Université Libre de Bruxelles

Biophysics II

BIOL-F-459

Task 2 - Modeling of the structure of Blo t 5 and Der p 5

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1 Models built with Modeller

We built 10 models for Der f 5 using the 3D structure of Blo t 5 (2JMH), 10 models for Der f 5 based on the structure of Der p 5 (chain A of 3MQ1) and 10 models for Blo t 5 also based on the structure of Der p 5. To compare the models, we used the normalized DOPE (Discrete Optimized Protein Energy) score. The DOPE score is an energy function based on atomic distance-dependent statistical potential calculated from a sample of native protein structures. So the lower the score, the closer the structure is close to a native one. The normalized DOPE score is a z-score, which means it the value of the DOPE score from which we deducted the mean value of alternatives solutions, then divided by the standard deviation of the distribution of the solutions. This allows us to compare the scores obtained between different structures obtained from different templates.

1.1 Der f 5 using the structure of Blo t 5 (2JMH)

```
>> Summary of successfully produced models:
Filename                          molpdf Normalized DOPE score
----------------------------------------------------------------
derf5.B99990001.pdb            492.10083        0.24513
derf5.B99990002.pdb            445.39008        0.23379
derf5.B99990003.pdb            483.56454        0.21047
derf5.B99990004.pdb            451.73026       -0.06076
derf5.B99990005.pdb            504.43655        0.19679
derf5.B99990006.pdb            483.38110        0.08034
derf5.B99990007.pdb            481.85413        0.35569
derf5.B99990008.pdb            505.64279        0.33380
derf5.B99990009.pdb            511.20584        0.15162
derf5.B99990010.pdb            523.12244        0.10939
```

Figure 1: Summary of the results of modeller for Der f 5 structure based on the structure of Blo t 5

To choose the best model for this point, we first selected the three best models based on their DOPE score (Fig 1), which are the numbers 4, 6 and 10. Then we analysed them with the website ProSA-web (`https://prosa.services.came.sbg.ac.at/prosa.php`) to assess the quality of the structures.

The structural difference between the models is the loop in the N-terminal part of the protein. The three models seems to be of the same quality when we look

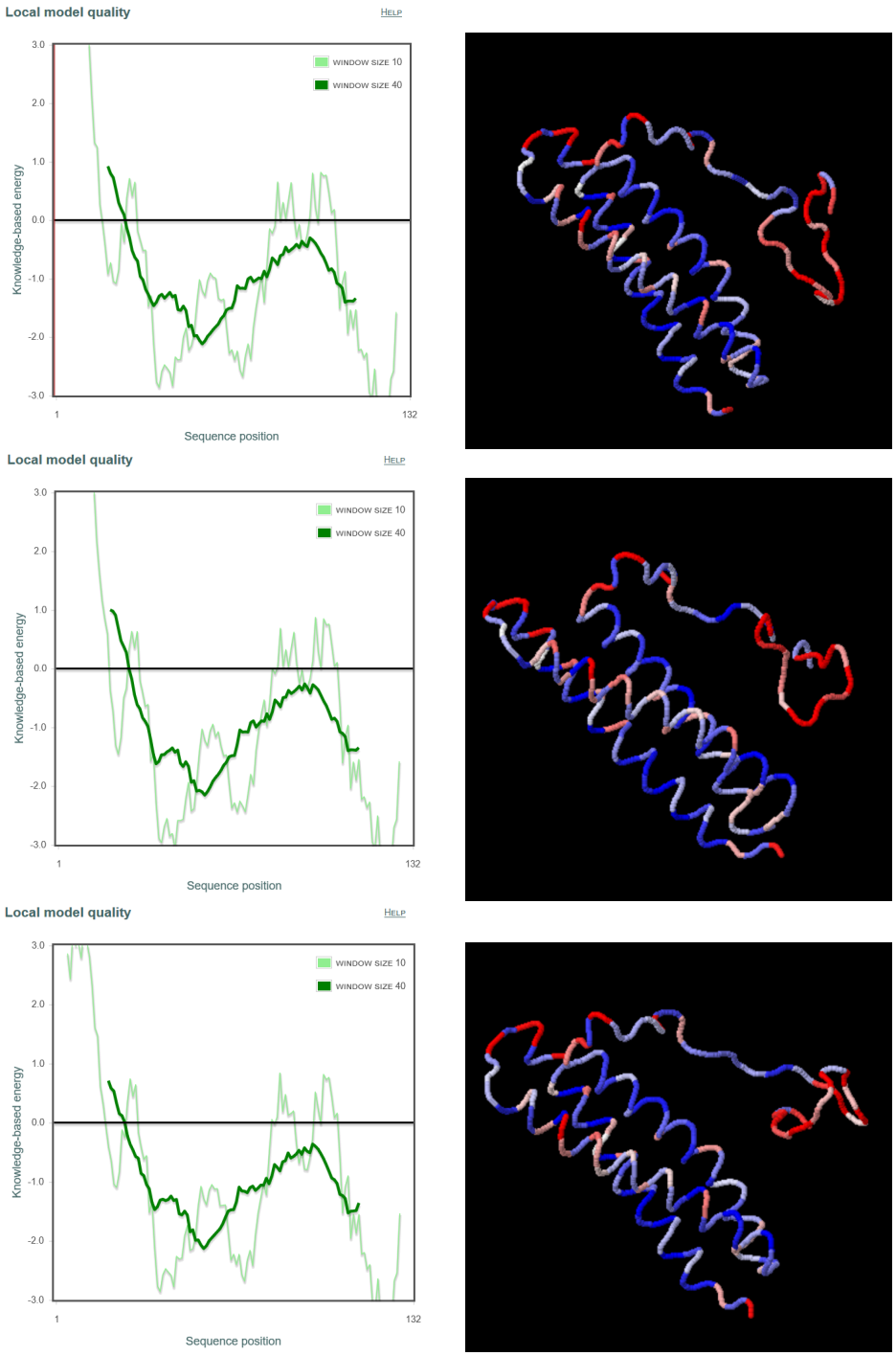


Figure 2: Analyse and structure obtained on ProSA-web of the three best models obtained with modeller of Der f 5 using the structure of Blo t 5, in the order structure 4, 6 and 10 of the previous list in Fig 1

with a window of 40 residues. However, when we reduce the size of the window, we observe differences in the local quality of the model, especially at the N-terminal part. The position of the loop in the model 10 seems to be of better quality than in the other models (Fig 2).

Nonetheless, we selected 4 as the best model because it had the best normalized DOPE score and a good overall quality of the model, despite the poor quality for the loop region.

1.2 Der f 5 using the structure of Der p 5 (chain A 3MQ1)

```
>> Summary of successfully produced models:
Filename                          molpdf Normalized DOPE score
----------------------------------------------------------------
derf5.B99990001.pdb            585.07709        0.48294
derf5.B99990002.pdb            631.03961        0.76444
derf5.B99990003.pdb            496.29663        0.48305
derf5.B99990004.pdb            558.13873        0.51111
derf5.B99990005.pdb            544.75000        0.51577
derf5.B99990006.pdb            541.17853        0.42484
derf5.B99990007.pdb            613.58765        0.47888
derf5.B99990008.pdb            905.83966        0.44716
derf5.B99990009.pdb            701.45526        0.61232
derf5.B99990010.pdb            607.09113        0.58526
```

Figure 3: Summary of the results of modeller for Der f 5 structure based on the structure of Der p 5

As done previously, we selected the three best models based on the DOPE normalized score obtained with modeller (Fig 3). The selected models are the models 6, 8 and 7. They were analysed on ProSA-web.

We observed the scores obtained for the models based on the chain A of 3MQ1 were considerably higher than the ones obtained with the structure of Blo t 5 (Fig 1 and 3). This could mean that Der f 5 is a monomer like Blo t 5.

The main structural difference can be observed at the N-terminal part of the protein (Fig 4). This part is essentially a loop that is differentially positioned in the three models.

We selected the model 6 as the best model.

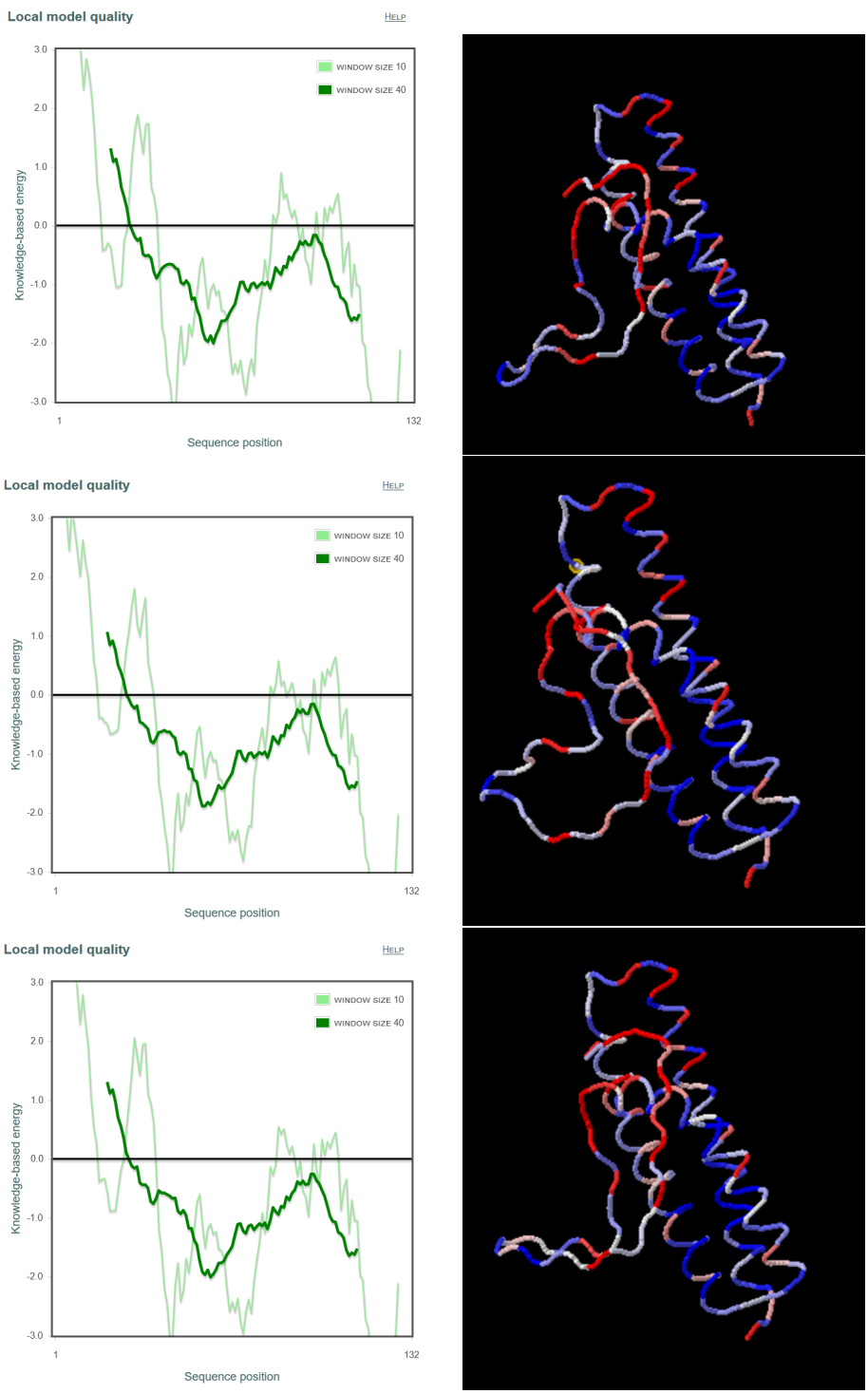


Figure 4: Analyse and structure obtained on ProSA-web of the three best models obtained with modeller of Der f 5 using the structure of Der p 5, in the order structure 6, 8 and 7 of the previous list in Fig 3

1.3 Blo t 5 using the structure of Der p 5 (chain A 3MQ1)

```
>> Summary of successfully produced models:
Filename                          molpdf Normalized DOPE score
----------------------------------------------------------------------
blot5.B99990001.pdb            427.86465        0.26543
blot5.B99990002.pdb            447.44385        0.31864
blot5.B99990003.pdb            352.05826        0.22860
blot5.B99990004.pdb            372.51822        0.17983
blot5.B99990005.pdb            412.56955        0.16108
blot5.B99990006.pdb            417.51050        0.37243
blot5.B99990007.pdb            406.15512        0.22307
blot5.B99990008.pdb            396.10434        0.43617
blot5.B99990009.pdb            363.36459        0.28762
blot5.B99990010.pdb            436.89822        0.39772
```

Figure 5: Summary of the results of modeller for Blo t 5 structure based on the structure of Der p 5

Technically, we already know the structure of Blo t 5 (PDB code 2JMH). However, since Blo t 5 is a monomer and Der p 5 an homodimer, building models of Blo t 5 based on the structure of Der p 5 could allow us to detect what could be the structural difference involved in dimerization and maybe to build a model of Blo t 5 able to dimerize.

We selected three models based on the normalized DOPE score : the models 5, 4 and 7 (Fig 5), and analysed them with ProSA-web.

Once more, the main structural difference observed is the loop which is positioned differently in each model (Fig 6).

We selected the model 5 as the best model.

We compared the model selected and the real structure of Blo t 5 to study the difference brought when Blo t 5 is built using the structure of Der p 5. We superimposed the structures with *PDBeFold* (`http://www.ebi.ac.uk/msd-srv/ssm/cgi-bin/ssmserver`) and visualized the superimposition on *Pymol*.

The analyses (Fig 7) showed that the structured had a rmsd of 2.2 and the sequence are quite well-matched with 97.5%. The secondary structures were matched at 100%. However, the P score is quite low (below 3), showing that the matching is not statistically relevant.

On *Pymol* (Fig 8), we could see that the structures were well superimposed, except for the first helix, which is slightly crooked. We saw in the previous task that

this structural particularity was found in the protein Der p 5 and was suspected to be relevant for the dimerization. We need to simulate dimerization with this new model of Blo t 5 to confirm if this structure is indeed involved in the process or not.

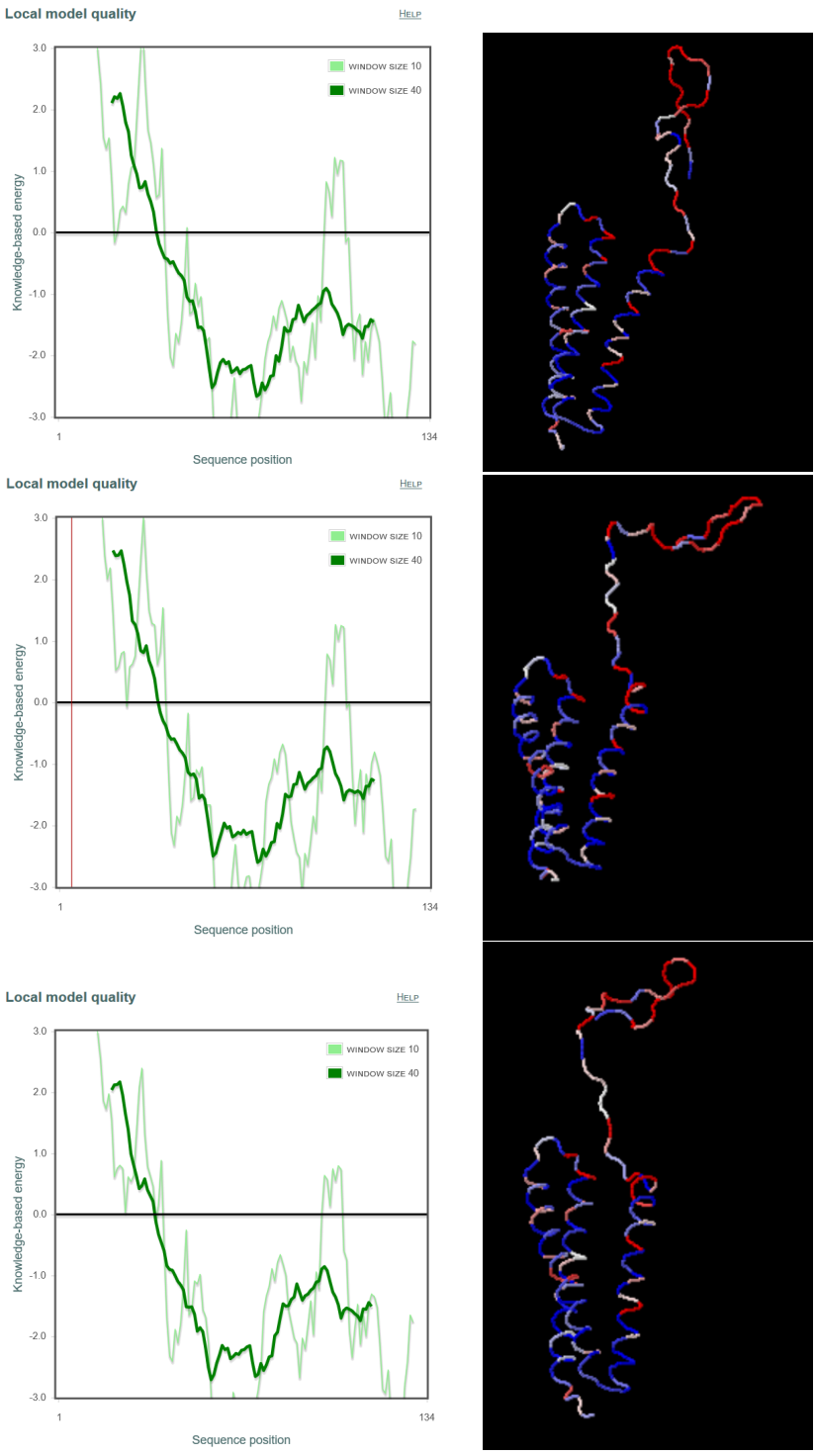
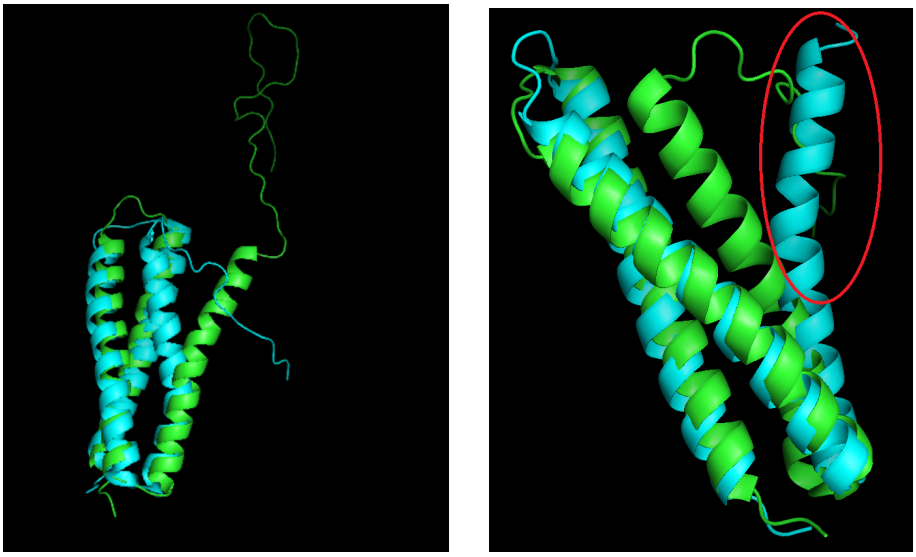


Figure 6: Analyse and structure obtained on ProSA-web of the three best models obtained with modeller of Blo t 5 using the structure of Der p 5, in the order structure 5, 4 and 7 of the previous list in Fig 5

Query blot5.B99990005.pdb:				Alignment (1 of 1)				Target PDB 2jmh:A			
N_{res}	$\%_{res}$	N_{SSE}	$\%_{SSE}$	Q	P	RMSD	N_{align}	N_{res}	$\%_{res}$	N_{SSE}	$\%_{SSE}$
134	60	3	100	0.264	1.49	2.218	80	117	68	3	100
---				$\%_{seq}$	Z	N_{SSE}	N_{gaps}	NMR SOLUTION STRUCTURE OF BLO T 5, A MAJOR MITE ALLERGEN FROM BLOMIA TROPICALIS			
				97.5	3.53	3	1				

Figure 7: Result of superimposition of the model 5 with the structure 2JMH on *PDBeFold*



(a) Superimposition on *Pymol* of the model 5 (green) with the structure 2JMH (blue)

(b) Superimposition on *Pymol* of the Blo t 5 (green) and Der p 5 (blue)

Figure 8: Comparison between the superimposition of Blo t 5, the model obtained with modeller and Der p 5

2 Mutation of residues in Der p 5

We mutated two residues (Val 85 and Val 88) into Alanine in Der p 5 which are considered essential in the stabilization of the dimerization due to the formation of a valine zipper. We did not observe any steric clash after the mutation in *Pymol* (Fig 9). Thus, further analysis are necessary to deem if the mutation of the two Val residues destabilizes the dimerization or not.

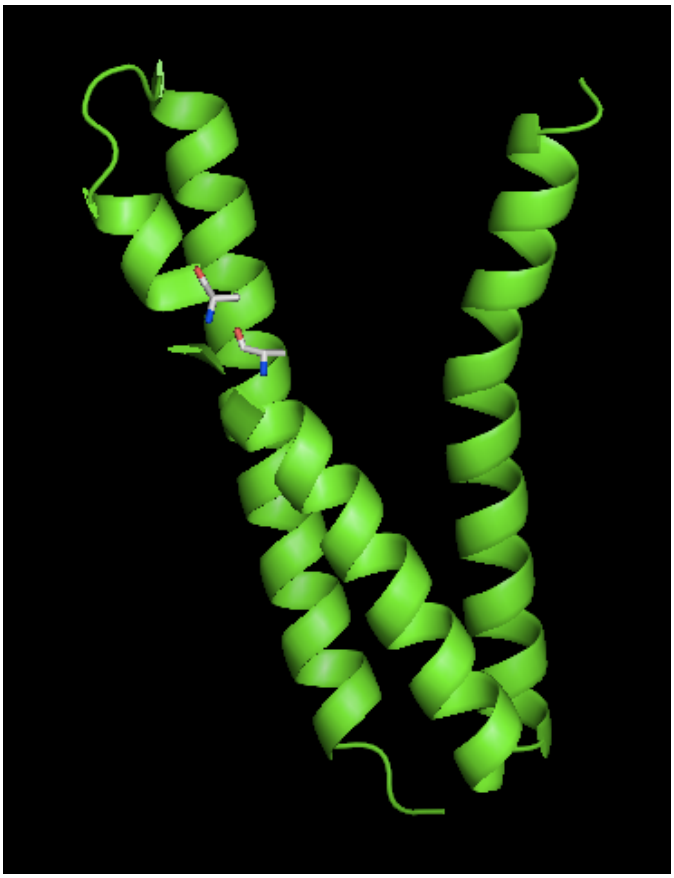


Figure 9: 3D structure of Der p 5 with the residues 85 and 88 mutated into alaline