Abstract for Public Release

Replacing high volume petroleum fuels with renewables could have a significant environmental impact even as energy consumption is projected to increase 28% by 2040. Replacing petroleum fuels is economically challenging as cost is critical in a high volume, low margin market—a challenge that has so far stymied most "New to Market" renewable fuels. Achieving stringent cost targets requires both extremely abundant, inexpensive biomass feedstocks and robust conversion into fuels with energy efficient product separation. So far, microbial conversion approaches for 2nd generation biofuels have not been able to achieve the stringent production parameters required to match the low petroleum fuels costs. Invizyne has successfully demonstrated a cell-free approach that uses enzymes to efficiently convert both pure and cellulosic sugars into isobutanol without having to worry about maintaining life processes. In this project, Invizyne will demonstrate the potential for economic and energy efficient cell-free production of the biofuel isobutanol by developing an optimized cell-free process that utilizes yellow dent corn starch coupled to energy efficient *in situ* separation and isolation of product, enabling the sustained production of isobutanol at high productivity over many days. Upon completion of this project, we expect to demonstrate a working prototype system that can be deployed at scale to provide a commercially competitive biofuel. Our ambitious targets are feasible and can lead not only to biofuels, but provide an alternative, viable route to cost-effective production of many other low value/high volume chemicals that compete with petroleum products. Success of the project will demonstrate that cell-free biomanufacturing is an important option that complements or replaces metabolic engineering, redefining the possibilities for bioconversion technologies.