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# Structural insights into a *Plasmodium falciparum* IMC1 protein using bioinformatics tools

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**Abstract**
Alveolates (dinoflagellates, ciliates and apicomplexans) possess a characteristic cortical structure termed as the 'pellicle'. Alveolate pellicle plays a structural role and helps define the cell shape in dinoflagellates and ciliates whereas in apicomplexans it plays an additional role in parasite motility and cytokinesis. The pellicle is composed of flattened membranous sacs which subtend immediately below the plasma membrane. These flattened sacs are termed as the 'inner membrane complex' (IMC) in apicomplexans. IMC proteins can be categorized according to their structural features into three groups namely multi-transmembrane proteins, alveolins and non-alveolins. Only a few members of IMC1 family of proteins are known in *Plasmodium falciparum* (Pf). Knock-out studies of IMC1 members in other *Plasmodium* species have demonstrated the role of these proteins in mechanical stability and motility of the parasite. Here, we used bioinformatics tools to gain insight into the structure of IMC1l. We predicted the partial 3 dimensional model of Pf IMC1l and propose that its α-helical region might be involved in binding to cytoskeletal proteins such as actin or myosin. The hydrophobic residues in the IMCp region of IMC1 proteins might be responsible for their attachment to the IMC membrane. PfIMC1 proteins were also found to have asparagine rich regions, N-or C-terminal palmitoylation sites and other motifs of functional significance. Being crucial structural elements of inner membrane complex, IMC1 proteins are likely to be potential antimalarial drug targets.

**Keywords:** Inner membrane complex, *Plasmodium falciparum*, secondary structure, motifs

## 1. Introduction
The super phylum Alveolata consists of three phyla *viz*. the Dinoflagellata, the Cilliata and the Apicomplexa. While dinoflagellates and ciliates are commonly known to exist in the aquatic niches, most apicomplexans have adopted the parasitic mode of nutrition for their survival. The phylum Apicomplexa consists of unicellular protozoan parasites which cause numerous diseases in humans and animals. This phylum includes species of *Plasmodium*, *Toxoplasma*, *Neospora*, and *Babesia* etc [1].

The malaria parasite has complex and a multistage life cycle that occurs within the two hosts: the mosquito and the vertebrate host. The development and the survival of the parasite in these hosts is accomplished by specialized proteins that enable the parasite to divide and grow in multiple host cell types, and also evade the immune response [2].

A characteristic feature common to all alveolates is the presence of cortically placed flattened membranous sacs termed as 'alveoli' in ciliates, 'amphiesmal vesicles' in dinoflagellates and the 'inner membrane complex' in apicomplexa. These sacs subtend below the plasma membrane and forms a three layered structure called as pellicle [3, 4]. The pellicle maintains the cell shape in dinoflagellates and ciliates [5] whereas the IMC in apicomplexa plays an additional role in parasite motility and cytokinesis [6].

Apart from its close association with cytoskeletal elements such as actin, myosin and microtubules, IMC on its cytoplasmic surface is lined by a meshwork of intermediate-filament like proteins. IMC serves to support the parasite's actin-myosin apparatus (located between the IMC and the plasma membrane) required for the host cell invasion [7, 8]. Furthermore, IMC also plays an important role in parasite's cell division and acts as a scaffold for organellogenesis and organelle partitioning [9]. Structurally, the IMC occurs as a single large flattened vesicle connected by a single suture in *Plasmodium* sporozoites, [10] whereas in other apicomplexans it consists of multiple flattened vesicles joined by a patchwork of sutures [11].

Till date only a handful of Pf IMC1 family of proteins has been identified. In contrast knock-out studies of proteins IMC1a, IMC1b, and IMC1h in *P. berghei* zoites have revealed the

participation of these proteins in parasite's motility and mechanical strength [12-14].

IMC1l (PF3D7_1417000/PF14_0168) is a Pf IMC protein that is highly conserved across the *Plasmodium* species. Mass spectrum data available on *www.Plasmodb.org* shows the expression of this protein during the salivary gland sporozoite stage of the parasite. We used bioinformatics tools to predict secondary structure and identify the domains and motifs of functional importance in PfIMC1 proteins. Further, the partial tertiary model of IMC1l was used to predict the binding site on this protein.

## 2. Material and methods

### 2.1 Multiple sequence alignment

The amino acid sequence of IMC1l and its orthologs in other *Plamsodium* species were obtained from *www.Plasomodb.org*.database. In order to determine the sequence conservation of IMC1l, we performed multiple sequence alignment using Clustal Omega tool accessed via EMBL-EBI (https://www.ebi.ac.uk/Tools/msa/clustalo/) [15].

### 2.2 Protein BLAST analysis

Homologs of IMC1l in species other than *Plasmodium* were identified by protein BLAST (BLASTp) (https://blast.ncbi.nlm.nih.gov/Blast.cgi) accessed via National Center for Biotechnology Information (NCBI). (https://www.ncbi.nlm.nih.gov/).

### 2.3 Secondary structure prediction of Pf IMC1 proteins

Secondary structure prediction of proteins of Pf IMC1 family members was performed by using PSIPRED v3.3 tool. (Position specific iterative-blast based secondary structure PREDiction) [16, 17]

### 2.4 Tertiary structure prediction of IMC1l

SWISS MODEL tool [18] was used to generate the 3D model of IMC1l. Further, model validation was performed using RAMPAGE [19].

### 2.5 Identification of motifs, domains and other post-translational modification sites in Pf IMC1 proteins.

ScanProsite tool [20] available through ExPASy (https://www.expasy.org/) server was used to identify biologically relevant residues such as active sites, binding sites and post-translational modification sites and InterPro67.0 [21] was used to identify the domains and motifs in Pf IMC1 proteins.

The STRING database [22] was used to predict the proteins likely to interact with IMC1l while TMPreD [23] was used to predict the trans-membrane regions in IMC1l.

## 3. Results and discussion

**3.1** Apicomplexan zoite stage (ookinete, sporozoites and merozoites) pellicle is composed of cortically distinct flattened membranous sacs called as 'inner membrane complex' which subtends immediately below the plasma membrane. On its cytoplasmic surface, the IMC is associated with the meshwork of 8-10 nm intermediate-filaments like proteins. These proteins form the membrane skeleton by constituting the indispensable component of the subpellicular network. The SPN supports the pellicular membranes and provides mechanical stability to the cell [8]. Apart from their important role in morphogenesis and tensile strength, *Plasmodium* IMC1 proteins expresses throughout the life cycle of *Plasmodium* suggesting their participation in parasite's development in both hosts and are also involved in gliding motility of the parasite probably through interaction with the components of the glidosome (actin, myosin, glidosome associated proteins 45 and 50) [12, 13]. Thirteen Pf IMC1 proteins (IMC1a-IMC1m) have been predicted till date [12, 24, 25]. The gene ID, the alternative names (if applicable), their chromosome number, and the number of amino acids in each these Pf IMC1 proteins are shown in Table 1.

**Table 1:** Pf IMC1proteins (IMC1a-IMC1m). NA: Not applicable

| Pf IMC1 protein | Gene ID | Alternative Names | Location (Chromosome Number) | Number of amino acids |
|---|---|---|---|---|
| IMC1a | PF3D7_0304000 | PFALV1 | 3 | 861 |
| IMC1b | PF3D7_1141900 | NA | 11 | 504 |
| IMC1c | PF3D7_1003600 | PFALV5 | 10 | 281 |
| IMC1d | PF3D7_0708600 | NA | 07 | 252 |
| IMC1e | PF3D7_0304100 | PFALV2 | 03 | 525 |
| IMC1f | PF3D7_1351700 | PFALV6 | 13 | 1318 |
| IMC1g | PF3D7_0525800 | NA | 05 | 300 |
| IMC1h | PF3D7_1221400 | PFALV3 | 12 | 524 |
| IMC1i | PF3D7_0823500 | NA | 08 | 502 |
| IMC1j | PF3D7_0621400 | PF77, PFALV7 | 06 | 664 |
| IMC1k | PF3D7_1341800 | NA | 13 | 462 |
| IMC1l | PF3D7_1417000 | NA | 14 | 382 |
| IMC1m | PF3D7_1028900 | NA | 10 | 369 |

Pf IMC1l protein has been found to be conserved across *Plasmodium* species [26]. Multiple sequence alignment enabled us to obtain the conserved regions of Pf IMC1l orthologs (Figure 1).

**Fig 1:** Multiple sequence alignment of IMC1l in *Plasmodium* species. P.gab: *Plasmodium gaboni*; Pre: *Plasmodium reichenowi*; Pf: *Plasmodium falciparum strain FCH/4*; Pca: *Plasmodium chabaudi adami*; Pcc: *Plasmodium chabaudi chabaudi*; Pk: *Plasmodium knowlesi*; Pv: *Plasmodium vivax*; Pg: *Plasmodium gallinaceum*; Pr: *Plasmodium relictum*; Pm: *Plasmodium malariae*; PoW: *Plasmodium ovale wallikeri* PoC: *Plasmodium ovale curtisi*. The conserved residues are marked with (*) in pink while the semi conserved (:) are colored as green.

Articulins are cytoskeletal proteins that were initially described in ciliates. This group of proteins possess conserved repeat motifs (VPVPV) strikingly similar to the residues found in IMCp region of the IMC1 proteins and has been found to contribute in maintaining the cellular shape and integrity of these organisms. Articulins may appear to be specific to alveolates but may have wide and therefore ancient distribution [27]. Two articulin proteins in *Euglena* interact to form its membrane skeletal system [28]. Additionally prokaryotic articulins have also been identified in *Caulobacter crescentus*, a bacterium that relies on membrane skeleton for maintaining its shape [29]. Multiple members of articulin family are known to be present in apicomplexans and display stage specific expression [12]. BLASTp results of Pf IMC1l revealed 42% sequence identity to an articulin of *Cryptosporidium* species (Accession number OII75513.1). This information thus clearly indicates that articulins and proteins alike (IMC1 proteins) exist widespread in nature [30] and may contribute their role in maintaining the cellular architecture.

### 3.2 Role of 'Asparagine rich domains' in *Plasmodium falciparum* proteins.

Most of the PfIMC1 proteins (except IMC1c, d, g, k and m) had asparagine rich regions (Figure 5).

Proteins with high number of amino acid repeats or the regions with low complexity tend to form loops or adopt disordered structures [31]. Many Pf asparagine rich proteins (ARPs) have been identified [32-34] and about 30% of the Pf proteome is composed of repeat regions primarily rich in asparagine. During the course of its life cycle, Pf is subjected to temperature variations in its two hosts. This heat shock may result in unfolding, misfolding or aggregation of proteins particularly ARPs [35]. Aggregation can lead to parasite death via abnormal protein interactions or cell membrane puncture [36] which may be avoided with the help of chaperones [37]. Although the exact cellular role of asparagine rich proteins in Pf has not been identified, these have been suggested to act as tRNA sponges [38] or molecules involved in immune evasion, antigenic variations [39, 40] or protein-protein interactions [41].

Other motifs that were identified by Scan Prosite include a *Nuclear localization signal/ sequence (NLS)* in IMC1j. This sequence tags the protein to be transported into the nucleus [42]. Usually NLS consists of one or more sequences composed of lysines or arginines exposed of the protein surface. IMC1j was found to have an NLS sequence rich in lysine residues suggesting that this protein has a nuclear or nuclear-associated function. Proteins IMC1a, IMC1b and IMC1i contain a *zinc finger related protein motif* indicative of their DNA binding properties. A *mycoplasma virulence signal region* was identified in IMC1c. This sequence contributes to the virulence in mycoplasma, and is likely to share a similar function in Pf.

### 3.3 Palmitoylation of Pf IMC1 proteins

Palmitoylation process is not only restricted to the membrane attachment of the proteins, but also associated with the regulation of protein-protein interactions [43]. Over 400 Pf proteins have been found to be palmitoylated including asexual stage proteins, erythrocyte invasion and those involved in the development of liver and mosquito stages of the parasite [44]. IMC1 proteins possess conserved amino- or carboxy-terminal cysteine motifs which may serve as the substrates for post-translational modification enzymes [45, 46] (Figure 5). Most palmitolation sites are difficult to predict. A study conducted to determine the function of palmitoylation of IMC1c in *P.berghei* indicated that this modification was not essential for protein stability or its membrane targeting [47] but could strengthen the cytoskeleton by enhancing connection between SPN and adjoining IMC. In contrast, the IMC1a cysteine mutants showed marked effects on parasite motility in *P.berghei* [45]. The effect of palmitoylation of cysteine residues in other Pf IMC1 proteins is yet to be determined.

### 3.4 Secondary structure prediction of Pf IMC1 proteins

PSIPRED tool was used to predict the secondary structure of all Pf IMC1 proteins. We found that these proteins predominantly contained beta pleated sheets and random coils with alpha helical stretches being absent in IMC1e, IMC1g and IMC1l. Moreover, we also found that the the IMCp regions (rich in hydrophobic amino acids) of all the Pf IMC1 proteins were rich in beta sheets interspersed by random coils (Table 2). According to previous findings, the snake venom toxins and some microbial peptides that contained hydrophobic stretches of amino acids form amphiphilic beta sheets or beta hair pins that enhance their interaction and promotes their anchorage to the membrane [48]. Here we predict that the hydrophobic region of IMCp might be responsible for the attachment of these proteins to the membrane via its beta sheet conformation.

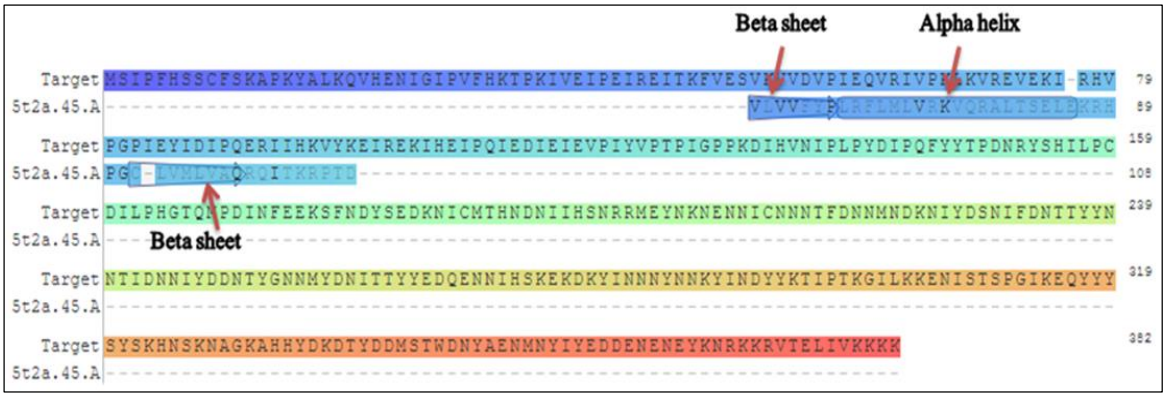
| IMC1 proteins | α-helix | β-sheets and random coils | IMCp region |
|---|---|---|---|
| IMC1a | 13-21, 437-457, 489-505, 532-534, 636-639, 645-649, 655-659, 681-693, 741-747, 763-765, 768-773, 789-807, 810-813, 831-846 | 1-12, 22-436, 458-488, 506-531, 535-635, 640-644, 650-654, 660-680, 694-740, 748-762, 766-767, 774-788, 808-809, 814-830, 847-861 | 103-189 |
| IMC1b | 345-367, 401-404, 409-412, 463-473, 488-501 | 1-344, 367-400, 405-408, 413-462, 474-487, 501-504 | 71-155 |
| IMC1c | 209-211, 233-237 | 1-208, 212-232, 230-280 | 68-152 |
| IMC1d | 89-96 | 1-88, 97-252 | Not predicted |
| IMC1e | Not predicted | 1-520 | 95-177, 153-230 |
| IMC1f | 11-24, 86-88, 127-130, 1296-1300 | 1-10, 25-85, 89-126, 131-1295, 1301-1318 | 576-664, 626-711, 664-774 |
| IMC1g | Not predicted | 1-300 | 48-127, 96-187 |
| IMC1h | 4-10, 16-27, 517-522 | 1-3, 11-15, 28- 516, 523-524 | 117-206, 172-263 |
| IMC1i | 296-298, 491-499 | 1-295, 299-490, 500-502 | 34-116, 84-170 |
| IMC1j | 339-351, 413-419, 536-540, 623-628 | 1-338, 352-412, 420-535, 541-622, 629-664 | 100-188 |
| IMC1k | 410-414, 439-442 | 1-409, 415-438, 443-462 | 185-272 |
| IMC1l | Not predicted | 1-382 | 14-74 |
| IMC1m | 207-214, 291-293, 300-305, 307-320 ,342-350 | 1-206, 215-290, 294-299, 306, 321-341, 351-360 | 52-126 |

**Table 2:** Secondary structure prediction of Pf IMC1 proteins. The stretches containing α helix, β-sheets and coils, and the IMCp region are shown.

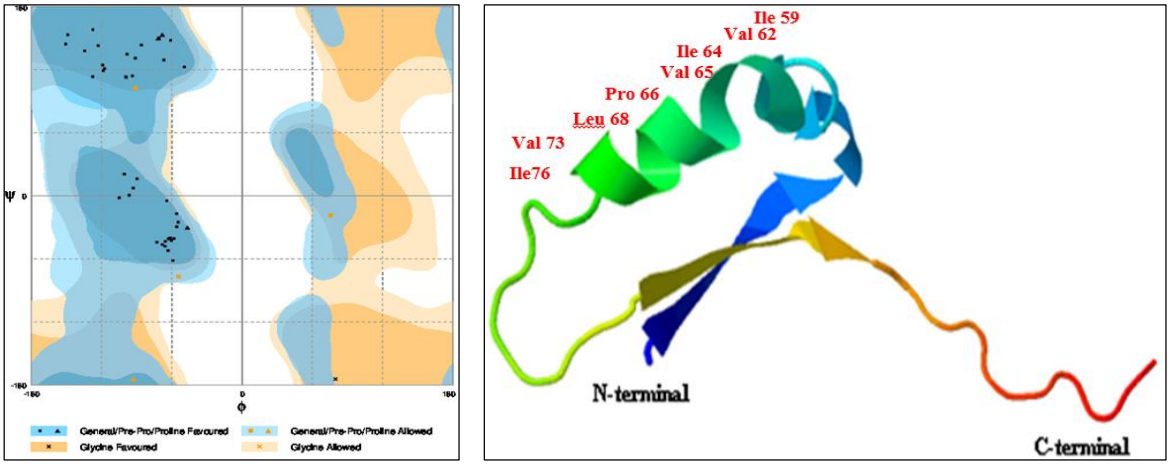
By using the TMPreD tool [23], we found that PfIMC1l does not contain any trans-membrane region. However, its association with the inner membrane complex suggests it to be a monotopic protein that maintain interactions with the membrane without spanning it [49].

### 3.5 Three dimensional (3D) model of PfIMC1l

Using homology modeling (SWISS modeling), the 3D structural model of IMC1l was built using the structure of *Leishmania donovani* 80S ribosome as a template (spanning residues 52-99) (PDB ID: 5T2A). The template had 21.28% sequence identity with IMC1l. As compared to our secondary structure analysis which predicted the occurrence of only beta sheets and coils in IMC1l, the 3D model generated showed the presence of two beta sheets and an alpha helix (Figure2A). Further, the model generated was validated by using RAMPAGE [19] which showed approximately 98.0% of the residues in the favored region while 2.0% in the allowed region and none in the outliers suggesting it to be a reliable model for IMC1l (Figure 2B). Figure 2C shows the 3D model of IMC1l.




**A**




**B** **C**

**Fig 2: A)** Alignment of IMC1l sequence with the template sequence obtained by SWISS-Model. **B)** Validation of the 3D model using RAMPAGE. **C)** 3D model of IMC1l showing probable hydrophobic amino acids (red) for binding to cytoskeletal proteins such as actin or myosin.

### 3.6 PfIMC1l is predicted to bind actin

It has been shown that some actin binding proteins (ABPs) such as gelsolin [50] (Figure 3), WASP homology domain 2 (WH2)-related proteins and [51] vitamin D binding proteins (DBP) [52] share a common actin-binding motif embedded in an α helix. This has a few exposed hydrophobic side chains which may be responsible for interaction with the cleft formed by the sub-domains 1 and 3 of actin [53].

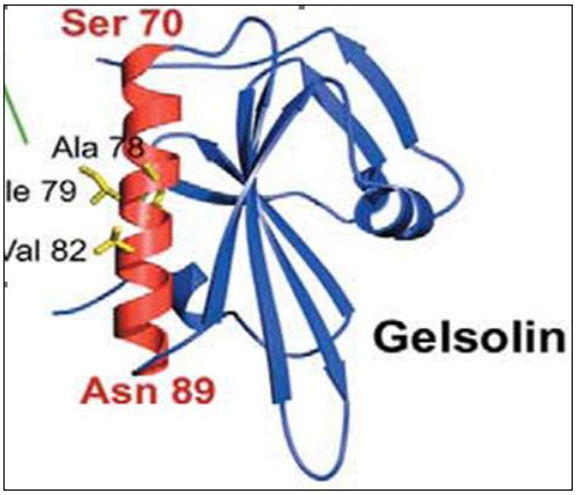



**Fig 3:** The α-helical region (red) of gelsolin (actin binding protein) that binds to actin.

Further, it has been reported that the *Pb*IMCa may directly or indirectly interact with glideosome components [12, 13]. These components are located between the outer leaflet of the IMC and the plasma membrane. The α helix predicted in the tertiary structure of IMC1l has hydrophobic amino acids such as Valine (Val), Isoleucine (Ile), Proline (Pro), and Leucine (Leu). It is possible that these amino acids might be responsible for the interaction of IMC1l to the cytoskeletal elements such as actin/myosin (Figure 2C).

### 3.7 IMC1l is predicted to be localized to the outer leaflet of IMC

We used STRING database to identify proteins that could potentially interact with IMC1l. A small heat shock protein (Pf-shsp) (MAL8P1.78), PF13_0248 and MAL13P1.350 were predicted to interact with IMC1l (Figure 4). Further analysis using BLASTp tool, revealed that a homolog of MAL8P1.78 in *Toxoplasma gondii* (Accession ID AAT66039) had 41% sequence identity with Pf-shsp. According to a previous study, this homolog in *Toxoplama* acts as a chaperone like protein associated with the outer leaflet of IMC, [54] suggesting IMC1l also to be associated with the outer leaflet. Since actin is also located between the outer leaflet of IMC and the plasma membrane of the parasite, it is likely that IMC1l and actin interact here.

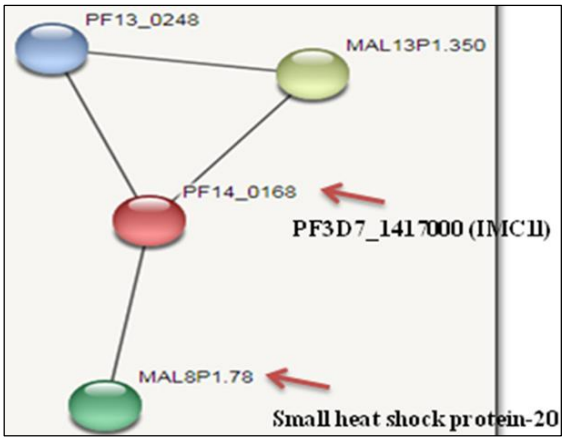



**Fig 4:** Prediction of interacting proteins with IMC1l by STRING database

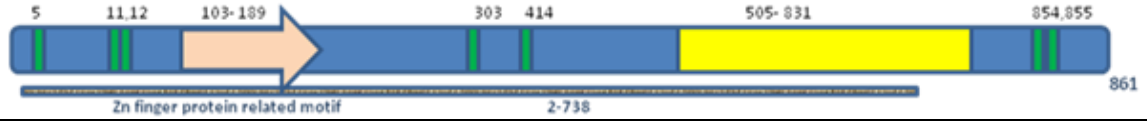
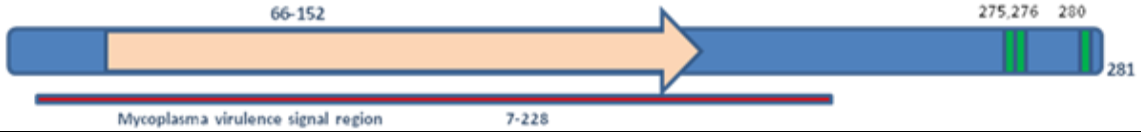
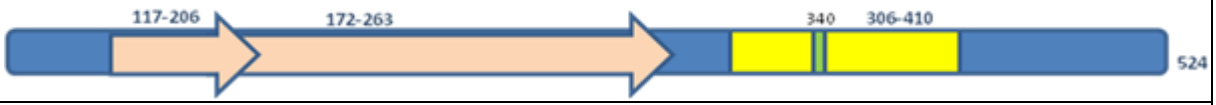
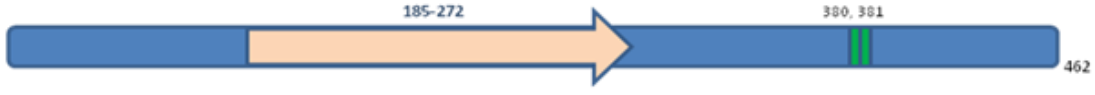
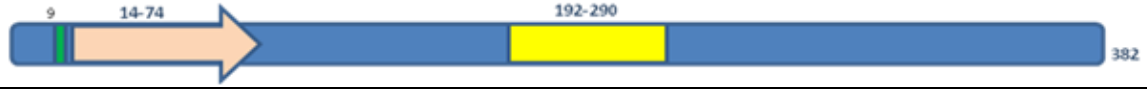
| | |
|---|---|
| Pf IMC1 proteins | **Motifs and domains**<br>: IMCp region in beta sheet conformation.<br>: Asparagine rich regions.<br>: Zinc finger protein related motif.<br>: Mycoplasma virulence signal region<br>: Palmitoylation sites<br>: Nuclear localization sequence /signal (NLS) |
| IMC1a |  |
| IMC1b |  |
| IMC1c |  |
| IMC1d |  |
| IMC1e |  |
| IMC1f |  |
| IMC1g |  |
| IMC1h |  |
| IMC1i |  |
| IMC1j |  |
| IMC1k |  |
| IMC1l |  |
| IMC1m |  |

**Fig 5:** Diagrammatic representation of Pf IMC1 proteins (IMC1a-IMC1m). The IMCp region, predicted domains and motifs with their respective positions are shown.

## 4. Conclusion

Bioinformatics tools are valuable in predicting several structural features of unannotated proteins and molecules. The present study has shed light on some of the structural features of IMC1 proteins. These proteins were found to be rich in beta-strands that might be associated with binding of these proteins to the IMC membrane. Further, we also predicted motifs and domains of functional importance in these proteins. Pf IMC1l expresses during the salivary gland stage of the sporozoites. We predicted the partial tertiary structure of IMC1l and hypothesize that its α-helical region might be involved in binding to cytoskeletal proteins such as actin or myosin. We also hypothesize that IMC1l might be localized to the outer leaflet of the inner membrane complex. Although these hypotheses require experimental validation, our results may form the basis for designing new antimalarial drugs using IMC1 proteins as targets.

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## 6. Competing interests

Authors declare that they have no competing interests.

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