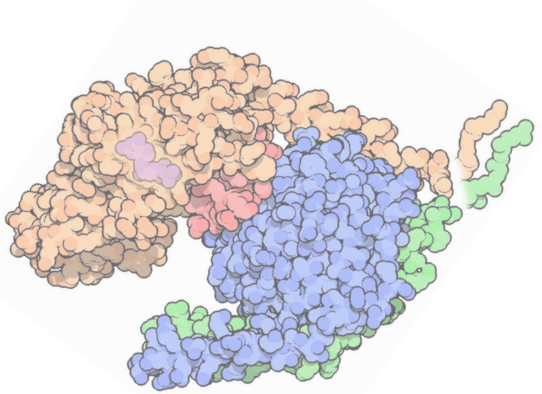
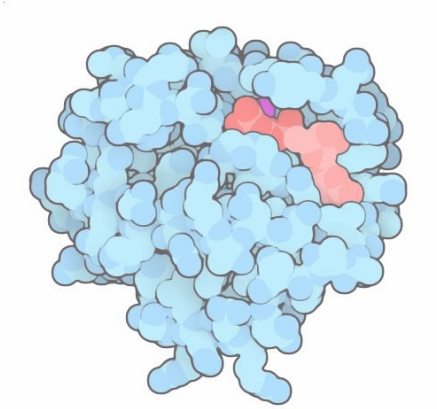
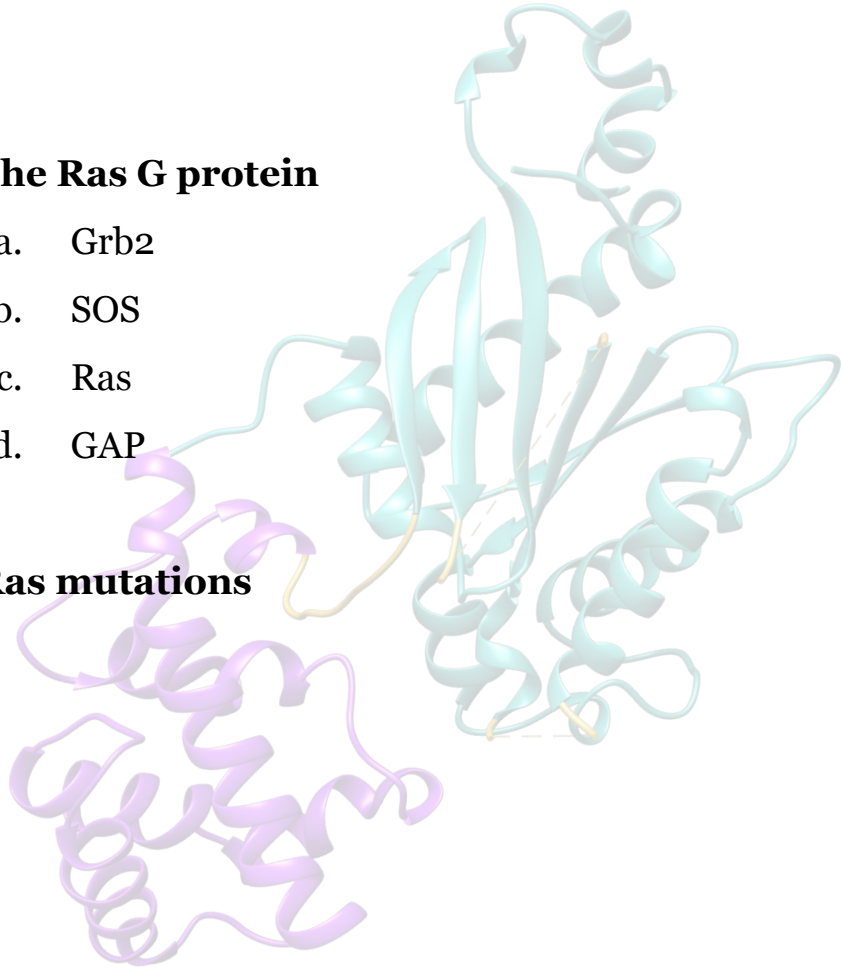


G proteins



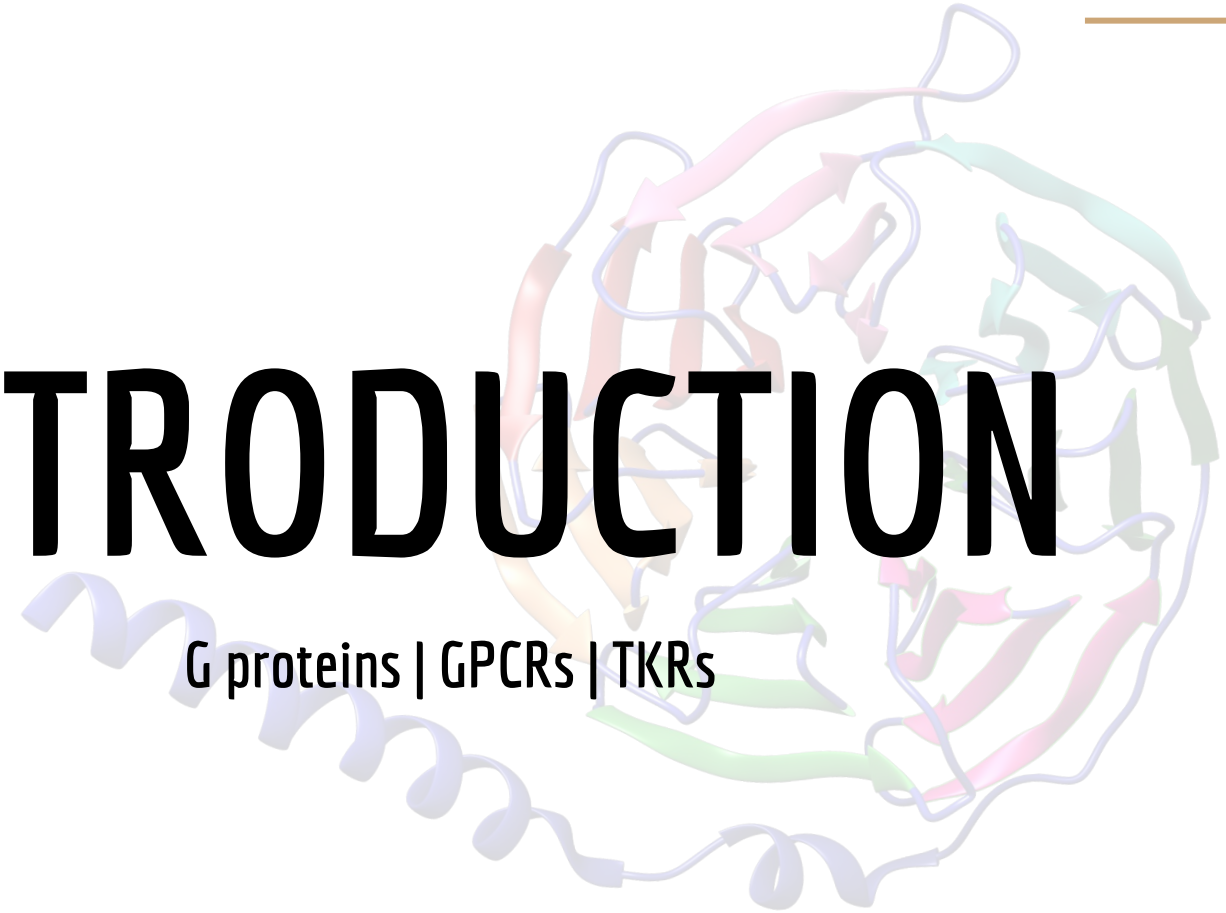
María Martínez, Sergi Mira,
Ana Pérez, Aina Rill and
Carolina Villaró

Contents



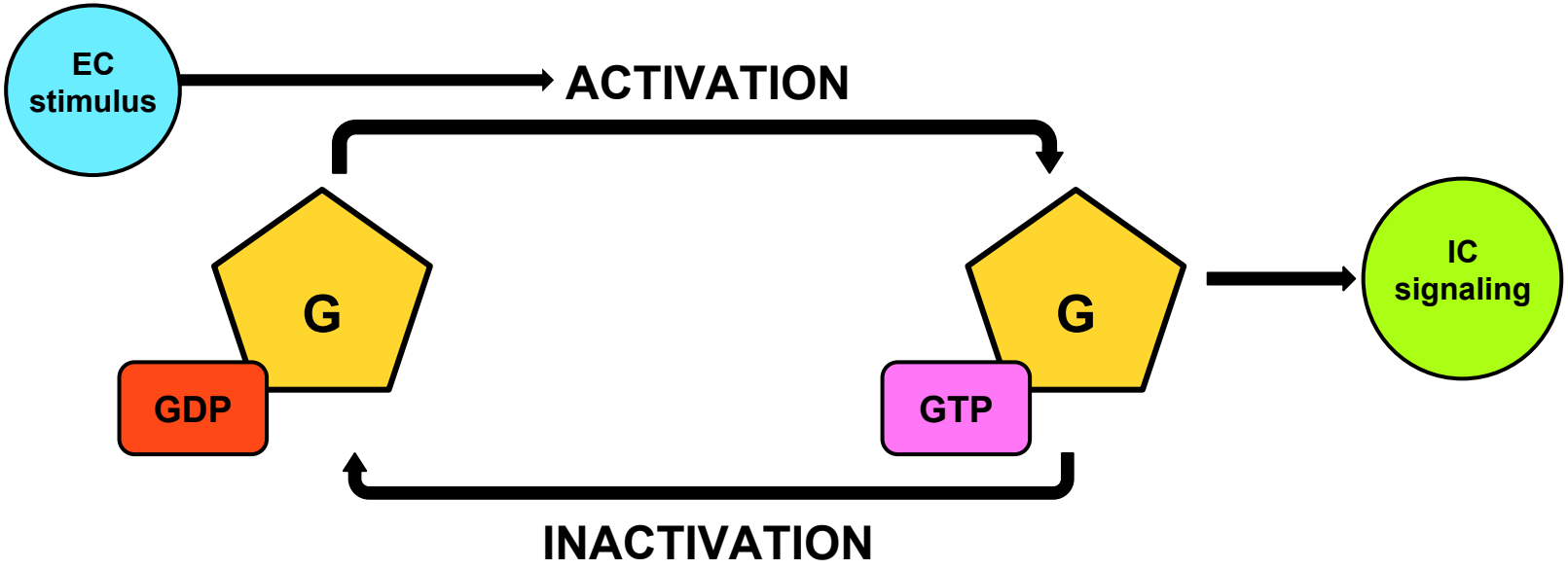
INTRODUCTION

G proteins | GPCRs | TKRs



G proteins

- **Guanine nucleotide-binding proteins**: they can bind GTP and hydrolize it to GDP.
- They act as a molecular switch: **active** when bound to **GTP**, **inactive** when bound to **GDP.**



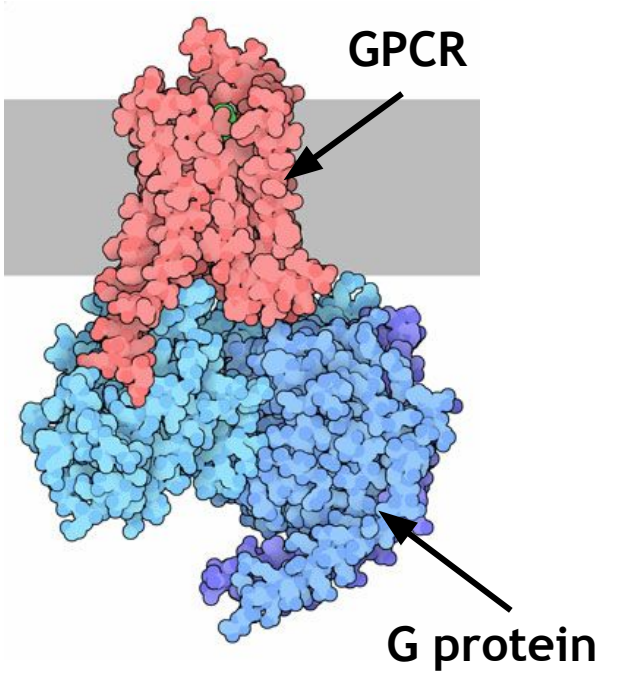
G proteins - Classification

Monomeric G proteins	Heterotrimeric G proteins
Ras superfamily of GTPases	Divided into 4 families: G_s, $G_{i/o}$, G_q, $G_{12/13}$
Small proteins (20-25 kDa)	Large complexes (~85 kDa)
Homologous to the α-subunit	3 subunits: α, ß, γ
Cytosolic	Membrane-bound (GPCRs)

G Protein-Coupled Receptors (GPCRs)

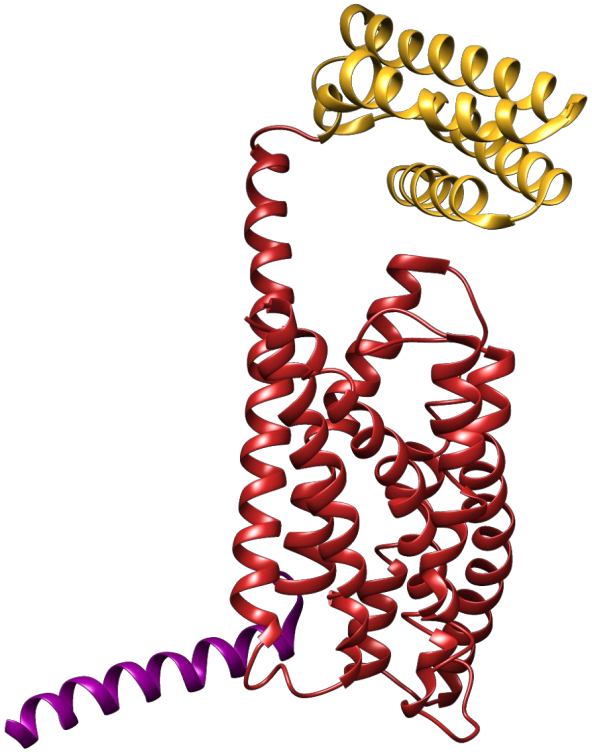
- Center of **signaling pathways** that control many essential processes, ranging from vision to carcinogenesis.
- Target of 20-50% of current drugs

GRAFS classification of GPCRs in vertebrates	
Glutamate family	12 GPCRs, including metabotropic glutamate receptors, $GABA_B$, and Ca^{2+} sensing receptors.
Rodhopsin family	719 GPCRs (~80%), including receptors for most hormones and neurotransmitters.
Adhesion family	33 GPCRs
Frizzled family	11 GPCRs, including receptors for the frizzled protein family and smoothened.
Secretin family	15 GPCRs.



PDB-101: GPCR (Molecule of the Month)

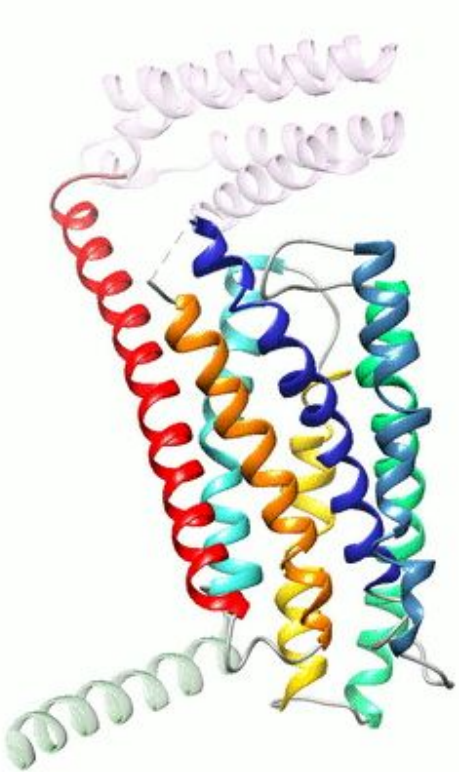
G Protein-Coupled Receptors (GPCRs)



N-terminus
(ectodomain)

7TM domain

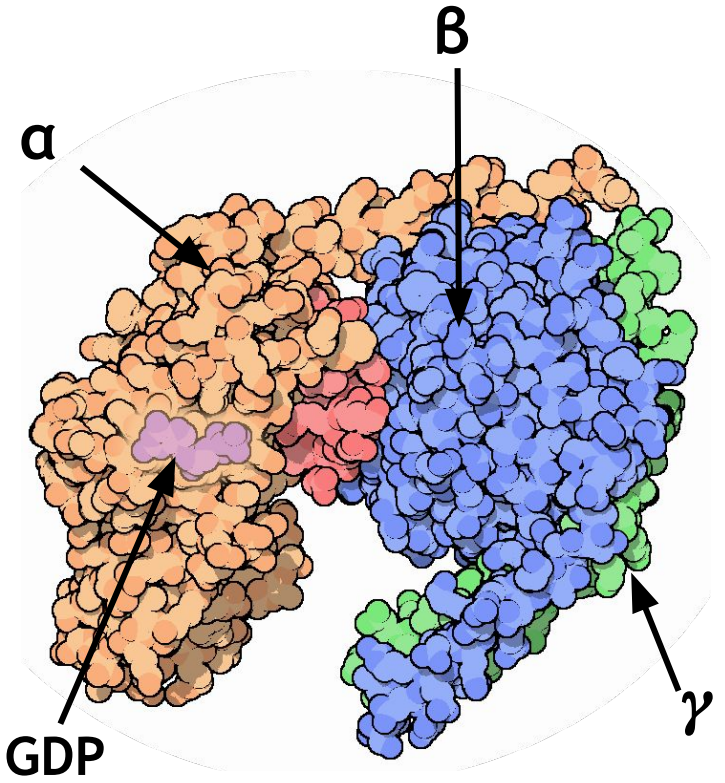
C-terminus
(endodomain)



Heterotrimeric G proteins

Upon activation of the **GPCR**, the α subunit exchanges GDP for GTP, **dissociates** from the ß/γ complex and goes on to regulate its **target.** The **ß/γ complex** can also act as a signaling molecule.

FAMILY	SIGNAL TRANSDUCTION
G_s	Activates adenylate cyclase
G_i	Inhibits adenylate cyclase
$G_{q/11}$	Activates phospholipase C
$G_{12/13}$	Activates Rho family of GTPases
ß/γ	Many proteins: PLA, Ca^{2+} channels, Ref



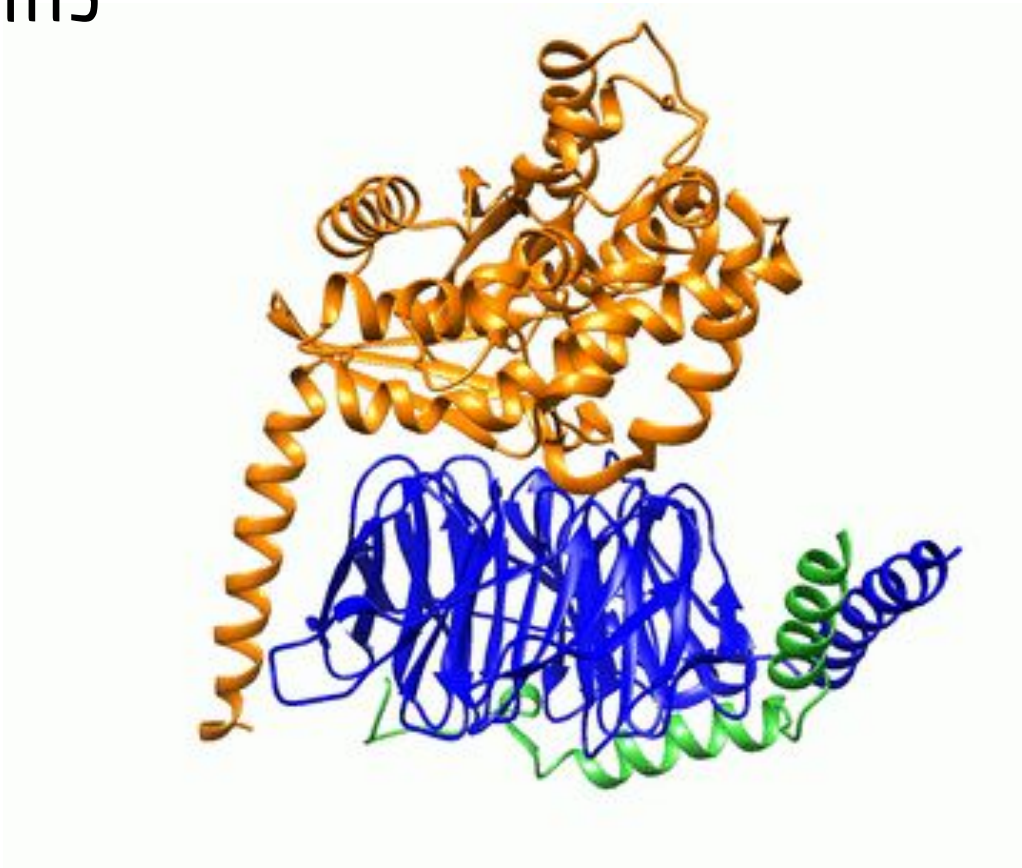
PDB-101: G-protein (Molecule of the Month)

Heterotrimeric G proteins

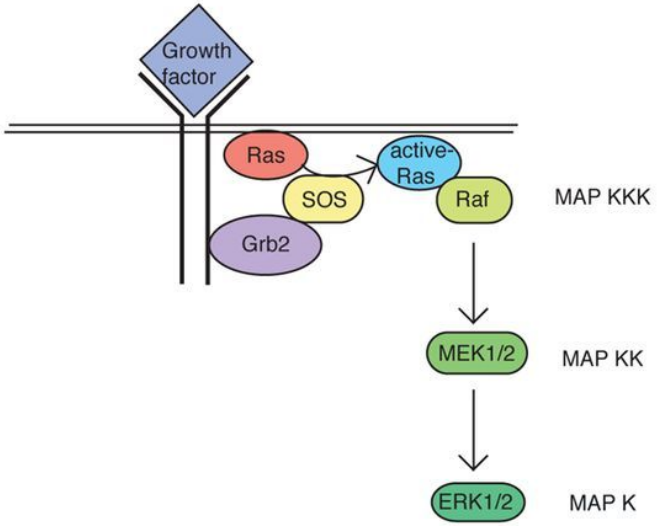
α-subunit

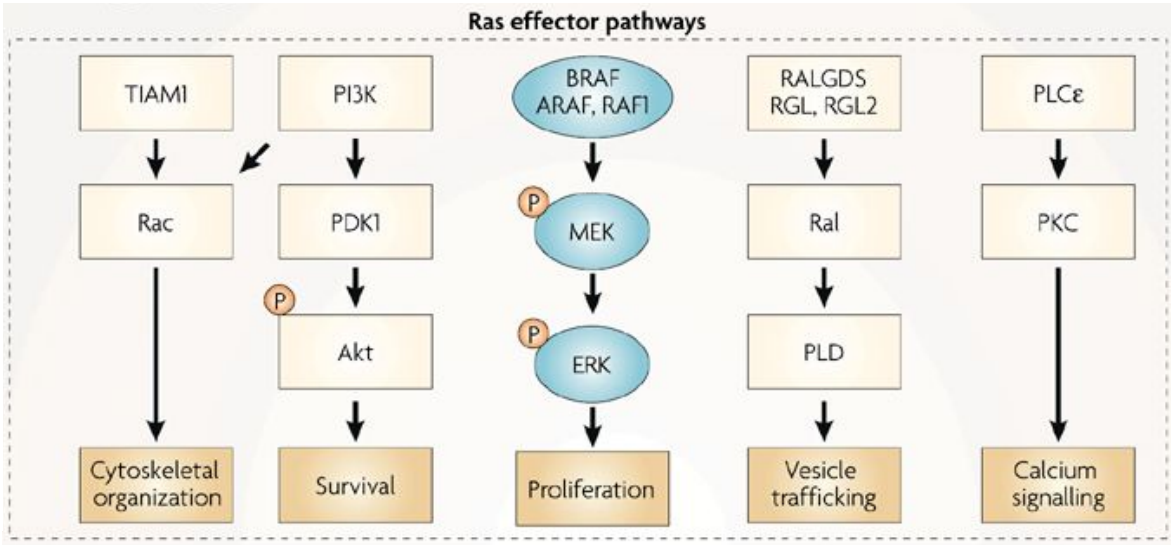
ß-subunit

γ-subunit



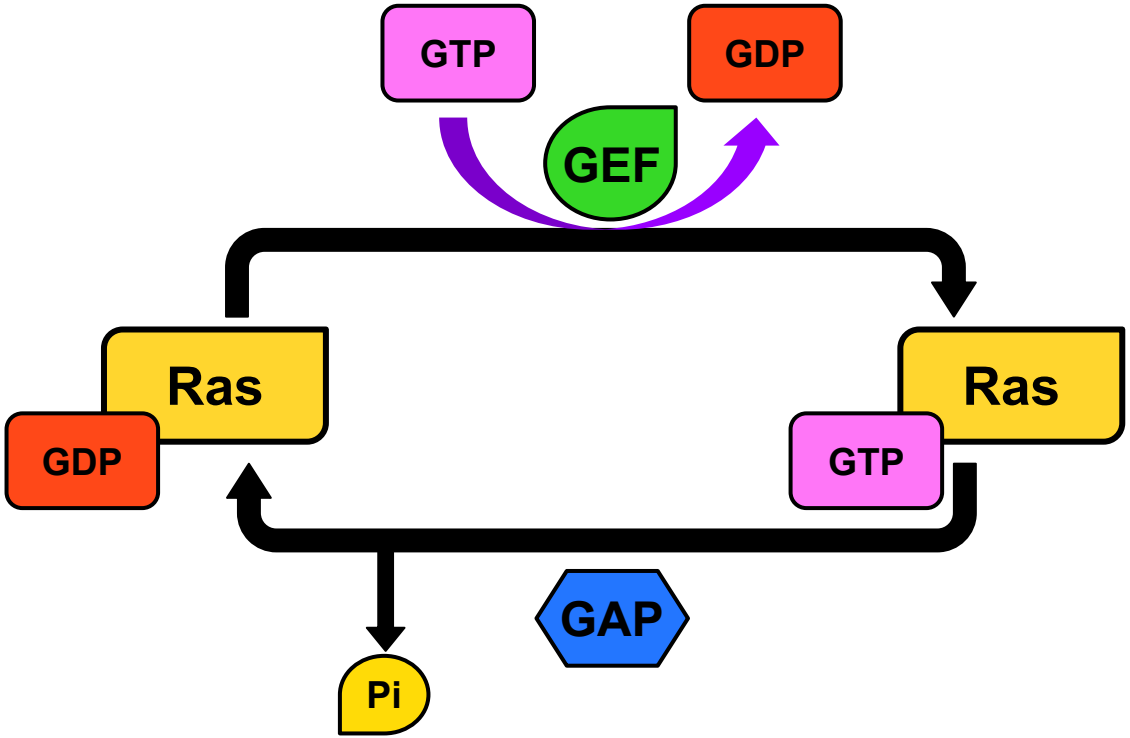
Monomeric G proteins - Ras





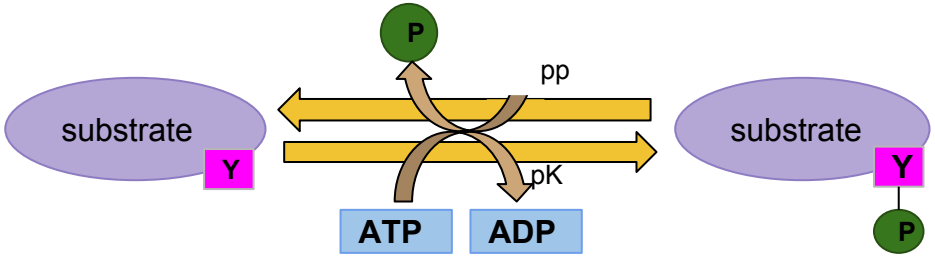
Schubbert S, Shannon K, Bollag GHyperactive Ras in developmental disorders and cancer. Nat Rev Cancer 7:295-308.

Monomeric G proteins - Ras



Tyrosine Kinase Receptors

- Tyrosine kinases catalyse the transfer of a phosphate of group ATP to a hydroxyl group of serine or threonine

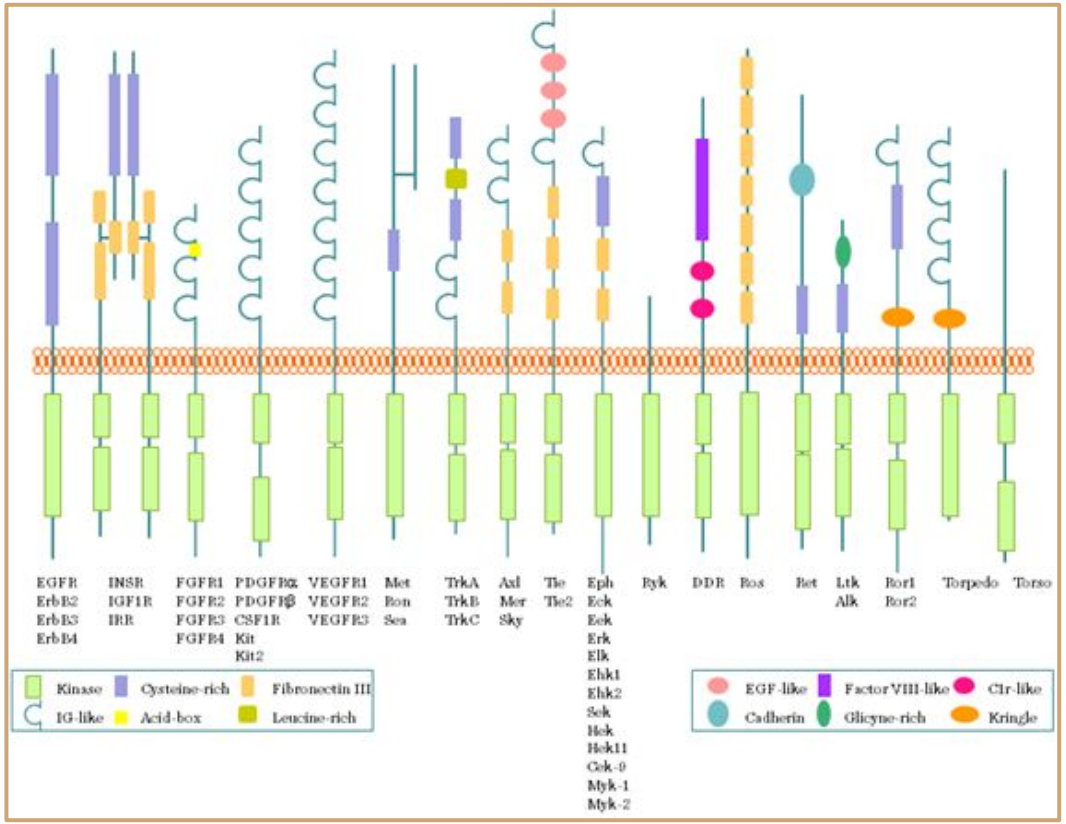


- Characterized by
  - Single transmembrane domain
  - Glycosylated N-terminal extracellular domain with a high number of disulfide bonds.

Class	Family name	Members	Molecular characteristics of the extracellular domains
I	EGFR	EGFR, ERBB2, ERBB3, ERBB4	2 cysteine-rich domains
II	Insulin R	INSR IGFR	2 chains α and β, with one cysteine-rich and 2 FNIII domains
III	PDGFR	PDGFRα, PDGFRβ, M-CSFR, KIT, FLT3L	5 Ig-like domains
IV	VEGFR	VEGFR1, VEGFR2, VEGFR3	7 Ig-like domains
V	FGFR	FGFR1, FGFR2, FGFR3, FGFR4	3 Ig-like domains, 1 acidic box
VI	CCK	CCK4	7 Ig-like domains
VII	NGFR	TRKA, TRKB, TRKC	2 Ig-like domains, rich leucin domains
VIII	HGFR	MET, RON	1 transmenbrane α chain linked with one extracellular β chain
IX	EPHR	EPHA1 to 6, EPHB1 to 6	1 Ig-like, 1 cysteine-rich and 2 FNIII-like domains
X	AXL	AXL, MER, TYRO3	2 Ig-line, 2 FNIII-like domains
XI	TIE	TIE, TEK	2 Ig-like, 1 EGF, and 3 FNIII-like domains
XII	RYK	RYK	1 transmenbrane β chain linked with one extracellular α chain
XIII	DDR	DDR1, DDR2	1 discoidin-like domain
XIV	RET	RET	1 cadherin-like domain
XV	ROS	ROS	6 FNIII-like domains
XVI	LTK	LTK, ALK	1 cysteine-rich domain
XVII	ROR	ROR1, ROR2	1 Ig-domain, 1 cysteine-rich domain and one kringle-like domains
XVIII	MUSK	MUSK	4 Ig-like and 1 cysteine-rich domains
XIX	LMR	AATYK1, AATYK2, AATYK3	A short extracellular domain
XX	Undetermined	RTK106	A short receptor chain with a short extracellular domain

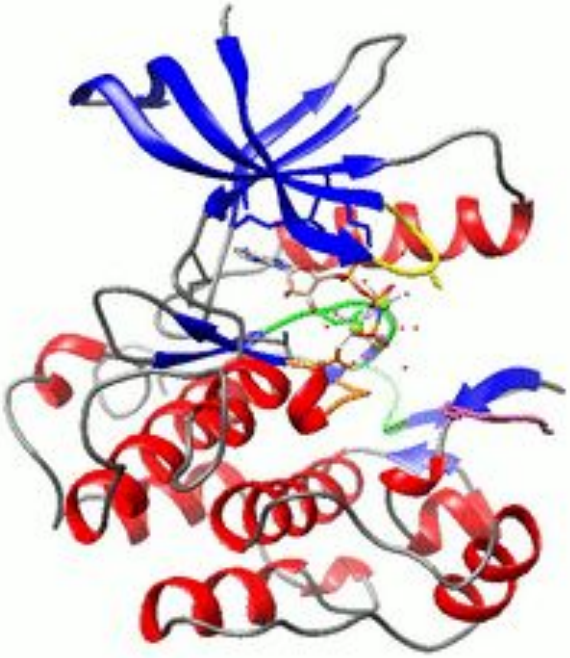
TKR - Extracellular domain

- Its dimerization is essential for ligand recognition
- Its composition defines ligand specificity

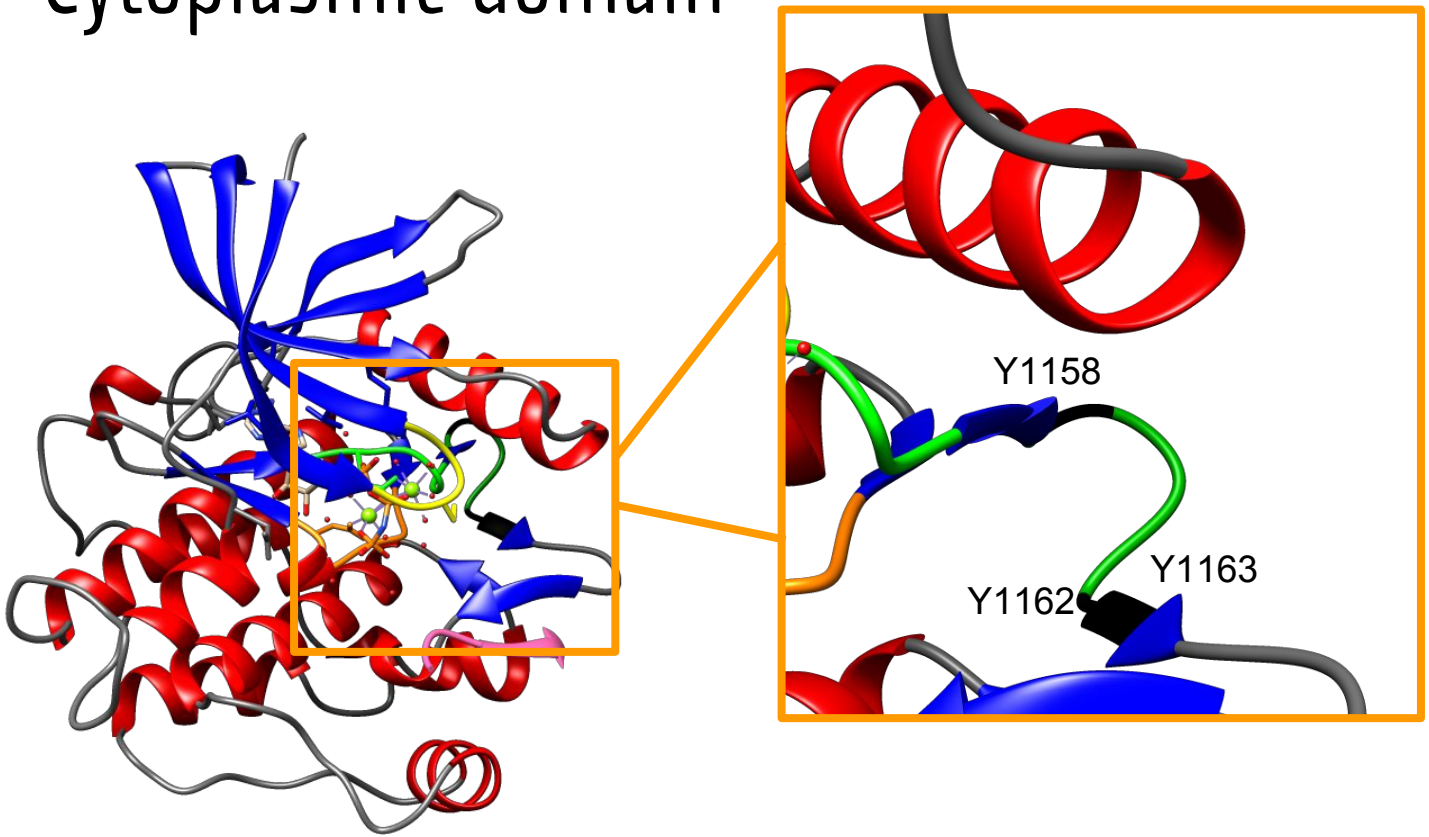


TKR - Cytoplasmic domain

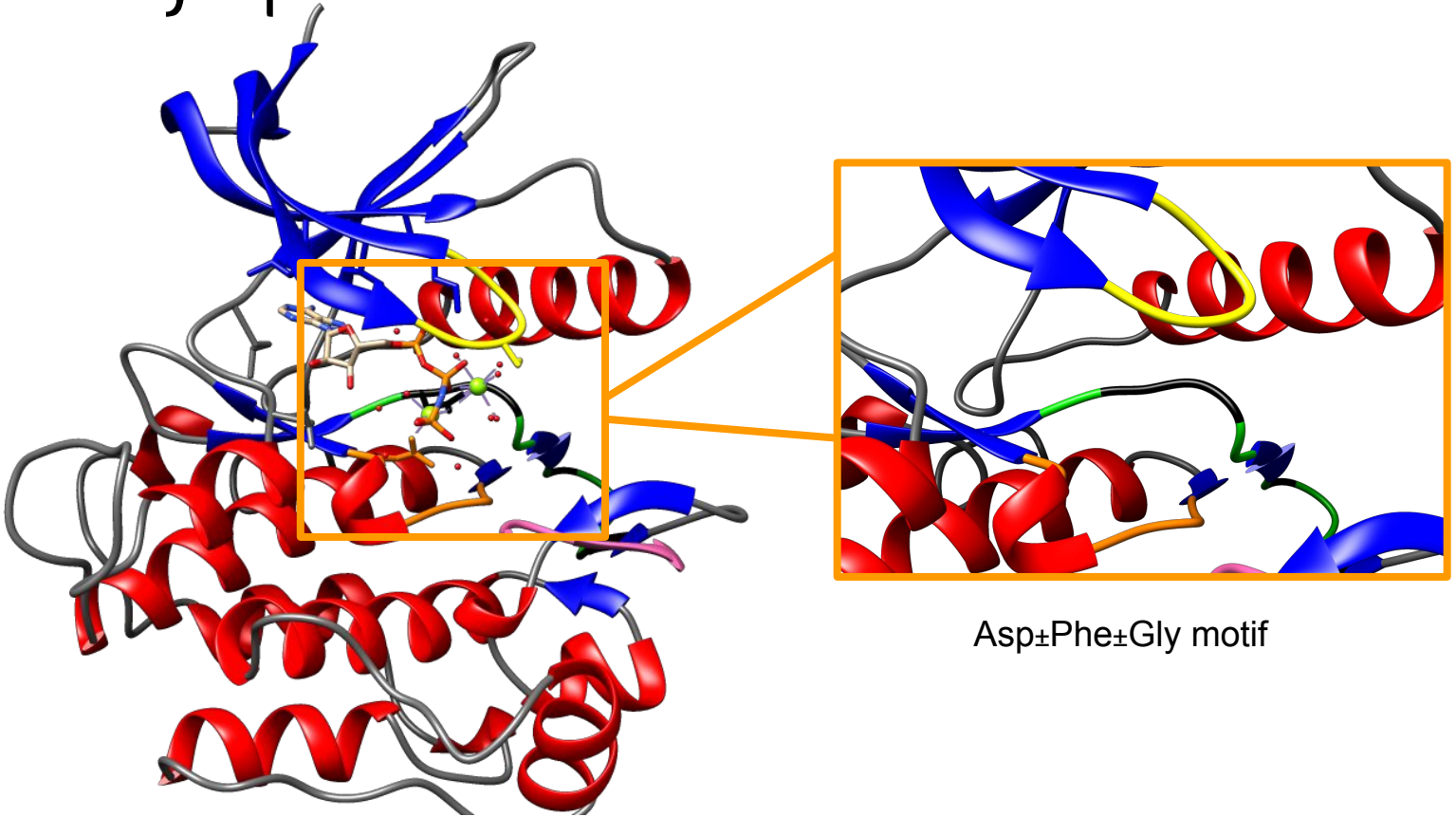
- With tyrosine kinase activity
- Formed by:
  - Amino-terminal lobe :
    5-stranded β-sheet + 1 α-helix
  - Carboxy-terminal lobe: α-helical
- Variable lengths amongst families → specificity of intracellular signals



TKR - Cytoplasmic domain

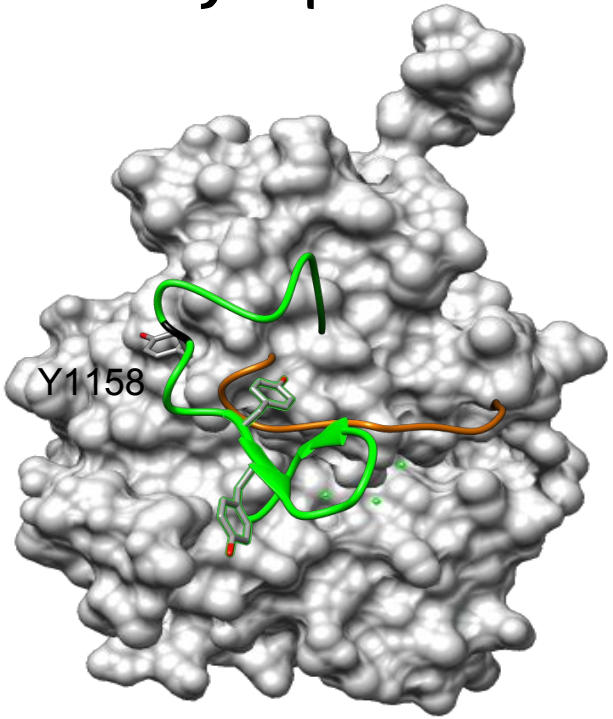


TKR - Cytoplasmic domain

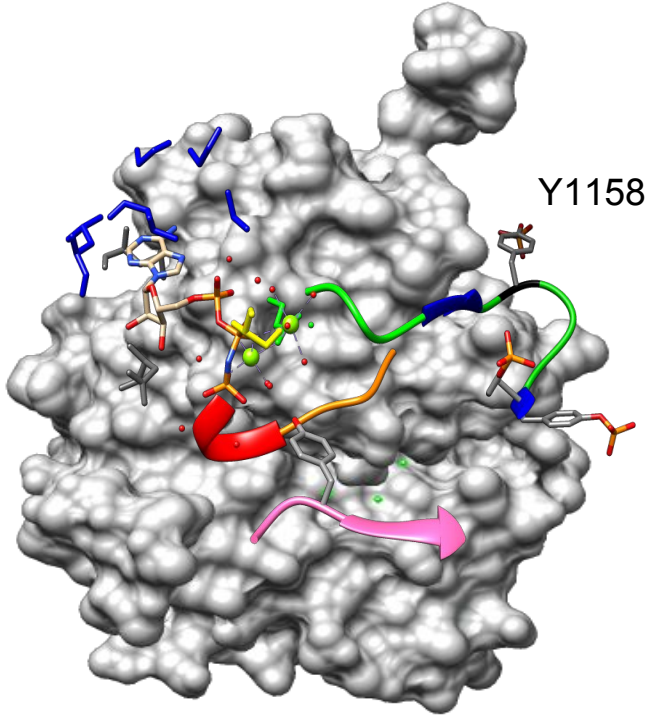


Asp±Phe±Gly motif

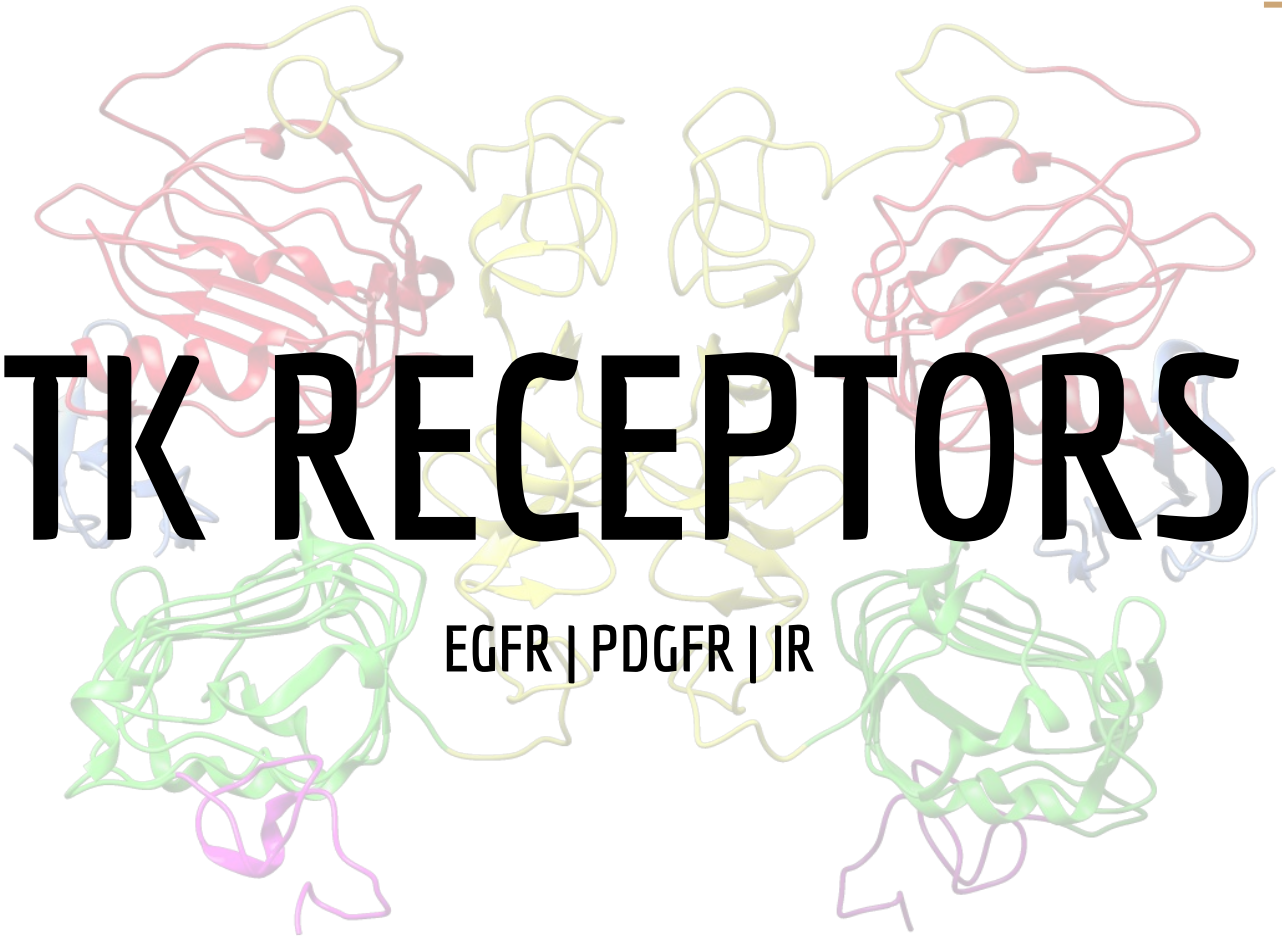
TKR - Cytoplasmic domain



IRK



IRK3P

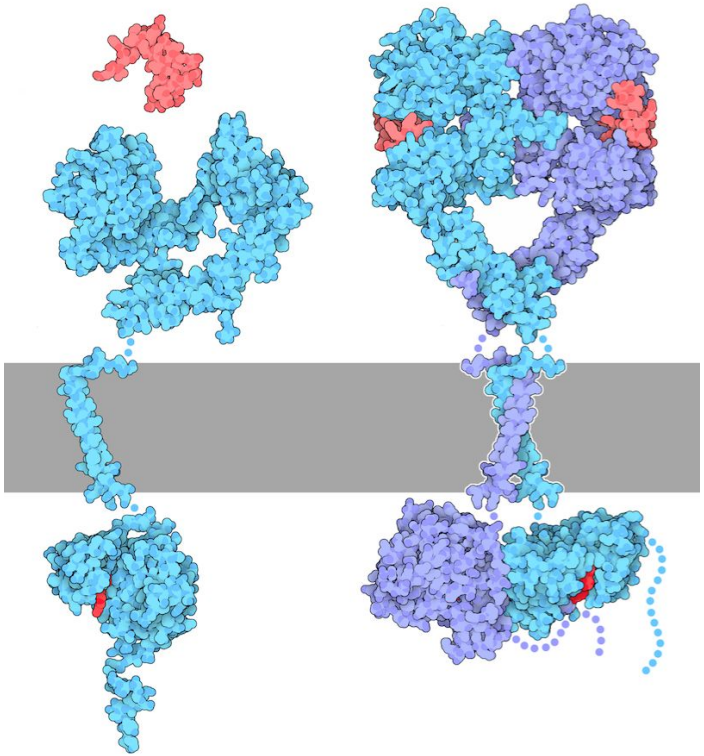


TK RECEPTORS

EGFR | PDGFR | IR

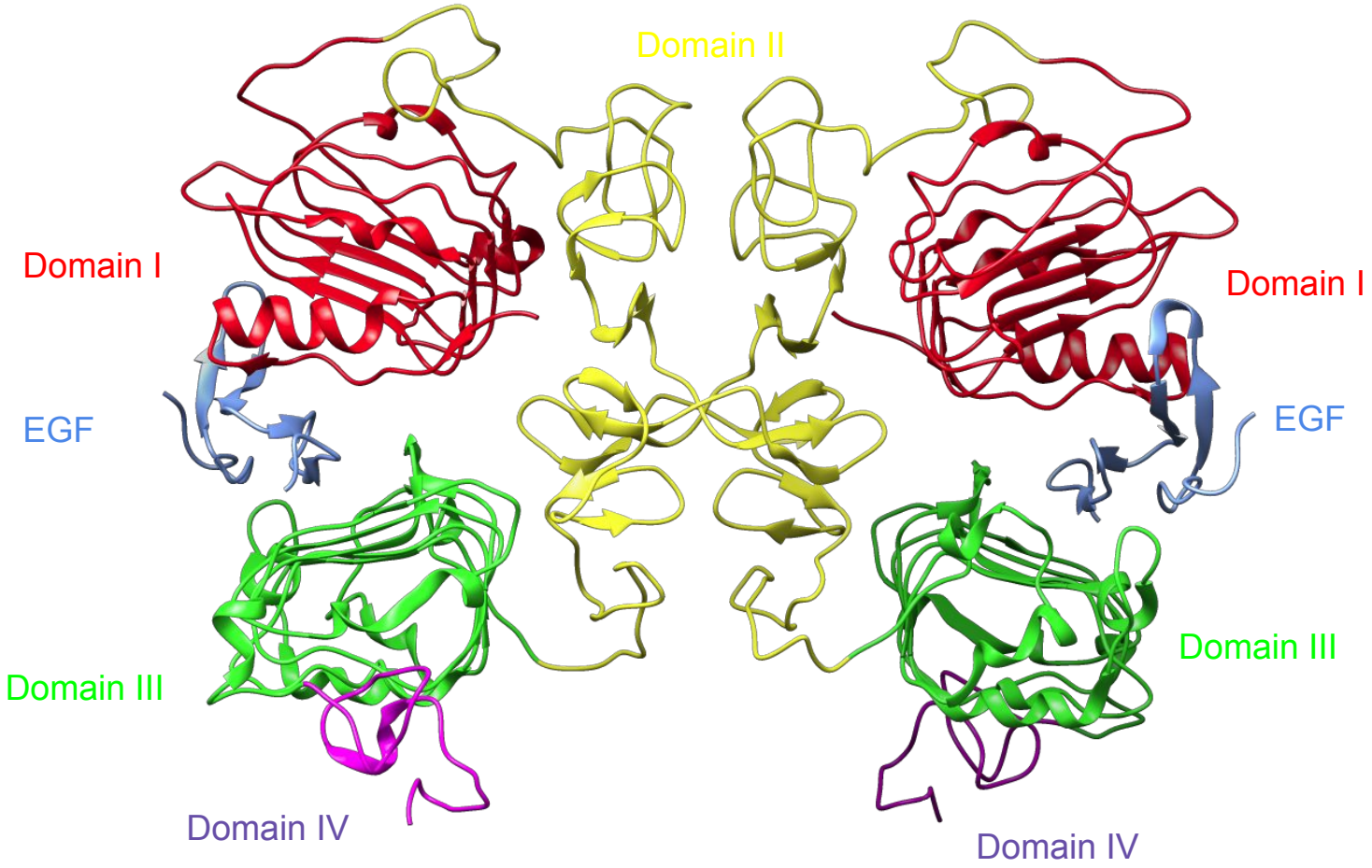
EGFR

- Family: ErbB receptors
- Subfamily:
  - EGFR
  - Her2/neu
  - Her3
  - Her4
- Important in epithelial and cardiac development and nervous system.

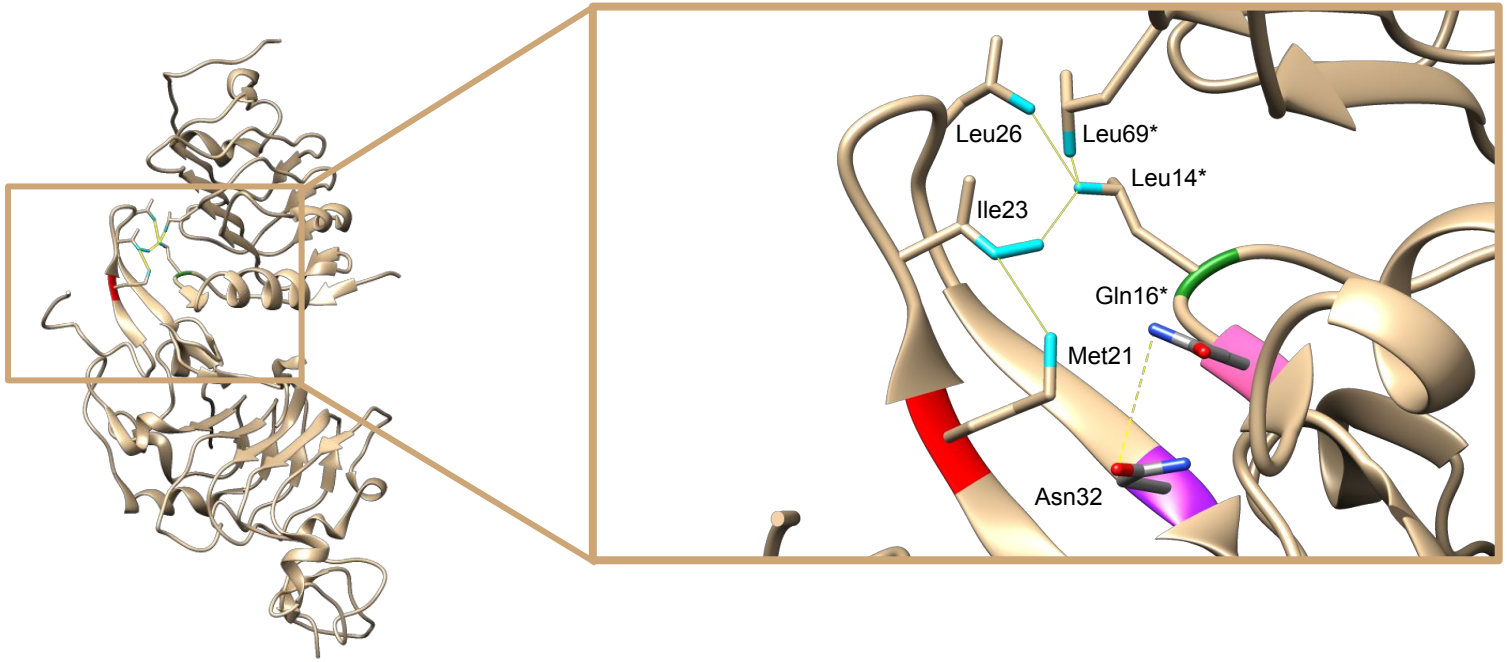


PDB-101: EGFR (Molecule of the Month)

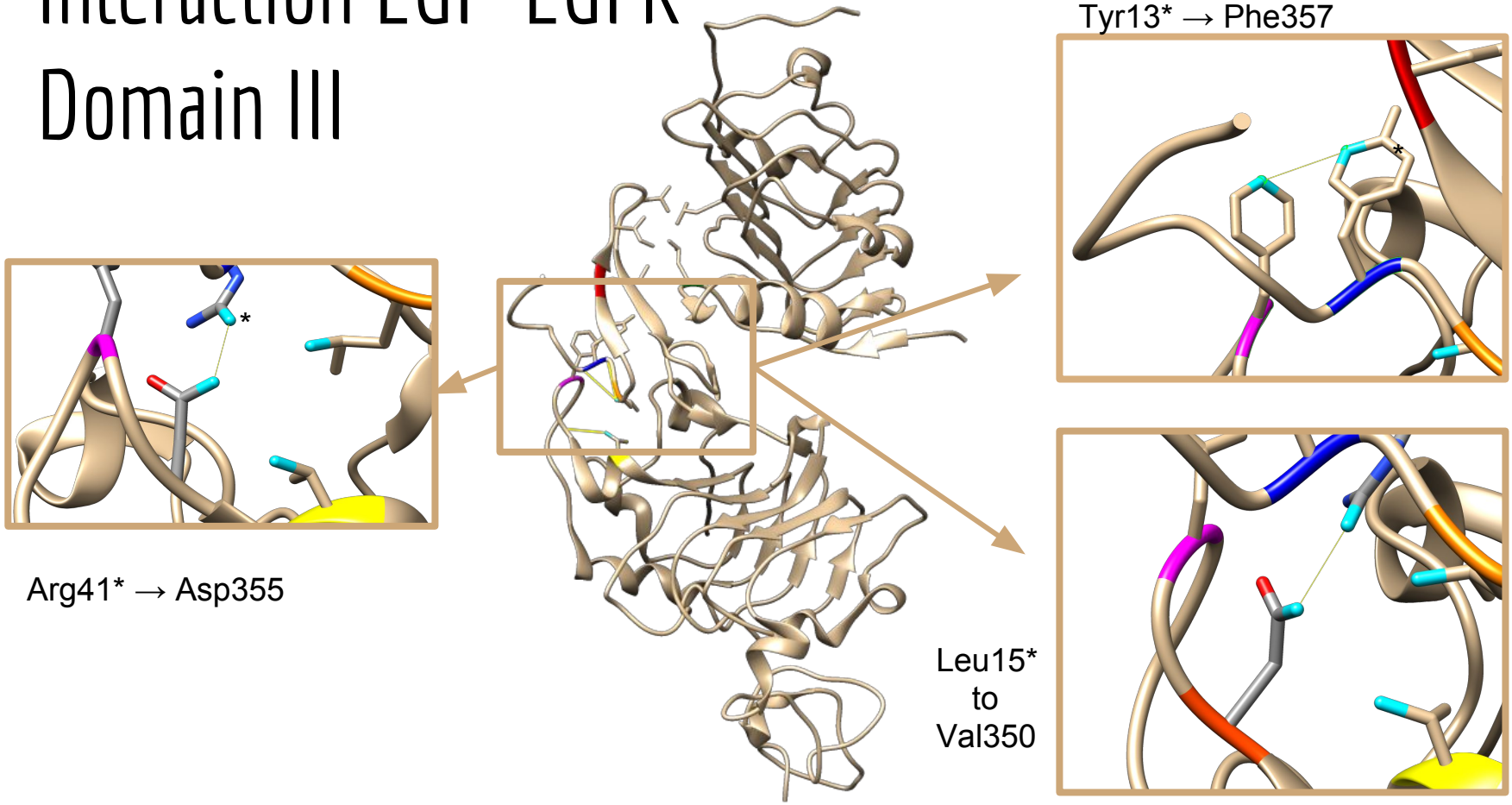
EGFR


Domain II
Domain I
Domain I
EGF
EGF
Domain III
Domain III
Domain IV
Domain IV

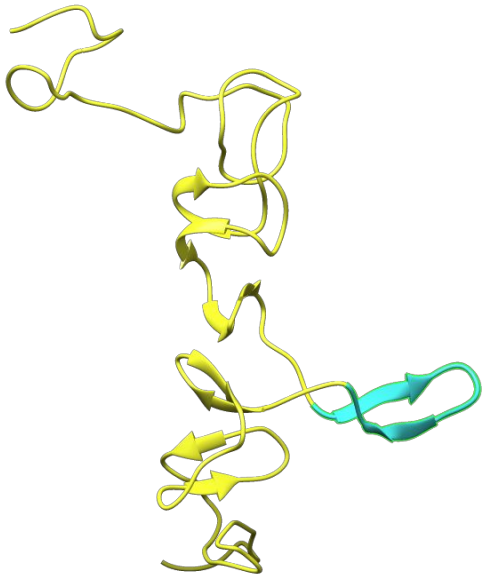
Interaction EGF-EGFR
Domain I



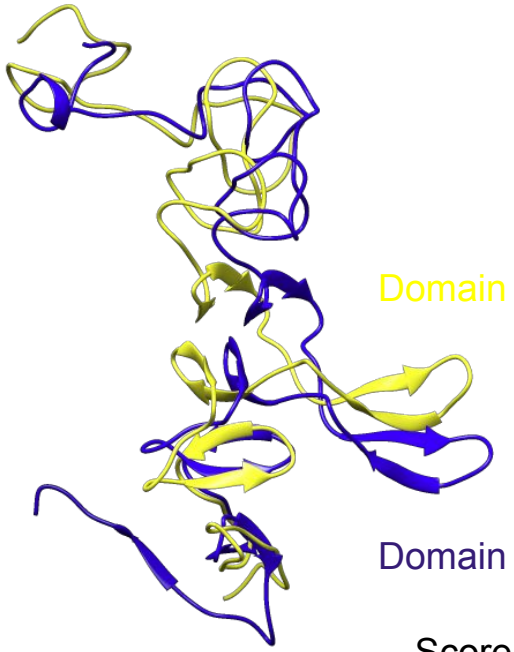
Interaction EGF-EGFR Domain III



Interaction Rc-Rc: β-Hairpin



Domain II extended

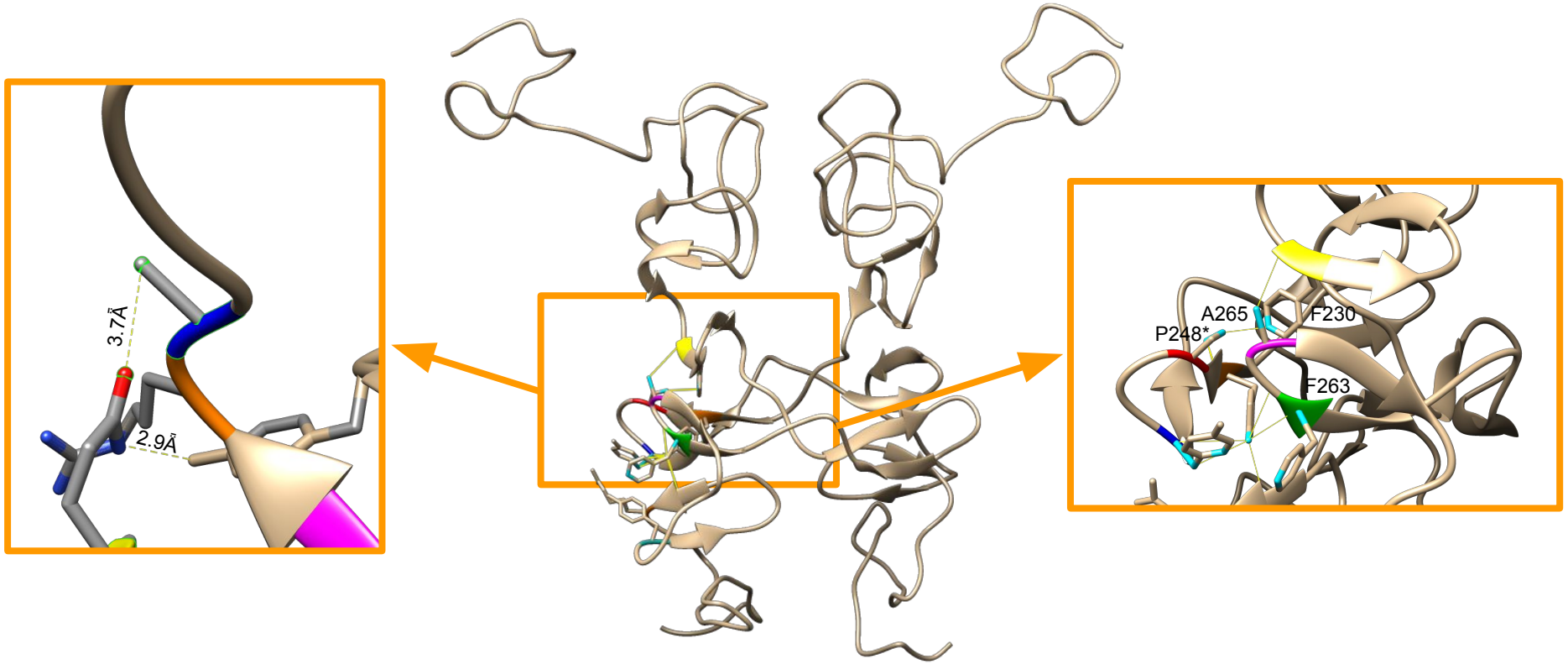


Domain II extended

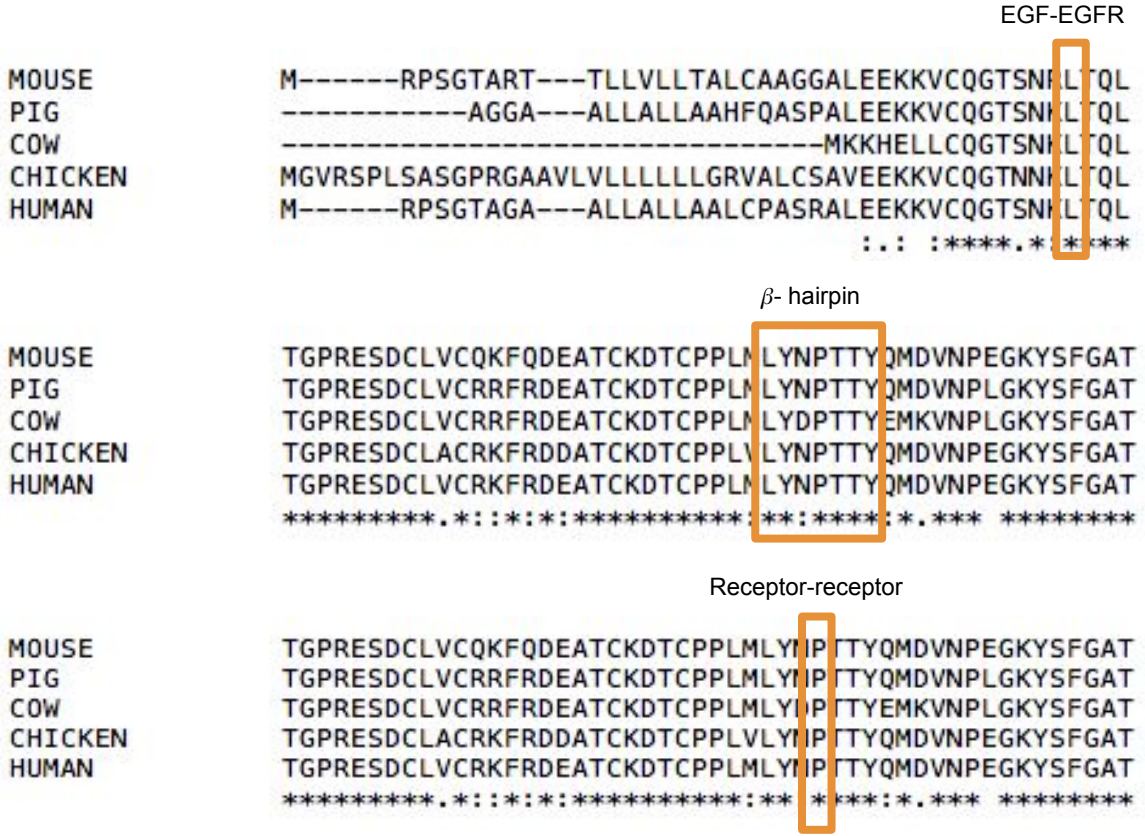
Domain II gathered

Score: 6,12
RMSD: 1,24

Interaction Receptor-Receptor

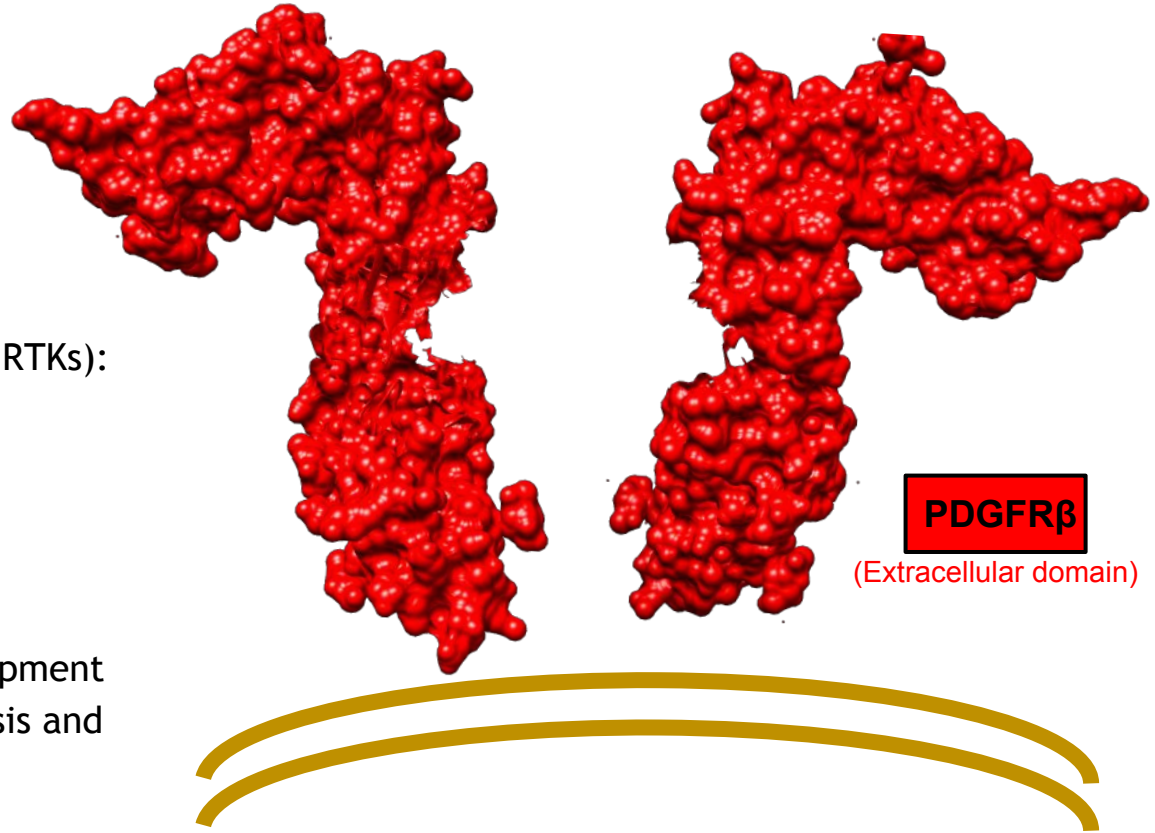


EGFR alignment

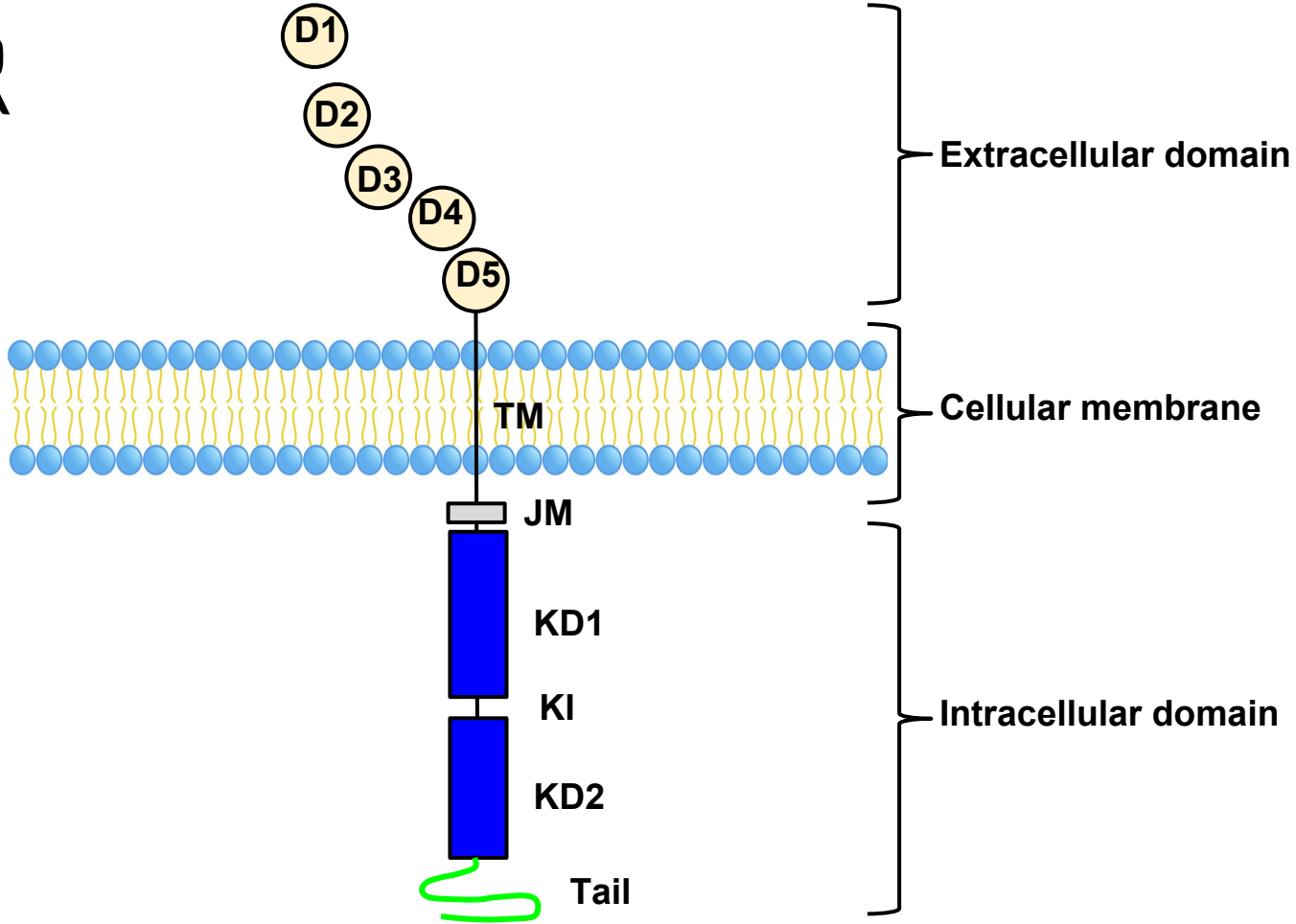


PDGFR

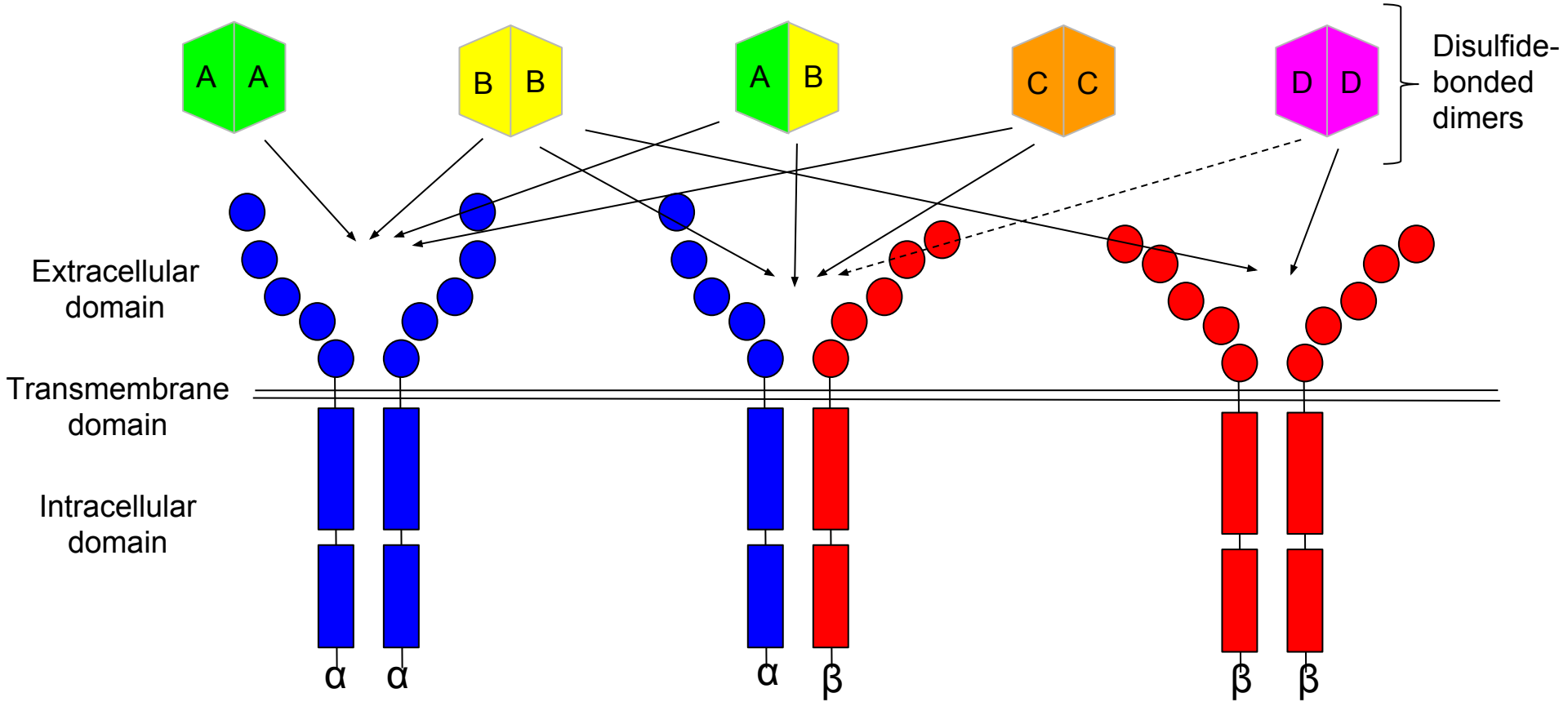
- PDGF receptors
- Class lll receptor tyrosine kinases (RTKs):
  - PDGFRα
  - PDGFRβ
  - KIT
  - FMS
  - FLT3
- Importance in gastrulation, development of many organs, early hematopoiesis and blood vessel formation.



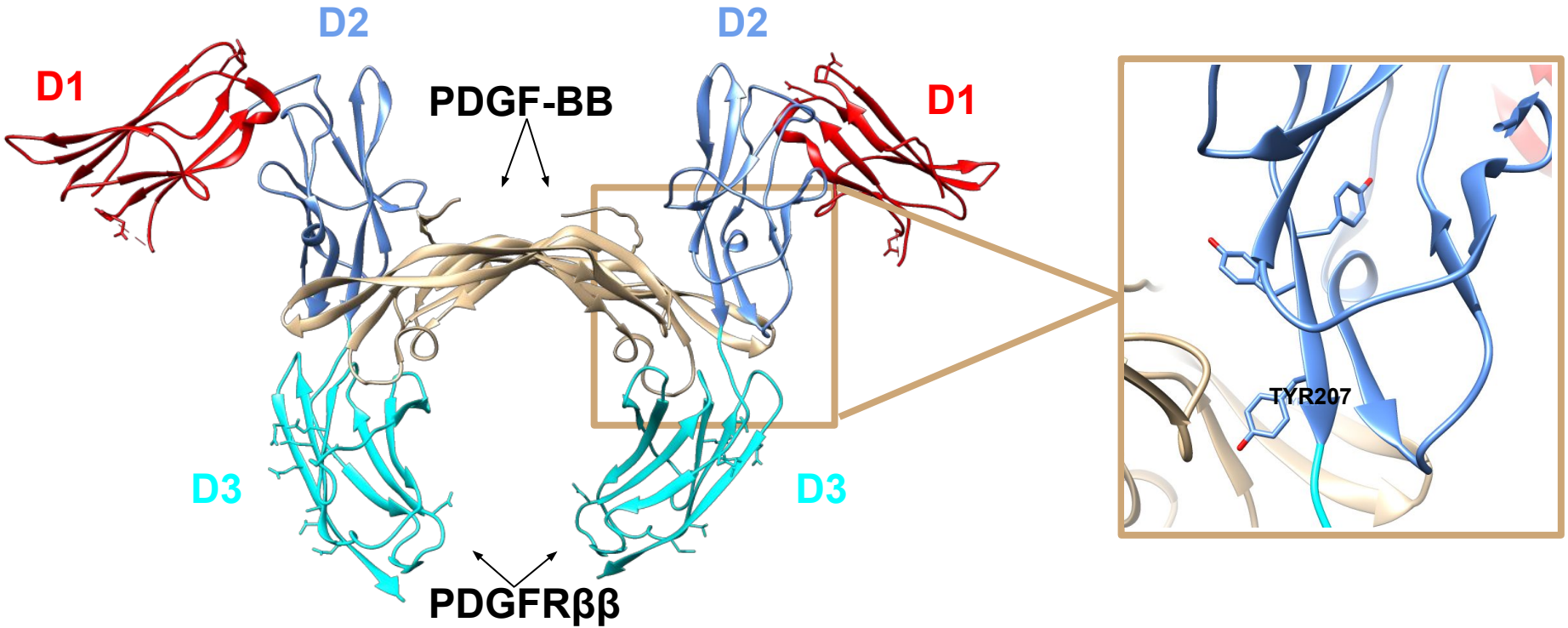
PDGFR


D1
D2
D3
D4
D5
TM
JM
KD1
KI
KD2
Tail
Extracellular domain
Cellular membrane
Intracellular domain

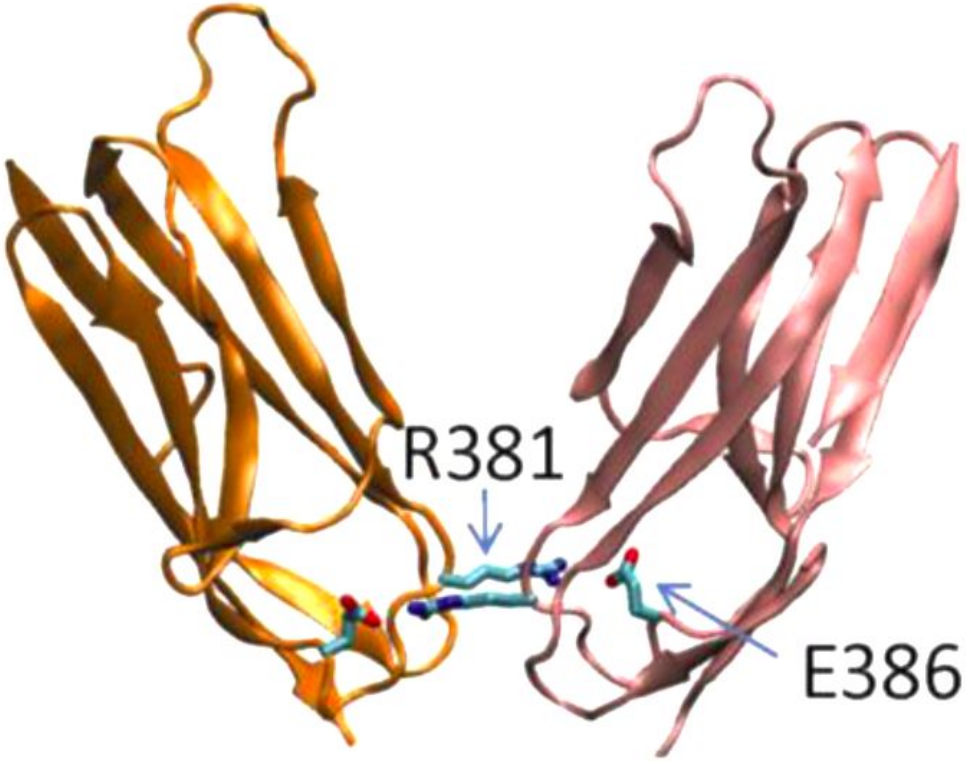
Interaction PDGF-PDGFR



Interaction PDGF-PDGFR



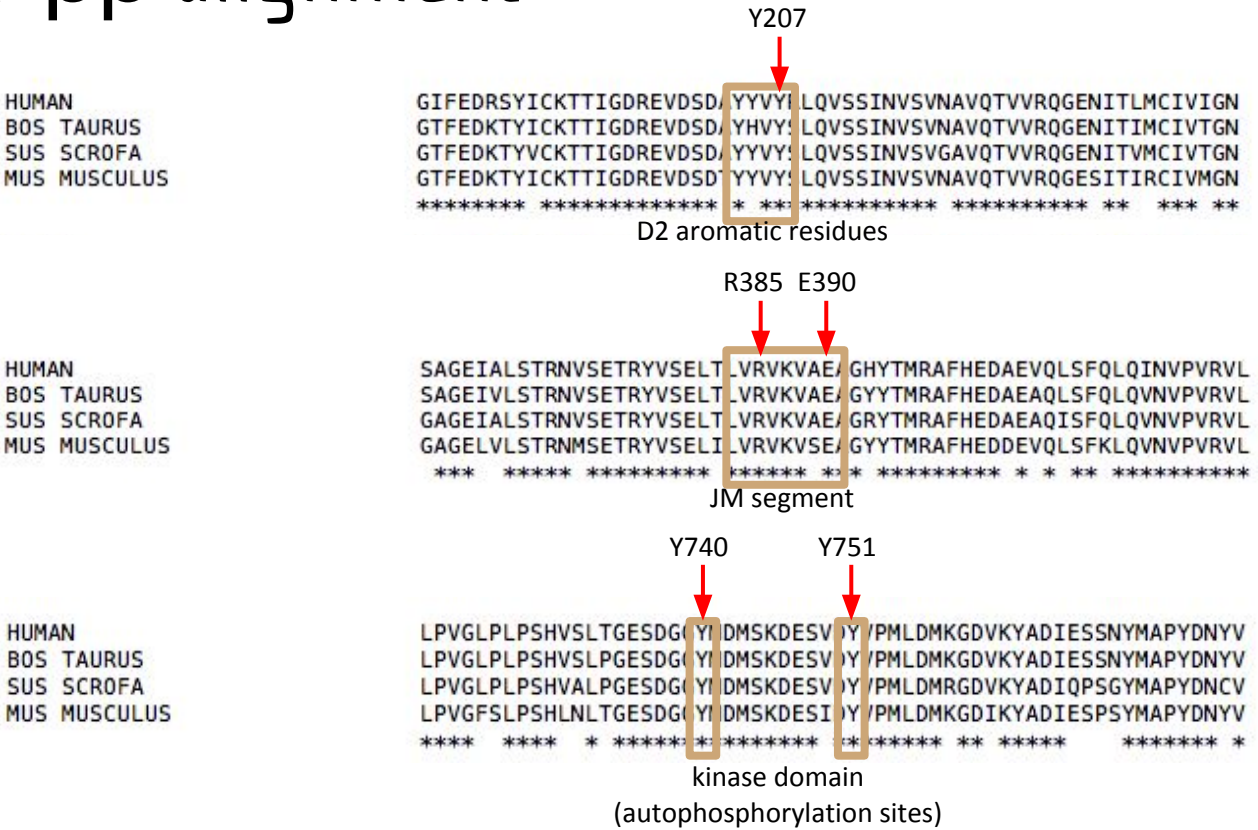
Interaction PDGFR-PDGFR



KIT

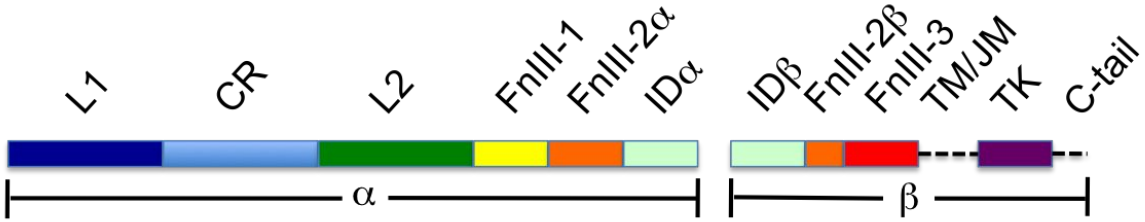
R385
E391

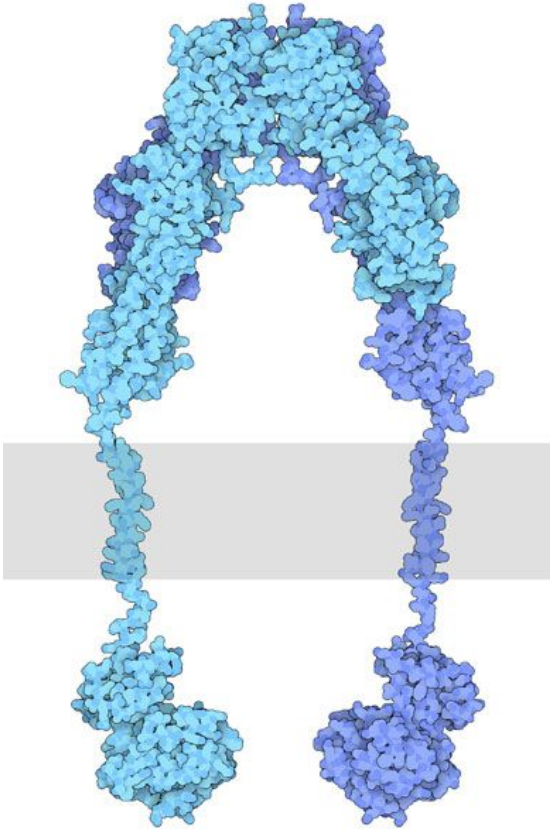
PDGFR-ββ alignment



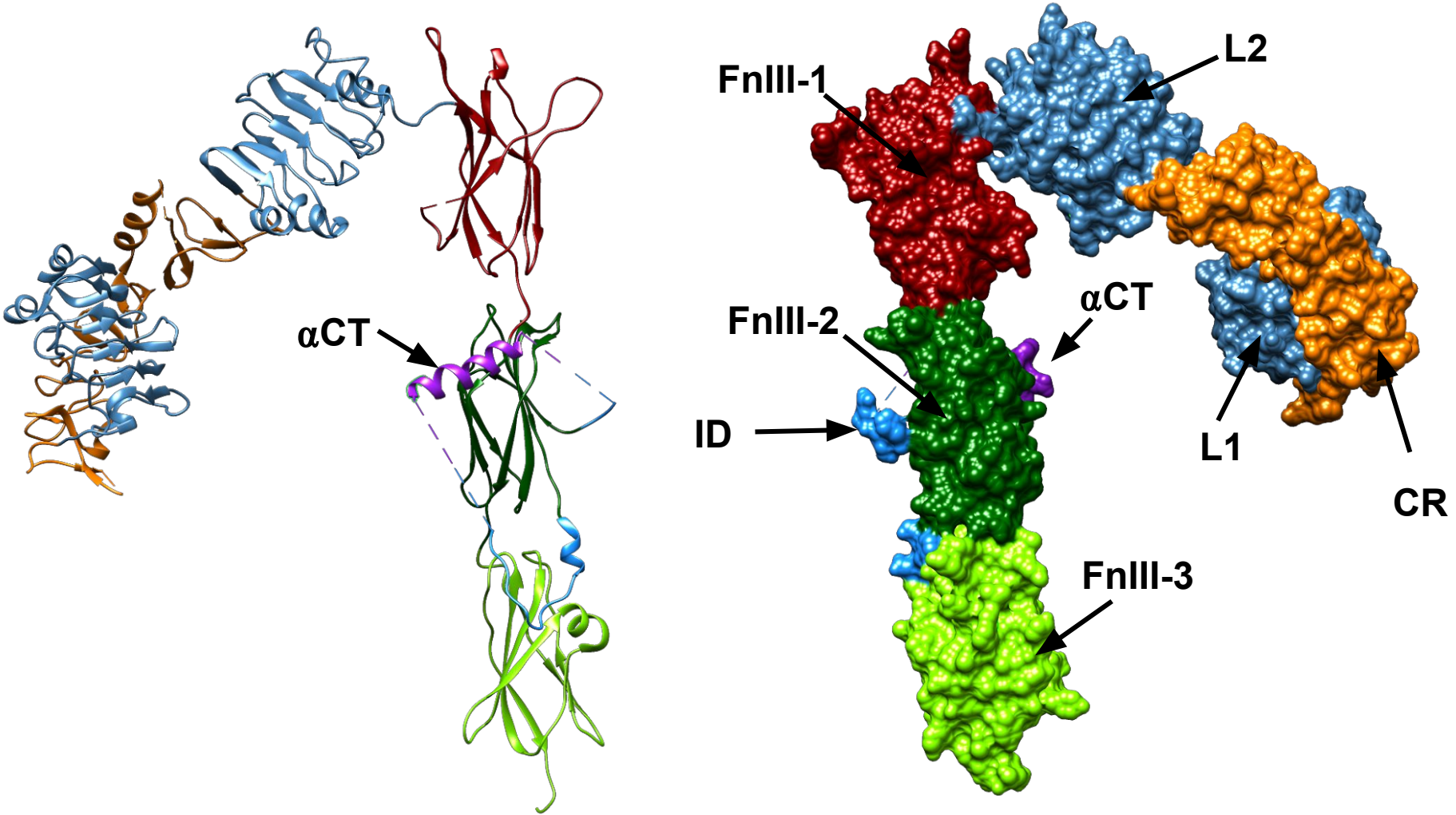
Insulin Receptor

- Activated by insulin, IGF-I and IGF-II.
- Key role in the regulation of glucose homeostasis.
- Dimer in the basal state.
- Activation of Ras through IRS.

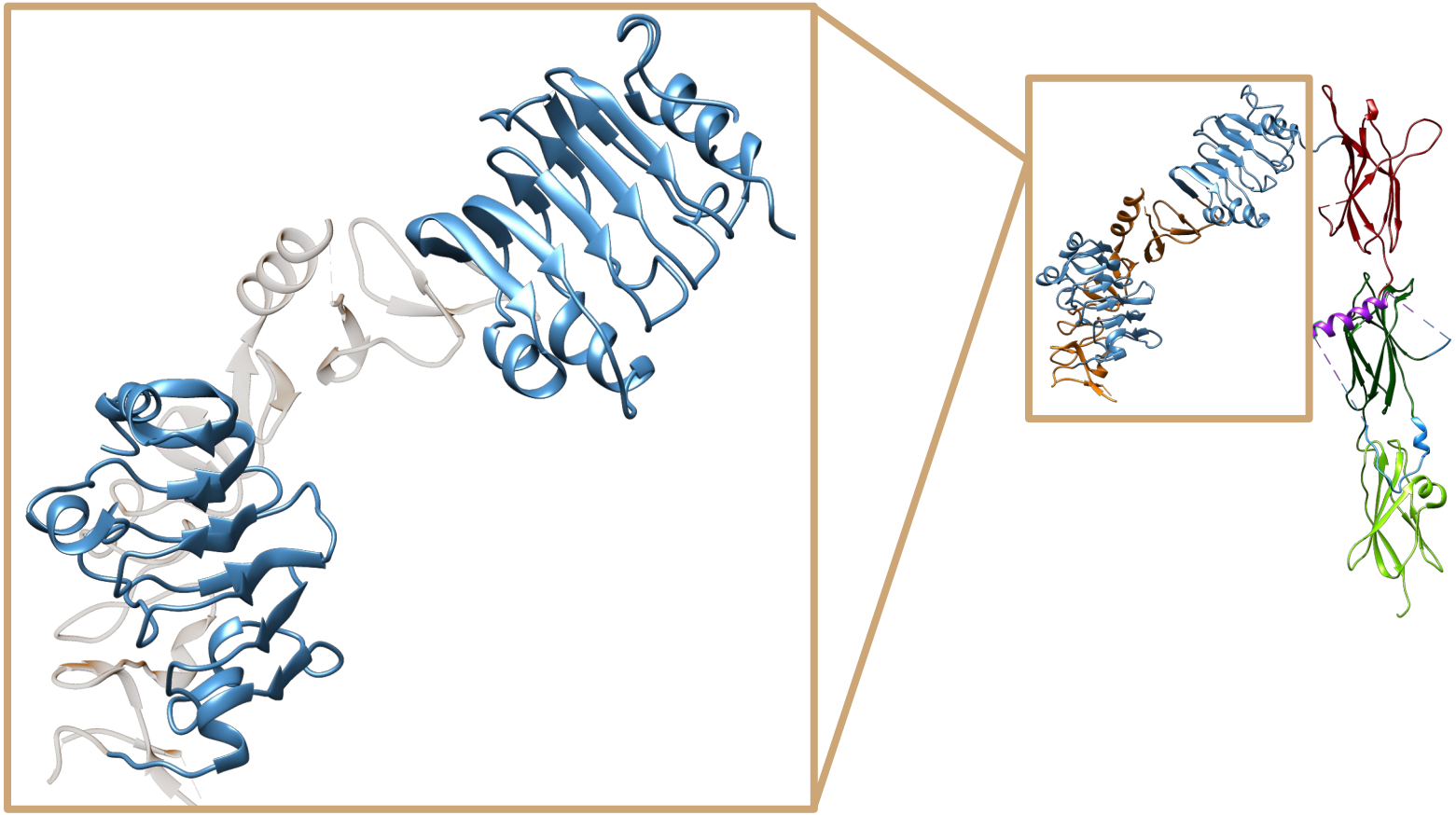




PDB-101: IR (Molecule of the Month)

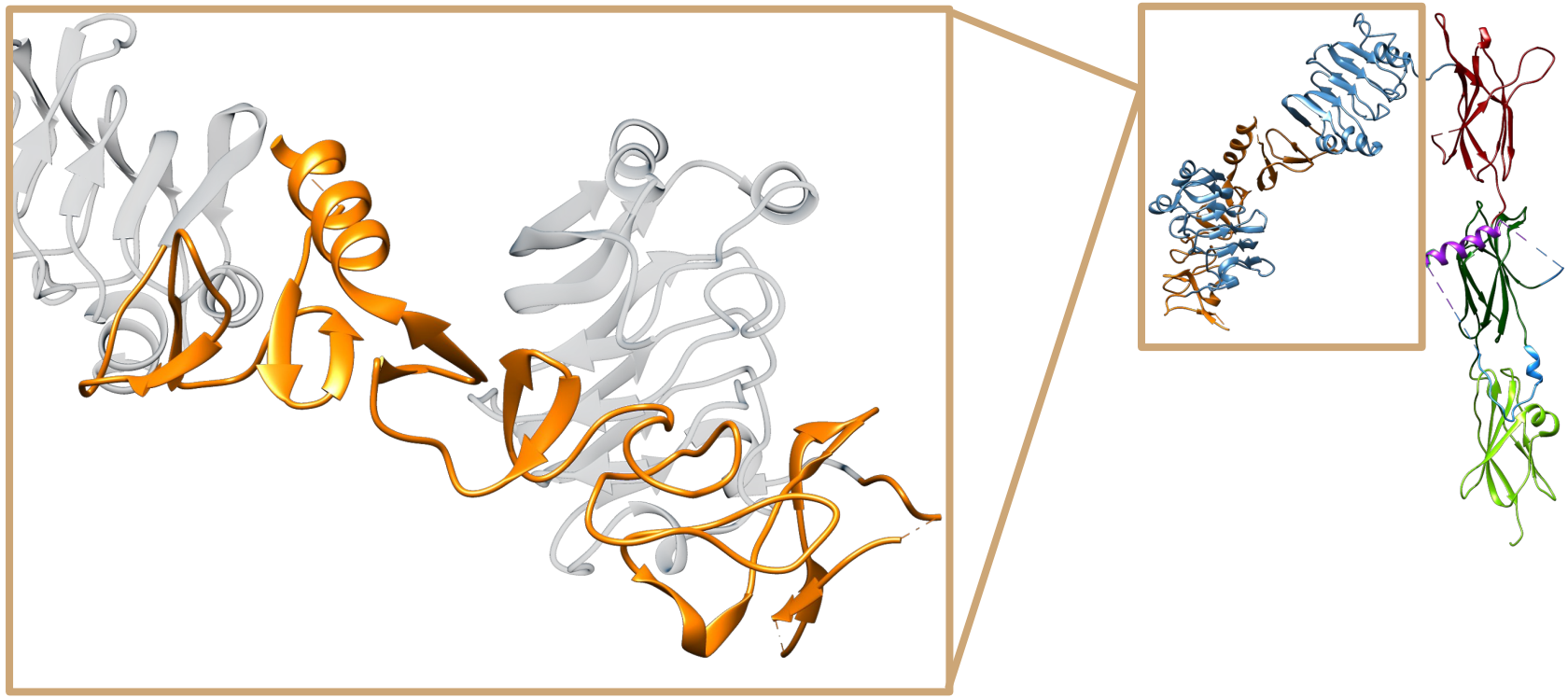

αCT
FnIII-1
L2
FnIII-2
αCT
ID
L1
CR
FnIII-3

IR



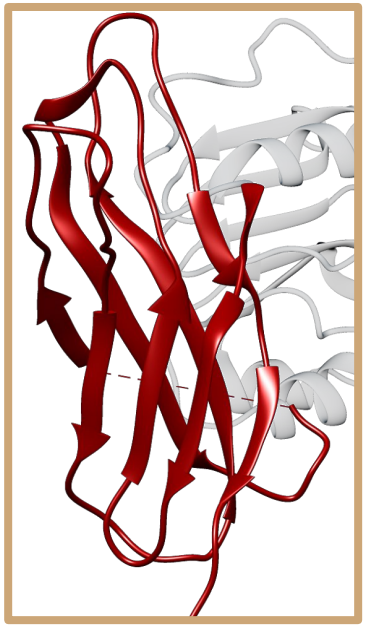
Leucine-rich domains

IR



Cysteine-rich domain

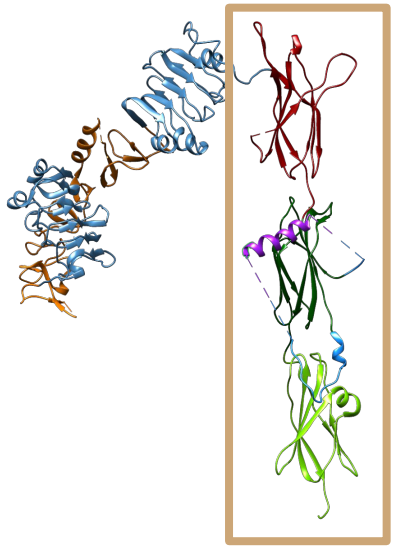
IR

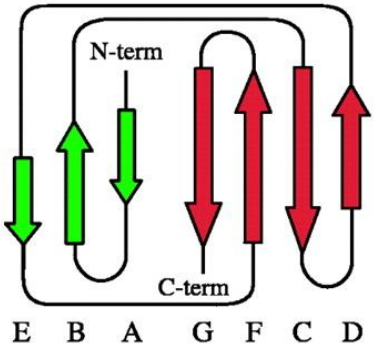


FnIII-1

FnIII-2

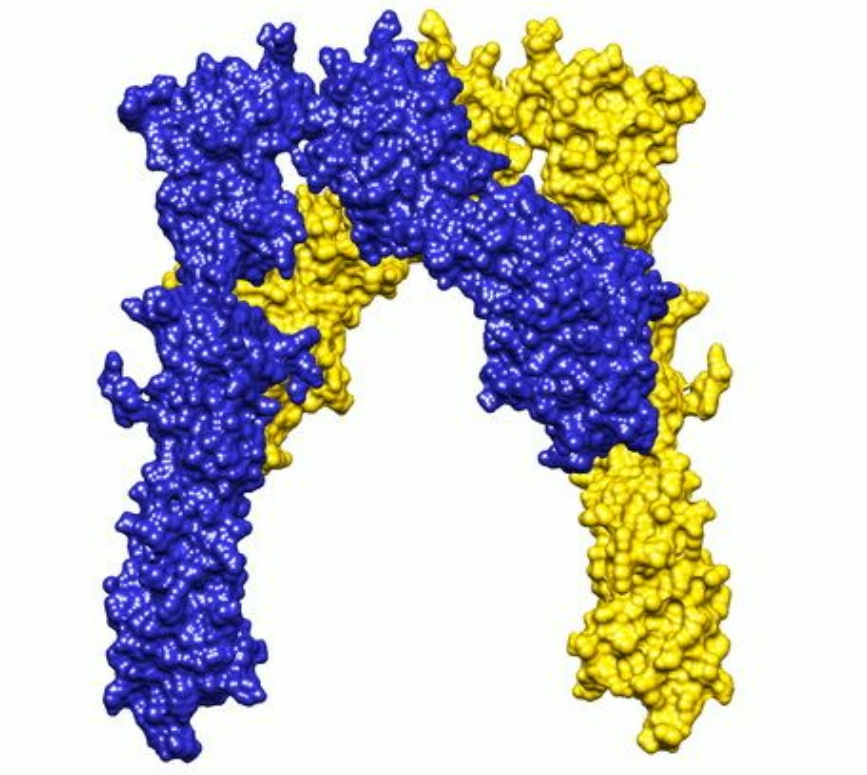
FnIII-3



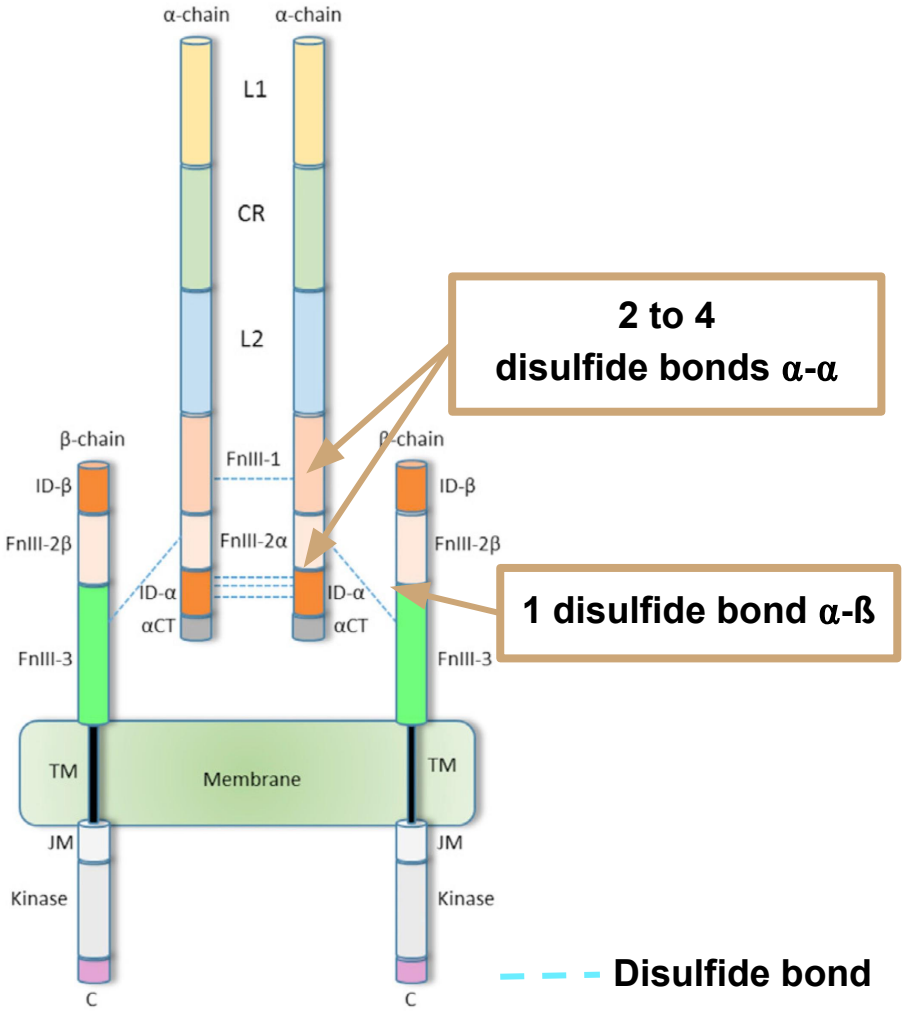


(ß-sandwich, 4-on-3)

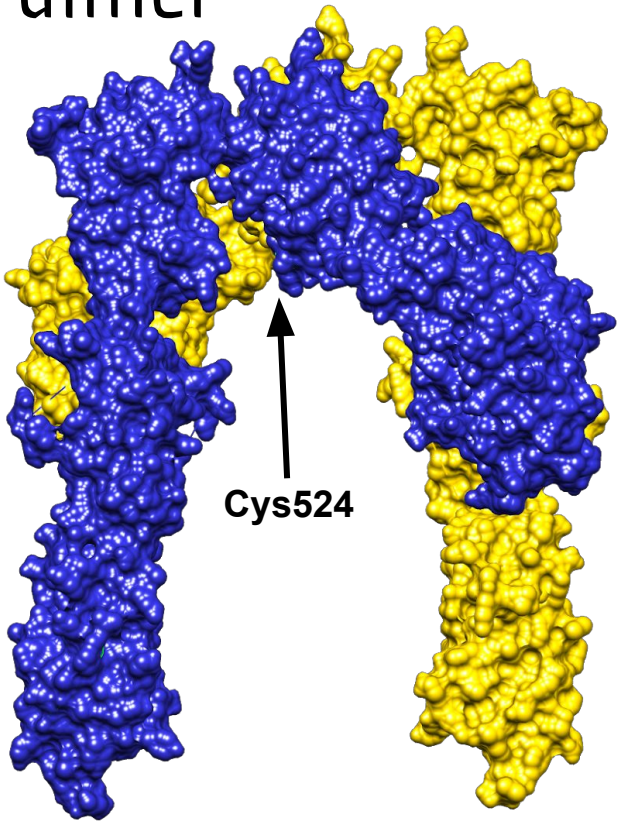
IR dimer

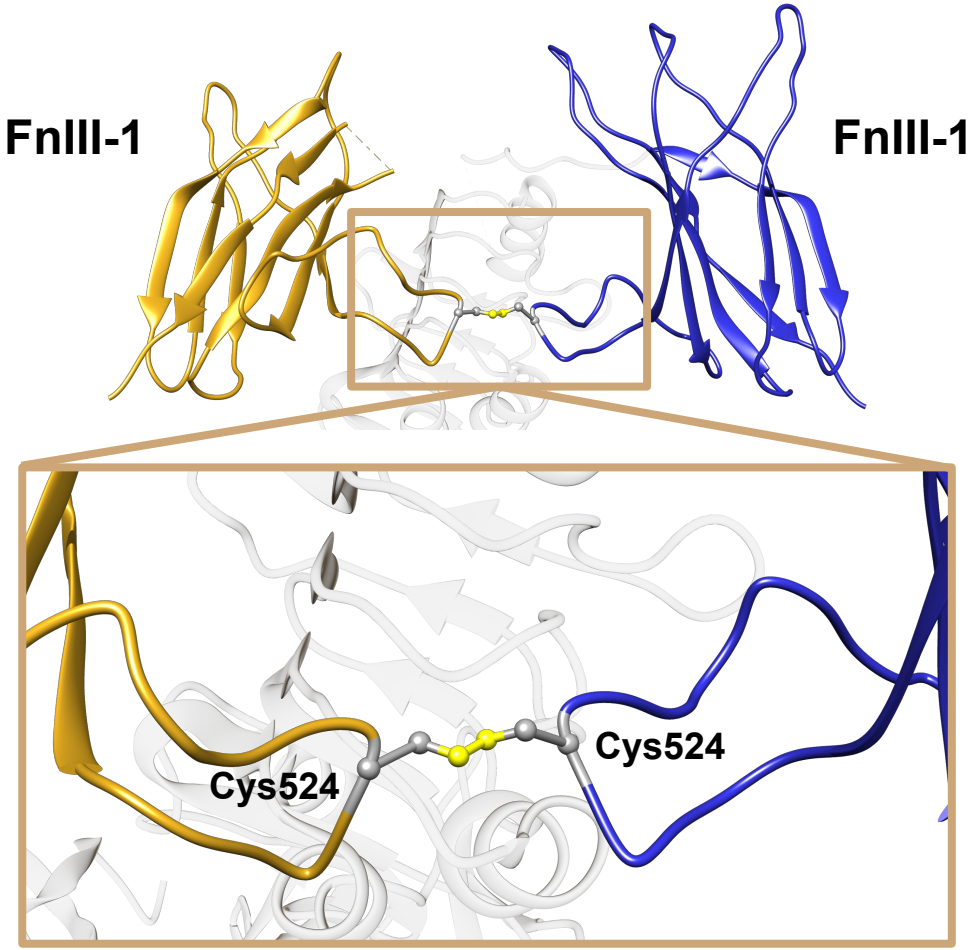


Interaction αCT-L1



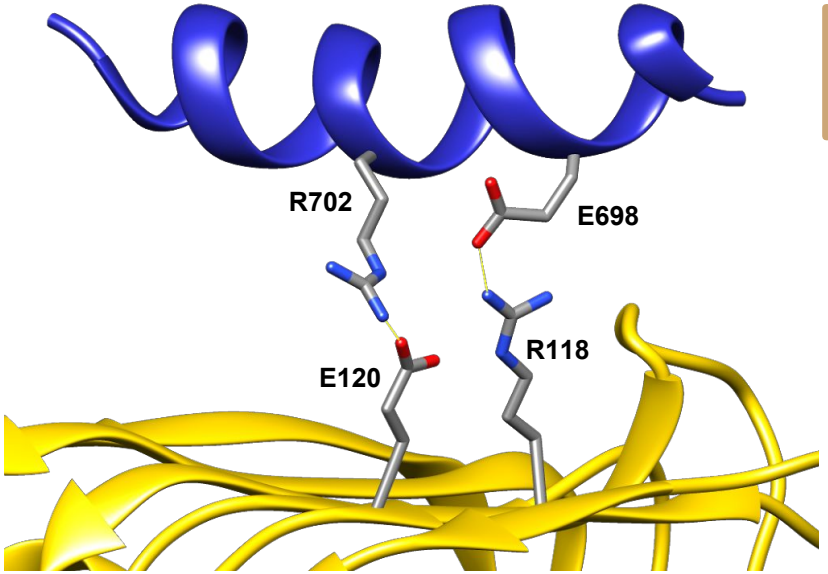
IR dimer





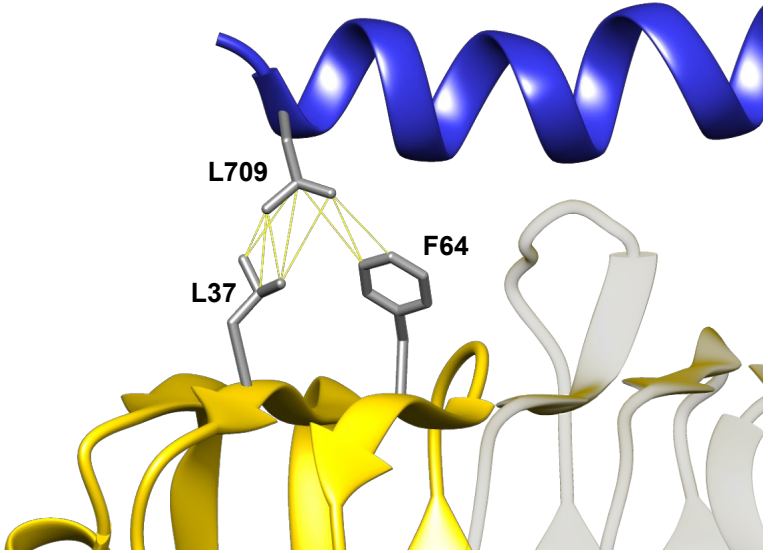
Triplet C682, C683, C685 - not represented in the PDB

IR dimer



R_1: αCT

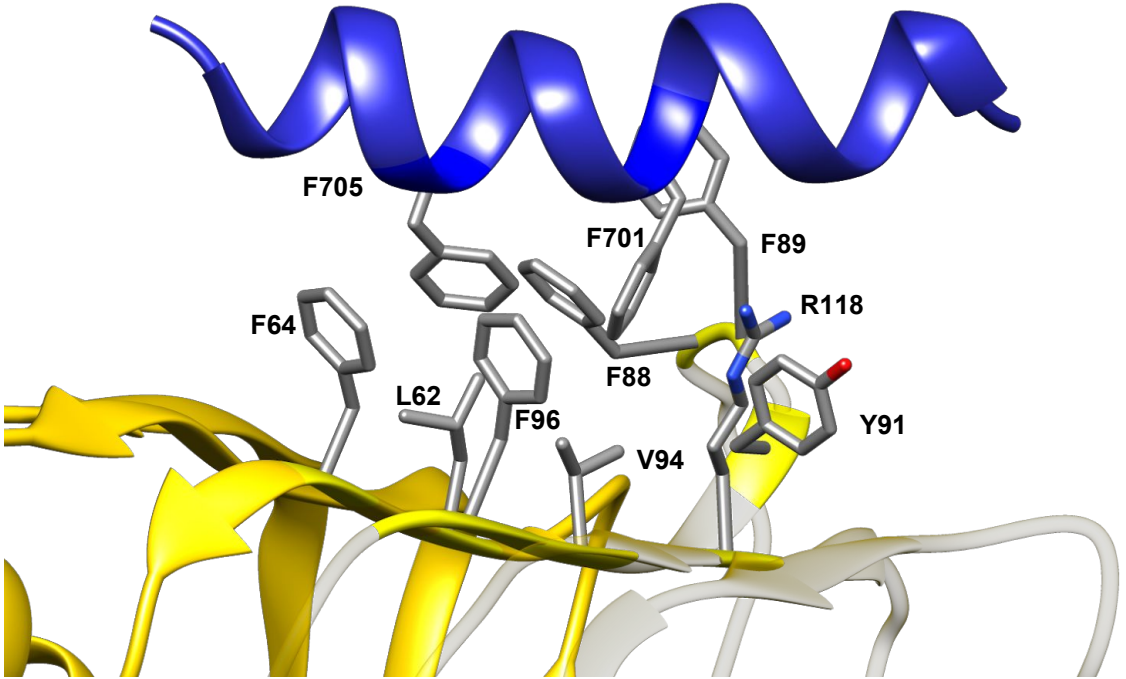
R_2: L1

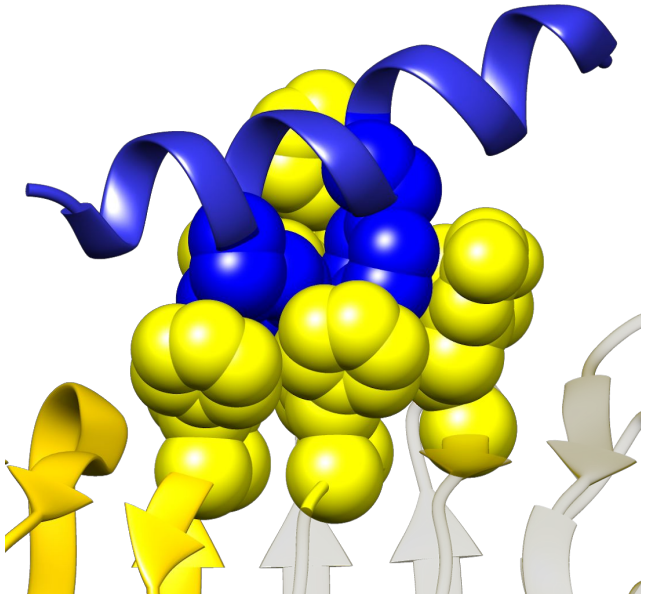


Electrostatic interactions
(charge-compensating cluster)

Hydrophobic interactions

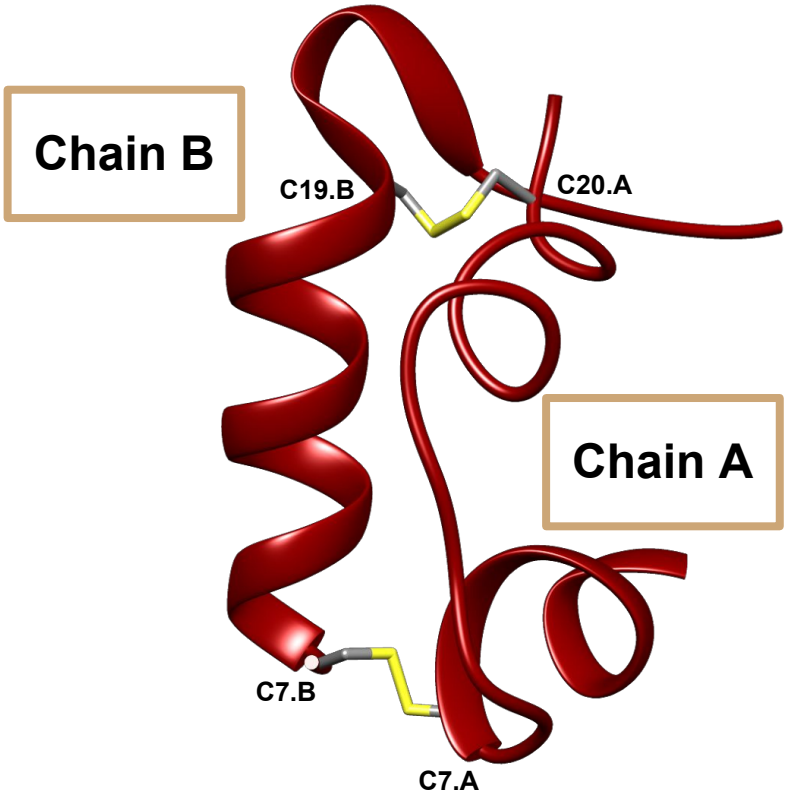
IR dimer

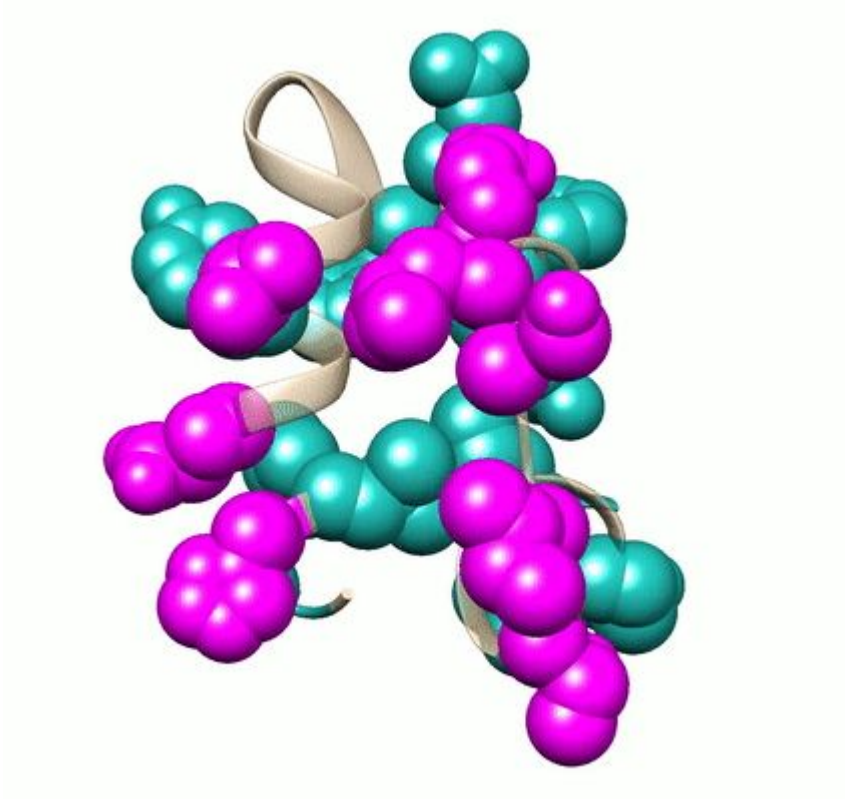




Hydrophobic pocket

Insulin binding



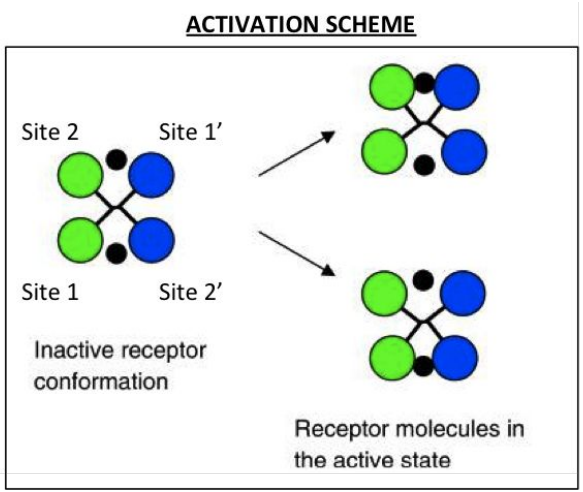


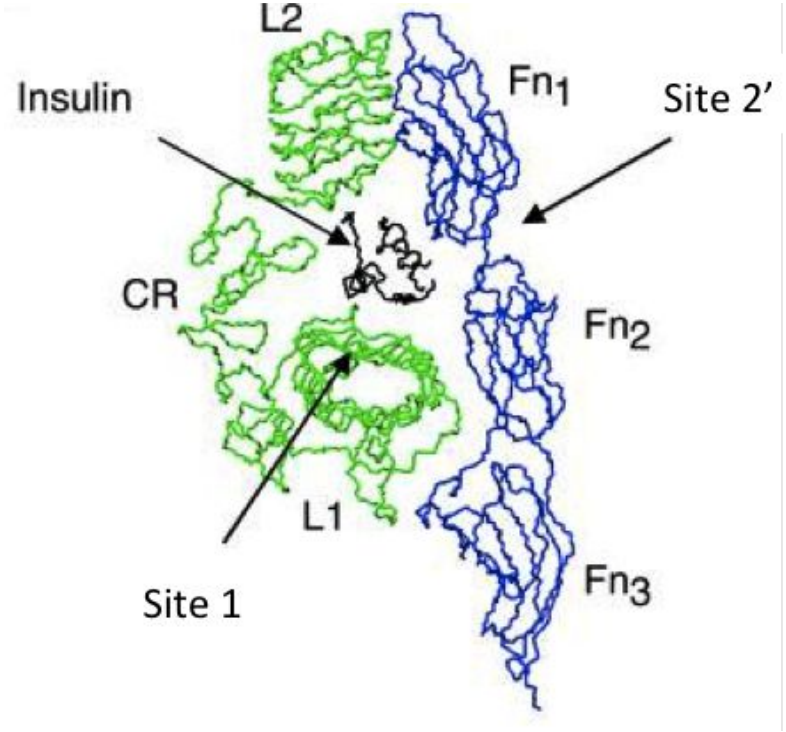
Site 1

Site 2

Insulin binding

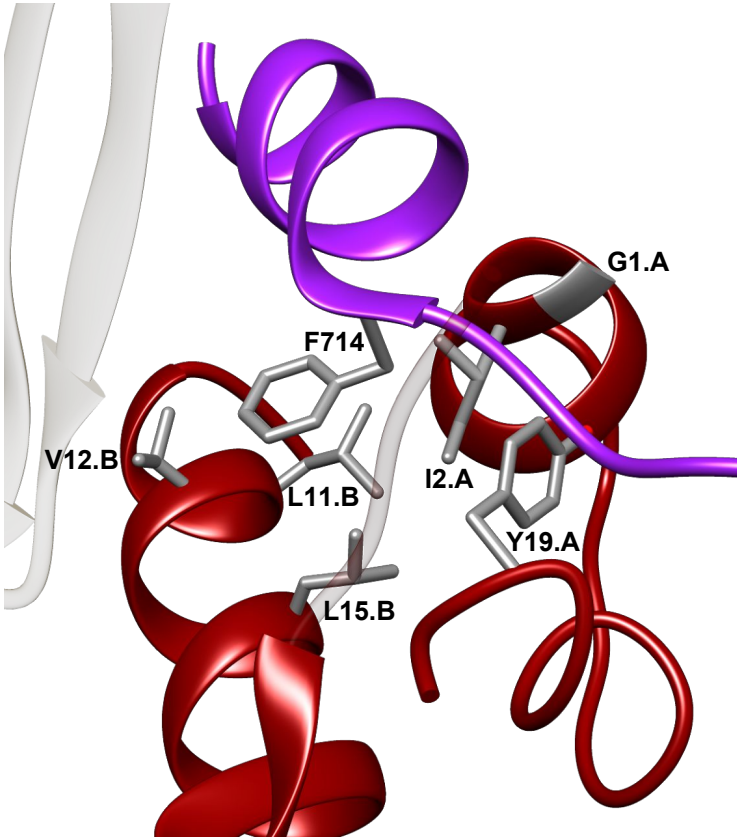
- 2 binding sites: αCT-L1 (site 1), junction of FnIII-1 and FnIII-2 (site 2).
- Induces a conformational change in the receptor that activates the TK domain.

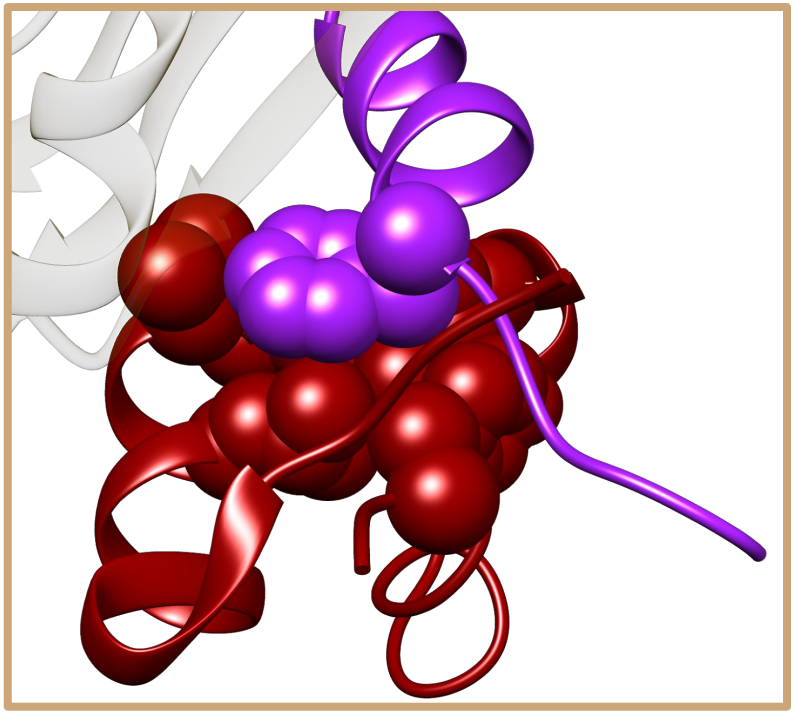




Kiselyov V, Versteyhe S, Gauguin L, De Meyts P. Harmonic oscillator model of the insulin and IGF1 receptors' allosteric binding and activation. Molecular Systems Biology. 2009;5.

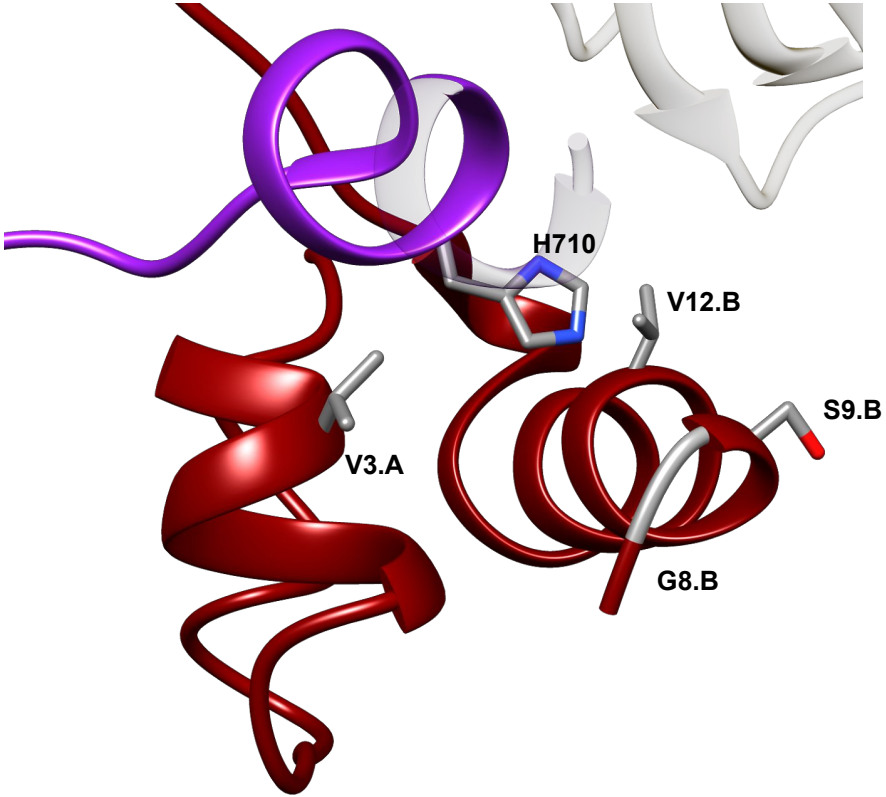
Insulin binding

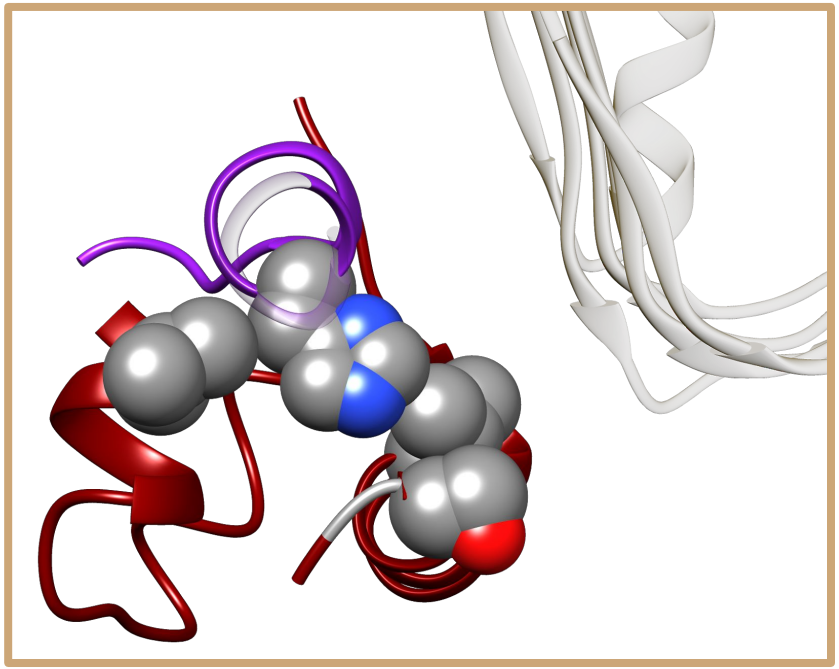




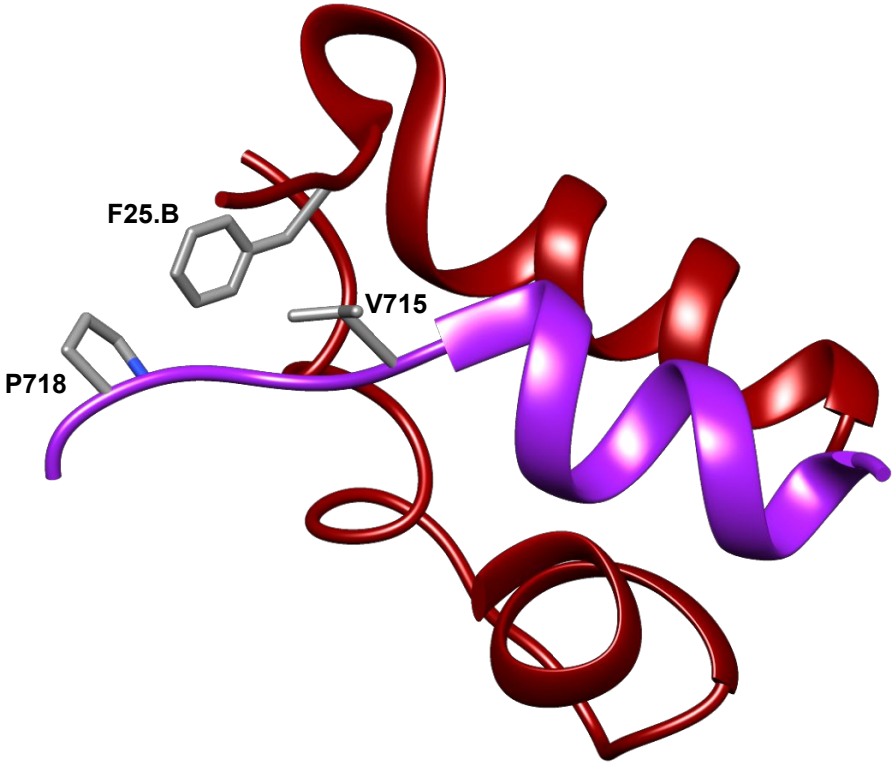
Hydrophobic pocket

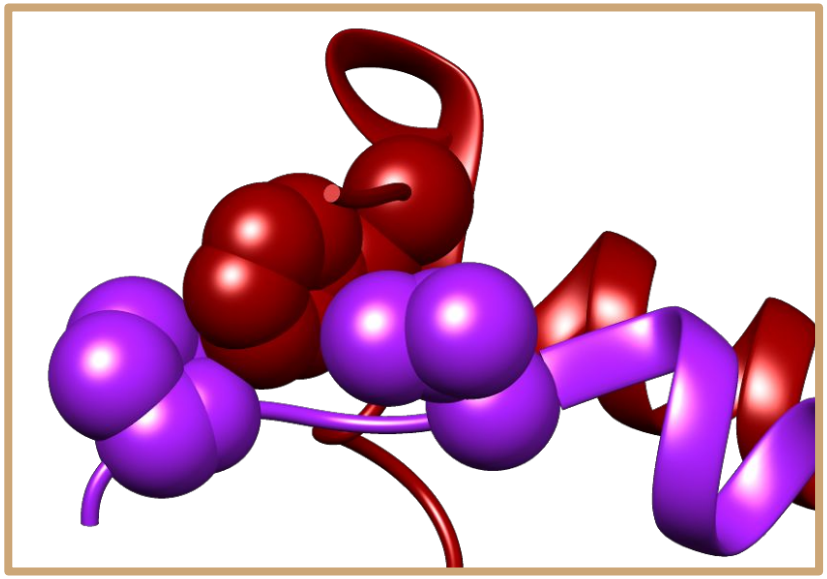
Insulin binding





Insulin binding





Hydrophobic pocket

IR species alignment

L1 domain

```
HUMAN           MATGGRRGAAAAPLLVAVAALLLGAAGHLYPGEVCPGMDIRNNLTRLHEL
PIG             MGAGGRRGAAALPLLVAVAALLVSAAGHLYPGEVCPGMDIRNNLTRLHEL
COW             MEGQN----------------------LESLCPGMDIRNNLTRLHEL
MOUSE           MGFGRGCETTAVPLLVAVAALLVGTAGHLYPGEVCPGMDIRNNLTRLHEL
CHICKEN         MGPRA-----AAAAVVAVAAVLAACGGR---AEICRSMDIRNNLTRLSLL
                *                                .:* .**********  *

HUMAN           ENCSVIEGHLQILLMFKTRPEDFRDLSFPKLIMITDYLLFRVYGLESLK
PIG             ANCSVIEGHLQILLMFKTRPEDFRDLSFPKLIMITDYLLFRVYGLESLK
COW             ANCSVIEGHLQILLMFKTRPEDFRDLSFPKLIMITDYLLFRVYGLESLK
MOUSE           ENCSVIEGHLQILLMFKTRPEDFRDLSFPKLIMITDYLLFRVYGLESLK
CHICKEN         ENCTVIEGHLQILLMFKTKPEDFRELSFPKLTMITDYLLFRVYGLESLK
                 **:**************:*****:****** ******************

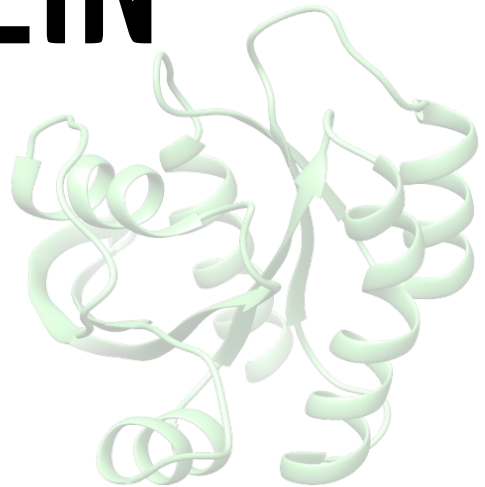
HUMAN           DLFPNLTVIRGSRLFFNYALVIFEMVHLKELGLYNLMNITRGSVRIEKNN
PIG             DLFPNLTVIRGSRLFFNYALVIFEMVHLKELGLYNLMNITRGAVRIEKNN
COW             DLFPNLTVIRGSRLFFNYALVIFEMVHLKELGLYNLMNITRGSVRIEKNN
MOUSE           DLFPNLTVIRGSRLFFNYALVIFEMVHLKELGLYNLMNITRGSVRIEKNN
CHICKEN         GLFPNLTVIRGTHLFFNYALVIFEMVHLKEIGLYNLMNITRGAVRIEKNN
                .**********::******************:***********:*******
```

ID + αCT domain

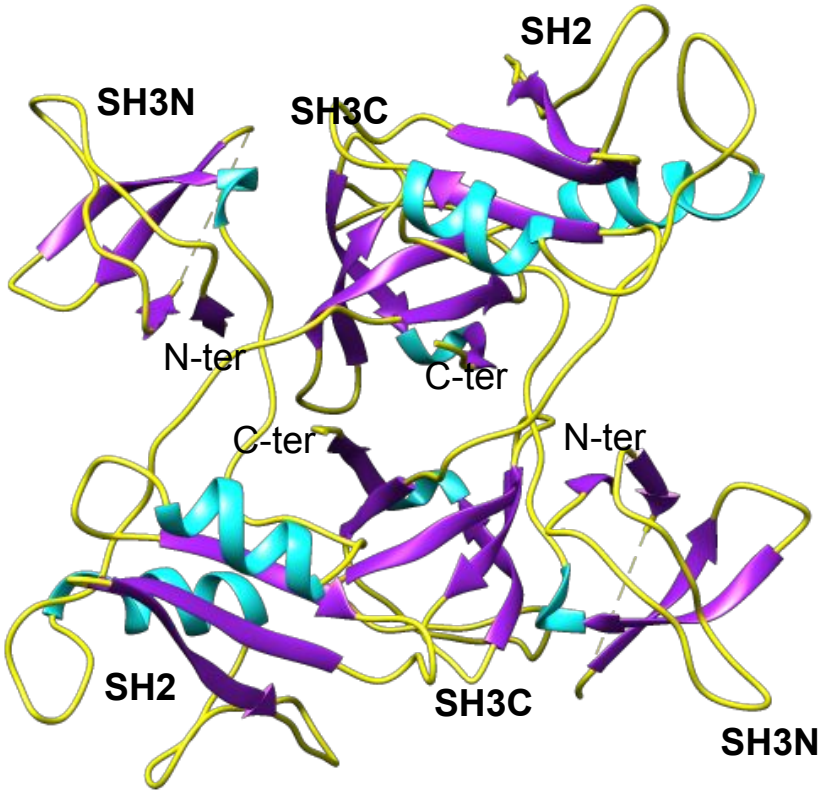
```
HUMAN           QSEYEDSAGECCSCPKTDSQILKELEESSFRKTFEDYLHNVVFVPRKTSS
PIG             QSEYEESSGECCSCPKTDSQILKELEESSFRKTFEDYLHNVVFIPRKSSS
COW             QSEYEESAGECCSCPKTDSQILKELEESSFRKTFEDYLHNVVFIPRKSSS
MOUSE           QSEYDDSASECCSCPKTDSQILKELEESSFRKTFEDYLHNVVFVP-----
CHICKEN         QSESEDVSGECCSCPKTDSQIQKELEESAFRKTFENYLHNEVFVP-----
                *** :: :.************ ******:******:**** **:*
```

THE RAS G PROTEIN

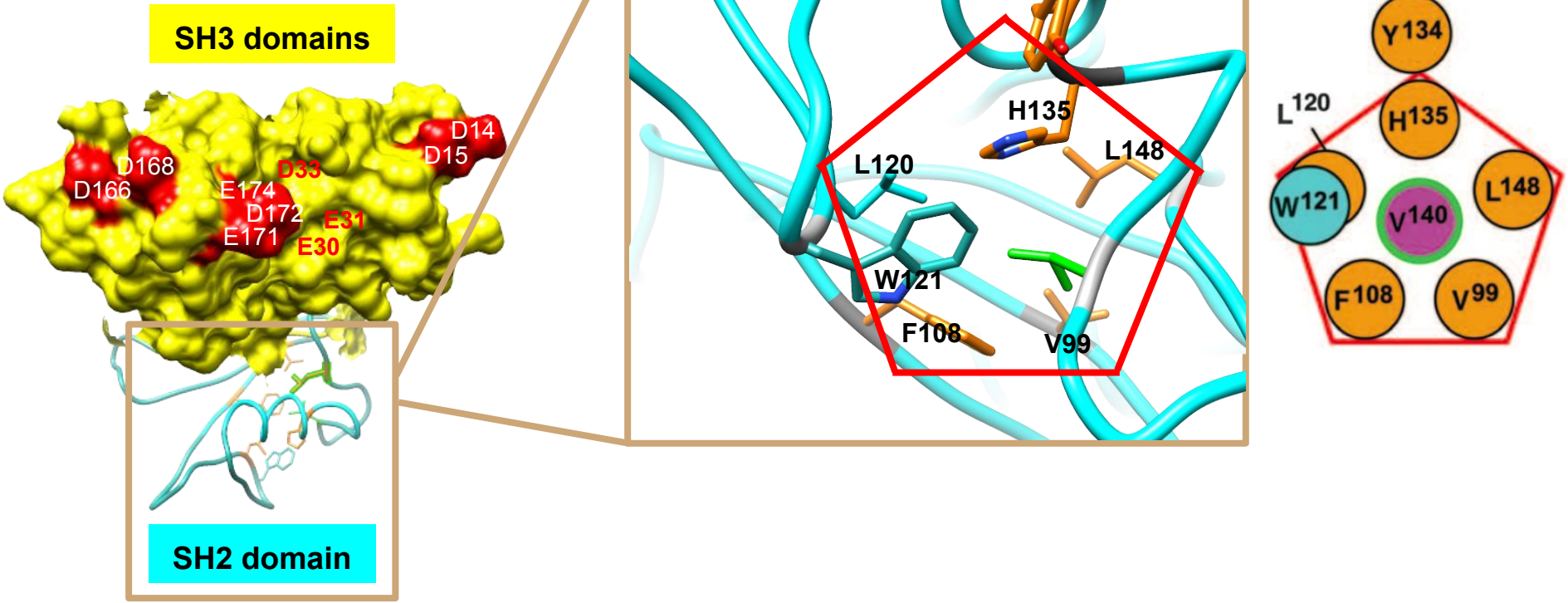
Grb2 | SOS | Ras | GAP



GRB2



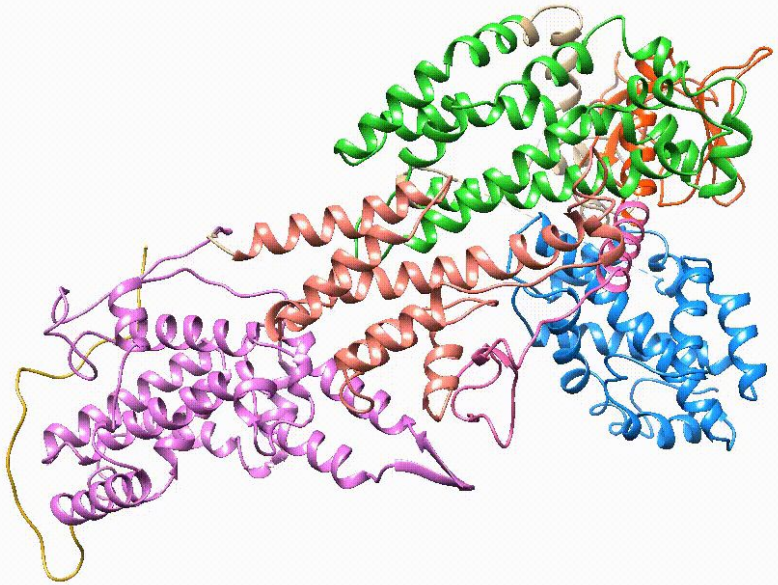
GRB2


SH3 domains
D14
D15
D168
D166
D33
E174
D172
E171
E31
E30
SH2 domain
Y134
H135
L120
L148
W121
F108
V99
L120
Y134
H135
L148
W121
V140
F108
V99

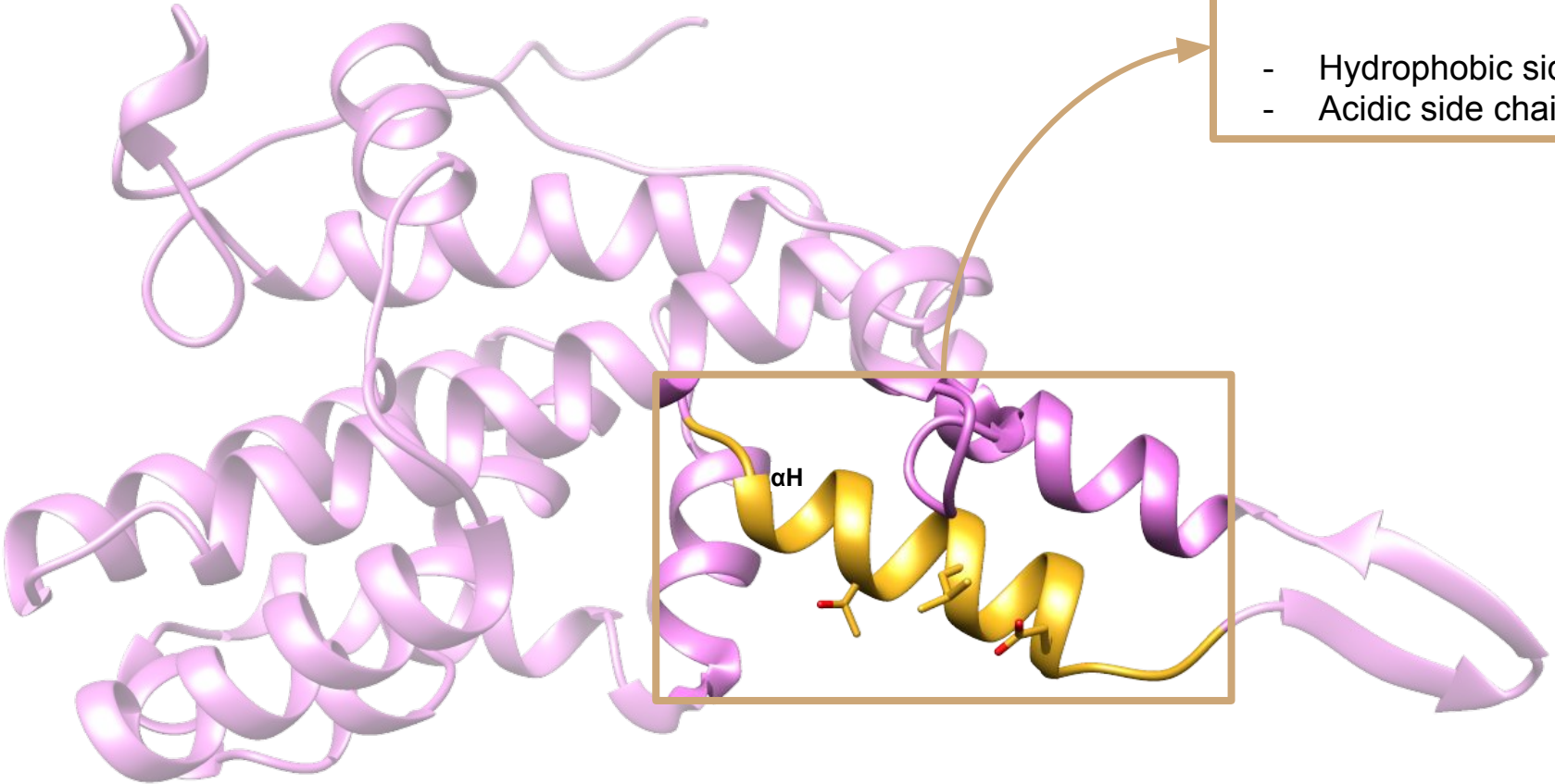
Son of Sevenless (SOS)

Sos protein consists of several domains:

- Histone-like domain (HD)
- Db1 homology domain (DH)
- Pleckstrin domain (PH)
- Helical linker domain (HL)
- Ras exchanger motif (REM)
- Catalytic GEF domain (CDC25)
- Proline-rich region



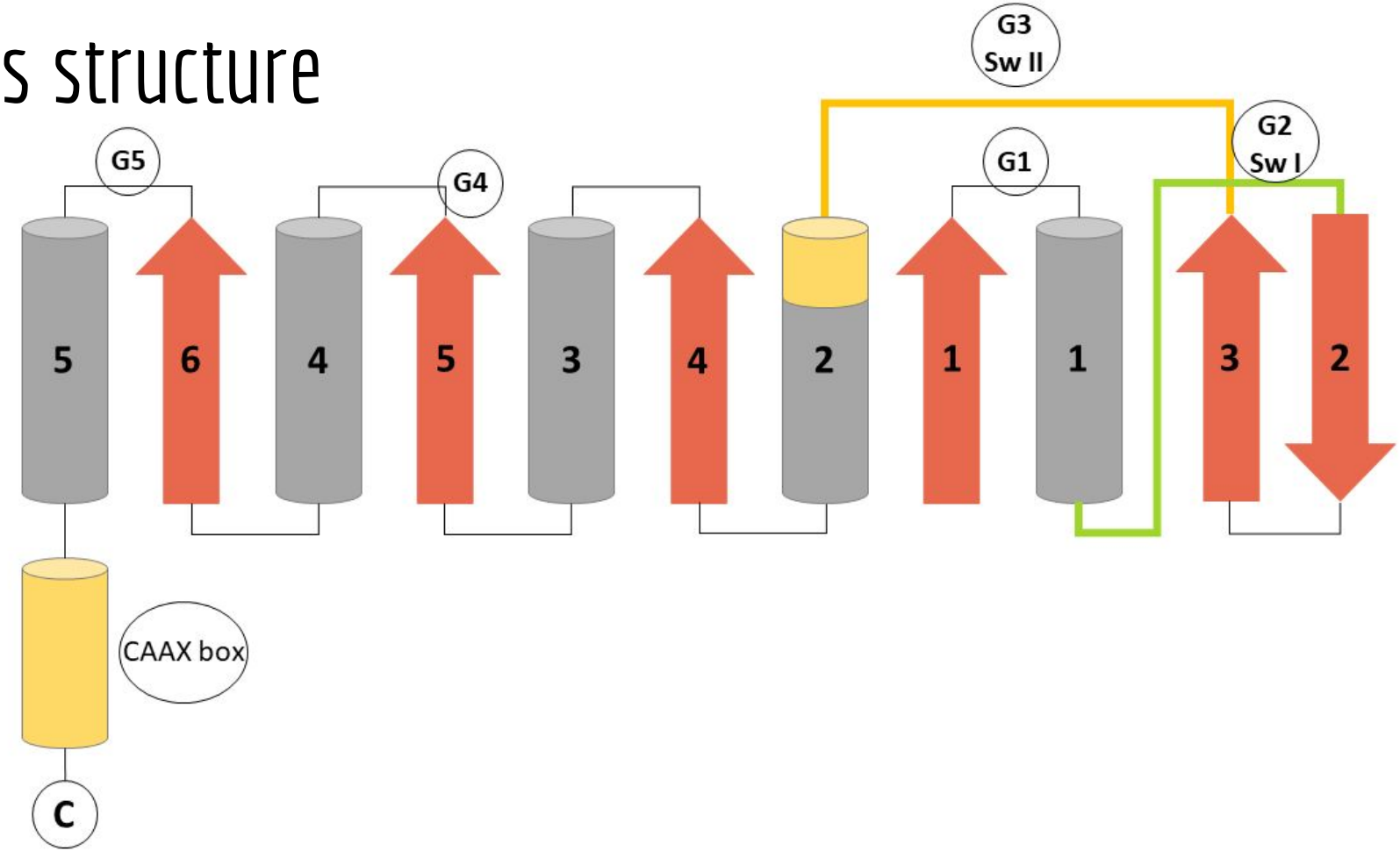
SOS - CDC25 domain



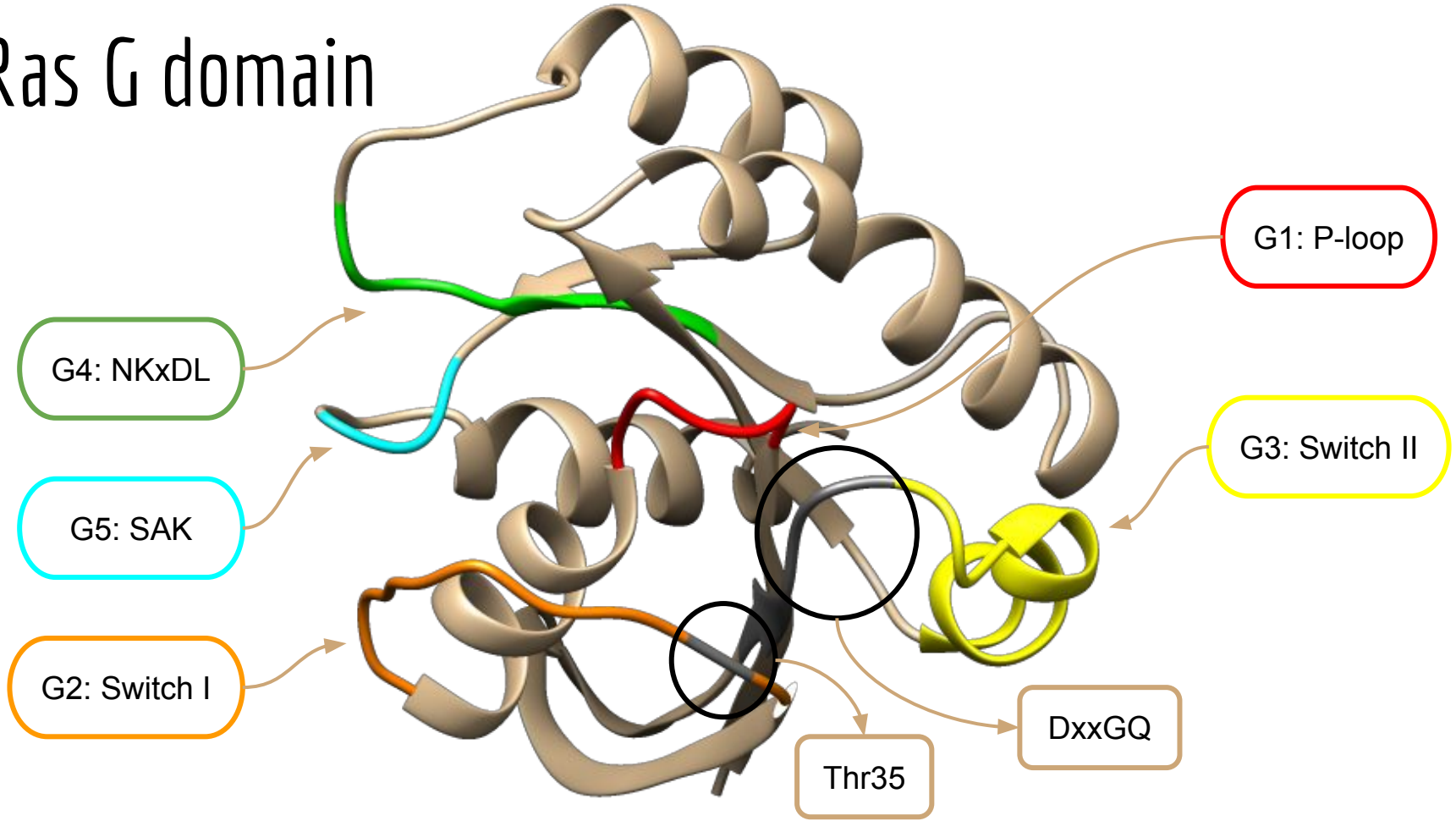
Helix αH of SOS:

- Hydrophobic side chain
- Acidic side chain

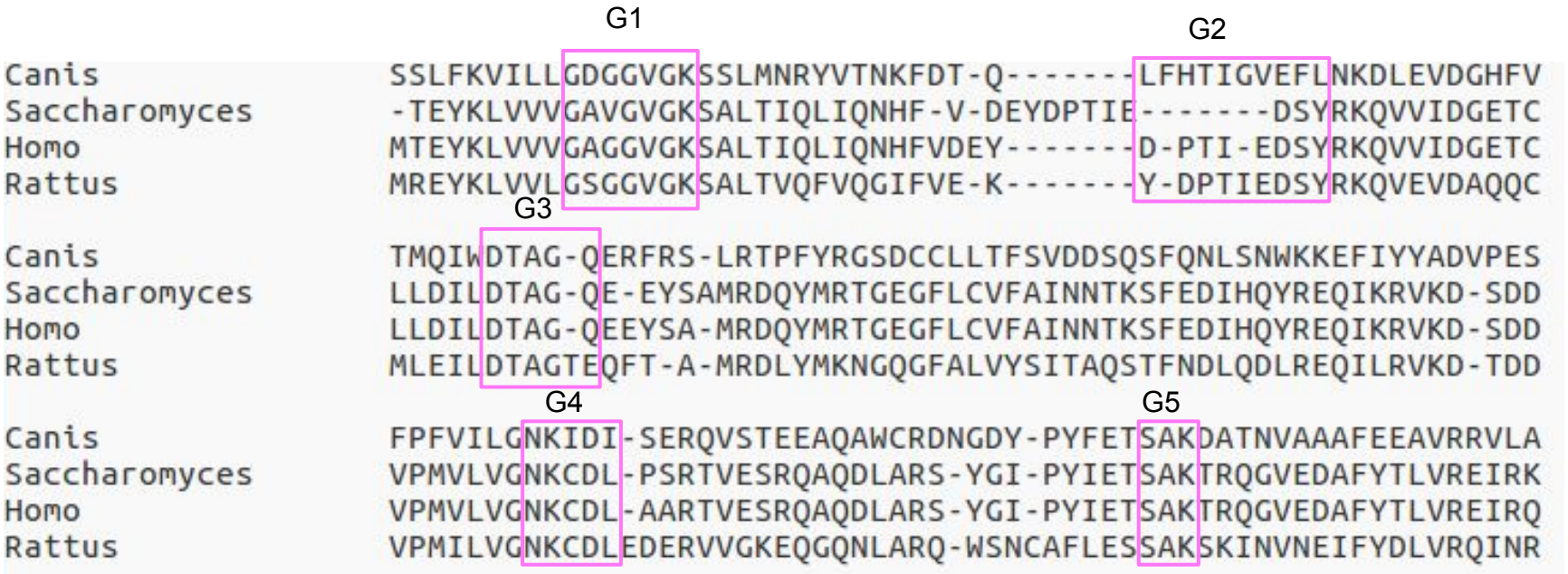
Ras structure



Ras G domain



Ras alignment



Ras alignment

```
STAMP Structural Alignment of Multiple Proteins

Version 4.4 (May 2010)
 by Robert B. Russell & Geoffrey J. Barton
 Please cite PROTEINS, v14, 309-323, 1992

Running roughfit.

    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  Canis           Homo            4.07   1.41    341  166   169  156 156    0  32.69 100.00 6.99e-15
Pair   2  Canis           Rattus          3.80   1.47    341  167   172  151 143    0  30.77 100.00 4.59e-12
Pair   3  Canis           Saccharomyces   3.58   1.33    341  165   177  144 143    0  33.57 100.00 1.35e-14
Pair   4  Homo            Rattus          8.66   1.05    166  167   169  159 157    0  56.05 100.00 2.57e-46
Pair   5  Homo            Saccharomyces   8.10   0.90    166  165   173  149 147    0  97.28 100.00 0.00e+00
Pair   6  Rattus          Saccharomyces   7.81   0.86    167  165   173  145 145    0  55.86 100.00 1.29e-42
Reading in matrix file sps.mat...
Doing cluster analysis...
Cluster:  1 (    Homo  &   Rattus ) Sc  8.66 RMS   1.05 Len 169 nfit 159
 See file sps.1 for the alignment and transformations
Cluster:  2 (Saccharomyces  &     Homo   Rattus ) Sc  8.60 RMS   0.81 Len 177 nfit 147
 See file sps.2 for the alignment and transformations
Cluster:  3 (   Canis  & Saccharomyces     Homo   Rattus ) Sc  6.04 RMS   1.55 Len 180 nfit 155
 See file sps.3 for the alignment and transformations
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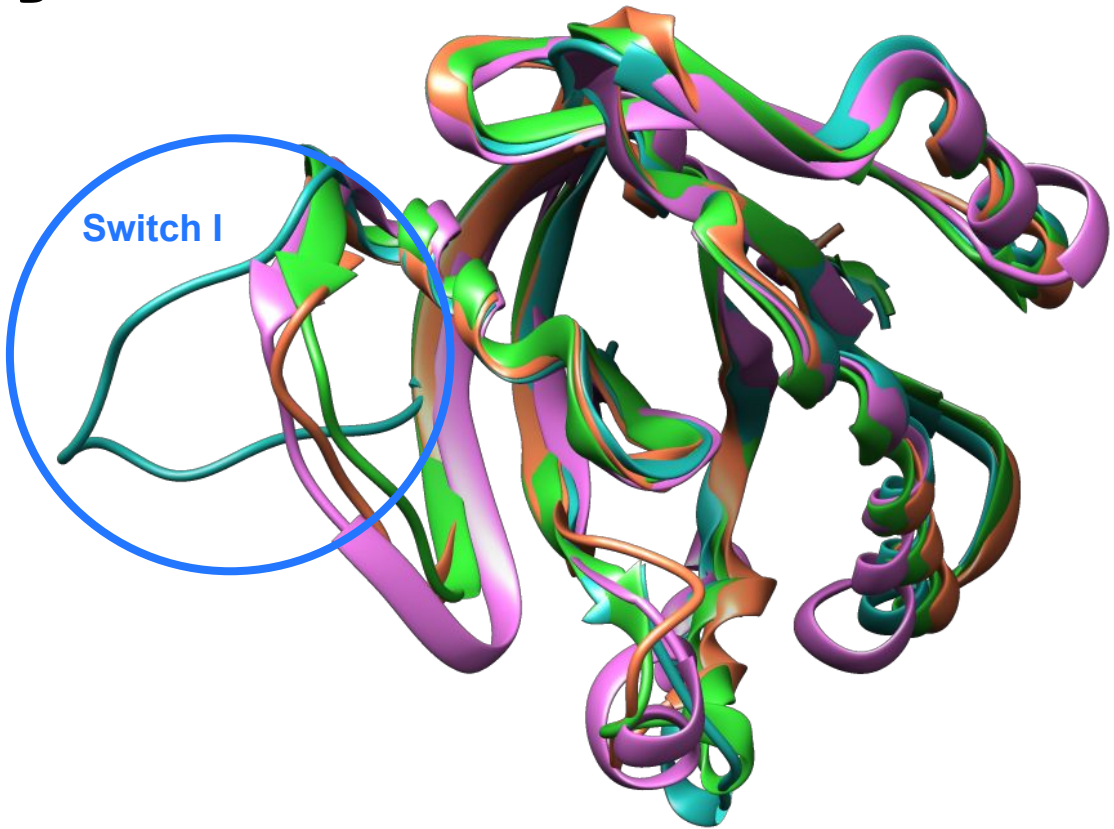
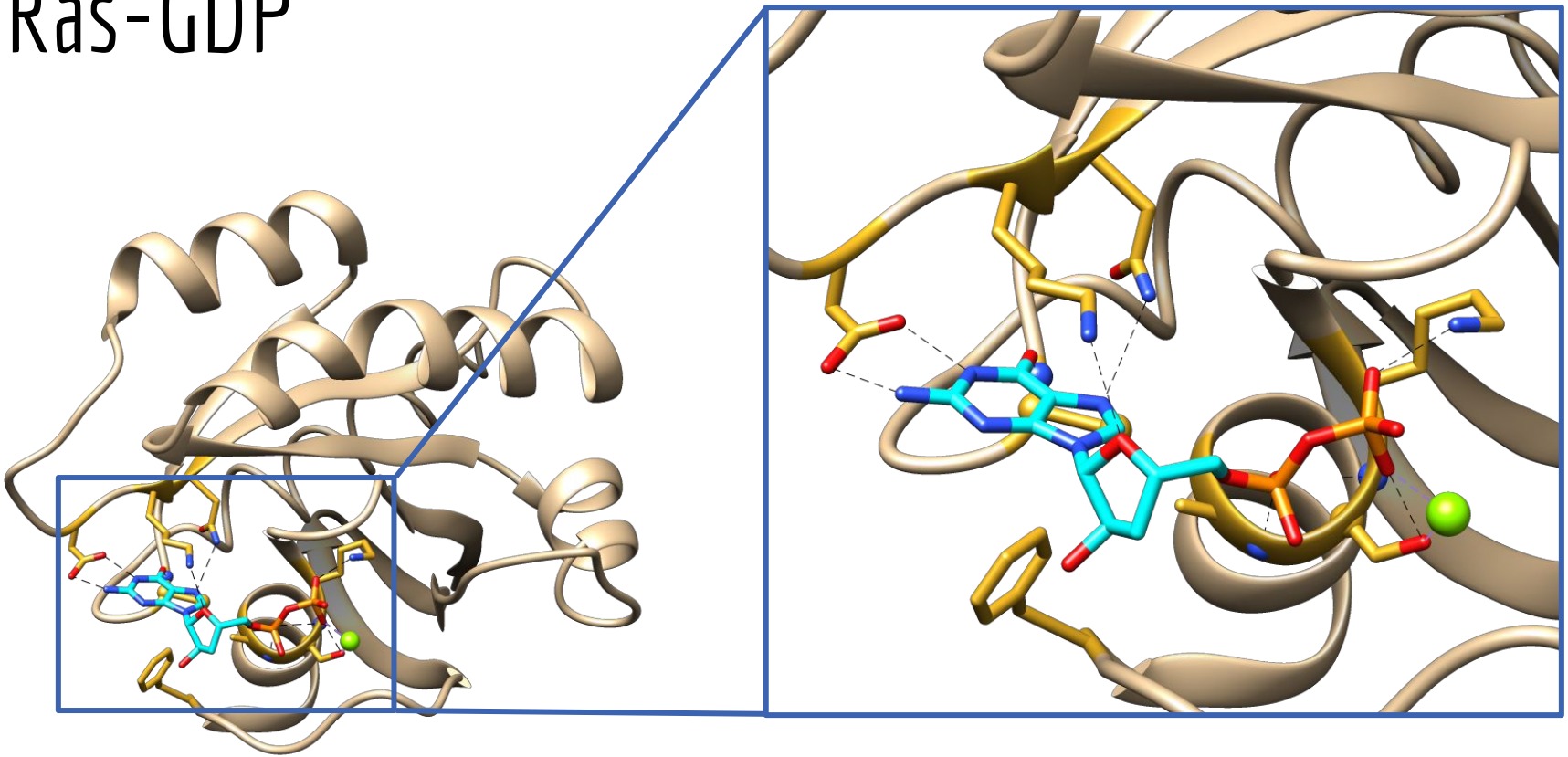
Ras alignment



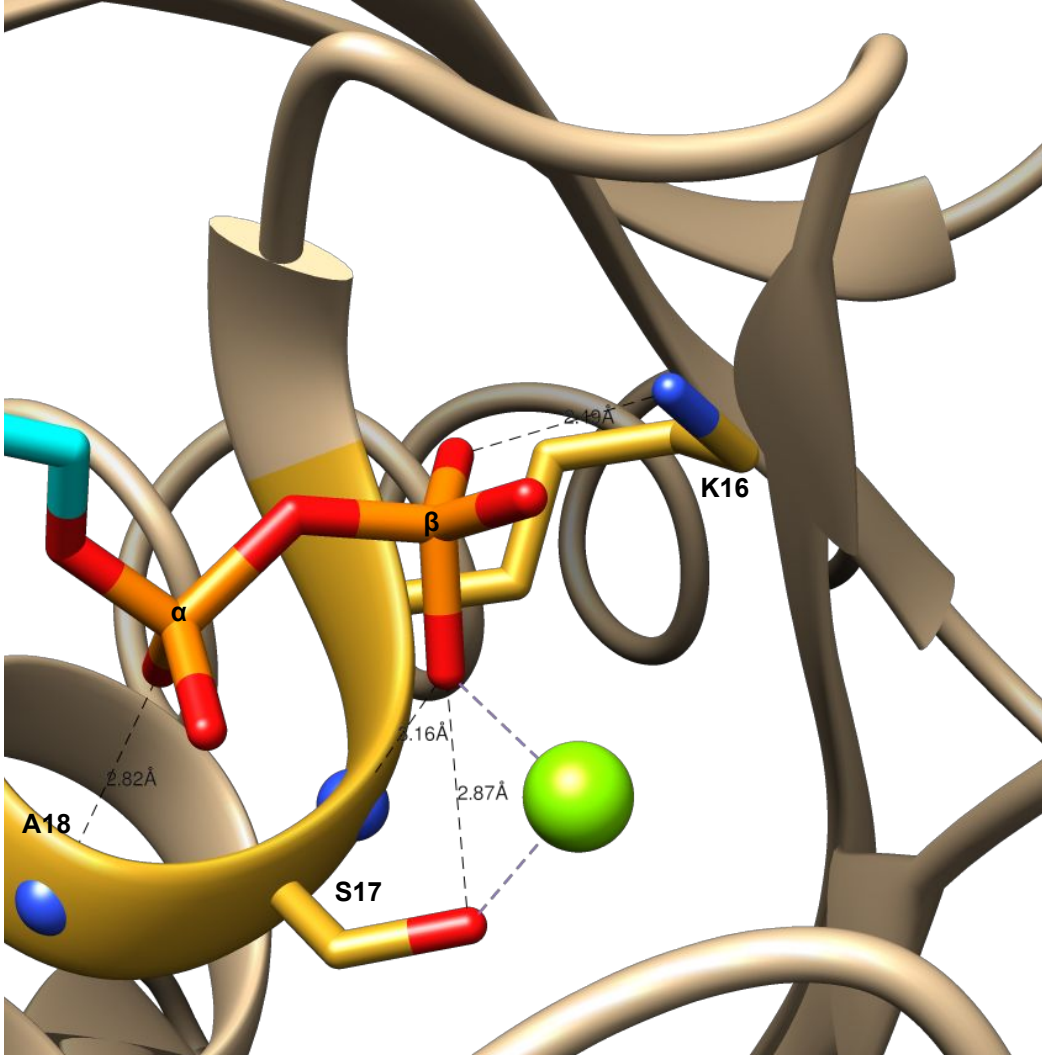
Ras-GDP



Ras-GDP

Hydrogen bonds

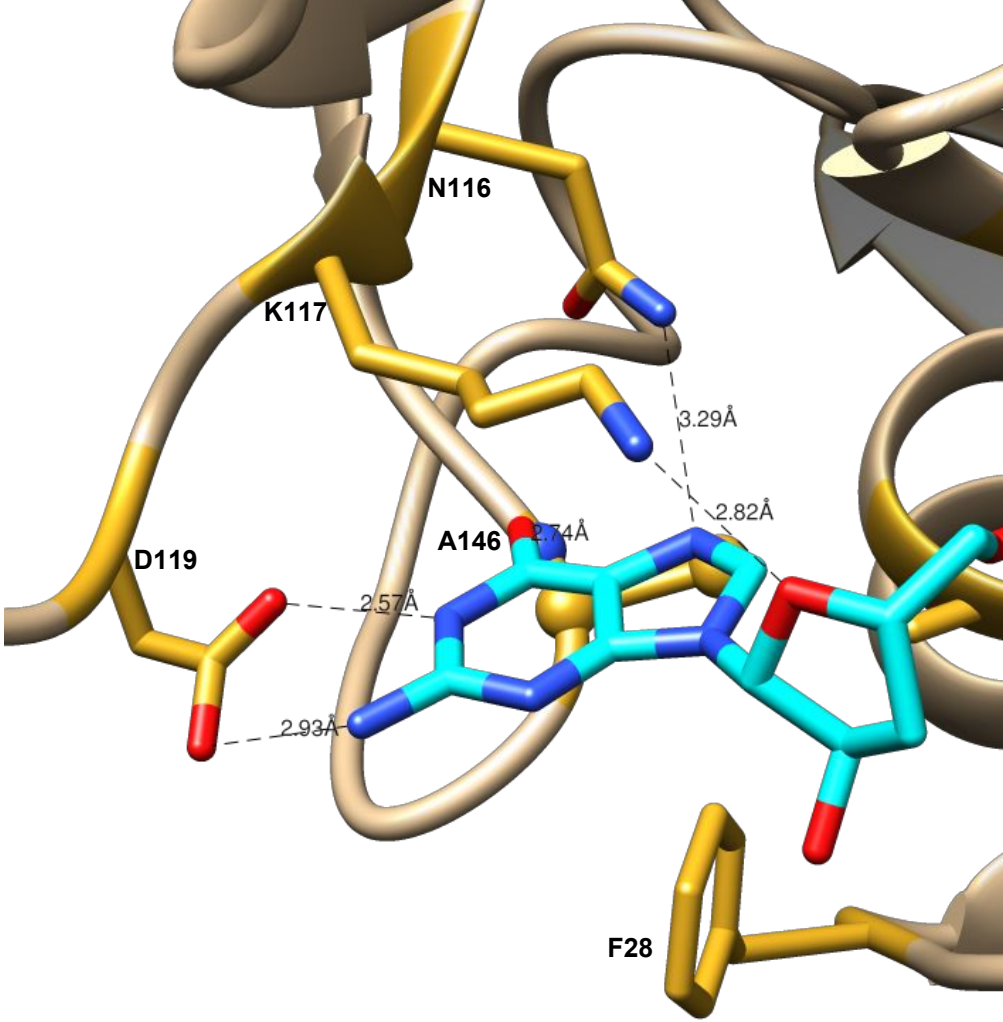
Lys 16.A NZ - GDP 274.A O1B - 2,49Å
Ser 17.A OG - GDP 274.A O3B - 2,87Å
Ser17.A N - GDP 274.A O3B - 3,16Å
Ala 18.A N - GDP 274.A O2A - 2,82Å



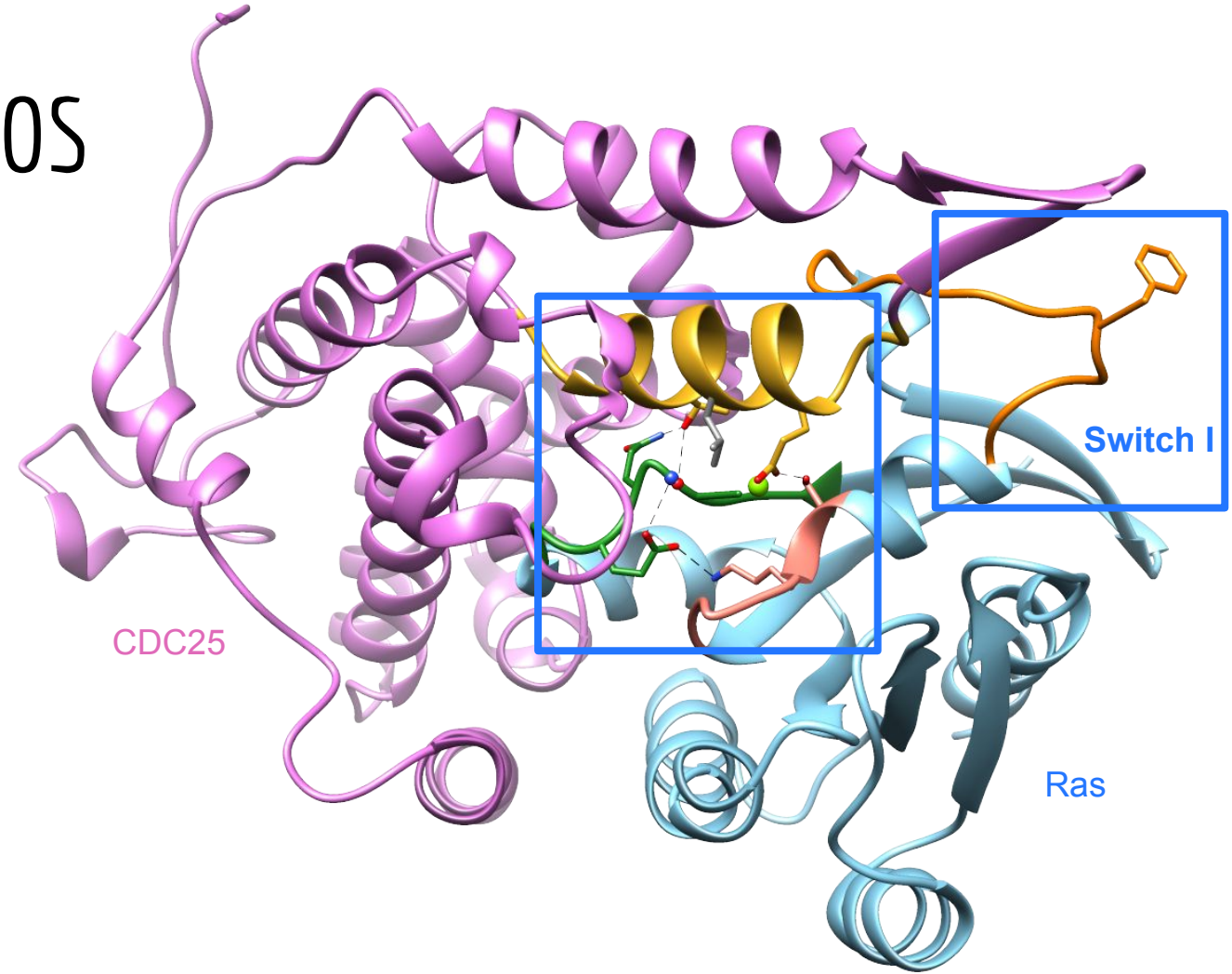
Ras-GDP

Hydrogen bonds

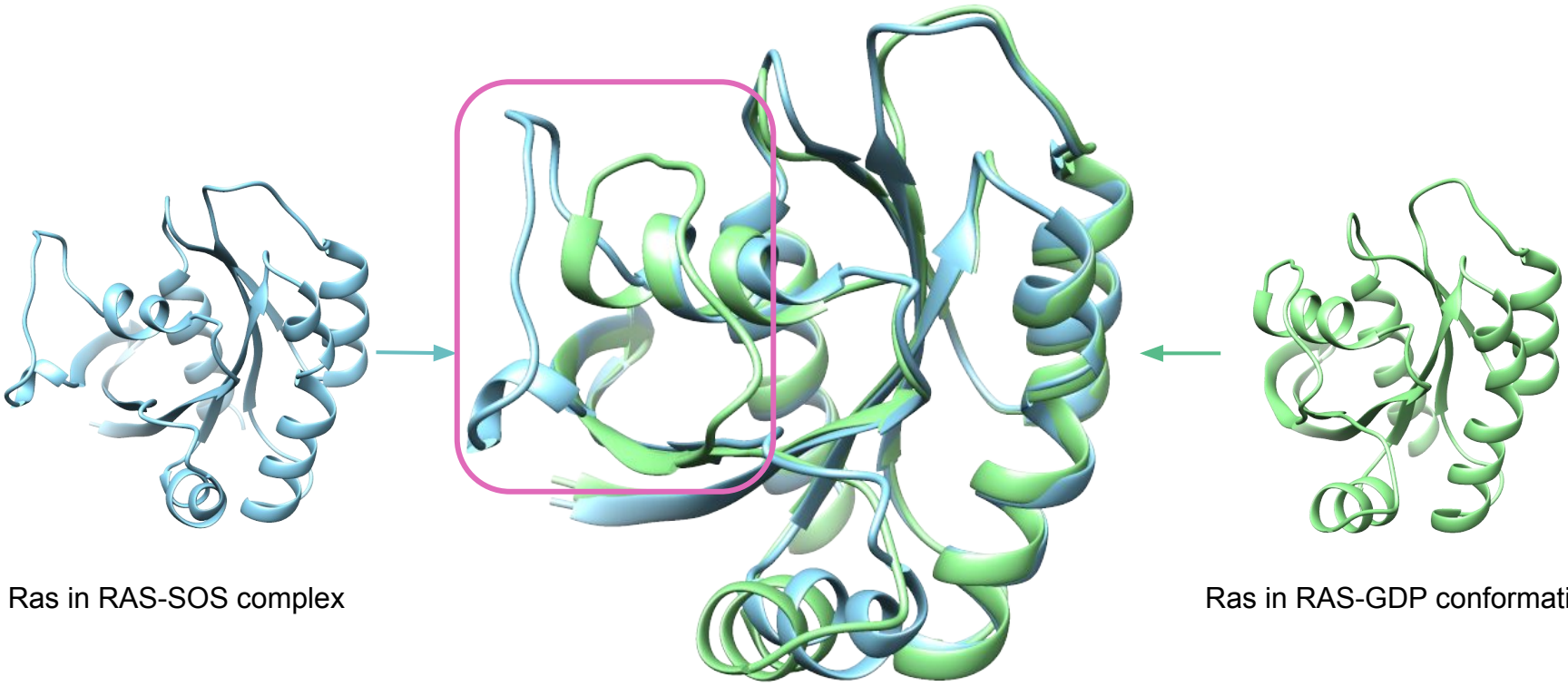
Asn 116.A ND2 - GDP 274.A N7 - 3,29Å
Lys 117.A NZ - GDP 274.A O4’ - 2,82Å
Asp 119.A OD1 - GDP 274.A N1 - 2,57Å
Asp 119.A OD2 - GDP 274.A N2 - 2,93Å
Ala 0146.A N - GDP 274.A O6 - 2,74Å



Ras-SOS



Ras-SOS



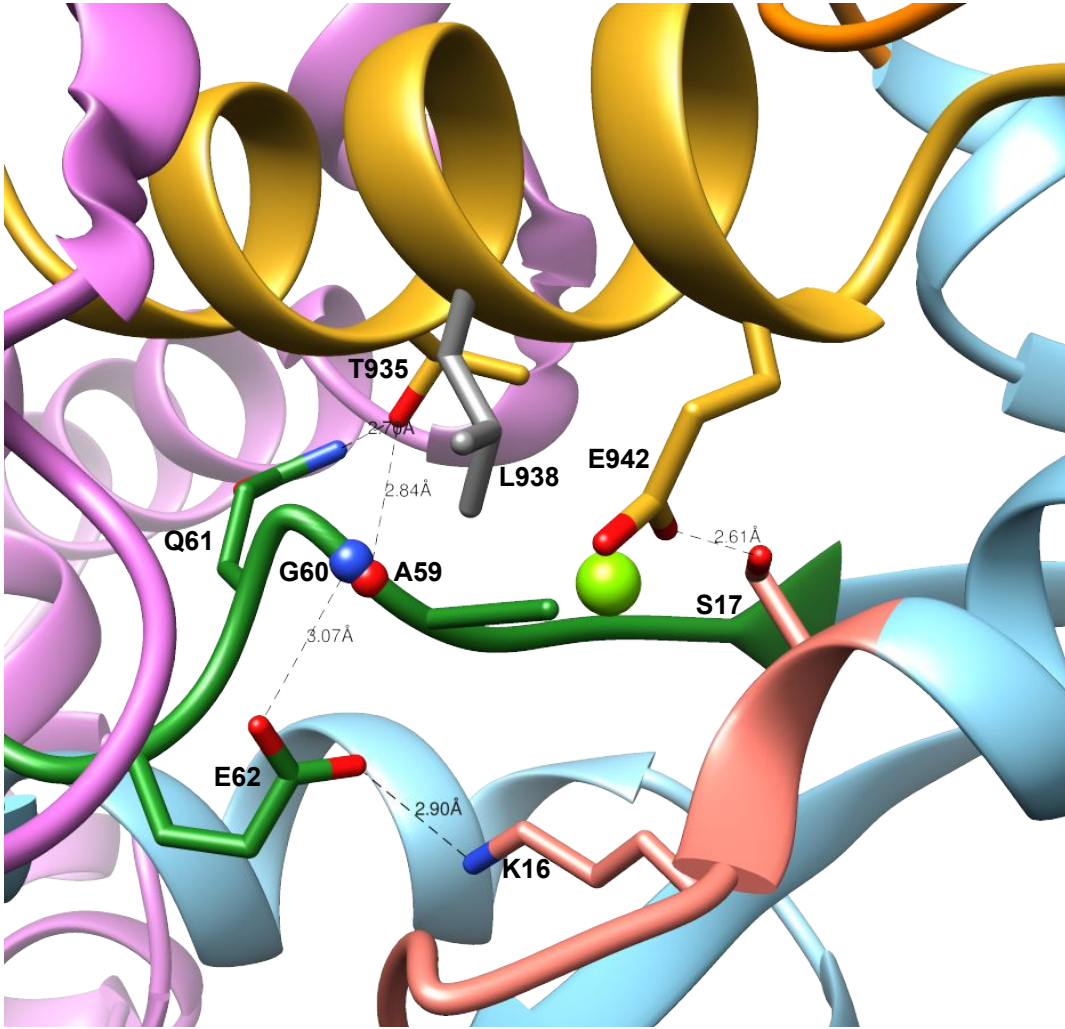
Ras in RAS-SOS complex

Ras in RAS-GDP conformation

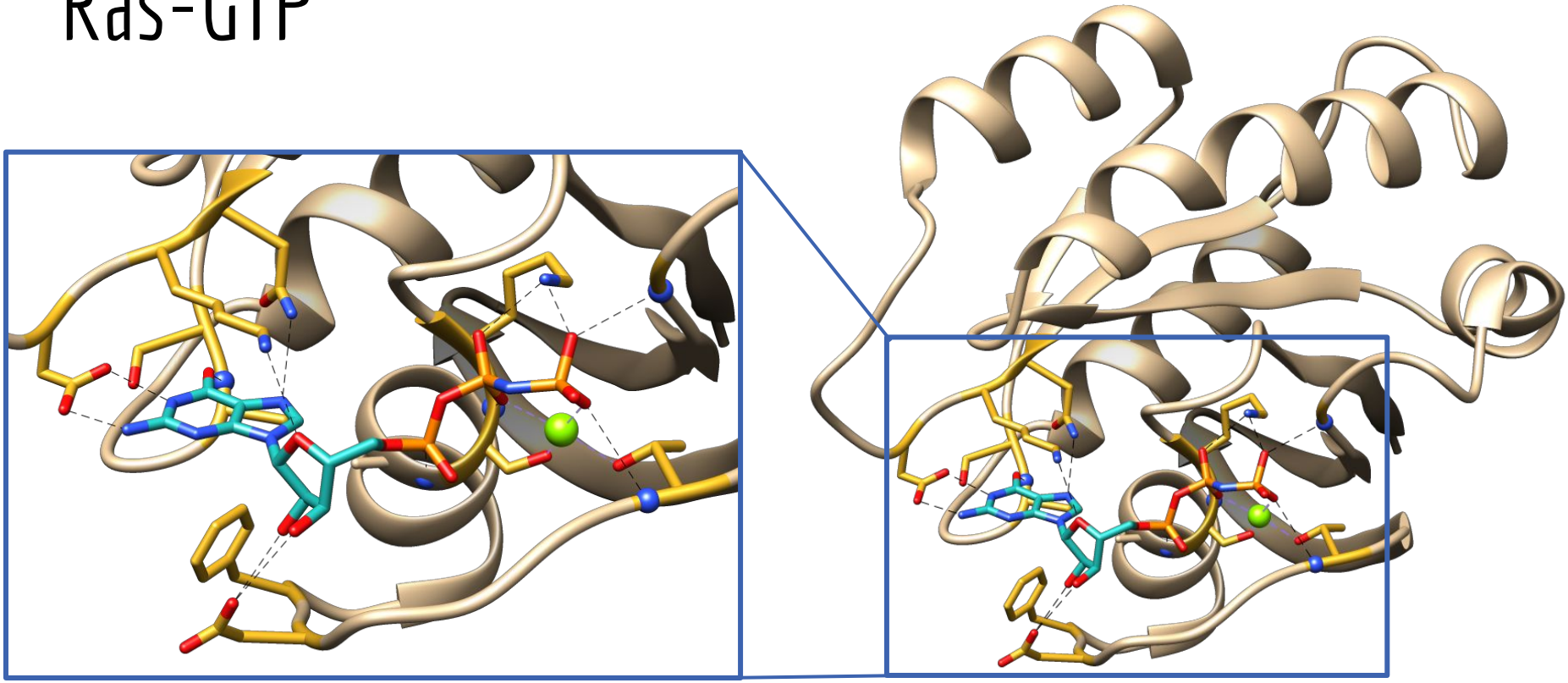
Ras-SOS

Hydrogen bonds

Ala 59.R O - Thr 935.S OG1 - 2,84Å
Gln 61.R NE2 - Thr 935.S OG1 - 2,70Å
Ser 17.R OG - Glu 942.S OE1 - 2,61Å
Lys 16.R NZ - Glu 62.R OE2 - 2,90Å
Glu 62.R. OE1 - Gly 60.R N - 3,07Å



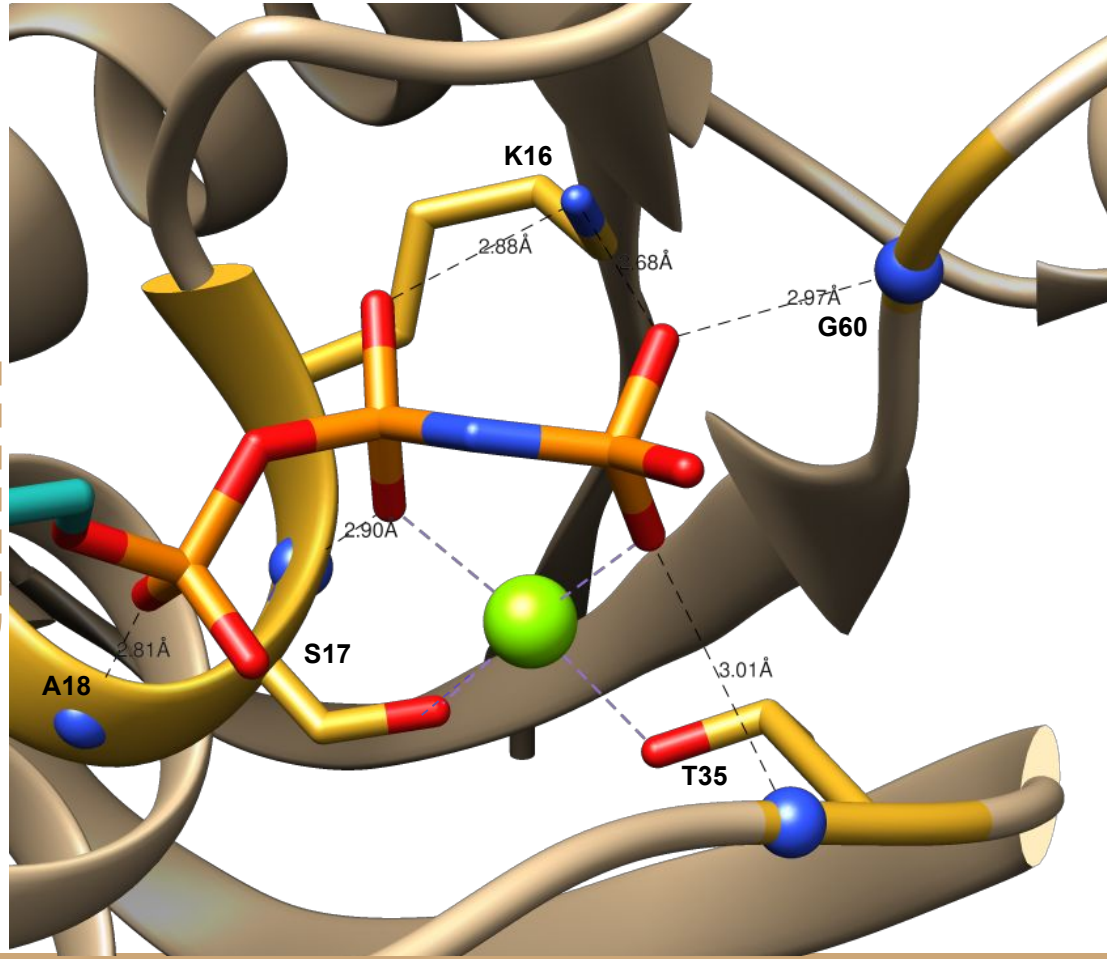
Ras-GTP



Ras-GTP

Hydrogen bonds

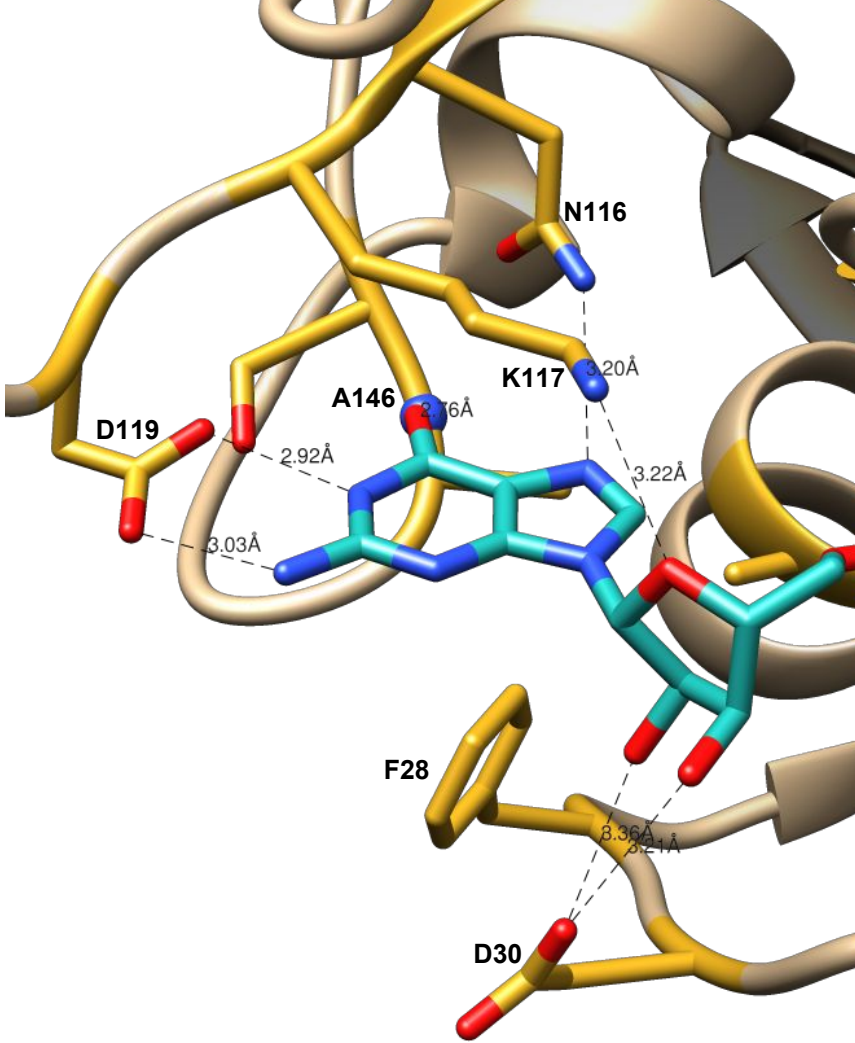
Lys 16.A NZ - GTP 167.A O1B - 2,88Å
Lys 16.A NZ - GTP 167.A O3G - 2,68Å
Ser 17.A ND2 - GTP 167.A O2B - 2,90Å
Ala 18.A N - GTP 167.A O1A - 2,81Å
Thr 35.A N - GTP 167.A O2G - 3,01Å
Gly 60.A N - GTP 167 O3G - 2,97Å



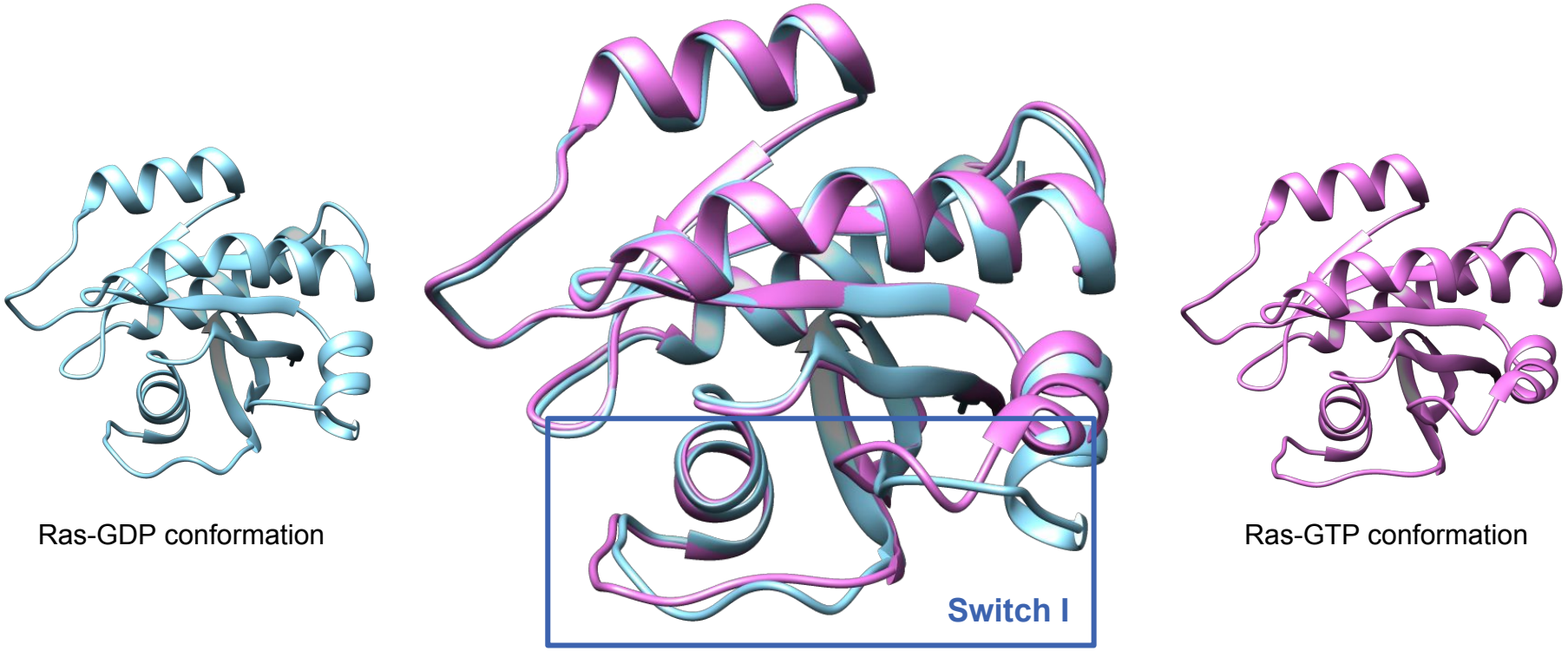
Ras-GTP

Hydrogen bonds

Asp 30.A OD1 - GTP 167.A O2’ - 3,36Å
Asp30.A OD1 - GTP 167.A O4’ - 3,22Å
Asn 116.A ND2 - GTP 167.A N7 - 3,20Å
Lys 117.A NZ - GTP 167.A O4’ - 3.22Å
Asp 119.A OD1 - GTP 167.A N1 - 2,92Å
Asp 119 OD2 - GTP 167.A N2 - 3,03Å
Ala 146.A N - GTP 167.A O5 - 2,76Å



Ras-GDP vs Ras-GTP

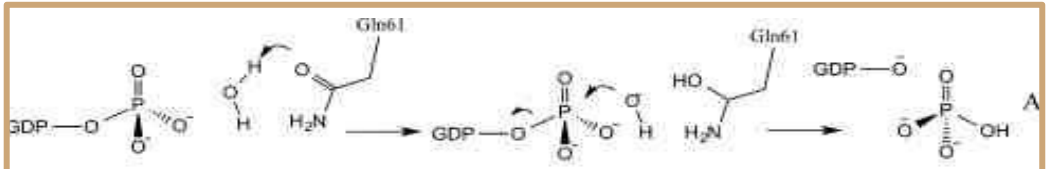


Ras-GDP conformation

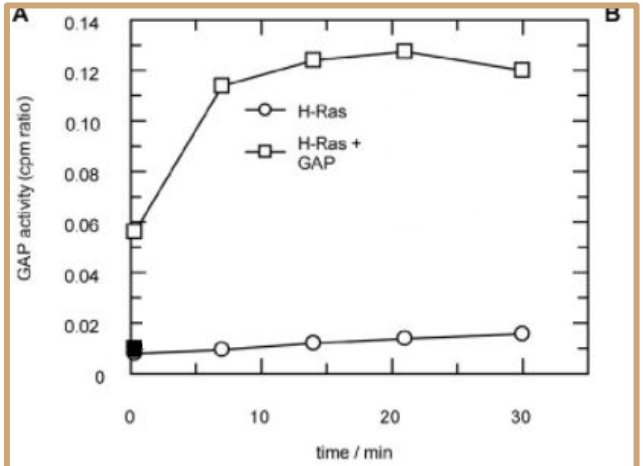
Ras-GTP conformation

Cluster: 1 (Ras-GTP & Ras-GDP) Sc 8.60 RMS 0.93

RAS GTPase activity

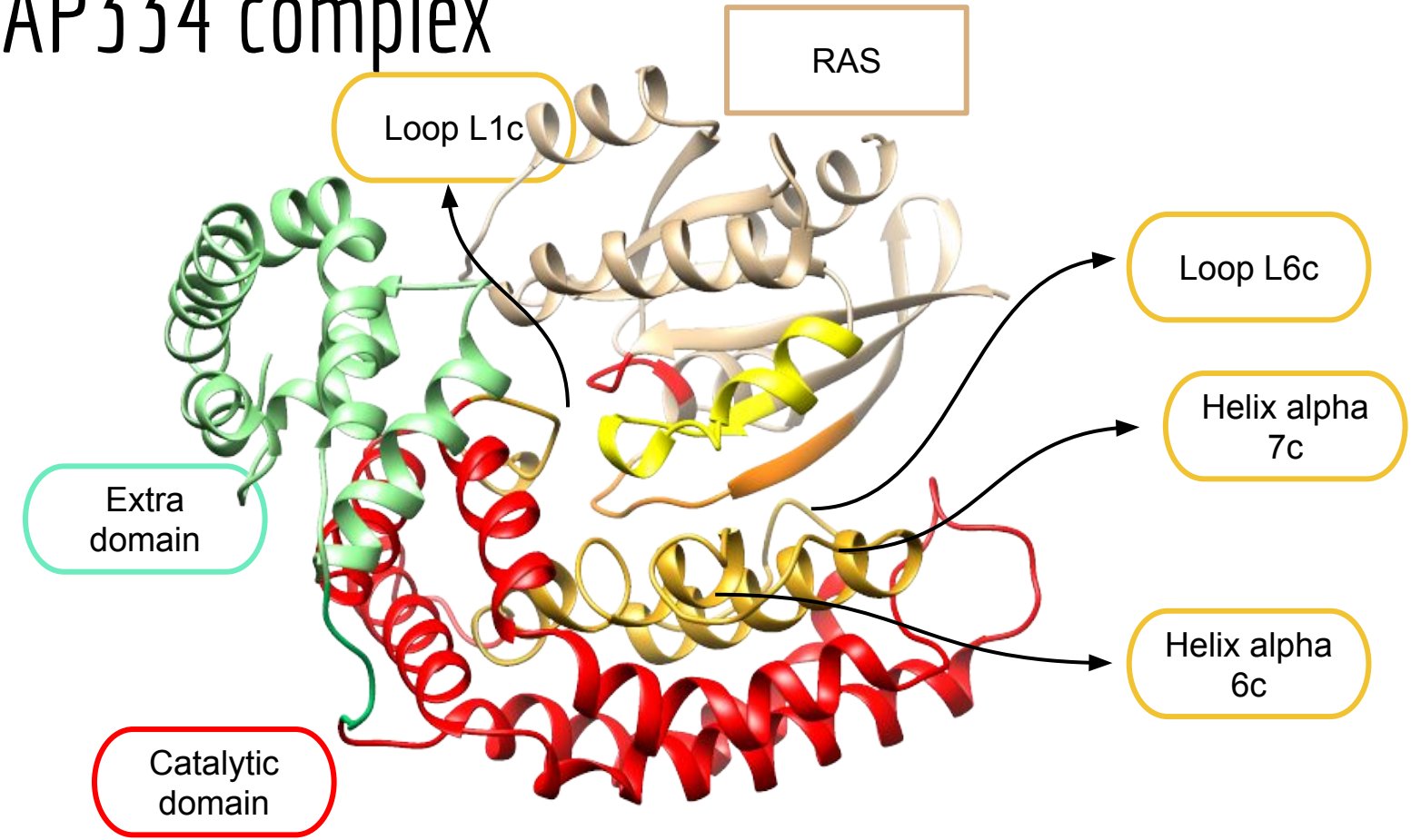


Carvalho A, Szeler K, Vavitsas K, Åqvist J, Kamerlin S. Modeling the mechanisms of biological GTP hydrolysis. Archives of Biochemistry and Biophysics. 2015;582:80-90.

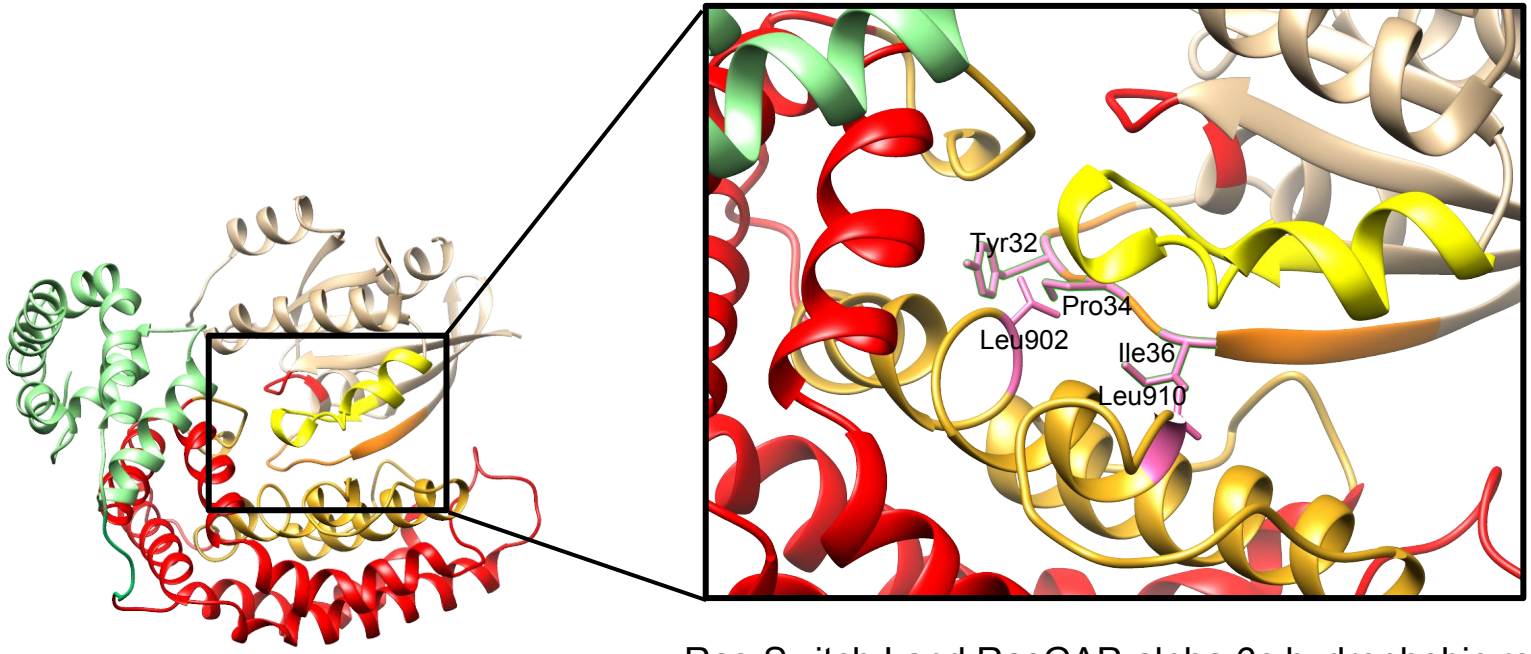


Mai A, Veltel S, Pellinen T, Padzik A, Coffey E, Marjomäki V et al. Competitive binding of Rab21 and p120RasGAP to integrins regulates receptor traffic and migration. The Journal of Cell Biology. 2011;194(2):291-306.

Ras-GAP334 complex

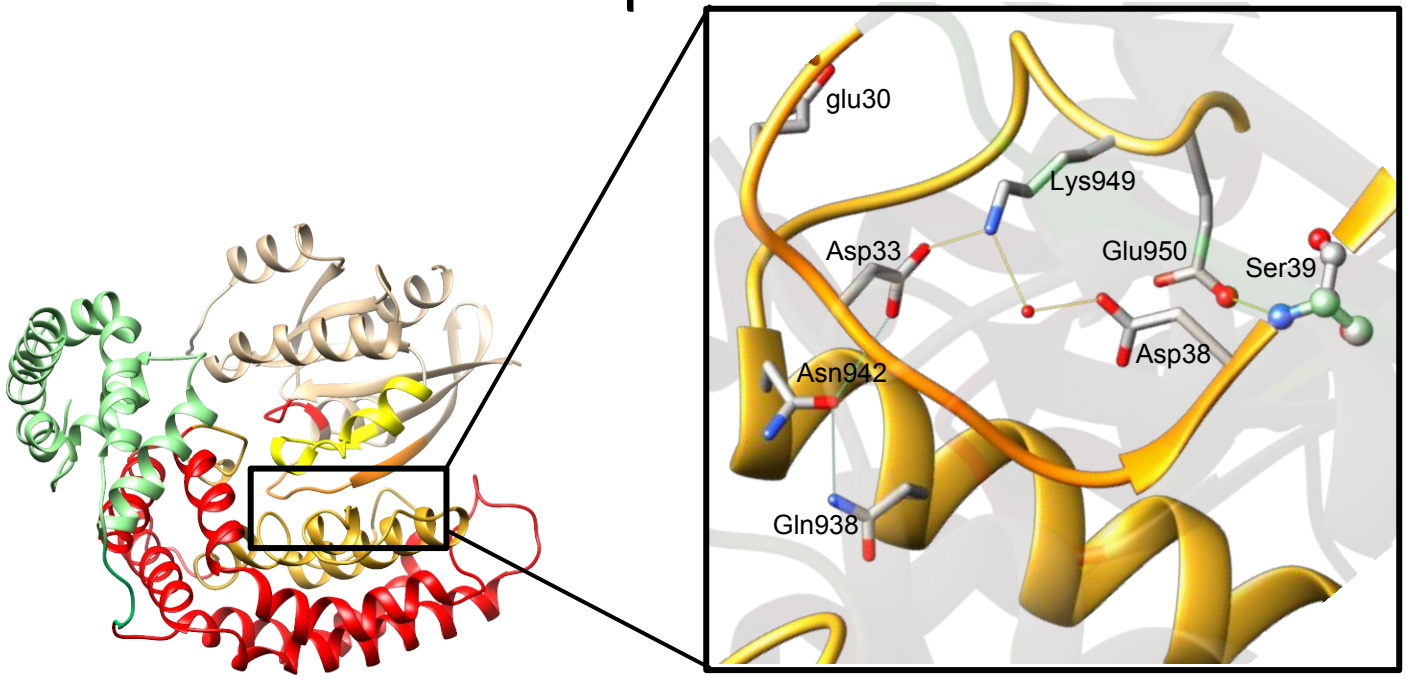


Ras-RasGAP complex



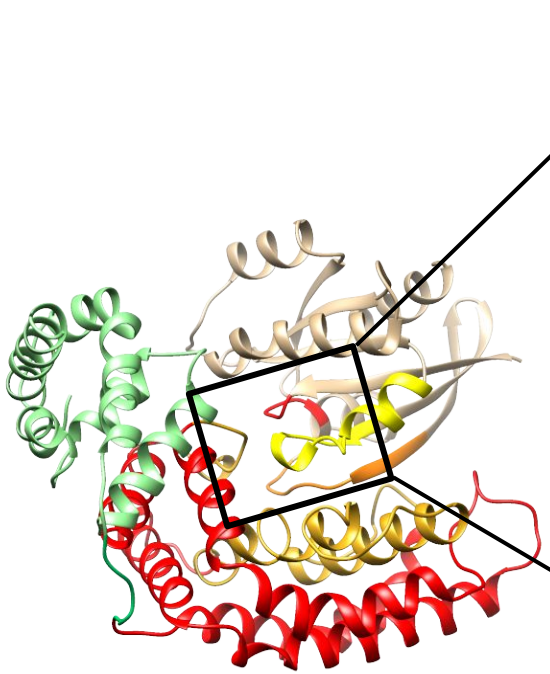
Ras-Switch I and RasGAP-alpha 6c hydrophobic residues

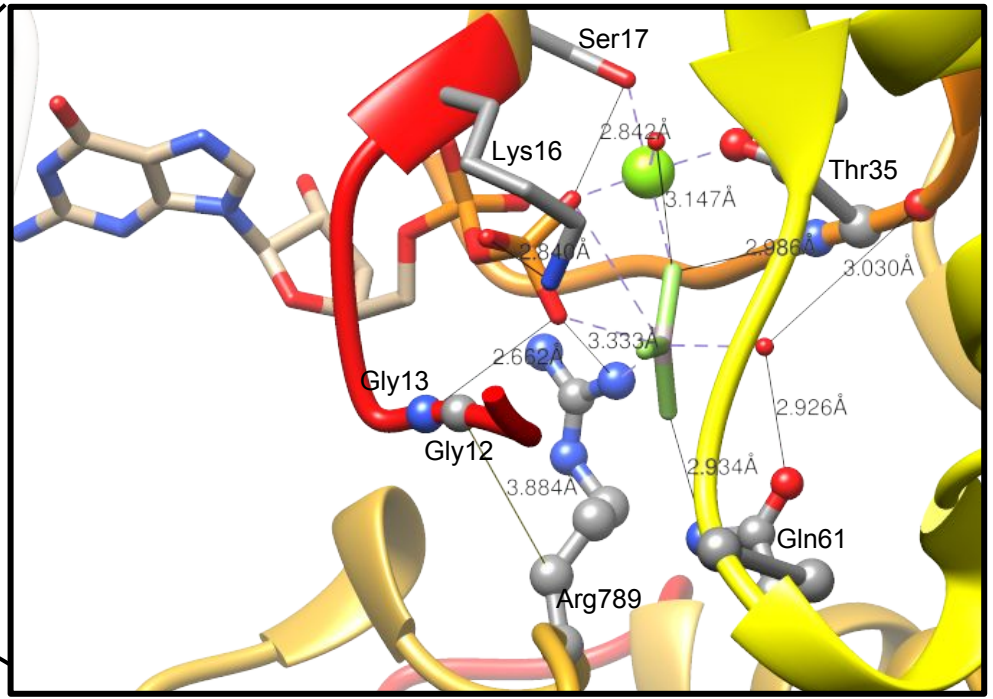
Ras-RasGAP complex



Ras-Switch I interactions with RasGAP helix alpha7c and loop L61

Ras-RasGAP complex



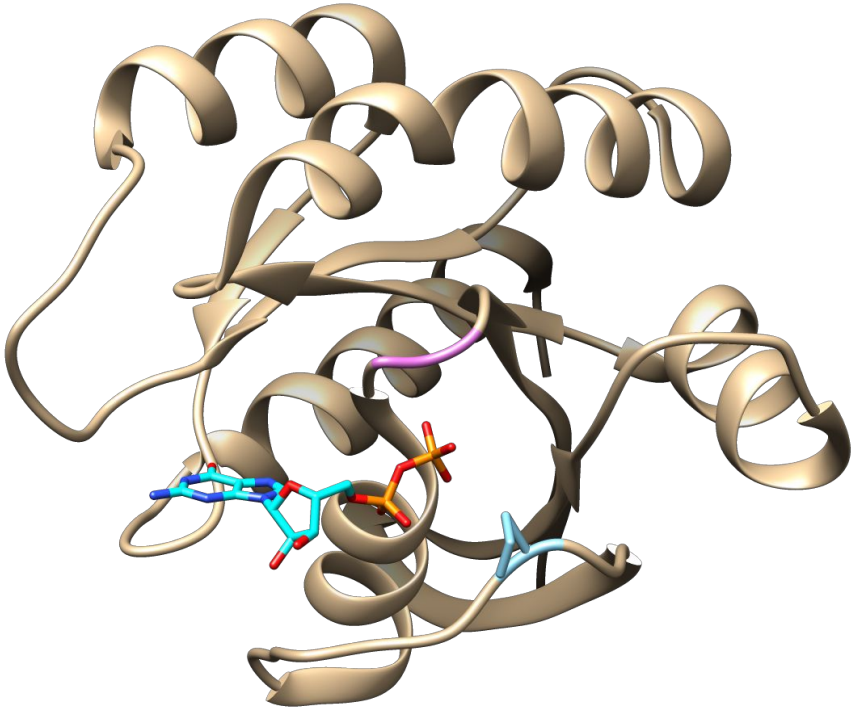


Ras-RasGAP interactions that stabilize Ras switch II and catalytic residues

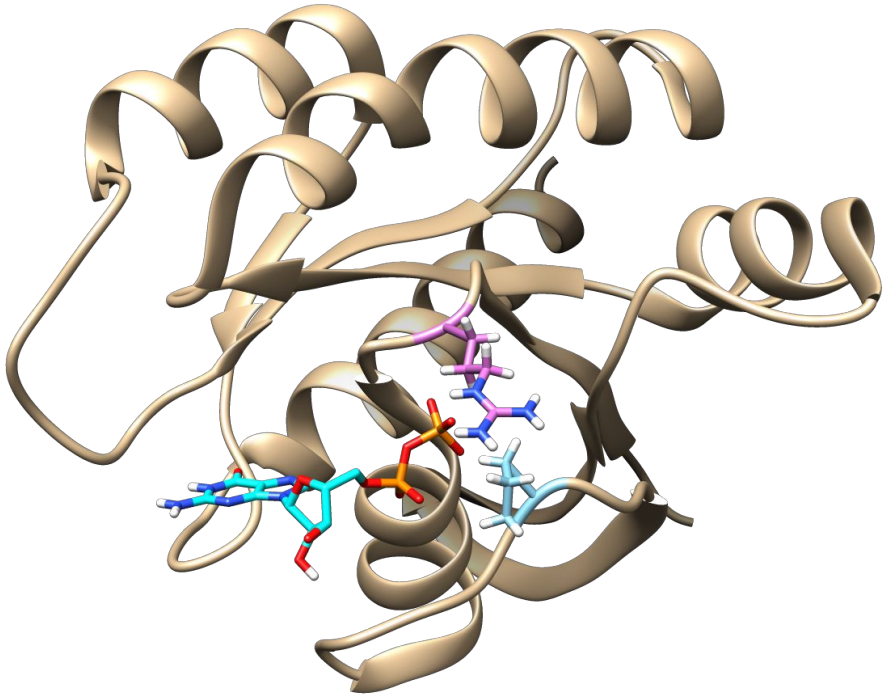
RAS MUTATIONS

Mutations that decrease GAP sensibility

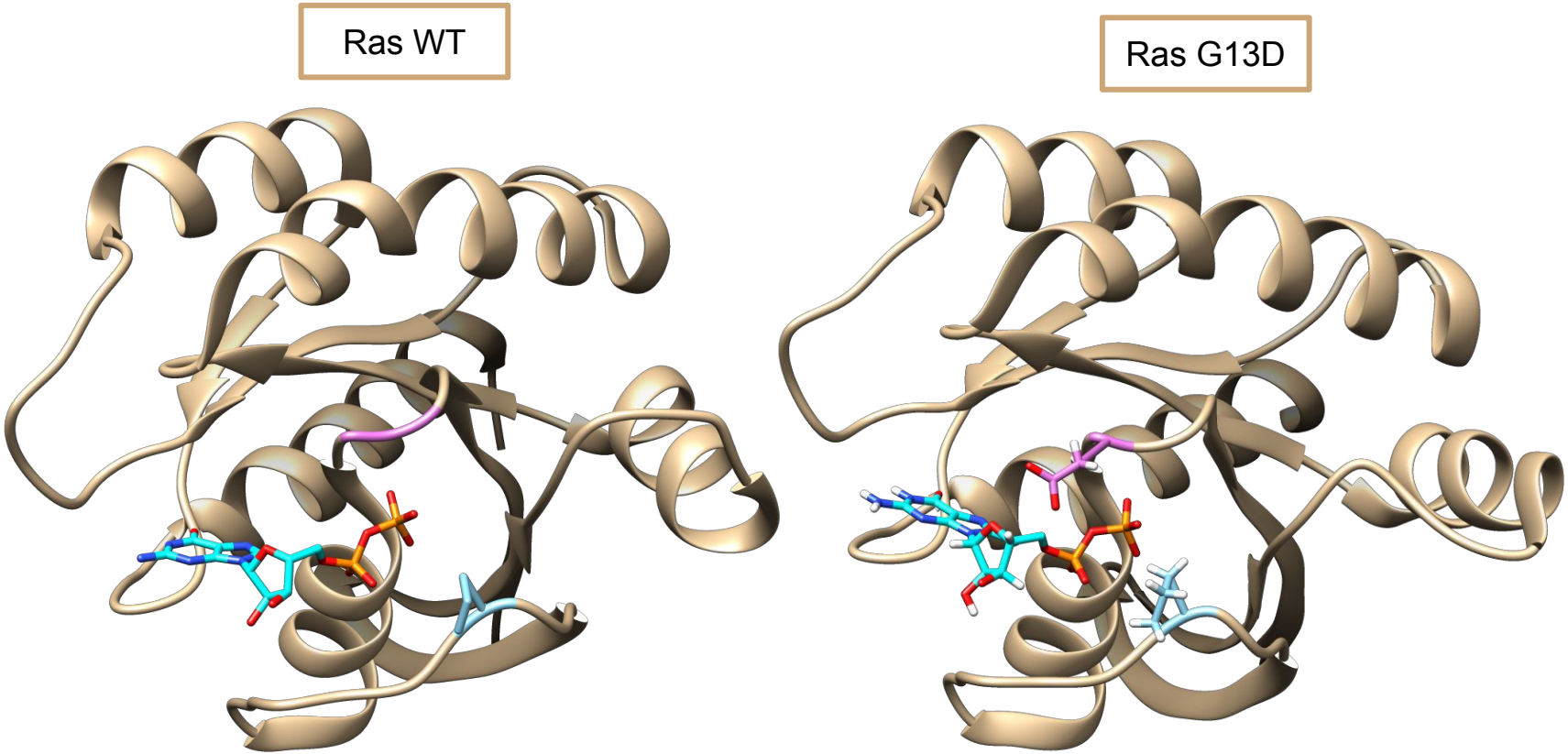
Ras WT



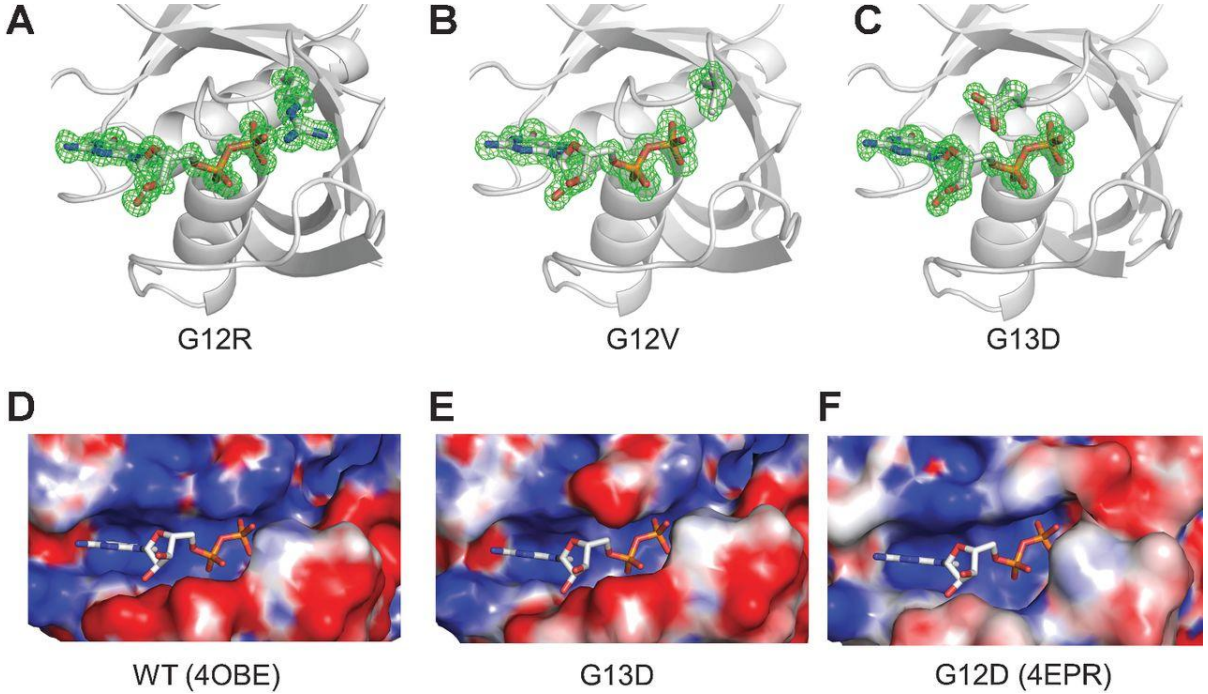
Ras G12R



Mutations that decrease GAP sensibility



Impact of mutations in charge distribution



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PEM questions

1. G protein-coupled receptors:
   a. Contain 7 transmembrane helices.
   b. Are involved in very few signaling pathways.
   c. Are not targeted by any drug.
   d. Inactivate the G protein when a ligand binds to them.
   e. Are only found in fish.

2. Choose the **correct** answers related to tyrosine kinases:
   a. Protein kinases catalyse the transfer of a phosphate group from ATP to a hydroxyl group of a serine or a threonine
   b. The insulin receptor is a tyrosine kinase receptor
   c. A and B are correct
   d. Asp±Phe±Gly motif interfere the ATP binding
   e. All of the answers are correct

3. Which of the domains of EGFR is in charge of the dimerization of the receptor?
   a. Domain I
   b. Domain III
   c. A and B are correct
   d. Domain II
   e. Domain IV

PEM questions

4. Choose the **wrong** answer related to the class III Receptor Tyrosine Kinases (RTKs):
   a. PDGFR alpha and beta belong to RTKs.
   b. They have a kinase insert between between two kinases domains.
   c. They are characterized by a 5-Ig-domain extracellular segment.
   d. The kinase domain is located inside the cell.
   e. Insulin Receptor belongs to class III RTKs.

5. Related to insulin binding to its receptor, choose the right statement:
   a. The receptor dimerizes after insulin binds to it.
   b. The alpha and beta subunits of the receptor are bound through multiple disulfide bonds.
   c. The insulin receptor has two binding sites for insulin.
   d. The fibronectin type III domains (FnIII) of the insulin receptor contain alpha-helices.
   e. The alpha-CT domain is not important for insulin binding.

6. PDGF receptors:
   a. They belong to the class IV Receptor Tyrosine Kinases (RTKs).
   b. PDGFRα signaling is not important in the development of many organs.
   c. The Juxtamembrane domain is widely studied.
   d. The residues involved in the dimerization process are well conserved between different species.
   e. PDGFRs need the dimerization of the receptor but they don't need any transphosphorylation.

PEM questions

7. Choose the correct answer related to SOS-Ras interaction:
   a. Pleckstrin domain is the one which binds G domain of Ras
   b. All the interactions are between hydrophobic residues
   c. There is no change in Switch I chain
   d. Alpha-H is the one that interacts with the G domain of Ras
   e. Leucine decreases the hydrophobicity of magnesium binding site

8. Ras proteins:
   a. They are composed by three domains
   b. Switch I is the most conserved motif
   c. They are small GTPase proteins
   d. Ras-GTP form is the inactivated one
   e. Threonine 35 binds the guanine base

9. Choose the **wrong** answer related to Ras inactivation:
   a. Ras proteins require GAP effectors to get inactivated.
   b. The inactivation consists of the hydrolysation of the gamma phosphate of GTP.
   c. Ras proteins do not have hydrolysis activity.
   d. The main catalytic residue is Gln61.
   e. A partial negative charge develops during the process.

10. Choose the correct statement of Ras mutations:
    a. They cause the same effects in GAP affinity than in GAP sensitivity.
    b. Almost every mutation on residue 12 is oncogenic.
    c. They can make up to a 50% decrease in the GAP-stimulated hydrolysis rate compared to wt Ras.
    d. They cause drastic changes on Ras structure.
    e. They only affect to Ras-RasGAP complex.

G proteins and GPCRs

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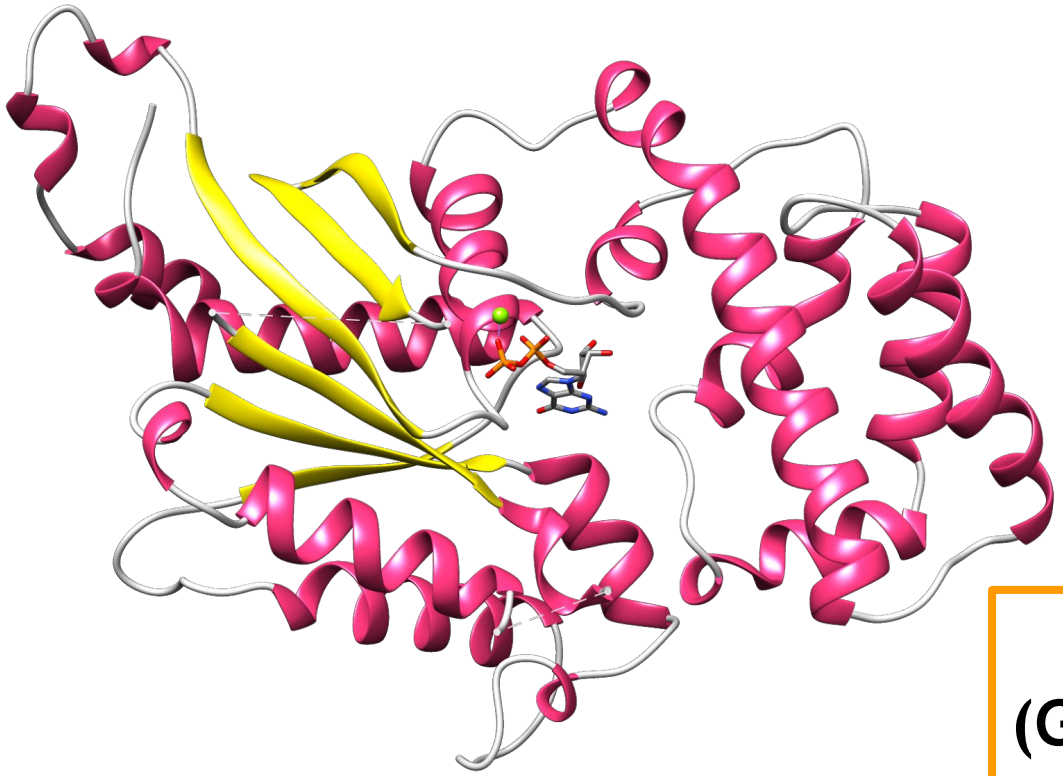
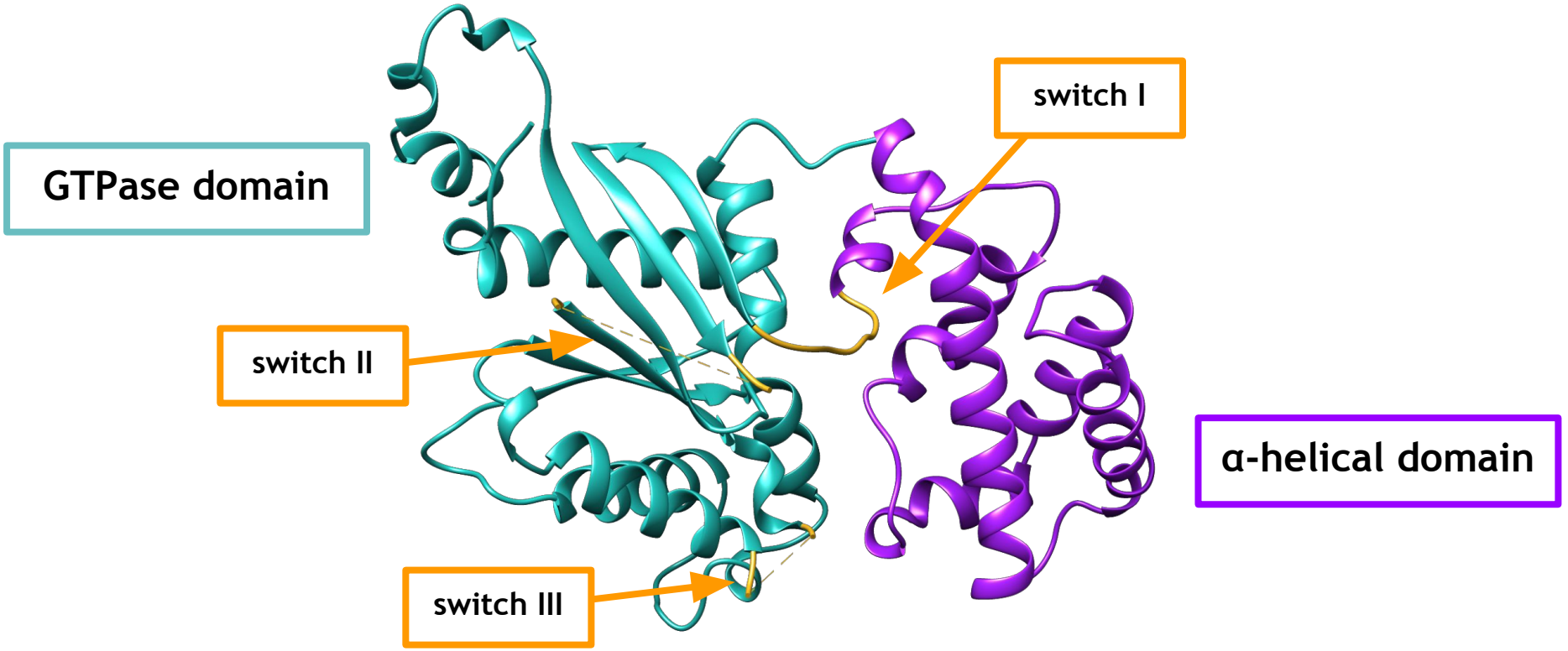
Annex - Structure of the G_{α} subunit



α-subunit
(Gα$_i$ GDP-bound)

Annex - Structure of the G_α subunit



Annex - Structure of the ß subunit

ß-subunit
(ß propeller)