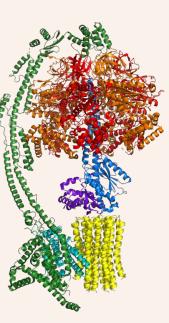
# Protein Structure



Bruno Álvarez Jan Izquierdo Jaume Jurado Marc Trujillo



#### PDB structures

We used psiblast to create a pssm of a protein of the family based on uniprot, and used the pssm to get a list of sequence structures from the pdb\_seq database.

Subunit F1 alpha

| _  |                            |            |              |                 |      |      |       |
|----|----------------------------|------------|--------------|-----------------|------|------|-------|
|    |                            |            |              |                 | 2    | соге | E     |
| 38 | Sequences producing signif | icant alig | nments:      |                 | (E   | its) | Value |
|    |                            |            |              |                 |      |      |       |
|    | 1vdz_A mol:protein lengt   |            |              |                 | 8    | 37   | 0.0   |
|    | 2qe7_F mol:protein lengt   |            |              |                 | 7    | 77   | 0.0   |
|    | 2qe7_E mol:protein lengt   | h:462 ATP  | synthase sub | unit beta       | 7    | 77   | 0.0   |
|    | 2qe7_D mol:protein lengt   | h:462 ATP  | synthase sub | unit beta       | 7    | 77   | 0.0   |
|    | 1kmh_B mol:protein lengt   | h:498 ATPa | se beta subu | nit             | 7    | 60   | 0.0   |
|    | 1fx0_B mol:protein lengt   |            |              | A CHAIN         | 7    | 60   | 0.0   |
|    | 1sky_E mol:protein lengt   |            |              |                 |      | '59  | 0.0   |
|    | 2f43_B mol:protein lengt   | h:479 ATP  | synthase bet | a chain, mitoch | on 7 | 46   | 0.0   |
|    | 1mab_B mol:protein lengt   | h:479 PROT | EIN (F1-ATPA | SE BETA CHAIN)  | 7    | 46   | 0.0   |
|    | 2v7q_F mol:protein lengt   | h:482 ATP  | SYNTHASE SUB | UNIT BETA       | 7    | 44   | 0.0   |
|    | 2v7q_E mol:protein lengt   |            |              |                 | 7    | 44   | 0.0   |
|    | 2v7q_D mol:protein lengt   | h:482 ATP  | SYNTHASE SUB | UNIT BETA       | 7    | 44   | 0.0   |
|    | 2jj2_M mol:protein lengt   | h:482 ATP  | SYNTHASE SUB | UNIT BETA       | 7    | 44   | 0.0   |
|    | 2jj2_L mol:protein lengt   | h:482 ATP  | SYNTHASE SUB | UNIT BETA       | 7    | 44   | 0.0   |
|    | 2jj2_K mol:protein lengt   | h:482 ATP  | SYNTHASE SUB | UNIT BETA       | 7    | 44   | 0.0   |
|    | 2jj2 F mol:protein lengt   |            |              | UNIT BETA       |      | 44   | 0.0   |
|    | 2obm A mol:protein lengt   |            |              |                 | 13   | 192  | 1e-55 |
|    | 2obl A mol:protein lengt   |            |              |                 |      | 192  | 1e-55 |
|    | 2dpy_B mol:protein lengt   |            |              |                 |      | 178  | 2e-49 |
|    | 2dpy_A mol:protein lengt   |            |              |                 |      | 178  | 2e-49 |
|    | 3b2q_B mol:protein lengt   |            |              |                 |      | 132  | 9e-33 |
|    | 3b2q_A mol:protein lengt   |            |              |                 |      | 132  | 9e-33 |
|    | 2rkw_B mol:protein lengt   |            |              |                 |      | 132  | 9e-33 |
|    | 2rkw_A mol:protein lengt   |            |              |                 |      | 132  | 9e-33 |
|    | 2c61_B mol:protein lengt   |            |              |                 |      | 131  | 1e-32 |
|    | 2c61_A mol:protein lengt   |            |              |                 |      | 131  | 1e-32 |
|    | 1kmh_A mol:protein lengt   |            |              |                 |      | 123  | 1e-29 |
|    | 1fx0_A mol:protein lengt   |            |              | HA CHAIN        |      | 123  | 1e-29 |
|    | 1sky_B mol:protein lengt   |            |              |                 |      | 120  | 1e-28 |
|    | 2v7q_C mol:protein lengt   |            |              |                 |      | 115  | 1e-26 |
|    | 2v7q_B mol:protein lengt   |            |              |                 |      | 115  | 1e-26 |
|    | 2v7q_A mol:protein lengt   |            |              |                 |      | 115  | 1e-26 |
|    | 2ii2 J mol:protein lengt   | h:510 ATP  | SYNTHASE SUB | UNIT ALPHA HEAL | RT   | 115  | 1e-26 |

Subunit F1 beta

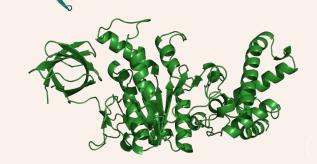
| 5<br>7 S | Sequences producing significant alignments:   |                    | Score<br>(Bits) | E<br>Valu |
|----------|---|--------------------|-----------------|-----------|
|          | 2qe7 F mol:protein length:462 ATP synthase s  | ubunit beta        | 833             | 0.0       |
|          | 2ge7 E mol:protein length:462 ATP synthase s  | ubunit beta        | 833             | 0.0       |
|          | 2ge7 D mol:protein length:462 ATP synthase s  | ubunit beta        | 833             | 0.0       |
|          | 1sky E mol:protein length:473 F1-ATPASE       |                    | 818             | 0.0       |
|          | 2v7q F mol:protein length:482 ATP SYNTHASE S  | UBUNIT BETA        | 795             | 0.0       |
|          | 2v7q E mol:protein length:482 ATP SYNTHASE S  | UBUNIT BETA        | 795             | 0.0       |
|          | 2v7q D mol:protein length:482 ATP SYNTHASE S  | UBUNIT BETA        | 795             | 0.0       |
|          | 2jj2 M mol:protein length:482 ATP SYNTHASE S  | UBUNIT BETA        | 795             | 0.0       |
|          | 2jj2 L mol:protein length:482 ATP SYNTHASE S  | UBUNIT BETA        | 795             | 0.0       |
|          | 2jj2 K mol:protein length:482 ATP SYNTHASE S  | UBUNIT BETA        | 795             | 0.0       |
|          | 2jj2_F mol:protein length:482 ATP SYNTHASE S  | UBUNIT BETA        | 795             | 0.6       |
|          | 2jj2 E mol:protein length:482 ATP SYNTHASE S  |                    | 795             | 0.6       |
|          | 3b2g B mol:protein length:469 V-type ATP synt | hase beta chain    | 405             | 6e-1      |
|          | 3b2q A mol:protein length:469 V-type ATP synt | hase beta chain    | 405             | 6e-1      |
|          | 2rkw B mol:protein length:469 V-type ATP synt | hase beta chain    | 405             | 6e-1      |
|          | 2rkw A mol:protein length:469 V-type ATP synt | hase beta chain    | 405             | 6e-1      |
|          | 2c61 B mol:protein length:469 A-TYPE ATP SYNT |                    | 404             | 3e-1      |
|          | 2c61 A mol:protein length:469 A-TYPE ATP SYNT | HASE NON-CATALYTIC | 404             | 3e-1      |
|          | 2dpy B mol:protein length:438 Flagellum-speci | fic ATP synthase   | 341             | 3e-1      |
|          | 2dpy A mol:protein length:438 Flagellum-speci | fic ATP synthase   | 341             | 3e-1      |
|          | 2obm_A mol:protein length:347 EscN            |                    | 312             | 3e-1      |
|          | 2obl_A mol:protein length:347 EscN            |                    | 312             | 3e-10     |
|          | 2qe7_C mol:protein length:502 ATP synthase su | bunit alpha        | 218             | 6e-6      |
|          | 2qe7_B mol:protein length:502 ATP synthase su | bunit alpha        | 218             | 6e-6      |
|          | 2qe7_A mol:protein length:502 ATP synthase su | bunit alpha        | 218             | 6e-6      |
|          | 1sky_B mol:protein length:502 F1-ATPASE       |                    | 212             | 1e-6      |
|          | 2r9v_A mol:protein length:515 ATP synthase su | bunit alpha        | 212             | 2e-62     |
|          | 1kmh A mol:protein length:507 ATPase alpha su | bunit              | 208             | 3e-6:     |

## Structure superimposition

We superimpose the structures against the protein used as psiblast query and measure the RMSD

We used A3CS71 as a query and as the alignment base for F1 alpha subunit

We used AORL95 as a query and as the alignment base for F1 beta subunit

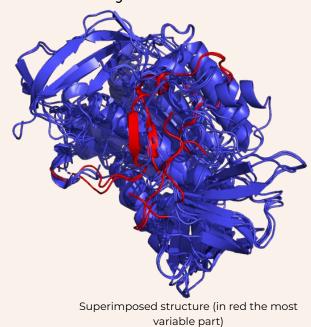


# High variability region

Using Pymol and the superimposition alignment we identify that:

Subunit F1 alpha presents high variability at the beginning of the sequence

| /1          |               | 11    |               |       |       | 31     |        |        |        | 51    |       | 61     | 66    |
|-------------|---------------|-------|---------------|-------|-------|--------|--------|--------|--------|-------|-------|--------|-------|
| MVA<br>1    |               |       | .VVADGI<br>16 | MKGAK | MYEVV | /RVGEL | .GLIGE | IIRLE  | EGDKAV | IQVYI | EETAG | /RPGEF | VVGTG |
| 0.11.5.2    | NPTTSD<br>6   |       |               |       |       |        |        |        |        |       |       |        | NL    |
| MRI<br>Z100 | NPTTSD<br>106 |       | LEKK-<br>116  |       |       |        |        |        |        |       |       |        | NL    |
| GSH<br>Z100 | KIRVGD        | ALLGE | LIDGI<br>116  | GRPME | SNIVE | PYLPE  | ERSLY  | 'AEPPI | OPLL   |       |       |        |       |
| . 100       | KIRVGD        |       |               |       |       |        |        |        |        |       |       |        |       |
| MAL         | LPAV          |       |               |       |       |        |        |        |        |       |       |        | RRY   |

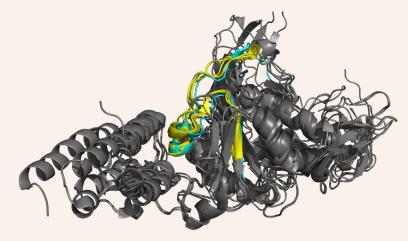


### Region conservation

We identify conserved regions in subunit F1 alpha using Pymol and the alignment, it can be seen that it presents 2 conserved regions, here is the first one

This is the first conserved region the blue region is from 1vdz\_A and the yellow is from the other structures.

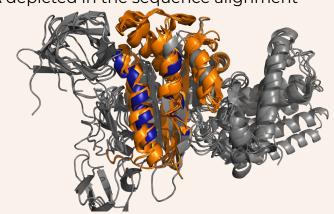
| 196        | 201   | 206 23          | 11 216   | 221       | 226 231  | 236         |
|------------|-------|-----------------|----------|-----------|----------|-------------|
|            |       |                 |          |           |          | GPFGSGK     |
| 1 136      |       |                 |          |           |          |             |
|            |       |                 |          |           |          | FGGA        |
|            |       |                 |          |           | 166      | 171<br>FGGA |
| пкононы    | Q L I |                 | 156 1    |           |          | 176         |
|            | R     | (V-IDQPF        | [LGVRAI] | GLLTCG    | IGQRIGI- | FA          |
|            |       |                 | 156 1    |           |          | 176         |
|            |       |                 |          |           |          | FA          |
| 1 146      |       |                 |          |           | 176      |             |
| -TIABLUBE- | ÜK-1  | <b>H-IEHALI</b> | TIGAKUTI | AHLE I AR | RGURMGL- | FA          |



### Region conservation

Here we can see the alignment of the second conserved region of F1 alpha, the grey overlapping zone belongs to the non-matching aligned region of Ivdz\_A depicted in the sequence alignment

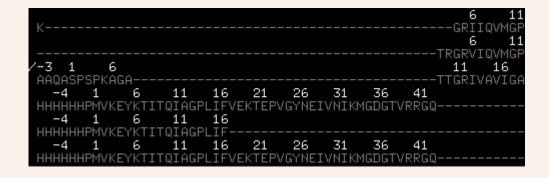
This is the second conserved region the blue region belongs to 1vdz\_A and the orange belongs to the other structures.

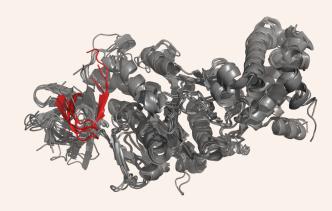


| 291 296 301 306 3               |              |                   | 361 366 371   |
|---------------------------------|--------------|-------------------|---|
|                                 |              |                   | A-YL-ASKLAEFYERAG   |
|                                 |              | 266 271 27        |   |
| HEQNIAESKVALVYGQMNE-PPGARMRVGLT |              |                   | FVQAGSEVSAL-LG-RMPSAVGYQP-TL-STEMGSLQERIT                                     |
| . 226 231 236 241 246 25        | 51 256 261   | 266 271 27        | 6 281 286 291 296 301 306 311   |
|                                 | TALTMAEYFR   | DVN-EQDVLLFIDNIFR | FVQAGSEVSAL-LG-RMPSAVGYQP-TL-STEMGSLQERIT                                     |
| 226 231 236 241 2               |              |                   | 271 276 281 286 291 296 301 306   |
|                                 |              |                   | YARAARDVGLA-S-GEPDVRGGFPPSVFSSLPKLLERAG                                       |
| 226 231 236 241 2               | 246 251 256  | 261 266           | 271 276 281 286 291 296 301 306<br>YARAARDVGLASGEPDVRGGFPPSV-F-SSLPKLLERAGPAF |
| DR-PALERMKAAFT                  | TATTIAEYFRDQ | GKNVLLMMDSVTR     | YARAARDVGLASGEPDVRGGFPPSV-F-SSLPKLLERAGPAF                                    |
| 231 236 241 246 25              | 51 256 261   | 266 271 2         | 76 281 286 291 296 301 306 311  |
| GRARSVVIAAPADV-SPLLRMQGAAY      | YATRIAEDFRD  | RGQHVLLIMDSLTR    | YAMAQREIALA-IG-EPPATKGYPP-SV-FAKLPALVERAG                                     |

# High variability region

Subunit F1 beta we can observe high variability at the beginning of the sequence



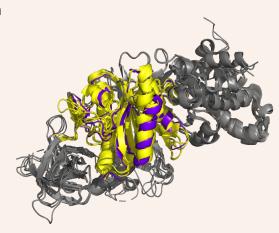


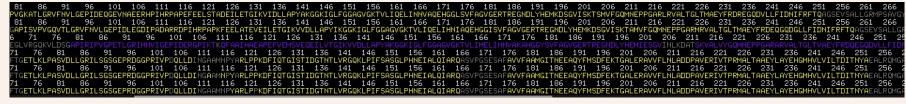
Superimposed structure (in red the most variable part)

### Region conservation

Using Pymol and the alignment it can be seen that subunit F1 beta presents 2 conserved regions, here is the first one.

The first conserved region, 2jj2\_E is represented in purple as the position of its conserved region differs slightly from the others, colored in yellow.

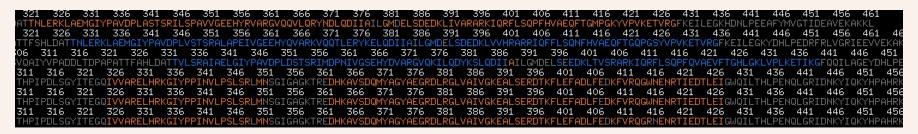




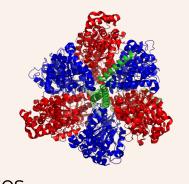
### Region conservation

This is the second conserved region of F1 beta, the grey-colored regions at the end of the longer helix belong to non-matching aligned regions of structures 3b2q\_B, 2rkw\_B and 2c61\_A

In this structure we can observe the blue region, that belongs to lsky\_E and 2jj2\_E, which have slightly different positions of conserved regions that the other structures, represented in orange.

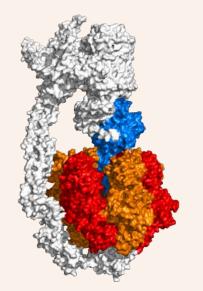


#### Regions for protein function

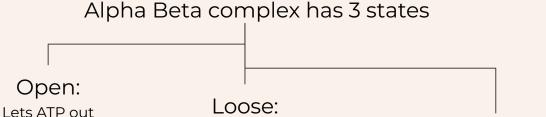


Tight:

**Synthesis** 



Gamma Alpha Beta



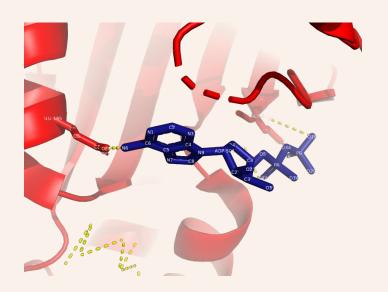
Lets ADP in and

prepares

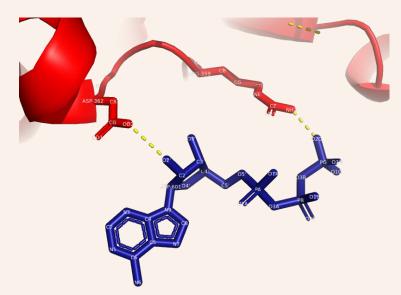
The cycle perpetuates because of Gamma protein spinnings produced by proton gradient

We will analyze Alpha and Beta interactions with Gamma among other interactions

## ATP binding and synthesis



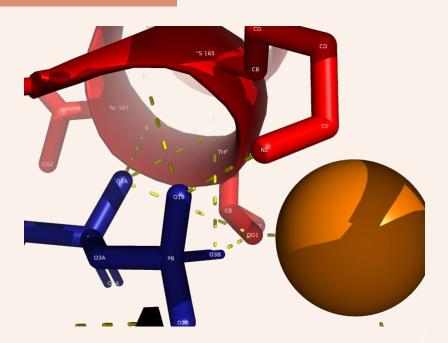
- Open state
- Binds to GLU-195



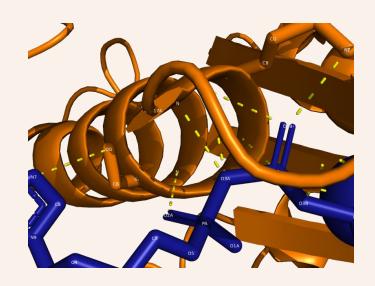
- Loose state
- ARG-359: retains the ATP
  - ASP-362: retains ATP

#### ADP catalytic site (aDP)

- Mg: stabilizes protein during reaction
- LYS-165: stabilizes protein to catalyze reaction
- THR-166: stabilizes protein to catalyze reaction

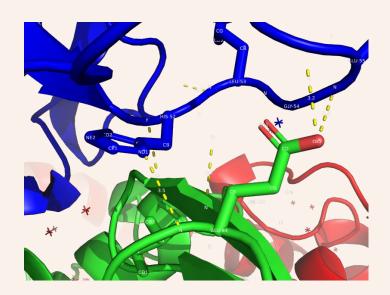


#### Interaction: beta chain -ATP

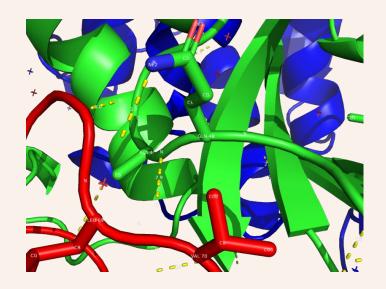


LYS-175 binds through N GLY-174 and SER-177 and Mg stabilize the reaction and proteins

### Interaction: alpha + beta

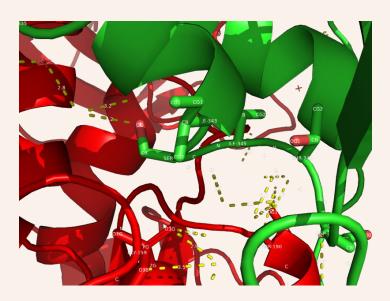


Glutamic acid of Alpha chain binds to Histidine with N and also interacts with the Beta chain



In this other case we observe a Leucine, Alanine and a Glutamine bond.

### Interaction: alpha + beta

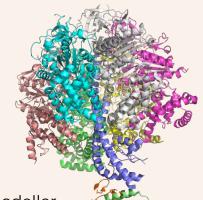


Serine + Isoleucine Bonding

#### Modeling the protein

Mutation: var\_088542; position 207 (R>H)

We used blast to find a template, we will use 2jdi



Then we model our structure with modeller

We separate the chains into different fasta files to use only chain A to align it with our mutation, we obtain a good alignment quality.

```
Sequence format is Pearson
Sequence 1: P25705 553 aa
Sequence 2: TemplateA 487 aa
Start of Pairwise alignments
Aligning...

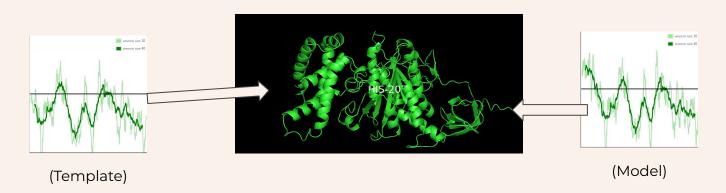
Sequences (1:2) Aligned. Score: 98
Guide tree file created: [mutation_template.dnd]

There are 1 groups
Start of Multiple Alignment
Aligning...

Group 1: Sequences: 2 Score:10394
Alignment Score 2849
```

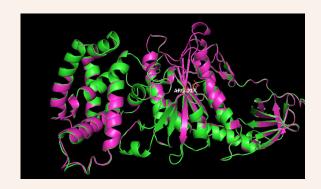
#### Model Analysis: Models in pymol

After putting our models in pymol we quickly realize what is that part that we have that gave us a peak when puting the model in prosa

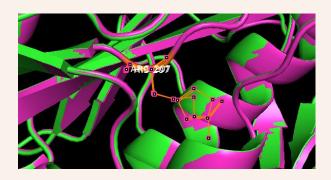


#### Model Analysis: Models in Pymol

To check for the differences between both models, we performed a superimposition to check if we can see the differences between the models



Superimposition: pink(mutation), green(template)



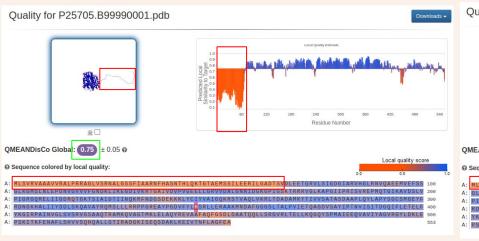
(Mutation)

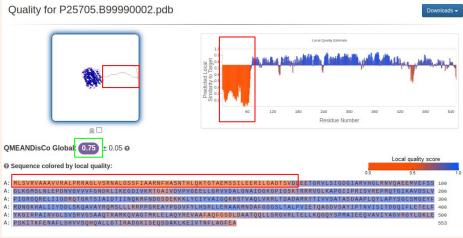
#### Model Analysis: Hypothesis

This mutation changes arginine (R) to histidine (H), both positively charged. However, histidine's ring structure suggests potential issues with DNA binding to the alpha helix, potentially affecting protein folding.

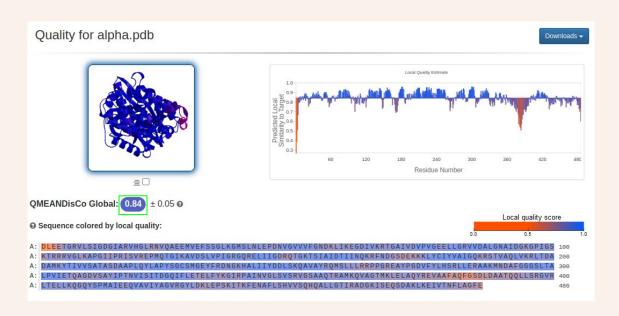
$$H_2N$$
 $H_2N$ 
 $H_2N$ 
 $H_3N$ 
 $H_2N$ 
 $H_3N$ 
 $H_3N$ 

#### Energy profile: mutant model

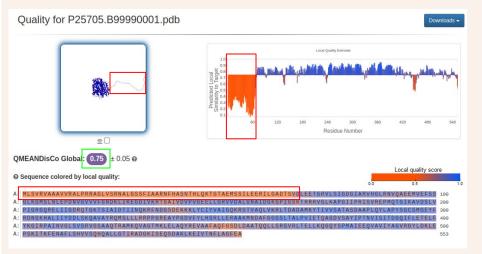


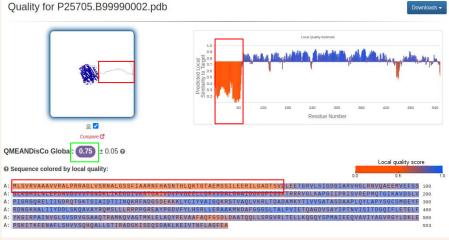


#### Energy profile: wild

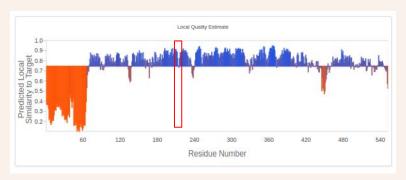


#### Energy profile wild model

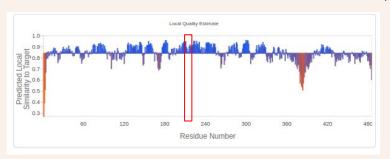


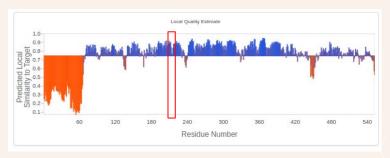


#### Comparison of profiles



#### Mutant model





Wild model 1