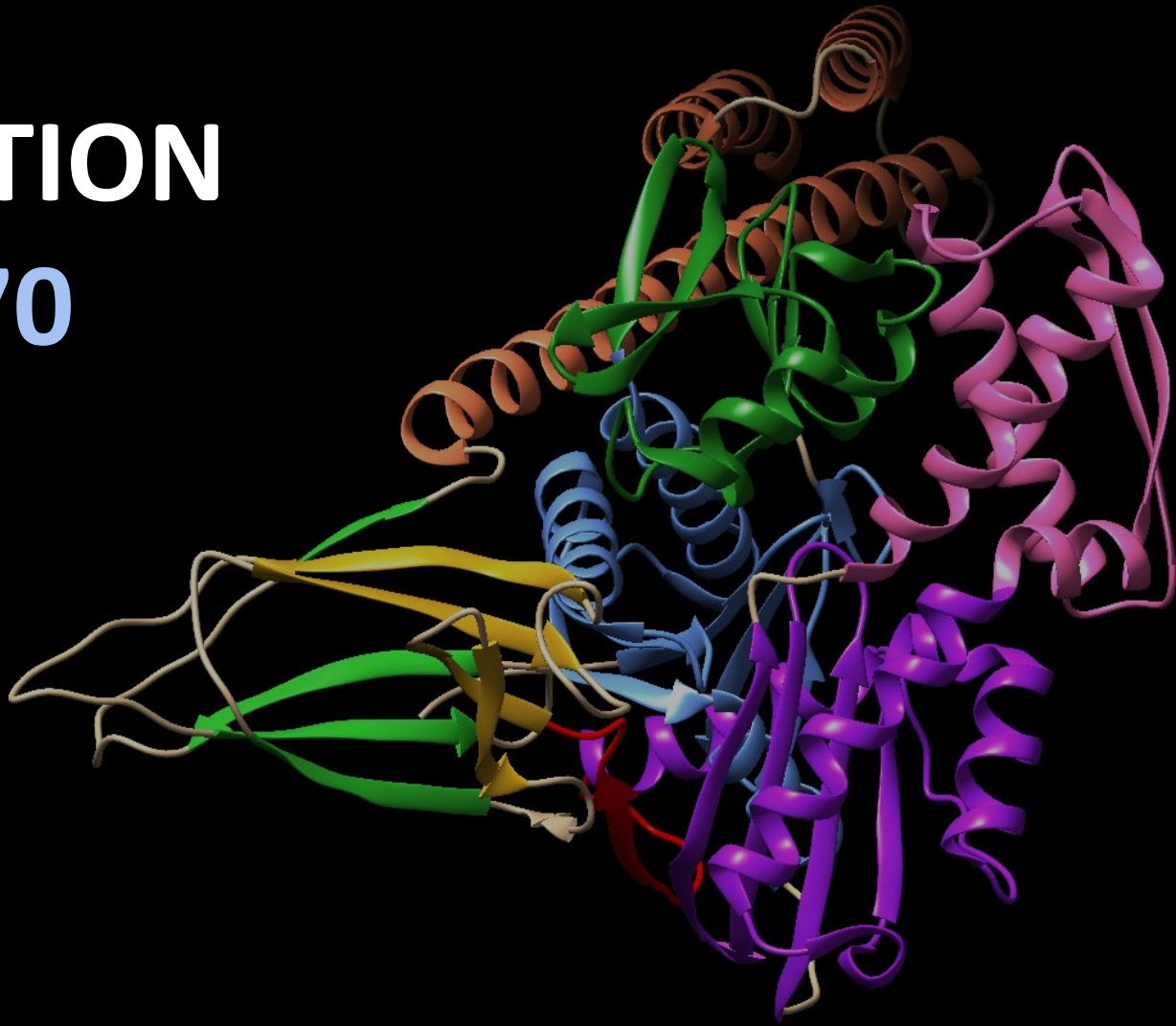
Chaperone involved in cell survival, cell cycle control, hormone signaling, and apoptosis.

Three main conserved domains that operated in an **ATP-dependent** manner:

1. N-terminal domain (NTD)
2. Middle domain (MD)
3. C-terminal domain (CTD)



INTRODUCTION TO HSP70



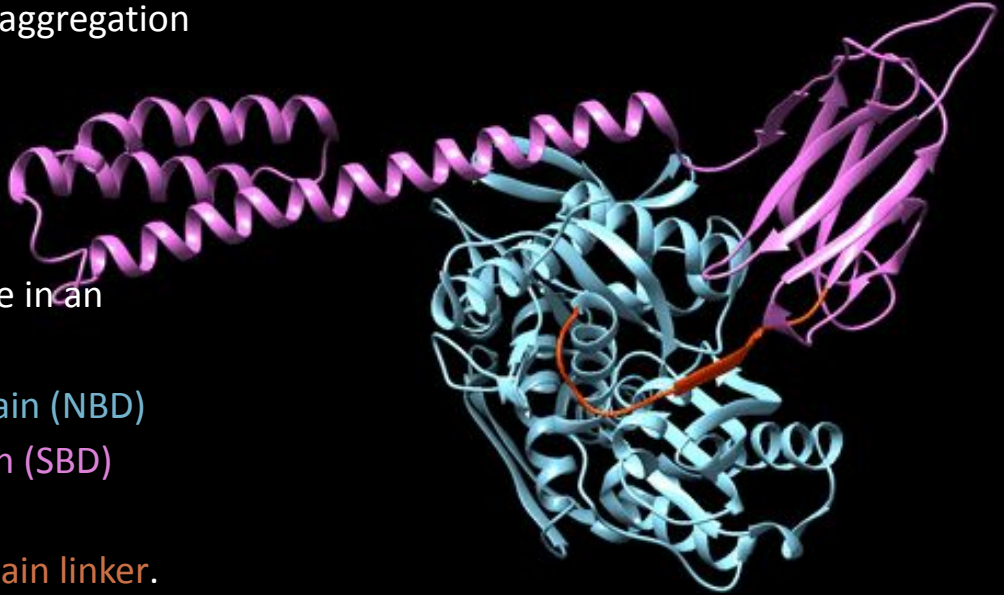
HSP70 FUNCTIONS AND DOMAINS

Involved in **proteostasis** by prevention of aggregation and refolding proteins to the native state.

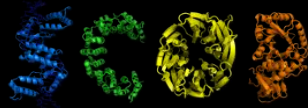
Two main functional domains that operate in an **ATP-dependent** manner:

1. N-terminal nucleotide binding domain (NBD)
2. C-terminal substrate binding domain (SBD)

Both domains connected by the **interdomain linker**.



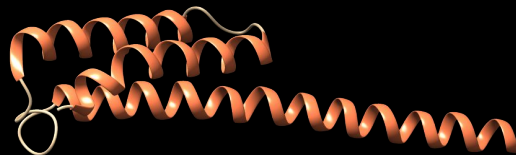
Movie 1. 3D representation of the *Homo sapiens*' Hsp70 in the ATP-state. PDB ID: 5E84. Created with Chimera.



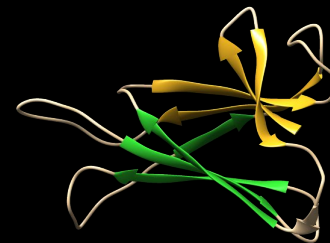
N-terminal NBD



C-terminal SBD -
C-terminal subdomain



C-terminal SBD -
N-terminal subdomain



Class 1000002
Alpha and beta proteins (a/b)

Class 1000000
All alpha proteins

Class 1000001
All beta proteins

Fold 2000067
Ribonuclease H-like motif

Fold 2000831
Immunoglobulin/albumin-binding
domain-like

Fold 2000420
HSP70, peptide-binding domain

Superfamily 3000092
Actin-like ATPases

Superfamily 3000668
HSP70, C-terminal subdomain

Superfamily 3000642
HSP70, peptide-binding domain

Family 4000313
Actin/HSP70

Family 4000583
HSP70, C-terminal
subdomain

Family 4000583
HSP70, peptide-binding domain

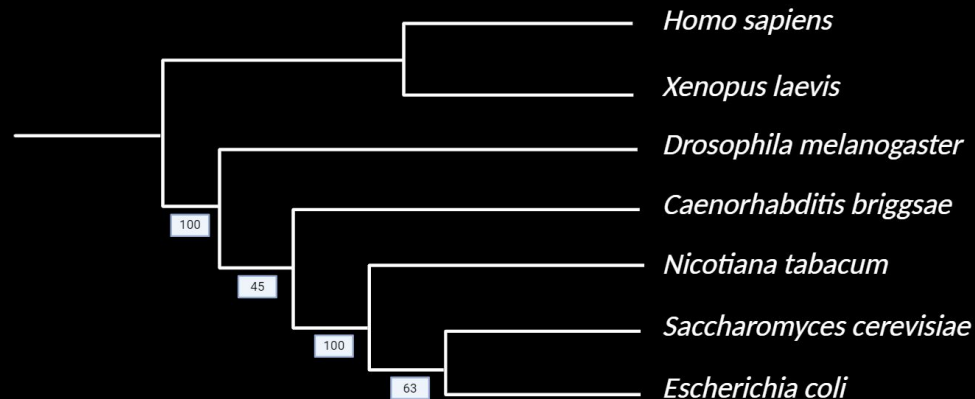
STRUCTURAL ANALYSIS OF HSP70



Multiple Sequence Alignment and Phylogeny

BLAST → UNIPROT: Selection of sequences from the species

Homo sapiens
Xenopus laevis
Drosophila melanogaster
Caenorhabditis briggsae
Nicotiana tabacum
Saccharomyces cerevisiae
Escherichia coli



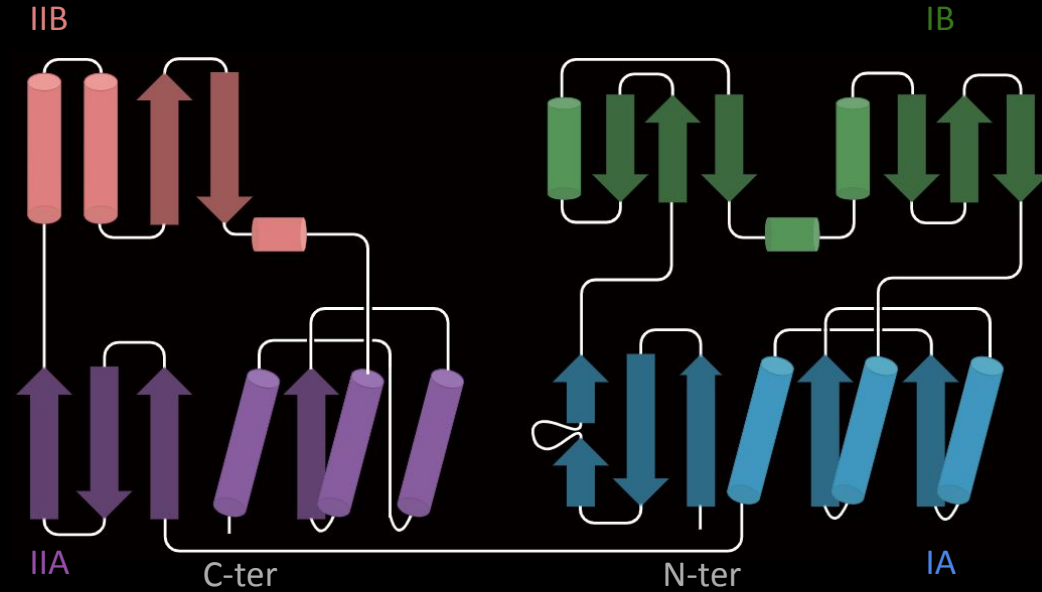
CLUSTALW

IQTREE

Homo vs. Xenopus: 93.43% identity
Homo vs. Drosophila: 80.43% identity
Homo vs. Caenorhabditis: 78.44% identity
Homo vs. Nicotiana: 69.42% identity
Homo vs. Saccharomyces: 65.60% identity
Homo vs. Escherichia: 48.12% identity

N-ter Nucleotide Binding Domain (NBD)

Two lobes with a **deep cleft** in between, where ATP/ADP will bind.
4 different subdomains:



IIANBD and IANBD are slightly more conserved.

	51	61	71	81	91
Consensus	TviGIDLGTT	YSCVgVYKNG	rveIIANDQG	NRITPSy	VAF Tpe - gERLIG
Conservation	■■■■■	■■■■■	■■■■■	■■■■■	■■■■■
Homo	TVVGIIDLGTT	YSCVGVYKNG	RVEIIANDQG	NRITPSYVAF	TPE - GERLIG
Xenopus	TVVGIIDLGTT	YSCVGVYKNG	RVEIIANDQG	NRITPSYVAF	TPE - GERLIG
Drosophila	TVIGIDLGTT	YSCVGVYKNG	RVEIIANDQG	NRITPSYVAF	TAD - GERLIG
Caenorhabditis	TVIGIDLGTT	YSCVGVYKNG	RVEIIANDQG	NRITPSYVAF	SGEQGDRRLIG
Nicotiana	TVIGIDLGTT	YSCVGVYKNG	HVEIIANDQG	NRITPSWVAF	TDG - -ERLIG
Saccharomyces	TVIGIDLGTT	YSCVAVMKNG	KTEILANEQG	NRITPSYVAF	TDD - -ERLIG
Escherichia	KIIGIDLGTT	NSCVAIMDGT	TPRVLENAEG	DRTTPIIAY	TQD - GETLVG

	101	111	121	131	141
Consensus	dAAKNQltsN	PeNTvFDaKR	LIGRkwnDps	VQqDIKhIPF	KvveKd - gKP
Conservation	■■■■■	■■■■■	■■■■■	■■■■■	■■■■■
Homo	DAAKNQLTSN	PENTVFDaKR	LIGRTWNDPS	VQQDIKFLPF	KVVERK - TKP
Xenopus	DAAKNQLTSN	PENTVFDaKR	LIGRTWNDPS	VQQDIKFLPF	KVVERK - TKP
Drosophila	DAAKNQLTSN	PENTVFDaKR	LIGREWSDTN	VQHDIKFFPF	KVVERK - SKP
Caenorhabditis	DAAKNQLTIN	PENTIFDaKR	LIGRDYNDKT	VQADIKHWPF	KVLDKS - NKP
Nicotiana	EAAKNLA AVN	PERTVFDV KR	LIGRKFD DKE	VQRDMKLV PY	KIVNKD - GKP
Saccharomyces	DAAKNQVAAN	PQNTIFDIKR	LIGLKYND RS	VQKDIKHL PF	NVVNKD - GKP
Escherichia	QPAKRQAVTN	PQNTLFAIKR	LIGRRFQDEE	VQRDVSIMPF	KIIAADNGDA

	151	161	171	181	191
Consensus	yieVki g - g q	tKtFaPEEIS	AMvLtKMKet	AEaYLGkkVt	hAVVTVPAYF
Conservation	■■■■■	■■■■■	■■■■■	■■■■■	■■■■■
Homo	YIQVDITGGGQ	TKTFAPPEIS	AMVLT KMKET	AEAYLGKKVT	HAVVTVPAYF
Xenopus	YIEVDIG - DQ	MKTFAPEEIS	AMVLVKMKET	AEAYLGRKVT	HAVVTVPAYF
Drosophila	HISVDTS - QG	AKVFAPEEIS	AMVLGKMKET	AEAYLGKKVT	HAVVTVPAYF
Caenorhabditis	SVEVKG - AD	NKQFTPEEVS	AMVLVKMKEI	AEAYLGKKEVK	HAVVTVPAYF
Nicotiana	YIQVKIKDGE	TKIFSPPEEIS	AMILT KMKET	AEAYLGKKIK	DAVVTVPAYF
Saccharomyces	AVEVSVK - GE	KKVFTPEEIS	GMILGKMKQI	AEDYLGTKVT	HAVVTVPAYF
Escherichia	WVEVKGQ - -	- - KMAPPOIS	AEVLKMKKKT	AEDYLGEPVT	EAVITVPAYF

	201	211	221	231	241
Consensus	NDAQRQATKD	AGtIAGLnVm	RIINEPTAAA	IAYGLDKkeG	EknIlVfDLG
Conservation	■■■■■	■■■■■	■■■■■	■■■■■	■■■■■
Homo	NDAAQRQATKD	AGTIAGLNVm	RIINEPTAAA	IAYGLDKKEG	EKNILVFDLG
Xenopus	NDAAQRQATKD	AGTIAGLNVm	RIINEPTAAA	IAYGLDKKEG	EKNILVFDLG
Drosophila	NDAAQRQATKD	AGV IAGLQVM	RIINEPTAAA	IAYGLDKKEG	EKNILVFDLG
Caenorhabditis	NDAAQRQATKD	AGTIAGLNVV	RIINEPTAAA	IAYGLDKKDG	ERNILVFDLG
Nicotiana	NDAAQRQATKD	AGV IAGLNVV	RIINEPTAAA	IAYGLDKKGG	EKNILVFDLG
Saccharomyces	NDAAQRQATKD	AGTIAGLNVV	RIVNEPTAAA	IAYGLDKSDK	EHQIIVYDLG
Escherichia	NDAAQRQATKD	AGR IAGLEVK	RIINEPTAAA	LAYGLDKGTG	NRTIAYVDLG

NBD - IA

NBD - IB

NBD - IIA

IA NBD sequence
interrupted by IB NBD
sequence.

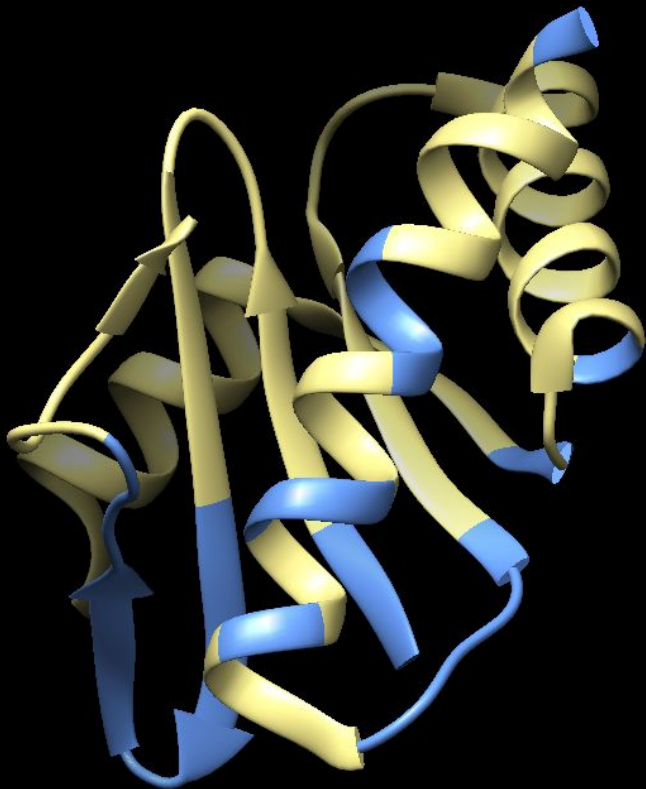
	251	261	271	281	291
Consensus	GGTFDVSILt	IDNG - - - VF	EVIATNGDTH	LGGEDFDqRv	me y f i k l y KK
Conservation					
Homo	GGTFDVSLLT	IDNG - - - VF	EVVATNGDTH	LGGEDFDQRV	MEHFIKLYKK
Xenopus	GGTFDVSLLT	IDNG - - - VF	EVVATNGDTH	LGGEDFDQRV	MEHFIKLYKK
Drosophila	GGTFDVSLLT	IDNG - - - VF	EVVATNGDTH	LGGEDFDQRV	MDHFIKLYKK
Caenorhabditis	GGTFDVSMLT	IDNG - - - VF	EVLATNGDTH	LGGEDFDQRV	MEYFIKLYKK
Nicotiana	GGTFDVSILT	IDNG - - - VF	EVLSTNGDTH	LGGEDFDQRI	MEYFIKLIKK
Saccharomyces	GGTFDVSLLS	IENG - - - VF	EVQATSGDTH	LGGEDFDYKI	VRQLIKAFKK
Escherichia	GGTFDISIIE	IDEVDGEKTF	EVLATNGDTH	LGGEDFDSRL	INYLVEEFKK
	301	311	321	331	341
Consensus	KhGkDvrkDn	rAvqKLrREV	EKAKRALSSq	hQtrIEIEsF	feGeD - - - f
Conservation					
Homo	KTGKDVRRKN	RAVQKLRRREV	EKAKRALSSQ	HQARTETESF	YEGED - - - F
Xenopus	KTGKDVRA DK	RAVQKLRRREV	EKAKRALSAQ	HQSRIEIESF	FEGED - - - F
Drosophila	KKGKD I R K D N	RAVQKLRRREV	EKAKRALSSG	HQVRIEIESF	FEGDD - - - F
Caenorhabditis	KSGKDLR K D K	RAVQKLRRREV	EKAKRALSTQ	HQTKVEIESL	FDGED - - - F
Nicotiana	KH GK D I S K D N	RALGKLRRREA	ERAKRALSSQ	HQVRVEIESL	FDGVD - - - F
Saccharomyces	KH G I D V S D N N	KALAKLKREA	EKAKRALSSQ	MSTRIEIDSF	VDGID - - - L
Escherichia	DQGI D L R N D P	LAMQRLKEAA	EKAKIE LSSA	QQT D V N L P Y I	TADATGPKHM
	351	361	371	381	391
Consensus	SEtLTRAkFE	ELN mDLFrst	mkPVqKvLeD	sdlkKsDide	IVLVGGSTR I
Conservation					
Homo	SETLTRAkFE	ELNMDLFRST	MKPVQKVLED	SDLKKS DIDE	IVLVGGSTR I
Xenopus	SETLTRAkFE	ELNMDLFRST	MKPVQKVLED	SDLKKS DIDE	IVLVGGSTR I
Drosophila	SETLTRAkFE	ELNLDLFRST	LKPVQKVLED	ADMNKKDVHE	IVLVGGSTR I
Caenorhabditis	SETLTRAkFE	ELNMDLFRAT	LKPVQKVLED	SDLK KDDVHE	IVLVGGSTR I
Nicotiana	SEPLTRARFE	ELNMDLFRKT	MGPVKVAMDD	AGLEKTQIDE	IVLVGGSTR I
Saccharomyces	SETLTRAkFE	ELNLDLFKKT	LKPVEKVLQD	SGLEKKDVDD	IVLVGGSTR I
Escherichia	NIKVTRAKLE	SLVEDLVNRS	IEPLKVALQD	AGLSVSDIDD	VILVGGQTRM
	401	411	421	431	441
Consensus	PKvQQLvkef	F nGKEPs rGi	NPDEAVAYGA	AVQaGVLS Ge	- - q d t g d i v L
Conservation					
Homo	PKIQQLVKEL	FNGKEPSRG I	NPDEAVAYGA	AVQAGVLSGD	- - QDTGDLVL
Xenopus	PKIQQLVKEL	FNGKEPSRG I	NPDEAVAYGA	AVQAGVLSGD	- - QDTGDLVL
Drosophila	PKVQQLVKDF	FNGKEPSRG I	NPDEAVAYGA	AVQAGVLSGE	- - QDTDAIVL
Caenorhabditis	PKVQQLVKEF	FNGKEPSRG I	NPDEAVAYGA	AVQGGVLSGE	- - EDTGEIVL
Nicotiana	PKVQQLLKDY	FDGKEPNKGV	NPDEAVAYGA	AVQGGVLSGE	GGDET KD ILL
Saccharomyces	PKVQQLLESY	FDGKKASKGI	NPDEAVAYGA	AVQAGVLSGE	- - EGVEDIVL
Escherichia	PMVQKKVAEF	F - GKEPRKDV	NPDEAVAIGA	AVQGGVLTGD	- - - - VKDVLL

NBD - IIA

NBD - IIB

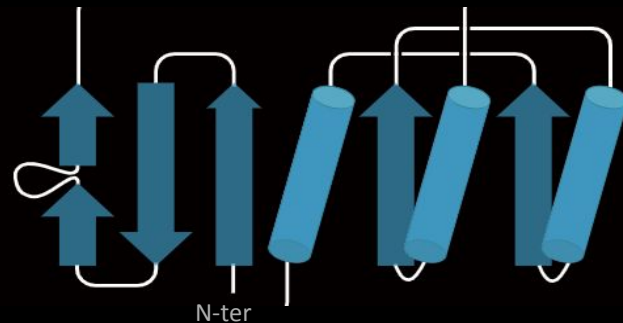
IIANBD sequence
interrupted by IIBNBD
sequence.

NBD - IA



Conserved regions:

- Closer to the deep cleft for **binding ATP/ADP.**
- Outer alpha helices for **binding SBD.**



Consensus	v i	G I D L G T T Y	S C V	g V y K N G r	v E I i A N d	Q G N	R I T P	a P E E I S A M v L
Conservation								
Homo	V V	G I D L G T T Y	S C V	G V F K N G R	V E I I A N D	Q G N	R I T P	A P E E I S A M V L
Xenopus	V V	G I D L G T T Y	S C V	G V F K N G R	V E I I A N D	Q G N	R I T P	A P E E I S A M V L
Drosophila	V I	G I D L G T T Y	S C V	G V Y K N G R	V E I I A N D	Q G N	R I T P	A P E E I S A M V L
Caenorhabditis	I I	G I D L G T T Y	S C V	G V Y K N G R	V E I I A N D	Q G N	R I T P	T P E E V S A M V L
Nicotiana	V I	G I D L G T T Y	S C V	G V Y K N G H	V E I I A N D	Q G N	R I T P	S P E E I S A M I L
Saccharomyces	V I	G I D L G T T Y	S C V	A V M K N G K	T E I L A N E	Q G N	R I T P	T P E E I S G M I L
Escherichia	I I	G I D L G T T N	S C V	A I M D G T T	P R V L E N A	E G D	R T T P	A P P Q I S A E V L

Consensus	t	K M K	e t	A E	a Y	L G	k k	V t h	A V V	T V P A Y F N D A Q	R Q A T K D A G	t i	A G L	n V m	R I I N	E P T A A A I A Y G	L D
Conservation																	
Homo	T	K M K	E T	A E	A Y	L G	K K	V T H	A V V	T V P A Y F N D A Q	R Q A T K D A G	T I	A G L	N V M	R I I N	E P T A A A I A Y G	L D
Xenopus	V	K M K	E T	A E	A Y	L G	R K	V T H	A V V	T V P A Y F N D A Q	R Q A T K D A G	T I	A G L	N V M	R I I N	E P T A A A I A Y G	L D
Drosophila	G	K M K	E T	A E	A Y	L G	K K	V T H	A V V	T V P A Y F N D A Q	R Q A T K D A G	V I	A G L	Q V M	R I I N	E P T A A A I A Y G	L D
Caenorhabditis	V	K M K	E I	A E	S Y	L G	K E	V K H	A V V	T V P A Y F N D A Q	R Q A T K D A G	T I	A G L	N V V	R I I N	E P T A A A I A Y G	L D
Nicotiana	T	K M K	E T	A E	A Y	L G	K K	I K D	A V V	T V P A Y F N D A Q	R Q A T K D A G	V I	A G L	N V A	R I I N	E P T A A A I A Y G	L D
Saccharomyces	G	K M K	Q I	A E	D Y	L G	T K	V T H	A V V	T V P A Y F N D A Q	R Q A T K D A G	T I	A G L	N V A	R I V N	E P T A A A I A Y G	L D
Escherichia	K	K M K	K T	A E	D Y	L G	E P	V T E	A V I	T V P A Y F N D A Q	R Q A T K D A G	R I	A G L	E V K	R I I N	E P T A A A I A Y G	L D

A 3D ribbon diagram of the 19S proteasome structure. The structure is composed of two main parts: a large green subunit and a smaller yellow subunit. The green subunit is a large, complex structure with multiple loops and turns, while the yellow subunit is a smaller, more compact structure. The two subunits are intertwined, forming a complex, cage-like structure. The background is black, making the protein structure stand out.

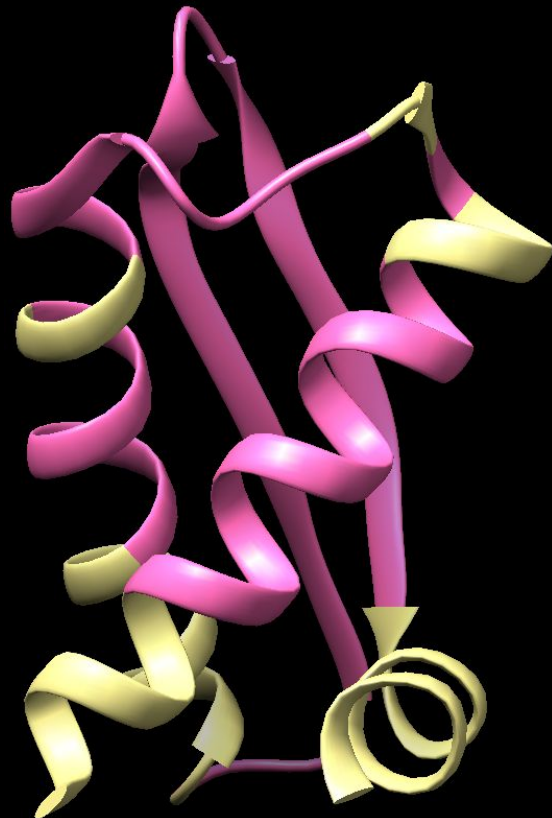
The diagram shows a quantum circuit with two parallel blocks of four gates each, connected by a CNOT gate. Each block contains a CNOT gate followed by three single-qubit rotation gates. The first block's gates are labeled R_1, R_2, R_3 and the second block's gates are labeled R_4, R_5, R_6 . The CNOT gate connects the two blocks, with the first qubit as the control and the second qubit as the target.

Consensus Conservation	V	A	F	T	P	e	-	G	E	R	L	I	G	D	A	A	K	N	Q	L	I	t	s	N	P	E	N	T	V	F	D	a	K	R	L	I	G	R	k	w	n	D	p	s	V	Q	Q	D	I	K	H	I	P	F	K	v	v	e	K	D	-	g	K	P	y	i	e	V	k	i	g	-	g	q	t	K	t	F		
Homo	V	A	F	T	P	E	-	G	E	R	L	I	G	D	A	A	K	N	Q	L	L	T	S	N	P	E	N	T	V	F	D	A	K	R	L	I	G	R	T	W	N	D	P	S	V	Q	Q	D	I	K	F	L	P	F	K	V	V	E	K	K	-	T	K	P	Y	I	Q	V	D	I	G	I	G	G	Q	T	K	T	F	
Xenopus	V	A	F	T	P	E	-	G	E	R	L	I	G	D	A	A	K	N	Q	L	L	T	S	N	P	E	N	T	V	F	D	A	K	R	L	I	G	R	T	W	N	D	P	S	V	Q	Q	D	I	K	F	L	P	F	K	V	I	E	K	K	-	T	K	P	Y	I	E	V	D	I	G	I	G	-	D	Q	M	K	T	F
Drosophila	V	A	F	T	A	D	-	G	E	R	L	I	G	D	A	A	K	N	Q	L	L	T	T	N	P	E	N	T	V	F	D	A	K	R	L	I	G	R	E	W	S	D	T	N	V	Q	H	D	I	K	F	F	P	F	K	V	E	K	N	-	S	K	P	H	I	S	V	D	T	S	-	Q	G	A	K	V	F			
Caenorhabditis	V	A	F	S	G	E	Q	G	D	R	L	I	G	D	A	A	K	N	Q	L	L	T	I	N	P	E	N	T	I	F	D	A	K	R	L	I	G	R	D	Y	N	D	K	T	V	Q	A	D	I	K	H	W	P	F	K	V	L	D	K	S	-	N	K	P	S	V	E	V	K	V	G	-	A	D	N	K	Q	F		
Nicotiana	V	A	F	T	D	G	-	-	E	R	L	I	G	D	A	A	K	N	L	A	L	A	V	N	P	E	R	T	V	F	D	V	K	R	L	I	G	R	K	F	D	D	K	E	V	O	R	D	M	K	L	V	P	Y	K	I	V	N	K	D	-	G	K	P	I	Q	V	K	I	K	D	G	E	T	K	I	F			
Saccharomyces	V	A	F	T	D	D	-	-	E	R	L	I	G	D	A	A	K	N	Q	L	V	A	A	N	P	Q	N	T	I	F	D	I	K	R	L	I	G	L	K	Y	N	D	D	R	S	V	Q	K	D	I	K	H	L	F	N	V	N	K	D	-	G	K	P	A	V	E	S	V	K	-	-	G	E	K	V	F				
Escherichia	I	A	Y	T	Q	D	-	G	E	T	L	V	G	Q	P	A	K	R	Q	A	A	V	T	N	P	Q	N	T	L	F	A	I	K	R	L	I	G	R	R	F	Q	D	E	E	V	O	R	D	V	S	I	M	P	F	K	I	A	A	D	N	G	D	A	W	E	V	K	G	Q	-	-	-	-	-	K	M				

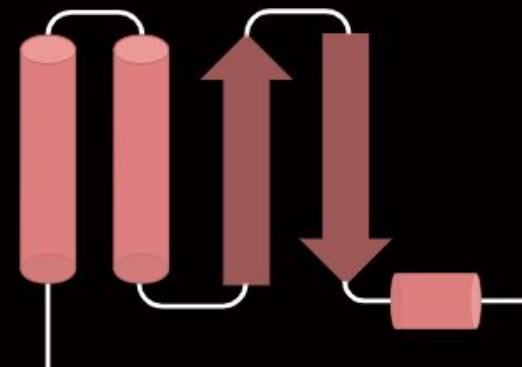
Consensus	E	k	n	I	V	f	DLG	GGT	F	D	V	S	I	L	t	I	D	N	G	-	-	-	V	F	E	V	I	A	T	N	G	D						
Conservation																																						
Homo	E	K	N	I	L	V	F	D	L	G	G	G	T	F	D	V	S	I	L	T	I	D	N	G	-	-	-	V	F	E	V	I	A	T	N	G	D	
Xenopus	E	K	N	I	L	V	F	D	L	G	G	G	T	F	D	V	S	L	L	T	I	D	N	G	-	-	-	V	F	E	V	I	A	T	N	G	D	
Drosophila	E	K	N	V	L	V	F	D	L	G	G	G	T	F	D	V	S	L	L	T	I	D	N	G	-	-	-	V	F	E	V	I	A	T	N	G	D	
Caenorhabditis	E	R	N	I	L	V	F	D	L	G	G	G	T	F	D	V	S	M	L	T	I	D	N	G	-	-	-	V	F	E	V	I	A	T	N	G	D	
Nicotiana	E	K	N	I	L	V	F	D	L	G	G	G	T	F	D	V	S	I	L	T	I	D	N	G	-	-	-	V	F	E	V	I	A	T	N	G	D	
Saccharomyces	E	H	Q	I	I	V	Y	D	L	G	G	G	T	F	D	V	S	L	L	S	I	E	N	G	-	-	-	V	F	E	V	I	A	T	S	G	D	
Escherichia	N	R	T	I	A	V	Y	D	L	G	G	G	T	F	D	I	S	I	I	E	I	D	E	V	D	G	E	K	T	F	E	V	I	A	T	N	G	D

Consensus	mDLFrSTmkP										VQKVLeDsdL					kKsDideIVL					VGGSTRIPKv					QQLvkeffNg					KEPsrGiNPD					EAVAYGAAVQ					aGVLS																																			
Conservation																																																																												
Homo	M	D	L	F	R	S	T	M	K	P	V	Q	K	V	L	E	D	S	D	L	K	K	S	D	I	D	E	I	V	L	V	G	G	S	T	R	I	P	K	I	Q	Q	L	V	K	E	F	F	N	G	K	E	P	S	R	G	I	N	P	D	E	A	V	A	Y	G	A	A	V	Q	A	G	V	L	S	
Xenopus	M	D	L	F	R	S	T	M	K	P	V	Q	K	V	L	D	D	S	D	L	K	K	S	D	I	D	E	I	V	L	V	G	G	S	T	R	I	P	K	I	Q	Q	L	V	K	E	L	F	F	N	G	K	E	P	S	R	G	I	N	P	D	E	A	V	A	Y	G	A	A	V	Q	A	G	V	L	S
Drosophila	L	D	L	F	R	S	T	L	K	P	V	Q	K	V	L	E	D	A	D	M	N	K	K	D	V	H	E	I	V	L	V	G	G	S	T	R	I	P	K	V	Q	Q	L	V	K	D	F	F	G	G	K	E	P	S	R	G	I	N	P	D	E	A	V	A	Y	G	A	A	V	Q	A	G	V	L	S	
Caenorhabditis	M	D	L	F	R	A	T	L	K	P	V	Q	K	V	L	E	D	S	D	L	K	K	D	V	H	E	I	V	L	V	G	G	S	T	R	I	P	K	V	Q	Q	L	I	K	E	F	F	N	G	K	E	P	S	R	G	I	N	P	D	E	A	V	A	Y	G	A	A	V	Q	G	G	V	I	S		
Nicotiana	N	D	L	F	R	K	T	M	K	P	V	K	K	A	M	D	A	G	L	E	K	T	Q	I	D	E	I	V	L	V	G	G	S	T	R	I	P	K	V	Q	Q	L	L	K	D	Y	F	D	G	K	E	P	N	K	V	N	P	D	E	A	V	A	Y	G	A	A	V	Q	G	G	I	L	S			
Saccharomyces	L	D	L	F	K	K	T	L	K	P	V	E	K	V	L	Q	D	S	G	L	E	K	K	D	V	D	I	V	L	V	G	G	S	T	R	I	P	K	V	Q	Q	L	L	E	S	Y	F	D	G	K	K	A	S	K	G	I	N	P	D	E	A	V	A	Y	G	A	A	V	Q	A	G	V	L	S		
Escherichia	E	D	L	V	N	R	S	I	E	P	L	K	V	A	L	Q	D	A	G	L	S	V	S	D	I	D	D	V	I	L	V	G	G	Q	T	R	M	P	M	V	Q	K	K	V	A	E	F	F	-	G	K	E	P	R	K	D	V	N	P	D	E	A	V	A	I	G	A	A	V	Q	G	G	V	L	S	

NBD - IIB

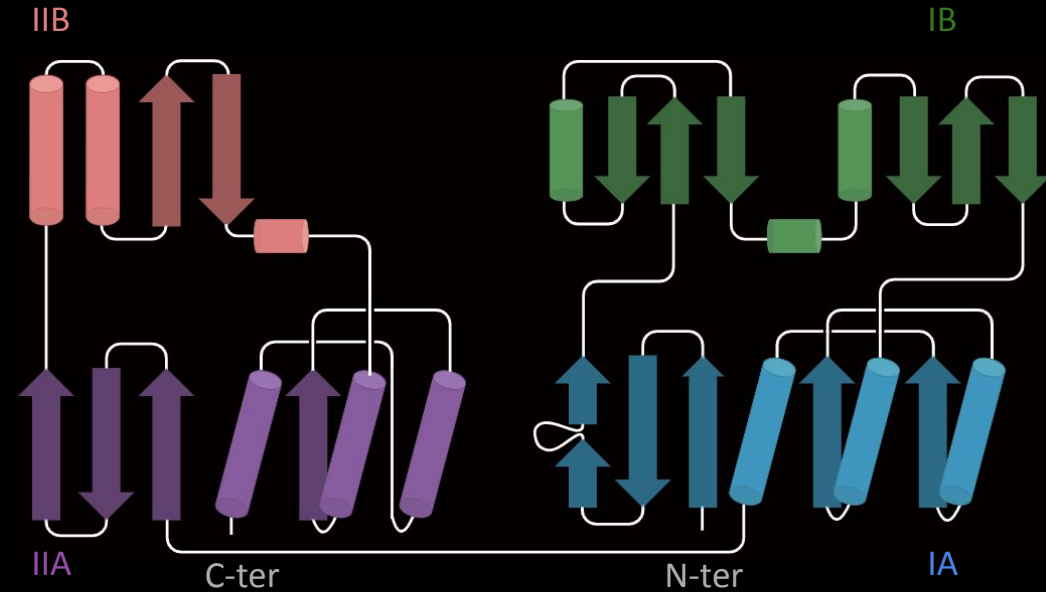
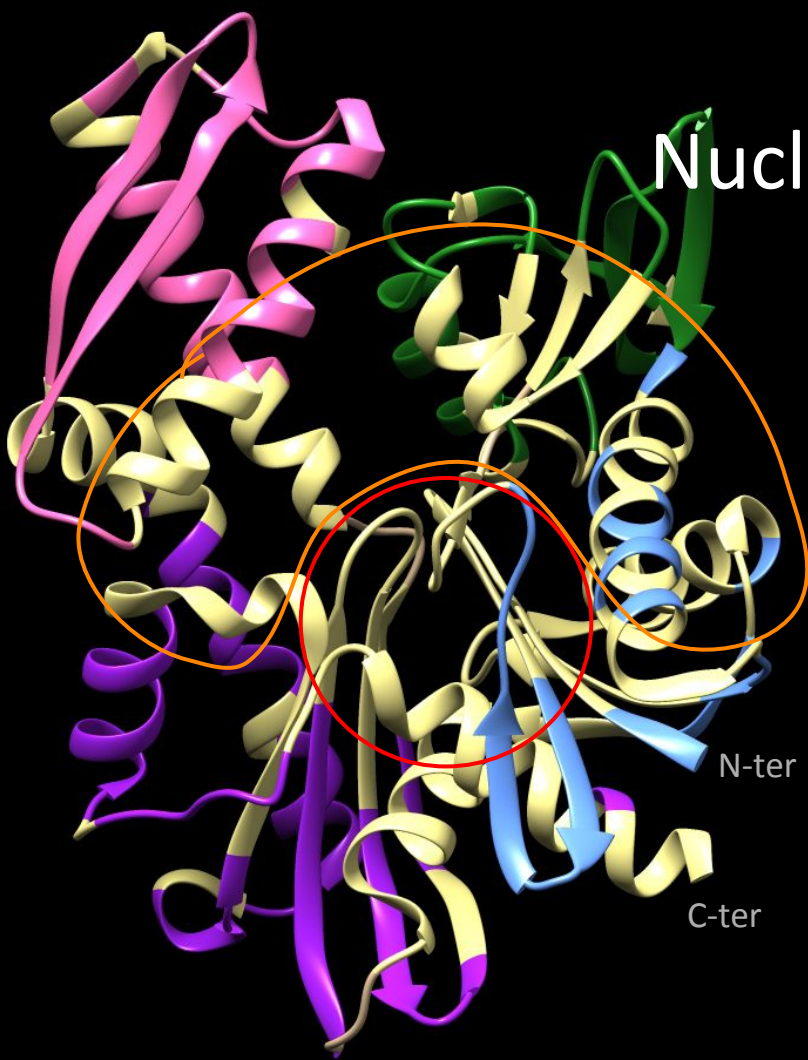


Regions closer to the deep cleft conserved for binding ATP/ADP.



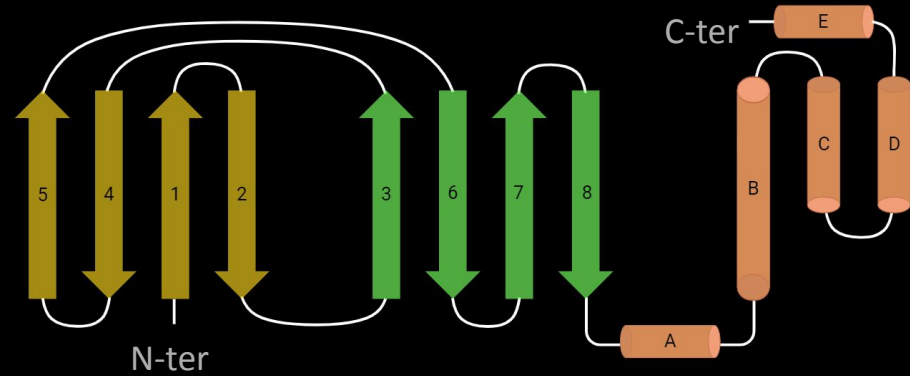
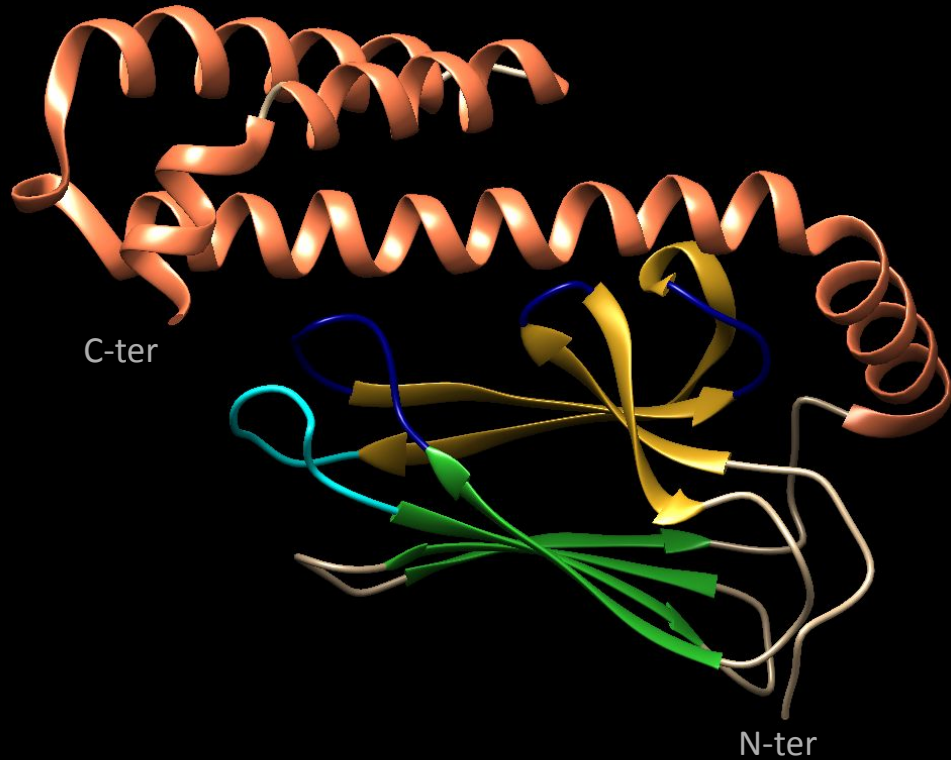
Consensus	GGEDFD										qRvm	eyfIKly	KKK
Conservation													
Homo	GGEDFD	QRVM	EHFIKLY	KKK									
Xenopus	GGEDFD	QRVM	EHFIKLY	KKK									
Drosophila	GGEDFD	QRVM	DHFIKLY	KKK									
Caenorhabditis	GGEDFD	QRVM	EYFIKLY	KKK									
Nicotiana	GGEDFD	QRIM	EYFIKLY	KKK									
Saccharomyces	GGEDFD	YKIV	RQLIKAF	KKK									
Escherichia	GGEDFD	SRLI	NYLVEEF	KKD									
Consensus	v r k D n r	A v q K L r R E v	E	K A K R A L S	s q h Q t r i E l e S f f	e G E D	- - - - f S	E t L	T R A K F E E	L N			
Conservation													
Homo	VRKDNr	AVQKLRRREVE	E	KAKRALs	SQHQAriEIESfY	EGED	- - - - FS	ETL	TRAKFEE	LN			
Xenopus	VRADKR	AVQKLRRREVE	E	KAKRALs	AQHQSRIEIESFF	EGED	- - - - FS	ETL	TRAKFEE	LN			
Drosophila	IRKDNr	AVQKLRRREVE	E	KAKRALs	GSHQVRiEIESfF	EGDD	- - - - FS	ETL	TRAKFEE	LN			
Caenorhabditis	LRKDKR	AVQKLRRREVE	E	KAKRALs	TQHQTkVEIESLF	DGED	- - - - FS	ETL	TRAKFEE	LN			
Nicotiana	ISKDNr	ALGKLRRREAE	E	RAKRALs	SQHQVRVEIESLF	DGVD	- - - - FS	EPL	TRARFEE	LN			
Saccharomyces	VSDNrk	ALAKLKREAE	E	KAKRALs	SQMSTRiEIDSFV	DGID	- - - - LS	ETL	TRAKFEE	LN			
Escherichia	LRNDPL	AMQRLKEAAE	E	KAKIELS	SAQQTdVNLpYIT	ADATGPKHMN		IKV	TRAKLES	LV			

Conserved regions of the Nucleotide Binding Domain (NBD)



Regions **closer to the deep cleft** conserved for **binding ATP/ADP**.
Other **regions** conserved for **binding SBD**.

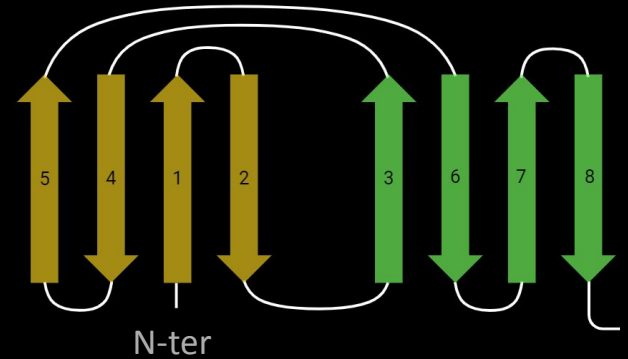
C-ter Substrate Binding Domain (SBD)



Two well differentiated subdomains:

- C-terminal alpha helical lid subdomain
- N-terminal twisted β -sandwich

N-terminal twisted β -sandwich



β -sandwich composed of eight **antiparallel β -strands**:

The four **upper** β -strands (**1, 2, 4, 5**) form an unusual structure $\rightarrow$ **β 1- β 2 tilt up 20°** compared to β 4- β 5.
The four **lower** β -strands (**3, 6, 7, 8**) have a regular twist.

Consensus	I G I E T v G G V M	T k L I p R N T v i	P T K K S Q v F S T	A s D N Q p T V T I	q V y E G E R p m t
Conservation					
Homo	L G I E T V G G V M	T K L I P R N T V V	P T K K S Q I F S T	A S D N Q P T V T I	K V Y E G E R P L T
Xenopus	L G I E T V G G V M	T K L I P R N T V V	P T K K S Q I F S T	A S D N Q P T V T I	K V Y E G E R P L T
Drosophila	M G I E T V G G V M	T K L I P R N T V I	P T K K S Q V F S T	A S D N Q H T V T I	Q V Y E G E R P M T
Caenorhabditis	M G I E T V G G V M	T K L I S R N T V I	P T K K S Q V F S T	A A D N Q P T V T I	Q V F E G E R P M T
Nicotiana	L G I E T V G G V M	T K L I P R N T V I	P T K K S Q V F T T	Y Q D Q Q T T V T I	Q V F E G E R S L T
Saccharomyces	L G I E T T G G V M	T P L I K R N T A I	P T K K S Q I F S T	A V D N Q P T V M I	K V Y E G E R A M S
Escherichia	L G I E T M G G V M	T T L I A K N T T I	P T K H S Q V F S T	A E D N Q S A V T I	H V L Q G E R K R A

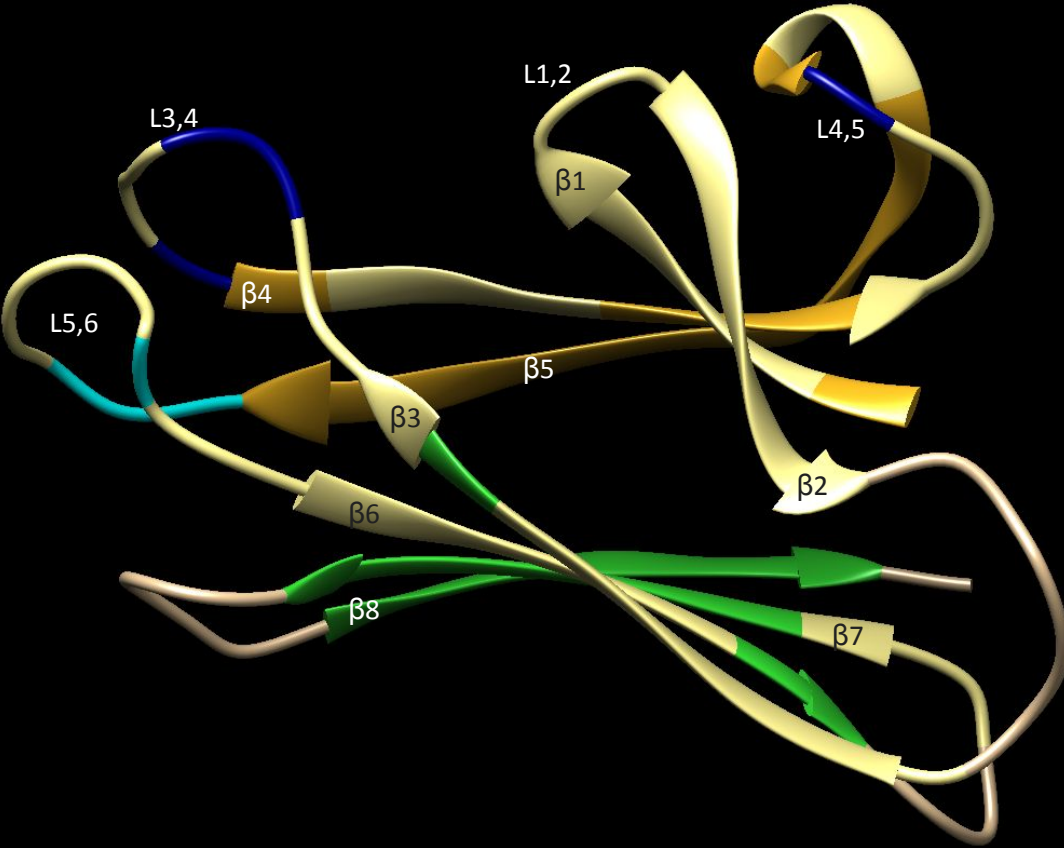
SBD - beta more conserved than

SBD - alpha

Consensus	K D N h I L G k F d	L T G I p P A P R G	v P Q I E V T F e i	D a N G I L h v s A	e D K g t G n k e K
Conservation					
Homo	K D N H L L G T F D	L T G I P P A P R G	V P Q I E V T F E I	D V N G I L R V T A	E D K G T G N K N K
Xenopus	K D N H L L G T F D	L T G I P P A P R G	V P Q I E V T F E I	D V N G I L R V T A	E D K G T G N K N K
Drosophila	K D N H L L G K F D	L T G I P P A P R G	I P Q I E V S F E I	D A N G I L Q V S A	E D K G T G N K E K
Caenorhabditis	K D N H Q L G K F D	L T G I P P A P R G	V P Q I E V T F E I	D V N G I L H V T A	E D K G T G N K N K
Nicotiana	K D C R L L G K F D	L T G I A P A P R G	T P Q I E V T F E V	D A N G I L N V K A	E D K A S G K S E K
Saccharomyces	K D N N L L G K F E	L T G I P P A P R G	V P Q I E V T F A L	D A N G I L K V S A	T D K G T G K S E S
Escherichia	A D N K S L G Q F N	L D G I N P A P R G	M P Q I E V T F D I	D A D G I L H V S A	K D K N S G K E Q K

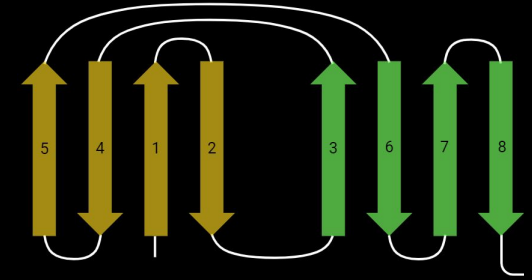
Consensus	I T I T N D q n r L	t p E e I e R M v r	d A E k F A e e D k	K I K E r i e t R N	e L E s Y a y s I k
Conservation					
Homo	I T I T N D Q N R L	T P E E I E R M V N	D A E K F A E E D K	K L K E R I D T R N	E L E S Y A Y S L K
Xenopus	I T I T N D Q N R L	T P E E I E R M V T	D A E K F A E E D K	K L K E R I D T R N	E L E S Y A Y S L K
Drosophila	I V I T N D Q N R L	T P E D I D R M I R	D A E K F A E E D K	K L K E R V E S R N	E L E S Y A Y S L K
Caenorhabditis	I T I T N D Q N R L	S P E D I E R M I N	D A E K F A E E D K	K V K E K A E A R N	E L E S Y A Y S L K
Nicotiana	I T I T N D K G R L	S Q E E I E R M V K	E A E E F A E E D K	K V K E R I D A R N	S L E T Y V Y N M R
Saccharomyces	I T I T N D K G R L	T Q E E I D R M V E	E A E K F A S E D A	S I K A K V E S R N	K L E N Y A H S L K
Escherichia	I T I K A S S G - L	N E D E I Q K M V R	D A E A N A E A D R	K F E E L V Q T R N	Q G D H L L H S T R

Consensus	N Q i g d k e k L G	g K L s s d d K e t	i E k A v e e a i e	W L e s n q d - A d	i E d f k e k k k e	l e a i v q P I i s
Conservation						
Homo	N Q I G D K E K L G	G K L S S E D K E T	M E K A V E E K I E	W L E S H Q D - A D	I E D F K A K K K E	L E E I V Q P I I S
Xenopus	N Q I G D K E K L G	G K L S S E D K E T	I E K A V E E K I E	W L E S H Q D - A D	I E D F K A K K K E	L E E I V Q P I V G
Drosophila	N Q I G D K D K L G	A K L S D D E K N K	L E S A I D E S I K	W L E Q N P D - A D	P E E Y K K Q K K D	L E A I V Q P V I A
Caenorhabditis	N Q I G D K E K L G	G K L S D D E K K T	I E E A V D E A I S	W L G S N A D - A S	A E E L K E Q K K E	L E G K V Q P I V S
Nicotiana	N Q I N D K D K L A	D K L E S D E K E K	I E T A T K E A L E	W L D D N Q S - A E	K E D Y E E K L K E	V E A V C N P I I T
Saccharomyces	N Q V N G - - D L G	E K L E E E D K E T	L L D A A N D V L E	W L D D N F E T A I	A E D F D E K F E S	L S K V A Y P I T S
Escherichia	K Q V E E A - - - G	D K L P A D D K T A	I E S A L T A L E T	A L K G E D K - A S	I E A K M Q E L A Q	V S Q K L M E I A Q



SBD - Beta sandwich

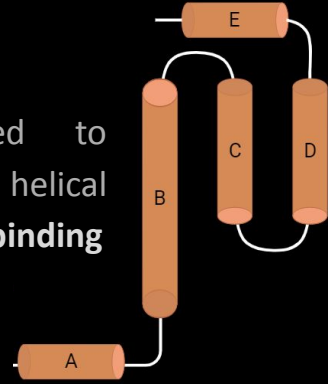
Regions conserved for binding the substrate →
hydrophobic substrate binding cavity.



Consensus	PTTKSQ		v	FST	As	DNQ	p	TVT	I	q	VY	EGER	p	m	t	KD	Nh	L	G	k	F	d	LT	G	I	p	P	A	P	R	G	v	P	Q	I	E	V	T	F	e	i	D	a	N	G	I	L	h	V	S	A	e	D	K	g	t	G	n	k	e	K	I	T	I	T																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																						
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A 3D ribbon diagram of the SBD domain, showing two alpha-helices in orange and yellow.

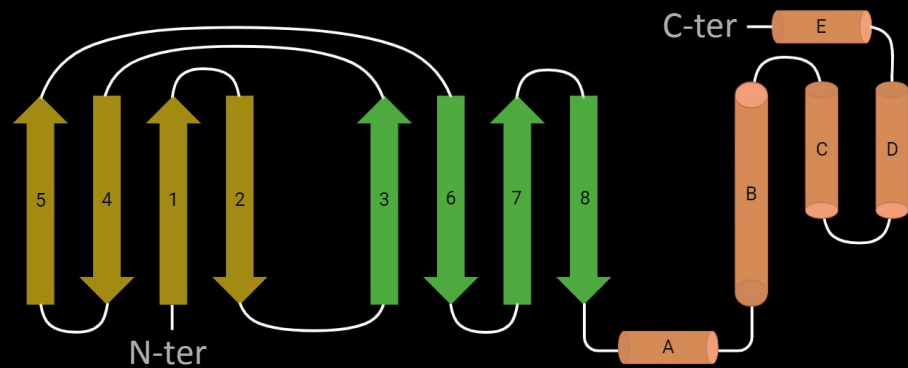
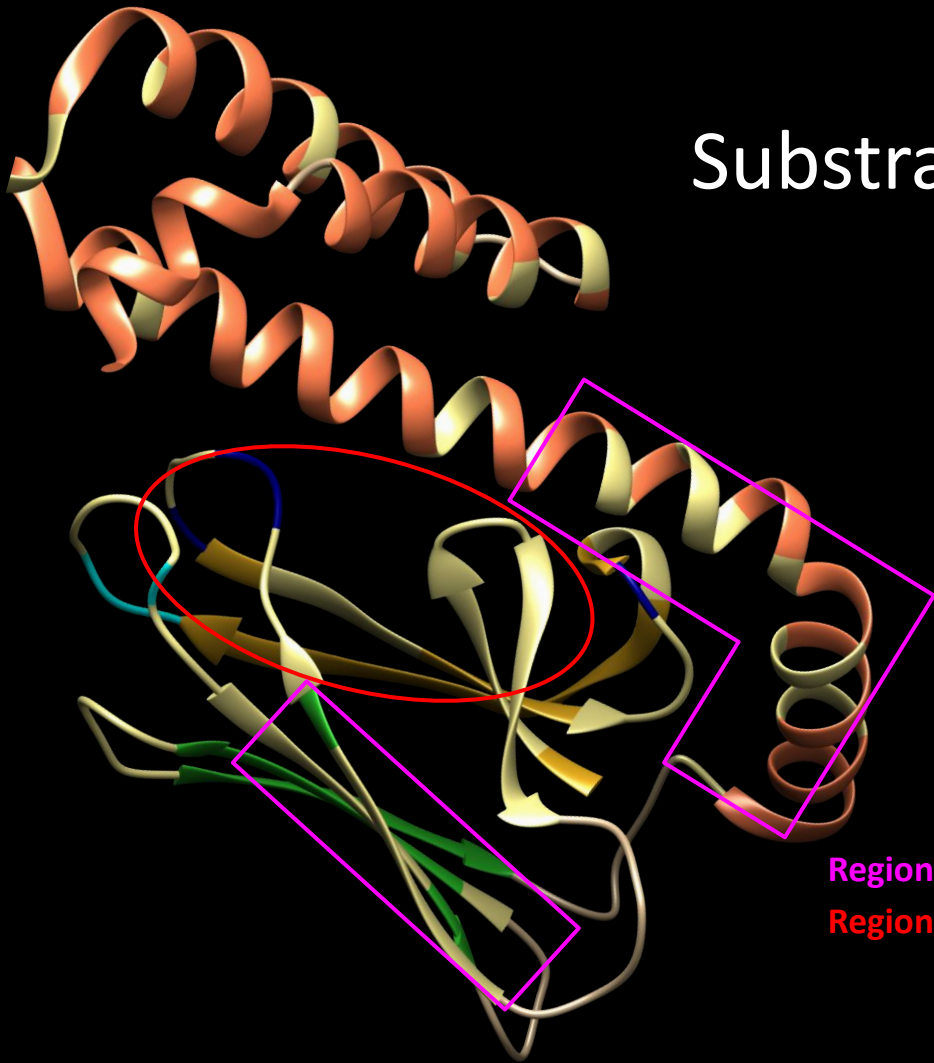
Regions conserved to ensure the helical structure and for **binding to the β -sandwich.**



Consensus	L	T	P	E	E	I	E	R	M	V	r	d	A	E	K	F	A	e	D	k	K	I	K	E	r	i	e	r	R	N	e	L	E	S	Y	A	Y	S	L	
Conservation																																								
Homo	L	T	P	E	E	I	E	R	M	V		N	D	A	E	K	F	A	e	D	K	K	L	K	E	R	I	D	T	R	N	E	L	E	S	Y	A	Y	S	L
Xenopus	L	T	P	E	E	I	E	R	M	V		T	D	A	E	K	F	A	e	D	K	K	L	K	E	R	I	D	T	R	N	E	L	E	S	Y	A	Y	S	L
Drosophila	L	T	P	E	D	I	D	R	M	I		R	D	A	E	K	F	A	e	D	K	K	L	K	E	R	V	E	S	R	N	E	L	E	S	Y	A	Y	S	L
Caenorhabditis	L	S	P	E	D	I	E	R	M	I		N	D	A	E	K	F	A	e	D	K	K	V	K	E	K	A	E	A	R	N	E	L	E	S	Y	A	Y	S	L
Nicotiana	L	S	Q	E	E	I	E	R	M	V		K	E	A	E	E	F	A	e	D	K	K	V	K	E	R	I	D	A	R	N	S	L	E	T	Y	V	Y	N	M
Saccharomyces	L	T	Q	E	E	I	D	R	M	V		E	E	A	E	K	F	A	e	D	A	S	I	K	A	K	V	E	S	R	N	K	L	E	N	Y	A	H	S	L
Escherichia	L	N	E	D	E	I	Q	K	M	V		R	D	A	E	A	N	A	e	A	R	K	F	E	E	L	V	Q	T	R	N	Q	G	D	H	L	L	H	S	T

[illegible]

Conserved regions of the Substrate Binding Domain (SBD)

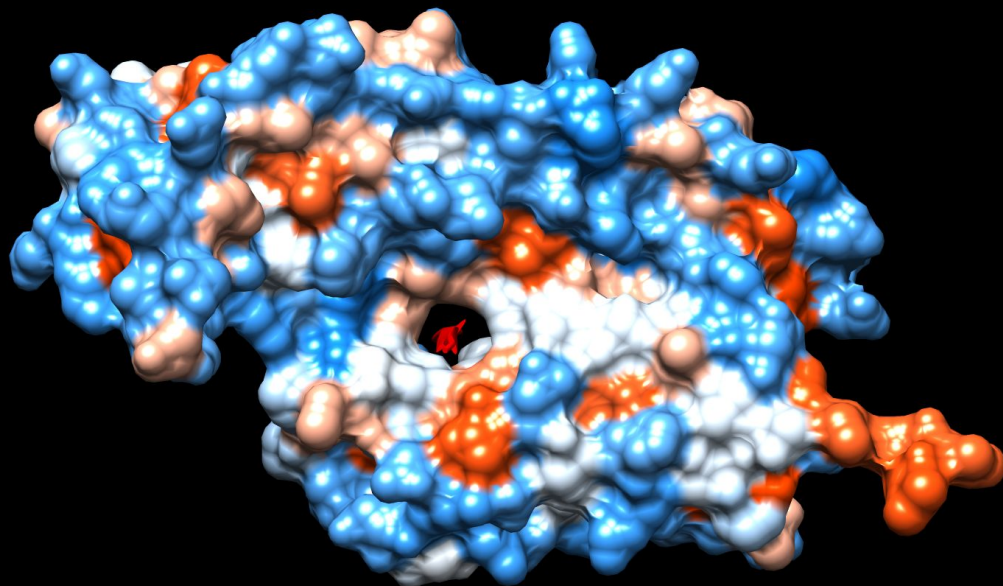
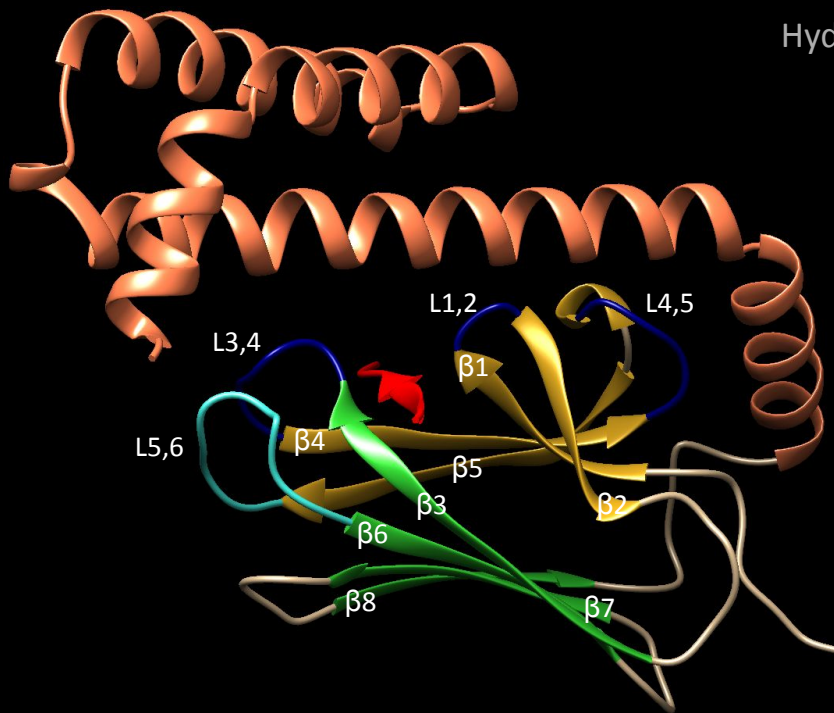


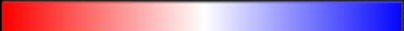
Regions conserved for **intramolecular bounds**.

Regions conserved to form the **hydrophobic substrate binding cavity**.

Substrate binding cavity

Hydrophobic cavity formed by the **loops L1,2 and L3,4** and stabilized by interactions with the helix B and the loops L4,5 and L5,6.



HYDROPHOBIC  HYDROPHILIC

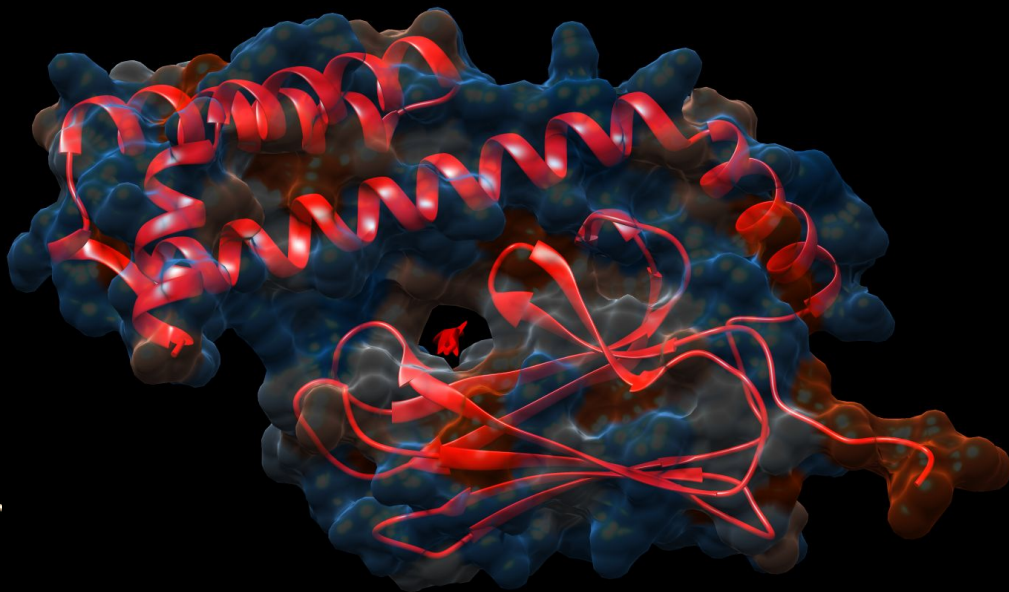
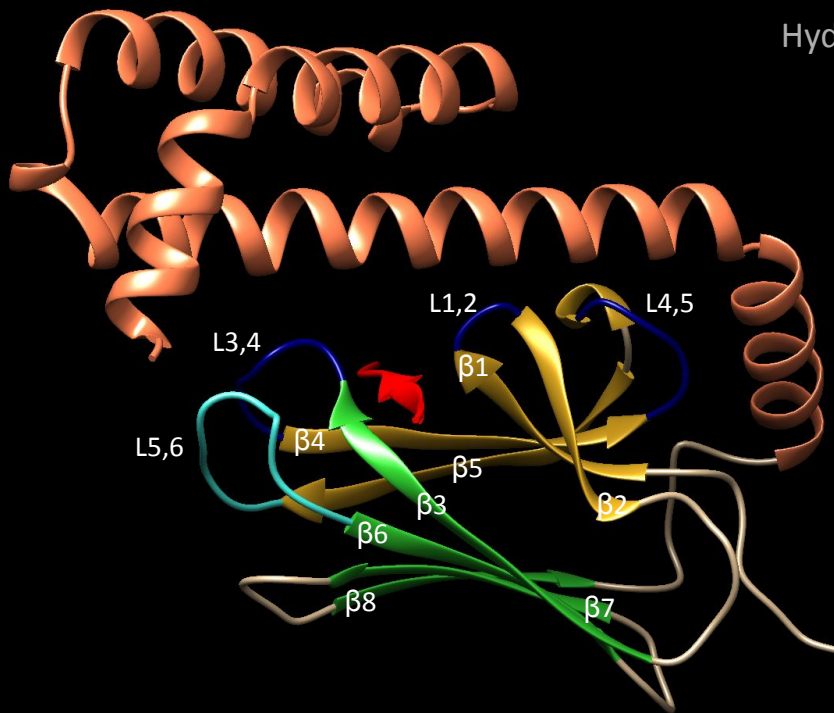
-3 -2 -1 0 +1 +2 +3

Sequence of the peptide: N R L L L T G

1DKX - *E. coli*

Substrate binding cavity

Hydrophobic cavity formed by the **loops L1,2 and L3,4** and stabilized by interactions with the helix B and the loops L4,5 and L5,6.

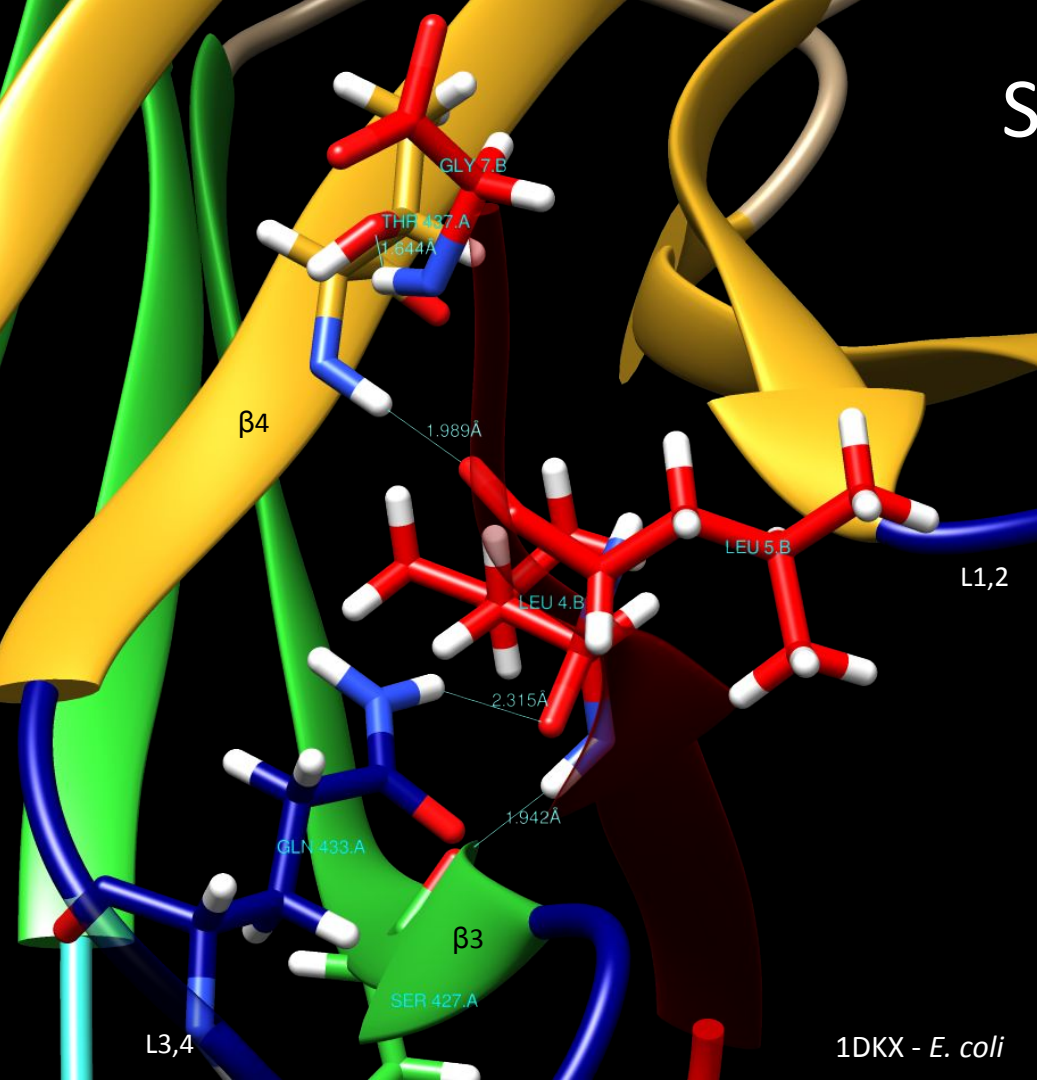


-3 -2 -1 0 +1 +2 +3

Sequence of the peptide: N R L L L T G

1DKX - *E. coli*

Substrate binding cavity



Amino acids involved

	Peptide	SBD	
Hydrogen bond	Leu 4	Ser 427	β3
Hydrogen bond	Leu 4	Gln 433	L3,4
Hydrogen bond	Leu 5	Thr 437	β4
Hydrogen bond	Gly 7	Thr 437	β4

427

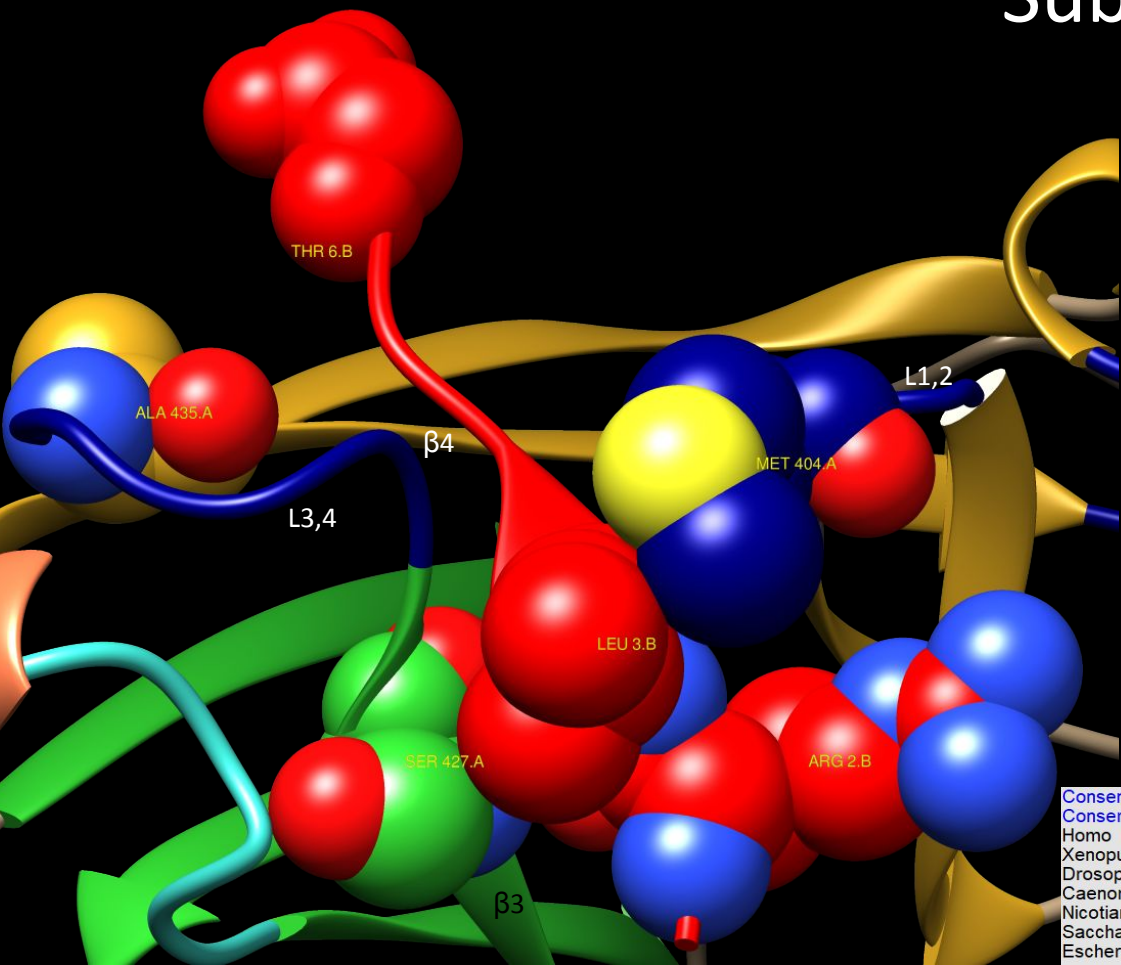
433

437

Consensus	v	G	G	V	M	T	k	L	I	p	R	N	T	v	i	P	T	K	K	S	Q	v	F	S	T	A	s	D	N	Q	p	T	V	T
Conservation																																		
Homo	V	G	G	V	M	T	K	L	I	P	R	N	T	V	V	P	T	K	K	S	Q	V	F	S	T	A	S	D	N	Q	P	T	V	T
Xenopus	V	G	G	V	M	T	K	L	I	P	R	N	T	V	V	P	T	K	K	S	Q	V	F	S	T	A	S	D	N	Q	P	T	V	T
Drosophila	V	G	G	V	M	T	K	L	I	P	R	N	T	V	I	P	T	K	K	S	Q	V	F	S	T	A	S	D	N	Q	P	T	V	T
Caenorhabditis	V	G	G	V	M	T	K	L	I	S	R	N	T	V	I	P	T	K	K	S	Q	V	F	S	T	A	A	D	N	Q	P	T	V	T
Nicotiana	V	G	G	V	M	T	K	L	I	P	R	N	T	V	I	P	T	K	K	S	Q	V	F	T	T	Y	Q	D	N	Q	P	T	V	T
Saccharomyces	T	G	G	V	M	T	P	L	I	K	R	N	T	A	I	P	T	K	K	S	Q	V	F	S	T	A	V	D	N	Q	P	T	V	M
Escherichia	M	G	G	V	M	T	T	L	I	A	K	N	T	T	I	P	T	K	H	S	Q	V	F	S	T	A	E	N	Q	S	A	V	T	

1DKX - *E. coli*

Substrate binding cavity

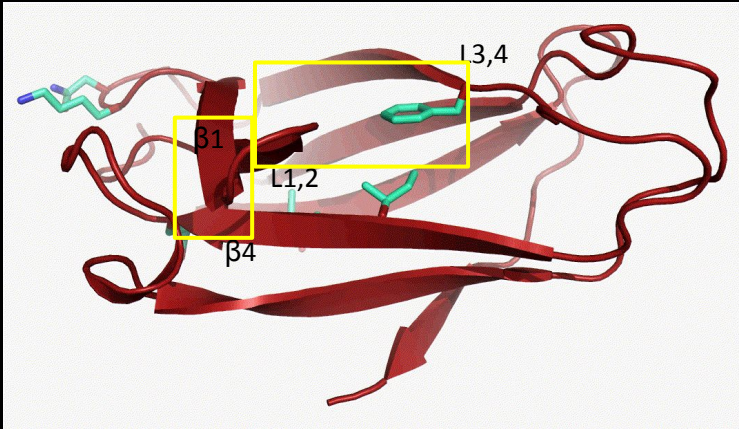


Van der Waals		
Peptide	SBD	
Arg 2	Ser 427	$\beta 3$
Leu 3	Met 404	L1,2
Thr 6	Ala 435	$\beta 4$

	404		427		435
Consensus	vGGVM	TkLIPRNTvi	PTKKSQvFST	AsDNQp	TVT
Conservation					
Homo	VGGVM	TKLIPRNTVV	PTKKSQVIFST	ASDNQPTVT	VTV
Xenopus	VGGVM	TKLIPRNTVV	PTKKSQVIFST	ASDNQPTVT	VTV
Drosophila	VGGVM	TKLIPRNTVI	PTKKSQVIFST	ASDNQPTVT	VTV
Caenorhabditis	VGGVM	TKLIPRNTVI	PTKKSQVIFST	ASDNQPTVT	VTV
Nicotiana	VGGVM	TKLIPRNTVI	PTKKSQVIFST	YQDQQT	VTV
Saccharomyces	TGGVM	TPLIKRNTAI	PTKKSQVIFST	AVDNQPTVM	
Escherichia	MGGVM	TTLIAKNTTI	PTKH	ESQV	FST

Conformational changes of the substrate binding cavity

L1,2 and L3,4 come closer due to the H bonds they form → **narrowing of the angle** between $\beta 1$ and $\beta 4$.



$\beta 3$ slightly moves apart.



Movies 2 and 3. Conformational changes of the substrate binding cavity [22].

Interdomain linker

Hydrophobic sequence conserved involved in the **allosteric control** of the NBD by the SBD.

	439																		457
Consensus	G	e	-	-	q	d	t	g	d	i	v	L	L	D	V	n	P	L	T
Conservation																			
Homo	G	D	-	-	Q	D	T	G	D	L	V	L	L	D	V	C	P	L	T
Xenopus	G	D	-	-	Q	D	T	G	D	L	V	L	L	D	V	C	P	L	T
Drosophila	G	E	-	-	Q	D	T	D	A	I	V	L	L	D	V	N	P	L	T
Caenorhabditis	G	E	-	-	E	D	T	G	E	I	V	L	L	D	V	N	P	L	T
Nicotiana	G	E	G	G	D	E	T	K	D	I	L	L	L	D	V	A	P	L	T
Saccharomyces	G	E	-	-	E	G	V	E	D	I	V	L	L	D	V	N	A	L	T
Escherichia	G	D	-	-	-	-	V	K	D	V	L	L	L	D	V	T	P	L	S

Interdomain linker

Hydrophobic sequence conserved involved in the **allosteric control** of the NBD by the SBD.

	439																457		
Consensus	G	e	-	-	q	d	t	g	d	i	v	L	L	D	V	n	P	L	T
Conservation																			
Homo	G	D	-	-	Q	D	T	G	D	L	V	L	L	D	V	C	P	L	T
Xenopus	G	D	-	-	Q	D	T	G	D	L	V	L	L	D	V	C	P	L	T
Drosophila	G	E	-	-	Q	D	T	D	A	I	V	L	L	D	V	N	P	L	T
Caenorhabditis	G	E	-	-	E	D	T	G	E	I	V	L	L	D	V	N	P	L	T
Nicotiana	G	E	G	G	D	E	T	K	D	I	L	L	L	D	V	A	P	L	T
Saccharomyces	G	E	-	-	E	G	V	E	D	I	V	L	L	D	V	N	A	L	T
Escherichia	G	D	-	-	-	-	V	K	D	V	L	L	L	D	V	T	P	L	S

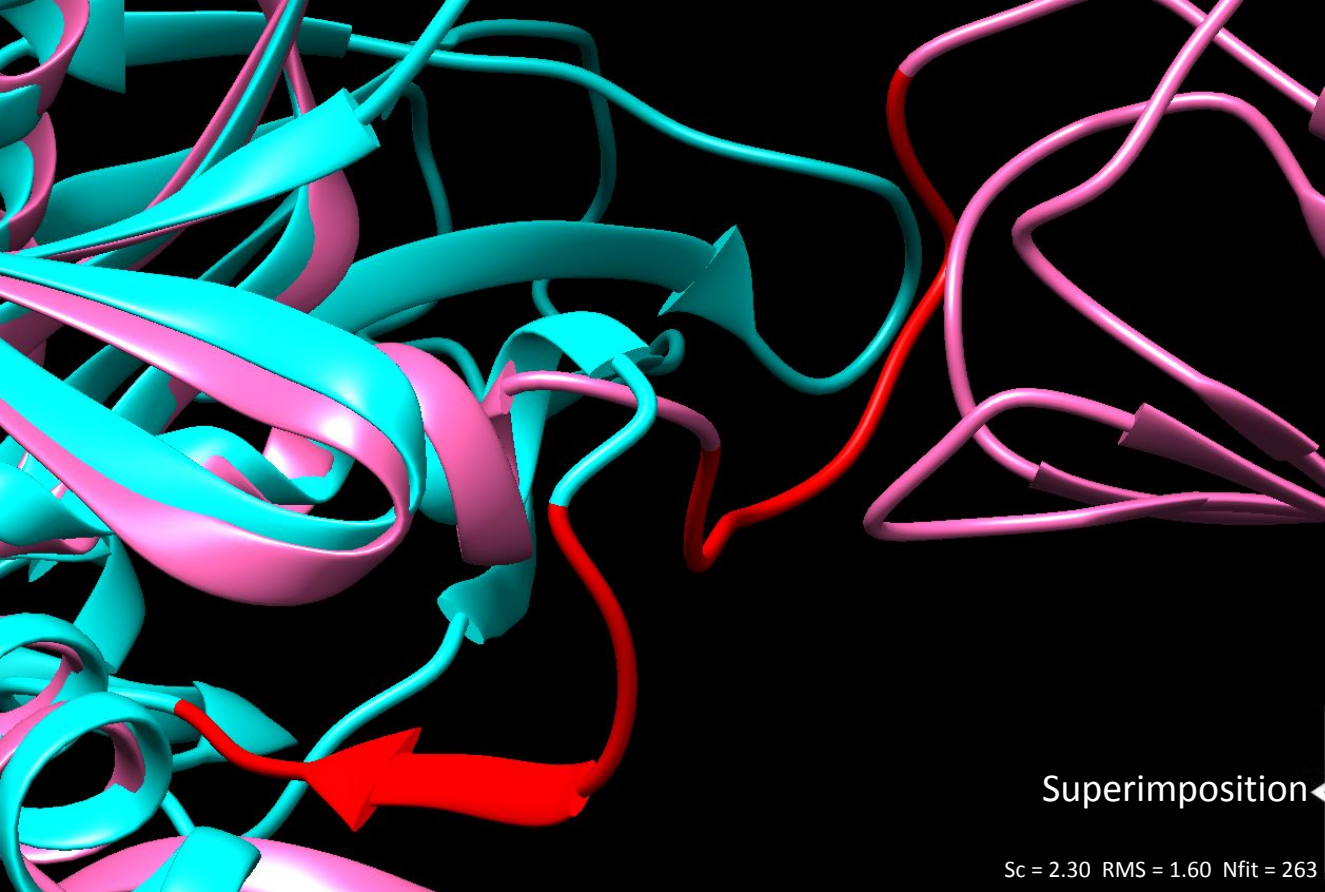
HYDROPHOBIC



HYDROPHILIC

5E84 - *Homo sapiens*

Conformational change in the interdomain linker



Interdomain linker only interacting with the SBD.

Interdomain linker fitted in the bottom groove between the two lobes of the NBD → β -sheet

Superimposition

ATP-bound

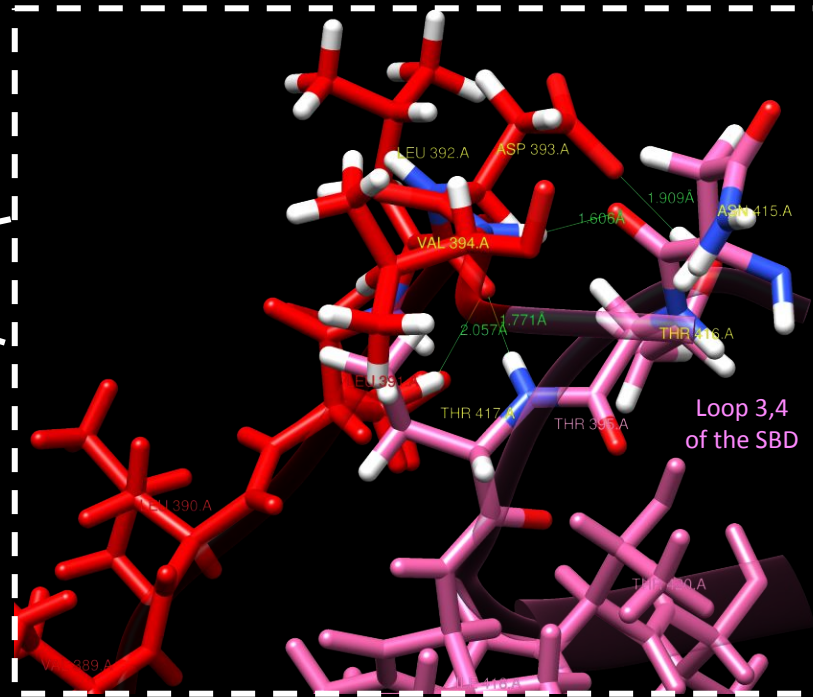
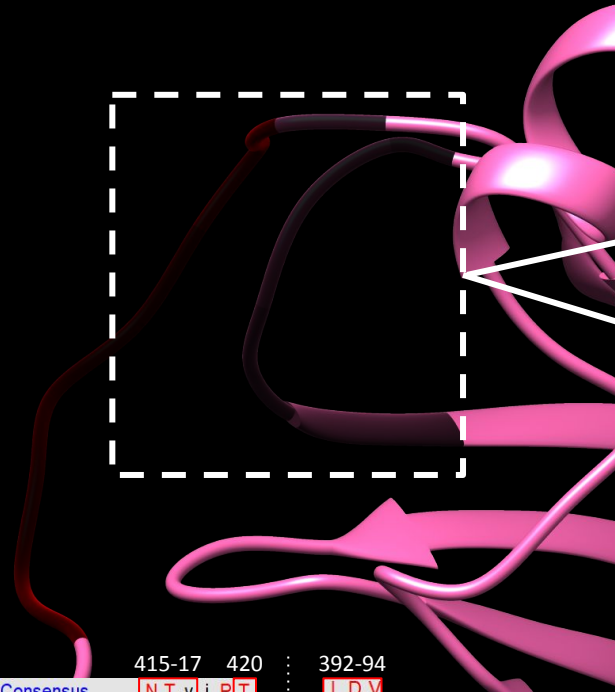
4B9Q - *E. coli*

ADP-bound

2KHO - *E. coli*

Sc = 2.30 RMS = 1.60 Nfit = 263

Interdomain linker bonds - ADP state

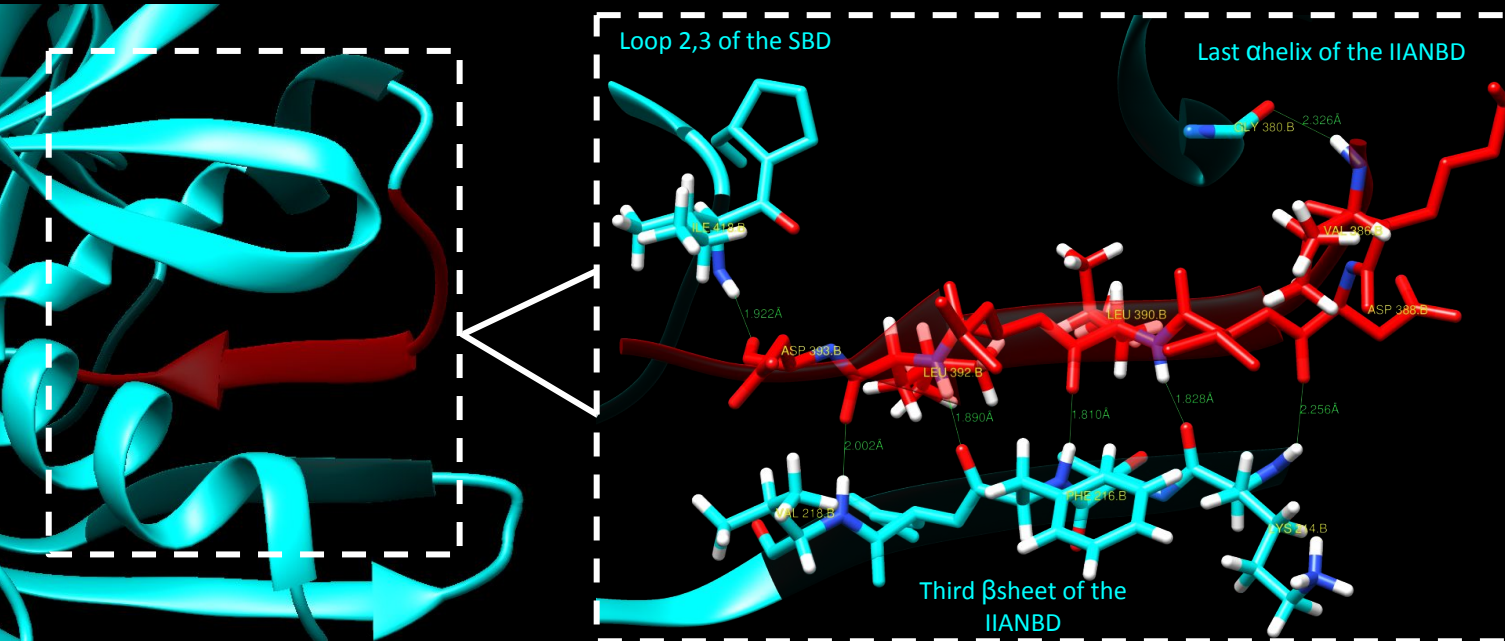


Hydrogen bonds

Linker	SBD
Leu 392 (O)	Thr 417 (H)
Leu 392 (O)	Thr 420 (H)
Asp 393 (O)	Thr 416 (H)
Val 394 (H)	Asn 415 (O)

	415-17	420	392-94
Consensus	NT v i PT		LDV
Conservation			
Homo	NT V V P T		LDV
Xenopus	NT V V P T		LDV
Drosophila	NT V I P T		LDV
Caenorhabditis	NT V I P T		LDV
Nicotiana	NT V I P T		LDV
Saccharomyces	NT A I P T		LDV
Escherichia	NT T I P T		LDV

Interdomain linker bonds - ATP state

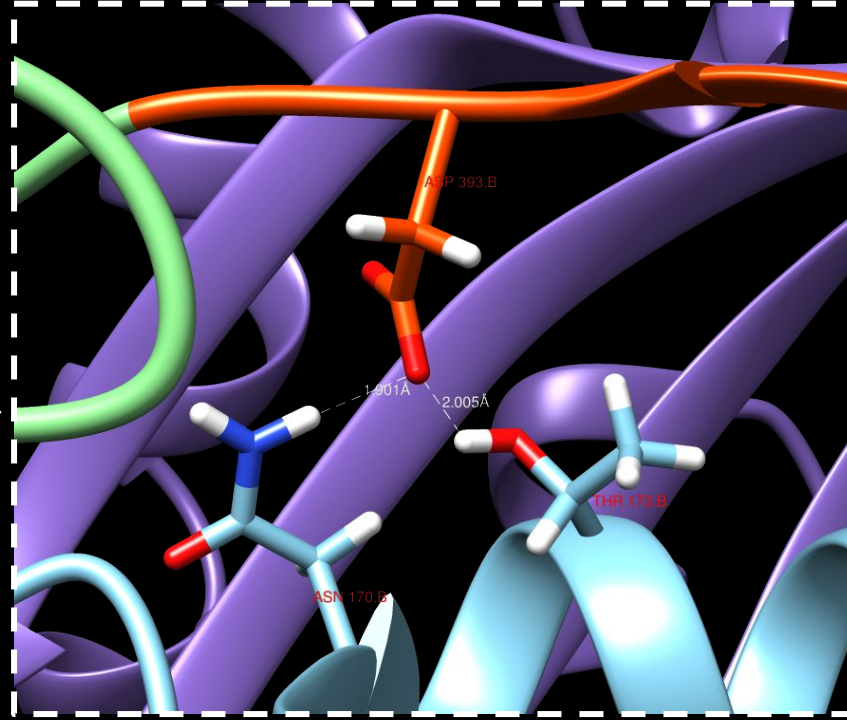


Hydrogen bonds	
Linker	SBD/NBD
Asp 393 (O)	Ile 418 (H)
Leu 392 (O)	Val 218 (H)
Leu 392 (H)	Phe 216 (O)
Leu 390 (O)	Phe 216 (H)
Leu 390 (H)	Lys 214 (O)
Asp 388 (O)	Lys 214 (H)
Val 386 (H)	Gly 380 (O)

+2 more H bonds

	214	216	218	380	418	386	388	390	392-93				
Consensus	-	V	F	E	V	I	A	G	V	L	v	i	P
Conservation	-	V	F	E	V	V	A	G	V	L	V	V	P
Homo	-	V	F	E	V	V	A	G	V	L	V	V	P
Xenopus	-	V	F	E	V	V	A	G	V	L	V	V	P
Drosophila	-	V	F	E	V	V	A	G	V	L	V	V	P
Caenorhabditis	-	V	F	E	V	V	A	G	V	L	V	V	P
Nicotiana	-	V	F	E	V	L	S	G	I	L	V	L	P
Saccharomyces	-	V	F	E	V	Q	A	G	V	L	A	I	P
Escherichia	K	T	F	E	V	L	A	G	V	L	T	I	P

A 3D ribbon diagram of a protein structure, likely a viral capsid, shown in cyan. A red arrow points to a specific region on the surface, which is highlighted by a dashed white box. The structure is complex, with many loops and helices. The red arrow points to a region that appears to be a potential binding site or a site of interest for the study.



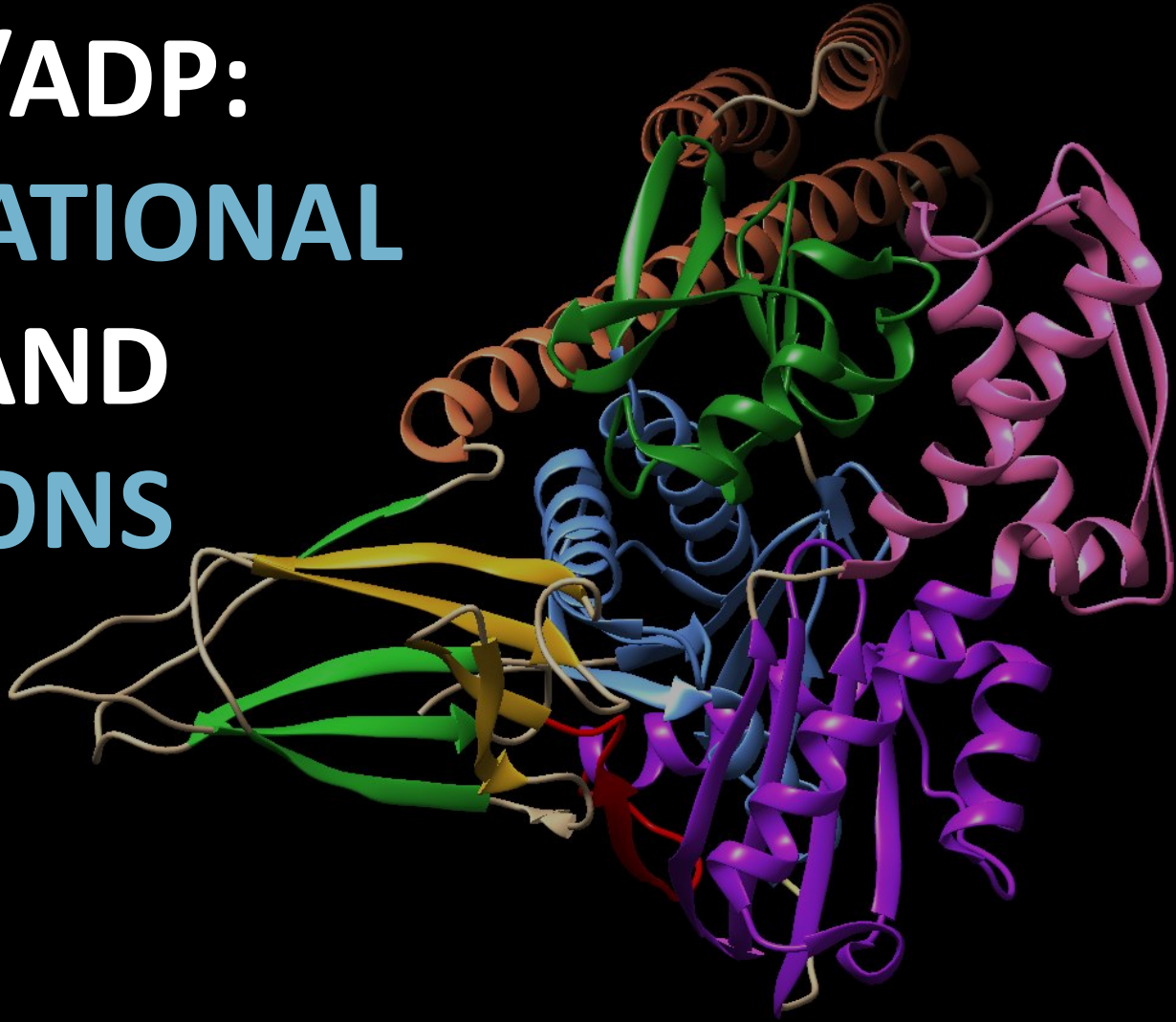
170 173

393

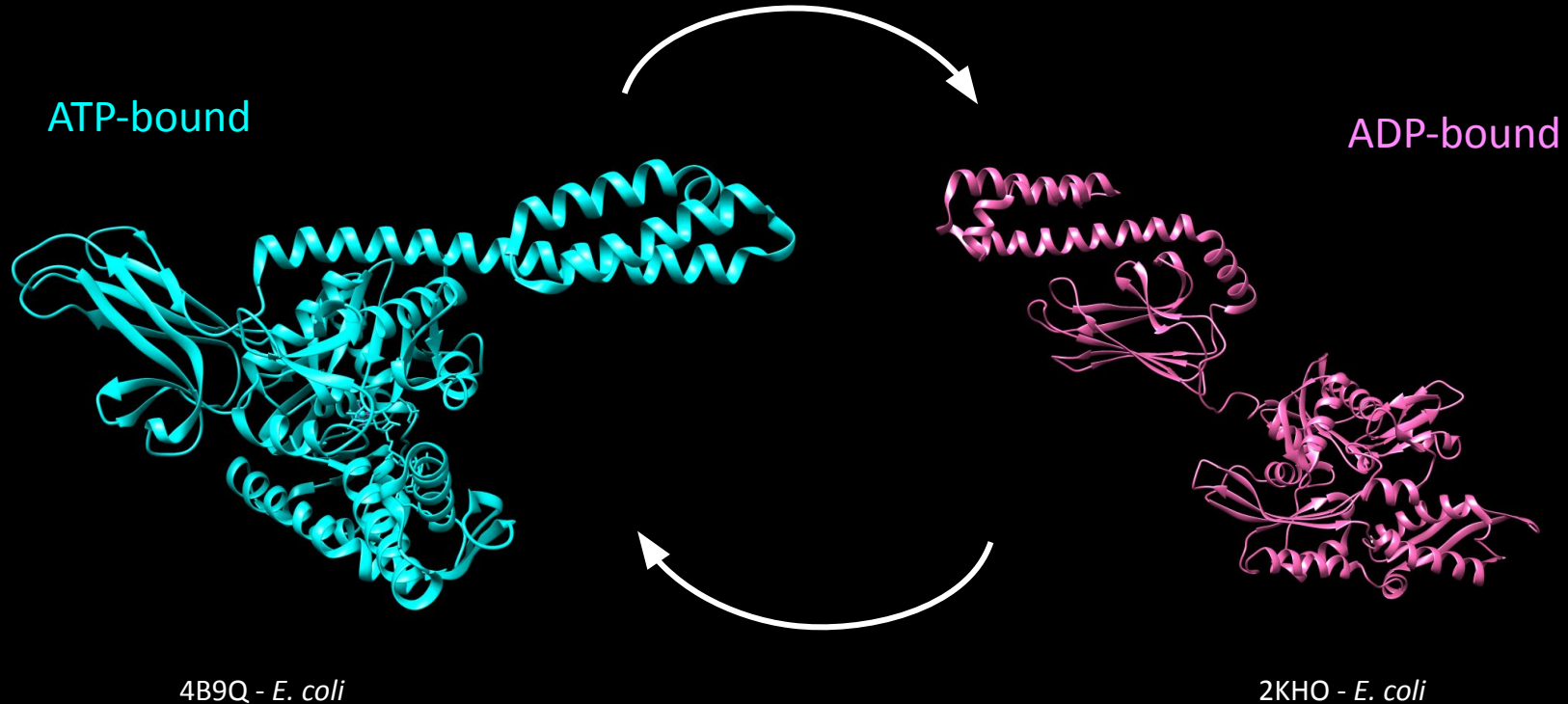
Consensus
Conservation
Homo
Xenopus
Drosophila
Caenorhabditis
Nicotiana
Saccharomyces
Escherichia

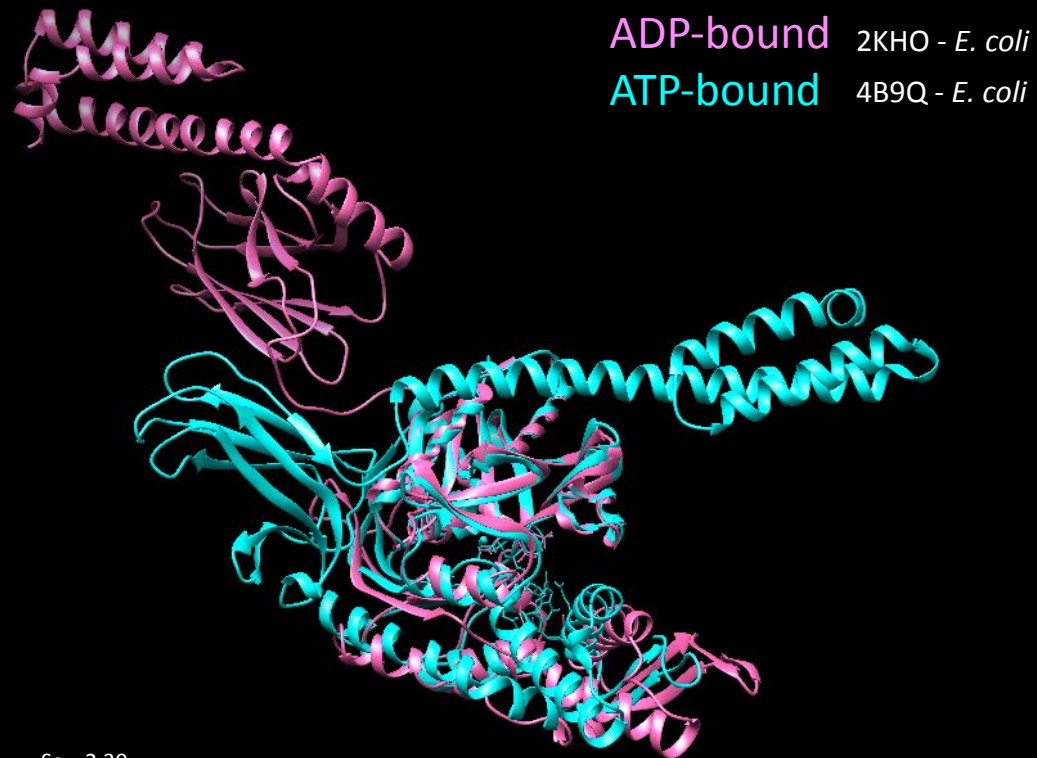
[illegible][illegible]4B9Q - *E. coli*

HSP70 ATP/ADP: CONFORMATIONAL CHANGES AND INTERACTIONS

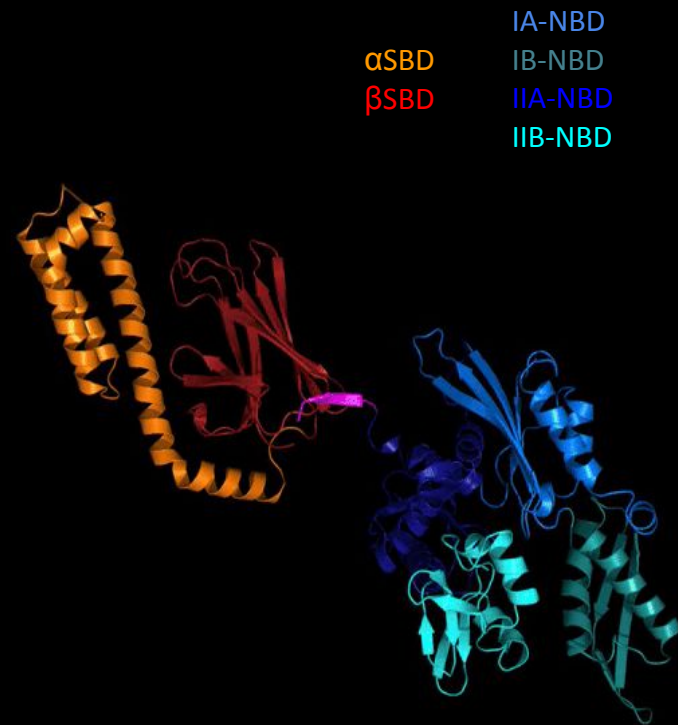


Hsp70 Cycle - ATP/ADP conformations



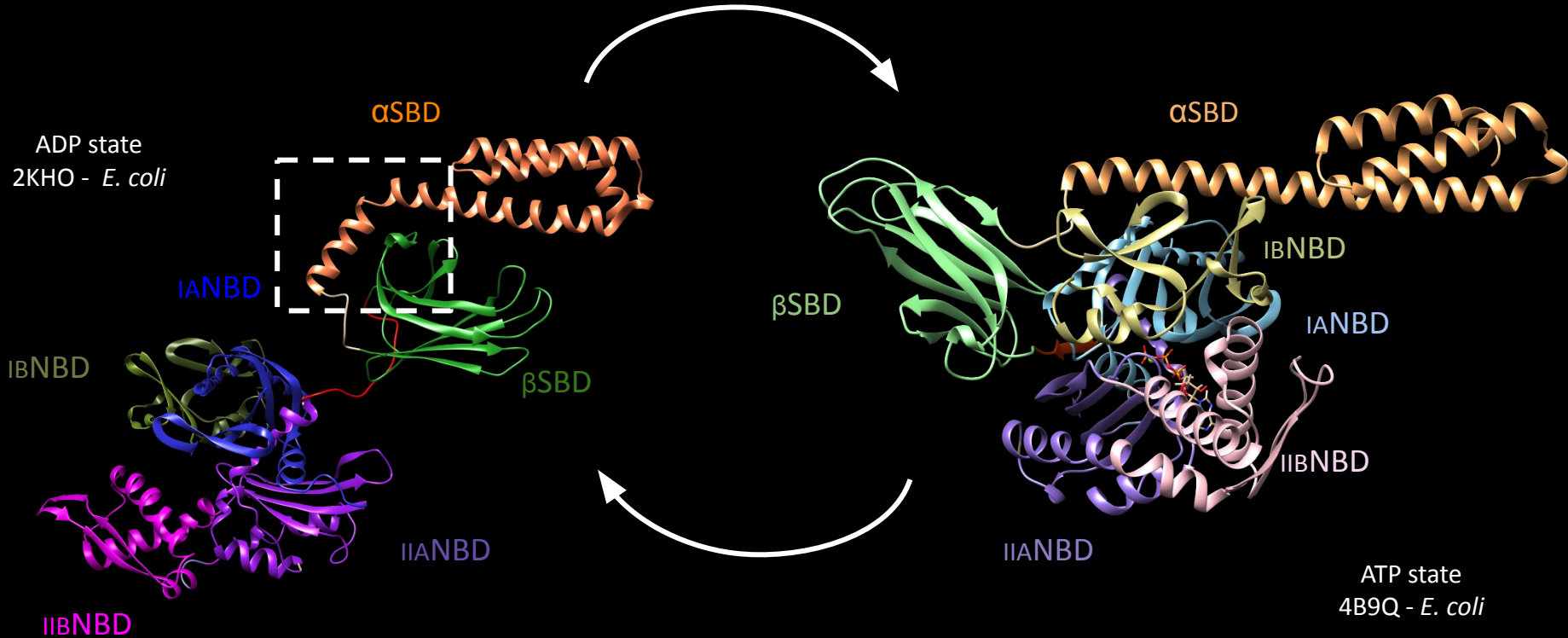


Sc = 2.30
RMS = 1.60
Nfit = 263

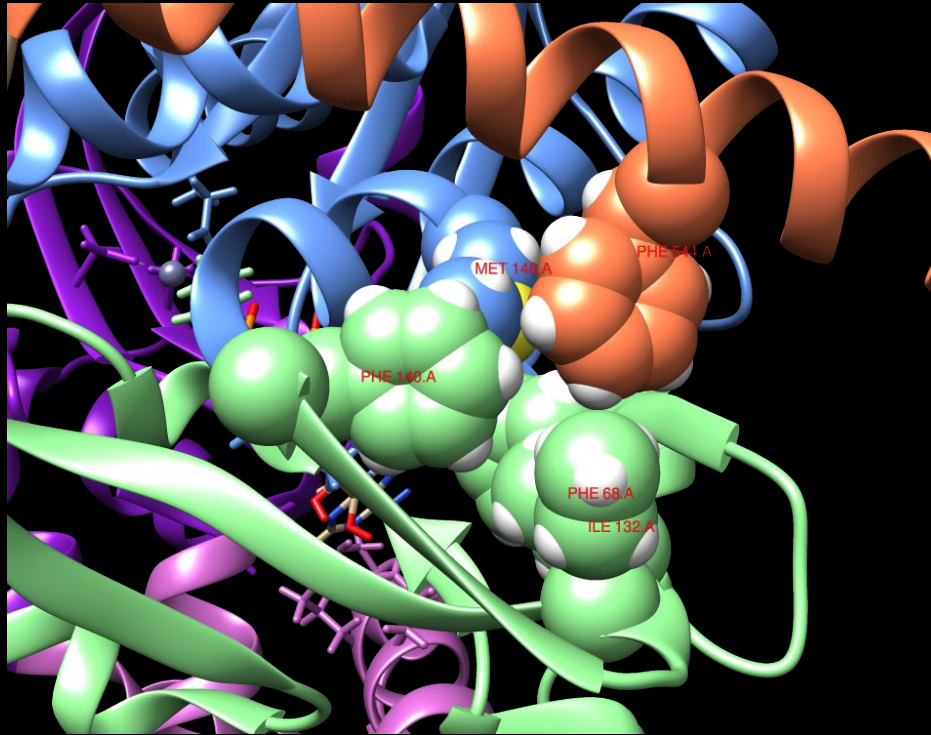


Movie 4. Illustration of the sequential transition from the ADP-state to the ATP-state. [24]

SBD - conformational changes



α SBD/NBD - interactions

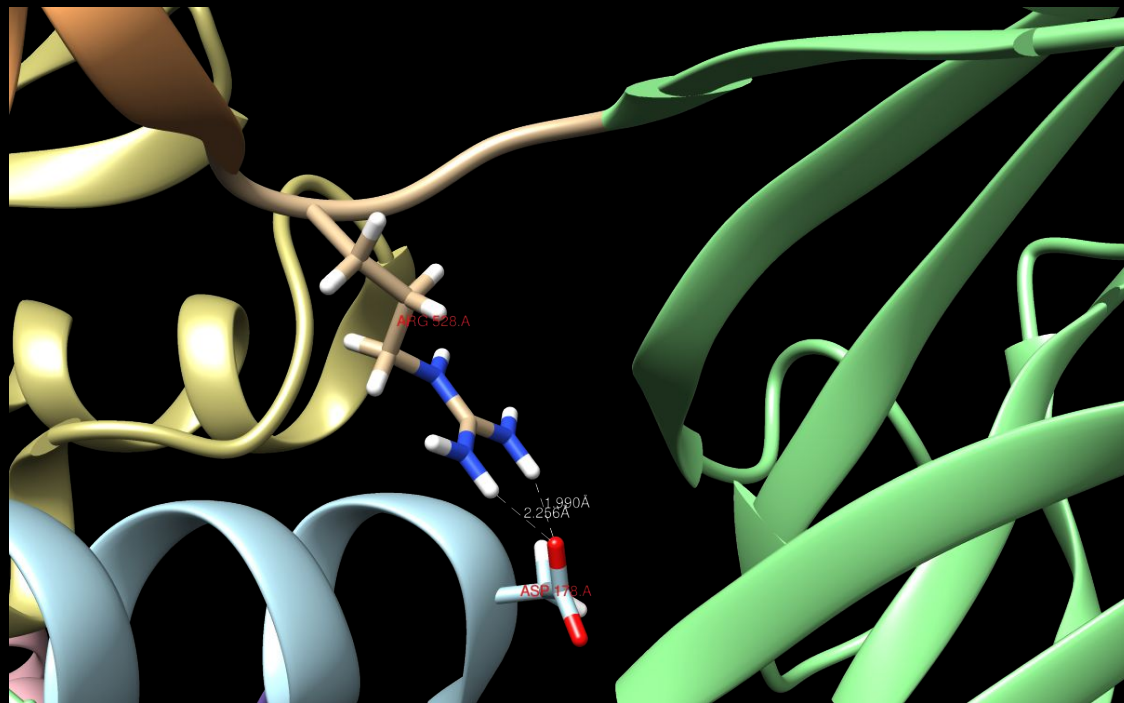


Hydrophobic contacts

α SBD	IBNBD	IANBD
Phe 544	Phe 68, Ile132, Phe140	Met148

	68	132	140	148	544
Consensus	AFT	k l g	t F a	A M v	k F A
Conservation					
Homo	AFT	D I G	T F A	A M V	K F A
Xenopus	AFT	D I G	T F A	A M V	K F A
Drosophila	AFT	D I G	V F A	A M V	K F A
Caenorhabditis	AFS	K V G	Q F T	A M V	K F A
Nicotiana	AFT	K I K	I F S	A M I	E F A
Saccharomyces	AFT	S V K	V F T	G M I	K F A
Escherichia	A Y T	K G Q	K M A	A E V	A N A

SBD/NBD - interactions



5E84 - *Homo sapiens*

Hydrogen bond

Linker SBD	IANBD
Arg 528	Asp 178

	178	528
Consensus	NDA	nRL
Conservation		
Homo	NDA	NRL
Xenopus	NDA	NRL
Drosophila	NDA	NRL
Caenorhabditis	NDA	NRL
Nicotiana	NDA	GRL
Saccharomyces	NDA	GRL
Escherichia	NDA	G-

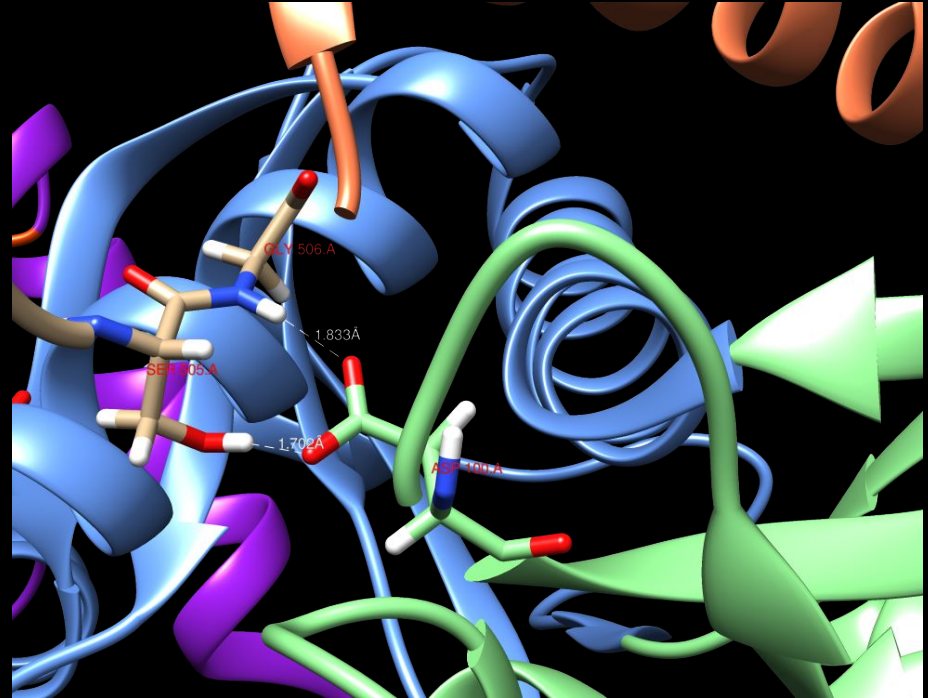
SBD/NBD - interactions

100 505 506

Consensus	K P	q n
Conservation		
Homo	K P	Q N
Xenopus	K P	Q N
Drosophila	K P	Q N
Caenorhabditis	K P	Q N
Nicotiana	K P	K G
Saccharomyces	K P	K G
Escherichia	D A	S G

Hydrogen bond

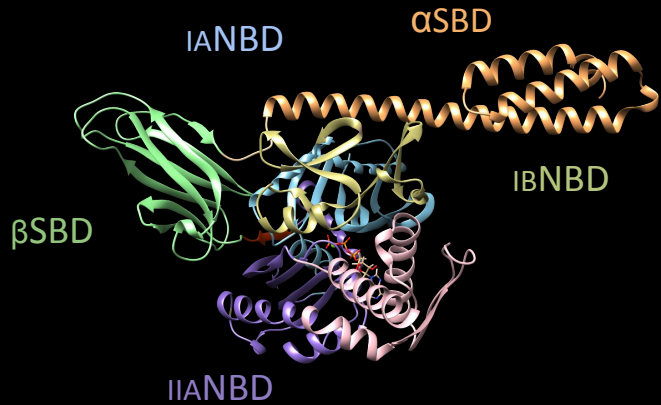
Linker	IBNBD	Distance
Ser 505 (H)	Asp 100 (O)	1.70
Gly 506 (H)	Asp 100 (O)	1.83



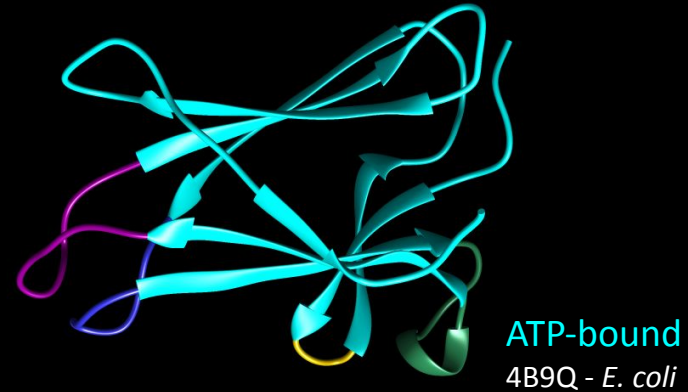
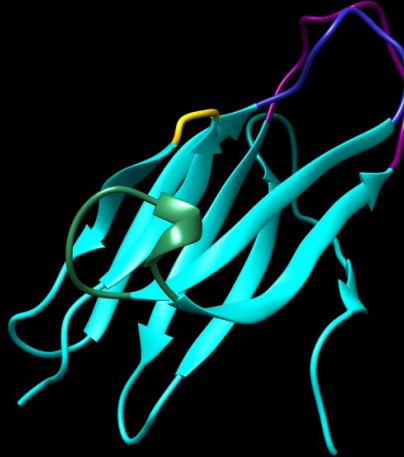
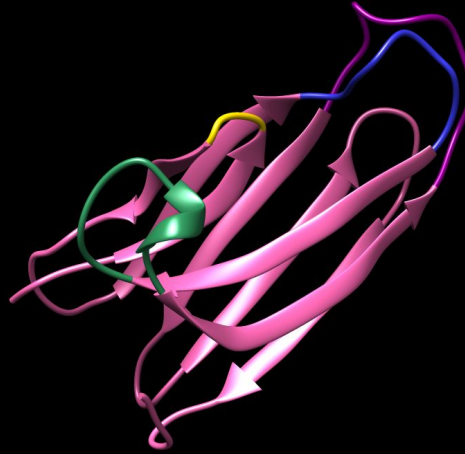
Linker - IBNBD

4B9Q - *E. coli*

β SBD - conformational changes



L1,2 - L3,4 - L4,5 - L5,6



β SBD/NBD - interactions

Hydrogen bond

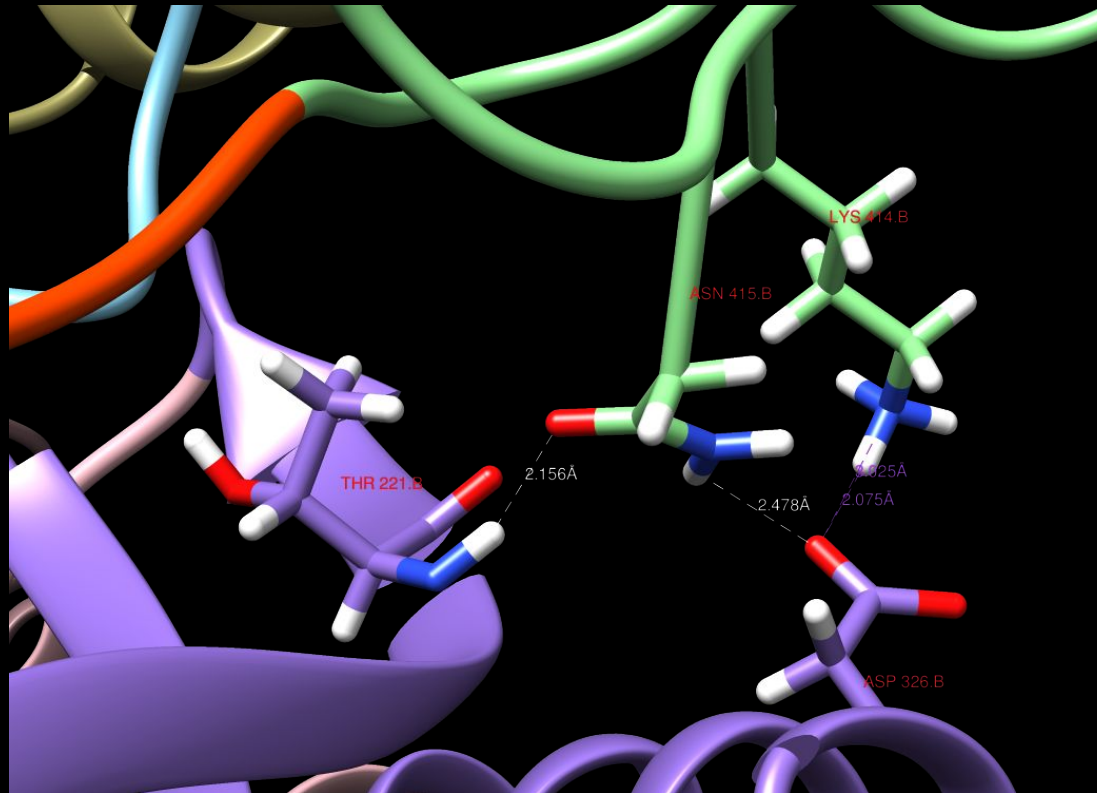
β SBD	IIANBD	Distance
Asn 415	Asp 326	2.48
Asn 415	Thr 221	2.16

Salt Bridge

Lys 414	Asp 326
---------	---------

414 415 221 326

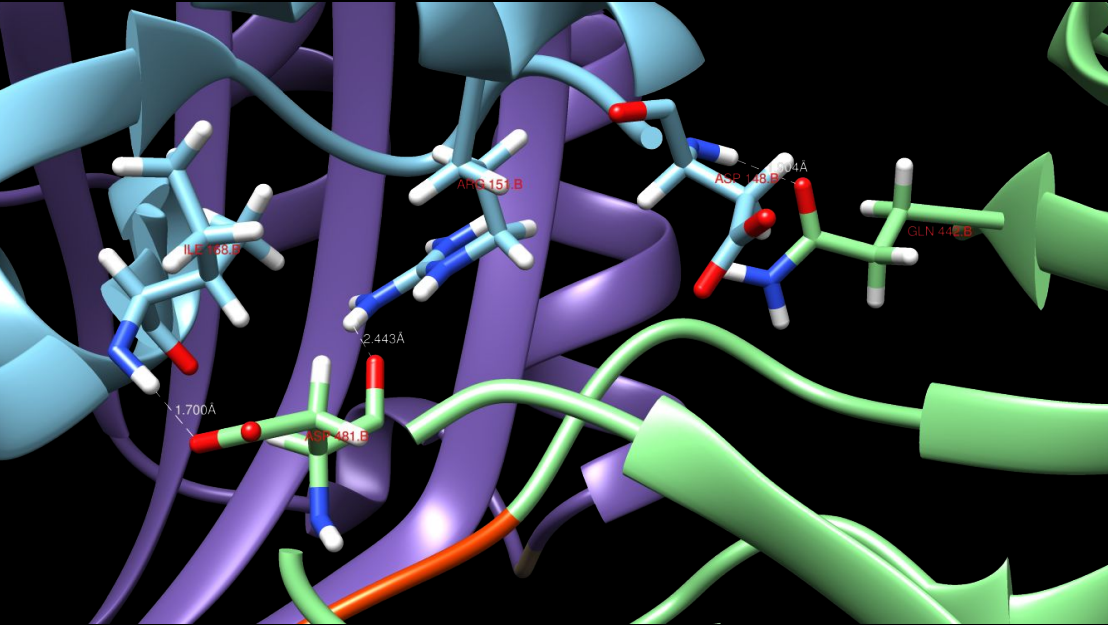
Consensus	R	N	T	D
Conservation				
Homo	R	N	T	D
Xenopus	R	N	T	D
Drosophila	R	N	T	D
Caenorhabditis	R	N	T	D
Nicotiana	R	N	T	D
Saccharomyces	R	N	T	D
Escherichia	K	N	T	D



β SBD - IIANBD

4B9Q - *E. coli*

βSBD/NBD - interactions



βSBD - 1ANBD

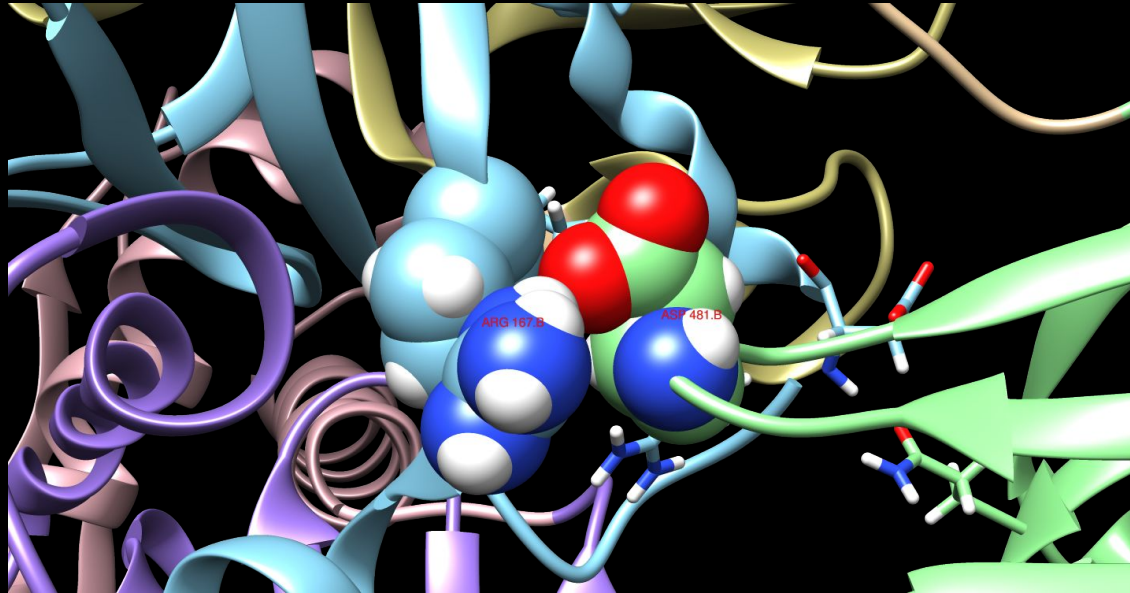
4B9Q - *E. coli*

Hydrogen bond

βSBD	1ANBD	Distance
Gln 442 (O)	Asp 148 (N-H)	1.80
Asp 481 (O)	Ile 168 (N-H)	1.70
Asp 481 (O)	Arg 151 (N-H)	2.44

	148	151	168	442	481
Consensus	D A Q R	R I I	E G	a N G	
Conservation					
Homo	D A Q R	R I I	E G	V N G	
Xenopus	D A Q R	R I I	E G	V N G	
Drosophila	D A Q R	R I I	E G	A N G	
Caenorhabditis	D A Q R	R I I	E G	V N G	
Nicotiana	D A Q R	R I I	E G	A N G	
Saccharomyces	D A Q R	R I V	E G	A N G	
Escherichia	D A Q R	R I I	Q G	A D G	

β SBD/NBD - interactions



β SBD - IANBD

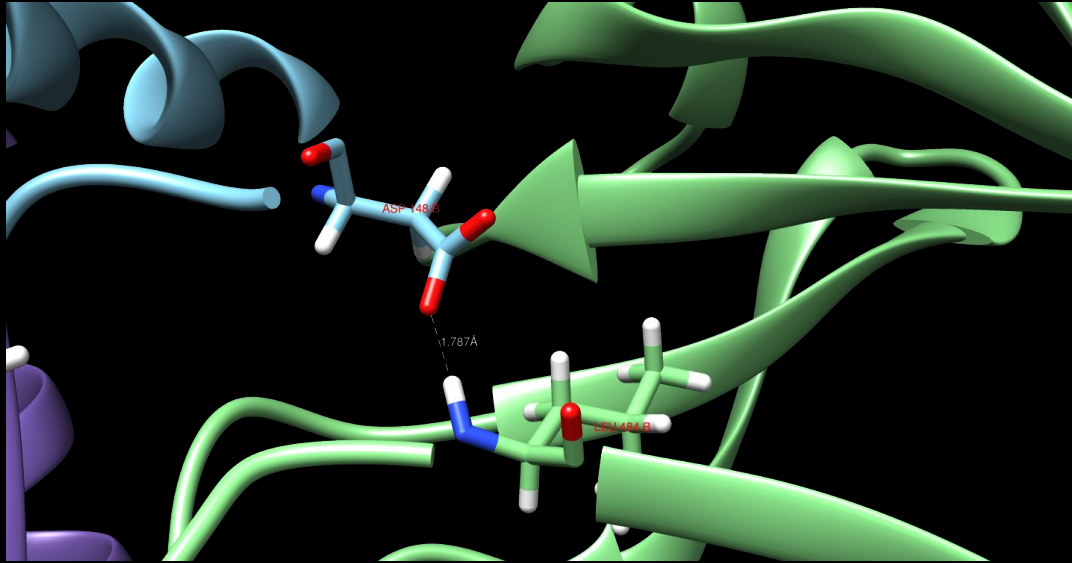
4B9Q - *E. coli*

	167	481
Consensus	R I I	a N G
Conservation	■ ■ ■	■ ■ ■
Homo	R I I	V N G
Xenopus	R R I I	V N G
Drosophila	R R I I	A N G
Caenorhabditis	R R I I	V N G
Nicotiana	R R I I	A N G
Saccharomyces	R R I V	A N G
Escherichia	R I I	A D G

Polar bond

β SBD	IANBD
Asp 481	Arg 167

βSBD/NBD - interactions



βSBD - IANBD

4B9Q - *E. coli*

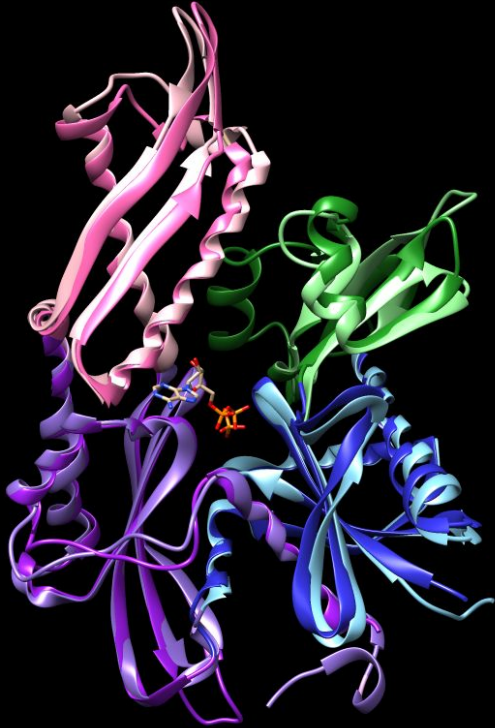
	148	484
Consensus	D A Q	G I L
Conservation		
Homo	D A Q	G I L
Xenopus	D A Q	G I L
Drosophila	D A Q	G I L
Caenorhabditis	D A Q	G I L
Nicotiana	D A Q	G I L
Saccharomyces	D A Q	G I L
Escherichia	D A Q	G I L

Hydrogen bond

βSBD	IANBD	Distance
Leu 484 (O)	Asp 148 (H)	1.79

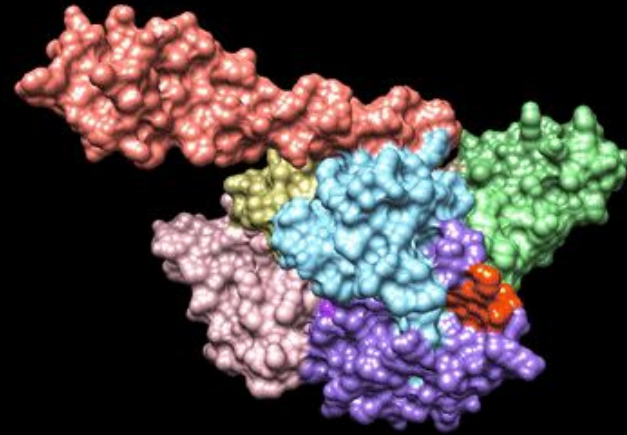
NBD - conformational changes

Sc = 7.70
RMS = 1.19
Nfit = 339



5OBW - *E. coli* → ATP: IA, IB, IIA, IIB

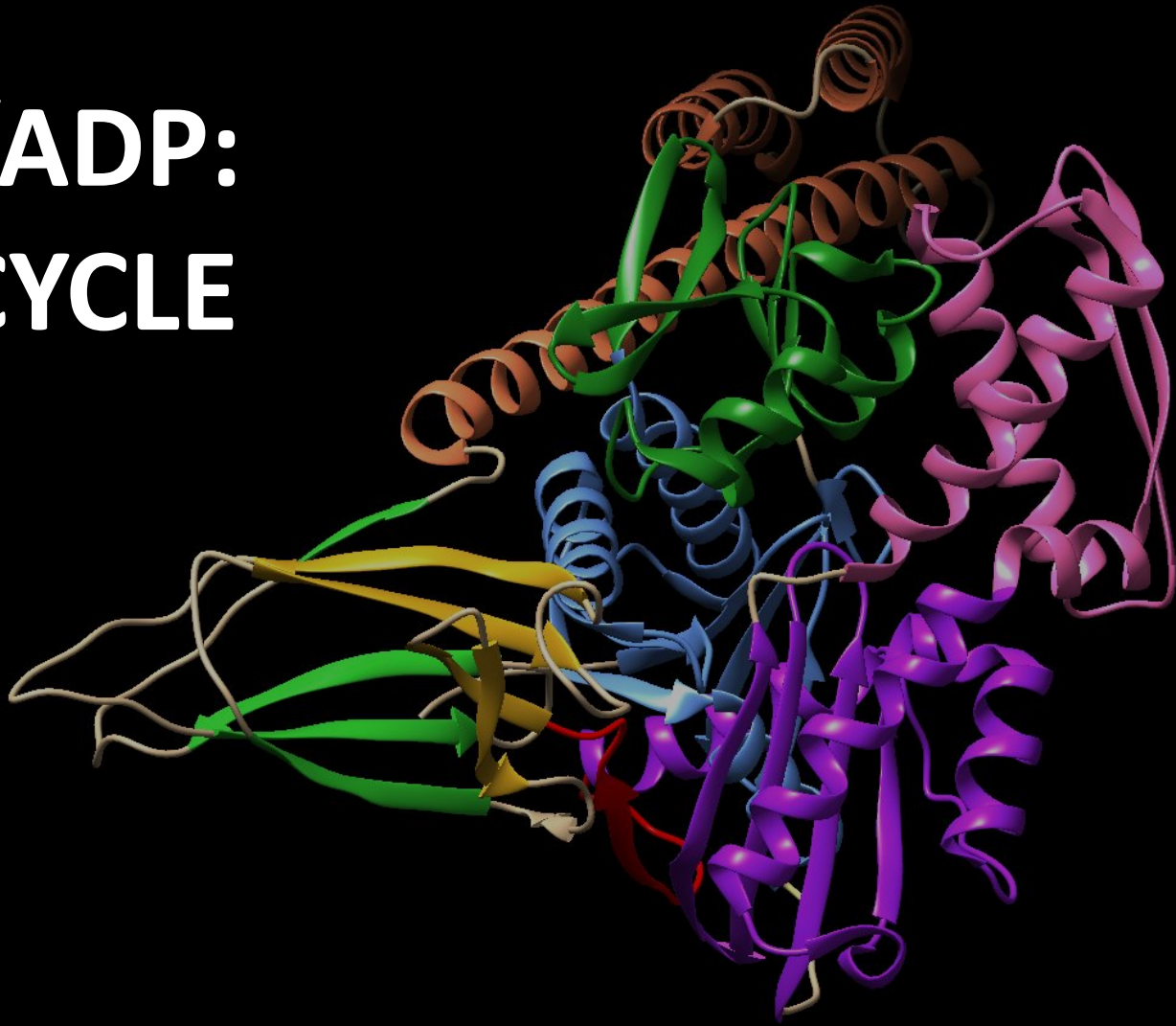
7N46 - *E. coli* → ADP: IA, IB, IIA, IIB



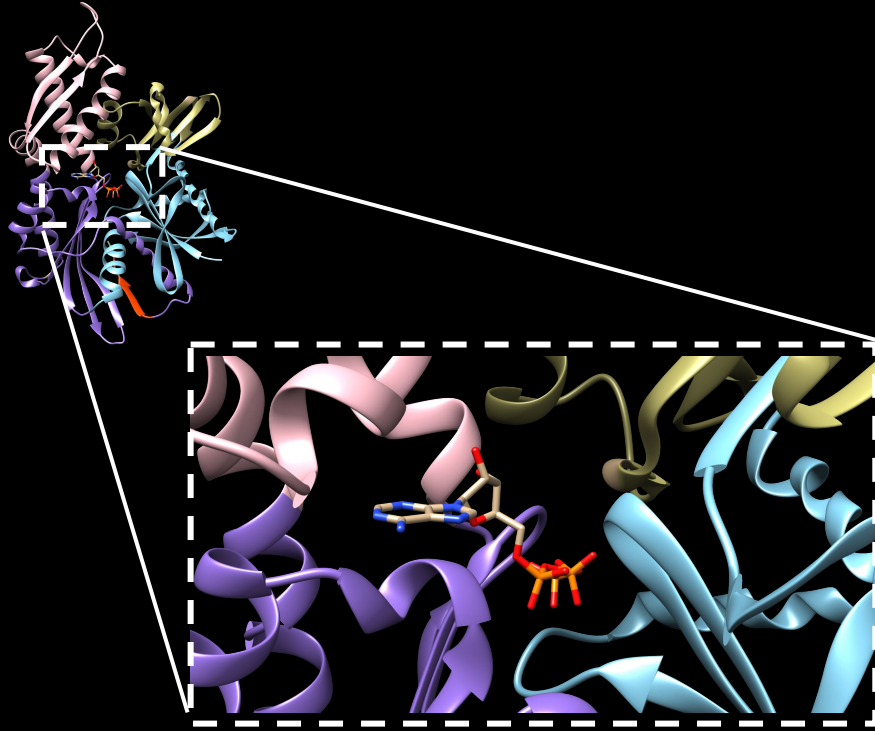
Movie 5. 3D representation of Hsp70 surface in the ATP-state. PDB ID: 4B9Q.
Created with Chimera.

4B9Q - *E. coli* → ATP

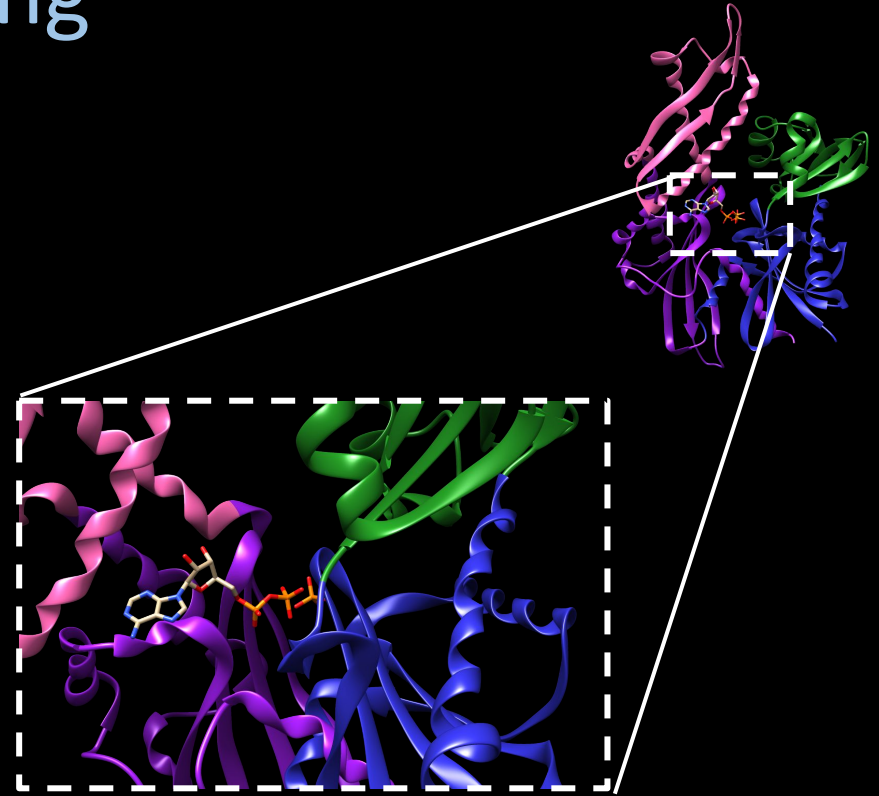
HSP70 ATP/ADP: BONDING CYCLE



Hsp70 - ATP/ADP binding

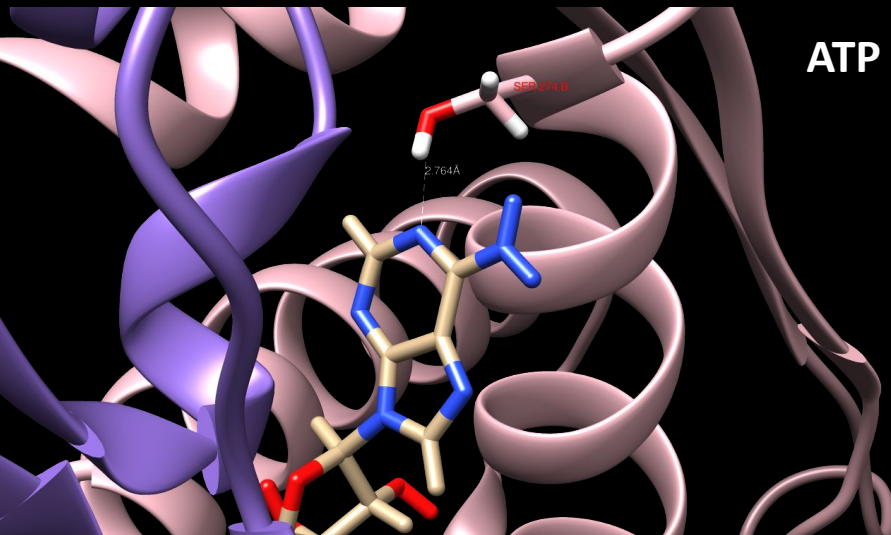


4B9Q - *E. coli*



7N46 - *E. coli*

ATP/ADP binding - Adenine base

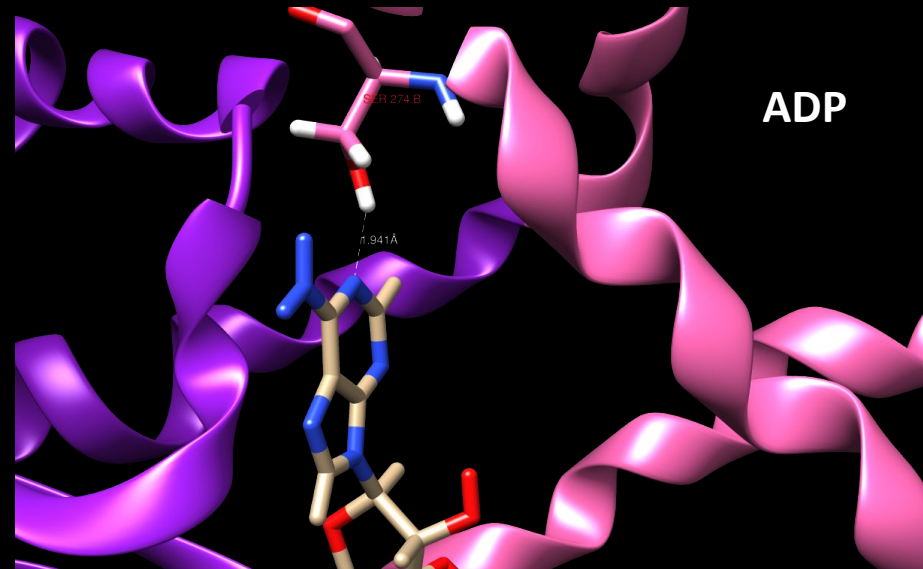


ATP

4B9Q - *E. coli*

Hydrogen Bond

Hsp70	Adenine base	Distance
Ser 274 (OG)	N1	2.76



ADP

7N46 - *E. coli*

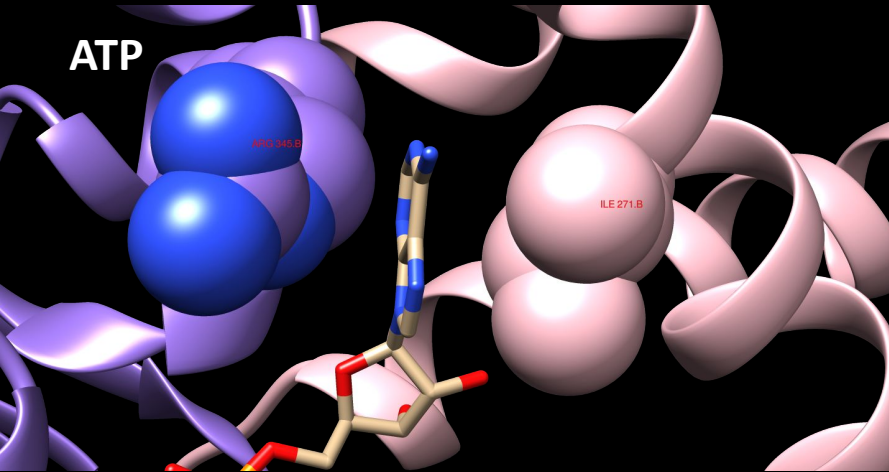
Hydrogen Bond

Hsp70	Adenine base	Distance
Ser 274 (OG)	N1	1.94

274

Consensus	L	S	S
Conservation			
Homo	L	S	S
Xenopus	L	S	A
Drosophila	L	S	G
Caenorhabditis	L	S	T
Nicotiana	L	S	S
Saccharomyces	L	S	S
Escherichia	L	S	S

ATP/ADP binding - Adenine base

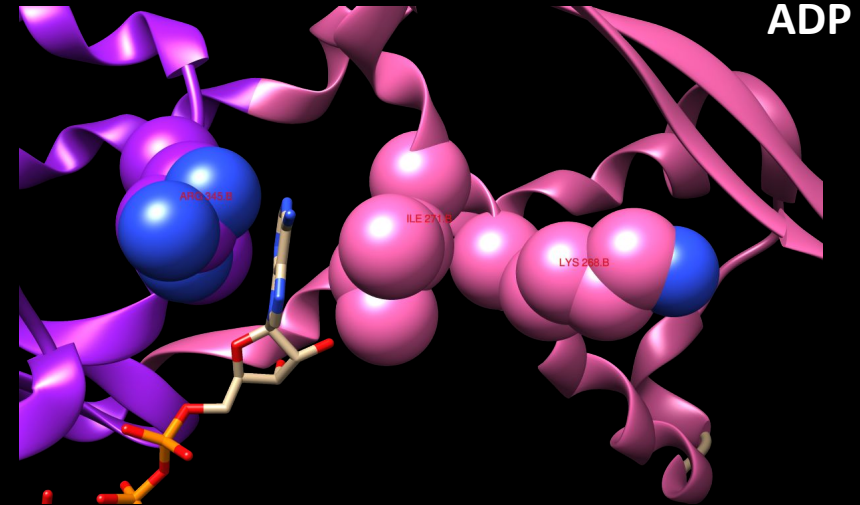


4B9Q - *E. coli*

Hydrophobic bond

Hsp70
Ile 271
Arg 345

	268	271	345
Consensus	E	K	A
Conservation	K	A	K
Homo	E	K	A
Xenopus	E	K	A
Drosophila	E	K	A
Caenorhabditis	E	K	A
Nicotiana	E	K	A
Saccharomyces	E	K	A
Escherichia	E	K	A



Hydrophobic bond

7N46 - *E. coli*

Hsp70
Ile 271
Lys 268
Arg 345

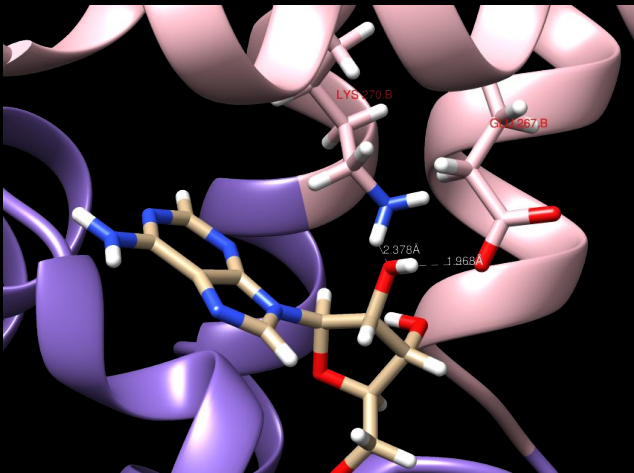
ADP binding- Ribose

Hydrogen Bond 4B9Q - *E. coli*

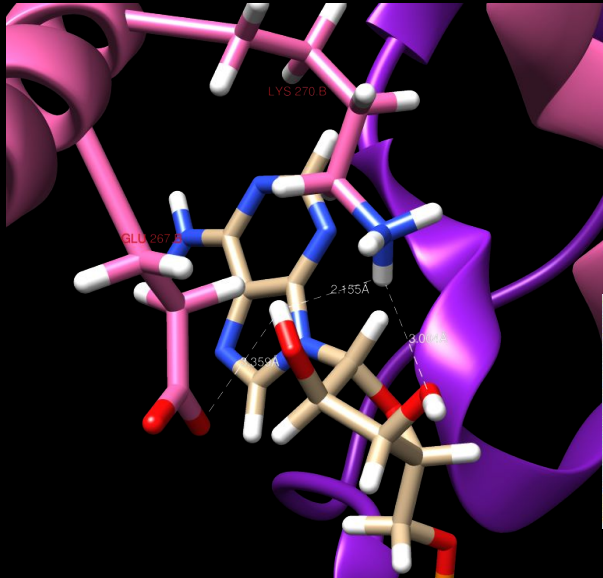
Binding	Ribose	Distance
Glu 267 (O)	O2-H	1.97
Lys 270 (N-H)	O2	2.34

Hydrogen Bond 7N46 - *E. coli*

Binding	Ribose	Distance
Glu 267 (O)	O2-H	3.36
Lys 270 (N-H)	O2	2.15
Lys 270 (N-H)	O3	3.00



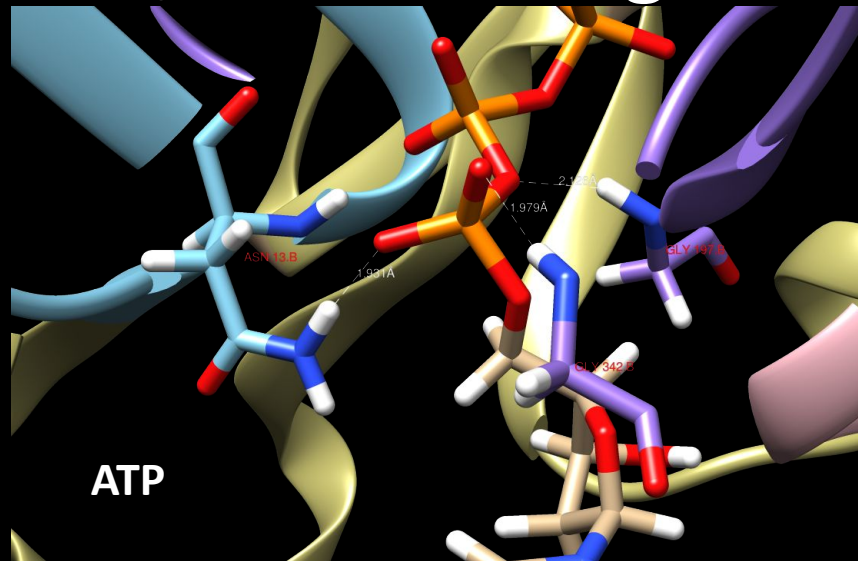
ATP



ADP

	267	270
Consensus	E	K A K R
Conservation		
Homo	E	K A K R
Xenopus	E	K A K R
Drosophila	E	K A K R
Caenorhabditis	E	K A K R
Nicotiana	E	R A K R
Saccharomyces	E	K A K R
Escherichia	E	K A K I

ATP/ADP binding- α P



Hydrogen Bond

4B9Q - *E. coli*

Hsp70	α P	Distance
Asn 13 (N-H)	O2	1.93
Gly 197 (N-H)	O3	2.12
Gly 342 (N-H)	O1	1.98

13

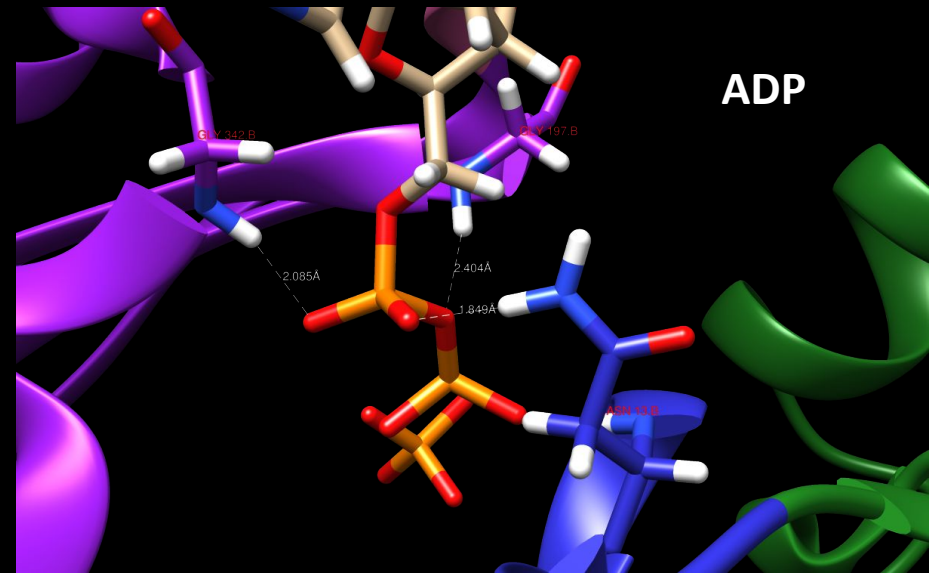
197

342

Consensus	Y	S	C
Conservation			
Homo	Y	S	C
Xenopus	Y	S	C
Drosophila	Y	S	C
Caenorhabditis	Y	S	C
Nicotiana	Y	S	C
Saccharomyces	Y	S	C
Escherichia	N	S	C

Consensus	G	G
Conservation		
Homo	G	G
Xenopus	G	G
Drosophila	G	G
Caenorhabditis	G	G
Nicotiana	G	G
Saccharomyces	G	G
Escherichia	G	G

Consensus	G	G	S
Conservation			
Homo	G	G	S
Xenopus	G	G	S
Drosophila	G	G	S
Caenorhabditis	G	G	S
Nicotiana	G	G	S
Saccharomyces	G	G	S
Escherichia	G	G	Q

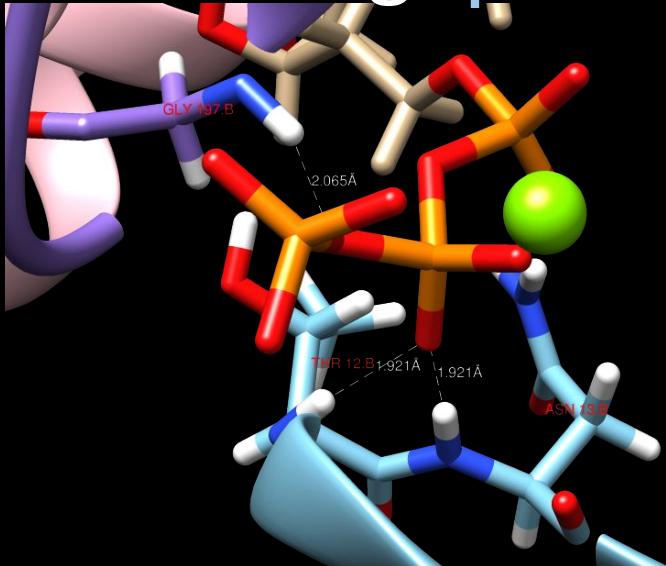


Hydrogen Bond

7N46 - *E. coli*

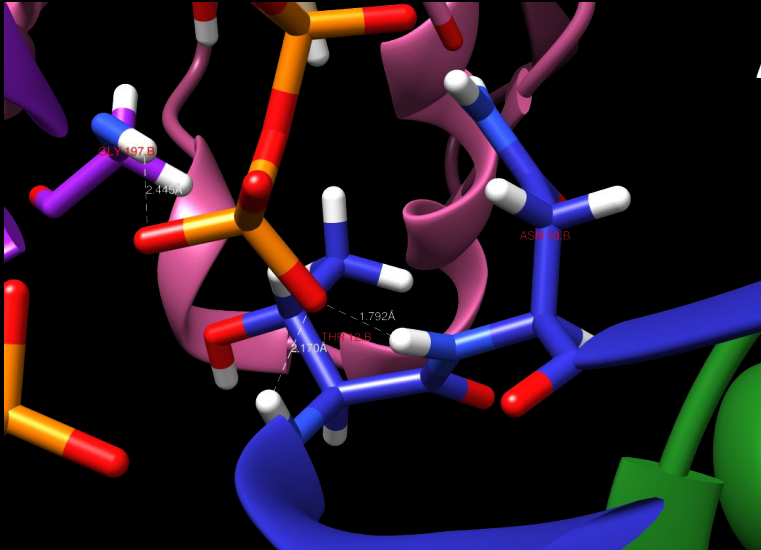
Hsp70	α P	Distance
Asn 13 (N-H)	O2	1.85
Gly 197 (N-H)	O3	2.40
Gly 342 (N-H)	O1	2.08

ATP binding- β P



Hydrogen Bond 4B9Q - *E. coli*

ATP



ADP

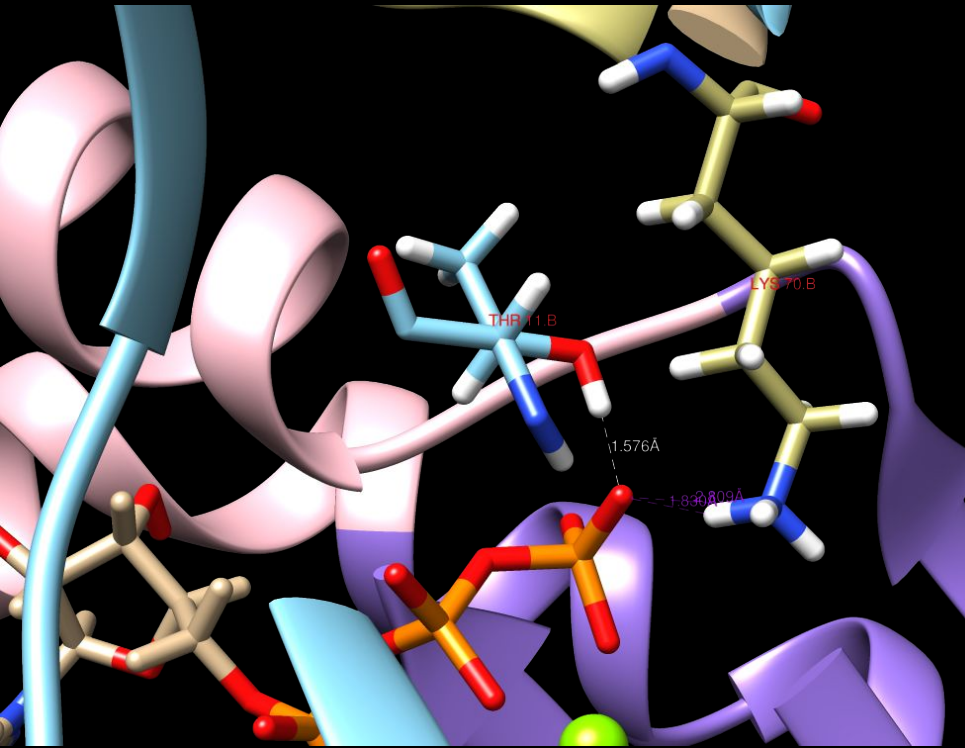
Hydrogen Bond 7N46 - *E. coli*

Binding	β P	Distance Å
Thr 12 (N-H)	O3	1.92
Asn 13 (N-H)	O1	1.92
GLY 197 (N-H)	O1	2.06

	12	13	197
Consensus	T	T	S
Conservation			
Homo	T	T	S
Xenopus	T	T	S
Drosophila	T	T	S
Caenorhabditis	T	T	S
Nicotiana	T	T	S
Saccharomyces	T	T	S
Escherichia	T	T	N

Binding	β P	Distance
Thr 12 (N-H)	O3	2.17
Asn 13 (N-H)	O3	1.79
Gly 197 (N-H)	O1	2.44

ATP binding- Py



4B9Q - *E. coli*

Hydrogen Bond

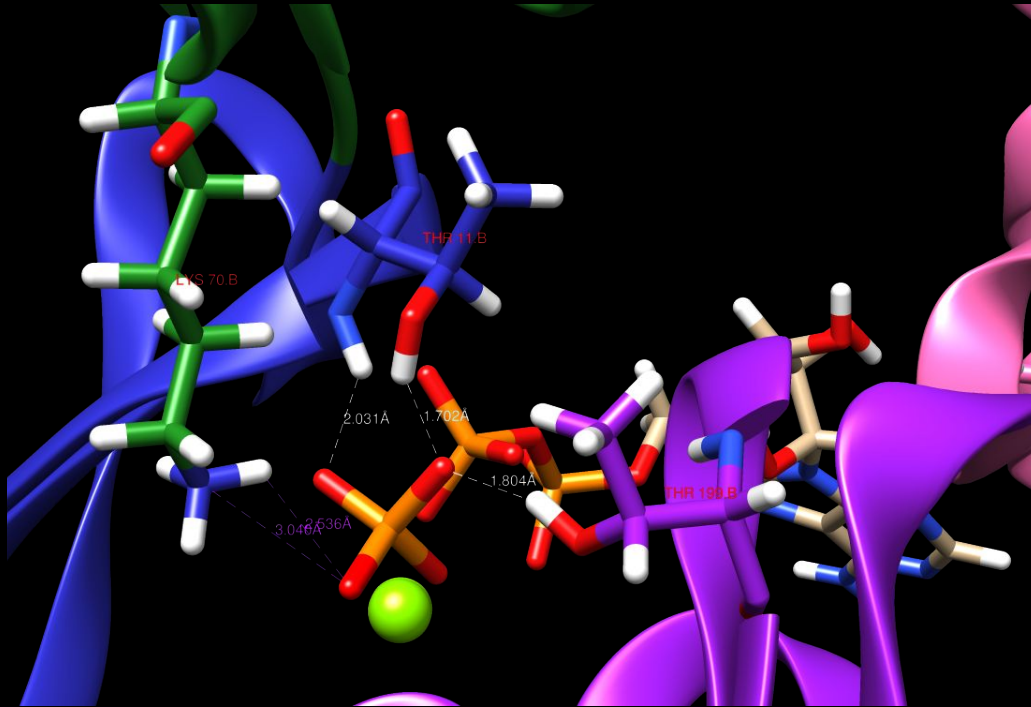
Hsp70	Pi	Distances
Thr 11 (O-H)	O1	1.57

Salt Bridge

Hsp70	Pi
Lys 70	O1

	11	70
Consensus	G T T	a K R
Conservation	■ ■ ■	■ ■ ■
Homo	G T T	A K R
Xenopus	G T T	A K R
Drosophila	G T T	A K R
Caenorhabditis	G T T	A K R
Nicotiana	G T T	V K R
Saccharomyces	G T T	I K R
Escherichia	G T T	I K R

ADP binding- Pi



7N46 - *E. coli*

Hydrogen Bond

Hsp70	Pi	Distances
Thr 11 (O-H)	O2	1.70
Thr 11 (N-H)	O4	2.03
Thr 199 (O-H)	O2	1.80

Salt Bridge

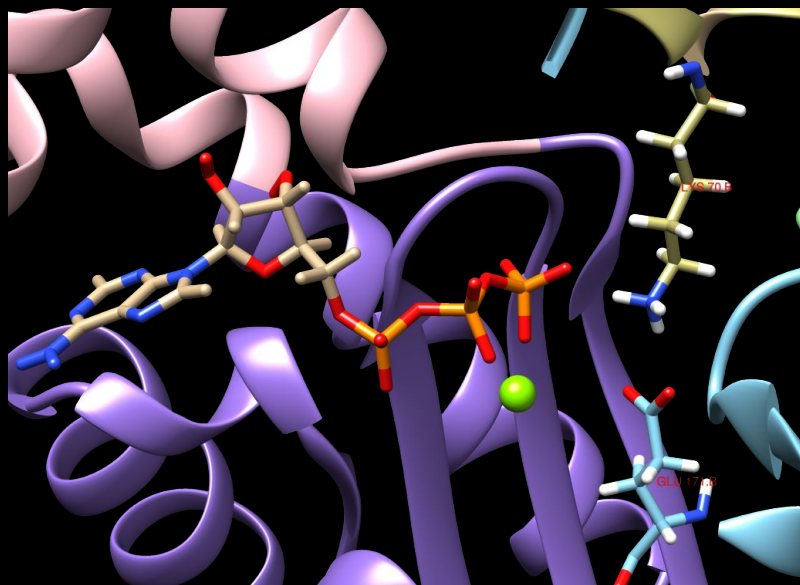
Hsp70	Pi
Lys 70	O4

Bridge	Pi (O3) -Mg+2 - βP (O2)
--------	-------------------------

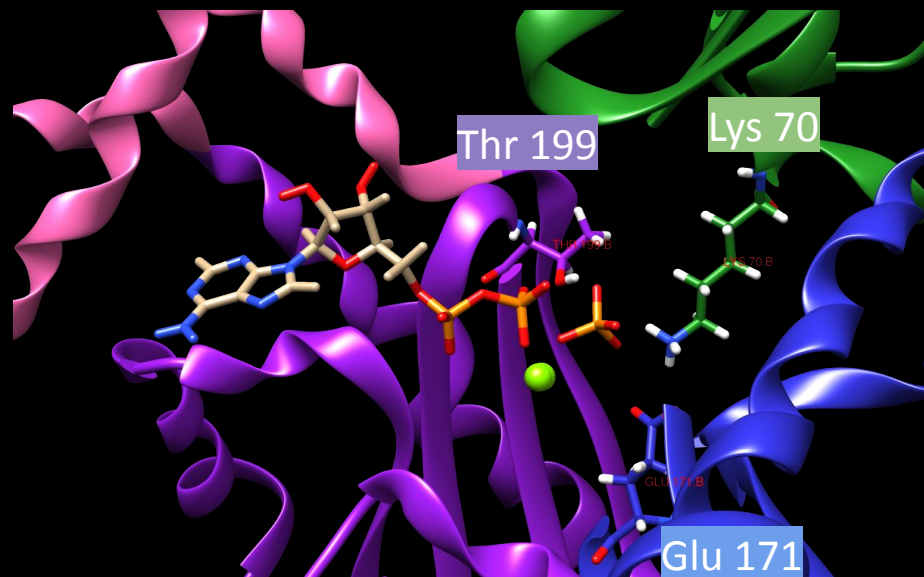
	11	70	199
Consensus	G T T	a K R	G T F
Conservation	■ ■ ■	■ ■ ■	■ ■ ■
Homo	G T T	A K R	G T F
Xenopus	G T T	A K R	G T F
Drosophila	G T T	A K R	G T F
Caenorhabditis	G T T	A K R	G T F
Nicotiana	G T T	V K R	G T F
Saccharomyces	G T T	I K R	G T F
Escherichia	G T T	I K R	G T F

ATP hydrolysis

	70	171	199
Consensus	aKR	NEP	GI
Conservation			
Homo	AKR	NEP	GI
Xenopus	AKR	NEP	GI
Drosophila	AKR	NEP	GI
Caenorhabditis	AKR	NEP	GI
Nicotiana	VKR	NEP	GI
Saccharomyces	IKR	NEP	GI
Escherichia	IKR	NEP	GI



4B9Q - *E. coli*

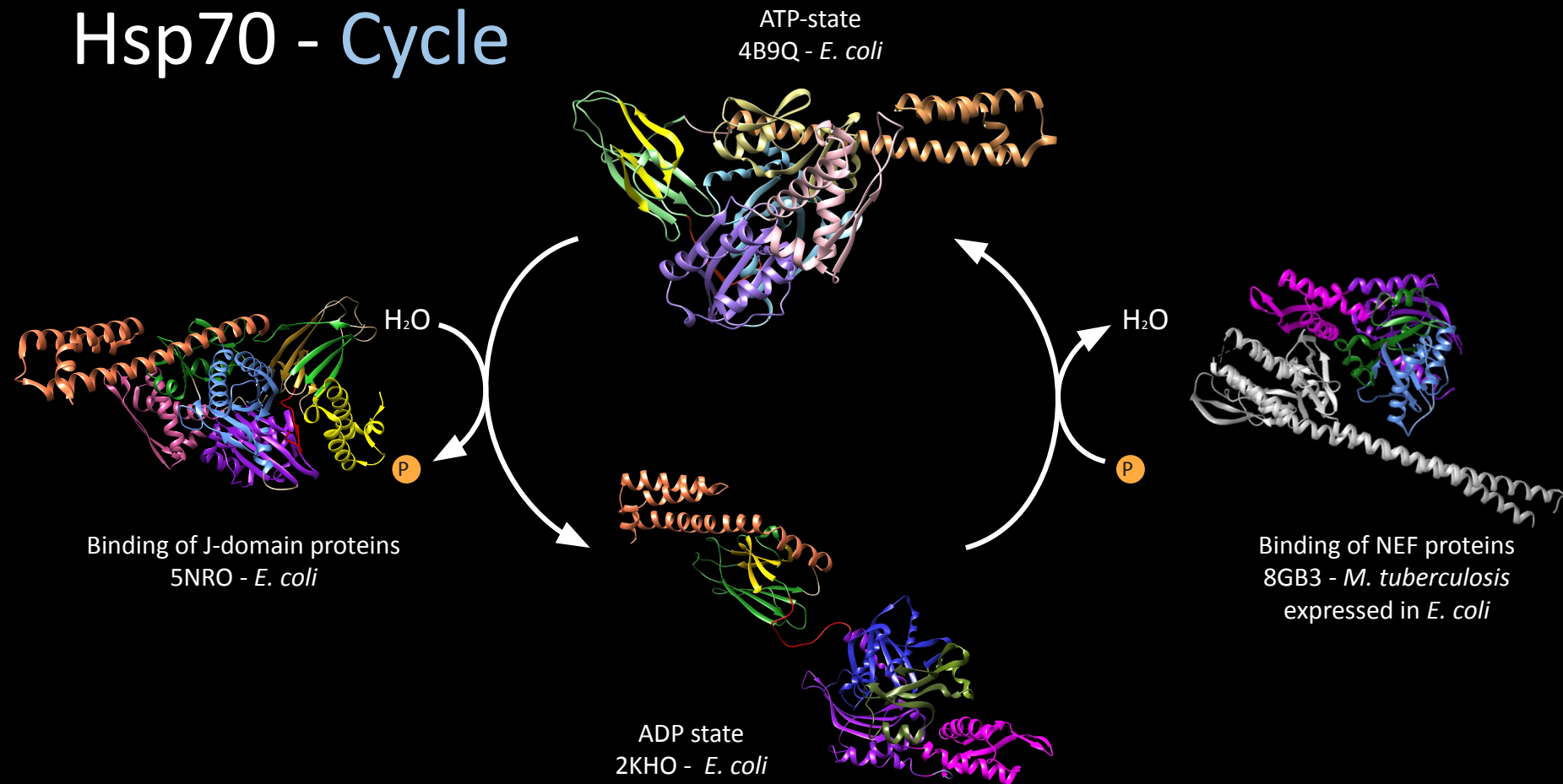


7N46 - *E. coli*

H₂O

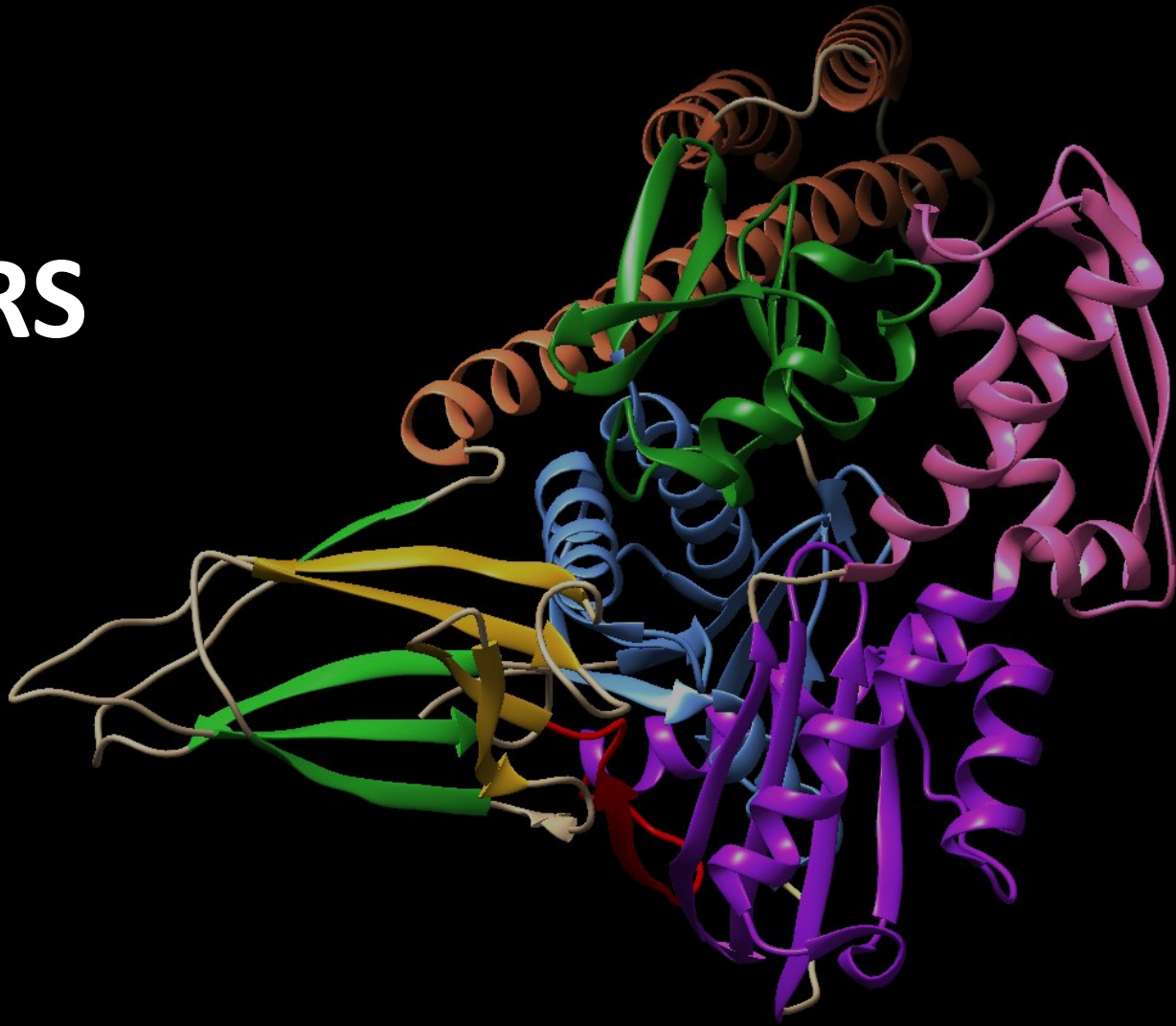
P

Hsp70 - Cycle

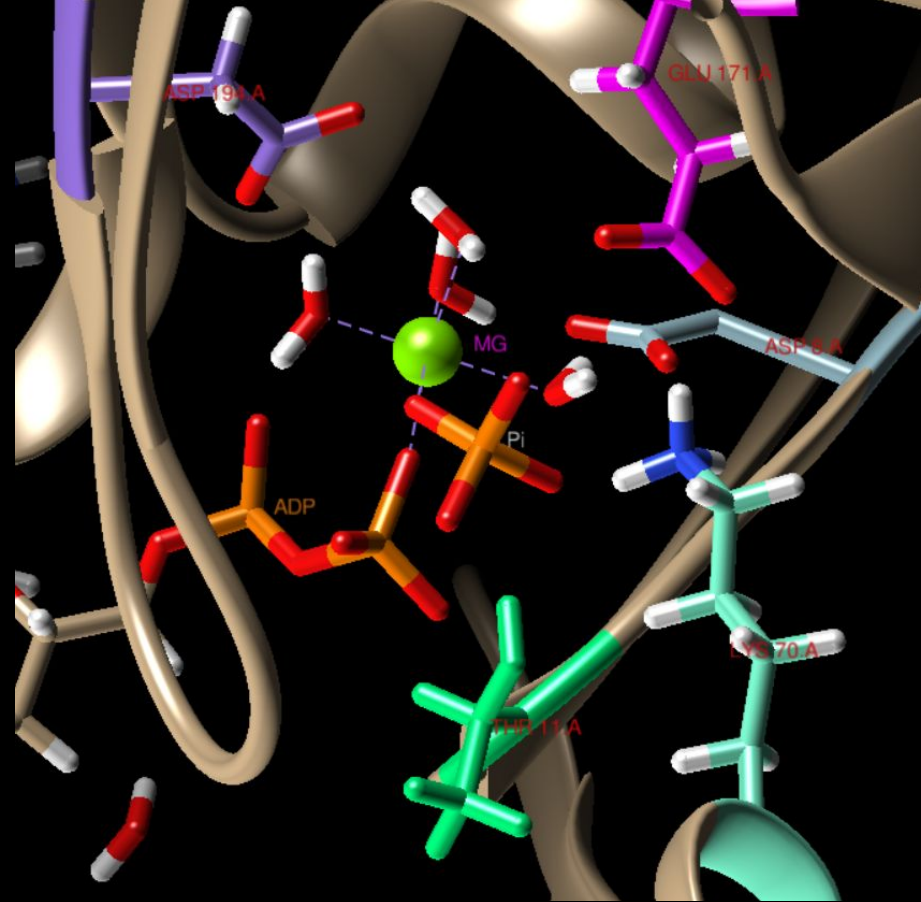


HSP70

CO-FACTORS

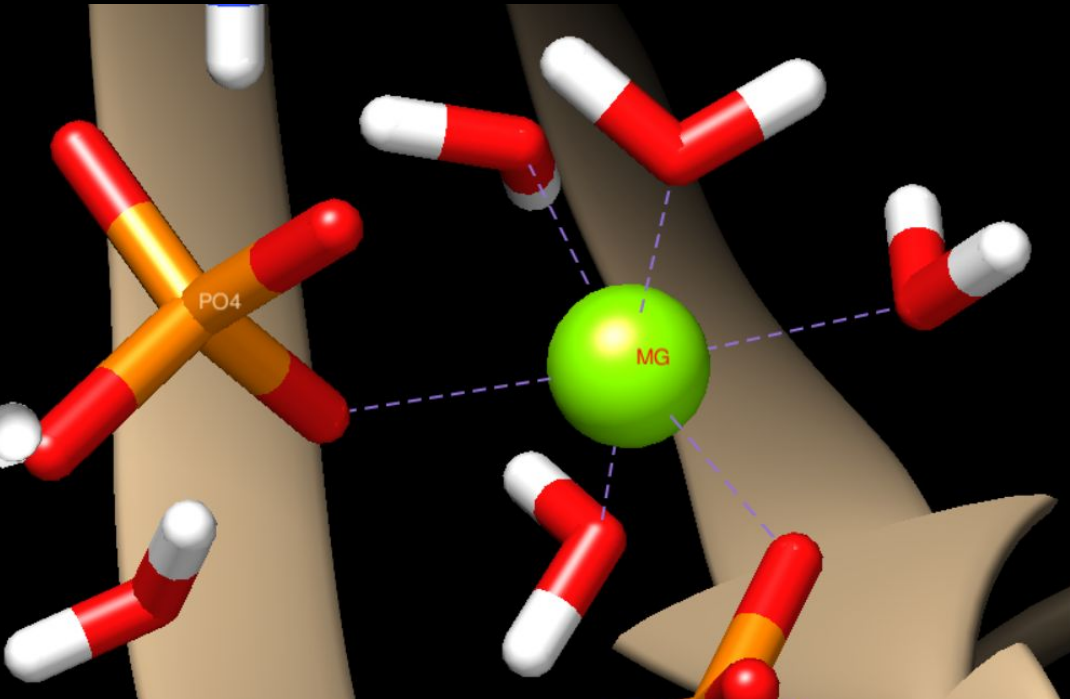


MAGNESIUM



7N46 - *E. coli*

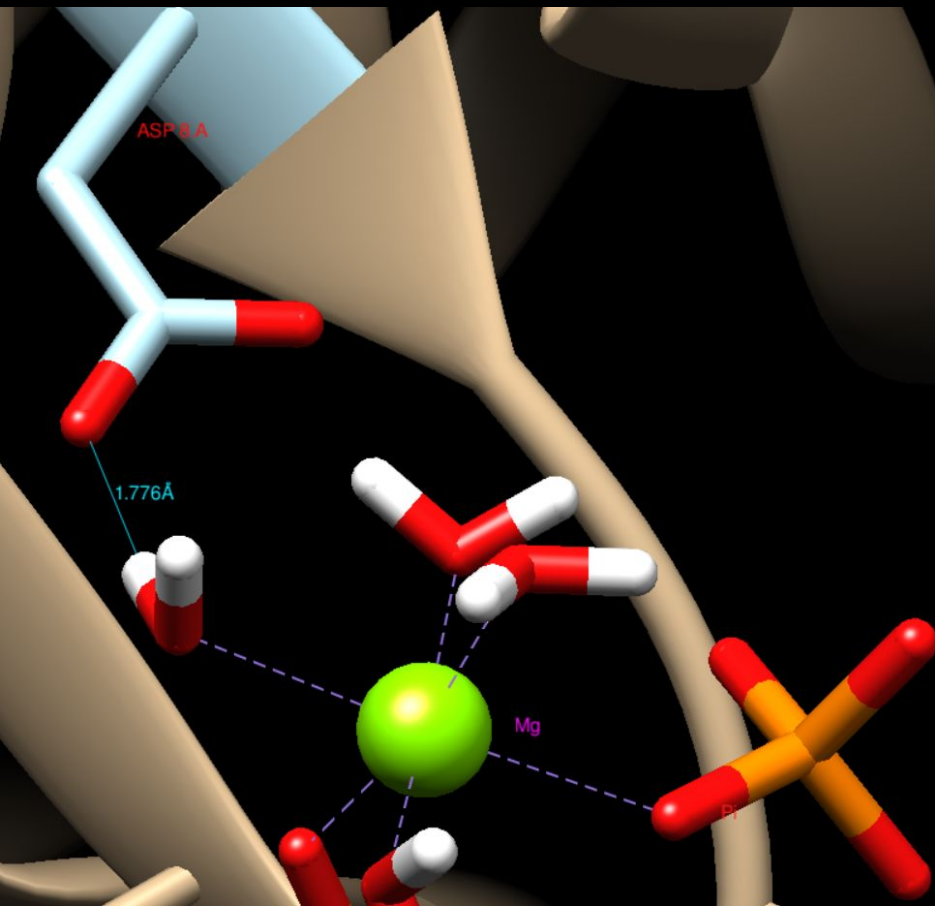
Hydration shell - Mg and water molecules



- Aqueous cellular environment
- Stabilization of conformational structure of Hsp70

Aspartic acid 8

Hydrogen bond



Residues	Distance
Asp8 (O) - H2O (H)	1.776

- Connected with the hydration shell

	8
Consensus	I D L
Conservation	I D L
Homo	I D L
Xenopus	I D L
Drosophila	I D L
Caenorhabditis	I D L
Nicotiana	I D L
Saccharomyces	I D L
Escherichia	I D L

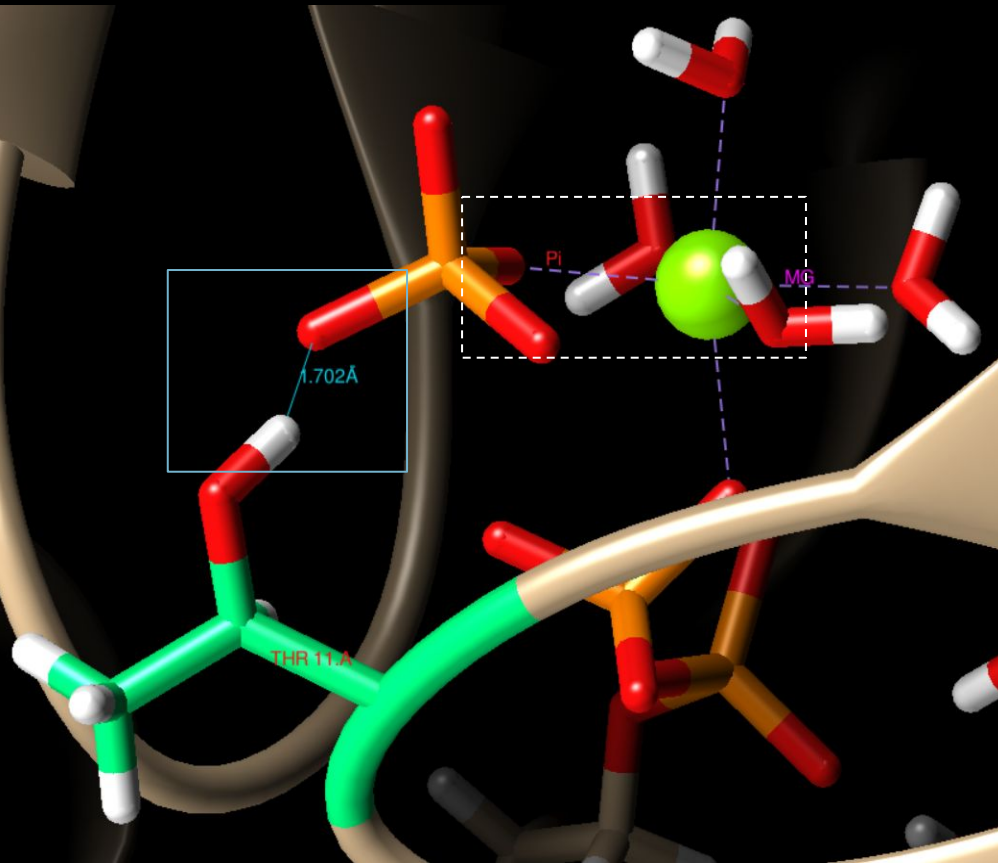
Threonine 11

Hydrogen bond

Residues	Distance
Thr11 (OH) - Pi	1.702

Magnesium phosphate complex

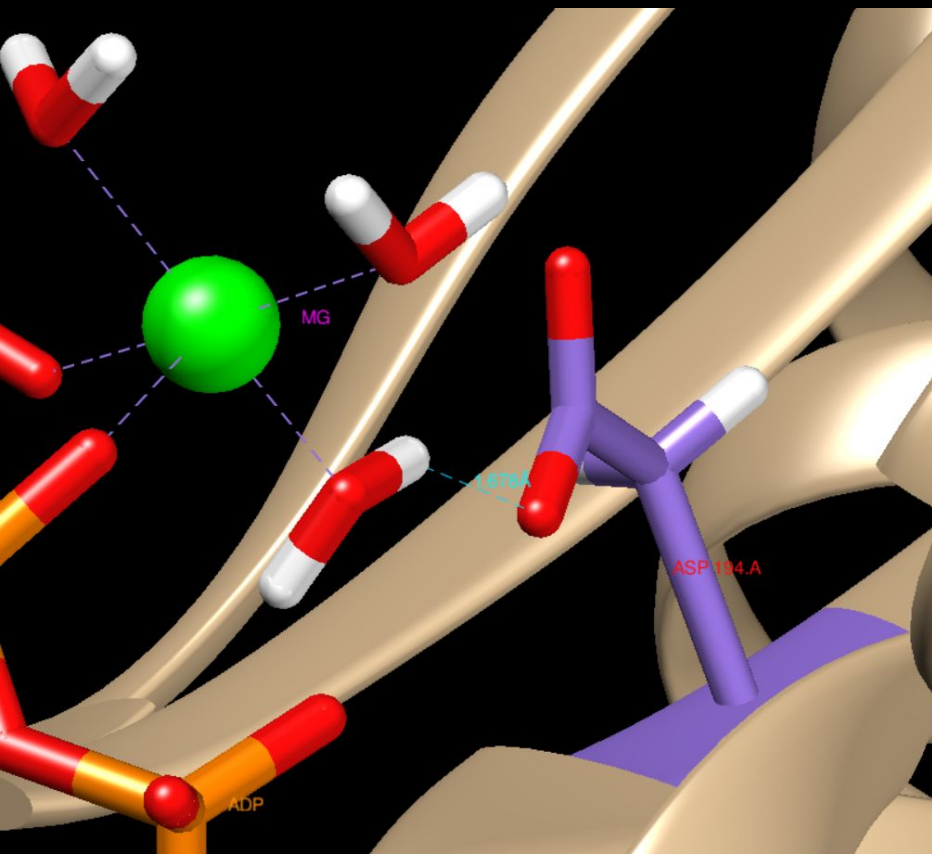
Stabilization of the molecule for facilitating ATP hydrolysis



11

	L	G	T
Consensus			
Conservation	L	G	T
Homo	L	G	T
Xenopus	L	G	T
Drosophila	L	G	T
Caenorhabditis	L	G	T
Nicotiana	L	G	T
Saccharomyces	L	G	T
Escherichia	L	G	T

Aspartic acid 194



Hydrogen bond

Residues	Distance
Asp (COO) - H2O	1.678

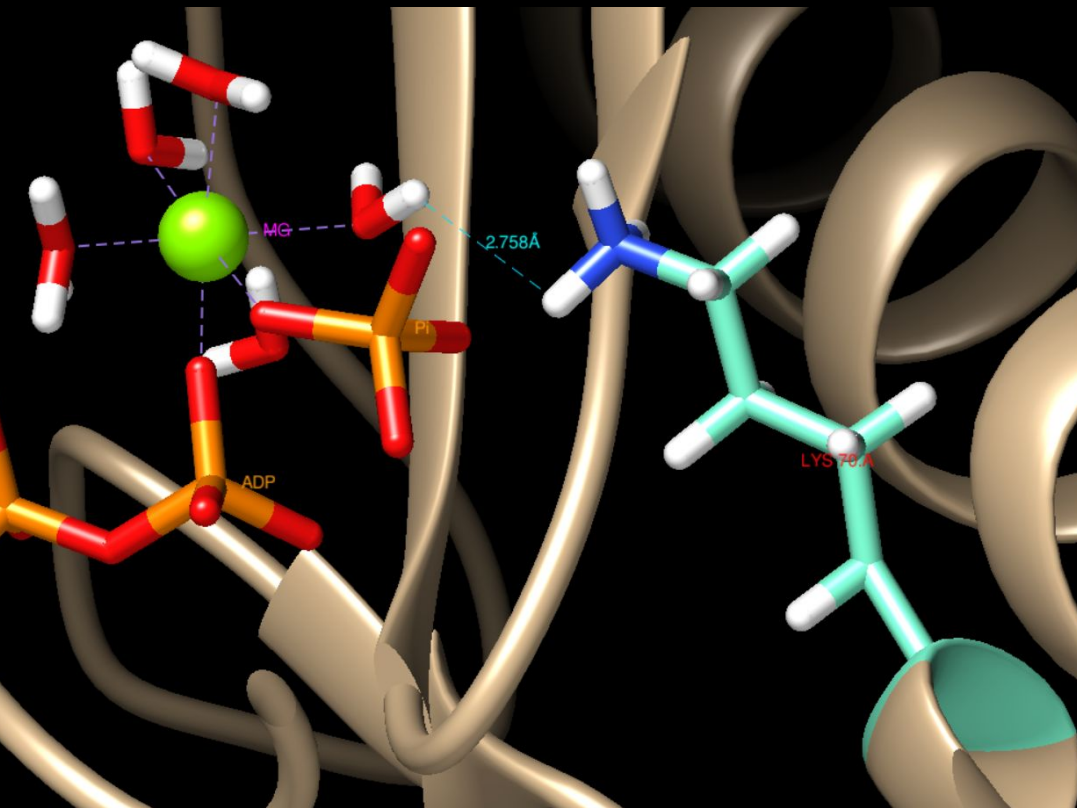
- Connected with the hydration shell

194

Consensus	V	f	D	L
Conservation				
Homo	V	F	D	L
Xenopus	V	F	D	L
Drosophila	V	F	D	L
Caenorhabditis	V	F	D	L
Nicotiana	V	F	D	L
Saccharomyces	V	Y	D	L
Escherichia	V	Y	D	L

Lysine 70

Hydrogen bond

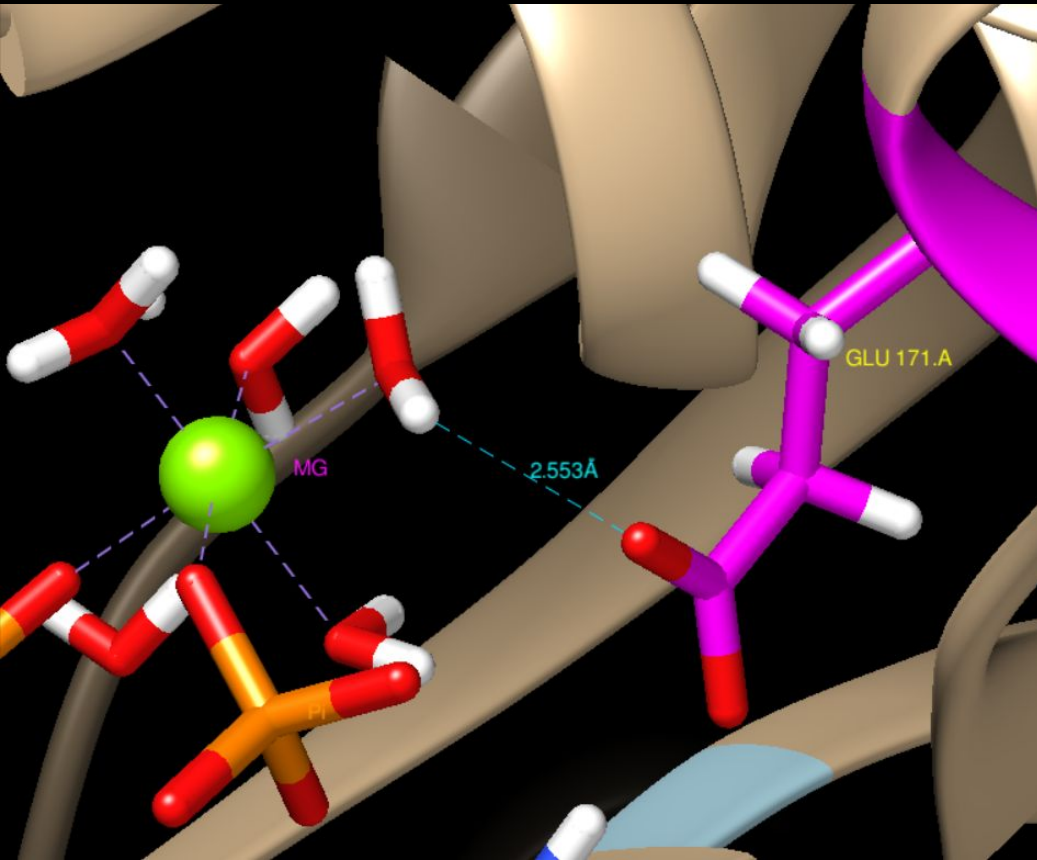


Residues	Distance
Lys70 (-NH ₂) - H ₂ O	2.758

- Connected with the hydration shell

	70
	D a K R
Consensus	
Conservation	D A K R
Homo	D A K R
Xenopus	D A K R
Drosophila	D A K R
Caenorhabditis	D A K R
Nicotiana	D V K R
Saccharomyces	D I K R
Escherichia	A I K R

Glutamic acid 171



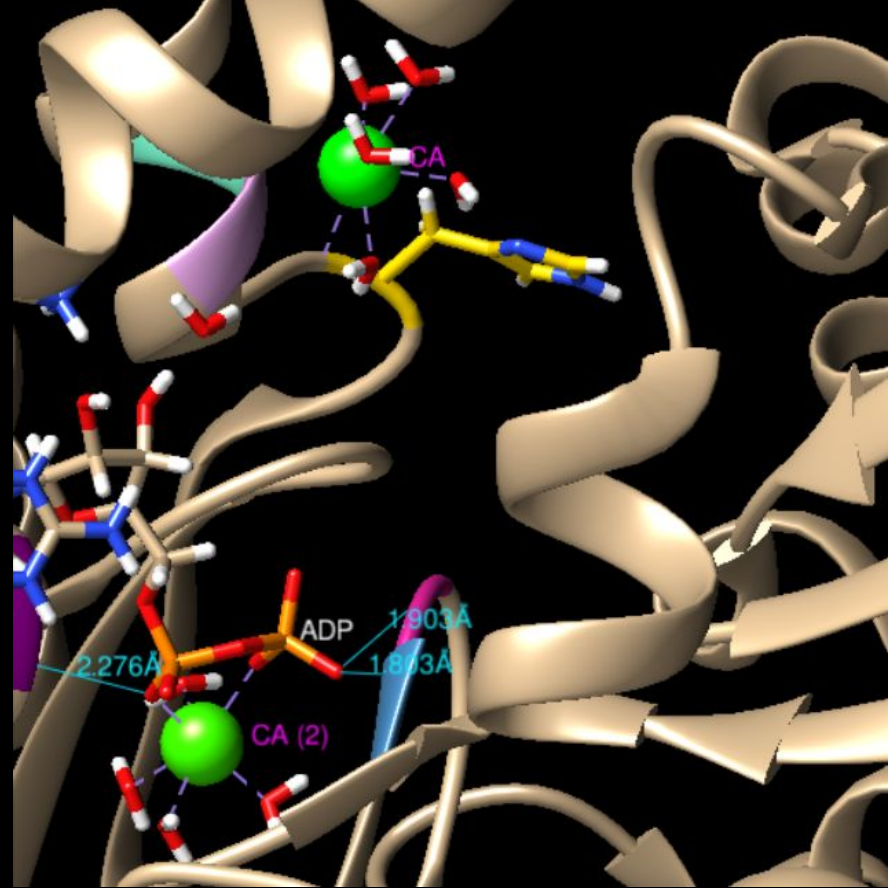
Hydrogen bond

Residues	Distance
Glu171 (COO-) - H2O	2.758

- Connected with the hydration shell

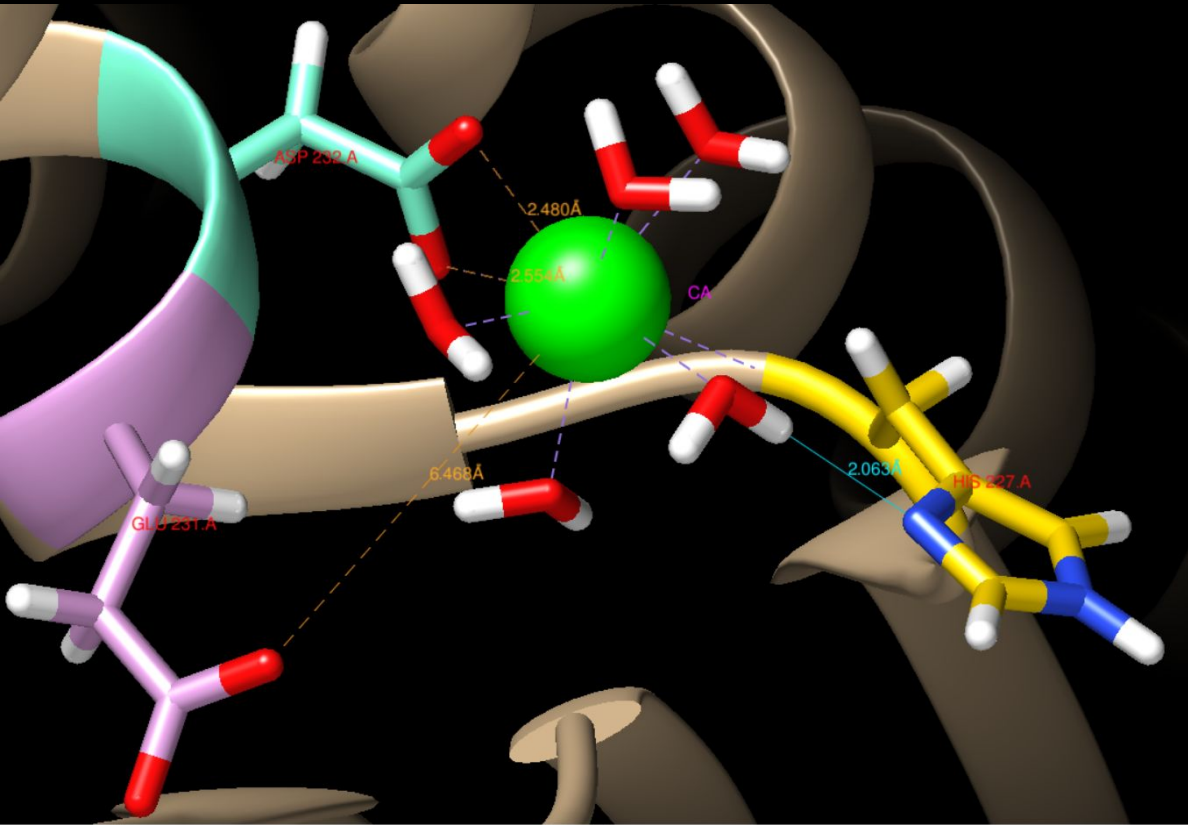
	171
	NEPT
Consensus	NEPT
Conservation	NEPT
Homo	NEPT
Xenopus	NEPT
Drosophila	NEPT
Caenorhabditis	NEPT
Nicotiana	NEPT
Saccharomyces	NEPT
Escherichia	NEPT

CALCIUM



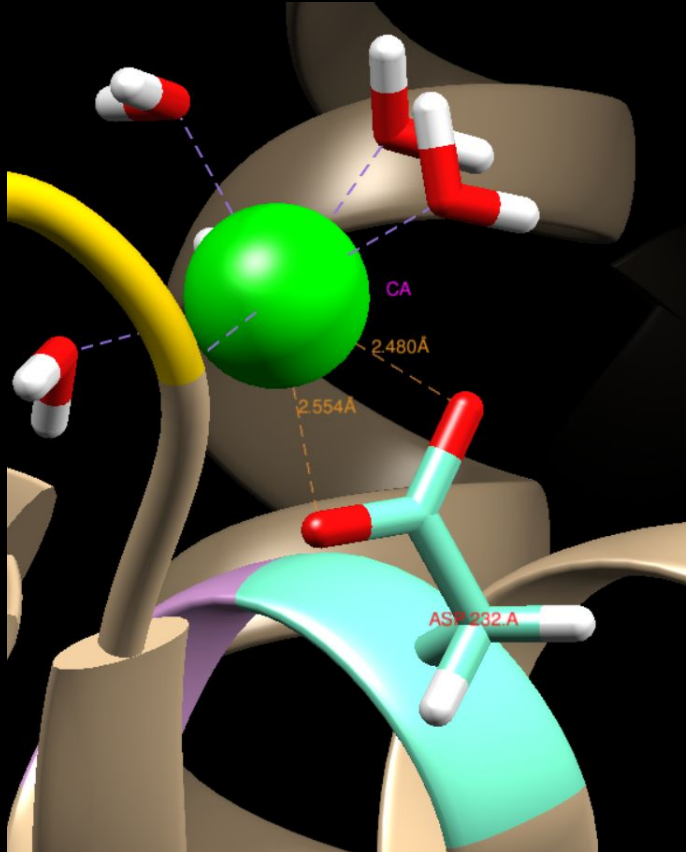
6ZYI - *E. coli*

FIRST CALCIUM BINDING SITE



6ZYI - *E. coli*

Aspartic acid 232

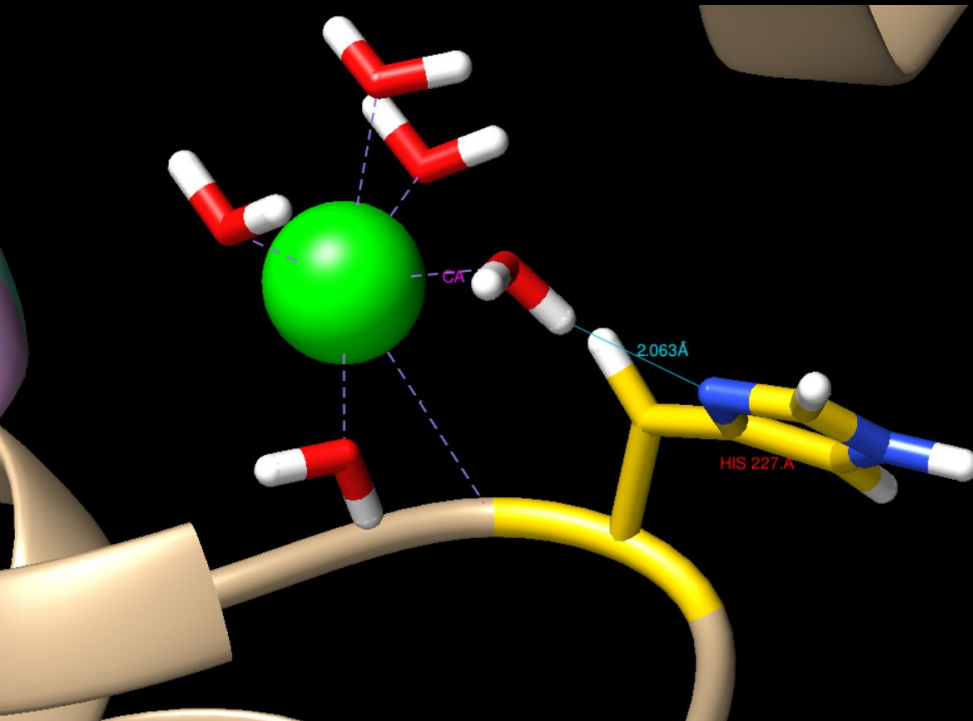


Salt bridges

Residues	Distance
Asp232 (COO-) - Ca ²⁺	2.480
Asp232 (COO-) - Ca ²⁺	2.554

	232
Consensus	G E D F
Conservation	— — — —
Homo	G E D F
Xenopus	G E D F
Drosophila	G E D F
Caenorhabditis	G E D F
Nicotiana	G E D F
Saccharomyces	G E D F
Escherichia	G E D F

Histidine 227



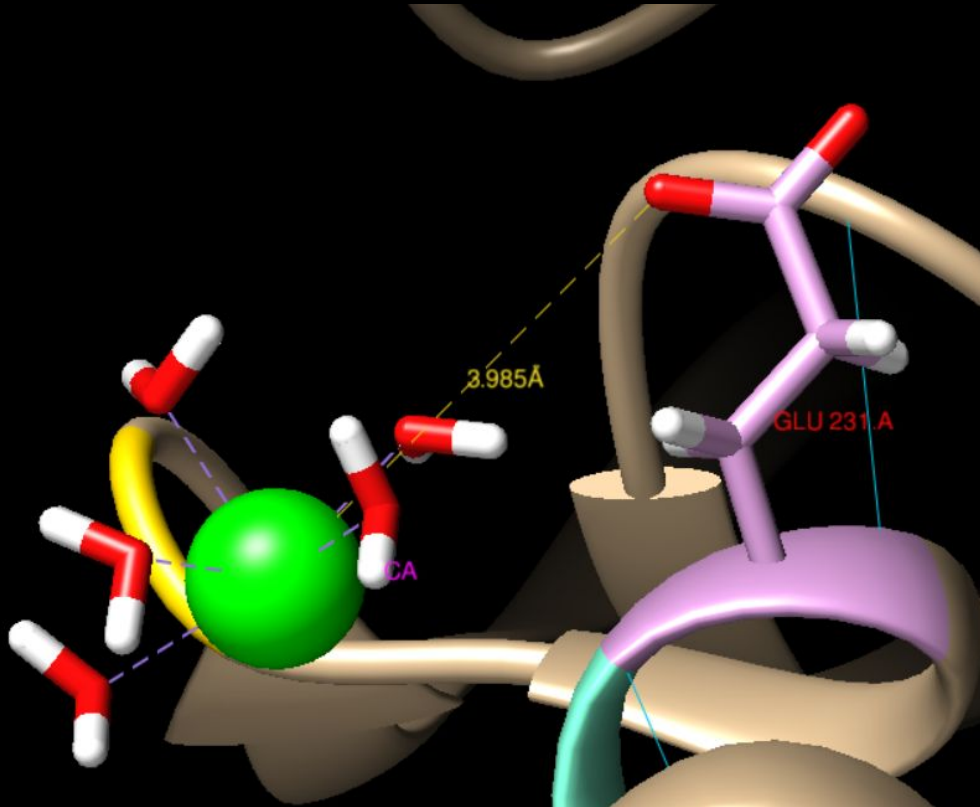
227

	T	H	L	G
Consensus				
Conservation	T	H	L	G
Homo	T	H	L	G
Xenopus	T	H	L	G
Drosophila	T	H	L	G
Caenorhabditis	T	H	L	G
Nicotiana	T	H	L	G
Saccharomyces	T	H	L	G
Escherichia	T	H	L	G

Hydrogen bonds

Residues	Distance
Hys (imidazole) - H ₂ O	2.063

Glutamic acid 231



231

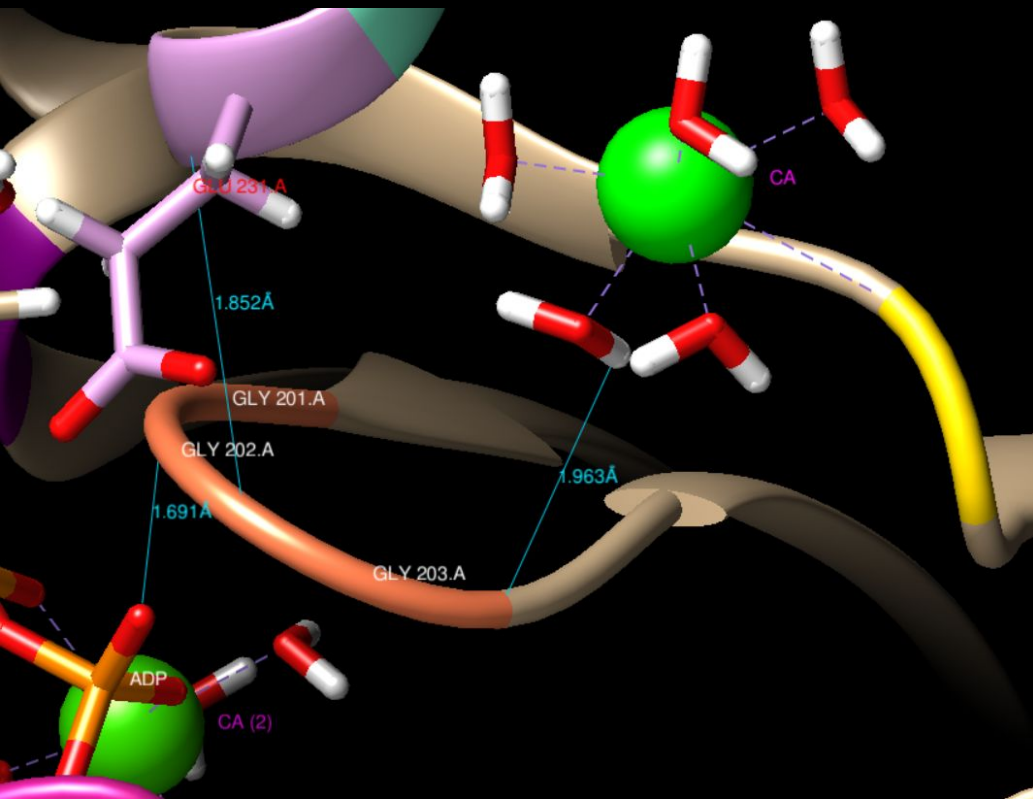
	G	E	D	F
Consensus	G	E	D	F
Conservation	G	E	D	F
Homo	G	E	D	F
Xenopus	G	E	D	F
Drosophila	G	E	D	F
Caenorhabditis	G	E	D	F
Nicotiana	G	E	D	F
Saccharomyces	G	E	D	F
Escherichia	G	E	D	F

Electrostatic interaction

Residues	Distance
Glu231 (COO-) Ca	3.985

Glycine 202 and 203 - Glycine loop

Hydrogen bond

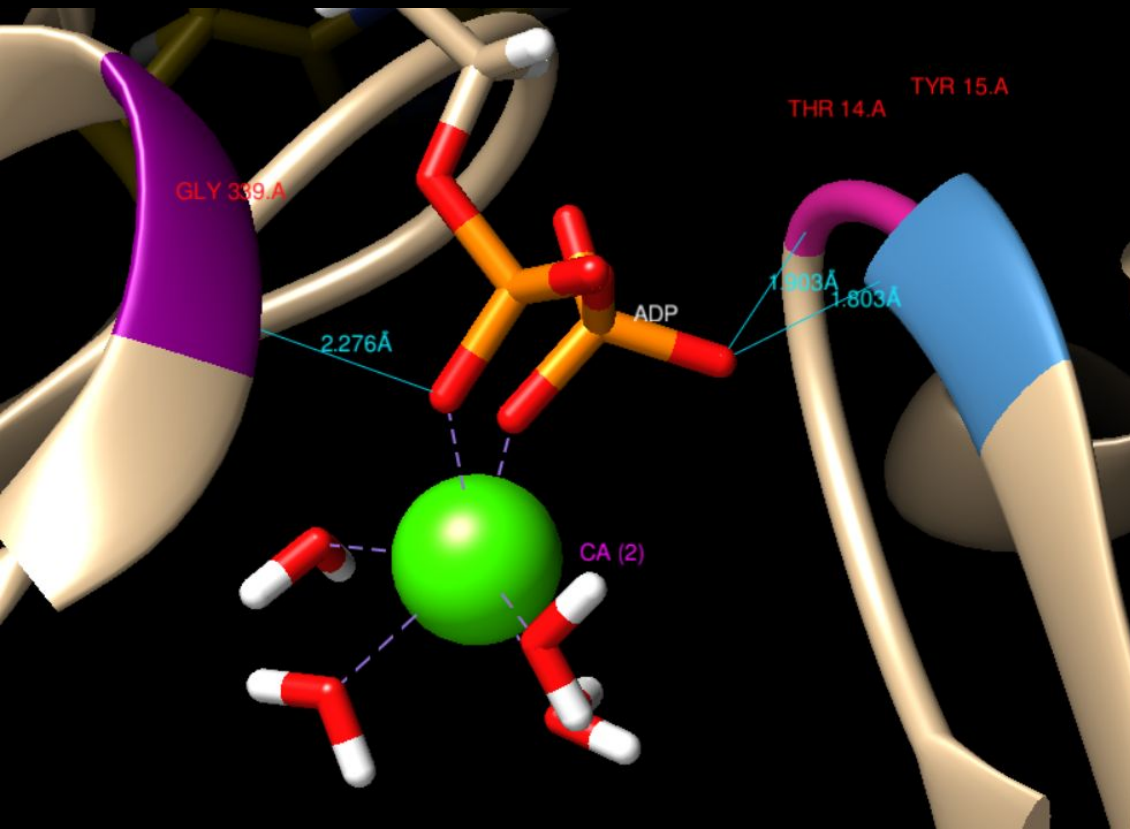


Residues	Distance
Gly202 - Glu231	1.852
Gly202 - ADP	1.691
Gly203 (COO-) - H2O	1.963

202 203

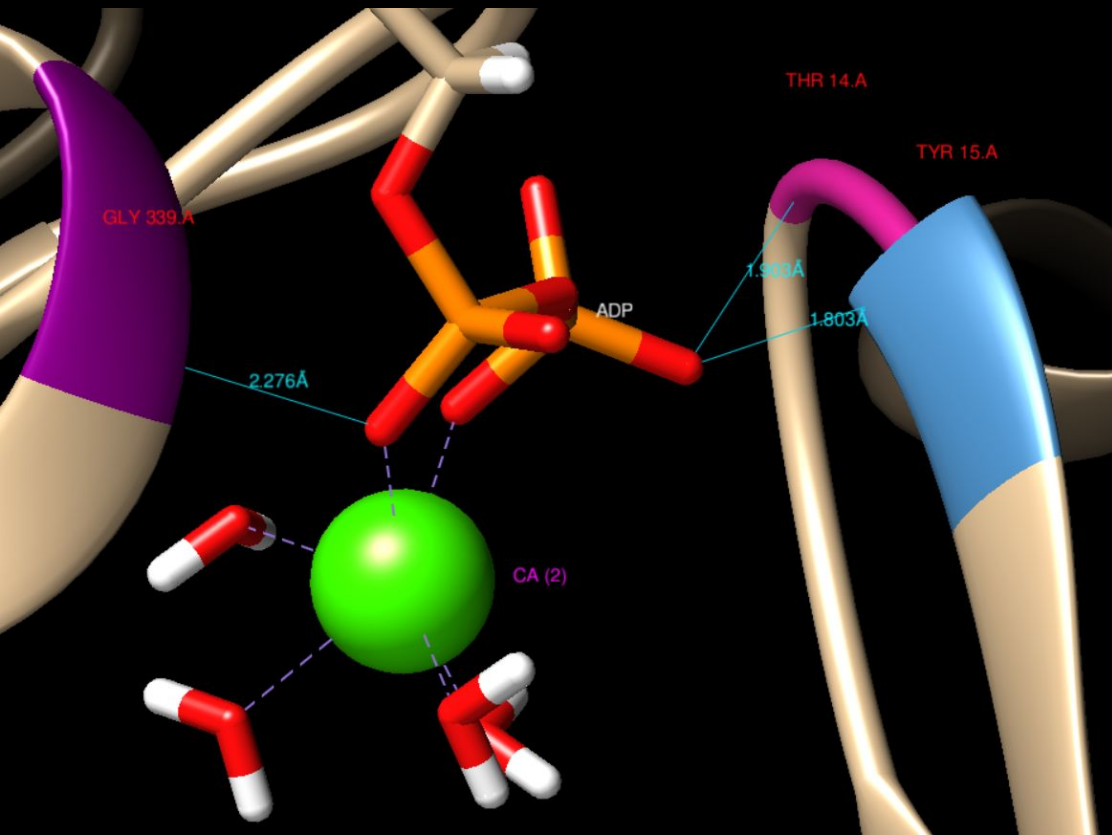
Consensus	G G T F
Conservation	■ ■ ■ ■
Homo	G G T F
Xenopus	G G T F
Drosophila	G G T F
Caenorhabditis	G G T F
Nicotiana	G G T F
Saccharomyces	G G T F
Escherichia	G G T F

SECOND CALCIUM BINDING SITE



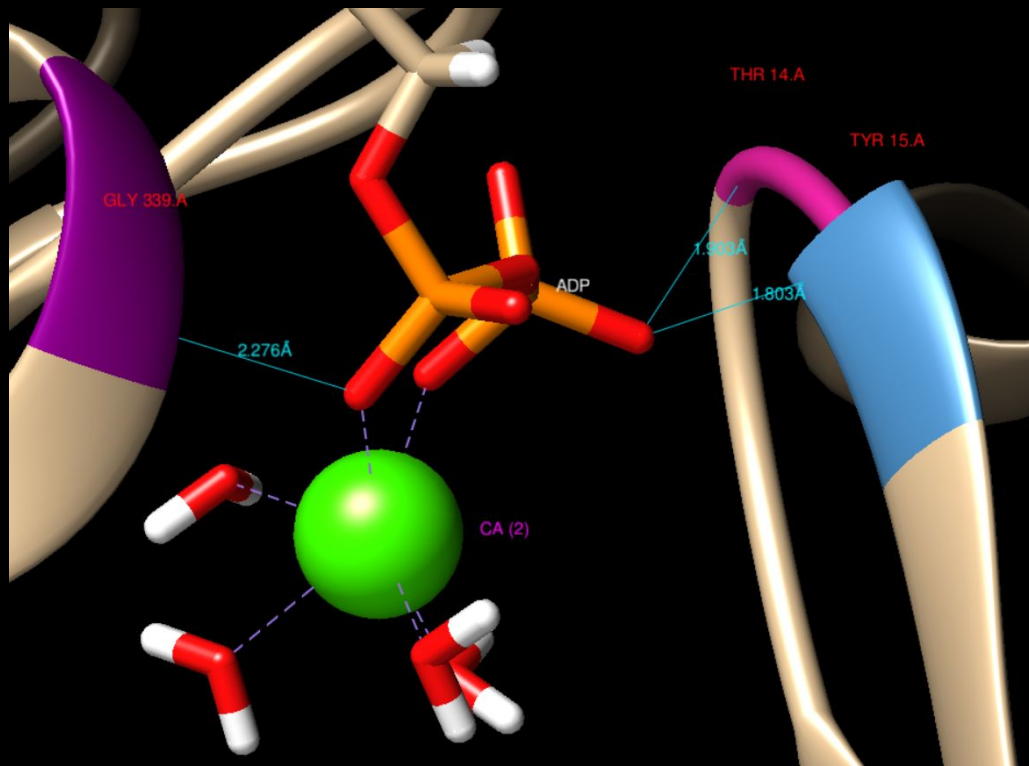
6ZYI - *E. coli*

Glycine 339, Threonine 14 and Threonine 15



Hydrogen bonds

Residues	Distance
Gly - ADP	2.276
ADP - Thr14	1.903
ADP (O) - Tyr15	1.803

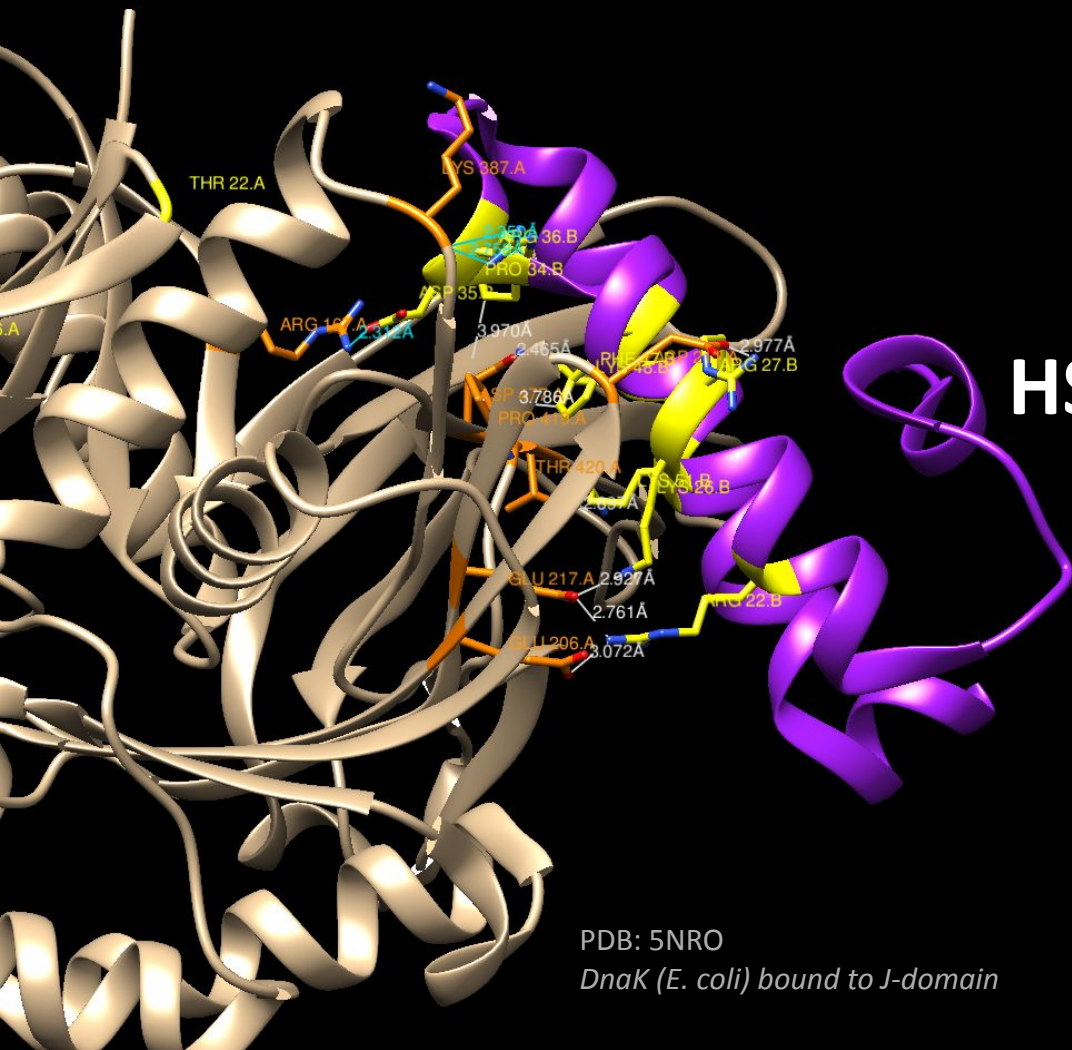


	339	14	15
Consensus	L V G G	G T T	Y S C
Conservation			
Homo	L V G G	G T T	Y S C
Xenopus	L V G G	G T T	Y S C
Drosophila	L V G G	G T T	Y S C
Caenorhabditis	L V G G	G T T	Y S C
Nicotiana	L V G G	G T T	Y S C
Saccharomyces	L V G G	G T T	Y S C
Escherichia	L V G G	G T T	Y S C

HSP70

CO-CHAPERONES





HSP70 CO-CHAPERONES:

J-Domain Proteins (JDPs)

PDB: 5NRO

DnaK (E. coli) bound to J-domain

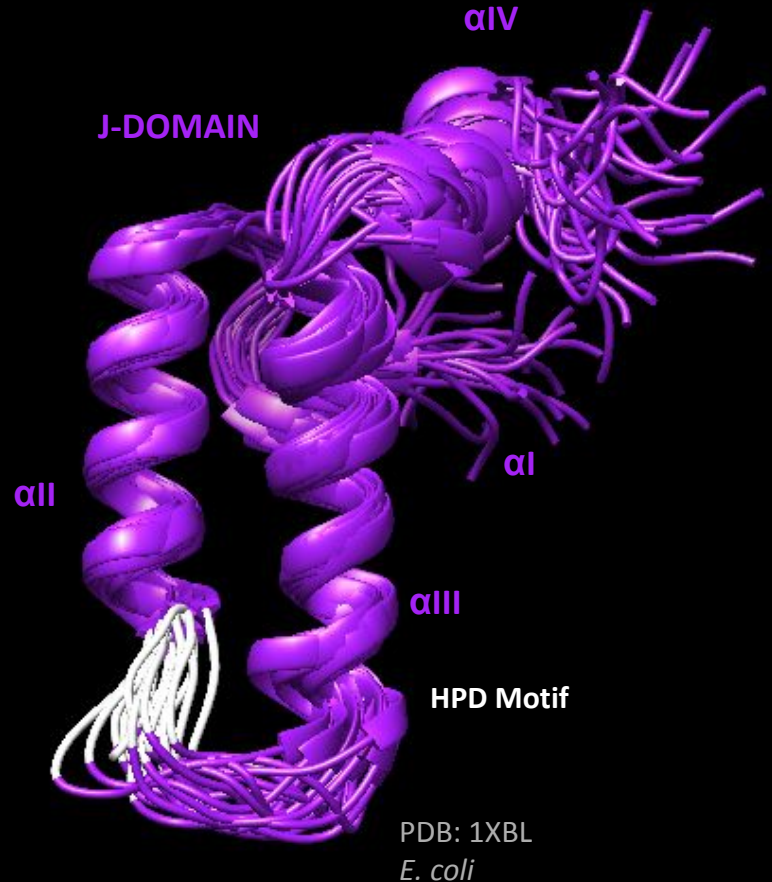
Co-Chaperone: J-Domain Protein

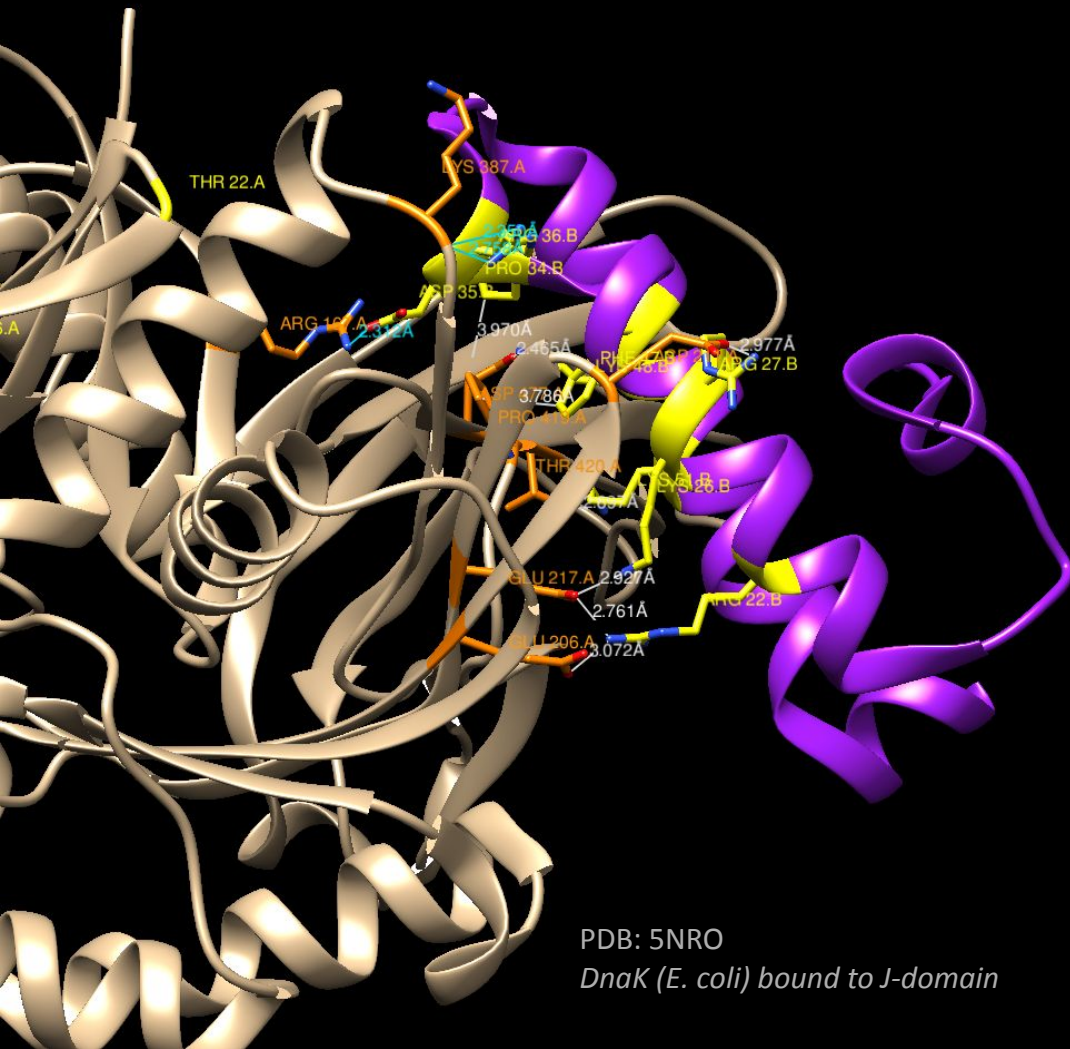
Structure:

- 4 α -helices
- HPD motif (His33-Pro34-Asp35)

Function:

Stimulates and accelerates the hydrolysis of Hsp70-bound ATP and stabilizes the interaction with substrate polypeptides.





JDPs - Binding to Hsp70

The J-domain interacts with the **open ATP-bound state**.

It binds on top of the linker contacting NBD and SBD β via **electrostatic and hydrophobic interactions**.

PDB: 5NRO

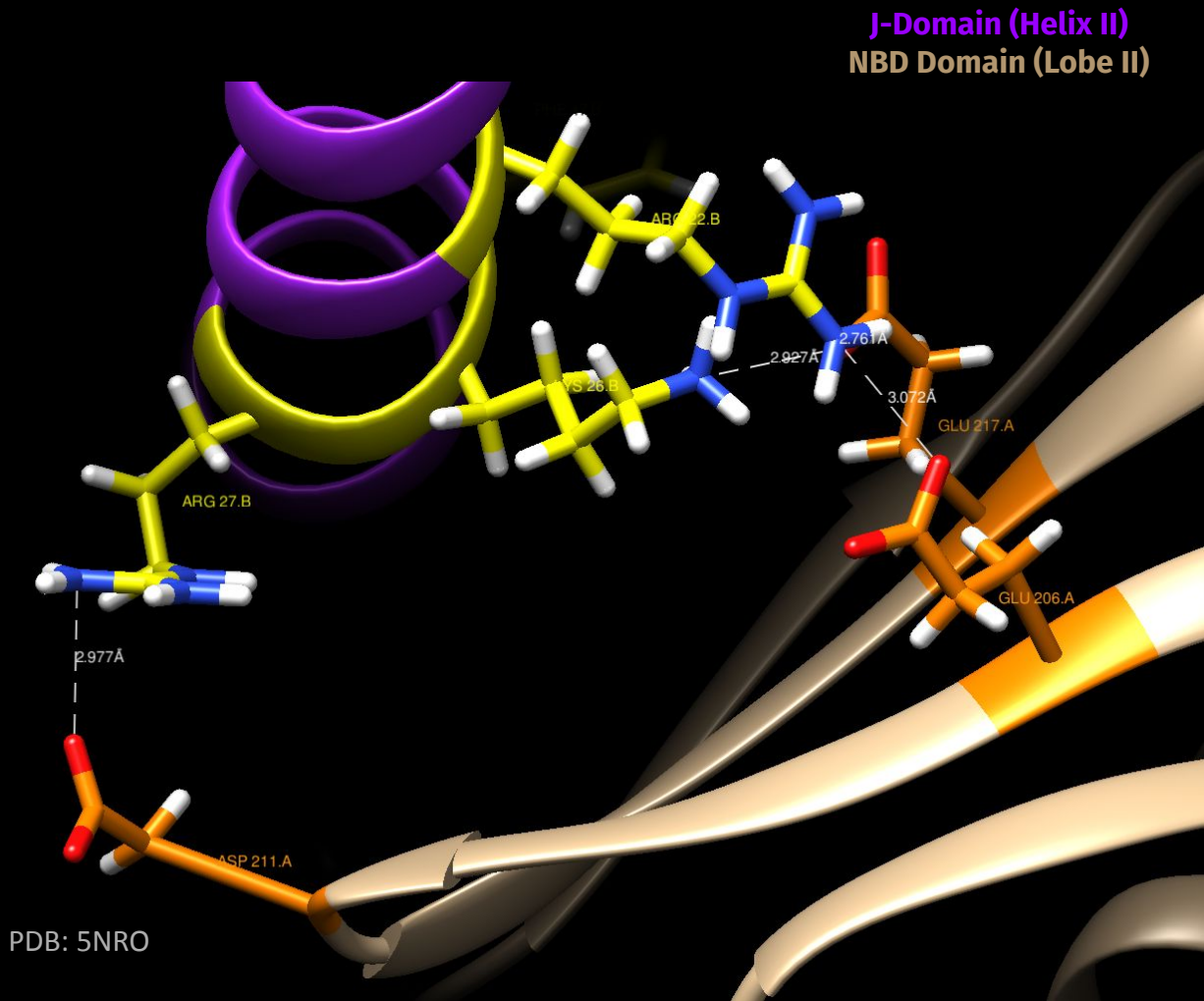
DnaK (E. coli) bound to J-domain

JDPs - First Interface

Electrostatic Interactions

Residues	Distance
Arg22 - Glu206	3.072
Arg22 - Glu217	2.761
Arg27 - Asp211	2.927
Lys26 - Glu217	2.977

	206	211	217
Consensus	L t	I D N G - - - V F	E V
Conservation	L T	I D N G - - - V F	E V
Homo	L T	I D N G - - - V F	E V
Xenopus	L T	I D N G - - - V F	E V
Drosophila	L T	I D N G - - - V F	E V
Caenorhabditis	L T	I D N G - - - V F	E V
Nicotiana	L T	I D N G - - - V F	E V
Saccharomyces	L S	I E N G - - - V F	E V
Escherichia	E	I D E V D G E K T F	E V

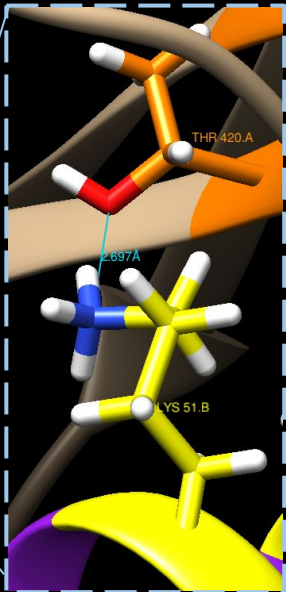
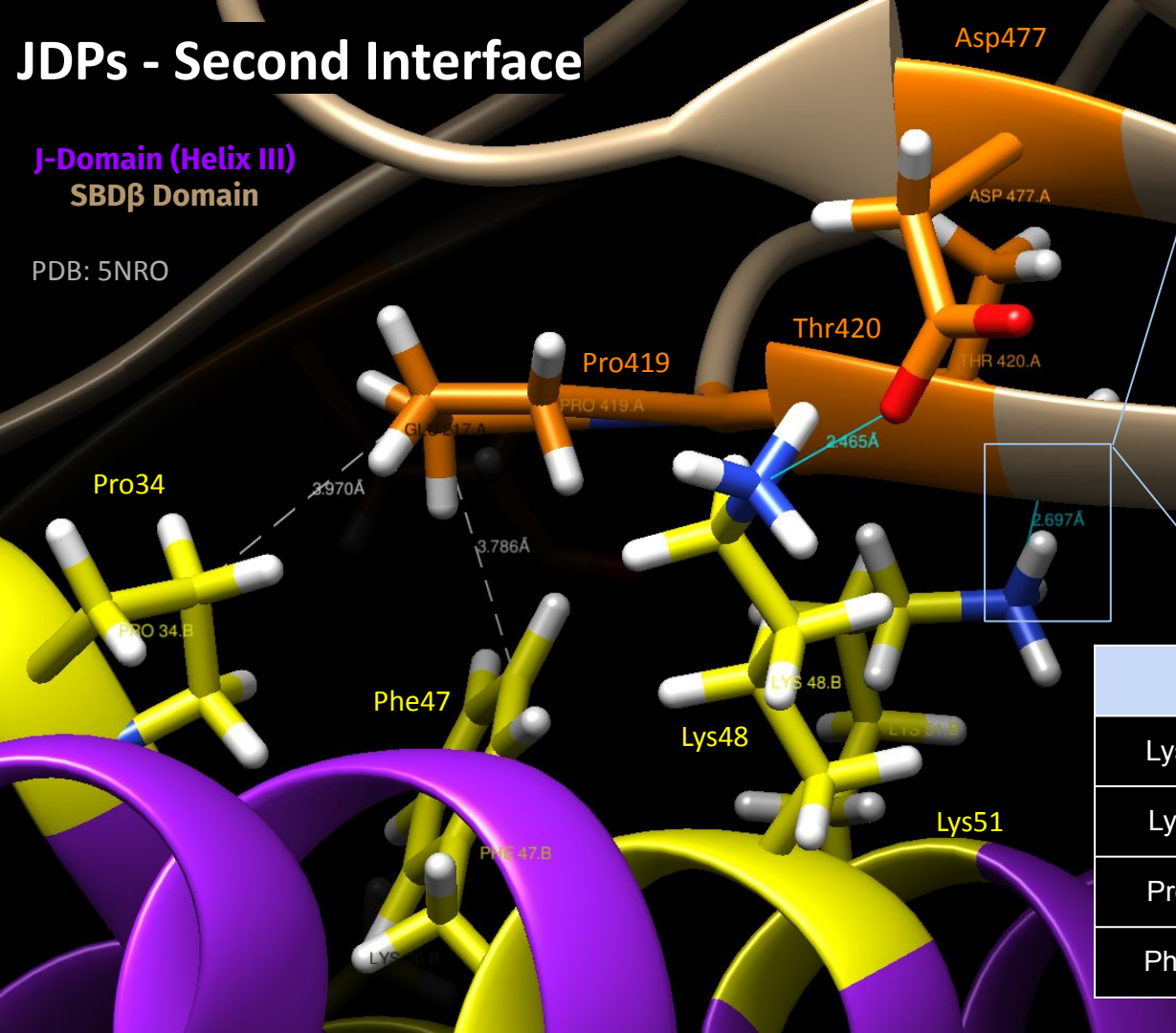


Electrostatic Interactions

JDPs - Second Interface

J-Domain (Helix III)
SBDβ Domain

PDB: 5NRO



Consensus Conservation
Homo
Xenopus
Drosophila
Caenorhabditis
Nicotiana
Saccharomyces
Escherichia

419/20

P	T	K
P	T	K
P	T	K
P	T	K
P	T	K
P	T	K
P	T	K
P	T	K

477

V	T	F	e	i	D	a
V	T	F	E	I	D	V
V	T	F	E	I	D	V
V	S	F	E	I	D	A
V	T	F	E	I	D	V
V	T	F	E	V	D	A
V	T	F	A	L	D	A
V	T	F	D	I	D	A

Residues	Distance	Interaction
Lys48 - Asp477	2.465	H-Bond
Lys51 - Thr420	2.697	H-Bond
Pro34 - Pro419	3.970	Hydrophobic
Phe47 - Pro419	3.786	Hydrophobic

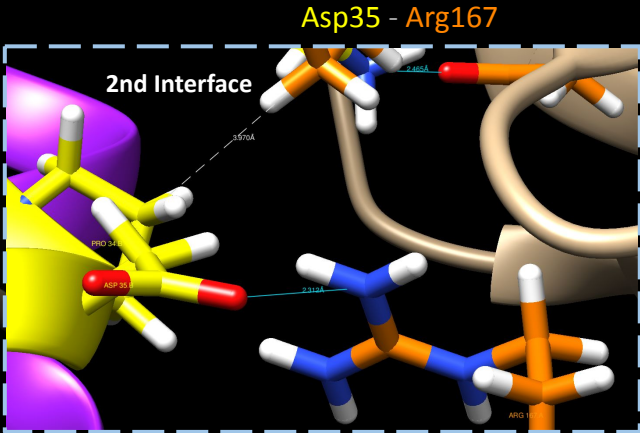
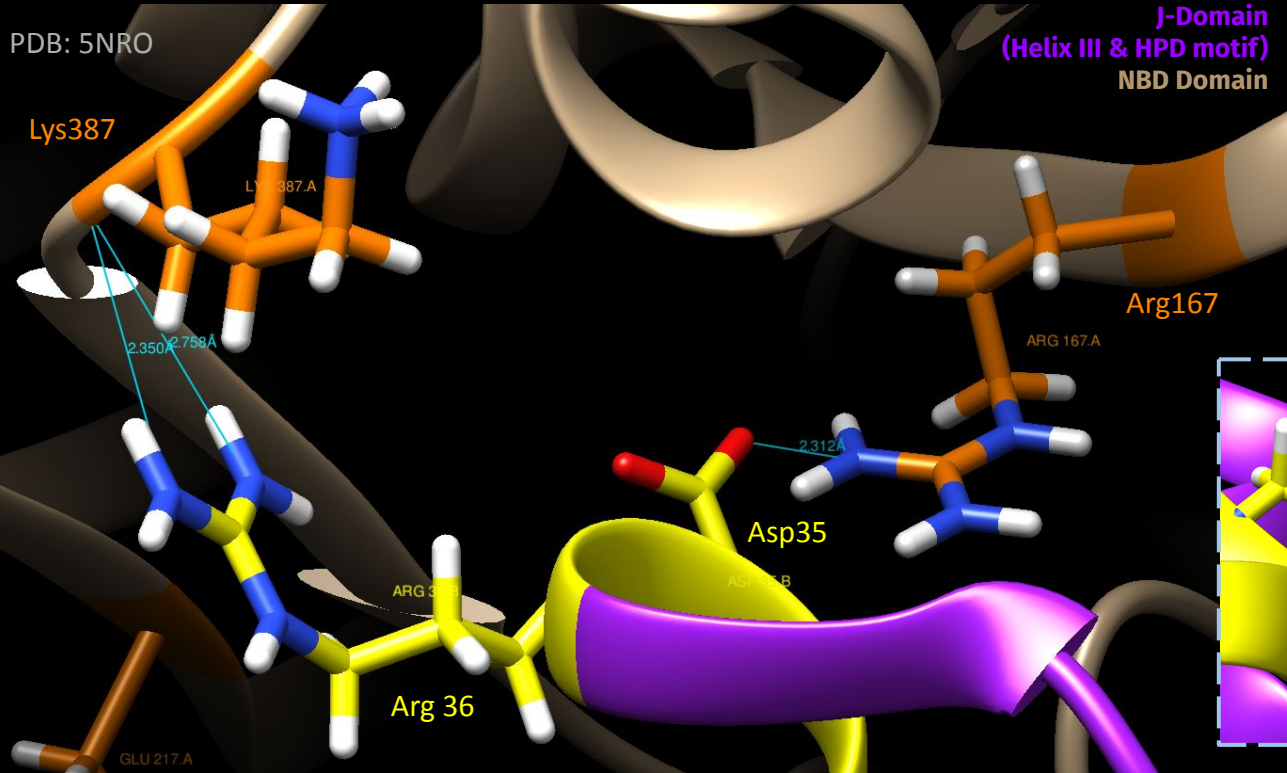
JDPs - Hydrogen bonds

Located around the HPD Motif

	167	387
Consensus	R	I
Conservation		
Homo	R	I
Xenopus	R	I
Drosophila	R	I
Caenorhabditis	R	I
Nicotiana	R	I
Saccharomyces	R	V
Escherichia	R	I

	-	q	d	t	g	d	i
	-	Q	D	T	G	D	L
	-	Q	D	T	G	D	L
	-	Q	D	T	D	A	I
	-	E	D	T	G	E	I
	G	D	E	T	K	D	I
	-	E	V	E	D	D	I
	-	-	-	V	K	D	V

Residues	Distance
Asp35 - Arg167	2.312
Arg36 - Lys 387	2.758
Arg36 - Lys 387	2.350

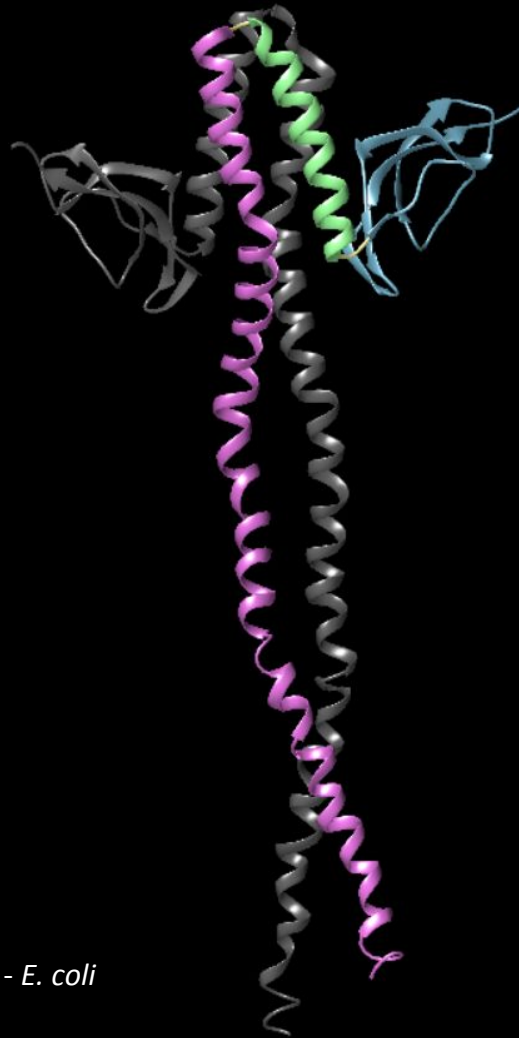




HSP70 CO-CHAPERONES: Nucleotide Exchange Factors (NEFs)

8GB3 - *M. tuberculosis* expressed in *E. coli*

GrpE structure



N- terminal alpha
helix

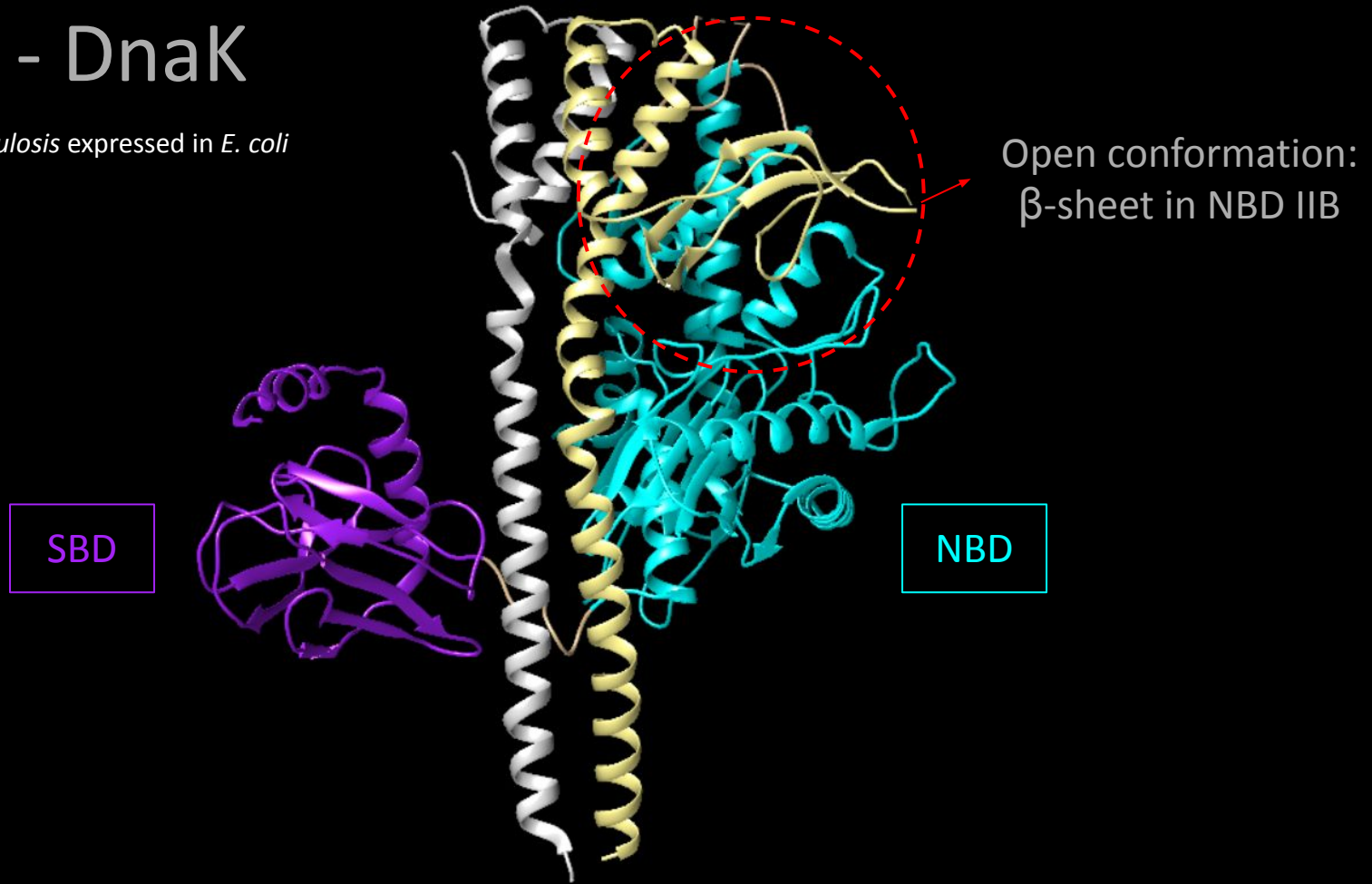
Central helix bundle

C- terminal beta
sheet

3A6M - *E. coli*

GrpE - DnaK

8GB3 - *M. tuberculosis* expressed in *E. coli*



Rotation of 14°



Sc = 8.76
RMS = 1.28
Nfit = 367

NBD IIB

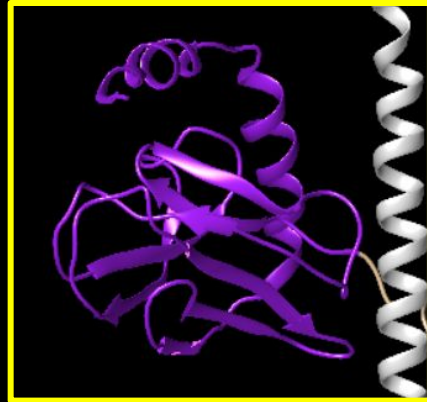


DnaK-ADP

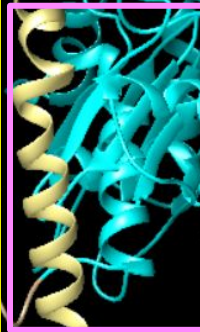
DnaK-GrpE

The 3 contact regions (CR)

CR 3



CR 2



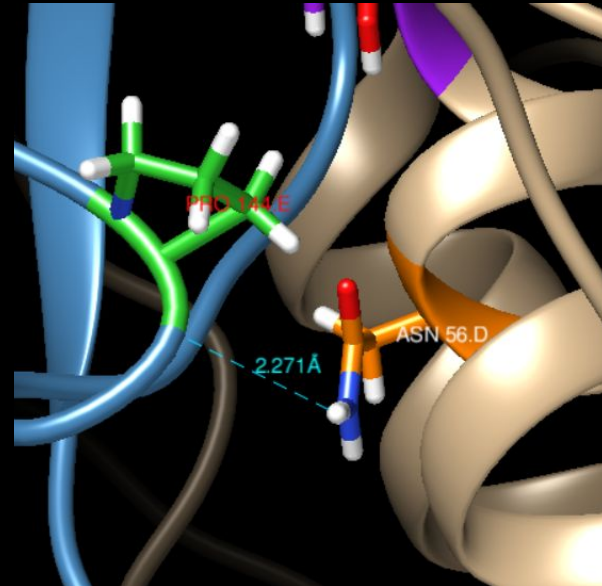
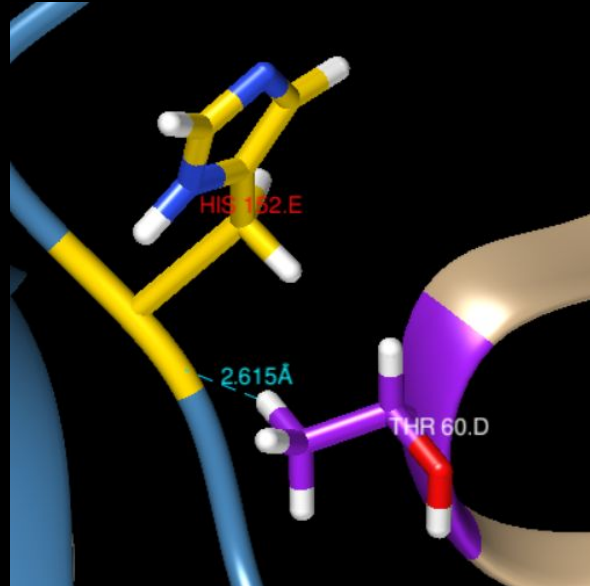
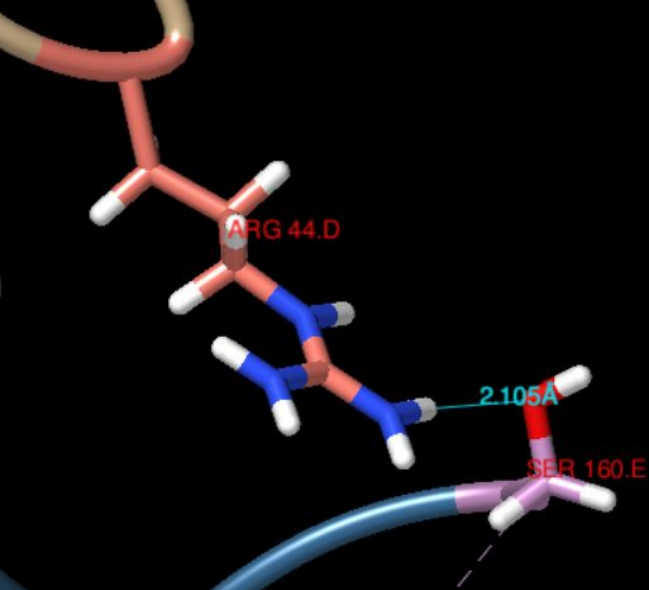
CR 1



Contact region 1 - NBD IB and IIB

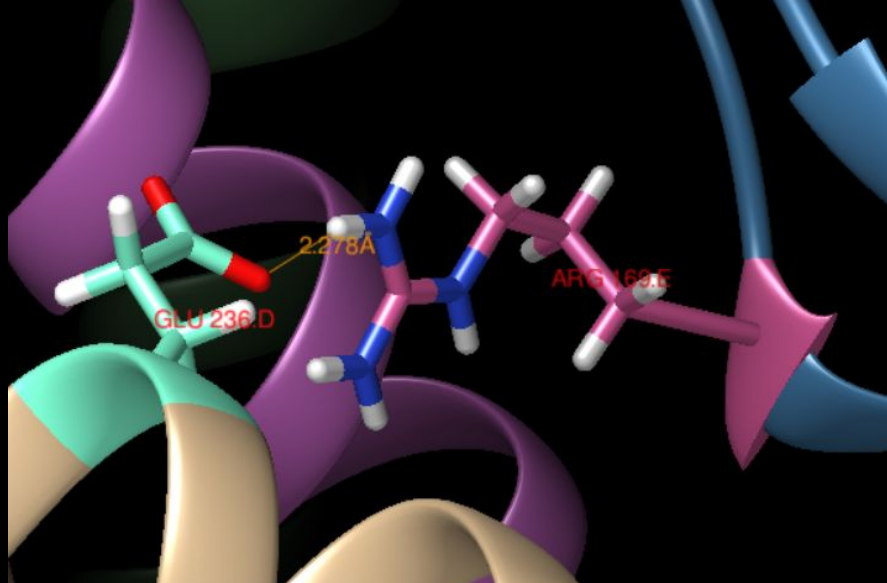


CR 1



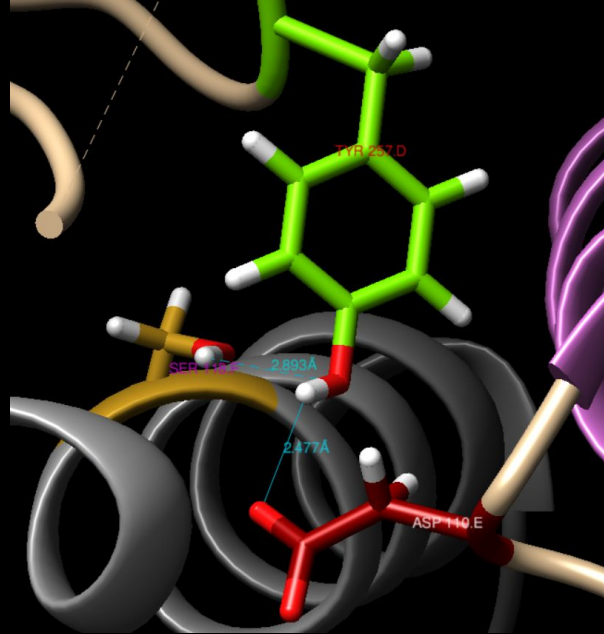
Hydrogen bonds

DnaK residue	GrpE residue	Distance
Arg44 (guanidinium)	Ser160 (OH)	2.105
Thr60 (H)	His152 (C=O)	2.615
Asn56 (H)	Pro144 (C=O)	2.271



Salt bridges

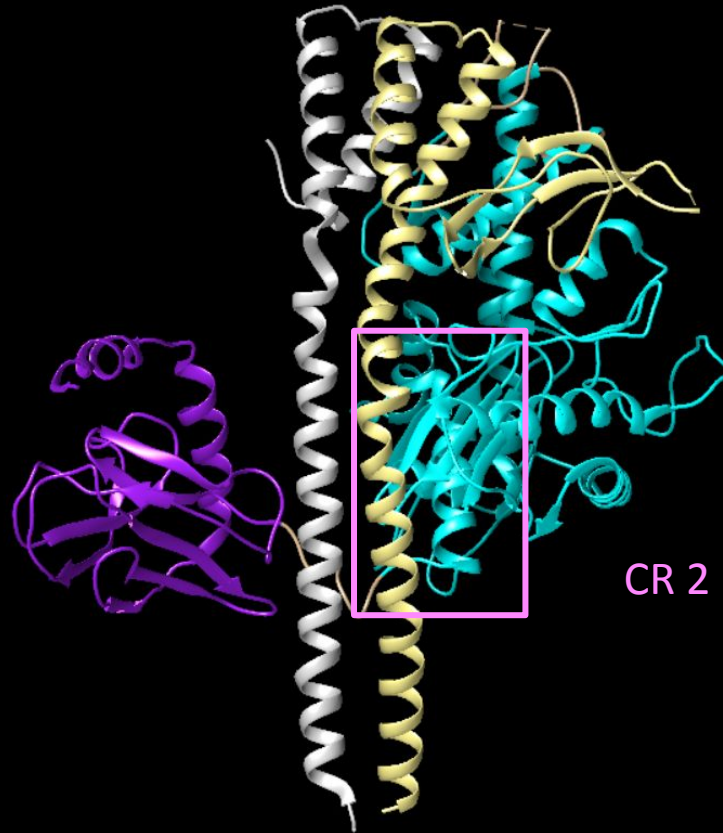
DnaK residue	GrpE residue	Distance
Glu236	Arg169	2.278

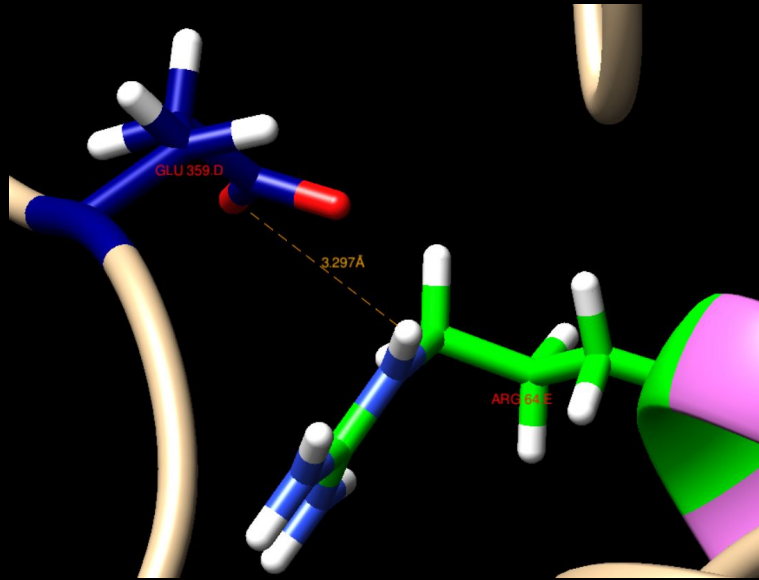


Hydrogen bonds

DnaK residue	GrpE residue	Distance
Tyr257 (OH)	Asp110 (COOH)	2.477
Tyr257 (OH)	Ser118 (OH)	2.893

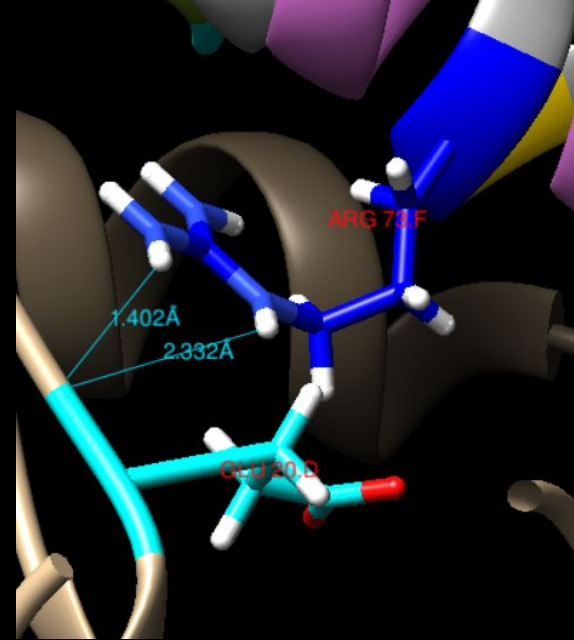
Contact region 2 - NBD IA and IIA





Salt bridges

DnaK residue	GrpE residue	Distance
Glu359 (-COO ⁻)	Arg64 (-NH ₂ ⁺)	3.297

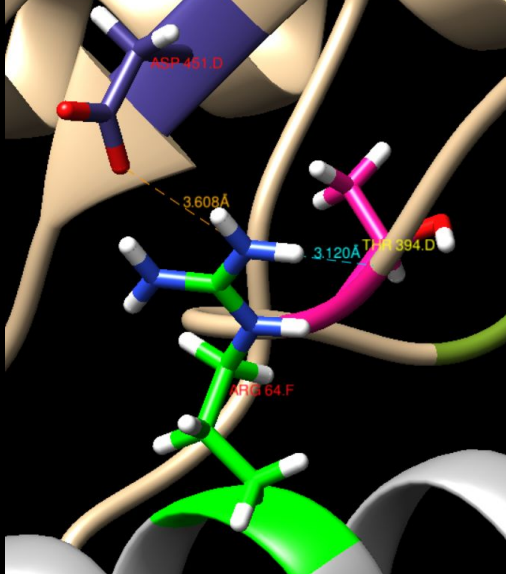


DnaK residue	GrpE residue	Distance
Glu20 (-COOH)	Arg73	2.332
Glu20 (-COOH)	Arg73	1.402

Hydrogen bonds

Contact region 3 - SBD



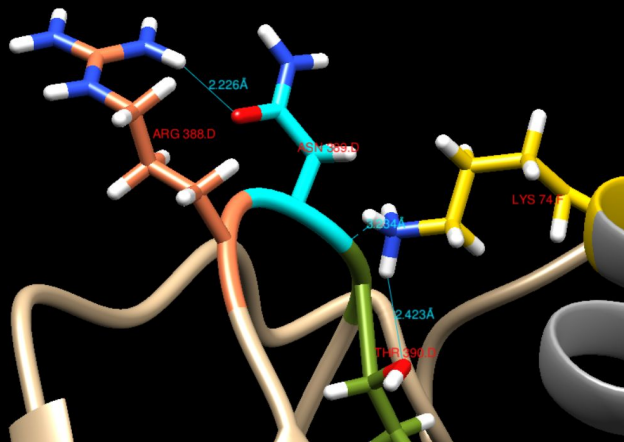


Salt bridges

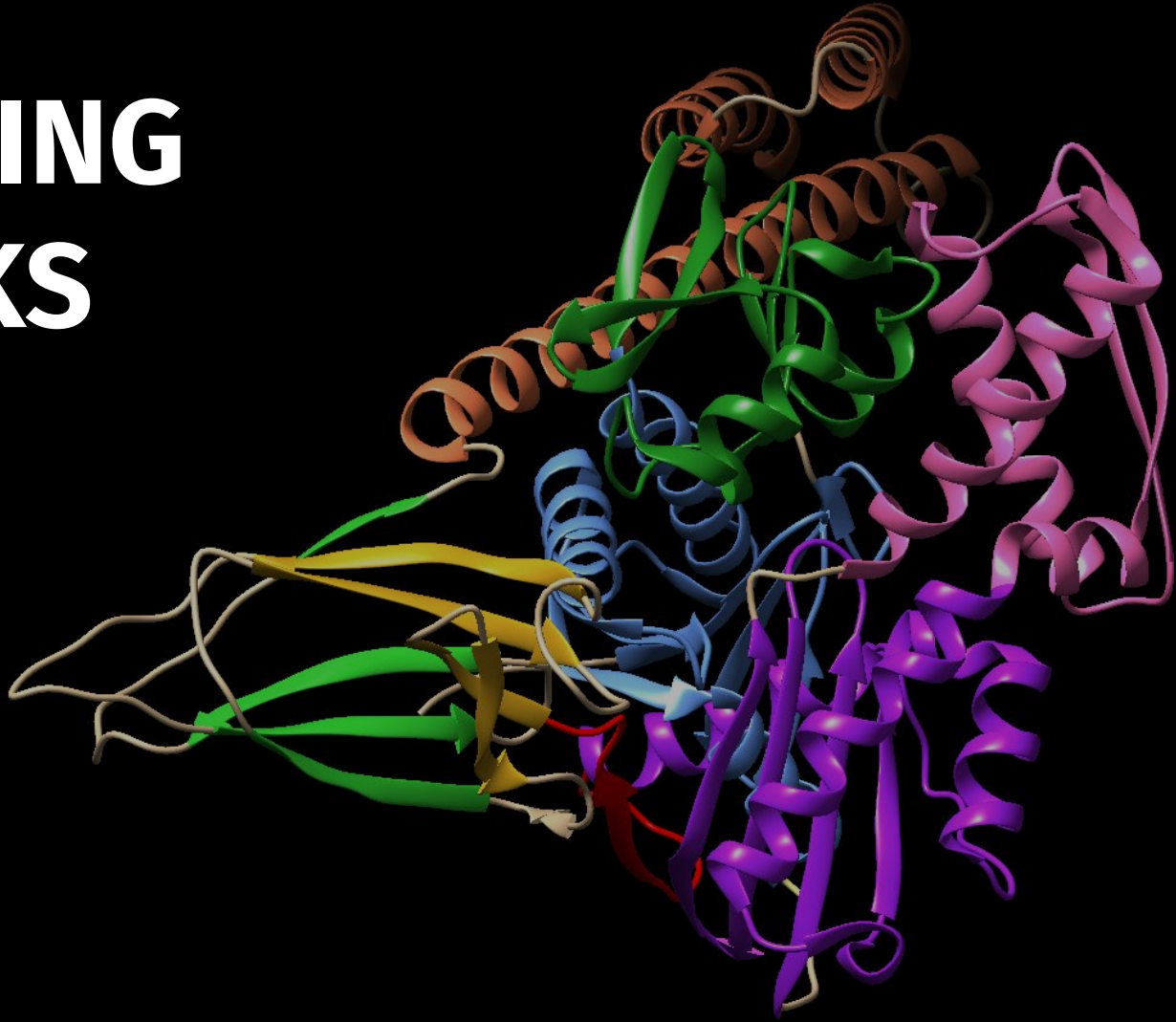
DnaK residue	GrpE residue	Distance
Asp451 (-COO-)	Arg64 (guanidinium)	3.608

Hydrogen bonds

DnaK residue	GrpE residue	Distance
Thr394	Arg64	3.120
Arg388 (guanidinium)	Asn389 (amide)	2.226
Asn389	Lys74 (amino)	3.234
Thr394 (hidroxyl)	Lys74 (amino)	2.423



CONCLUDING REMARKS



FROM

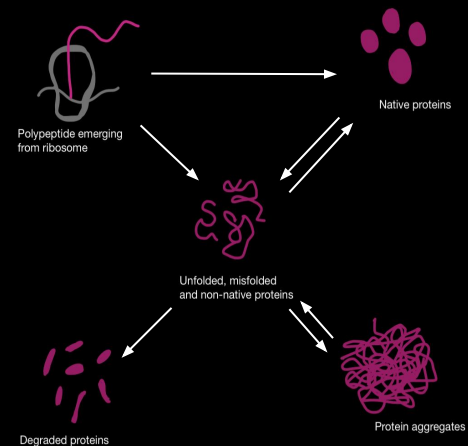
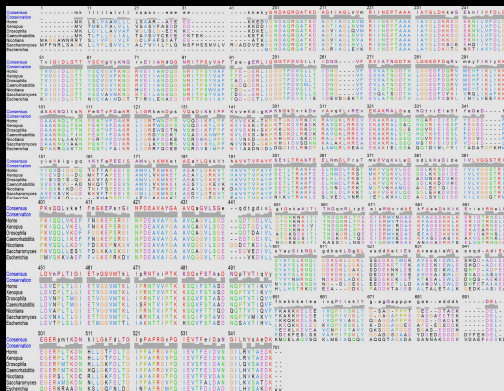
Sequence

to

Structure

to

Function



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PEM QUESTIONS

- 1) Which regions in J-domain proteins are relevant for binding to Hsp70?
 - a) Helix I, II and III
 - b) Helix IV and the HPD motif
 - c) Helix II and IV
 - d) Helix II, III and the HPD motif
 - e) HPD motif
- 2) How does the binding of J-domain proteins influence Hsp70s activity?
 - a) It accelerates ATP hydrolysis.
 - b) It stimulates substrate and ATP release.
 - c) It inhibits ATP binding.
 - d) It enhances ATP binding.
 - e) It accelerates the conformational change to the open ATP-bound state.
- 3) Which of the following is true about the interdomain linker of Hsp70?
 - a) It has the same length for all the species.
 - b) Always present a β -sheet conformation.
 - c) Has a hydrophilic sequence for binding to the NBD.
 - d) Adopts a β -sheet conformation only in the ATP-state.
 - e) Is about 50 residues long.
- 4) Which of the following is true about the domains of Hsp70?
 - a) The N-terminal domain is the Substrate Binding Domain.
 - b) The C-terminal domain is the Nucleotide Binding Domain.
 - c) The N-terminal domain is subdivided into 4 subdomains.
 - d) The C-terminal domain is subdivided into 3 subdomains.
 - e) They are directly connected, without a linker.
- 5) The protein Hsp70:
 - a) Has a well-conserved sequence in all species.
 - b) The regions of higher conservation correspond to the ones of ATP/ADP binding.
 - c) The two answers above are true.
 - d) The structure of the protein differs a lot between species.
 - e) All answers are correct.
- 6) Which of the following species had the greatest percentage of identity with the human Hsp70?
 - a) Escherichia coli
 - b) Xenopus laevis
 - c) Saccharomyces cerevisiae
 - d) Nicotiana tabacum
 - e) Drosophila melanogaster
- 7) Which of the following affirmations about Hsp70 is true:
 - a) The ADP-state has a higher affinity for substrate binding.
 - b) Substrate binding stimulates ATP hydrolysis.
 - c) Both are correct.
 - d) In the ATP-state, SBD and NBD do not interact with each other.
 - e) All are correct.
- 8) In the Hsp70 ATP-state, with NBD subdomains does not interact with the SBD ?
 - a) IA-NBD
 - b) IB-NBD
 - c) IIA-NBD
 - d) IIB-NBD
 - e) None of the above, all the NBD subdomains interact with the SBD
- 9) The principal function of NEFs in the Hsp70 cycle is:
 - a) To stabilize Hsp70.
 - b) To accelerate ATP hydrolysis.
 - c) To accelerate the exchange from ADP to ATP.
 - d) To inhibit ATP binding.
 - e) To inhibit ADP binding.
- 10) When GrpE binds to Hsp70, the conformational change is:
 - a) A rigid body motion of 14° of NBD subdomain.
 - b) There is no conformational change.
 - c) A rigid body motion of 90° of NBD subdomain.
 - d) The protein rotates 360° .
 - e) None of the above are correct.

An insight into Hsp70

Structural Biology Project 23/24

Berta Armangué, Tess Ley, Gemma Piqué and Paula Sánchez

