Supplemental Information

The structure and interactions of periplasmic domains of crucial MmpL membrane proteins from *Mycobacterium tuberculosis*.

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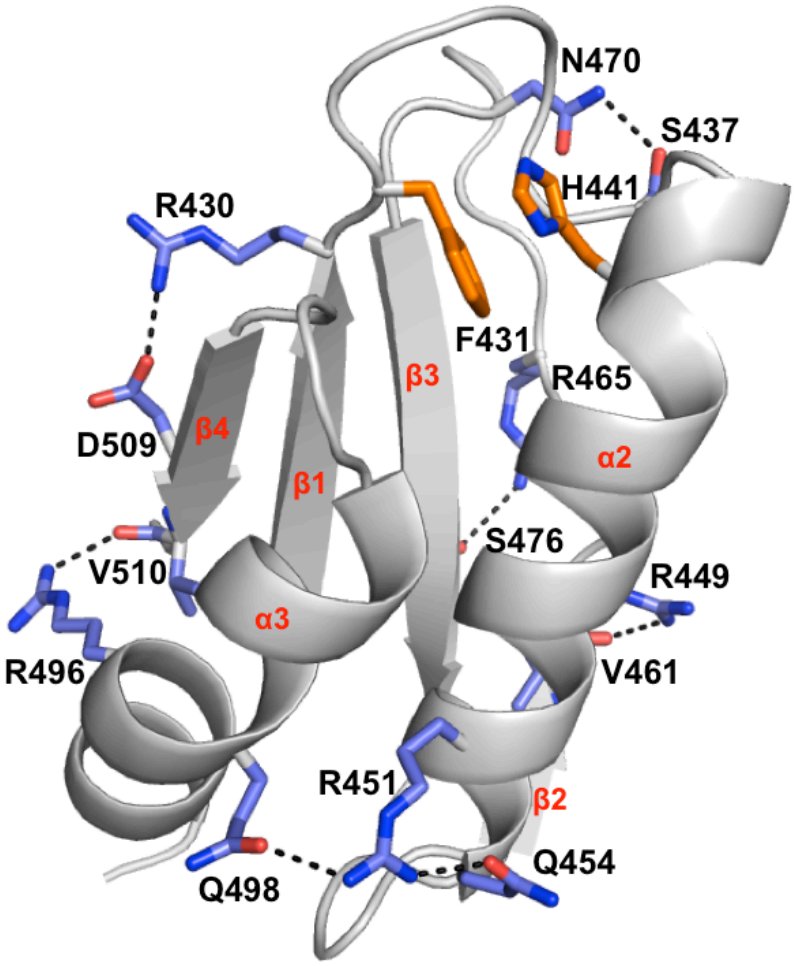

Figure S1, related to Figure 2 Cartoon representation of MmpL11-D2 structure in grey. Residues involved in ion-pair interactions (dashed lines) and π-π stacking are shown as sticks and colored lilac and orange, respectively. Oxygen and nitrogen atoms within the stick residues are colored red and blue, respectively.

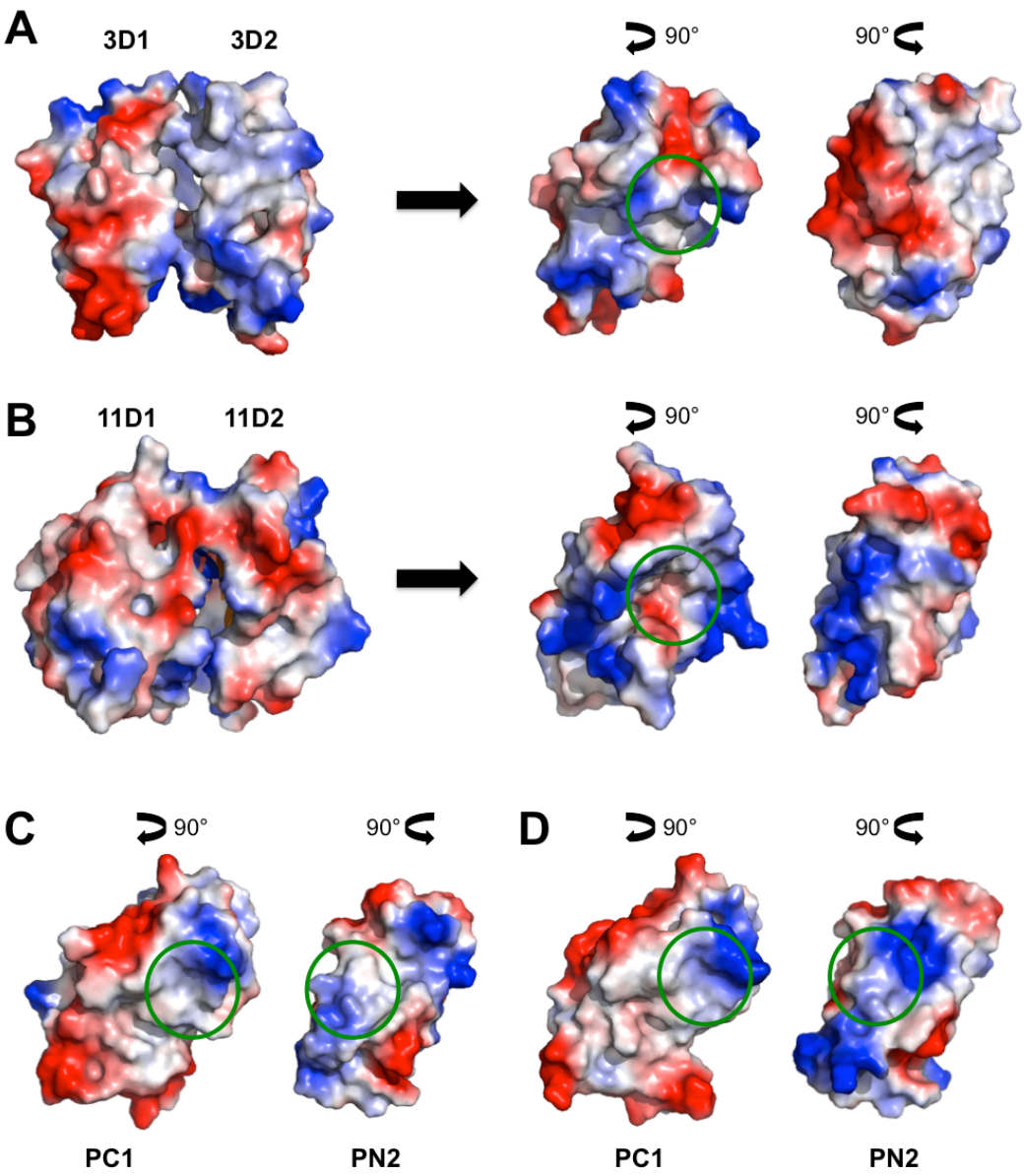

Figure S2, related to Figure 5 Electrostatics potential maps of (A) MmpL3-D1-D2 and (B) MmpL11-D1-D2 heterodimer models where, on the right, each domain is rotated 90° to expose their binding interface. The orientations of both heterodimer models are identical to that of Figure 4A (top). Electrostatics potential maps of (C) AcrB (PDB code: 3W9H) and (D) MexB (PDB code: 2V50) PC1 and PN2 porter subdomains, where their binding interfaces are oriented similar to (A) and (B). Green circles in MmpL3/11 D1 domains signify potential binding sites that correspond to the binding sites of AcrB and MexB.

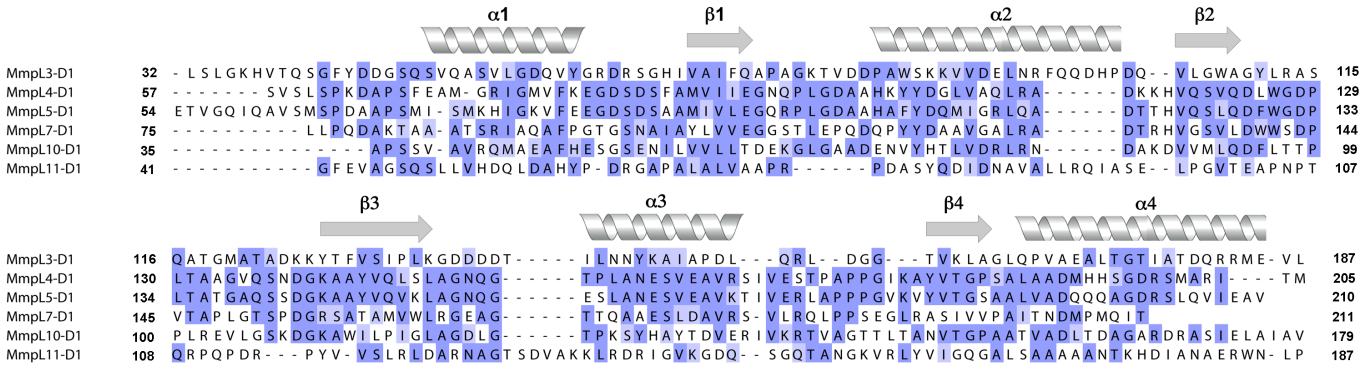

Figure S3, related to Figure 6 MmpL proteins D1 domains topology prediction (Krogh, et al., 2001) and alignment based on secondary structural prediction. The predicted secondary structural elements are shown and conserved residues are colored according to the BLOSUM matrix.

Table S1, related to Figure 2. Structural homologs of MmpL11-D2 determined by DALI (Holm and Rosenstrom, 2010)

Homolog	PDB identifier	Z-score	RMSD (Å)[1]	Lali[2]	nres[3]	%id[4]
CusA	4DNT	9.5	2.3	80	1031	11
ArcB	3W9H	8.8	2.4	82	1033	15
CusA	3T56	8.7	2.2	80	1027	14
MexB	2V50	8.6	2.6	81	1012	15
ZneA	4K0E	8.4	2.2	80	942	16
MtrD	4MT1	8.3	2.5	81	1023	15

[1]RMSD over αC atoms
[2]Lali is the number of residues the search structure aligns to
[3]nres the number of residues in the protein
[4]%id is the sequence identity between aligned proteins.

Table S2, related to Figure 4&5. Intermolecular crosslinked peptides between MmpL3 D1 and D2 domains

3D1 – 3D2	m/z	z	Score	Score Diff
AYAVGASK(504)*DPSVR – TVDDPAWSK(88)*K (2X)	868.7828 651.8394	3 4	28.1 28.7	9.6 12.7
AYAVGASK(504)*DPSVR – ASQATGMATADK(125)*K	685.1018	4	28.4	15.2
AYAVGASK(504)*DPSVR – G(N-term)*SHMLSLGK (2X)	796.4180 597.5668	3 4	26.7 18.3	7.6 5.6
G(N-term)*SHMLGGISEK – G(N-term)*SHMLSLGK	546.2835	4	24.8	15.6
AYAVGASK(504)*DPSVR – K(89)*VVDELNR	608.3311	4	20.7	7.0

Table S3, related to Experimental Procedures. List of MmpL3 and MmpL11 D1 and D2 domain constructs with corresponding primers. Restriction enzyme sequences are italicized.

Construct			Primers
Protein	Domain	Boundary	
MmpL3	D1	32 – 187	For: 5' G*CATATG*CTGAGCCTCGGCAAGCACGTCACGCA 3' Rev: 5' CG*AAGCTT*CTACAGCACTTCCATTCGCCGTTGGTCGG 3'
	D1 Δα4	42 – 168	For: 5' CG*GGATCC*GGCTTCTACGACGACGGCAGCCAATCGGTGCAAG 3' Rev: 5' GC*CTCGAG*TCACGGTTGCAGCCCGGCGAGCTTCACC 3'
	D1 Δα1α4	67 – 168	For: 5' CG*GGATCC*GGTCACATCGTCGCGATCTTCCAAGCCCCAGC 3' Rev: 5' GC*CTCGAG*TCACGGTTGCAGCCCGGCGAGCTTCACC 3'
	D2	419 – 560	For: 5' G*CATATG*TTGGGCGGGATCAGCGAGAAGTACTTGCCGC 3' Rev: 5' CG*AAGCTT*CTACTACGGCATCTTCGCGAACAGGCCGTG 3'
	D2 Δα4	419 – 545	For: 5' G*CATATG*TTGGGCGGGATCAGCGAGAAGTACTTGCCGC 3' Rev: 5' GC*CTCGAG*TCAGGGAGTTCCACCGACCAAGACCGTGATTCCTTTGG 3'
	D2 Δα1α4	446 – 545	For: 5' CG*GGATCC*GGATACCGCACCAATCCGCTGACACTGGTGATCCAG 3' Rev: 5' GC*CTCGAG*TCAGGGAGTTCCACCGACCAAGACCGTGATTCCTTTGG 3'
MmpL11	D1	41 – 187	For: 5' CG*GGATCC*GGTTTCGAAGTCGCCGGGTCGCAGTCATTGCTGGT 3' Rev: 5' GC*CTCGAG*TCAAGGCAGGTTCCAGCGTTCGGCGTTGGCAATATCGTGT 3'
	D1 Δα4	41 – 166	For: 5' CG*GGATCC*GGTTTCGAAGTCGCCGGGTCGCAGTCATTGCTGGT 3' Rev: 5' GC*CTCGAG*TCACAGCGCGCCCTGCCCGATGACATAGAGC 3'
	D1 Δα1α4	64 – 166	For: 5' CG*GGATCC*GGAGCCCCGGCGCTGGCGCTG 3' Rev: 5' GC*CTCGAG*TCACAGCGCGCCCTGCCCGATGACATAGAGC 3'
	D2	390 – 529	For: 5' G*CATATG*GTGCTGGGCAACAGCTTGCTGCGCCAGTTC 3' Rev: 5' CG*AAGCTT*CTACTACGGTTGCGTCGCGGACACCCG 3'
	D2 Δα4	390 – 514	For: 5' G*CATATG*GTGCTGGGCAACAGCTTGCTGCGCCAGTTC 3' Rev: 5' GC*CTCGAG*TCAGGTCGGCCCGCCGACGTCCACTTG 3'
	D2 Δα1α4	423 – 514	For: 5' CG*GGATCC*GGCCCGGTTCAGGTGCTGGTCAGGTTTGACG 3' Rev: 5' GC*CTCGAG*TCAGGTCGGCCCGCCGACGTCCACTTG 3'

References

Holm, L., and Rosenstrom, P. (2010). Dali server: conservation mapping in 3D. Nucleic Acids Res 38, W545-549.

Krogh, A., Larsson, B., von Heijne, G., and Sonnhammer, E.L. (2001). Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes. Journal of molecular biology 305, 567-580.