The development of leucine consuming strains as therapeutics for Maple Syrup Urine Disease

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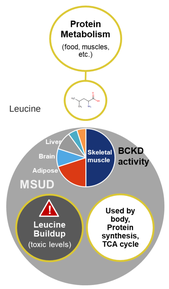
Abstract

MSUD is caused by a defect in the multi-enzyme complex called branched-chain alpha-ketoacid dehydrogenase complex (BCKDC) responsible to catalyze the oxidative decarboxylation of branched-chain amino acids (BCAA) leucine, isoleucine and valine in humans. Defects in BCKDC in MSUD result in the abnormal accumulation of all three BCAAs along with their various byproducts throughout the body causing poor feeding, vomiting, dehydration, lethargy, hypotonia, seizures, hypoglycemia, ketoacidosis, pancreatitis, coma and neurological decline. To date, the only existing treatments are dietary restriction and liver transplantation; no approved therapy exists, resulting in a significant unmet medical need. Synlogic is using its proprietary engineering technology to produce engineered bacteria (Synthetic Biotic™ medicines) capable of rapidly degrading BCAAs in the gut, thereby preventing their toxic accumulation.

Ginkgo Bioworks sought to improve Synlogic's probiotic bacterial therapeutic for MSUD by optimizing the three-step conversion of leucine to isopentanol in Synlogic's chassis strain. Ginkgo's Foundry uses software and automation to support genetic engineering at an unprecedented scale. Over the course of dozens of organism engineering programs, Ginkgo has also developed an unmatched genetic Codebase of re-usable biological data and physical assets that provide many diverse routes to success.

The collaboration between Synlogic and Ginkgo Bioworks resulted in a strain with greater-than-10 fold improvement of *in vitro* BCAA consumption, with scalability up to 3 Liter fermentor, as compared to the prototype strain. Characterization using *in vitro* gut simulating system and healthy non-human primates also showed significant improvements versus the prototype strain.

MSUD – toxic amino acid accumulation resulting in severe health impacts



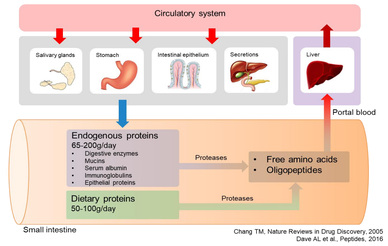
Maple Syrup Urine Disease (MSUD):

- Overall incidence 1:185,000 (higher in certain population e.g. Mennonites 1:380)*
- Genetic defect in multi-enzyme complex found in mitochondria called branched chain α-ketoacid dehydrogenase "BCKDC"
- BCKDC mutations prevent the decarboxylation of the α-ketoacids of branched-chain amino acids (BCAA: leucine, isoleucine and valine) causing them to accumulate
- Symptoms: include poor feeding, vomiting, dehydration, lethargy, hypotonia, seizures, hypoglycemia, ketoacidosis, pancreatitis, coma and neurological decline
- Current treatment limited to dietary restriction and liver transplantation

* Genetics Home Reference *www.ghr.nlm.nih.gov*

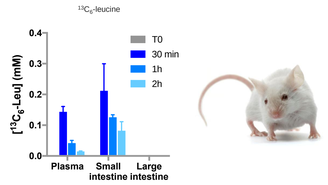
The gut can access the circulating leucine pool

Schematic of mechanism

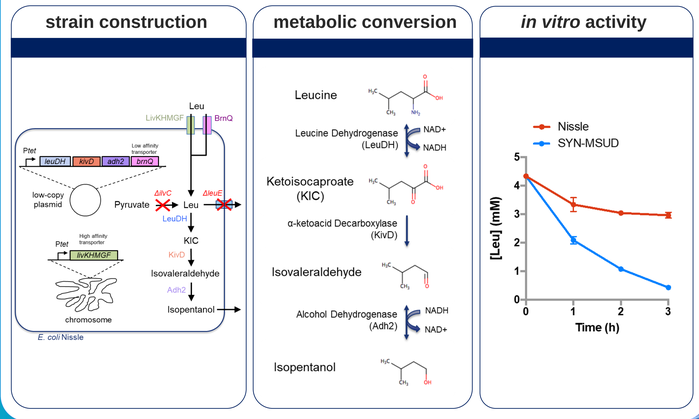


***In vivo* verification of enterorecirculation**

- Subcutaneous injection of ^{13}C-labeled leucine in mice (0.1mg/g BW)
- Early appearance of labeled leucine in the intestinal lumen after injection demonstrates the rapid recirculation of the amino acid into the GI tract



Prototype strain consumes BCAA *in vitro*



BCAA-consuming pathway optimization

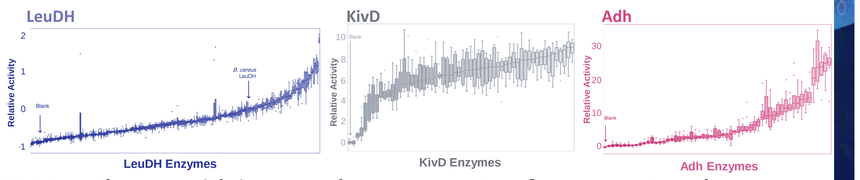
Superior enzymes were identified by screening large metagenomic libraries

To improve Synlogic's prototype leucine-consuming BCAA pathway, Ginkgo Bioworks sought to identify LeuDH, KivD, and Adh pathway enzymes with superior activity relative to the prototype pathway enzymes found in the SYN1980 pathway.

Ginkgo built a library of ~1,300 enzyme variants for each pathway enzyme using metagenomic sourcing (see Sequence similarity networks). Hits were selected from each ~1,300-member through two rounds of *in vitro* enzyme screening.

Enzyme sequence similarity networks demonstrate diversity of each enzyme library: *Each spot represents an amino acid sequence, and the distance between spots indicates the relatedness.*

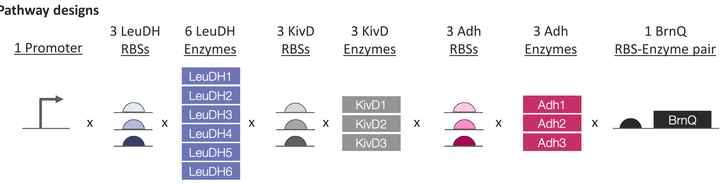
Enzyme activity measured in high throughput: *The top enzymes from the initial screen were re-screened to rank enzymes by activity, and the data are shown below. Enzyme activity is reported relative to the activity of the prototype pathway enzyme. The prototype KivD and Adh enzymes did not exhibit activity greater than the background lysate activity, so KivD and Adh enzyme activities are reported relative to the non-zero background lysate activity.*



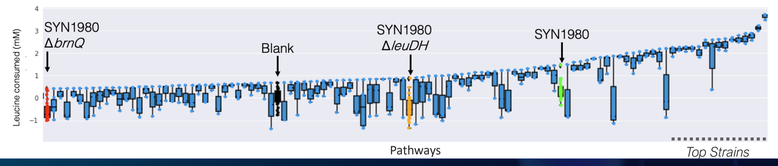
BCAA pathways with improved enzymes outperform parent pathway

Ginkgo selected superior enzymes from each library to design ~500 full BCAA pathway variants. Enzyme identity and enzyme expression were varied in the partial combinatorial library. The order of the enzymes and the BrnQ transporter were held constant. The pathway library was transformed into Synlogic's BCAA pathway chassis and screened for Leu consumption. The top pathways were further analyzed by Synlogic.

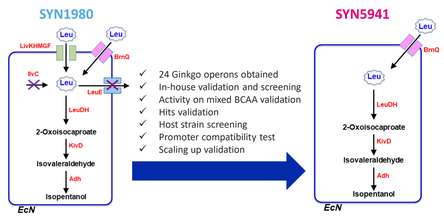
Pathway designs



Pathway screening: *Pathways were screened for Leu consumption in triplicate and ranked according to the highest single replicate. The prototype pathway (SYN1980) and variants of SYN1980 were included as controls.*

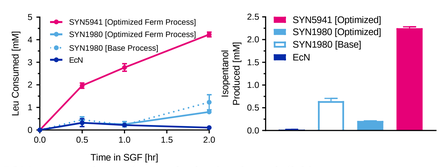


Leucine consumption is improved with optimized strain



- ✓ 24 Ginkgo operons obtained
- ✓ In-house validation and screening
- ✓ Activity on mixed BCAA validation
- ✓ Hits validation
- ✓ Host strain screening
- ✓ Promoter compatibility test
- ✓ Scaling up validation

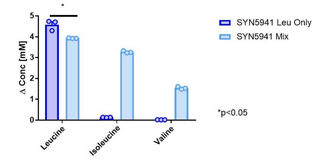
Leucine consumption in simulated gastric fluid system (7% to 4% O_2)



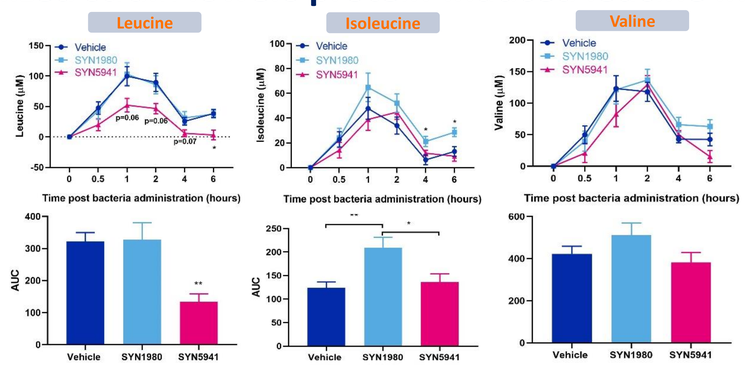
***In vitro* leucine consumption rates of fermentation materials**

Strain	Leucine Consumption Rate μmol/10^9cells/h
	Optimized PD Process (normalized by CFU)
SYN5941	**2.69**
SYN1980	**0.17**

BCAA consumption preference in simulated gastric fluid system

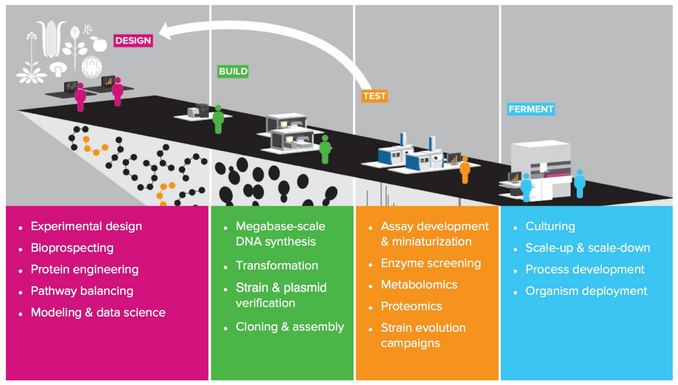


Optimized strain lowers protein-induced leucine in NHP



To assess the efficacy of SYN1980 and SYN5941 in healthy non-human primates, monkeys received a single dose of vehicle or bacteria concomitantly with the meat digest peptone. Branched-chain amino acid levels in serum peaked approximately 1 hour post-peptone administration in vehicle-treated animals to 99.7 ± 16.5 μM, 47.7 ± 9.3 μM and 123.2 ± 21.4 μM, respectively. SYN1980 did not significantly lower the levels of leucine, isoleucine or valine in this model, but a trend was observed with SYN5941 to lower leucine levels, with a statistically significant decreased area under the curve for this metabolite

Organism engineering at Ginkgo Bioworks



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In this collaboration, Ginkgo leveraged this platform to design, build, and test new component parts (pathway enzymes) to improve independent steps of the branched-chain amino acid (BCAA) pathway.

Conclusion

MSUD is a rare metabolic disease caused by the accumulation of BCAAs with no therapeutic options available. Screening of large metagenomic libraries resulted in the identification of an optimized strain with greater potential for BCAA consumption. Indeed, the resulting optimized strain, SYN5941, demonstrated improved BCCA consumption *in vitro*, and translational studies in healthy non-human primates, resulted in enhanced activity over the prototype strain. The pilot process development work indicated that process changes could further improve *in vitro* potency of the strain. Collectively, this work suggests that, with further optimization, an engineered bacteria consuming BCAAs could become a viable approach to treat MSUD.