MeMBrane: MEmbrane Modulation for BiopRocess enhANcEment

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The global economy has an unsustainable dependence on fossil materials with demand for raw material inputs to industry growing steadily. Concerns about environmental sustainability are becoming more acute; thus, alternatives to traditional, fossil-fuel based chemical production are urgently required. Cell factories, which use microorganisms to produce materials from renewable biomass, are an attractive alternative, and an increasing number of platform chemicals are being produced at industrial scale using engineered microorganisms. These are expected to have a transformative impact in industrial biotechnology, but, first, we must meet the challenges of designing and optimizing high-yield cell factory strains that can produce commercially viable amounts of product. One reason for poor product output is that the production conditions are ultimately toxic to the producing cells. In addition to damage to intracellular components such as enzymes, the lipid cell membrane and associated proteins are vulnerable to biomolecules e.g. ethanol and propionate, as well as to physical parameters during production such as osmotic stress, pH, and temperature. An approach whereby membranes can be “tuned”, in terms of their lipid and protein content, to become more resistant to stresses brought about by toxicity would revolutionise the field. Additionally, expression of efficient membrane transporters to export ‘toxic’ products can mitigate intracellular damage. These approaches will ultimately enable production of higher concentrations of the desired molecules or cells making the bioprocesses more efficient, increasing product yield,

reducing cost, and help to drive the move away from fossil-based raw materials. An adoption of such “green” processes and avoidance of depletion of non-renewable carbon sources will bring huge social and environmental benefits. Products and processes which are currently economically unviable due to toxicity can be rendered profitable by even small increases in the resistance of strains and concomitant yield increases.

This collaborative project will ultimately engineer robust cell factories (yeast and *Propionibacterium*) that overcome existing toxicity challenges, improve efficiency and allow their effective commercialization following demonstration at pilot scale. The strategies developed within this project will be applicable across the sector to facilitate rational strain engineering with far-reaching benefits.

The project is divided into seven interconnected, iterative work packages (WPs) with a well-established build-test-analyse approach. Initial analysis of –omics data will identify key alterations in membrane protein and lipid content of both microbes subjected to stresses associated with bioproduction and those strains known to be somewhat resistant to such stresses (WP1). *In vitro* and in *silico* approaches will be used to rapidly delineate the roles of these alterations and rationally design more resistant membranes (WP2). Using synthetic biology and strain evolution approaches, we will alter the membrane composition of microbes to reflect the “optimal” membranes determined in WP2 (WP3). Optimal strains will be identified in a high throughput manner and subjected to large-scale testing to ensure that the changes made translate to the industrial setting (WP4). Following this, another iteration of the cycle will further optimise the strains. WP5 will evaluate the environmental and social sustainability of the innovative production processes and the final products. WP6 will develop and implement a strategy for the dissemination and exploitation of research results to different stakeholders. WP7 involves consortium management, project governance, communication activities and administrative oversight to ensure maximum impact of the project.