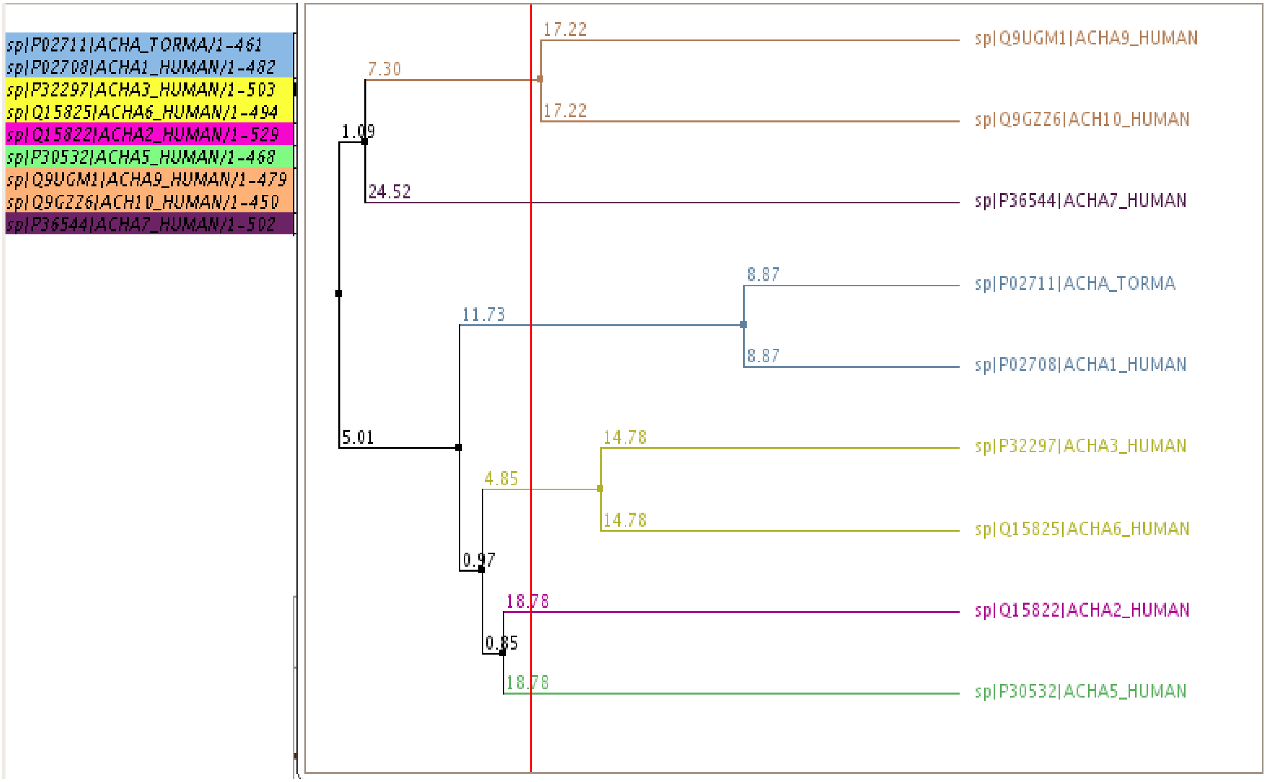
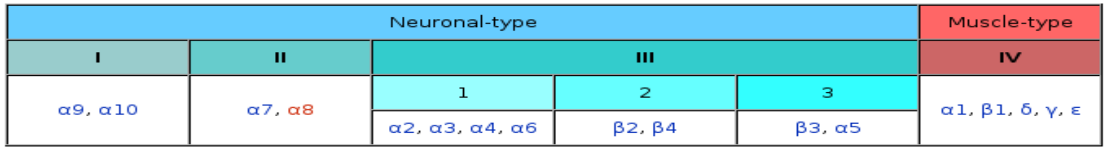
EVOLUTION OF α SUBUNITS IN CYS-LOOP FAMILIES

- HUMAN NICOTINIC α SUBUNITS
- HUMAN α SUBUNITS (EXTENDED)

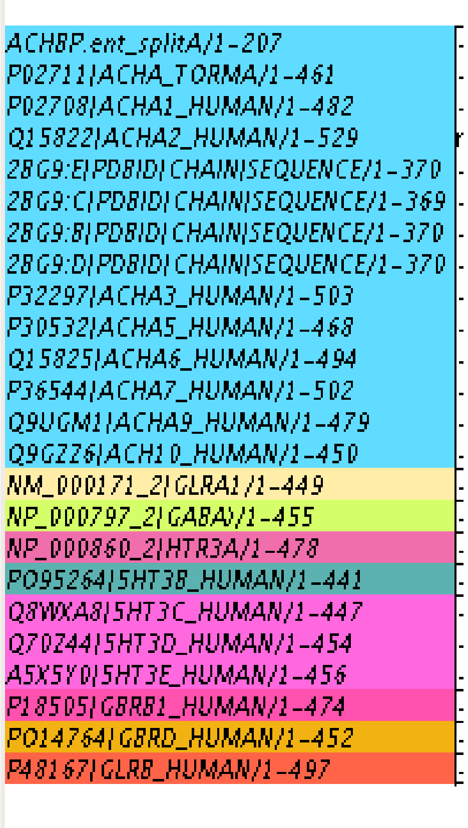
HUMAN NICOTINIC α SUBUNITS



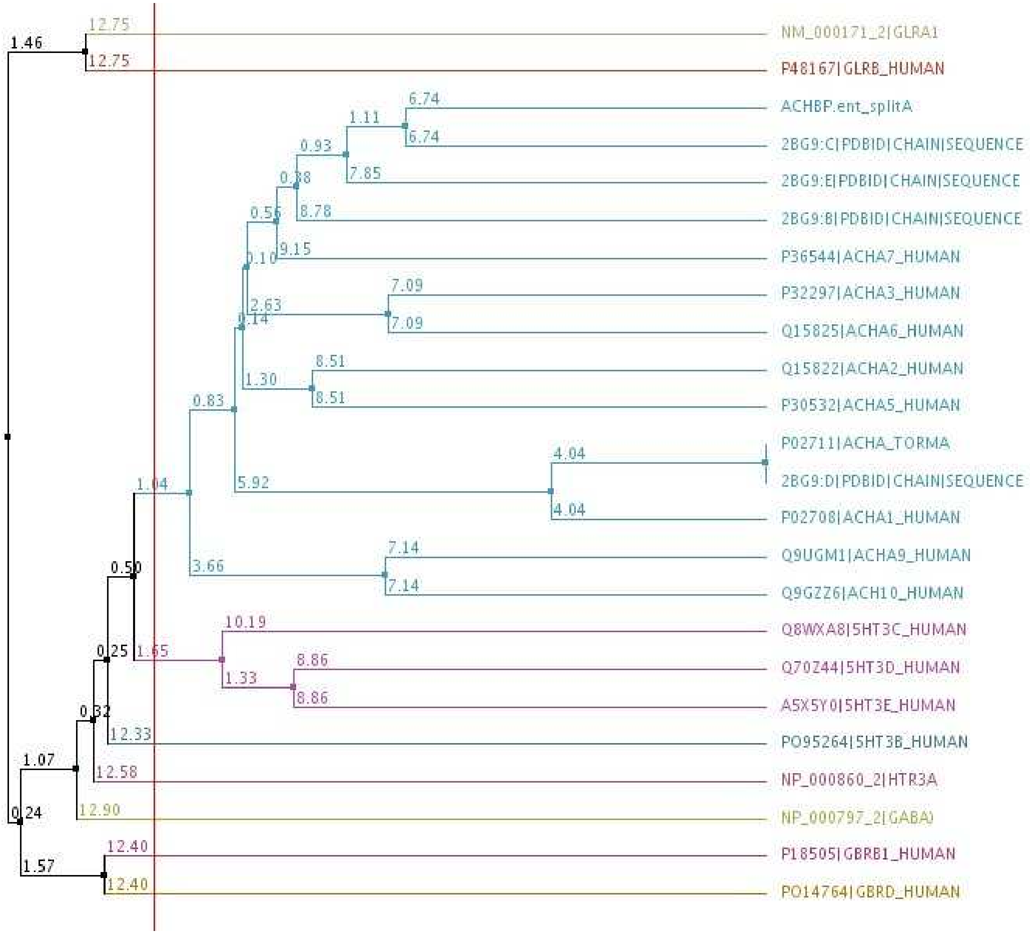


Neuronal-type					Muscle-type
I	II	III			IV
α9, α10	α7, α8	1	2	3	α1, β1, δ, γ, ε
		α2, α3, α4, α6	β2, β4	β3, α5	

HUMAN α SUBUNITS (EXTENDED)



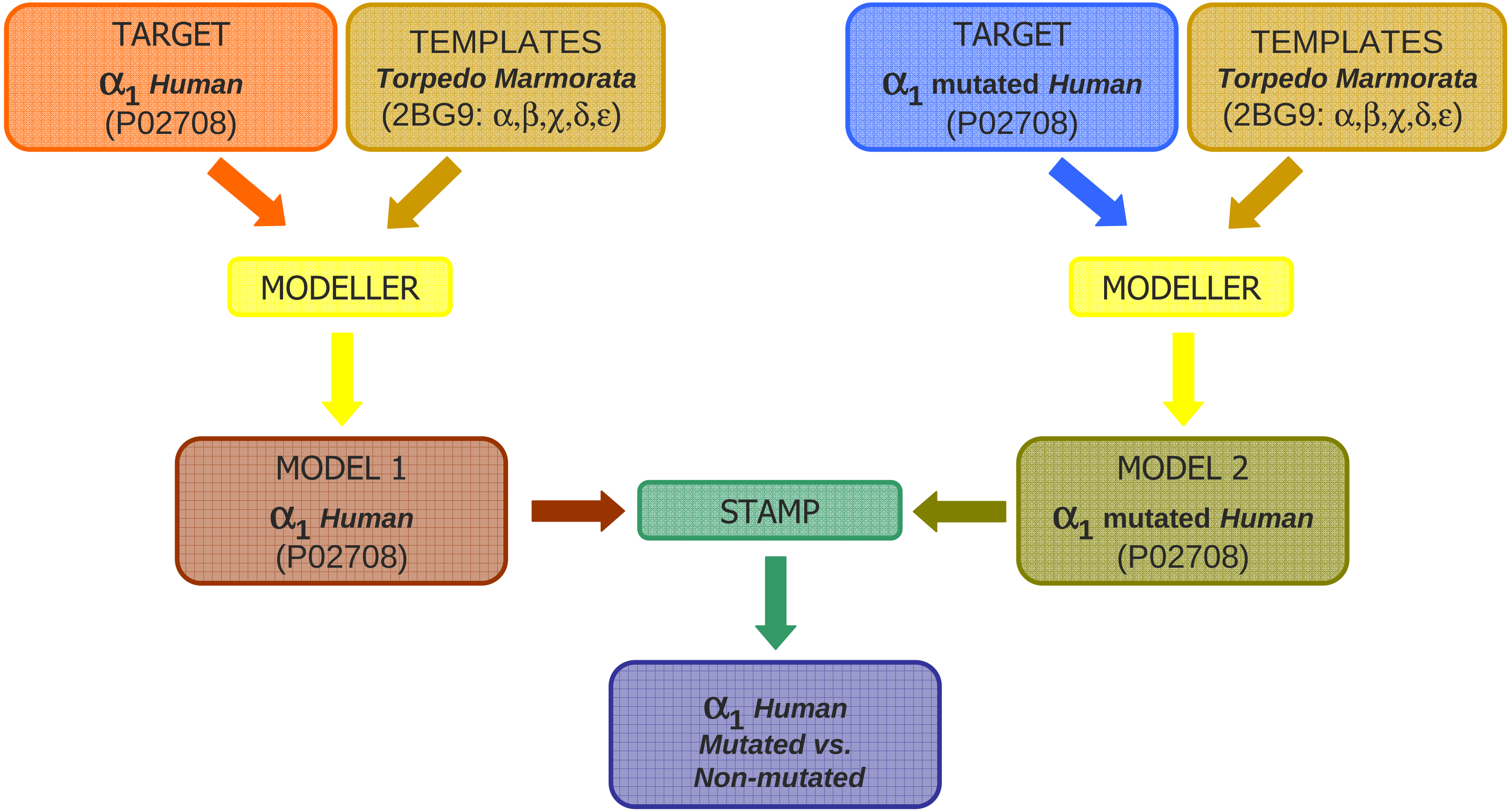
ACHBP.ent_splitA/1-207
P02711|ACHA_TORMA/1-461
P02708|ACHA1_HUMAN/1-482
Q15822|ACHA2_HUMAN/1-529
2BG9:E|PDBID|CHAIN|SEQUENCE/1-370
2BG9:C|PDBID|CHAIN|SEQUENCE/1-369
2BG9:B|PDBID|CHAIN|SEQUENCE/1-370
2BG9:D|PDBID|CHAIN|SEQUENCE/1-370
P32297|ACHA3_HUMAN/1-503
P30532|ACHA5_HUMAN/1-468
Q15825|ACHA6_HUMAN/1-494
P36544|ACHA7_HUMAN/1-502
Q9UGM1|ACHA9_HUMAN/1-479
Q9GZZ6|ACH10_HUMAN/1-450
NM_000171_2|GLRA1/1-449
NP_000797_2|GABA)/1-455
NP_000860_2|HTR3A/1-478
PO95264|5HT3B_HUMAN/1-441
Q8WXA8|5HT3C_HUMAN/1-447
Q70Z44|5HT3D_HUMAN/1-454
A5X5Y0|5HT3E_HUMAN/1-456
P18505|GBRB1_HUMAN/1-474
PO14764|GBRD_HUMAN/1-452
P48167|GLRB_HUMAN/1-497



STUDY OF PUNCTUAL MUTATIONS IN NICOTINIC α SUBUNITS

- Work Flow
- Human α subunit model
- Human α mutated subunit models
- Comparison of α-mutated vs. α-non-mutated region

STUDY OF MUTATIONS: WORK FLOW



TARGET
α_1 ***Human***
(P02708)

TEMPLATES
Torpedo Marmorata
(2BG9: α,β,χ,δ,ε)

MODELLER

MODEL 1
α_1 ***Human***
(P02708)

TARGET
α_1 **mutated *Human***
(P02708)

TEMPLATES
Torpedo Marmorata
(2BG9: α,β,χ,δ,ε)

MODELLER

MODEL 2
α_1 **mutated *Human***
(P02708)

STAMP

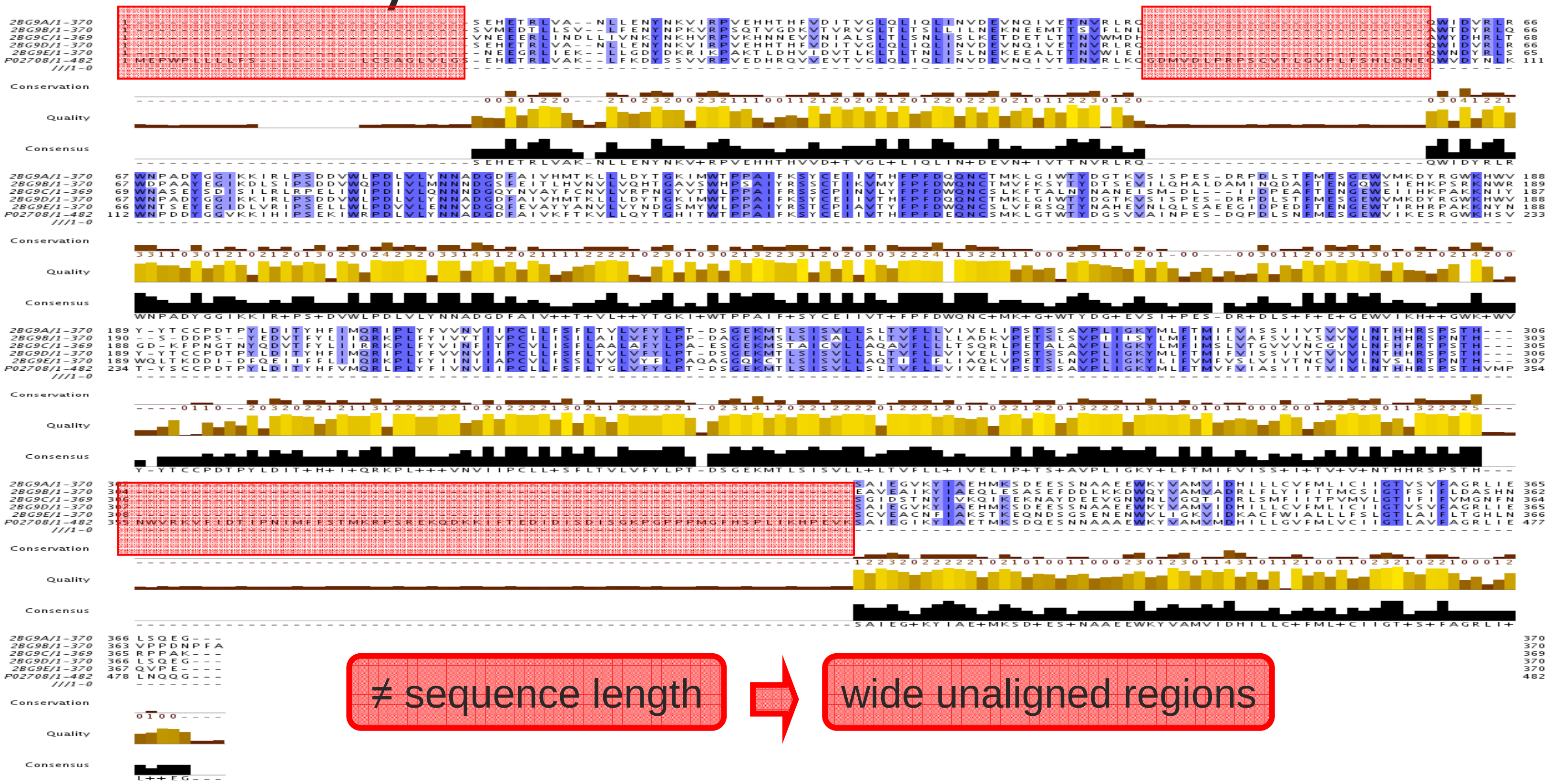
α_1 ***Human***
Mutated vs.
Non-mutated

STUDY OF MUTATIONS

HUMAN α SUBUNIT MODEL

ALIGNMENT 2BG9-HUMAN α_1 MODEL :

70% Identity



≠ sequence length

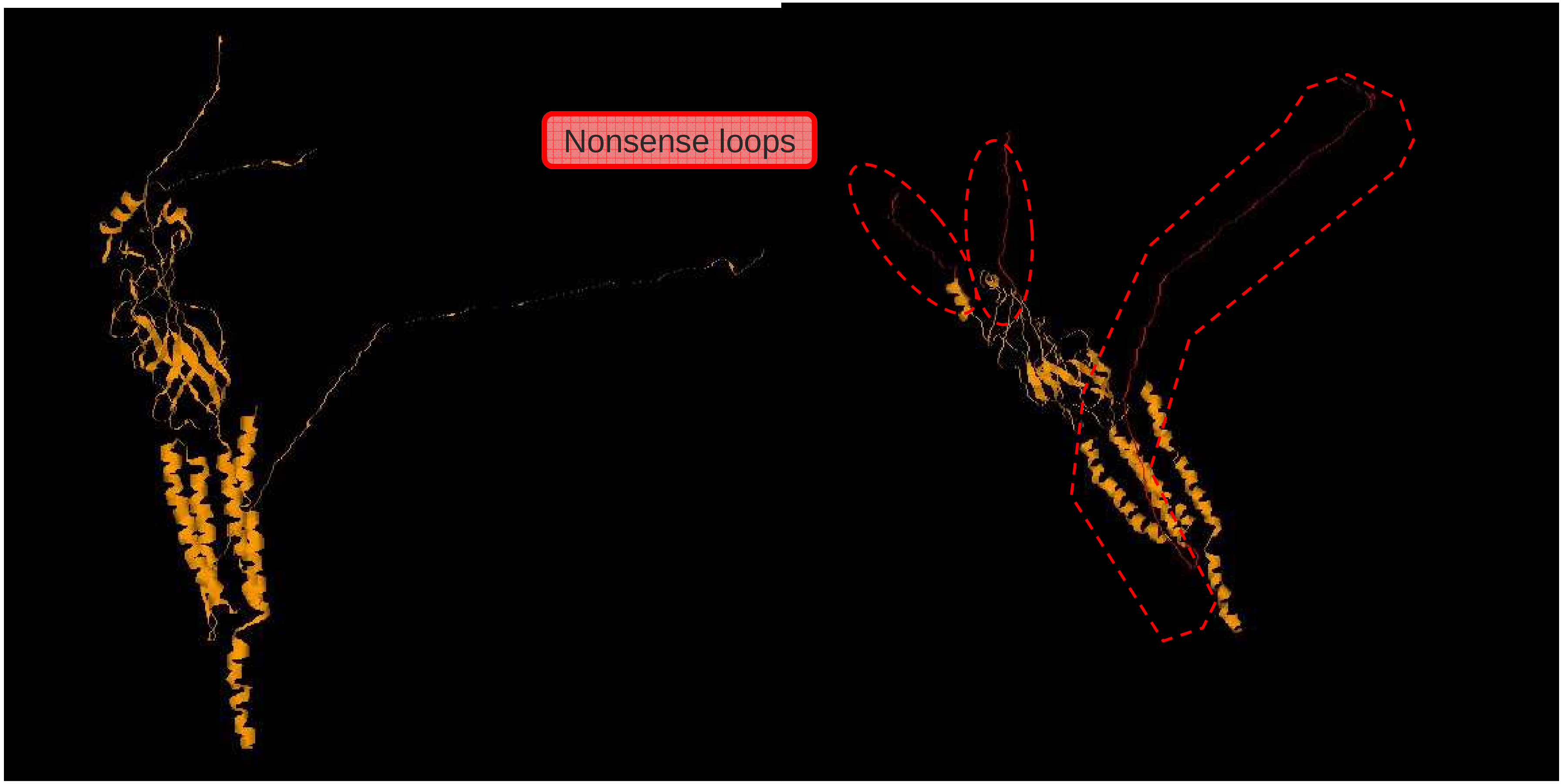
wide unaligned regions

STUDY OF MUTATIONS

HUMAN α SUBUNIT MODEL

3D STRUCTURE:

≠ sequence length ⇨ wide unaligned regions



Nonsense loops

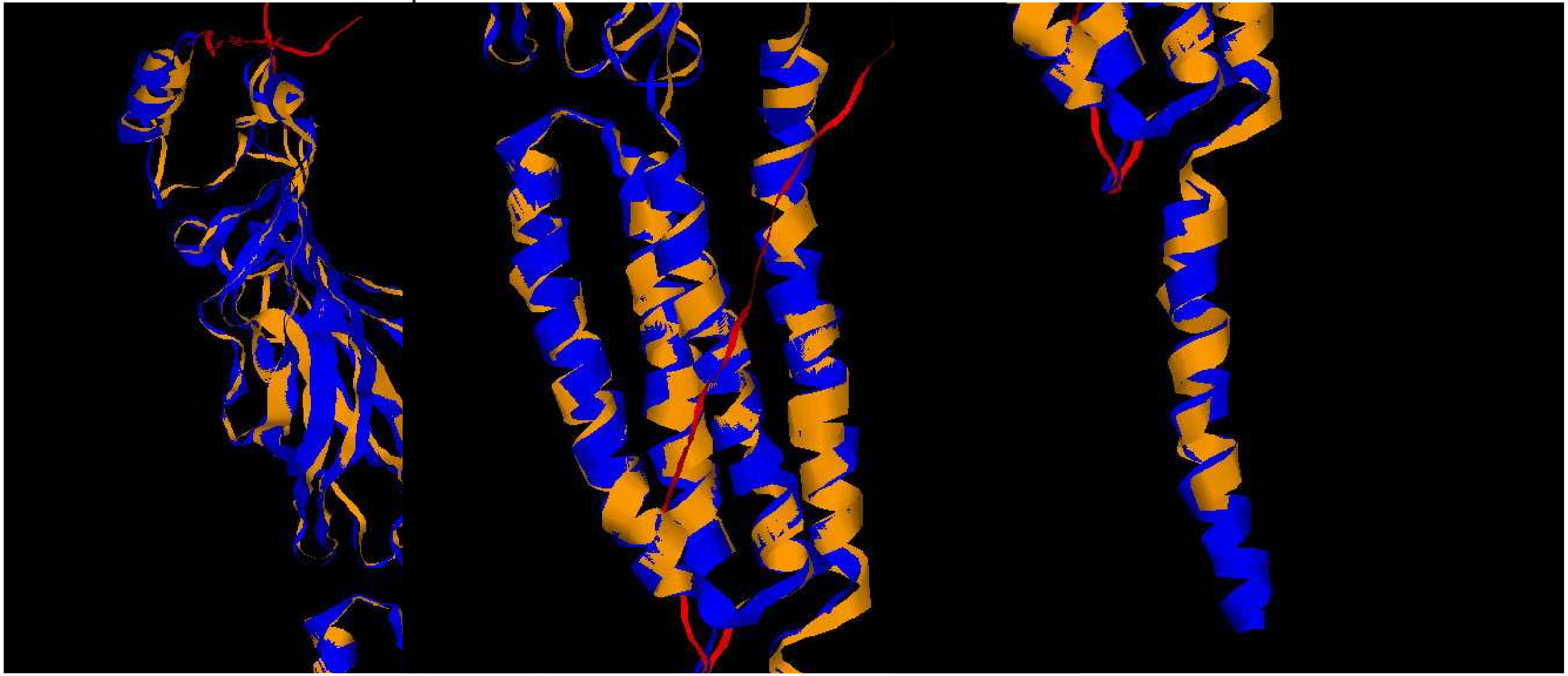
STUDY OF MUTATIONS

HUMAN α SUBUNIT MODEL

SUPERPOSITION

Legend: 2BG9_A HUMAN Model

HUMAN α_1 SUBUNIT MODEL vs 2BG9 CHAIN A

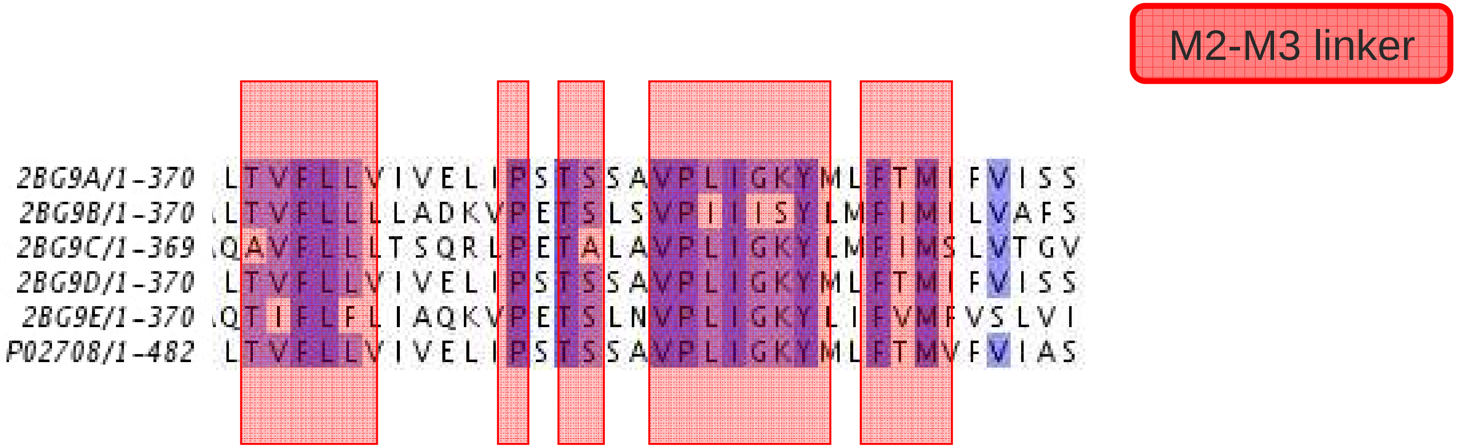


STUDY OF MUTATIONS

HUMAN α SUBUNIT MODEL

SUPERPOSITION

HUMAN α_1 SUBUNIT MODEL vs 2BG9: M2-M3 linker



```
2BG9A/1-370  LTVFLLVIVELIPSTSSAVPLIGKYMLFTMIFVISS
2BG9B/1-370  LTVFLLLLADKVPETSLSVPIIISYLMFIMILVAFS
2BG9C/1-369  QAVFLLLTSQRLPETALAVPLIGKYLMFIMSLVTGV
2BG9D/1-370  LTVFLLVIVELIPSTSSAVPLIGKYMLFTMIFVISS
2BG9E/1-370  QTIFLFLIAQKVPETSLNVPLIGKYLIFVMFVSLVI
P02708/1-482 LTVFLLVIVELIPSTSSAVPLIGKYMLFTMVFVIAS
```

STUDY OF MUTATIONS

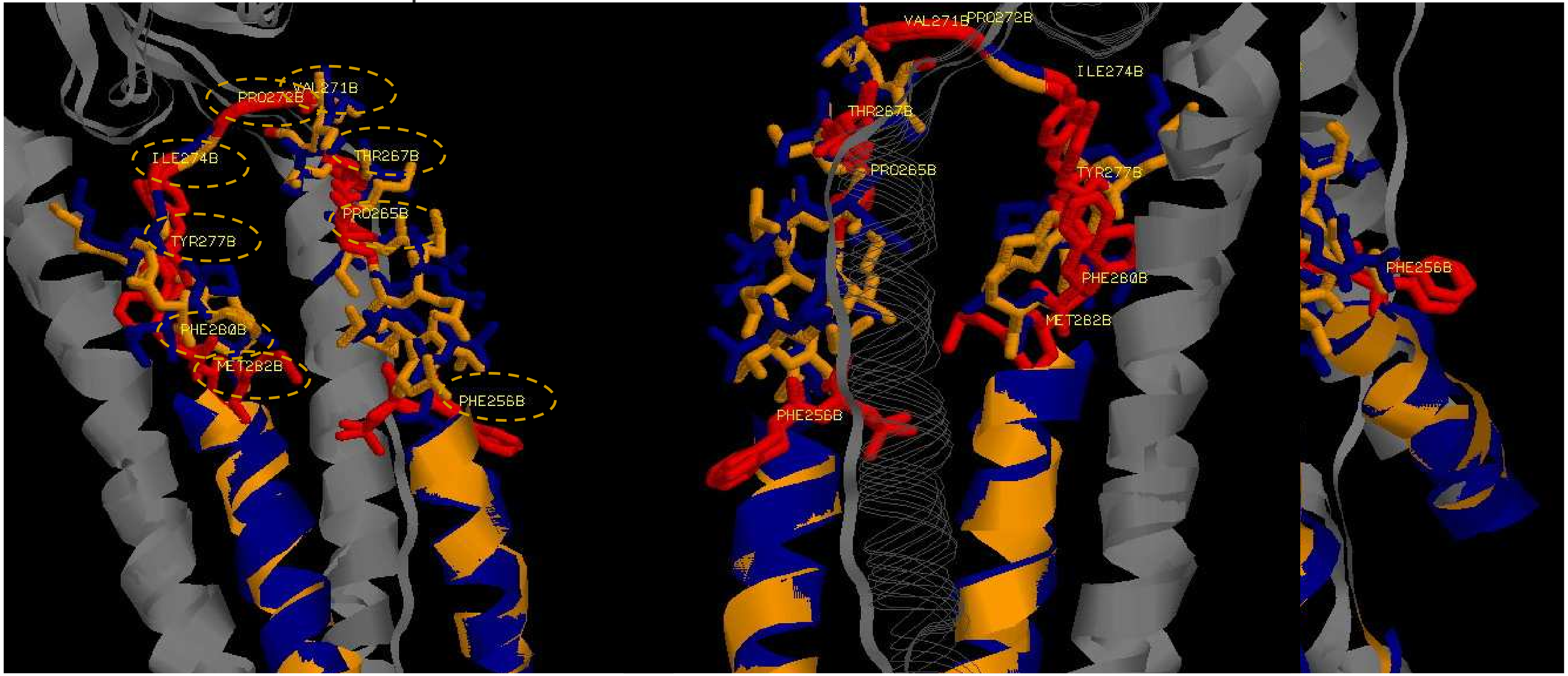
HUMAN α SUBUNIT MODEL

Legend: 2BG9_A HUMAN Model

Fully Conserved

SUPERPOSITION

HUMAN α_1 SUBUNIT MODEL vs 2BG9: M2-M3 linker



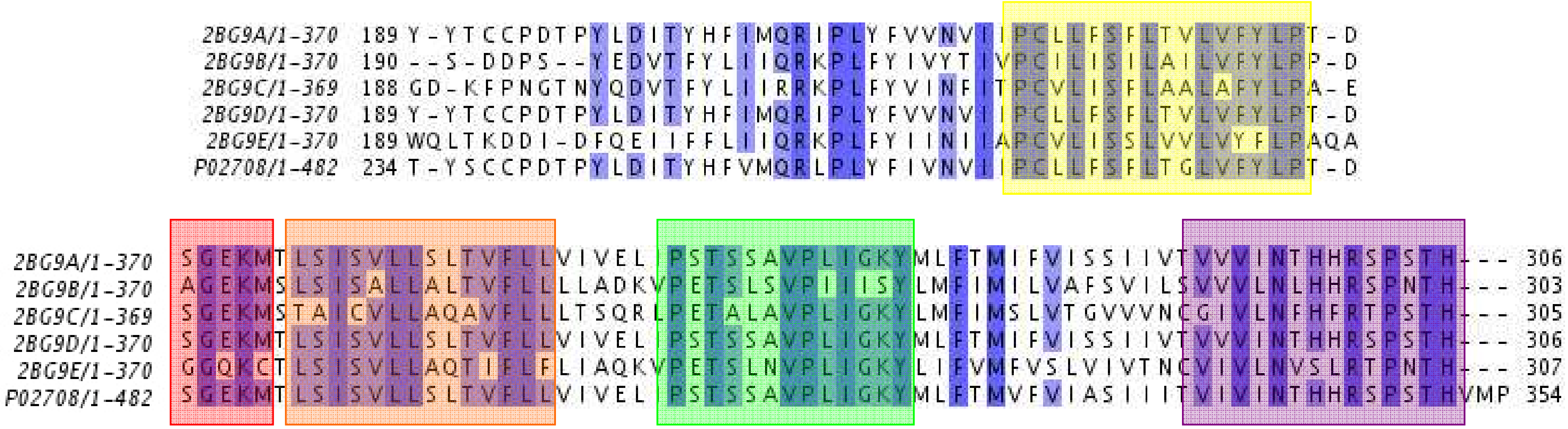
STUDY OF MUTATIONS

HUMAN α SUBUNIT MODEL

SUPERPOSITION

HUMAN α_1 SUBUNIT MODEL vs 2BG9: Opening Mech.

Legend: M1 | M1-M2 linker | M2 | M2-M3 linker | M3



```
2BG9A/1-370  189 Y-YTCCPDTPYLDITYHFIMQRIPLYFVVNVIIPCLLFSFLTVLVFYLPT-D
2BG9B/1-370  190 --S-DDPS--YEDVTFYLIIQRKPLFYIVYTIVPCILISILAILVFYLPP-D
2BG9C/1-369  188 GD-KFPNGTNYQDVTFYLIIRRKPLFYVINFITPCVLISFLAALAFYLPA-E
2BG9D/1-370  189 Y-YTCCPDTPYLDITYHFIMQRIPLYFVVNVIIPCLLFSFLTVLVFYLPT-D
2BG9E/1-370  189 WQLTKDDI-DFQEIIFFLIIQRKPLFYIINIIAPCVLISSLVVLVYFLPAQA
P02708/1-482 234 T-YSCCPDTPYLDITYHFVMQRLPLYFIVNVIIPCLLFSFLTGLVFYLPT-D

2BG9A/1-370  SGEKMTLSISVLLSLTVFLLVIVELIPSTSSAVPLIGKYMLFTMIFVISSIIVTVVVINTHHRSPSTH--- 306
2BG9B/1-370  AGEKMSLSISALLALTVFLLLLADKVPETSLSVPIIISYLMFIMILVAFSVILSVVVLNLHHRSPNTH--- 303
2BG9C/1-369  SGEKMSTAICVLLAQAVFLLLTSQRLPETALAVPLIGKYLMFIMSLVTGVVVNCGIVLNFHFRTPSTH--- 305
2BG9D/1-370  SGEKMTLSISVLLSLTVFLLVIVELIPSTSSAVPLIGKYMLFTMIFVISSIIVTVVVINTHHRSPSTH--- 306
2BG9E/1-370  GGQKCTLSISVLLAQTIFLFLIAQKVPETSLNVPLIGKYLIFVMFVSLVIVTNCVIVLNVSLRTPNTH--- 307
P02708/1-482 SGEKMTLSISVLLSLTVFLLVIVELIPSTSSAVPLIGKYMLFTMVFVIASIIITVIVINTHHRSPSTHVMP 354
```

STUDY OF MUTATIONS

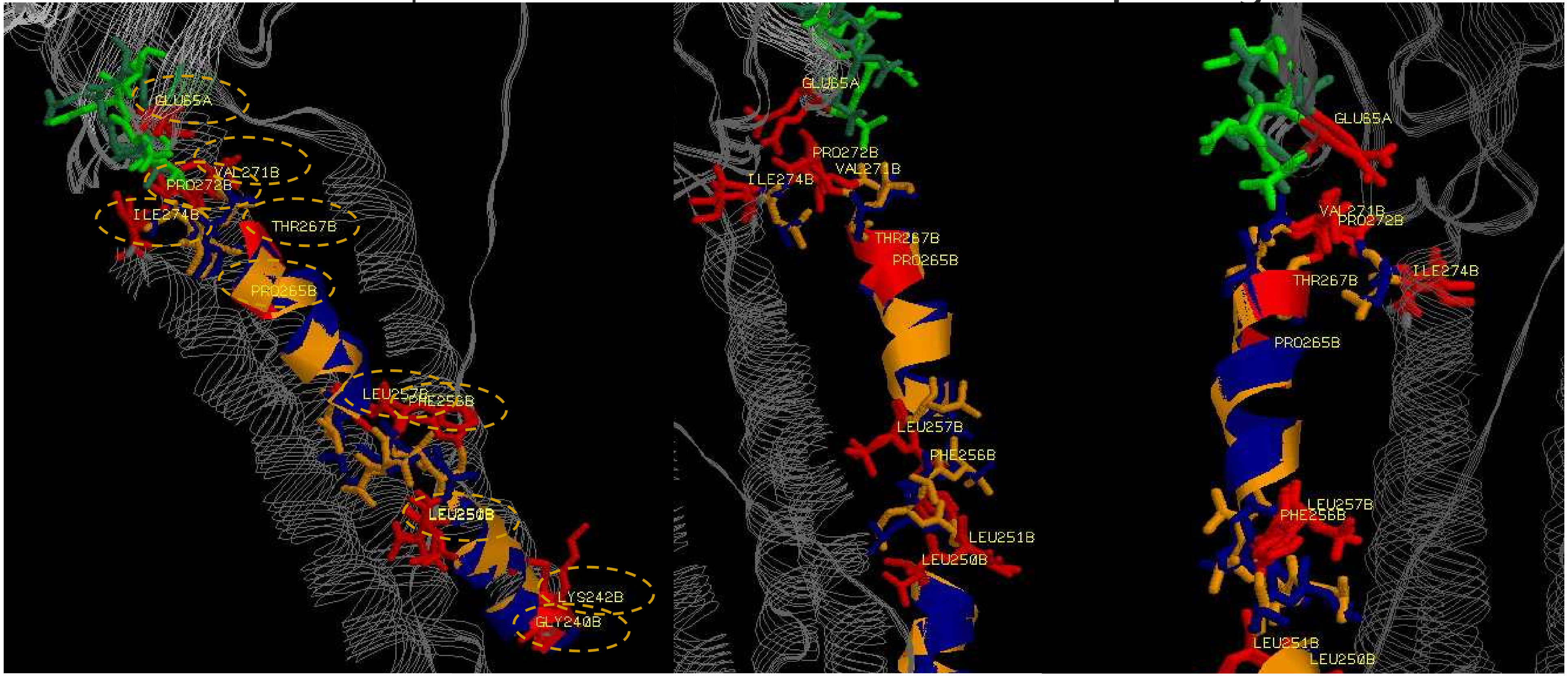
HUMAN α SUBUNIT MODEL

Legend: HUMAN Model | L2-loop Human

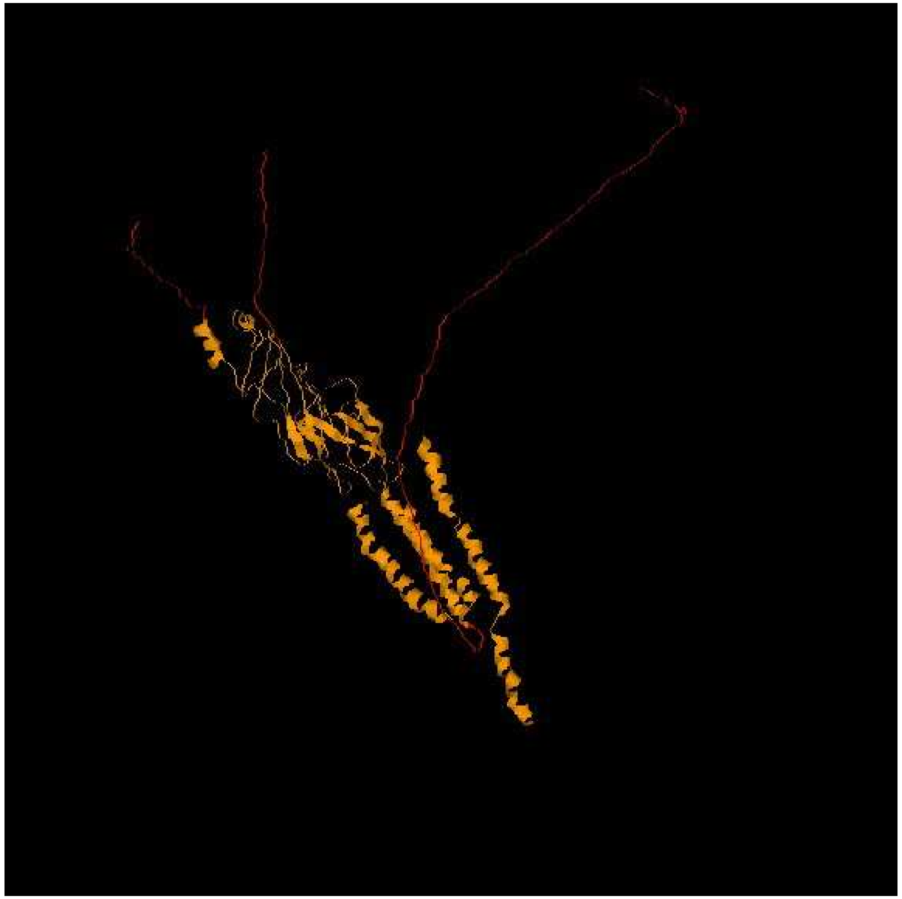
SUPERPOSITION

Fully Conserved | 2BG9_A | L2-loop 2BG9

HUMAN α_1 SUBUNIT MODEL vs 2BG9: Opening Mech.



MODELLING BY HOMOLOGY:
CUSTOM MODEL vs. SWISS-MODEL



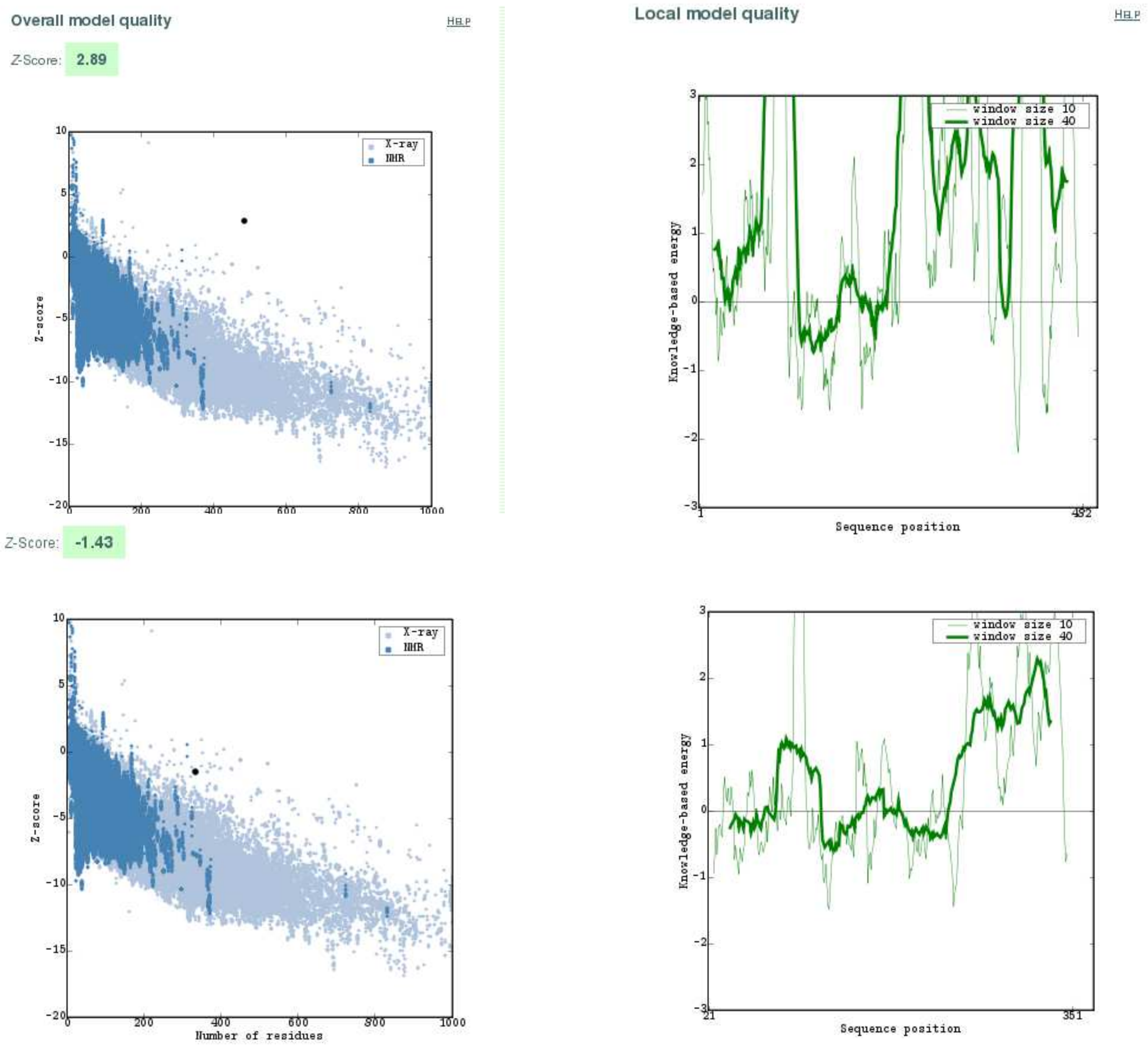
CUSTOM MODEL

SWISS-MODEL

MODEL OF h-α1

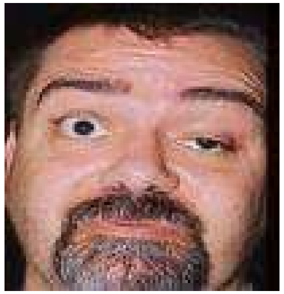
CUSTOM MODEL

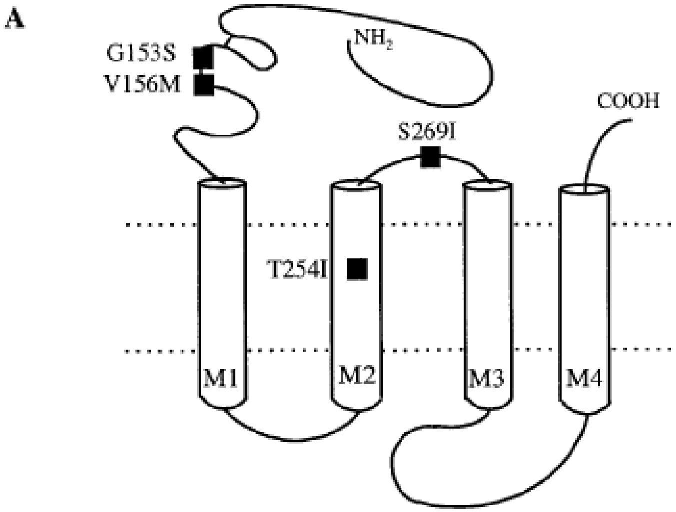
SWISS-MODEL



MUTATIONS

Congenital myasthenic syndromes : slow-channel congenital myasthenic syndrome (SCCMS)





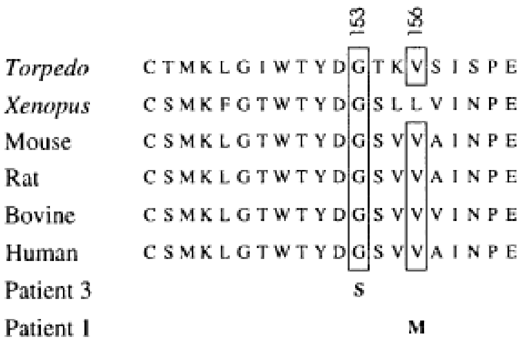
G153S : near binding site

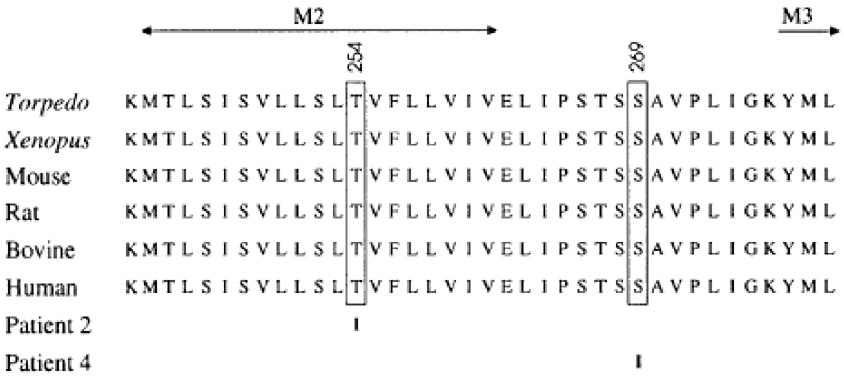
T254I : in the porus (M2)

S269I : in the loop M2-M3

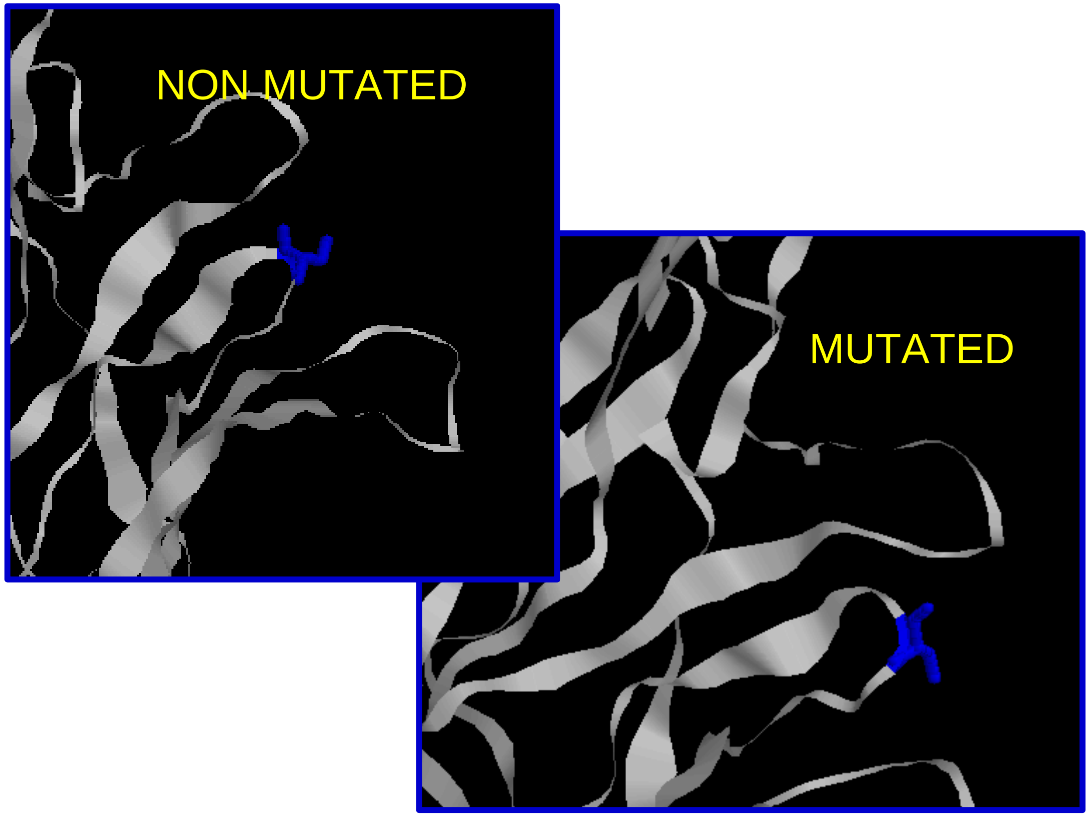
MUTATIONS

- Conserved positions: 153, 254, 269

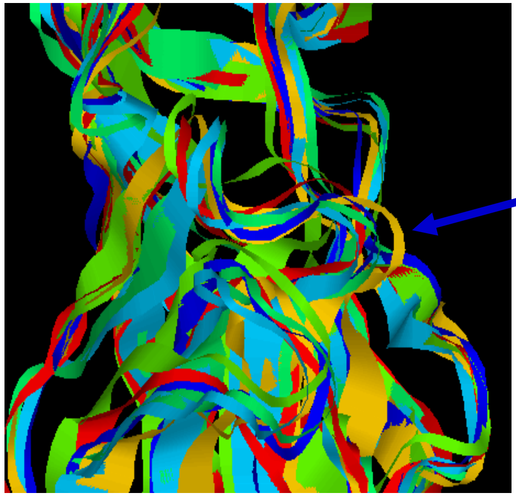




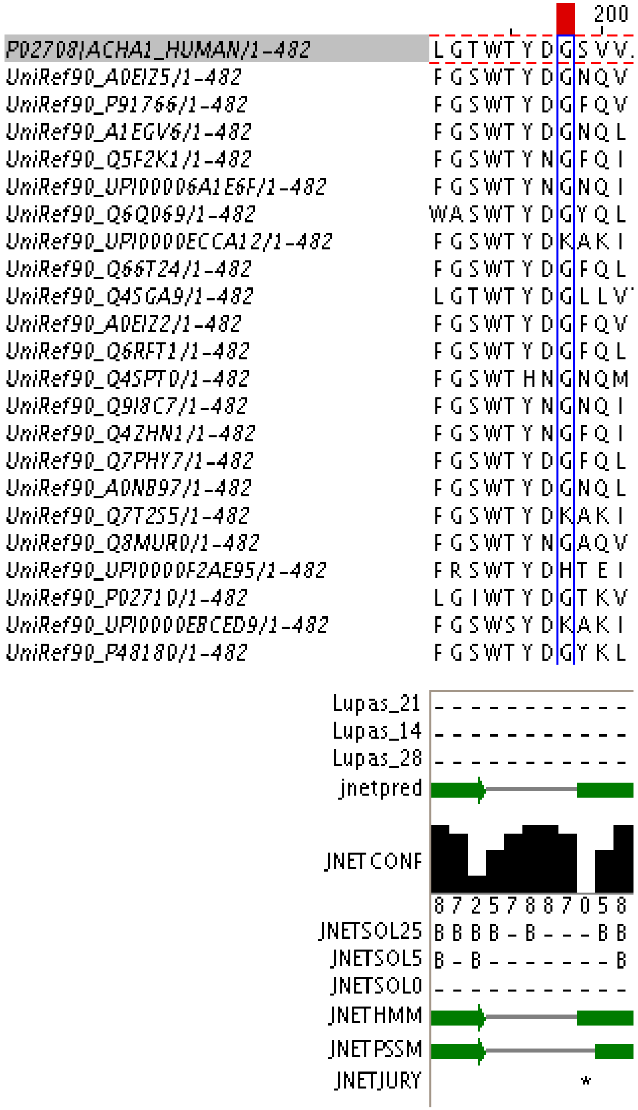
MUTATION G153S



MUTATION G153S

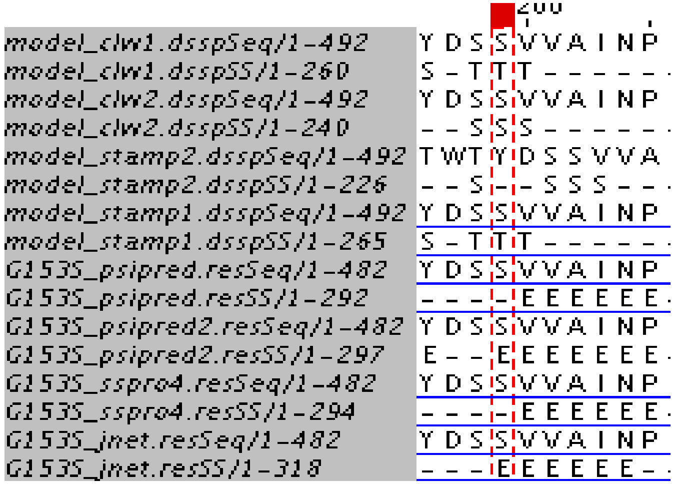


β7---*β8



```
                                              200
P02708|ACHA1_HUMAN/1-482           L G T WT Y D G S V V.
UniRef90_A0EIZ5/1-482              F G S WT Y D G N Q V
UniRef90_P91766/1-482              F G S WT Y D G F Q V
UniRef90_A1EGV6/1-482              F G S WT Y D G N Q L
UniRef90_Q5F2K1/1-482              F G S WT Y N G F Q I
UniRef90_UPI00006A1E6F/1-482       F G S WT Y N G N Q I
UniRef90_Q6Q069/1-482              WA S WT Y D G Y Q L
UniRef90_UPI0000ECCA12/1-482       F G S WT Y D K A K I
UniRef90_Q66T24/1-482              F G S WT Y D G F Q L
UniRef90_Q4SGA9/1-482              L G T WT Y D G L L V
UniRef90_A0EIZ2/1-482              F G S WT Y D G F Q V
UniRef90_Q6RFT1/1-482              F G S WT Y D G F Q L
UniRef90_Q4SPT0/1-482              F G S WT H N G N Q M
UniRef90_Q9I8C7/1-482              F G S WT Y N G N Q I
UniRef90_Q4ZHN1/1-482              F G S WT Y N G F Q I
UniRef90_Q7PHY7/1-482              F G S WT Y D G F Q L
UniRef90_A0N897/1-482              F G S WT Y D G N Q L
UniRef90_Q7T2S5/1-482              F G S WT Y D K A K I
UniRef90_Q8MUR0/1-482              F G S WT Y N G A Q V
UniRef90_UPI0000F2AE95/1-482       F R S WT Y D H T E I
UniRef90_P02710/1-482              L G I WT Y D G T K V
UniRef90_UPI0000EBCED9/1-482       F G S WS Y D K A K I
UniRef90_P48180/1-482              F G S WT Y D G Y K L

      Lupas_21   - - - - - - - - - - -
      Lupas_14   - - - - - - - - - - -
      Lupas_28   - - - - - - - - - - -
      jnetpred
      JNETCONF
                 8 7 2 5 7 8 8 7 0 5 8
    JNETSOL25    B B B B - B - - - B B
     JNETSOL5    B - B - - - - - - - B
     JNETSOL0    - - - - - - - - - - -
      JNETHMM
     JNETPSSM
     JNETJURY                    *
```

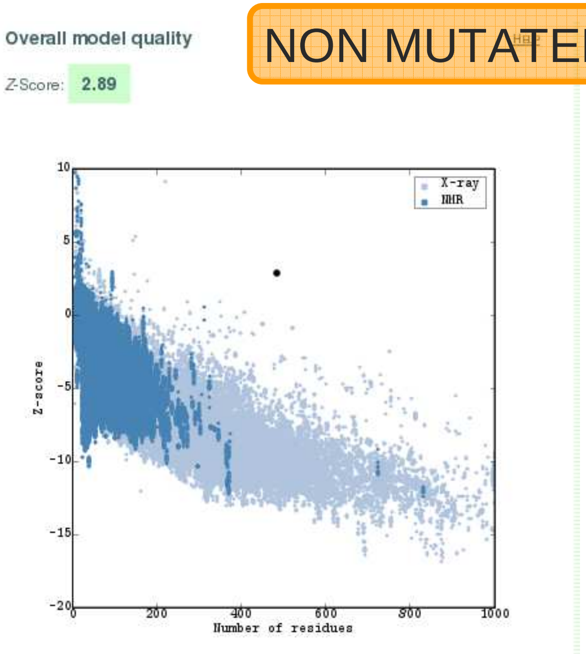


```
                                    200
model_clw1.dsspSeq/1-492        Y D S S V V A I N P
model_clw1.dsspSS/1-260         S - T T T - - - - - -
model_clw2.dsspSeq/1-492        Y D S S V V A I N P
model_clw2.dsspSS/1-240         - - S S S - - - - - -
model_stamp2.dsspSeq/1-492      T WT Y D S S V V A
model_stamp2.dsspSS/1-226       - - S - - S S S - - -
model_stamp1.dsspSeq/1-492      Y D S S V V A I N P
model_stamp1.dsspSS/1-265       S - T T T - - - - - -
G153S_psipred.resSeq/1-482      Y D S S V V A I N P
G153S_psipred.resSS/1-292       - - - - E E E E E E
G153S_psipred2.resSeq/1-482     Y D S S V V A I N P
G153S_psipred2.resSS/1-297      E - - E E E E E E E
G153S_sspro4.resSeq/1-482       Y D S S V V A I N P
G153S_sspro4.resSS/1-294        - - - - E E E E E E
G153S_jnet.resSeq/1-482         Y D S S V V A I N P
G153S_jnet.resSS/1-318          - - - E E E E E E -
```

T = hyd bonded turn
S=bend
E=strand

MUTATED G153S: Custom model

NON MUTATED

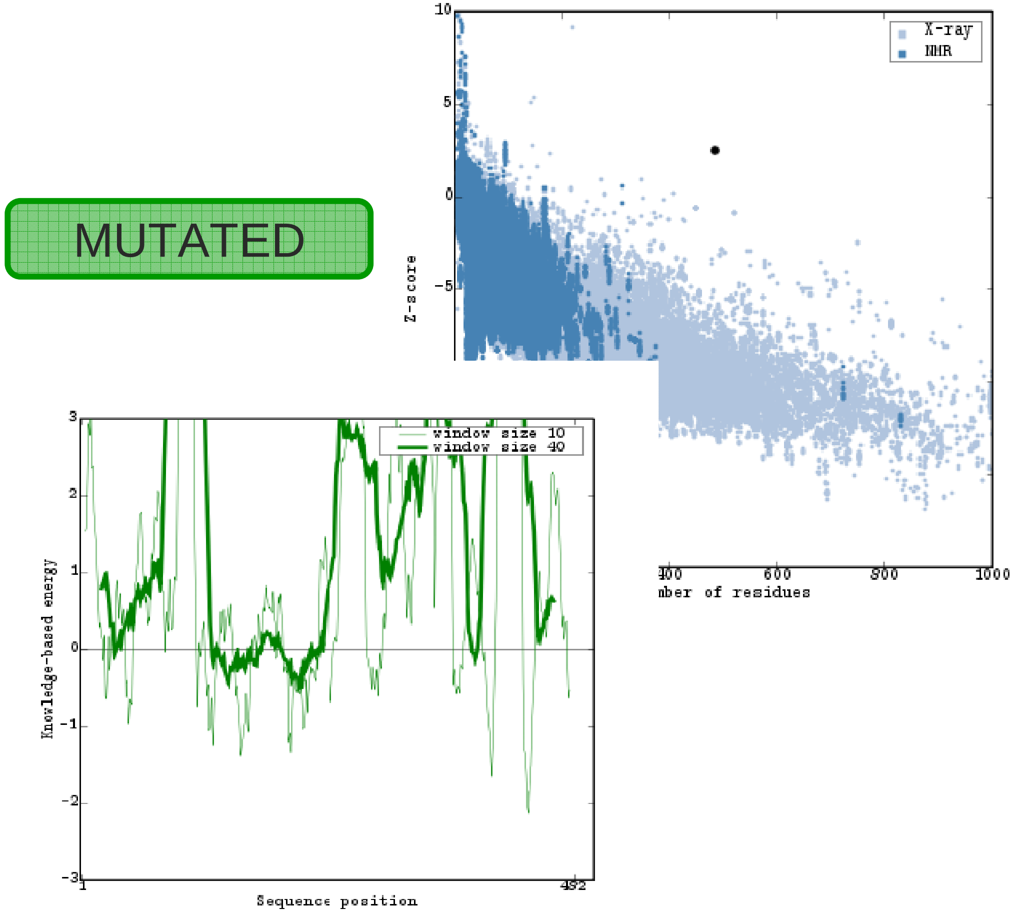


Overall model quality

Z-Score: 2.89

MUTATED

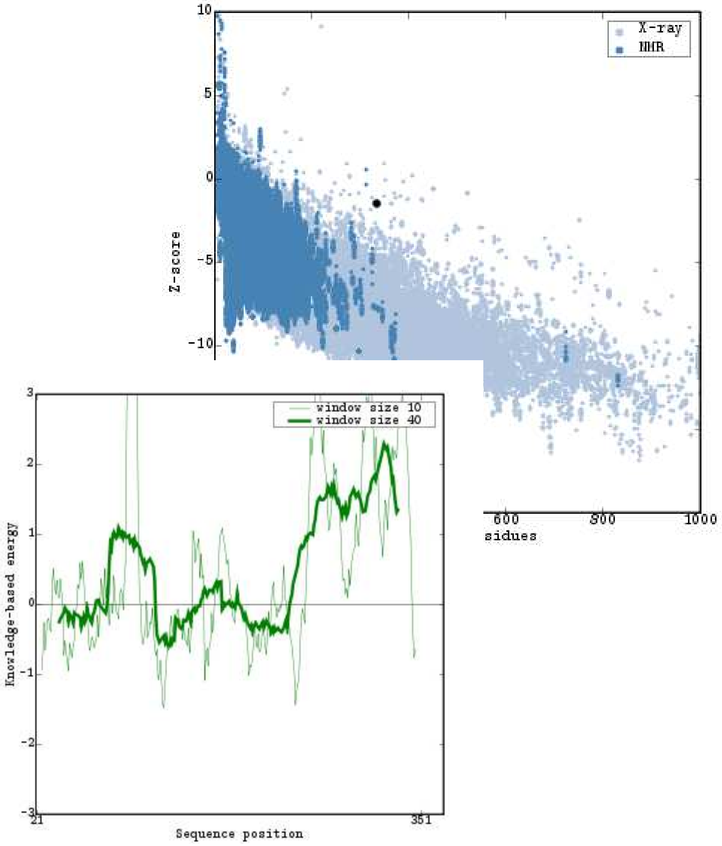
Z-score : 2.54



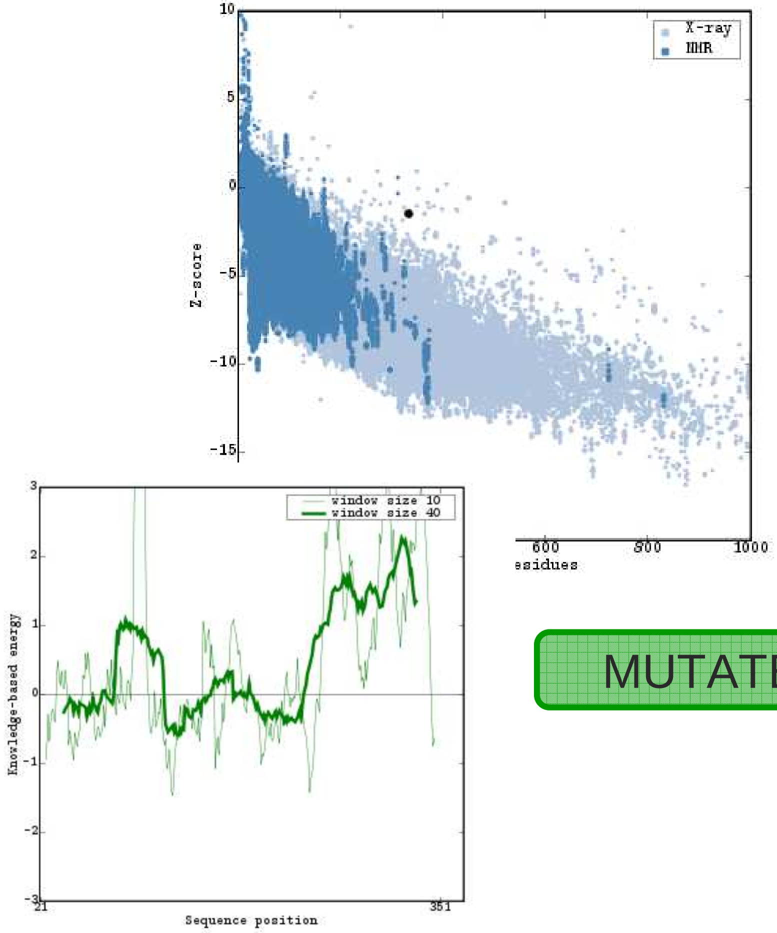
MUTATED G153S: Swiss model

Z-Score: **-1.43**

NON MUTATED

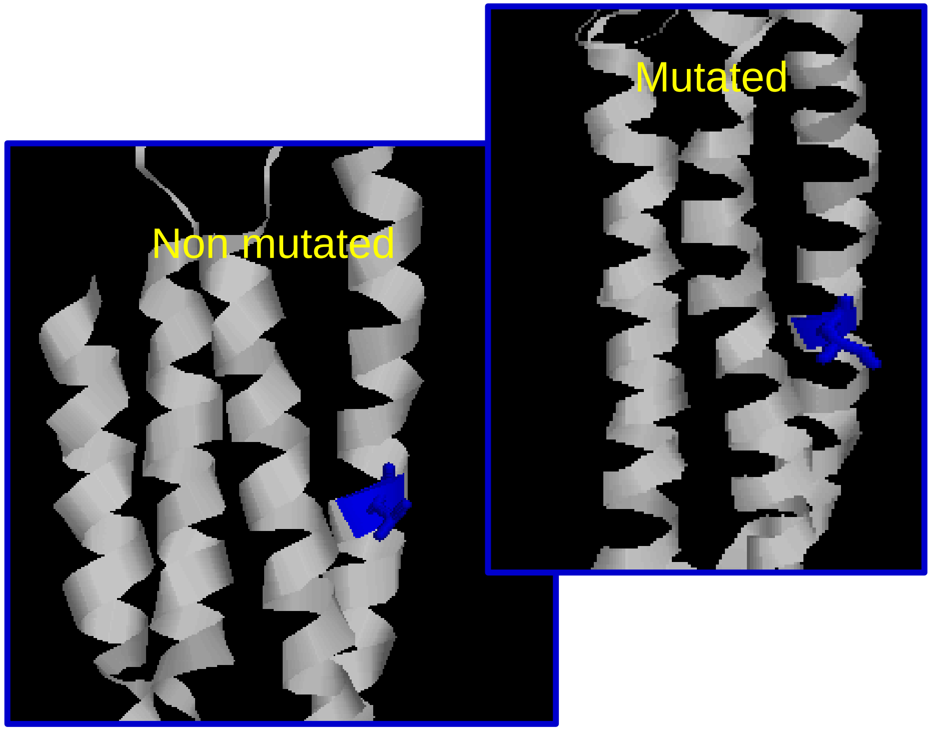


Z-Score: **-1.45**

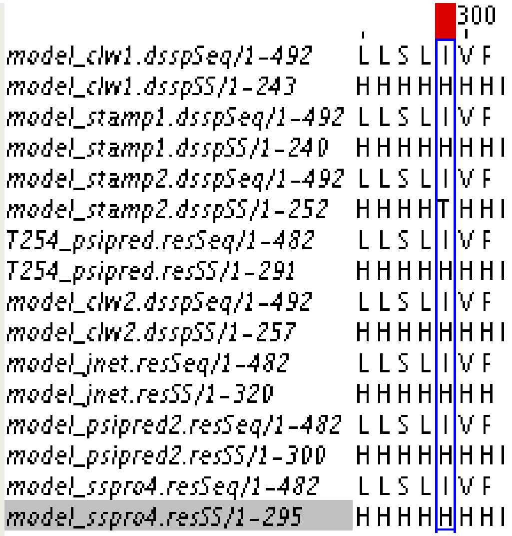


MUTATED

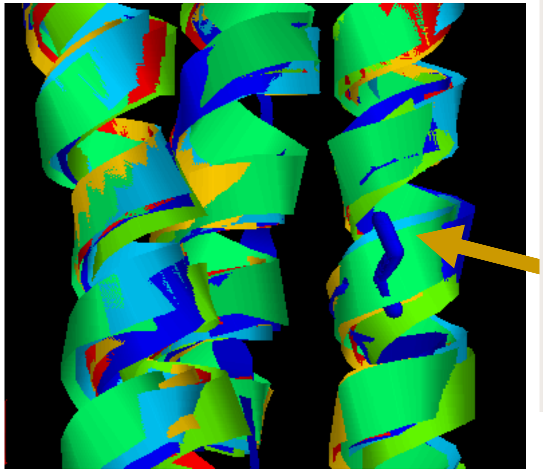
MUTATION T254I

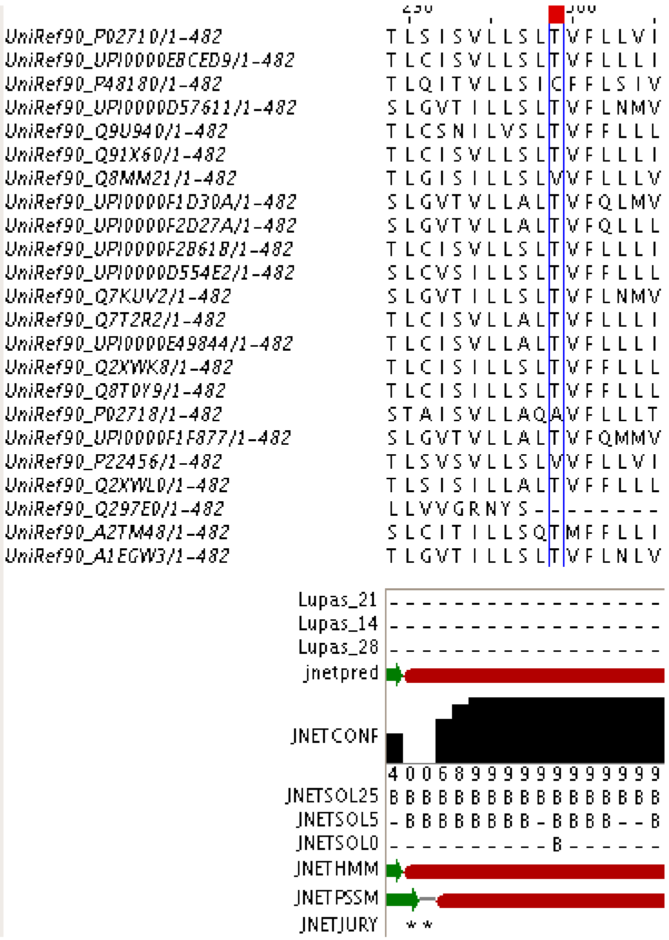


MUTATION T254I



```
                                   300
model_clw1.dsspSeq/1-492     L L S L I V F
model_clw1.dsspSS/1-243     H H H H H H H I
model_stamp1.dsspSeq/1-492   L L S L I V F
model_stamp1.dsspSS/1-240   H H H H H H H I
model_stamp2.dsspSeq/1-492   L L S L I V F
model_stamp2.dsspSS/1-252   H H H H T H H I
T254_psipred.resSeq/1-482    L L S L I V F
T254_psipred.resSS/1-291    H H H H H H H I
model_clw2.dsspSeq/1-492     L L S L I V F
model_clw2.dsspSS/1-257     H H H H H H H I
model_jnet.resSeq/1-482      L L S L I V F
model_jnet.resSS/1-320      H H H H H H H
model_psipred2.resSeq/1-482  L L S L I V F
model_psipred2.resSS/1-300  H H H H H H H I
model_sspro4.resSeq/1-482    L L S L I V F
model_sspro4.resSS/1-295    H H H H H H H I
```





```
UniRef90_P02710/1-482          T L S I S V L L S L T V F L L V I
UniRef90_UPI0000EBCED9/1-482   T L C I S V L L S L T V F L L L I
UniRef90_P48180/1-482          T L Q I T V L L S I C F F L S I V
UniRef90_UPI0000D57611/1-482   S L G V T I L L S L T V F L N M V
UniRef90_Q9U940/1-482          T L C S N I L V S L T V F F L L L
UniRef90_Q91X60/1-482          T L C I S V L L S L T V F L L L I
UniRef90_Q8MM21/1-482          T L G I S I L L S L V V F L L L V
UniRef90_UPI0000F1D30A/1-482   S L G V T V L L A L T V F Q L M V
UniRef90_UPI0000F2D27A/1-482   S L G V T V L L A L T V F Q L L L
UniRef90_UPI0000F2861B/1-482   T L C I S V L L S L T V F L L L I
UniRef90_UPI0000D554E2/1-482   S L C V S I L L S L T V F F L L L
UniRef90_Q7KUV2/1-482          S L G V T I L L S L T V F L N M V
UniRef90_Q7T2R2/1-482          T L C I S V L L A L T V F L L L I
UniRef90_UPI0000E49844/1-482   T L C I S V L L A L T V F L L L I
UniRef90_Q2XWK8/1-482          T L C I S I L L S L T V F F L L L
UniRef90_Q8T0Y9/1-482          T L C I S I L L S L T V F F L L L
UniRef90_P02718/1-482          S T A I S V L L A Q A V F L L L T
UniRef90_UPI0000F1F877/1-482   S L G V T V L L A L T V F Q M M V
UniRef90_P22456/1-482          T L S V S V L L S L V V F L L V I
UniRef90_Q2XWL0/1-482          T L S I S I L L A L T V F F L L L
UniRef90_Q297E0/1-482          L L V V G R N Y S - - - - - - - -
UniRef90_A2TM48/1-482          S L C I T I L L S Q T M F F L L I
UniRef90_A1EGW3/1-482          T L G V T I L L S L T V F L N L V

     Lupas_21  - - - - - - - - - - - - - - - - -
     Lupas_14  - - - - - - - - - - - - - - - - -
     Lupas_28  - - - - - - - - - - - - - - - - -
     jnetpred

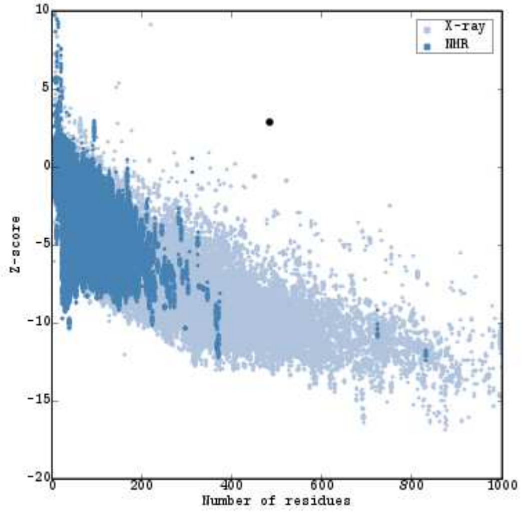
     JNETCONF
               4 0 0 6 8 9 9 9 9 9 9 9 9 9 9 9 9
   JNETSOL25   B B B B B B B B B B B B B B B B B
    JNETSOL5   - B B B B B B B B - B B B B - - B
    JNETSOL0   - - - - - - - - - - B - - - - - -
    JNETHMM
    JNETPSSM
    JNETJURY     * *
```

MUTATION T254I : custom model

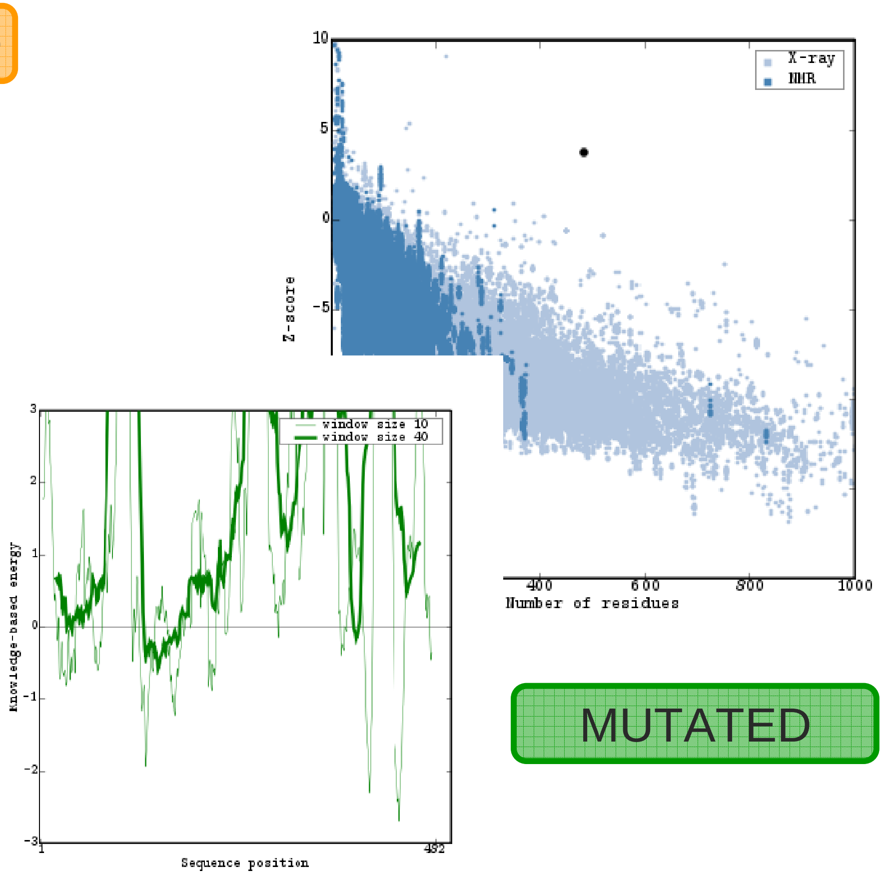
NON MUTATED

Overall model quality

Z-Score: **2.89**



Z-score : 3.75

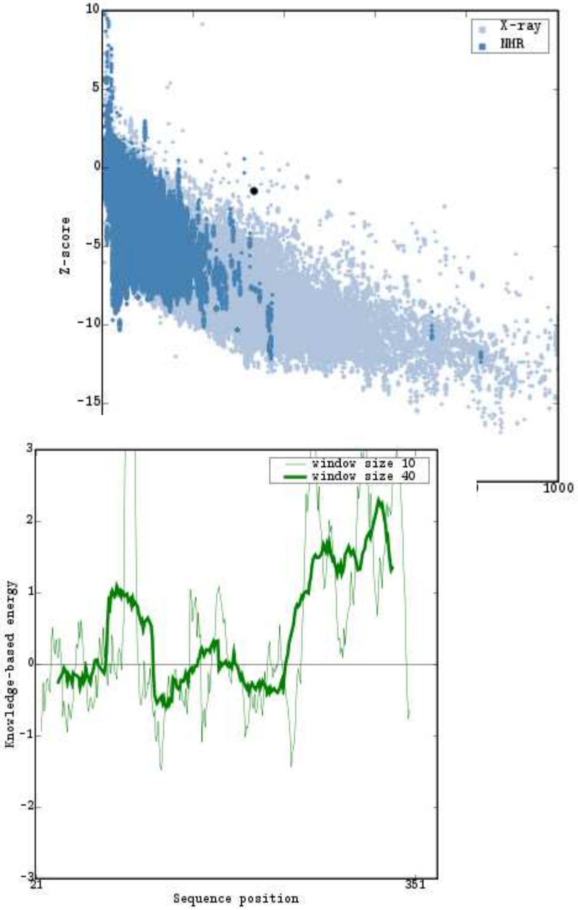


MUTATED

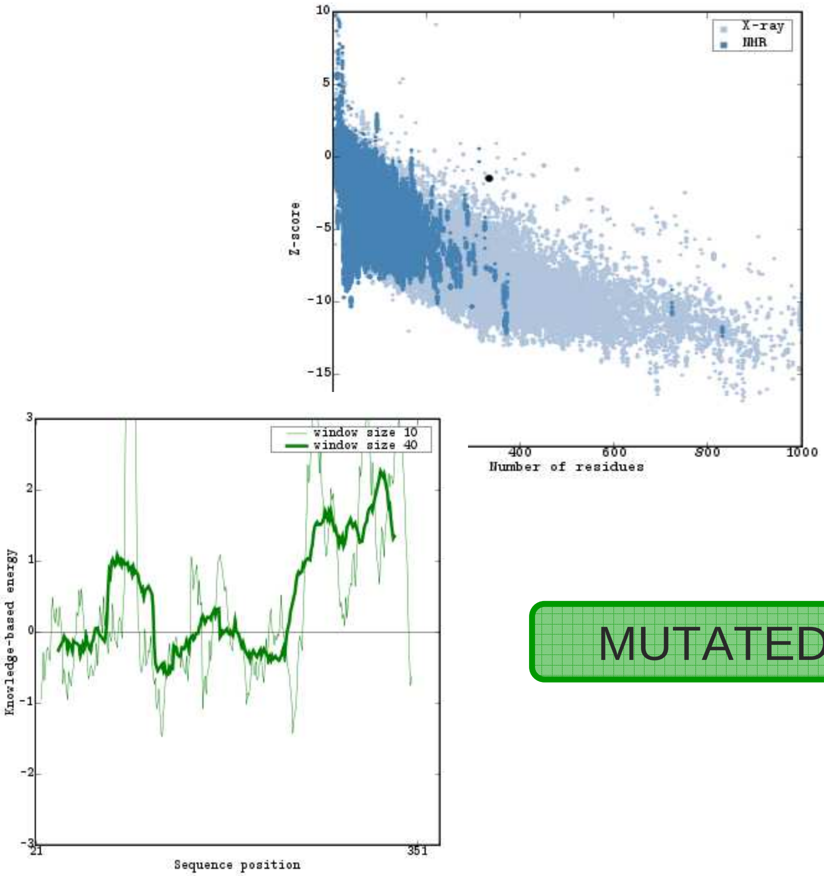
MUTATION T254I : Swiss-model

NON MUTATED

Z-Score: -1.43



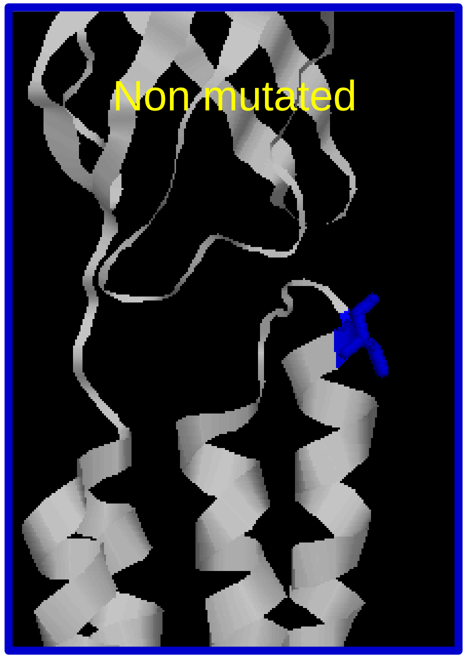
Z-Score: -1.45

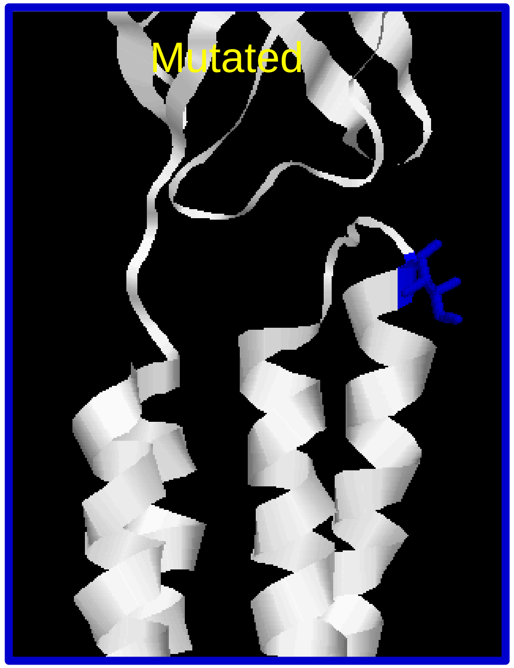


MUTATED

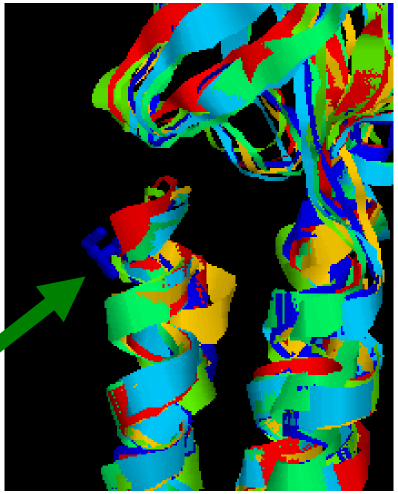
MUTATION S269I

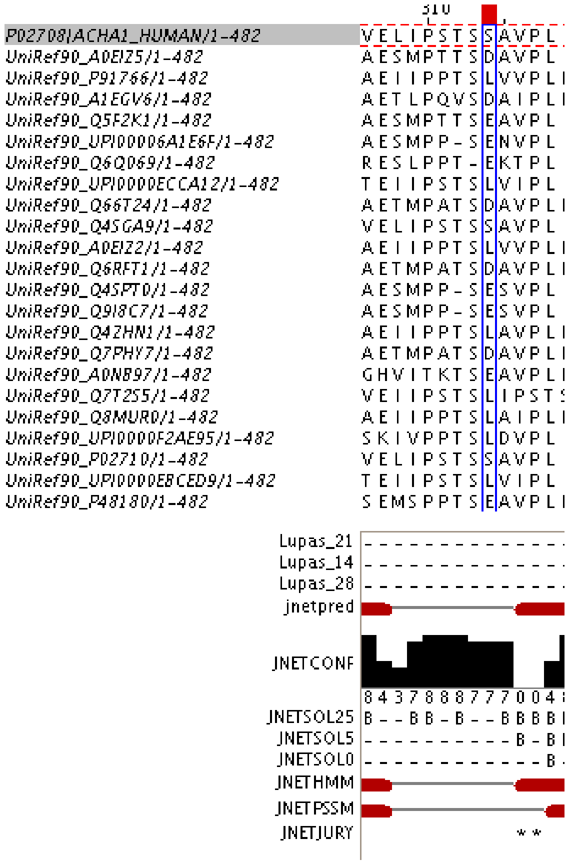
Can change the sensitivity to opening_closing mechanisms





MUTATION S269I





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                                    310
P02708|ACHA1_HUMAN/1-482            V E L I P S T S S A V P L
UniRef90_A0EIZ5/1-482               A E S M P T T S D A V P L
UniRef90_P91766/1-482               A E I I P P T S L V V P L
UniRef90_A1EGV6/1-482               A E T L P Q V S D A I P L
UniRef90_Q5F2K1/1-482               A E S M P T T S E A V P L
UniRef90_UPI00006A1E6F/1-482        A E S M P P - S E N V P L
UniRef90_Q6Q069/1-482               R E S L P P T - E K T P L
UniRef90_UPI0000ECCA12/1-482        T E I I P S T S L V I P L
UniRef90_Q66T24/1-482               A E T M P A T S D A V P L
UniRef90_Q45GA9/1-482               V E L I P S T S S A V P L
UniRef90_A0EIZ2/1-482               A E I I P P T S L V V P L
UniRef90_Q6RFT1/1-482               A E T M P A T S D A V P L
UniRef90_Q4SPT0/1-482               A E S M P P - S E S V P L
UniRef90_Q9I8C7/1-482               A E S M P P - S E S V P L
UniRef90_Q4ZHN1/1-482               A E I I P P T S L A V P L
UniRef90_Q7PHY7/1-482               A E T M P A T S D A V P L
UniRef90_A0N897/1-482               G H V I T K T S E A V P L
UniRef90_Q7T2S5/1-482               V E I I P S T S L I P S T
UniRef90_Q8MUR0/1-482               A E I I P P T S L A I P L
UniRef90_UPI0000F2AE95/1-482        S K I V P P T S L D V P L
UniRef90_P02710/1-482               V E L I P S T S S A V P L
UniRef90_UPI0000EBCED9/1-482        T E I I P S T S L V I P L
UniRef90_P48180/1-482               S E M S P P T S E A V P L

    Lupas_21   - - - - - - - - - - - - - -
    Lupas_14   - - - - - - - - - - - - - -
    Lupas_28   - - - - - - - - - - - - - -
    jnetpred

    JNETCONF
               8 4 3 7 8 8 8 7 7 7 0 0 4
  JNETSOL25    B - - B B - B - - B B B B
   JNETSOL5    - - - - - - - - - - B - B
   JNETSOL0    - - - - - - - - - - - - B
    JNETHMM
   JNETPSSM
   JNETJURY                        * *
```

```
model_clw1.dsspSeq/1-492         V E L I P S T S I A
model_clw1.dsspSS/1-237          H H H H H H H T T T
model_clw2.dsspSeq/1-492         L V I V E L I P S T
model_clw2.dsspSS/1-229          H H H H T T S T T S
model_stamp1.dsspSeq/1-492       I V E L I P S T S I
model_stamp1.dsspSS/1-243        H H H H S T T T S S
model_stamp2.dsspSeq/1-492       V E L I P S T S I A
model_stamp2.dsspSS/1-232        H H H H H H H T T T
S269I_psipred.resSeq/1-482       V E L I P S T S I A
S269I_psipred.resSS/1-297        H H H - - - - - - -
S269I_psipred2.resSeq/1-482      V E L I P S T S I A
S269I_psipred2.resSS/1-298       H H H - - - - - - -
S269I_jnet.resSeq/1-482          V E L I P S T S I A
S269I_jnet.resSS/1-321           E E E E - - - - H H
S269I_sspro4.resSeq/1-482        V E L I P S T S I A
S269I_sspro4.resSS/1-295         H H H - - - - H H H
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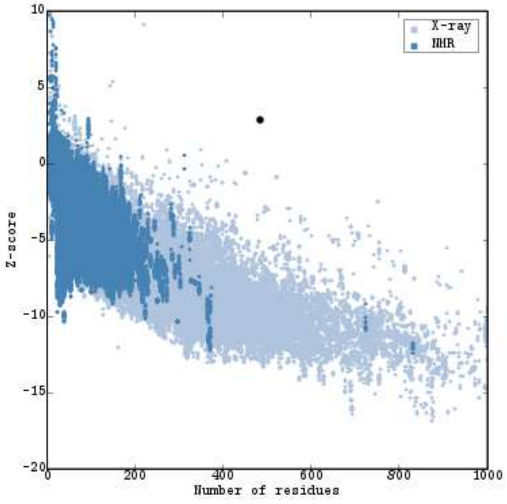
T = hyd bonded turn
H = alpha helix

MUTATION S269I : custom model

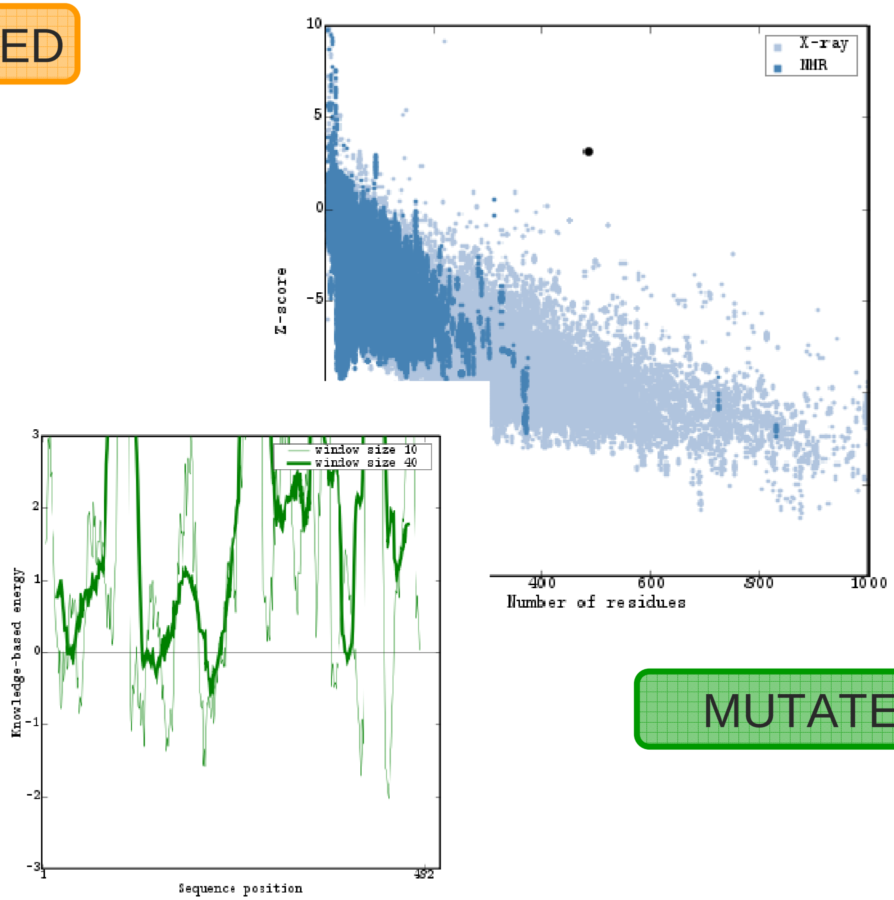
NON MUTATED

Overall model quality

Z-Score: **2.89**



Z-score : 3.17

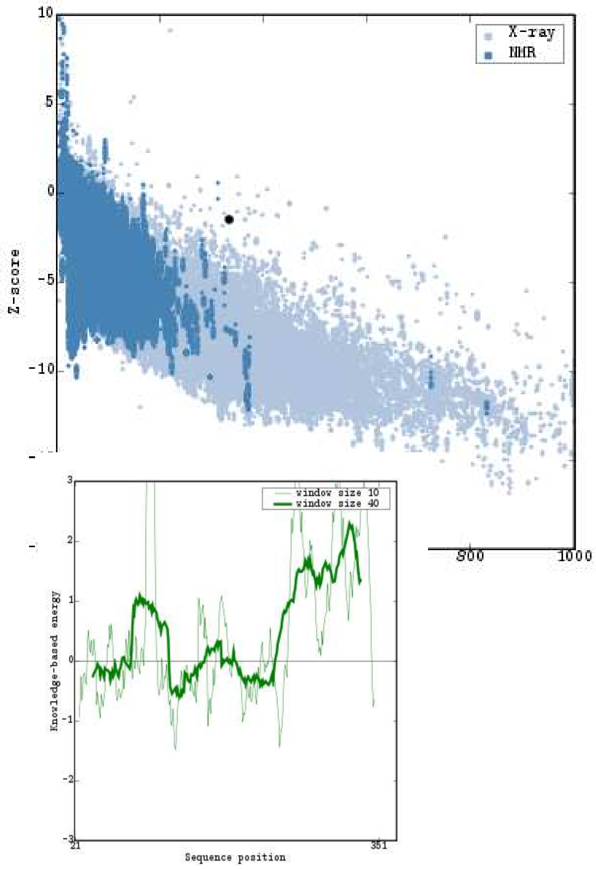


MUTATED

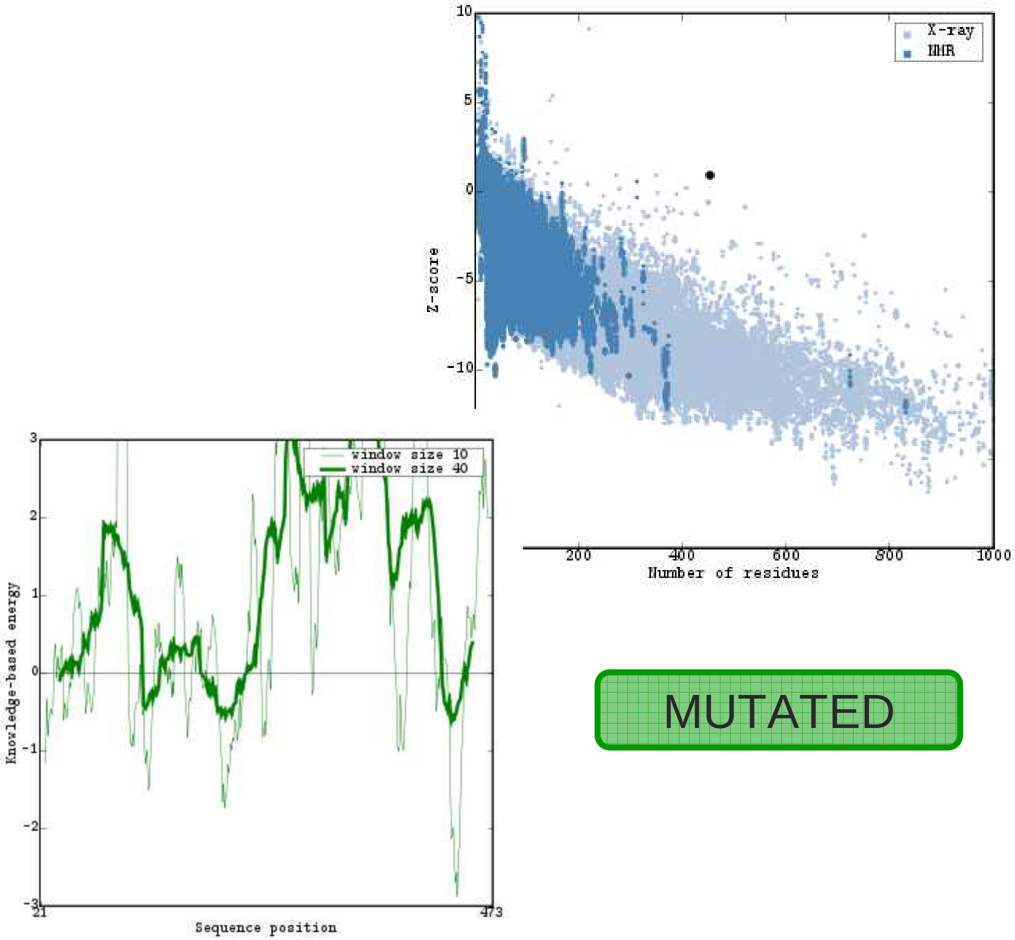
MUTATION S269I : Swiss-model

NON MUTATED

Z-Score: -1.43



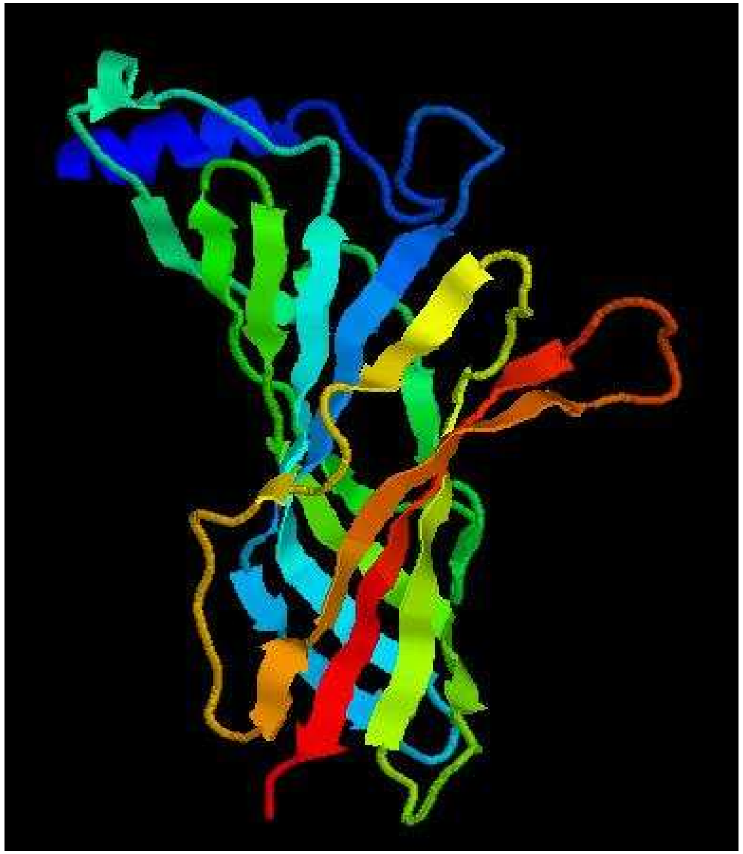
Z-Score: 0.9



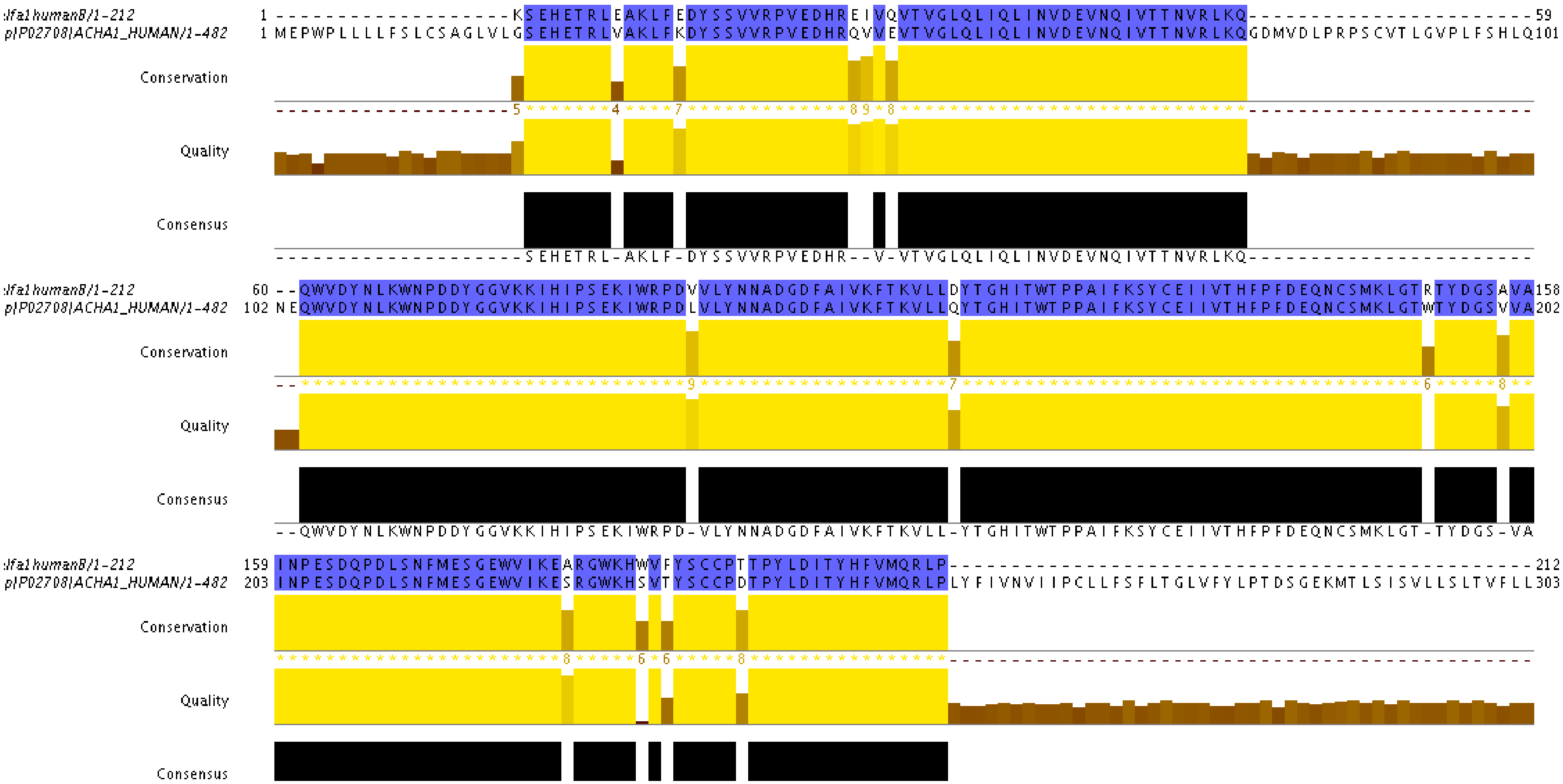
MUTATED

MOUSE NICOTINIC α-1 SUBUNIT

MOUSE NICOTINIC α_1 SUBUNIT



CLUSTALW α_1 MOUSE-α_1 HUMANA



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p|P02708|ACHA1_HUMAN/1-482       1 MEPWPLLLLFSLCSAGLVLGSEHETRLVAKLFKDYSSVVRPVEDHRQVVEVTVGLQLIQLINVDEVNQIVTTNVRLKQGDMVDLPRPSCVTLGVPLFSHLQ101
Conservation                       -------------------5*******4****7*************89*8****************************-----------------------
Quality
Consensus                          --------------------SEHETRL-AKLF-DYSSVVRPVEDHR--V-VTVGLQLIQLINVDEVNQIVTTNVRLKQ-----------------------

:lfa1human8/1-212               60 --QWVDYNLKWNPDDYGGVKKIHIPSEKIWRPDVVLYNNADGDFAIVKFTKVLLDYTGHITWTPPAIFKSYCEIIVTHFPFDEQNCSMKLGTRTYDGSAVA158
p|P02708|ACHA1_HUMAN/1-482     102 NEQWVDYNLKWNPDDYGGVKKIHIPSEKIWRPDLVLYNNADGDFAIVKFTKVLLQYTGHITWTPPAIFKSYCEIIVTHFPFDEQNCSMKLGTWTYDGSVVA202
Conservation                       --*******************************9********************7*************************************6*****8**
Quality
Consensus                          --QWVDYNLKWNPDDYGGVKKIHIPSEKIWRPD-VLYNNADGDFAIVKFTKVLL-YTGHITWTPPAIFKSYCEIIVTHFPFDEQNCSMKLGT-TYDGS-VA

:lfa1human8/1-212              159 INPESDQPDLSNFMESGEWVIKEARGWKHWVFYSCCPTTPYLDITYHFVMQRLP-----------------------------------------------212
p|P02708|ACHA1_HUMAN/1-482     203 INPESDQPDLSNFMESGEWVIKESRGWKHSVTYSCCPDTPYLDITYHFVMQRLPLYFIVNVIIPCLLFSFLTGLVFYLPTDSGEKMTLSISVLLSLTVFLL303
Conservation                       ***********************8*****6*6*****8****************-----------------------------------------------
Quality
Consensus
```