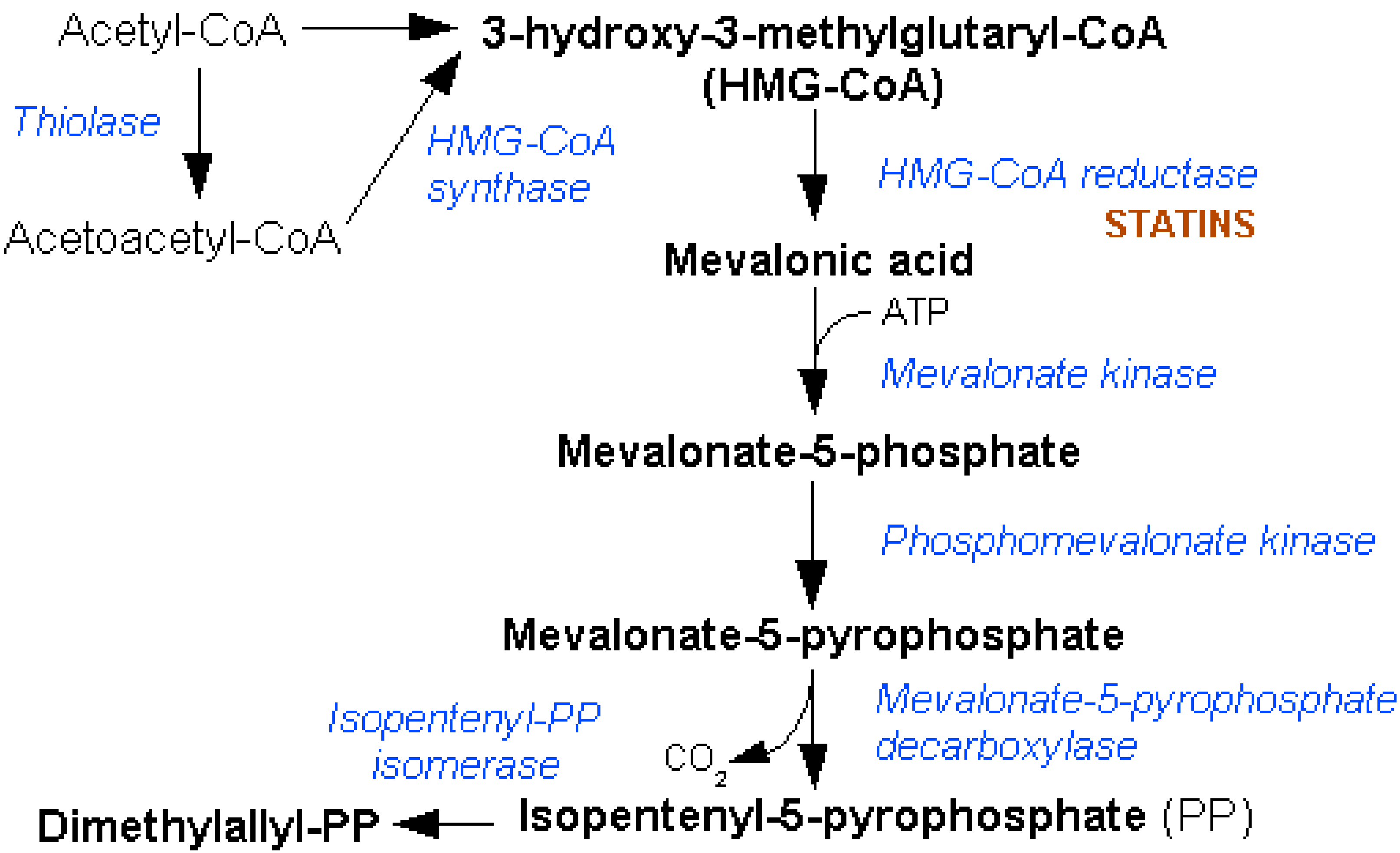
12.158 Lecture 3

- Polyisoprenoid lipids
  - Structural diversity and biosynthesis
  - Hydrocarbons
  - Complex lipids in archaea
  - Isoprenoids of plants and algae
  - Polyisoprenoids as environment and process indicators
    - Lacustrine environments – botryococcenes etc
    - Methanogenesis
    - Anaerobic oxidation of methane
  - Fossil record of Archaea

2- carbon molecule be the major building block for the complex 27- carbon, 4- ringed structure of the cholesterol molecule?

BLOCH, LYNEN, AND THE CORNFORTH / POPJAK TEAM

In the late 1930s, another young Jewish émigré from Germany, Konrad Bloch, joined Clarke‟s department as a graduate student. Bloch had already completed most of his thesis research at the University of Basel and had published two papers on that research. Still, the Basel faculty rejected it as "insufficient" (10). Bloch many years later learned that only one examiner on his committee had objected and that was on the grounds that the thesis failed to cite some important references – papers authored by that examiner! Looking back, Bloch realized that this may have been providential. Had he passes he decided to stay on in Germany. At any rate, when Bloch came to New York in 1936, Clarke, a guardian angel to refugee scientists, admitted him to his program and the Ph.D. was awarded about 2 years later. At that point, Schoenheimer offered a Bloch position in his



Acetyl-CoA
3-hydroxy-3-methylglutaryl-CoA
(HMG-CoA)
Thiolase
HMG-CoA
synthase
Acetoacetyl-CoA
HMG-CoA reductase
STATINS
Mevalonic acid
ATP
Mevalonate kinase
Mevalonate-5-phosphate
Phosphomevalonate kinase
Mevalonate-5-pyrophosphate
Isopentenyl-PP
isomerase
CO_2
Mevalonate-5-pyrophosphate
decarboxylase
Dimethylallyl-PP
Isopentenyl-5-pyrophosphate (PP)

Biochem. J. (1996) **316**, 73–80 (Printed in Great Britain)

Biosynthesis of isoprenoids (carotenoids, sterols, prenyl side-chains of chlorophylls and plastoquinone) via a novel pyruvate/glyceraldehyde 3-phosphate non-mevalonate pathway in the green alga *Scenedesmus obliquus**

Jörg SCHWENDER†||, Myriam SEEMANN‡, Hartmut K. LICHTENTHALER† and Michel ROHMER§

†Botanisches Institut II, Universität Karlsruhe, D-76128 Karlsruhe, Germany, ‡Ecole Nationale Superiéure de Chimie de Mulhouse, 3 rue Alfred Werner, F68093 Mulhouse Cedex, France, and §Université Louis Pasteur/CNRS, Institut Le Bel, 4 rue Blaise Pascal, 67070 Strasbourg Cedex, France

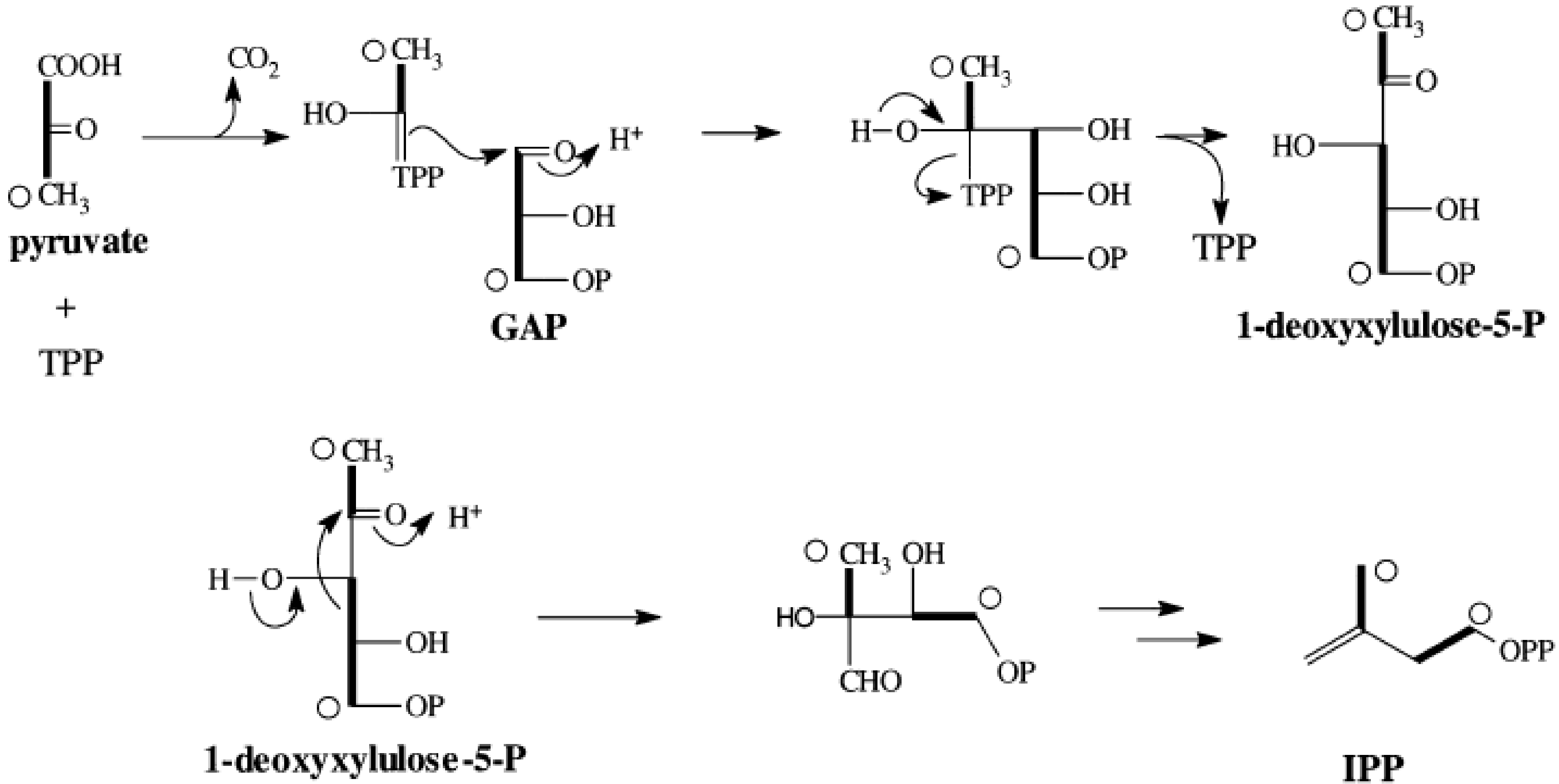
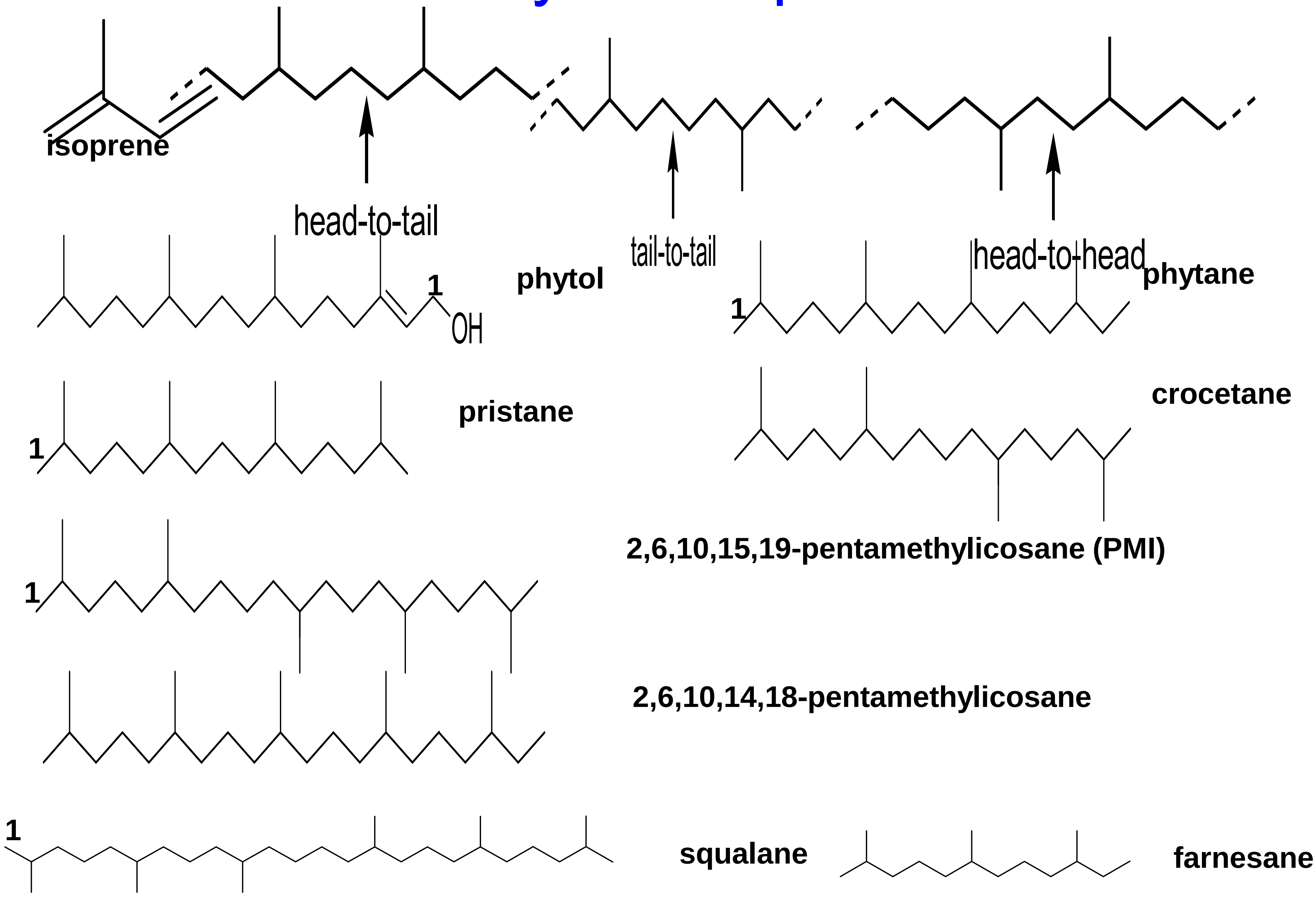


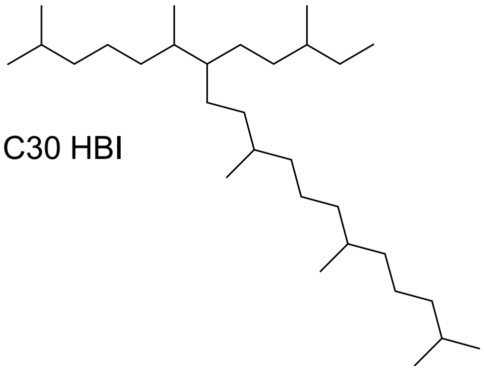
Figure 4 Hypothetical scheme for the biosynthesis of IPP from pyruvate and GAP in *Scenedesmus*

○, label from [1-^{13}C]glucose. TPP, thiamin diphosphate.

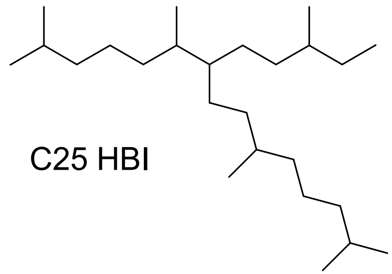
Common Acyclic Isoprenoids



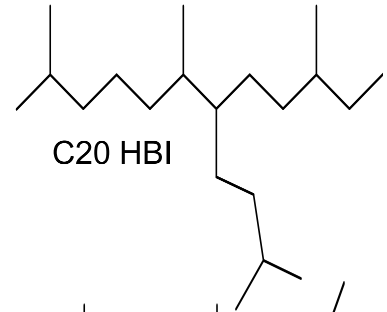
Less Common Acyclic Isoprenoids



C30 HBI

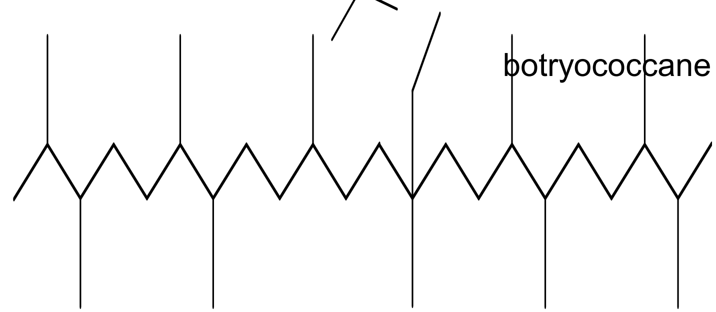


C25 HBI



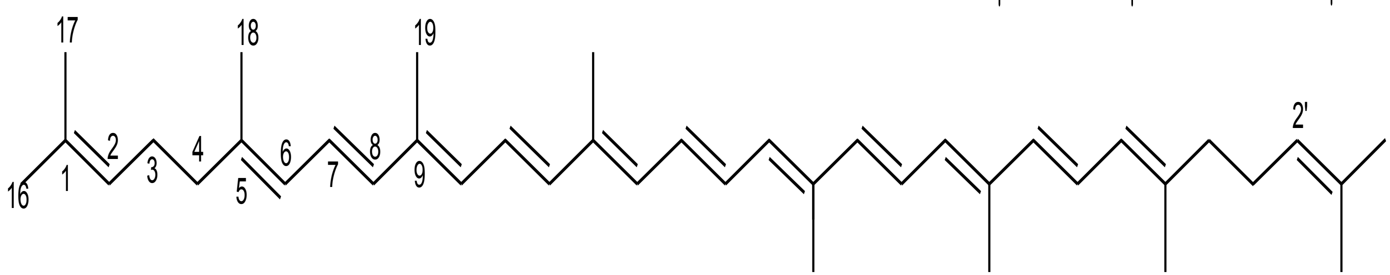
C20 HBI

Diatom sources



botryococcane

Botryococcus braunii

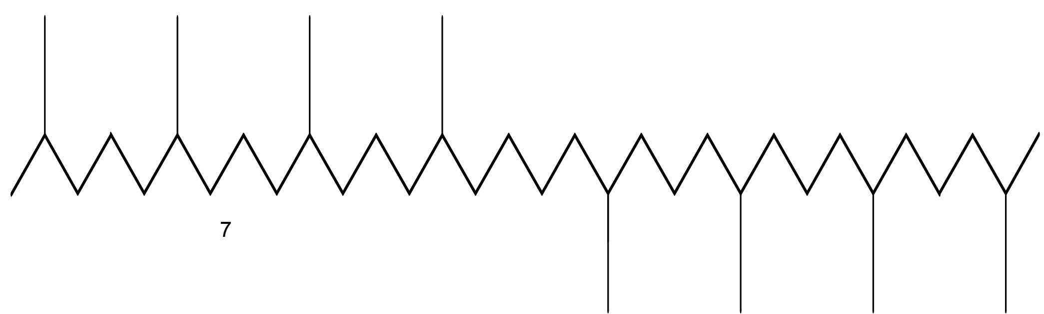


lycopene

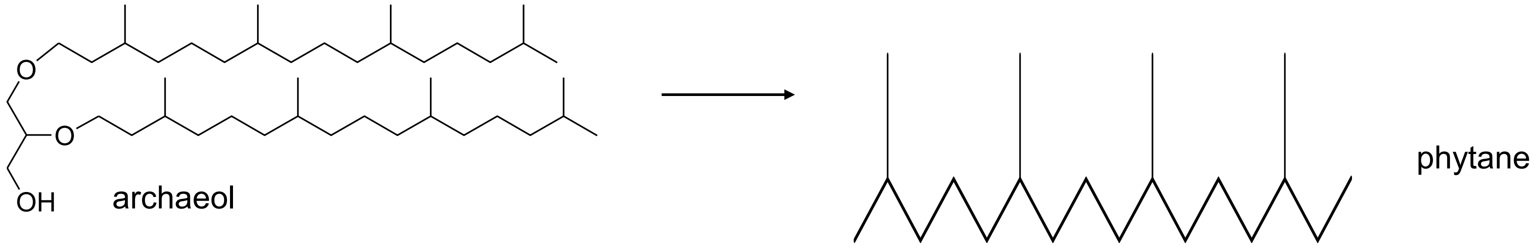
tomato carotenoid

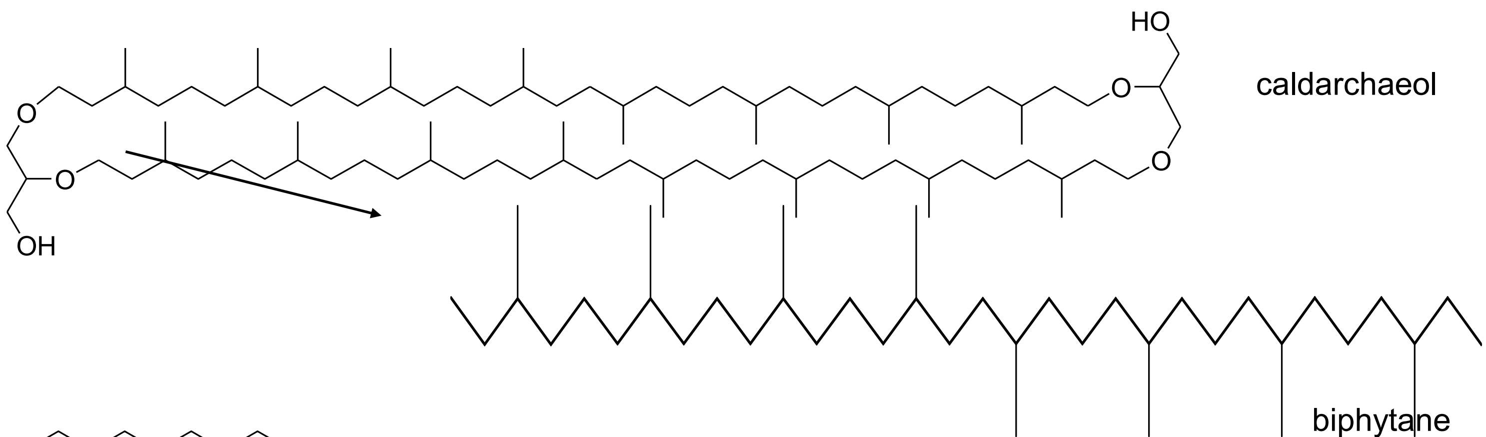
Probable algal hydrocarbon
? from lycapodiene

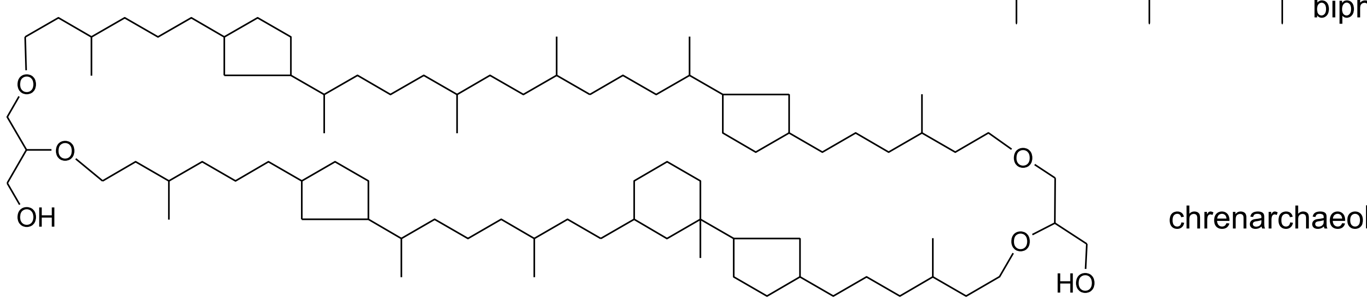
lycopane

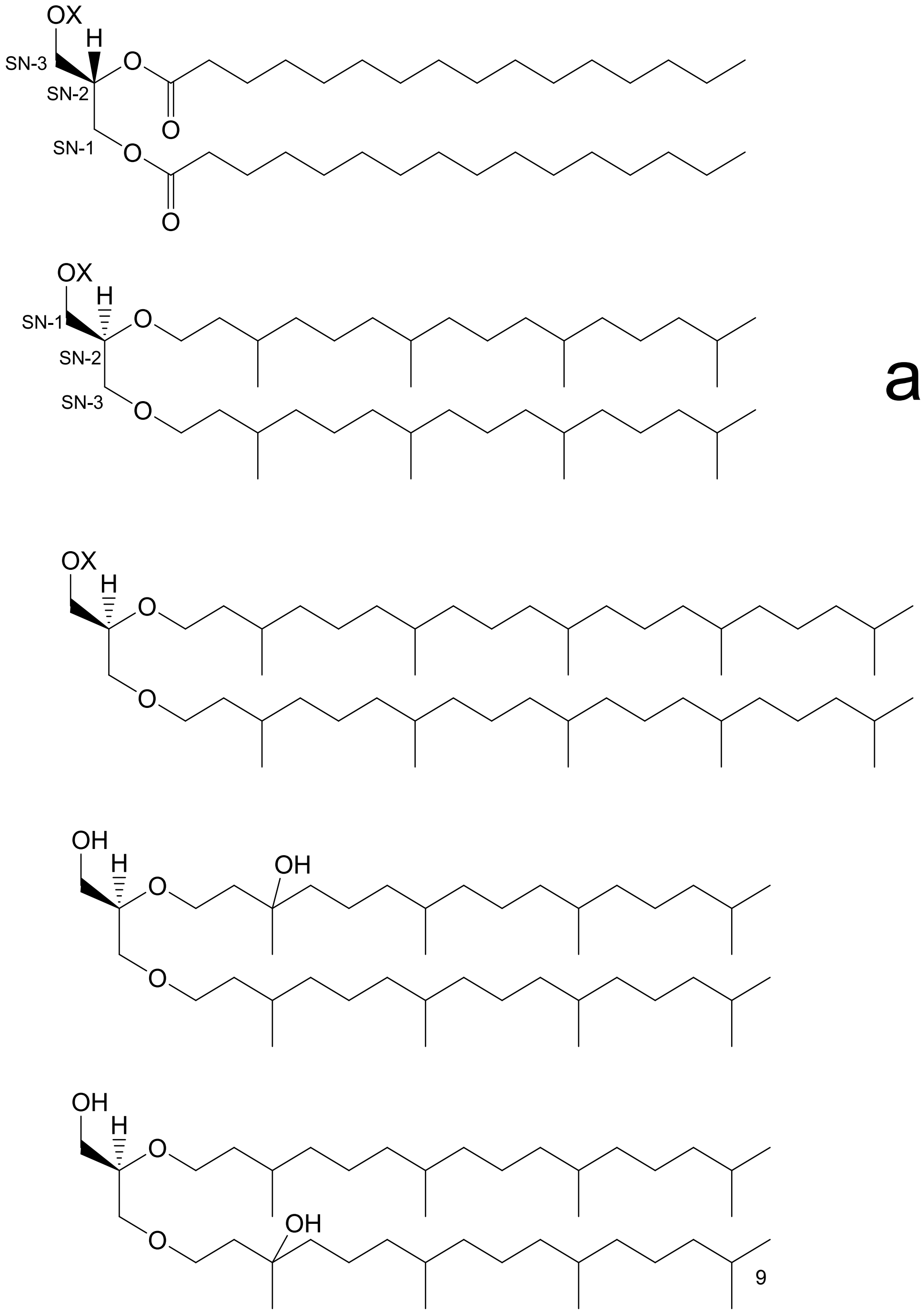


Polar Lipid Precursors of Acyclic Isoprenoids

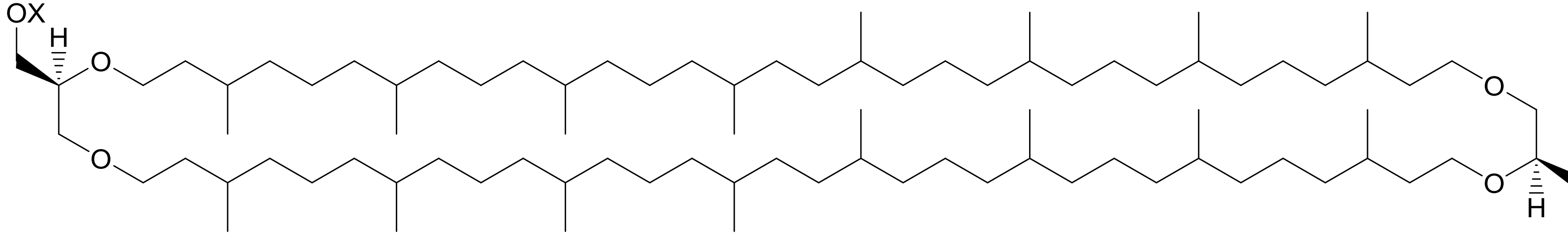




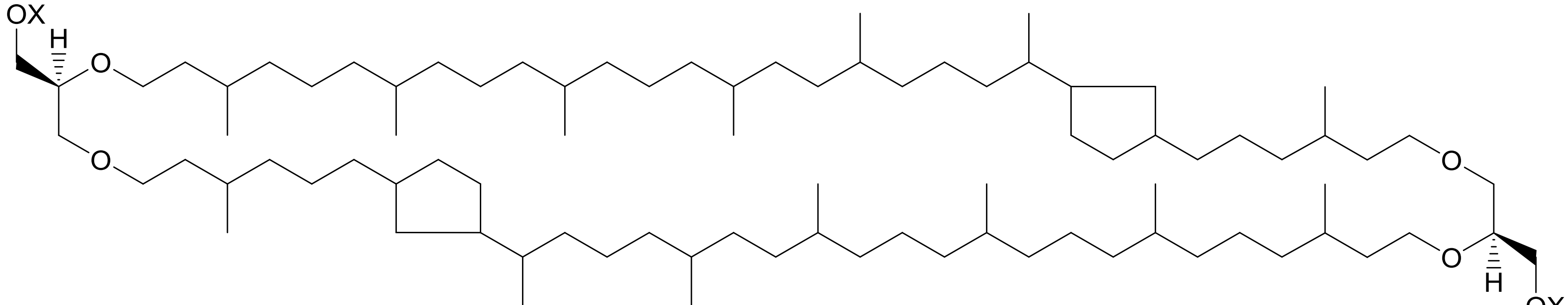




Stereochemistry of archaeal and bacterial lipids



33



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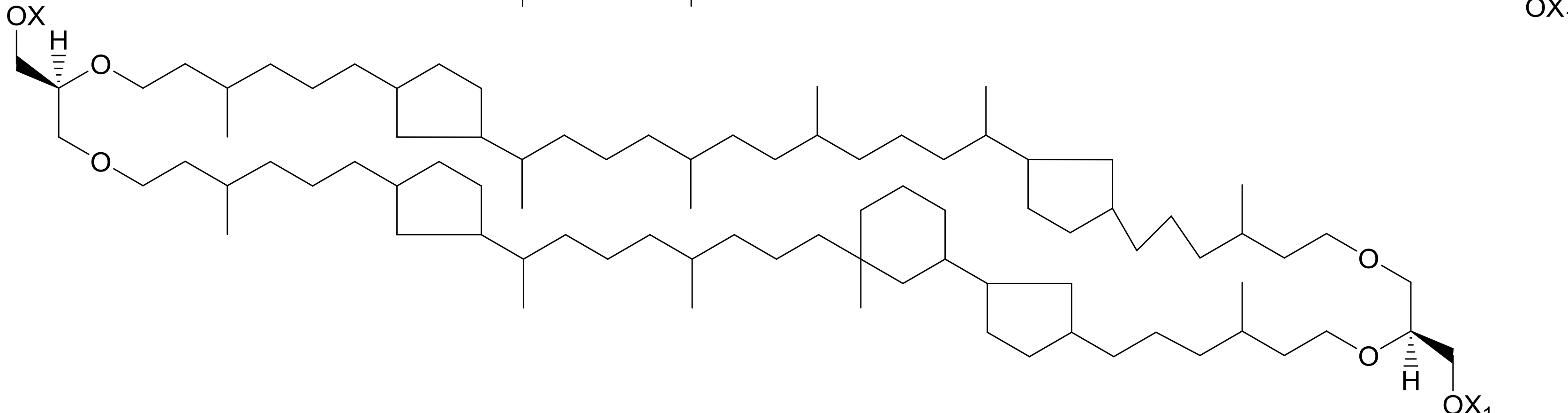

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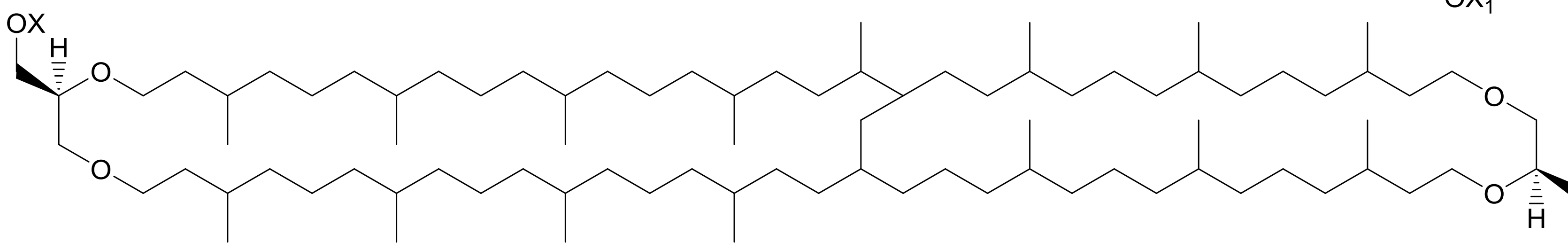

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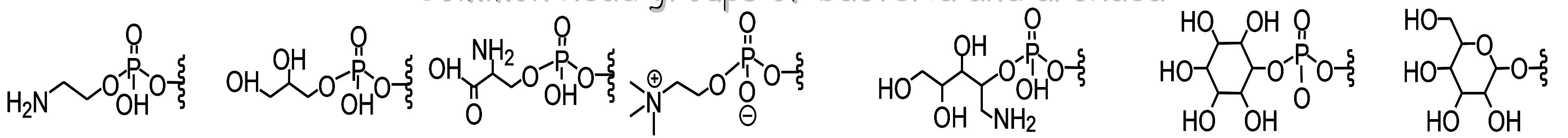
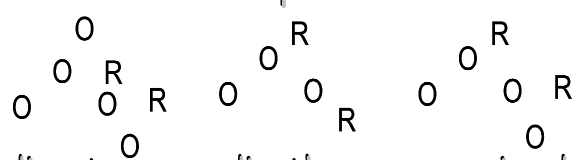
Polar Lipid Precursors of Acyclic Isoprenoids

Common head groups of bacteria and archaea



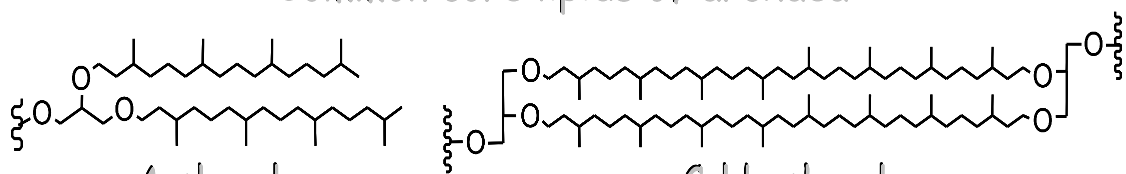
ethanolamine (PE) glycerol (PG) serine (PS) choline (PC) aminopentanetetrol (APT) inositol (PI) hexose (archaea)

Common core lipids of bacteria



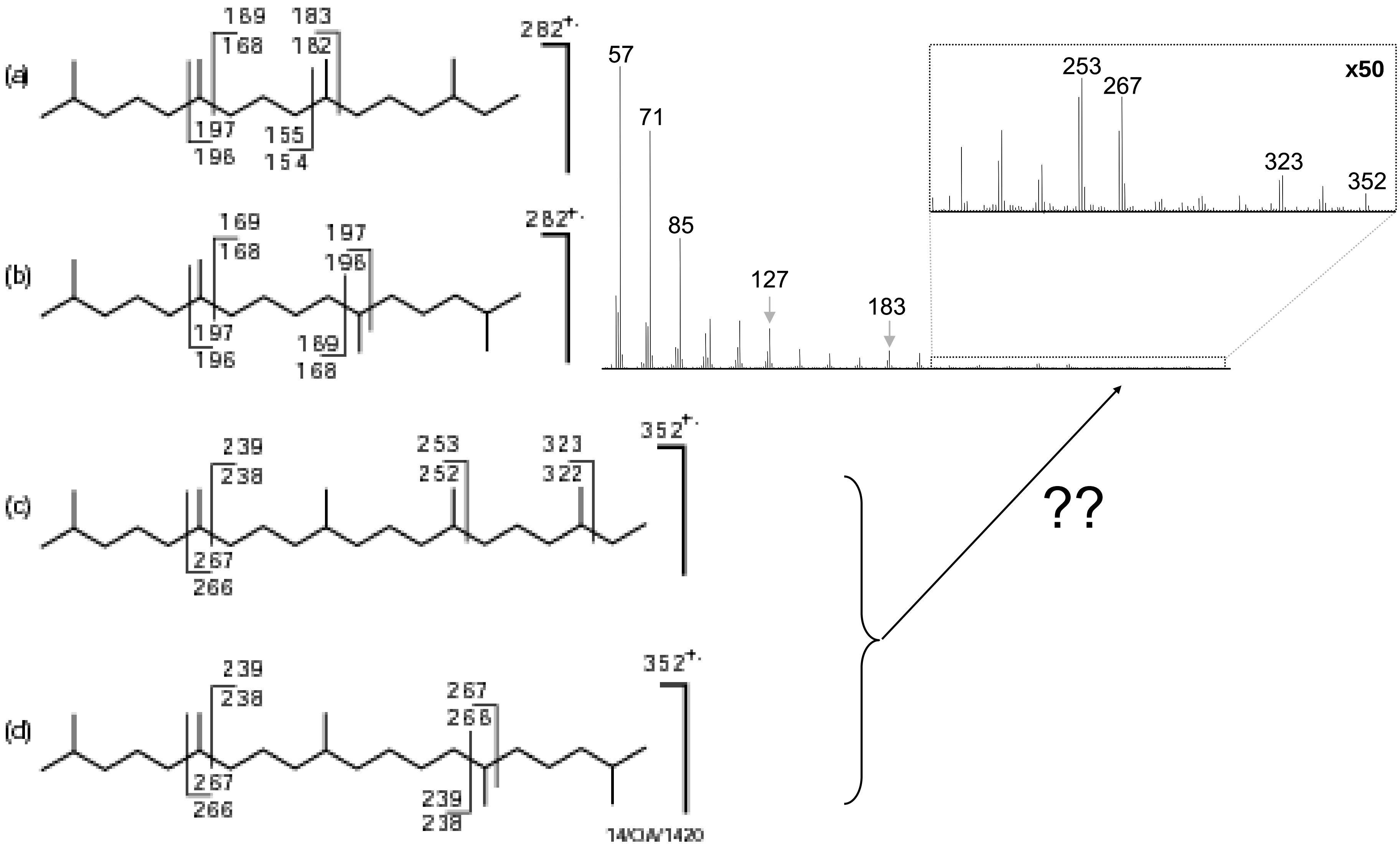
di-ester di-ether mixed

Common core lipids of archaea

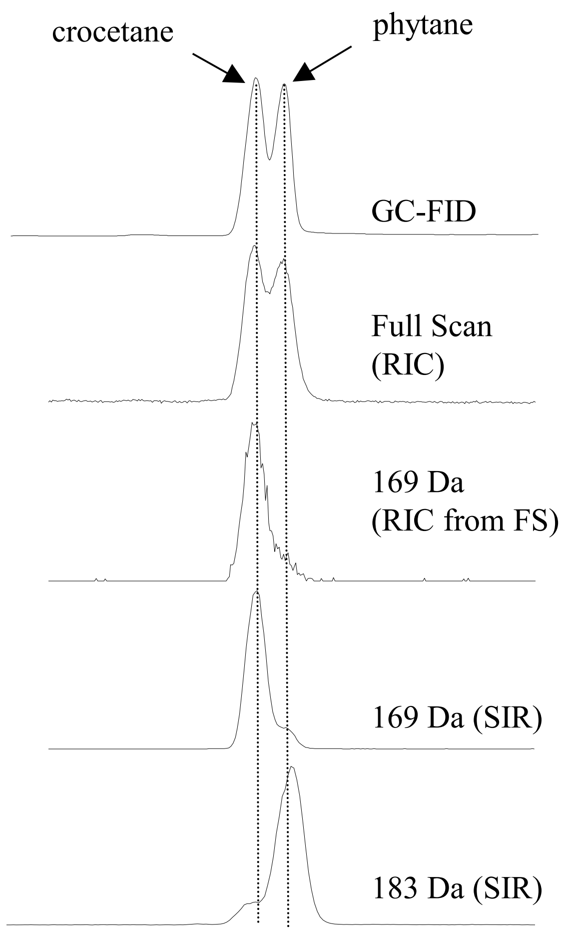


Archaeol Caldarchaeol

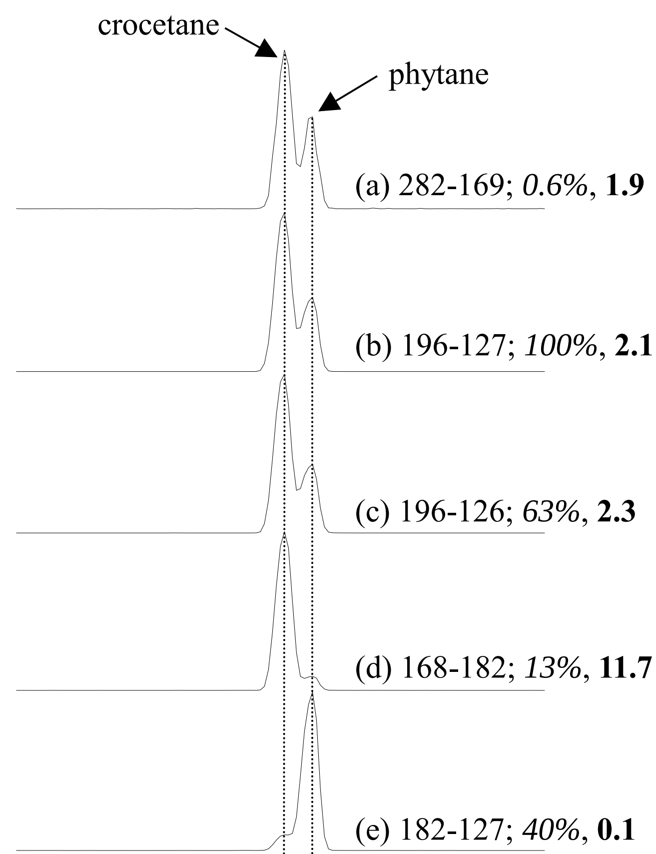
Favored Mass Spectrometric Fragmentations



Crocetane – Phytane Distinction

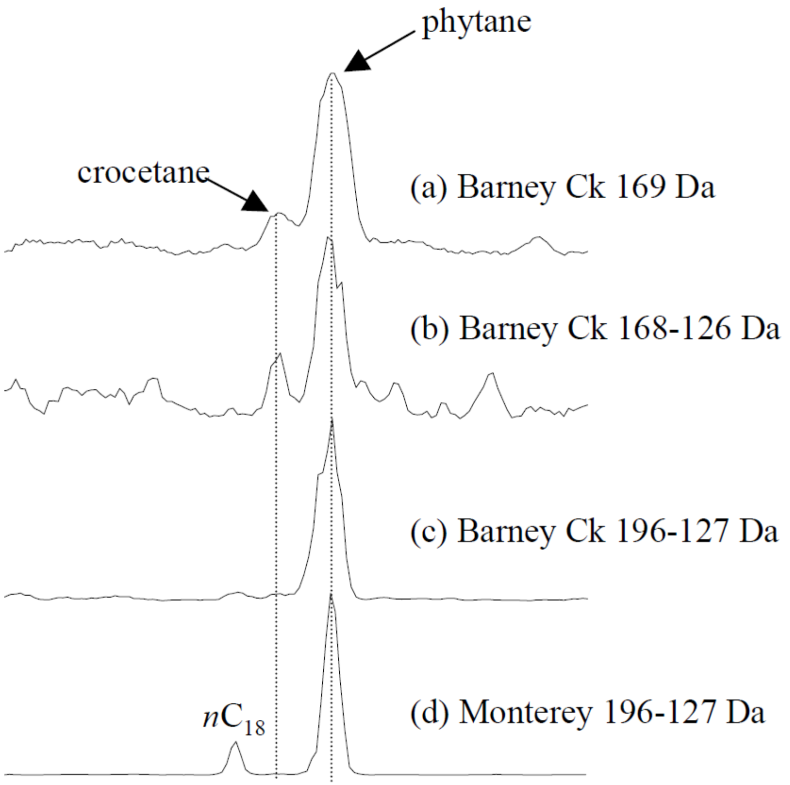


GC and GC-MS (SIR)

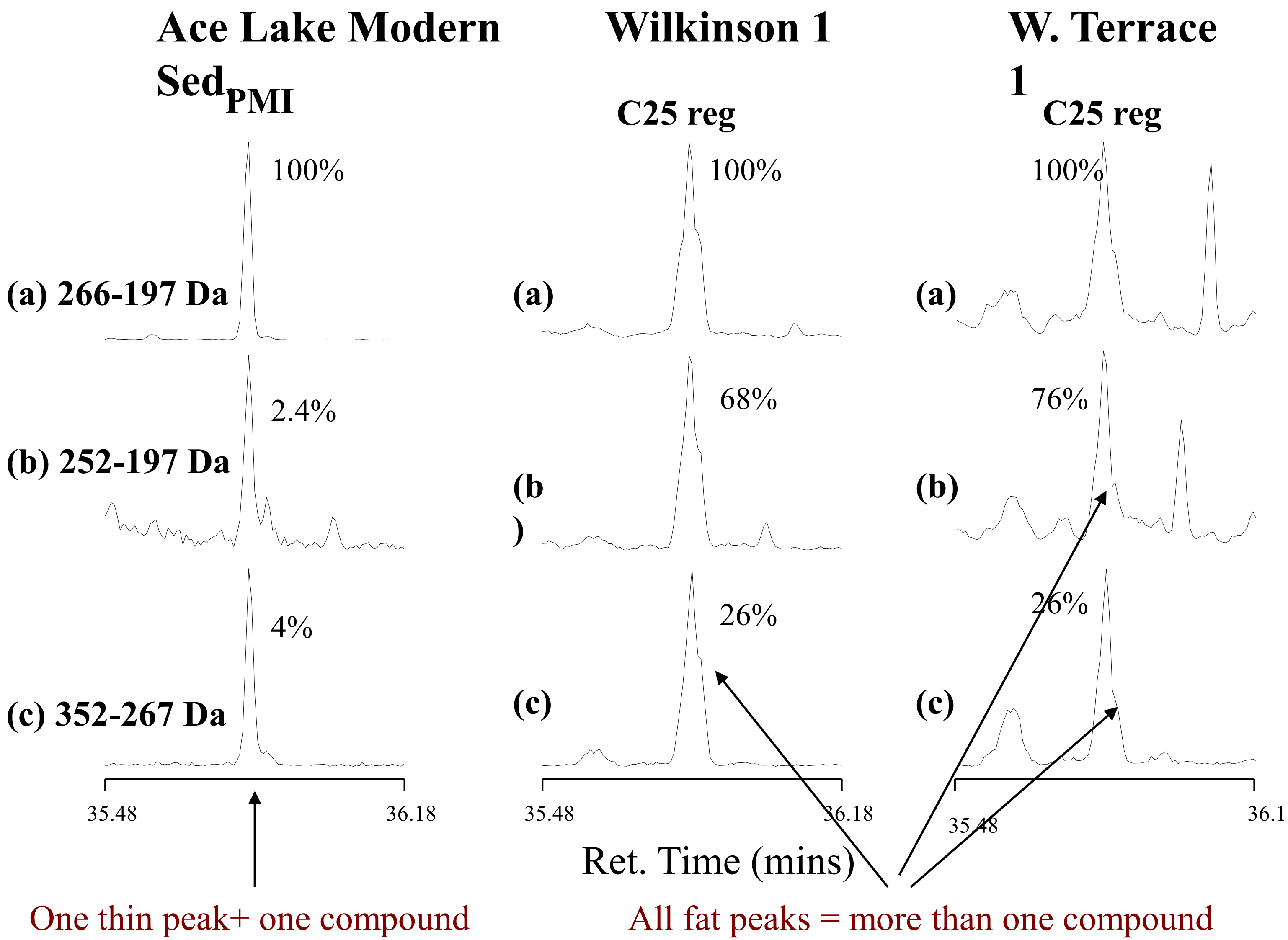


GC-MS-MS

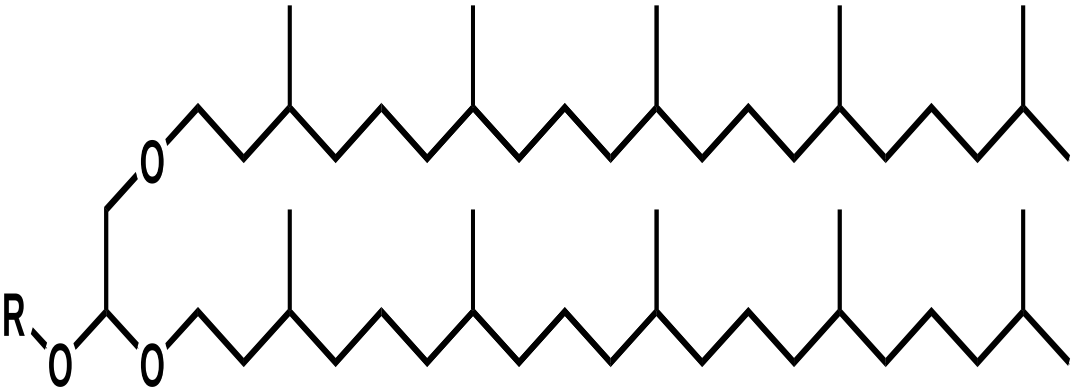
Crocetane – Phytane Distinction



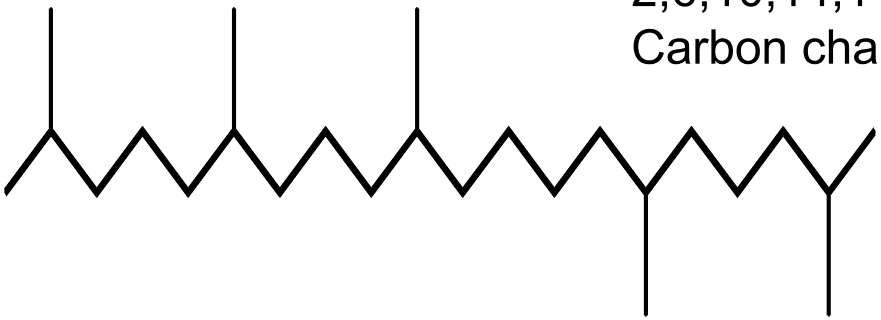
Regular C_{25} vs PMI Distinction



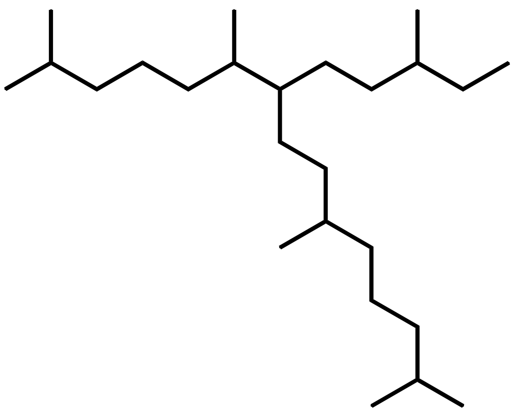
Regular C_{25} vs PMI & HBI Distinction



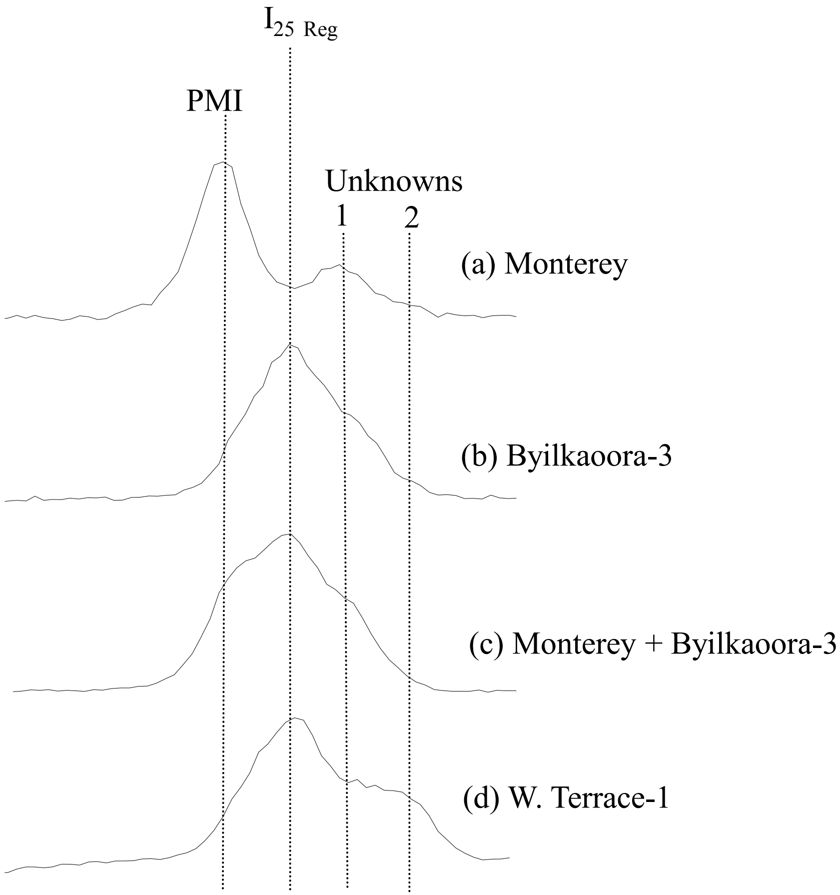
2,6,10,14,14-pentamethylicosane
Carbon chains of *Halobacterium* core lipid



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



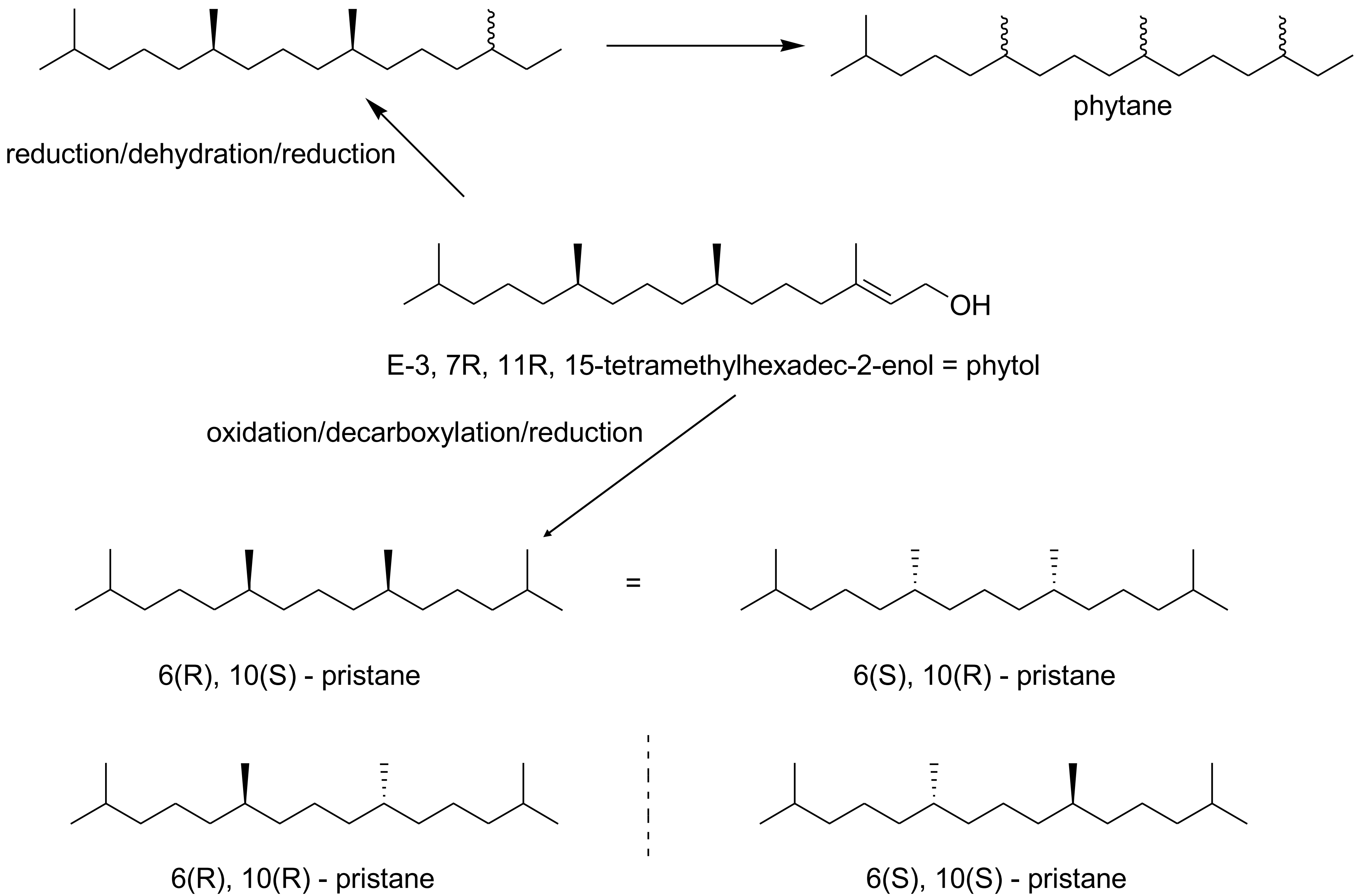
A „highly branched isoprenoid" (HBI)
from a diatom



Distinguishing C25 Isoprenoids

note peak shapes

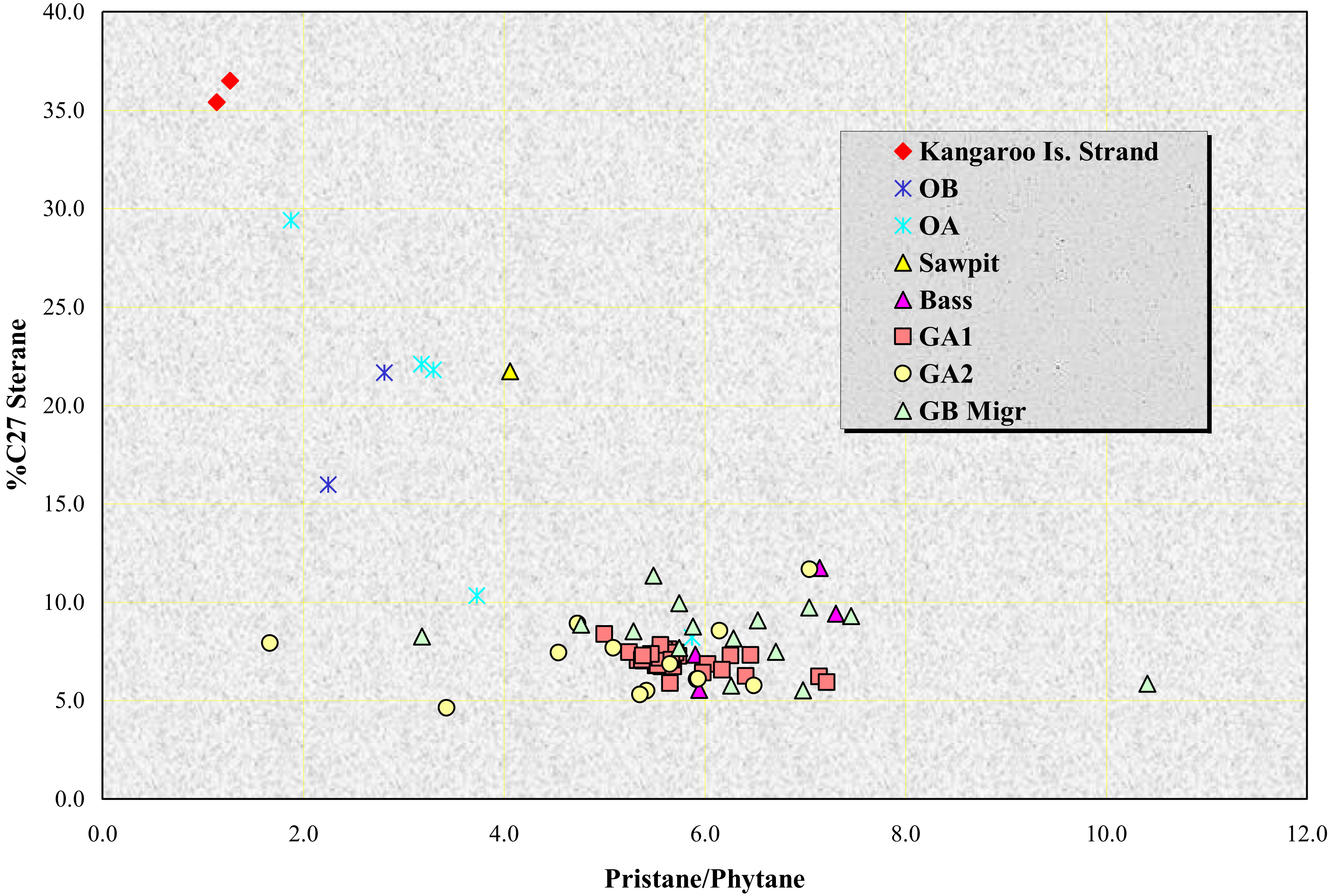
Partial 183 Da (SIR) chromatograms of (a) Monterey Formation showing elution position of PMI; (b) Byilkaoora-3 showing elution position of I25 reg; (c) Monterey + Byilkaoora-3 mixture showing relative elution order of PMI and I25 reg isomers (NB. only partially resolved); (d) West Terrace-1 which has 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.

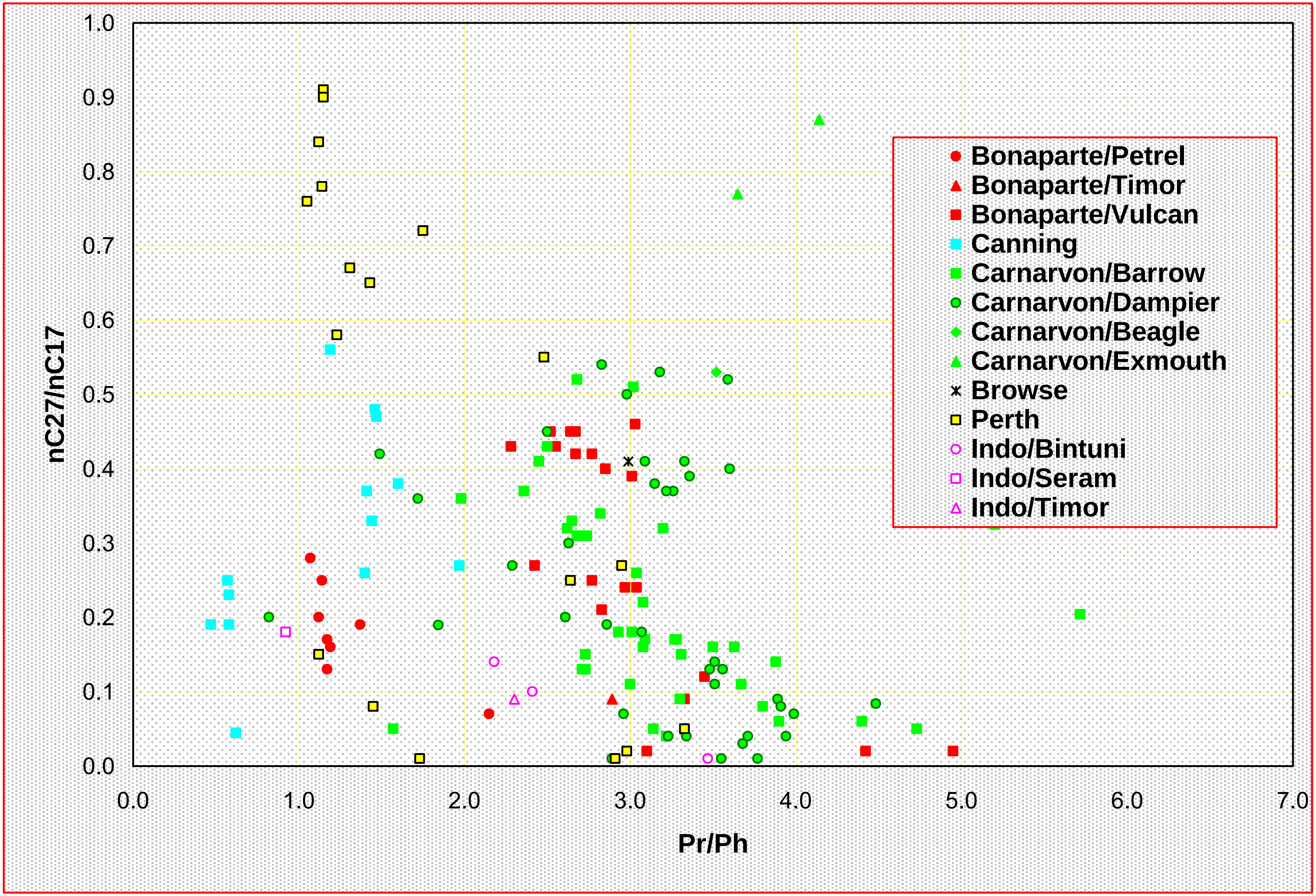

phytane
reduction/dehydration/reduction
OH
E-3, 7R, 11R, 15-tetramethylhexadec-2-enol = phytol
oxidation/decarboxylation/reduction
=
6(R), 10(S) - pristane
6(S), 10(R) - pristane
6(R), 10(R) - pristane
6(S), 10(S) - pristane

Pristane to Phytane Ratio Pr/Ph

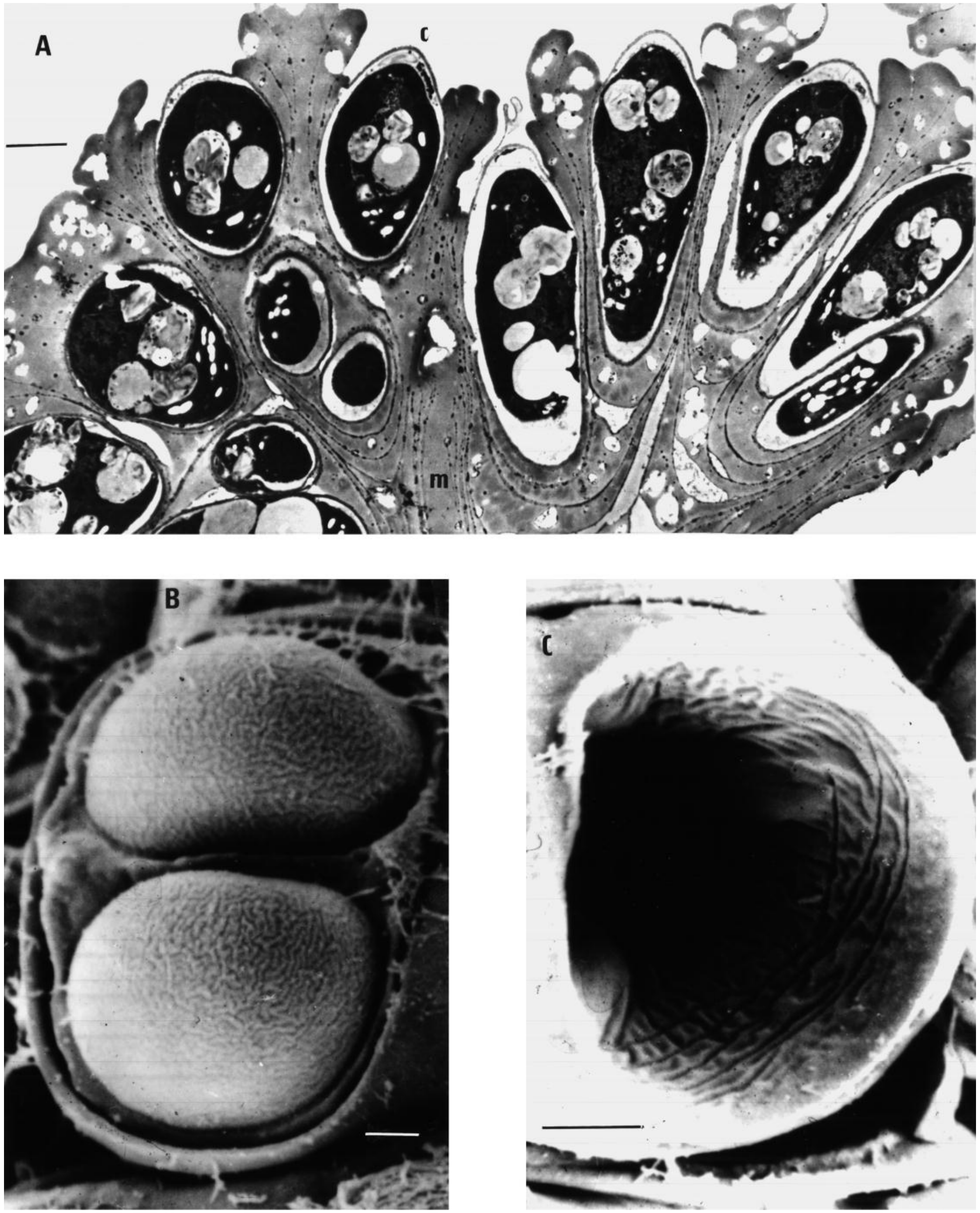
- An empirical parameter that was originally used to classify Australian oils; high in oils from land plant OM (Powell & McKirdy, 1973)
- Empirical correlation with depositional environment (Didyk et al., 1978)
  - <1 → strongly reducing or evaporitic environments (correlates with Gammacerane)
  - 1-4 reducing marine and lacustrine environments
  - >4 terrestrial aquatic environments

    $\delta^{13}C$ of Pr and Ph generally similar
- Pr/Ph probably reflects redox control on diagenesis of phytol


40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
%C27 Sterane
0.0
2.0
4.0
6.0
8.0
10.0
12.0
Pristane/Phytane
Kangaroo Is. Strand
OB
OA
Sawpit
Bass
GA1
GA2
GB Migr

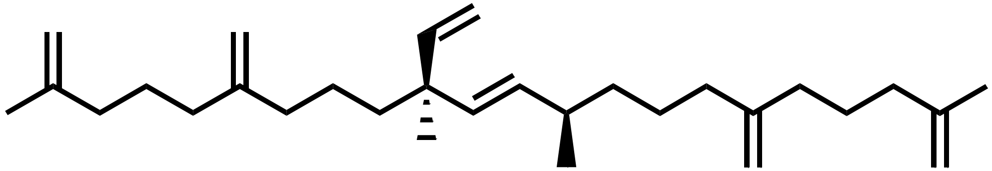


nC27/nC17
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0.0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
Pr/Ph
Bonaparte/Petrel
Bonaparte/Timor
Bonaparte/Vulcan
Canning
Carnarvon/Barrow
Carnarvon/Dampier
Carnarvon/Beagle
Carnarvon/Exmouth
Browse
Perth
Indo/Bintuni
Indo/Seram
Indo/Timor

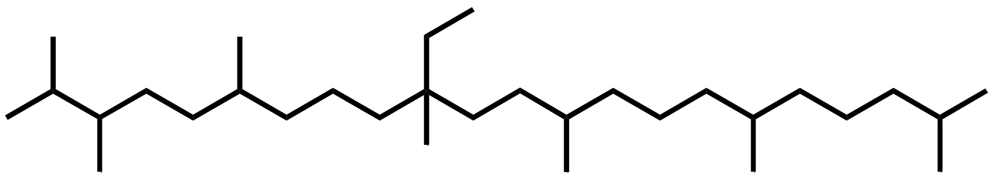


Botryococcus braunii isoprenoids

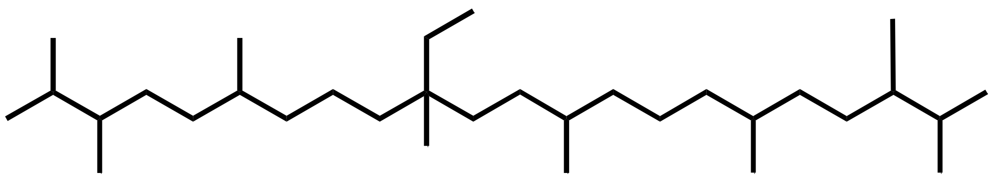
C_{30} Botryococcene C_{31}-C_{33} Botryoccanes



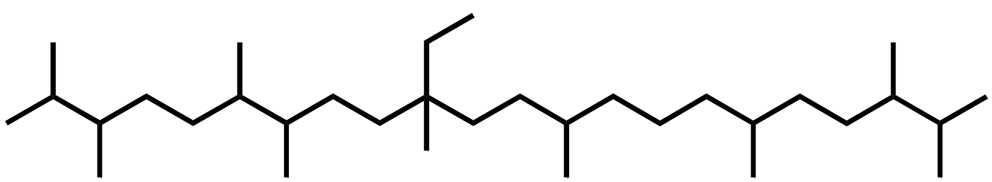
C$_{30}$



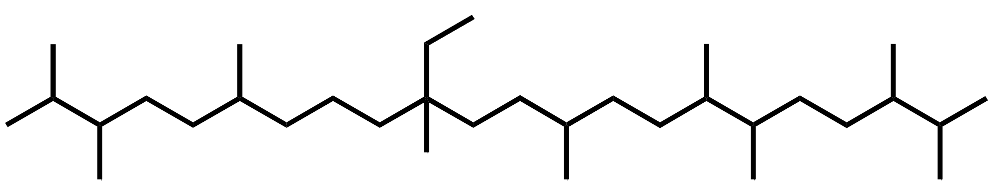
C$_{31}$



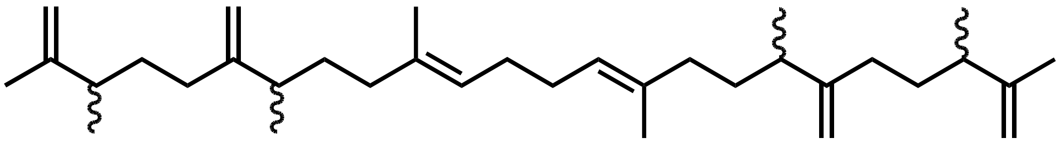
C$_{32}$



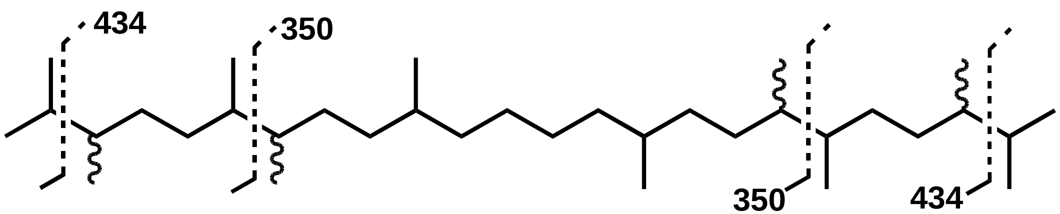
C$_{33}$



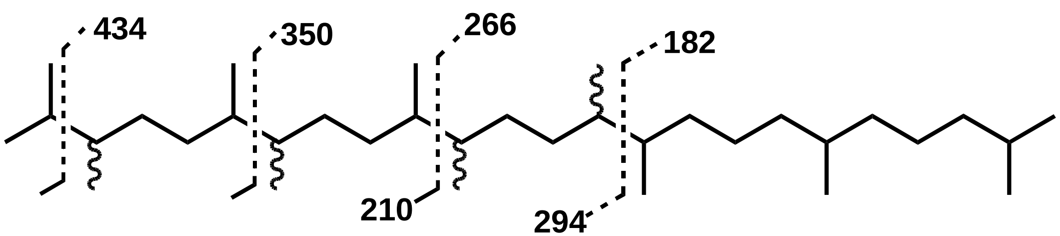
C$_{33}$



some cultured *B braunii* strains



lake sediments (Maoming) and
Oils (Duri of Sumatra)



lake sediments (Maniguin) and
Oils (Minas and Duri of Sumatra)

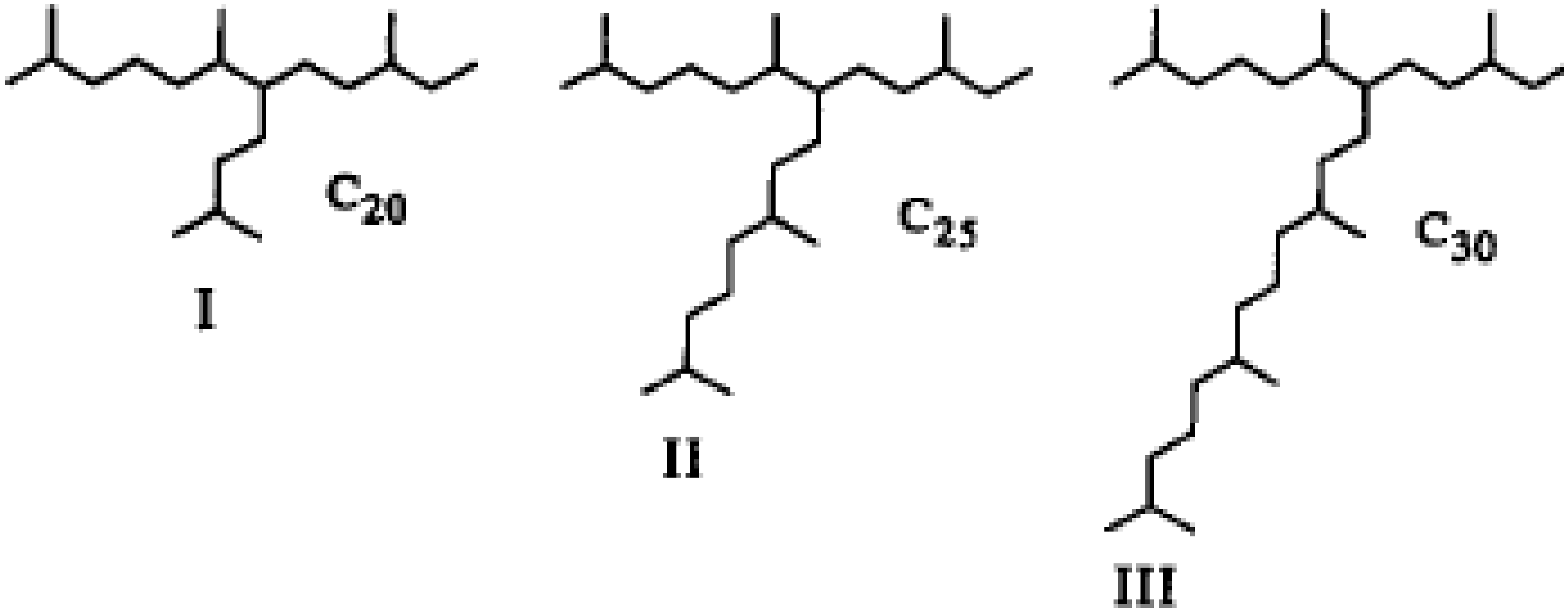
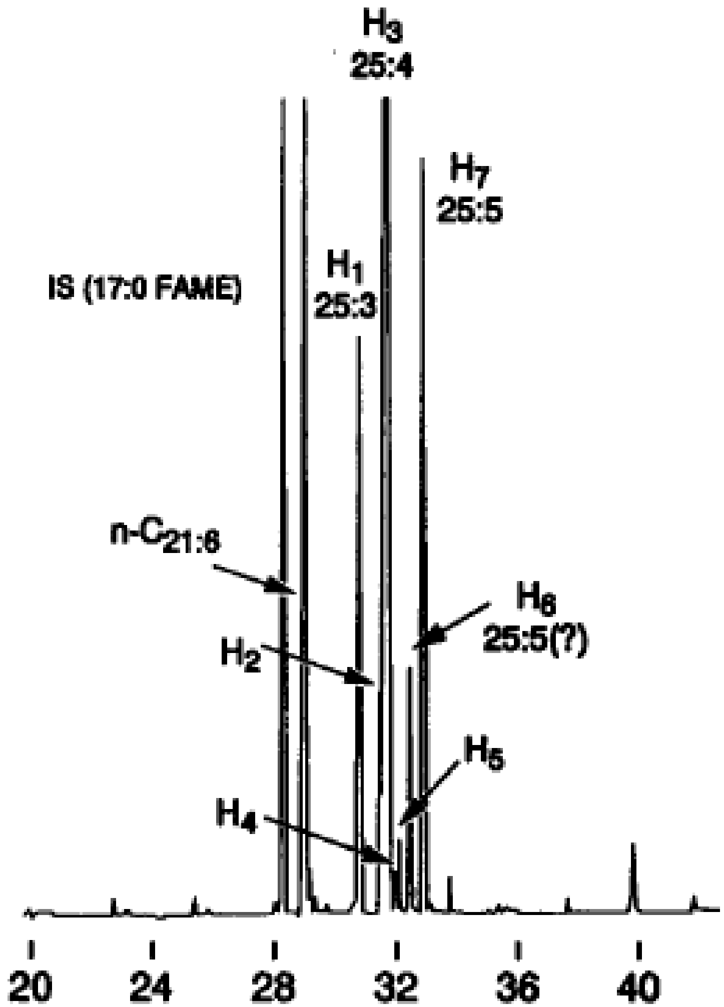
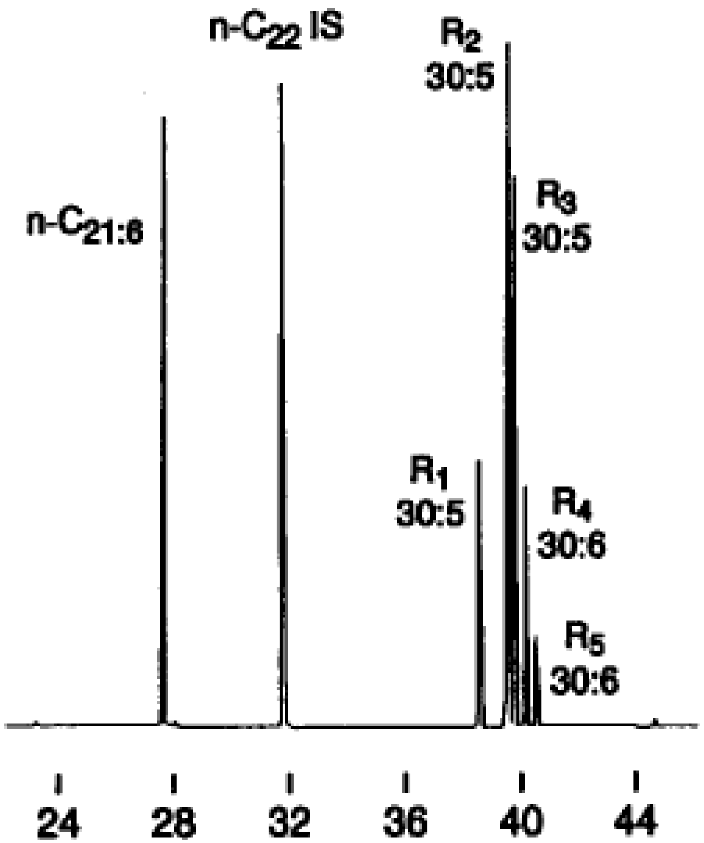


Fig. 1. Structures of the parent C_{20}, C_{25} and C_{30} carbon skeletons. The C_{20} alkane is a major constituent of the hydrocarbons isolated from Rozel Point crude oil. Its structure was elucidated by Yon *et al.* (1982). In marine sediments and seawater, the C_{25} and C_{30} hydrocarbons mainly occur as highly unsaturated alkenes.


H3
25:4
H7
25:5
H1
25:3
IS (17:0 FAME)
n-C21:6
H6
25:5(?)
H2
H5
H4
20
24
28
32
36
40


n-C22 IS
R2
30:5
n-C21:6
R3
30:5
R1
30:5
R4
30:6
R5
30:6
24
28
32
36
40
44

Science 23 April 2004:
Vol. 304. no. 5670, pp. 584 – 587

The Rise of the Rhizosolenid Diatoms

Jaap S. Sinninghe Damsté,[1*] Gerard Muyzer,[1,2] Ben Abbas,[1] Sebastiaan W. Rampen,[1] Guillaume Massé,[3] W. Guy Allard,[3] Simon T. Belt,[3] Jean-Michel Robert,[4] Steven J. Rowland,[3] J. Michael Moldowan,[5] Silvana M. Barbanti,[5,6] Frederick J. Fago,[5] Peter Denisevich,[5] Jeremy Dahl,[5] Luiz A. F. Trindade,[6] Stefan Schouten[1]

The 18S ribosomal DNA molecular phylogeny and lipid composition of over 120 marine diatoms showed that the capability to biosynthesize highly branched isoprenoid (HBI) alkenes is restricted to two specific phylogenetic clusters, which independently evolved in centric and pennate diatoms.

This image has been removed due to copyright restrictions. Please see Figure 1 on http://www.sciencemag.org/cgi/content/full/304/5670/584.

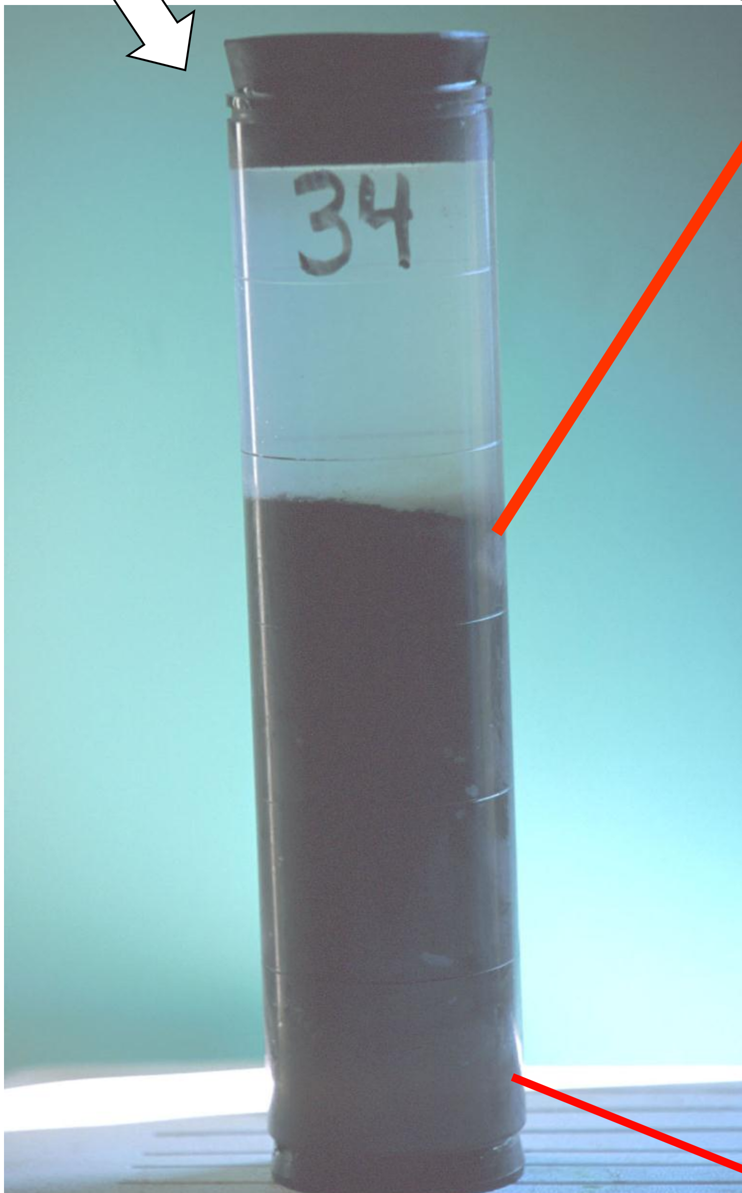
Fig. 1. Neighbor-joining phylogenetic tree based on nearly complete 18S rRNA sequences of diatoms. Some of the sequences were published before (5); 86 others (see table S1 for details) were determined in this study. The sequences of Coccoid haptophyte and Emiliania huxleyi were used as outgroups but were pruned from the tree. Bolidomonas mediterranea is a sister group of the diatoms. The tree was created with the use of the Jukes Cantor model. HBI-biosynthesizing strains are indicated in red. Diatoms in green were tested but did not contain HBI alkenes; diatoms in black were not tested for the presence of HBI alkenes. The scale bar indicates 10% sequence variation. The inset shows the structure of C25 HBI alkane (27) and parent skeleton of C25 HBI unsaturated alkenes (7–11) produced by diatoms. Note that the odd non HBI-biosynthesizing Rhizosolenia strain, R. robusta, falls completely out of the Rhizosolenia phylogenetic cluster, indicating that its morphological classification as a Rhizosolenia diatom is probably wrong.

Methane seeps:
Anaerobic oxidation of methane (AOM)

Image courtesy of Victoria Orphan. Used with permission.

Sediment Core from a methane-rich Monterey cold seep

This is a chemistry "profile" from the core



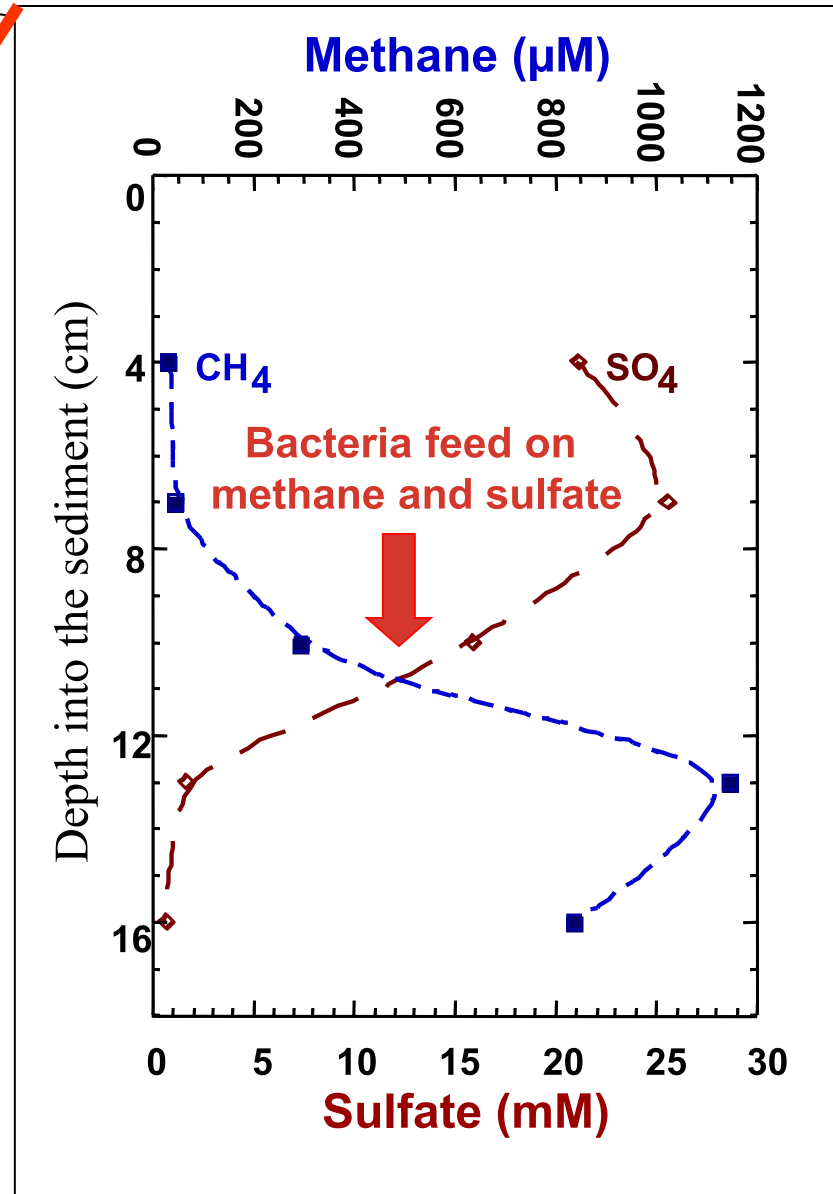
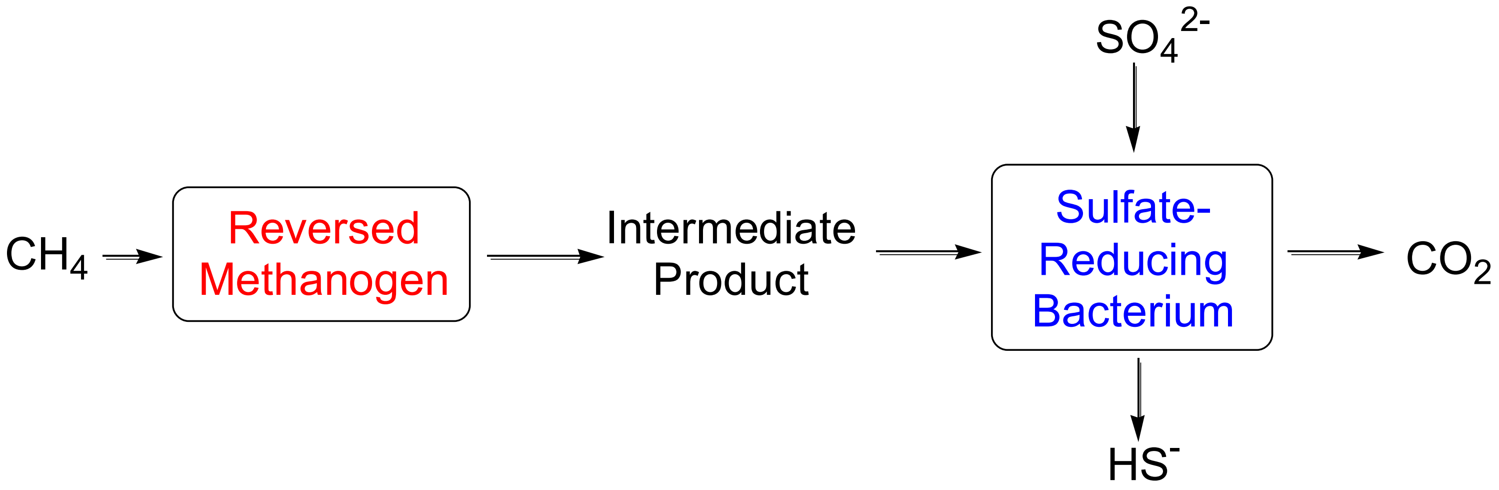


Image courtesy of Victoria Orphan. Used with permission.

Anaerobic oxidation of methane

The "consortium hypothesis"



Hoehler *et al.*, *Global Biogeochemical Cycles* **8**, 451-463 (1994)

Geochim. Cosmochim. Acta **62**, 1745-1756 (1998)

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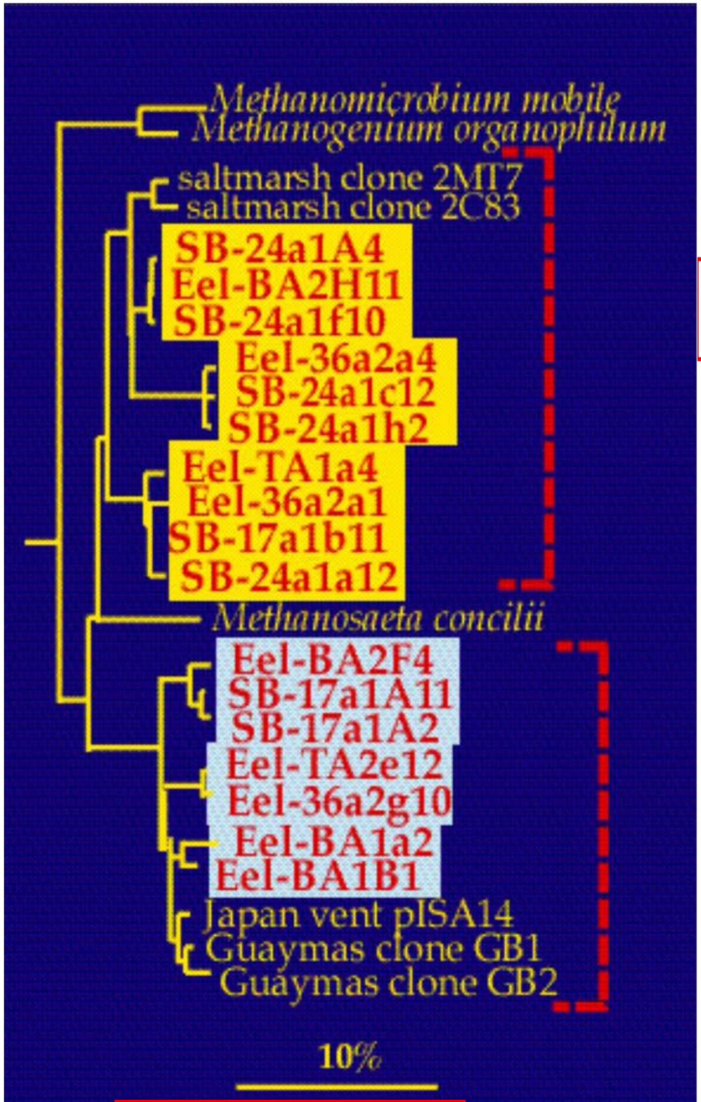
Please see Figure 1 on http://www.nature.com/nature/journal/v398/n6730/full/398802a0.html.

Reconstructed-ion-current chromatograms of trimethylsilylated total lipid extracts from (A) a sample 13±15 cm below the sediment surface at a site of active methane seepage (ERB-PC26) and (B) a control sample 33±36 cm below the sediment surface in the same basin but remote from any site of methane release (ERB-HPC5). Analytical conditions for both sediment extracts were identical (similar amounts of extracted sediment, identical dilutions prior injection into the GC). Compound 1=archaeol, compound 2=sn-2-hydroxyarchaeol.

Bacterial 16S rRNA methane seep otypes affiliated with AOM



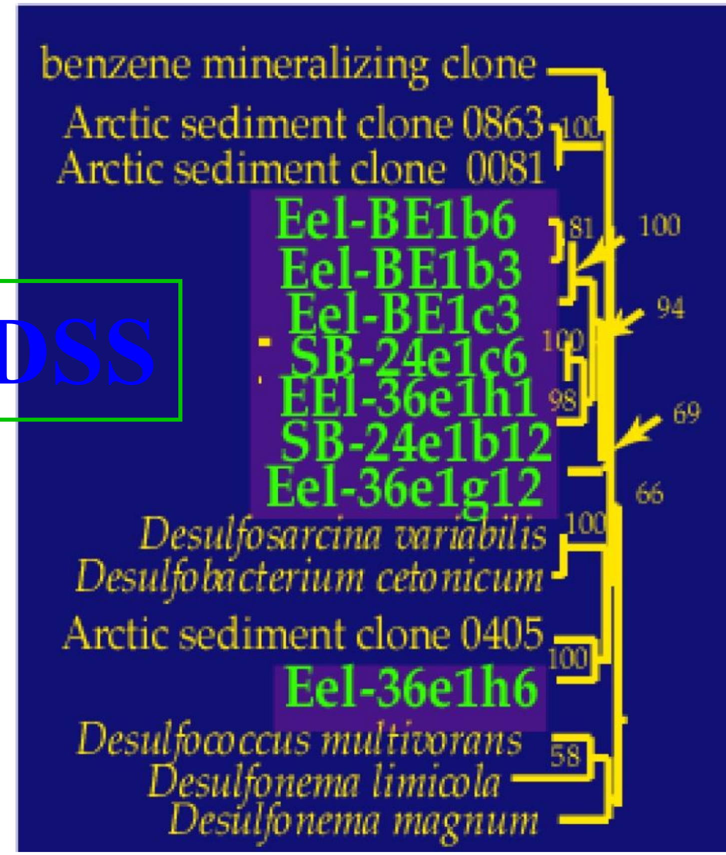
ANME-2

ANME-1

Archaea

Image courtesy of Victoria Orphan.
Used with permission.

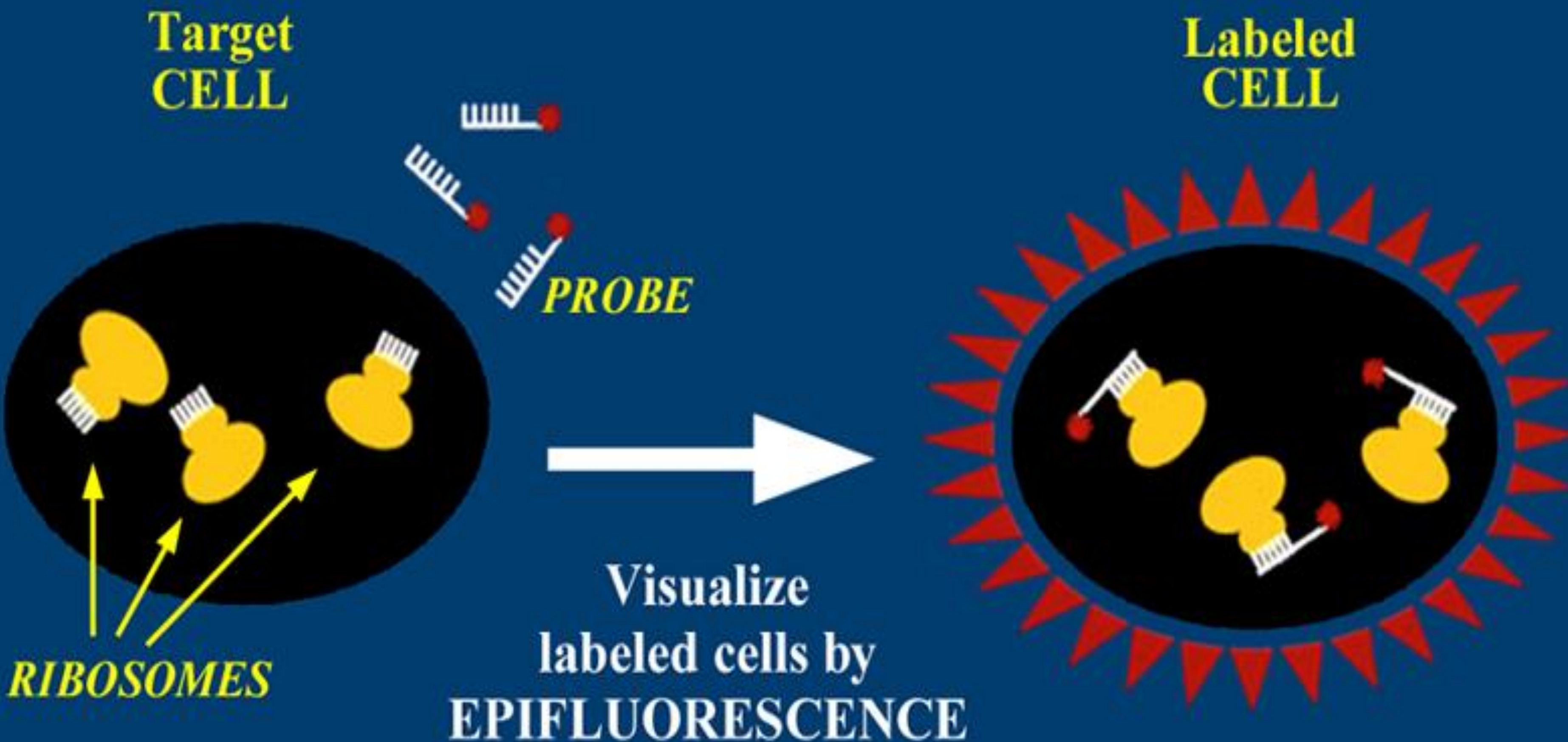
DSS



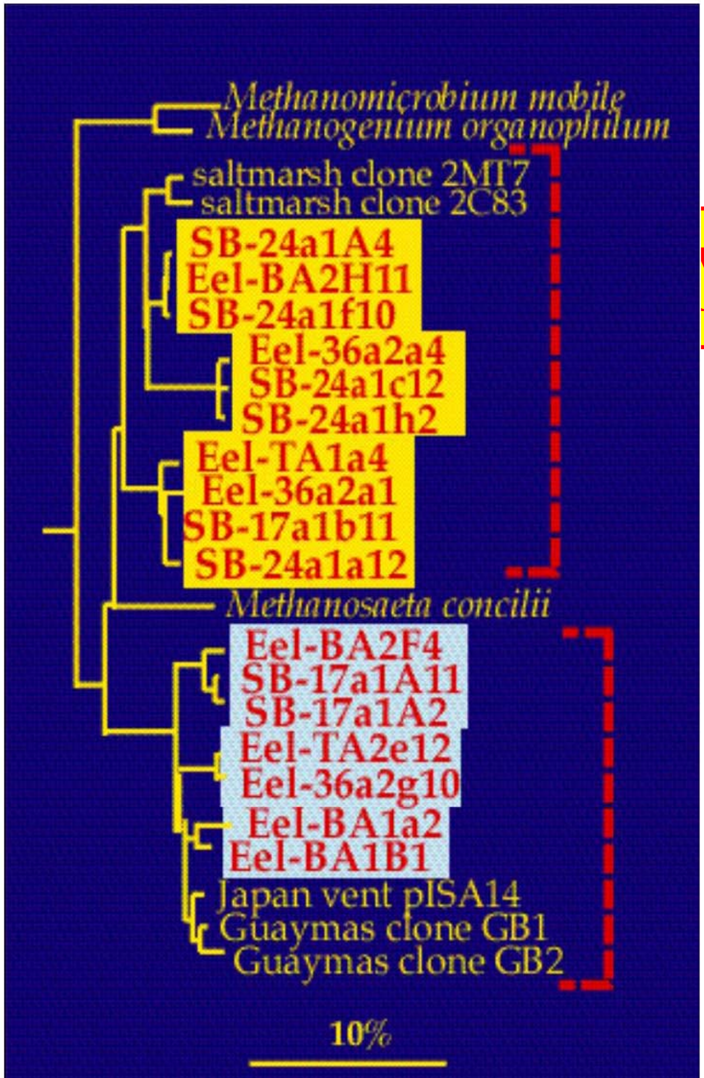
Bacteria (Desulfosarcina)

modified from Orphan et al. (2001)

Fluorescent *In Situ* Hybridization (FISH)

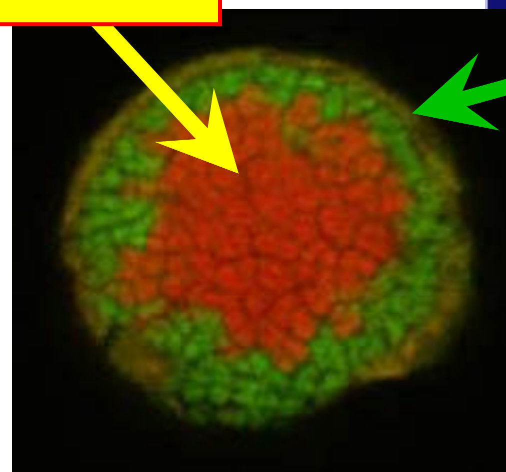


cterial 16S rRNA methane seep
ypes affiliated with AOM



Archaea

ME-2



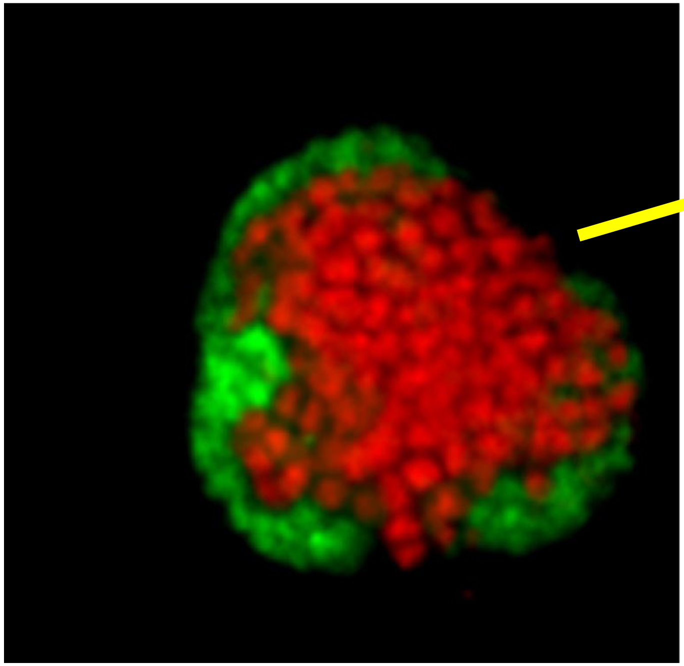
DSS

Bacteria (Desulfosarcina)

Image courtesy of Victoria Orphan.
Used with permission.

Distribution of anaerobic methane-oxidizing consortia

ANME-2 / Desulfosarcina



Hydrate Ridge, Oregon

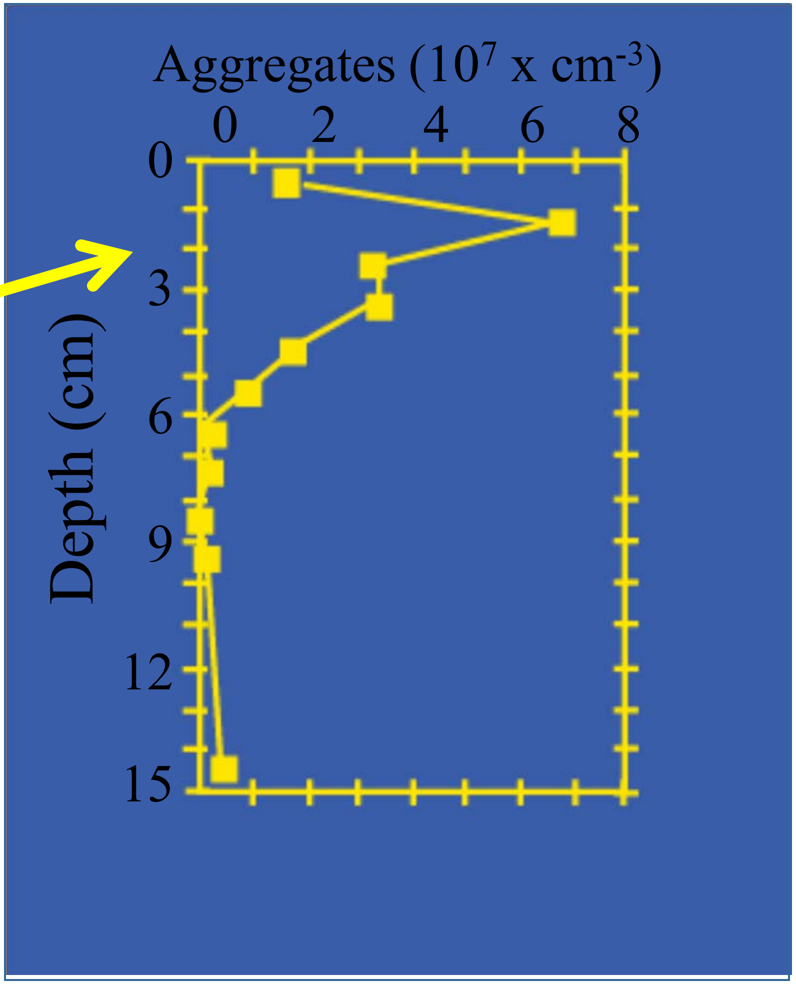
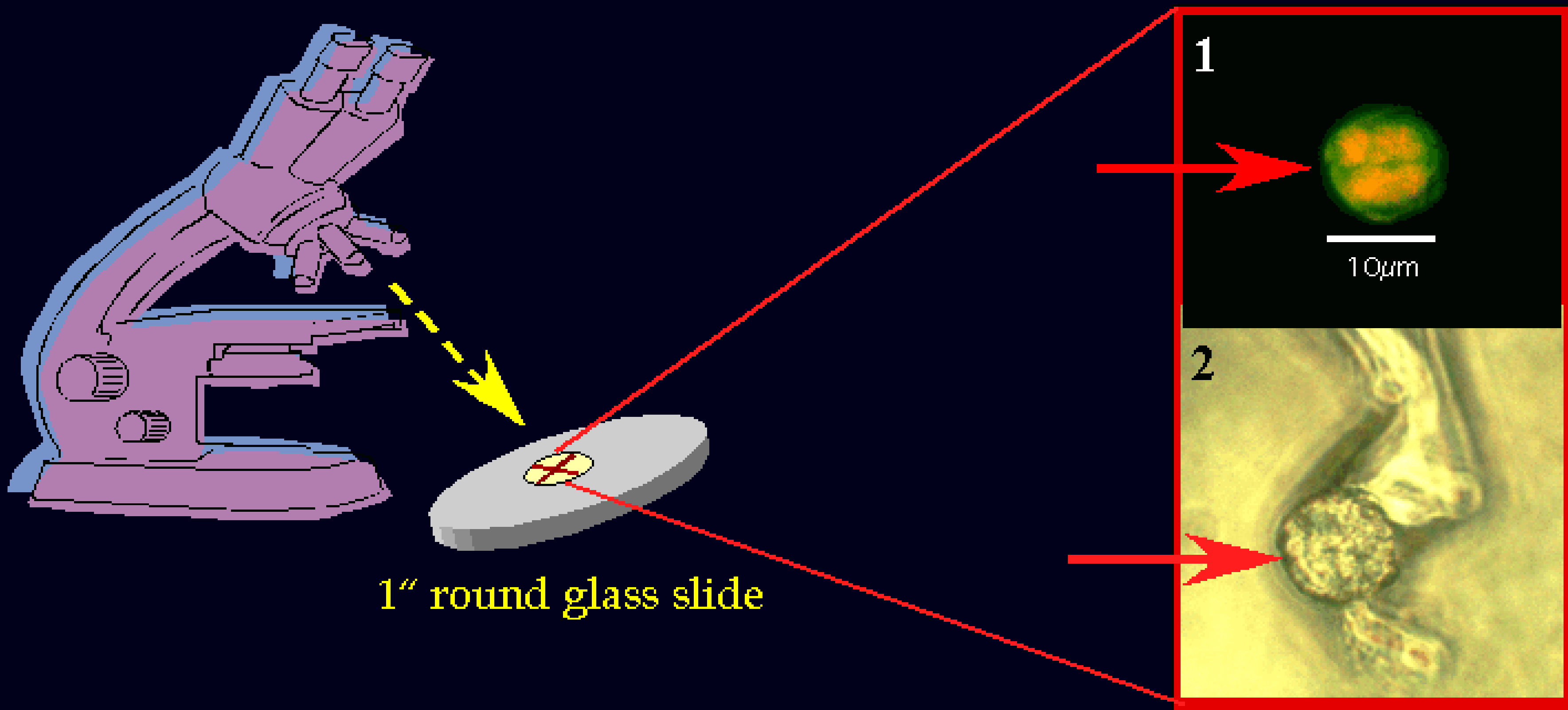


Image courtesy of Victoria Orphan. Used with permission.

Up to 80% total biomass in sample

FISH-SIMS

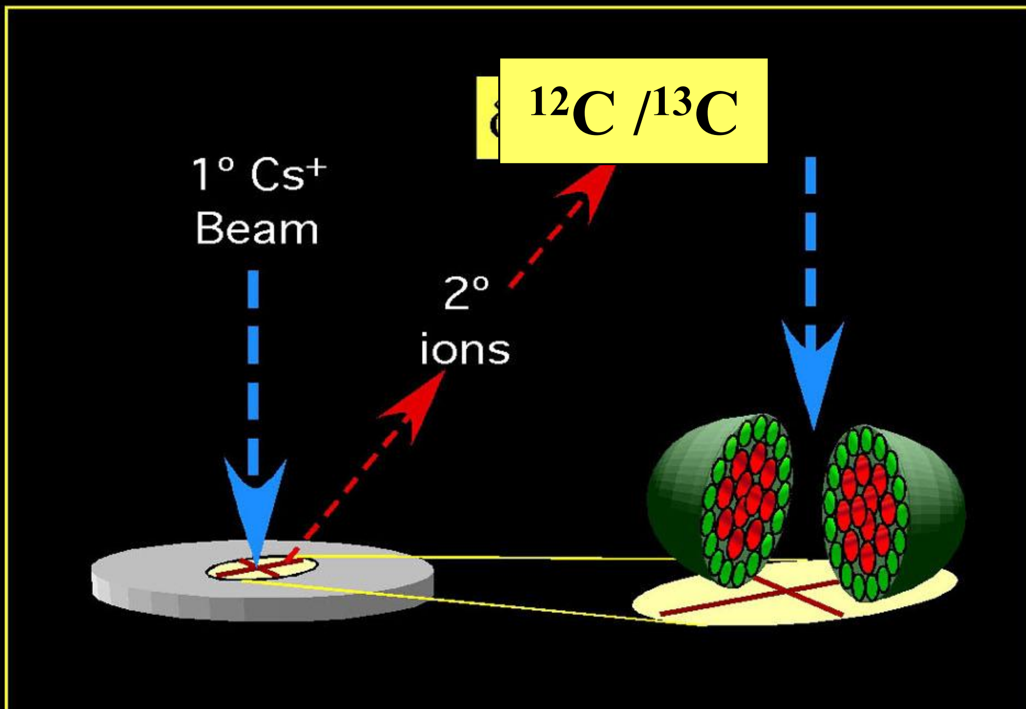


1) identify target cells using FISH and epi-fluorescence microscopy

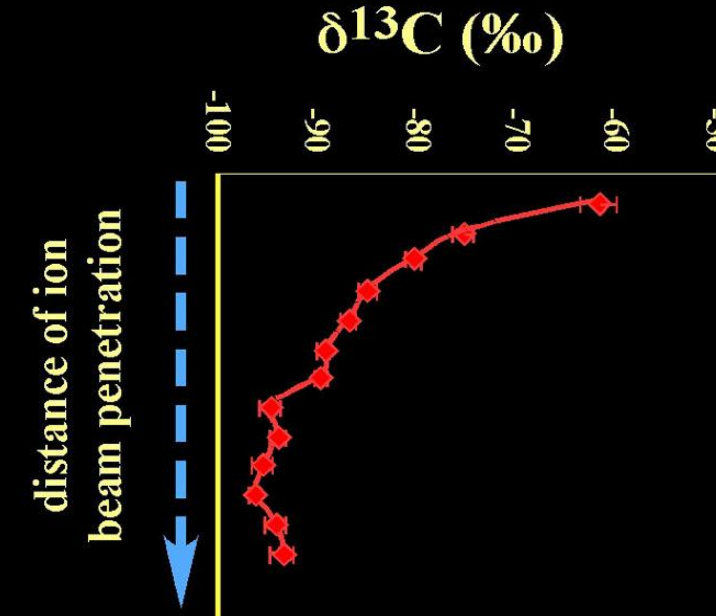
2) map and photo document aggregate location using light microscopy

Secondary Ion Mass Spectrometry (SIMS)

CAMECA ims-1270



SEM of "burned" targets

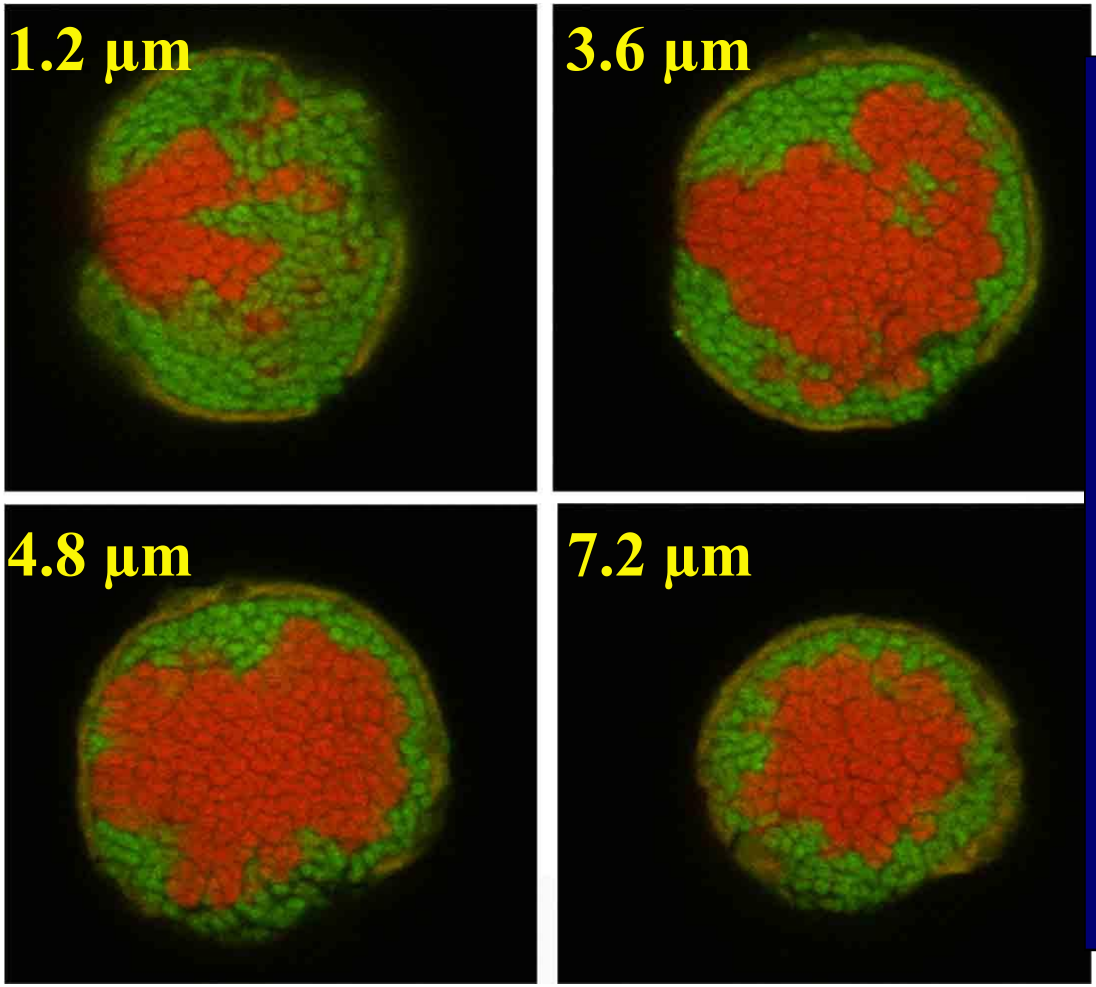


3) relocate target (reflected light) in CAMECA ims-1270.

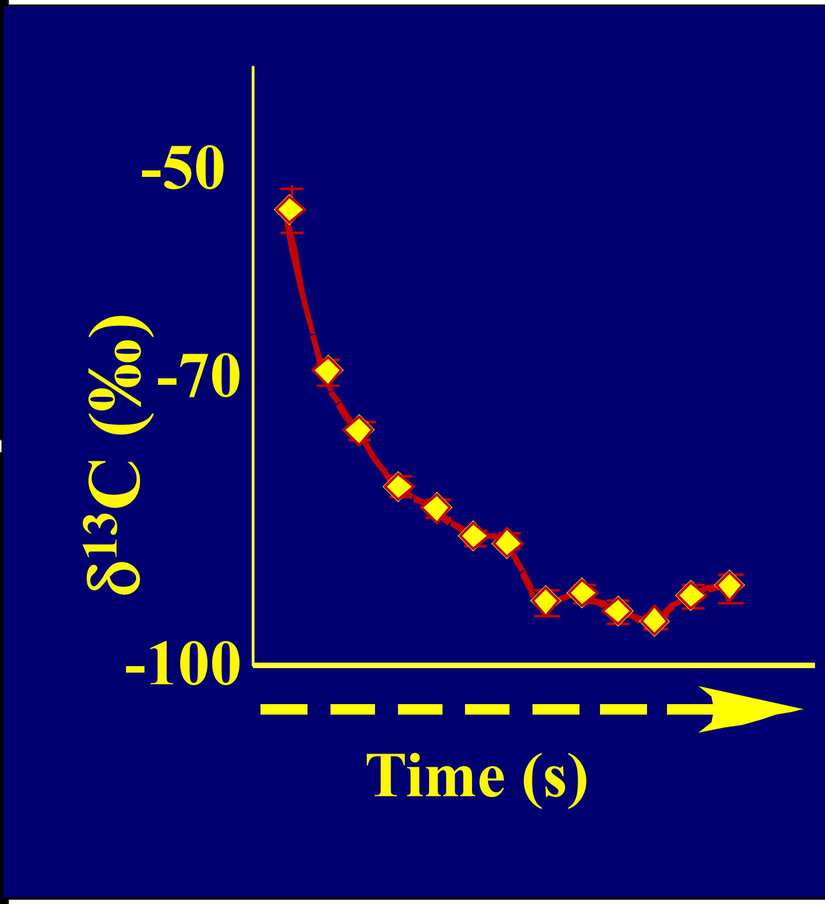
Sputter sample with Cs^+ beam.

4) measure $\delta^{12}C$ and $\delta^{13}C$ for target cells vs. time.

Heterogeneous composition of ANME-2 archaea and Desulfosarcina in AOM aggregates



Depth profile ANME-2/DSS aggregate
1.2 µm optical sections (confocal)



Distance of ion beam penetration (µm)

^{13}C compositions of archaeal lipids from different marine sedimentary environments

AOM Environment	Archaeol	OH-archaeol	Crocetane	PMI	Phytanol
Eel River Basin	-100	-106	-92	-92	-88
Santa Barbara	-119	-128	-119	-129	-120
Hydrate Ridge	-114	-133	-118	-114	---
Guaymas Basin	-81	-85	---	---	---
Kattegat	---	---	-100	-47	---
Mediterranean mud volcanoes	-96	-77	-64	-91	---

Hinrichs et al (1999); Hinrichs et al (2000); Boetius et al (2000); Bian et al (1994); Pancost et al (2000); Orphan et al (2001); Teske et al (2002)

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1934.2001 Apr. 2001, p. 1922–1934 Vol. 67, No. 4

Comparative Analysis of Methane-Oxidizing Archaea and
Sulfate-Reducing Bacteria in Anoxic Marine Sediments

V. J. ORPHAN,[1]* K.-U. HINRICHS,[2] W. USSLER III,[1] C. K. PAULL,[1] L. T. TAYLOR,[1]
S. P. SYLVA,[2] J. M. HAYES,[2] AND E. F. DELONG[1]*
Monterey Bay Aquarium Research Institute, Moss Landing, California 95039,[1] *and Department of Geology and Geophysics, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543*[2]
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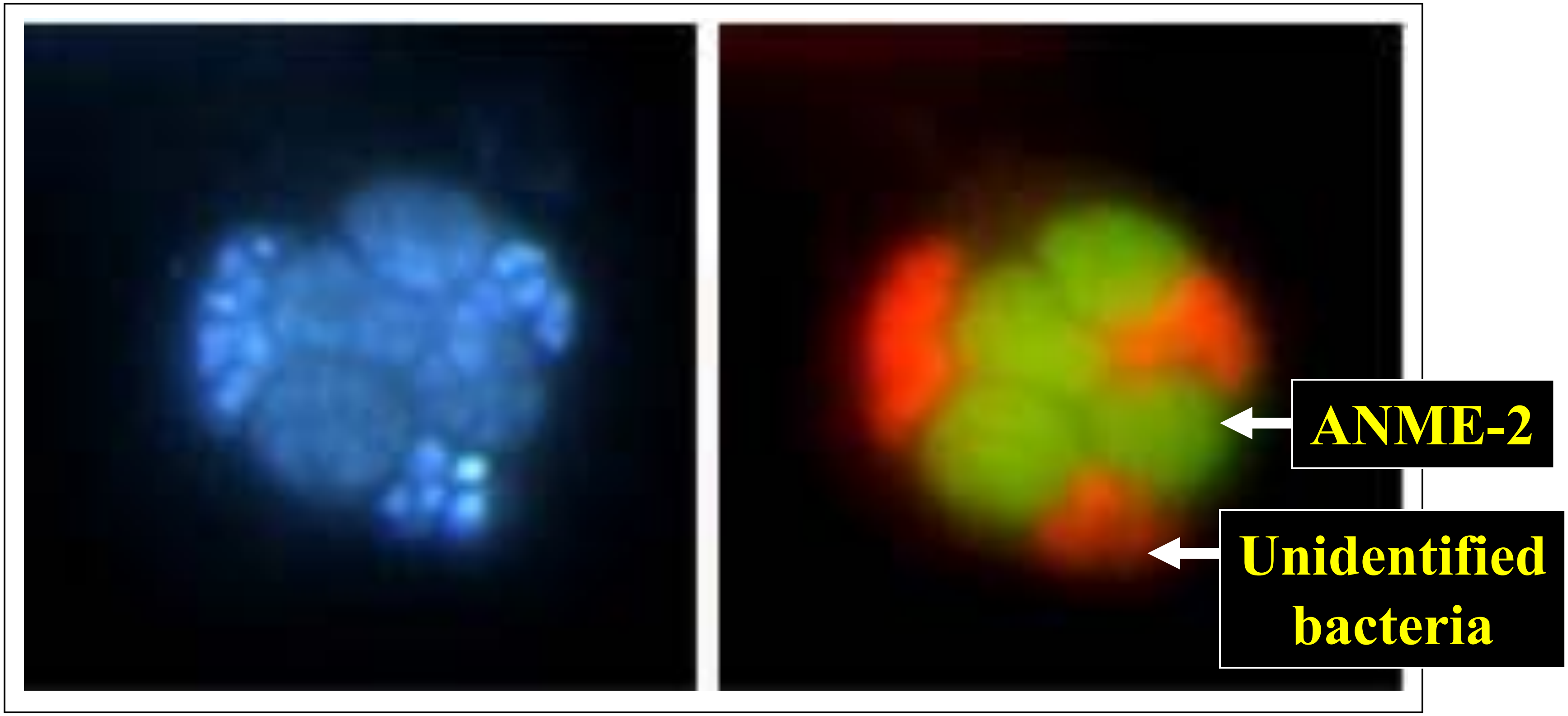
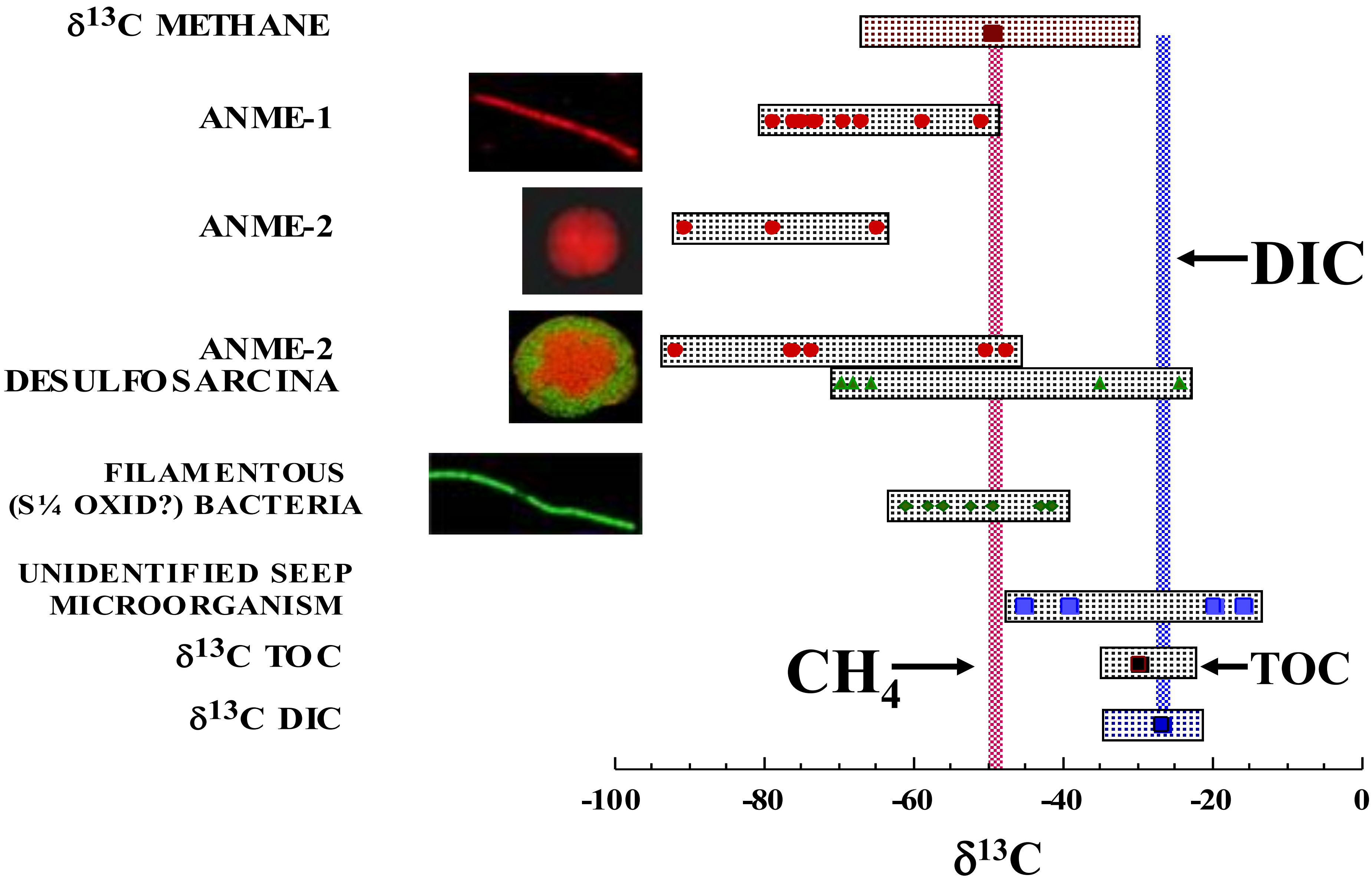
The oxidation of methane in anoxic marine sediments is thought to be mediated by a consortium of methane-consuming archaea and sulfate-reducing bacteria. In this study, we compared results of rRNA gene (rDNA) surveys and lipid analyses of archaea and bacteria associated with methane seep sediments from several different sites on the Californian continental margin. Two distinct archaeal lineages (ANME-1 and ANME-2), peripherally related to the order *Methanosarcinales*, were consistently associated with methane seep marine sediments. The same sediments contained abundant 13C-depleted archaeal lipids, indicating that one or both of these archaeal groups are members of anaerobic methane-oxidizing consortia. 13C-depleted lipids and the signature 16S rDNAs for these archaeal groups were absent in nearby control sediments. Concurrent surveys of bacterial rDNAs revealed a predominance of d-proteobacteria, in particular, close relatives of *Desulfosarcina variabilis*. Biomarker analyses of the same sediments showed bacterial fatty acids with strong 13C depletion that are likely products of these sulfate-reducing bacteria. Consistent with these observations, whole-cell fluorescent in situ hybridization revealed aggregations of ANME-2 archaea and sulfate-reducing *Desulfosarcina* and *Desulfococcus* species. Additionally, the presence of abundant 13C-depleted ether lipids, presumed to be of bacterial origin but unrelated to ether lipids of members of the order *Desulfosarcinales*, suggests the participation of additional bacterial groups in the methane-oxidizing process. Although the *Desulfosarcinales* and ANME-2 consortia appear to participate in the anaerobic oxidation of methane in marine sediments, our data suggest that other bacteria and archaea are also involved in methane oxidation in these environments.



DAPI (DNA stain)

ANME-2/Desulfosarcina/ Bacteria probes


δ13C METHANE
ANME-1
ANME-2
ANME-2
DESULFOSARCINA
FILAMENTOUS
(S¼ OXID?) BACTERIA
UNIDENTIFIED SEEP
MICROORGANISM
δ13C TOC
δ13C DIC
DIC
CH4
TOC
-100
-80
-60
-40
-20
0
δ13C

Marmorito (Miocene) Hydrocarbons

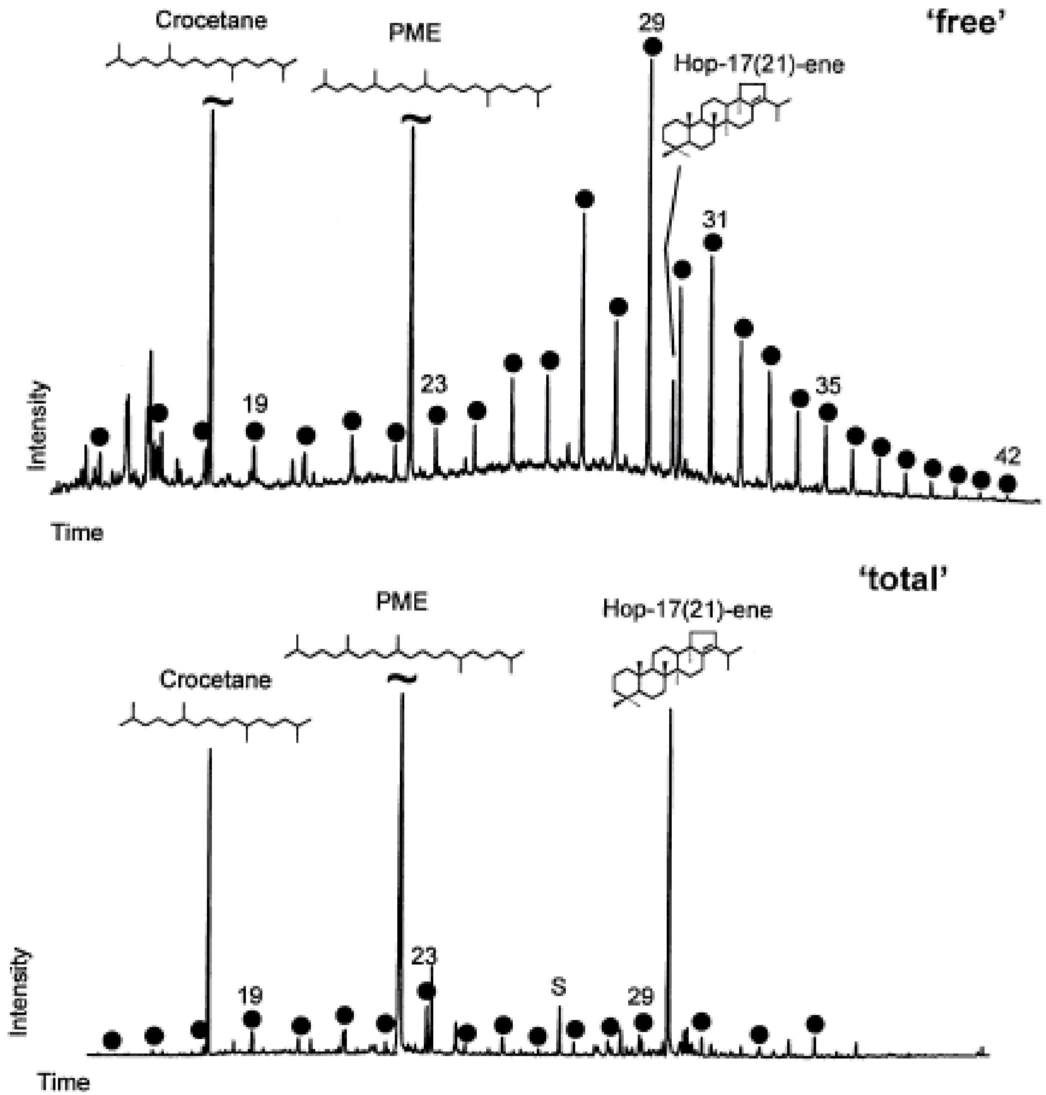


Fig. 1. Gas chromatograms of the hydrocarbon fractions extracted from the total sediment ("free") and from the residual matter after decalcification ("total"). ● = *n*-alkanes (selected carbon numbers denoted); S, 2,6,10,15,19,23-hexamethyltetracosane (squalane). Peak heights of crocetane and PME in the "free" fraction are cut at 75%; the peak of PME in the GC trace of the "total" fraction is cut at 50% peak height.

Thiel V., Peckmann J., Seifert R., Wehrung P., Reitner J., and Michaelis W. (1999) Highly isotopically depleted isoprenoids: molecular markers for ancient methane venting. *Geochim. Cosmochim. Acta* **63**(23/24), 3959-3966.

Table 1. Isotopic composition of selected biomarkers ($\delta^{13}C$ [‰] vs. PDB).

Compound	$\delta^{13}C$ [‰] Free	Total
Hydrocarbons		
n-C_{18}	n.a.	−44.2
Crocetane	−108.3	−115.6
PME	−105.5	−112.2
n-C_{26}	−30.3	−32.0
n-C_{29}	−30.4	−38.4
Hop-17(21)-ene	n.a.	−83.2
Alcohols		
Anteiso-C_{15}	n.a.	−88.3
n-C_{16}	n.a.	−87.6
10-Methyl-C_{16}	n.a.	−87.8
Phytanol	n.a.	−108.5
n-C_{18}	n.a.	−66.8
n-C_{26}	n.a.	−51.3
Ether lipid*	n.a.	−108.2

Standard deviations (σ) are below ±1‰ for all compounds except *n*-octadecanol (±6.5‰), and *n*-hexacosanol (±2.8‰); 'free' = compounds extractable from the untreated rock; 'total' = compounds obtained from the total rock after carbonate dissolution; n.a. = not analysed. *: 'Ether lipid' refers to the compound tentatively assigned as 1-O-hexadecyl-2-O-phytanylglycerol.

Table 3. Biomarkers for Which Isotopic Compositions Vary With Depth[a]

Depth, cm	ΣC_{20}isop[b] δ, ‰	Crocetane[c] δ, ‰	Crocetane[c] f_{Cr}	PMI[d] δ, ‰	Ether-Bound Carbon Skeletons: Phytane δ, ‰	Ether-Bound Carbon Skeletons: Biphytane δ, ‰
40	−29.8 ± 0.3	absent	0	abs.	−26.8 ± 0.5	abs.
80	−30.1 ± 0.1	absent	0	−30.4 ± 0.3	−26.1 ± 0.1	−26.4 ± 0.2
120	−30.0 ± 0.6	absent	0	−31.2 ± 0.3	−25.6 ± 0.3	−26.0 ± 0.3
170	−31.5 ± 0.1	absent	0	−30.0 ± 0.3	−27.3 ± 0.4	−26.4
175	−31.8 ± 0.5	absent	0	−30.8 ± 1.4	n.d.	n.d.
180	−31.9 ± 1.1	absent	0	−31.9 ± 0.8	n.d.	n.d.
185	−40.0 ± 0.5	−67.0 ± 4.7	0.25	−32.3 ± 0.6	n.d.	n.d.
190	−47.6 ± 0.5	−90.3 ± 5.5	0.28	−32.7 ± 0.5	−26.0 ± 0.4	−25.9 ± 1.4
195	−78.2 ± 0.6	−100.4 ± 3.0	0.68	−35.0 ± 0.9	−26.7 ± 0.5	−27.1
220	−40.1 ± 0.3	−84.5 ± 8.3	0.17	−36.0 ± 0.6	−28.6 ± 0.1	−27.4 ± 1.1
245	−52.6 ± 0.5	−91.0 ± 4.4	0.36	−47.3 ± 0.1	−28.7 ± 0.3	−27.1

[a]Here n.d., not determined.

[b]Sum of C_{20} acyclic isoprenoid alkanes: 40–180 cm, phytane; 185–245 cm, phytane + crocetane.

[c]Isotopic compositions of crocetane calculated from those of the C_{20} isoprenoid peaks and f_{Cr}, assuming $\delta_{Ph} = -31.0‰$. Values of f_{Cr} determined from mass spectra of C_{20} isoprenoid peaks, as described in text. Uncertainties in δ_{Cr} calculated by propagation of errors, assigning $\sigma_{\delta Ph} = 1.0‰$ and estimating that the mass spectral peak intensities of which f_{Cr} is based were measured with a constant standard deviation equal to 2% of full scale.

[d]2,6,10,15,19-pentamethylicosane

Science 21 February 2003:
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DOI: 10.1126/science.1079601

Molecular Fossil Record of Elevated Methane Levels in Late Pleistocene Coastal Waters

Kai-Uwe Hinrichs, Laura R. Hmelo, Sean P. Sylva

Accumulating evidence suggests that methane has been released episodically from hydrates trapped in sea floor sediments during many intervals of rapid climate warming. Here we show that sediments from the Santa Barbara Basin deposited during warm intervals in the last glacial period contain molecular fossils that are diagnostic of aerobic and anaerobic methanotrophs. Sediment intervals with high abundances of these compounds indicate episodes of vigorous methanotrophic activity in methane-laden water masses. Signals for anaerobic methanotrophy in 44,100-year-old sediment are evidence for particularly intense methane emissions and suggest that the basin's methane cycle can profoundly affect oxygen budgets in the water column.

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Please see caption on next page.

Fig. 1. Records of (A) carbon isotopic composition of benthic (left) and planktonic (right) foraminifera (5) in comparison to (B) abundance (left) and carbon isotopic composition (right) of the molecular biomarker diplopterol (hopan-22-ol, structure shown) in sediments deposited between 37 and 44.2 ka at ODP Site 893 (14). Light-brown shading marks periods of deposition of predominantly laminated sediments that coincide with relatively warm interstadials (15). Purple shading designates the four excursions in the carbon isotopic record of planktonic foraminifera that had previously been interpreted as evidence for particularly large releases of methane (5). Benthic foraminifera are as follows: Bolivina tumida, B. argentea, Uvigerina peregrina, Buliminella tenuata, and Rutherfordoides rotundata.

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Figure 2. Carbon isotopic composition of the archaeal ether lipid archaeol (structure shown) in sediments deposited 43 and 44.2 ka (shading as in Fig. 1). The minimum isotopic composition of archaeol in the 44.1-ka horizon indicates contributions from methanotrophic archaea. In addition, three 13C-depleted dialkylglycerolethers with non-isoprenoidal alkyl moieties, presumed to represent bacterial members of anaerobic methanotrophic communities (16, 21), were detected in this sample only (fig. S1).

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Figure 2. Carbon isotopic composition of the archaeal etherlipid archaeol (structureshown) in sediments deposited 43,000 and 44,200 years before present (shading as in Figure 1). Concentrations of archaeol are uniform throughout this interval (~ 150 ng/g dry sediment; data not shown). Like strongly 13C-depleted archaeol, three dialkylglycerolethers were detected in the 44.1-kyr horizon only (structural type shown).