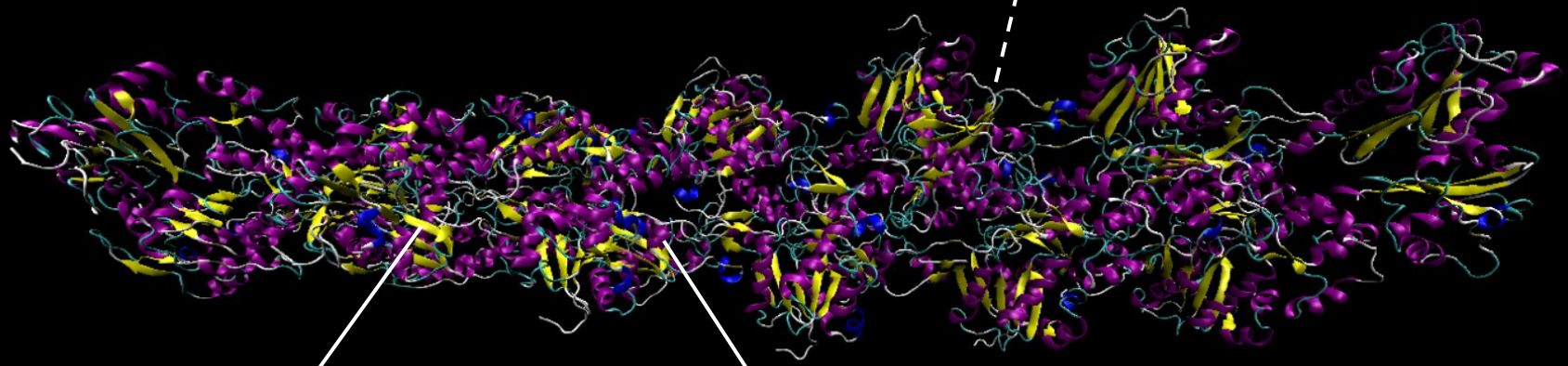
ACTIN FILAMENT



Universitat Pompeu Fabra

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Anna Castellet
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Structural Biology
March 2022

Introduction: Actin

- Most abundant protein in eukaryotic cells
- Highly conserved
- Transition between G-actin and F-actin
- Involved in: cellular movement, cytoskeleton conformation, cytokinesis, cell adhesion, cell signalling, muscle contraction…

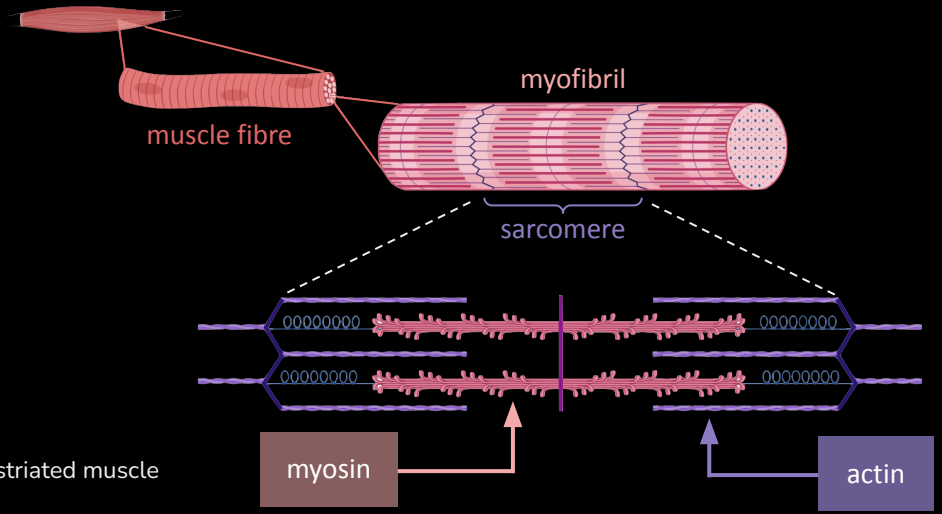
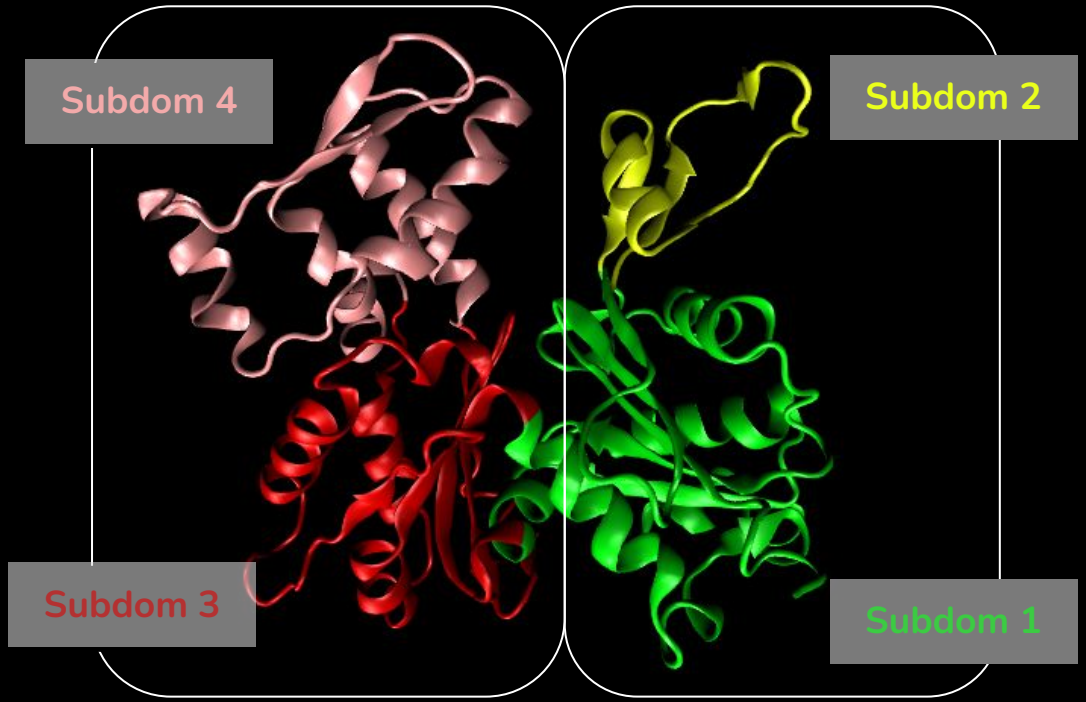


Fig. Structure of striated muscle
Own source

G-ACTIN

G-Actin



375 residues ≃ 42 kDa

PDB ID: 1ATN
Resolution: 2.80 Å

class 1000002
α and β proteins (a/b)

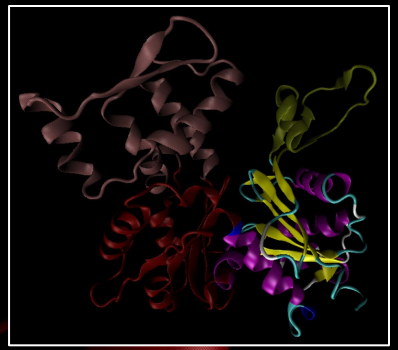
fold 2000067
ribonuclease H-like motif

superfamily 3000092
actin-like ATPases

family 4000313
actin/HSP70

G-Actin: Subdomain 1

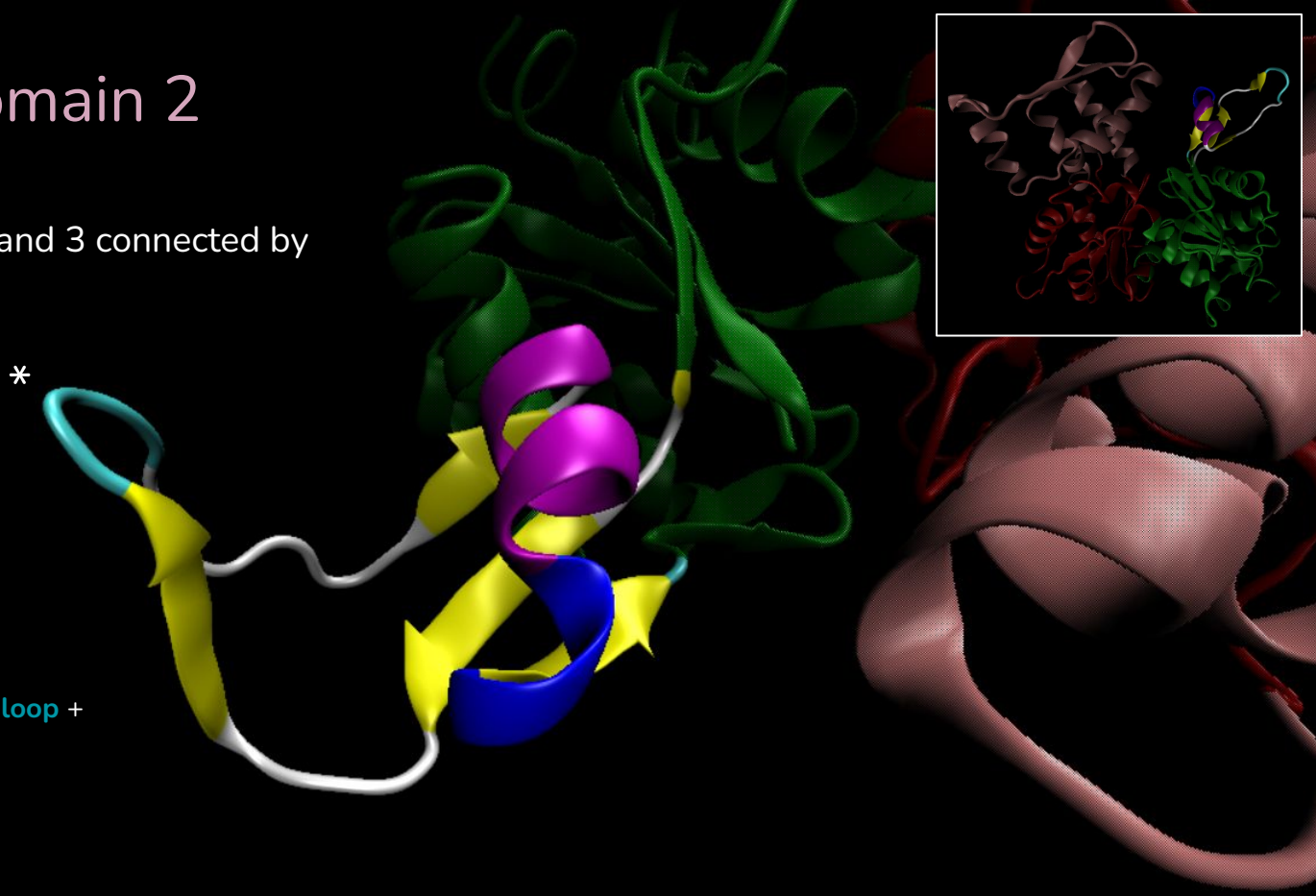
Three-layered sandwich
5 β-strands surrounded by
5 α-helices



PDB ID: 1ATN
Resolution: 2.80 Å

G-Actin: Subdomain 2

α/β domain
3 antiparallel β-strands, 1 and 3 connected by
1 α-helix

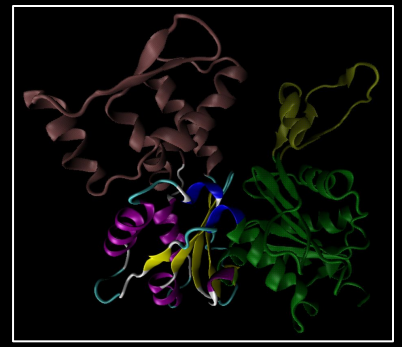


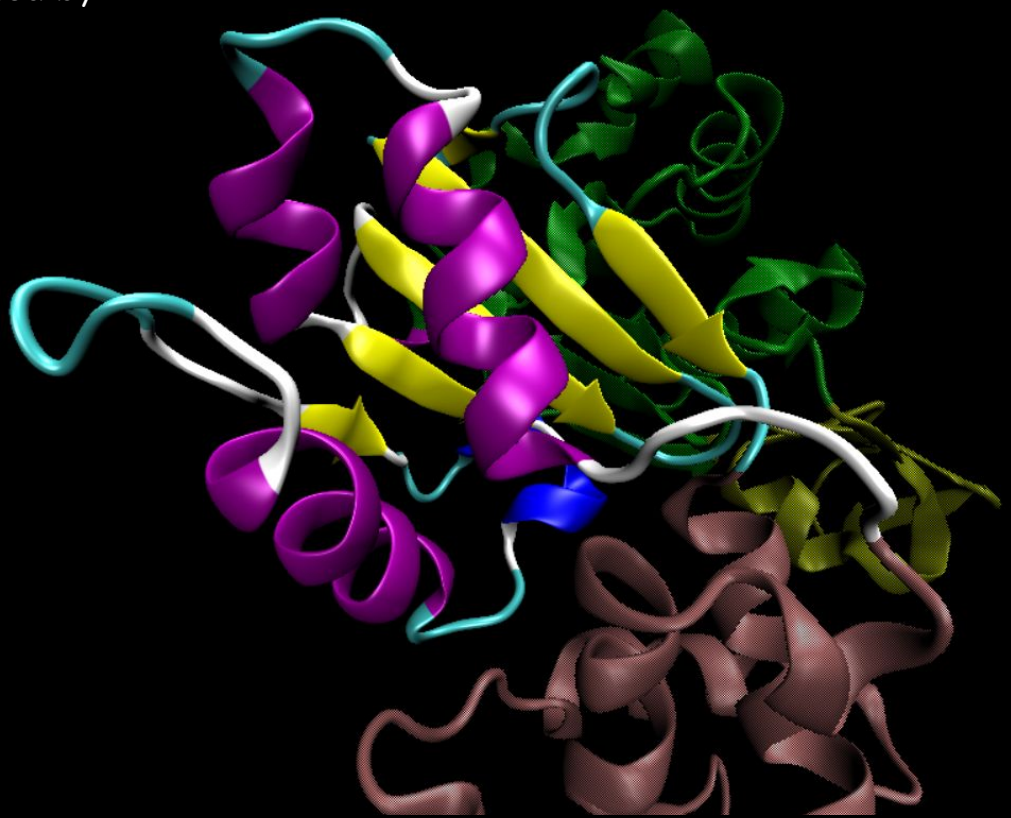
* β-strands 1 and 2 connected by a **loop** +
extra **β-strand**

PDB ID: 1ATN
Resolution: 2.80 Å

G-Actin: Subdomain 3

Two-layered sandwich
5 β-strands surrounded by
3 α-helices

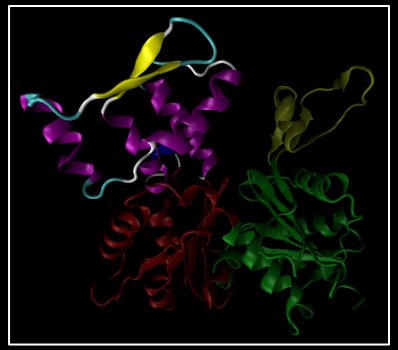




PDB ID: 1ATN
Resolution: 2.80 Å

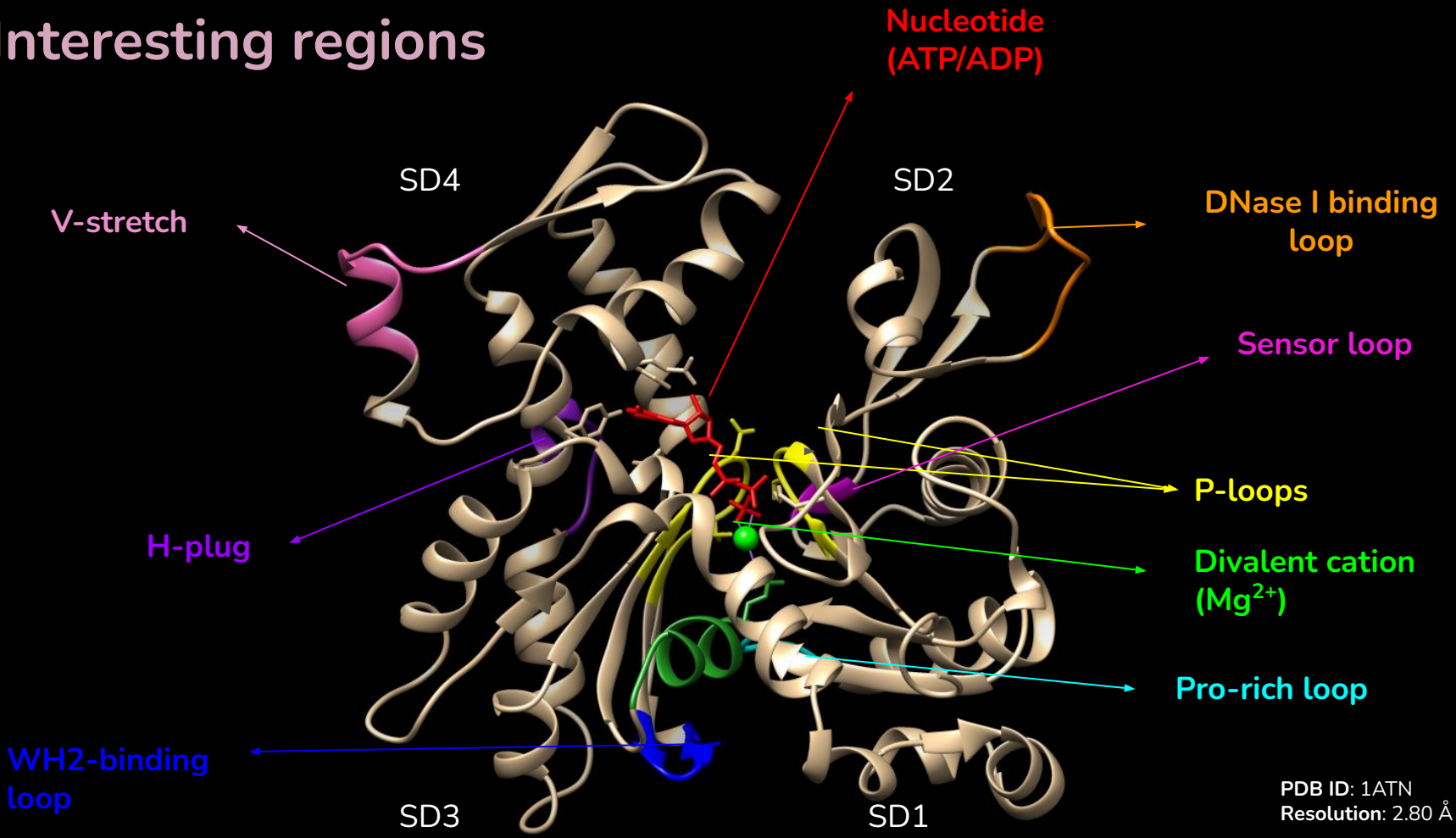
G-Actin: Subdomain 4

α/β domain
2 antiparallel β-strands and
4 α-helices



PDB ID: 1ATN
Resolution: 2.80 Å

Interesting regions



PDB ID: 1ATN
Resolution: 2.80 Å

Interesting regions

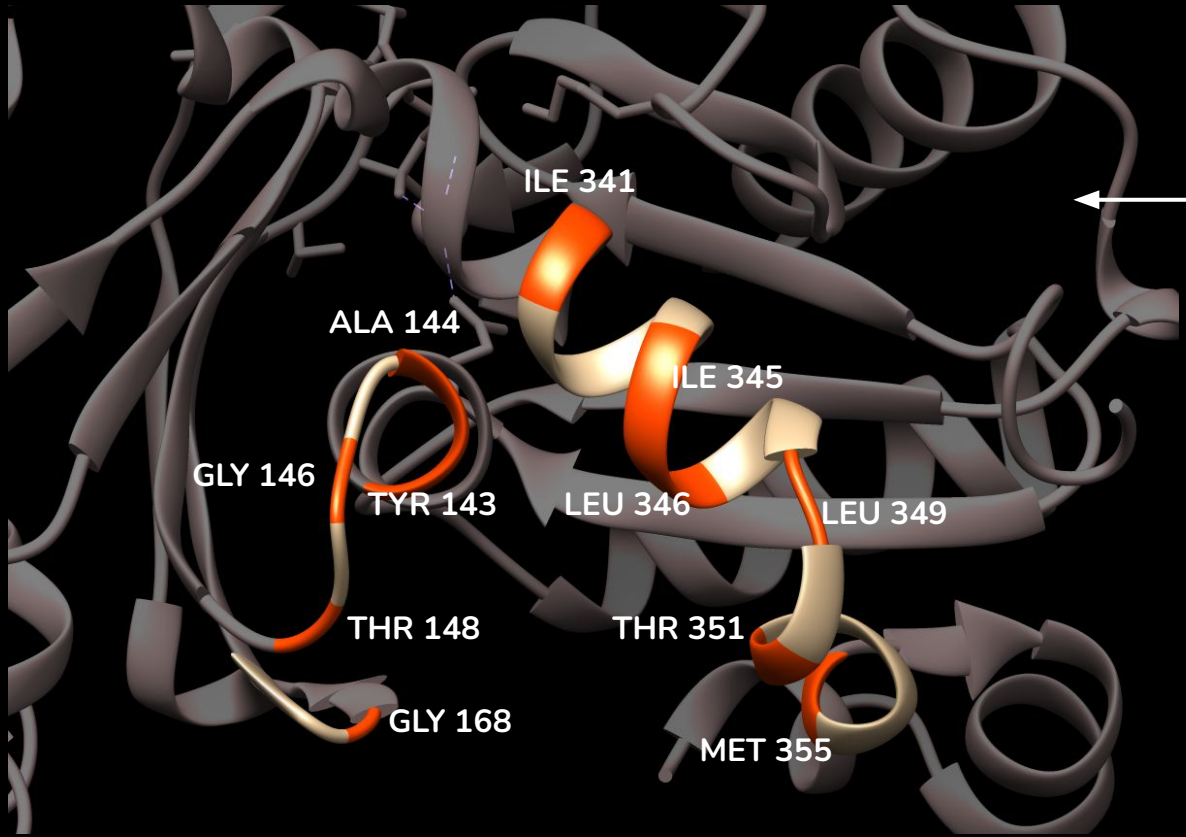
Nucleotide binding cleft

Hinge region

Target binding cleft
(ABPs, small macrolide toxins)

PDB ID: 1ATN
Resolution: 2.80 Å

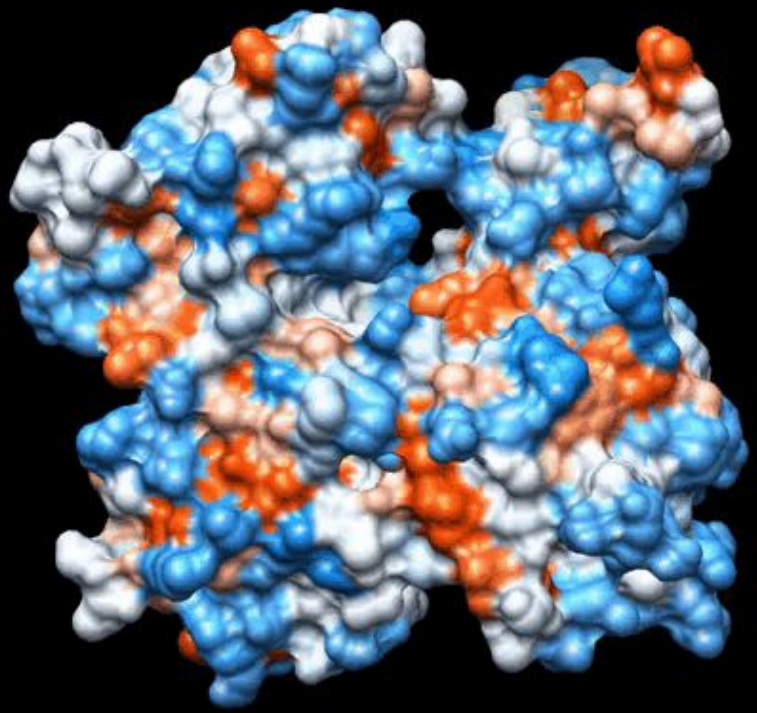
Interesting regions: Target binding site

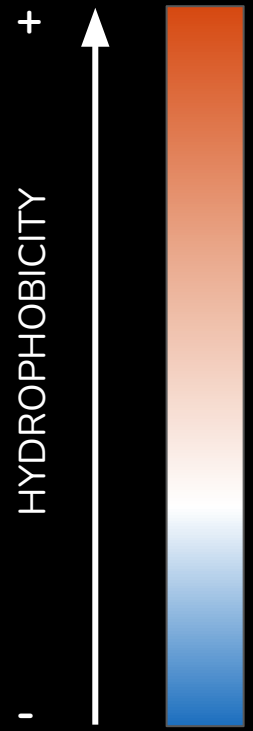


Target binding site or
hydrophobic cleft

PDB ID: 1ATN
Resolution: 2.80 Å

Interesting regions: Hydrophobic residues

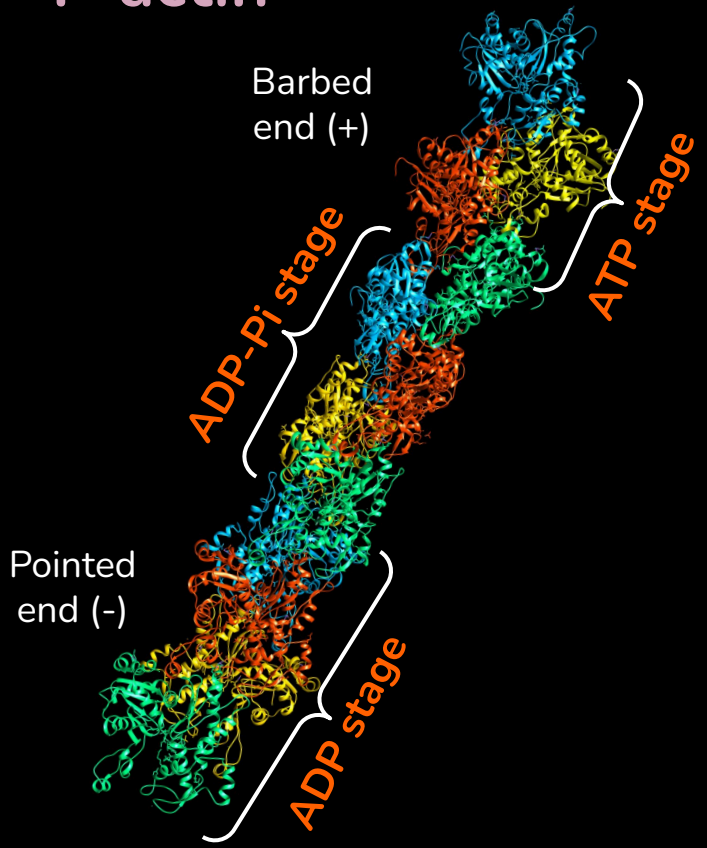




PDB ID: 1ATN
Resolution: 2.80 Å

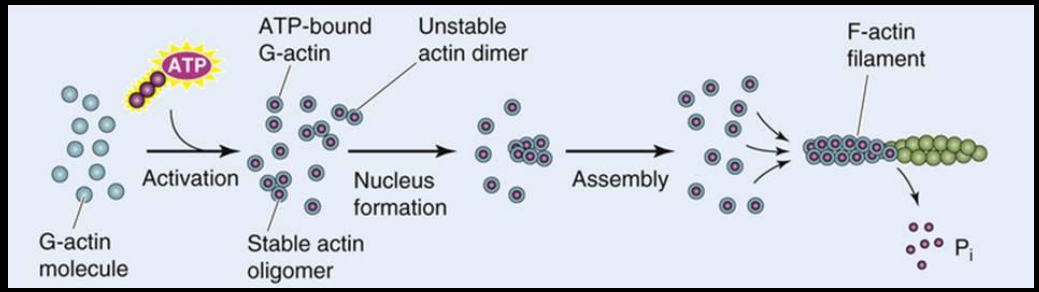
G-ACTIN TO F-ACTIN

F-actin



PDB ID: 3G37
Resolution: 6 Å

A. Formation of F-actin



B. Treadmilling reaction

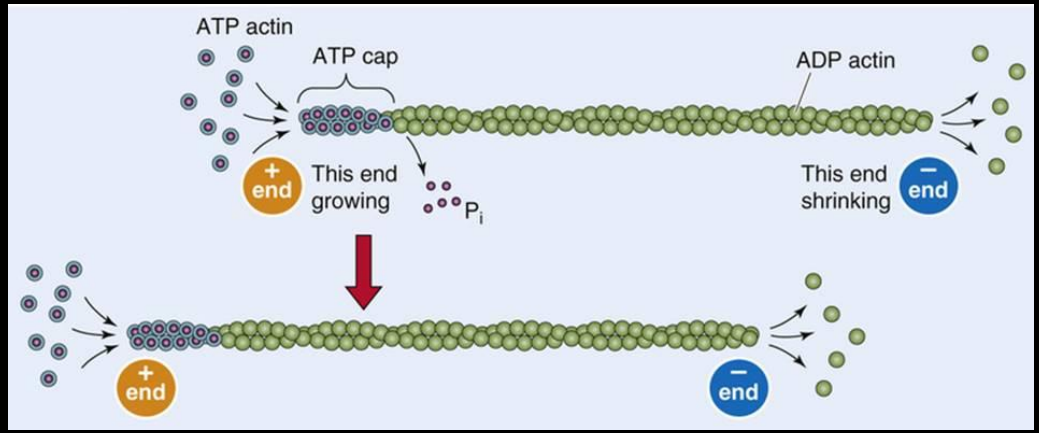


Fig. Formation and treadmilling of F-actin
Source: Carlier, et al. (2017)

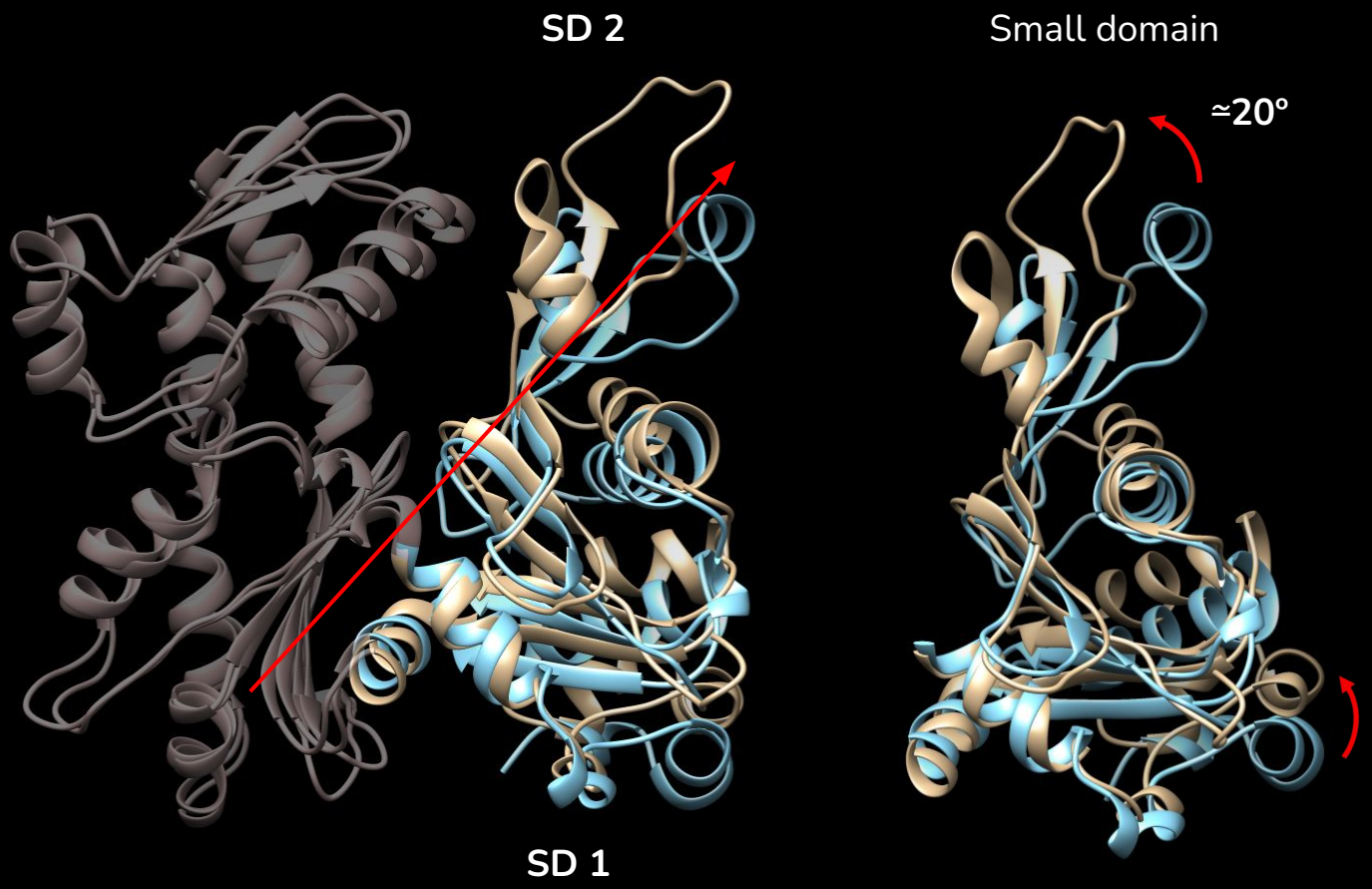
G-actin to F-actin transition

F-actin

G-actin

PDB ID: 1ATN, 2ZWH
Resolution: 2.80 Å, 3,30 Å

G-actin to F-actin transition

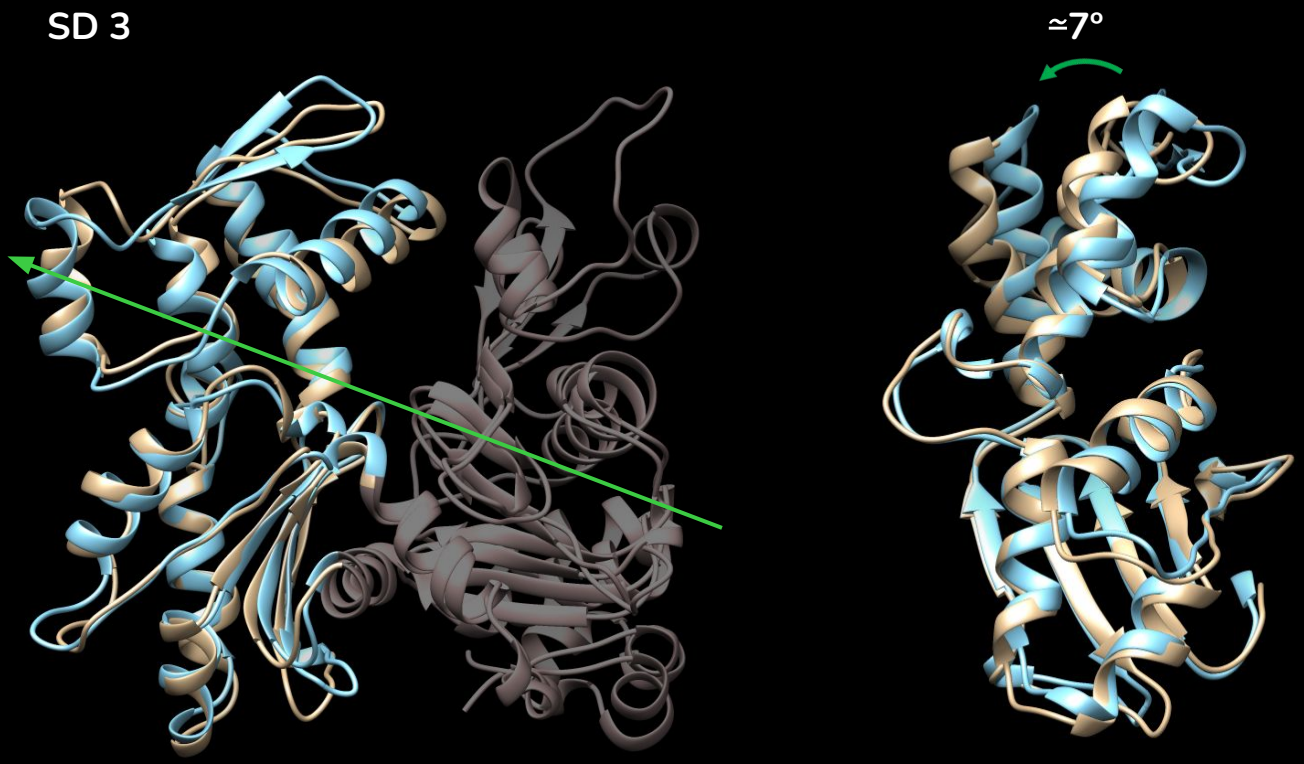


F-actin

G-actin

PDB ID: 1ATN, 2ZWH
Resolution: 2.80 Å, 3,30 Å

G-actin to F-actin transition

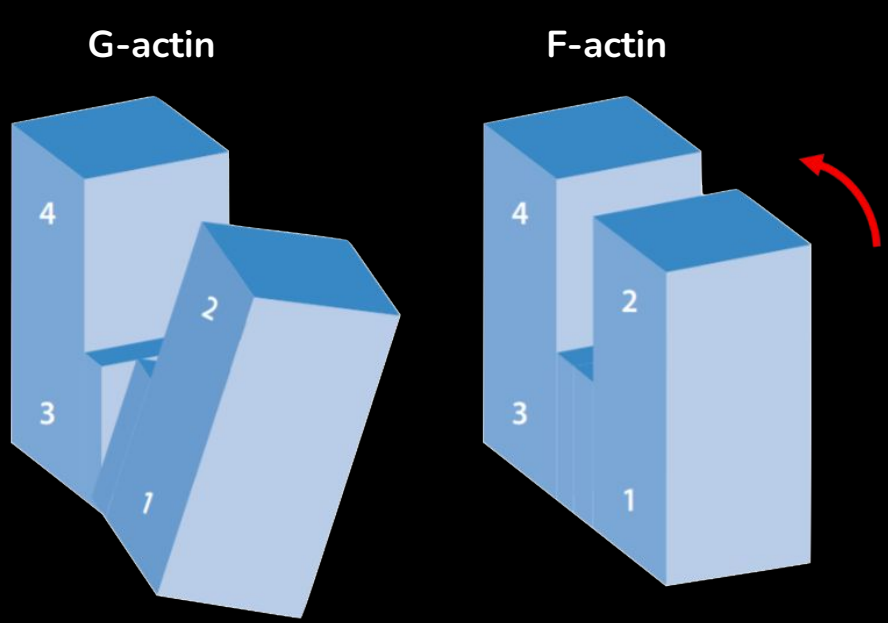


F-actin

G-actin

PDB ID: 1ATN, 2ZWH
Resolution: 2.80 Å, 3,30 Å

G-actin to F-actin transition



Flattening of the actin molecule

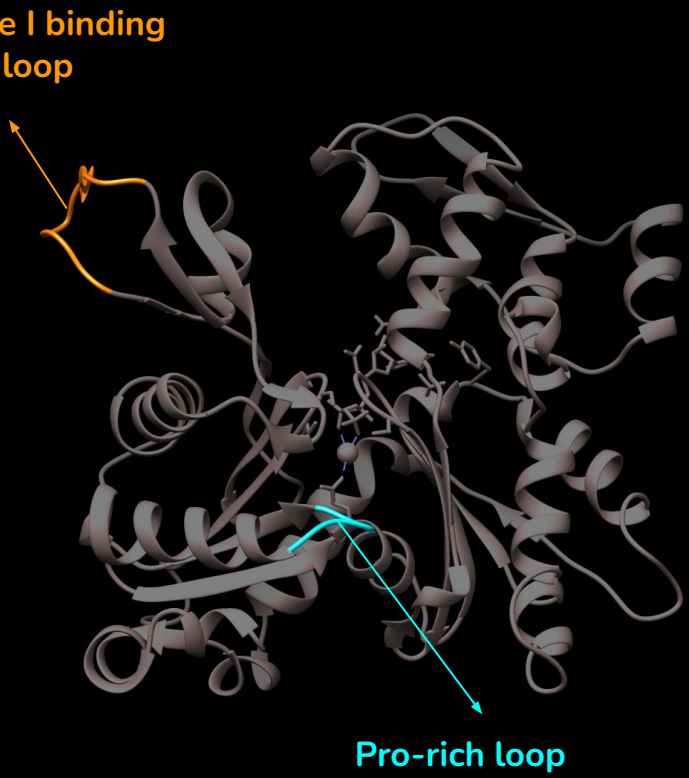
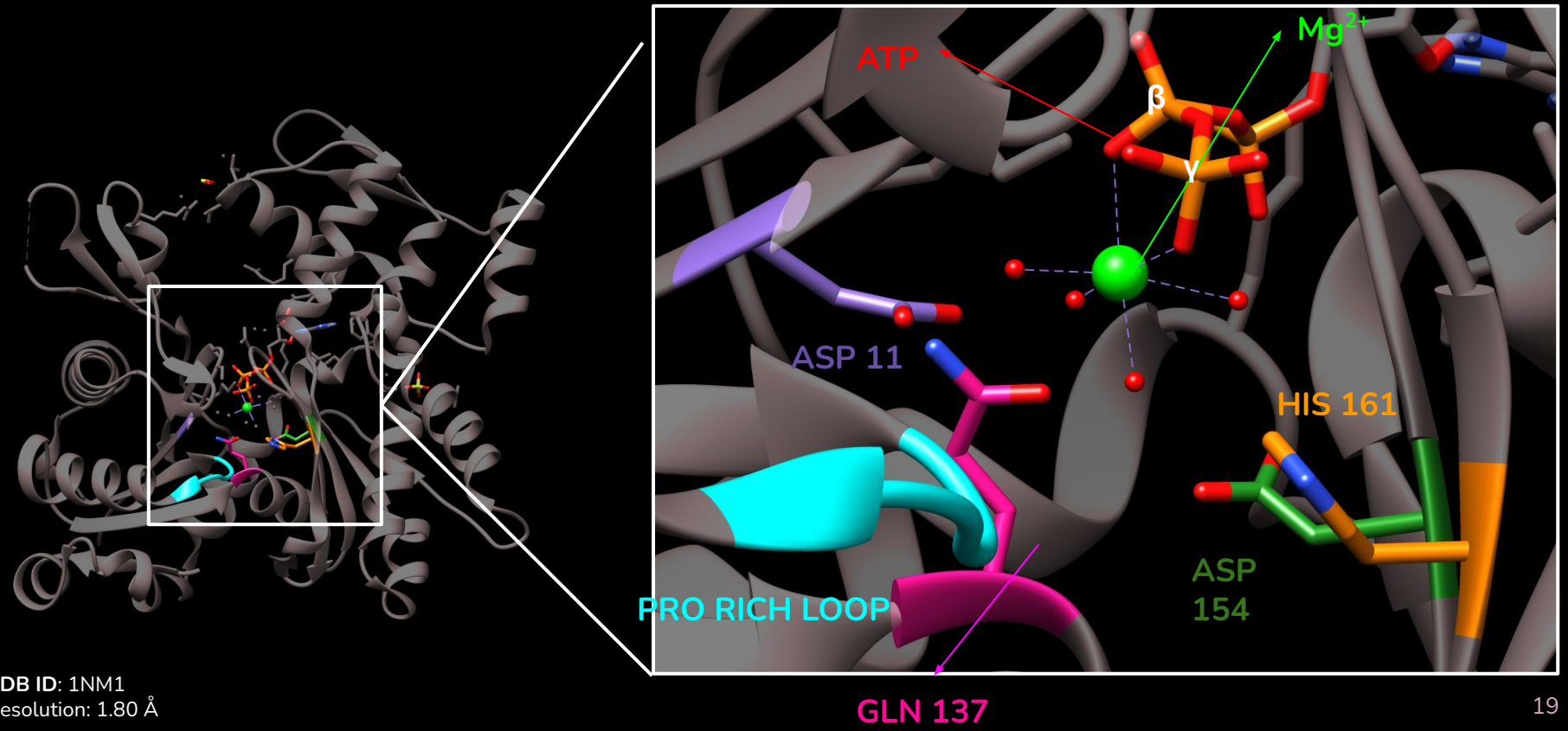


Fig. Essence of G-actin to F-actin transition
Source: Dominguez, et al. (2011)

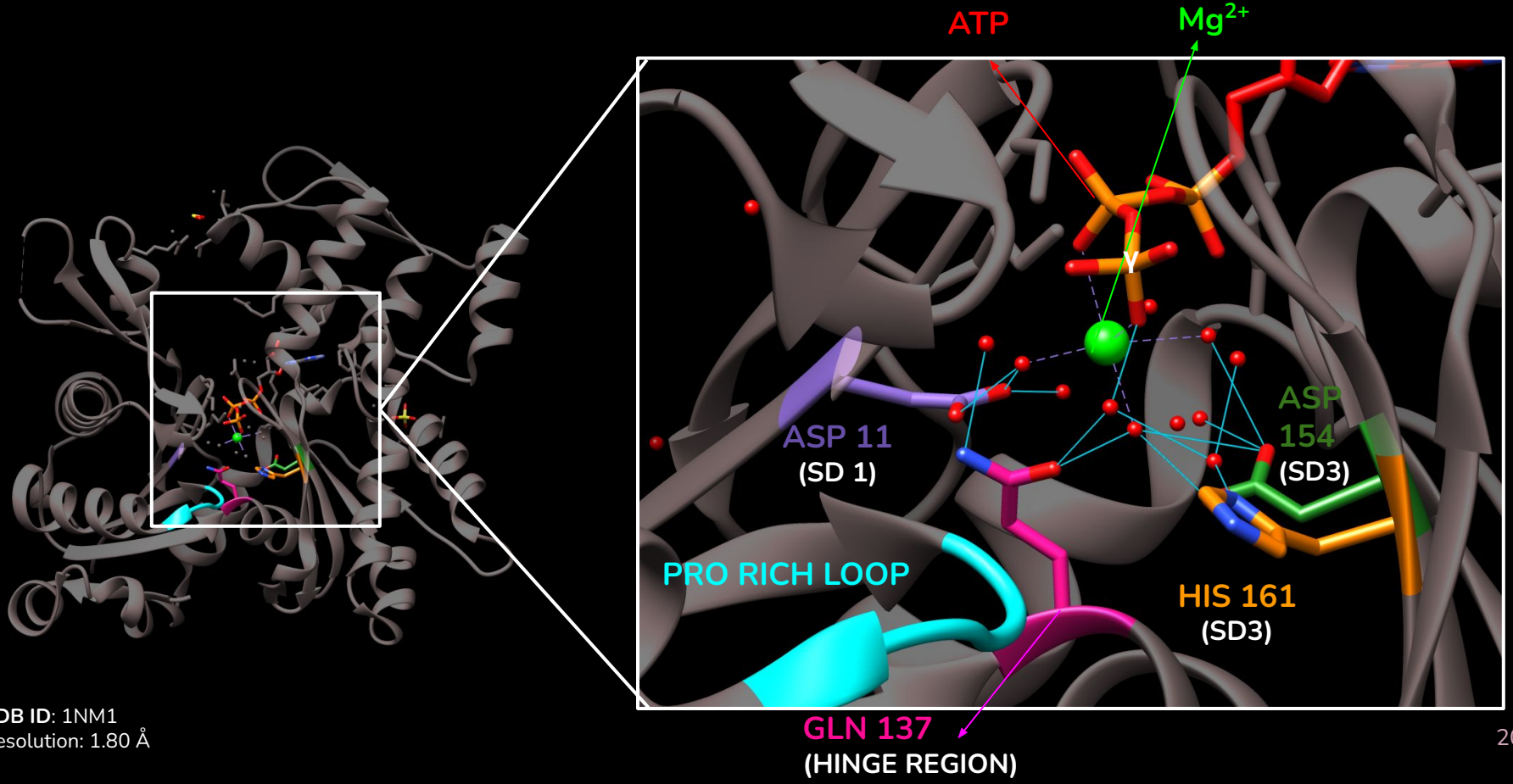
PDB ID: 1ATN
Resolution: 2.80 Å

ATP hydrolysis: ATP-binding site



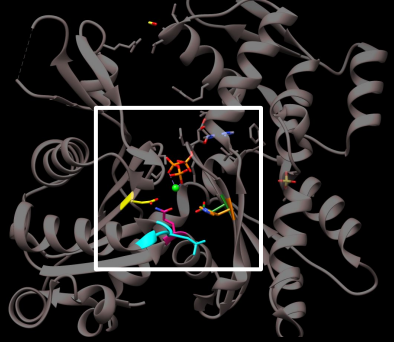
PDB ID: 1NM1
Resolution: 1.80 Å

ATP hydrolysis: ATP-binding site

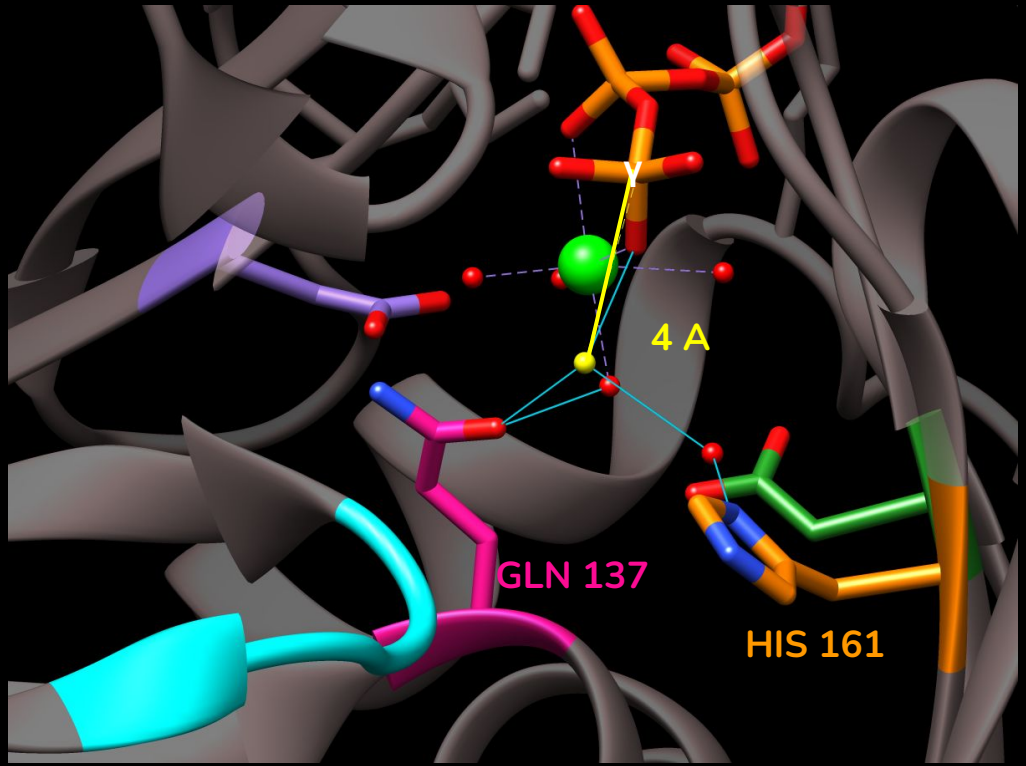


PDB ID: 1NM1
Resolution: 1.80 Å

ATP hydrolysis: ATP-binding site



G ACTIN

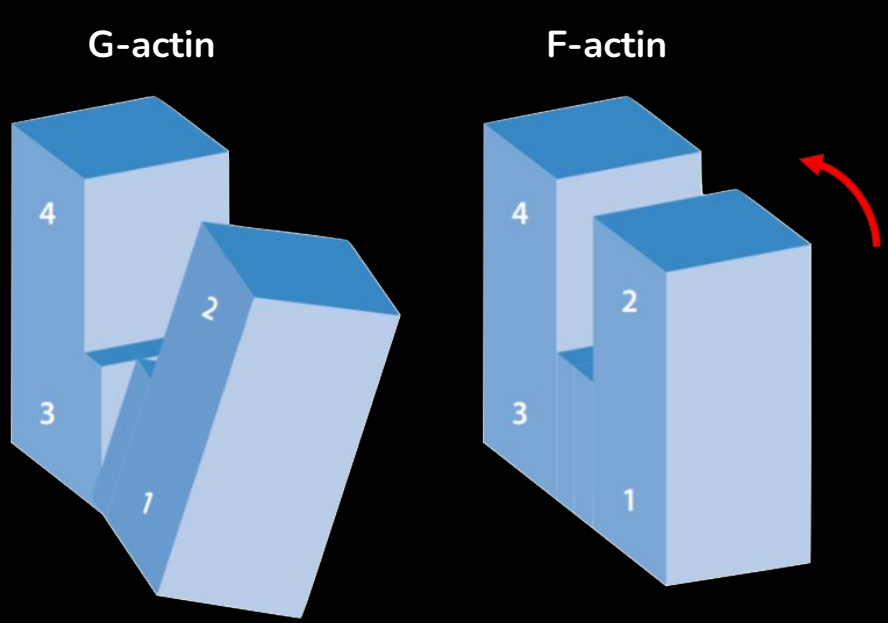


Distance between NA and gamma phosphate

Nucleophilic water (NA)

PDB ID: 1NM1
Resolution: 1.80 Å

G-actin to F-actin transition



Flattening of the actin molecule

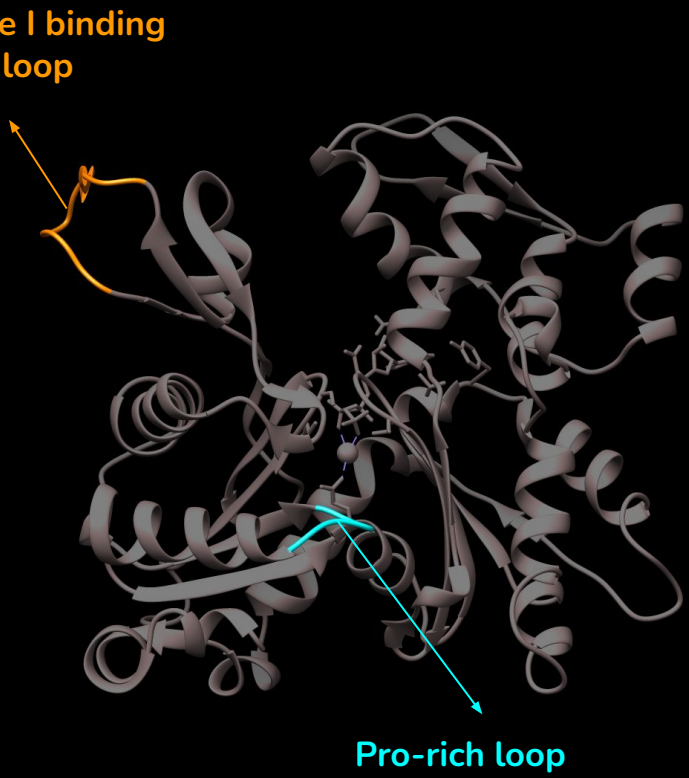


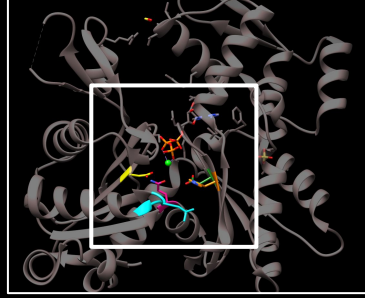
Fig. Essence of G-actin to F-actin transition
Source: Dominguez, et al. (2011)

PDB ID: 1ATN
Resolution: 2.80 Å

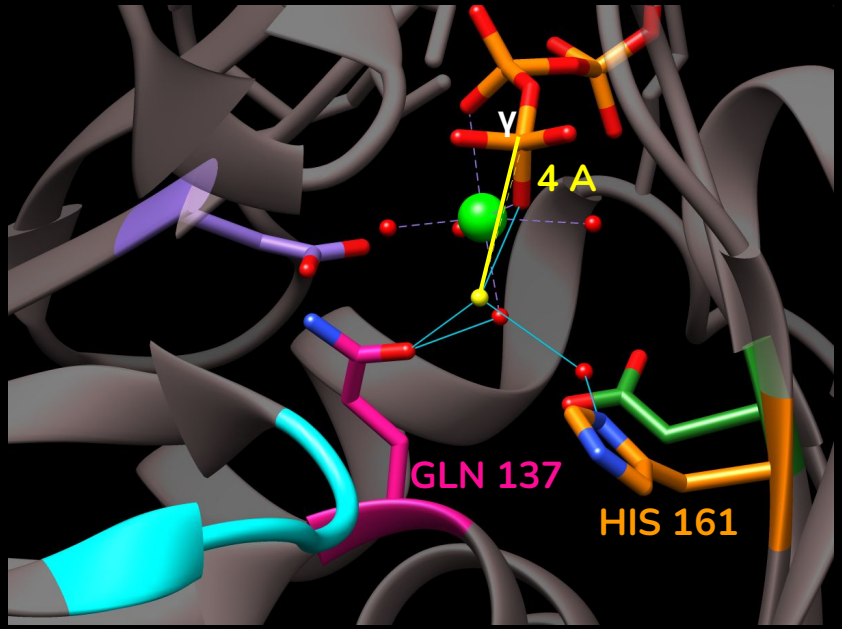
ATP hydrolysis: ATP-binding site

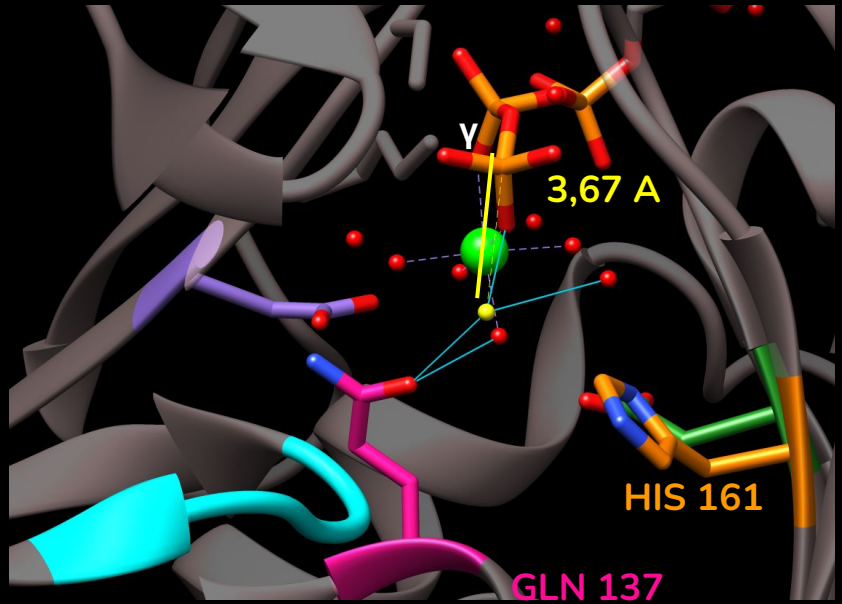
Nucleophilic water (NA)

Distance between NA and gamma phosphate



G ACTIN → F ACTIN





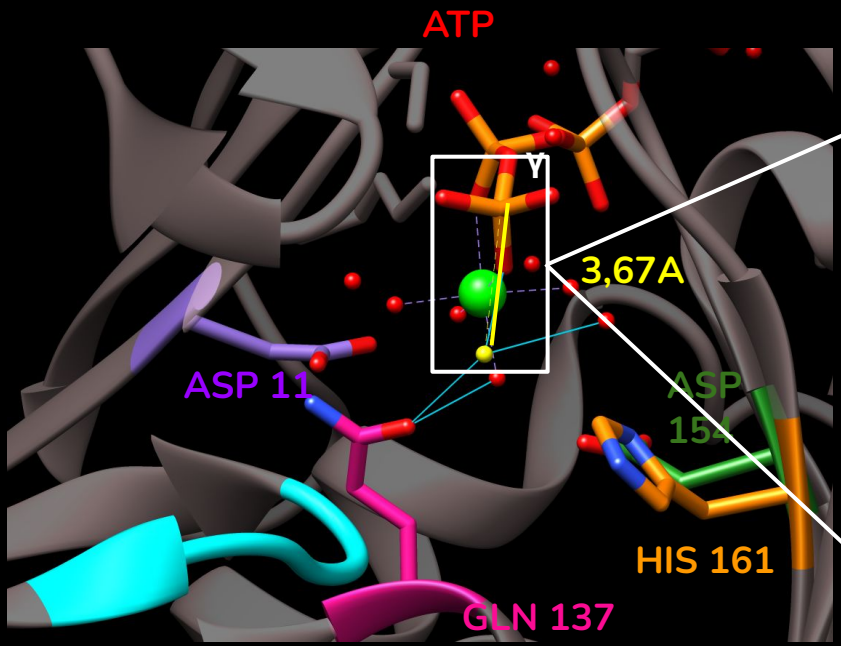
PDB ID: 1NM1
Resolution: 1.80 Å

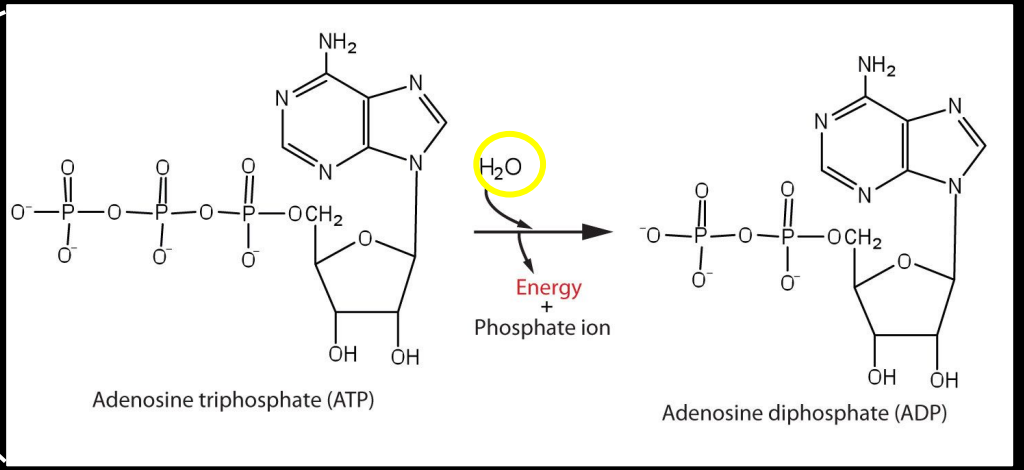
HIS 161 - PRORICH LOOP
HIS 161 - NA
GLN137- NA

GLN137- NA

PDB ID: 3A5M
Resolution: 2,40A

ATP hydrolysis: mechanism

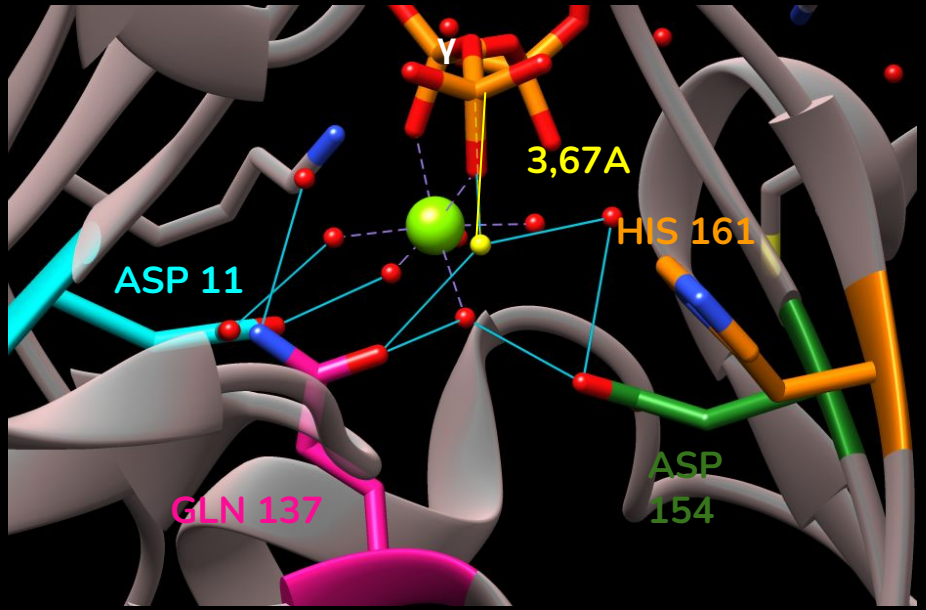




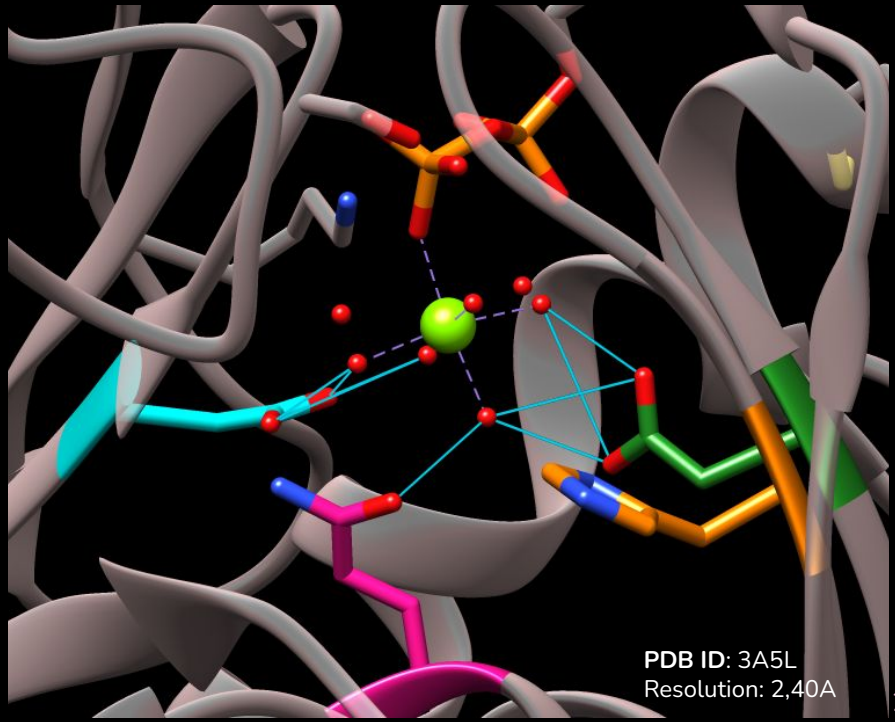
PDB ID: 3A5M
Resolution: 2.40 Å

ATP hydrolysis: mechanism

ATP → ATP HYDROLYSIS → ADP



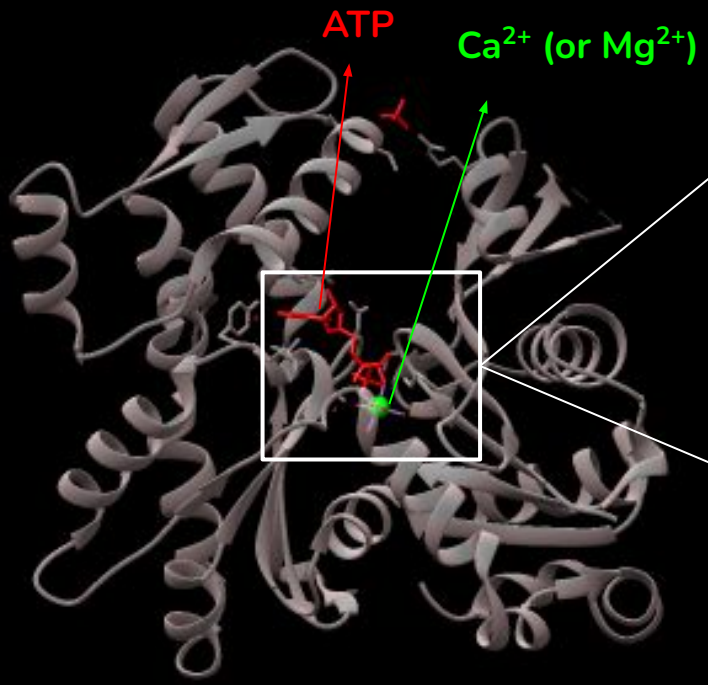
PDB ID: 3A5M
Resolution: 2.40 Å

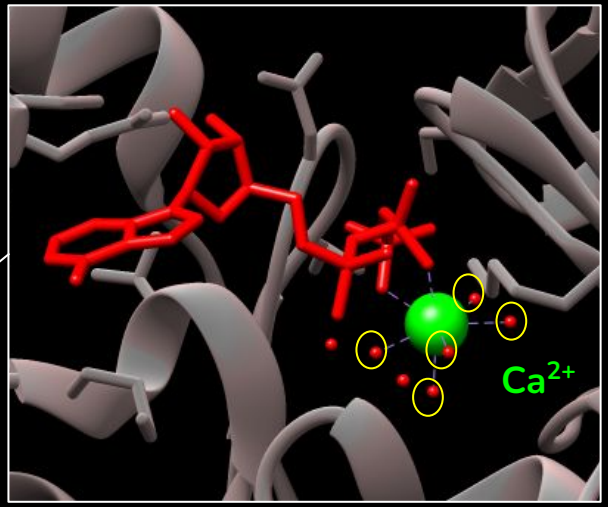


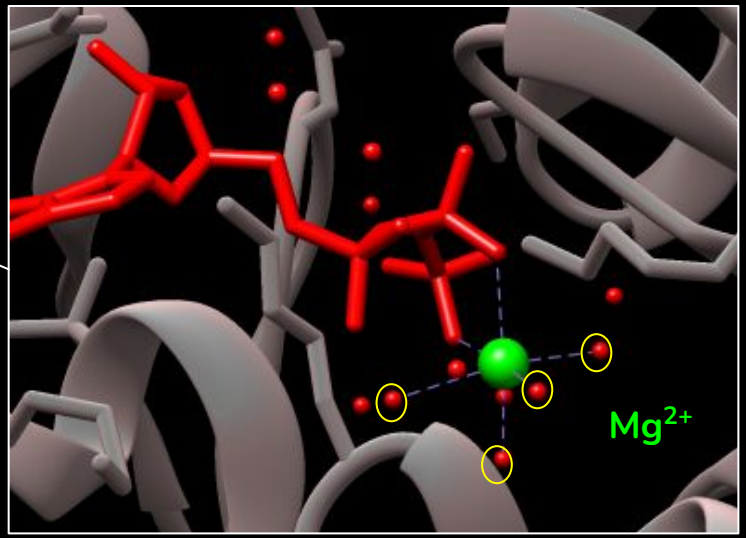
PDB ID: 3A5L
Resolution: 2,40A

ATP hydrolysis

Differences between Mg^{2+} and Ca^{2+}





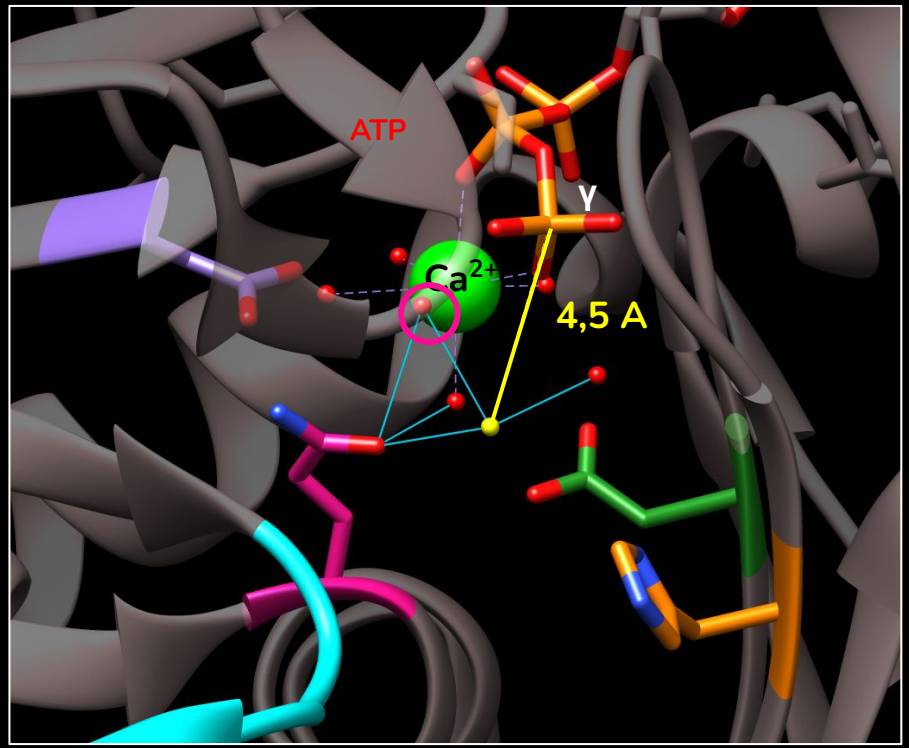


PDB ID: 3HBT
Resolution: 2.70 Å

water molecules

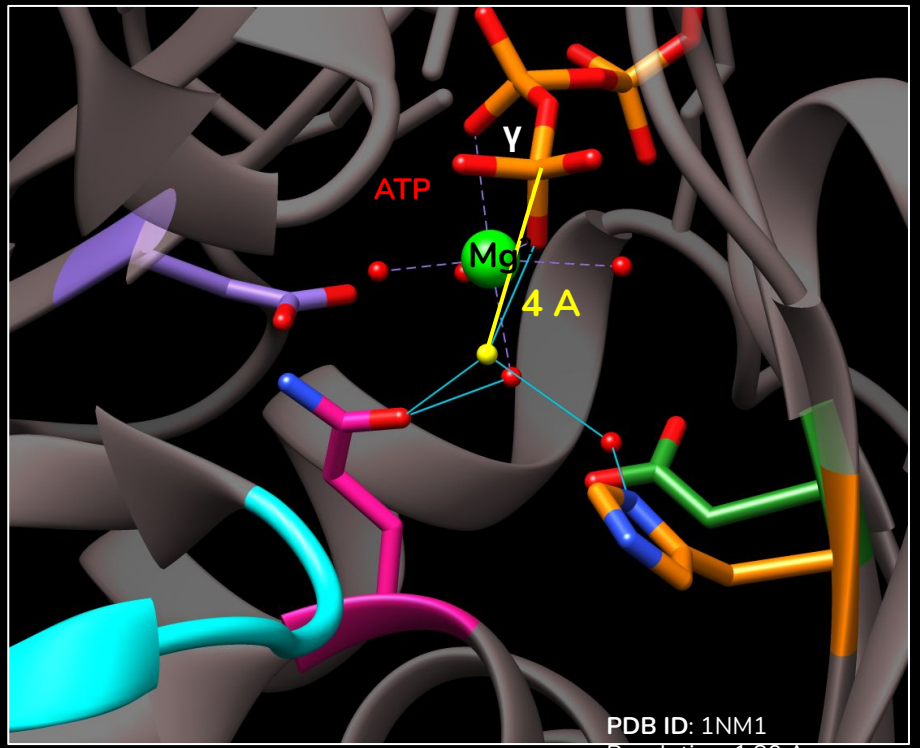
ATP hydrolysis: ATP-binding site

ACTIN-Ca^{2+}



PDB ID: 3A5O
Resolution: 2.40 Å

ACTIN-Mg^{2+}



PDB ID: 1NM1
Resolution: 1,80 A

Distance between NA and gamma phosphate

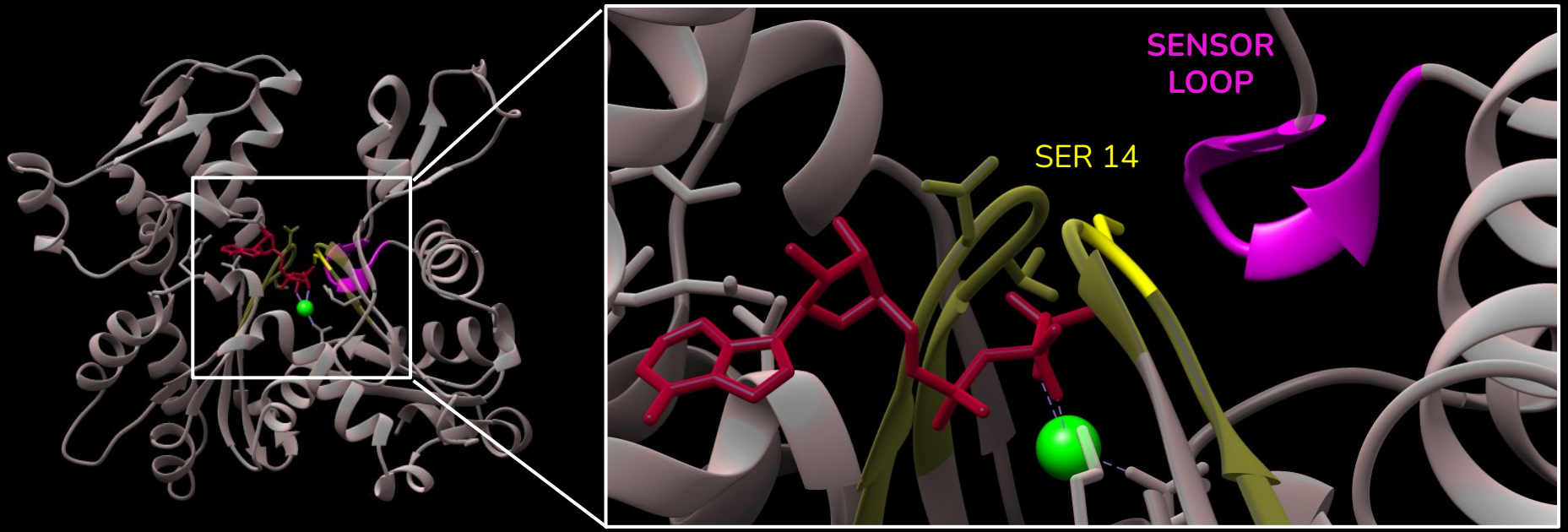
Nucleophilic water (NA)

The polymerization process involves changes in actin that allow ATP hydrolysis to be activated

ATP → ADP transition produces conformational changes in some regions of the actin protein

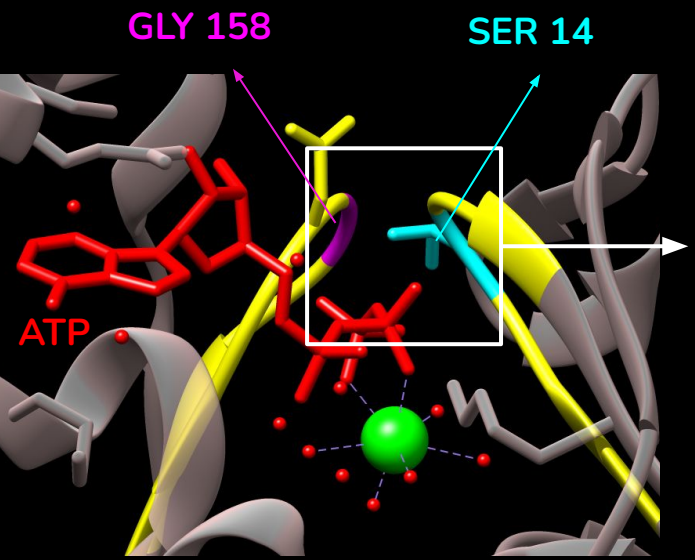
Nucleotide-dependent conformational states

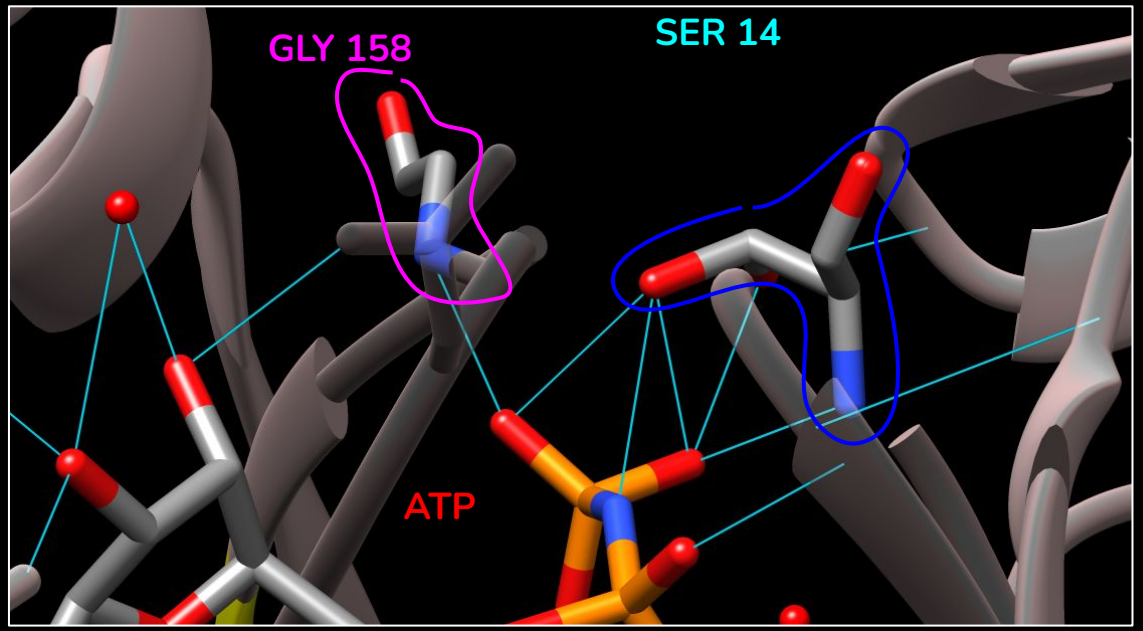
The difference between the ATP- and ADP-bound states involve primarily two loops: the Ser14 beta-hairpin loop and the sensor loop.



Nucleotide-dependent conformational states

P-loops	Sensor loop	D-loop





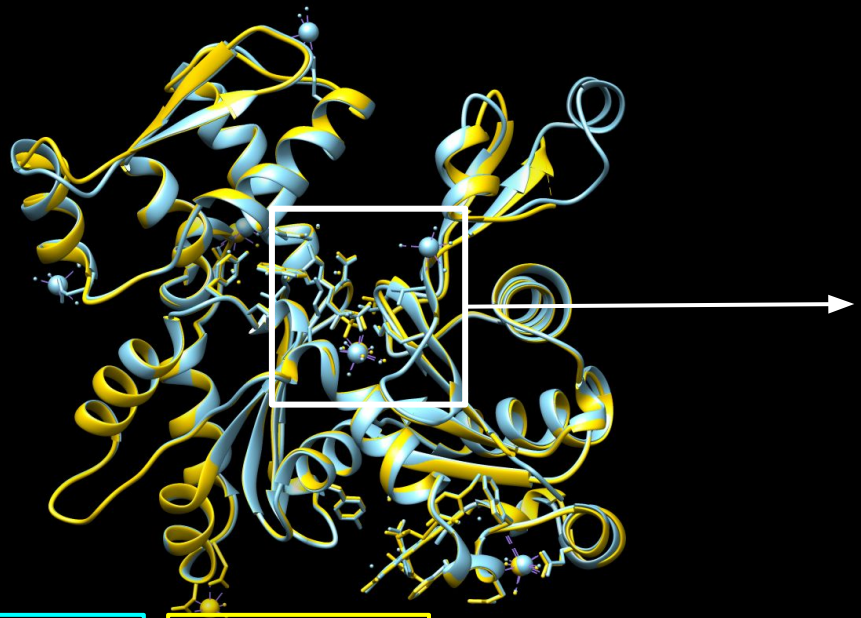
ATP serves as a bridge connecting the p-loops with a network of hydrogen bonds

PDB ID: 1NWK
Resolution: 1.85 Å

Nucleotide-dependent conformational states

P-loops	Sensor loop	D-loop

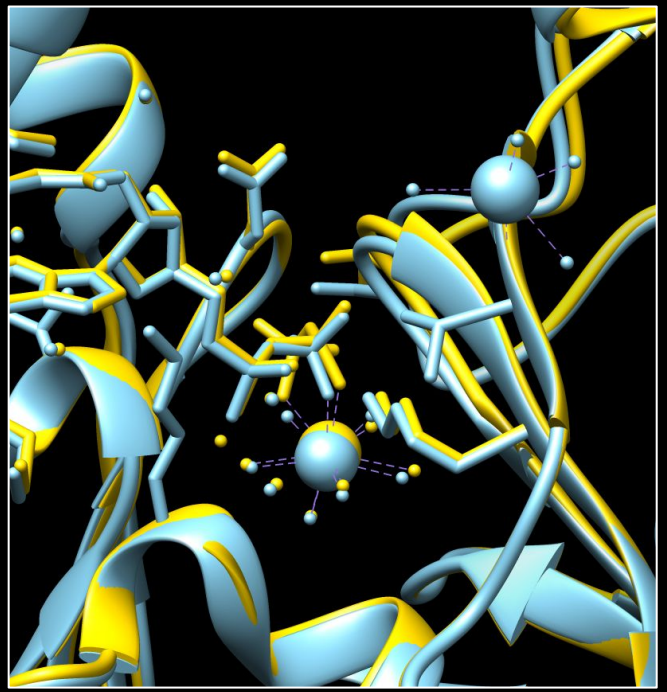
Superimposition ATP and ADP-actin



PDB ID: 1J6Z
Resolution: 1.54 Å

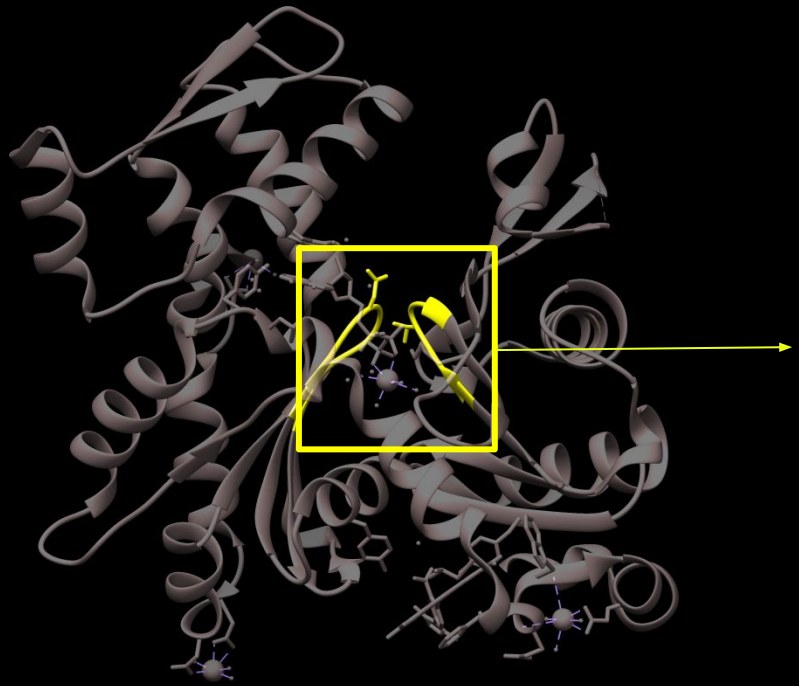
PDB ID: 1NWK
Resolution: 1.85 Å

P-loops are perfectly superimposed

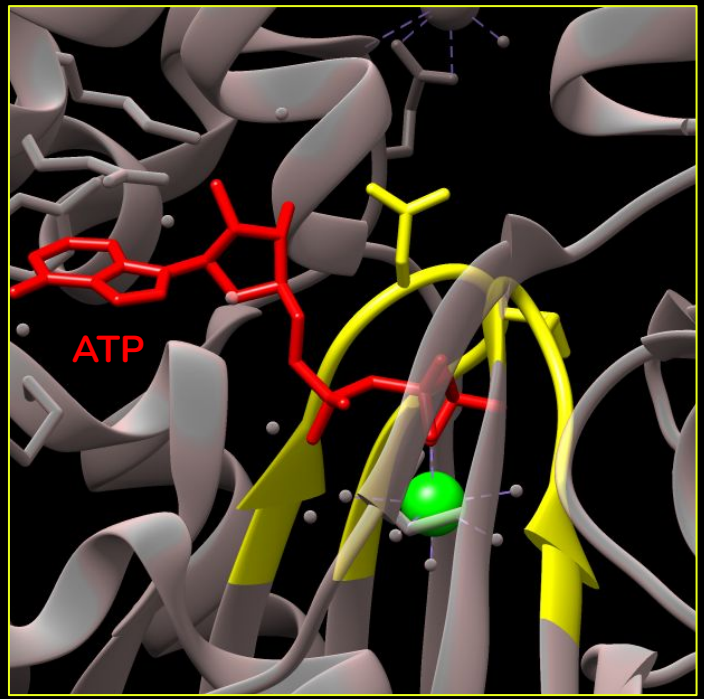


Nucleotide-dependent conformational states

P-loops	Sensor loop	D-loop

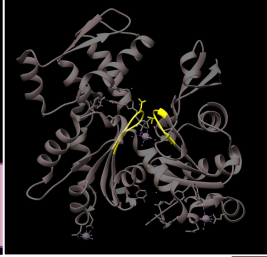


P-loops form beta-hairpins in the ADP and ATP state



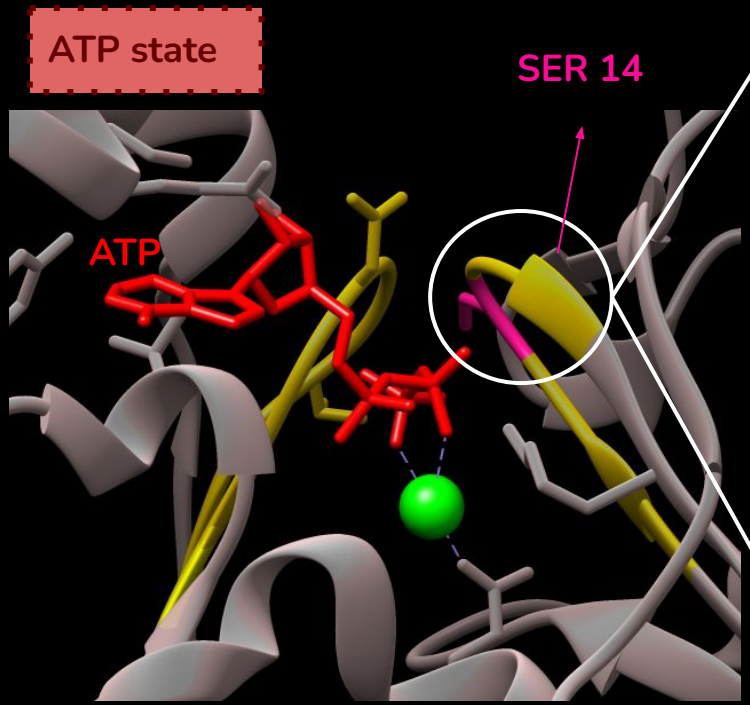
PDB ID: 1NWK
Resolution: 1.85 Å

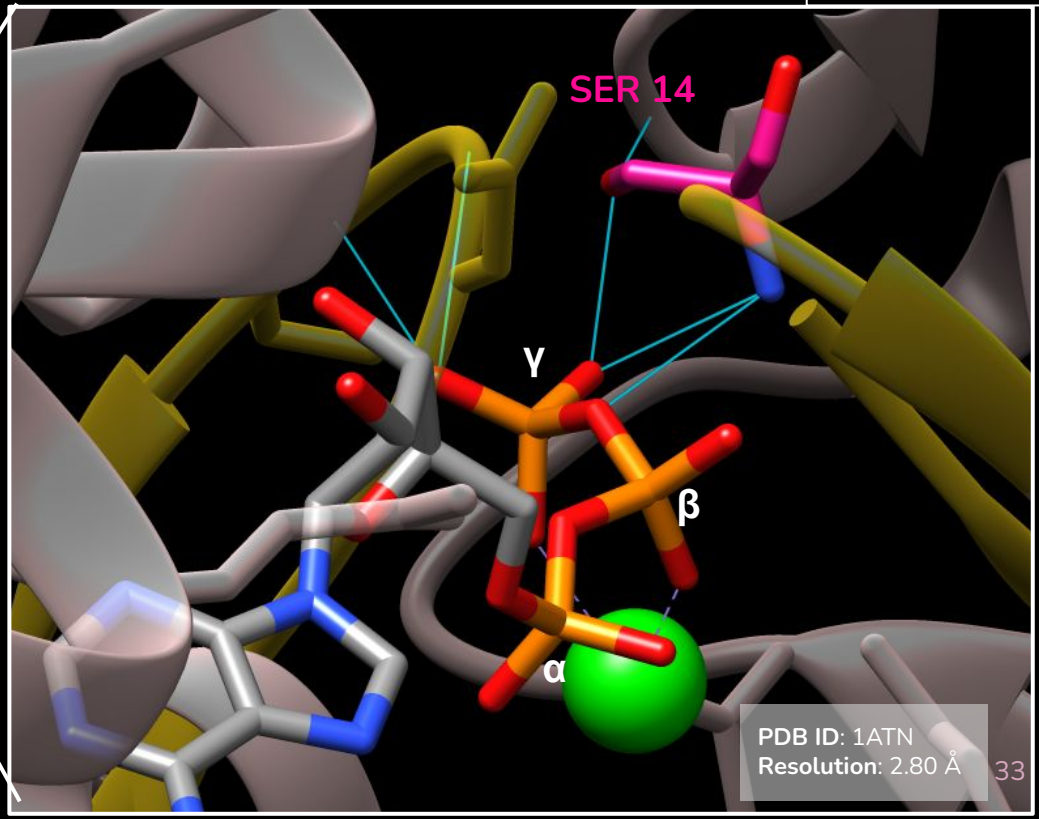
Nucleotide-dependent conformational states



P-loops	Sensor loop	D-loop

S14 (p-loop) as a direct sensor of ATP hydrolysis

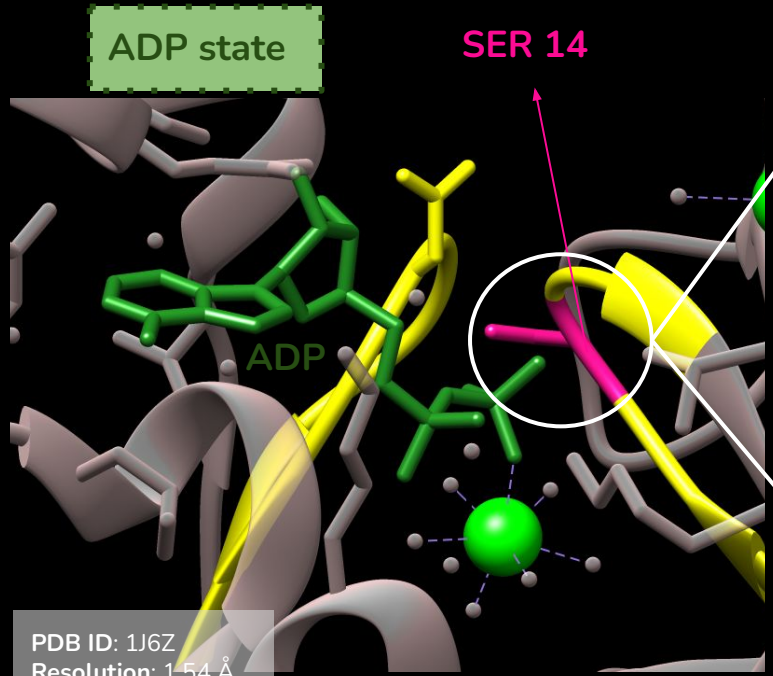


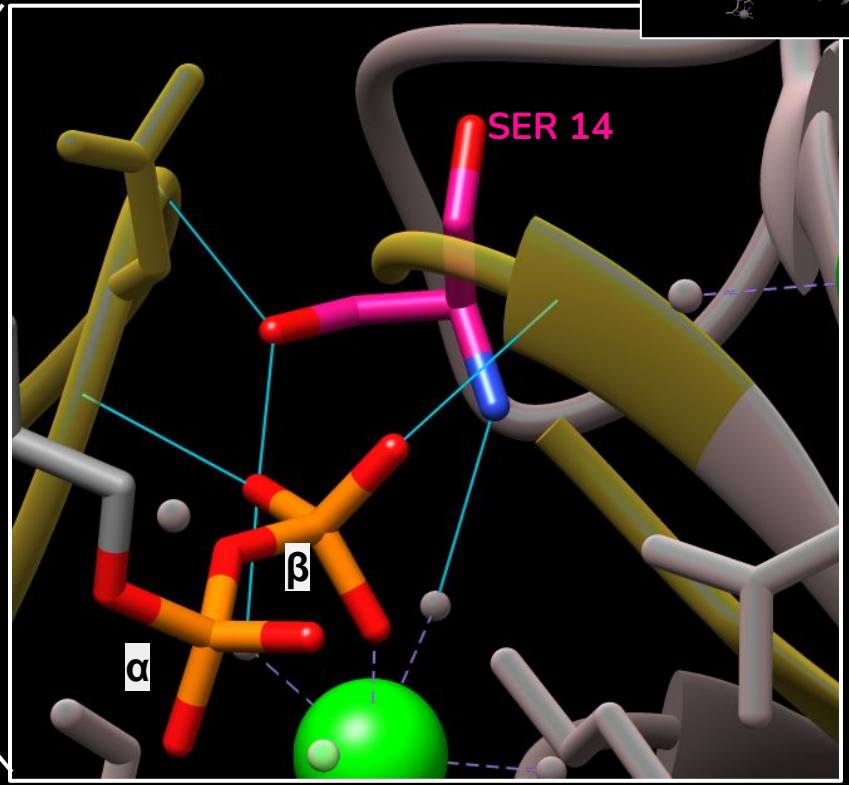


Nucleotide-dependent conformational states

P-loops	Sensor loop	D-loop

S14 (p-loop) as a direct sensor of ATP hydrolysis



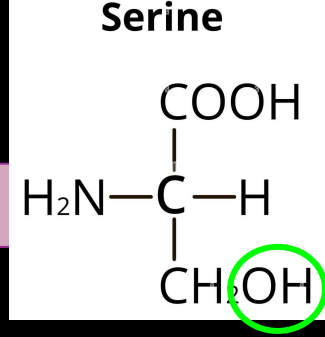


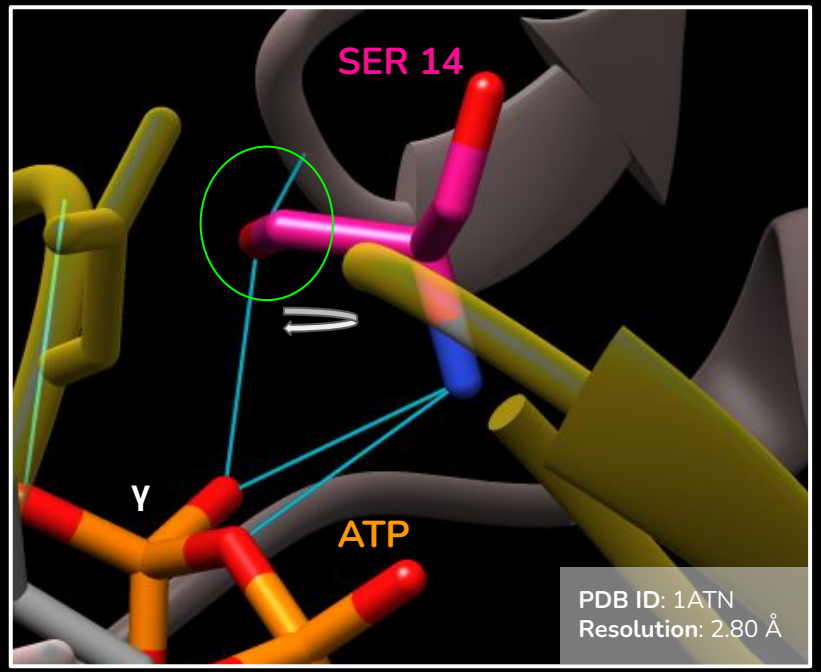
PDB ID: 1J6Z
Resolution: 1.54 Å

Nucleotide-dependent conformational states

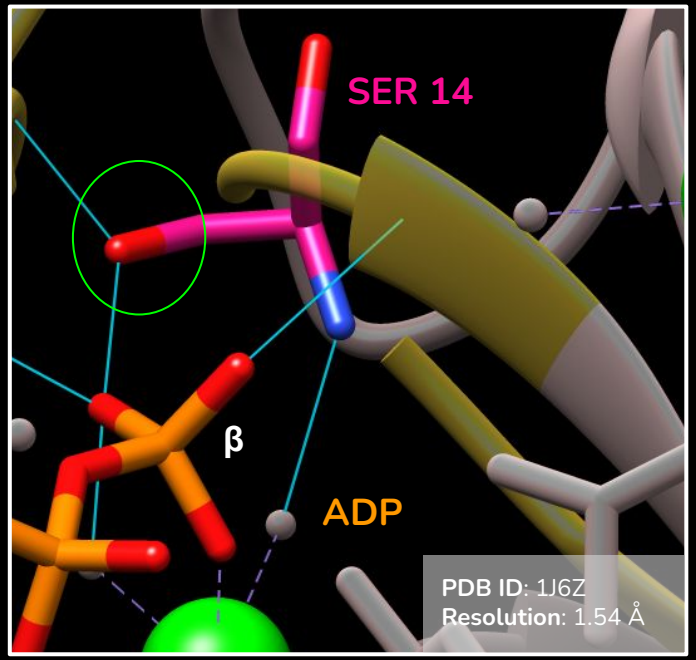
P-loops	Sensor loop	D-loop

ATP state → ATP hydrolysis → ADP state

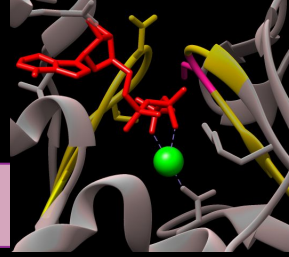




130°

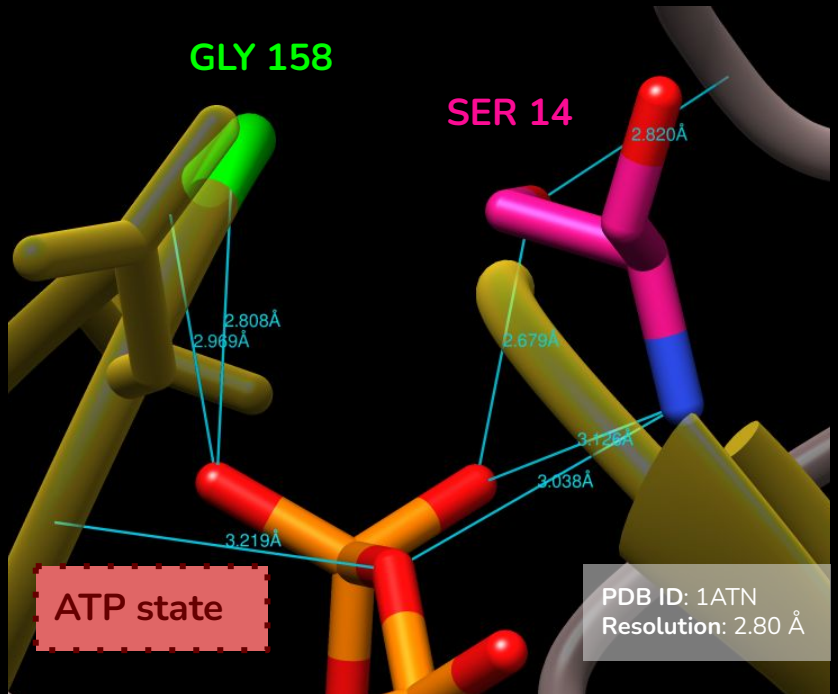


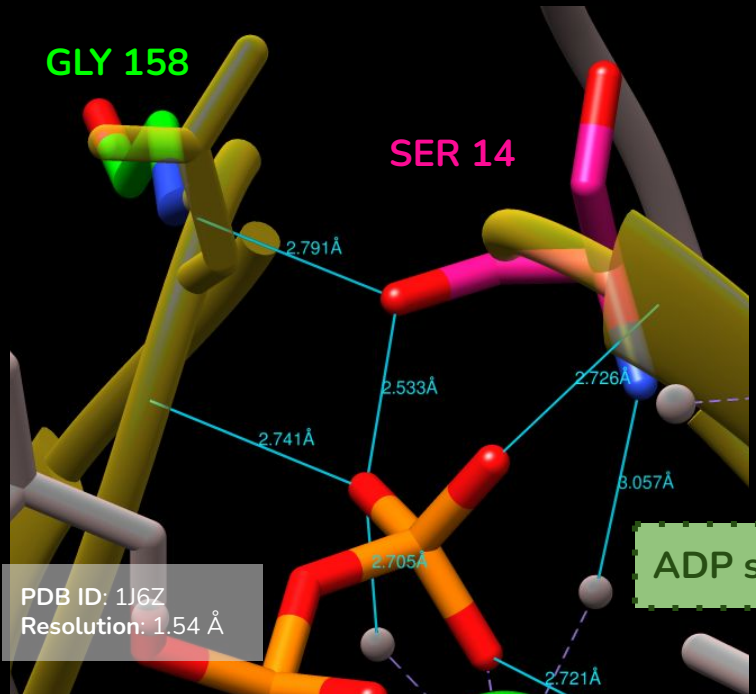
Nucleotide-dependent conformational states



P-loops	Sensor loop	D-loop

The presence of a γ phosphate of ATP initiates a series of structural transitions extending from the active site outward to the sensor loop

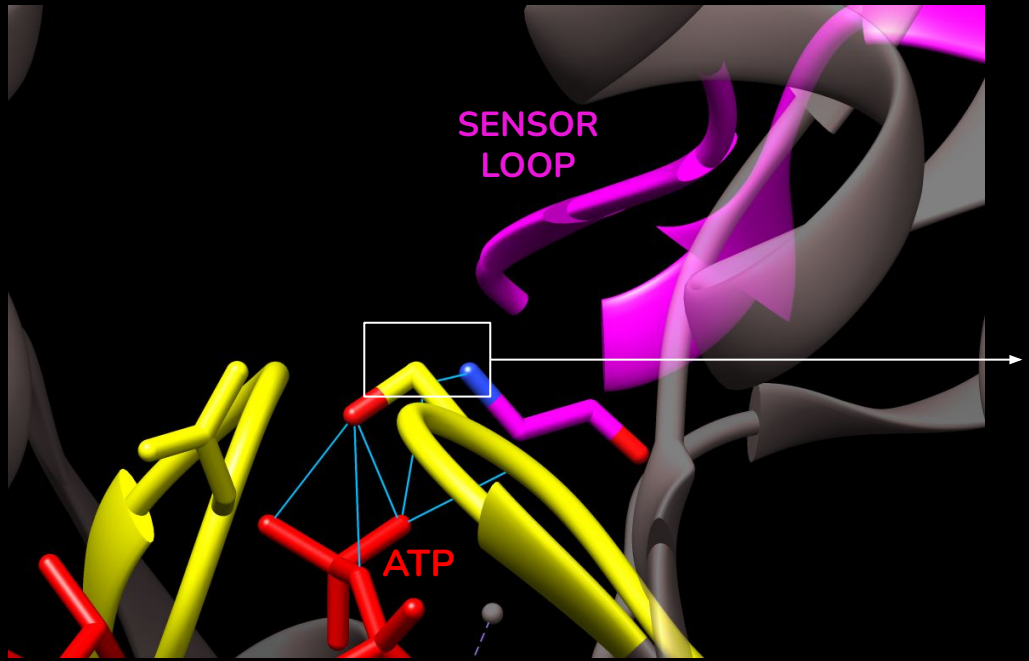


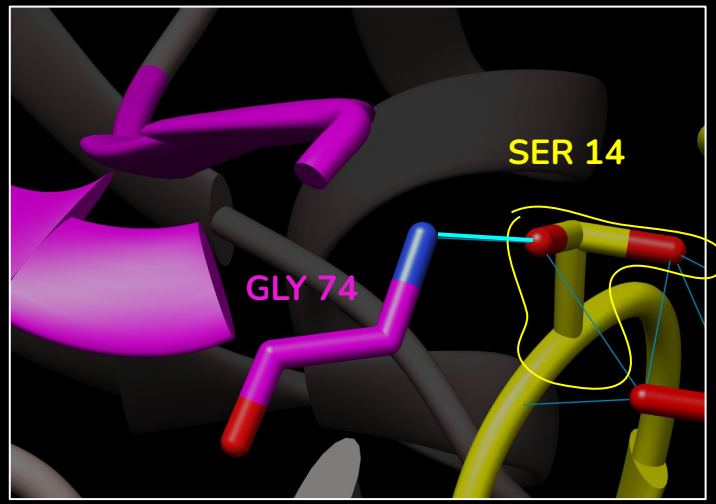


Nucleotide-dependent conformational states

P-loops	Sensor loop	D-loop

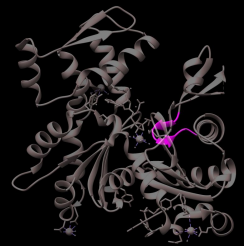
This network of hydrogen bonds links together the p-loops and sensor loop, holding the nucleotide-binding cleft in a closed conformation





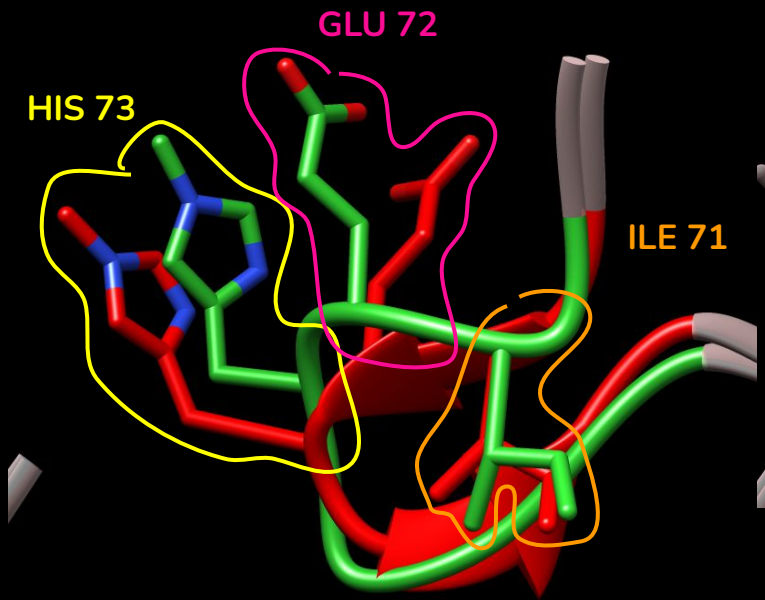
PDB ID: 1NWK
Resolution: 1.85 Å

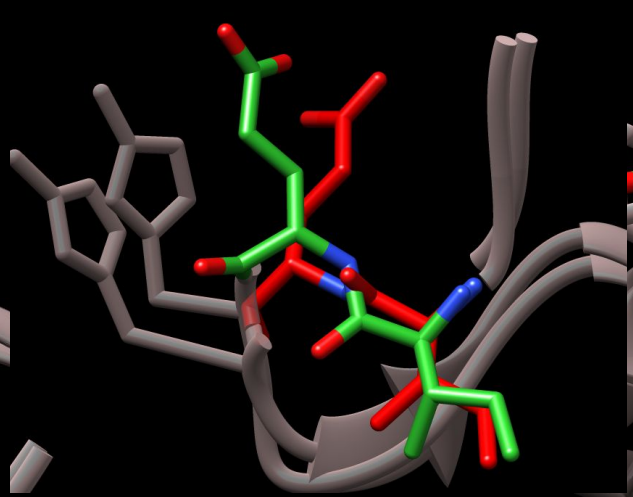
Nucleotide-dependent conformational states

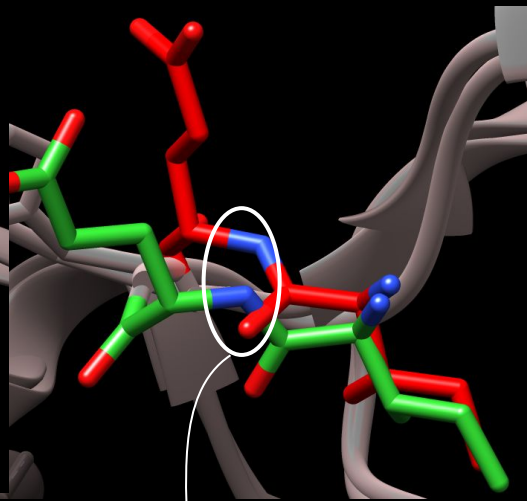


P-loops	Sensor loop	D-loop

The presence of a γ phosphate of ATP initiates a series of structural transitions extending from the active site outward to the sensor loop

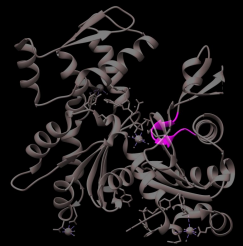






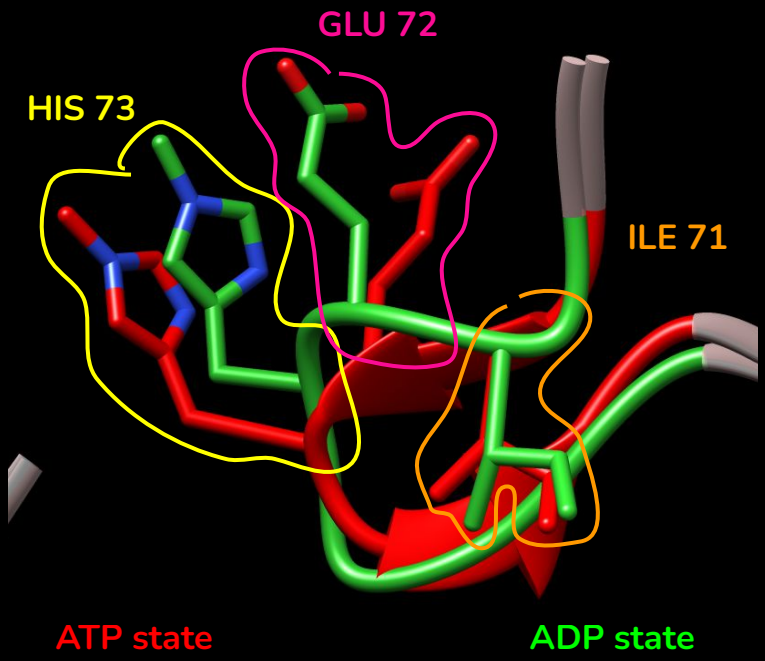
Upon Pi release, Ser-14 rotates and the polypeptide linkage GLU 72 and ILE 71 of the loops rotates (90°)

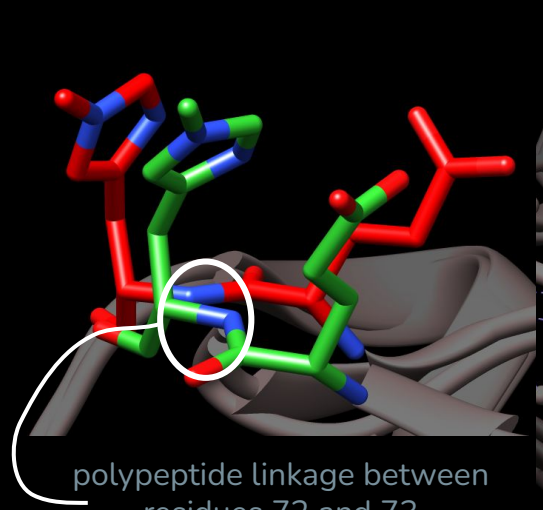
Nucleotide-dependent conformational states

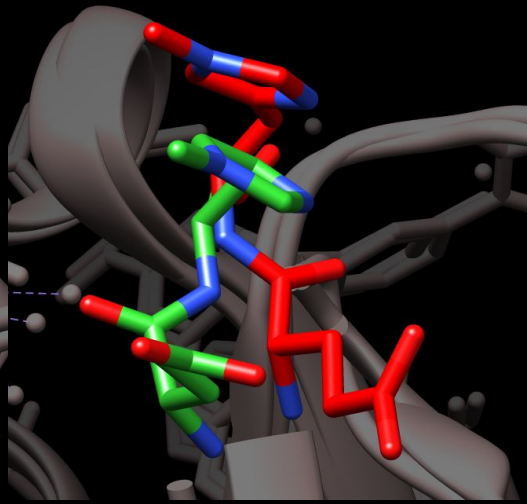


P-loops	Sensor loop	D-loop

The presence of a γ phosphate of ATP initiates a series of structural transitions extending from the active site outward to the sensor loop



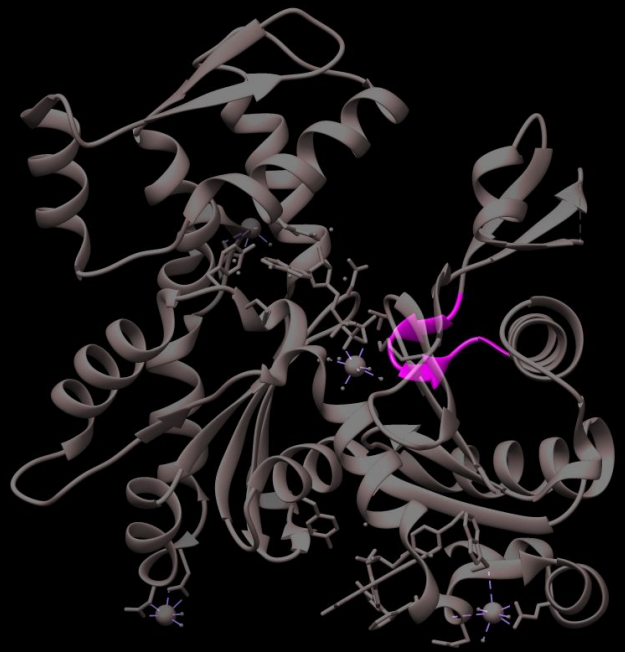




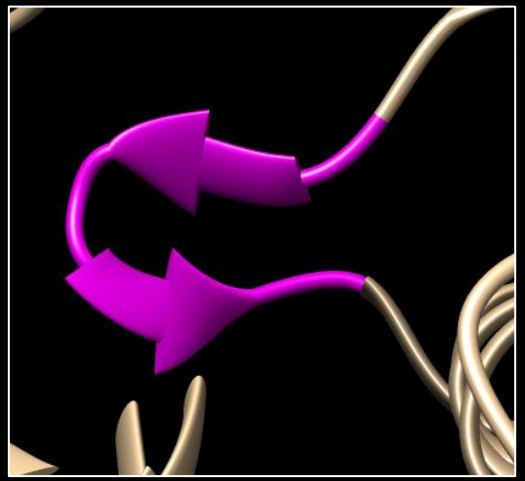
Upon Pi release, Ser-14 rotates and the polypeptide linkage GLU 72 and HIS 73 of the loops flips around (180° flip)

Nucleotide-dependent conformational states

P-loops	Sensor loop	D-loop



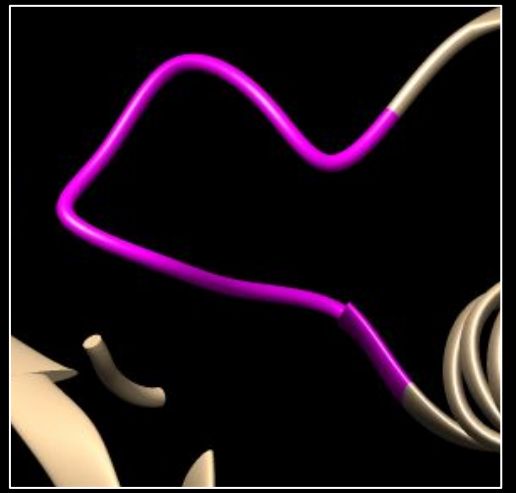
ATP and ADP-Pi state



PDB ID: 1NWK
Resolution: 1.85 Å

PDB ID: 6DJN
Resolution: 3.1 Å

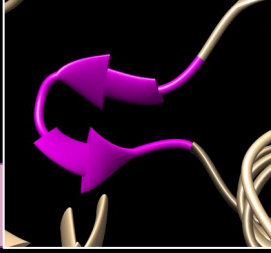
ADP state



PDB ID: 1J6Z
Resolution: 1.54 Å

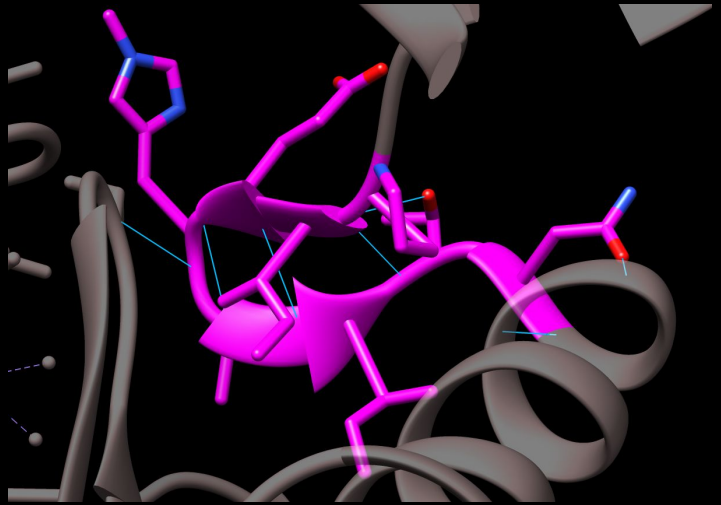
Nucleotide-dependent conformational states

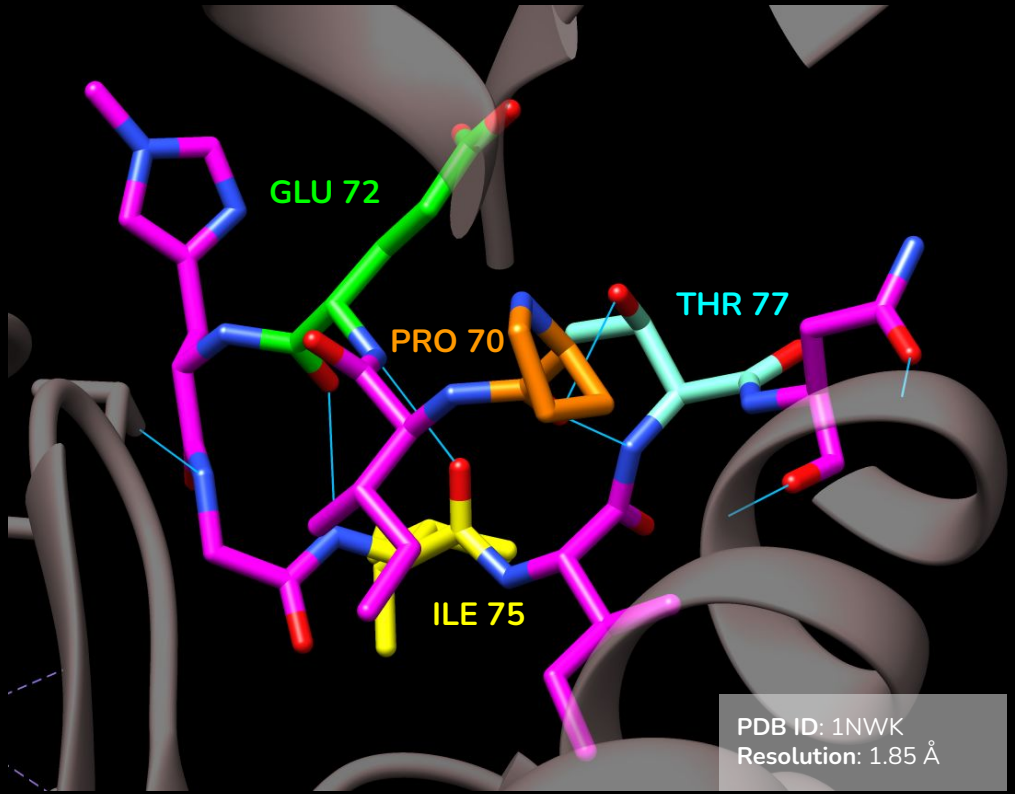
P-loops	Sensor loop	D-loop



ATP state

HB PRO70 - THR77
HB ILE75 - GLU72





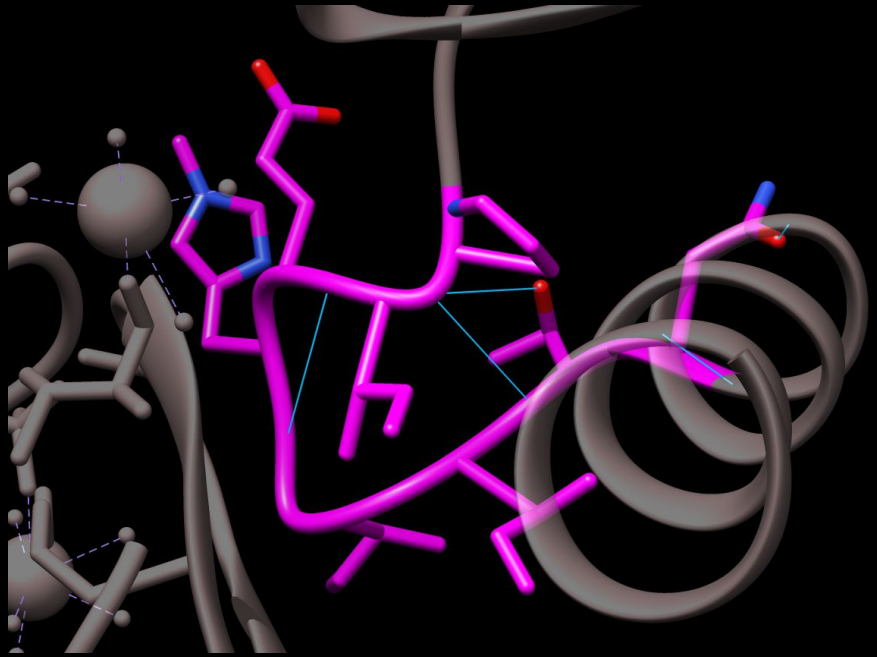
PDB ID: 1NWK
Resolution: 1.85 Å

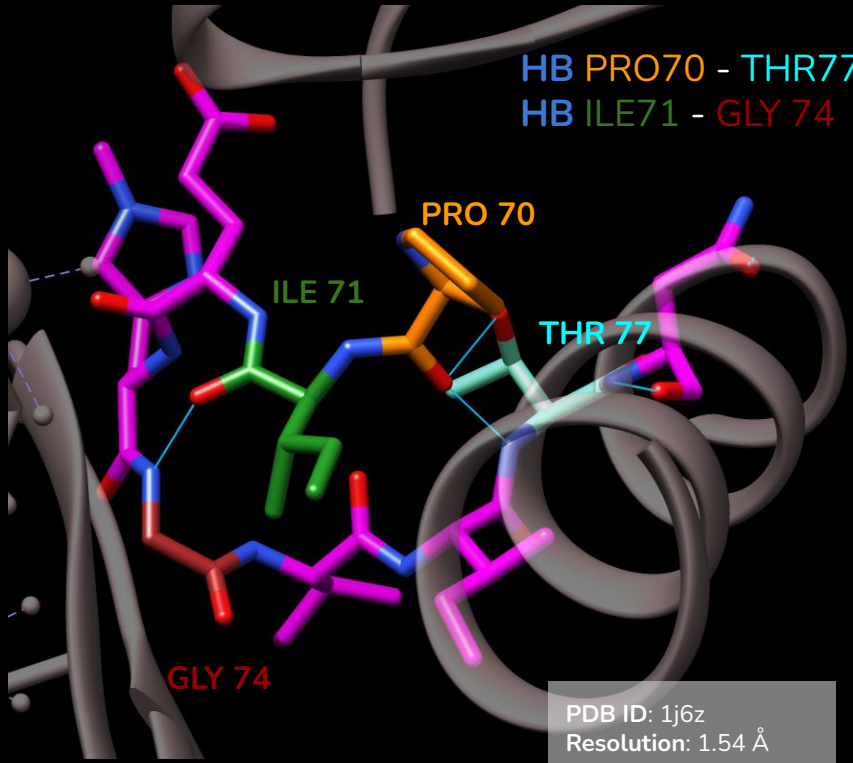
Nucleotide-dependent conformational states

P-loops	Sensor loop	D-loop

ADP state

Most of the hydrogen bonds are broken

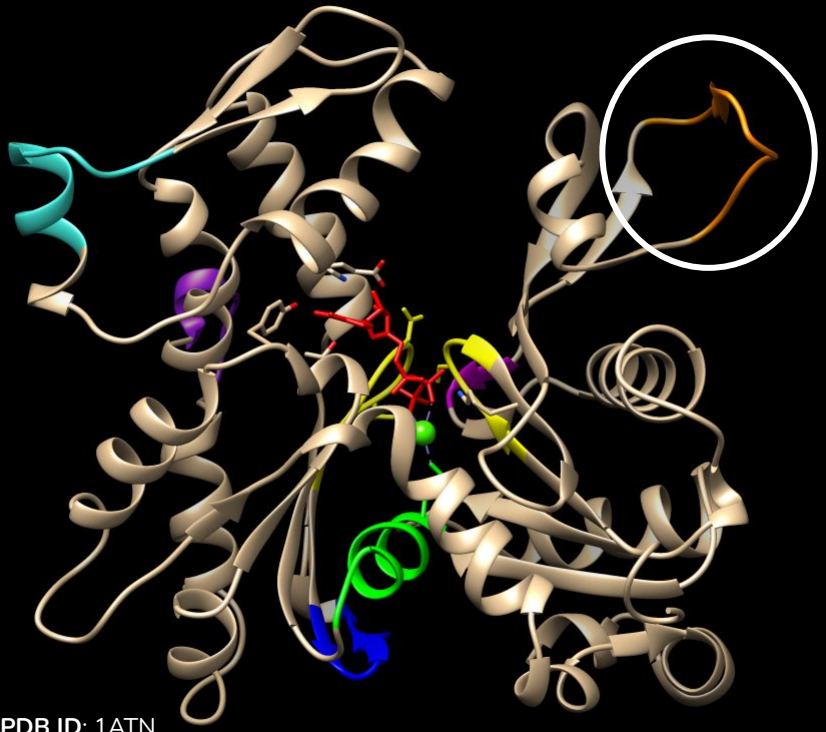




PDB ID: 1j6z
Resolution: 1.54 Å

Nucleotide-dependent conformational states

P-loops	Sensor loop	D-loop



PDB ID: 1ATN
Resolution: 2.80 Å

CONTROVERSY

Nucleotide-dependent conformational states

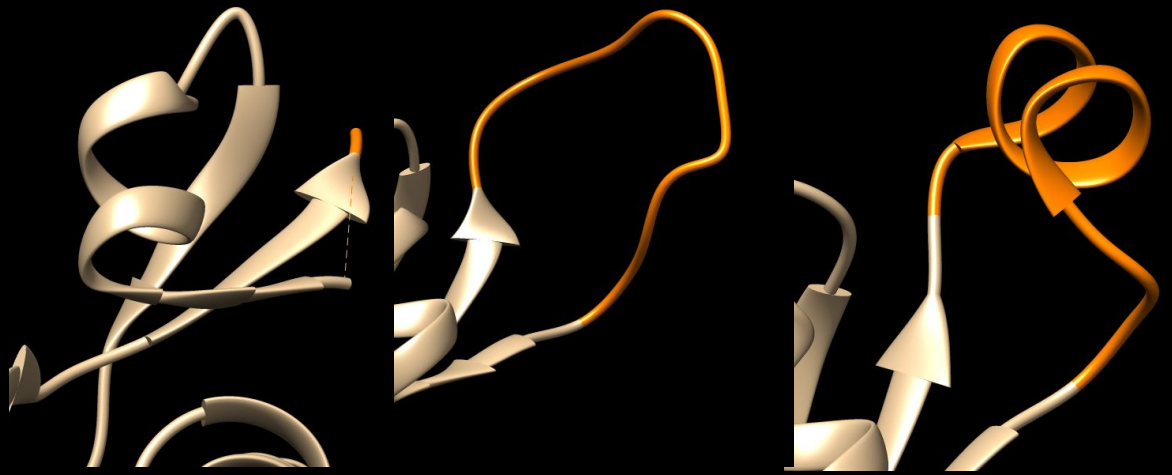
P-loops	Sensor loop	D-loop

- Domínguez lab (2001)

- Zheng lab (2007)

- Rould lab (2006)

Nucleotide dependent conformational changes in sensor loop were proposed to be translated into structural changes in D-loop



ATP state

PDB ID: 1NWK
Resolution: 1.85 Å

ADP-Pi state

PDB ID: 6DJN
Resolution: 3.1 Å

ADP state

PDB ID: 1J6Z
Resolution: 1.54 Å

F-ACTIN

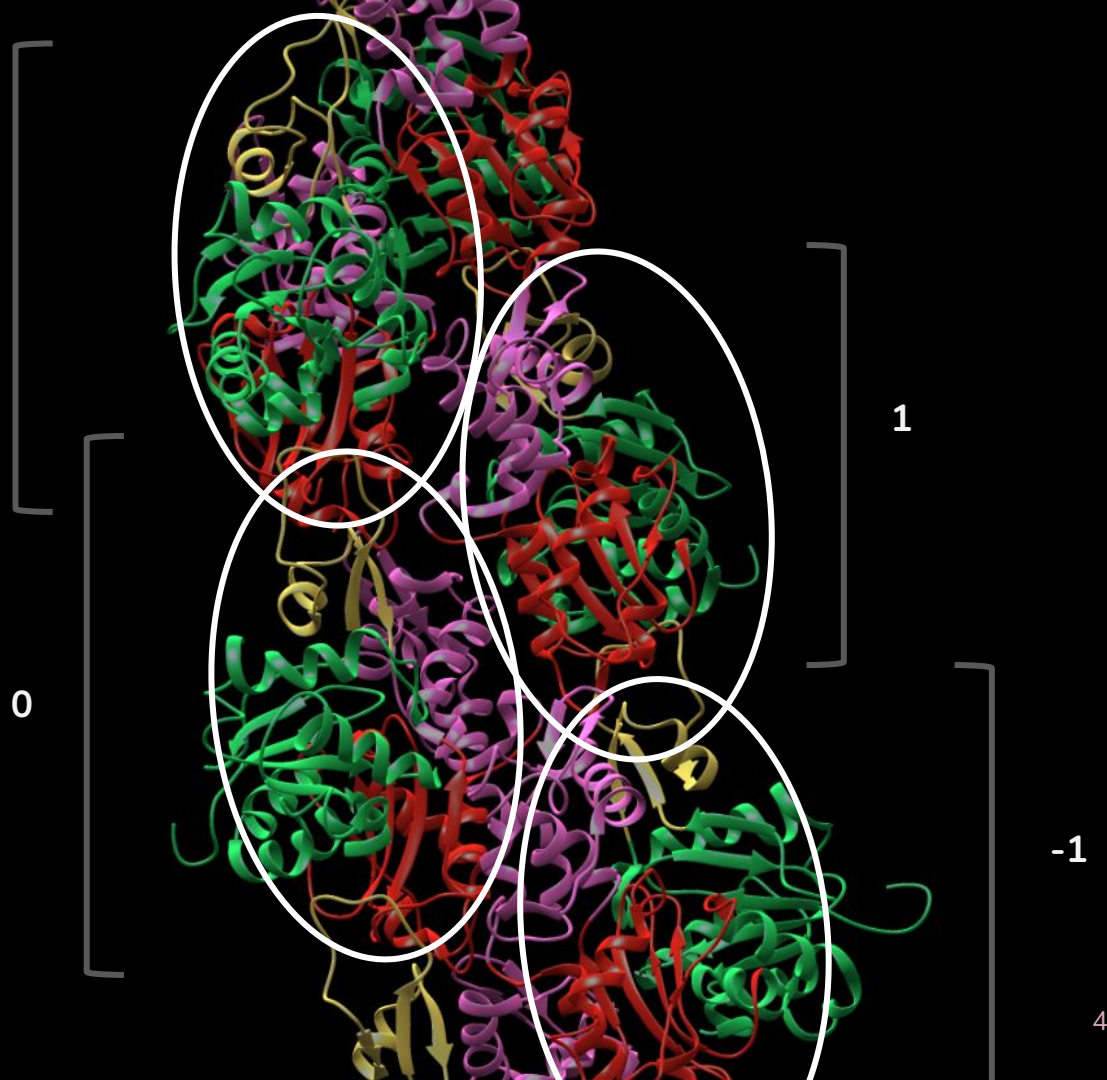
F-actin interactions

SUBDOMAIN 1

SUBDOMAIN 2

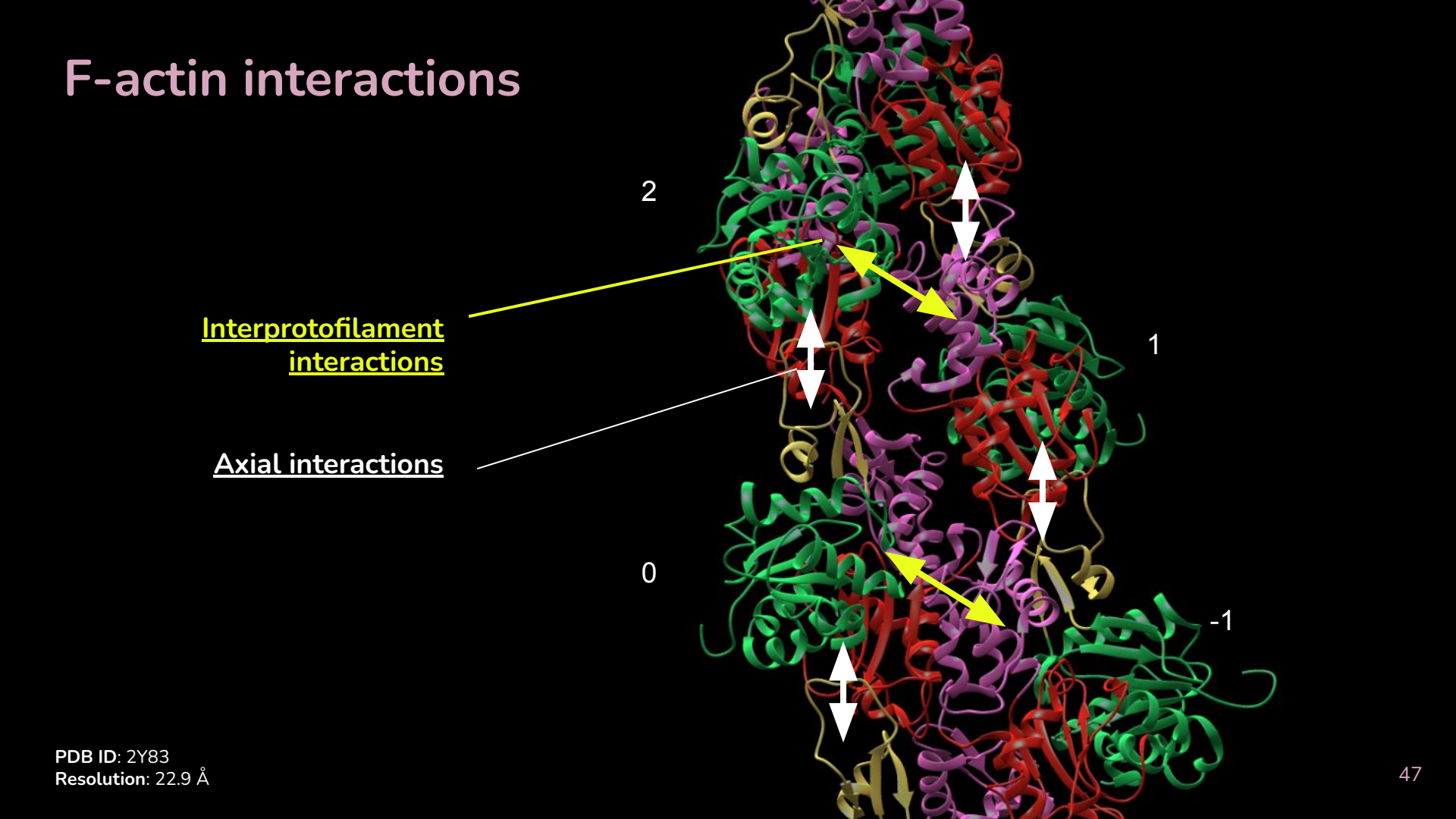
SUBDOMAIN 3

SUBDOMAIN 4



PDB ID: 2Y83
Resolution: 22.9 Å

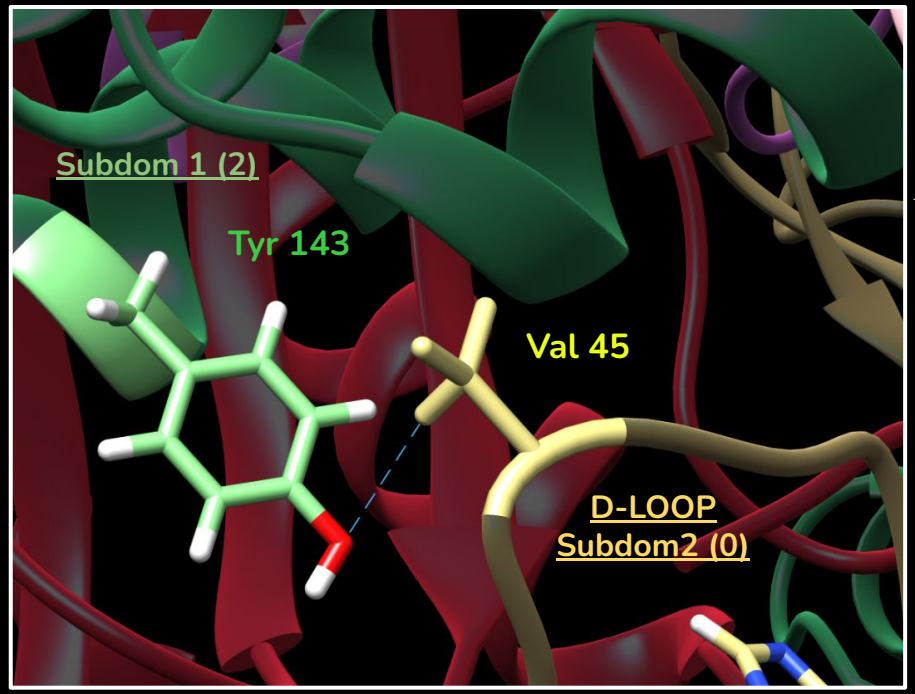
F-actin interactions



PDB ID: 2Y83
Resolution: 22.9 Å

F-actin interactions

Axial	Interprotofilament



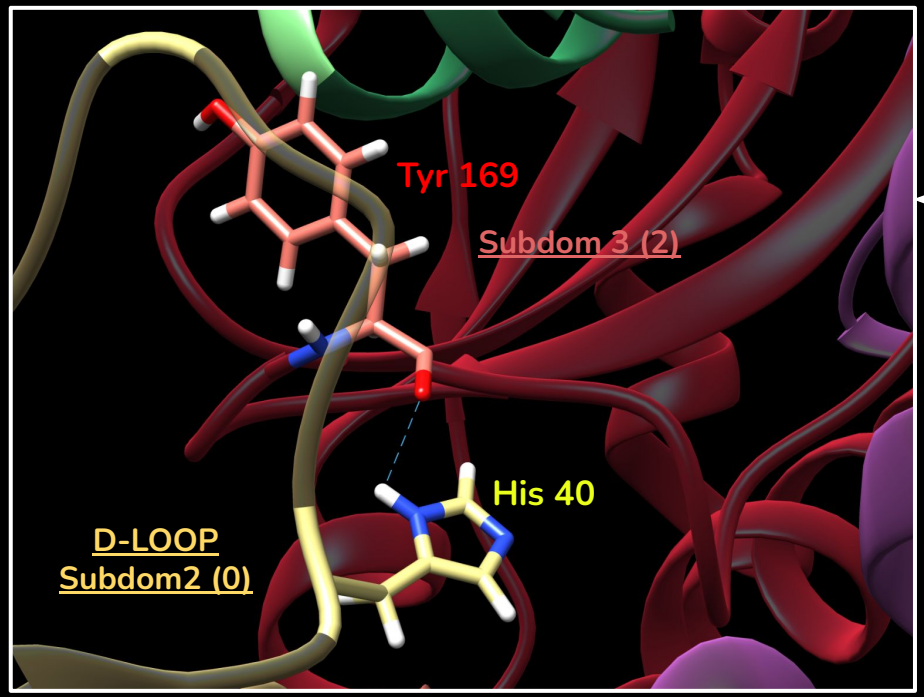
3
2
1
0
-1

D-loop (0) ↔ Subdom 1,3 (2)
Subdom 2 (0) ↔ Subdom 3 (2)
Subdom 4 (0) ↔ Subdom 3 (2)

PDB ID: 2Y83
Resolution: 22.9 Å

F-actin interactions

Axial	Interprotofilament



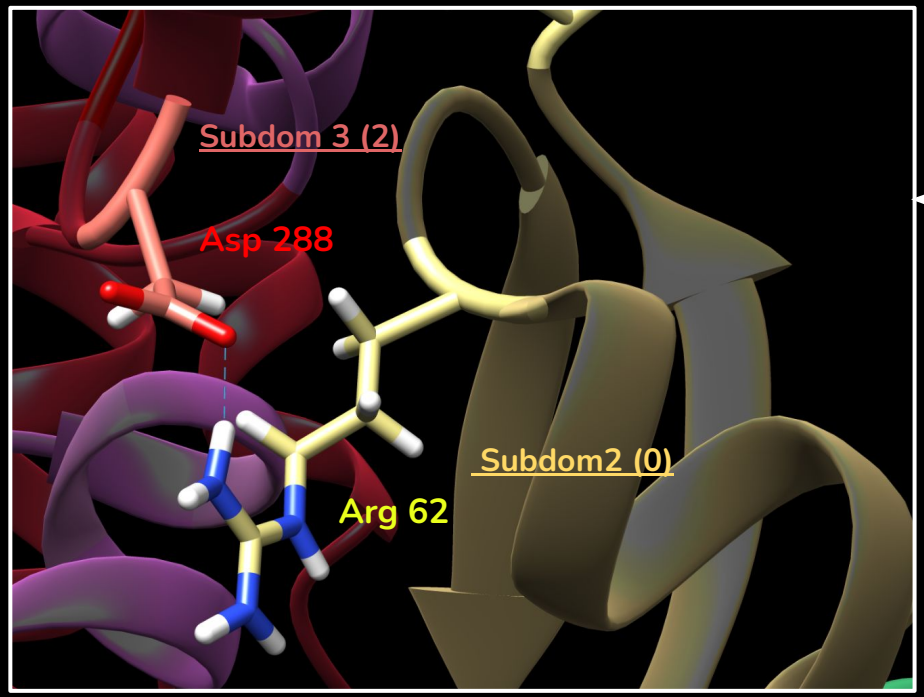
PDB ID: 2Y83
Resolution: 22.9 Å

3
2
1
0
-1

D-loop (0) ↔ Subdom 1,3 (2)
Subdom 2 (0) ↔ Subdom 3 (2)
Subdom 4 (0) ↔ Subdom 3 (2)

F-actin interactions

Axial	Interprotofilament



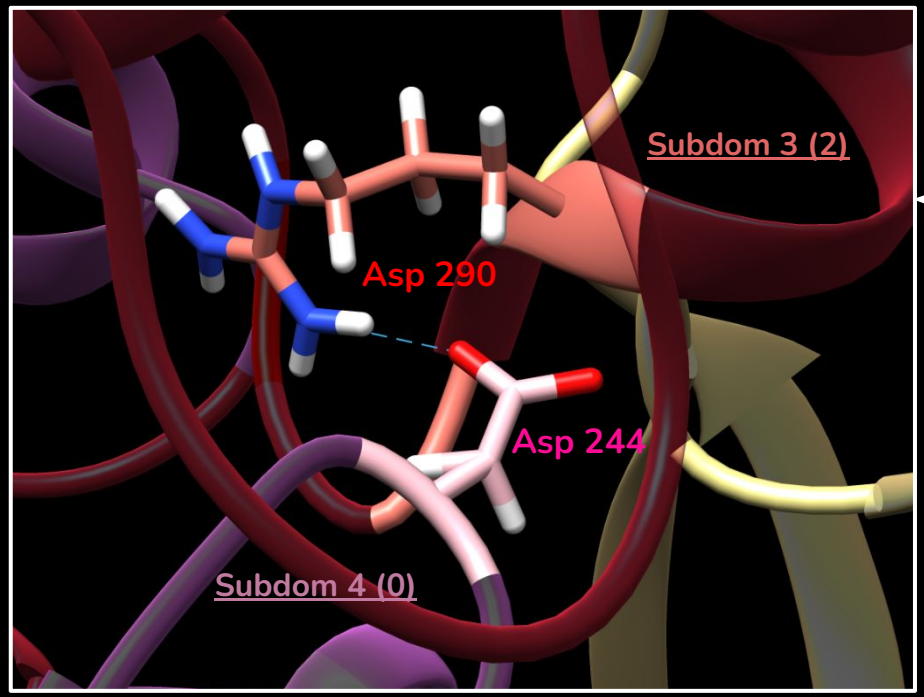
3
2
1
0
-1

D-loop (0) ↔ Subdom 1,3 (2)
Subdom 2 (0) ↔ Subdom 3 (2)
Subdom 4 (0) ↔ Subdom 3 (2)

PDB ID: 2Y83
Resolution: 22.9 Å

F-actin interactions

Axial	Interprotofilament



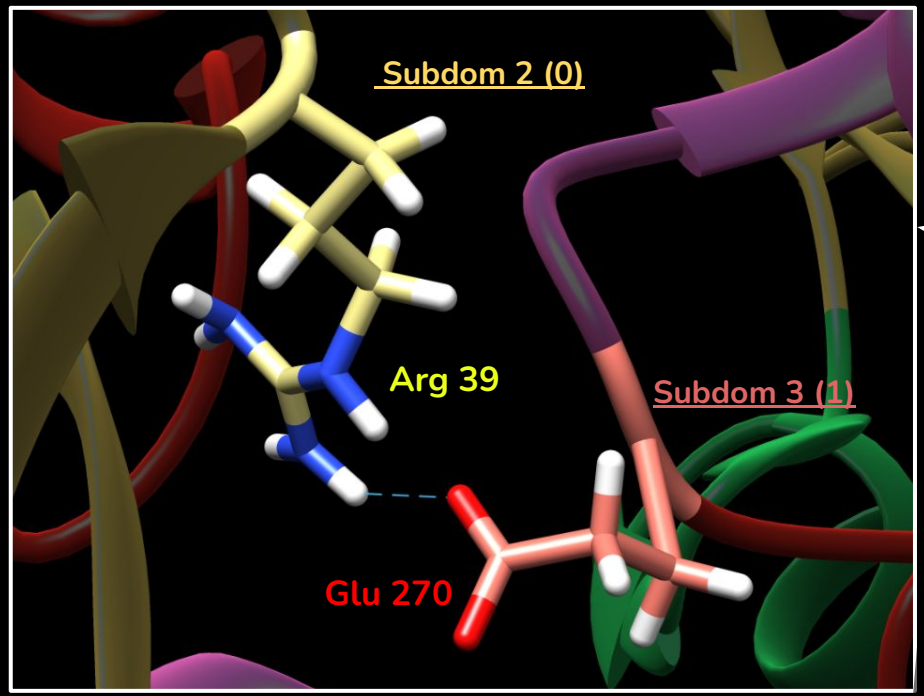
3
2
1
0
-1

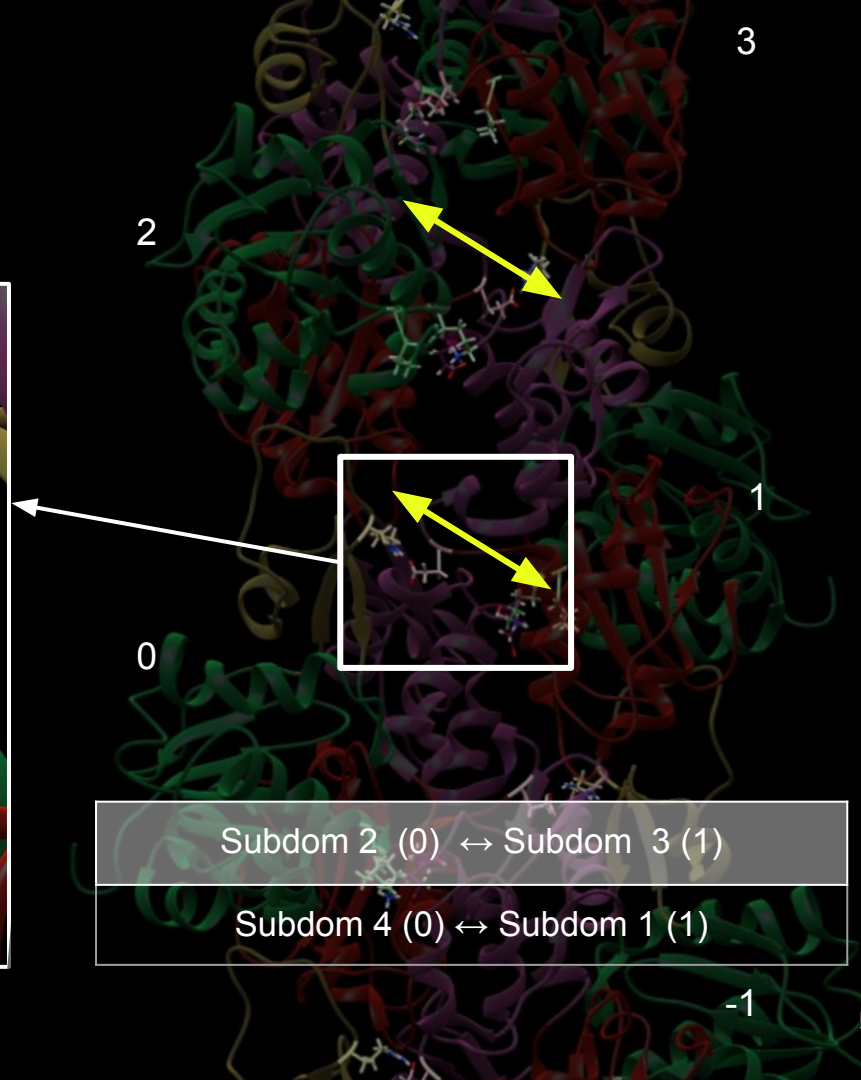
D-loop (0) ↔ Subdom 1,3 (2)
Subdom 2 (0) ↔ Subdom 3 (2)
Subdom 4 (0) ↔ Subdom 3 (2)

PDB ID: 2Y83
Resolution: 22.9 Å

F-actin interactions

Axial	Interprotofilament



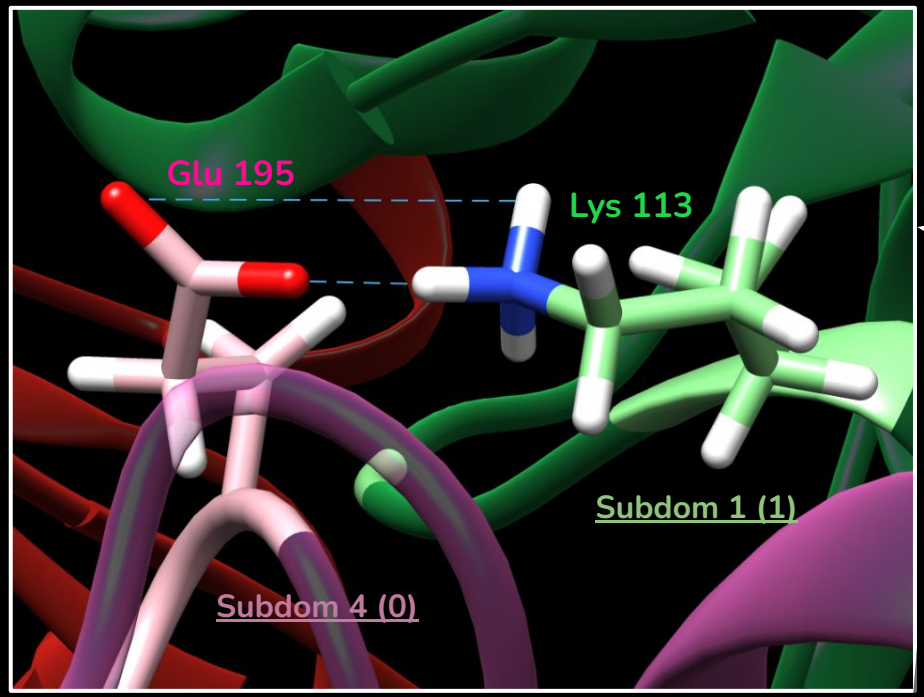


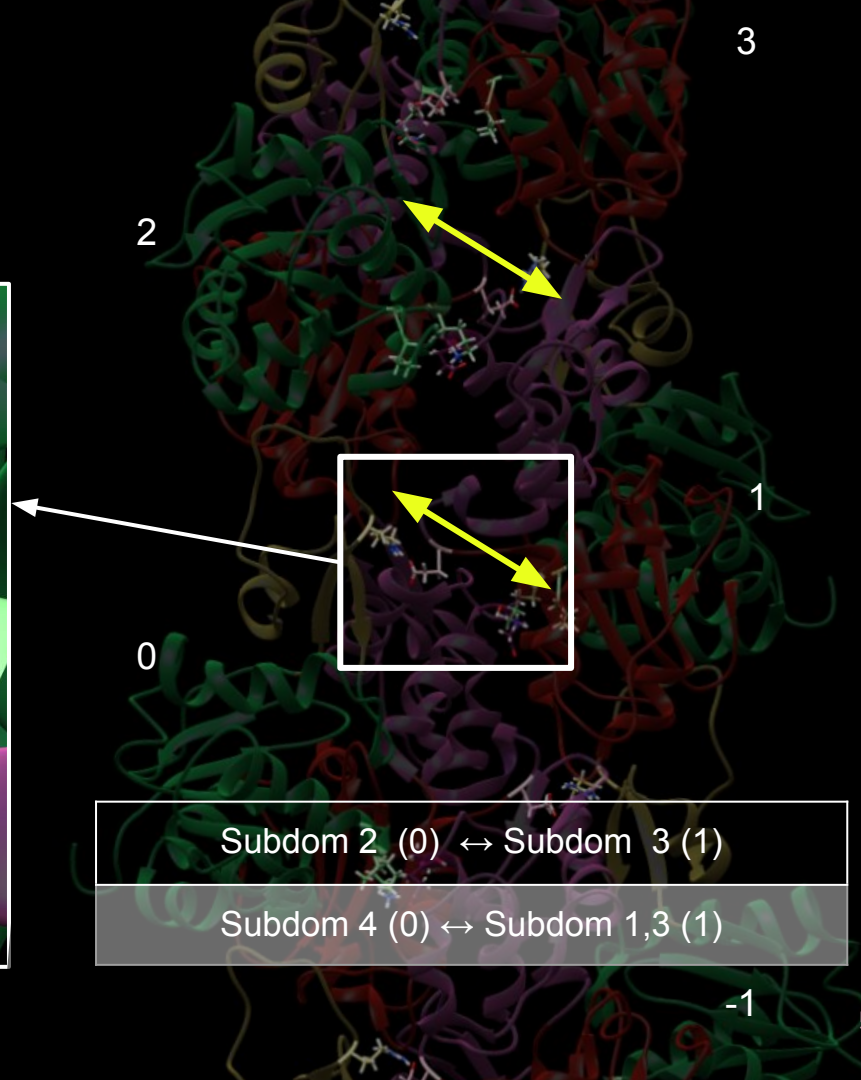
Subdom 2 (0) ↔ Subdom 3 (1)
Subdom 4 (0) ↔ Subdom 1 (1)

PDB ID: 2Y83
Resolution: 22.9 Å

F-actin interactions

Axial	Interprotofilament



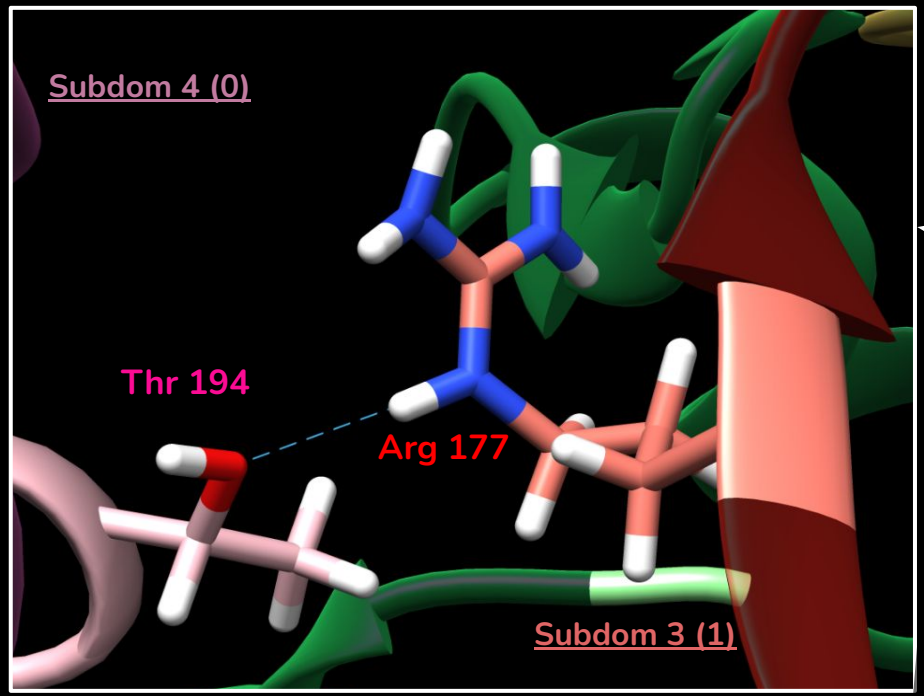


Subdom 2 (0) ↔ Subdom 3 (1)
Subdom 4 (0) ↔ Subdom 1,3 (1)

PDB ID: 2Y83
Resolution: 22.9 Å

F-actin interactions

Axial	Interprotofilament



3

2

1

0

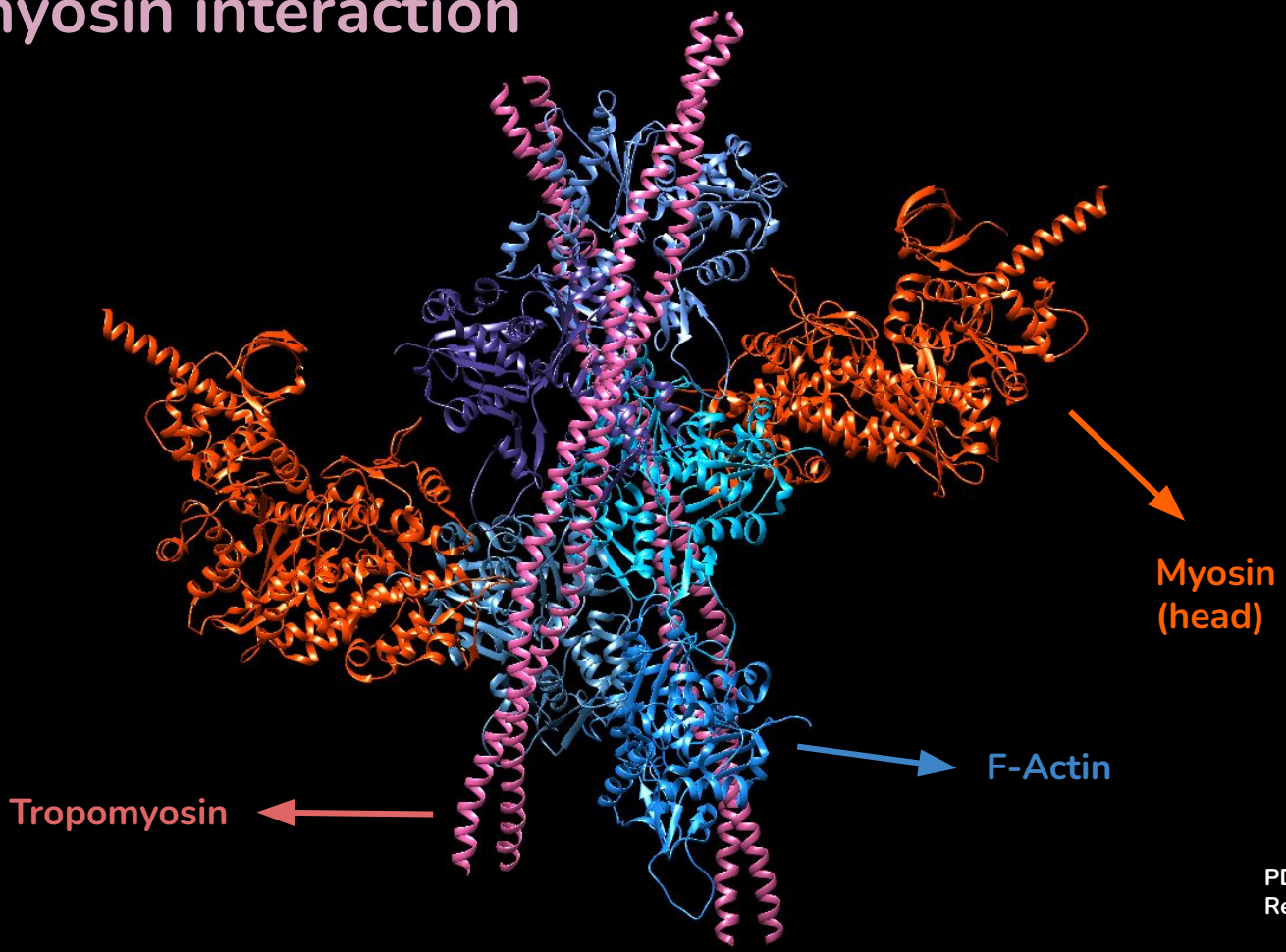
-1

Subdom 2 (0) ↔ Subdom 3 (1)
Subdom 4 (0) ↔ Subdom 1,3 (1)

PDB ID: 2Y83
Resolution: 22.9 Å

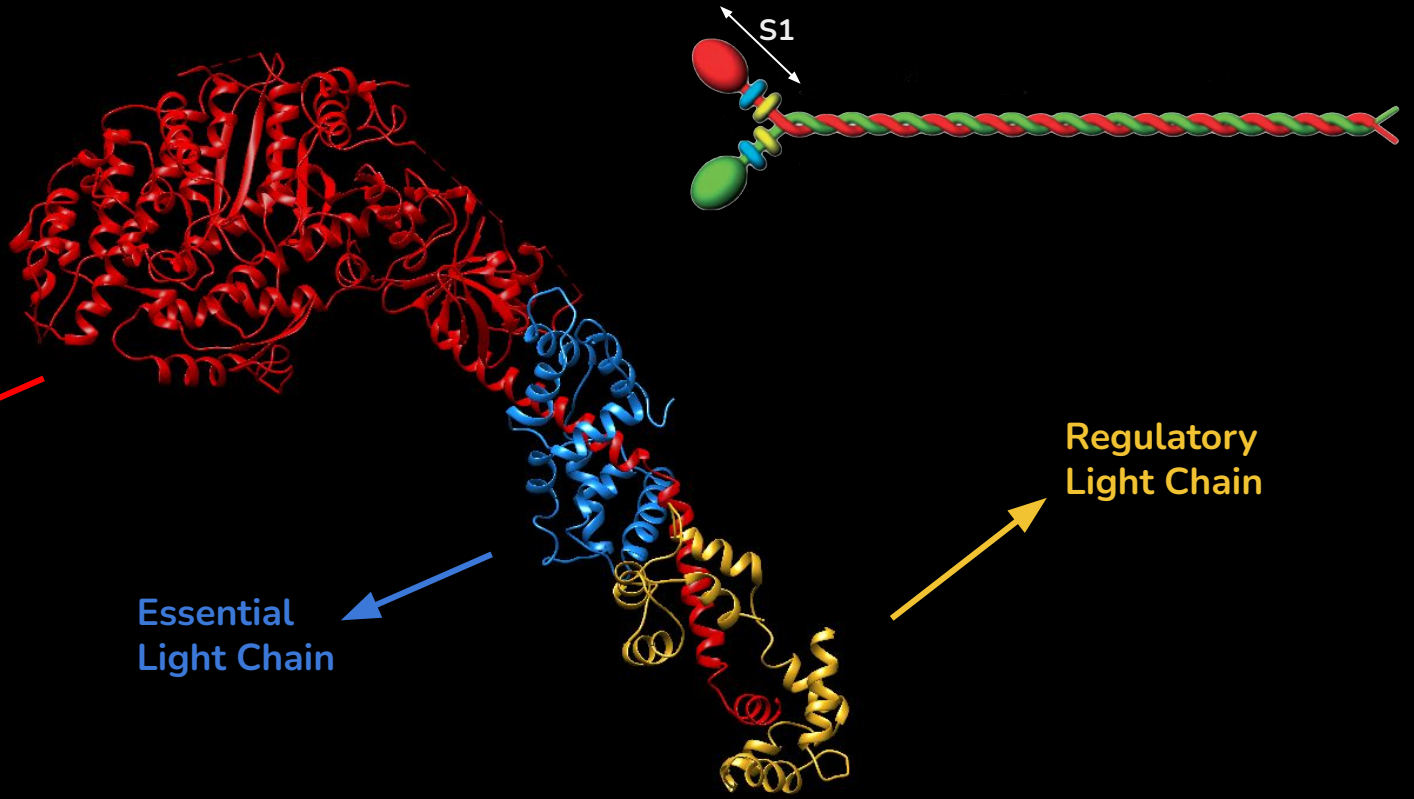
ACTIN - MYOSIN

Actin-myosin interaction



PDB ID: 5JLH
Resolution: 3.90 Å

Actin-myosin interaction: Myosin

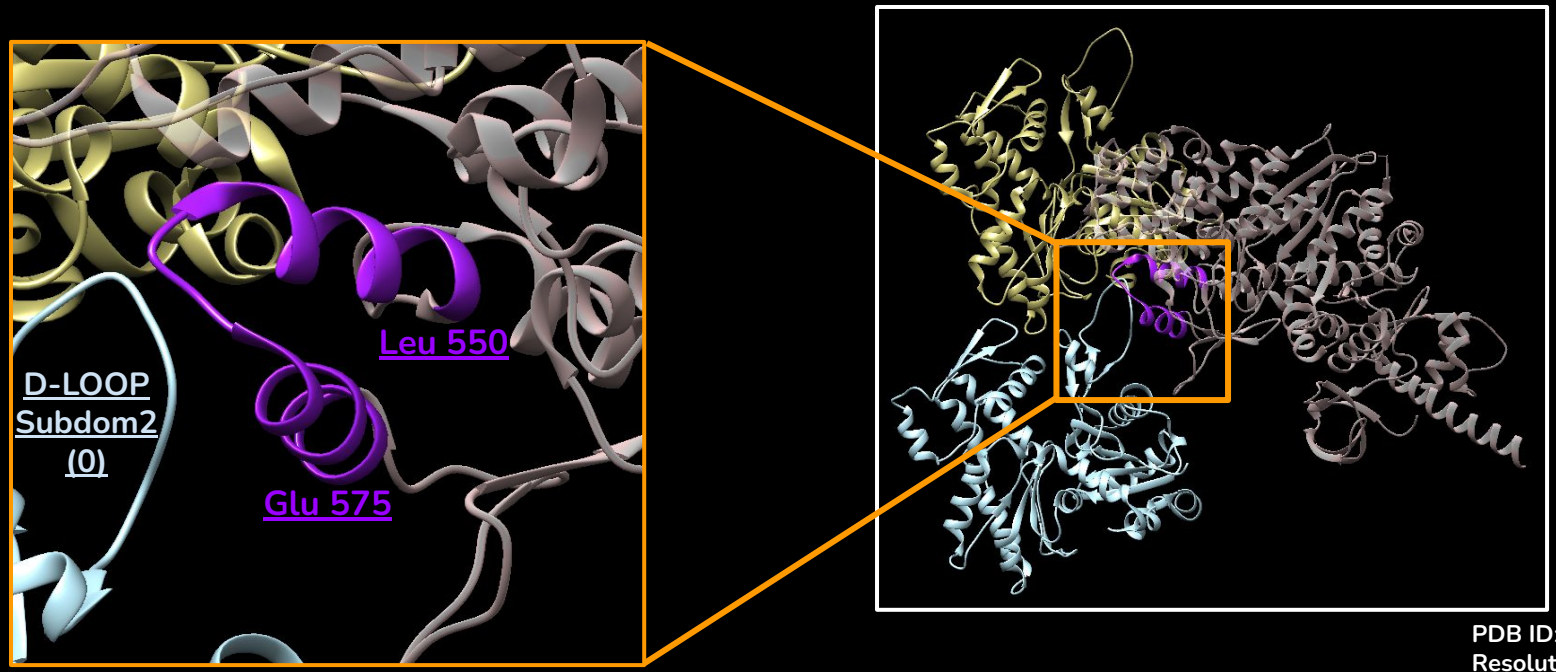


PDB ID: 1B7T
Resolution: 2.50 Å

Actin-myosin interaction

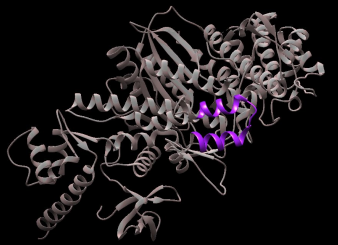
HLH	CM-Loop	Loop-2	Activation loop

Helix-Loop-Helix motif (Leu 550 - Glu 575) establishes hydrophobic and electrostatic interactions



PDB ID: 5jlh
Resolution: 3.90 Å

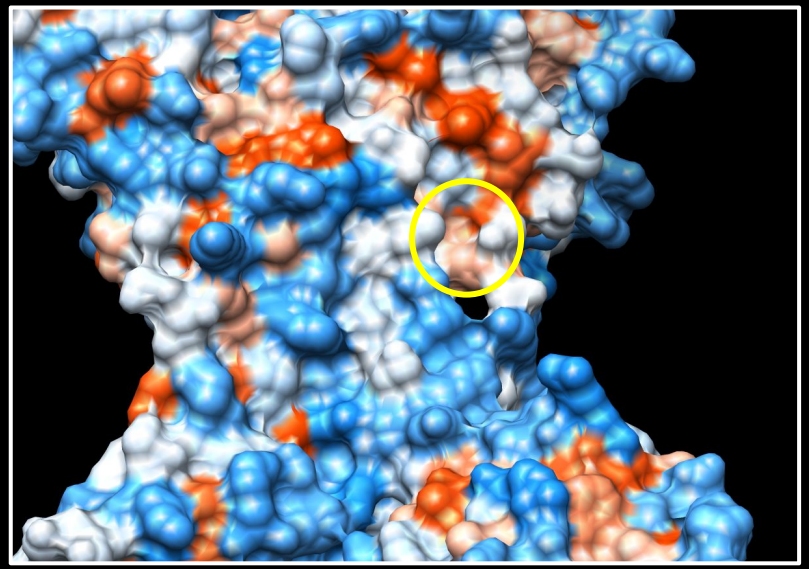
Actin-myosin interaction

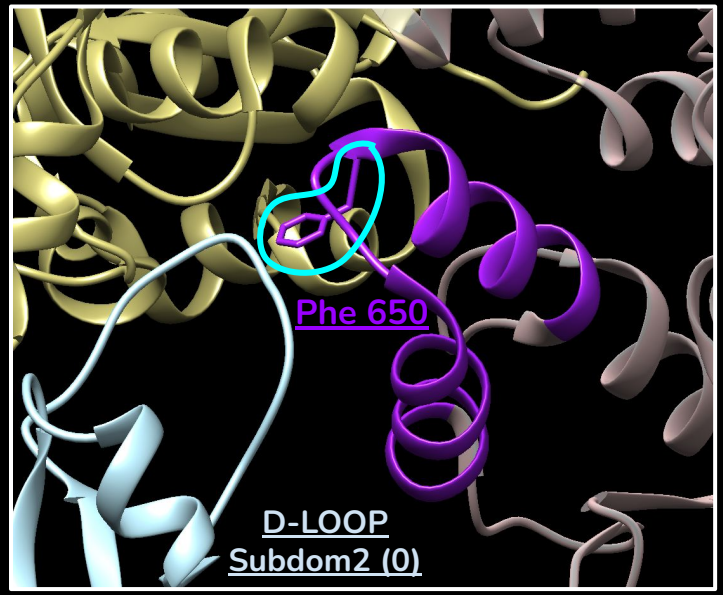


HLH	CM-Loop	Loop-2	Activation loop

Hydrophobic interactions:

- Actin hydrophobic cleft (SD1, SD3 and D-loop)
- HLH Phe 650

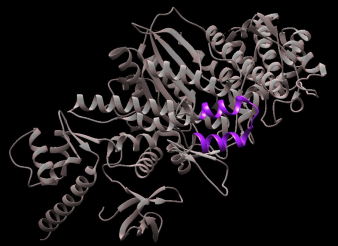




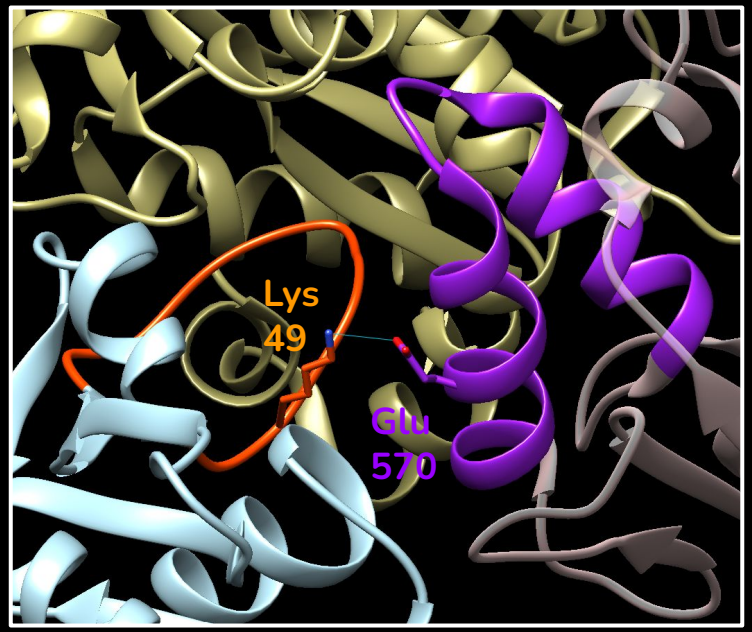
PDB ID: 5JLH
Resolution: 3.90 Å

Actin-myosin interaction

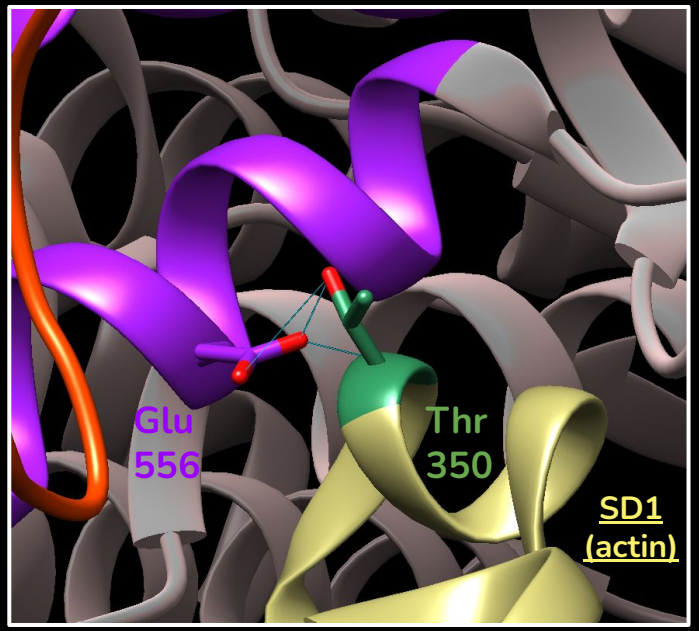
HLH	CM-Loop	Loop-2	Activation loop



We can find two electrostatic interactions (probably salt bridges):



Distance: 3,479 Å



Distance: 3,29 and 2,27 Å

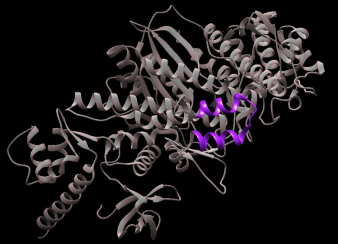
Mutation E556Q

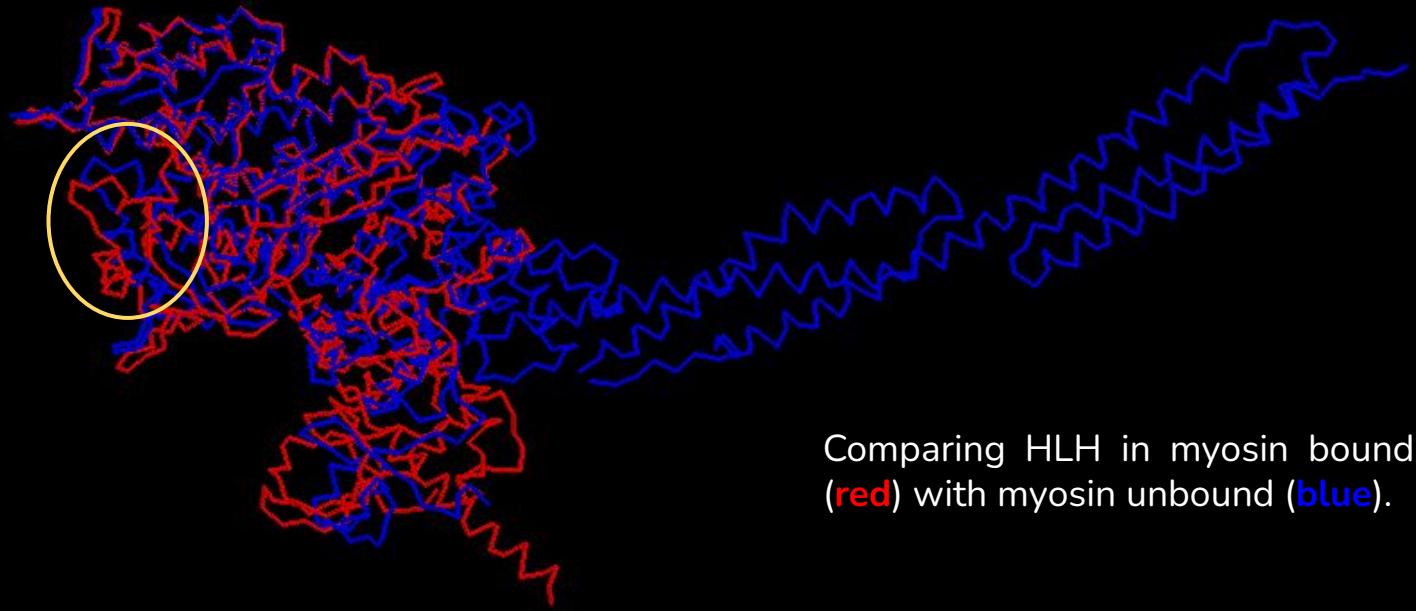
- Reduction of the F-actin binding affinity

PDB ID: 5JLH
Resolution: 3.90 Å

Actin-myosin interaction

HLH	CM-Loop	Loop-2	Activation loop

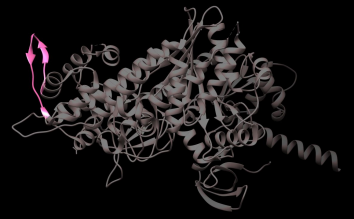




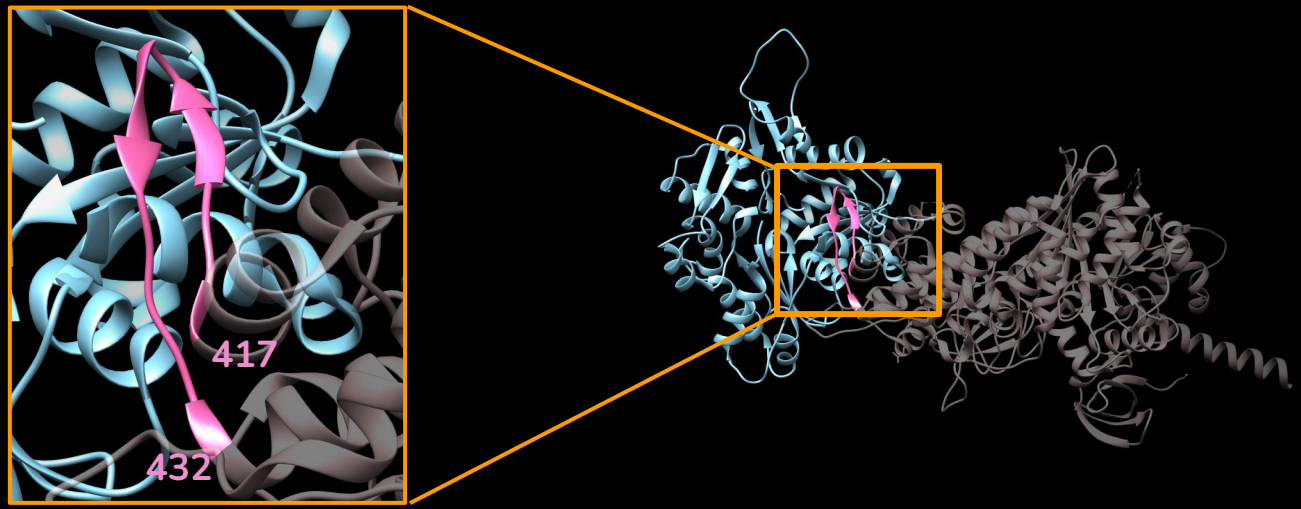
Comparing HLH in myosin bound to actin (red) with myosin unbound (blue).

PDB ID: 5JLH / 5I4E
Resolution: 3.90 / 2.25 Å

Actin-myosin interaction



HLH	CM-Loop	Loop-2	Activation loop

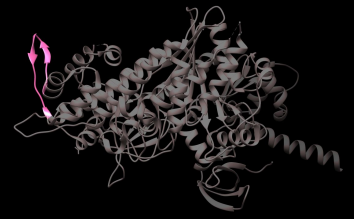


The Cardiomyopathy loop (Thr 417 - Thr 432) is formed by an antiparallel β-strand pair.

We find hydrophobic and electrostatic interactions

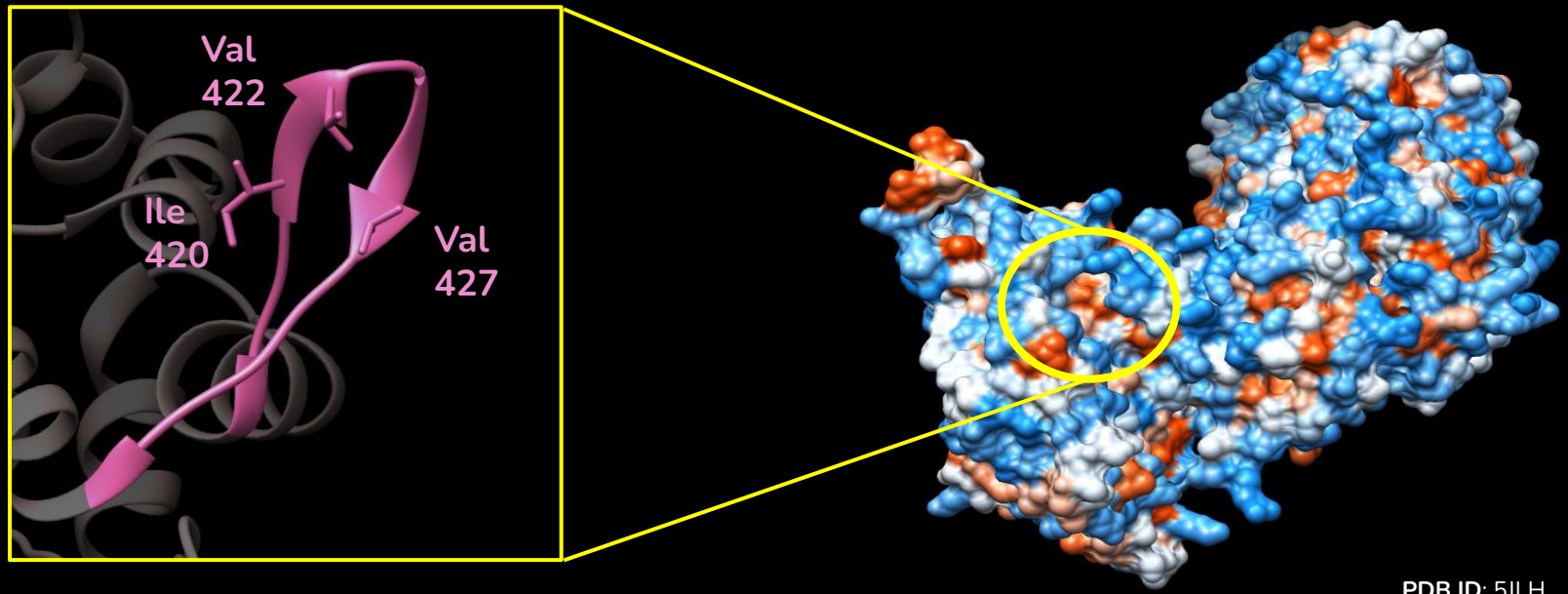
PDB ID: 5JLH
Resolution: 3.90 Å

Actin-myosin interaction



HLH	CM-Loop	Loop-2	Activation loop

Hydrophobic interactions:



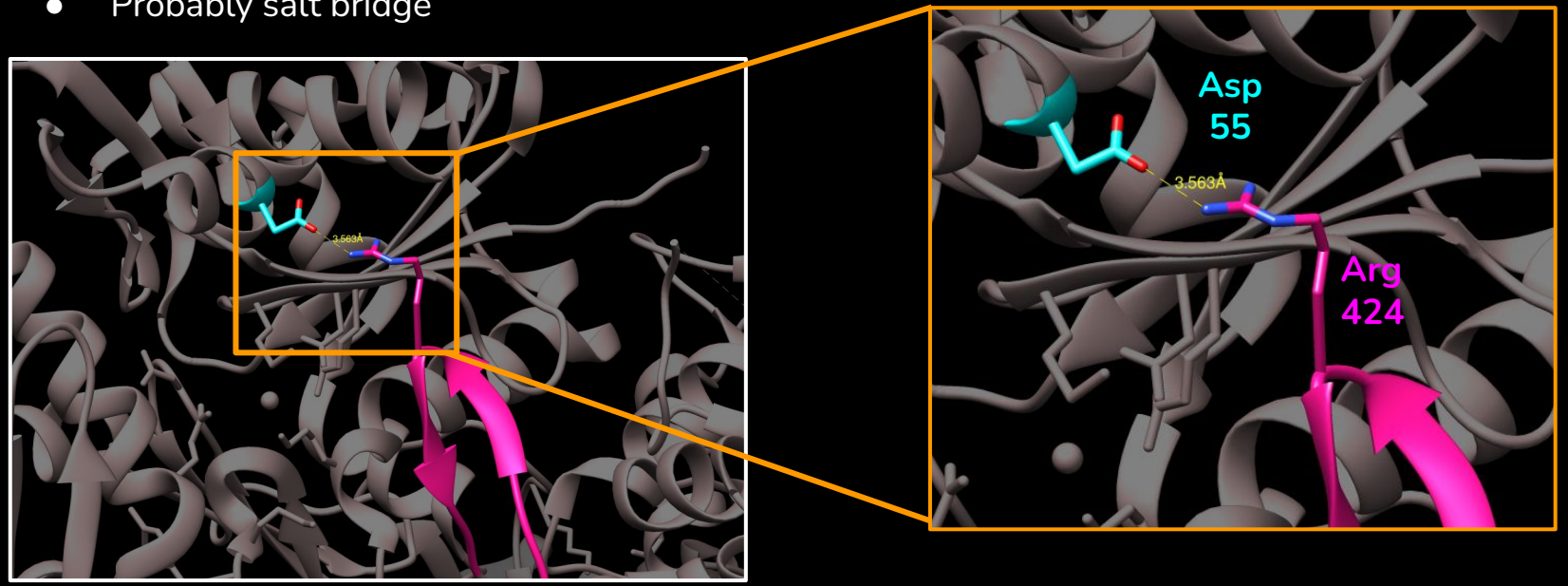
PDB ID: 5JLH
Resolution: 3.90 Å

Actin-myosin interaction

HLH	CM-Loop	Loop-2	Activation loop

Electrostatic interactions

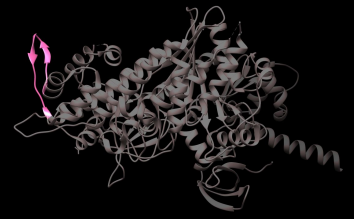
- Probably salt bridge



Distance: 3,563 Å

PDB ID: 5JLH
Resolution: 3.90 Å

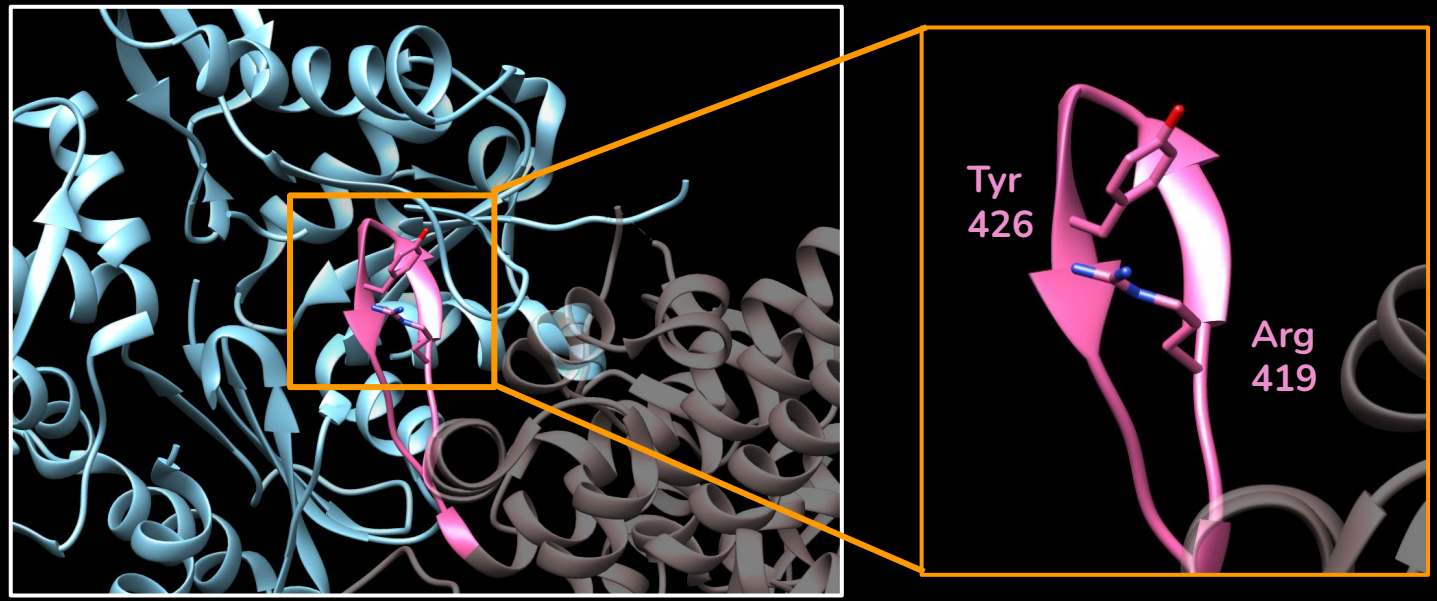
Actin-myosin interaction



HLH	CM-Loop	Loop-2	Activation loop

Special mention: Arg 419

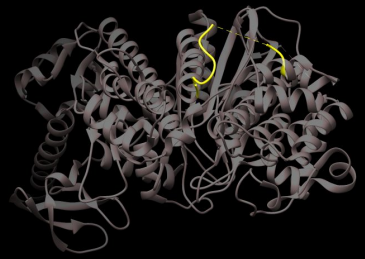
- Highly conserved → interaction with actin?



PDB ID: 5JLH
Resolution: 3.90 Å

Actin-myosin interaction

HLH	CM-Loop	Loop-2	Activation loop



The Loop-2 (Trp 638 – Thr 669) is crucial in initial binding with F-actin.

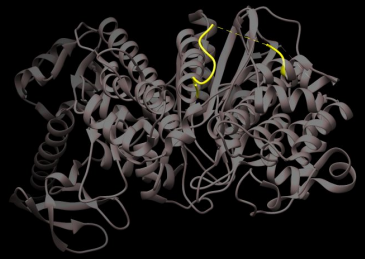
Hydrophobic interactions (SD1)

Base of the loop is ordered, but the rest is more flexible (Leu 647 to Arg 663 are not well resolved).

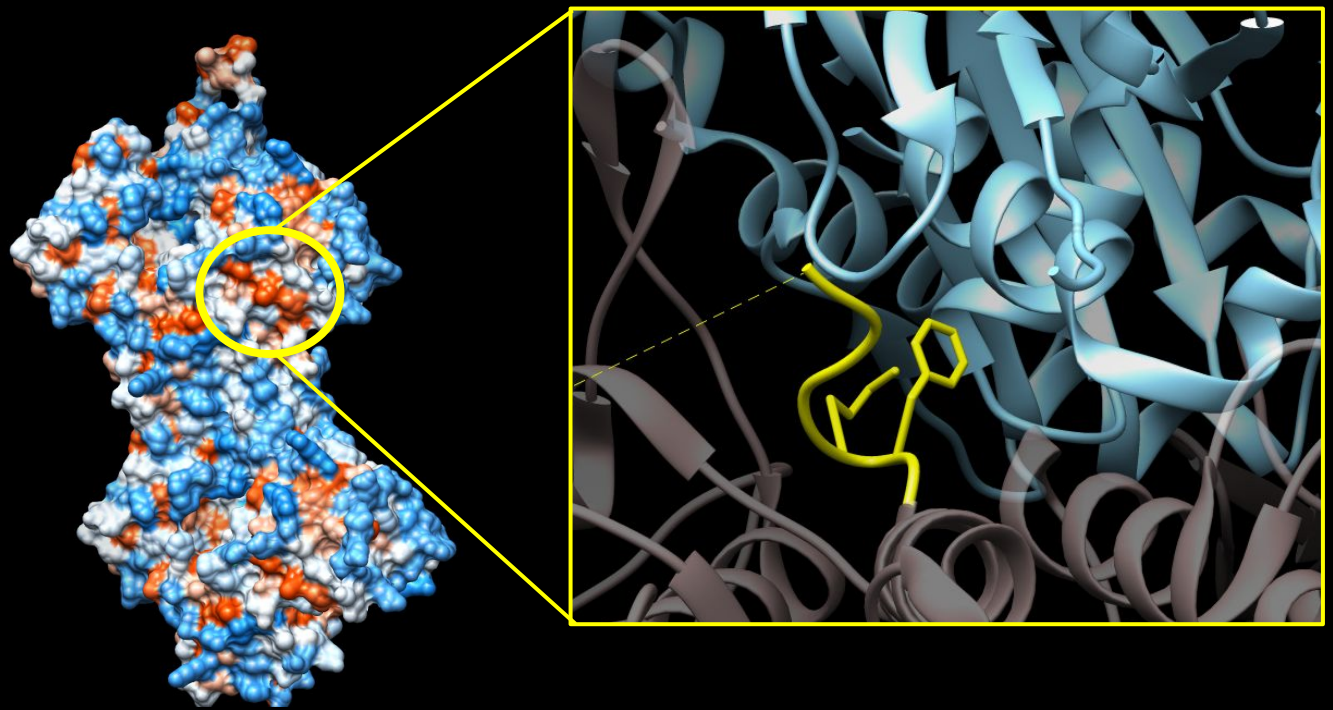
PDB ID: 5I4E
Resolution: 2.25 Å

Actin-myosin interaction

HLH	CM-Loop	Loop-2	Activation loop



Hydrophobic interaction:



Conserved hydrophobic patch:

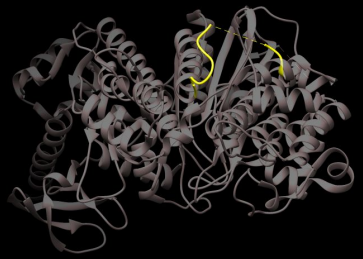
- Met 666
- Phe 667

Actin SD1 domain

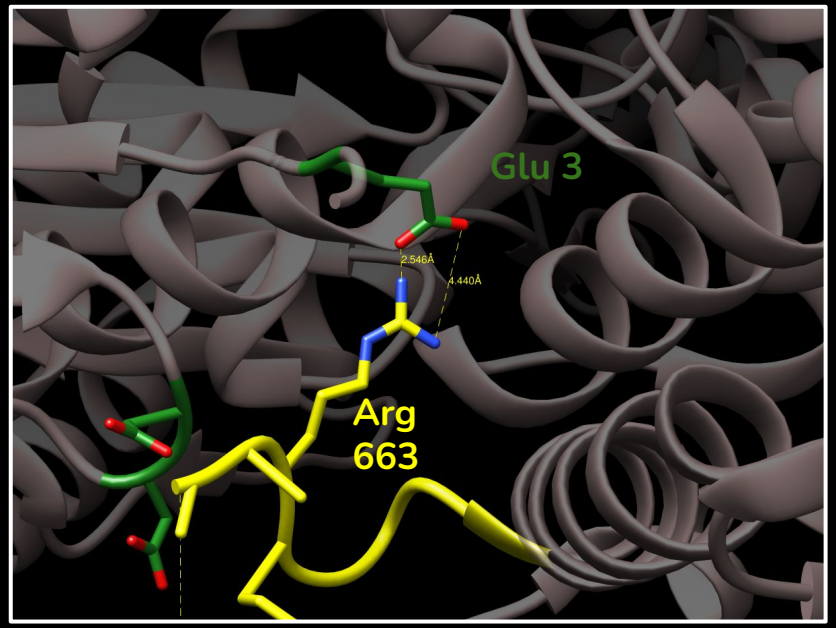
PDB ID: 5JLH
Resolution: 3.90 Å

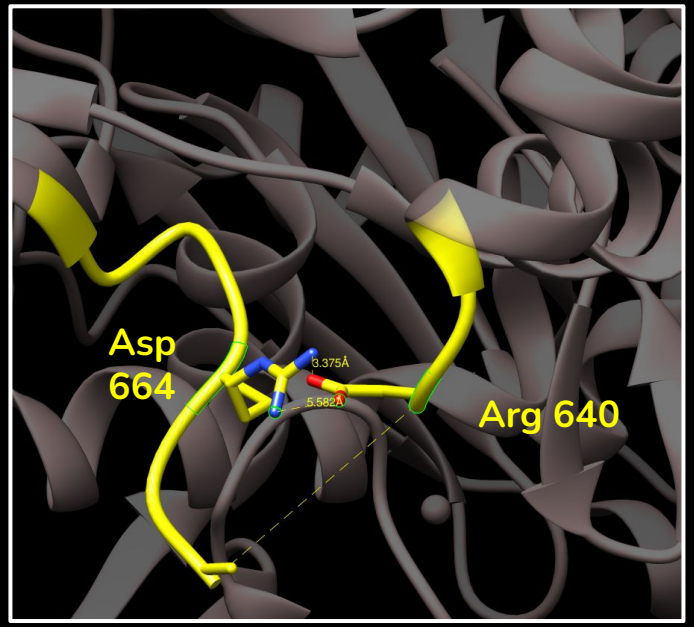
Actin-myosin interaction

HLH	CM-Loop	Loop-2	Activation loop



Electrostatic interaction:



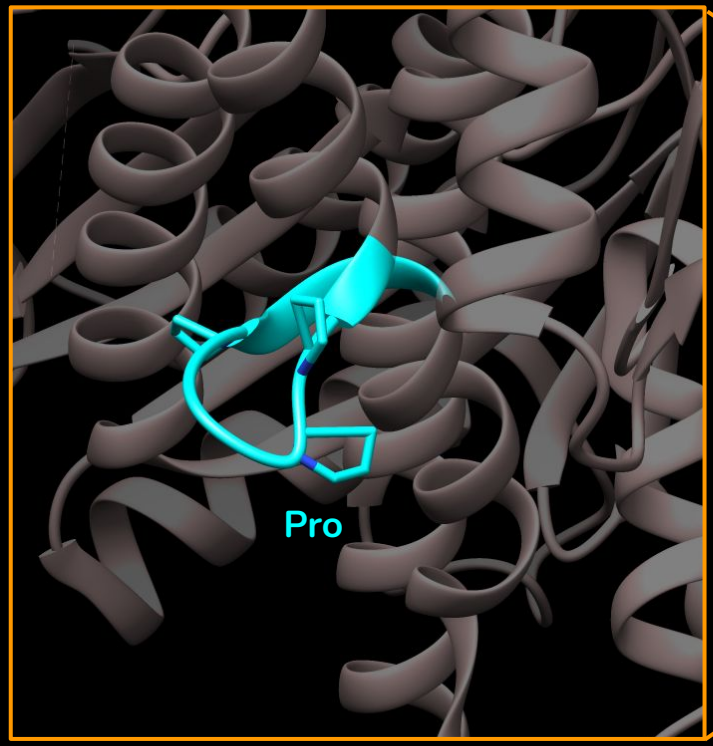


They probably form a salt bond, but again … we can not know for sure.

PDB ID: 5JLH
Resolution: 3.90 Å

Actin-myosin interaction

HLH	CM-Loop	Loop-2	Activation loop

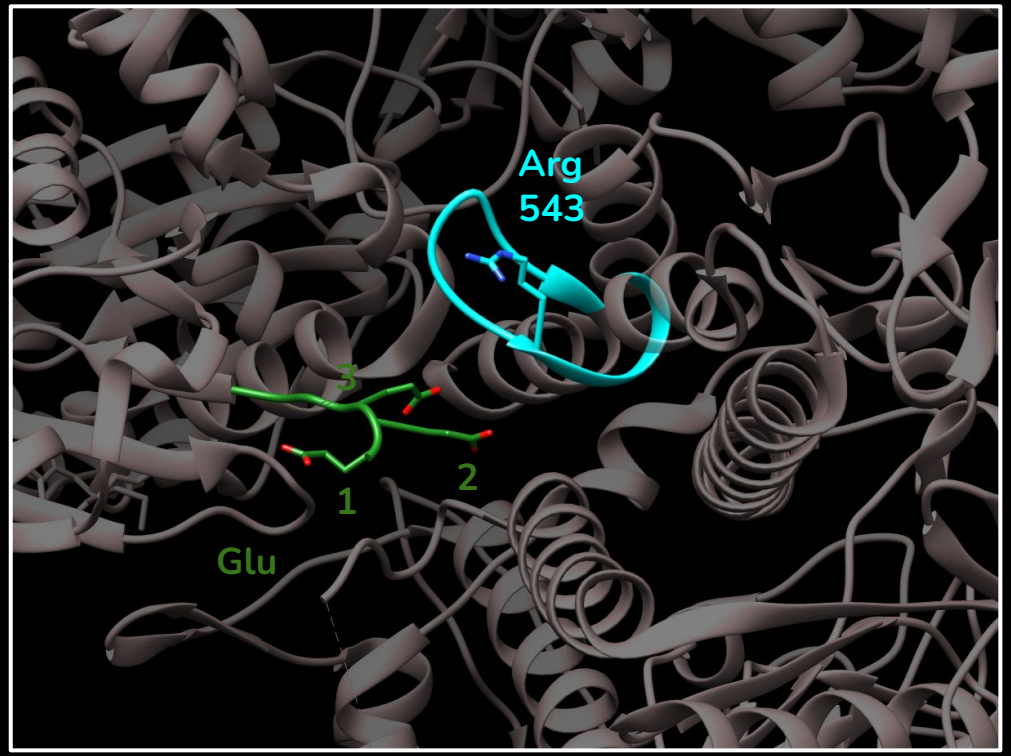


The “Activation loop” (Ile 541– Gly 549) is a Proline-rich group → activation of myosin?

PDB ID: 5JLH
Resolution: 3.90 Å

Actin-myosin interaction

HLH	CM-Loop	Loop-2	Activation loop

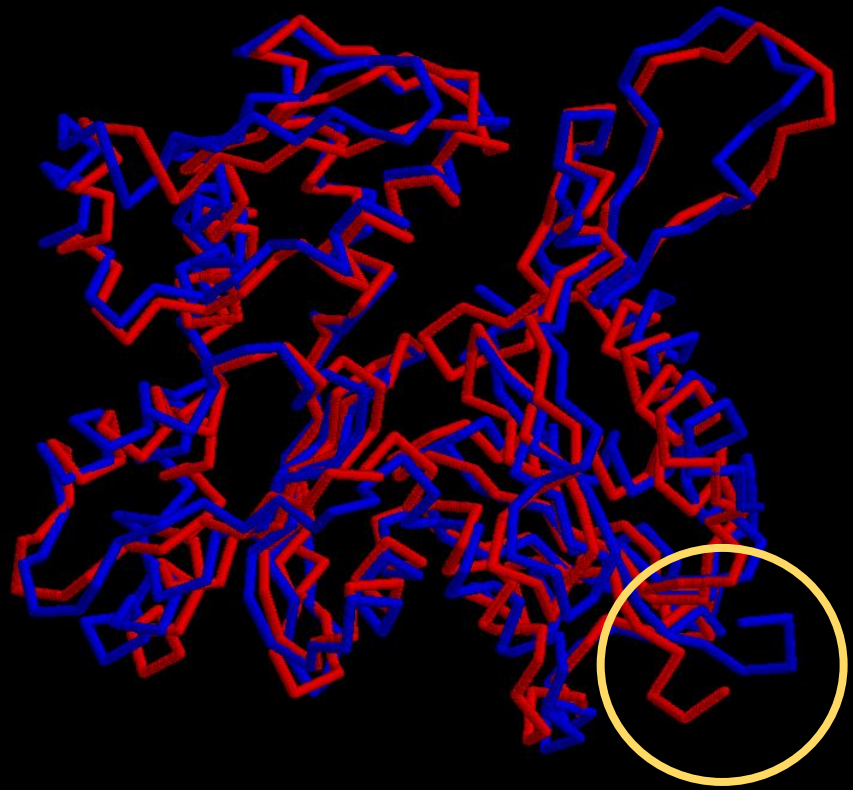


Activation loop → **Supporting loop**

1. It forms a positively charged basin (with Loop-2) that interacts with N terminus of actin (negatively charged).
2. Arg 543 (positively charged) points away from the N-terminal domain → Supporting loop.
3. No actin induced conformational changes (not shown) after binding

PDB ID: 5JLH
Resolution: 3.90 Å

Myosin induced conformational changes



The binding of myosin to F-actin induces a conformational change in the N-terminal domain

Minimal overall changes:

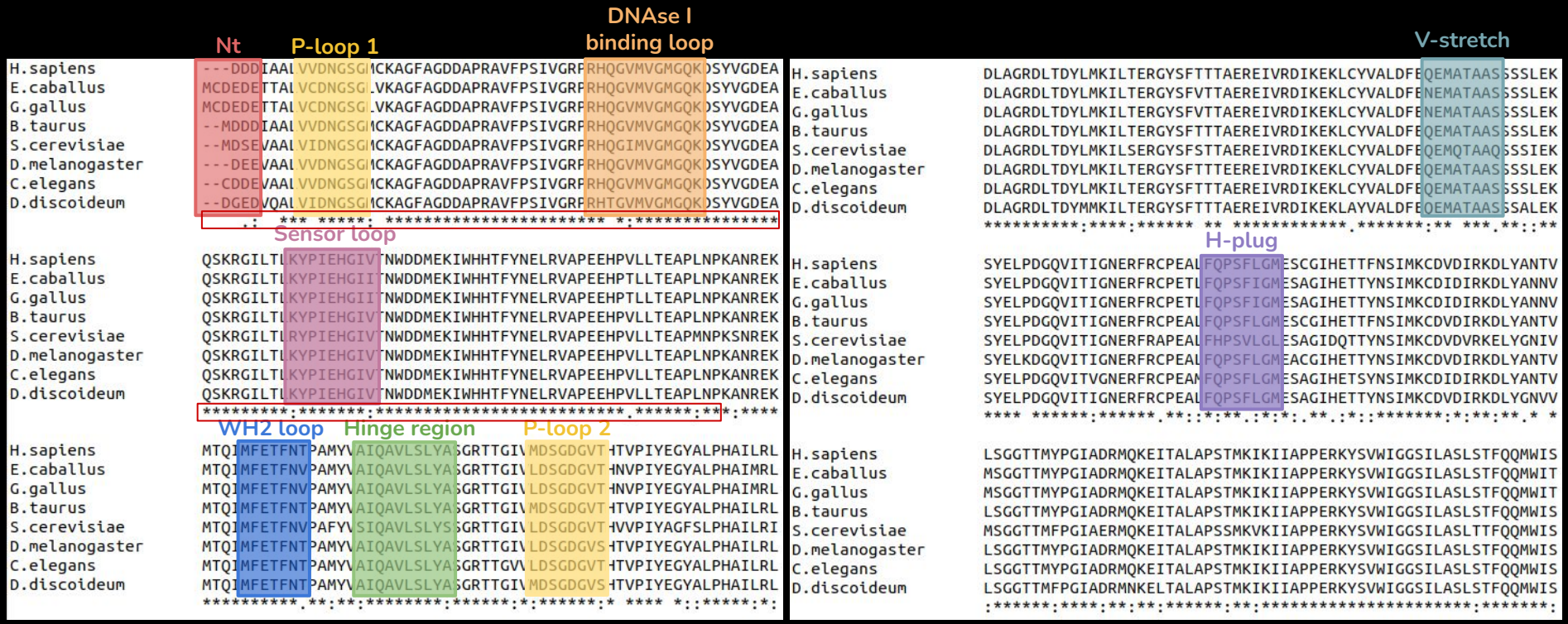
- Actin N-terminal domain is pulled (Arg in Loop-2 interacting with Glu 3)
- Partially resolved in F-actin but completely ordered in this structure

F-actin not bound to myosin
F-actin bound to myosin

PDB ID: 5JLH / 3EL2
Resolution: 3.90 / 2.50 Å

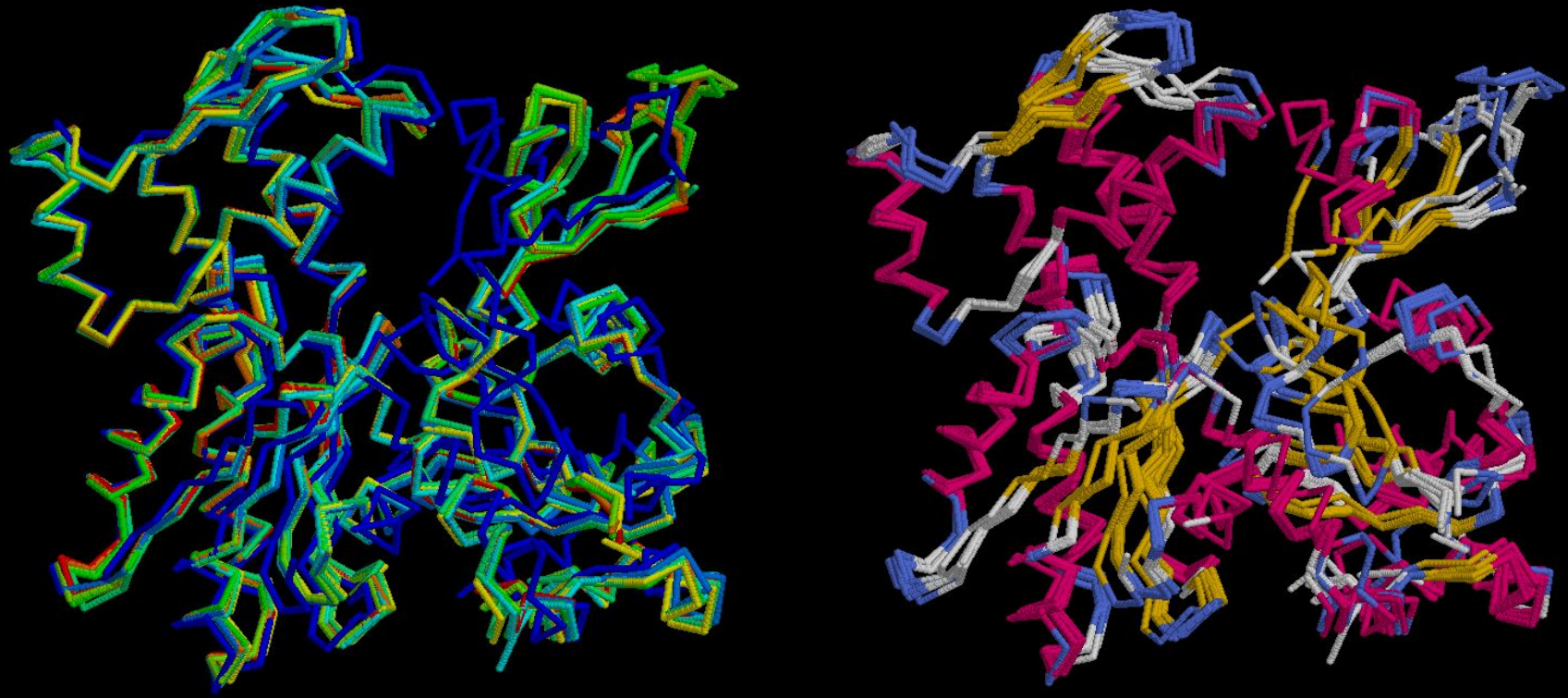
SEQUENCE AND STRUCTURAL ANALYSIS

Actin: Sequence alignment (ClustalW)



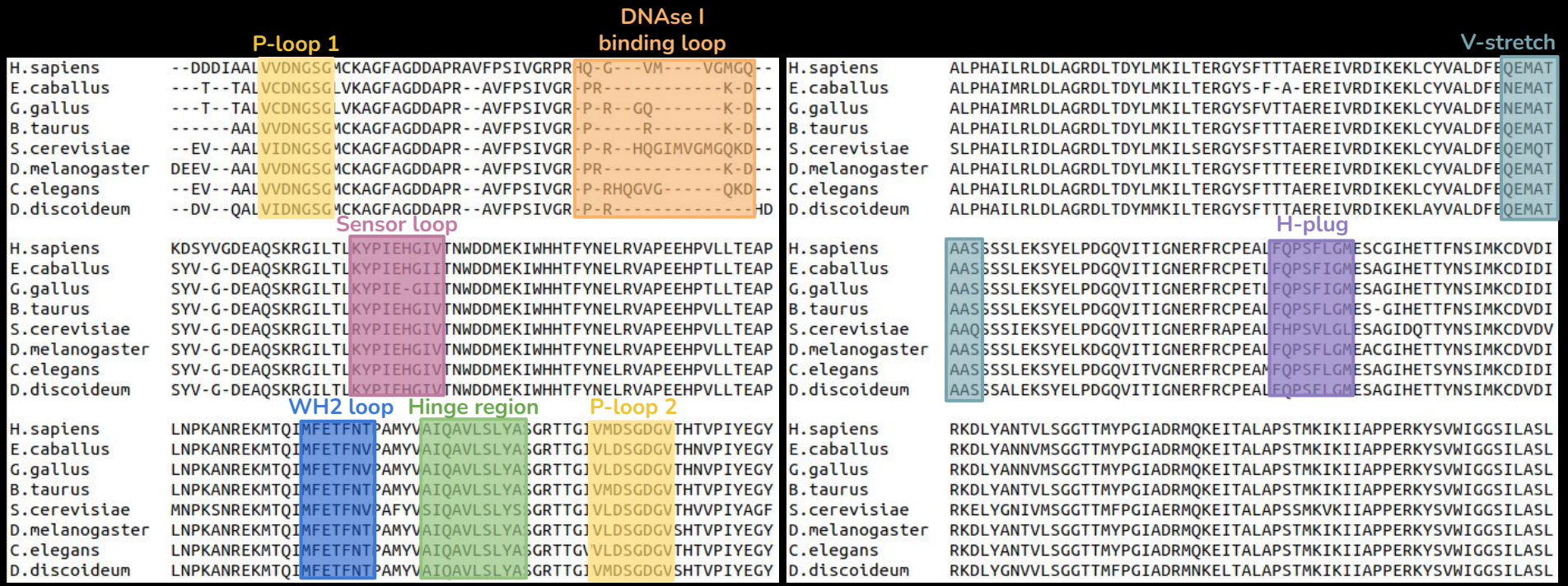
PDB ID: 1D4X, 2OAN, 1NMD, 1YAG, 1RGI, 1MDU, 2HF4, 3BYH
Resolution: 1.75 Å, 2.61 Å, 1.90 Å, 1.90 Å, 3.00 Å, 2.20 Å, 1.80 Å, 12 Å

Actin: Structural alignment



Superimposition of actin monomers of 8 different species using RasMol.
Colouring based on ‘Chain’ (left) and ‘Structure’ (right).

Actin: Structural alignment (STAMP)



Actin: Structural alignment (STAMP)

DNAse I binding loop

```
H.sapiens          --DDDIAALVVDNGSGMCKAGFAGDDAPRAVFPSIVGRPRHQ-G---VM----VGMGQ--
E.caballus         ---T--TALVCDNGSGLVKAGFAGDDAPR--AVFPSIVGR-PR----------K-D--
G.gallus           ---T--TALVCDNGSGLVKAGFAGDDAPR--AVFPSIVGR-P-R--GQ------K-D--
B.taurus           ------AALVVDNGSGMCKAGFAGDDAPR--AVFPSIVGR-P-----R------K-D--
S.cerevisiae       --EV--AALVIDNGSGMCKAGFAGDDAPR--AVFPSIVGR-P-R--HQGIMVGMGQKD--
D.melanogaster     DEEV--AALVVDNGSGMCKAGFAGDDAPR--AVFPSIVGR-PR----------K-D--
C.elegans          --EV--AALVVDNGSGMCKAGFAGDDAPR--AVFPSIVGR-P-RHQGVG-----QKD--
D.discoideum       --DV--QALVIDNGSGMCKAGFAGDDAPR--AVFPSIVGR-P-R----------HD

H.sapiens          KDSYVGDEAQSKRGILTLKYPIEHGIVTNWDDMEKIWHHTFYNELRVAPEEHPVLLTEAP
E.caballus         SYV-G-DEAQSKRGILTLKYPIEHGIITNWDDMEKIWHHTFYNELRVAPEEHPTLLTEAP
G.gallus           SYV-G-DEAQSKRGILTLKYPIE-GIITNWDDMEKIWHHTFYNELRVAPEEHPTLLTEAP
B.taurus           SYV-G-DEAQSKRGILTLKYPIEHGIVTNWDDMEKIWHHTFYNELRVAPEEHPVLLTEAP
S.cerevisiae       SYV-G-DEAQSKRGILTLRYPIEHGIVTNWDDMEKIWHHTFYNELRVAPEEHPVLLTEAP
D.melanogaster     SYV-G-DEAQSKRGILTLKYPIEHGIVTNWDDMEKIWHHTFYNELRVAPEEHPVLLTEAP
C.elegans          SYV-G-DEAQSKRGILTLKYPIEHGIVTNWDDMEKIWHHTFYNELRVAPEEHPVLLTEAP
D.discoideum       SYV-G-DEAQSKRGILTLKYPIEHGIVTNWDDMEKIWHHTFYNELRVAPEEHPVLLTEAP

H.sapiens          LNPKANREKMTQIMFETFNTPAMYVAIQAVLSLYASGRTTGIVMDSGDGVTHTVPIYEGY
E.caballus         LNPKANREKMTQIMFETFNVPAMYVAIQAVLSLYASGRTTGIVLDSGDGVTHNVPIYEGY
G.gallus           LNPKANREKMTQIMFETFNVPAMYVAIQAVLSLYASGRTTGIVLDSGDGVTHNVPIYEGY
B.taurus           LNPKANREKMTQIMFETFNTPAMYVAIQAVLSLYASGRTTGIVMDSGDGVTHTVPIYEGY
S.cerevisiae       MNPKSNREKMTQIMFETFNVPAFYVSIQAVLSLYSSGRTTGIVLDSGDGVTHVVPIYAGF
D.melanogaster     LNPKANREKMTQIMFETFNTPAMYVAIQAVLSLYASGRTTGIVLDSGDGVSHTVPIYEGY
C.elegans          LNPKANREKMTQIMFETFNTPAMYVAIQAVLSLYASGRTTGVVLDSGDGVTHTVPIYEGY
D.discoideum       LNPKANREKMTQIMFETFNTPAMYVAIQAVLSLYASGRTTGIVMDSGDGVSHTVPIYEGY
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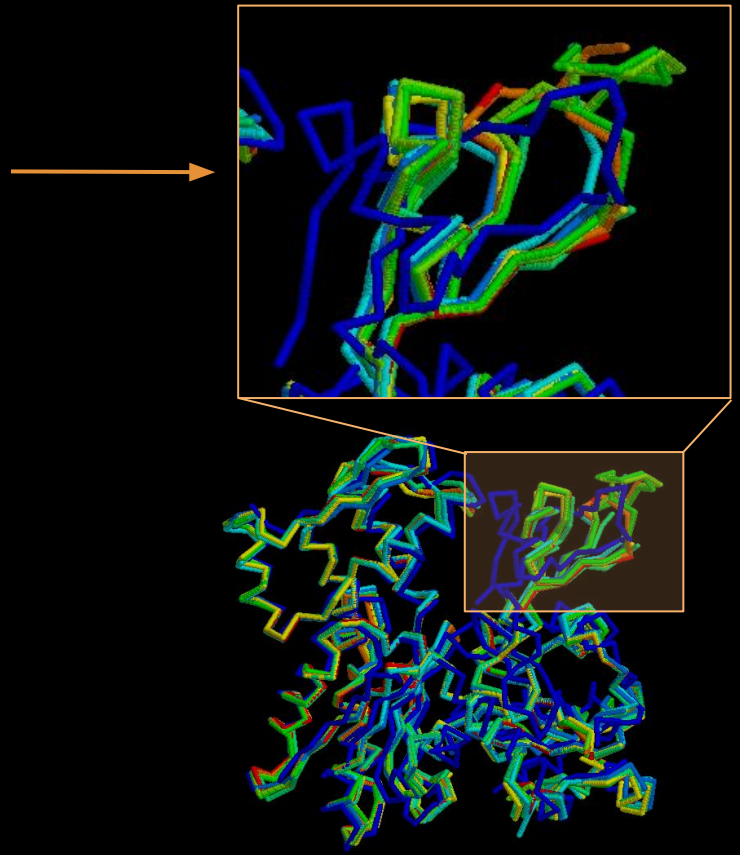


Actin: Structural alignment (STAMP)

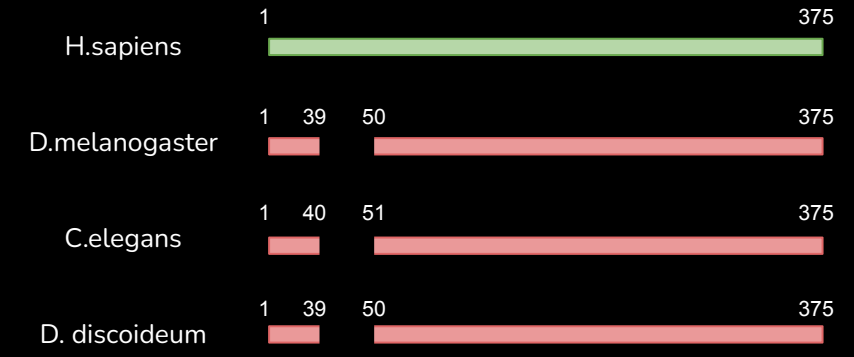
DNAse I binding loop

```
H.sapiens          --DDDIAALVVDNGSGMCKAGFAGDDAPRAVFPSIVGRPR-HQ-G---VM----VGMGQ--
E.caballus         ---T--TALVCDNGSGLVKAGFAGDDAPR--AVFPSIVGR-PR------------K-D--
G.gallus           ---T--TALVCDNGSGLVKAGFAGDDAPR--AVFPSIVGR-P-R--GQ-------K-D--
B.taurus           ------AALVVDNGSGMCKAGFAGDDAPR--AVFPSIVGR-P-----R-------K-D--
S.cerevisiae       --EV--AALVIDNGSGMCKAGFAGDDAPR--AVFPSIVGR-P-R--HQGIMVGMGQKD--
D.melanogaster     DEEV--AALVVDNGSGMCKAGFAGDDAPR--AVFPSIVGR-PR------------K-D-- *
C.elegans          --EV--AALVDNGSGMCKAGFAGDDAPR--AVFPSIVGR-P-RHQGVG------QKD-- *
D.discoideum       --DV--QALVIDNGSGMCKAGFAGDDAPR--AVFPSIVGR-P-R--------------HD *

H.sapiens          KDSYVGDEAQSKRGILTLKYPIEHGIVTNWDDMEKIWHHTFYNELRVAPEEHPVLLTEAP
E.caballus         SYV-G-DEAQSKRGILTLKYPIEHGIITNWDDMEKIWHHTFYNELRVAPEEHPTLLTEAP
G.gallus           SYV-G-DEAQSKRGILTLKYPIE-GIITNWDDMEKIWHHTFYNELRVAPEEHPTLLTEAP
B.taurus           SYV-G-DEAQSKRGILTLKYPIEHGIVTNWDDMEKIWHHTFYNELRVAPEEHPVLLTEAP
S.cerevisiae       SYV-G-DEAQSKRGILTLRYPIEHGIVTNWDDMEKIWHHTFYNELRVAPEEHPVLLTEAP
D.melanogaster     SYV-G-DEAQSKRGILTLKYPIEHGIVTNWDDMEKIWHHTFYNELRVAPEEHPVLLTEAP
C.elegans          SYV-G-DEAQSKRGILTLKYPIEHGIVTNWDDMEKIWHHTFYNELRVAPEEHPVLLTEAP
D.discoideum       SYV-G-DEAQSKRGILTLKYPIEHGIVTNWDDMEKIWHHTFYNELRVAPEEHPVLLTEAP

H.sapiens          LNPKANREKMTQIMFETFNTPAMYVAIQAVLSLYASGRTTGIVMDSGDGVTHTVPIYEGY
E.caballus         LNPKANREKMTQIMFETFNVPAMYVAIQAVLSLYASGRTTGIVLDSGDGVTHNVPIYEGY
G.gallus           LNPKANREKMTQIMFETFNVPAMYVAIQAVLSLYASGRTTGIVLDSGDGVTHNVPIYEGY
B.taurus           LNPKANREKMTQIMFETFNTPAMYVAIQAVLSLYASGRTTGIVMDSGDGVTHTVPIYEGY
S.cerevisiae       MNPKSNREKMTQIMFETFNVPAFYVSIQAVLSLYSSGRTTGIVLDSGDGVTHVVPIYAGF
D.melanogaster     LNPKANREKMTQIMFETFNTPAMYVAIQAVLSLYASGRTTGIVLDSGDGVSHTVPIYEGY
C.elegans          LNPKANREKMTQIMFETFNTPAMYVAIQAVLSLYASGRTTGVVLDSGDGVTHTVPIYEGY
D.discoideum       LNPKANREKMTQIMFETFNTPAMYVAIQAVLSLYASGRTTGIVMDSGDGVSHTVPIYEGY
```

PDB actin length (residues)



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251 REMARK 465 MISSING RESIDUES
252 REMARK 465 THE FOLLOWING RESIDUES WERE NOT LOCATED IN THE
253 REMARK 465 EXPERIMENT. (M=MODEL NUMBER; RES=RESIDUE NAME; C=CHAIN
254 REMARK 465 IDENTIFIER; SSSEQ=SEQUENCE NUMBER; I=INSERTION CODE.)
255 REMARK 465
256 REMARK 465   M RES C SSSEQI
257 REMARK 465     HIS A    40
258 REMARK 465     GLN A    41
259 REMARK 465     GLY A    42
260 REMARK 465     VAL A    43
261 REMARK 465     MET A    44
262 REMARK 465     VAL A    45
263 REMARK 465     GLY A    46
264 REMARK 465     MET A    47
265 REMARK 465     GLY A    48
266 REMARK 465     GLN A    49
```

Actin: Structural alignment (STAMP)

DNAse I binding loop

```
H.sapiens          --DDDIAALVVDNGSGMCKAGFAGDDAPRAVFPSIVGRPR-HQ-G---VM----VGMGQ--
E.caballus         ---T--TALVCDNGSGLVKAGFAGDDAPR--AVFPSIVGR-PR-----------K-D--
G.gallus           ---T--TALVCDNGSGLVKAGFAGDDAPR--AVFPSIVGR-P-R--GQ-------K-D--
B.taurus           ------AALVVDNGSGMCKAGFAGDDAPR--AVFPSIVGR-P-----R-------K-D--
S.cerevisiae       --EV--AALVIDNGSGMCKAGFAGDDAPR--AVFPSIVGR-P-R--HQGIMVGMGQKD--
D.melanogaster     DEEV--AALVVDNGSGMCKAGFAGDDAPR--AVFPSIVGR-PR-----------K-D--  *
C.elegans          --EV--AALVVDNGSGMCKAGFAGDDAPR--AVFPSIVGR-P-RHQGVG-----QKD--  *
D.discoideum       --DV--QALVIDNGSGMCKAGFAGDDAPR--AVFPSIVGR-P-R----------HD     *

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E.caballus         SYV-G-DEAQSKRGILTLKYPIEHGIITNWDDMEKIWHHTFYNELRVAPEEHPTLLTEAP
G.gallus           SYV-G-DEAQSKRGILTLKYPIE-GIITNWDDMEKIWHHTFYNELRVAPEEHPTLLTEAP
B.taurus           SYV-G-DEAQSKRGILTLKYPIEHGIVTNWDDMEKIWHHTFYNELRVAPEEHPVLLTEAP
S.cerevisiae       SYV-G-DEAQSKRGILTLRYPIEHGIVTNWDDMEKIWHHTFYNELRVAPEEHPVLLTEAP
D.melanogaster     SYV-G-DEAQSKRGILTLKYPIEHGIVTNWDDMEKIWHHTFYNELRVAPEEHPVLLTEAP
C.elegans          SYV-G-DEAQSKRGILTLKYPIEHGIVTNWDDMEKIWHHTFYNELRVAPEEHPVLLTEAP
D.discoideum       SYV-G-DEAQSKRGILTLKYPIEHGIVTNWDDMEKIWHHTFYNELRVAPEEHPVLLTEAP

H.sapiens          LNPKANREKMTQIMFETFNTPAMYVAIQAVLSLYASGRTTGIVMDSGDGVTHTVPIYEGY
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G.gallus           LNPKANREKMTQIMFETFNVPAMYVAIQAVLSLYASGRTTGIVLDSGDGVTHNVPIYEGY
B.taurus           LNPKANREKMTQIMFETFNTPAMYVAIQAVLSLYASGRTTGIVMDSGDGVTHTVPIYEGY
S.cerevisiae       MNPKSNREKMTQIMFETFNVPAFYVSIQAVLSLYSSGRTTGIVLDSGDGVTHVVPIYAGF
D.melanogaster     LNPKANREKMTQIMFETFNTPAMYVAIQAVLSLYASGRTTGIVLDSGDGVSHTVPIYEGY
C.elegans          LNPKANREKMTQIMFETFNTPAMYVAIQAVLSLYASGRTTGVVLDSGDGVTHTVPIYEGY
D.discoideum       LNPKANREKMTQIMFETFNTPAMYVAIQAVLSLYASGRTTGIVMDSGDGVSHTVPIYEGY
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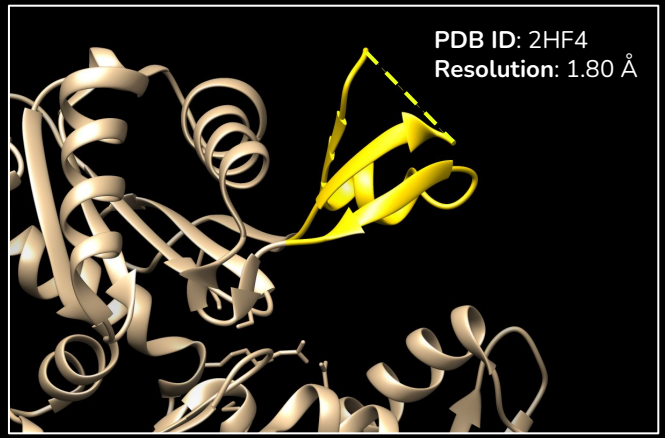

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250 REMARK 465
251 REMARK 465 MISSING RESIDUES
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253 REMARK 465 EXPERIMENT. (M=MODEL NUMBER; RES=RESIDUE NAME; C=CHAIN
254 REMARK 465 IDENTIFIER; SSSEQ=SEQUENCE NUMBER; I=INSERTION CODE.)
255 REMARK 465
256 REMARK 465   M RES C SSSEQI
257 REMARK 465     HIS A    40
258 REMARK 465     GLN A    41
259 REMARK 465     GLY A    42
260 REMARK 465     VAL A    43
261 REMARK 465     MET A    44
262 REMARK 465     VAL A    45
263 REMARK 465     GLY A    46
264 REMARK 465     MET A    47
265 REMARK 465     GLY A    48
266 REMARK 465     GLN A    49
```

CONCLUSIONS

Conclusions

- Transition of actin monomer to a filament is a dynamic process mediated basically by nucleotide exchange
- Interactions between actin subunits in the filament are mostly electrostatic or hydrophilic
- Upon polymerization, ATP hydrolysis is activated and thus, conformational changes occur in some regions (Ser14-β hairpin and sensor loop)
- Positively charged N-terminal domain of actin is highly conserved and plays an important role on myosin activation
- Actin sequence is highly conserved amongst eukaryotic organisms

PEM test

According to actin, choose the correct answer:

a) A network of Van der Waals interactions holds the nucleotide binding site in a open conformation
b) A network of hydrogen bonds interactions holds the nucleotide binding site in a close conformation
c) Both are correct
d) A network of salt bridges interactions holds the nucleotide binding site in a open conformation
e) None of the above are correct

According to actin, choose the false statement:

a) Actin is a ATPase, so it is in charge of ATP hydrolysis
b) Actin has three isoforms
c) Actin has two major domains and 4 subdomains
d) It is involved in muscle contraction, cell movement, cytokinesis,etc
e) It's not really conserved throughout evolution

Regarding, ATP hydrolysis, it is NOT true that:

a) Polymerization enhances ATP hydrolysis
b) Mg^{2+} binds 4 molecules of water and Ca^{2+} five
c) Mg^{2+} has higher affinity for nucleotide than Ca^{2+}
d) Polymerization allows the nucleophilic water to move away from the gamma phosphate of ATP and therefore allows hydrolysis to occur.
e) The ATP hydrolysis rate is much faster than the phosphate dissociation rate.

PEM test

According to actin, choose the correct answer:

a) Transition between G-actin and F-actin it's necessary for it's assembly
b) 20º structural change is needed for the DNAse-loop to interact with the actin above
c) Monomer actin is always bound to ATP
d) Actin filament has polarity
e) All of them are correct

According to actin, choose the correct answer:

a) Actin - Actin interactions are mainly electrostatic
b) F-actin interacts only with the molecule at it's side
c) F-actin's D-loop doesn't interact with any residue in the filament
d) G-actin has exactly the same interactions as F-actin
e) Non of the above are correct

Regarding to G-actin monomer, choose the false statement:

a) It is formed by 4 subdomains
b) It has 3 different isoforms, which are α, β, γ
c) It is involved in different cell processes like muscle interaction, cell movement or cell signaling
d) It is an all α structure
e) It is thought that subdomain 1 and 3 come from a gene duplication

PEM test

Actin-myosin interface in rigor state is generated:

a) Exclusively by hydrophobic interactions
b) Bt hydrophobic and electrostatic interactions
c) These two proteins do not interact
d) Exclusively by electrostatic interactions
e) None of the above are correct

Which region of myosin does not interact with actin:

a) Cardiomyopathy loop
b) Helix-Loop-Helix motif
c) Loop-2
d) Light regulatory chain
e) Activation loop

Select the correct/s option/s regarding Arginine 419:

a) It is found in the Cardiomyopathy loop
b) It stabilizes the Cardiomyopathy loop
c) a and b are correct
d) It directly interacts with actin
e) All of them are correct

Regarding actin's sequence conservation between species:

a) Only the region that binds with myosin is highly conserved
b) It is highly conserved between eukaryotic cells
c) There are no regions conserved among the sequence
d) Residue's conservation depends on how much exercise is done
e) All of the above are false

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