Three-dimensional structures of Lipoproteins from *Streptococcus pneumoniae* and *Staphylococcus aureus*

Sergio G. Bartual¹, Martín Alcorlo, Siseth Martínez-Caballero, Rafael Molina, Juan A. Hermoso*

Department of Crystallography and Structural Biology, Institute of Physical Chemistry “Rocasolano”, CSIC, 28006 Madrid, Spain

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ABSTRACT

Bacterial lipoproteins (Lpp) compose a large family of surface-exposed proteins that are involved in diverse, but critical, cellular functions spanning from fitness to virulence. All of them present a common signature, a sequence motif, known as LipoBox, containing an invariant Cys residue that allows the protein to be covalently bound to the membrane through a thioether linkage. Despite the abundance and relevance of Lpp, there is a scarcity of structural and functional information for this family of proteins. In this review, the updated structural and functional data for Lpp from two Gram-positive pathogenic model organisms, *Staphylococcus aureus* and *Streptococcus pneumoniae* is presented. The available structural information offers a glimpse over the Lpp functional mechanisms. Their relevance in bacterial fitness, and also in virulence and host-pathogen interactions, reveals lipoproteins as very attractive targets for designing of novel antimicrobials, and interesting candidates as novel vaccine antigens.

1. Introduction

A common feature of all bacterial groups is the presence of a single or double membrane that serves, among other functions, as an anchor point for proteins. Membrane proteins can be classified according to their interaction with the membrane as integral membrane proteins, when they are embedded in the phospholipid bilayer by transmembrane segments, or peripheral membrane proteins, when they remain attached to the membrane surface but not inserted. Bacterial lipoproteins (Lpp) are classified as peripheral membrane proteins that have been shown to be directly involved in diverse, but critical, cellular functions like: cell fitness, division, signal transduction, motility, redox and antibiotic resistance, extracytoplasmic folding of proteins, conjugation, sporulation, adhesion and virulence (Abdullah et al., 2014; Alloing et al., 1994; Kohler et al., 2016; Nguyen and Gotz, 2016; Saleh et al., 2013; Schmalzer et al., 2009; Shahmirzadi et al., 2016) (Fig. 1A). Indeed, Lpp contribute directly to the pathogen virulence by promoting colonization, invasion, and survival in the host bloodstream (Kovacs-Simon et al., 2011; Pribyl et al., 2014; Saleh et al., 2013).

Gram-negative Lpp could be placed in any of the two membranes but normally are attached to the inner leaflet of the outer membrane (Narita et al., 2004). In Gram-positive bacteria Lpp are translocated from the inner face of the plasma membrane to the extracellular surface (Hutchings et al., 2009). Furthermore, due to the lack of a second

membrane, Gram-positive bacteria avoid the diffusion of many vital proteins into the external space by turning them into Lpp.

Bacterial Lpp modification, normally, includes the attachment of an N-Acyl Diacyl Glycerol group that allows Lpp to remain attached to the membranes by direct interaction with the phospholipids polar heads. Some Gram-positive bacteria like *S. aureus* can, in addition to the N-Diacyl Glycerol, attach N-Acyl Triacyl Glycerol depending on environmental conditions (Nakayama et al., 2012). Lpp typically consist of an N-terminus signal peptide, containing positively-charged residues, a hydrophobic sequence and the LipoBox motif [LVI] [ASTVI] [GAS] [C] (Sankaran and Wu, 1994) (Fig. 1B). After synthesis, the N-terminal signal peptide allows prelipoproteins to be translocated across the cytoplasmic membrane by either a Sec or Tat secretory pathways (Driessen and Nouwen, 2008). Then is anchored to the cell wall by the addition of diacylglycerol moieties (lipidation) to the thiol group of an invariant cysteine residue in the lipobox motif via the lipoprotein diacylglycerol transferase (Ltg) (Kohler et al., 2016; Oudega et al., 1993). Finally, a type II signal peptidase (Lps) cleaves the N-terminal signal peptide close to the Cys/diglyceride bond generating the mature lipoprotein.

Interestingly, in many Gram-positive pathogenic bacteria, such as *Streptococcus pneumoniae* or *Staphylococcus aureus*, most Lpp are required for sustaining virulence in pathogen-host interactions (Johnston et al., 2004; Saleh et al., 2013; Shahmirzadi et al., 2016). However,

* Corresponding author.

E-mail address: xjuan@iqfr.csic.es (J.A. Hermoso).

¹ Present address: School of Life Sciences, University of Dundee, DD1 5HL, Dundee, UK.

Fig. 1. (A) Schematic representation of the biological roles exerted by bacterial Lipoproteins. Figure adapted from <http://www.mrc-lmb.cam.ac.uk/genomes/dolop/> (Babu et al., 2006; Madan Babu and Sankaran, 2002). (B) Schematic cartoon of the modular arrangement found in bacterial Lipoproteins. The lipobox contains the invariant Cys that is lipid-modified and the –3 position is mainly Leu. The consensus sequence [LVI][ASTVI][GAS][C] is shown in red. Apart from the lipobox the N-terminal 5–7 residues with mostly two positively charged Lys or Arg residues and the intervening stretch of hydrophobic and uncharged residues of 7–22 amino acid length are important (yellow box). (C) Biosynthesis of lipoproteins in Gram-positive bacteria. The precursor of lipoproteins is the prelipoprotein, with an N-terminal signal peptide including the consensus sequence of the lipobox at the C-terminal region of the signal peptide (shown in red). During lipoprotein maturation, the thiol group of the invariant cysteine in the lipobox is modified by a diacylglyceryl moiety (in blue) by lipoprotein diacylglyceryl transferase (Lgt), which serves as a membrane anchor. After lipidation, lipoprotein signal peptidase (Lsp) cleaves the signal peptide, leaving the cysteine as the new amino-terminal residue forming the mature lipoprotein in Gram-positive bacteria. M stands for the initial methionine in the precursor forms. Figure adapted from (Kovacs-Simon et al., 2011). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

despite their attractiveness as potential Gram-positive antimicrobial targets and novel vaccine antigens, little is known about their three-dimensional structures. There are different facts that could explain the scarcity of structural information in Lpp family. Among them, the hydrophobic nature of the full-length protein (all the up to now reported crystal structures of Lpp are obtained after deletion of the LipoBox region), the usual presence of a flexible linker connecting the lipidated Cys with the functional domain(s) that hamper crystallization of Lpp, and finally, the insufficient information about *in vivo* functions for many of these Lpp (few of them presenting a catalytic activity), could explain why these proteins have been typically neglected in structural studies. I.e. within the disease model organisms *S. aureus* USA300 and *Pneumococcus* TIGR4, 83% and 49% of their respective Lpp remains structurally uncharacterized. Furthermore, among all the available Lpp structures only half of them have been functional and biochemically characterized. This fact reflects the huge gap in knowledge for this group of critical membrane proteins.

During the last years, there has been intensive bioinformatics analysis of bacterial Lpp. An example of this is the development of DOLOP, a knowledge base for bacterial lipoproteins (<http://www.mrc-lmb.cam.ac.uk/genomes/dolop/>) that provides a list of functional classification, predictive algorithm for query sequences, primary sequence analysis and lists of predicted lipoproteins from different completed bacterial genomes (Babu et al., 2006; Madan Babu and Sankaran, 2002). DOLOP, accessed on July 2017, contains information of 199 distinct lipoproteins identified among 43 bacterial genomes and classified according to their cellular functions. Although it is a powerful computational tool, DOLOP still doesn't offer functional or structural correlation to published data.

As all the reported three-dimensional structures of Lpp refer to the protein part, and typically just their functional domains, this review focus on the comparison of the Lpp roles and associated three-dimensional structures for two main Gram-positive human pathogens, *S. pneumoniae* TIGR4 and the *S. aureus* MRSA USA300.

2 Overview of the solved *S. pneumoniae* and *S. aureus* lipoproteomes

Lipoproteome constitutes a minimal fraction of the total bacterial genome, 1.76% in the case of *S. pneumoniae* TIGR4 and 2.04% for the bigger *S. aureus* complete genome (Babu et al., 2006). Due to their exposed location and the critical functions under their command, Lpp are being considered targets for the generation of new antimicrobials and interesting candidates for novel vaccine antigens. However, to achieve this goal a deep structural and functional knowledge is required.

Evaluation of the protein data bank (PDB, <https://www.rcsb.org/pdb/home/home.do>) accessed on July 2017, reveals that, in the case of *S. aureus* USA300, only one third of lipoproteins have been, up to now, structurally characterized. PDB database contains only 18 out of a total of the 67 lipoproteins reported in the strain USA300 that accounts for 0.7% of the total genome (Shahmirzadi et al., 2016). Interestingly, among all the deposited bacterial Lpp structures, there is no structural information of the lipobox or the attached lipid, thus only Lpp catalytic protein cores can be structurally analysed. A large proportion of the staphylococcal Lpp is involved in the uptake of essential ions and nutrients, 24 Lpp are distributed among metal, anion, amino acid and sugar transporters (Fig. 2A). Of them, only 9 have been structurally determined: 4 iron transporters (FhuD2, SirA, SirF and HstA), one manganese and one nickel transporters (MntC and NikA, respectively), one nitrate ABC binding protein, two amino acid transporters (OpuCc and GmpC) and one maltose binding protein (Table 1A). The rest of the *S. aureus* Lpp are divided into other 7 functional categories (Biosynthesis, Respiration, Protein folding, Protein translocation, Phage and plasmid Lpp, Lpl cluster and the unknown Lpp) that accounts for the 62.68% of the total lipoproteome. However, the structures of only 11% of them have been solved (Table 1A). The Biosynthesis category has a single Lpp involved in plasmid uptake whose structure is reported (CamS). Respiration category contains two Lpp but no structure is reported so far. The Protein folding category contains two Lpp of known

Fig. 2. Statistical analysis of the *Staphylococcus aureus* and *Streptococcus pneumoniae* lipoproteomes. (A) *S. aureus* sector representation of lipoprotein proportion in each functional category with respect to the total *S. aureus* lipoproteome. (B) *S. pneumoniae* sector representation of lipoprotein proportion in each functional category respect the total *S. pneumoniae* lipoproteome.

structure (PrsA and DsbA). No structural information has been reported for the only one Lpp (YidC) involved in translocation mechanisms. Two proteins have been classified into the Phage and Plasmid encoded Lpp (PhiSLT and BlaZ) being BlaZ the only structurally characterized (Chen and Herzberg, 2001). Furthermore, USA300 strain carries relatively high number of tandem repeated Lpp in the ν Sac genomic island (22% of the total Lpp) (Nguyen et al., 2015a,b). Among them, nine homologous lpp, referred to as “lipoprotein-like” (lpl) play a role in pathogenicity and are encoded by the ν Sac genomic island (Babu et al., 2006); however, their exact function is still unknown. Only two structures have been reported up to date (Csa 1A and Csa 1B). Finally, 28% of the staphylococcal lipoproteome is classified as unknown. Two structures have been reported for this group, although biochemical validation is not yet available (Table 1A).

In contrast with the *S. aureus* USA300 case, approximately 50% of the *S. pneumoniae* TIGR4 lipoproteome is structurally characterized (20 out of 43 Lpp). Although, in general, pneumococcus has a significant less number of ABC transporters compared with an average *S. aureus* strain, the number of available structures among metal, cation, anion, amino acid and sugar ABC transporters is quite similar in both organisms; 10 in *S. pneumoniae* TIGR4 vs 9 in *S. aureus* USA300. Two, out of 3 iron transporters (PiaA and PiuA), a manganese (PsaA) and a Zinc binding protein (AdcAII) completes the picture of the structurally known metal transporters in pneumococci. It is worth to notice that although the structures of the two putative phosphate transporters Lpp (PstS1 and PstS2) have been solved, none of them has, up to date, any associated biochemical analysis. Four out of 9 amino acids transporters have been structurally characterized (AbpA, GlnH, AatB and LivJ) and finally, all the 5 sugar transporters (FcsSBP, RafE, MalX, NgtS and FusA) are available in the PDB (Table 1B). Due to its specific life style, *S. pneumoniae* TIGR4 has a functional cluster of Lpp dedicated to ROS (reactive oxygen species) resistance with 3 out of 3 redox proteins whose structure is known (Etrx1, Etrx2 and GshT). Furthermore, it is interesting to notice a higher number of biosynthetic Lpp in pneumococci; 5 Lpp compared with just one in *S. aureus*. Two out of 5 Lpp in this biosynthetic category have solved structures (DacB and SP0198). Two chaperone Lpp are also present in *S. pneumoniae* and both have reported 3D structures (SrtD and PrsA). None of the protein translocation Lpp have reported structures so far. Finally, the 3D structures for 3 Lpp from the Unknown categories have been determined (Table 1B).

In this review, we have grouped the three-dimensional structures of Lpp according to their established, or predicted, function. Thus, Lpp are classified as substrate-binding proteins in ABC transporters (including Iron, Cation, Anion and Sugar transporters), redox and respiration proteins, biosynthetic and processing proteins, chaperon and foldase proteins, Lpp involved in protein translocation, miscellaneous (Lpl

cluster of antigens, or ν Sac genomic island, and proteins from phage or plasmid origin) and, finally, the hotchpotch category of Unknown Lpp (Table 1).

3 Three-dimensional structures of Lpp associated to ABC transporters

ABC transporters function as protein complexes that are able to translocate specific substrates across the phospholipid bilayer of the cell membrane. All ABC transporters share a common protein complex architecture composed of two permease components, two nucleotide binding components (ATPases) and one specific cargo protein which in bacteria is often one Lpp substrate-binding protein (SBP) (Khandavilli et al., 2008). SBPs have a highly conserved three-dimensional structural fold, even though they often have little or no sequence similarity and can bind quite different types of substrates. They consist of two α/β domains, each of them with a central β -sheet core with 4–6 β -strands flanked by α -helical periphery, and the substrate binds between the two domains.

Traditionally, the SBPs have been grouped based on their substrate specificity, their sequence similarity or the topological arrangements of the β -sheets (Fukami-Kobayashi et al., 1999). In 2010, a new classification of SBPs was proposed based on the 3D alignments of the available protein structures. Six different classes known as clusters A to F were proposed (Bertsson et al., 2010). Very recently, a new cluster, G, was incorporated to make a total of 7 clusters (Scheepers et al., 2016) (Table 2). The main structural differences among these clusters are the presence of different interconnecting elements between the two domains that act as a hinge region (a long rigid α -helix, one or several β -sheets with different length, etc.) and the presence of additional domains. Another structural difference is found concerning the binding of the substrate. While in most SBPs, ligand binding induces a substantial domain rotation resulting in closure of the two domains (the so called “venus fly-trap” mechanism (Mao et al., 1982)), in the case of ionic transporters no large movement is observed upon substrate binding. Below the available 3D structures of the SBP are reviewed according to the function of the associated ABC transporter.

3.1. Iron and cation transporters

As explained before, this group is the most represented in the PDB. Iron is an essential nutrient for all organisms, thus vertebrate hosts uses efficient iron sequesters (the so-called siderophores), like transferrin, to prevent bacterial invading pathogens proliferation by neglecting them access to available Fe(II) (Hammer and Skaar, 2011). In response, *S. aureus*, has evolved a sophisticated mechanism to respond to iron

Table 1A
S. aureus USA300 Lpp.

Locus Uniprot	Other locus	Name	Function ^(b)	PDB ^a	References
A0A0H2XGM1 (303 aa)	SAUSA300_1978	FhuD1	Fe Transport Ferric hydroxamate receptor		(Sebulsky and Heinrichs, 2001; Sebulsky et al., 2004)
A0A0H2XFG0 (303 aa)	SAUSA300_2235	FhuD2	Fe ABC transporter	4FKM, 4FMNA, 4FIL, 4B8Y	(Mariotti et al., 2013; Podkowa et al., 2014; Sebulsky and Heinrichs, 2001; Sebulsky et al., 2004)
A0A0H2XKM7 (342 aa)	SAUSA300_0721	SstD, YclQ, CeuE	Transferrin receptor		
A0A0H2XHK0 (330)	SAUSA300_0117	SirA	Fe ABC transporter	3MWF, 3MWG	(Heinrichs et al., 1999)
Q7A652 (292 aa)	SAUSA300_1032	LsdE, SirF	Fe ABC transporter	2Q8P, 2Q8Q	(Grigg et al., 2007)
Q2FJS0 (284 aa)	SAUSA300_0344	Efem, Efeo	Efem/Efeo family lipoprotein lipidation		
A0A0H2XEB0 (327 aa)	SAUSA300_2136	HstA	Fe ABC transporter	3EIX, 3EIW, 3LI2, 3LHS	(Beasley et al., 2009)
A0A0H2XH19 (322 aa)	SAUSA300_0219		Iron Binding Protein		
A0A0H2XI48 (309 aa)	SAUSA300_0618	MntC (SitC)	Other cation transporters Manganese-binding protein	4K3 V	(Gribenko et al., 2013; Gribenko et al., 2016)
A0A0H2XDI1 (516 aa)	SAUSA300_2351	AdcAII-like	Zinc-binding		
A0A0H2XH66 (532 aa)	SAUSA300_2411	Opp1A	Cobalt and nickel transporter Cnt		(Remy et al., 2013)
A0A0H2XG18 (491 aa)	SAUSA300_0231	NikA	Nickel ABC transporter	4XKR, 4XKQ, 4XKP, 4XKN, 4OFJ, 3RQT	(Lebrette et al., 2015)
A0A0H2XIF6 (591 aa)	SAUSA300_0203		Nickel-Peptide/transporter substrate-binding protein-		
A0A0H2XGY9 (260 aa)	SAUSA300_2230	ModA	Molybdenum ABC transporter		(Neubauer et al., 1999)
Q2FH48 (327 aa)	SAUSA300_1283	PstS	Anion Transporters Phosphate ABC transporter		
A0A0H2XKE4 (318 aa)	SAUSA300_0145		Phosphonate ABC transporter		
A0A0H2XJJ1 (324 aa)	SAUSA300_0175		Nitrate ABC transporter substrate-binding protein	3UN6	
A0A0H2XIQ0 (313 aa)	SAUSA300_2391	OpuCc	AA peptide transport Glycine betaine/carnitine/ choline ABC transporter	3O66	
A0A0H2XK42 (259 aa)	SAUSA300_2359	–	Amino acid ABC transporter		
A0A0H2XIR7 (517 aa)	SAUSA300_0073	–	Peptide ABC transporter		
A0A0H2XIJ5 (551 aa)	SAUSA300_0891	Opp3A	Oligopeptide ABC transporter		
A0A0H2XGK4 (571 aa)	SAUSA300_0892	Opp4A	Oligopeptide ABC transporter		
A0A0H2XFT9 (280 aa)	SAUSA300_0437	GmpC	NLPA/D-Methionine binding	1P99	(Williams et al., 2004)
A0A0H2XEA1 (273 aa)	SAUSA300_0798	–	D-Methionine ABC transporter		
A0A0H2XEQ7 (423 aa)	SAUSA300_0209		Sugar transport Maltose ABC transporter	4HS7, 4HW8	
A0A0H2XG95 (399 aa)	SAUSA300_1884	CamS	Biosynthesis CamS sex pheromone biosynthesis	2QX2	
Q2FI17 (366 aa)	SAUSA300_0963	QoxA	Respiration Quinol oxidase, subunit II		
A0A0H2XH45 (146 aa)	SAUSA300_0693	SaeP	Electron transfer domain		
Q2FFQ5 (320 aa)	SAUSA300_1790	PrsA	Chaperone/Foldase Foldase protein	2JZV	(Heikkinen et al., 2009)
Q9S446 (206 aa)		SrtA	Sortase.	1IJA, 1T2P, 1T2W	(Ilangoan et al., 2001; Zong et al., 2004)
A0A0H2XFS3 (199 aa)	SAUSA300_2354	DsbA	Thioredoxin/Protein disulfide-isomerase	3BCI, 3BD2, 3BCK	(Heras et al., 2008)
Q2FF36 (290 aa)	SAUSA300_2046	YidC (OxaA)	Protein Translocation YidC (OxaA)–essential protein		
A0A0H2XE22 (401 aa)	SAUSA300_1438	PhiSLT	Phage and Plasmid encoded Lpp		
pUSA300_HOUMR0011	pUSA300_HOUMR0011	BlaZ	ORF144-like Membrane bound penicillinase	3BLM, 1PIO, 1KGF, 1DJC, 1DJB, 1DJA, 1KGE, 1OME, 1KGG, 1GHP, 1GHM, 1GHI	(Chen and Herzberg, 2001; Chen et al., 1996)
Q2FJK4 (257 aa)	SAUSA300_0410		Lpl cluster		
Q2FJK3 (266 aa)	SAUSA300_0411		Lpl-1 ν Sa α specific		(Nguyen et al., 2015a,b)
Q2FJK2 (266 aa)	SAUSA300_0413		Lpl-2 ν Sa α specific		(Nguyen et al., 2015a,b)
Q2FJK1 (256 aa)	SAUSA300_0414		Lpl-3 ν Sa α specific		(Nguyen et al., 2015a,b)
Q2FJK0 (263 aa)	SAUSA300_0415		Lpl-4 ν Sa α specific		(Nguyen et al., 2015a,b)
Q2FJJ9 (258 aa)	SAUSA300_0416		Lpl-5 ν Sa α specific		(Nguyen et al., 2015a,b)
Q2FJJ8 (270 aa)	SAUSA300_0417		Lpl-6 ν Sa α specific		(Nguyen et al., 2015a,b)
			Lpl-7 ν Sa α specific		(Nguyen et al., 2015a,b)

(continued on next page)

Table 1A (continued)

Locus Uniprot	Other locus	Name	Function (^b)	PDB ^a	References
Q2FJJ7 (261 aa)	SAUSA300_0418		Lpl-8 ν Saa specific		(Nguyen et al., 2015a,b)
Q2FJJ6 (270 aa)	SAUSA300_0419		Lpl-9 ν Saa specific		(Nguyen et al., 2015a,b)
Q2FE15 (261 aa)	SAUSA300_2429		Tandem lpp		
Q2FE14 (257 aa)	SAUSA300_2430		Tandem lpp		
Q2FKG1 (255 aa)	SAUSA300_0100	Csa1A	Conserved staphylococcal antigen 1A	4BIH	(Nguyen et al., 2015a,b)
Q2FKG0 (256 aa)	SAUSA300_0101	Csa1B	conserved staphylococcal antigen 1B	4BIG	(Nguyen et al., 2015a,b)
Q2FKF9 (255 aa)	SAUSA300_0102		Tandem lpp		
Q2FKF8 (255 aa)	SAUSA300_0103		Tandem lpp		
			Other/unknown		
A0A0H2XFS1 (181 aa)	SAUSA300_0079	YdhK-like	Unknown		
A0A0H2XH82 (190 aa)	SAUSA300_0372	PepSY	Unknown		
A0A0H2XHE2 (208 aa)	SAUSA300_0377		Unknown		
A0A0H2XGW7 (193 aa)	SAUSA300_1492		Unknown		
A0A0H2XIX8 (208 aa)	SAUSA300_0992		Unknown	2AP3	
A0A0H2XG58 (151 aa)	SAUSA300_2403		Unknown		
A0A0H2XIF2 (292 aa)	SAUSA300_0724		Unknown		
Q2FEC8 (209 aa)	SAUSA300_2315		Unknown		
A0A0H2XEH9 (374 aa)	SAUSA300_2614		Unknown		
A0A0H2XGB5 (131 aa)	SAUSA300_0663		Unknown		
A0A0H2XF17 (317 aa)	SAUSA300_1106		Unknown		
A0A0H2XGB8 (124 aa)	SAUSA300_0303		Unknown		
A0A0H2XHH5 (118 aa)	SAUSA300_1478		Unknown		
A0A0H2XIE6 (304 aa)	SAUSA300_1376		Unknown		
A0A0H2XK53 (305 aa)	SAUSA300_1379		Unknown		
A0A0H2XET3 (289 aa)	SAUSA300_1440		Unknown		
A0A0H2XFX0 (156 aa)	SAUSA300_1742		Unknown		
A0A0H2XJH2 (51 aa)	SAUSA300_1741		Unknown		
A0A0H2XHH4 (242 aa)	SAUSA300_0769		Unknown	3QFG	

^a Protein data bank (PDB) (<https://www.rcsb.org/pdb/home/home.do>) access on 17/07/2017.

^b Depending on the protein, Lpp function has been experimentally or putatively assigned as indicated in the associated reference.

starvation that includes switching to fermentative metabolism, to decrease the local pH, to force the release of iron from host transferrin or to increase the number of expressed iron Lpp scavengers (Friedman et al., 2006; Hammer and Skaar, 2011). Interestingly, the situation for *S. pneumoniae* is quite different. Iron uptake in *S. pneumoniae* bacterium depends entirely on the orphan response regulator RitR. However, although deletion of *ritR* affects pneumococci growth, this defect can be completely suppressed by the addition of manganese (Ong et al., 2013). In contrast with *S. aureus*, *S. pneumoniae* does not depend on iron but requires a highly efficient Mn^{2+} transporter, like PsaA. Moreover, Mn^{2+} plays a key role in the oxidative stress protection for this catalase negative pathogen that uses H_2O_2 as a chemical weapon against niche competitors and that colonizes the respiratory tract (Selva et al., 2009). Indeed, lack of PsaA makes the strain unable to deal with redox stress (Tseng et al., 2002).

The structures of four *S. aureus* SBP Lpp involved in iron uptake have been reported (FhuD2, SirA, HtsA, IsdE) (Fig. 3A, Table 1B). FhuD2 (Fe ABC transporter) and FhuD1 (Ferric hydroxamate receptor) are homologous SBPs (41% sequence identity) that, interestingly, exhibit different activities. FhuD2 is conserved in other bacterial species and binds to a broad spectrum of Fe chelators. However, FhuD1 seems to be restricted to staphylococcal species and only binds ferrichrome and ferrioxamine B. Although FhuD1 structure remains unknown, the X-ray structures of FhuD2 in complex with ferrichrome and ferrioxamine-B reveals the characteristic bilobate fold of cluster A, that accommodates the ligands between both lobes (PDB 4B8Y and PDB 4FIL), (Mariotti et al., 2013). SirA, the staphylo-ferrin B (SB) receptor, belongs to the same SBP cluster A. Its 3D structure (PDB 3MWF) presents the N- and C-terminal lobes bridged by a single α -helix that, together with both lobes, defines the siderophore (FeSB in this case) binding site (Grigg et al., 2010a). SirA undergoes conformational change upon FeSB

binding, affecting the conformation of two loops from the C-terminal domain to enclose FeSB. The staphylococcal HtsA is homologous to SirA (rmsd 2.11 Å over 179 C α alignment) and its crystal structure represents the first for a Gram-positive siderophore-receptor complex (PDB 3EIW, PDB 3EIX and 3LI2) (Beasley et al., 2009). In a similar way to SirA, HtsA exhibits two different conformations depending on the presence or absence of bound ferric ligand into the binding site that is recognized through interaction with a six-arginine patch (Grigg et al., 2010b). However, in HtsA the siderophore-protein contacts are almost entirely different, and siderophore binding also induces distinct conformational changes affecting different loops. Thus, although both SirA and HtsA bind siderophores from the same α -hydroxycarboxylate class, the structural features of each receptor provide an explanation for their distinct specificity (Grigg et al., 2010b). Finally, the staphylococcal IsdE (also named SirF) structure (PDB 2Q8P) was reported in complex with heme, in a hexa-coordinated fashion mode by Met78 and His229 (Grigg et al., 2007).

S. pneumoniae has two Lpp iron uptake SBP of known structure, PiaA and PiuA (Fig. 3A, Table 1B). Both SBPs share a similar folding containing the same structural elements found in SBPs from cluster A. In the PiaA structure (PDB 4HMQ), a molecule of ferrichrome is stabilized by several highly-conserved residues at the inter-domain cleft (Cheng et al., 2013). Upon ferrichrome binding, two highly flexible segments at the entrance of the cleft undergo significant conformational changes, indicating their contribution to the binding and/or release of ferrichrome. Furthermore, two conserved PiaA residues: Glu119 and Glu262 are proposed to form salt bridges with two arginines of the permease subunits (Fig. 3A). As expected, similar motifs are found for the PiuA SBP (PDB 4JCC).

Table 1B
S. pneumoniae TIGR4 Lpp.

Uniprot_locus	Other locus	Name	Function (^b)	PDB ^a	References
A0A0H2UPT5 (341 aa)	SP1032	PiaA	Fe Transport Iron-compound ABC transporter Ion.	4HMO, 4HMP, 4HMQ	(Cheng et al., 2013; Tettelin et al., 2001)
A0A0H2URP0 (318 aa)	SP1870		Iron-compound ABC transporter, pernease protein.		(Hoskins et al., 2001; Tettelin et al., 2001)
A0A0H2URK5 (321 aa)	SP1872	PiuA	iron uptake ABC transporter	4JCC	(Hoskins et al., 2001; Tettelin et al., 2001)
P0A4G2 (309 aa)	SP1650	PsaA	Other cation transporters Manganese ABC transporter, manganese-binding adhesion lipoprotein or Pneumococcal surface adhesin A	1PSZ, 3ZTT, 3ZK7, 3ZK8, 3ZK9, 3ZKA, 3ZTT, 4UTO, 4UTP	(Begg et al., 2015; Counago et al., 2014; Dintilhac and Claverys, 1997)
O05703 (501 aa)	SP2169	AdcA	Zinc ABC transporter		
A0A0H2UPY0 (305 aa)	SP1002	AdcAII	Zn binding protein	3CX3	(Hoskins et al., 2001; Tettelin et al., 2001)
Q97Q31 (292 aa)	SP1400	PstS1	Anion Transporters Phosphate ABC transporter, phosphate-binding protein, putative	4LAT	
P0C2M5 (291 aa)	SP2084	PstS2	Phosphate ABC transporter	4H1X	(Orihuela et al., 2001)
A0A0H2UMZ3 (268 aa)	SP0112	AbpA	AA peptide transport Amino acid ABC transporter.	4I62	(Tettelin et al., 2001)
A0A0H2UN92 (284 aa)	SP0149		Methionine transporter		
P35592 (661 aa)	SP0366	AliA exp1, PlpA	ABC transport system. Oligopeptide-binding protein AliA		(Hoskins et al., 2001; Tettelin et al., 2001)
A0A0H2UP02 (266 aa)	SP0620		Amino acid ABC transporter.		(Hoskins et al., 2001; Tettelin et al., 2001)
A0A0H2UQR1 (271 aa)	SP1394	GlnH	Amino acid ABC transporter	4H5F, 4H5G	(Tettelin et al., 2001)
A0A0H2UQY7 (278 aa)	SP1500	AatB	Amino acid ABC transporter	4OHN	(Hoskins et al., 2001; Tettelin et al., 2001)
P0A4G0 (652 aa)	SP1527	AliB	Oligopeptide ABC transporter		(Hoskins et al., 2001; Tettelin et al., 2001)
P18791 (659 aa)	SP1891	AmiA	Oligopeptide ABC transporter		(Hoskins et al., 2001; Tettelin et al., 2001)
A0A0H2UPD6 (386 aa)	SP0749	LivJ	ABC transport system	4GNR	(Tettelin et al., 2001)
A0A0H2URD1 (442 aa)	SP1683		Sugar transport Sugar ABC transporter		(Tettelin et al., 2001)
A0A0H2URJ7 (419 aa)	SP1897	RafE	Sugar transporter, sugar-binding protein.	2HEU, 2HFB, 2HQ0, 2I58	(Tettelin et al., 2001)
P59213 (423 aa)	SP2108	MalX	Maltose/maltodextrin ABC transporter, maltose/maltodextrin-binding protein	2XD2, 2XD3	(Abbott et al., 2010)
A0A0H2URD6 (538 aa)	SP1796	FusA	ABC transporter	5G5Y, 5G5Z, 5G60, 5G61, 5G62	(Culurgioni et al., 2017a; Tettelin et al., 2001)
A0A0B7M2T5 (430 aa)		FcsSBP	ABC transporter	2W7Y	(Higgins et al., 2009)
A0A0T7JX40 (491 aa)	SP0092	NgtS	ABC transporter	5SUO, 5SWA, 5SWB	(Culurgioni et al., 2017b; Robb et al., 2017)
A0A0H2UMY0 (491 aa)				5MLT	
A0A0H2UNA1 (152 aa)	SP0198		Biosynthesis/processing Putative D-stereospecific aminopeptidase.	3GE2	(Tettelin et al., 2001)
A0A0H2UP74 (238 aa)	SP0629	DacB	L,D-carboxypeptidase.	4NT9, 4D0Y	(Abbott et al., 2010; Tettelin et al., 2001)
A0A0H2UPH6 (267 aa)	SP0771	PpiA	Peptidyl-propyl <i>cis</i> - <i>trans</i> isomerase, cyclophilin-type		(Hoskins et al., 2001; Tettelin et al., 2001)
Q97Q95 (233 aa)	SP1330	NanE	N-acetylmannosamine-6-phosphate epimerase (N-acetylneuraminate degradation)		(Tettelin et al., 2001)
P65520 (232 aa)	SP1685	nanE2	N-acetylmannosidase-6-phosphate epimerase (NanE)		(Hoskins et al., 2001; Tettelin et al., 2001)
A0A0H2UN58 (276 aa)	SP0148	GshT	Redox ABC transporter Glutathione-Binding Protein	4EQ9	(Tettelin et al., 2001)
A0A0H2UP26 (188 aa)	SP0659	Etrx1	Thioredoxin family protein. Oxido stress resistance	4EVM, 4HQS	(Tettelin et al., 2001)
A0A0H2UPR5 (185 aa)	SP1000	Etrx2	Thioredoxin family protein. Oxido stress resistance	2YP6, 4HQZ	(Tettelin et al., 2001)
A0A0H2UNN1 (283 aa)	SP0468	SrtC-3 or SrtD	Chaperone/Foldase Sortase.	2W1 K	(Manzano et al., 2008; Tettelin et al., 2001)
Q97R51 (313 aa)	SP0981	PrsA	Protease maturation protein, putative foldase	5TVL	(Tettelin et al., 2001)
Q97NP5 (308 aa)	SP1975	YidC1	Protein Translocation/insertion SpoIIIJ family protein. Chaperone		(Tettelin et al., 2001)
Q97NI6 (274 aa)	SP2041	YidC2	SpoIIIJ family protein.		(Tettelin et al., 2001)
A0A0H2UN92 (284 aa)	SP0149		Other/unknown Lipoprotein.		(Tettelin et al., 2001)
A0A0H2UNC7 (189 aa)	SP0191		Putative uncharacterized protein.	2MVB	(Tettelin et al., 2001)
A0A0H2UPF3 (350 aa)	SP0845		Substrate binding Lipoprotein.		(Tettelin et al., 2001)

(continued on next page)

Table 1B (continued)

Uniprot_locus	Other locus	Name	Function (^b)	PDB ^a	References
A0A0H2UPN3 (307 aa)	SP0859		DUF979 superfamily. Unknown function		(Tettelin et al., 2001)
A0A0H2URB7 (445 aa)	SP1690		ABC transporter		(Tettelin et al., 2001)
A0A0H2URL4 (355 aa)	SP1826		ABC transporter		(Tettelin et al., 2001)
A0A0H2URR9 (167 aa)	SP1916		PAP2 family protein		
A0A0H2URP6 (206 aa)	SP1945		Hypothetical Protein		
A0A0H2US66 (335 aa)	SP2197		ABC transporter		(Tettelin et al., 2001)

^a Protein data bank (PDB) (<https://www.rcsb.org/pdb/home/home.do>) access on 17/07/2017.

^b Depending on the protein, Lpp function has been experimentally or putatively assigned as indicated in the associated reference.

3.2. Other cation transporters

Pneumococcal Zn^{2+} AdcAII receptor and, in particular, the Mn^{2+} PsaA receptor are critical for a non-strong iron dependent pathogen survival (Tseng et al., 2002). Indeed, these proteins are widespread and named laminin-binding proteins in other bacterial species. The three-dimensional structures of AdcAII (PDB 3CX3 (Loisel et al., 2008)) and PsaA (PDB 3ZTT (Lawrence et al., 1998)) have been reported (Fig. 3B, Table 1B). Both proteins present a similar fold typical from cluster A, the main difference being the conformation of a flexible loop that is located at the entrance of the binding site. In both structures a Zn^{2+} cation is found in the interdomain cleft, stabilized by a tetrahedral coordination through three His and one Glu residues. Despite the first reported crystal structure of PsaA contains Zn^{2+} , *in vivo* and *in vitro* data indicated that Mn^{2+} is the natural substrate for this transport system (Dintilhac and Claverys, 1997). The structure of metal-free PsaA reveals an open conformation with the metal-binding site completely exposed to the solvent (PDB 3ZK7, PDB 3ZK8, PDB 3ZK9 (Counago et al., 2014)). The structure of PsaA in complex with Mn^{2+} is also available (PDBID 3ZKA, (Counago et al., 2014)). The inability of open-state PsaA to satisfy the preferred coordination chemistry of Mn^{2+} enables the protein to undergo the conformational changes required for cargo release to the Psa permease. Despite this difference in metal ion affinities, high-resolution structures of PsaA in complex with Mn^{2+}

(PDBID 3ZTT (McDevitt et al., 2011)) (Fig. 3B) or Zn^{2+} showed almost no difference. In addition, the structure of PsaA in complex with Cd^{2+} has also been reported (PDB 4UTP, PDB 4UTO (Begg et al., 2015)).

S. aureus presents other Lpps that are involved in cation transport. Besides iron transporters there are cation transporters for Co, Cu, Mn, Mo, Ni, Zn ions, which become important when these trace elements are limited. In some cases, they are crucial in infection. Such is the case of MntC, (also named SitC) one of the most abundant Lpp in *S. aureus*. MntC is the metal-binding protein component of the Mn^{2+} -specific mntABC transporter and belongs to the SBP cluster A. The protein is expressed during the early stages of infection and is currently being tested in human clinical trials as a component of a multi antigen vaccine for the prevention of *S. aureus* infections (Gribenko et al., 2013). The crystal structure suggests two potential metal binding modes, which may lead to reversible as well as irreversible metal binding (PDBID 4K3 V (Gribenko et al., 2013)). The X-ray structure of MntC was also solved in complex with two different antibody fragments that were selected because they prevent the capture and transport of Mn^{2+} , a key element that the pathogen uses to evade host immunity (Fig. 3B) (Gribenko et al., 2016).

Nickel is a transition metal required as a cofactor for several bacterial enzymes. *S. aureus* possesses two canonical ABC-importers dedicated to nickel acquisition: the NikABCDE and the CntABCDF systems. The structure of the extracytoplasmic nickel-binding component Nika

Table 2

Correlation of the structural motif acting as hinge region with SBP cluster type, ligand specificity and receptor or transport mechanism. Table 2 has been adapted from (Scheepers et al., 2016). Very recently, at least five new SBPs with different structural arrangement have been assigned to a new cluster, cluster G.

Cluster	Structural motif acting as hinge region	Substrate	Associated with
A-I A-II	Single α -helix	Metal ions	Type II ABC transporter
B-I	Three interconnecting strands	Siderophores, hemes, vitamin B12, molybdate, tungstate Sugars, sugar alcohols, autoinducer 2	Type I ABC transporter, 2 component sensor kinase, transcription factor
B-II B-III B-IV	Second interconnecting strand interrupted by one α -helix Second interconnecting strand interrupted by two α -helices One α -helix interrupting the strand hinge. In addition, this cluster shows a glycan moiety	Amino acids Aromatic acids Natriuretic Compounds	Type I ABC transporter
B-V C	Two small α -helices interrupting the strand hinge An additional domain is present in between the two lobes	Glutamate Di- and Oligopeptides, cellobiose, opine, nickel	G-protein-coupled receptor Type I ABC transporter
D-I D-I D-IIIa D-IIIb D-IV E-I	The hinge-region consists of two short strands, 4–5 amino acids long One large α -helix	Sugars, sugar alcohols, spermidine/putrescine Thiamine, spermidine/putrescine Phosphate, molybdate, tungstate Molybdate, tungstate Iron ions	Type I ABC transporter Type I ABC transporter, transcription factor Type I ABC transporter
E-II F-I F-II F-III F-IV	Two strand hinges that almost double the length compared with cluster D	Organic acids, amino alcohols, dipeptides, glycerol-3-phosphate Amino acids Thiamine, pyrimidines, sulfonates, bicarbonate Methionine Compatible solutes Amino acids	TRAP transporter TT transporter Type I ABC transporter
G	The hinge region is formed by three loops. In addition, there is a regulatory EF-hand-like calcium-binding site	Oligosaccharides	Type I ABC transporter, ligand-gated ion channel, G-protein-coupled receptor Type I ABC transporter

Fig. 3. Three-dimensional structures of Lpp ABC transporters SBPs. (A) Iron Lpp ABC transporters. In all cases the linking residues are colored in pale green. Iron ion, when present in PDB, is depicted as a smudge green sphere. Ferrichrome, Heme and FeSB groups are represented as yellow sticks. (B) Other cation ABC transporters. Within cluster A, Zn^{2+} ion (AdcII) is depicted dark green sphere, Mn^{2+} is colored red sphere (PsaA and MntC) and Nickel is represented as green sphere (Nika). Cluster D Lpp PstS1 is shown in complex with phosphate. The linking sheet is colored in pale green and the phosphate is depicted as red spheres (C) Amino acid ABC transporters are, in all cases except AatB, shown with its bounded substrates as yellow sticks. (D) Sugars ABC transporters are, in all cases shown with its bounded substrates as yellow sticks. Lipobox plus its linker is schematically represented in all structures as a short helix fused to a dash line laying on top of the membrane. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

shows that is able to bind either a $Ni(L-His)_2$ (PDB 4XKQ, PDB 4XKP, PDB 4OFJ, PDB 3RQT and PDB 4XKN) complex or a $Ni(L-His)$ (2-methyl-thiazolidine dicarboxylate) (PDB 4XKR) complex (Fig. 3A). The protein binds specifically nickel complexes, in an opportunistic manner: if histidine is present in the bacteria's environment, the protein binds preferably $Ni(L-His)_2$. Otherwise, it binds nickel chelated by available products of the metabolism (Lebrette et al., 2015). In addition to Nika, Cnt (previously annotated as Opp1A) is a novel identified cobalt and nickel transporter (Remy et al., 2013) (Table 1A).

3.3. Anion and amino acid transporters

Despite the suggested critical impact over bacterial virulence of ABC amino acid transporters, such as the pneumococcal putative methionine

Lpp Sp0149 transporter (Basavanna et al., 2009) or the PstS phosphate transporters (Zheng et al., 2016), few structural information is currently available for this kind of Lpp (Table 1). Two phosphate transporters in *S. pneumoniae* (PstS1, PstS2) have been structurally characterized (Table 1, Fig. 3B). PstS1 and PstS2 structures (PDBs 4LAT and 4H1X respectively) are included as members of the cluster D that contains a β -hinge connecting the two lobes of the protein. The phosphate moiety is accommodated between the two lobes. No biochemical or functional data is yet available. Based on genetic annotation, the staphylococcal Lpp USA300_0175 is involved in nitrate transport. Its structure is available (PDB 3UN6), which has allowed its classification into sub-cluster F-I, which contains a β -hinge similar like cluster D but with almost double the length creating more flexibility inside the SBP.

The pneumococcal Lpp SBP amino acid components, ApbA, GlnH

and AatB (PDBs 4I62, 4H5F and 4OHN respectively) present the two lobes connected by two longer strands, 8–10 amino acids long, a characteristic from cluster F (Berntsson et al., 2010; Scheepers et al., 2016). In these structures, the binding site is located between the two lobes just below of the connecting strands (Fig. 3C). Other example of predicted amino acid transporter is the pneumococcal LivJ (PDB 4GNR), which belong to cluster B. The three-dimensional structure reported for this protein is characterized by the presence of three interconnecting strands between the two domains and the presence of one α -helix that interrupts the second interconnecting strand (Fig. 3B). However, no biochemical characterization is yet available to define its substrate specificity.

S. aureus has two amino acid ABC transporters of reported structure, GmpC (PDB 1P99) and OpuCc (PDB 3O66) belonging to clusters F-II and F-III respectively (Fig. 3C, Table 1A). According to this classification, GmpC should bind D-methionine, however GmpC binds glycylmethionine dipeptide instead. A comparative analysis of the substrate-binding pocket within the family members reflects the modifications in the hydrophobic nature of GmpC active site allowing accommodation of the larger methionine-containing dipeptide (Williams et al., 2004). No associated study exists for OpuCc (PDB 3O66).

3.4. Sugar transporters

The α -glucans are believed to be just a carbon source for “free-living” bacteria with little or no use in commensalism or pathogeny (Henrissat et al., 2002). However, the use of α -glucan in soft tissue infections by bacterial pathogens has been reported (Graham et al., 2006). Interestingly, *S. pneumoniae* and *S. aureus* possess Lpp specialized in using α -glucans in virulence (MalX in *S. pneumoniae* and protein encoded by gene SAUSA300_0209 in *S. aureus*). Furthermore, MalX has been shown to be critical for *S. pneumoniae* cells grow using exogenous host glycogen (Abbott et al., 2010). Indeed, Glycogen is a precursor in the synthesis of the surfactant by the alveolar type II cells and this might be correlated with the preference of *S. pneumoniae* to colonize this kind of cells (Cundell and Tuomanen, 1994).

Pneumococcal FcsSBP, RaffE and MalX belong to the cluster D. The discernible feature of these proteins is their 4–5 amino-acids long hinge-region consisting of two short strands (Fig. 3D). FcsSBP was crystallized in complex with a A-trisaccharide (PDB 2W7Y) (Higgins et al., 2009) and MalX with maltopentaose (PDB 2XD3) (Abbott et al., 2010). Structural analysis and isothermal titration calorimetry (ITC) experiments revealed that FcsSBP binding is limited to trisaccharides, specifically to the soluble group A/B trisaccharides (Higgins et al., 2009). MalX bind glucooligosaccharides of different lengths with different affinities. A comparison of maltopentaose-MalX (PDB 2XD3) (Table 1B, Fig. 3D) with the apo-MalX (PDB 2XD2) demonstrated that recognition of longer maltooligosaccharides requires structural plasticity in this protein (Abbott et al., 2010). The only *S. aureus* maltose transporter available in the PDB (SAUSA300_029) (PDB 4HW8) is classified as D cluster member presenting the characteristic two lobes linked by a hinge-region. However, this structure has not been yet associated with any publication.

The Pneumococcal sugars transporters NgtS and FusA belong to the cluster G, presenting relative larger molecular weight, additional structural elements and a metal-binding site, which is located close to the SBP-membrane domain interface (Scheepers et al., 2016).

NgtS was co-crystallized with the Man1GlcNAc (PDB 5SWA) and Man5GlcNAc (PDB 5SWB); both complexes are in a “closed” conformation relative to the “open” conformation obtained in the absence of ligand (PDB 5SUO). As with other SBPs, the ligand-binding site was found at the interface of the two domains and ligand recognition involves a transition from the open, unbound form to a ligand-stabilized closed form (Robb et al., 2017). In parallel, Culurgioni and co-workers reported the monomeric and oligomeric species to NgtS. The

tridimensional structure revealed that the residues 361–491 are positioned in different conformation (Culurgioni et al., 2017a) compared with the structure without ligand reported by Robb and co-workers (Robb et al., 2017). This region is interacting with other symmetry-related monomer producing a domain-swapped dimer, that could be used as an oligomerization mechanism to regulate carbohydrate binding (Culurgioni et al., 2017a). The FusA (Fig. 3B) presents high affinity for longer fructooligosaccharides (tetrasaccharides and larger). The structural superposition of the complexes of FusA with kestose (PDB 5G5Z), nystose (PDB 5G60), and fructofuranosyl-nystose (PDB 5G61) showed a similar overall conformation compared with apo-FusA (PDB 5G5Y), thus, indicating the absence of conformational changes triggered by ligand. The structural analysis of the three FusA complexes revealed the conserved binding site (Culurgioni et al., 2017b).

4 Three-dimensional structures of Lpp involved in redox processes and respiration

One of the most interesting aspects of the pneumococcal biology is its ability to survive under highly oxidizing conditions. Thus, on top of other mechanisms, pneumococci have evolved to have specific Lpp to deal with ROS stress. This bacterium, lacking catalase (the enzyme catalysing the decomposition of hydrogen peroxide to water and oxygen), presents in its lipoproteome two thioredoxin-like Lpp, Etrx1 and Etrx2, both critical for virulence (Saleh et al., 2013), and the Glutathione scavenger GshT. In contrast, *S. aureus* has a Quinol oxidase (QoxA), an electron transporter component (SaeP) and a thioredoxin Lpp (DsbA). However, none of them seems to be related with redox resistance but instead with respiration and protein folding mechanisms (Table 1A and 1B, Fig. 4A).

No structural or functional characterization of *S. aureus* Lpp respiration proteins QoxA and SaeP was available at the date of query. Luckily, all pneumococcal Lpp redox proteins have been structurally and functionally characterized (Saleh et al., 2013). Pneumococcus tolerates high concentrations of ROS during the infection process, and even produces by itself large quantities of H_2O_2 . This specific pneumococcal feature has a beneficial impact in niche competition. It has been reported that pneumococci use ROS particles to kill bacterial competitors as *S. aureus* (Selva et al., 2009). Furthermore, H_2O_2 plays a critical role in the infectious process allowing pneumococcal cells to invade lungs (Duane et al., 1993) and brain tissues (Braun et al., 2002). To deal with the challenge of surviving its own H_2O_2 , pneumococci has developed an extremely robust ROS defence mechanism that requires the combined action of cytoplasmic, membrane embedded proteins, secreted proteins and Lpp (Ezraty et al., 2017; Yesilkaya et al., 2013). There are three Lpp involved in such a defense mechanism, two thioredoxin-like lipoproteins (Etrx1, Etrx2), that are essential for external oxidative protein damage repair (Saleh et al., 2013), and a Glutathione-binding protein ABC transporter GshT that might contribute to provide protection against redox stress (Potter et al., 2012). The structures of Etrx1 and Etrx2 present a α/β thioredoxin-like fold (Fig. 4A). Etrx2 is highly similar to Etrx1 but embellished by a central $\alpha\beta$ insertion and an N-terminal β -hairpin. In both structures, the loop connecting $\beta 3$ with $\alpha 2$ contains the catalytic CxxC motif. Although structurally similar, the active site of Etrx1 exhibit a more polar character while Etrx2 is mainly hydrophobic that could account for specific recognition of the A and B catalytic domains of the Methionine sulfoxide reductase MsrA/MsrB 2 (MsrAB2) during repairing of the damaged proteins (Saleh et al., 2013). Considering that Etrx2 was crystallized in complex with either Cyclofos-3™ detergent or with 2-Hydroxyethyl Disulfide (HED) ligands, both bound to the active site. This result opens the gate in the search for Etrx inhibitors (Fig. 4A).

The crystal structure of GshT has been solved (PDB code 4EQ9) but not associated publication is already available. GshT is an α/β protein containing two globular domains fused by a flexible linker. Interestingly, the interface between both domains defines the active

Fig. 4. Three-dimensional structures of the rest of Lpp categories. (A) Three-dimensional structures of Lpp involved redox/respiration. Etrx1 is displayed in its apo form, Etrx2 and GshT are displayed in complex with cyclofos-3 and Glutathione, respectively. Both ligands are represented as red sticks. Domains composing GshT is colored differently. (B, C and D) miscellaneous families. (B) Biosynthetic/Processing Lpp. DacB Zinc ion is depicted as red sphere and phosphate is depicted in sticks. (C) Chaperone/Foldases. The partial structure of *S. aureus* PrsA (red) is shown close to the full length pneumococcal one for comparison (green). DsbA includes a thioredoxin domain which is colored in light blue and a helical insertion in dark blue. (D) Other/Lpp/Plasmid encoded Lpp groups. BlaZ is represented with two colors to reflect the bilobular architecture. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

site, which in the reported GshT structure is complexed with Glutathione (Fig. 4A). However, no description of its functional properties is, hitherto, available.

5 Three-dimensional structures of Lpp involved in biosynthesis and processing

Bacterial lipoproteins are also involved in biosynthetic and processing pathways. Inside this group, the only reported structure from *S. aureus* is the so-called sex pheromone CamS (PDB code: 2QX2) (Fig. 4B). This Lpp is literally responsible for the transmission of plasmids between *S. aureus* strains being a critical virulence factor responsible for the acquisition of antibiotic resistance (Flanagan and Clewell, 2002). Its structure presents a novel folding composed by two domains: the N- and C-terminal domains. N-terminal domain has a core of four β -sheets surrounded by two long α -helices, whereas C-terminal domain is composed by six β -sheets and two long α -helices. In *S. pneumoniae* biosynthetic Lpp are related with nutrient processing and turnover of the peptidoglycan having a considerable impact on the microorganism virulence. This is the case of the pneumococcal L,D-carboxypeptidase DacB that, besides its role in maintaining cell shape, is also relevant in cell adhesion and in susceptibility against vancomycin (Abdullah et al., 2014). The crystal structure of DacB (PDB code: 4D0Y) (Fig. 4B), presents a mononuclear Zn^{2+} catalytic center located in the middle of a large and fully exposed groove (Abdullah et al., 2014). Two different conformations were found presenting a different arrangement of the active site topology. The critical residues for catalysis and substrate specificity were identified. Comparison with DacA (the pneumococcal D,D-carboxypeptidase) revealed different regulatory mechanism: while DacA presents a fully open active site and a rigid structure, DacB lipoprotein presents a long flexible loop connecting the membrane with different regions of the cell-wall and two different conformations for the active site (Abdullah et al., 2014). Other example of *S. pneumoniae* biosynthetic Lpp is SP0198 (PDB code: 3GE2) (Fig. 4B). Although this protein has not been functionally characterized, its crystal structure revealed a beta barrel motif pointed by CATH database as a putative D-stereospecific aminopeptidase. This protein holds the DUF3642 domain, a major virulence factor from gram-negative bacteria (Punta et al., 2012), suggesting that this lipoprotein could be

involved in host-pathogen interactions in *S. pneumoniae*.

6 Three-dimensional structures of Lpp involved in protein folding

PrsA foldases are essential proteins ubiquitous within Gram-positive bacteria (Vitikainen et al., 2004) performing the *cis-trans* isomerization of the proline preceding peptide bonds acting as a folding chaperone (Fanghanel and Fischer, 2004). In this sense, staphylococcal PrsA has been reported as an essential factor for secreted proteins in *S. aureus* (Heikkinen et al., 2009) acting also as an auxiliary factor of oxacillin resistance in *S. aureus* (Jousselin et al., 2015). The Staphylococcal reported PrsA structure, comprising a shorter 110 residues construct (PDB 2JZV), overlaps perfectly with the longest *S. pneumoniae* structure (274 residues) (PDB 5TVL). Pneumococcal PrsA structure contains a parvulin-type peptidyl-prolyl *cis-trans* isomerase domain (Fig. 4C).

Sortases are catalyse the covalent anchor of proteins to the peptidoglycan by cleaving between the threonine and glycine residues of the conserved LPXTG motif (where X represents any amino acid) in substrate proteins (Fischetti et al., 1990; Schneewind et al., 1992). There are two Lpp sortases structurally determined, one from *S. aureus* (SrtA, PDB 1LJA, 1T2P) and one from *S. pneumoniae* (SrtC-3, PDB 2W1 K) (Table 1A and 1B). Both structures are formed by a β -barrel structure composed of eight anti-parallel β -strands linked by multiple α - and 3_{10} helices (Ilangoan et al., 2001; Manzano et al., 2008; Zong et al., 2004). The main difference between them is the additional α -helix present at the SrtC-3C terminus and the presence of a “lid” over the active site region.

Other example of Lpp structurally reported within this category is the disulphide isomerase protein DsbA (PDB 3BCI) from *S. aureus*, which is involved in catalysing the disulphide bonds of the secreted proteins (Table 1A). Its crystal structure comprises a thioredoxin domain and an embedded helical domain (Heras et al., 2008) (Fig. 4C)

7 Three-dimensional structures of miscellaneous Lpp

A huge portion of the *S. aureus* lipoproteome (22%) is classified as the specific *S. aureus* Lpl cluster whose only properly characterized members are the Csa1A (PDB 4BIH) and Csa1 B (PDB 4BIG), (Fig. 4D) antigens. These two proteins, with low structural similarity to any other

known protein, have been suggested to be protective antigens with a clear role in pathogeny (Schluepen et al., 2013). As expected due to their high sequence identity (75%), the overall fold of Csa1A is almost identical with that of Csa1 B (rms of 0.718 Å over 144 α aligned). Both structures exhibit a single α/β domain arranged as a large ten-stranded antiparallel β -sheet that closely resembles a β -half-barrel fold (Schluepen et al., 2013).

Within this miscellaneous category the only crystal structure reported is that of BlaZ from *S. aureus* (PDB 1PIO) (Fig. 4D). BlaZ is a β -lactamase composed by two close-packed domains where the polypeptide chain crosses twice from one domain to the other. One domain is formed by a five-stranded antiparallel beta-sheet with three helices packing against a face of the sheet. The second domain is formed mostly by helices that pack against the second face of the sheet. The active site is located in the interface between the two domains (Herzberg and Moutl, 1987).

8 Three-dimensional structures of Lpp with unknown function

It is worth to mention that 28% of the *S. aureus* and 21.4% of the pneumococcal lipoproteome is largely unknown. Although some efforts have been done to structurally characterise this group of proteins, only one structure from *S. pneumoniae* (PDB code 2MVB) and two structures from *S. aureus* (PDB codes: 2AP3 and 3QFG) have been deposited in the but no available functional characterization is reported so far. Their structural features are awaiting classification.

9 Concluding remarks

Although, the average fraction of the lipoproteome in the context of the full bacterial genome is very low, just 1.7% of the *S. pneumoniae* TIGR4 genome and no more than 2.57% for the MRSA *S. aureus* strain USA300 (Madan Babu and Sankaran, 2002; Shahmirzadi et al., 2016), the impact of this limited set of exposed proteins in the adaptability and pathogenic capabilities of these two Gram-positive pathogens is huge. This review summarizes and brings together the functional and structural knowledge concerning this group of peripheral membrane proteins.

Despite fragmentary information we dispose for many of these Lpp, the few cases in which a more in depth, functional and structural, knowledge is available reveals that Lpp are essential in regulation of quite diverse processes. This regulation depends on specific protein-ligand and protein-protein interactions. Understanding of the molecular basis of these interactions, and thus, of the regulatory processes mediated by Lpp, is essential. In an era of multidrug resistance emergence, the critical impact of the small Lpp group in bacterial survival makes them extremely attractive candidates as novel vaccine antigens and antibiotic targets to control infections caused by bacterial pathogens. In this sense, more structural and functional studies in the Lpp family are required.

Another important challenge for the near future is structural characterization of the full-length lipoprotein in which both the lipid and protein components can be visualized. What could be the effect for the complete structure of Lpp when attached to the membrane? As previously mentioned, different kind of lipids have been found in Lpp, besides, most lipoproteins present a linker of variable length connecting their functional modules to the membrane; therefore, it is expected that, at least, for those Lpp having a short linker and/or functional domains lying close to the membrane, the full-length structure in the presence of membrane-mimicking molecules (detergents, amphipols, lipids, nanodiscs) would provide unexpected and important details key to understand *in vivo* functioning of Lpp.

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