Supplemental Material: The structures of penicillin binding protein 4 (PBP4) and PBP5 from *Enterococci* provide structural insights into β–lactam resistance

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Supplementary Tables

Table S1. Impact of E. faecium PBP5 aa substitutions on ampicillin and ceftriaxone MICs

Plasmid[a]	**Amino acid at the following position in PBP5**				**MIC (µg/mL)**		**Reference**
	485	**499**	**629**	**S466'**	**Ampicillin**	**Ceftriaxone**	
pCWR624 (wt, susceptible PBP5)	M	I	E	–	38	2150	(1)
pCWR666[b]	A	T	V	+	185	6350	(1)

[a]*plasmid constructs in strain D344SRF; ampicillin MICs for D344SRF and D344SRF containing the vector plasmid pTCV-lac were repeatedly 2 µg/ml.*
[b]*pCWR666 contains mutations in addition to those designated (F497G, R598Q, P667S) and contains the* pbp5 *gene from highly resistant clinical strain E. faecium C68.*

Table S2. Structural similarities between PBP4 and PBP5 domains

PBP domain	**PBP4 residues**	**PBP5 residues**	**RMSD (Å)**
N1	nv	39-174 314-339	nd
N2	193-260	191-258	0.9
nPB	177-188 267-315 342-350 386-411	175-186 265-313 340-348 384-409	0.75
TP	349-383 410-678	351-385 412-680	1.1

nv, *no interpretable electron density*
nd*, not determined*

Table S3. Structural similarities between the free and acylated TP domains of PBP4 and PBP5

PBP	**RMSD (Å)**
PBP4 (compared to apo)	
penG	0.40
imipenem	0.31
ceftaroline	0.53
PBP5 (compared to apo)	
penG	0.36
imipenem	0.34

Table S4. Overlay of PBP2a with PBP5

PBP domain	PBP5 residues	PBP2a residues	RMSD (Å)
N1	87-126 132-139 147-150 162-165 314-339	66-105 110-117 125-128 140-143 294-319	2.5
N2	187-200 209-240	165-178 182-213	0.8
TP	349-383 410-576 577-597 613-654	330-364 391-557 559-579 593-634	2.3

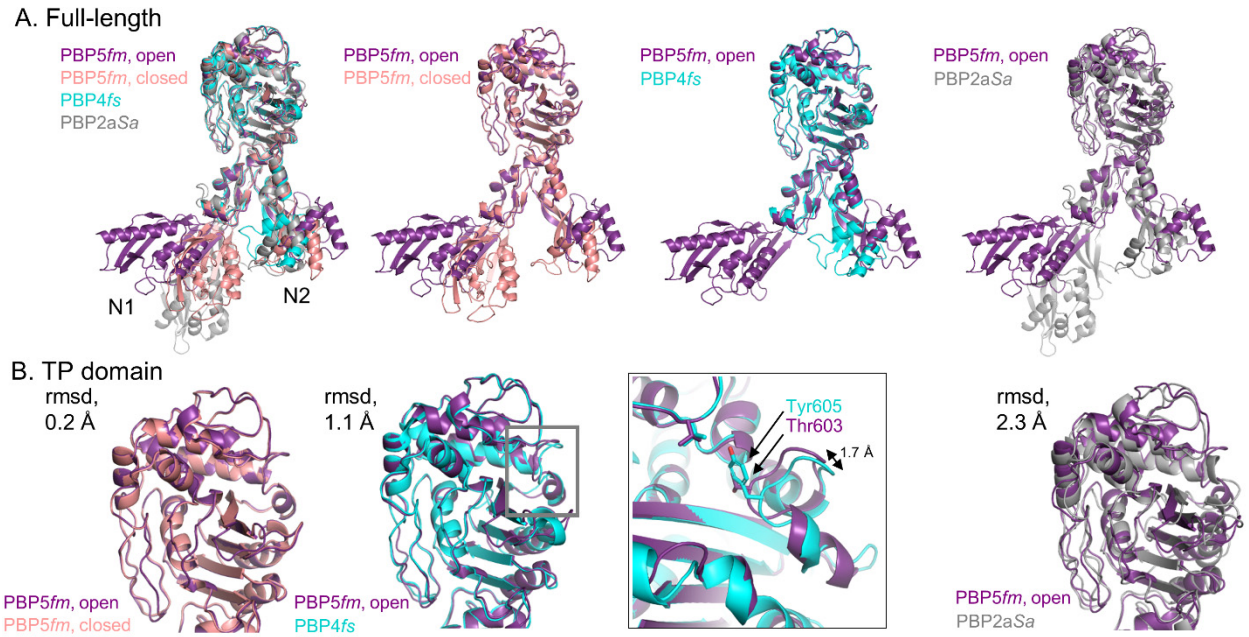

Figure S1. Overlays of the low-affinity PBP family via the TP domains. **A**. PBP5-open (magenta), PBP5-closed (beige), PBP4 (cyan) and PBP2a (grey) superimposed on one another via their TP domains illustrates the range of conformations that can be adopted by the N1 and N2 domains (labeled). **B**. Same overlays and colors as in 'A', but showing only the TP domains. The calculated RMSDs are reported.

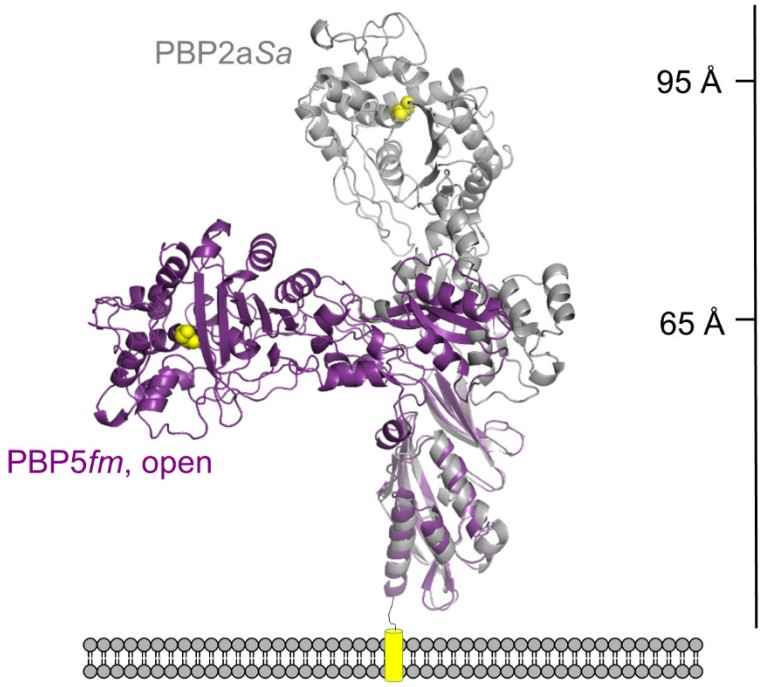

Figure S2 Overlay of PBP5 and PBP2a via the N1 domain. Assuming the N-terminal helix is co-linear with transmembrane helix results in the distances of the transpeptidase domain active sites being either ~65 Å or 95 Å away from the membrane.

Supplementary References

1. Rice, L. B., Bellais, S., Carias, L. L., Hutton-Thomas, R., Bonomo, R. A., Caspers, P., Page, M. G. P., and Gutmann, L. (2004) Impact of specific pbp5 mutations on expression of beta-lactam resistance in Enterococcus faecium. *Antimicrob. Agents Chemother.* **48**, 3028–3032