



Application of synthetic biology to bioregenerative life support for human spaceflight

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ABSTRACT

The conversion of carbon dioxide into higher value products is a key challenge for the development of closed-loop life support systems for human space flight. Much of the past research on bioregenerative life support systems has focused on plant growth chambers as a solution for CO₂ removal and O₂ generation, but photosynthetic microorganisms may also have a role to play in these functions. Cyanobacteria have the advantages of relatively high CO₂ fixation rates and fairly well-developed molecular biology tools, allowing for genetic engineering approaches to strain improvement.

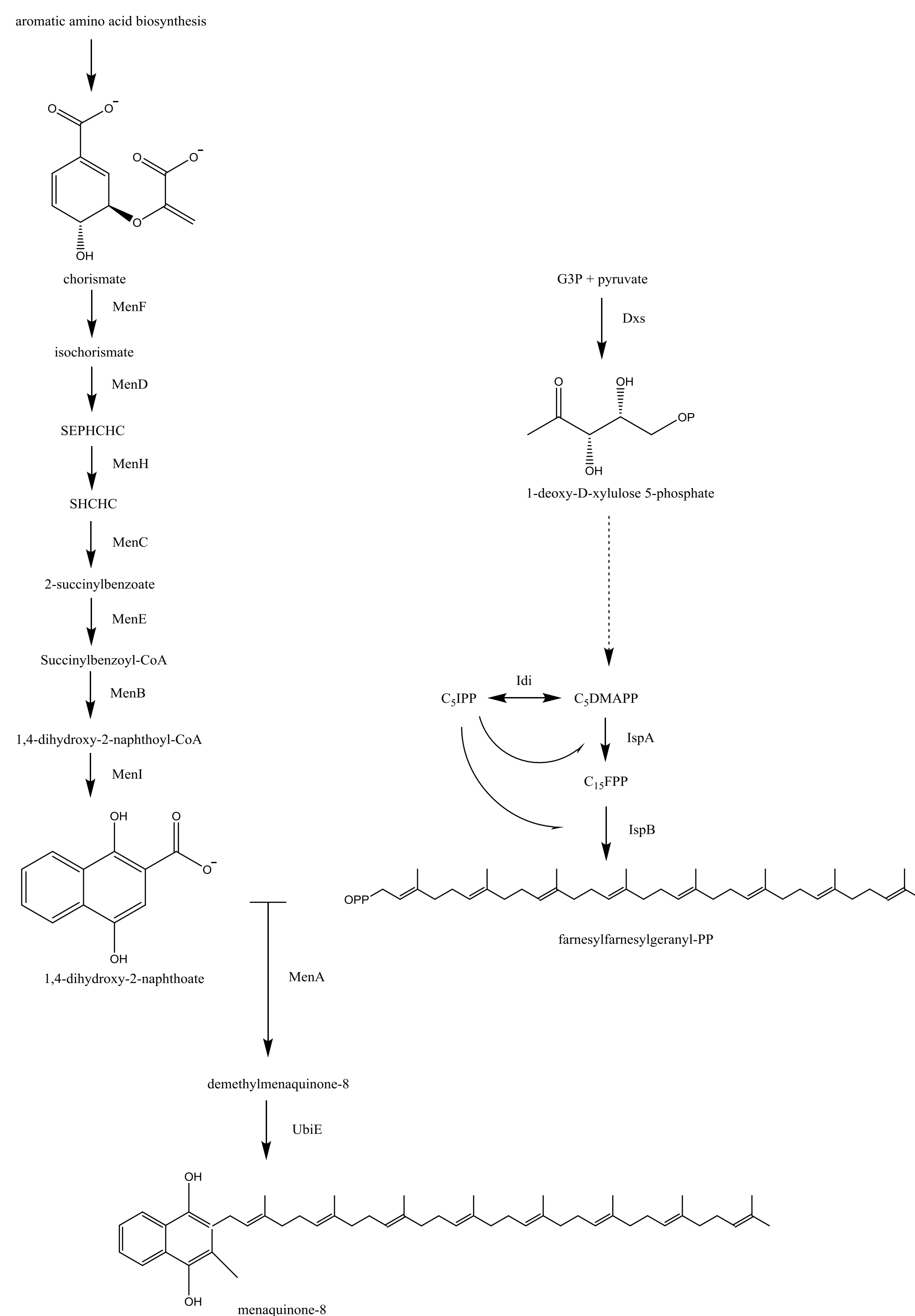
Manned missions to Mars or other targets beyond low Earth orbit will require advances in the nutritional systems for life support on these longer duration missions. A key challenge will likely be supplementing pre-packaged meals with specific nutrients that will be deficient due to problems in long-term storage or low abundance. Vitamin K is one such nutrient that may be important as a supplement. Production of vitamin K for nutrient supplementation during spaceflight will likely require genetic engineering of microorganisms to increase vitamin titers.

A microbial bioreactor system that could efficiently convert CO₂ to nutritional supplements would be a valuable component for a future advanced life support system. We are exploring biological systems to determine the feasibility of using bioreactors to convert CO₂ to higher-value products. We are examining the performance of photosynthetic bacteria engineered to produce sugars, determining rates of production and reliability. We are also engineering microbes to produce higher titers of vitamin K and other potentially important nutrients. The results of this research will offer demonstrations of potential technologies that could be developed further in the future. This work will also provide valuable information for understanding basic science questions about the use of genetically engineered microbes in the microgravity environment.

BACKGROUND

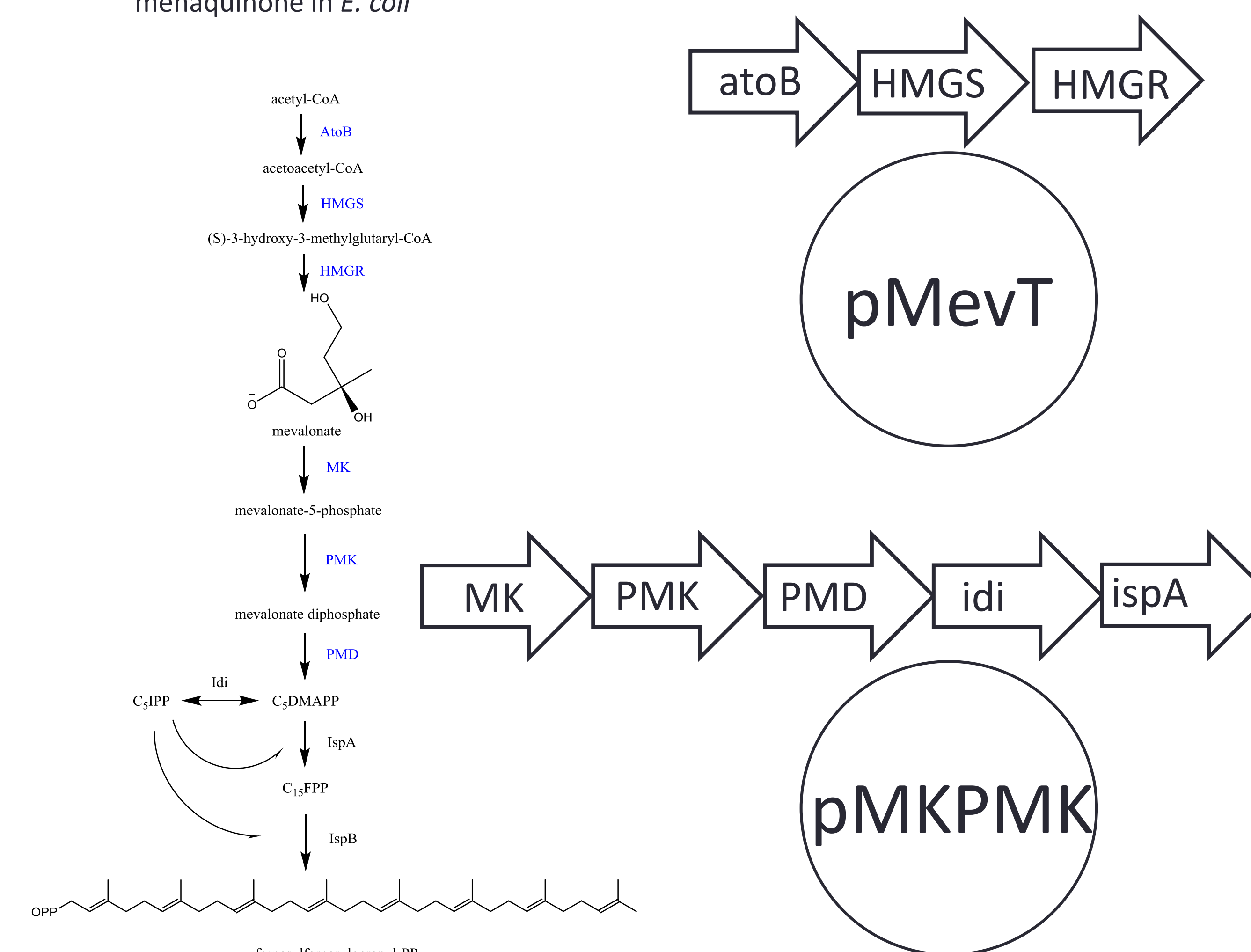
Menaquinone biosynthesis in *E. coli*

Menaquinone (Vitamin K2) is naturally synthesized by *E. coli*, functioning in the electron transport chain during anaerobic respiration.



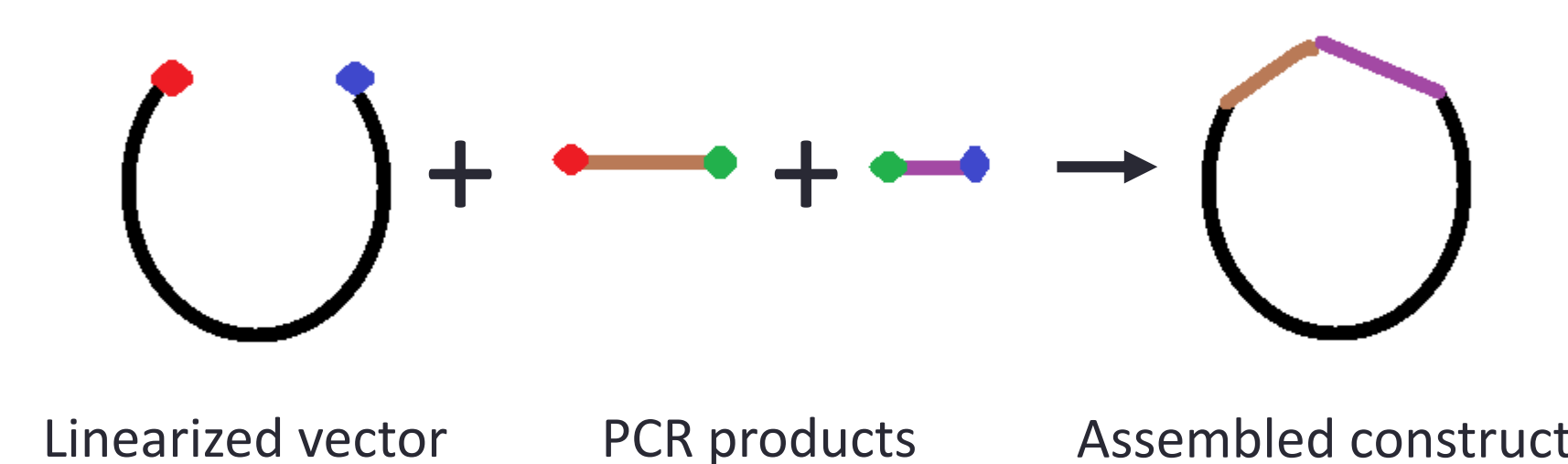
PROPOSED DESIGN

- Replace native *E. coli* isoprenoid pathway with mevalonate pathway
 - The Keasling lab at UC Berkeley has successfully engineered the mevalonate pathway into *E. coli*
 - This modification allowed for increased production of the isoprenoid, amorphadiene, a precursor of the malaria drug artemisinin
 - Using similar strains *should* allow for increased production of the isoprenoid portion of menaquinone in *E. coli*



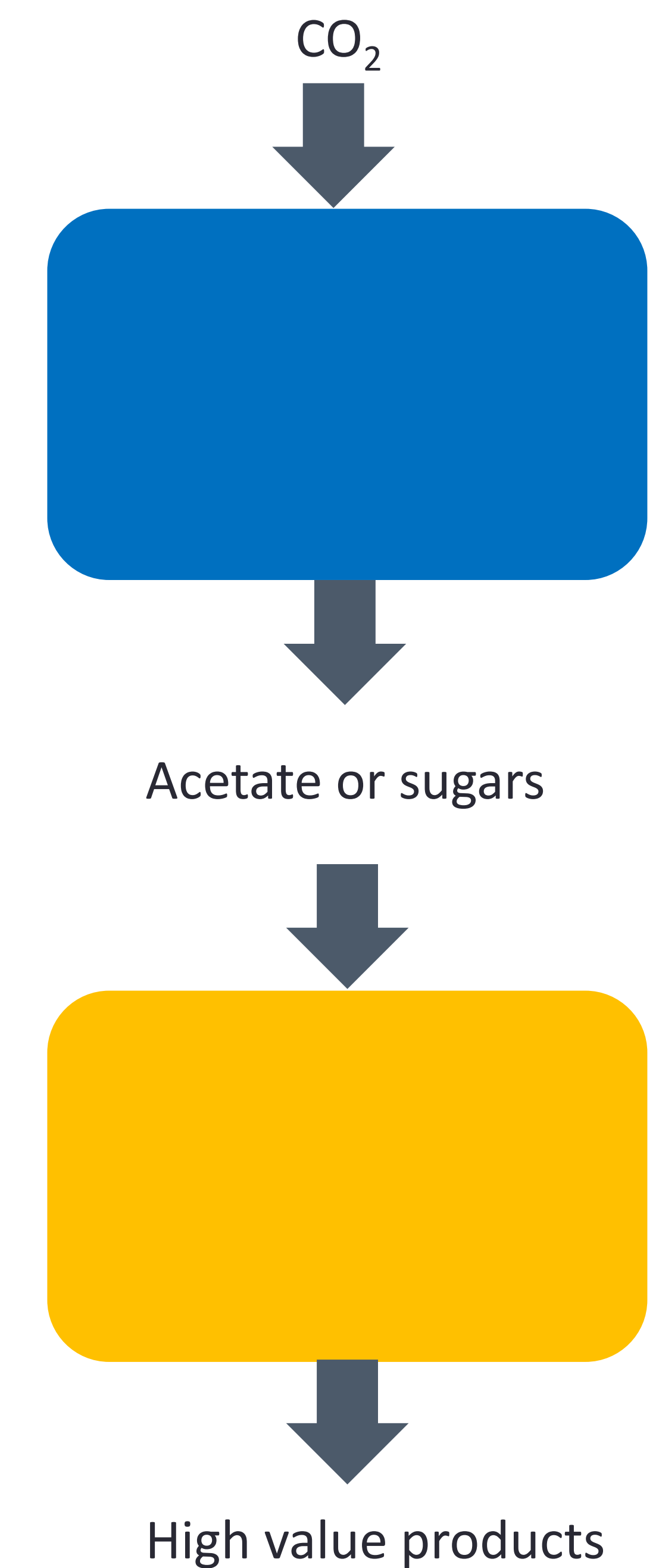
- Overexpress the *men* genes in *E. coli* engineered with mevalonate pathway
 - A plasmid will be constructed with the 8 *men* genes plus *ubiE*
 - Golden Gate cloning methodology will be used, allowing scarless, multi-part DNA assembly
 - The expression levels of both parts of the menaquinone pathway will be tuned to maximize production

5'GGTCTCN'NNNNN3'
3'CCAGAGNNNNN5'
BsaI site



APPLICATION TO LIFE SUPPORT

- A microbial bioreactor containing engineered organisms producing essential nutrients such as vitamin K could be used as part of a modular bioreactor system converting CO₂ to higher value compounds
- The first autotrophic bioreactor would convert CO₂ to a feedstock compound
 - Acetogens have been described that can convert CO₂ to acetate in a bioelectrochemical system
 - Engineered cyanobacteria have been described that convert CO₂ to secreted sugars, such as glucose or fructose, photosynthetically
- The second bioreactor would convert the feedstock chemical to higher value compounds
 - Nutrients like vitamin K or lutein
 - Other products – bioplastics?



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