Development of SYNB1353, A Synthetic Biotic Engineered to Consume Methionine for the Treatment of Homocystinuria

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Introduction

- Homocystinuria (HCU) is a rare autosomal recessive disease caused by a loss of function of cystathionine β-synthase, leading to an accumulation of homocysteine (Hcy) in the plasma.
- Patients with high levels of Hcy are at risk for thromboembolism, lens dislocation, skeletal abnormalities, developmental delay, and intellectual disability. Current treatment options are limited due to efficacy and tolerability.
- Many patients must adhere to a heavily methionine (Met) restricted diet, however lifelong compliance is challenging.
- Here, we present an engineered Synthetic Biotic bacteria designed to consume methionine in the gut as a potential therapeutic for the treatment of HCU.

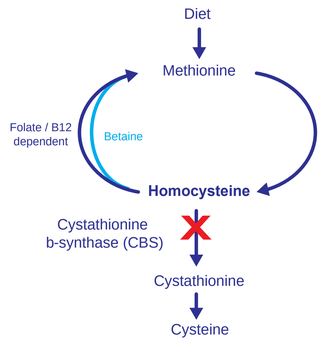

Figure 1. Diagram of dietary methionine cycle. This simplified diagram illustrates the cycling of dietary Met and conversion into homocysteine. Normally, homocysteine is converted into cystathionine by the cystathionine β-synthase enzyme. In HCU patients, this enzyme is absent or nonfunctional (represented by the red X) leading to the accumulation of homocysteine in the plasma.

Results

Development of a methionine consuming prototype strain of *E. coli* Nissle

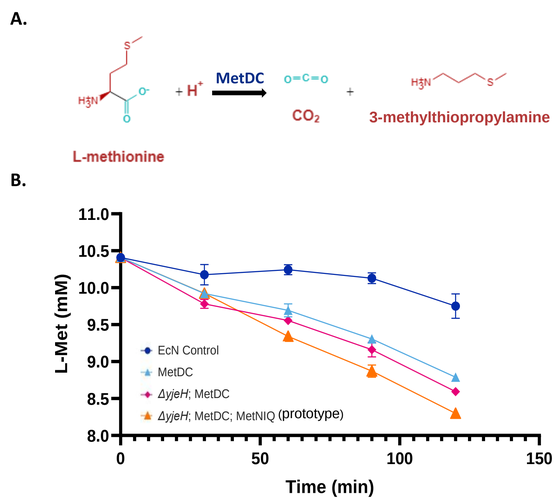

Figure 2. A) Met degradation via methionine decarboxylase (MetDC) from *Streptomyces* sp 590 converts L-methionine to CO_2 and 3-methylthiopropylamine (3-MTP) B) Met consumption by an *E. coli* Nissle (EcN) strain heterologously expressing *Streptomyces* MetDC. Deletion of the methionine exporter, *yjeH,* and overexpression of the endogenous methionine importer, MetNIQ, additively increase activity of a MetDC expressing strain.

Discovery of a high performing MetDC via high throughput screening of metagenomic and protein engineered libraries

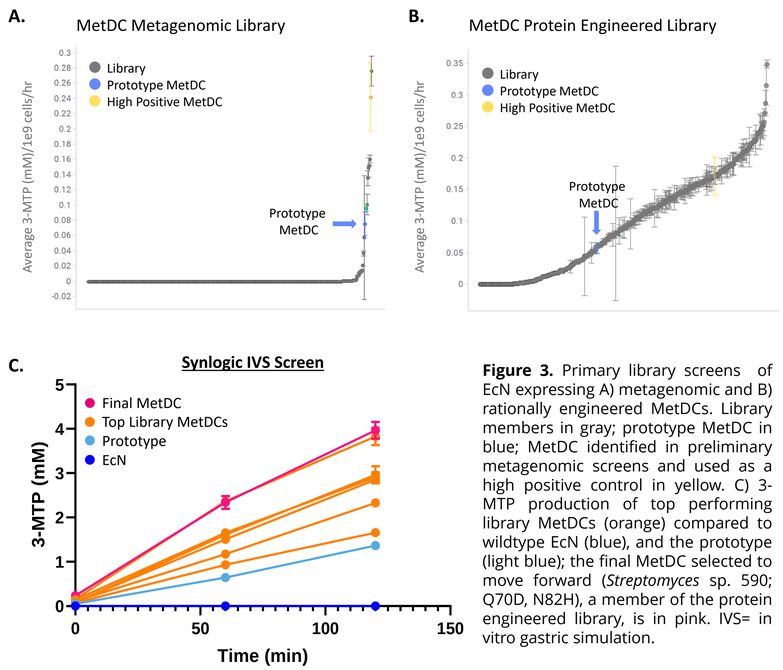

Figure 3. Primary library screens of EcN expressing A) metagenomic and B) rationally engineered MetDCs. Library members in gray; prototype MetDC in blue; MetDC identified in preliminary metagenomic screens and used as a high positive control in yellow. C) 3-MTP production of top performing library MetDCs (orange) compared to wildtype EcN (blue), and the prototype (light blue); the final MetDC selected to move forward (*Streptomyces* sp. 590; Q70D, N82H), a member of the protein engineered library, is in pink. IVS= in vitro gastric simulation.

Screening of methionine importers via metagenomic sourcing of MetPs and protein engineering of MetNIQ.

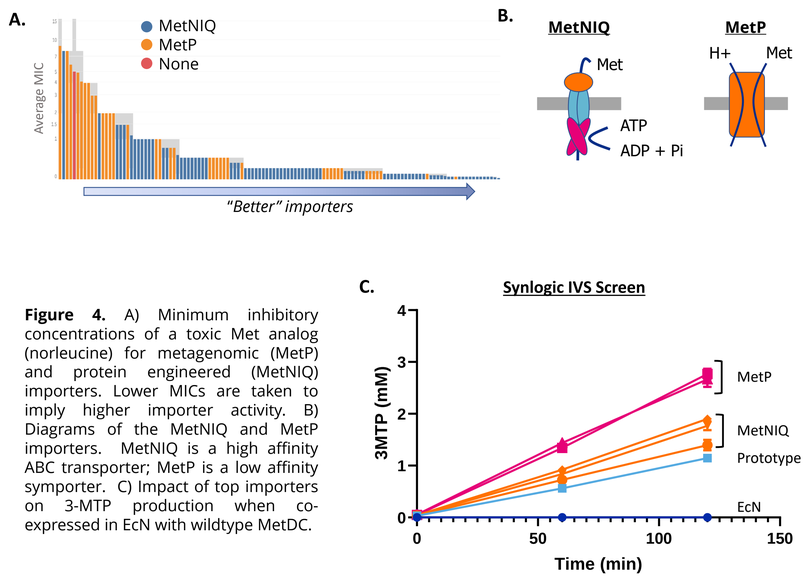

Figure 4. A) Minimum inhibitory concentrations of a toxic Met analog (norleucine) for metagenomic (MetP) and protein engineered (MetNIQ) importers. Lower MICs are taken to imply higher importer activity. B) Diagrams of the MetNIQ and MetP importers. MetNIQ is a high affinity ABC transporter; MetP is a low affinity symporter. C) Impact of top importers on 3-MTP production when co-expressed in EcN with wildtype MetDC.

Optimized components are integrated into an EcN chassis resulting in the high performing strain SYNB1353

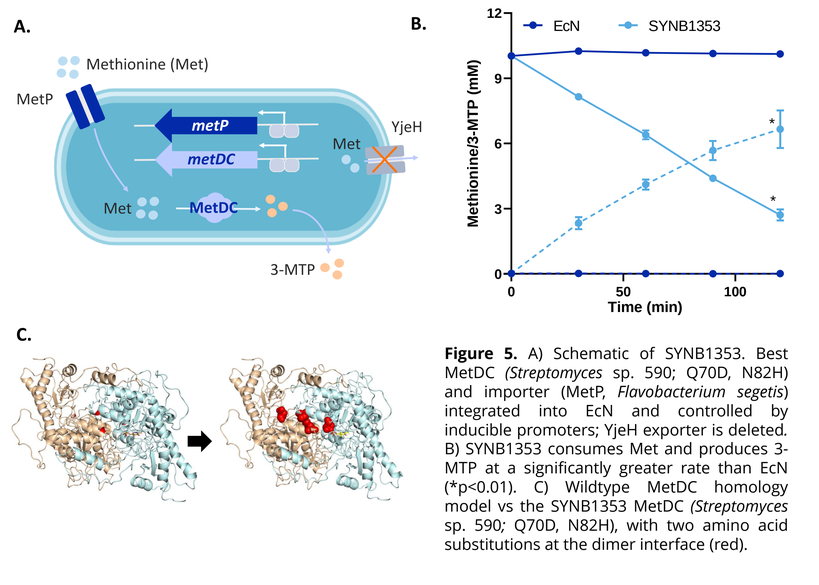

Figure 5. A) Schematic of SYNB1353. Best MetDC *(Streptomyces* sp. 590; Q70D, N82H) and importer (MetP, *Flavobacterium segetis*) integrated into EcN and controlled by inducible promoters; YjeH exporter is deleted. B) SYNB1353 consumes Met and produces 3-MTP at a significantly greater rate than EcN (*p<0.01). C) Wildtype MetDC homology model vs the SYNB1353 MetDC *(Streptomyces* sp. 590*;* Q70D, N82H), with two amino acid substitutions at the dimer interface (red).

SYNB1353 consumes Met and produces 3-MTP in nonhuman primates

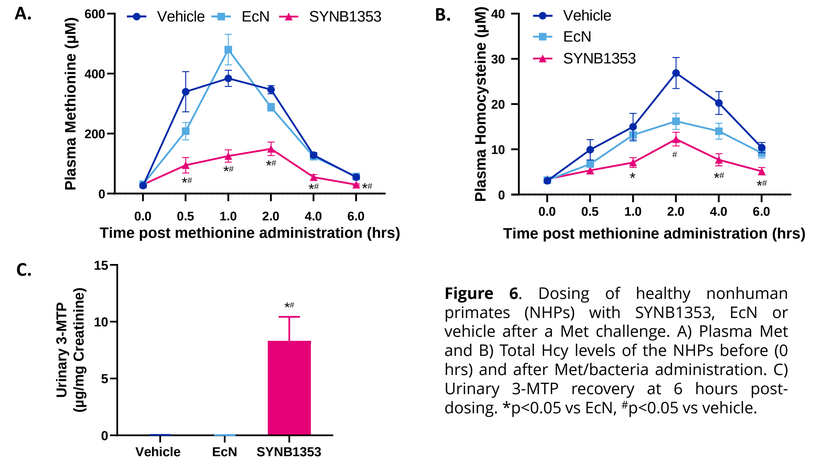

Figure 6. Dosing of healthy nonhuman primates (NHPs) with SYNB1353, EcN or vehicle after a Met challenge. A) Plasma Met and B) Total Hcy levels of the NHPs before (0 hrs) and after Met/bacteria administration. C) Urinary 3-MTP recovery at 6 hours post-dosing. *p<0.05 vs EcN, #p<0.05 vs vehicle.

Conclusions

- Metagenomic analysis and protein engineering identified MetDCs and importers with greater activity than wildtype.
- These parts were combined with the deletion of YjeH to engineer the clinical candidate strain SYNB1353.
- Data from NHPs suggests that dosing with SYNB1353 decreases plasma Met and blunts the resulting increase of Hcy, showing the potential of SYNB1353 as a novel therapeutic for the treatment of HCU.