

Table S1: Flux studies

Compound	K ⁺ Fluxed (%)	Glutamate Fluxed (%)
011	17*	ND
011A	26**	14*
120	31**	ND

* P<0.05; ** p<0.01; ND not detectable

Methods:**Steady State Glutamate.**

The MJF612 strain was used carrying either the pb10d empty vector or expressing WT Eco-MscL in the same pb10d vector. Overnight cultures were inoculated from a single colony in K10 media. The next day cultures were diluted 1:100 in the same K10 media and expression was induced with 1 mM IPTG for 1 hour after an OD₆₀₀ of 0.2 was reached. The cultures were then split in two; mock (DMSO only) or treated with compounds 120 at 5 μm, 011 or 011A at 40 μm with a final DMSO concentration of 0.9% for all samples. Cultures were grown overnight for 17 hours in a 37°C shaker when the final OD₆₀₀ was recorded. For each sample 6 ml was pelleted. Pellets were brought up in the same media adjusting volume for OD and sonicated for 1 min. Glutamate measurements were performed using the EnzyChrom Glutamate Assay Kit (BioAssay Systems, Hayward, CA, US) per the manufacturer's instructions. Viability assays were done in conjunction n = 3.

Steady State K⁺.

The MJF612 strain was used for the K⁺ steady state experiments carrying either the pb10d empty vector or expressing WT Eco-MscL in the same pb10d vector. Overnight cultures grown in K10 media from a single colony were diluted 1:25 into the same media, grown to an OD₆₀₀ of 0.2, when expression was induced with 1 mM IPTG for 1 hour. Cultures were then split into; mock (DMSO only), or treated with one of the three compounds 120 at 5 μm, 011 at 40 μm or 011A at 40 μm, with a final DMSO concentration of 0.9%. Cultures were then grown for 17 hours in a 37°C shaker. Final OD₆₀₀ was then recorded and the amount of cells to be used was adjusted for OD. Typical OD₆₀₀ values ranged from 1.0 to 1.3 and approximately 950 μl-1.2 ml of the cultures were passed through a 0.45-μm filter and washed with K0 media (K10 with the KCL replaced by NaCl) having the same osmolality, without ampicillin or supplements. Filters were placed in a plastic beaker, covered with foil and dried in an 80°C oven overnight. The next day 3 ml of double distilled water was added to each beaker and K⁺ was measured using a Jenway flame photometer (Cole-Palmer, East Norwalk, CT) n = 3.

Table S2: Binding free energies between MscL and 011A, all energies in kcal/mol

E _{vdw}	E _{EEL}	ΔG_{pol}^{PB}	$\Delta G_{nonpolar}^{SAS}$	TΔs	ΔG _{Binding}
-30.59 ± 0.26	-4.61 ± 0.35	9.21 ± 1.02	-2.18 ± 0.00	-17.85 ± 0.03	-10.32