

# A novel method to induce solvent tolerance in bacteria

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*Biobutanol has vast potential as a drop-in alternative to fossil fuel (1, 2). Butanol can be produced biologically by Clostridium beijerinckii and Clostridium acetobutylicum although biological production is uneconomical due to low process yields which account for high recovery costs by distillation (2-4). Poor microbiological solvent tolerance underlies poor process yields in biological solventogenesis, including butanol production. Solvents are known to compromise the integrity of biological membrane, accordingly research endeavours to develop solvent tolerant microbes have focused on shoring up membrane integrity in instances of solvent challenge using engineering, metabolic and adaptive approaches (2, 5-10).*

*Membrane Insertion Molecules (MIMs) are molecules that modify cell function or stability by intercalating into the phospholipid bilayer of microbial membranes. There are two types of MIM; those designed to act as antimicrobial agents, killing cells by destabilizing the lipid bilayer, and enhancement MIMs, which are designed to improve cell function (11).*

*We show that a proprietary MIM can increase butanol tolerance in E. coli K12 which, when treated, can outgrow an untreated control in concentrations up to 3.5% v/v butanol. Using a combination of molecular dynamic modelling and single molecule tracking in model membranes treated with MIM<sub>ST</sub>, along with membrane leakage and cell/butanol partitioning assays, we propose a mechanism for how MIM<sub>ST</sub> treatment may increase solvent tolerance in microbes. We discuss the benefits and shortcomings of this technique and the relationship between solvent tolerance and solvent yield in solventogenesis.*

**Keywords**—butanol, tolerance, biofuels, membranes, modification

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