structural analysis of

G-Proteins

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Carla Fontanet,
Carolina Pazos

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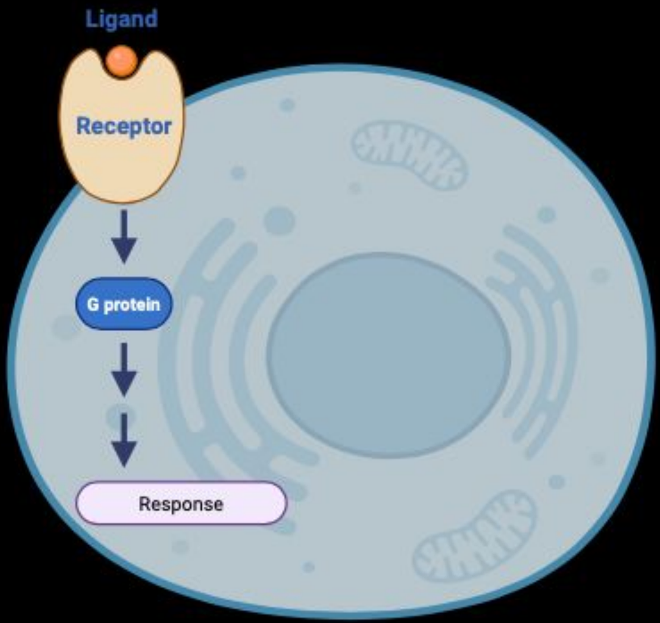
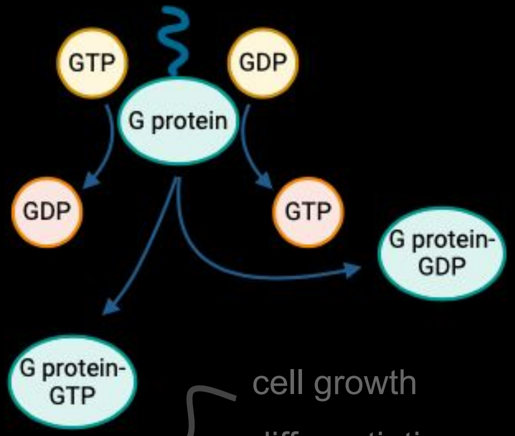
G-Proteins



transmission of signals from outside the cell to the inside



cell growth

differentiation

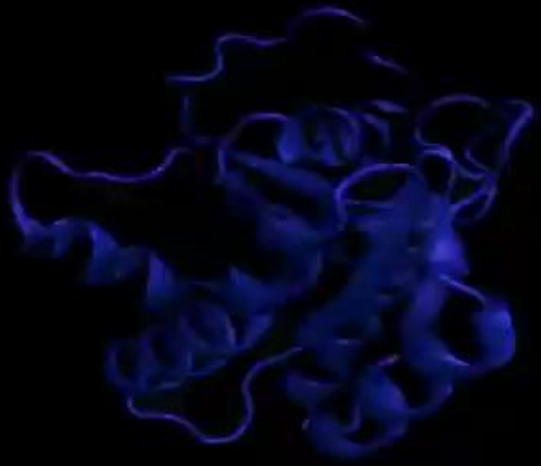
neurotransmission

hormone signaling

G-Proteins Classification

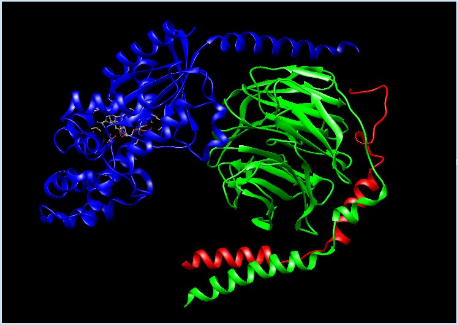
Heterotrimeric G-Proteins

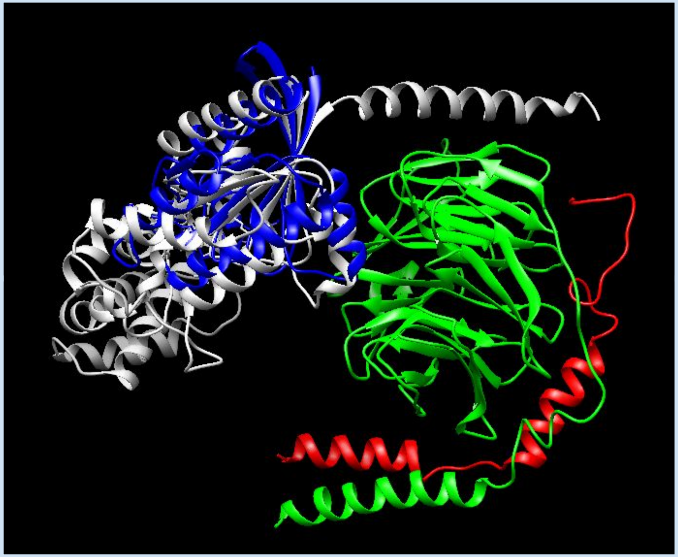
Monomeric G-proteins



Heterotrimeric vs Monomeric

heterotrimeric





alignment score Sc = 3.459473
RMSD = 1.251992

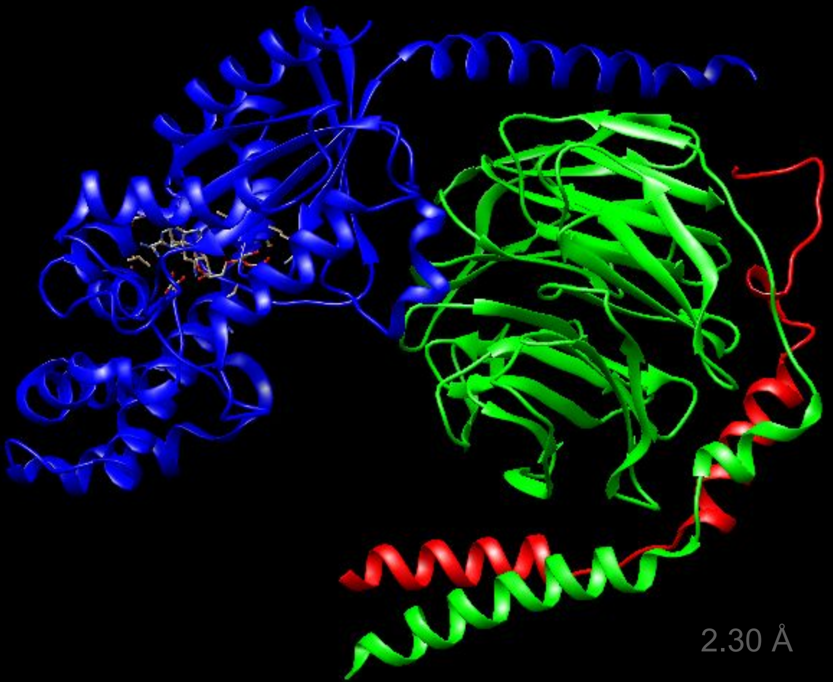
monomeric

Heterotrimeric G-Proteins

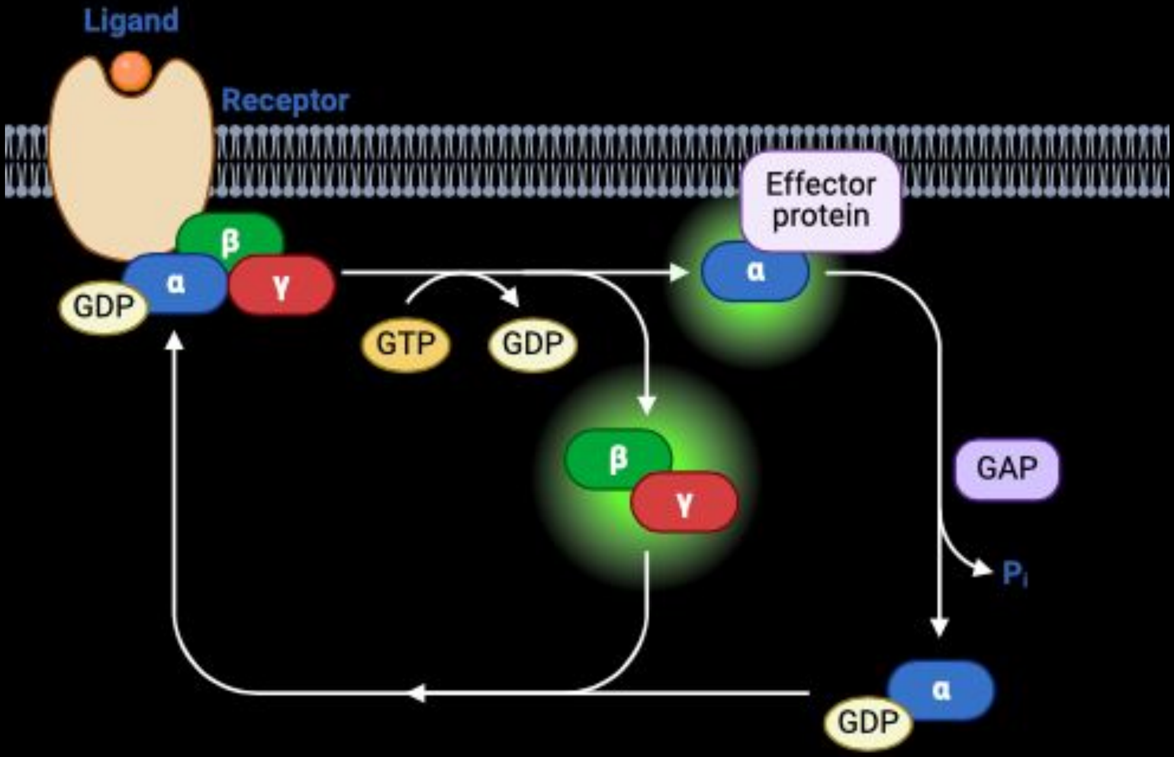
molecular weight: 86 kDa

3 subunits

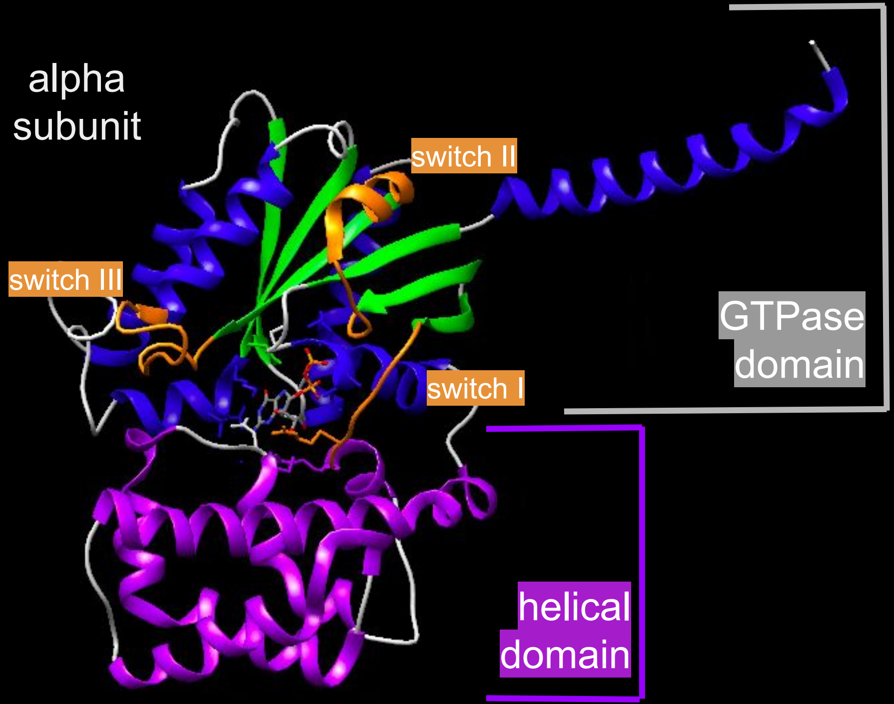
alpha	beta	gamma
binding to GNP	bind to downstream molecules	

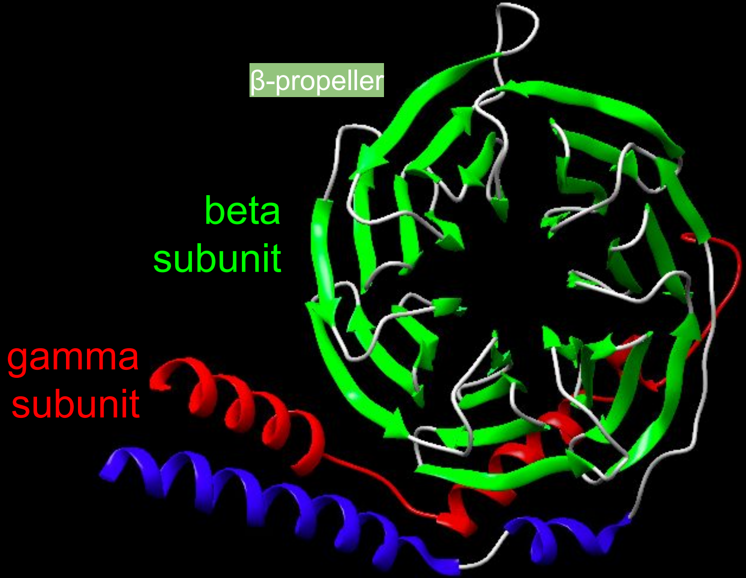


Heterotrimeric G-Proteins



Heterotrimeric G-Proteins

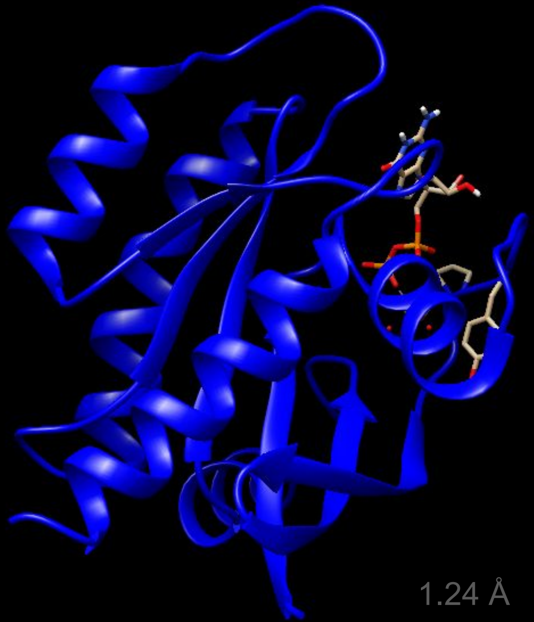




Monomeric G-Proteins

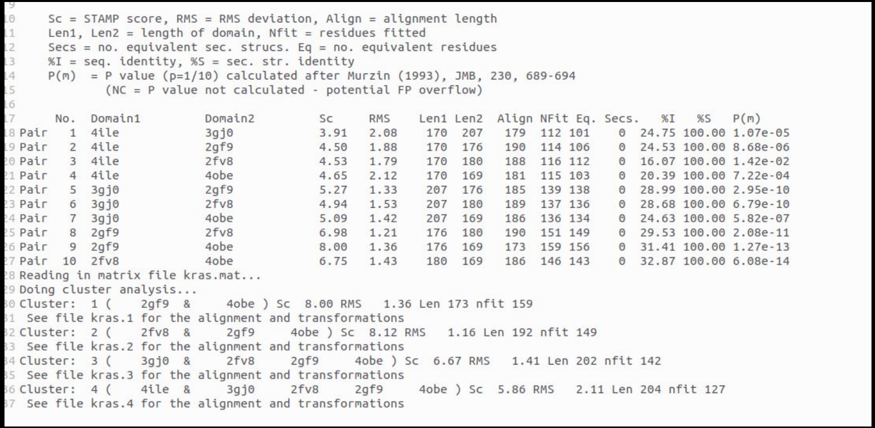
molecular weight: 20 kDa

- superfamily of G-Proteins
  - Ras
    - proliferation
    - differentiation
    - survival
  - Rho
    - cytoskeleton
    - morphology
  - Rab
    - vesicular transport
  - Arf
    - coating vesicles
  - Ran
    - nuclear transport



1.24 Å

Superimposition between families



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    Sc = STAMP score, RMS = RMS deviation, Align = alignment length
    Len1, Len2 = length of domain, Nfit = residues fitted
    Secs = no. equivalent sec. strucs. Eq = no. equivalent residues
    %I = seq. identity, %S = sec. str. identity
    P(m)  = P value (p=1/10) calculated after Murzin (1993), JMB, 230, 689-694
              (NC = P value not calculated - potential FP overflow)

     No.  Domain1         Domain2         Sc     RMS    Len1 Len2  Align NFit Eq. Secs.    %I    %S   P(m)
Pair   1  4ile            3gj0            3.91   2.08    170  207   179  112 101    0  24.75 100.00 1.07e-05
Pair   2  4ile            2gf9            4.50   1.88    170  176   190  114 106    0  24.53 100.00 8.68e-06
Pair   3  4ile            2fv8            4.53   1.79    170  180   188  116 112    0  16.07 100.00 1.42e-02
Pair   4  4ile            4obe            4.65   2.12    170  169   181  115 103    0  20.39 100.00 7.22e-04
Pair   5  3gj0            2gf9            5.27   1.33    207  176   185  139 138    0  28.99 100.00 2.95e-10
Pair   6  3gj0            2fv8            4.94   1.53    207  180   189  137 136    0  28.68 100.00 6.79e-10
Pair   7  3gj0            4obe            5.09   1.42    207  169   186  136 134    0  24.63 100.00 5.82e-07
Pair   8  2gf9            2fv8            6.98   1.21    176  180   190  151 149    0  29.53 100.00 2.08e-11
Pair   9  2gf9            4obe            8.00   1.36    176  169   173  159 156    0  31.41 100.00 1.27e-13
Pair  10  2fv8            4obe            6.75   1.43    180  169   186  146 143    0  32.87 100.00 6.08e-14
Reading in matrix file kras.mat...
Doing cluster analysis...
Cluster:  1 (    2gf9  &     4obe ) Sc  8.00 RMS   1.36 Len 173 nfit 159
 See file kras.1 for the alignment and transformations
Cluster:  2 (    2fv8  &     2gf9     4obe ) Sc  8.12 RMS   1.16 Len 192 nfit 149
 See file kras.2 for the alignment and transformations
Cluster:  3 (    3gj0  &     2fv8     2gf9     4obe ) Sc  6.67 RMS   1.41 Len 202 nfit 142
 See file kras.3 for the alignment and transformations
Cluster:  4 (    4ile  &     3gj0     2fv8     2gf9     4obe ) Sc  5.86 RMS   2.11 Len 204 nfit 127
 See file kras.4 for the alignment and transformations
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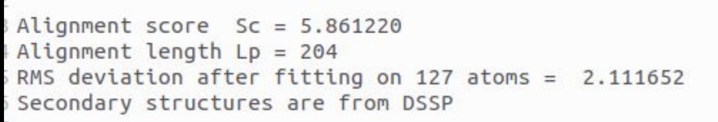


```
Alignment score  Sc = 5.861220
Alignment length Lp = 204
RMS deviation after fitting on 127 atoms =  2.111652
Secondary structures are from DSSP
```

Ras: k-Ras4B

Rho: RhoB

Rab: Rab3

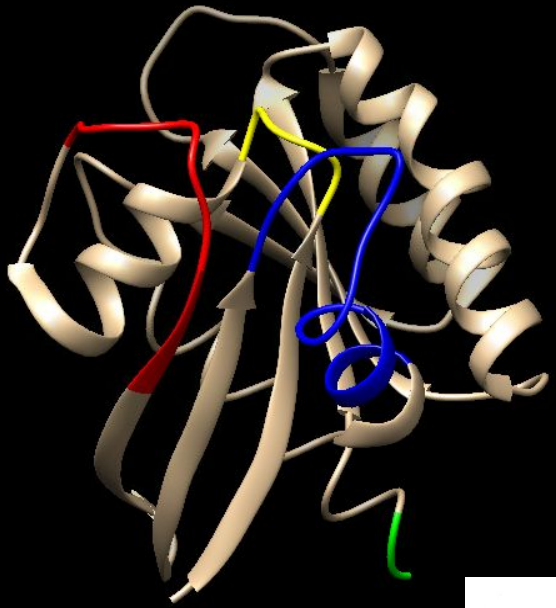
Arf: Arf8A

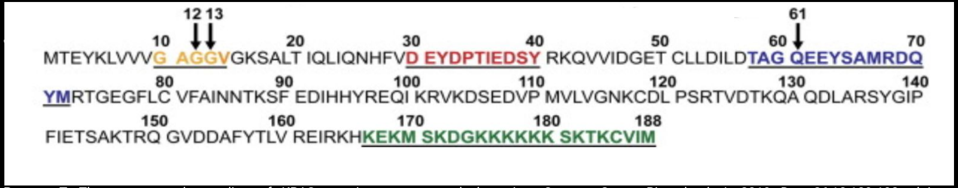
Ran: Rab3

Ras Family

Ras structure

- GTPase domain (residues 1–166)
  - ○ Switch-I (residues 30-40)
  - ○ Switch-II (residues 58-72)
  - ○ P-loop (residues 10–14)
  - ○ N-terminal
- C-terminal or the hypervariable region (residues 167–188)





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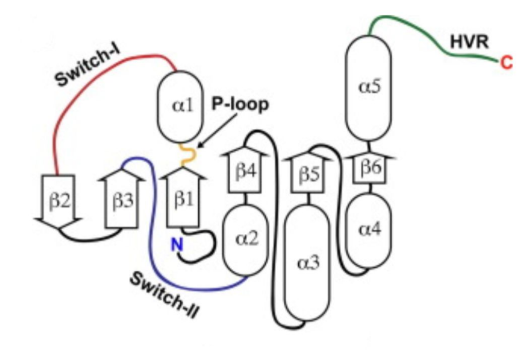
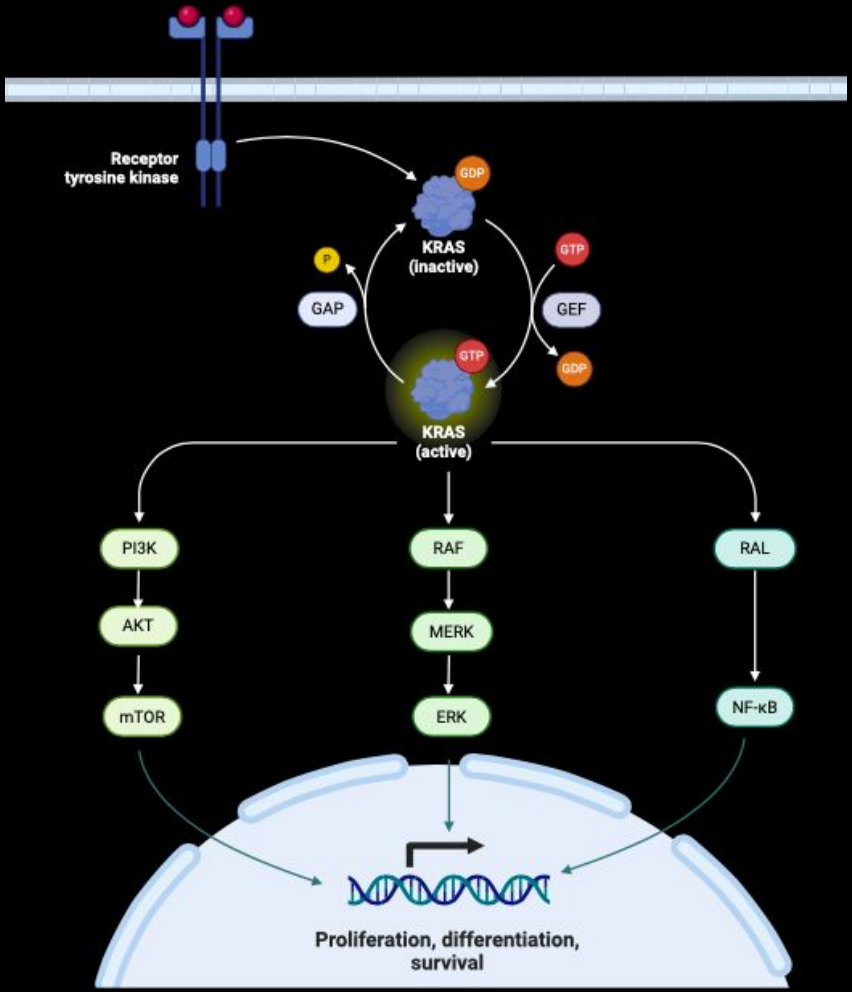
FIETSAKTRQ GVDDAFYTLV REIRKHKEKM SKDGKKKKKK SKTKCVIM

Pantsar T. The current understanding of KRAS protein structure and dynamics. Comput Struct Biotechnol J. 2019 Dec 26;18:189-198. doi: 10.1016/j.csbj.2019.12.004. PMID: 31988705; PMCID: PMC6965201.

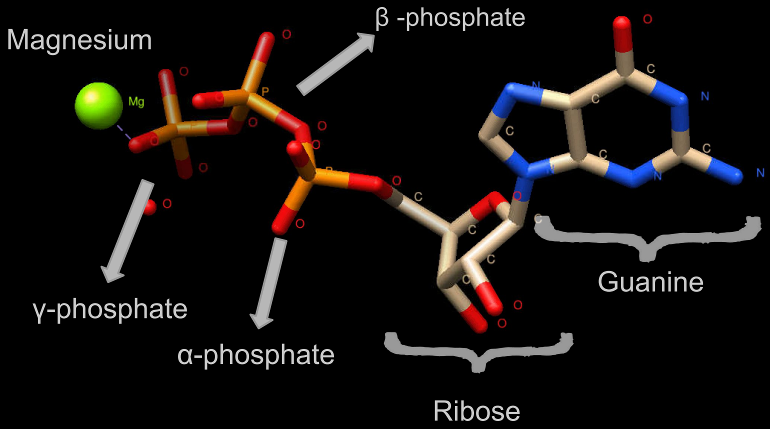


Ras cycle

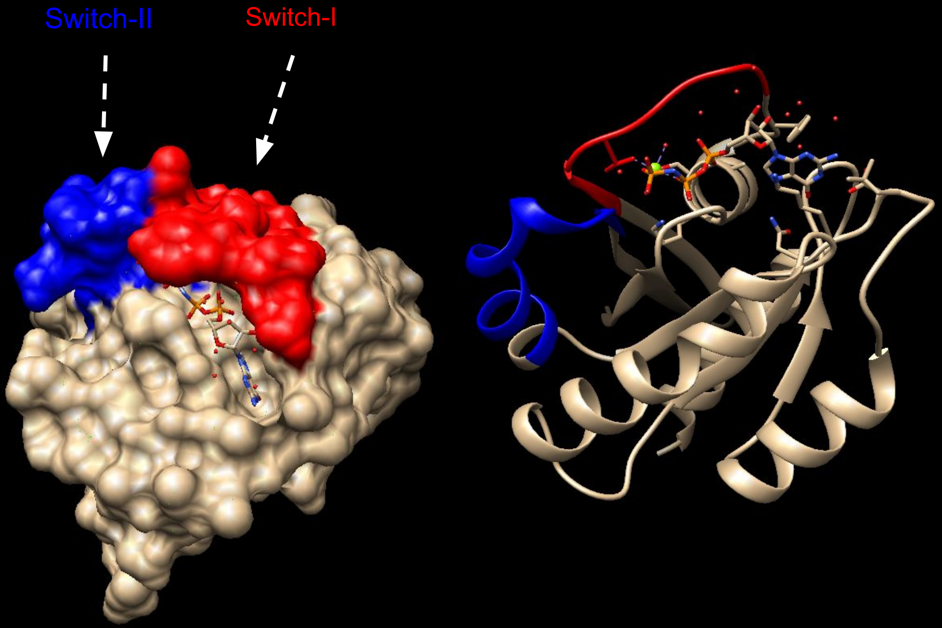

Receptor tyrosine kinase
GDP
KRAS (inactive)
P
GAP
GTP
GEF
GTP
GDP
KRAS (active)
PI3K
AKT
mTOR
RAF
MERK
ERK
RAL
NF-κB
Proliferation, differentiation, survival

Ras interaction with GTP

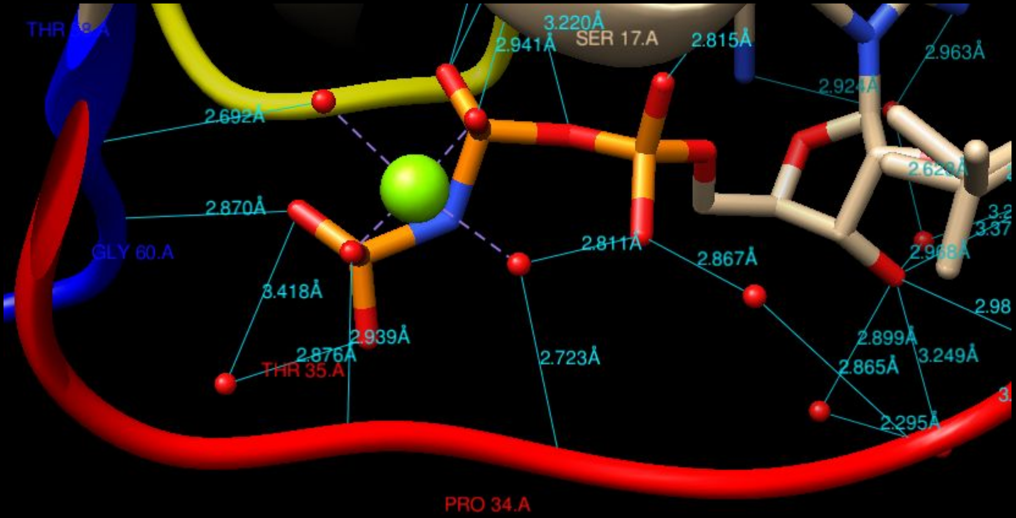
GTP structure

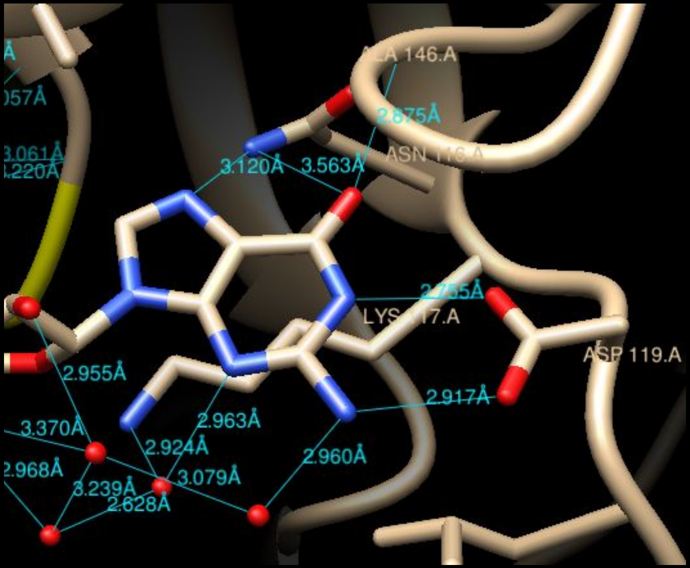


Activated Ras, GTP-bound



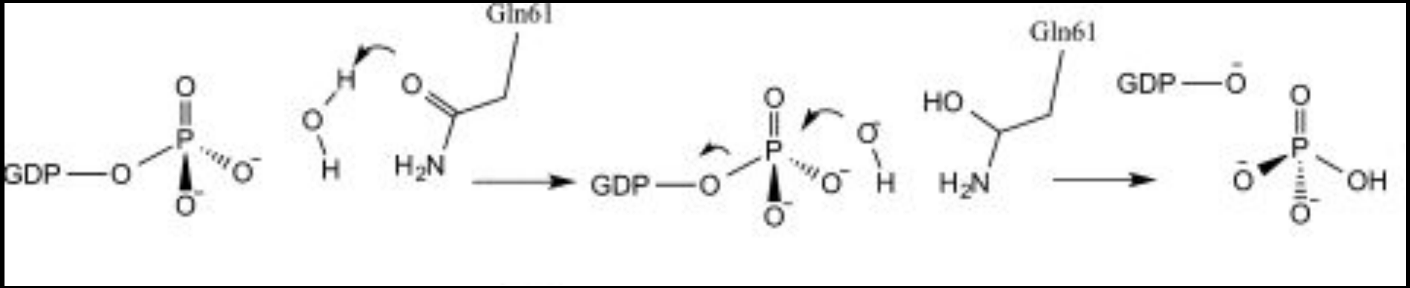
Ras interaction with GTP





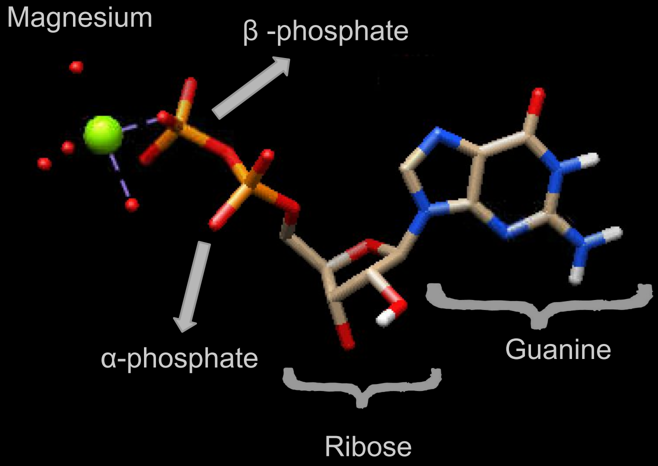
Nucleophilic attack

A water molecule acts as a nucleophile → The oxygen donates a pair of electrons to the γ-phosphate → Cleavage of the phosphodiester bond

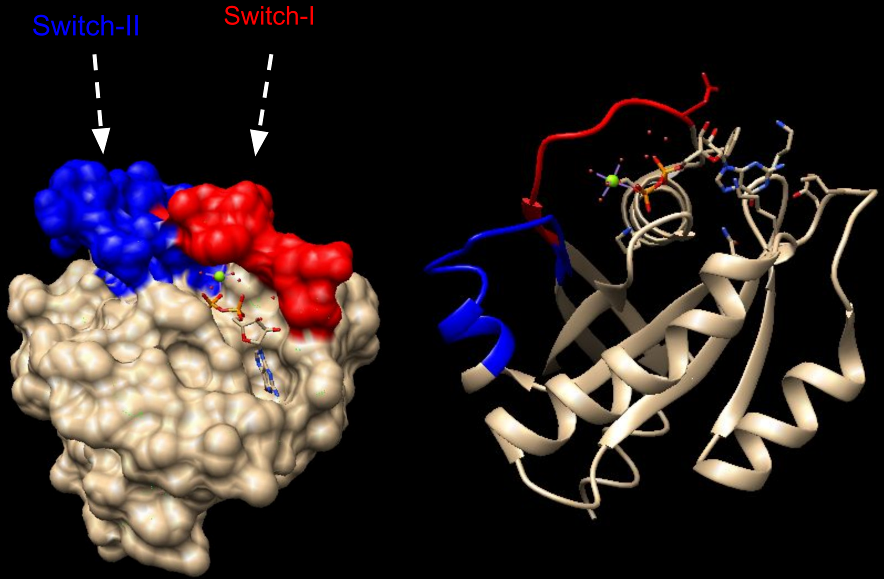


Ras interaction with GDP

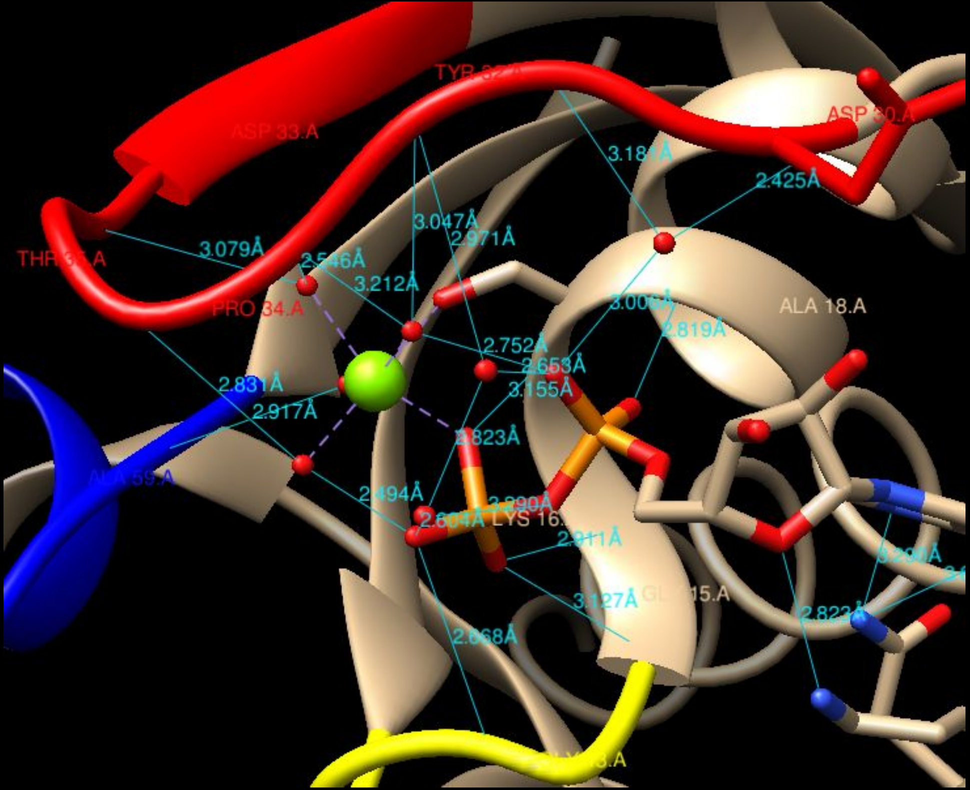
GDP structure

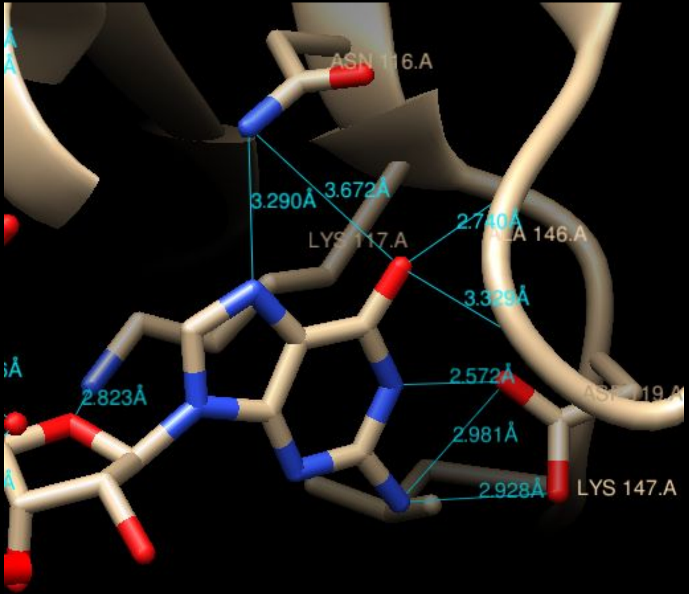


Inactivated Ras, GDP-bound



Ras interaction with GDP





Ras interaction with GEF: overview

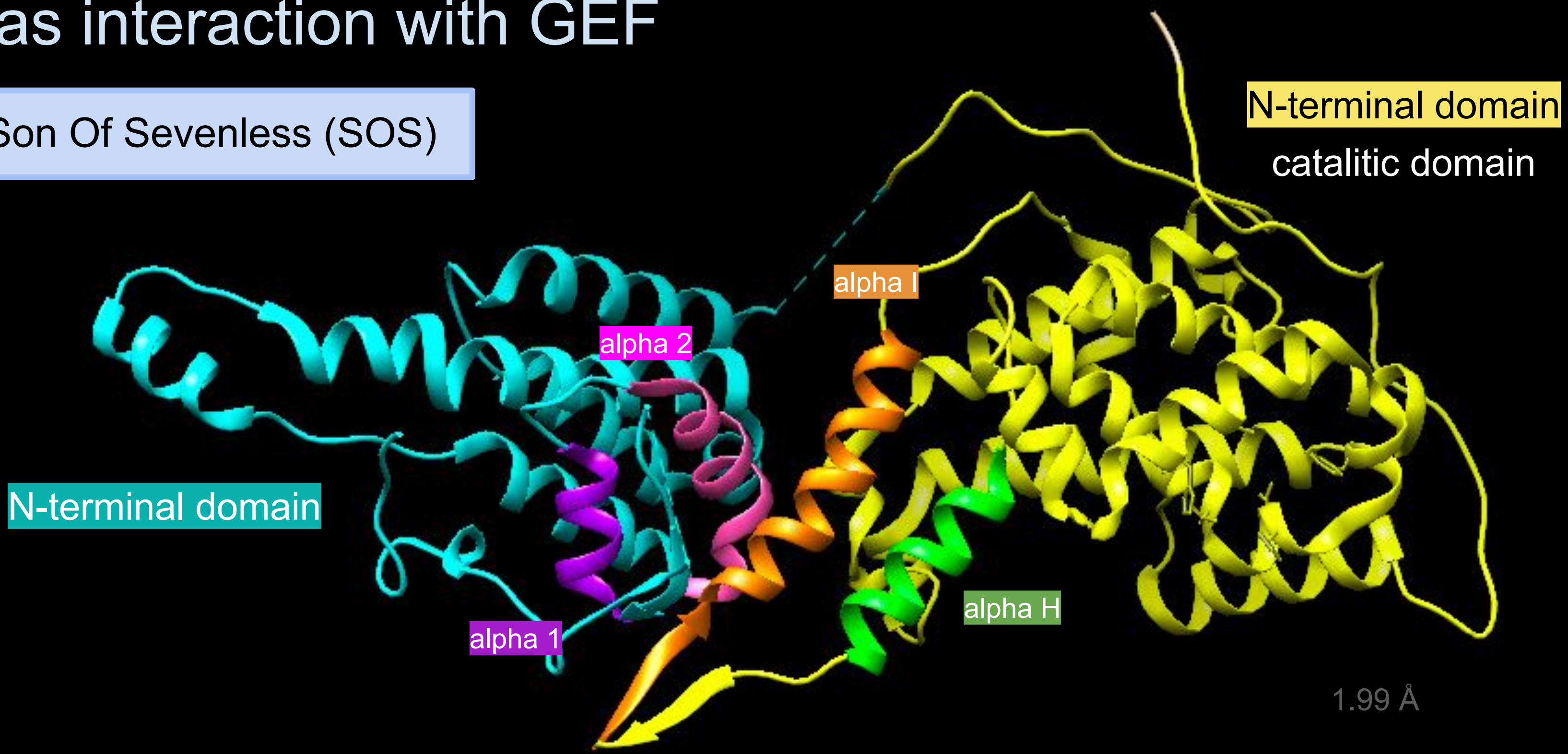
- The insertion into Ras of an alpha-helix from Sos results in the displacement of the Switch 1 region of Ras, opening up the nucleotide-binding site.

- Side chains presented by the alpha-helix and by a distorted conformation of the Switch 2 region of Ras alter the chemical environment of the binding site for the phosphate groups of the nucleotide and the associated magnesium ion, so that their binding is no longer favored.

So the Ras–Sos complex adopts a structure that allows nucleotide release and rebinding.

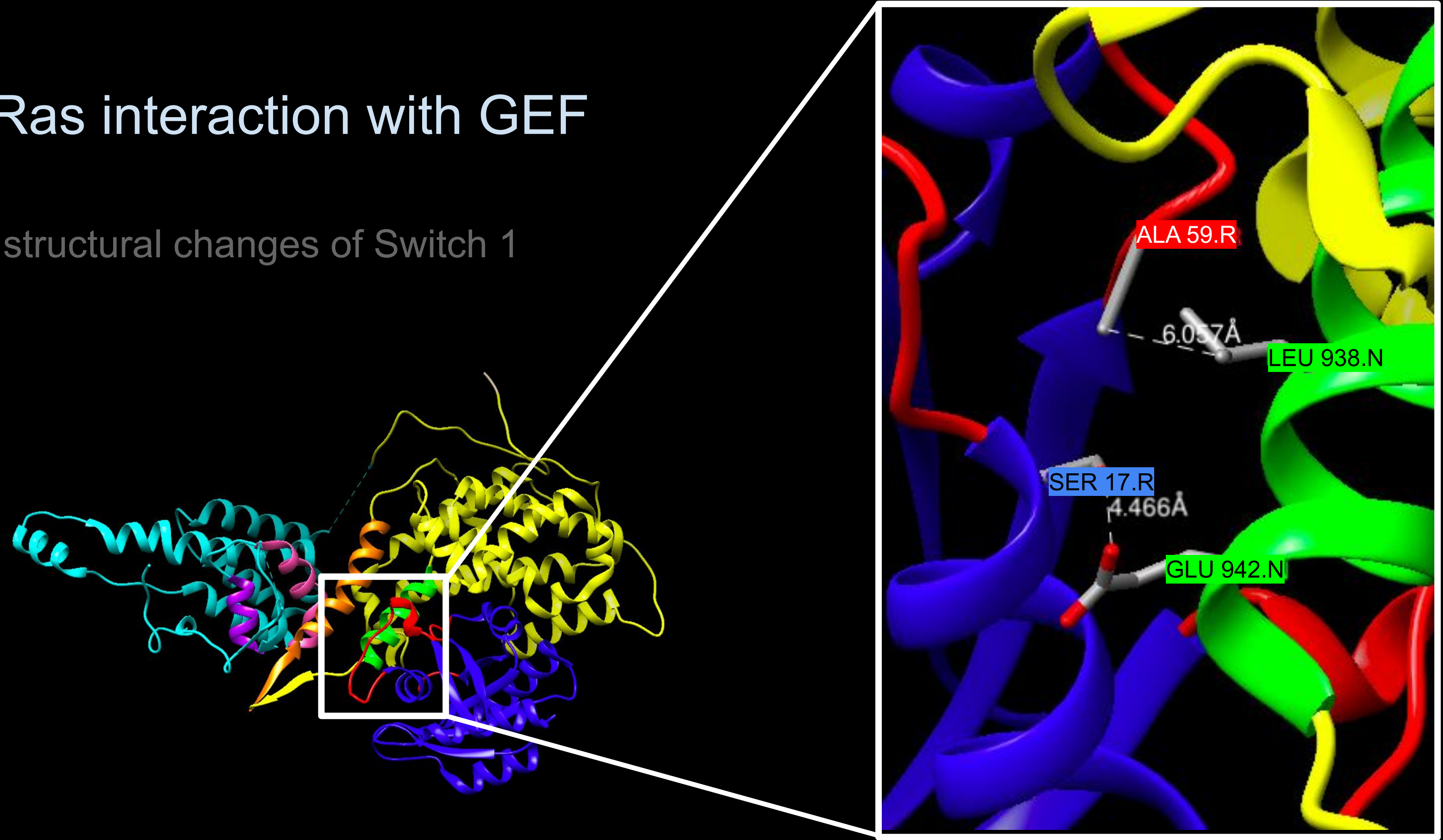
Ras interaction with GEF

Son Of Sevenless (SOS)



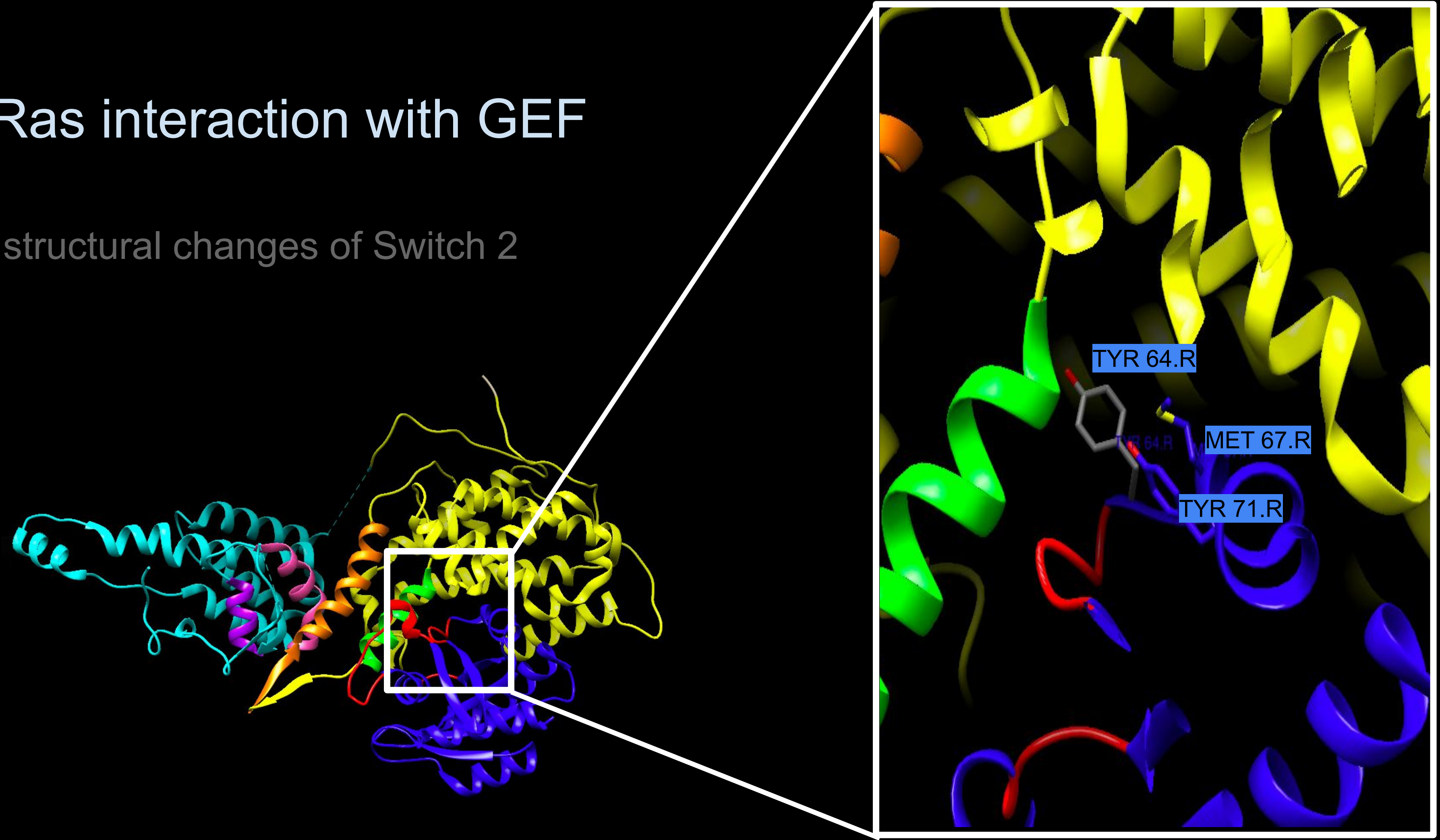
Ras interaction with GEF

structural changes of Switch 1



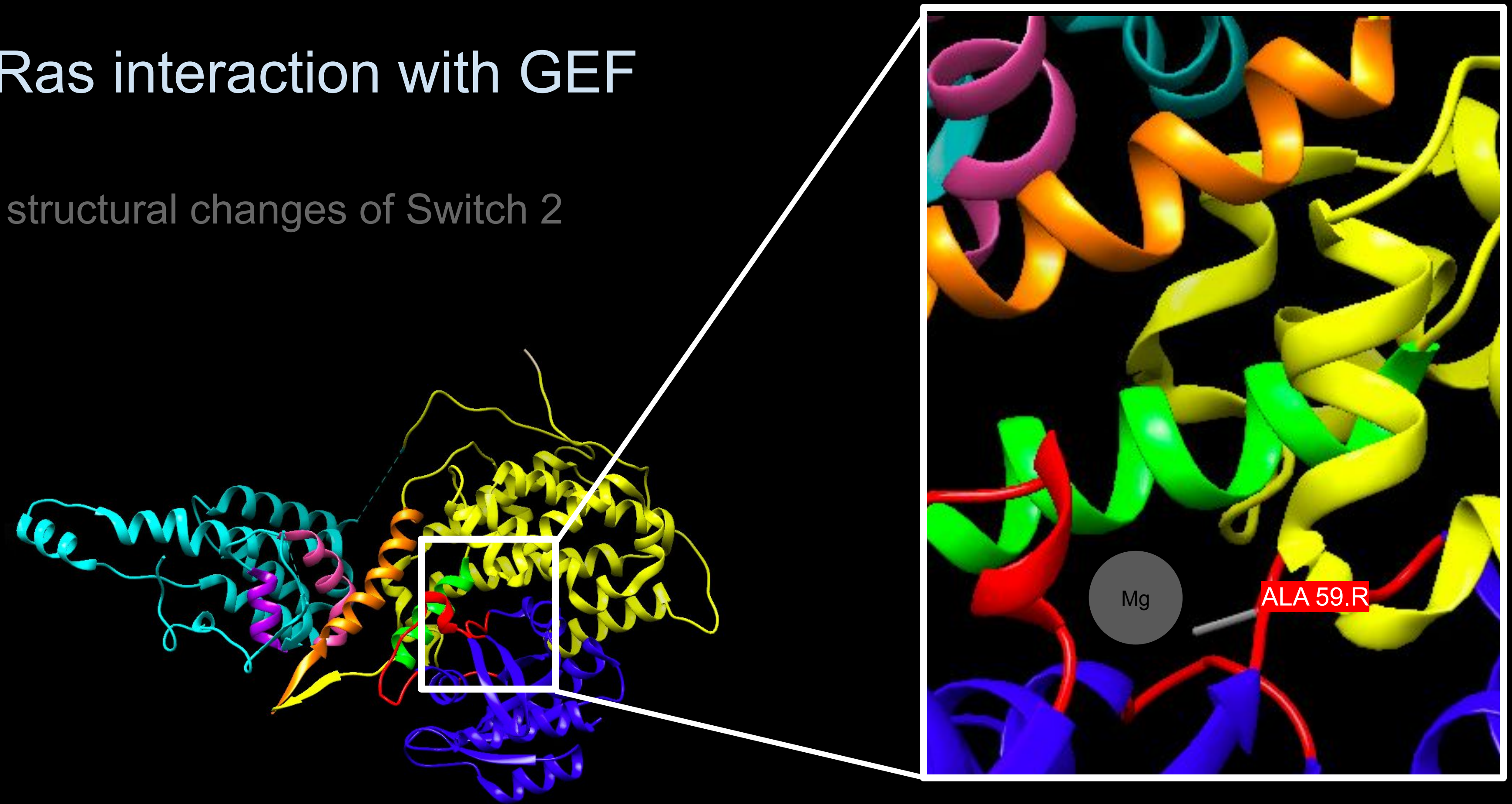
Ras interaction with GEF

structural changes of Switch 2



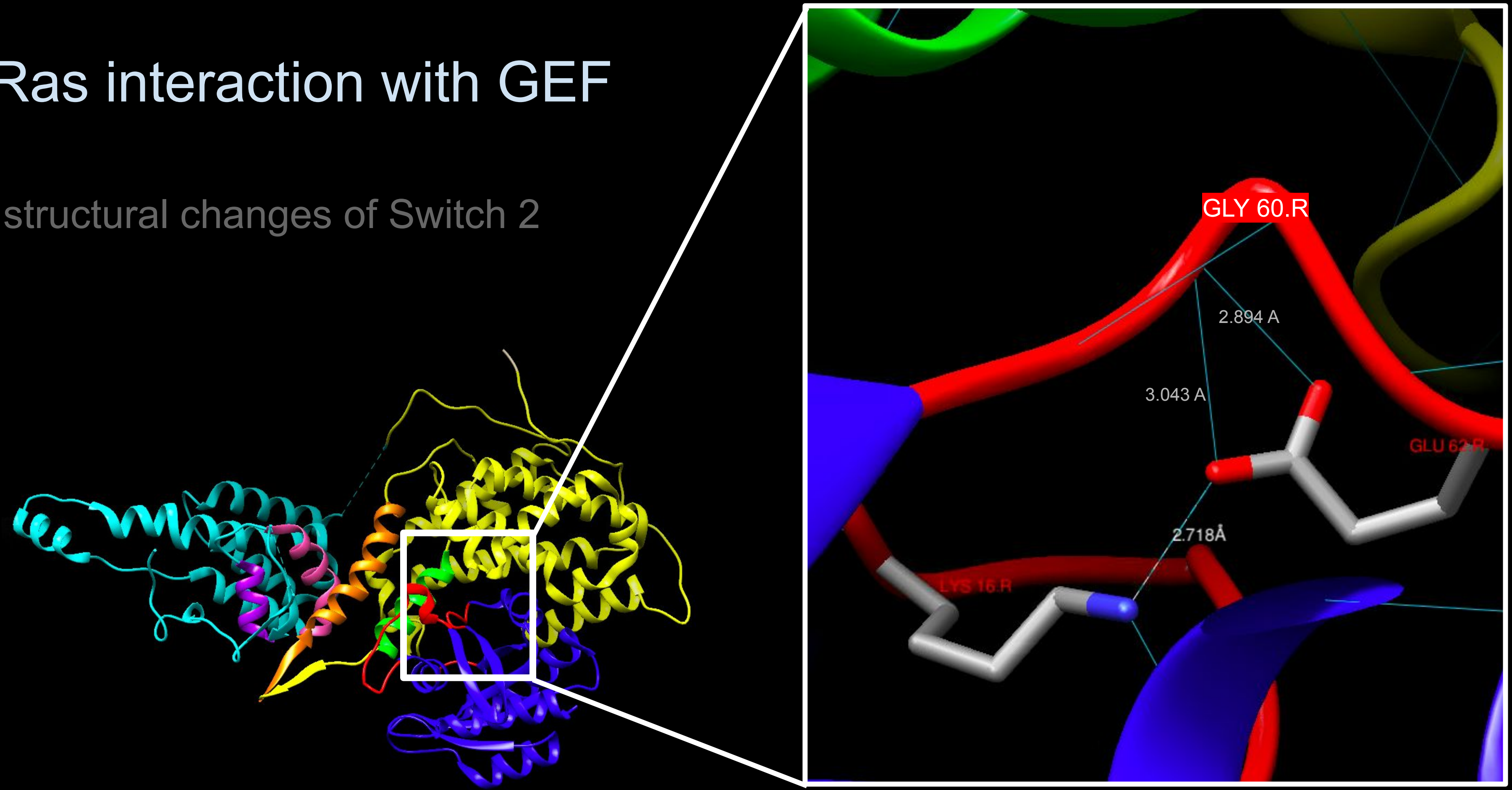
Ras interaction with GEF

structural changes of Switch 2



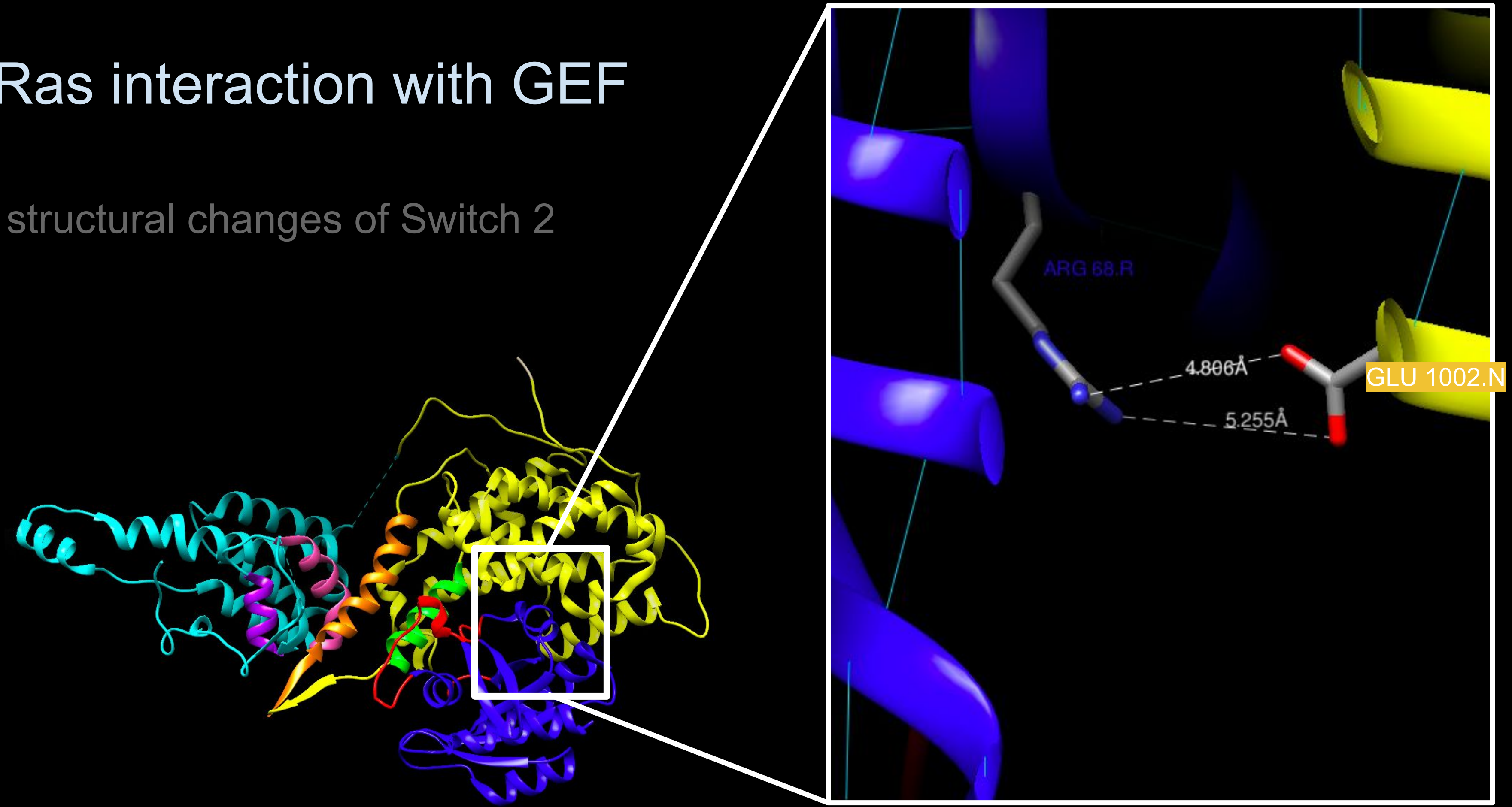
Ras interaction with GEF

structural changes of Switch 2

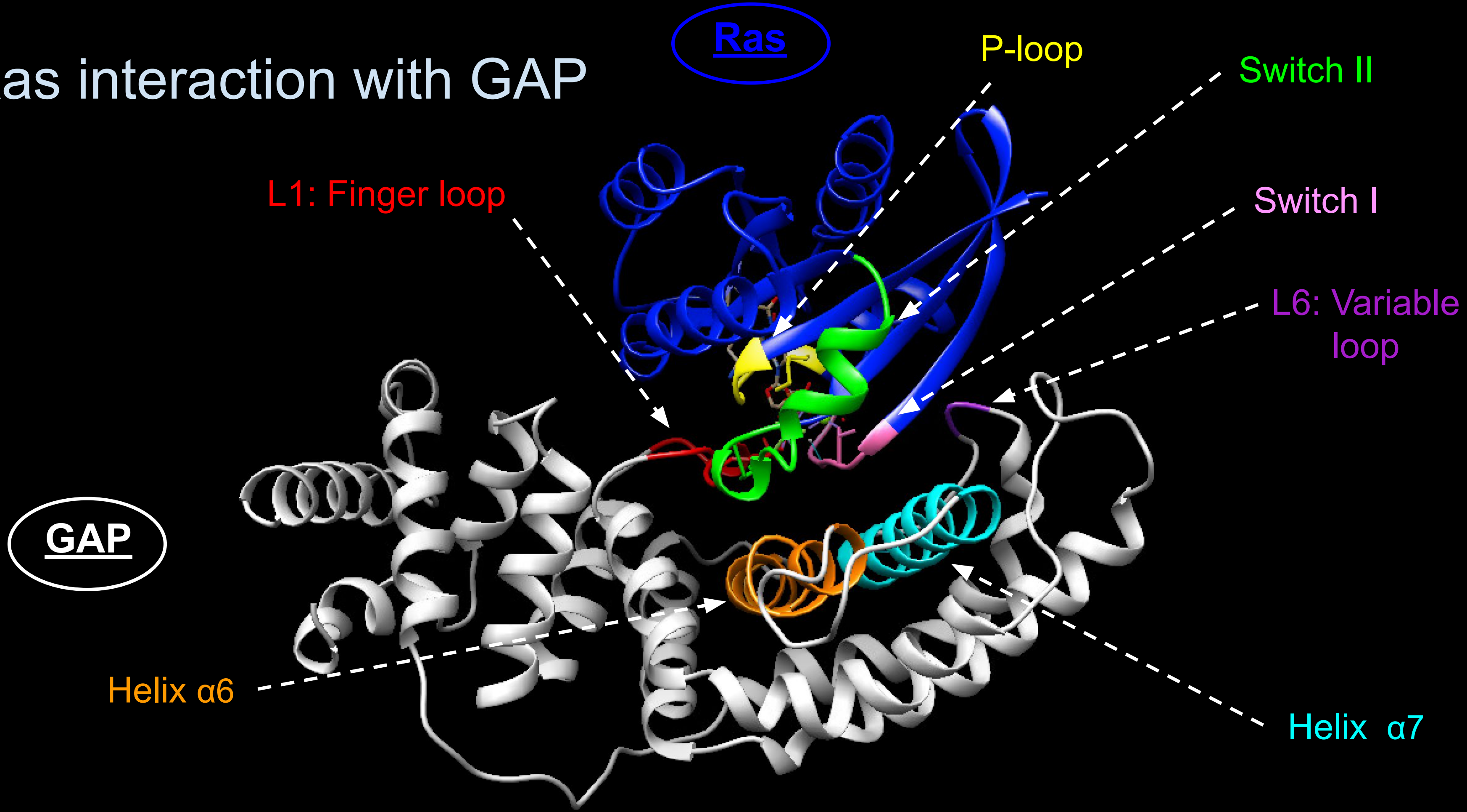


Ras interaction with GEF

structural changes of Switch 2

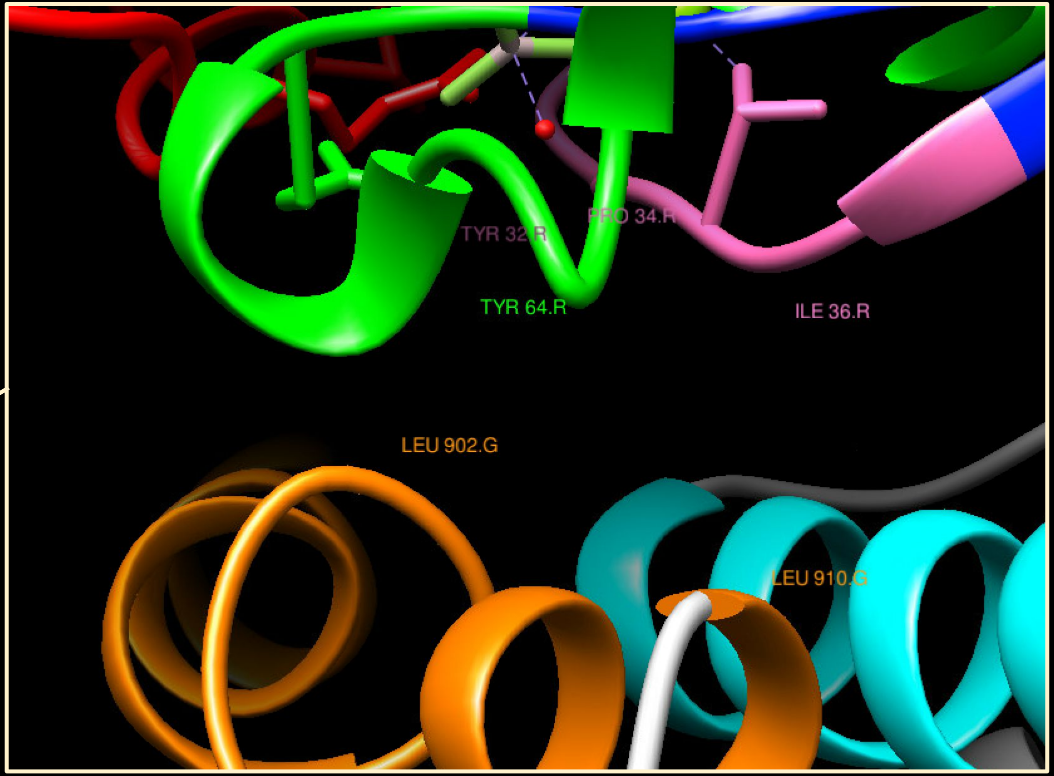


Ras interaction with GAP



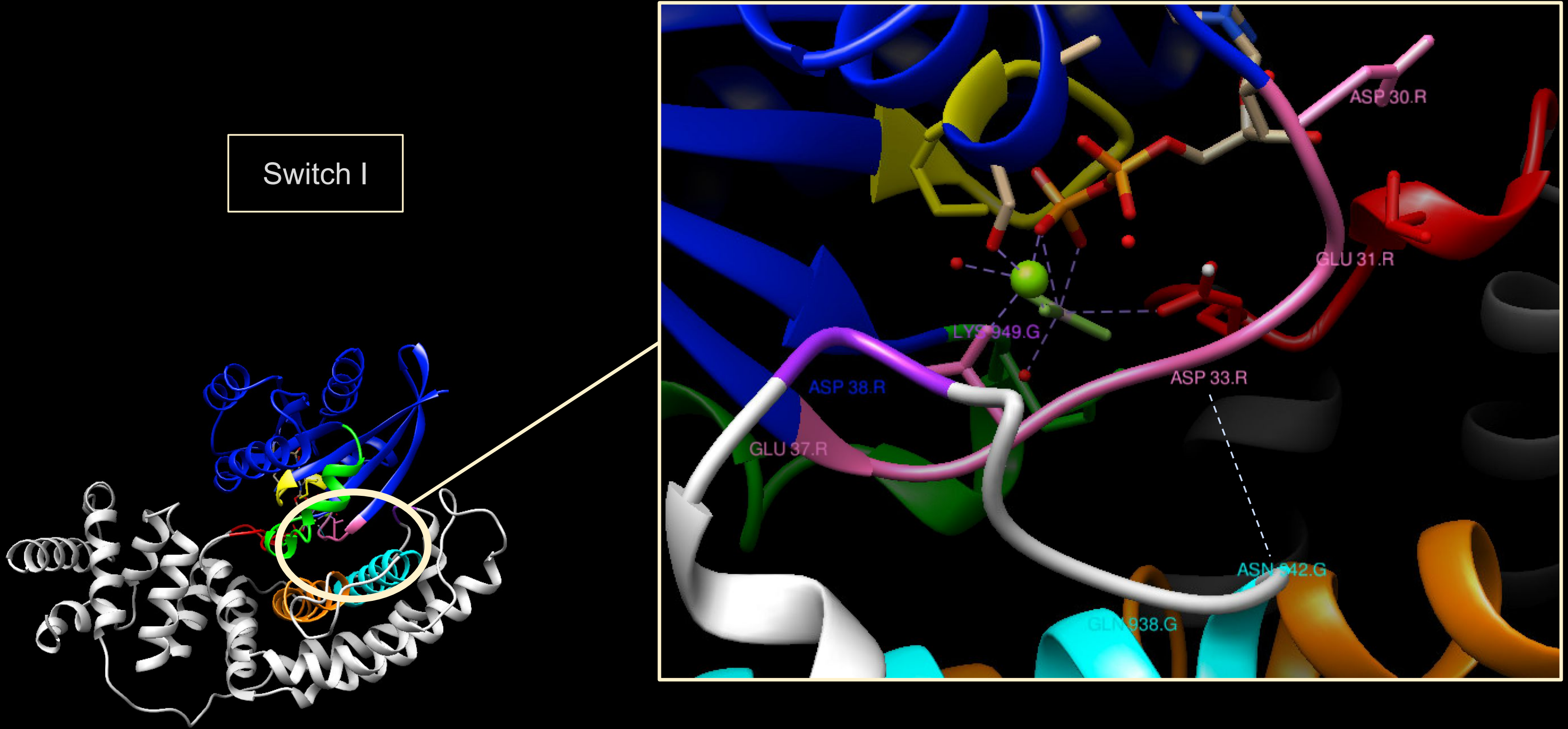
Ras interaction with GAP

Hydrophobic interactions



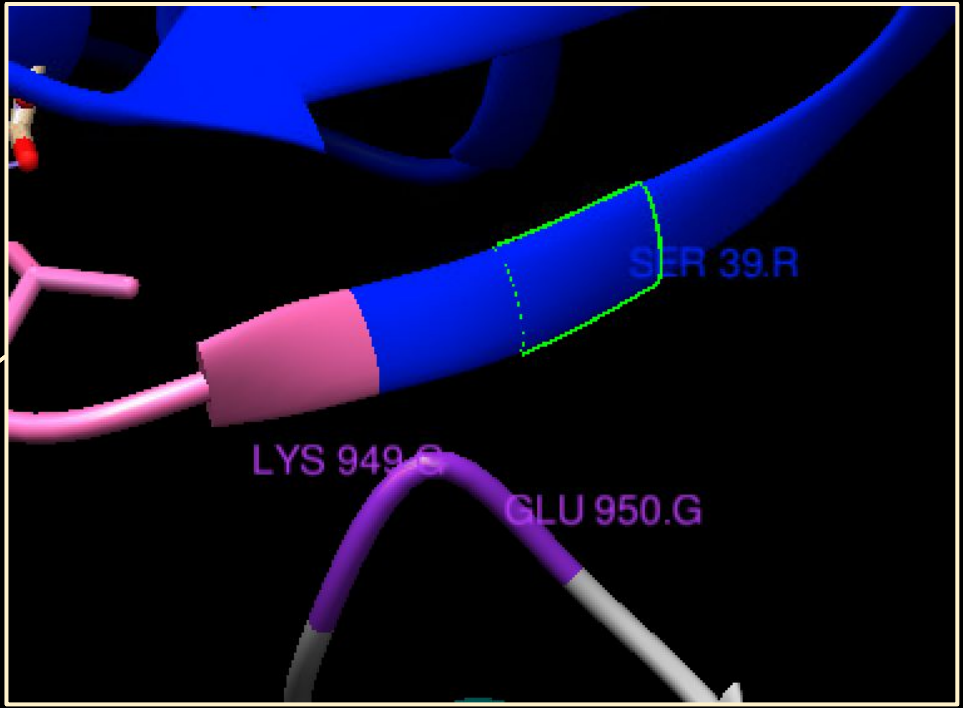
Ras interaction with GAP

Switch I



Ras interaction with GAP

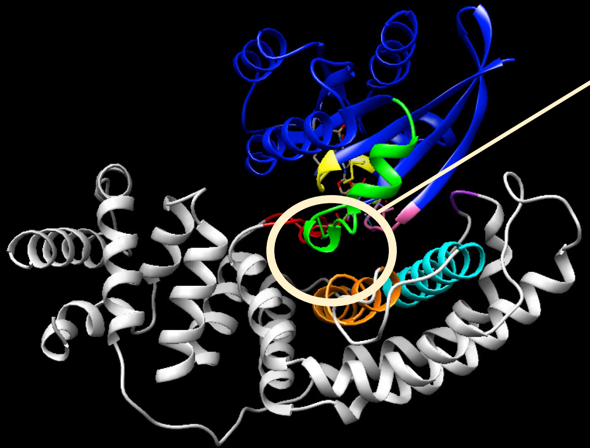
Variable loop: L6

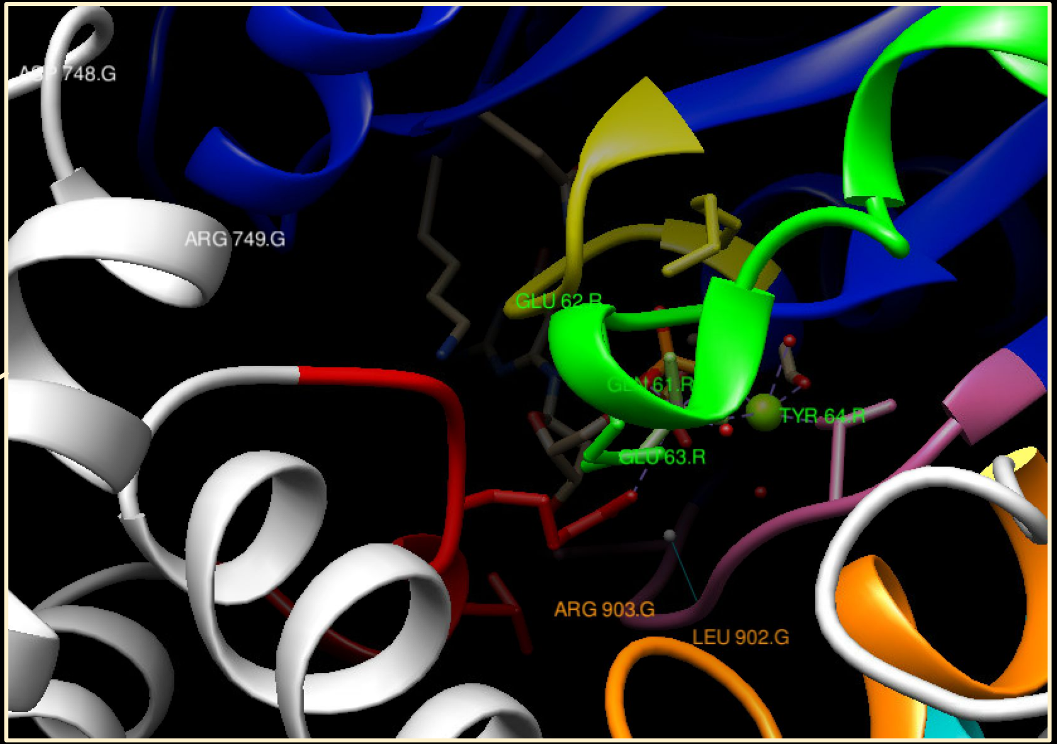


Ras interaction with GAP

Switch II

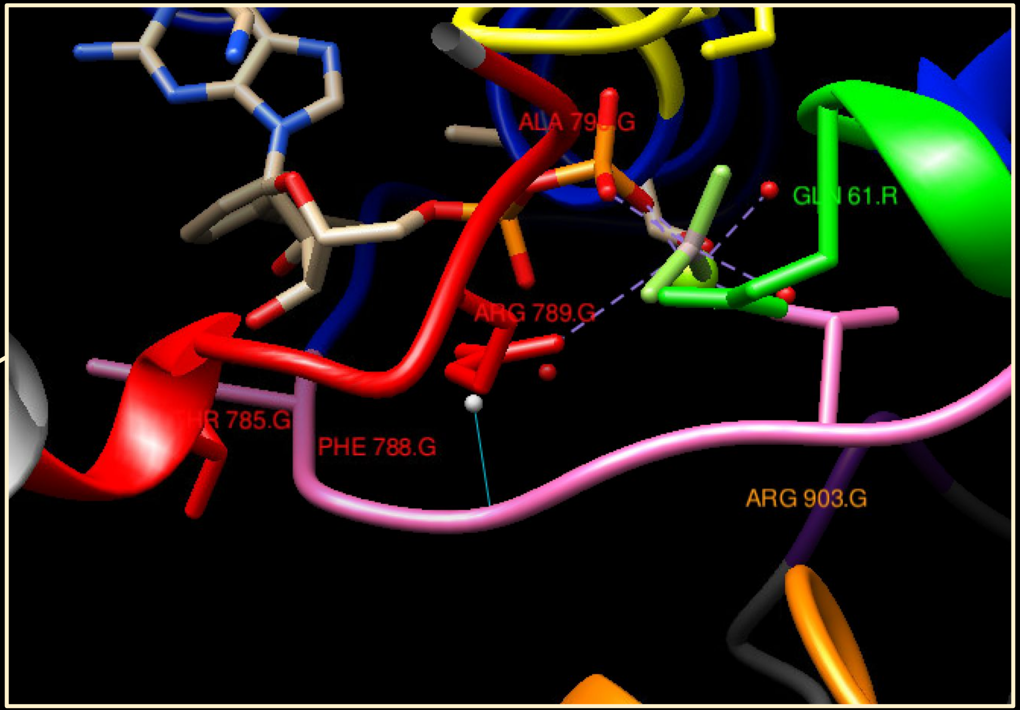
Glu61 - Arg789	Stabilizes switch II
Glu62 - Arg749	
Glu63 - Arg903	



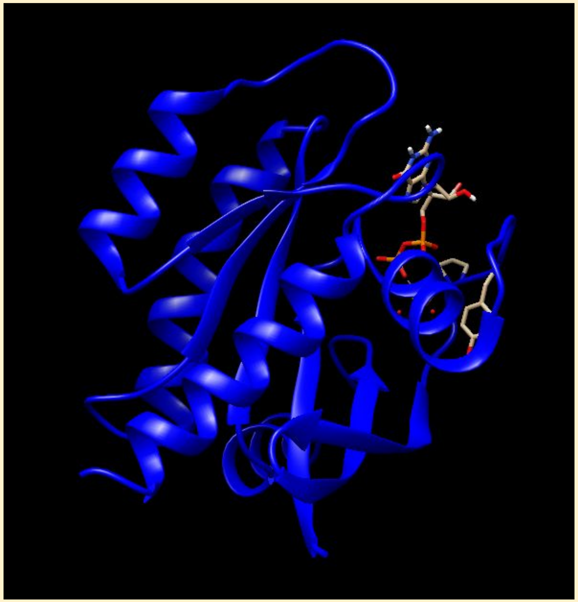


Ras interaction with GAP

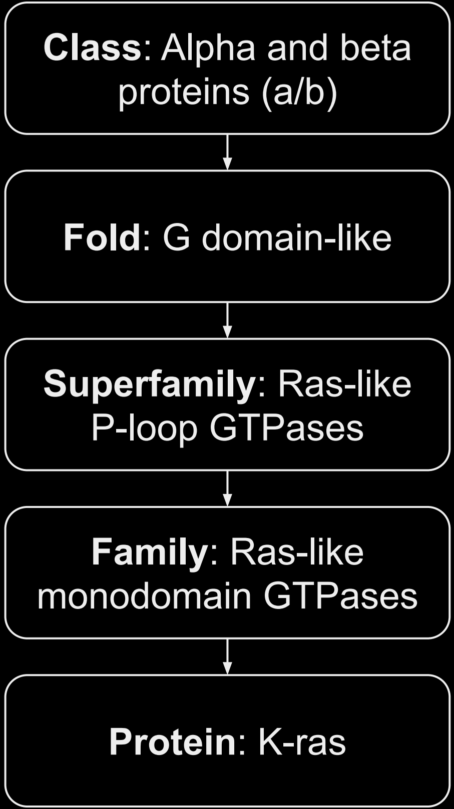
Finger loop



SCOP classification



6 beta-sheets surrounded by 5 alpha helices



Class: Alpha and beta proteins (a/b)

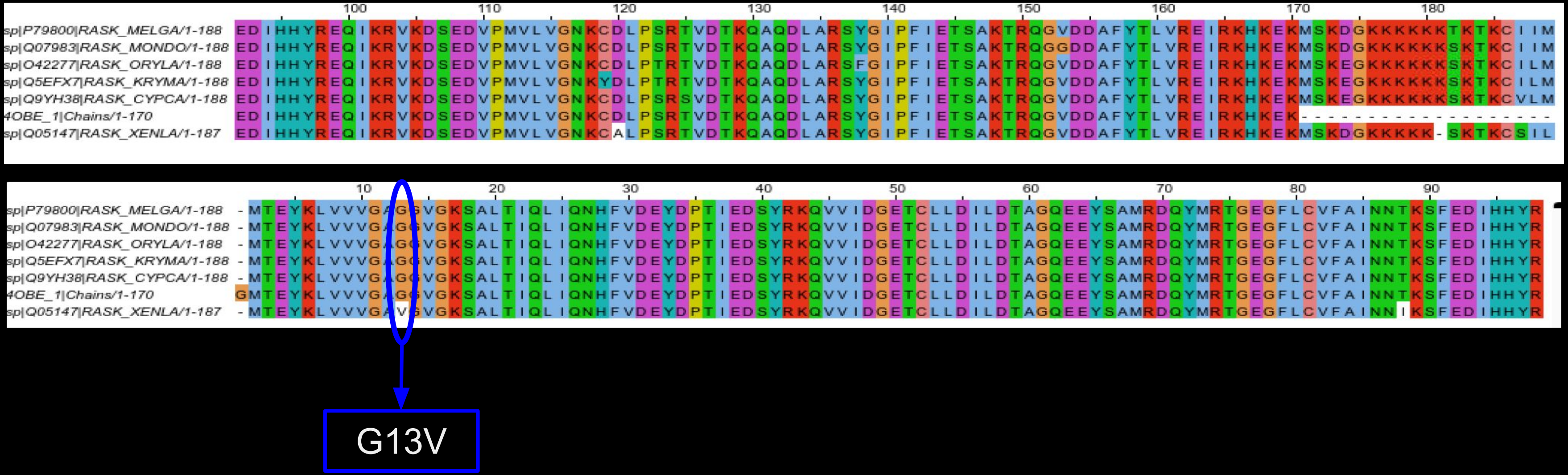
Fold: G domain-like

Superfamily: Ras-like P-loop GTPases

Family: Ras-like monodomain GTPases

Protein: K-ras

Species alignment: K-ras



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sp|Q07983|RASK_MONDO/1-188 EDIHHYREQIKRVKDSEDVPMVLVGNKCDLPSRTVDTKQAQDLARSYGIPFIETSAKTRQGGDDAFYTLVREIRKHKEKMSKDGKKKKKKSKTKCIIM
sp|O42277|RASK_ORYLA/1-188 EDIHHYREQIKRVKDSEDVPMVLVGNKCDLPTRTVDTKQAQDLARSFGIPFIETSAKTRQGVDDAFYTLVREIRKHKEKMSKEGKKKKKKSKTKCILM
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sp|Q9YH38|RASK_CYPCA/1-188 EDIHHYREQIKRVKDSEDVPMVLVGNKCDLPSRSVDTKQAQDLARSYGIPFIETSAKTRQGVDDAFYTLVREIRKHKEKMSKEGKKKKKKSKTKCVLM
4OBE_1|Chains/1-170        EDIHHYREQIKRVKDSEDVPMVLVGNKCDLPSRTVDTKQAQDLARSYGIPFIETSAKTRQGVDDAFYTLVREIRKHKEK-------------------
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                                    10        20        30        40        50        60        70        80        90
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sp|O42277|RASK_ORYLA/1-188 -MTEYKLVVVGAGGVGKSALTIQLIQNHFVDEYDPTIEDSYRKQVVIDGETCLLDILDTAGQEEYSAMRDQYMRTGEGFLCVFAINNTKSFEDIHHYR
sp|Q5EFX7|RASK_KRYMA/1-188 -MTEYKLVVVGAGGVGKSALTIQLIQNHFVDEYDPTIEDSYRKQVVIDGETCLLDILDTAGQEEYSAMRDQYMRTGEGFLCVFAINNTKSFEDIHHYR
sp|Q9YH38|RASK_CYPCA/1-188 -MTEYKLVVVGAGGVGKSALTIQLIQNHFVDEYDPTIEDSYRKQVVIDGETCLLDILDTAGQEEYSAMRDQYMRTGEGFLCVFAINNTKSFEDIHHYR
4OBE_1|Chains/1-170        GMTEYKLVVVGAGGVGKSALTIQLIQNHFVDEYDPTIEDSYRKQVVIDGETCLLDILDTAGQEEYSAMRDQYMRTGEGFLCVFAINNTKSFEDIHHYR
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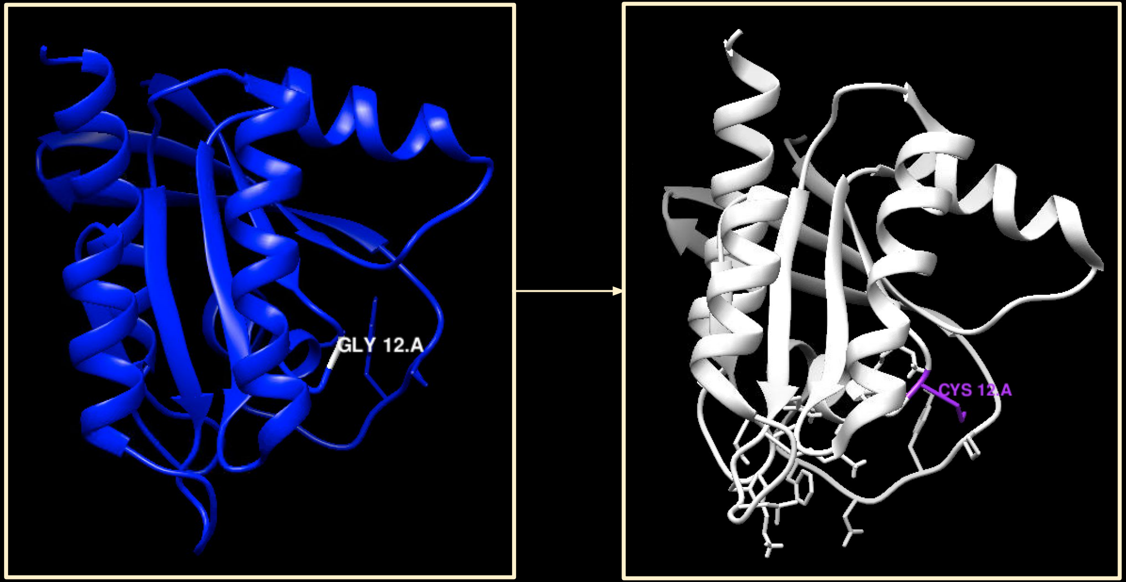
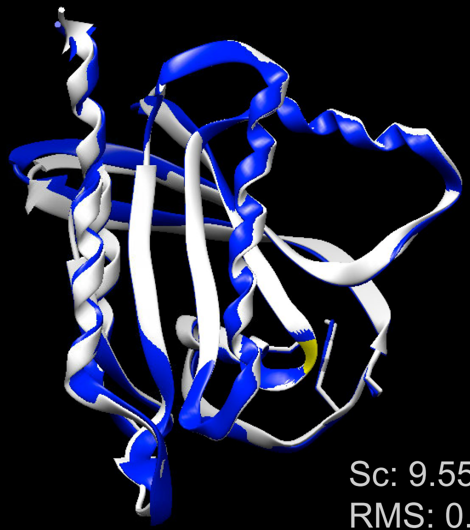
```

Cancer: G12C K-Ras

- KRAS is the most oncogenic RAS proteins.
- It is found predominantly in lung, pancreatic, and colorectal cancer.
- Gly12 interacts with Finger loop of GAP.

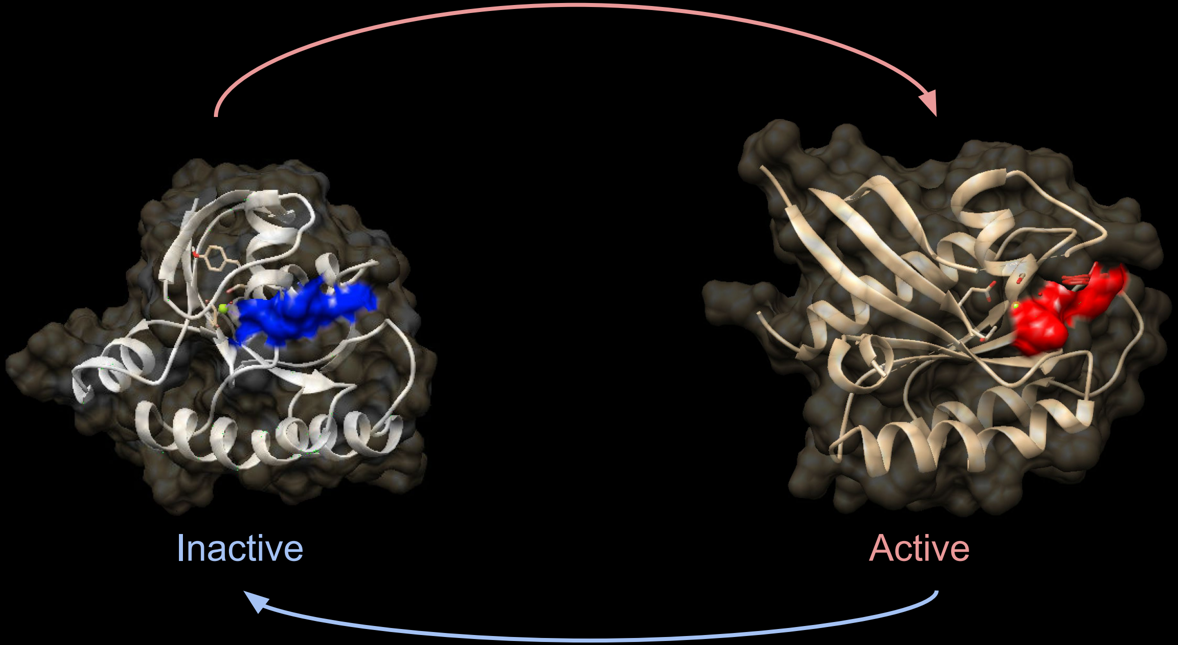


Superimposition



Sc: 9.55
RMS: 0.56

Cancer: G12C K-Ras



Exchange → GEFs

Inactive

Active

Hydrolysis - -x- -→GAPs

Bibliography

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PEM

Monomeric G proteins

a) structural and functional homology with the alpha subunit of the heterotrimeric g proteins
b) only functional homology with the alpha subunit of the heterotrimeric g proteins
c) only structural homology with the alpha subunit of the heterotrimeric g proteins
d) structural and functional homology with the beta subunit of the heterotrimeric g proteins
e) only structural homology with the alpha subunit of the heterotrimeric g proteins

The beta subunit of heterotrimeric G proteins has the structure of

a) TIM barrel
b) Rossmann fold
c) beta propeller
d) greek key
e) Jelly roll

Monomeric G proteins are active when bound to

a) ATP
b) ADP
c) GDP
d) GTP
e) the beta and gamma subunits

The binding interface of ras is formed by:

a) The Hypervariable region
b) The P-loop
c) Switch I region
d) Switch I and Switch II
e) None of the above

Upon GTP binding, Ras proteins:

a) Dissociates from the membrane
b) Become unactivated
c) Adopt a more open conformation
d) Adopt a more closed conformation
e) Translocate to the nucleus

Which of the following ions interacts with GTP and GDP?

a) Ca
b) Zn
c) Na
d) Mg
e) Fe

Ras interacts with GTP and GDP mainly through:

a) Van der Waals bonds
b) Ionic bonds
c) Covalent bonds
f) Metallic bonds
g) Hidrogen bonds

What regions of Ras are involved in the interaction with its GTPase-activating protein (GAP)?

a) Switch I.
b) Loop L1.
c) Switch II.
d) Helices alpha-6 and alpha-7.
e) All of the above.

What is the role of Arg789 in loop L1 in the interaction between Ras and its GAP?

a) Stabilizes switch II.
b) Forms a hydrogen bond with Asn942.
c) Acts as the arginine finger.
d) Interacts with Tyr-64.
e) Stabilizes loop L6.

What structural changes in the Ras-Sos interface are primarily responsible for preventing the coordination of magnesium and phosphate in the nucleotide-binding site?

a) Rearrangement of the Switch I region.
b) Formation of the alpha 1-alpha 2 pocket in the N-domain.
c) Interaction between Leu 938 and Glu 942.
d) Compression of the backbone in Switch II.
e) Protrusion of a helical hairpin from the catalytic domain.