STRUCUTRAL BIOLOGY

A MONOMERİC G-PROTEİN: RAC1 (RAS-RELATED C3 BOTULİNUM TOXİN SUBSTRATE 1)

Adriana Rodríguez, Laia Uyà i Karen Vay

Rac1

1. CLASSIFICATION
2. BIOLOGICAL STRUCTURE
3. CONSERVATION
4. INTRAMOLECULAR AND INTERMOLECULAR INTERACTIONS

Mg2+ , water, GDP/GTP, GAP/GEF/GDI, WAVE

SCOP classification

CLASS Alpha and beta proteins (a/b)

1

2

3

4

SCOP classification

1

2

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CLASS Alpha and beta proteins (a/b)

FOLD G domain-like

A specific topology where the strands form a central sheet surrounded by helices.



SCOP classification

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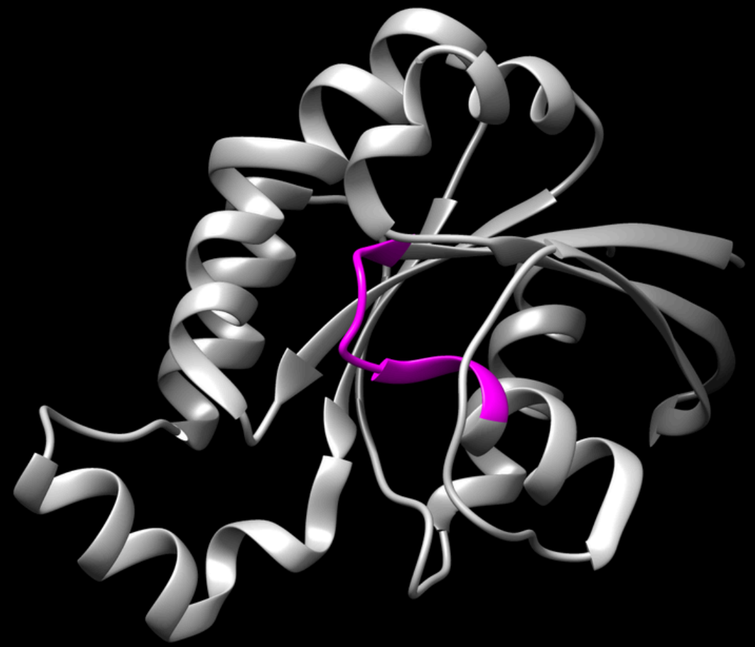
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CLASS Alpha and beta proteins (a/b)

FOLD G domain-like

SUPERFAMILY Ras-like P-loop GTPases

- *Share a distant common ancestor.*
- *P-loop: Phosphate-binding loop (Walker A) - a specific region that interacts with GTP/GDP*



SCOP classification

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CLASS	Alpha and beta proteins (a/b)
FOLD	G domain-like
SUPERFAMILY	Ras-like P-loop GTPases
FAMILY	Ras-like monodomain GTPases



Small monomeric GTP-binding proteins acting as molecular switches and sharing the conserved five G-motifs.

SCOP classification

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4

CLASS	Alpha and beta proteins (a/b)
FOLD	G domain-like
SUPERFAMILY	Ras-like P-loop GTPases
FAMILY	Ras-like monodomain GTPases
DOMAIN	8057405

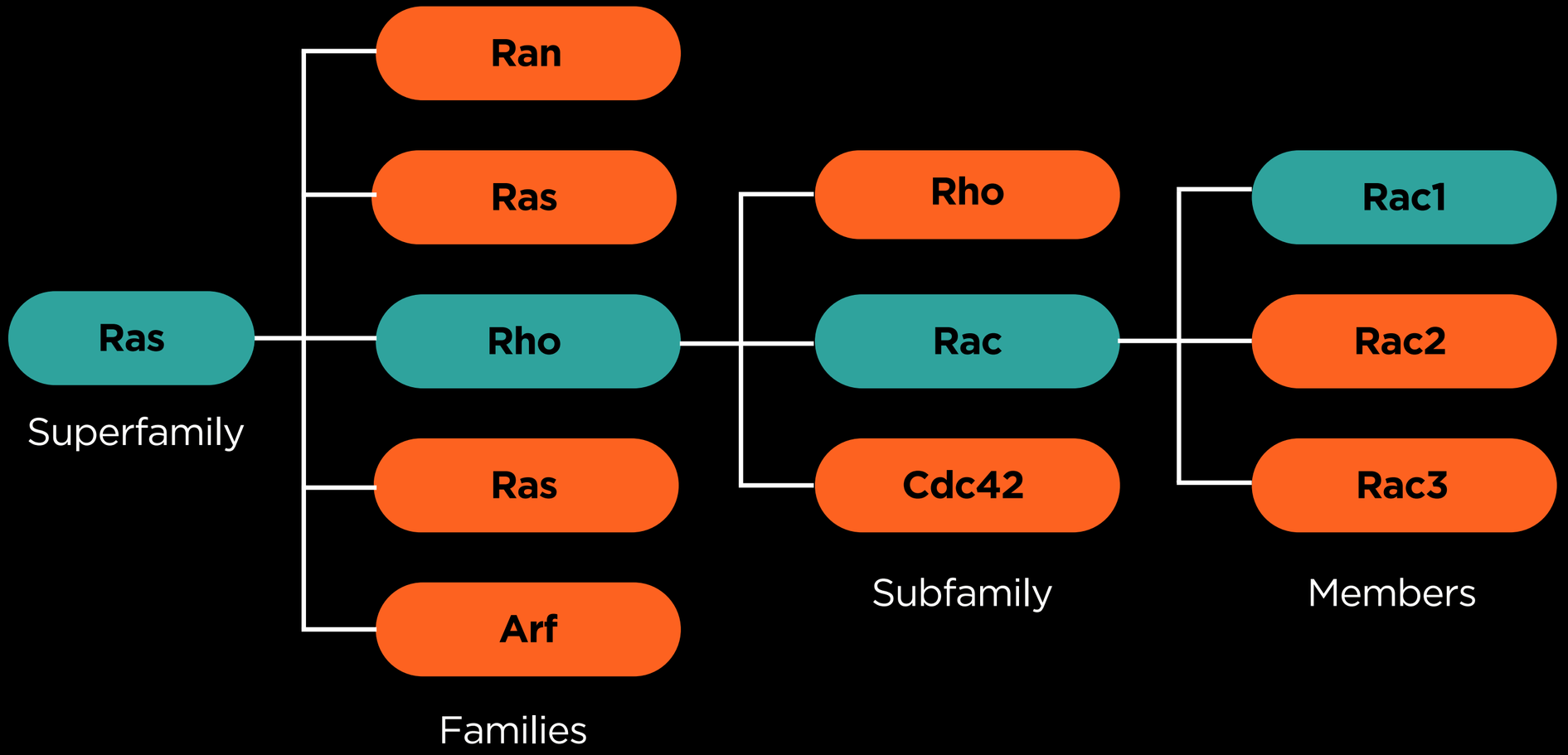
Rac1 classification

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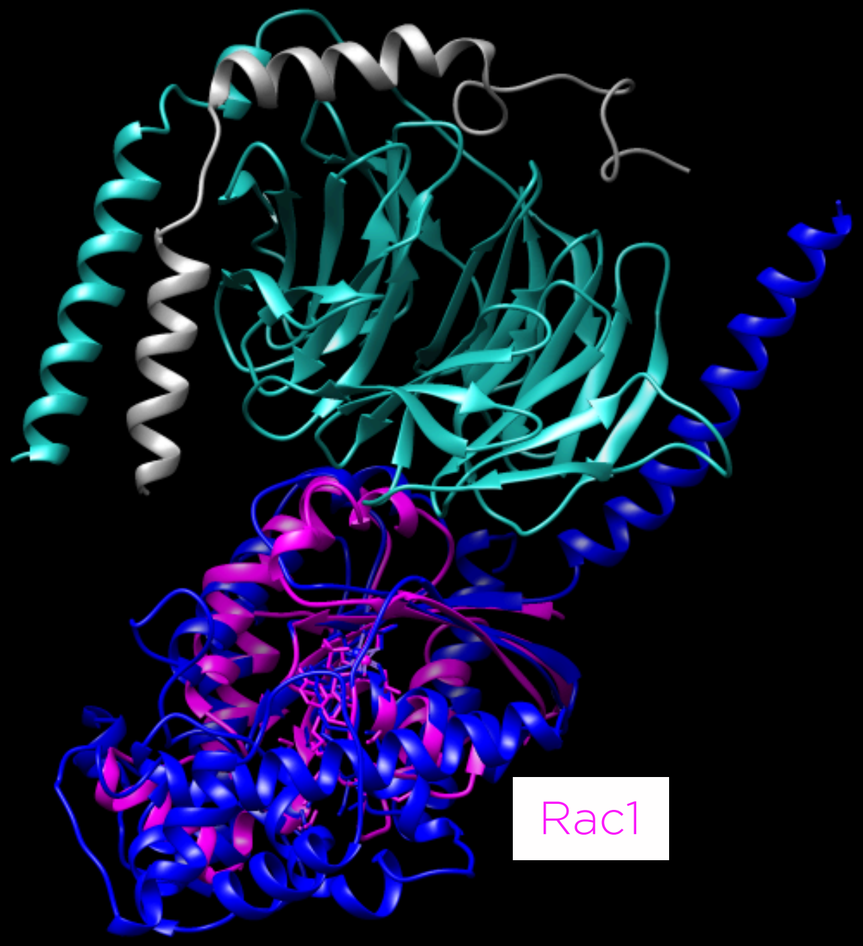
G-proteins

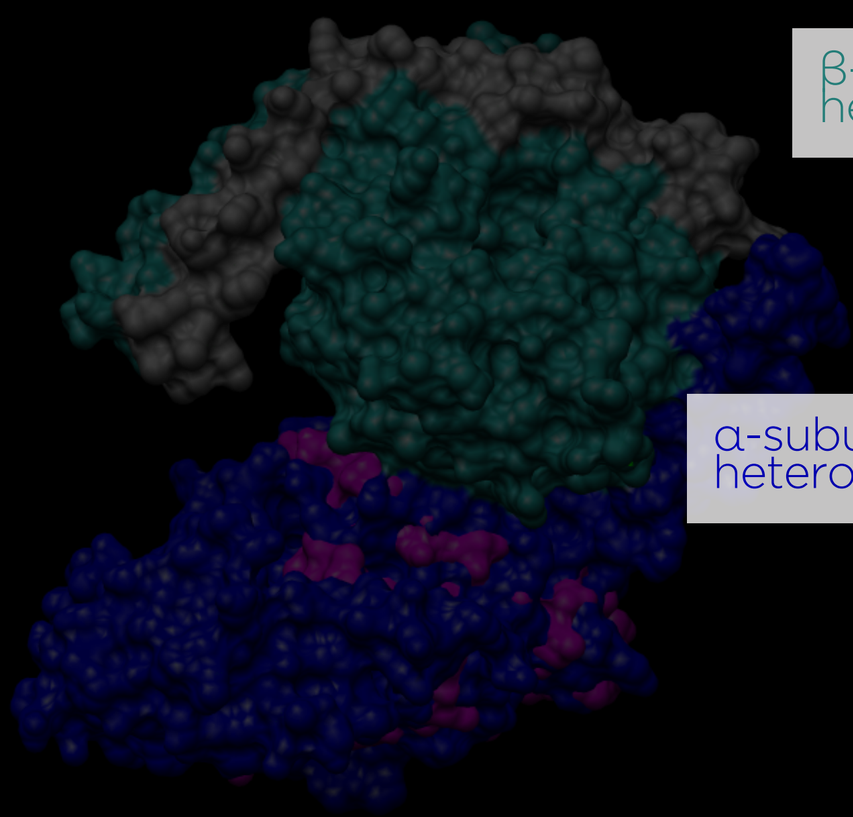
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G-proteins

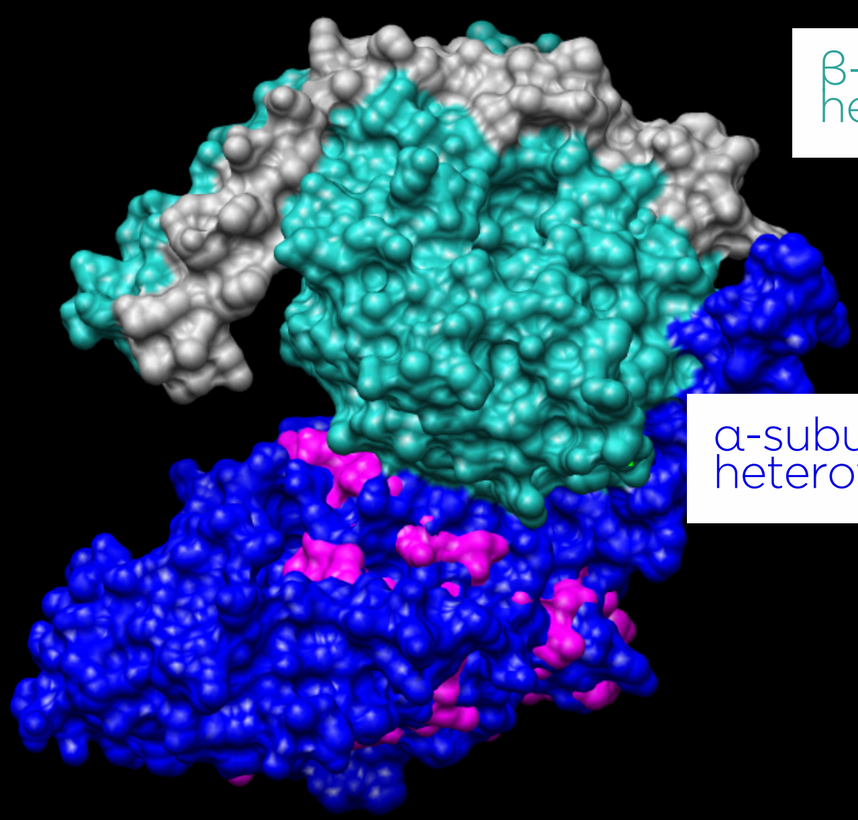
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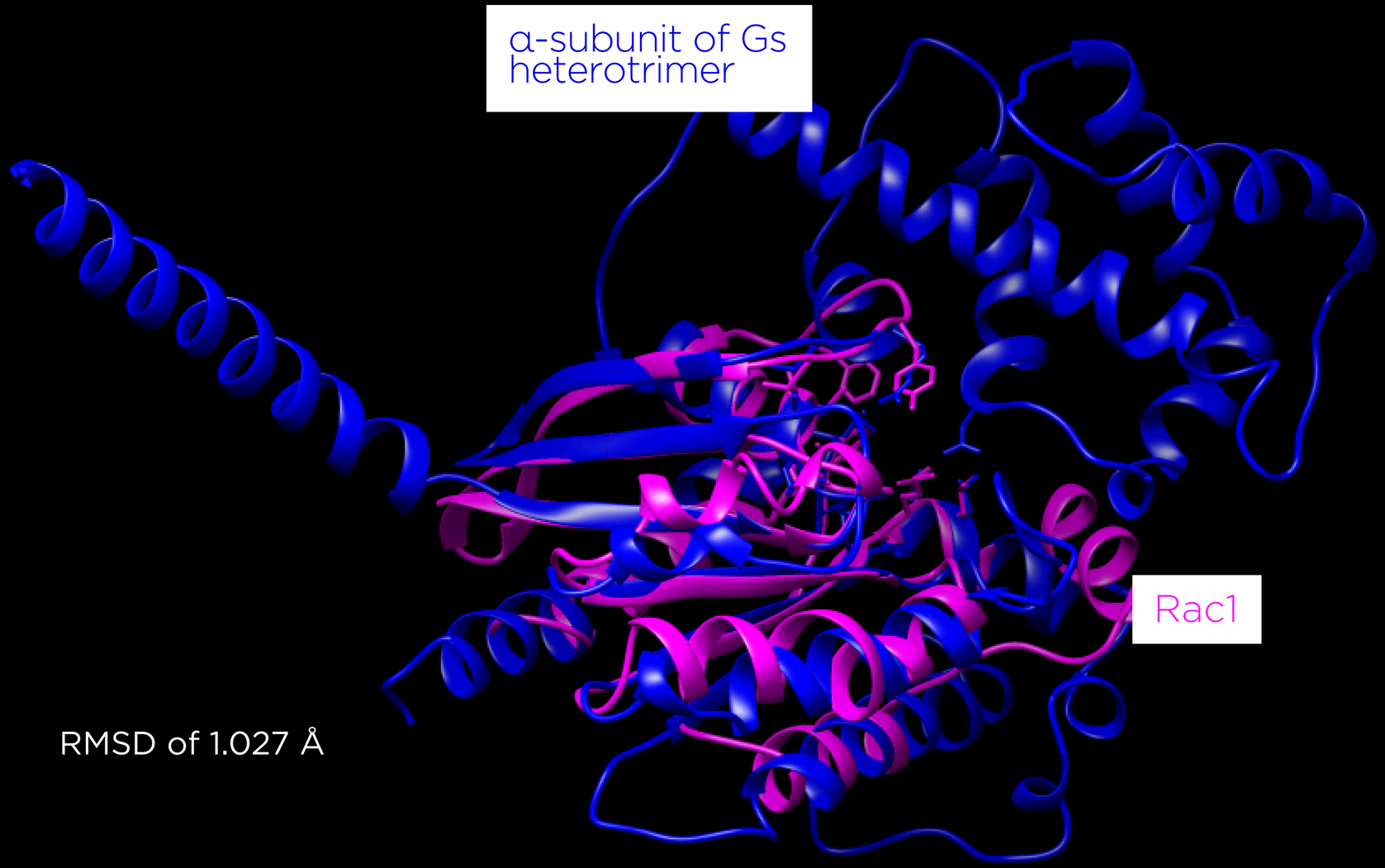
G-proteins

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RMSD of 1.027 Å

RAC1

Rac1 is involved in modulation of the cytoskeleton which plays a role in multiple cellular functions such as:

- phagocytosis and pinocytosis.
- formation of synapses.
- formation of actin-rich membrane protrusions, such as lamellipodia and filopodia.

It has **2 conditions**:

- To be anchored at plasma membrane to activate its primary effectors.
- To be bound to GTP (it’s the only conformational state compatible with the interaction of most effectors)

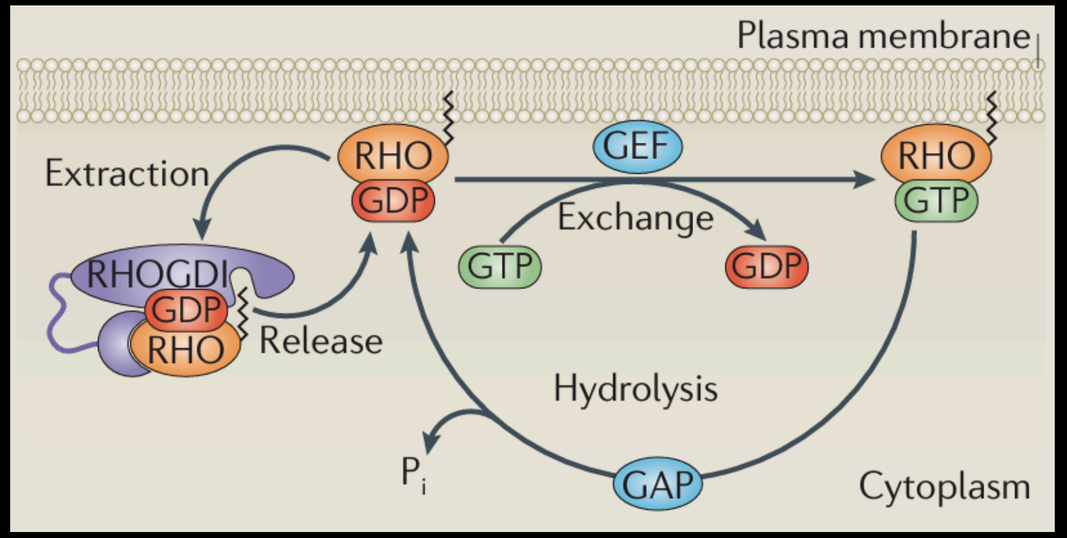
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RAC1 activity



The 'invisible hand': regulation of RHO GTPases by RHOGDIs

Mediated by:

- GDIs (guanine nucleotide dissociation inhibitor) binds to Rac1-GDP RhoGDI
- GEFs (guanine nucleotide exchange factors) GDP release Tiam1
- GAPs (GTPase-activating proteins) GTP hydrolysis RacGAP1

RAC1 models of study:

1

2

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3TH5

Rac1-GTP isolated protein structural representation

METHOD	X-RAY	**RESOLUTION**	2.30 Å
WEIGHT	46.56 kDa	**RESIDUES**	204
SPECIES	*Homo sapiens*		

2RMK

Rac1 sequence analysis and alignments **has a point mutation G61L*

METHOD	SOLUTION NMR	**RESOLUTION**	-
WEIGHT	31.03 kDa	**RESIDUES**	192
SPECIES	*Homo sapiens*		

5N6O

Rac1-GDP isolated protein structural representation

METHOD	X-RAY	**RESOLUTION**	2.59 Å
WEIGHT	40.09 kDa	**RESIDUES**	176
SPECIES	*Homo sapiens*		

1GRN

CDC42/CDC42GAP ~ Rac1 /GAP structural representation

METHOD	X-RAY	**RESOLUTION**	2.30 Å
WEIGHT	46.56 kDa	**RESIDUES**	204
SPECIES	*Homo sapiens*		

RAC1 models of study:

1

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1FOE

Rac1/Tiam (GEF) structural representation

METHOD	X-RAY	**RESOLUTION**	2.80 Å
WEIGHT	254.54 kDa	**CHAINS**	2
SPECIES	*Homo sapiens*		

1HH4

Rac/RhoGDI structural representation

METHOD	X-RAY	**RESOLUTION**	2.70 Å
WEIGHT	90.73 kDa	**CHAINS**	2
SPECIES	*Homo sapiens*		

7USE

Rac1/WAVE structual representation

METHOD	Electron microscopy	**RESOLUTION**	3.00 Å
WEIGHT	381.24 kDa	**CHAINS**	7
SPECIES	*Homo sapiens*		

6EG8

Gs heterotrimer - GDP structural representation

METHOD	X-RAY	**RESOLUTION**	2.80 Å
WEIGHT	361.5 kDa	**RESIDUES**	797
SPECIES	*Homo sapiens*		

Rac1 primary structure

Swiss-Prot P63000

- Length: 192 amino acids
- Mass (KDa): 21,619

1 2 3 4

MQAIKCVVVG DGAVGKTCLL ISYTTNAFPG EYIPTVFDNY SANVMVDGKP
1 10 20 30 40 50

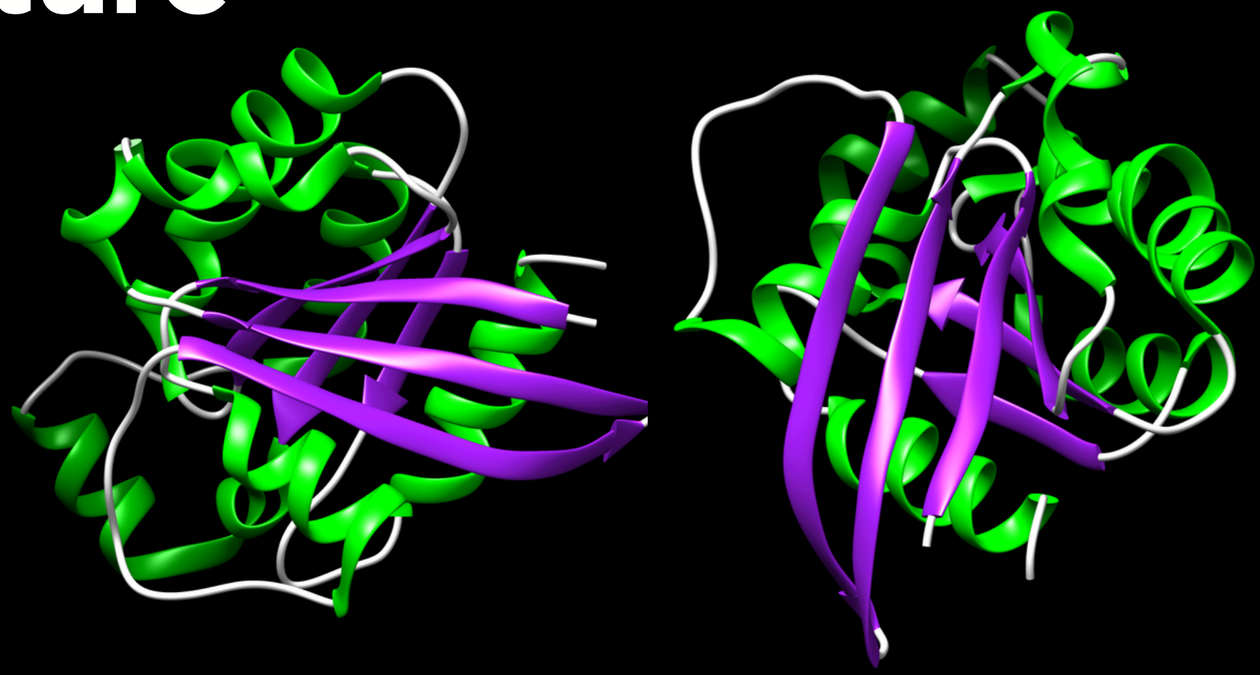
VNLGLWDTAG QEDYDRLRPL SYPQTDVFLI CFSLVSPASF ENVRAKWYPE
60 70 80 90 100

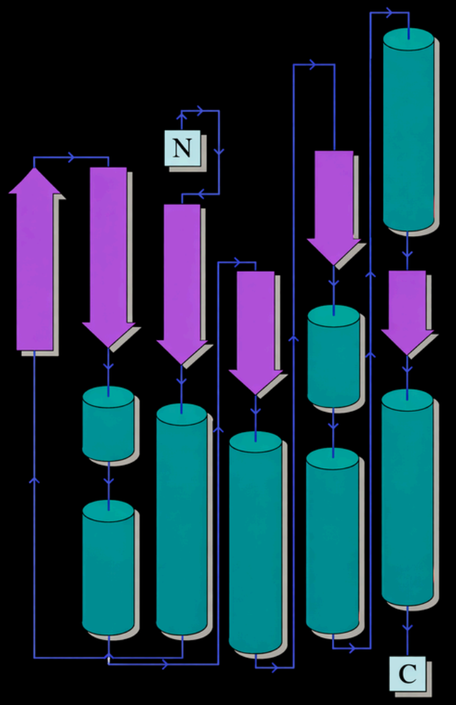
135

VRHHCPNTPI ILVGTKLDLR DDKDTIEKLK EKKLTPITYP QGLAMAKEIG
110 120 130 140 150

AVKYLECSAL TQRGLKTVFD EAIRAVLCPP PVKKRKRKCL LL
160 170 180 190 192

RAC1 supersecondary structure





Modified from EMBL-EBI. PDBsum

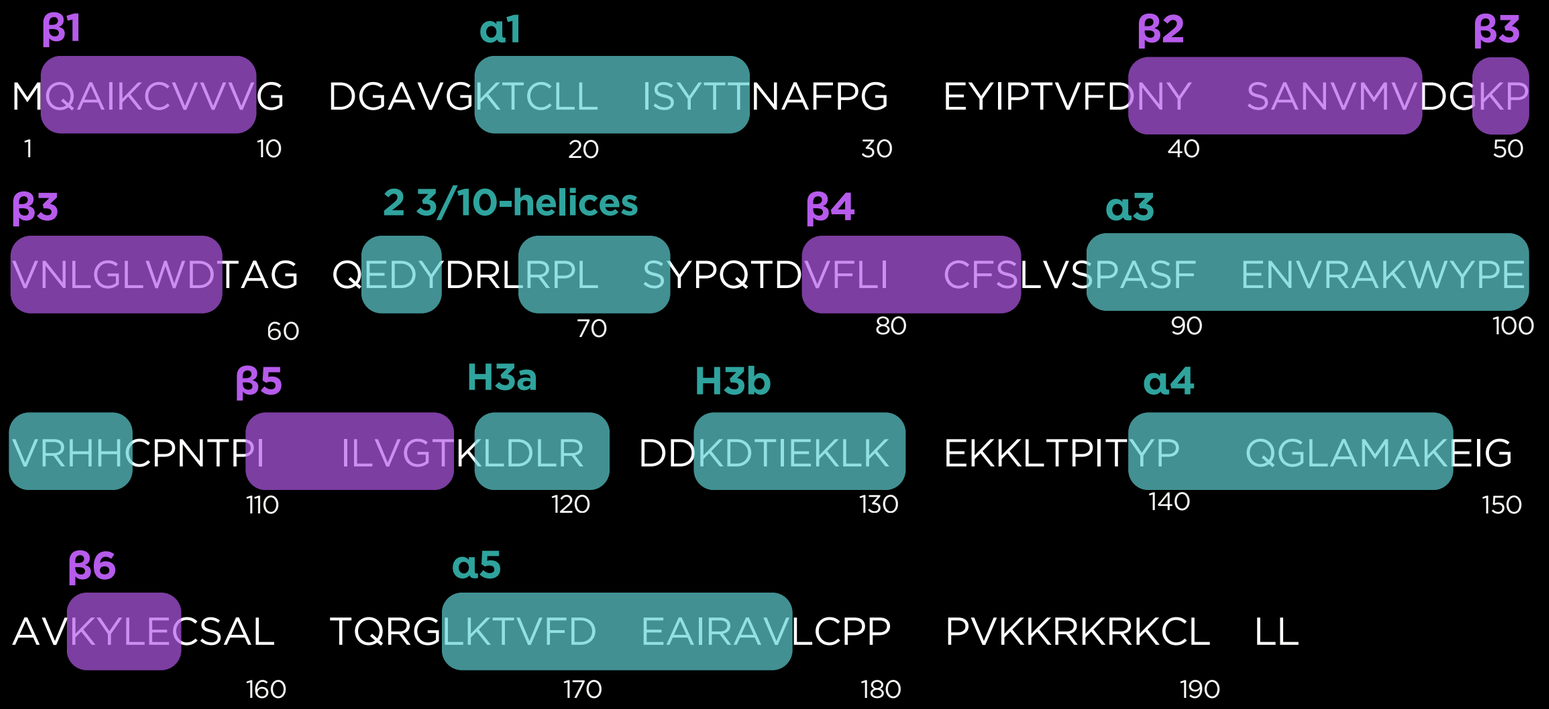
G-domain fold - variant of the Rossmann fold

- 5 α-helixs surrounding the core
- 6 β-sheets (5 parallel + 1 anti-parallel -β2) forming the core

+

- 2 3/10 helixs
- αi - Inserted Region

Rac1 secondary structure



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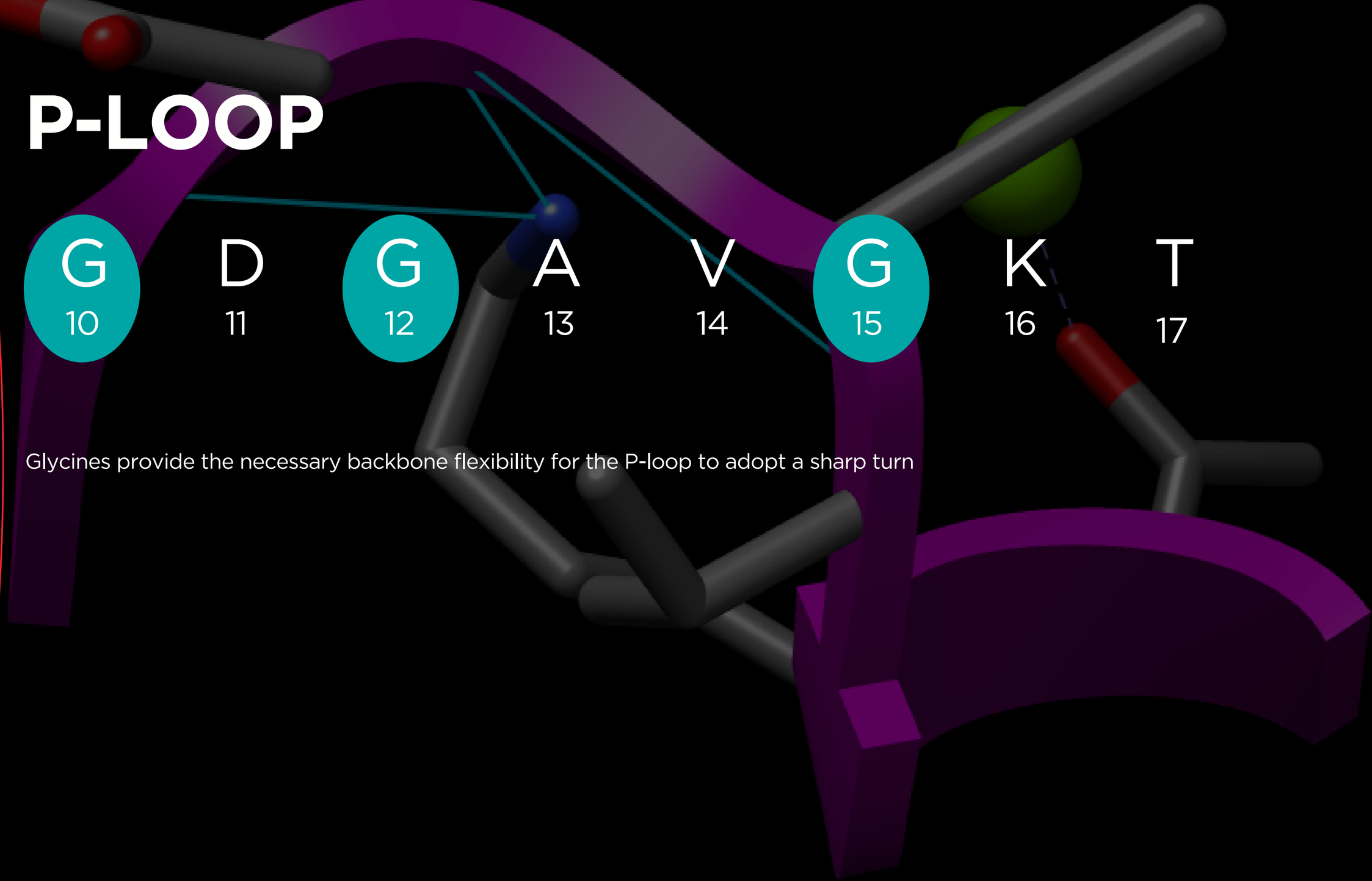
Rac1 secondary structure

N-terminal residues: 1–19

P-loop residues: 10–17

P-LOOP

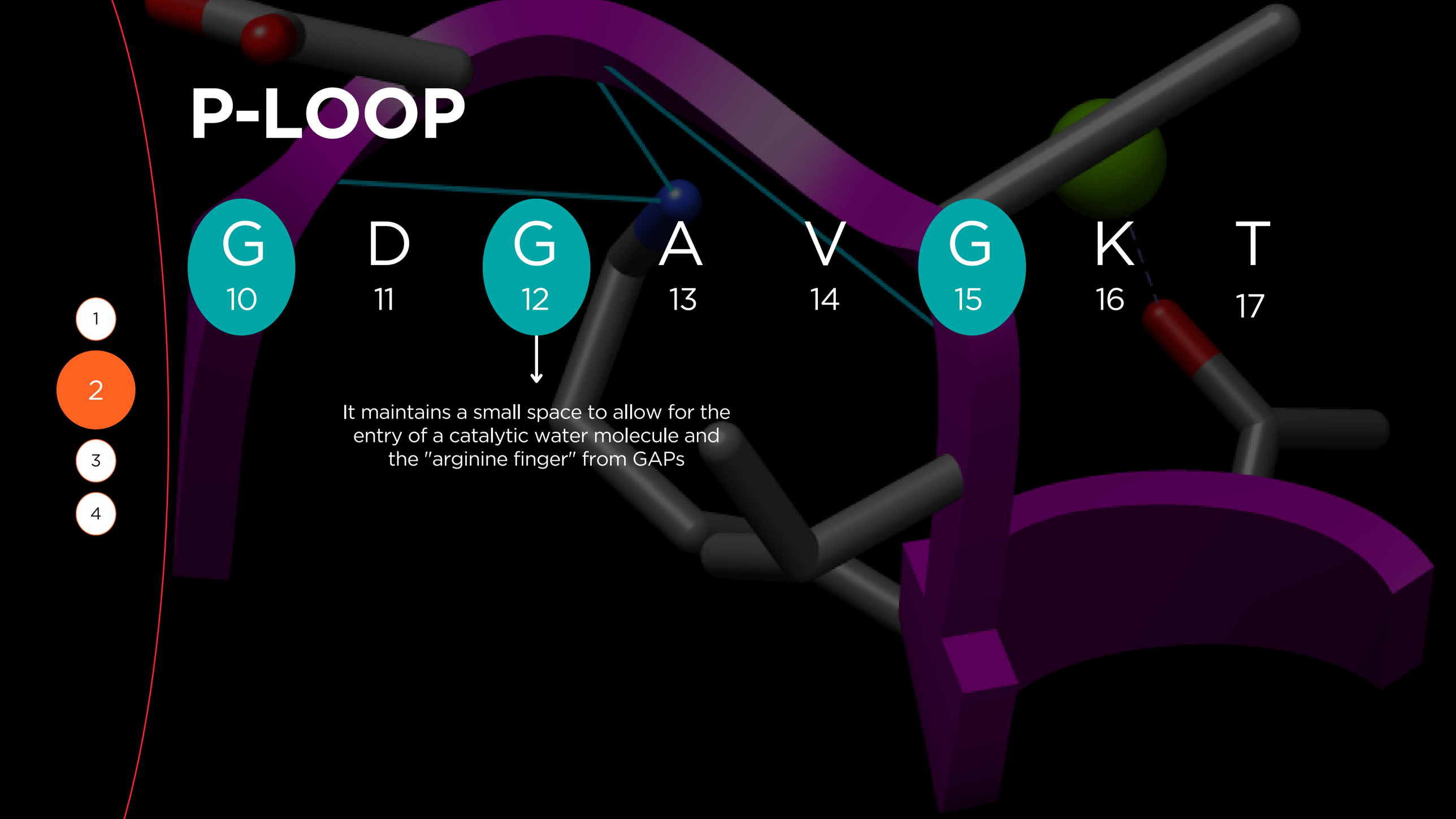
G	D	G	A	V	G	K	T
10	11	12	13	14	15	16	17



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Glycines provide the necessary backbone flexibility for the P-loop to adopt a sharp turn

P-LOOP



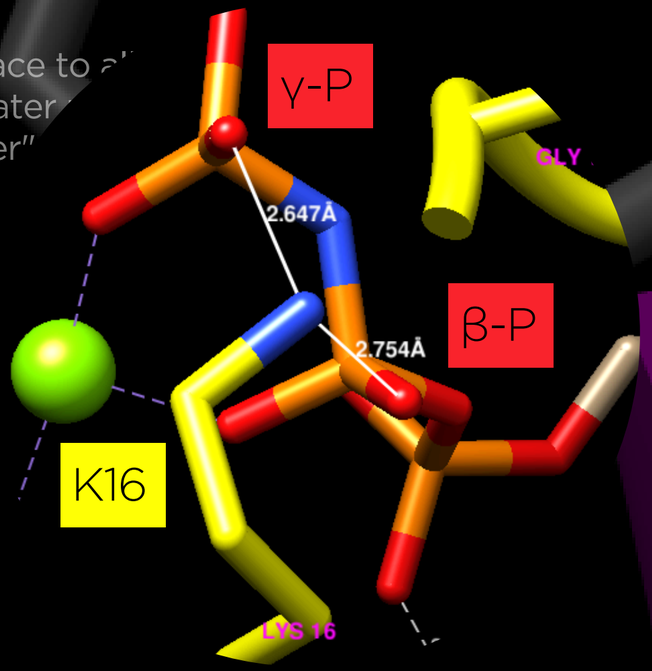
G	D	G	A	V	G	K	T
10	11	12	13	14	15	16	17

It maintains a small space to allow for the entry of a catalytic water molecule and the "arginine finger" from GAPs

P-LOOP

G	D	G	A	V	G	K	T
10	11	12	13	14	15	16	17

It maintains a small space to a
entry of a catalytic water
the "arginine finger"

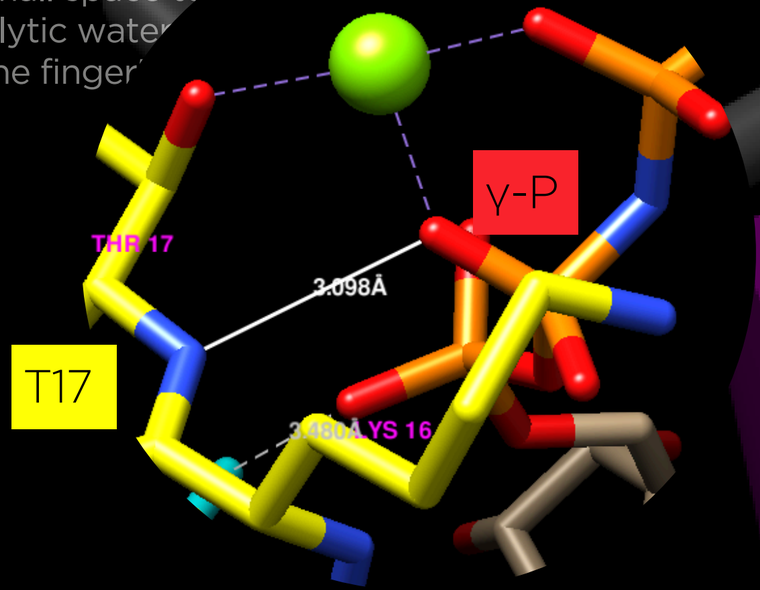


Neutralizes the negative charge of β and γ phosphates

P-LOOP

G	D	G	A	V	G	K	T
10	11	12	13	14	15	16	17

It maintains a small space to
entry of a catalytic wate
the "arginine finger"



Neutralizes the negative
charge of β and γ phosphates

- coordinates Mg2+
- its NH forms hydrogen bonds with the γ phosphate of GTP

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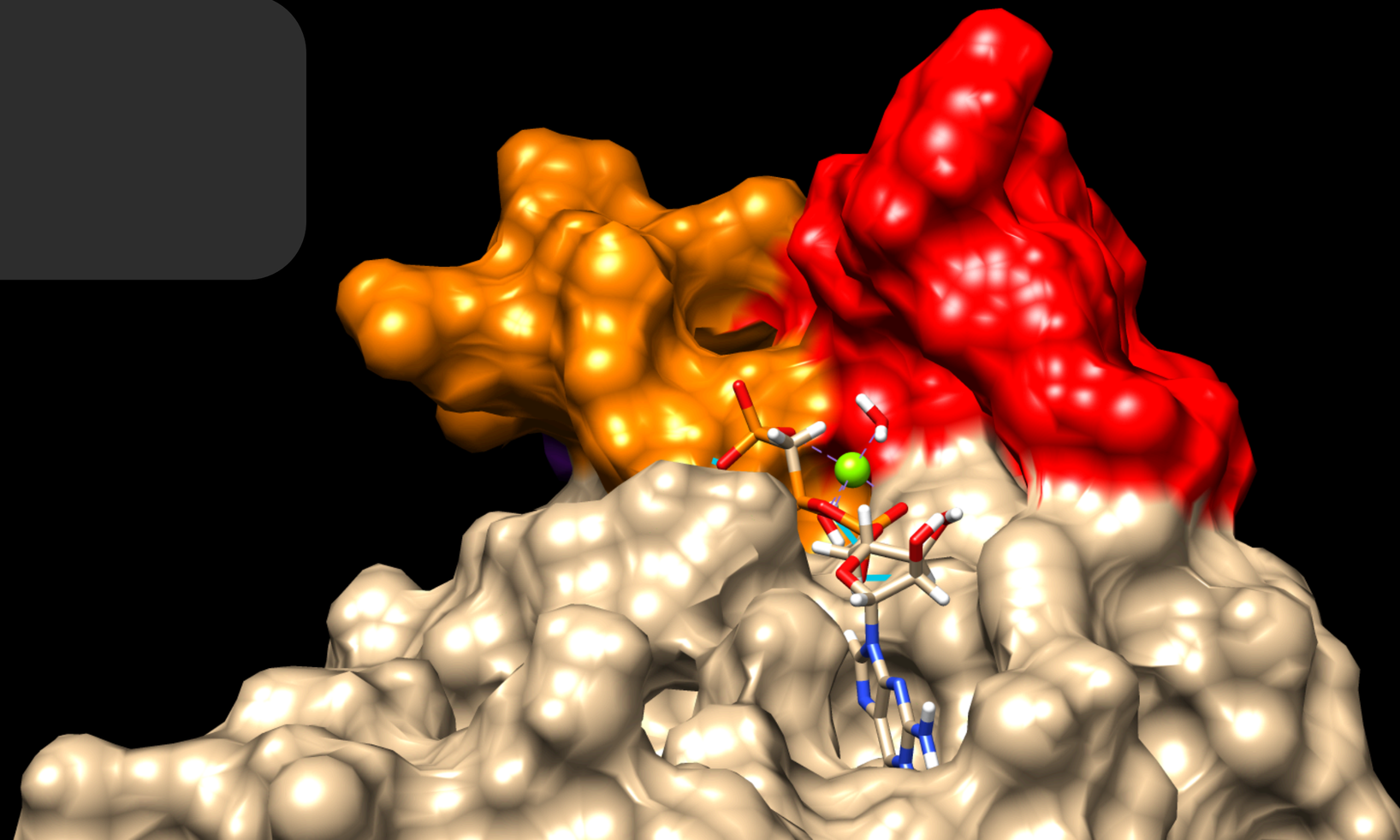
Rac1 secondary structure

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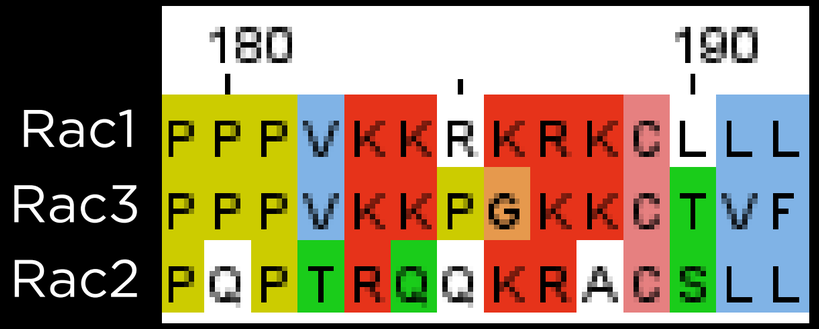
GTPase domain

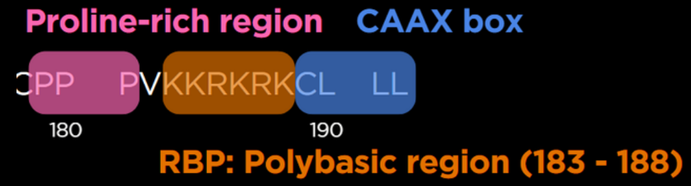
Switch I residues: 29-44

Switch II residues: 56-75



Rac1 secondary structure



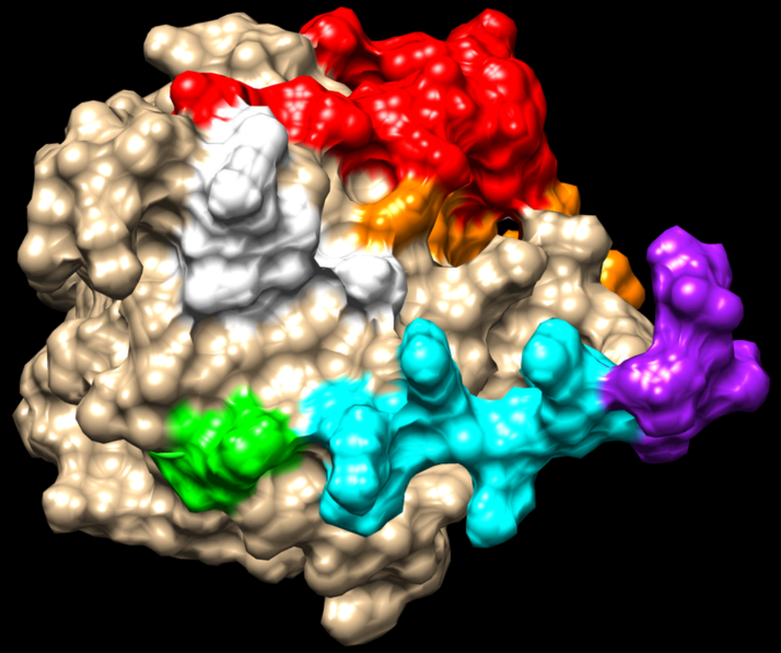


C-terminal residues: 179 - 192

Proline-rich region (PRR) residues: 179 - 181

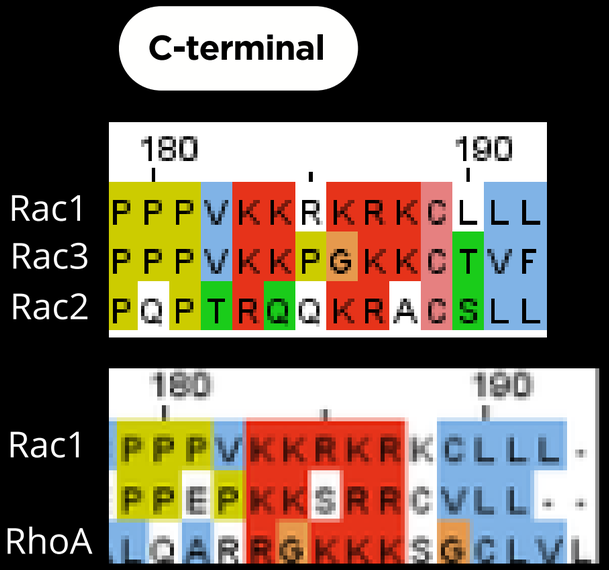
Polybasic region (PBR) residues: 183 - 188

CAAX box residues: 189 - 192



Rac1 secondary structure

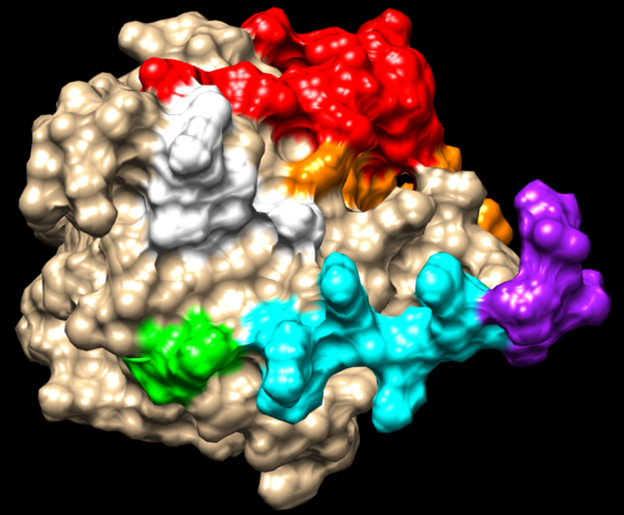
- Rigidity and GEF binding
- Prolines bend the chain → “type II helix” → proteins with SH3 domains (such as Nck) binding
- Direct Rac1 to frontal part of cell (lamellipodia)
- Are often accompanied by GEF → causing Rac1 activation



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C-terminal residues: 179 - 192

- **Proline-rich region (PRR)** residues: 179 - 181
- **Polybasic region (PBR)** residues: 183 - 188
- **CAAX box** residues: 189 - 192

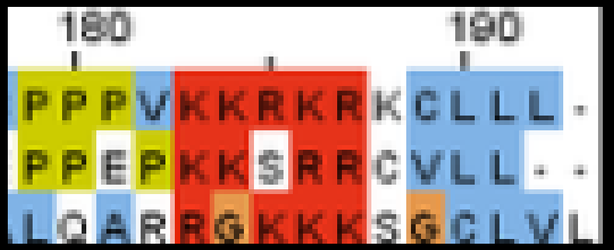


Rac1 secondary structure

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- Rich in lysines and arginines which have positive charges
- Determination of: membrane binding site, the strength and dimer formation
- During human bacterial infections, MARTX toxins → acylation of L186 blocking its interactions with effectors.

Rac1
Cdc42
RhoA

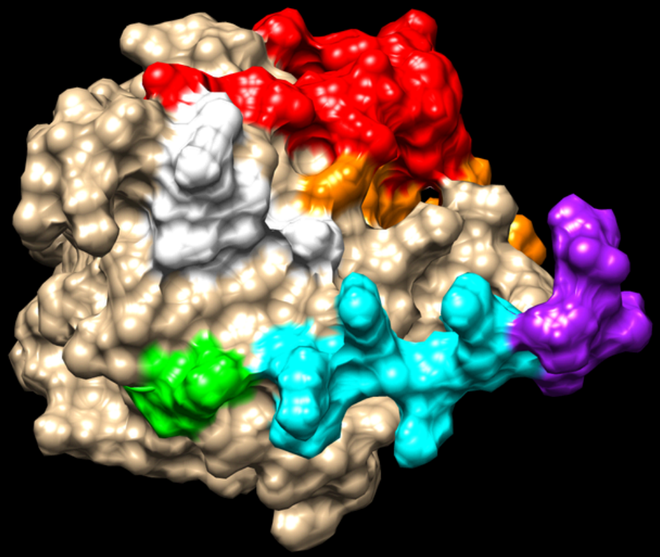


C-terminal residues: 179 - 192

Proline-rich region (PRR) residues: 179 - 181

Polybasic region (PBR) residues: 183 - 188

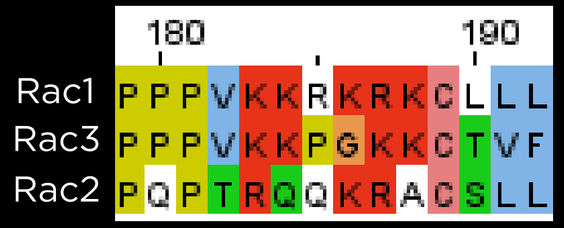
CAAX box residues: 189 - 192



Rac1 secondary structure

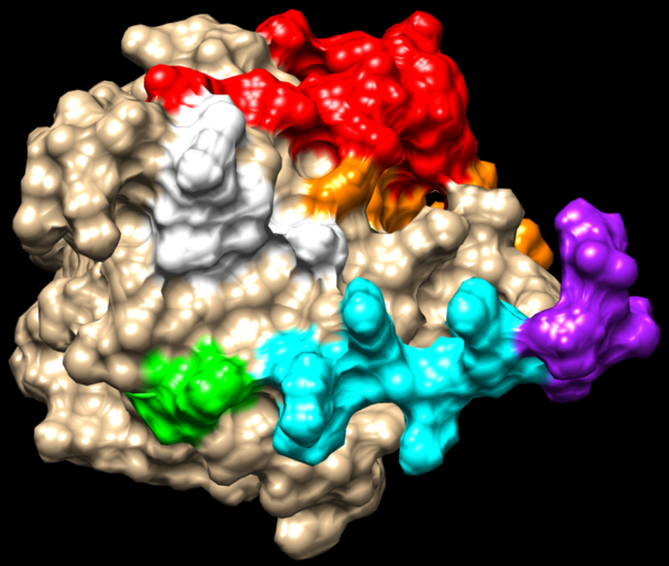
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- Prenylation: attachment of prenyl group to CAAX box → allowing protein adhesion to the membrane.
- Prenyl group + PBR → strong binding of Rac1 to the membrane.



C-terminal residues: 179 - 192

- **Proline-rich region (PRR)** residues: 179 - 181
- **Polybasic region (PBR)** residues: 183 - 188
- **CAAX box** residues: 189 - 192



Nucleotide binding site

P-loop

Switch I

MQAIKCVVVG DGAVGKTCLL ISYTTNAFPG EYIPTVFDNY SANVMVDGKP

1 10 20 30 40 50

Switch II

VNLGLWDTAG QEDYDRLRPL SYPQTDVFLI CFSLVSPASF ENVRAKWYPE

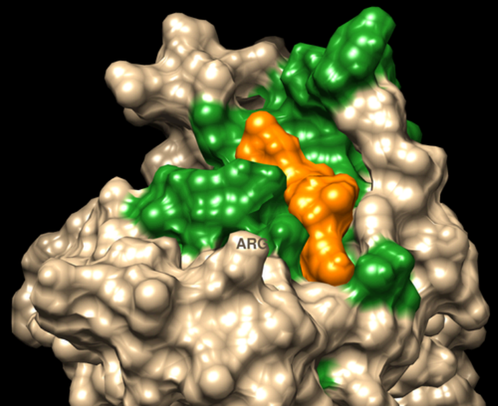
60 70 80 90 100

VRHHCPNTPI ILVGTKLDLR DDKDTIEKLK EKKLTPITYP QGLAMAKEIG

110 120 130 140 150

AVKYLECSAL TQRGLKTVFD EAIRAVLCPP PVKKRKRKCL LL

160 170 180 190



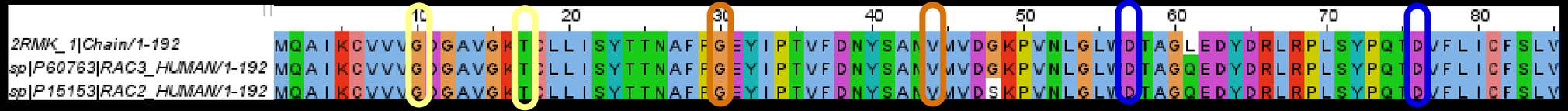
Nucleotide binding site



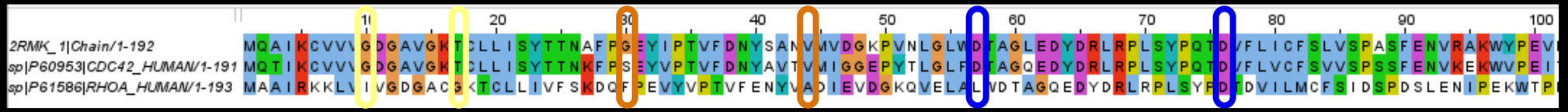
Conservation of nucleotide binding site

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Alignment between Racs (Rac1, Rac2, Rac3)

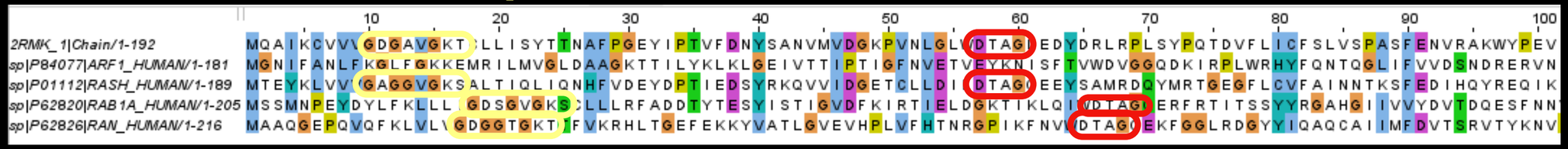


Alignment between Rho (Rac1, Cdc42, RhoA)

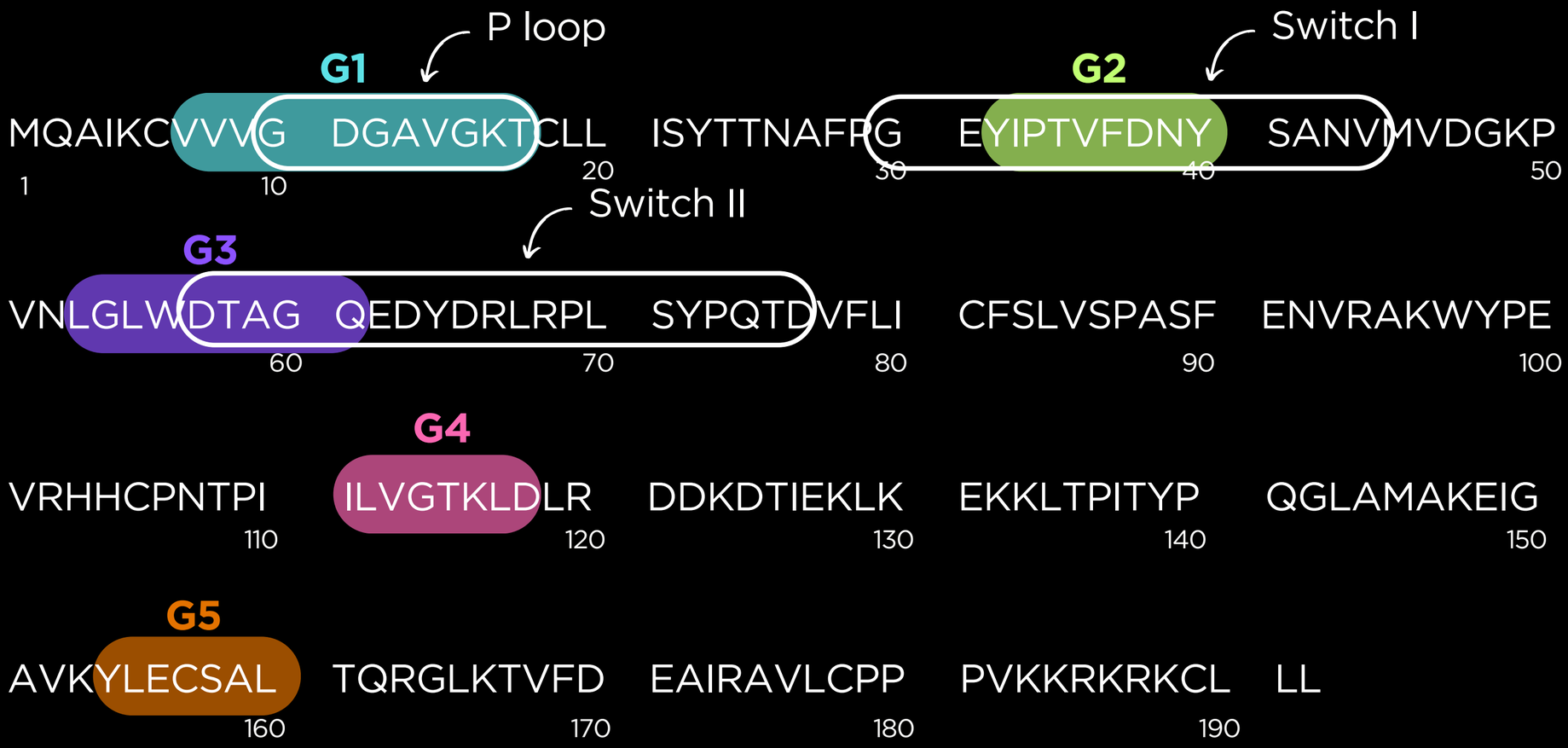


- Position 15: Gly and Ala with similar properties
- Position 30: Switch I interacts with each specific effector
- Position 57: Switch II → hydrophobic L also interacts with specifics GEFs → stress fibers

Alignment between Ras superfamily (Ras, Ran, Rab, Rac, Arf)

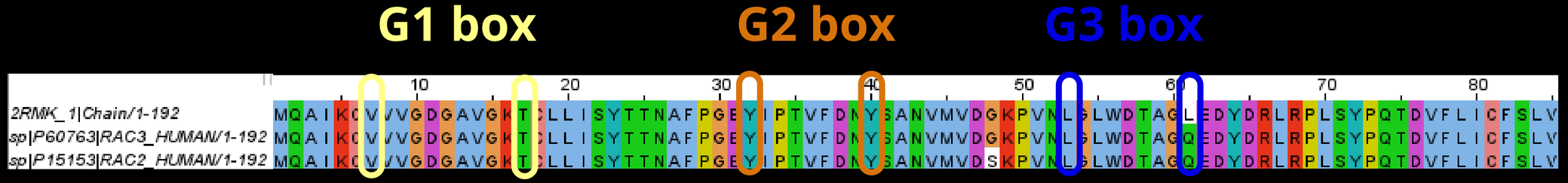


G box sequence



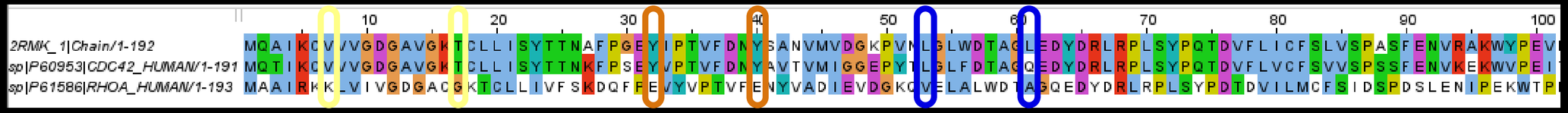
Conservation of G boxes

Alignment between Racs (Rac1, Rac2, Rac3)



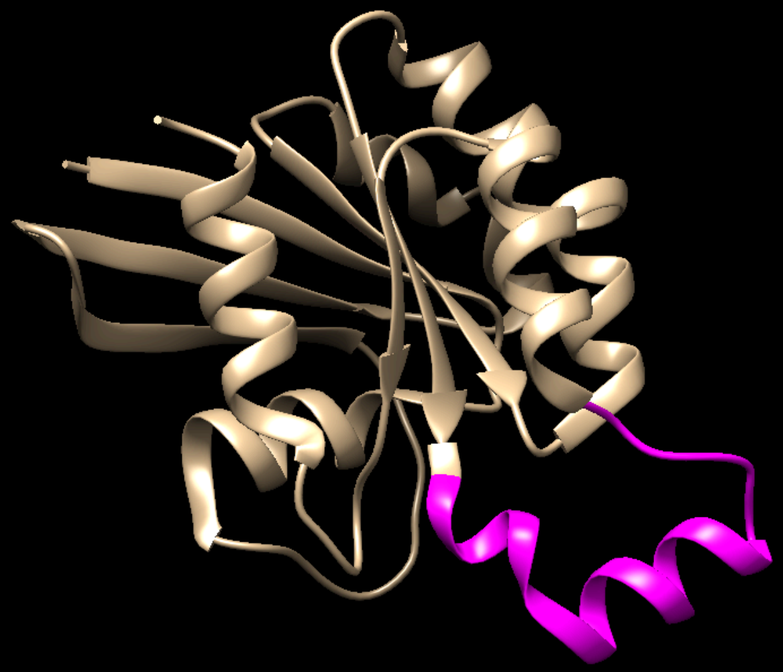
**2MKD had a mutation on Gln61

Alignment between Rho (Rac1, Cdc42, RhoA)



- G1 box: some changes are compensated by other amino acids around them.
- G3 box: V and L have similar properties

Inserted region



117 137

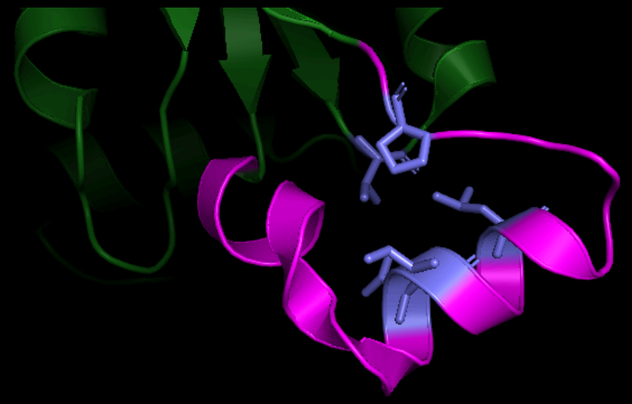
LDLR DD**KDTIEKLK EKKLT**PI

123 13-aa insertion 135

Two α-helices, H3a and H3b, followed by an extended loop

It is implicated in effector regulation

Residues Thr 125, Ile 126, Leu 129 and Pro 136 together with Val 85 form a loosely packed hydrophobic core.

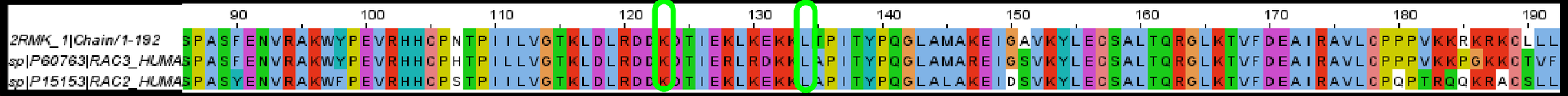


Conservation of inserted domain

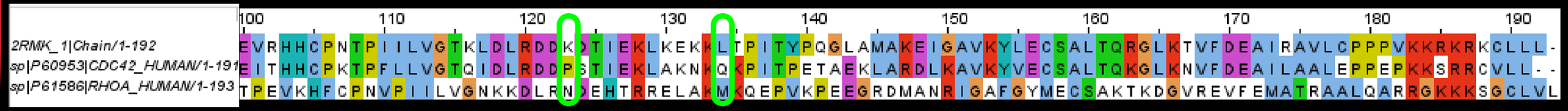
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Alignment between Racs (Rac1, Rac2, Rac3)

Inserted domain



Alignment between Rho (Rac1, Cdc42, RhoA)

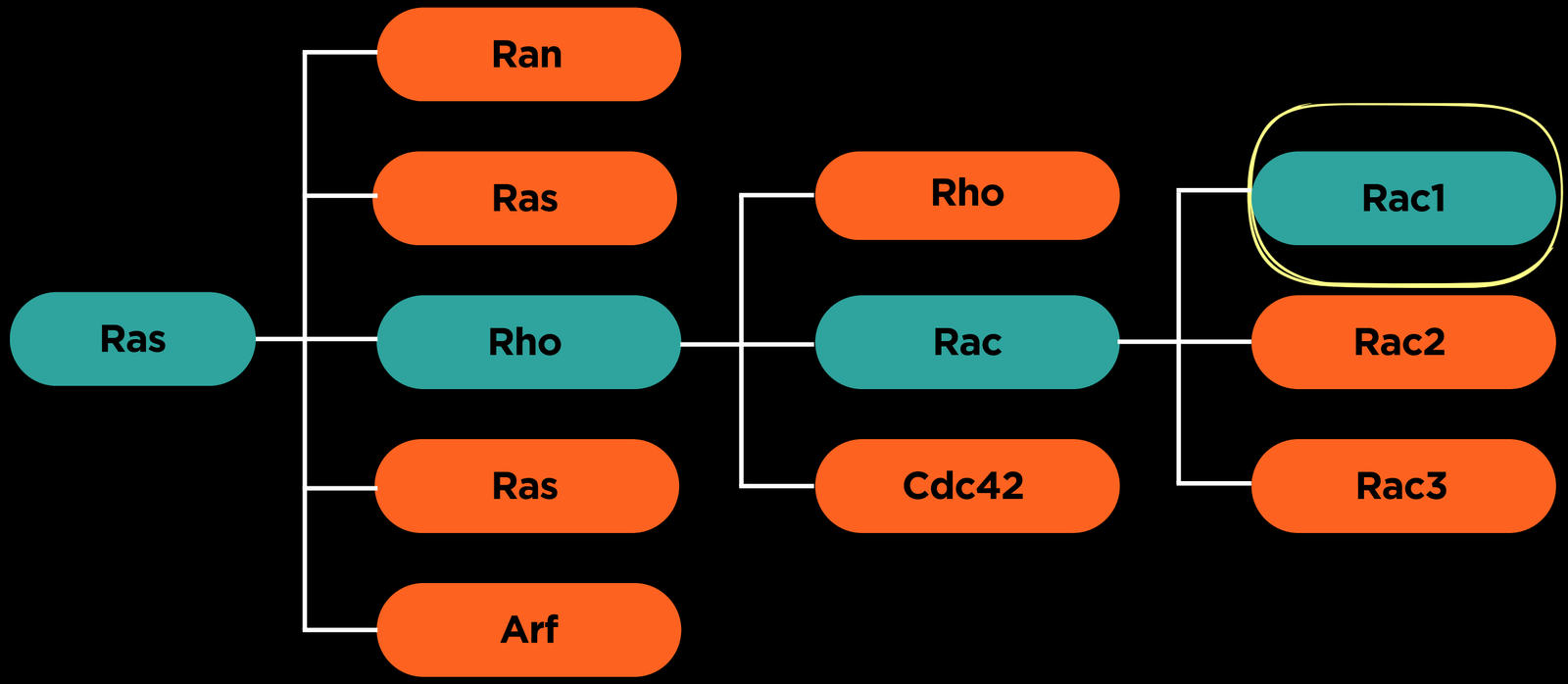


Alignment between Ras family (Ras, Ran, Rab, Rac, Arf)



MSAs

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Between Rac1a and Rac1b from:

- Homo sapiens

Alignment between Rac1a and Rac1b

```
2RMK_1|Chain                MQAIKCVVVGDGAVGKTCLLISYTTNAFPGEYIPTVFDNYSANVMVDGKP
sp|P63000-2|RAC1_HUMAN      MQAIKCVVVGDGAVGKTCLLISYTTNAFPGEYIPTVFDNYSANVMVDGKP
                            **************************************************

2RMK_1|Chain                VNLGLWDTAGLEDYDRLRPLSYPQT------------------DVFLIC
sp|P63000-2|RAC1_HUMAN      VNLGLWDTAGQEDYDRLRPLSYPQTVGETYGKDITSRGKDKPIADVFLIC
                            ********** **************                 ******

2RMK_1|Chain                FSLVSPASFENVRAKWYPEVRHHCPNTPIILVGTKLDLRDDKDTIEKLKE
sp|P63000-2|RAC1_HUMAN      FSLVSPASFENVRAKWYPEVRHHCPNTPIILVGTKLDLRDDKDTIEKLKE
                            **************************************************

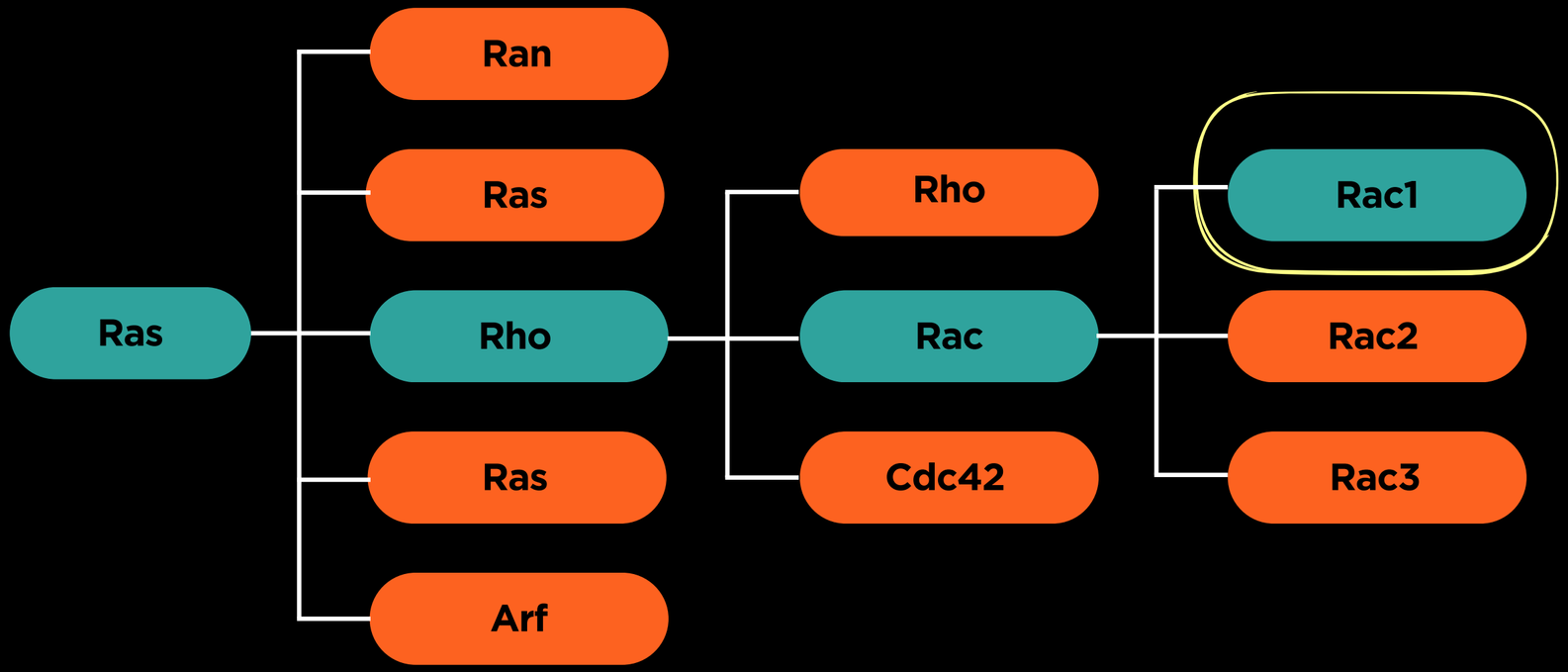
2RMK_1|Chain                KKLTPITYPQGLAMAKEIGAVKYLECSALTQRGLKTVFDEAIRAVLCPPP
sp|P63000-2|RAC1_HUMAN      KKLTPITYPQGLAMAKEIGAVKYLECSALTQRGLKTVFDEAIRAVLCPPP
                            **************************************************

2RMK_1|Chain                VKKRKRKCLLL
sp|P63000-2|RAC1_HUMAN      VKKRKRKCLLL
                            ***********
```

- Rac1b: 19 amino acids extra after residue 75
- Rac1b more active and higher membrane binding capacity (compared with Rac1a)
- No interaction with RhoGDI
- Its expression is increased in tumors

MSAs

1
2
3
4



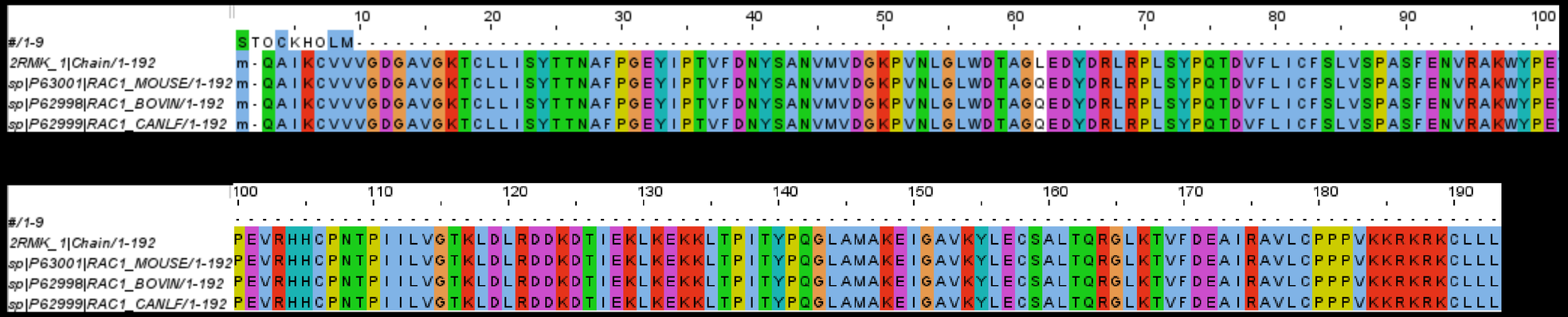
Between different species:

1st:

- Homo sapiens
- Dog
- Mouse
- Bovine

Alignment between Rac1 from different species

1
2
3
4

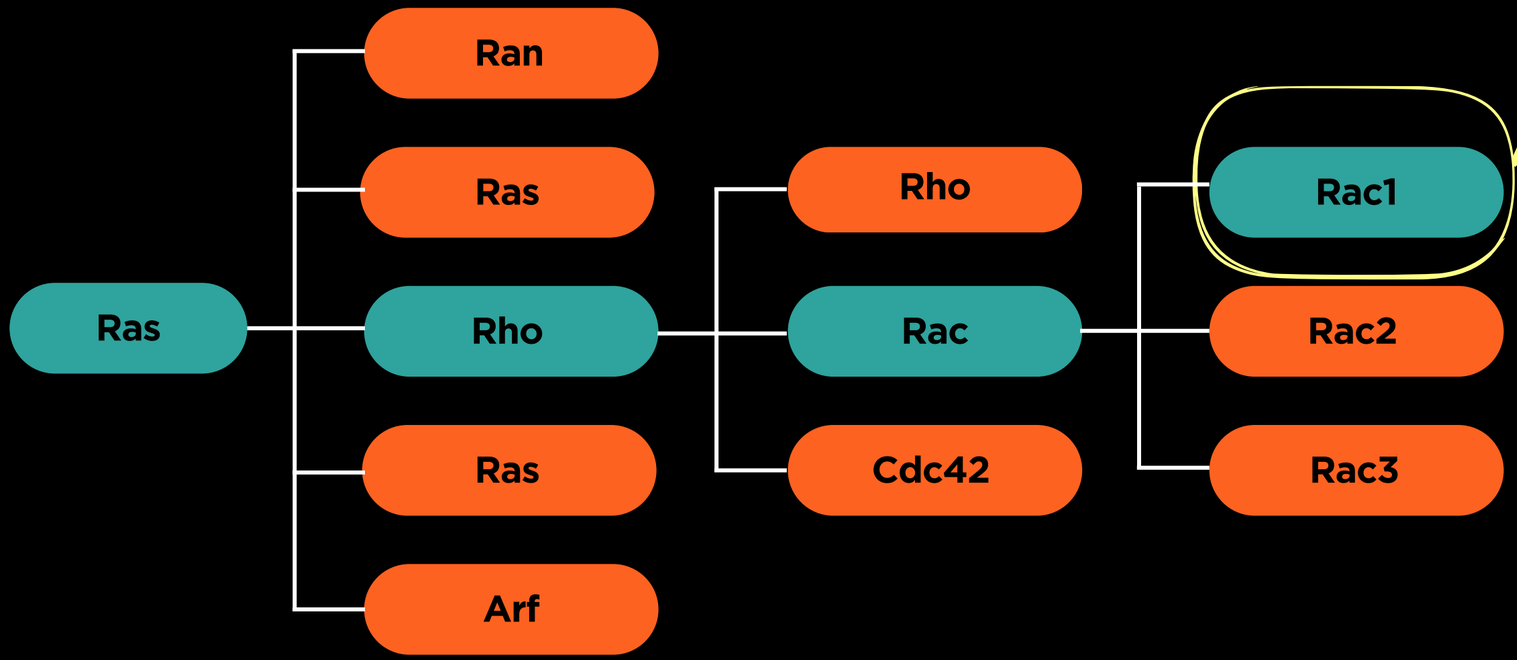


E-value	score	bias	E-value	score	bias	exp	N	Sequence	Description
5.8e-83	281.9	0.0	6.4e-83	281.8	0.0	1.0	1	sp|P62998|RAC1_BOVIN	Ras-related C3 botulinum toxin substra
5.8e-83	281.9	0.0	6.4e-83	281.8	0.0	1.0	1	sp|P62999|RAC1_CANFA	Ras-related C3 botulinum toxin substra
5.8e-83	281.9	0.0	6.4e-83	281.8	0.0	1.0	1	sp|P63000|RAC1_HUMAN	Ras-related C3 botulinum toxin substra
5.8e-83	281.9	0.0	6.4e-83	281.8	0.0	1.0	1	sp|P63001|RAC1_MOUSE	Ras-related C3 botulinum toxin substra

They have almost a 100% of identity

MSAs

1
2
3
4



Between different species:

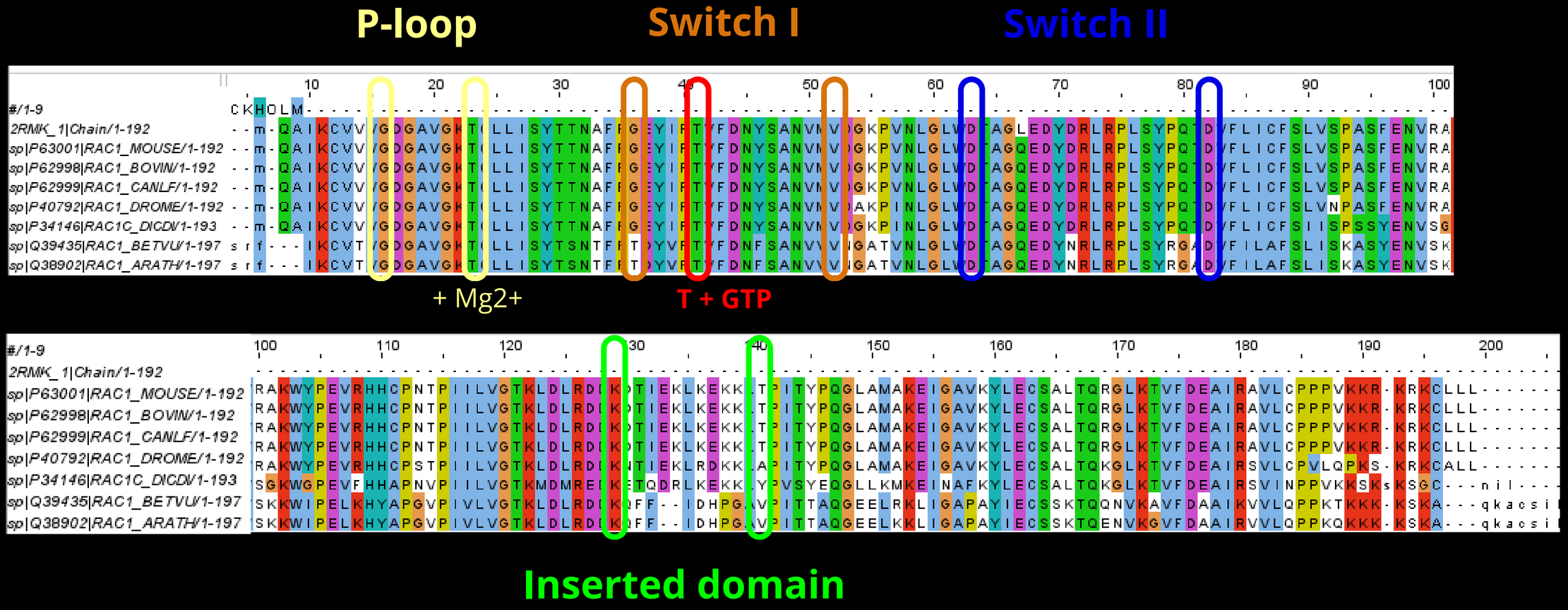
1st: Homo sapiens, dog, mouse, bovine
2nd we added:

- Animal (drosophila)
- Plants (sugar been and arabidopsis thaliana)
- Protists (one amoeba)

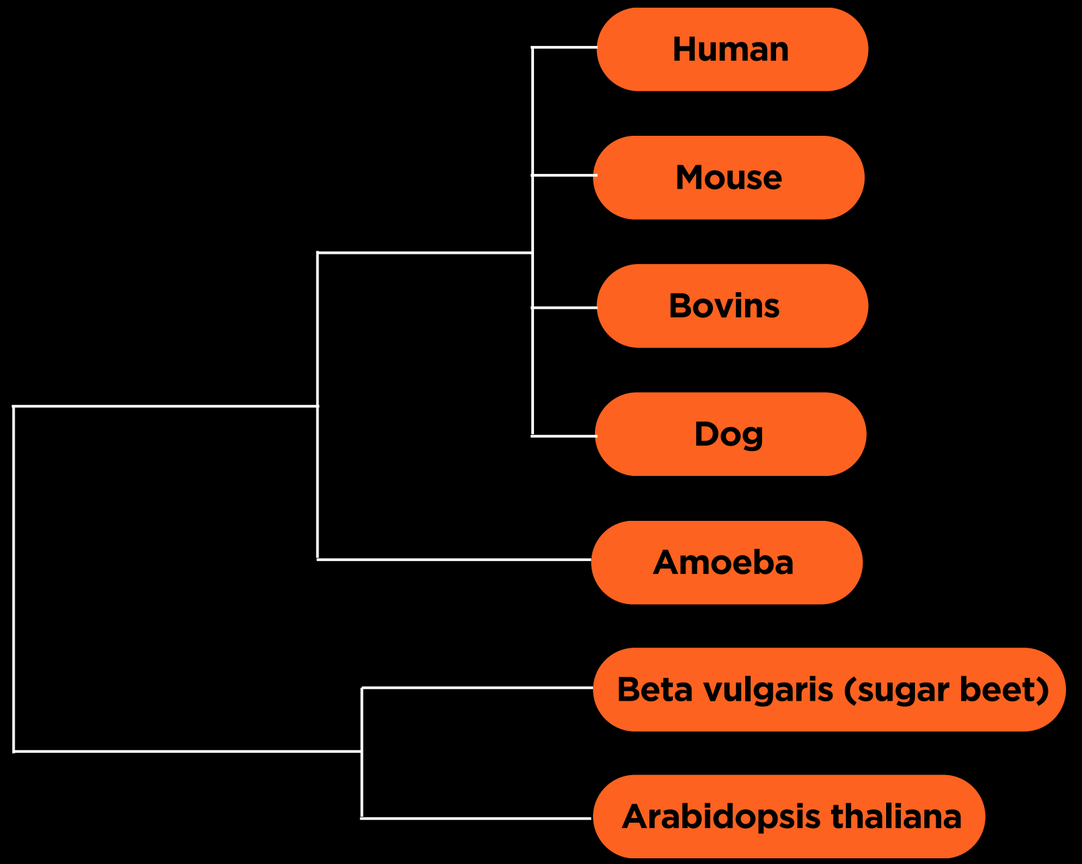
There’s no Rac1 in bacteria

Alignment between Rac1 from different species

1
2
3
4



Dendogram between Rac1 from different species

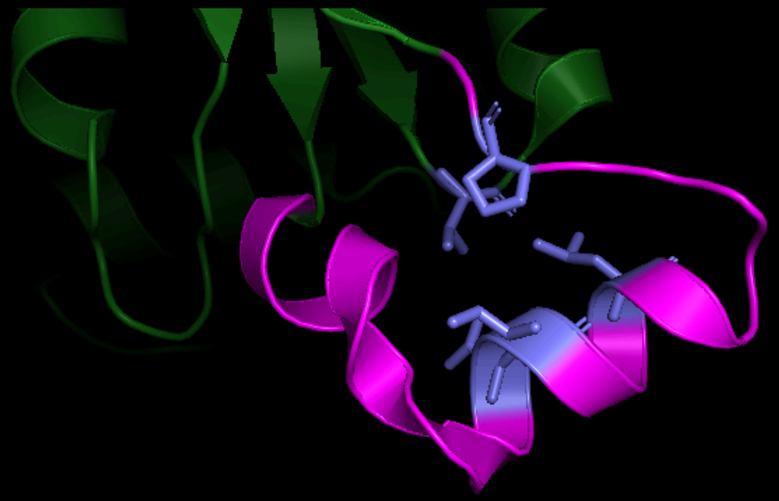


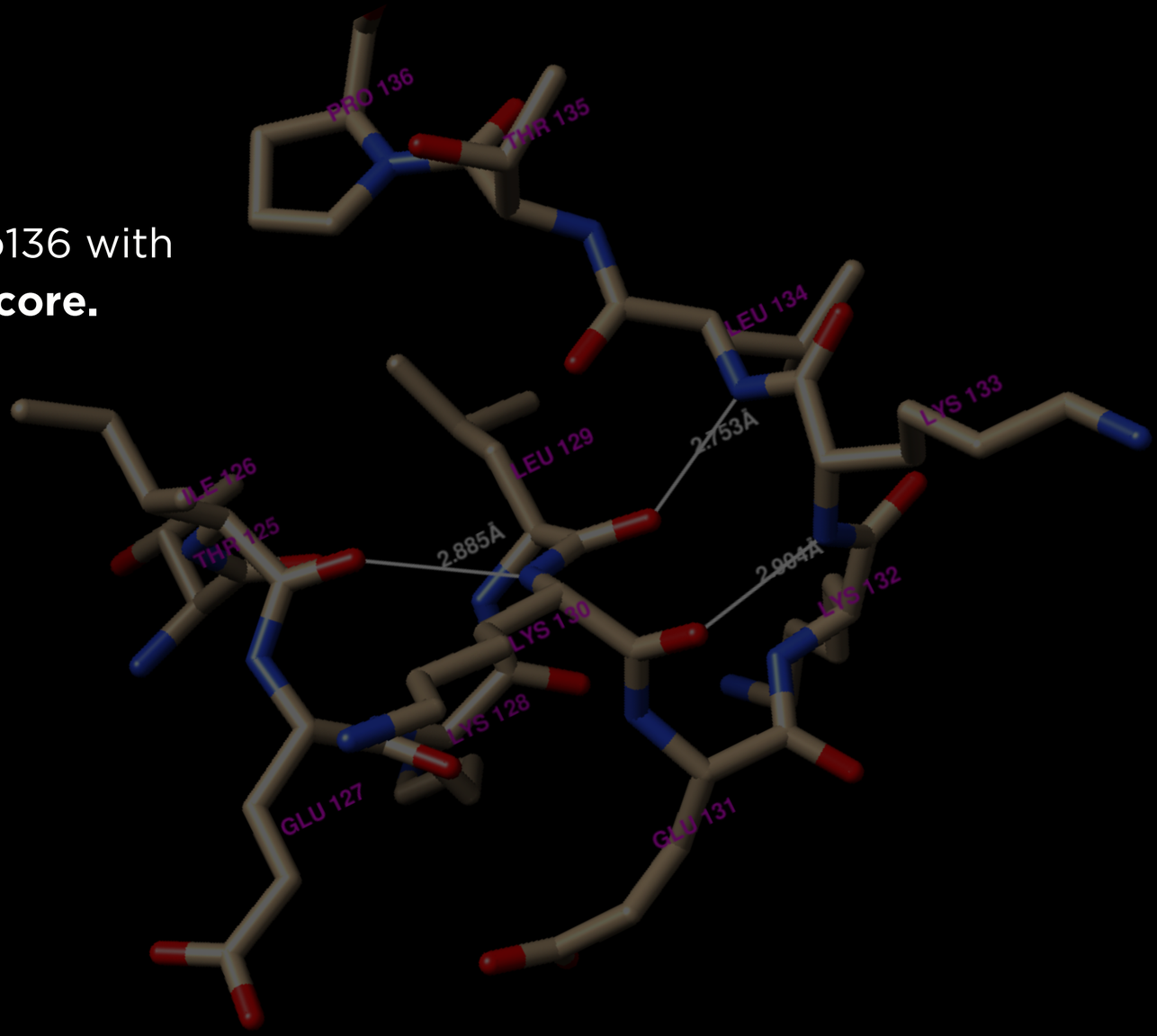
Intramolecular interactions

Inserted domain

1
2
3
4

Residues Thr 125, Ile 126, Leu 129 and Pro136 with Val 85 are forming the **hydrophobic core.**



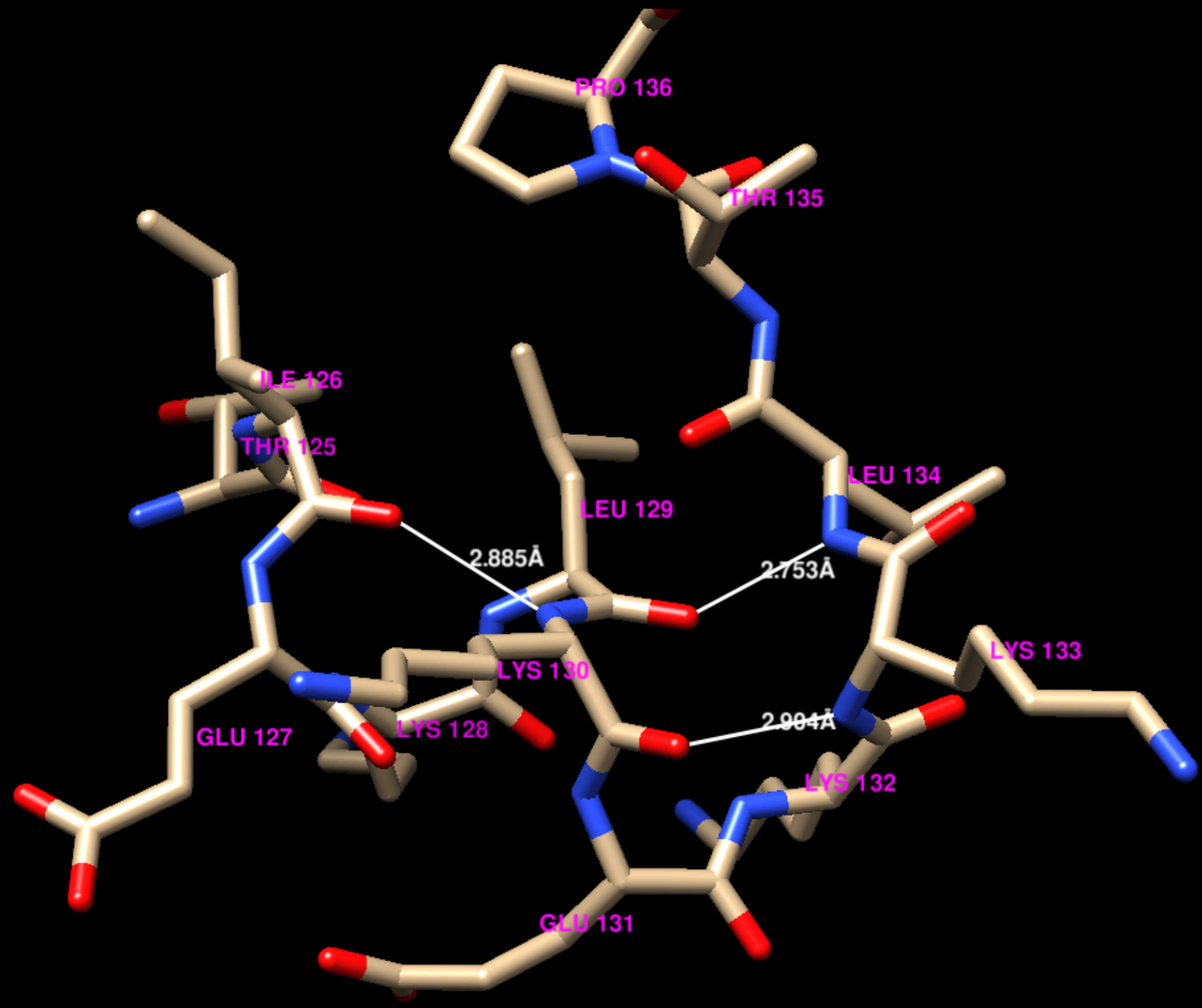


Intramolecular interactions

Inserted domain

1
2
3
4

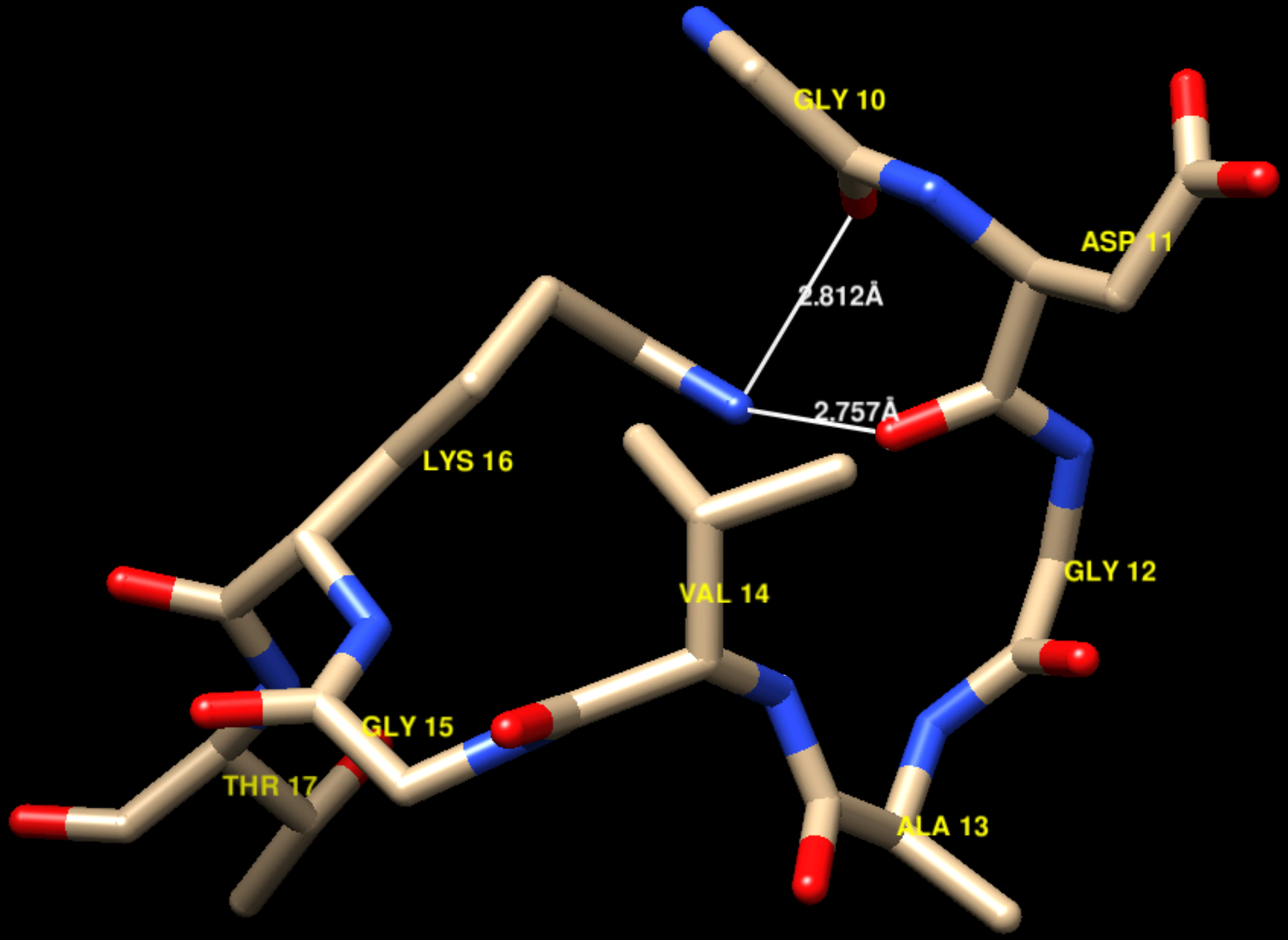
- Lys 130 N - Ile 126 O
- Lys 133 N - Lys 130 O
- Leu 134 N - Leu 129 O



Intramolecular interactions

P-loop

- Lys 16 NZ - Gly 10 O
- Lys 16 NZ - Asp 11 O

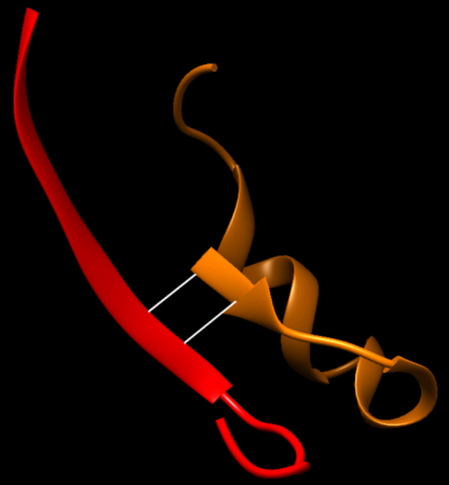


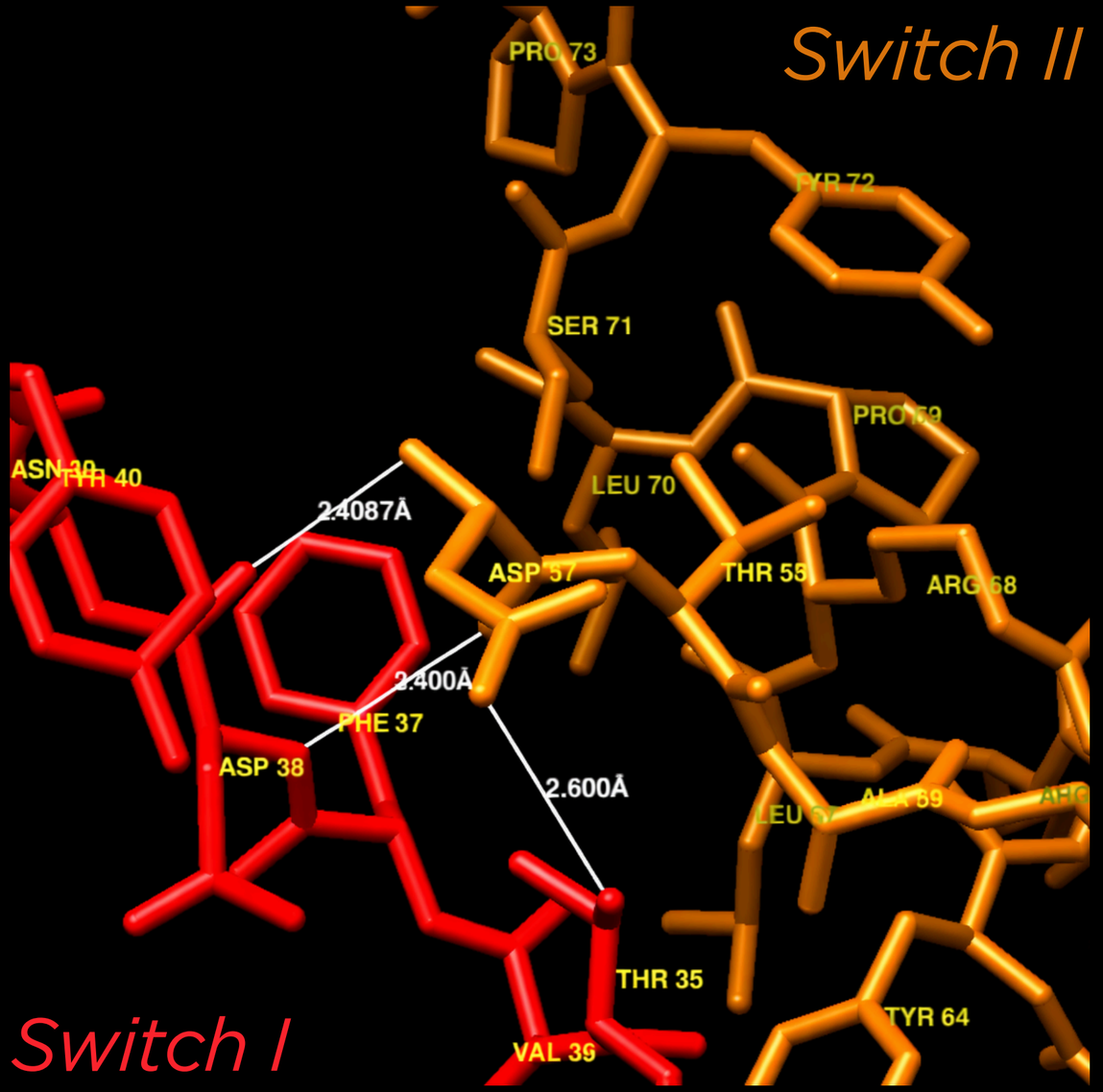
Intramolecular interactions

Switch I and II

1
2
3
4

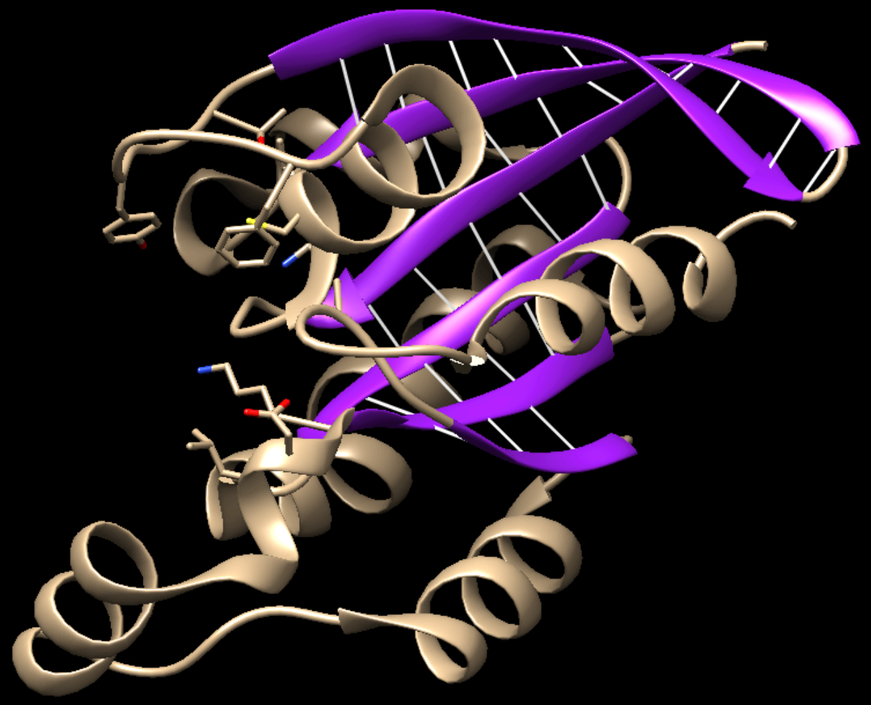
- Thr 35 OG1 - Asp 57 OD2
- Asp 38 N - Asp 57 O
- Asp 57 N - Asp 38 O



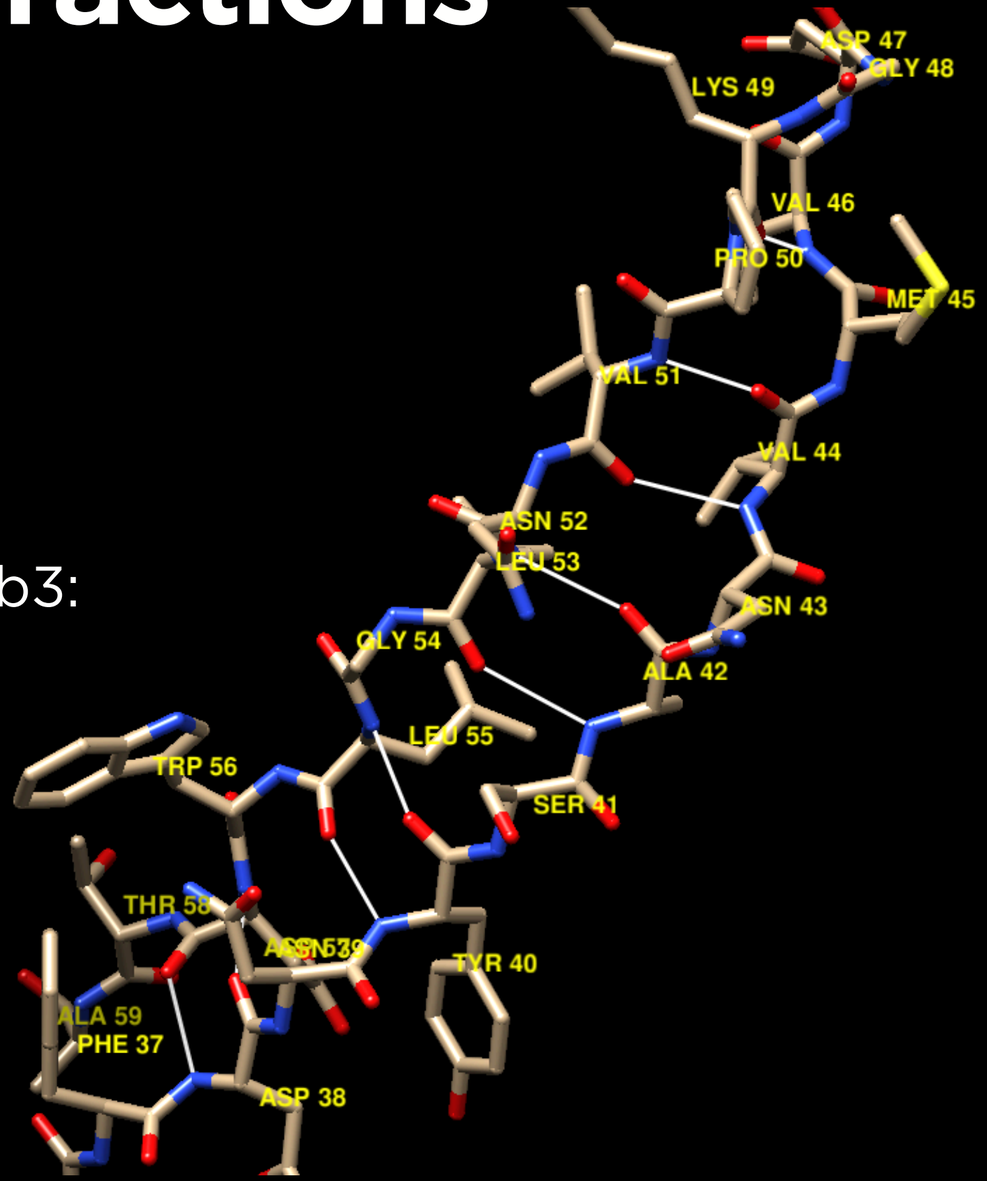


Intramolecular interactions

Between beta strands



b2-b3:

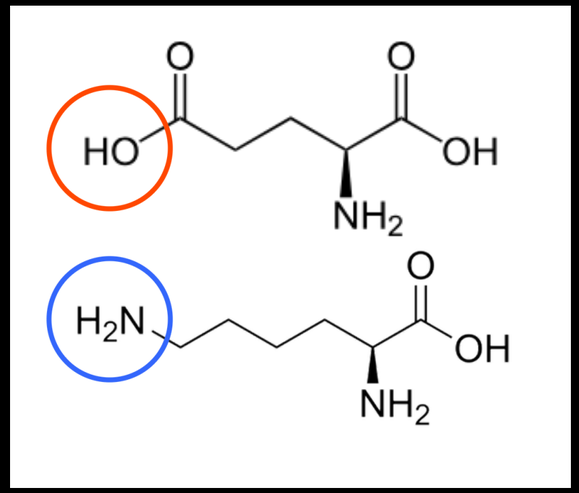


Intramolecular interactions

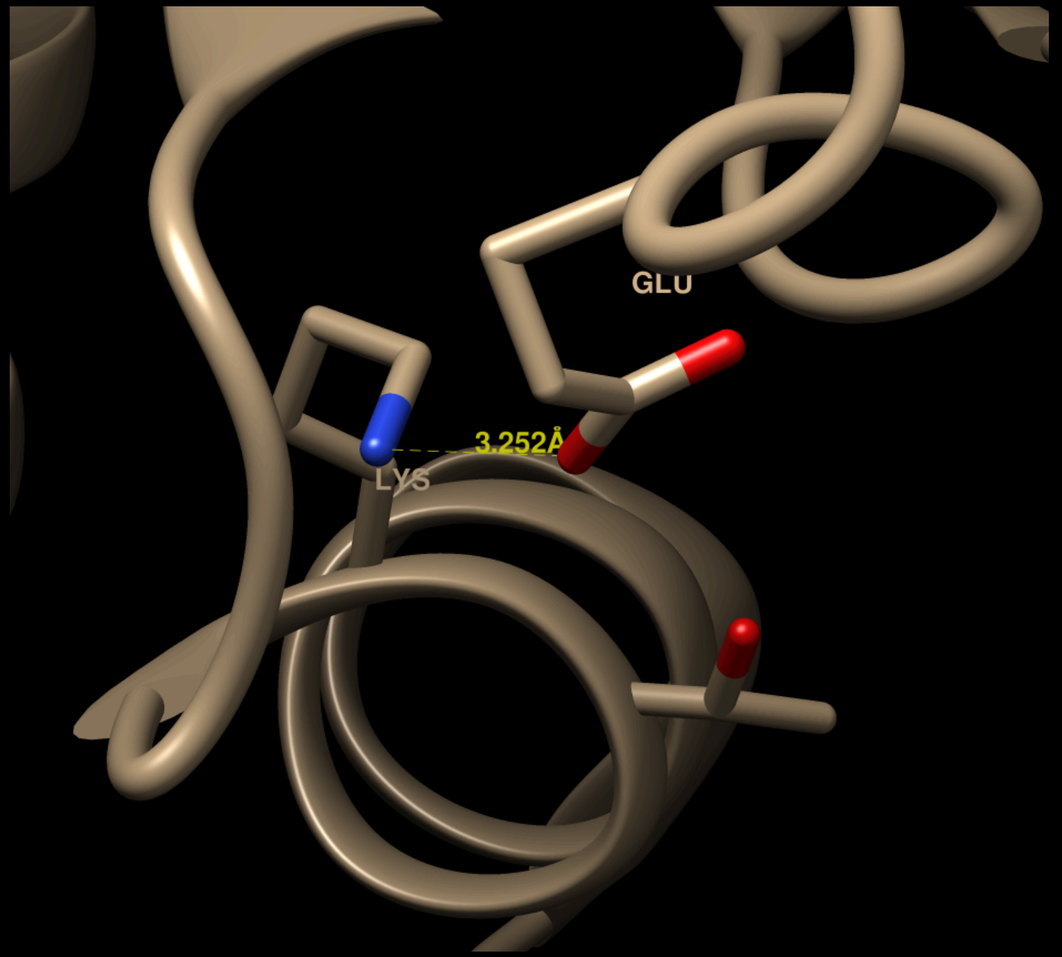
Between P-loop and Switch II

1
2
3
4

Glutamic acid



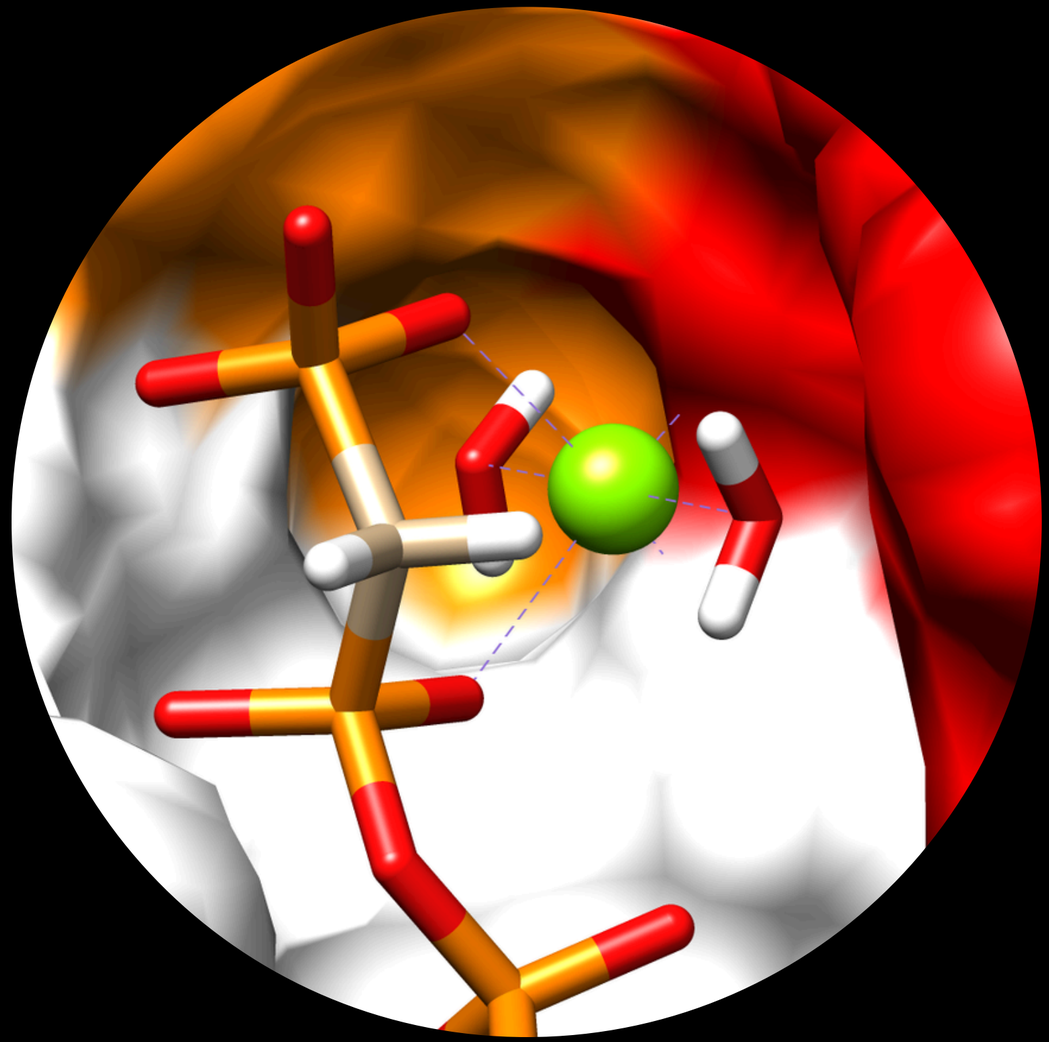
Lysine



Mg^{2+}: essential catalytic cofactor

- Functions:
  - Holds GTP/GDP within the binding pocket.
  - Stabilizes the negative charges.

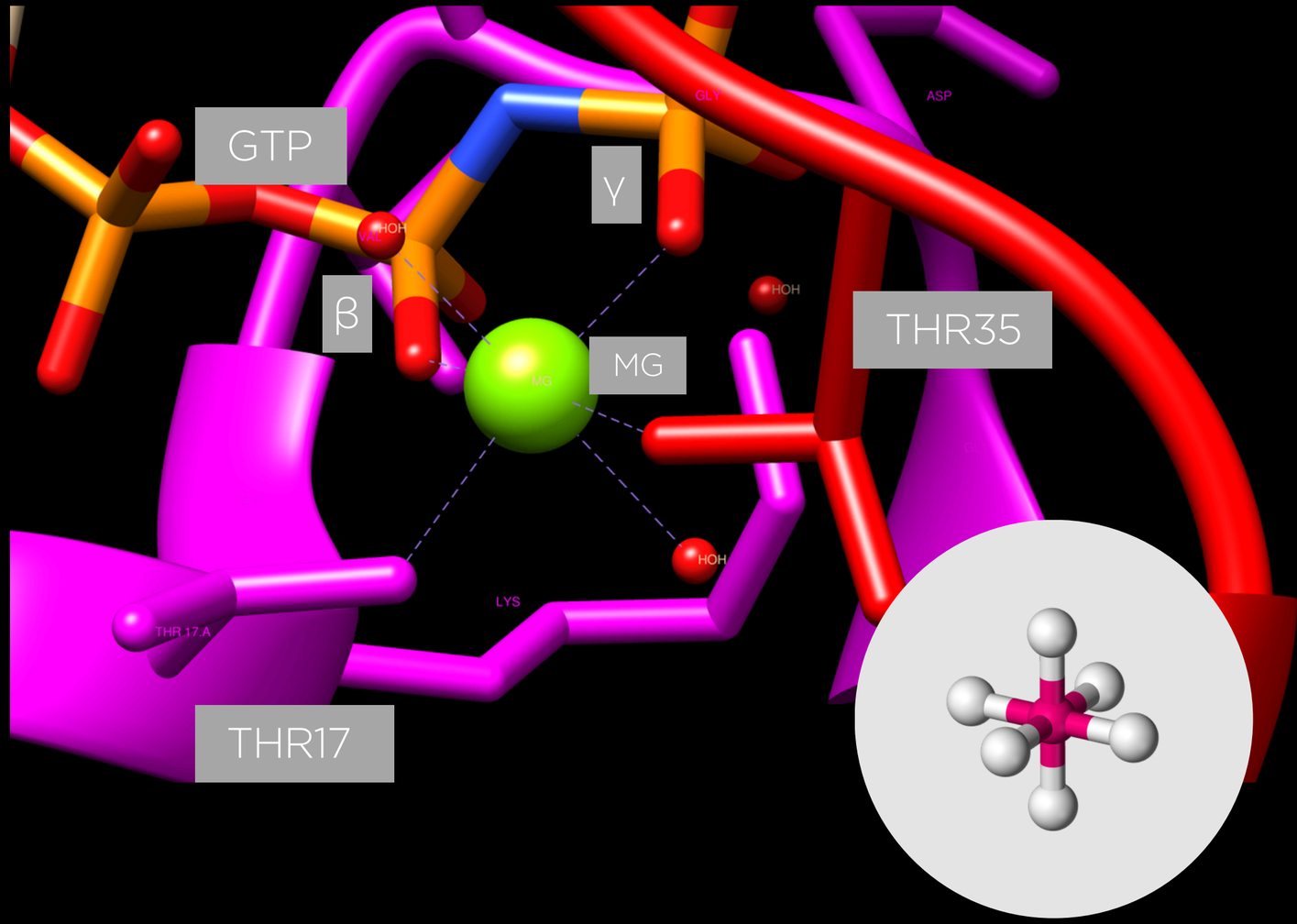
- Octaedric coordination that involves 6 oxygen atoms from:
  - Thr17 (P-loop)
  - Thr35 (Switch I)
  - 2 water molecules
  - β phosphate of GTP
  - γ phosphate of GTP



Mg^{2+}: essential catalytic cofactor

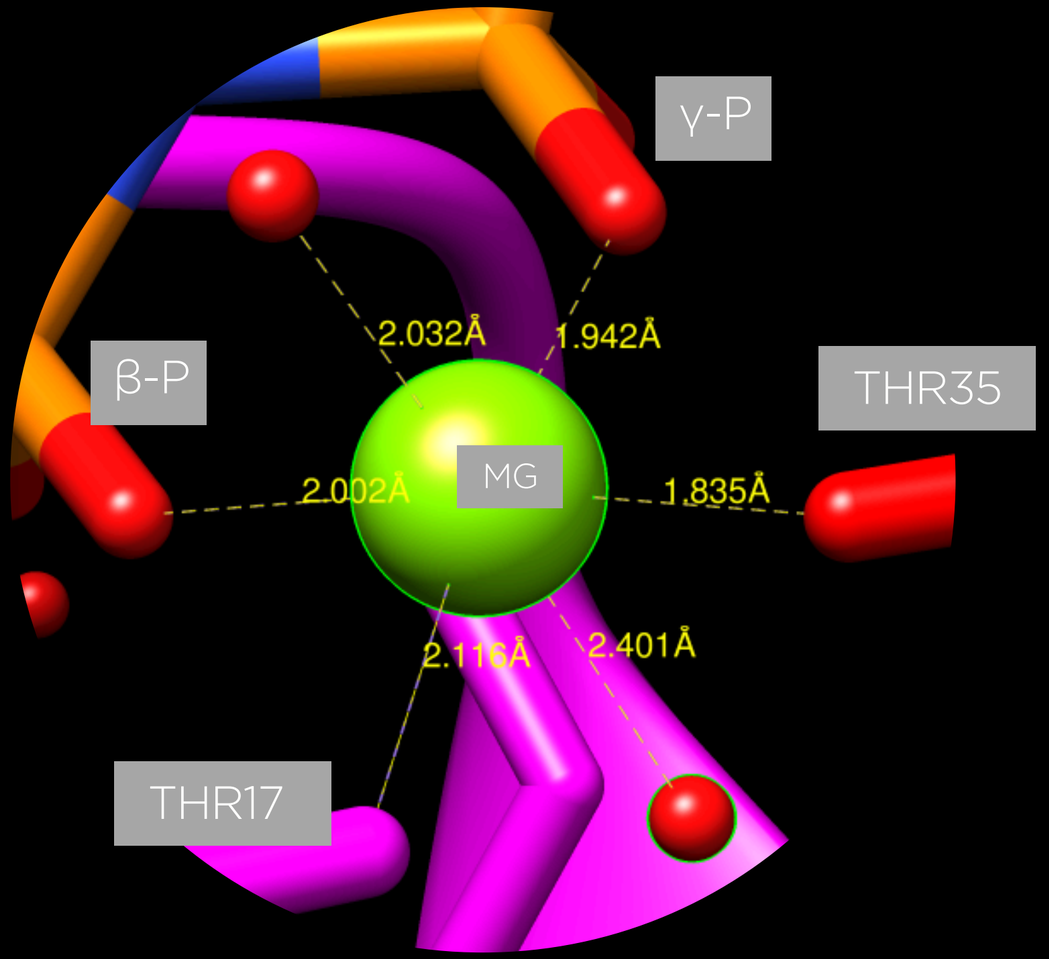
- Functions:
  - Holds GTP/GDP within the binding pocket.
  - Stabilizes the negative charges.

- Octaedric coordination that involves 6 oxygen atoms from:
  - Thr17 (P-loop)
  - Thr35 (Switch I)
  - 2 water molecules
  - β phosphate of GTP
  - γ phosphate of GTP



1
2
3
4

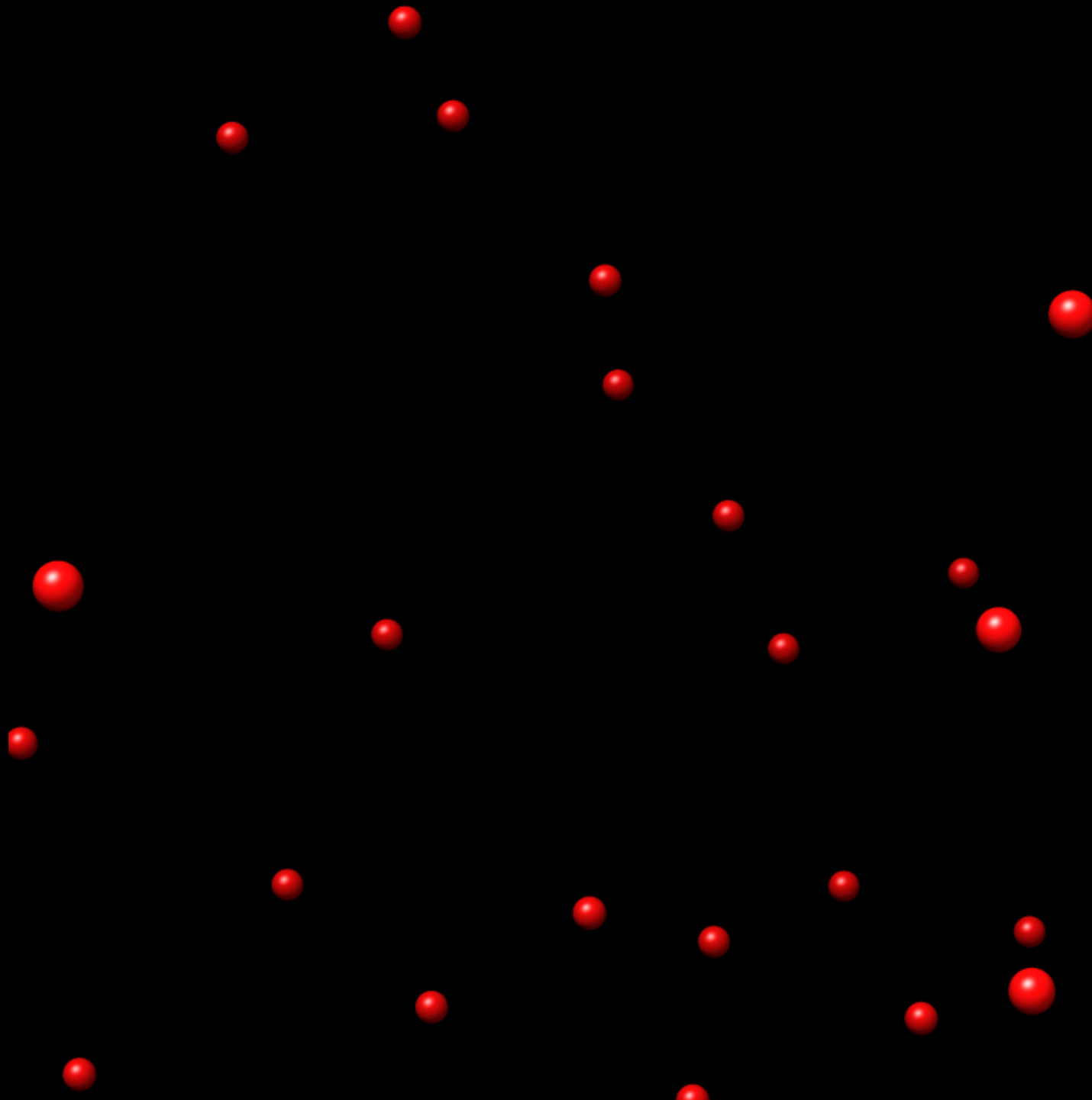
Mg^{2+}: essential catalytic cofactor



H_2O

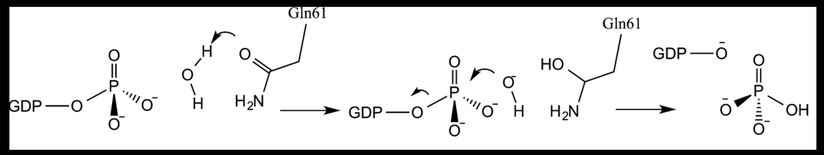
- Roles
  - Nucleophilic water
  - Active-site solvation
  - Support to switch I and switch II
  - GAP-assisted catalysis
  - Binding specificity of effectors
  - Stabilizes CO and NH side-chain residues of P-loop

1
2
3
4

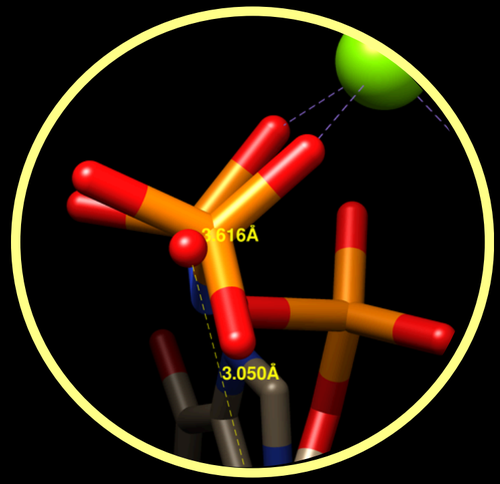


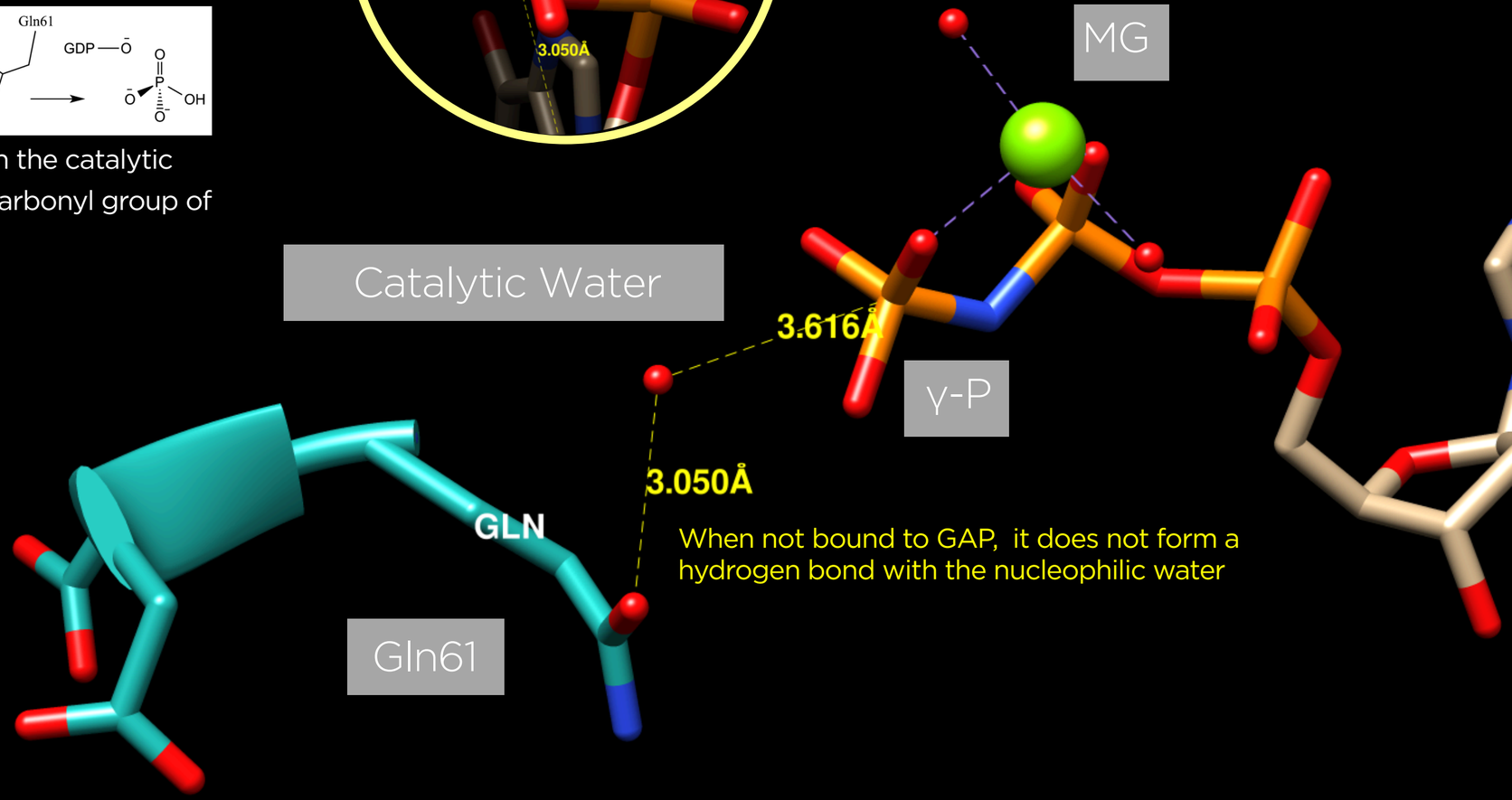
H_2O

Nucleophilic water



A base-catalyzed mechanism in which the catalytic water molecule is deprotonated by the carbonyl group of the Gln 61 sidechain.





When not bound to GAP, it does not form a hydrogen bond with the nucleophilic water

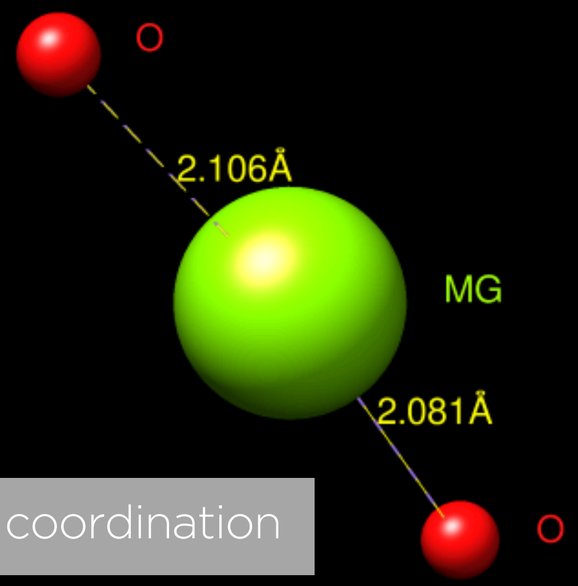
1
2
3
4

H_2O

Active-site solvation

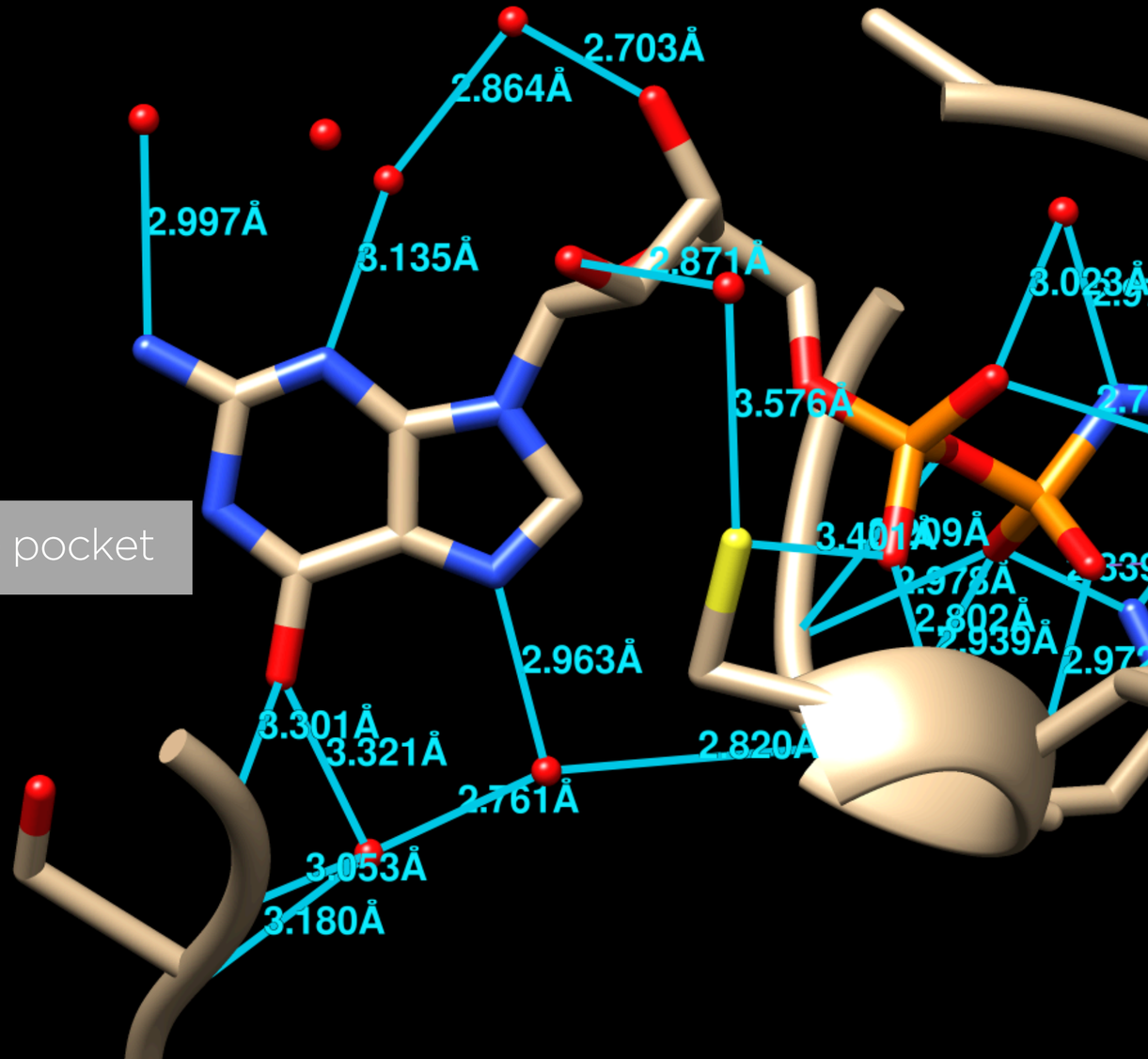
Structured water networks stabilize GTP/GDP and Mg^{2+} coordination, optimizing electrostatics and geometry of the catalytic site and lowering the activation barrier.

1
2
3
4



Mg2+ coordination

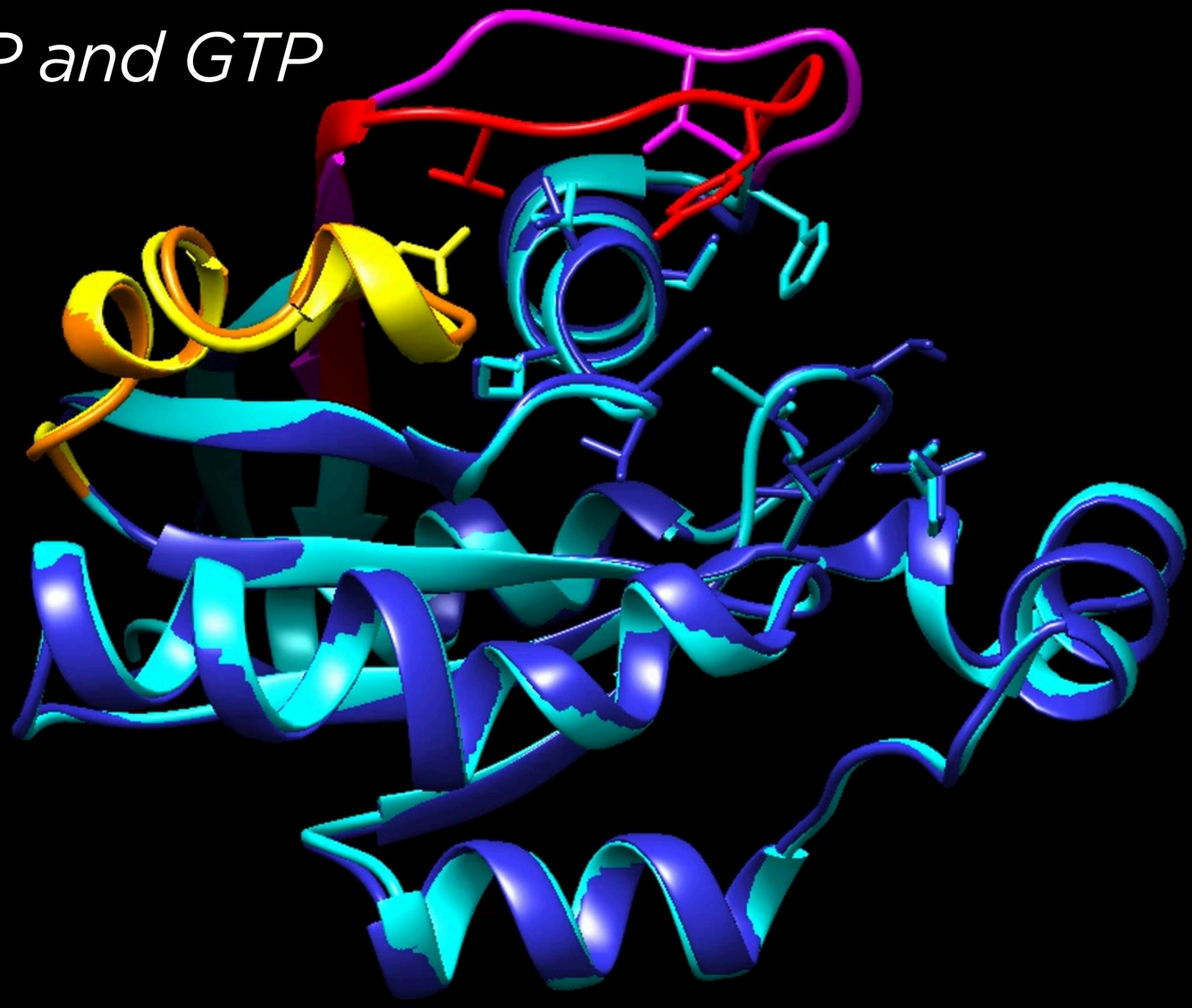
Guanine pocket



GDP/GTP bound state

Superimposition of GDP and GTP bound state

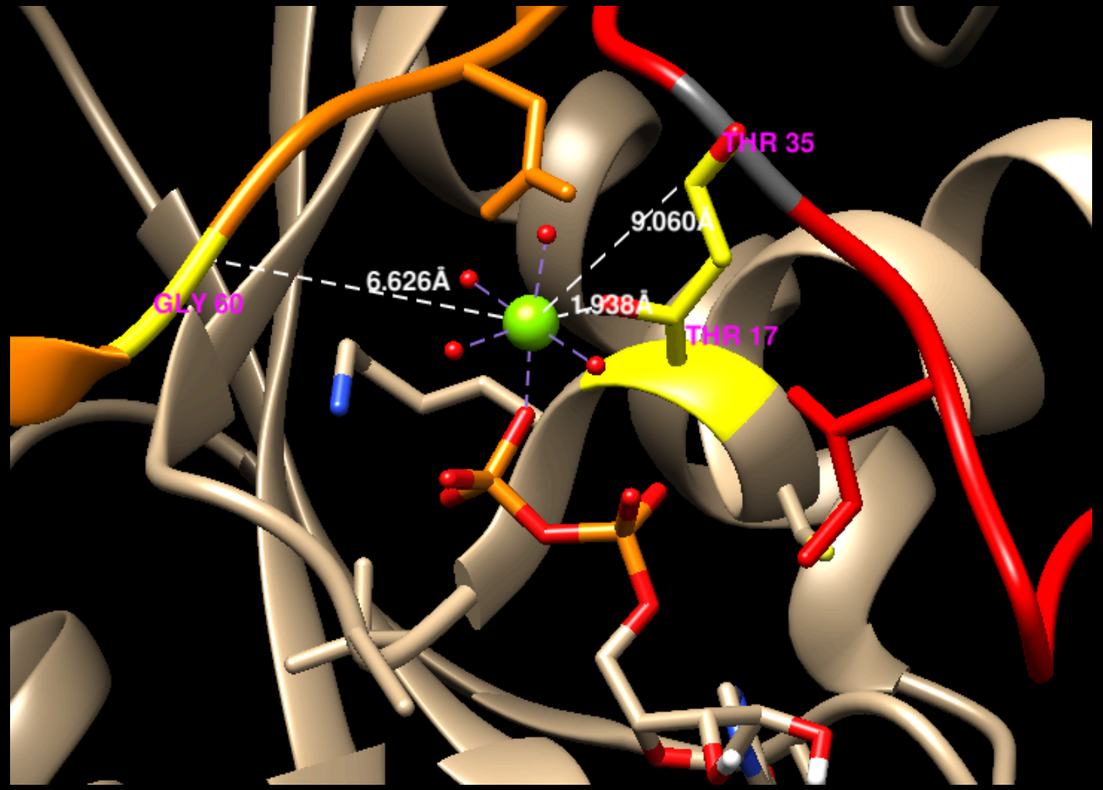
RMSD: 0.474Å



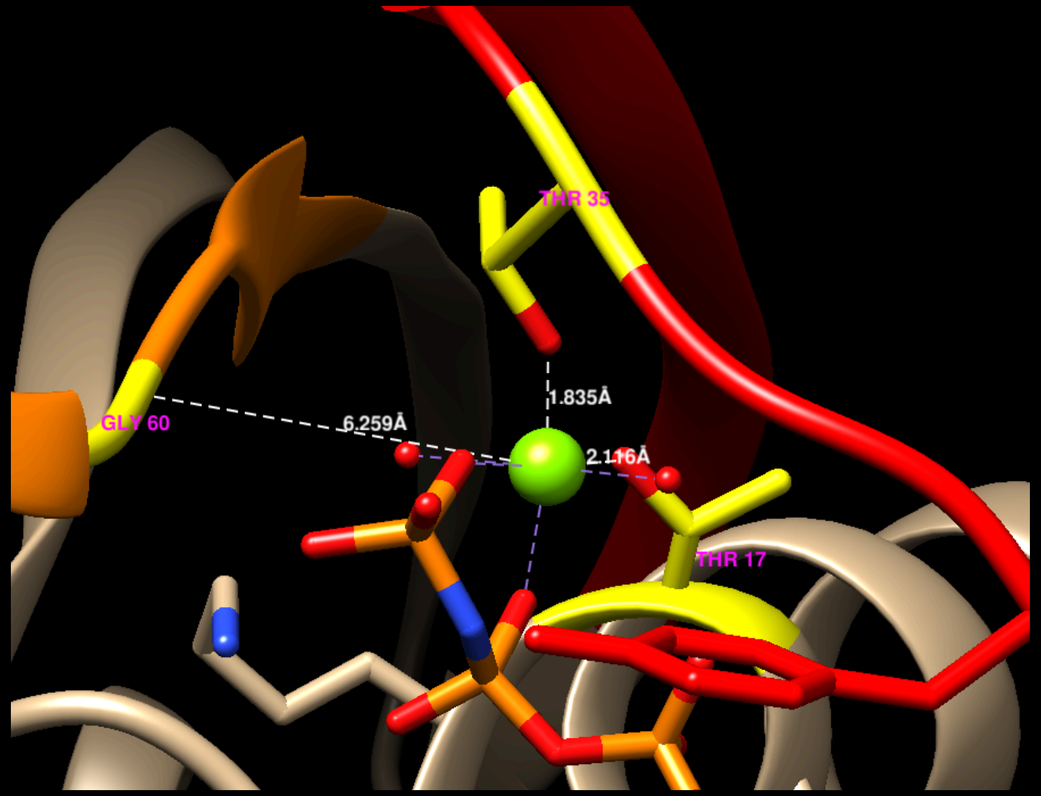
GDP/GTP bound state

1
2
3
4

GDP-bound state



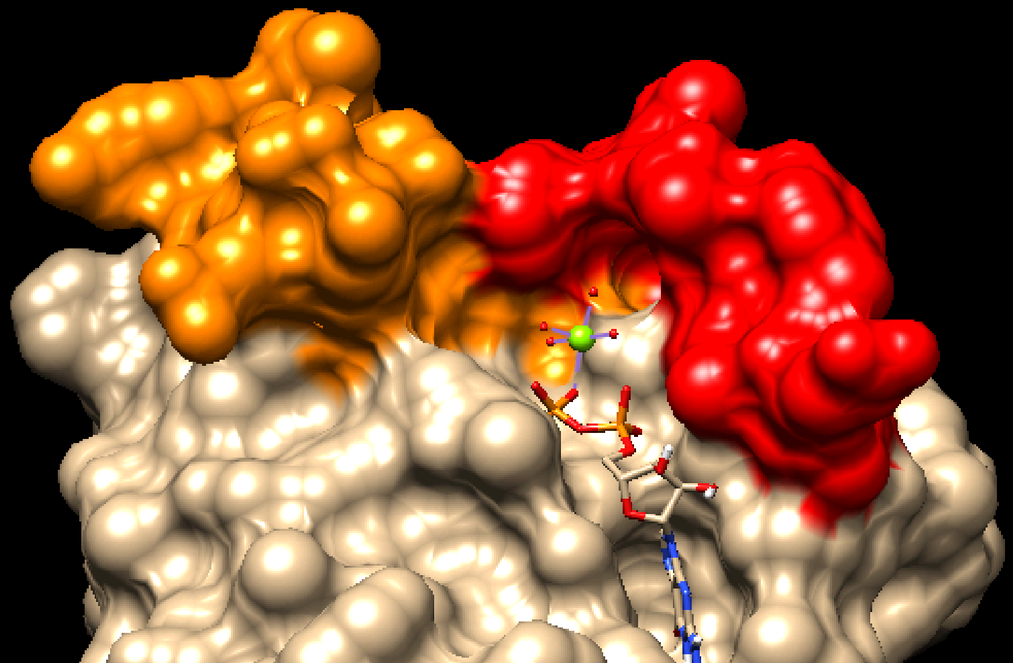
GTP-bound state



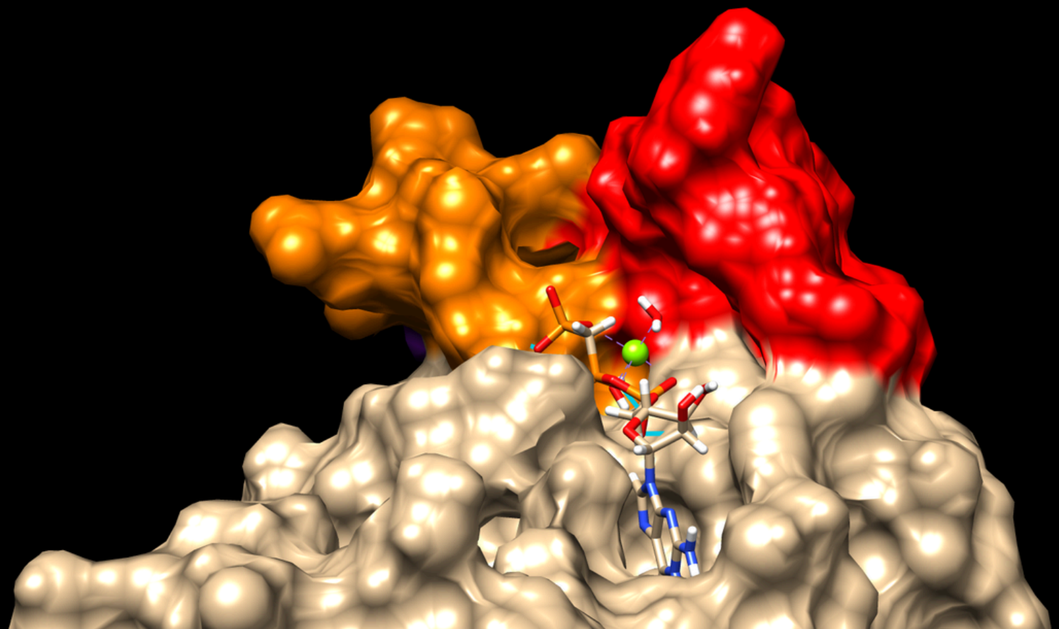
GDP/GTP bound state

1
2
3
4

GDP-bound state



GTP-bound state

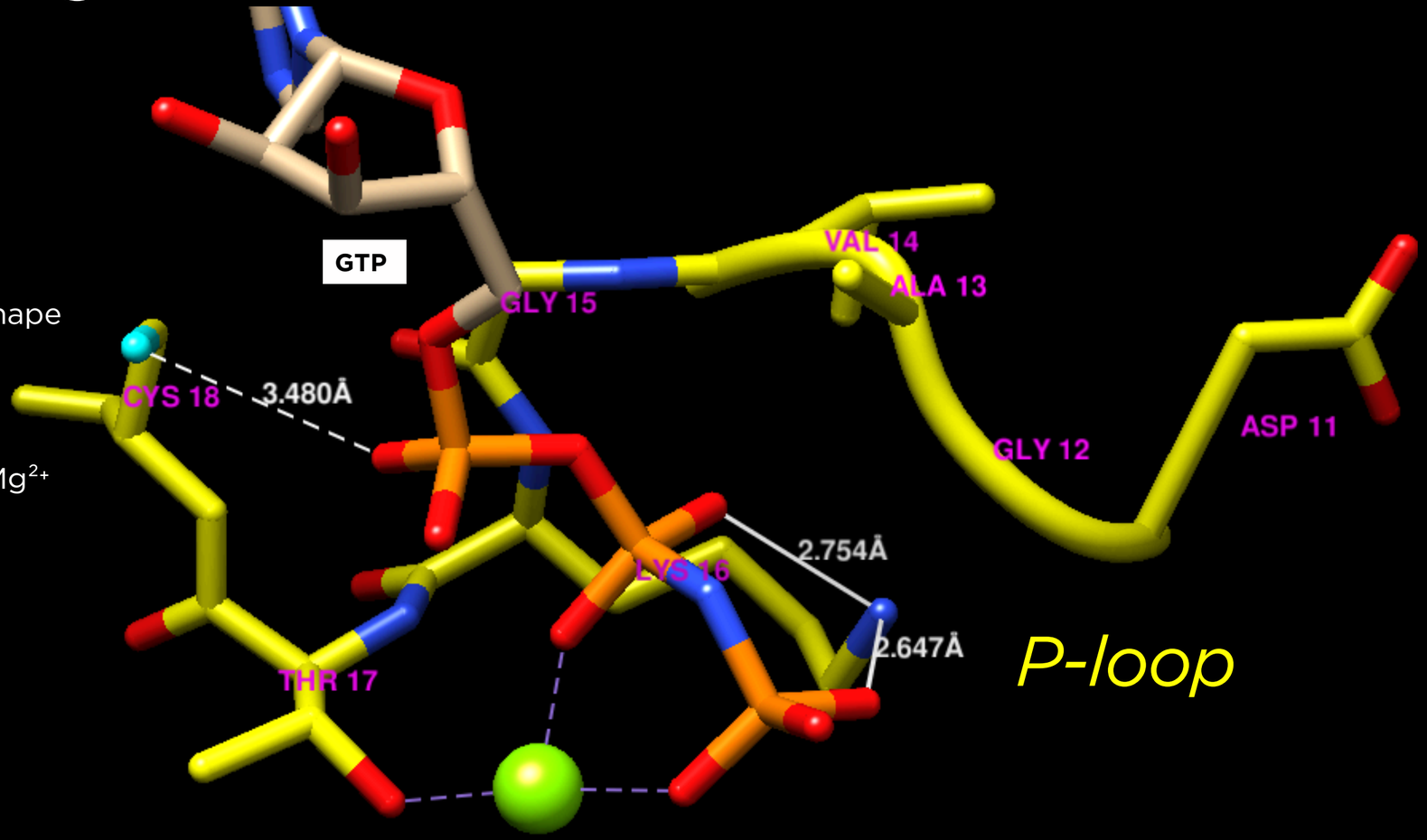


GDP/GTP bound state

Residues interacting with GTP

1
2
3
4

- GLY15: essential for binding shape
- LYS 16: Salt bridges
  - **NZ+** - **O1B-**
  - **NZ+** - **O1G-**
- THR 17: OG1 → Coordinates Mg^{2+}
- CYS 18: Dipol-dipol
  - **SG** - O1A

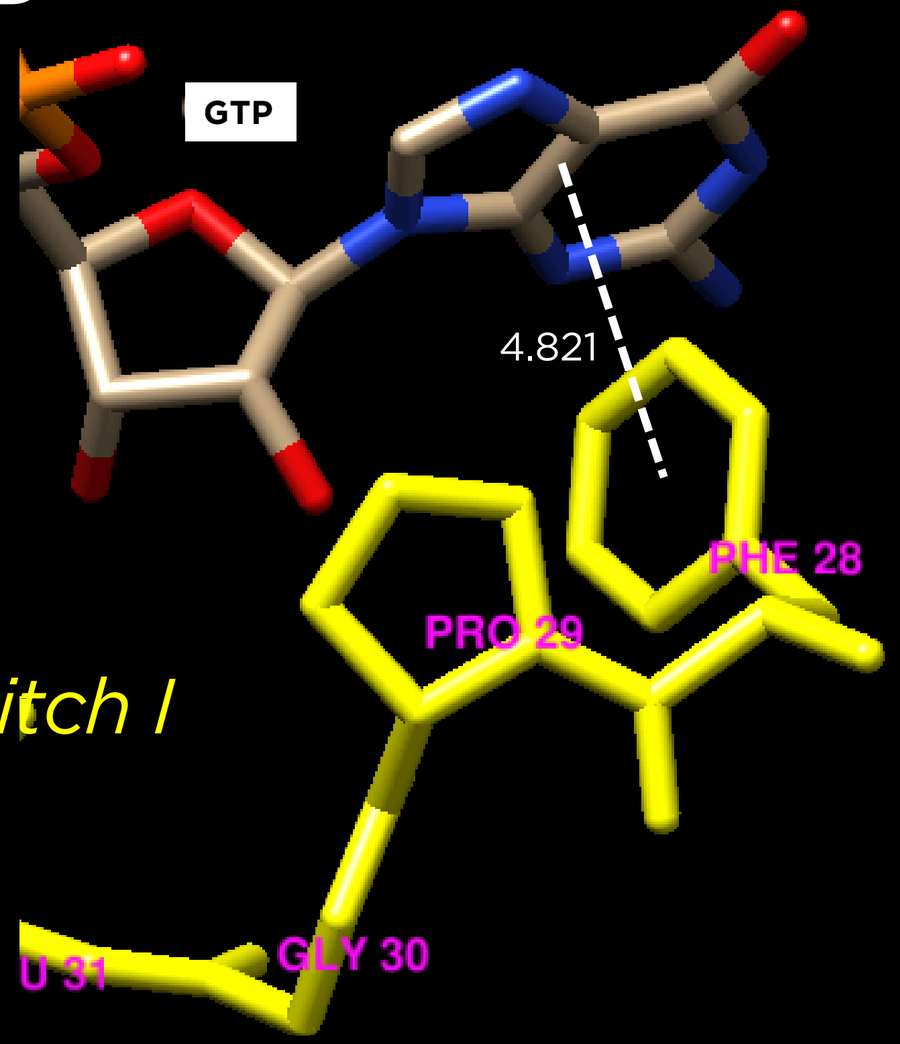


GDP/GTP bound state

Residues interacting with GTP

1
2
3
4

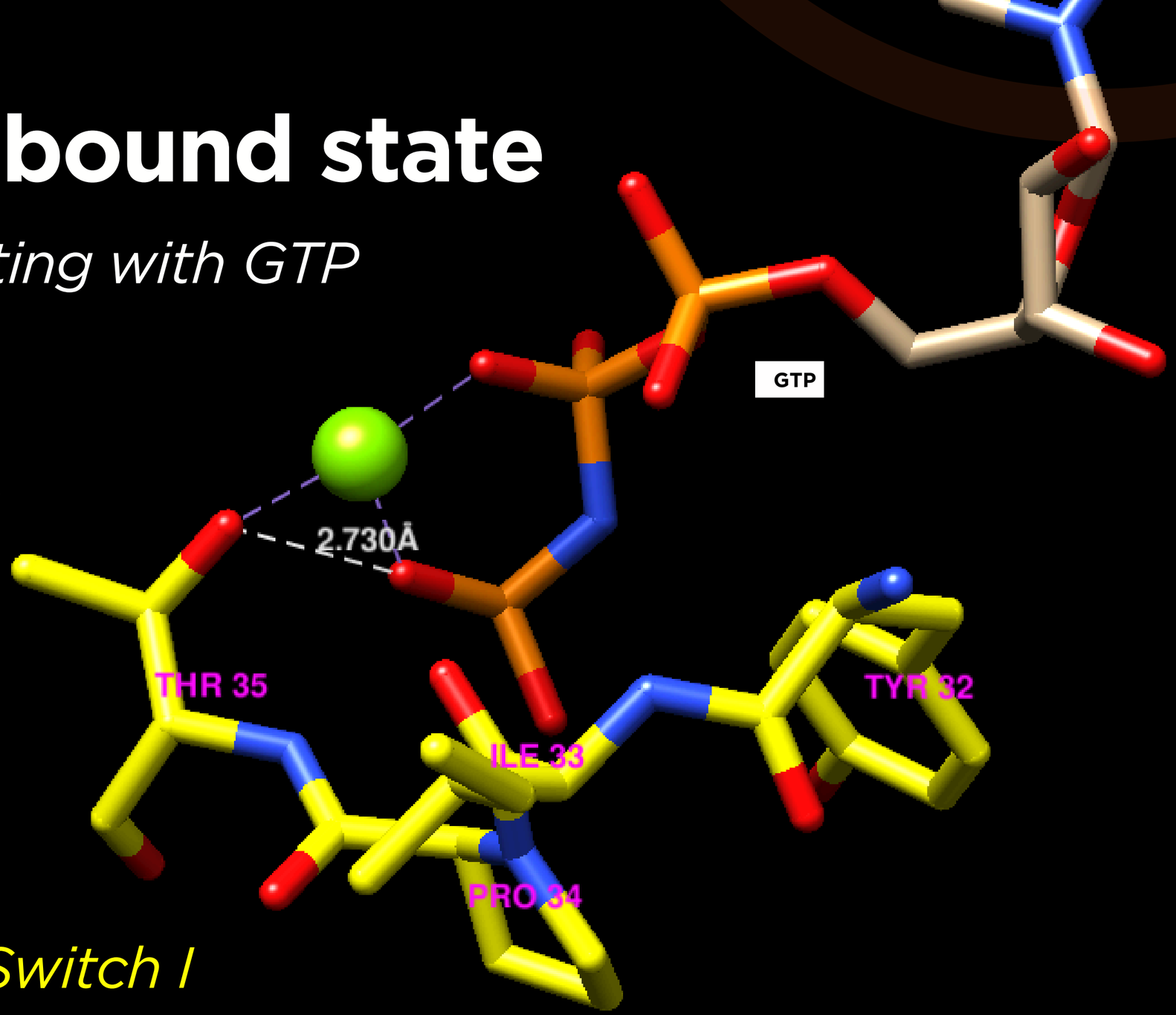
- PHE28: π–π stacking with guanine ring
  - primary recognition for the guanine ring
- PRO 29: side chain is directed toward the nucleotide binding pocket



GDP/GTP bound state

Residues interacting with GTP

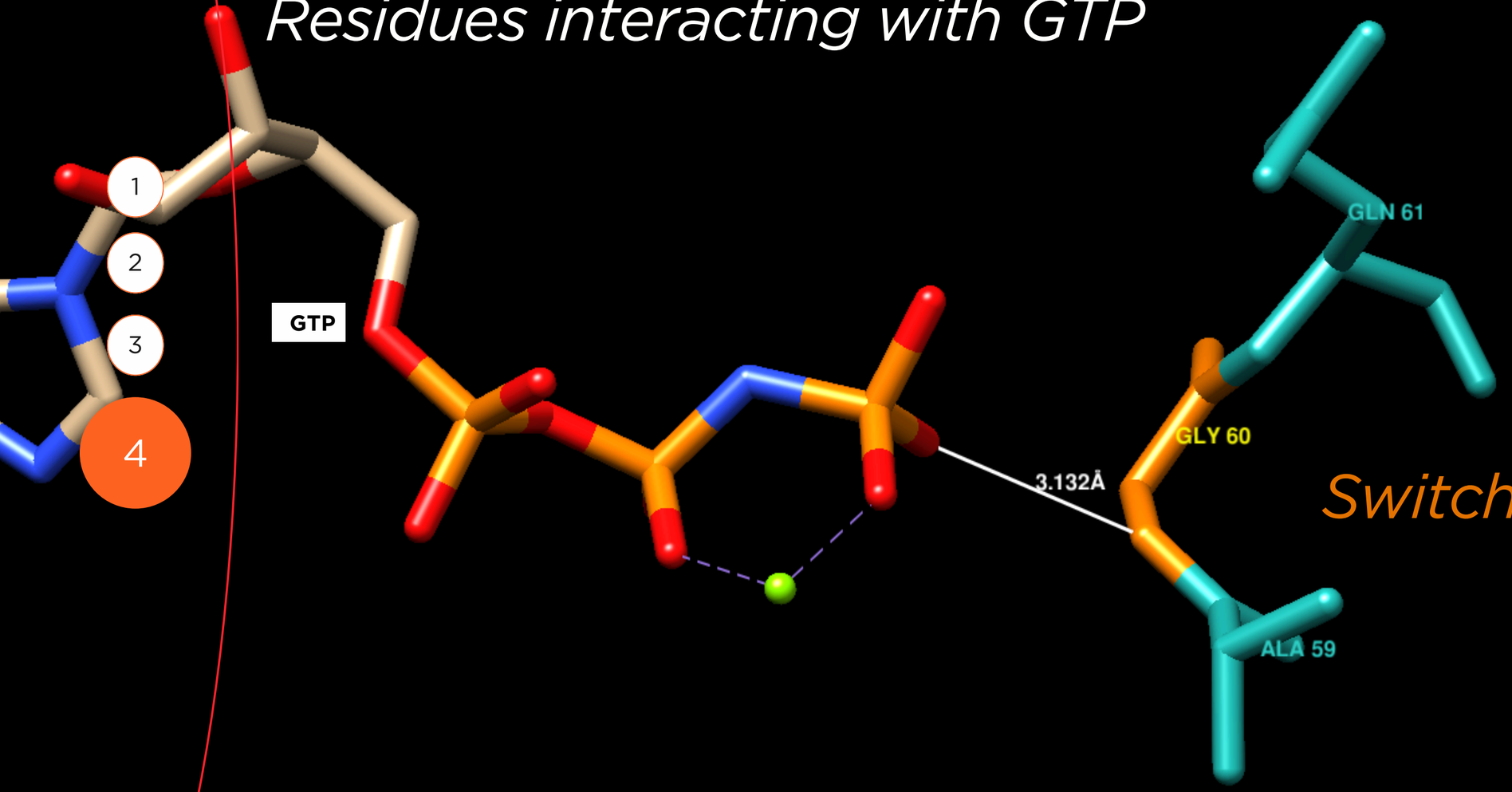
- THR 35
  - H bond OG1 - OG
  - Coordination: OG1 - Mg2+



Switch I

GDP/GTP bound state

Residues interacting with GTP

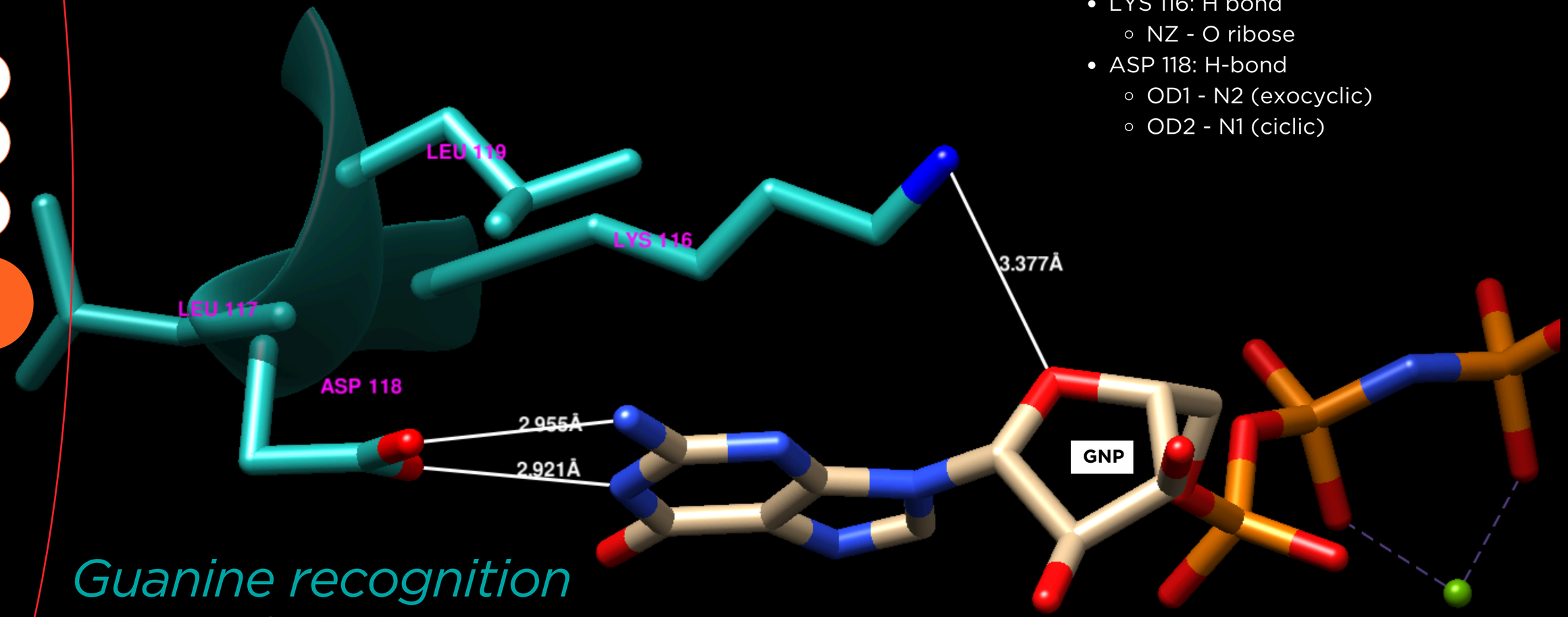


- GLY 60: H bond
  - Backbone NH - O1G

GDP/GTP bound state

Residues interacting with GTP

- LYS 116: H bond
  - NZ - O ribose
- ASP 118: H-bond
  - OD1 - N2 (exocyclic)
  - OD2 - N1 (ciclic)



1
2
3
4

Guanine recognition

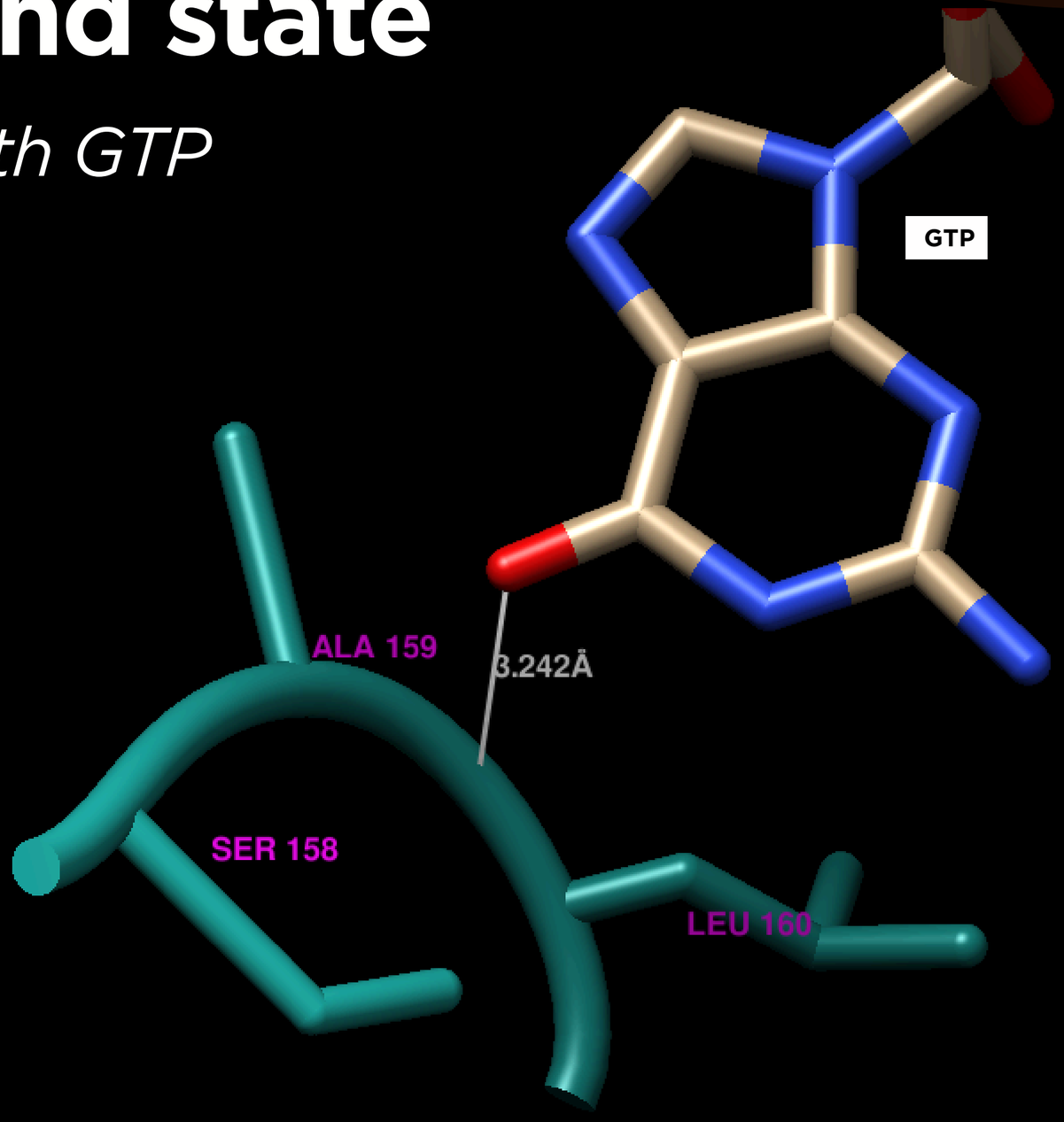
TKLD motif (NKxD)

GDP/GTP bound state

Residues interacting with GTP

1
2
3
4

- ALA 159 and LEU 160 form hydrophobic interactions with the guanine ring



Guanine stabilization

SAL motif

1

2

3

4

GAP (RacGAP1)

GTPase-activating proteins

GAP-RAC1

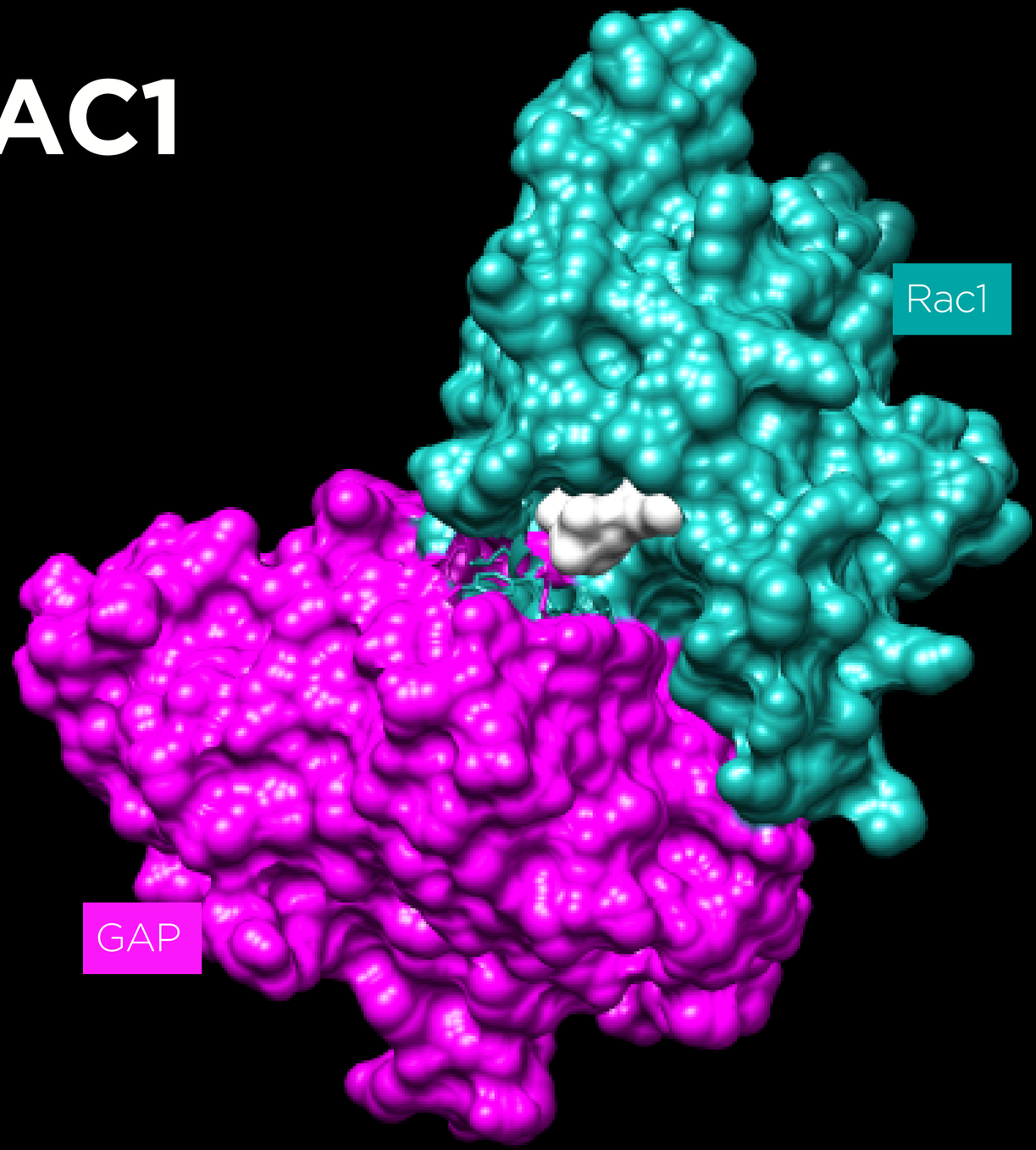


GAP-RAC1



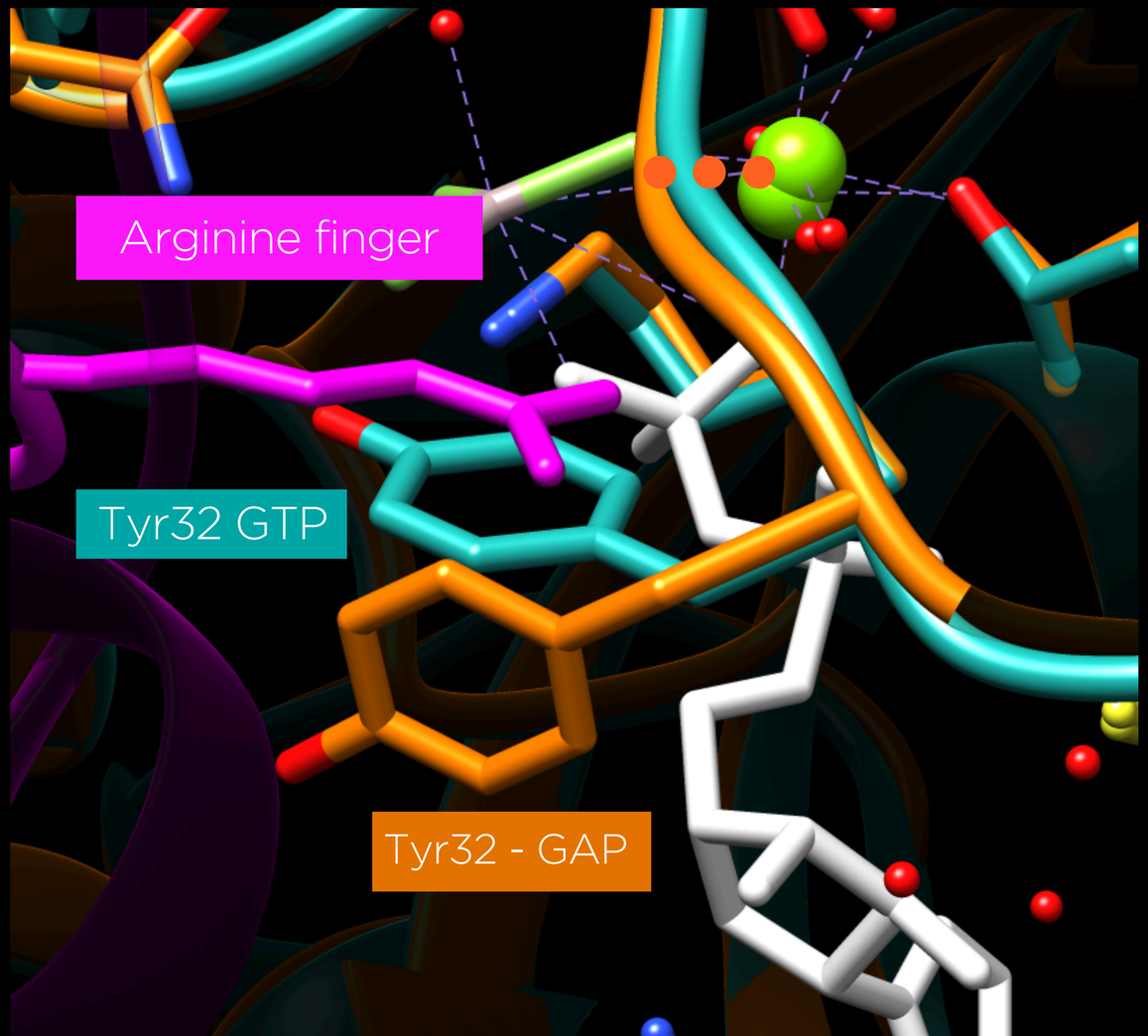
1
2
3
4

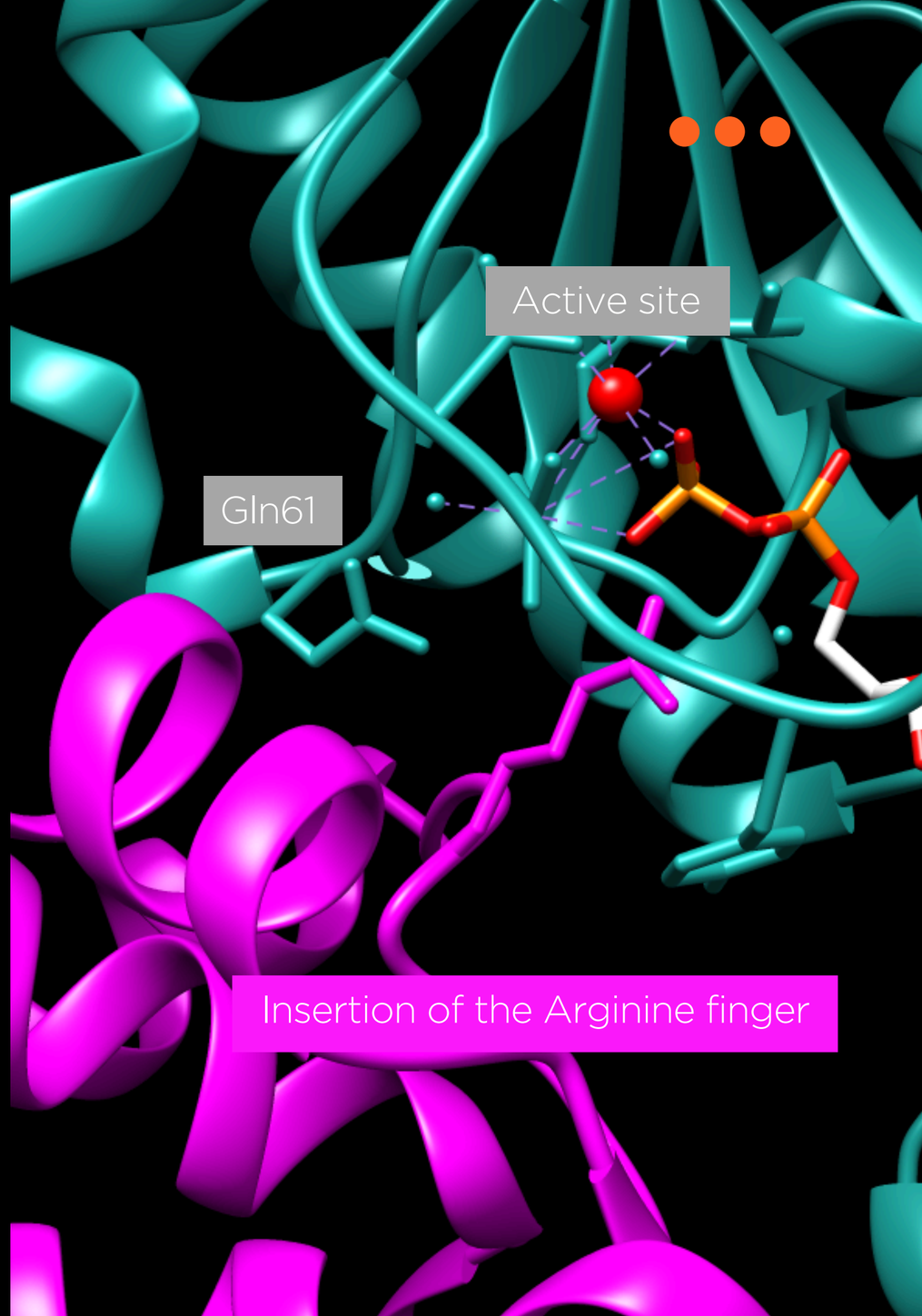
GAP-RAC1



1
2
3
4

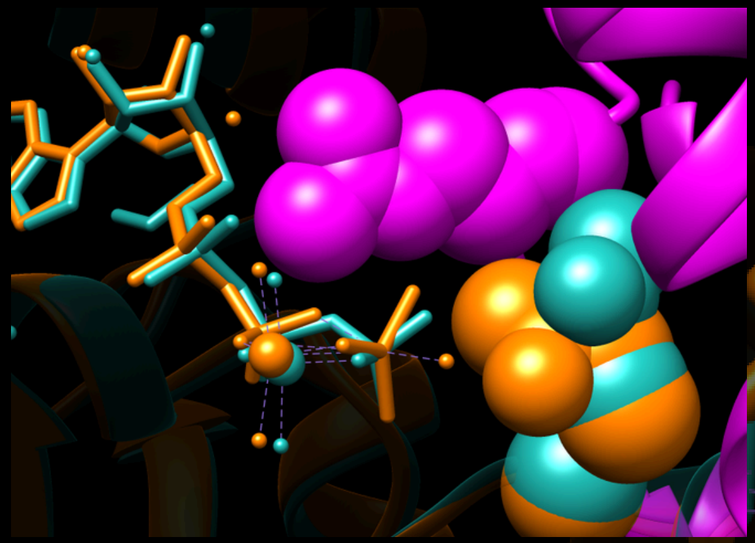
GAP-RAC1


Arginine finger
Tyr32 GTP
Tyr32 - GAP


Active site
Gln61
Insertion of the Arginine finger

GAP-RAC1

Insertion of the Arginine finger



Active site

Positions Gln61

1

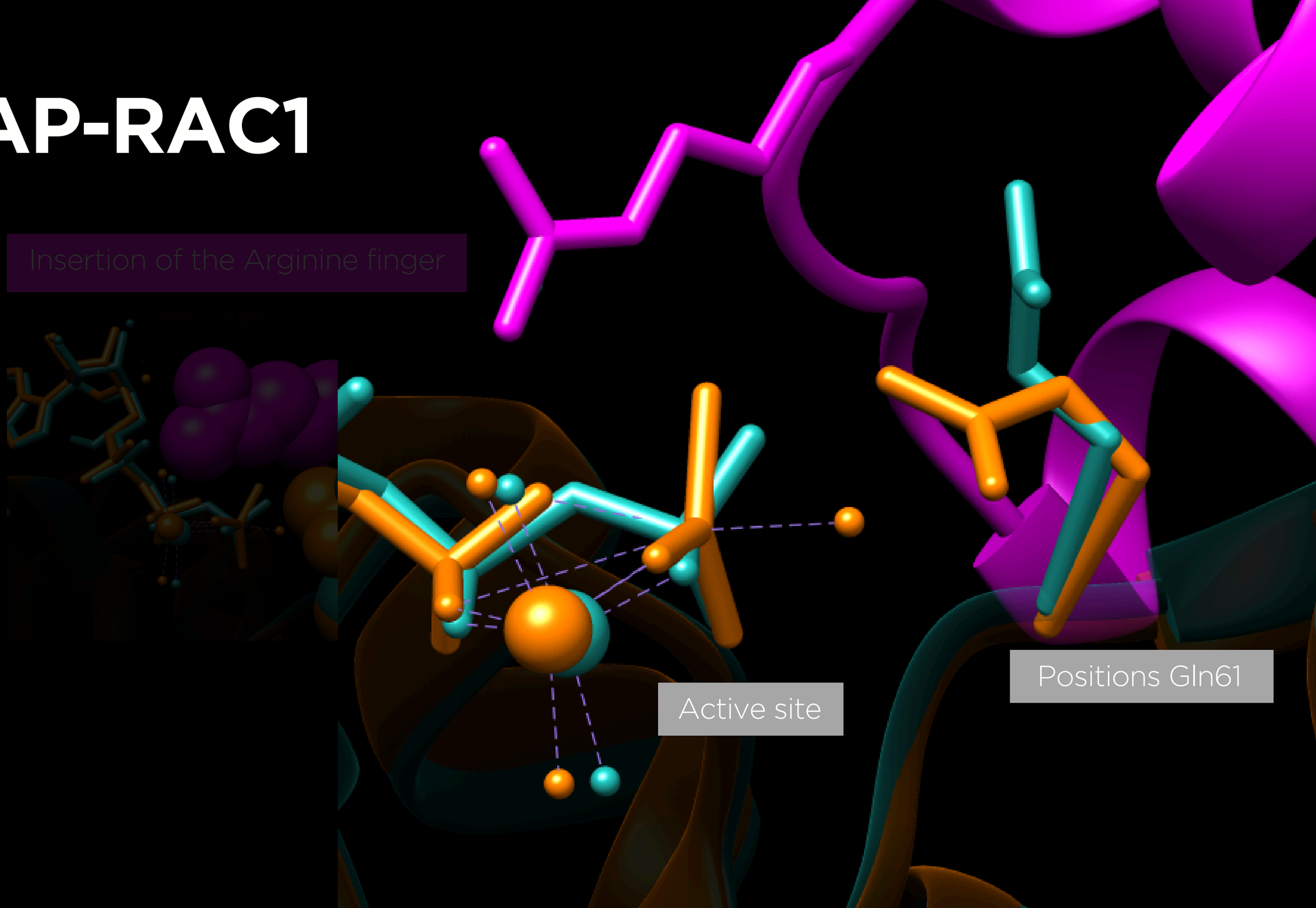
2

3

4

GAP-RAC1

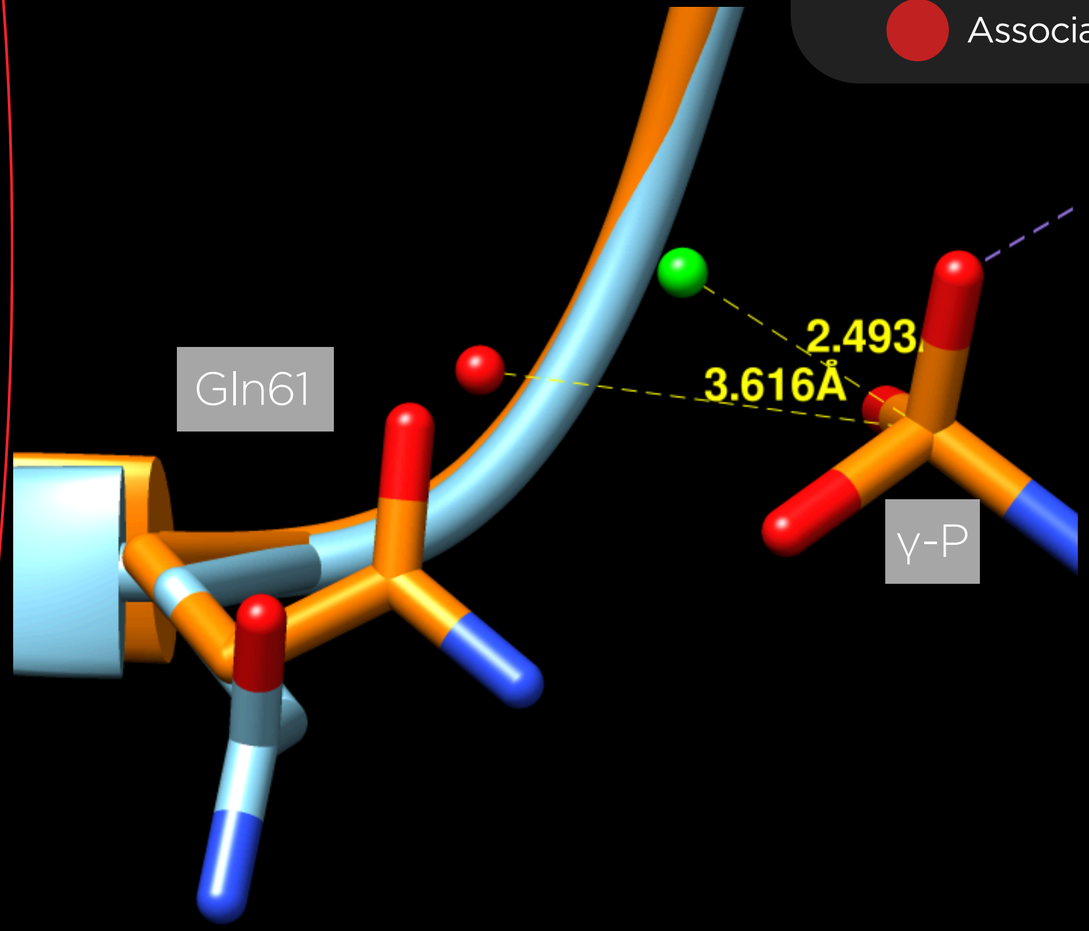
1
2
3
4



GAP-RAC1

Change of conformation of Gln61

1
2
3
4

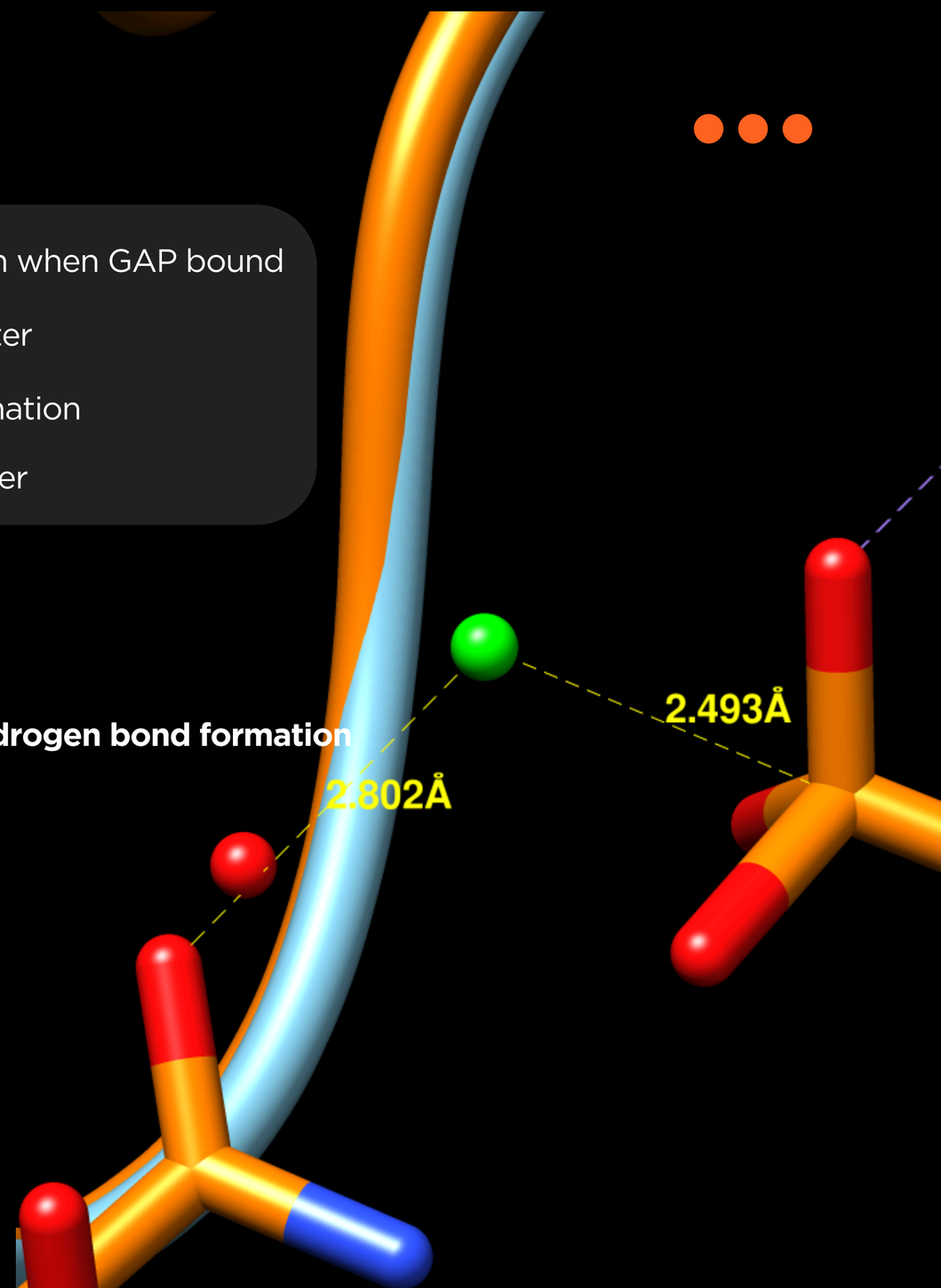


Gln61 conformation when GAP bound

Associated water

Gln61 GTP conformation

Associated water

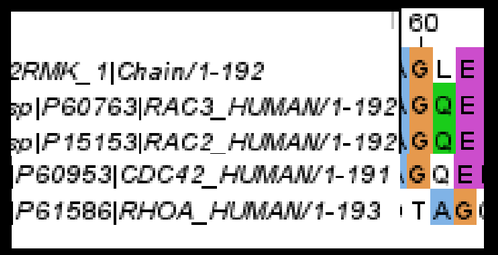


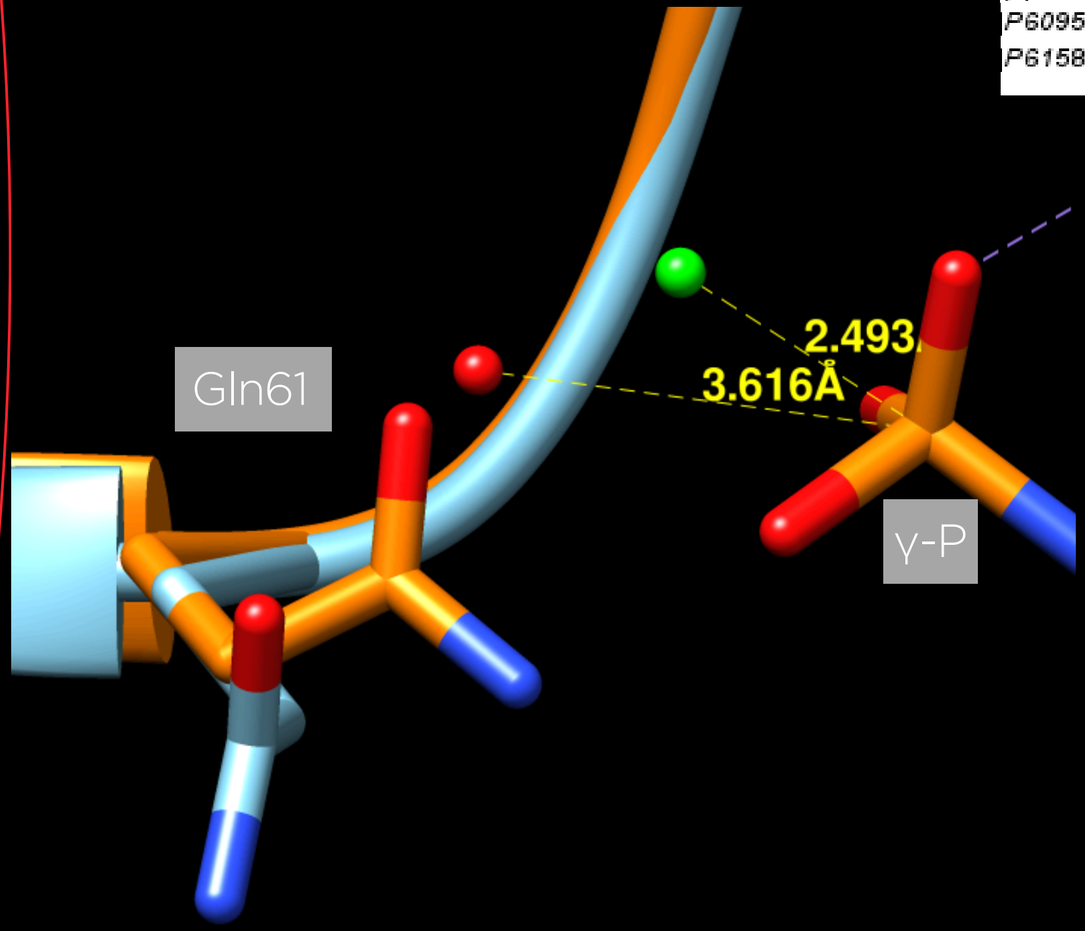
GAP-RAC1

Change of conformation of Gln61

1
2
3
4

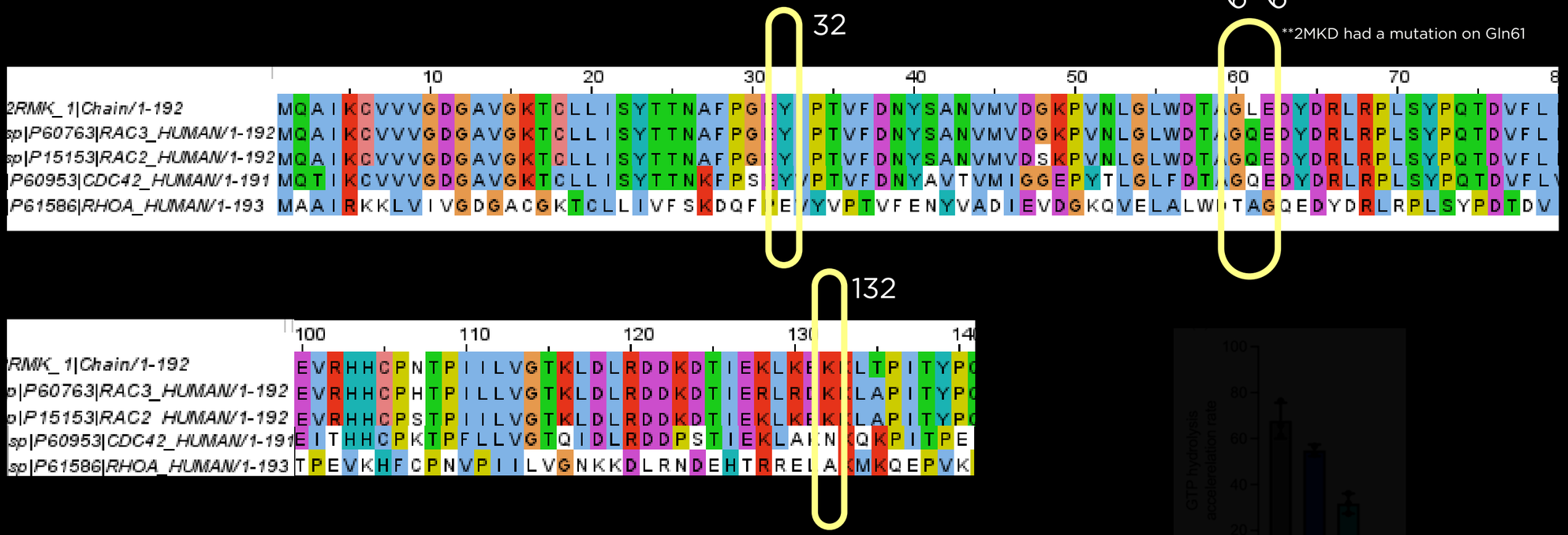
2RMK_1 (Rac1) had a mutation on Q61





GAP-RAC1

RacGAP1 differential specificity for Rho GTPases



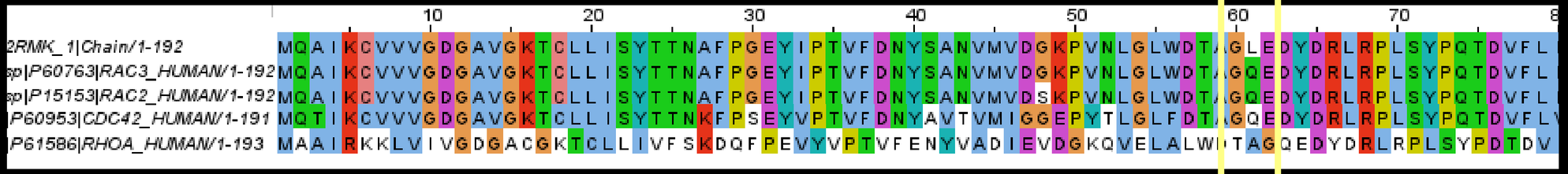
The cytokines is regulator RacGAP1 is a Rac1-specific GAP on membranes

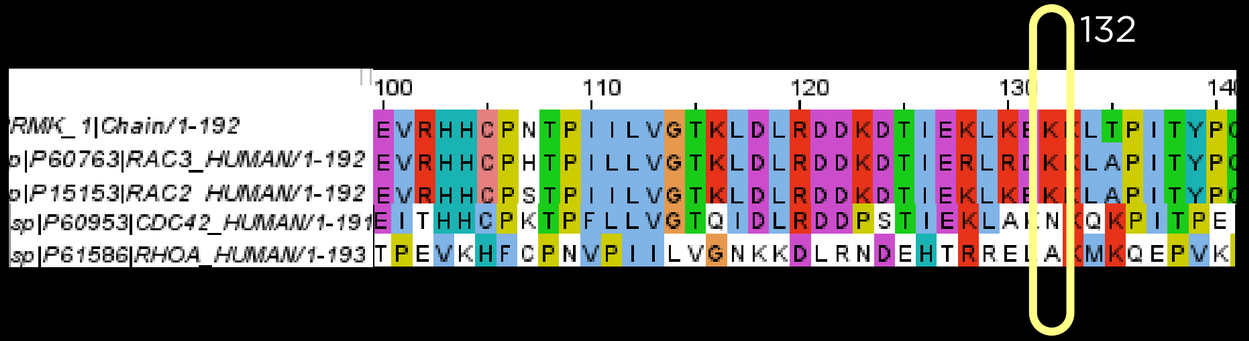
RacGAP1 can bind multiple Rho GTPases but is functionally specific for Rac1 due to optimal conformational and membrane-dependent alignment enabling efficient GAP activity.

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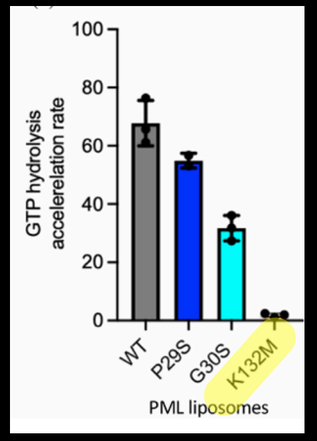
GAP-RAC1

RacGAP1 differential specificity for Rho GTPases





RacGAP1 can bind multiple Rho GTPases but is functionally specific for Rac1 due to optimal conformational and membrane-dependent alignment enabling efficient GAP activity.

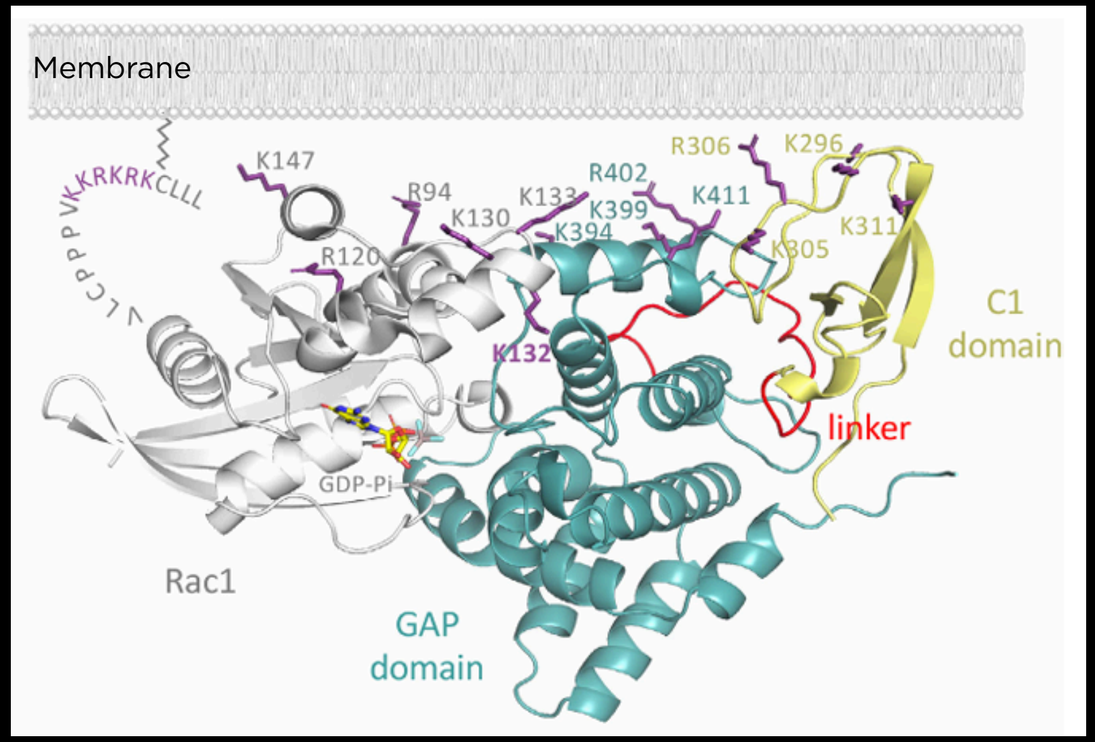


The cytokines is regulator RacGAP1 is a Rac1-specific GAP on membranes

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GAP-RAC1

Rac1-RacGAP1 complex associated with membranes



The cytokines is regulator RacGAP1 is a Rac1-specific GAP on membranes
***The structure of C1GAP was generated by Alphafold 3*

Non-specific electrostatic contacts (salt bridges)

Between anionic lipids (such as phosphatidylserine or PIP_2) and polybasic surfaces rich in K and R on Rac1 and RacGAP1.

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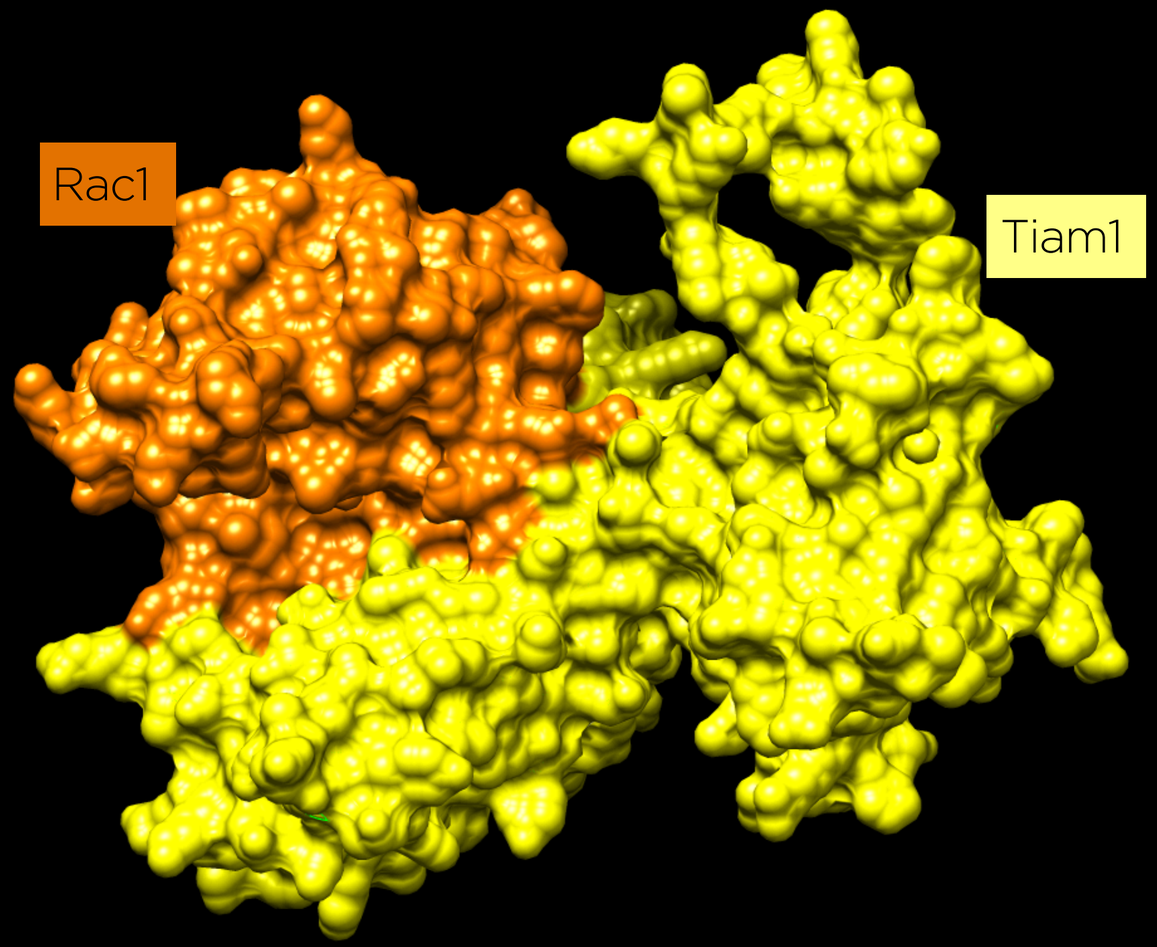
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GEF (Tiam1)

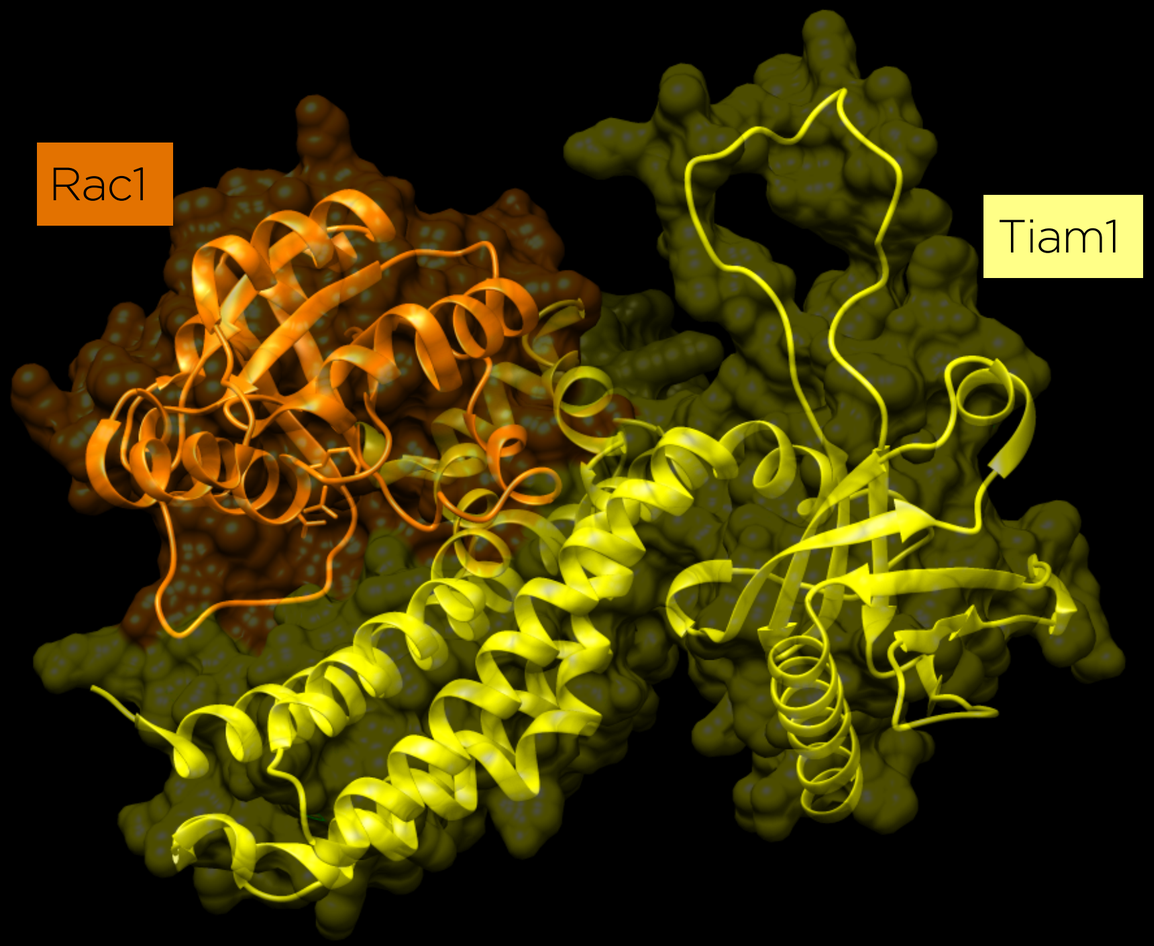
Guanine nucleotide exchange factor (GEF)
T-lymphoma invasion and metastasis factor 1 (Tiam1)

GEF



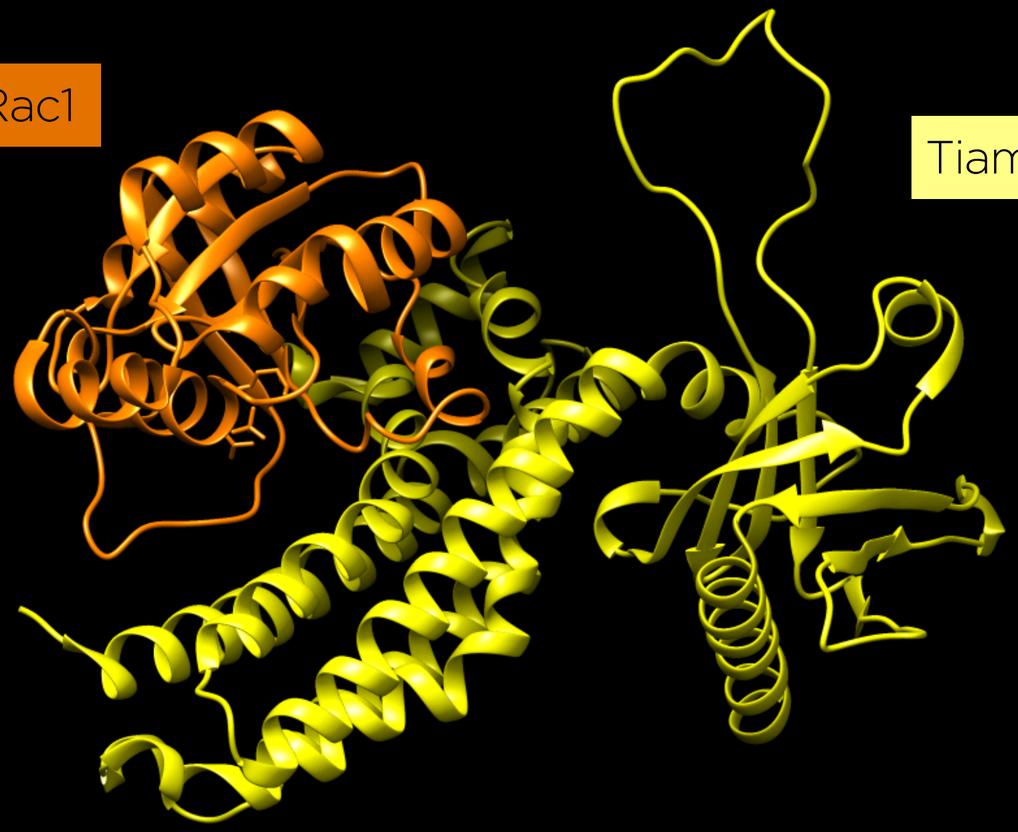
GEF

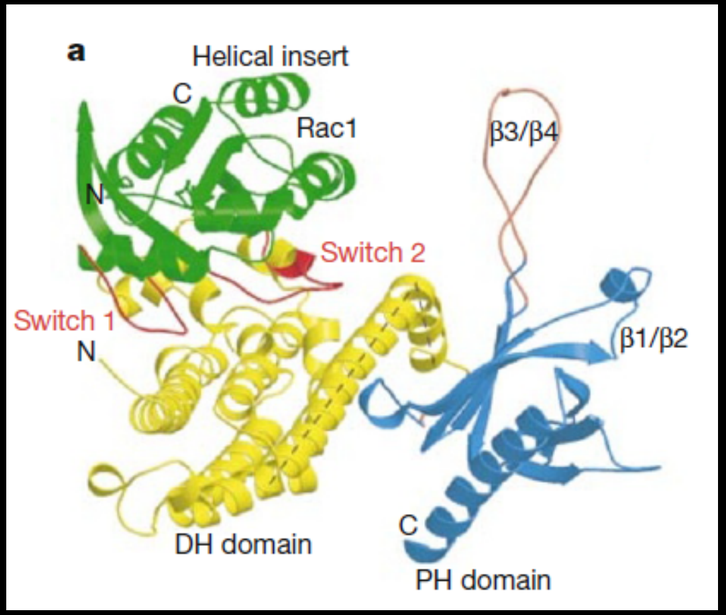
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GEF

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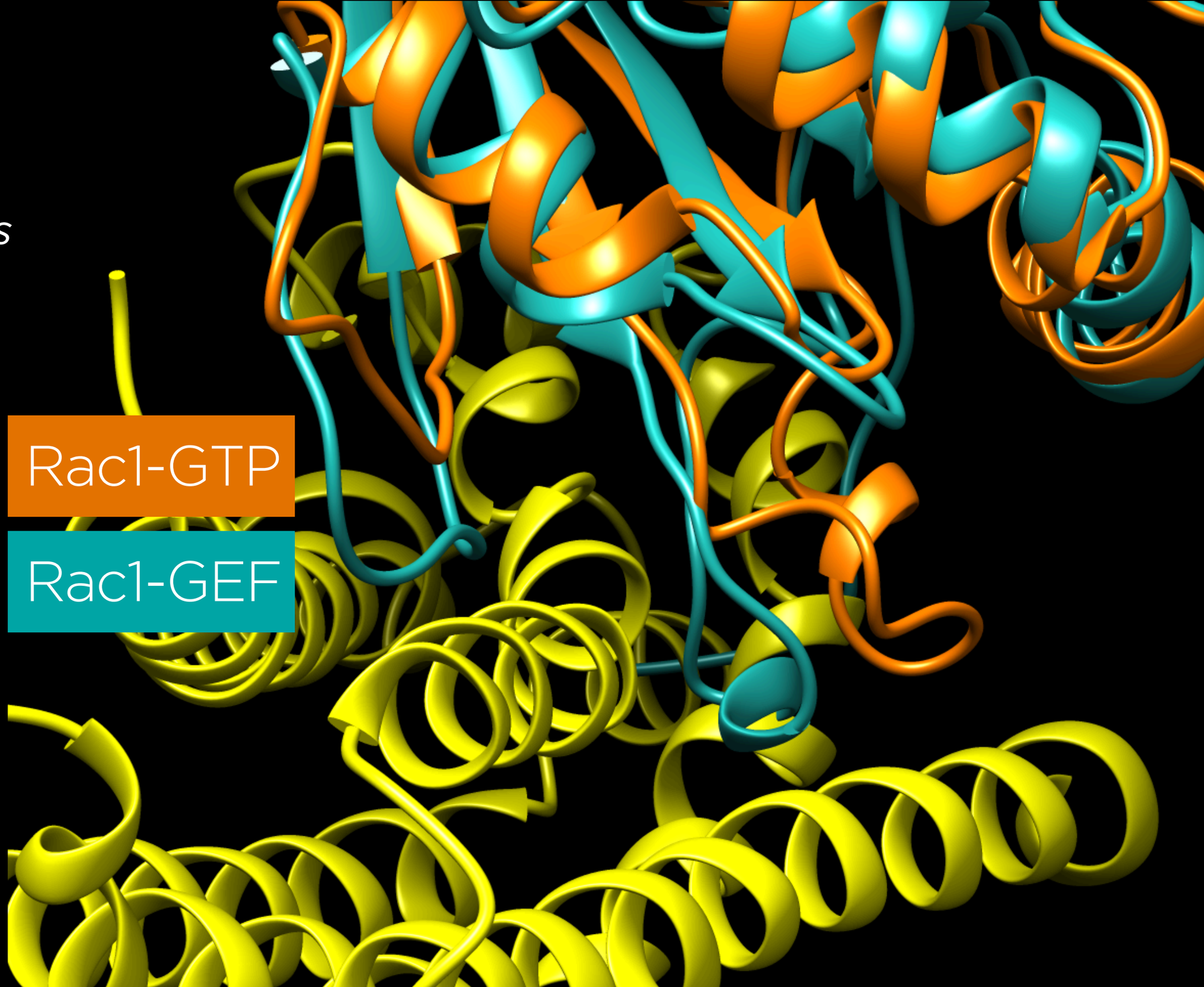


Crystal structure of Rac1 in complex with the guanine nucleotide exchange region of Tiam1

GEF

Shift of switches

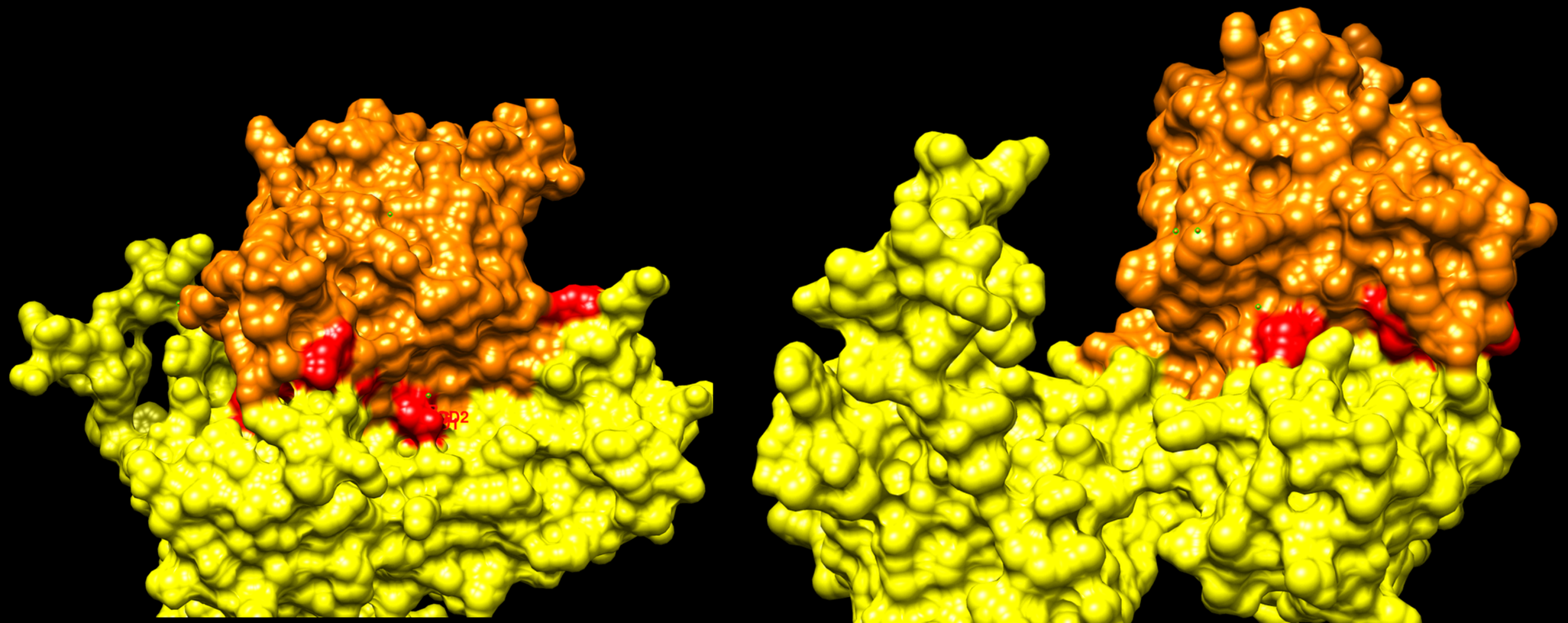
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GEF

Selective coupling of Rac1 with DH domains

Ala 3, Cys18, Glu 31, Thr35, Val36, Asp 38, Ser 41, Asn 43, Asn 52, Gly 54, Arg66, Trp 56 and Gln 74



GEF

Rac1/Tiam1

Rac1/GTP

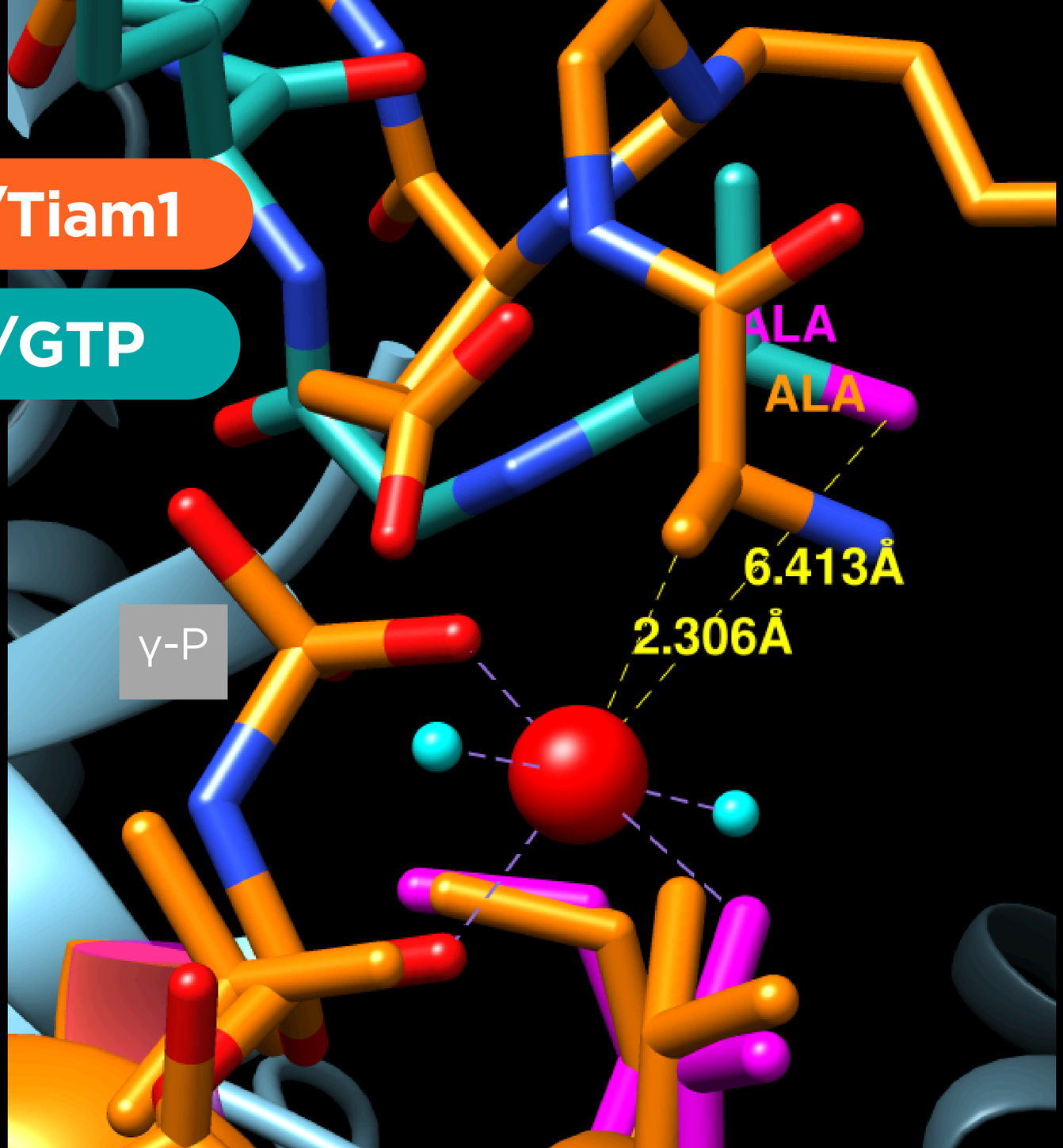
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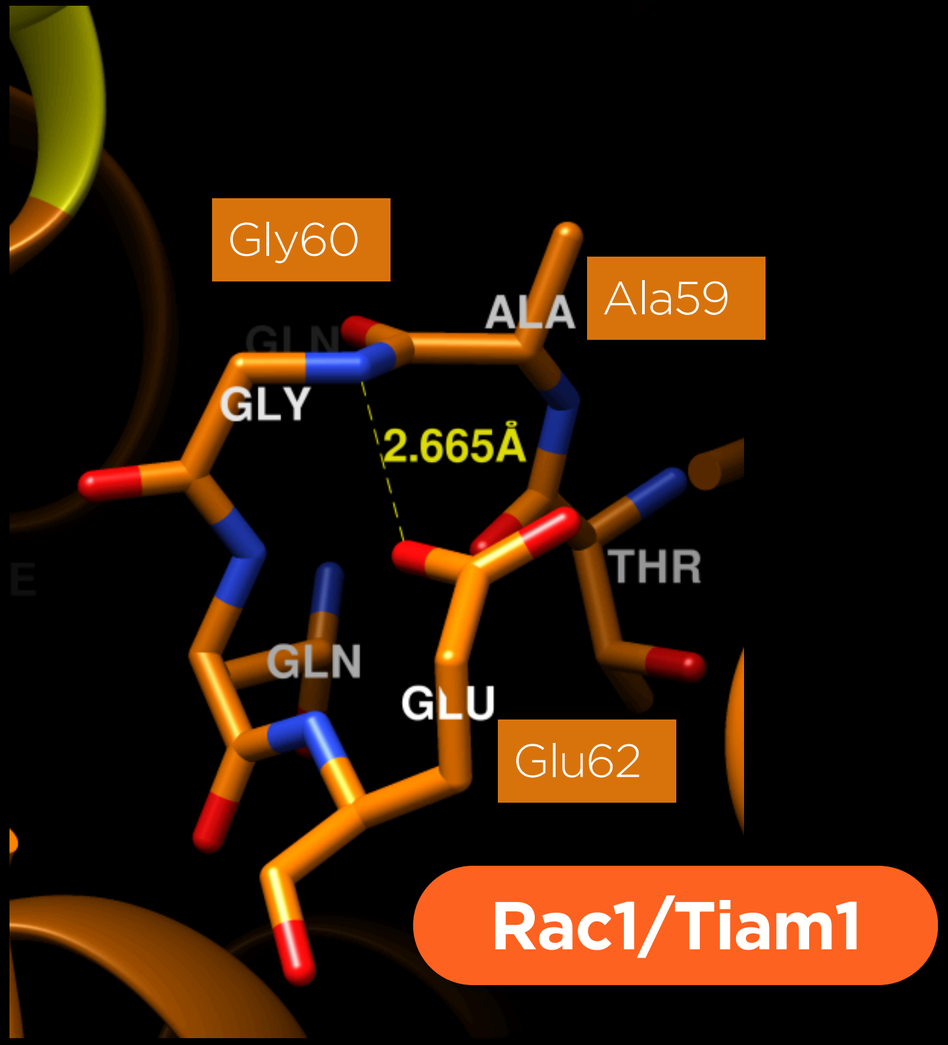
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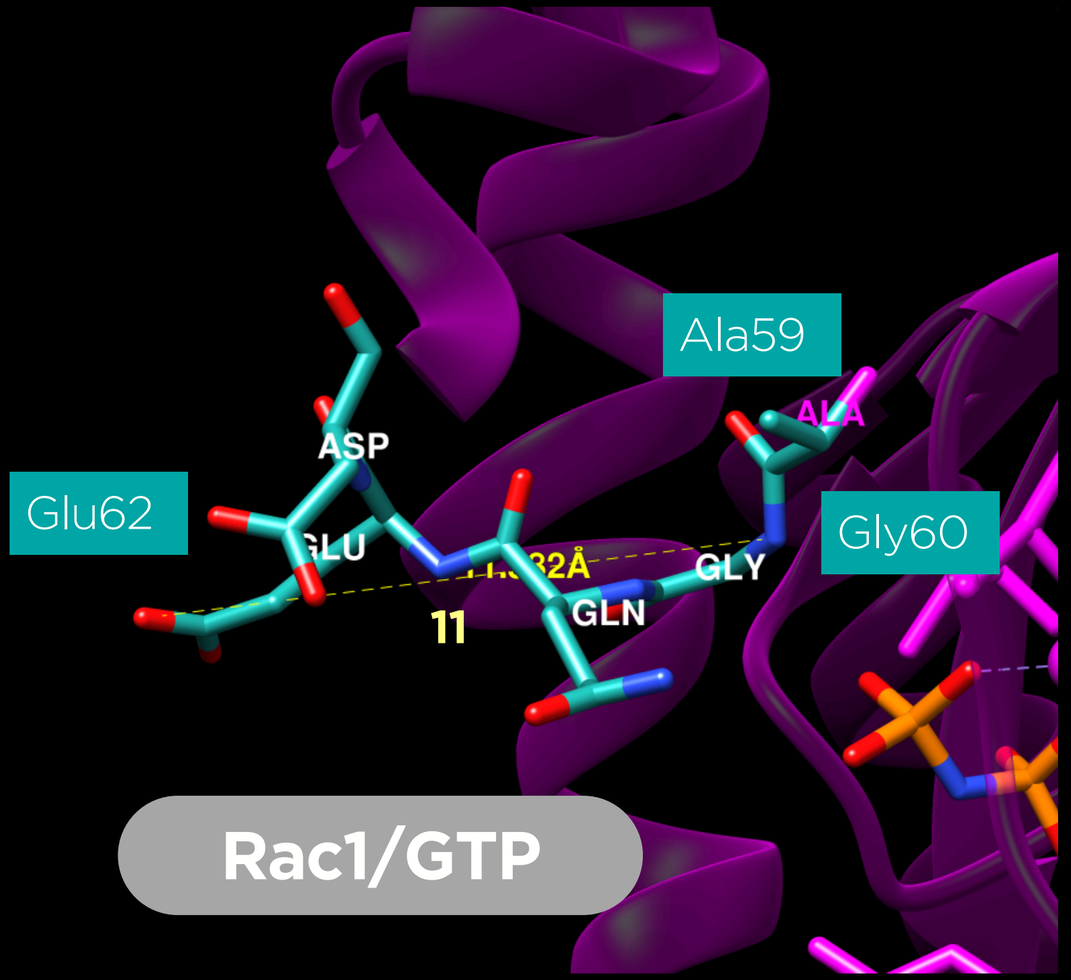
Ala59 intrusion into the Mg2+ binding site



GEF



Rac1/Tiam1

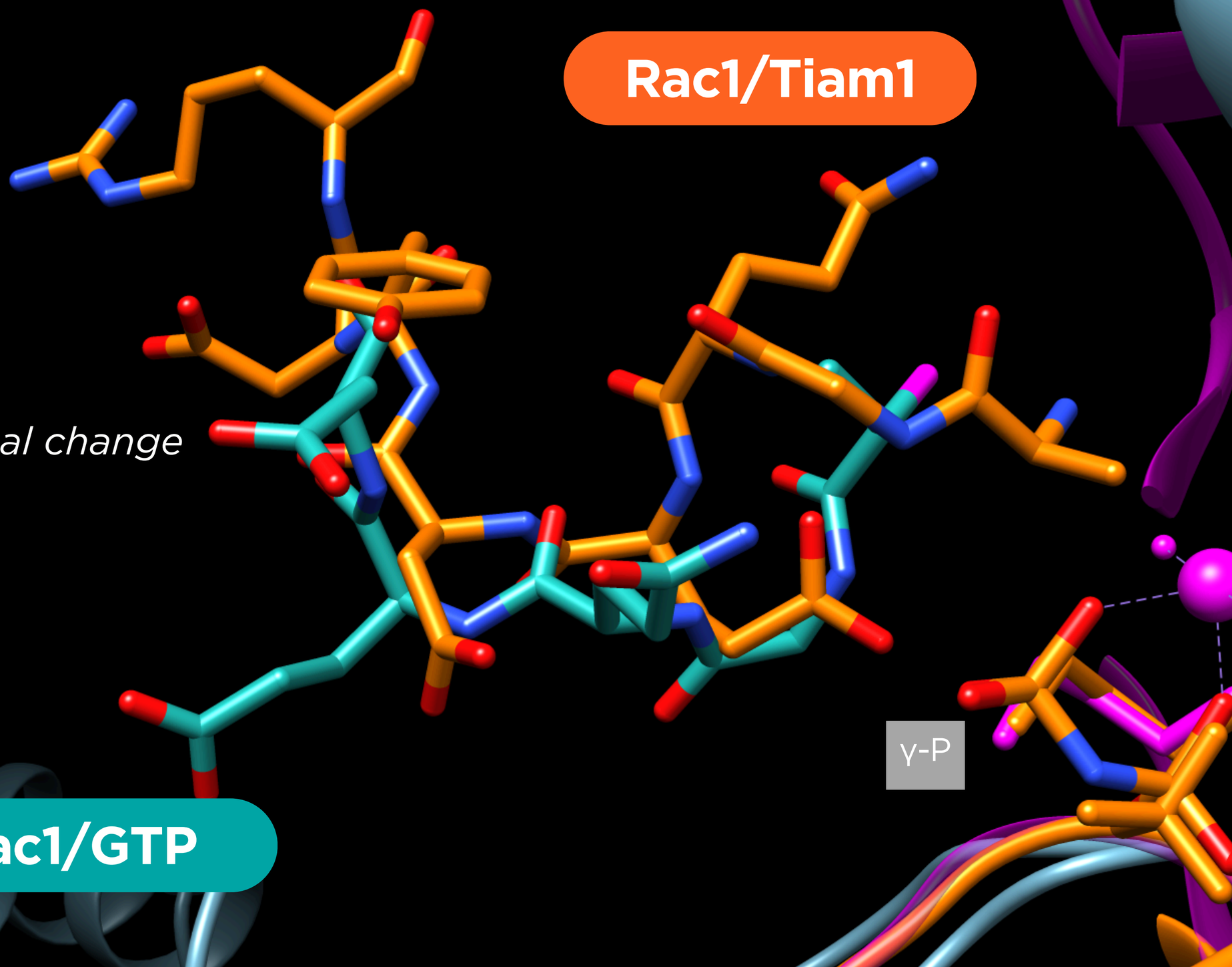


Rac1/GTP

GEF

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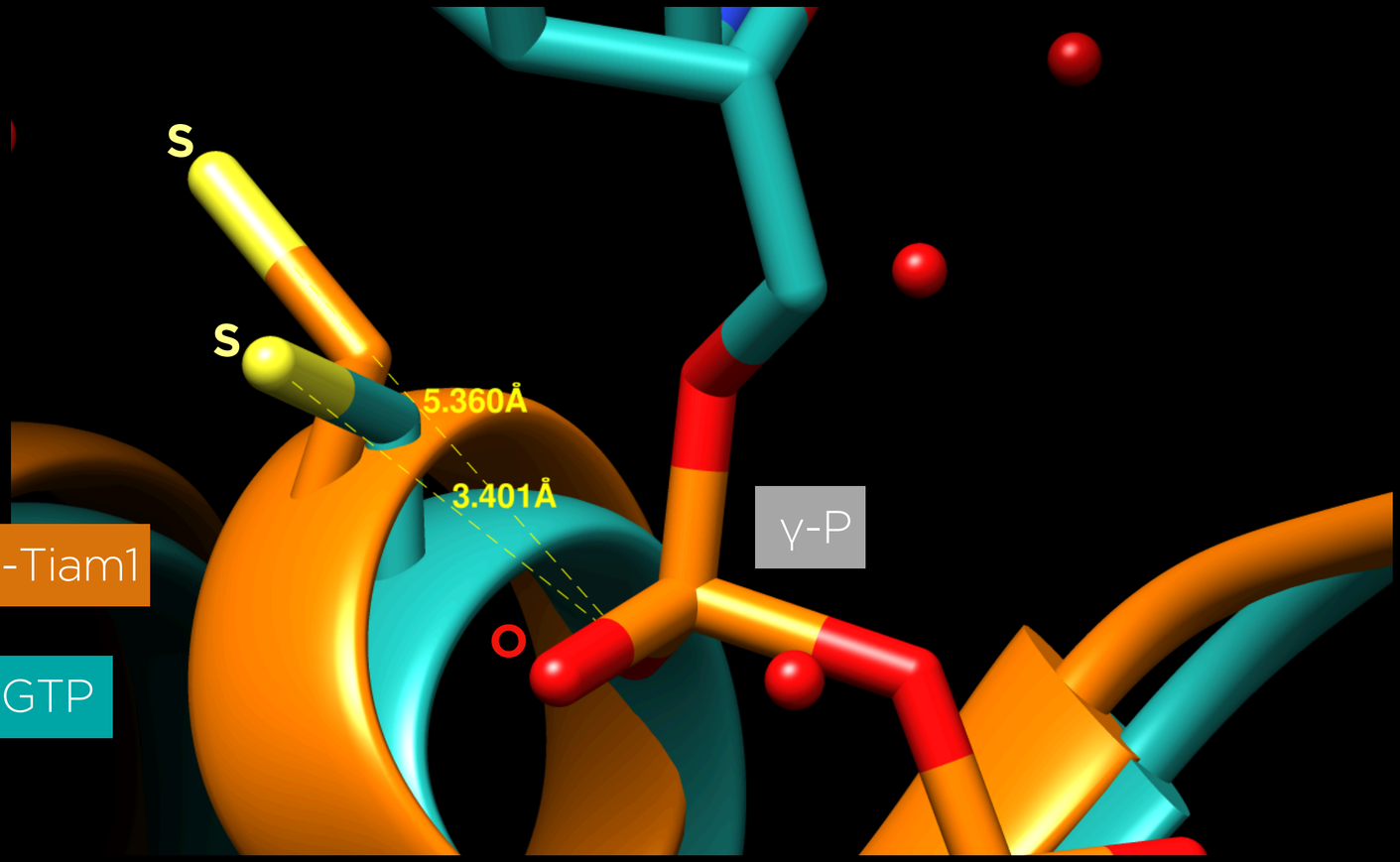
Conformational change



GEF

Rac1-geranyl - N-terminal regulatory arm GDI

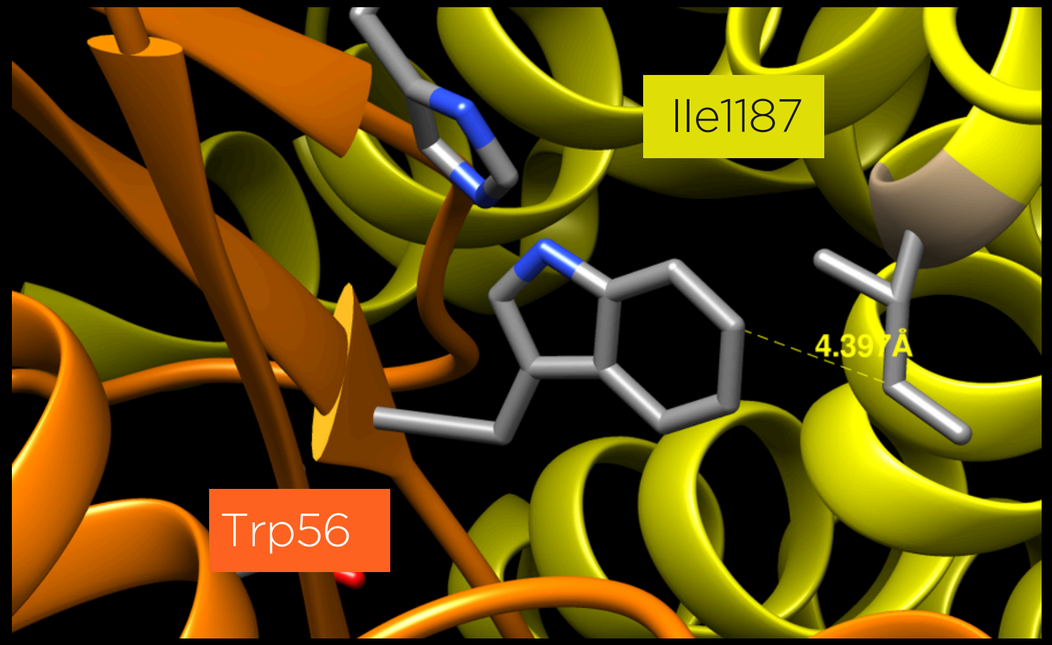
Dipol-Dipol



GEF

Rac1-geranyl - N-terminal regulatory arm GDI

Non-covalent hydrophobic contact

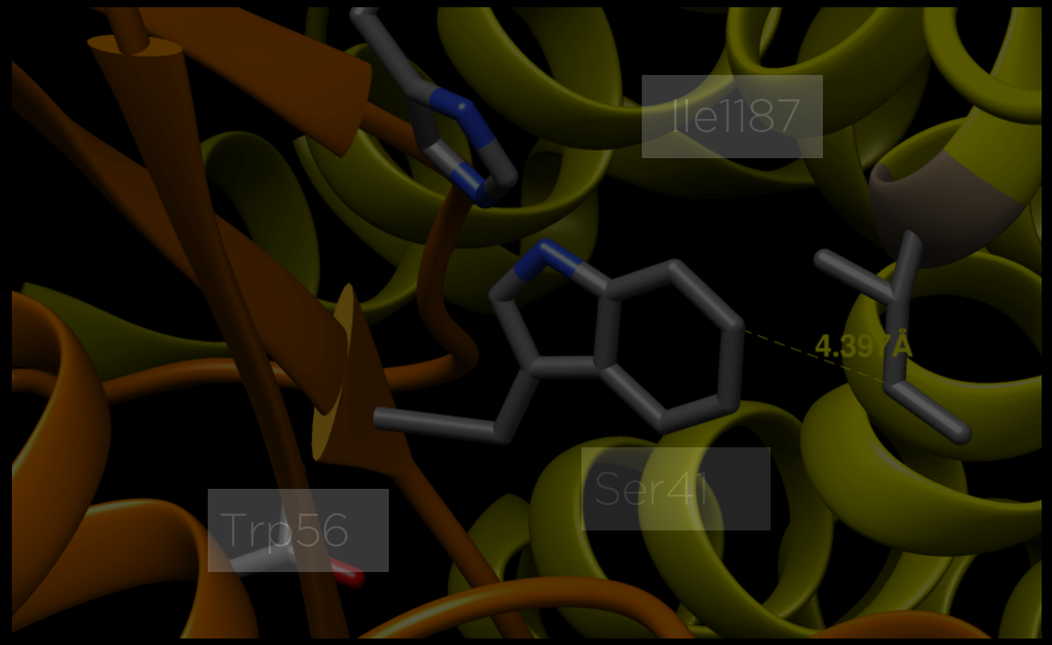


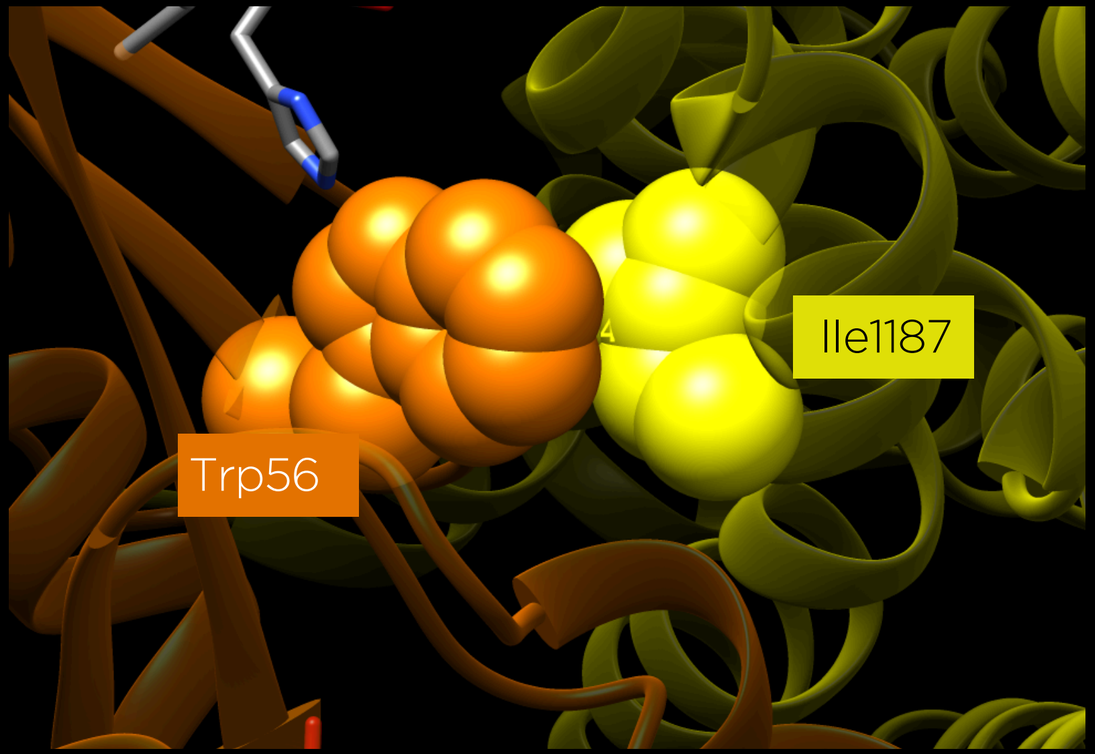


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GEF

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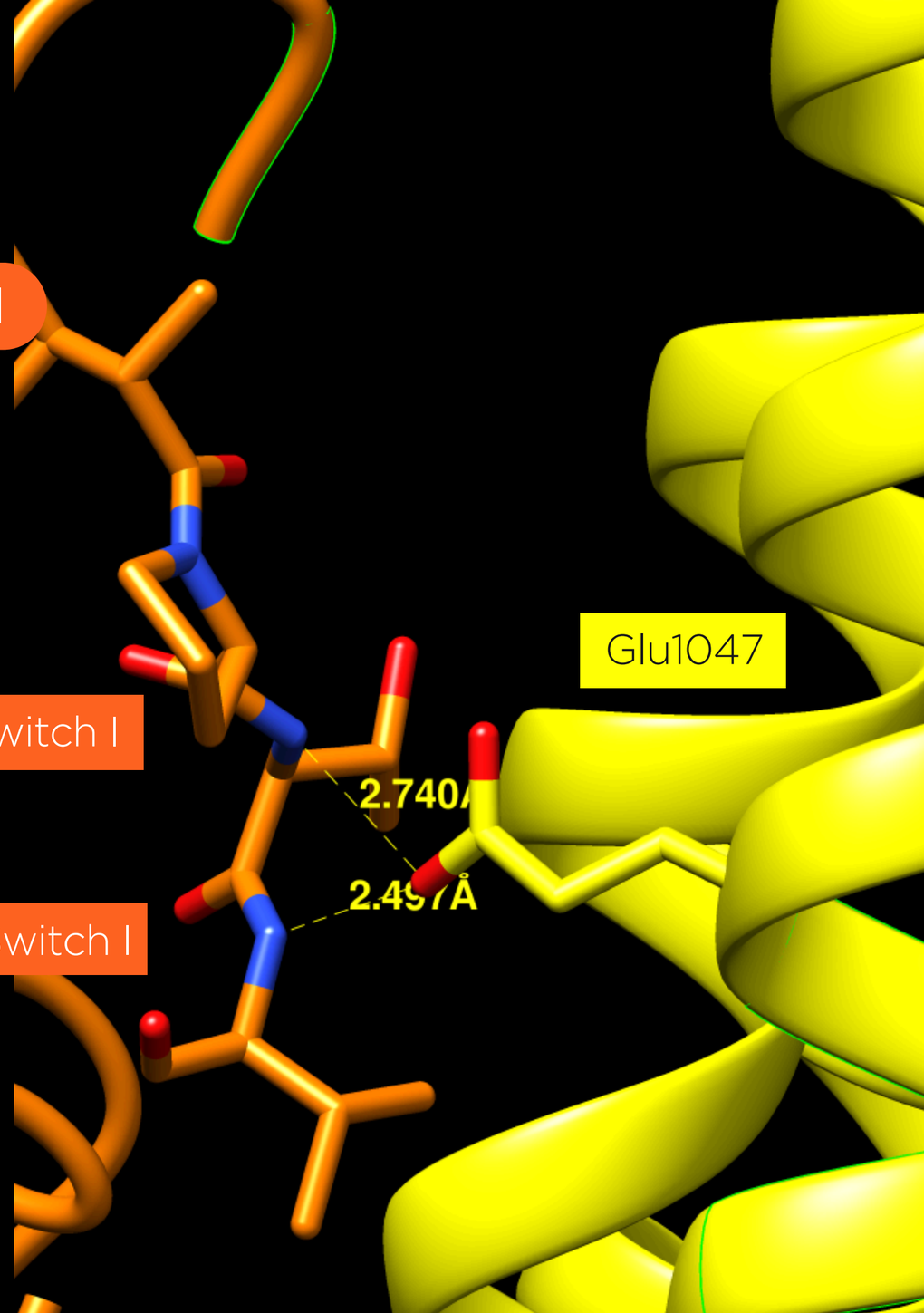


GEF

Rac1-geranyl - N-terminal regulatory arm GDI

Hydrogen bond



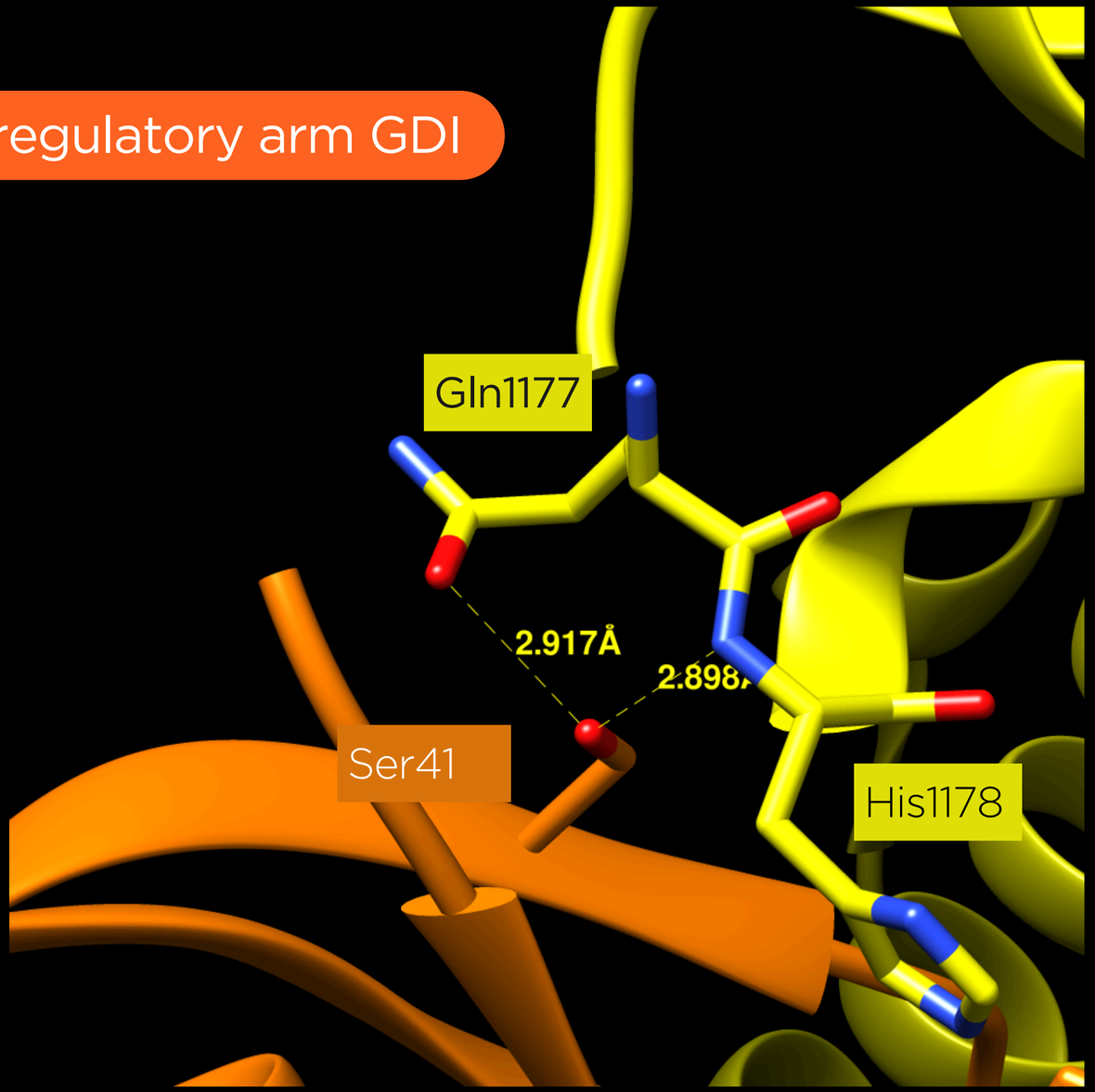


GEF

Rac1-geranyl - N-terminal regulatory arm GDI

Hydrogen bond

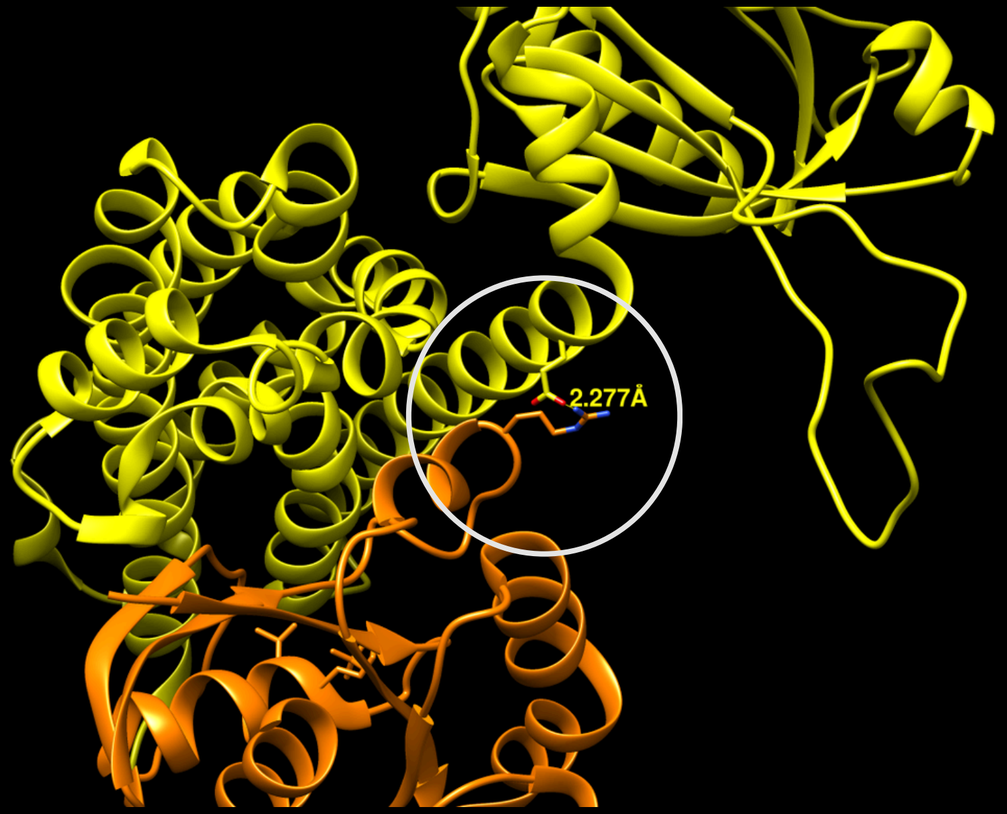
Inherently selective

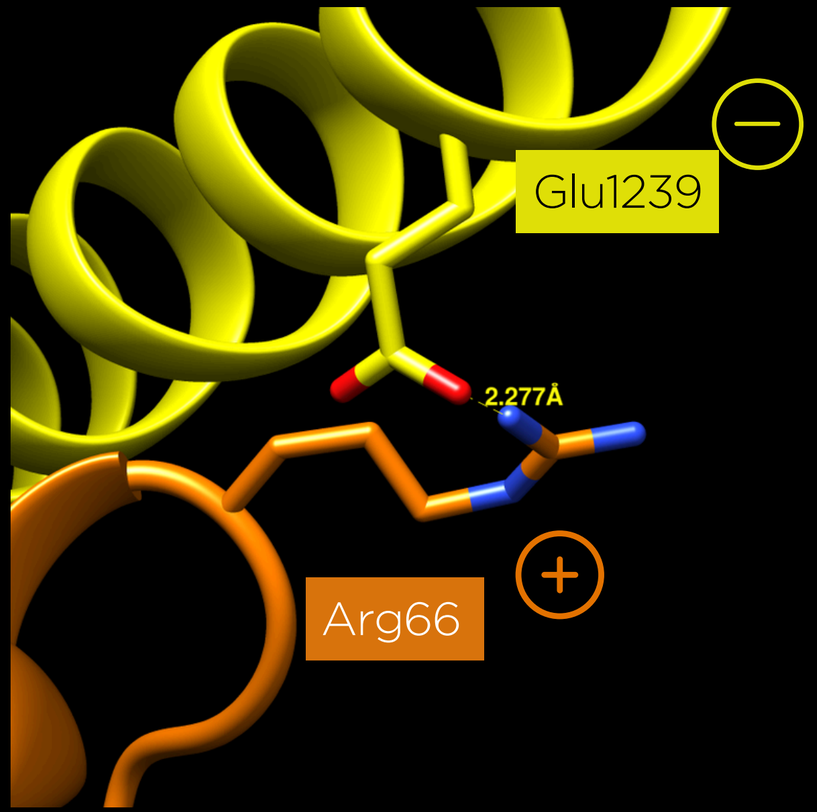


GEF

Rac1-geranyl - N-terminal regulatory arm GDI

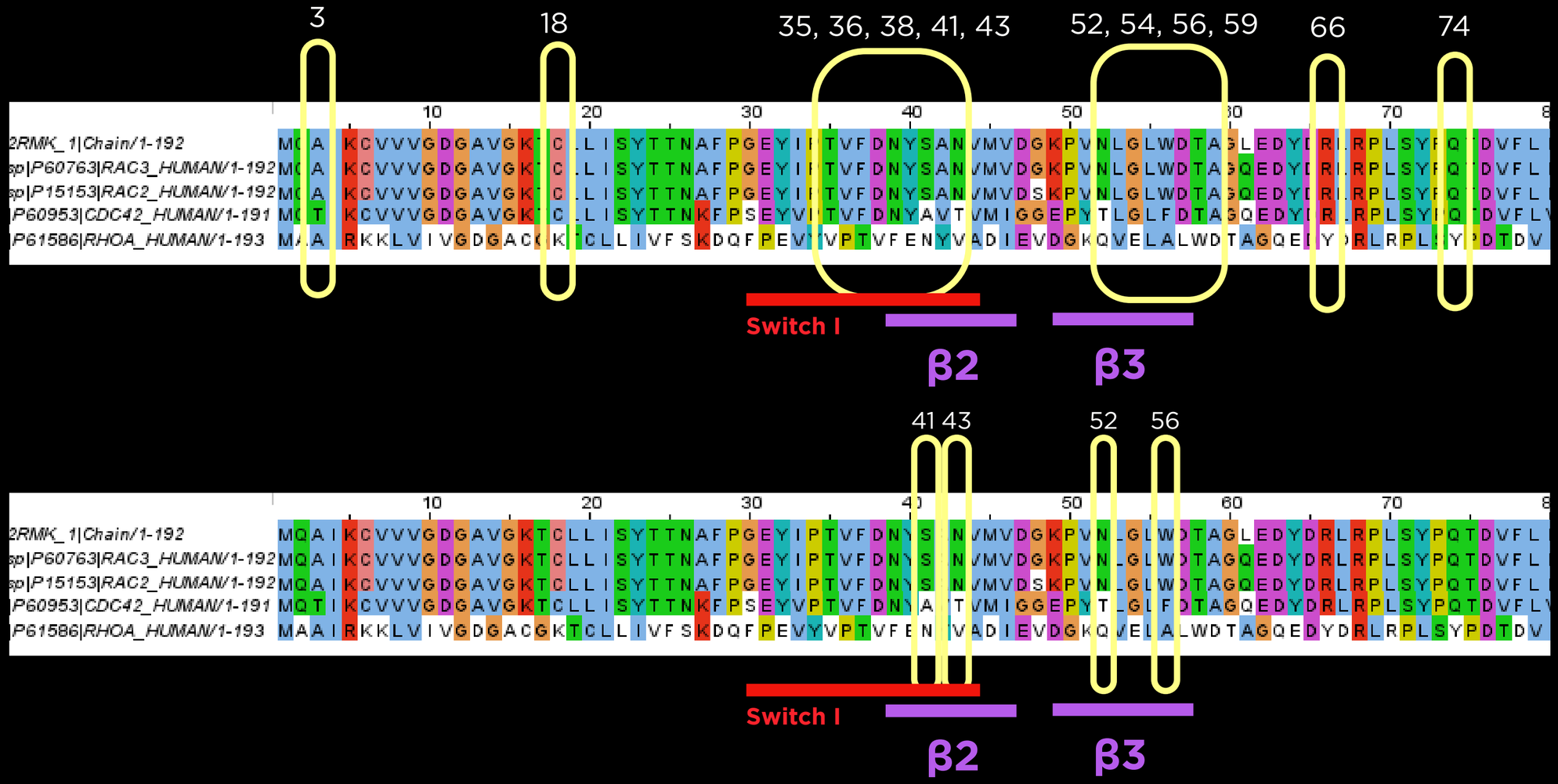
Salt brigde





GEF

Ala 3, Cys18, Glu 31, Thr35, Val36, Asp 38, Ser 41, Asn 43, Asn 52, Gly 54, Arg66, Trp 56 and Gln 74



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GDI (RhoGDI)

Guanosine dissociation inhibitor

GDI

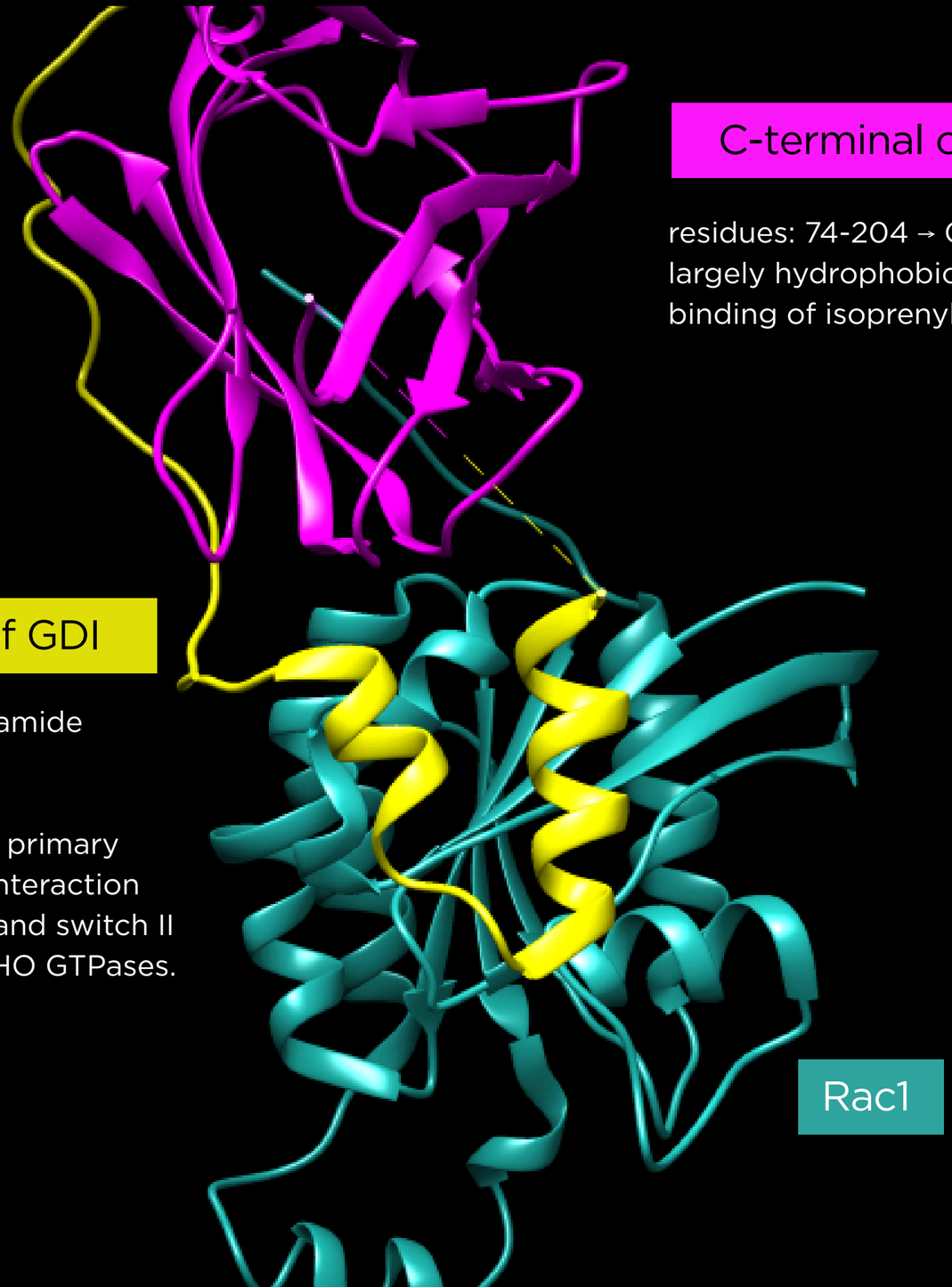
Domains

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C-terminal of GDI

residues: 74-204 → GDI forms a largely hydrophobic cavity for the binding of isoprenyl group of Rac1

N-terminal of GDI

- residues: 22-41 → amide chemical shift
- residues: 45-60 → primary site of Rac1-GDI interaction with the switch I and switch II domains in the RHO GTPases.

Rac1

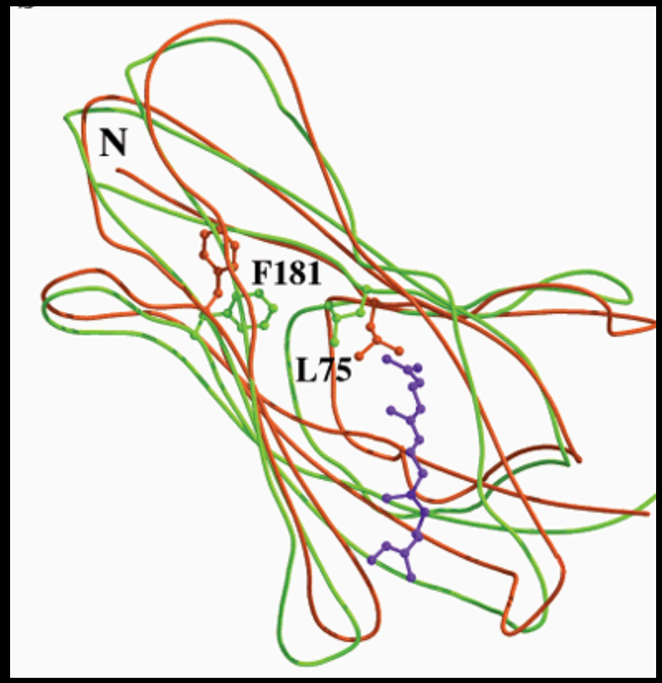
GDI

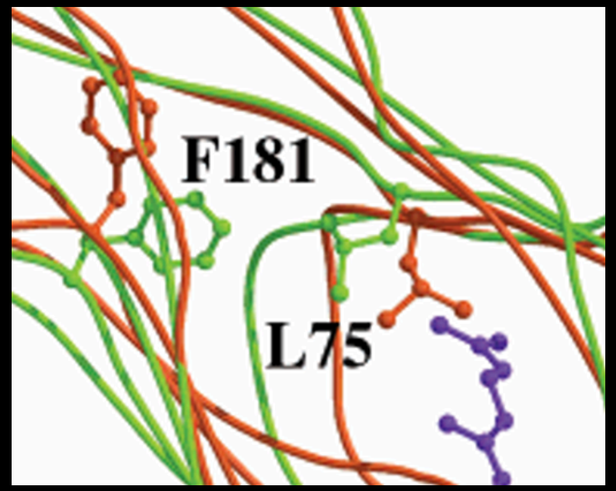


Rac1-geranyl - C-terminal GDI

Hydrophobic interaction:
Geranylgeranyl-binding pocket of GDI

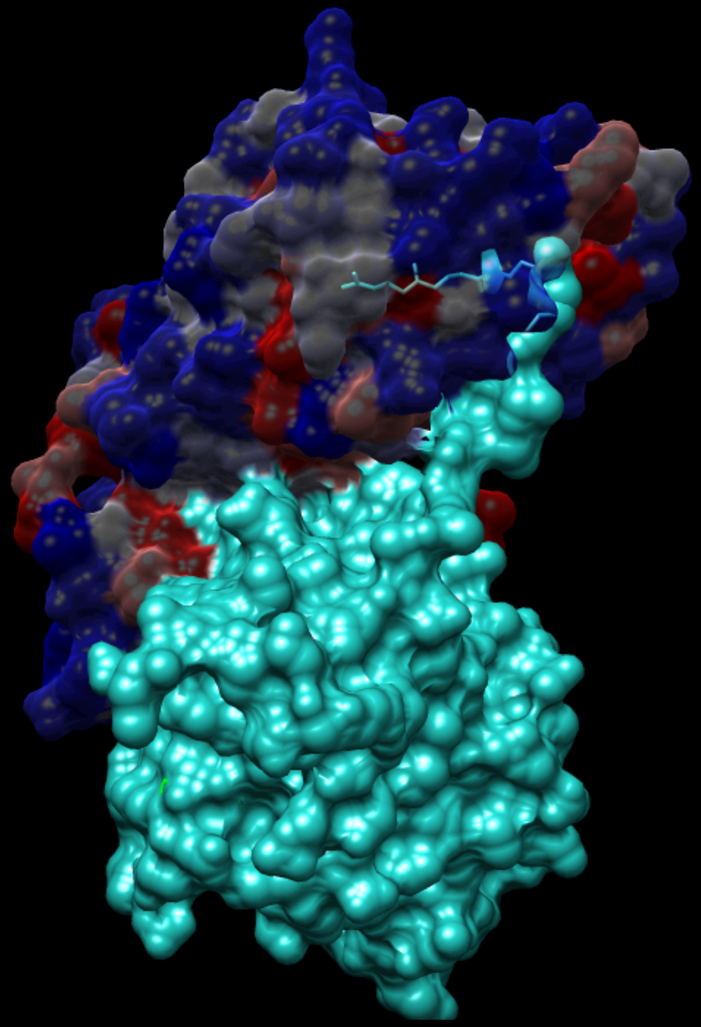
A mechanism that covers the lipid tail, shielding it from the aqueous cytosol and keeping the GTPase away from the membrane.





Crystal Structure of the Rac1-RhoGDI Complex Involved in NADPH Oxidase Activation

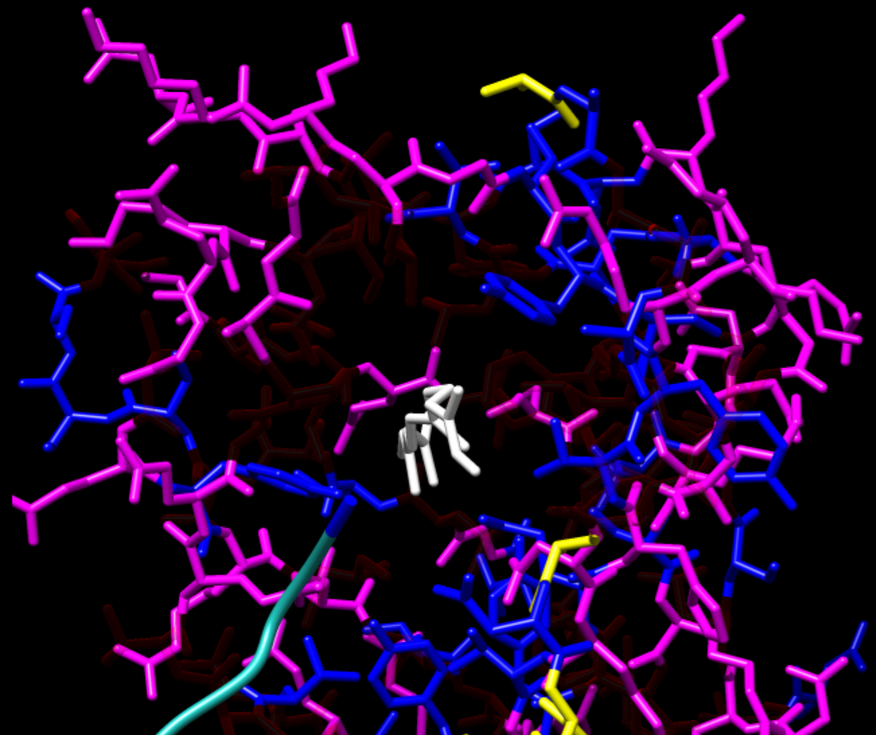
GDI



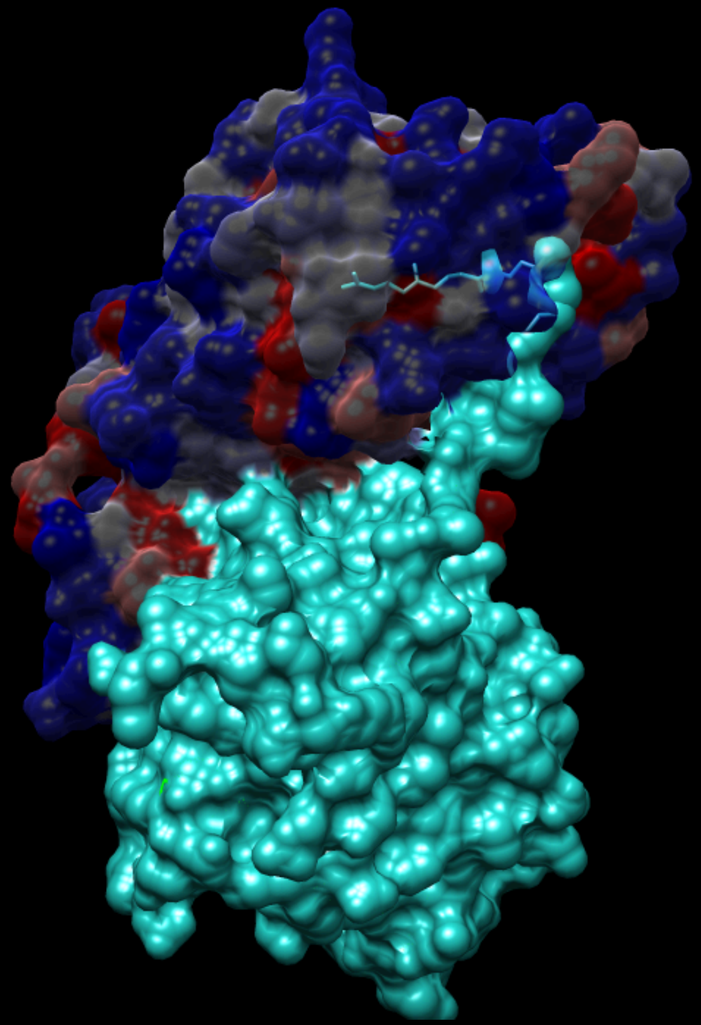
Rac1-geranyl - C-terminal GDI

Deep hydrophobic cavity

- Hydrophobic aminoacids: A V L I M F W P
- Neutral aminoacids
- Hydrophilic aminoacids: S T N Q Y C



GDI



Rac1-geranyl - C-terminal GDI

Deep hydrophobic cavity

- Hydrophobic aminoacids: A V L I M F W P
- Neutral aminoacids
- Hydrophilic aminoacids: S T N Q Y C

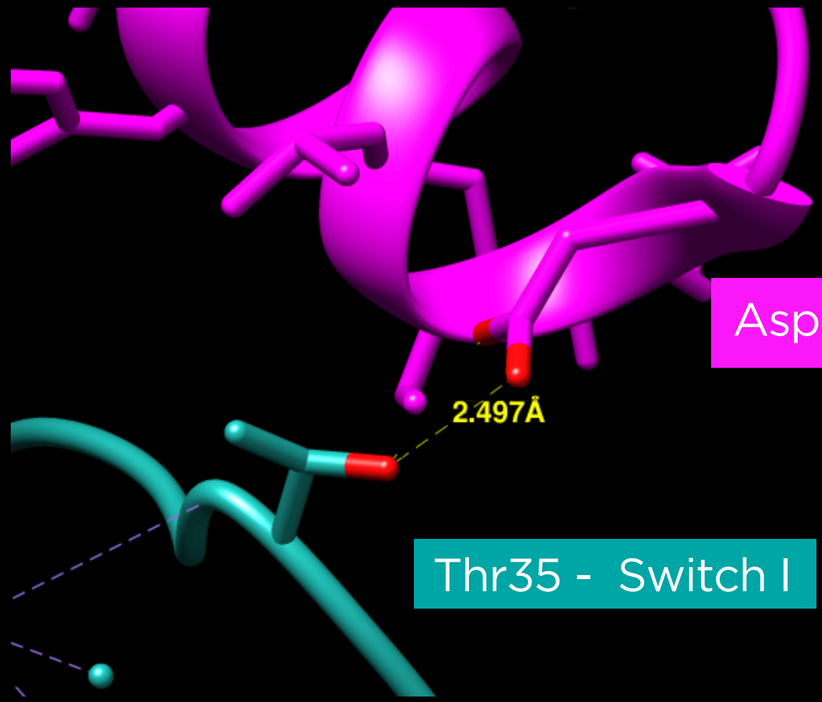


GDI

Rac1-geranyl - N-terminal regulatory arm GDI

Hydrophobic interaction





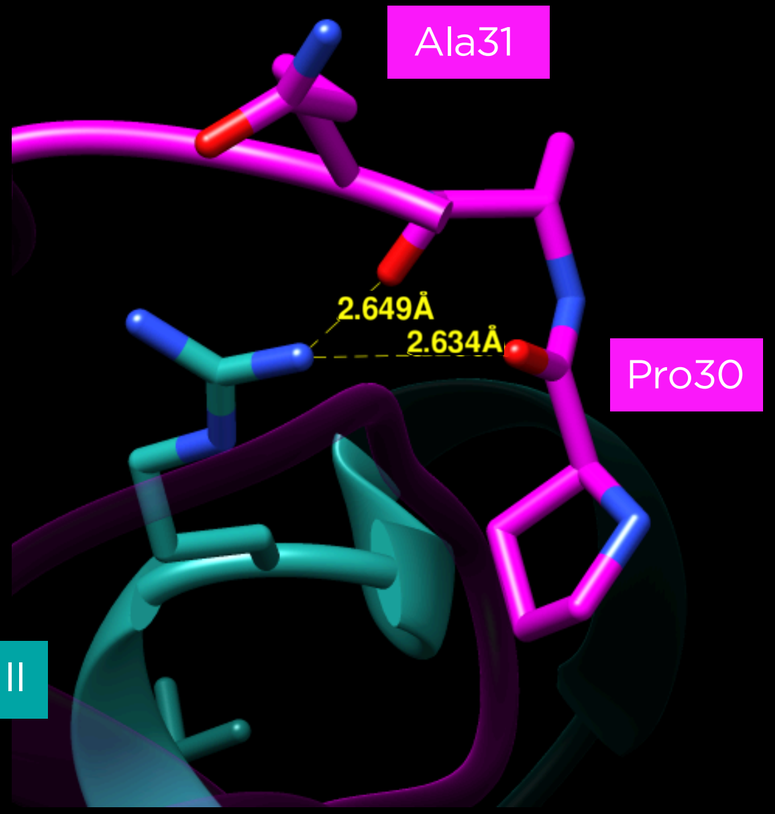
Important for the inhibition of the GDP-GTP exchange.

GDI

Rac1-geranyl - N-terminal regulatory arm GDI

Hydrophobic interaction





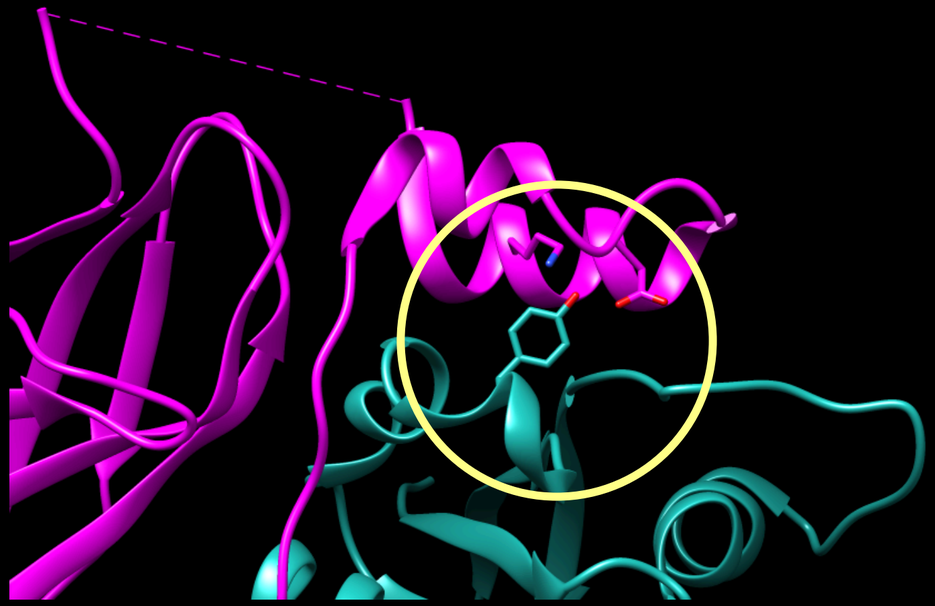
Arg66 - Switch II

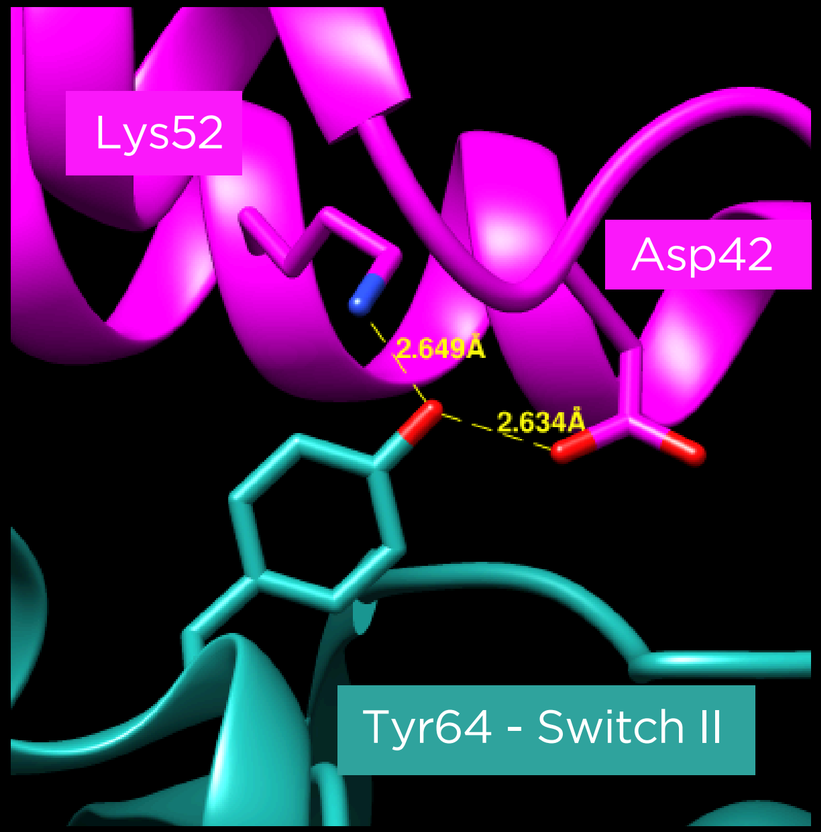
Important for complex formation → R66G abolishes the interaction

GDI

Rac1-geranyl - N-terminal regulatory arm GDI

Hydrophobic interaction

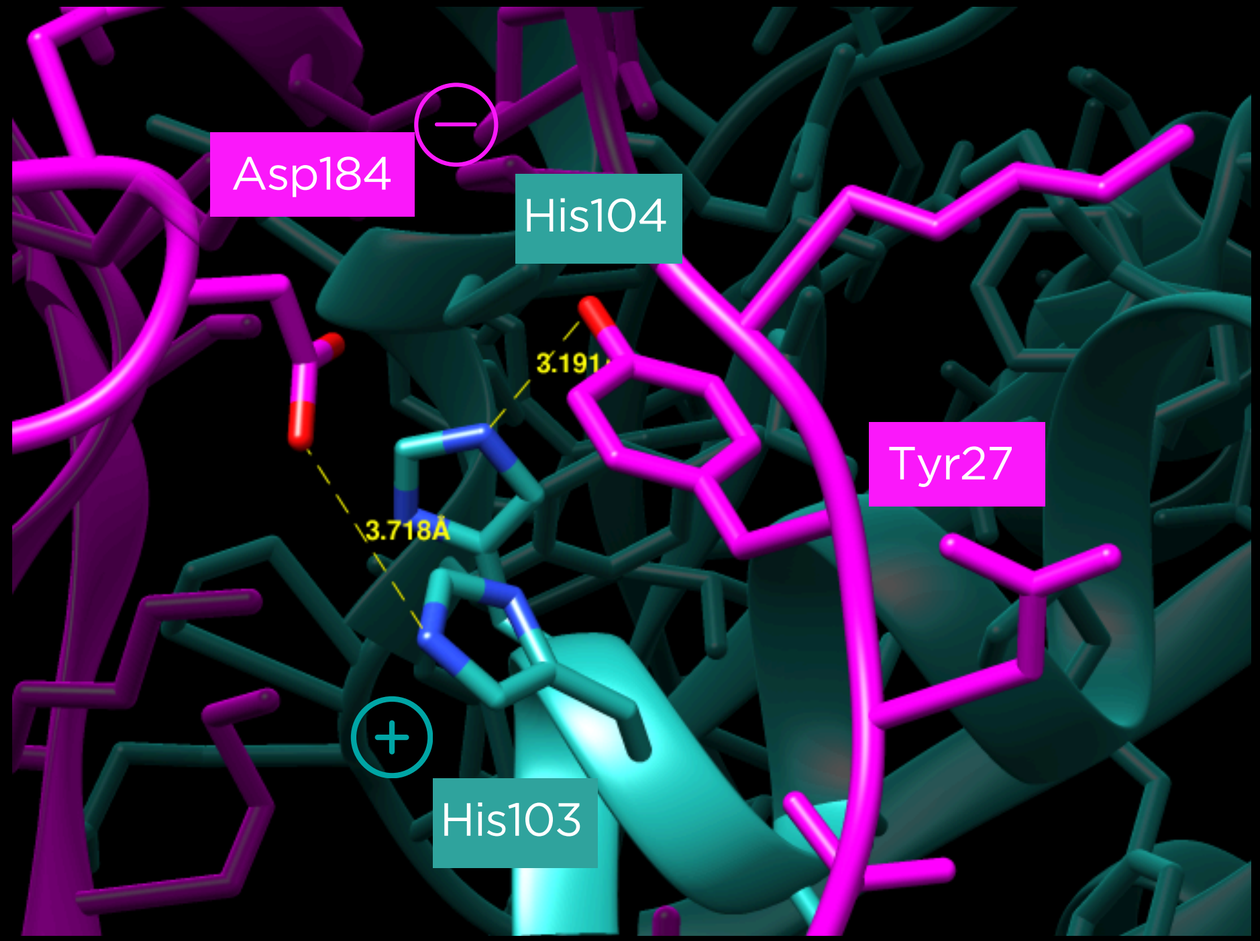




GDI

Rac1-geranyl - N-terminal regulatory arm GDI

Salt brigdes and hydrogen bonds



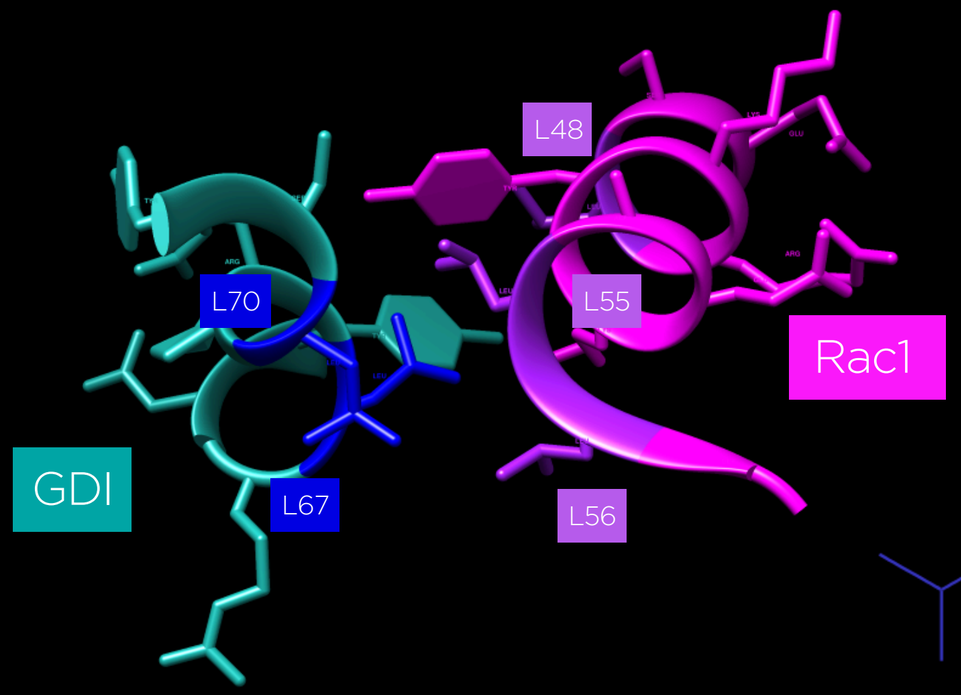
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GDI

Rac1-geranyl - N-terminal regulatory arm GDI

Hydrophobic interaction







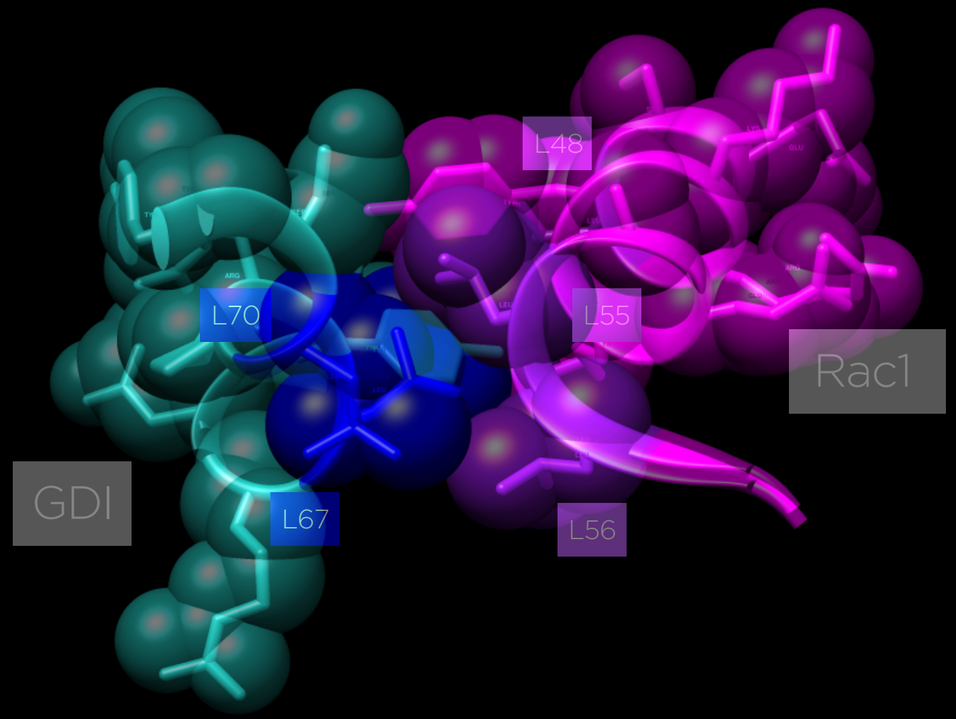
Van der Waals forces

GDI

Rac1-geranyl - N-terminal regulatory arm GDI

Hydrophobic interaction





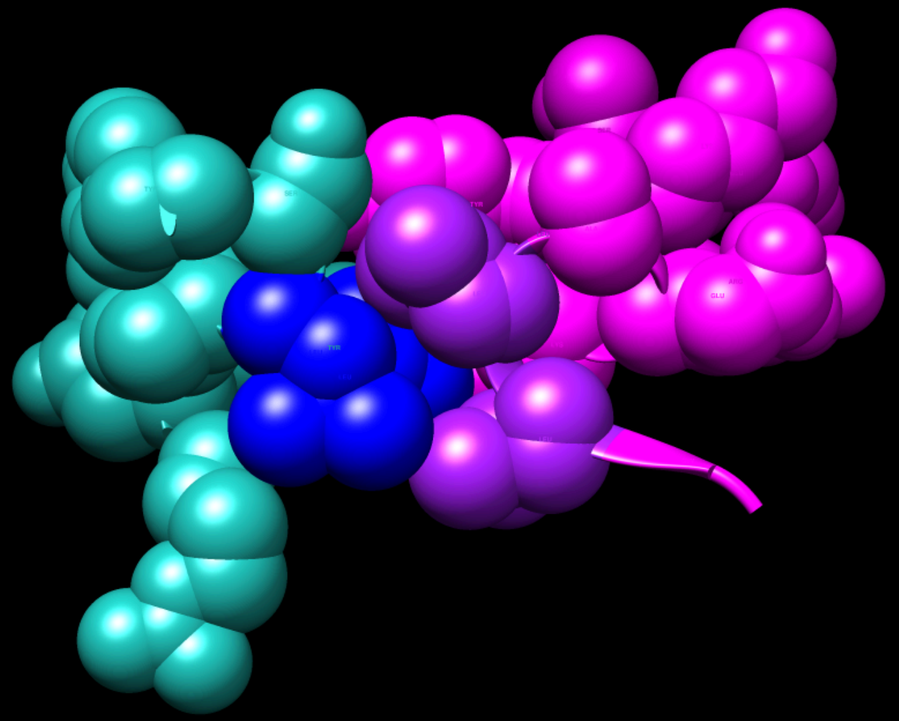
Van der Waals forces

GDI

Rac1-geranyl - N-terminal regulatory arm GDI

Hydrophobic interaction

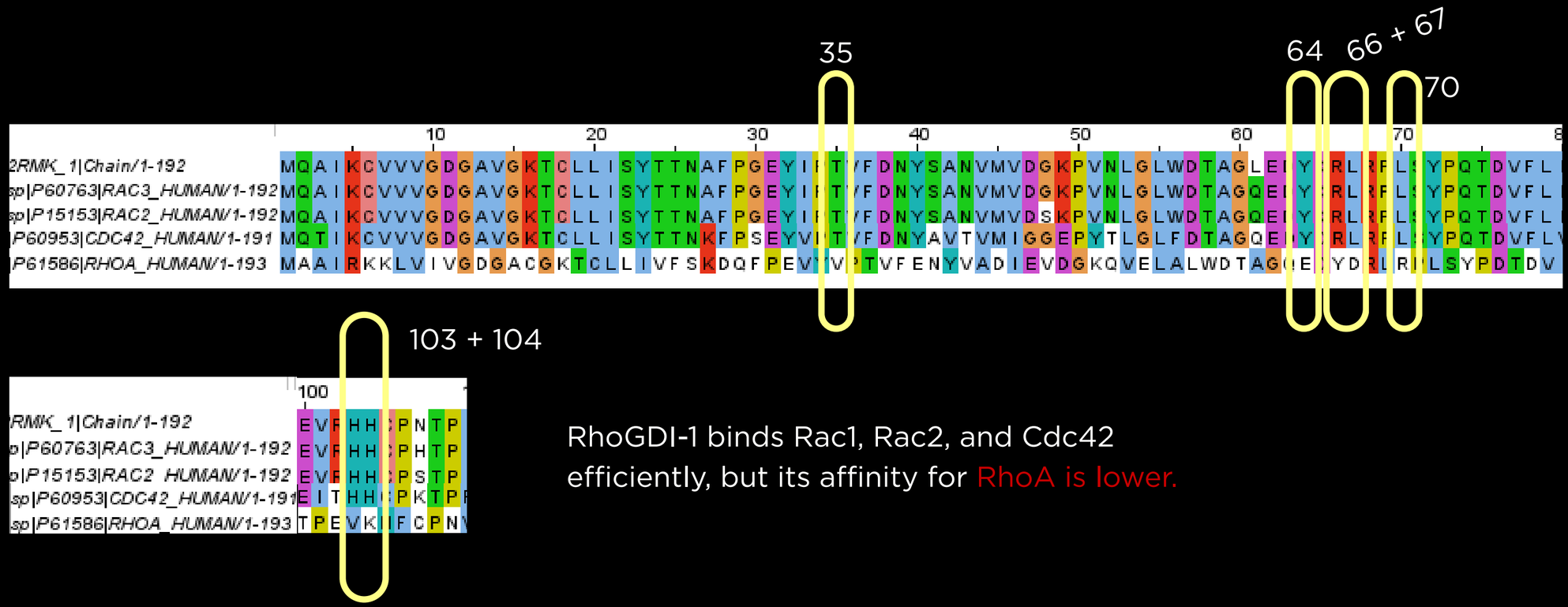




Van der Waals forces

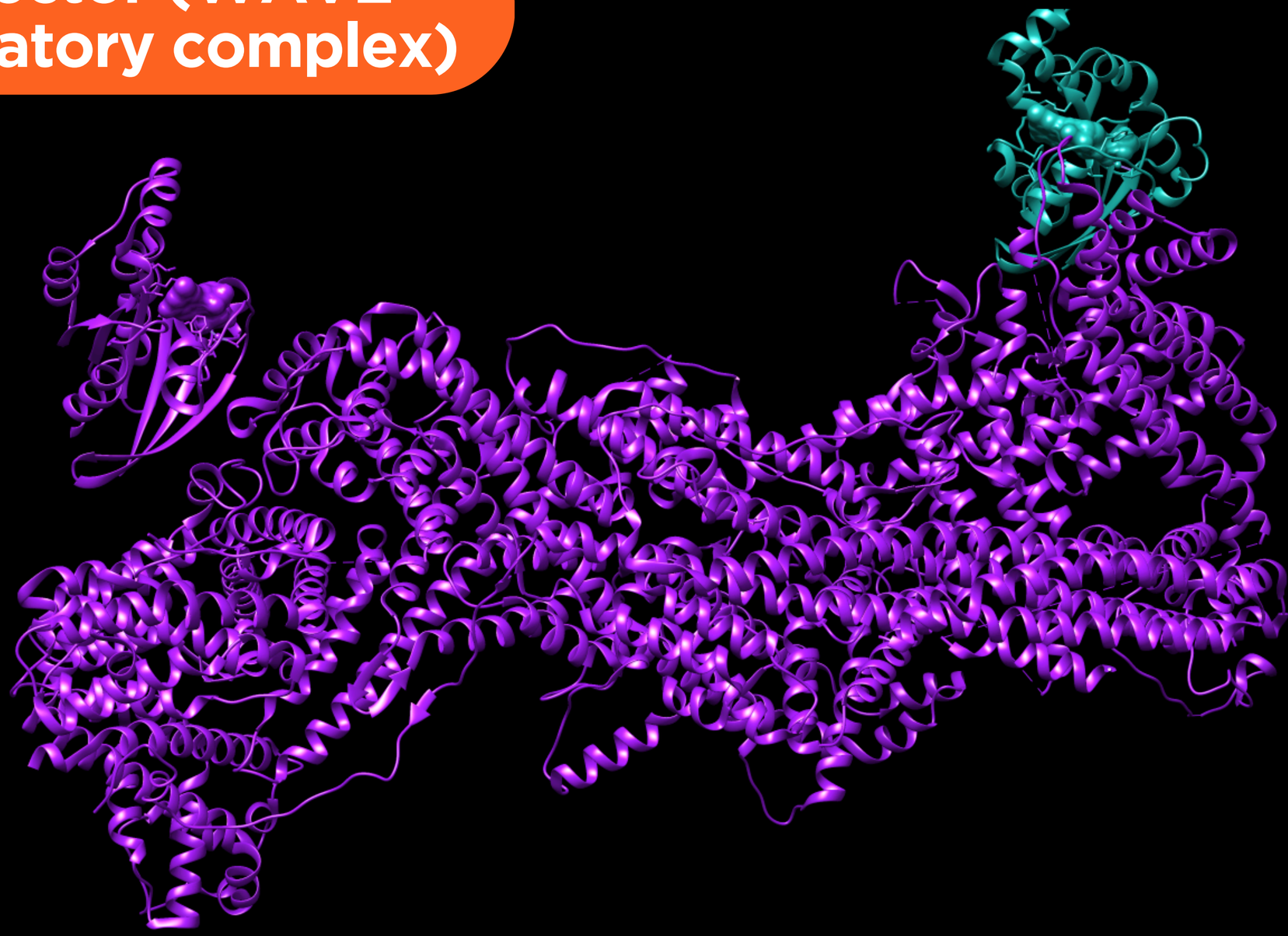
GDI

RhoGDIs differential specificity for Rho GTPases



RhoGDI-1 binds Rac1, Rac2, and Cdc42 efficiently, but its affinity for RhoA is lower.

Effector (WAVE regulatory complex)



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Conclusions

- Studies where mutations of concrete residues are done, are essencial for discovering the role of key residues and interactions.

- The activation of Rac1 is driven by a mechanical conformation change to the presence of the γ-phosphate of GTP.

- G4 motif and hydrogen bonds there ensure that the protein binds specifically to GTP.
- Coordination of Mg2+ ion stabilizes the high-energy state of the nucleotide and organizes the active site for catalysis.

- We could prove that Rac1 protein is highly conserved between species showing that Rac1 is an essential ancestral protein in eukaryotes.

- Although some differences are observed among members of the Ras superfamily, this is expected due to the variation in protein length and functional diversity.

Thanks for your attention

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PEM questions

1. Which are the regions implicated on the GTP/GDP binding of Rac1?

A. The C-terminal polybasic region (PBR) and the CAAX motif.
B.The Switch I, Switch II regions, and the conserved P-loop.
C.The Dbl-homology (DH) domain and the Pleckstrin-homology (PH) domain.
D. The Src-homology 3 (SH3) domain and the proline-rich region.
E. The GDI-interfacing domain located exclusively at the N-terminus.

2. How GAP interact with Rac1?

A. By physically blocking the binding site of downstream effectors through steric hindrance at the C-terminal CAAX motif.
B. By replacing GDP with GTP in the P-loop.
C. By inserting a conserved "arginine finger" into the active site to stabilize the transition state of GTP hydrolysis.
D. By phosphorylating the Switch II region, which triggers the spontaneous release of the inorganic phosphate.
E. By sequestering Rac1 in the cytosol and preventing its degradation.

3. Which one is a function of the inserted domain of Rac1

A. To facilitate the assembly of the NADPH oxidase complex.
B. To promote nuclear translocation via a polybasic sequence.
C. To increase the membrane affinity
D. It has no relevant function
E. To modulate the effector Wave Regulatory Complex (WRC)

4. How is Mg2+ stabilized in Rac1?

A. By anchoring to the beta and gamma phosphate groups
B. By forming a coordination sphere with an octahedral geometry
C. By directly binding to the invariant Lysine (Lys16)
D. By coordinating with two specific water molecules
E. By forming 8 hydrogen bonds with different water molecules and side chains

PEM questions

5. What is a unique structural and functional feature of the "Inserted Domain" in Rac1?

A. it is a highly conserved region found in all members of the Ras superfamily.
B. It is an extra alpha-helix that provides a unique surface for specific effector interactions.
C. It is found in the "G5 motif"
D. It unfolds completely when the protein is bound to GDP.
E. It is found in the N-terminal domain.

6. During the activation of Rac1, what is the most significant conformational change that occurs upon swapping GDP for GTP?

A. The protein undergoes dimerization to transport the signal to the nucleus.
B. The binding pocket expands to allow the entry of the GTP.
C. Switch I and Switch II transition from a open state to a closed conformation.
D. The Magnesium ion is released from the active site to trigger signaling.
E. Only Switch II suffers conformational changes.

7. Which residues are primarily responsible for sensing the presence of the GTP gamma-phosphate and triggering the conformational change of the Switches?

A. Only the residues in the P-loop, as they hold the phosphates in place.
B. Thr35 and Gly60, which form hydrogen bonds with the gamma-phosphate to pull Switch I and Switch II into the active state.
C. Asn116 and Asp118, because they recognize the guanine ring and position the rest of the protein.
D. Any hydrophobic residue in the core, as they stabilize the protein's overall shape.
E. Residues from the C-terminal.

8. We can talk about high conservation in Rac1 between:

A. The Rac subfamily (Rac1, Rac2, and Rac3) since they are paralogues.
B. Different species, especially in mammals such as cattle, mice, and humans.
C. The Ras superfamily (Ran, Rac, Rab, Rho, and Arf).
D. All of the above
E. We cannot speak of conservation in Rac1 since each species uses different Rac proteins

PEM questions

9. Rac1b differs from Rac1a because…

A. It has a 19-amino acid insertion after Switch II.
B. It has higher activation and affinity for the plasma membrane.
C. It doesn't bind to RhoGDI
D. It doesn't appear in healthy tissues
E. All the above

10. The polybasic region of the C-terminal from Rac1 consists of:

A. A region rich in lysines and arginines
B. A regions which allows Rac1 to form dimers
C. The two above
D. A region rich in prolines that bend the chain forming a "type II helix"
E. All the above