

# synlogic

## **A Genetically-Engineered Probiotic Designed to Consume Methionine for the Treatment of Homocystinuria**

Analise Reeves, PhD

June 1, 2023



**2023 SYNTHETIC BIOLOGY:  
Engineering, Evolution & Design**



# Synlogic Posters at SEED

**Robust Performance and Rapid Construction of Live Bacterial Therapeutics Lacking the Colibactin Gene Cluster**

Jackie Thompson, John Bonaguidi, Robert Evers, Jason Russell, Amy Redburn, Sean Collins, Brian Moran, Brita Gonzalez, Vincent Juballa, Mylene Perreault, David L. Hoyle, and Andrew Rosen  
Synlogic Inc., Cambridge MA, USA

**Introduction**

- Synlogic Biotic utilizes non-pathogenic probiotic *E. coli* Strain (ECo) as the chassis organism for engineering strains with drug-like properties designed to perform therapeutic functions in patients.
- Safety concerns have been raised regarding the native plasmid cluster in ECo, including the genotoxin colibactin, prompting an evaluation of removing the plasmid from Synlogic Biotics.
- Here, we report that ΔColECo Biotics maintain engineered activity and display no growth disadvantage when tested in in vitro assays and in vivo preclinical mouse and non-human primate models. Following this, 24 clinical trials, including the Universal Cholecystectomy, were unimpeded to enable the rapid construction of future Synlogic Biotics.

**Synlogic Biotic engineer for glioblastoma and evaluate degradation of its activity with universal cholecystectomy**

**No change in breast kinetics or glioblastoma degradation activity between WT ECo and ΔColECo for both models**

**Universal Cholecystectomy enables rapid construction of Synlogic Biotics**

**No growth disadvantage observed in ΔColECo ECo compared to WT ECo in in vitro assays**

**WT ECo and ΔColECo ECo show similar breast kinetics in naive (NOD) and congenitally treated mouse models (giga)**

**Figure 1. Rapid construction of Synlogic Biotics.** The Synlogic Biotic chassis organism, E. coli Strain (ECo), is a well-tolerated chassis organism and will be included in all Synlogic Biotics. The Universal Cholecystectomy enables construction of clinically relevant strains in as little as 4-6 weeks.

Jackie Thompson  
Tuesday May 30  
Poster 38A



**Engineering Synthetic Biotics to Secrete Therapeutic Proteins**

Jian Hong Gao, Chun-Chen Chao, Jenny Shi, Doug Kenny, Isabel Matoska, Jacklyn Thompson, Doree Hava, Analisa Reeves  
Synlogic Inc., Cambridge MA, USA

**Summary**

- Synlogic Biotic utilizes non-pathogenic *E. coli* Strain (ECo) chassis designed with drug-like properties.
- Synlogic's synthetic biology platform allows the engineering of probiotic strains to express and secrete single or multiple proteins with immunomodulatory functions (e.g., colibactin, Clostridia toxin (CTX) and lipoteichoic acid).
- Secretion tags to enhance using signal peptide libraries, protein fusions, or membrane to improve stability and activity.
- Library of secretion tags enabled enhanced secretory efficiency.
- Chassis strains are genetically modified.
- Expansion of therapeutic secretion system.
- Chassis development (e.g., membrane growth optimization).

**Engineered ECo secretes a high level of bioactive IL2**

**IL2 secretion improved by membrane fusion with stability tag**

**ECo secretes bioactive IL2, promoted expansion of primary CD8<sup>+</sup> and CD4<sup>+</sup> T cells**

**ECo secretion improved by biosynthesis optimization**

**ECo secretes multiple therapeutic agents for combination therapy**

**ECo secretion of a bioactive anti-IL1β antibody**

**Conclusions**

- ECo is a versatile chassis organism for secretion of therapeutic proteins.
- Protein stability and activity are enhanced by a variety of engineering strategies to improve secretion, which can be tailored to the protein, tag, and host organism, as well.
- These innovations are key to the development of a new class of probiotic therapeutics in the gut, both as immunomodulatory based disease.

JR Gao  
Wednesday May 31  
Poster 51B



**SYNB1353, a Synthetic Biotic engineered for the treatment of HCU, metabolizes methionine and lowers homocysteine in preclinical and clinical models**

Jillian Means, Michael James, Mary McDonald, Julie Blomberg, Neal Sondheimer, Analisa Reeves, Mylene Perreault, David Lubkowitz, David Hava  
Synlogic Inc., Cambridge MA, USA

**Introduction**

- Homocysteinuria (HCU) is a rare autosomal recessive disease caused by a loss of function of cystathionine β synthase, leading to an accumulation of homocysteine that is toxic to the patient.
- Patients with high levels of Hcy are at risk for thromboembolism, lens dislocation, cerebral atrophy, developmental delay, and intellectual disability. Current treatment options are limited due to efficacy and tolerability.
- Many patients that adhere to healthy methionine (Met) restricted diet, however, finding compliance is challenging.
- Here, we present preclinical and clinical results for SYNB1353, an engineered Synlogic Biotic, designed to consume methionine in the gut as a potential therapeutic for the treatment of HCU.

**Results**

**Protein abundance of MetHcy<sub>1353</sub> is increased and exhibits minimal feedback inhibition by Hcy**

**SYNB1353 reduces plasma Met and Hcy in healthy non-human primates in a dose dependent manner**

**SYNB1353 metabolizes methionine and lowers homocysteine in healthy volunteers**

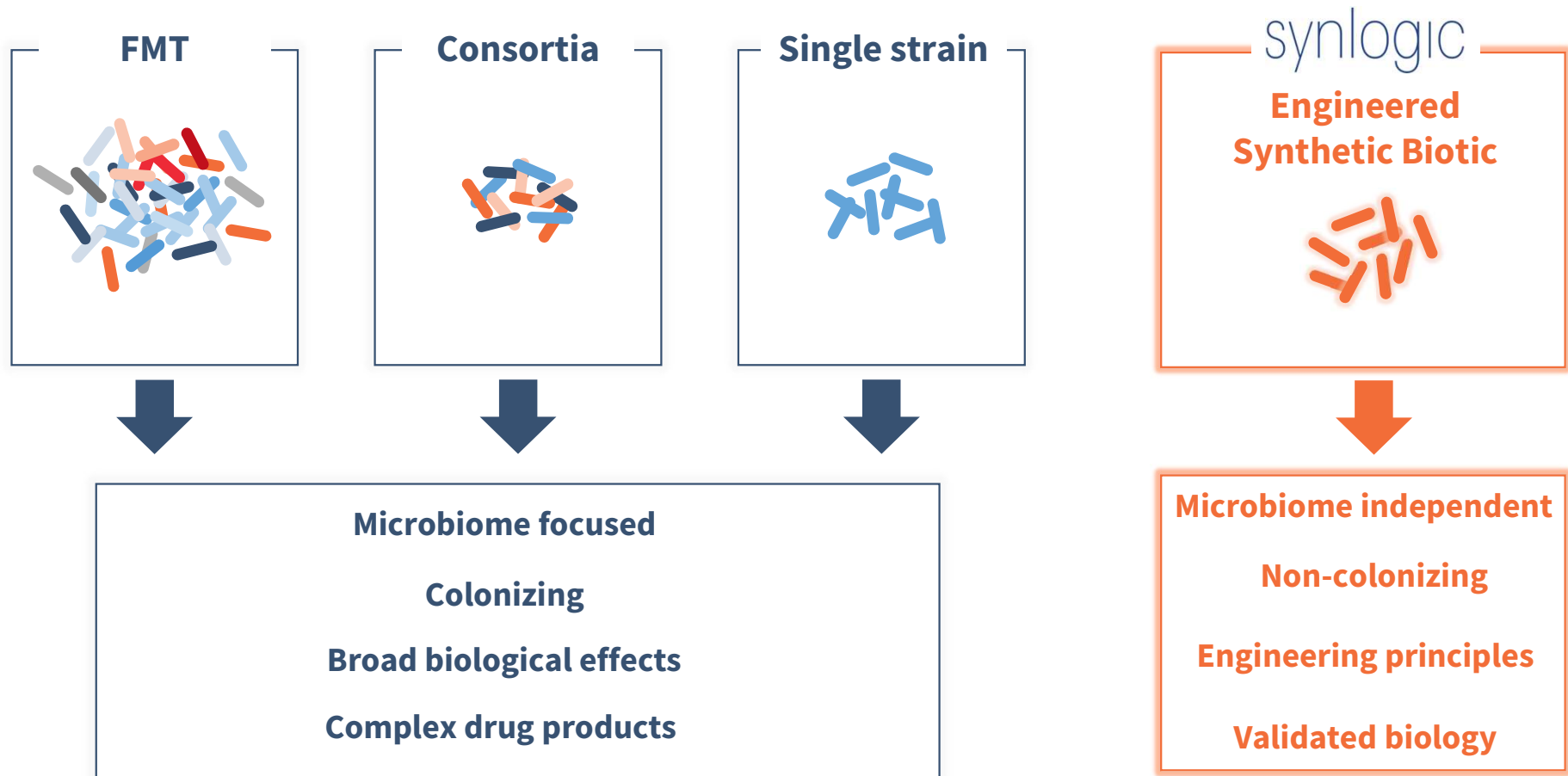
**Conclusions**

- The engineered MetHc enzyme in SYNB1353 exhibits an increase in protein abundance and minimal feedback inhibition by Hcy.
- SYNB1353 reduces plasma Met and Hcy in a dose dependent manner in a preclinical NHP model.
- In a double blind, placebo-controlled Phase 1 study, SYNB1353 was generally safe and well-tolerated and lowered Met absorption in healthy volunteers after a Met challenge.

Jill Means  
Thursday June 1  
Poster 51C



# Synthetic Biotics: Precise Engineering to Address Specific Biology

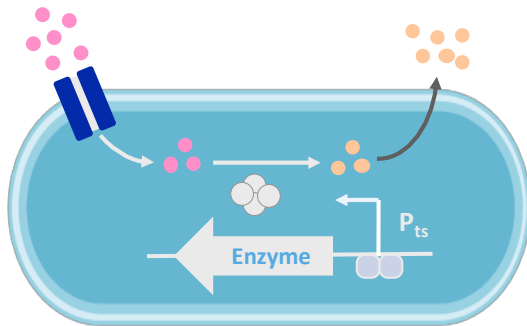


# Advancing a New Paradigm of Biotherapeutics

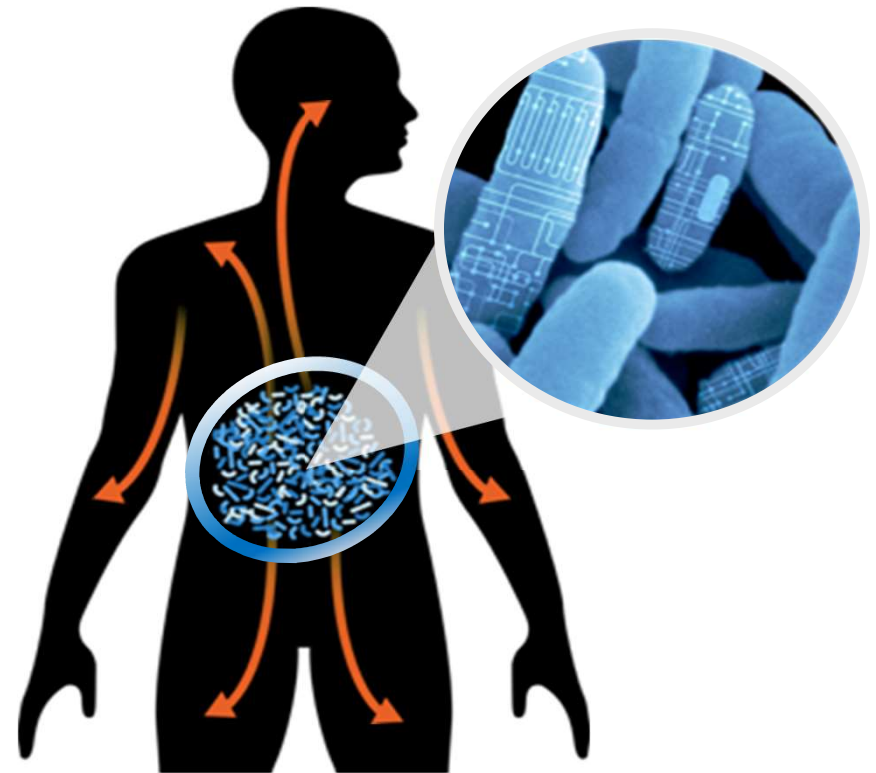
Goal: To use engineered probiotic bacteria to deliver therapeutic function from within the GI tract

## Synthetic Biotics

Well-characterized **probiotic chassis** + Programmable **genetic engineering**



**Convert disease-causing targets to harmless metabolites**



# Probiotics as a Delivery Vehicle for Therapies

Application of synthetic biology to probiotic “chassis”



100 years of *E. coli* strain Nissle 1917

*Ulrich Sonnenborn, 2017*

## Why *E. coli* Nissle?

- Available over the counter in many countries
- Leverage >100 year safety profile
- Amenable to genetic manipulation
- Easy to manufacture

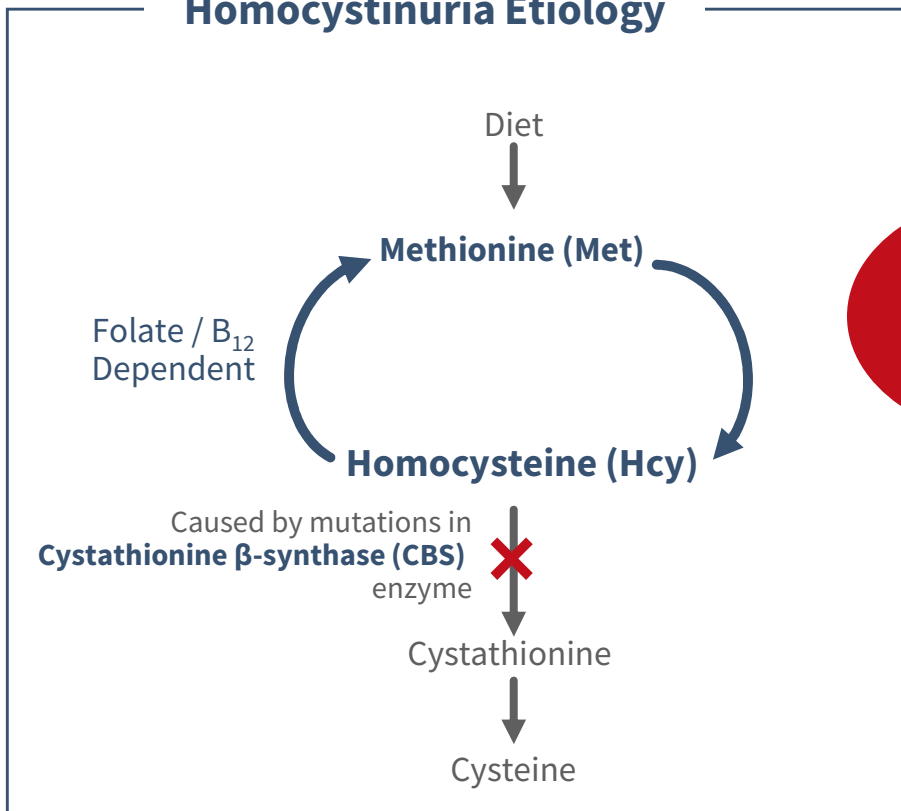


# Engineering a probiotic to metabolize methionine

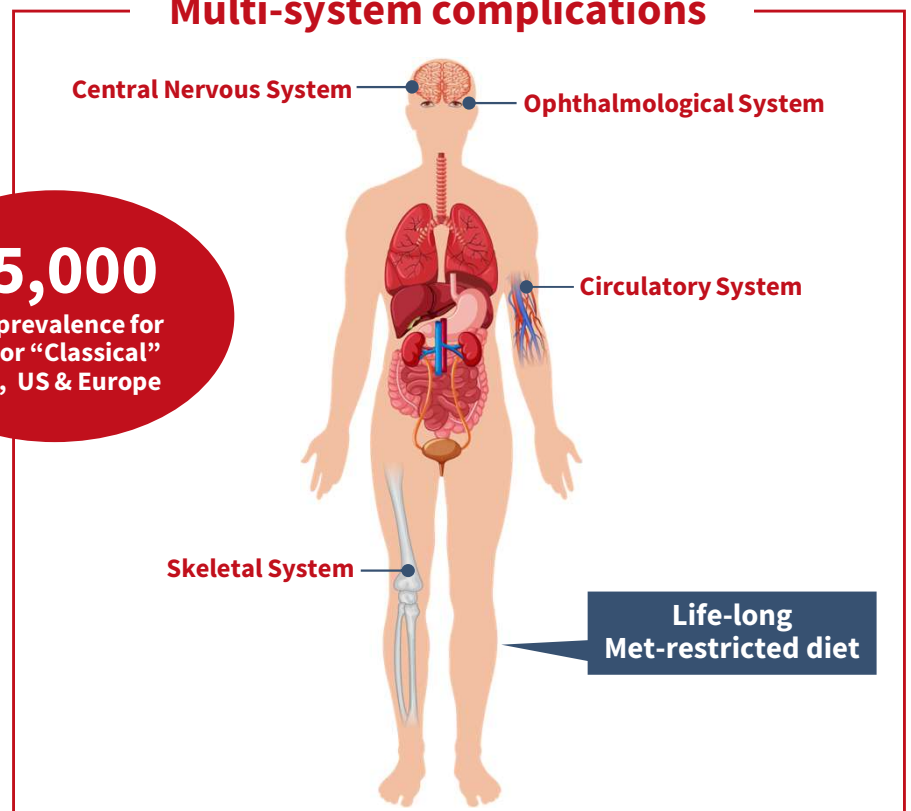


# Homocystinuria, a rare metabolic disease with unmet need

## Homocystinuria Etiology

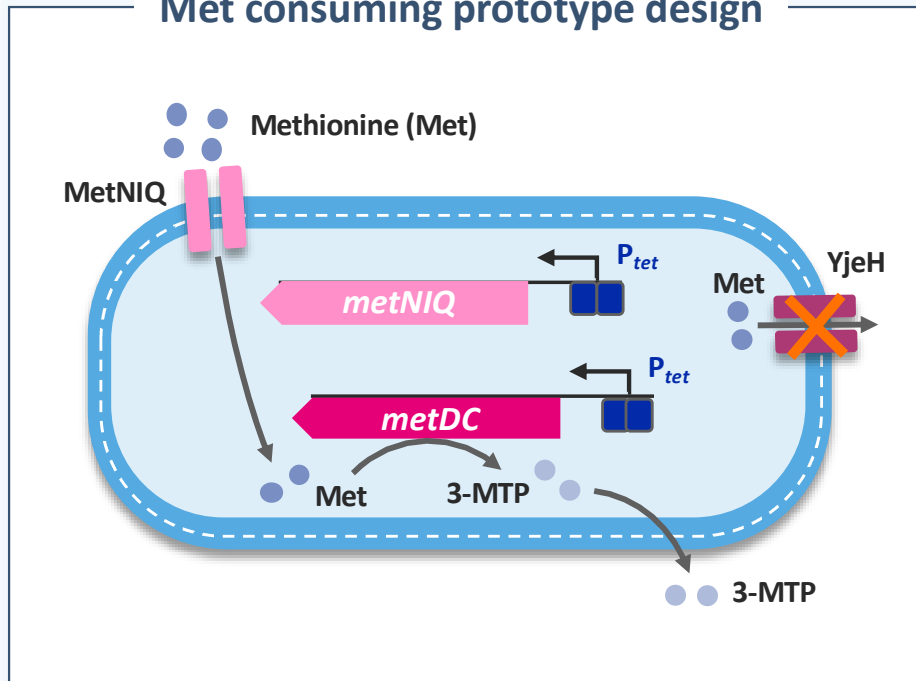


## Multi-system complications

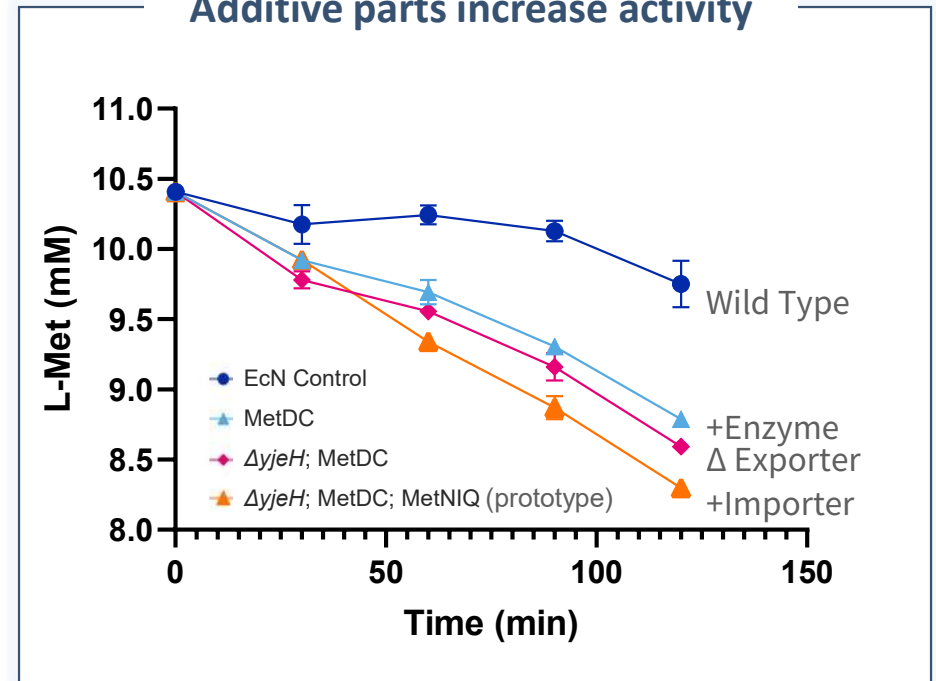


# Engineered prototypes consume methionine in vitro

Met consuming prototype design



Additive parts increase activity



- Key design elements:
- 1 Methionine decarboxylase enzyme, MetDC, *Streptomyces*
  - 2 Methionine Importer: MetNIQ, *E. coli*
  - 3 Methionine Exporter: YjeH, *E. coli*



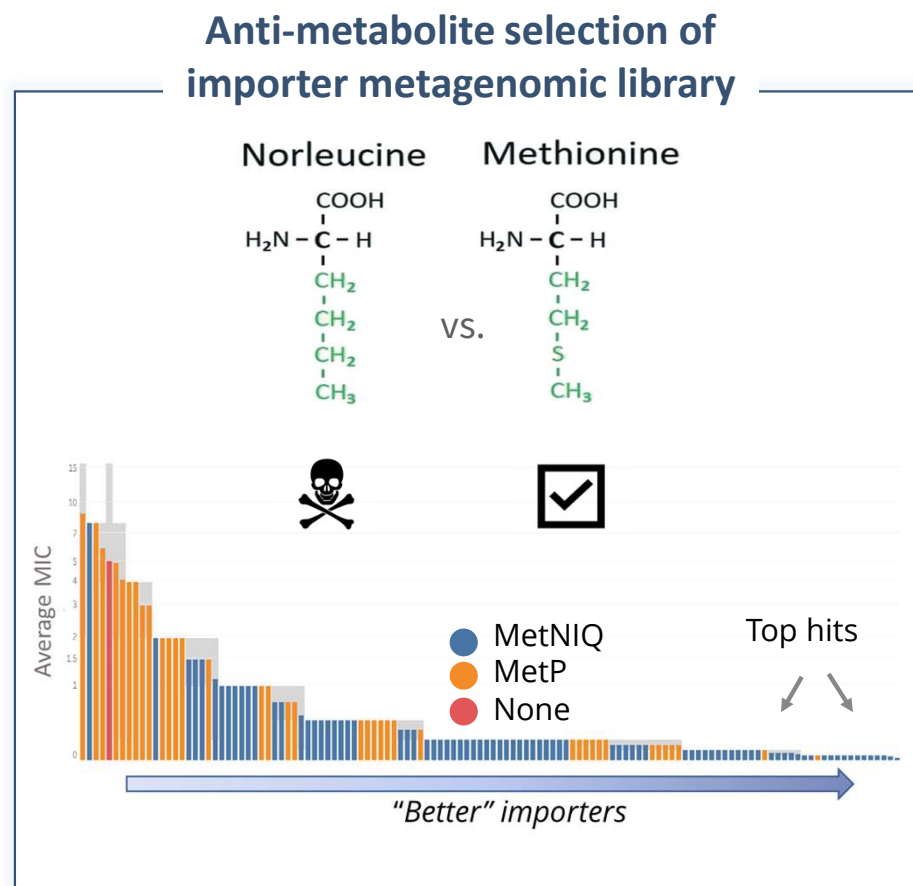
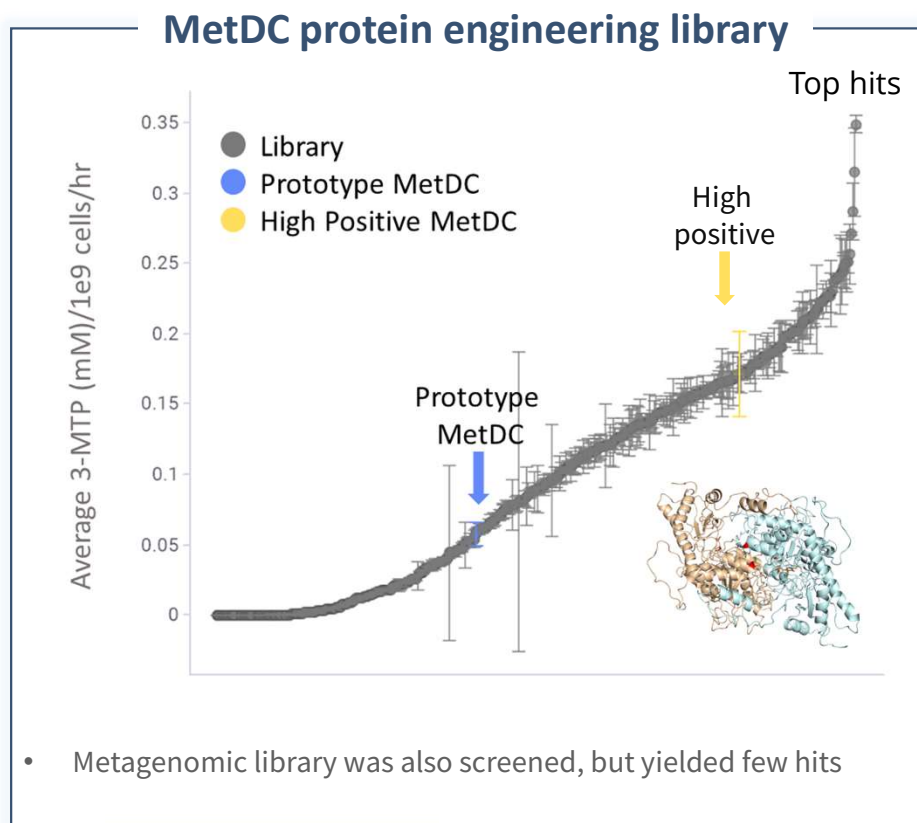


**GINKGO BIOWORKS**  
THE ORGANISM COMPANY



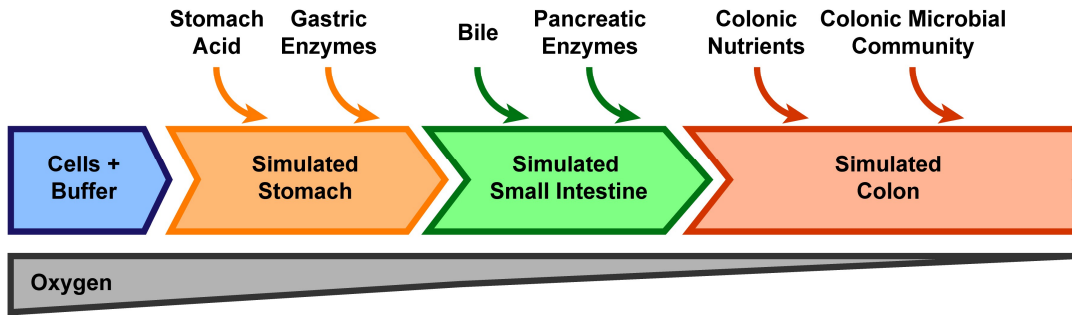
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# High Throughput screening identifies top performing proteins



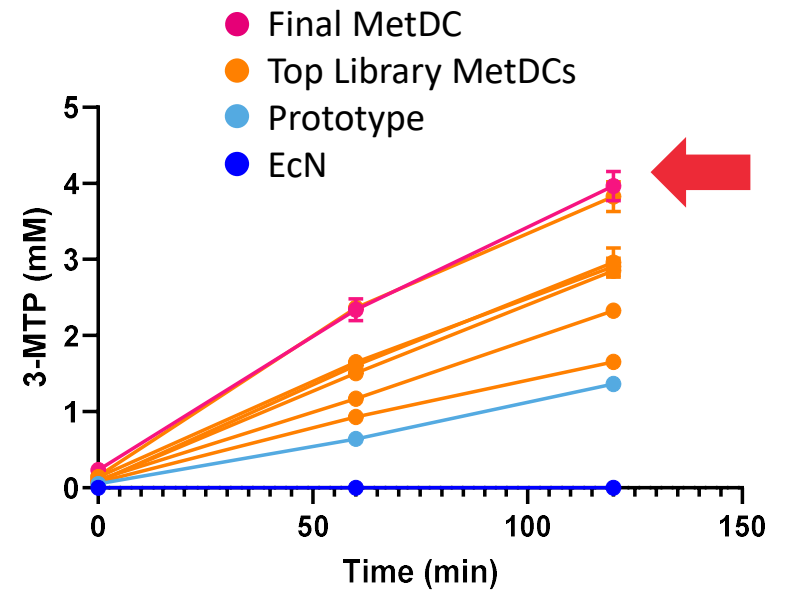
# Secondary screen identifies lead MetDC with improved activity

## In vitro simulation (IVS) assay

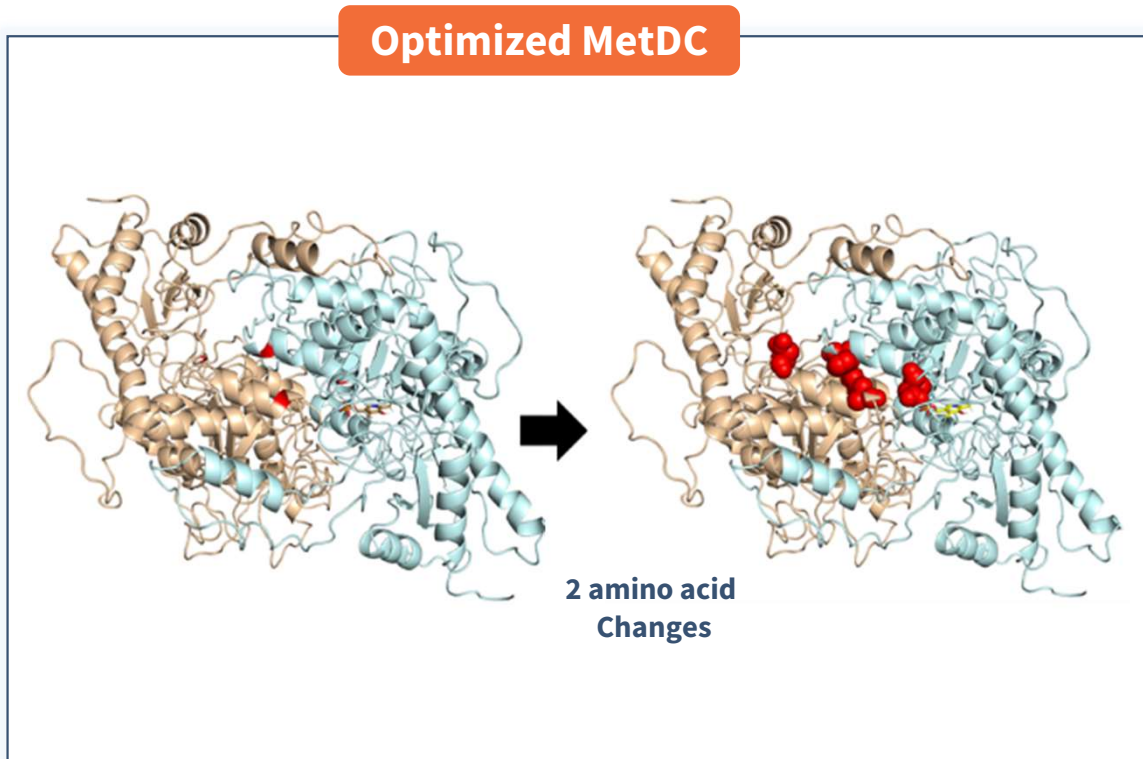


Models transit through the gut

## Lead 3MTP Production

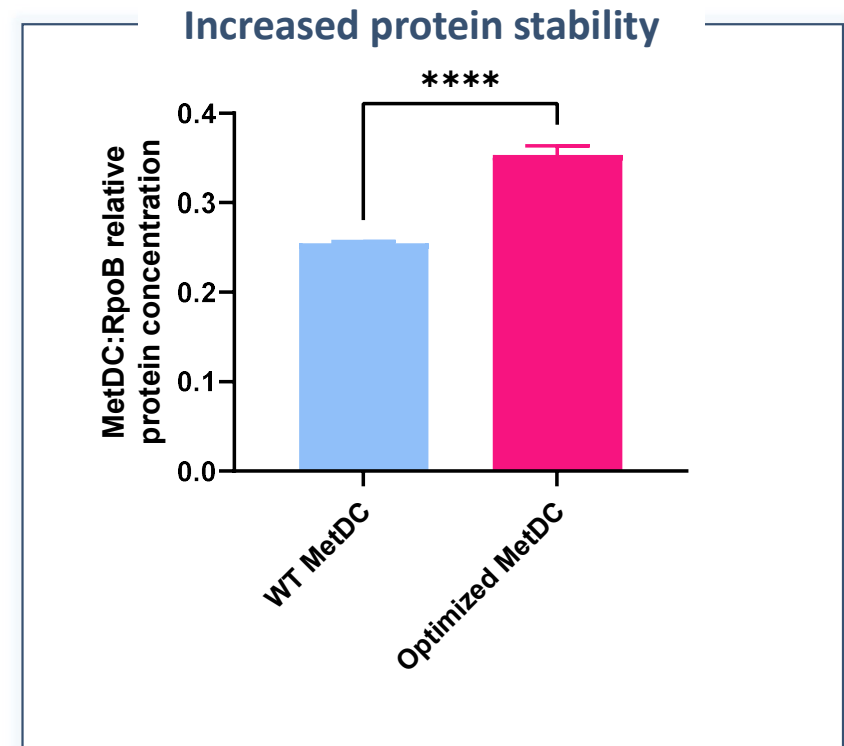


# Secondary screen identifies lead MetDC with improved activity



Prototype MetDC

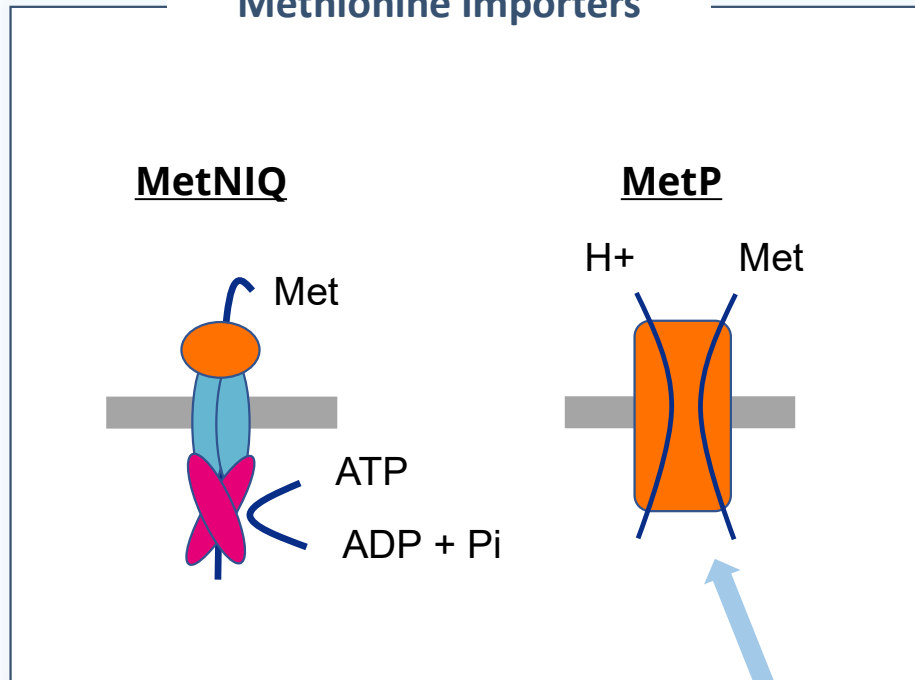
Optimized MetDC  
(Q70D, N82H)



Possible contributing mechanism

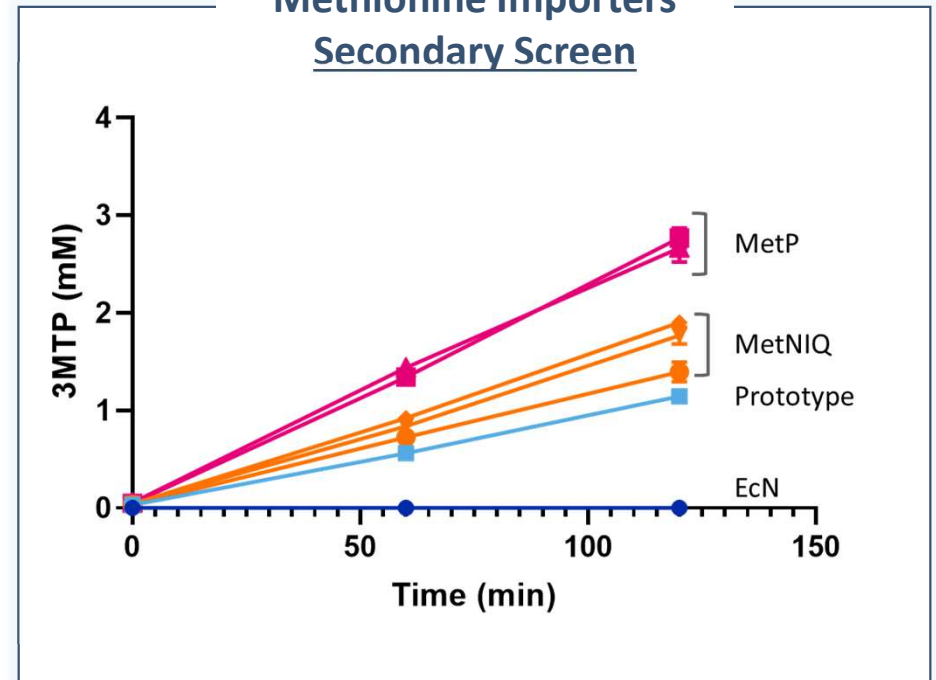
# Identification of more active Met importers

## Methionine Importers

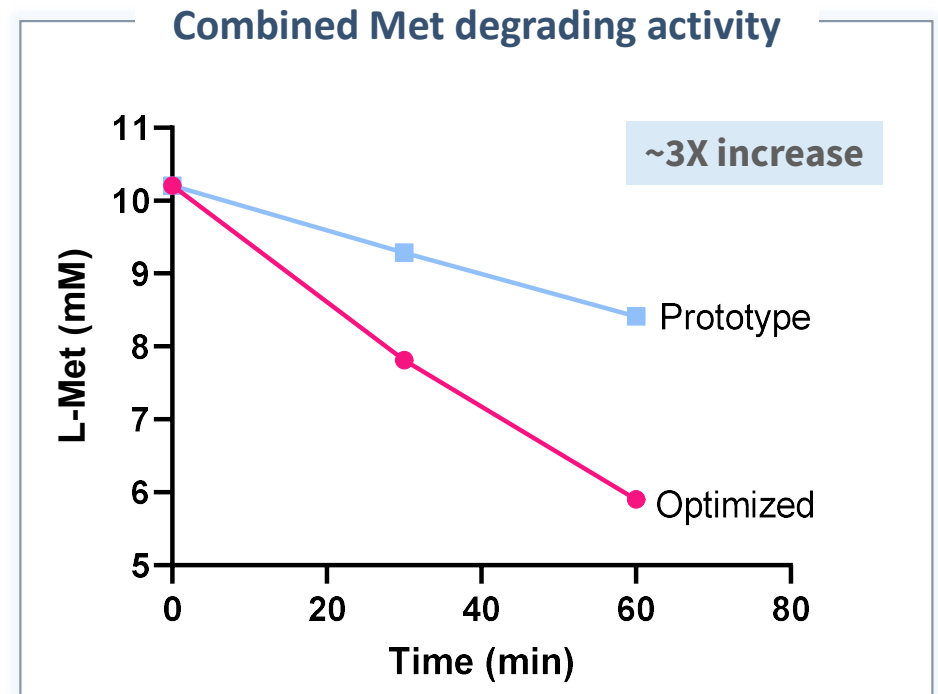
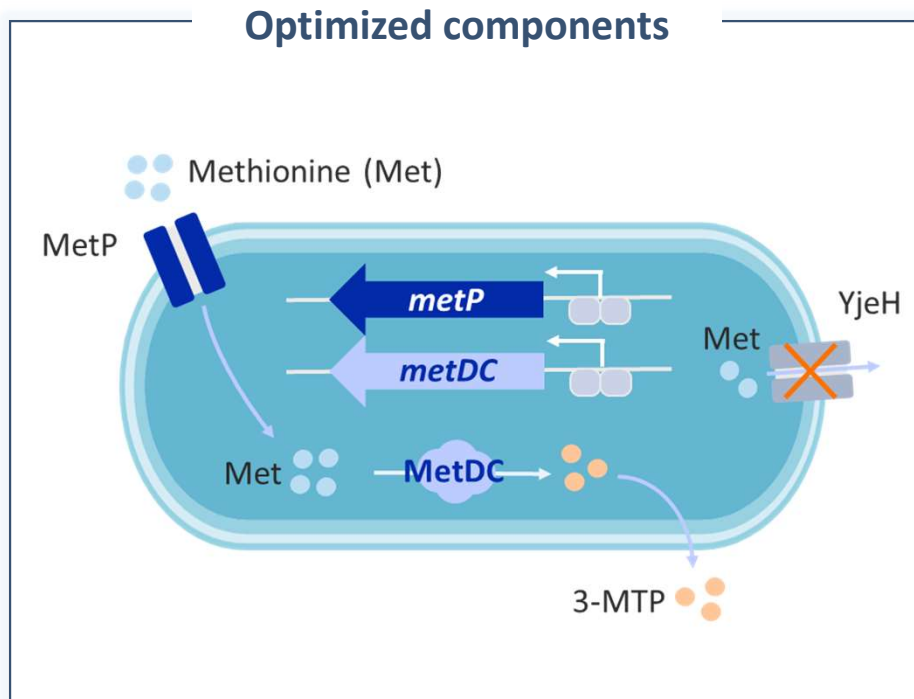


Alternate  
mechanism

## Methionine Importers Secondary Screen



# Combining optimized components improves activity



- Improved Parts:
- 1 Methionine decarboxylase enzyme, **MetDC (Q70D, N82H)** *Streptomyces*
  - 2 Methionine Importer: **MetP, *Flavobacterium segetis***
  - 3 Methionine Exporter: YjeH, *E. coli*

# SYNB1353, a methionine metabolizing Synthetic Biotic

## Naming SYNB1353

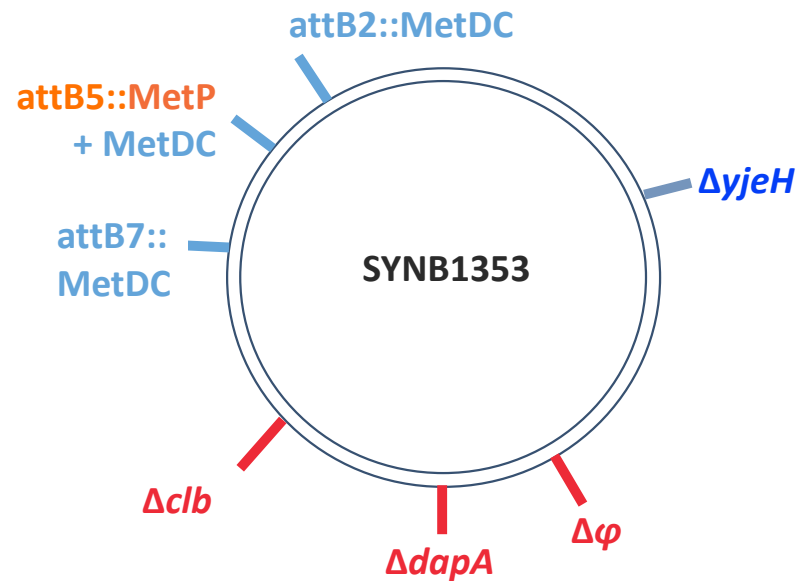
**Akhenaten** was an ancient Egyptian pharaoh

Speculated that he suffered from **HCU**

His ruling started in **1353** b.c.



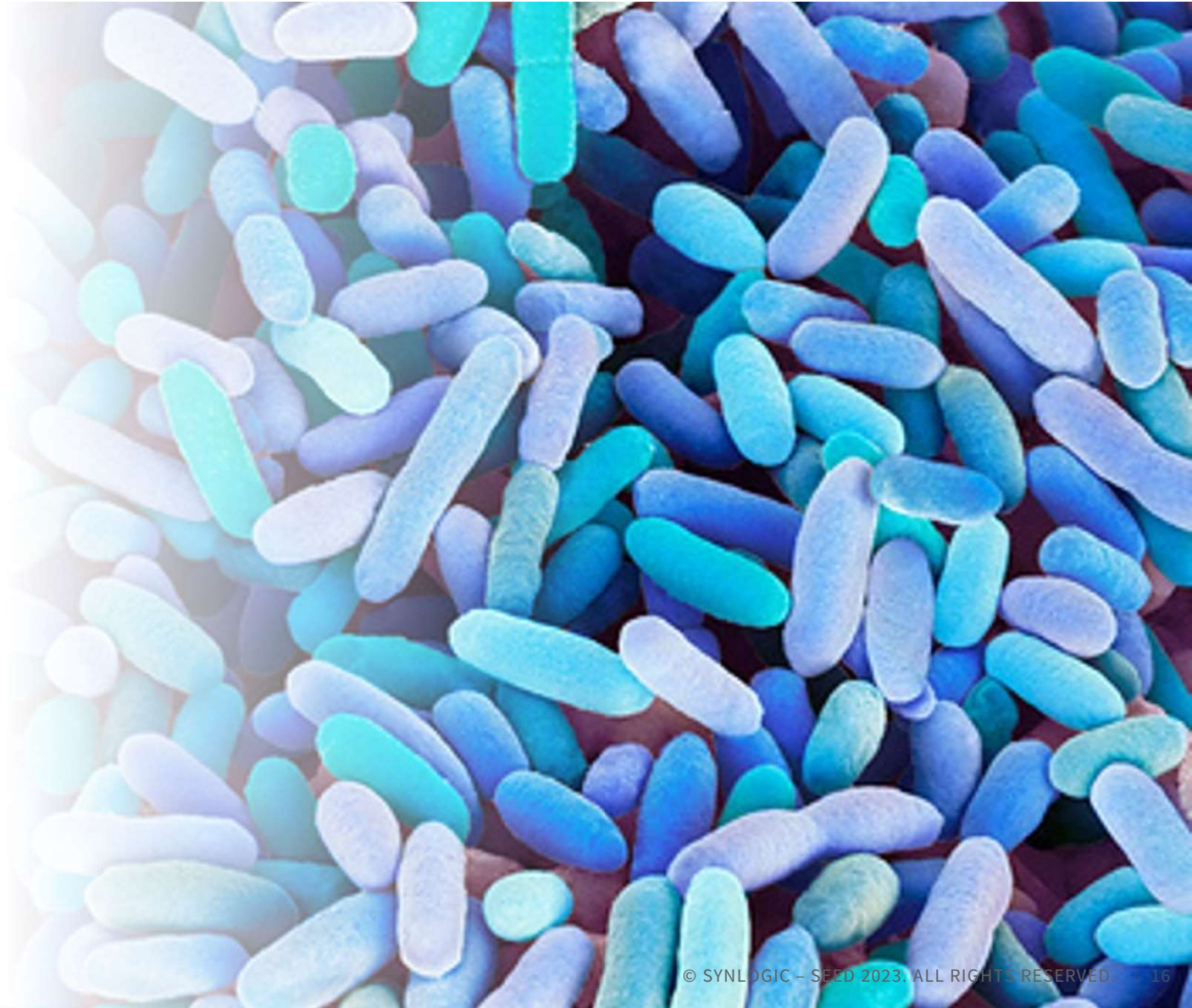
## Chromosomal Map



Clinic ready features:

- $\Delta dapA$  – containment
- $\Delta \varphi$  – manufacturing
- $\Delta clb$  – safety

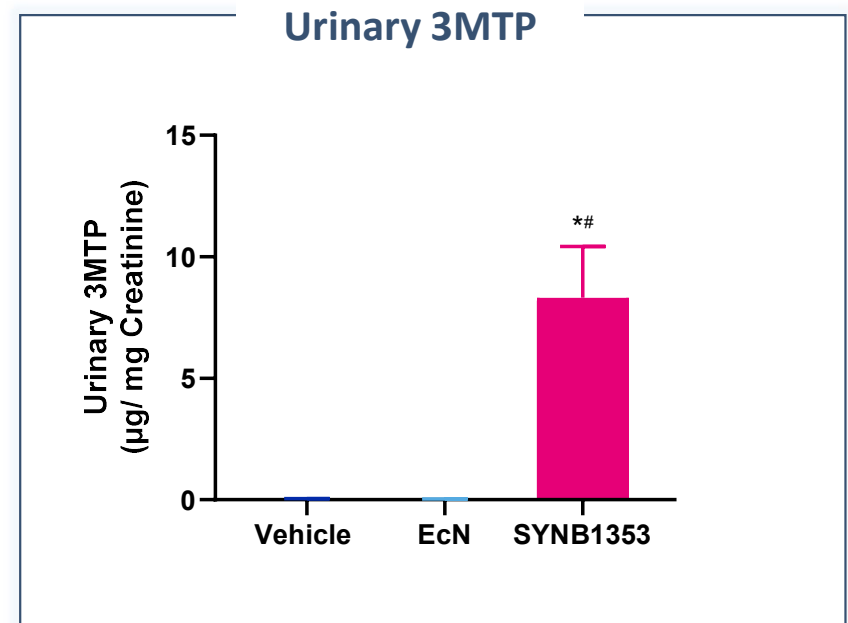
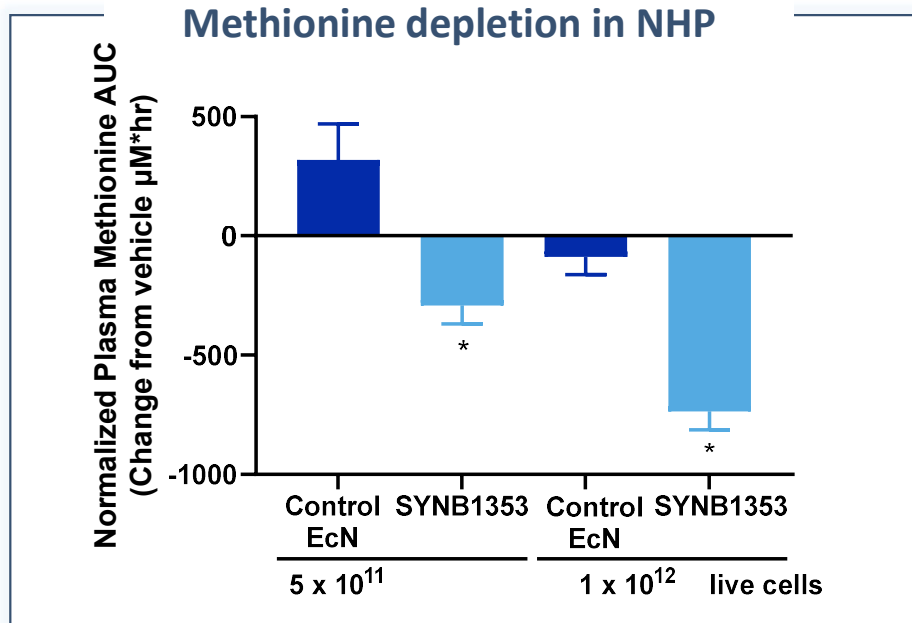
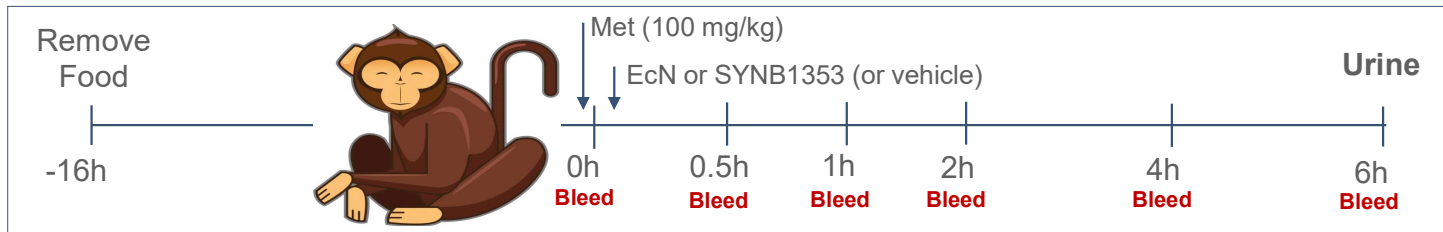
## **In vivo testing of SYNB1353**

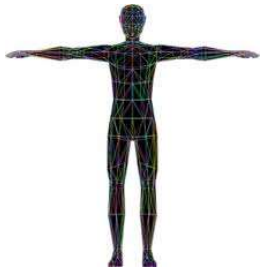




# In vivo efficacy of SYN1353 in healthy NHPs

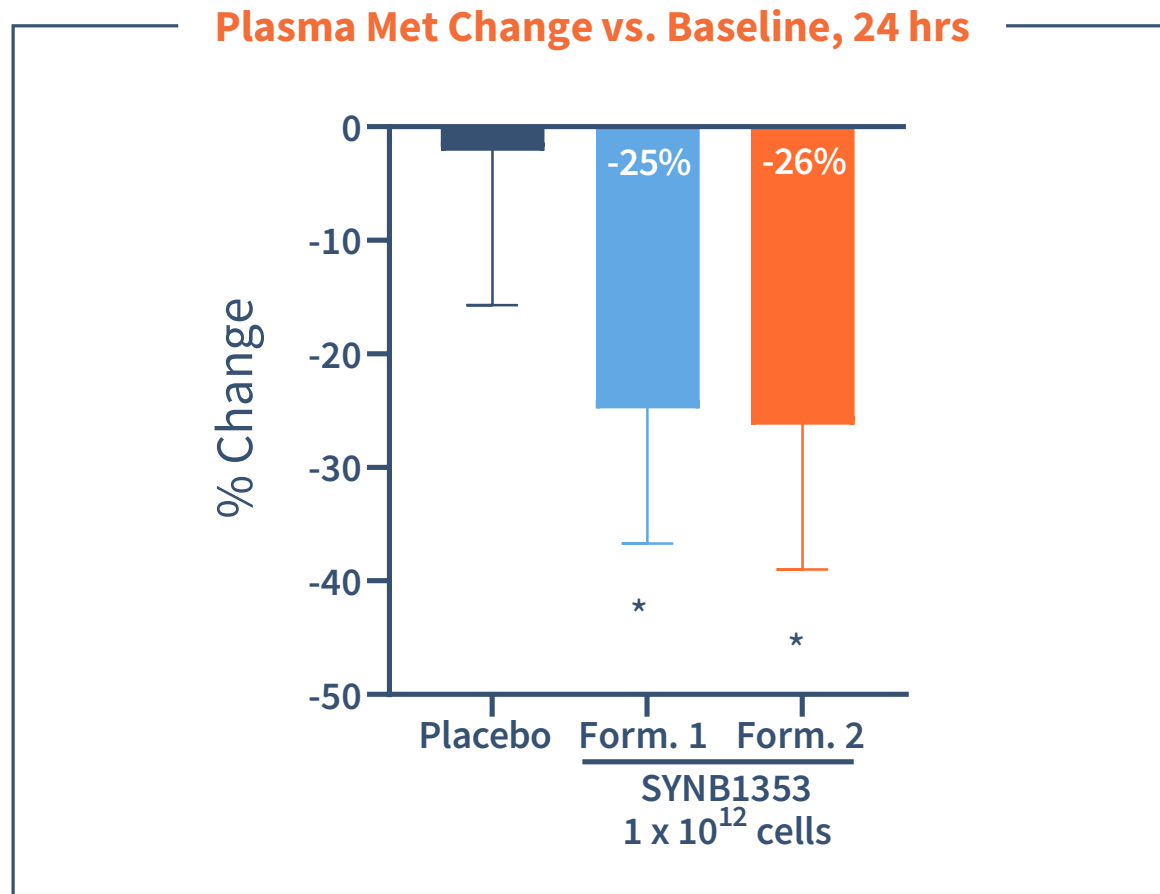
SYN1353 results in significant blunting in serum Met and Hcy following challenge





Phase 1

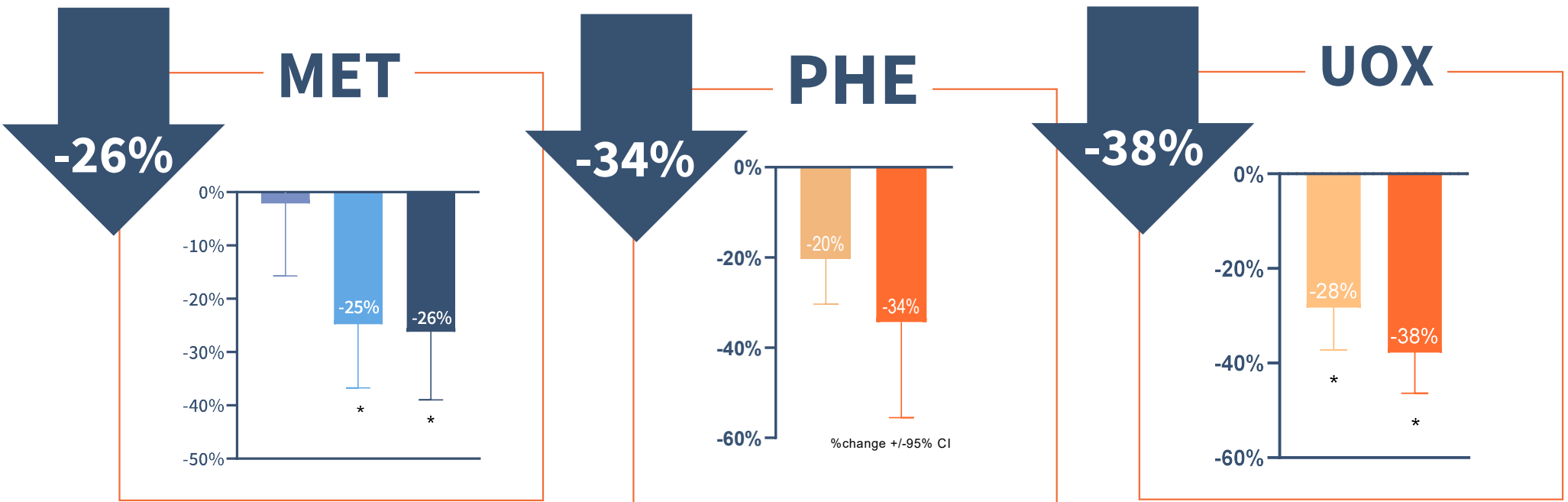
# SYNB1353: Proof-of-Mechanism Achieved



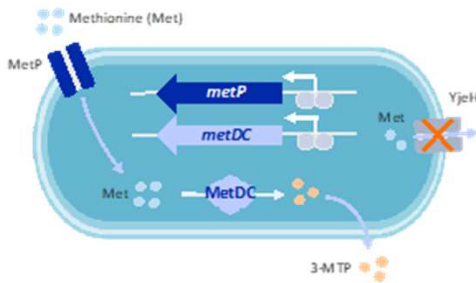
LS mean change, 95% CI  
\**p* < 0.05

Form = formulation; placebo n=8, 1x10<sup>12</sup> form 1 n=6, 1x10<sup>12</sup> form 2 n=5

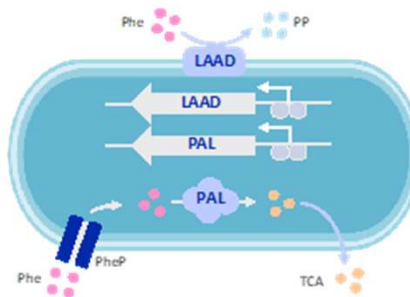
# A synthetic biology platform with multiple hits



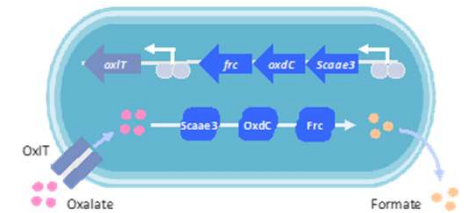
SYNB1353



SYNB1934



SYNB8802



# Advancing a New Class of Biotherapeutics

Exploratory

Preclinical

IND-  
Enabling  
Studies

Phase 1

Phase 2

Phase 3

**Metabolic**

**Phenylketonuria (PKU)**

**SYNB1934**

RPDD

US - ODD

EU - OD

**Homocystinuria (HCU)**

**SYNB1353**

RPDD

US - ODD

FT

**Enteric Hyperoxaluria**

**SYNB8802**

**Gout**

**SYNB2081**

**Immunology**

**Inflammatory Bowel Disease (IBD)**

**IBD Program - Single Target**



RPDD = Rare Pediatric Disease Designation granted by FDA  
 EU - OD = Orphan Designation granted by EMA  
 US - ODD = Orphan Drug Designation granted by FDA  
 FT = Fast Track granted by FDA

# Acknowledgements

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## Discovery

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