

# Activity of SYN1353, an Investigational Methionine-Consuming Synthetic Biotic Medicine, in an Acute Nonhuman Primate Model of Homocystinuria



Mylene Perreault<sup>1</sup>, Erik Gerson<sup>1</sup>, Jillian Means<sup>1</sup>, Michael James<sup>1</sup>, Ted Moore<sup>2</sup>, Analise Reeves<sup>1</sup>, David Lubkowicz<sup>1</sup>, and David Hava<sup>1</sup>

<sup>1</sup>Synlogic Inc, Cambridge, MA, USA <sup>2</sup>Ginkgo Bioworks Boston, MA, USA.

## Introduction

Homocystinuria (HCU) is a recessive inherited disorder caused by a defect in cystathionine  $\beta$ -synthase (CBS), which results in abnormal methionine metabolism and leads to an accumulation of homocysteine (Hcy) in the body (Figure 1). Elevated Hcy levels are associated with impairments of the eye, skeletal system, vascular system, and central nervous system. In patients with residual CBS activity (~50% of HCU population), vitamin B6 (pyridoxine) is effective at reducing Hcy levels. For pyridoxine unresponsive patients, betaine (involved in remethylation of Hcy to methionine) and a low-methionine diet that is very low in natural protein are the current therapeutic options. Early initiation of a low-methionine diet significantly lowers the risk of developing complications in HCU patients, but compliance to low protein diet is difficult.

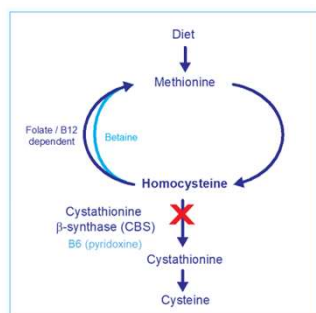


Figure 1. In HCU patients, mutations in the CBS gene result in accumulation of Hcy. Pharmacotherapeutic options for the treatment of HCU consist of vitamin B6 (pyridoxine), which can lower Hcy levels in B6-responsive patients, and betaine, which is involved in Hcy remethylation to methionine.

## Study Design

The probiotic *E. coli* Nissle (EcN) was engineered to metabolize methionine within the gastrointestinal (GI) tract via the methionine decarboxylase (MetDC) pathway (Figure 2). Using proprietary codebase and metagenomic libraries, combined with protein engineering strategies, MetDC from *Streptomyces sp. 590* and methionine importer MetP from *Flavobacterium segetis* were identified by metagenomic screen and MetDC was further optimized via protein engineering. Genes encoding these proteins were chromosomally integrated under the control of a chemically inducible promoter,  $P_{lac}$ , which is induced by isopropyl  $\beta$ -D-1-thiogalactopyranoside (IPTG). To prevent the release of methionine in the GI tract once it enters the cell, the *yjeH* gene that encodes a methionine/branched chain amino acid exporter was deleted. The resulting strain, SYN1353, converts methionine to carbon dioxide ( $CO_2$ ) and 3-methylthiopropylamine (3-MTP), which is used as a biomarker of strain activity.

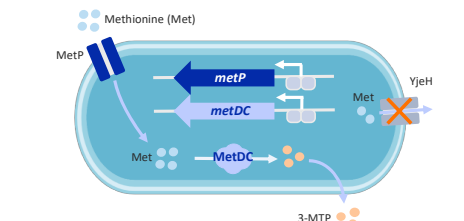


Figure 2. Schematic of engineered *E. coli* Nissle SYN1353 with its components. Optimal metP and metDC were identified using proprietary metagenomic, codebase and protein engineering libraries.

## Results

### Engineered EcN SYN1353 consumes methionine and produces 3-MTP *in vitro*

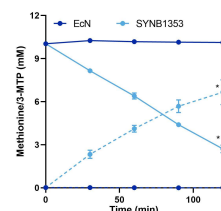


Figure 3. *In vitro* methionine consumption (solid line) and 3-MTP production (dotted line) by EcN (control bacteria) or SYN1353. Cells were incubated for the indicated time with 10 mM methionine at 37°C, and supernatants were collected. \* $p < 0.05$  versus EcN.

### An oral methionine load increases plasma methionine (A) and plasma total Hcy (B) in healthy cynomolgus monkeys

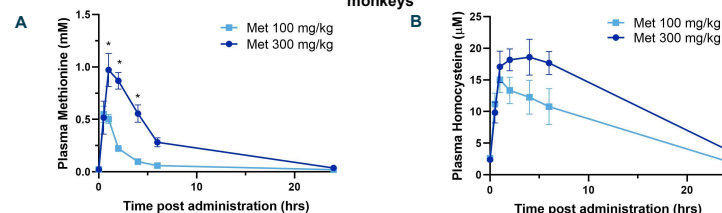


Figure 4. Monkeys were fasted overnight and received a single oral dose of methionine (100 or 300 mg/kg). Blood was collected for methionine and Hcy measurements. Data presented as mean  $\pm$  SEM (n = 6/group). Statistical analysis was performed using two-way repeated ANOVA with Sidak's multiple comparison test. \* $p < 0.05$  versus 100 mg/kg methionine.

### SYN1353 is active in a nonhuman primate model of acute homocystinuria

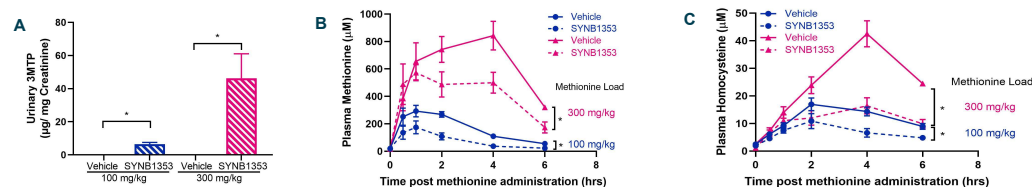


Figure 5. Monkeys were fasted overnight and received a single oral dose of methionine (100 or 300 mg/kg) with vehicle or SYN1353 ( $1 \times 10^{12}$  live cells). Urine was collected for 3-MTP, and blood was collected for methionine and Hcy measurements. Data presented as mean  $\pm$  SEM (n=12/group for 100 mg/kg methionine, n=6/group for 300 mg/kg methionine). Statistical analysis was performed using unpaired t-test with Welch's correction (A) and two-way ANOVA with Sidak's multiple comparison test (B-C). \* $p < 0.05$ .

### SYN1353 dose-dependently increases urinary recovery of 3-MTP (A) and decreases plasma methionine (B) and plasma homocysteine (C) in a nonhuman primate model of acute homocystinuria

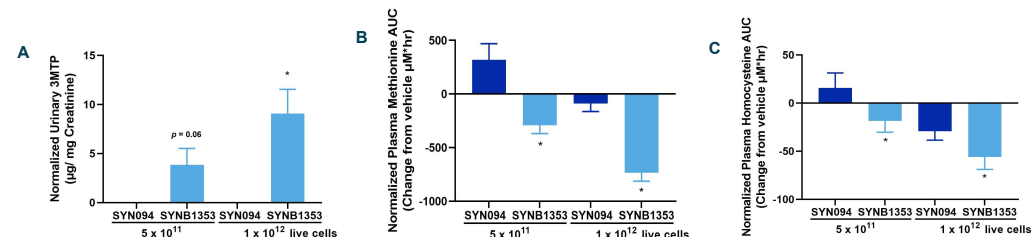


Figure 6. Monkeys were fasted overnight and received a single oral dose of methionine (100 mg/kg) with vehicle, EcN (control strain) or SYN1353 ( $5 \times 10^{11}$  or  $1 \times 10^{12}$  live cells). Urine was collected for 3-MTP, and blood was collected for methionine and Hcy measurements. Data was normalized to the study-respective vehicle and presented as mean SEM (n = 12/group). Statistical analysis was performed using paired t-test. \* $p < 0.05$ .

## Conclusions

SYN1353 is an engineered *E. coli* Nissle strain capable of metabolizing methionine and producing 3-MTP *in vitro*.

Concomitant administration of SYN1353 with an oral load of methionine blunts the appearance of plasma methionine and plasma total homocysteine in nonhuman primates. Thus, SYN1353 represents a promising approach for the treatment of HCU.