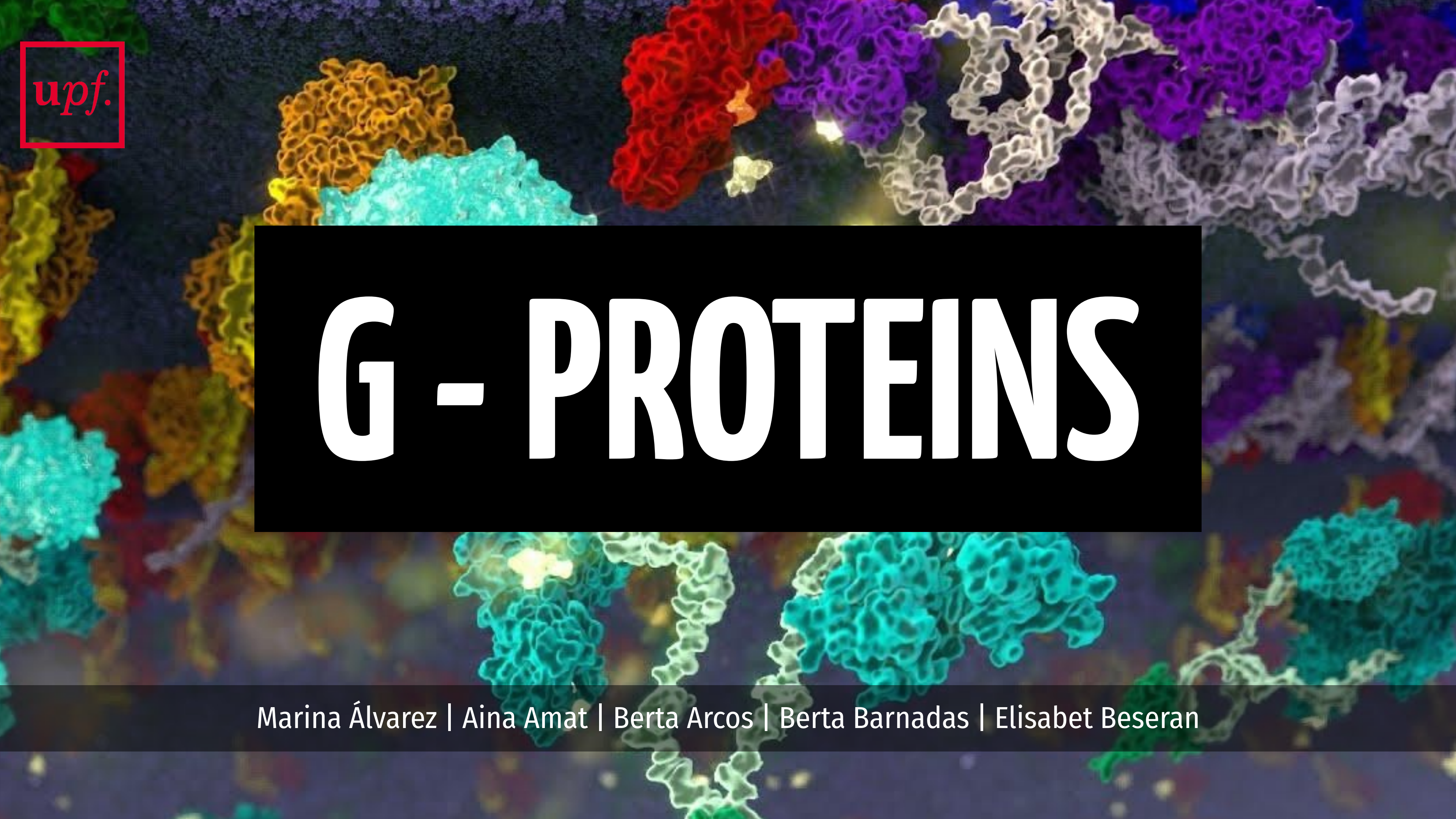


G - PROTEINS

Marina Álvarez | Aina Amat | Berta Arcos | Berta Barnadas | Elisabet Beseran

INDEX

INTRODUCTION (G PROTEINS)

- History
- Classification : MONOMERIC + TRIMERIC
- Evolution

RAB FAMILY

TAKE HOME MESSAGES

BIBLIOGRAPHY

HISTORY

Cells communicate with each other

How the cell manages to convert external signals into the cell?

1994- Nobel Prize in Physiology or Medicine

Alfred G. Gilman and **Martin Rodbell** received the Nobel Prize in Physiology or Medicine for their discovery of "G-proteins and the role of these proteins in signal transduction in cells"

TRIMERIC G - PROTEINS

Signalling transduction processes

3 subunits

Alpha	Beta	Gamma

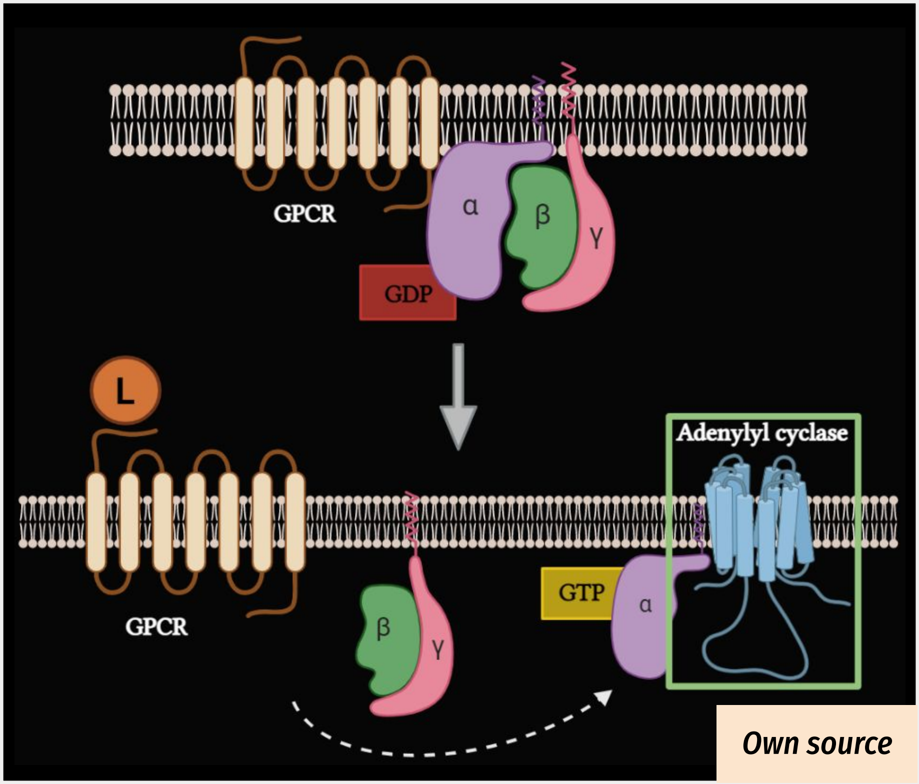
Gα: GTPase intrinsic activity

20% conservation	Variability

Classification

$G\alpha_s$	$G\alpha_i$	$G\alpha_q$	$G\alpha_{12}$

GTPase domain	Homology



Own source

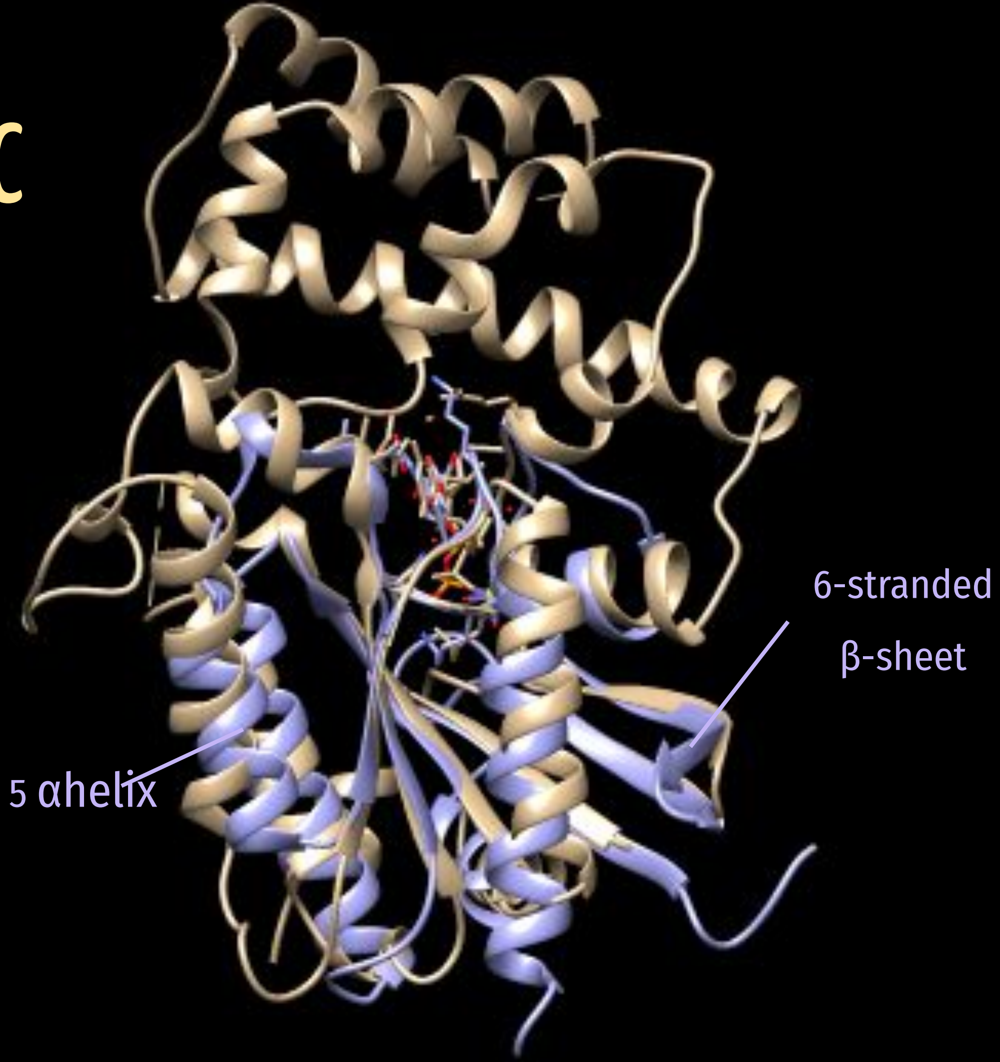
MONOMERIC - TRIMERIC

🟪 RRAS

🟨 G - α-l1

RAS domain

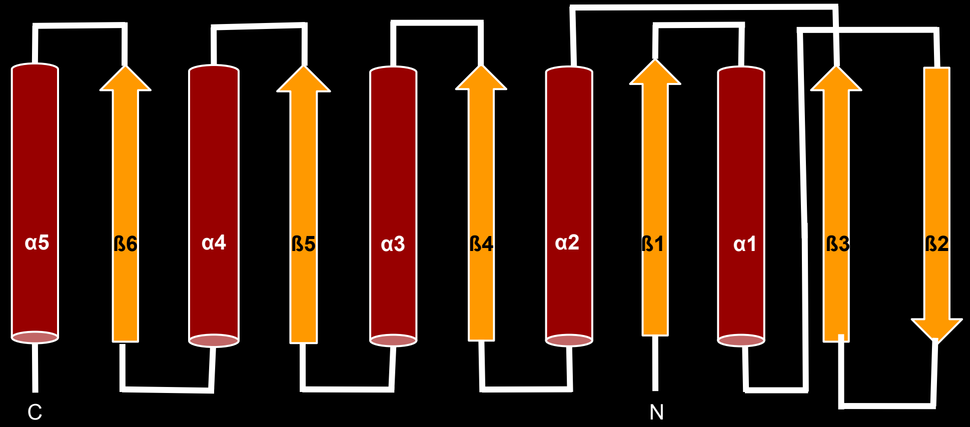
(GTP binding domain)



Resolution = 0,936 Å

MONOMERIC G - PROTEINS (or RAS SUPERFAMILY)

- **Small GTPases** (21 - 30 kD)
- Common core structure (G domain) : **5 helix + 6-stranded β-sheet**
- 5 families (Post translational modifications)
- Cytosol/membrane



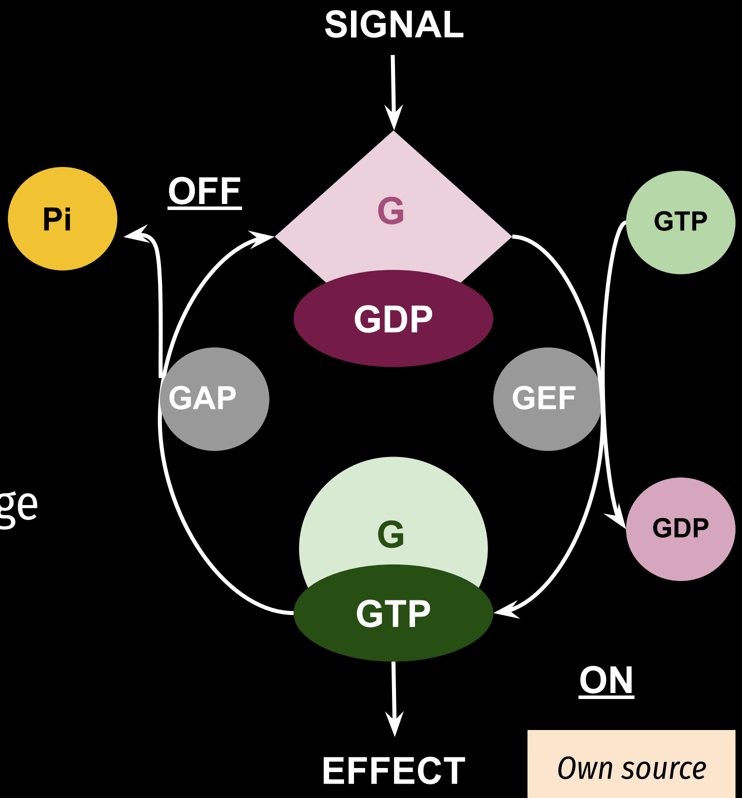
Own source

MONOMERIC G - PROTEINS (or RAS SUPERFAMILY)

- SWITCHES :
  - ON (GTP)
  - OFF (GTP hydrolyzed to GDP)

- Switch I + Switch II
- Activation by proteins → conformational change
- RGS = Regulators of G protein Signalling

(GAP + GEF)



Own source

MONOMERIC G - PROTEINS

FAMILY	SUBFAMILIES	FUNCTION
RAS	HRAS, NRAS, KRAS, RRAS, RAP, RAL, RIT...	Cell growth (growth factor signal cascades)
RHO	RHOA,B,C,D...RAC, ROCK, RND...	Cytoskeletal dynamics = cell adhesion and migration
RAB	RAB3A, RABL, RAB1A, RAB14, RAB35...	Protein trafficking pathways, regulation of vesicle formation, actin-tubulin dependent movement and membrane fusion
RAN	RAN	Transport of proteins into and out of the nucleus (localisation of prot)
ARF	ARF1, ARF3, ARF4, ARF5...ARL 4, ARFRP, ARL, SARA...	Vesicle biogenesis, recycling and trafficking

EVOLUTION OF G PROTEINS

TRIMERIC : GPCR signalling system

Most of the gene families of this system were already present in the LECA

Some species have GPCRs without G proteins and vice versa

Different parts of the GPCR signalling system evolved independently

The system is very plastic

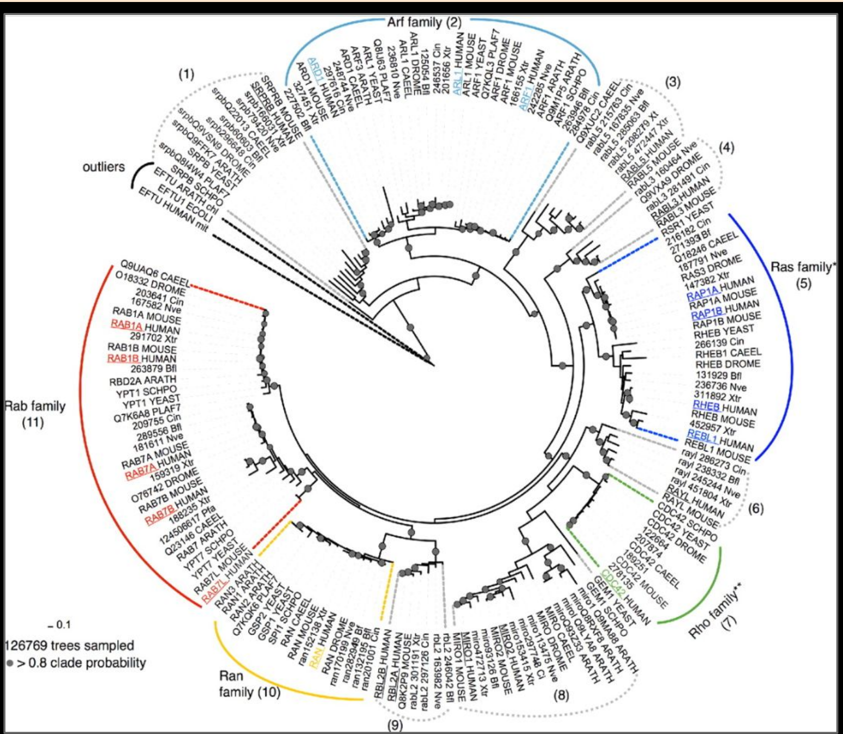
The expansion of the GPCR receptors could be motivated by the emergence of multicellularity in metazoans

A key to developing multicellularity complexy

EVOLUTION OF G PROTEINS

MONOMERIC : Ras superfamily

- The Ras superfamily is divided into five families: Ras, Rho, Arf/Sar, Ran, and Rab.
- The separation between families was an early evolutionary event that predated the expansion of eukaryotes.
- Arf family is the possible founding member.



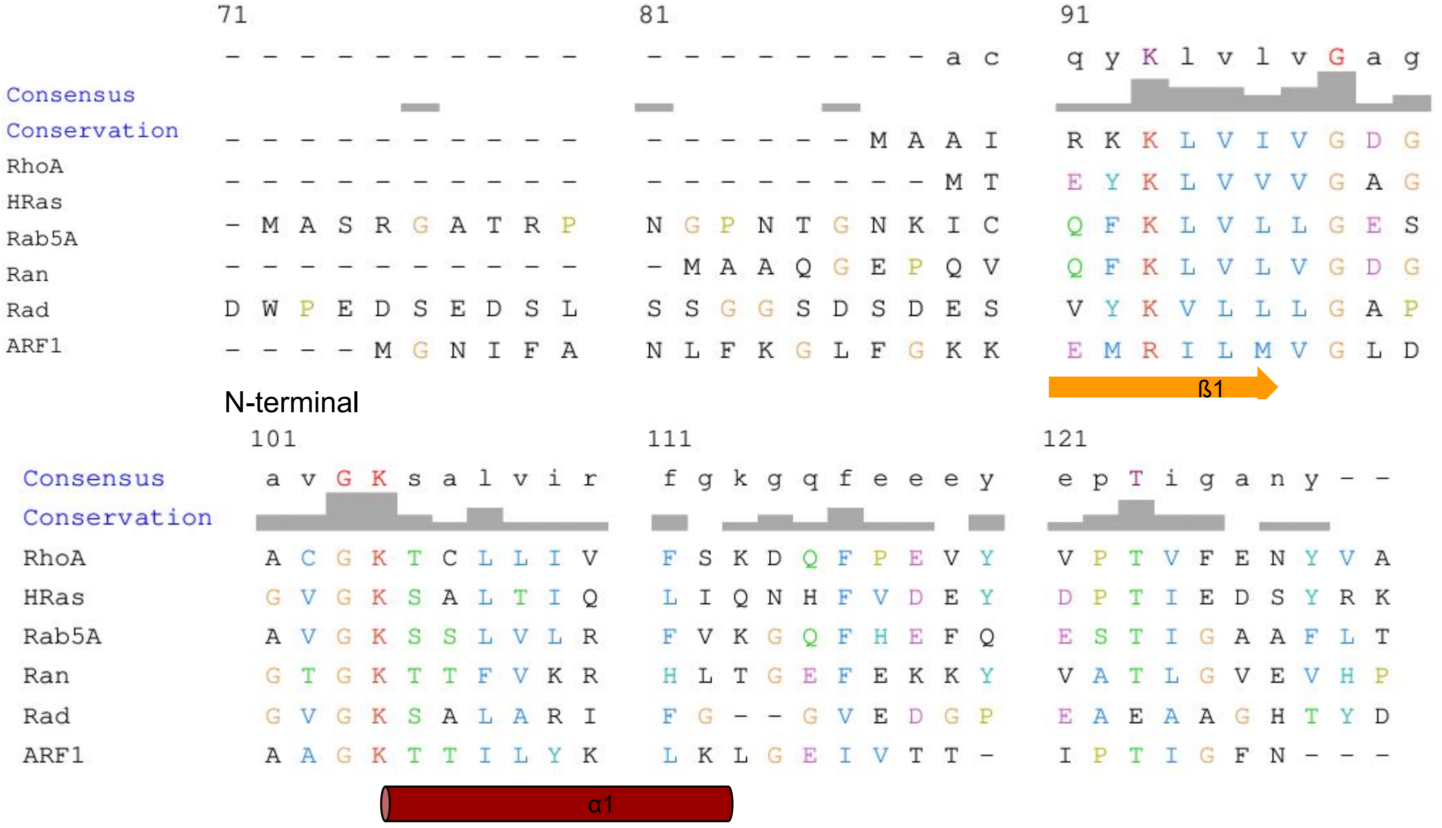
Phylogenetic tree of selected Ras superfamily members rooted with outliers. Rojas A, Fuentes G, Rausell A, Valencia A. The Ras protein superfamily: Evolutionary tree and role of conserved amino acids. *J Cell Biol*. 2012;196(2):189-201.

MONOMERIC G - PROTEINS : CLUSTALW

Are the human superfamily ras proteins similar between them?

SwissProt (fasta files) → { **All studied sequences** } → **Clustalw** → **Chimera**

MULTIPLE SEQUENCE ALIGNMENT (MSA) OF HUMAN RAS SUPERFAMILY

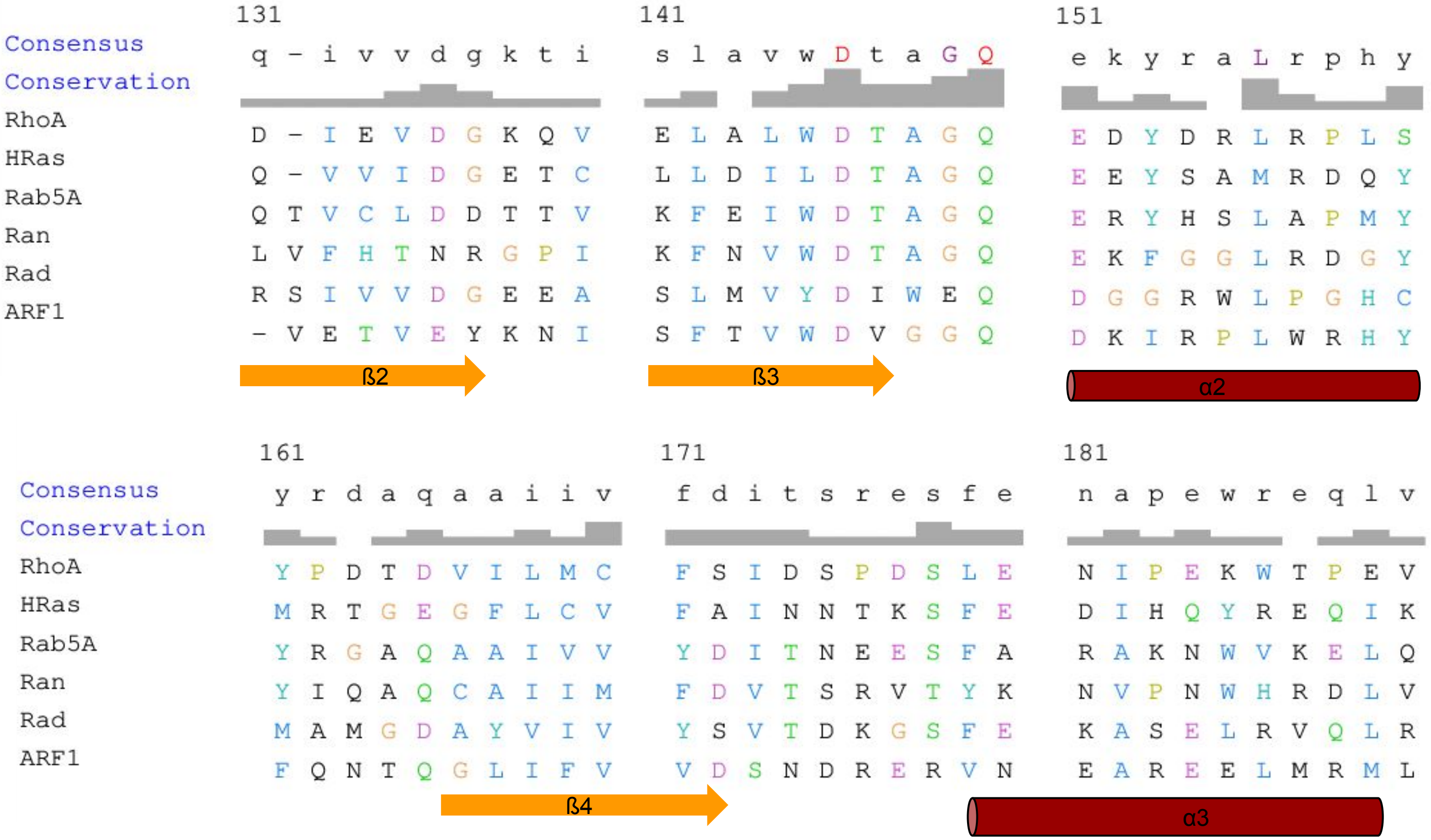


	71	81	91
Consensus	- - - - - - - - - -	- - - - - - - - a c	q y K l v l v G a g
Conservation			
RhoA	- - - - - - - - - -	- - - - - - M A A I	R K K L V I V G D G
HRas	- - - - - - - - - -	- - - - - - - - M T	E Y K L V V V G A G
Rab5A	- M A S R G A T R P	N G P N T G N K I C	Q F K L V L L G E S
Ran	- - - - - - - - - -	- M A A Q G E P Q V	Q F K L V L V G D G
Rad	D W P E D S E D S L	S S G G S D S D E S	V Y K V L L L G A P
ARF1	- - - - M G N I F A	N L F K G L F G K K	E M R I L M V G L D

	101	111	121
Consensus	a v G K s a l v i r	f g k g q f e e e y	e p T i g a n y - -
Conservation			
RhoA	A C G K T C L L I V	F S K D Q F P E V Y	V P T V F E N Y V A
HRas	G V G K S A L T I Q	L I Q N H F V D E Y	D P T I E D S Y R K
Rab5A	A V G K S S L V L R	F V K G Q F H E F Q	E S T I G A A F L T
Ran	G T G K T T F V K R	H L T G E F E K K Y	V A T L G V E V H P
Rad	G V G K S A L A R I	F G - - G V E D G P	E A E A A G H T Y D
ARF1	A A G K T T I L Y K	L K L G E I V T T -	I P T I G F N - - -

Color code

- 🟪 Charge -
- 🟥 Charge +
- 🟩 Polar
- 🟦 Hydrophobic
- 🟩 Prolines
- 🟧 Glycines

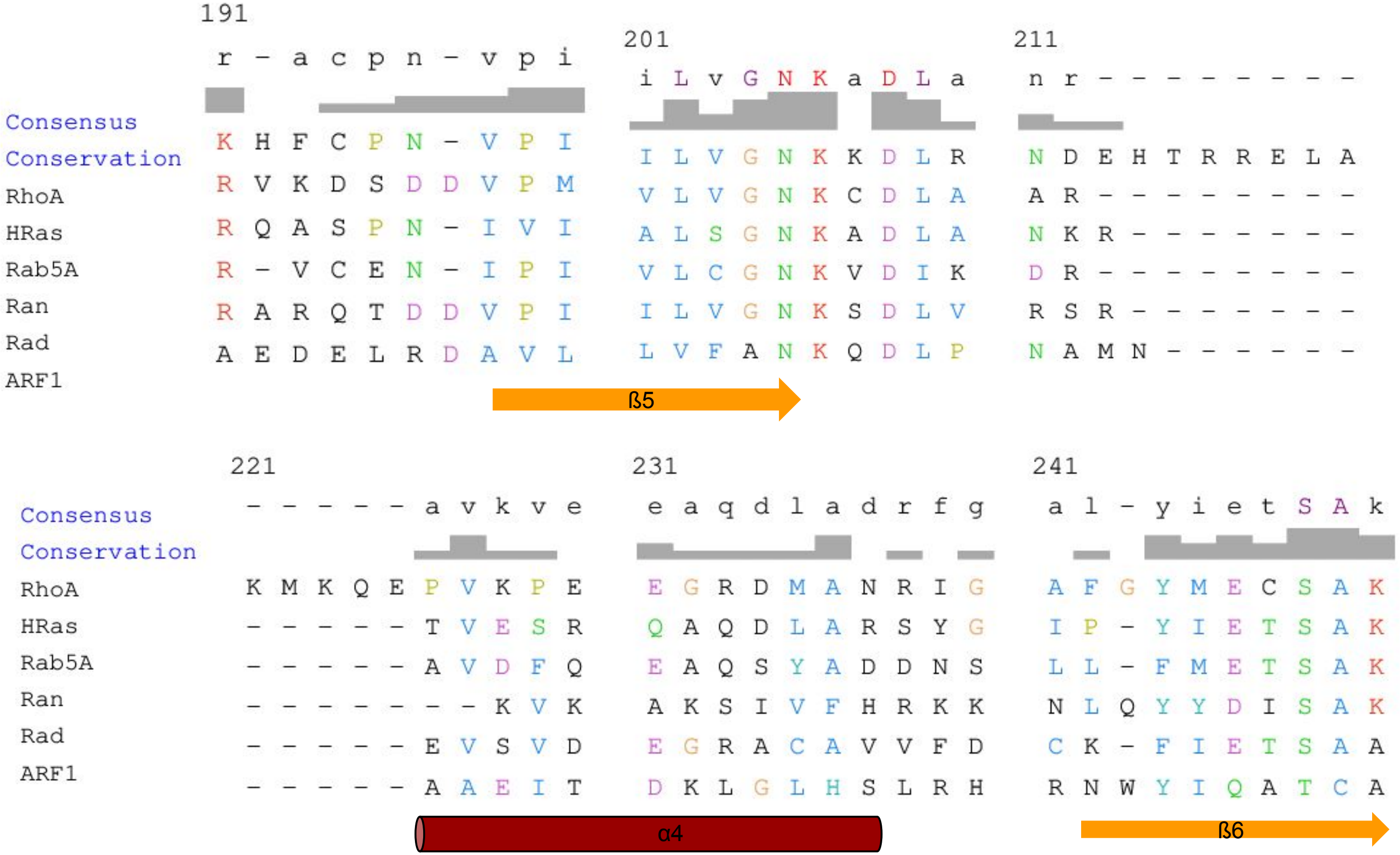


	131	141	151
Consensus	q - i v v d g k t i	s l a v w D t a G Q	e k y r a L r p h y
Conservation			
RhoA	D - I E V D G K Q V	E L A L W D T A G Q	E D Y D R L R P L S
HRas	Q - V V I D G E T C	L L D I L D T A G Q	E E Y S A M R D Q Y
Rab5A	Q T V C L D D T T V	K F E I W D T A G Q	E R Y H S L A P M Y
Ran	L V F H T N R G P I	K F N V W D T A G Q	E K F G G L R D G Y
Rad	R S I V V D G E E A	S L M V Y D I W E Q	D G G R W L P G H C
ARF1	- V E T V E Y K N I	S F T V W D V G G Q	D K I R P L W R H Y
	ß2	ß3	α2

	161	171	181
Consensus	y r d a q a a i i v	f d i t s r e s f e	n a p e w r e q l v
Conservation			
RhoA	Y P D T D V I L M C	F S I D S P D S L E	N I P E K W T P E V
HRas	M R T G E G F L C V	F A I N N T K S F E	D I H Q Y R E Q I K
Rab5A	Y R G A Q A A I V V	Y D I T N E E S F A	R A K N W V K E L Q
Ran	Y I Q A Q C A I I M	F D V T S R V T Y K	N V P N W H R D L V
Rad	M A M G D A Y V I V	Y S V T D K G S F E	K A S E L R V Q L R
ARF1	F Q N T Q G L I F V	V D S N D R E R V N	E A R E E L M R M L
	ß4		α3

Color code

🟪	Charge -
🟥	Charge +
🟩	Polar
🟦	Hydrophobic
🟩	Prolines
🟧	Glycines

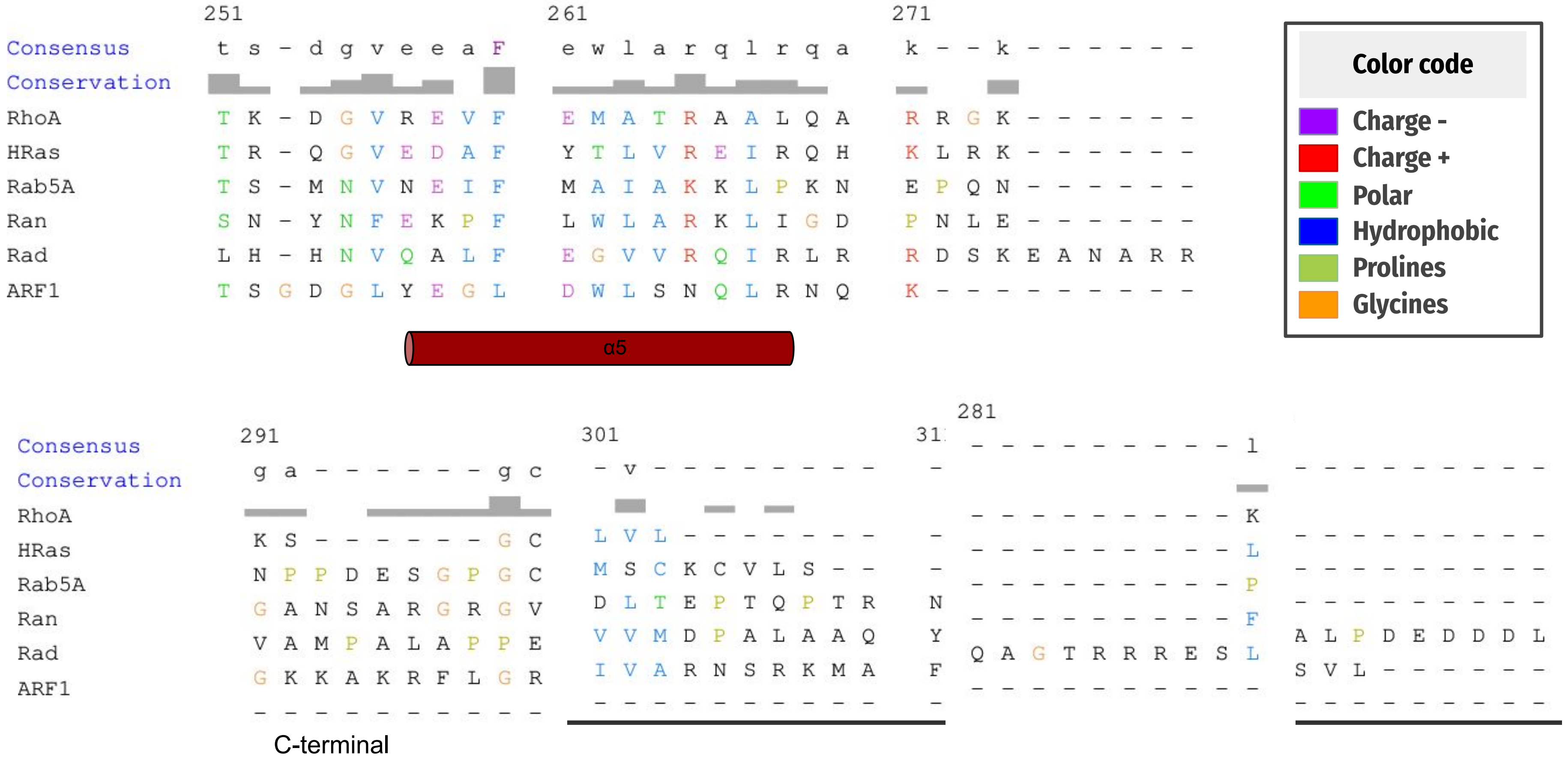


	191										201										211									
Consensus	r	-	a	c	p	n	-	v	p	i	i	L	v	G	N	K	a	D	L	a	n	r	-	-	-	-	-	-	-	-
RhoA	K	H	F	C	P	N	-	V	P	I	I	L	V	G	N	K	K	D	L	R	N	D	E	H	T	R	R	E	L	A
HRas	R	V	K	D	S	D	D	V	P	M	V	L	V	G	N	K	C	D	L	A	A	R	-	-	-	-	-	-	-	-
Rab5A	R	Q	A	S	P	N	-	I	V	I	A	L	S	G	N	K	A	D	L	A	N	K	R	-	-	-	-	-	-	-
Ran	R	-	V	C	E	N	-	I	P	I	V	L	C	G	N	K	V	D	I	K	D	R	-	-	-	-	-	-	-	-
Rad	R	A	R	Q	T	D	D	V	P	I	I	L	V	G	N	K	S	D	L	V	R	S	R	-	-	-	-	-	-	-
ARF1	A	E	D	E	L	R	D	A	V	L	L	V	F	A	N	K	Q	D	L	P	N	A	M	N	-	-	-	-	-	-

	221										231										241									
Consensus	-	-	-	-	-	a	v	k	v	e	e	a	q	d	l	a	d	r	f	g	a	l	-	y	i	e	t	S	A	k
RhoA	K	M	K	Q	E	P	V	K	P	E	E	G	R	D	M	A	N	R	I	G	A	F	G	Y	M	E	C	S	A	K
HRas	-	-	-	-	-	T	V	E	S	R	Q	A	Q	D	L	A	R	S	Y	G	I	P	-	Y	I	E	T	S	A	K
Rab5A	-	-	-	-	-	A	V	D	F	Q	E	A	Q	S	Y	A	D	D	N	S	L	L	-	F	M	E	T	S	A	K
Ran	-	-	-	-	-	-	-	K	V	K	A	K	S	I	V	F	H	R	K	K	N	L	Q	Y	Y	D	I	S	A	K
Rad	-	-	-	-	-	E	V	S	V	D	E	G	R	A	C	A	V	V	F	D	C	K	-	F	I	E	T	S	A	A
ARF1	-	-	-	-	-	A	A	E	I	T	D	K	L	G	L	H	S	L	R	H	R	N	W	Y	I	Q	A	T	C	A

Color code

🟪	Charge -
🟥	Charge +
🟩	Polar
🟦	Hydrophobic
🟩	Prolines
🟧	Glycines



```
              251                   261                   271
Consensus     t s - d g v e e a F   e w l a r q l r q a   k - - k - - - - - -
RhoA          T K - D G V R E V F   E M A T R A A L Q A   R R G K - - - - - -
HRas          T R - Q G V E D A F   Y T L V R E I R Q H   K L R K - - - - - -
Rab5A         T S - M N V N E I F   M A I A K K L P K N   E P Q N - - - - - -
Ran           S N - Y N F E K P F   L W L A R K L I G D   P N L E - - - - - -
Rad           L H - H N V Q A L F   E G V V R Q I R L R   R D S K E A N A R R
ARF1          T S G D G L Y E G L   D W L S N Q L R N Q   K - - - - - - - - -

              281                   291                   301                   31
Consensus     - - - - - - - - - l   g a - - - - - - g c   - v - - - - - - - - -   - - - - - - - - -
RhoA          - - - - - - - - - K   K S - - - - - - G C   L V L - - - - - - - -   - - - - - - - - -
HRas          - - - - - - - - - L   N P P D E S G P G C   M S C K C V L S - - -   - - - - - - - - -
Rab5A         - - - - - - - - - P   G A N S A R G R G V   D L T E P T Q P T R N   - - - - - - - - -
Ran           - - - - - - - - - F   V A M P A L A P P E   V V M D P A L A A Q Y   A L P D E D D D L
Rad           Q A G T R R R E S L   G K K A K R F L G R   I V A R N S R K M A F   S V L - - - - - -
ARF1          - - - - - - - - - -   - - - - - - - - - -   - - - - - - - - - - -   - - - - - - - - -
```

Color code

- 🟪 Charge -
- 🟥 Charge +
- 🟩 Polar
- 🟦 Hydrophobic
- 🟩 Prolines
- 🟧 Glycines

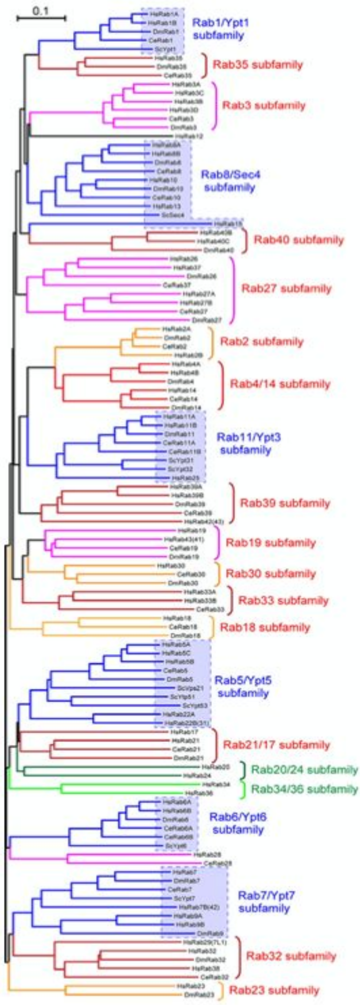
INTRODUCTION
(G PROTEINS)

RAB FAMILY
- Introduction + Structure
- Evolution
- Interactions

TAKE HOME MESSAGES

BIBLIOGRAPHY

INTRODUCTION TO RAB FAMILY



Largest small GTPases 200-250 amino acids

70 Rabs and 44 subfamilies

They modulate vesicle trafficking and protein transport of eukaryotes

Little is known about regulation and function of a large number of rab proteins.

Phylogenetic analysis of Rabs. Homma Y, Hiragi S, Fukuda M. Rab family of small GTPases: an updated view on their regulation and functions. FEBS J. 2021 Jan;288(1):36-55.

RAB SCOPE CLASSIFICATION

STRUCTURAL CLASS → Alpha and beta proteins

SUPERFAMILY → Ras like protein P loop GTPases

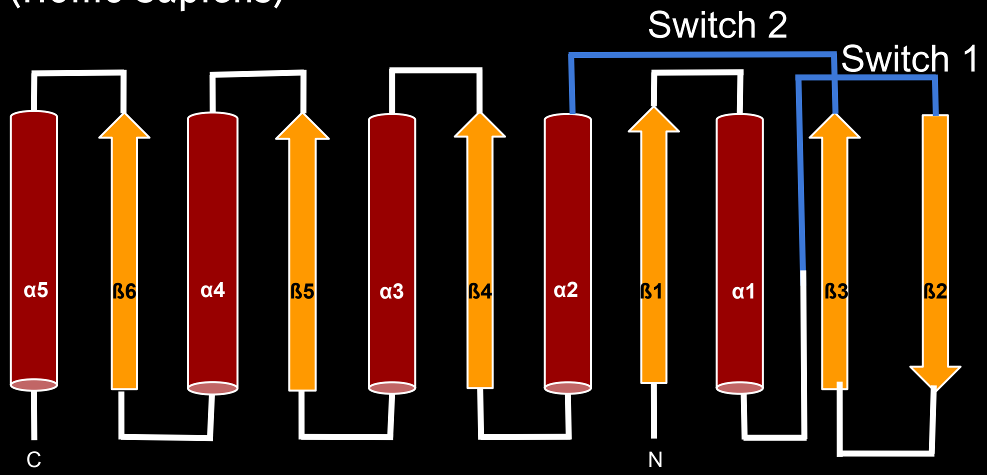
SUBFAMILY→Rab14, Rab7

SPECIES → Human (Homo Sapiens)

FOLD → G domain like

FAMILY → Ras like monodomain GTPases (Rab)

PROTEIN →Rab14, Rab7A, Rab7B



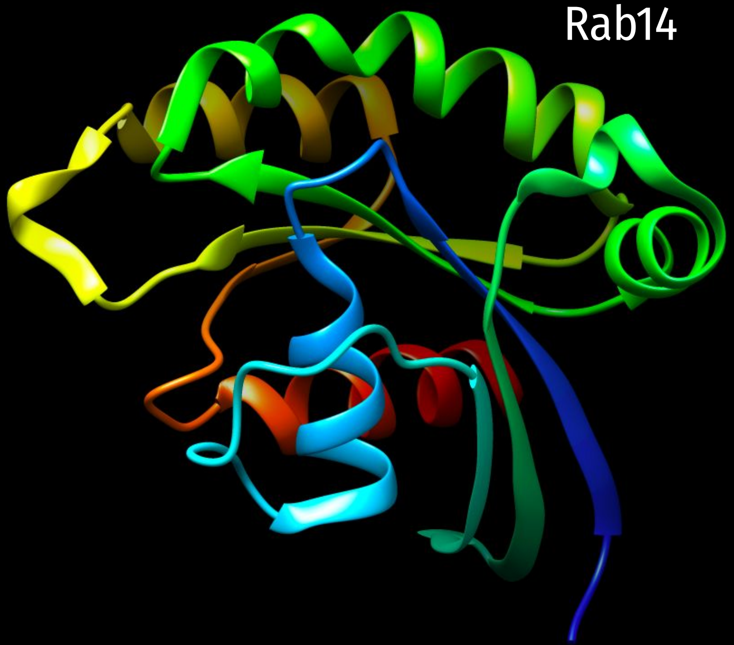
Own source

GENERAL STRUCTURE

SECONDARY STRUCTURE

6 stranded ß-sheet
(with five parallel strands and one antiparallel)

5 α-helices

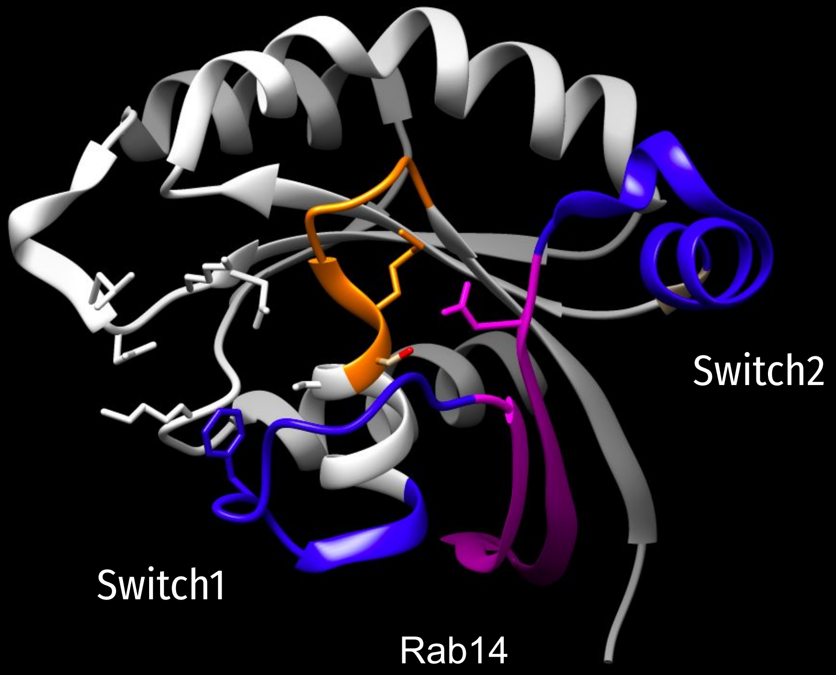


GENERAL STRUCTURE

STRUCTURAL ELEMENTS

- 🟧 P-loop
- 🟦 Switch I
- 🟪 InterSwitch
- 🟦 Switch II

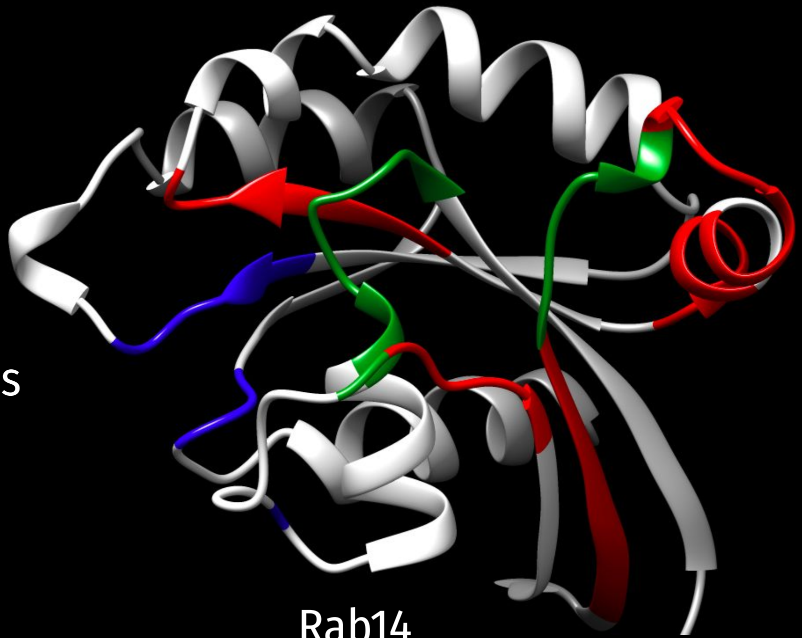
COOH-terminal → hypervariable region (CAAX boxes: aprox 35–40 aa)



GENERAL STRUCTURE

SEQUENCE MOTIFS

- 🟥 Conserved regions (F1-F5)
- 🟩 Phosphate/magnesium-binding motifs (PM1-PM3) → P-loop NTPases
- 🟦 Guanine-binding motifs (G1-G3)

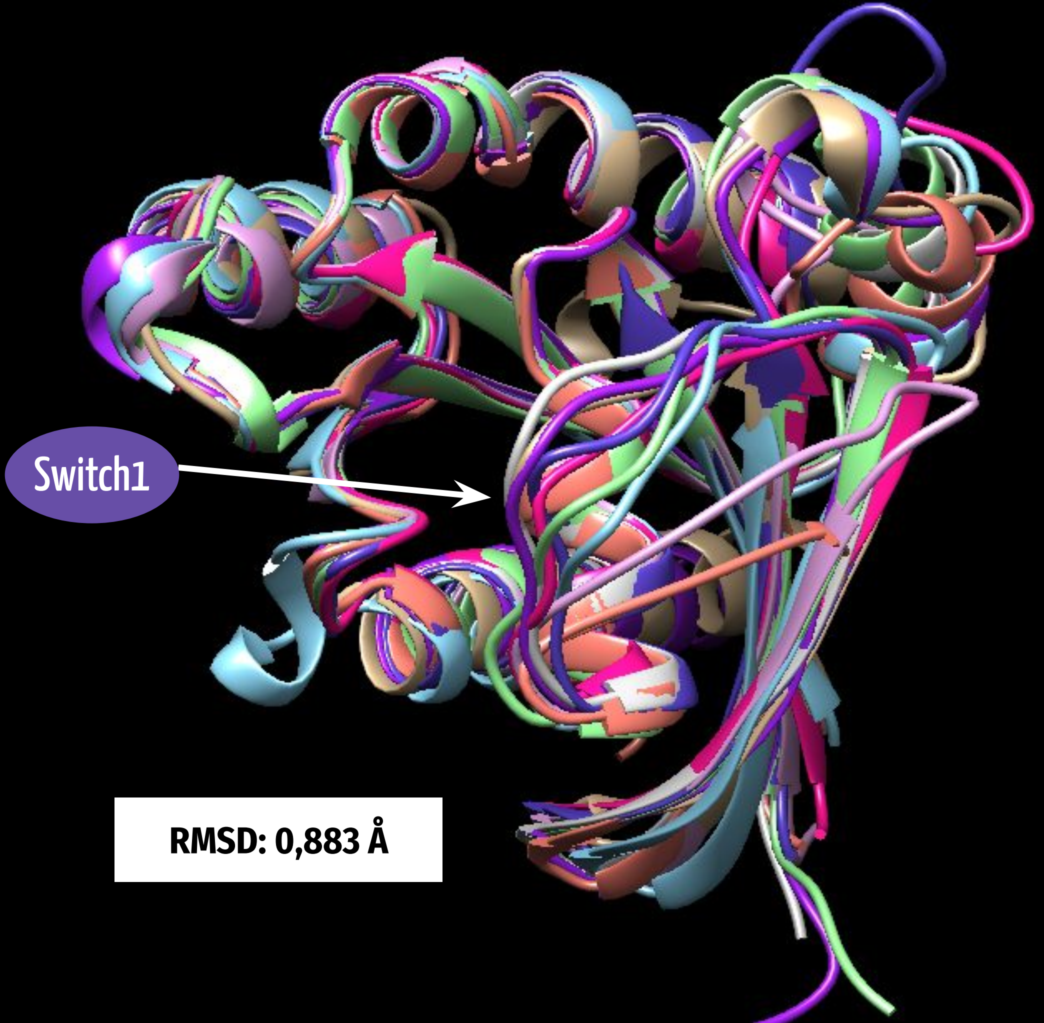


Rab14

SUPERIMPOSITION

(of HUMAN Rab proteins)

- Rab14
- Rab2A
- Rab1B
- Rab4A
- Rab33
- Rab7
- Rab35
- Rab11
- Rab43



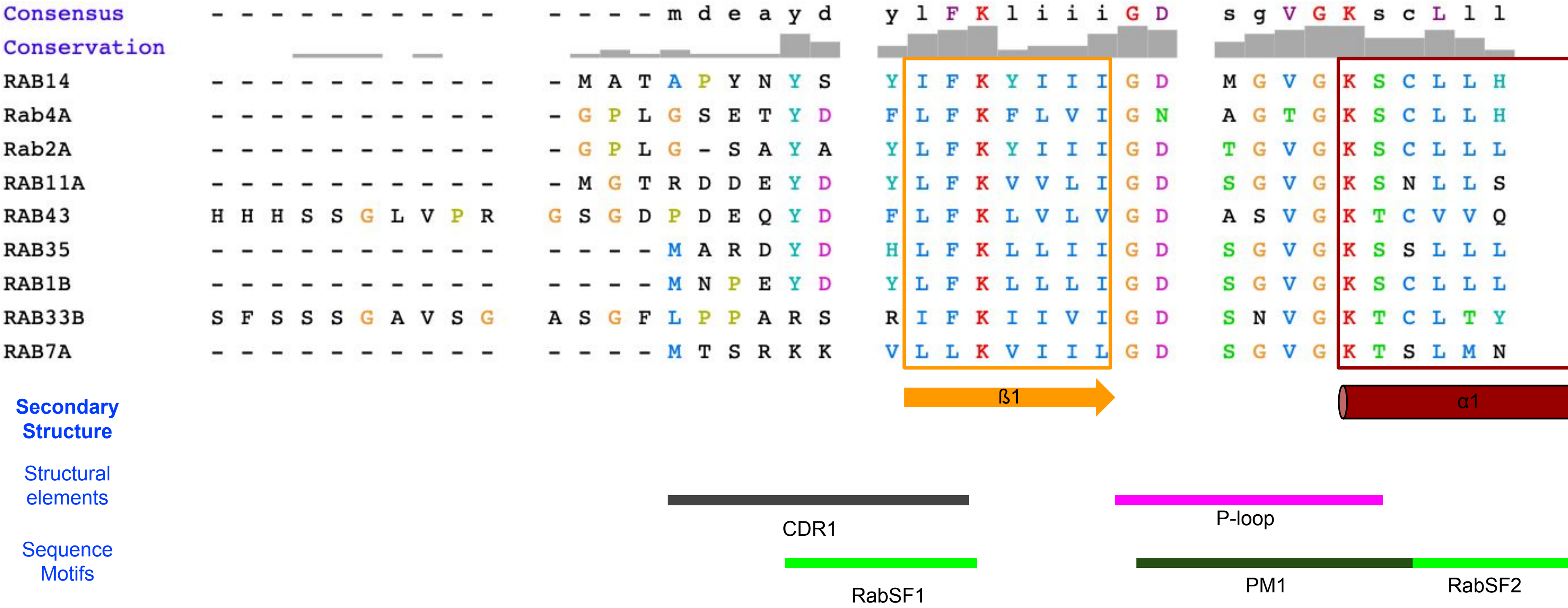
RMSD: 0,883 Å

EVOLUTION

CLUSTALW
of human Rabs proteins

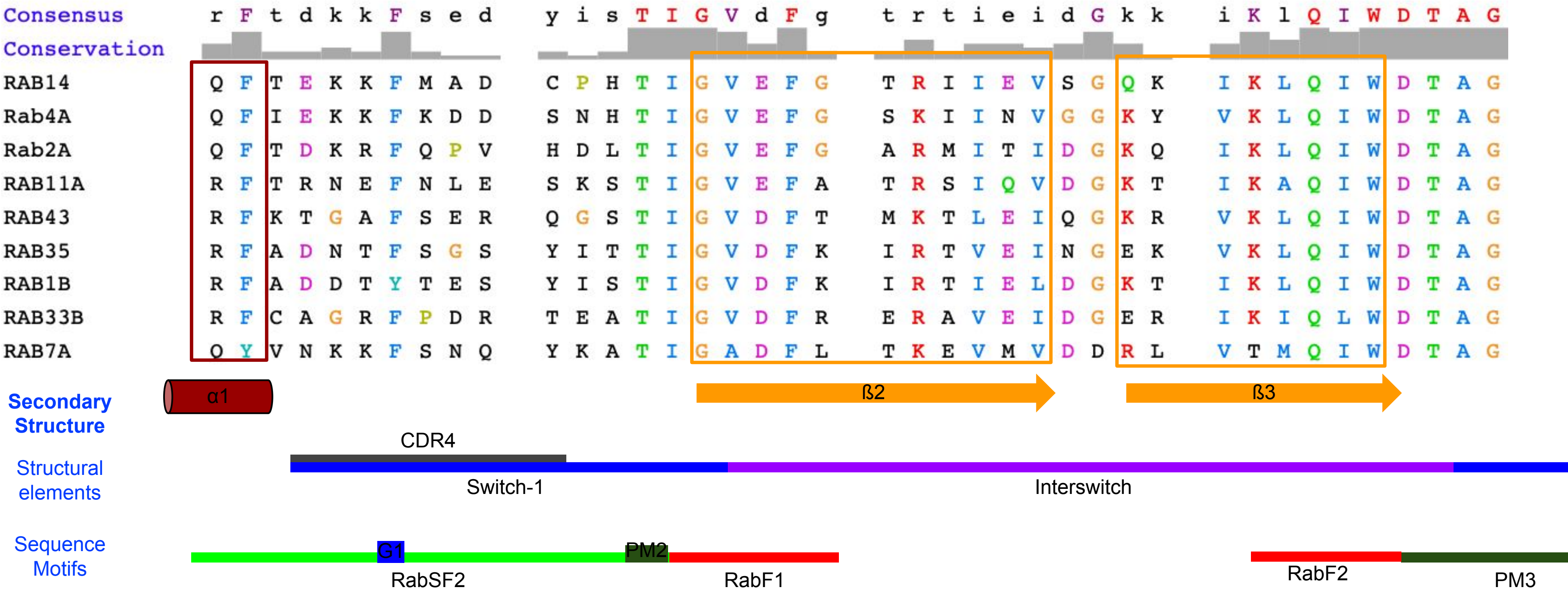
- Rab14
- Rab4A
- Rab2A
- Rab11A
- Rab43
- Rab3
- Rab1B
- Rab33B
- Rab7A

MULTIPLE SEQUENCE ALIGNMENT (MSA) OF HUMAN RAB PROTEINS



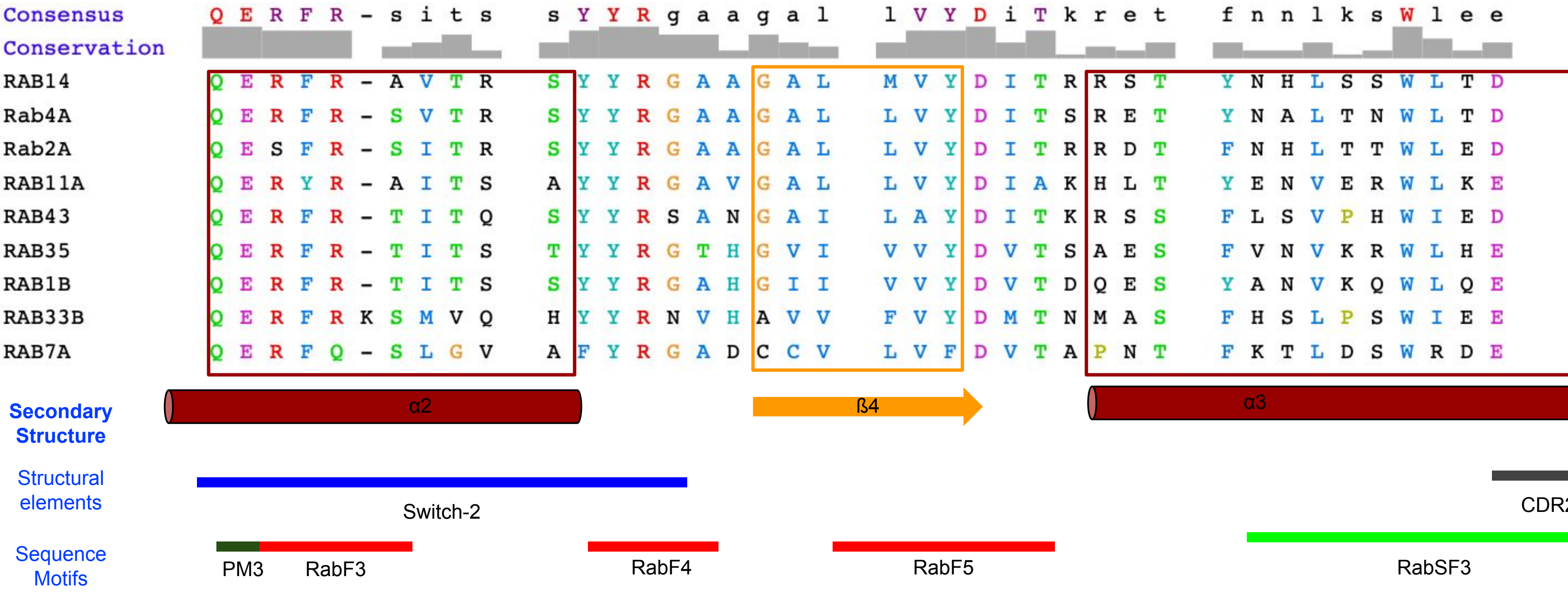
Consensus	----------	----mdeayd	ylFKliiiGD	sgVGKscLll
Conservation				
RAB14	----------	-MATAPYNYS	YIFKYIIIGD	MGVGKSCLLH
Rab4A	----------	-GPLGSETYD	FLFKFLVIGN	AGTGKSCLLH
Rab2A	----------	-GPLG-SAYA	YLFKYIIIGD	TGVGKSCLLL
RAB11A	----------	-MGTRDDEYD	YLFKVVLIGD	SGVGKSNLLS
RAB43	HHHSSGLVPR	GSGDPDEQYD	FLFKLVLVGD	ASVGKTCVVQ
RAB35	----------	----MARDYD	HLFKLLIIGD	SGVGKSSLLL
RAB1B	----------	----MNPEYD	YLFKLLLIGD	SGVGKSCLLL
RAB33B	SFSSSGAVSG	ASGFLPPARS	RIFKIIVIGD	SNVGKTCLTY
RAB7A	----------	----MTSRKK	VLLKVIILGD	SGVGKTSLMN

MULTIPLE SEQUENCE ALIGNMENT (MSA) OF HUMAN RAB PROTEINS



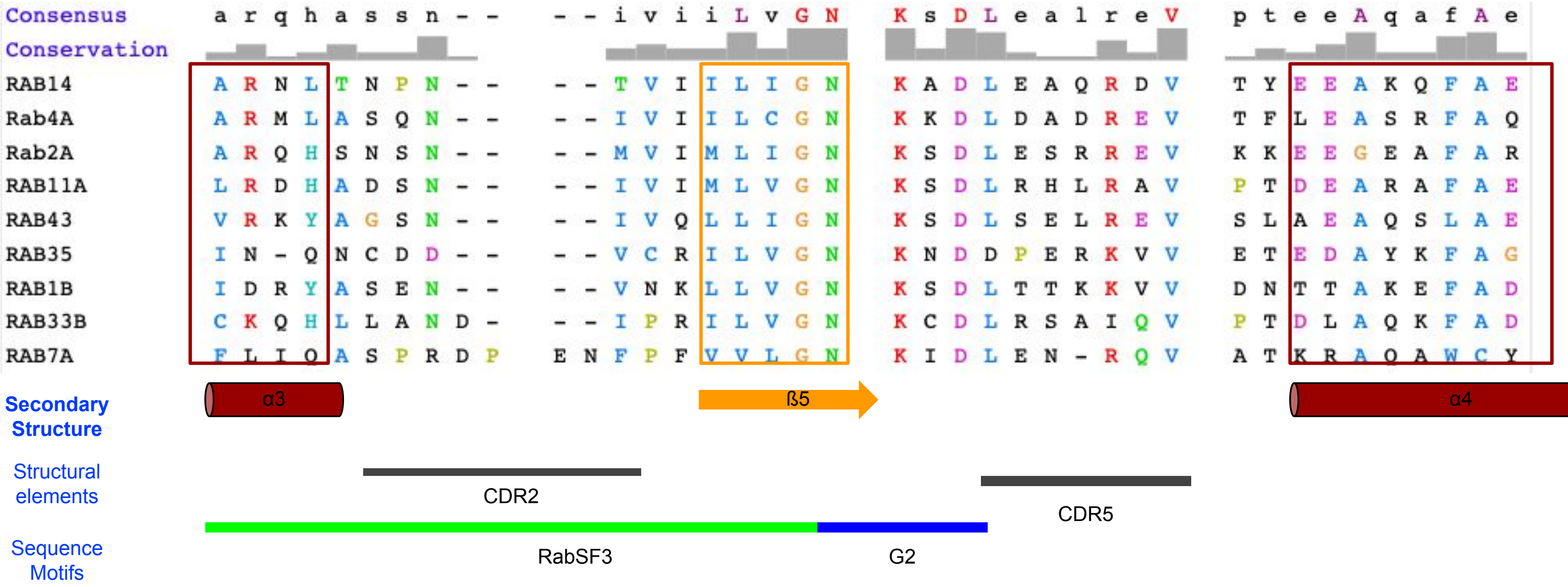
```
Consensus      r F t d k k F s e d   y i s T I G V d F g   t r t i e i d G k k   i K l Q I W D T A G
Conservation
RAB14          Q F T E K K F M A D   C P H T I G V E F G   T R I I E V S G Q K   I K L Q I W D T A G
Rab4A          Q F I E K K F K D D   S N H T I G V E F G   S K I I N V G G K Y   V K L Q I W D T A G
Rab2A          Q F T D K R F Q P V   H D L T I G V E F G   A R M I T I D G K Q   I K L Q I W D T A G
RAB11A         R F T R N E F N L E   S K S T I G V E F A   T R S I Q V D G K T   I K A Q I W D T A G
RAB43          R F K T G A F S E R   Q G S T I G V D F T   M K T L E I Q G K R   V K L Q I W D T A G
RAB35          R F A D N T F S G S   Y I T T I G V D F K   I R T V E I N G E K   V K L Q I W D T A G
RAB1B          R F A D D T Y T E S   Y I S T I G V D F K   I R T I E L D G K T   I K L Q I W D T A G
RAB33B         R F C A G R F P D R   T E A T I G V D F R   E R A V E I D G E R   I K I Q L W D T A G
RAB7A          Q Y V N K K F S N Q   Y K A T I G A D F L   T K E V M V D D R L   V T M Q I W D T A G
```

MULTIPLE SEQUENCE ALIGNMENT (MSA) OF HUMAN RAB PROTEINS



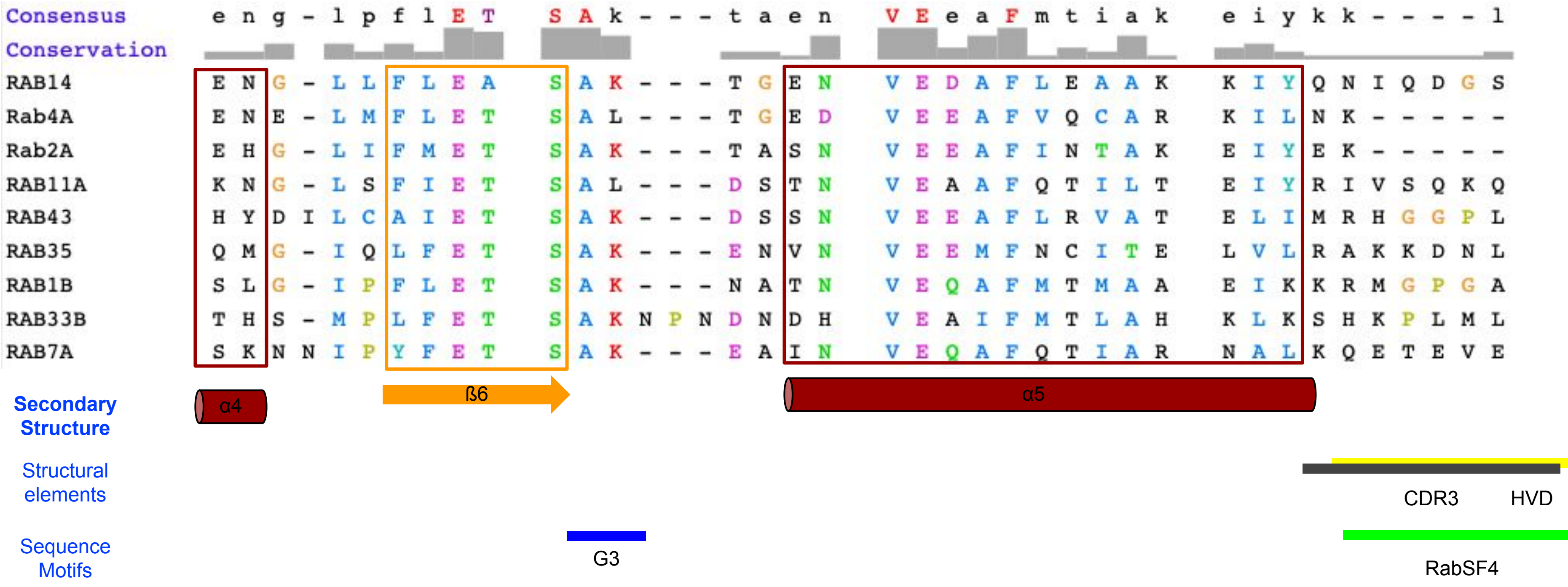
Consensus	Q	E	R	F	R	-	s	i	t	s	s	Y	Y	R	g	a	a	g	a	l	l	V	Y	D	i	T	k	r	e	t	f	n	n	l	k	s	W	l	e	e
RAB14	Q	E	R	F	R	-	A	V	T	R	S	Y	Y	R	G	A	A	G	A	L	M	V	Y	D	I	T	R	R	S	T	Y	N	H	L	S	S	W	L	T	D
Rab4A	Q	E	R	F	R	-	S	V	T	R	S	Y	Y	R	G	A	A	G	A	L	L	V	Y	D	I	T	S	R	E	T	Y	N	A	L	T	N	W	L	T	D
Rab2A	Q	E	S	F	R	-	S	I	T	R	S	Y	Y	R	G	A	A	G	A	L	L	V	Y	D	I	T	R	R	D	T	F	N	H	L	T	T	W	L	E	D
RAB11A	Q	E	R	Y	R	-	A	I	T	S	A	Y	Y	R	G	A	V	G	A	L	L	V	Y	D	I	A	K	H	L	T	Y	E	N	V	E	R	W	L	K	E
RAB43	Q	E	R	F	R	-	T	I	T	Q	S	Y	Y	R	S	A	N	G	A	I	L	A	Y	D	I	T	K	R	S	S	F	L	S	V	P	H	W	I	E	D
RAB35	Q	E	R	F	R	-	T	I	T	S	T	Y	Y	R	G	T	H	G	V	I	V	V	Y	D	V	T	S	A	E	S	F	V	N	V	K	R	W	L	H	E
RAB1B	Q	E	R	F	R	-	T	I	T	S	S	Y	Y	R	G	A	H	G	I	I	V	V	Y	D	V	T	D	Q	E	S	Y	A	N	V	K	Q	W	L	Q	E
RAB33B	Q	E	R	F	R	K	S	M	V	Q	H	Y	Y	R	N	V	H	A	V	V	F	V	Y	D	M	T	N	M	A	S	F	H	S	L	P	S	W	I	E	E
RAB7A	Q	E	R	F	Q	-	S	L	G	V	A	F	Y	R	G	A	D	C	C	V	L	V	F	D	V	T	A	P	N	T	F	K	T	L	D	S	W	R	D	E

MULTIPLE SEQUENCE ALIGNMENT (MSA) OF HUMAN RAB PROTEINS



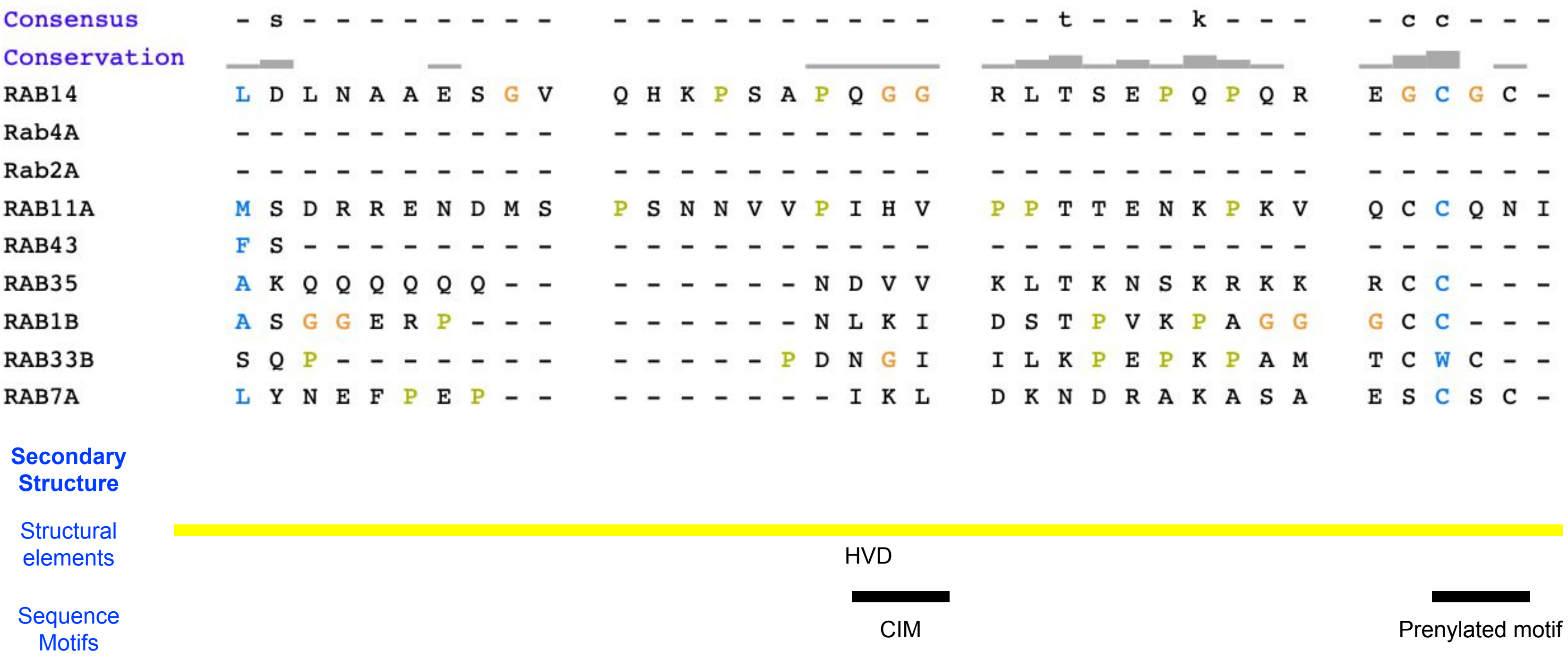
Consensus	a r q h a s s n - -	- - i v i i L v G N	K s D L e a l r e V	p t e e A q a f A e
Conservation				
RAB14	A R N L T N P N - -	- - T V I I L I G N	K A D L E A Q R D V	T Y E E A K Q F A E
Rab4A	A R M L A S Q N - -	- - I V I I L C G N	K K D L D A D R E V	T F L E A S R F A Q
Rab2A	A R Q H S N S N - -	- - M V I M L I G N	K S D L E S R R E V	K K E E G E A F A R
RAB11A	L R D H A D S N - -	- - I V I M L V G N	K S D L R H L R A V	P T D E A R A F A E
RAB43	V R K Y A G S N - -	- - I V Q L L I G N	K S D L S E L R E V	S L A E A Q S L A E
RAB35	I N - Q N C D D - -	- - V C R I L V G N	K N D D P E R K V V	E T E D A Y K F A G
RAB1B	I D R Y A S E N - -	- - V N K L L V G N	K S D L T T K K V V	D N T T A K E F A D
RAB33B	C K Q H L L A N D -	- - I P R I L V G N	K C D L R S A I Q V	P T D L A Q K F A D
RAB7A	F L I Q A S P R D P	E N F P F V V L G N	K I D L E N - R Q V	A T K R A Q A W C Y

MULTIPLE SEQUENCE ALIGNMENT (MSA) OF HUMAN RAB PROTEINS



Consensus	e	n	g	-	l	p	f	l	E	T	S	A	k	-	-	-	t	a	e	n	V	E	e	a	F	m	t	i	a	k	e	i	y	k	k	-	-	-	-	l
Conservation																																								
RAB14	E	N	G	-	L	L	F	L	E	A	S	A	K	-	-	-	T	G	E	N	V	E	D	A	F	L	E	A	A	K	K	I	Y	Q	N	I	Q	D	G	S
Rab4A	E	N	E	-	L	M	F	L	E	T	S	A	L	-	-	-	T	G	E	D	V	E	E	A	F	V	Q	C	A	R	K	I	L	N	K	-	-	-	-	-
Rab2A	E	H	G	-	L	I	F	M	E	T	S	A	K	-	-	-	T	A	S	N	V	E	E	A	F	I	N	T	A	K	E	I	Y	E	K	-	-	-	-	-
RAB11A	K	N	G	-	L	S	F	I	E	T	S	A	L	-	-	-	D	S	T	N	V	E	A	A	F	Q	T	I	L	T	E	I	Y	R	I	V	S	Q	K	Q
RAB43	H	Y	D	I	L	C	A	I	E	T	S	A	K	-	-	-	D	S	S	N	V	E	E	A	F	L	R	V	A	T	E	L	I	M	R	H	G	G	P	L
RAB35	Q	M	G	-	I	Q	L	F	E	T	S	A	K	-	-	-	E	N	V	N	V	E	E	M	F	N	C	I	T	E	L	V	L	R	A	K	K	D	N	L
RAB1B	S	L	G	-	I	P	F	L	E	T	S	A	K	-	-	-	N	A	T	N	V	E	Q	A	F	M	T	M	A	A	E	I	K	K	R	M	G	P	G	A
RAB33B	T	H	S	-	M	P	L	F	E	T	S	A	K	N	P	N	D	N	D	H	V	E	A	I	F	M	T	L	A	H	K	L	K	S	H	K	P	L	M	L
RAB7A	S	K	N	N	I	P	Y	F	E	T	S	A	K	-	-	-	E	A	I	N	V	E	Q	A	F	Q	T	I	A	R	N	A	L	K	Q	E	T	E	V	E

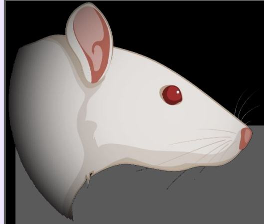
MULTIPLE SEQUENCE ALIGNMENT (MSA) OF HUMAN RAB PROTEINS



Consensus	-	s	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	t	-	-	-	k	-	-	-	-	c	c	-	-	-
Conservation																																				
RAB14	L	D	L	N	A	A	E	S	G	V	Q	H	K	P	S	A	P	Q	G	G	R	L	T	S	E	P	Q	P	Q	R	E	G	C	G	C	-
Rab4A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rab2A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
RAB11A	M	S	D	R	R	E	N	D	M	S	P	S	N	N	V	V	P	I	H	V	P	P	T	T	E	N	K	P	K	V	Q	C	C	Q	N	I
RAB43	F	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
RAB35	A	K	Q	Q	Q	Q	Q	Q	-	-	-	-	-	-	-	-	N	D	V	V	K	L	T	K	N	S	K	R	K	K	R	C	C	-	-	-
RAB1B	A	S	G	G	E	R	P	-	-	-	-	-	-	-	-	-	N	L	K	I	D	S	T	P	V	K	P	A	G	G	G	C	C	-	-	-
RAB33B	S	Q	P	-	-	-	-	-	-	-	-	-	-	-	-	P	D	N	G	I	I	L	K	P	E	P	K	P	A	M	T	C	W	C	-	-
RAB7A	L	Y	N	E	F	P	E	P	-	-	-	-	-	-	-	-	-	I	K	L	D	K	N	D	R	A	K	A	S	A	E	S	C	S	C	-

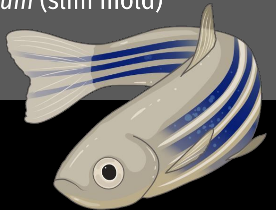
EVOLUTION

CLUSTALW
of Rab14 proteins

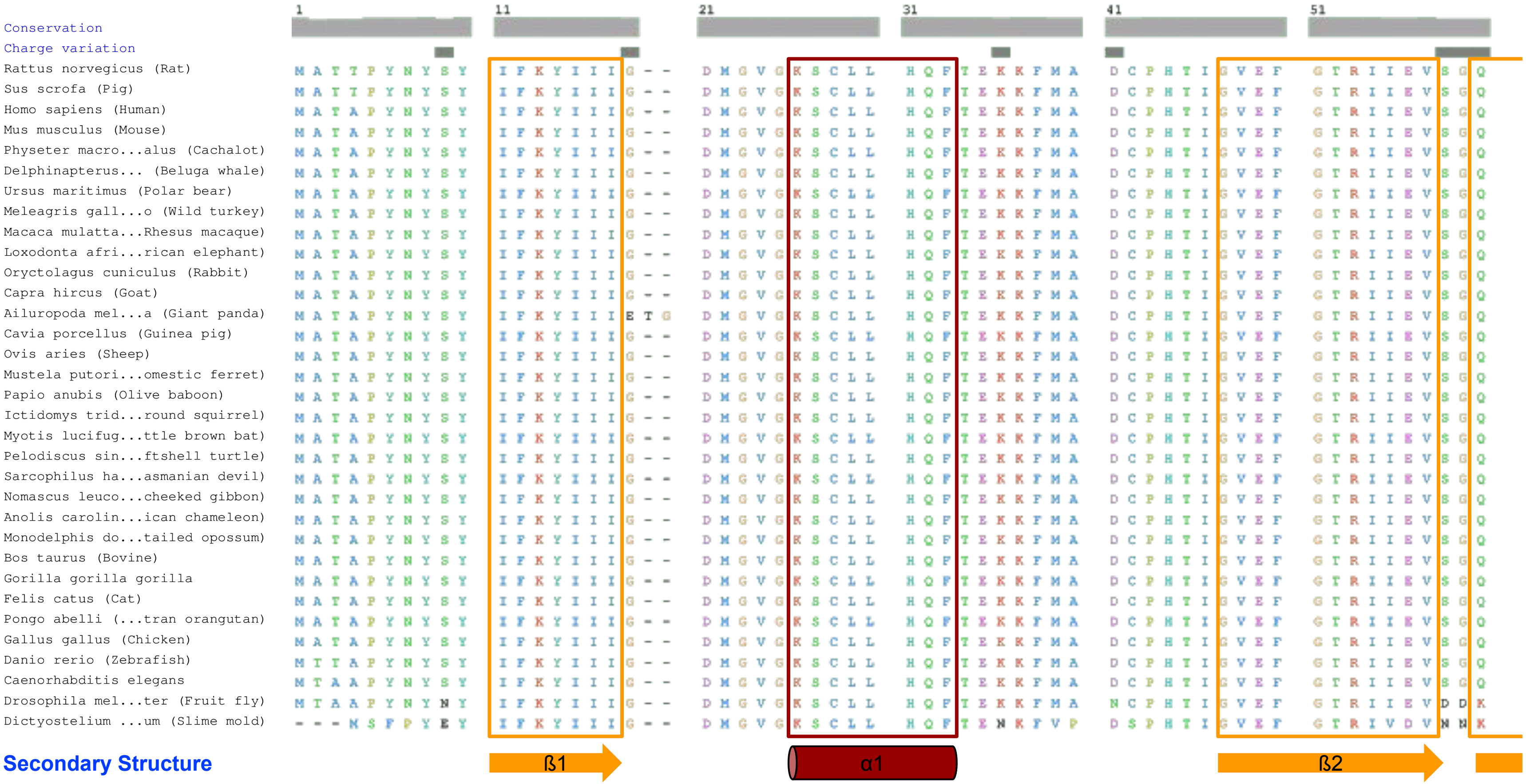


Homo sapiens (Human)
Rattus norvegicus (Rat)
Sus scrofa (Pig)
Mus musculus (Mouse)
Physeter macrocephalus (Cachalot)
Delphinapterus leucas (Beluga whale)
Ursus maritimus (Polar bear)
Meleagris gallopavo (Wild turkey)
Loxodonta africana (African elephant)
Oryctolagus cuniculus (Rabbit)
Capra hircus (Goat)
Ailuropoda melanoleuca (Giant panda)
Cavia porcellus (Guinea pig)
Ovis aries (Sheep)
Mustela putorius furo (Domestic ferret)
Papio anubis (Olive baboon)

Ictidomys tridecemlineatus (Thirteen-lined ground squirrel)
Myotis lucifugus (Little brown bat)
Pelodiscus sinensis (Chinese softshell turtle)
Sarcophilus harrisii (Tasmanian devil)
Nomascus leucogenys (Northern white-cheeked gibbon)
Anolis carolinensis (American chameleon)
Monodelphis domestica (Gray short-tailed opossum)
Bos taurus (Bovine)
Gorilla gorilla gorilla
Felis catus (Cat)
Pongo abelli (Sumatran orangutan)
Gallus gallus (Chicken)
Danio rerio (Zebrafish)
Caenorhabditis elegans
Drosophila melanogaster (Fruit fly)
Dictyostelium discoideum (slim mold)

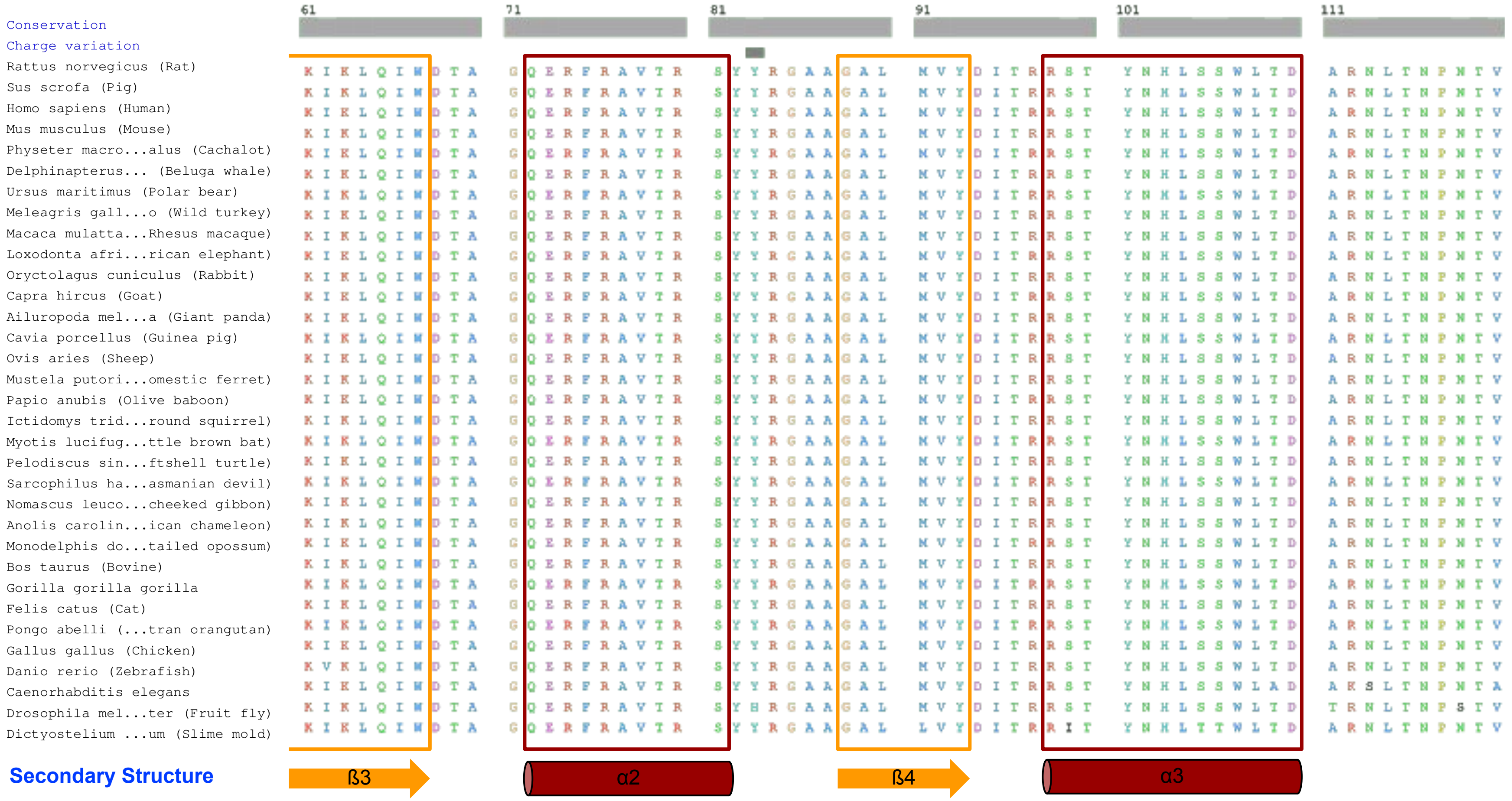


MULTIPLE SEQUENCE ALIGNMENT (MSA) OF RAB14 PROTEINS



	1	11	21	31	41	51
Conservation						
Charge variation						
Rattus norvegicus (Rat)	MATTPYNYSY	IFKYIIIG--	DMGVGKSCLL	HQFTEKKFMA	DCPHTIGVEF	GTRIIEVSGQ
Sus scrofa (Pig)	MATTPYNYSY	IFKYIIIG--	DMGVGKSCLL	HQFTEKKFMA	DCPHTIGVEF	GTRIIEVSGQ
Homo sapiens (Human)	MATAPYNYSY	IFKYIIIG--	DMGVGKSCLL	HQFTEKKFMA	DCPHTIGVEF	GTRIIEVSGQ
Mus musculus (Mouse)	MATAPYNYSY	IFKYIIIG--	DMGVGKSCLL	HQFTEKKFMA	DCPHTIGVEF	GTRIIEVSGQ
Physeter macro...alus (Cachalot)	MATAPYNYSY	IFKYIIIG--	DMGVGKSCLL	HQFTEKKFMA	DCPHTIGVEF	GTRIIEVSGQ
Delphinapterus... (Beluga whale)	MATAPYNYSY	IFKYIIIG--	DMGVGKSCLL	HQFTEKKFMA	DCPHTIGVEF	GTRIIEVSGQ
Ursus maritimus (Polar bear)	MATAPYNYSY	IFKYIIIG--	DMGVGKSCLL	HQFTEKKFMA	DCPHTIGVEF	GTRIIEVSGQ
Meleagris gall...o (Wild turkey)	MATAPYNYSY	IFKYIIIG--	DMGVGKSCLL	HQFTEKKFMA	DCPHTIGVEF	GTRIIEVSGQ
Macaca mulatta...Rhesus macaque)	MATAPYNYSY	IFKYIIIG--	DMGVGKSCLL	HQFTEKKFMA	DCPHTIGVEF	GTRIIEVSGQ
Loxodonta afri...rican elephant)	MATAPYNYSY	IFKYIIIG--	DMGVGKSCLL	HQFTEKKFMA	DCPHTIGVEF	GTRIIEVSGQ
Oryctolagus cuniculus (Rabbit)	MATAPYNYSY	IFKYIIIG--	DMGVGKSCLL	HQFTEKKFMA	DCPHTIGVEF	GTRIIEVSGQ
Capra hircus (Goat)	MATAPYNYSY	IFKYIIIG--	DMGVGKSCLL	HQFTEKKFMA	DCPHTIGVEF	GTRIIEVSGQ
Ailuropoda mel...a (Giant panda)	MATAPYNYSY	IFKYIIIETG	DMGVGKSCLL	HQFTEKKFMA	DCPHTIGVEF	GTRIIEVSGQ
Cavia porcellus (Guinea pig)	MATAPYNYSY	IFKYIIIG--	DMGVGKSCLL	HQFTEKKFMA	DCPHTIGVEF	GTRIIEVSGQ
Ovis aries (Sheep)	MATAPYNYSY	IFKYIIIG--	DMGVGKSCLL	HQFTEKKFMA	DCPHTIGVEF	GTRIIEVSGQ
Mustela putori...omestic ferret)	MATAPYNYSY	IFKYIIIG--	DMGVGKSCLL	HQFTEKKFMA	DCPHTIGVEF	GTRIIEVSGQ
Papio anubis (Olive baboon)	MATAPYNYSY	IFKYIIIG--	DMGVGKSCLL	HQFTEKKFMA	DCPHTIGVEF	GTRIIEVSGQ
Ictidomys trid...round squirrel)	MATAPYNYSY	IFKYIIIG--	DMGVGKSCLL	HQFTEKKFMA	DCPHTIGVEF	GTRIIEVSGQ
Myotis lucifug...ttle brown bat)	MATAPYNYSY	IFKYIIIG--	DMGVGKSCLL	HQFTEKKFMA	DCPHTIGVEF	GTRIIEVSGQ
Pelodiscus sin...ftshell turtle)	MATAPYNYSY	IFKYIIIG--	DMGVGKSCLL	HQFTEKKFMA	DCPHTIGVEF	GTRIIEVSGQ
Sarcophilus ha...asmanian devil)	MATAPYNYSY	IFKYIIIG--	DMGVGKSCLL	HQFTEKKFMA	DCPHTIGVEF	GTRIIEVSGQ
Nomascus leuco...cheeked gibbon)	MATAPYNYSY	IFKYIIIG--	DMGVGKSCLL	HQFTEKKFMA	DCPHTIGVEF	GTRIIEVSGQ
Anolis carolin...ican chameleon)	MATAPYNYSY	IFKYIIIG--	DMGVGKSCLL	HQFTEKKFMA	DCPHTIGVEF	GTRIIEVSGQ
Monodelphis do...tailed opossum)	MATAPYNYSY	IFKYIIIG--	DMGVGKSCLL	HQFTEKKFMA	DCPHTIGVEF	GTRIIEVSGQ
Bos taurus (Bovine)	MATAPYNYSY	IFKYIIIG--	DMGVGKSCLL	HQFTEKKFMA	DCPHTIGVEF	GTRIIEVSGQ
Gorilla gorilla gorilla	MATAPYNYSY	IFKYIIIG--	DMGVGKSCLL	HQFTEKKFMA	DCPHTIGVEF	GTRIIEVSGQ
Felis catus (Cat)	MATAPYNYSY	IFKYIIIG--	DMGVGKSCLL	HQFTEKKFMA	DCPHTIGVEF	GTRIIEVSGQ
Pongo abelli (...tran orangutan)	MATAPYNYSY	IFKYIIIG--	DMGVGKSCLL	HQFTEKKFMA	DCPHTIGVEF	GTRIIEVSGQ
Gallus gallus (Chicken)	MATAPYNYSY	IFKYIIIG--	DMGVGKSCLL	HQFTEKKFMA	DCPHTIGVEF	GTRIIEVSGQ
Danio rerio (Zebrafish)	MTTAPYNYSY	IFKYIIIG--	DMGVGKSCLL	HQFTEKKFMA	DCPHTIGVEF	GTRIIEVSGQ
Caenorhabditis elegans	MTAAPYNYSY	IFKYIIIG--	DMGVGKSCLL	HQFTEKKFMA	DCPHTIGVEF	GTRIIEVSGQ
Drosophila mel...ter (Fruit fly)	MTAAPYNYNY	IFKYIIIG--	DMGVGKSCLL	HQFTEKKFMA	NCPHTIGVEF	GTRIIEVDDK
Dictyostelium ...um (Slime mold)	---MSFPYEY	IFKYIIIG--	DMGVGKSCLL	HQFTENKFVP	DSPHTIGVEF	GTRIVDVNNK
Secondary Structure		ß1	α1			ß2

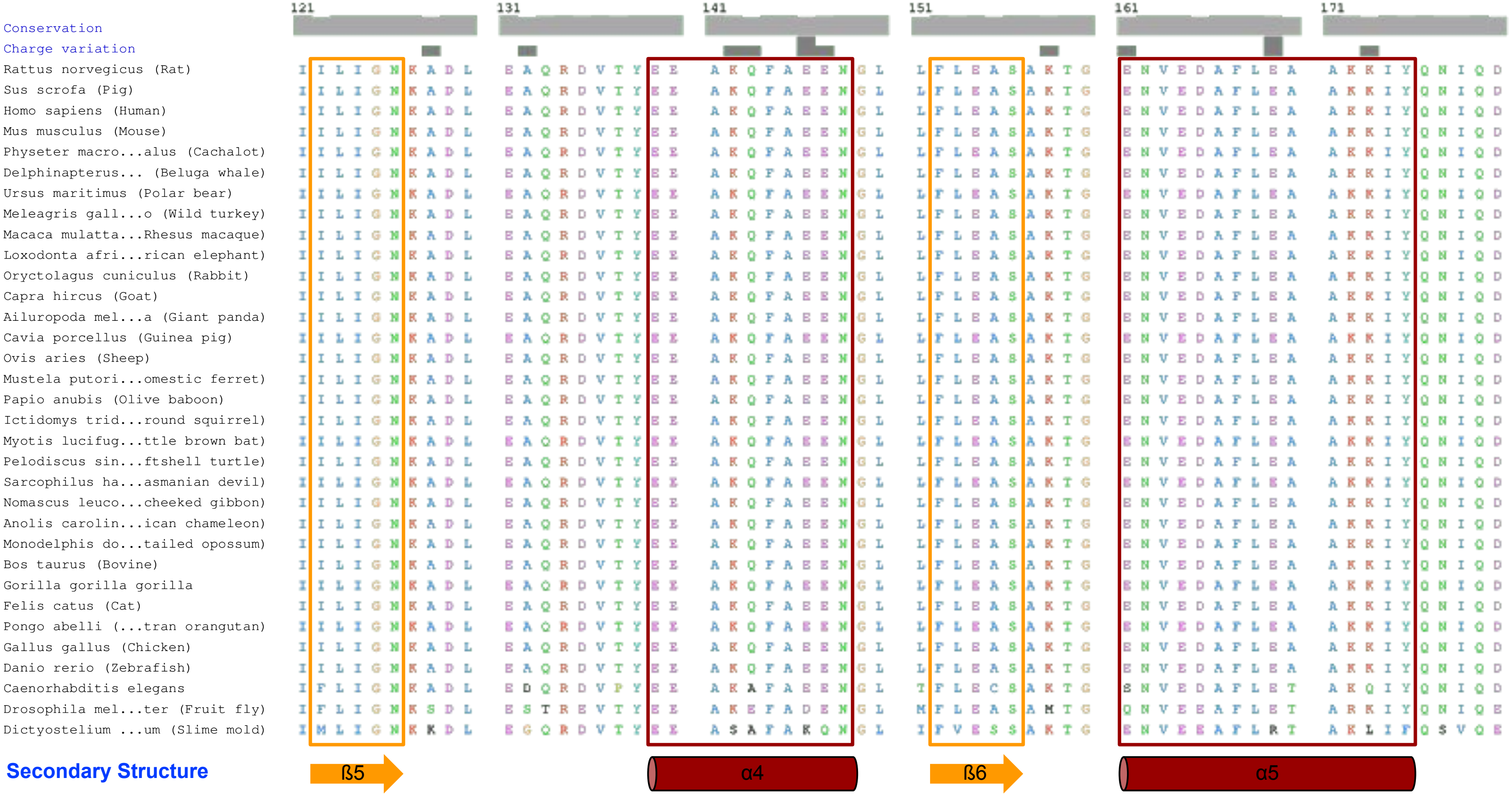
MULTIPLE SEQUENCE ALIGNMENT (MSA) OF RAB14 PROTEINS



	61	71	81	91	101	111
Conservation						
Charge variation						
Rattus norvegicus (Rat)	KIKLQIWDTA	GQERFRAVTR	SYYRGAAGAL	MVYDITRRST	YNHLSSWLTD	ARNLTNPNTV
Sus scrofa (Pig)	KIKLQIWDTA	GQERFRAVTR	SYYRGAAGAL	MVYDITRRST	YNHLSSWLTD	ARNLTNPNTV
Homo sapiens (Human)	KIKLQIWDTA	GQERFRAVTR	SYYRGAAGAL	MVYDITRRST	YNHLSSWLTD	ARNLTNPNTV
Mus musculus (Mouse)	KIKLQIWDTA	GQERFRAVTR	SYYRGAAGAL	MVYDITRRST	YNHLSSWLTD	ARNLTNPNTV
Physeter macro...alus (Cachalot)	KIKLQIWDTA	GQERFRAVTR	SYYRGAAGAL	MVYDITRRST	YNHLSSWLTD	ARNLTNPNTV
Delphinapterus... (Beluga whale)	KIKLQIWDTA	GQERFRAVTR	SYYRGAAGAL	MVYDITRRST	YNHLSSWLTD	ARNLTNPNTV
Ursus maritimus (Polar bear)	KIKLQIWDTA	GQERFRAVTR	SYYRGAAGAL	MVYDITRRST	YNHLSSWLTD	ARNLTNPNTV
Meleagris gall...o (Wild turkey)	KIKLQIWDTA	GQERFRAVTR	SYYRGAAGAL	MVYDITRRST	YNHLSSWLTD	ARNLTNPNTV
Macaca mulatta...Rhesus macaque)	KIKLQIWDTA	GQERFRAVTR	SYYRGAAGAL	MVYDITRRST	YNHLSSWLTD	ARNLTNPNTV
Loxodonta afri...rican elephant)	KIKLQIWDTA	GQERFRAVTR	SYYRGAAGAL	MVYDITRRST	YNHLSSWLTD	ARNLTNPNTV
Oryctolagus cuniculus (Rabbit)	KIKLQIWDTA	GQERFRAVTR	SYYRGAAGAL	MVYDITRRST	YNHLSSWLTD	ARNLTNPNTV
Capra hircus (Goat)	KIKLQIWDTA	GQERFRAVTR	SYYRGAAGAL	MVYDITRRST	YNHLSSWLTD	ARNLTNPNTV
Ailuropoda mel...a (Giant panda)	KIKLQIWDTA	GQERFRAVTR	SYYRGAAGAL	MVYDITRRST	YNHLSSWLTD	ARNLTNPNTV
Cavia porcellus (Guinea pig)	KIKLQIWDTA	GQERFRAVTR	SYYRGAAGAL	MVYDITRRST	YNHLSSWLTD	ARNLTNPNTV
Ovis aries (Sheep)	KIKLQIWDTA	GQERFRAVTR	SYYRGAAGAL	MVYDITRRST	YNHLSSWLTD	ARNLTNPNTV
Mustela putori...omestic ferret)	KIKLQIWDTA	GQERFRAVTR	SYYRGAAGAL	MVYDITRRST	YNHLSSWLTD	ARNLTNPNTV
Papio anubis (Olive baboon)	KIKLQIWDTA	GQERFRAVTR	SYYRGAAGAL	MVYDITRRST	YNHLSSWLTD	ARNLTNPNTV
Ictidomys trid...round squirrel)	KIKLQIWDTA	GQERFRAVTR	SYYRGAAGAL	MVYDITRRST	YNHLSSWLTD	ARNLTNPNTV
Myotis lucifug...ttle brown bat)	KIKLQIWDTA	GQERFRAVTR	SYYRGAAGAL	MVYDITRRST	YNHLSSWLTD	ARNLTNPNTV
Pelodiscus sin...ftshell turtle)	KIKLQIWDTA	GQERFRAVTR	SYYRGAAGAL	MVYDITRRST	YNHLSSWLTD	ARNLTNPNTV
Sarcophilus ha...asmanian devil)	KIKLQIWDTA	GQERFRAVTR	SYYRGAAGAL	MVYDITRRST	YNHLSSWLTD	ARNLTNPNTV
Nomascus leuco...cheeked gibbon)	KIKLQIWDTA	GQERFRAVTR	SYYRGAAGAL	MVYDITRRST	YNHLSSWLTD	ARNLTNPNTV
Anolis carolin...ican chameleon)	KIKLQIWDTA	GQERFRAVTR	SYYRGAAGAL	MVYDITRRST	YNHLSSWLTD	ARNLTNPNTV
Monodelphis do...tailed opossum)	KIKLQIWDTA	GQERFRAVTR	SYYRGAAGAL	MVYDITRRST	YNHLSSWLTD	ARNLTNPNTV
Bos taurus (Bovine)	KIKLQIWDTA	GQERFRAVTR	SYYRGAAGAL	MVYDITRRST	YNHLSSWLTD	ARNLTNPNTV
Gorilla gorilla gorilla	KIKLQIWDTA	GQERFRAVTR	SYYRGAAGAL	MVYDITRRST	YNHLSSWLTD	ARNLTNPNTV
Felis catus (Cat)	KIKLQIWDTA	GQERFRAVTR	SYYRGAAGAL	MVYDITRRST	YNHLSSWLTD	ARNLTNPNTV
Pongo abelli (...tran orangutan)	KIKLQIWDTA	GQERFRAVTR	SYYRGAAGAL	MVYDITRRST	YNHLSSWLTD	ARNLTNPNTV
Gallus gallus (Chicken)	KIKLQIWDTA	GQERFRAVTR	SYYRGAAGAL	MVYDITRRST	YNHLSSWLTD	ARNLTNPNTV
Danio rerio (Zebrafish)	KVKLQIWDTA	GQERFRAVTR	SYYRGAAGAL	MVYDITRRST	YNHLSSWLTD	ARNLTNPNTV
Caenorhabditis elegans	KIKLQIWDTA	GQERFRAVTR	SYYRGAAGAL	MVYDITRRST	YNHLSSWLAD	AKSLTNPNTA
Drosophila mel...ter (Fruit fly)	KIKLQIWDTA	GQERFRAVTR	SYHRGAAGAL	MVYDITRRST	YNHLSSWLTD	TRNLTNPSTV
Dictyostelium ...um (Slime mold)	KIKLQIWDTA	GQERFRAVTR	SYYRGAAGAL	LVYDITRRIT	YNHLTTWLTD	ARNLTNPNTV

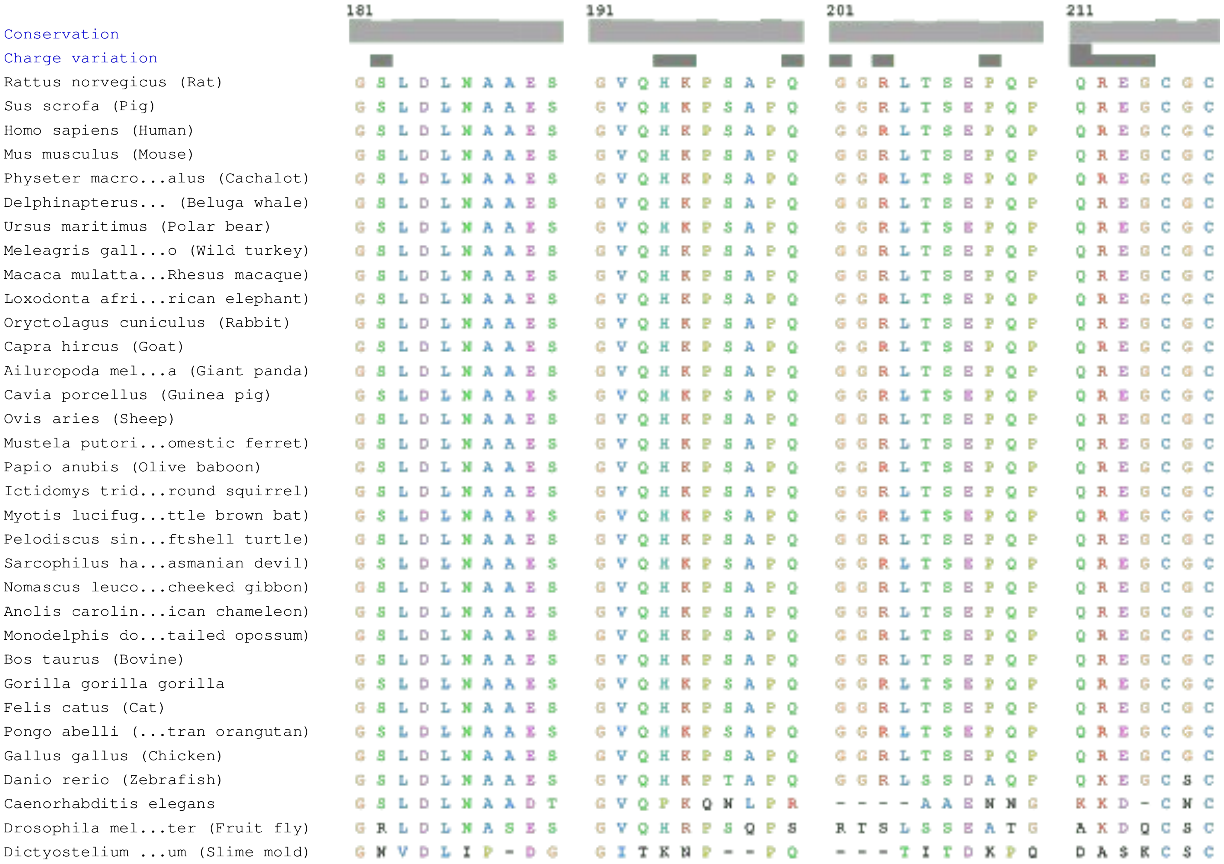
Secondary Structure

MULTIPLE SEQUENCE ALIGNMENT (MSA) OF RAB14 PROTEINS



	121	131	141	151	161	171
Rattus norvegicus (Rat)	I I L I G N K A D L	E A Q R D V T Y E E	A K Q F A E E N G L	L F L E A S A K T G	E N V E D A F L E A	A K K I Y Q N I Q D
Sus scrofa (Pig)	I I L I G N K A D L	E A Q R D V T Y E E	A K Q F A E E N G L	L F L E A S A K T G	E N V E D A F L E A	A K K I Y Q N I Q D
Homo sapiens (Human)	I I L I G N K A D L	E A Q R D V T Y E E	A K Q F A E E N G L	L F L E A S A K T G	E N V E D A F L E A	A K K I Y Q N I Q D
Mus musculus (Mouse)	I I L I G N K A D L	E A Q R D V T Y E E	A K Q F A E E N G L	L F L E A S A K T G	E N V E D A F L E A	A K K I Y Q N I Q D
Physeter macro...alus (Cachalot)	I I L I G N K A D L	E A Q R D V T Y E E	A K Q F A E E N G L	L F L E A S A K T G	E N V E D A F L E A	A K K I Y Q N I Q D
Delphinapterus... (Beluga whale)	I I L I G N K A D L	E A Q R D V T Y E E	A K Q F A E E N G L	L F L E A S A K T G	E N V E D A F L E A	A K K I Y Q N I Q D
Ursus maritimus (Polar bear)	I I L I G N K A D L	E A Q R D V T Y E E	A K Q F A E E N G L	L F L E A S A K T G	E N V E D A F L E A	A K K I Y Q N I Q D
Meleagris gall...o (Wild turkey)	I I L I G N K A D L	E A Q R D V T Y E E	A K Q F A E E N G L	L F L E A S A K T G	E N V E D A F L E A	A K K I Y Q N I Q D
Macaca mulatta...Rhesus macaque)	I I L I G N K A D L	E A Q R D V T Y E E	A K Q F A E E N G L	L F L E A S A K T G	E N V E D A F L E A	A K K I Y Q N I Q D
Loxodonta afri...rican elephant)	I I L I G N K A D L	E A Q R D V T Y E E	A K Q F A E E N G L	L F L E A S A K T G	E N V E D A F L E A	A K K I Y Q N I Q D
Oryctolagus cuniculus (Rabbit)	I I L I G N K A D L	E A Q R D V T Y E E	A K Q F A E E N G L	L F L E A S A K T G	E N V E D A F L E A	A K K I Y Q N I Q D
Capra hircus (Goat)	I I L I G N K A D L	E A Q R D V T Y E E	A K Q F A E E N G L	L F L E A S A K T G	E N V E D A F L E A	A K K I Y Q N I Q D
Ailuropoda mel...a (Giant panda)	I I L I G N K A D L	E A Q R D V T Y E E	A K Q F A E E N G L	L F L E A S A K T G	E N V E D A F L E A	A K K I Y Q N I Q D
Cavia porcellus (Guinea pig)	I I L I G N K A D L	E A Q R D V T Y E E	A K Q F A E E N G L	L F L E A S A K T G	E N V E D A F L E A	A K K I Y Q N I Q D
Ovis aries (Sheep)	I I L I G N K A D L	E A Q R D V T Y E E	A K Q F A E E N G L	L F L E A S A K T G	E N V E D A F L E A	A K K I Y Q N I Q D
Mustela putori...omestic ferret)	I I L I G N K A D L	E A Q R D V T Y E E	A K Q F A E E N G L	L F L E A S A K T G	E N V E D A F L E A	A K K I Y Q N I Q D
Papio anubis (Olive baboon)	I I L I G N K A D L	E A Q R D V T Y E E	A K Q F A E E N G L	L F L E A S A K T G	E N V E D A F L E A	A K K I Y Q N I Q D
Ictidomys trid...round squirrel)	I I L I G N K A D L	E A Q R D V T Y E E	A K Q F A E E N G L	L F L E A S A K T G	E N V E D A F L E A	A K K I Y Q N I Q D
Myotis lucifug...ttle brown bat)	I I L I G N K A D L	E A Q R D V T Y E E	A K Q F A E E N G L	L F L E A S A K T G	E N V E D A F L E A	A K K I Y Q N I Q D
Pelodiscus sin...ftshell turtle)	I I L I G N K A D L	E A Q R D V T Y E E	A K Q F A E E N G L	L F L E A S A K T G	E N V E D A F L E A	A K K I Y Q N I Q D
Sarcophilus ha...asmanian devil)	I I L I G N K A D L	E A Q R D V T Y E E	A K Q F A E E N G L	L F L E A S A K T G	E N V E D A F L E A	A K K I Y Q N I Q D
Nomascus leuco...cheeked gibbon)	I I L I G N K A D L	E A Q R D V T Y E E	A K Q F A E E N G L	L F L E A S A K T G	E N V E D A F L E A	A K K I Y Q N I Q D
Anolis carolin...ican chameleon)	I I L I G N K A D L	E A Q R D V T Y E E	A K Q F A E E N G L	L F L E A S A K T G	E N V E D A F L E A	A K K I Y Q N I Q D
Monodelphis do...tailed opossum)	I I L I G N K A D L	E A Q R D V T Y E E	A K Q F A E E N G L	L F L E A S A K T G	E N V E D A F L E A	A K K I Y Q N I Q D
Bos taurus (Bovine)	I I L I G N K A D L	E A Q R D V T Y E E	A K Q F A E E N G L	L F L E A S A K T G	E N V E D A F L E A	A K K I Y Q N I Q D
Gorilla gorilla gorilla	I I L I G N K A D L	E A Q R D V T Y E E	A K Q F A E E N G L	L F L E A S A K T G	E N V E D A F L E A	A K K I Y Q N I Q D
Felis catus (Cat)	I I L I G N K A D L	E A Q R D V T Y E E	A K Q F A E E N G L	L F L E A S A K T G	E N V E D A F L E A	A K K I Y Q N I Q D
Pongo abelli (...tran orangutan)	I I L I G N K A D L	E A Q R D V T Y E E	A K Q F A E E N G L	L F L E A S A K T G	E N V E D A F L E A	A K K I Y Q N I Q D
Gallus gallus (Chicken)	I I L I G N K A D L	E A Q R D V T Y E E	A K Q F A E E N G L	L F L E A S A K T G	E N V E D A F L E A	A K K I Y Q N I Q D
Danio rerio (Zebrafish)	I I L I G N K A D L	E A Q R D V T Y E E	A K Q F A E E N G L	L F L E A S A K T G	E N V E D A F L E A	A K K I Y Q N I Q D
Caenorhabditis elegans	I F L I G N K A D L	E D Q R D V P Y E E	A K A F A E E N G L	T F L E C S A K T G	S N V E D A F L E T	A K Q I Y Q N I Q D
Drosophila mel...ter (Fruit fly)	I F L I G N K S D L	E S T R E V T Y E E	A K E F A D E N G L	M F L E A S A M T G	Q N V E E A F L E T	A R K I Y Q N I Q E
Dictyostelium ...um (Slime mold)	I M L I G N K K D L	E G Q R D V T Y E E	A S A F A K Q N G L	I F V E S S A K T G	E N V E E A F L R T	A K L I F Q S V Q E

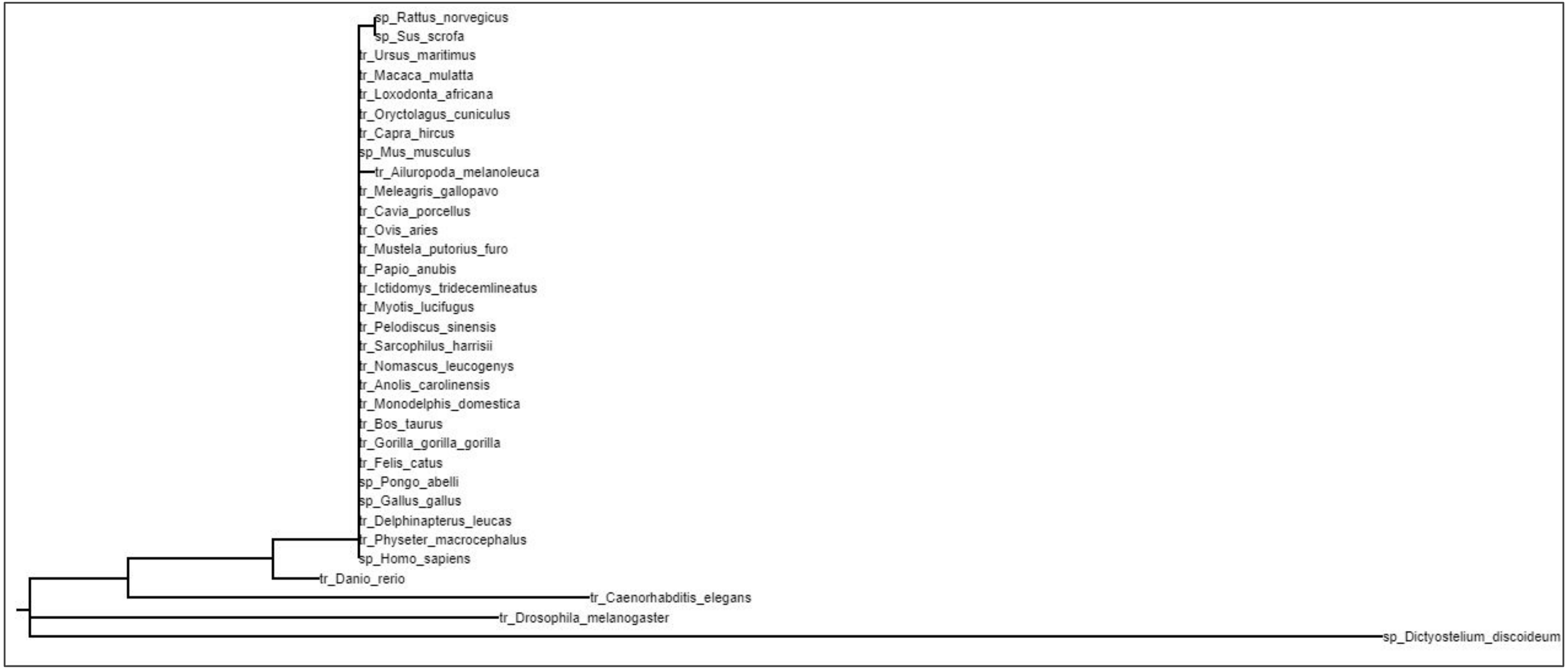
MULTIPLE SEQUENCE ALIGNMENT (MSA) OF RAB14 PROTEINS



	181	191	201	211
Conservation				
Charge variation				
Rattus norvegicus (Rat)	G S L D L N A A E S	G V Q H K P S A P Q	G G R L T S E P Q P	Q R E G C G C
Sus scrofa (Pig)	G S L D L N A A E S	G V Q H K P S A P Q	G G R L T S E P Q P	Q R E G C G C
Homo sapiens (Human)	G S L D L N A A E S	G V Q H K P S A P Q	G G R L T S E P Q P	Q R E G C G C
Mus musculus (Mouse)	G S L D L N A A E S	G V Q H K P S A P Q	G G R L T S E P Q P	Q R E G C G C
Physeter macro...alus (Cachalot)	G S L D L N A A E S	G V Q H K P S A P Q	G G R L T S E P Q P	Q R E G C G C
Delphinapterus... (Beluga whale)	G S L D L N A A E S	G V Q H K P S A P Q	G G R L T S E P Q P	Q R E G C G C
Ursus maritimus (Polar bear)	G S L D L N A A E S	G V Q H K P S A P Q	G G R L T S E P Q P	Q R E G C G C
Meleagris gall...o (Wild turkey)	G S L D L N A A E S	G V Q H K P S A P Q	G G R L T S E P Q P	Q R E G C G C
Macaca mulatta...Rhesus macaque)	G S L D L N A A E S	G V Q H K P S A P Q	G G R L T S E P Q P	Q R E G C G C
Loxodonta afri...rican elephant)	G S L D L N A A E S	G V Q H K P S A P Q	G G R L T S E P Q P	Q R E G C G C
Oryctolagus cuniculus (Rabbit)	G S L D L N A A E S	G V Q H K P S A P Q	G G R L T S E P Q P	Q R E G C G C
Capra hircus (Goat)	G S L D L N A A E S	G V Q H K P S A P Q	G G R L T S E P Q P	Q R E G C G C
Ailuropoda mel...a (Giant panda)	G S L D L N A A E S	G V Q H K P S A P Q	G G R L T S E P Q P	Q R E G C G C
Cavia porcellus (Guinea pig)	G S L D L N A A E S	G V Q H K P S A P Q	G G R L T S E P Q P	Q R E G C G C
Ovis aries (Sheep)	G S L D L N A A E S	G V Q H K P S A P Q	G G R L T S E P Q P	Q R E G C G C
Mustela putori...omestic ferret)	G S L D L N A A E S	G V Q H K P S A P Q	G G R L T S E P Q P	Q R E G C G C
Papio anubis (Olive baboon)	G S L D L N A A E S	G V Q H K P S A P Q	G G R L T S E P Q P	Q R E G C G C
Ictidomys trid...round squirrel)	G S L D L N A A E S	G V Q H K P S A P Q	G G R L T S E P Q P	Q R E G C G C
Myotis lucifug...ttle brown bat)	G S L D L N A A E S	G V Q H K P S A P Q	G G R L T S E P Q P	Q R E G C G C
Pelodiscus sin...ftshell turtle)	G S L D L N A A E S	G V Q H K P S A P Q	G G R L T S E P Q P	Q R E G C G C
Sarcophilus ha...asmanian devil)	G S L D L N A A E S	G V Q H K P S A P Q	G G R L T S E P Q P	Q R E G C G C
Nomascus leuco...cheeked gibbon)	G S L D L N A A E S	G V Q H K P S A P Q	G G R L T S E P Q P	Q R E G C G C
Anolis carolin...ican chameleon)	G S L D L N A A E S	G V Q H K P S A P Q	G G R L T S E P Q P	Q R E G C G C
Monodelphis do...tailed opossum)	G S L D L N A A E S	G V Q H K P S A P Q	G G R L T S E P Q P	Q R E G C G C
Bos taurus (Bovine)	G S L D L N A A E S	G V Q H K P S A P Q	G G R L T S E P Q P	Q R E G C G C
Gorilla gorilla gorilla	G S L D L N A A E S	G V Q H K P S A P Q	G G R L T S E P Q P	Q R E G C G C
Felis catus (Cat)	G S L D L N A A E S	G V Q H K P S A P Q	G G R L T S E P Q P	Q R E G C G C
Pongo abelli (...tran orangutan)	G S L D L N A A E S	G V Q H K P S A P Q	G G R L T S E P Q P	Q R E G C G C
Gallus gallus (Chicken)	G S L D L N A A E S	G V Q H K P S A P Q	G G R L T S E P Q P	Q R E G C G C
Danio rerio (Zebrafish)	G S L D L N A A E S	G V Q H K P T A P Q	G G R L S S D A Q P	Q K E G C S C
Caenorhabditis elegans	G S L D L N A A D T	G V Q P K Q N L P R	- - - - A A E N N G	K K D - C N C
Drosophila mel...ter (Fruit fly)	G R L D L N A S E S	G V Q H R P S Q P S	R T S L S S E A T G	A K D Q C S C
Dictyostelium ...um (Slime mold)	G N V D L I P - D G	G I T K N P - - P Q	- - - T I T D K P Q	D A S K C S C

Secondary Structure

EVOLUTION: DENDROGRAM OF RAB14 PROTEINS



Own source

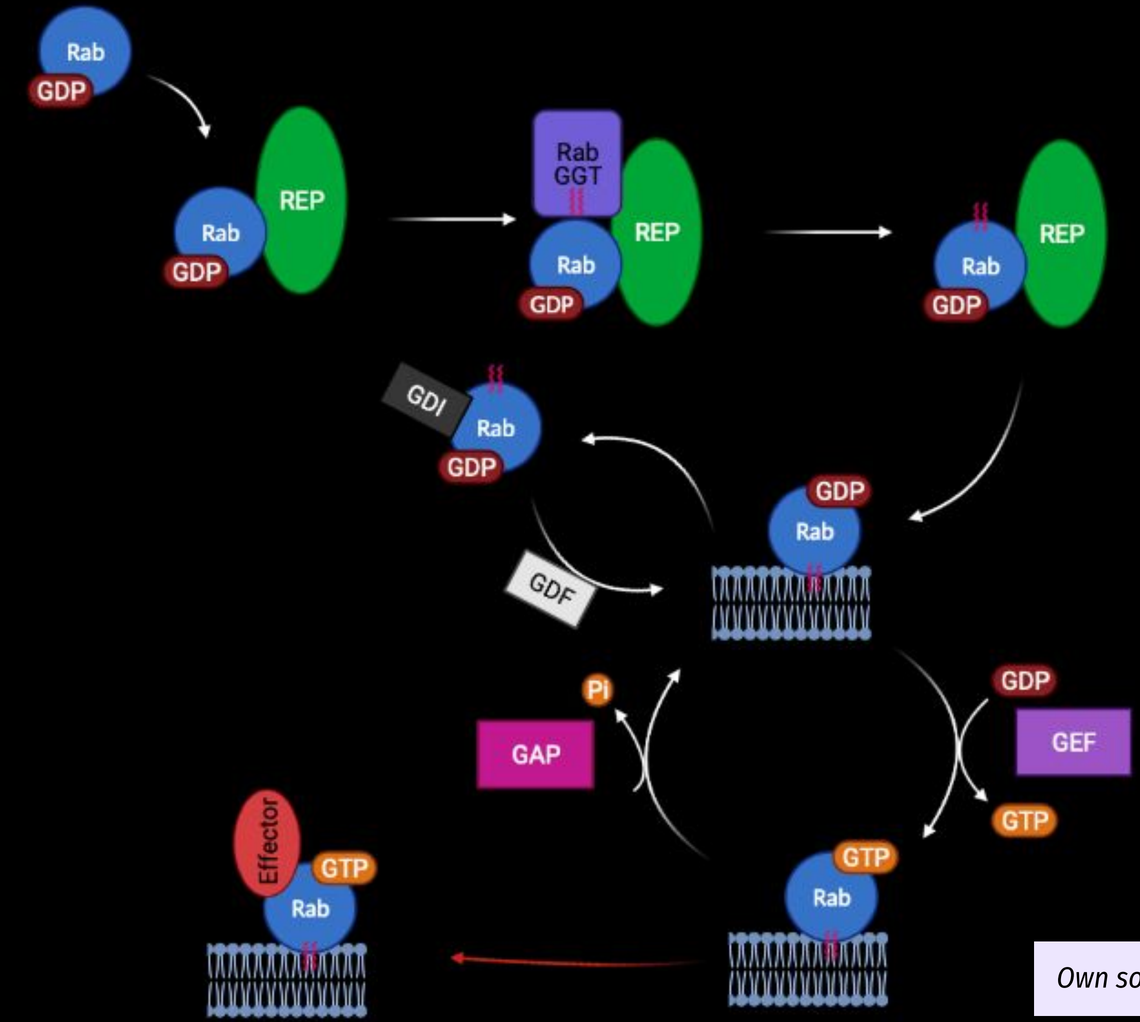
FUNCTION OF RAB PROTEINS

They regulate intracellular membrane trafficking by orchestrating the **biogenesis, transport, tethering, and fusion of membrane-bound organelles and vesicles**.

TWO STATES

- **Active state** (GTP-loaded)
- **Inactive state** (GDP-loaded)

Rab GTPase cycle



Own source

INTRODUCTION
(G PROTEINS)

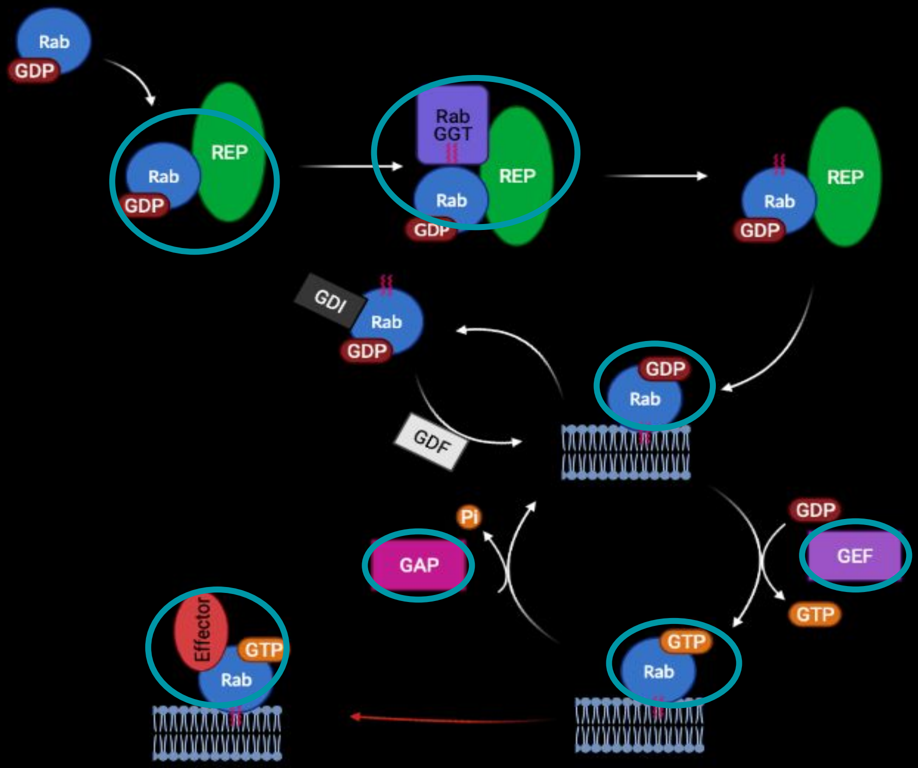
RAB FAMILY
- Introduction + Structure
- Evolution
- INTERACTIONS

TAKE HOME MESSAGES

BIBLIOGRAPHY

RAB FAMILY : INTERACTIONS

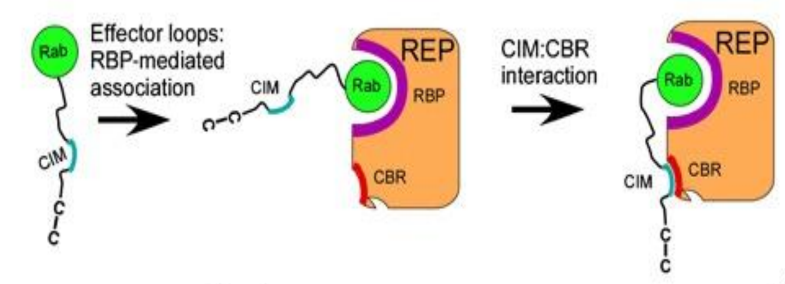
- Rab- REP
- REB- Rab- RabGGTase
- Rab- GDP/GTP
- Rab- GEF
- Rab-GAP
- Effectors



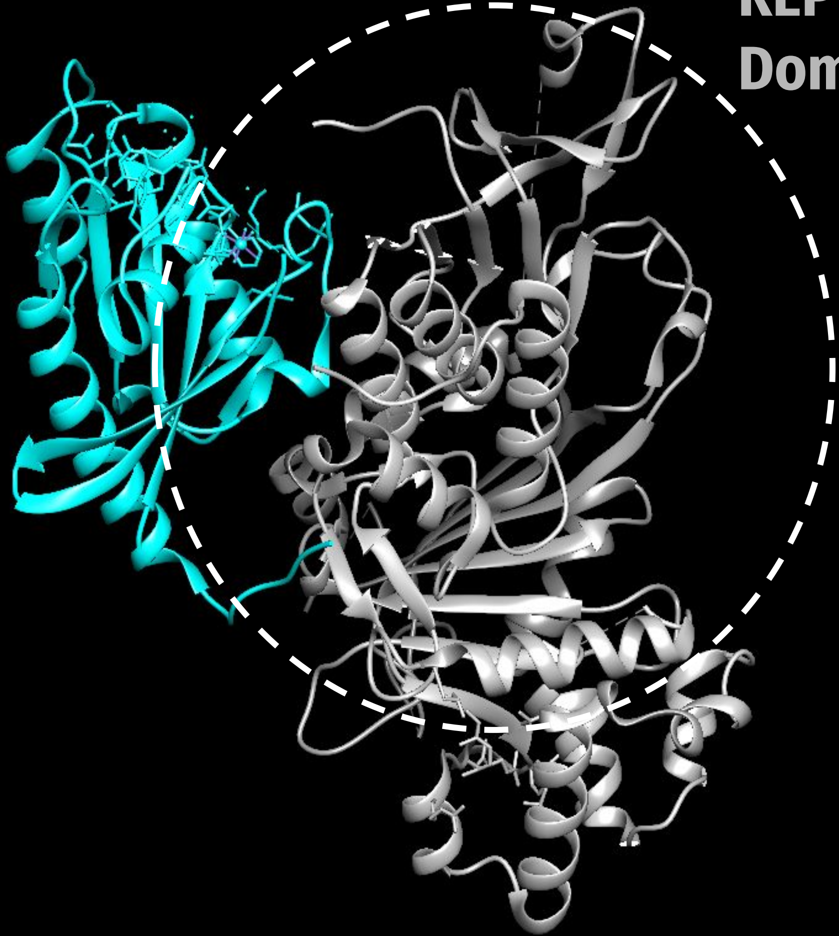
Rab7:REP1 INTERFACE

Rab

REP 1



Mechanistic model of RabGGTase-mediated protein prenylation. Guo Z, Wu Y, Das D, Delon C, Cramer J, Yu S et al. Structures of RabGGTase–substrate/product complexes provide insights into the evolution of protein prenylation. *EMBO J*. 2008; 27(18): 2444-2456.



REP 1 Domain I

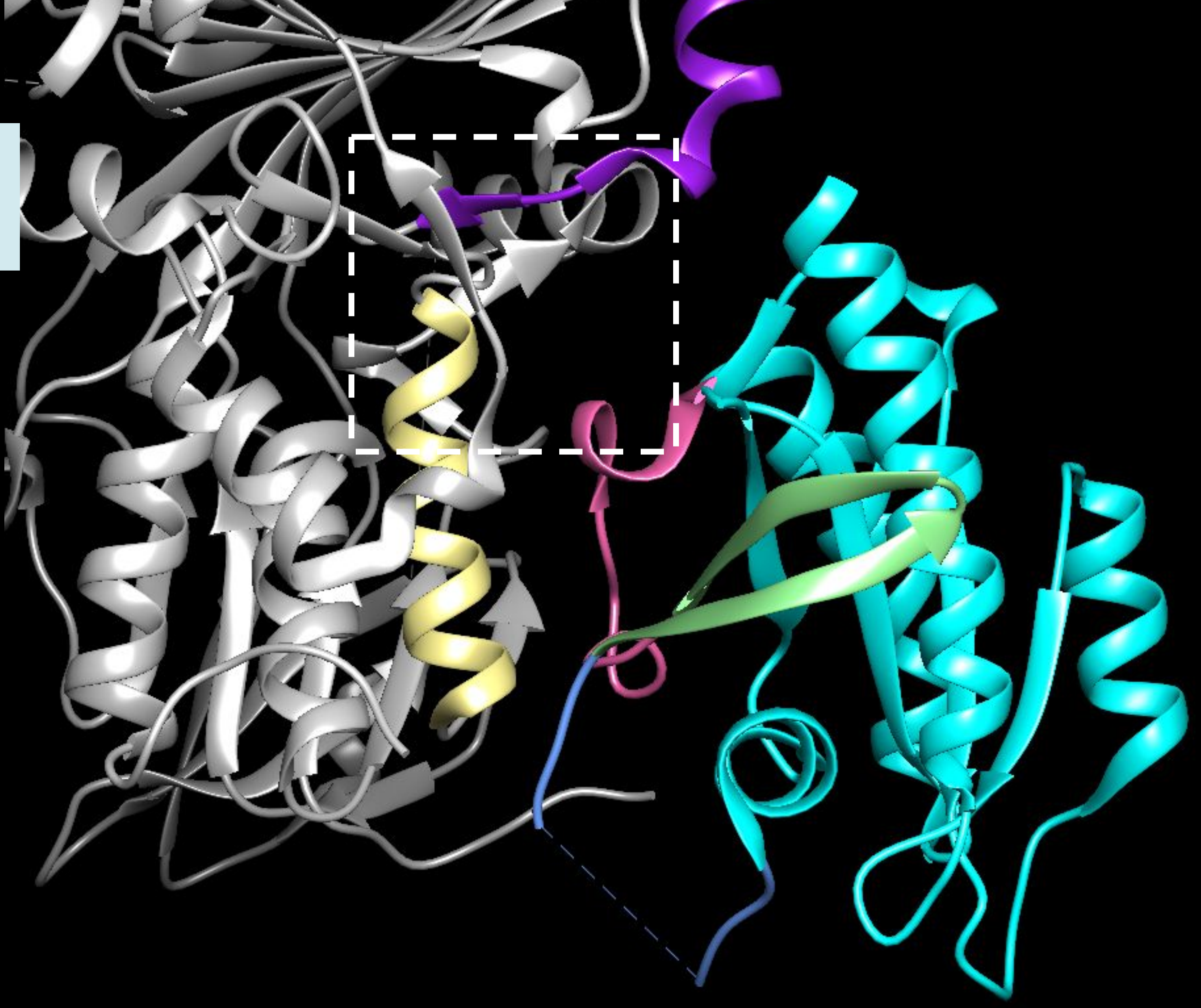
RAB7: REP1 INTERFACE

RAP7

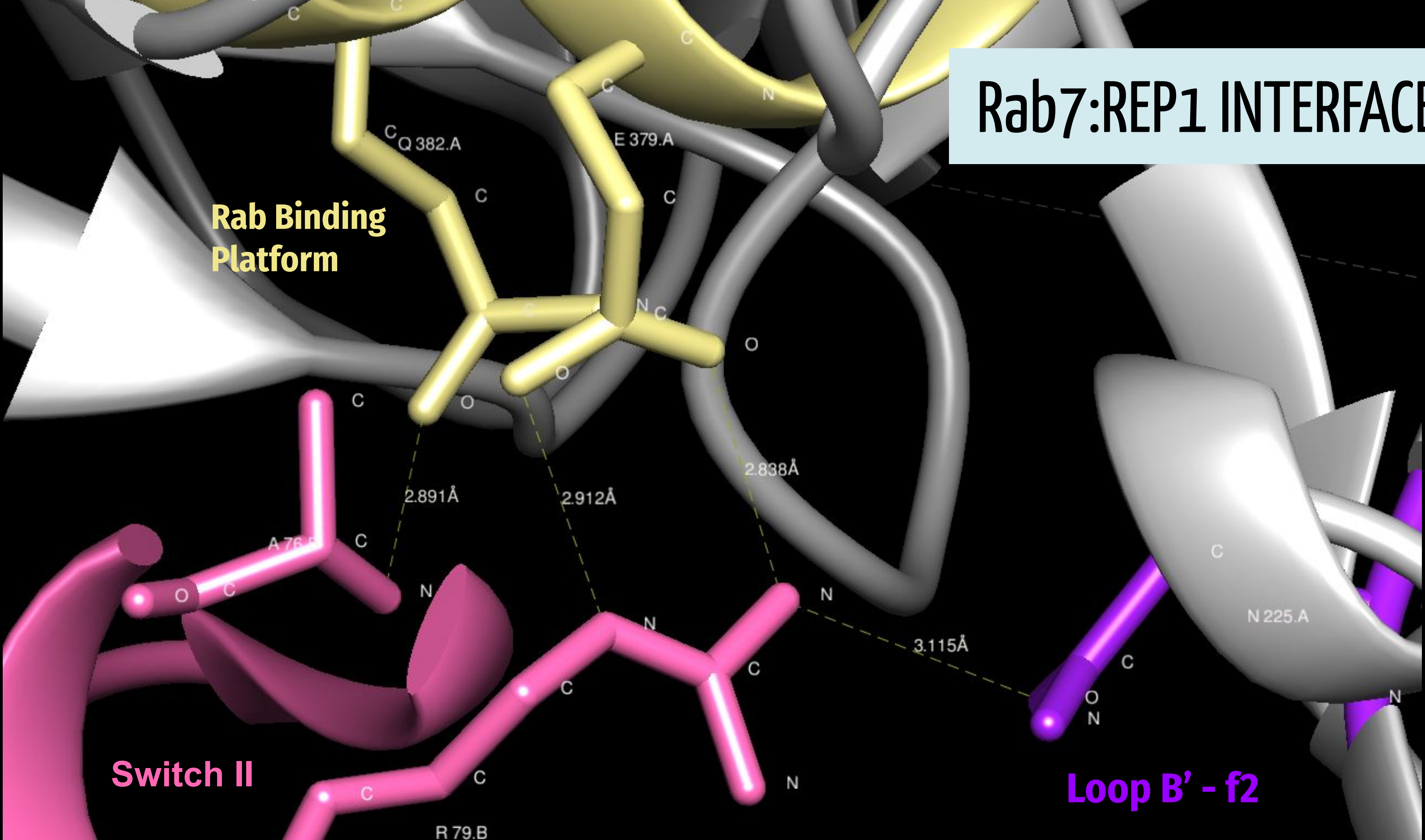
🟦 Switch I
🟥 Switch II
🟩 Interswitch

REP1

🟨 Rab Binding Platform
🟪 Loop B' - f2



Rab7:REP1 INTERFACE



Rab7:REP1 INTERFACE

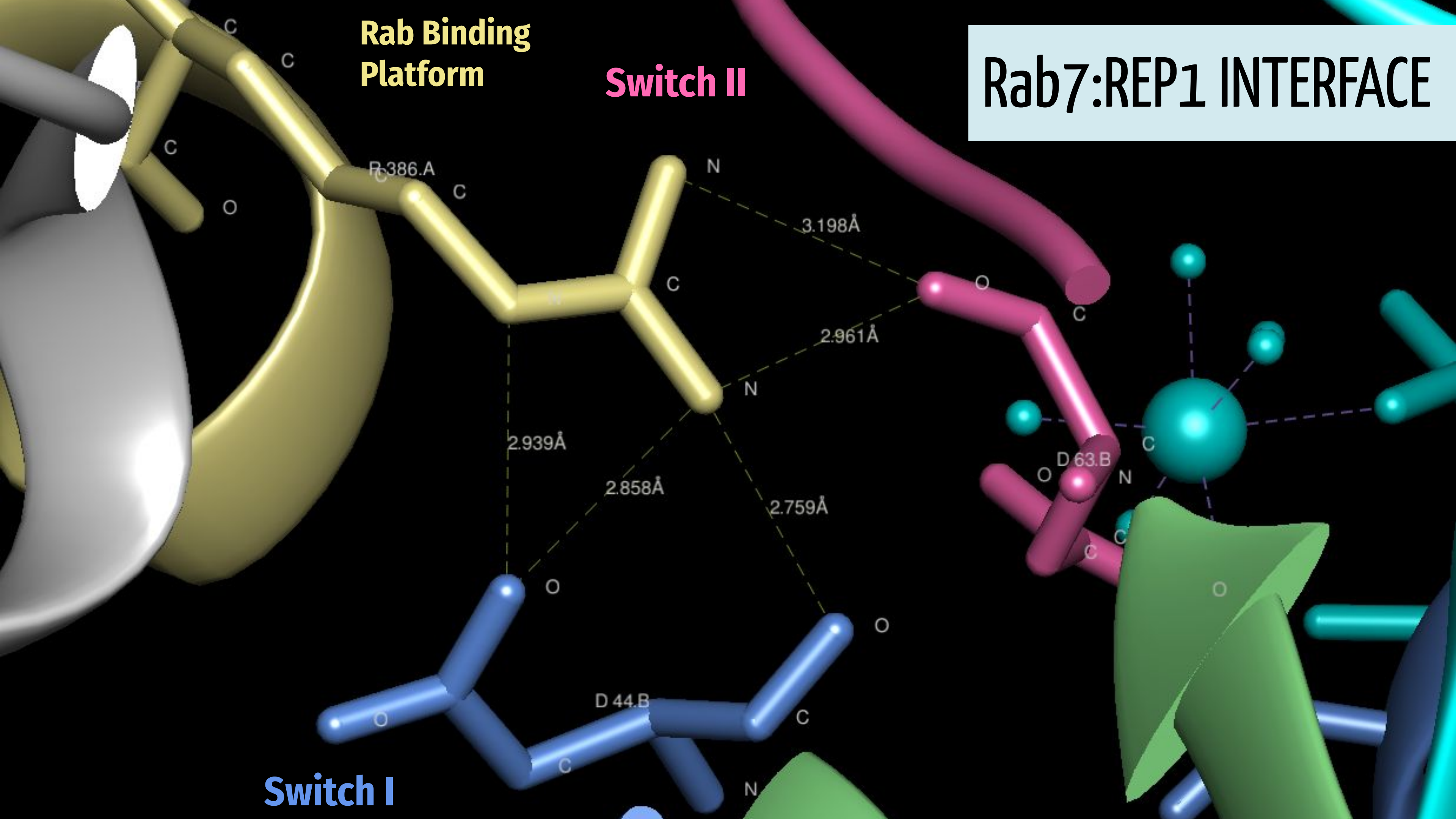
RAP7

- 🟦 Switch I
- 🟥 Switch II
- 🟩 Interswitch

REP1

- 🟨 Rab Binding Platform
- 🟪 Loop B’ - f2

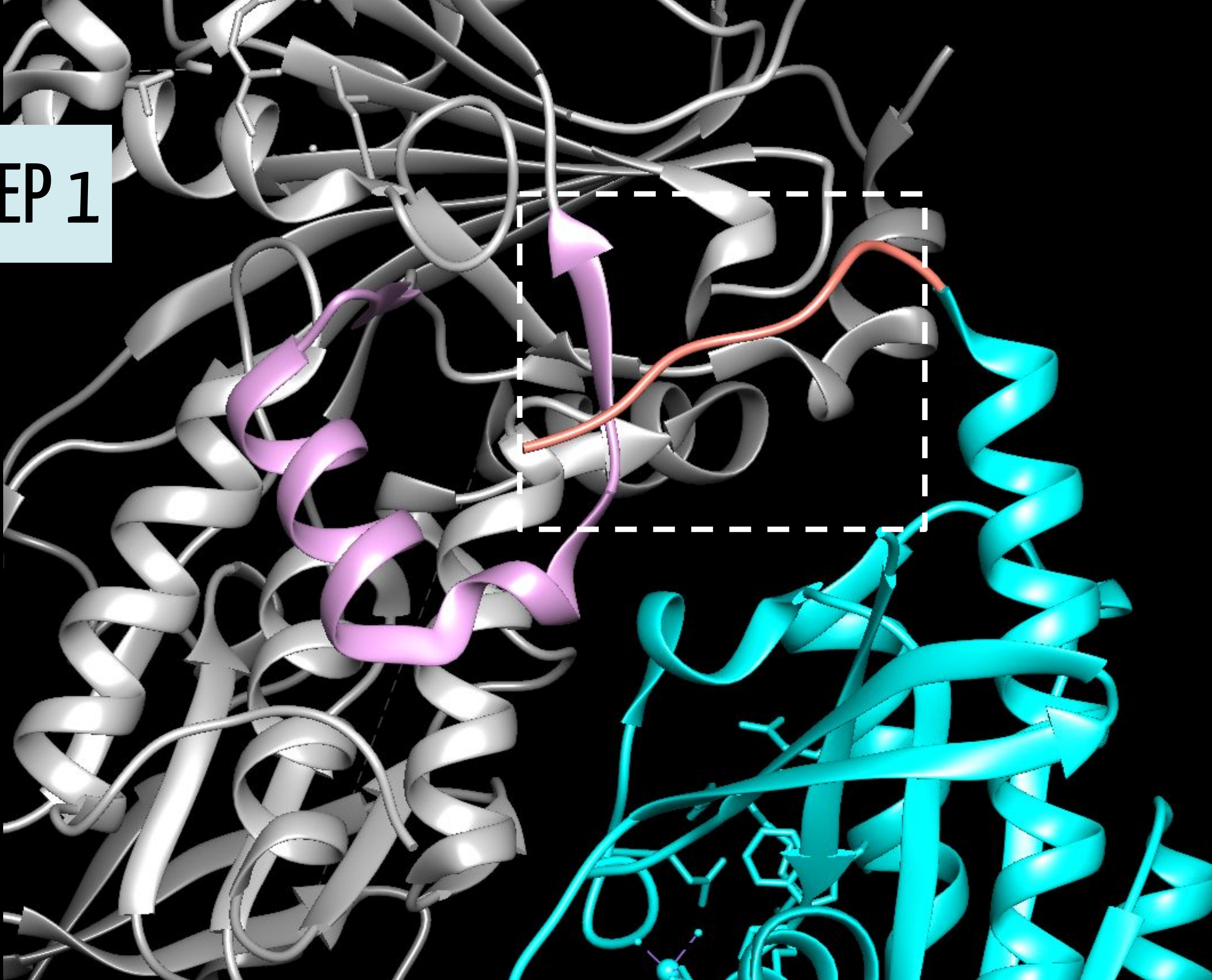
Rab7:REP1 INTERFACE



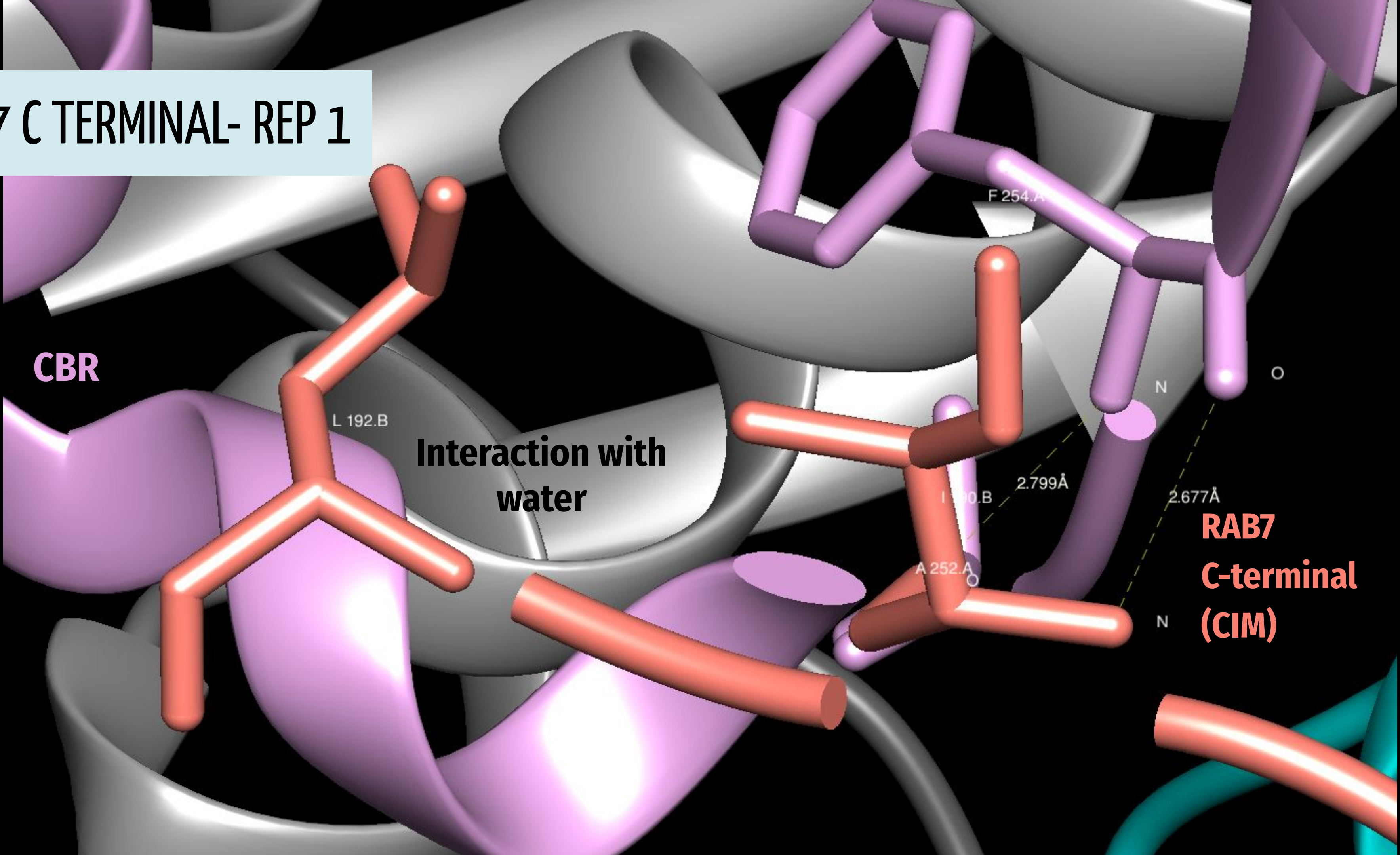
RAB7 C TERMINAL- REP 1

RAB7 C terminal (CIM)

REP1 C-terminus binding region (CBR)



RAB7 C TERMINAL- REP 1

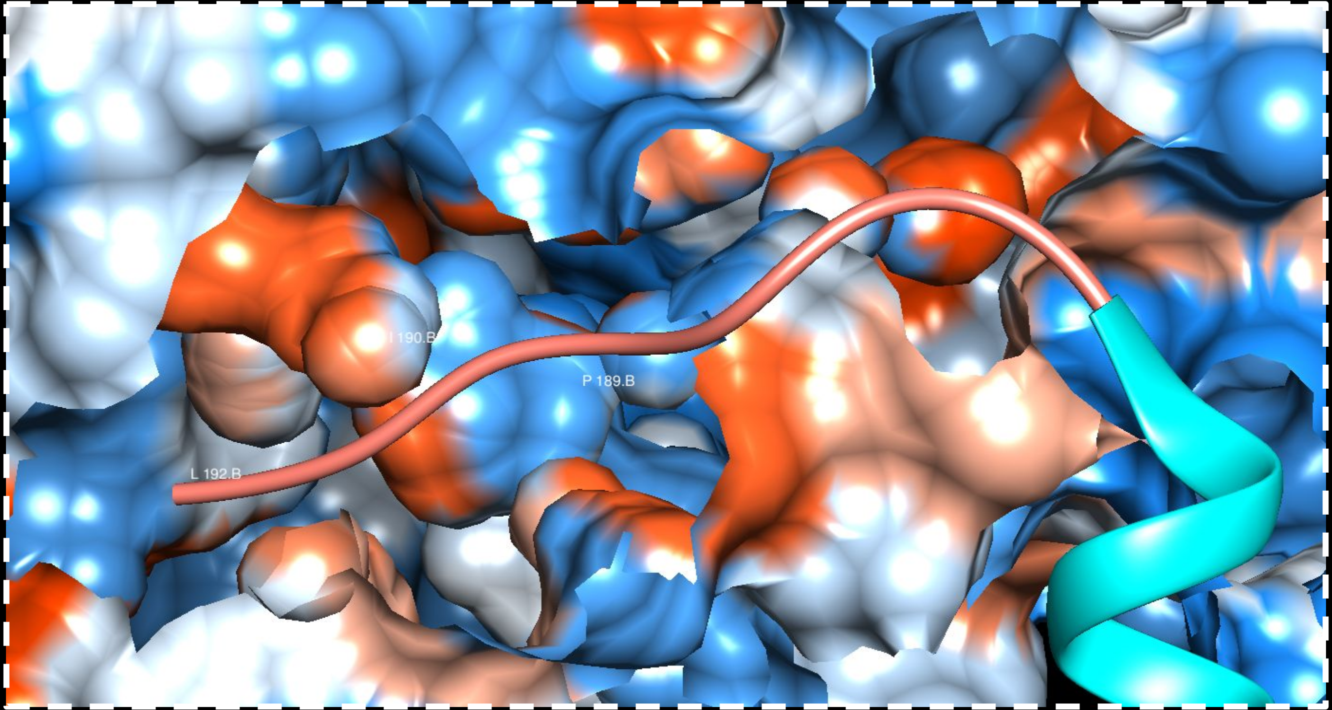


CBR

Interaction with water

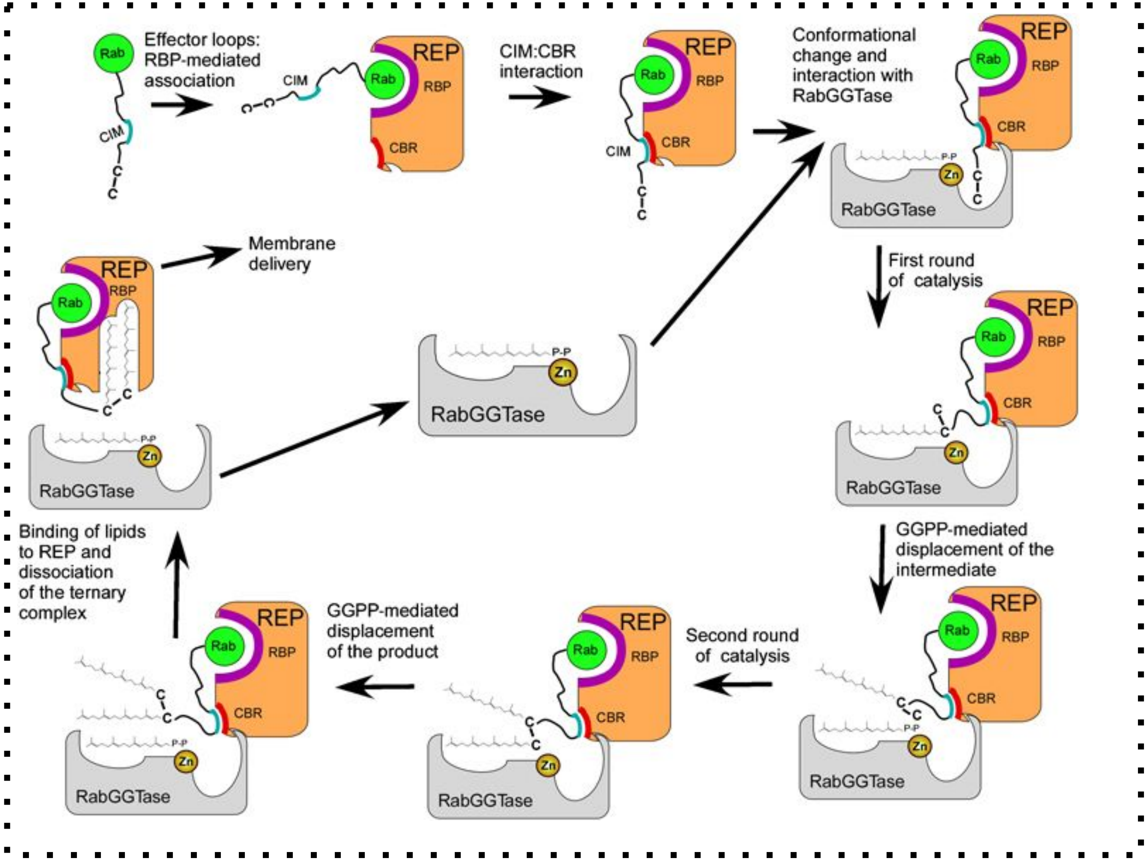
RAB7 C-terminal (CIM)

RAB7 C TERMINAL - REP 1



Rab C terminal (CIM) → I K L

REP - Rab- RabGGTase



Mechanistic model of RabGGTase-mediated protein prenylation. Guo Z, Wu Y, Das D, Delon C, Cramer J, Yu S et al. Structures of RabGGTase–substrate/product complexes provide insights into the evolution of protein prenylation. *EMBO J*. 2008; 27(18): 2444-2456.

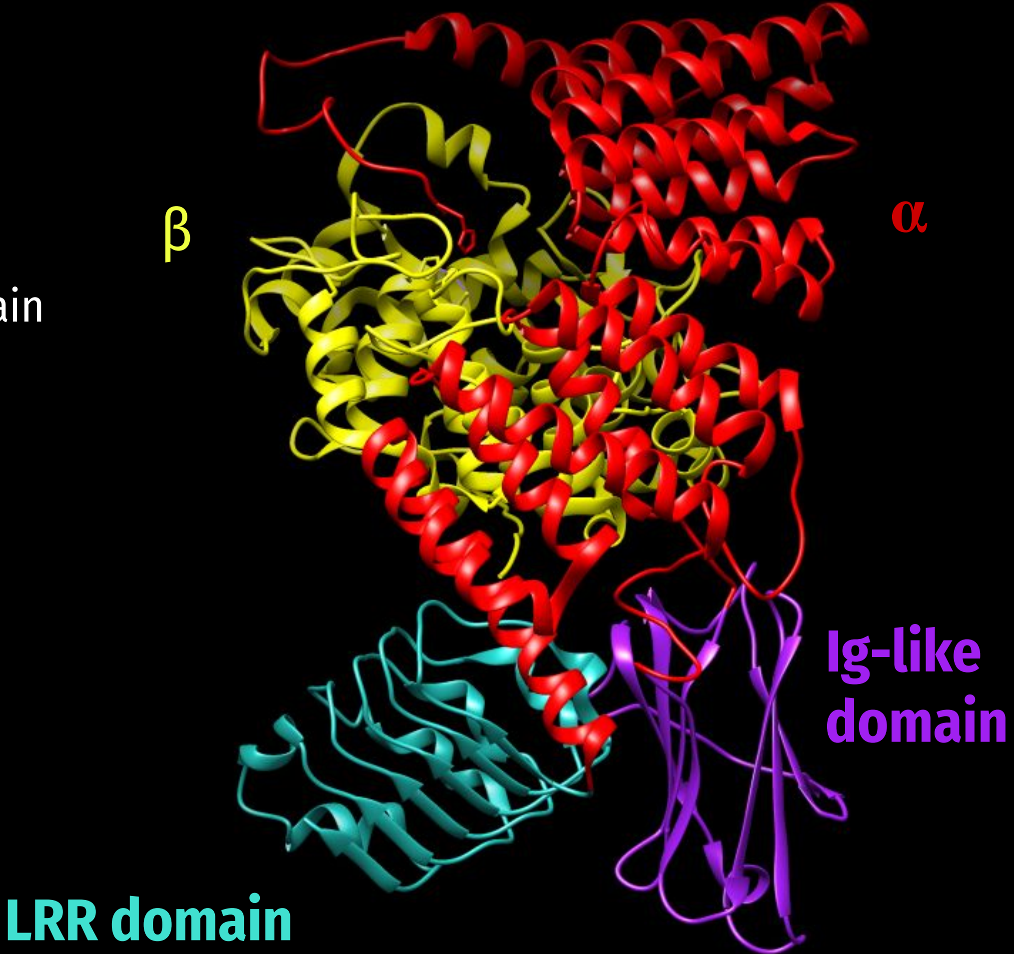
RabGGTase

Alpha subunit: interacts with REP II domain

- LRR domain
- Ig-like domain
- His2: coordinates a zinc ion

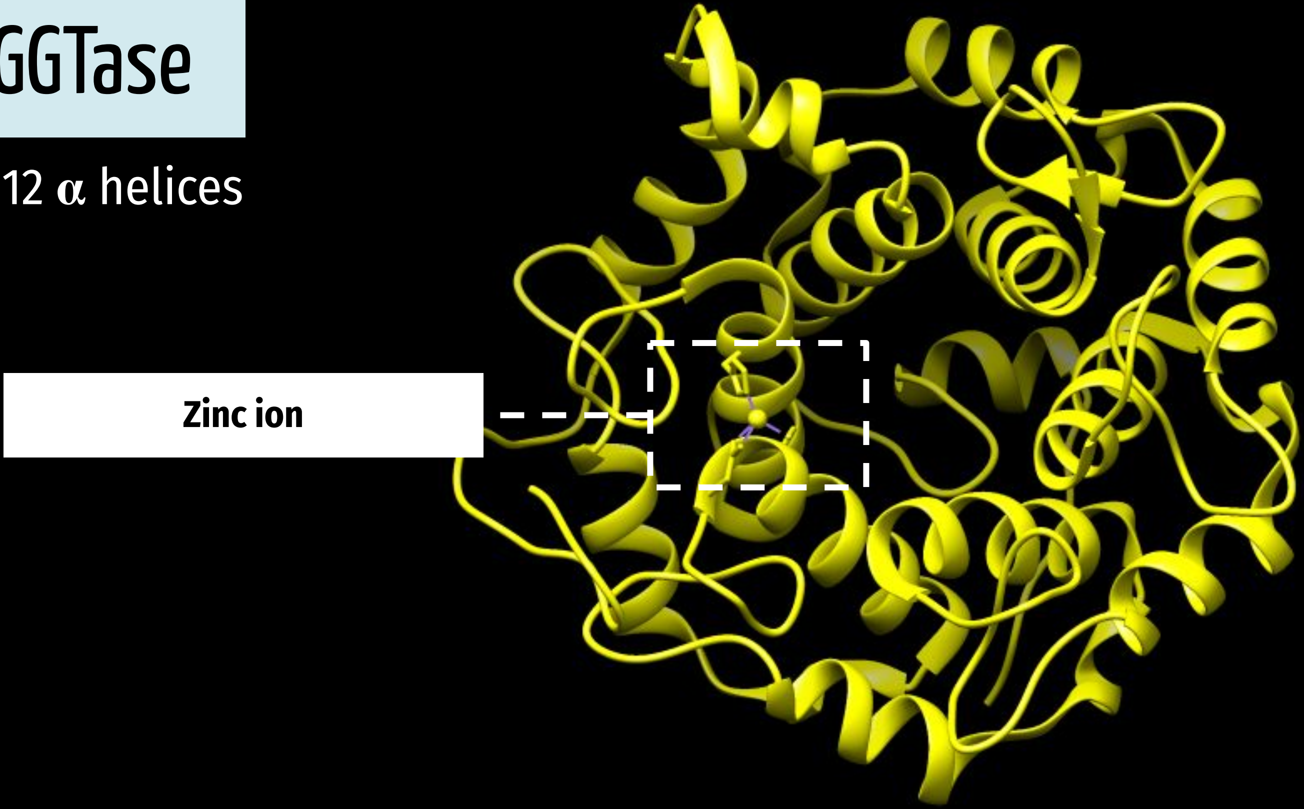
Beta subunit: has the active site

- Coordinates a zinc ion
- GGPP-binding site

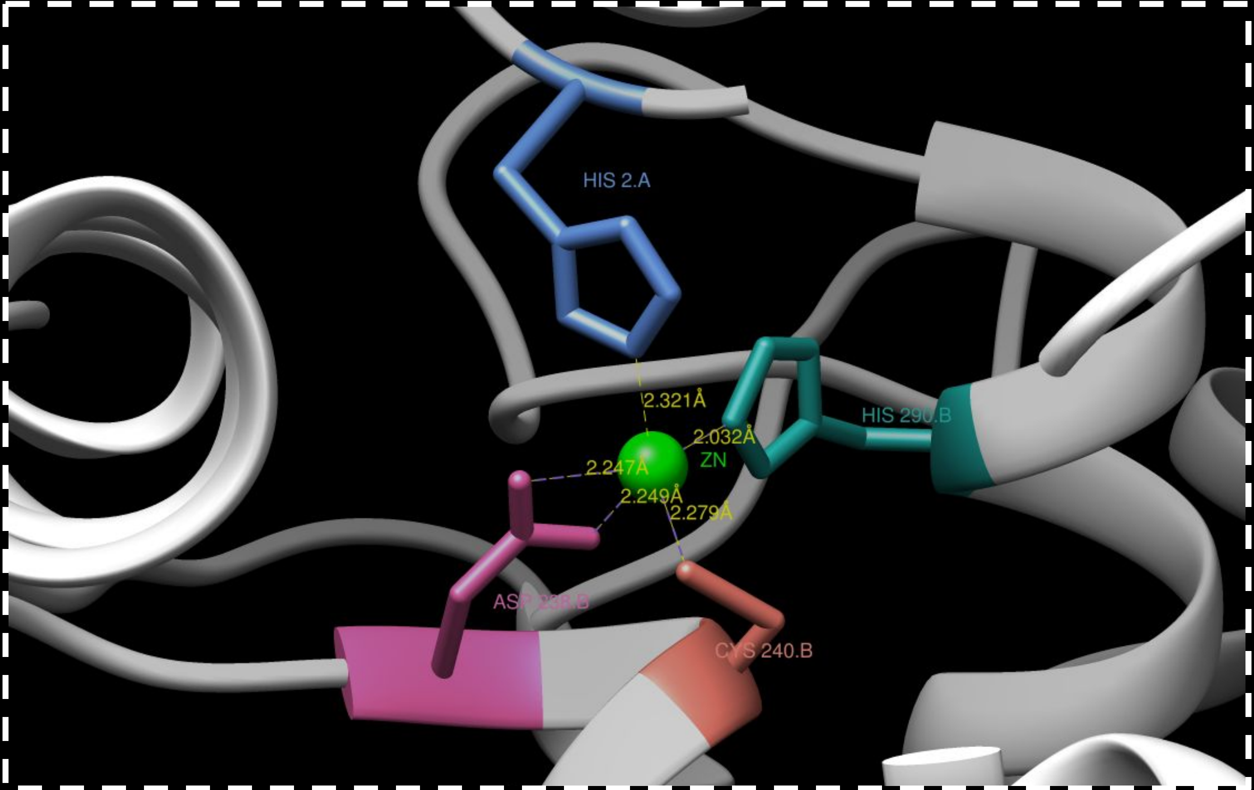


□ subunit RabGGTase

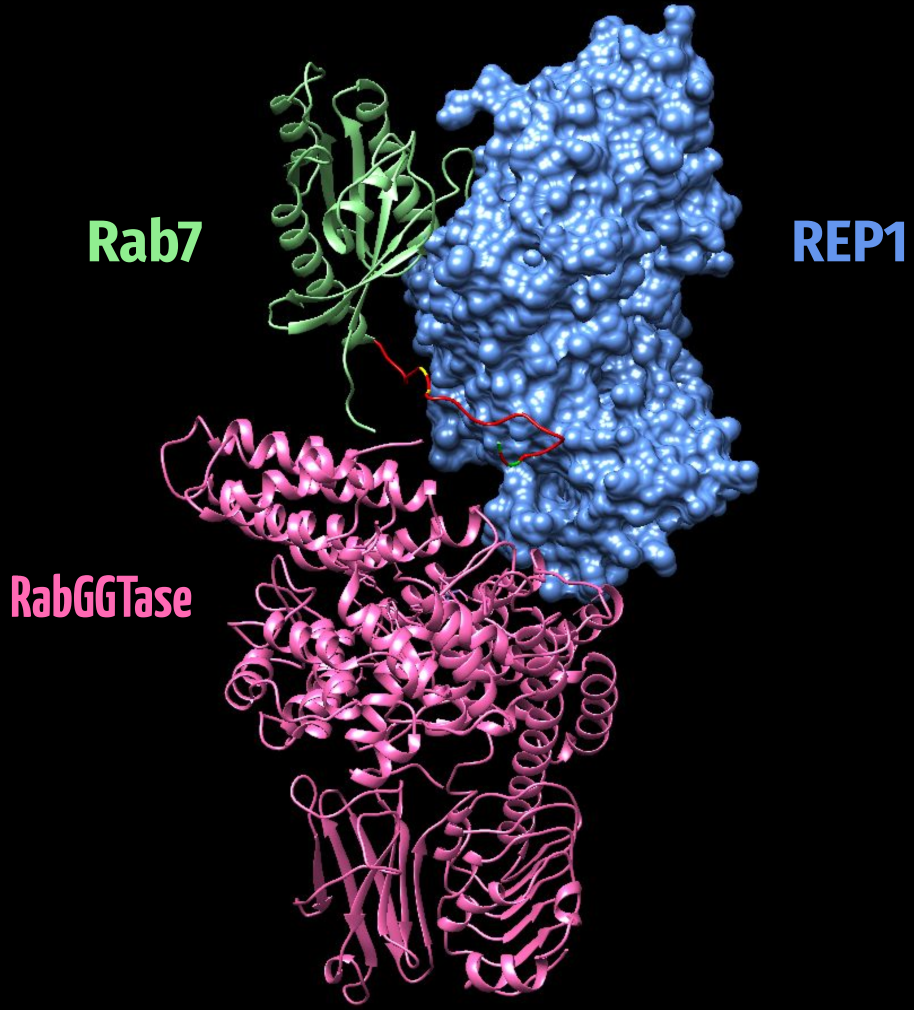
α-α barrel made up of 12 α helices



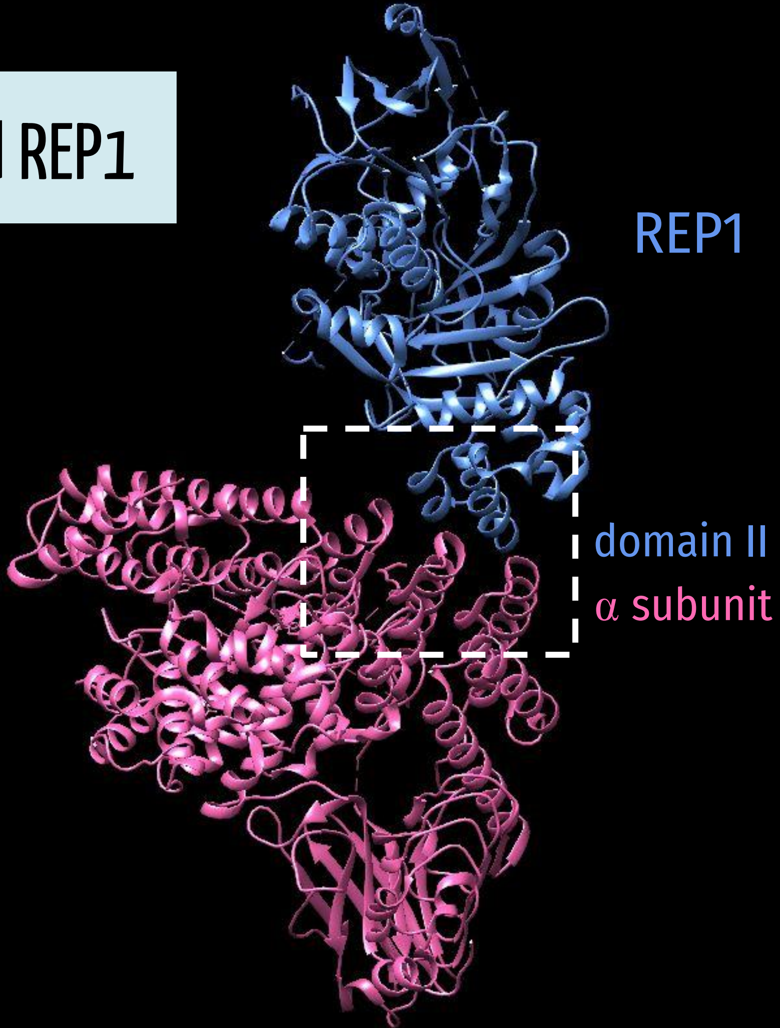
Catalytic Zn^{2+} ion



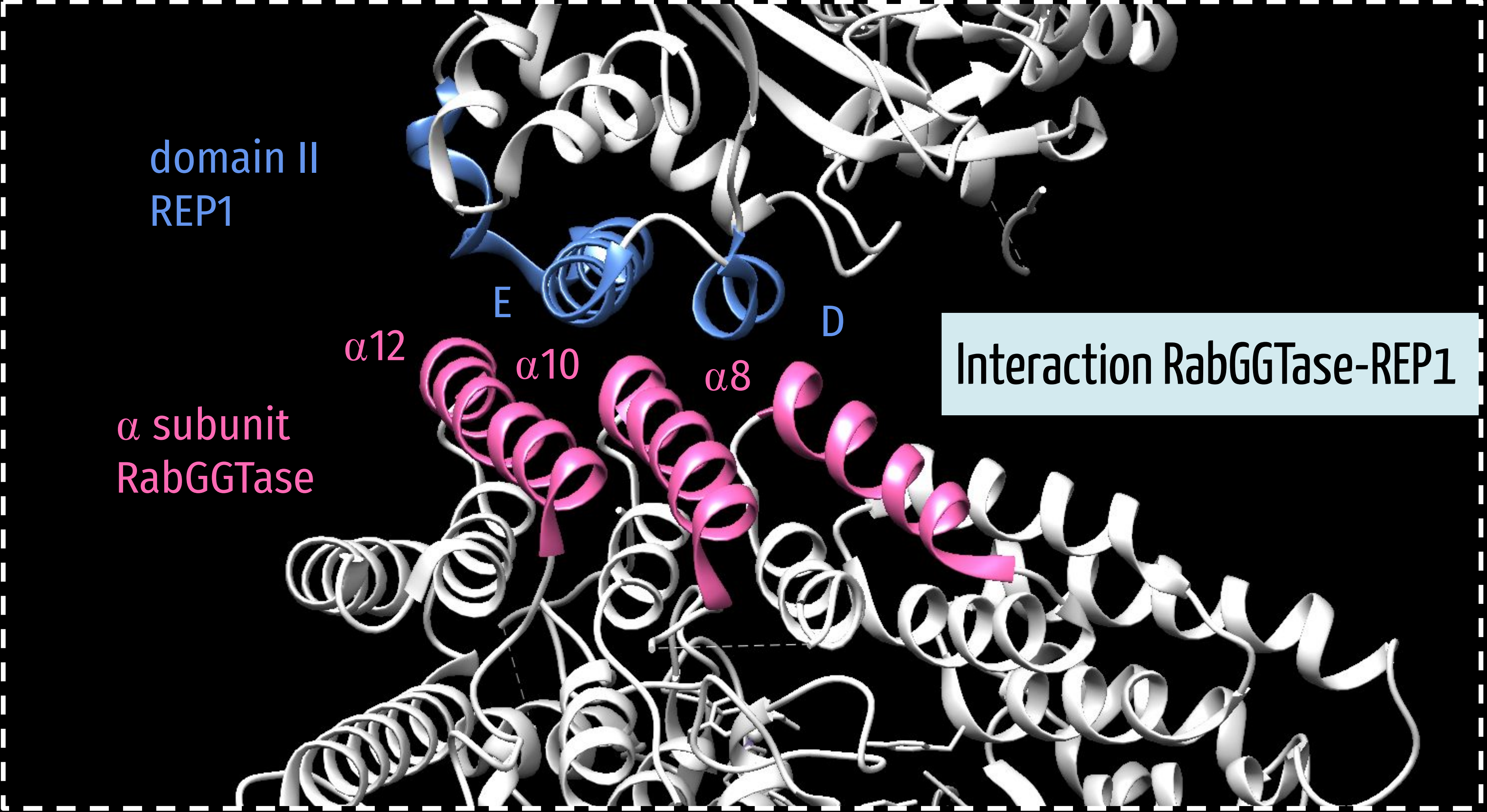
Ternary complex



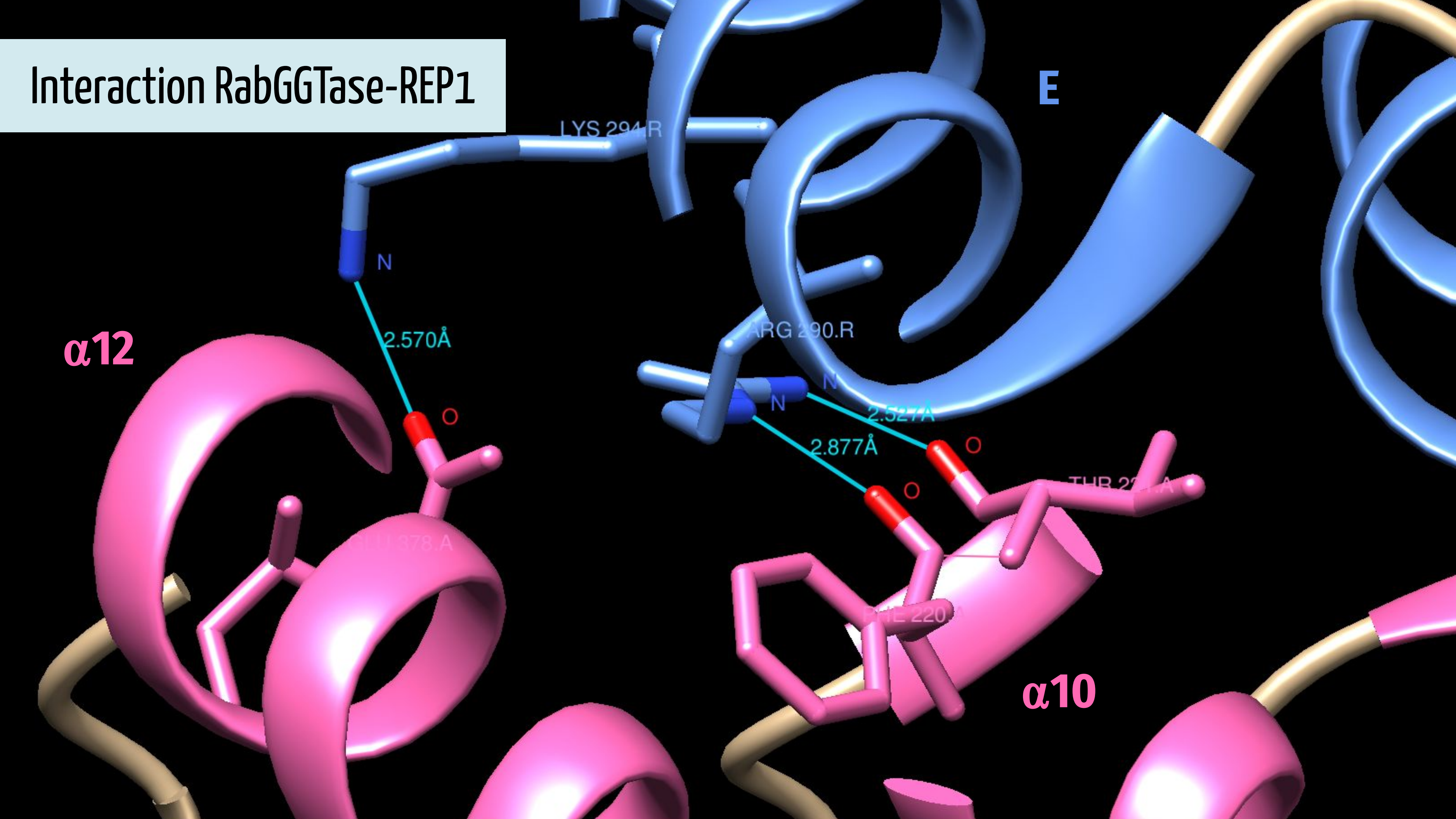
Interaction between RabGGTase and REP1

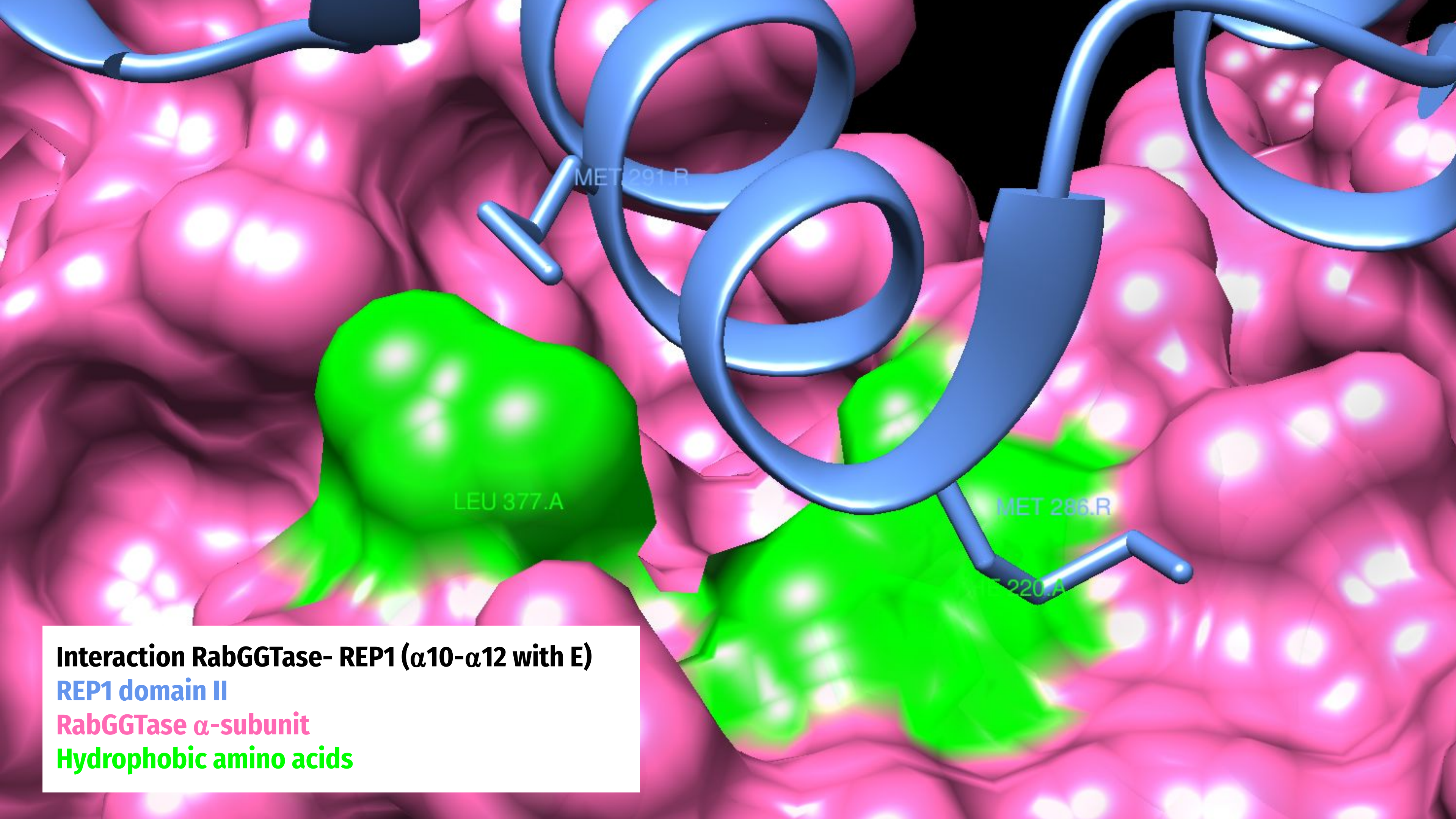


Interaction RabGGTase-REP1



Interaction RabGGTase-REP1



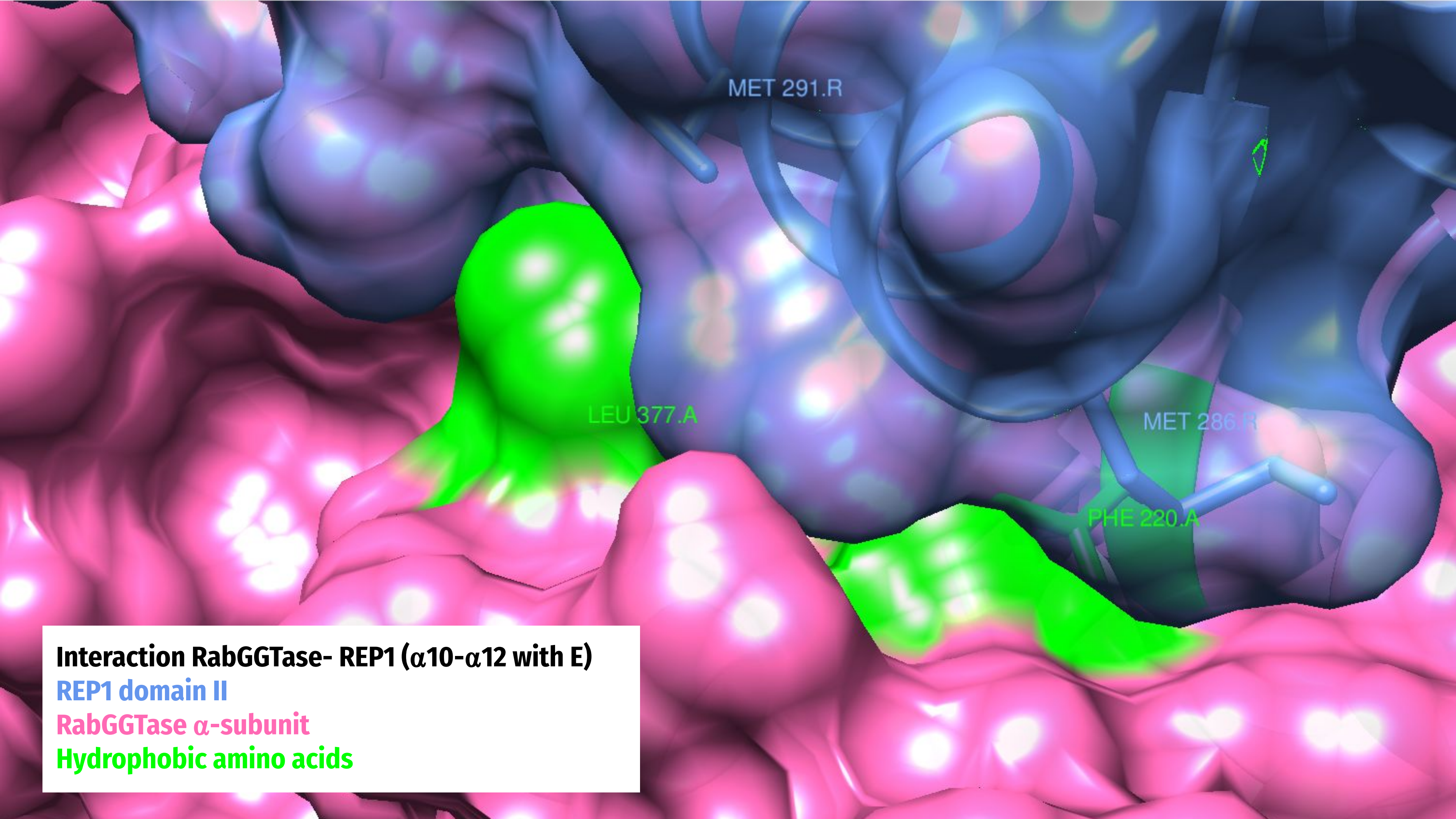


Interaction RabGGTase- REP1 (α10-α12 with E)

REP1 domain II

RabGGTase α-subunit

Hydrophobic amino acids

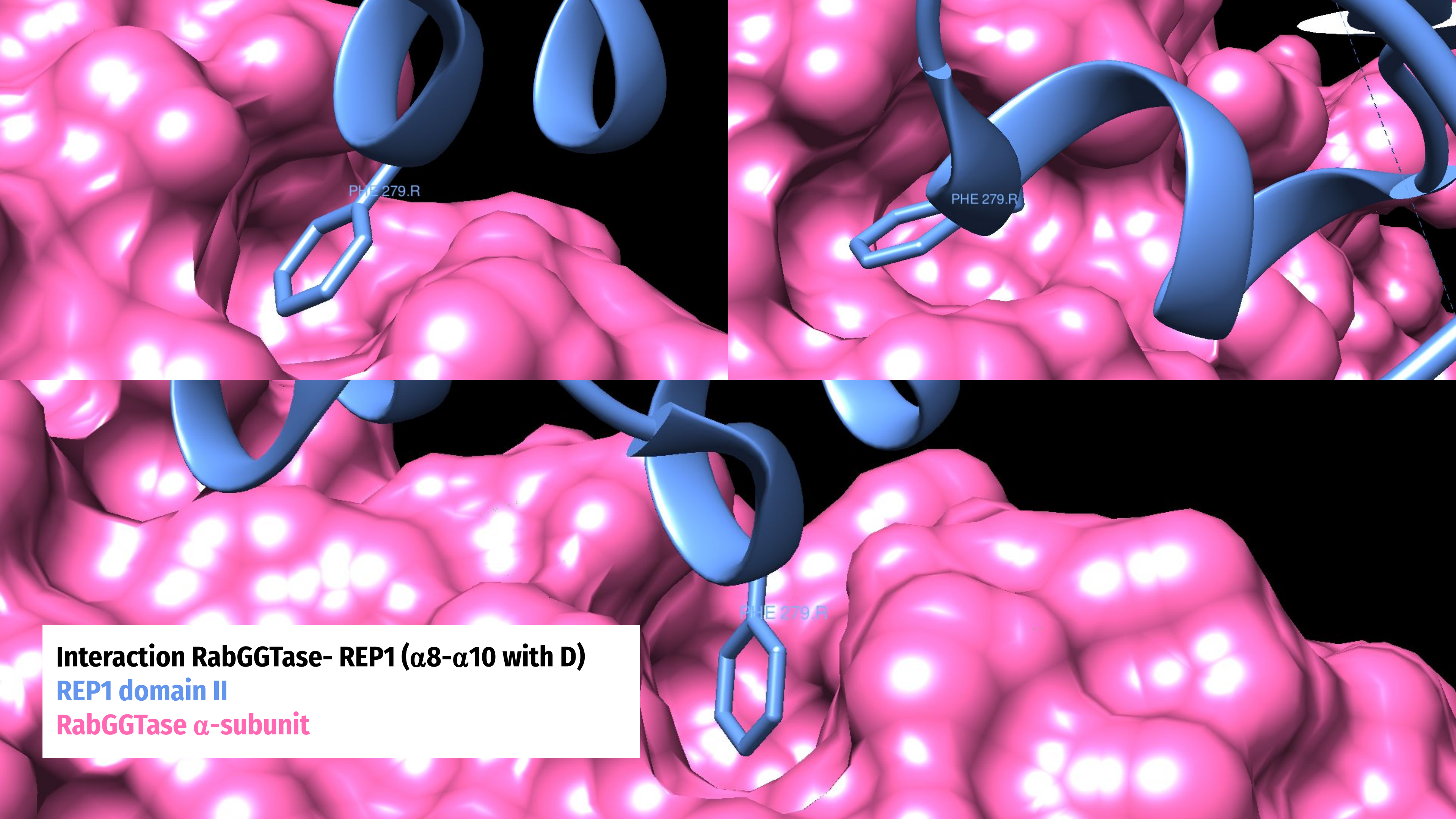


Interaction RabGGTase- REP1 (α10-α12 with E)

REP1 domain II

RabGGTase α-subunit

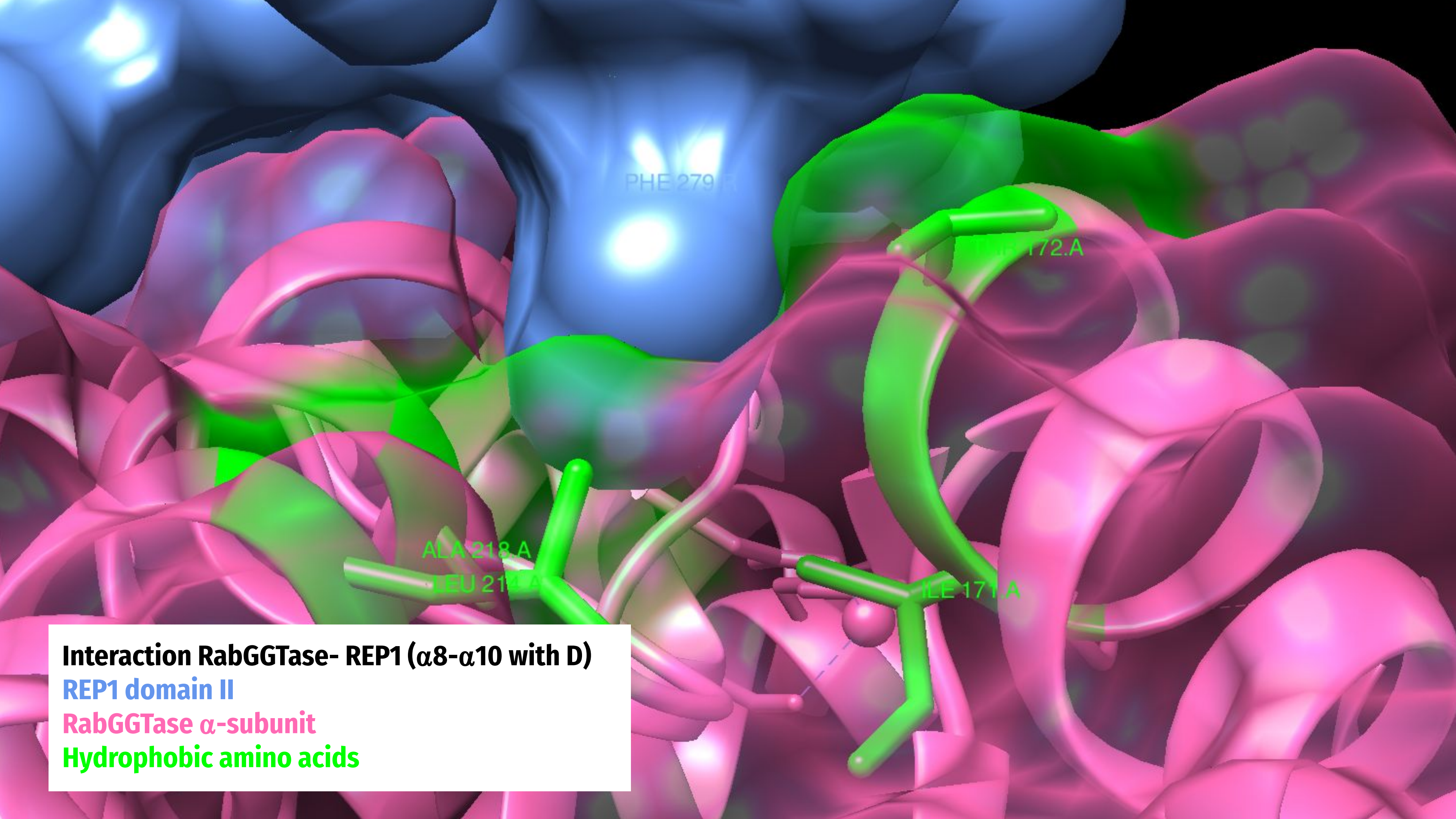
Hydrophobic amino acids



Interaction RabGGTase- REP1 (α8-α10 with D)

REP1 domain II

RabGGTase α-subunit



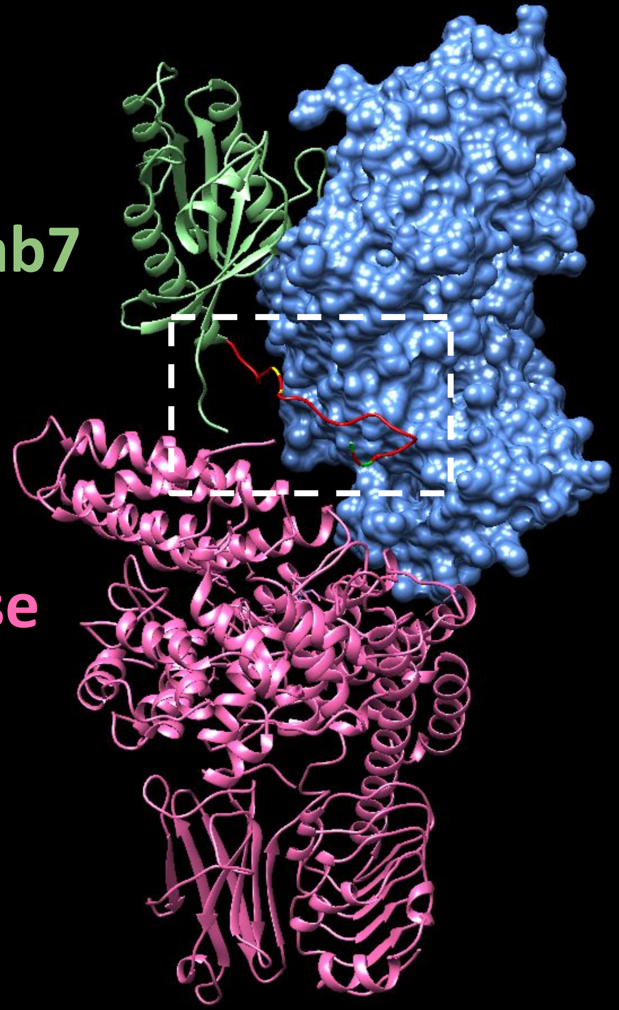
Interaction RabGGTase- REP1 (α8-α10 with D)

REP1 domain II

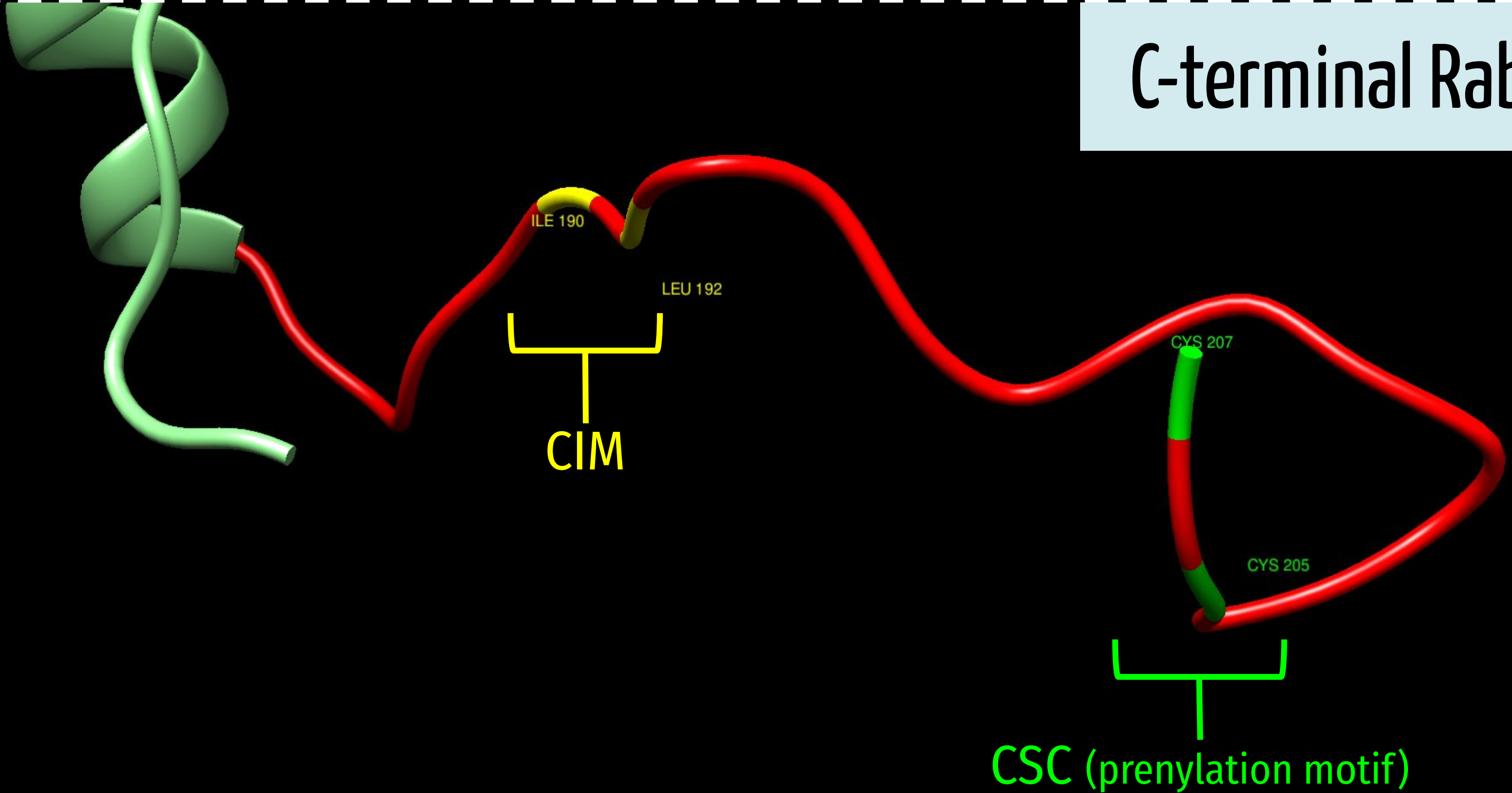
RabGGTase α-subunit

Hydrophobic amino acids

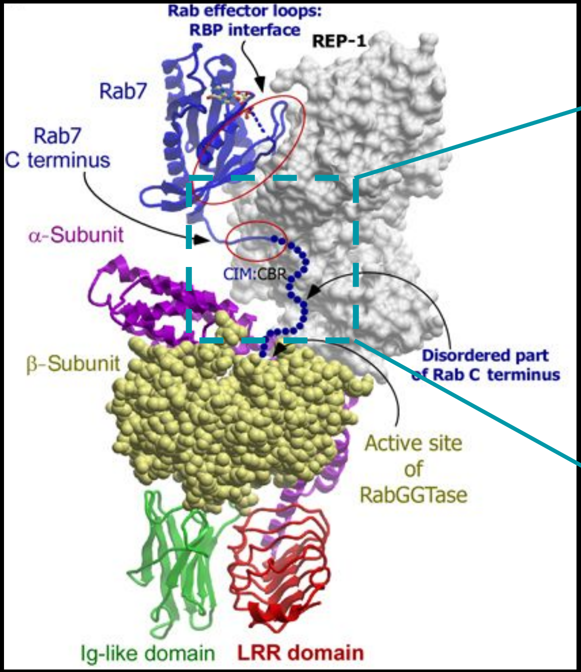
Ternary complex



C-terminal Rab7



Rab7 - RabGGTase



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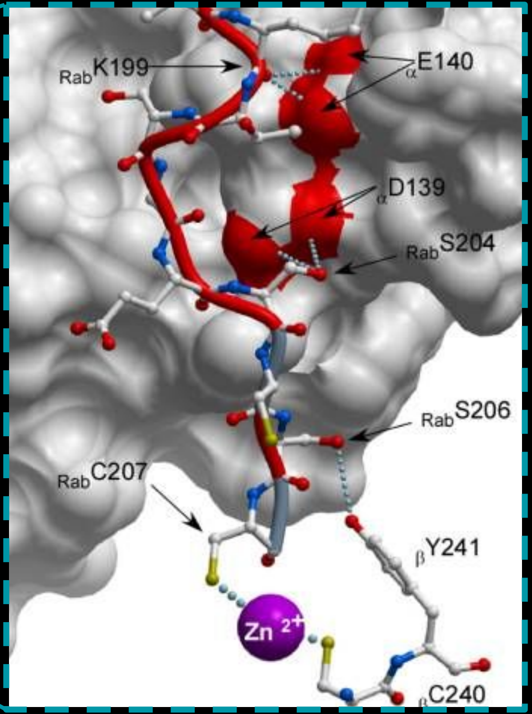

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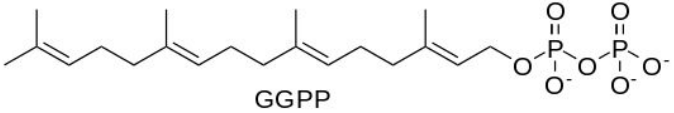
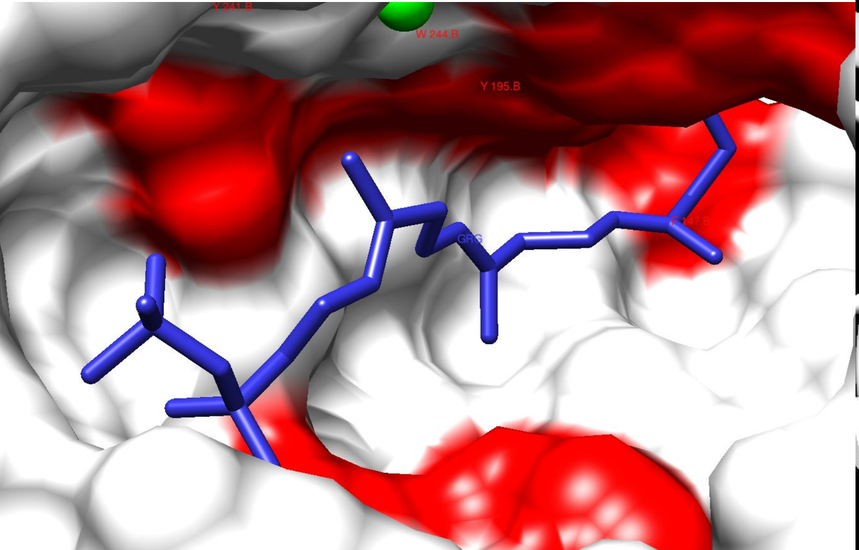
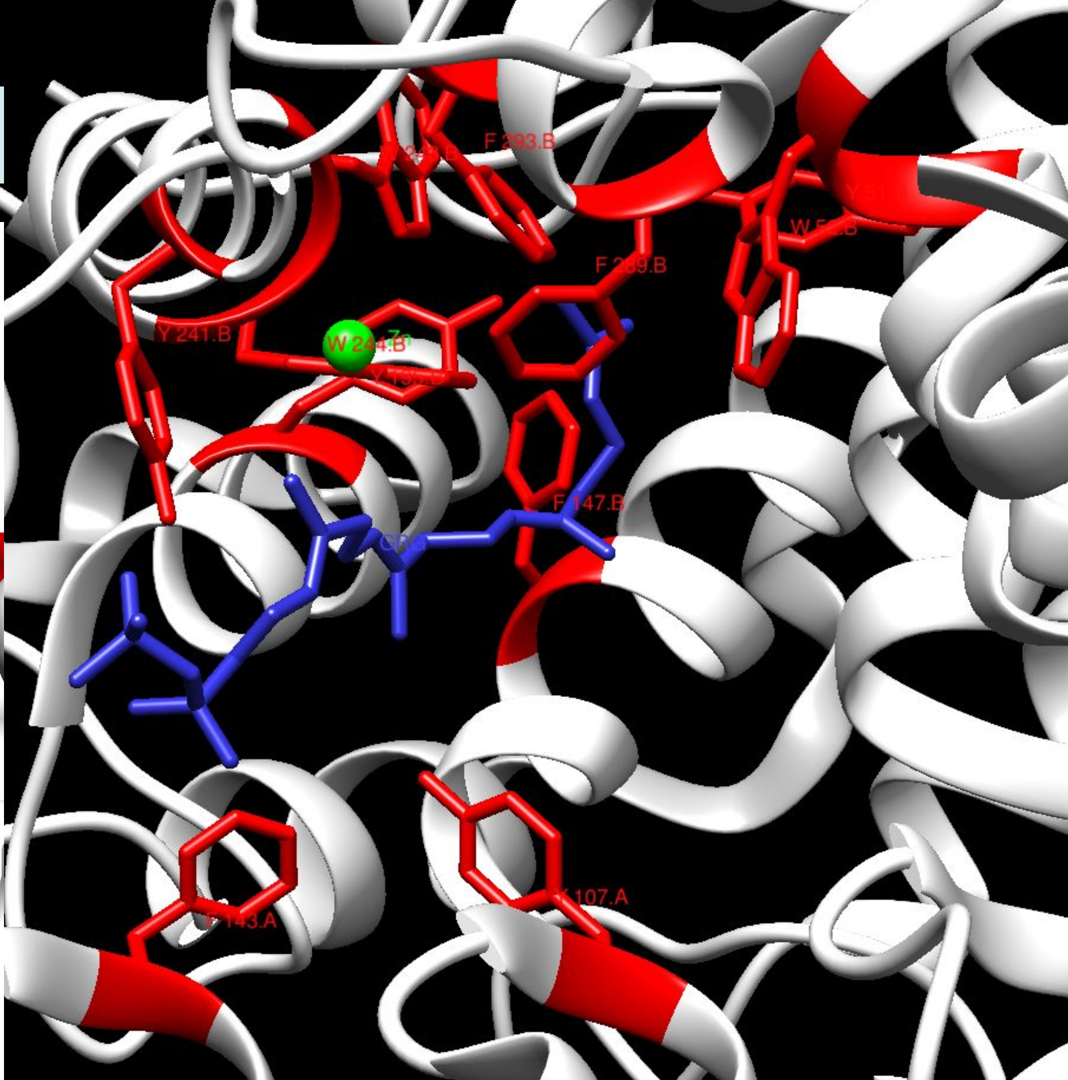
Prenylation: interaction RabGGTase-GGPP

GGPP
Hydrophobic amino acids
Zinc ion

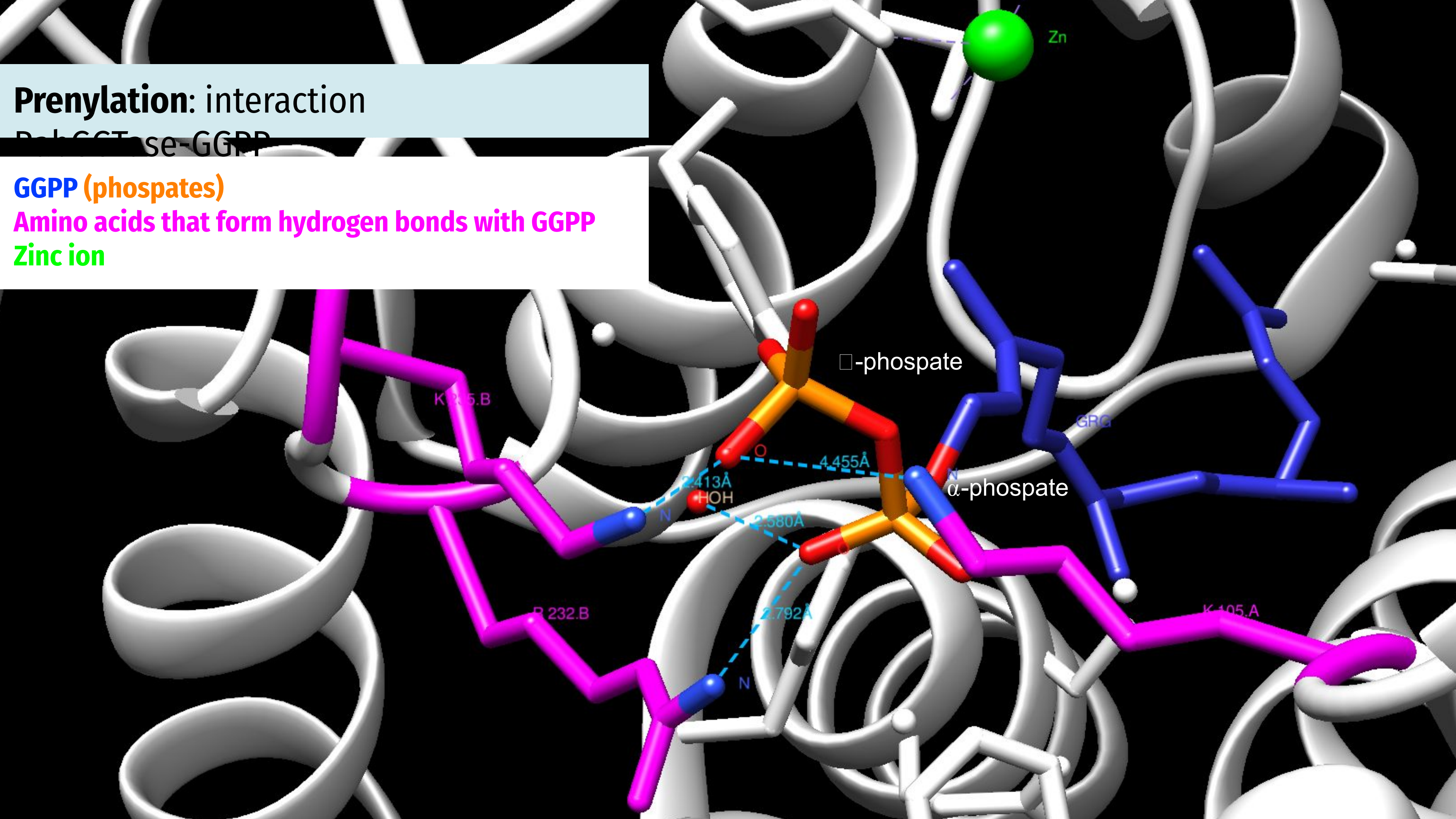




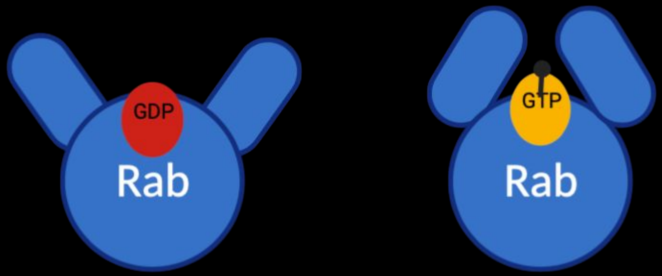


Prenylation: interaction RabGGTase-GGPP

GGPP (phospates)
Amino acids that form hydrogen bonds with GGPP
Zinc ion



GDP-Rab & GTP-Rab



INACTIVE

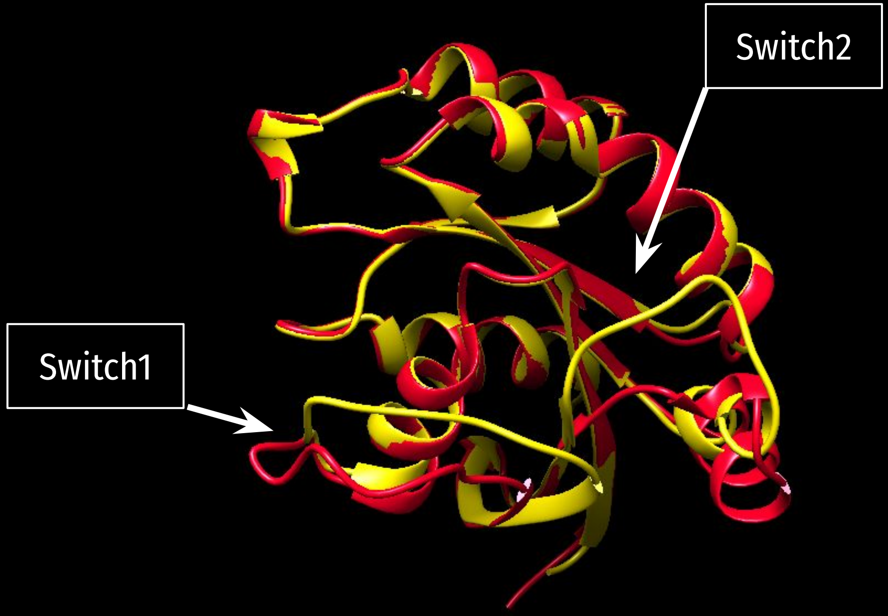
↓

High degree of flexibility and disorder for the Switch1 and Switch2 region

ACTIVE

↓

Restricted conformational flexibility

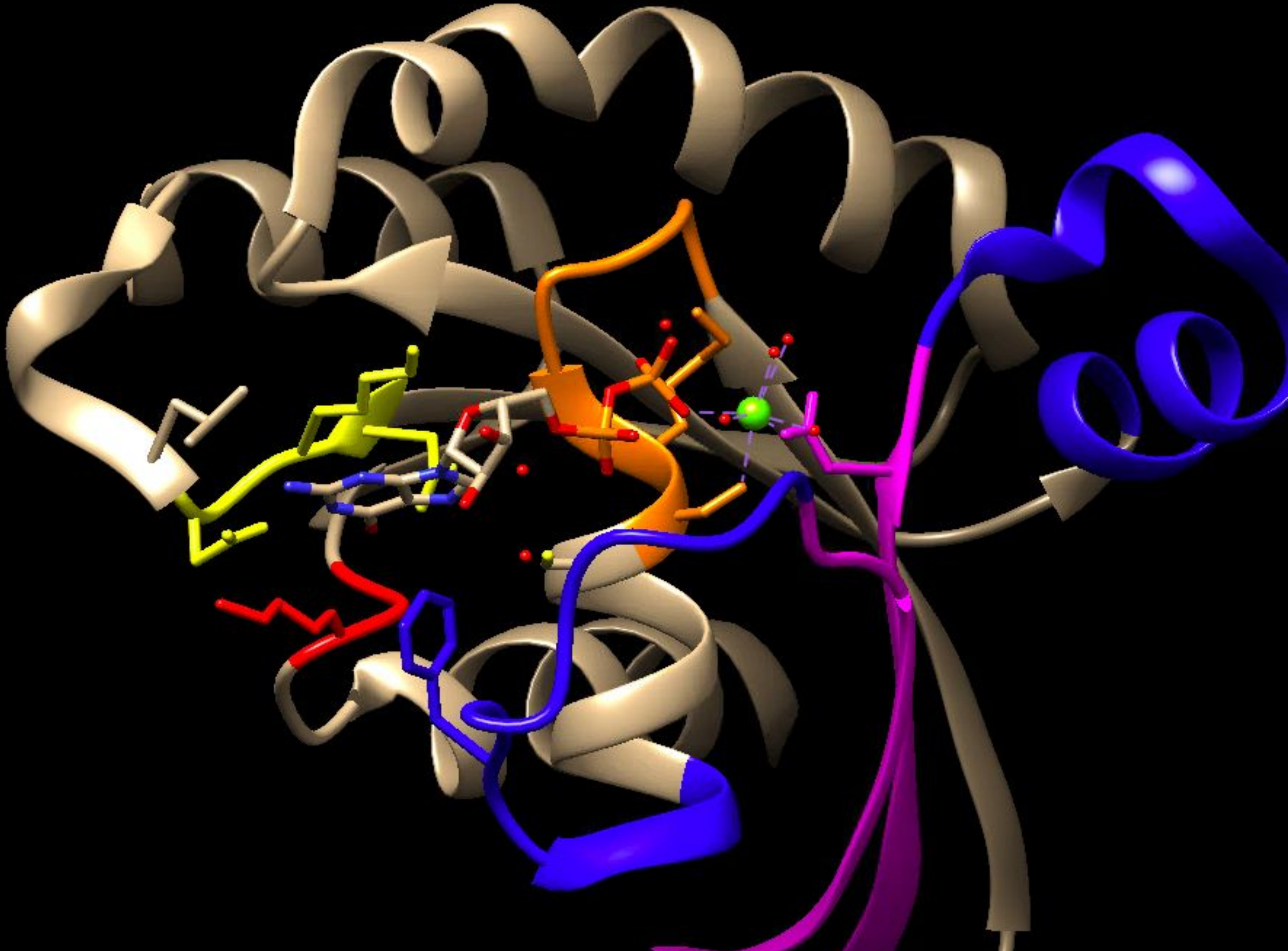


🟥 GDP 🟨 GTP

RMSD: 0,506 Å

Video

Rab - GDP



P-loop

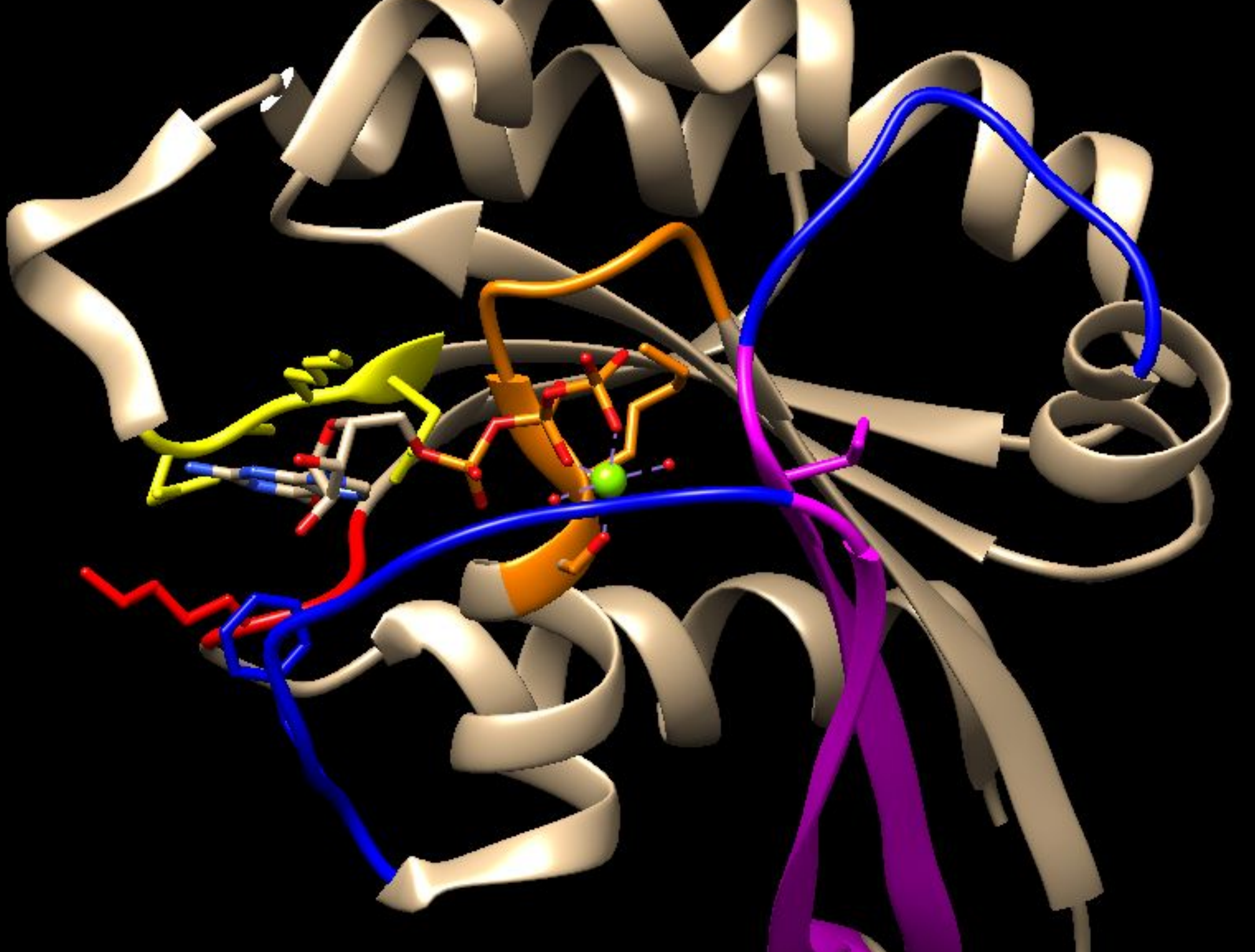
G2

G3

Switch (I & II)

Interswitch

Rab - GTP



P-loop

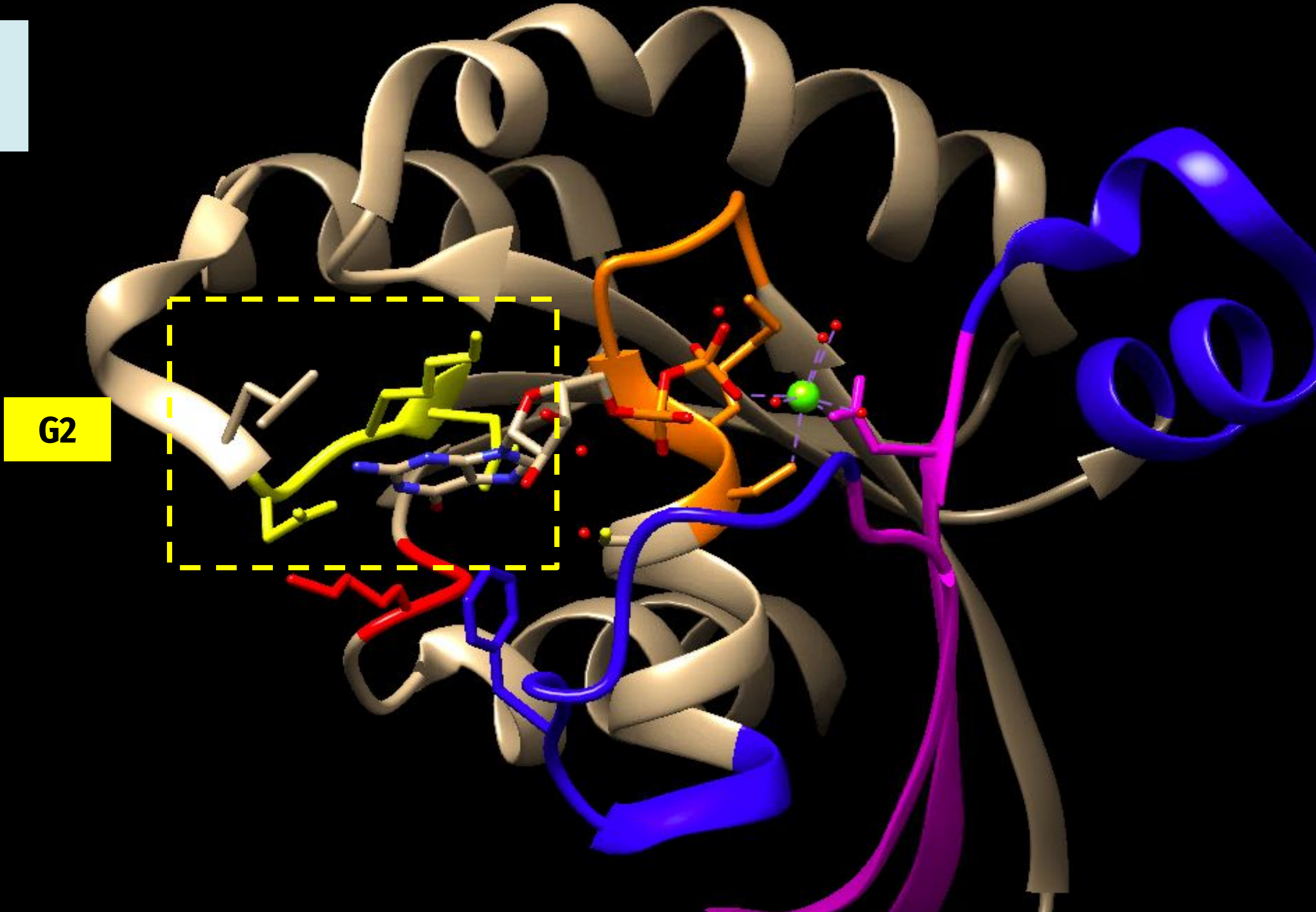
G2

G3

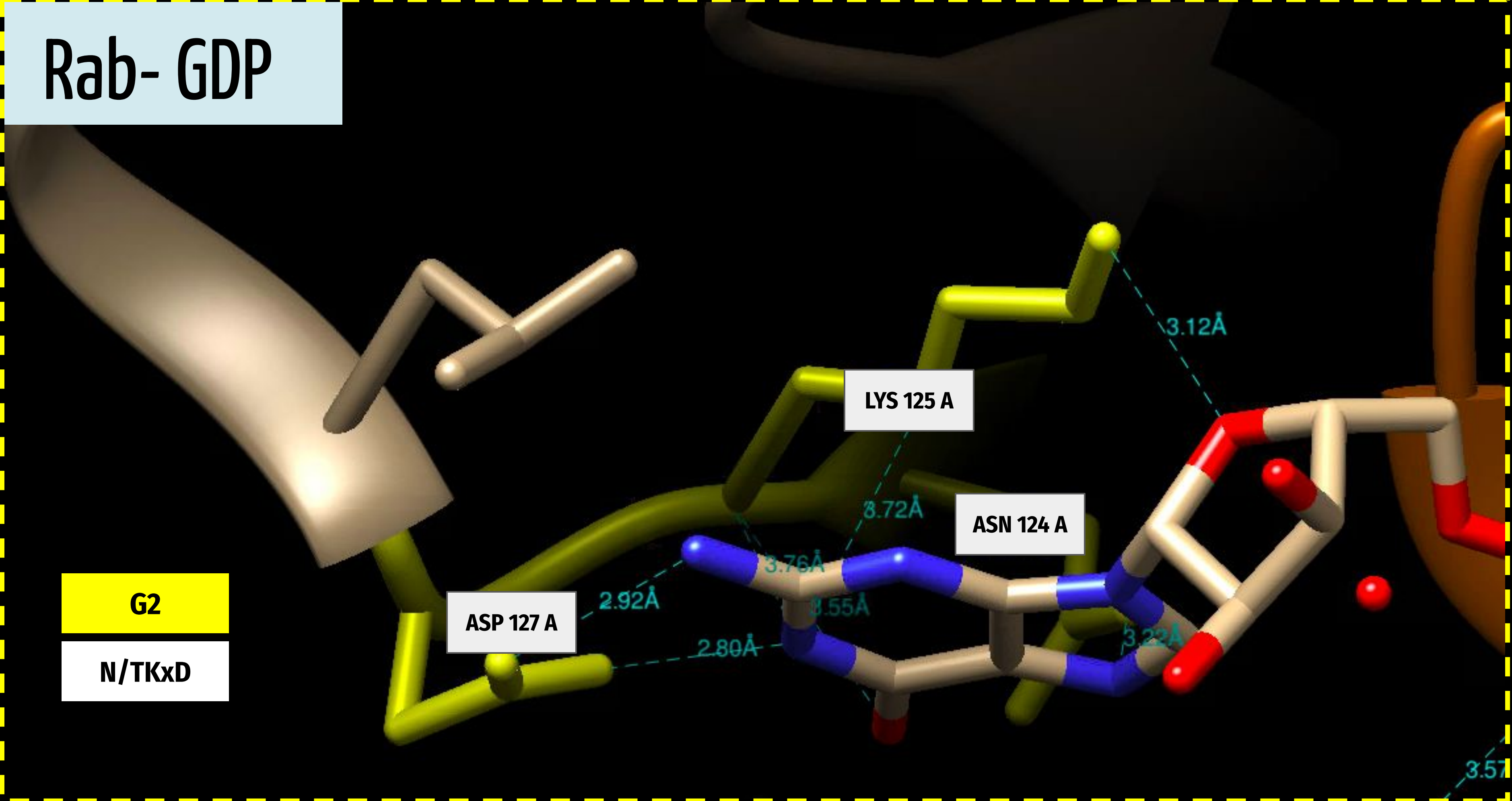
Switch (I & II)

Interswitch

Rab - GDP



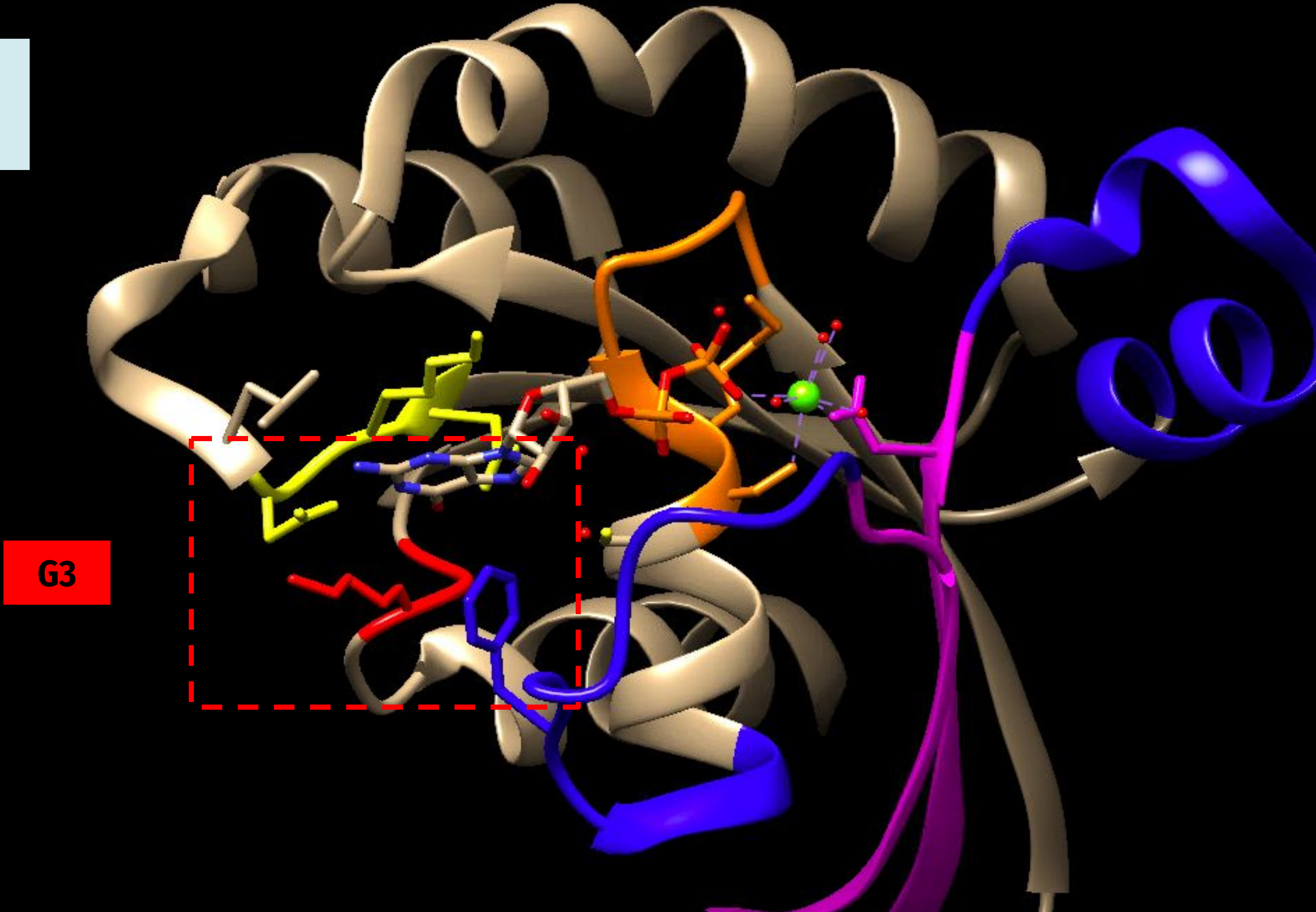
Rab- GDP



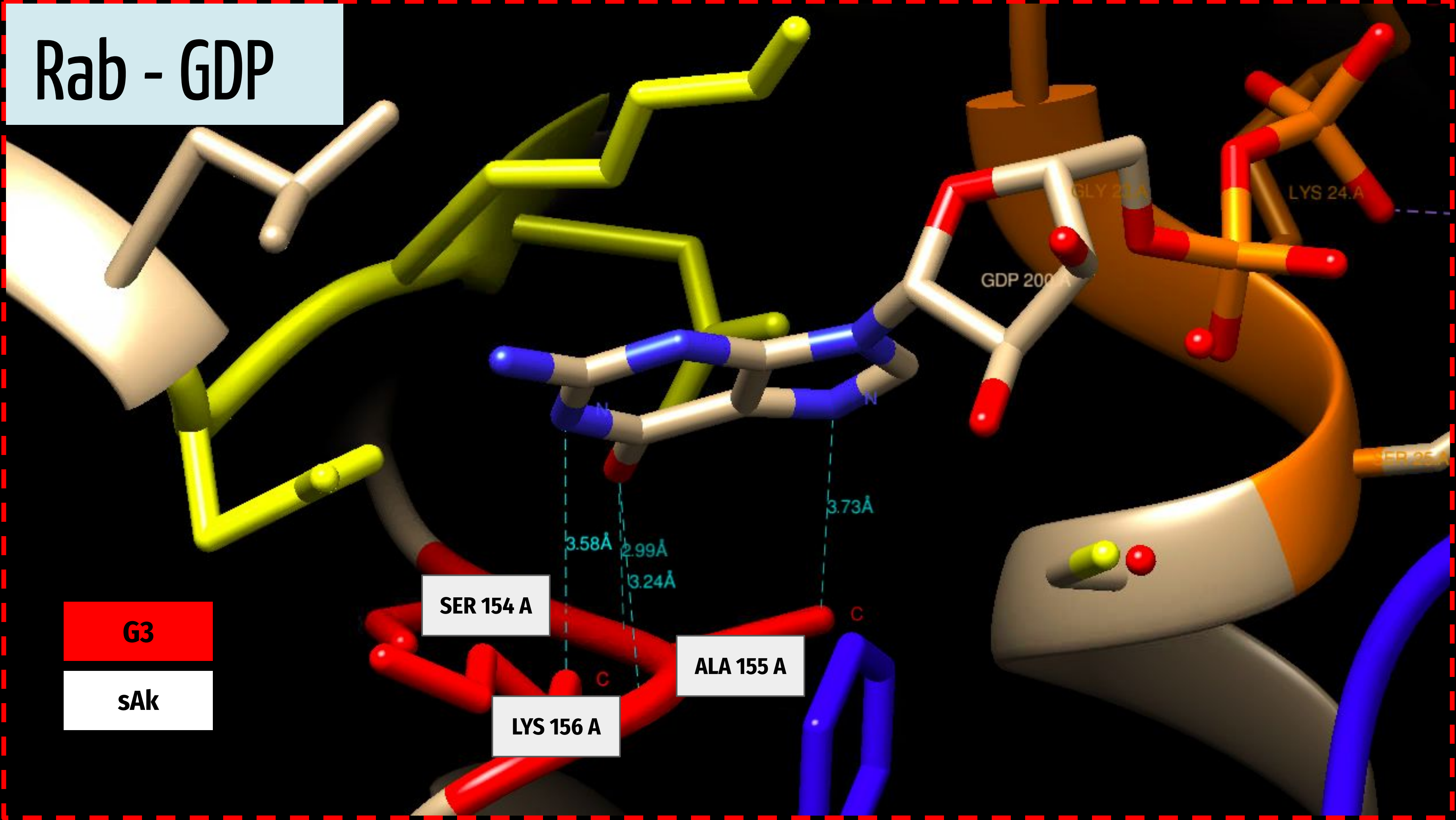
G2

N/TKxD

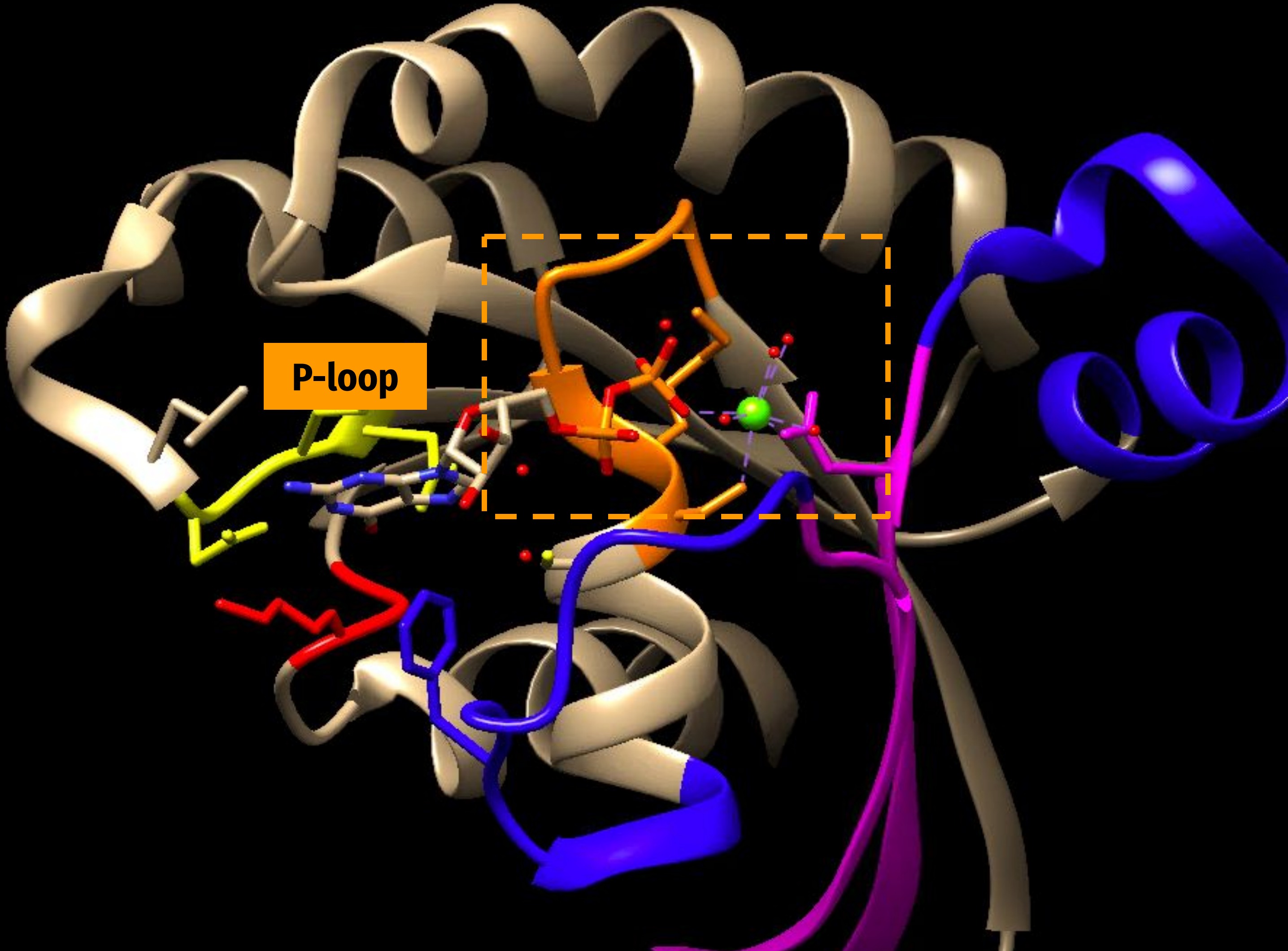
Rab - GDP



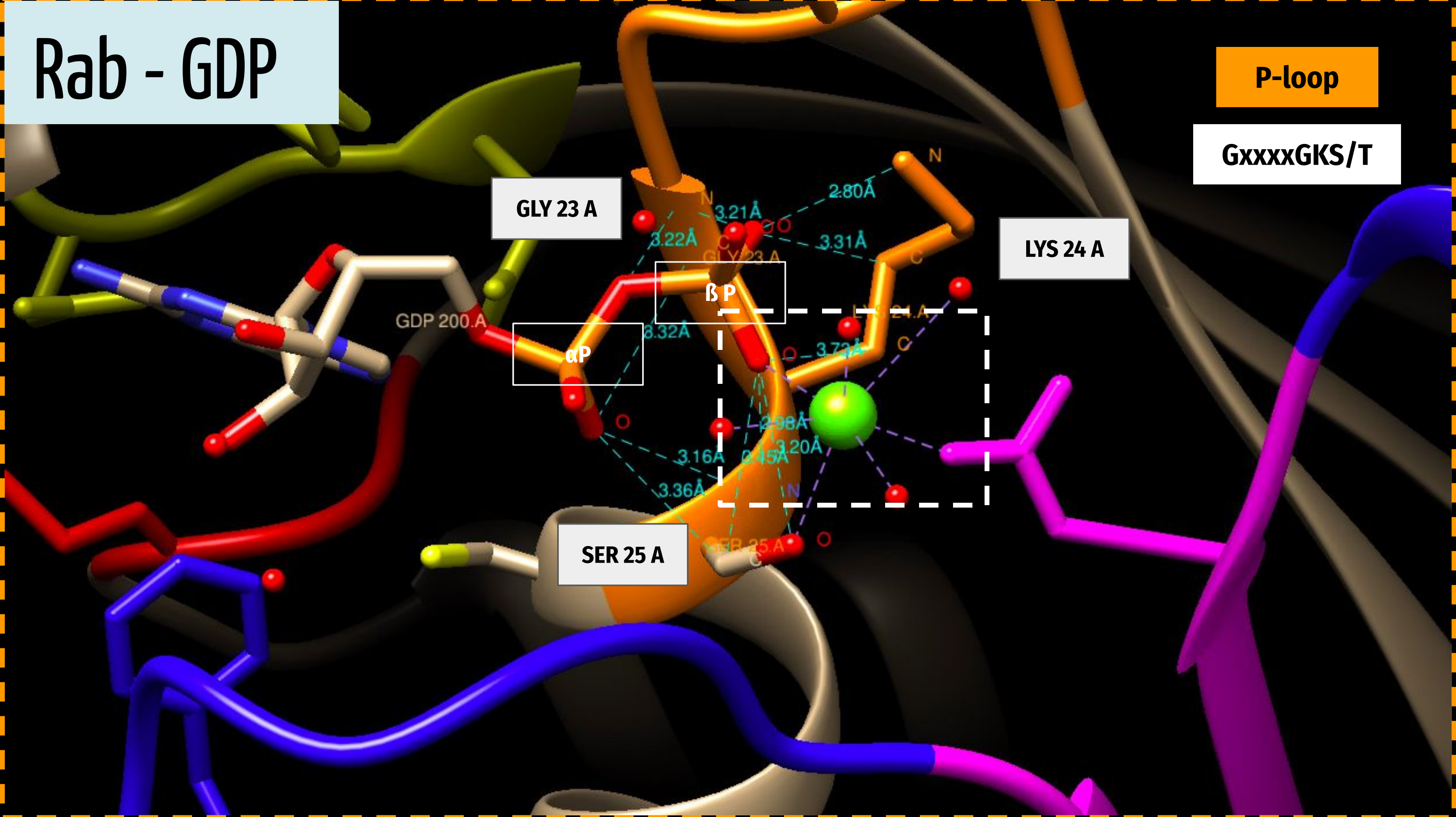
Rab - GDP


GDP 200.A
GLY 23.A
LYS 24.A
ER 25.A
N
N
3.73Å
3.58Å
2.99Å
3.24Å
C
C
SER 154 A
ALA 155 A
LYS 156 A
G3
sAk

Rab - GDP



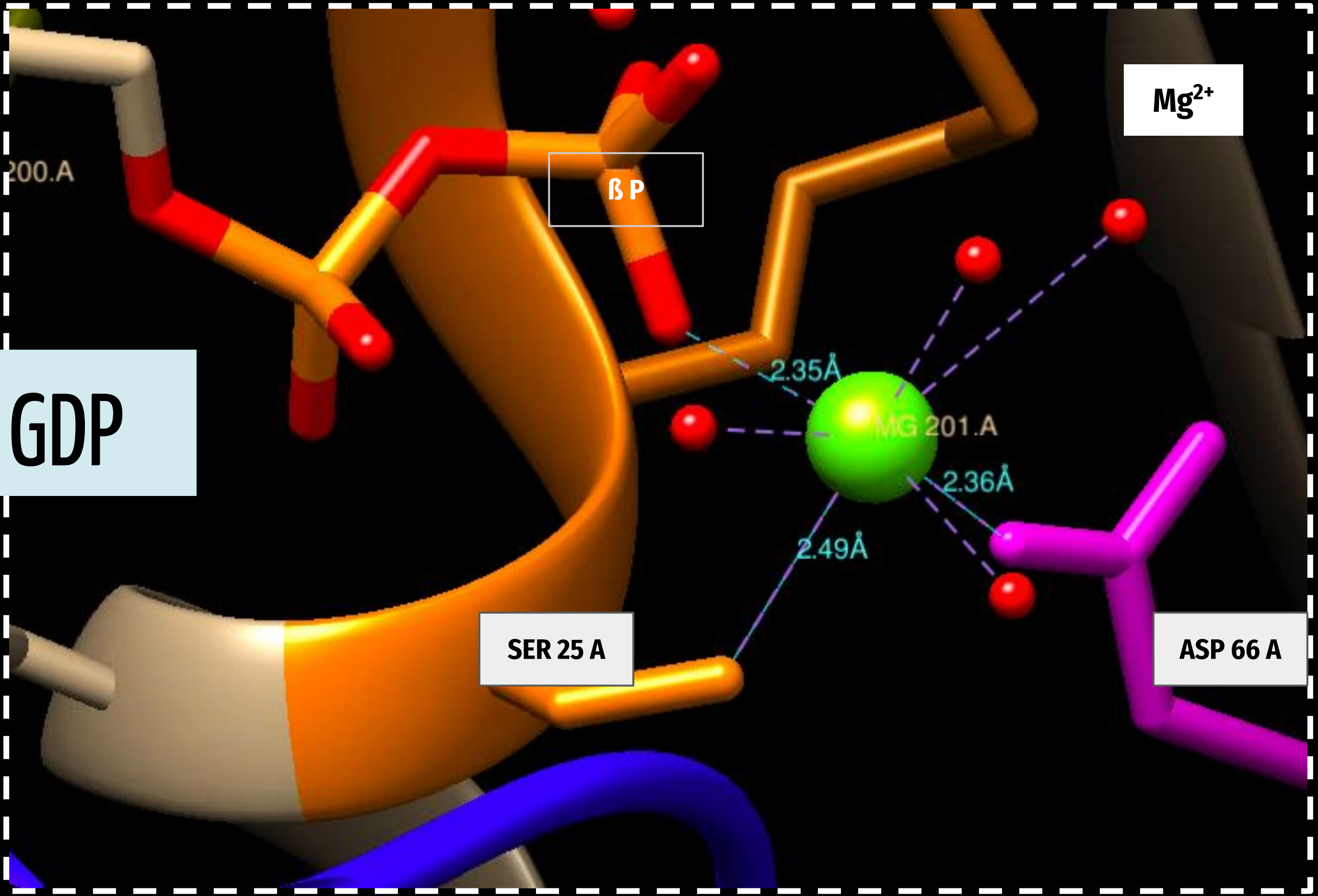
Rab - GDP



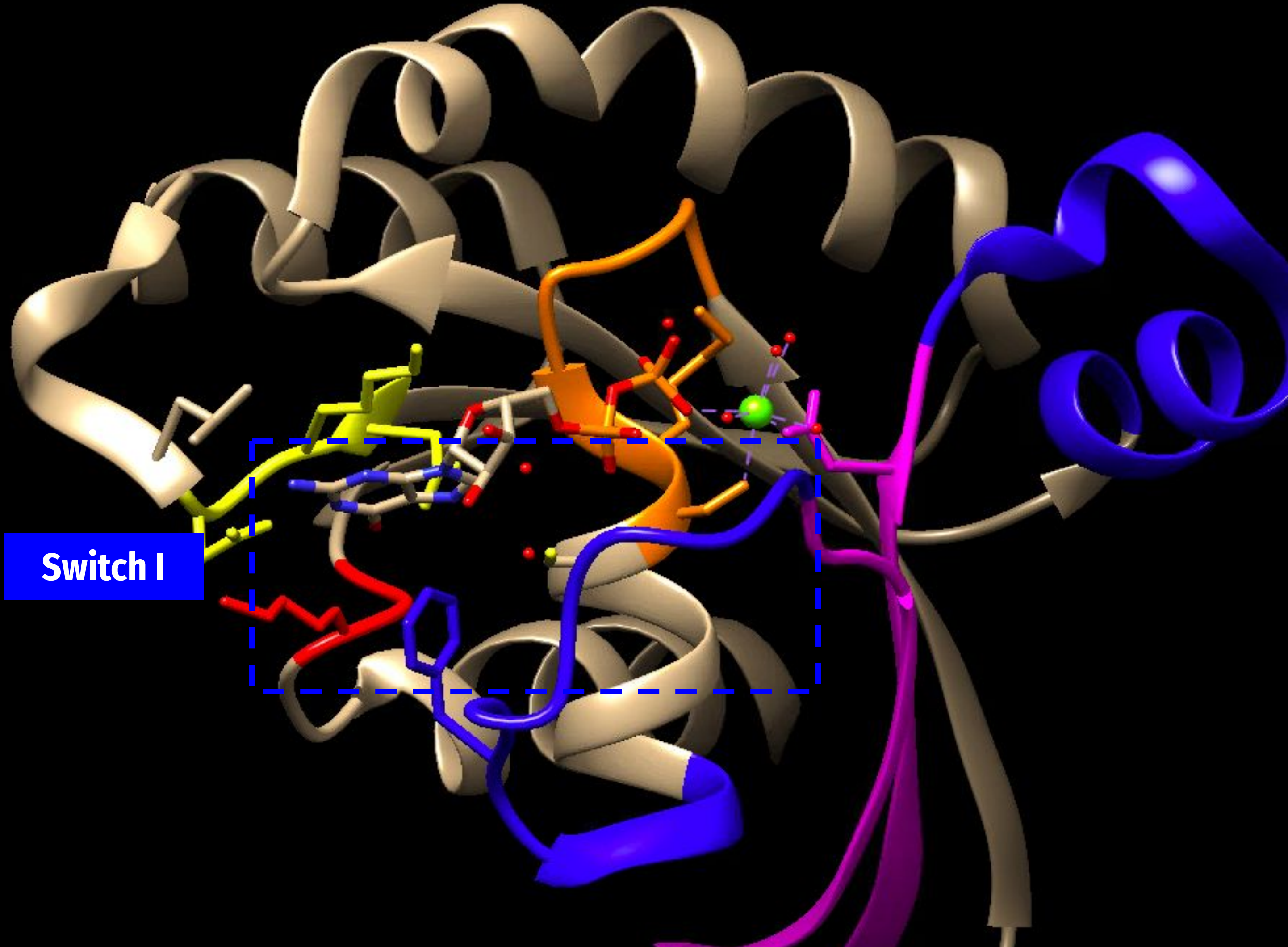
P-loop

GxxxxGKS/T

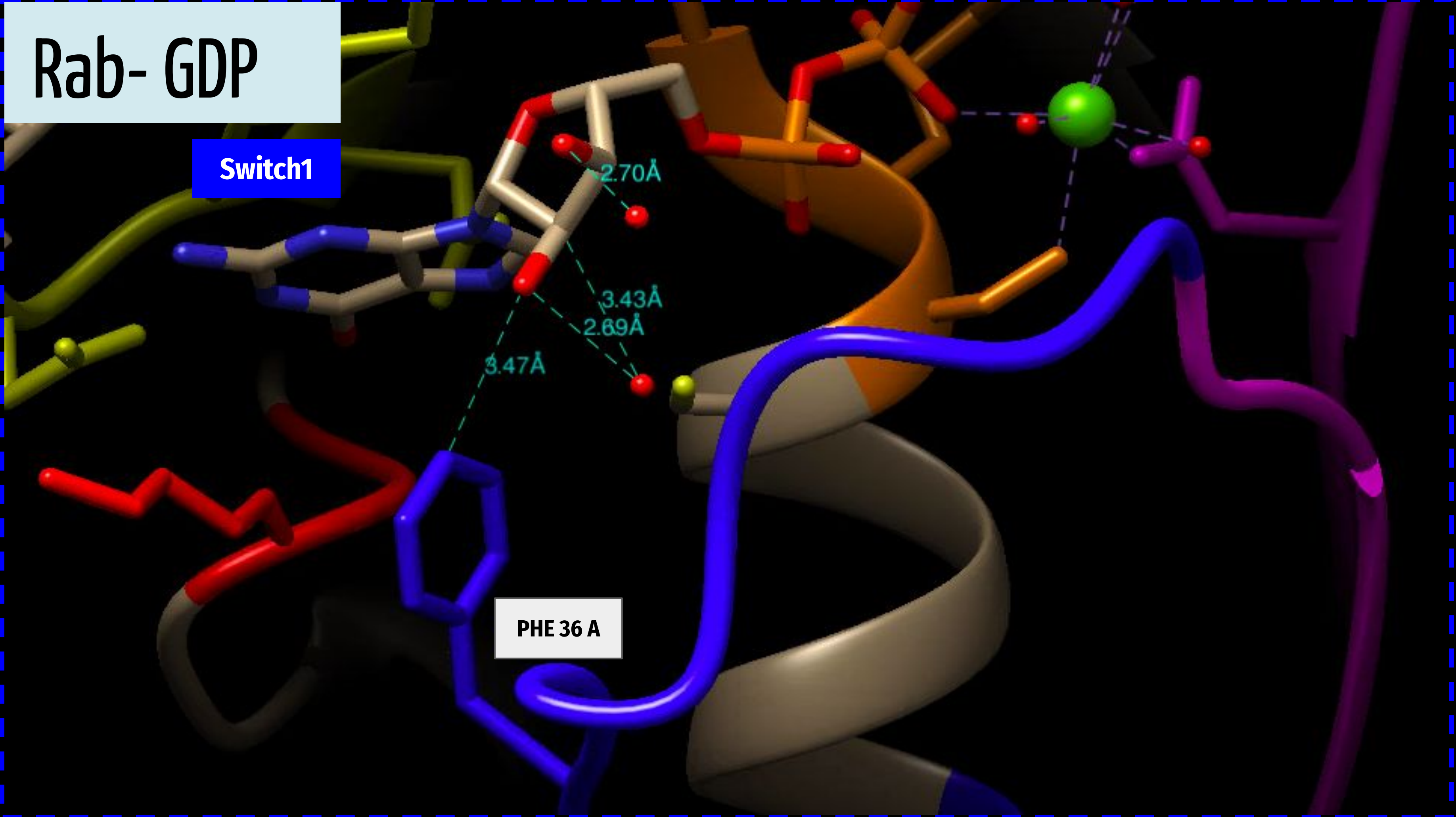
Rab - GDP



Rab - GDP



Rab- GDP

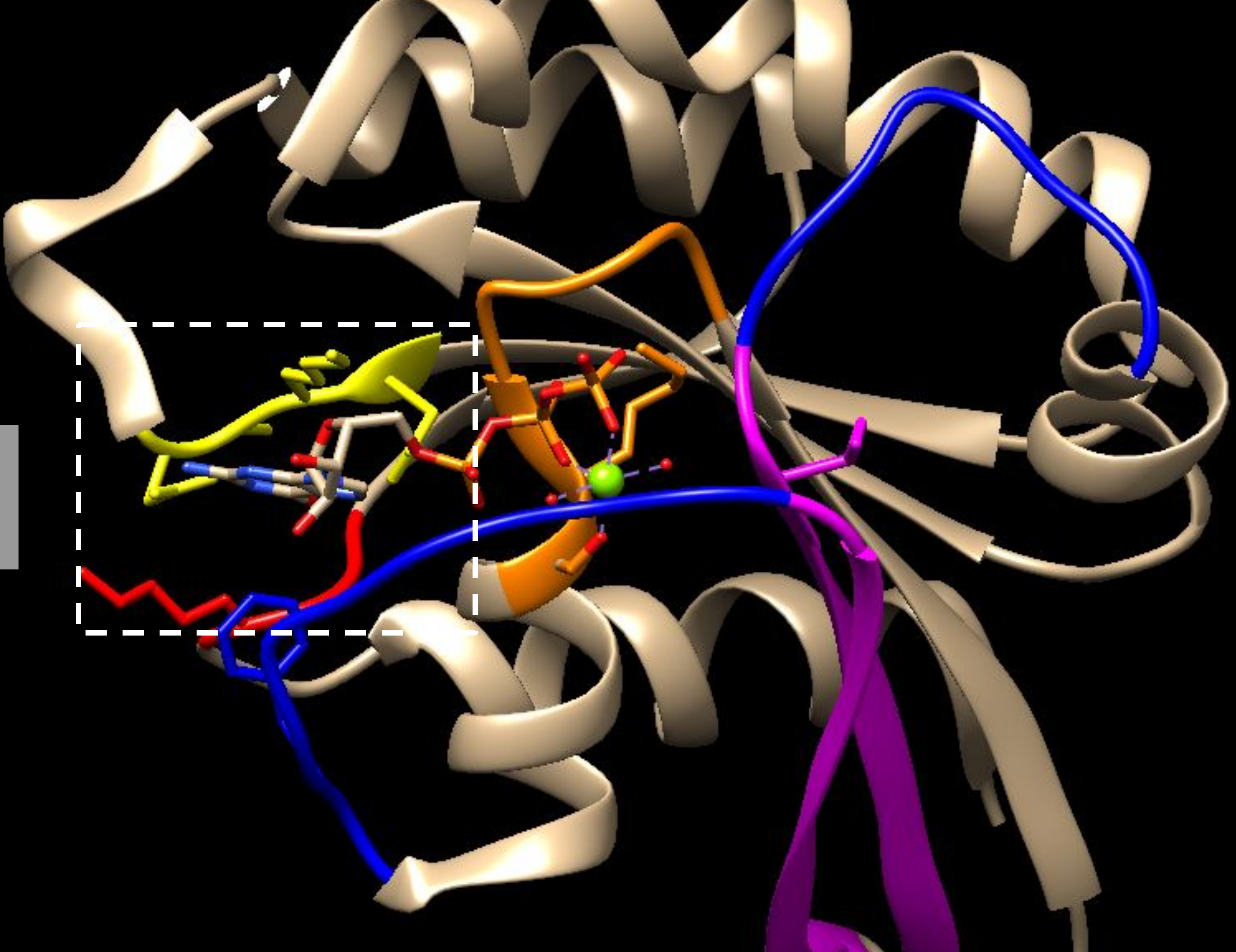


Switch1

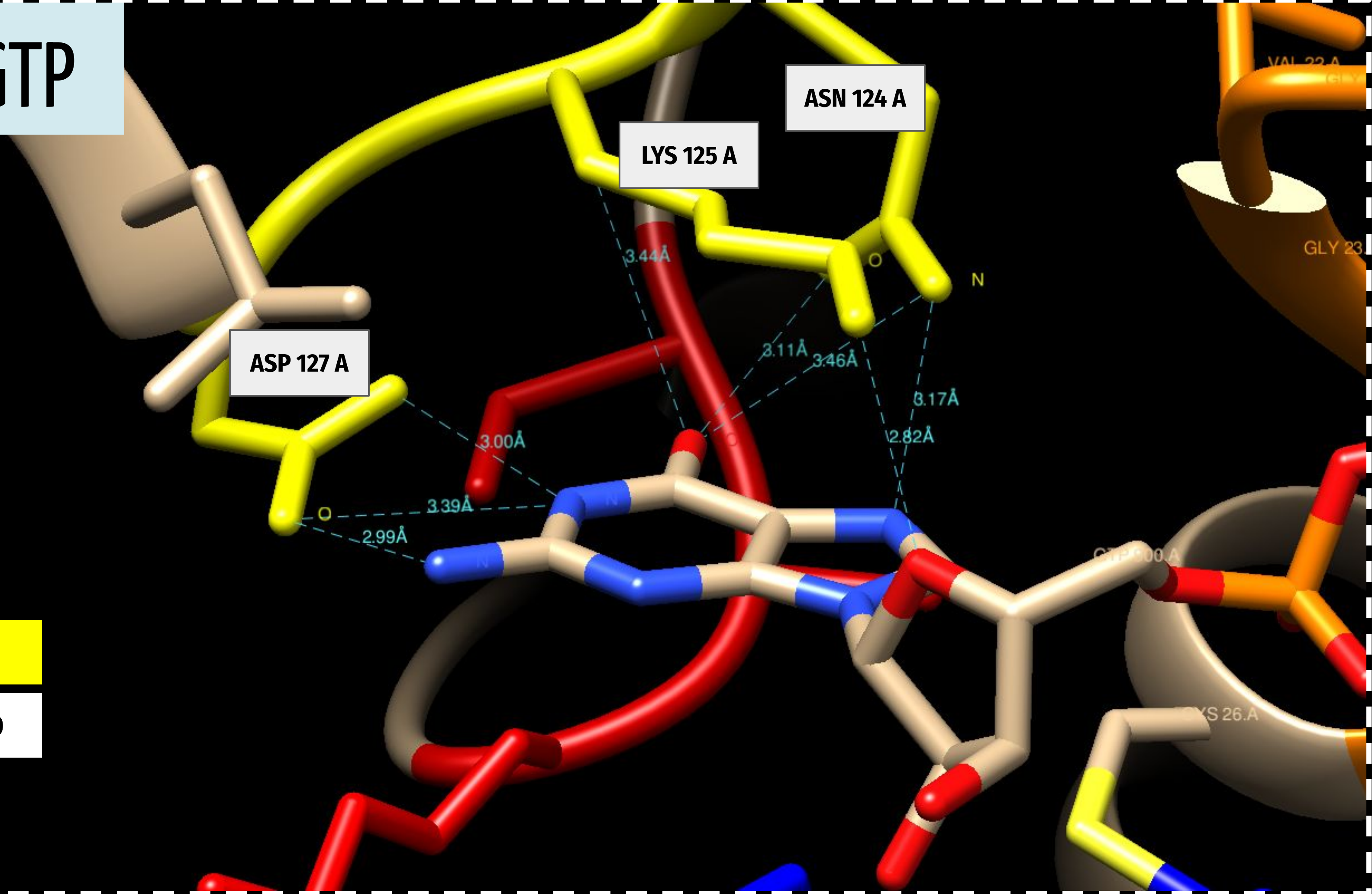
PHE 36 A

Rab - GTP

Rab - GTP



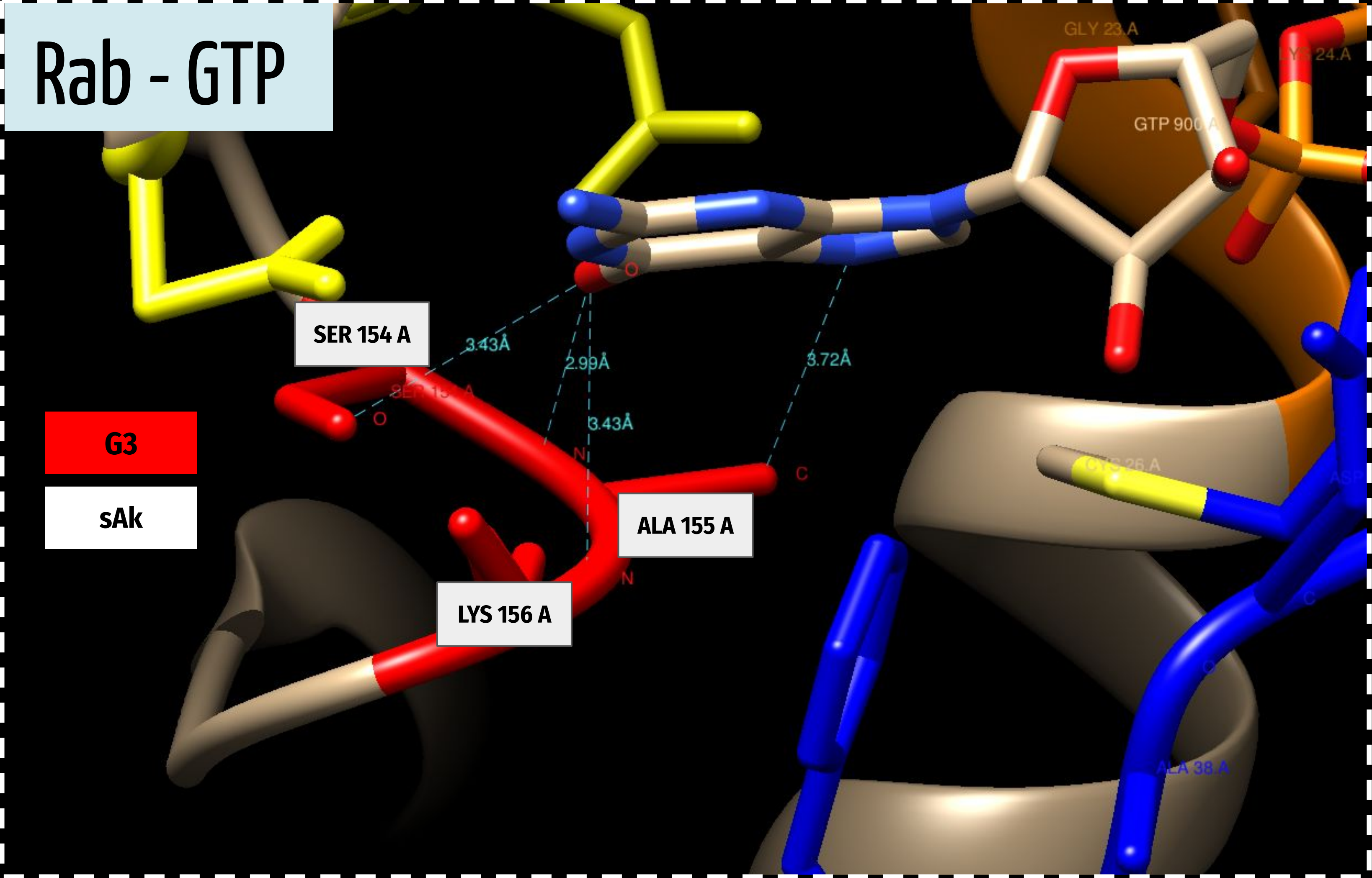
Rab - GTP



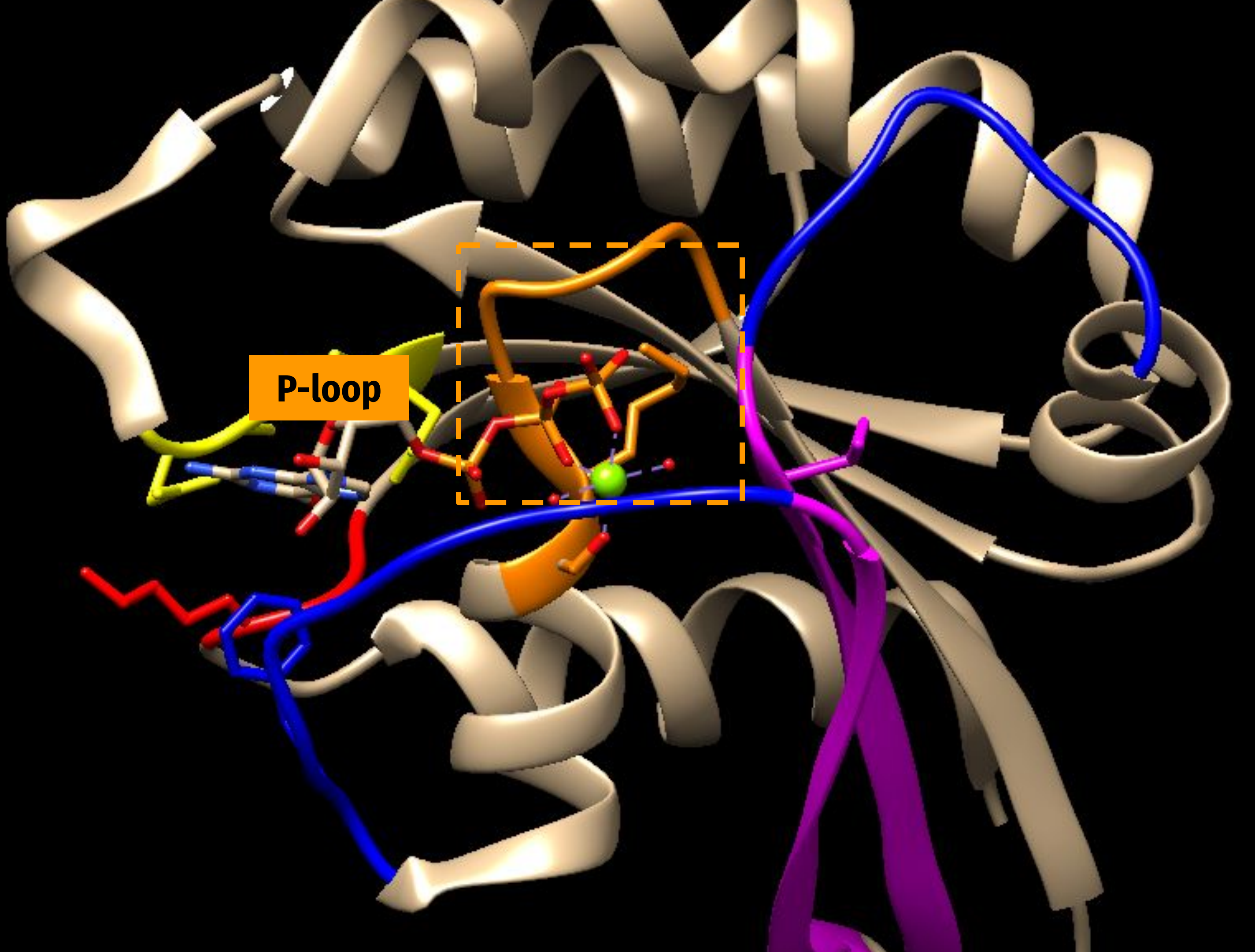
🟨 **G2**

⬜ **N/TKxD**

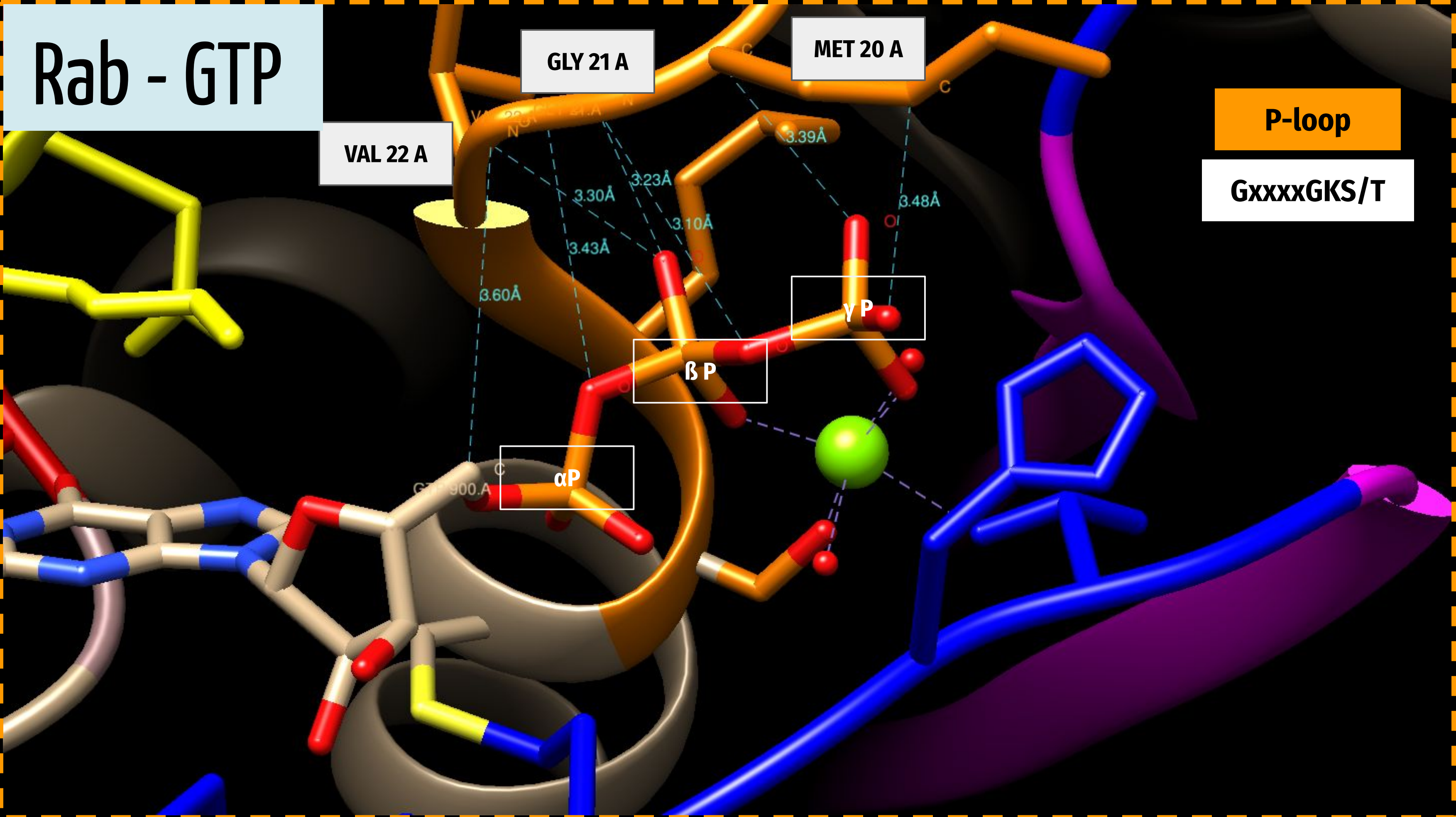
Rab - GTP


G3
sAk
SER 154 A
ALA 155 A
LYS 156 A
3.43Å
2.99Å
3.43Å
3.72Å
GLY 23.A
LYS 24.A
GTP 900.A
CYS 26.A
ALA 38.A
O
O
N
C
N

Rab - GTP



Rab - GTP



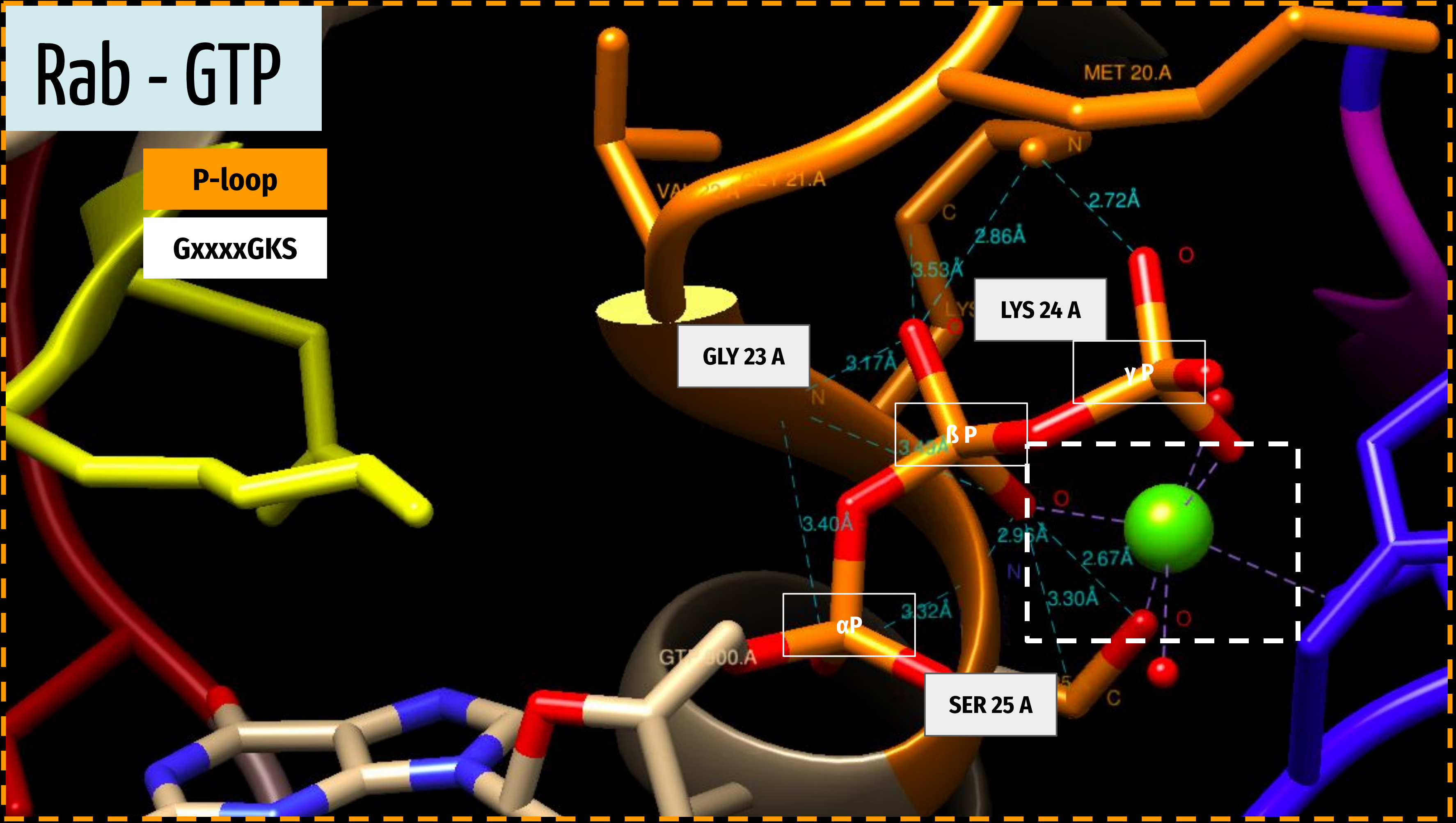
P-loop

GxxxxGKS/T

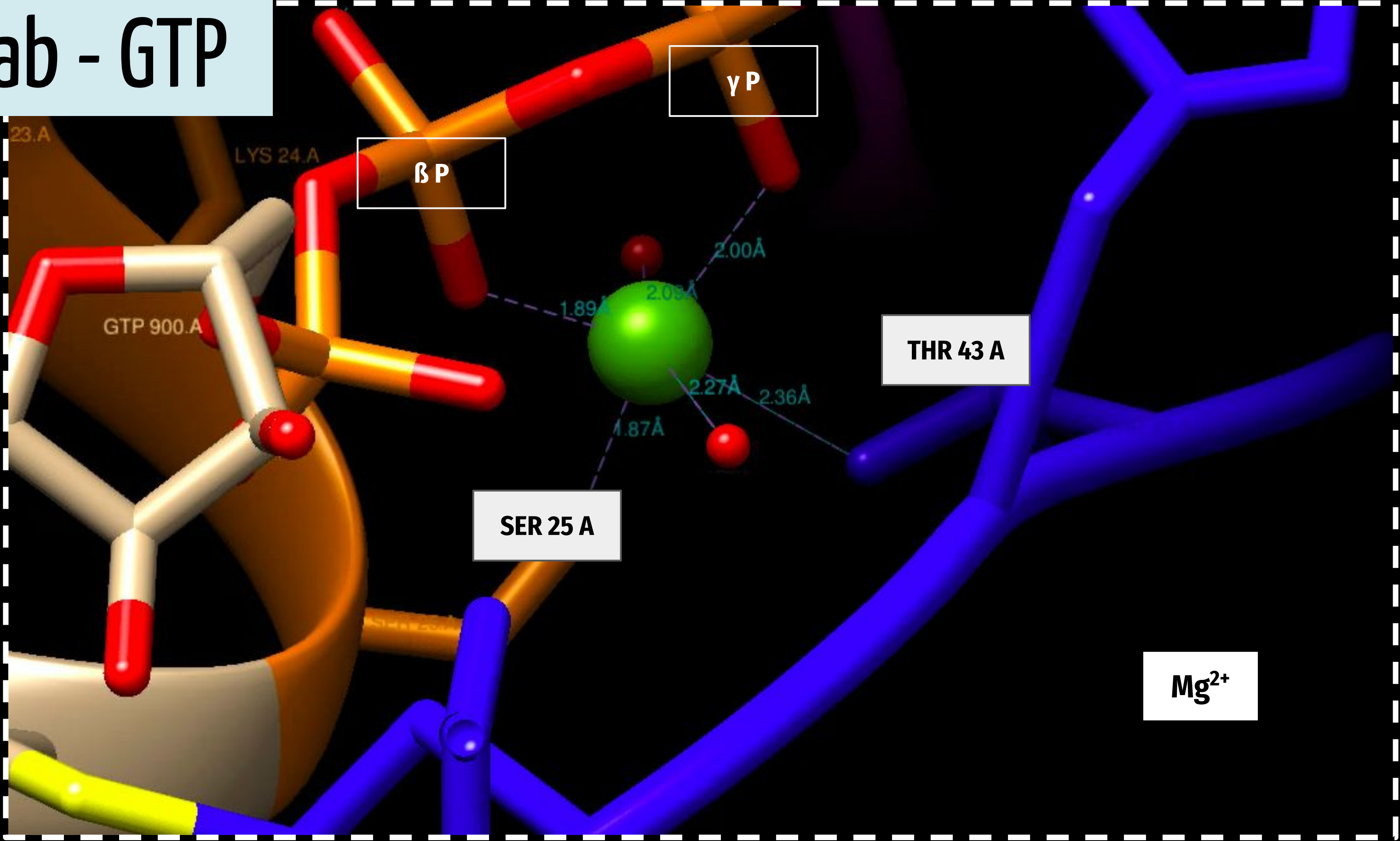
Rab - GTP

P-loop

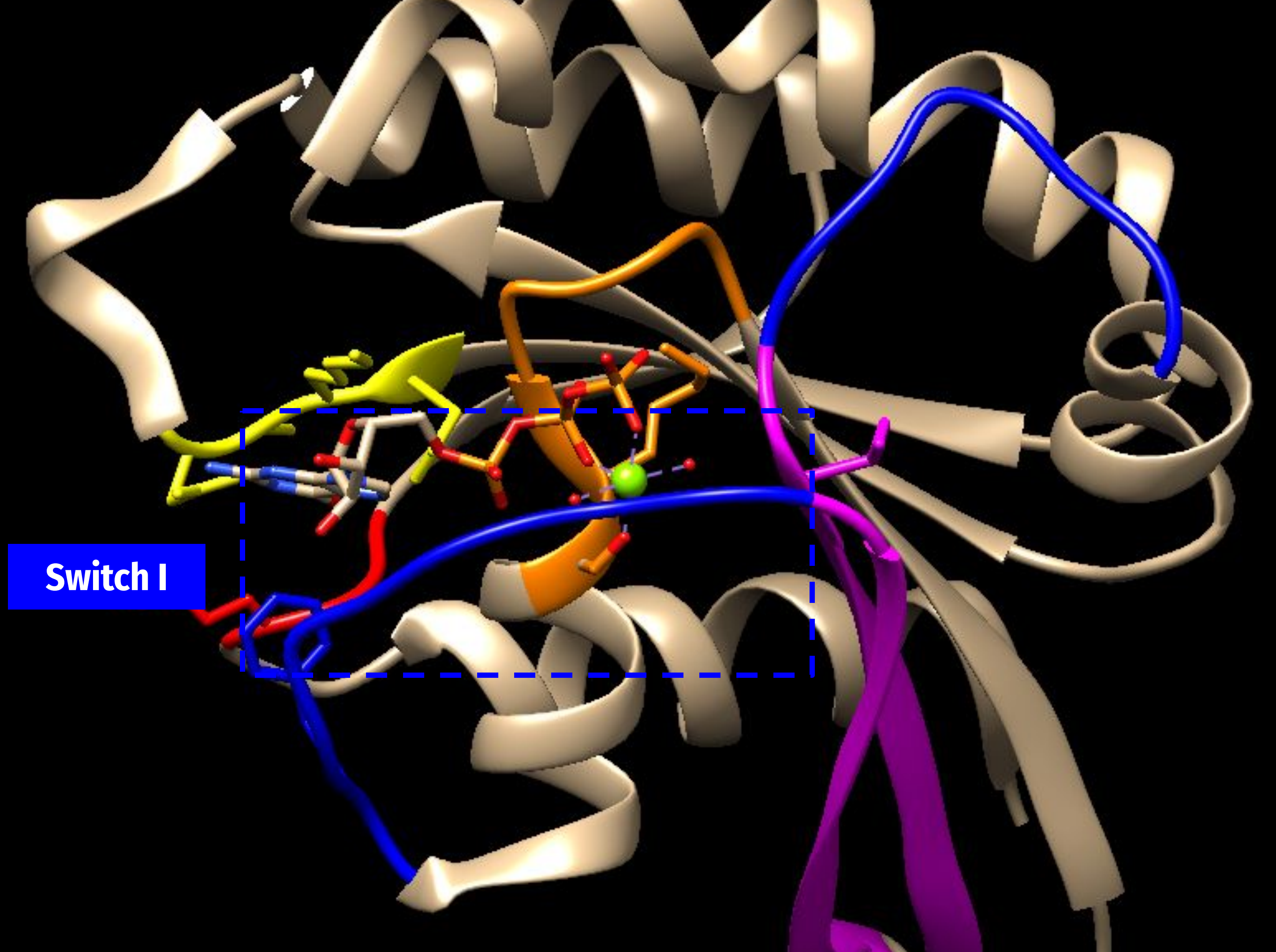
GxxxxGKS



Rab - GTP

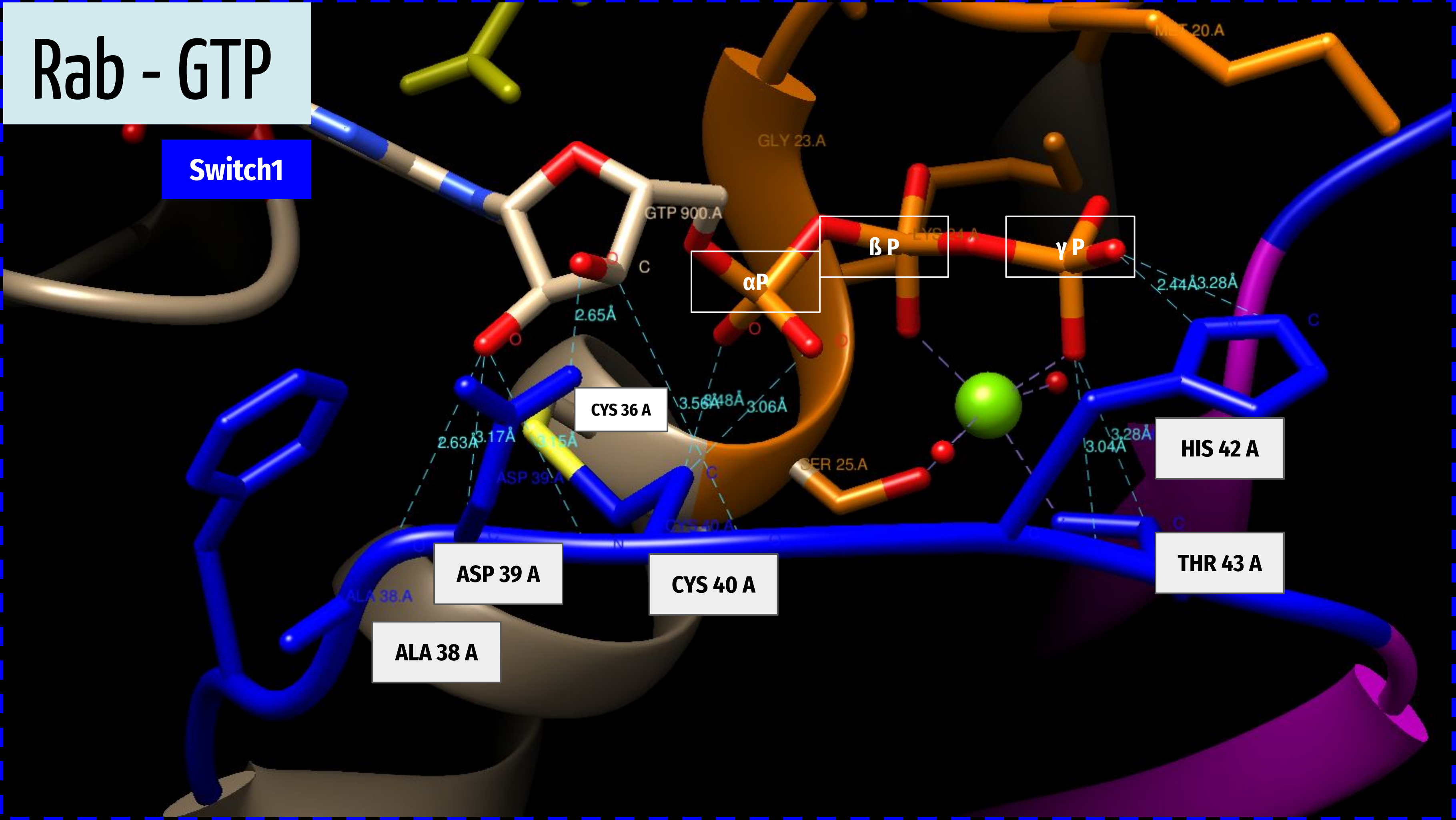

γ P
ß P
23.A
LYS 24.A
GTP 900.A
2.00Å
2.09Å
1.89Å
2.27Å
2.36Å
1.87Å
THR 43 A
SER 25 A
Mg^{2+}

Rab- GTP



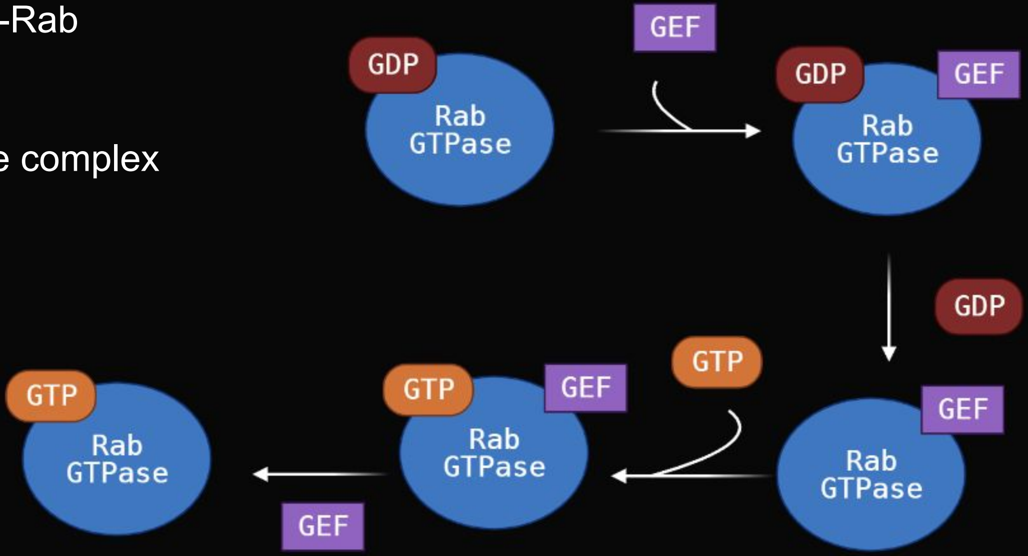
Rab - GTP

Switch1



Rab-GDP <-> GEF

1) GEFs destabilizes the ternary GDP-Rab
2) GDP is released
3) Nucleotide free-Rab + GEF = stable complex
4) GTP binding (higher concentration)
5) Destabilization = GEF released
6) Rab ACTIVE



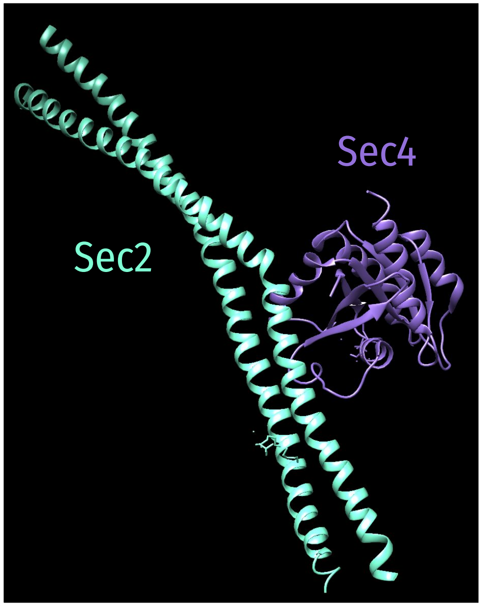
Own source

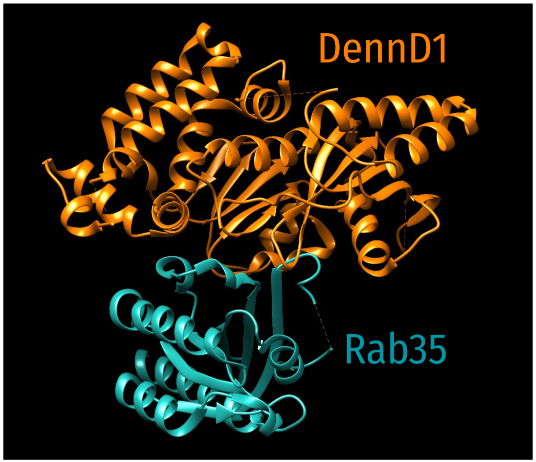
*Catalytic mechanism → variety

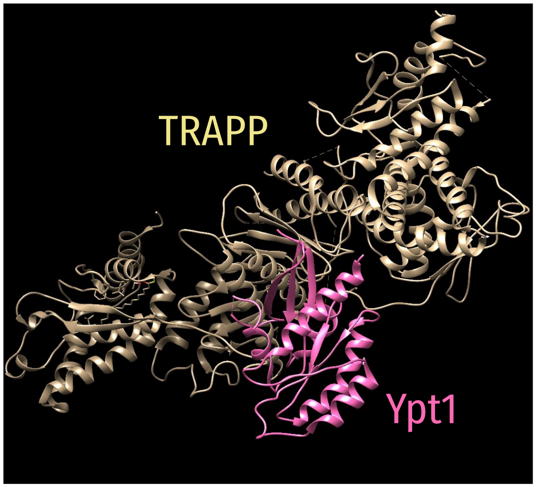
*Residues conservation GAP > GEF

*Opening of the nucleotide binding pocket + projection residues of Mg binding

Rab-GDP <-> GEF

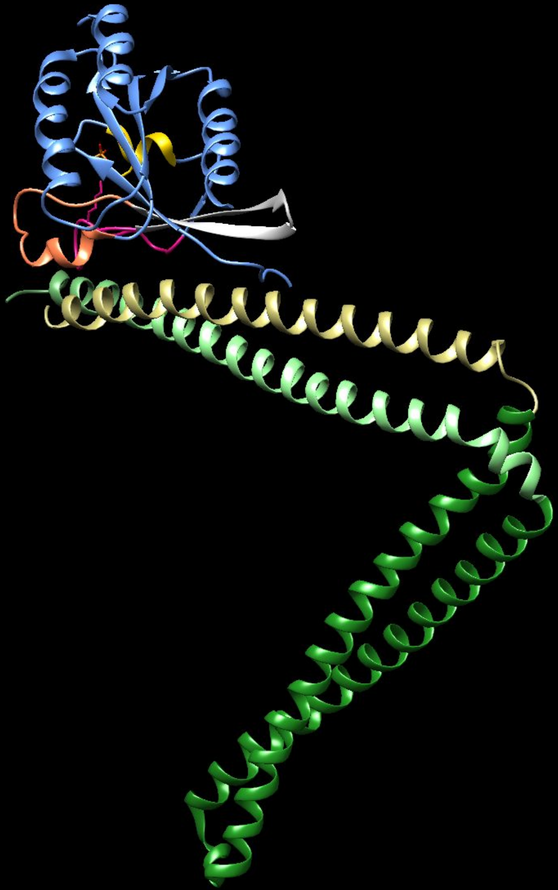


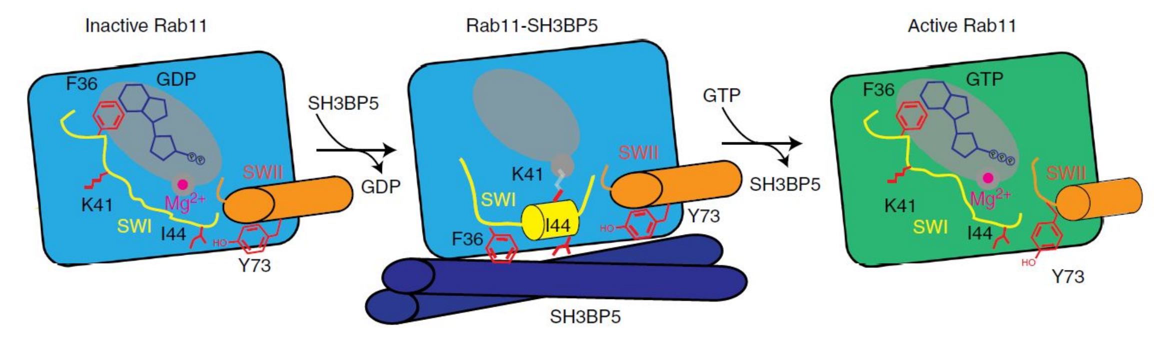




GEFs	Rab
DENN proteins	The largest class **Rab3, Rab27, Rab14, Rab35** u-DENN, c-DENN, d-DENN
VSP9 proteins	Vacuolar protein **Rab5, Rab21, Rab22** Alpha helix
Sec2 proteins	**Sec4** (yeast homologue of Rab8)
TRAPP complexes	Multi-subunit 3 forms (I, II, III) **Ypt1** (Rab1 homologue)
Heterodimer GEFS	Mon1A-Ccz1, HPS1-HPS4, Ric1-Rgp1, Rab3GAP1-Rab3GAP2 **Rab7, 32/38, 6, 18**
SH3BP5	**Rab11**

Rab11a INTERACTION WITH SH3BP5 (GEF)



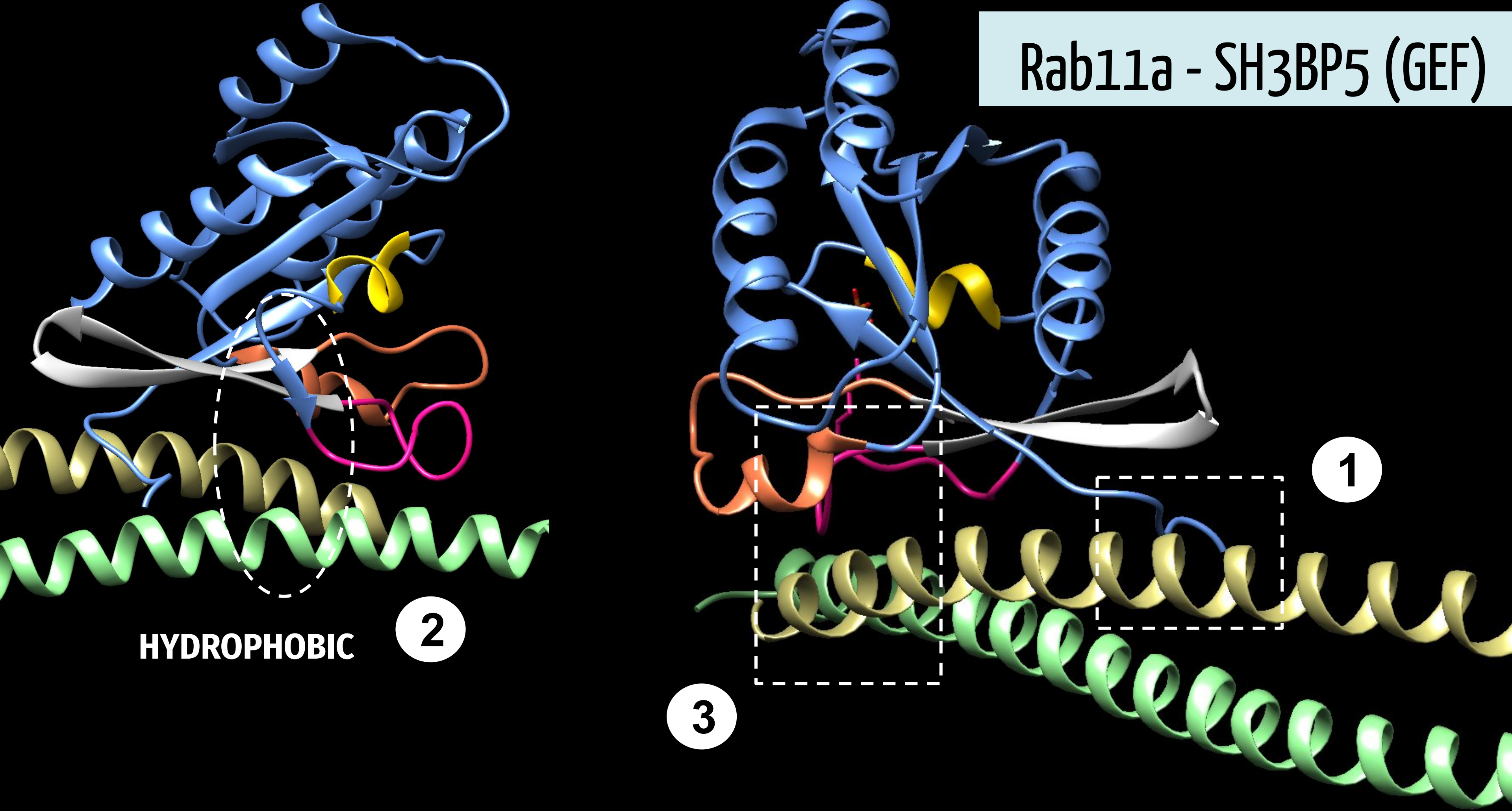


Jenkins M, Margaria J, Stariha J, Hoffmann R, McPhail J, Hamelin D et al. Structural determinants of Rab11 activation by the guanine nucleotide exchange factor SH3BP5. Nature Communications. 2018;9(1).

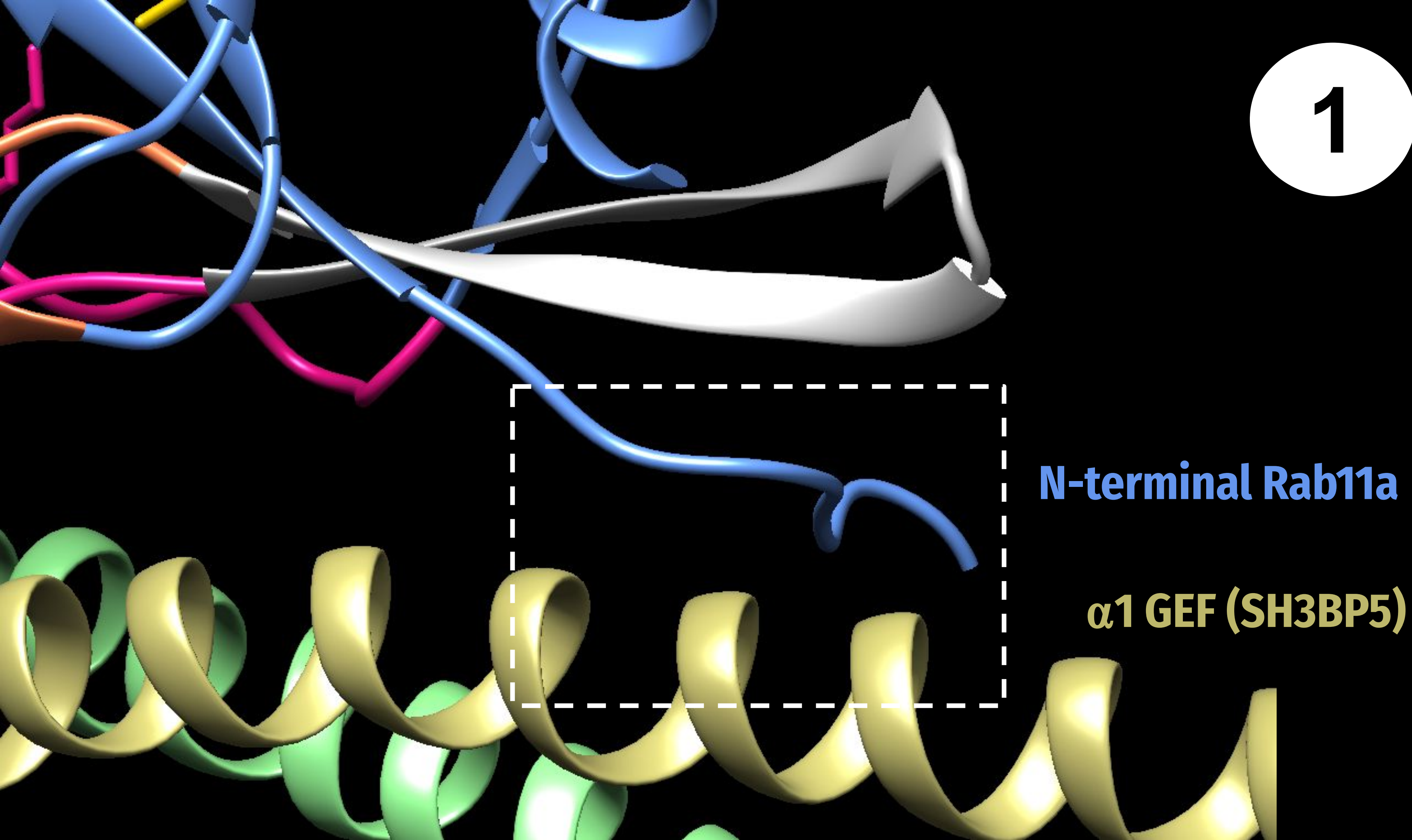
SH3BP5 (GEF) : α1 + α2 + α3 + α4

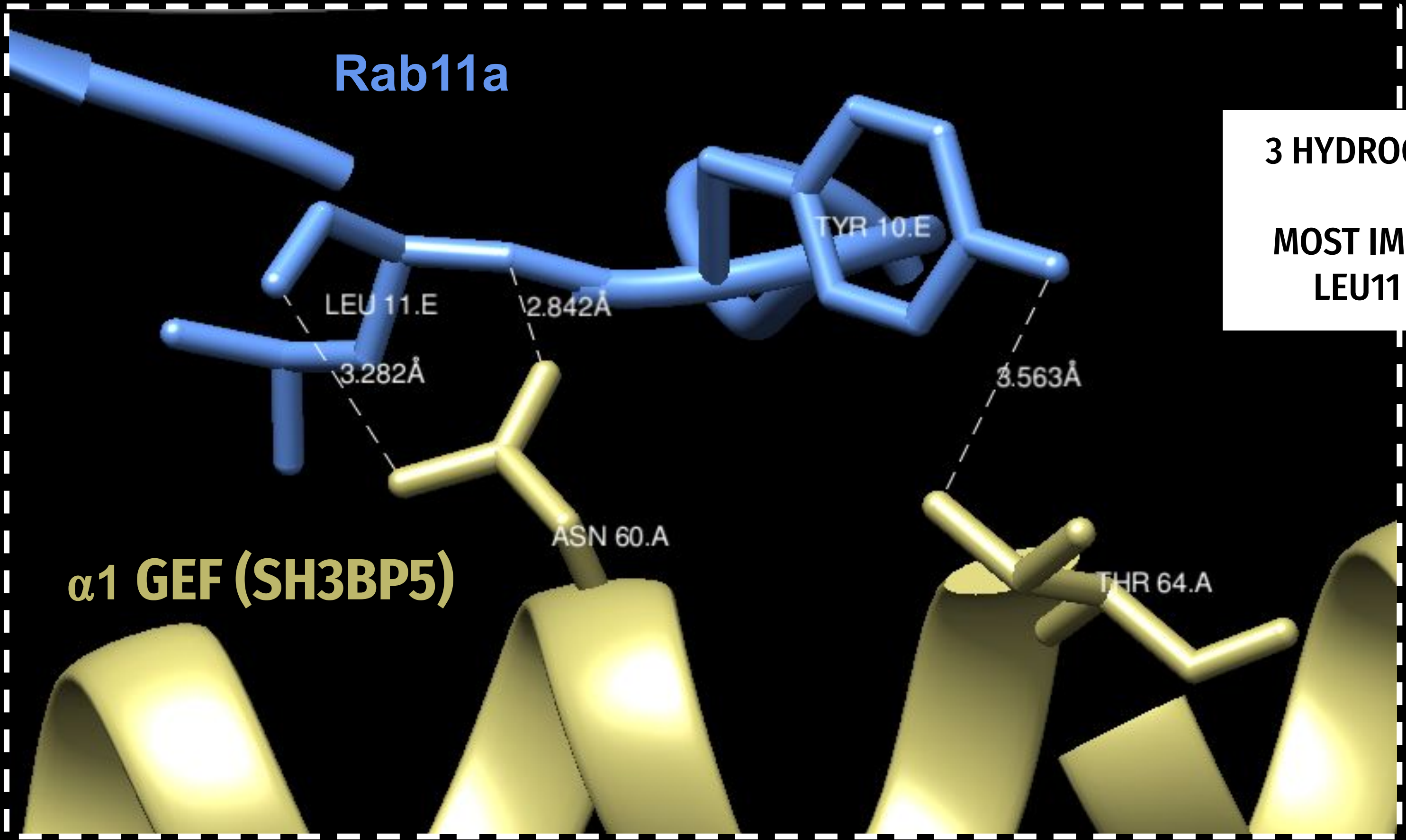
Rab11a :
P-loop + Switch I + Switch II + Interswitch

Rab11a - SH3BP5 (GEF)



①



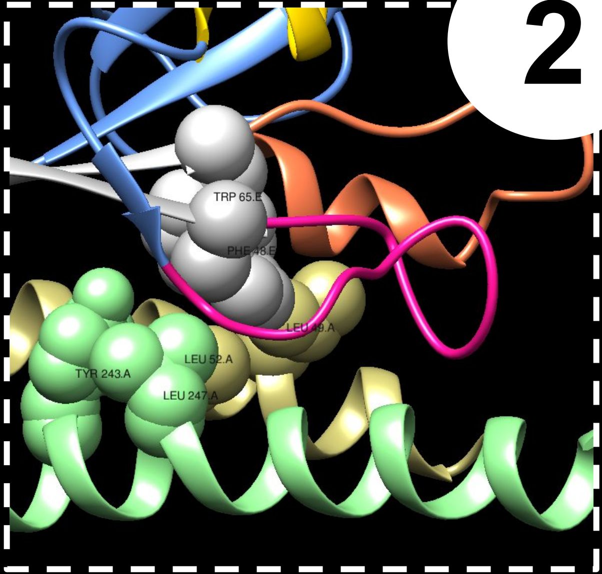


3 HYDROGEN BONDS

MOST IMPORTANTS:
LEU11 - ASN60

HYDROPHOBIC INTERACTION

INTERSWITCH Rab11 + α1 α4 GEF (SH3BP5)

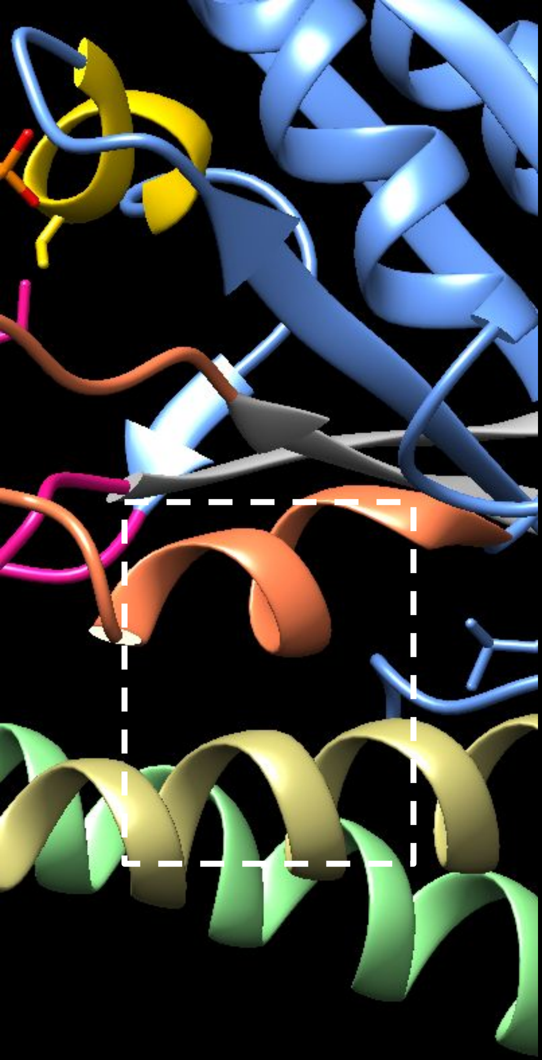


PHE48 + TRP65

LEU49 + LEU 52

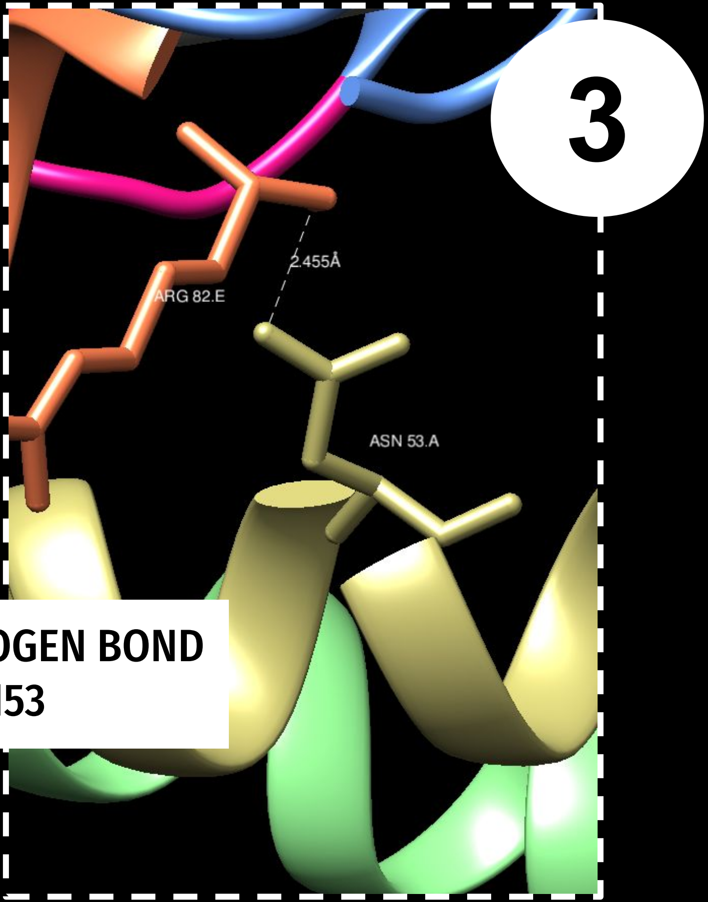
TYR 243 + LEU 247

\+ **DISPENSABLE H.BOND GLN63 - TYR243**



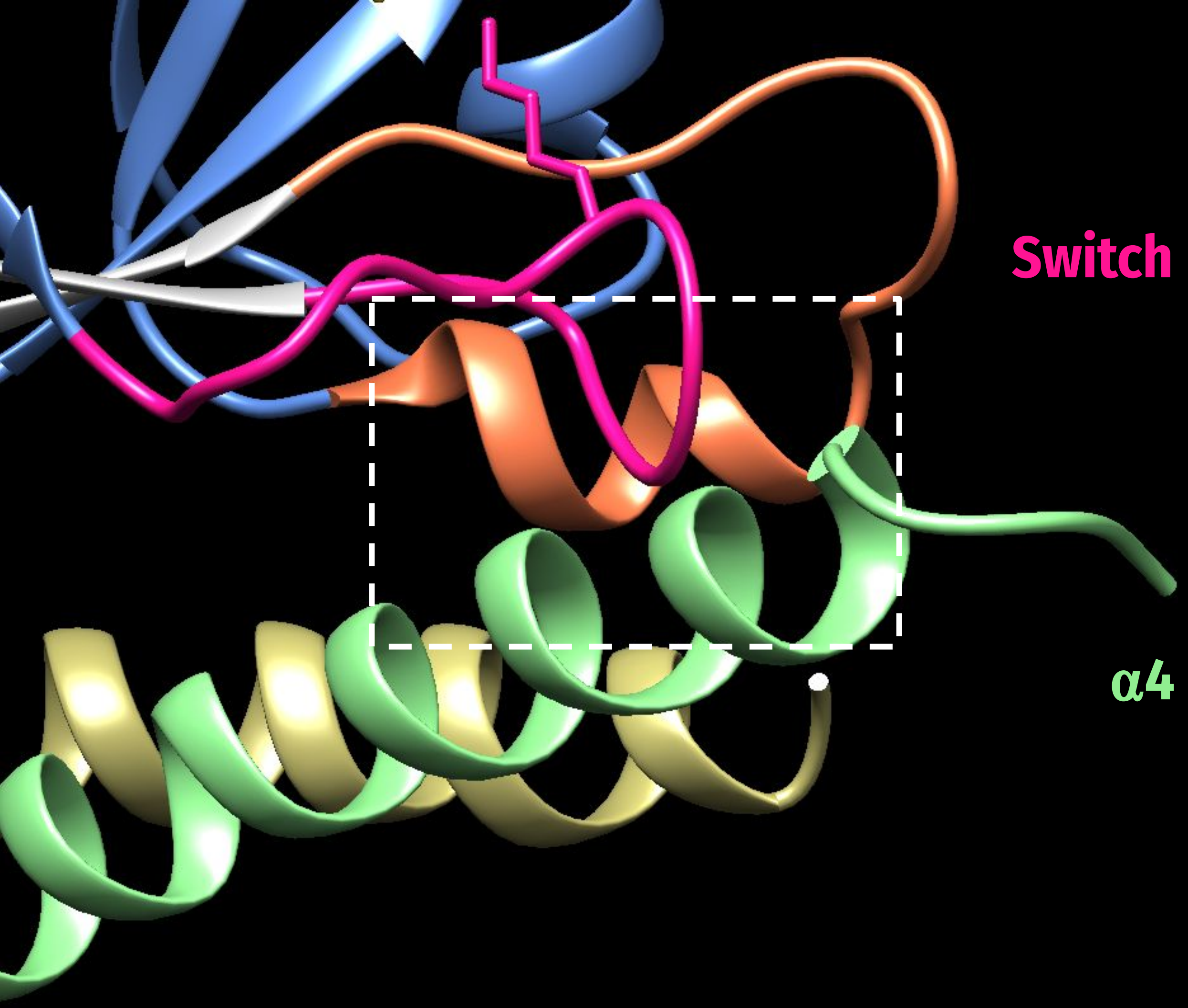
Switch II Rab11a

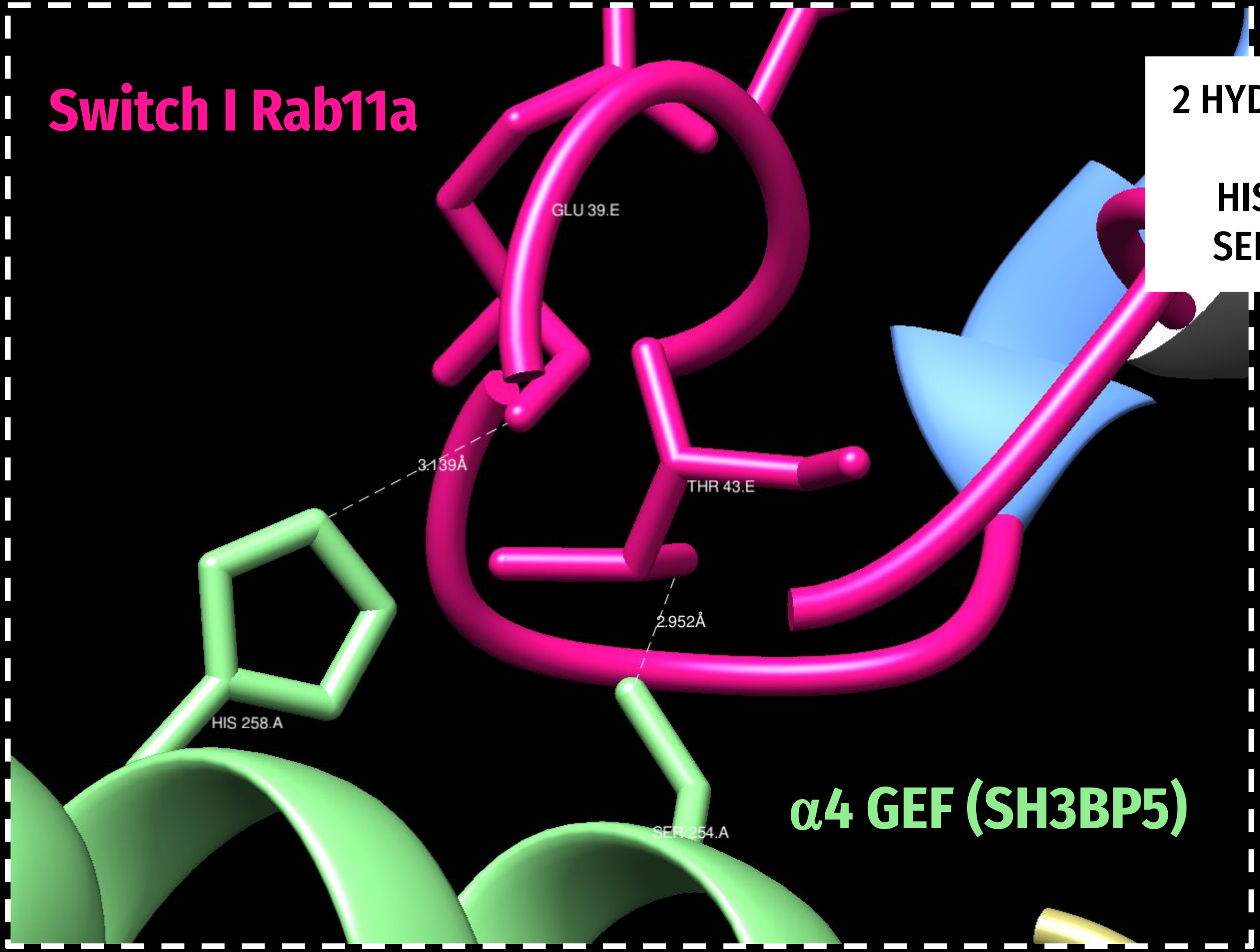
α1 GEF (SH3BP5)



AN ESSENTIAL HYDROGEN BOND
ARG82 - ASN53

③



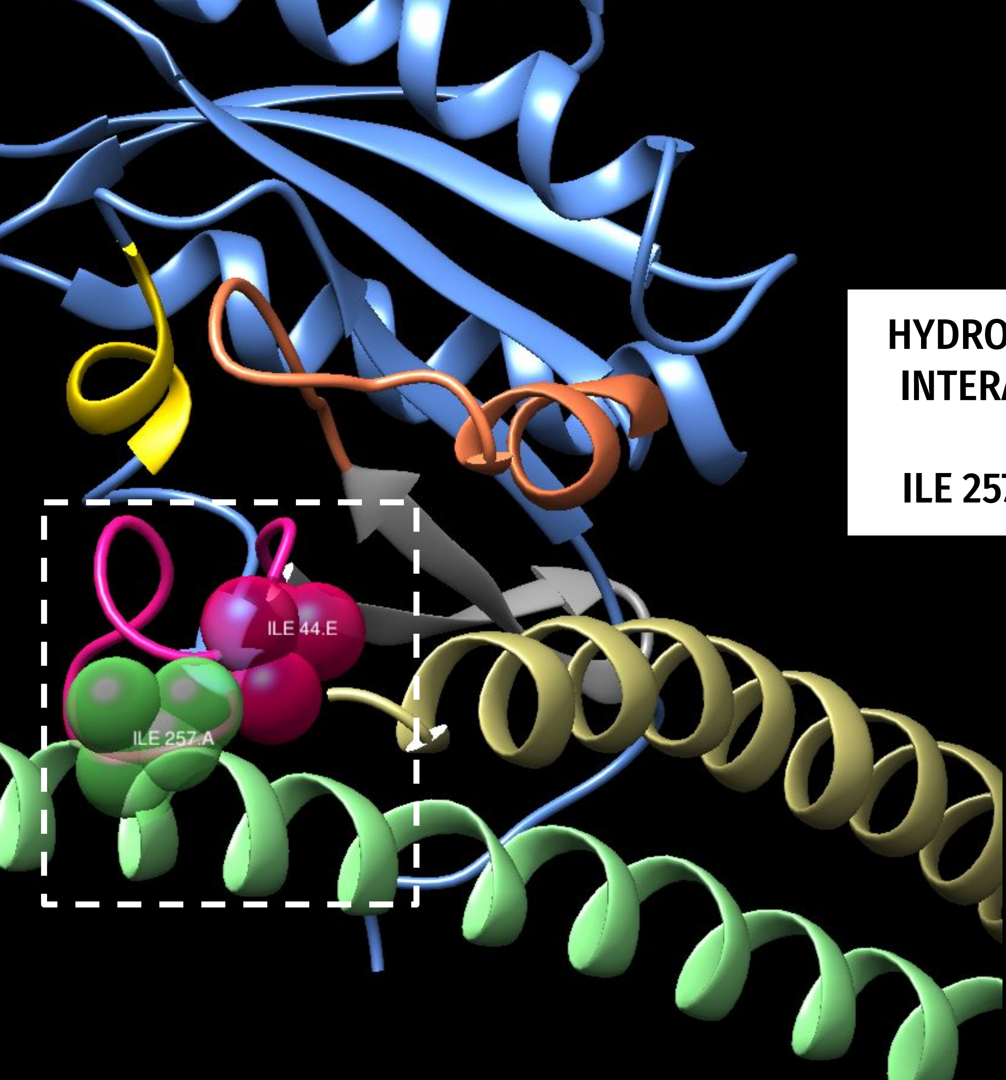


2 HYDROGEN BONDS

HIS258 - GLU39
SER254 - THR43

THR43 coordinates Mg^{2+} in the nucleotide-bound Rab11a

3



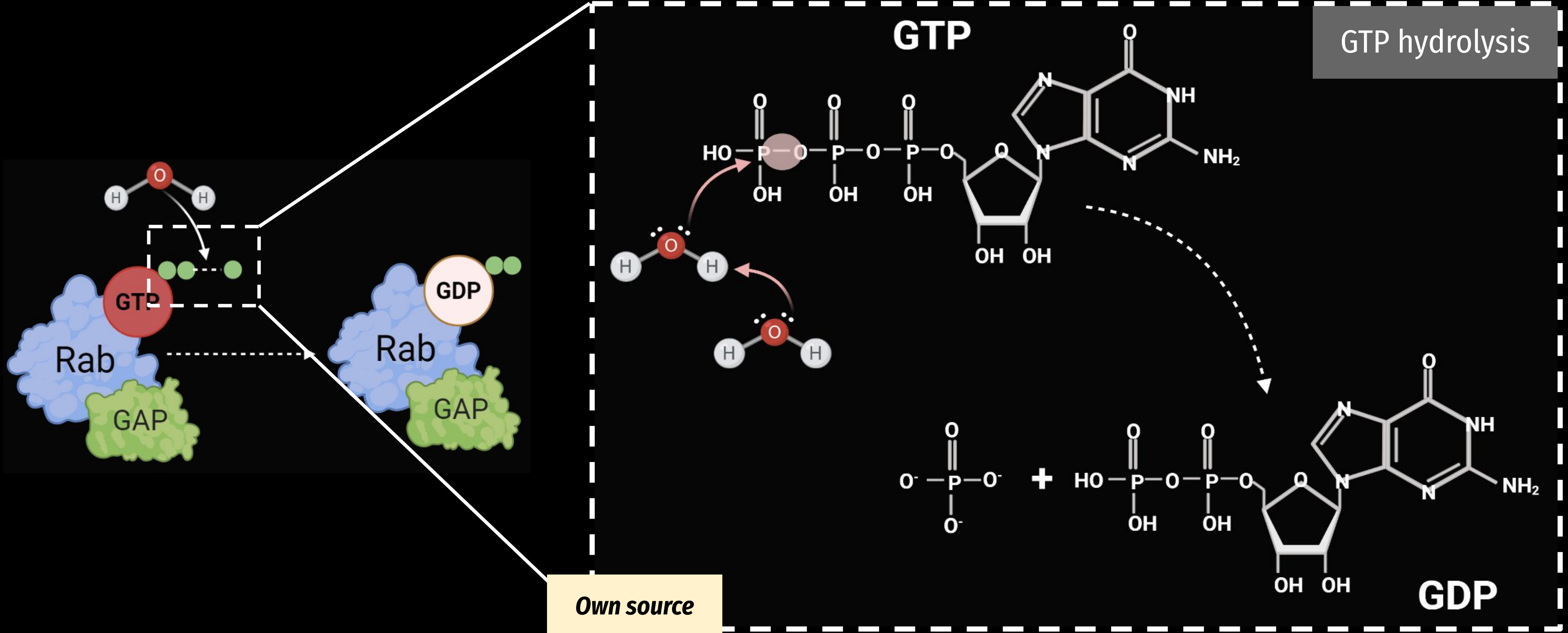
HYDROPHOBIC INTERACTION

ILE 257-ILE44

Switch I Rab11a

α4 GEF (SH3BP5)

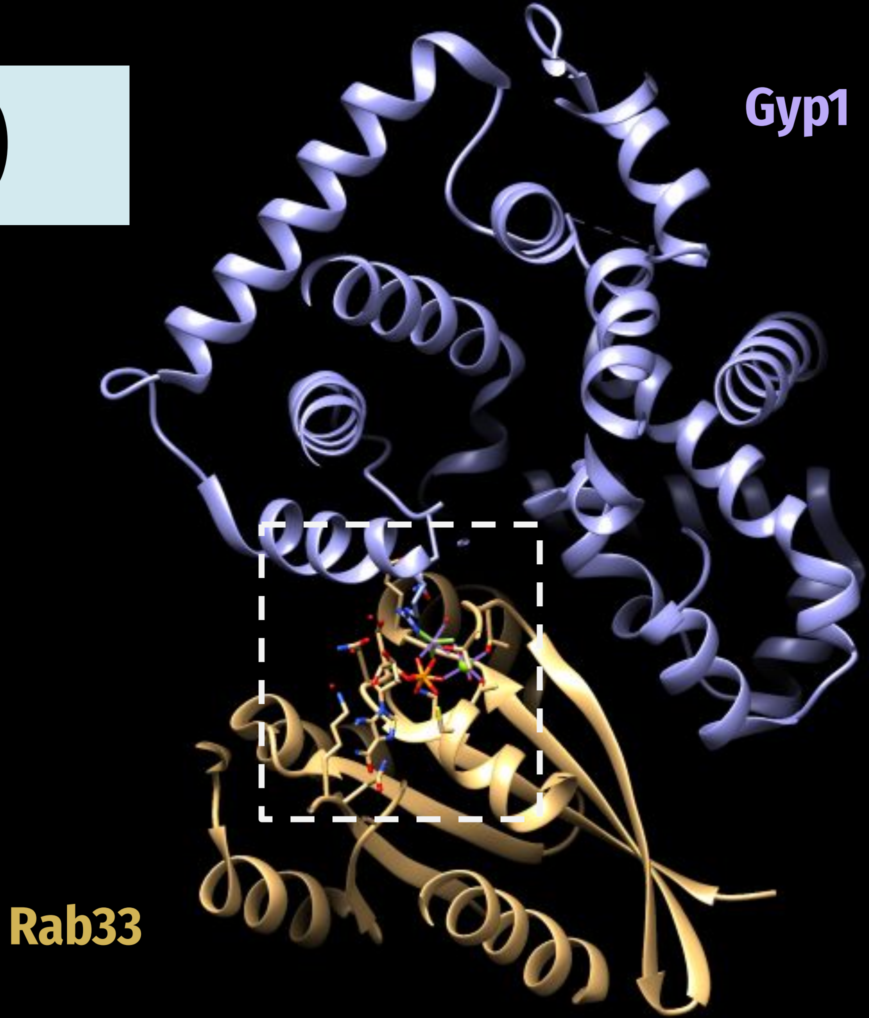
GTPase Activating Protein (GAP)- GTP Hydrolysis



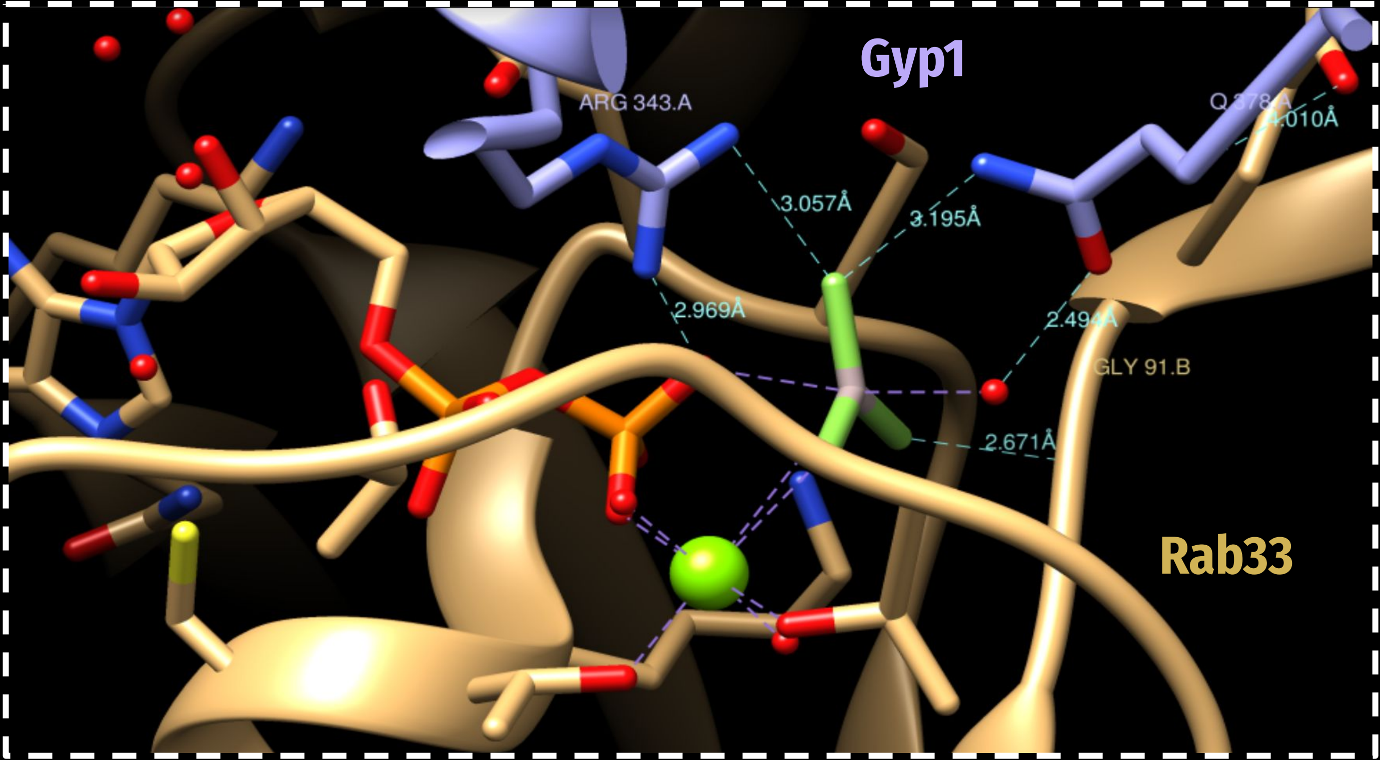
Own source

GTPase Activating Protein (GAP)

- Rab GAPs have a very conserved structure
- TBC domain (Tre2, Bub2, Cdc16)
- Arginine Finger
- Glycine Finger
- Gyps (GAP for Ypt proteins)



Rab33 INTERACTIONS WITH Gyp1 (GAP)



Arginine Finger

Glycine Finger

Aluminium Fluoride

GDP molecule

Glycine 91B

EFFECTORS

Rab protein	Effector	Function/Membrane Traffic Pathway
Rab14	FIP2,RCP, Rip11, D-AKAP2	TGN/RE to plasma membrane; apical membrane targeting
Rab11a	Sec15, Rab11-FIP1 to FIP5	TGN/RE to plasma membrane
Rab7A	Vps 35/29/26 complex (retromer), Rabring7, proteasome alpha-subunit PSMA7, Vps34/p150 PI3-kinase complex	Late endosome to lysosome

TAKE HOME MESSAGES

1. Rab proteins are a huge family
2. There’s a conservation through the evolution and the phylogeny between species
3. The interaction REP1-Rab-GGTase is essential to its anchorage at the membrane
4. The active state of a G protein is mediated by GAP and GEF (the protein will be active in the presence of GTP and inactive with GDP)
5. G proteins have a common structure
6. There’s homology between the alpha region of trimeric G proteins and monomeric G proteins
7. A mutation in a G protein cycle or structure implies a disease

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- Europe P. Ligand GTP bound in PDB entry 4d0g ‹ Protein Data Bank in Europe (PDBe) ‹ EMBL-EBI [Internet]. Ebi.ac.uk. 2021. Available from: https://www.ebi.ac.uk/pdbe/entry/pdb/4d0g/bound/GTP

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THANK YOU FOR YOUR ATTENTION!

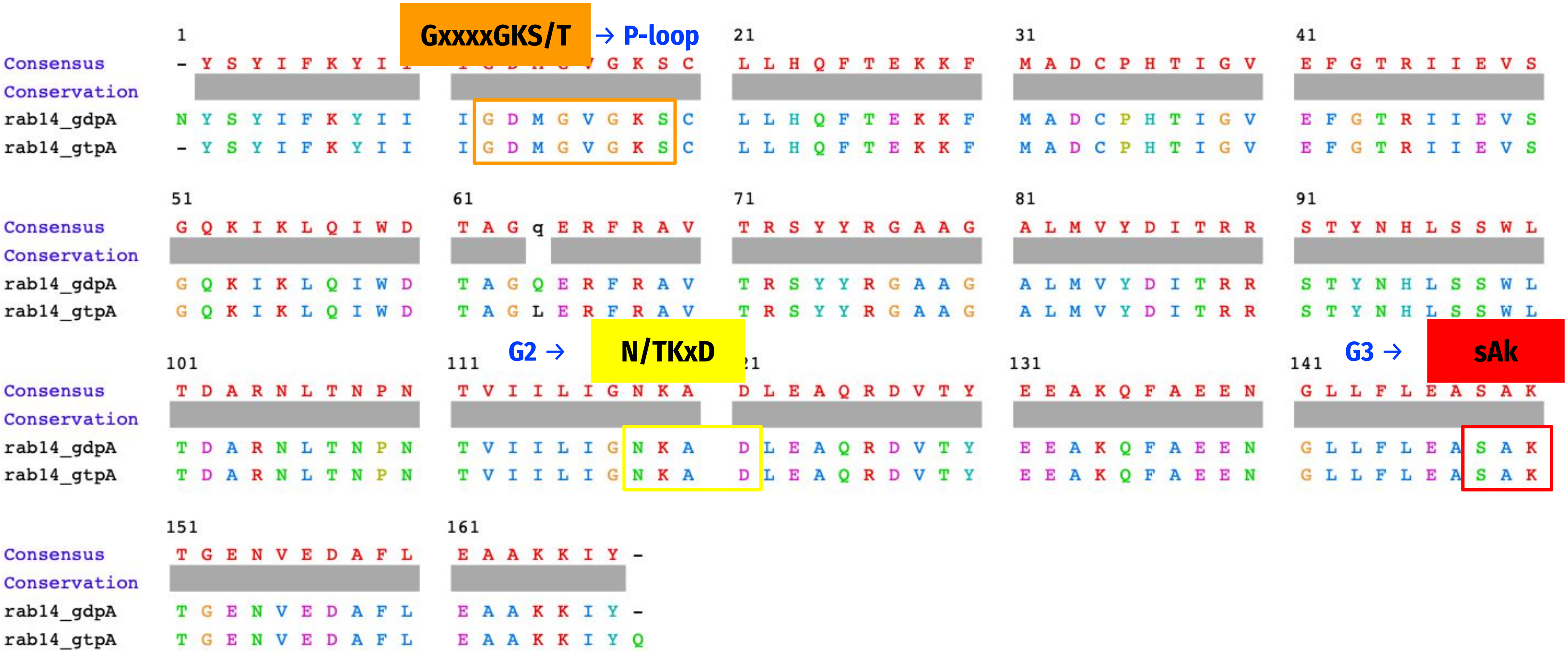
EXTRA

WHY THIS INTERACTION IS WEAK?

Rabs wt/mutants	C-terminal sequence	*Kd* of binary complex, REP-1 (*nM*)	*Kd* of ternary complex, RabGGTase (*nM*)
Rab7wt	EFPEP**I**K**L**DKNDRAKTSAES**CSC**	7.5 ± 2.7	130 ± 9.3
Rab7Δ3	EFPEP**I**K**L**DKNDRAKTSAES	16.1 ± 1.0	191 ± 22
Rab7Δ14	EFPEP**I**K**L**D	15.8 ± 2.1	321 ± 11
Rab7Δ22	E	381 ± 37	491 ± 31
Rab7-5A	EFPEP**I**K**L**DKNDRAAAAAA**CSC**	21.5 ± 1.1	188 ± 45

Summary of dissociation constants for interaction between Rab7wt/mutants and REP and RabGGTase. Adapted from: Wu Y, Goody R, Abagyan R, Alexandrov K. Structure of the Disordered C Terminus of Rab7 GTPase Induced by Binding to the Rab Geranylgeranyl Transferase Catalytic Complex Reveals the Mechanism of Rab Prenylation. *J Biol Chem*. 2009; 284(19): 13185-13192.

Rab-GTP vs Rab -GDP : IMPORTANT MOTIFS



GxxxxGKS/T → P-loop

G2 → N/TKxD

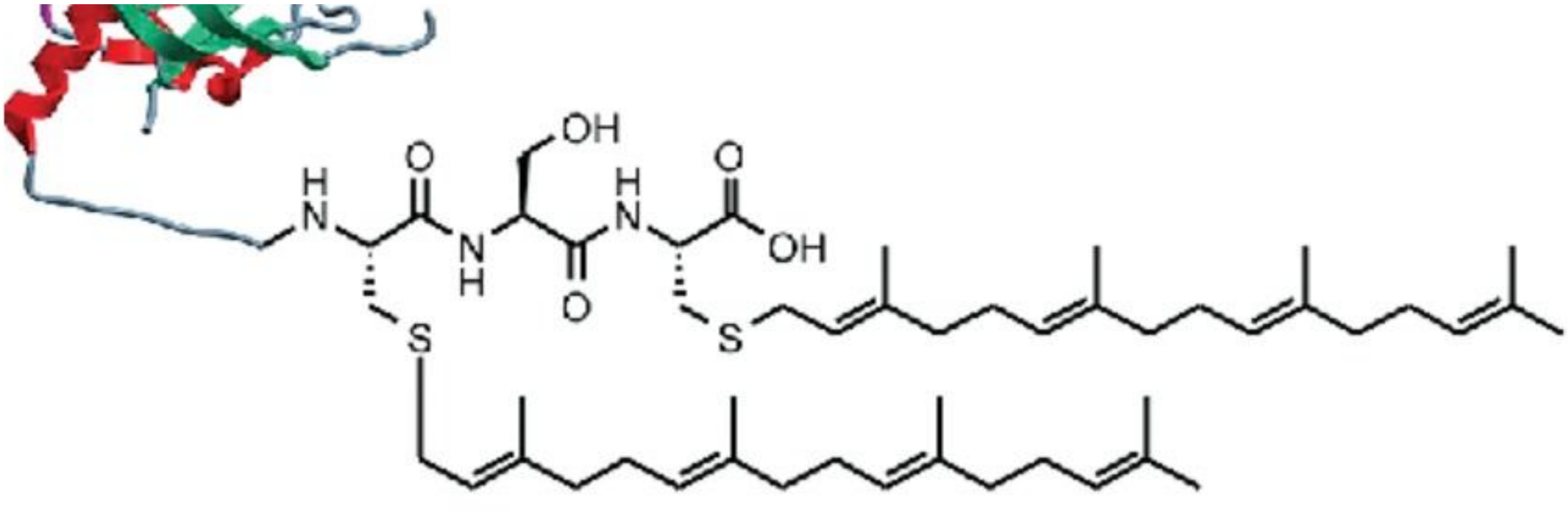
G3 → sAk

```
              1                     11                    21                    31                    41
Consensus     - Y S Y I F K Y I I   I G D M G V G K S C   L L H Q F T E K K F   M A D C P H T I G V   E F G T R I I E V S
Conservation
rab14_gdpA    N Y S Y I F K Y I I   I G D M G V G K S C   L L H Q F T E K K F   M A D C P H T I G V   E F G T R I I E V S
rab14_gtpA    - Y S Y I F K Y I I   I G D M G V G K S C   L L H Q F T E K K F   M A D C P H T I G V   E F G T R I I E V S

              51                    61                    71                    81                    91
Consensus     G Q K I K L Q I W D   T A G q E R F R A V   T R S Y Y R G A A G   A L M V Y D I T R R   S T Y N H L S S W L
Conservation
rab14_gdpA    G Q K I K L Q I W D   T A G Q E R F R A V   T R S Y Y R G A A G   A L M V Y D I T R R   S T Y N H L S S W L
rab14_gtpA    G Q K I K L Q I W D   T A G L E R F R A V   T R S Y Y R G A A G   A L M V Y D I T R R   S T Y N H L S S W L

              101                   111                   121                   131                   141
Consensus     T D A R N L T N P N   T V I I L I G N K A   D L E A Q R D V T Y   E E A K Q F A E E N   G L L F L E A S A K
Conservation
rab14_gdpA    T D A R N L T N P N   T V I I L I G N K A   D L E A Q R D V T Y   E E A K Q F A E E N   G L L F L E A S A K
rab14_gtpA    T D A R N L T N P N   T V I I L I G N K A   D L E A Q R D V T Y   E E A K Q F A E E N   G L L F L E A S A K

              151                   161
Consensus     T G E N V E D A F L   E A A K K I Y -
Conservation
rab14_gdpA    T G E N V E D A F L   E A A K K I Y -
rab14_gtpA    T G E N V E D A F L   E A A K K I Y Q
```



di-geranylgeranylated Rab

QUESTIONS

1. **Choose the correct affirmation about small GTPases:**
   a) In the transition of active and inactive state switch regions are known to undergo large conformational changes.
   b) In the inactive state it has been demonstrated that its structure has a high degree of flexibility and disorder for the Switch1 and Switch2 regions
   c) Both a and c are correct.
   d) In the activate state their structures show a more restricted conformational flexibility compared with the inactive form.
   e) All of them are correct.

2. **Which functions are regulated by Rab proteins?**

1. Vesicle biogenesis
2. Vesicle transport
3. Vesicle tethering
4. Vesicle fusion
   a) 1,2,3
   b) 2 i 4
   c) 1 i 3
   d) 4
   e) 1,2,3 i 4

QUESTIONS

3. Mark the incorrect answer:

a) The Ras superfamily is divided into five families: Ras, Rho, Arf/Sar, Ran, and Rab.
b) The separation between G protein families was an early evolutionary event that predated the expansion of eukaryotes.
c) G proteins have not a common structure.
d) The original function of these proteins may have been related to the regulation of membrane trafficking.
e) Some species have GPCRs without G proteins and vice versa.

4. Choose the correct answer related with the interaction Rab-REP-RabGGTase (ternary complex):

a) The prenylation (the addition of hydrophobic molecules to a protein) of Rab is optional for membrane anchoring.
b) There is a Na^+ ion in the active site of RabGGTase.
c) The CIM (C-terminal interacting motif) of Rab interacts with REP.
d) There is not any hydrophobic interaction in the assembly of the ternary complex.
e) RabGGTase can recognize directly the Rab C-terminal peptide as a substrate.

QUESTIONS

5. Which structure of Rab11 doesn't interact with GEF?

a. P-loop
b. Switch I
c. Switch II
d. Interswitch
e. N-terminal

6. Choose the correct affirmation(s) about GEF:

1. GEFs destabilize the GTPase interaction with GDP
2. Each GEF interacts with the Rab surface differently
3. GEF plays an important role in activating Rab proteins
4. GEF stabilizes the intermediate nucleotide-free state of small GTPases

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

QUESTIONS

7. The most important sequence motifs in Rab proteins are:

a. Alpha helices and beta sheets
a. Conserved regions (F1-F5), phosphate/magnesium-binding motifs (PM1-PM3) and guanine-binding motifs (G1-G3)
b. P-loop, switch I, interSwitch and switch II
c. Switch III and Switch IV

8. Which is the secondary conserved structure of Ras superfamily?

b. 6 stranded ß-sheet (with six parallel strands) and 5 α-helices
c. 6 stranded ß-sheet (with five parallel strands and one antiparallel) and 5 α-helices
d. 6 stranded ß-sheet (with six parallel strands and one antiparallel) and 4 α-helices
e. 5 stranded ß-sheet (with four parallel strands and one antiparallel) and 4 α-helices

QUESTIONS

9. Which of these affirmations about GTPase Activating Protein (GAP) is correct:

a. Rab GAPs have a very conserved structure
b. GAP hydrolyzes GTP to GDP
c. a) and b) are correct
d. GAP exchanges GDP for GTP
e. All are incorrect

10. Choose the correct affirmation(s) about trimeric G-proteins:

1. Trimeric G-proteins have two β subunits and an α one.
2. The Gβ subunit has GTPase intrinsic activity.
3. The GTPase domain isn't structurally homologous to the monomeric G-proteins family.
4. The Gα subunit is the most heavy subunit.

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