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Supplementary Information

Peptide-based efflux pump inhibitors of the small multidrug resistance protein from *Pseudomonas aeruginosa*

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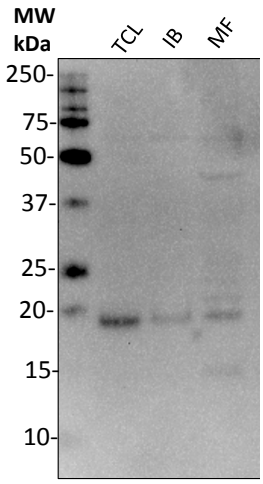
Methods

Membrane fractionation and immunoblot

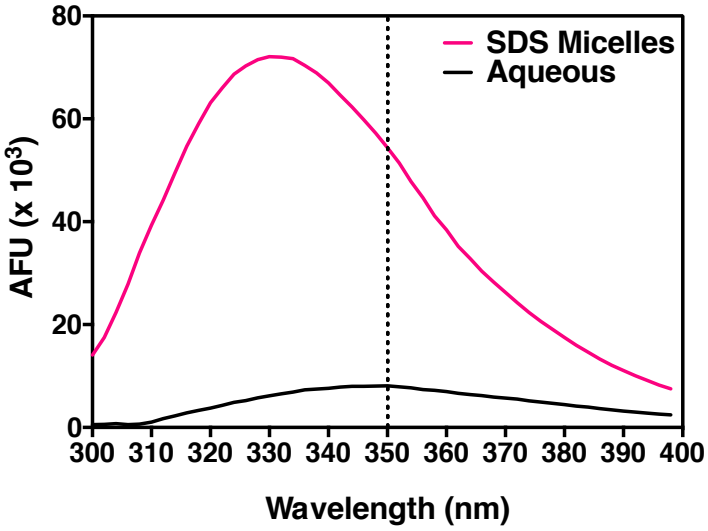
E. coli cells with cloned with His-Myc tagged PAsmr were grown in Luria-Bertani (LB) broth overnight. 1.5 mL of overnight culture was inoculated into 50 mL fresh LB. Cells were grown to an OD of 0.4, similar to conditions of the efflux assay, and were then grown for an additional 2 hrs. Cells were resuspended in lysis buffer (100 μg/mL lysozyme, 5 μg/mL DNAseI, and 1μM PMSF) and incubated on ice for 30 min, followed by sonication. Inclusion bodies were spun out at 17,000 rcf and were resuspended in 8 M urea. The membrane fraction was then spun out using an ultra-centrifuge, the supernatant was spun at 85,000 rcf at 4°C for 90 minutes. The pellets were then resuspended in lysis buffer. The total cell lysate (TCL), inclusion bodies (IB) and membrane fractions (MF) were separated on a 12% acrylamide gel, transferred to a PDVF membrane, and probed with an anti-His antibody.

Tryptophan fluorescence

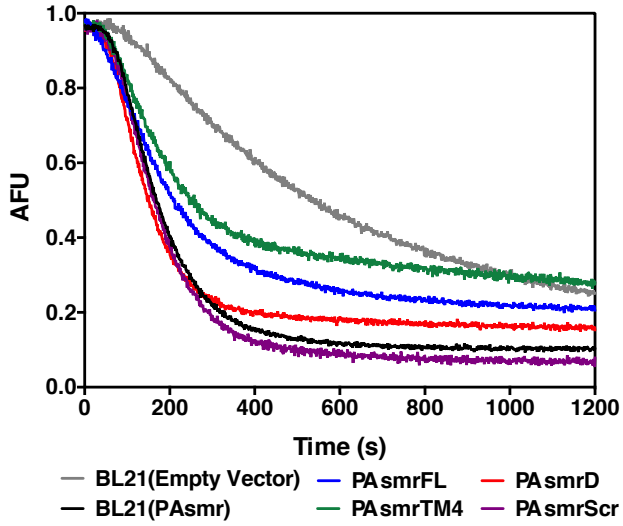
The spectrum for PsmrW was recorded on a Photon Technology International fluorimeter. The spectrum was recorded in an aqueous solution (5 μM peptide, 10 mM Tris, 10 mM NaCl, pH 7.2) and membrane-mimetic environment (5 μM peptide, 5mM SDS 10 mM Tris, 10 mM NaCl, pH 7.2). Tryptophan was excited at 280 nm and the emission was recorded for three separate samples at 300 nm – 400 nm with background subtracted.



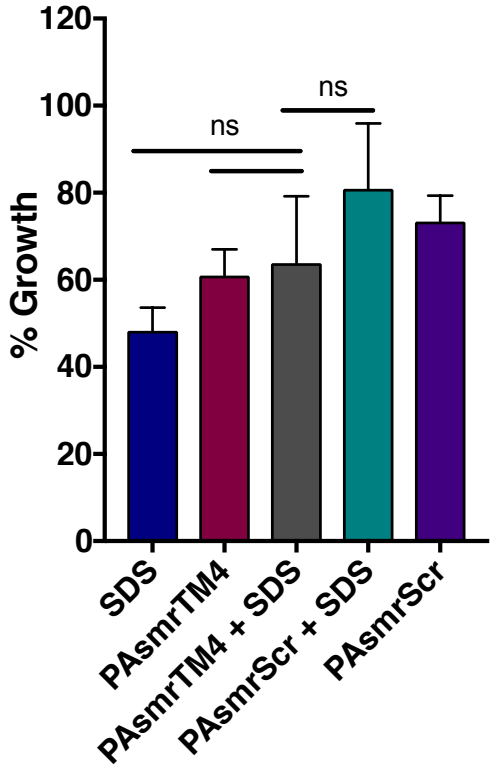
Supplementary Figure 1. Membrane fractionation and immunoblot analysis of PAsmr. *E.coli* cells with cloned PAsmr were grown for two hours after an OD of 0.4 was reached. Membrane fractionation was completed, inclusion bodies were resuspended in 8 M urea, and membrane fractions then were resuspended in lysis buffer. Total cell lysate (TCL), inclusion bodies (IB) and membrane fractions (MF) were separated on a 12% acrylamide gel and transferred to a PVDF membrane and probed with an anti-His antibody.



Supplementary Figure 2: Tryptophan fluorescence spectra of PsmrW. 5 μM of peptide was dissolved in 10 mM Tris-Cl buffer to represent an aqueous environment (black). 5 μM peptide in 10 mM Tris-Cl buffer and 5 mM SDS, 1:1000 peptide/detergent ratio, represents a membrane environment (pink). Peptide blue shift compared to emission maxima of free Trp in buffer is depicted as vertical dashed line at 350 nm (n = 3).



Supplemental Figure 3. Peptide mediated efflux inhibition of *E. coli* cells expressing PAsmr. *E. coli* cells expressing PAsmr were incubated with 1 μg/mL of EtBr and fluorescence decay was measured at 600 nm for 20 minutes. Baseline efflux was measured for *E. coli* cells alone (gray curve), and for those expressing PAsmr (black). Cells were then incubated with 4 μM PAsmrFL (blue), PAsmrTM4 (green), PAsmrScr (purple), and PAsmrD (red) (n = 3).



Supplemental Figure 4. BL21 cells expressing PAsmr do not resensitize to non-substrate sodium dodecyl sulfate (SDS) upon treatment with PAsmrTM4 peptide. Cells were treated with a sub-lethal dose of non-substrate detergent SDS as determined for *E. coli* cells expressing PAsmr (blue bar); for the PAsmrTM4 peptide alone (red); and for the PAsmrScr peptide alone (purple). Cells were plated with a combination of a sublethal dose of SDS (500 μg/mL) along with the addition of 2 μM of either PAsmrTM4 (gray), or 2 μM of PAsmrScr (turquoise). After 20 hr incubation under all conditions shown, the OD_{600} was measured to determine cell growth. No significance is indicated by ns (n = 3).