
Supplementary information

Structural basis of broad-spectrum β -lactam resistance in *Staphylococcus aureus*

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Structural basis of broad spectrum β -lactam resistance in *Staphylococcus aureus*

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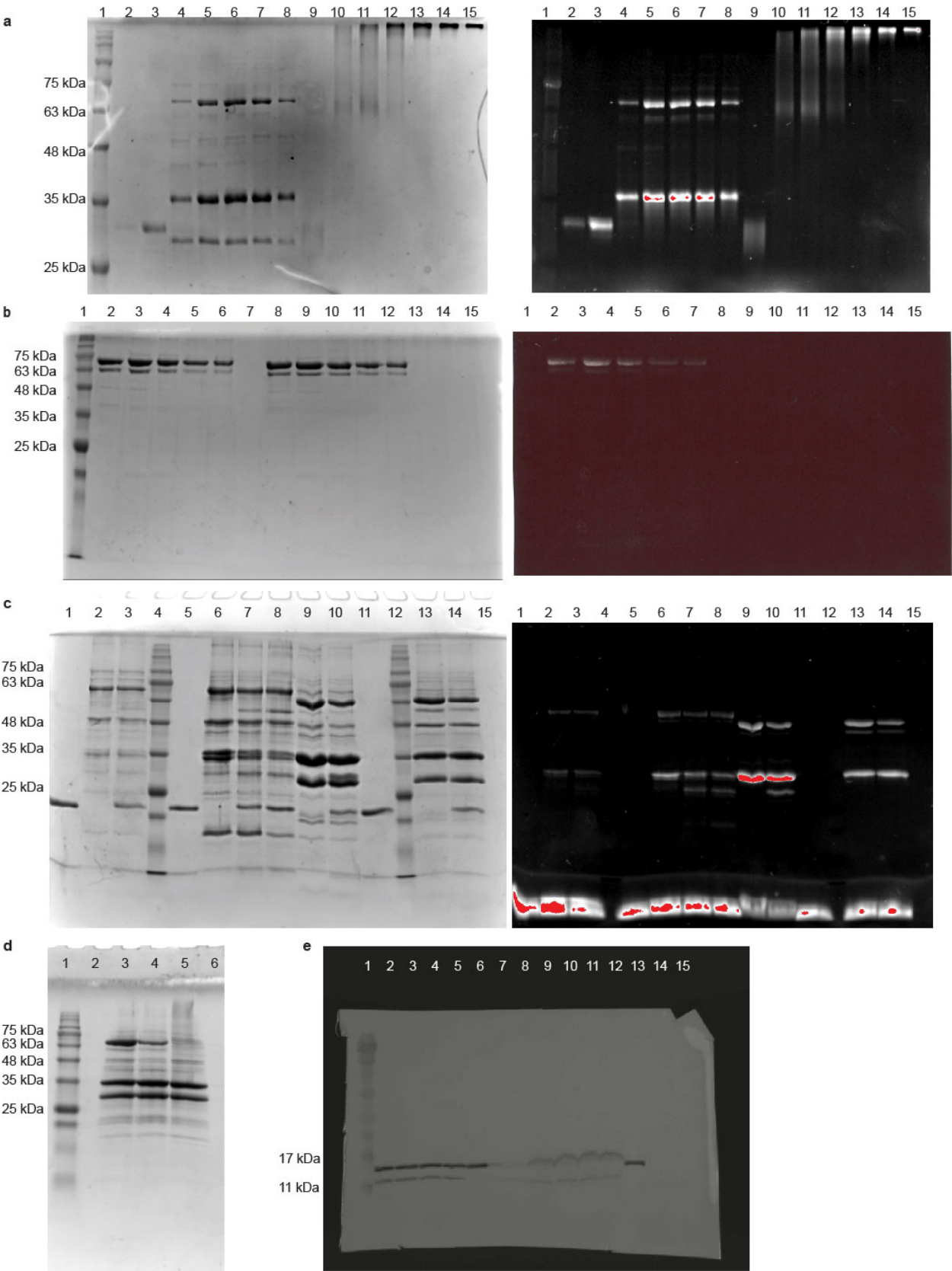
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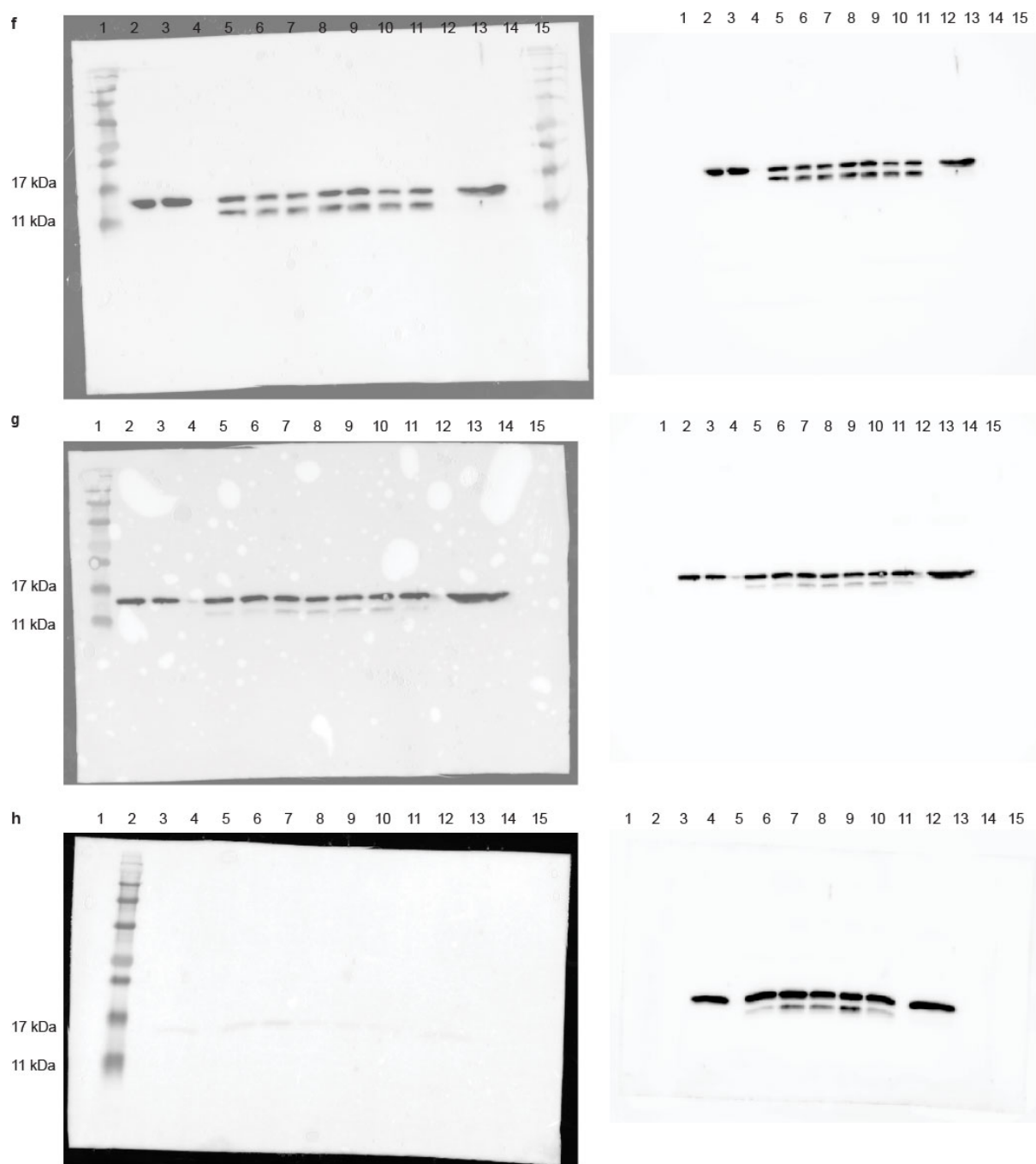
Supplementary Information contains:

- Supplementary Figure 1
- Supplementary Tables 1-5

Supplementary Information



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Supplementary Figure 1: Uncropped gels and blots. **a**, Coomassie stained (left) and fluorescent imaged (right) SDS-PAGE gels shown in Extended Data Fig. 1c. Figure cropped to show lanes 1-8. Marker is in lane 1. **b**, Coomassie stained (left) and fluorescent imaged (right) SDS-PAGE gels shown in Extended Data Fig. 1e. Figure shows full gels. Marker is in lane 1. **c**, Coomassie stained (left) and fluorescent imaged (right) SDS-PAGE gels shown in Extended Data Fig. 8g. Figure cropped to show lanes 4 (marker), 5, 9, 10, 13, 14. **d**, Coomassie stained SDS-PAGE gel shown in Extended Data Fig. 1g. Figure shows cropped lanes 1-6. **e**, Fluorescent imaged Western blot membrane shown in Extended Data Fig. 1a. Figure shows cropped lanes 1 (marker), 9, 13, 14. **f**, Colorimetric (left) and fluorescent (right) imaged Western blot membrane shown in Extended Data Fig. 8d. Fluorescent image cropped to show region around 11-17 kDa for lanes 3-14. **g**, Colorimetric (left) and fluorescent (right) imaged Western blot membrane shown in Extended Data Fig. 8e. Fluorescent image cropped to show region around 11-17 kDa for lanes 3-14. **h**, Colorimetric (left) and fluorescent (right) imaged Western blot membrane shown in Extended Data Fig. 8d. Fluorescent image cropped from to show region around 11-17 kDa for lanes 4-13.

Supplementary Information

Supplementary Table 1: ICP-MS measurement of zinc in BlaR1

Sample	[BlaR1-F284] (nM)	[Zn] (ppb)	[Zn] (nM)	Zn : Protein Ratio (mol:mol)
Measured BlaR1-F284A (Concentration corrected from buffer)	700	52 (50)	790 (760)	1.1
Buffer	-	2	30	-

Supplementary Table 2: M17BEM components prior to autoclaving

Component	Mass per litre (g)	Supplier
Glucose	10	Sigma, G8270
Bio-Tryptone	5	BioShop, TRP402
Bacto Soytone	5	BD, 243620
BBL Beef extract powder	5	BD, 212303
Yeast extract	2.5	BioShop, YEX401.205
Ascorbic acid	0.5	Bio Basic, AB0021
MgSO ₄ (anhydrous)	0.25	Fisher, M65-500

Supplementary Information

Supplementary Table 3: List of strains used for *S. aureus* growth assay.

Strain	Description	Reference
RN4220	Laboratory strain	BEI Resources
SF8300ermS	SF8300 strain cured of the plasmid containing <i>BlaZ-blaR1-blaI</i> and EM resistance	²
RN4220 <i>pTxΔ16</i>	RN4220 with the <i>pTxΔ16</i> empty vector	
RN4220 <i>pTxΔ [blaR1-blaI]</i>	RN4220 with <i>pTxΔ</i> containing a functional <i>blaR1</i> and <i>blaI</i>	This Study
RN4220 <i>pTxΔ [blaR1(null)-blaI]</i>	RN4220 with <i>pTxΔ</i> containing a functional <i>blaI</i> and a <i>blaR1</i> null mutant.	This Study
RN4220 <i>pTxΔ [blaR1(null)-blaI(null)]</i>	RN4220 with <i>pTxΔ</i> that contains null mutants of <i>blaR1</i> and <i>blaI</i> .	This Study
RN4220 <i>pTxΔ [blaR1(F284A)-blaI]</i>	RN4220 with <i>pTxΔ</i> that contains a functional <i>blaR1</i> with a F284A mutation and a WT <i>blaI</i> .	This Study
SFermS <i>pTxΔ 16</i>	SFermS with the <i>pTxΔ16</i> empty vector.	This Study
SFermS <i>pTxΔ [blaR1-blaI]</i>	SFermS with <i>pTxΔ</i> containing a functional <i>blaR1</i> and <i>blaI</i> .	This Study
SFermS <i>pTxΔ [blaR1(null)-blaI]</i>	SFermS with <i>pTxΔ</i> containing a functional <i>blaI</i> and a <i>blaR1</i> null mutant.	This Study
SFermS <i>pTxΔ [blaR1(null)-blaI(null)]</i>	SFermS with <i>pTxΔ</i> that contains null mutants of <i>blaR1</i> and <i>blaI</i> .	This Study
SFermS <i>pTxΔ [blaR1(F284A)-blaI]</i>	SFermS with <i>pTxΔ</i> that contains a functional <i>blaR1</i> with a F284A mutation and a WT <i>blaI</i> .	This Study

Supplementary Information

Supplementary Table 4: List of plasmids used for *S. aureus* growth assay.

Plasmid	Description	Reference
<i>pTX_Δ</i> 16	Empty vector	³
<i>pTX_Δ</i> [<i>blaR1-blaI</i>]	<i>pTX_Δ</i> containing a functional <i>blaR1</i> and <i>blaI</i>	This study
<i>pTX_Δ</i> [<i>blaR1</i> (null)- <i>blaI</i>]	<i>pTX_Δ</i> containing a functional <i>blaI</i> and a <i>blaR1</i> null mutant.	This study
<i>pTX_Δ</i> [<i>blaR1</i> (null)- <i>blaI</i> (null)]	RN4220 with <i>pTX_Δ</i> that contains null mutants of <i>blaR1</i> and <i>blaI</i>	This study
<i>pTX_Δ</i> [<i>blaR1</i> (F284A)- <i>blaI</i>]	<i>pTX_Δ</i> that contains a functional <i>blaR1</i> with a F284A mutation and a WT <i>blaI</i> .	This study

Supplementary Table 5: List of primers used for *S. aureus* growth assay.

Primer	Sequence	Purpose
<i>blaR1</i> -BamH1-for	ttaGGatCCatatttgaagaagggtgcaa aatggc	Cloning of <i>blaR1-blaI</i> into <i>pTX_Δ</i>
<i>pTX_Δ</i> - <i>blaR1</i> -I-Mlu1-rev	Cloning of <i>blaR1-blaI</i> into ptxD	Cloning of <i>blaR1-blaI</i> into <i>pTX_Δ</i>
<i>pTX_Δ</i> - <i>blaR1</i> -null-F	ttaGGatCCatatttgaagaagggtgcaa aacggctaaattac	Cloning of <i>blaR1-blaI</i> into ptxD, introduction of a M1T null mutation in <i>blaR1</i>
<i>blaI</i> -null-rev	ttcaacttgctattggtcgtttaaaccacca ttcc	Introduction of a M1T null mutation in <i>blaI</i>
<i>blaI</i> -null-for	ttttaaacgaccaataagcaagttgaaatat ctatgg	Introduction of a null mutation in <i>blaI</i>
<i>blaR1</i> _F284A-Rev	ttttaccattTGCtgaatggcttaatatatttt gttgg	Introduction of F284A mutation in <i>blaR1</i>
<i>blaR1</i> _F284A-For	atatattaagccattcaGCAaatggtaaaa aatcattactc	Introduction of F284A mutation in <i>blaR1</i>