



# 3<sup>rd</sup> Paris NASH Symposium

French-US Meetings

July 6 & 7, 2017

Institut Pasteur - Paris

Organized by  
Arun Sanyal & Lawrence Serfaty

Virginia Commonwealth University School of Medicine, Richmond, Virginia, US  
Hôpital Saint-Antoine, APHP, Inserm, Université Pierre & Marie Curie, Paris, France

With the partnership of





# synlogic

**Powering the Microbiome**  
with synthetic biotics to correct metabolic dysregulation  
throughout the body

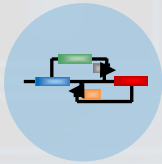


**3<sup>rd</sup> Paris NASH Symposium**

**July 6-7, 2017**

**Dean Falb, Ph.D.**  
**Co-Founder and Chief Technical Officer**

# Synthetic Biotics: A New Class of Engineered Medicines that Operate from Our Natural Microbiome



## Synthetic

- Engineered bacteria
- With **designed genetic circuits**
- To **degrade metabolites** that induce disease or **synthesize substances** to treat disease

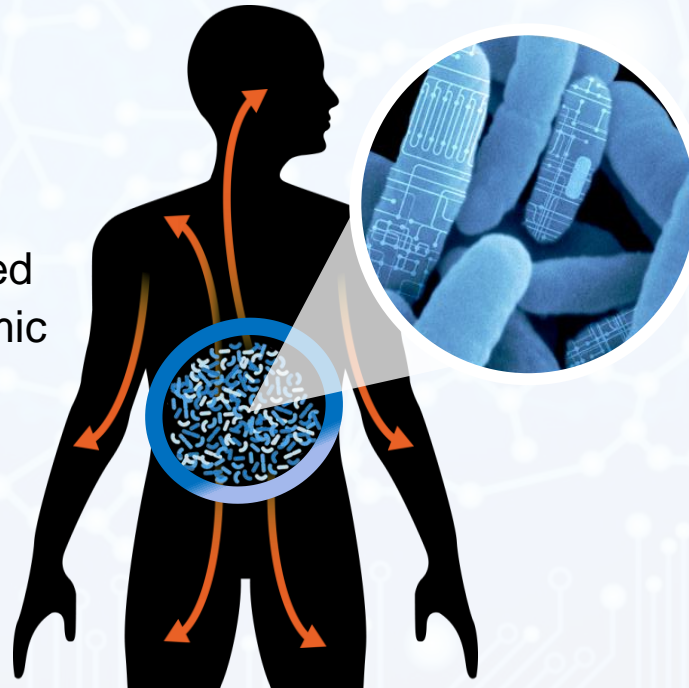


## Biotics: *E. coli* Nissle as chassis:

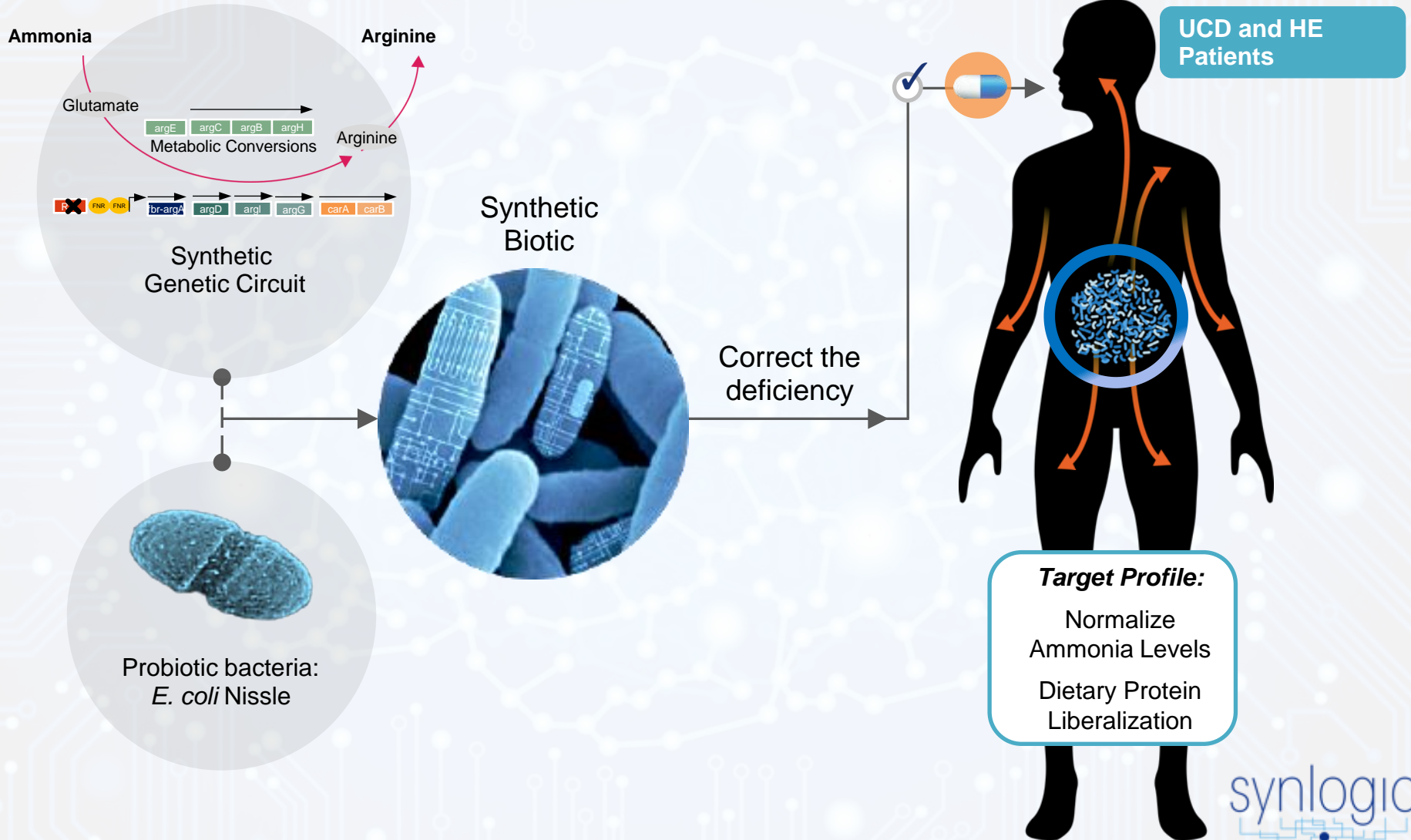
- Widely-used **oral probiotic**
- **Leverage the safety** of probiotic
- Found within natural human **microbiome**
- **Amenable to genetic manipulation**

**Synthetic Biology + Bacteria = Synthetic Biotic**

**Therapeutic** delivered locally to treat systemic diseases

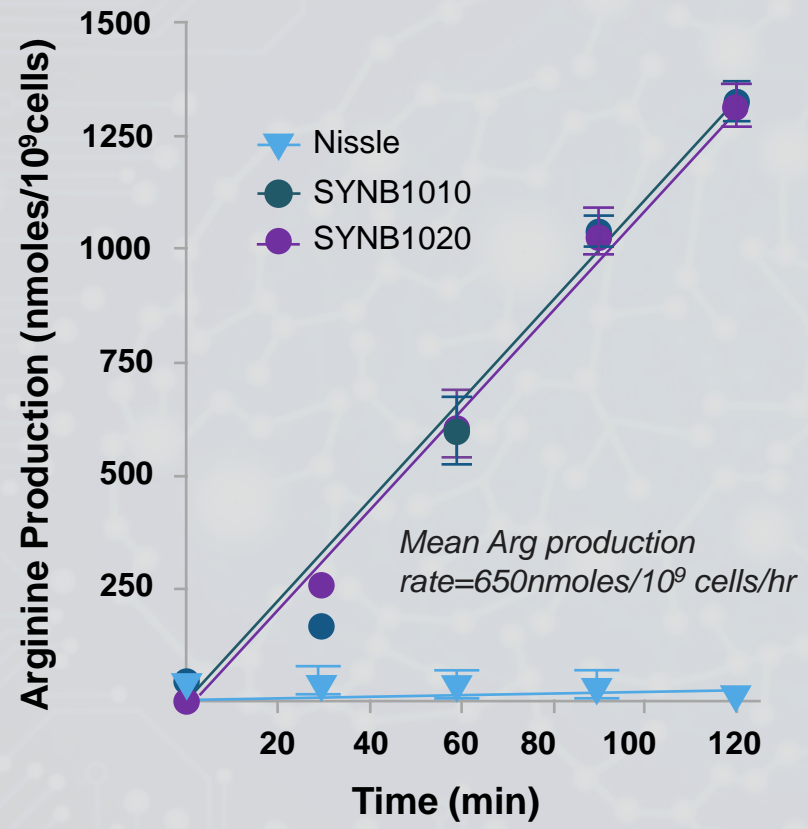


# SYNB1020: Conversion of Toxic Ammonia into Beneficial Arginine for the Treatment of UCD and HE



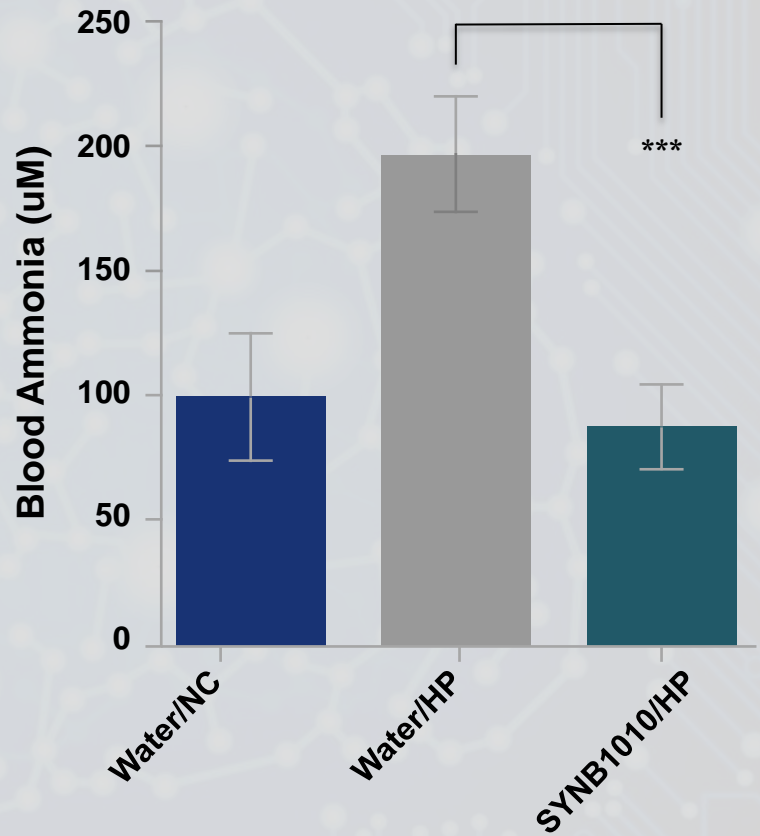
# SYNB1020: Efficient Ammonia Conversion by Synthetic Biotic *In Vitro* and *In Vivo*

## Ammonia to Arginine Conversion *in vitro*



Nissle – *E. coli* Nissle 1917 *Strep* resistant control strain  
 SYNB1010 – arginine producing, Thy A auxotrophy, *Kan* resistant  
 SYNB1020, arginine producing, Thy A auxotrophy, clinical candidate

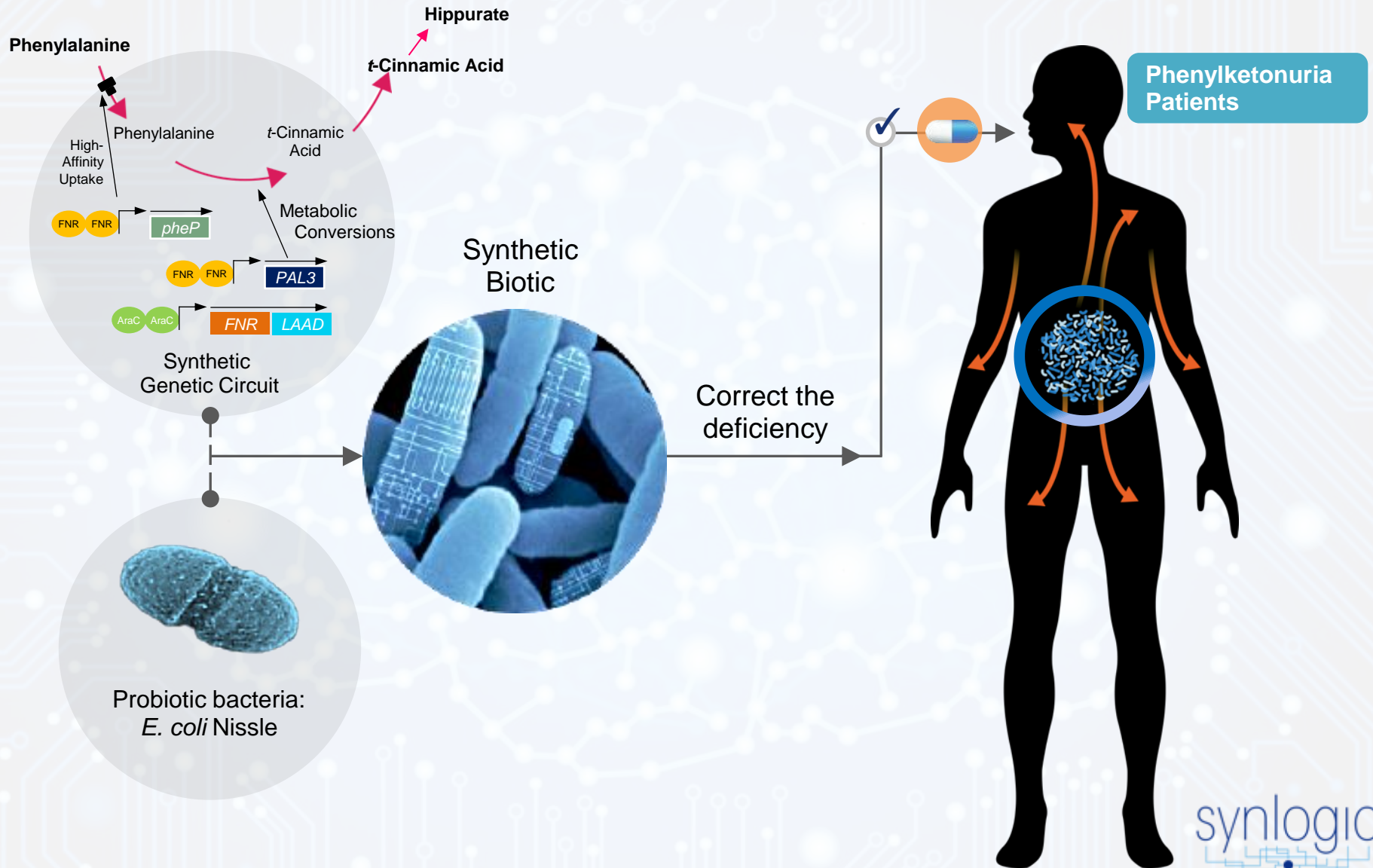
## Blood Ammonia Reduced *in vivo* (spf-ash hyperammonia/UCD model on High Protein Diet)



NC – Normal chow, HP – High protein chow  
 SYNB1010 – arg producing, Thy A auxotrophy, *Kan* resistant (a kanamycin resistant version of SYNB1020 clinical candidate)

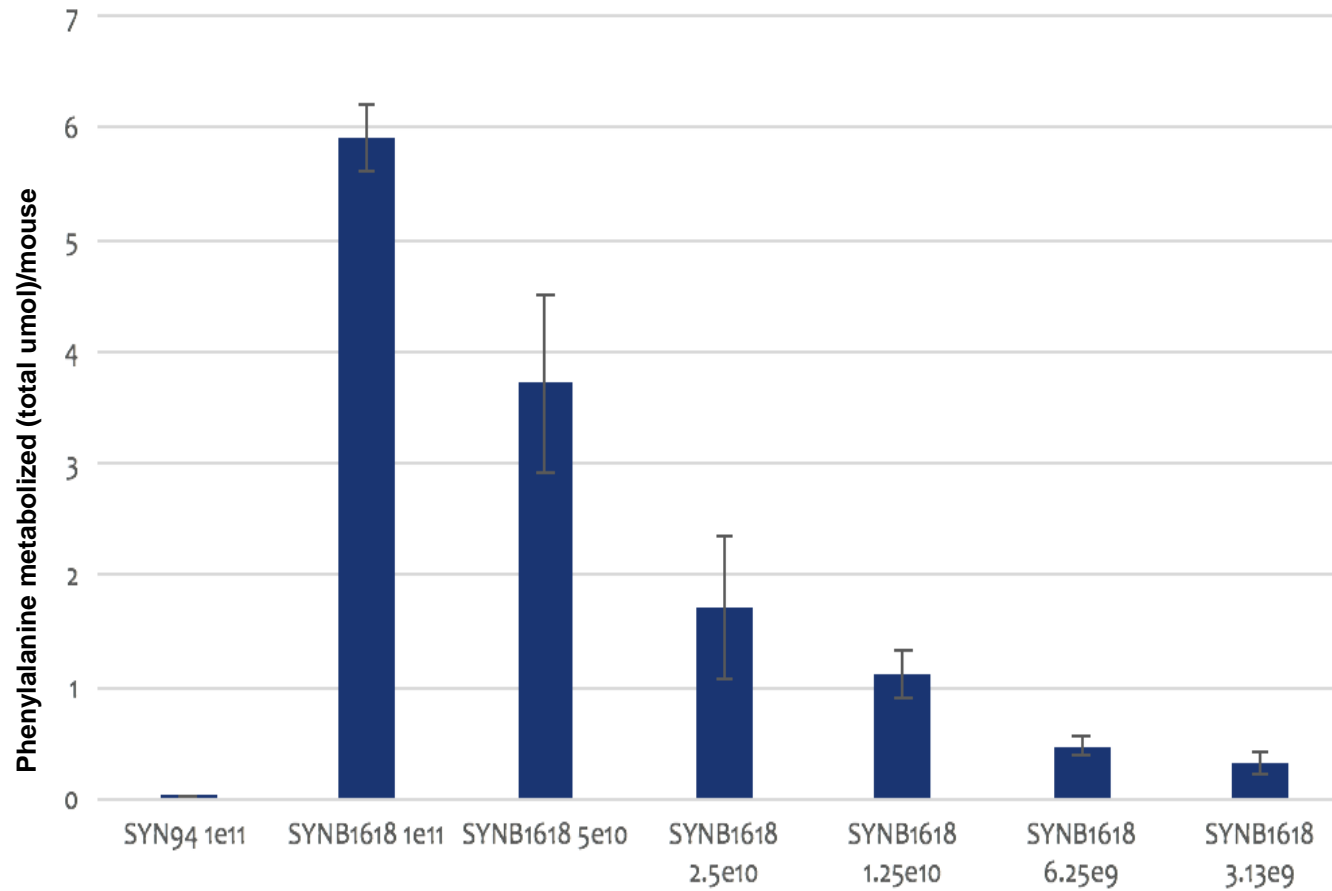


# SYNB1618: Degradation of Toxic Phenylalanine for the Treatment of PKU



# SYNB1618: Efficient Phe Degradation *In Vitro* and *In Vivo*

## Dose Response of SYNB1618 in PKU Mice



Each bar represents an average of n=9 mice/group

# Metabolic Disease and the Gut Microbiome

- FMT study in obese patients with Metabolic Syndrome
- Placebo-controlled study in 18 male patients
- Treatment group (n=9) received allogeneic microbiota from lean donors
- Placebo group (n=9) received autologous microbiota

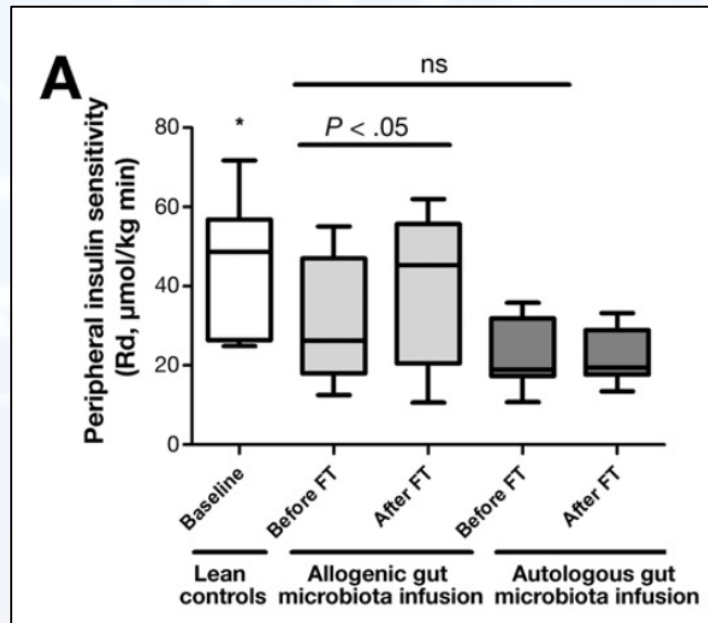
GASTROENTEROLOGY 2012;143:913-916

## BRIEF REPORT

### Transfer of Intestinal Microbiota From Lean Donors Increases Insulin Sensitivity in Individuals With Metabolic Syndrome

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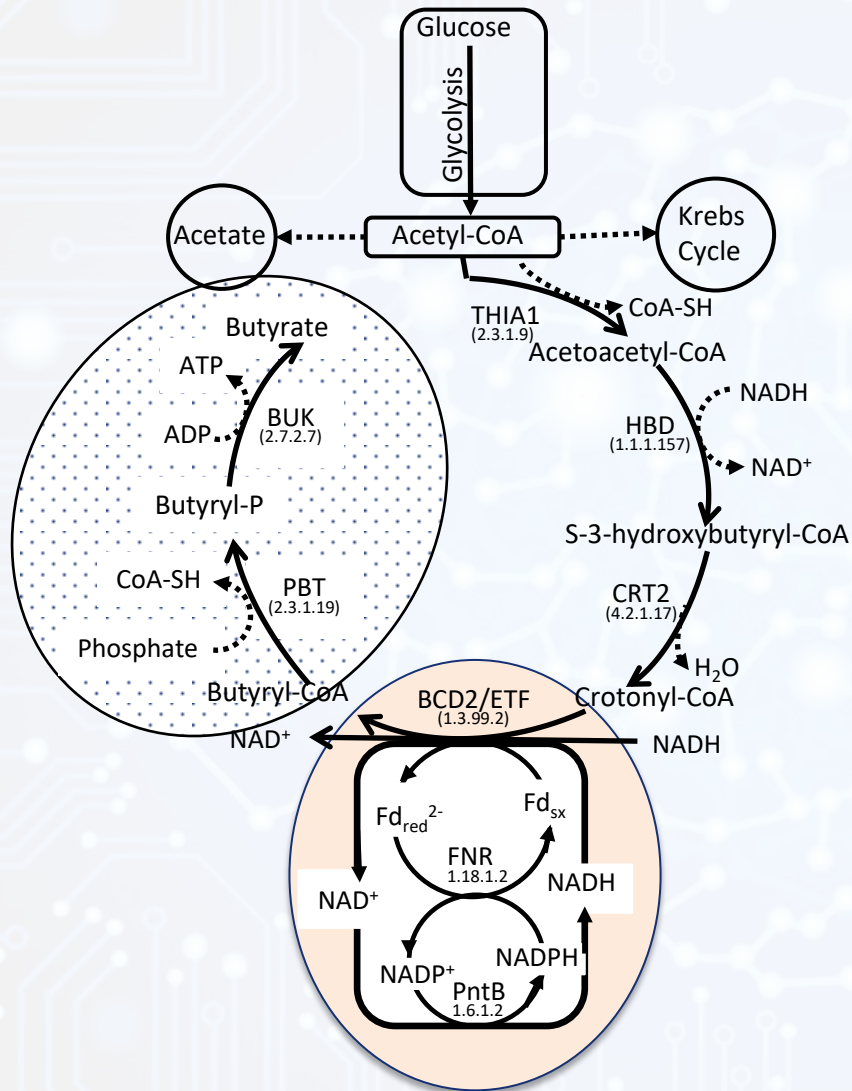
# Synthetic Biotic Approaches to Treating NASH

## NASH Pathology:

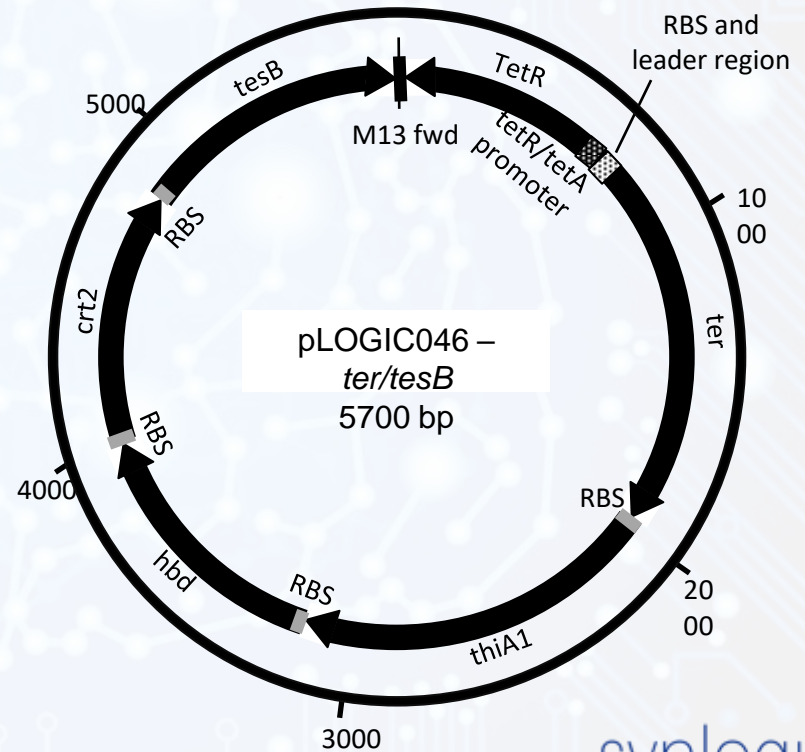
- Inflammation
- Fibrosis
- Insulin resistance
- Hyperlipidemia
- Obesity

- **SCFA Production**
  - Improves barrier function
  - Induces Treg differentiation
  - Lowers inflammation
- **GLP-1 Secretion**
  - Improves insulin secretion
  - Promotes weight loss
  - Improves lipid profiles
- **Bile salt modulation**
  - Lower lipid absorption
  - Lowers bile acid concentrations

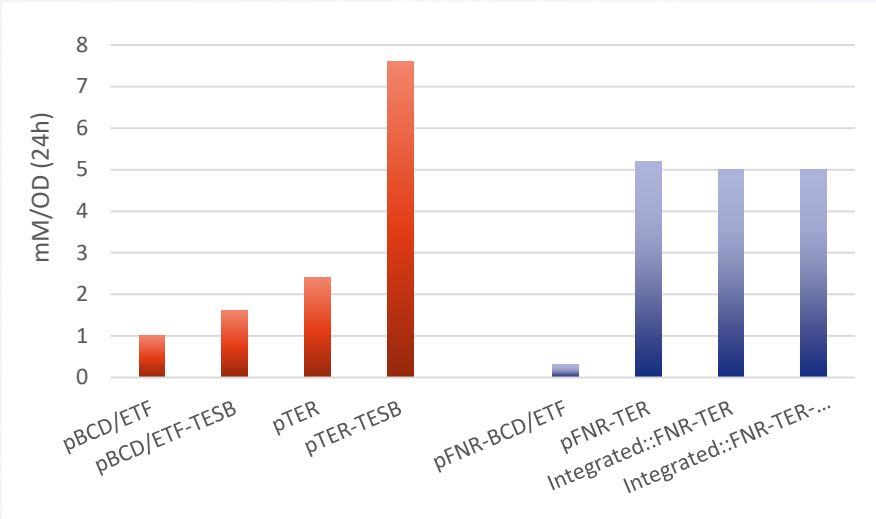
# Butyrate Production in *E. coli* Nissle



- Iterative pathway improvements increased butyrate yield and reduced circuit size
- Replacement of P<sub>tet</sub> with P<sub>fnrS</sub> retains high level production



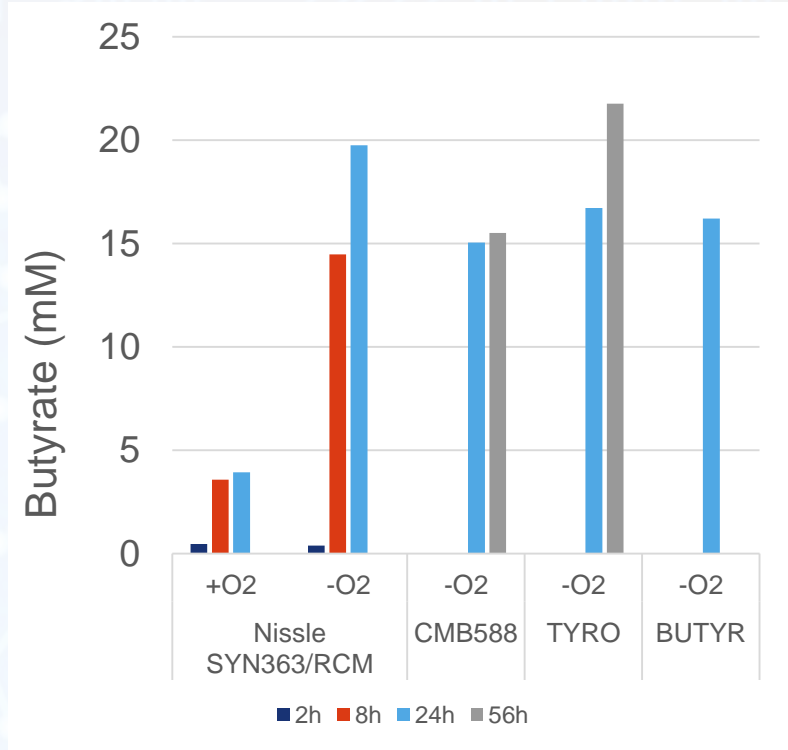
# Lead Optimization of Butyrate Producing Synthetic Biotic Strains



Iterative engineering on plasmid vectors increases butyrate yield



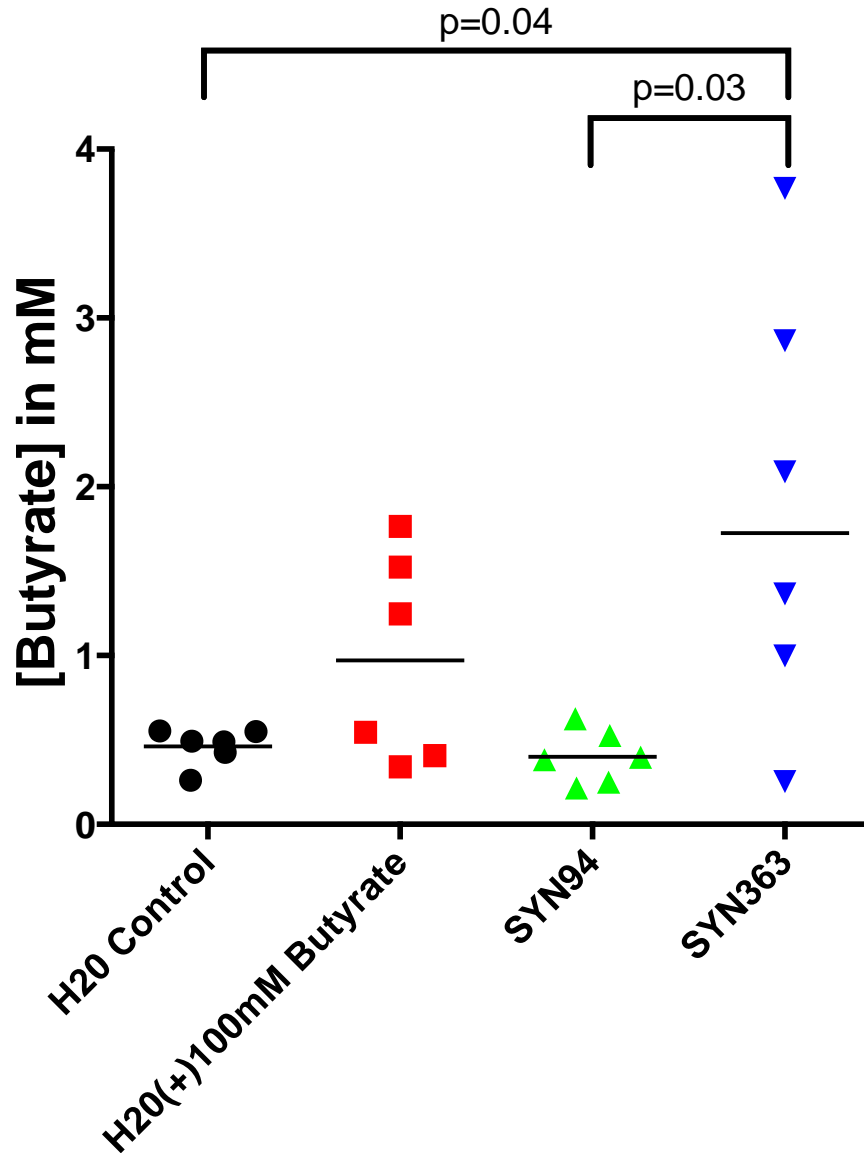
Conversion of Tet to FNR promoters with subsequent integration retains strong production



Anaerobically-induced Synlogic strain produces butyrate at similar levels to naturally-producing *Clostridia* strains (CMB588, *C. tyrobutylicum* (TYRO), *C. butyricum* (BUTYR))



# Administration of SYN363 results in significant increases in fecal butyrate levels

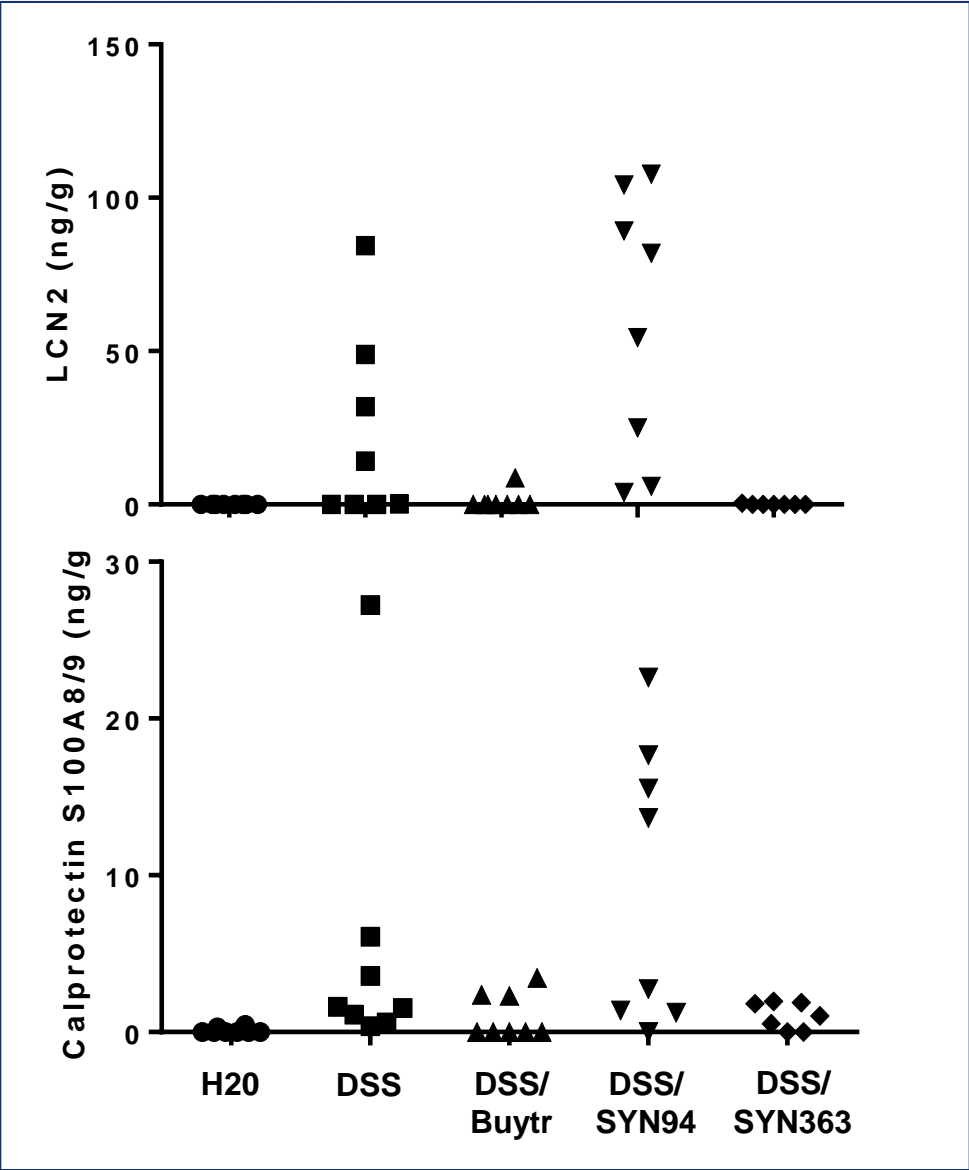


# Butyrate Effects in DSS IBD Model

- Calprotectin and LCN2 measured in fecal samples
- Produced by activated neutrophils in the mucosa
- Surrogate for gut permeability
- Used for clinical assessment of IBD

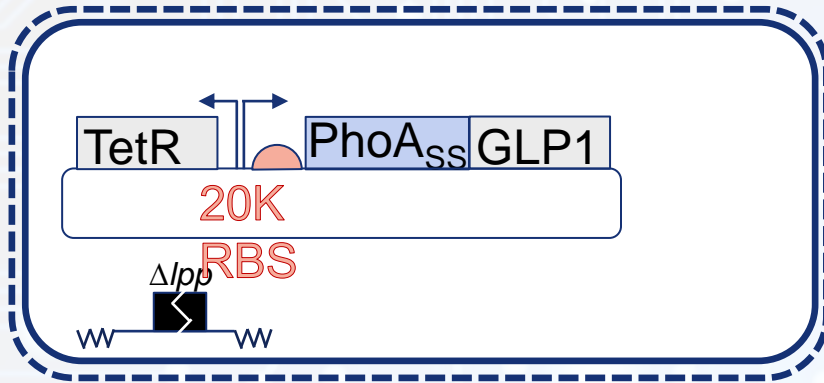
### Groups

- H<sub>2</sub>O control
- DSS alone
- DSS + oral butyrate
- DSS + SYN94 (native probiotic)
- DSS + SYN363 (butyrate strain)

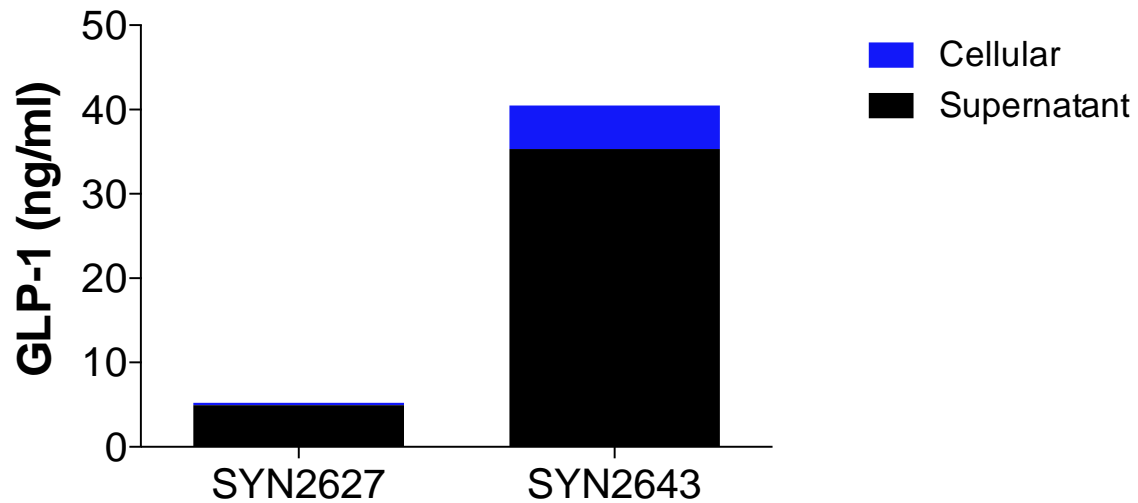
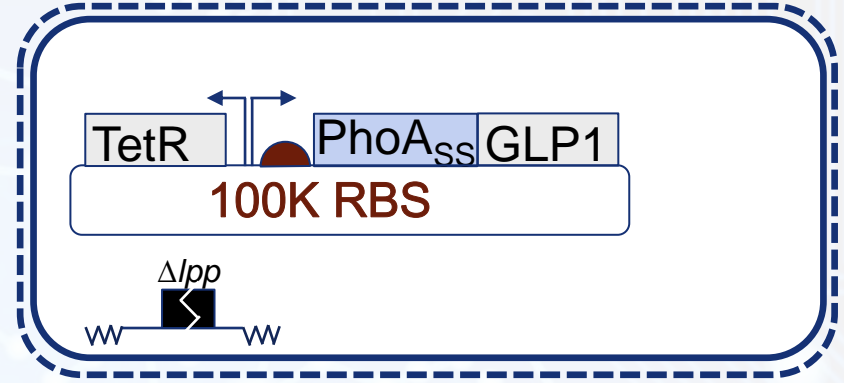


# Modulating GLP1 secretion levels with RBS

SYN2627



SYN2643



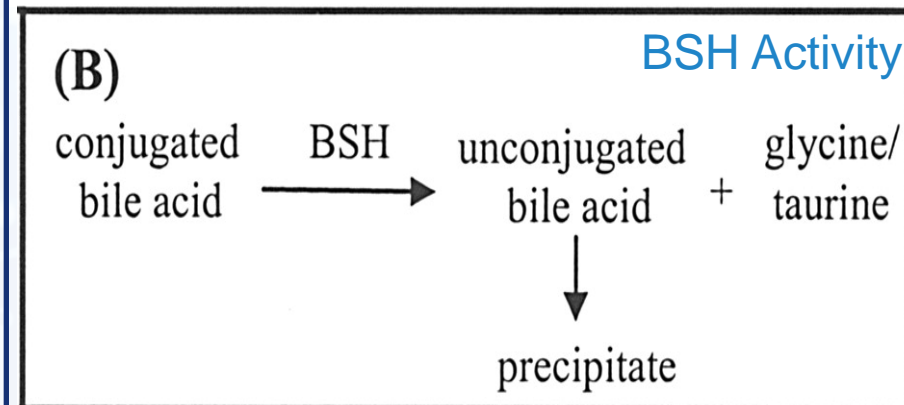
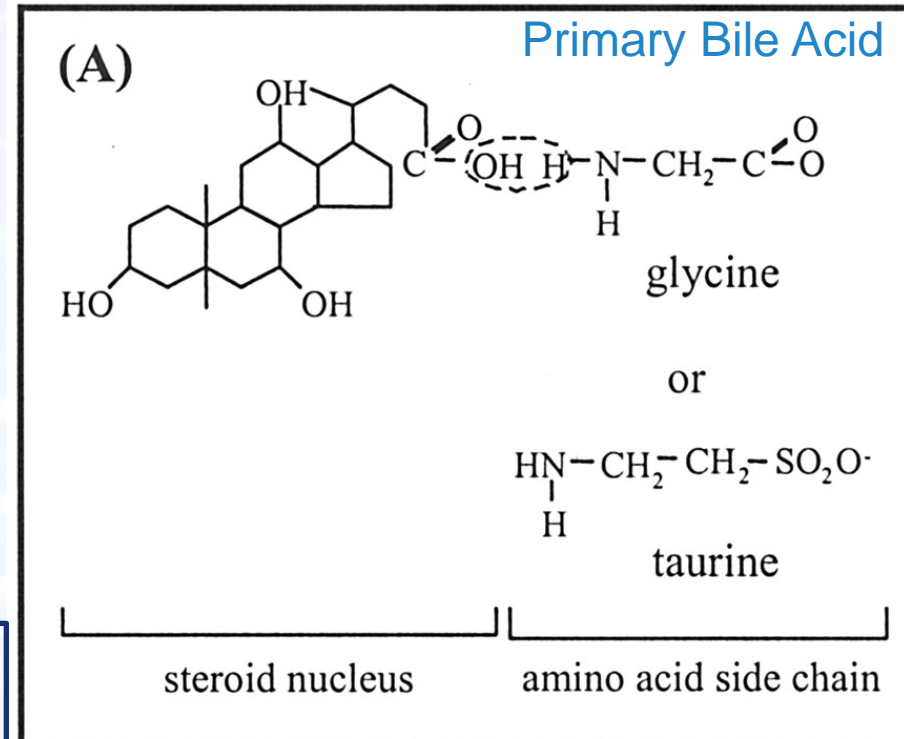
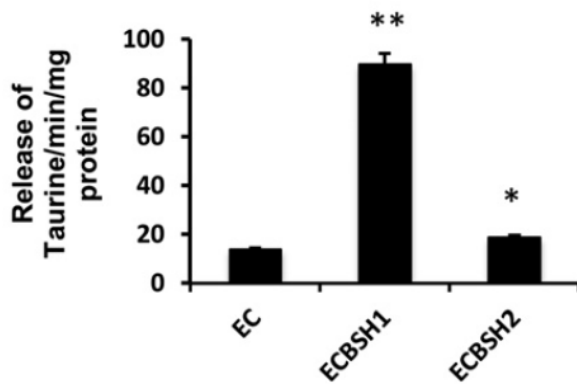
# Bile Salt Hydrolases: Consuming Primary Bile Salts

NASH patients have **elevated blood bile salt concentration**, which may be involved with liver damage.

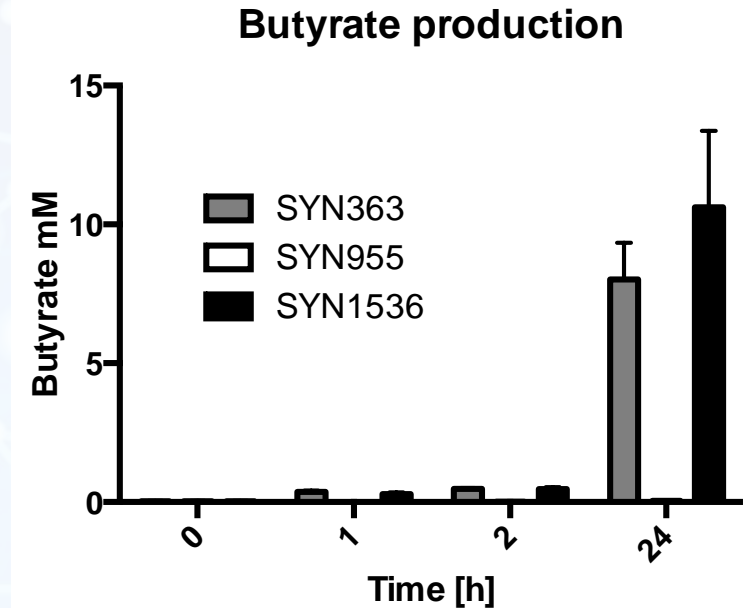
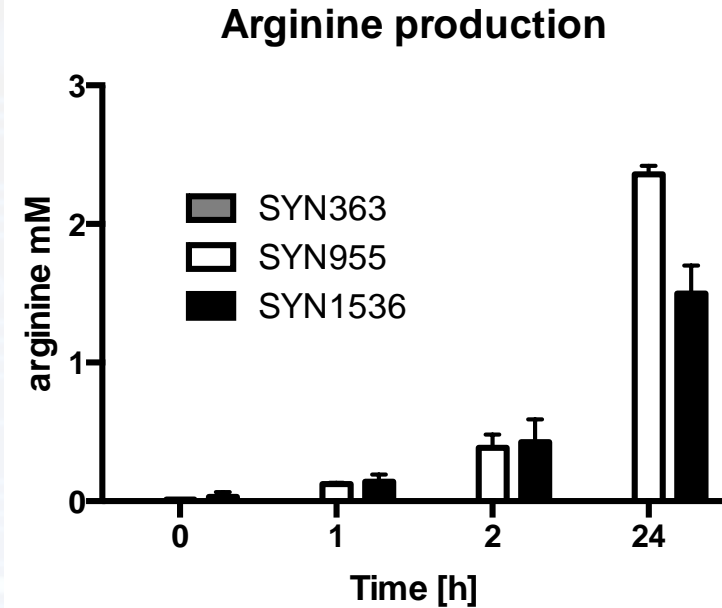
Inducible bile salt hydrolase operon



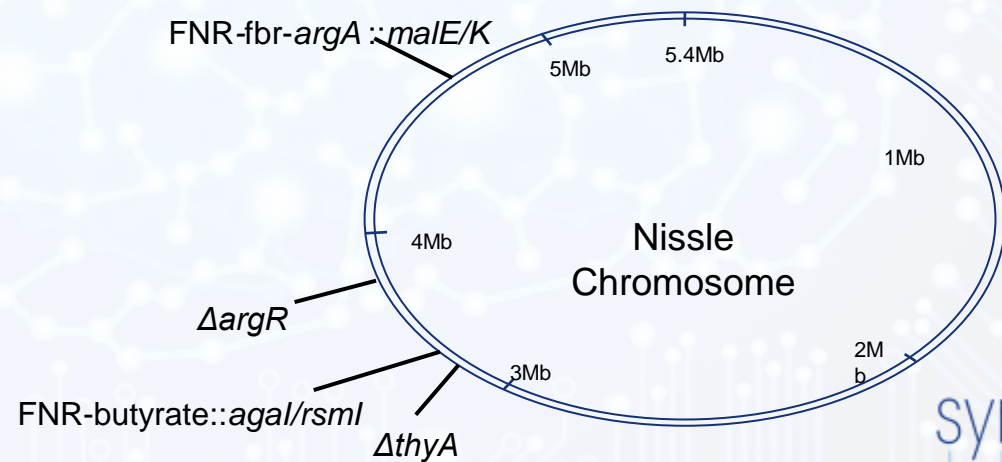
**BSH1 and BSH2 activity relative to *E. coli*-only control; measured as rate of taurine release**



# Simultaneous and efficient performance of an Integrated $\text{NH}_4^+$ /Butyrate Dual Synthetic Biotic

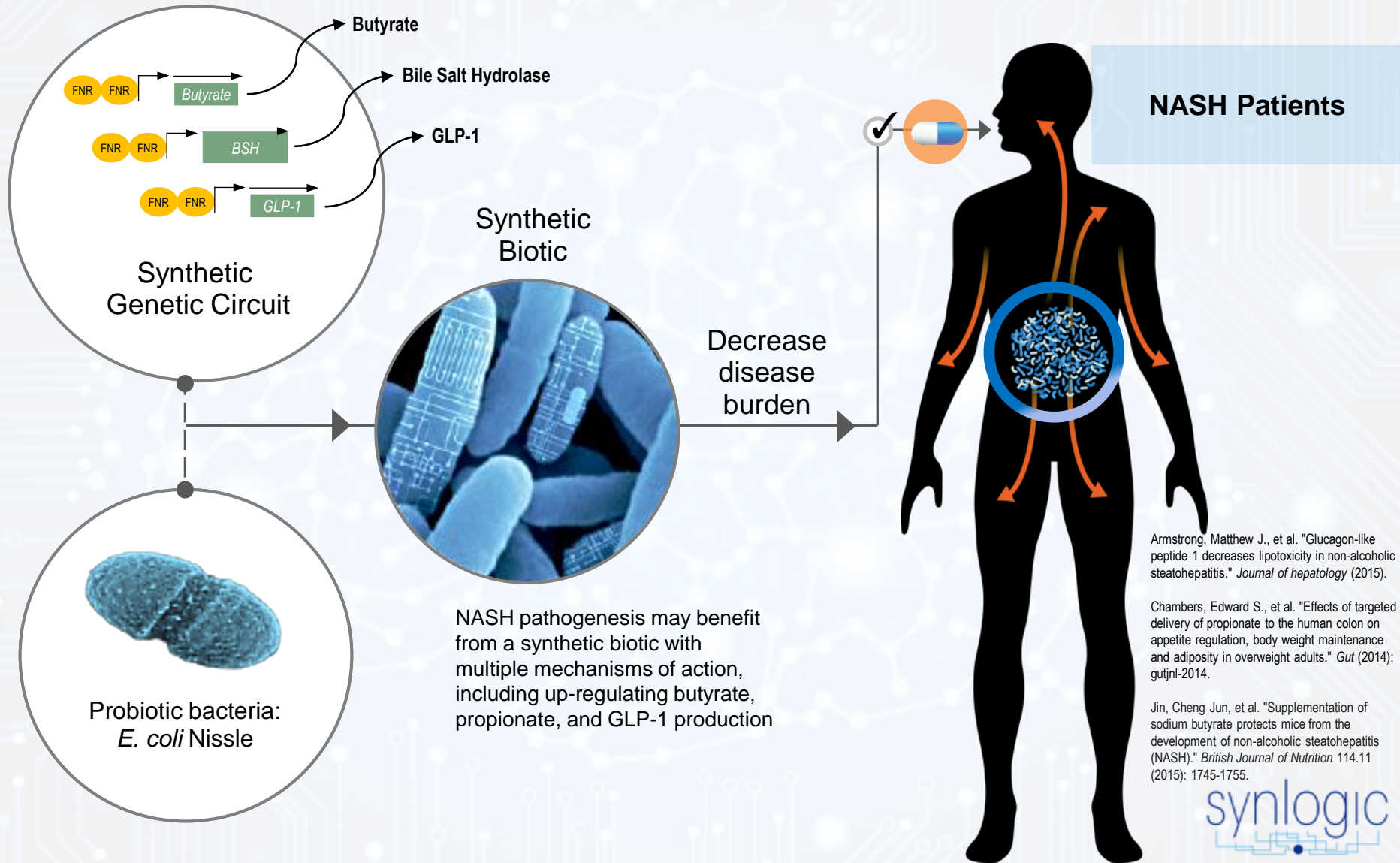


**SYN363** – E. coli Nissle harboring butyrate plasmid (P<sub>fnr</sub>-ter-pbt-buk)  
**SYN955** - E. coli Nissle  $\Delta$ ArgR; maleEK:P<sub>fnr</sub>S-fbrArgA; thyA::f<sub>rt</sub>  
**SYN1536** – SYN955 harboring butyrate plasmid (P<sub>fnr</sub>-ter-pbt-buk)





# Synthetic Biotic for NASH/PBC



# Questions?

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Synlogictx.com

