Intact Polar Lipids (IPLs)

Analytical techniques and applications of IPLs as biomarkers for viable microbial communities

Molecular Biogeochemistry Course

Winter Semester 2011

Structural diversity of intact polar lipids (IPLs)

Phospholipid biosynthetic pathways

- The archaeal and bacterial lipid synthesis is fundamentally different in terms of stereochemistry.
- The head group in **bacteria** is attached at **sn-3** of the glycerol
- The head group in **archaea** is attached at **sn-1** of the glycerol
- The pathways in archaeal lipid biosynthesis are not yet all eluciated.
- Cytidine diphospho (CDP)diacylgylcerol is the precursor of all phospholipids (with the exception of PC) in bacteria. In Archaea it is CDP archaeol.

PA - phosphatidic acid
PE - phospatidyl ethanolamine
PG - phospatidyl glyerol
PC - phospatidyl choline
PS - phospatidyl serine
PI - phospatidyl inositol

Daiyasu et al., 2005

Chemotaxonomic specificy of IPLs

Archaeal

diakylglycerol / isoprenoids

Morris Kates
Langworthy
Goldfine
DeRosa

Bacterial (eukaryotic)

diacylglycerol / fatty acids

Marine crenarchaeota

Methanotrophs

Methanogens

Algae / Cyanobacteria

Sulfate-reducing bacteria

Planctomycetes

Diversity of archaeal IPLs in methanogen cultures

Lipid based tree of life

Using polar lipids to study microbial communities in the environment: PLFAs (phospho-/polar lipid fatty acids) – the traditional approach

Oecologia (berl.) 40, 51-62 (1979)

Oecologia

Determination of teh Sedimentary Microbial Biomass by Extractible Lipid Phosphate

D. C. White[1], W. M. Davis, J. S. Nickels, J. D. King and R. J. Bobbie
Department of Biological Science, Florida State University, Tallahassee, Florida 32306, USA

- Seminal paper showing that phospholipids degrade rapidly upon cell death.
- Phospholipid decay was analyzed via the release of ^{32}P phosphate (which was added initially to build up phospholipids).
- For many years PLFAs were successfully applied as biomarkers for viable organisms in environmental studies.
- No methods developed yet to readily analyze the intact molecule.

Analysis of IPLs by thin layer chromatography (TLC)

Silica gel plates

A - Chloroform:methanol:water
(25:10:1 ; v/v)

B - Chloroform:methanol:acetic acid water
(25:15:4:2 ; v/v)
Skipski et al., 1964

C - diisobutyl:ketone-acetic acid:water
(40:25:3:7 ; v/v)
Nichols et al., 1963

Until the establishment of HPLC-MS this method was applied for a variety of bacterial and archaeal cultures but not for analysis of environmental samples.

Shortcomings: **Time consuming**
no direct structural information
high background signal for soil/sediment samples

Slide: Julius Lipp

Advance in analytical techniques: from PLFAs to IPLs

ELSEVIER Journal of Microbiological Methods 33 (1998) 23-35

Journal of Microbiological Methods

Structural determination and quantitative analysis of bacterial Phospholipids using liquid chromatography/electrospray ionization/ mass spectrometry

Jaisong Fang*, Michael J. Barcelona

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Received 24 November 1997; received in revised form 13 March 1998; accepted 15 March 1998

Allowed for rapid screening of environmental samples for bacterial phospholipids.

Extended the method to the analysis of archaeal lipids.

All three domains of life in one analytical window!

RAPID COMMUNICATIONS IN MASS SPECTROMETRY

Rapid Commun. Mass Spectrom. 2004; 18: 617-628

Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/rcm.1378

RCM

Intact polar mambrane lipids in prokaryotes and sediments deciphered by high-performance liquid chromatography/electrospray ionization multistage mass spectrometry-new biomakers for biogeochemistry and microbial ecology.

Helen F. Sturt[1], Roger E. Summons[2], Kristin Smith[1], Marcus Elvert[3] And Kai-Uwe Hinrichs[1,3*]

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Received 27 October 2003; Revised 3 December 2003; Accepted 8 December 2003

Advantage of combining PLFA + head group information

PERGAMON

Organic
Geochemistry

A direct comparision between fatty acid analysis and intact phospholipid profiling for microbial identification

Jaisong fang [a,*], Michael J . Barcelona a, Pedro J.J. Alvarez b

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b *Department of Civil and Environmental Engineering. The university of Iowa, Iowa City, IA 52242-15272, USA*

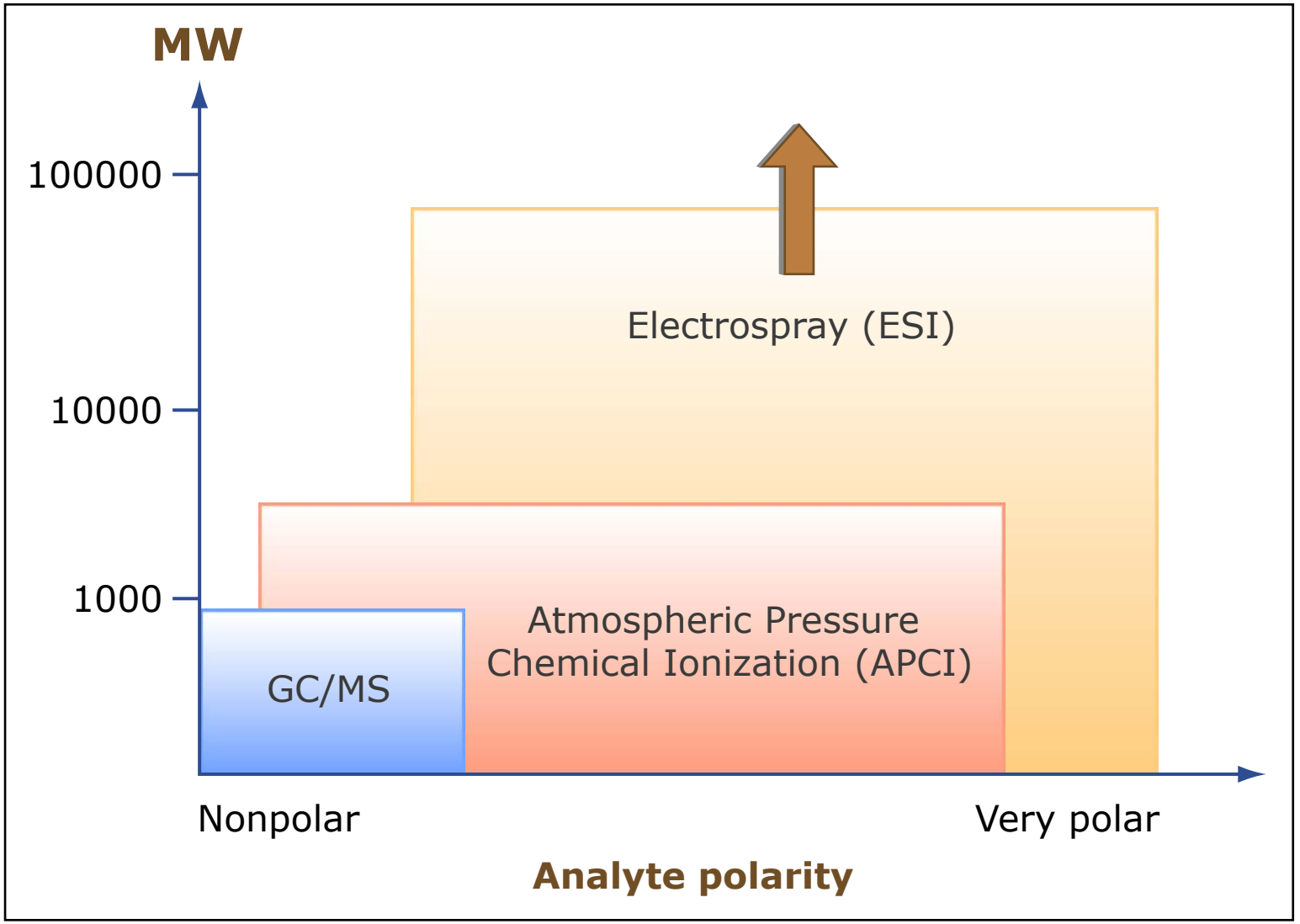
Received 25 October 1999; accepted 5 April 2000
(returned to author for revision 21 December 1999)

- Study of cultures
  (5 Pseudomonas strains)
- Combining the information of PLFA analysis with the head group type provides a more detailed information than just the PLFA analyses

IPLs

Analytical Techniques

Relative applicability of GC/MS vs. LC/MS



Slide: Ellen Hopman

Instrument Design: GC/MS vs. LC/MS

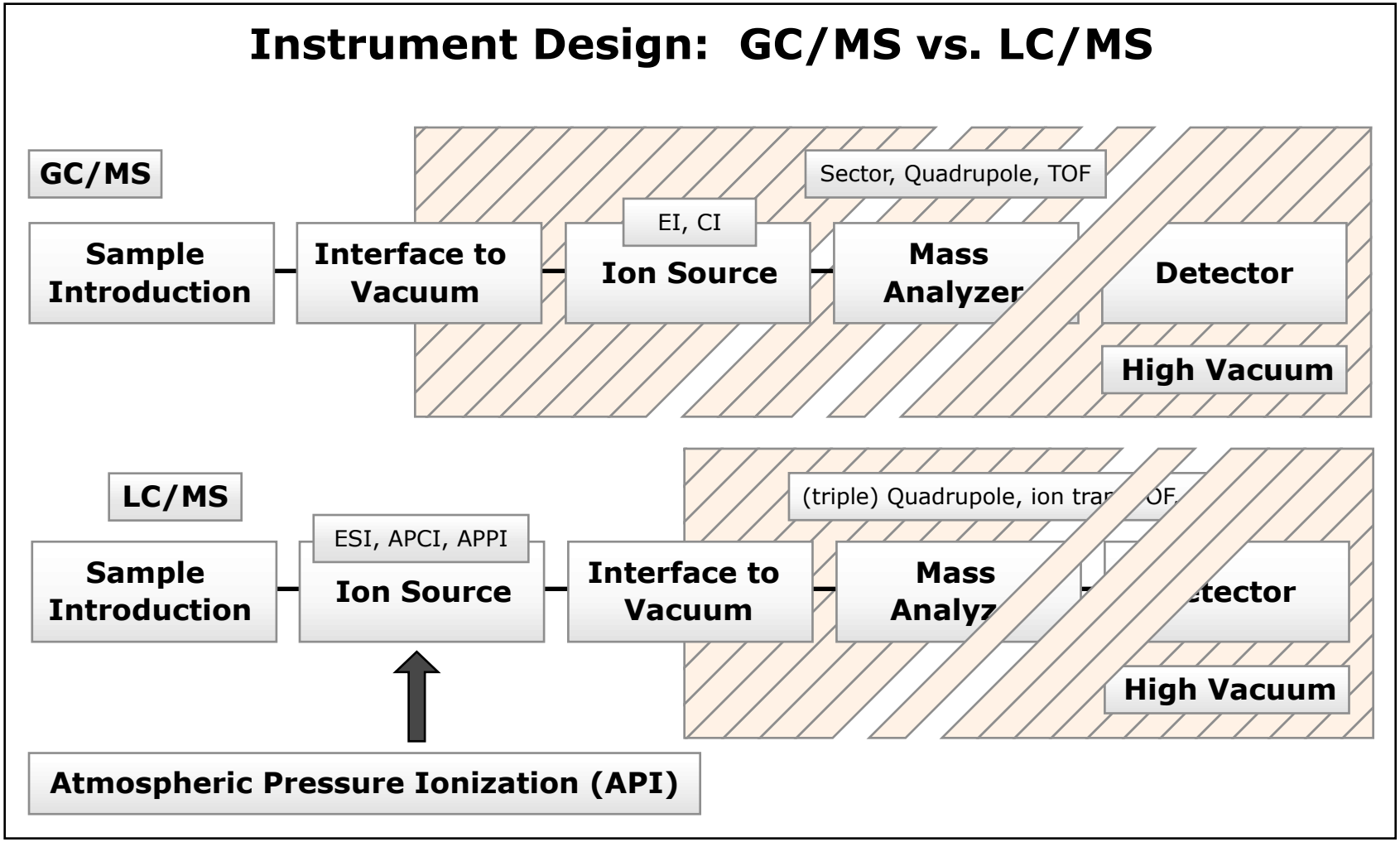
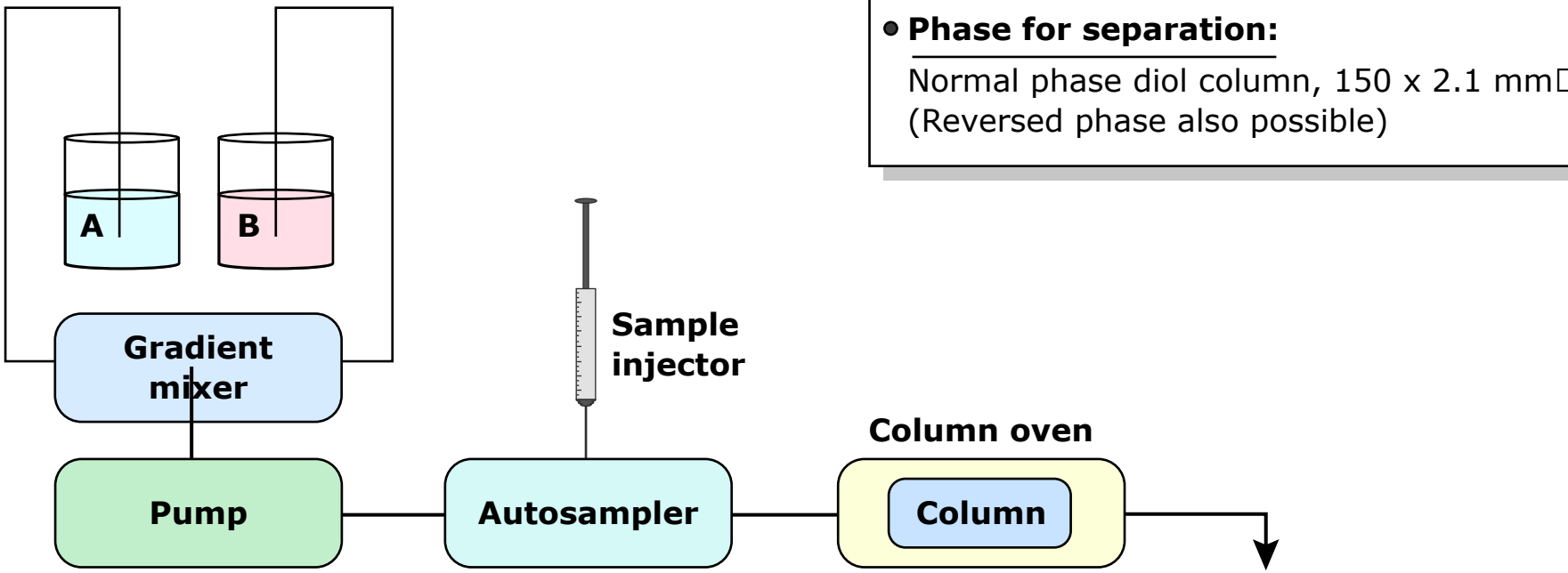


Image by MIT OpenCourseWare.

Slide: Ellen Hopmans

IPL Analysis: HPLC Separation

- **Phase for separation:**
  Normal phase diol column, 150 x 2.1 mm
  (Reversed phase also possible)



- **Pressure:** 20-100 bar
- **Eluent flow rate:** 0.2 ml/min
- **Gradient elution:** 0% B to 65% in 45 min
- **Eluents:**
  (A): 79% Hexane, 20% Isopropanol
  (B): 89% Isopropanol, 10% Water
  both include Ammonium formate buffer

Chromatogram

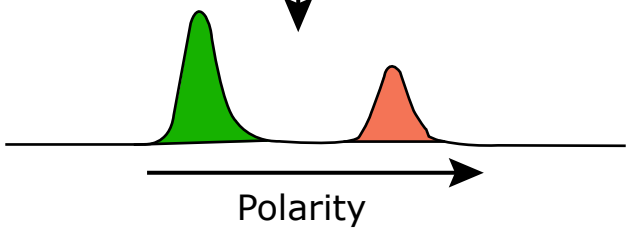


Image by MIT OpenCourseWare.

Slide: Julius Lipp

Electrospray Ionization (ESI)

http://penyfan.ugent.be/labo/joelv/Esquire.html

- High electric field (3-5 kV/cm) produces a fine mist of highly charged droplets
- Ion evaporation process produces analyte ions (Coulomb explosion: the magnitude of the charge is sufficient to overcome the surface tension holding the droplet together)

Positive species formed: $[M+H]^+$, $[M+NH4]^+$, $[M+Na]^+$
Negative species formed: $[M-H]^-$, $[M+HCOO]^-$, $[M+OAc]^-$

M. Yamashita and J. B. Fenn, J. Phys. Chem., 88, 4451-4459 (1984)
Kebarle and Tang, Aral. Chem., 65, 2, 972A-985A (1993)

Compound detection: Single quadrupole mass spectrometry

- DC (direct current) and Rf (radio frequency) voltages are applied to the rods creating an electrical field in which the ions oscillate.

- As Rf and DC voltages are ramped up, ions of successively higher *m/z* have a stable trajectory and are allowed to pass.

Single Quad: Gives only information on molecular mass and intensity, but with high sensitivity in SIM (selected ion monitoring) mode. No structural information!

Single Quad analysis

Isobaric IPL molecules with *m/z* 706

C18/C15 PE-DAG

C17/C16 PE-DAG

C16/C17 PE-DAG

C16/C16 PME-DAG

C16/C15 PDME-DAG

C15/C15 PC-DAG

C16/C15 PC-AEG

- **How can those isobaric molecules be distinguished in the mass spectrometer?**
- **MS1 information is not enough**
- **Retention time can help if there are structural differences**

Slide: Julius Lipp

Ion Trap mass spectrometry

- Isolation of ions of interest (e.g. 700 Da)
- Trapped ions can be fragmented by application of energy
- Fragments can be analyzed or isolated and fragmented again

- **Higher order MS^n** is possible in this type of mass spectrometer, which enables better structural elucidations.

MS analysis: data dependent mode

MS analysis: Ion trap MS^n

Quadrupole Time-of-Flight mass spectrometry (QToF-MS)

Slide: Julius Lipp

Triple Quadrupole mass spectrometry

MRM and **SRM** possible = multiple and selected reaction monitoring. This method focuses on one parent ion and it's specific fragmentation. It is very good for **quantification** as it entails a **high sensitivity**.

Mass spectrometric fragmentation pathways of IPLs

Slide: Julius Lipp

Typical phospholipid fragmentation pattern ➔ loss of head group

Glycolipid fragmentation pattern ➔ loss of head group/acyl side

Typical IPL fragmentation patterns

Common Reactions of Selected Phospholipids Under MS/MS Conditions in both Positive and Negative Ion Mode

Headgroup	Positive Ion Mode $[M+H]^+$		Negative Ion Mode [M-H]	
	AEG, DAG	DEG	AEG, DAG	DEG
PE	141 Da Loss (Phosphoethanolamine)	43 Da Loss (Ethanolamine)		43 Da Loss (Ethanolamine)
APT	231 Da Loss (Phospho-APT)	133 Da Loss (APT)	AEG-P; Loss of sn-2 fatty acid	133 Da Loss (APT)
PG	189 Da Loss (phosphoglycerol + NH_4^+ adduct)	75 Da Loss (Glycerol)	DAG-P; Loss of head group + sn-2 fatty acid	75 Da Loss (Glycerol)
PI	162 Da Loss (Hexose)			Major ion *m/z* 241 (Phosphoglycosy1 -H_2O)
PS	185 Da Loss (Phosphoserine)	87 Da Loss (Serine)		87 Da Loss (Serine)
PC	All give major Ion *m/z* 184 (Phosphocholine)		All show 60 Da Loss (CH_3 + $HCOO^-$ adduct)	

Sturt et al., 2004, RCM

Rapid Commun. Mass Spectrom. **2011**, *25*, 3563–3574
(wileyonlinelibrary.com) DOI: 10.1002/rcm.5251

Systematic fragmentation patterns of archaeal intact polar lipids by high-performance liquid chromatography/electrospray ionization ion-trap mass spectrometry

Marcos Y. Yoshinaga[1,2*], Matthias Y. Kellermann[1,2], Pamela E. Rossel[1,2†], Florence Schubotz[1,2‡], Julius S. Lipp[1,2] and Kai-Uwe Hinrichs[1,2]

Typically, two analyses are ideal: positive & negative ion mode
→ Complementary information that aid structural assignments

Problems of quantification: response factors & ion suppression

Ion suppression when working with sediment extracts

Pronounced reduction of response due to matrix effect

➔ Problem for quantification!

Can be partly solved by adding an internal or injection standard to the samples

Slide: Julius Lipp

Injection of calibration mixture with multiple compounds

Different response for different compounds

→ Ionization efficiency

Slide: Julius Lipp

Varying response factors of IPL compounds

IPLs

Application in environmental samples

PLFA / IPL application in the Wadden Sea

- **Ground-breaking application in environmental samples**
- **PLFA and IPL seem to reflect same pool**
- **IPLs decrease rapidly after 10cm**
- **IPLs still present at deeper depth = reflects presence of viable cells**
- **composition of IPLs: mainly PC, PG and other phospholipids, could be partly linked to SRBs**

Application of IPLs in environmental samples

Available online at www.sciencedirect.com

PERGAMON

Organic Geochemistry 34 92003) 755-769

Organic Geochemistry

www.elsevier.com/locate/orggeochem

Intact phospholipids-microbial "life markers" in marine deep subsurface sediments

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GeoForschungsZentrum Potsdam, Sektion 4.3, Telegrafenberg, 14473 Potsdam, Germany

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Received 22 July 2002; accepted 21 January 2003
(returned to author for revision 25 October 2002)

- **Detection of phospholipids in sediments up to depths of 799 m**
- **Alternative method to quantify biomass to gene-based methods.**

- **Changes in IPL composition reflect geochemical zonation**
- **The assignments to specific source organisms is, however, not straightforward**

ELSEVIER

Contents lists available at ScienceDirect

Organic Geochemistry

journal homepage: www.elsevier.com/locate/orggeochem

Vertical distribution of microbial lipids and functional genes In chemical distinct layers of a highly polluted meromictic lake

Tobias F. Ertefai[a,*], Meredith C. Fisher[b], Helen F. Fredricks[d], Julius S. Lipp[a], Ann Pearson[c], Daniel Birgel[a], Kai M. Udert[e], Colleen M. Cavanaugh[b], Philip M. Gschwend[e], Kai-Uwe Hinrich[a]

IPL composition follows water column stratification: Black Sea

Schubotz et al., EM (2009)

IPL composition follows water column stratification: Black Sea

Suboxic: anoxygenic phototrophs
Ammonium oxidizing crenarchaea

Schubotz et al., EM (2009)

IPL composition follows water column stratification: Black Sea

Anoxic: sulfate-reducing bacteria, unknown anaerobic bacteria

Schubotz et al., EM (2009)

Archaeal IPLs in the Black Sea water column and sediments

Schubotz et al., EM (2009)

IPL analysis of the marine crenarchaea *Nitrosopumilus maritimus*

Vol. 74, No. 8

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Apr. 2008, p. 2433-2440
0099-2240/08/$08.00+0 doi: 10.1128/AEM.01709-07

Intact Membrane Lipids of "*Candidatus* Nitrosopumilus maritimus," a Cultivated Representative of the Cosmopolitan Mesophilic Group I Crenarchaeota ▽

Stefan Schouten,[1]* Ellen C. Hopmans,[1] Marianne bass,[1] Henry Boumann,[1] Sonja Standfest,[2] Martin Könneke,[2] David A. Stahl,[3] and Jaap S. Sinninghe Damsté[1]

IPLs

Biggest problem: Identifying biological sources for IPLs

IPLs have a limited level of chemotaxonomic specificy.

There is need for more IPL analysis in environmentally relevant cultures. For many of the IPL observed in the environment we don't know who the sources might be.

Solutions:

Always combine with DNA data if available. Geochemical parameters are also important.

Target very specific IPLs that are characteristic for only certain groups of organisms (e.g. crenarchaeol, ladderane lipids…)

Combine the IPL analysis with stable isotope data to gain insights on the metabolic activities of the organisms.

Investigate more environmentally relevant cultures.

Targeted IPL analysis – investigating specific biomarkers in the N cycle

The ISME Journal (2011), 1-9

www.nature.com/ismej

archaea

ORIGINAL ARTICLE

Niche segregation of ammonia-oxidizing archaea and anammox bacteria in the Arabian Sea oxygen minimum zone

Angela Pitcher [1,3], Laura Villanueva [1,3], Ellen c Hopmans [1], Stefan Schoutan [1,2], Gert-Jan Reichart [2] and Jaap S Sinninghe Damsté [1,2]

bacteria

Marine crenarchaea: nitrification

Anammox bacteria: Anaerobic oxidation of ammonium

IPLs are not only community markers but also reflect the physiological state of the microbial community

Prochlorococcus 9312

North Pacific Subtropical Gyre

„Phospholipid substitution are fundamental biochemical mechanisms that allow phytoplankton to maintain growth in the face of phosphorous limitation.“

Van Mooy et al., 2006 PNAS

- **Heterotrophic bacteria do not have the ability to substitute for phospholipids**
- **Cynobacteria do not synthesize nitrogen containing lipids, while eukaryotic phytoplankton is „burdened“ with an extra nitrogen requirement during lipid synthesis.**

Van Mooy et al., 2009 Nature

IPLs from consortia that mediate the anaerobic oxidation of methane(AOM)

IPL distribution of AOM communities around the world

Which environmental factors control AOM consortia dominance?

Which Environmental Factors Control AOM Consortia Dominance?

	ANME-1	*ANME-2a/DSS*	*ANME-3/DBB*
Archaeal IPLs	Gly-GDGTs P-GDGTs	P-AR based IPLs Gly-AR based IPLs	P-AR based IPLs
Bacterial IPLs	Low abundance	High abundance (PC, PG, PE)	High abundance (esp. PDME, PME)
Temperature	Medium	Low	Low
O_2-conc. in bottom waters	Low (or anoxic)	High	High
Sulfate	Less supply of sulfate	Need of efficient supply of sulfate	----------

Image by MIT OpenCourseWare.

Rossel et al., GCA, 2011

Carbon flow tracked by stable isotopes $\delta^{13}C$

Fixation pathway

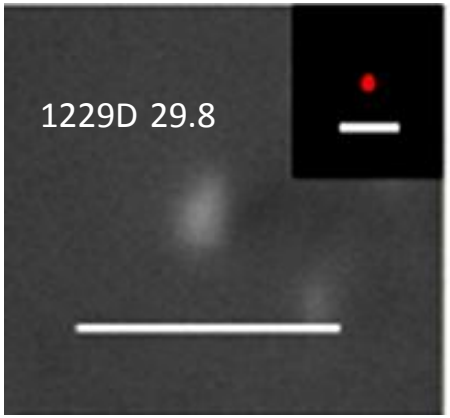
$\delta^{13}C$ analysis at methane seep areas

ODP Leg 201: Deep sulfate-methane transition zones (SMTZ)

Reaction: $CH_4 + SO_4^{2-} \rightarrow HCO_3^- + HS^- + H_2O$ + ^{13}C-depleted biomass

ODP Leg 201: Archaea in deeply buried sulfate-methane interfaces

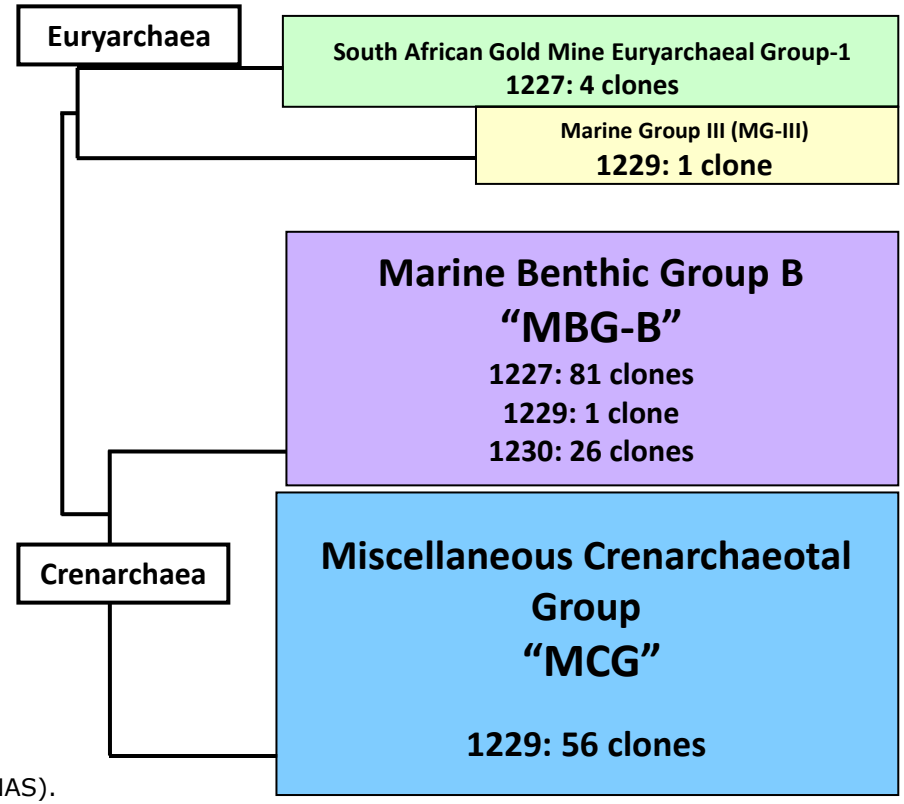
- Detection of archaeal tetraether and diether IPLs
- No bacterial lipids detected



- FISH probes Arch915 and Eub338 used
- Detection of archaeal cells with less than 0.5 µm in diameter

Image courtesy of National Academy of Sciences (PNAS).
Used with permission

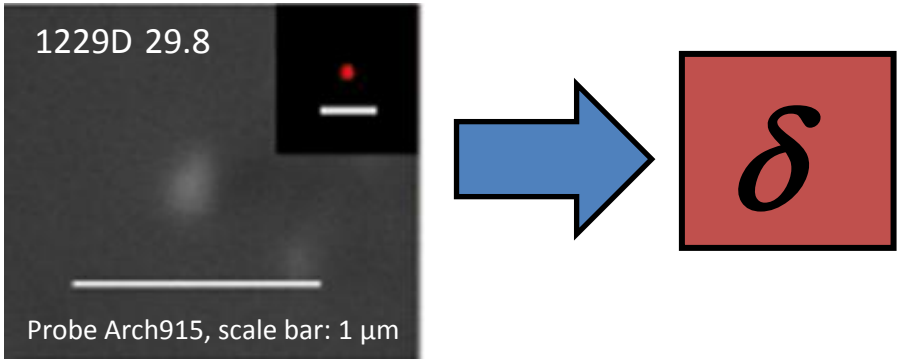
Probe Arch915, scale bar: 1 µm



- 16S rRNA: selecting for active Archaea
- No ANMEs detected, only *Benthic Archaea*

Biddle, Lipp *et al.*, PNAS (2006)

Archaea in deeply buried SMTZs: what is feeding them?



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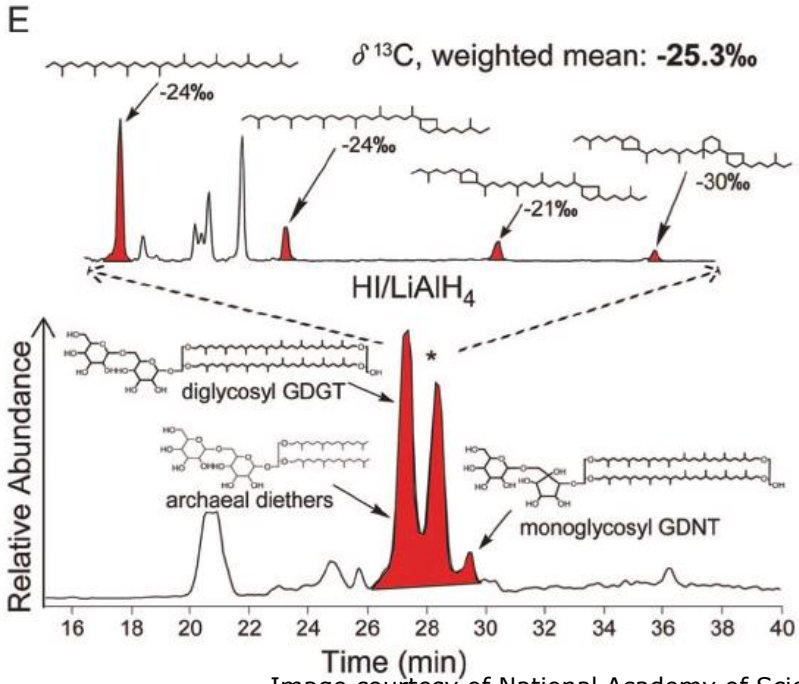

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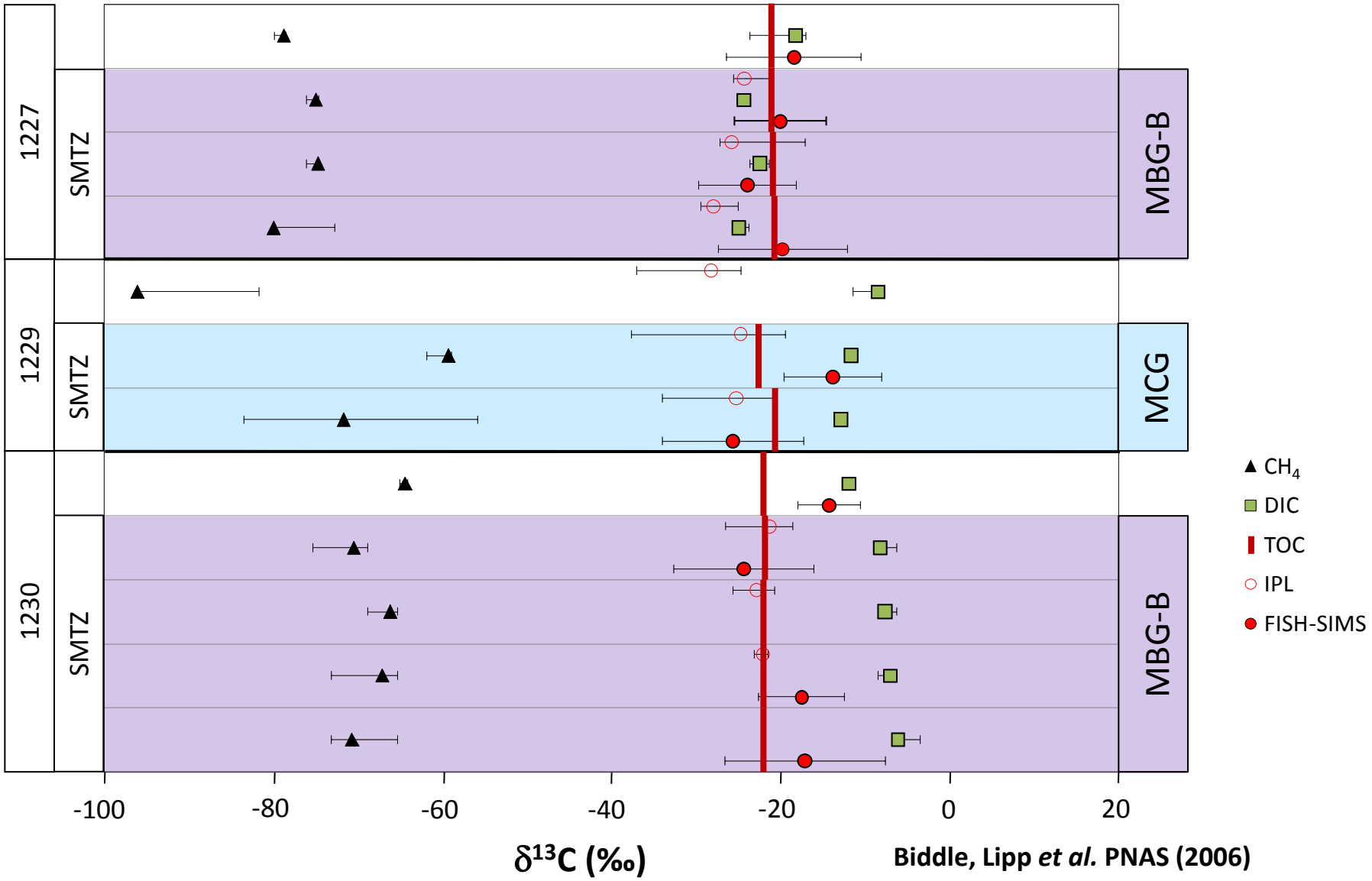
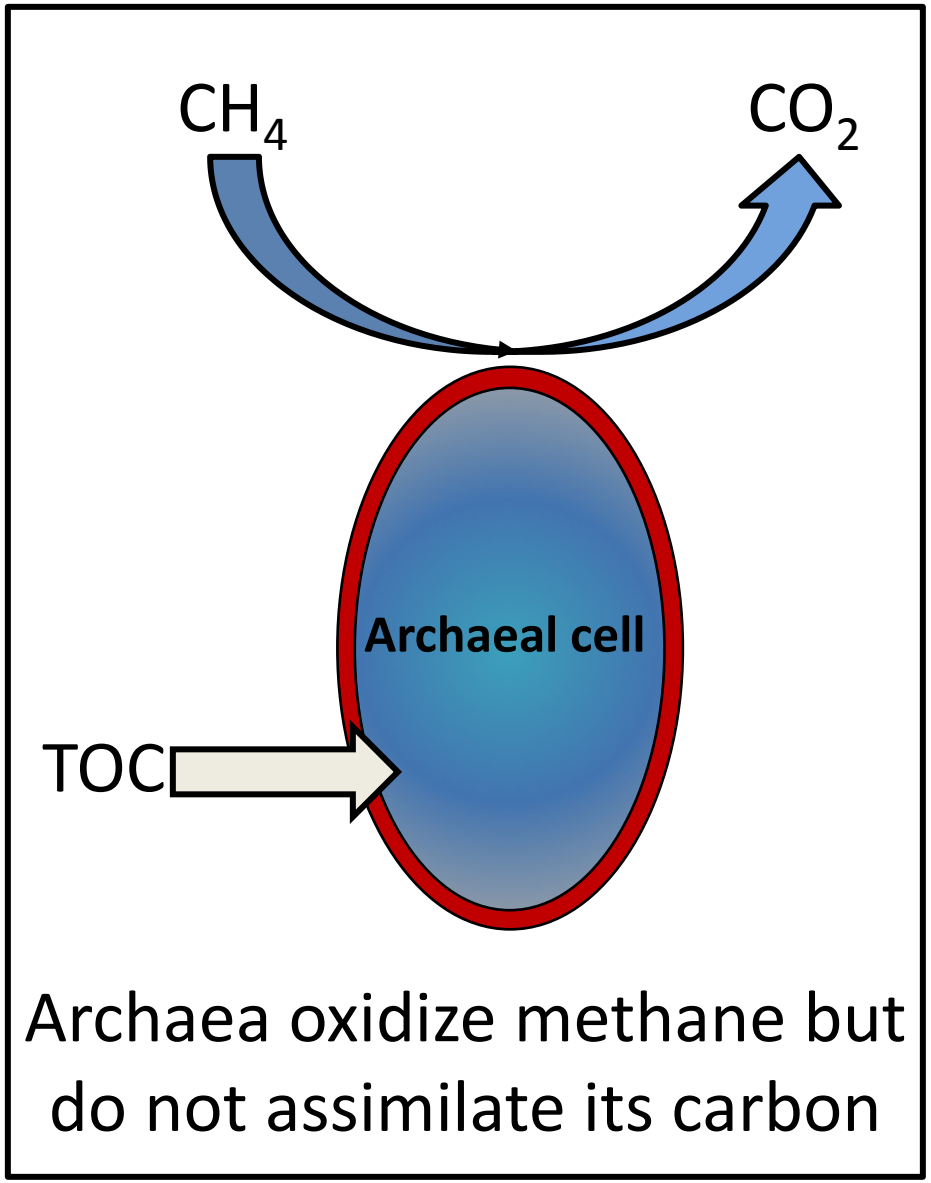
Biddle, Lipp et al., PNAS (2006)

ODP Leg 201: $\delta^{13}C$ of sedimentary carbon pools



Biddle, Lipp *et al.* PNAS (2006)

ODP Leg 201: $\delta^{13}C$ of sedimentary carbon pools



Archaea oxidize methane but do not assimilate its carbon

Methane assimilation should be reflected in the isotopic signature

12.158 Molecular Biogeochemistry

Fall 2011