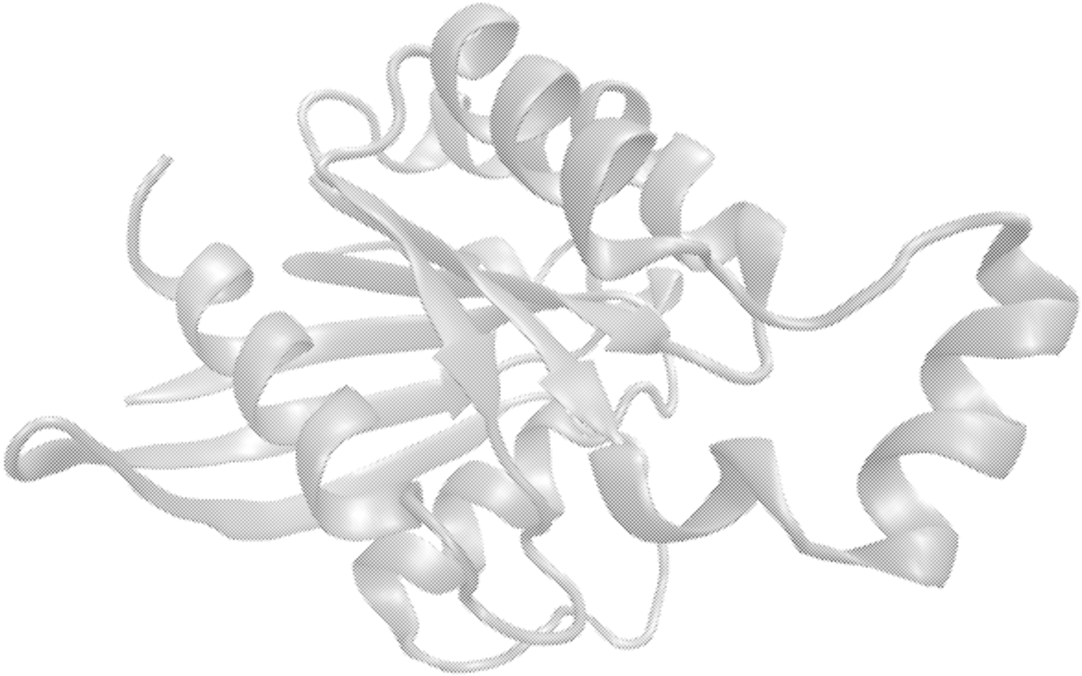

G PROTEINS

Carla Canal Capdevila

Mar Fernández Rubio

Maria Miquel Sedo

Eva Vendrell Almero

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G proteins

- Mechanism of action, states, types

Ras superfamily

- Structure
- Alignments
- Superimpositions

RhoA

- Introduction
- Alignments
- Structure
- Interactions

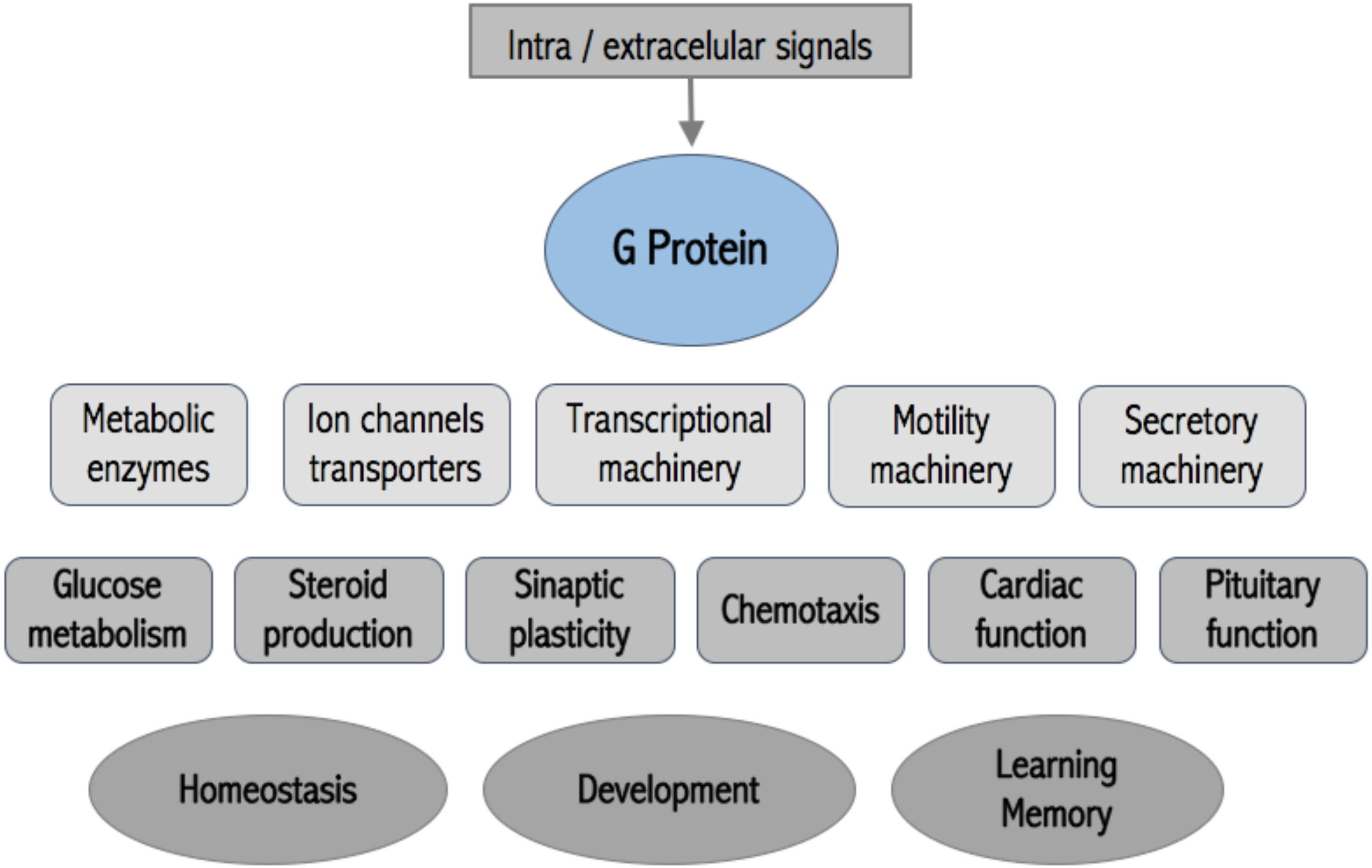
Diseases

- Recurrent RhoA mutations in human cancer

G PROTEINS

INTRODUCTION – G PROTEINS

G proteins are a family that has an important role on the cellular signaling process



Intra / extracelular signals

G Protein

Metabolic enzymes

Ion channels transporters

Transcriptional machinery

Motility machinery

Secretory machinery

Glucose metabolism

Steroid production

Sinaptic plasticity

Chemotaxis

Cardiac function

Pituitary function

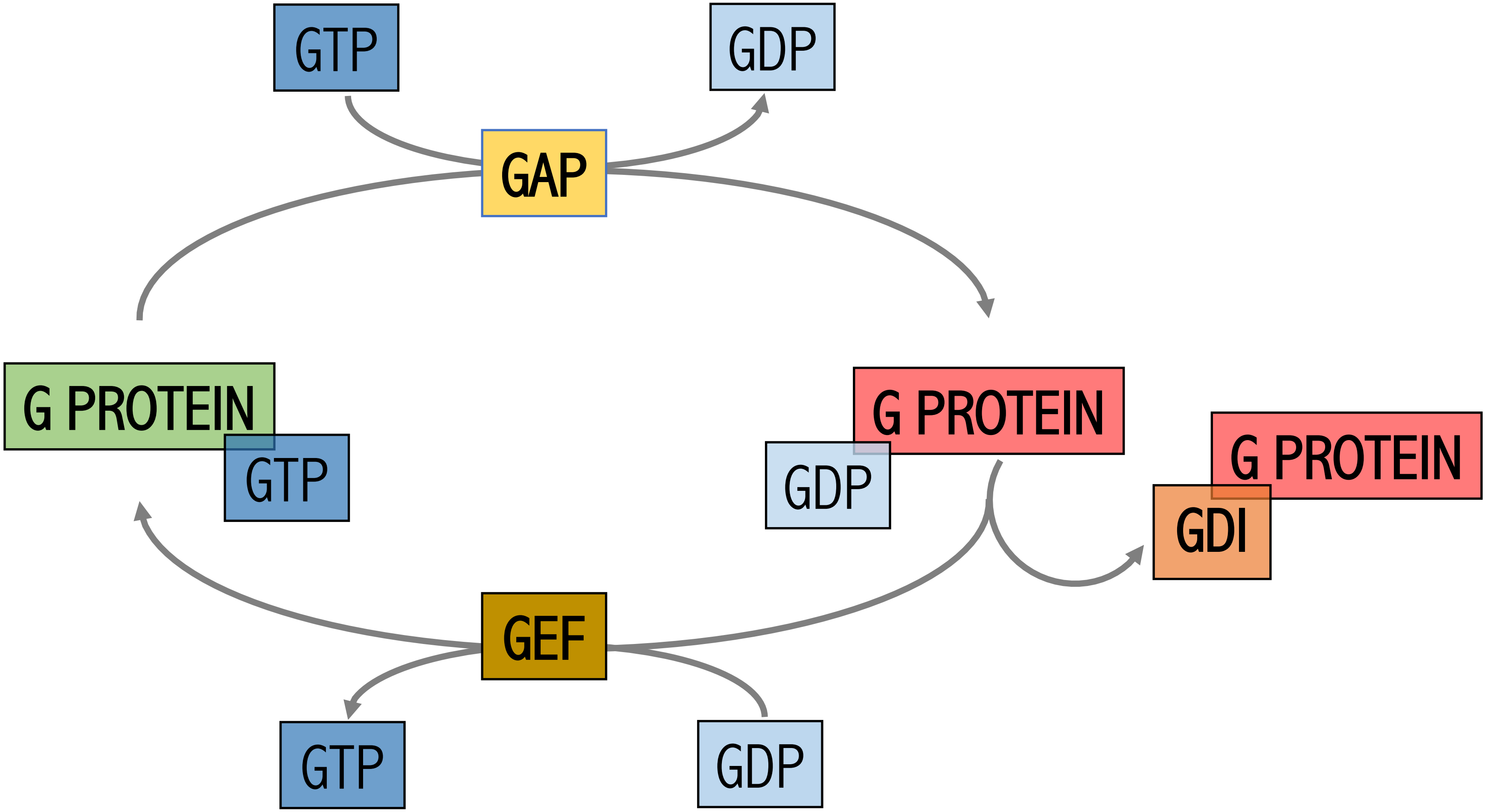
Homeostasis

Development

Learning Memory

Adapted from: . Neves S. G Protein Pathways. Science. 2002;296(5573):1636-1639.

MECHANISM OF ACTION



GTP

GDP

GAP

G PROTEIN

GTP

G PROTEIN

GDP

G PROTEIN

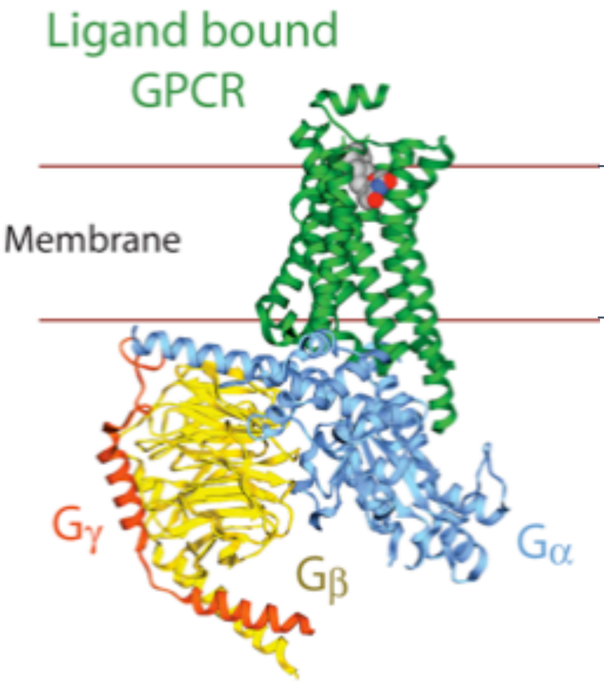
GDI

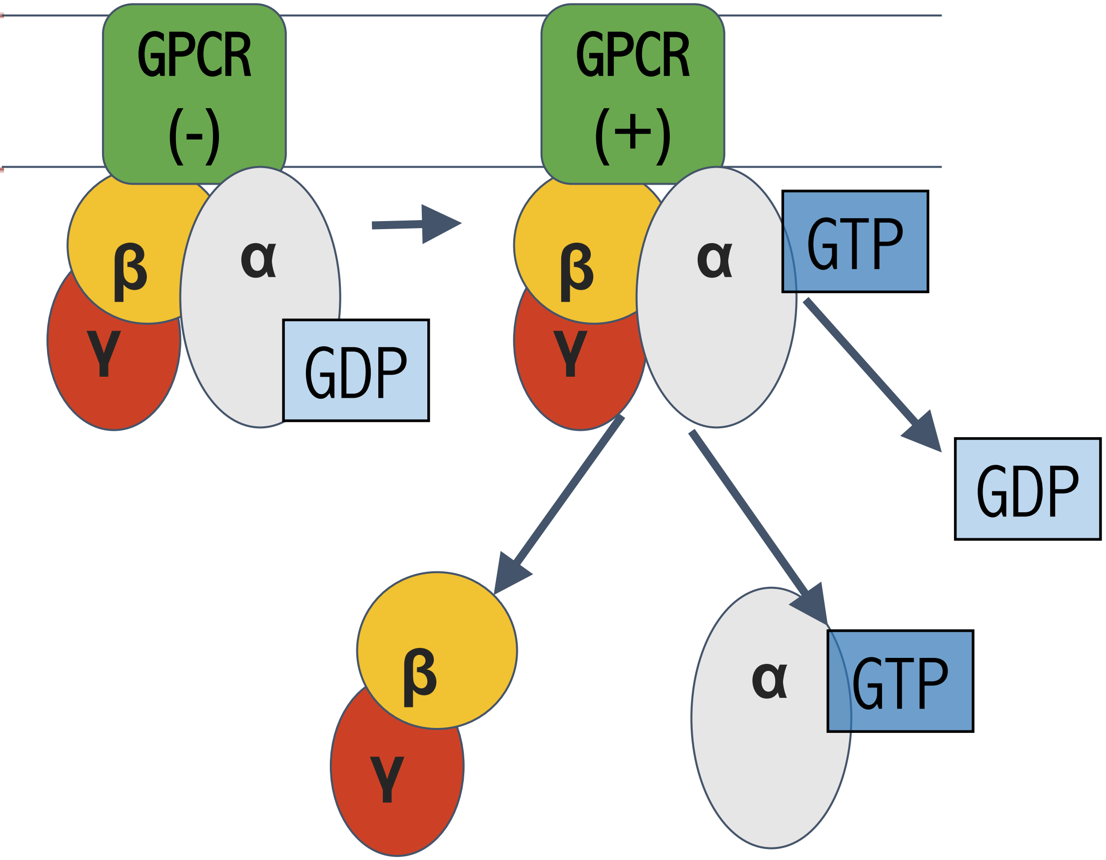
GEF

GTP

GDP

G PROTEIN STATES

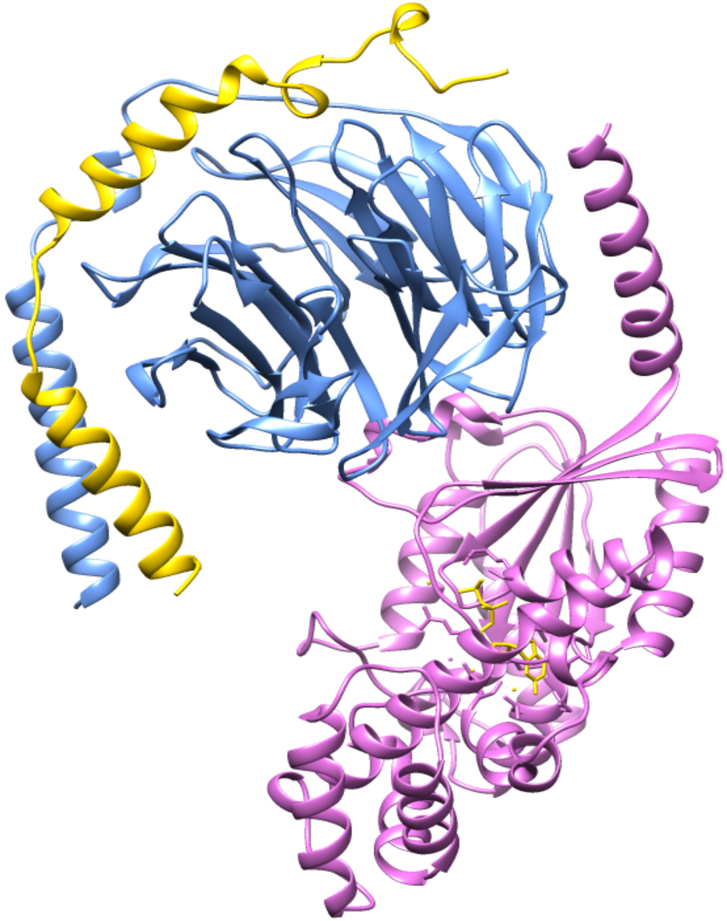




TYPES OF G PROTEINS

HETEROTRIMERIC

- **Location:** Cell membrane.
- Coupled by GPCRs.
- **3 Subunits:** Alpha, beta-gamma.
- α: Binds GTP, dissociates and interacts with effector.



HETEROTRIMERIC G PROTEINS

Families: Gi, Gs, Gq, and G12/13

Domains:

- GTPase: binding and hydrolysis of GTP
- Helical domain
- Switches: I,II, III
- NTD, CTD: Activation directs protein-protein interactions.

Ligands:

- **GEF**s (Guanine Nucleotide Exchange Factors)
- **GAP**s (GTPase-activating proteins)
- **GDI**s (Guanine nucleotide dissociation inhibitors)

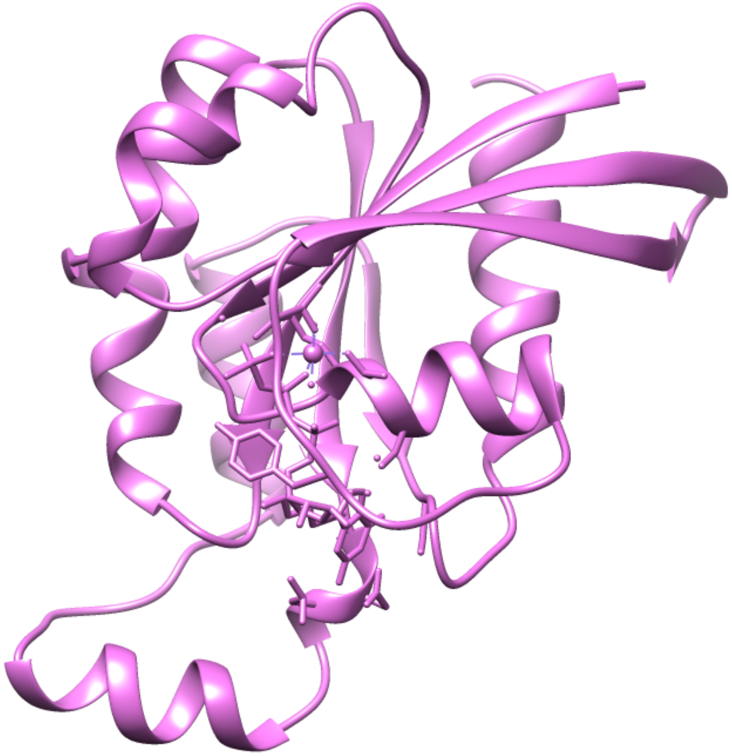
Efectors:

- AC, PLC, Phosphodiesterase, Ion channels.

TYPES OF G PROTEINS

MONOMERIC

- **Location:** Cytosol.
- **Structure:** Galpha homologous.
- Bind GTP.
- Catalyse hydrolysis of the terminal phosphate group (inactive GDP-bound conformation) .



MONOMERIC G PROTEINS

Families: Ras superfamily.

Domains:

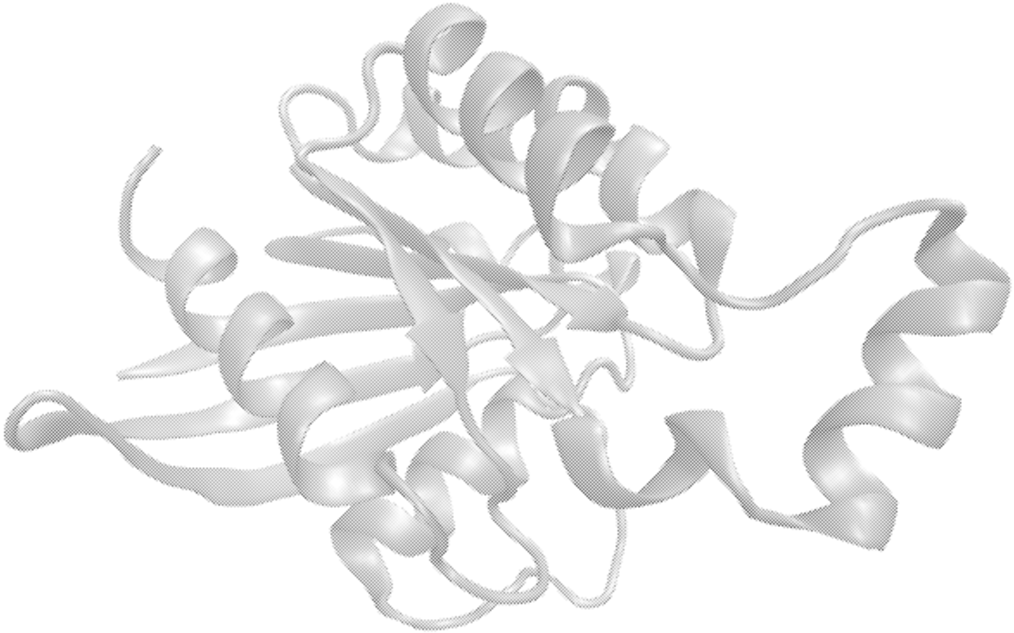
- G domain /G motif / Core effector / Membrane targeting
- Switches: I, II
- NTD, CTD: little known: activation directs protein-protein interactions

Ligands:

- **GEF**s (Guanine Nucleotide Exchange Factors)
- **GAP**s (GTPase-activating proteins)
- **GDI**s (Guanine nucleotide dissociation inhibitors)

Effectors:

- AC, PLC, Phosphodiesterase, Ion channels

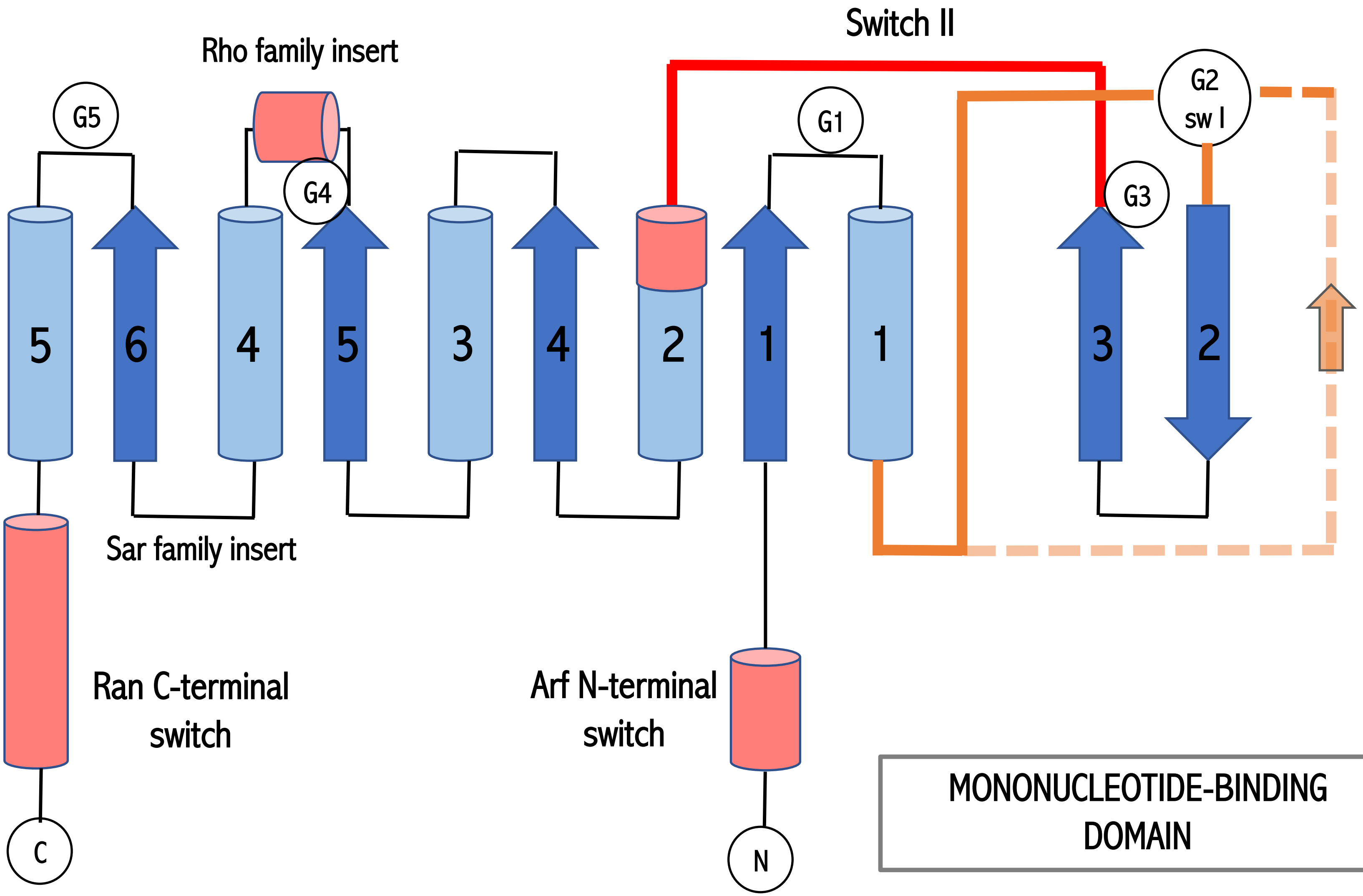


RAS SUPERFAMILY

RAS SUPERFAMILY

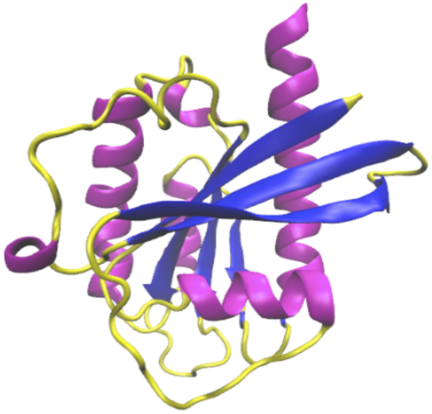
FAMILY	FUNCTION	MEMBERS
Ras (Ras sarcoma oncoproteins)	Cell proliferation	H-Ras, K-Ras, M-Ras, N-Ras, R-Ras, RalA, RalB, Rap1, Rap2, TC21, Rin, Rit
Rab (Ras like proteins in brain)	Vesicules trafic	Rab-1, Rab63
Ran (Ras like nuclear protein)	Nuclear traffic	Ran
Arf (ADP-ribosylation factor)	Vesicules traffic	Arf1, Arf2, Arf3, Arf4, Arf5, Afr6
Rho (Ras homologous proteins)	Cell morphology	RhoA, RhoB, RhoC, RhoD, RhoE, RhoG, RhoH, Rac1, Rac2, Rac3, Cdc4, Rnd1, Rnd2, RIF, CHP, WRCH1, TC10

RAS GENERAL STRUCTURE

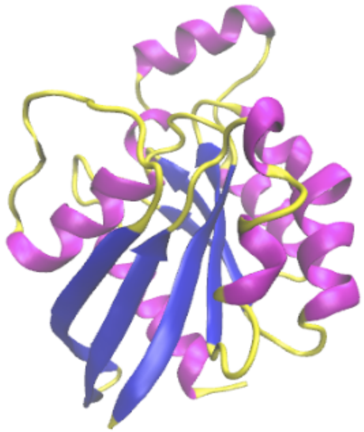


MONONUCLEOTIDE-BINDING DOMAIN

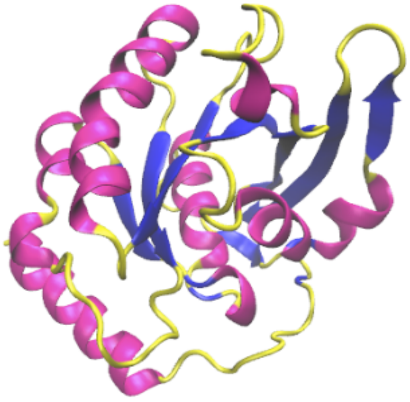
RAS SUPERFAMILY



RAB

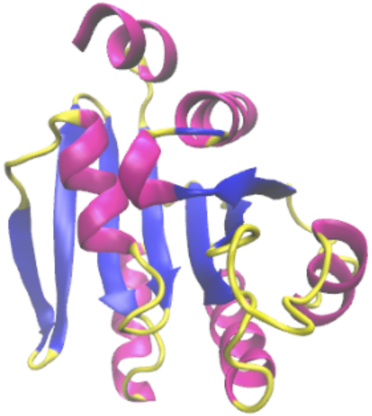


RHO



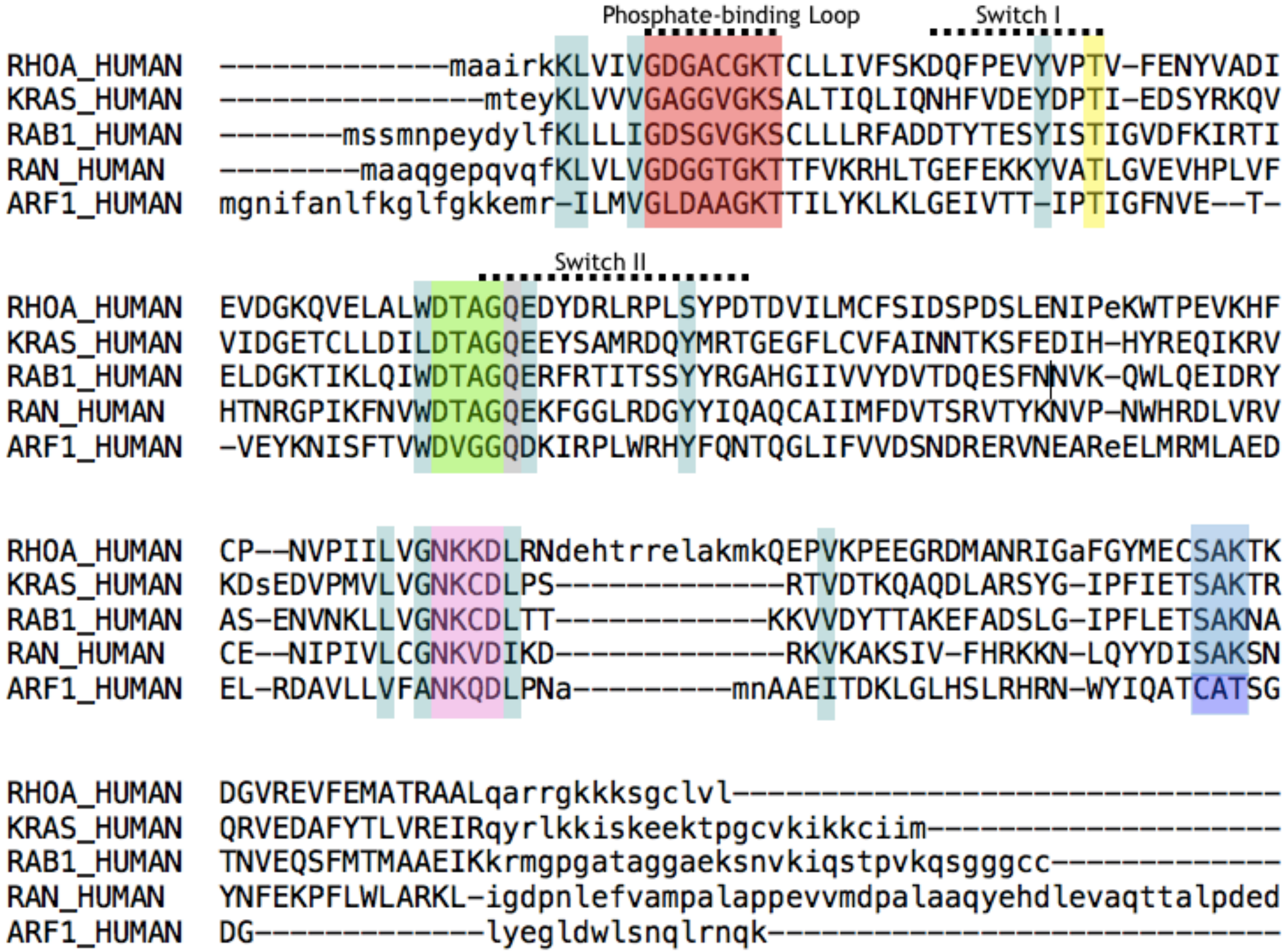
RAN

RAS



ARF

ALINGNMENT RAS



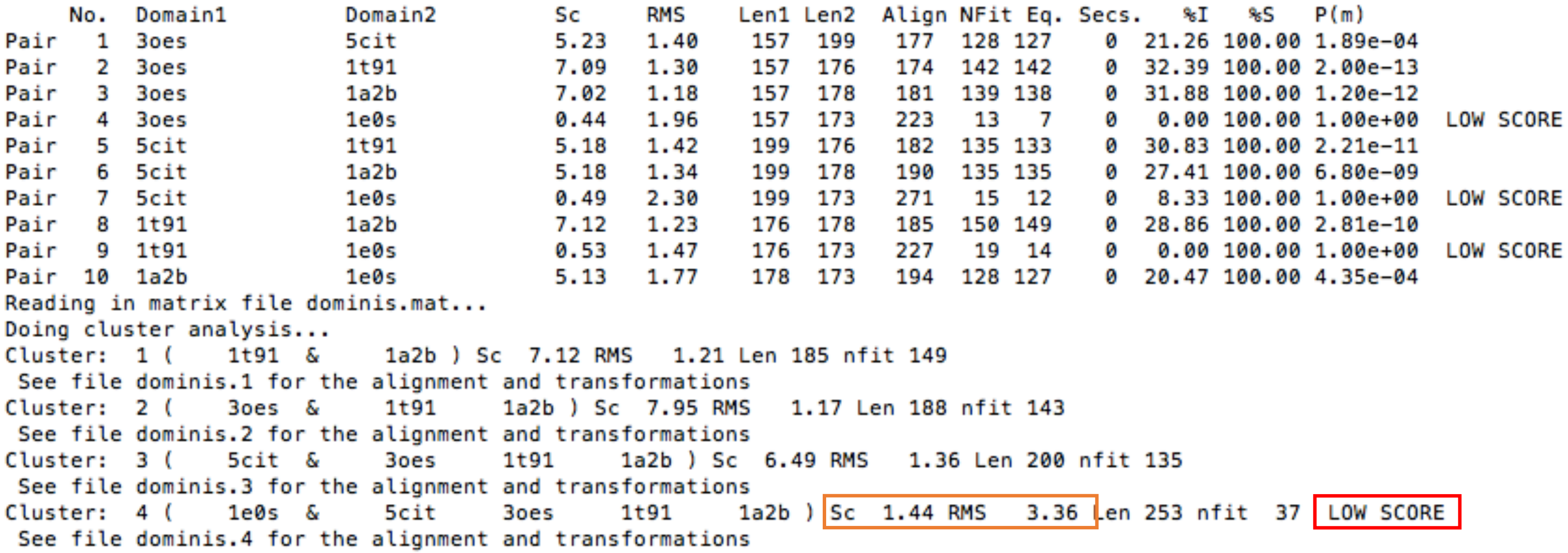
```
RHOA_HUMAN  -------------maairkKLVIVGDGACGKTCLLIVFSKDQFPEVYVPTV-FENYVADI
KRAS_HUMAN  ---------------mteyKLVVVGAGGVGKSALTIQLIQNHFVDEYDPTI-EDSYRKQV
RAB1_HUMAN  -------mssmnpeydylfKLLLIGDSGVGKSCLLLRFADDTYTESYISTIGVDFKIRTI
RAN_HUMAN   --------maaqgepqvqfKLVLVGDGGTGKTTFVKRHLTGEFEKKYVATLGVEVHPLVF
ARF1_HUMAN  mgnifanlfkglfgkkemr-ILMVGLDAAGKTTILYKLKLGEIVTT-IPTIGFNVE--T-

RHOA_HUMAN  EVDGKQVELALWDTAGQEDYDRLRPLSYPDTDVILMCFSIDSPDSLENIPeKWTPEVKHF
KRAS_HUMAN  VIDGETCLLDILDTAGQEEYSAMRDQYMRTGEGFLCVFAINNTKSFEDIH-HYREQIKRV
RAB1_HUMAN  ELDGKTIKLQIWDTAGQERFRTITSSYYRGAHGIIVVYDVTDQESFNNVK-QWLQEIDRY
RAN_HUMAN   HTNRGPIKFNVWDTAGQEKFGGLRDGYYIQAQCAIIMFDVTSRVTYKNVP-NWHRDLVRV
ARF1_HUMAN  -VEYKNISFTVWDVGGQDKIRPLWRHYFQNTQGLIFVVDSNDRERVNEAReELMRMLAED

RHOA_HUMAN  CP--NVPIILVGNKKDLRNdehtrrelakmkQEPVKPEEGRDMANRIGaFGYMECSAKTK
KRAS_HUMAN  KDsEDVPMVLVGNKCDLPS-------------RTVDTKQAQDLARSYG-IPFIETSAKTR
RAB1_HUMAN  AS-ENVNKLLVGNKCDLTT------------KKVVDYTTAKEFADSLG-IPFLETSAKNA
RAN_HUMAN   CE--NIPIVLCGNKVDIKD-------------RKVKAKSIV-FHRKKN-LQYYDISAKSN
ARF1_HUMAN  EL-RDAVLLVFANKQDLPNa---------mnAAEITDKLGLHSLRHRN-WYIQATCATSG

RHOA_HUMAN  DGVREVFEMATRAALqarrgkkksgclvl-------------------------------
KRAS_HUMAN  QRVEDAFYTLVREIRqyrlkkiskeektpgcvkikkciim--------------------
RAB1_HUMAN  TNVEQSFMTMAAEIKkrmgpgataggaeksnvkiqstpvkqsgggcc-------------
RAN_HUMAN   YNFEKPFLWLARKL-igdpnlefvampalappevvmdpalaaqyehdlevaqttalpded
ARF1_HUMAN  DG-------------lyegldwlsnqlrnqk-----------------------------
```

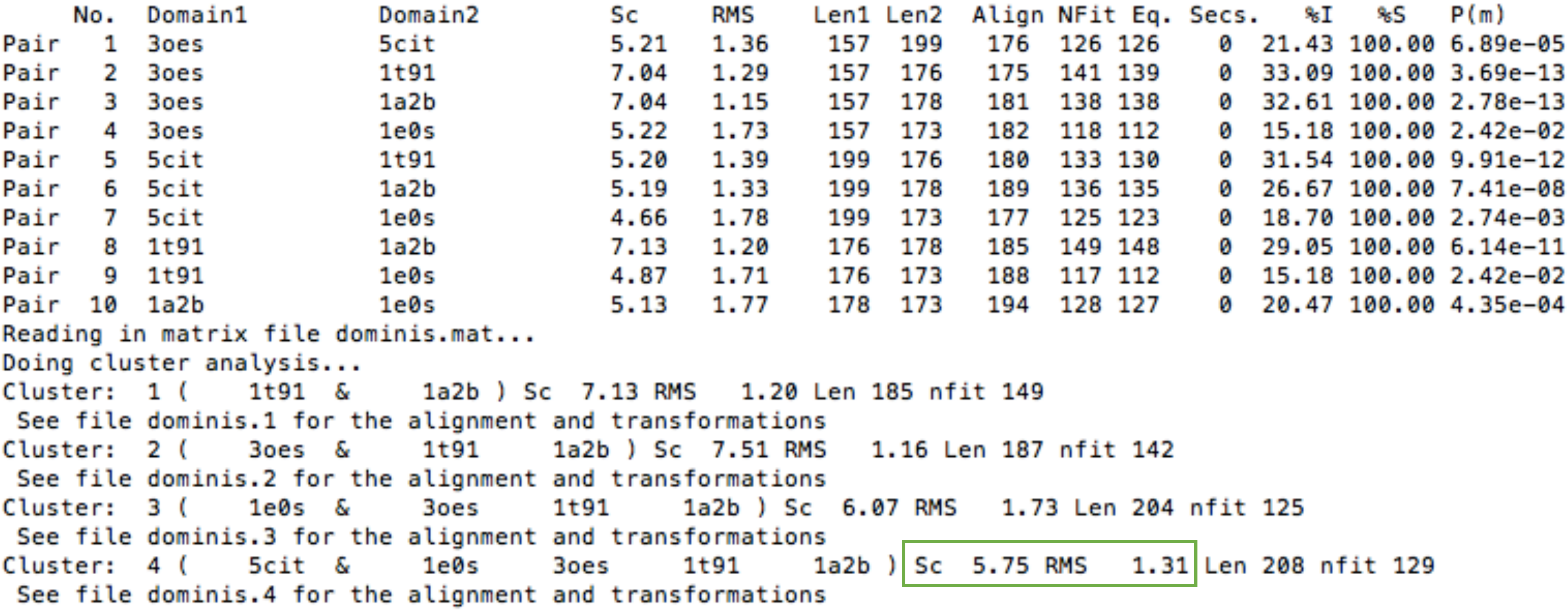
RAS SUPERFAMILY (STAMP)



```
     No.  Domain1         Domain2         Sc     RMS    Len1 Len2  Align NFit Eq. Secs.    %I    %S    P(m)
Pair   1  3oes            5cit            5.23   1.40    157  199   177  128 127     0  21.26 100.00 1.89e-04
Pair   2  3oes            1t91            7.09   1.30    157  176   174  142 142     0  32.39 100.00 2.00e-13
Pair   3  3oes            1a2b            7.02   1.18    157  178   181  139 138     0  31.88 100.00 1.20e-12
Pair   4  3oes            1e0s            0.44   1.96    157  173   223   13   7     0   0.00 100.00 1.00e+00  LOW SCORE
Pair   5  5cit            1t91            5.18   1.42    199  176   182  135 133     0  30.83 100.00 2.21e-11
Pair   6  5cit            1a2b            5.18   1.34    199  178   190  135 135     0  27.41 100.00 6.80e-09
Pair   7  5cit            1e0s            0.49   2.30    199  173   271   15  12     0   8.33 100.00 1.00e+00  LOW SCORE
Pair   8  1t91            1a2b            7.12   1.23    176  178   185  150 149     0  28.86 100.00 2.81e-10
Pair   9  1t91            1e0s            0.53   1.47    176  173   227   19  14     0   0.00 100.00 1.00e+00  LOW SCORE
Pair  10  1a2b            1e0s            5.13   1.77    178  173   194  128 127     0  20.47 100.00 4.35e-04
Reading in matrix file dominis.mat...
Doing cluster analysis...
Cluster:  1 (     1t91  &      1a2b ) Sc  7.12 RMS    1.21 Len 185 nfit 149
 See file dominis.1 for the alignment and transformations
Cluster:  2 (     3oes  &      1t91      1a2b ) Sc  7.95 RMS    1.17 Len 188 nfit 143
 See file dominis.2 for the alignment and transformations
Cluster:  3 (     5cit  &      3oes      1t91      1a2b ) Sc  6.49 RMS    1.36 Len 200 nfit 135
 See file dominis.3 for the alignment and transformations
Cluster:  4 (     1e0s  &      5cit      3oes      1t91      1a2b ) Sc  1.44 RMS    3.36 Len 253 nfit   37  LOW SCORE
 See file dominis.4 for the alignment and transformations
```

ALIGNFIT in order to improve the results

RAS SUPERFAMILY (STAMP)

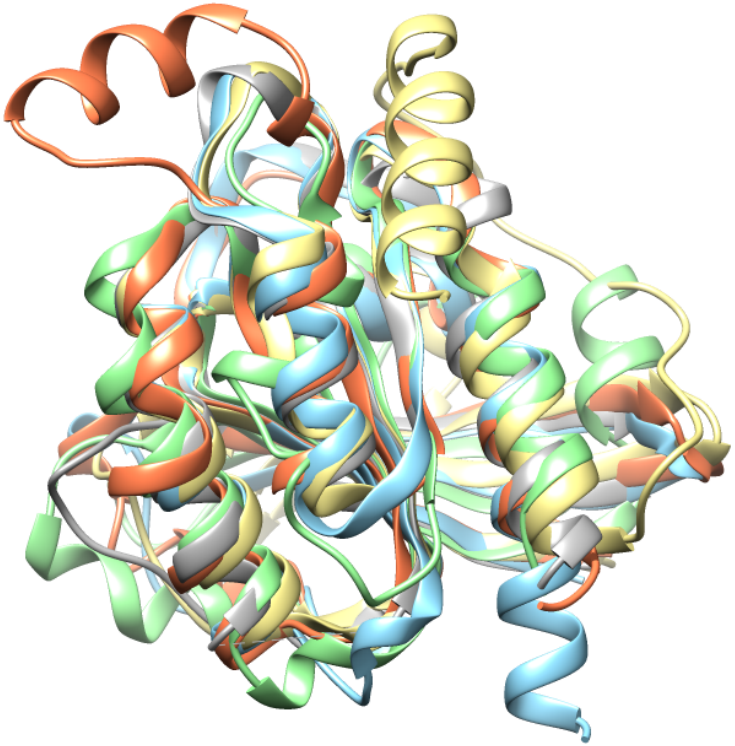


```
     No.  Domain1          Domain2          Sc      RMS      Len1 Len2  Align NFit Eq. Secs.    %I    %S    P(m)
Pair   1  3oes             5cit             5.21    1.36      157  199   176  126 126     0  21.43 100.00 6.89e-05
Pair   2  3oes             1t91             7.04    1.29      157  176   175  141 139     0  33.09 100.00 3.69e-13
Pair   3  3oes             1a2b             7.04    1.15      157  178   181  138 138     0  32.61 100.00 2.78e-13
Pair   4  3oes             1e0s             5.22    1.73      157  173   182  118 112     0  15.18 100.00 2.42e-02
Pair   5  5cit             1t91             5.20    1.39      199  176   180  133 130     0  31.54 100.00 9.91e-12
Pair   6  5cit             1a2b             5.19    1.33      199  178   189  136 135     0  26.67 100.00 7.41e-08
Pair   7  5cit             1e0s             4.66    1.78      199  173   177  125 123     0  18.70 100.00 2.74e-03
Pair   8  1t91             1a2b             7.13    1.20      176  178   185  149 148     0  29.05 100.00 6.14e-11
Pair   9  1t91             1e0s             4.87    1.71      176  173   188  117 112     0  15.18 100.00 2.42e-02
Pair  10  1a2b             1e0s             5.13    1.77      178  173   194  128 127     0  20.47 100.00 4.35e-04
Reading in matrix file dominis.mat...
Doing cluster analysis...
Cluster:  1 (     1t91  &      1a2b ) Sc  7.13 RMS   1.20 Len 185 nfit 149
 See file dominis.1 for the alignment and transformations
Cluster:  2 (     3oes  &      1t91      1a2b ) Sc  7.51 RMS   1.16 Len 187 nfit 142
 See file dominis.2 for the alignment and transformations
Cluster:  3 (     1e0s  &      3oes      1t91      1a2b ) Sc  6.07 RMS   1.73 Len 204 nfit 125
 See file dominis.3 for the alignment and transformations
Cluster:  4 (     5cit  &      1e0s      3oes      1t91      1a2b ) Sc  5.75 RMS   1.31 Len 208 nfit 129
 See file dominis.4 for the alignment and transformations
```

1t91: Rab **1a2b**: Rho **3oes**: Ras **1e0s**: Arf **5cit**: Ran

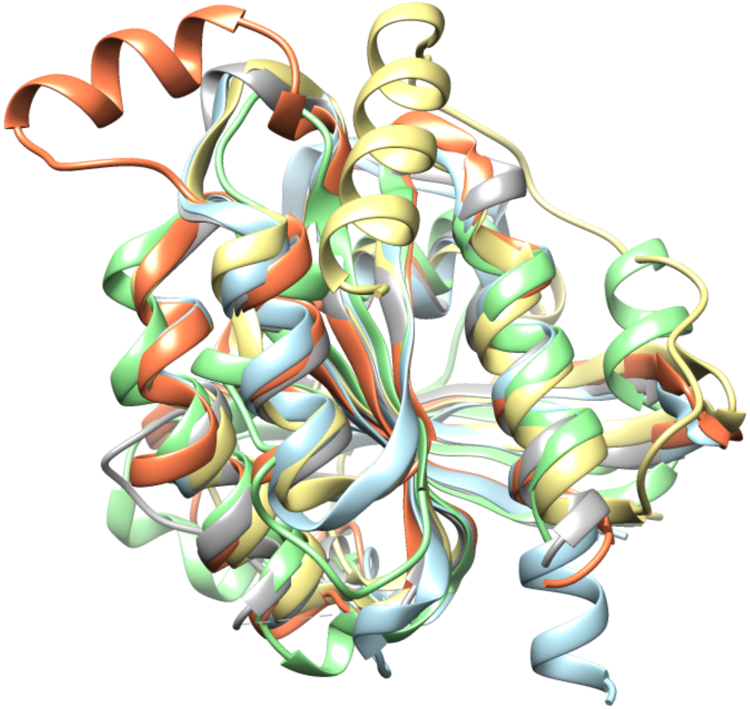
SUPERIMPOSITION

🟦 Rab
🟩 Arf
🟨 Ran
🟧 Rho
⬜ Ras



WITHOUT ALIGNFIT

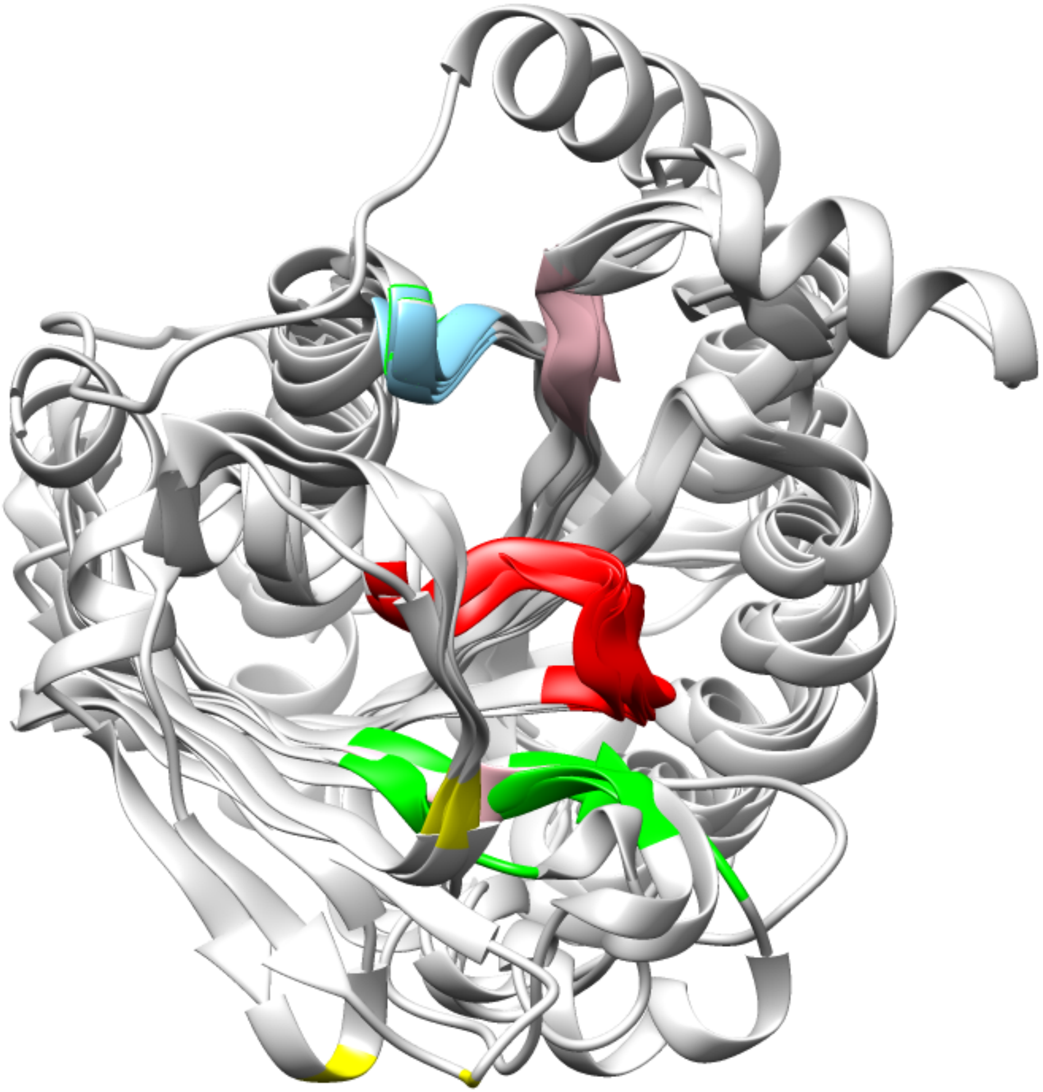
RMS: 3,36
Score: 1,44



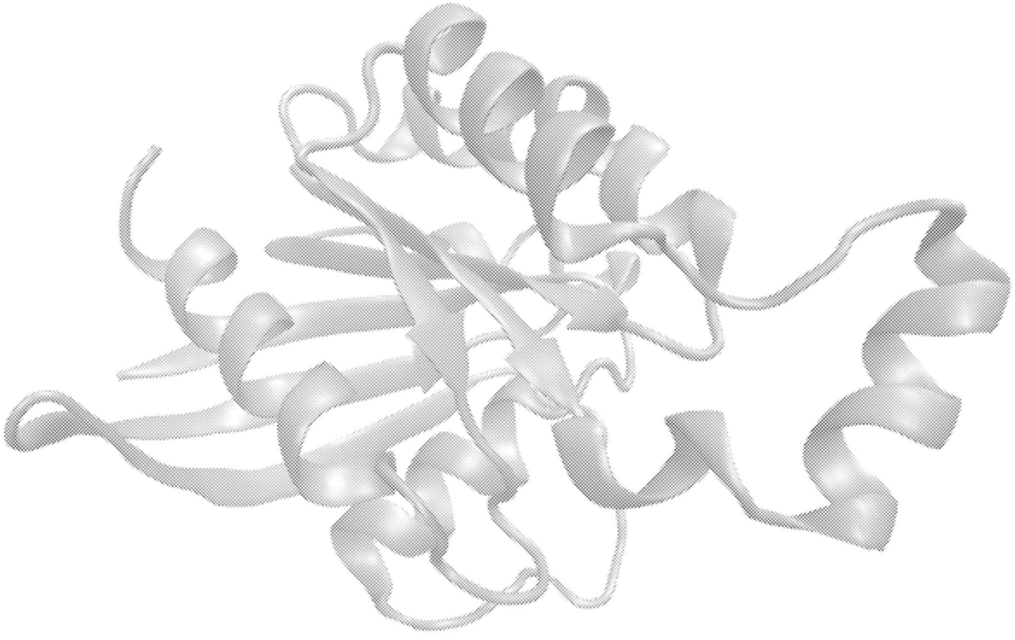
ALIGNFIT

RMS: 1,31
Score: 5,75

G DOMAINS



🟥 Domain 1
🟨 Domain 2
🟩 Domain 3
🟪 Domain 4
🟦 Domain 5



RHO A

RHO FAMILY

Function: Regulation of actin cytoskeletal organization.

- Small (~21 kDa)
- All eukaryotic kingdoms

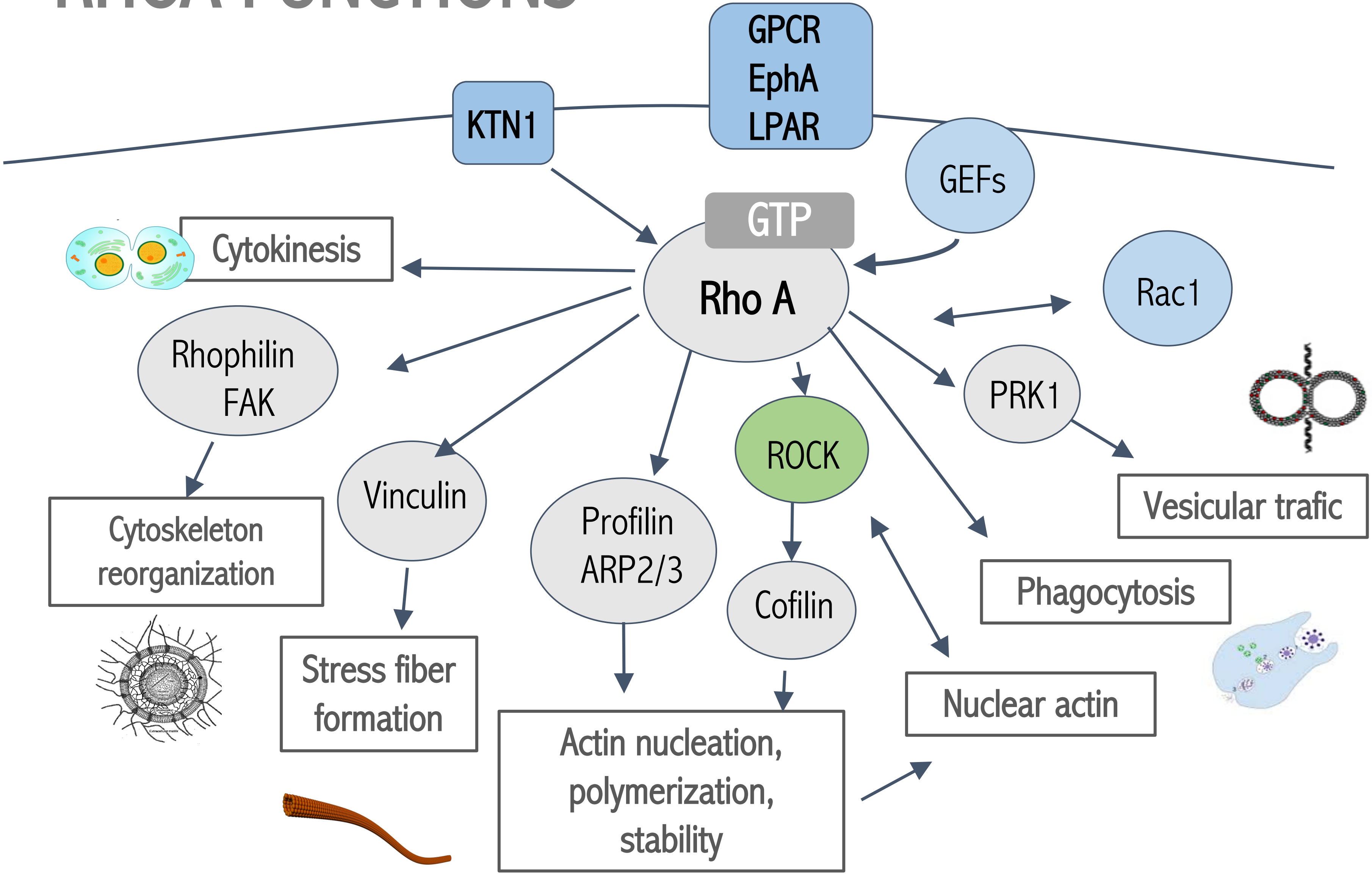
- Molecular switches
- Cell migration
- Secretion and endocytosis
- Proliferation and transformation

RHO FAMILY

RHO GTPASE	EFFECTORS	FUNCTION IN CELL BIOLOGY
Rho A, B, C	ROCK I, II	Actimyosin contractility, cell migration
Rac 1, 2, 3, Rho G	IRSp53	Actin polymeration, lamellopodia formation, driving force in cell migration
Cdc42 TC10, TCL, Chip, Wrch-1	WASP/N-WASP	Actin polymerization, filopodia formation, sensor role in cell migration

Other: RhoE/Rnd3, RhoH/TTF, Rif, RhoBTB1, RhoBTB2, Miro-1, Miro-2, RhoD, Rnd1 and Rnd2

RHOA FUNCTIONS



KTN1

GPCR
EphA
LPAR

GEFs

GTP

Rho A

Rac1

Cytokinesis

Rhophilin
FAK

Cytoskeleton reorganization

Vinculin

Stress fiber formation

Profilin
ARP2/3

ROCK

Cofilin

Actin nucleation, polymerization, stability

PRK1

Vesicular trafic

Phagocytosis

Nuclear actin

Adapted from: Kloc M, Li XC, Ghobrial RM. RhoA Cytoskeletal Pathway to Transplantation. J Immunol Clin Res. 2014; 2(1): 1012.

ALINGNMENT RHO

```
RAC1_HUMAN    ----------------------------------------------mqaiKCVVVGDGAV
RAC2_HUMAN    ----------------------------------------------mqaiKCVVVGDGAV
RAC3_HUMAN    ----------------------------------------------mqaiKCVVVGDGAV
RHOG_HUMAN    ----------------------------------------------mqsiKCVVVGDGAV
RHOJ_HUMAN    ----------------------------mnckegtdsscgcrgndekkmlKCVVVGDGAV
RHO1_HUMAN    ----------------------------------------mahgpgalmlKCVVVGDGAV
CDC42_HUMAN   ----------------------------------------------mqtiKCVVVGDGAV
RHOU_HUMAN    mppqqgdpafpdrceappvpprrerggrggrgpgepggrgraggaegrgvKCVLVGDGAV
RHOV_HUMAN    -------------------mpprelseaeppplraptppprrsappelgiKCVLVGDGAV
RHOH_HUMAN    ---------------------------------------------mlssiKCVLVGDSAV
RHOA_HUMAN    --------------------------------------------maairkKLVIVGDGAC
RHOC_HUMAN    --------------------------------------------maairkKLVIVGDGAC
RHOB_HUMAN    --------------------------------------------maairkKLVVVGDGAC
RHOD_HUMAN    --------------------------------mtaaqaageeappgvrsvKVVLVGDGGC
RHOF_HUMAN    ------------------------------mdapgalaqtaapgpgrkelKIVIVGDGGC
RND3_HUMAN    --------------------------mkerrasqklssksimdpnqnvkcKIVVVGDSQC
RND2_HUMAN    ------------------------------------------megqsgrcKIVVVGDAEC
RND1_HUMAN    ------------------------------------mkerrapqpvvarcKLVLVGDVQC

RAC1_HUMAN    GKTCLLISYTTNAFPGEYIPTVFDNYSANVMVDGKPVNLGLWDTAGQEDYDRLRPLSYPQ
RAC2_HUMAN    GKTCLLISYTTNAFPGEYIPTVFDNYSANVMVDSKPVNLGLWDTAGQEDYDRLRPLSYPQ
RAC3_HUMAN    GKTCLLISYTTNAFPGEYIPTVFDNYSANVMVDGKPVNLGLWDTAGQEDYDRLRPLSYPQ
RHOG_HUMAN    GKTCLLICYTTNAFPKEYIPTVFDNYSAQSAVDGRTVNLNLWDTAGQEEYDRLRTLSYPQ
RHOJ_HUMAN    GKTCLLMSYANDAFPEEYVPTVFDHYAVTVTVGGKQHLLGLYDTAGQEDYNQLRPLSYPN
RHO1_HUMAN    GKTCLLMSYANDAFPEEYVPTVFDHYAVSVTVGGKQYLLGLYDTAGQEDYDRLRPLSYPM
CDC42_HUMAN   GKTCLLISYTTNKFPSEYVPTVFDNYAVTVMIGGEPYTLGLFDTAGQEDYDRLRPLSYPQ
RHOU_HUMAN    GKTSLVVSYTTNGYPTEYIPTAFDNFSAVVSVDGRPVRLQLCDTAGQDEFDKLRPLCYTN
RHOV_HUMAN    GKSSLIVSYTCNGYPARYRPTALDTFSVQVLVDGAPVRIELWDTAGQEDFDRLRSLCYPD
RHOH_HUMAN    GKTSLLVRFTSETFPEAYKPTVYENTGVDVFMDGIQISLGLWDTAGNDAFRSIRPLSYQQ
RHOA_HUMAN    GKTCLLIVFSKDQFPEVYVPTVFENYVADIEVDGKQVELALWDTAGQEDYDRLRPLSYPD
RHOC_HUMAN    GKTCLLIVFSKDQFPEVYVPTVFENYIADIEVDGKQVELALWDTAGQEDYDRLRPLSYPD
RHOB_HUMAN    GKTCLLIVFSKDEFPEVYVPTVFENYVADIEVDGKQVELALWDTAGQEDYDRLRPLSYPD
RHOD_HUMAN    GKTSLLMVFADGAFPESYTPTVFERYMVNLQVKGKPVHLHIWDTAGQDDYDRLRPLFYPD
RHOF_HUMAN    GKTSLLMVYSQGSFPEHYAPSVFEKYTASVTVGSKEVTLNLYDTAGQEDYDRLRPLSYQN
RND3_HUMAN    GKTALLHVFAKDCFPENYVPTVFENYTASFEIDTQRIELSLWDTSGSPYYDNVRPLSYPD
RND2_HUMAN    GKTALLQVFAKDAYPGSYVPTVFENYTASFEIDKRRIELNMWDTSGSSYYDNVRPLAYPD
RND1_HUMAN    GKTAMLQVLAKDCYPETYVPTVFENYTACLETEEQRVELSLWDTSGSPYYDNVRPLCYSD
```

🟥 Domain 1
🟨 Domain 2
🟩 Domain 3
🟪 Domain 4
🟦 Domain 5
⬜ Conserved
▨ Almost conserved

ALINGNMENT RHO

- 🟥 Domain 1
- 🟨 Domain 2
- 🟩 Domain 3
- 🟪 Domain 4
- 🟦 Domain 5
- ⬜ Conserved
- ▨ Almost conserved

```
RAC1_HUMAN    TDVFLICFSLVSPASFENVRaKWYPEVRHHCP-NTPIILVGTKLDLRDdkdtie-klkek
RAC2_HUMAN    TDVFLICFSLVSPASYENVRaKWFPEVRHHCP-STPIILVGTKLDLRDdkdtie-klkek
RAC3_HUMAN    TDVFLICFSLVSPASFENVRaKWYPEVRHHCPH-TPILLVGTKLDLRDdkdtie-rlrdk
RHOG_HUMAN    TNVFVICFSIASPPSYENVRhKWHPEVCHHCP-DVPILLVGTKKDLRAqpdtlr-rlkeq
RHOJ_HUMAN    TDVFLICFSVVNPASYHNVQeEWVPELKDCMPH-VPYVLIGTQIDLRDdpktla-rllym
RHO1_HUMAN    TDVFLICFSVVNPASFQNVKeEWVPELKEYAP-NVPFLLIGTQIDLRDdpktla-rlndm
CDC42_HUMAN   TDVFLVCFSVVSPSSFENVKeKWVPEITHHCPK-TPFLLVGTQIDLRDdpstie-klakn
RHOU_HUMAN    TDIFLLCFSVVSPSSFQNVSeKWVPEIRCHCPK-APIILVGTQSDLREdvkvli-eldkc
RHOV_HUMAN    TDVFLACFSVVQPSSFQNITeKWLPEIRTHNPQ-APVLLVGTQADLRDdvnvliqldqgg
RHOH_HUMAN    ADVVLMCYSVANHNSFLNLKnKWIGEIRSNLPC-TPVLVVATQTDQREmg--------ph
RHOA_HUMAN    TDVILMCFSIDSPDSLENIPeKWTPEVKHFCP-NVPIILVGNKKDLRNdehtrr-elakm
RHOC_HUMAN    TDVILMCFSIDSPDSLENIPeKWTPEVKHFCP-NVPIILVGNKKDLRQdehtrr-elakm
RHOB_HUMAN    TDVILMCFSVDSPDSLENIPeKWVPEVKHFCP-NVPIILVANKKDLRSdehvrt-elarm
RHOD_HUMAN    ASVLLLCFDVTSPNSFDNIFnRWYPEVNHFCKK-VPIIVVGCKTDLCKdkslvn-klrrn
RHOF_HUMAN    THLVLICYDVMNPTSYDNVLiKWFPEVTHFCR-GIPMVLIGCKTDLRKdkeqlr-klraa
RND3_HUMAN    SDAVLICFDISRPETLDSVLkKWKGEIQEFCP-NTKMLLVGCKSDLRTdvstlv-elsnh
RND2_HUMAN    SDAVLICFDISRPETLDSVLkKWQGETQEFCP-NAKVVLVGCKLDMRTdlatlr-elskq
RND1_HUMAN    SDAVLLCFDISRPETVDSALkKWRTEILDYCP-STRVLLIGCKTDLRTdlstlm-elshq
```

ALINGNMENT RHO

```
RAC1_HUMAN    kLTPITYPQGLAMAKEIGaVKYLECSALTQ-R-G-LKTVFDEAIRAVLcpppvkkrkrkc
RAC2_HUMAN    kLAPITYPQGLALAKEIDsVKYLECSALTQ-R-G-LKTVFDEAIRAVLcpqptrqqkrac
RAC3_HUMAN    kLAPITYPQGLAMAREIGsVKYLECSALTQ-R-G-LKTVFDEAIRAVLcpppvkkpgkkc
RHOG_HUMAN    gQAPITPQQGQALAKQIHaVRYLECSALQQ-D-G-VKEVFAEAVRAVLnptpikrgrsci
RHOJ_HUMAN    kEKPLTYEHGVKLAKAIGaQCYLECSALTQ-K-G-LKAVFDEAILTI-fhpkkkkkrcse
RHO1_HUMAN    kEKPICVEQGQKLAKEIGaCCYVECSALTQ-K-G-LKTVFDEAIIAI-ltpkkhtvkkri
CDC42_HUMAN   kQKPITPETAEKLARDLKaVKYVECSALTQ-K-G-LKNVFDEAILA--aleppepkksrr
RHOU_HUMAN    kEKPVPEEAAKLCAEEIKaASYIECSALTQ-K-N-LKEVFDAAI----vagiqysdtqqq
RHOV_HUMAN    rEGPVPQPQAQGLAEKIRaCCYLECSALTQ-K-N-LKEVFDSAIL---saiehkarlekk
RHOH_HUMAN    rASCVNAMEGKKLAQDVRaKGYLECSALSN-R-G-VQQVFECAVRTA-vnqarrrnrrrl
RHOA_HUMAN    kQEPVKPEEGRDMANRIGaFGYMECSAKTK-D-G-VREVFEMATRAALqarrgkkksgcl
RHOC_HUMAN    kQEPVRSEEGRDMANRISaFGYLECSAKTK-E-G-VREVFEMATRA--glqvrknkrrrg
RHOB_HUMAN    kQEPVRTDDGRAMAVRIQaYDYLECSAKTK-E-G-VREVFETATRAALqkrygsqngcin
RHOD_HUMAN    gLEPVTYHRGQEMARSVGaVAYLECSARLH-D-N-VHAVFQEAAEVA-lssrgrnfwrri
RHOF_HUMAN    qLEPITYMQGLSACEQIRaALYLECSAKFR-E-N-VEDVFREAAKVA-lsalkkaqrqkk
RND3_HUMAN    rQTPVSYDQGANMAKQIGaATYIECSALQS-E-NsVRDIFHVA-----tlacvnktnknv
RND2_HUMAN    rLIPVTHEQGTVLAKQVGaVSYVECSSRSS-ErS-VRDVFHVA-----tvaslgrghrql
RND1_HUMAN    kQAPISYEQGCAIAKQLGaEIYLEGSAFTSeK-S-IHSIFRTA-----smlclnkpsplp

RAC1_HUMAN    lll------------------------------------
RAC2_HUMAN    sll------------------------------------
RAC3_HUMAN    tvf------------------------------------
RHOG_HUMAN    ll-------------------------------------
RHOJ_HUMAN    ghsccsii-------------------------------
RHO1_HUMAN    gsrcincclit----------------------------
CDC42_HUMAN   cvll-----------------------------------
RHOU_HUMAN    pkksksrtpdkmknlskswwkkyccfv------------
RHOV_HUMAN    lnakgvrtlsrcrwkkffcfv------------------
RHOH_HUMAN    fsineckif------------------------------
RHOA_HUMAN    vl-------------------------------------
RHOC_HUMAN    cpil-----------------------------------
RHOB_HUMAN    cckvl----------------------------------
RHOD_HUMAN    tqgfcvvt-------------------------------
RHOF_HUMAN    rrlclll--------------------------------
RND3_HUMAN    krnksqratkrishmpsrpelsavatdlrkdkaksctvm
RND2_HUMAN    rrtdsrrgmqrsaqlsgrpdrgnegeihkdrakscnlm-
RND1_HUMAN    qkspvrslskrllhlpsrselisstfkkekakscsim--
```

- 🟥 Domain 1
- 🟨 Domain 2
- 🟩 Domain 3
- 🟪 Domain 4
- 🟦 Domain 5
- ⬜ Conserved
- ▨ Almost conserved

ALIGNMENT RHOA

```
                    Phosphate-binding Loop                Switch I
RHOA_HUMAN  maairkKLVIVGDGACGKTCLLIVFSKDQFPEVYVPTVFENYVADIEVDGKQVELALWDT
RHOA_MOUSE  maairkKLVIVGDGACGKTCLLIVFSKDQFPEVYVPTVFENYVADIEVDGKQVELALWDT
RHOA_CHICK  maairkKLVIVGDGACGKTCLLIVFSKDQFPEVYVPTVFENYVADIEVDGKQVELALWDT
RHOA_XENLA  maairkKLVIVGDGACGKTCLLIVFSKDQFPEVYVPTVFENYVADIEVDSKQVELALWDT
RHOA_DANRE  maairkKLVIVGDGACGKTCLLIVFSKDQFPEVYVPTVFENYVADIEVDSKQVELALWDT
RHOA_DROME  mttirkKLVIVGDGACGKTCLLIVFSKDQFPEVYVPTVFENYVADIEVDGKQVELALWDT
RHOA_CAEEL  maairkKLVIVGDGACGKTCLLIVFSKDQFPDVYVPTVFENYVADIEVDGKQVELALWDT
RHOA_EMENI  maeirrKLVIVGDGACGKTCLLIVFSKGTFPEVYVPTVFENYVADVEVDGKHVELALWDT

                 Switch II
RHOA_HUMAN  AGQEDYDRLRPLSYPDTDVILMCFSIDSPDSLENIPeKWTPEVKHFCPNVPIILVGNKKD
RHOA_MOUSE  AGQEDYDRLRPLSYPDTDVILMCFSIDSPDSLENIPeKWTPEVKHFCPNVPIILVGNKKD
RHOA_CHICK  AGQEDYDRLRPLSYPDTDVILMCFSIDSPDSLENIPeKWTPEVKHFCPNVPIILVGNKKD
RHOA_XENLA  AGQEDYDRLRPLSYPDTDVILMCFSIDSPDSLENIPeKWTPEVKHFCPNVPIILVGNKKD
RHOA_DANRE  AGQEDYDRLRPLSYPDTDVILMCFSIDSPDSLENIPeKWTPEVKHFCPNVPIILVGNKKD
RHOA_DROME  AGQEDYDRLRPLSYPDTDVILMCFSVDSPDSLENIPeKWTPEVKHFCPNVPIILVGNKKD
RHOA_CAEEL  AGQEDYDRLRPLSYPDTDVILMCFSIDSPDSLENIPeKWTPEVRHFCPNVPIILVGNKRD
RHOA_EMENI  AGQEDYDRLRPLSYPDSHVILICFAVDSPDSLDNVQeKWISEVLHFCQGLPIILVGCKKD

RHOA_HUMAN  LRNdehtrrelakmkQEPVKPEEGRDMANRIGaFGYMECSAKTKDGVREVFEMATRAALq
RHOA_MOUSE  LRNdehtrrelakmkQEPVKPEEGRDMANRIGaFGYMECSAKTKDGVREVFEMATRAALq
RHOA_CHICK  LRNdehtrrelakmkQEPVKPEEGRDMANRIGaFGYMECSAKTKDGVREVFEMATRAALq
RHOA_XENLA  LRNdehtrreltkmkQEPVKPEEGRDMANRISaYGYMECSAKTKDGVREVFELATRAALq
RHOA_DANRE  LRNdehtrrelqkmkQEPVKPEEGRDMANRINaFGYLECSAKTKEGVREVFEMATRAALq
RHOA_DROME  LRNdpntirdlakmkQEPVKPQEGRAMAEKINaFAYLECSAKSKEGVRDVFETATRAALq
RHOA_CAEEL  LRSdpqtvrelakmkQEPVKPEQGRAIAEQIGaFAYLECSAKTKDGIREVFEKATQAALq
RHOA_EMENI  LRHdpktieelnktsQKPVTPEQGEEVRKKIGaYKYLECSARTNEGVREVFEAATRAA-l

RHOA_HUMAN  arrgkkksgclvl-
RHOA_MOUSE  arrgkkksgclil-
RHOA_CHICK  arrgkkksgclll-
RHOA_XENLA  arrgkkkttclli-
RHOA_DANRE  akkrgkknacall-
RHOA_DROME  vkkrkktrclll--
RHOA_CAEEL  qkkkkkskcmil--
RHOA_EMENI  ltkthkskkkcsil
```

SCOP

- **Class: Alpha and beta proteins (a/b)**

  Mainly parallel beta sheets (beta-alpha-beta units).
- **Fold: P-loop containing nucleoside triphosphate hydrolases**

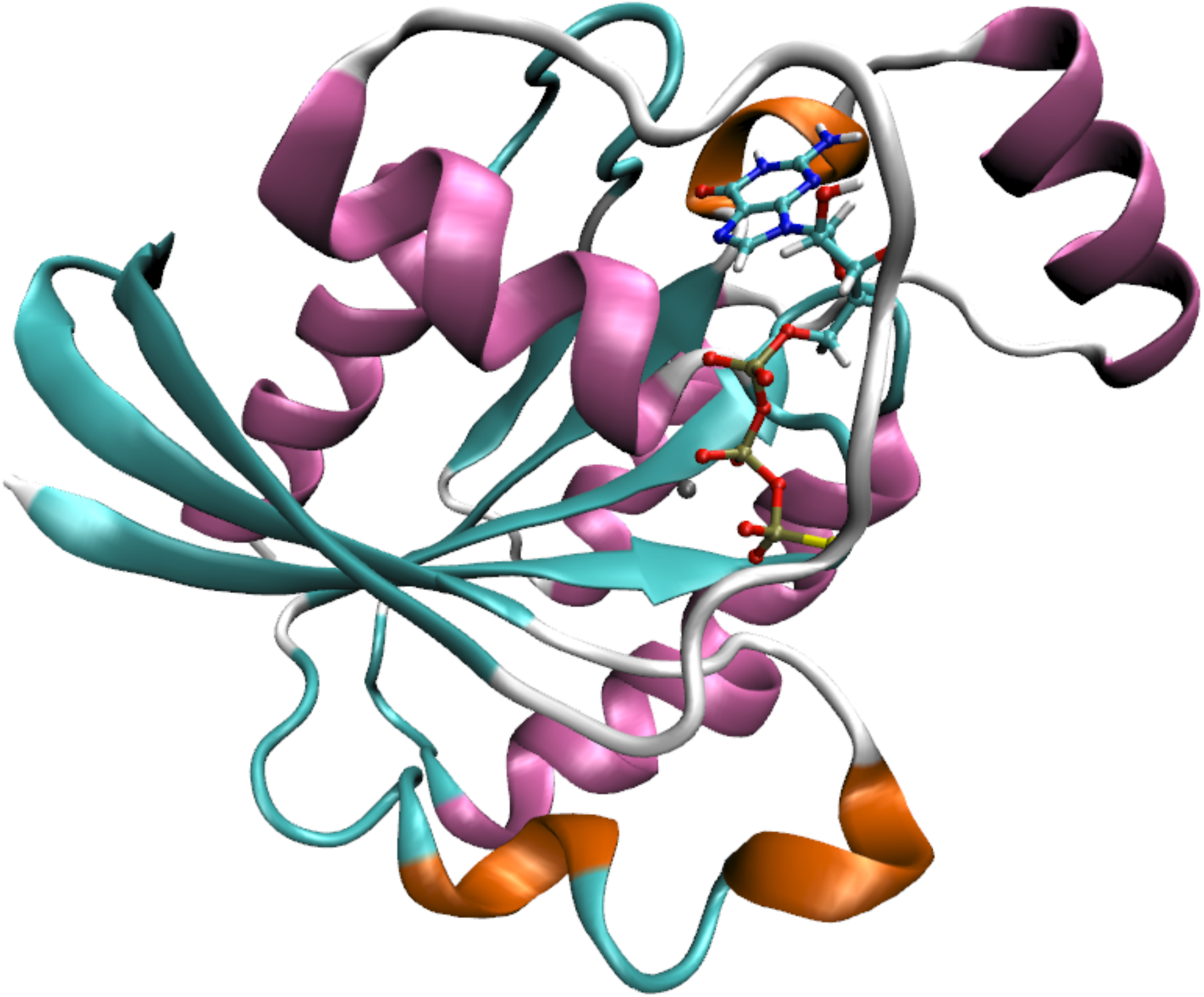
  3 layers: a/b/a, parallel or mixed beta-sheets of variable sizes
- **Superfamily: P-loop containing nucleoside triphosphate hydrolases**

  Division into families based on beta-sheet topologies.
- **Family: G proteins**

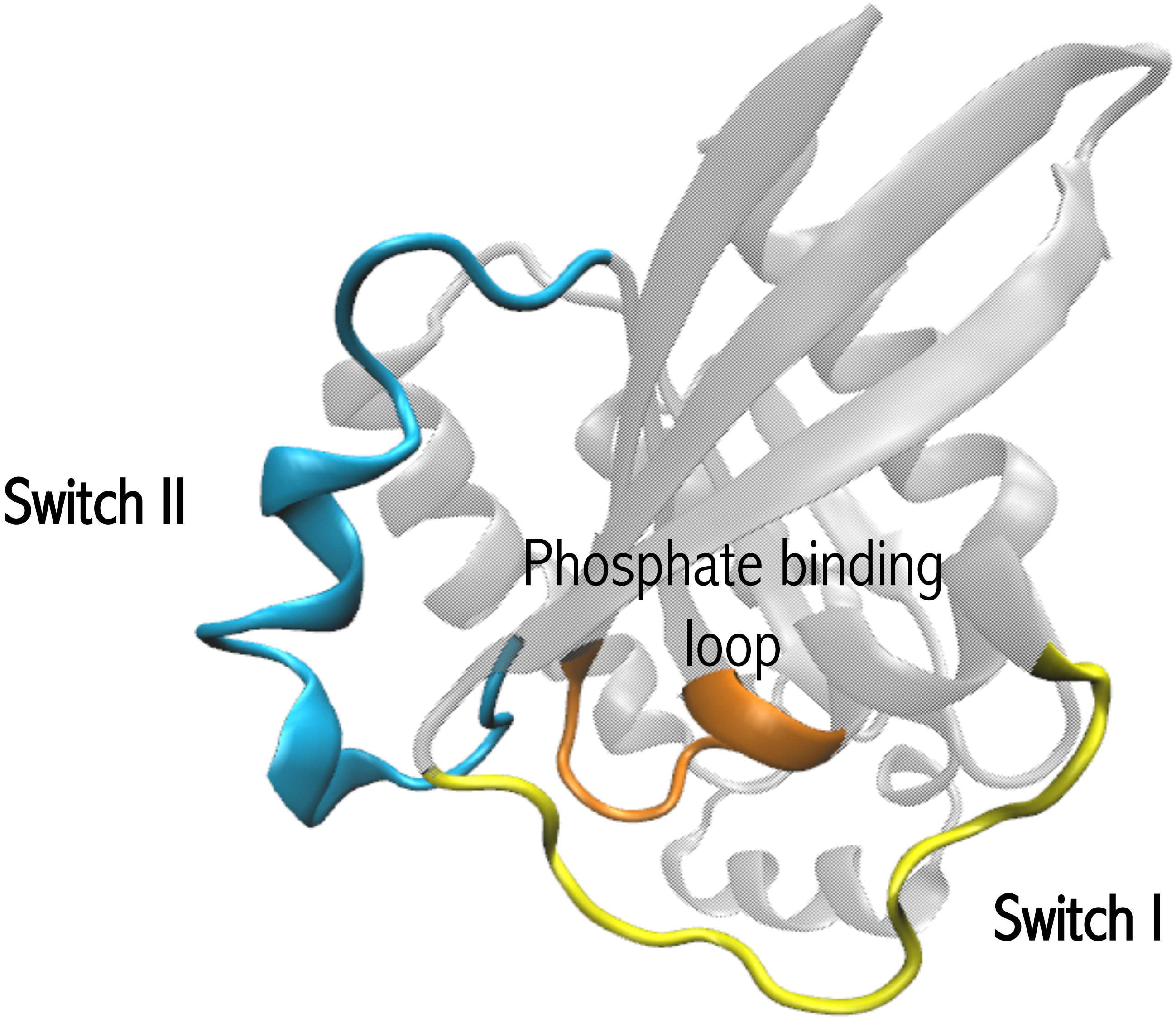
  Core: Mixed beta-sheet of 6 strands, order 231456; strand 2 is antiparallel to the rest.
- **Protein: Rho A**
- **Species: Human (Homo sapiens)**

  SQ P61586

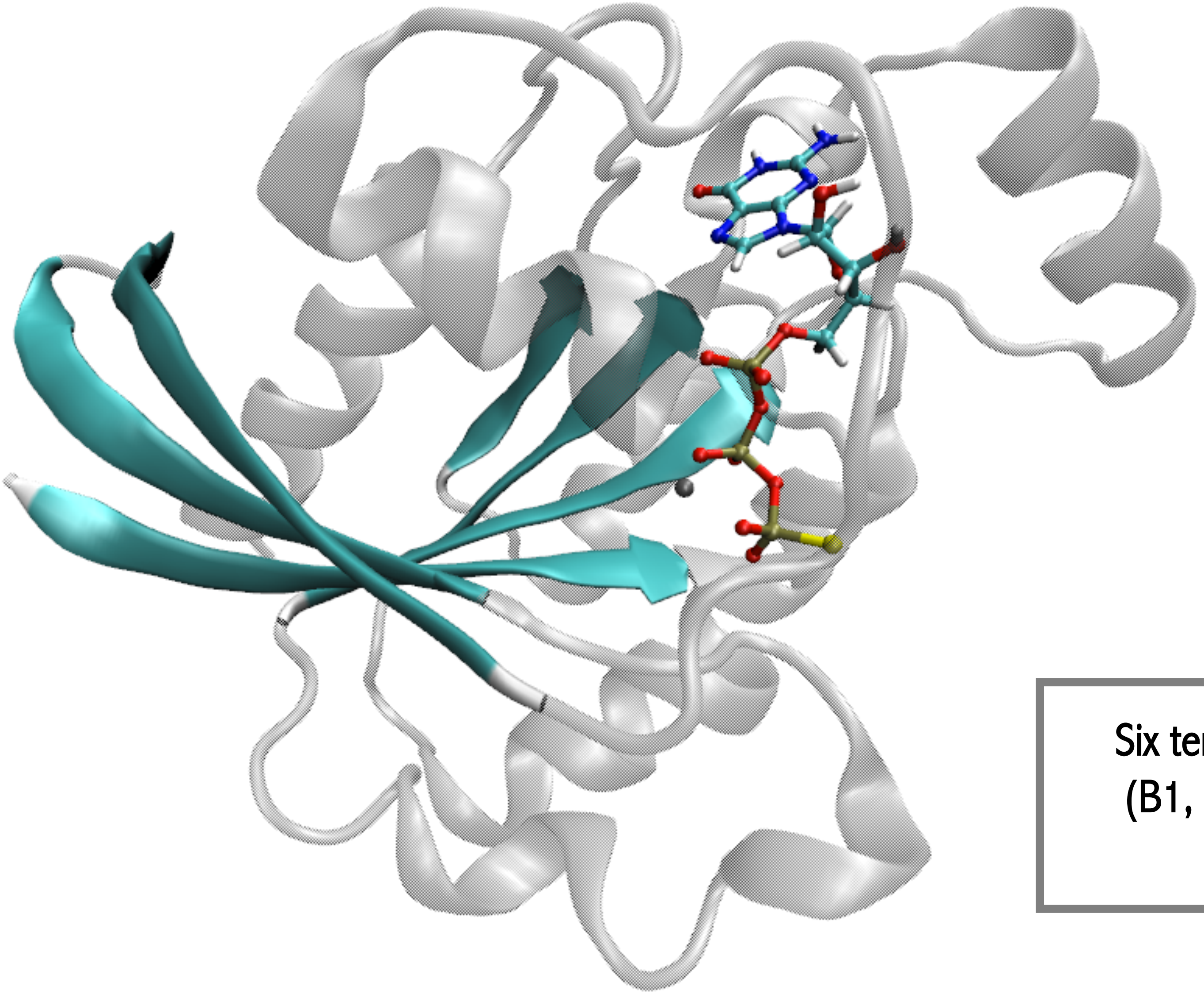
OVERALL STRUCTURE OF RHOA



RHOA SWITCH REGIONS

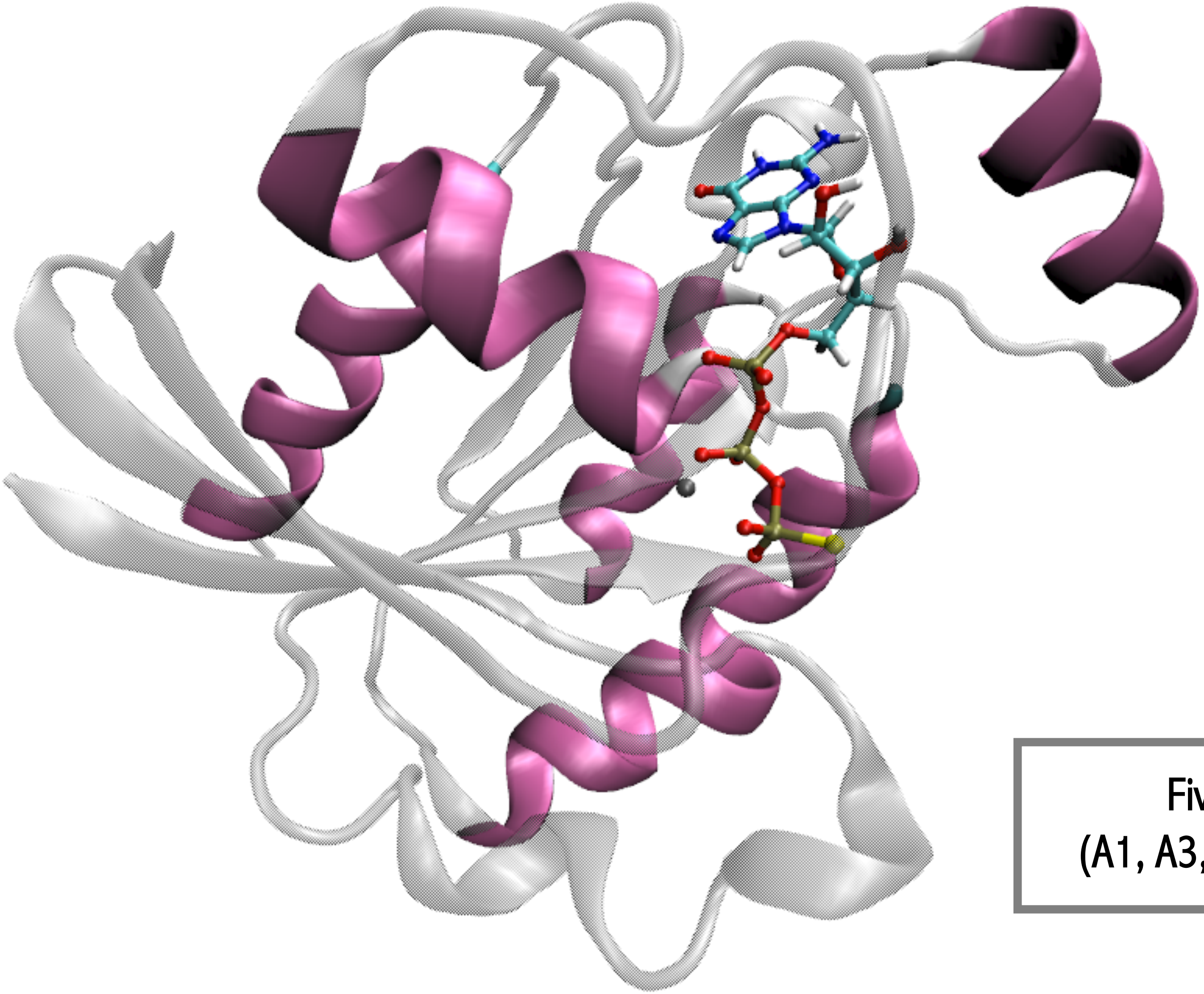


OVERALL STRUCTURE OF RHOA



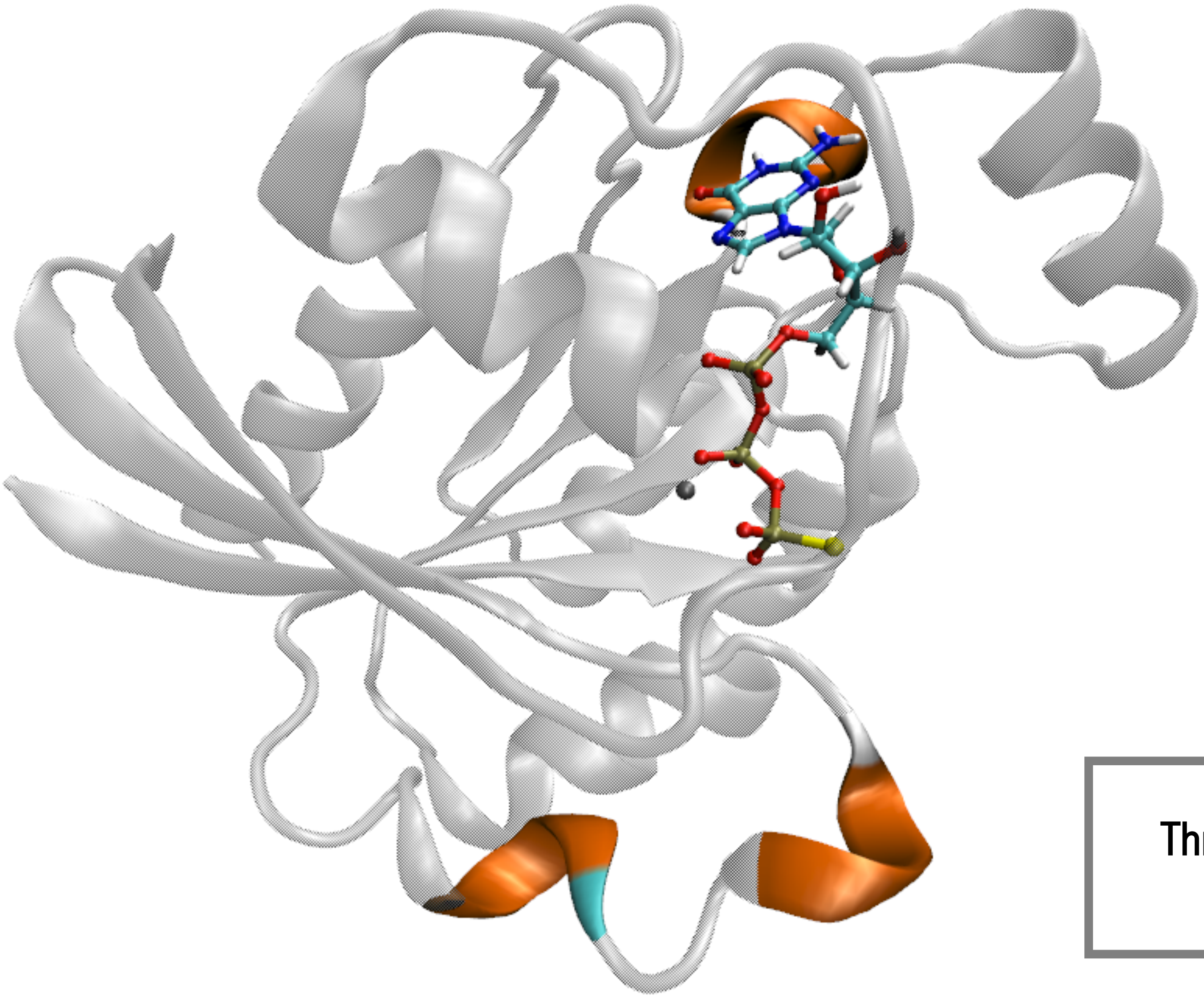
Six tended *β*-strands
(B1, B2, B3 B4, B5
and B6)

OVERALL STRUCTURE OF RHOA



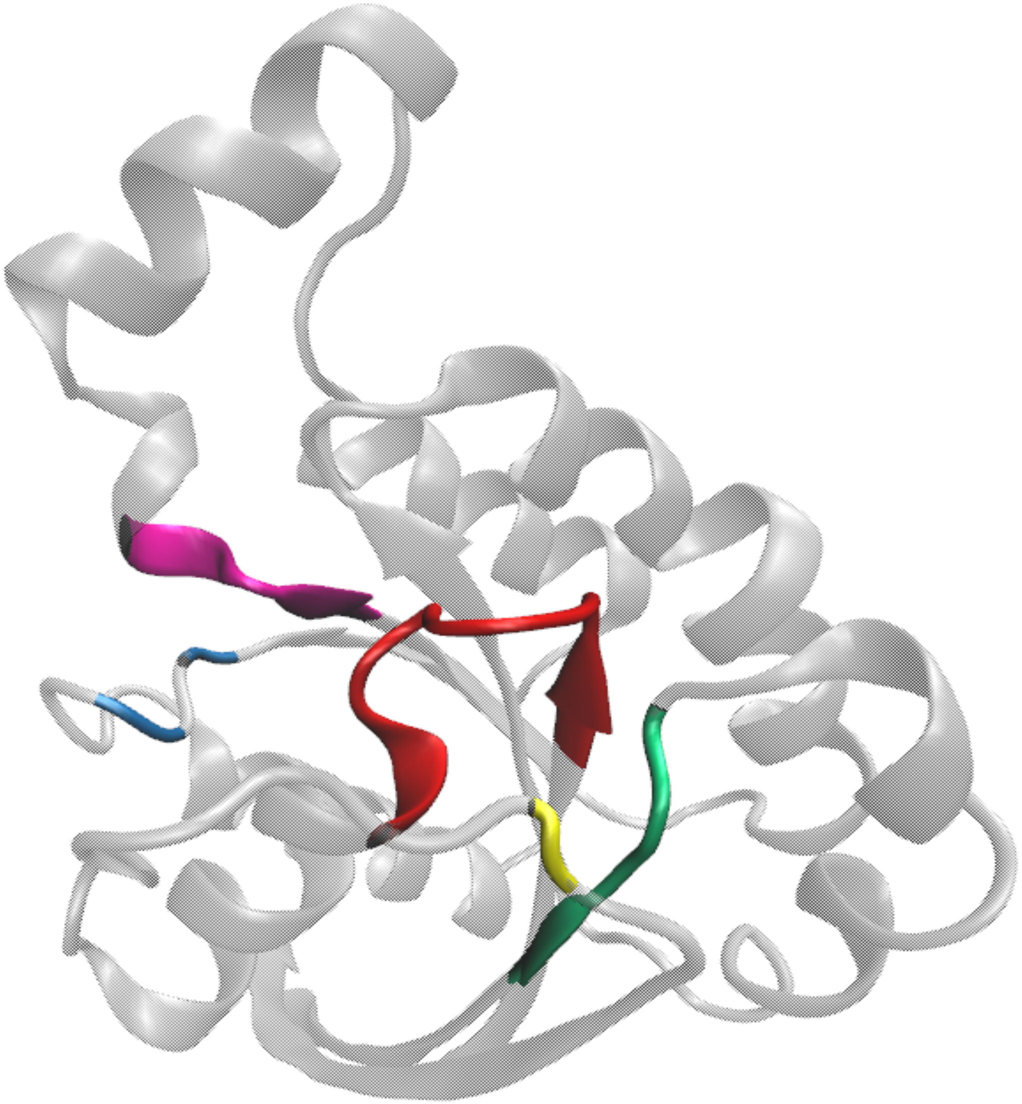
Five α-helices
(A1, A3, A3’, A4 and A5)

OVERALL STRUCTURE OF RHOA



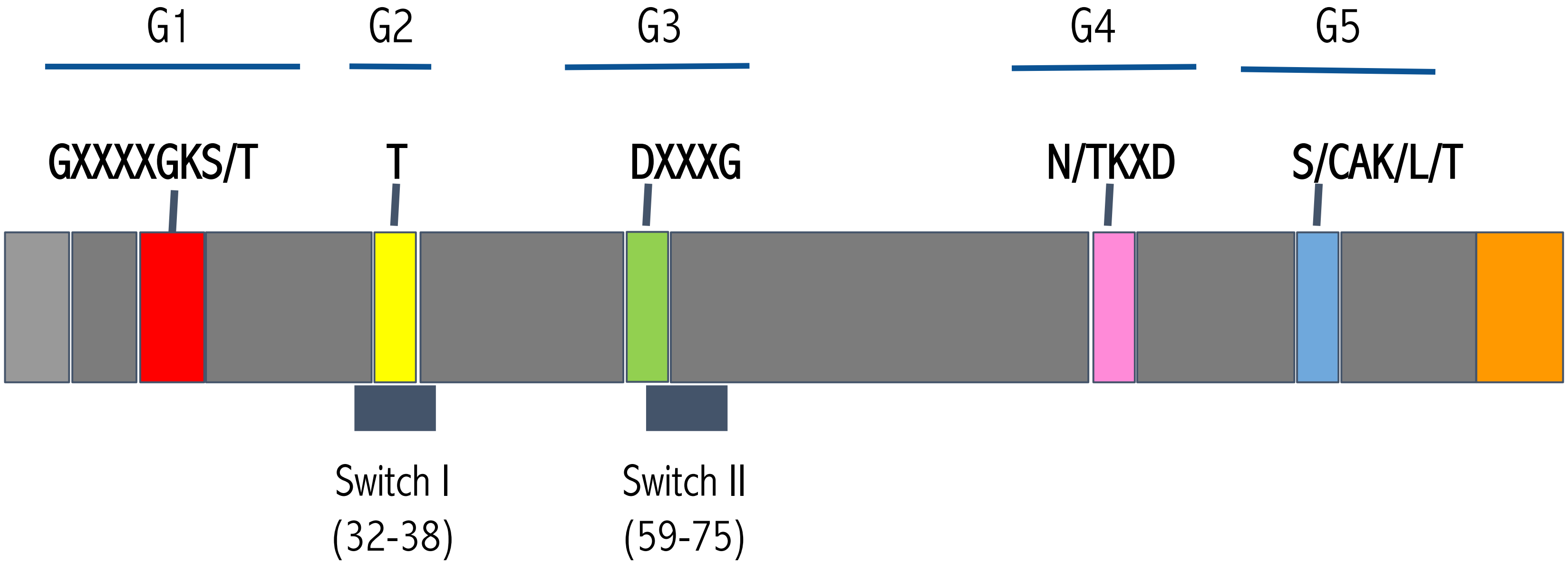
Three 3_{10} helices (H1, H2 and H3)

G DOMAINS



- 🟥 Domain 1
- 🟨 Domain 2
- 🟩 Domain 3
- 🟪 Domain 4
- 🟦 Domain 5

MONOMERIC GTPASE STRUCTURE



G1 G2 G3 G4 G5

GXXXXGKS/T **T** **DXXXG** **N/TKXD** **S/CAK/L/T**

Switch I
(32-38)

Switch II
(59-75)

◼ G Domain (5-166)

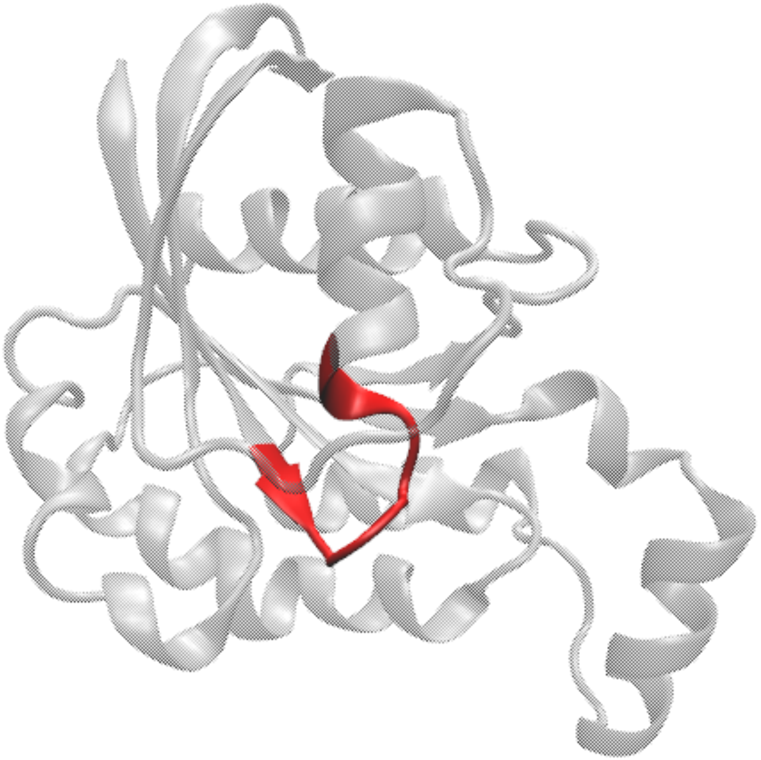
🟧 Membrane targeting sequence

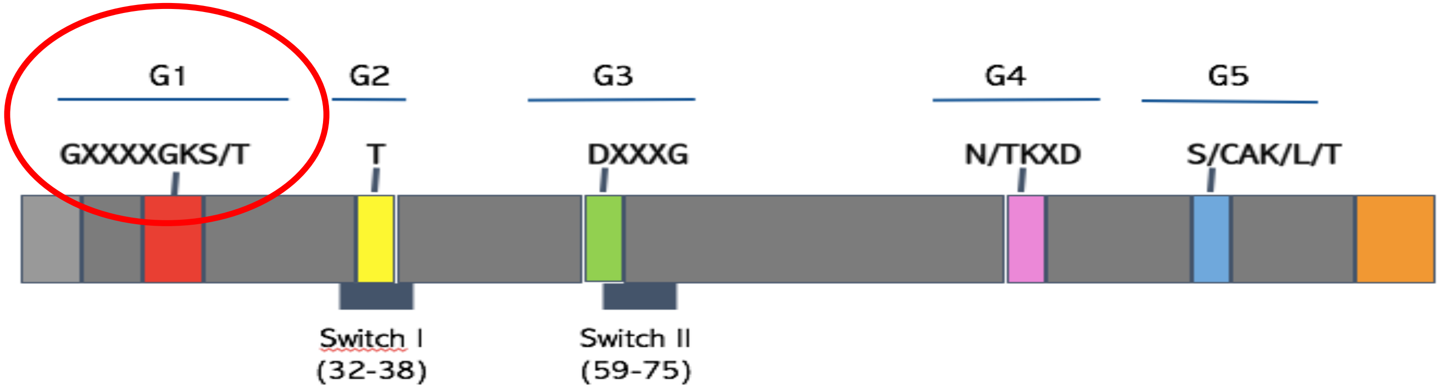
🟨 Core effector domain (32-40)

RHOA: G1 DOMAIN

$_{12}\text{GxxxxGK[S/T]}_{19}$

Phosphate binding effector loop.

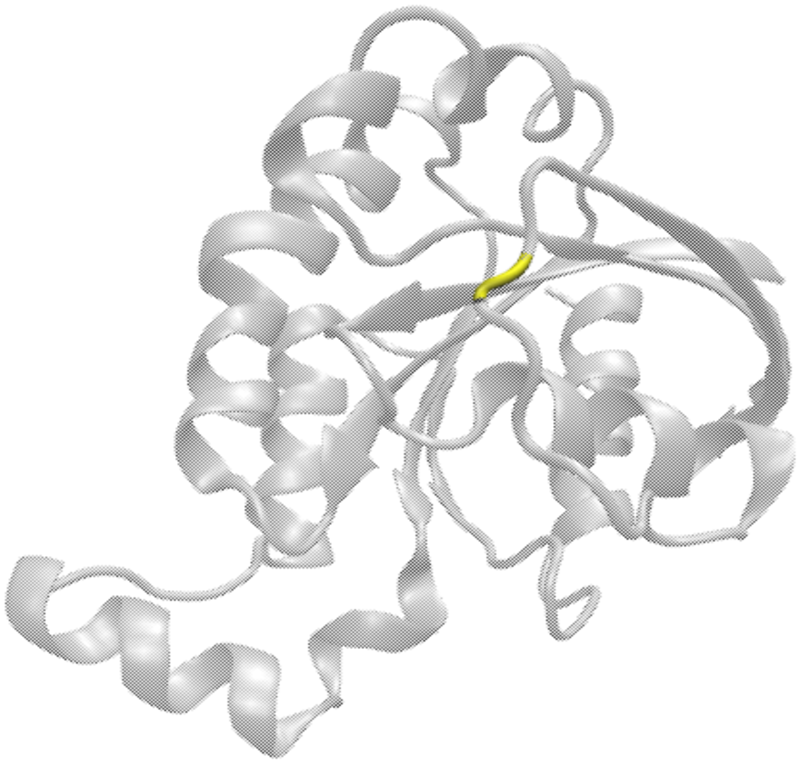


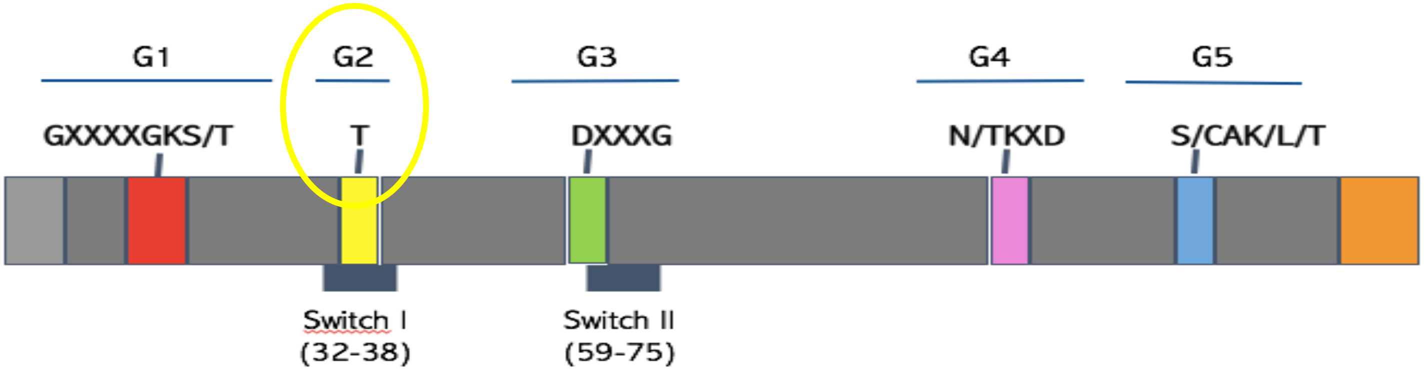


RHOA: G2 DOMAIN

T_{37}

Provides major components of the effector binding surface

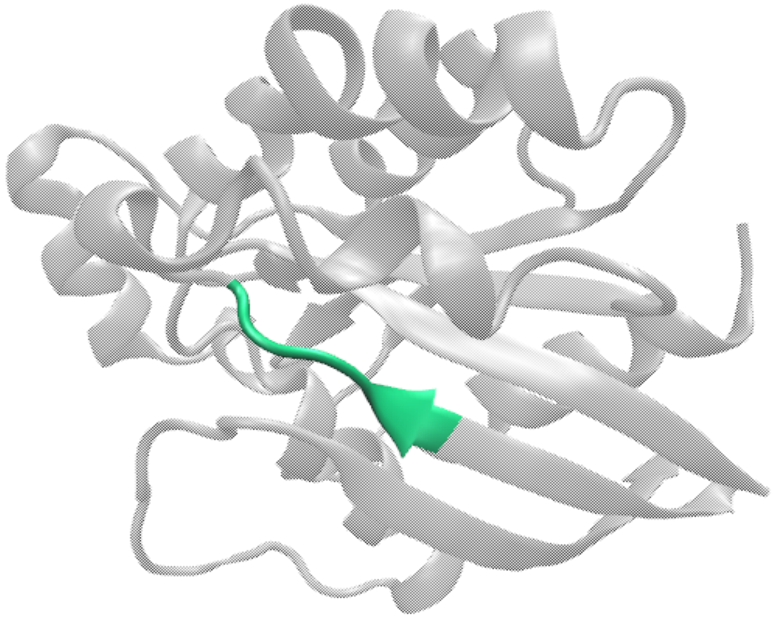


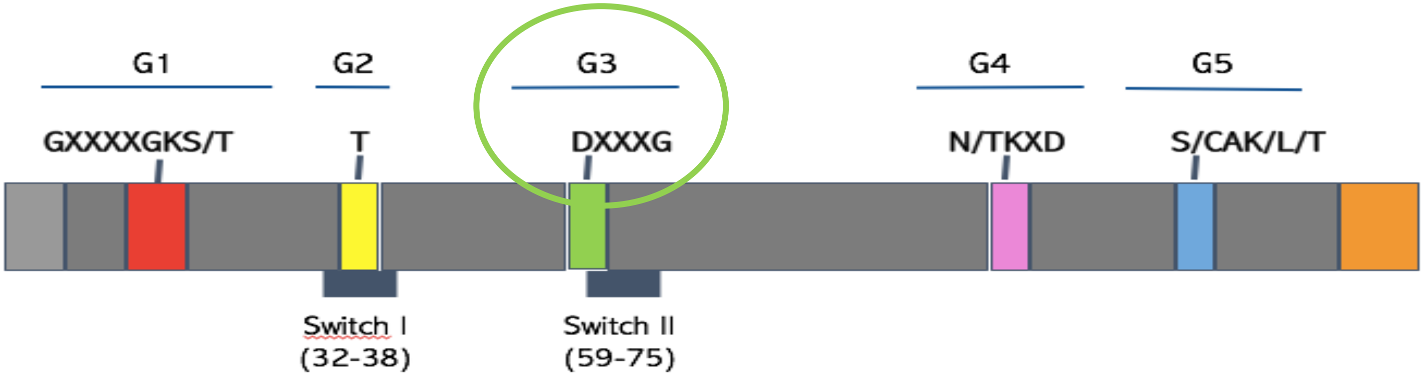


RHOA: G3 DOMAIN

$_{59}\mathrm{DxxG}_{62}$

Involved in binding a nucleotide – associated Mg^{2+} ion by water contact. Important role in GEFs and GAPs exchange

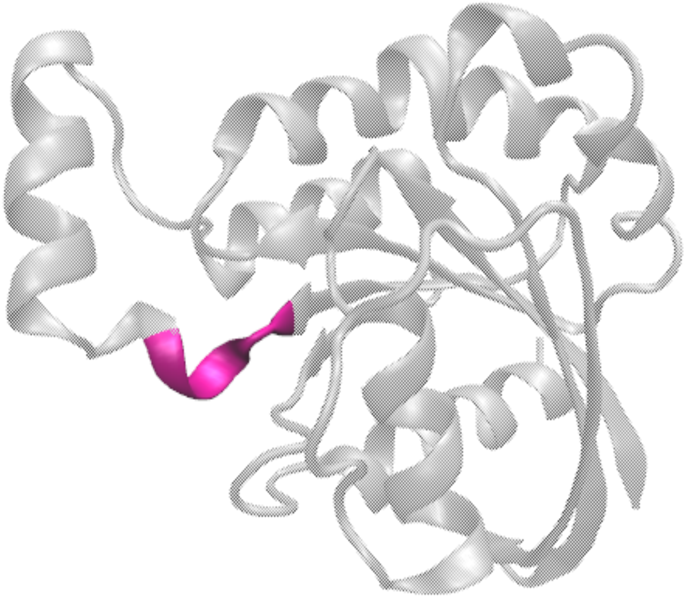


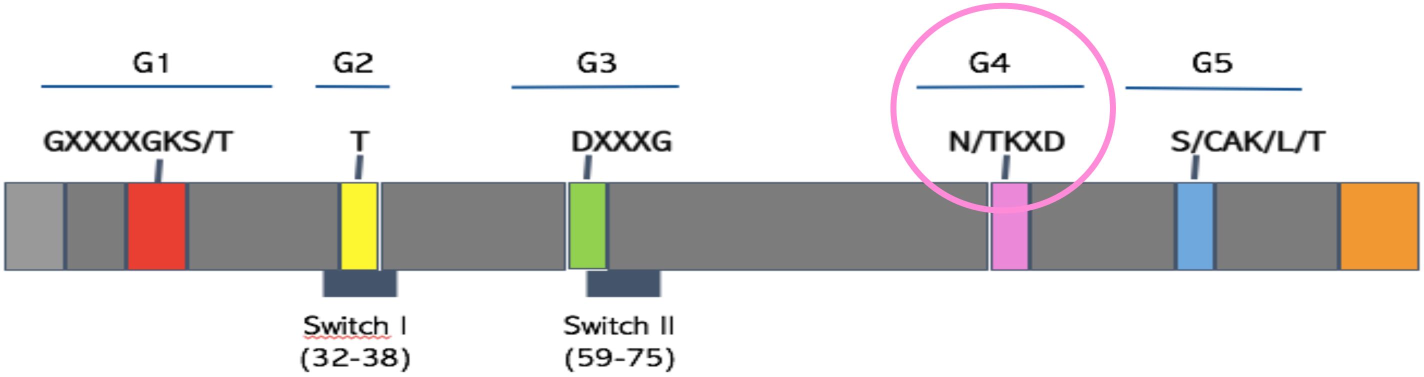


RHOA: G4 DOMAIN

${}_{118}[N/T]KxD_{121}$

Confers specificity to GTP over ATP

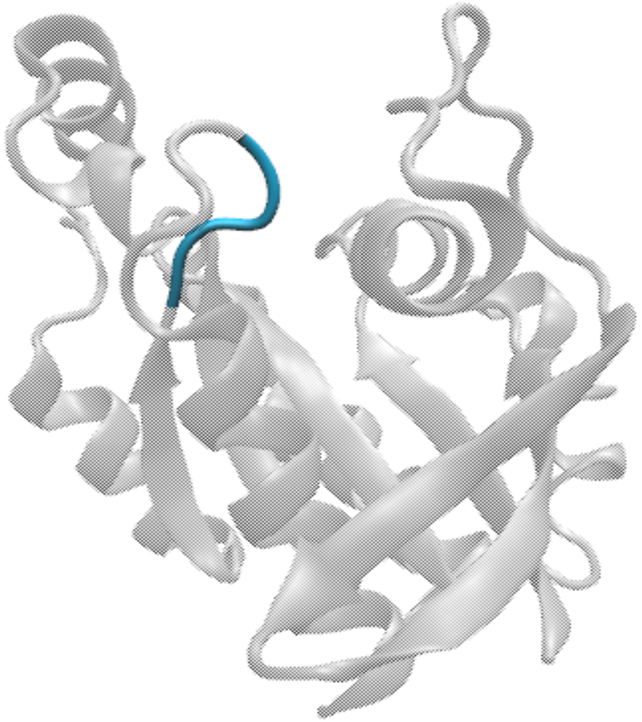


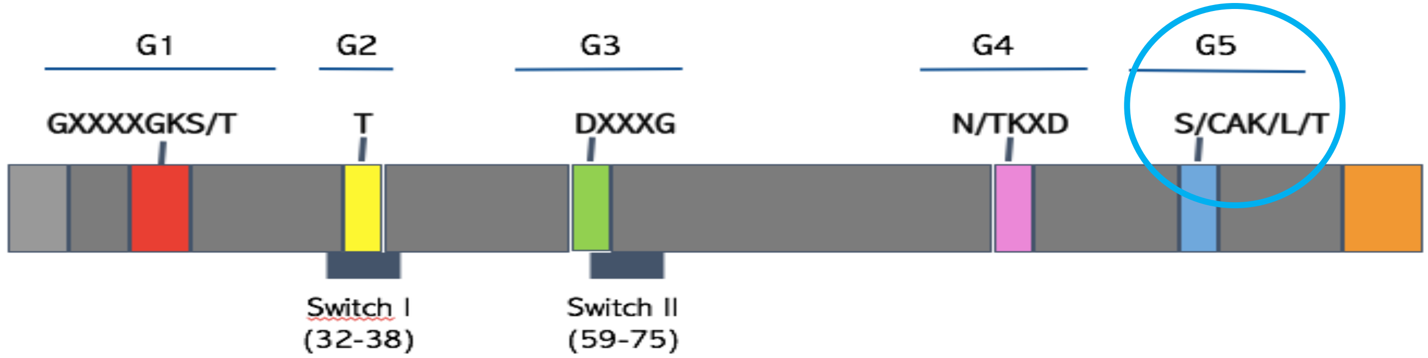


RHOA: G5 DOMAIN

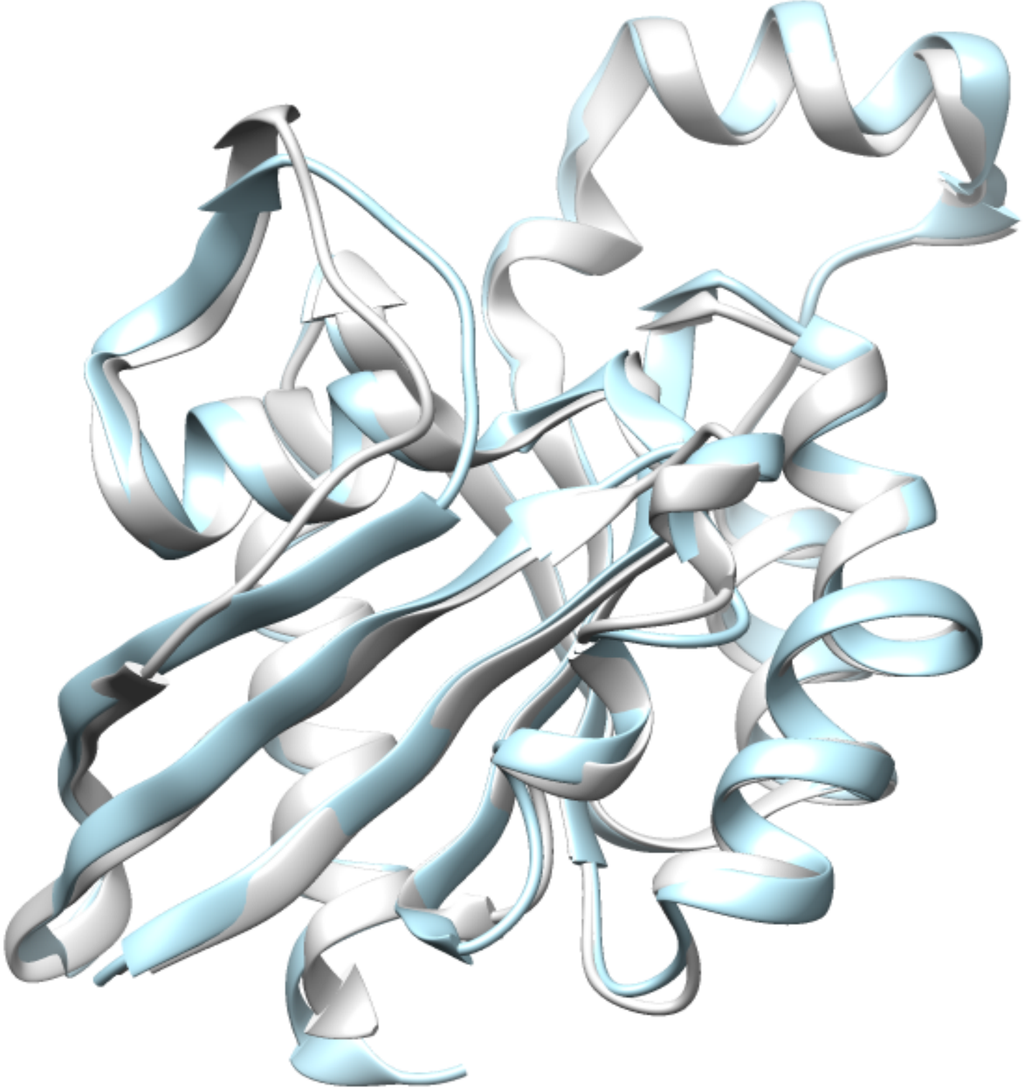
$_{160}\mathrm{SAK}_{162}$

Makes indirect associations with the guanine nucleotide





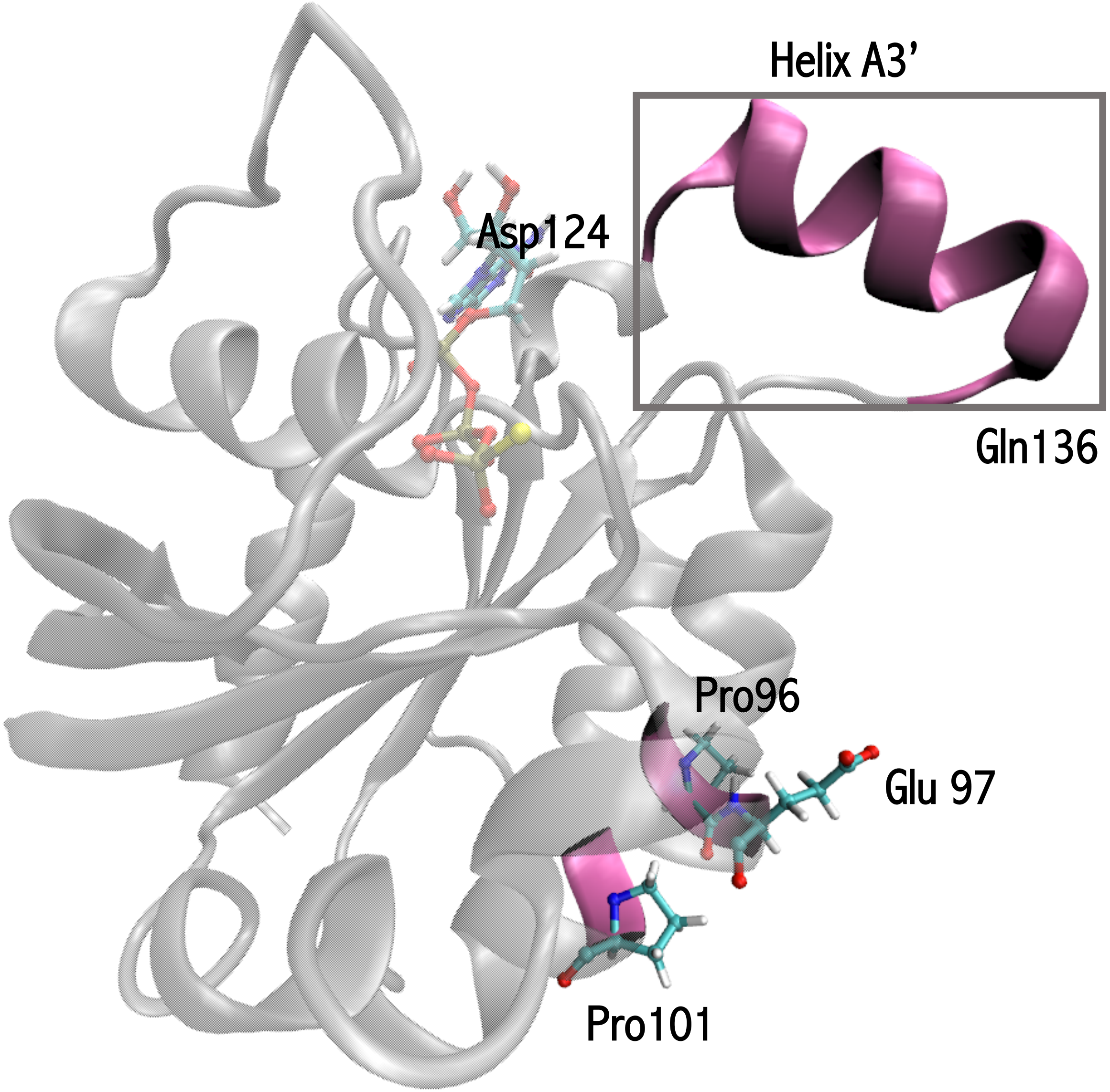
SUPERIMPOSITION RHOA-GTP RHOA-GDP

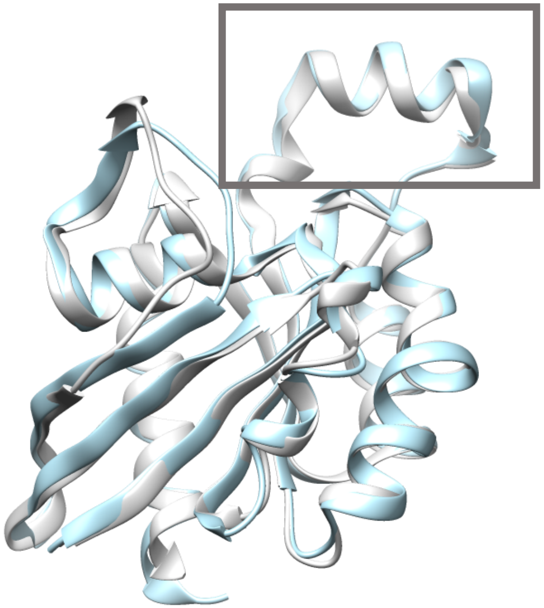


RMS: 0,94
Score: 9,00

🟦 RhoA - GTP
⬜ RhoA - GDP

INSERT REGIONS





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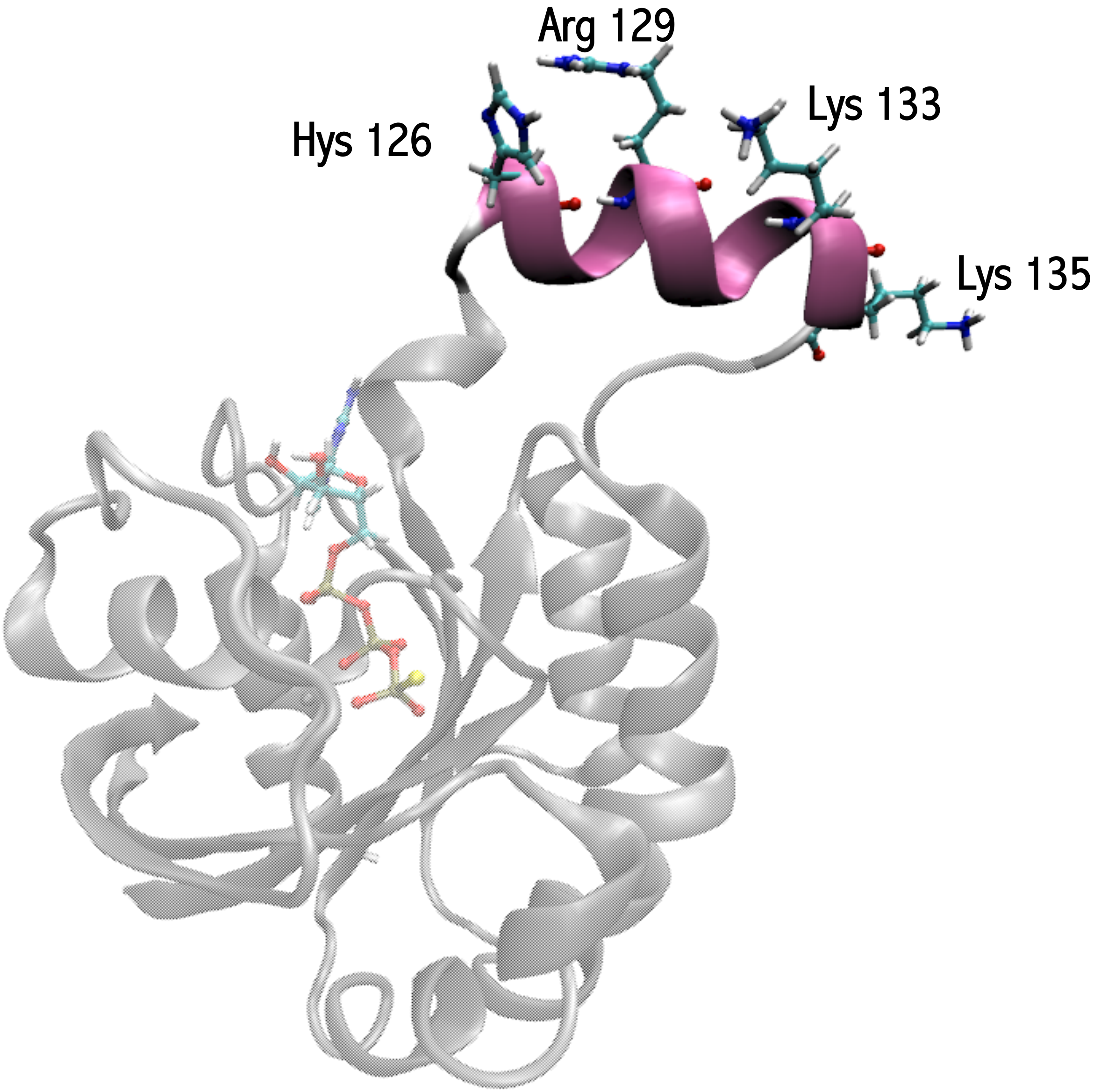
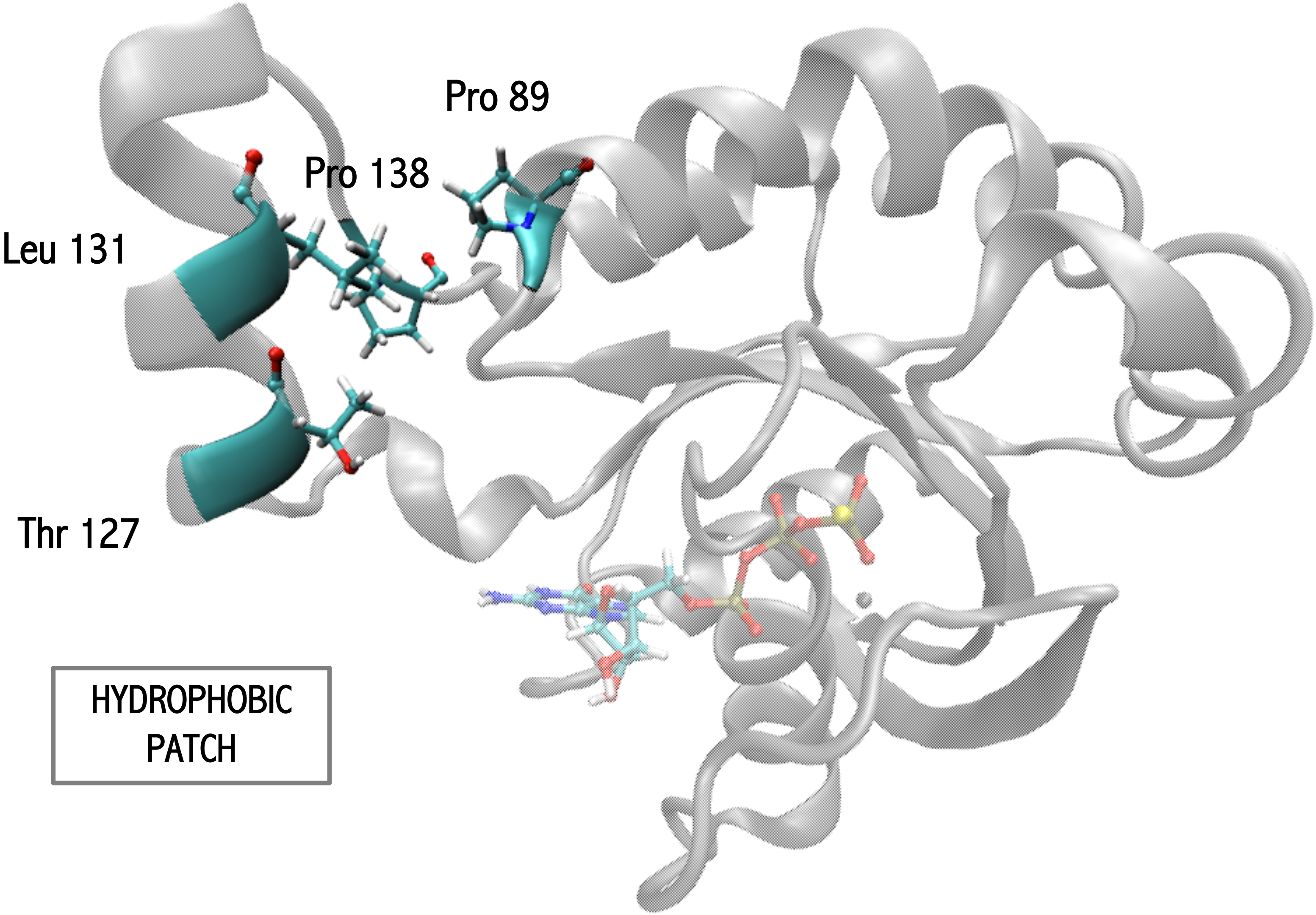
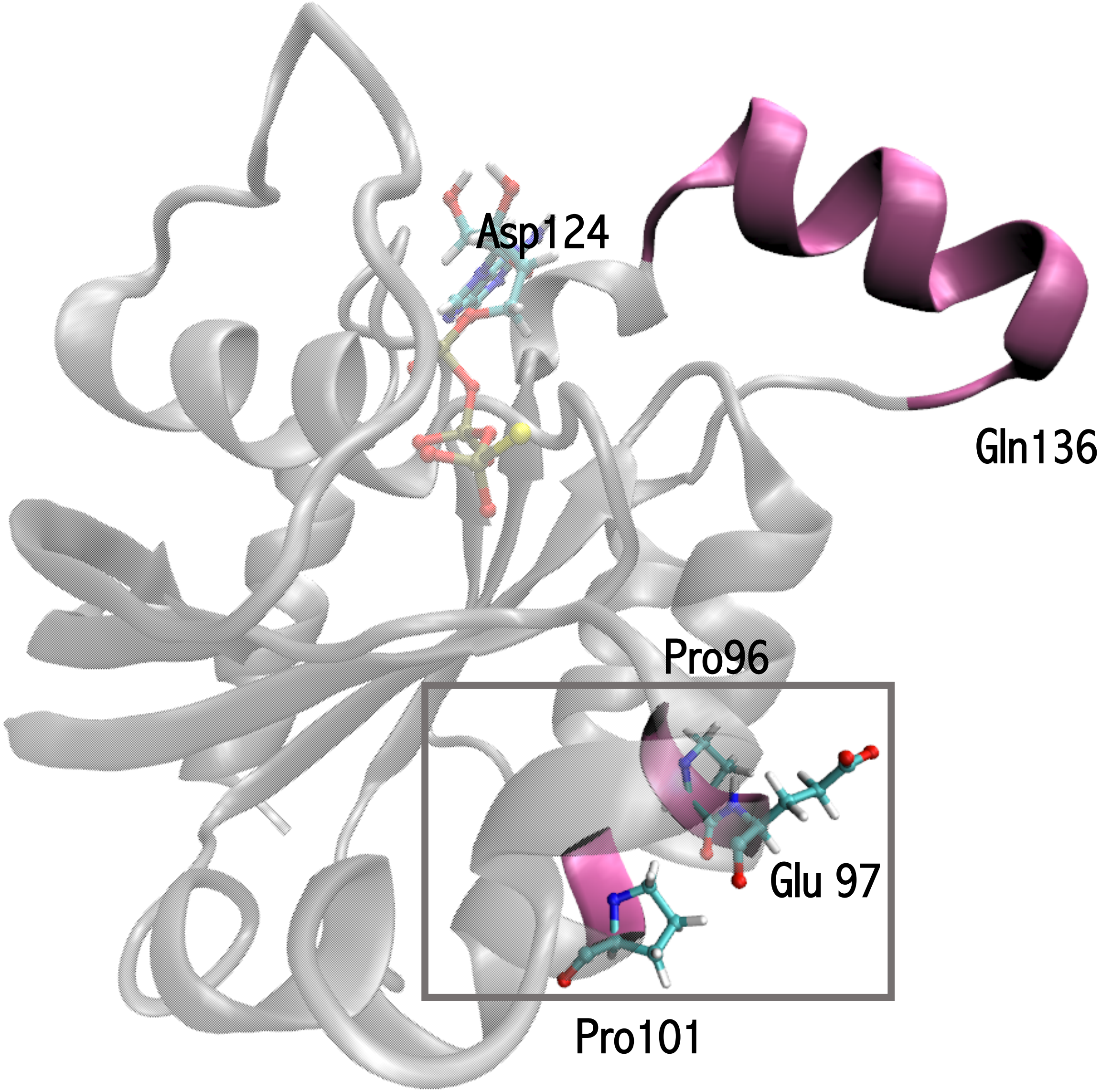


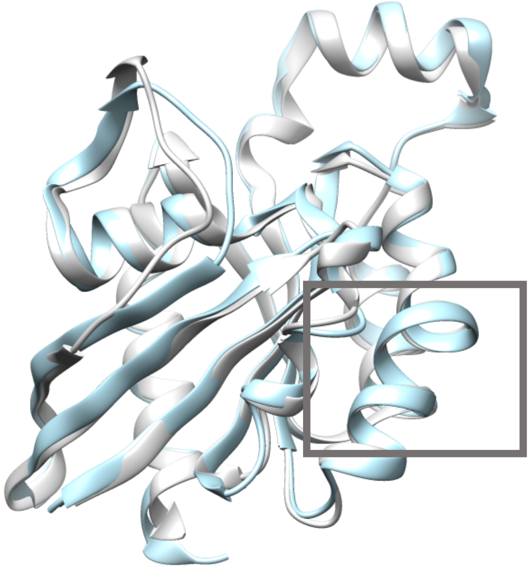
INSERT REGIONS



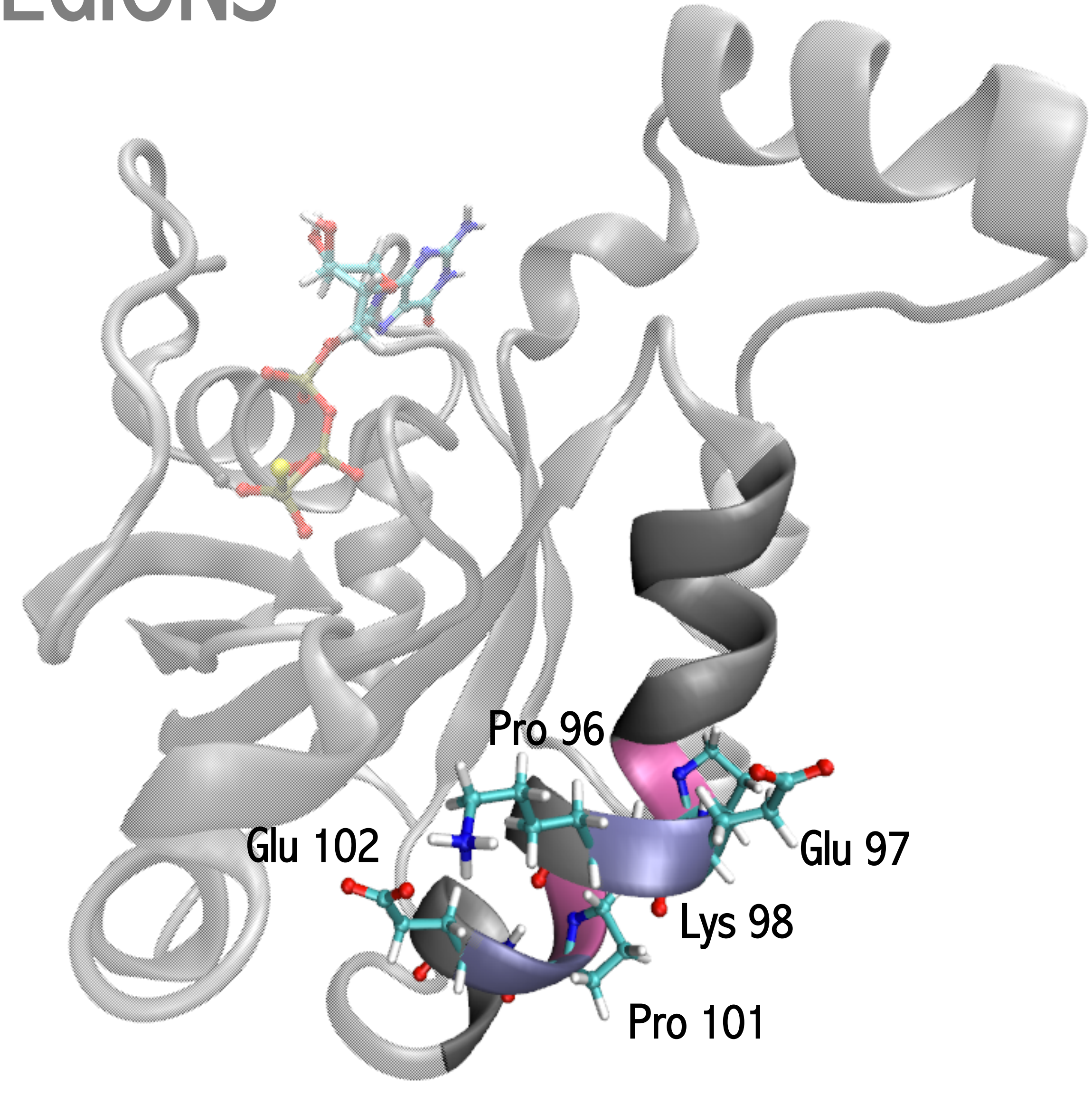
HYDROPHOBIC
PATCH

INSERT REGIONS



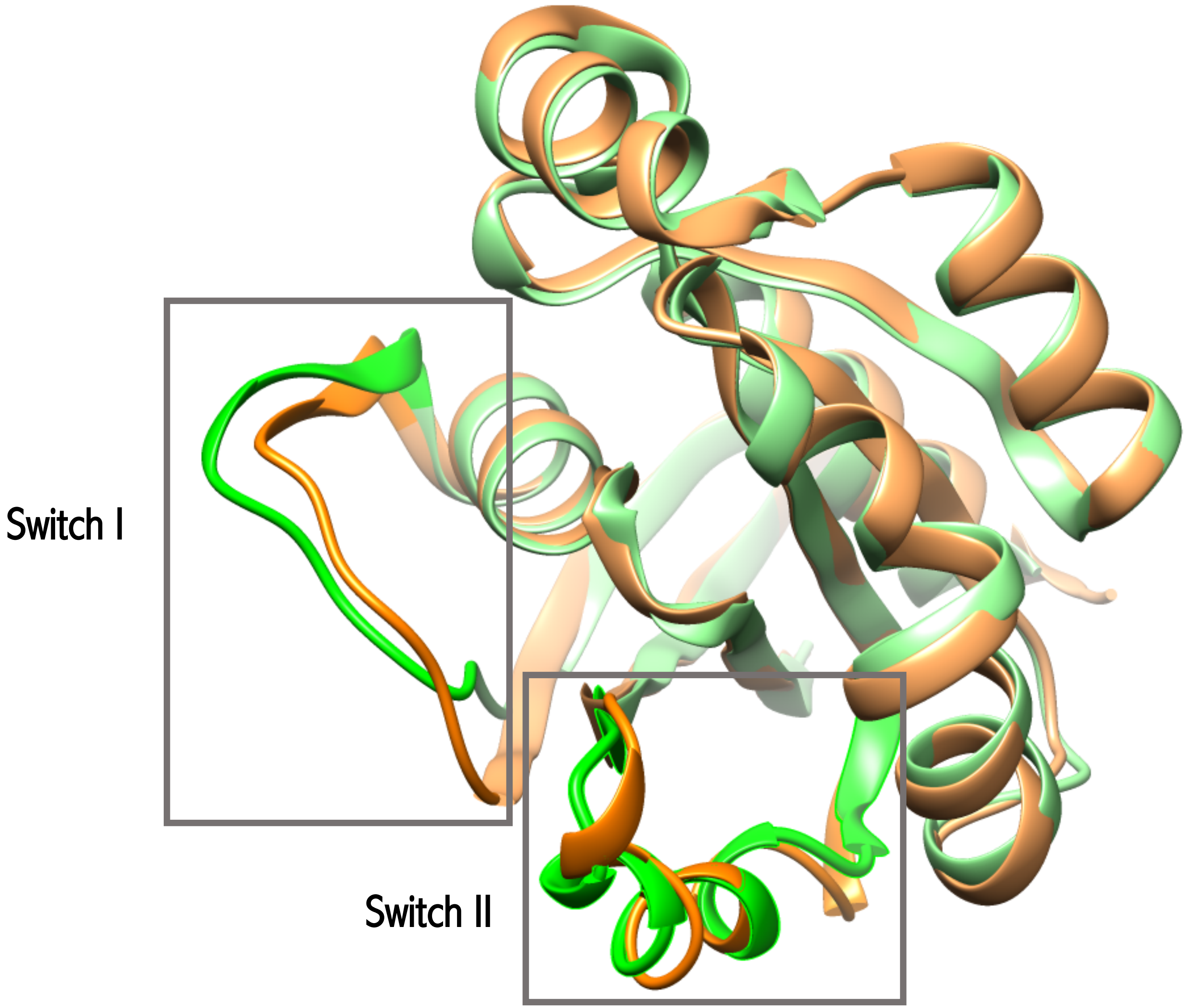


INSERT REGIONS

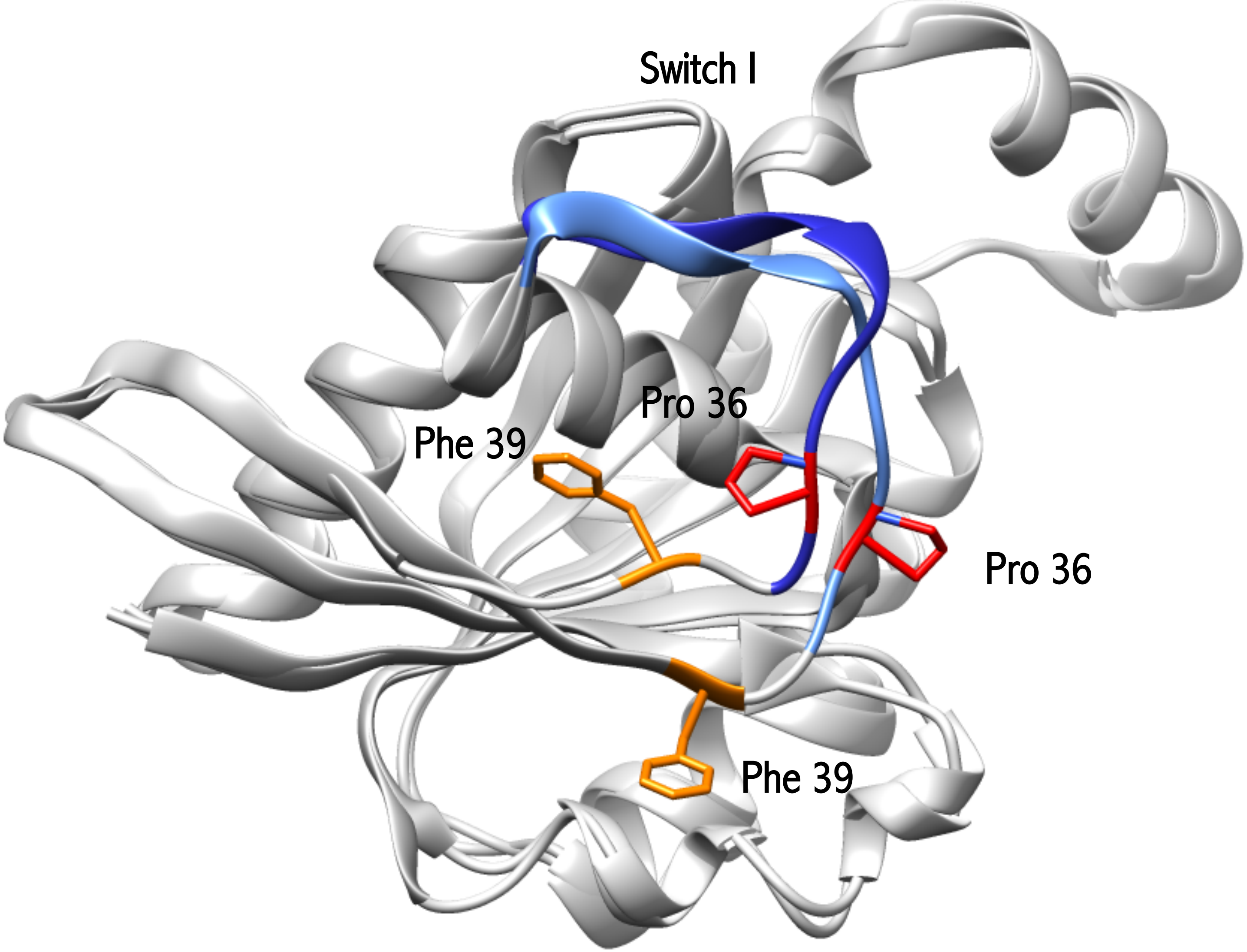


RHOA SWITCH REGIONS

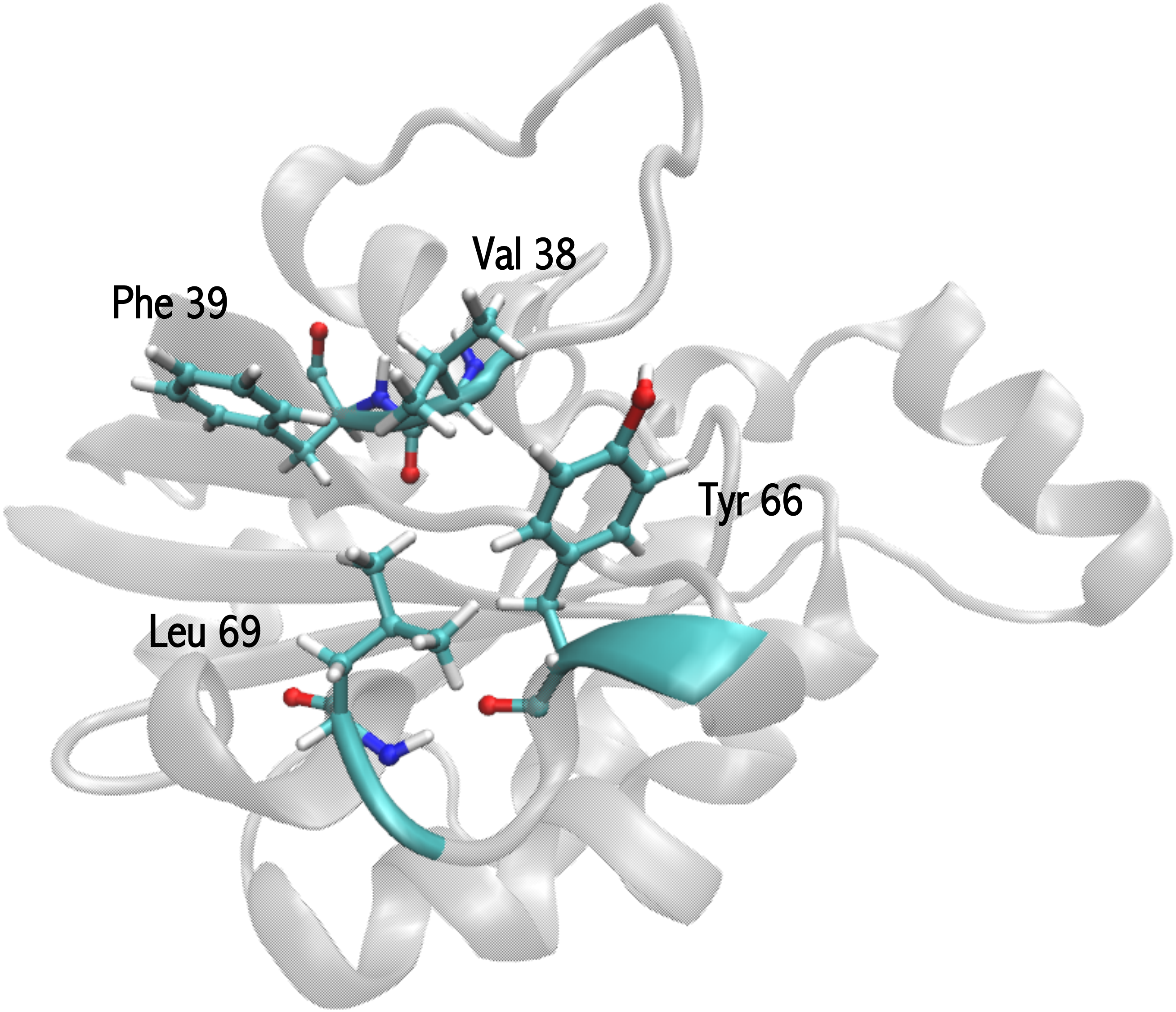
🟩 Rho A-GTP
🟧 Rho A-GDP



RHOA SWITCH REGIONS



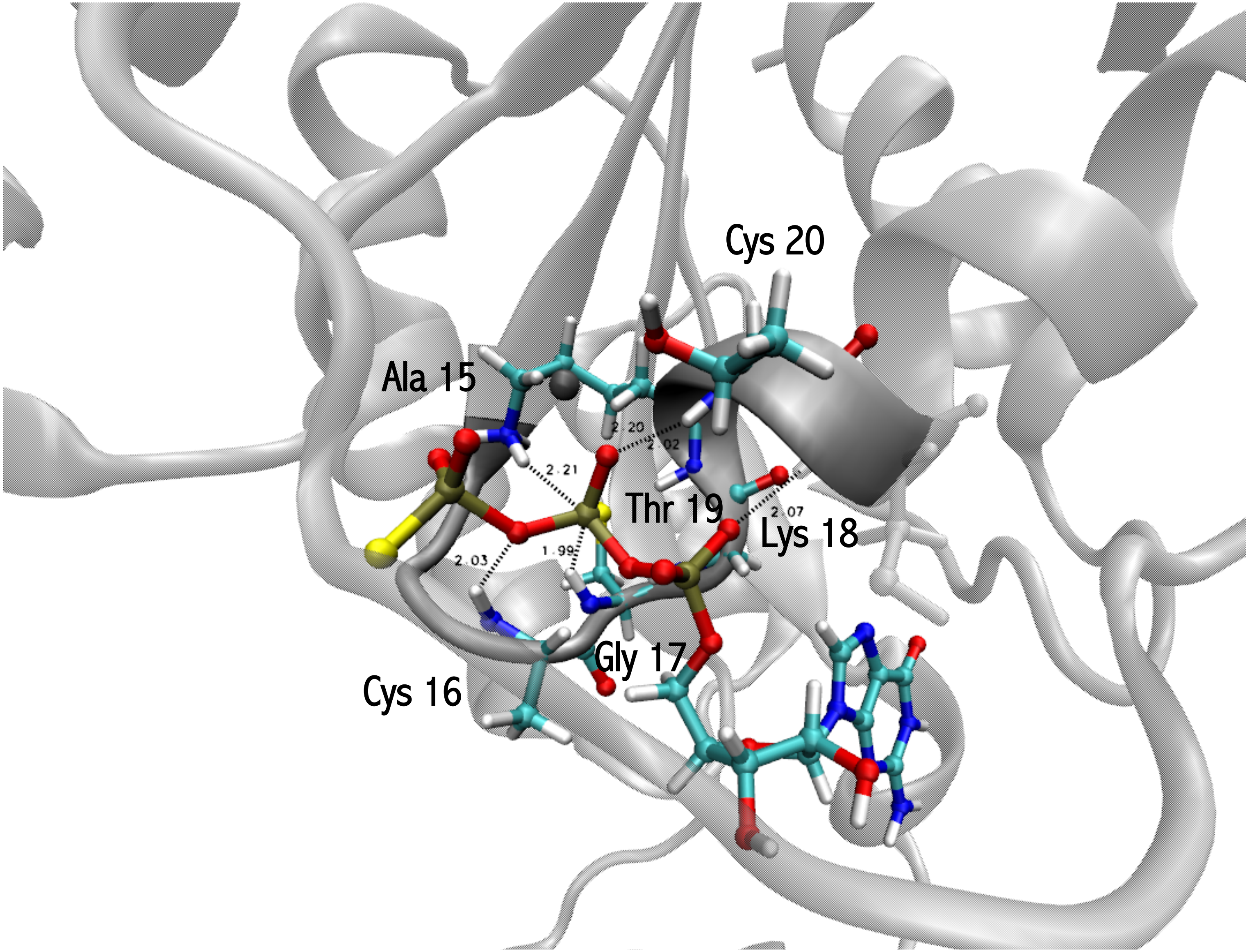
RHOA SWITCH REGIONS



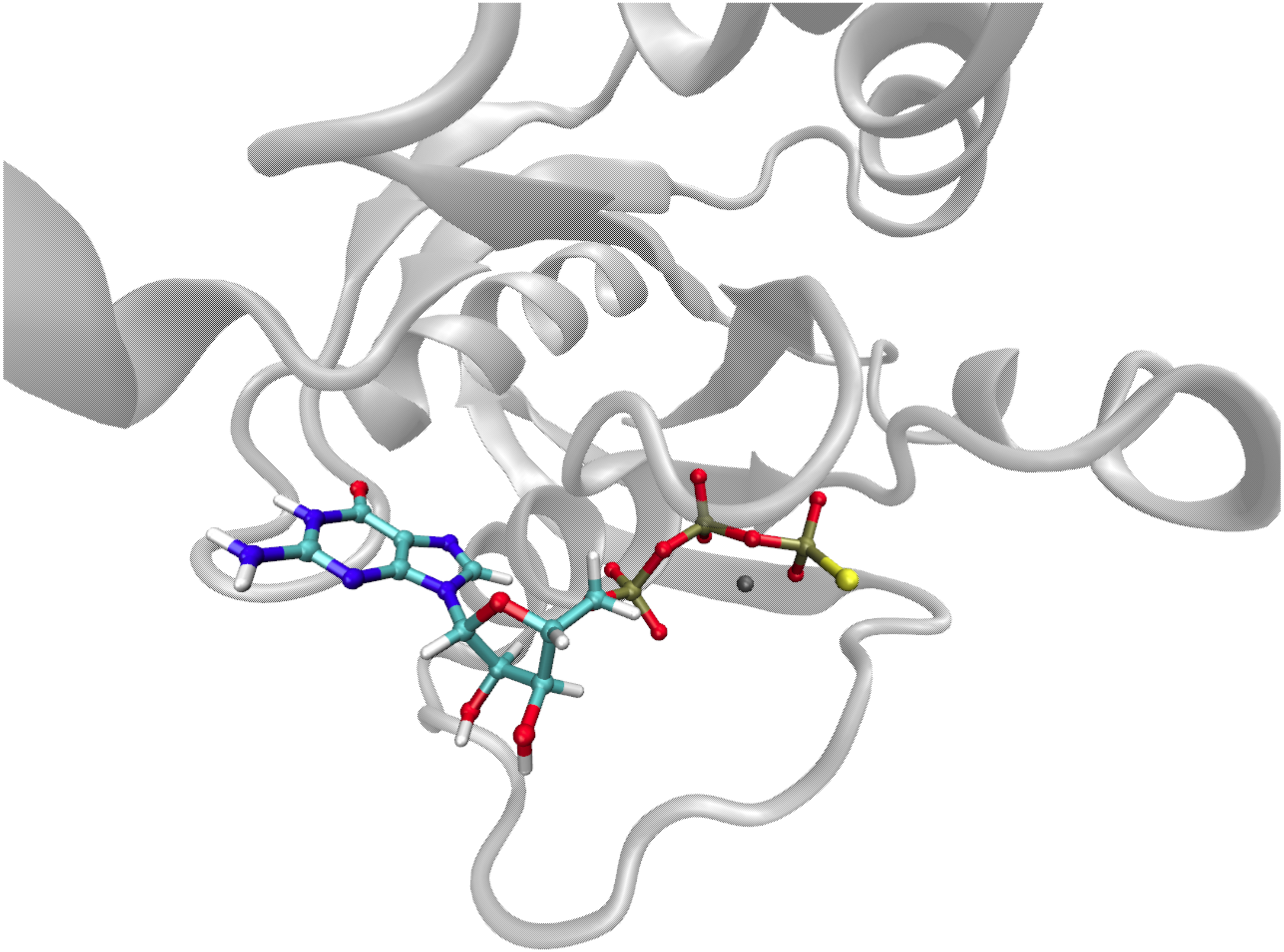
RHOA INTERACTIONS GTP / GDP

COMMON	GTP - SPECIFIC	GDP - SPECIFIC
ASP 120 LYS 118 LYS 18 GLY 17 ALA 15 THR 19 CYS 20	ALA 161 CYS 16 THR 37	LYS 162

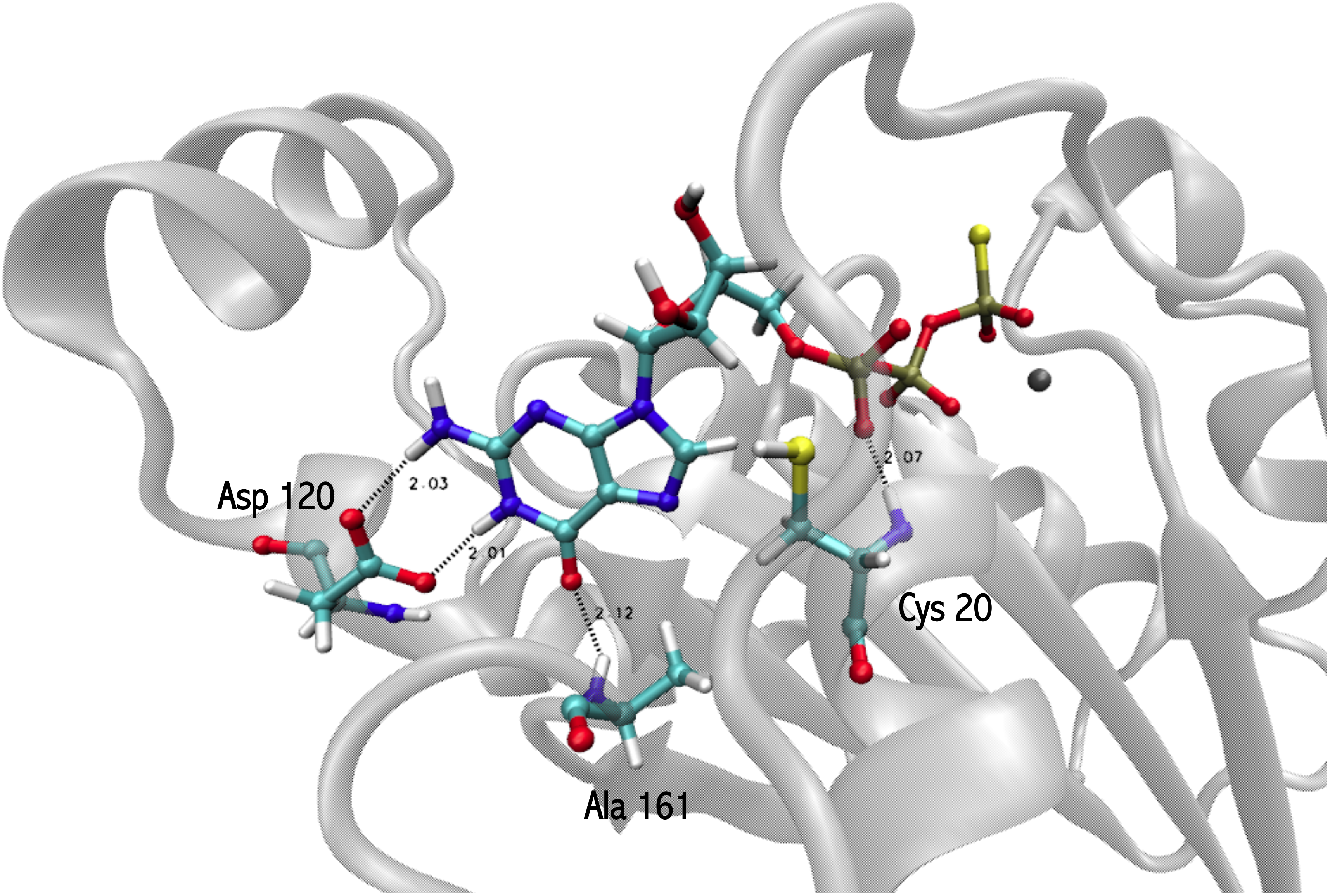
PHOSPHATE BINDING LOOP



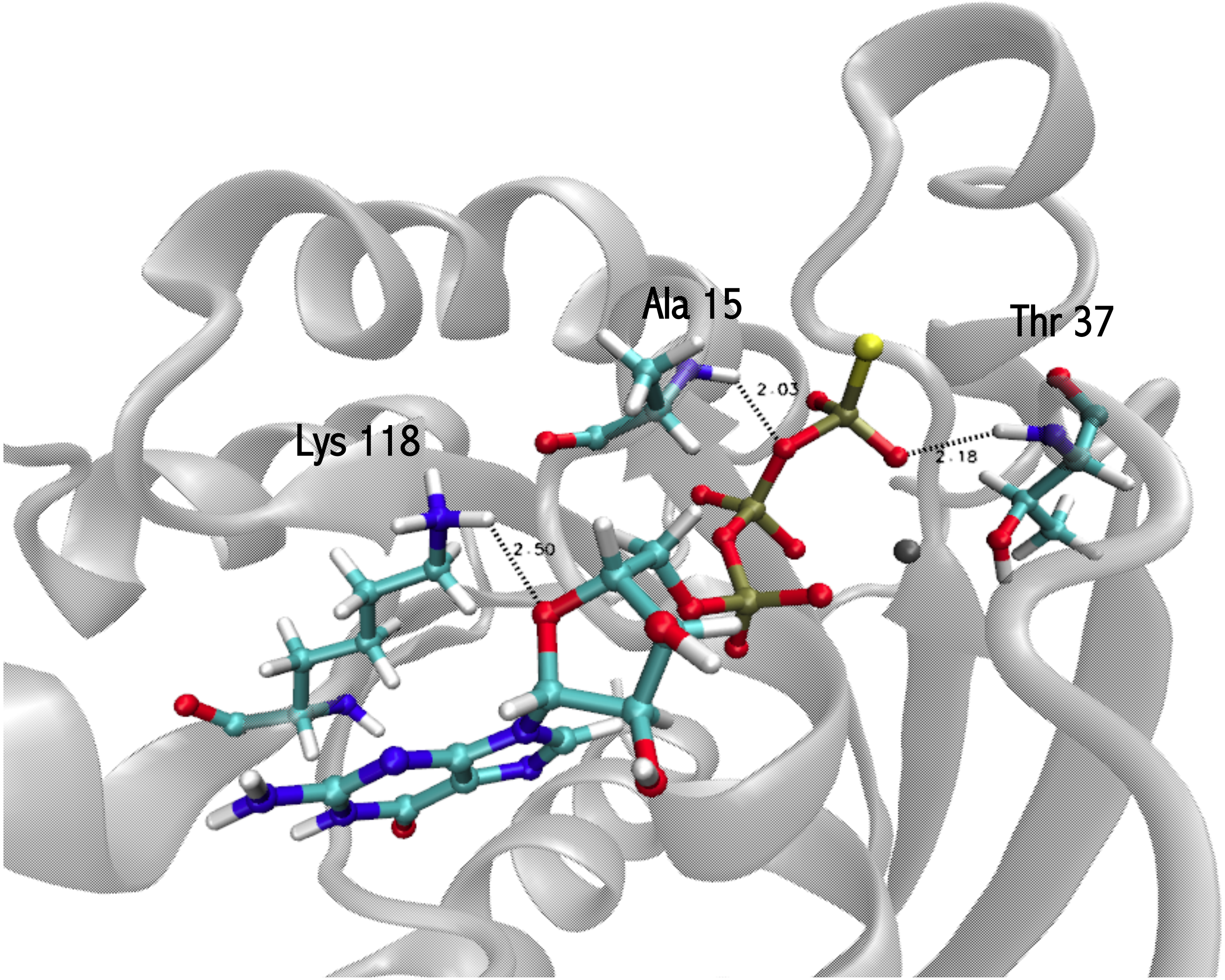
RHOA INTERACTIONS GTP



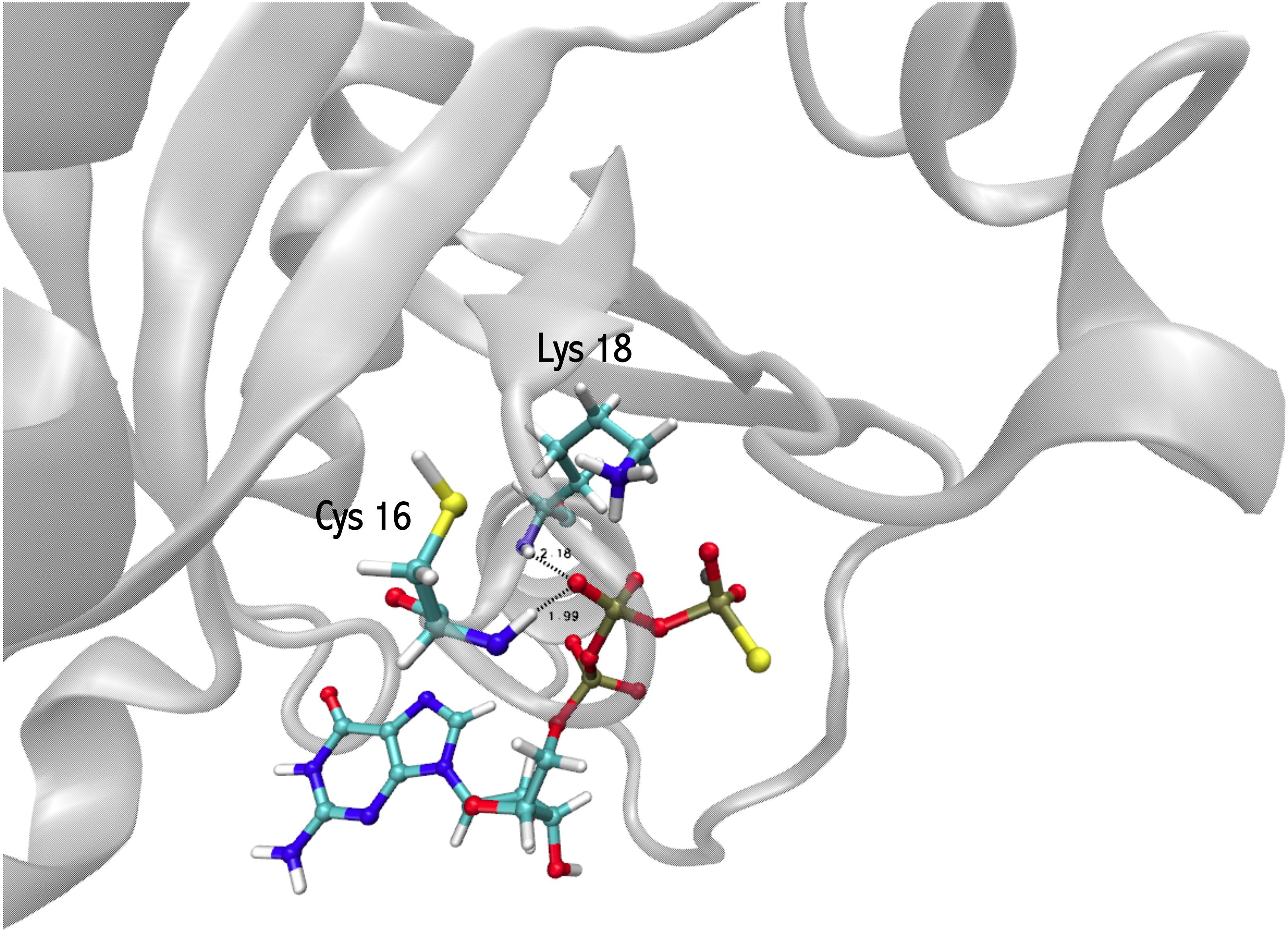
RHOA INTERACTIONS GTP



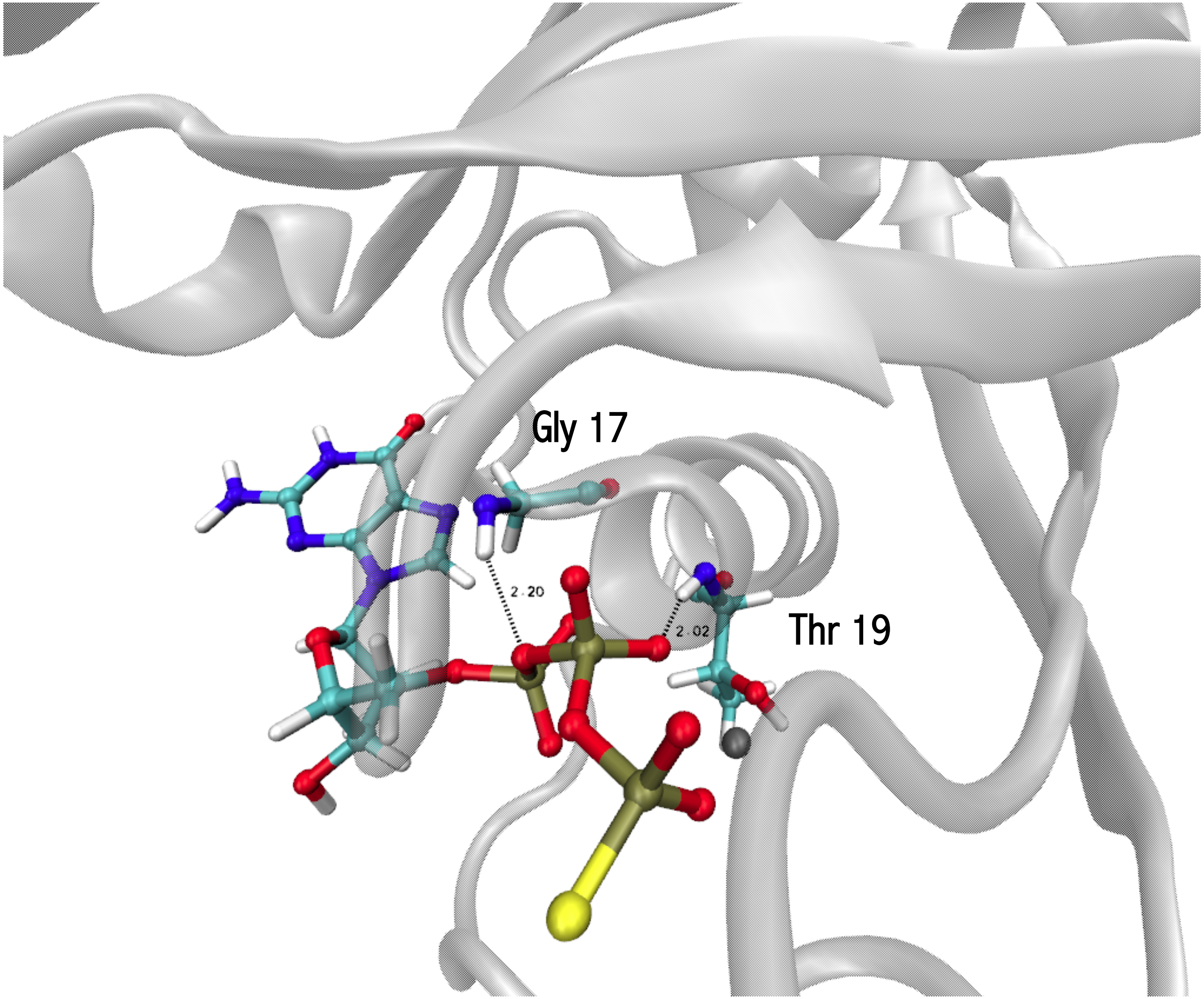
RHOA INTERACTIONS GTP



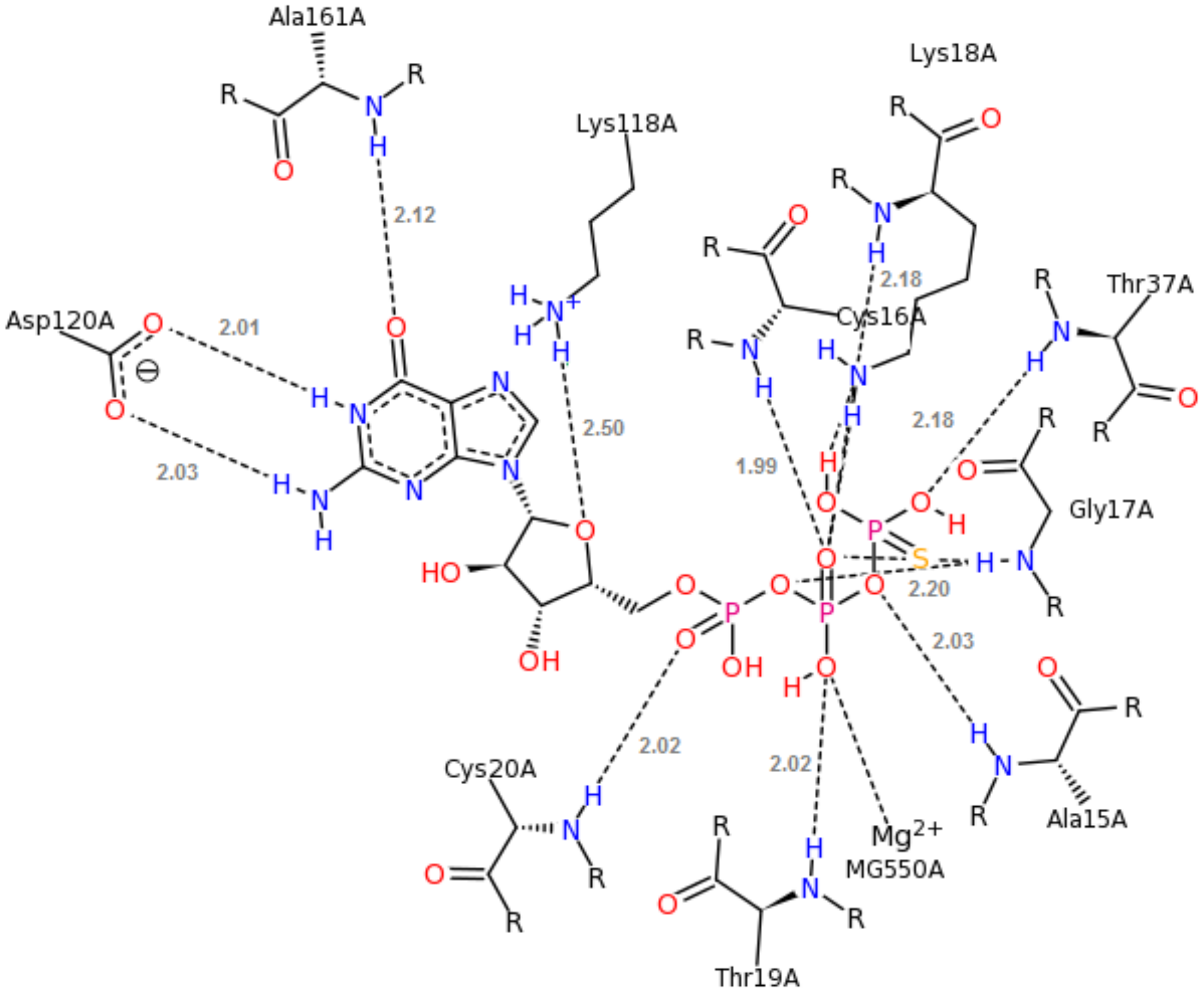
RHOA INTERACTIONS GTP



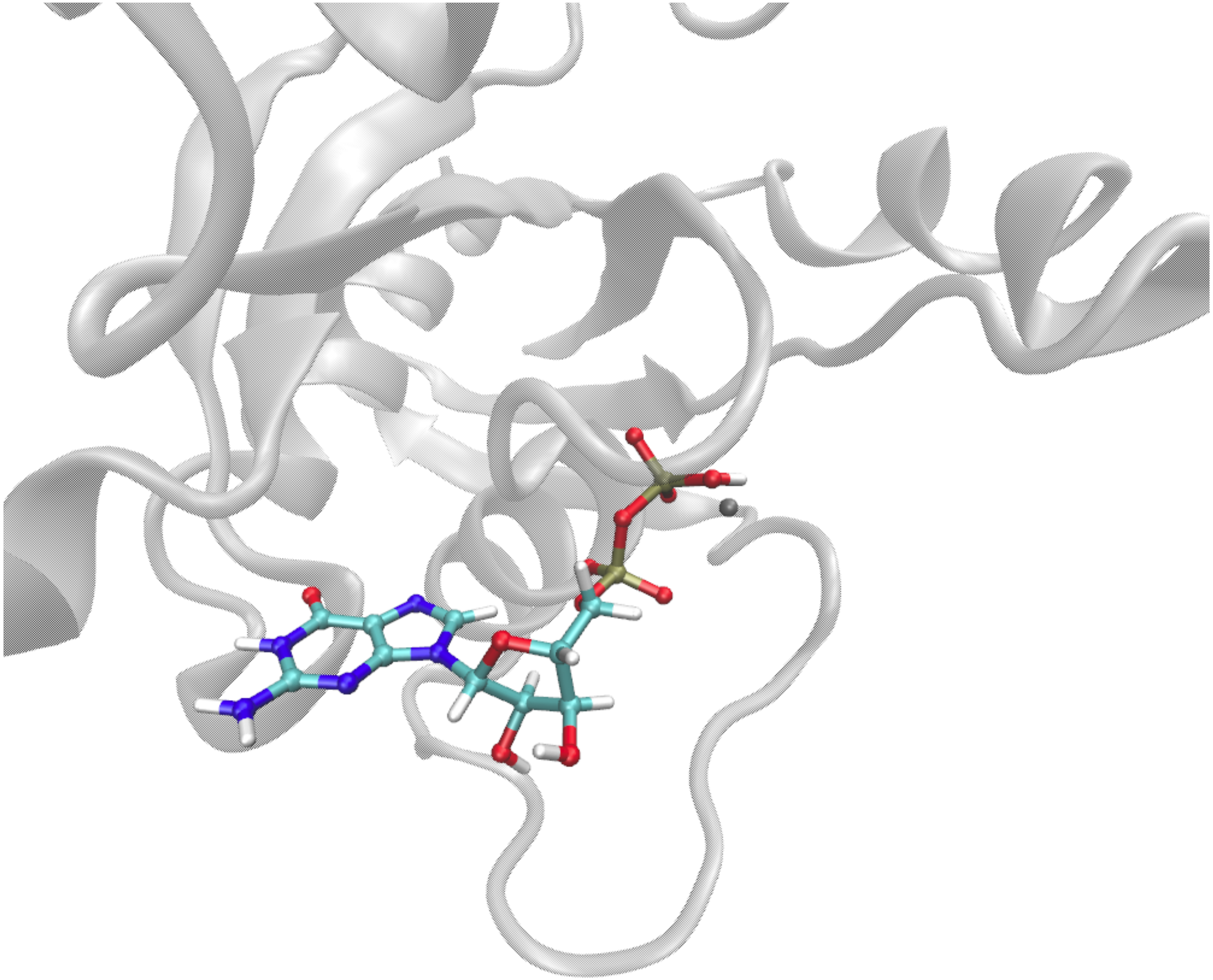
RHOA INTERACTIONS GTP



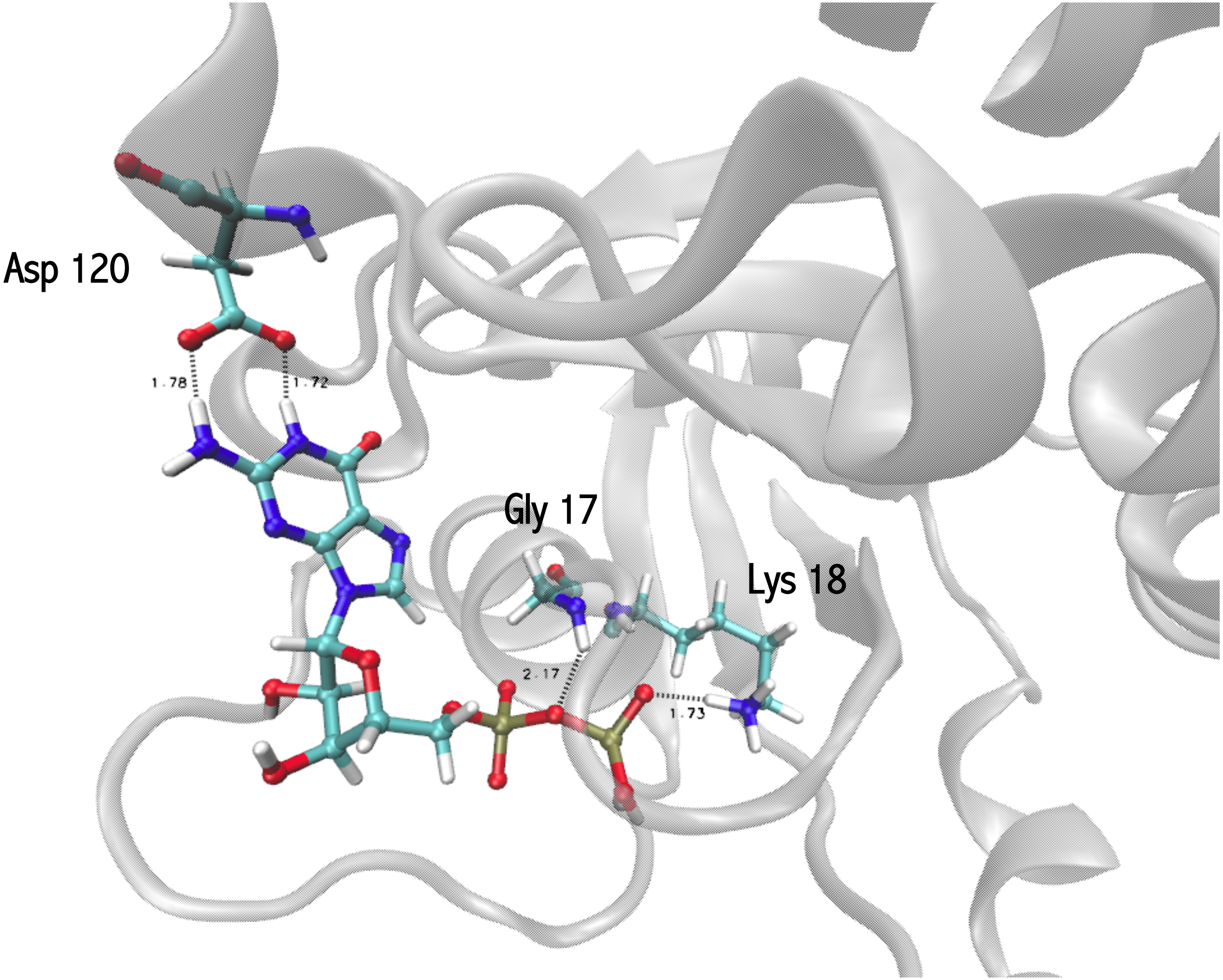
RHOA INTERACTIONS GTP



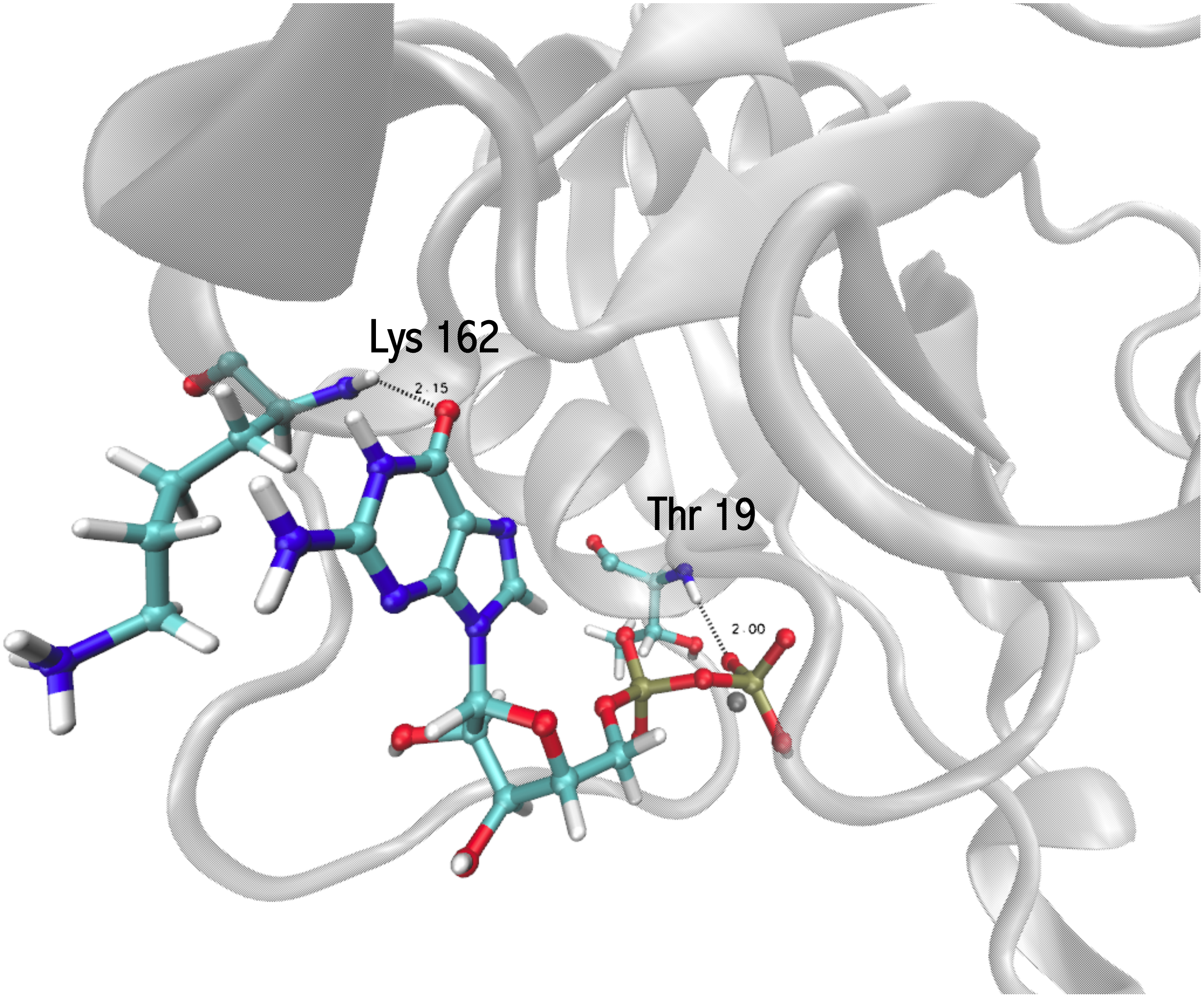
RHOA INTERACTIONS GDP



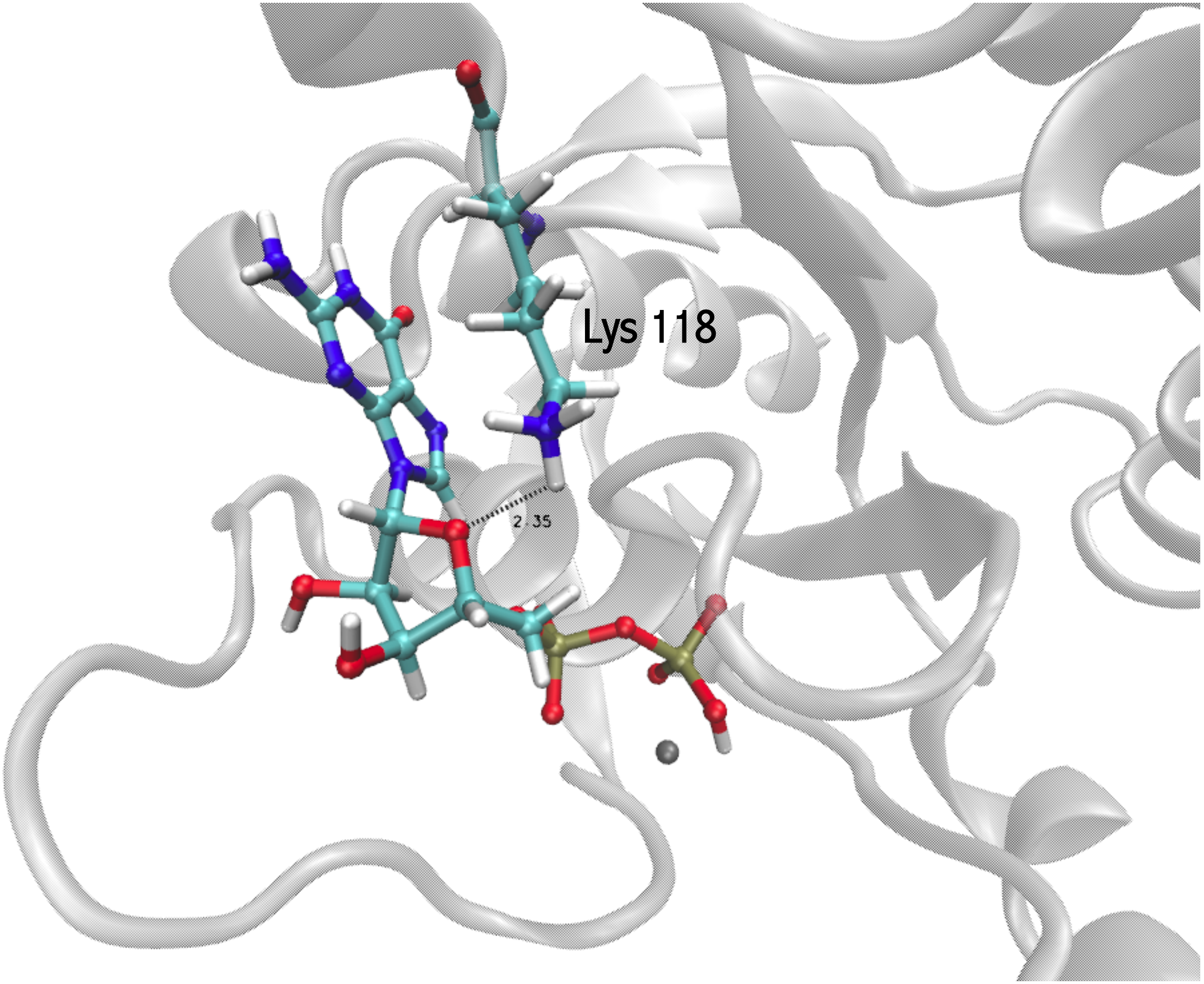
RHOA INTERACTIONS GDP



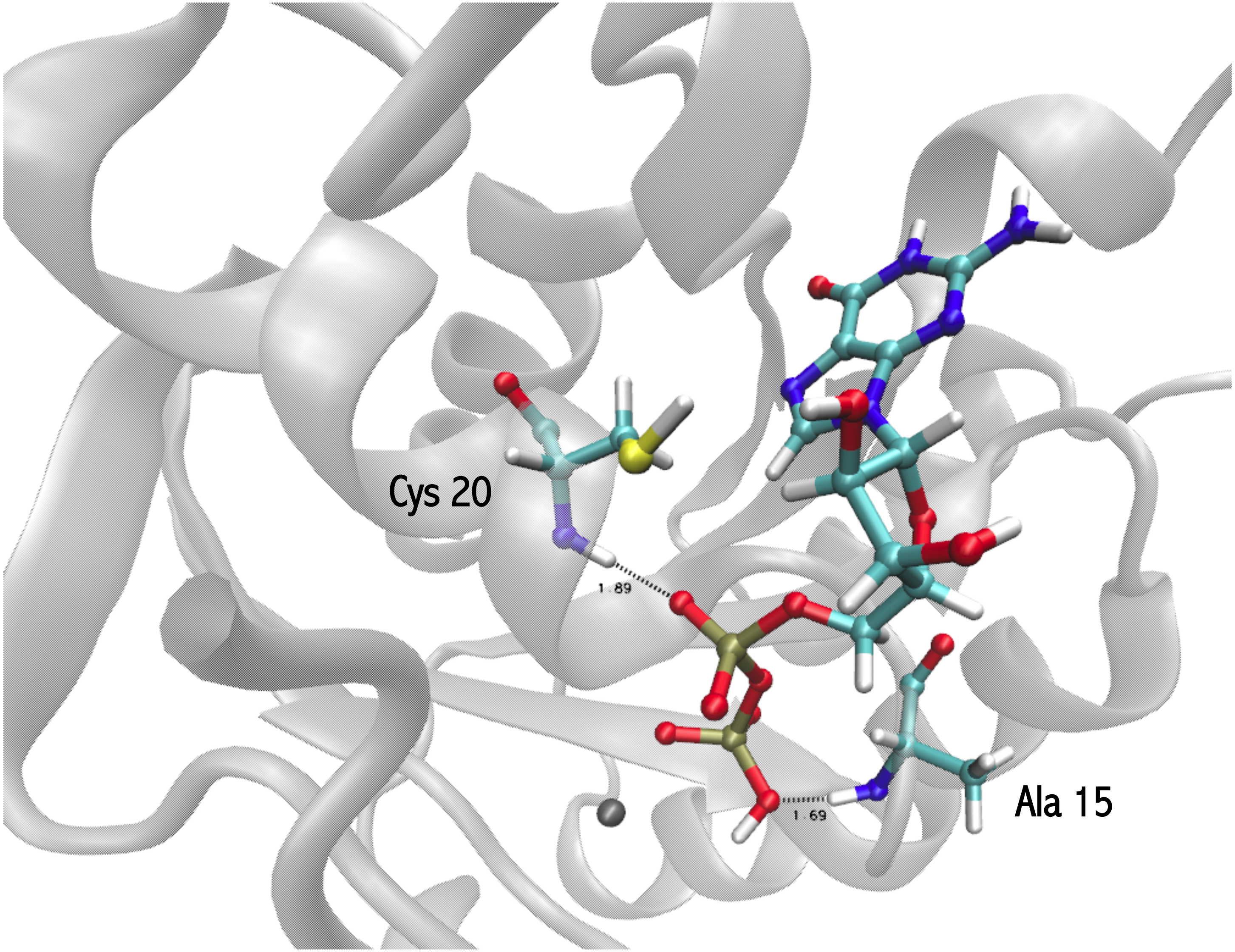
RHOA INTERACTIONS GDP



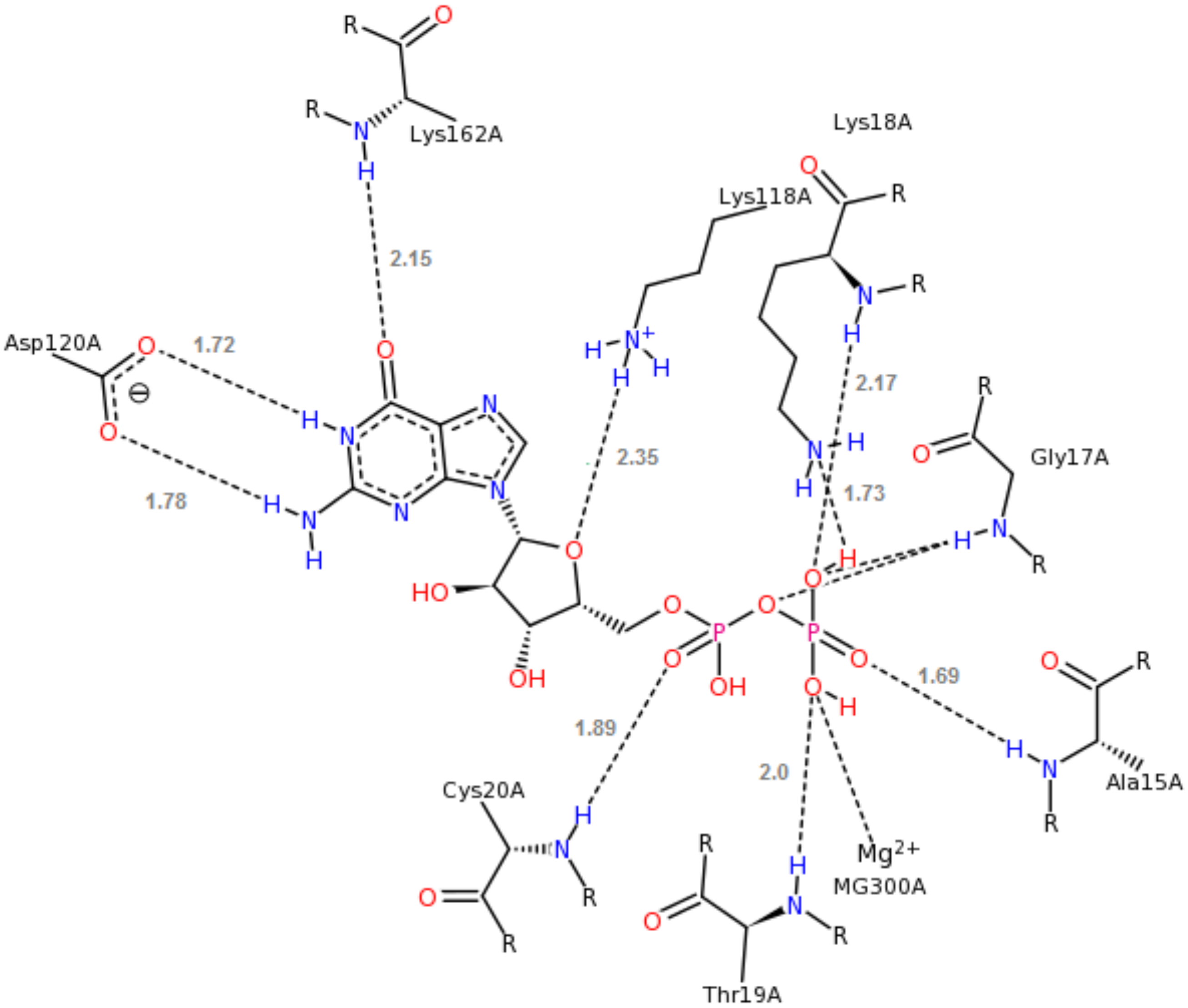
RHOA INTERACTIONS GDP



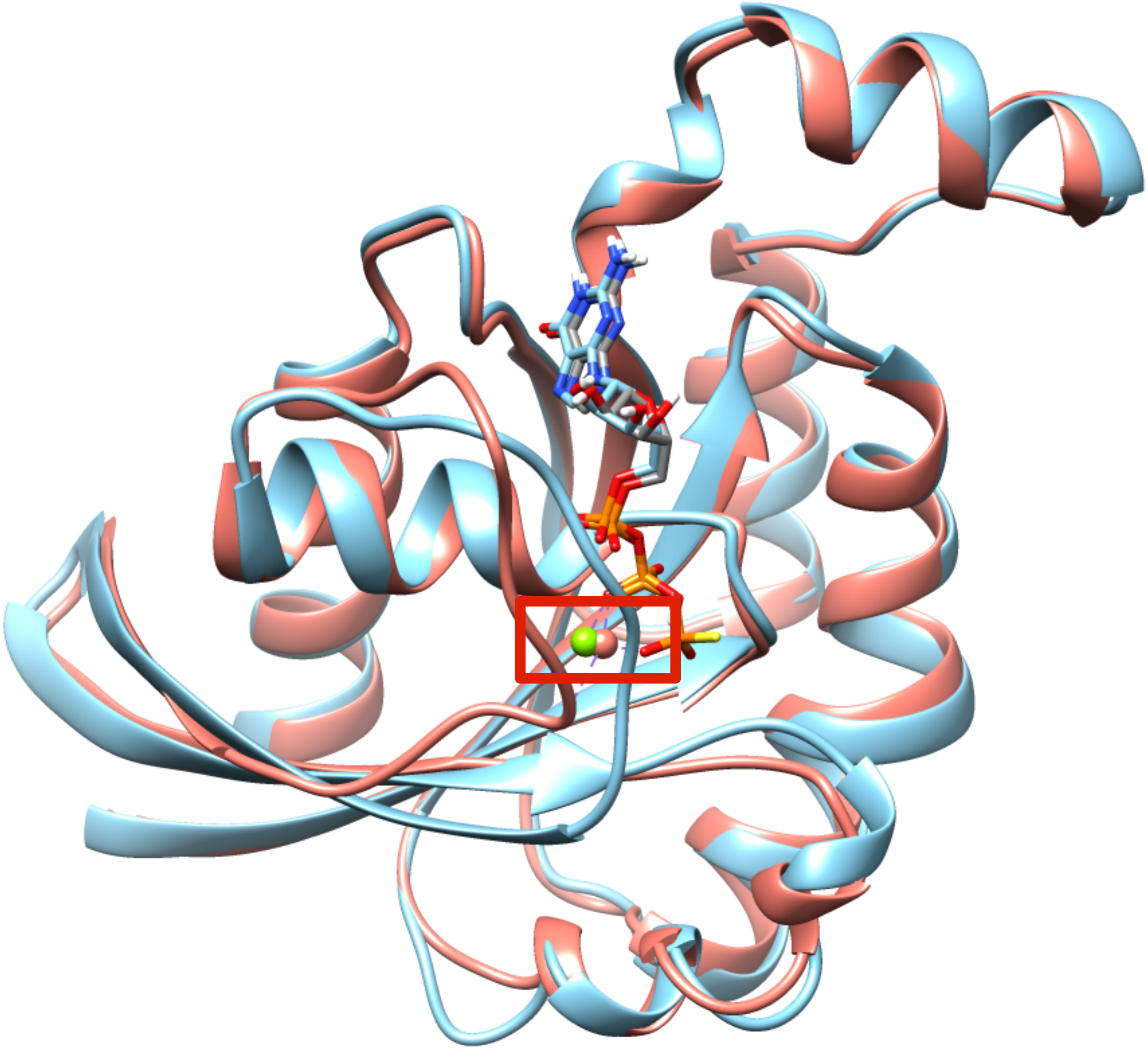
RHOA INTERACTIONS GDP



RHOA INTERACTIONS GDP



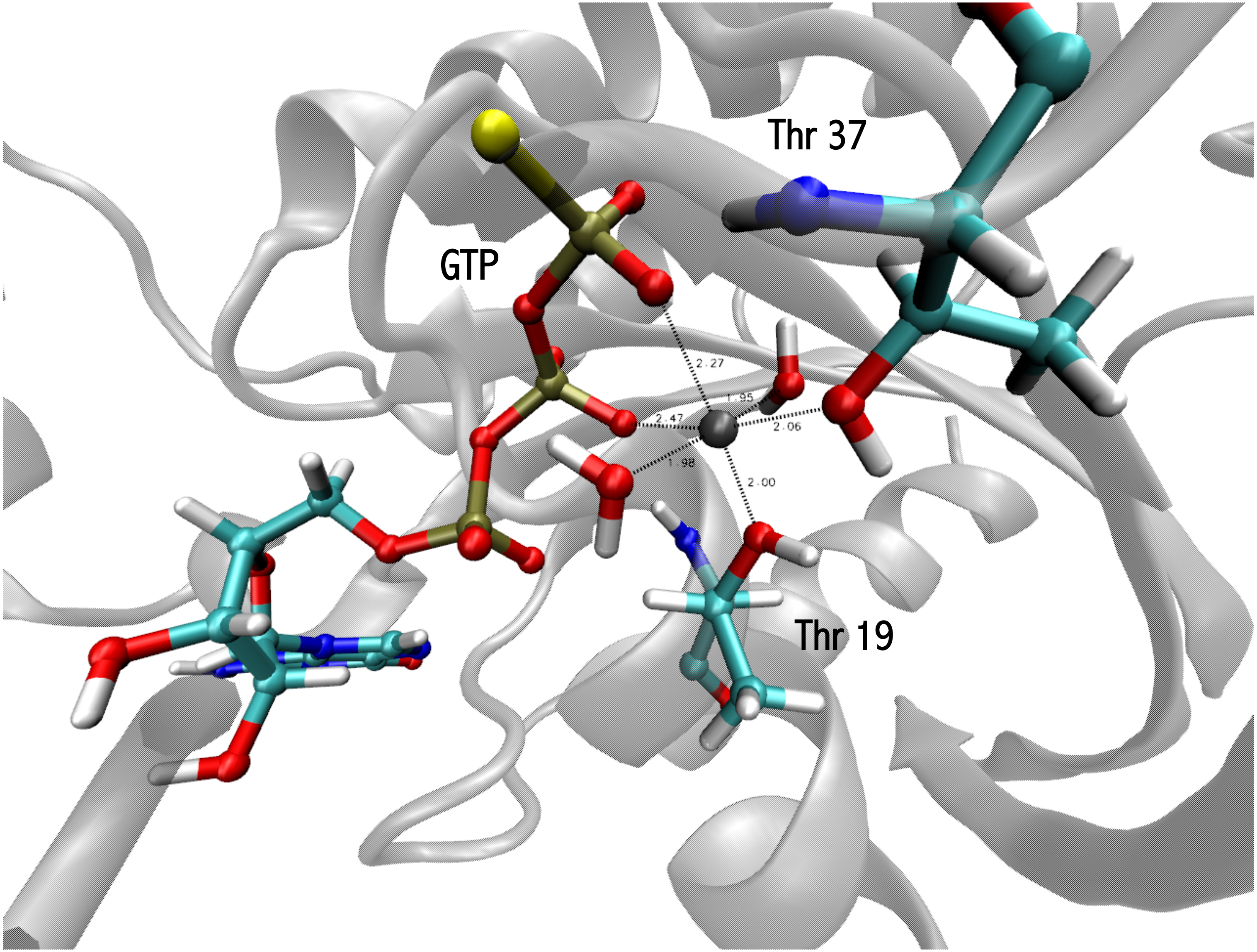
RHOA INTERACTIONS Mg^{2+}



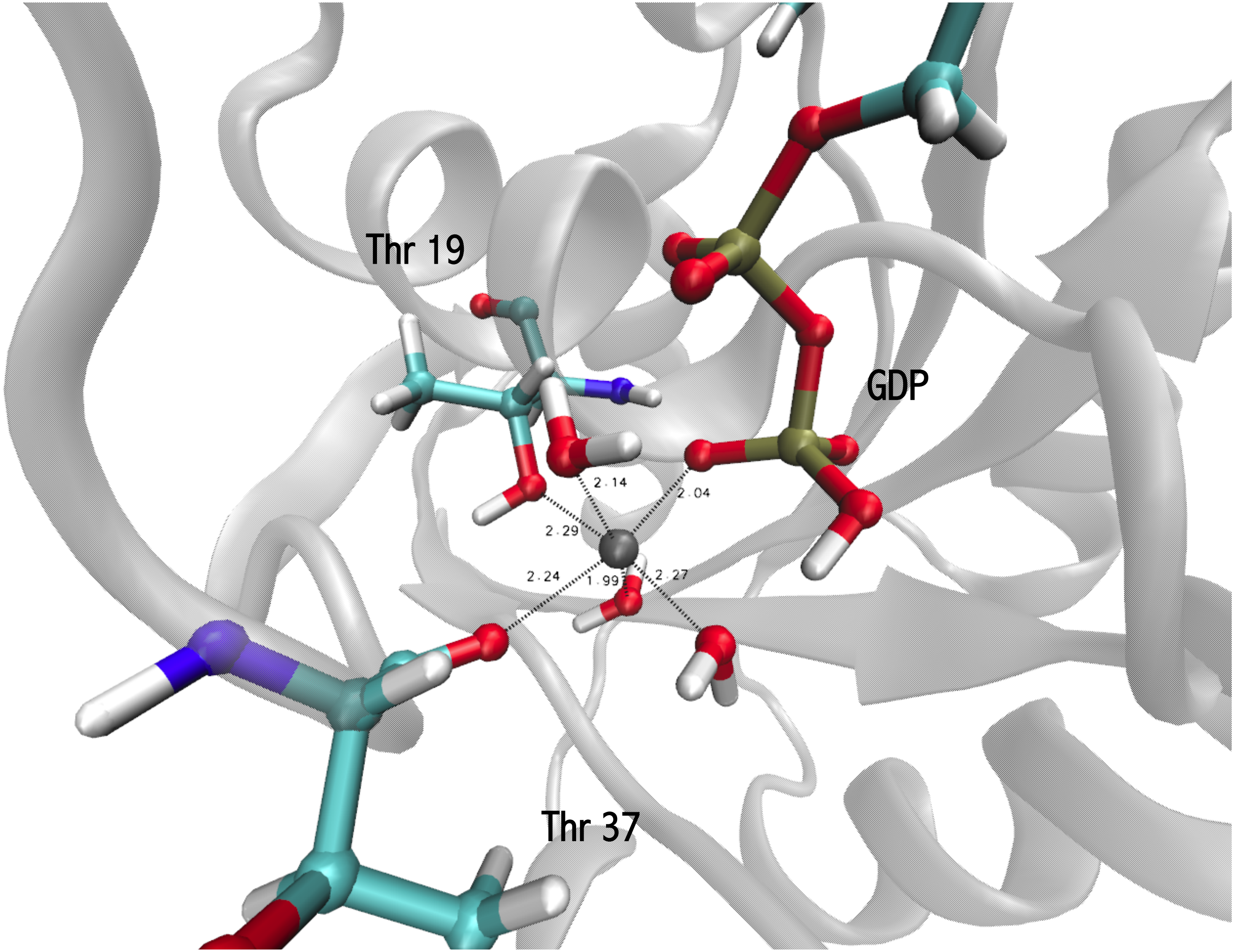
🟦 RhoA - GDP
🟥 RhoA - GTP

DISTANCE
0,66

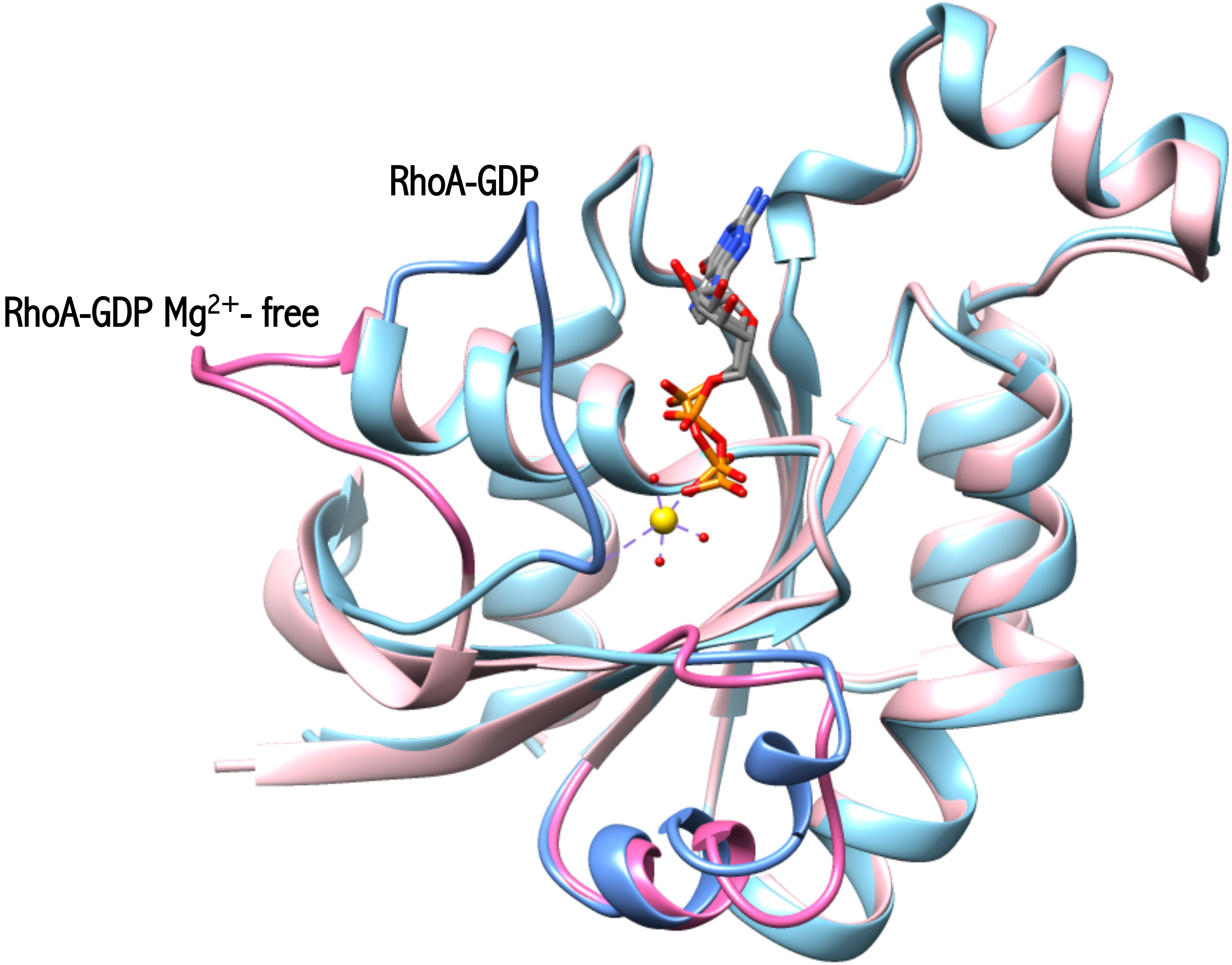
RHOA - GTP INTERACTIONS Mg^{2+}



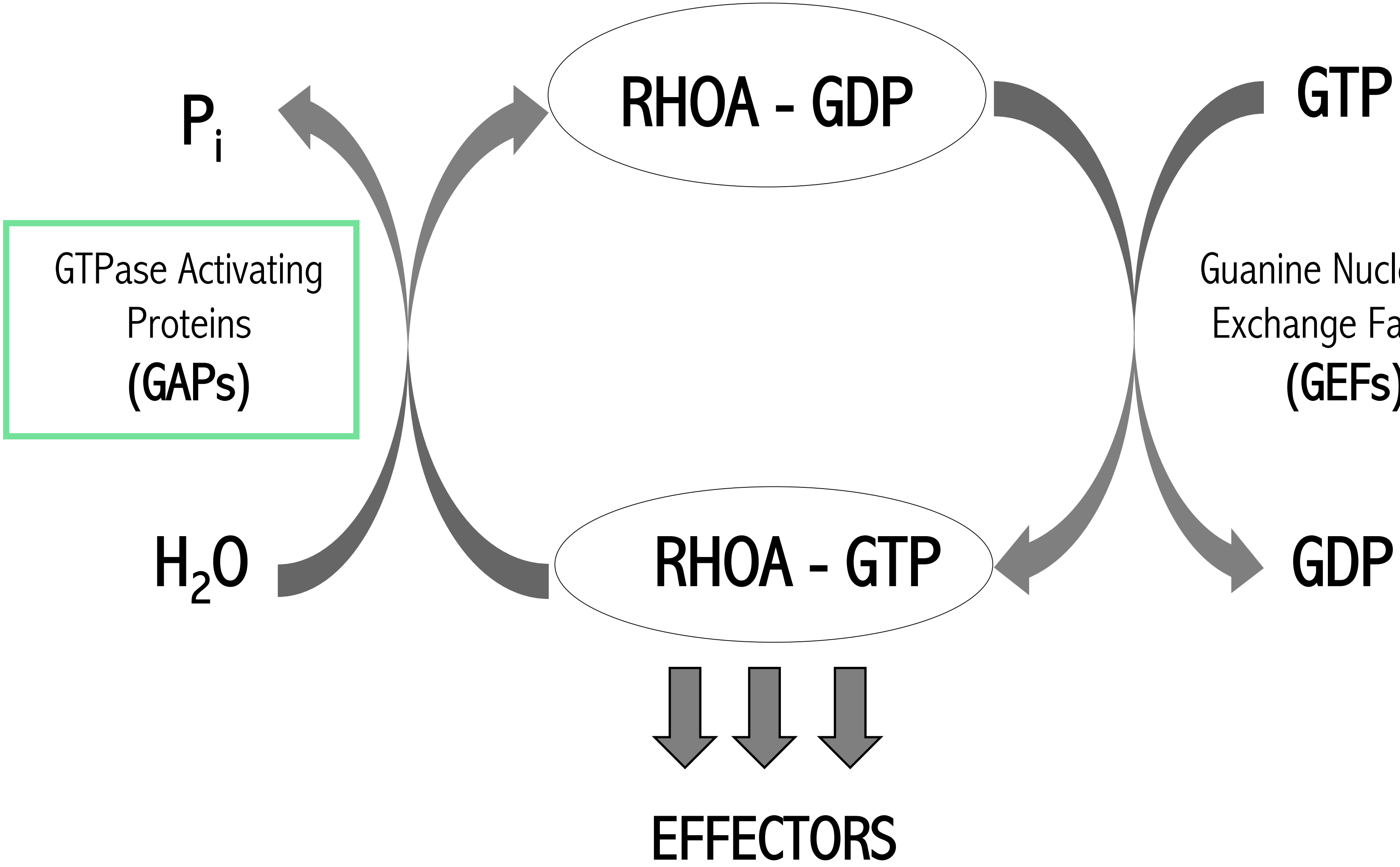
RHOA - GDP INTERACTIONS Mg^{2+}



RHOA - GDP INTERACTIONS Mg^{2+}



RHOA INTERACTIONS WITH GAP



P_i

RHOA - GDP

GTP

GTPase Activating Proteins
(GAPs)

Guanine Nucleotide Exchange Factors
(GEFs)

H_2O

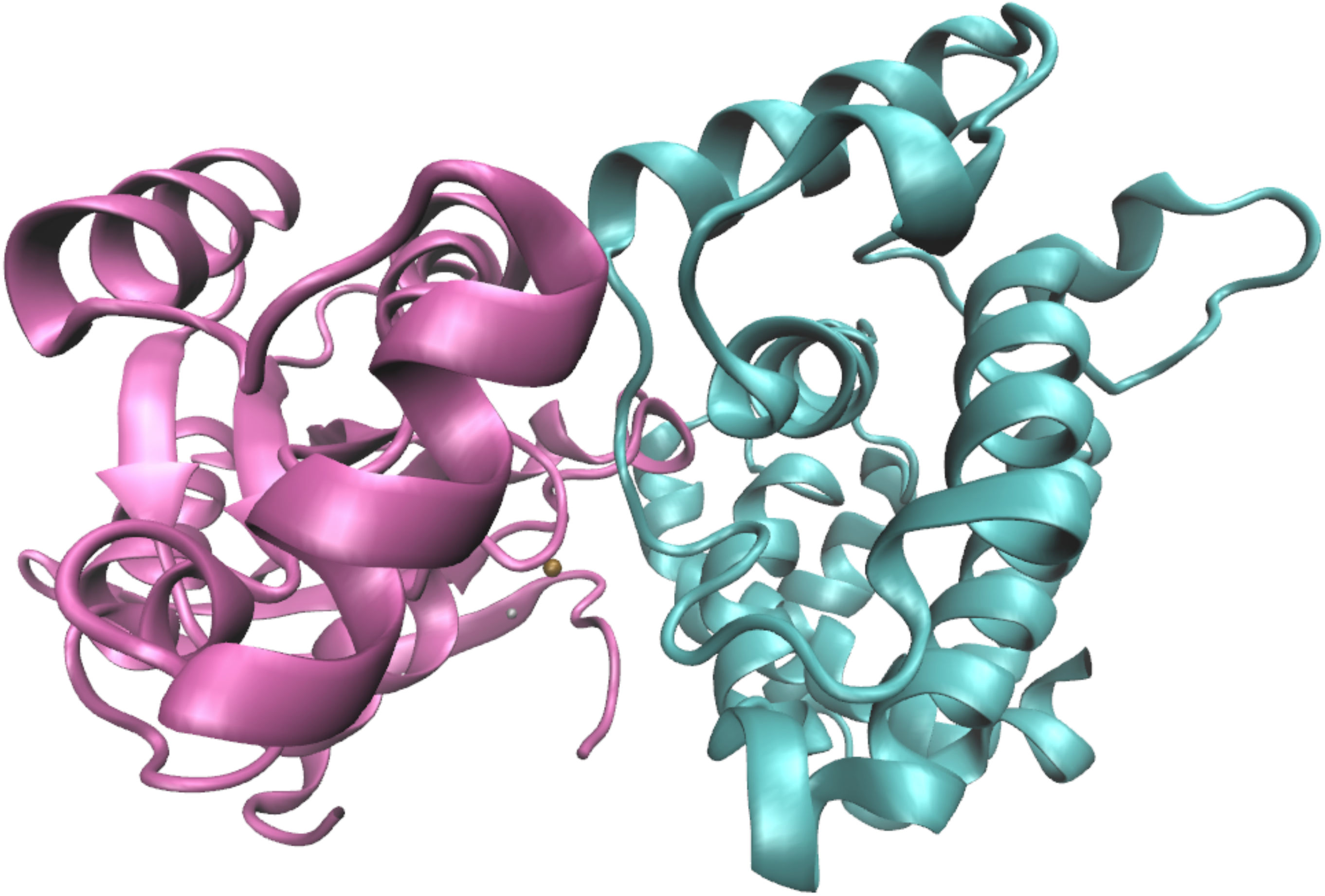
RHOA - GTP

GDP

EFFECTORS

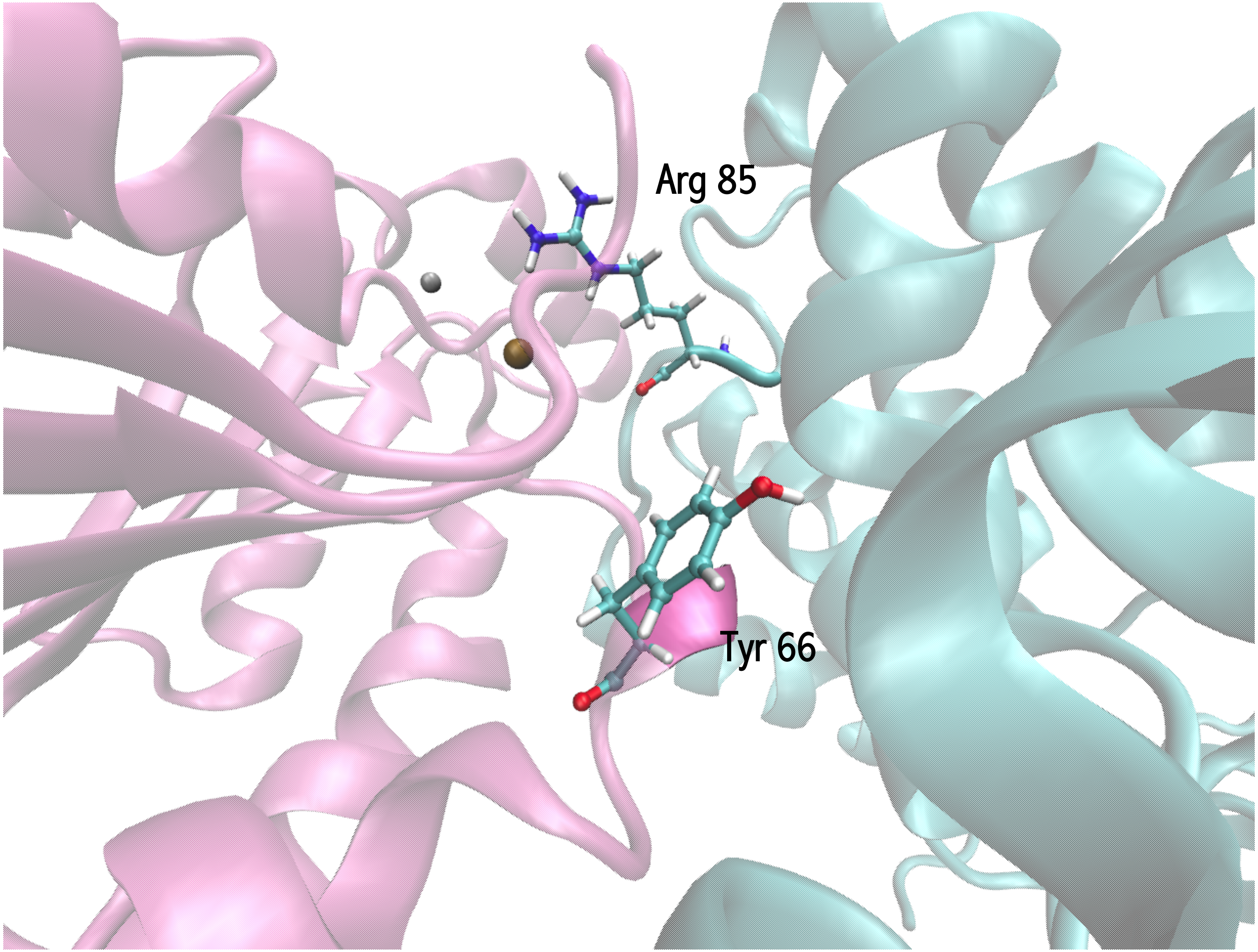
RHOA — GAP

🟥 Rho A
🟦 GAP



RHOA – GAP

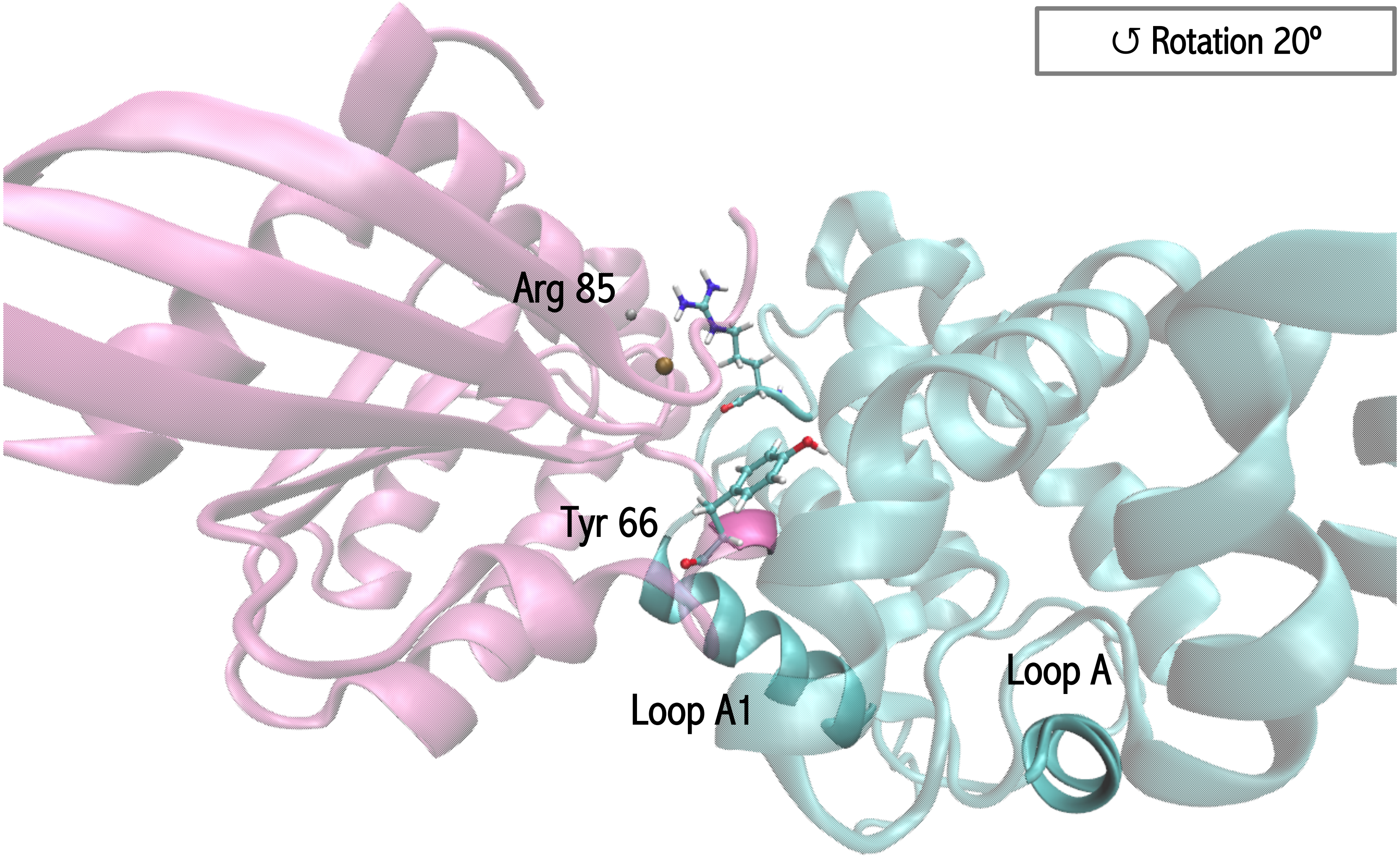
🟪 Rho A
🟦 GAP



RHOA – GAP

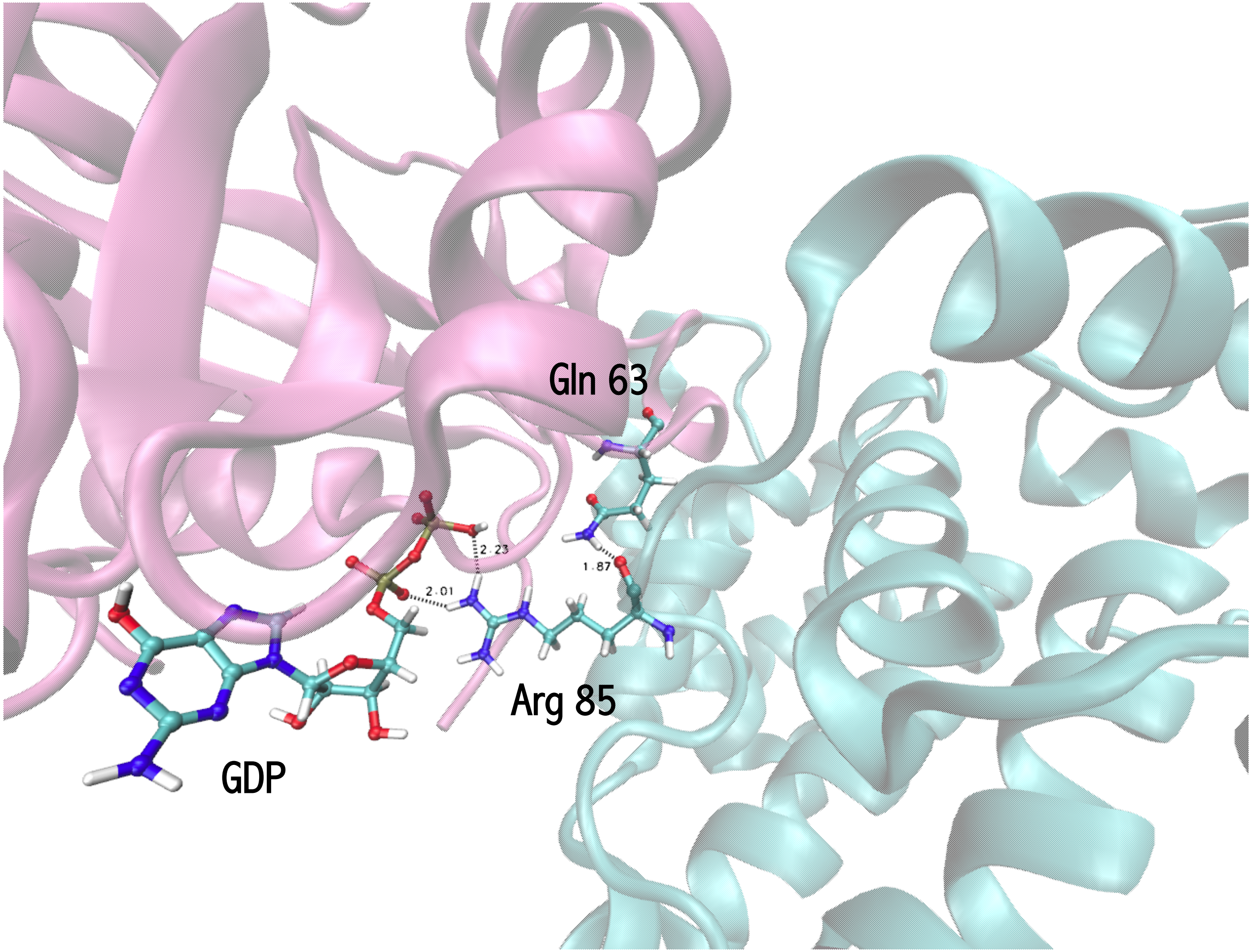
■ Rho A
■ GAP

↺ Rotation 20º



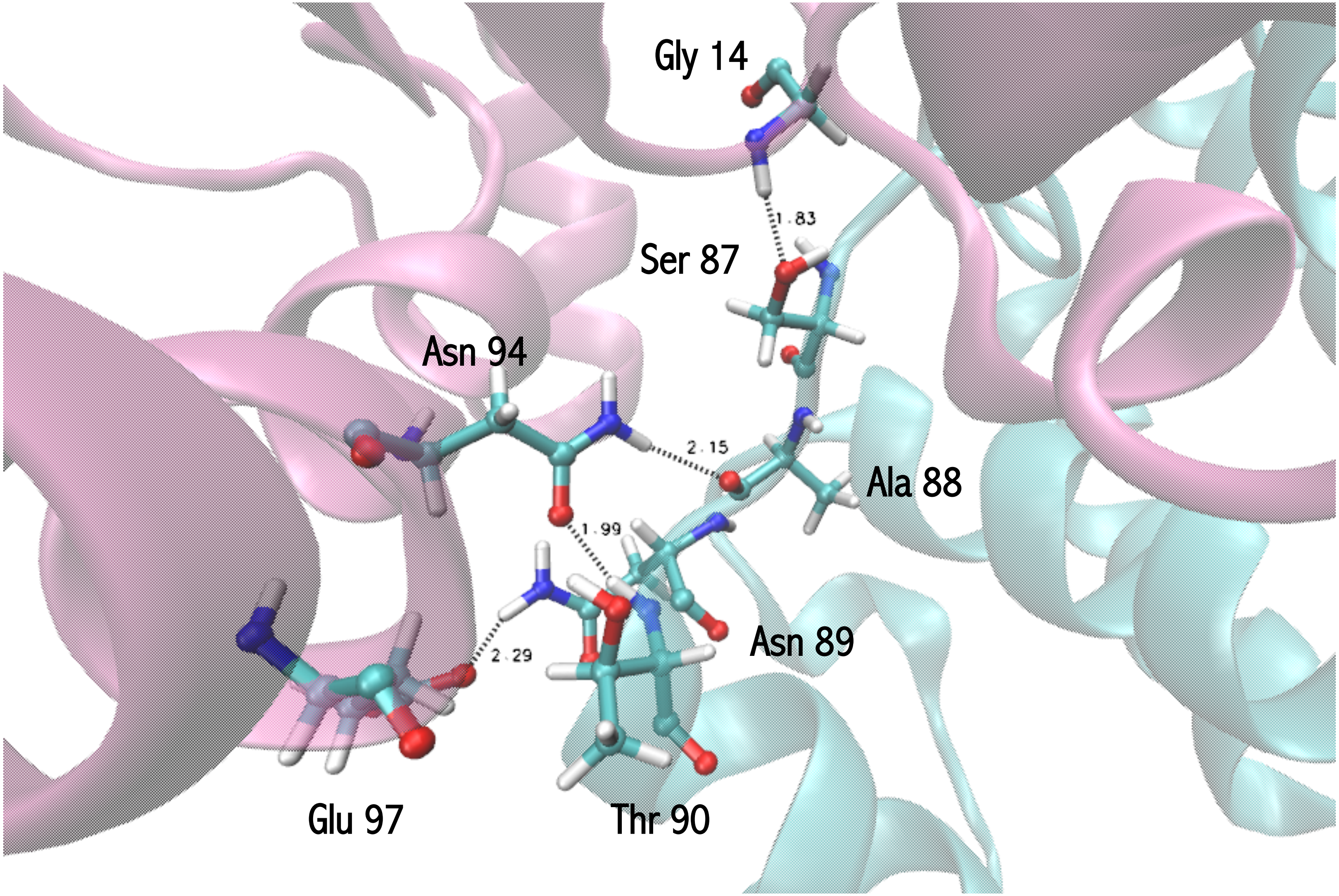
RHOA – GAP

🟥 Rho A
🟦 GAP



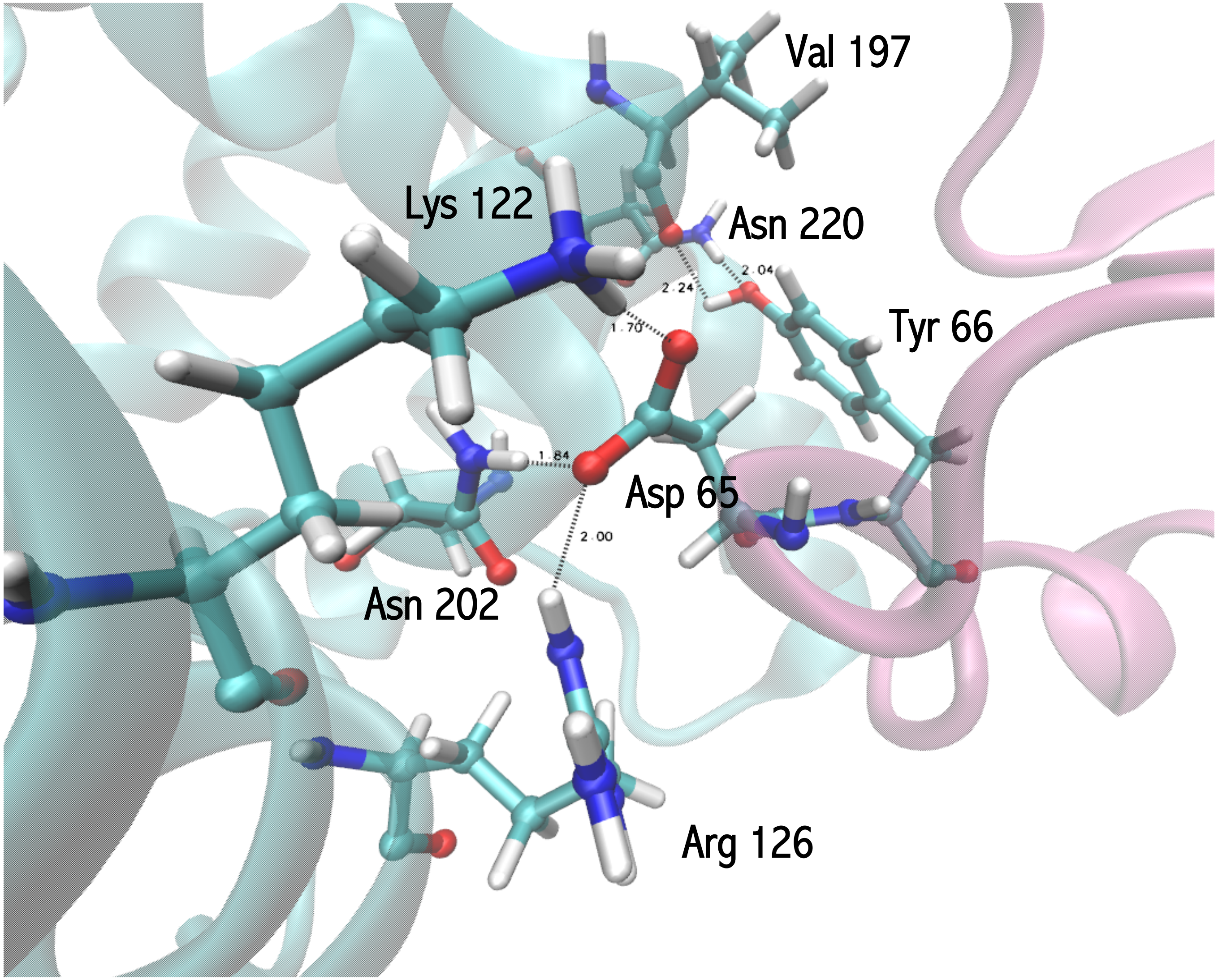
RHOA – GAP

■ Rho A
■ GAP



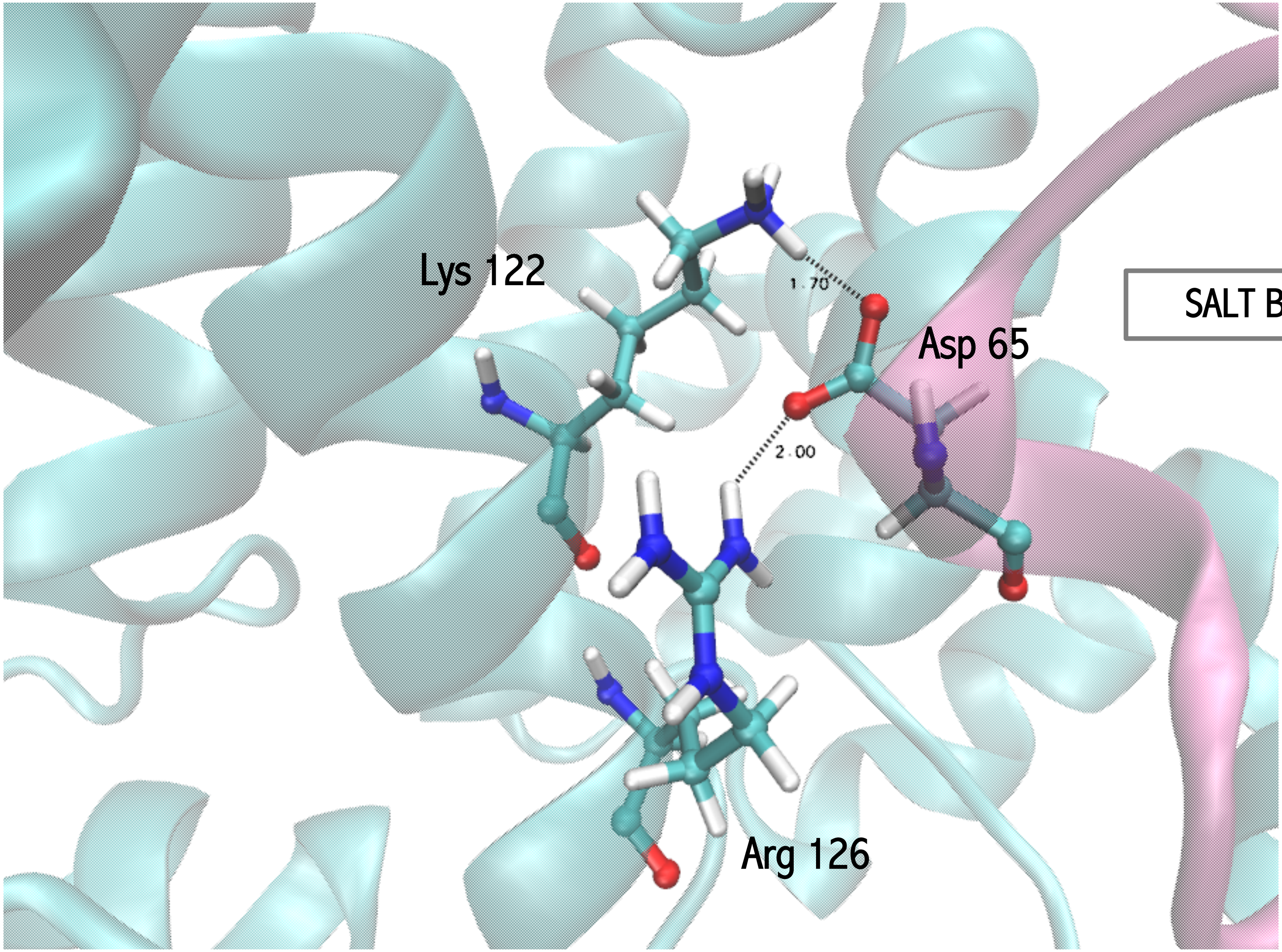
RHOA – GAP

🟪 Rho A
🟦 GAP



RHOA – GAP

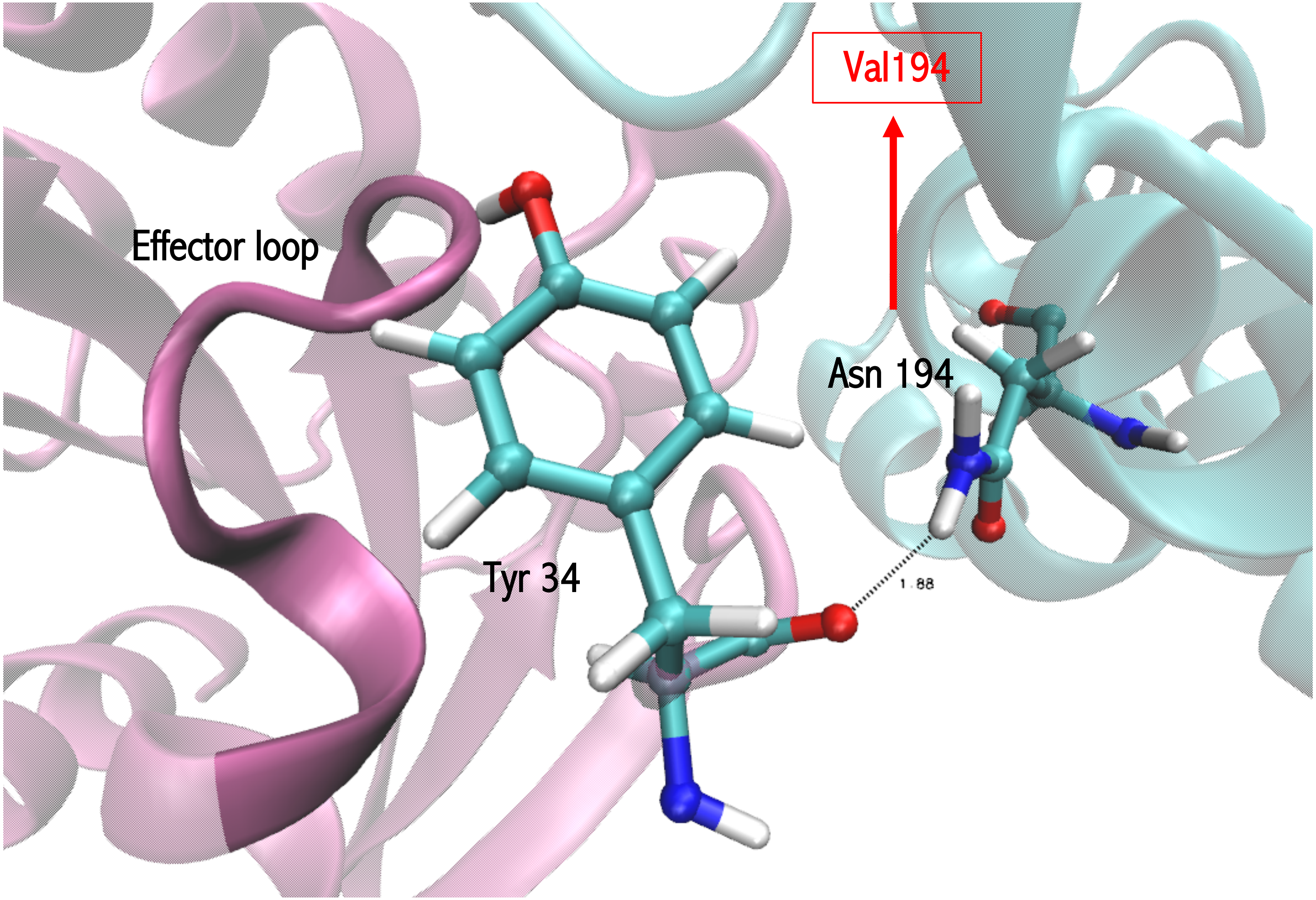
🟥 Rho A
🟦 GAP



SALT BRIDGE

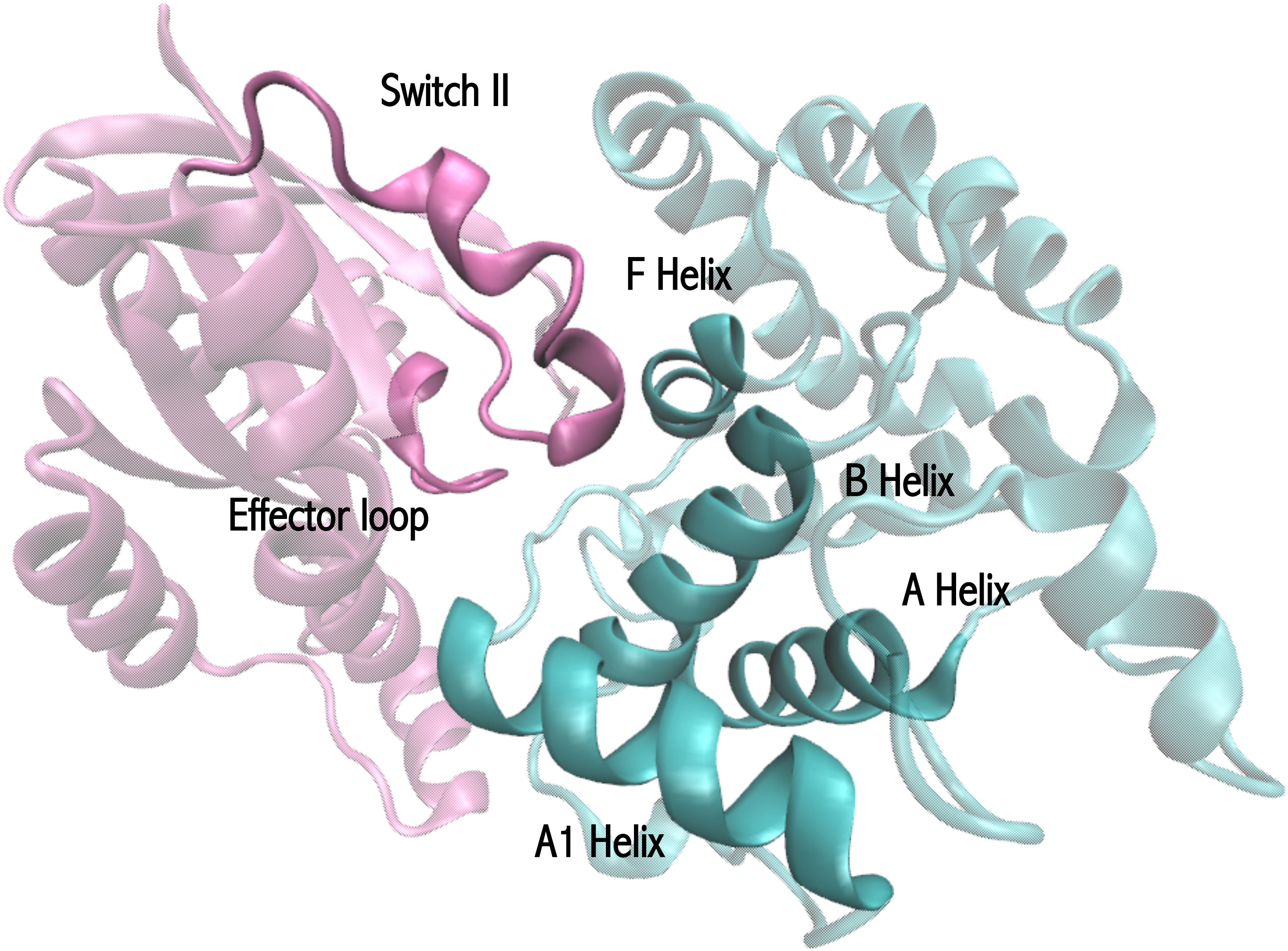
RHOA – GAP

🟪 Rho A
🟦 GAP



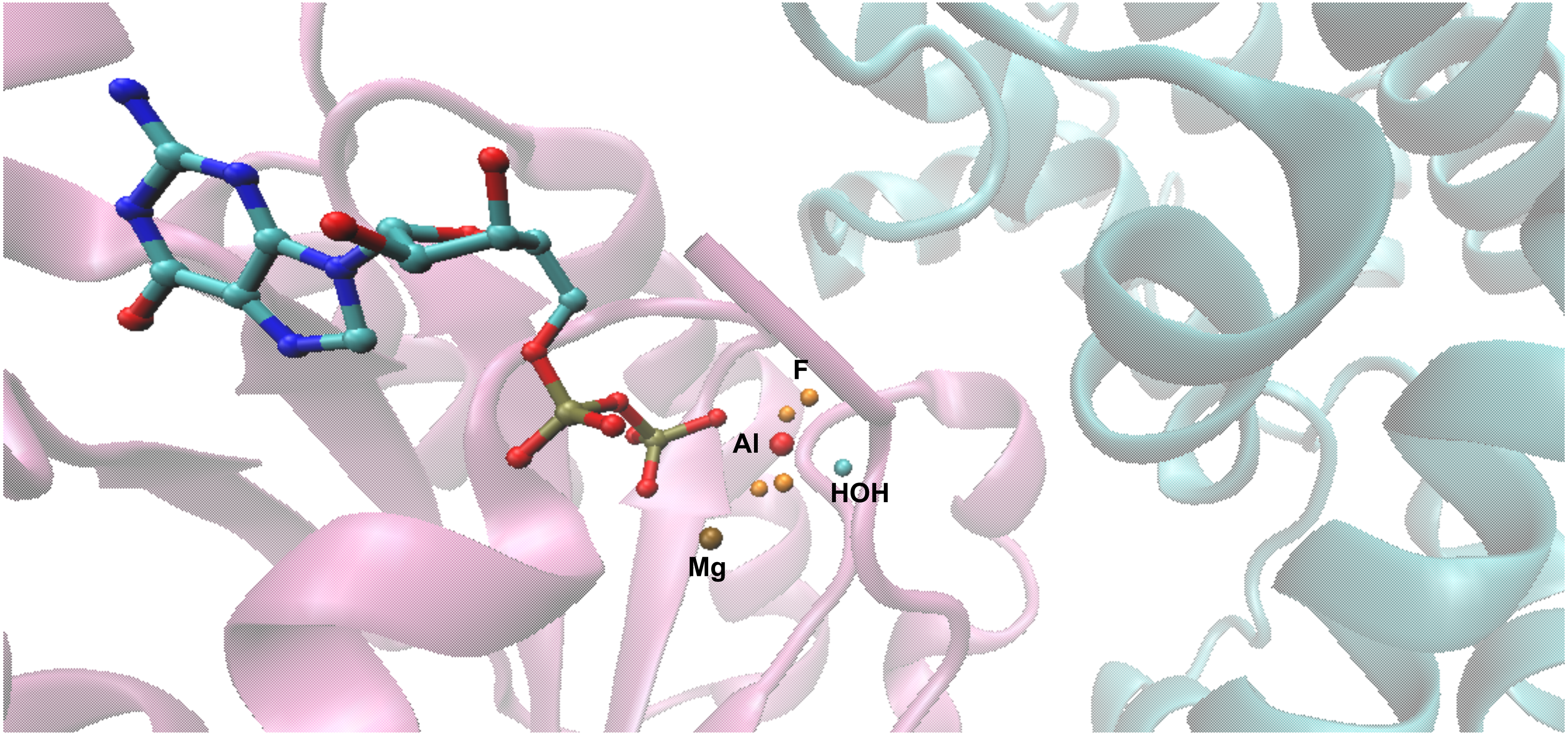
RHOA – GAP

🟥 Rho A
🟦 GAP



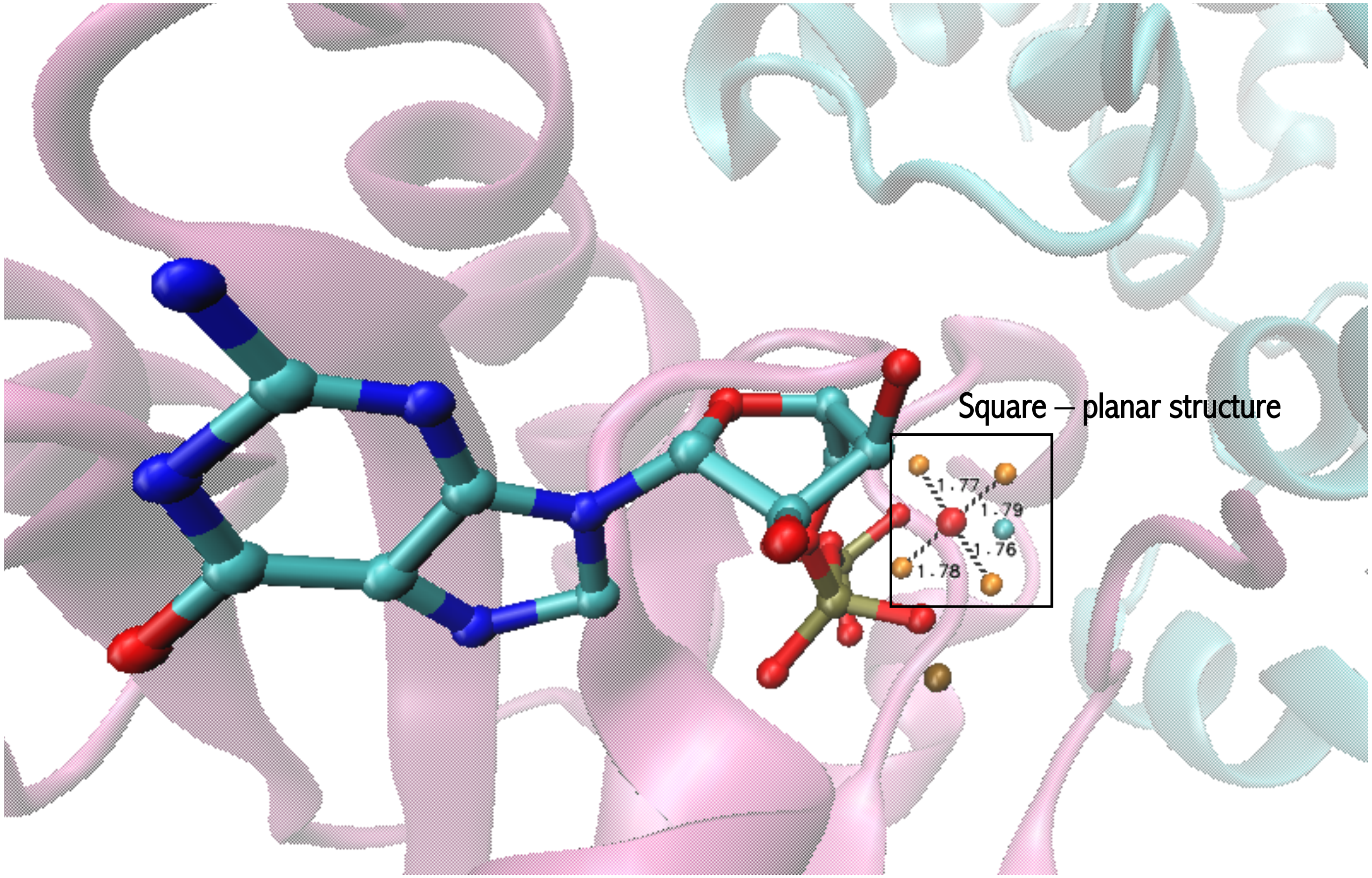
RHOA – GAP

🟥 Rho A
🟦 GAP



RHOA – GAP

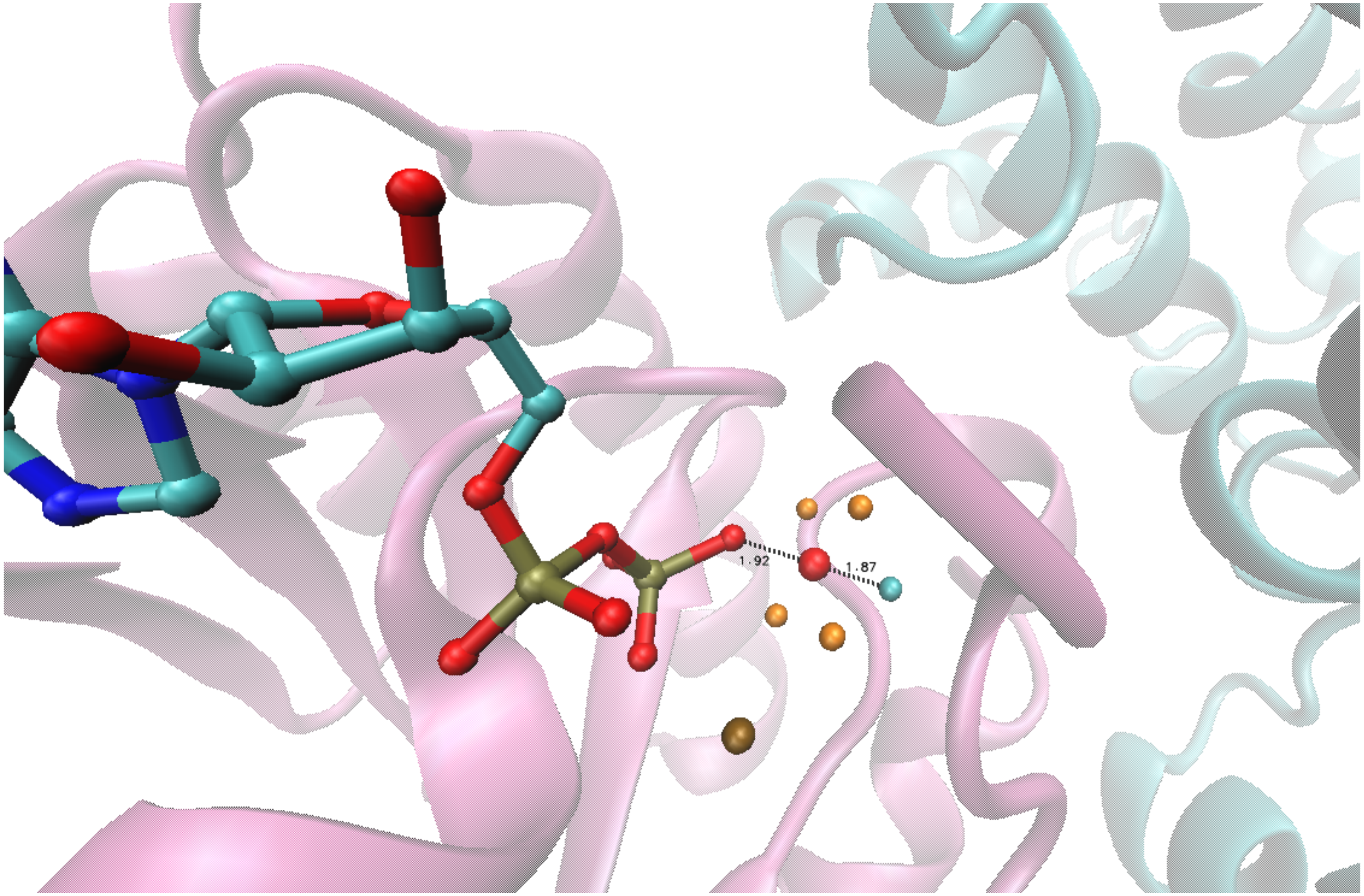
🟪 Rho A
🟦 GAP



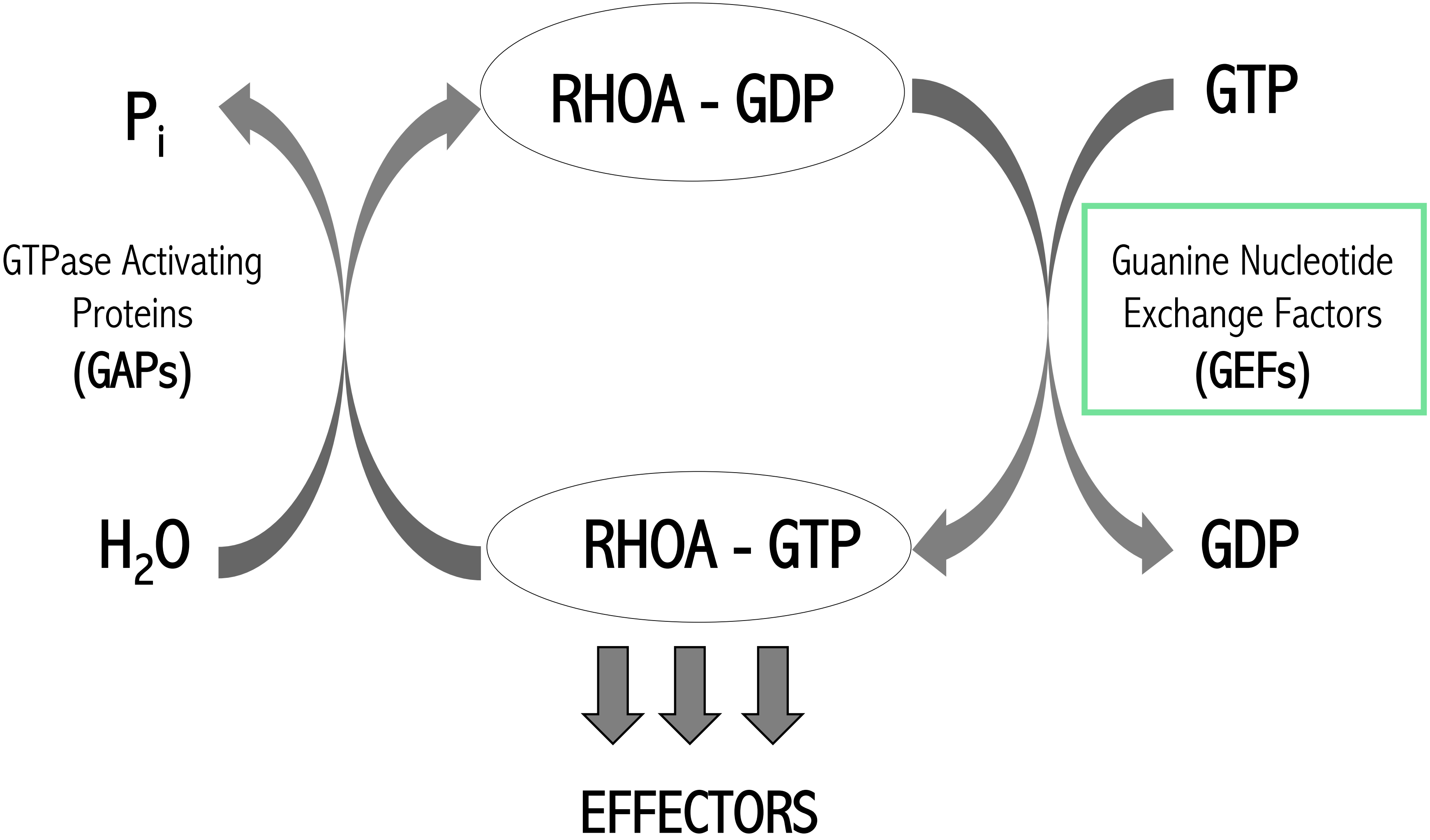
Square – planar structure

RHOA – GAP

🟪 Rho A
🟦 GAP



RHOA INTERACTIONS WITH GEF



P_i

RHOA - GDP

GTP

GTPase Activating Proteins
(GAPs)

Guanine Nucleotide Exchange Factors
(GEFs)

H_2O

RHOA - GTP

GDP

EFFECTORS

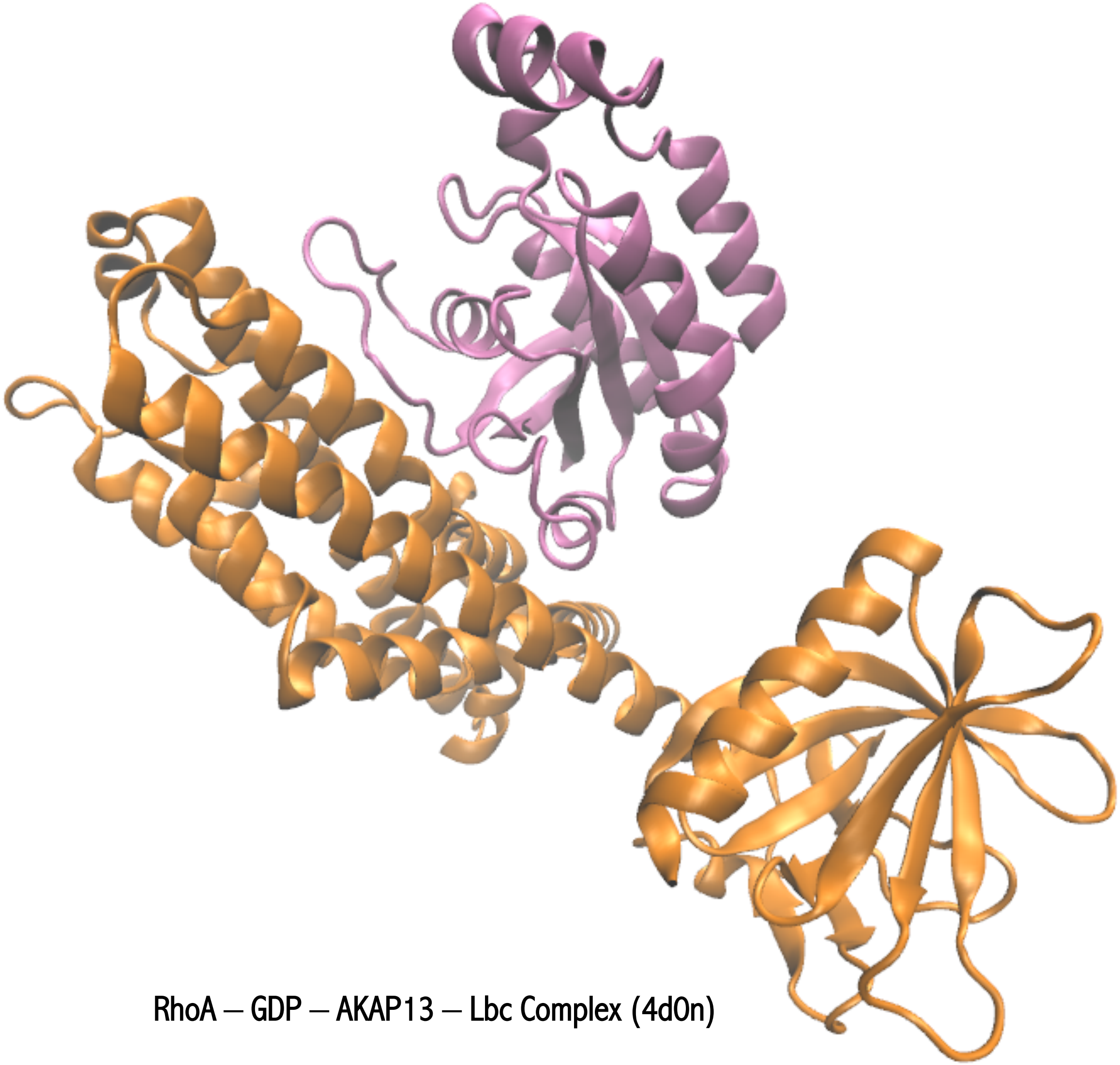
RHOA INTERACTIONS WITH GEF

GEFs from RHO family

ARGH	ABR	TRIO	OBSCN
FARP	NET	KALRN	ECT
AKAP13	MCF	SPATA	DNMBP
PLEK	BCR	FGD	ITSN

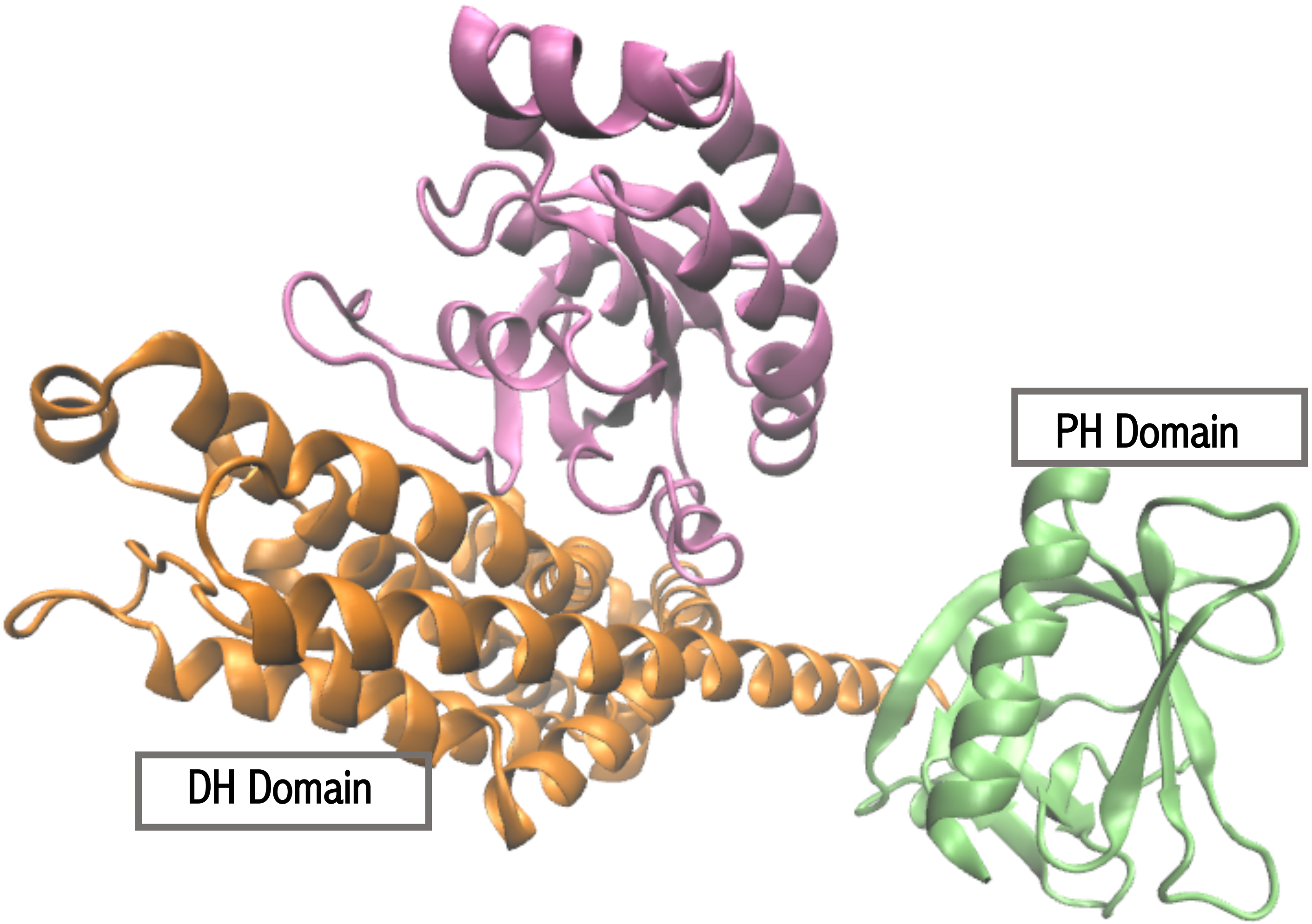
RHOA – GEF

🟪 Rho A
🟧 GEF



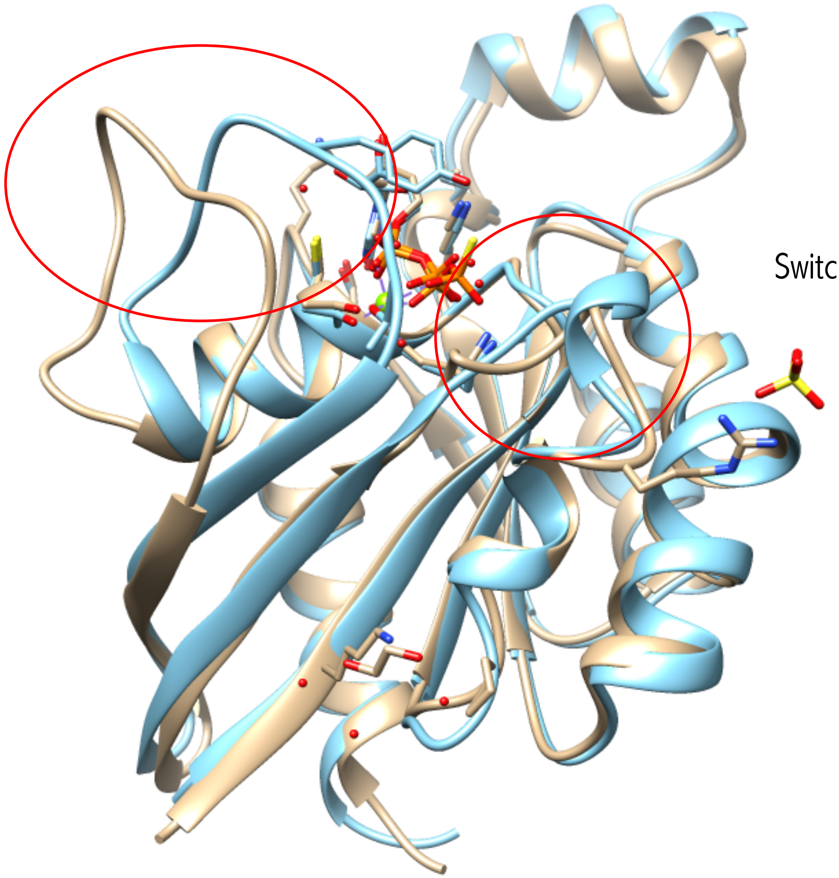
RhoA – GDP – AKAP13 – Lbc Complex (4d0n)

RHOA – GEF



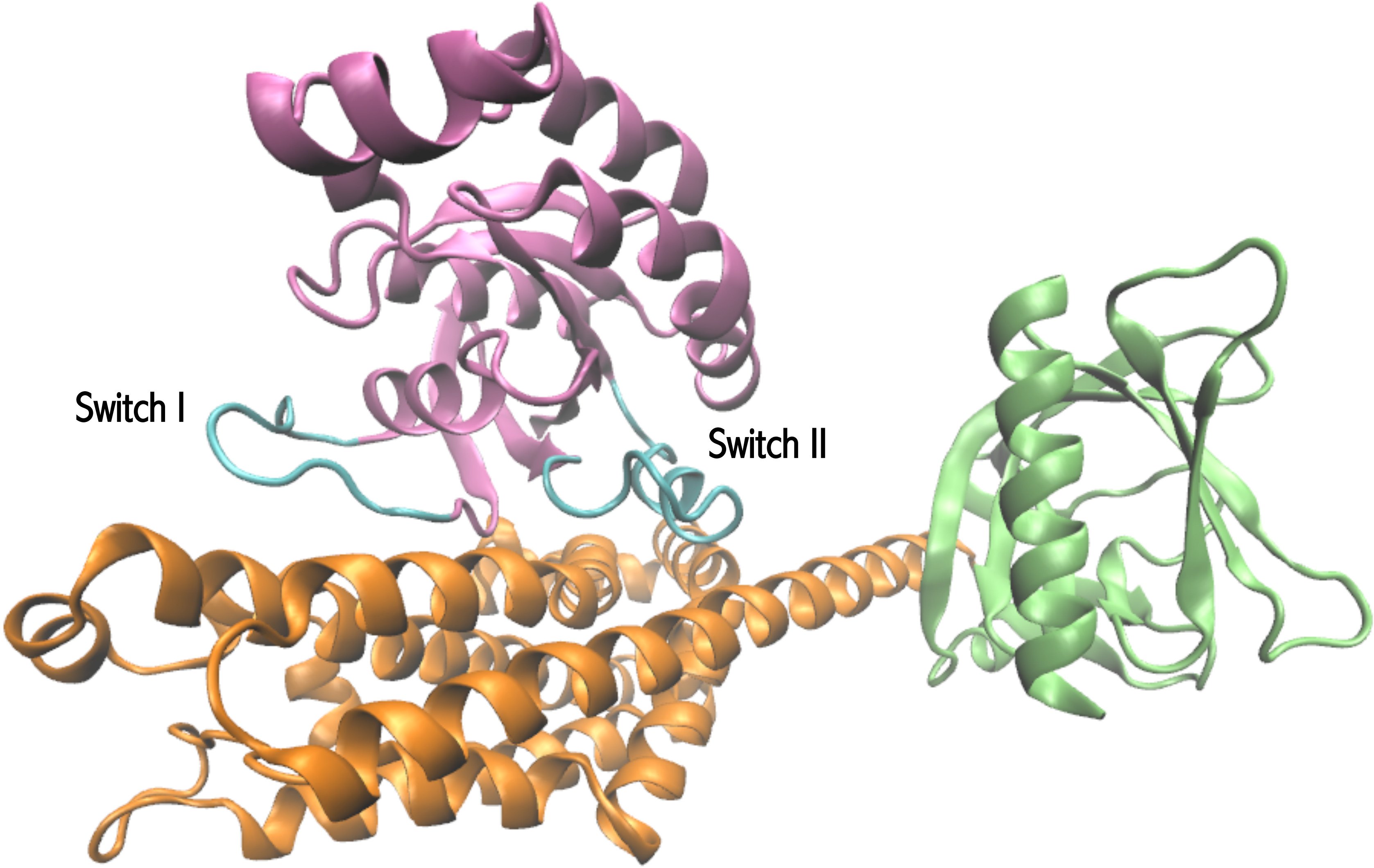
RHOA – GEF

🟦 RhoA
🟧 Rho A - GEF



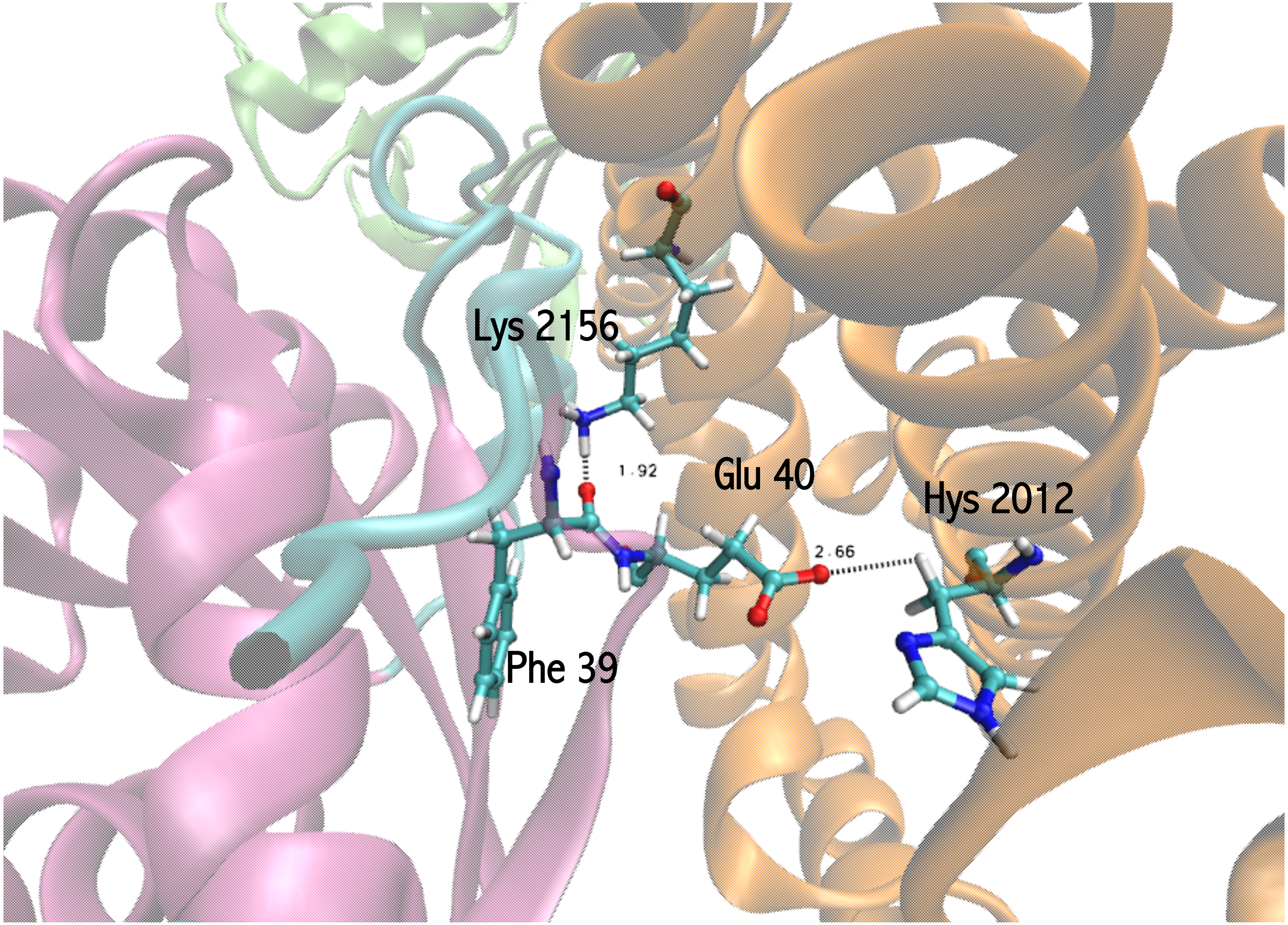
Push and Pull mechanism

RHOA – GEF



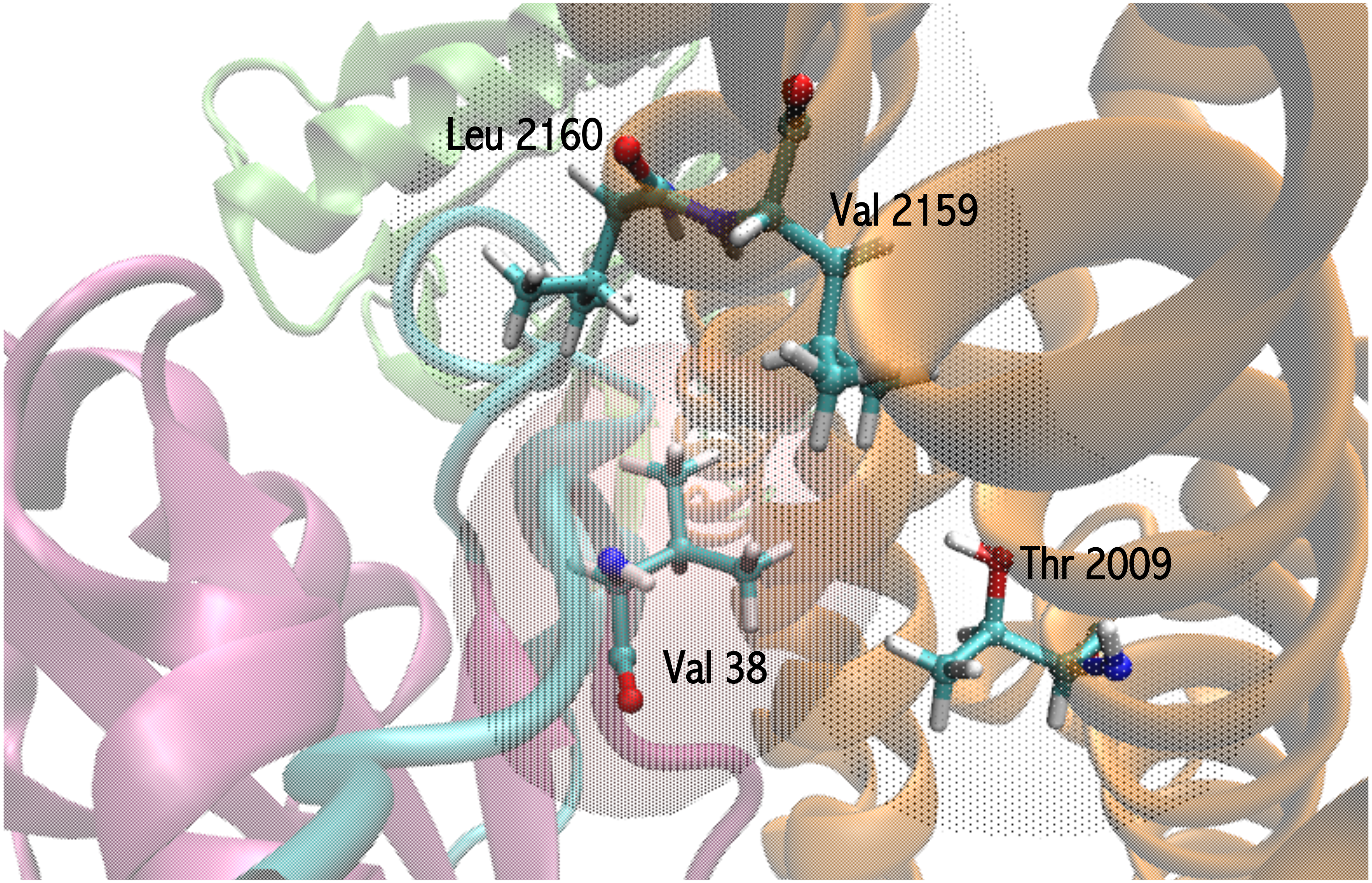
RHOA – GEF

Switch I



RHOA – GEF

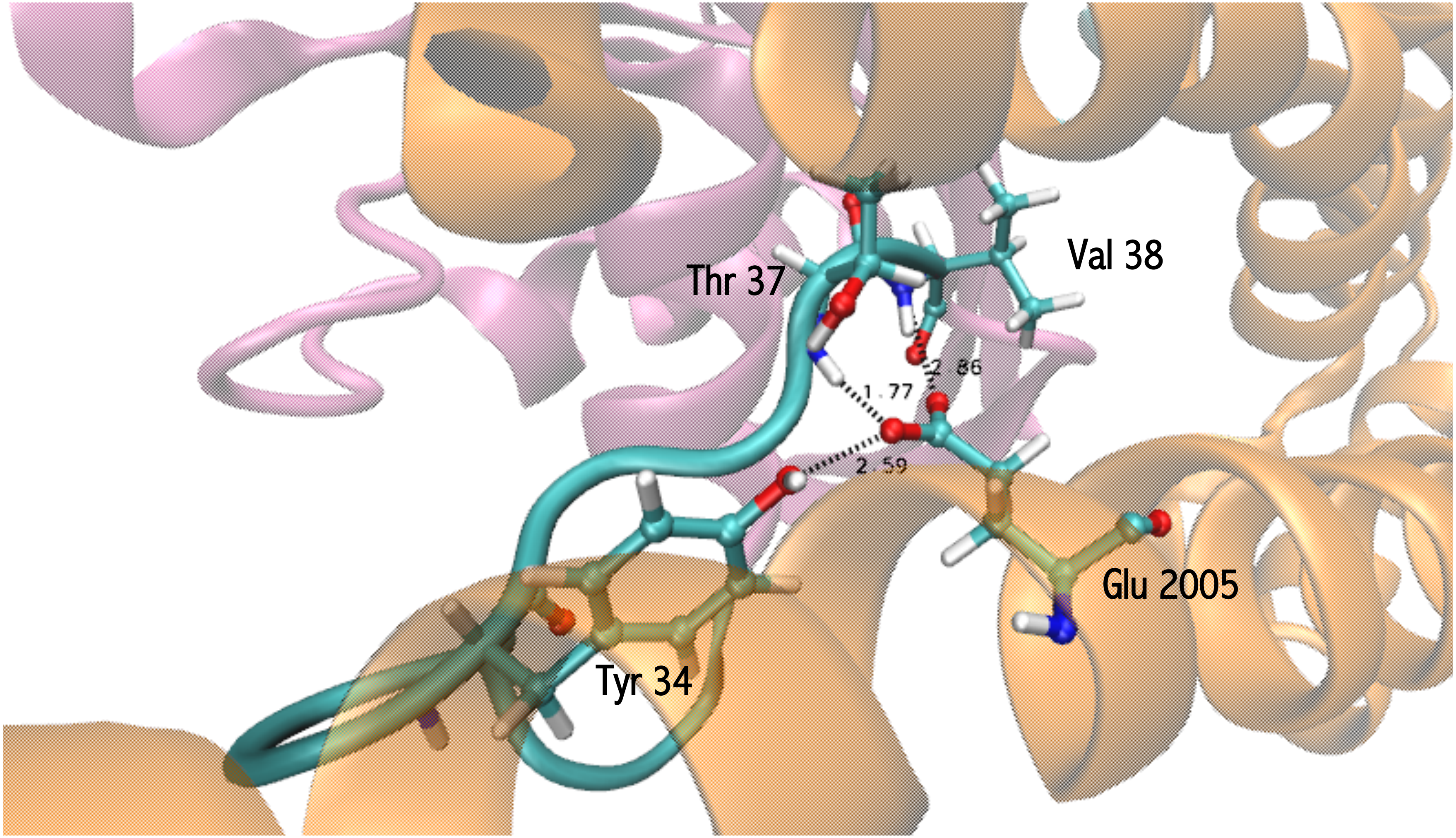
Switch I



HYDROPHOBIC PATCH

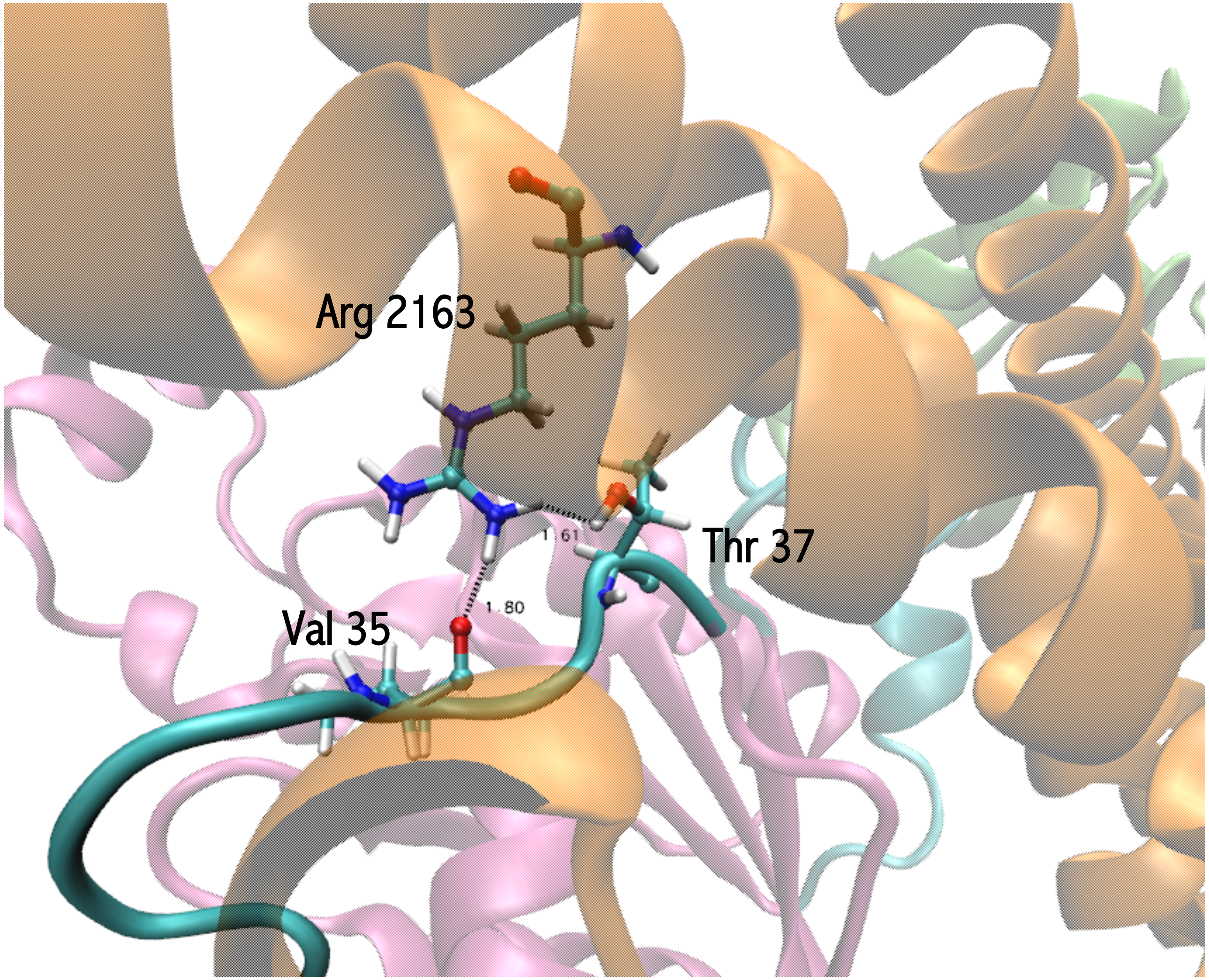
RHOA – GEF

Switch I



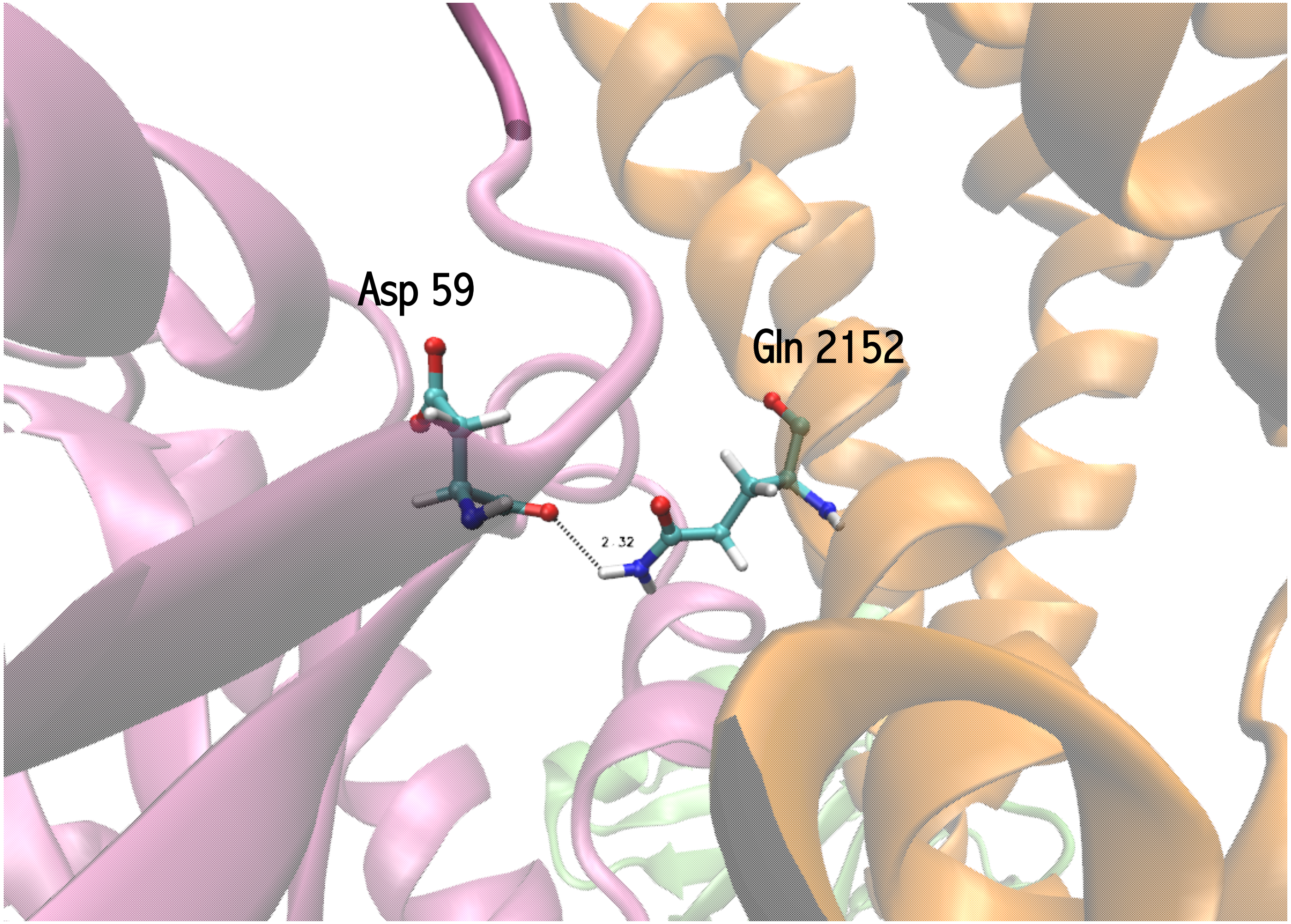
RHOA – GEF

Switch I



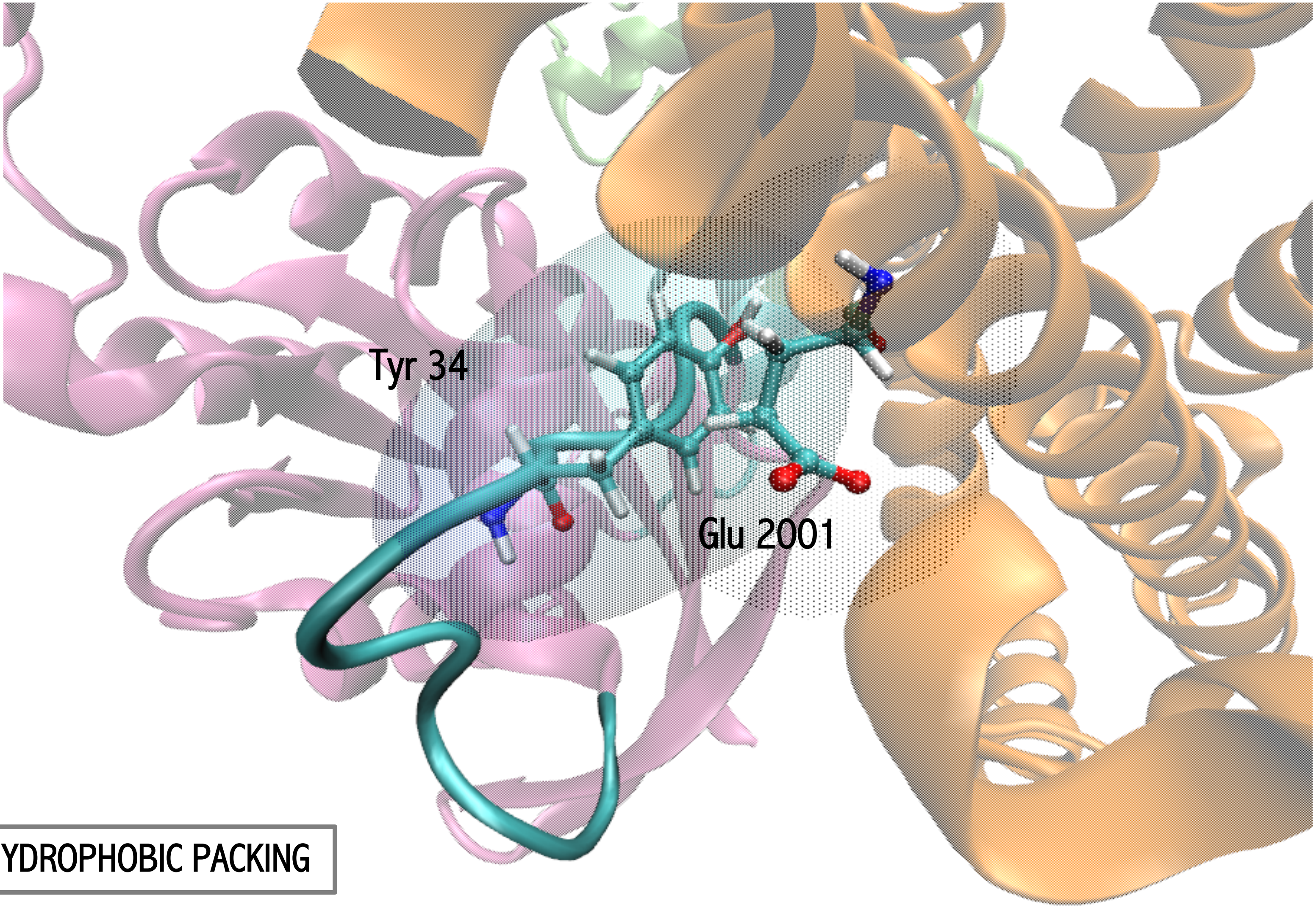
RHOA – GEF

Switch I



RHOA – GEF

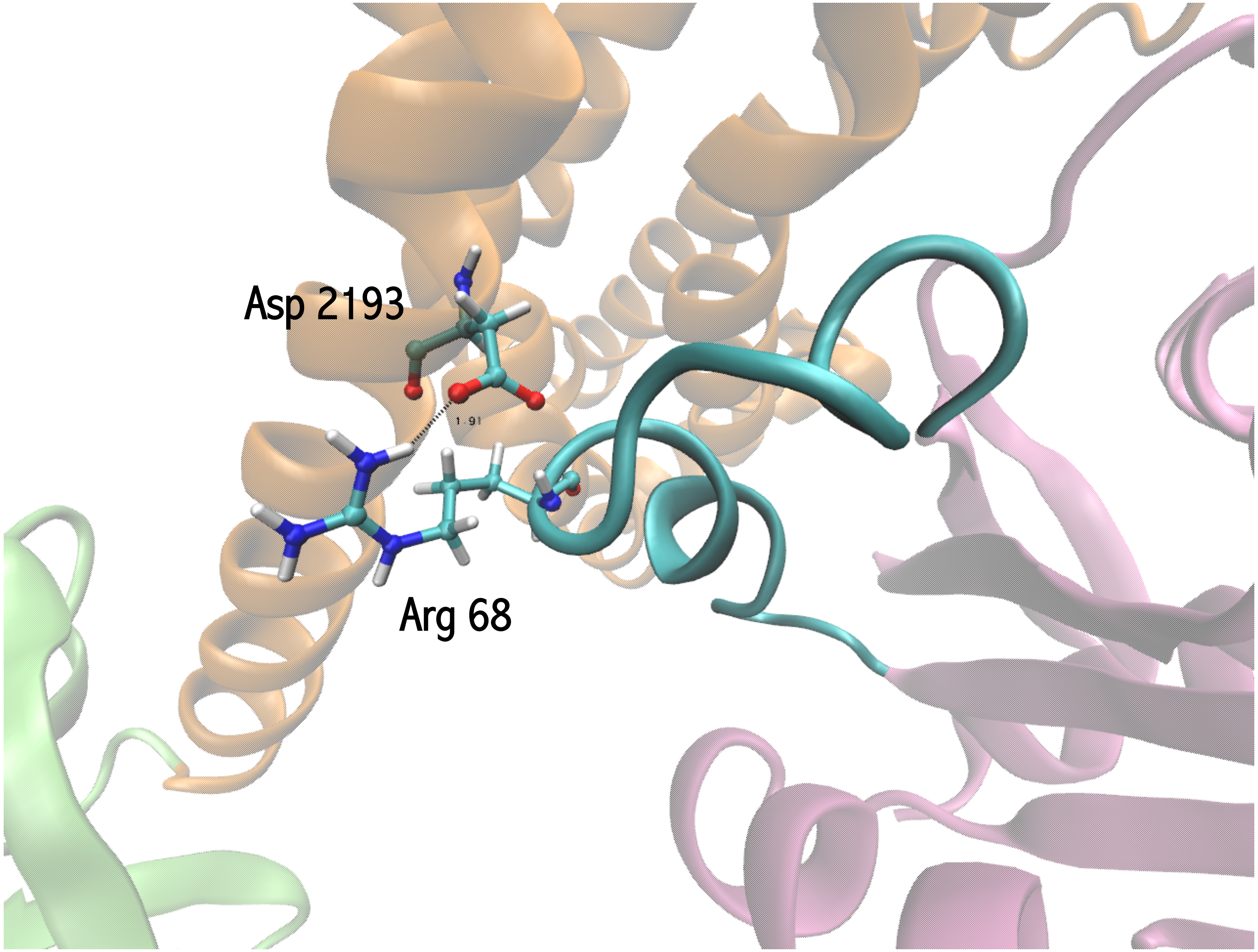
Switch I



HYDROPHOBIC PACKING

RHOA – GEF

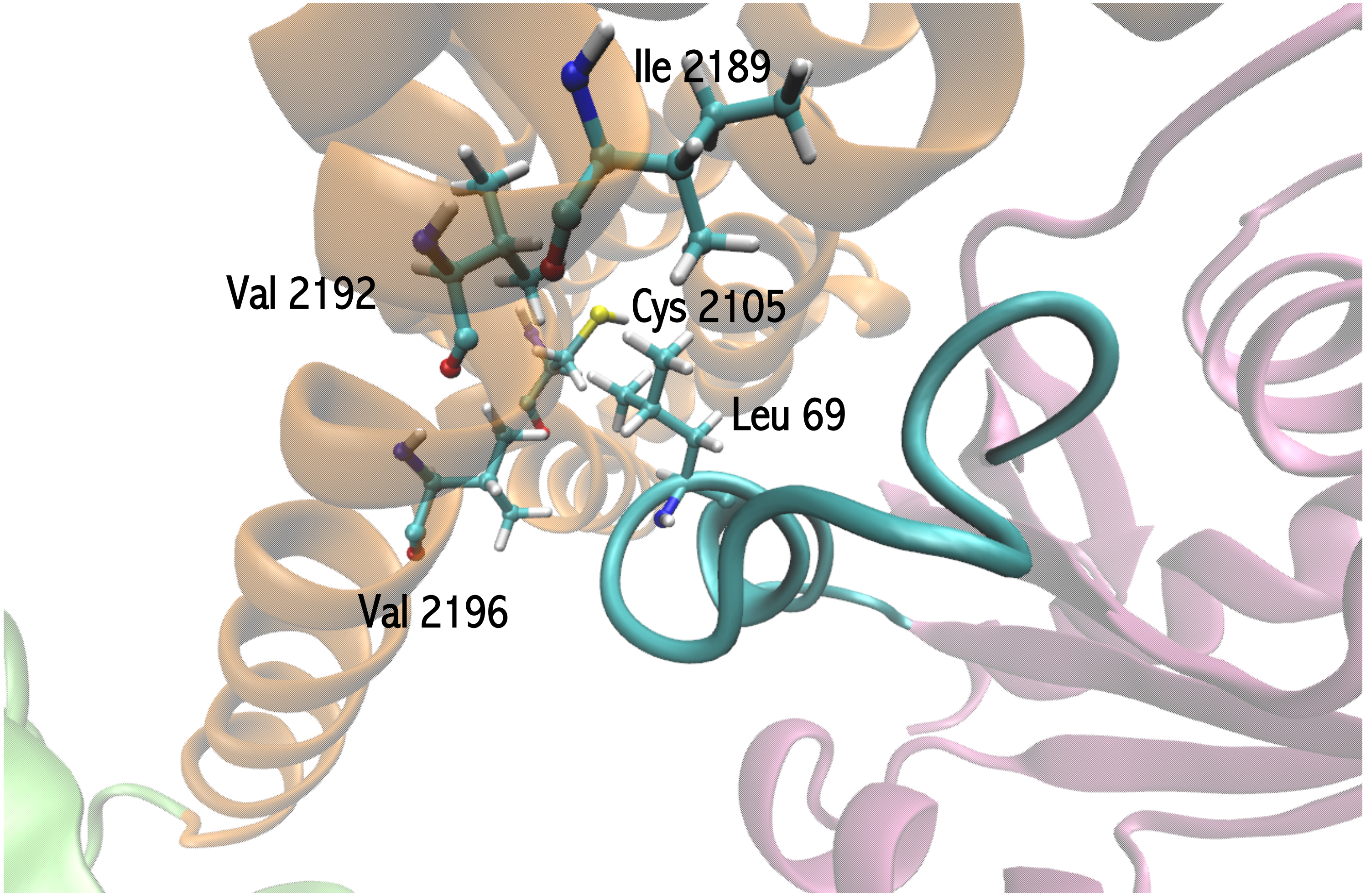
Switch II



POLAR INTERACTION

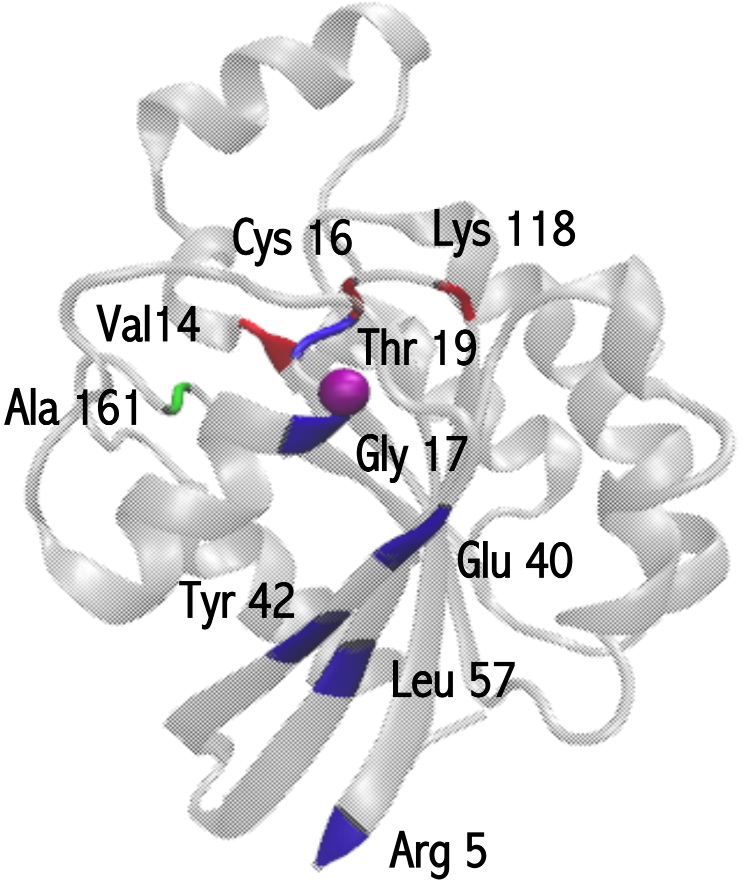
RHOA – GEF

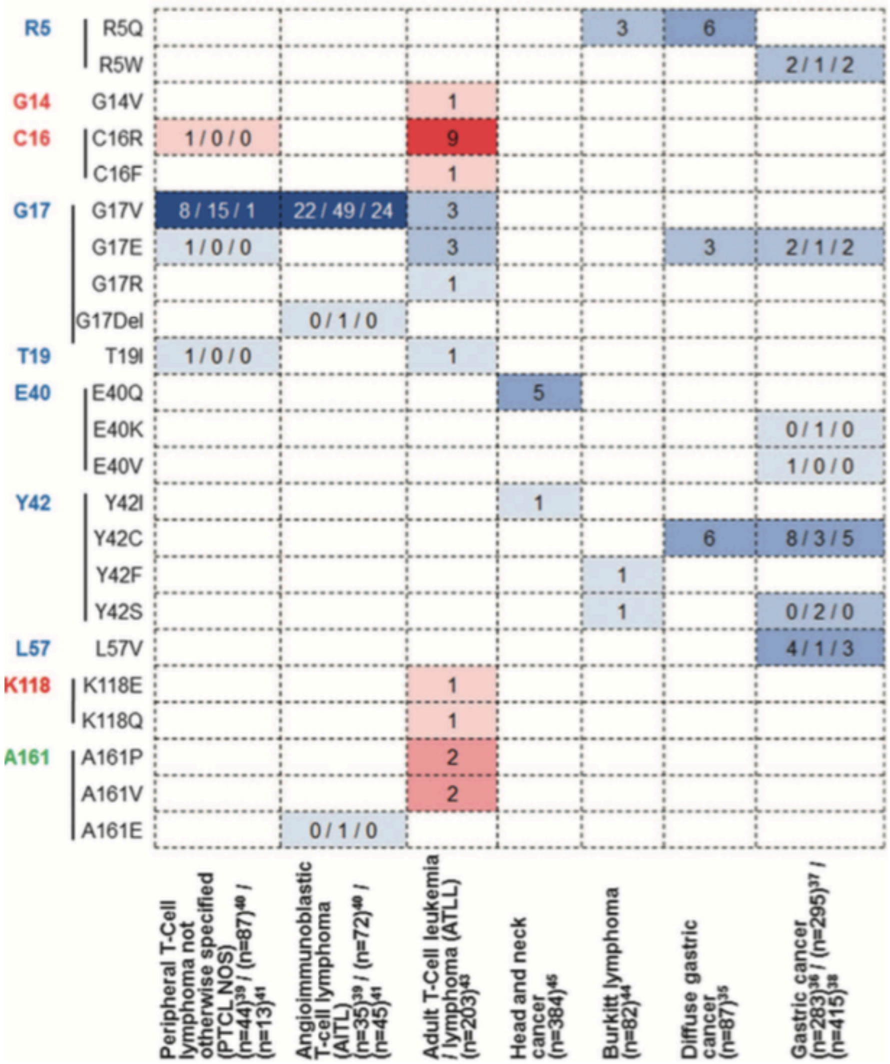
Switch II



HYDROPHOBIC PACKING

RECURRENT RHOA MUTATIONS IN HUMAN CANCER





		Peripheral T-Cell lymphoma not otherwise specified (PTCL NOS) (n=44)[39] / (n=87)[40] / (n=13)[41]	Angioimmunoblastic T-cell lymphoma (AITL) (n=35)[39] / (n=72)[40] / (n=45)[41]	Adult T-Cell leukemia / lymphoma (ATLL) (n=203)[43]	Head and neck cancer (n=384)[45]	Burkitt lymphoma (n=82)[44]	Diffuse gastric cancer (n=87)[35]	Gastric cancer (n=283)[36] / (n=295)[37] / (n=415)[38]
R5	R5Q					3	6	
	R5W							2 / 1 / 2
G14	G14V			1				
C16	C16R	1 / 0 / 0		9				
	C16F			1				
G17	G17V	8 / 15 / 1	22 / 49 / 24	3				
	G17E	1 / 0 / 0		3			3	2 / 1 / 2
	G17R			1				
	G17Del		0 / 1 / 0					
T19	T19I	1 / 0 / 0		1				
E40	E40Q				5			
	E40K							0 / 1 / 0
	E40V							1 / 0 / 0
Y42	Y42I				1			
	Y42C						6	8 / 3 / 5
	Y42F					1		
	Y42S					1		0 / 2 / 0
L57	L57V							4 / 1 / 3
K118	K118E			1				
	K118Q			1				
A161	A161P			2				
	A161V			2				
	A161E		0 / 1 / 0					

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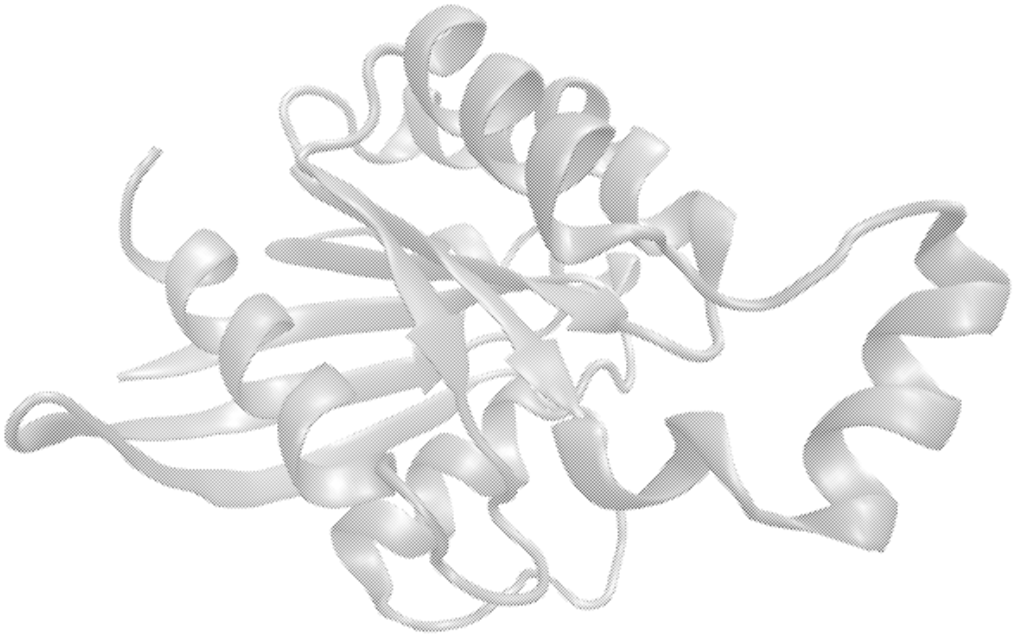
CONCLUSIONS

- G Domains are conserved in the different monomeric G – proteins.
- The interactions between Rho A and the nucleotide is essential for its GTPase activity.
- Magnesium ion is a relevant element from the structure and the function of the protein.
- The key residues in the interaction between GAP and Rho A are Arg85, Asn194 from GAP and Tyr66 from Rho A whereas the interaction between GEF and Rho A needs the presence of Switch regions.
- Taking into account gain/loss of function mutations of Rho A and Cdc42.



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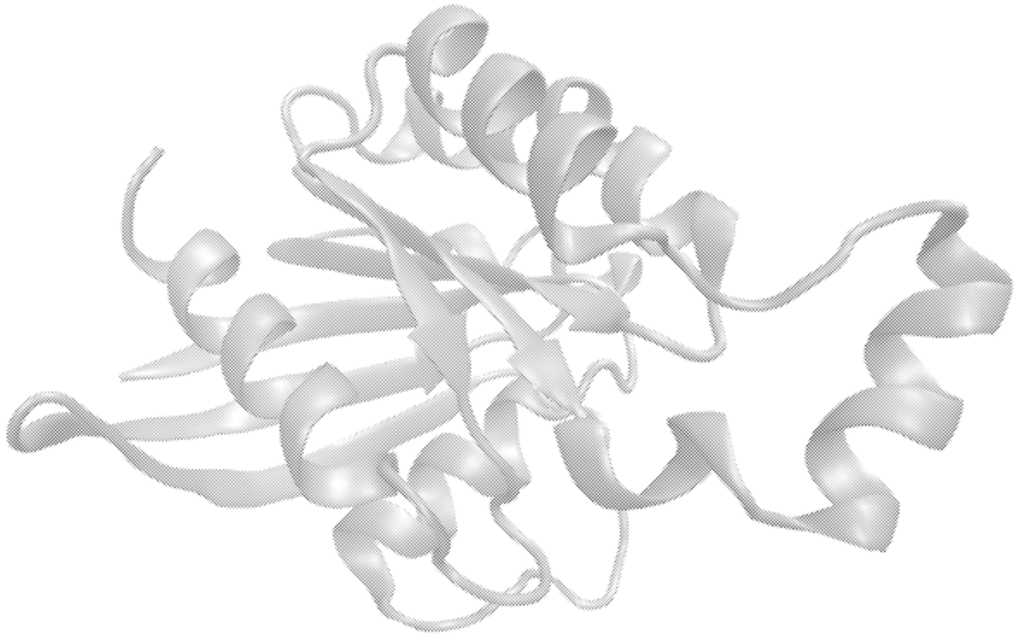


QUESTIONS

QUESTIONS

1. Which ion has a pivotal role in G protein affinity to the GTP/GDP molecules:

a) Mg^{2+}
b) Fe^{2+}
c) K^+
d) Cl^-
e) Mn^+

2. Choose the **correct** answer

a) RhoA is involved in Huntington and Alzheimer disease.
b) RhoA is involved in several types of cancer.
c) Once GDP is bound, the alpha subunit assumes its activated conformation and dissociates from both the receptor and the By dimer.
d) Alpha and beta subunits from trimeric G proteins works as a dimer.
e) GAPs (GTPase activation protein) helps on the exchange from GTP to GDP.

3. Choose the **incorrect** answer

a) Heterotrimeric G proteins function as molecular switches.
b) Monomeric G proteins function as molecular switches.
c) Monomeric G proteins have three subunits, alpha, beta and gamma.
d) When a G protein is bound to GTP is in its active form.
e) The alpha subunit of trimeric G proteins functions as an hydrolase.

4. In relation to the Ras superfamily choose the **correct** answer:

a) Ras family is involved in cell vesicular trafic.
b) Ras superfamily is divided in five families: Ran, Rab, Ras, Rho, Rif.
c) Arf and Rab families are involved on vesicle trafficking.
d) Ras superfamily is divided in seven families: Ran, Rho, Ras, Rac, Rap, Rab, Ros.
e) Ras superfamily is the most known heterotrimeric G proteins.

QUESTIONS

5. Choose the **correct** answers
1. Heterotrimeric G proteins are mainly located in the cell membrane.
2. Alpha subunit binds GTP, dissociates and interacts with effector.
3. The ligands of G proteins are GEFs, GAPs and GDI.
4. Monomeric G proteins are coupled by GPCRs.

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

6. In relation with monomeric G proteins, choose the **incorrect** answer:

a) They are located in the cytosol.
b) Its structure is homologous to Gbeta.
c) They catalyse the hydrolysis of the terminal phosphate group.
d) AC, PLC and Ion channels are its typical effectors.
e) These proteins are characterized by a very conserved fold known as the G DOMAIN formed by five domains.

7. Regarding RhoA signalling, choose the **incorrect** answer

a) RhoA is activated by growth factors and cytokines.
b) RhoA is activated by GPCRs and hormones.
c) GPCRs, EphA, activate RhoA through the function of GEFs.
d) KTN1 can activate RhoA directly.
e) GPCRs, EphA, activate RhoA through the function of GDIs.

QUESTIONS

8. Choose the **incorrect** answer

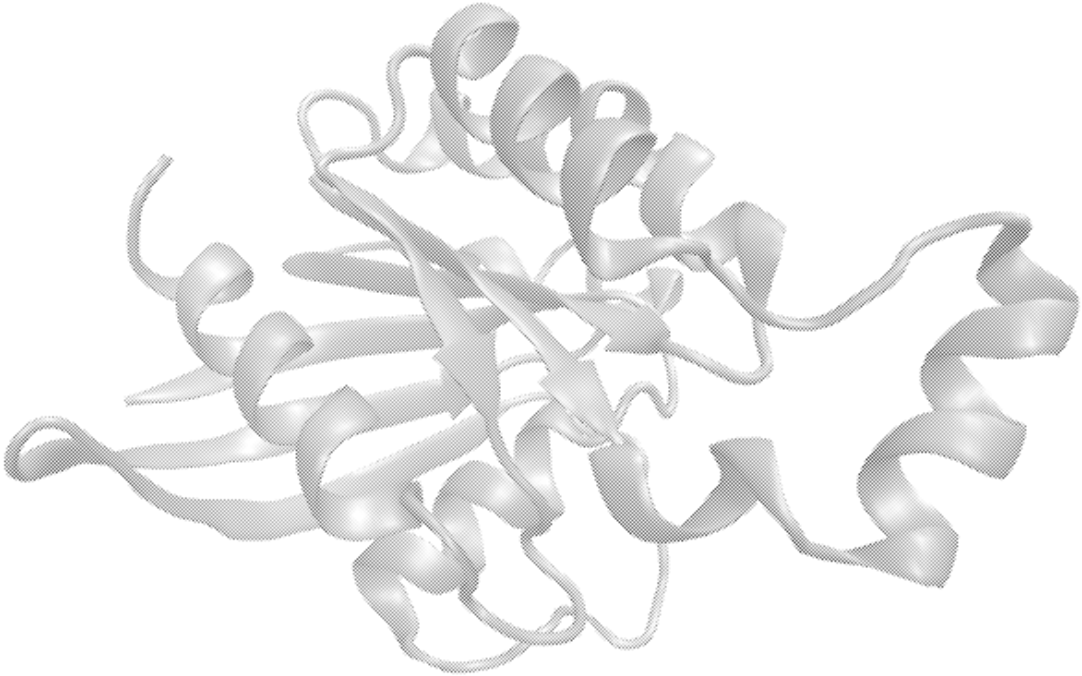
a) Rho GTPase signalling is important in Ras pathways and other cancer-driven mechanisms.
b) Multiple Rho GTPases have been found to be potential anti-neoplastic targets in a wide variety of cancers.
c) Rho GTPases may function as pro-oncogenes but never as tumour suppressors.
d) Recent studies show that Rho GTPases, RhoA and Cdc42 in particular, may also behave as ‘tumour suppressors’ in certain cancer and defined circumstances.
e) Loss- and gain-of-function mutations of RhoA may endow the tumour cells selective advantage in early vs late stages of the cancer progression, respectively.

9. Regarding Rho family choose the **correct** answer:

a) Rac 1 effectors are ROCK I and II.
b) Rac effectos are WASP/N-WASP.
c) Cdc42 effector is IRSp53.
d) Rac effextors are ROCK I and II.
e) Rho effectors are ROCK I and II.

10. In relation to the G DOMAIN (G1-G5), choose the **correct** answer:

a) Together the 5 domains make up an ~20 kDa G domain that has a conserved structure and biochemistry shared by all Ras superfamily proteins.
b) G5 is the less prevalent domain.
c) a and b are correct.
d) All G DOMAINS are constituted by 7 amino acids.
e) All answers are correct.



G PROTEINS

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