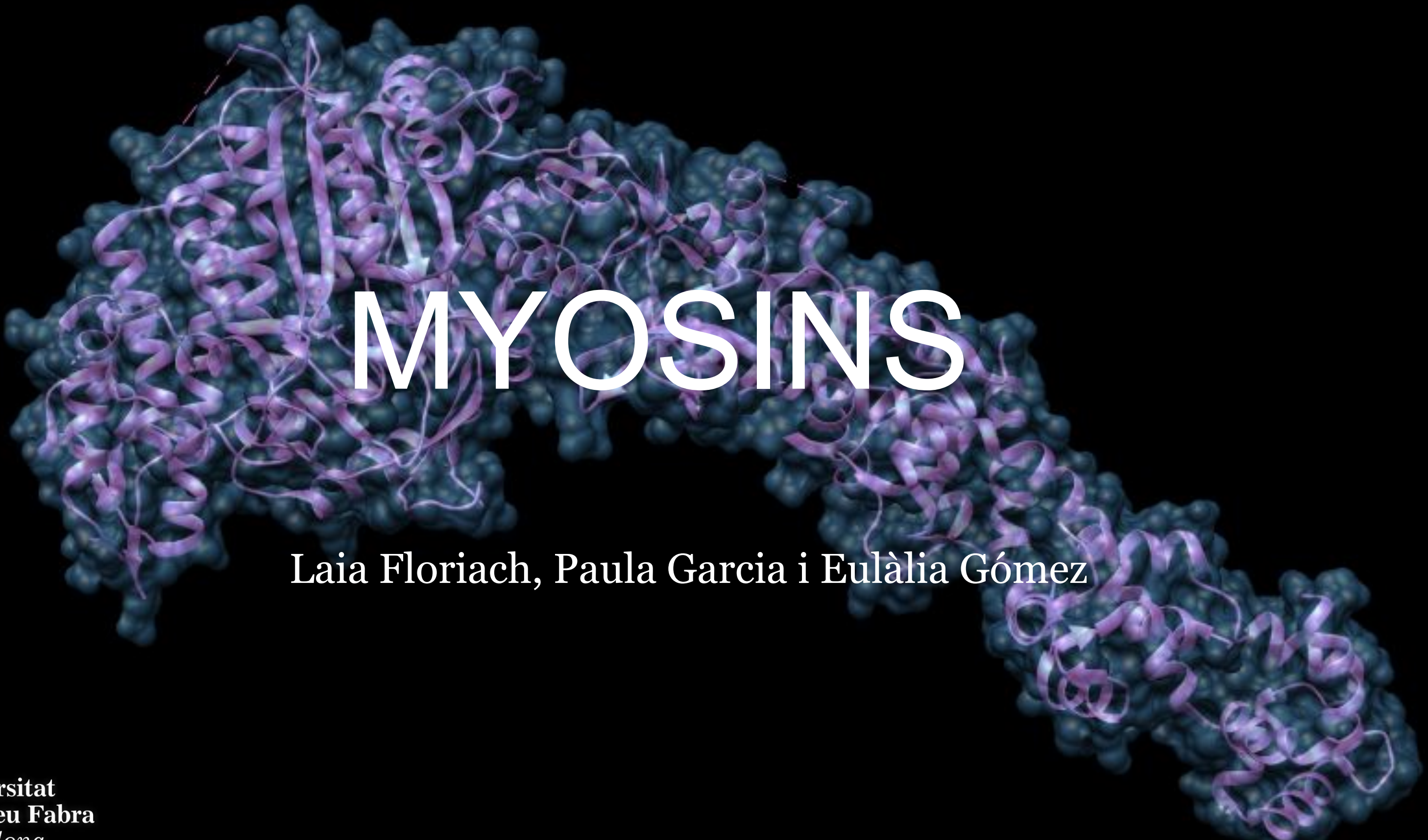


MYOSINS

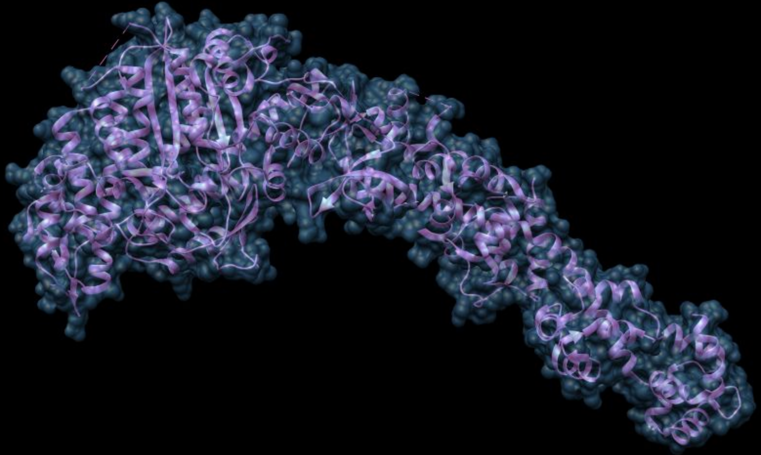
Laia Floriach, Paula Garcia i Eulàlia Gómez

Universitat
Pompeu Fabra
Barcelona

Structural Biology - Course 2023/2024

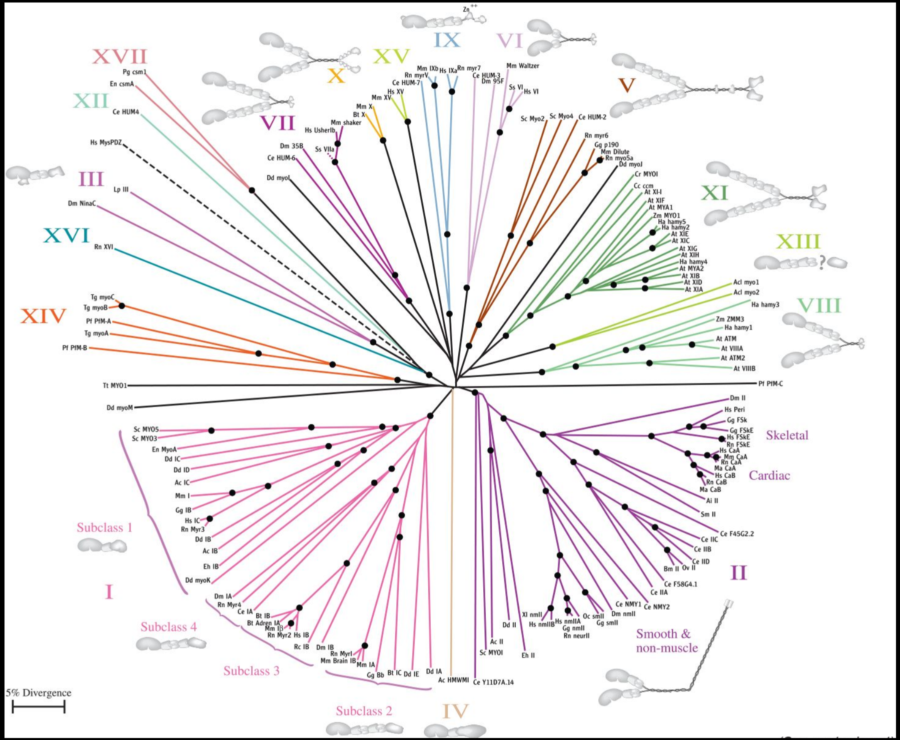
TABLE OF CONTENT

INTRODUCTION



GENERAL INFORMATION

- Superfamily of motor proteins
- 40 different myosin genes that encode different myosin isoforms
- All eukaryotic cells contain myosin isoforms



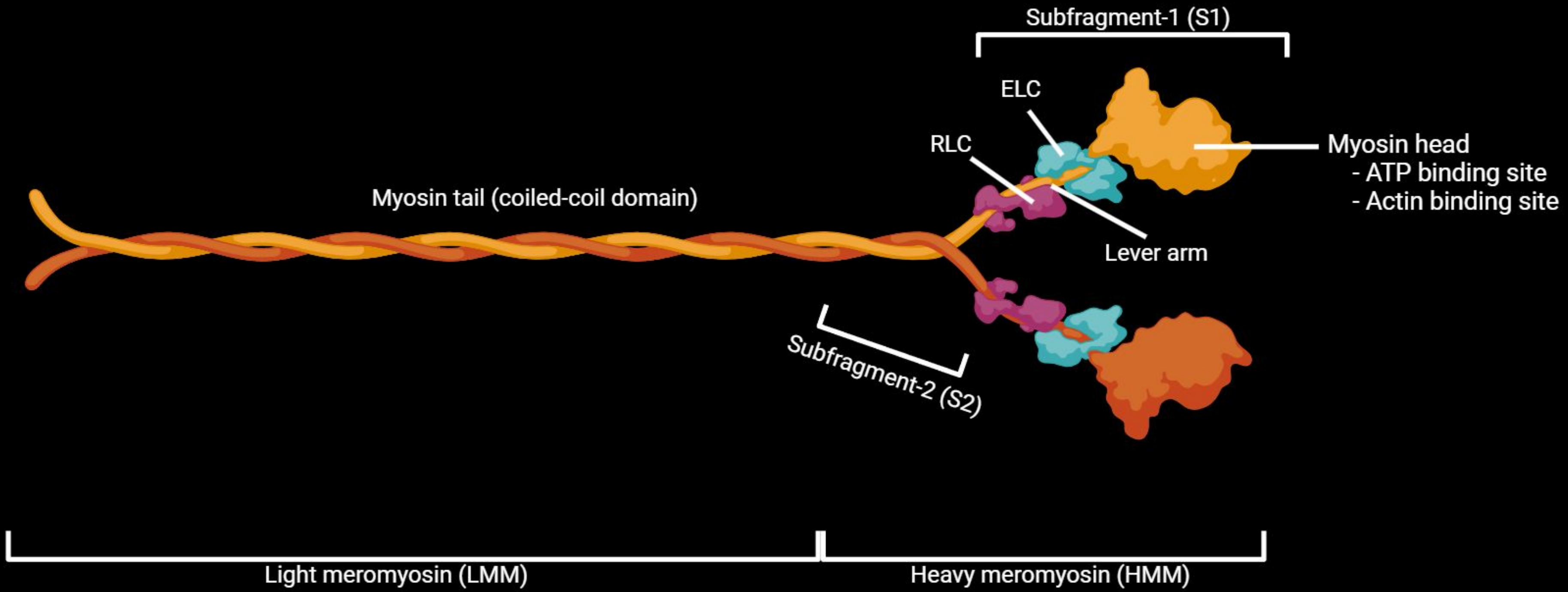
Phylogenetic tree of the myosin superfamily

Hodge, T., and Cope, M. J. (2000) A myosin family tree, J Cell Sci 113 Pt 19, 3353- 3354.

GENERAL INFORMATION

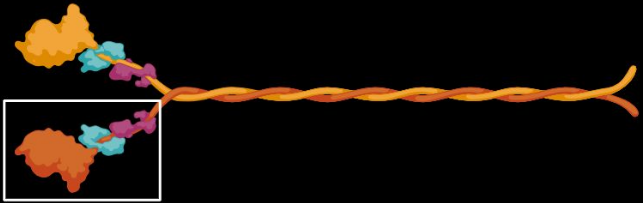
- Conventionals myosins consists in:
  - 2 myosin heavy chains (MHCs)
  - 2 regulatory light chains (RLCs)
  - 2 essential light chains (ELCs)
- According to cleavage with different proteases, the myosin molecule is broken down into:
  - Heavy meromyosin (HMM) → in turn is fragmented into:
    - Domain S1: motor domain
    - Domain S2: helps to dimerize the two MHCs and allows myosin flexibility.
  - Light meromyosin (LMM) → large coiled-coil tail

GENERAL INFORMATION



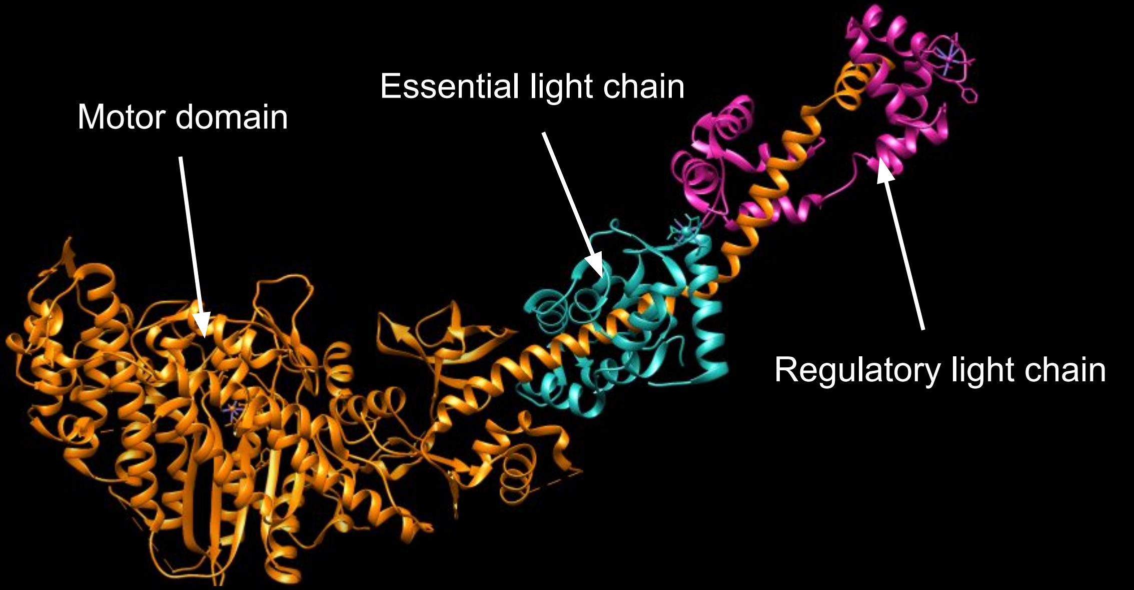
Made with BioRender

SCOP CLASSIFICATION



Motor Domain

- CLASS: Alpha and beta proteins (a/b)
- FOLD: P-loop containing nucleoside triphosphate hydrolases
- SUPERFAMILY: P-loop containing nucleoside triphosphate hydrolases
- FAMILY: motor proteins

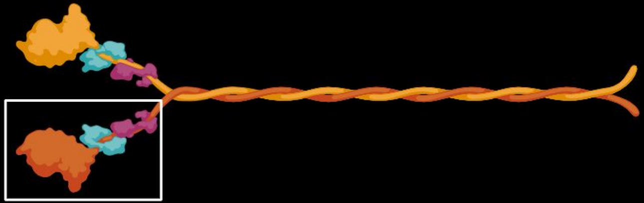


P-loop: G x x x x GK[T/S]

↳ GESGA**GKT**

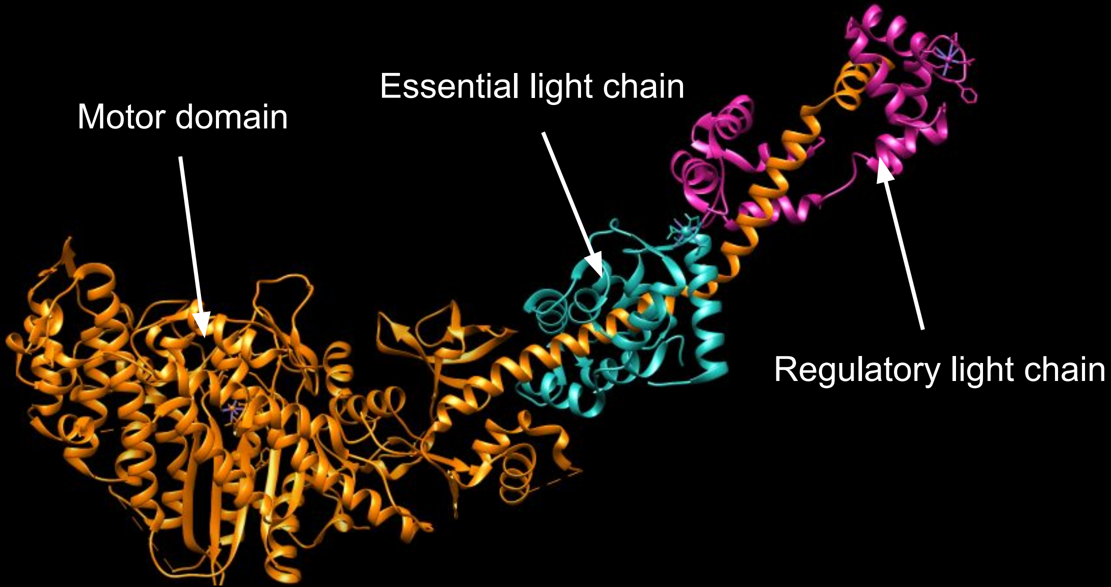
ID: 1KK8
2.30 Å

SCOP CLASSIFICATION



Essential light chain

- CLASS: All alpha proteins
- FOLD: EF Hand-like
- SUPERFAMILY: EF-hand
- FAMILY: Calmodulin-like



ID: 1KK8
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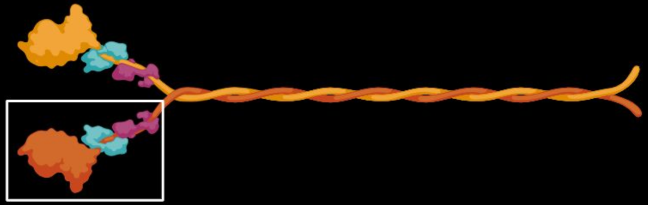
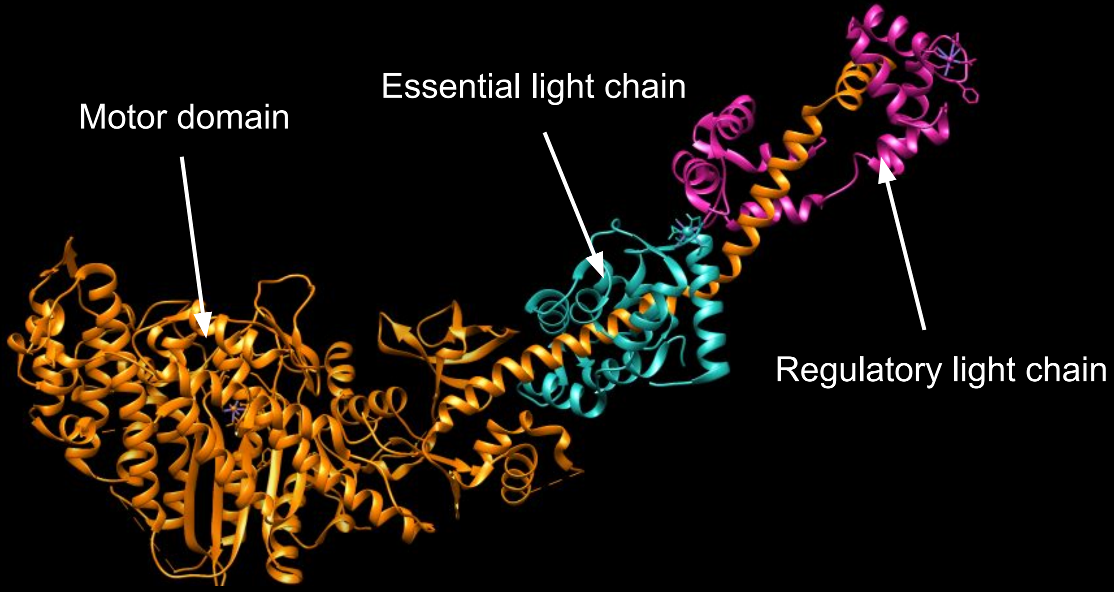


Regulatory light chain
- CLASS: All alpha proteins
- FOLD: EF Hand-like
- SUPERFAMILY: EF-hand
- FAMILY: Calmodulin-like



ID: 1KK8
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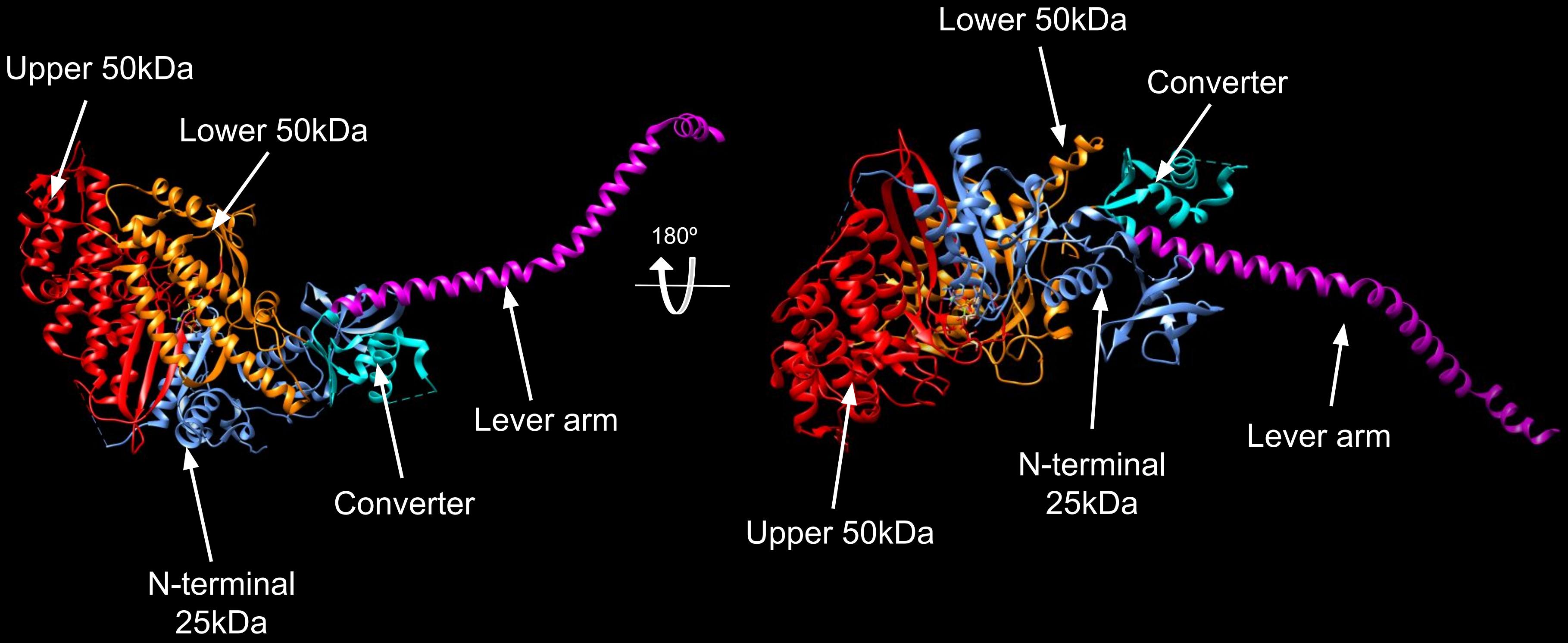
MOTOR DOMAIN SUBDOMAINS



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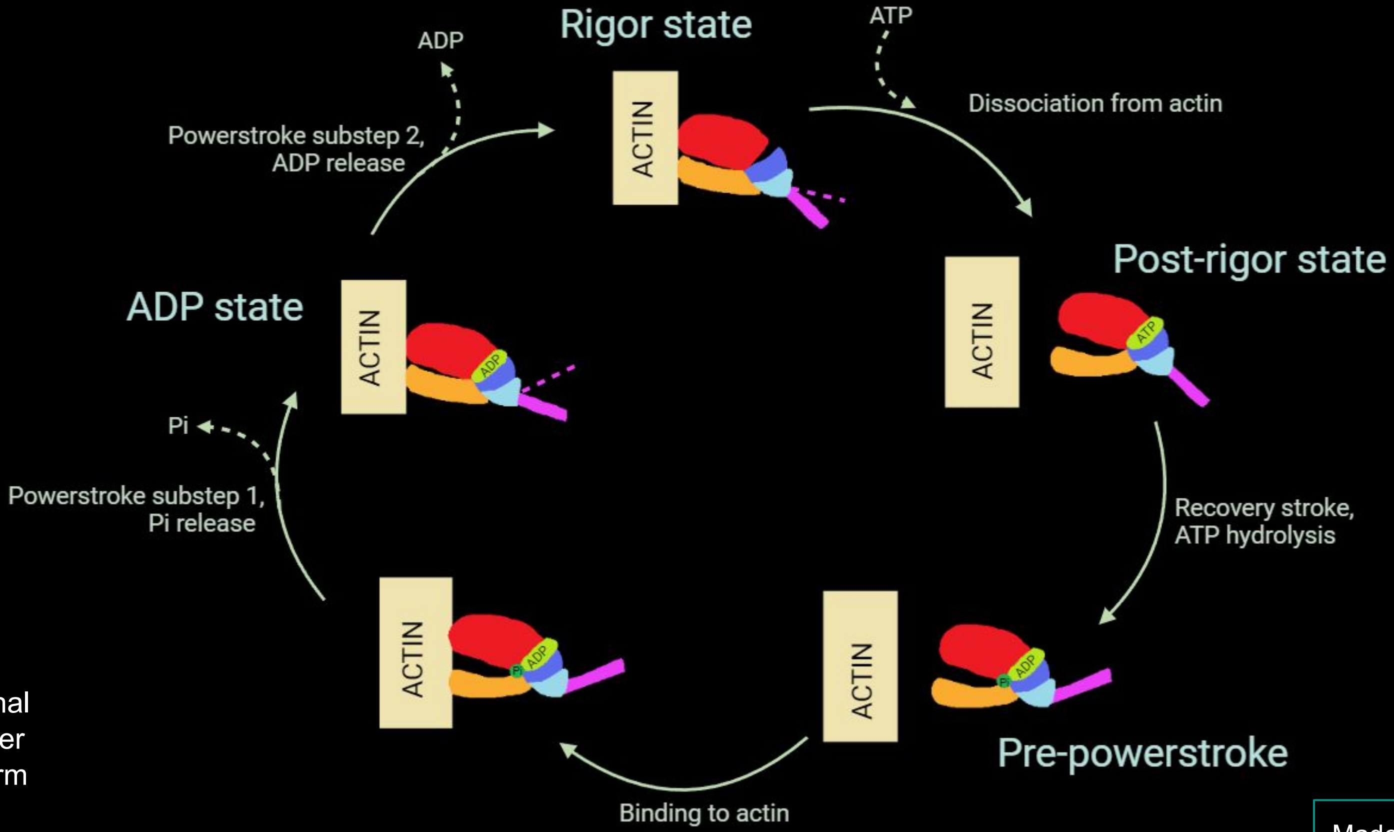
MYOSIN ATPASE CYCLE



Rigor state

ATP

Dissociation from actin

Post-rigor state

Recovery stroke,
ATP hydrolysis

Pre-powerstroke

Binding to actin

Powerstroke substep 1,
Pi release

Pi

ADP state

Powerstroke substep 2,
ADP release

ADP

- 🟥 U50
- 🟧 L50
- 🟦 N-terminal
- ● Converter
- 🟪 Lever arm

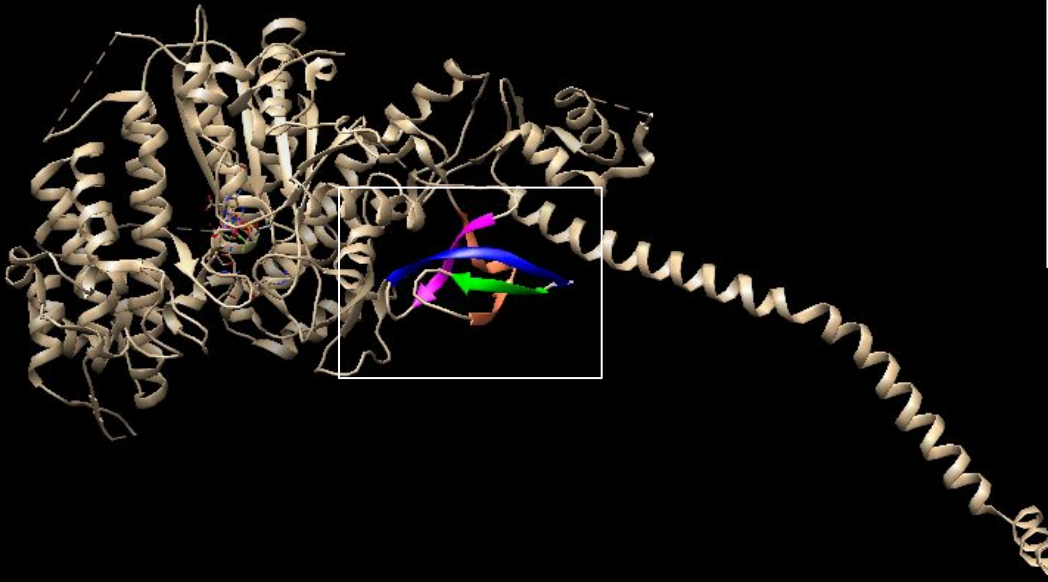
Made with BioRender

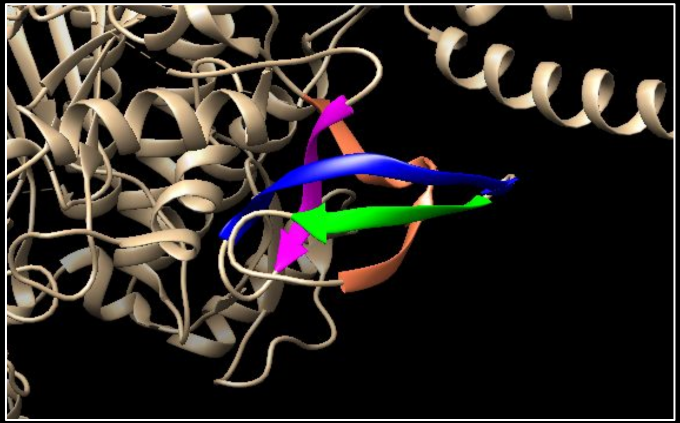
MOTOR DOMAIN MOTIFS

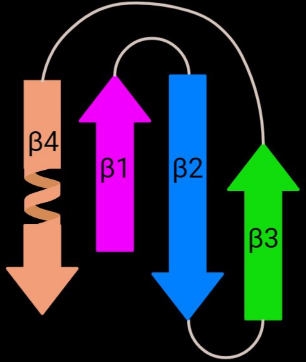
- 🟩 7 stranded β-sheet
- 🟦 SH3-like barrel

ID: 1KK8
2.30 Å

MOTOR DOMAIN MOTIFS



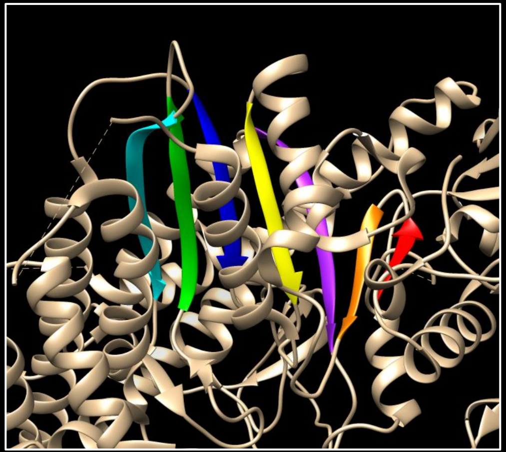


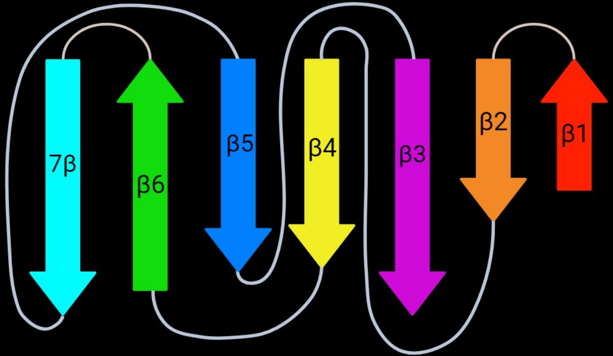


SH3-like barrel

Barrel, partly opened, n*=4, meander, the last strand is interrupted by a turn of 3-10 helix

MOTOR DOMAIN MOTIFS

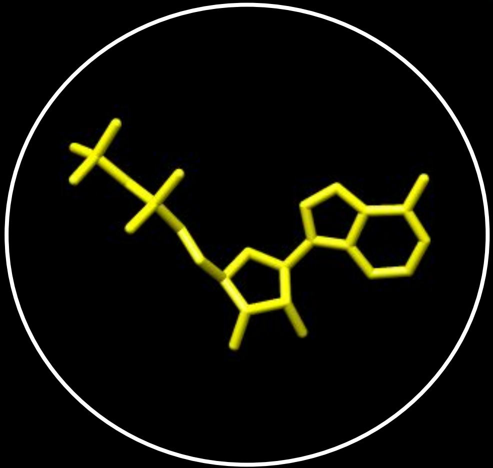




7 stranded β-sheet

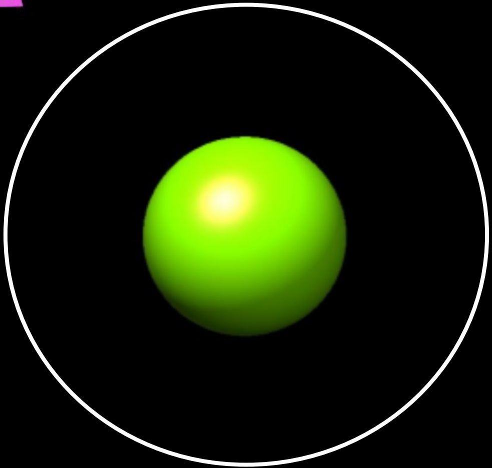
MYOSIN INTERACTIONS

1



ADP

2

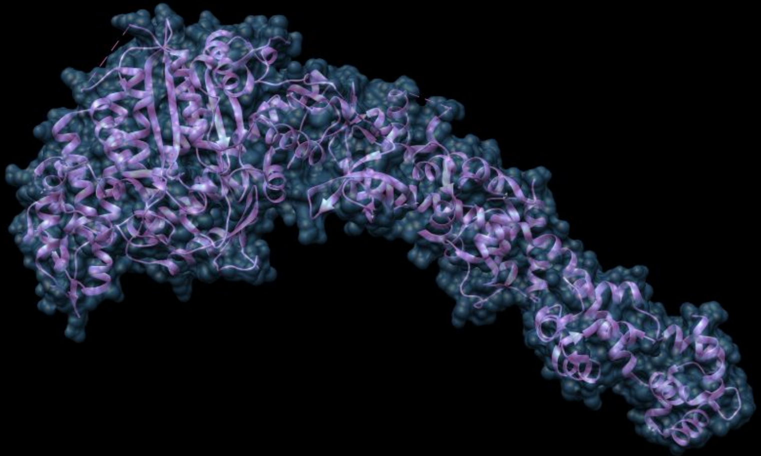


Mg2+

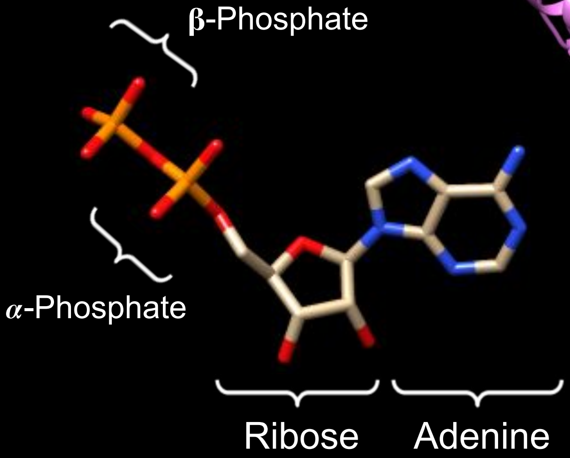
3

Actin

MYOSIN-NUCLEOTIDE INTERACTIONS



ADP STATE



Motor domain

Light chain

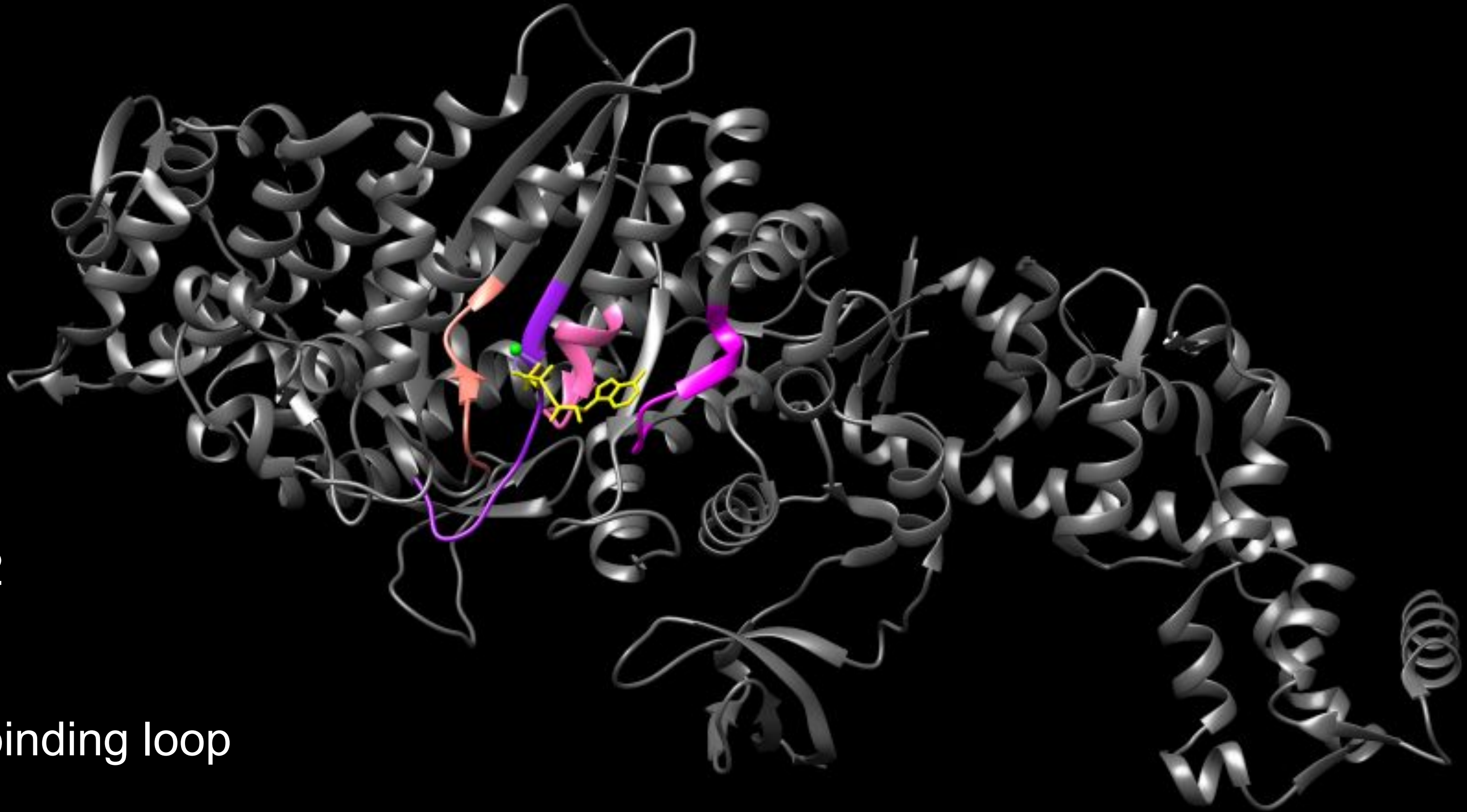
ADP

MG+

ID: 8EFE

Resolution: 3.80Å

NUCLEOTIDE BINDING POCKET



- ● Switch 1
- ● Switch 2
- ● P-loop
- ● Purine-binding loop

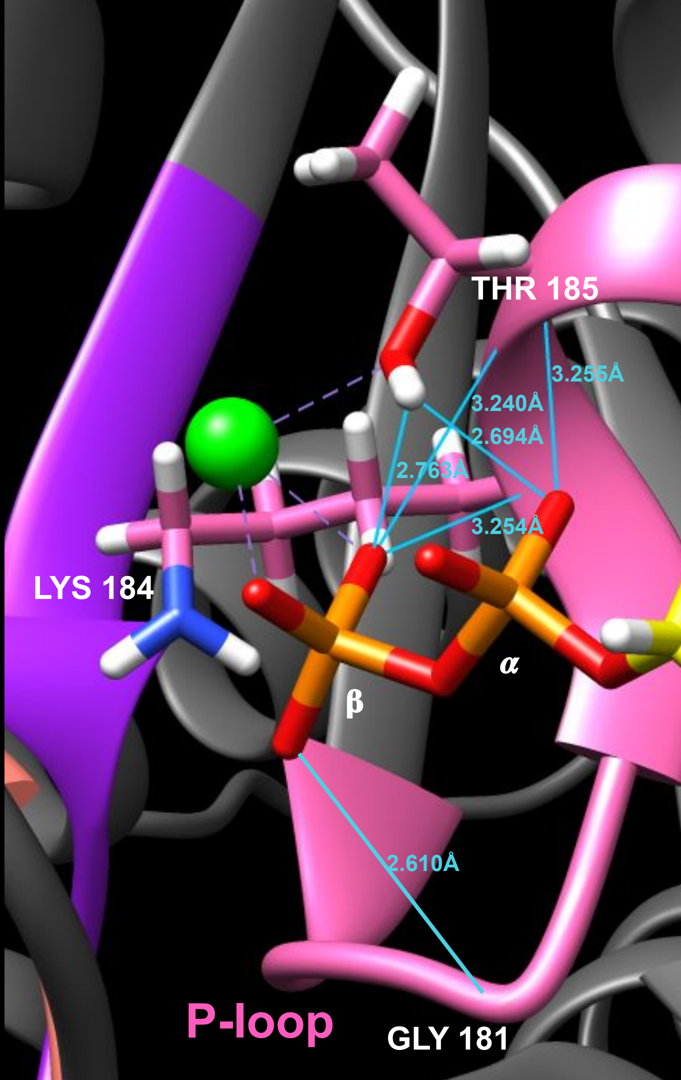
ALPHA AND BETA PHOSPHATES

Protein residue	**Atom**	**β-Phosphate**
GLY 181	**N**	**O2B**
LYS 184	**N**	**O1B**
THR 185	**OG1**	**O1B**
THR 185	**N**	**O1B**

Protein residue	**Atom**	***α*-Phosphate**
THR 185	**OG1**	**O1A**
THR 185	**N**	**O1A**

— Hydrogen bonds

P-loop: GxxxxGK[T/S]
↳ GESGA**GKT**

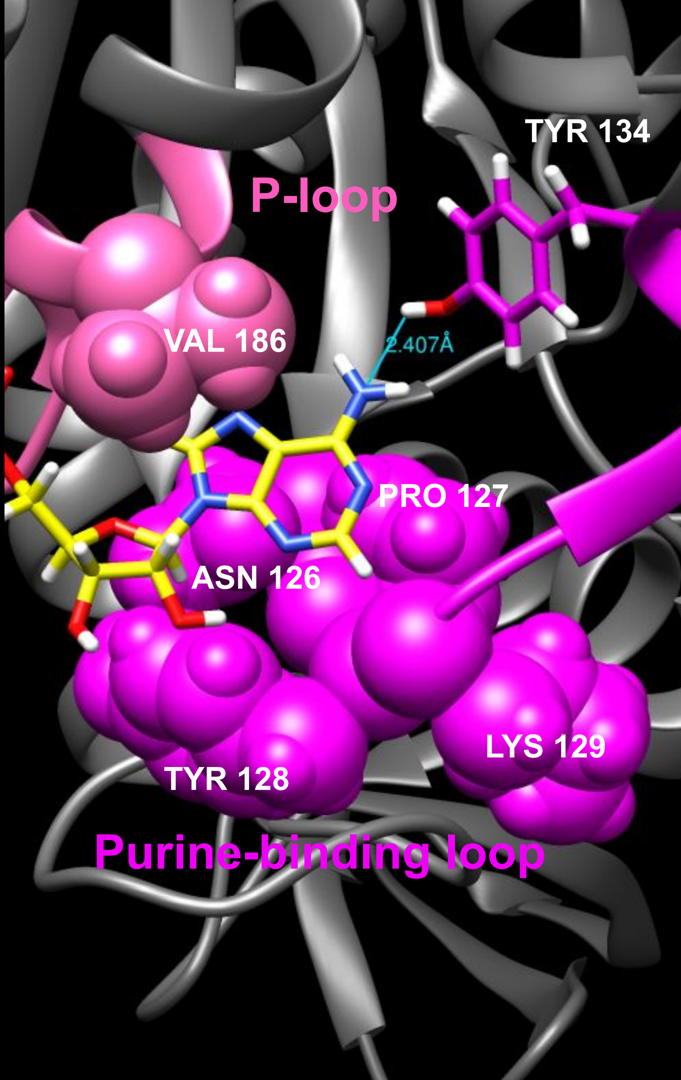


ADP RIBOSE AND ADENINE

Residue	Atom	ADP
TYR 134	OH	N6

— Hydrogen bonds

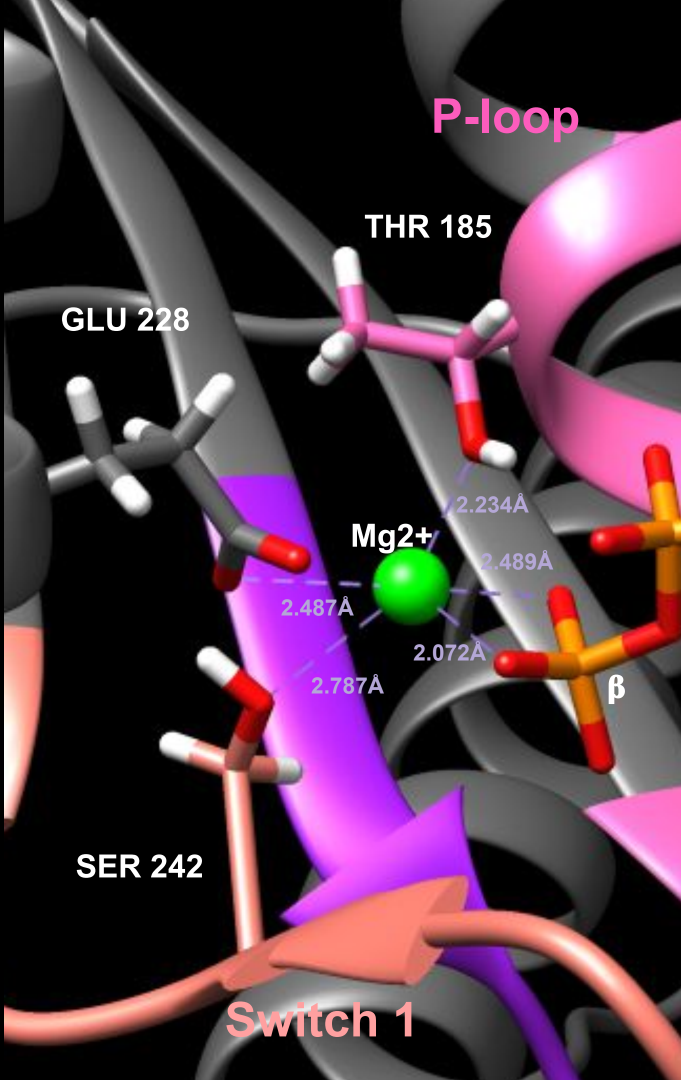
● Hydrophobic pocket



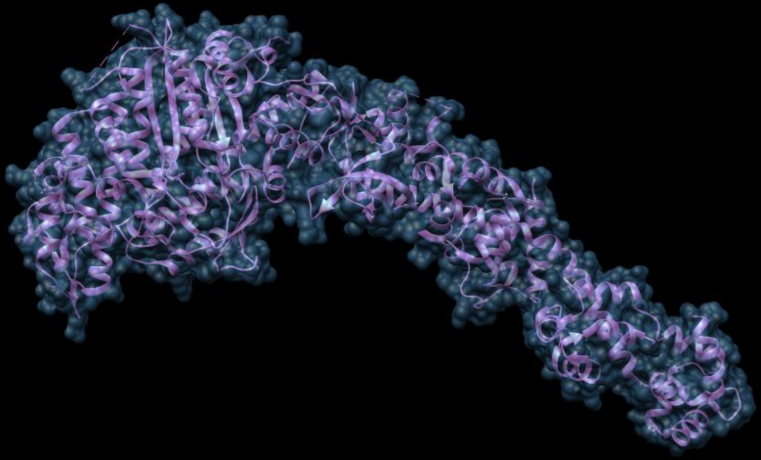
MAGNESIUM ION

Residue	**Atom**	**Metal**
THR 185	**OG1**	**Mg2+**
GLU 228	**OE2**	**Mg2+**
SER 242	**OG**	**Mg2+**
β-Phosphate	**O1B**	**Mg2+**
β-Phosphate	**O3B**	**Mg2+**

— — — Metal coordinations

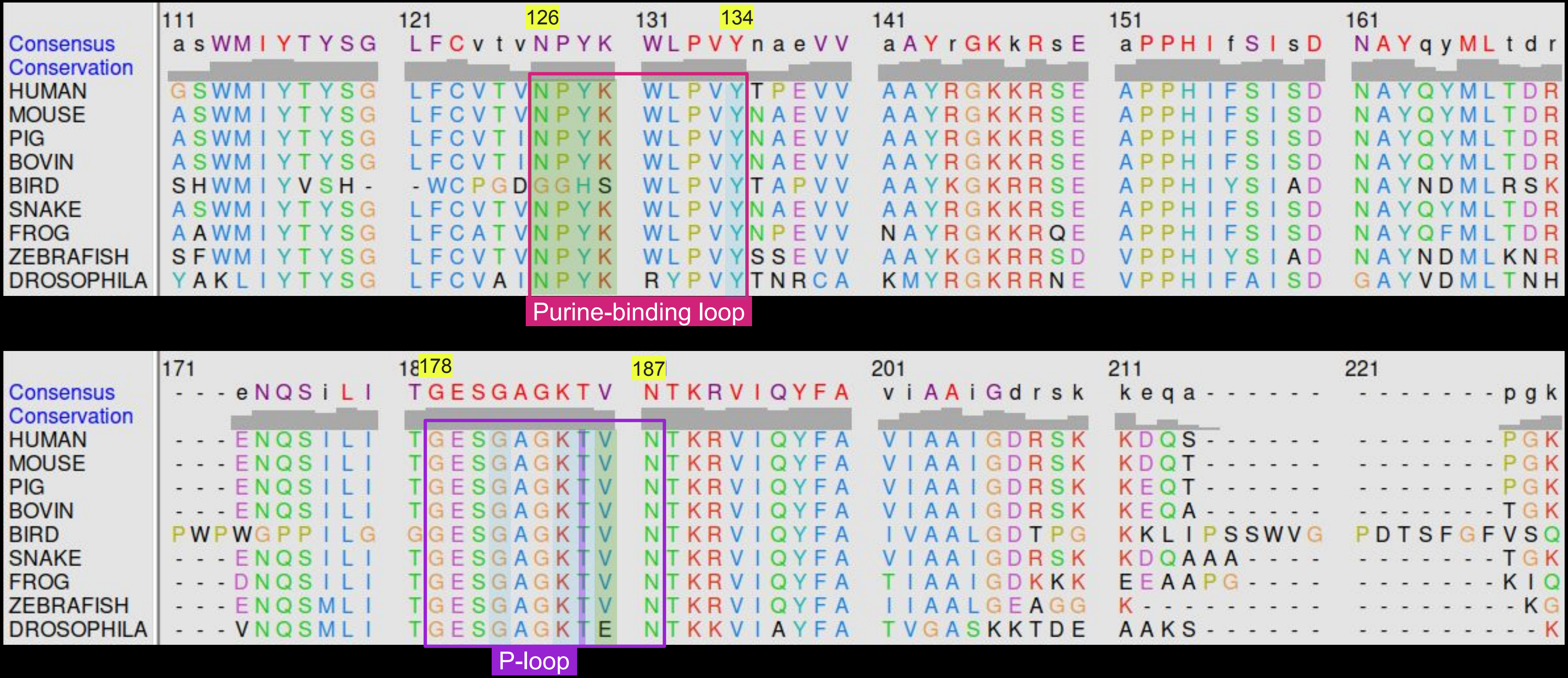


CONSERVATION BETWEEN SPECIES



SEQUENCE ALIGNMENT

Hydrophobic interactions
Hydrogen bounds
Metal coordination

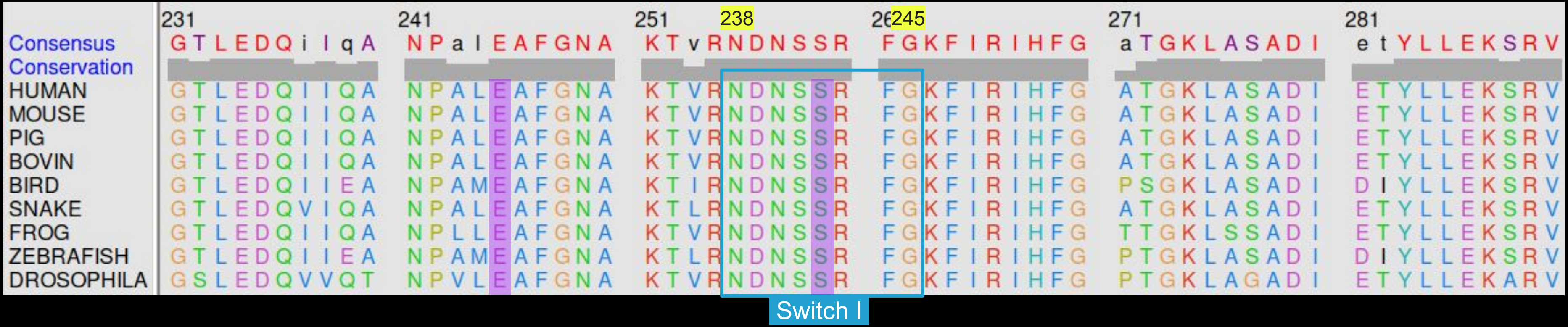


	111	121	131	141	151	161
Consensus	a s W M I Y T Y S G	L F C v t v N P Y K	W L P V Y n a e V V	a A Y r G K k R s E	a P P H I f S I s D	N A Y q y M L t d r
Conservation						
HUMAN	G S W M I Y T Y S G	L F C V T V N P Y K	W L P V Y T P E V V	A A Y R G K K R S E	A P P H I F S I S D	N A Y Q Y M L T D R
MOUSE	A S W M I Y T Y S G	L F C V T V N P Y K	W L P V Y N A E V V	A A Y R G K K R S E	A P P H I F S I S D	N A Y Q Y M L T D R
PIG	A S W M I Y T Y S G	L F C V T I N P Y K	W L P V Y N A E V V	A A Y R G K K R S E	A P P H I F S I S D	N A Y Q Y M L T D R
BOVIN	A S W M I Y T Y S G	L F C V T I N P Y K	W L P V Y N A E V V	A A Y R G K K R S E	A P P H I F S I S D	N A Y Q Y M L T D R
BIRD	S H W M I Y V S H -	- W C P G D G G H S	W L P V Y T A P V V	A A Y K G K R R S E	A P P H I Y S I A D	N A Y N D M L R S K
SNAKE	A S W M I Y T Y S G	L F C V T V N P Y K	W L P V Y N A E V V	A A Y R G K K R S E	A P P H I F S I S D	N A Y Q Y M L T D R
FROG	A A W M I Y T Y S G	L F C A T V N P Y K	W L P V Y N P E V V	N A Y R G K K R Q E	A P P H I F S I S D	N A Y Q F M L T D R
ZEBRAFISH	S F W M I Y T Y S G	L F C V T V N P Y K	W L P V Y S S E V V	A A Y K G K R R S D	V P P H I Y S I A D	N A Y N D M L K N R
DROSOPHILA	Y A K L I Y T Y S G	L F C V A I N P Y K	R Y P V Y T N R C A	K M Y R G K R R N E	V P P H I F A I S D	G A Y V D M L T N H

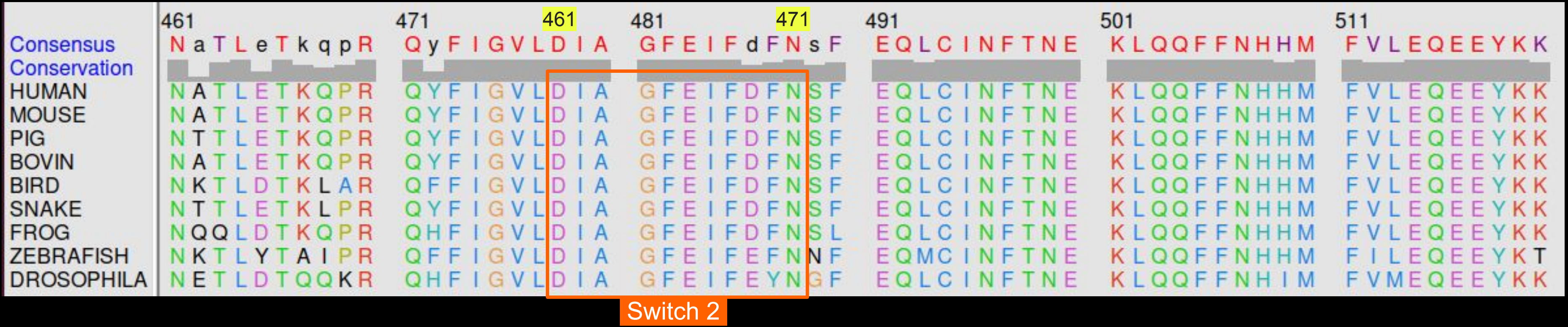
	171	18		201	211	221
Consensus	- - - e N Q S i L I	T G E S G A G K T V	N T K R V I Q Y F A	v i A A i G d r s k	k e q a - - - - - -	- - - - - - - p g k
Conservation						
HUMAN	- - - E N Q S I L I	T G E S G A G K T V	N T K R V I Q Y F A	V I A A I G D R S K	K D Q S - - - - - -	- - - - - - - P G K
MOUSE	- - - E N Q S I L I	T G E S G A G K T V	N T K R V I Q Y F A	V I A A I G D R S K	K D Q T - - - - - -	- - - - - - - P G K
PIG	- - - E N Q S I L I	T G E S G A G K T V	N T K R V I Q Y F A	V I A A I G D R S K	K E Q T - - - - - -	- - - - - - - P G K
BOVIN	- - - E N Q S I L I	T G E S G A G K T V	N T K R V I Q Y F A	V I A A I G D R S K	K E Q A - - - - - -	- - - - - - - T G K
BIRD	P W P W G P P I L G	G G E S G A G K T V	N T K R V I Q Y F A	I V A A L G D T P G	K K L I P S S W V G	P D T S F G F V S Q
SNAKE	- - - E N Q S I L I	T G E S G A G K T V	N T K R V I Q Y F A	V I A A I G D R S K	K D Q A A A - - - -	- - - - - - - T G K
FROG	- - - D N Q S I L I	T G E S G A G K T V	N T K R V I Q Y F A	T I A A I G D K K K	E E A A P G - - - -	- - - - - - - K I Q
ZEBRAFISH	- - - E N Q S M L I	T G E S G A G K T V	N T K R V I Q Y F A	I I A A L G E A G G	K - - - - - - - - -	- - - - - - - - K G
DROSOPHILA	- - - V N Q S M L I	T G E S G A G K T E	N T K K V I A Y F A	T V G A S K K T D E	A A K S - - - - - -	- - - - - - - - - K

SEQUENCE ALIGNMENT

Hydrophobic interactions
Hydrogen bounds
Metal coordination

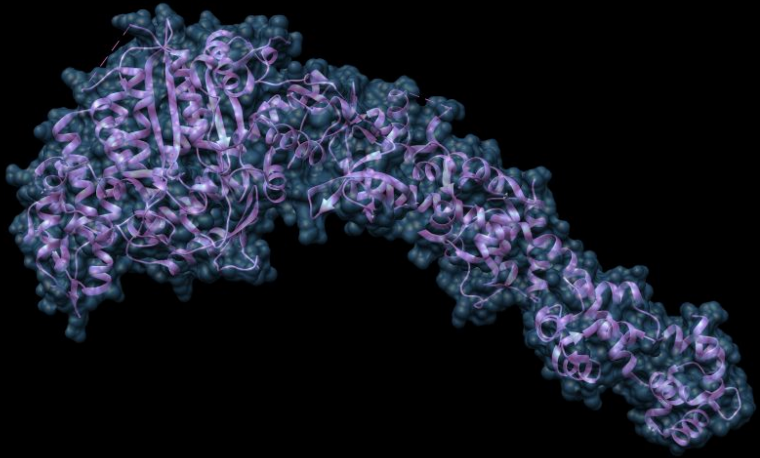


	231	241	251 238	26245	271	281
Consensus	G T L E D Q i I q A	N P a l E A F G N A	K T v R N D N S S R	F G K F I R I H F G	a T G K L A S A D I	e t Y L L E K S R V
Conservation						
HUMAN	G T L E D Q I I Q A	N P A L E A F G N A	K T V R N D N S S R	F G K F I R I H F G	A T G K L A S A D I	E T Y L L E K S R V
MOUSE	G T L E D Q I I Q A	N P A L E A F G N A	K T V R N D N S S R	F G K F I R I H F G	A T G K L A S A D I	E T Y L L E K S R V
PIG	G T L E D Q I I Q A	N P A L E A F G N A	K T V R N D N S S R	F G K F I R I H F G	A T G K L A S A D I	E T Y L L E K S R V
BOVIN	G T L E D Q I I Q A	N P A L E A F G N A	K T V R N D N S S R	F G K F I R I H F G	A T G K L A S A D I	E T Y L L E K S R V
BIRD	G T L E D Q I I E A	N P A M E A F G N A	K T I R N D N S S R	F G K F I R I H F G	P S G K L A S A D I	D I Y L L E K S R V
SNAKE	G T L E D Q V I Q A	N P A L E A F G N A	K T L R N D N S S R	F G K F I R I H F G	A T G K L A S A D I	E T Y L L E K S R V
FROG	G T L E D Q I I Q A	N P L L E A F G N A	K T V R N D N S S R	F G K F I R I H F G	T T G K L S S A D I	E T Y L L E K S R V
ZEBRAFISH	G T L E D Q I I E A	N P A M E A F G N A	K T L R N D N S S R	F G K F I R I H F G	P T G K L A S A D I	D I Y L L E K S R V
DROSOPHILA	G S L E D Q V V Q T	N P V L E A F G N A	K T V R N D N S S R	F G K F I R I H F G	P T G K L A G A D I	E T Y L L E K A R V

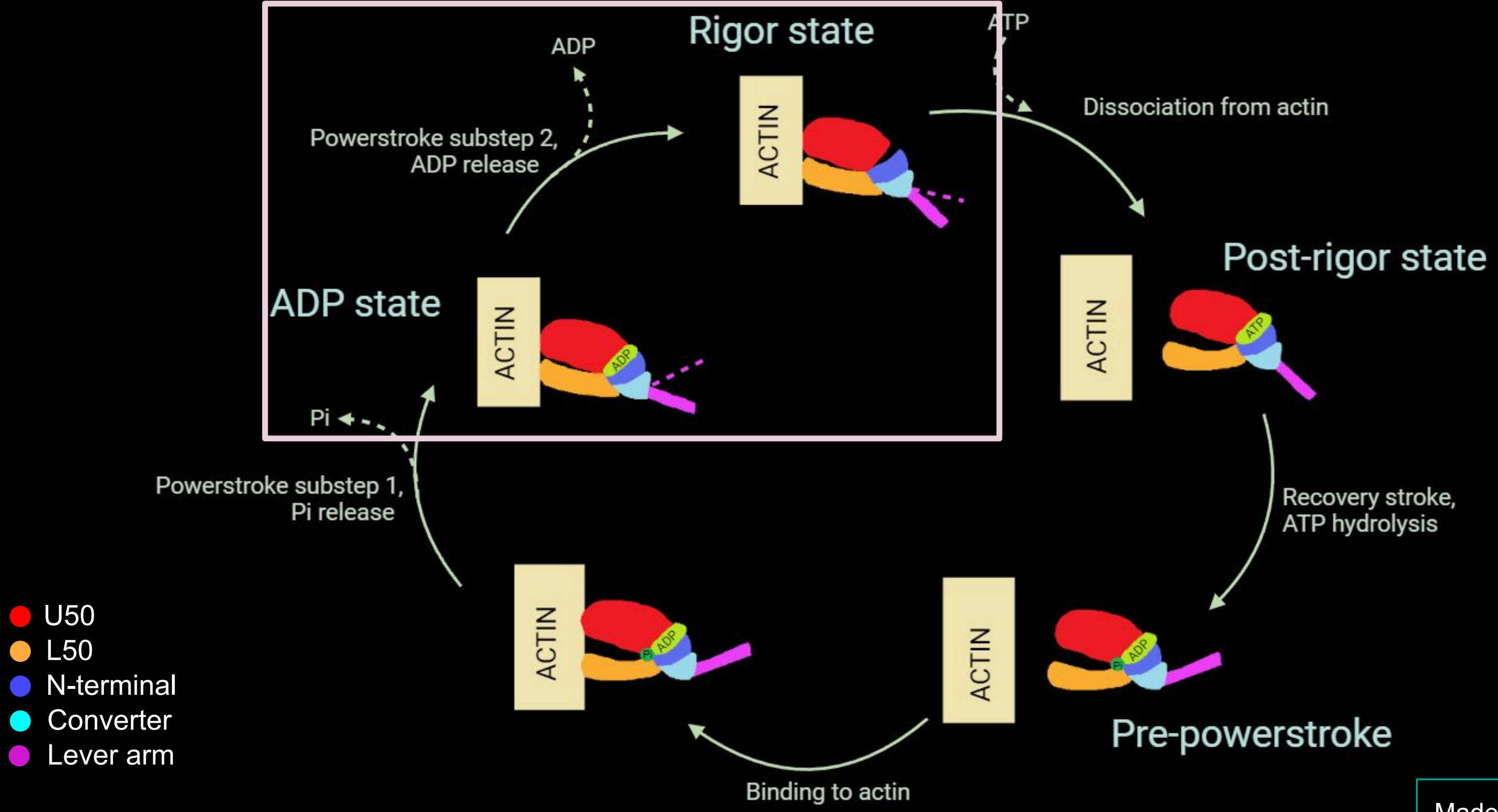


	461	471 461	481 471	491	501	511
Consensus	N a T L e T k q p R	Q y F I G V L D I A	G F E I F d F N s F	E Q L C I N F T N E	K L Q Q F F N H H M	F V L E Q E E Y K K
Conservation						
HUMAN	N A T L E T K Q P R	Q Y F I G V L D I A	G F E I F D F N S F	E Q L C I N F T N E	K L Q Q F F N H H M	F V L E Q E E Y K K
MOUSE	N A T L E T K Q P R	Q Y F I G V L D I A	G F E I F D F N S F	E Q L C I N F T N E	K L Q Q F F N H H M	F V L E Q E E Y K K
PIG	N T T L E T K Q P R	Q Y F I G V L D I A	G F E I F D F N S F	E Q L C I N F T N E	K L Q Q F F N H H M	F V L E Q E E Y K K
BOVIN	N A T L E T K Q P R	Q Y F I G V L D I A	G F E I F D F N S F	E Q L C I N F T N E	K L Q Q F F N H H M	F V L E Q E E Y K K
BIRD	N K T L D T K L A R	Q F F I G V L D I A	G F E I F D F N S F	E Q L C I N F T N E	K L Q Q F F N H H M	F V L E Q E E Y K K
SNAKE	N T T L E T K L P R	Q Y F I G V L D I A	G F E I F D F N S F	E Q L C I N F T N E	K L Q Q F F N H H M	F V L E Q E E Y K K
FROG	N Q Q L D T K Q P R	Q H F I G V L D I A	G F E I F D F N S L	E Q L C I N F T N E	K L Q Q F F N H H M	F V L E Q E E Y K K
ZEBRAFISH	N K T L Y T A I P R	Q F F I G V L D I A	G F E I F E F N N F	E Q M C I N F T N E	K L Q Q F F N H H M	F I L E Q E E Y K T
DROSOPHILA	N E T L D T Q Q K R	Q H F I G V L D I A	G F E I F E Y N G F	E Q L C I N F T N E	K L Q Q F F N H I M	F V M E Q E E Y K K

ADP RELEASE



MYOSIN ATPASE CYCLE



Rigor state

ATP

Dissociation from actin

Post-rigor state

Recovery stroke,
ATP hydrolysis

Pre-powerstroke

Binding to actin

Powerstroke substep 1,
Pi release

Pi

ADP state

Powerstroke substep 2,
ADP release

ADP

- ● U50
- ● L50
- ● N-terminal
- ● Converter
- ● Lever arm

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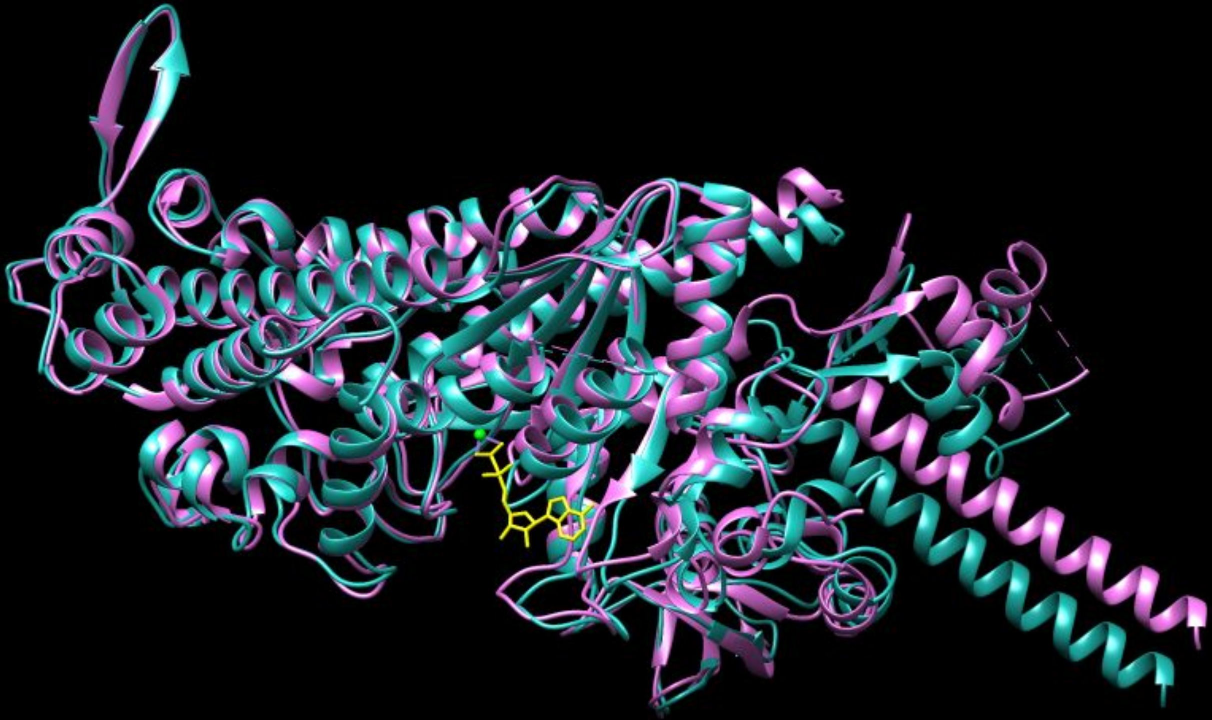
ADP RELEASE SUPERIMPOSITION

● ADP state (8EFE)

● Rigor state (8EFD)

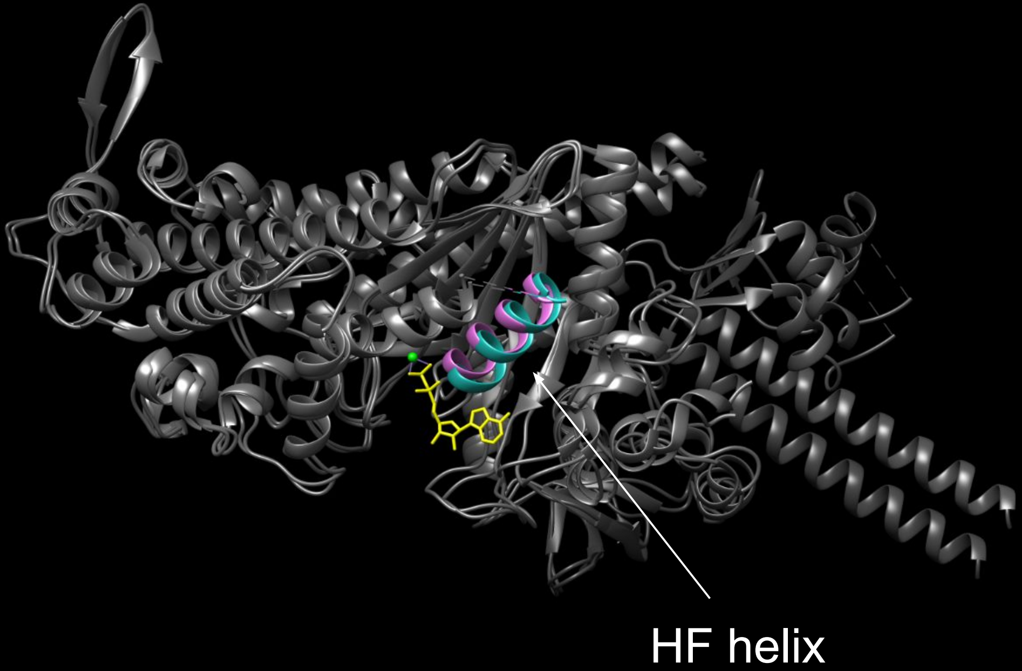
Sc	8.27
RMSD	1.44
Len	749
nfit	686

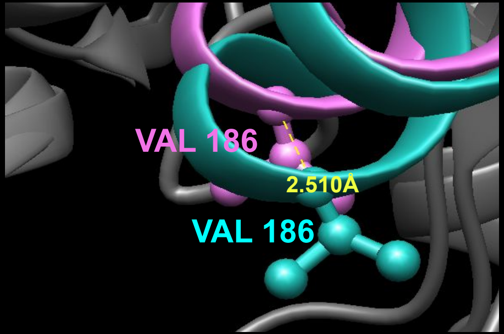


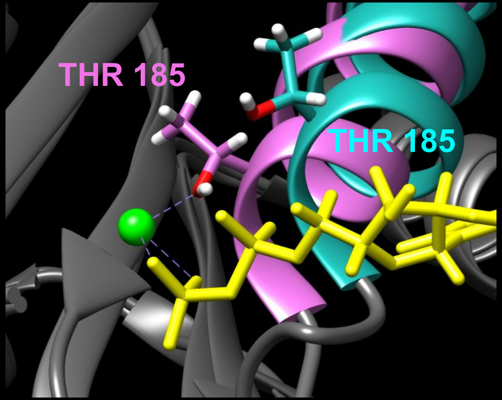
STRUCTURAL CHANGES
Binding domain

● ADP state

● Rigor state





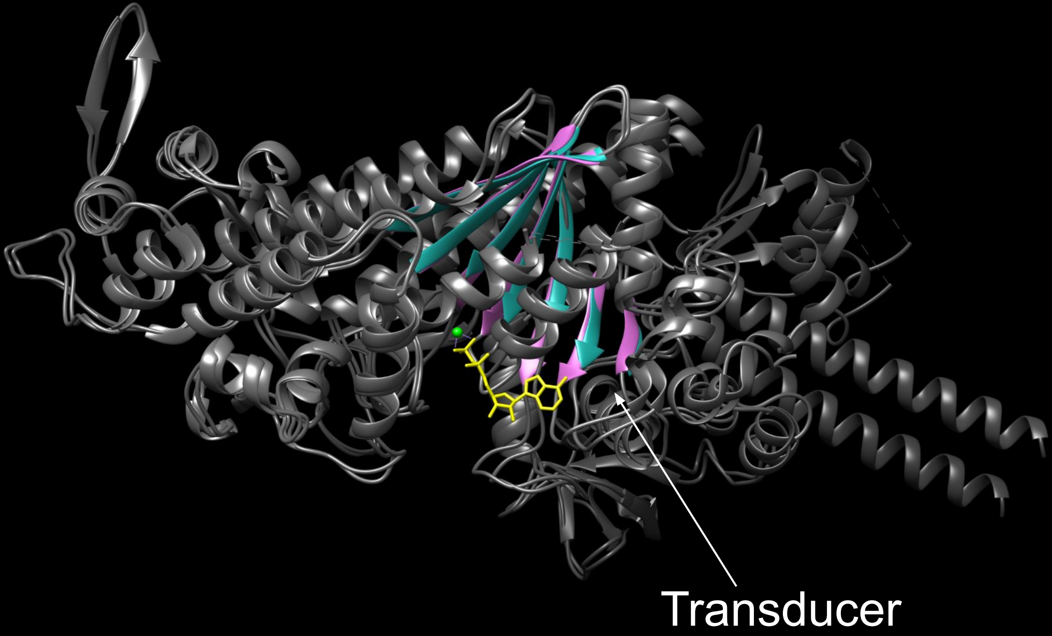


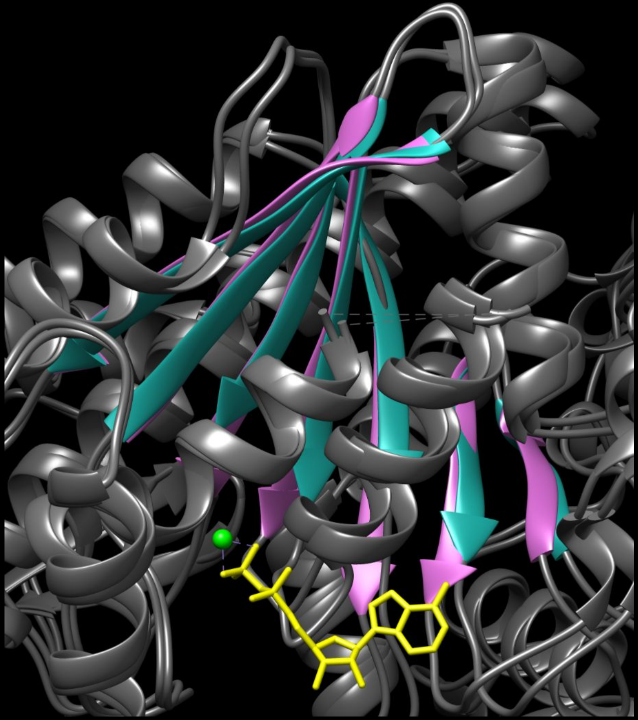
STRUCTURAL CHANGES

Binding domain

● ADP state

● Rigor state



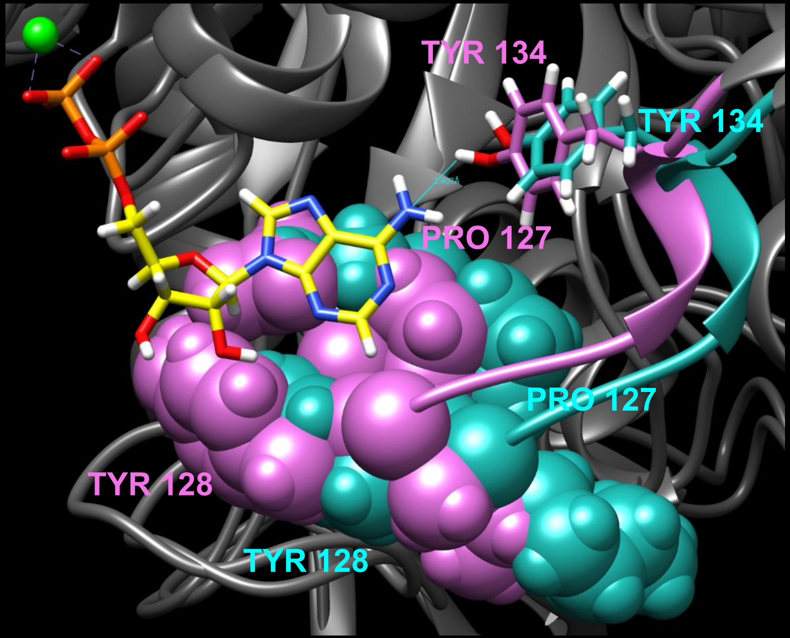


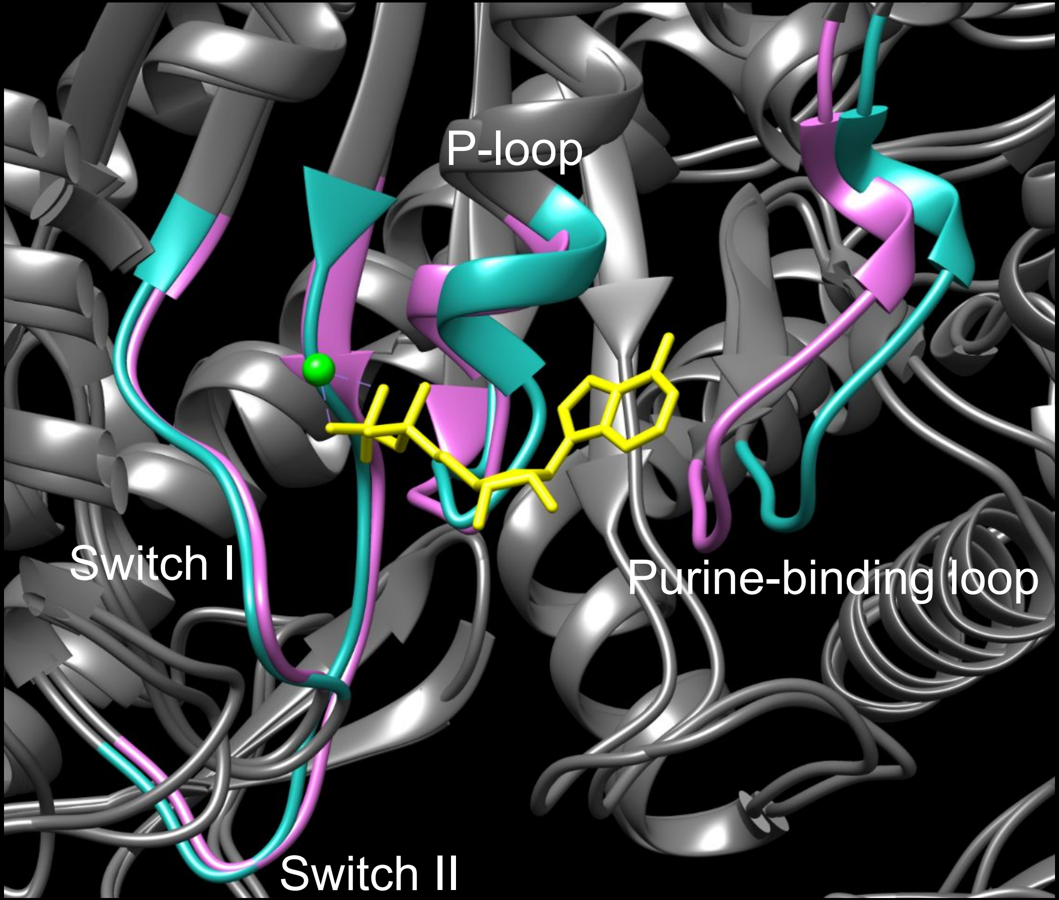
STRUCTURAL CHANGES
Binding domain

● ADP state

● Rigor state


Purine-binding loop





● ADP state
Close conformation

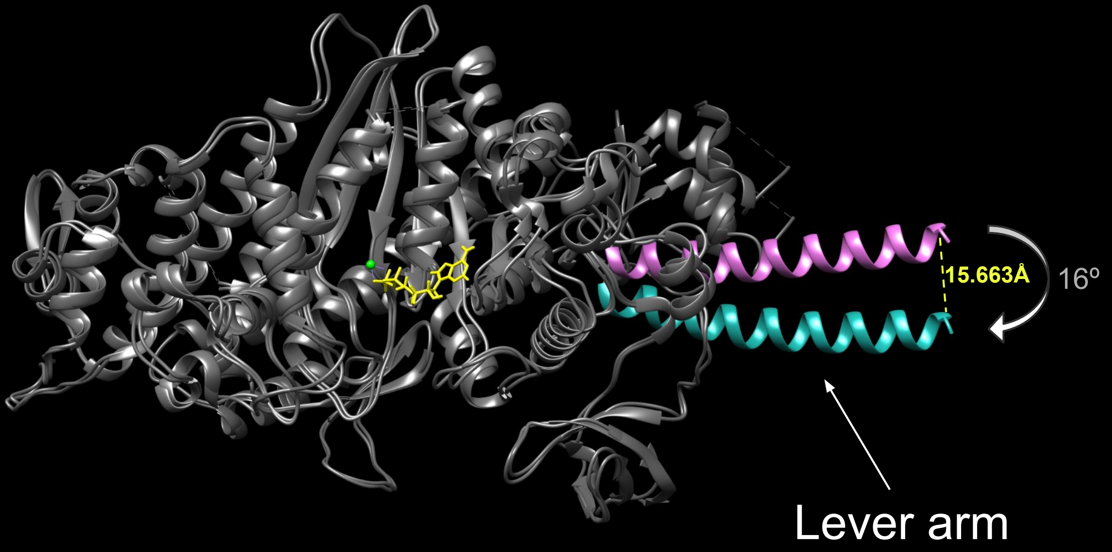
● Rigor state
Open conformation

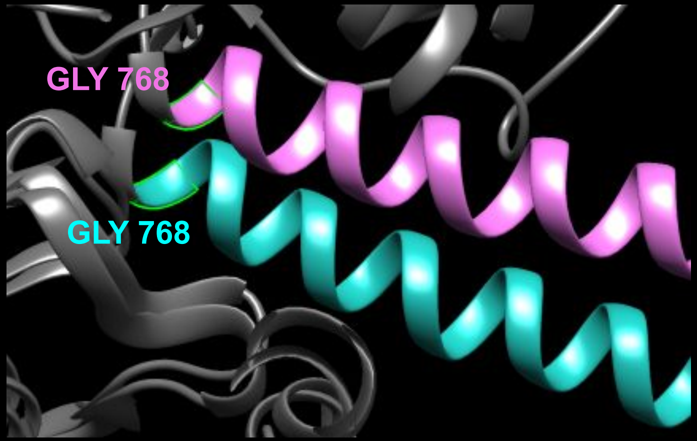
STRUCTURAL CHANGES

Large-scale

● ADP state

● Rigor state

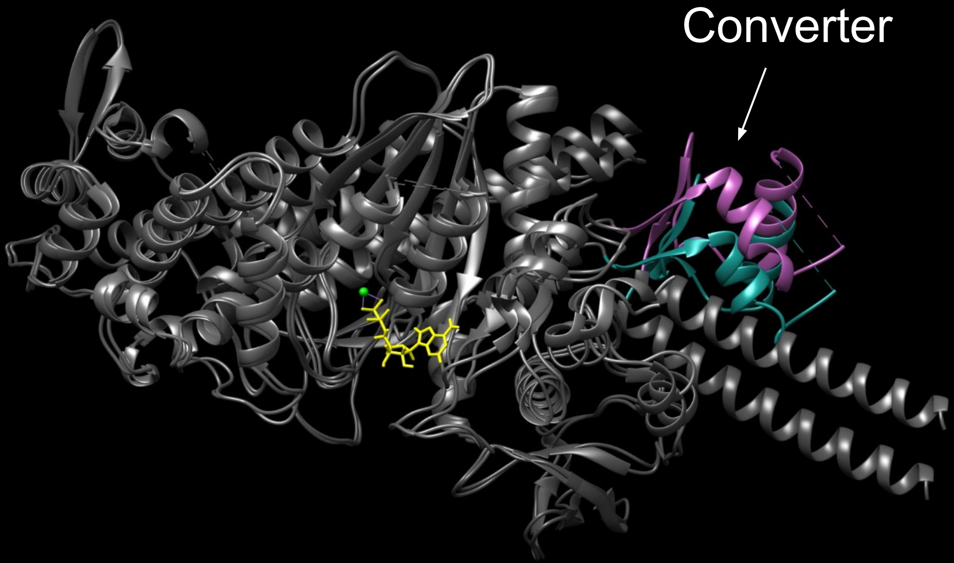


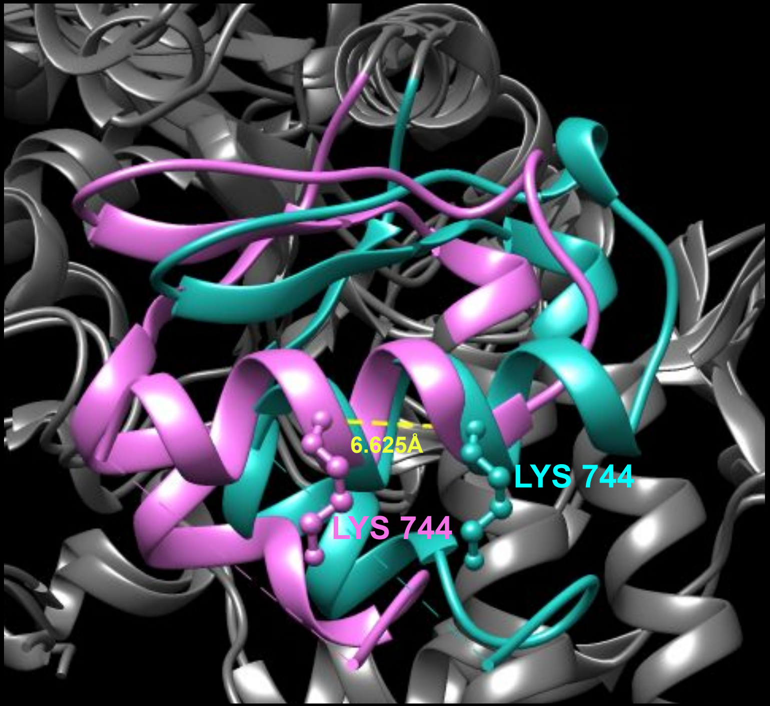


STRUCTURAL CHANGES
Large-scale

● ADP state

● Rigor state

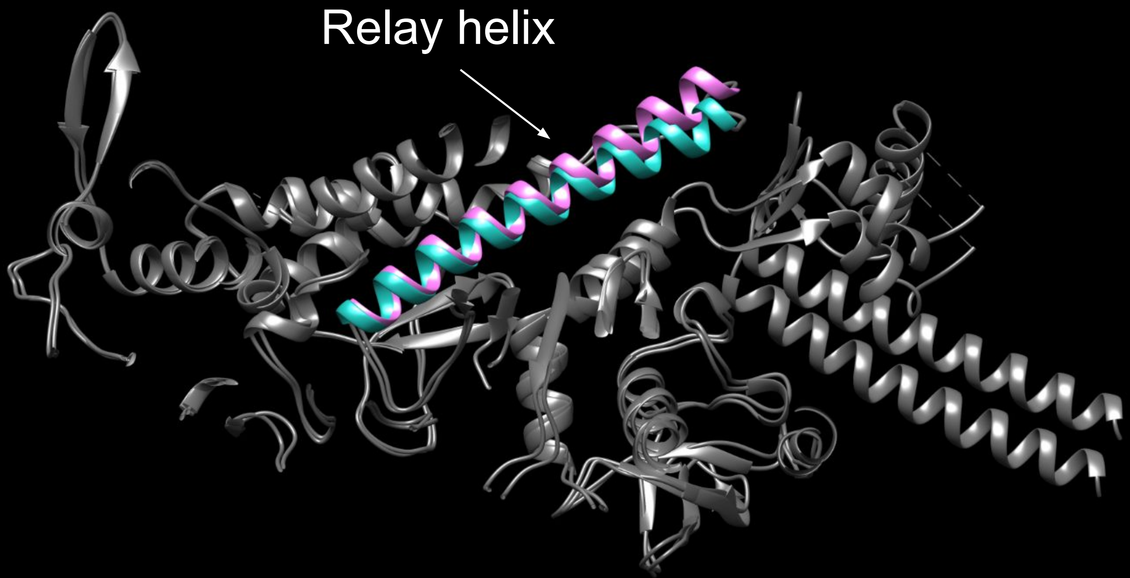


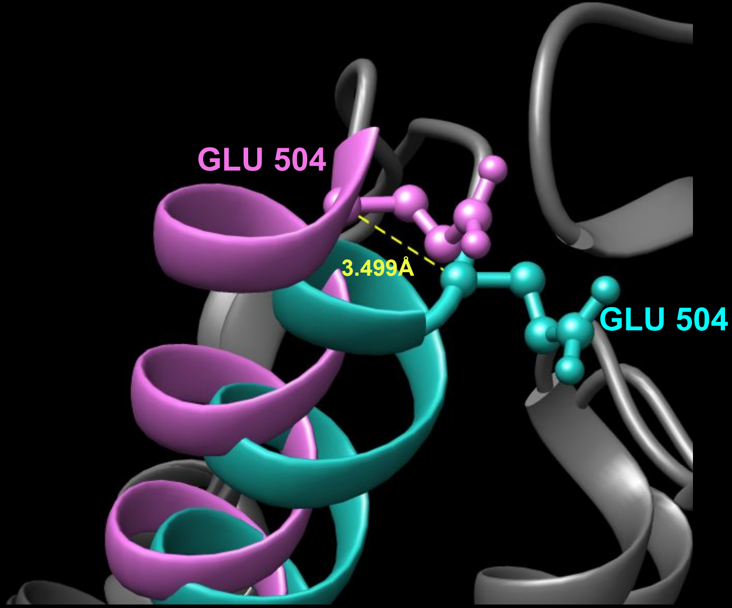


STRUCTURAL CHANGES
Large-scale

● ADP state

● Rigor state





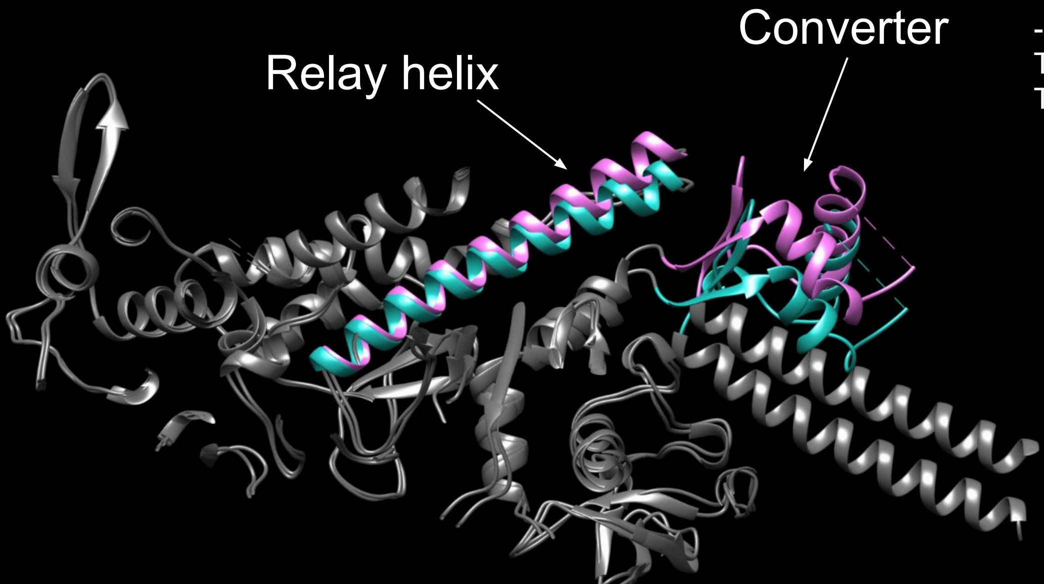
STRUCTURAL CHANGES
Large-scale

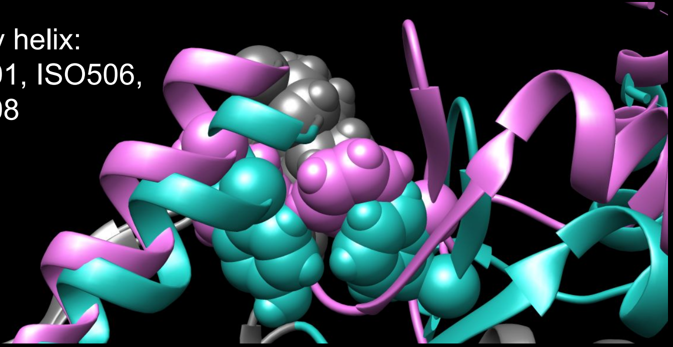
● ADP state

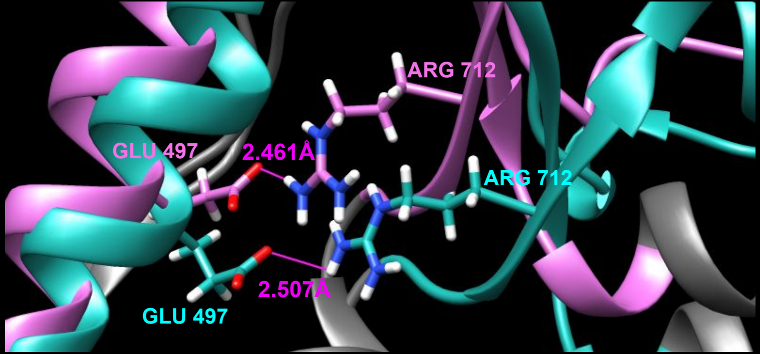
● Rigor state

- Converter: PHE764

- Relay helix: TYR501, ISO506, TRP508







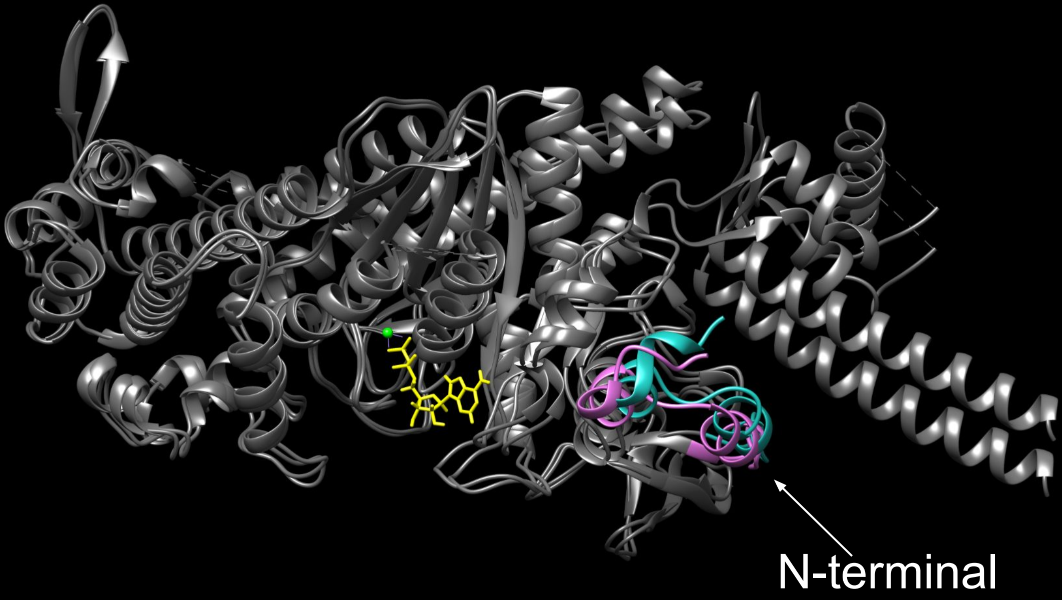
— Salt bridge

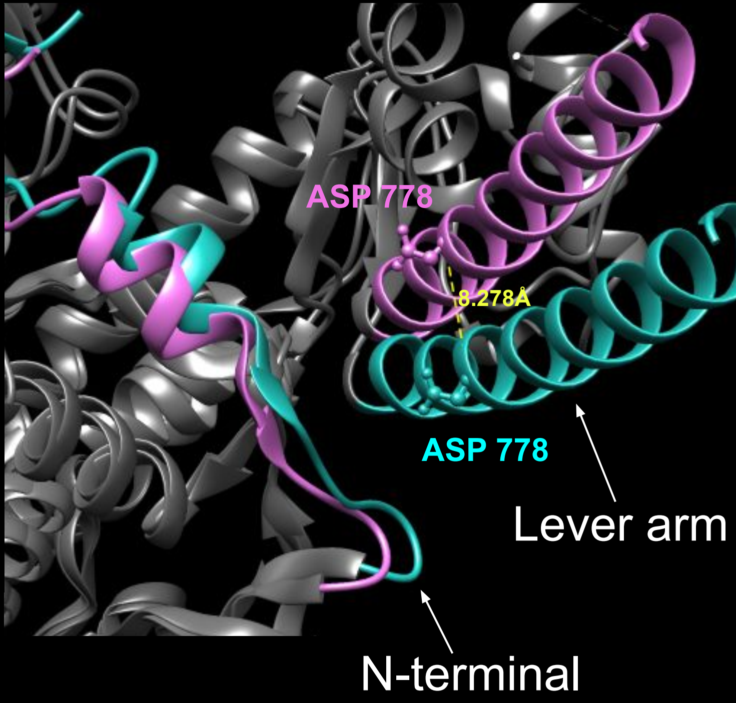
●● Hydrophobic pocket

STRUCTURAL CHANGES
Large-scale

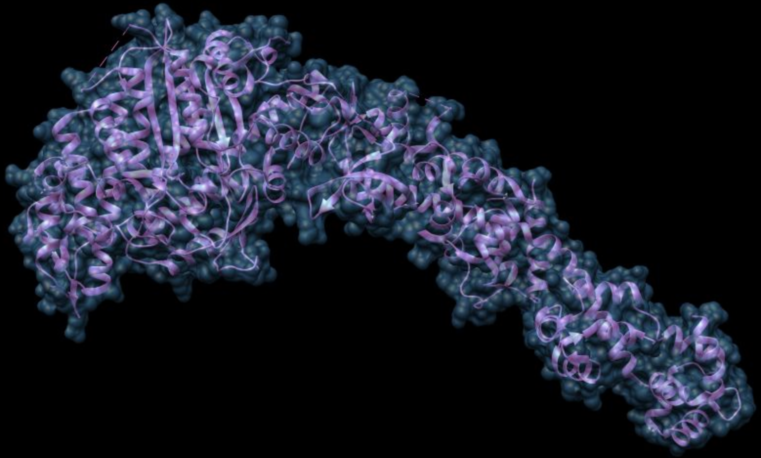
● ADP state

● Rigor state

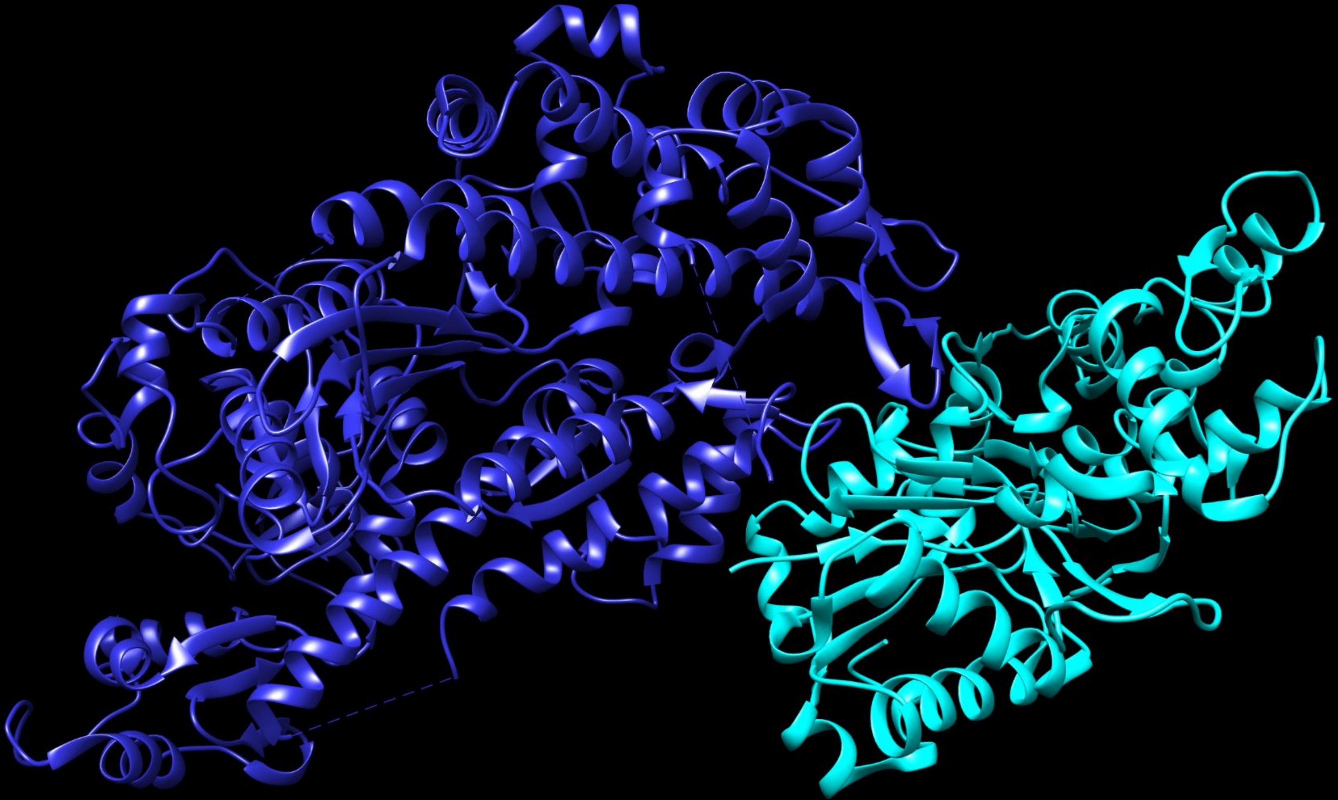




MYOSIN - ACTIN INTERACTION



ACTIN-MYOSIN INTERACTION



ACTIN
MYOSIN

ID: 7JH7
Resolution: 3.80Å

MYOSIN INTERACTING LOOPS

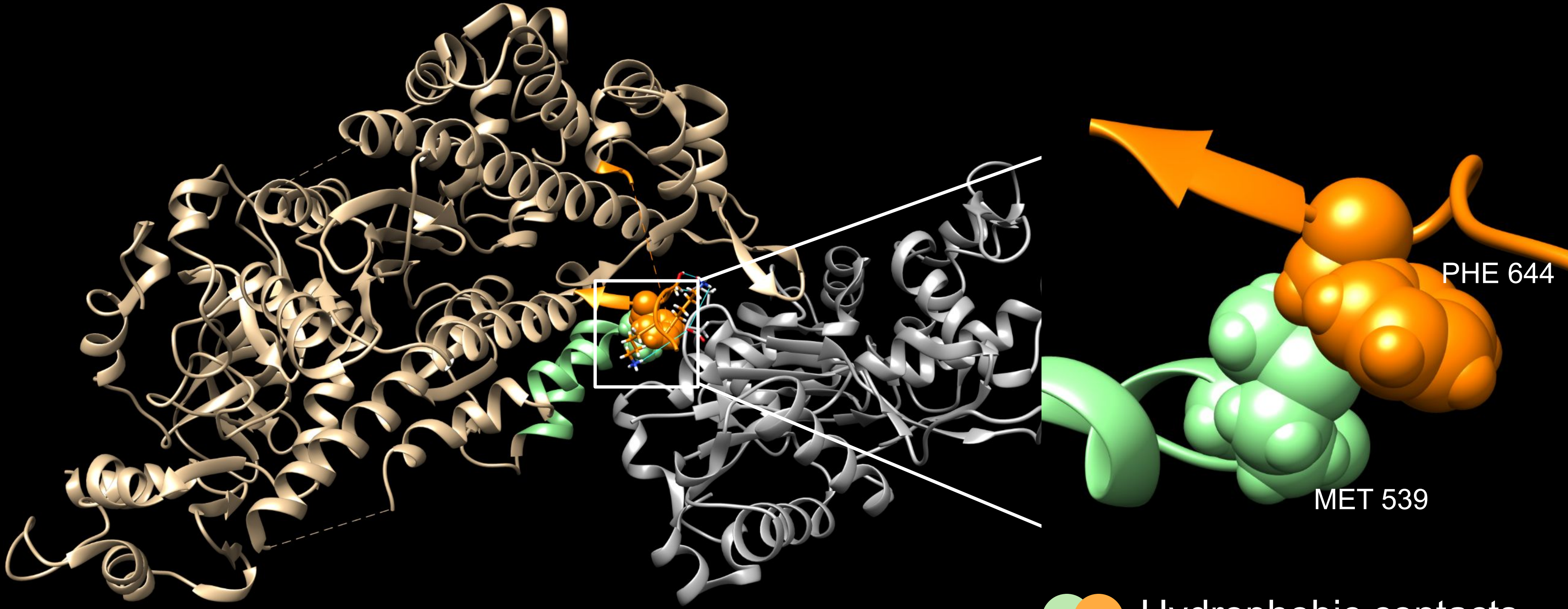
LOOP 2
L620-T646

C LOOP
N361-E379

CM LOOP
H401-N416

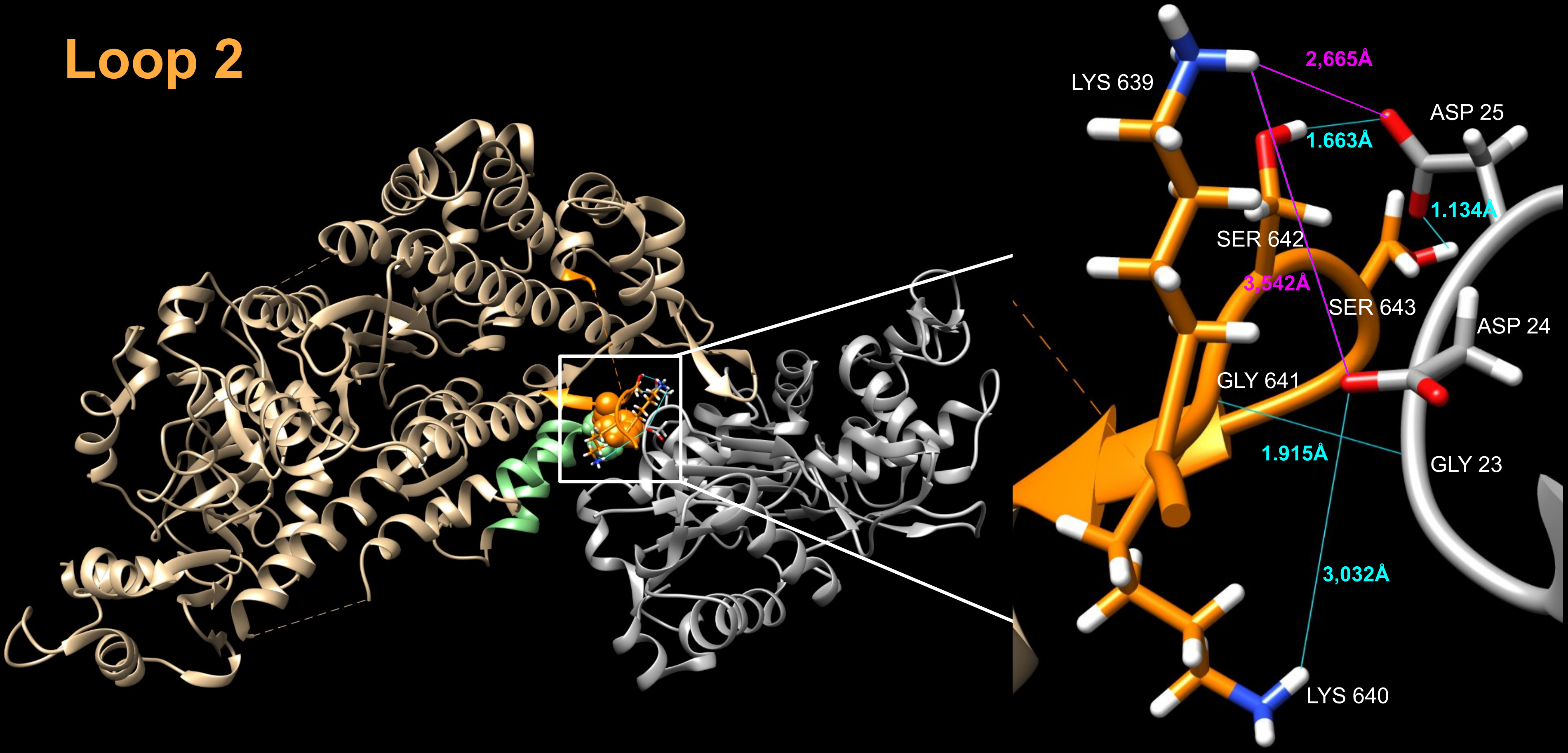
HLH MOTIF
I530-H556

Loop 2

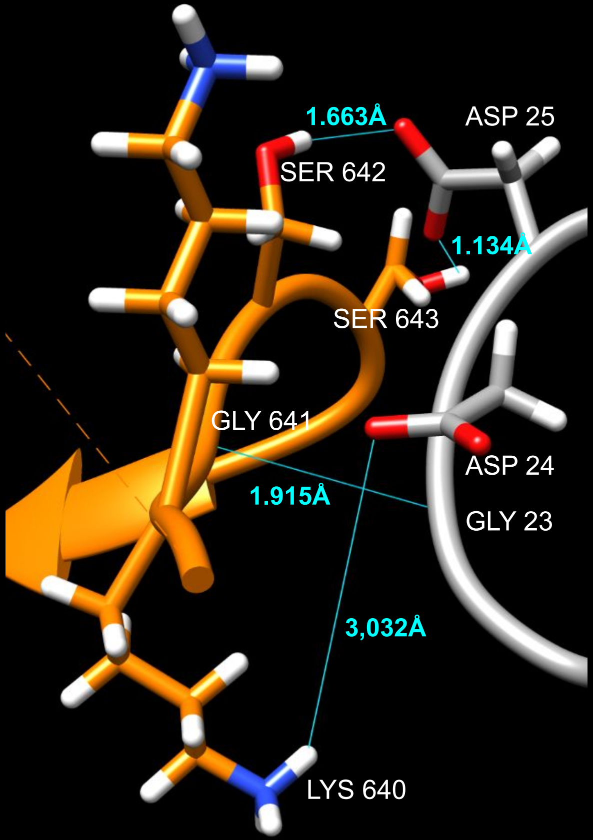


🟢🟠 Hydrophobic contacts

Loop 2


2,665Å
LYS 639
ASP 25
1.663Å
1.134Å
SER 642
3,542Å
SER 643
ASP 24
GLY 641
1.915Å
GLY 23
3,032Å
LYS 640

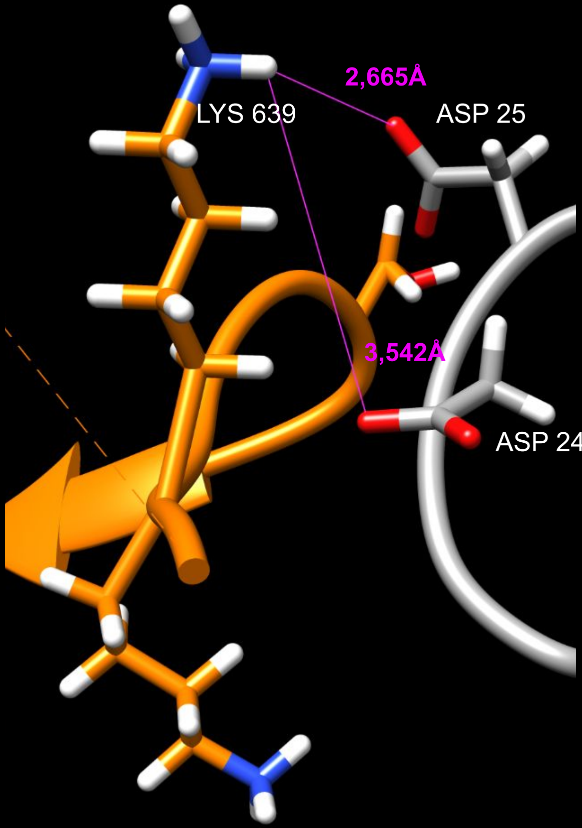
Loop 2



Residue Myosin	Atom	Residue Actin	Atom
GLY 641	**N**	GLY 23	**O**
LYS 640	**N**	ASP 24	**O**
SER 642	**OH**	ASP 25	**O**
SER 643	**OH**	ASP 25	**O**

▬ Hydrogen bonds

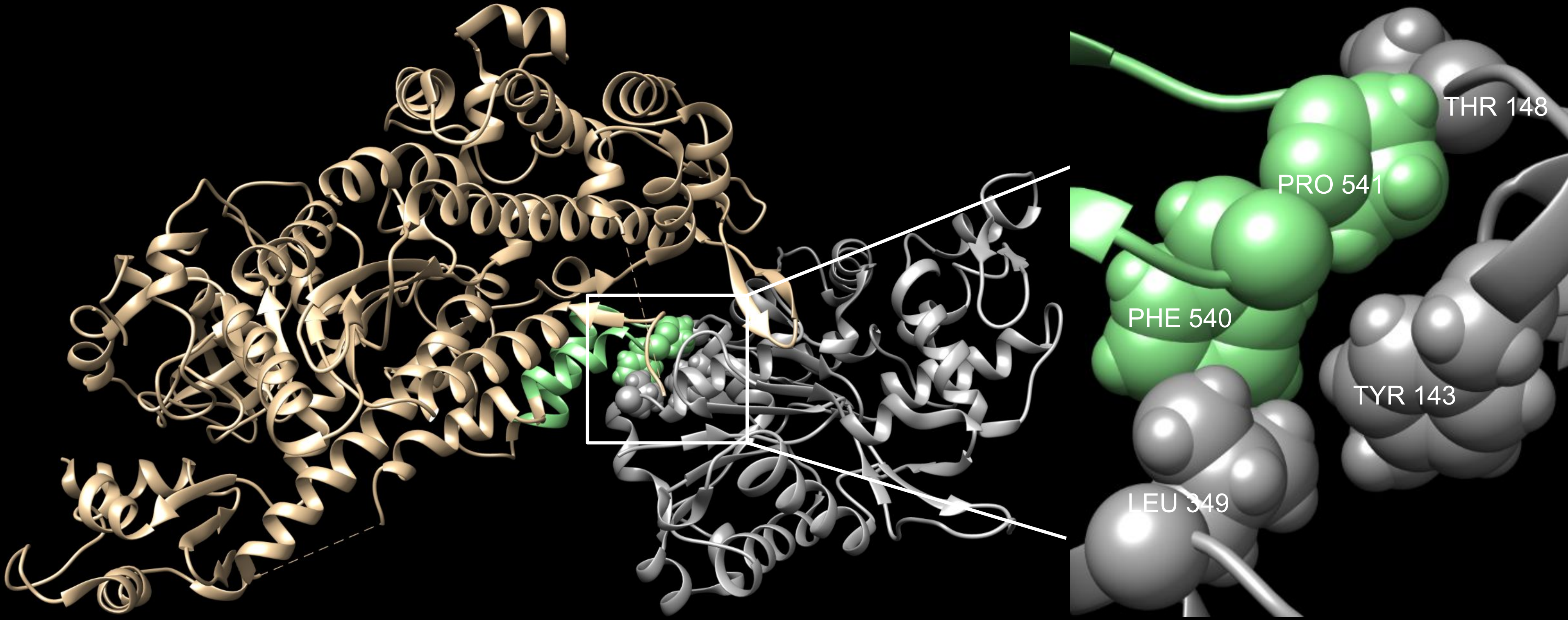
Loop 2



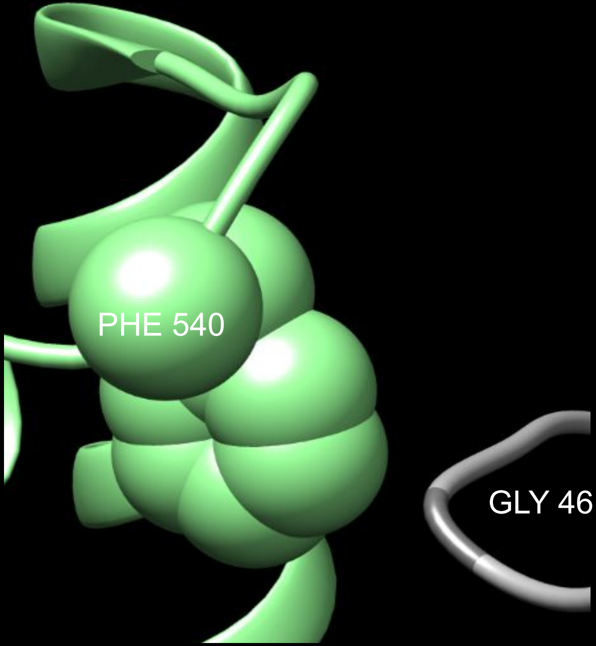
Residue Myosin	**Atom**	**Residue Actin**	**Atom**
LYS 639	**N**	ASP 24	**O**
LYS 639	**N**	ASP 25	**O**

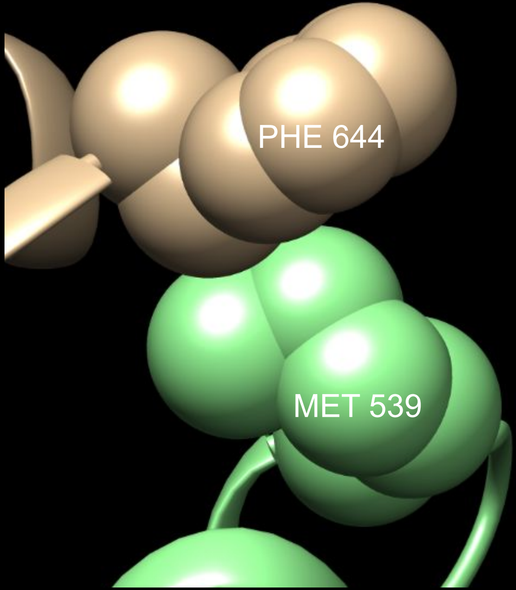
▬ Salt bridge

HLH motif

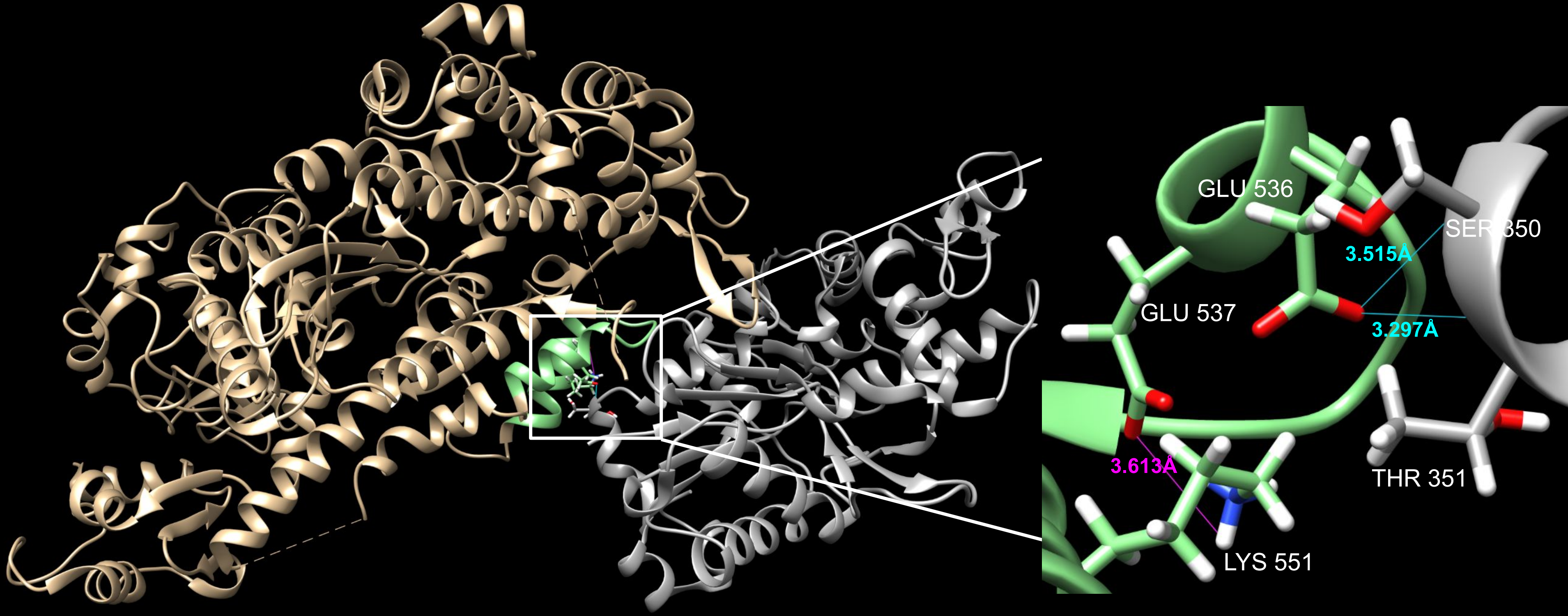


HLH motif



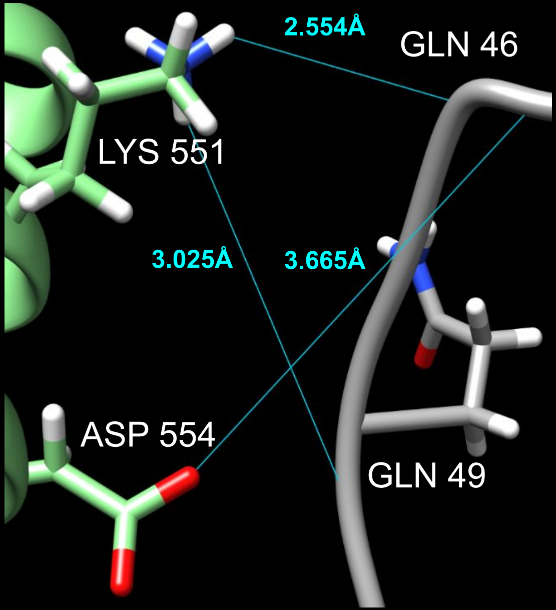


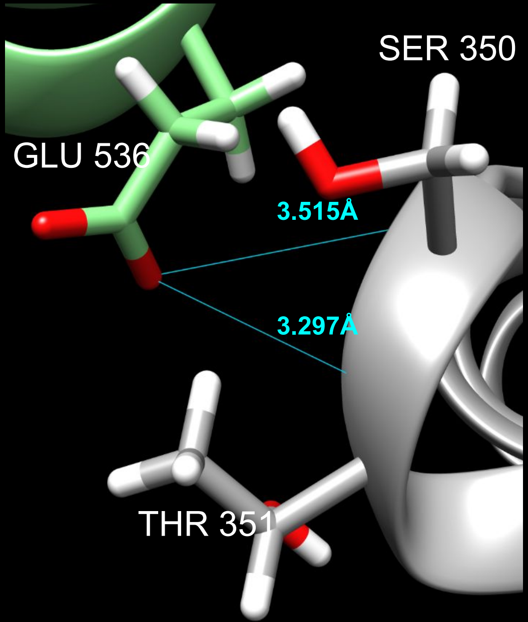
HLH motif



HLH motif

Residue Myosin	Atom	Residue Actin	Atom
GLU 536	**O**	SER 350	**N**
GLU 536	**O**	THR 351	**N**
LYS 551	**N**	GLN 49	**O**
LYS 551	**N**	GLY 46	**O**
ASP 554	**O**	GLY 46	**N**

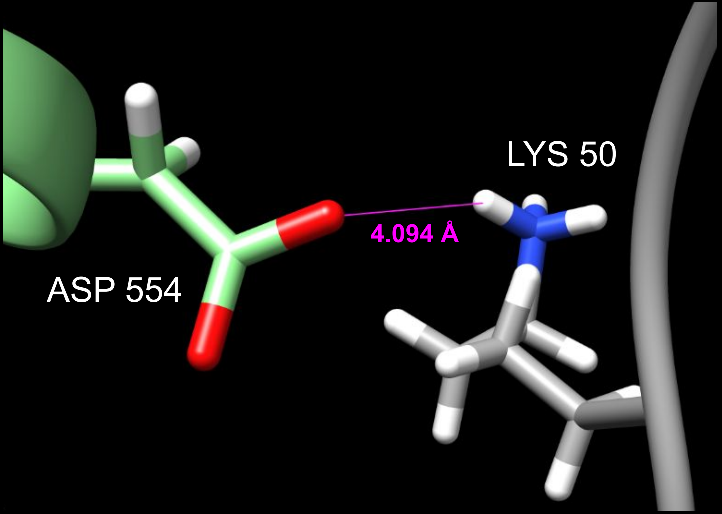


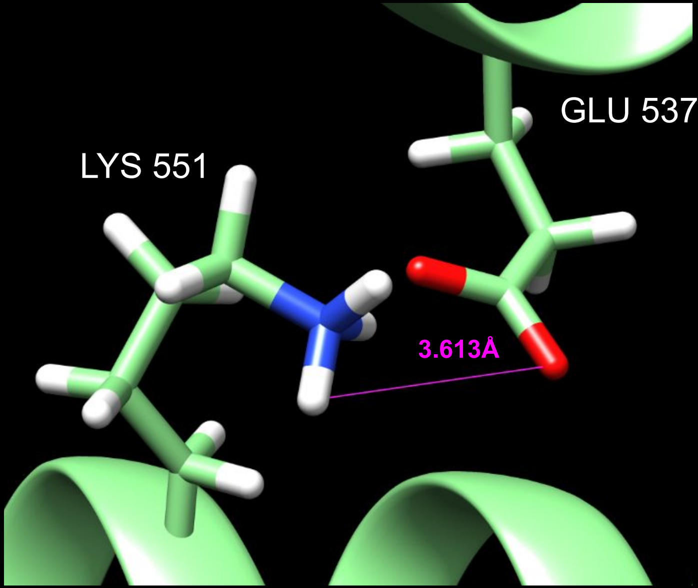


▬ Hydrogen bonds

— Salt bridge

HLH motif

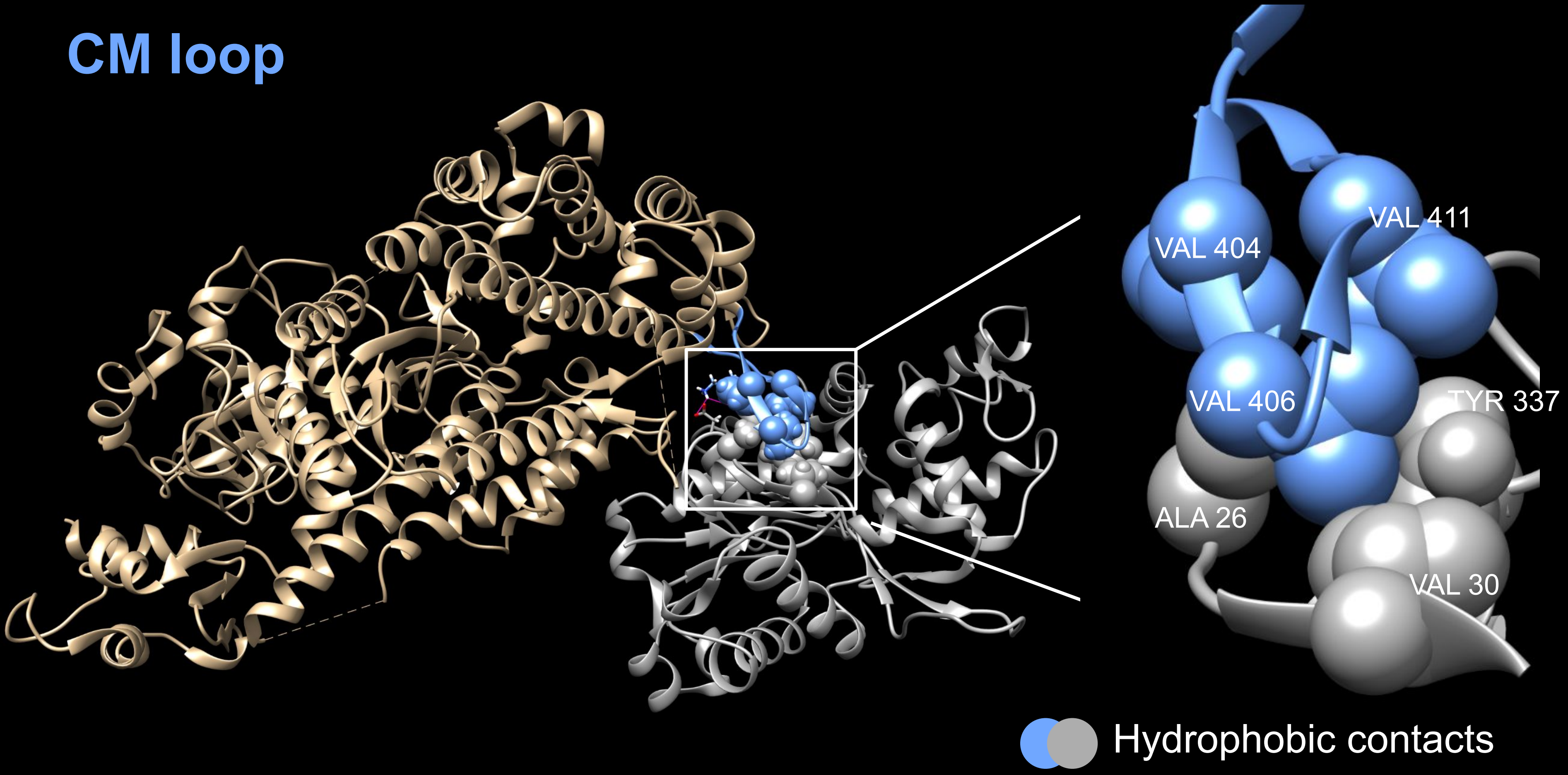




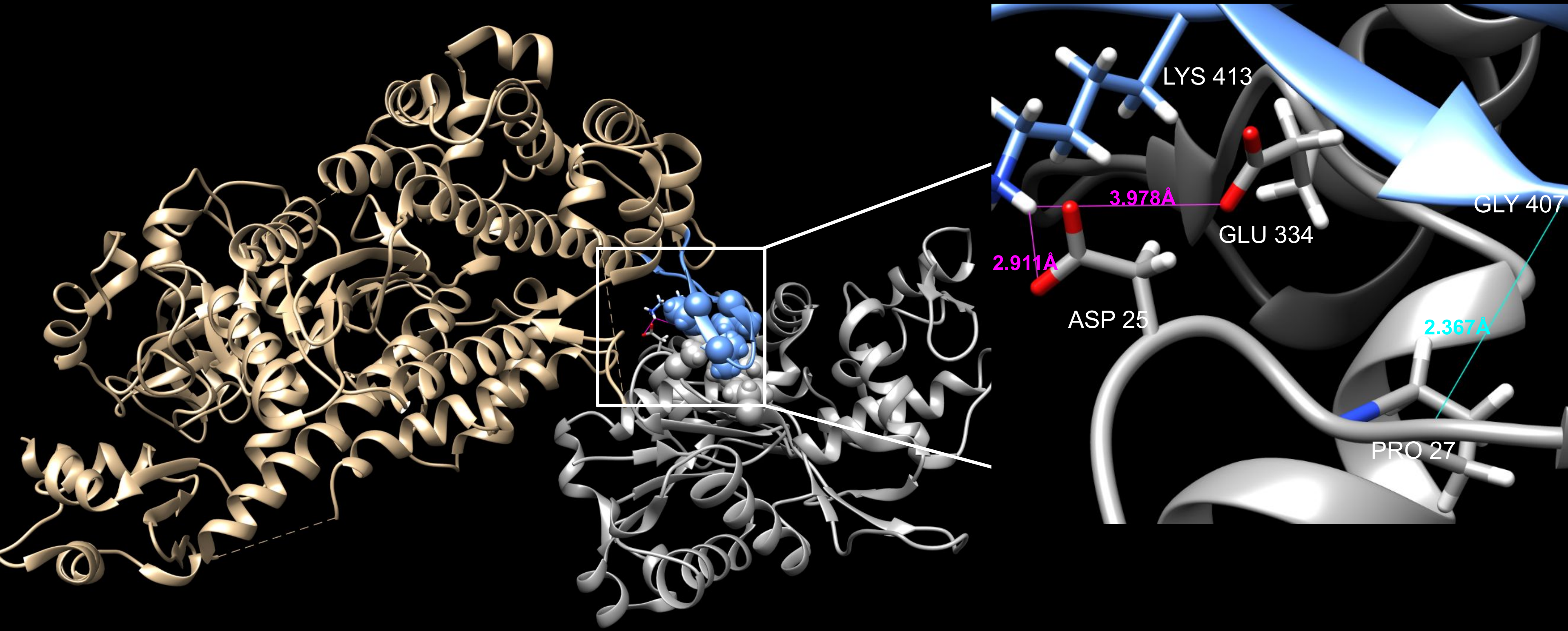
Residue Myosin	Atom	Residue Actin	Atom
ASP 554	**O**	LYS 50	**N**

Residue Myosin	Atom
GLU 537	**O**
LYS 551	**N**

CM loop

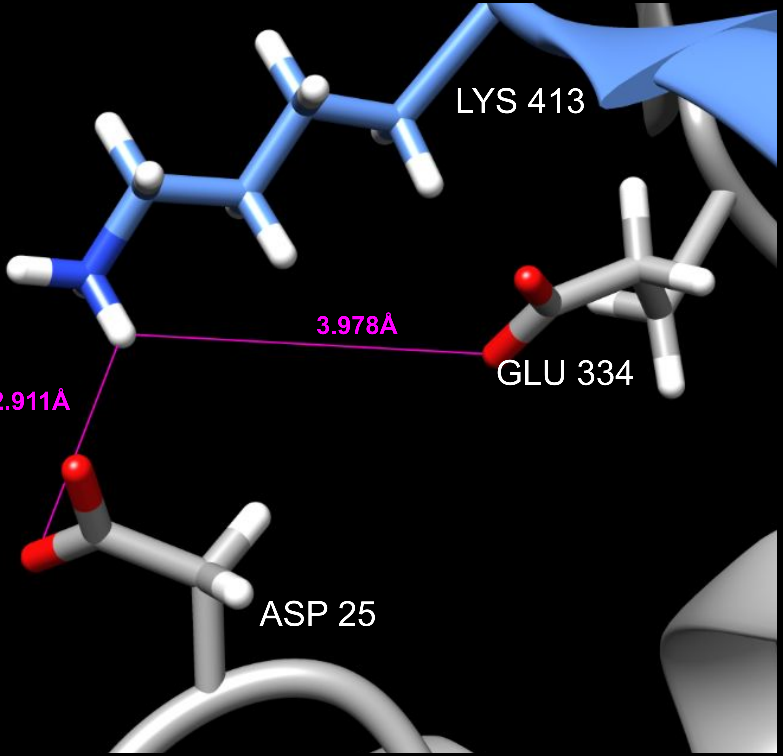

VAL 404
VAL 411
VAL 406
TYR 337
ALA 26
VAL 30
Hydrophobic contacts

CM loop



CM loop

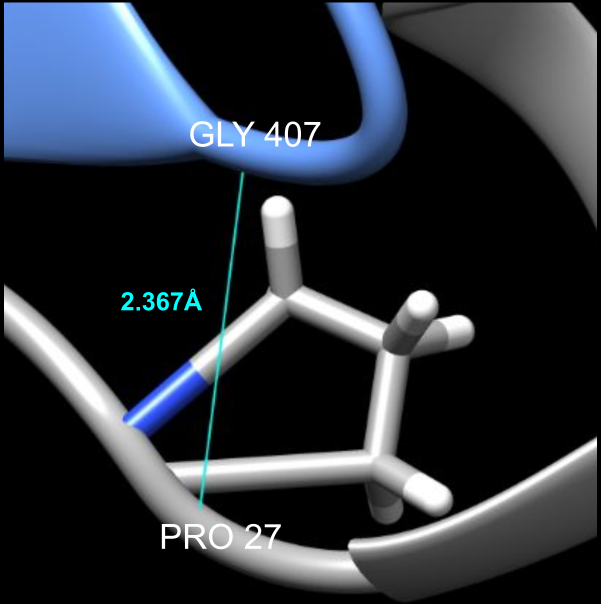
Residue Myosin	Atom	Residu Actin	Atom
LYS 413	**N**	ASP 25	**O**
LYS 413	**N**	GLU 334	**O**



▬ Salt bridge

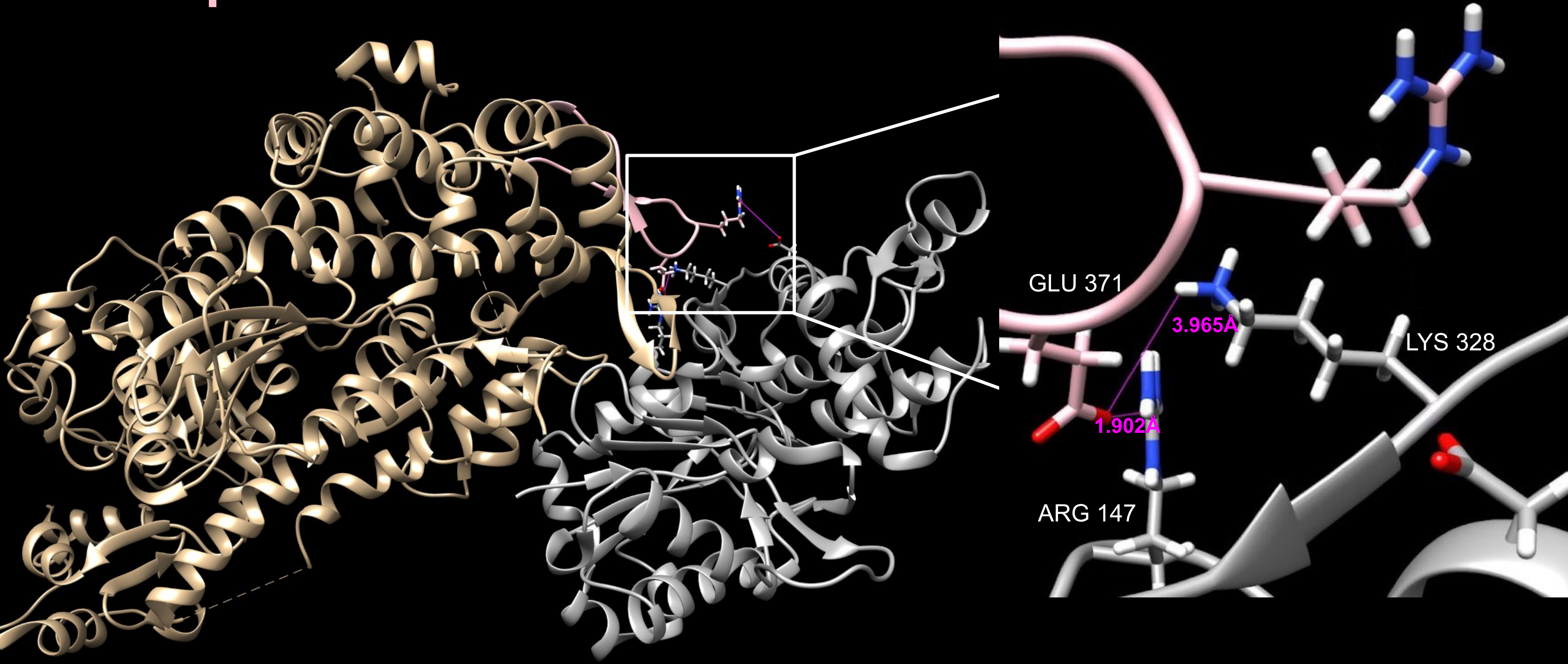
CM loop

Residue Myosin	**Atom**	**Residu Actin**	**Atom**
GLY 407	**N**	PRO 27	**O**



▬ Hydrogen bonds

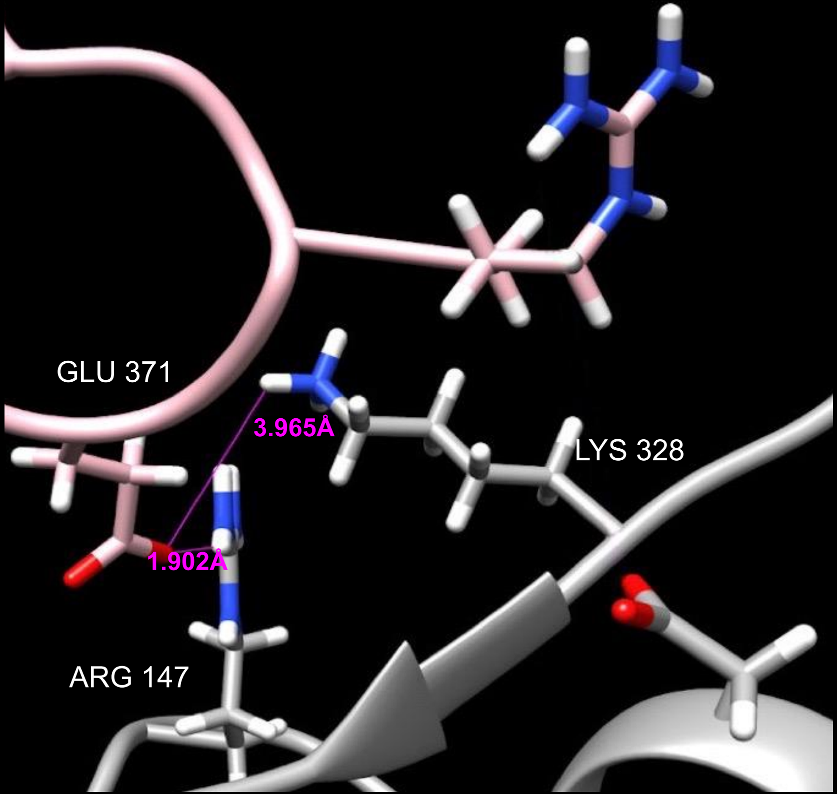
C loop



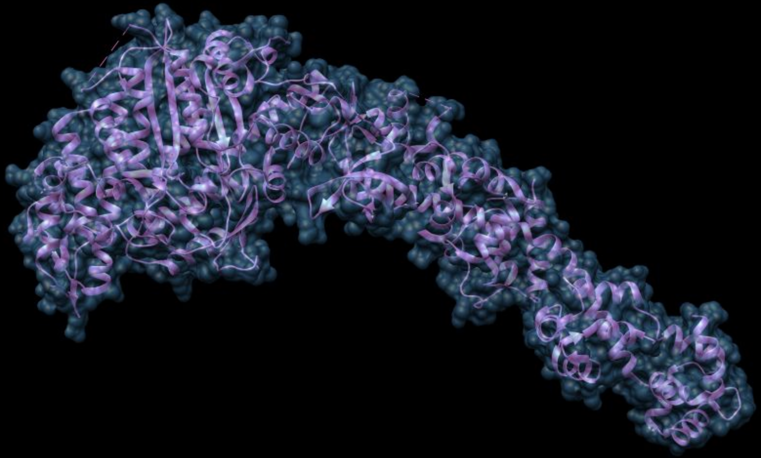
C loop

Residue Myosin	Atom	Residu Actin	Atom
GLU 371	**O**	ARG 147	**N**
GLU 371	**O**	LYS 328	**N**

━ Salt bridge

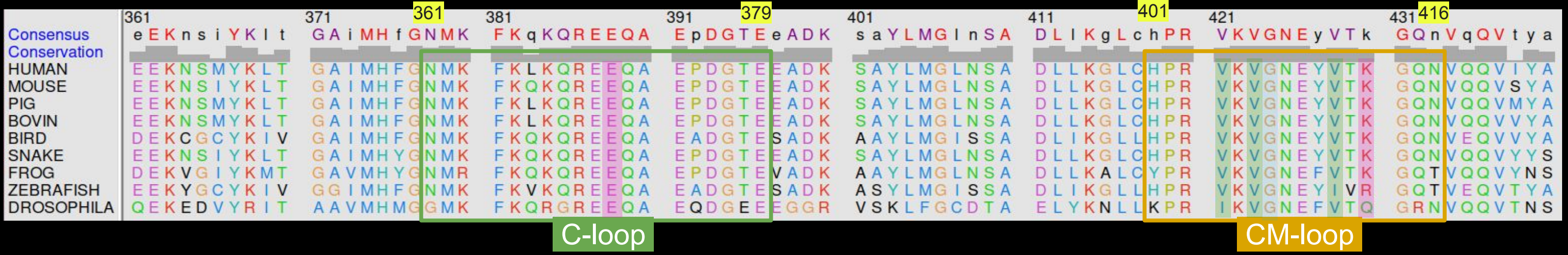


CONSERVATION OF MYOSIN - ACTIN INTERACTION BETWEEN SPECIES



SEQUENCE ALIGNMENT

Hydrophobic interactions
Hydrogen bounds
Salt bridges



```
              361        371        381        391        401        411        421        431
Consensus     eEKnsiYKlt GAiMHfGNMK FKqKQREEQA EpDGTEeADK saYLMGlnSA DLlKgLchPR VKVGNEyVTk GQnVqQVtya
Conservation
HUMAN         EEKNSMYKLT GAIMHFGNMK FKLKQREEQA EPDGTEEADK SAYLMGLNSA DLLKGLCHPR VKVGNEYVTK GQNVQQVIYA
MOUSE         EEKNSIYKLT GAIMHFGNMK FKQKQREEQA EPDGTEEADK SAYLMGLNSA DLLKGLCHPR VKVGNEYVTK GQNVQQVSYA
PIG           EEKNSMYKLT GAIMHFGNMK FKLKQREEQA EPDGTEEADK SAYLMGLNSA DLLKGLCHPR VKVGNEYVTK GQNVQQVMYA
BOVIN         EEKNSMYKLT GAIMHFGNMK FKLKQREEQA EPDGTEEADK SAYLMGLNSA DLLKGLCHPR VKVGNEYVTK GQNVQQVVYA
BIRD          DEKCGCYKIV GAIMHFGNMK FKQKQREEQA EADGTESADK AAYLMGISSA DLIKGLLHPR VKVGNEYVTK GQNVEQVVYA
SNAKE         EEKNSIYKLT GAIMHYGNMK FKQKQREEQA EPDGTEEADK SAYLMGLNSA DLLKGLCHPR VKVGNEYVTK GQNVQQVYYS
FROG          DEKVGIYKMT GAVMHYGNMR FKQKQREEQA EPDGTEVADK AAYLMGLNSA DLLKALCYPR VKVGNEFVTK GQTVQQVYNS
ZEBRAFISH     EEKYGCYKIV GGIMHFGNMK FKVKQREEQA EADGTESADK ASYLMGISSA DLIKGLLHPR VKVGNEYIVR GQTVEQVTYA
DROSOPHILA    QEKEDVYRIT AAVMHMGGMK FKQRGREEQA EQDGEEEGGR VSKLFGCDTA ELYKNLLKPR IKVGNEFVTQ GRNVQQVTNS
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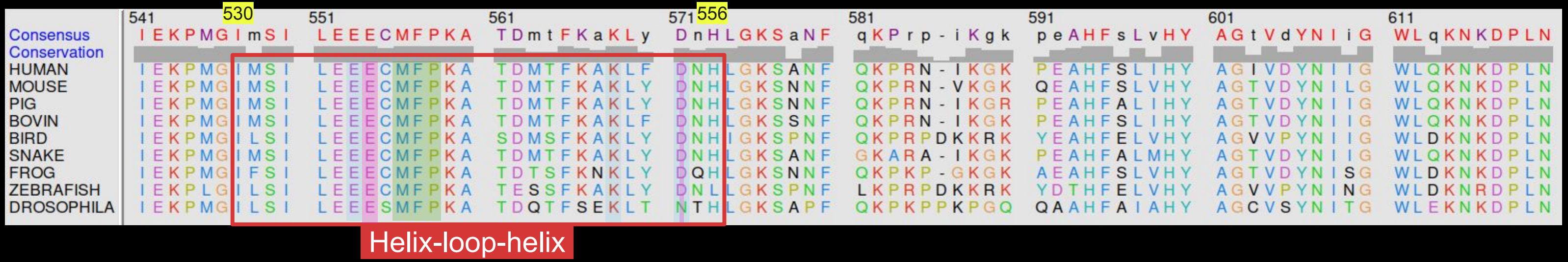

```
              541        551        561        571        581        591        601        611
Consensus     IEKPMGImSI LEEECMFPKA TDmtFKaKLy DnHLGKSaNF qKPrp-iKgk peAHFsLvHY AGtVdYNIiG WLqKNKDPLN
Conservation
HUMAN         IEKPMGIMSI LEEECMFPKA TDMTFKAKLF DNHLGKSANF QKPRN-IKGK PEAHFSLIHY AGIVDYNIIG WLQKNKDPLN
MOUSE         IEKPMGIMSI LEEECMFPKA TDMTFKAKLY DNHLGKSNNF QKPRN-VKGK QEAHFSLVHY AGTVDYNILG WLQKNKDPLN
PIG           IEKPMGIMSI LEEECMFPKA TDMTFKAKLY DNHLGKSNNF QKPRN-IKGR PEAHFALIHY AGTVDYNIIG WLQKNKDPLN
BOVIN         IEKPMGIMSI LEEECMFPKA TDMTFKAKLF DNHLGKSSNF QKPRN-IKGK PEAHFSLIHY AGTVDYNIIG WLQKNKDPLN
BIRD          IEKPMGILSI LEEECMFPKA SDMSFKAKLY DNHIGKSPNF QKPRPDKKRK YEAHFELVHY AGVVPYNIIG WLDKNKDPLN
SNAKE         IEKPMGIMSI LEEECMFPKA TDMTFKAKLY DNHLGKSANF GKARA-IKGK PEAHFALMHY AGTVDYNIIG WLQKNKDPLN
FROG          IEKPMGIFSI LEEECMFPKA TDTSFKNKLY DQHLGKSNNF QKPKP-GKGK AEAHFSLVHY AGTVDYNISG WLDKNKDPLN
ZEBRAFISH     IEKPLGILSI LEEECMFPKA TESSFKAKLY DNLLGKSPNF LKPRPDKKRK YDTHFELVHY AGVVPYNING WLDKNRDPLN
DROSOPHILA    IEKPMGILSI LEEESMFPKA TDQTFSEKLT NTHLGKSAPF QKPKPPKPGQ QAAHFAIAHY AGCVSYNITG WLEKNKDPLN
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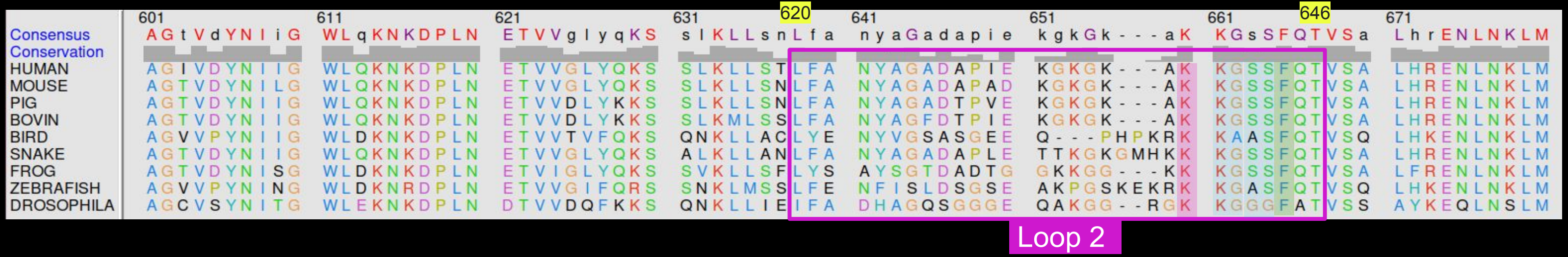


```
              601        611        621        631        641        651        661        671
Consensus     AGtVdYNIiG WLqKNKDPLN ETVVglyqKS slKLLsnLfa nyaGadapie kgkGk---aK KGsSFQTVSa LhrENLNKLM
Conservation
HUMAN         AGIVDYNIIG WLQKNKDPLN ETVVGLYQKS SLKLLSTLFA NYAGADAPIE KGKGK---AK KGSSFQTVSA LHRENLNKLM
MOUSE         AGTVDYNILG WLQKNKDPLN ETVVGLYQKS SLKLLSNLFA NYAGADAPAD KGKGK---AK KGSSFQTVSA LHRENLNKLM
PIG           AGTVDYNIIG WLQKNKDPLN ETVVDLYKKS SLKLLSNLFA NYAGADTPVE KGKGK---AK KGSSFQTVSA LHRENLNKLM
BOVIN         AGTVDYNIIG WLQKNKDPLN ETVVDLYKKS SLKMLSSLFA NYAGFDTPIE KGKGK---AK KGSSFQTVSA LHRENLNKLM
BIRD          AGVVPYNIIG WLDKNKDPLN ETVVTVFQKS QNKLLACLYE NYVGSASGEE Q---PHPKRK KAASFQTVSQ LHKENLNKLM
SNAKE         AGTVDYNIIG WLQKNKDPLN ETVVGLYQKS ALKLLANLFA NYAGADAPLE TTKGKGMHKK KGSSFQTVSA LHRENLNKLM
FROG          AGTVDYNISG WLDKNKDPLN ETVIGLYQKS SVKLLSFLYS AYSGTDADTG GKKGG---KK KGSSFQTVSA LFRENLNKLM
ZEBRAFISH     AGVVPYNING WLDKNRDPLN ETVVGIFQRS SNKLMSSLFE NFISLDSGSE AKPGSKEKRK KGASFQTVSQ LHKENLNKLM
DROSOPHILA    AGCVSYNITG WLEKNKDPLN DTVVDQFKKS QNKLLIEIFA DHAGQSGGGE QAKGG--RGK KGGGFATVSS AYKEQLNSLM
```

STRUCTURE ALIGNMENT

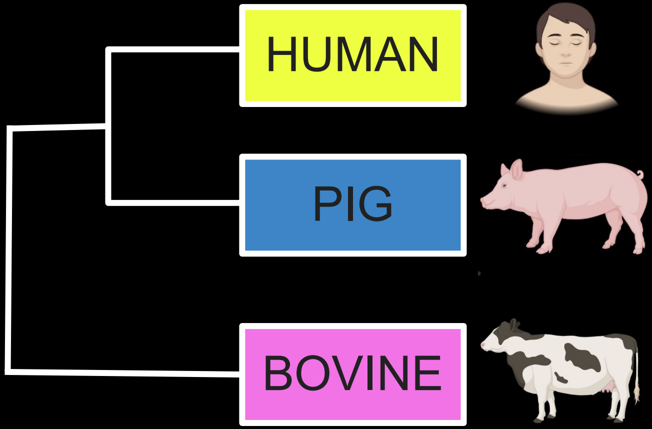
SUPERIMPOSITION OF MYH7 IN RIGOR STATE

Human (8efd)
Pig (7jh7)
Bovine (6x5z)

Score (Sc)	8,93
RMSD	1.51

STRUCTURE ALIGNMENT

SUPERIMPOSITION OF MYH7 IN RIGOR STATE

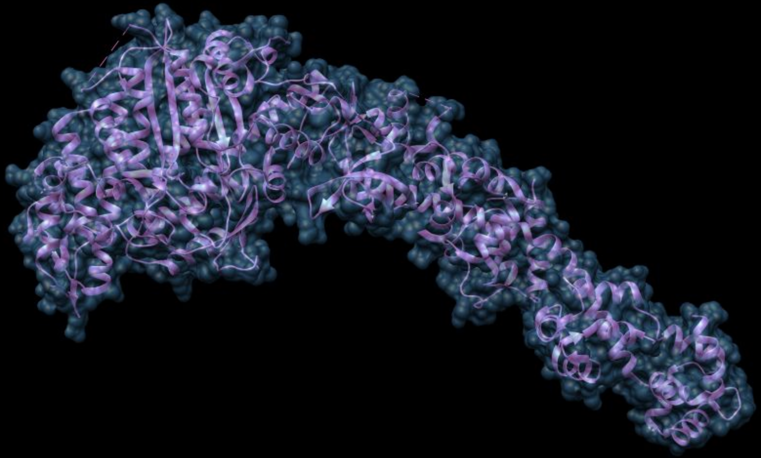


HUMAN

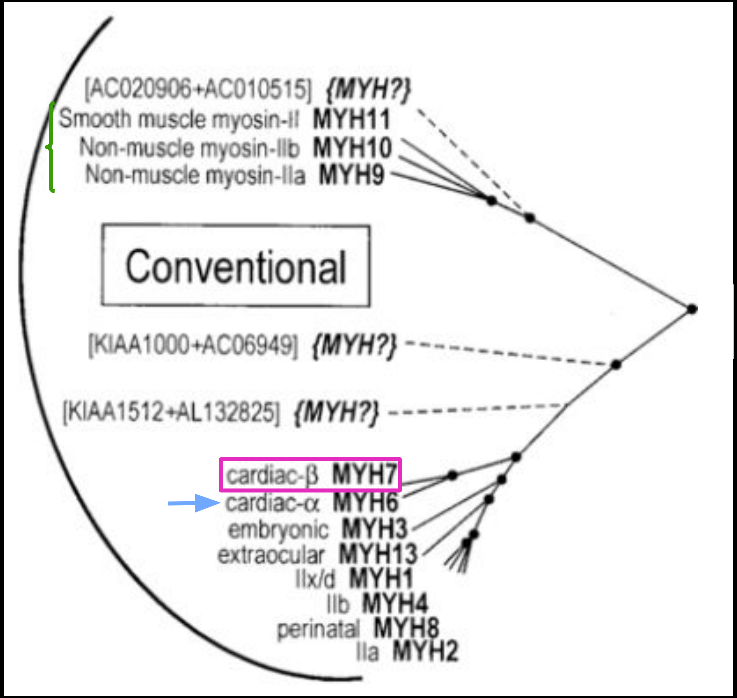
PIG

BOVINE

CONSERVATION OF MYOSIN BETWEEN FAMILIES



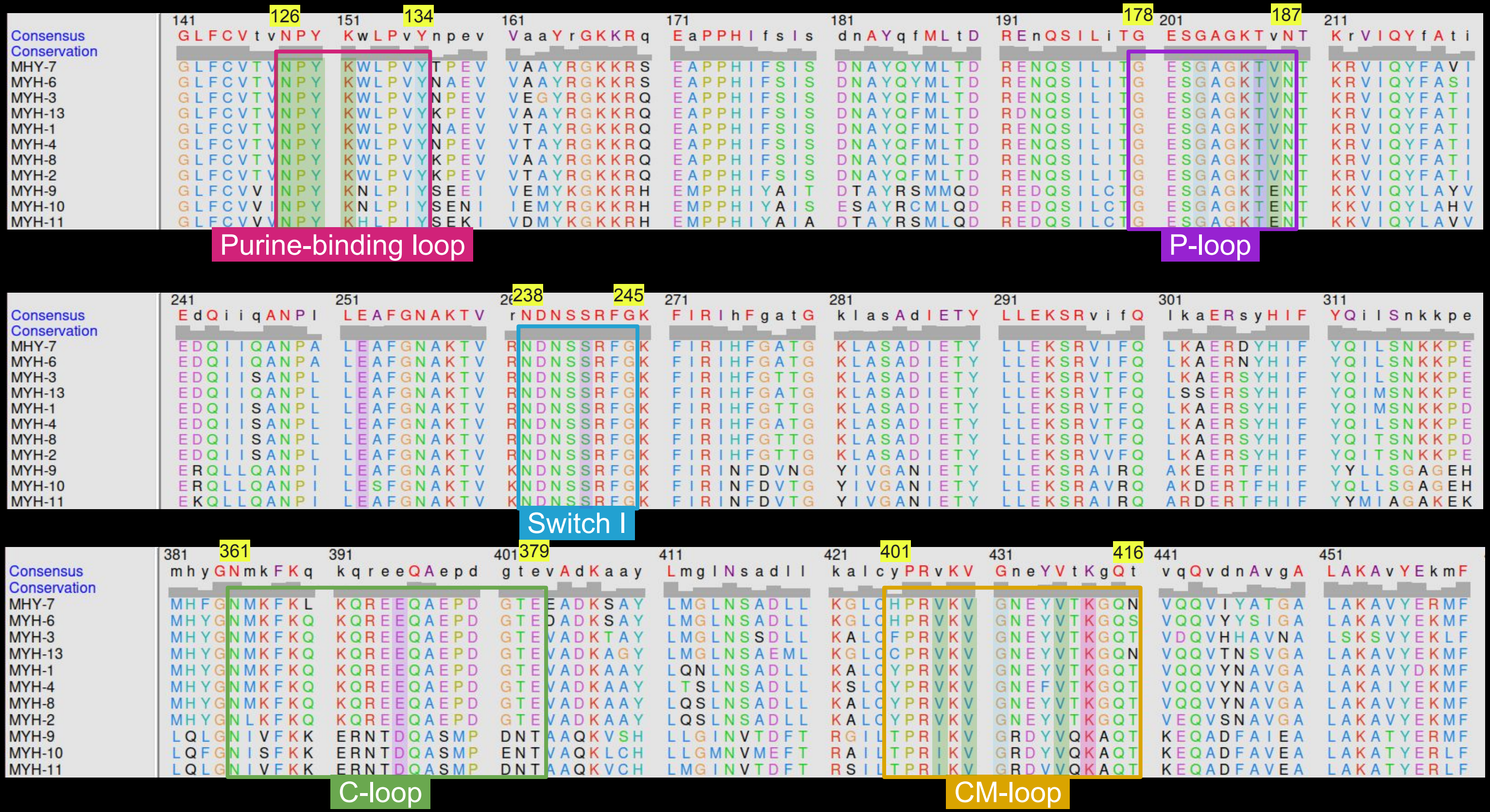
CONVENTIONAL MYOSINS OVERVIEW



Conventional myosins
Berg JS, Powell BC, Cheney RE. A Millenial Myosin Census. Molecular Biology of the Cell. 2001 Apr 1; 12(4): 780-794.

SEQUENCE ALIGNMENT

Hydrophobic interactions
Hydrogen bounds
Metal coordination
Salt bridges



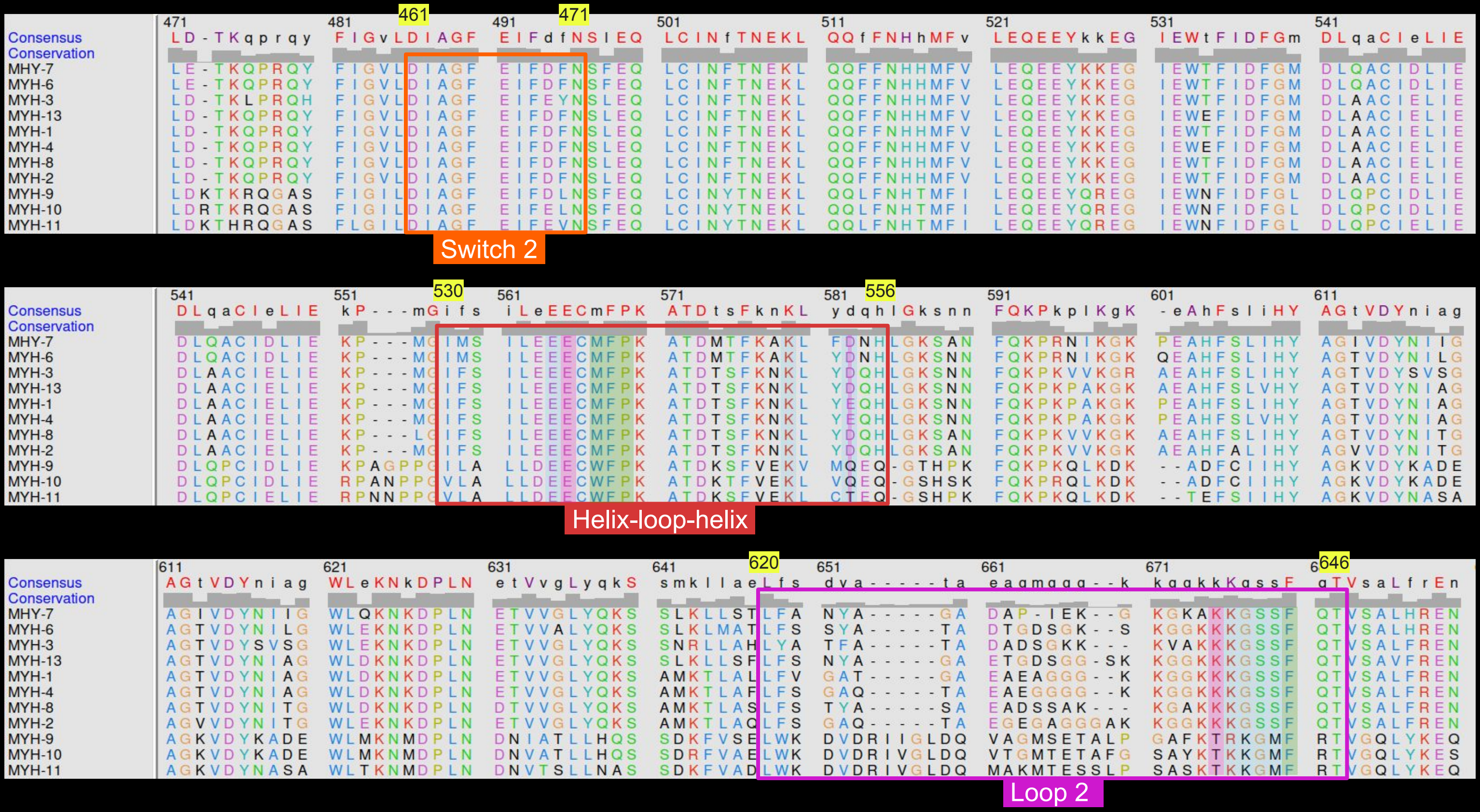
	141	151	161	171	181	191	201	211
Consensus	GLFCVtvNPY	KwLPvYnpev	VaaYrGKKRq	EaPPHIfsIs	dnAYqfMLtD	REnQSILiTG	ESGAGKTvNT	KrVIQYfAti
Conservation								
MHY-7	GLFCVTVNPY	KWLPVYTPEV	VAAYRGKKRS	EAPPHIFSIS	DNAYQYMLTD	RENQSILITG	ESGAGKTVNT	KRVIQYFAVI
MYH-6	GLFCVTVNPY	KWLPVYNAEV	VAAYRGKKRS	EAPPHIFSIS	DNAYQYMLTD	RENQSILITG	ESGAGKTVNT	KRVIQYFASI
MYH-3	GLFCVTVNPY	KWLPVYNPEV	VEGYRGKKRQ	EAPPHIFSIS	DNAYQFMLTD	RENQSILITG	ESGAGKTVNT	KRVIQYFATI
MYH-13	GLFCVTVNPY	KWLPVYKPEV	VAAYRGKKRQ	EAPPHIFSIS	DNAYQFMLTD	RDNQSILITG	ESGAGKTVNT	KRVIQYFATI
MYH-1	GLFCVTVNPY	KWLPVYNAEV	VTAYRGKKRQ	EAPPHIFSIS	DNAYQFMLTD	RENQSILITG	ESGAGKTVNT	KRVIQYFATI
MYH-4	GLFCVTVNPY	KWLPVYNPEV	VTAYRGKKRQ	EAPPHIFSIS	DNAYQFMLTD	RENQSILITG	ESGAGKTVNT	KRVIQYFATI
MYH-8	GLFCVTVNPY	KWLPVYKPEV	VAAYRGKKRQ	EAPPHIFSIS	DNAYQFMLTD	RENQSILITG	ESGAGKTVNT	KRVIQYFATI
MYH-2	GLFCVTVNPY	KWLPVYKPEV	VTAYRGKKRQ	EAPPHIFSIS	DNAYQFMLTD	RENQSILITG	ESGAGKTVNT	KRVIQYFATI
MYH-9	GLFCVVINPY	KNLPIYSEEI	VEMYKGKKRH	EMPPHIYAIT	DTAYRSMMQD	REDQSILCTG	ESGAGKTENT	KKVIQYLAYV
MYH-10	GLFCVVINPY	KNLPIYSENI	IEMYRGKKRH	EMPPHIYAIS	ESAYRCMLQD	REDQSILCTG	ESGAGKTENT	KKVIQYLAHV
MYH-11	GLFCVVVNPY	KHLPIYSEKI	VDMYKGKKRH	EMPPHIYAIA	DTAYRSMLQD	REDQSILCTG	ESGAGKTENT	KKVIQYLAVV

	241	251	26	271	281	291	301	311
Consensus	EdQiiqANPl	LEAFGNAKTV	rNDNSSRFGK	FIRIhFgatG	klasAdIETY	LLEKSRvifQ	lkaERsyHIF	YQilSnkkpe
Conservation								
MHY-7	EDQIIQANPA	LEAFGNAKTV	RNDNSSRFGK	FIRIHFGATG	KLASADIETY	LLEKSRVIFQ	LKAERDYHIF	YQILSNKKPE
MYH-6	EDQIIQANPA	LEAFGNAKTV	RNDNSSRFGK	FIRIHFGATG	KLASADIETY	LLEKSRVIFQ	LKAERNYHIF	YQILSNKKPE
MYH-3	EDQIISANPL	LEAFGNAKTV	RNDNSSRFGK	FIRIHFGTTG	KLASADIETY	LLEKSRVTFQ	LKAERSYHIF	YQILSNKKPE
MYH-13	EDQIIQANPL	LEAFGNAKTV	RNDNSSRFGK	FIRIHFGATG	KLASADIETY	LLEKSRVTFQ	LSSERSYHIF	YQIMSNKKPE
MYH-1	EDQIISANPL	LEAFGNAKTV	RNDNSSRFGK	FIRIHFGTTG	KLASADIETY	LLEKSRVTFQ	LKAERSYHIF	YQIMSNKKPD
MYH-4	EDQIISANPL	LEAFGNAKTV	RNDNSSRFGK	FIRIHFGATG	KLASADIETY	LLEKSRVTFQ	LKAERSYHIF	YQILSNKKPE
MYH-8	EDQIISANPL	LEAFGNAKTV	RNDNSSRFGK	FIRIHFGTTG	KLASADIETY	LLEKSRVTFQ	LKAERSYHIF	YQITSNKKPD
MYH-2	EDQIISANPL	LEAFGNAKTV	RNDNSSRFGK	FIRIHFGTTG	KLASADIETY	LLEKSRVVFQ	LKAERSYHIF	YQITSNKKPE
MYH-9	ERQLLQANPI	LEAFGNAKTV	KNDNSSRFGK	FIRINFDVNG	YIVGANIETY	LLEKSRAIRQ	AKEERTFHIF	YYLLSGAGEH
MYH-10	ERQLLQANPI	LESFGNAKTV	KNDNSSRFGK	FIRINFDVTG	YIVGANIETY	LLEKSRAVRQ	AKDERTFHIF	YQLLSGAGEH
MYH-11	EKQLLQANPI	LEAFGNAKTV	KNDNSSRFGK	FIRINFDVTG	YIVGANIETY	LLEKSRAIRQ	ARDERTFHIF	YYMIAGAKEK

	381	391	401	411	421	431	441	451
Consensus	mhyGNmkFKq	kqreeQAepd	gtevAdKaay	LmglNsadll	kalcyPRvKV	GneYVtKgQt	vqQvdnAvgA	LAKAvYEkmF
Conservation								
MHY-7	MHFGNMKFKL	KQREEQAEPD	GTEEADKSAY	LMGLNSADLL	KGLCHPRVKV	GNEYVTKGQN	VQQVIYATGA	LAKAVYERMF
MYH-6	MHYGNMKFKQ	KQREEQAEPD	GTEDADKSAY	LMGLNSADLL	KGLCHPRVKV	GNEYVTKGQS	VQQVYYSIGA	LAKAVYEKMF
MYH-3	MHYGNMKFKQ	KQREEQAEPD	GTEVADKTAY	LMGLNSSDLL	KALCFPRVKV	GNEYVTKGQT	VDQVHHAVNA	LSKSVYEKLF
MYH-13	MHYGNMKFKQ	KQREEQAEPD	GTEVADKAGY	LMGLNSAEML	KGLCCPRVKV	GNEYVTKGQN	VQQVTNSVGA	LAKAVYEKMF
MYH-1	MHYGNMKFKQ	KQREEQAEPD	GTEVADKAAY	LQNLNSADLL	KALCYPRVKV	GNEYVTKGQT	VQQVYNAVGA	LAKAVYDKMF
MYH-4	MHYGNMKFKQ	KQREEQAEPD	GTEVADKAAY	LTSLNSADLL	KSLCYPRVKV	GNEFVTKGQT	VQQVYNAVGA	LAKAIYEKMF
MYH-8	MHYGNMKFKQ	KQREEQAEPD	GTEVADKAAY	LQSLNSADLL	KALCYPRVKV	GNEYVTKGQT	VQQVYNAVGA	LAKAVYEKMF
MYH-2	MHYGNLKFKQ	KQREEQAEPD	GTEVADKAAY	LQSLNSADLL	KALCYPRVKV	GNEYVTKGQT	VEQVSNAVGA	LAKAVYEKMF
MYH-9	LQLGNIVFKK	ERNTDQASMP	DNTAAQKVSH	LLGINVTDFT	RGILTPRIKV	GRDYVQKAQT	KEQADFAIEA	LAKATYERMF
MYH-10	LQFGNISFKK	ERNTDQASMP	ENTVAQKLCH	LLGMNVMEFT	RAILTPRIKV	GRDYVQKAQT	KEQADFAVEA	LAKATYERLF
MYH-11	LQLGNIVFKK	ERNTDQASMP	DNTAAQKVCH	LMGINVTDFT	RSILTPRIKV	GRDVVQKAQT	KEQADFAVEA	LAKATYERLF

SEQUENCE ALIGNMENT

Hydrophobic interactions
Hydrogen bounds
Metal coordination
Salt bridges

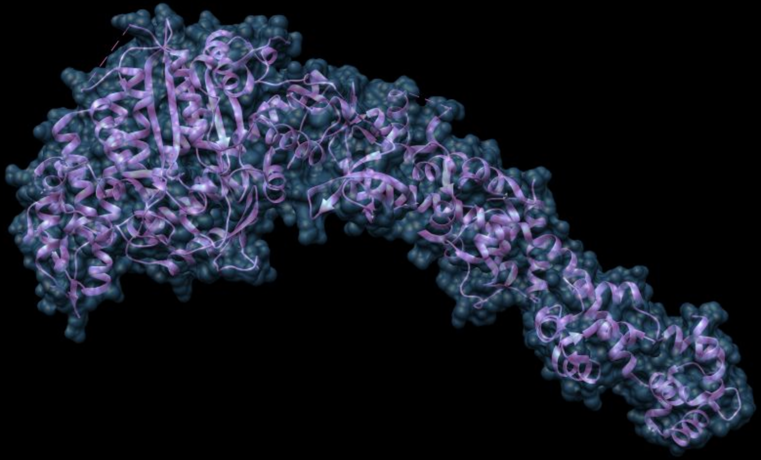


	471	481	491	501	511	521	531	541
Consensus	LD-TKqprqy	FIGvLDIAGF	EIFdfNSlEQ	LCINfTNEKL	QQfFNHhMFv	LEQEEYkkEG	IEWtFIDFGm	DLqaCIeLIE
Conservation								
MHY-7	LE-TKQPRQY	FIGVLDIAGF	EIFDFNSFEQ	LCINFTNEKL	QQFFNHHMFV	LEQEEYKKEG	IEWTFIDFGM	DLQACIDLIE
MYH-6	LE-TKQPRQY	FIGVLDIAGF	EIFDFNSFEQ	LCINFTNEKL	QQFFNHHMFV	LEQEEYKKEG	IEWTFIDFGM	DLQACIDLIE
MYH-3	LD-TKLPRQH	FIGVLDIAGF	EIFEYNSLEQ	LCINFTNEKL	QQFFNHHMFV	LEQEEYKKEG	IEWTFIDFGM	DLAACIELIE
MYH-13	LD-TKQPRQY	FIGVLDIAGF	EIFDFNSLEQ	LCINFTNEKL	QQFFNHHMFV	LEQEEYKKEG	IEWEFIDFGM	DLAACIELIE
MYH-1	LD-TKQPRQY	FIGVLDIAGF	EIFDFNSLEQ	LCINFTNEKL	QQFFNHHMFV	LEQEEYKKEG	IEWTFIDFGM	DLAACIELIE
MYH-4	LD-TKQPRQY	FIGVLDIAGF	EIFDFNSLEQ	LCINFTNEKL	QQFFNHHMFV	LEQEEYKKEG	IEWEFIDFGM	DLAACIELIE
MYH-8	LD-TKQPRQY	FIGVLDIAGF	EIFDFNSLEQ	LCINFTNEKL	QQFFNHHMFV	LEQEEYKKEG	IEWTFIDFGM	DLAACIELIE
MYH-2	LD-TKQPRQY	FIGVLDIAGF	EIFDFNSLEQ	LCINFTNEKL	QQFFNHHMFV	LEQEEYKKEG	IEWTFIDFGM	DLAACIELIE
MYH-9	LDKTKRQGAS	FIGILDIAGF	EIFDLNSFEQ	LCINYTNEKL	QQLFNHTMFI	LEQEEYQREG	IEWNFIDFGL	DLQPCIDLIE
MYH-10	LDRTKRQGAS	FIGILDIAGF	EIFELNSFEQ	LCINYTNEKL	QQLFNHTMFI	LEQEEYQREG	IEWNFIDFGL	DLQPCIDLIE
MYH-11	LDKTHRQGAS	FLGILDIAGF	EIFEVNSFEQ	LCINYTNEKL	QQLFNHTMFI	LEQEEYQREG	IEWNFIDFGL	DLQPCIELIE

	541	551	561	571	581	591	601	611
Consensus	DLqaCIeLIE	kP---mGifs	iLeEECmFPK	ATDtsFknKL	ydqhlGksnn	FQKPkplKgK	-eAhFsliHY	AGtVDYniag
Conservation								
MHY-7	DLQACIDLIE	KP---MGIMS	ILEEECMFPK	ATDMTFKAKL	FDNHLGKSAN	FQKPRNIKGK	PEAHFSLIHY	AGIVDYNIIG
MYH-6	DLQACIDLIE	KP---MGIMS	ILEEECMFPK	ATDMTFKAKL	YDNHLGKSNN	FQKPRNIKGK	QEAHFSLIHY	AGTVDYNILG
MYH-3	DLAACIELIE	KP---MGIFS	ILEEECMFPK	ATDTSFKNKL	YDQHLGKSNN	FQKPKVVKGR	AEAHFSLIHY	AGTVDYSVSG
MYH-13	DLAACIELIE	KP---MGIFS	ILEEECMFPK	ATDTSFKNKL	YDQHLGKSNN	FQKPKPAKGK	AEAHFSLVHY	AGTVDYNIAG
MYH-1	DLAACIELIE	KP---MGIFS	ILEEECMFPK	ATDTSFKNKL	YEQHLGKSNN	FQKPKPAKGK	PEAHFSLIHY	AGTVDYNIAG
MYH-4	DLAACIELIE	KP---MGIFS	ILEEECMFPK	ATDTSFKNKL	YEQHLGKSNN	FQKPKPAKGK	PEAHFSLVHY	AGTVDYNIAG
MYH-8	DLAACIELIE	KP---LGIFS	ILEEECMFPK	ATDTSFKNKL	YDQHLGKSAN	FQKPKVVKGK	AEAHFSLIHY	AGTVDYNITG
MYH-2	DLAACIELIE	KP---MGIFS	ILEEECMFPK	ATDTSFKNKL	YDQHLGKSAN	FQKPKVVKGK	AEAHFALIHY	AGVVDYNITG
MYH-9	DLQPCIDLIE	KPAGPPGILA	LLDEECWFPK	ATDKSFVEKV	MQEQ-GTHPK	FQKPKQLKDK	--ADFCIIHY	AGKVDYKADE
MYH-10	DLQPCIDLIE	RPANPPGVLA	LLDEECWFPK	ATDKTFVEKL	VQEQ-GSHSK	FQKPRQLKDK	--ADFCIIHY	AGKVDYKADE
MYH-11	DLQPCIELIE	RPNNPPGVLA	LLDEECWFPK	ATDKSFVEKL	CTEQ-GSHPK	FQKPKQLKDK	--TEFSIIHY	AGKVDYNASA

	611	621	631	641	651	661	671	6
Consensus	AGtVDYniag	WLeKNkDPLN	etVvgLyqkS	smkllaeLfs	dva-----ta	eagmggg--k	kggkkKgssF	qTVsaLfrEn
Conservation								
MHY-7	AGIVDYNIIG	WLQKNKDPLN	ETVVGLYQKS	SLKLLSTLFA	NYA-----GA	DAP-IEK--G	KGKAKKGSSF	QTVSALHREN
MYH-6	AGTVDYNILG	WLEKNKDPLN	ETVVALYQKS	SLKLMATLFS	SYA-----TA	DTGDSGK--S	KGGKKKGSSF	QTVSALHREN
MYH-3	AGTVDYSVSG	WLEKNKDPLN	ETVVGLYQKS	SNRLLAHLYA	TFA-----TA	DADSGKK---	KVAKKKGSSF	QTVSALFREN
MYH-13	AGTVDYNIAG	WLDKNKDPLN	ETVVGLYQKS	SLKLLSFLFS	NYA-----GA	ETGDSGG-SK	KGGKKKGSSF	QTVSAVFREN
MYH-1	AGTVDYNIAG	WLDKNKDPLN	ETVVGLYQKS	AMKTLALLFV	GAT-----GA	EAEAGGG--K	KGGKKKGSSF	QTVSALFREN
MYH-4	AGTVDYNIAG	WLDKNKDPLN	ETVVGLYQKS	AMKTLAFLFS	GAQ-----TA	EAEGGGG--K	KGGKKKGSSF	QTVSALFREN
MYH-8	AGTVDYNITG	WLDKNKDPLN	DTVVGLYQKS	AMKTLASLFS	TYA-----SA	EADSSAK---	KGAKKKGSSF	QTVSALFREN
MYH-2	AGVVDYNITG	WLEKNKDPLN	ETVVGLYQKS	AMKTLAQLFS	GAQ-----TA	EGEGAGGGAK	KGGKKKGSSF	QTVSALFREN
MYH-9	AGKVDYKADE	WLMKNMDPLN	DNIATLLHQS	SDKFVSELWK	DVDRIIGLDQ	VAGMSETALP	GAFKTRKGMF	RTVGQLYKEQ
MYH-10	AGKVDYKADE	WLMKNMDPLN	DNVATLLHQS	SDRFVAELWK	DVDRIVGLDQ	VTGMTETAFG	SAYKTKKGMF	RTVGQLYKES
MYH-11	AGKVDYNASA	WLTKNMDPLN	DNVTSLLNAS	SDKFVADLWK	DVDRIVGLDQ	MAKMTESSLP	SASKTKKGMF	RTVGQLYKEQ

CONCLUSIONS



CONCLUSIONS

1. Myosins are a very large superfamily of motor proteins involved in several functions (not limited to muscle contraction).
2. To carry out their functions, they attach to actin and a nucleotide.
3. The contraction cycle involves different conformational states and interactions so it is very dynamic and complex.
4. Myosin has the ability to efficiently couple small changes in its catalytic ATPase site to large conformational changes in both the actin-binding and the distant force-generating domains.
5. Our sequence and structural analysis indicates that both actin and nucleotide binding regions are highly conserved among eukaryotic organisms and conventional myosins.

PEM QUESTIONS

Regarding the myosin superfamily, which sentence is true?

a) All myosins have one essential light chain and one regulatory light chain.
b) Myosins are only present in muscle cells.
c) A and B are correct.
d) The motor domain belongs to the P-loop containing nucleoside triphosphate hydrolases fold.
e) All of them are correct.

Mark the correct answer:

a) After doing the structure alignment, it can be affirmed that the human beta cardiac myosin is more similar to the bovine protein than the pig one.
b) Myosins present high conservation between species.
c) Actin interacts with loop 2, c-loop, HLH motif and lever arm of myosin.
d) There are not any salt bridges between actin and myosin.
e) An important hydrogen bond is present between M539 of the HLH motifs and F644 of Loop 2.

The second powerstroke step consists of:

a) ATP hydrolysis
b) Inorganic phosphate release
c) ADP hydrolysis
d) ADP release
e) ATP union

PEM QUESTIONS

Myosin binding to actin is important for:

a) Muscle contraction
b) Cell division
c) A and B are true
d) Cell motility
e) All the above are true

What subdomain of the beta cardiac myosin performs a 70º rotation during the powerstroke:

a) Lever arm
b) Converter
c) Upper 50kDa
d) Lower 50kDa
e) Rotator

How many beta strands is the myosin converter composed of:

a) 5
b) 6
c) 7
d) 8
e) 9

PEM QUESTIONS

Which structures form the nucleotide-binding site of myosins:

a) Switch 1, switch 2, P-loop and purine-binding loop
b) Switch 1, switch 2, P-loop and c-loop
c) Switch 1, P-loop, HLH motif and c-loop
d) P-loop, purine-binding loop, HLH motif and c-loop
e) P-loop, purine-binding loop, CM loop and HLH motif

The interactions between the nucleotide-binding site and the magnesium ion are:

a) Hydrogen bonds
b) Metal coordinations
c) A and B are true
d) Salt bridges
e) All the above are true

Choose the incorrect statement:

a) According to SCOP myosin light chains belong to the calmodulin-like family.
b) The lever arm connects the motor domain to the tail domain of myosins.
c) ADP alpha and beta phosphates are stabilized in the nucleotide-binding site with hydrophobic contacts.
d) The CM loop is involved in transmitting conformational changes during the myosin ATPase cycle.

e) The HLH motif consists of two alpha helices connected by a loop region. Each alpha helix is approximately 20 amino acids long.

PEM QUESTIONS

What state goes after ADP release in the myosin ATPase cycle:

a) Pi release state
b) ADP state
c) Pre-powerstroke state
d) Rigor state
e) Non is correct

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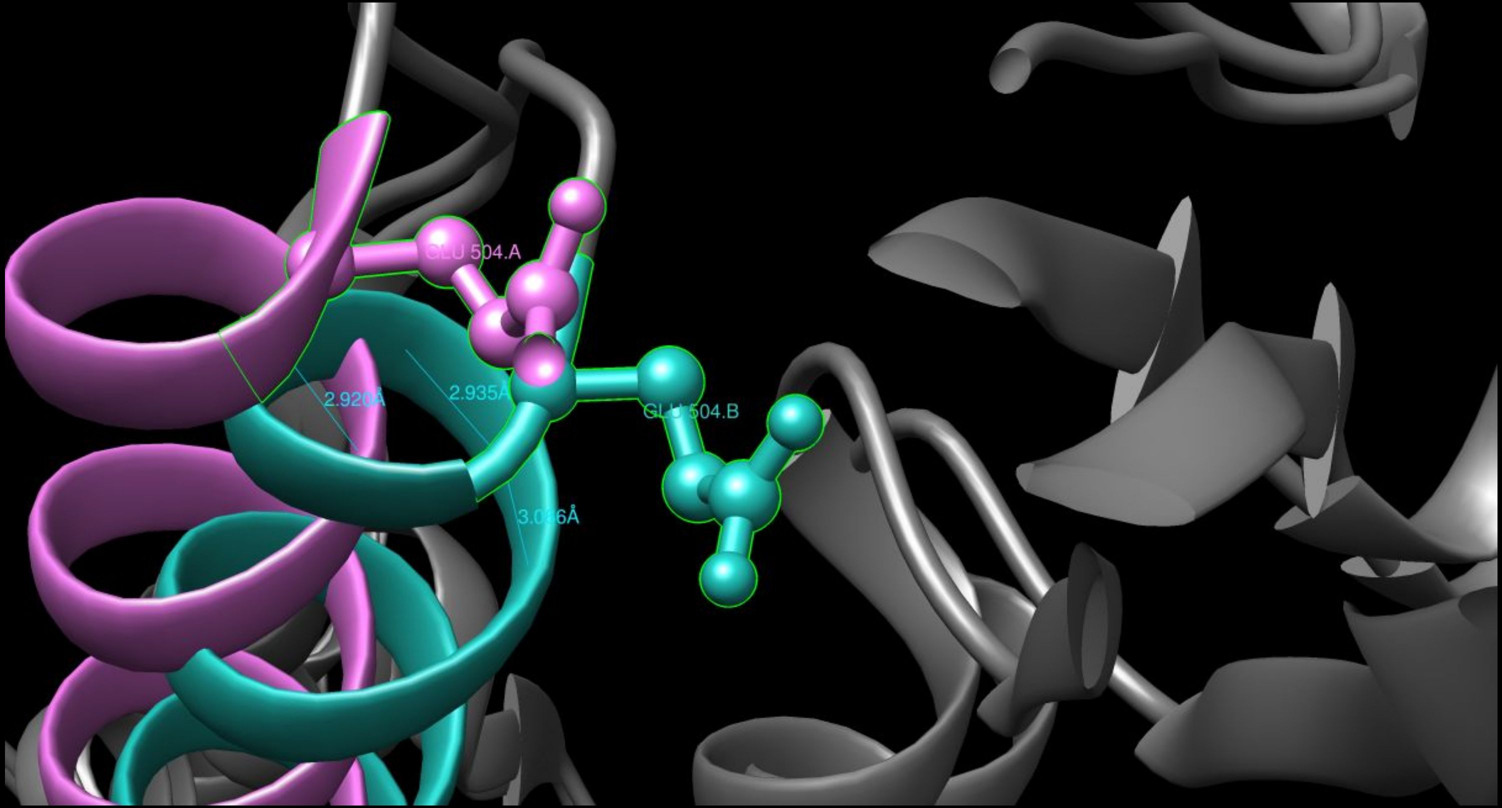
THANKS FOR YOUR ATTENTION

BACKUP SLIDES

Backup slides



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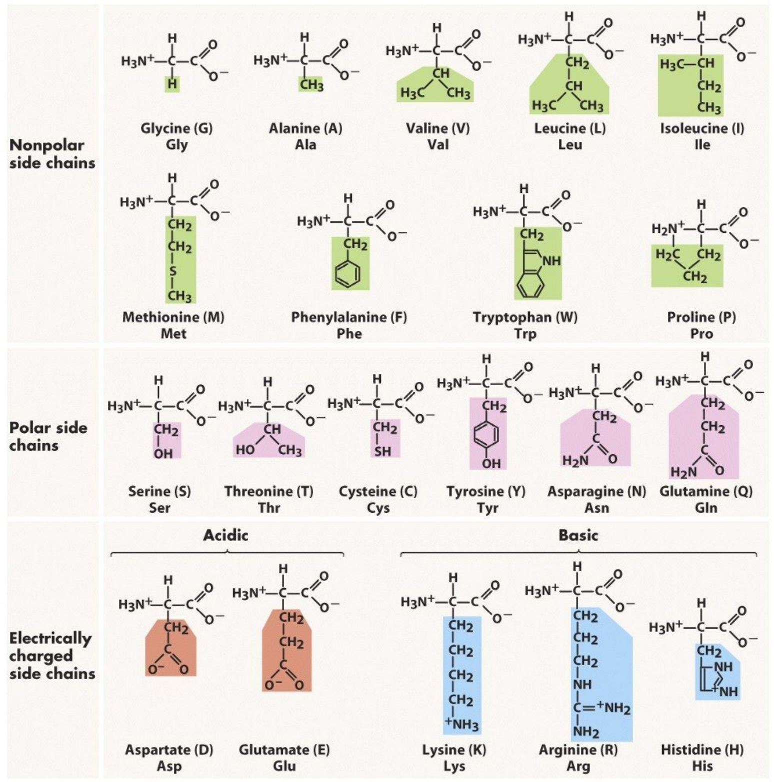

Figure 3-5 Biological Science, 2/e

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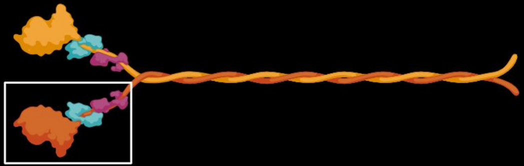
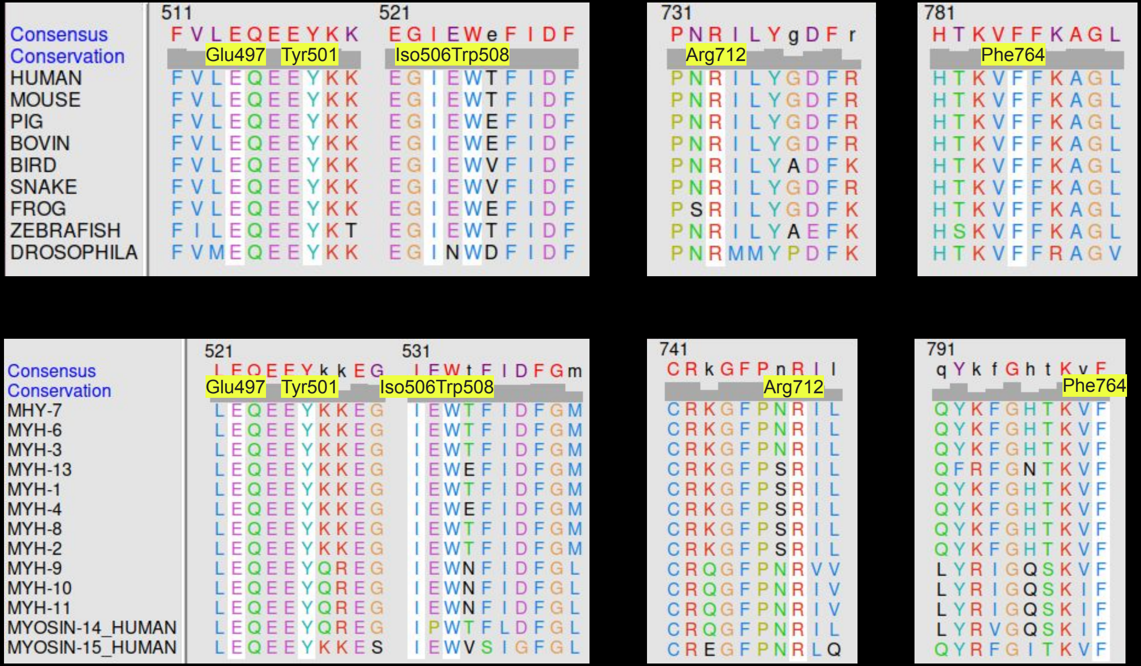


ESSENTIAL LIGHT CHAIN (ELC)	REGULATORY LIGHT CHAIN (RLC)
- Involved in stabilizing the myosin motor domain, it helps in maintaining the structural integrity of myosin head. - Plays a role in the regulation of myosin's enzymatic activity. It can modulate the speed and force of muscle contraction by influencing the kinetics of the myosin ATPase activity.	- Involved in the regulation of myosin activity through phosphorylation. - Phosphorylation of the RLC by specific kinases can activate the myosin motor activity → RLC phosphorylation promotes a fluctuation of myosin heads, facilitating weak-to-strong cross-linking of actin. - The level of phosphorylation can be modulated in response to various cellular signals, allowing for precise control of muscle contraction and relaxation.

Backup slides



	511	521	731	781
Consensus	F V L E Q E E Y K K	E G I E W e F I D F	P N R I L Y g D F r	H T K V F F K A G L
Conservation	Glu497 Tyr501	Iso506Trp508	Arg712	Phe764
HUMAN	F V L E Q E E Y K K	E G I E W T F I D F	P N R I L Y G D F R	H T K V F F K A G L
MOUSE	F V L E Q E E Y K K	E G I E W T F I D F	P N R I L Y G D F R	H T K V F F K A G L
PIG	F V L E Q E E Y K K	E G I E W E F I D F	P N R I L Y G D F R	H T K V F F K A G L
BOVIN	F V L E Q E E Y K K	E G I E W E F I D F	P N R I L Y G D F R	H T K V F F K A G L
BIRD	F V L E Q E E Y K K	E G I E W V F I D F	P N R I L Y A D F K	H T K V F F K A G L
SNAKE	F V L E Q E E Y K K	E G I E W V F I D F	P N R I L Y G D F R	H T K V F F K A G L
FROG	F V L E Q E E Y K K	E G I E W E F I D F	P S R I L Y G D F K	H T K V F F K A G L
ZEBRAFISH	F I L E Q E E Y K T	E G I E W T F I D F	P N R I L Y A E F K	H S K V F F K A G L
DROSOPHILA	F V M E Q E E Y K K	E G I N W D F I D F	P N R M M Y P D F K	H T K V F F R A G V

	521	531	741	791
Consensus	L E Q E E Y k k E G	I E W t F I D F G m	C R k G F P n R I l	q Y k f G h t K v F
Conservation	Glu497 Tyr501	Iso506Trp508	Arg712	Phe764
MHY-7	L E Q E E Y K K E G	I E W T F I D F G M	C R K G F P N R I L	Q Y K F G H T K V F
MYH-6	L E Q E E Y K K E G	I E W T F I D F G M	C R K G F P N R I L	Q Y K F G H T K V F
MYH-3	L E Q E E Y K K E G	I E W T F I D F G M	C R K G F P N R I L	Q Y K F G H T K V F
MYH-13	L E Q E E Y K K E G	I E W E F I D F G M	C R K G F P S R I L	Q F R F G N T K V F
MYH-1	L E Q E E Y K K E G	I E W T F I D F G M	C R K G F P S R I L	Q Y K F G H T K V F
MYH-4	L E Q E E Y K K E G	I E W E F I D F G M	C R K G F P S R I L	Q Y K F G H T K V F
MYH-8	L E Q E E Y K K E G	I E W T F I D F G M	C R K G F P S R I L	Q Y K F G H T K V F
MYH-2	L E Q E E Y K K E G	I E W T F I D F G M	C R K G F P S R I L	Q Y K F G H T K V F
MYH-9	L E Q E E Y Q R E G	I E W N F I D F G L	C R Q G F P N R V V	L Y R I G Q S K V F
MYH-10	L E Q E E Y Q R E G	I E W N F I D F G L	C R Q G F P N R I V	L Y R I G Q S K I F
MYH-11	L E Q E E Y Q R E G	I E W N F I D F G L	C R Q G F P N R I V	L Y R I G Q S K I F
MYOSIN-14_HUMAN	L E Q E E Y Q R E G	I P W T F L D F G L	C R Q G F P N R I L	L Y R V G Q S K I F
MYOSIN-15_HUMAN	L E Q E E Y K K E S	I E W V S I G F G L	C R E G F P N R L Q	Q Y R F G I T K V F

Backup slides