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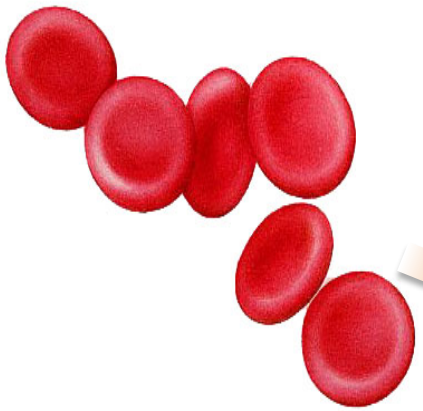
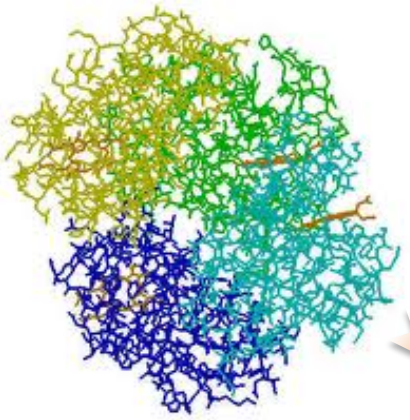
UtahStateUniversity

Biliverdin and Mesobiliverdin: Gold from Green

Erythrocyte scenescence

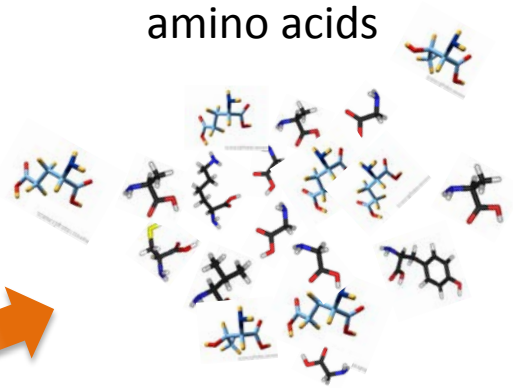


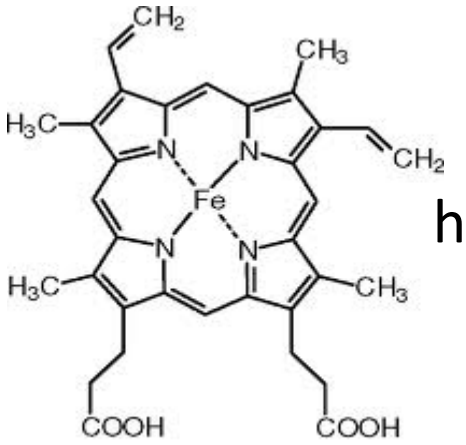
macrophages



hemoglobin

amino acids





heme

toxic

CO, Fe

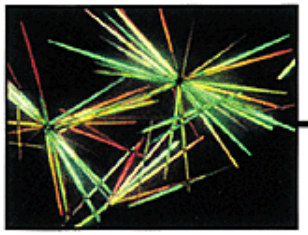
HEME

HO

Heme Oxygenase

NADPH

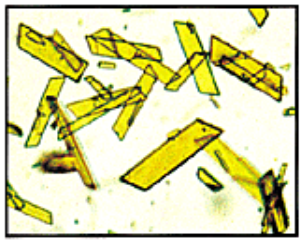
O_2



BVR

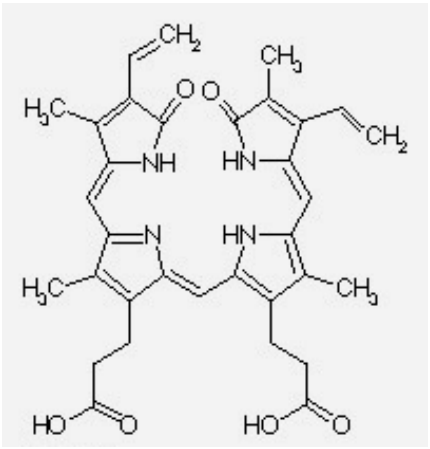
Biliverdin Reductase

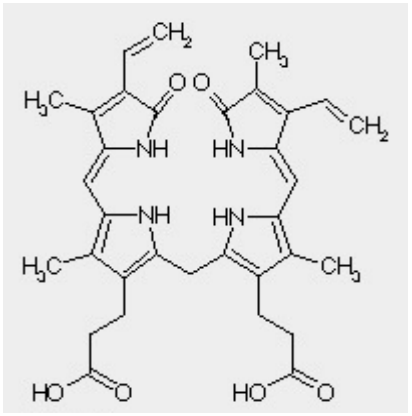
NADPH



Biliverdin

Bilirubin

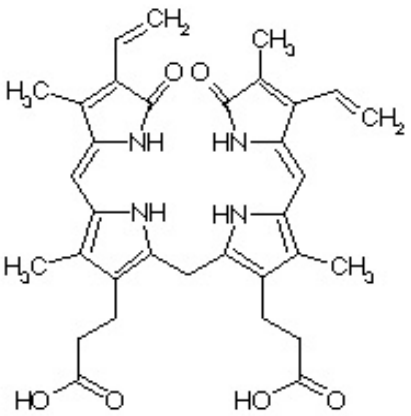




+ sugar

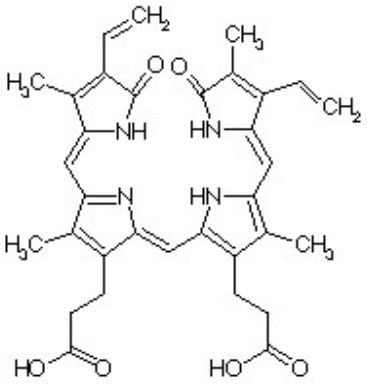
Conjugated Bilirubin

excreted



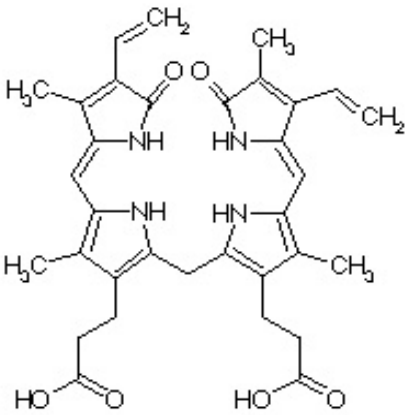
bilirubin

BVR



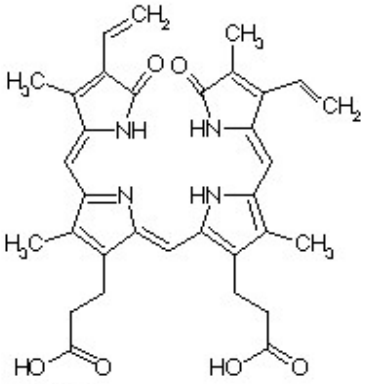
biliverdin

also
anti-oxidants

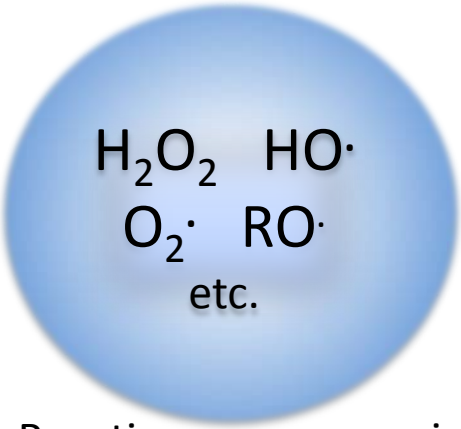


bilirubin

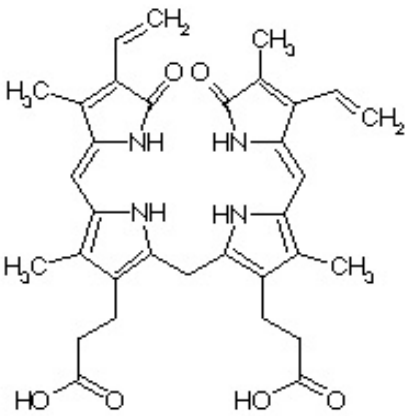
BVR



biliverdin

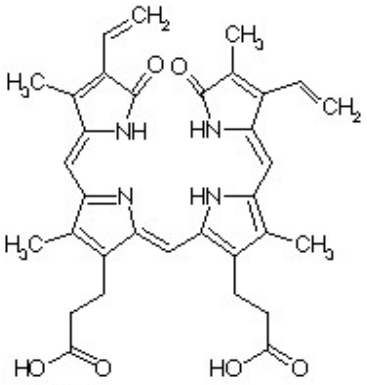


Reactive oxygen species



bilirubin

BVR



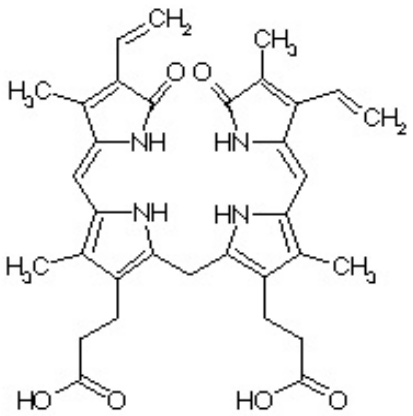
biliverdin

toxins,
injury,
trauma,
disease

H_2O_2 $HO\cdot$
$O_2\cdot$ $RO\cdot$
etc.

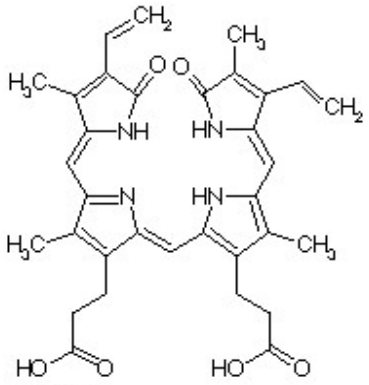
Reactive oxygen species

oxidative stress
(cell death, necroses,
acute and chronic
inflammation)



bilirubin

BVR



biliverdin

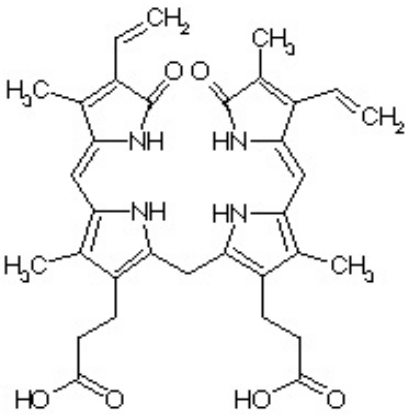
toxins,
injury,
trauma,
disease

X

H_2O_2 $HO\cdot$
$O_2\cdot$ $RO\cdot$
etc.

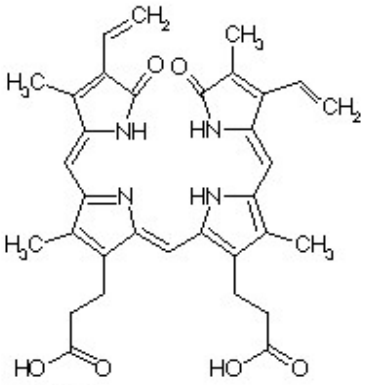
Reactive oxygen species

oxidative stress
(cell death, necroses,
acute and chronic
inflammation)



bilirubin

BVR



biliverdin

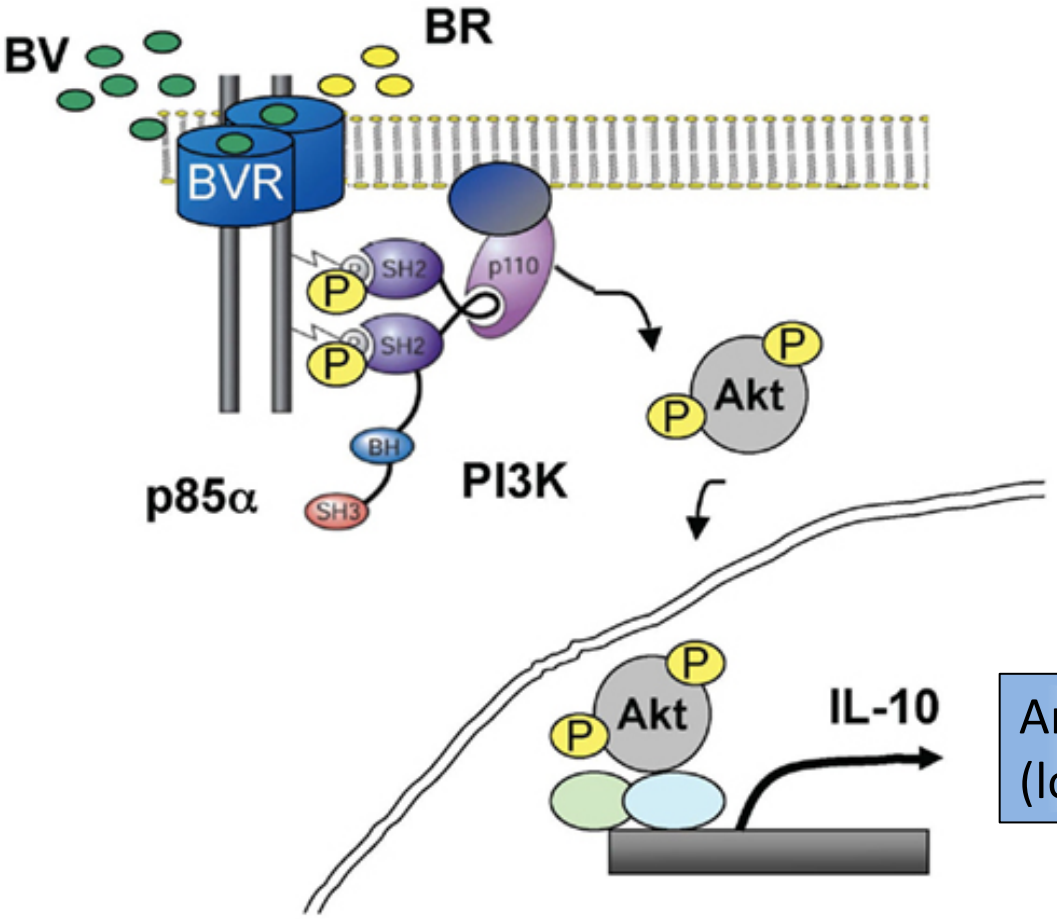
→ oxidative stress (cell death, necroses, acute and chronic inflammation)

Bilirubin & biliverdin are powerful anti-oxidants.

- As low as 10 nM protects against H_2O_2 at 10^4 times higher concentrations .
- Provides better protection against lipid peroxidation than α-tocopherol.
- Abundant natural anti-oxidants in mammalian tissues.

Biliverdin activates anti-inflammatory mechanisms

through BVR

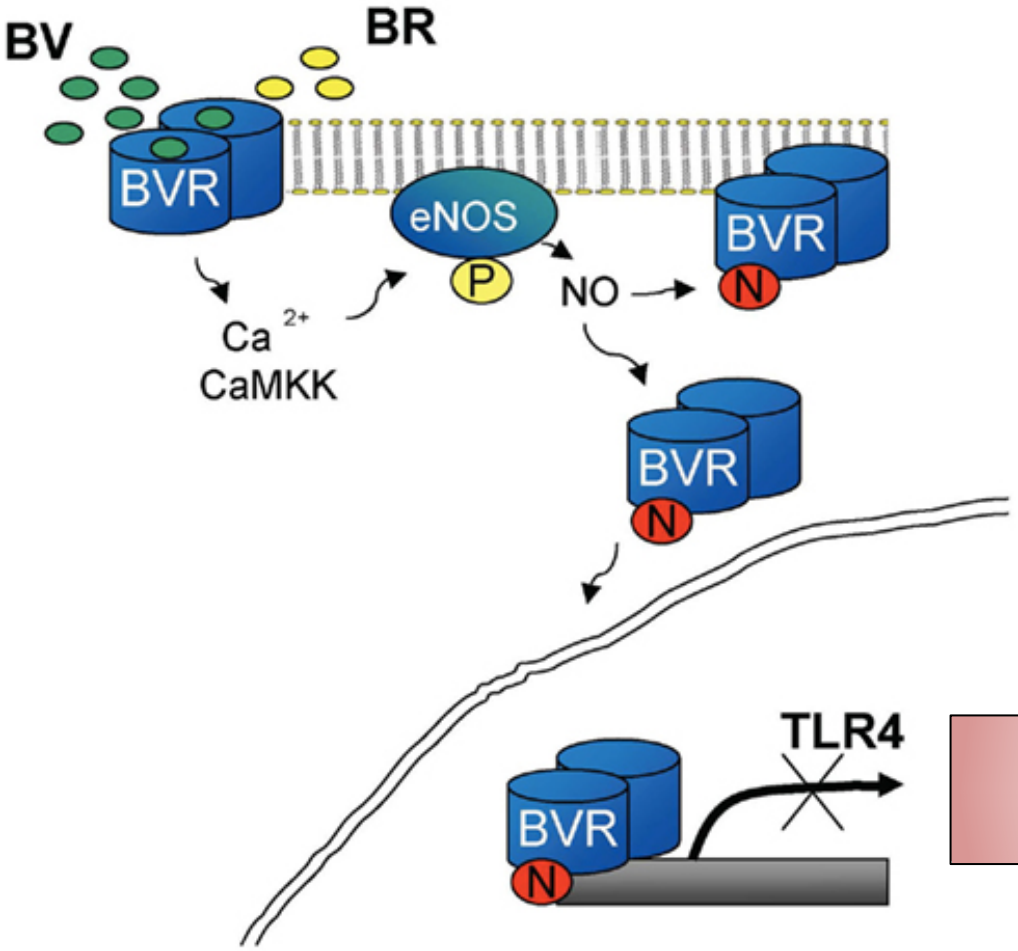


Anti-inflammatory response
(lowers IL-1,TNFα, IL-6)

Wegiel, B., C. J. Baty, et al. (2009). J Biol Chem **284**(32): 21369-21378.

Biliverdin suppresses pro-inflammatory mechanisms

through BVR



Decrease TLR4-mediated inflammatory responses

Biliverdin has dual anti-inflammatory mechanisms:

- scavenges ROS directly
- anti-inflammatory/pro-inflammatory pathway regulation by BVR (biliverdin reductase)

Biliverdin cytoprotective effects:

e.g. vascular injuries (intimal hyperplasia, vascular endothelial dysfunction), organ (liver, kidney, cardiac, small bowel, lung) transplantation, ischemia/reperfusion injuries, graft rejection, corneal epithelial injury, hepatitis C infection, endotoxic shock, type 2 diabetes, pancreatic islet β-cell apoptosis,

(*Kapitulnik, J., and M. D. Maines. Frontiers in Pharmacology,* July 13, 2012.)

Haihang Industry Co., Ltd.

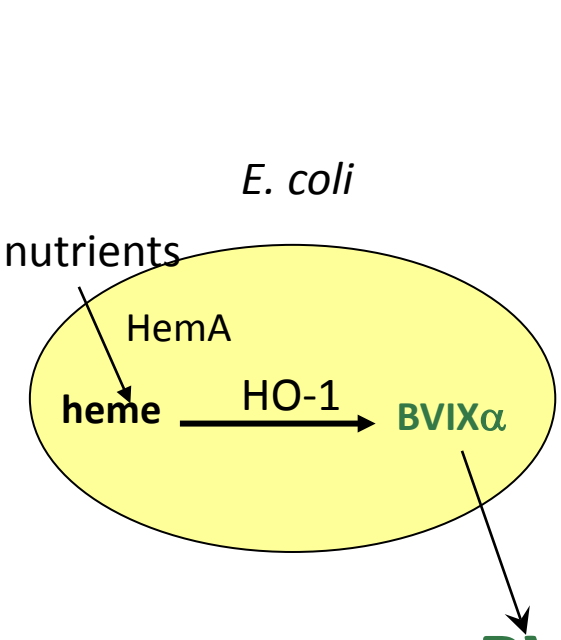
Biliverdin sources:

- derived from animal bile bilirubin
    - possibly contaminated with TSE prions
    - contains isomers

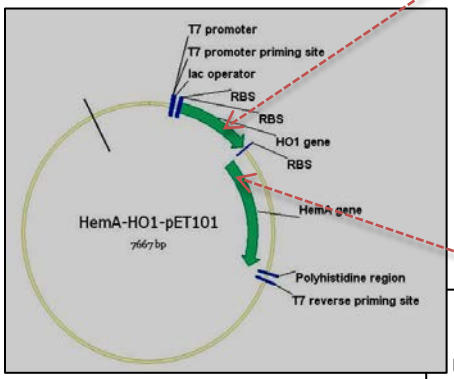
(Biliverdin IXa is the major, active isomer)

- supply is limited

Engineering *E. coli* to produce biliverdin IXα



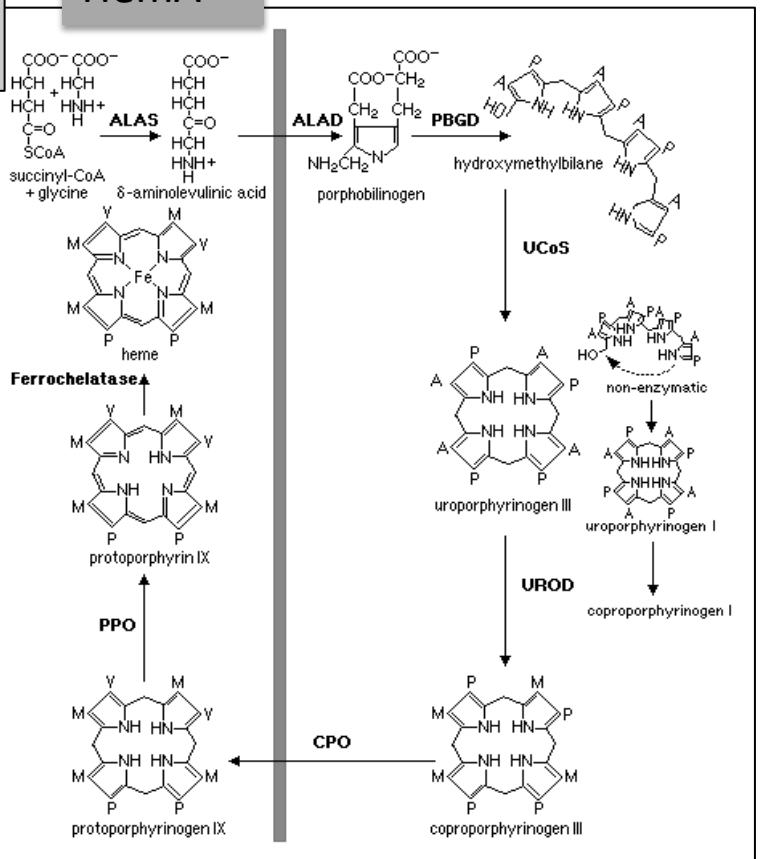
BVIXα



HO-1 (heme oxygenase) gene

HemA

heme biosynthesis



Dong Chen (SBC)

Biliverdin IXα bioproduction

Progress & Problems

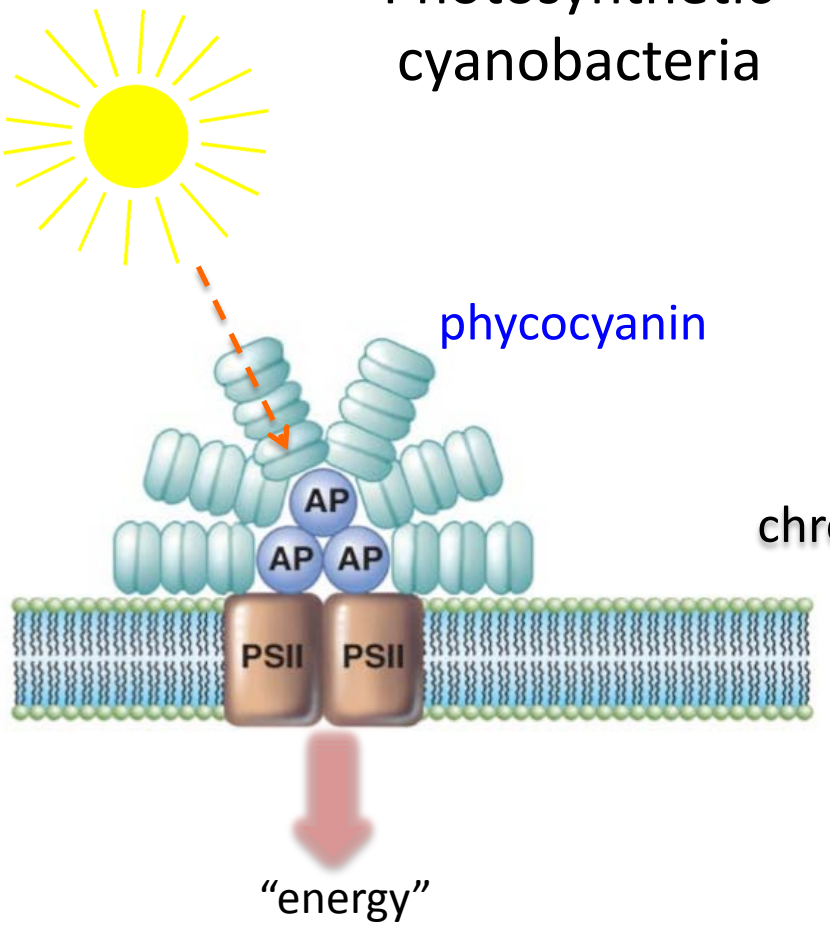
- High quality (>98% purity) BVIXα

  (Currently available animal-derived BVIXα is < 90% purity)

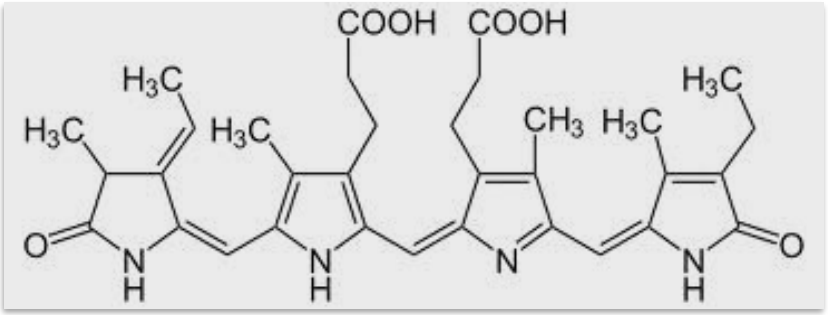
- Consistent production (~40 mg per L *E. coli* culture)
- Substrate for human biliverdin reductase
- Requires endotoxin (*E. coli* lipopolysaccharide) clean-up
- Expensive ($275 per 50 mg)

Another path to biliverdin IXα

Photosynthetic cyanobacteria

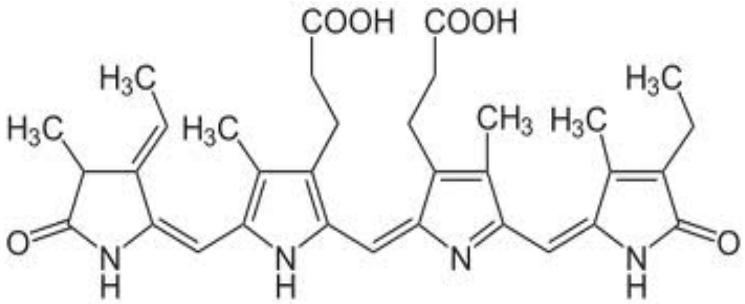


chromophore: phycocyanobilin

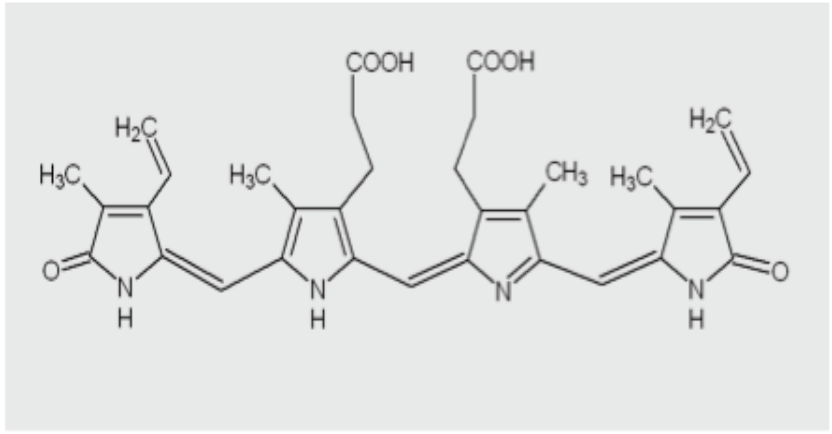


Tom Chang

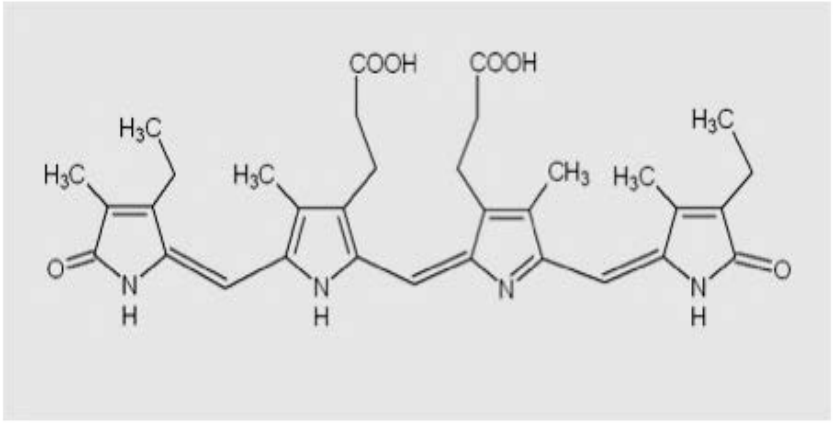
?



phycocyanobilin

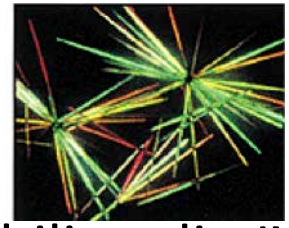


biliverdin IXα



mesobiliverdin IXα

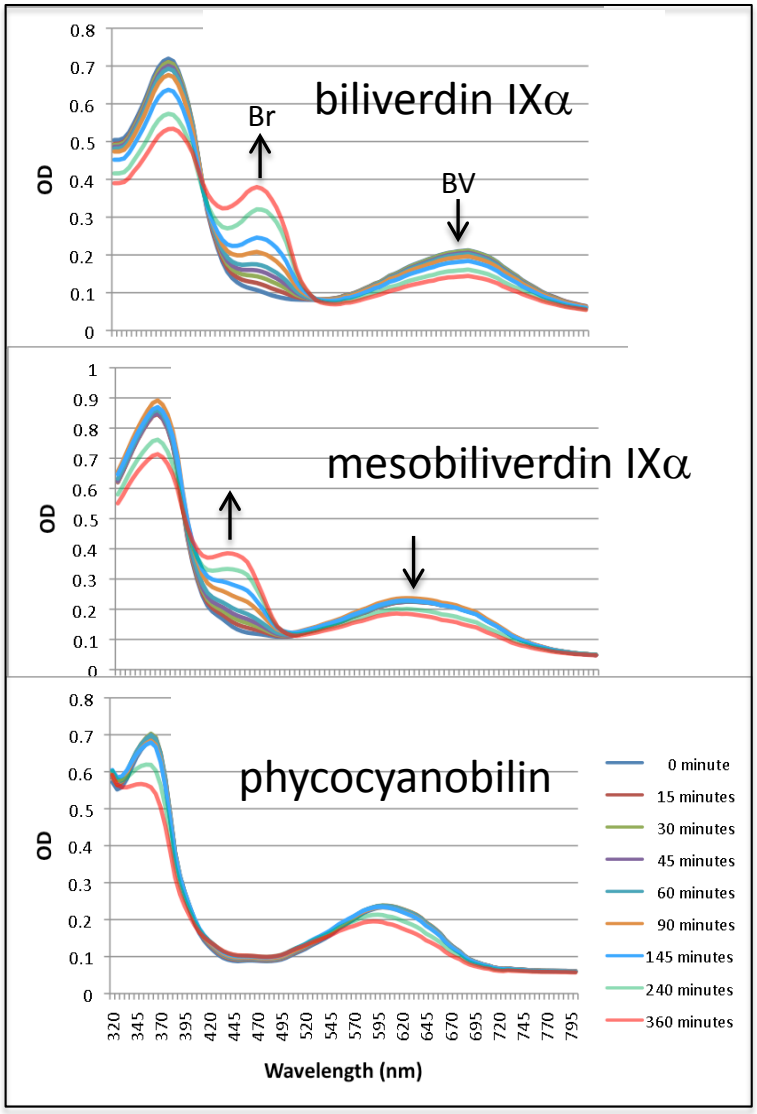
Mesobiliverdin IXα is a substrate for biliverdin reductase



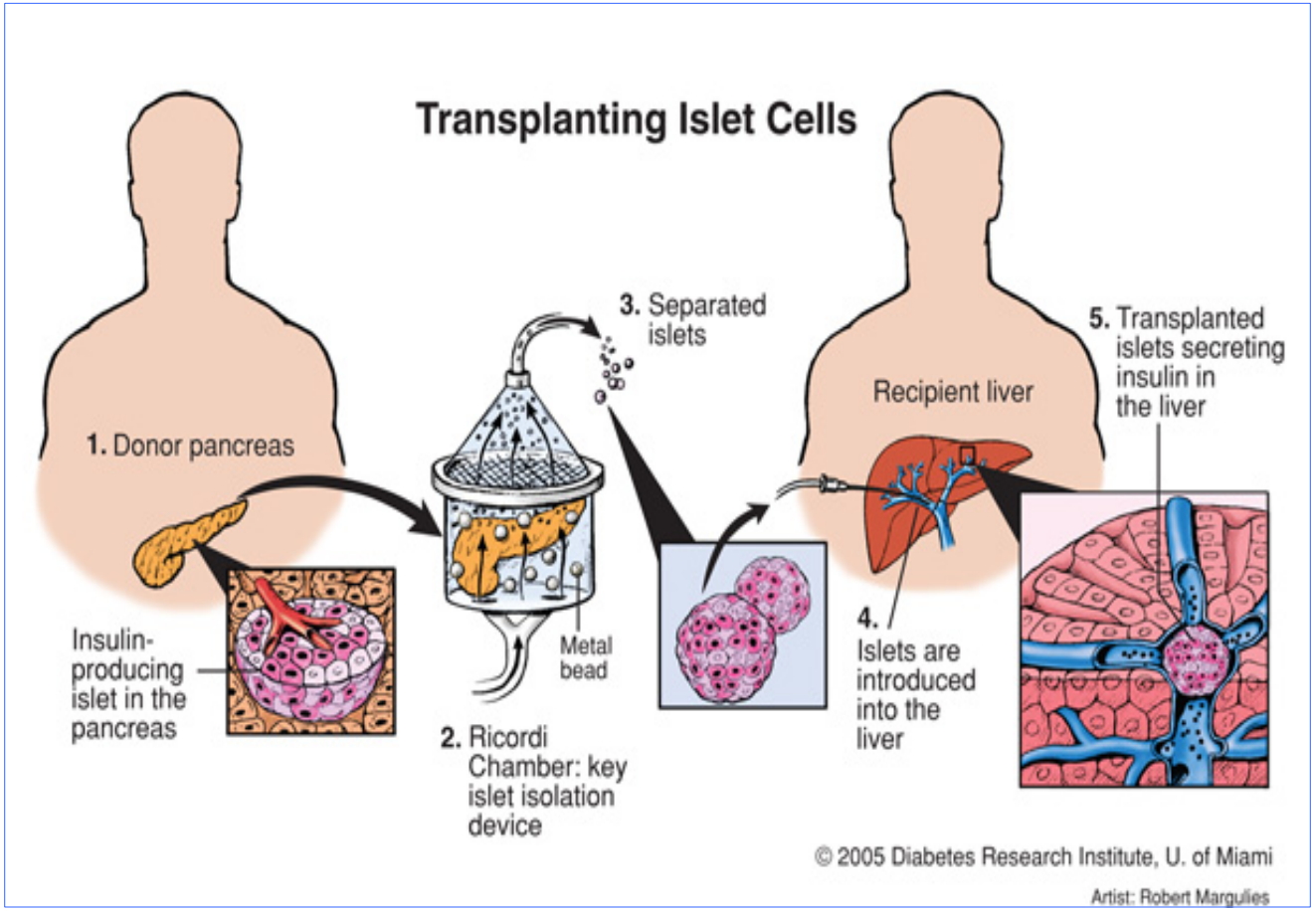
biliverdin IXα

biliverdin reductase

bilirubin IXα



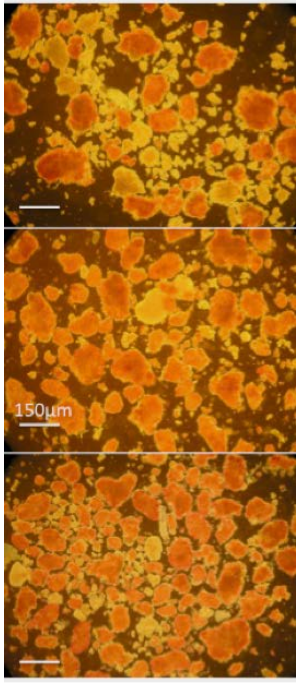
Mesobiliverdin IXα protects rat pancreatic islet β-cells from oxidative stress



Pancreatic islet allograft transplantation for type 1 diabetes

Mesobiliverdin IXα protects rat pancreatic islet β-cells from oxidative stress

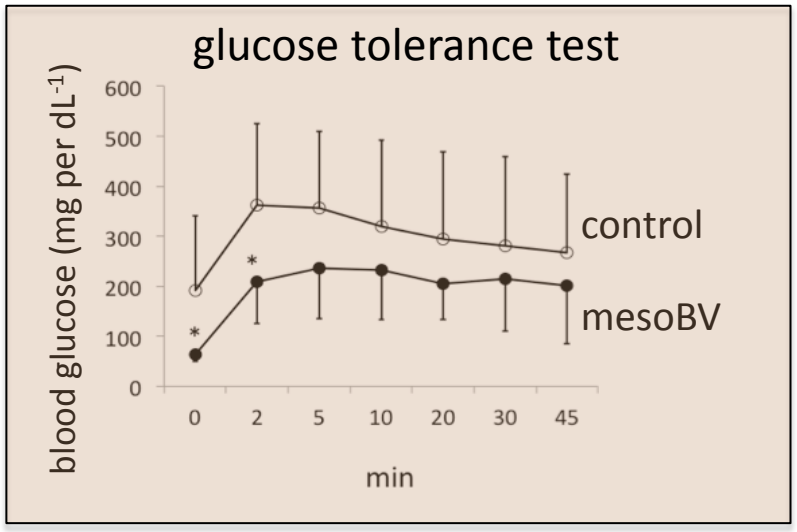
dithizone staining (red) of viable, insulin producing islets



control

E.coli BV

mesoBV



Viable islet yield after pancreatic ductal administration of biliverdin IXα and mesobiliverdin IXα

Treatment	# Viable Islets	% Increase
1µM BV_{FS} 100µM BV_{FS} control	1328 (358) 1527 (403) 1193 (223)	11.3 28
1µM BV_{EC} 100µM BV_{EC} control	1345 (629) 1759 (703 1289 (559)	4.3 36.5
1µM mesoBV 100µM mesoBV control	1599 (475) 1535 (287) 856 (229)	86.8 79.3
p38IH control	2100 1510	39.1

- 7 to 9 organs per infusion treatment
- # viable islets g^{-1} pancreatic tissue

Conclusion

Hypothesis: Heme/HO-derived metabolites and analogs that are substrate oxidants for BVR are powerful anti-inflammatories.

Reasoning: They have dual and complementary anti-inflammatory mechanisms:

1) Directly scavenge ROS
2) Activate anti-inflammatory and/or suppress pro-inflammatory cell signaling pathways

SYNTHETIC BIOPRODUCTS CENTER
UtahStateUniversity

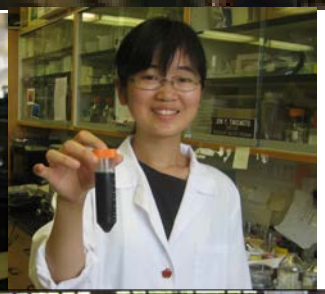
Dong Chen

Tom Chang

Jason Brown

Gilbert Nelson

Christine Dhiman



Yukie Kawasaki

NathalyCarranza

Garrett Hinton

Susie Frisby

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Reactants		Products	
$O_2 + e^-$	$\rightarrow$	O_2^-	Superoxide
$O_2^- + e^- + 2\,H^+$	$\rightarrow$	H_2O_2	Hydrogen peroxide
$H_2O_2 + e^- + H^+$	$\rightarrow$	$H_2O + OH\bullet$	Hydroxyl radical
$OH\bullet + e^- + H^+$	$\rightarrow$	H_2O	Water

Outcome:

$$O_2 + 4\,e^- + 4\,H^+ \rightarrow 2\,H_2O$$

(a) Catalase

(b) Peroxidase

(c) Superoxide dismutase

(d) Superoxide dismutase/catalase in combination

(e) Superoxide reductase