# Improving Bioprocess Robustness by Cellular Noise Engineering

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**Project Goals: This project sets out to develop an integrative workflow combining genome-wide editing, multi-omic and single-cell analyses, and computational models to enable cellular noise engineering and, thus, the design of robust biological systems that maintain function beyond standard laboratory conditions. In this context, cellular noise engineering bestows a non-genetic division of labor, where distinct subpopulations express pathways that maximize function under key environmental conditions. Specifically, the project focuses on improving the robustness of cellulosic oil and alkane production in *Yarrowia lipolytica* under genuine industrial conditions of time-varying toxic lignocellulosic hydrolysate inhibitor concentration and temperatures. To this end, our approach involves *Y. lipolytica* evolution, multi-omics and single-cell analyses, and construction of genome-wide kinetic models that will guide the noise engineering efforts.**

Growing global energy demands, environmental concerns, and the need to reduce dependency on decreasing fossil fuel reserves drive the pursuit of identifying alternative energy sources. The trucking, shipping, and aviation industries require heavy liquid fuels that can presently only be supplied by fossil fuels. Alternative forms of heavy fuels, such as microbial oils and alkanes, are gathering increasing interest; however, despite the increasing efficiency of microorganisms, such as *Y. lipolytica*, in converting sugars to microbial oils, the cost of glucose feedstocks is still very high. Lignocellulosic plant matter represents an inexpensive feedstock with an ample supply that does not compete with food resources. However, lignocellulosic biomass pretreatment increases not only the amount of released sugars but also the levels of toxic byproducts. Specialized methods to reduce the resulting toxicity levels come at high costs and processing complexity. Recently, we reported a more cost-effective and scalable approach to reduce hydrolysate toxicity by engineering the fermenting microorganism to reduce furans to furan alcohols[1], for which tolerance strategies are already available[2].

Our project aims to develop a similar phenotype in *Y. lipolytica* that can tolerate common lignocellulosic hydrolysate inhibitors, as well as efficiently produce cellulosic oils and alkanes by harnessing all available hydrolysate sugars. Our approach involves evolving *Y. lipolytica* to obtain variants that can tolerate, grow, and efficiently synthesize biofuel precursors under steady-state, albeit stressful conditions pertaining to both toxic hydrolysates and high temperatures. Multi-omics analyses of the evolved strains will inform about the dynamic control of key genes required for coping with varying stressful conditions and aid the construction of genome-wide metabolic kinetic models. These models will guide the selection of gene targets for engineering noise, which we will accomplish by replacing the native promoters of these genes with synthetic ones that confer specific noise levels. Testing in programmable microfluidics will validate the effects of noise on robustness before undertaking similar validation efforts in bioreactors under industrially relevant conditions for producing cellulosic oils and, after further adaptation, cellulosic alkanes[3]. Overall, we anticipate that strains exhibiting optimal levels of cellular noise will also demonstrate robustness that maintains production under time-varying stresses and both laboratory and simulated industrial-scale conditions.

**References**


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3. Li, J. *et al.* Synthesis of high-titer alka(e)nes in *Yarrowia lipolytica* is enabled by a discovered mechanism. *Nat. Commun.* **11**, 6198 (2020).



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