The global *Treponema pallidum* OMPeome: a structural platform for deciphering stealth pathogenicity and developing a syphilis vaccine with worldwide efficacy

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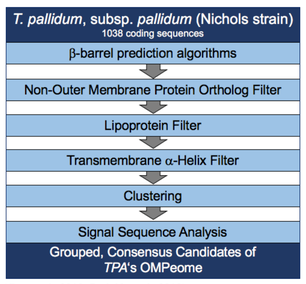
Abstract

The outer membrane (OM) of *Treponema pallidum* (*Tp*) serves as the permeability barrier and interface between the syphilis spirochete, an extracellular bacterium with extremely limited biosynthetic capacity, and its obligate human host. It is also the key to developing a syphilis vaccine with worldwide efficacy. The molecular architecture and composition of the *Tp* OM differ markedly from those of prototypical Gram-negative bacteria; recently we have used structural and bioinformatics computational tools to delineate the repertoire of β-barrel forming outer membrane proteins ('OMPeome') in the Nichols strain. The *Tp* OMPeome consists of two 'stand-alone' proteins (BamA and LptD) involved in OM biogenesis and four paralogous families (8-stranded barrel, long-chain fatty acid transport protein (FadL), OM factor of efflux pump (OMF), and *Tp* repeat protein (Tpr) OMPs) involved in influx and efflux of small molecules. The β-barrel assembly machinery (BAM) in *E. coli* consists of BamA and four accessory lipoproteins (BamB/C/D/E). *T. pallidum*'s BAM differs markedly. *Tp* has a BamA (TP0326) but lacks orthologs for BamB/C/D/E; this is consistent with (i) the presence of a gain-of-function mutation (*E. coli* E470 → TP0326 K484) in the BamA β-barrel domain, (ii) the absence of BamB/C/D/E interacting residues in BamA's polypeptide transport-associated motifs (POTRA)1-5 and (iii) a hybrid BAM system in which POTRA1-5 interacts with the DUF domain of TamB (TP0325). SAXS analysis of TP0326 POTRA1-5 revealed a three-state ensemble of compact, bent, and intermediate conformations, highlighting this region's flexibility and providing a model of BamA POTRA-TamB interactions. Although *Tp* does not produce lipopolysaccharide, surprisingly, its genome encodes a nearly complete Lpt-like apparatus for transport and insertion of an unidentified OM constituent, presumably glycolipids. Interestingly, the *Tp* LptD ortholog (TP0515) contains a large unstructured C-terminal domain, which models unbiasedly inside its β-barrel, like LptE of prototypes. *Tp* has four sequentially unique eight-stranded β-barrels containing positively charged extracellular loops (ECLs) believed to aid the spirochete in dissemination. Surprisingly, five orthologs of FadL (14-stranded β-barrel) were found in the *Tp* genome; all have hatch domains and NPA motifs inside the barrel, characteristic features of Gram-negative FadLs. The *Tp* genome encodes Mac and RND tripartite efflux pumps that achieve remarkable combinatorial diversity by co-expression of four paralogous OMFs. Lastly, we confirmed the 'bipartitism' of Tpr paralogs by solving the solution structure of N-terminal periplasmic domains of TprK and *T. denticola* MOSP, the parental ortholog of the Tpr family, and building 3D models of C-terminal β-barrel domains from ten Tprs. Overall, *Tp* appears to have evolved a unique OMP repertoire that balances the spirochete's physiological needs with its parasitic strategy as a stealth pathogen. The Nichols OMPeome provides a structural platform to (i) elucidate *Tp*'s enigmatic parasitic strategies for 'making a living' in the human host, (ii) decipher the targets of natural immunity, and (iii) select candidate vaccinogens for the development of a broadly protective syphilis vaccine for a spirochete that has afflicted humankind for centuries.

Introduction

Importance of *Tp* rare outer membrane proteins for protective immunity

Tp's outer membrane consists of a unique molecular architecture, with a paucity of unidentified OMPs observed by freeze fracture microscopy. Importantly, human syphilitic serum contains antibodies which lead to successful phagocytosis of the spirochete by human phagocytes and likely leads to successful clearance of treponemes during infection. It is believed that decoding *Tp* OMPs that are targeted by the host's natural immune response, paired with a clear understanding of each OMP's topology, will lead to the identification of vaccine candidates.

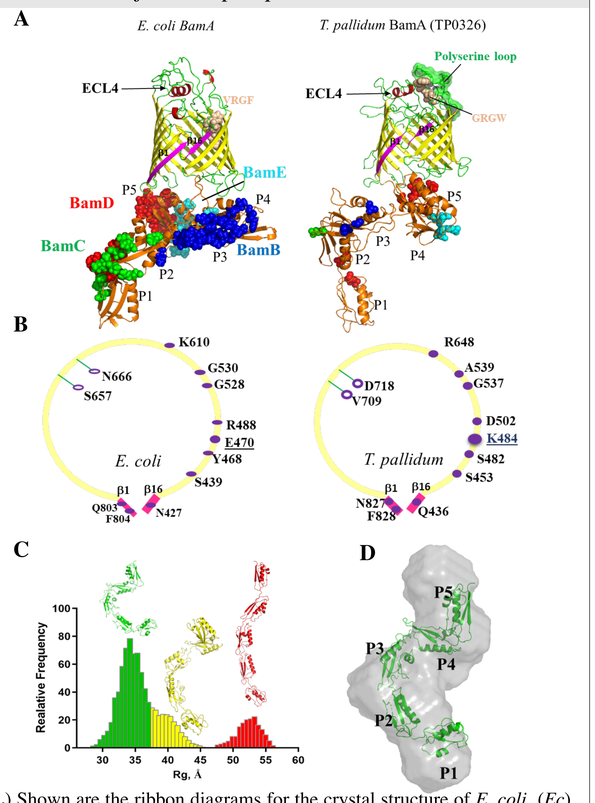


TPA's OMPeome		
***T. pallidum* repeat proteins (Tpr) OMP group**		
Subfamily I		
TprC (*TP0117*)	TprD/D2 (*TP0131*)	TprI (*TP0620*)
Subfamily II		
TprE (*TP0313*)	TprG (*TP0317*)	TprJ (*TP0621*)
Subfamily III		
TprB (*TP0011*)	TprH (*TP0610*)	TprL (*TP1031*)
Non-Tpr protein OMP group		
BamA (*TP0326*)	FadL (*TP0548*)	FadL (*TP0856*)
FadL (*TP0858*)	FadL (*TP0859*)	FadL (*TP0865*)
LptD (*TP0515*)	OmpW1 (*TP0126*)	OmpW2 (*TP0733*)
OprJ (*TP0966*)	OprN (*TP0967*)	TolC (*TP0969*)

(Cox *et al.*, 2010; Radolf *et al.*, 2018)

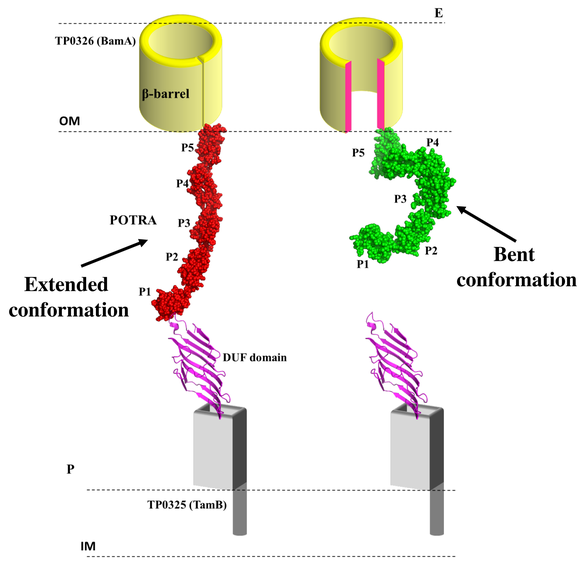
Results

T. pallidum BamA (TP0326) possesses an inherent gain-of-function (E470K) form of the β-barrel domain and a flexible periplasmic POTRA arm.

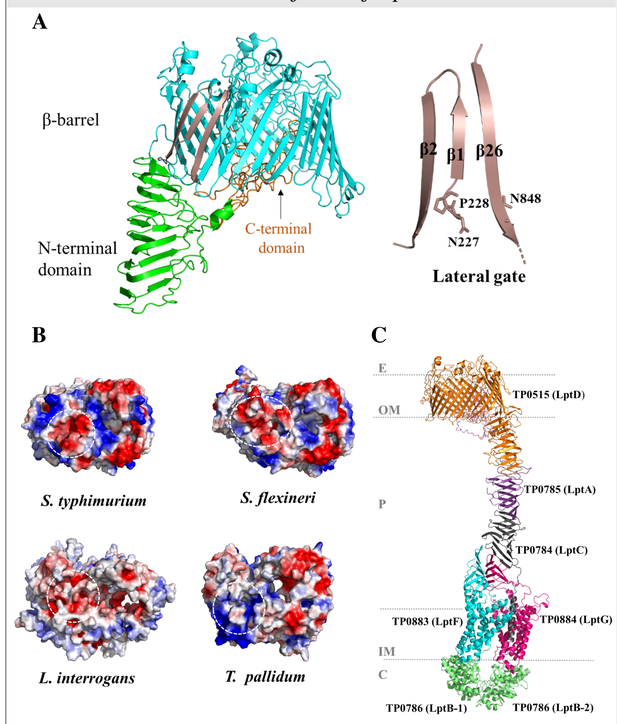


A.) Shown are the ribbon diagrams for the crystal structure of *E. coli* (*Ec*) BamA and the structural model of TP0326. The 16-stranded β-barrel domain and periplasmic POTRA arm (P1-P5) are shown as yellow and orange, respectively. **B.**) Shown are the schematic of β-barrel interior. The critical residues of *Ec* BamA, required for the folding of the β-barrel substrate, (left panel) and their equivalent amino acids in the structurally-aligned TP0326 (right panel) are shown as purple circles. The gain of function residue (E470) of *Ec* BamA and its corresponding amino acid (K489) in TP0326 are underlined. **C.**) Rg distribution of EOM-selected major conformations in *Tp*_POTRA1-5. **D.**) *Ab initio* reconstruction of the molecular envelope (gray surface) of *Tp*_POTRA1-5 calculated from the experimental SAXS data.

Proposed model of TP0326's mechanics.

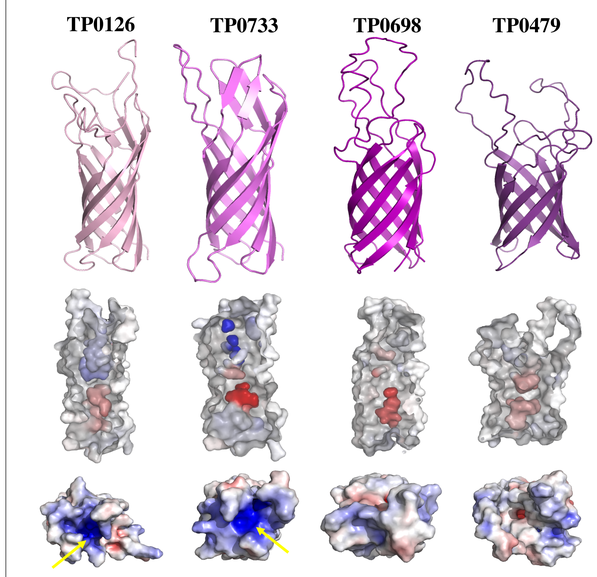


The structural model of TP0515 revealed a non-canonical form of LptD.



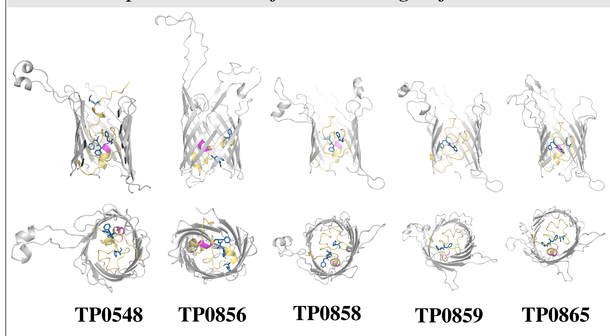
A.) The I-TASSER model of the TP0515. The β-jelly roll, N-terminal domain and 26-stranded β-barrel domain are shown in cyan and green, respectively. The C-terminal domain of TP0515 is modeled inside barrel's lumen (orange ribbon). The lateral gate, composed of strands, β1 and β26, is shown in brown. **B.**) Shown are the electrostatics of LptDs of *Salmonella typhimurium*, *Shigella flexineri*, *Leptospira interrogans* and TP0515. The dashed circle indicates the predicted LPS exit pore in each β-barrel domain. **C.**) Shown is the ribbon diagram of the assembled structural model of complete *Tp* Lpt machinery.

Tp has four 8-stranded β-barrels, potential surface molecules to interact with the extracellular matrix .



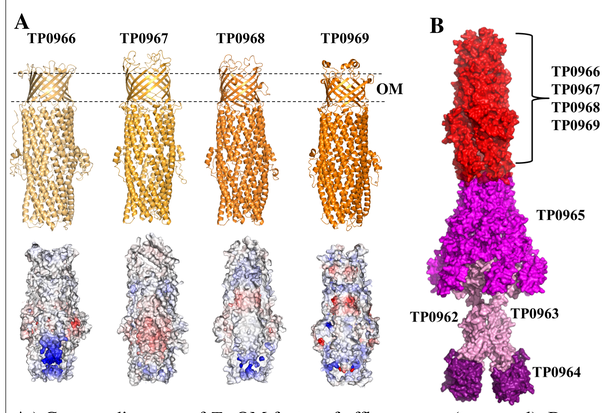
A.) Cartoon diagrams of *Tp's* eight-stranded β-barrels. **B.**) Representation of the inside of β-barrels showing the electrostatic potentials. The surface is colored *blue and red* for positive and negative charges, respectively. **C.**) View from the extracellular surface. Arrows indicate the positively charged ECLs of TP0126 and TP0733, the possible regions to interact with the extracellular matrix proteins laminin and fibronectin.

T. pallidum has five orthologs of FadL.



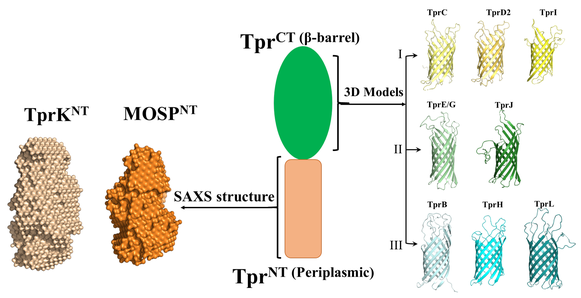
The structural models of *Tp* five FadL orthologs. *Tp*'s FadL orthologs (TP0548, TP0856, TP0858, TP0859, TP0865) are believed to transports hydrophobic substrates, such as fatty acids. The group of proteins consists of 14-stranded β-barrels with 7 ECLs. All five FadLs have an N-terminal hatch domain (yellow), which plugs the barrel. The classic N-P/A-A motif is colored as magenta in the hatch. Conserved Phe residues are shown as cyan sticks. *Tp* is an extreme fatty acid auxotroph and must get the carbon sources from the host; perhaps this is the reason why *Tp* has five FadL-like proteins.

OM factors of efflux pump in T. pallidum.

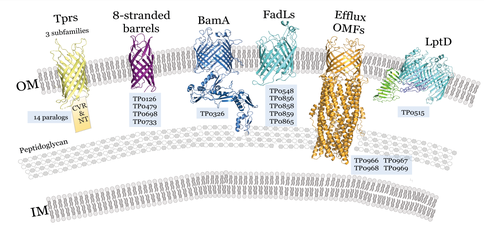


A.) Cartoon diagrams of *Tp* OM factor of efflux pumps (top panel). Bottom panel represents the inside of β-barrels showing the electrostatic potentials. The surface is colored *blue and red* for positive and negative charges, respectively. **B.**) Shown is the surface diagram of the assembled structural model of complete *Tp* efflux pump.

All Tprs have an OM-embedded C-terminal (CT) β-barrel and water-soluble N-terminal (NT) domain.



T. pallidum OM molecular architecture (v.2020).



Funding: This work was supported by NIAID grants R01 AI26756, U19 AI144177, and research funds provided by Connecticut Children's.