Mechanisms of Acute Toxicity

Dan Wilson, PhD, DABT
Science Leader – Cheminformatics
The Dow Chemical Company
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Acknowledgements

- Barun Bhhatarai
- Ed Carney
- Amanda Parks
- Paul Price

Pop quiz: Put these in order of lethality

- Which substance has **lowest** lethal dose? (i.e. the most ‘toxic’)

Agent	Toxicity ranking	LD50 (/kg bw)	GHS Cat
Caffeine	?	?	?
Arsenic	?	?	?
Aspirin	?	?	?
Salt (NaCl)	?	?	?
Ethanol	?	?	?
Nicotine	?	?	?
Botulism toxin	?	?	?

Pop quiz: Put these in order of lethality

- Which substance has **lowest** lethal dose? (i.e. the most 'toxic')

Agent	Toxicity ranking	LD50 (/kg bw)	GHS Cat
Caffeine	4	130-320 mg	3
Arsenic	3	46 mg	2
Aspirin	5	1000 mg	4
Salt (NaCl)	6	3000 mg	5
Ethanol	7	14000 mg	5
Nicotine	2	1 mg	1
Botulism toxin	1	0.02 ng	1

Acute classification categories

Regulatory Agency (Authorizing Act)	Animals	Endpoint	Classification
EPA (FIFRA)	Use current EPA or OECD protocol	Death[1]	I - LD_{50} ≤50 mg/kg II - 50 < LD_{50} ≤500 mg/kg III - 500 < LD_{50} ≤5000 mg/kg IV - LD_{50} >5000 mg/kg
CPSC (Federal Hazardous Substances Act)	White rats, 200-300 g	Death[1] within 14 days for ≥ half of a group of ≥10 animals	Highly toxic - LD_{50} ≤50 mg/kg Toxic - 50 mg/kg < LD_{50} <5 g/kg
OSHA (Occupational Safety and Health Act)	Albino rats, 200-300 g	Death[1], duration not specified.	Highly toxic - LD_{50} ≤50 mg/kg Toxic - 50 < LD_{50} <500 mg/kg
DOT (Federal Hazardous Material Transportation Act)	Male and female young adult albino rats	Death[1] within 14 days of half the animals tested. Number of animals tested must be sufficient for statistically valid results.	Packing Group 1 - LD_{50} ≤5 mg/kg Packing Group II - 5 < LD_{50} ≤50 mg/kg Packing Group III - LD_{50} <500 mg/kg (liquid) LD_{50} <200 mg/kg (solid)
OECD Guidance for Use of GHS (2001b)	Protocols not specified	Not specified	I - LD_{50} ≤5 mg/kg II - 5 < LD_{50} ≤50 mg/kg III - 50 < LD_{50} ≤300 mg/kg IV - 300 < LD_{50} ≤2000 mg/kg V - 2000 < LD_{50} ≤5000 mg/kg Unclassified - LD_{50} >5000 mg/kg

Abbreviations: EPA=U.S. Environmental Protection Agency; OECD=Organisation for Economic Co-operation and Development; LD_{50}=Dose producing death in 50% of the animals tested; CPSC=U.S. Consumer Product Safety Commission; FIFRA=Federal Insecticide, Fungicide, and Rodenticide Act; OSHA=U.S. Occupational Safety and Health Administration; DOT=U.S. Department of Transportation; GHS=Globally Harmonized System of Classification and Labelling of Chemicals (UN 2005).

Requirement is to classify by LD/LC_{50}

- This can be done using guideline animal studies
- Do in vitro alternative methods offer a replacement?
- Do in silico alternative methods offer a replacement?

In silico approaches - QSAR

QSAR Tools

- TOPKAT
- ChemBench
- DEREK
- EPA-TEST
- OASIS-beta
- Terra-QSAR
- ACD/ToxSuite
- ADMET Predictor

Global QSAR models not as applicable for compounds acting via highly specific mechanisms

Why study acute mechanisms?

- In vitro and in silico approaches not yet a total replacement
- May direct in vitro HTS assays and enable building of QSARs
- The ‘future’ is mechanism- (AOP-) based
- Better enable read-across
- Focus on identify compounds of high inherent toxicity
- Important in poisoning cases
- Acute mechanism *in scope* for repeat-dose studies
- Understand if animal data relevant to humans
- Understanding *mechanisms* makes us better toxicologists and better able to interpret and troubleshoot studies

Challenges of identifying acute MOAs

- A workshop like *THIS* has never been held…
- Not a guideline study requirement
- Study doesn’t include organ weights, histopath or clin path
- DBs of LD/LC50 values don’t contain other mechanistic info
- Studies often conducted at CROs blinded to TM identity
- Specific mechanisms rarely examined
- Relationship of mechanistic effect to apical effect not clear
- Risk assessors didn’t consider acute toxicity ‘sexy’ – rare focus
- Mechanistic in vitro HTS assays may only look above cytotoxicity noise level yet the MOA may drive cytotoxicity

Facts about acute data

- Distribution of GHS classification not evenly distributed for oral route - most compounds are GHS 4-5

	GHS 1	GHS 2	GHS 3	GHS 4	GHS 5
Daphnia	498	456	970	484	
Fish	640	565	830	580	
Rat	311	828	1885	5189	3284

- Provides information on inherent toxicity
- IV Data
  - Compounds average 40x more toxic by iv than oral route
  - Sometimes it's the only data you have, especially for highly insoluble compounds
  - Compounds that pass limit dose orally may cause lethality in seconds intravenously
  - Directly applicable for medical devices

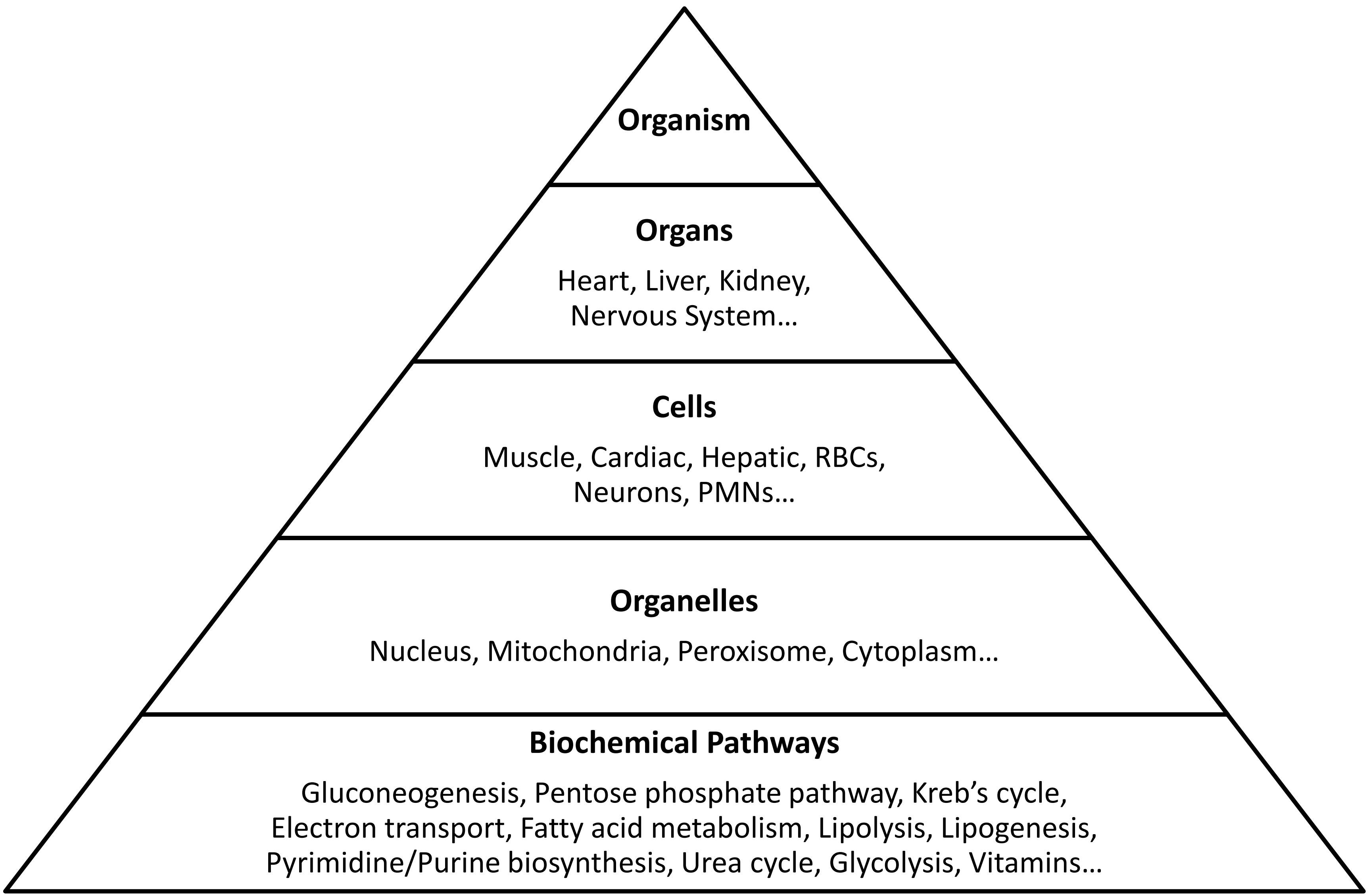
Ways to identify potential mechanisms

- Determine whether ‘reactive’ or ‘pharmacologic’
- 3-D crystalline protein structure mapping
- HT Gene expression data-mining
- Identify protein targets using wet-lab binding interactions
- Examine pathology and clinical pathology data
- Consider *Time-to-death*
- Examine relationship of acute toxicity to HTS data results
- Similarity to compounds with known mechanisms
- Years of experience resulting in a logical ‘hunch’
- Use systems biology approach
- Focus on critical targets for high acute toxicity

Chemical reactivity

- Electrophilicity
- Hardness (HOMO/LUMO)
- Acylation
- Schiff base formation
- Michael addition reaction
- SN1 mechanism
- SN2 mechanism
- SNAr mechanism
- Polarizability
- Molecular wt
- Protein/DNA binding
- Substructures
- Solubility
- pKa
- Log KoW

Systems biology approach

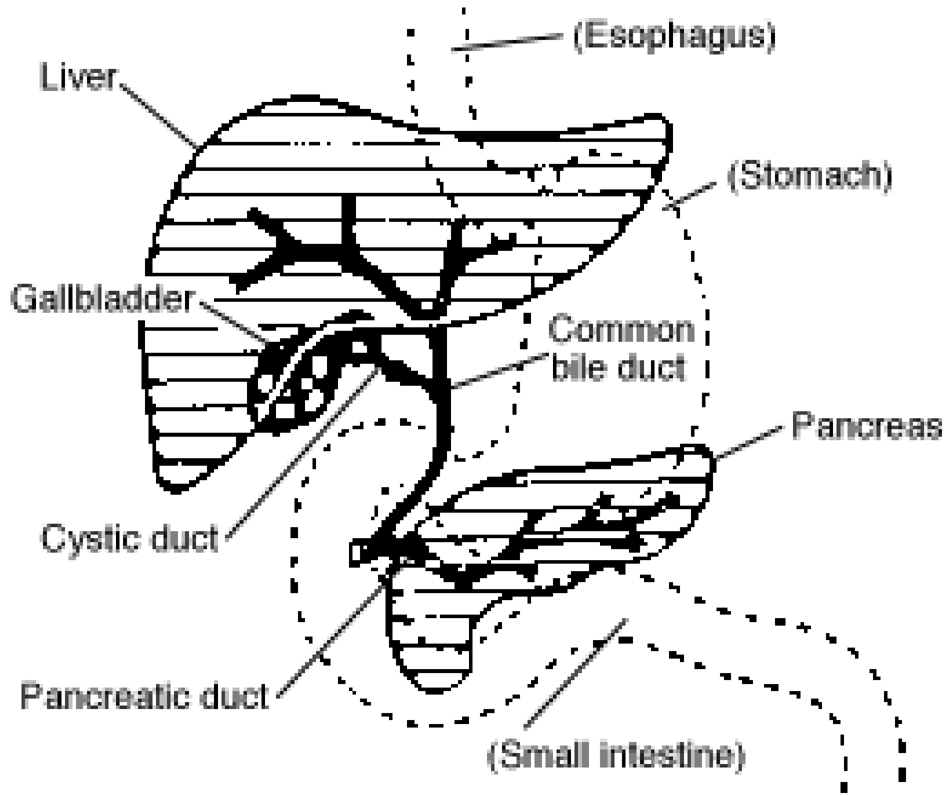


Some mechanisms of acute toxicity

- Inhibit energy production
- Antimetabolites
- Anticoagulants
- Chelants
- Inhibit signal transduction
- Ion channel blockers
- Inhibit Na+/K+ ATPase
- Protein synthesis inhibitors
- Non-specific high chemical reactivity
- Physico-chemical properties
  - Acids, Bases
  - Surfactants
  - Accept protons and uncouple mitochondrial during diffusion

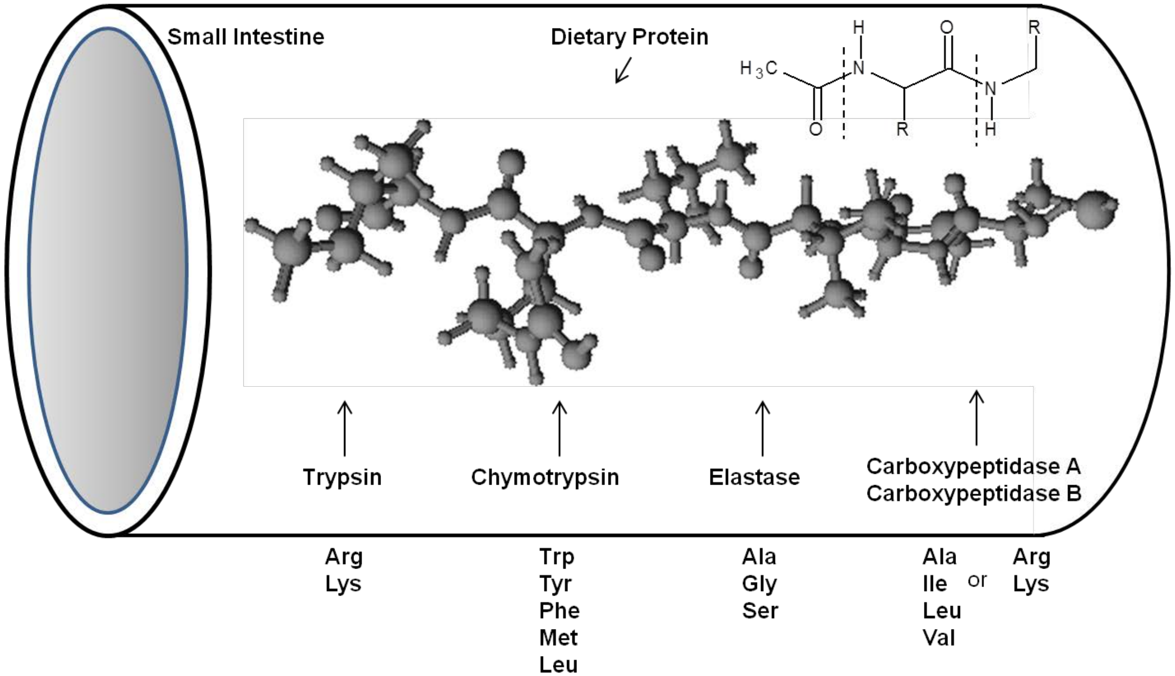
Metabolism - Bioavailability

- Physical
  - Mucous
  - Chewing
  - Mixing/churning
  - Acid
  - Emulsification
- Hormones
- Enzymes



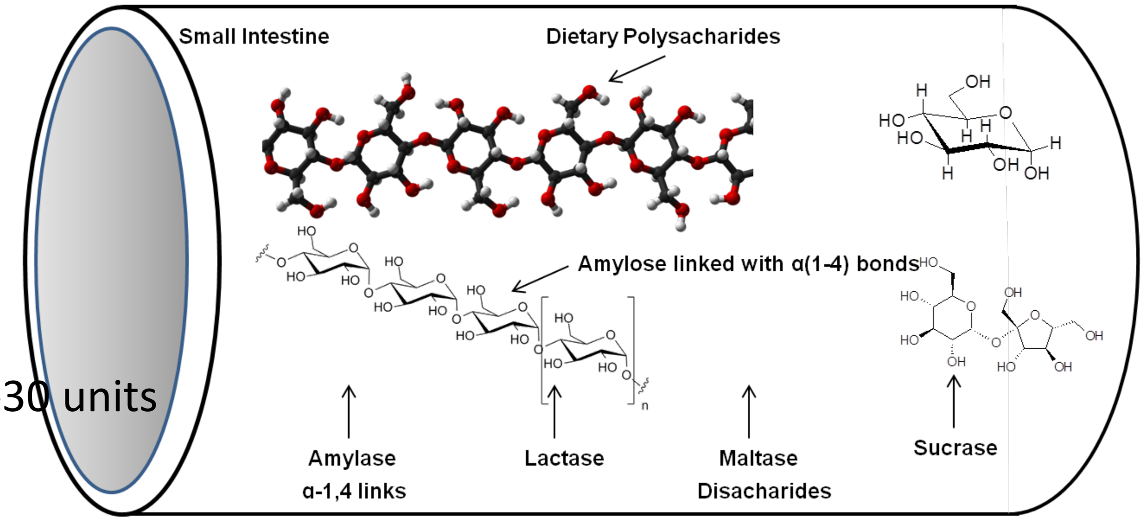
Protein Digestion

- Stomach
  - HCl denatures
  - Pepsinogen → pepsin
- Small Intestine
  - Hormones
    - Cholecystokinin
    - Secretin
  - Pancreatic enzymes
    - Trypsin, peptidases, elastase
- Amino acids ↑ insulin, ↓ glucagon
- <u>No storage form for protein</u>
  - amino acids → protein; carbons → carbohydrate/lipid; amino “N” as urea

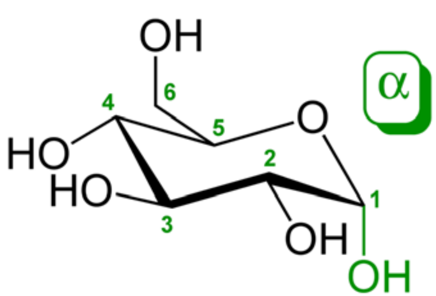


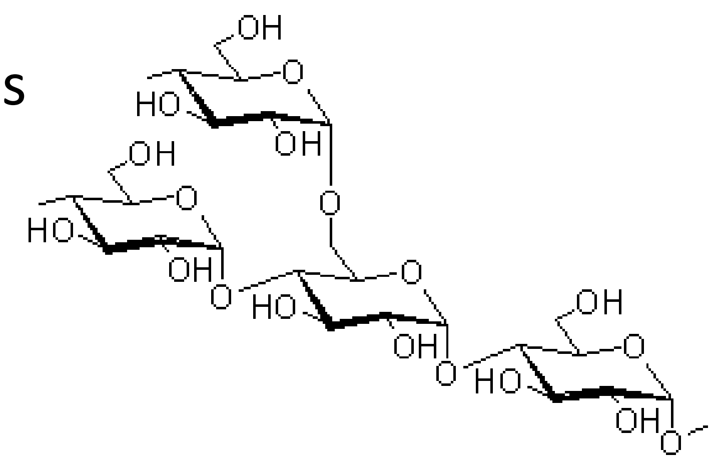
Carbohydrate Digestion

- Starch: glucose polymer α(1→4) glycosidic bonds
  - Amylose
    - linear, 100's glucoses
  - Amylopectin
    - branched, 1000's units
    - linear α(1→4)
    - branch α(1→6) each 24-30 units
  - Glycogen
    - branch each 8-12 units



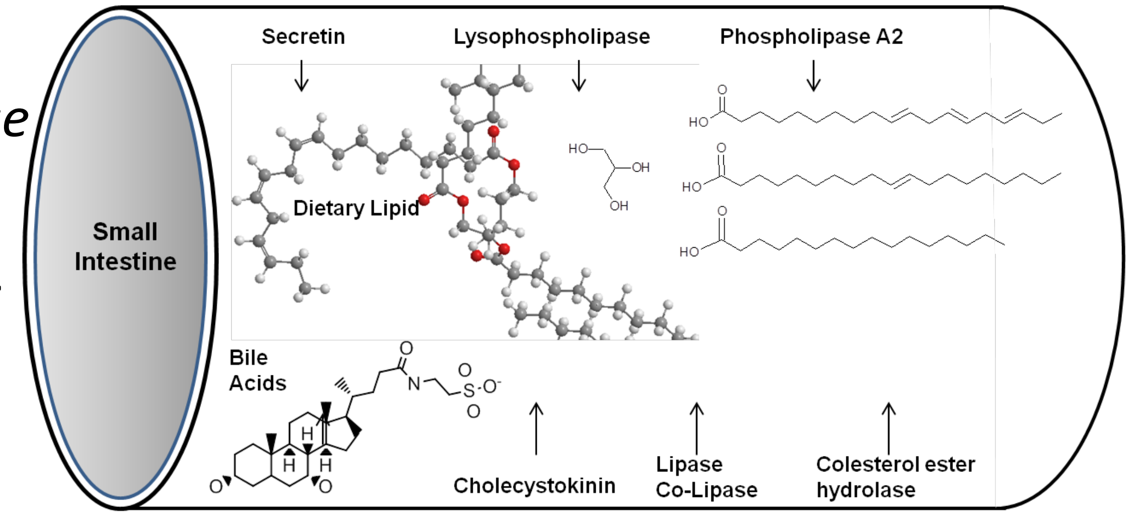
- Pancreatic *amylase* breaks α-1,4-bonds



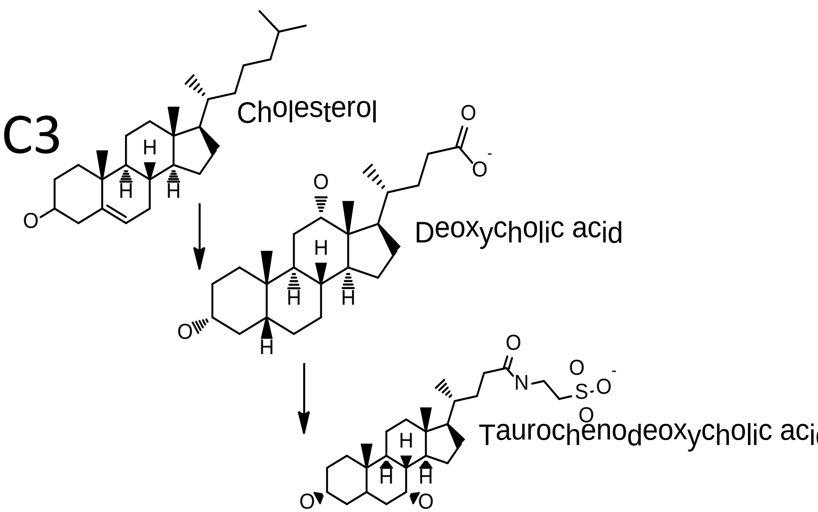


Lipid Digestion

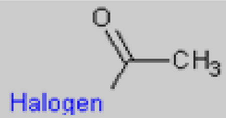
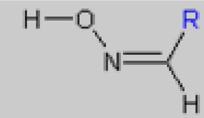
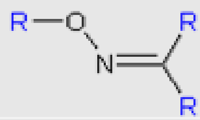
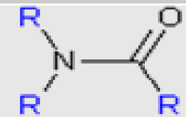
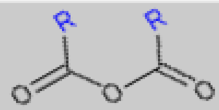
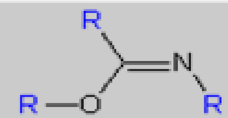
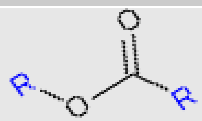
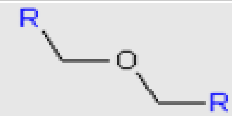
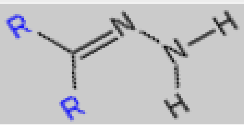
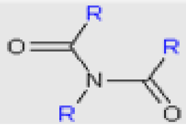
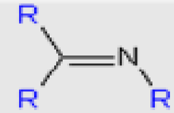
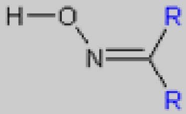
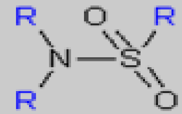
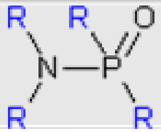
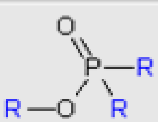
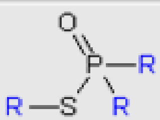
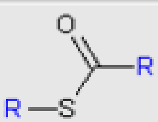
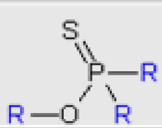
- Stomach
  - *Lingual and Gastric Lipase*
- Small intestine
  - Cystokinin → gallbladder
    - ↓ gastric motility
  - Secretin → pancreas
    - bicarbonate neutralizes pH
  - Emulsification → Bile saltss
  - *Pancreatic Lipase* → FA at C1 and C3
  - *Colipase* → stabilizes *Lipase*
  - *Cholesteryl ester hydrolase*
  - *Phospholipase A2* → FA at C2
  - *Lysophospholipase* → C2



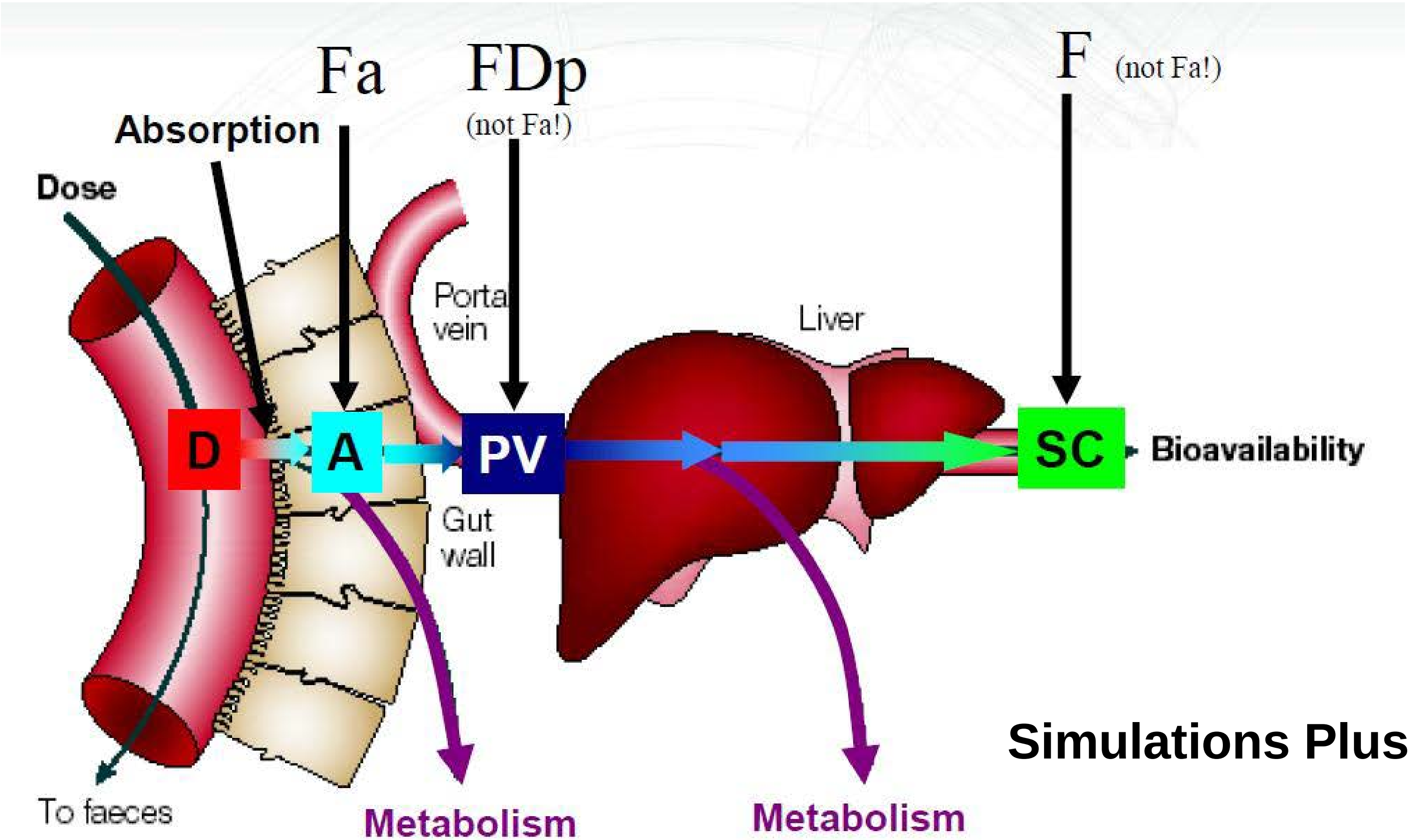
Lipid emulsified by bile acids, hydrolized by pancreatic enzymes



Potentially labile subfragments

Compound Name	Structures	Compound Name	Structures
Acyl Halides		Aldoxime	
O-Alkyloxime		Amides	
Anhydride		Carboximidate	
Ester		Ether	
Hydrazone		Imine-Hydrazone	
Imides		Imine	
Ketoxime		Sulphonamide	
Phosphoramide		Organohosphates (thiophosphates, etc)	   

Modeling Systemic Bioavailability

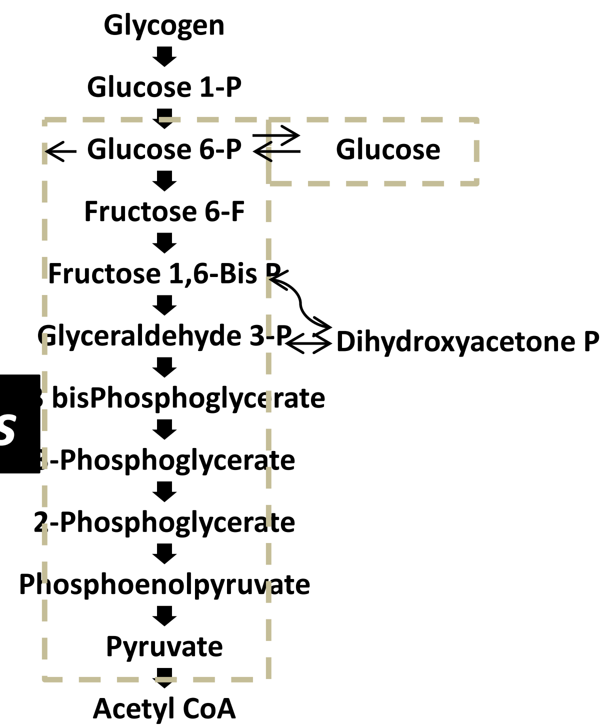


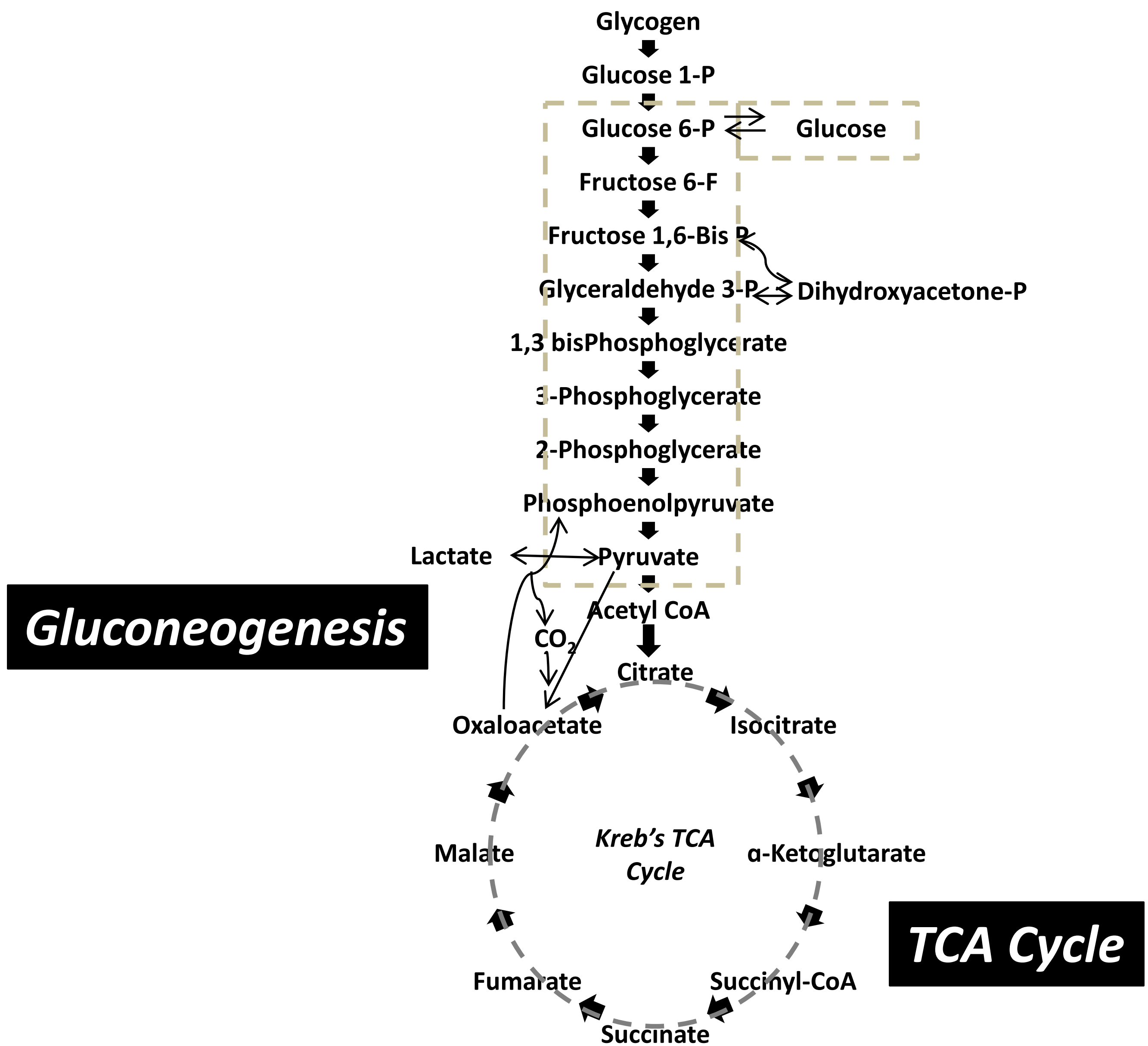
Simulations Plus

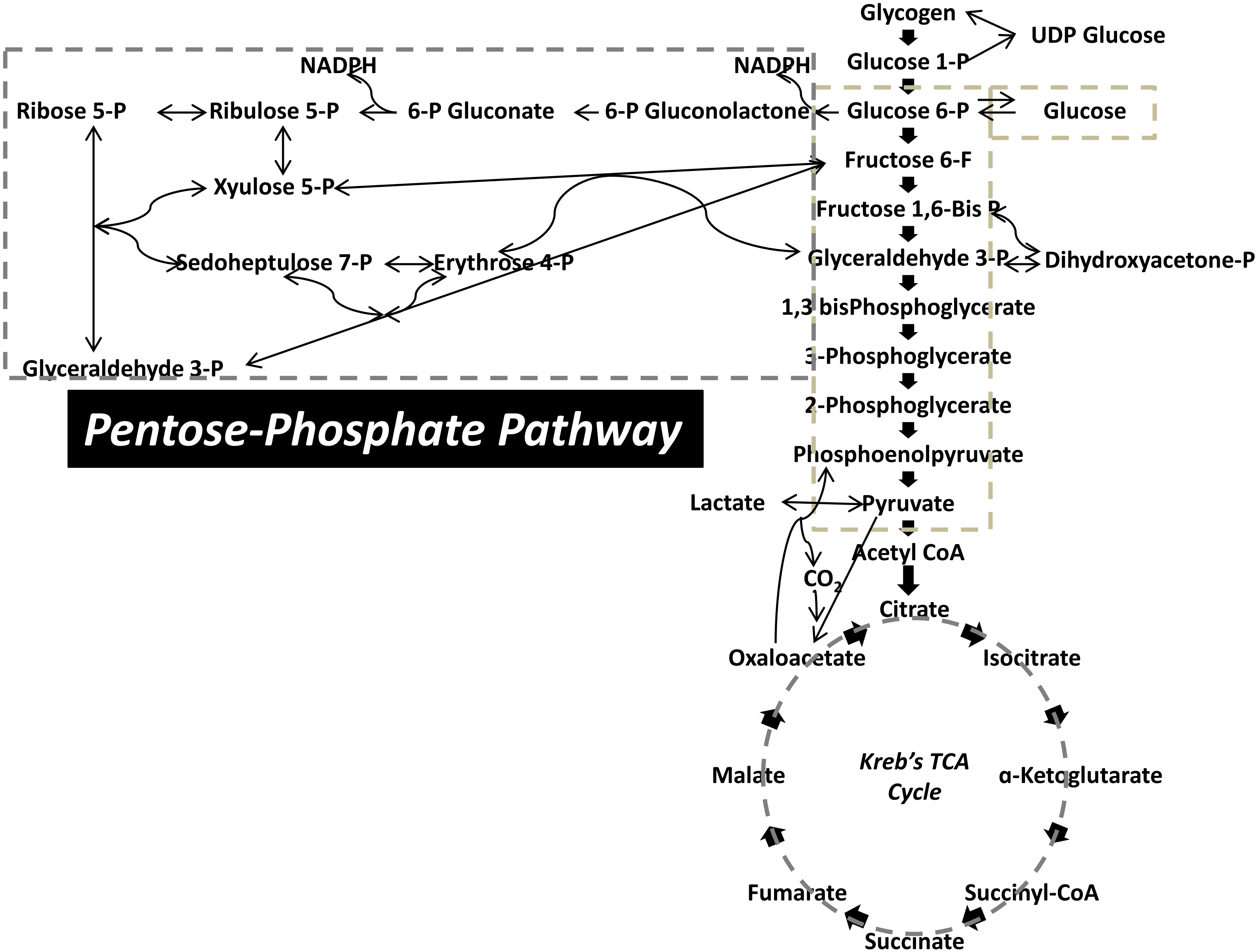
Energy production

- **Adenosine Triphosphate (ATP)** used for most energy requiring reactions (e.g., active transport). Isn't stored, consumption closely follows synthesis
- 1 kg created-recycled/hr. A cell uses 10 million ATP molecules/sec and recycles all of its ATP every 20-30 sec
- **Guanosine Triphosphate (GTP)** equivalent to ATP in energy content and preferentially used in some cellular reactions
- **Flavin Adenine Dinucleotide (FAD)** a cofactor reduced to FADH2, an energy-rich molecule
- **Nicotinamide Adenine Dinucleotide (NADH):** a cofactor; reducing potential converted to ATP through the electron transport chain
- **Nicotinamide adenine dinucleotide phosphate (NADPH)** is used in fatty acid and nucleic acid synthesis
- **Phosphocreatine** is used to replenish ATP from creatine and ADP

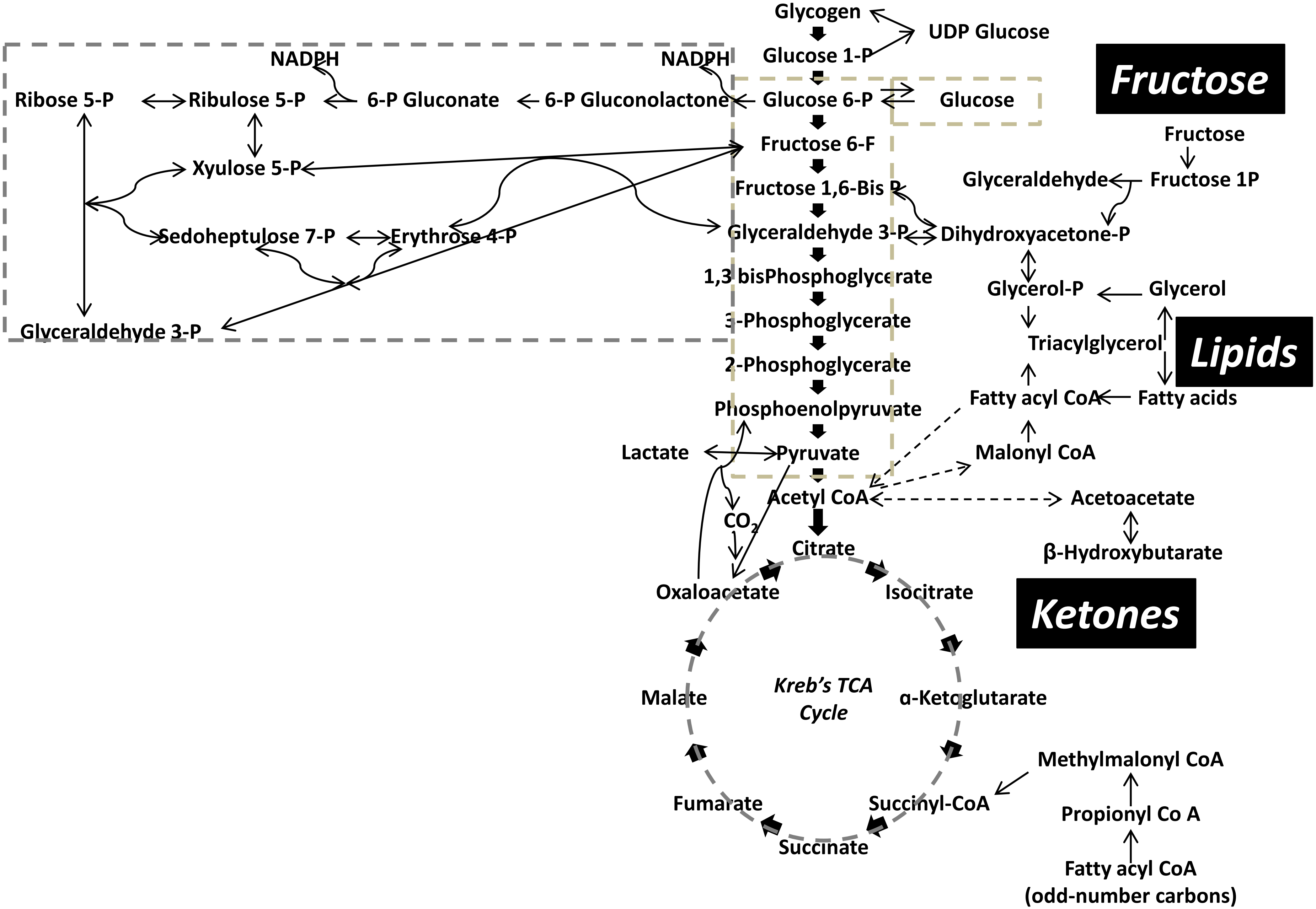
Glycolysis


Glycogen
Glucose 1-P
Glucose 6-P
Glucose
Fructose 6-F
Fructose 1,6-Bis P
Glyceraldehyde 3-P
Dihydroxyacetone P
bisPhosphoglycerate
-Phosphoglycerate
2-Phosphoglycerate
Phosphoenolpyruvate
Pyruvate
Acetyl CoA

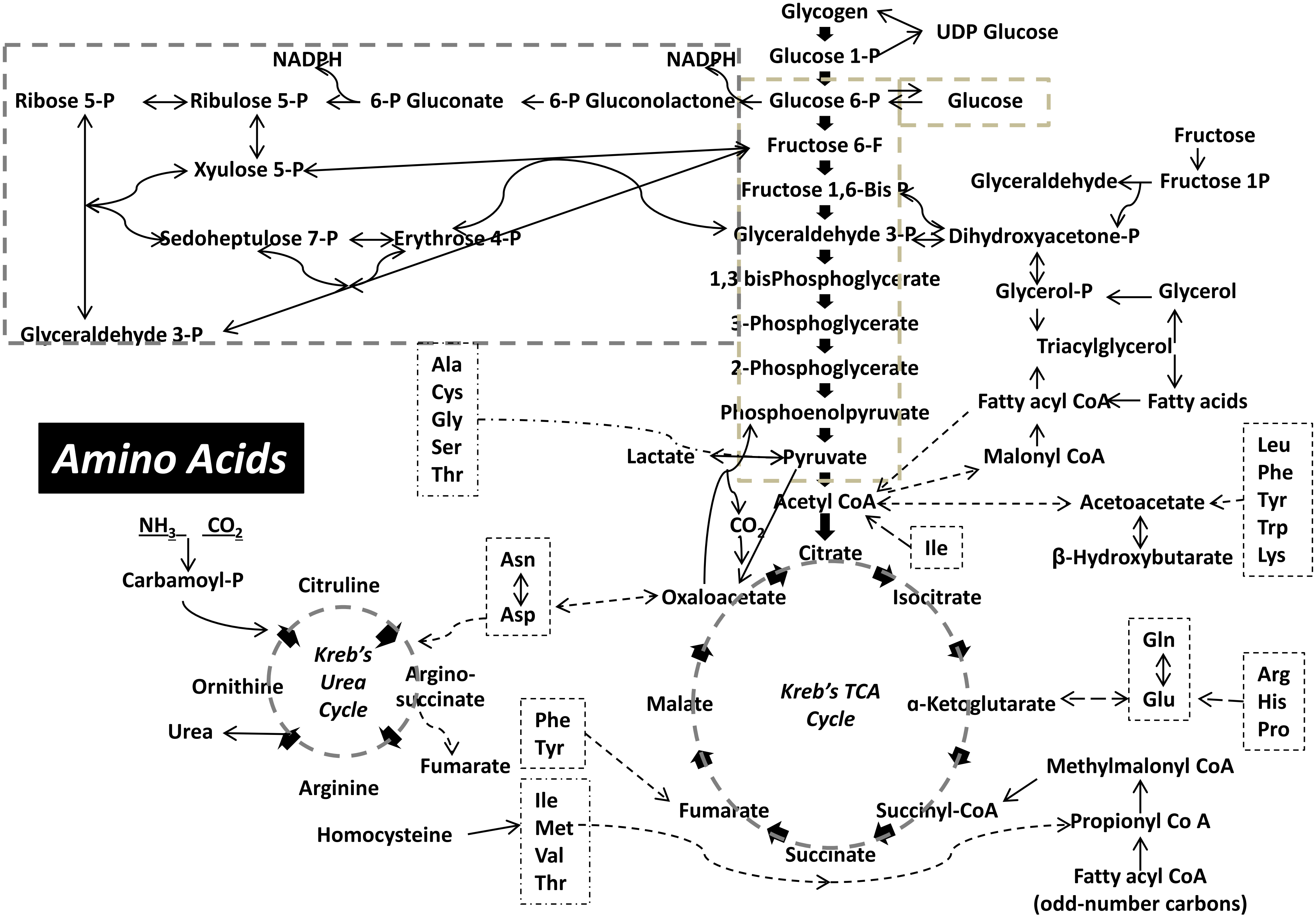

Glycogen
Glucose 1-P
Glucose 6-P
Glucose
Fructose 6-F
Fructose 1,6-Bis P
Glyceraldehyde 3-P
Dihydroxyacetone-P
1,3 bisPhosphoglycerate
3-Phosphoglycerate
2-Phosphoglycerate
Phosphoenolpyruvate
Lactate
Pyruvate
Acetyl CoA
CO_2
Citrate
Oxaloacetate
Isocitrate
Gluconeogenesis
Kreb's TCA Cycle
Malate
α-Ketoglutarate
Fumarate
Succinyl-CoA
Succinate
TCA Cycle

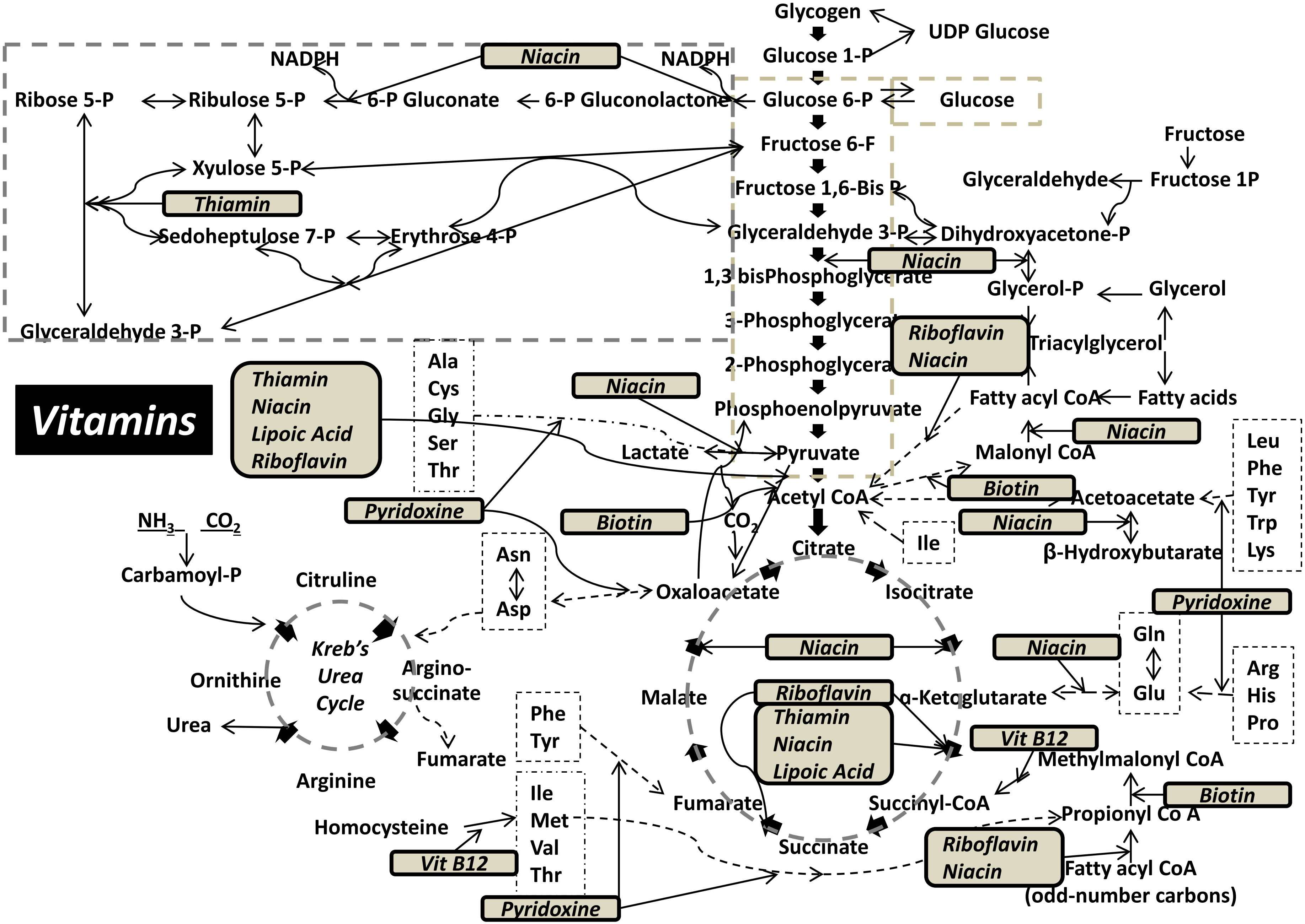


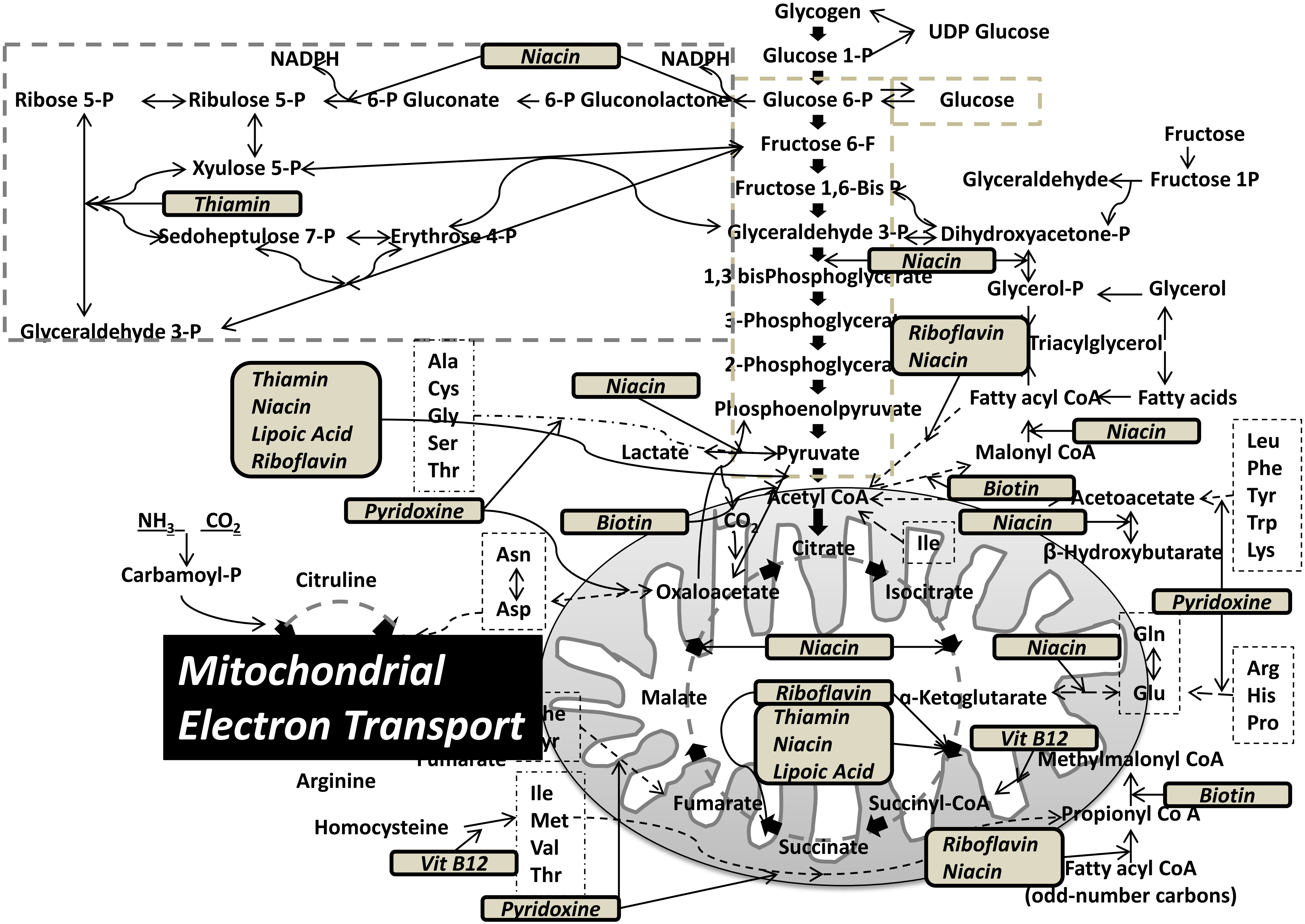
Pentose-Phosphate Pathway
Glycogen
UDP Glucose
Glucose 1-P
NADPH
NADPH
Ribose 5-P
Ribulose 5-P
6-P Gluconate
6-P Gluconolactone
Glucose 6-P
Glucose
Fructose 6-F
Xyulose 5-P
Fructose 1,6-Bis P
Sedoheptulose 7-P
Erythrose 4-P
Glyceraldehyde 3-P
Dihydroxyacetone-P
1,3 bisPhosphoglycerate
3-Phosphoglycerate
Glyceraldehyde 3-P
2-Phosphoglycerate
Phosphoenolpyruvate
Lactate
Pyruvate
Acetyl CoA
CO_2
Citrate
Oxaloacetate
Isocitrate
Kreb's TCA Cycle
Malate
α-Ketoglutarate
Fumarate
Succinyl-CoA
Succinate



Glycogen
UDP Glucose
Glucose 1-P
Fructose
NADPH
NADPH
Ribose 5-P
Ribulose 5-P
6-P Gluconate
6-P Gluconolactone
Glucose 6-P
Glucose
Fructose 6-F
Fructose
Xylulose 5-P
Fructose 1,6-Bis P
Glyceraldehyde
Fructose 1P
Glyceraldehyde 3-P
Dihydroxyacetone-P
Sedoheptulose 7-P
Erythrose 4-P
1,3 bisPhosphoglycerate
Glycerol-P
Glycerol
3-Phosphoglycerate
Glyceraldehyde 3-P
Triacylglycerol
Lipids
2-Phosphoglycerate
Fatty acyl CoA
Fatty acids
Phosphoenolpyruvate
Lactate
Pyruvate
Malonyl CoA
Acetyl CoA
Acetoacetate
CO_2
Citrate
β-Hydroxybutarate
Oxaloacetate
Isocitrate
Ketones
Malate
Kreb's TCA Cycle
α-Ketoglutarate
Methylmalonyl CoA
Fumarate
Succinyl-CoA
Propionyl Co A
Succinate
Fatty acyl CoA (odd-number carbons)

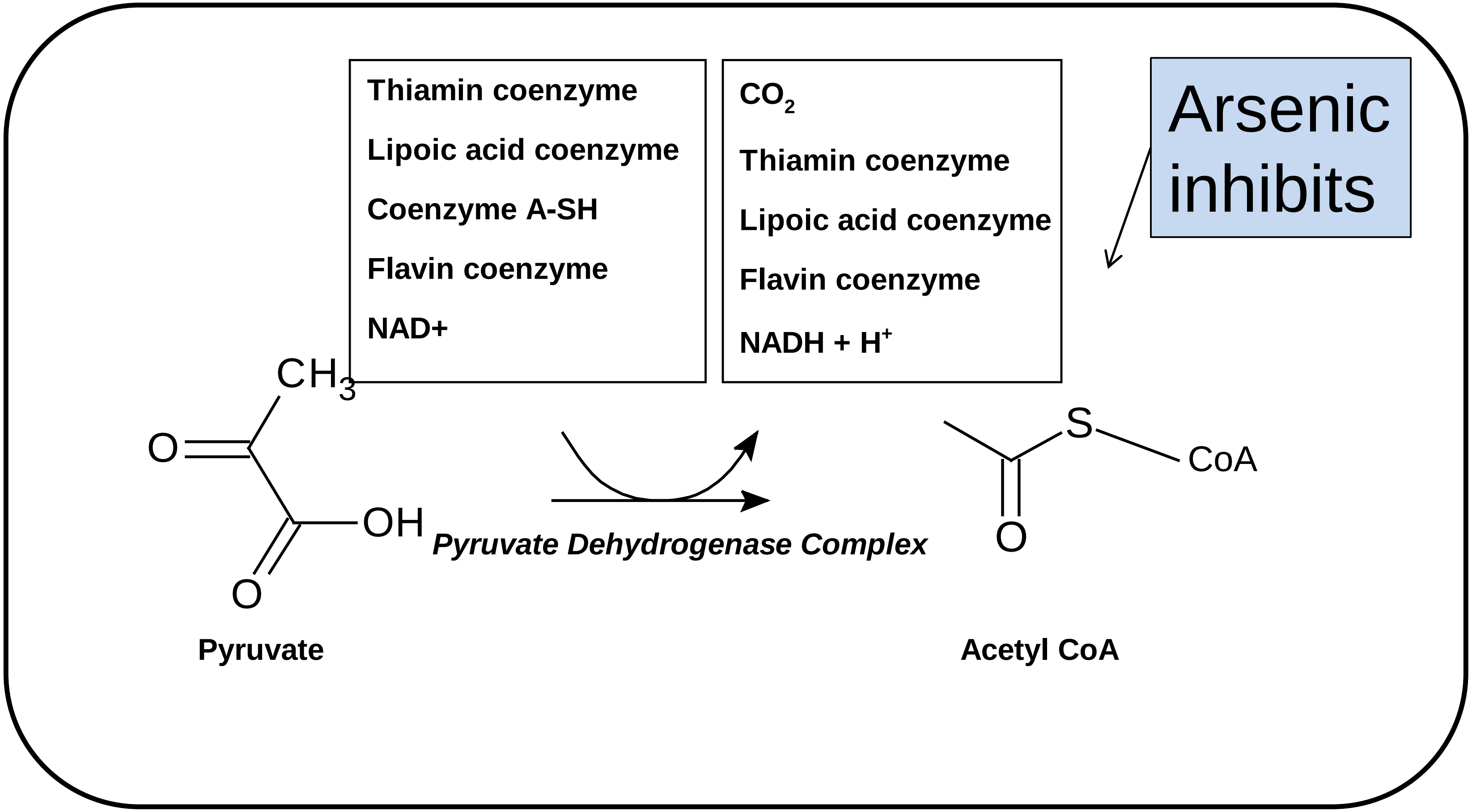

Amino Acids
Glycogen
UDP Glucose
Glucose 1-P
NADPH
NADPH
Ribose 5-P
Ribulose 5-P
6-P Gluconate
6-P Gluconolactone
Glucose 6-P
Glucose
Fructose 6-F
Fructose
Xylulose 5-P
Fructose 1,6-Bis P
Glyceraldehyde
Fructose 1P
Sedoheptulose 7-P
Erythrose 4-P
Glyceraldehyde 3-P
Dihydroxyacetone-P
1,3 bisPhosphoglycerate
Glycerol-P
Glycerol
Glyceraldehyde 3-P
3-Phosphoglycerate
Triacylglycerol
Ala
Cys
Gly
Ser
Thr
2-Phosphoglycerate
Fatty acyl CoA
Fatty acids
Phosphoenolpyruvate
Lactate
Pyruvate
Malonyl CoA
Leu
Phe
Tyr
Trp
Lys
Acetyl CoA
Acetoacetate
NH_3 CO_2
CO_2
Ile
β-Hydroxybutarate
Citrate
Carbamoyl-P
Citruline
Asn
Asp
Oxaloacetate
Isocitrate
Kreb's Urea Cycle
Ornithine
Argino-succinate
Malate
Kreb's TCA Cycle
α-Ketoglutarate
Gln
Glu
Arg
His
Pro
Urea
Phe
Tyr
Fumarate
Arginine
Methylmalonyl CoA
Ile
Met
Val
Thr
Homocysteine
Fumarate
Succinyl-CoA
Propionyl Co A
Succinate
Fatty acyl CoA
(odd-number carbons)


Vitamins
Glycogen
UDP Glucose
Glucose 1-P
Niacin
NADPH
NADPH
Ribose 5-P
Ribulose 5-P
6-P Gluconate
6-P Gluconolactone
Glucose 6-P
Glucose
Fructose 6-F
Xylulose 5-P
Fructose
Thiamin
Fructose 1,6-Bis P
Glyceraldehyde
Fructose 1P
Sedoheptulose 7-P
Erythrose 4-P
Glyceraldehyde 3-P
Dihydroxyacetone-P
Niacin
1,3 bisPhosphoglycerate
Glycerol-P
Glycerol
Glyceraldehyde 3-P
3-Phosphoglycerat
Riboflavin
Niacin
Triacylglycerol
Thiamin
Niacin
Lipoic Acid
Riboflavin
Ala
Cys
Gly
Ser
Thr
Niacin
2-Phosphoglycera
Fatty acyl CoA
Fatty acids
Phosphoenolpyruvate
Niacin
Malonyl CoA
Leu
Phe
Tyr
Trp
Lys
Lactate
Pyruvate
Biotin
Acetyl CoA
Acetoacetate
Pyridoxine
Biotin
CO_2
Niacin
Ile
β-Hydroxybutarate
NH_3
CO_2
Citrate
Carbamoyl-P
Citruline
Asn
Asp
Oxaloacetate
Isocitrate
Pyridoxine
Kreb's
Urea
Cycle
Niacin
Niacin
Gln
Glu
Ornithine
Argino-
succinate
Malate
Riboflavin
α-Ketoglutarate
Arg
His
Pro
Thiamin
Niacin
Lipoic Acid
Urea
Phe
Tyr
Vit B12
Methylmalonyl CoA
Fumarate
Arginine
Ile
Met
Val
Thr
Fumarate
Succinyl-CoA
Biotin
Homocysteine
Propionyl Co A
Riboflavin
Niacin
Succinate
Vit B12
Fatty acyl CoA
(odd-number carbons)
Pyridoxine



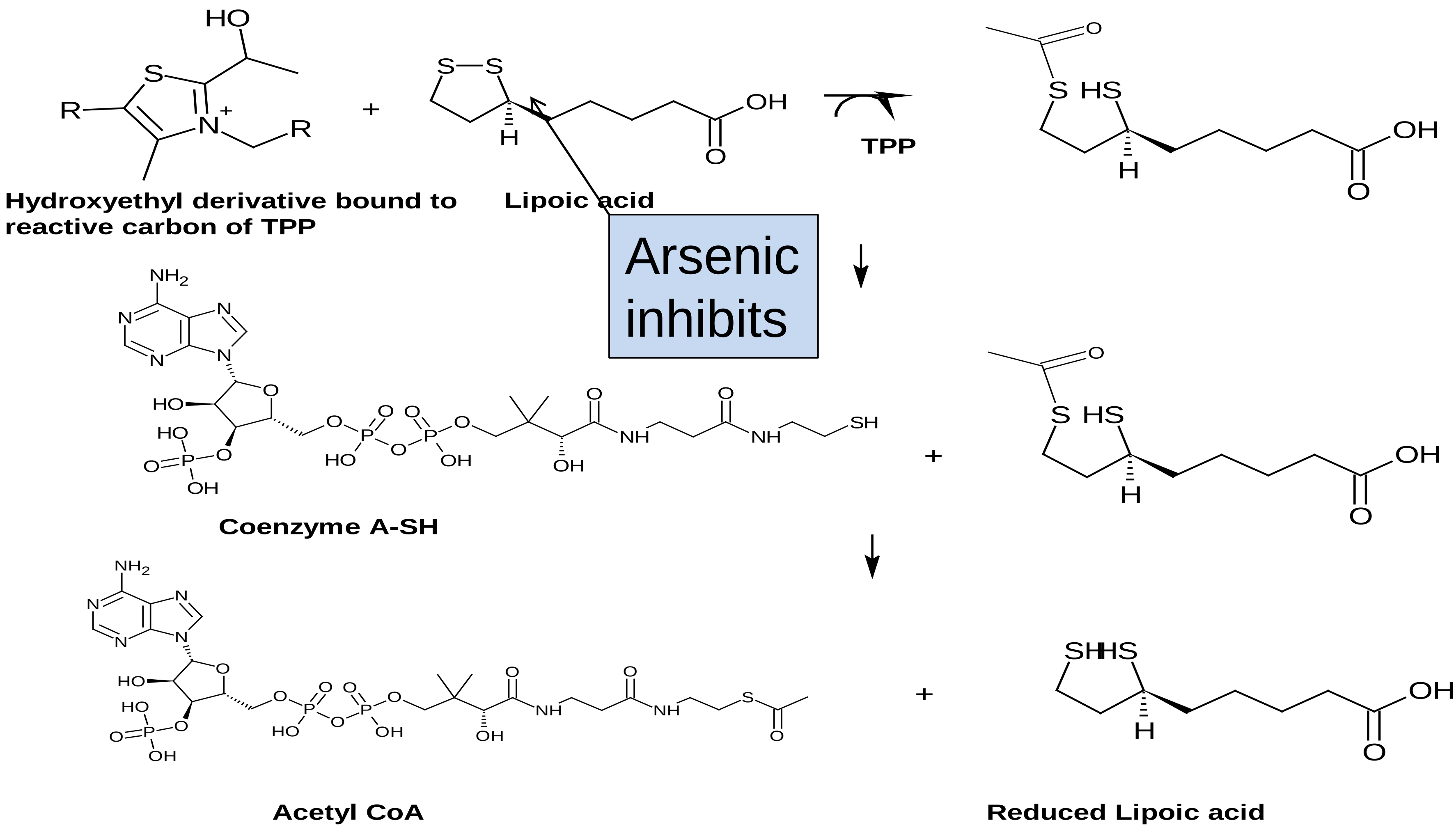
Glycogen
UDP Glucose
Glucose 1-P
Niacin
NADPH
NADPH
Ribose 5-P
Ribulose 5-P
6-P Gluconate
6-P Gluconolactone
Glucose 6-P
Glucose
Fructose 6-F
Xylulose 5-P
Fructose
Fructose 1,6-Bis P
Glyceraldehyde
Fructose 1P
Thiamin
Sedoheptulose 7-P
Erythrose 4-P
Glyceraldehyde 3-P
Dihydroxyacetone-P
Niacin
1,3 bisPhosphoglycerate
Glycerol-P
Glycerol
3-Phosphoglycerat
Riboflavin
Niacin
Glyceraldehyde 3-P
Triacylglycerol
2-Phosphoglycera
Thiamin
Niacin
Lipoic Acid
Riboflavin
Ala
Cys
Gly
Ser
Thr
Niacin
Phosphoenolpyruvate
Fatty acyl CoA
Fatty acids
Niacin
Lactate
Pyruvate
Malonyl CoA
Leu
Phe
Tyr
Trp
Lys
Biotin
Acetoacetate
Pyridoxine
Acetyl CoA
Biotin
Niacin
NH_3
CO_2
CO_2
Ile
β-Hydroxybutarate
Carbamoyl-P
Citrate
Citruline
Asn
Asp
Oxaloacetate
Isocitrate
Pyridoxine
Mitochondrial
Electron Transport
Niacin
Niacin
Gln
Glu
Arg
His
Pro
Malate
Riboflavin
Thiamin
Niacin
Lipoic Acid
α-Ketoglutarate
Vit B12
Methylmalonyl CoA
Arginine
Fumarate
Succinyl-CoA
Biotin
Propionyl Co A
Ile
Met
Val
Thr
Homocysteine
Vit B12
Succinate
Riboflavin
Niacin
Fatty acyl CoA
(odd-number carbons)
Pyridoxine

Pyruvate Dehydrogenase Complex



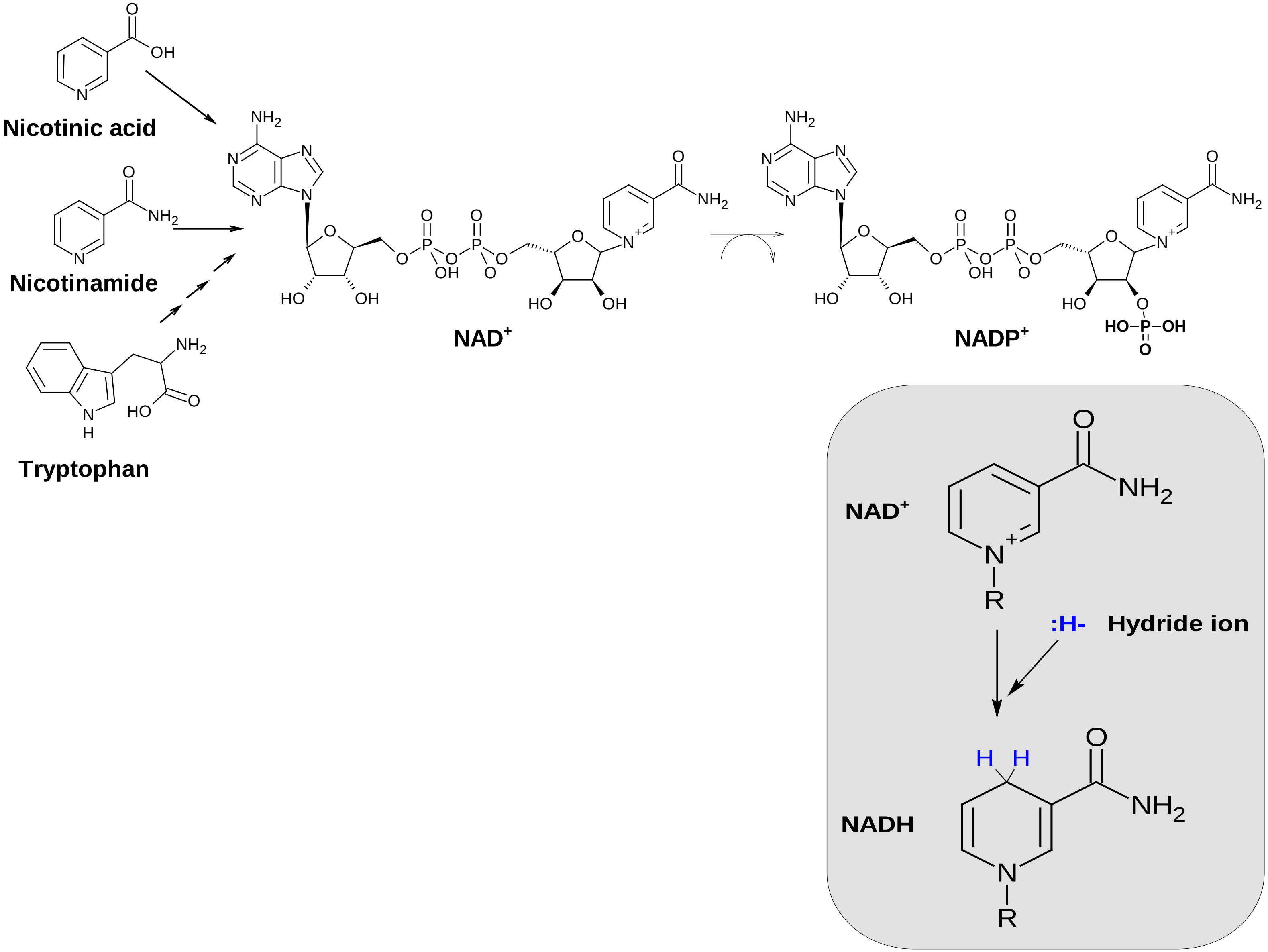
Mitochondria

Lipoic Acid: Cofactor for 2nd Enzyme

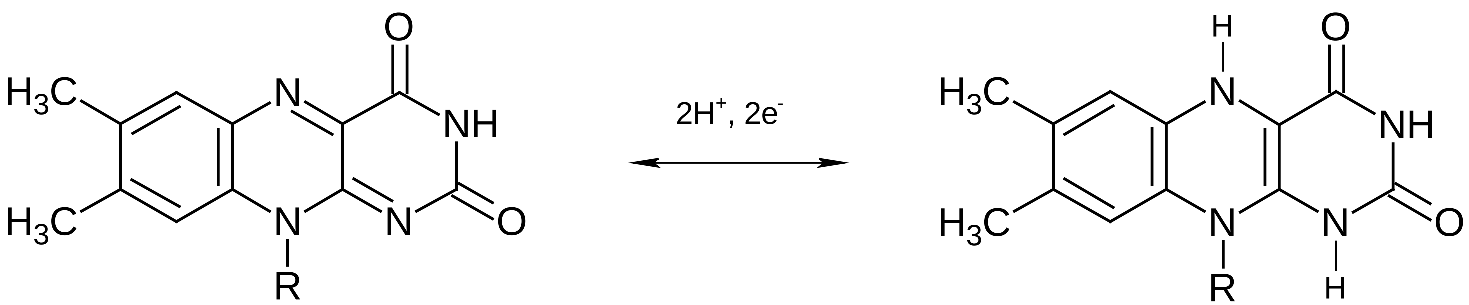


Dihydrolipoyl transacetylase

Niacin – Vitamin B3



Riboflavin – Vit B2

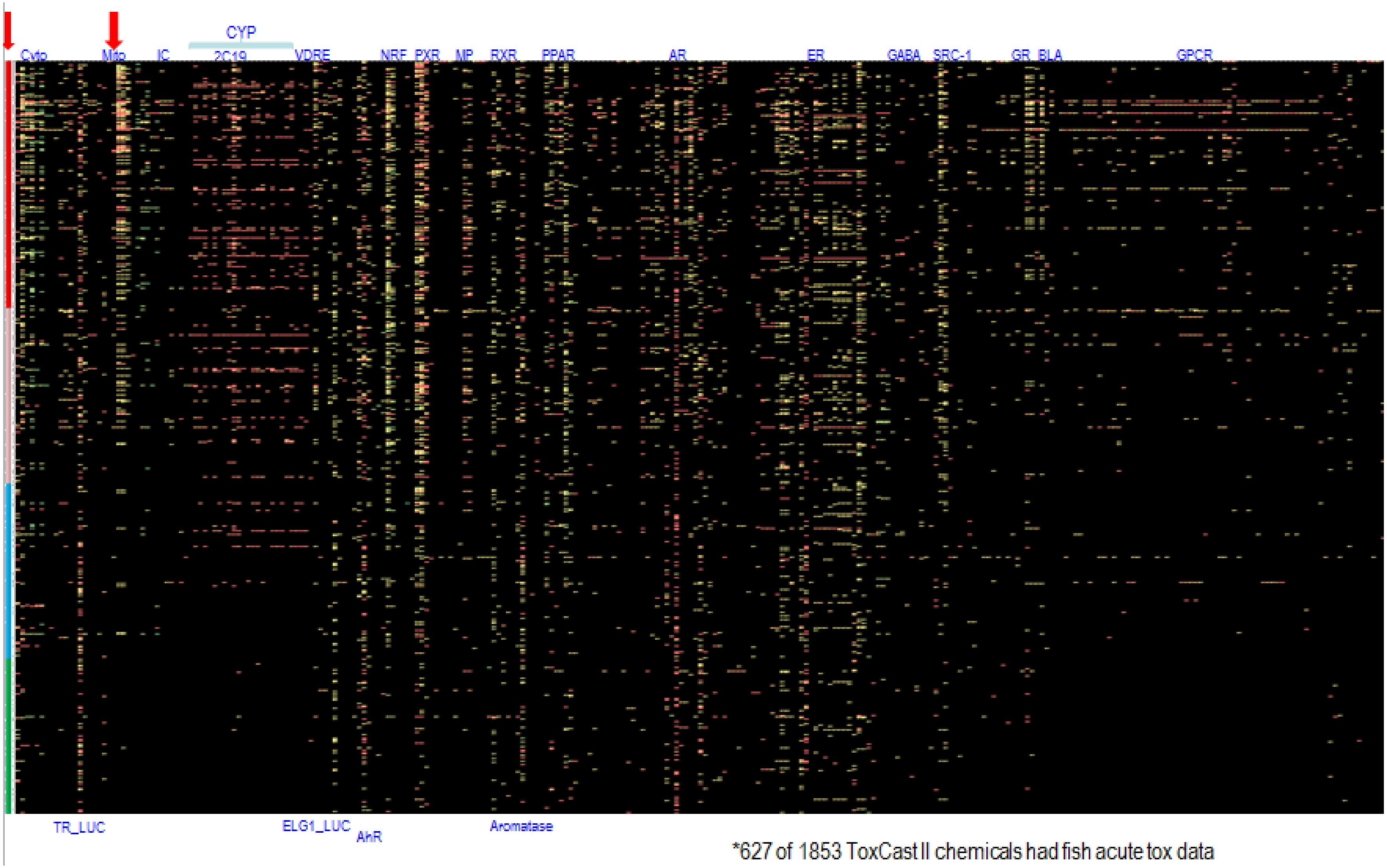


FAD (oxidized quinone form)

$FADH_2$ (reduced hydroquinone form)

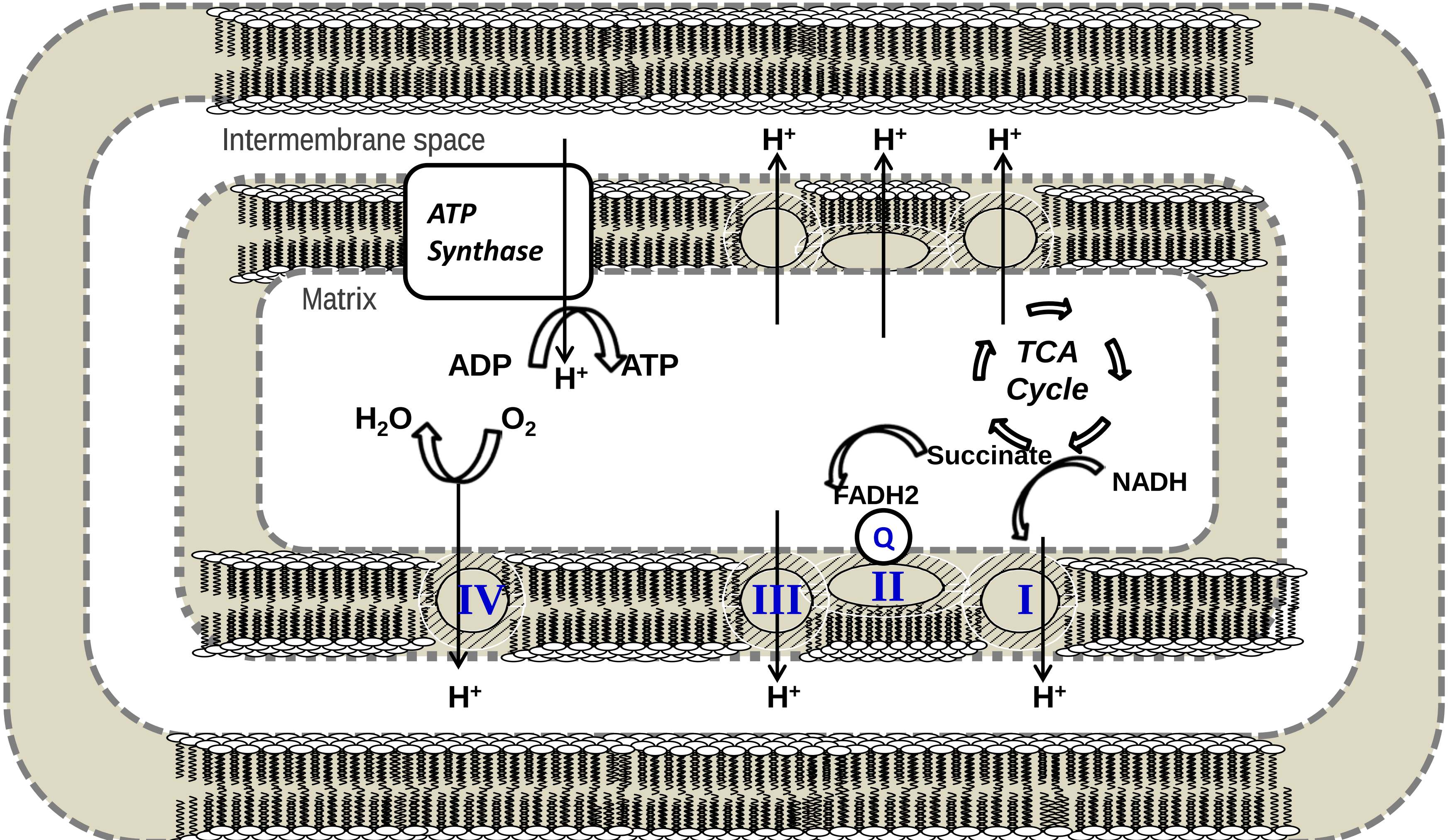
FMN and FAD tightly bound to enzymes that catalyze oxidation or reduction

Fish acute toxicity vs. ToxCast HTS

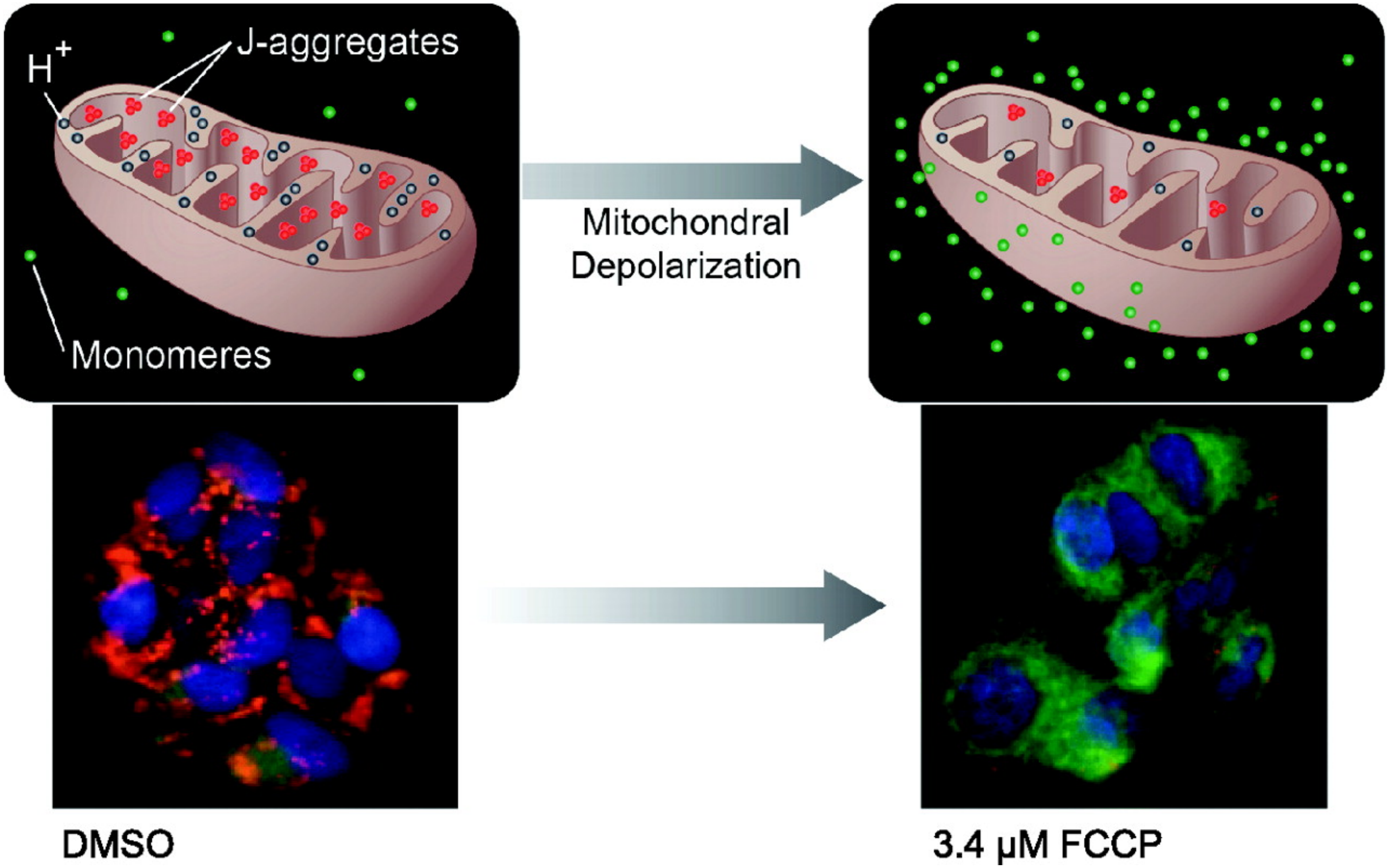


*627 of 1853 ToxCast II chemicals had fish acute tox data

Mitochondrial Electron Transport

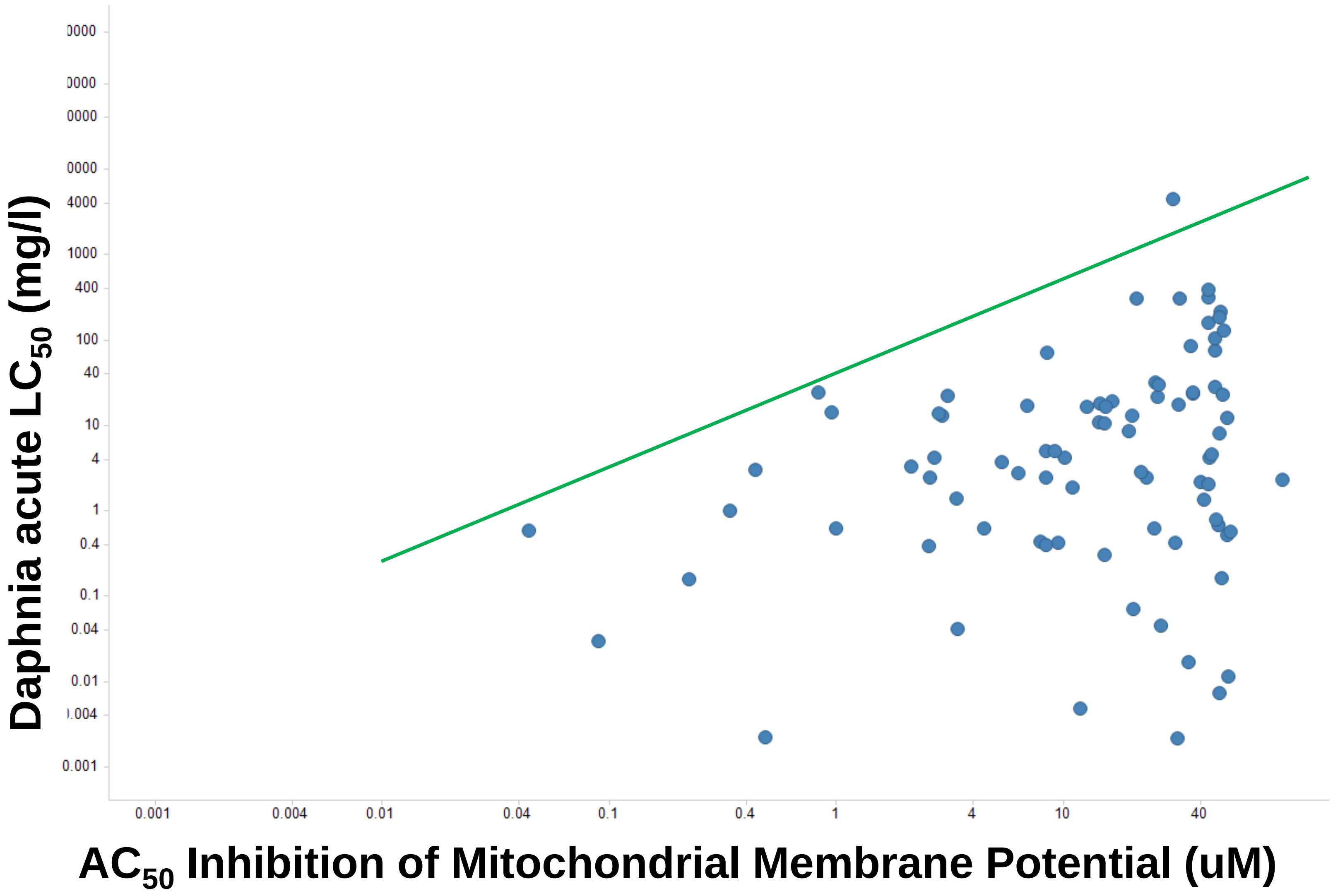


Mitochondria membrane potential assay

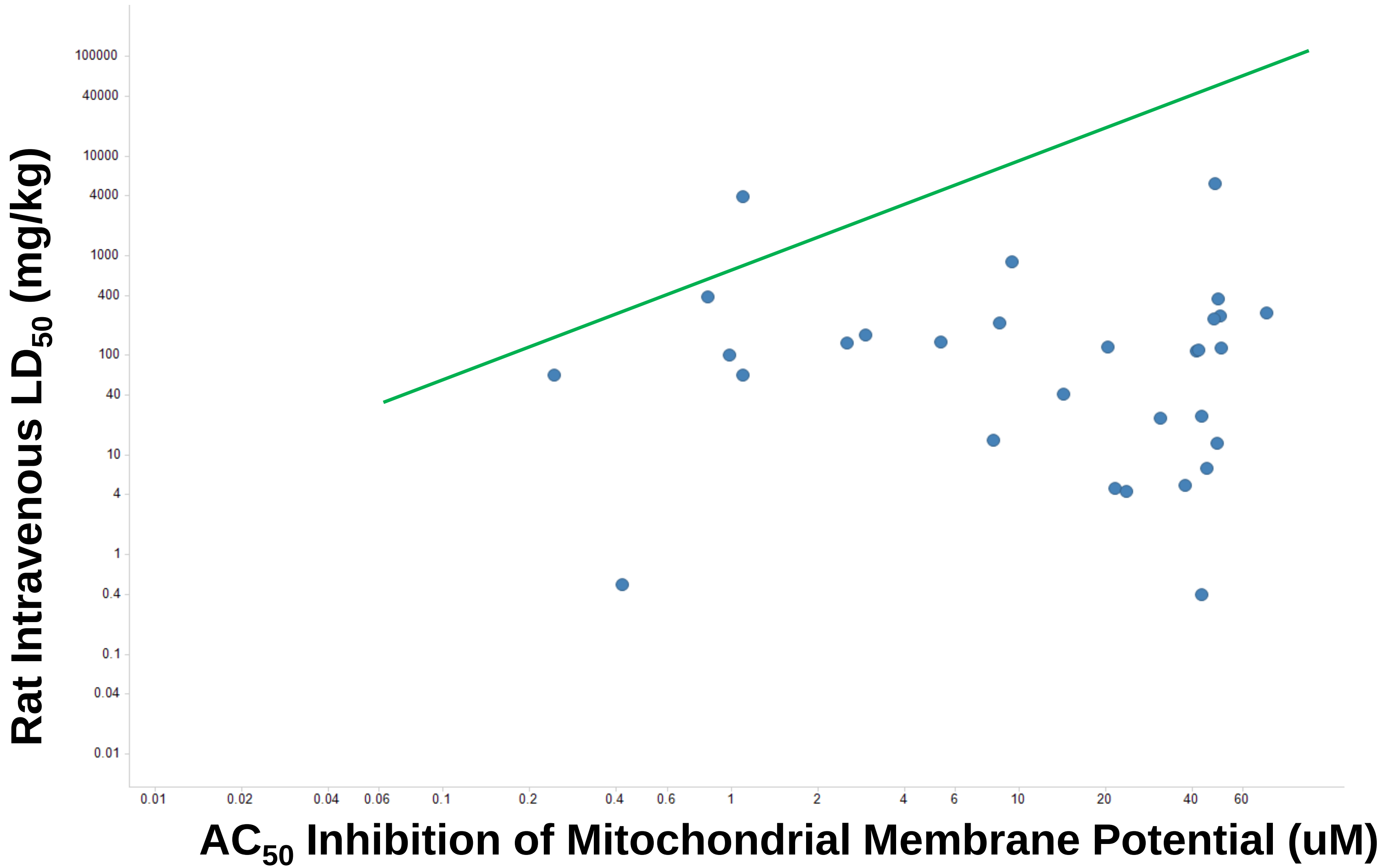


Sakamuru S et al. Physiol. Genomics 2012;44:495-503

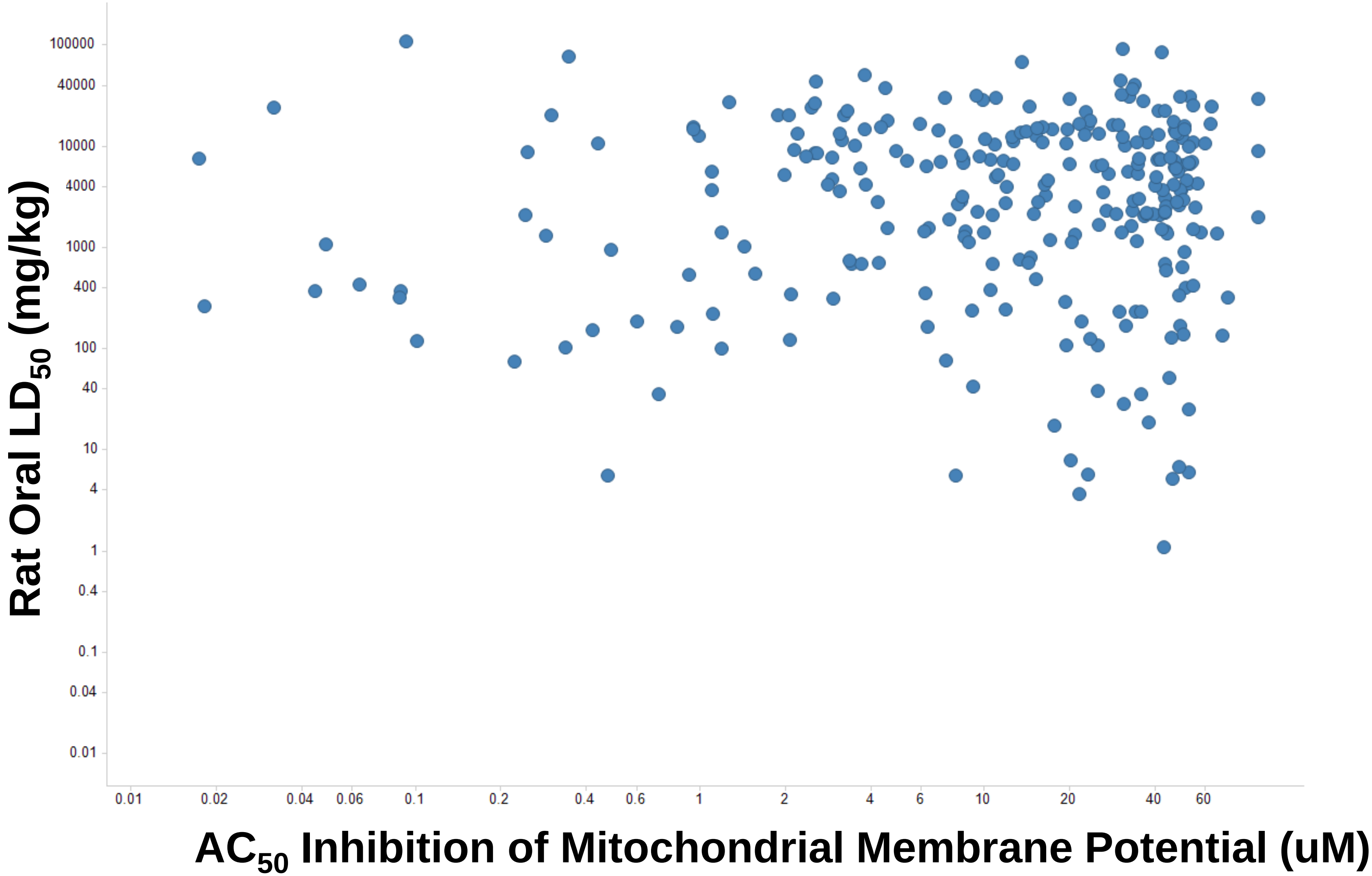
Mitochondrial tox predicts upper boundary to Daphnia toxicity



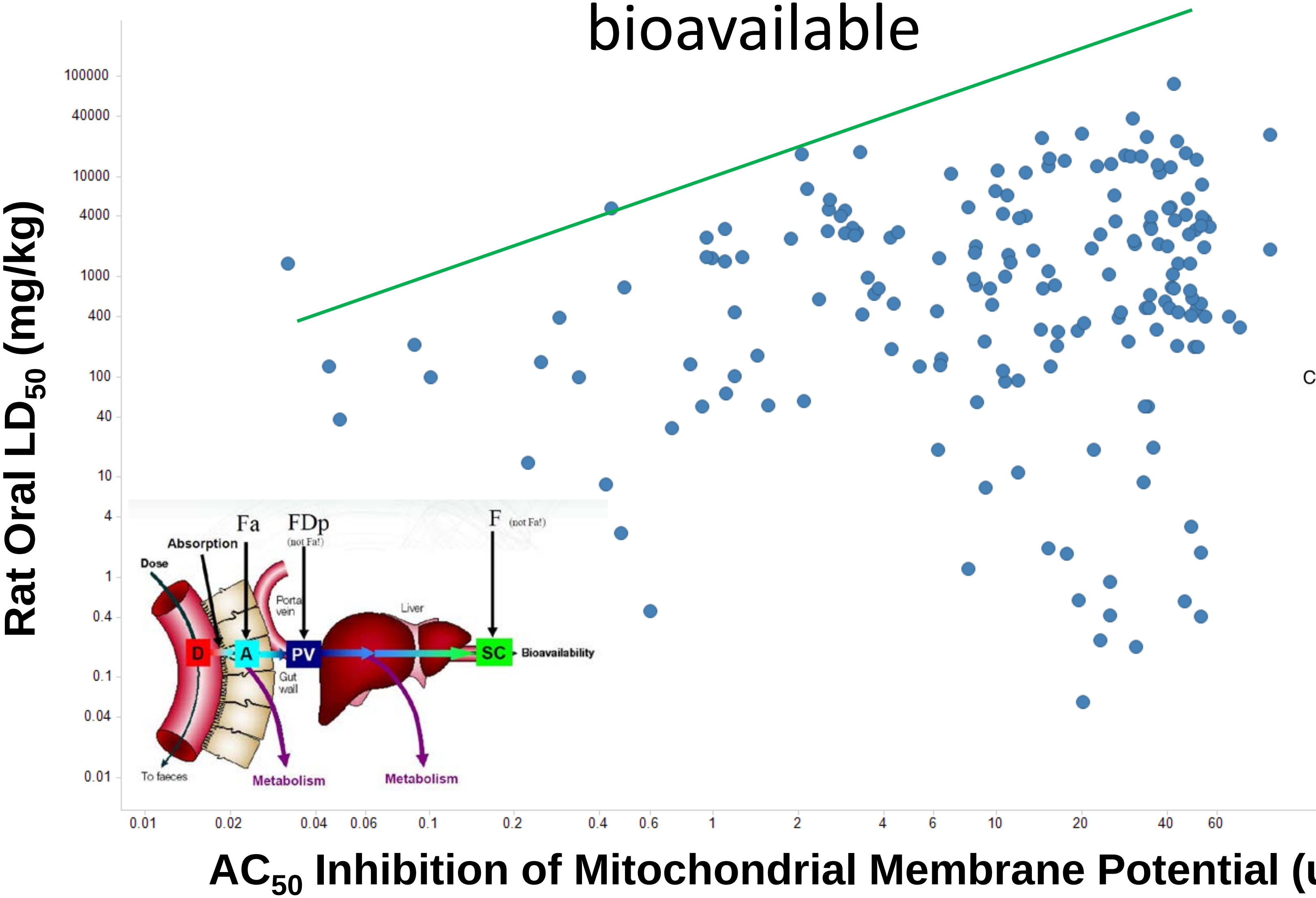
Mitochondrial toxicity predicts upper bound to acute intravenous toxicity

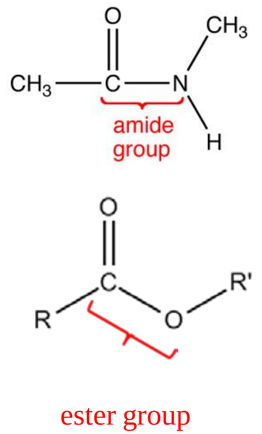


Mitochondrial toxicity doesn't predict upper bound to oral rat toxicity

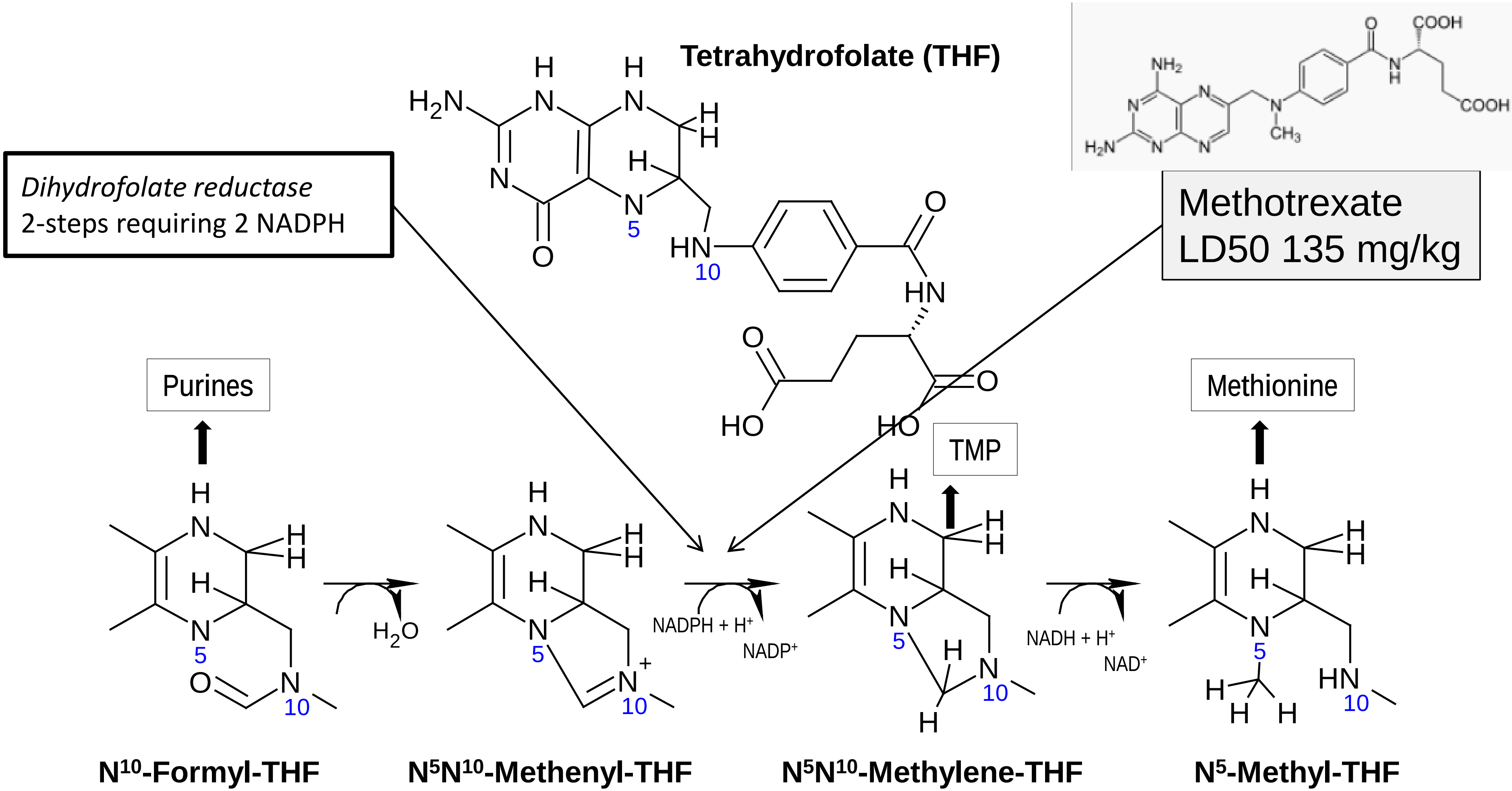


LD_{50} values adjusted downward by % bioavailable

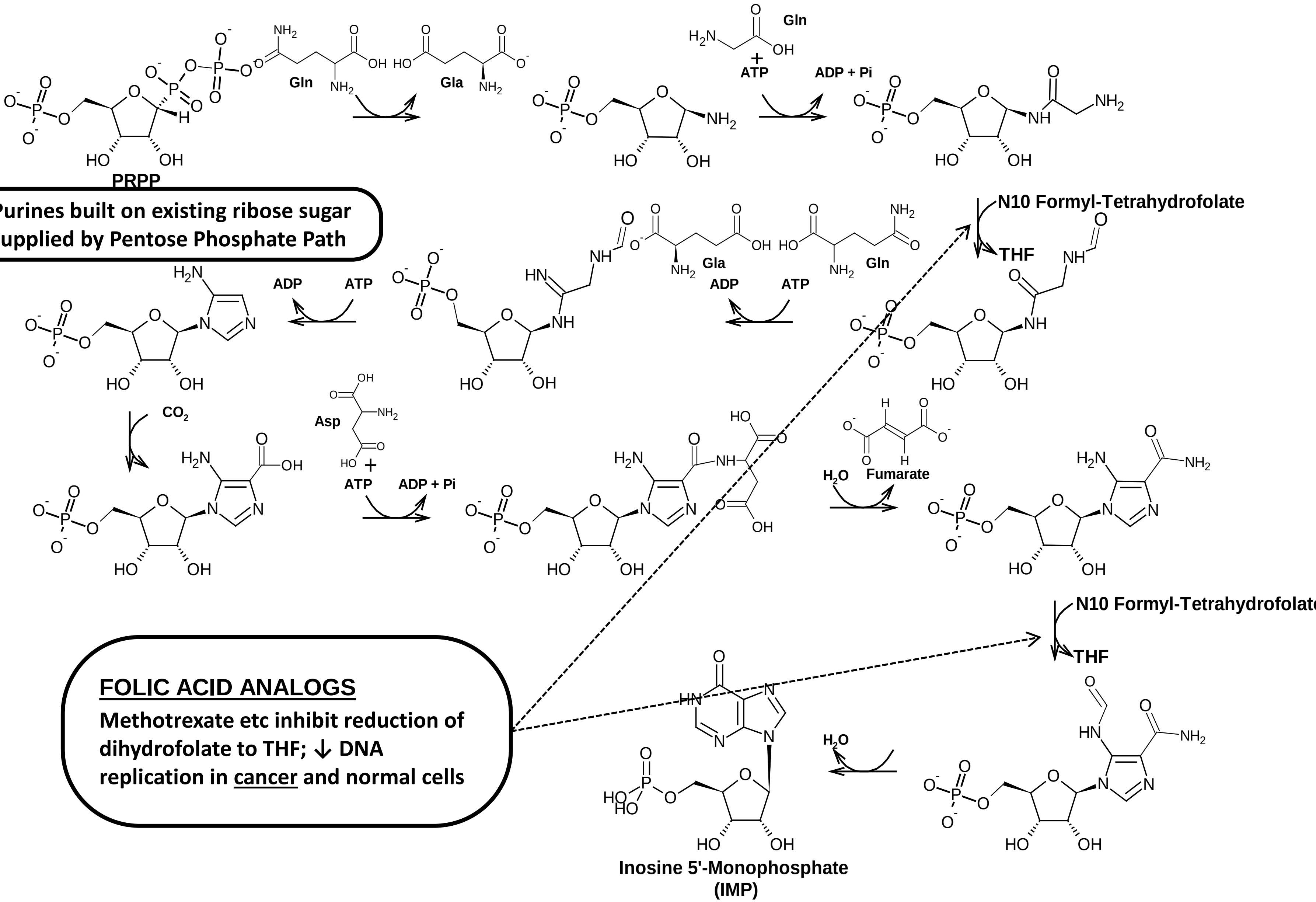




Antimetabolites



Purine synthesis

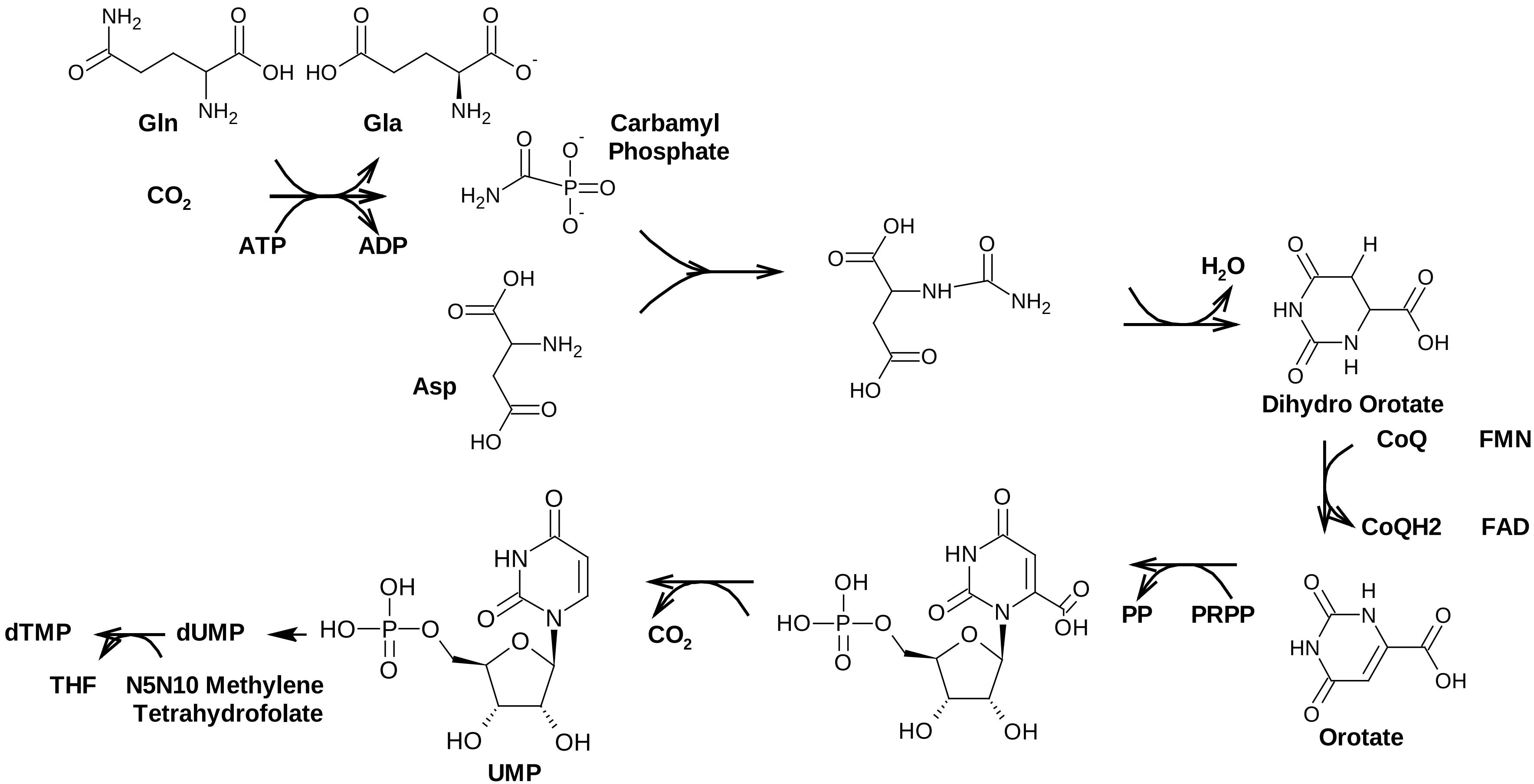


Purines built on existing ribose sugar supplied by Pentose Phosphate Path

FOLIC ACID ANALOGS

Methotrexate etc inhibit reduction of dihydrofolate to THF; ↓ DNA replication in cancer and normal cells

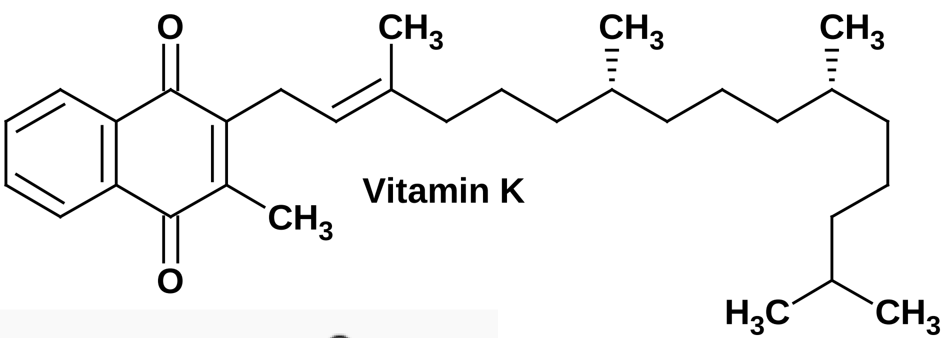
Pyrimidine Biosynthesis



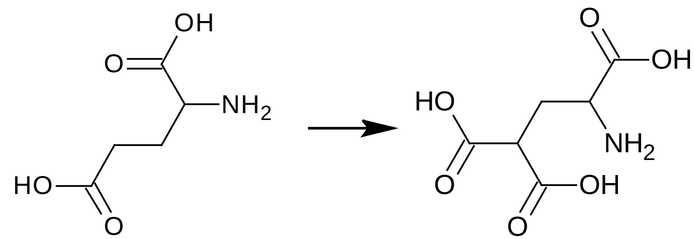
Base synthesized then added to preformed ribose

Anticoagulants

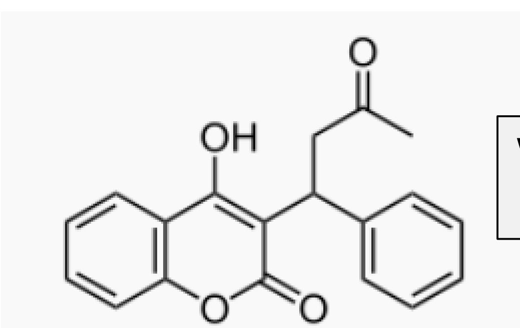
- Cofactor of enzyme that carboxylates γ-glutamyls in Prothrombin and Factors VII, IX and X
- Without carboxylation, don't bind membrane phospholipids
- Deficiency in infants - hemorrhagic disease of the newborn
- Natural K vitamins free of toxic side effects



Vitamin K

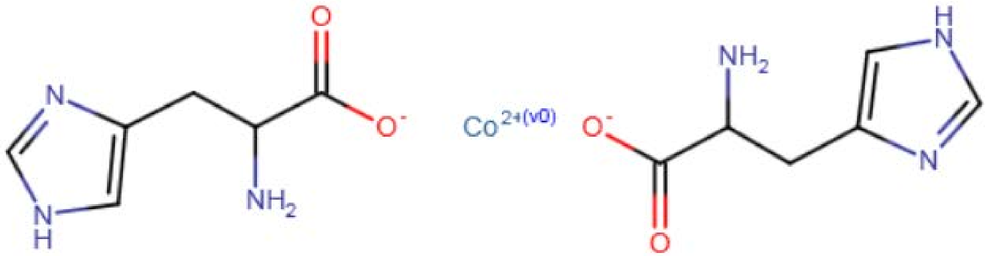


Glutamate → Carboxyglutamate



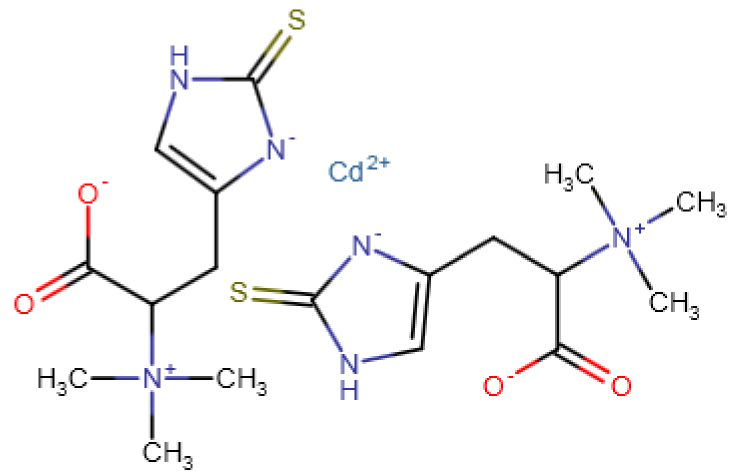
Warfarin is structural analog of Vit K
LD50 8.7 mg/kg

Chelators

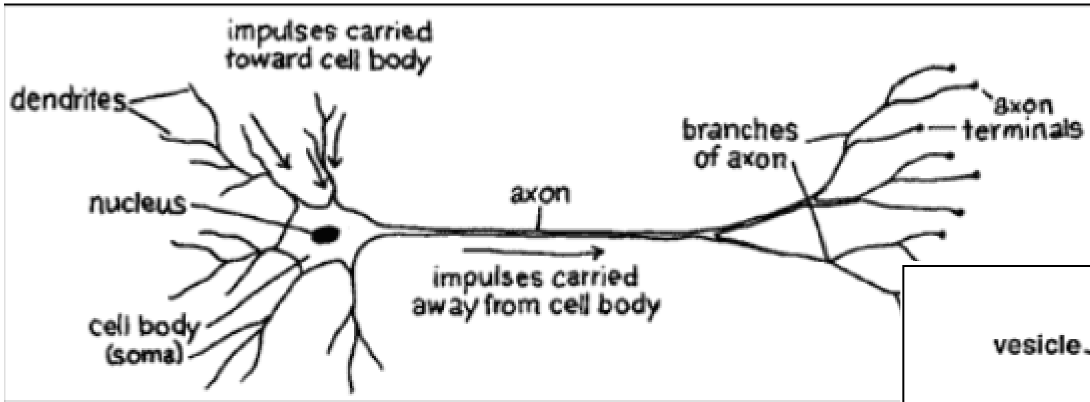


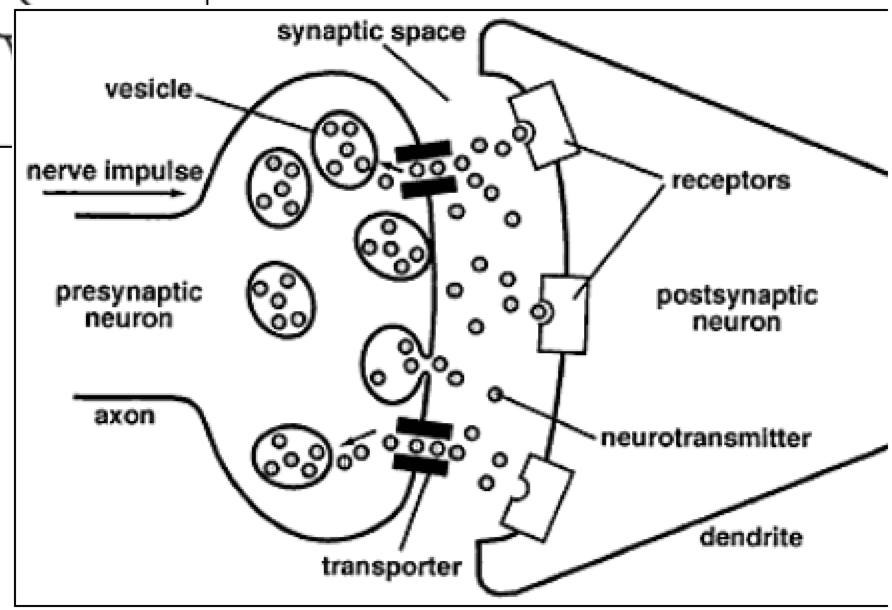
104mg/kg rat iv

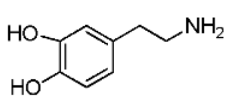
280 ug/kg rat iv

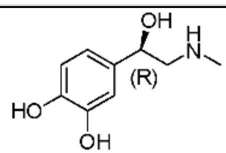


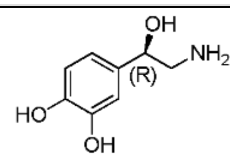
Signal transduction

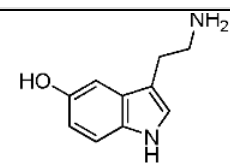


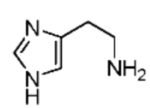


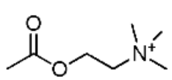

Dopamine

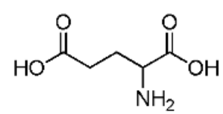

Epinephrine

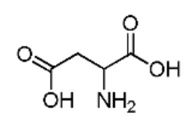

Norepinephrine

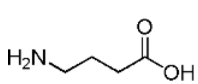

Serotonin

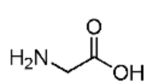

Histamine

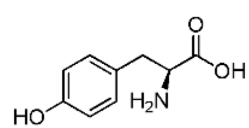

Acetylcholine

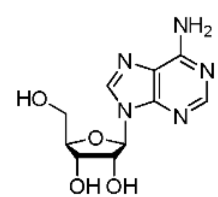

Glutamic Acid


Aspartic Acid

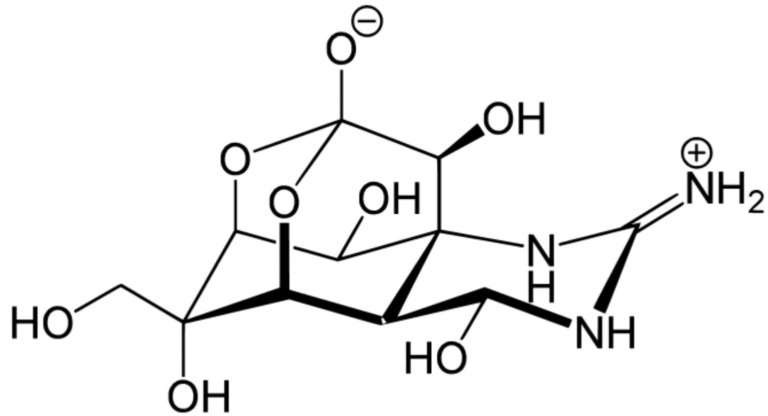

GABA


Glycine

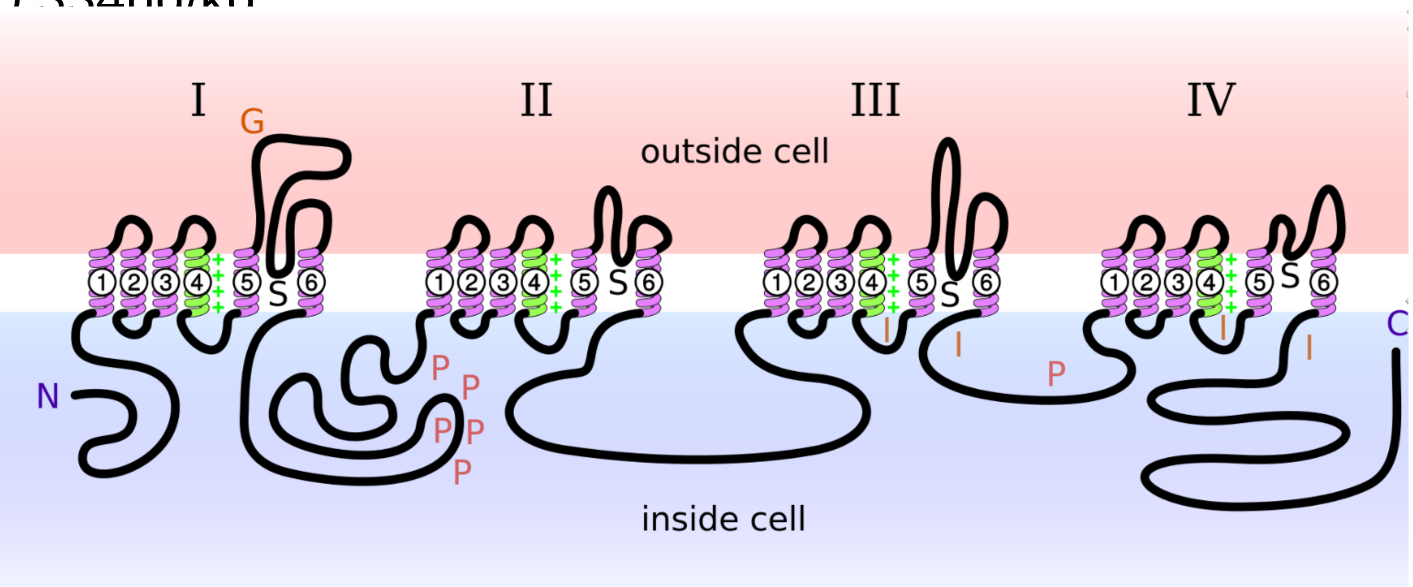

Tyrosine


Adenosine

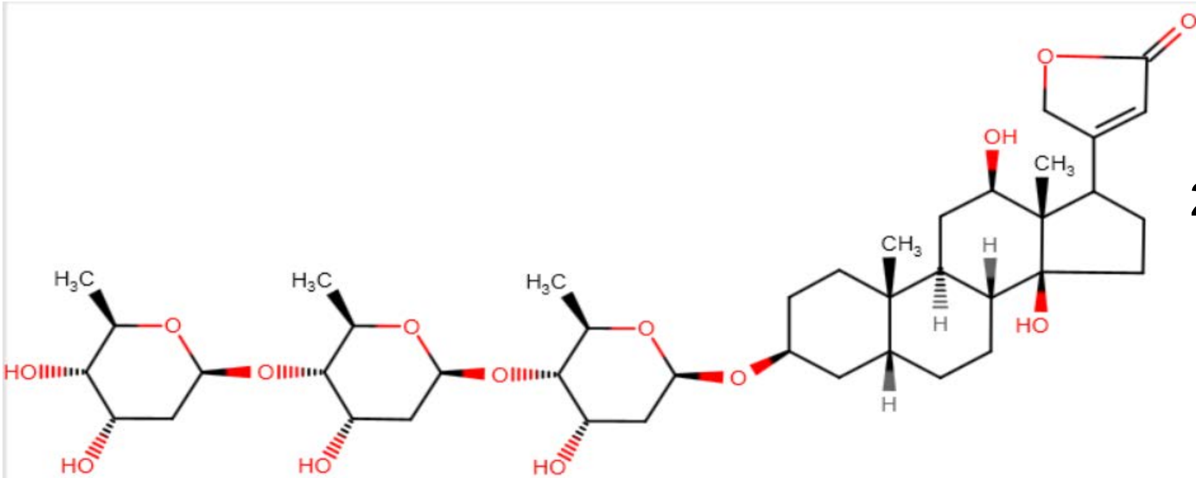
Tetrodotoxin



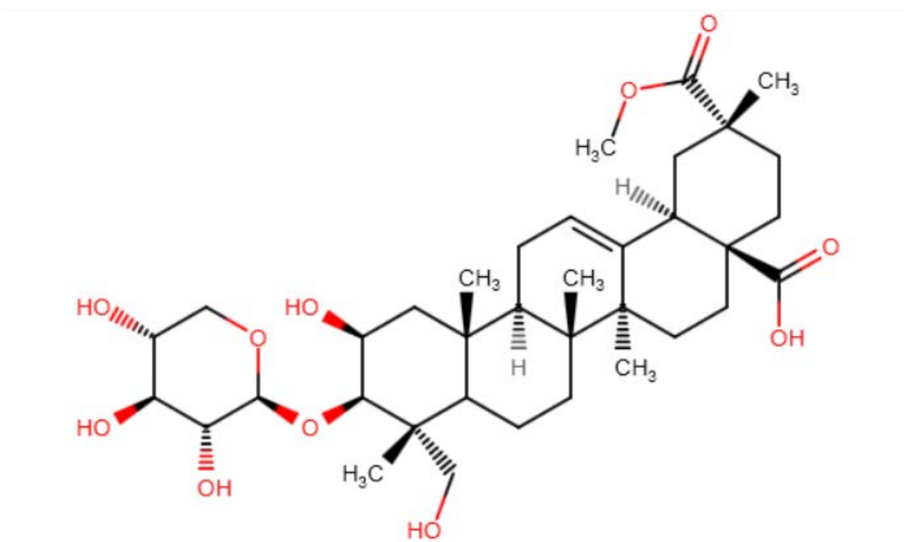
Inhibits voltage-gated sodium channels
Oral LD50 334µg/kg



Cardiac glycosides (Inh Na+/K+ ATPase)

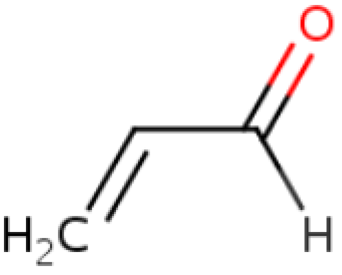


28.3 mg/kg rat LD50 oral



10.8 mg/kg rat LD50 iv

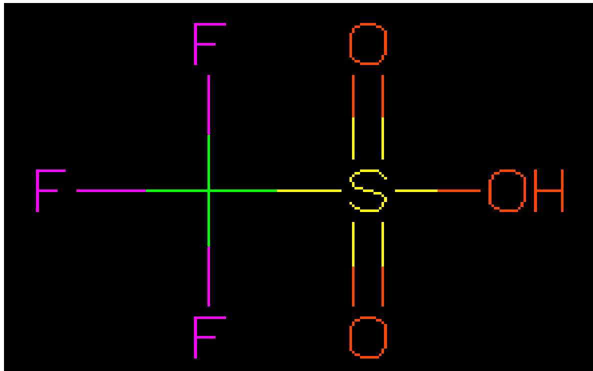
Michael acceptors



Acrolein
LD50 26 mg/kg

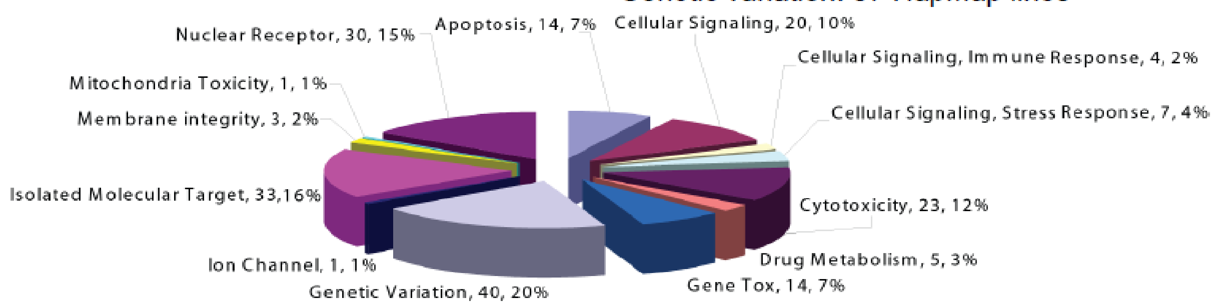
Acids

- Trifluoromethanesulfonic acid
- pH of 10% solution = 0.1
- Acute oral LD50: 1605.3 mg/kg bw
  - GHS Cat 4; H302: Harmful if swallowed
- Acute dermal LD50: > 50 mg/kg bw
  - test results inconclusive because of severe local effects on skin at 2000 mg/kg bw
- Acute inhalation LC50: ????
  - study scientifically unjustified



Future mechanistic approaches…

- Phenotypic readouts
  - Cytotoxicity
  - Apoptosis: caspase 3/7, 8, 9)
  - Membrane integrity: LDH, protease release
  - Mitochondrial toxicity (membrane potential)
  - Gene tox: p53, ELG1, DNA damage gene deficient lines (DT40 lines and mouse)
- Cell Signaling
  - Stress response: ARE, ESRE, HSP, Hypoxia, AP-1
  - Immune response: IL-8, TNFα, TTP
  - Other: AP-1, CRE, ERK, HRE, JNK3, NFkB, LDR
- Drug metabolism
  - CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4
- Target specific assays
  - Nuclear receptors: AR, AhR, ERα, FXR, GR, LXR, PPARα, PPARδ, PPARγ, PXR, RXR, TRβ, VDR, RORα, RORγ
  - hERG channel
  - Isolated molecular targets: 12hLO, 15hLO1, 15hLO2, ALDH1A1, HADH560, HPGD, HSD17b4, α-Glucosidase, α-Galactosidase, Glucocerebrosidase, APE1, TDP1, DNA polymerase III, RECQ1 helicase, RGS4, BRCA, IMPase, O-Glc NAc Transferase, Caspase-1/7, CBFβ-RUNX1, PK, Tau, Cruzain, β-Lactamase, PRX, YjeE , NPS, Proteasome, SF1, SMN2, beta-globin splicing, Anthrax Lethal Factor, TSHR
- Genetic variation: 87 HapMap lines



New Robot Can Test 10,000 Chemicals

Print Comment Share:

This high-speed robotic tester is revolutionizing routine chemical toxicity studies by screening thousands of chemicals each week for potential human toxicity

Questions?