Structural and functional characterization of outer membrane protein N in *Edwardsiella ictaluri*: A bioinformatic approach

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ABSTRACT

Outer membrane protein (OMP) N is involved in the translocation of solutes across the outer membrane of Gram negative bacteria. It is also involved in adhesion and invasion that bestow virulence attribute to the bacteria. *Edwardsiella ictaluri* is a Gram negative bacterium which causes enteric septicemia of catfish. It is a devastating disease causing huge economic loss to fish farmers. Studies are in progress to design suitable vaccine against *E. ictaluri*. OMP N gene occurs three times in the whole genome sequence of *E. ictaluri*. Since these proteins occur in the outer surface of bacterium, they are potential vaccine candidates. Towards this, understanding the structure and function of these proteins is highly important. Hence, a bioinformatics based study for characterizing the structure and function of these OMPs are carried out. The physiochemical characteristics of these OMPs were determined. The signal peptide and transmembrane regions of the proteins were identified. A multiple sequence alignment was done to locate the conserved regions in the proteins. The structure was obtained through homology modeling. The structure obtained was validated using different servers. Finally the functions of the OMPs were predicted. The study revealed that OMP N proteins of *E. ictaluri* are porins and have sixteen transmembrane strands traversing through the inner and outer membrane. The proteins are possibly involved in translocation of sugars, signal transduction, anchoring the flagellum and also in various cellular processes.

Keywords: *Edwardsiella ictaluri*, Outer membrane protein, Homology modeling, Bioinformatics

INTRODUCTION

Outer membrane proteins (OMPs) are a class of proteins residing in the outer membrane of Gram-negative bacterial cells. These proteins protect the bacteria in a hostile environment and also help in a number of tasks including translocation of solute across the impermeable outer membrane and signal transduction [1]. They also function as receptors for bacteriophages and bacteriocins. They aid in the translocation of solutes across the impermeable outer membrane of Gram negative bacteria. These proteins are also involved in adhesion and invasion of the bacteria [1]. Since these proteins are located in the outer surface of the membrane, they can act as epitopes and hence are potential vaccine candidates.

The Gram-negative bacteria *Edwardsiella ictaluri* causes enteric septicemia of catfish. The disease was first identified in moribund catfish in Georgia and Alabama in 1976 [2]. Although channel catfish [3, 4, 5] are the most susceptible to infection, yellow catfish [6], white catfish [7], and walking catfish [8] can also be infected by *E. ictaluri*. Natural outbreaks have also been reported in nonictalurid species, including Danio [9] and rosy barbs [10]. The disease can be best described as an acute, rapidly progressive septicemia in exposed or inoculated healthy fish. Fish can be seen swimming erratically in tight circles or hanging listlessly in the water column in a head up and tail down position. They normally stop eating shortly after becoming infected [11, 12]. The disease caused huge economic loss to cat fish industry.

Efforts on OMPs as vaccine candidate against this bacterium are in progress. Towards this knowledge about the structure and function of the OMP is essential. A whole genome analysis of *E. ictaluri* revealed that OMP N gene occurs thrice in the whole genome. In this study the structure and function of these OMPs of *E. ictaluri* for the purpose of using them as potential vaccine molecules was undertaken.

MATERIALS AND METHODS

Datasets

The OMP N protein sequences in the whole genome of *E. ictaluri*, (NC_012779) namely OMP N1, OMP N2 and OMP N3 (NCBI protein ID: YP_002932251.1, YP_002933286.1 and YP_002935188.1) were retrieved from NCBI GenBank (http://www.ncbi.nlm.nih.gov/).

Physicochemical analysis

Theoretical pI and molecular weight was determined using the ExPASy (Expert Protein Analysis System) proteomics server of the Swiss Institute of Bioinformatics (http://expasy.org/tools/pi_tool.html) [13, 14].

Transmembrane region prediction

The transmembrane beta strands of these OMPs were predicted using the web server Pred TMBB (http://biophysics.biol.uoa.gr/PRED-TMBB/) [15].

Signal peptide prediction

The signal peptide present in OMP N1, N2 and N3 of *E. ictaluri* was predicted by SignalP 3.0 server (http://www.cbs.dtu.dk/services/SignalP/) [16].

Multiple sequence alignment

The multiple sequence alignment between OMP N1, N2 and N3 of *E. ictaluri* was studied using the CLUSTAL W programme (www.ebi.ac.uk/clustalw/) [17].

Homology Modeling

The approach of homology modelling was used in constructing 3D models of *E. ictaluri* OMP N proteins. The proteins were modelled based on the alignment between the target and template. NCBI Protein BLAST of *E. ictaluri* OMP N proteins (target) was done against PDB database to obtain a structurally similar protein namely 'template'. The target - template sequence alignment was done using CLUSTAL W. The resulting alignment file was provided to SWISS MODEL server (http://swissmodel.expasy.org/) [18] with the alignment input format as CLUSTAL W to obtain a 3D model for the protein. This model was viewed using the visualization software PYMOL (The PyMOL Molecular Graphics System, Version 1.2r3pre, Schrödinger, LLC.) and the UCSF Chimera package from the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco (supported by NIH P41 RR001081). The structural superimposition of the proteins was also done using Chimera.

Structure validation

The homology modeled structure of the proteins was validated using PROCHECK (http://www.ebi.ac.uk/thornton-srv/software/PROCHECK/)[19] and WHAT IF servers (http://swift.cmbi.ru.nl/servers/html/index.html) [20]. The phi-psi torsion angles for all residues in the structure were plotted in the Ramchandran Plot at PROCHECK. Here the combinations of phi-psi angles are distributed into various regions of the plot namely the most favoured, allowed and disallowed regions. The What If server checks various parameters including nomenclature, packing quality control, anomalous bond lengths and bond angles, omega angles and proline puckering in the structures.

Function prediction

The structural motifs found in functionally important regions of the protein structure were obtained from ProFunc server (http://www.ebi.ac.uk/thornton-srv/databases/ProFunc/)[21]. The fingerprints, which are a group of conserved domains used to characterize a protein were obtained from PRINTS server (http:// www. bioinf. Manchester .ac .uk / dbbrowser/PRINTS/index.php) [22].

RESULTS AND DISCUSSION

The OMP N1 gene occurred in the complementary strand of *E. ictaluri* DNA and was flanked by a penicillin binding protein at one end and a hypothetical protein at the other end. The OMP N2 gene also occurred in the

complementary strand of *E. ictaluri* and was flanked by beta-ketoadipate enol-lactone hydrolase at one end and phosphofructo kinase at the other. Unlike OMP N1 and OMP N2 genes, OMP N3 gene was found in the positive strand of the DNA and was flanked by a HAD super family protein and a putative tRNA (uracil-5-)-methyltransferase.

Physicochemical analysis

OMP N1 was a 402 amino acid protein with molecular weight of 4.41 kDa and isoelectric point of 5.30. OMP N2 was a 373 amino acid protein with molecular weight of 4.10 kDa and isoelectric point of 5.39. OMP N3 had 376 aminoacids with molecular weight of 4.2 kDa and isoelectric point of 8.95.

Transmembrane region prediction

There were 16 transmembrane beta strands in OMP N proteins of *E. ictaluri*. The transmembrane regions are depicted in Fig. 1, 2 and 3.

Signal peptide prediction

The signal peptide of OMP N1 was "MKYKYLAVAIPVLLAAGMANS" and the cleavage occurs between the 21^{st} serine and 22^{nd} alanine residues. In OMP N2 the signal peptide cleavage occurs between the 21^{st} alanine and 22^{nd} alanine residues. The signal sequence was "MKRNLLAAVIPALLVAGAANA". The signal sequence of OMP N3 was "MNTIRSLVALSTIGCIGIFPLISHA" and cleavage occurs between 25^{th} alanine and 26^{th} alanine residues.

Multiple sequence alignment

The multiple sequence alignment of OMP N1, N2 and N3 by CLUSTAL W is shown in Fig. 4. Most of the conserved region in the MSA occurs in the porin signature or in the transmembrane strands.

Homology Modeling

The BLAST P analysis of OMP N proteins against PDB database was done to obtain structurally similar templates. The particulars of the templates used are detailed in table 1. The 3D model of OMP N1, N2 and N3 are shown in Fig. 5, 6 and 7. The structure revealed 16 antiparallel beta strands traversing the inner and outer membrane. The loops that connect the beta strands are shorter towards the periplasmic space, but longer towards the extracellular side. A comparison of the structures by means of structural superimposition (Fig. 8) revealed that variability occurs mostly in the extracellular space when compared to the protein region in the periplasmic space.

Structure validation

The structure was further validated using PROCHECK and WHAT IF servers. The Ramachandran Plot obtained from the PROCHECK server is shown in Fig. 9, 10 and 11. The Name check feature of WHAT IF server checks the accuracy of torsion angle nomenclature in the structures. The optimum chi^2 value was between -90 and +90. Except in 112^{th} tyrosine and 100^{th} phenylalanine amino acid residues in OMP N1, 303^{rd} tyrosine in OMP N2 and the deviation in 96^{th} tyrosine, 23^{rd} phenylalanine and 304^{th} aspartic acid residues in OMP N3 chi^2 values were within the expected range. The average Coarse Packing Quality Control (CPQC) for OMP N1 N2 and N3 amino acids were -1.209, -1.290 and -0.948 respectively which was well above the cut off value of -5. Hence, it is apparent that the CPQC of the proteins were good. Anomalous bond lengths are the bond lengths that deviate more than four sigma from the normal. A check for anomalous bond lengths showed that OMP N1, N2 and N3 had a RMS Z-score of 0.747, 0.809 and 0.697 with RMS-deviation of 0. 016, 0.018 and 0.014 respectively. The bond lengths were found to deviate normally from the standard bond lengths. The results of the fine packing quality control of OMP N1, OMP N2 and OMP N3 are listed in table 2. The standard deviation of the Omega angles of OMP N1, N2 and N3 was found to be 6.275, 4.332 and 6.804 respectively. The expected average value of standard deviation was approximately 5.5. Hence the standard deviation of the Omega angels of OMP N1, N2 and N3 were in the expected range. The puckering amplitude of all the proline residues of OMP N1 was within the normal range (0.20 to 0.45) except in the 66^{th} proline where it was 0.49. In OMP N2 the puckering amplitude of the entire proline residue was within the normal range but in OMP N3, 109^{th} and 111^{th} proline had high puckering amplitude of 0.45 and 0.56. The RMS Z score and RMS deviation for bond angles for OMP N1 was 1.427 and 2.765 while for OMP N2 it was 1.132 and 2.053 and for OMP N3 was 1.220 and 2.243 respectively. All these results show that the structure of OMP N1, N2 and N3 obtained by homology modelling is a valid one.

Function prediction

The ProFunc server detected five structural motifs in OMP N1 and N3 while N2 had four structural motifs in functionally important regions of the protein. The details of these motifs are presented in table 3. The fingerprint signatures found in these proteins are given in Fig. 1, 2 and 3.

OMP N1

The presence of a galactokinase family signature in OMP N1 indicates that the protein may be involved in the translocation of sugars, phosphorylation or in signal transduction. The porin signatures confirm the protein to be a porin. The Flagellar P-ring protein signature in OMP N1 suggests that the protein is involved in anchoring the flagellum in the peptidoglycan layer between the inner and outer membrane. The presence of EDG-6 sphingosine 1-phosphate receptor signature shows the involvement of the protein in signal transmission and cell recognition. The potassium channel present suggests the association of the protein in cellular processes.

OMP N2

OMP N2 is a porin and is involved in the virulence *of E. ictaluri*. The GPR6 orphan receptors suggest the role of this protein in signal transduction pathways and initiating cellular responses. The role of plant protein signatures namely Gliadin and LMW glutenin superfamily signature, plant beta-amylase signature and 2-S globulin family signature are yet to be determined. We suggest the possibility of an evolutionary significance.

OMP N3

The presence of epithelial membrane protein signature in OMP N3 suggests the role of this protein in the control of cell growth. The protein is also involved in iron acquisition as there is a transferrin signature present in the protein. OMP N3 is also a porin.

CONCLUSION

The homology based structure reveals that all the three OMPs *viz.* N1, N2 and N3 are porins with sixteen transmembrane beta strands. The validity of the structures was confirmed using web servers. These proteins possibly are involved in several functions such as translocation of sugars, phosphorylation and signal transduction, initiating cellular response control of cell growth and iron acquisition. The structural and functional prediction of OMP N proteins of *E. ictaluri* could help in vaccine design and identifying drug target against the bacterium.

ACKNOWLEDGEMENT

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FIGURE LEGENDS

Fig. 1. Nucleotide-Amino acid sequence of OMP N1 depicting functionally important regions
Fig. 2. Nucleotide-Amino acid sequence of OMP N2 depicting functionally important regions
Fig. 3. Nucleotide-Amino acid sequence of OMP N3 depicting functionally important regions
Fig. 4. Multiple sequence analysis of OMP N1, OMP N2 and OMP N3
Fig. 5. Homology modelled structure of OMP N1, OMP N2 and OMP N3
Fig. 6. Superimposed image of OMP N1 (blue), OMP N2 (Yellow) and OMP N3 (cyan)
Fig. 7. Ramachandran plot for OMP N1
Fig. 8. Ramachandran plot for OMP N2
Fig. 9. Ramachandran plot for OMP N3

Table 1: Details of template used in homology modelling

Target	Template PDB ID	Template description	Sequence Identity [%]

OMP N1	1phoA	Porin from *Escherichia coli*	45.855
OMP N2	1osmA	Osmoporin from *Klebsiella pneumoniae*	68
OMP N3	2xe1A	OMP C from *Escherichia coli*	41.389

Table 2: Fine packing quality control

Protein	BB-BB contacts (Z score)	BB-SC contacts (Z score)	SC-BB contacts (Z score)	SC-SC contacts (Z score)
OMP N1	0.44	-3.11	-1.14	-2.51
OMP N2	0.43	-3.84	-1.38	-2.76
OMP N3	1.17	-2.90	-0.85	-2.65

* BB-Back bone, SC-Side chain

Table 3: Structural motifs present in functionally important regions of OMP N1, N2 and N3

Sl. No	OMP N1	OMP N2	OMP N3
1	Asp249, Ala250,Gln251	Asn357,Ser358,Ile359	Gln111,Tyr112,Gly113
2	Asn289,Met290,Thr291	Ser91,Asn92,Phe93	Asn360,Gly361,Ile362
3	Ser344,Val345,Lys346	Asn266,Met267,Thr268	Asp313,Leu314,Lys315, Gly316, Asn317
4	Gly130,Asp131,Ser132	His227,Gly228,sn229	Asn167,Leu168,Val169
5	Met140,Thr141,Gly142		Asn271, Met272, His273

```
 1 atgaaatataaataccttgcagtggcgattccagttctgctggcg
   M   K   Y   K   Y   L   A   V   A   I   P   V   L   L   A
46 gcgggcatggccaattcggctgagatatataataagaatggcaat
   A   G   M   A   N   S   A   E   I   Y   N   K   N   G   N
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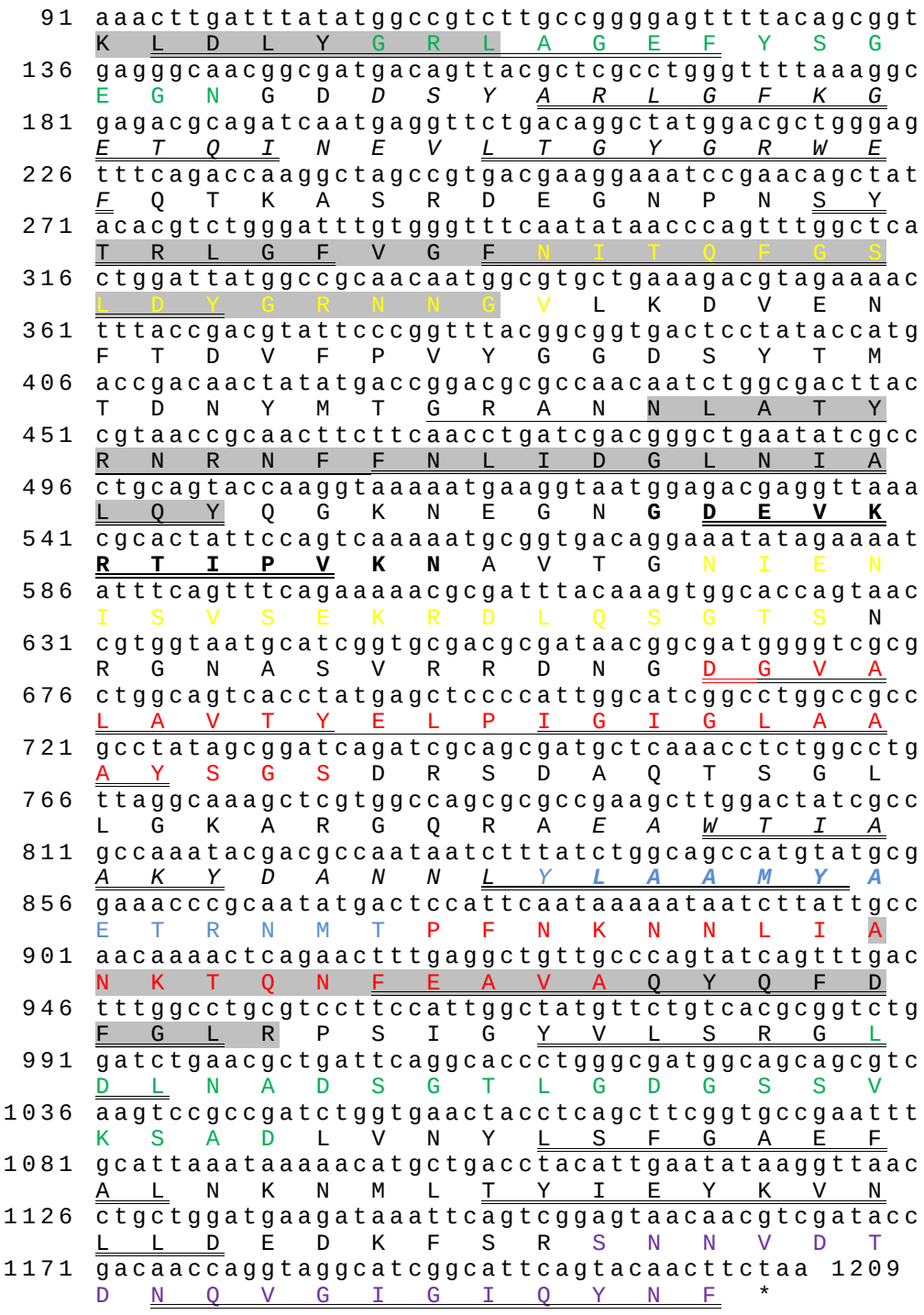

Fig. 1. Nucleotide-Amino acid sequence of OMP N1 depicting functionally important regions

Grey color highlight: E. coli porin signature, Under line: Galactokinase family signature, Italics: Neisseria sp. porin signature, Bold: Zeta tubulin signature, Red color: Beta Tubulin signature, Green color: EDG-6 sphingosine 1-phosphate receptor signature, Yellow color: Flagellar P-ring protein signature, Blue color: EAG/ELK/ERG potassium channel family signature, Purple color: Repair protein Rad1/Rec1 family signature and Double underline: Trans-membrane region

```
  1 atgaaacgtaatctgctggcagccgttattcctgctctgttagtt
    M  K  R  N  L  L  A  A  V  I  P  A  L  L  V
 46 gcaggcgcagccaacgctgcggaaatctacaacaaagacggcaac
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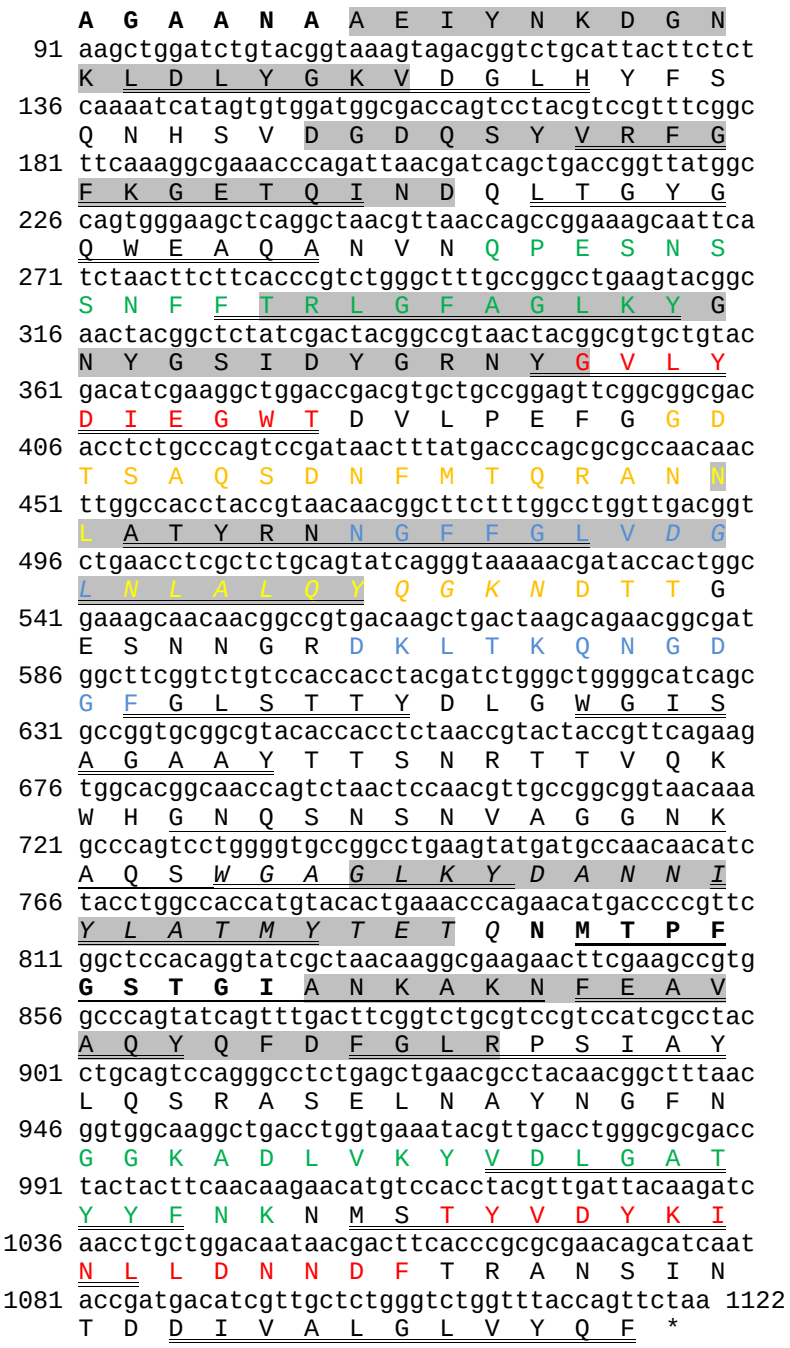
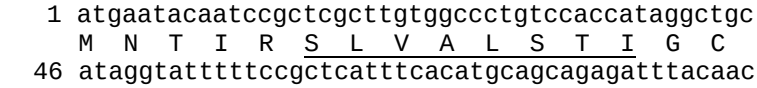


Fig. 2. Nucleotide-Amino acid sequence of OMP N2 depicting functionally important regions

Grey color highlight: E-coli porin signature, Under line: GPR6 orphan receptor signature, Italics: Enterobacterial virulence outer membrane protein signature, Bold: Gliadin and LMW glutenin superfamily signature, Red color: Plant beta-amylase signature, Green color: 2-S globulin family signature, Yellow color: P2Y1 purinoceptor signature, Blue color: Adenovirus fibre protein signature, Double underline: Trans-membrane region



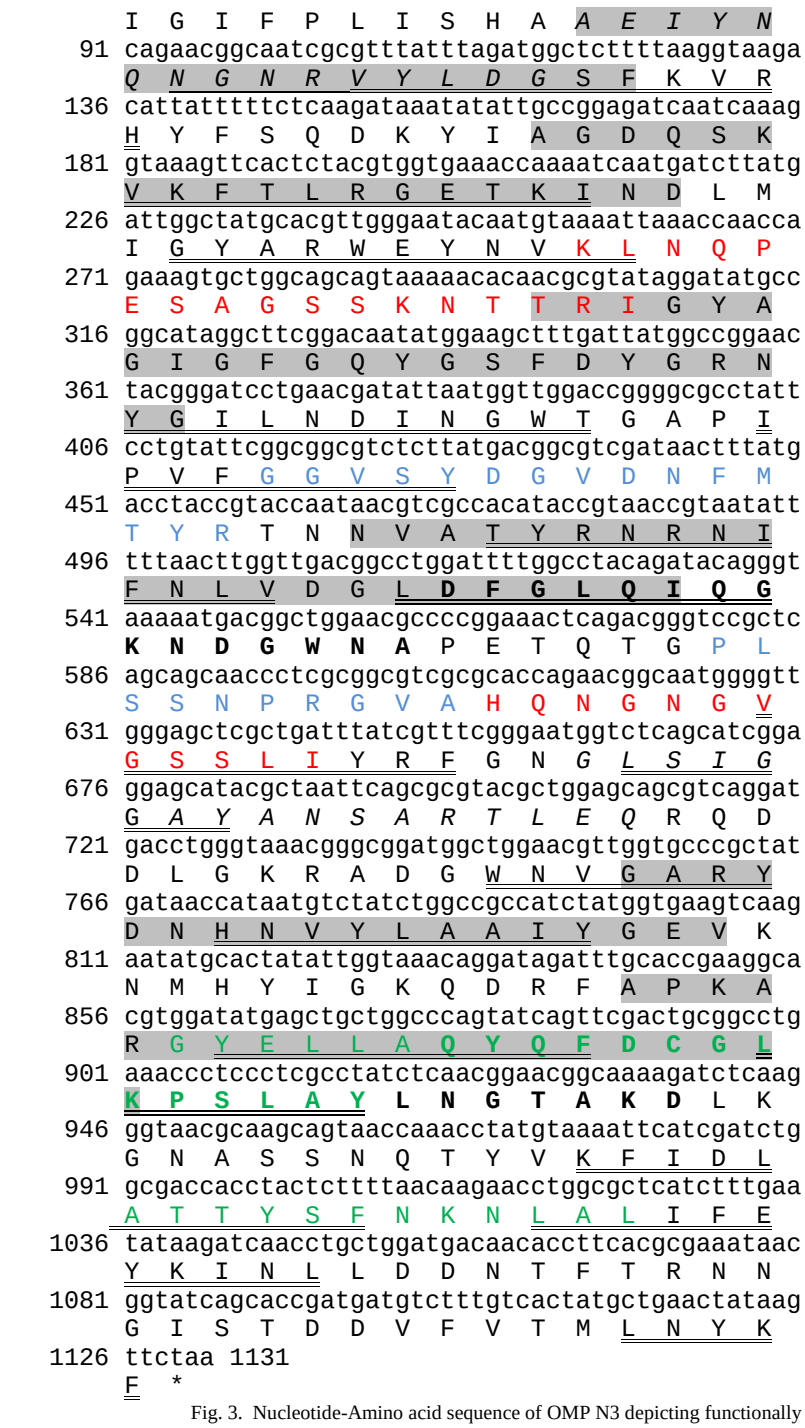


Fig. 3. Nucleotide-Amino acid sequence of OMP N3 depicting functionally important regions

Grey color highlight: E-coli porin signature, Under line: Epithelial membrane protein (EMP/PMP22/LMIP) family signature, Italics: Transferrin signature, Bold: Interleukin-1 beta precursor signature, Red color: Cholecystokinin type A receptor signature, Green color: Tumour necrosis factor c (lymphotoxin-beta) signature, blue color: Ependymin signature, Double under line: Trans-membrane region

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ompN1           MKYKYLAVAIPVLLAAG----MANSAEIYNKNGNKLDLYGRLAGEFYSGEGN---GDDSY 53
ompN2           MKRNLLAAVIPALLVAG----AANAAEIYNKDGNKLDLYGKVDGLHYFSQNHSVDGDQSY 56
ompN3           MNTIRSLVALSTIGCIGIFPLISHAAEIYNQNGNRVYLDGSFKVRHYFSQDKYIAGDQSK 60
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                *:     ..:..:   *     :::*****::**:: * * .   .* .:.:   **:*

ompN1           ARLGFKGETQINEVLTGYGRWEFQTKASRDE-GNP-NSYTRLGFVGFNITQFGSLDYGRN 111
ompN2           VRFGFKGETQINDQLTGYGQWEAQANVNQPE-SNSSNFFTRLGFAGLKYGNYGSIDYGRN 115
ompN3           VKFTLRGETKINDLMIGYARWEYNVKLNQPESAGSSKNTTRIGYAGIGFGQYGSFDYGRN 120
                .:: ::***:**: : **.:** :.: .: * ... :  **:*:.*:   ::**:*****

ompN1           NGVLKDVENFT-DVFPVYGGDSYTMTDNYMTGRANNLATYRNRNFFNLIDGLNIALQYQG 170
ompN2           YGVLYDIEGWT-DVLPEFGGDTSAQSDNFMTQRANNLATYRNNGFFGLVDGLNLALQYQG 174
ompN3           YGILNDINGWTGAPIPVFGGVSYDGVDNFMTYRTNNVATYRNRNIFNLVDGLDFGLQIQG 180
                 *:* *::.:*   :* :** :    **:** *:**:*****..:*.*:***::.** **

ompN1           KNEGNGDEVKRTIPVKNAVTGNIENISVSEKRDLQSGTSNRGNASVRRDNGDGVALAVTY 230
ompN2           KND-------------------------------TTGESNNGRDKLTKQNGDGFGLSTTY 203
ompN3           KNDGWN-----------------------APETQTGPLSSNPRGVAHQNGNGVGSSLIY 216
                **:                                 :*  . .   : ::**:*.. :  *

ompN1           ELPIGIGLAAAYSGSDRSDAQTSG----LLGKARGQRAEAWTIAAKYDANNLYLAAMYAE 286
ompN2           DLGWGISAGAAYTTSNRTTVQKWHGNQSNSNVAGGNKAQSWGAGLKYDANNIYLATMYTE 263
ompN3           RFGNGLSIGGAYANSARTLEQRQD--------DLGKRADGWNVGARYDNHNVYLAAIYGE 268
                 :  *:. ..**: * *:  *             *::*:.*  . :** :*:***::* *

ompN1           TRNMTPFNKNNLIANKTQNFEAVAQYQFDFGLRPSIGYVLSRGLDLNADSGTLGDGSSVK 346
ompN2           TQNMTPFG-STGIANKAKNFEAVAQYQFDFGLRPSIAYLQSRASELNAYNGFNGG----- 317
ompN3           VKNMHYIGKQDRFAPKARGYELLAQYQFDCGLKPSLAYLNGTAKDLKGNASSNQT----- 323
                .:**  :. .  :* *::.:* :****** **:**:.*: . . :*:.  .

ompN1           SADLVNYLSFGAEFALNKNMLTYIEYKVNLLDEDKFSRSNNVDTDNQVGIGIQYNF 402
ompN2           KADLVKYVDLGATYYFNKNMSTYVDYKINLLDNNDFTRANSINTDDIVALGLVYQF 373
ompN3           ---YVKFIDLATTYSFNKNLALIFEYKINLLDDNTFTRNNGISTDDVFVTMLNYKF 376
                    *:::.:.: : :***:   .:**:****:: *:* *.:.**: .   : *:*
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Fig. 4. Multiple sequence analysis of OMP N1, OMP N2 and OMP N3

‘*’ residues in that column are identical in all sequences in the alignment
‘:’ conserved substitutions
‘.’ semi-conserved substitutions

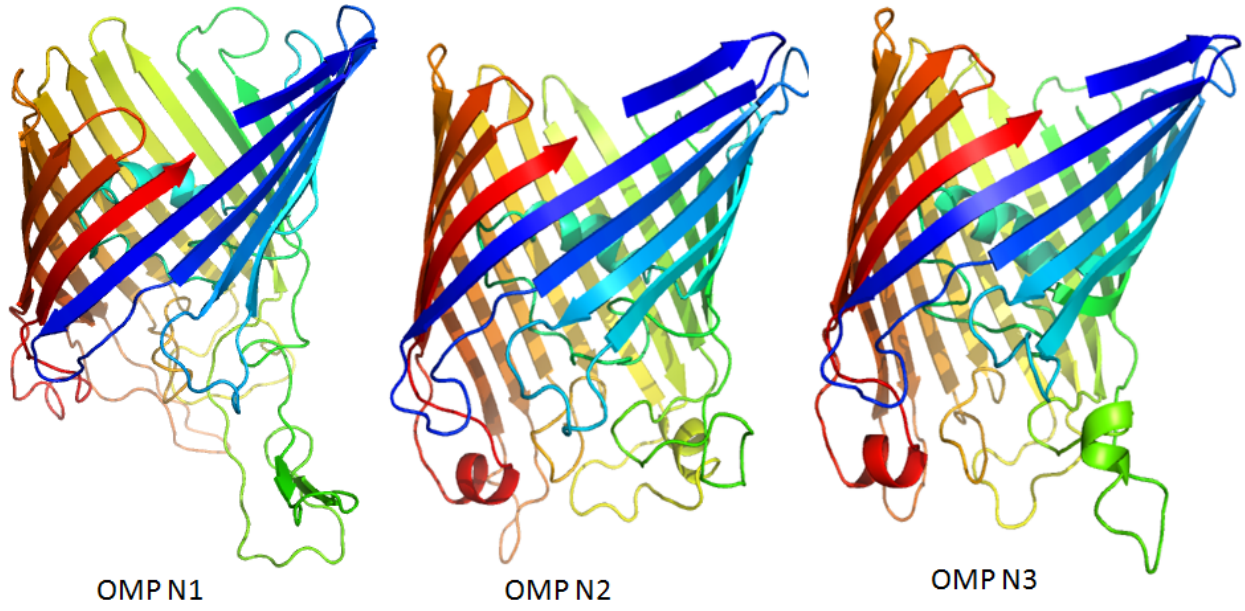

Fig. 5. Homology modelled structure of OMP N1, OMP N2 and OMP N3

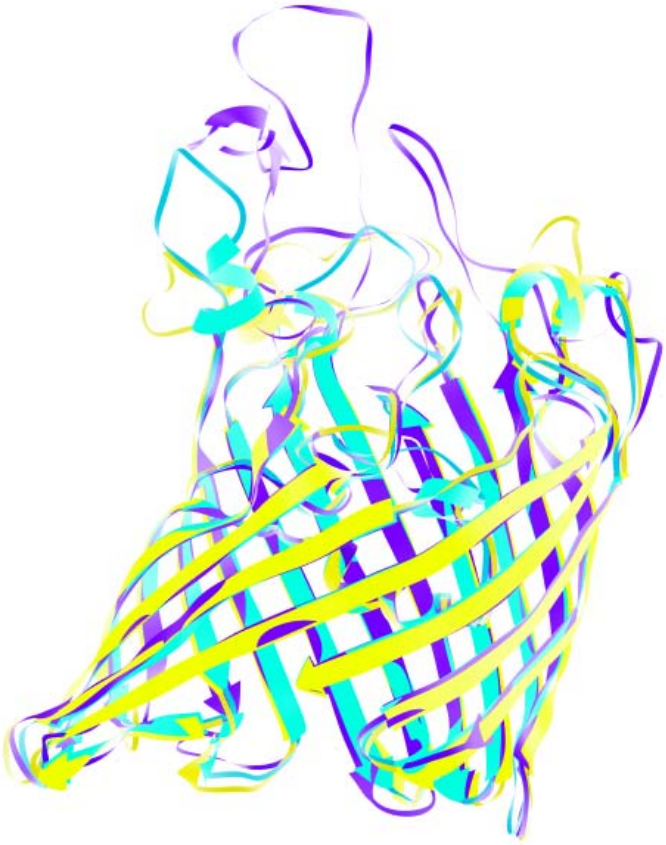

Fig. 6. Superimposed image of OMP N1 (blue), OMP N2 (Yellow) and OMP N3 (cyan)

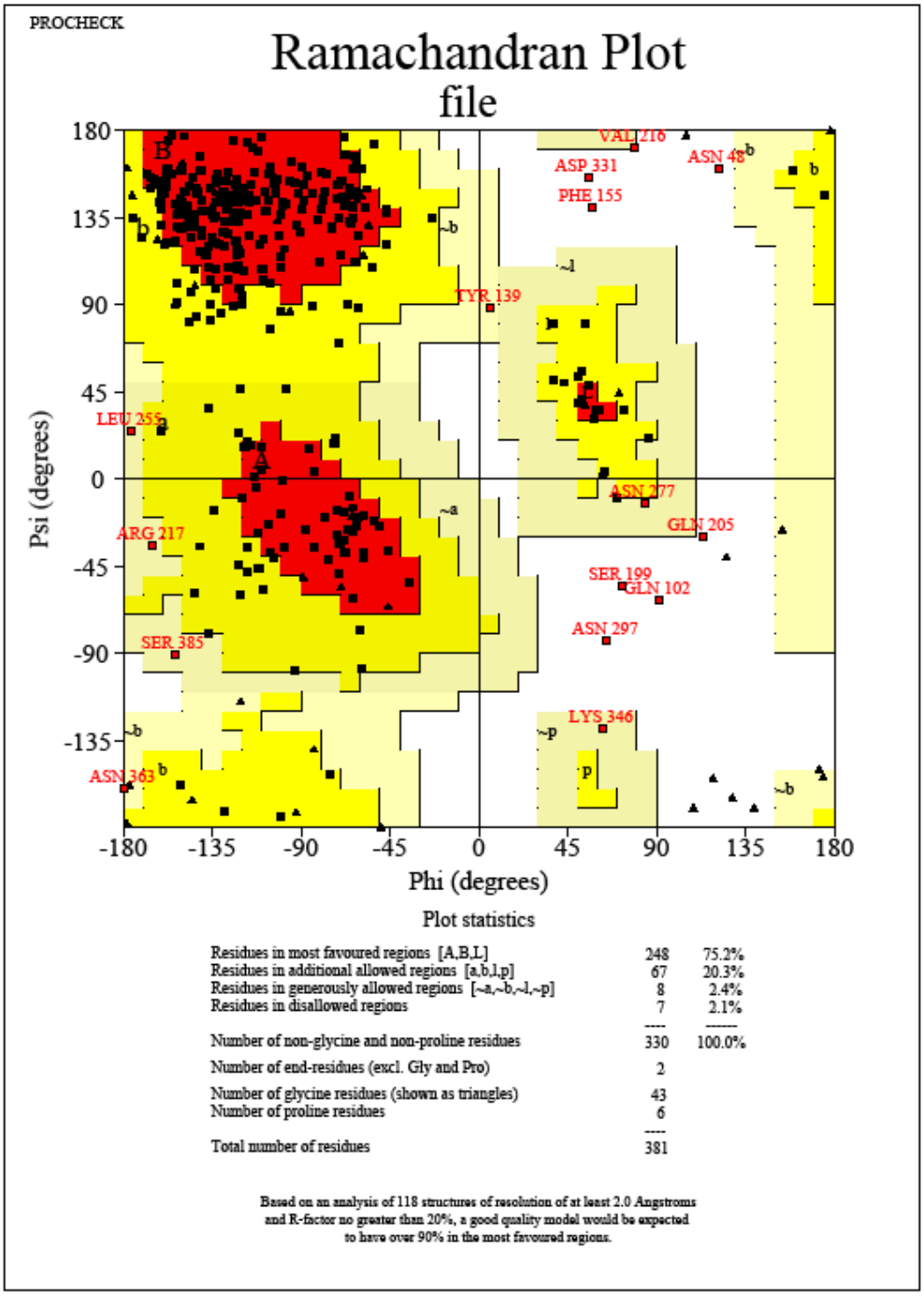

Fig. 7. Ramachandran plot for OMP N1

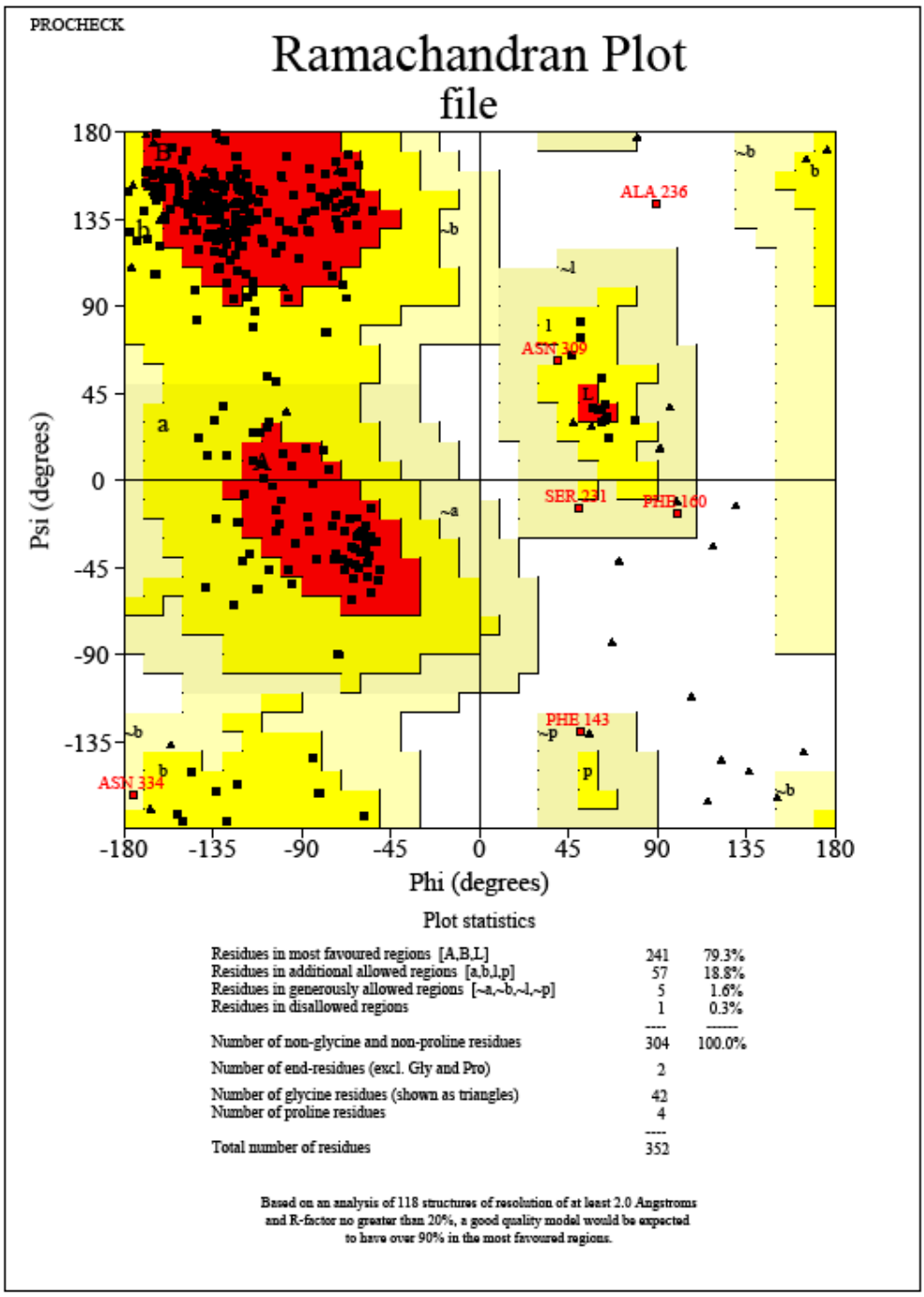

Fig. 8. Ramachandran plot for OMP N2

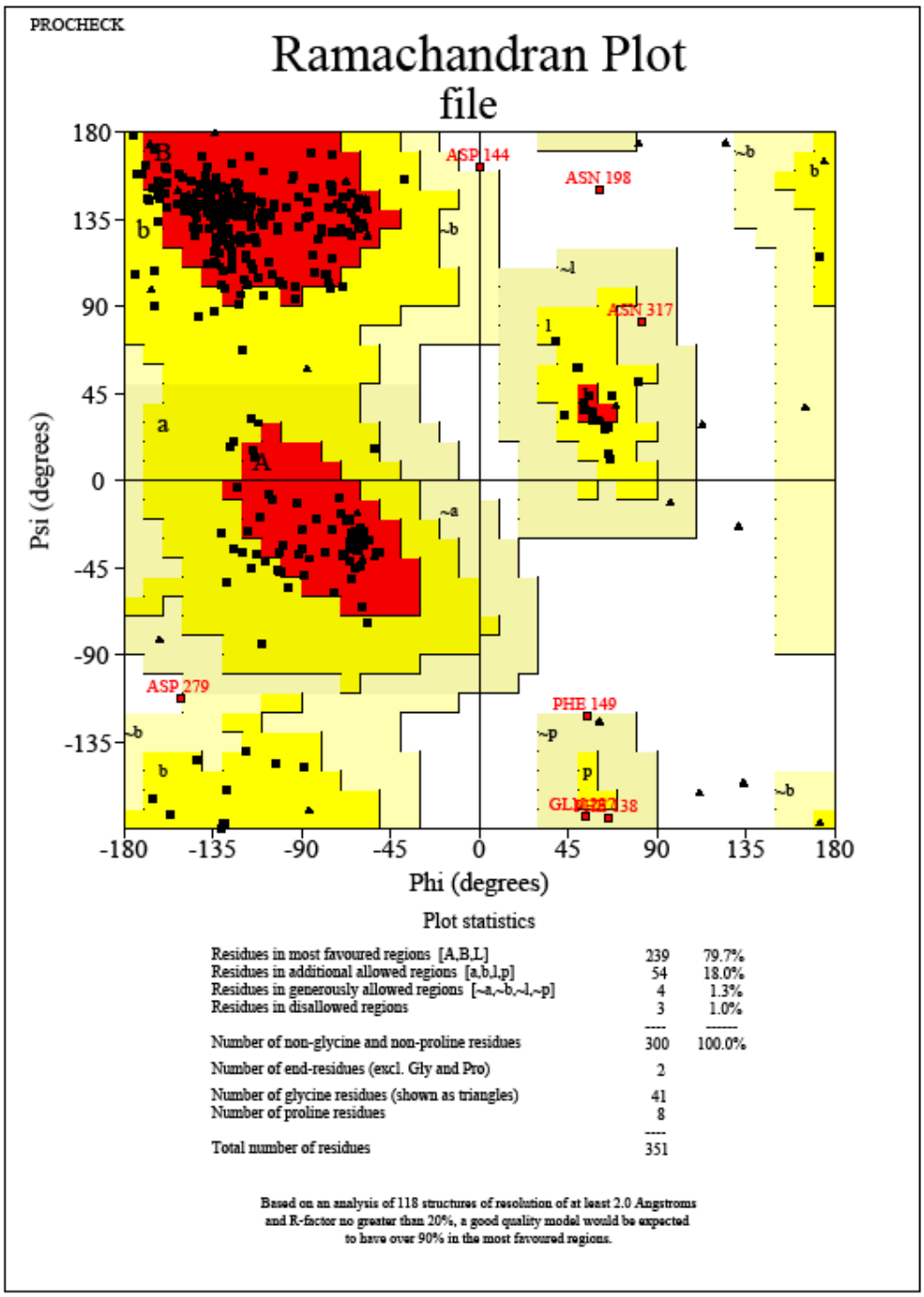

Fig. 9. Ramachandran plot for OMP N3