



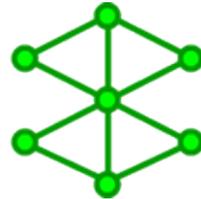
School of Biotechnology and Biomolecular Sciences

Unusual DPANN Archaea from a radioactive legacy site



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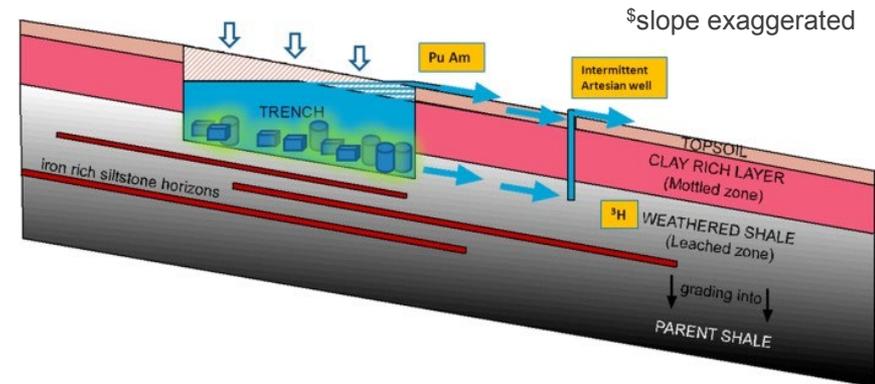
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Little Forest Legacy Site (LFLS)

- Radioactive legacy disposal site 1960-68
- 3 m deep trenches
- Mixed waste including $^{239+240}\text{Pu}$, ^{241}Am ,...
- *Alternating raining/drying periods → dynamic redox conditions



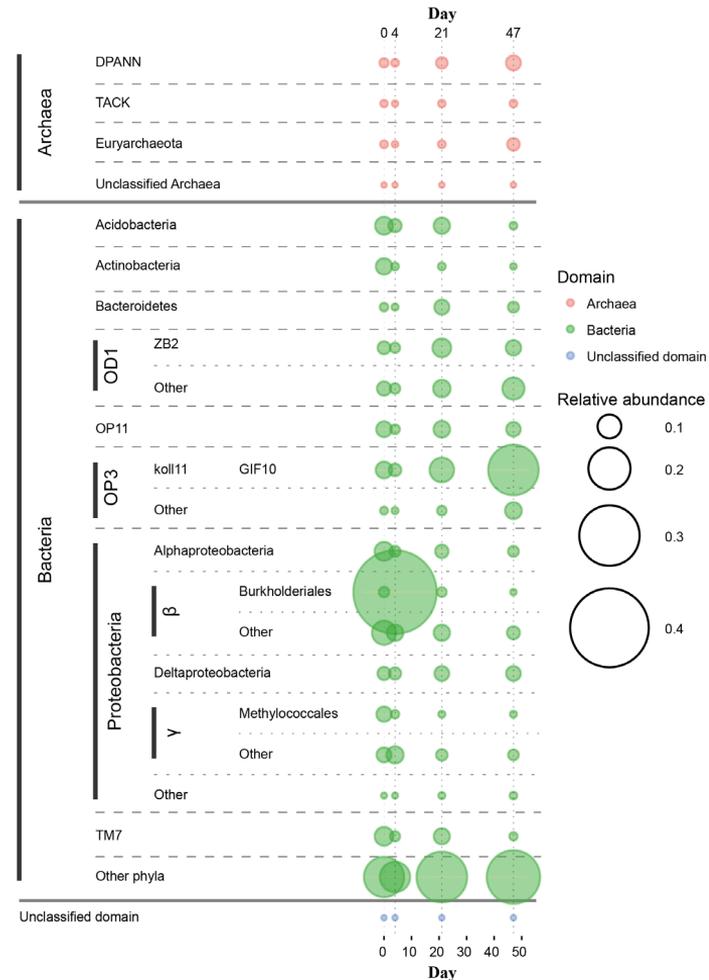
Payne. ANSTO E-780. 2012.



Payne et al. *Environ Sci Technol* 2013; 47: 13284–13293.

Background – Archaea

- *Highly anoxic periods, Archaea > 10%, based on GraftM (16S):
 - Max. 37.2% Euryarchaea
 - Mostly Methanomicrobia, incl. a lot of ANME-2d
 - Up to 55.8% DPANN
 - Max. 28.8% TACK (mainly *Nitrosotalea*-related)
- Relatively small dataset compared to Bacteria
- Many *trendy* Archaea
- Archaea are cool!



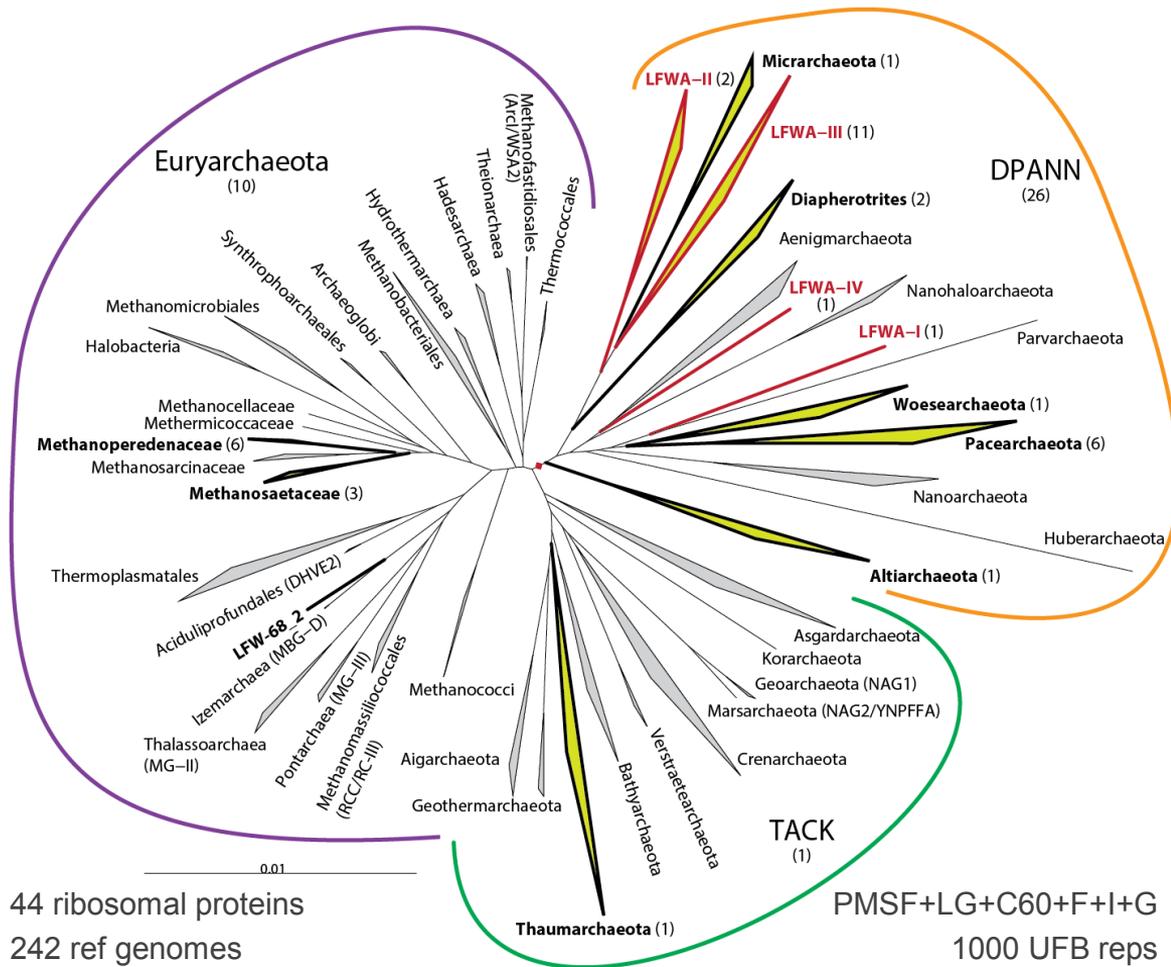
* Vázquez-Campos *et al. Appl Environ Microbiol* 2017; **83**: e00729.

General findings

- 37 archaeal bins:
 - $\geq 50\%C$ / $< 10\%R$

	DPANN	Euryarchaeota	TACK
Cov (%)	74.57%	22.16%	3.27%
	(56.19-82.50%)	(13.33-42.78%)	(1.03-4.88%)
Bins (%)	70.27%	27.03%	2.70%

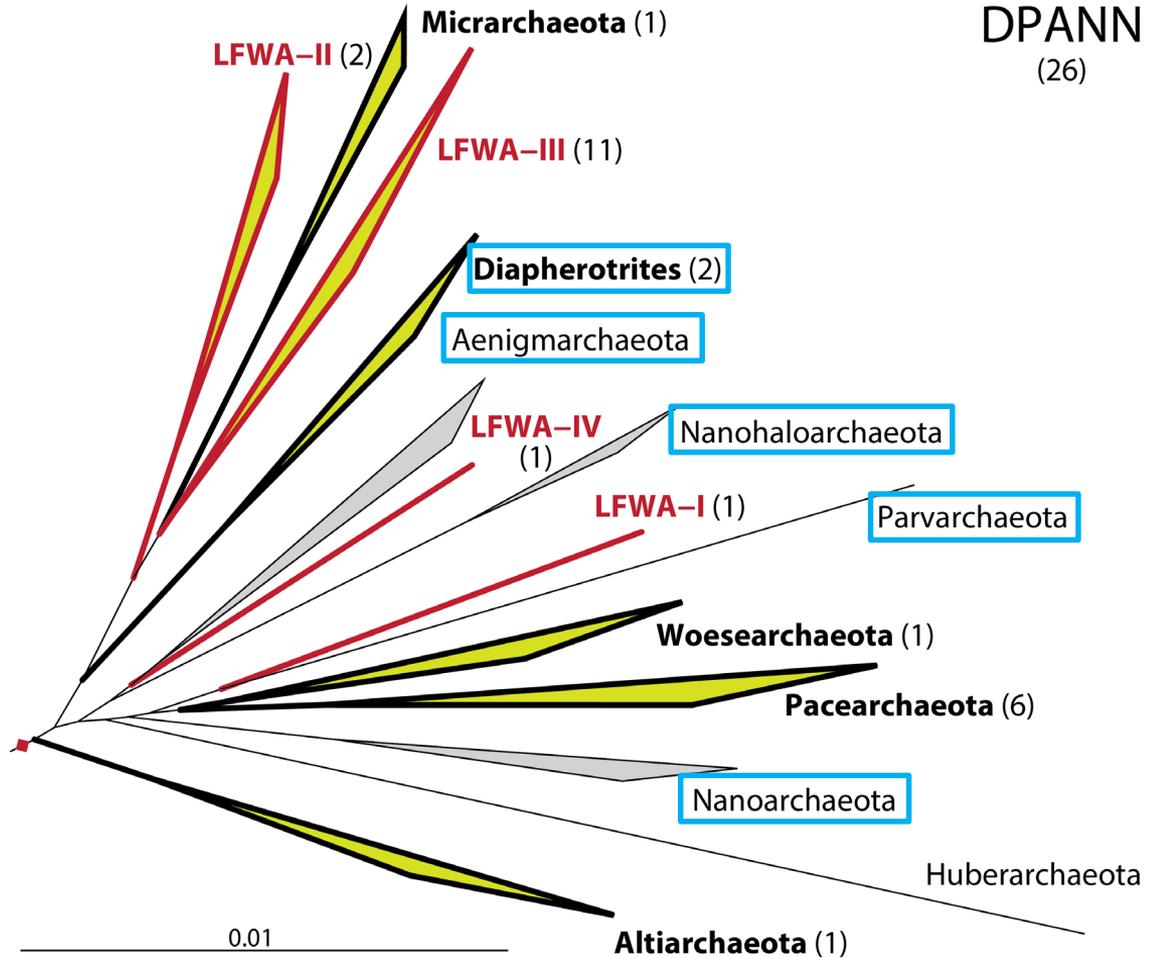
- Low variability bins:
 - most < 0.1 SNV/kbp



DPANN

- Present in diverse environments
- Small genomes: <1.5 Mbp
 - Except Altiarchaeota
- Lack of biosynthetic pathways
 - amino acids
 - purine/pyrimidines
 - vitamins...
- Fermenters

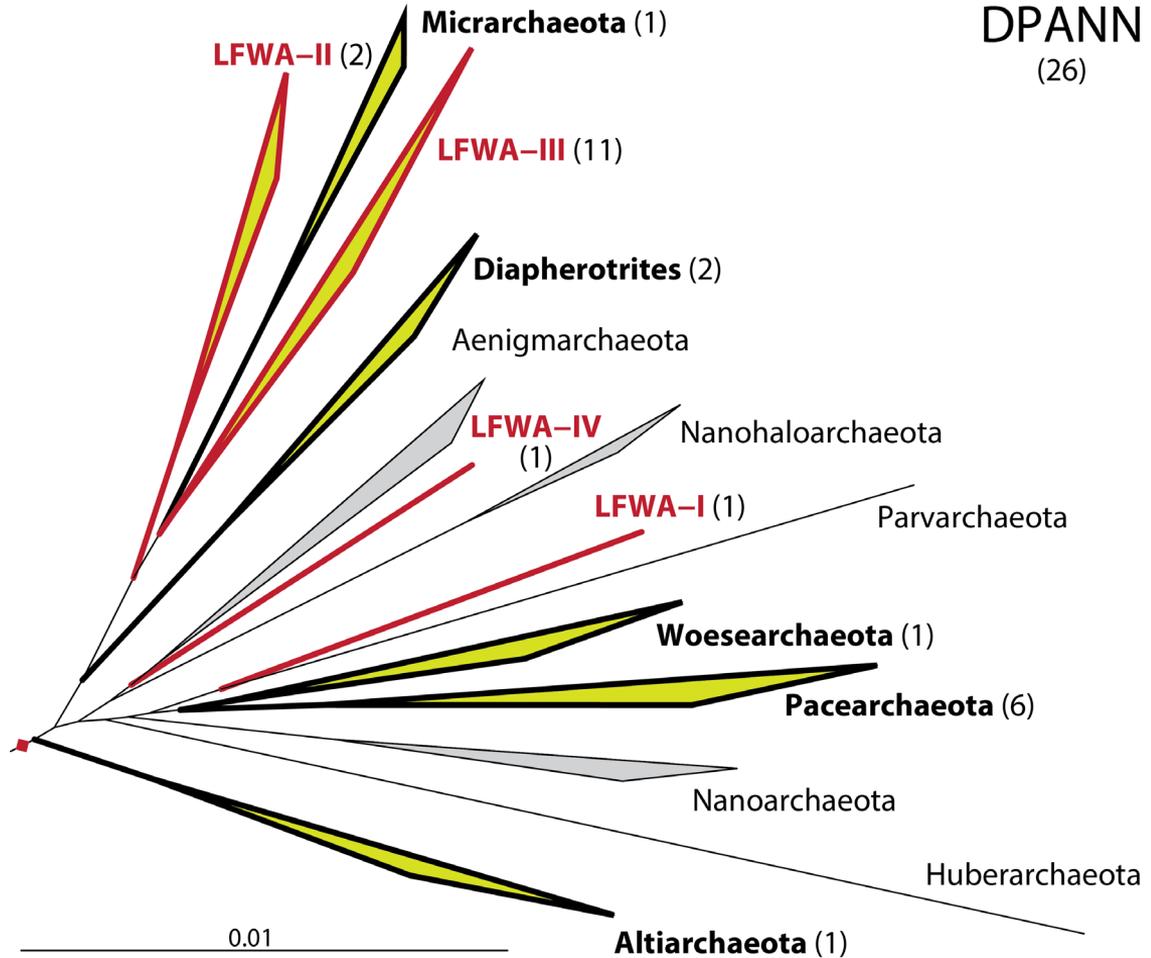
DPANN
(26)



DPANN

- Very diverse genomes
- No N or S dissimilatory pathways
- No C-fixation: RuBisCO III or II/III, if any.

DPANN
(26)

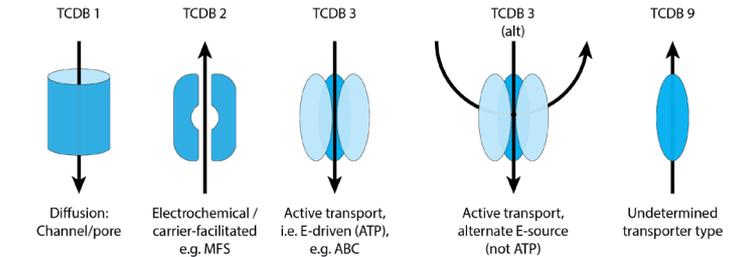
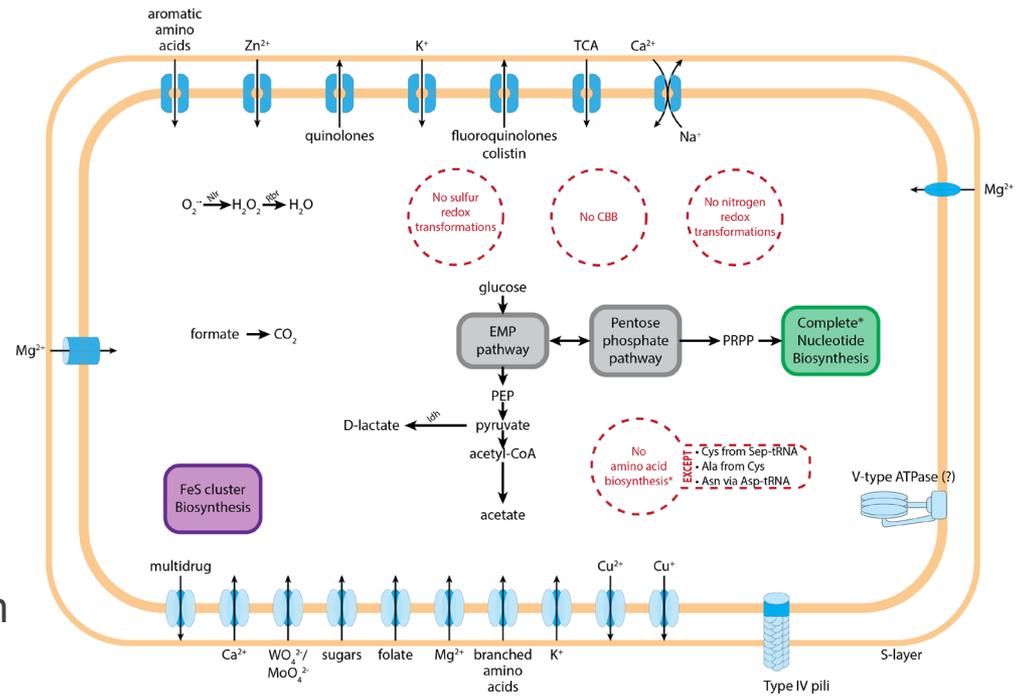


New lineages

- Biosynthesis of Fe-S clusters.
- Type IV pili but no archaeal flagella.
- S-layer.
- **Most** only able to synthesise a few amino acids.

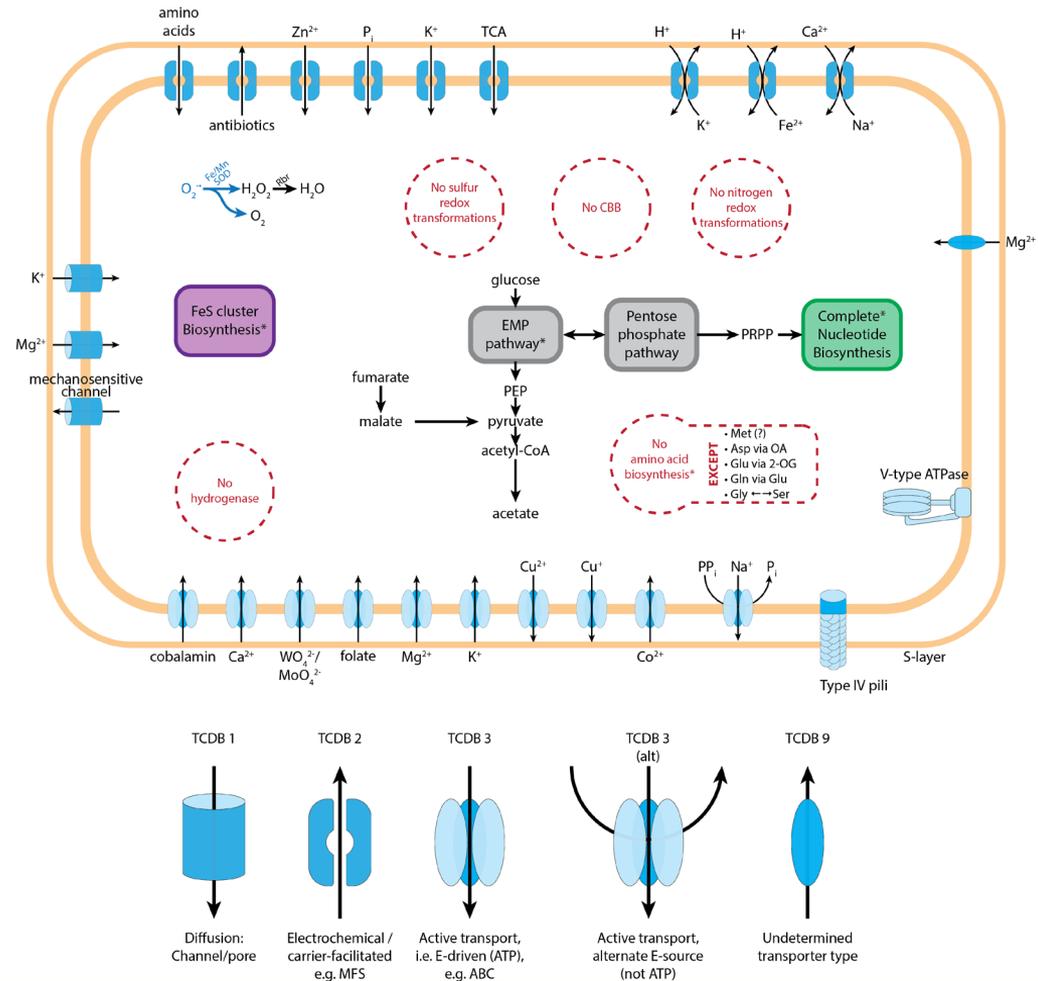
LFWA-I

- ‘*Ca. Tiddalikarchaeum anstoanum*’
- Closest relative to Parvarchaeota
 - 16S: <75% identity
- Mostly complete purine/pyrimidine biosynthesis pathways
- ROS detox: neelaredoxin + rubredoxin



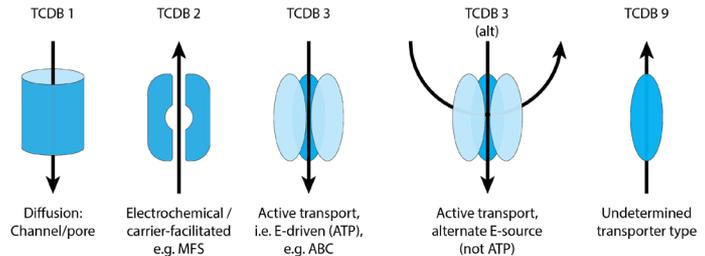
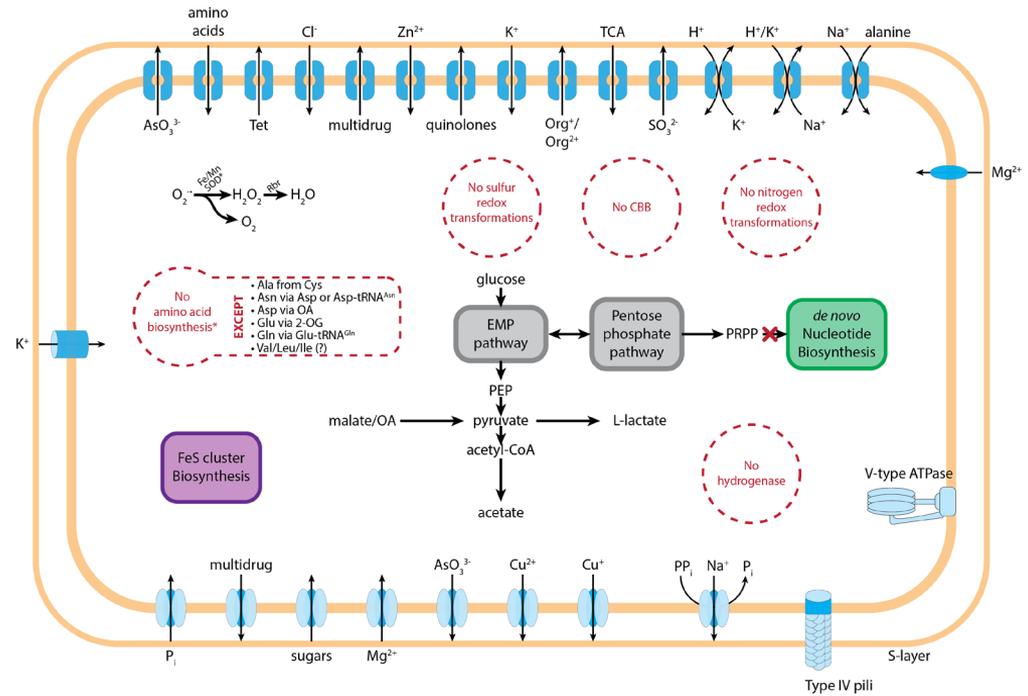
LFWA-II

- ‘*Ca. Wianamatarchaeum fermentum*’
- Sister to (Micrarchaeota, LFWA-III)
- Mostly complete purine/pyrimidine biosynthesis pathways
- ROS detox: Fe/Mn SOD + rubredoxin



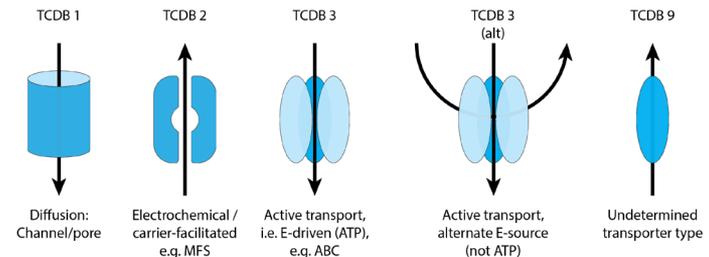
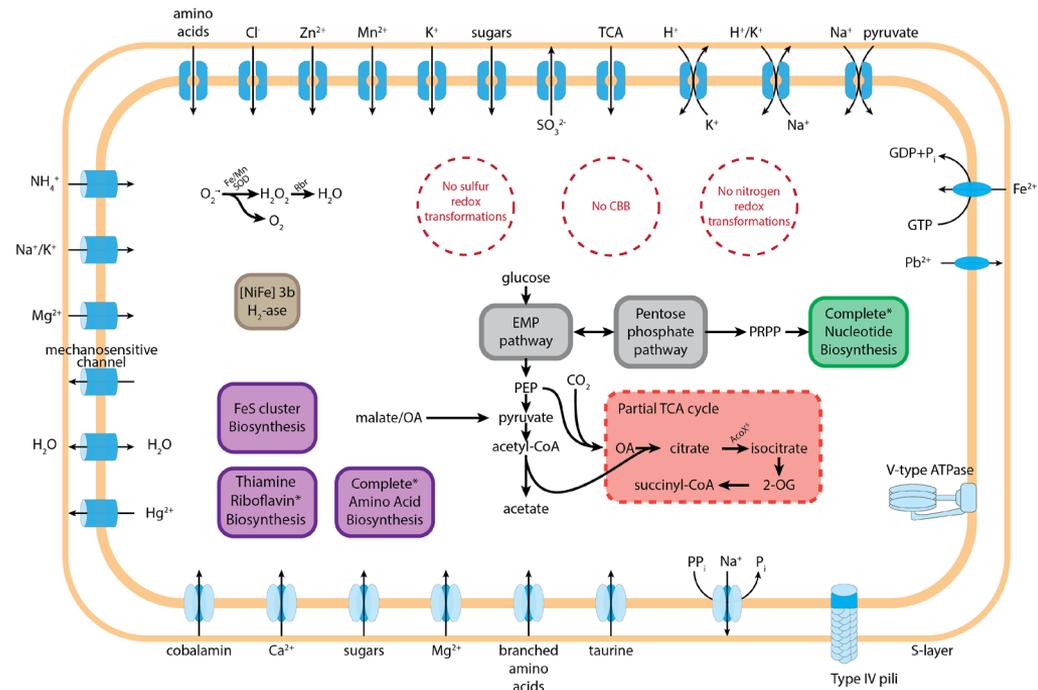
LFWA-IV

- Basal to Aenigmarchaeota
- No *de novo* purine/pyrimidine biosynthesis pathways
- ROS detox: Fe/Mn SOD + rubredoxin
- Wide variety of drug resistance exporting mechanisms, incl. organocations (e.g. cationic surfactants)



LFWA-III

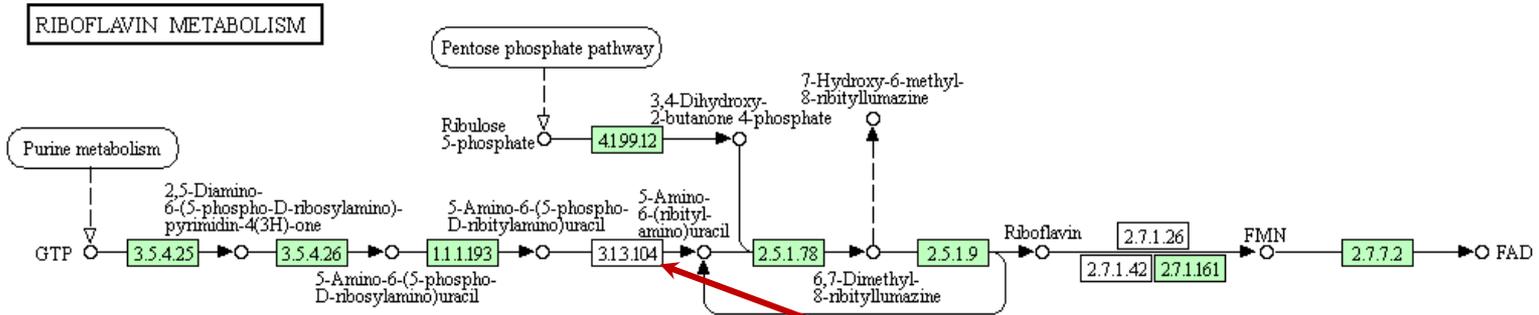
- ‘*Ca. Gugararchaeum adminiculabundum*’
- AAI: 10 bins, 9 gen, single fam
- Closest to *Micrarchaeota s. stricto*
 - ~75% id to 16S of *M. acidiphilum*
- ROS detox: Fe/Mn SOD + rubredoxin
- Thiamine biosynthesis
- [NiFe] 3b hydrogenase
 - H₂ evolving, O₂ resistant



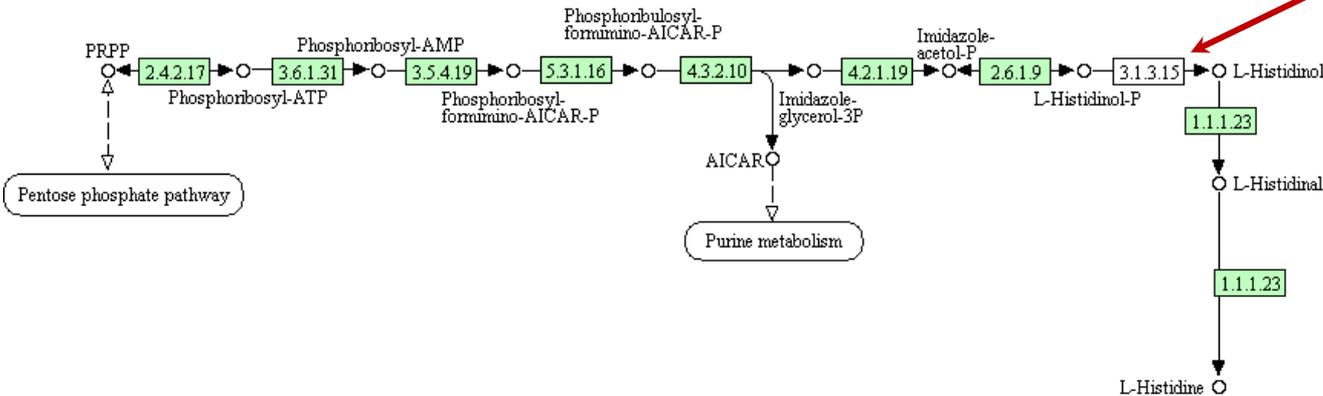
LFWA-III (cont.)

- Many “*gappy*” biosynthetic pathways almost complete:
 - Partial TCA cycle
 - Riboflavin biosynthesis
 - Many amino acid pathways
- Other DPANN, pathways often completely absent, not missing 1-2 enzymes
- Why the gaps?
 - Missing in assembly from metagenomes?
 - Not known enzymes in Archaea?
 - No model DPANN → Evol. far from other Archaea → Annotations unreliable/ambiguous
 - Promiscuous enzymes do it?
 - All of the above?

Example



HISTIDINE METABOLISM



phosphatases

Ex. Riboflavin biosynthesis

- Missing phosphatase in riboflavin biosynthesis
- In Bacteria:
 - YcsE in *Bacillus subtilis*, COG0561
 - YigB/YbjI in *Escherichia coli*, COG1011
 - Convergent specificity within the HAD superfamily from 2 different COG
- HAD phosphatases are promiscuous and redundant, e.g. Cof-like phosphatases family:
 - *E. coli* (4.5-5.5 Mbp): 7 paralogues for 5 substrates (overlapping activities)
 - 'Ca. *Blochmannia pennsylvanicus*' (ant endosymbiont, 0.78 Mbp): only 1 paralogue, uses 4 out of 5 same substrates
- LFWA-III has HAD phosphatases, incl. unknown / unclear specificity
- Digging through lit → Enzyme not known in Archaea

Summary

- DPANN dominate the archaeal community at LFLS... even when methanotrophs and *Methanoperedens* flourish
- New DPANN lineages, typical metabolism... mostly
- LFWA-III may be best candidates for *bona fide* free-living DPANN *sensu stricto*

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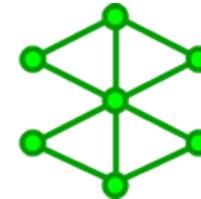
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