

Supporting information

Stress-based High-throughput Screening Assays to Identify Inhibitors of Cell Envelope Biogenesis

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Escherichia coli, high-throughput screening, antibiotics, potentiators, SigmaE, heat-shock, Cpx, Rcs

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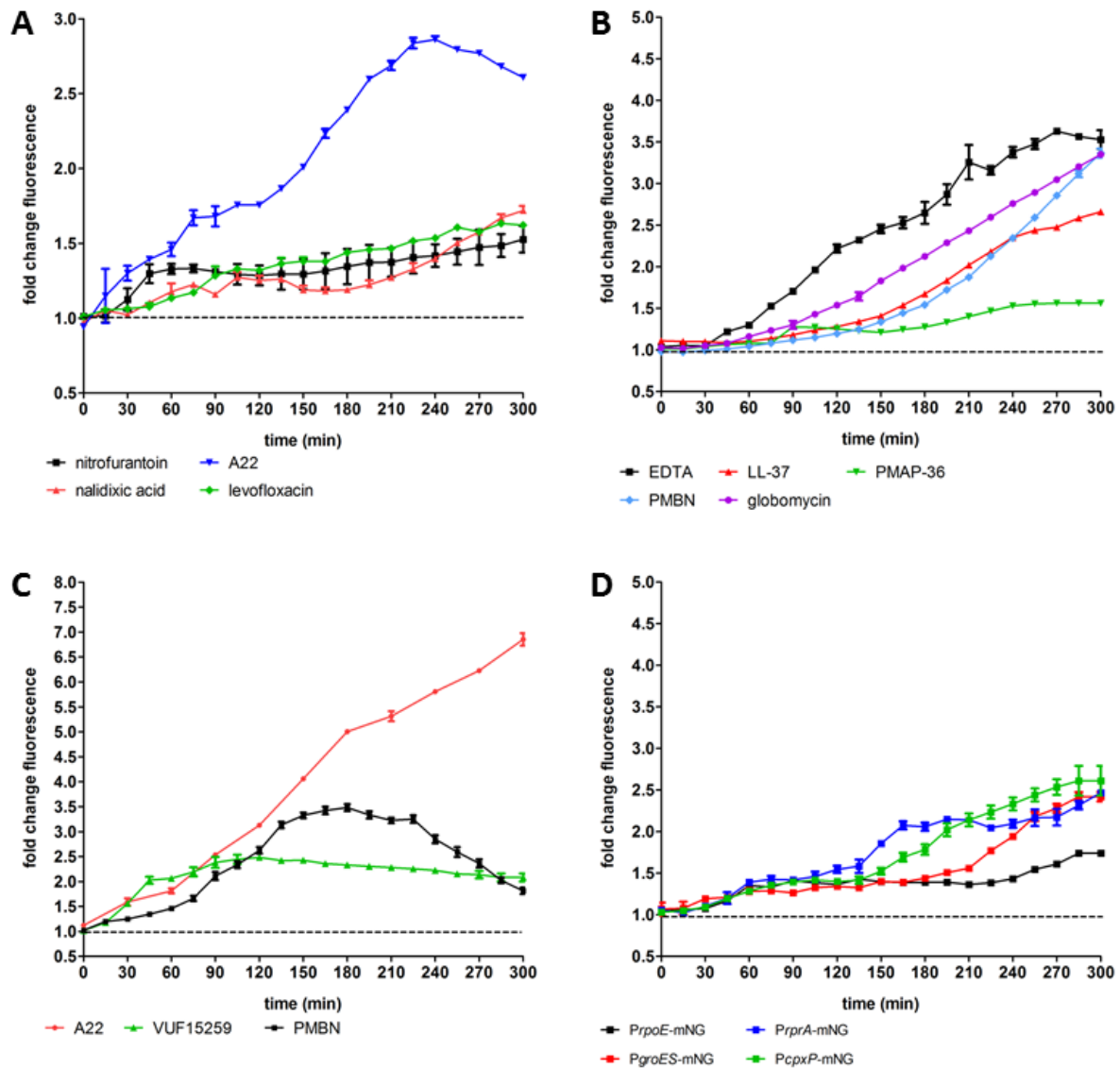


Figure S1. Real-time monitoring of heat-shock, Cpx and σ^E stress activation in response to selected antibacterial agents.

E. coli TOP10F' cells, harboring the *PgroES*-mNG, *PcpxP*-mNG or *PrpoE*-mNG reporter constructs to visualize (A) heat-shock, (B) Cpx and (C) σ^E stress respectively, were grown in M9 in a 96-well plate and exposed to 0.5xMIC of the indicated agents. mNG fluorescence was measured in time, corrected for growth (OD₆₀₀) and plotted as fold-change compared to untreated cells (set to 1, dashed line). (D) Cells were grown as described above and σ^E , Rcs, heat-shock and Cpx stress activation was measured in response to 2.5% ethanol using the reporter constructs as indicated, respectively. Error bars represent the standard deviation of duplicate samples. The figure shows a representative example of three independent experiments.

Table S1. Overview of the strains used in this study.

Plasmid	Description	Reference
<i>E. coli</i> TOP10F'	Cloning and expression strain	Thermo Fisher Scientific
<i>E. coli</i> MC4100	Cloning and expression strain	¹

Table S2. Overview of the plasmids used in this study.

Plasmid	Description	Reference
pUC66-RprA-GFPmut	P_{rprA} - <i>neongreen</i>	Matylda Zietek (EMBL)
pBAD22-DjlA	P_{BAD22} - <i>DjlA</i>	³
pABCON2-fhuA $\Delta C/\Delta 4L$	P_{OXB11} - <i>fhuA</i> $\Delta C/\Delta 4L$	⁴
pABCON2	Empty vector	⁴
pUA66-RpoE-mNG	P_{rpoE} - <i>neongreen</i>	⁵
pUA66-GroES-mNG	P_{groES} - <i>neongreen</i>	⁵
pUA66-RprA-mNG	P_{rprA} - <i>neongreen</i>	This study
pUA66-CpxP-mNG	P_{cpxP} - <i>neongreen</i>	This study
pEH3-Hbp	P_{lac} -hbp	⁶
pEH3-Hbp110C/348C	P_{lac} -hbp110C/348C	⁶

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