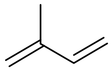
# Topics

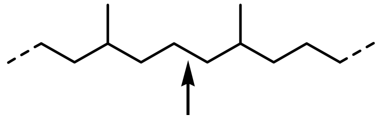
## Complex lipids of extant archaea

- Structural peculiarities of archaeal lipids
- LCMS methods
- Molecular Signatures of HT Methanogens
- Low temperature Chrenarchaea
- *Ignicoccus* sp., *Nanoarchaeum equitans*

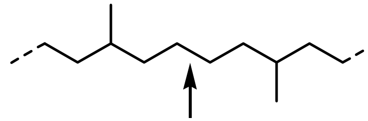
# Common Acyclic Isoprenoids



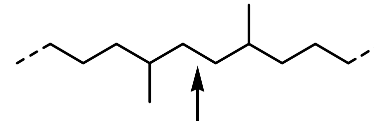
**isoprene**



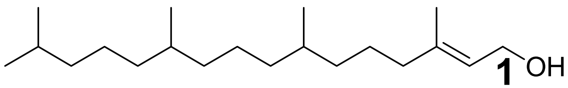
head-to-tail



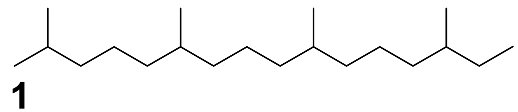
tail-to-tail



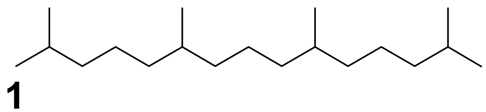
head-to-head



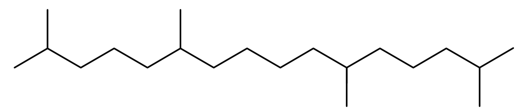

**phytol**



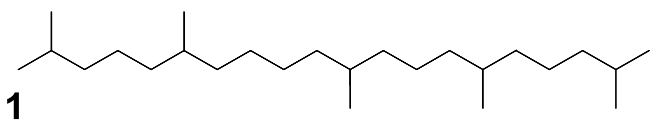

**phytane**



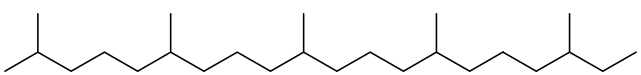

**pristane**



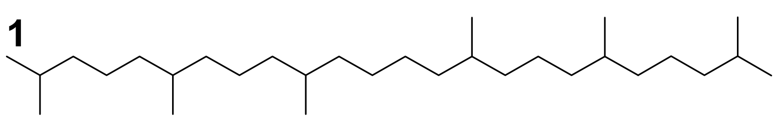
**crocetane**



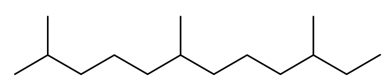

**2,6,10,15,19-pentamethylicosane (PMI)**



**2,6,10,14,18-pentamethylicosane**

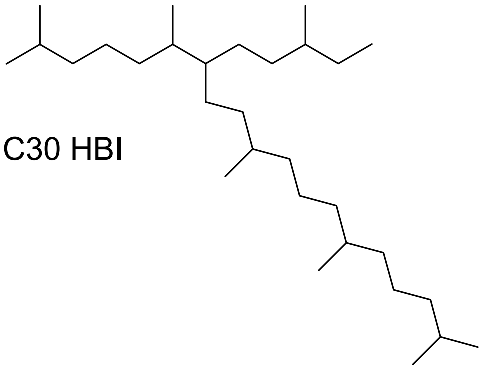



**squalane**

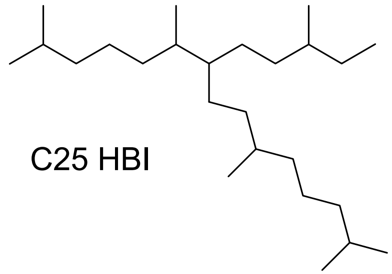


**farnesane**

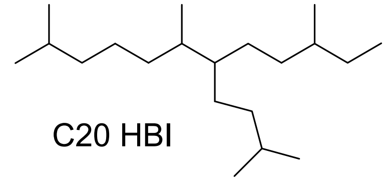
# Less Common Acyclic Isoprenoids



C30 HBI



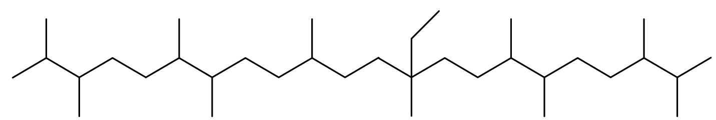
C25 HBI



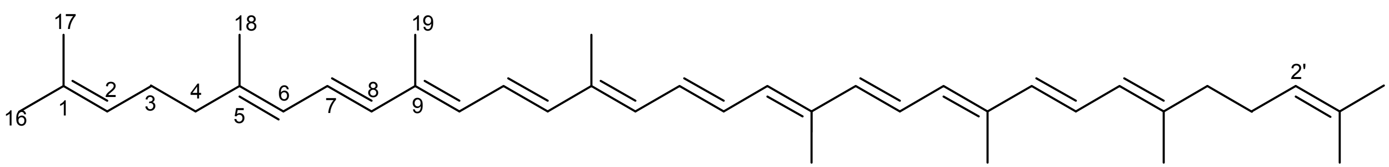
C20 HBI

Diatom sources

*Botryococcus braunii*



botryococcane

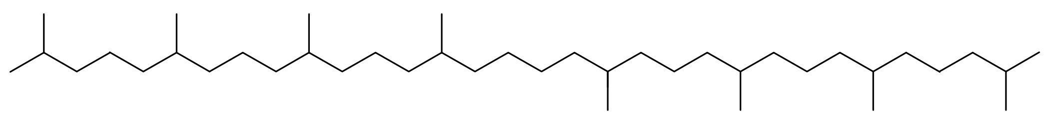



lycopene

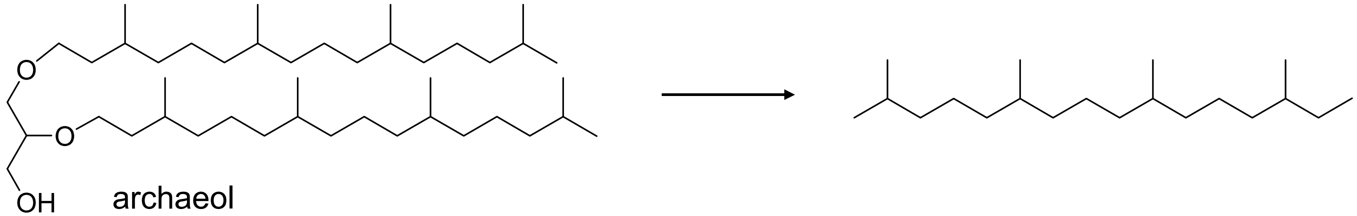
tomato carotenoid

Probable algal hydrocarbon
? from lycapodiene

lycopane

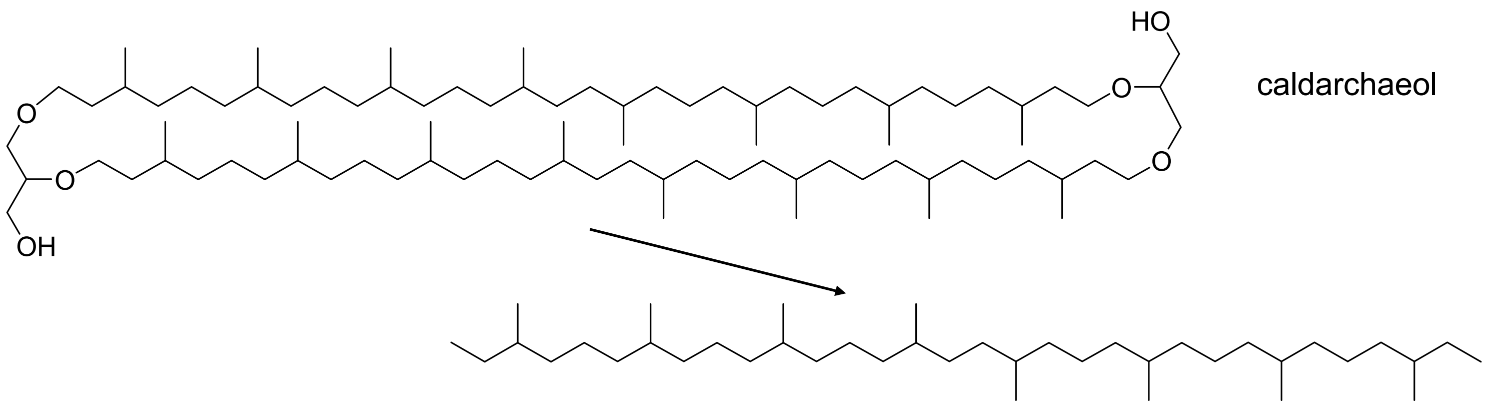


# Polar Lipid Precursors of Acyclic Isoprenoids



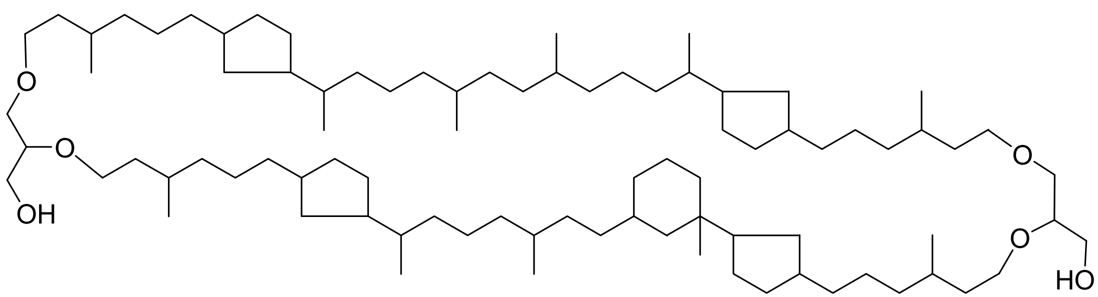
archaeol

phytane



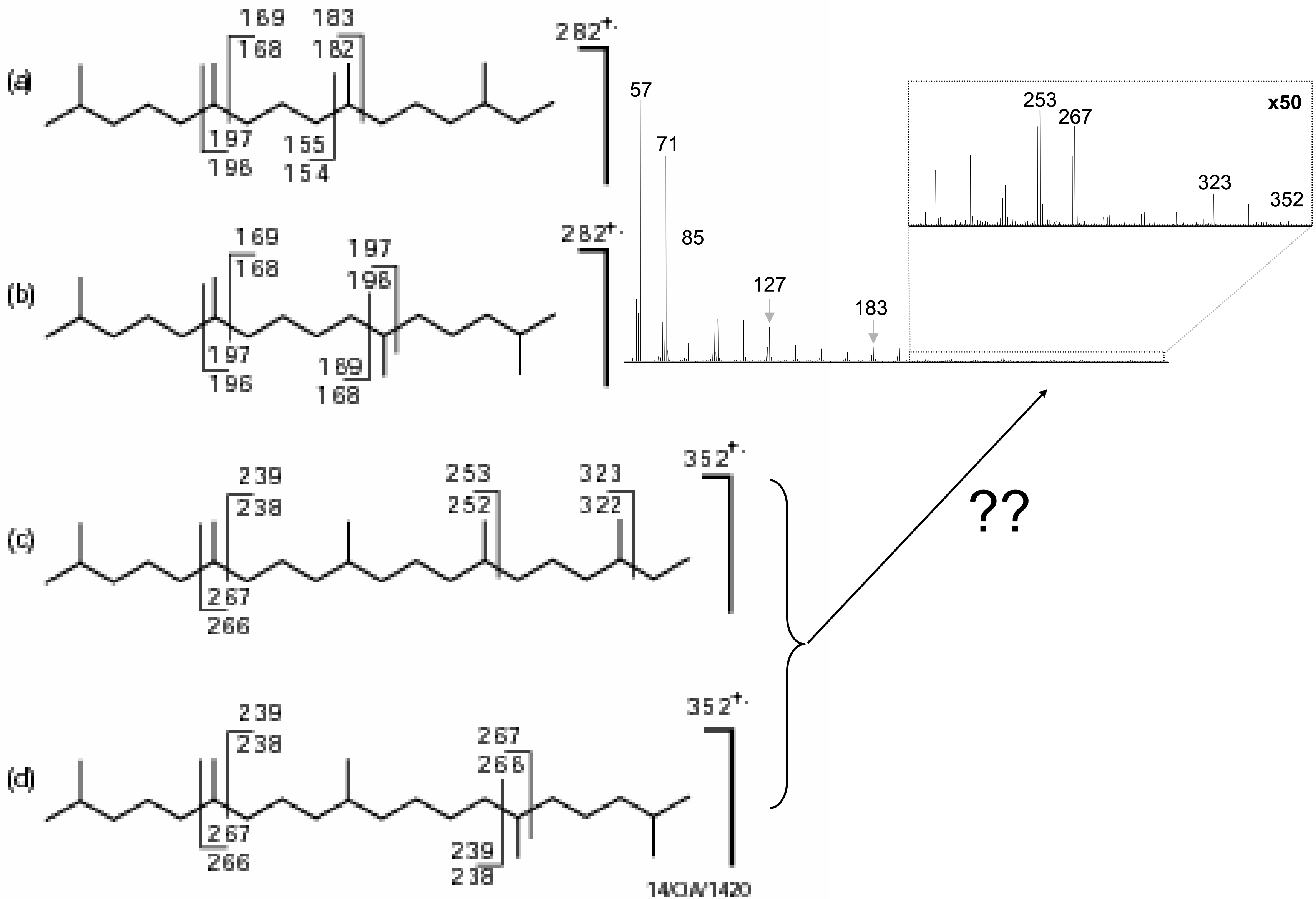
caldarchaeol

biphytane

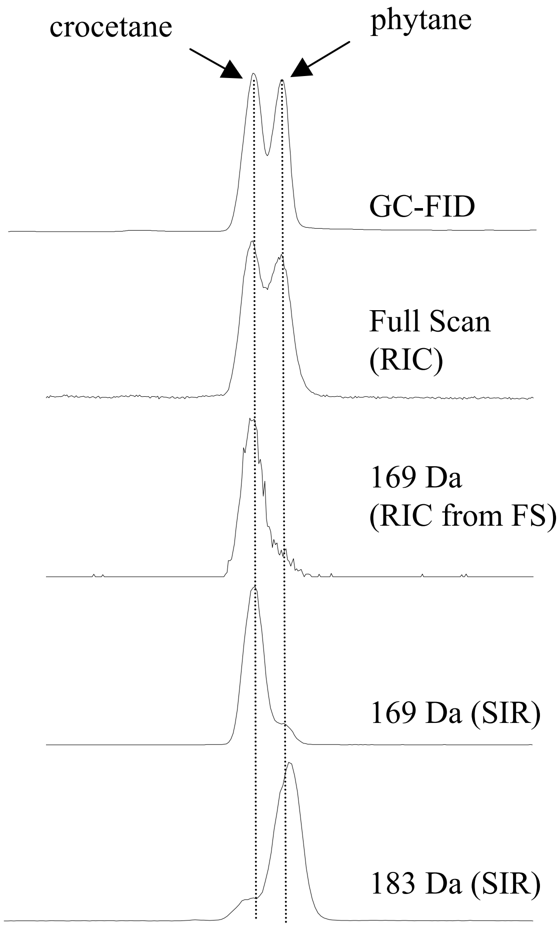


chrenarchaeol

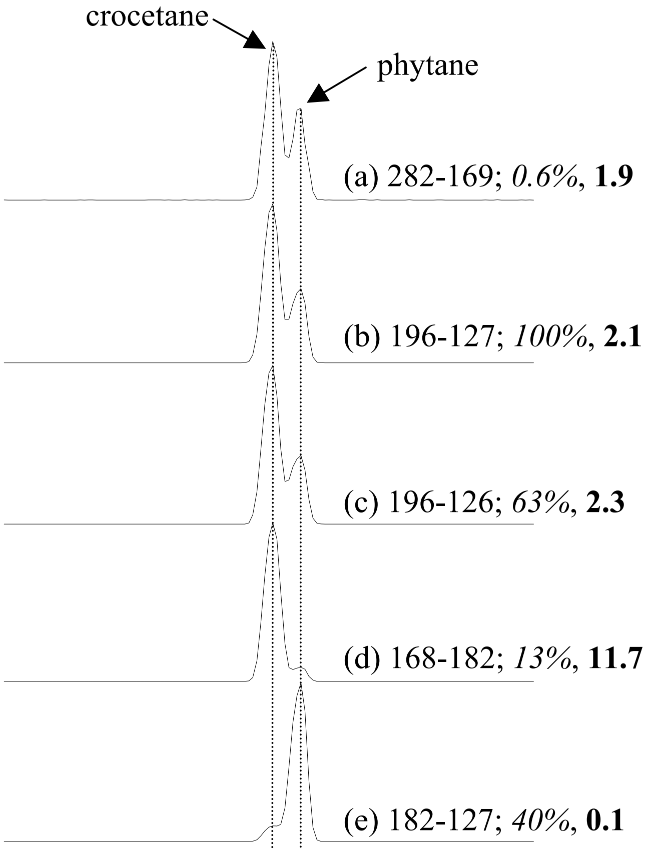
# Favored Mass Spectrometric Fragmentations



# Crocetane – Phytane Distinction




GC and GC-MS (SIR)




GC-MS-MS

# Crocetane – Phytane Distinction

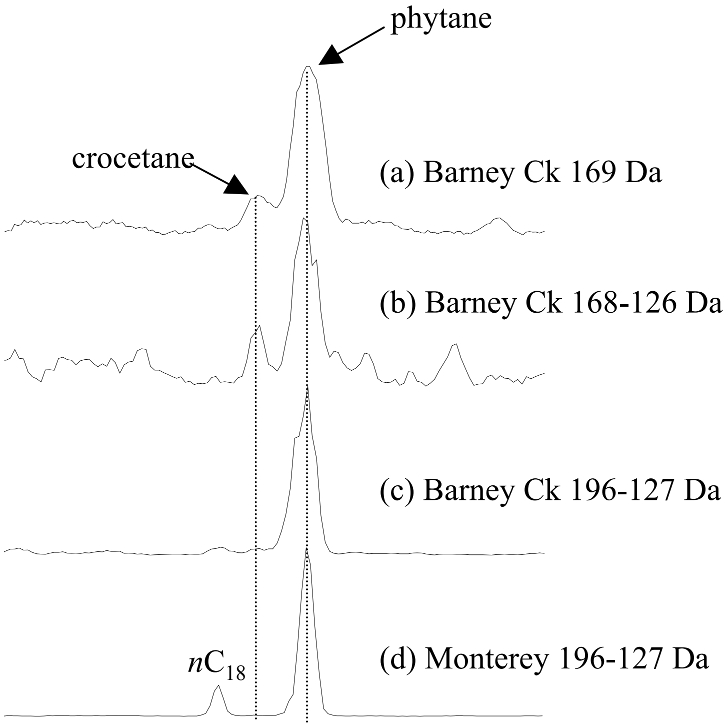
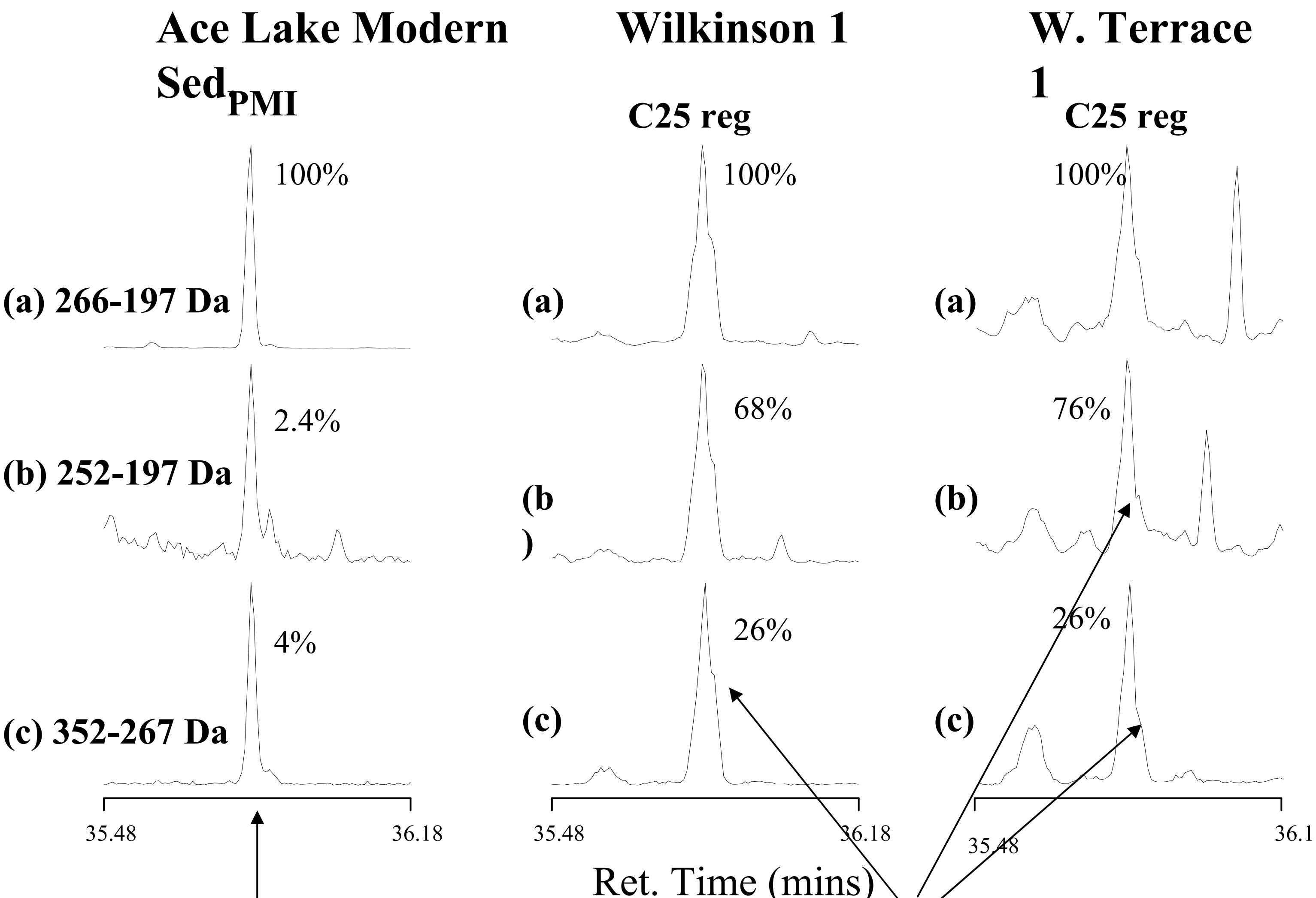



Figure 4

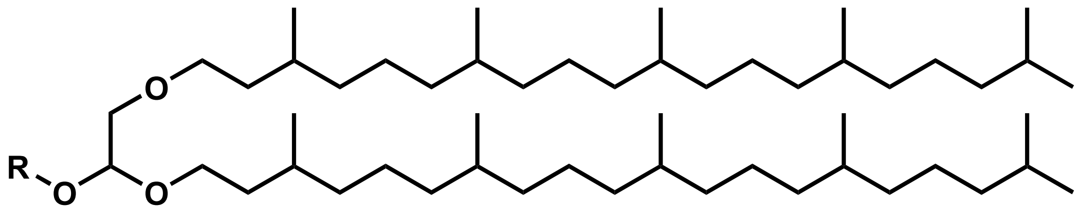
# Regular $C_{25}$ vs PMI Distinction



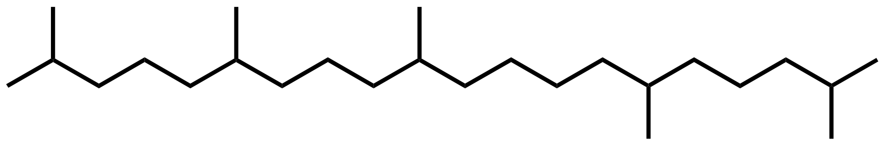

One thin peak+ one compound

All fat peaks = more than one compound

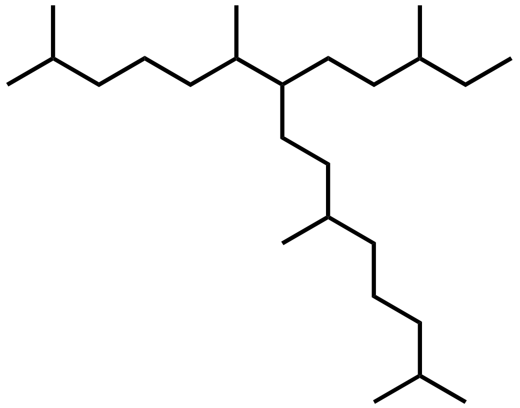
# Regular $C_{25}$ vs PMI Distinction




2,6,10,14,14-pentamethylicosane
Carbon chains of *Halobacterium* core lipid



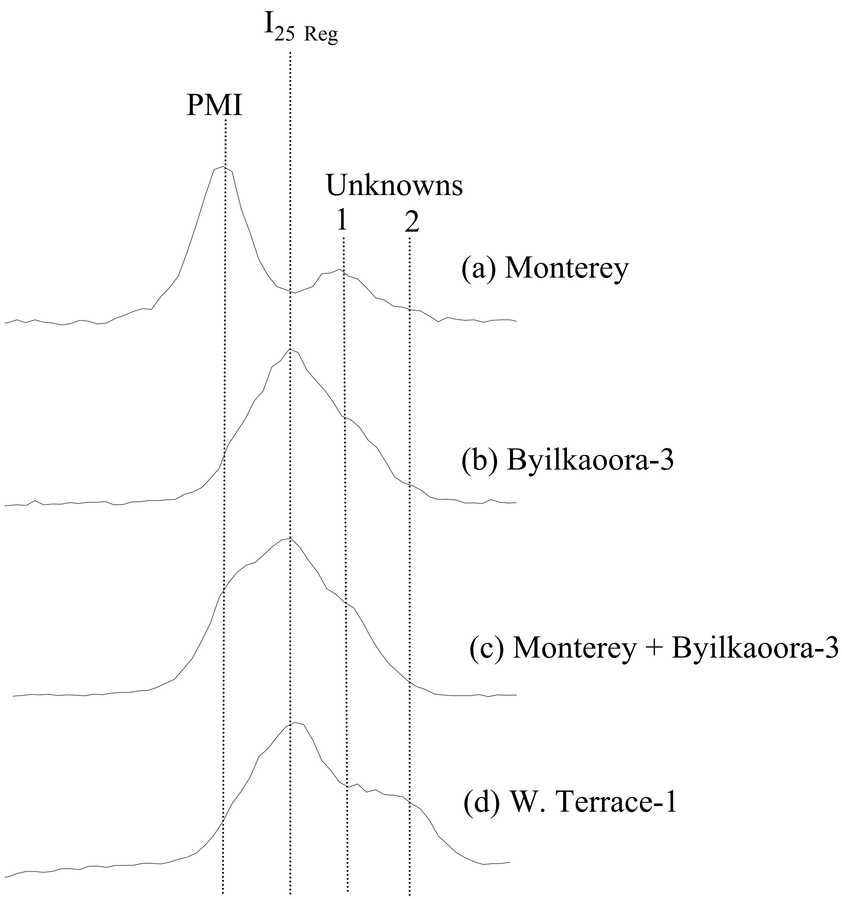
2,6,10,15,19-pentamethylicosane (PMI)
Found as a free hydrocarbon in some methanogens



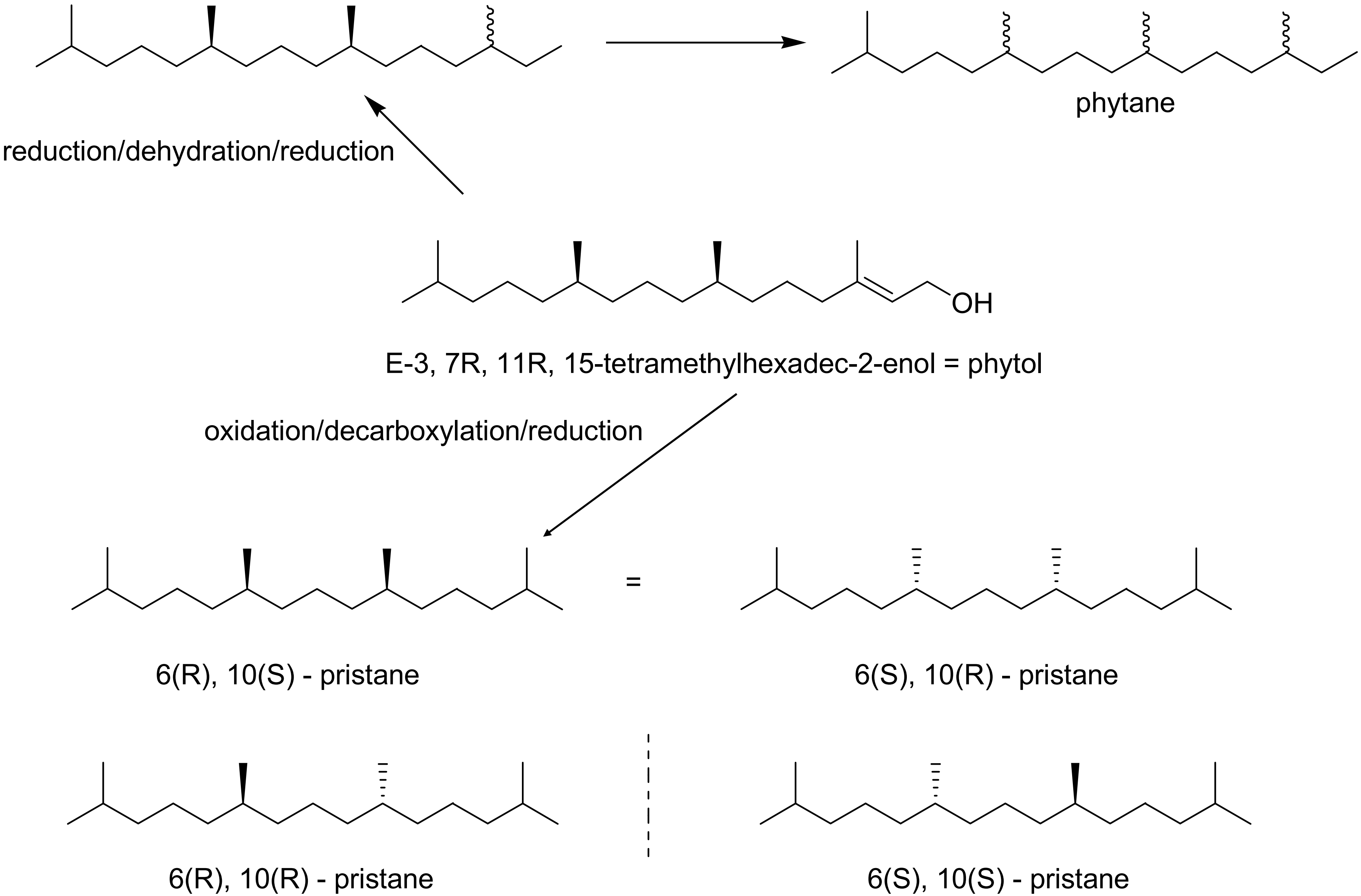
A ‘highly branched isoprenoid’ (HBI)
from a diatom

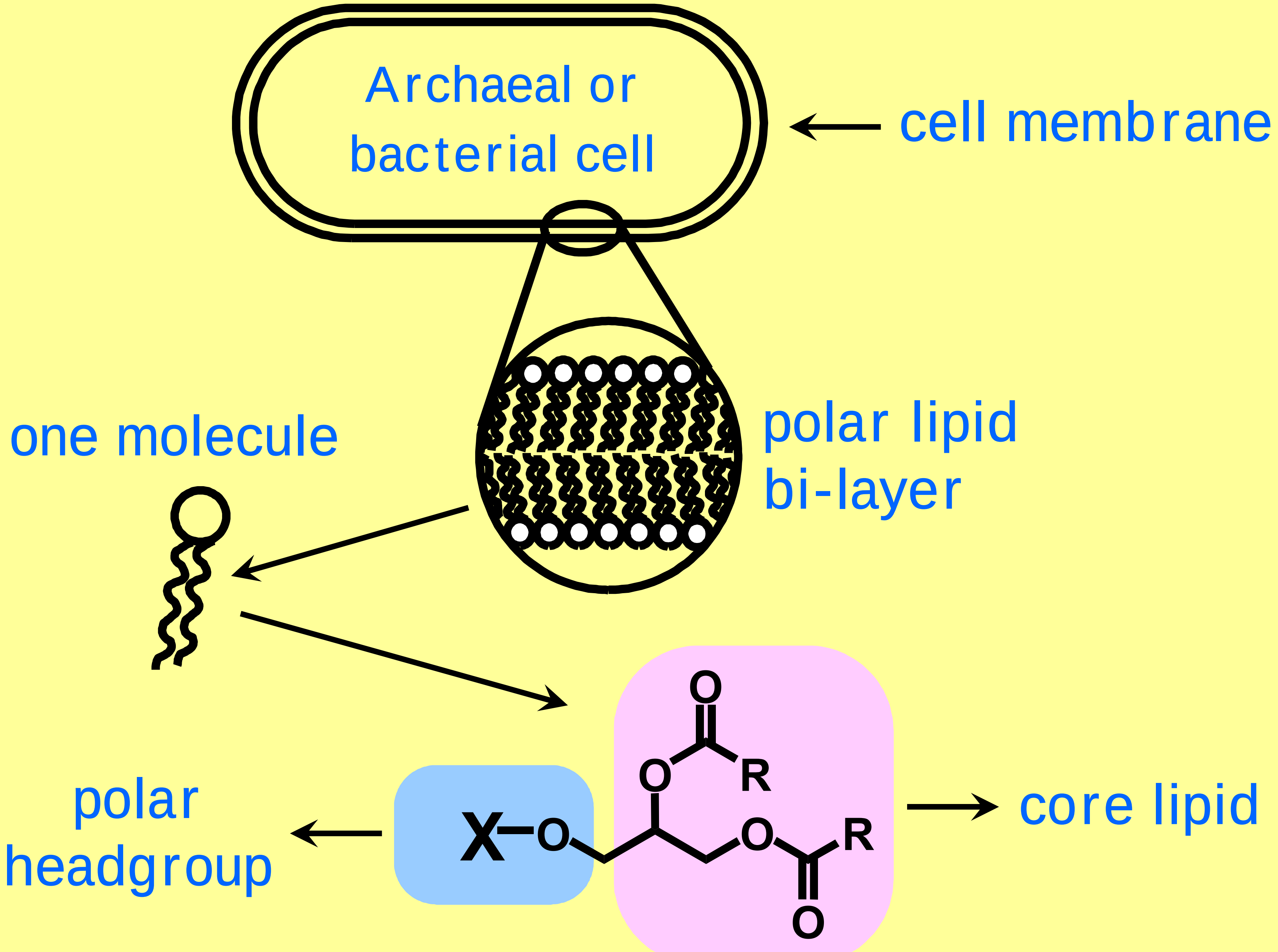
# Distinguishing C25 Isoprenoids

note peak shapes




Partial 183 Da (SIR) chromatograms of (a) Monterey Formation showing elution position of PMI; (b) Byilkaoora-3 showing elution position of I25 reg; (c) Monterey + Byilkaoora-3 mixture showing relative elution order of PMI and I25 reg isomers (NB. only partially resolved); (d) West Terrace-1 which has a peak at the same position as the I25 reg isomer and no peak at the earlier retention time of PMI. Unknown peaks 1 (Monterey) and 2 (West Terrace-1) elute after I25 reg. Chromatogram time range = 36 sec.

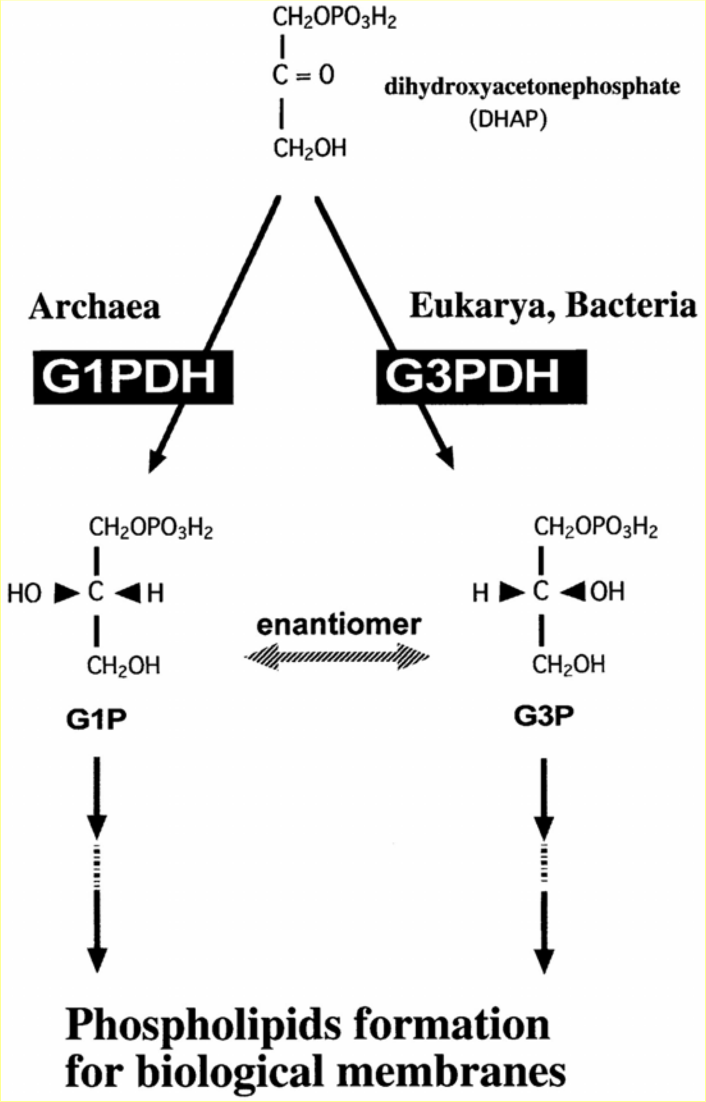

phytane
reduction/dehydration/reduction
OH
E-3, 7R, 11R, 15-tetramethylhexadec-2-enol = phytol
oxidation/decarboxylation/reduction
=
6(R), 10(S) - pristane
6(S), 10(R) - pristane
6(R), 10(R) - pristane
6(S), 10(S) - pristane




Courtesy of Helen Fredricks. Used with permission.

# Glycerol Stereochemistry ?

*sn*-glycerol-1-phosphate dehydrogenase (G1PDH)

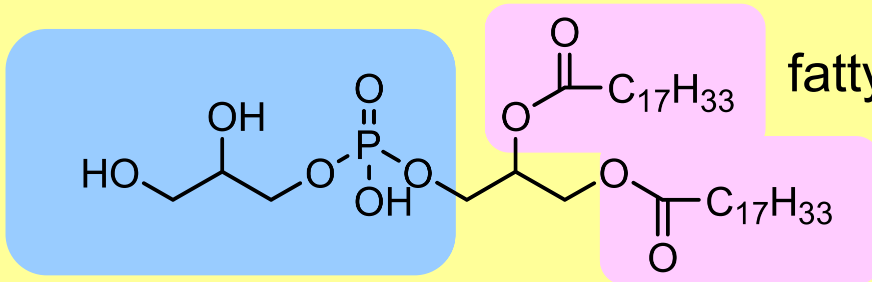



*sn*-glycerol-3-phosphate dehydrogenase (G3PDH)

# IPL variety analyzed by HPLC-ESI-$MS^n$

## bacterial

10+ different headgroups identified…




fatty acids ~$C_{12}$ to $C_{24}$

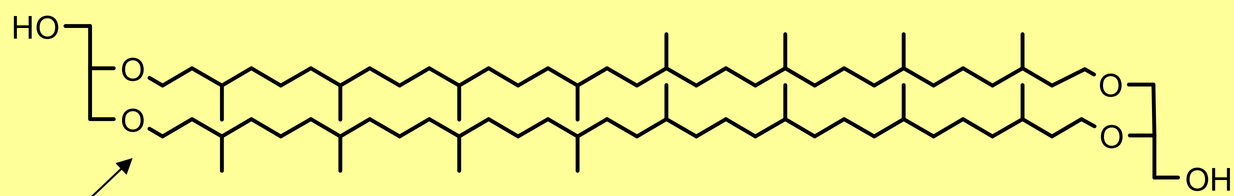
may also be alcohols

phospholipids & glycolipids, some highly specific

## archaeal

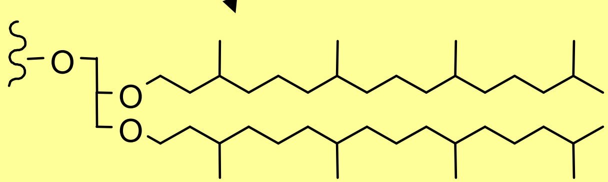
similar variety of headgroups but highly specific core lipids

2 basic types
with some variations




**caldarchaeol (GDGT: glycerol dialkyl glycerol tetraether)**

**archaeol**




Data on IPL distributions in biogeochemically relevant prokaryotes are very sparse

# *Ignicoccus islandicus & Ignicoccus pacificus*

**Sources of samples.** At the Kolbeinsey Ridge, north of Iceland, eight samples of submarine sandy sediments and venting water (original temperatures around 90 °C) were taken by the research submersible `Geo' at depths between 103 and 106 m (Fricke *et al*., 1989; Burggraf *et al*., 1990).

Furthermore, black smoker samples were obtained during dive 3072 of the submersible `Alvin' at the East PaciÆc Rise at 9° N, 104° W at a depth of 2500 m.

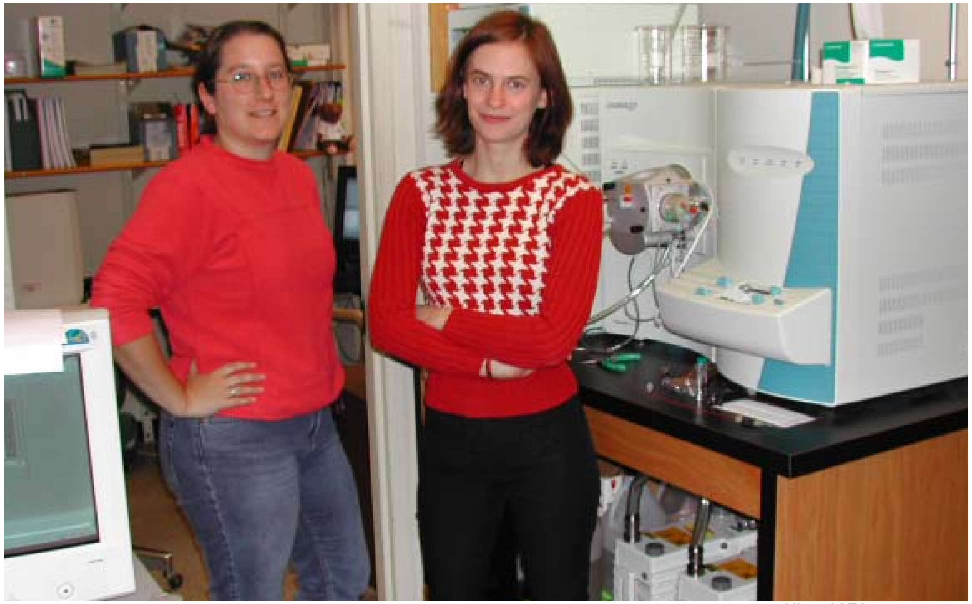
Arch Microbiol (2004) 182: 404-413
DOI 10.1007/s00 203-004-0725-x

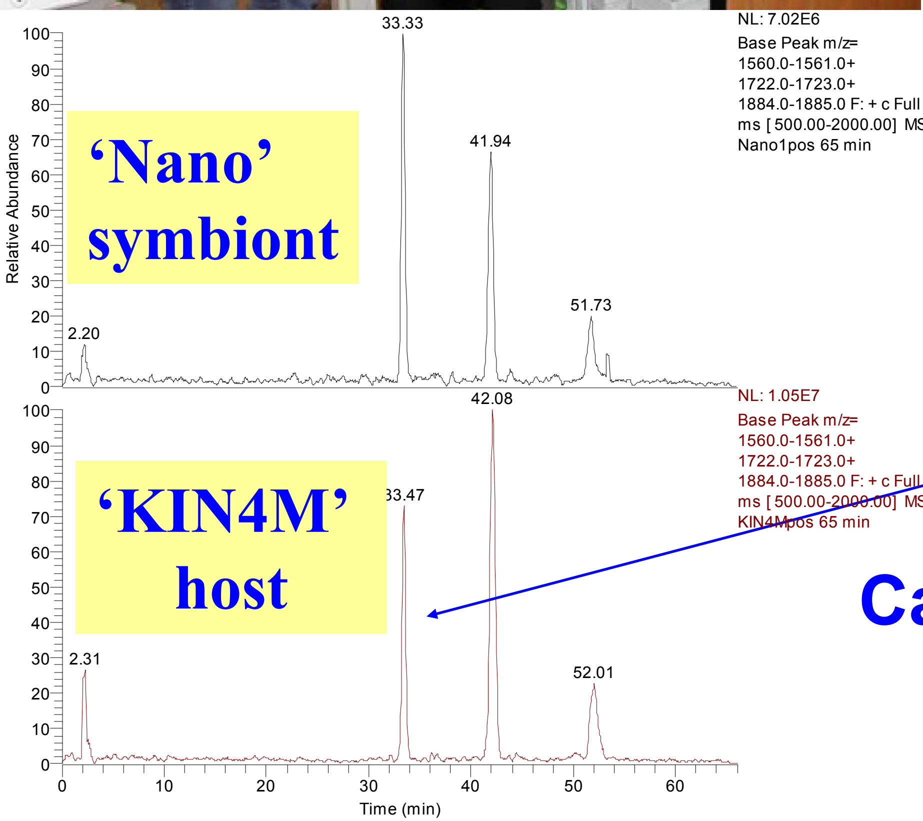
ORIGINAL PAPER

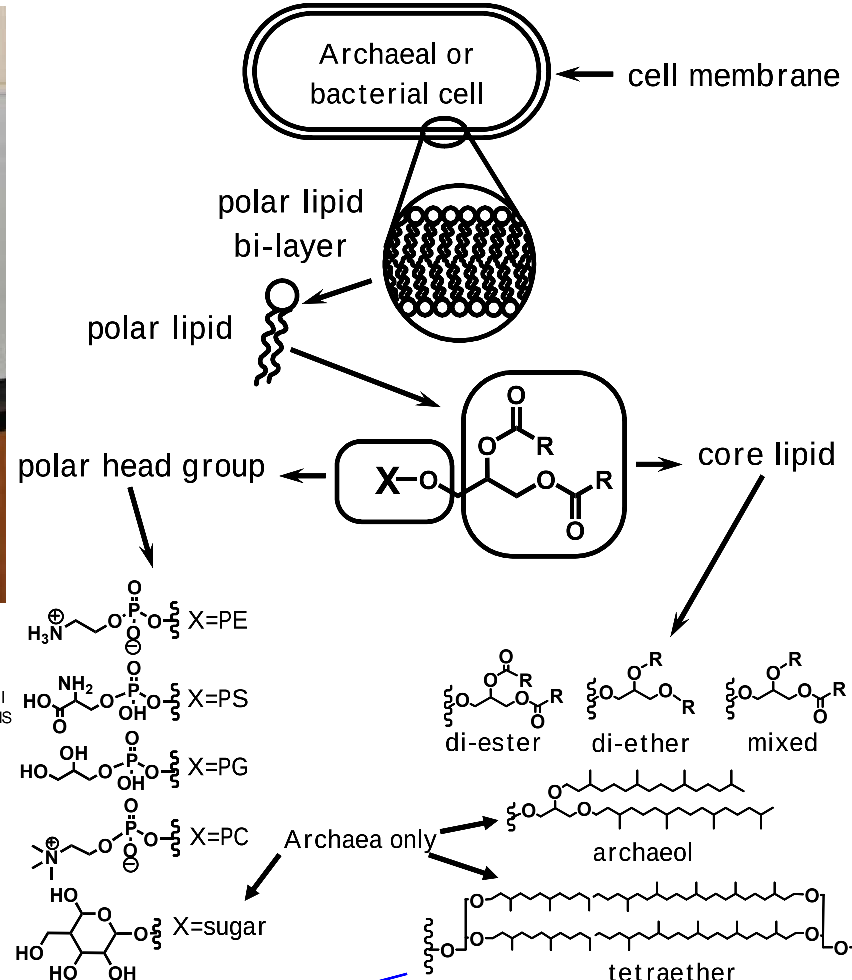
**Ulrike Jahn, Roger Summons, Helen Sturt**
**Emmanuelle Grosjean, Harald Huber**

# Composition of the lipids of *Nanoarchaeum equitans* and their origin from its host *Ignicoccus* sp. strain KIN4/I

Received: 22 January 2004 / Revised: 30 June 2004 / Accepted: 5 August 2004 /
Published online: 14 September 2004 






# Caldarchaeol glycolipid

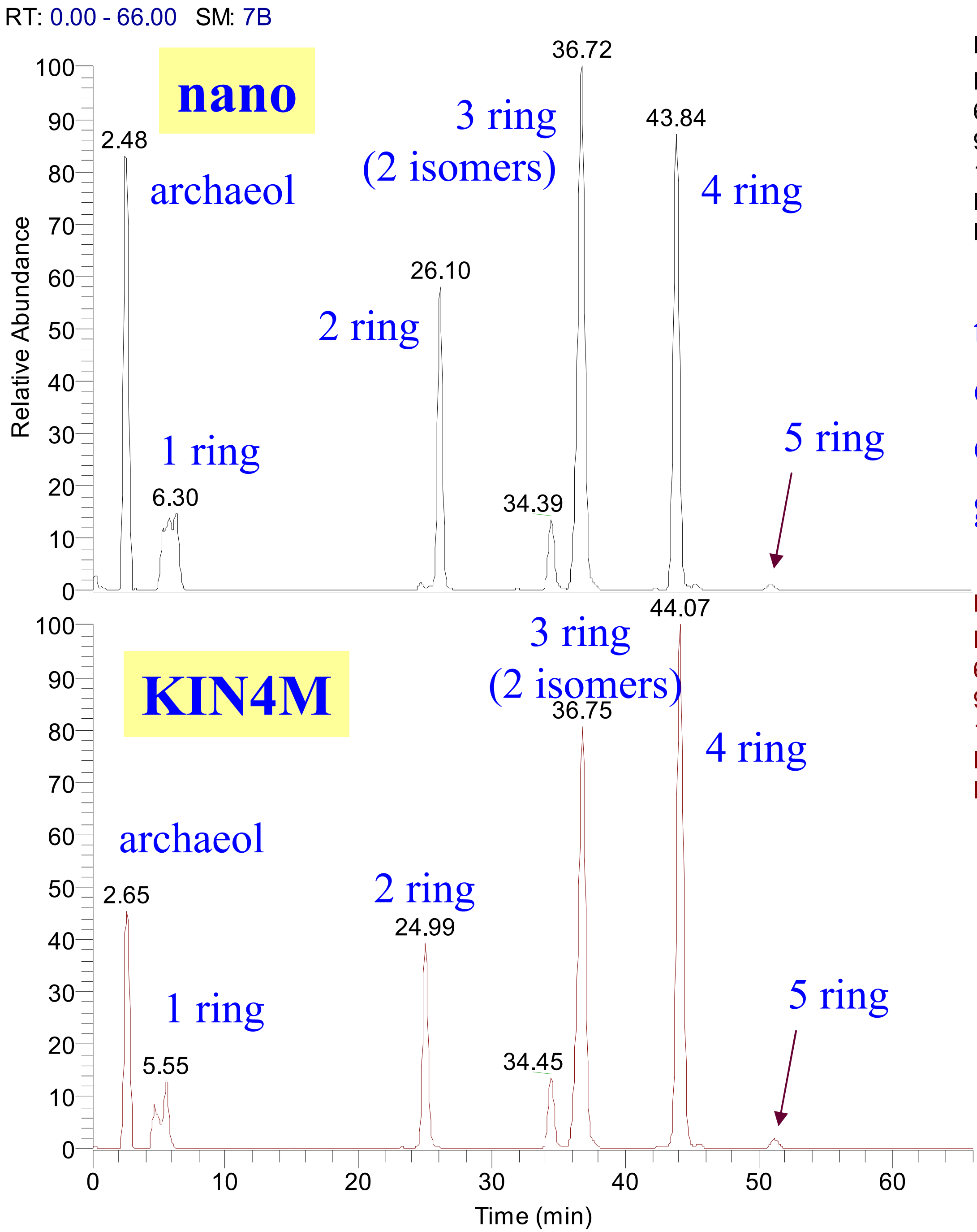
**Genome Structure**

*Nanoarchaeum* has a genome with only 490 kb, which represents the smallest archaeal genome to date. Comparing ss rRNA sequences, it was noted that sequence identities were more like archaeon than bacterial species. There was no difference, however, in the sequence identity to the Crenarchaeota, Euryarchaeota, and 'Korarchaeota', indicating it represents a new archaeal phylum.

**Cell Structure and Metabolism**

These coccus cells are only 400 nm in diameter and are covered by an S-layer. They require cell-cell contact with an actively growing *Ignicoccus* cell in order to grow.

Other nanoarchaeotal 16S rRNA genes have been obtained from the East Pacific Rise (pH 6.5), the Obsidian Pool in Yellowstone National Park (80oC, pH 6.0), and Caldera Uzon in Kamchatka, Russia (85oC, pH 5.5).




**this is an extracted ion chromatogram showing only archaeol and the glyco caldarchaeols**

positive ion mode
65 minute run
time

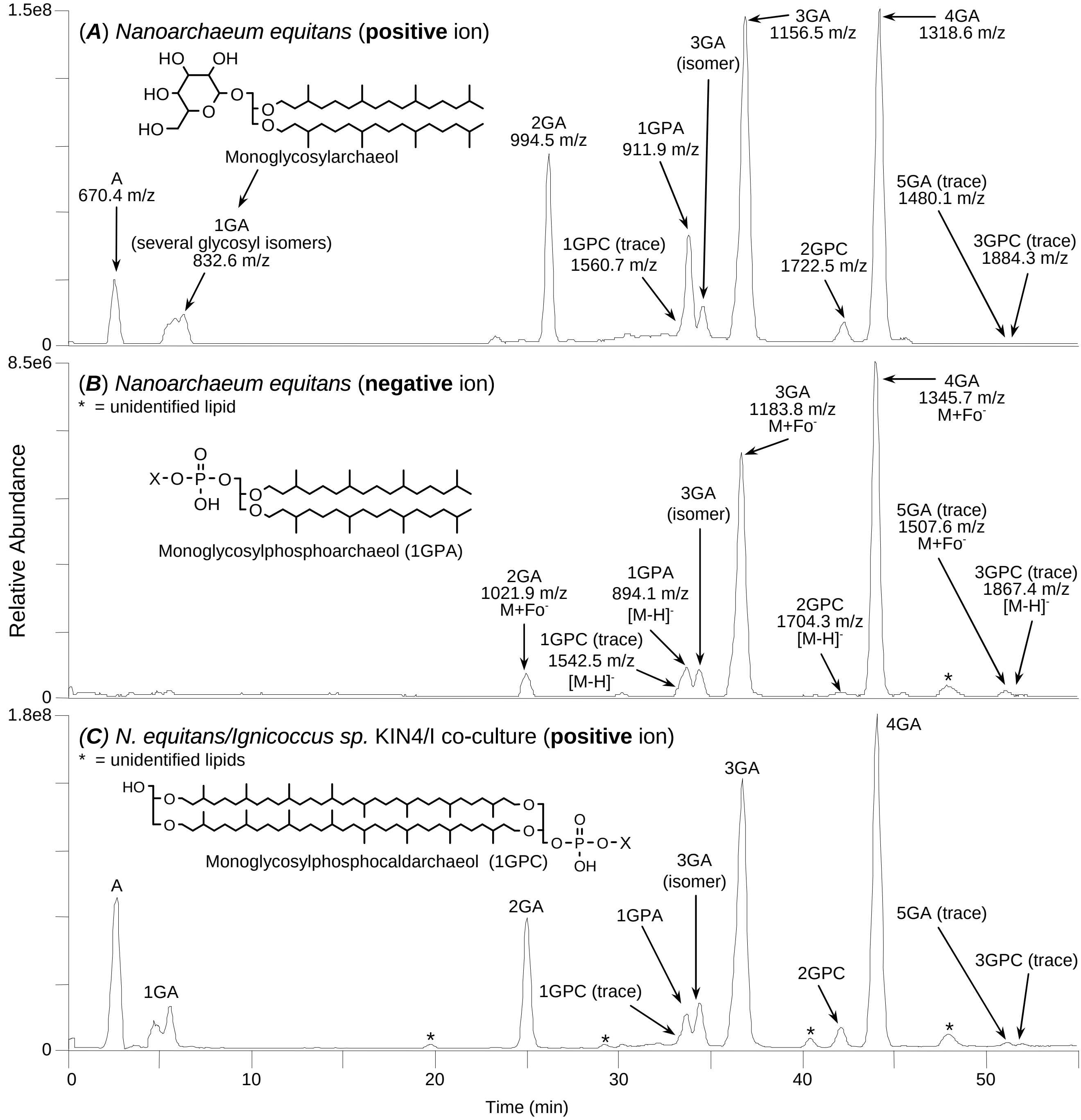



**Fig. 2** Base peak chromatograms of full-scan (650-2,000 *m/z*) mass spectra of

*N. equitans* (**a** positive ion; **b** negative ion) and a *N. equitans/ignicoccus* sp. strain KIN4/I co-culture (**c** positive ion). Identifications were made on the basis of $MS^N$ spectra in both positive and negative ion modes from LC-MS and from direct infusion of total lipid extracts. Peaks are labeled as follows: *I-5* number of glycosyl units, *G* glycolipid, *GP* phospholipid, *A* archaeol lipid core, *C* caldarchaeol lipid core and * unidentified lipid. The dominant lipids are glycolipids with an archaeol core, with smaller amounts of phospholipids which consist of a phosphate group with glycosyl headgroup and either an archaeol or caldarchaeol lipid core.

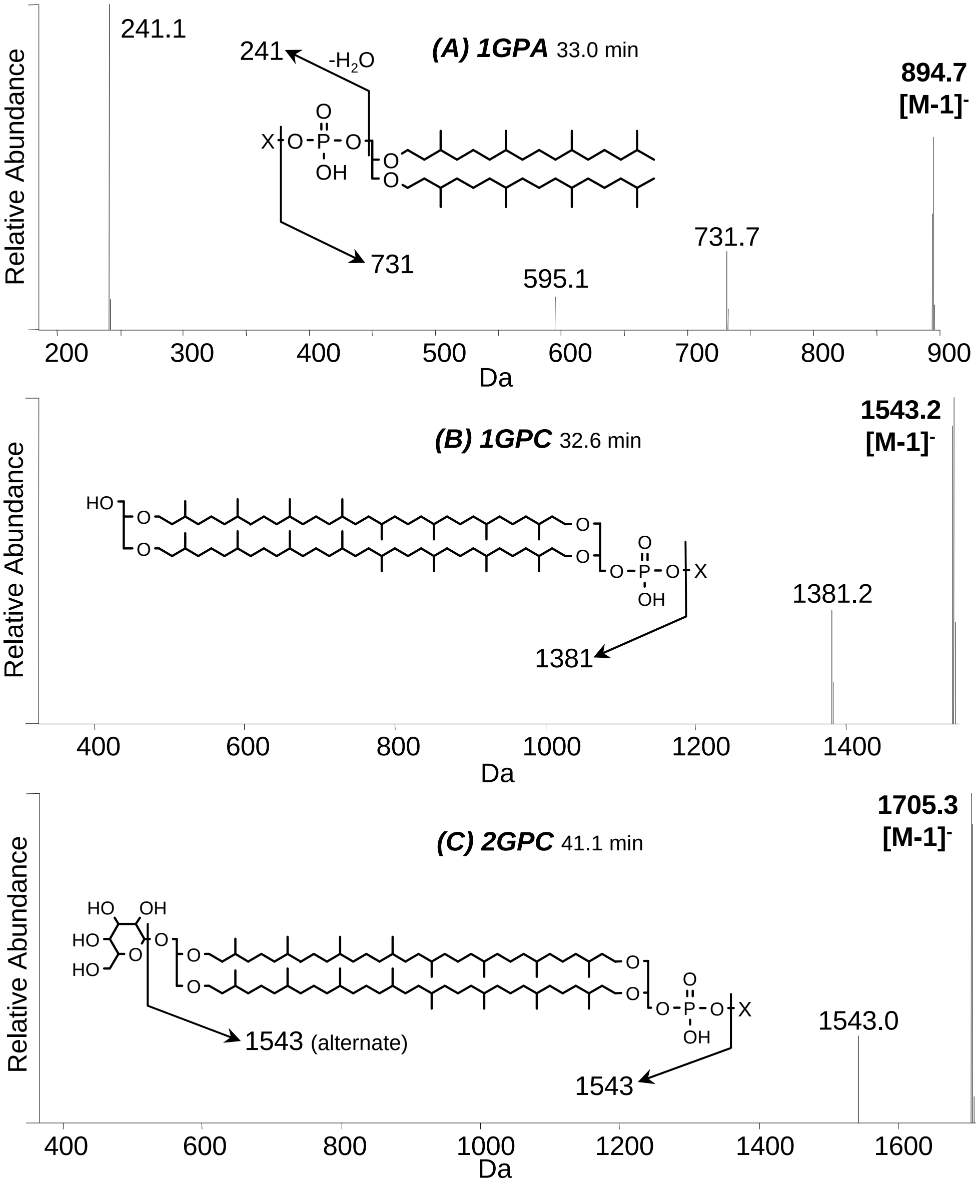



**Fig. 4** Negative ion mode MS/MS spectra of three glycosylbearing phospholipid species observed in the total lipid extracts of both *N. equitans* (shown above) and *Ignicoccus* sp. strain KIN4/I. Unlike glycolipids, these phospholipids do not form formate adducts and are directly observed as [M-H]-ions. Spectrum a is identified as a glycosylphosphoarchaeol (at 33 min in Fig. 2), since the 241-Da ion

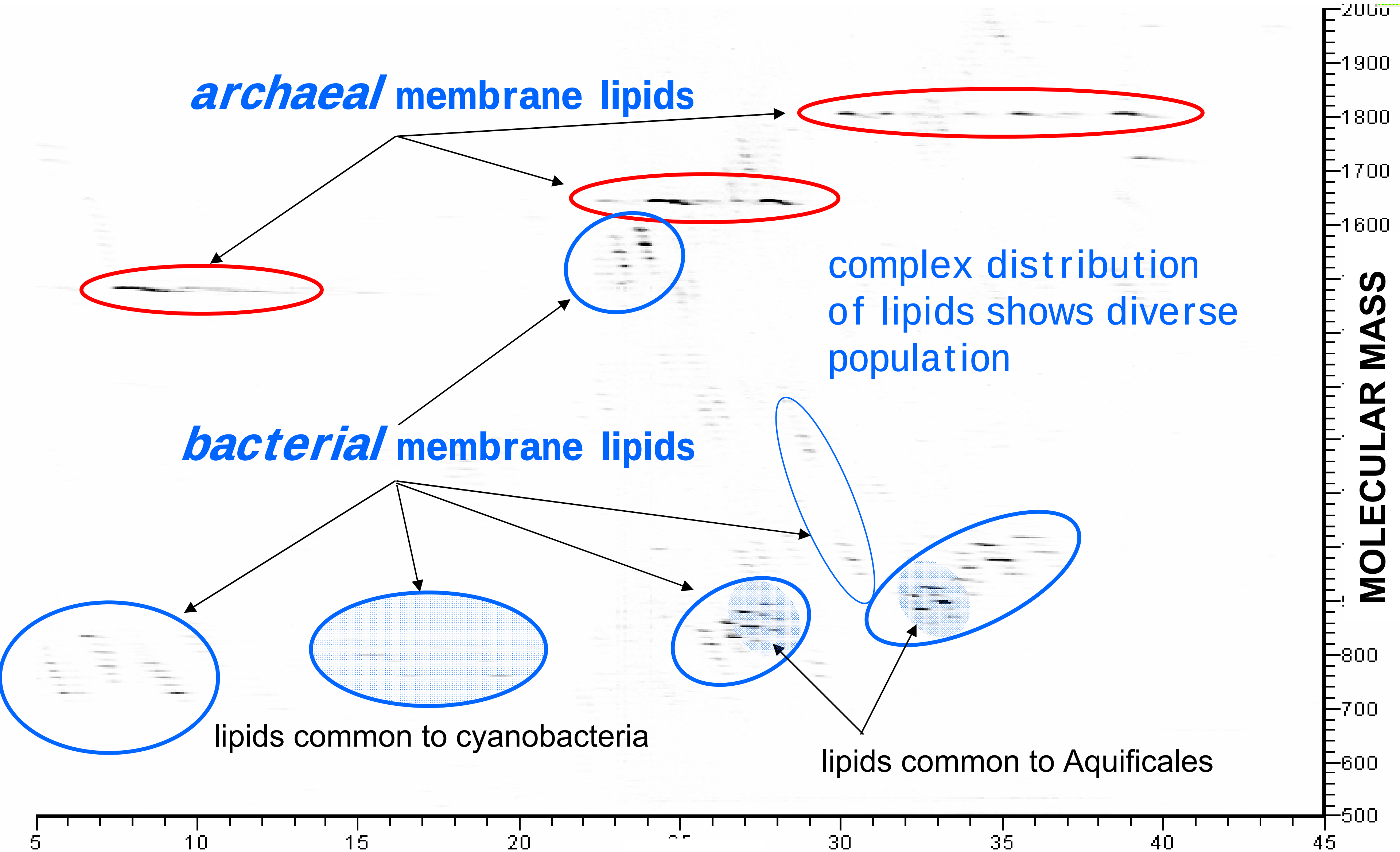
is diagnostic for phosphoinositol lipids and corresponds to the dehydrated glycophosphate headgroup. The 731-Da ion corresponds to an archaeol phosphate ion and is observed in phosphoarchaeol standards with different headgroups. The exact structure of the glycosyl unit attached to the phosphate is unknown since these $MS^N$ analyses do not give detailed structural information on the nature of the glycosyl units. Spectra **b, c** are identified as phosphoglycosyl caldarchaeols with one phosphate and one (at 32.6 min in Fig. 2) or two (at 41.1 min in Fig. 2) glycosyl groups, respectively, attached either via the phosphate group or the glycerol moiety.

# Co-culture Isotopes

| | phytane | biphytane |
|---|---|---|
| **nano** | -16.1185 | -16.0045 |
| **igni** | -16.023 | -16.297 |
| | | |
| **igni 77C** | -15.219 | -15.249 |
| **igni 90C** | -42.087 | -43.603 |
| **igni 95C** | -18.623 | -19.767 |

# Yellowstone National Park 'Ojo Caliente' hot spring

sample of biomass from a 'streamer' community




Data; Fredricks, Summons, Hinrichs

APPLIED AND ENVIRONMENTAL MICROBIOLOGY,
0099-2240/97/$04.0010 Jan. 1997, p. 50–56 Vol. 63, No. 1


# Vertical Distribution and Phylogenetic Characterization of Marine Planktonic *Archaea* in the Santa Barbara Channel

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Received 1 August 1996/Accepted 11 October 1996

**Newly described phylogenetic lineages within the domain *Archaea* have recently been found to be significant components of marine picoplankton assemblages. To better understand the ecology of these microorganisms, we investigated the relative abundance, distribution, and phylogenetic composition of *Archaea* in the Santa Barbara Channel. Significant amounts of archaeal rRNA and rDNA (genes coding for rRNA) were detected in all samples analyzed.**

# A Few Cosmopolitan Phylotypes Dominate Planktonic Archaeal Assemblages in Widely Different Oceanic Provinces

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*Institut de Cie`ncies del Mar, CSIC, 08039 Barcelona, Catalunya, Spain,*1 *and Monterey Bay Aquarium Research Institute, Moss Landing, California 95039*2
Received 4 October 1999/Accepted 2 February 2000

**We compared the phylogenetic compositions of marine planktonic archaeal populations in different marine provinces…………**

**letters to nature**
*Nature* **371**, 695 - 697 (2002); doi:10.1038/371695a0

# High abundance of Archaea in Antarctic marine picoplankton

Edward F. DeLong, Ke Ying Wu, Barbara B. Prézelin & Raffael V. M. Jovine

**ARCHAEA (archaebacteria) constitute one of the three major evolu-tionary lineages of life on Earth[13]. Previously these prokaryotes were thought to predominate in only a few unusual and disparate niches, characterized by hypersaline, extremely hot, or strictly anoxic conditions[47]. Recently, novel (uncultivated) phylotypes of Archaea have been detected in coastal[8] and subsurface[9,10] marine waters, but their abundance, distribution, physiology and ecology remain largely undescribed. Here we report exceptionally high archaeal abundance in frigid marine surface waters of Antarctica. Pelagic Archaea constituted up to 34% of the prokaryotic biomass in coastal Antarctic surface waters, and they were also abundant in a variety of other cold, pelagic marine environments. Because they can make up a significant fraction of picoplankton biomass in the vast habitats encompassed by cold and deep marine waters, these pelagic Archaea represent an unexpectedly abundant component of the Earth's biota.**

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, 0099-2240/98/$04.0010
Mar. 1998, p. 1133–1138 Vol. 64, No. 3 

# Dibiphytanyl Ether Lipids in Nonthermophilic Crenarchaeotes

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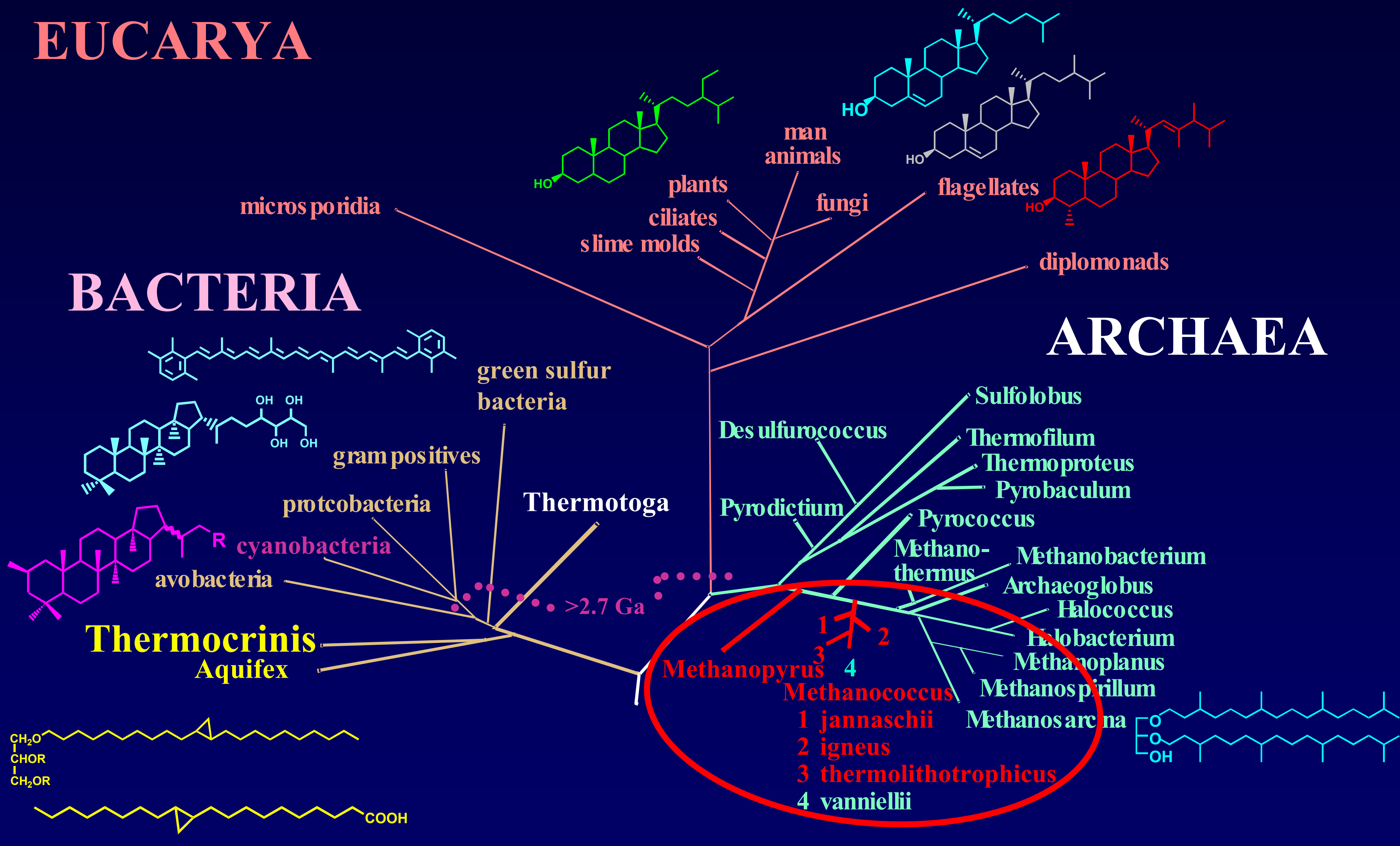
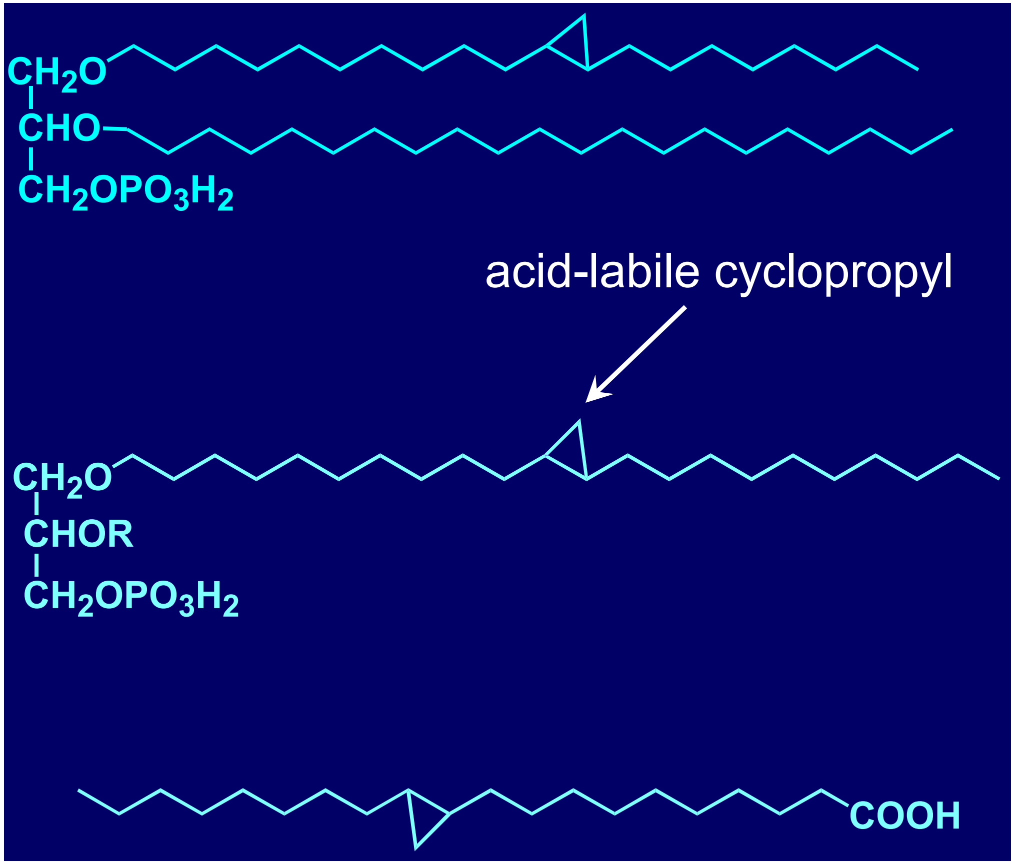
Received 30 September 1997/Accepted 23 December 1997

**The kingdom *Crenarchaeota* is now known to include archaea which inhabit a wide variety of low-temperature environments. We report here lipid analyses of nonthermophilic crenarchaeotes, which revealed the presence of cyclic and acyclic dibiphytanylglycerol tetraether lipids. Nonthermophilic crenarchaeotes appear to be a major biological source of tetraether lipids in marine planktonic environments.**

# Parallel Molecular Signatures



# Acid-labile cyclopropyl lipids from Aquificales




**Alkaline methanolysis OK for FAME but Mild Acid Hydrolysis needed to liberate ether lipids**