Directed evolution of a protein-based sensor for anaerobic biological methane activation

Abstract

We have engineered an *Escherichia coli* strain capable of anaerobic activation of short-chain alkanes (C3-C6) via fumarate addition. The resulting alkylsuccinate products can be metabolically converted into building block metabolites for the biosynthesis of biofuels or other value-added chemicals. Such bioprocesses offer opportunities for small scale deployment with drastically reduced CapEx, as compared to other GTL technologies. While fumarate addition to methane is biologically feasible and believed to occur in nature, no corresponding enzymes have been identified. With the aim of engineering methane activation by an alkylsuccinate synthase, resulting in production of the intermediate methylsuccinate, here I propose to design a sensor/reporter system that will enable high-throughput screening for fumarate addition to methane. Regulatory protein ItcR naturally relieves transcriptional repression in response to methylenesuccinate (itaconic acid). Despite the subtle difference between methylsuccinate and methylenesuccinate (methyl vs methylene group), ItcR displays 100-fold reduced response to methylsuccinate. Using directed evolution we will evolve ItcR variants with progressively increasing sensitivity to methylsuccinate. The result of this work (a sensor/reporter of methylsuccinate production) will provide a powerful tool to advertise while proposing research to engineer an anaerobic, biological process for methane conversion to fuels and chemicals via fumarate addition.