Minimal Metabolome: The Canonical Network of Autotrophic Metabolism and Its Analysis

Vijay Srinivasan
Krasnow Institute for Advanced Study
George Mason University
Fairfax, VA

vsriniva@ gmu.edu

Hierarchy in Biology:

- Prebiotic Chemistry
- Intermediary Metabolism
- Cellularity
- Prokaryotes
- Eukaryotes
- Multicellularity

Chemolithoautotrophs

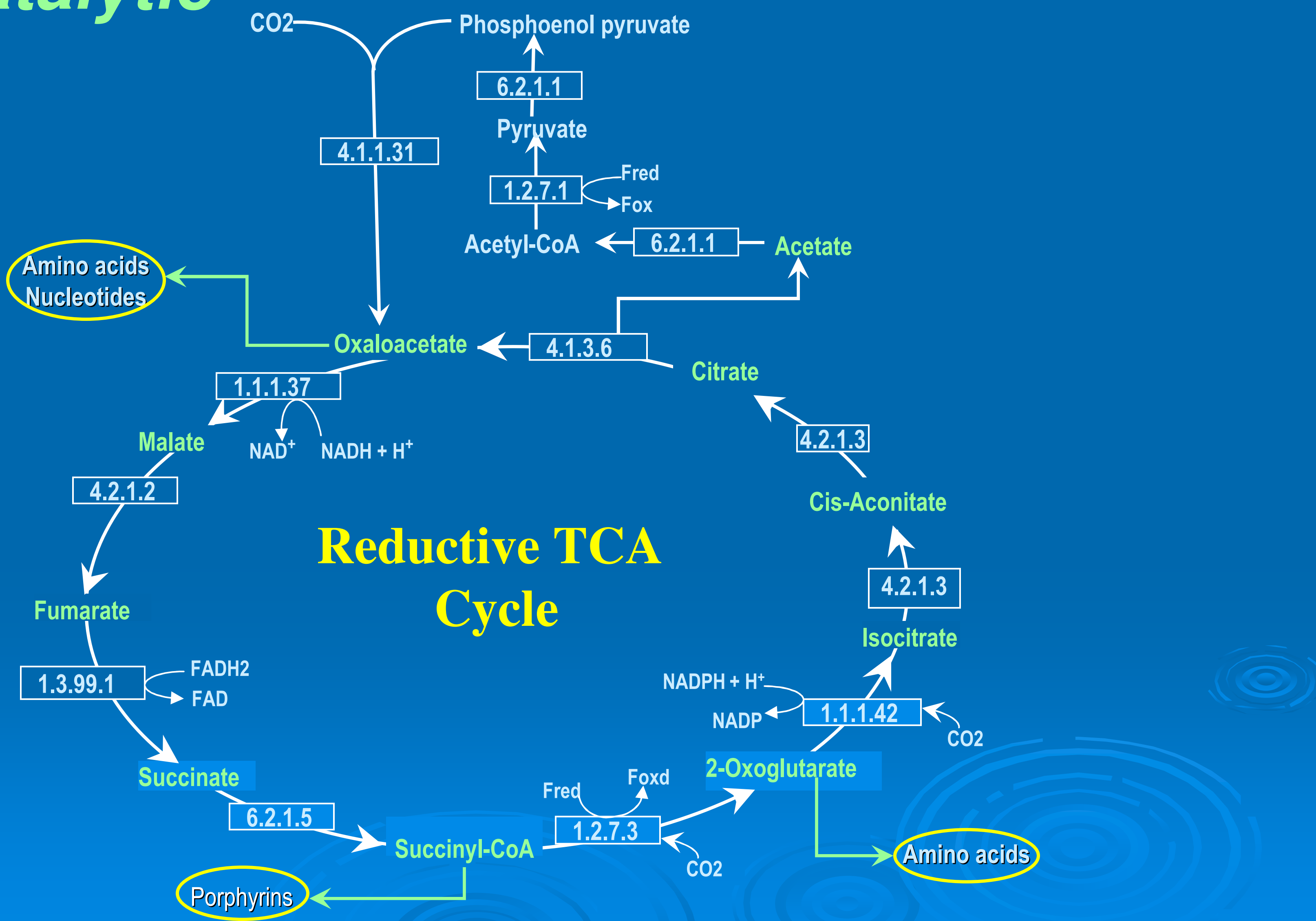
Hierarchy - Complexity

- Reductive world precedes Oxidative environment
- Autotrophs preceding heterotrophs
- Chemolithoautotrophs preceding phototrophs
- Minimal Metabolome and Chart of Autotrophic Metabolism

Core Intermediary Metabolism is Universal & Robust

Robust	Variable
Atoms	Gene (DNA) Sequence
Building Blocks (Metabolites)	RNA Sequence
Cofactors	Protein Sequence
Core Network	
Hydrophobic membrane	

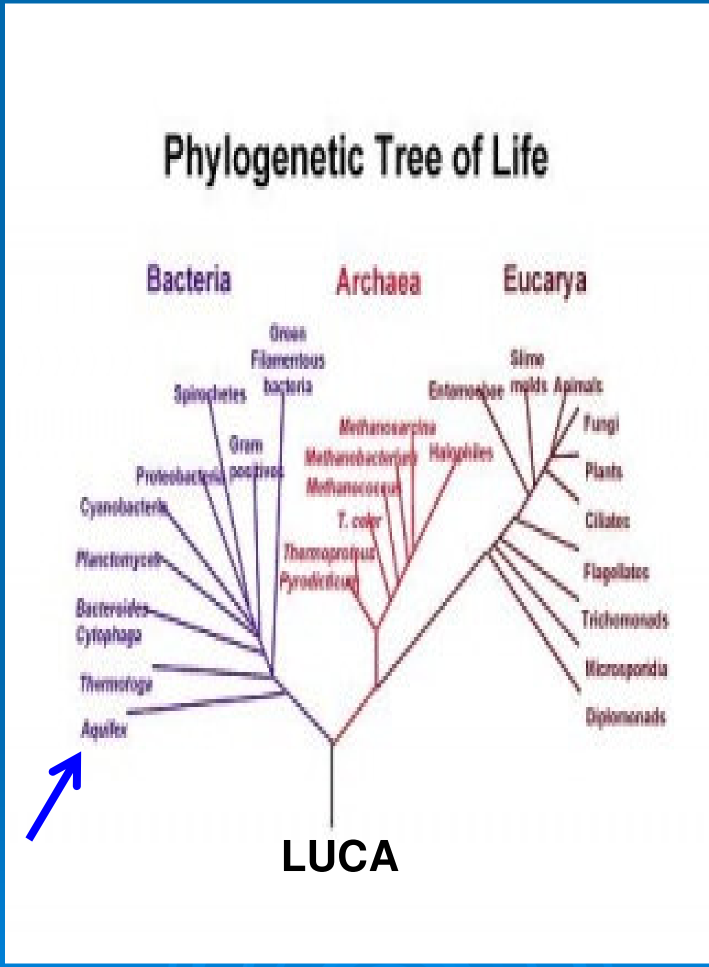
Reductive Citric Acid Cycle is Network Autocatalytic



Intermediary Metabolism of an Early Autotroph

- **Phylogenetic profiling places hyperthermophilic bacteria at the deep end**

- ***Aquifex aeolicus*:**
  - **Hyperthermophilic, Reductive**
  - **Chemolithoautotrophic,**
  - **Anabolic …**
  - **Small genome : 1,551,335 bp**
  - **1560 ORFs**



Autotrophic Intermediary Metabolism

- **Aim:** To Construct a Canonical Chart of Intermediary Metabolism

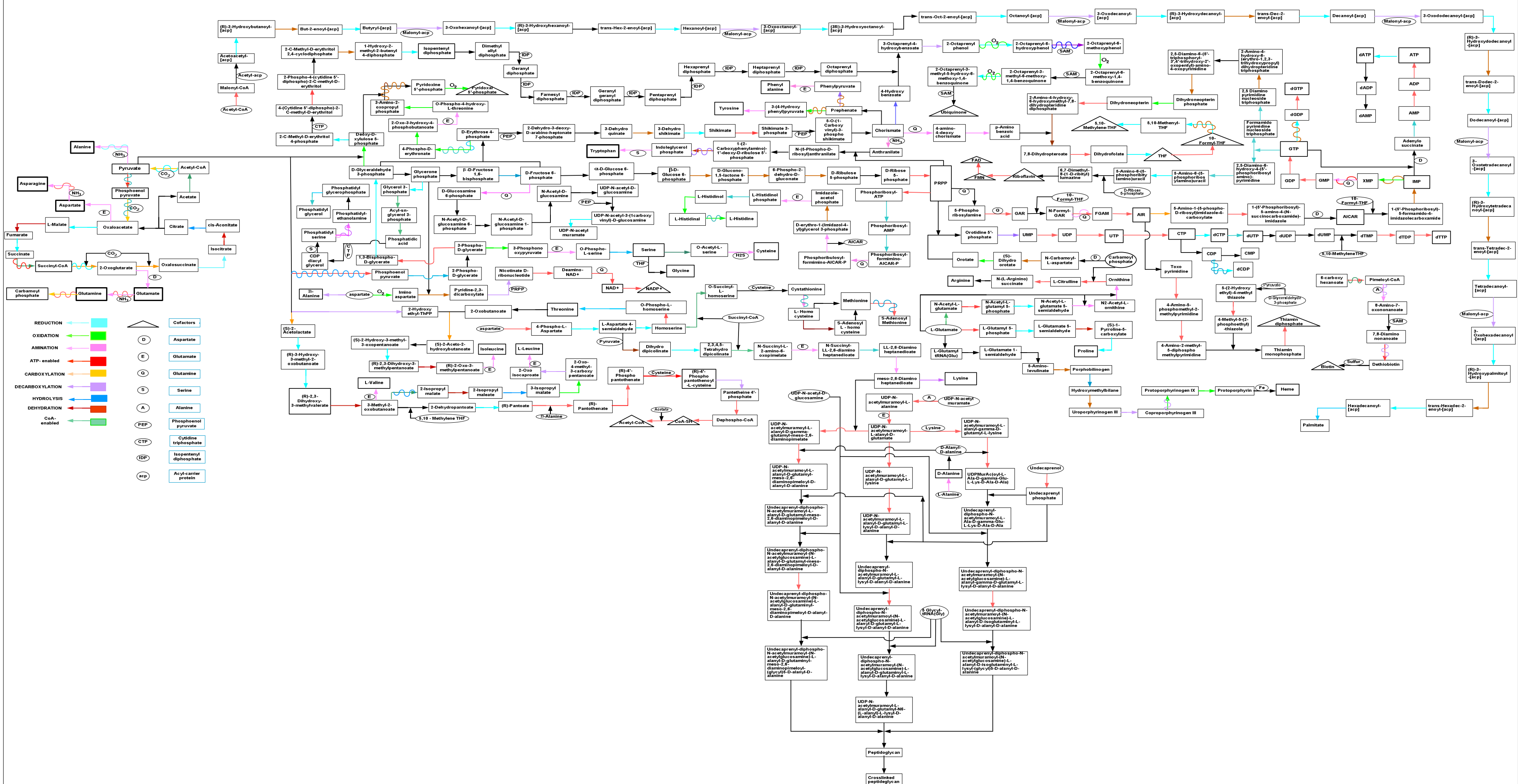
- **Strategy**: To datamine biological databases for metabolic pathway information
- KEGG, NCBI– Pubmed, Genome, Pubchem, Metacyc, BRENDA

- **Organisms:** *Aquifex aeolicus, Hydrogenobacter thermophilus, Thiomicrospira denitrificans, Chlorobium limicola*

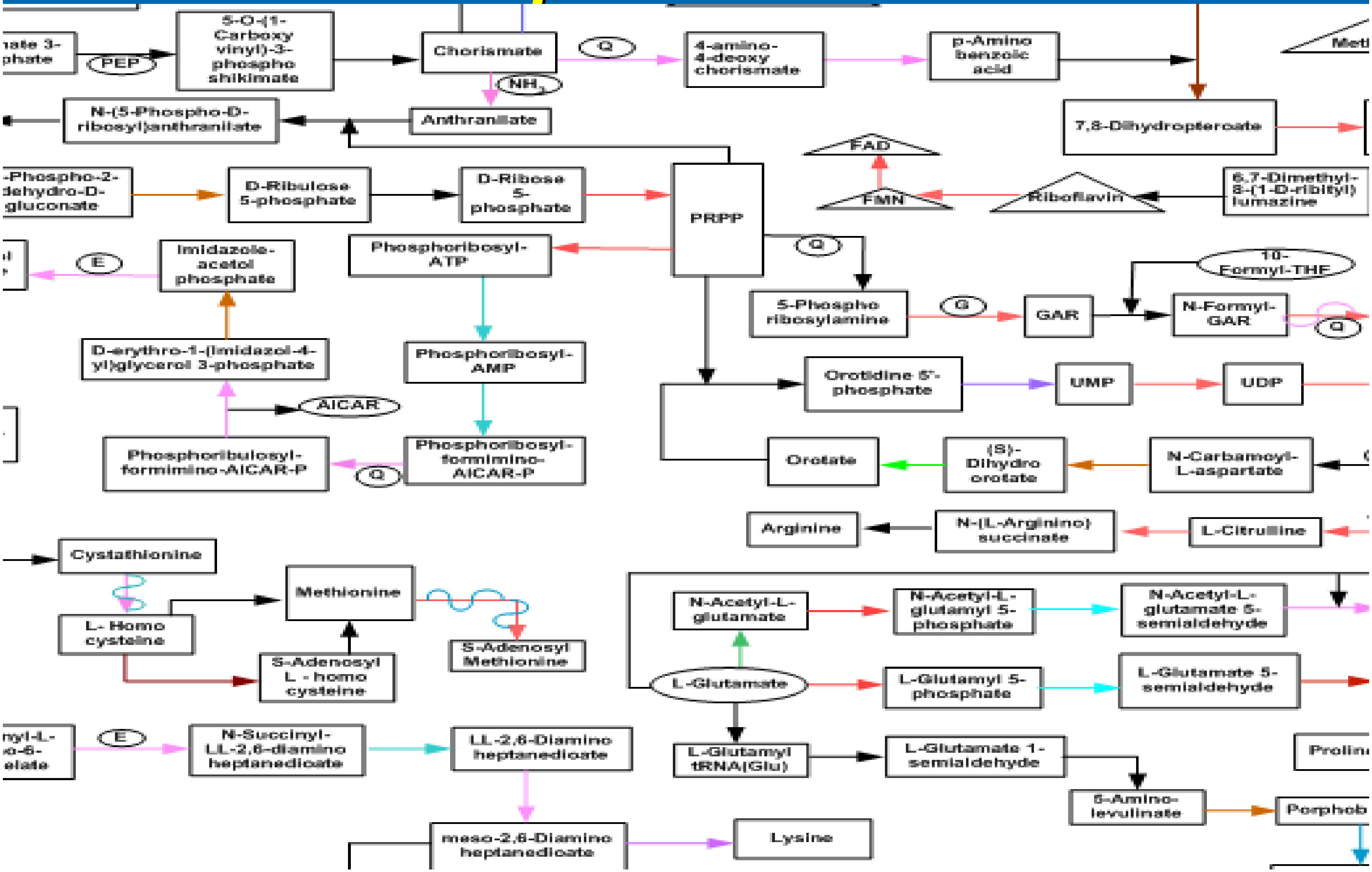
Metabolic Reconstruction

- Data-mining: KEGG & other Databases
- Reactions of individual metabolic and sub-metabolic pathways
- Reactants, Products and Intermediates of
  - rTCA cycle – Carbon fixation
  - Glycolysis/Gluconeogenesis
  - Amino acid biosynthesis
  - Purine & Pyrimidine biosynthesis
  - Fatty acid, Sterol and Lipid & Peptidoglycan biosynthesis
  - Cofactor biosynthesis

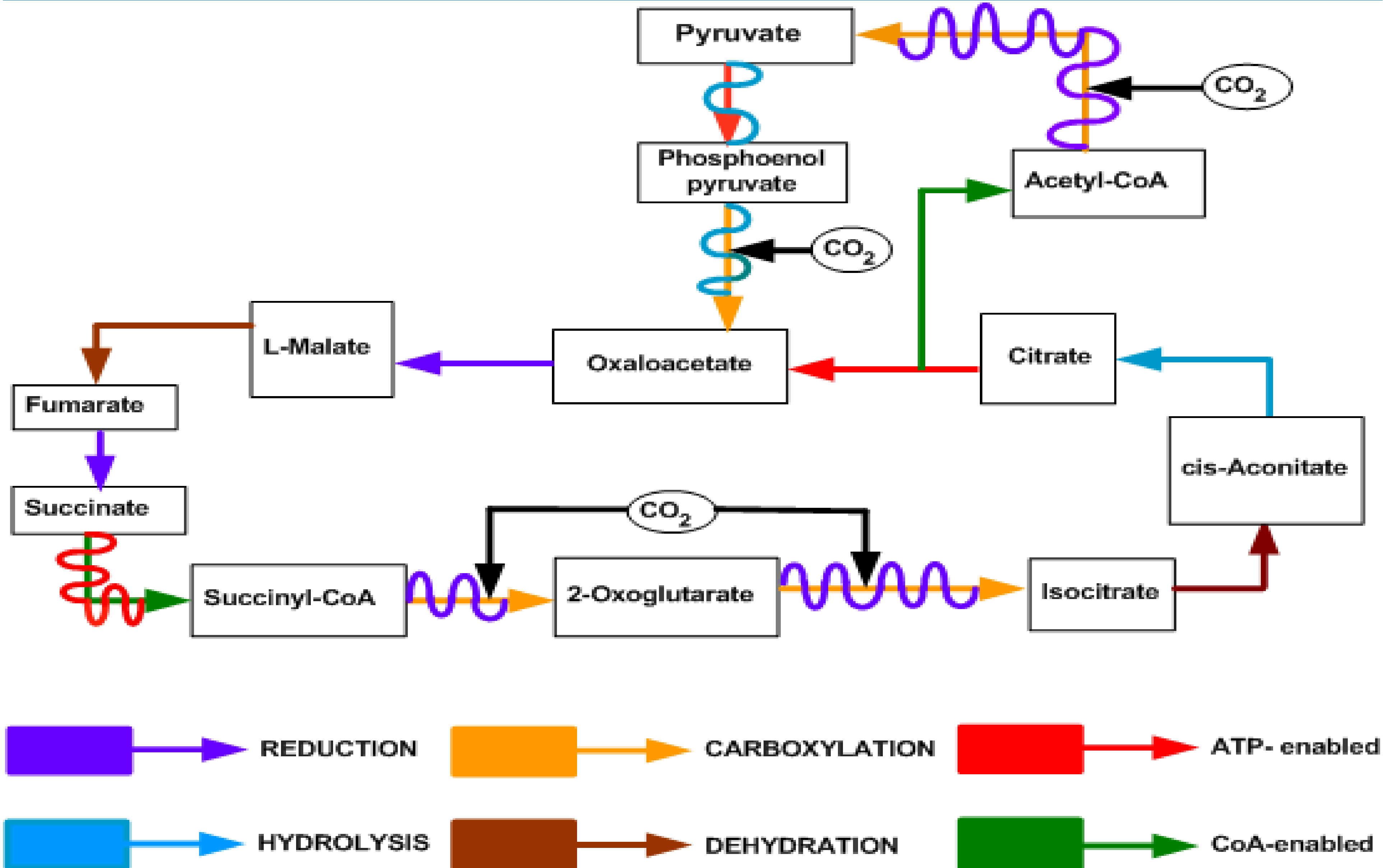
Complete Metabolic Chart Of a Reductive Chemoautotroph



Complete Metabolic Chart – Reductive Chemoautotroph



Pathway Reactions – rTCA cycle



Metabolome of a Reductive Chemoautotroph

Summary:

- Rudimentary Organic Chemical Reactions – 4 general types: oxidation/reduction, addition/elimination, hydrolysis and decarboxylation (Petsko & Dagmar, 2004)

- **287** Unique Compounds

- Compound Categories: Monomers and Intermediates

- Sub-classification in the context of 'Nodes in the metabolic network'

Metabolome of a Reductive Chemoautotroph

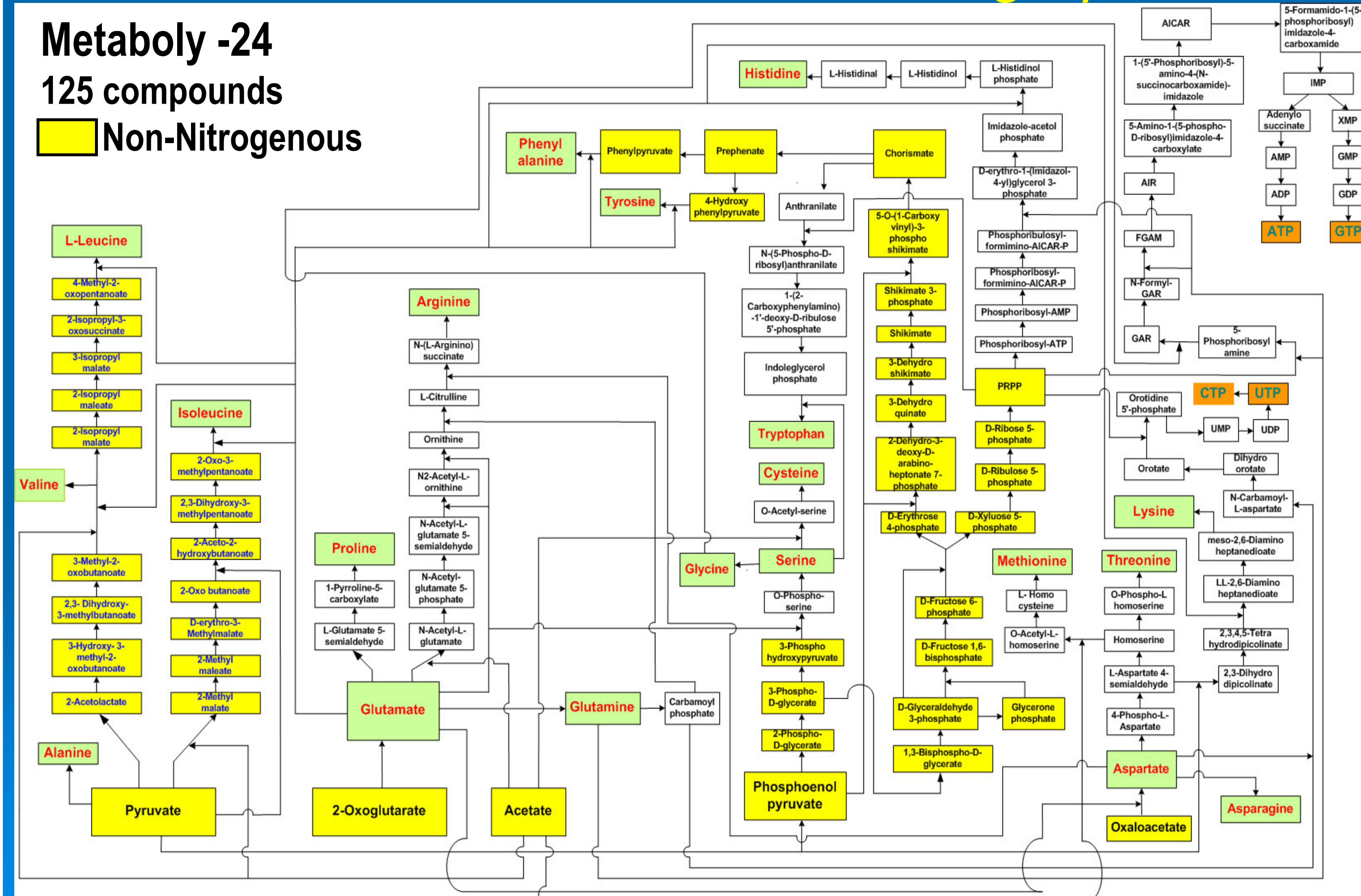
Core (rTCA outputs)	6
Nodal Core (Pyr, 2-OXG, OXA)	7
Intermediate (utilized once)	169
Nodal Intermediate (utilized > once)	20
Precursor to (amino acids, dNTPs) Polymerization	18
Nodal Precursor (amino acids, rNTPs) to Polymerization	13
CoFactor	12
Lipid Intermediate	35
Lipid Component	7
Total	**287**

Network Analysis & Generalizations –
Deconstruction of Network into Subgraphs

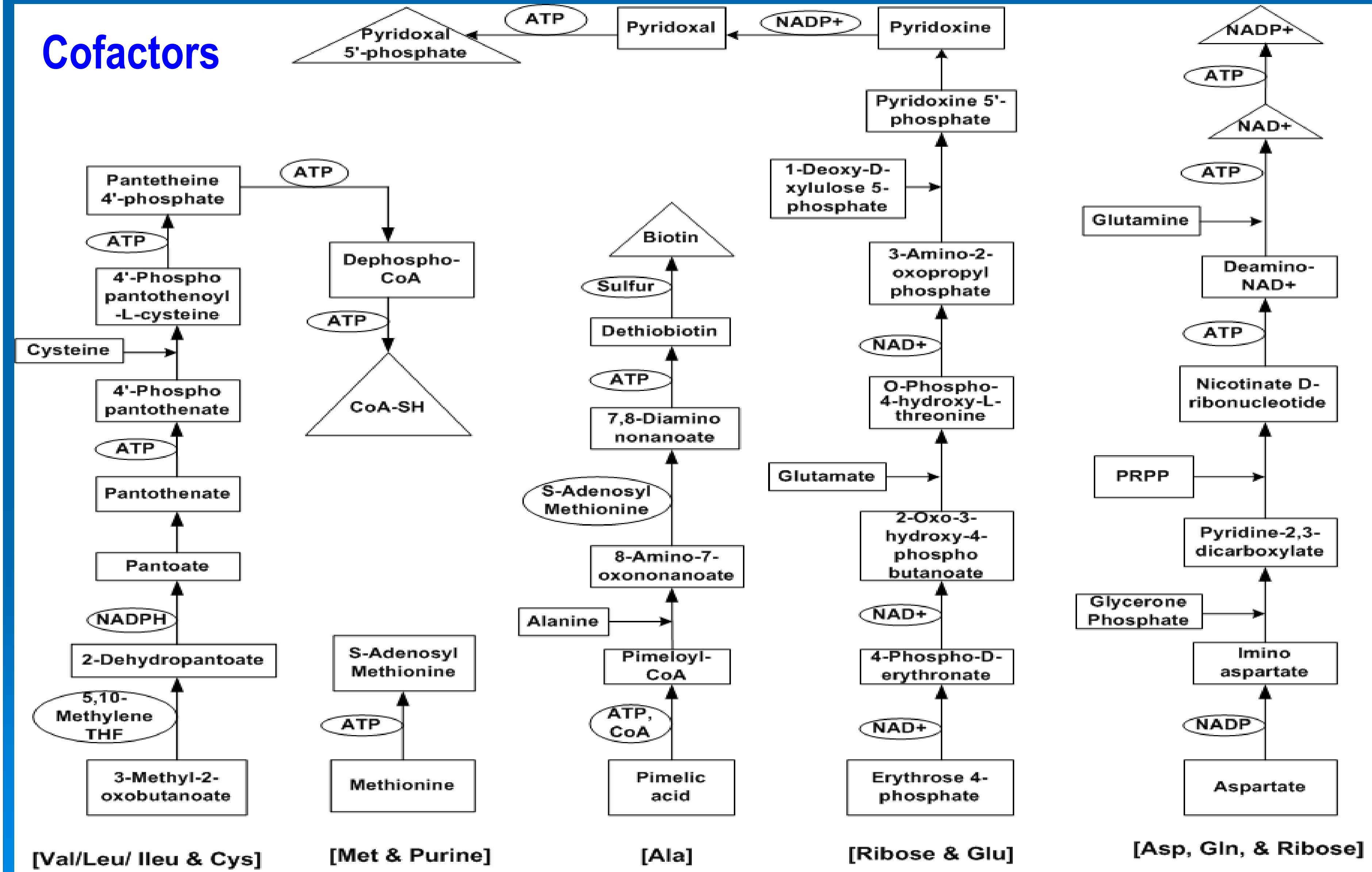
Metaboly -24

125 compounds

Non-Nitrogenous

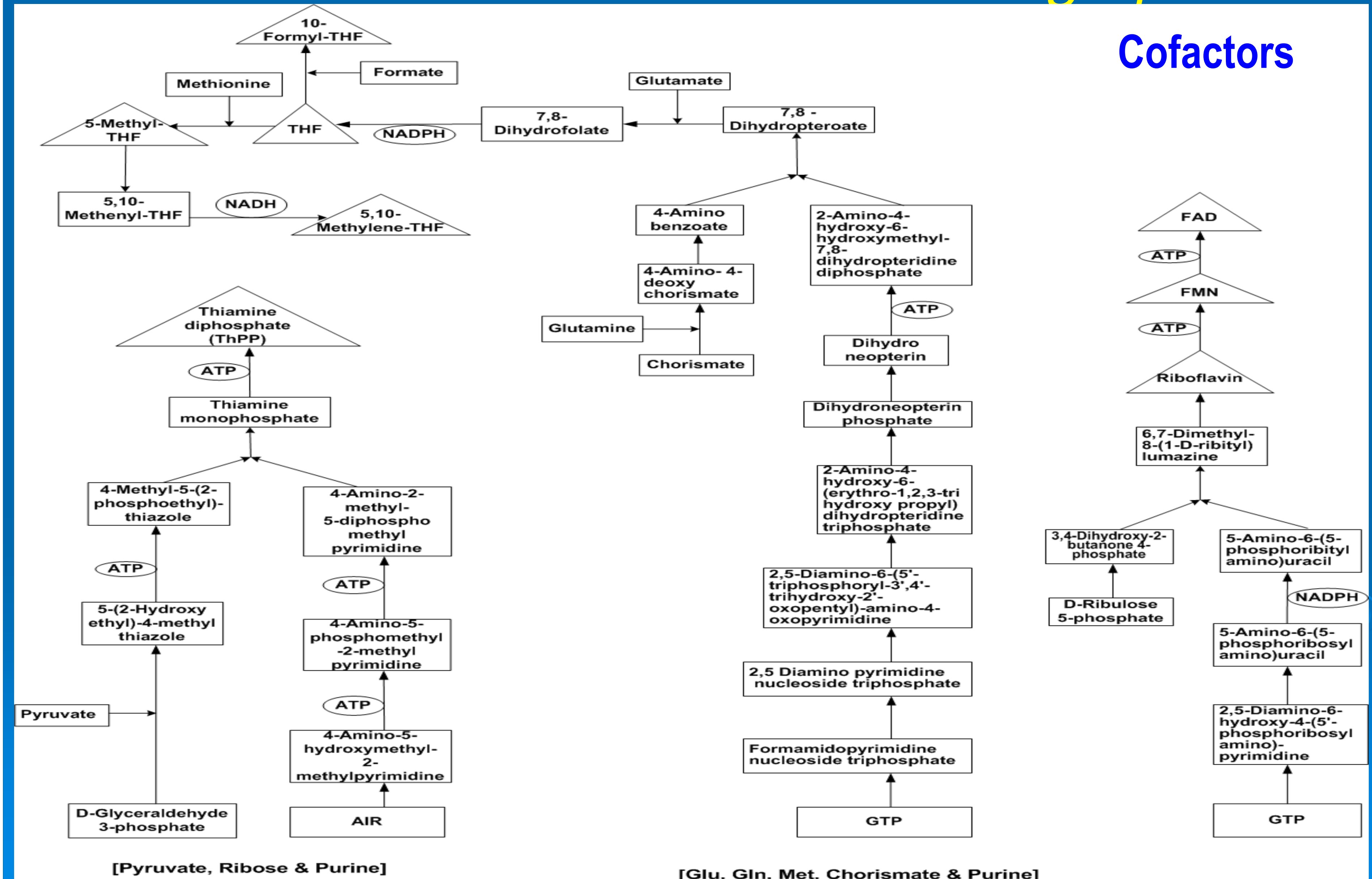


Network Analysis & Generalizations –
Deconstruction of Network into Subgraphs



Network Analysis & Generalizations –

Deconstruction of Network into Subgraphs



Network Analysis & Generalizations

- **1.** The universal atomic constituents of metabolism are **C**arbon, **H**ydrogen, **N**itrogen, **O**xygen, **P**hosphorous, and **S**ulfur

  Wald, G. 1962. "Life in the Second and Third Periods; Why Phosphorus and Sulfur for High-Energy Bonds?" In 'Horizons in Biochemistry' ed. M. Kasha and B. Pullman. Academic Press, New York.

- 2. All pathways are anabolic

- ***3. No Molecule Left Behind*** **–** When a pathway involves a splitting of a molecule, both parts enter into anabolic pathways

Network Analysis & Generalizations – No Molecule Left Behind

	Compound	Reaction	Production Pathway	Feedback Pathway
1	Pyruvate	Chorismate + NH3 <=> Anthranilate + **Pyruvate** + H2O	TRP, FOLATE	rTCA
2	Fumarate	N-(L-Arginino)succinate <=> **Fumarate** + L-Arginine	ARG, PUR	rTCA
3	2-Oxo glutarate	4-Methyl-2-oxopentanoate + L-Glutamate <=> L-Leucine + **2-Oxoglutarate**	LEU, VAL, ILEU, SER, LYS,HIS	rTCA
4	L-Glutamate	L-Glutamine + PRPP + H2O<=> 5-Phosphoribosylamine + Pyrophosphate + **L-Glutamate**	PUR,PYR, HIS	GLN, LEU, VAL, ILEU, SER, LYS,HIS

Network Analysis & Generalizations – *No Molecule Left Behind*

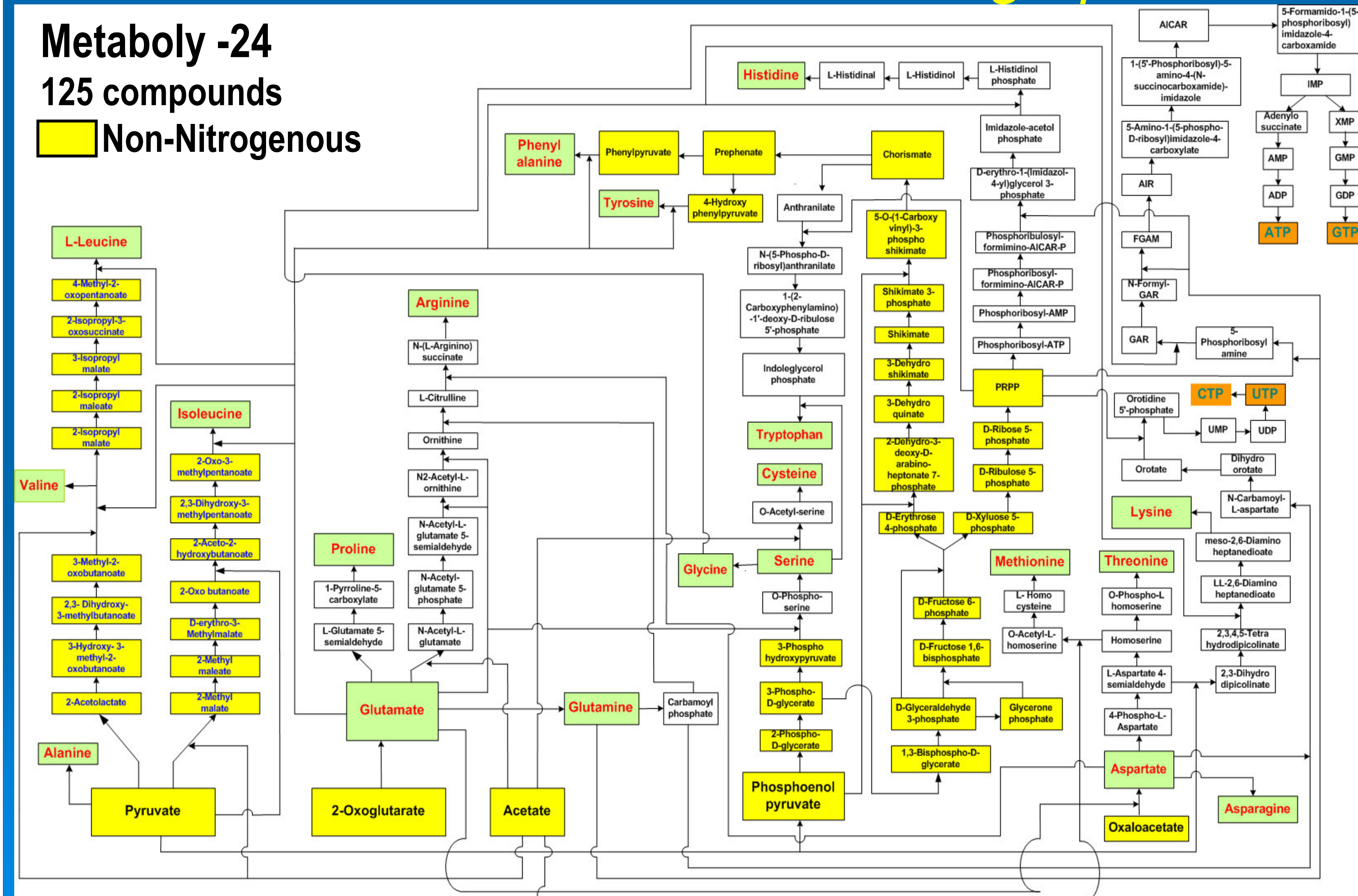
5	**Glyceraldehyde 3-phosphate**	L-serine + Indoleglycerol phosphate <=> L-tryptophan + **Glyceraldehyde 3-phosphate** + H_2O	**TRP**	**RIBOSE**
6	**D-Erythrose 4-phosphate**	D-Fructose 6-phosphate + D-Glyceraldehyde 3-phosphate <=> D-Xylulose 5-phosphate + **D-Erythrose 4-phosphate**	**RIBOSE**	**PHE, TYR, TRP**
7	**AICAR**	Phosphoribulosyl-formimino-AICAR-P + L-Glutamine <=> D-erythro-1-(Imidazol-4-yl)glycerol 3-phosphate + **AICAR** + L-Glutamate	**HIS**	**PURINE**

Network Analysis & Generalizations – Five Pillars of Anabolism

- Core-24 network has five starting termini leading to 20 amino acids and 4 ribonucleotides

- These five compounds are universal termini for all autotrophic metabolism
  - **Acetate (acetyl-CoA)**
  - **Pyruvate**
  - **Phosphoenolpyruvate**
  - **Oxaloacetate**
  - **2-Oxoglutarate**

Network Analysis & Generalizations – *Deconstruction of Network into Subgraphs*



Network Analysis & Generalizations – Five Pillars of Anabolism

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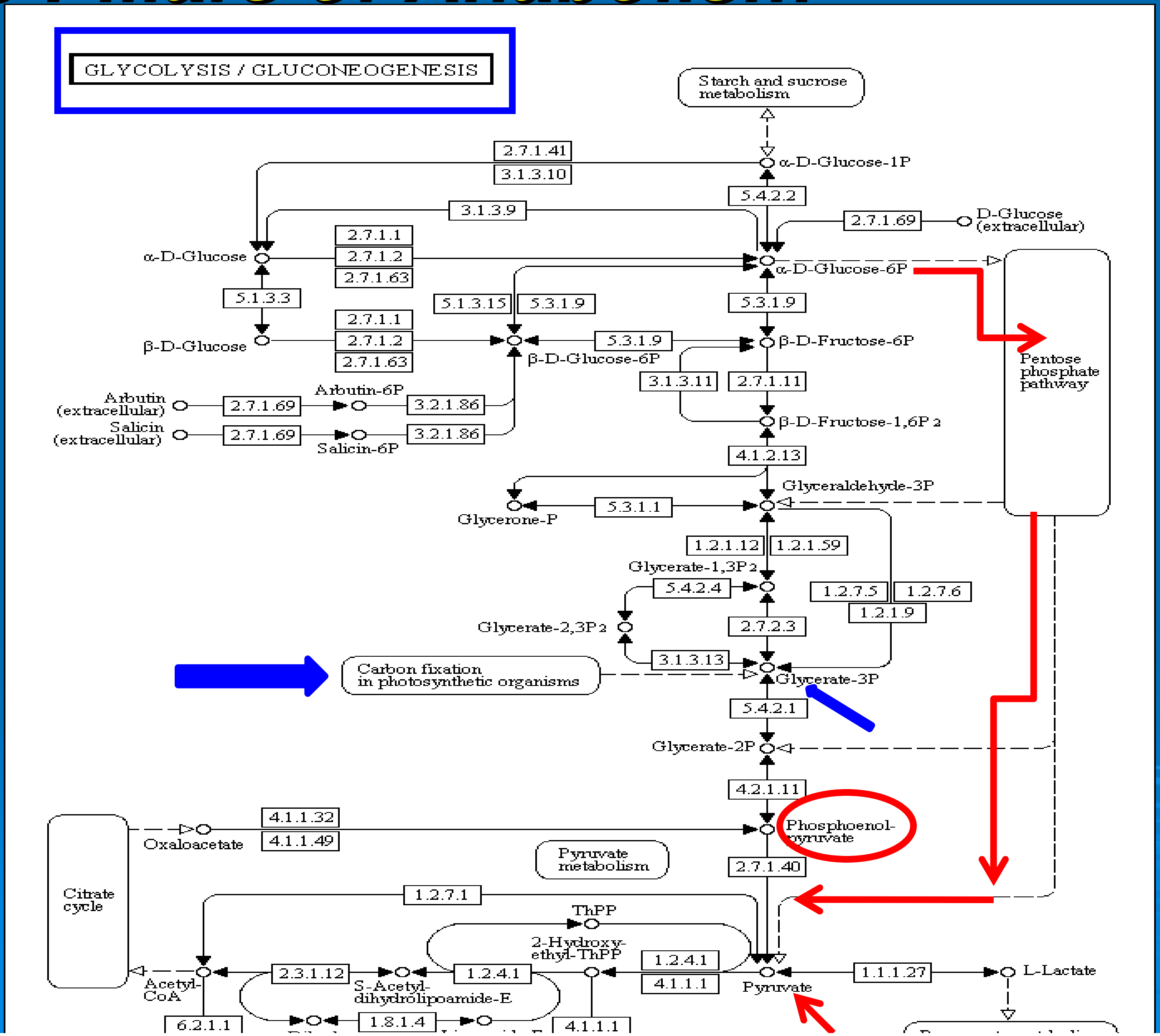
  - **Pyruvate**
  - **Phosphoenolpyruvate**
  - **Oxaloacetate**
  - **2-Oxoglutarate**

Network Analysis & Generalizations – Five Pillars of Anabolism

- Irrespective of the Carbon Fixation pathways these five compounds serve as universal termini in all autotrophic metabolism

1. rTCA cycle
2. Reductive acetyl-CoA pathway
3. 3- Hydroxypropianate cycle
4. 4-hydroxybutyrate cycle
5. Reductive Pentose Pathway

Network Analysis & Generalizations – Five Pillars of Anabolism

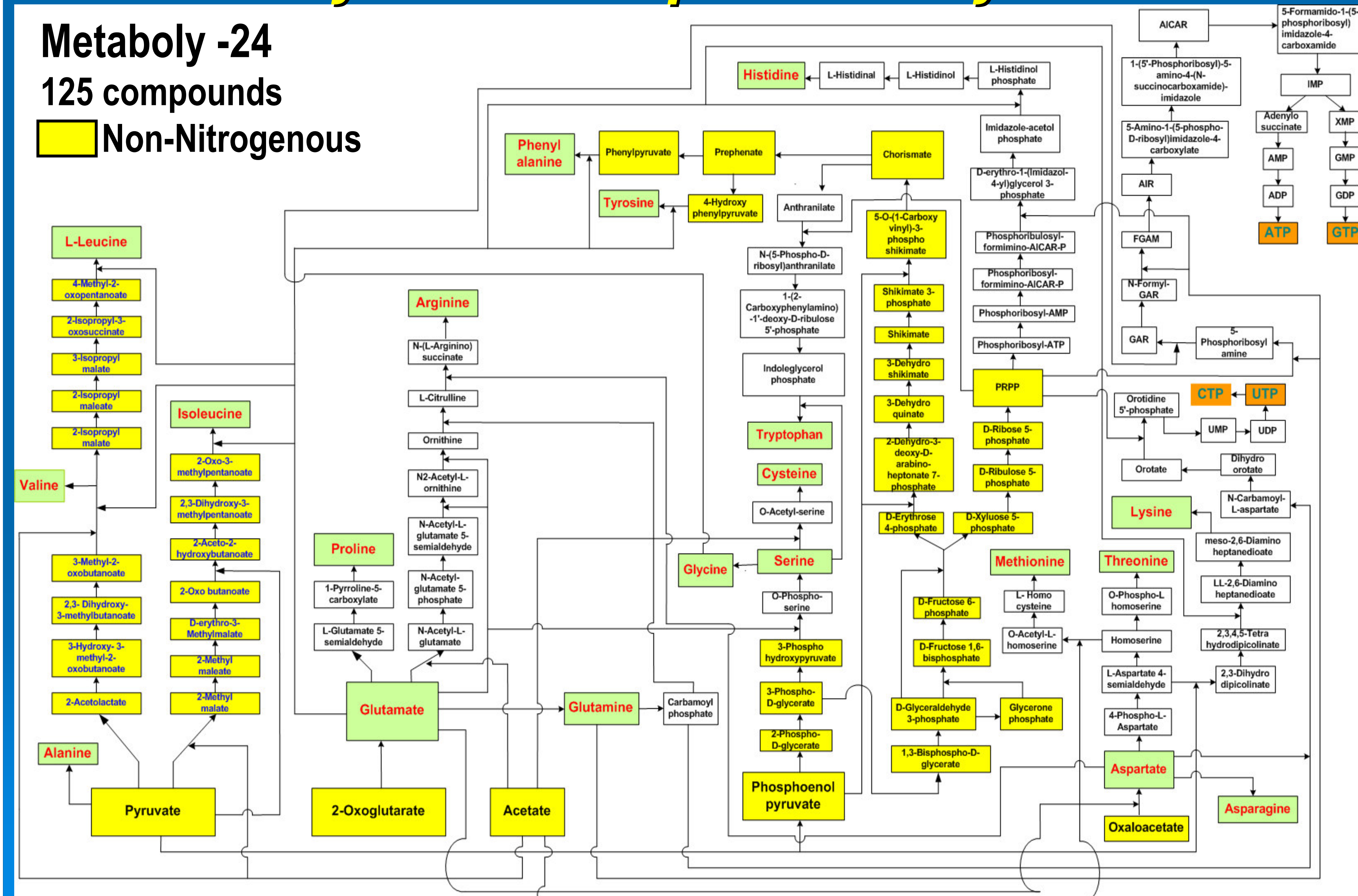


Network Analysis & Generalizations – Universality in autotrophic eco systems

Metaboly -24

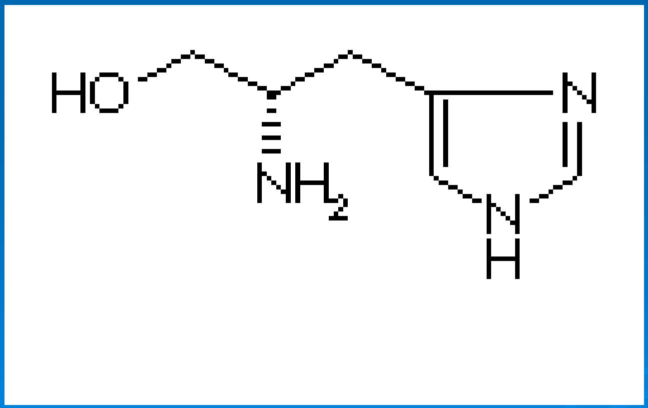
125 compounds

Non-Nitrogenous

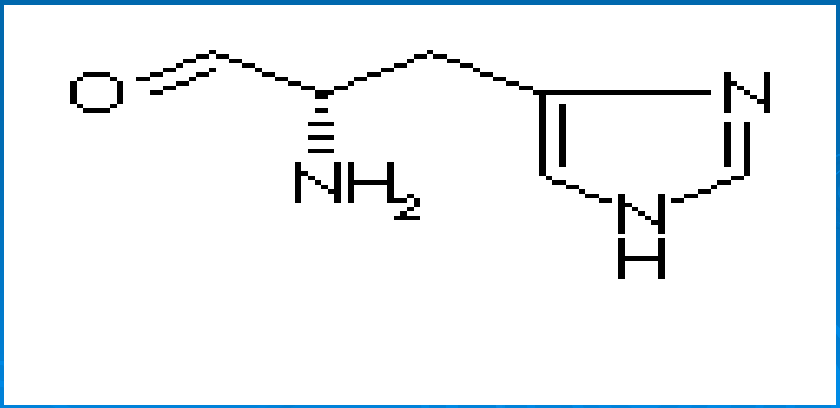


Network Analysis & Generalizations – Acid Derivatives

- 5. All core molecules contain either carboxylic or phosphoric acid moieties or both.
- ✓ The possible exceptions, histidinal and histidinol, may more appropriately be regarded as part of a coenzyme pathway



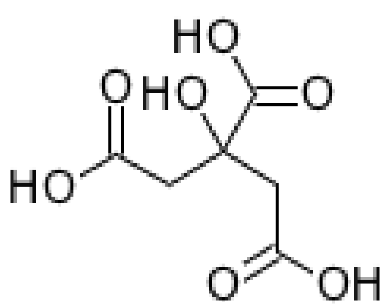
HISTIDINOL



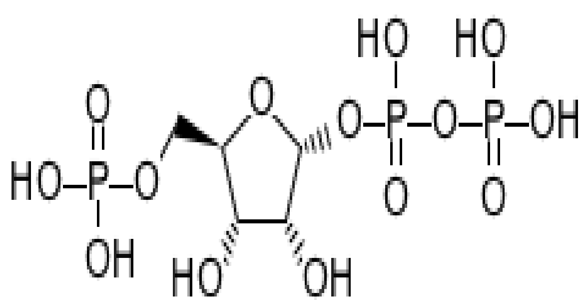
HISTIDINAL

Network Analysis & Generalizations – Acid Derivatives

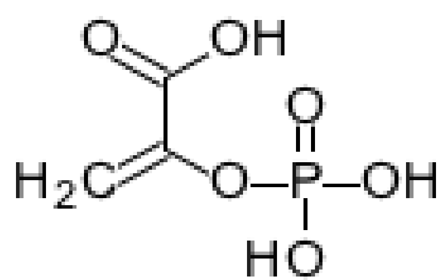
- 5. All core molecules contain either carboxylic or phosphoric acid moieties or both.
- R-COOH, R - O–P(=O)(OH)–OH



Citrate



PRPP



PEP

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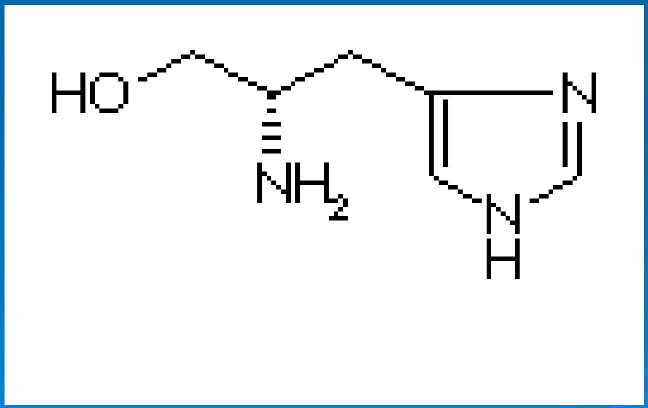
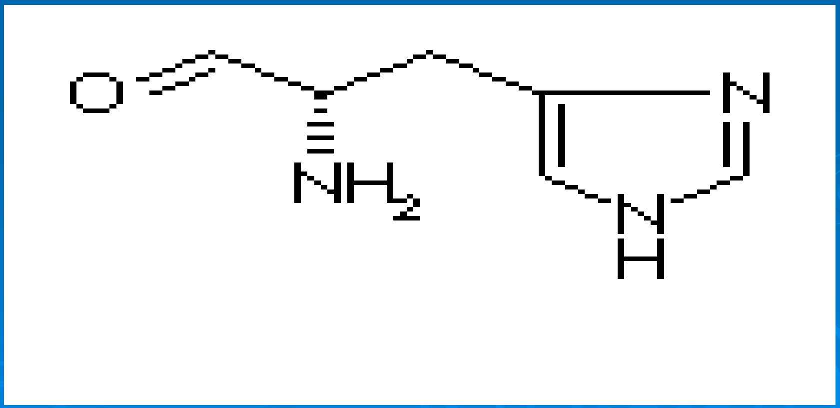


HISTIDINOL

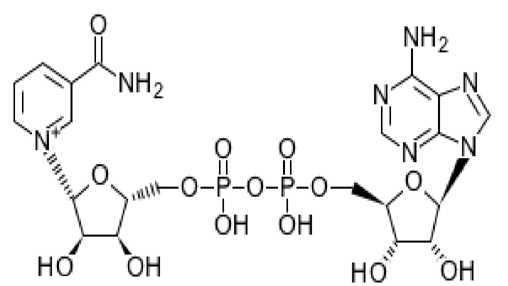


HISTIDINAL

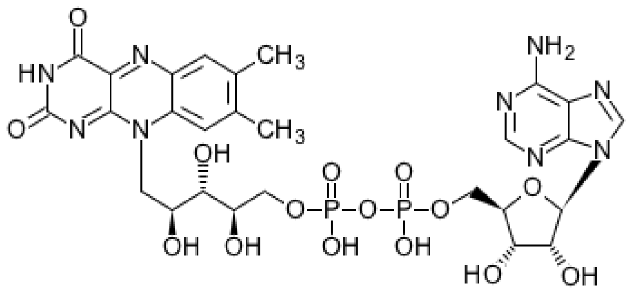
Histidine – a Vestigial Cofactor ?

- Most cofactors contain nitrogenous heterocyclic rings. Histidine has an imidazole heterocyclic ring
- Histidine mediates acid-base catalyzed reactions
- Histidine is at the active site of a vast majority of enzymes
- Of the   enyzymes catalyzing the biosynthesis of the core metabolome,   contain Histidine at the active site
- Self- cleavage of the Ribozyme His-84 is exclusively dependent on Histidine (Nuc. Acid Res.Symp 50, 241, 2006)
- RNA cleavage by a Deoxy Ribozyme is enhanced over 10^6 times by the addition of Histidine (Proc. Natl. Acad. Sci. 95, 6027 (1998)

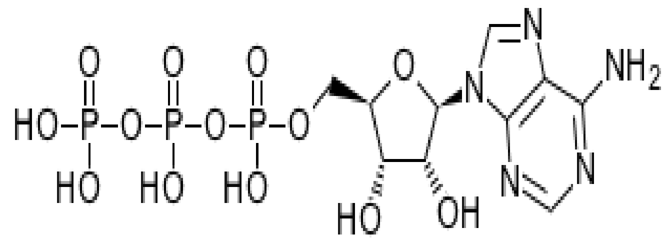
Cofactors – Nitrogenous heterocyclics



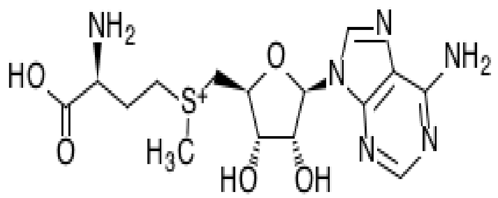
CoA-SH



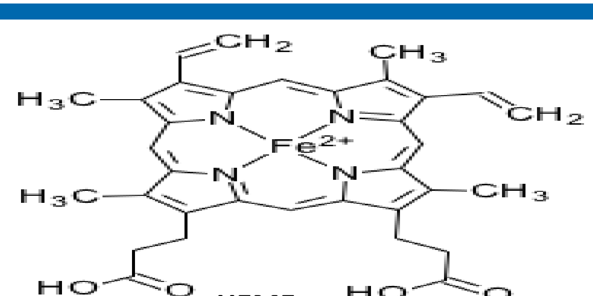
FAD



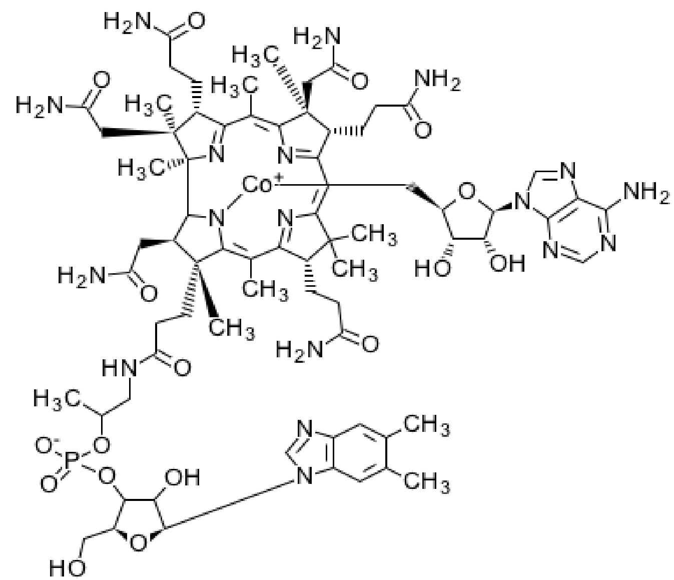
ATP



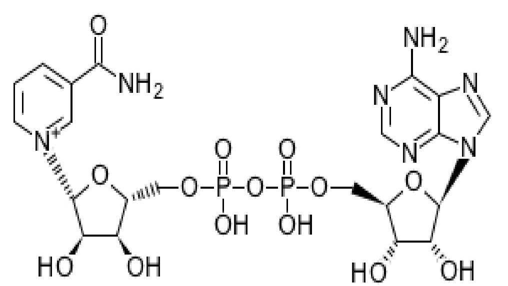
SAM



HEME

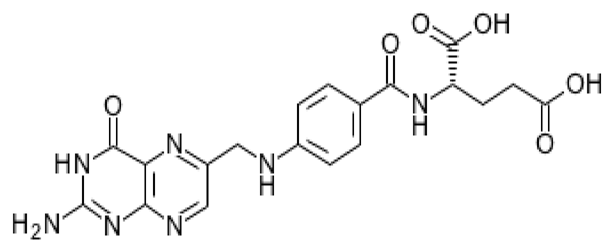


COBALAMIN



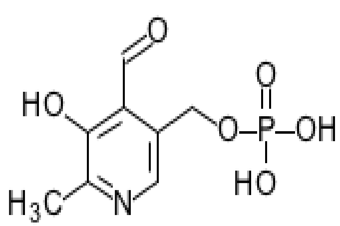
C00003

NAD

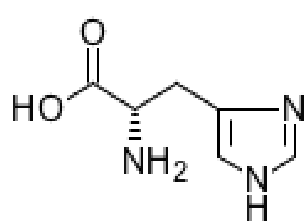


C00504

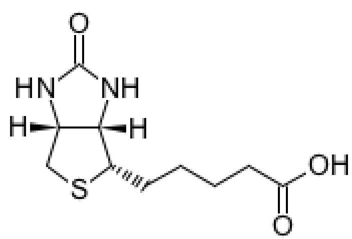
FOLATE



PYRIDOXAL PHOSPHATE



HISTIDINE



BIOTIN

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Network Analysis & Generalizations – Acid Derivatives

- 5. All core molecules contain either carboxylic or phosphoric acid moieties or both
- 6. All sugars are phosphorylated
- All compounds of the core metabolome are charged molecules - 'intrinsic barrier to diffusion'

Distribution: 65 – Carboxylic
45 – Phosphoric
10 - Both

Network Analysis & Generalizations – Stability

- 7. The core metabolic network is both brittle and robust

Brittleness: Any break in the core-24 network would result in the inability to make one or more of the 24 building blocks

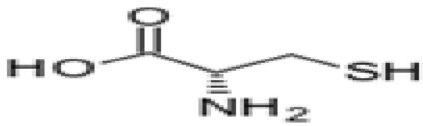
Robustness : Ubiquity and persistence of the network

Network Analysis & Generalizations –
Hierarchy of Synthesis and Structure

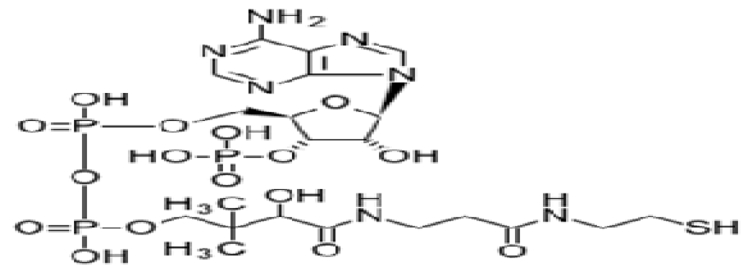
- The hierarchical order of synthesis produces:

1. Monomers
2. Polymers
3. Chimeromers
4. Repeatomers
5. Super chimeromers such as peptidoglycan
6. Coacervates and other structures held together by non covalent bonds

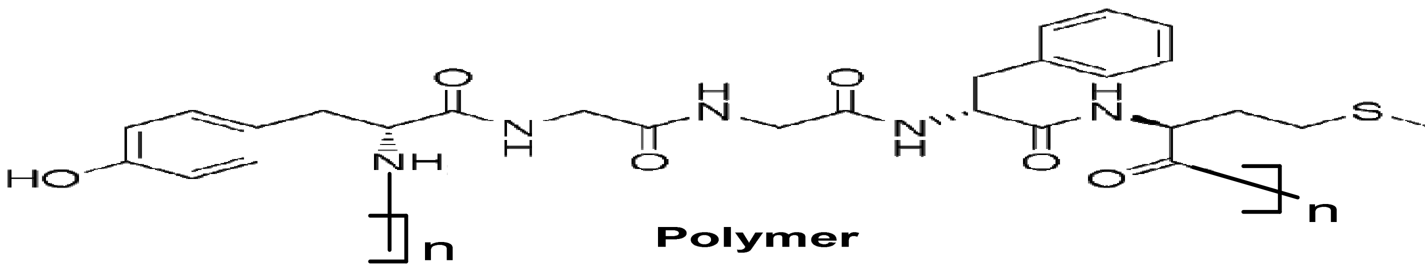
Network Analysis & Generalizations –
Hierarchy of Synthesis and Structure



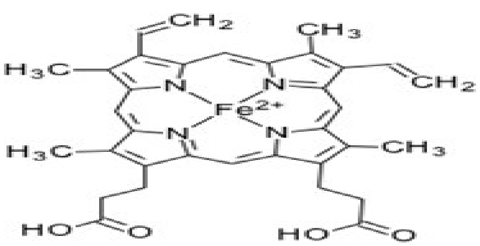
Monomer



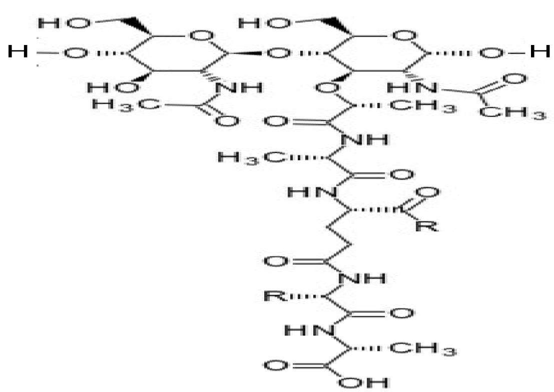
Chimeromer



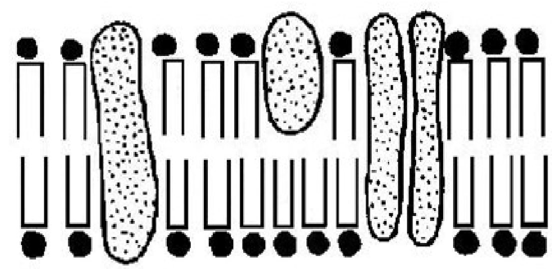
Polymer



Repeatomer



Superchimeromer



Aggregate

Network Analysis & Generalizations –
Hierarchy of Synthesis and Structure

Reaction Type	Building Blocks Pathways
Oxidation	8
Reduction	13
Amination /Transamination*	5 + 16*
Hydration	19
Dehydration	17
Phosphorylation	12
Dephosphorylation	17

Network Analysis & Generalizations –
Hierarchy of Synthesis and Structure

Decarboxylation	9
Carboxylation	2
Isomerization	9

- **11 types of Chemical Transformations:**

  Oxidation, Reduction, Amination, Hydration, Dehydration, Phosphorylation, Dephosphorylation

  Group transfer, Carboxylation, Decarboxylation, Isomerization

Network Analysis & Generalizations – Sparseness

- Estimation of covalently bonded CHNOPS with molecular weight of ~300 daltons > millions
- Core -24 is125 molecules **$C_{15}H_{25}N_5O_{20}P_4S$**
- Limited diversity in types of chemical transformations recursively used to generate a strikingly sparse set of 125 compounds
- How this selection is achieved ? What are the pruning rules?

Network Analysis & Generalizations – Small Molecule Catalysis

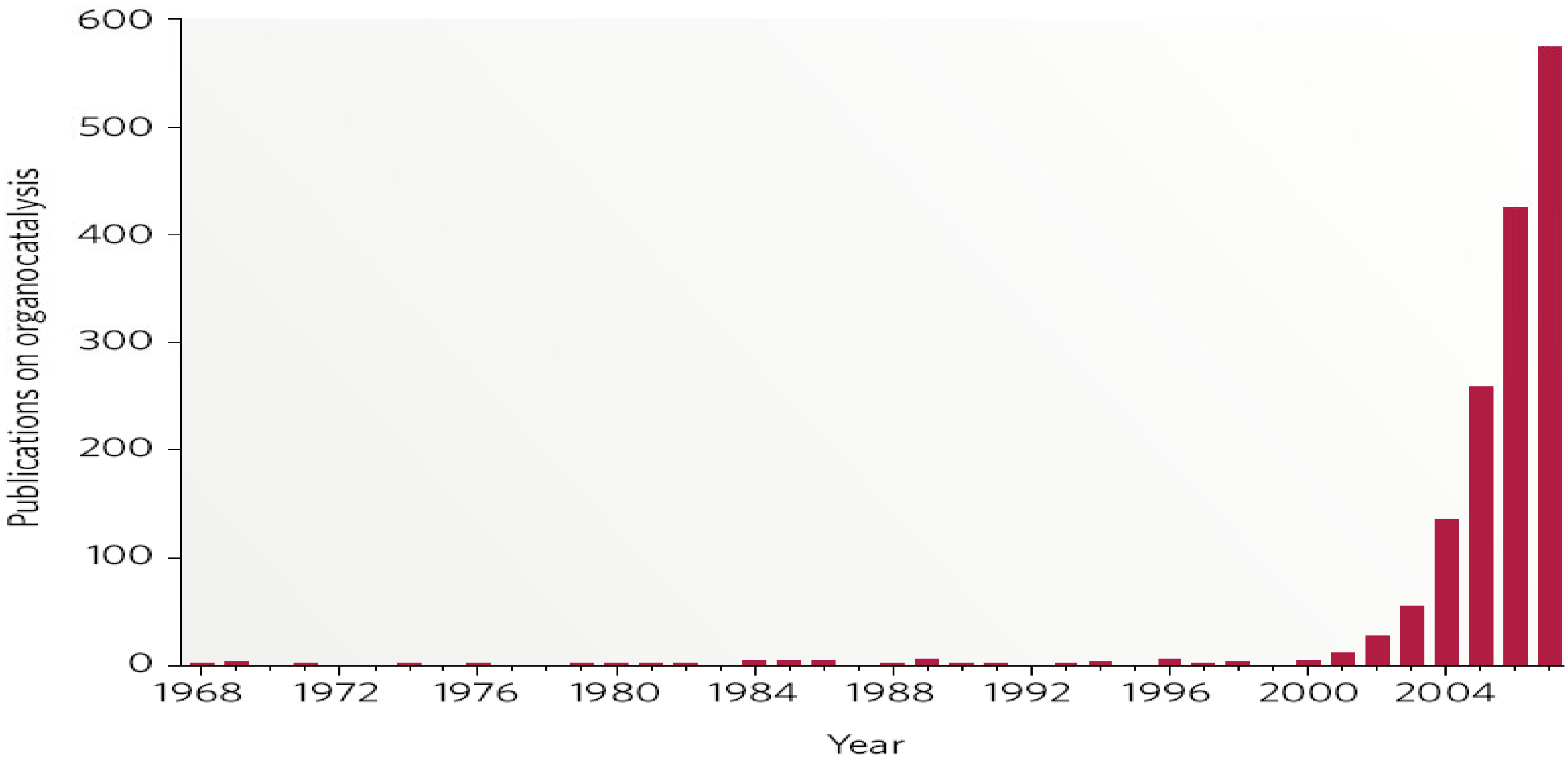


Figure 1 | An explosion of interest. The number of publications on the topic of organocatalysis has recently increased markedly. Data were obtained by a search of the ISI Web of Knowledge in May 2008 for the

Network Analysis & Generalizations – Small Molecule Catalysis

Organocatalysis

DOI: 10.1002/anie.200702

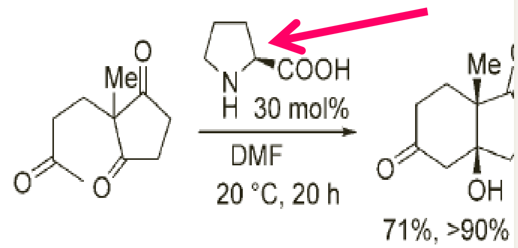
Organocatalysis Lost: Modern Chemistry, Ancient Chemistry, and an Unseen Biosynthetic Apparatus

*Carlos F. Barbas III**

aldolases · asymmetric synthesis · biosynthesis · catalytic antibodies · organocatalysis

In memory of Frank H. Westheimer (1912–2007)

Since the year 2000 there has been explosive growth in an area of catalytic asymmetric synthesis now known as organocatalysis, catalysis mediated solely by small organic molecules.[1] A large aldol and Robinson annulation,[2] the Hajos–Wiechert[3] reaction (1971) provides the proper foreshadowing. The MacMillan iminium ion based Diels–Alder reaction[4] is foreshadowed by the



"...I suggest that organocatalysis may be a yet-to-be-discovered biosynthetic mechanism at work in living organisms today."

Network Analysis & Generalizations – Small Molecule Catalysis

COMPLEXITY 2009 Vol. 14, No. 6

Revolution in Organic Chemistry and Its Implication in Biogenesis

Two articles appearing in 2008 [1, 2] point to the explosive emergence of a field of organic chemistry designated as organocatalysis, catalysis mediated solely by small organic molecules. Publication in this domain has gone from one or two articles a year in the late 1900s to over 500 articles in 2007. For a variety of reasons including stability, chirality, low cost, and nontoxicity these catalysts have presented organic chemists with an entirely new paradigm of synthetic organic chemistry. As a consequence of these developments, biochemists are called on to examine the role of small molecule catalysis in metabolism. This is especially true since enamines and iminium ions, two of the major organocatalysts, seem as intermediates in several metabolic networks. The concept of small molecule catalysis also provides us with a fresh point of view from which to look at biogenesis.

Small molecule catalysis was not part of traditional biochemistry because of the hypothesis that all reactions taking place in a cell are mediated by macromolecular protein enzymes and ribozymes. This permits a large number of chemical reactions to take place simultaneously in a small volume without mutual interference. This view must now be re-examined in some detail. The catalytic potential of a cell, determined by its genome and all the catalysts so encoded, may be insufficient to describe the cell's activity if some of the small molecules generated by the cell's metabolism are themselves catalytic for reactions that are part of the

HAROLD J. MOROWITZ,
VIJAYASARATHY SRINIVASAN,
AND ERIC SMITH

Minimal Autotroph

Experimental Search for Minimal Organisms and the Last Universal Common Ancestor

Reconstructing the Ur-Organism

Two questions that should be closely related have historically been studied with very different approaches. One is what constitutes a minimal living system, whether minimal cell or minimal self-contained ecosystem. The other is what actual system was the last universal common ancestor (LUCA) of all modern cells. As the LUCA is supposed to have been a bottleneck through which all life passed before diversifying into modern forms, it is treated as a self-sufficient organism and would be a candidate for a minimal cell.

Attempted reconstructions of the LUCA have largely been inferences in molecular phylogeny. Modern genes are grouped by common function and where possible by sequence homology, and primordial forms are traced back through the tree of life. In contrast, the search for minimal organisms has been mostly experimental, based on survey of short natural genomes and further random removal of genes. Current understanding of metabolism and control is still too primitive for theoretical approaches to have significantly affected this program.

The experimental search for a minimal microbial genome began in the early 1960s, culminating with *Mycoplasma genitalium*, which has only 482 protein-coding

Eric Smith, Harold J. Morowitz, and Vijayasarathy Srinivasan

Eric Smith is at the Santa Fe Institute, 1399 Hyde Park Road, Santa Fe, NM 87501. Harold J. Morowitz and Vijayasarathy Srinivasan are at the Krasnow Institute for Advanced Study,

Balch's Growth Media

TABLE 1. *Compositions of standard media*[a]

Medium	Mineral 1[b] (ml)	Mineral 2[c] (ml)	Mineral 3[d] (ml)	NaCl (g)	NH_4Cl (g)	Trace minerals[e] (ml)	Trace vitamins[f] (ml)	$FeSO_4 \cdot 7H_2O$ (g)	$Fe(NH_4)_2(SO_4)_2 \cdot 7H_2O$ (g)	$NaHCO_3$ (g)	Sodium acetate (g)	Sodium formate (g)	Yeast extract (Difco) (g)	Trypticase (BBL)	L-Cysteine hydrochloride·H_2O (g)	$Na_2S \cdot 9H_2O$ (g)
1	50	50				10	10	0.002		5.0	2.5	2.5	2.0	2.0	0.5	0.5
2		25			1.25	10	10		0.02	7.5					0.6	0.6
3			500	18		10	10		0.002	5.0	1.0		2.0	2.0	0.5	0.5

[a] Ingredients are added to distilled water to give a final volume of 1 liter. Cysteine and Na_2S are added after boiling the medium under an 80% N_2–20% CO_2 gas mixture, the final gas phase of tubed medium being an 80% H_2–20% CO_2 gas mixture at two atmospheres of pressure.

[b] Contains 6 g of K_2HPO_4 per liter of distilled water.

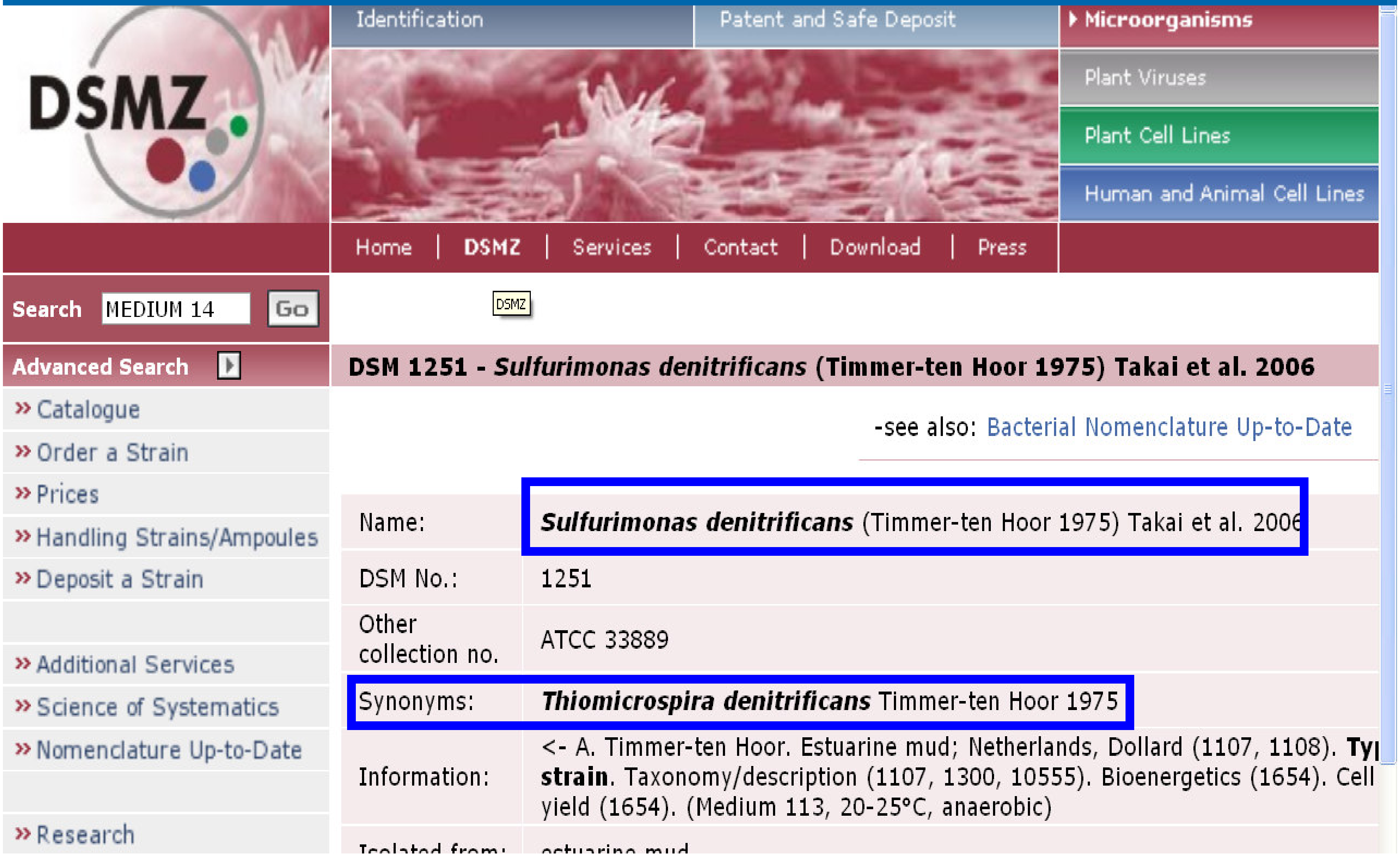
[c] Contains, in grams per liter of distilled water: KH_2PO_4, 6; $(NH_4)_2SO_4$, 6; NaCl, 12; $MgSO_4 \cdot 7H_2O$, 2.6; $CaCl_2 \cdot 2H_2O$, 0.16.

[d] Contains, in grams per liter of distilled water: KCl, 0.67; $MgCl_2 \cdot 2H_2O$, 5.5; $MgSO_4 \cdot 7H_2O$, 6.9; NH_4Cl, 0.5; $CaCl_2 \cdot 2H_2O$, 0.28; K_2HPO_4, 0.28.

[e] Contains, in grams per liter of distilled water (pH to 7.0 with KOH): nitrilotriacetic acid, 1.5; $MgSO_4 \cdot 7H_2O$, 3.0; $MnSO_4 \cdot 2H_2O$, 0.5; NaCl, 1.0; $FeSO_4 \cdot 7H_2O$, 0.1; $CoSO_4$ or $CoCl_2$, 0.1; $CaCl_2 \cdot 2H_2O$, 0.1; $ZnSO_4$, 0.1; $CuSO_4 \cdot 5H_2O$, 0.01; $AlK(SO_4)_2$, 0.01; H_3BO_3, 0.01; $Na_2MoO_4 \cdot 2H_2O$, 0.01. Dissolve nitrilotriacetic acid with KOH to pH 6.5; then proceed to add minerals.

[f] Contains, in milligrams per liter of distilled water: biotin, 2; folic acid, 2; pyridoxine hydrochloride, 10; thiamine hydrochloride, 5; riboflavin, 5; nicotinic acid, 5; DL-calcium pantothenate, 5; vitamin B_{12}, 0.1; *p*-aminobenzoic acid, 5; lipoic acid, 5.

Minimal Autotroph



Identification | Patent and Safe Deposit | Microorganisms

Plant Viruses

Plant Cell Lines

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DSMZ

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DSM 1251 - *Sulfurimonas denitrificans* (Timmer-ten Hoor 1975) Takai et al. 2006

-see also: Bacterial Nomenclature Up-to-Date

Name:	***Sulfurimonas denitrificans*** (Timmer-ten Hoor 1975) Takai et al. 2006
DSM No.:	1251
Other collection no.	ATCC 33889
Synonyms:	***Thiomicrospira denitrificans*** Timmer-ten Hoor 1975
Information:	<- A. Timmer-ten Hoor. Estuarine mud; Netherlands, Dollard (1107, 1108). **Ty strain**. Taxonomy/description (1107, 1300, 10555). Bioenergetics (1654). Cell yield (1654). (Medium 113, 20-25°C, anaerobic)
Isolated from:	estuarine mud

Minimal Autotroph

Microorganisms

DSMZ

Trace element solution:

Nitrilotriacetic acid	1.500	g
$MgSO_4 \times 7\ H_2O$	3.000	g
$MnSO_4 \times H_2O$	0.500	g
NaCl	1.000	g
$FeSO_4 \times 7\ H_2O$	0.100	g
$CoSO_4 \times 7\ H_2O$	0.180	g
$CaCl_2 \times 2\ H_2O$	0.100	g
$ZnSO_4 \times 7\ H_2O$	0.180	g
$CuSO_4 \times 5\ H_2O$	0.010	g
$KAl(SO_4)_2 \times 12\ H_2O$	0.020	g
H_3BO_3	0.010	g
$Na_2MoO_4 \times 2\ H_2O$	0.010	g
$NiCl_2 \times 6\ H_2O$	0.025	g

8.27 x 11.69 in

2 of 2

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Fin
pH 7.0 (with KOH).

Vitamin solution:

Biotin	2.000	mg
Folic acid	2.000	mg
Pyridoxine-HCl	10.000	mg
Thiamine-HCl x 2 H_2O	5.000	mg
Riboflavin	5.000	mg
Nicotinic acid	5.000	mg
D-Ca-pantothenate	5.000	mg
Vitamin B_{12}	0.100	mg
p-Aminobenzoic acid	5.000	mg
Lipoic acid	5.000	mg
Distilled water	1000.000	ml

Minimal Autotroph – Growth Medium

Medium data

Searched about grmd=[356].

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356 MJ BASAL MEDIUM

MJ(-N) synthetic sea water (see Medium No. 268)	1.0	L
NH_4Cl	0.25	g
$NaHCO_3$	1.5	g
$Na_2S_2O_3 \cdot 5H_2O$	1.5	g
Trace vitamins (see Medium No. 197)	1.0	ml

Mix ingredients, except $NaHCO_3$, $Na_2S_2O_3 \cdot 5H_2O$ and the trace vitamins, and autoclave. Fi (w/v) $NaHCO_3$, 10% (w/v) $Na_2S_2O_3 \cdot 5H_2O$ and the trace vitamins solutions and add to the the gas phase with a N_2-CO_2-O_2 (77:17:6, v/v) gas mixture, and pressurize to 200 kPa.

Minimal Autotroph

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-see also: Bacterial Nomenclature Up-to-Date

Name:	***Sulfurimonas denitrificans*** (Timmer-ten Hoor 1975) Takai et al. 2006
DSM No.:	1251
Other collection no.	ATCC 33889
Synonyms:	***Thiomicrospira denitrificans*** Timmer-ten Hoor 1975
Information:	<- A. Timmer-ten Hoor. Estuarine mud; Netherlands, Dollard (1107, 1108). **Ty[pe] strain**. Taxonomy/description (1107, 1300, 10555). Bioenergetics (1654). Cell yield (1654). (Medium 113, 20-25°C, anaerobic)
Isolated from:	estuarine mud
Medium:	113, 20-25°C, anaerobic
Literature:	1107, 1108, 1300, 1654, 10555

Minimal Autotroph

113. THIOBACILLUS DENITRIFICANS MEDIUM

Solution A:

KH_2PO_4	2.0	g
KNO_3	2.0	g
NH_4Cl	1.0	g
$MgSO_4 \times 7\ H_2O$	0.8	g
Trace element solution SL-4 (see medium 14)	2.0	ml
Distilled water	940.0	ml

Adjust pH to 7.0 with NaOH.

Solution B:

Minimal Autotroph – Growth Medium

Trace element solution SL-4:

EDTA	0.50	g
$FeSO_4 \times 7 H_2O$	0.20	g
Trace element solution SL-6 (see medium 27)	100.00	ml
Distilled water	900.00	ml

First dissolve EDTA in distilled water by adjusting the pH to 7.0 - 8.0 using a 2 M solution of NaOH; then add ferrous sulfate and the trace element solution SL-6.

Minimal Autotroph – Growth Medium

Trace element solution SL-6:

$ZnSO_4 \times 7\ H_2O$	0.10	g
$MnCl_2 \times 4\ H_2O$	0.03	g
H_3BO_3	0.30	g
$CoCl_2 \times 6\ H_2O$	0.20	g
$CuCl_2 \times 2\ H_2O$	0.01	g
$NiCl_2 \times 6\ H_2O$	0.02	g
$Na_2MoO_4 \times 2\ H_2O$	0.03	g
Distilled water	1000.00	ml

Continued on next page

JCM Database (medium) | Medium data

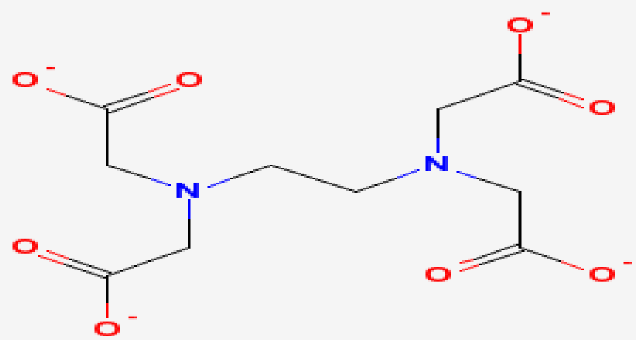
$CaCl_2$	0.75	g
KH_2PO_4	0.5	g
$(NH_4)_2Ni(SO_4)_2 \cdot 6H_2O$	2.0	mg
Distilled water	1.0	L

Trace minerals:

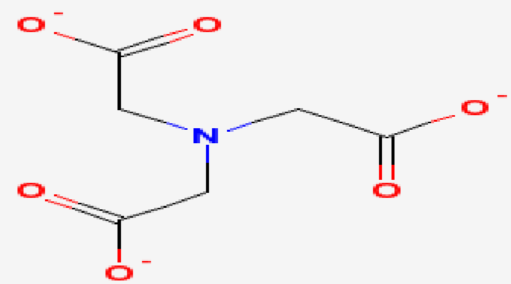
Nitrilotriacetic acid	1.5	g
$MgSO_4 \cdot 7H_2O$	3.0	g
$MnSO_4 \cdot xH_2O$	0.5	g
NaCl	1.0	g
$FeSO_4 \cdot 7H_2O$	0.1	g
$CoSO_4 \cdot 7H_20$	0.1	g
$CaCl_2 \cdot 2H_2O$	0.1	g
$ZnSO_4 \cdot 7H_2O$	0.1	g
$CuSO_4 \cdot 5H_2O$	0.01	g
$AlK(SO_4)_2$	0.01	g
H_3BO_3	0.01	g
$Na_2MoO_4 \cdot 2H_2O$	0.01	g
Distilled water	1.0	L

Dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH solution. Then proceed to add minera

Multidentate Metal Chelators

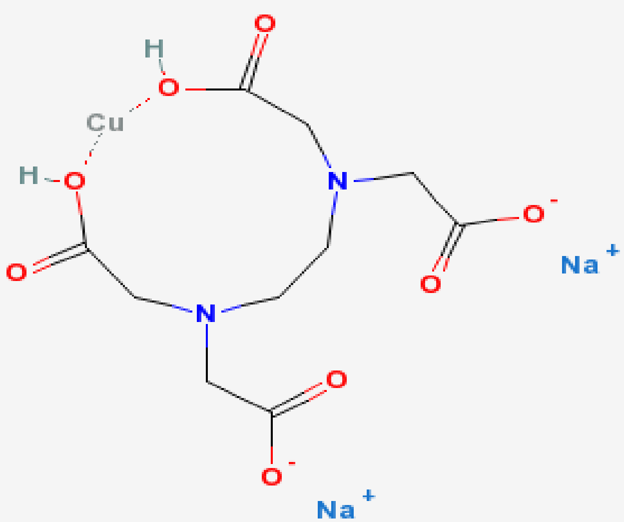


EDTA

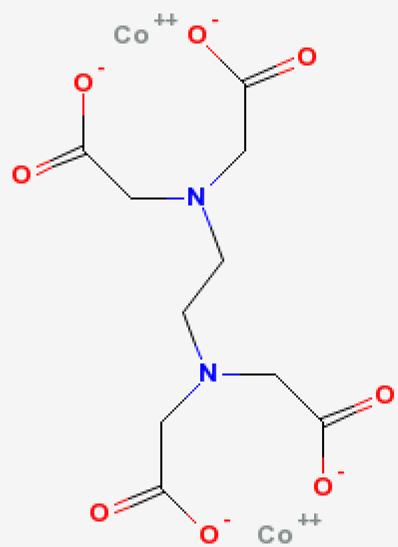


NTA

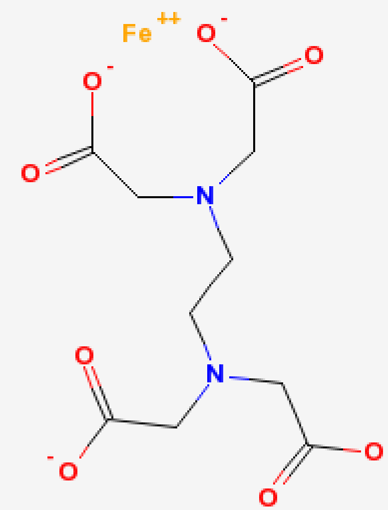
Copper



Cobalt



Iron



Fate of EDTA & NTA in growth media

- What happens to EDTA and NTA ? Can they get metabolized?
- That is, do they get catabolized /degraded /split into smaller carbon compounds?
- What happens to those compounds? Do they get modified into some metabolite?
- That is if those carbons get incorporated into metabolic compounds, could they be considered as additional source for anabolic synthesis?
- If so, wouldn't it then cause ambiguity towards the very identity of an autotroph?

entiality of Transition Metals in wth Medium

Trace element solution SL-6:

$ZnSO_4 \times 7\ H_2O$	0.10	g
$MnCl_2 \times 4\ H_2O$	0.03	g
H_3BO_3	0.30	g
$CoCl_2 \times 6\ H_2O$	0.20	g
$CuCl_2 \times 2\ H_2O$	0.01	g
$NiCl_2 \times 6\ H_2O$	0.02	g
$Na_2MoO_4 \times 2\ H_2O$	0.03	g
Distilled water	1000.00	ml

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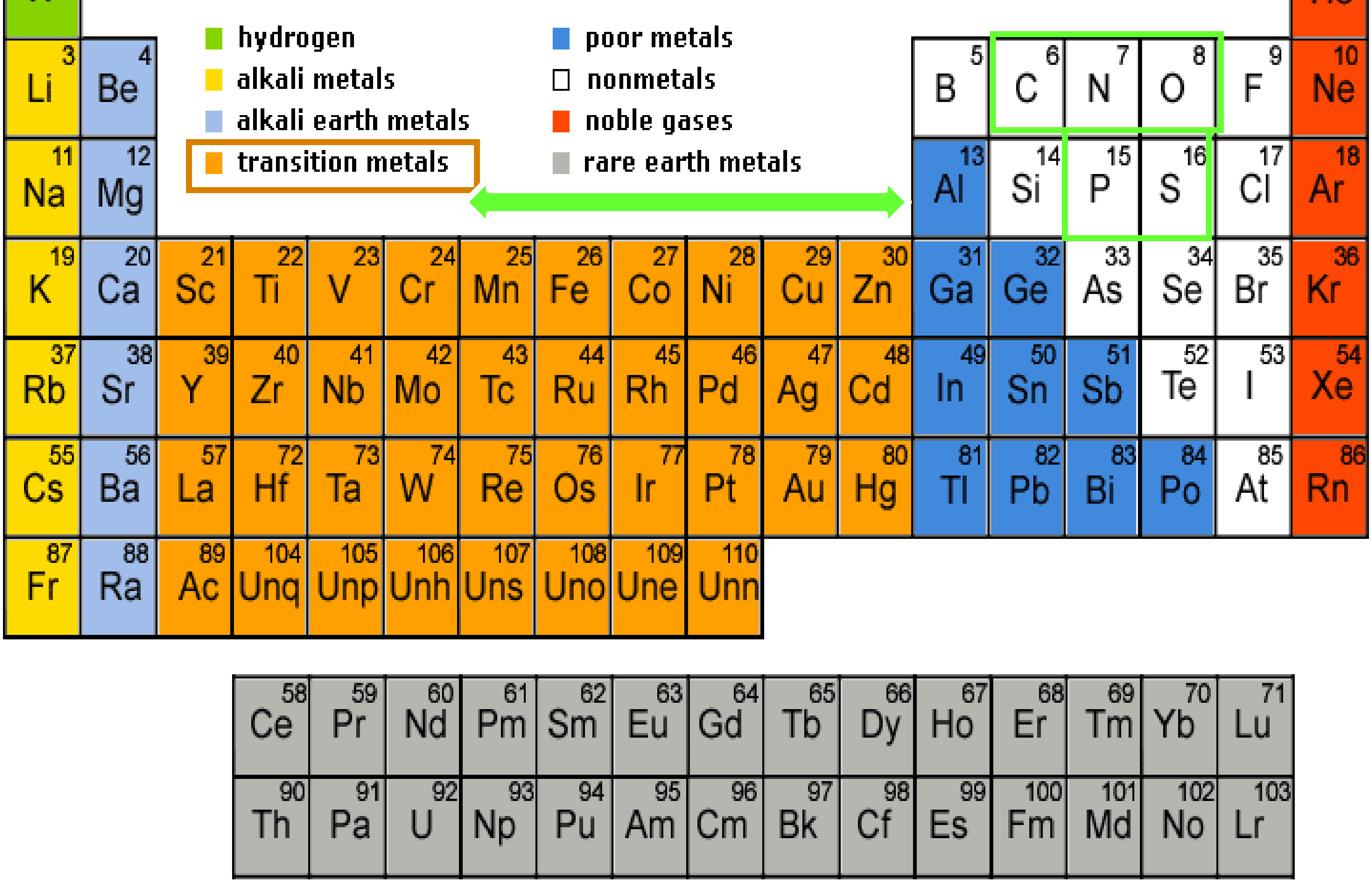
Periodic Table of the Elements



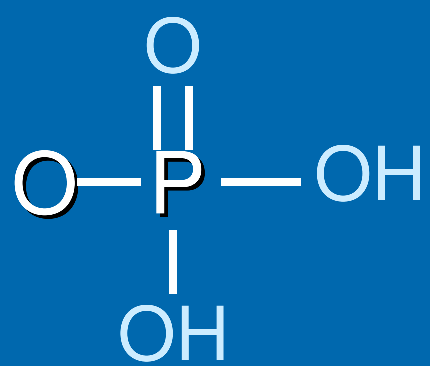
TRANSITION METALS IN EXTANT LIFE

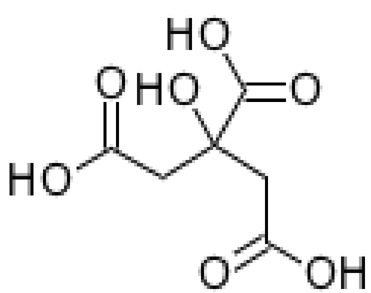
- **Iron is present in heme and cytochromes**
- **The enzyme Nitrogenase needed to fix Nitrogen in the global eco system requires Molybdenum as an essential cofactor**
- **Cobalt is a must for Vitamin B12 in all mammalian systems**
- **In archaea, the cofactors of methanogens need Nickel, Cobalt and Molybdenum and Iron complexed to their cofactors**
- **Many enzymes and coenzymes need Iron, Cobalt, Nickel, Copper, Manganese, Chromium, Vanadium, Molybdenum, Tungsten**

Transition metals in sea water

Metal	Concentration in nanomoles/liter
Iron	179
Nickel	91
Copper	47
Vanadium	39
Manganese	39
Cobalt	4.5
Chromium	0.9

Transition Metals & the Core Metabolome

- 5. All core molecules contain either carboxylic or phosphoric acid moieties or both.
- R-COOH, 



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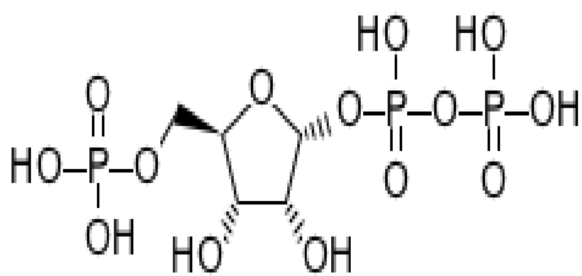

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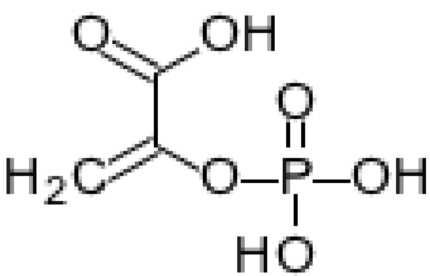

C00074

Transition Metals & the Core metabolome

- In addition to the Oxygen atoms of the carboxylic & phosphate groups, majority of the core compounds contain at least one or more Nitrogen atoms
- Both the Oxygens and Nitrogens can combine with the transition metals to form metallo-complexes
- These metallo-complexes can act as small molecule catalysts in the core network.
- Such an enrichment process may be a selection mechanism for the enrichment and emergence of the core metabolome

Acknowledgements

- We (VS and Harold J Morowitz) are grateful to NSF and William Melton for the funding support of these studies

MDL Keys & Feature Space

- Descriptors – Molecular features
- Descriptors encoded into binary "keybits"
- Historically, MDL Information Systems designed 'keys' for substructure searching in chemical compounds libraries/ databases
- Three sets: 166 , 324 and 960 keybits