synlogic

Therapeutic Programming of Synthetic Biotic Medicines

Synthetic Biotic™ medicines to perform and deliver critical therapeutic functions to treat diseases throughout the body

An Engineered *E. coli* Nissle for the treatment of Phenylketonuria (PKU)

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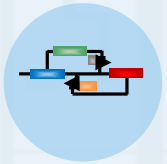
Synthetic Biotic Medicines:
A Novel Class of Living Medicines



Synthetic

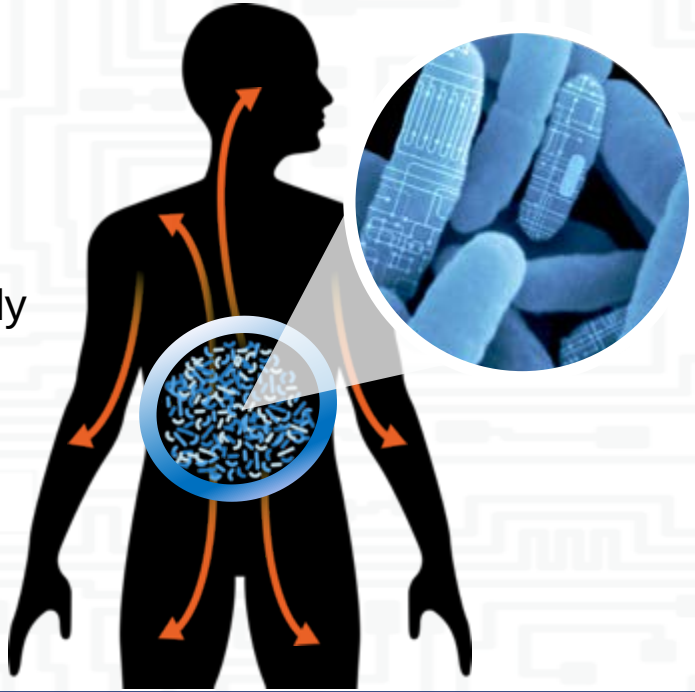
- **Engineered** bacteria
- With **designed genetic circuits**
- To **degrade metabolites** that induce disease or **synthesize substances** to treat disease

Biotic: *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 Medicine

Therapeutic delivered locally to treat systemic diseases



Synlogic Synthetic Biotic Platform:

Bringing Rational Drug Development to the Microbiome

Build Potency

Rational design:

- Synthetic biology tools applied
- Engineer potency
- Exceed endogenous bacterial activity

Apply Pharmacological Principles

Pharmacologically tractable:

- Non-colonizing
- Measurable dose-response

Develop Reliable Manufacturing

GMP manufacturing:

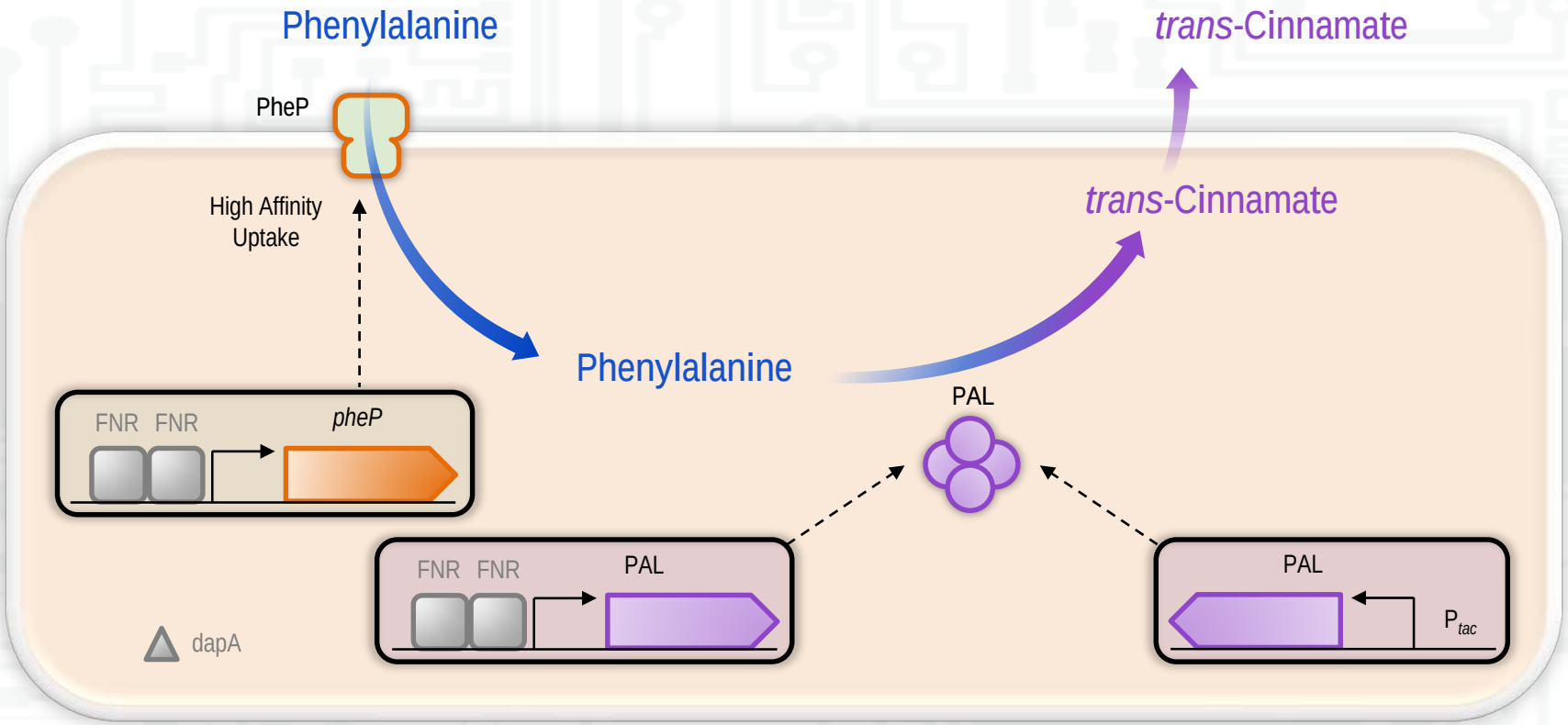
- Single strain
- Reproducible yield
- Formulation & delivery

SYNB1618 for Phenylketonuria (PKU):
Facilitating Normalization of Plasma Phe Levels

- **Rare Inherited amino acid metabolism disorder**
  - Build up of amino acid phenylalanine (Phe) in the blood and organs caused by mutation/loss of function of Phenylalanine hydroxylase (PAH), which normally converts Phe to Tyr
- **Diagnosed:** 16,500 in US, similar in Europe
  - If left untreated, symptoms include cognitive impairment, convulsions, behavior problems, skin rash, musty body odor
- **Treatment:**
  - Low protein diet (no meat, dairy, nuts, eggs)
    - Difficult to maintain lifelong compliance
  - Kuvan: PAH cofactor (Only for patients with some residual PAH activity)
    - Cofactor of PAH enzyme (20-40% of patients are responsive)

SYNB1618 Mechanism of Action:

Designed to Convert Toxic Phenylalanine to non-toxic metabolites

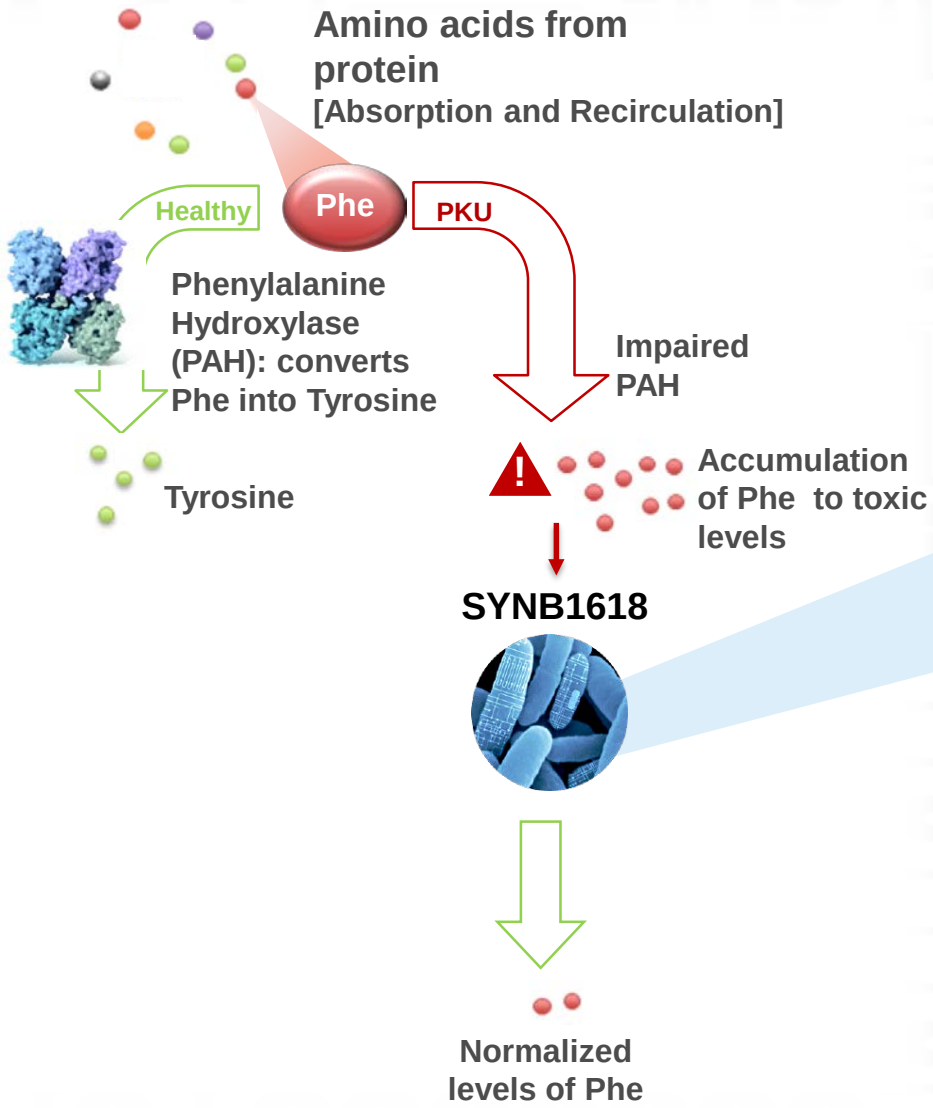


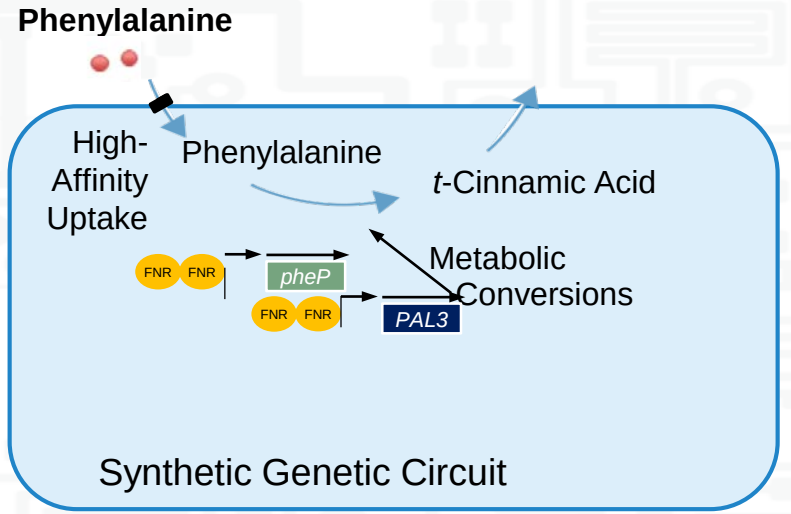
Key strain design elements:

- **PAL (Phenylalanine ammonia lyase)** – Breaks down Phe to non toxic byproduct, *trans*-cinnamate (TCA)
- ***pheP*** – High affinity Phe transporter – increase rate of Phe uptake into engineered cells, alleviating transport bottleneck
- **FNR (fumarate and nitrate reductase regulator)** promoter – Activates transcription of payload *in vivo*
- ***ΔdapA*** auxotrophy as biocontainment element

SYNB1618 Mechanism of Action:

Designed to Convert Toxic Phenylalanine to *Trans*-cinnamic Acid



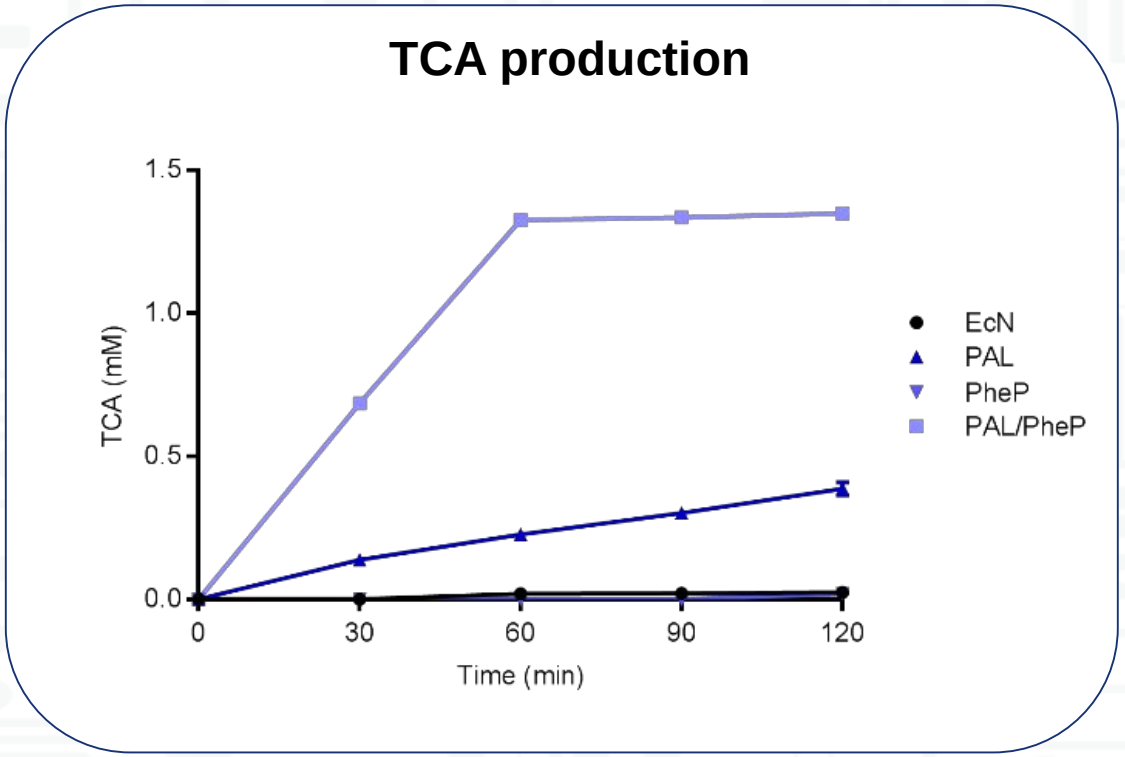


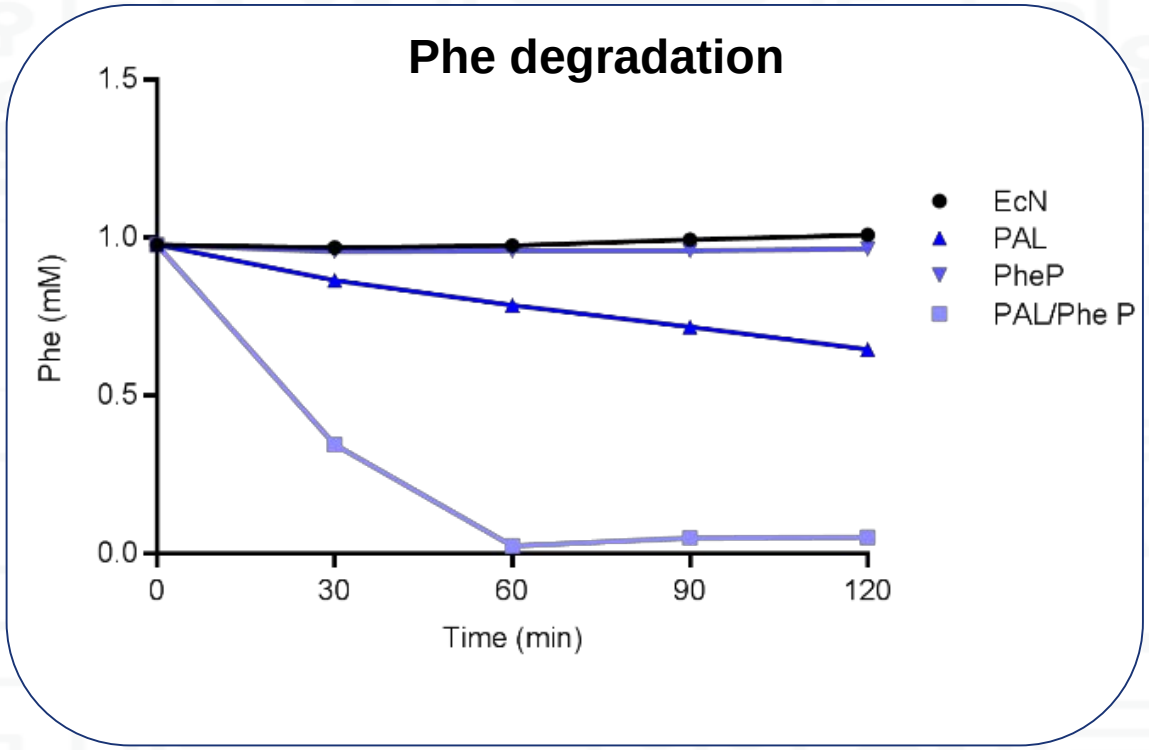
Probiotic bacteria: E. Coli Nissle

When Phe is not efficiently metabolized (PKU) **SYNB1618** provides an alternative mechanism

Mechanism of Action:

Functional analysis of PAL and *pheP* in vitro in *E. coli* Nissle





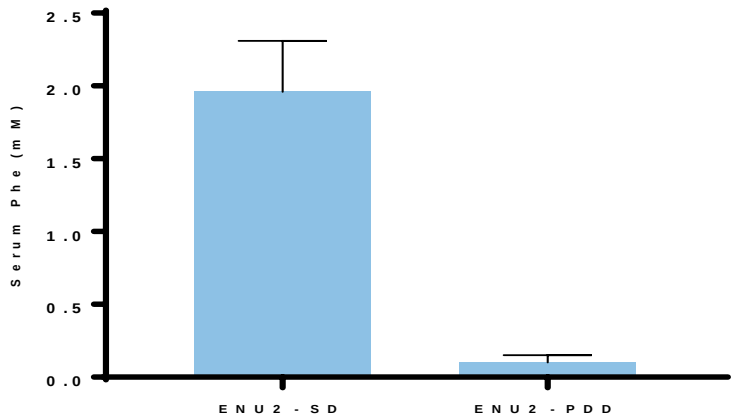
- Expression of PAL leads to production of *trans*-cinnamate (TCA) as a product of Phe degradation
- Uptake of Phe is rate-limiting; Expression of transporter, *pheP*, led to a 7-fold increase in the rate of TCA production/Phe degradation

Pah$^{enu2/enu2}$: A mouse model of PKU

Profiling the mouse model and the small intestine as a “Phe sink”

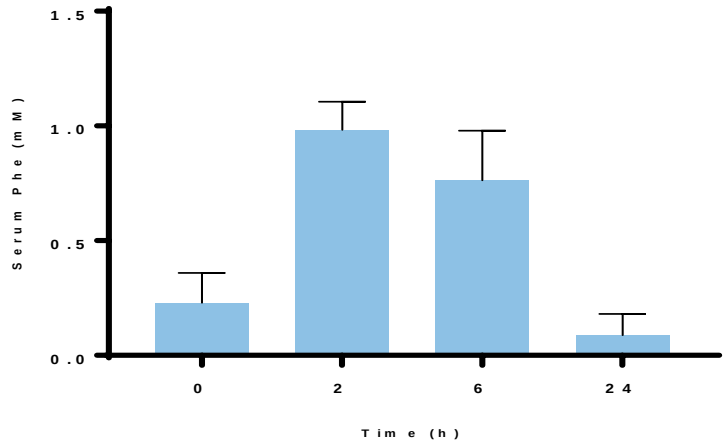
A

Standard diet (SD) vs. Phe-deficient diet (PDD) in *Pah*$^{enu2/enu2}$ *mice*



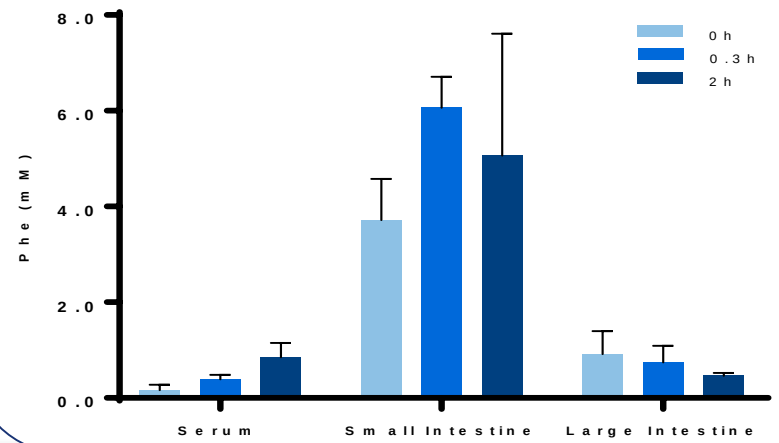
B

Stable elevation of Phe post-SQ injection in *Pah*$^{enu2/enu2}$ mice



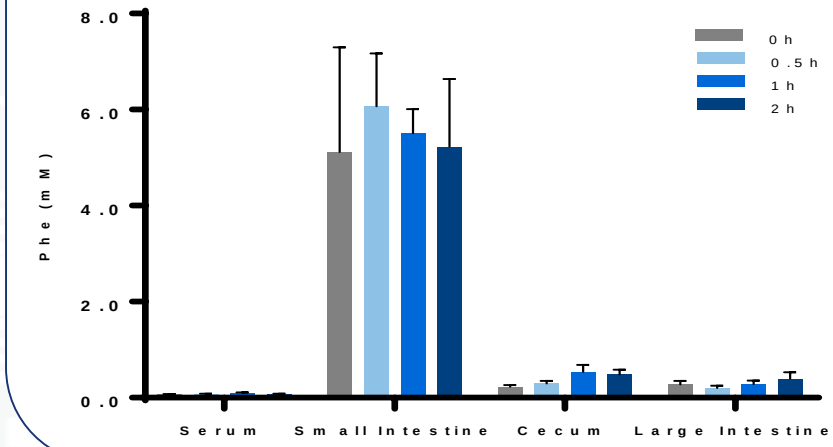
C

High Phe in small intestine of *Pah*$^{enu2/enu2}$ mice



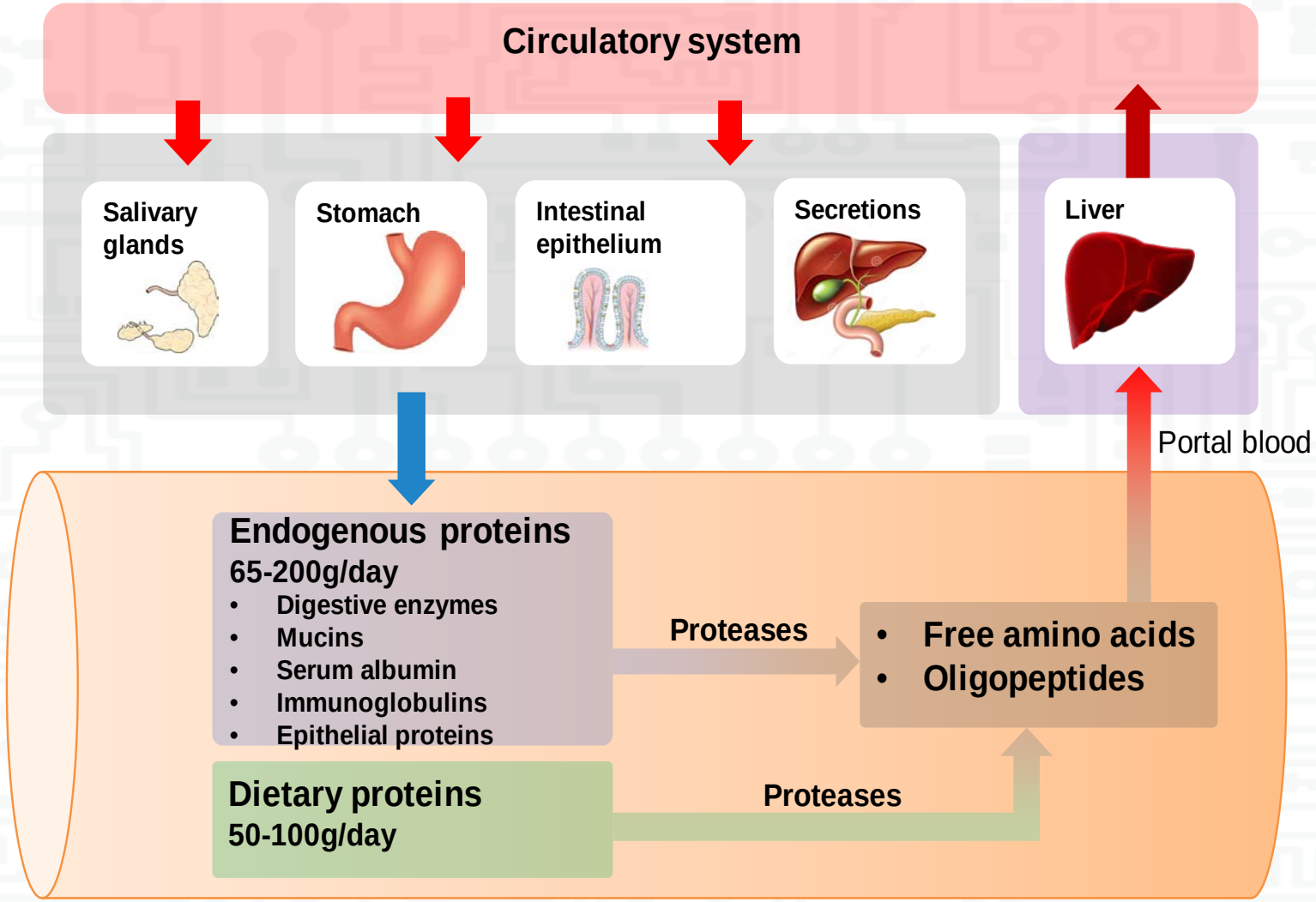
D

High Phe in small intestine of C57BL/6 mice



Dietary vs Non-Dietary sources of Phe

Enterorecirculation as a source of free Phe



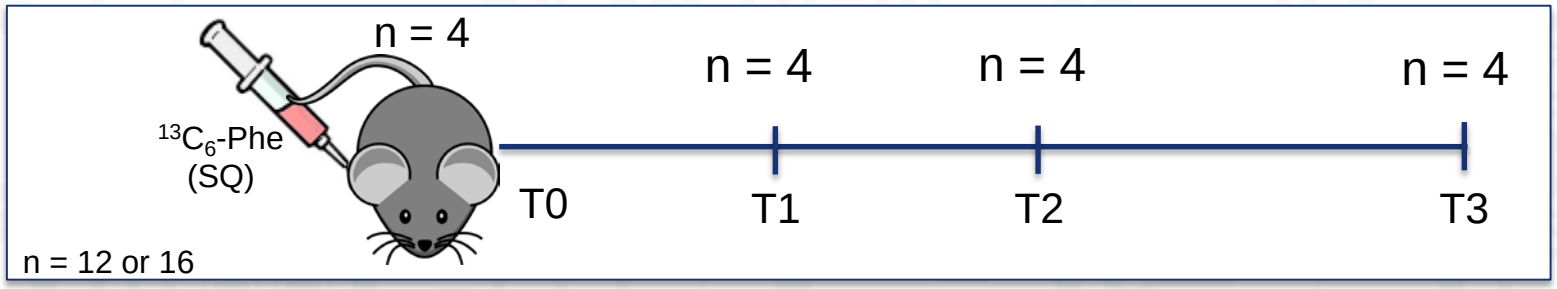
Chang TM, Nature Reviews in Drug Discovery, 2005
Dave AL et al., Peptides, 2016

Enterorecirculation and Phe:

- Dietary protein is not the only source of Phe in small intestine
- Amino acids recycled into the GI tract for reabsorption
- High steady-state levels of free Phe in the small intestine

Enterorecirculation of Phe in mice

Isotopically-labeled Phe in blood appears in the GI tract



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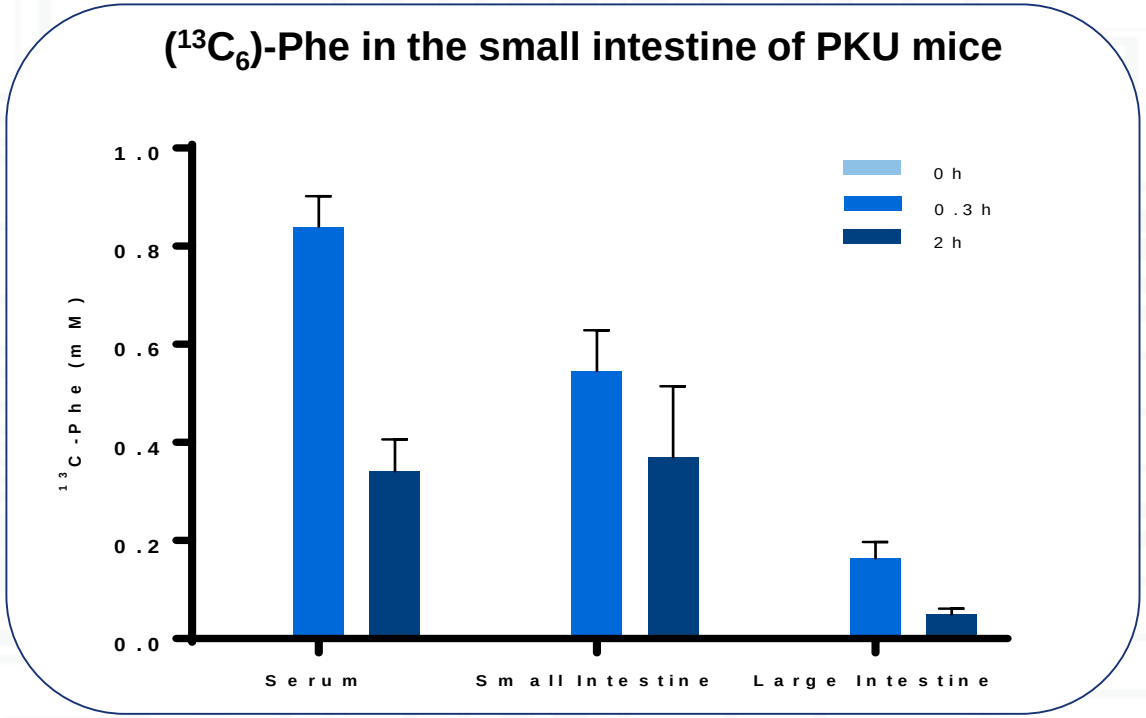

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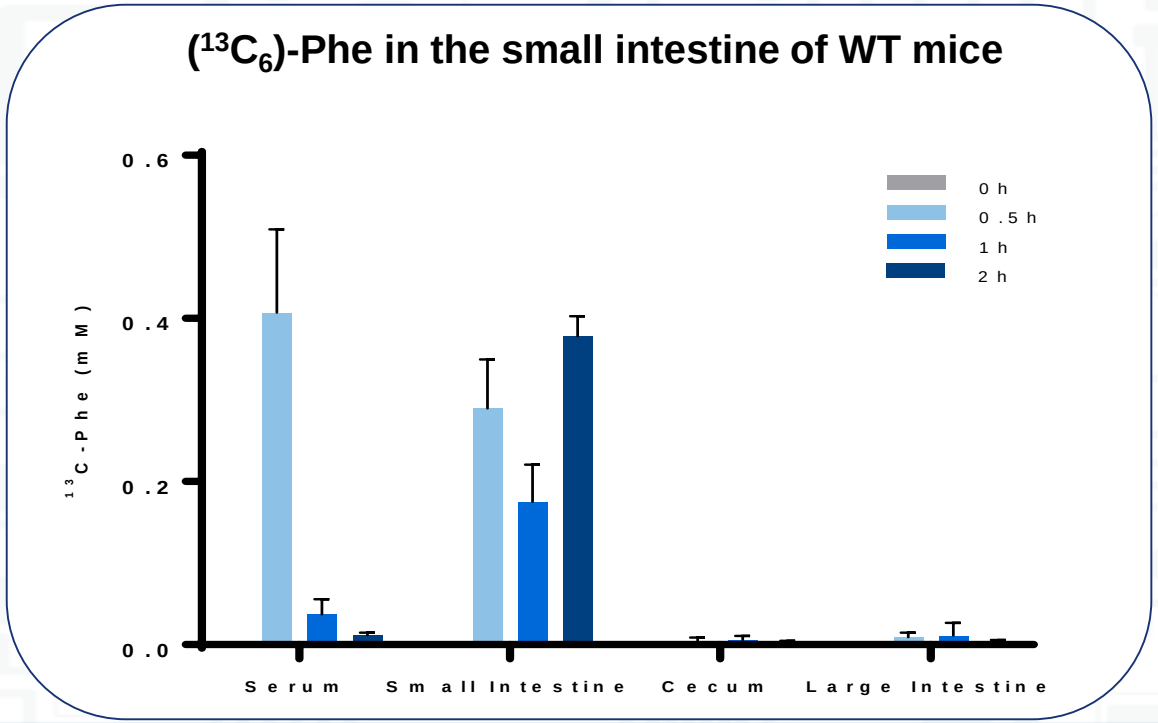
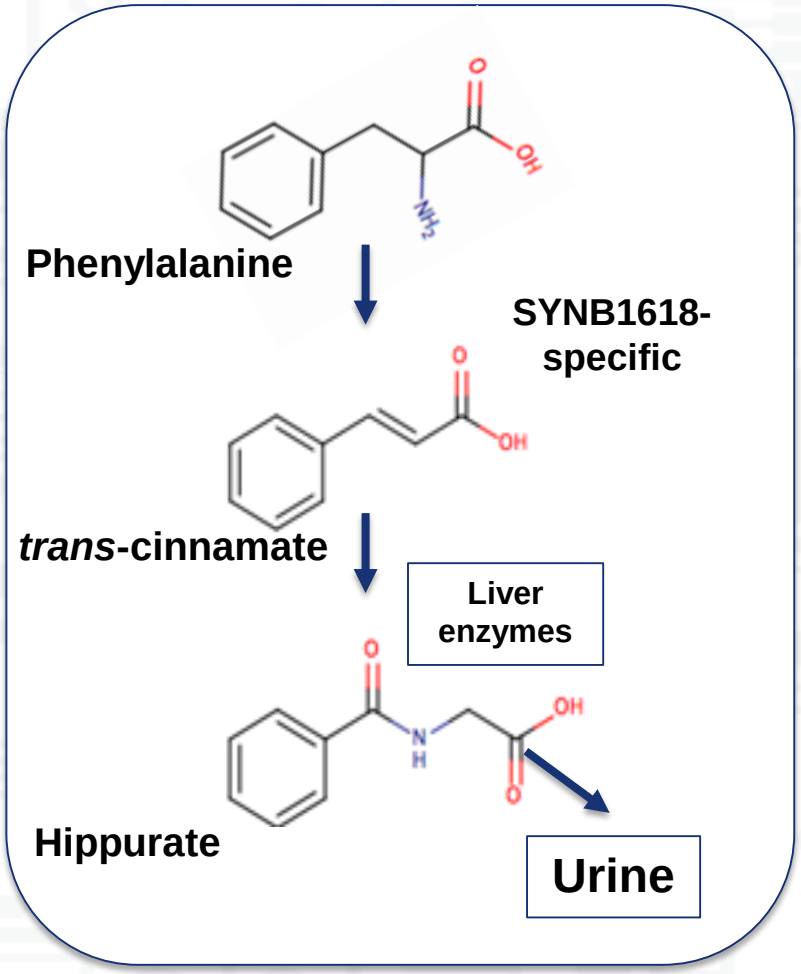


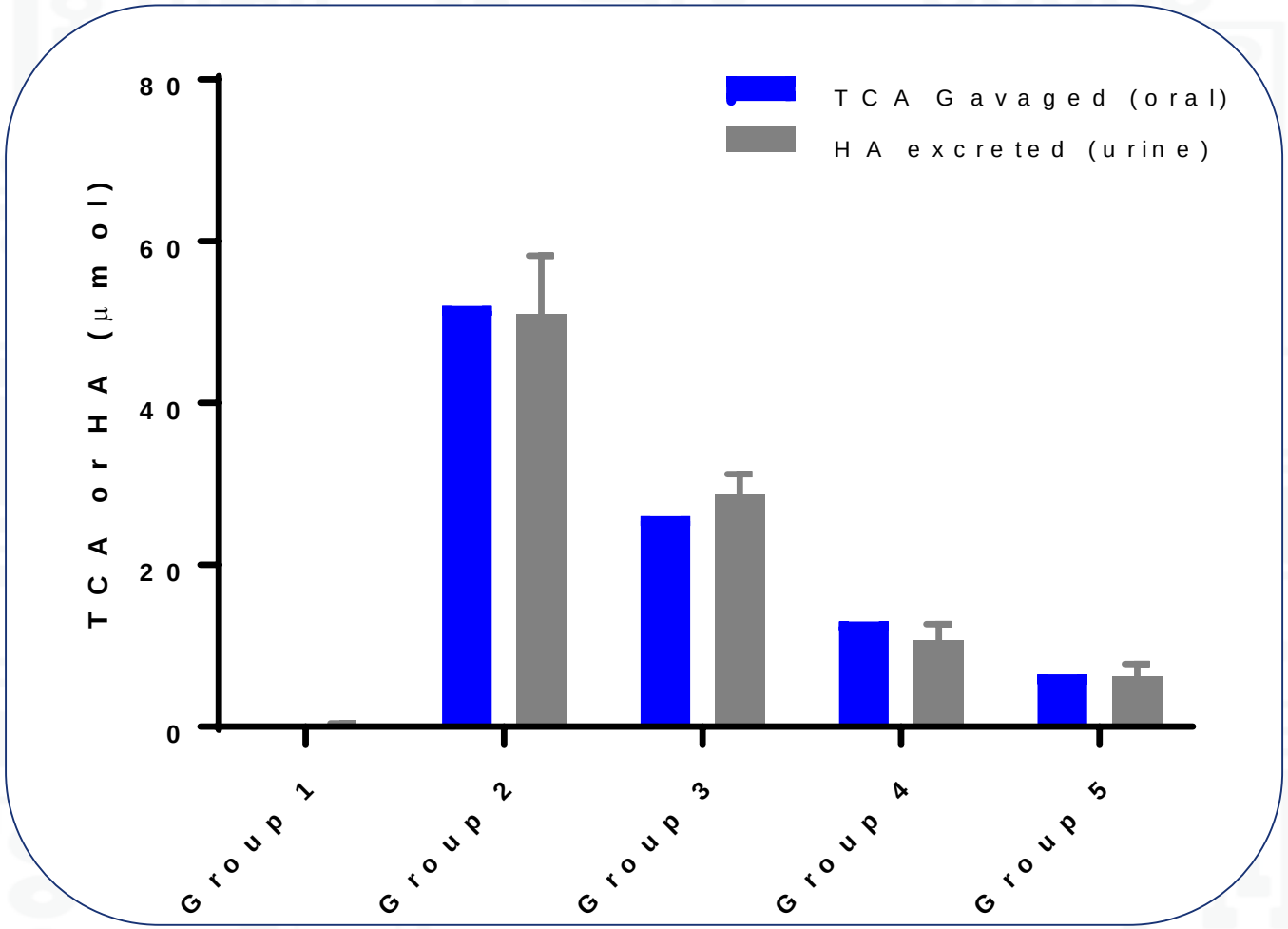
Enterorecirculation and Phe:

- Phe delivered to the blood was found in the small intestine of both PKU and WT mice as early as 20 min

Hippurate (HA): A biomarker of SYNB1618 activity in vivo

The fate of orally dosed TCA



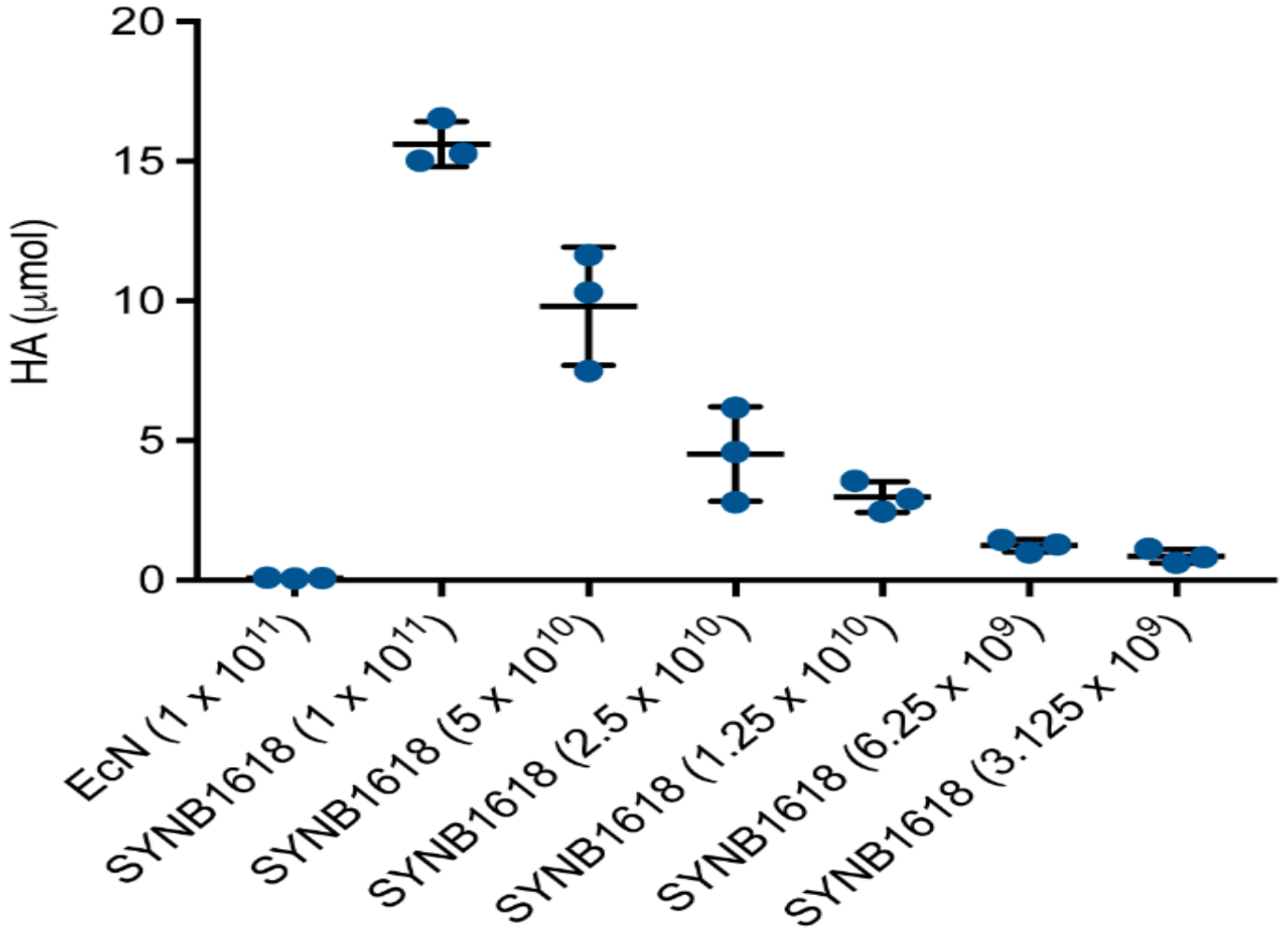


- Essentially all orally dosed TCA recovered as urinary hippurate (HA)
- HA could serve as a biomarker of SYNB1618 activity in vivo

Dose-dependent activity of SYNB1618 in *Pah*$^{enu2/enu2}$ mice:

HA is a biomarker of SYNB1618 activity in vivo

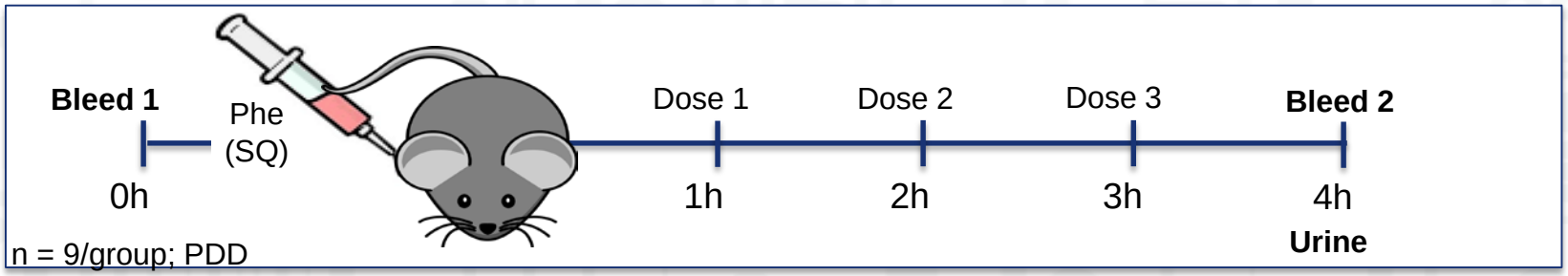
Urine collection over 4h for determination of HA recovery



Each dot represents urine collected from a metabolic cage of 3 mice/cage

In vivo efficacy of SYNB1618 in *Pah*$^{enu2/enu2}$ mice

SYNB1618 reduces enterorecirculating Phe with concomitant production of urinary HA



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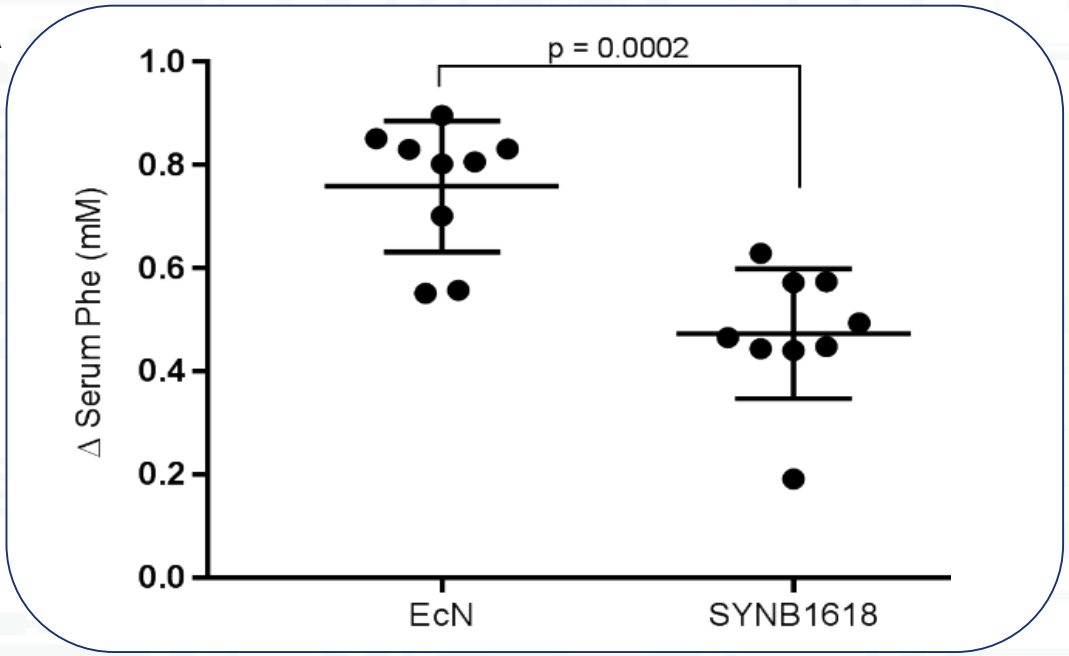
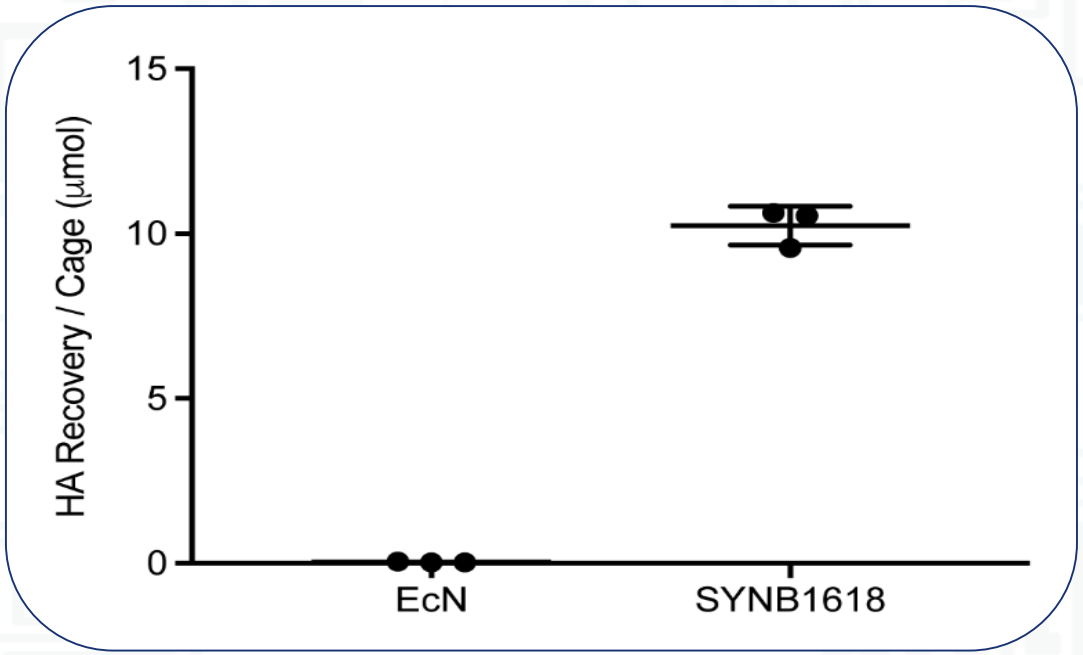


Each dot represents an individual PKU mouse

B



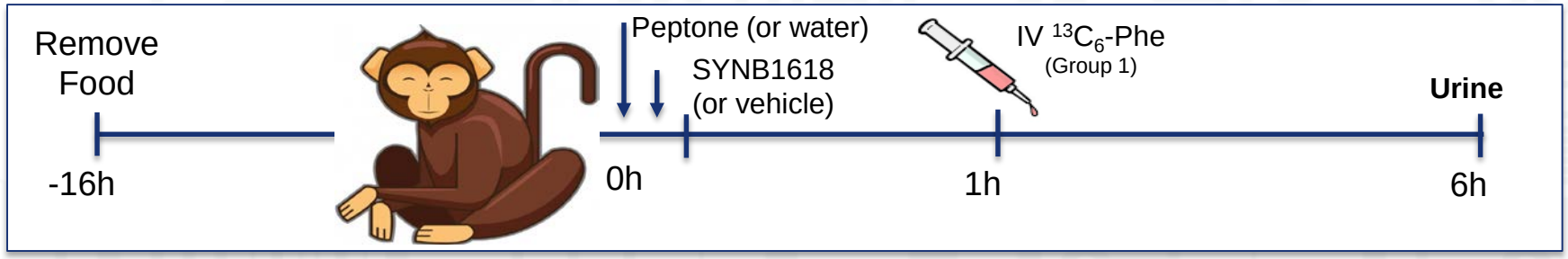
Each dot represents collection from a metabolic cage of 3 mice/cage

Results:

- Enterorecirculating (non-dietary) Phe is accessible within the GI tract; its degradation can lead to significant serum Phe reduction

In vivo activity of SYNB1618 in healthy non-human primates

Evidence for a “Phe sink” and enterorecirculation in a primate model



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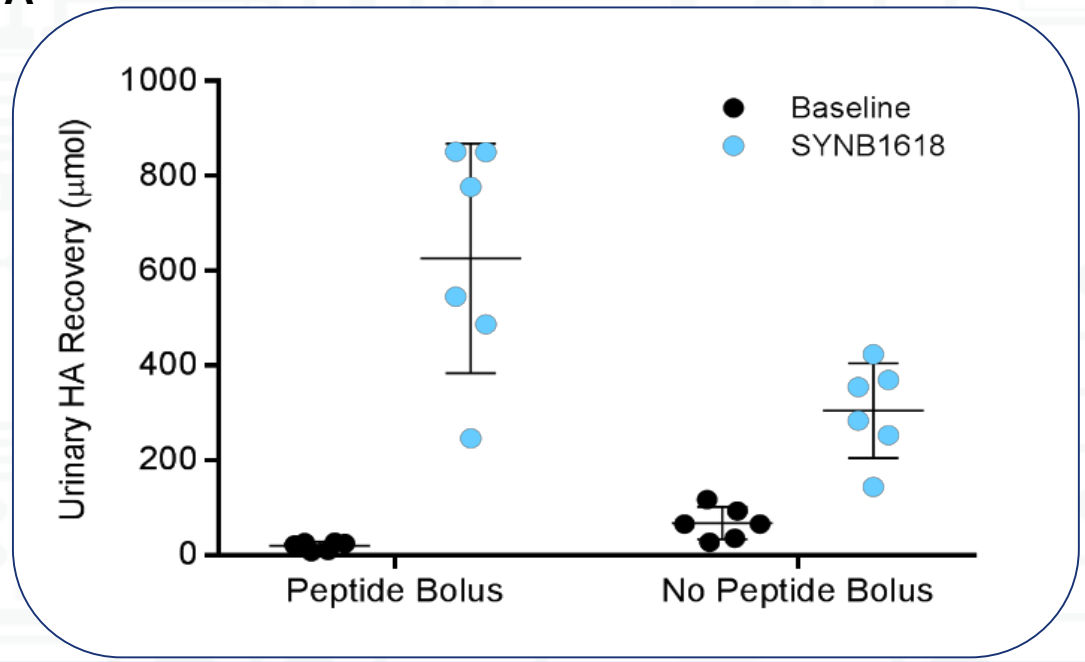

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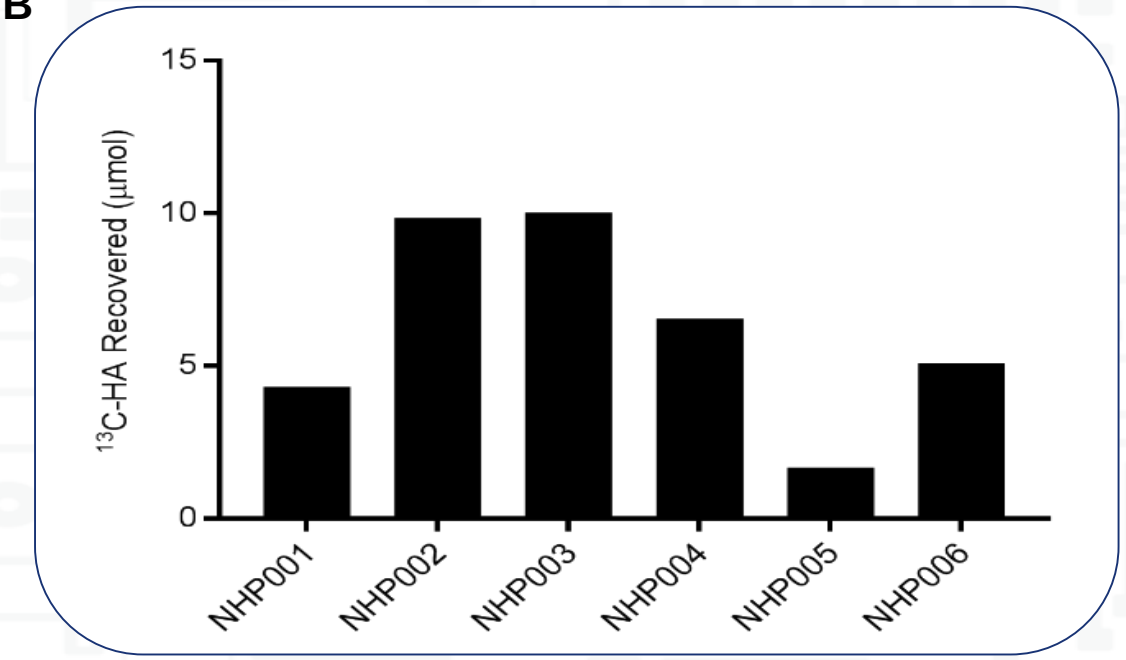
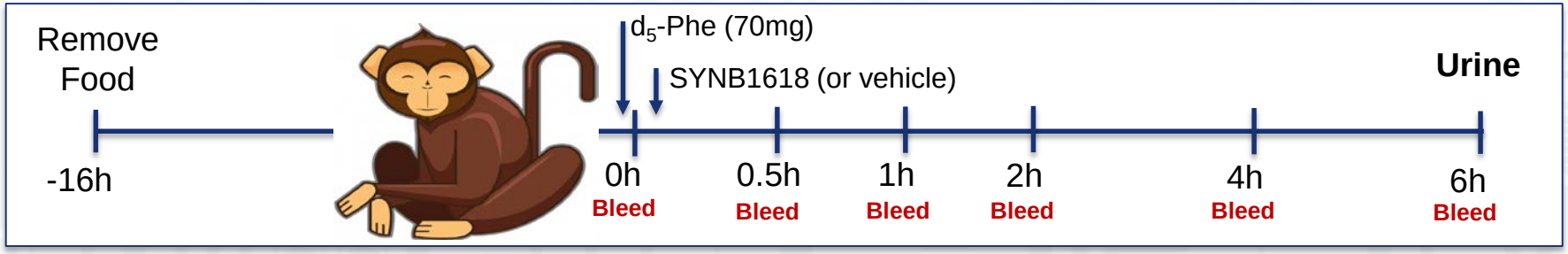


Results:

- Significant HA recovered from fasted animals, even those that did not receive a peptide bolus
- IV $^{13}C_6$-Phe was recovered in the urine as $^{13}C_6$-HA, demonstrating enterorecirculation and SYNB1618 activity

In vivo efficacy of SYNB1618 in healthy NHPs

SYNB1618 results in significant blunting in serum Phe following challenge



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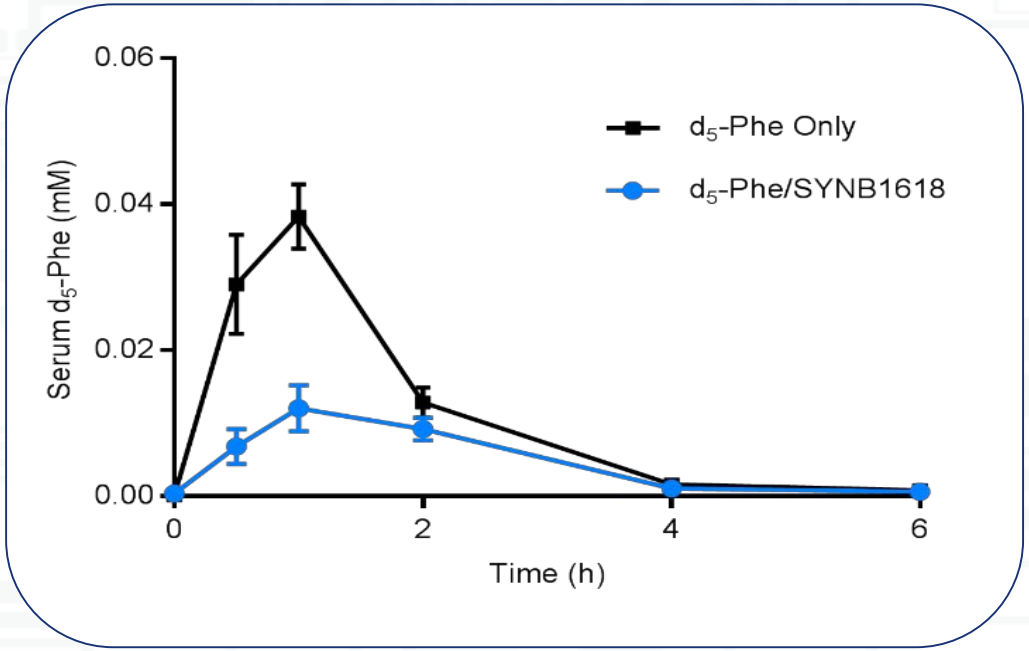

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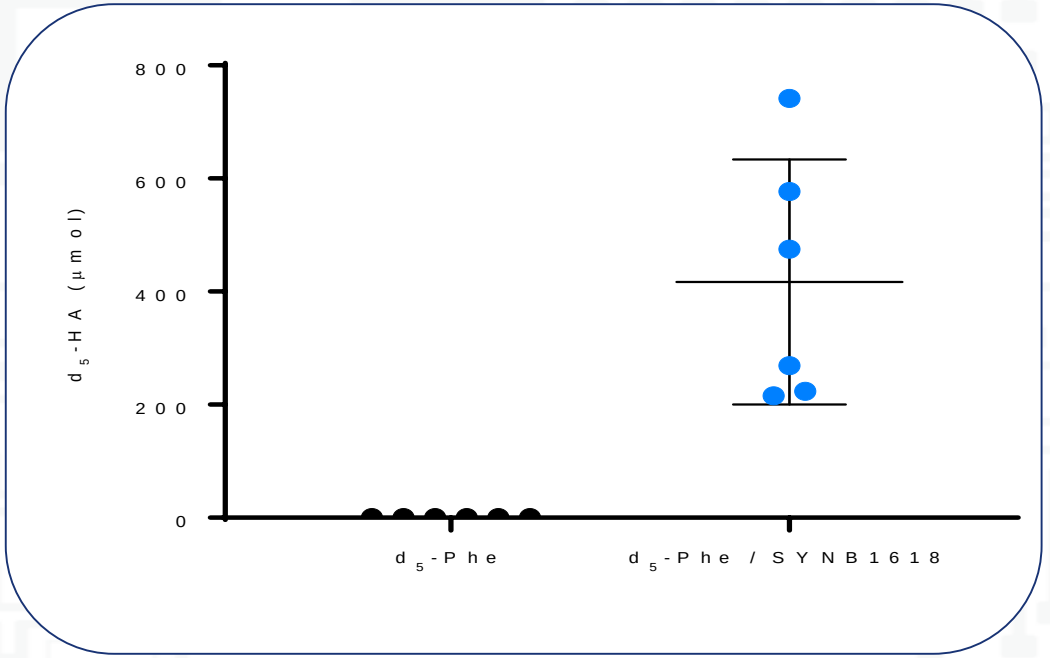
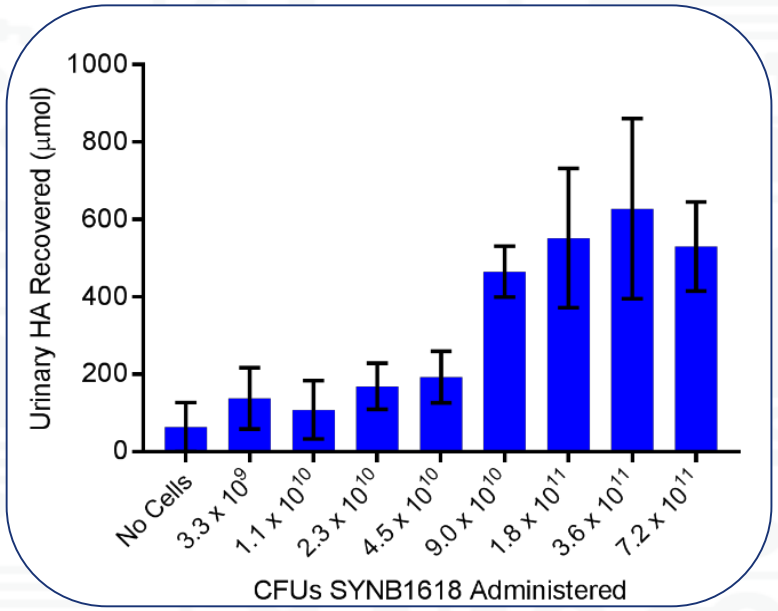


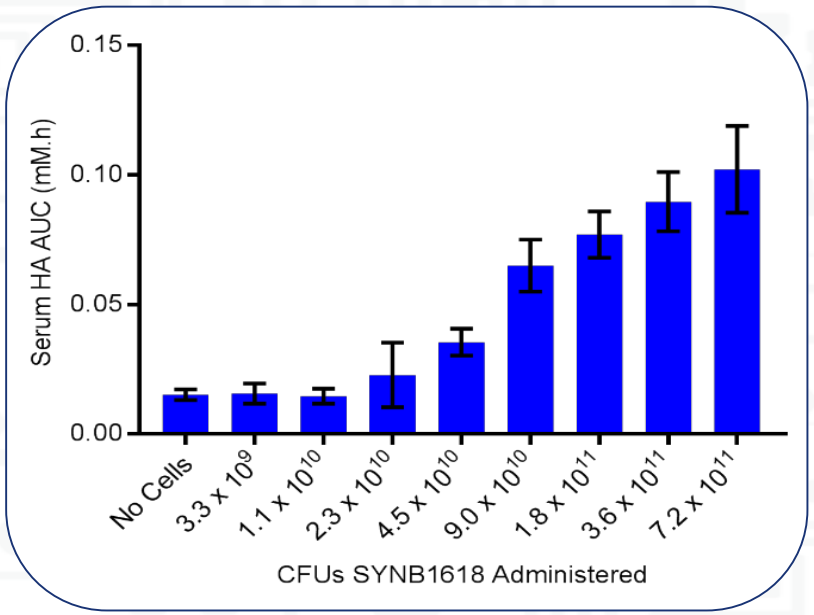
Results:

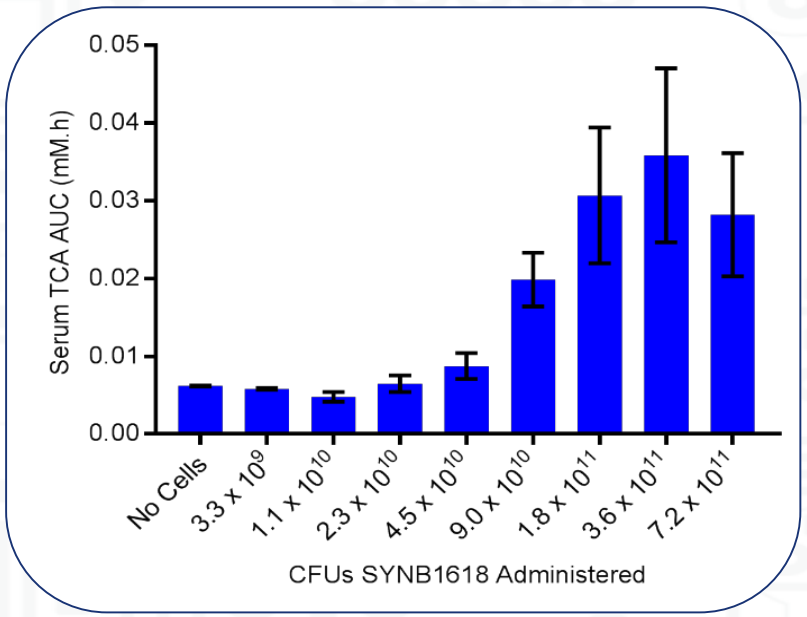
- SYNB1618 administration led to significant decrease (p = 0.015) in d_5-Phe AUC with corresponding increase in d_5-HA recovered in the urine

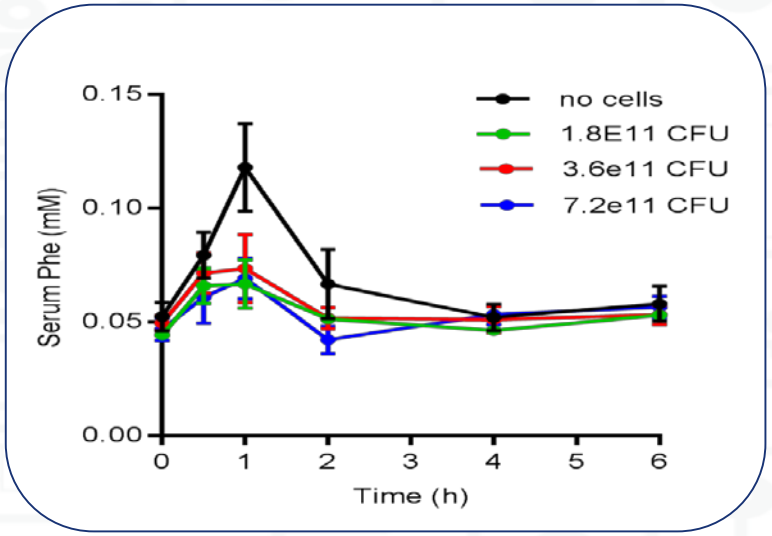
Dose-responsive activity of SYNB1618 in healthy NHPs

SYNB1618 exhibits dose-dependent pharmacokinetics









Results:

- Dose-responsive recovery of urinary HA
- Dose-responsive serum AUC for TCA and HA
- Significant blunting of serum Phe elevation at the 3 highest doses of SYNB1618 ($p < 0.05$)

Conclusions

- **A chromosomally integrated, modified *E. coli* Nissle strain, SYNB1618, was created and could degrade Phenylalanine to the non-toxic product *trans*-cinnamate**
  - **Activity of the strain could be enhanced by co-expression of high affinity transporter, *pheP***
- **Phenylalanine is abundant in the small intestine**
  - **Both dietary and non-dietary Phe make up a “reservoir” of Phe in the GI tract**
  - **Phe from the blood can re-enter the GI tract through enterorecirculation**
- **The product of SYNB1618, *trans*-cinnamate, is converted to hippurate and excreted in urine, which can be used as a quantitative biomarker of in vivo strain activity**
- **SYNB1618, administered orally, can result in significant decreases in serum Phe in both mice and NHP**
  - **SYNB1618 also exhibits dose-responsive pharmacokinetics**
- **SYNB1618 has entered Phase 1 trials in healthy volunteers**

Acknowledgements

Discovery
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- Chris Bergeron

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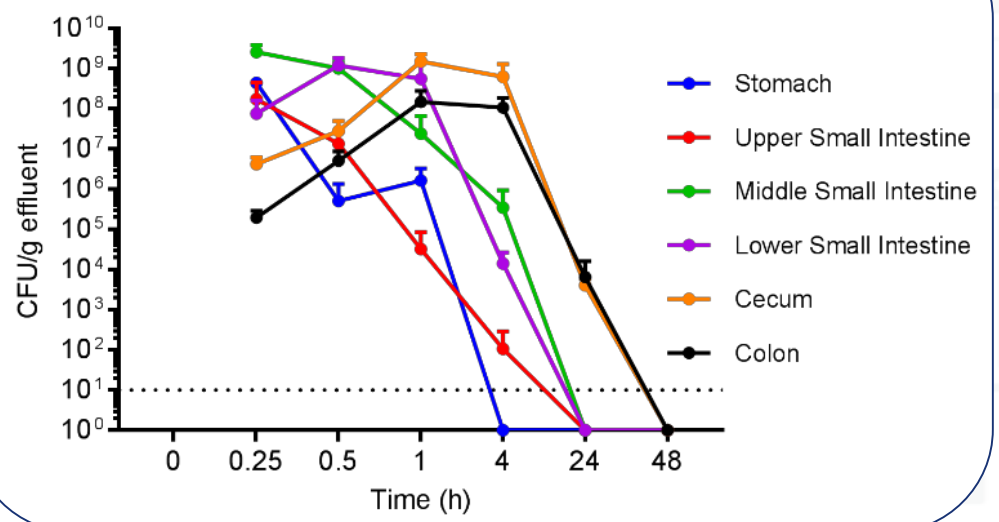
- Dean Falb
- Liz Wolffe
- Aoife Brennen

Back Up

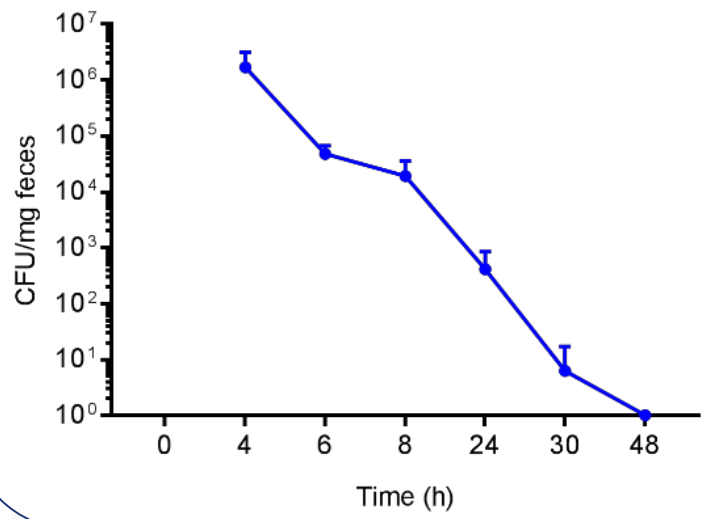
Pharmacokinetics of SYNB1618 in mice

SYNB1618 exhibits rapid transit

GI transit



Fecal excretion



- SYNB1618 gavaged to C57BL/6 mice and GI compartments plated over time
- Complete clearance from all animals within 48h
- Transit of SYNB1618 through the small intestine was rapid

- **Progression to primates anticipated to be a more ideal translation model**