

## A potent protein antibiotic kills *Pseudomonas aeruginosa* by inhibiting the BAM complex.

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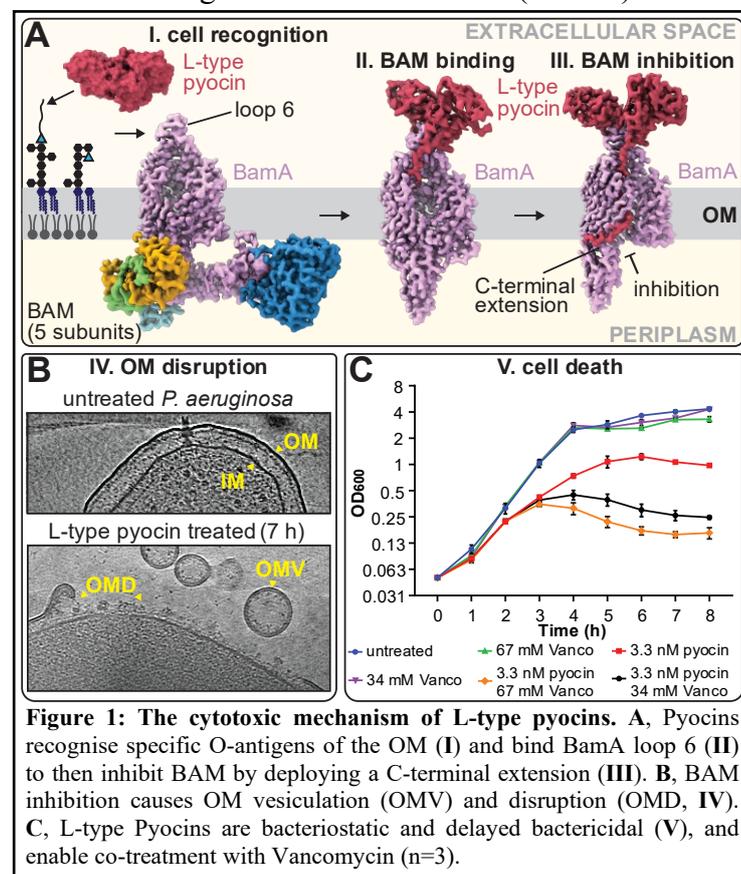
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*Pseudomonas aeruginosa* is a high-priority antibiotic-resistant pathogen with few new validated druggable targets. This is in part due to the Gram-negative bacterial outer membrane (OM), a permeability barrier for molecules >600 Da. L-type pyocins are potent protein antibiotics that selectively kill *P. aeruginosa*, but their mechanism of action has remained elusive. Here, we show with cryo-EM for the first time that L-type pyocins exploit a critical vulnerability in the OM by targeting the essential  $\beta$ -barrel assembly machinery (BAM) complex (Fig. 1A). We report the first structures of *P. aeruginosa* apo BAM (2.75 Å) and BAM in complex with L-type pyocins in two distinct binding states: Pre-inhibition (2.84 Å) and full inhibition (3.18 Å). Importantly, L-type



pyocins function without entering cells, making them immune to many bacterial defence mechanisms, such as efflux pumps. With cryo-ET, we show that BAM inhibition triggers envelope stress, leading to catastrophic loss of membrane integrity and cell death (Fig. 1B). Genetic and multi-omics approaches reveal that  $\beta$ -barrel assembly of OM proteins is halted and that intoxicated cells employ competitive self-preservation strategies, for example by upregulating the type VI secretion system. Lastly, the treatment of *P. aeruginosa* with L-type pyocins makes cells susceptible to co-treatment with the antibiotic Vancomycin, which cannot overcome a healthy OM (Fig. 1C). These findings provide key insights into the L-type pyocin cytotoxic mechanism and the cellular consequences of BAM inhibition, validating the BAM complex as a high-value therapeutic target and informing future antibiotic development.