The small multidrug resistance (SMR) family contains the smallest membrane transport proteins found in both bacteria and archaea. The family is functionally dominated by two major subtypes: the quaternary ammonium compound subtype (Qac) which transports a chemically diverse range of biocides, and the guanidinium exporter subtype (Gdx) which selectively transports the essential bacterial metabolite, guanidinium. Using high throughput mutagenesis, directed evolution, electrophysiology, and cell resistance assays, identified key molecular determinants of the specificity differences between the Gdx and Qac subtypes.