# Proof of Mechanism Studies with Bitopertin, a Selective Glycine Transporter 1 Inhibitor Under Development for the Treatment of Erythropoietic Protoporphyria (EPP) and X-linked Protoporphyria (XLPP)

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Disc Medicine

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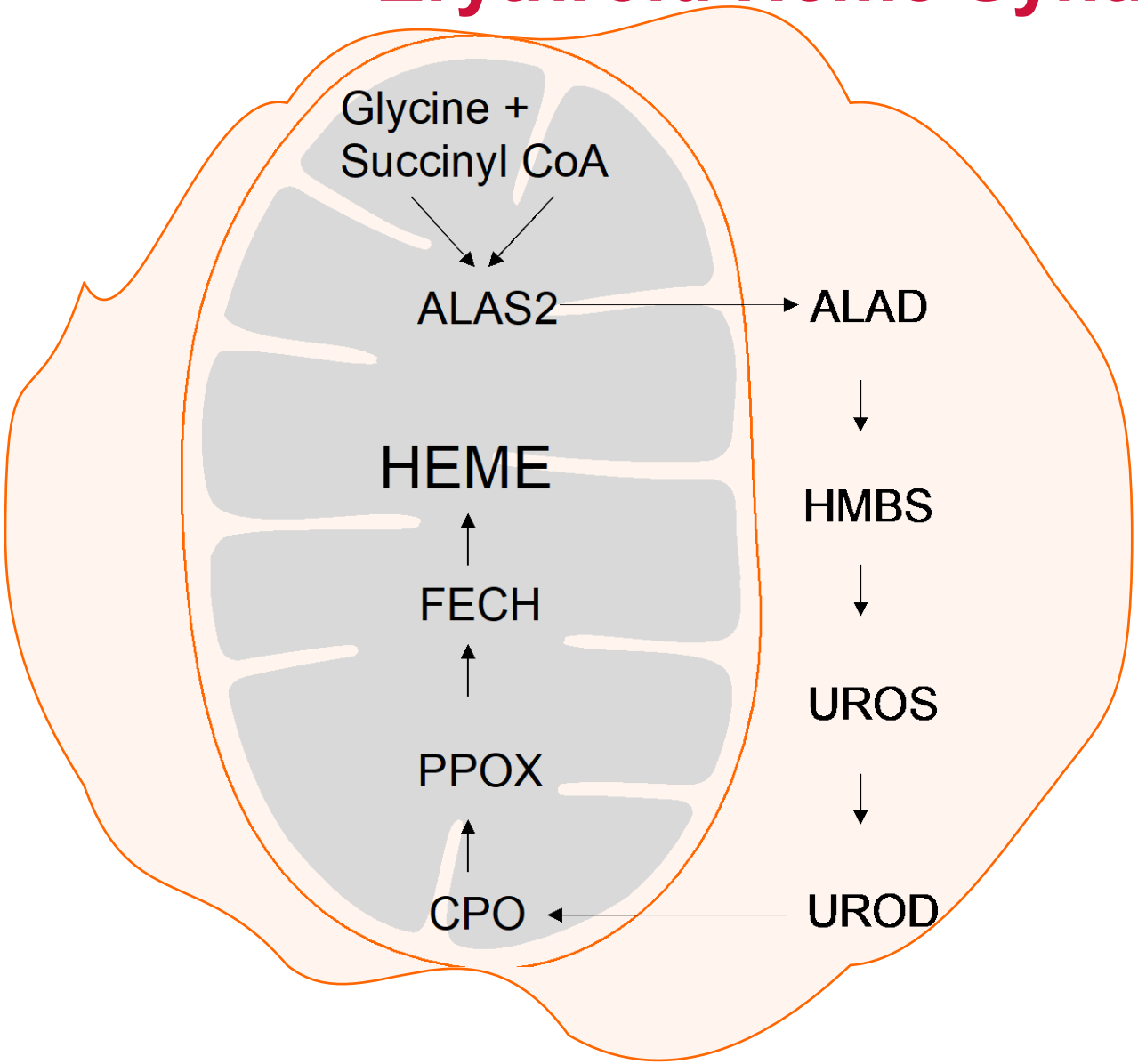
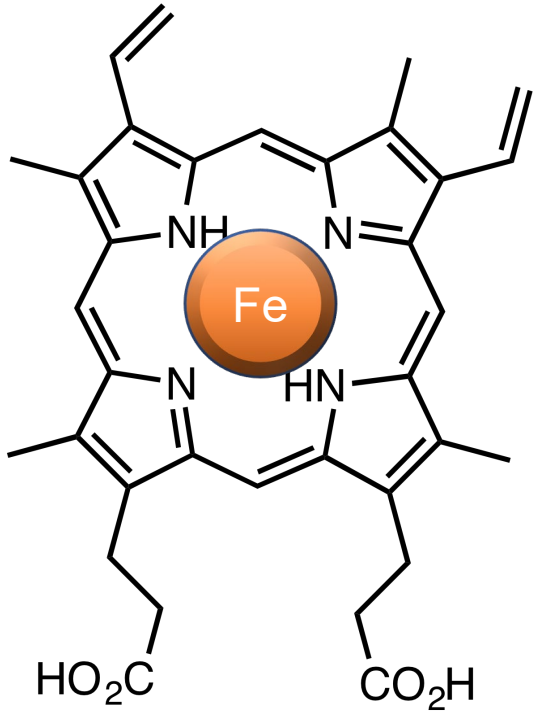
# Disclosure

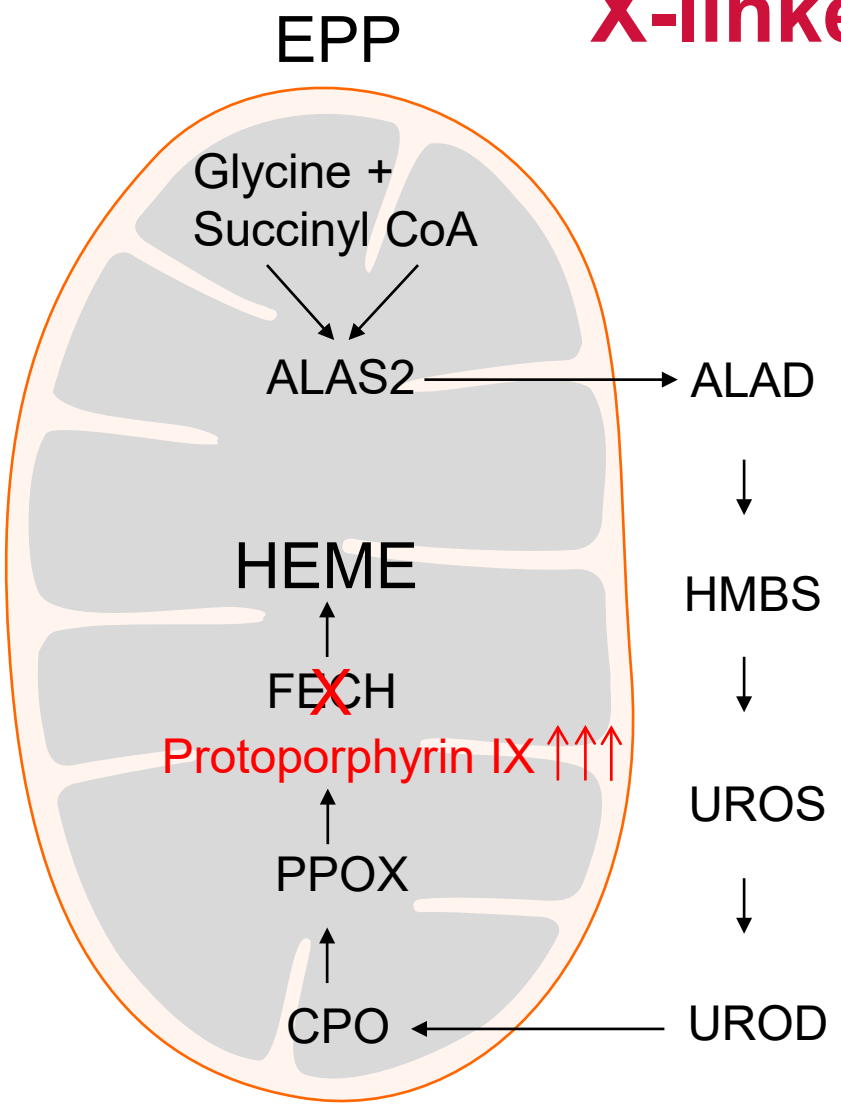
Disc Medicine provided research funding

# Erythroid Heme Synthesis pathway



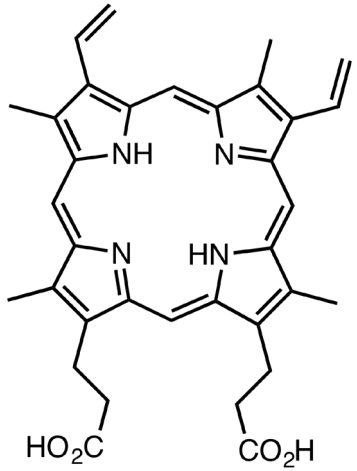



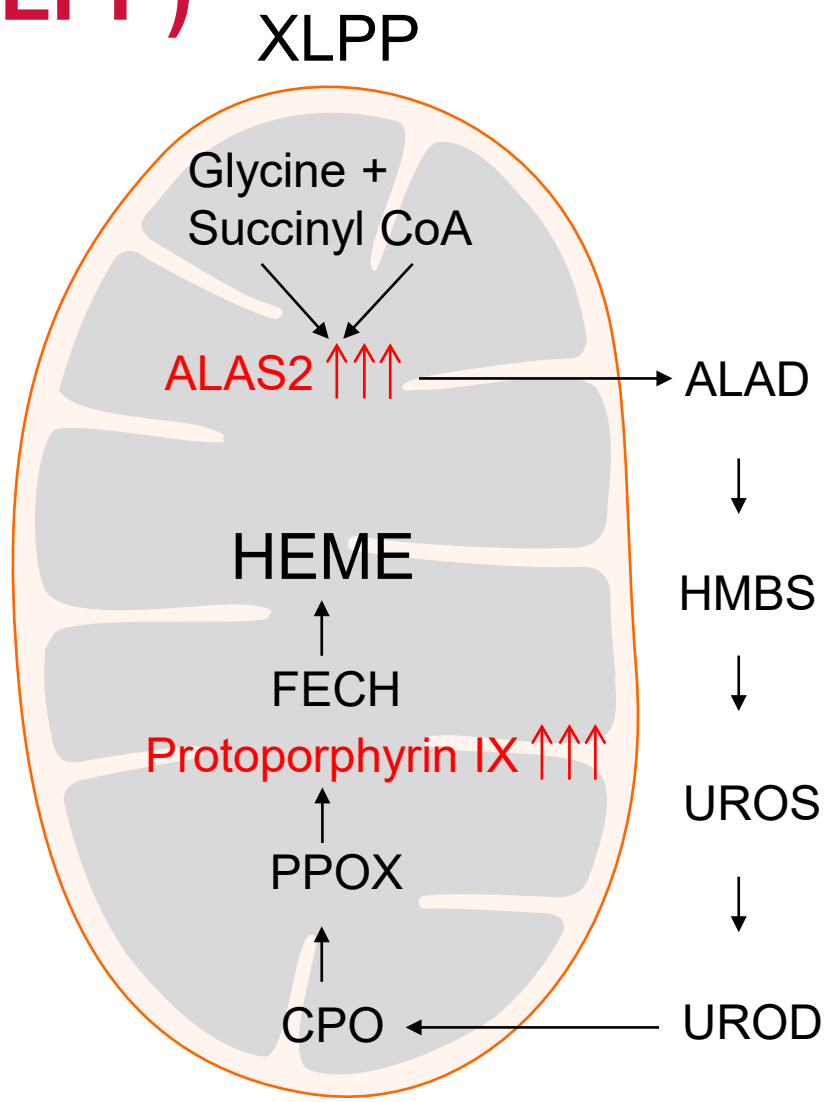
# Erythropoietic Protoporphyria (EPP) and X-linked Protoporphyria (XLPP)




**EPP and XLPP Patients**
Free PPIX in erythrocytes causes photosensitivity and rarely liver disease

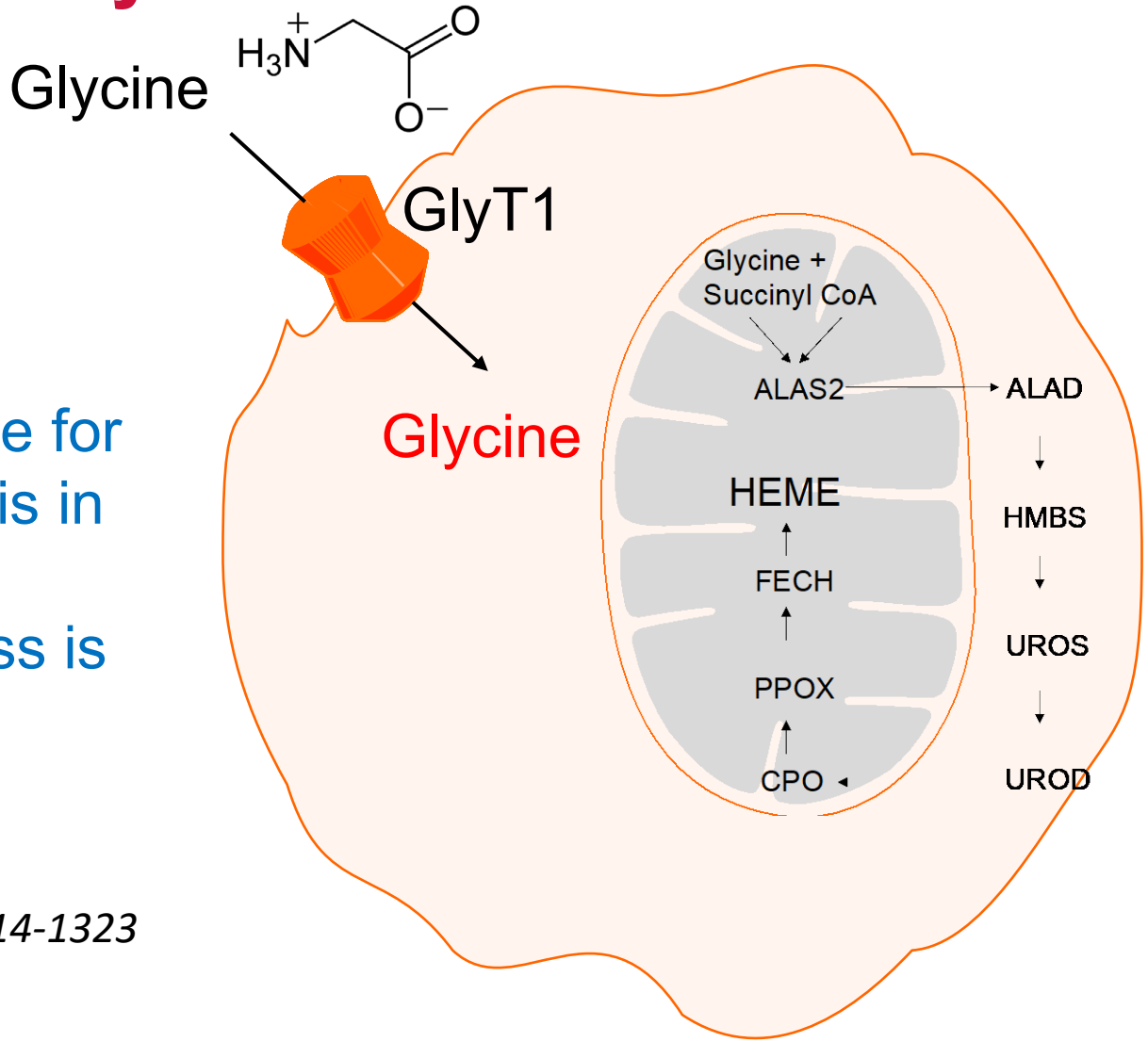
FECH loss-of-function or ALAS2 gain-of-function mutations result in accumulation of supra-physiological levels of free PPIX



# SLC6A9 (GlyT1) is the Glycine Importer in Early Erythroid cells

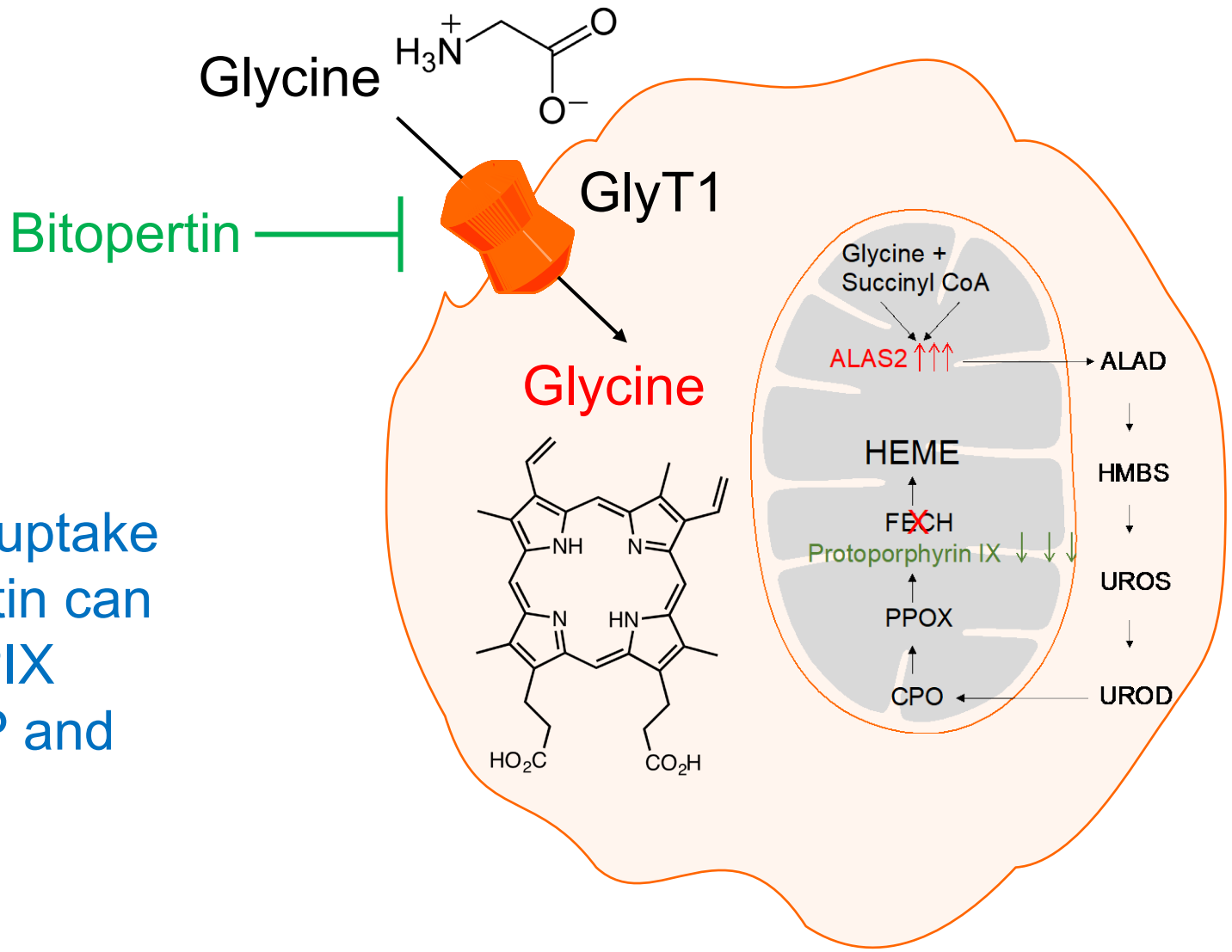
- GlyT1 supplies extracellular glycine for the initial step of heme biosynthesis in erythroid cells
- The role GlyT2 plays in this process is uncertain



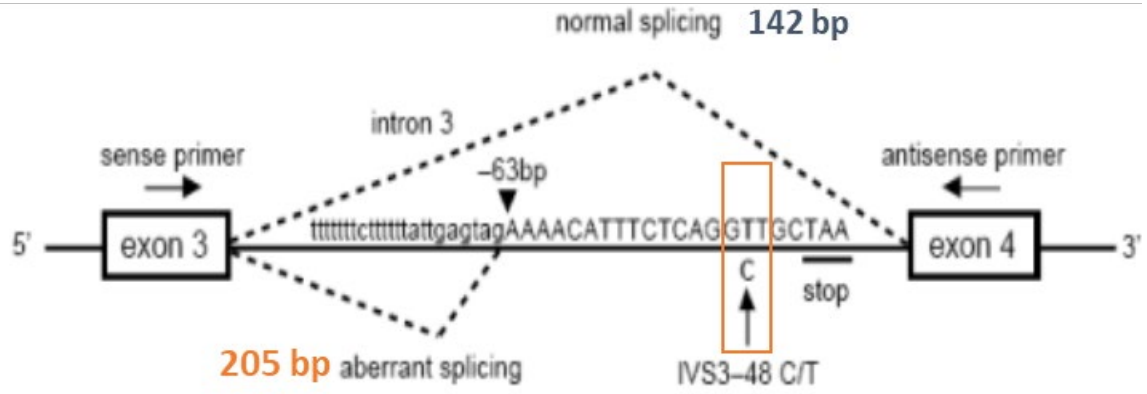

*Garcia-Santos et al, Haematologica 2017;102(8):1314-1323*

# Bitopertin: GlyT1 inhibitor

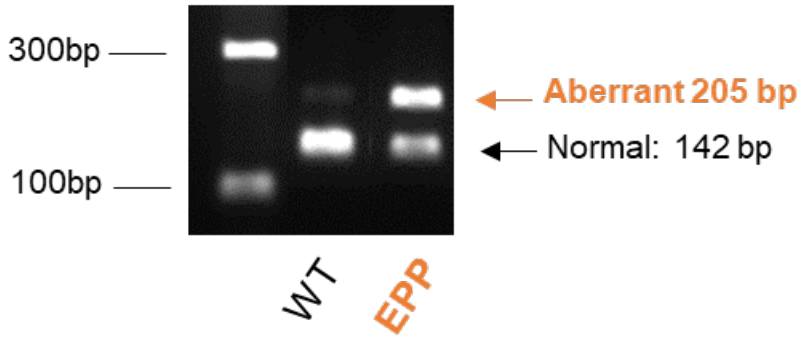
- Well-established tolerability profile in 4,000+ human subjects
- Established modulation of heme synthesis
- Hypothesis: by inhibiting glycine uptake into erythroid precursors, bitopertin can reduce the disease-causative PPIX accumulation in the blood of EPP and XLPP patients

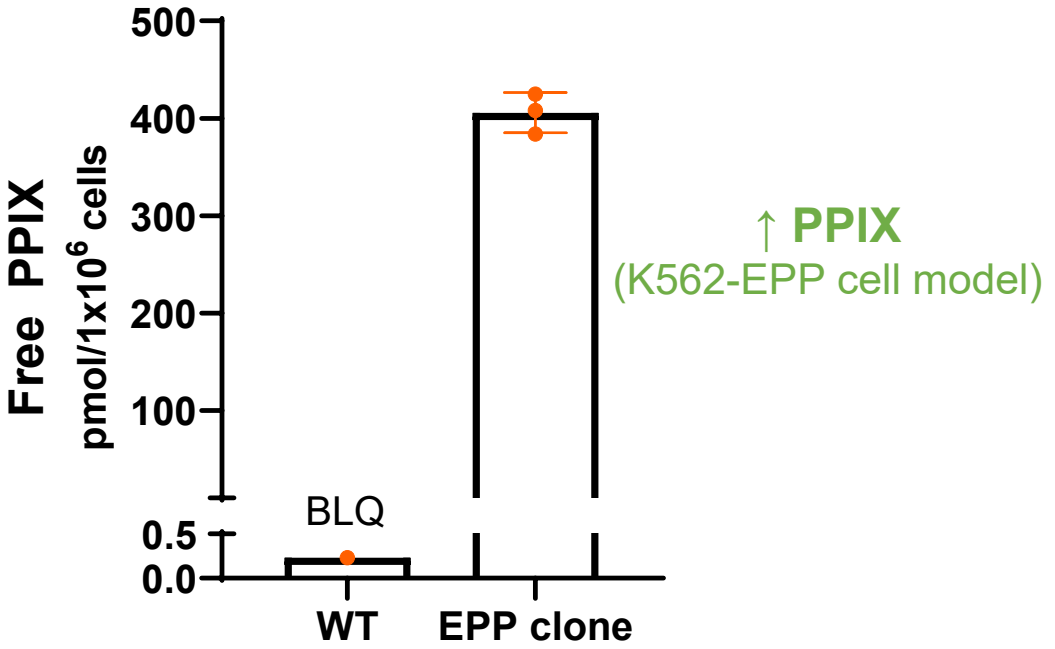


# Generation of a K562-EPP Cell Model




Modified from: *Gouya et al, Nat Genet* ***30,*** *27–28 (2002)*

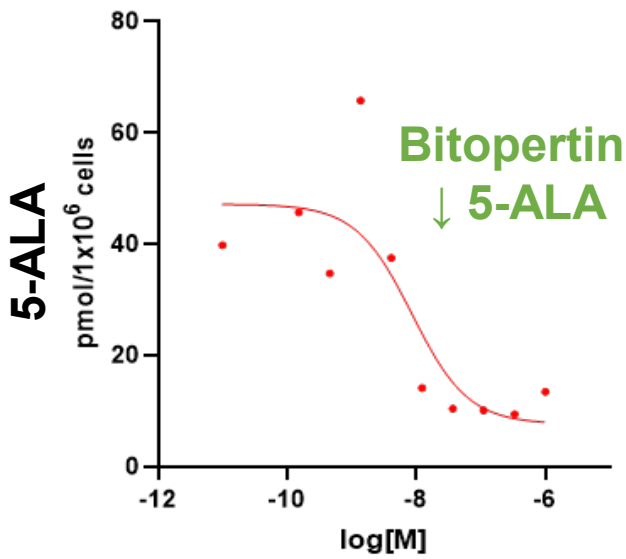


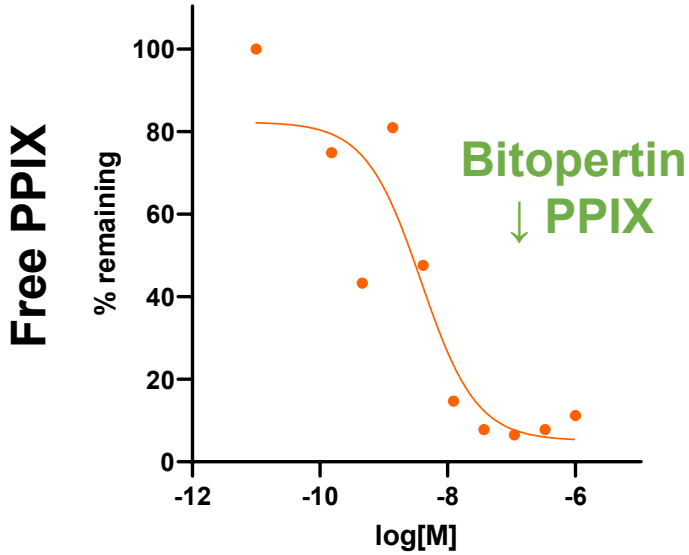



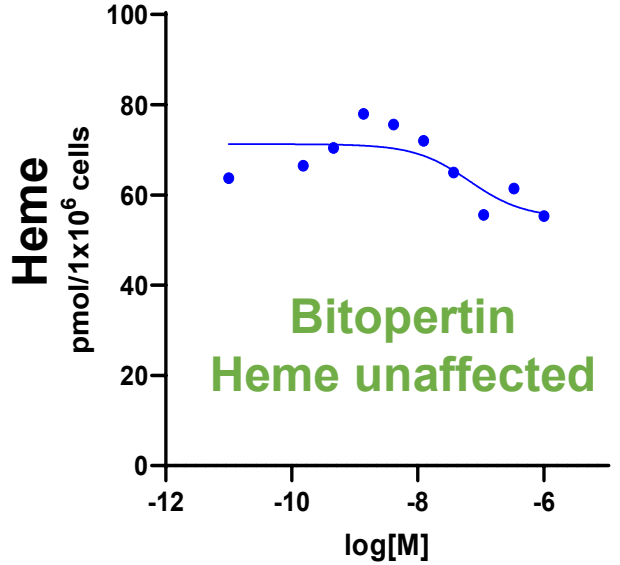

- K562-EPP clone with IVS3-48C/KO genotype was generated by CRISPR-Cas9
- LC-MS/MS was employed to measure PPIX in mutant cells

**Vu Hong**

# Bitopertin Reduced PPIX in a K562-EPP Cell Model




- Effects of Bitopertin on 5-ALA, PPIX and heme were measured by LC-MS/MS
- Blocking uptake of glycine modulates heme synthesis without affecting heme production

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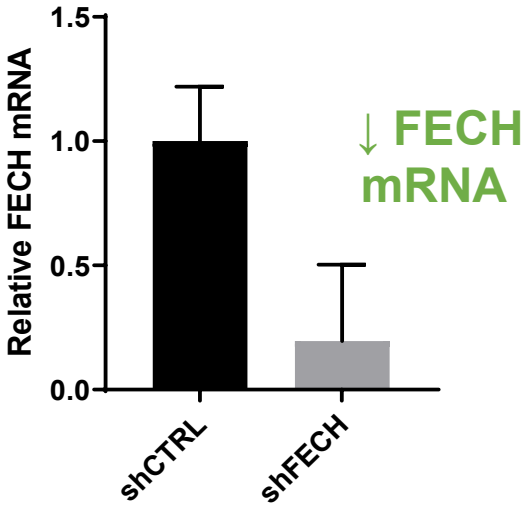
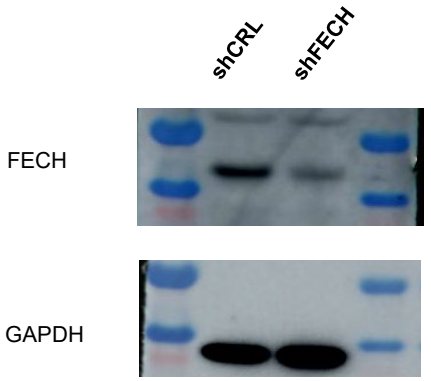
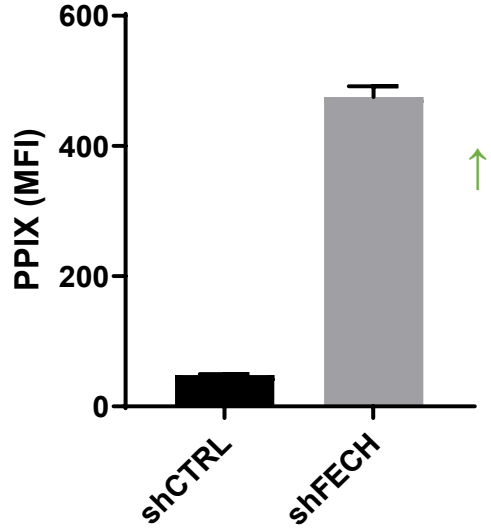
# Generation of a CD34+ Model of EPP




↓ FECH mRNA




↓ FECH protein

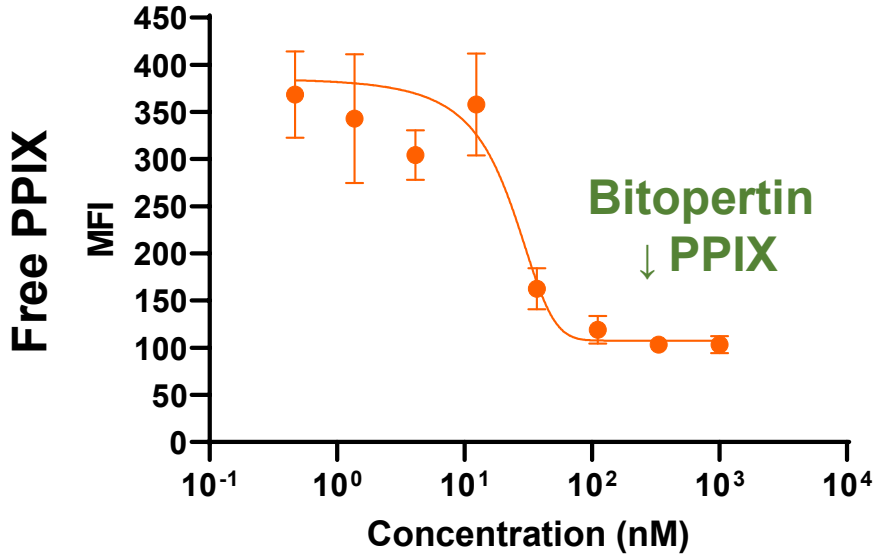



↑ PPIX

- Human cord blood CD34+ cells transduced with lentiviral vectors expressing shRNA of FECH
- ~75% reduction in FECH mRNA and protein levels
- Accumulation of PPIX in FECH shRNA treated cells as assessed by flow cytometry

**Vu Hong**

# Bitopertin Reduced PPIX in a hCD34+ Model of EPP

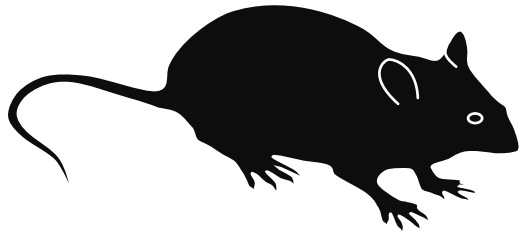


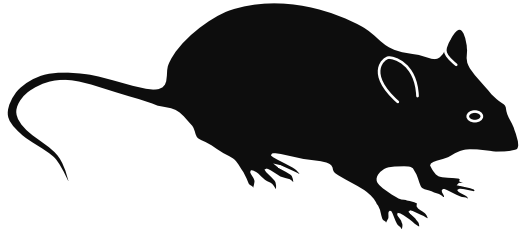

Bitopertin, $EC_{50}$ = 32nM

- Transduced cells were treated for 9 days with Bitopertin or GlyT2 inhibitor ORG-25543 in the presence of erythroid differentiation media
- Bitopertin reduced PPIX with an $EC_{50}$ of 32 nM
- Minimal effect with GlyT2 inhibitor ORG-25543

**Vu Hong**

# Bitopertin Treatment in Fech$^{m1pas}$ and Alas2$^{Q548X}$ Mice





- *Fech$^{m1Pas}$* allele is an ethylnitrosourea (ENU)-induced missense mutation that retains approximately 5% residual ferrochelatase activity

- *Tutois et al, J Clin Invest. 1991; 88: p1730*

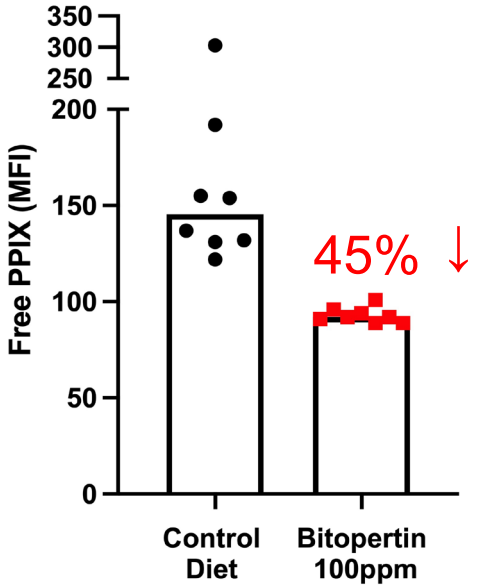
- The *Alas2$^{Q548X}$* animals were generated by employing CRISPR-Cas9 editing technology to introduce a known human gain-of-function mutation (**Sarah Ducamp**).

  NIDDK Cooperative Center of Excellence in Hematology – Boston Children's Hospital (Yuko Fujiwara)

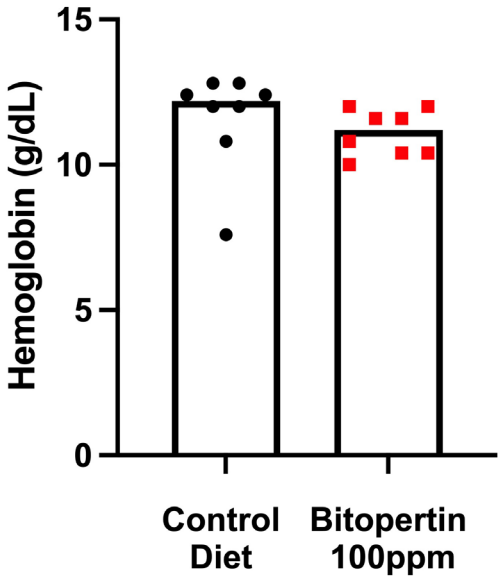
**925 Pyridoxine Response in Mouse Alas2 Knock-in Models of X-Linked Sideroblastic Anemia and X-Linked Protoporphyria**
Program: Oral and Poster Abstracts
Session: 101. Red Cells and Erythropoiesis, Excluding Iron: Poster I
Hematology Disease Topics & Pathways:
Fundamental Science, Genetic Disorders, Diseases

# Bitopertin Treatment in Fech$^{m1pas}$ and Alas2$^{Q548X}$ Mice

## EPP mouse model (Fech$^{m1pas}$ /Fech$^{m1pas}$)

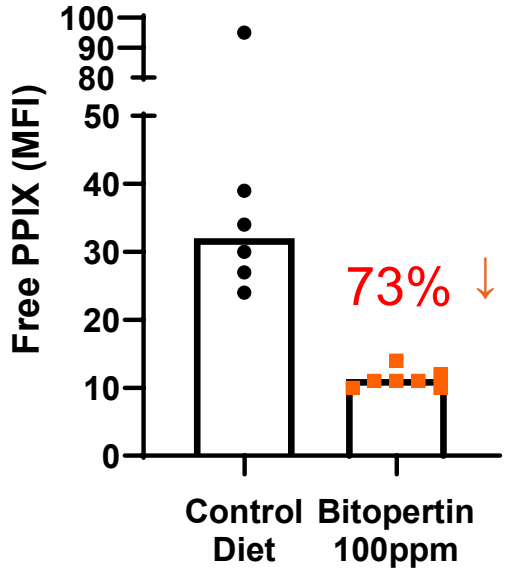



↓ Free PPIX
Flow Cytometry

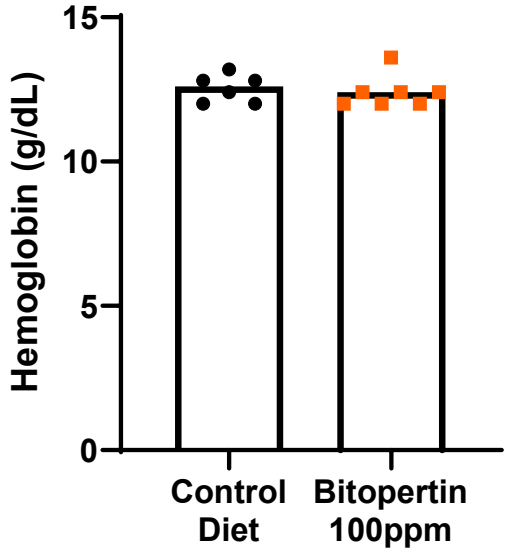



Hemoglobin

## XLPP mouse model (Alas2$^{Q548X/Y}$ )




↓ Free PPIX
Flow Cytometry




Hemoglobin

- 0 or 100 ppm bitopertin diet for 8 weeks starting at 6 weeks of age
- Bitopertin diminished PPIX in both mouse models with no effect on hemoglobin


**Sarah Ducamp**
**Dean Campagna**

# Conclusions

- Bitopertin is a selective GlyT1 inhibitor with a well-established tolerability profile in human clinical trials
- Bitopertin reduced free PPIX in K562 and CD34+ cellular models of EPP
- Bitopertin reduced free PPIX in mouse models of EPP and XLPP without diminishing hemoglobin
- Bitopertin has the potential to improve light tolerance and reduce hepatobiliary injury in EPP and XLPP patients by decreasing PPIX in erythrocytes