Estuaries in a changing world

May 18 – 22, 2008
Xiamen, China
2008年5月18－22日
中国　厦门

厦门欢迎您！

Xiamen Welcomes You!

High-precision frequency measurements: indisensable tools at the core of molecular-level analysis of complex systems

N. Hertkorn, et al.

HelmholtzZentrum münchen
Deutsches Forschungszentrum für Gesundheit und Umwelt

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85758 Neuherberg, Germany

outline

this presentation is a **conceptual overview** about **novel high-resolution analytical tools** which provide the capacity to entirely change our **perception of complexity** in any natural system

detailed application of these tools to the molecular-level structural characterization of natural organic matter (NOM) will be demonstrated in the ***poster session***

outline

this presentation is a

about **novel high-res**

which provide the

change our **perce**

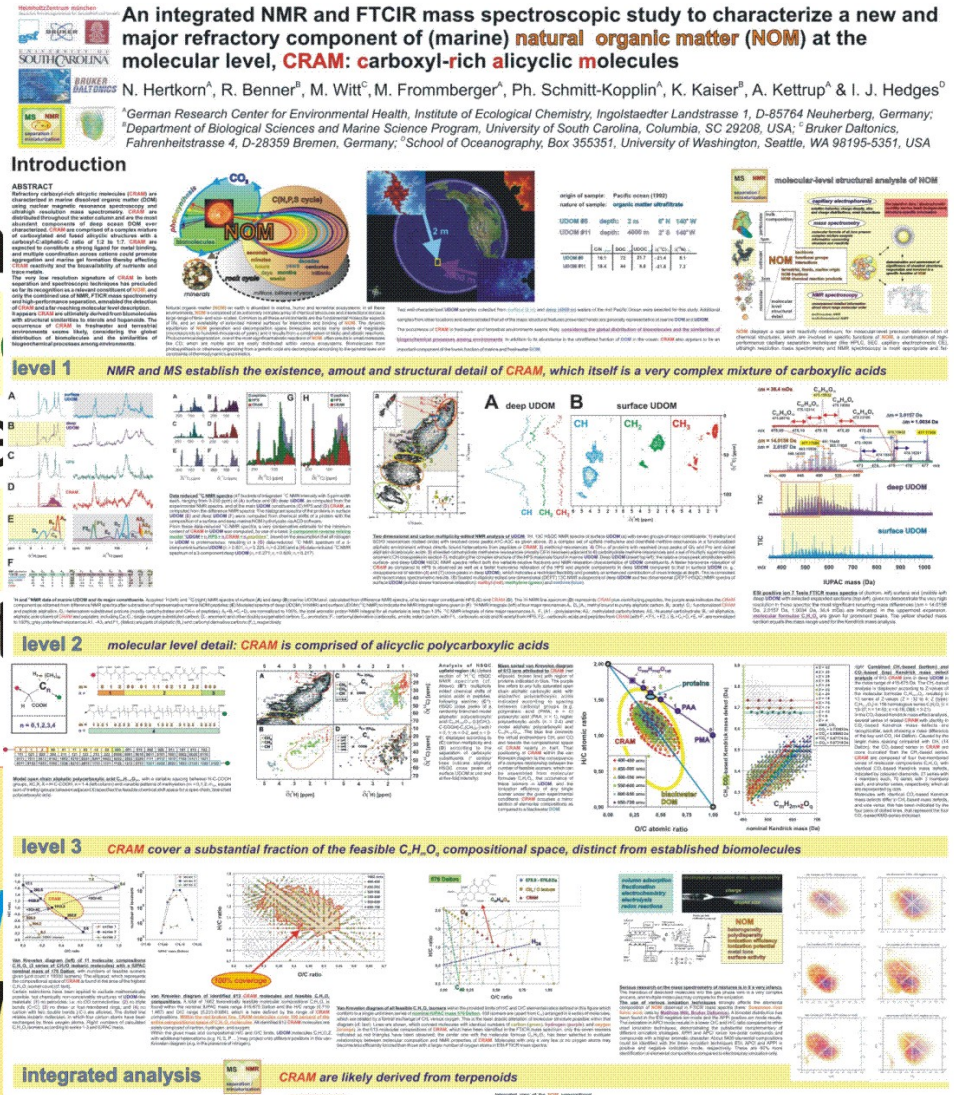
in any nat

detailed applicatio

molecular-level stru

of natural organic

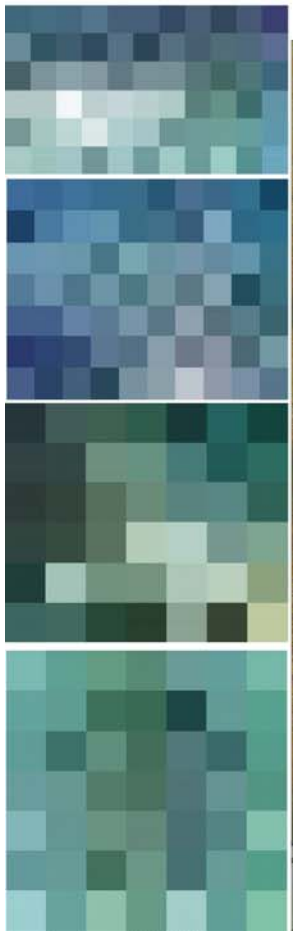
demonstrated in

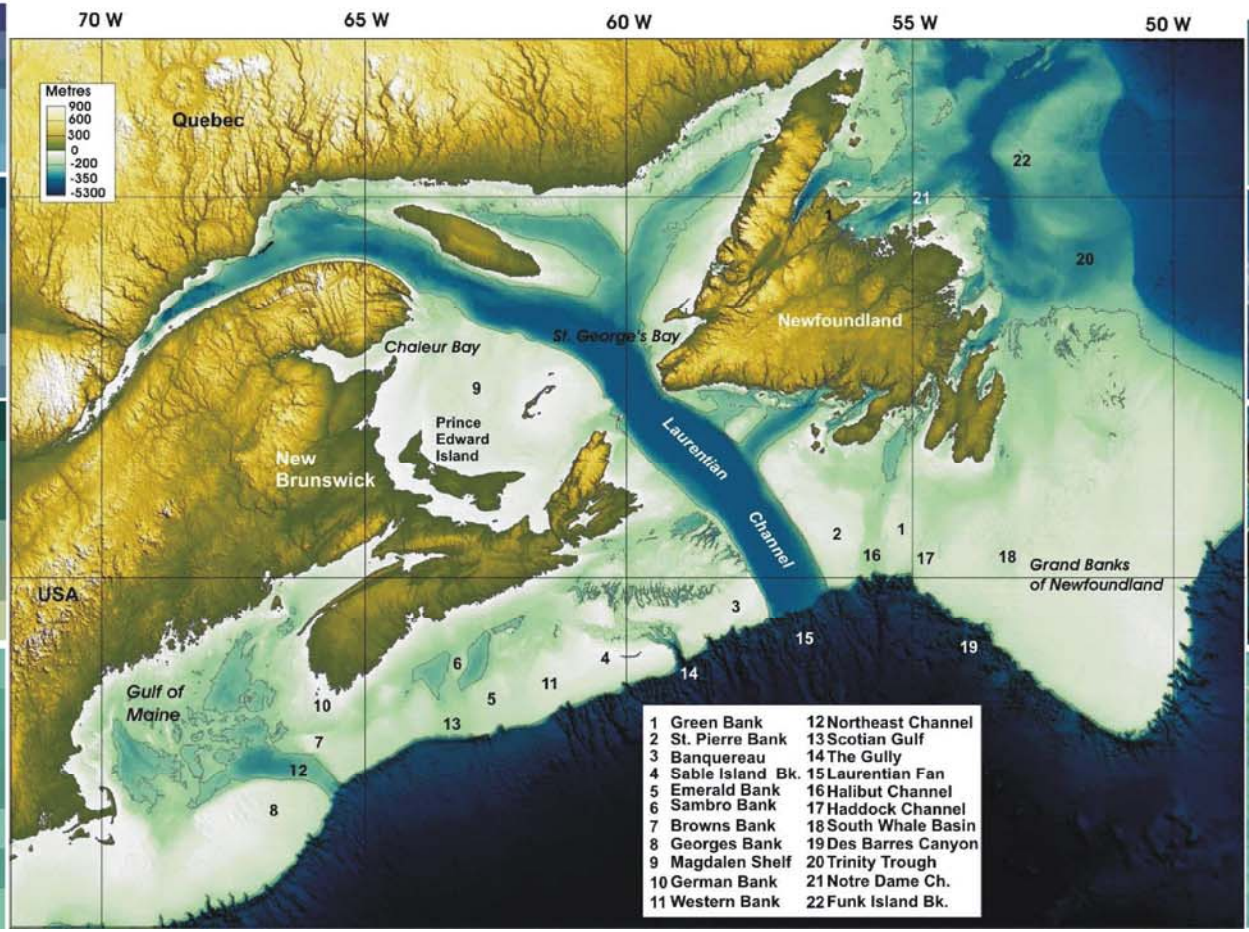


ten-year-vision:

authentic molecular representation of complex natural systems

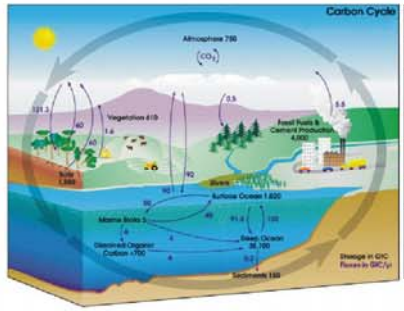
2005 → **2015**

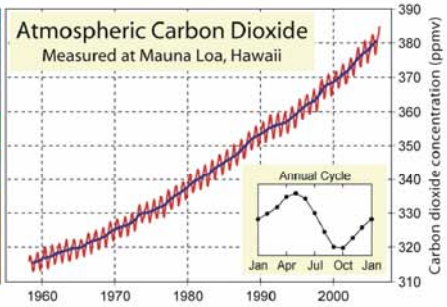




my “outside view”

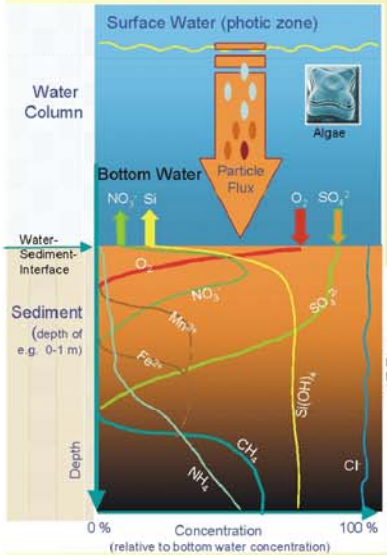
Joint IMBER / LOICZ
Continental Margins
Open Science Conference

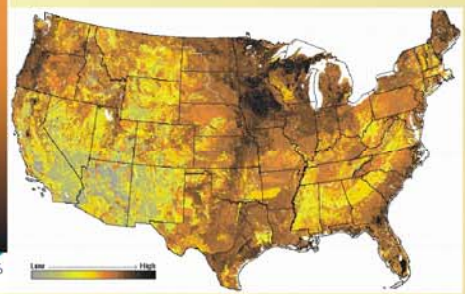




CO_2, N_2O, trace gases, FCKW, metal ions

→ **detailed, rich data and models are available**





terrestrial, freshwater, marine and atmospheric natural organic matter (NOM)

→ **rather pictorial descriptions of the BIG unknown mystery**

spatially resolved target analysis ⟷ **models**

determination of atmospheric trace gases
(e.g., CO_2, N_2O, CH_4)

molecularly resolved non-target analysis

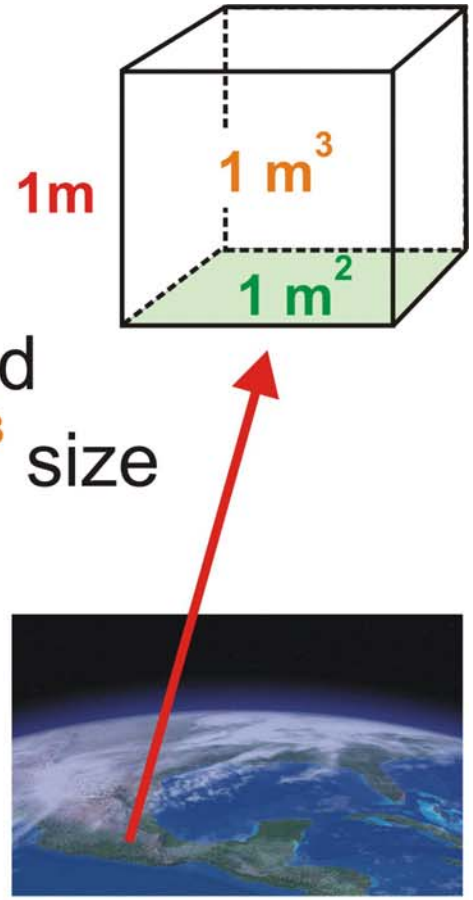
inventory ↕ **models**

?

assessing molecular composition and
chemical structures of atmospheric, terrestrial, limnic
and marine organic matter

spatially resolved target analysis

earth surface: 510.000.000 km²



dividing the surface in units of 1*1 m² and
expanding this area into cubes with 1 m³ size
and reaching for 15 km from the surface

$7.65 * 10^{19}$ cubes

determination of concentration in relative units of 10^{-4}

$7.65 * 10^{19+4} = 7.65 * 10^{23}$

options to depict dissimilarity

molecularly-resolved non-target analysis

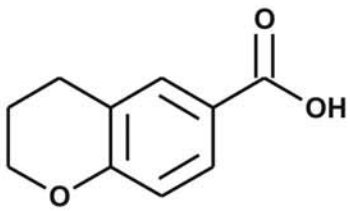
molecular diversity:

number of feasible isomeric molecules in one cubic meter

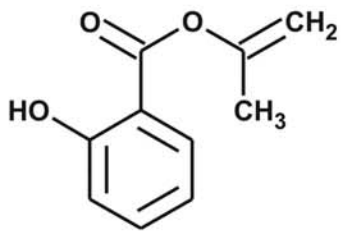
a typical molecule with nominal mass 178 Dalton $C_{10}H_{10}O_3$ (six DBE):

count of chemically relevant isomers: $1.1133 * 10^7$

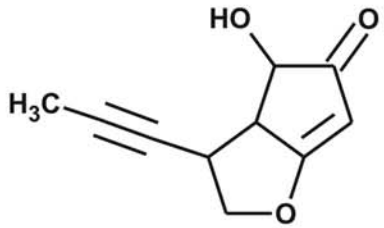
typical van der Waals volume of $C_{10}H_{10}O_3$: 160 $Å^3$ $\xrightarrow{V^{-1}}$ $0.63 *10^{28} m^{-3}$



153.4 $Å^3$



164.3 $Å^3$

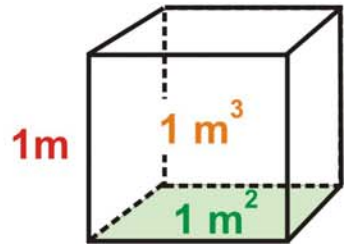


$C_{10}H_{10}O_3$

160.3 $Å^3$

"diversity index" of $C_{10}H_{10}O_3$ molecules in one cubic meter

$1.1133 * 10^7$ * $0.63 *10^{28} m^{-3}$ = ***$7.01 * 10^{34}$***



introduction

$hfs(^{133}Cs) = 9.192.631.770\ Hz$

high precision frequency measurements are manna from heaven for molecular-level resolution structural analysis

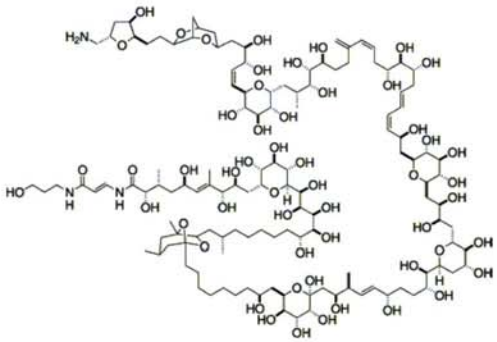
Nobel price 2005 (MPI Munich)

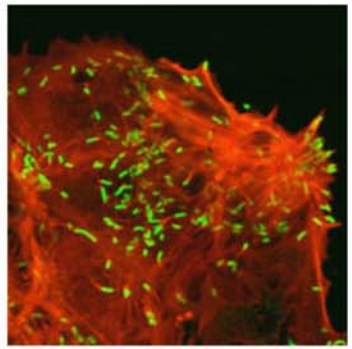
today:

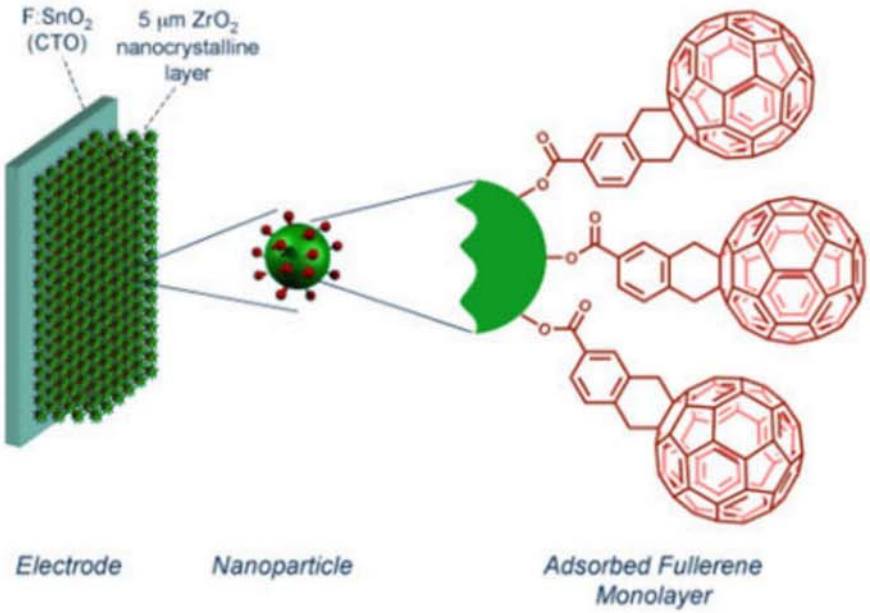
molecular-level perception of natural ecosystems requires educated handling of huge data sets in excess of 10^9 "pixels"

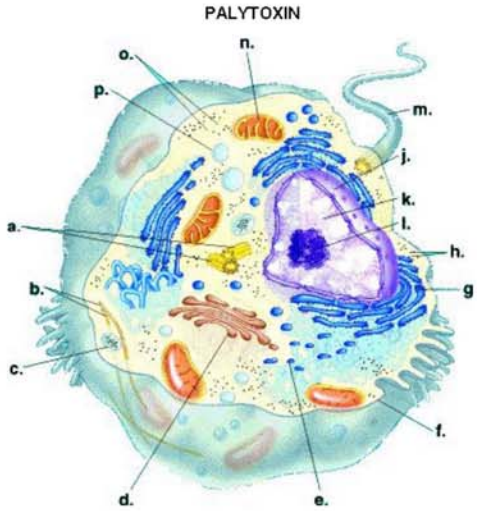
complex systems

any "*non-repetitive*" *non-protein*
natural or synthetic material









aspects of molecular complexity

atomic signature

molecular signature

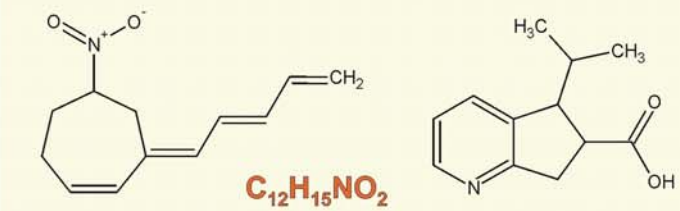
compositional

molecular formula

$C_{12}H_{15}NO_2$

FTICR mass spectrometry

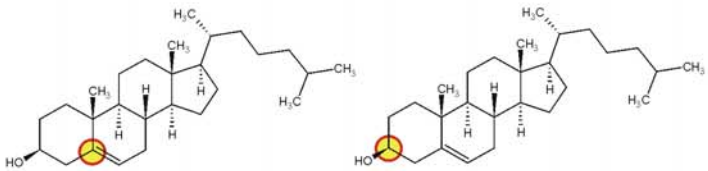
isomeric structures



atomic connectivities and spatial orientation

NMR spectroscopy

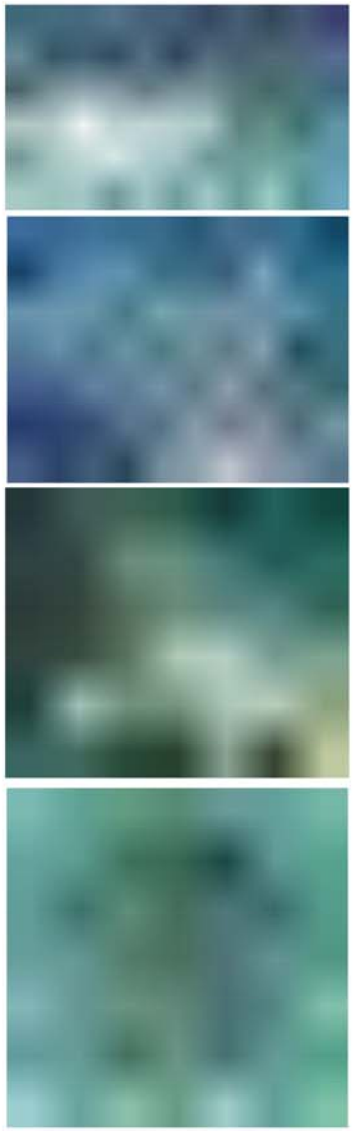
isotopomers



positions of (stable) isotopes within molecules

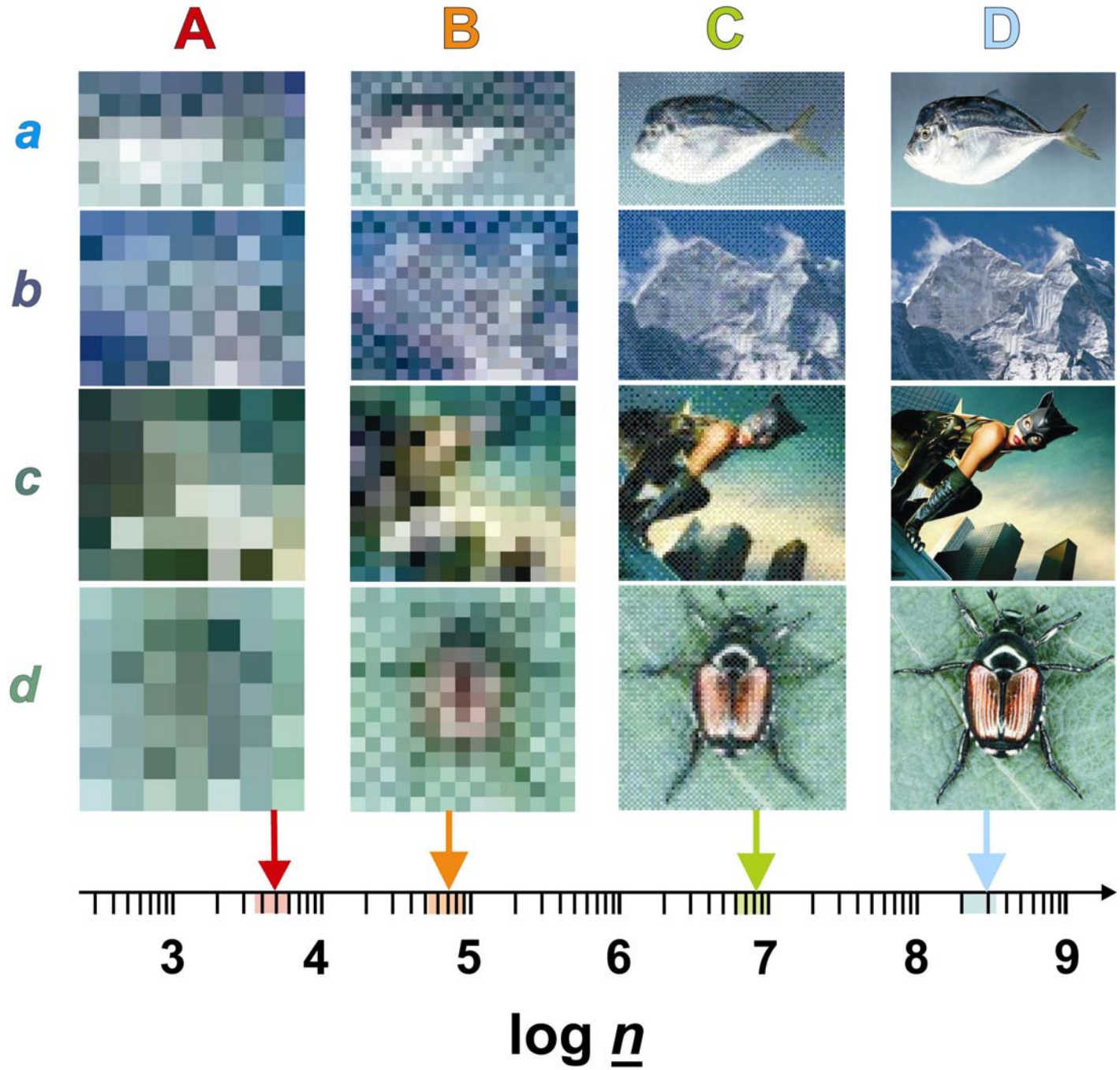
NMR spectroscopy

intrinsic averaging in low resolution analytical characterization of NOM

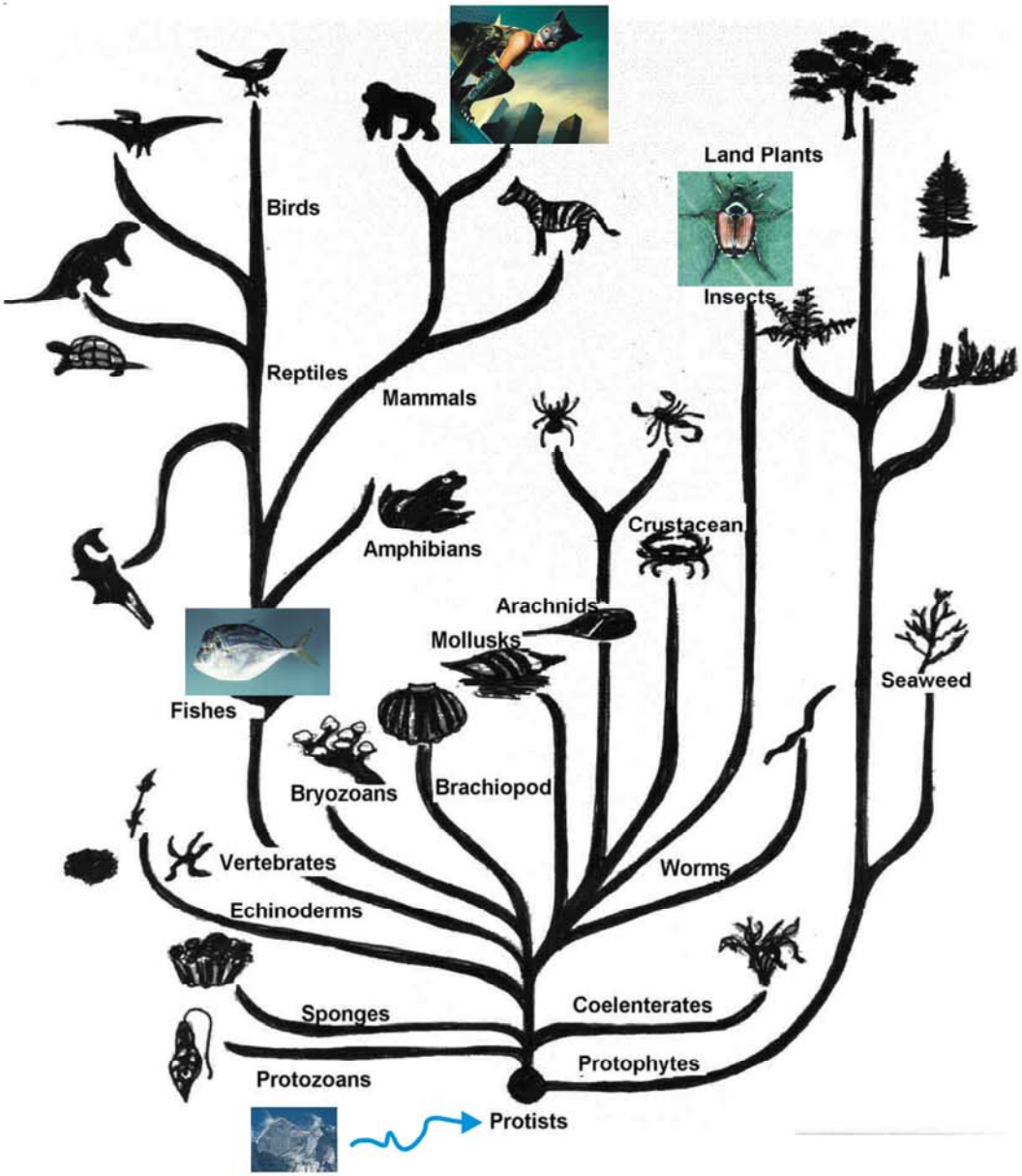


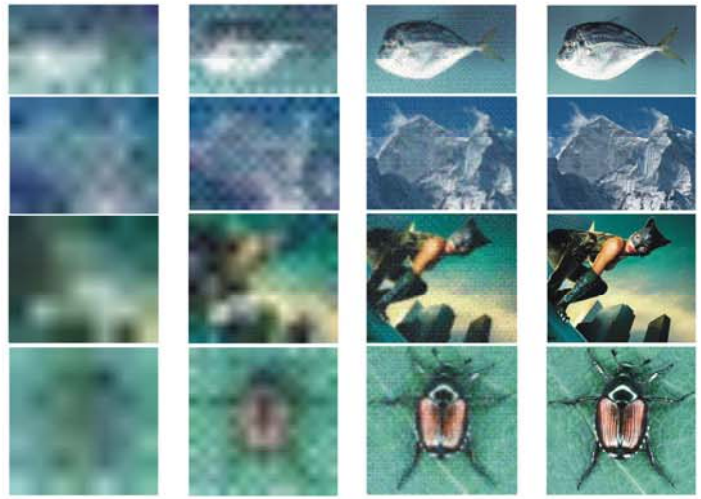
UV/VIS titration
elemental analysis

why do all NOM appear to be so similar ?


A
B
C
D
a
b
c
d
3
4
5
6
7
8
9
log n

sufficient resolution allows meaningful analysis of processes





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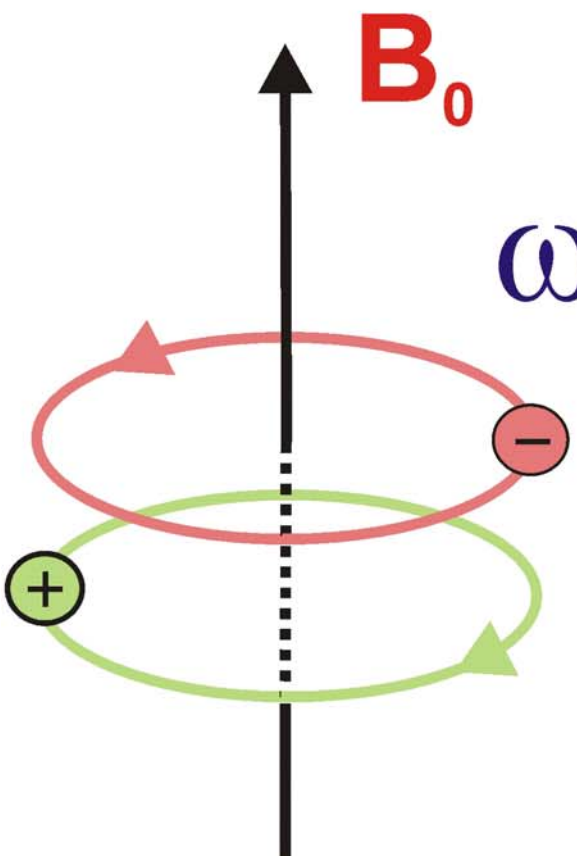
FTICR mass spectrometry / molecular process

orbital frequency ν_C directly relates to m / z

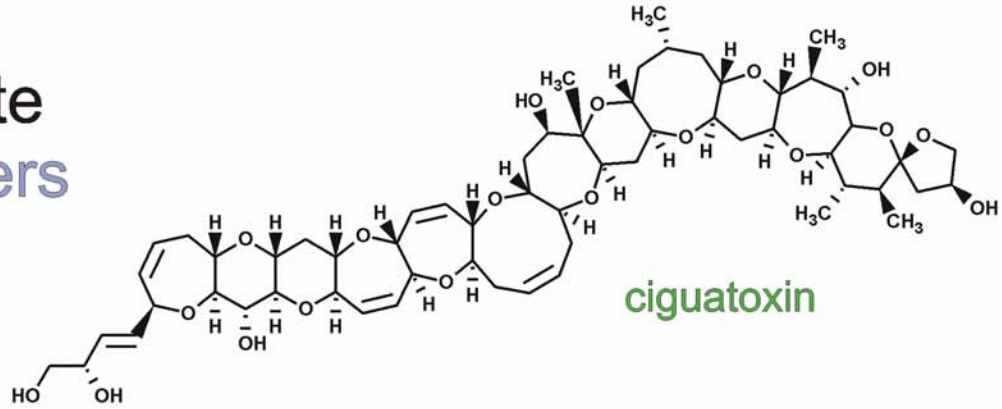


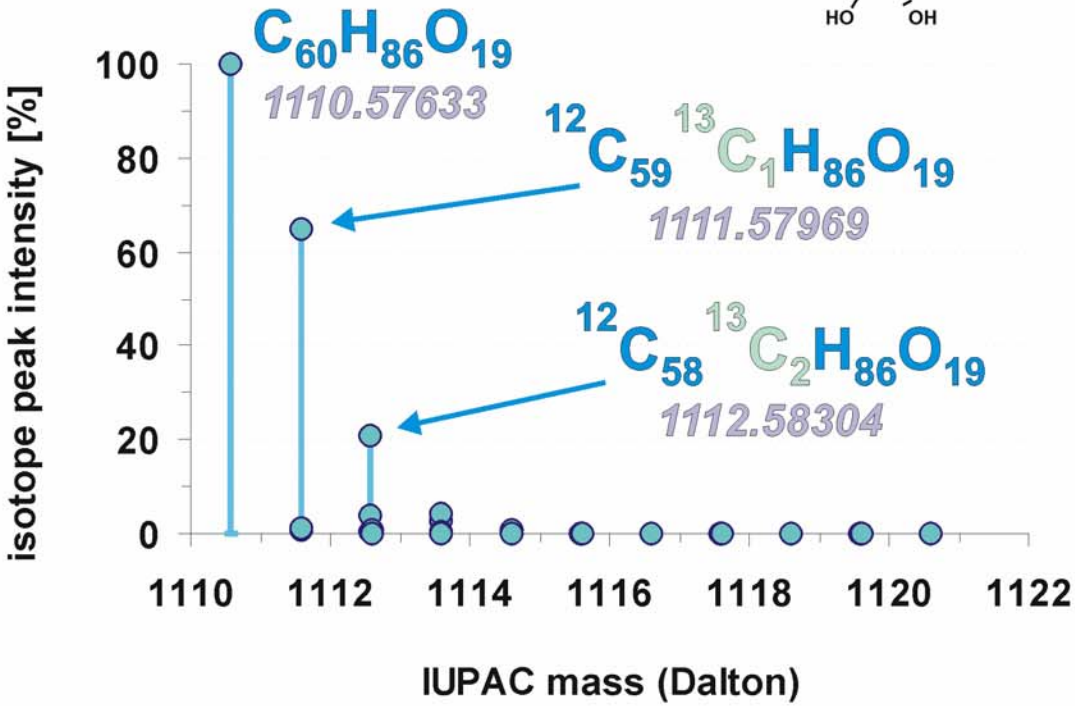
$$\omega_c = z \cdot B_0 / m$$

$$\omega_c = \frac{\nu_C}{2\pi} = \frac{1.535611\ B_0 \cdot z}{m}$$

FTICR mass spectrometry / molecular process

FTICR mass spectra indicate molecular ions of isotopomers

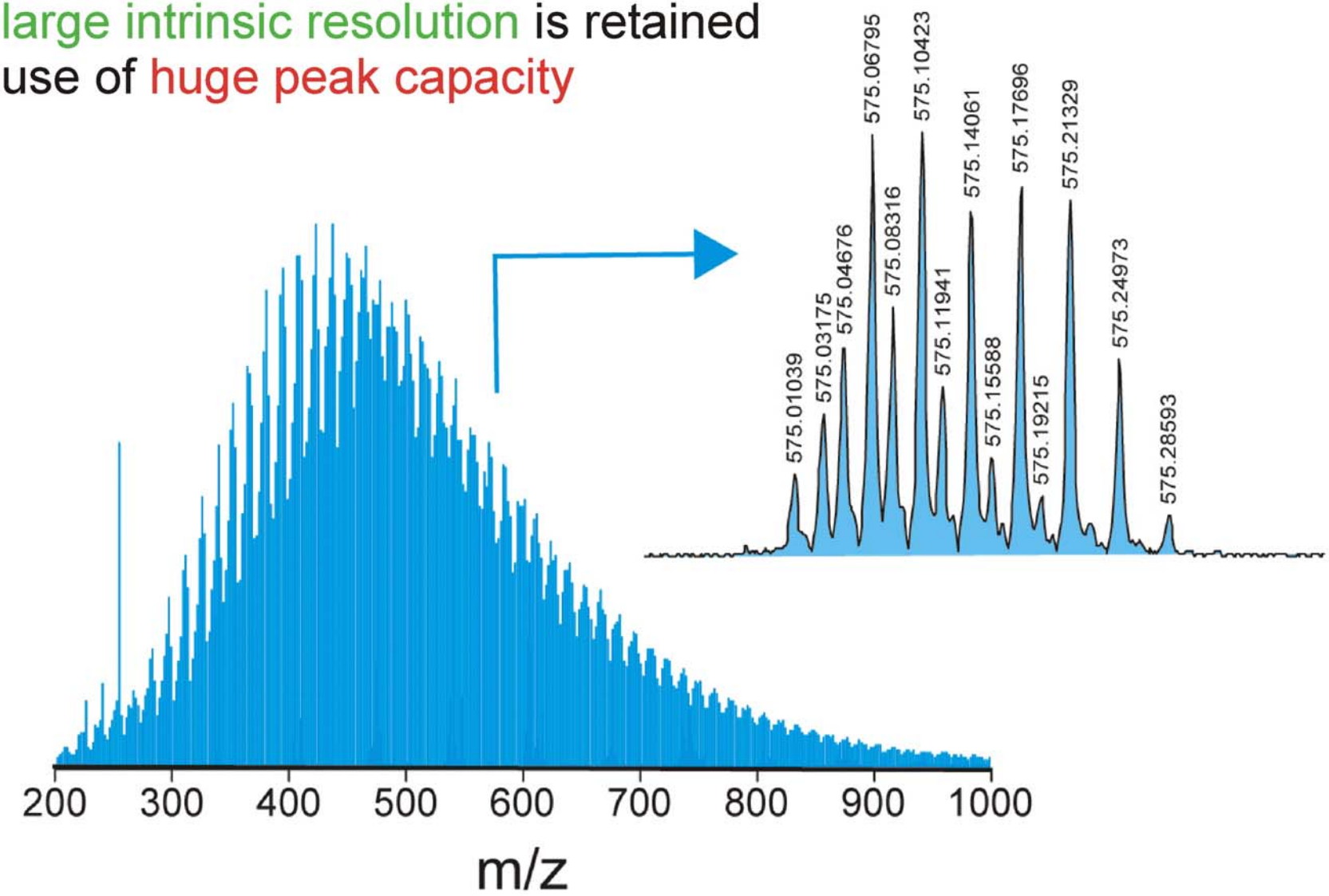




IUPAC mass of ciguatoxin $C_{60}H_{86}O_{19}$ = 1111.313 Da (reflecting average isotopic composition)

FTICR mass spectrometry / complex system

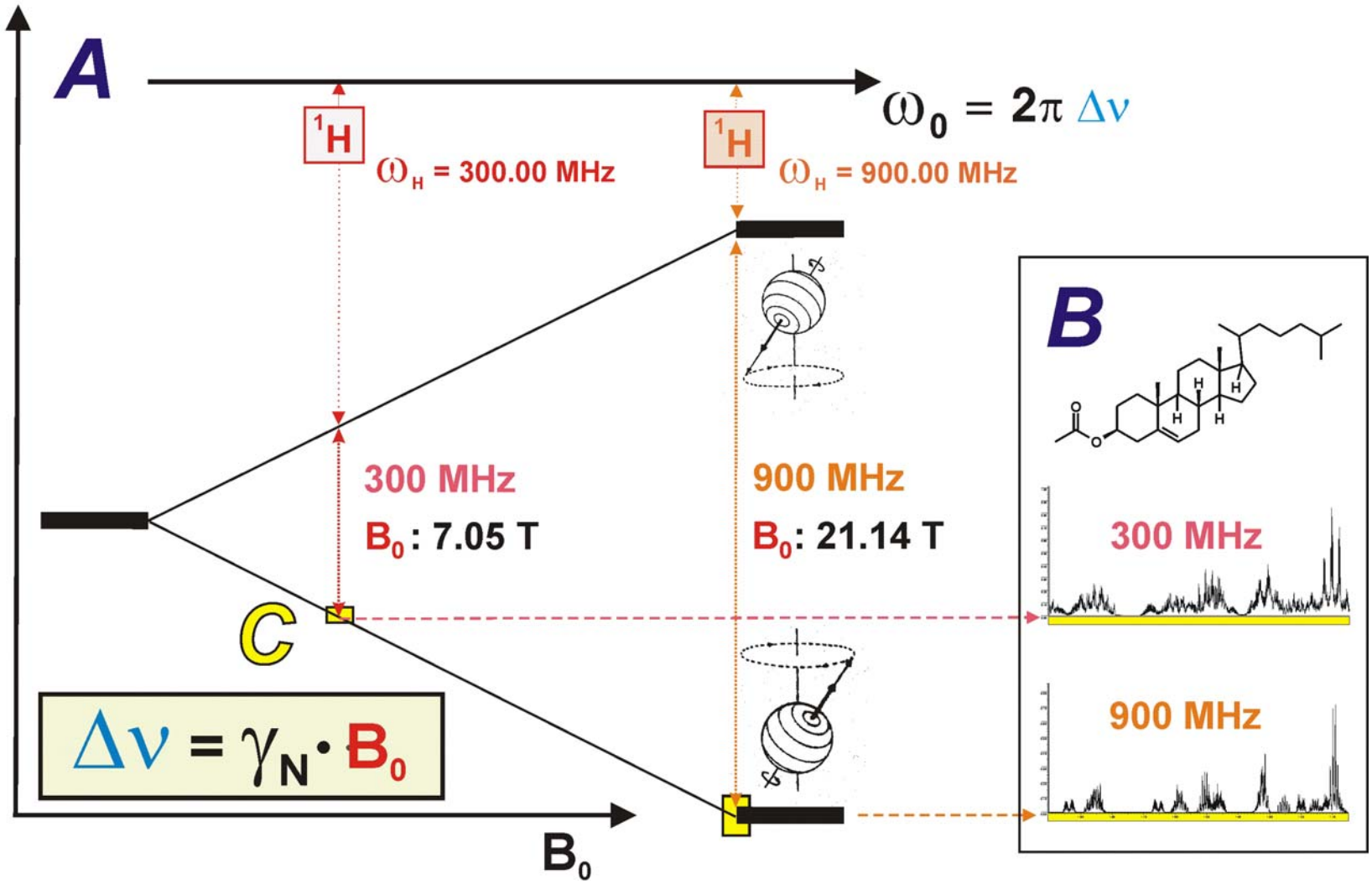
very large intrinsic resolution is retained
because of huge peak capacity



NMR spectroscopy / atomic process

transitions among individual atomic energy niveaus

$\Delta E = h\,\Delta\nu$



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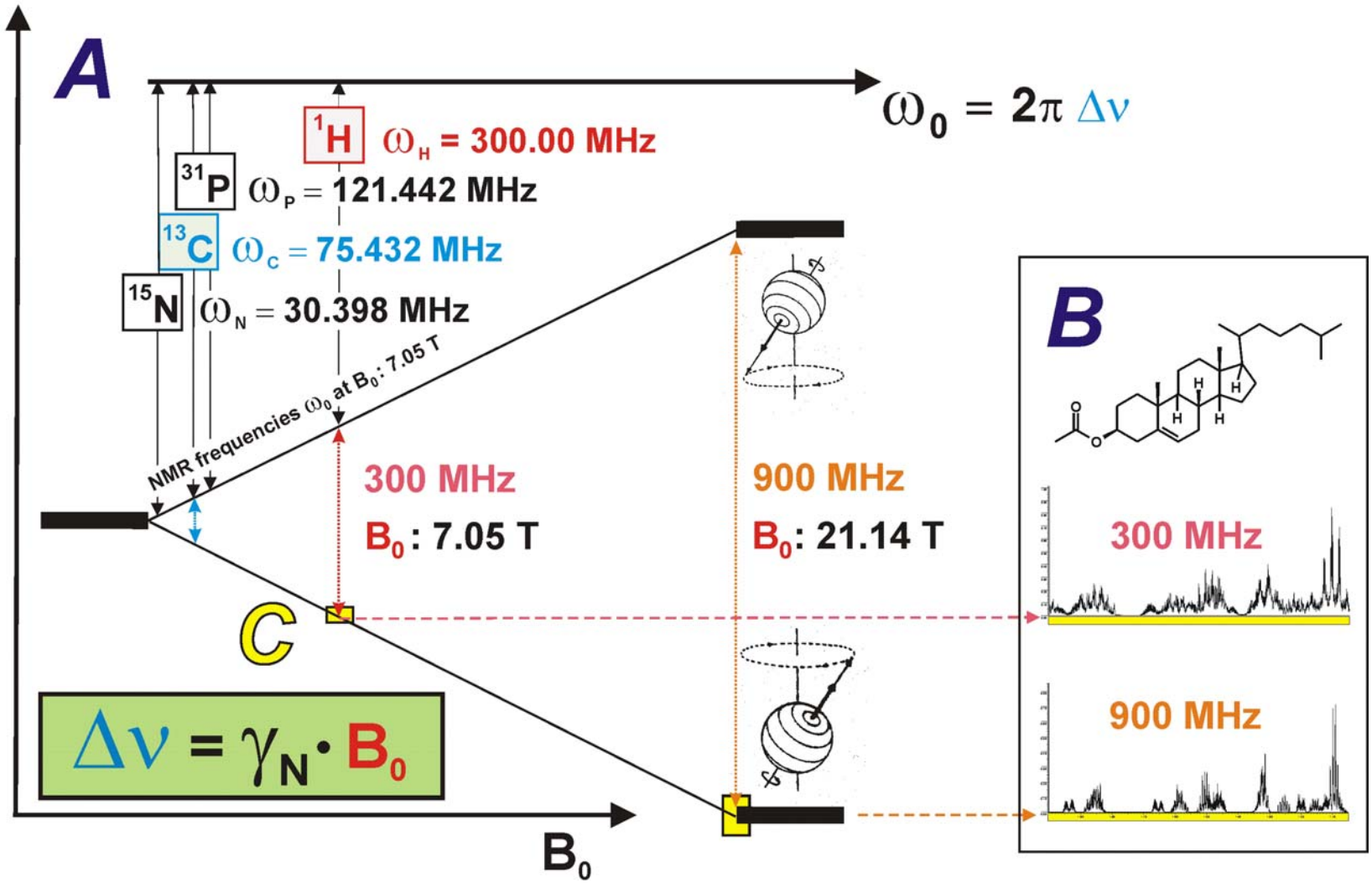
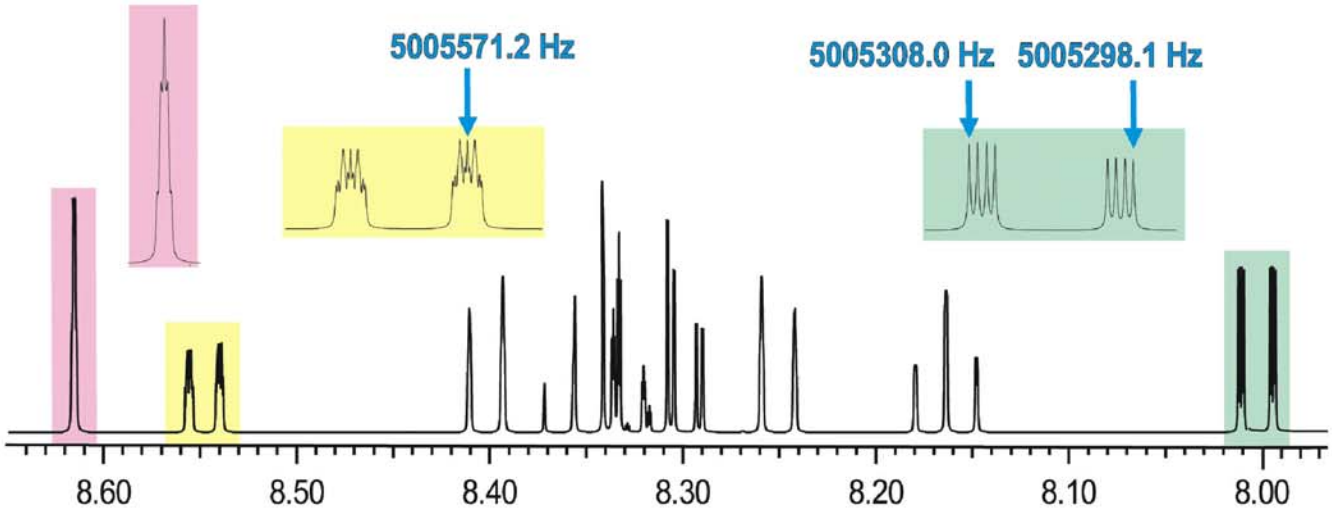


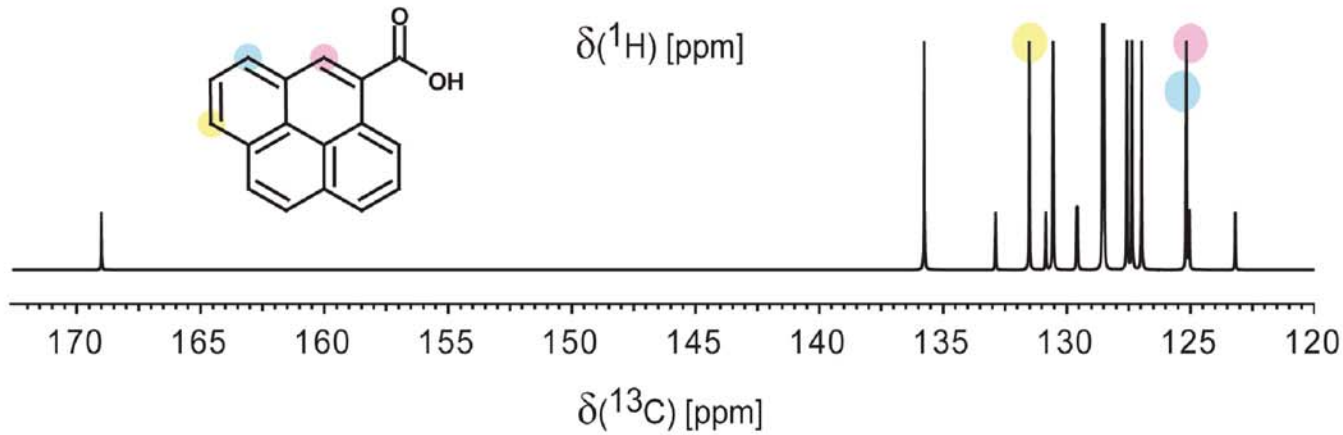
NMR spectroscopy / molecular process

atomic signatures in molecules allow
unambiguous assembly of (isomeric) structures

^{1}H NMR



^{13}C NMR



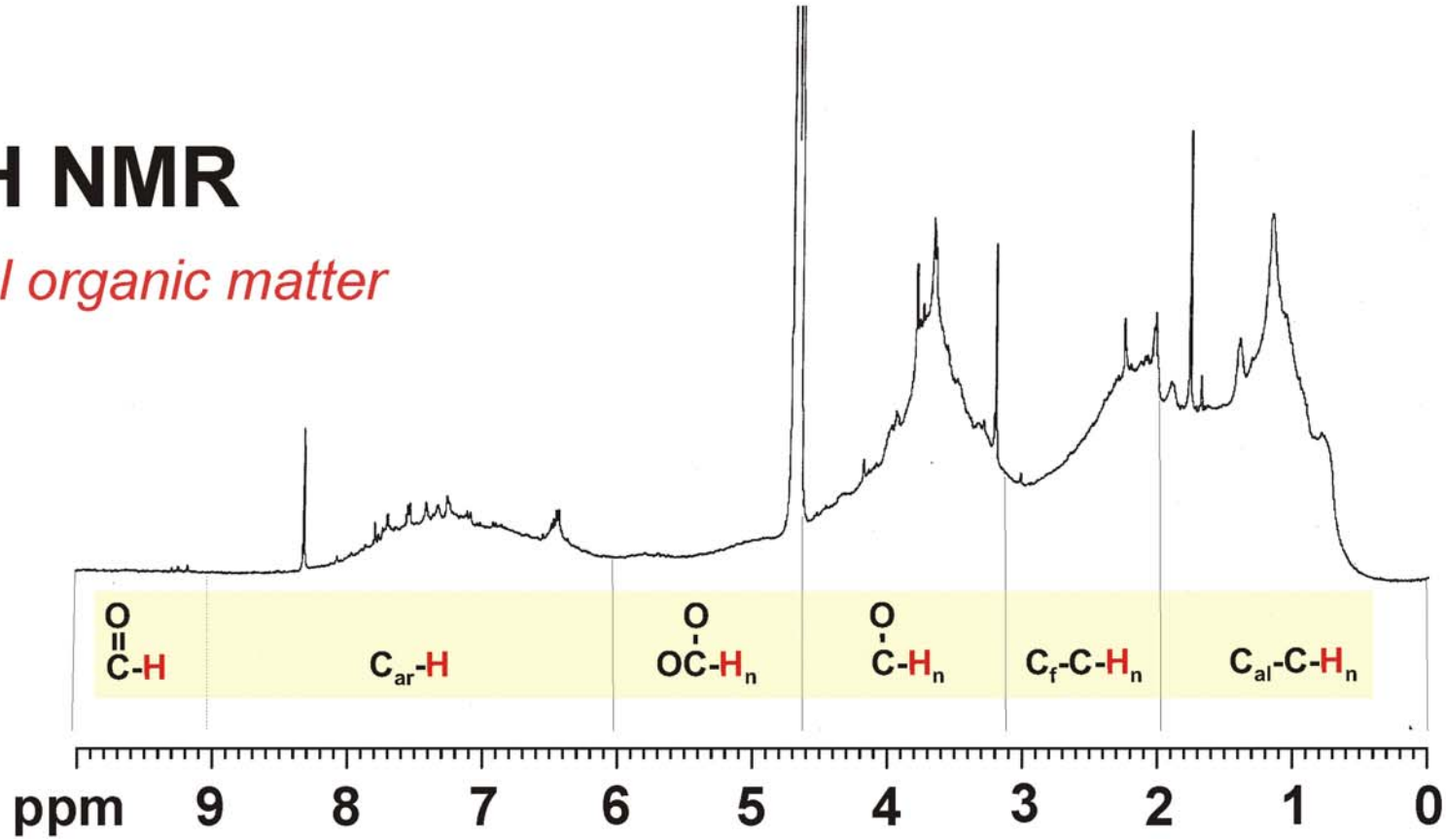
NMR spectroscopy / complex systems

NMR section integrals indicate **fundamental substructure regimes**

massive overlap ***interferes with*** ***resolution***

^{1}H NMR

natural organic matter

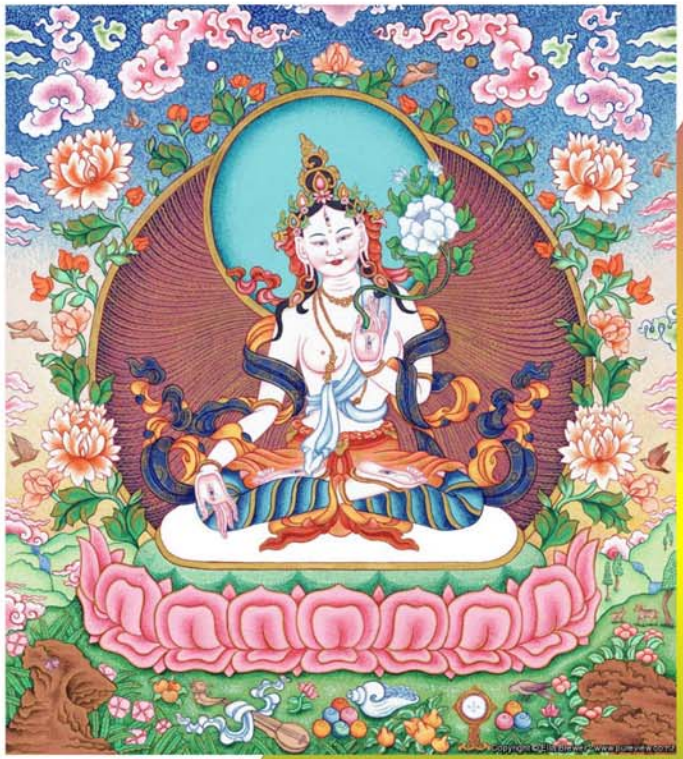


complex systems

high-precision frequency measurements are at the core of the ***two most insightful techniques***, which provide molecular resolution information about organic molecules

mass spectrometry

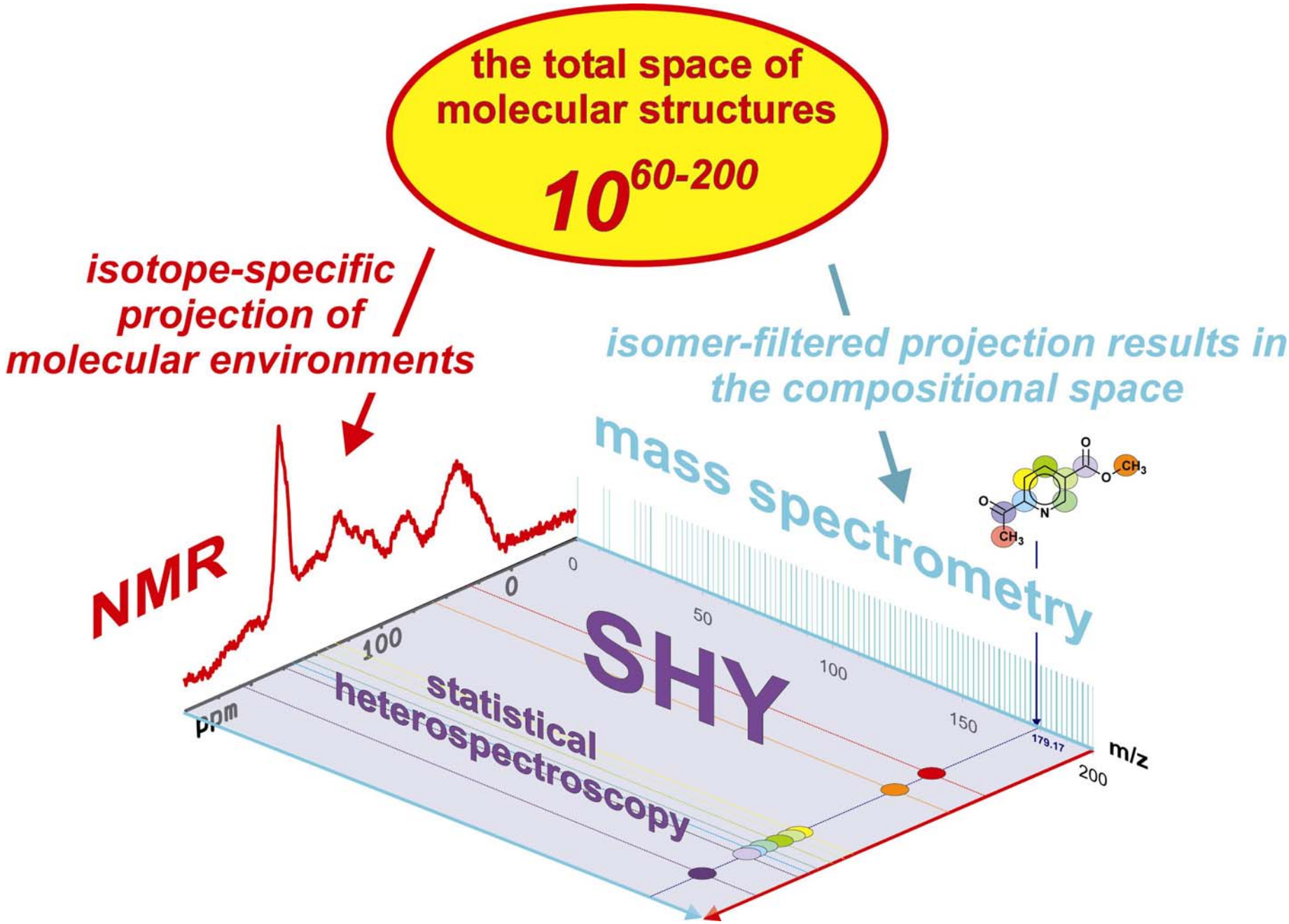
data reduced



$$e^{i\pi} = -1$$

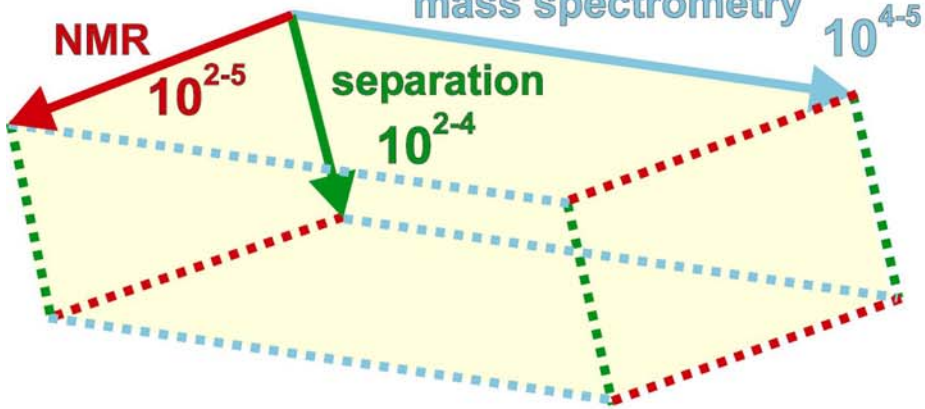
NMR spectroscopy

information-rich

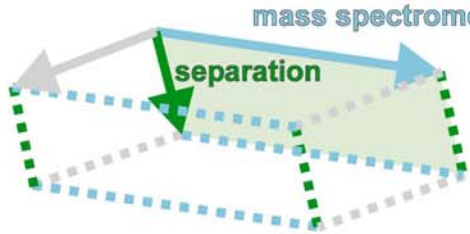

the total space of
molecular structures
$10^{60\text{-}200}$
isotope-specific
projection of
molecular environments
isomer-filtered projection results in
the compositional space
NMR
mass spectrometry
SHY
statistical
heterospectroscopy
ppm
0
0
50
100
100
150
179.17
m/z
200
O
O
CH3
O
N
CH3

$10^{60\text{-}200}$

structure space

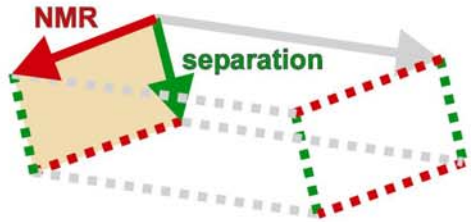


significant (analytical) bucket space: $10^{8\text{-}14}$



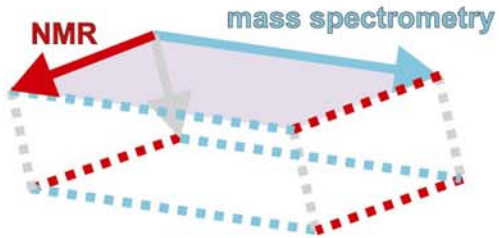
$10^{6\text{-}9}$

LC(CE) / MS



$10^{4\text{-}9}$

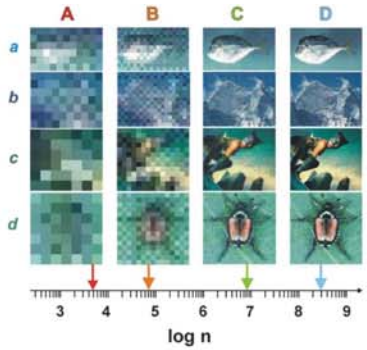
LC (CE) / NMR

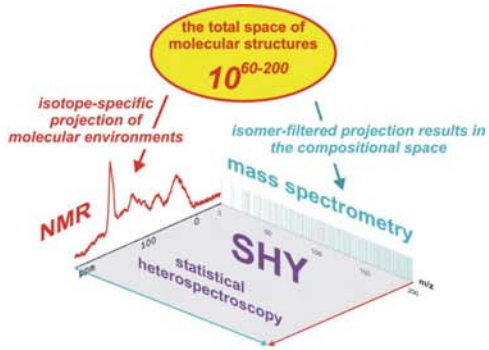


$10^{6\text{-}10}$

NMR / MS statistical hetero-spectroscopy (SHY)

...what is the utility of these novel tools....?





High-resolution organic structural spectroscopy already has advanced crucial paradigm shifts in structural characterization of natural organic matter (NOM)

Hertkorn et al., Anal. Bioanal. Chem. 2007, 389, 1311-1327

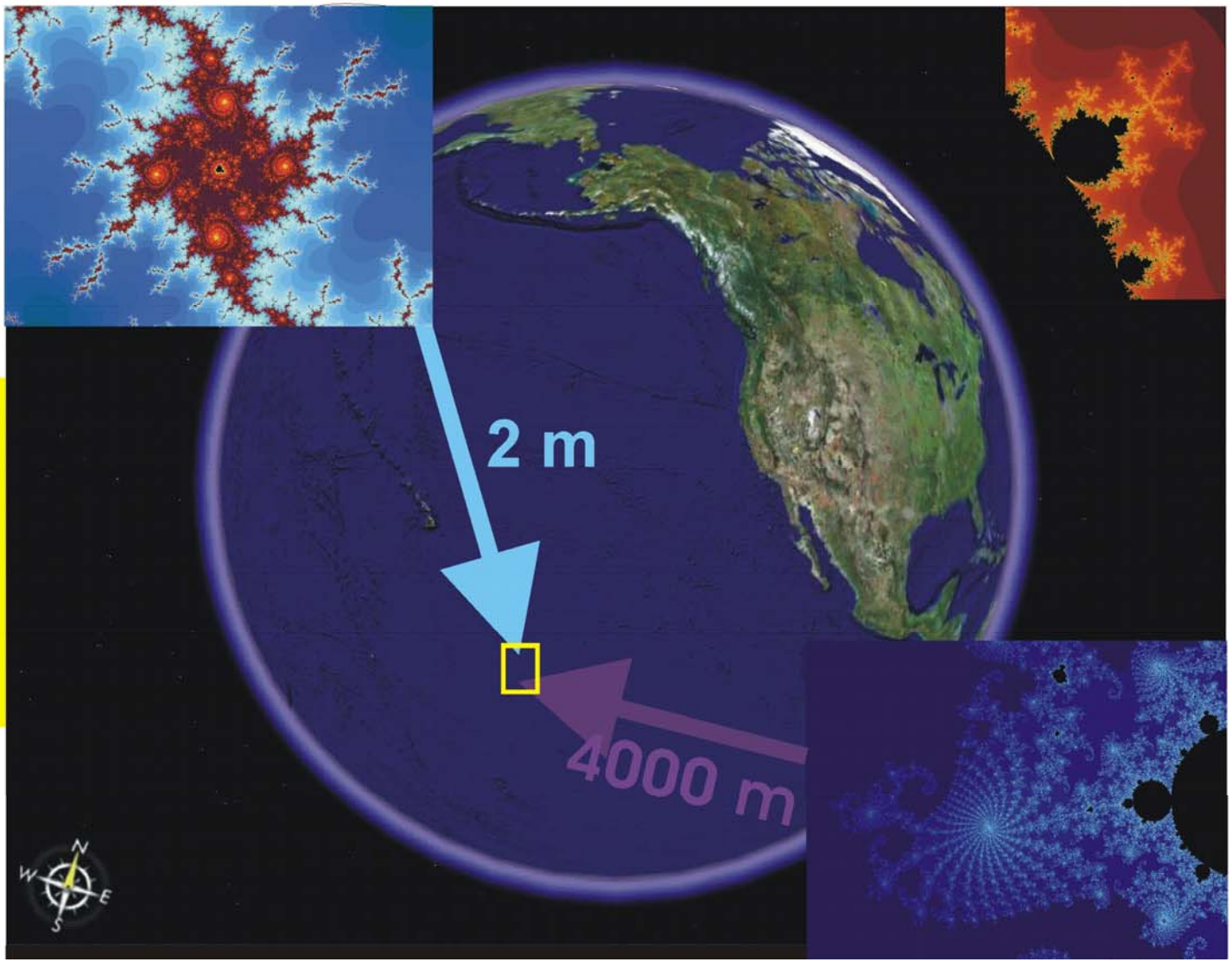
- discovery of carboxyl-rich alicyclic molecules (CRAM) as major constituents of NOM

Hertkorn et al., GCA 2006, 71, 2995-3010
Lam et al., Environ. Sci. Technol. 2007, 54, 8240-8247

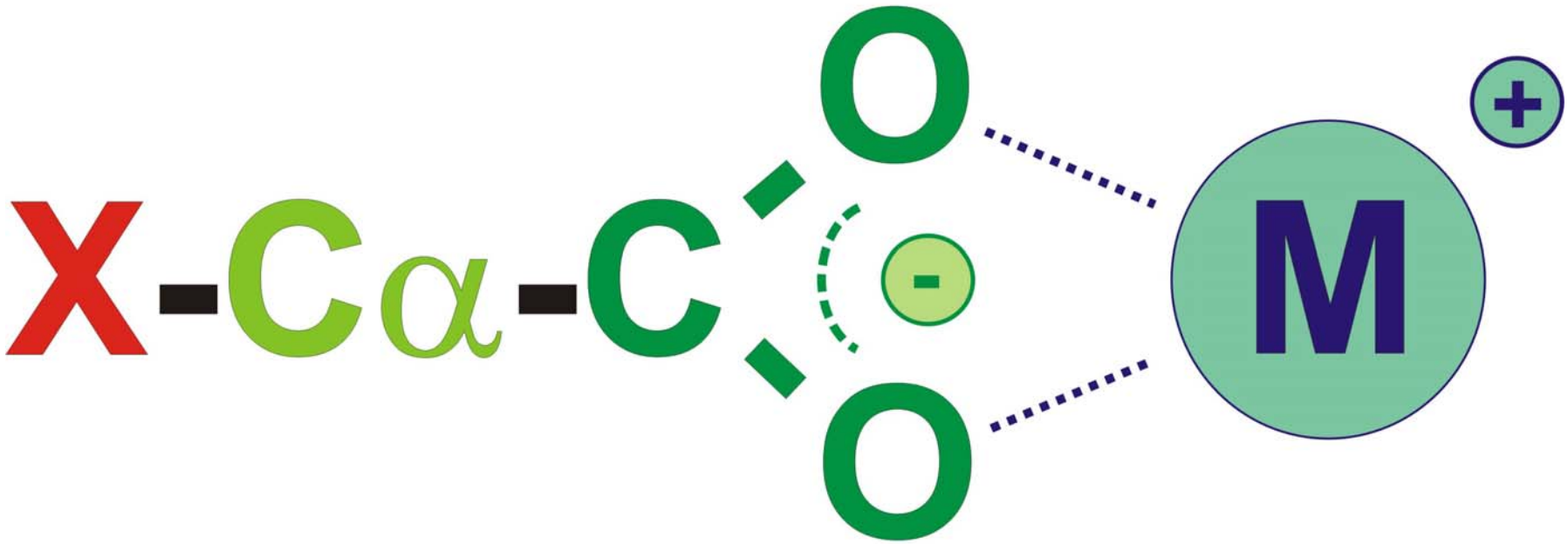
- large-scale molecular turnover of NOM on short time scales

Einsiedl et al., GCA, 2007, 71, 5474-5482

CRAM (carboxyl-rich alicyclic molecules) have been first identified in the surface and deep Pacific ocean; there is good reason to postulate CRAM as a major constituent of any NOM



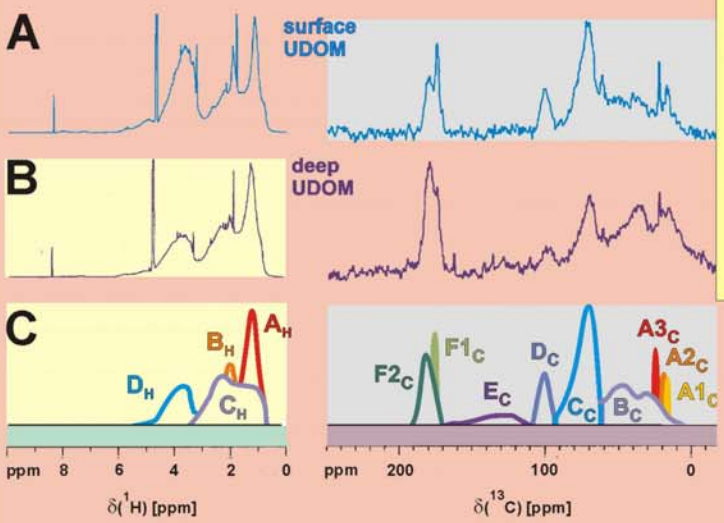
aliphatic polycarboxylic acid

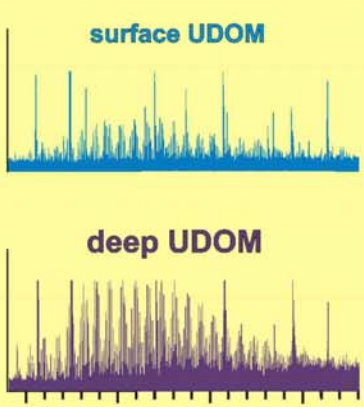


carboxyl-rich alicyclic molecules CRAM

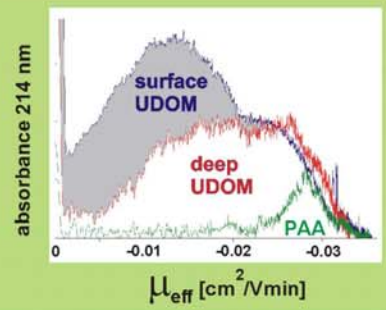
complementary techniques have led to the identification of CRAM (carboxylic-rich alicyclic molecules) in NOM

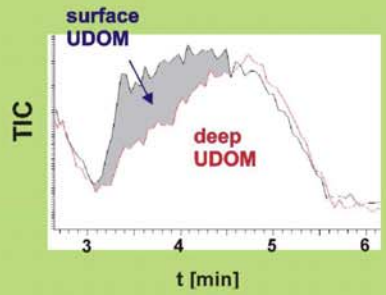
NMR
quantification





mass spectrometry
resolution

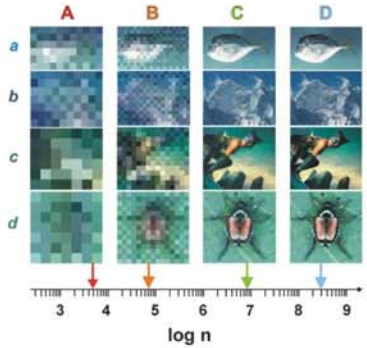


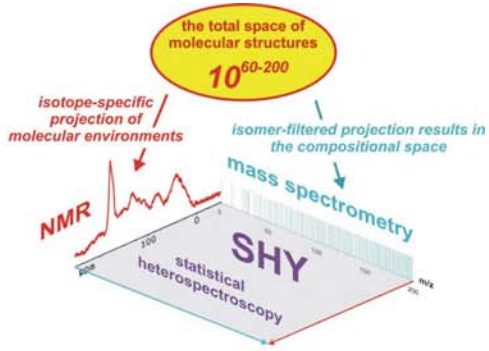


capillary electrophoresis
validation

ACS_slide_compl_method.cdr

...what is the utility of these novel tools....?





High-resolution organic structural spectroscopy already has advanced crucial paradigm shifts in structural characterization of natural organic matter (NOM)

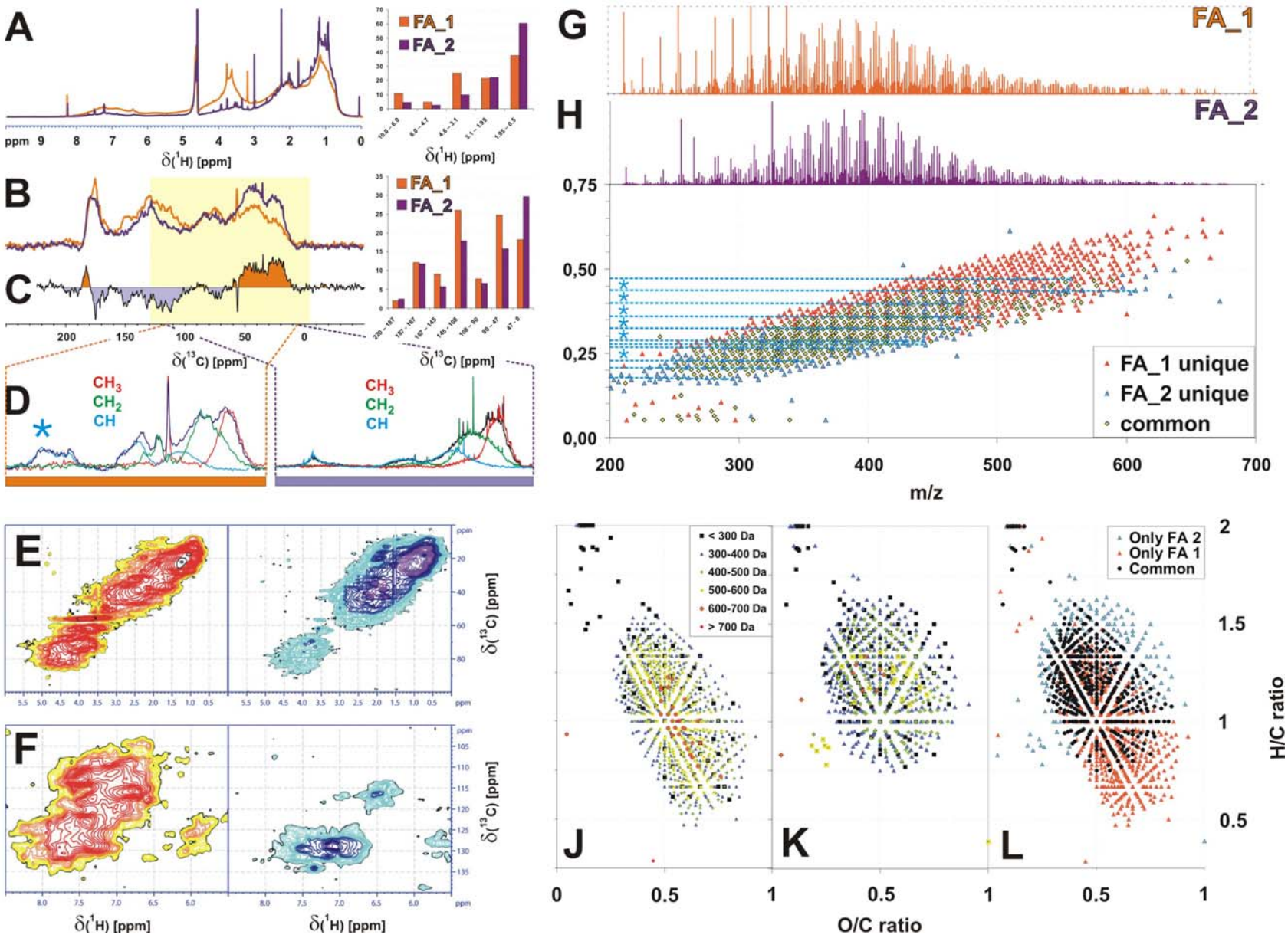
Hertkorn et al., Anal. Bioanal. Chem. 2007, 389, 1311-1327

- discovery of carboxyl-rich alicyclic molecules (CRAM) as major constituents of NOM

Hertkorn et al., GCA 2006, 71, 2995-3010
Lam et al., Environ. Sci. Technol. 2007, 54, 8240-8247

- large-scale molecular turnover of NOM on short time scales

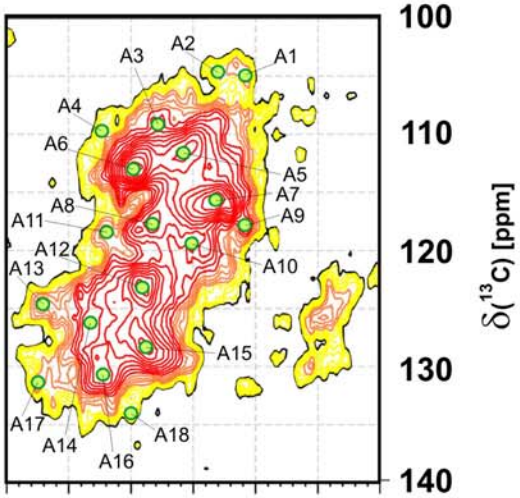
Einsiedl et al., GCA, 2007, 71, 5474-5482


A
B
C
D
E
F
G
H
J
K
L
FA_1
FA_2
ppm
δ(¹H) [ppm]
δ(¹³C) [ppm]
CH3
CH2
CH
FA_1 unique
FA_2 unique
common
m/z
0,00
0,25
0,50
0,75
200
300
400
500
600
700
< 300 Da
300-400 Da
400-500 Da
500-600 Da
600-700 Da
> 700 Da
Only FA 2
Only FA 1
Common
O/C ratio
H/C ratio
0
0.5
1
1.5
2

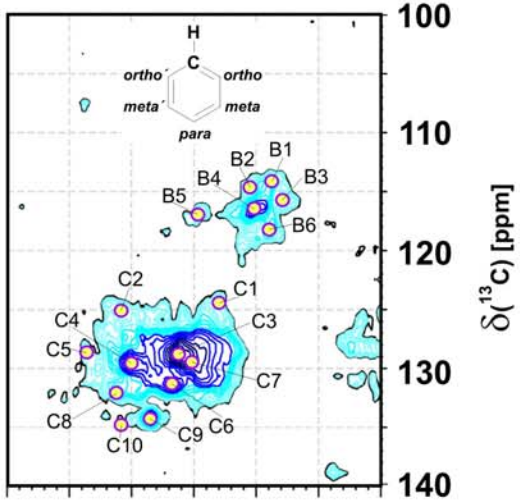
evolution of groundwater during 60 years

SPARIA analysis (Substitution Patterns in Aromatic Rings by Increment Analysis)

FA1
0 y



FA2
60y



FA1

peak	δ(¹H) [ppm]	δ(¹³C) [ppm]	ortho	meta	para	meta´	ortho´	percent	ne
A1	6.57	104.6						27	67
A2	6.79	104.4						45	20
A3	7.28	108.7						37	45
A4	7.73	109.5						50	2
A5	7.08	111.4						32	38
A6	7.49	112.6						41	59
A7	6.82	115.5						19	52
A8	7.33	117.4						17	59
A9	6.57	117.6						33	36
A10	7.13	120.2						31	78
A11	7.39	118.2						17	78
A12	7.42	122.8						14	114
A13	8.21	124.2						25	75
A14	7.83	126.0						14	122
A15	7.38	128.0						28	43
A16	7.74	130.3						33	812
A17	8.25	131.1						41	87
A18	7.50	133.6						52	48

FA2

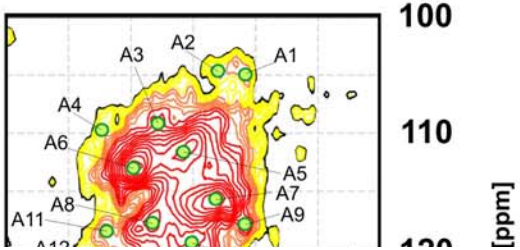
peak	δ(¹H) [ppm]	δ(¹³C) [ppm]	ortho	meta	para	meta´	ortho´	percent	ne
B1	6.39	113.9						36	33
B2	6.53	114.6						36	29
B3	6.29	115.3						100	10
B4	6.52	116.0						23	26
B5	6.97	116.6						24	104
B6	6.39	117.9						36	14
C1	6.79	124.1						33	30
C2	7.58	124.8						21	63
C3	7.12	128.3						19	36
C4	7.51	129.1						29	59
C5	7.86	128.3						28	97
C6	7.17	131.0						28	42
C7	7.05	128.9						43	30
C8	7.62	131.6						33	93
C9	7.34	133.8						36	56
C10	7.59	134.4						87	15

	aromatic substitution, composed of hydrogen
	aromatic substitution, composed of neutral carbon substituents
	aromatic substitution, composed of electron-withdrawing carbonyl derivative substituents
	aromatic substitution, composed of electron-donating oxygen substituents

evolution of groundwater during 60 years

SPARIA analysis (Substitution Patterns in Aromatic Rings by Increment Analysis)

FA1



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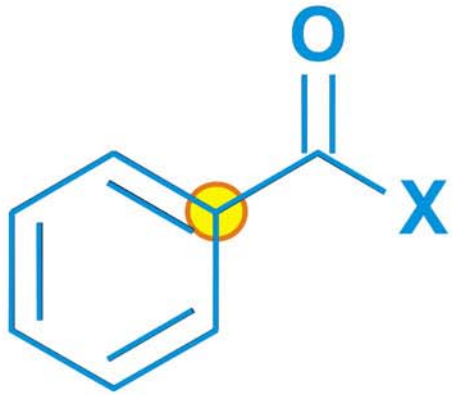
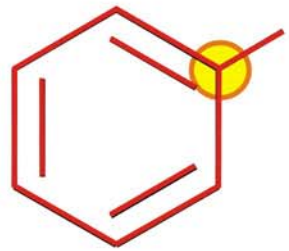
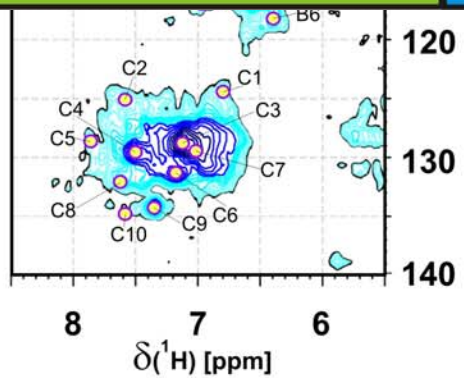




FA2
60y



B6	6.39	117.9						36	14
C1	6.79	124.1						33	30
C2	7.58	124.8						21	63
C3	7.12	128.3						19	36
C4	7.51	129.1						29	59
C5	7.86	128.3						28	97
C6	7.17	131.0						28	42
C7	7.05	128.9						43	30
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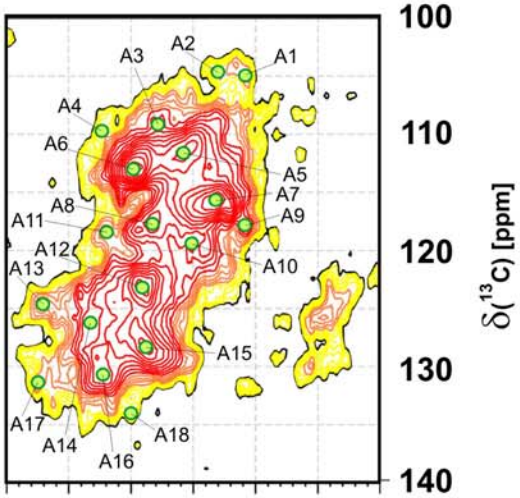
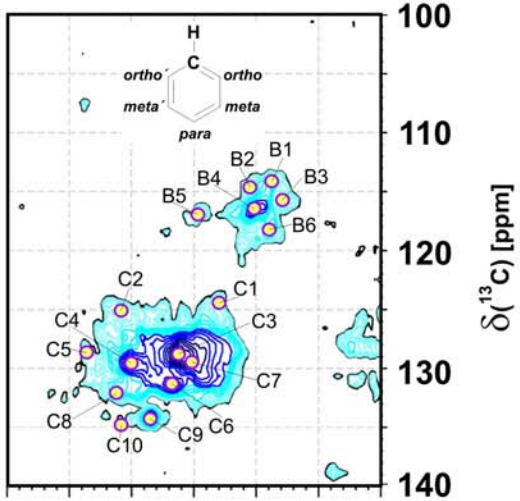


FA2
60y



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	aromatic substitution, composed of electron-donating oxygen substituents

conclusion

natural organic matter (NOM) is NOT refractory on the molecular level

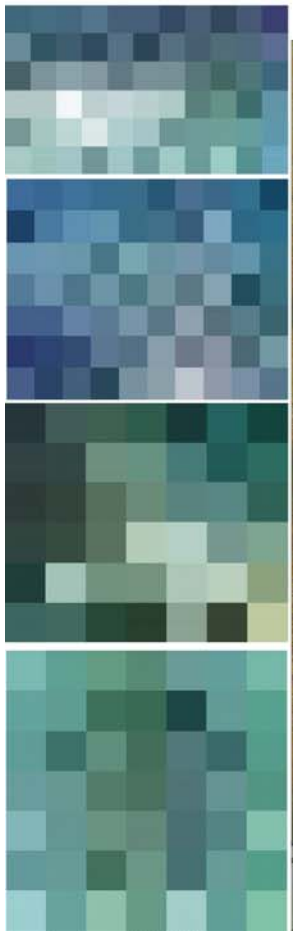
the current perception of NOM appearing refractory is mainly an inevitable consequence of intrinsic averaging produced by low-resolution analytical methods

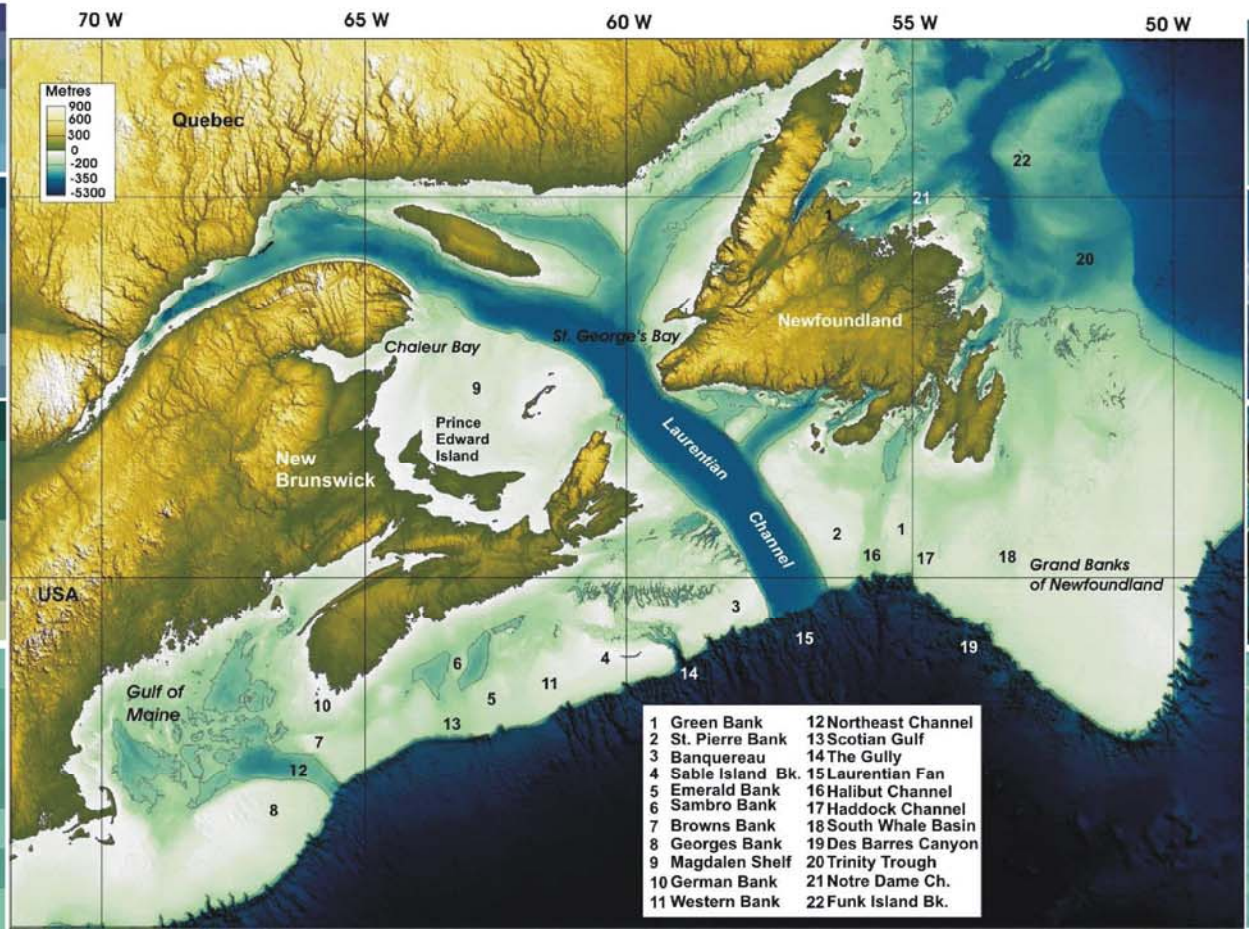
.....and most often likely false

ten-year-vision:

authentic molecular representation of complex natural systems

2005 → **2015**





peeking beyond the compositional space

$10^{60\text{-}200}$

chemistry

MS NMR

$e^{i\pi} = -1$

separation / miniaturization

4000000 $

$10^{8\text{-}14}$

$10^{4\text{-}5}$

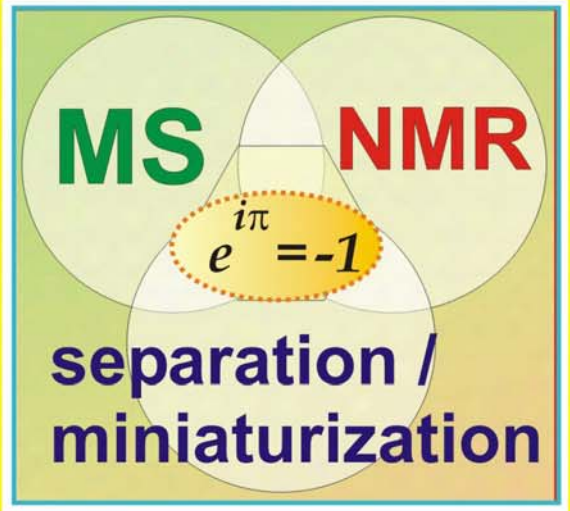
FTICR mass spectrometry

integrated systems analysis

complementary

*.omics approach

molecular-level structural analysis of non-repetitive complex unknowns



4000000 $

NMR — *quantification*

mass spectrometry — *resolution*

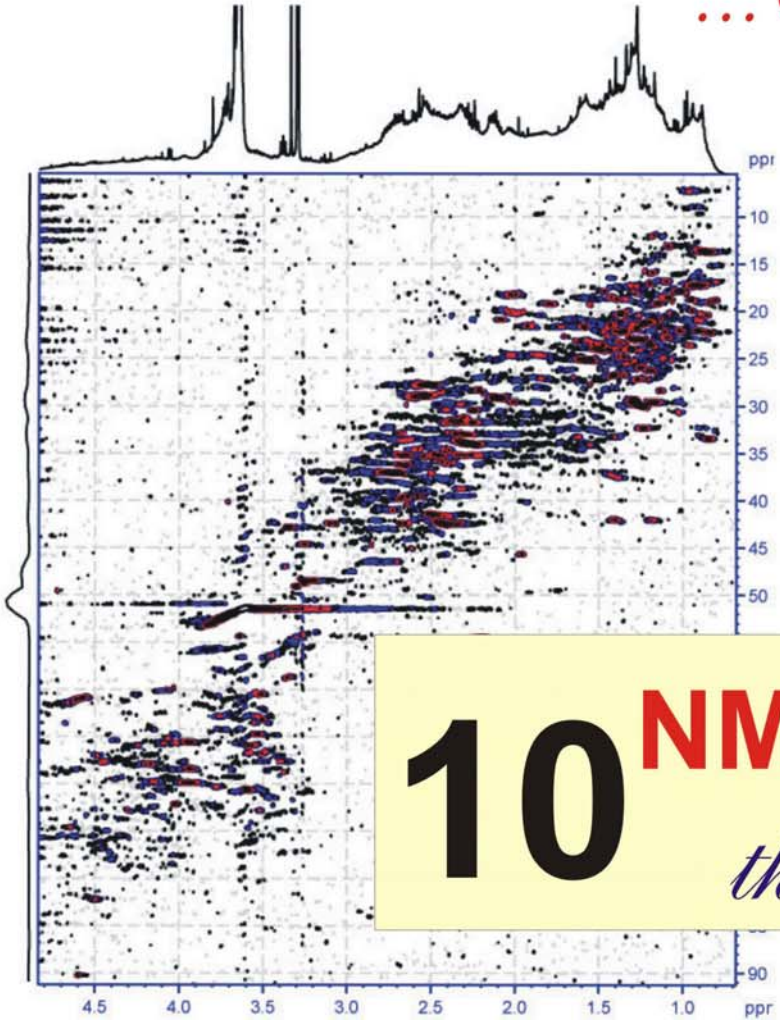
high-performance separation — *validation*

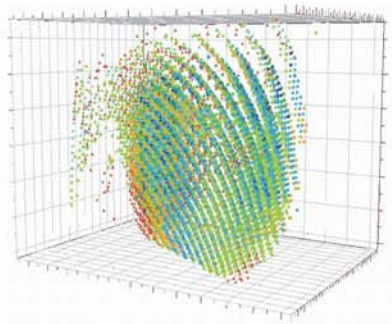
integrated mathematical systems analytics

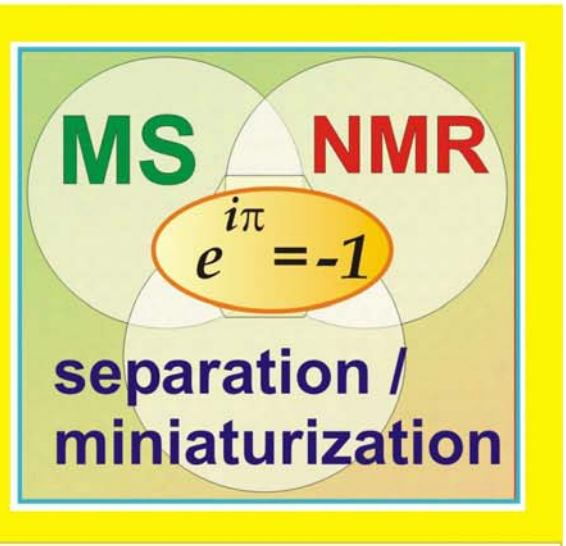
complementary techniques, aspects, brains

conclusion

... I want to stay in NMR







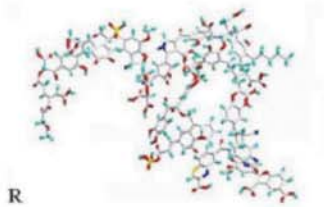
10 NMR+FTMS+sep+ $e^{i\pi}=-1$

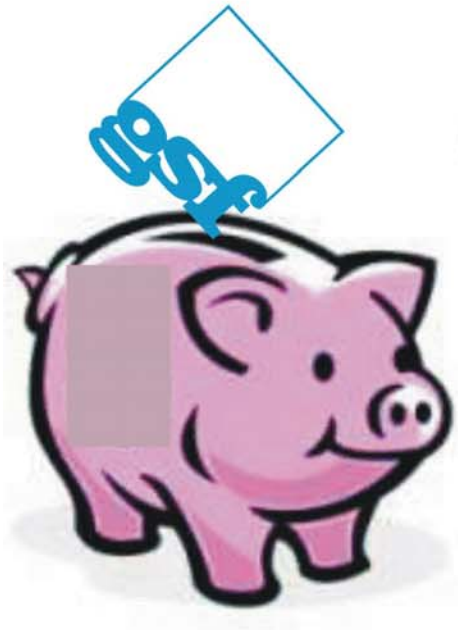
the unbeatable combination

acknowledgements

S. Schulte-Hostede
A. Kettrup *for generous and wonderful freedom*

our worldwide partners

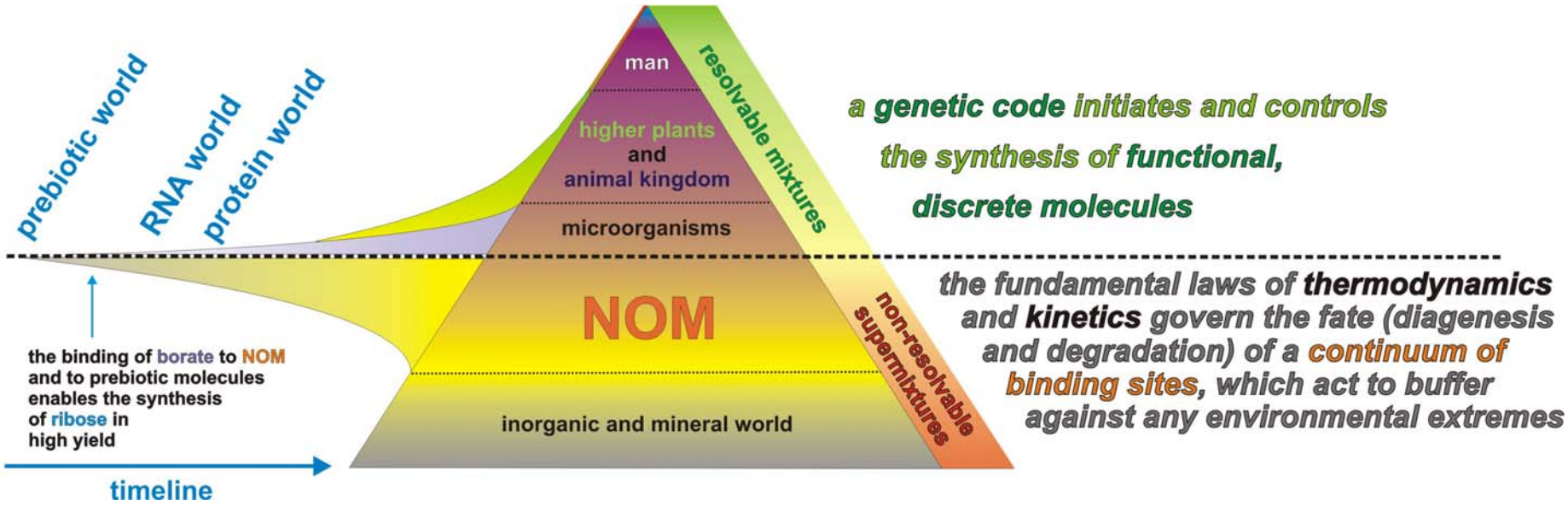




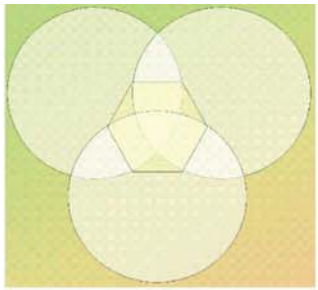
support: Alexander von Humboldt-Stiftung,
DFG, EU, DAAD
NSF, GIF, GSF

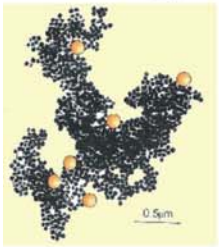
group BioGeomics

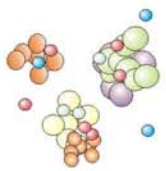
coevolution of biochemistry and natural organic matter (NOM)



molecular-level structural analysis of NOM







particulate

bulk composition

1µm

colloidal

NOM

backbone
functional groups
interactions

terrestrial, limnic, marine origin
NOM fractions
NOM chemical reaction products

3nm

molecular

molecular level structural detail

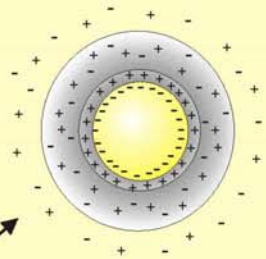
capillary electrophoresis

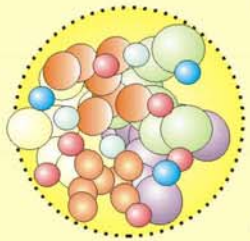
solution behavior, charge density, size and charge distributions, weak interactions

the retention time / electrophoretic mobility carries itself (independent) structure-specific information

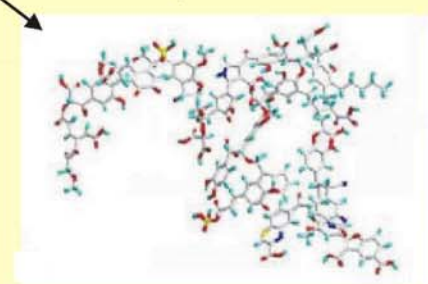
mass spectrometry

molecular formula of all ions present
complex mixture analysis
information concerning structure and reactivity





determination and assessment of significance of chemical structures, responsible and involved in a specific function of NOM



NMR spectroscopy

unsurpassed detailed information about short range molecular order

multinuclear NMR
multidimensional NMR

molecularly-resolved non-target analysis

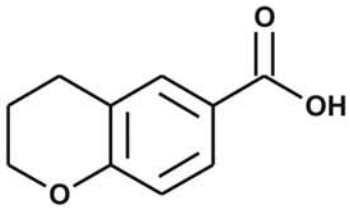
molecular diversity:

number of feasible isomeric molecules in one cubic meter

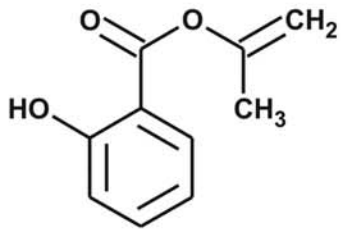
a typical molecule with nominal mass 178 Dalton $C_{10}H_{10}O_3$ (six DBE):

count of chemically relevant isomers: **$1.1133 * 10^7$**

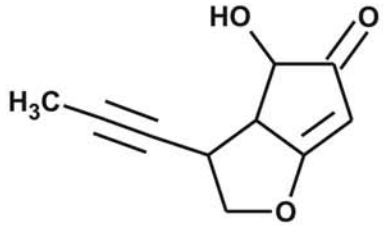
typical van der Waals volumes of $C_{10}H_{10}O_3$ isomers:



153.4 Å^3



164.3 Å^3

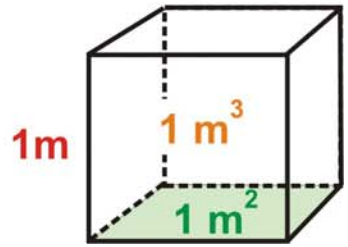


$C_{10}H_{10}O_3$

160.3 Å^3

feasible count of $C_{10}H_{10}O_3$ molecules in one cubic meter

$1 / 160\ Å^3 \xrightarrow{V^{-1}} 6.3 * 10^{27}\ m^{-3}$

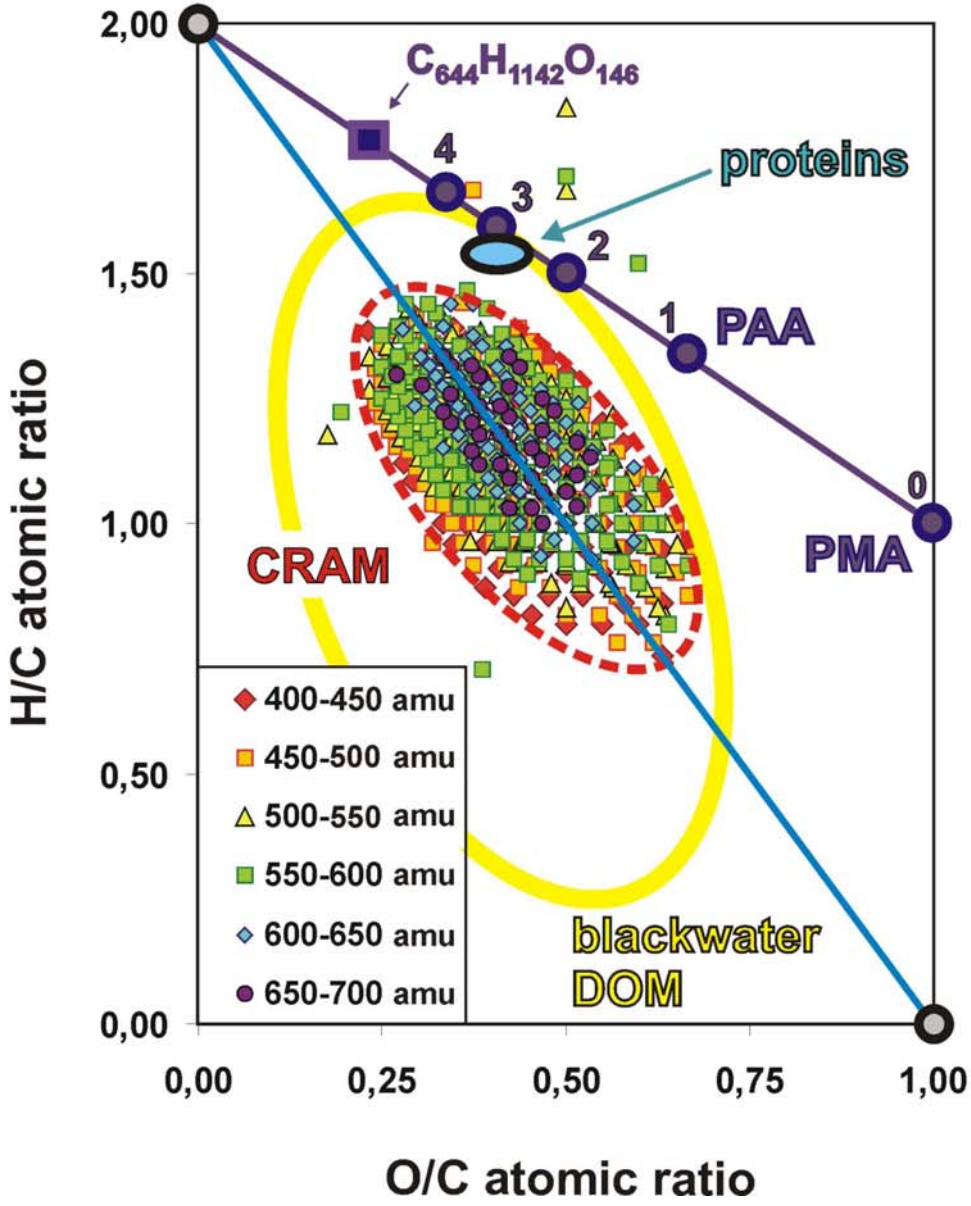


van Krevelen diagram

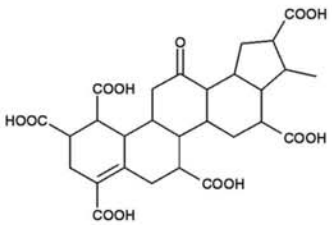
*Visser, ES&T, **17** (1983) 412-417.*

a powerful visual representation of complex mass spectra, with far reaching implications for the structural analysis of NOM

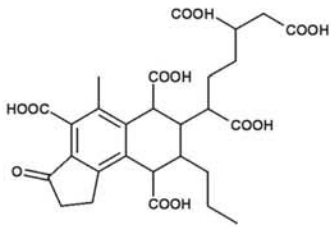
*indicates unsaturation of **CRAM** in excess of carboxylic groups*



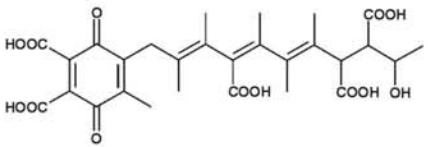
NMR properties serve to discriminate between (*classes of*) $C_{28}H_{32}O_{13}$ isomers (IUPAC mass: 576.546 Da)



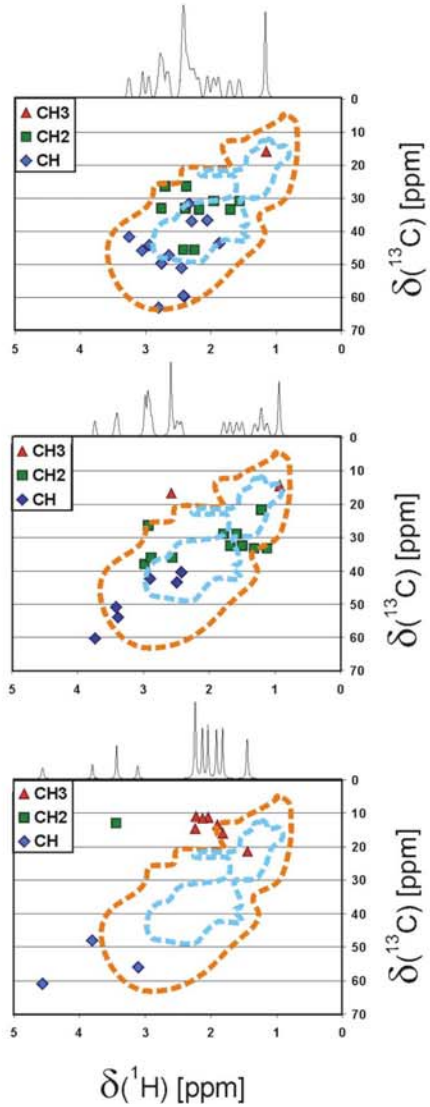
isomer I

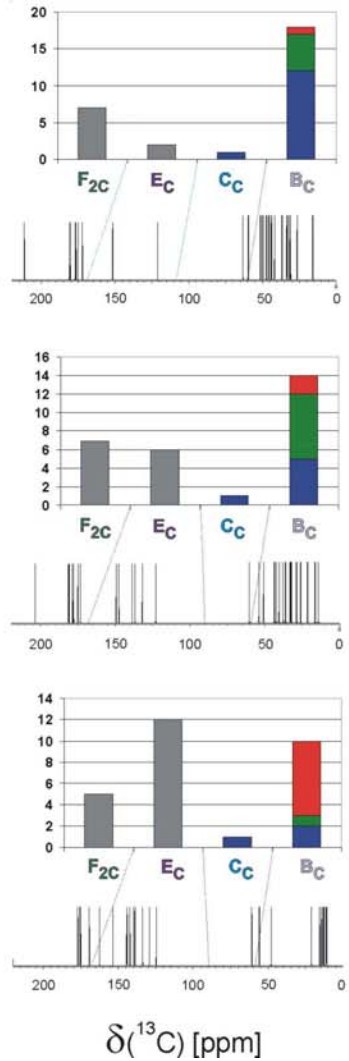


isomer II



isomer III





potential significance of CRAM

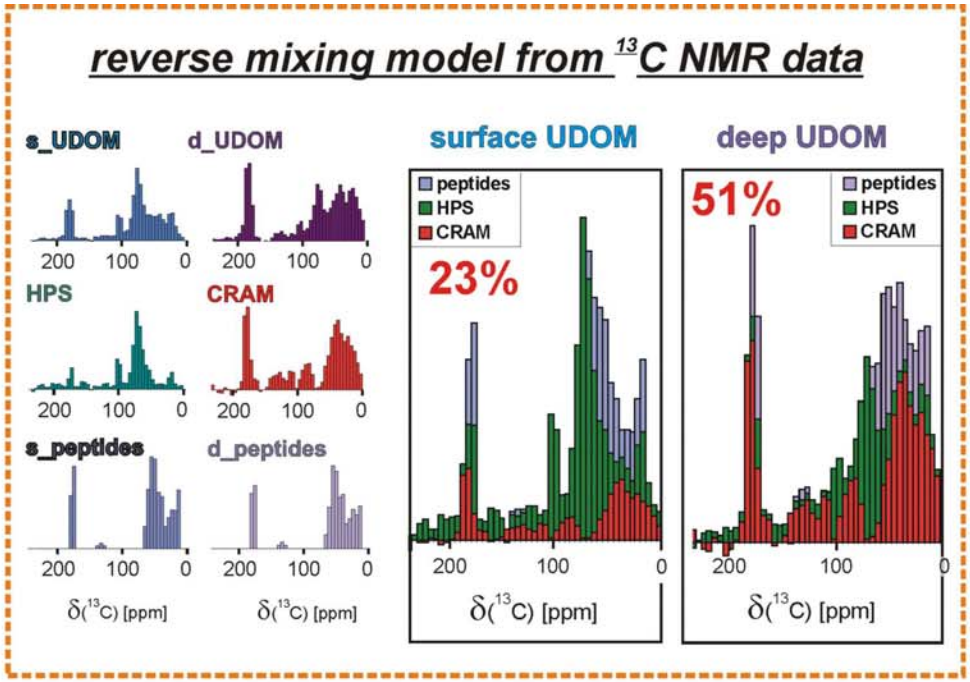
major constituent of marine UDOM and of the global carbon cycle

strong ligand for metal binding

may strongly assist in marine gel formation

controls bioavailability of

- trace and nutrient metals
- affects reactivity of marine organic matter



CRAM is compositionally and structurally more heterogeneous than other marine UDOM constituents; this contributes to ist resistance against biodegradation and to its refractory nature

possible precursors of CRAM

terpenoids

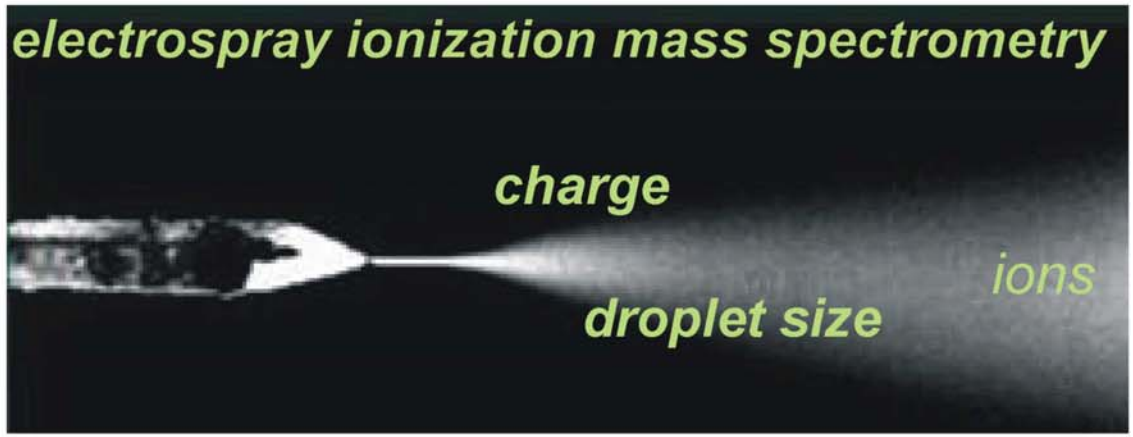
(oxidized black carbon)

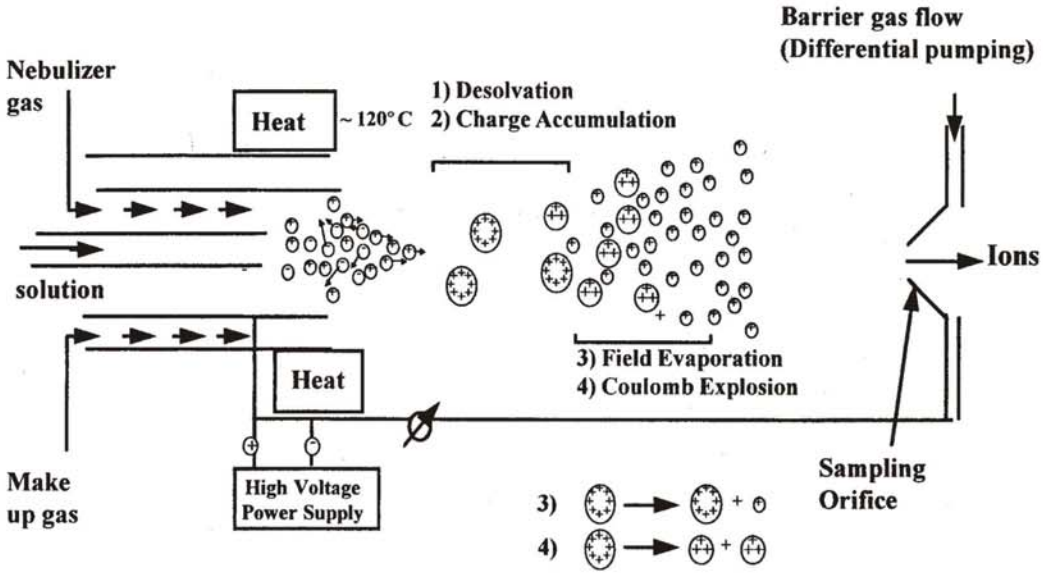
((((dimethylsulphonopropionate [DMSP]))))

the occurrence of CRAM in freshwater and terrestrial environments seems likely, considering the global distribution of biomolecules and the similarity of biogeochemical processes among environments

serious research on the mass spectrometry of mixtures is in it´s very infancy

column adsorption
fractionation
electrochemistry
electrolysis
redox reactions





NOM

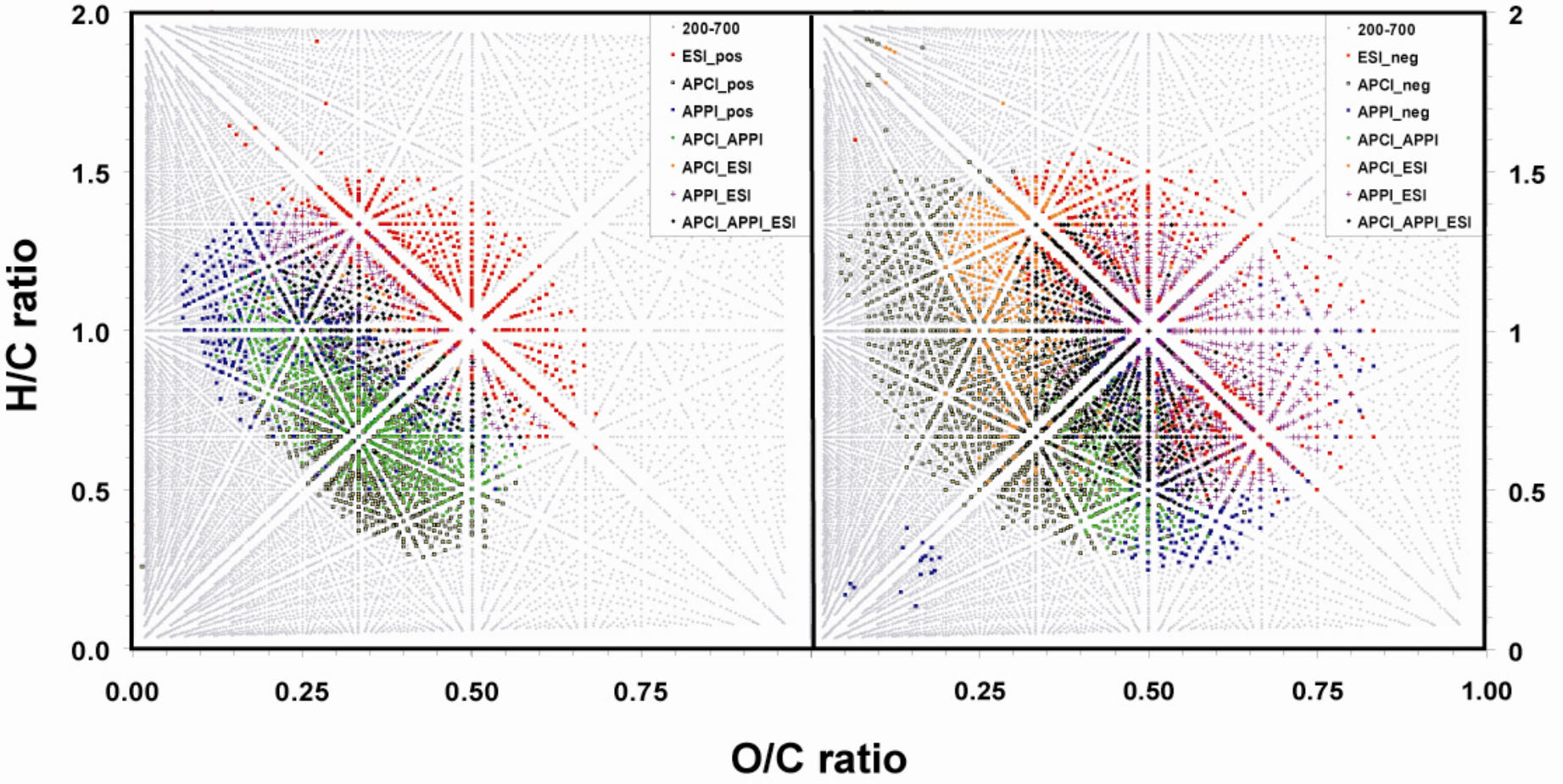
heterogeneity
polydispersity
ionization efficiency
ionization potential
metal ions
surface activity

FTICR mass spectrometry / complex systems

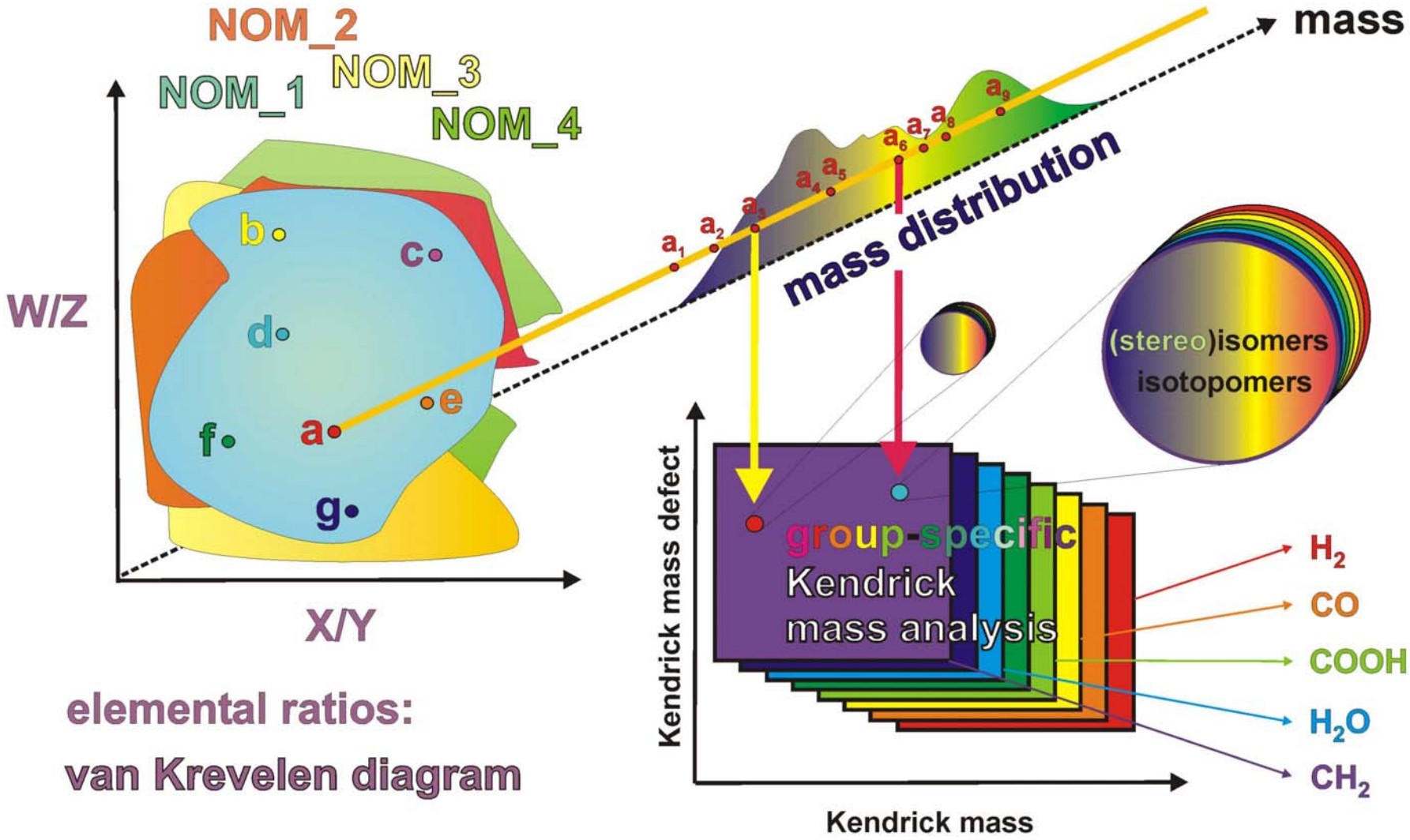
selectivity of ionization

positive

negative



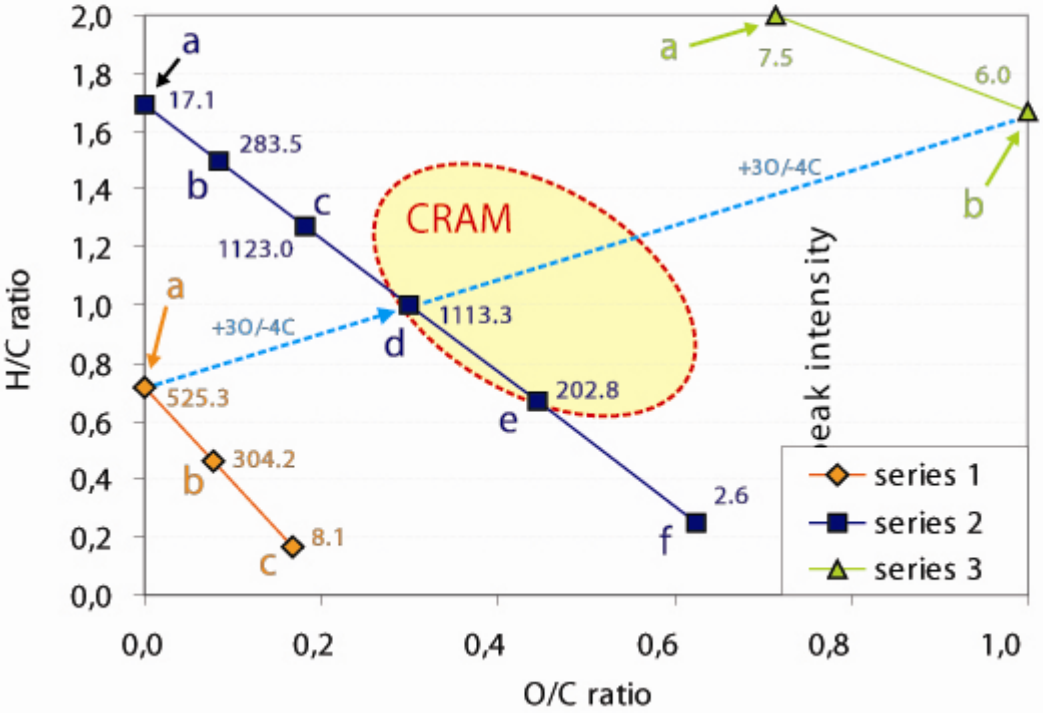
mass spectra confirm the immense complexity of NOM

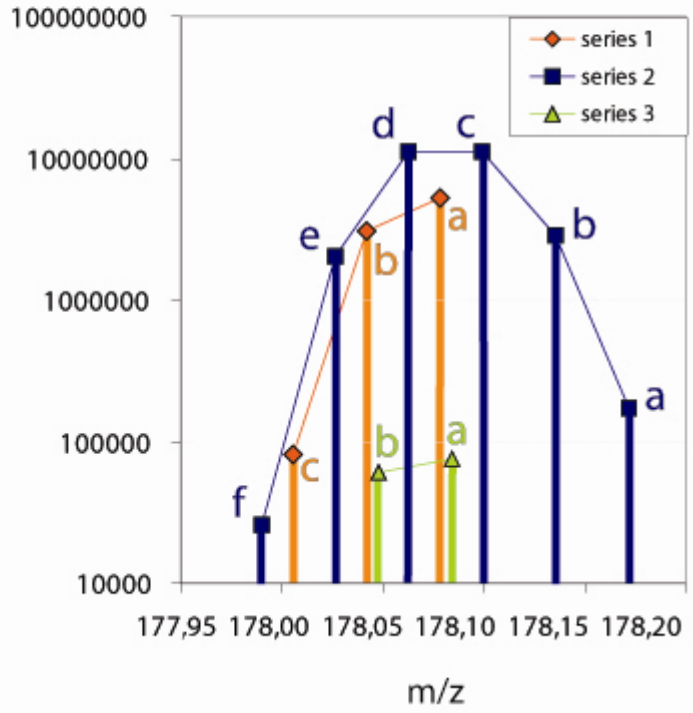


Ohio_NOM_complex_krev_new_a.cdr

FTICR mass spectrometry / complex systems

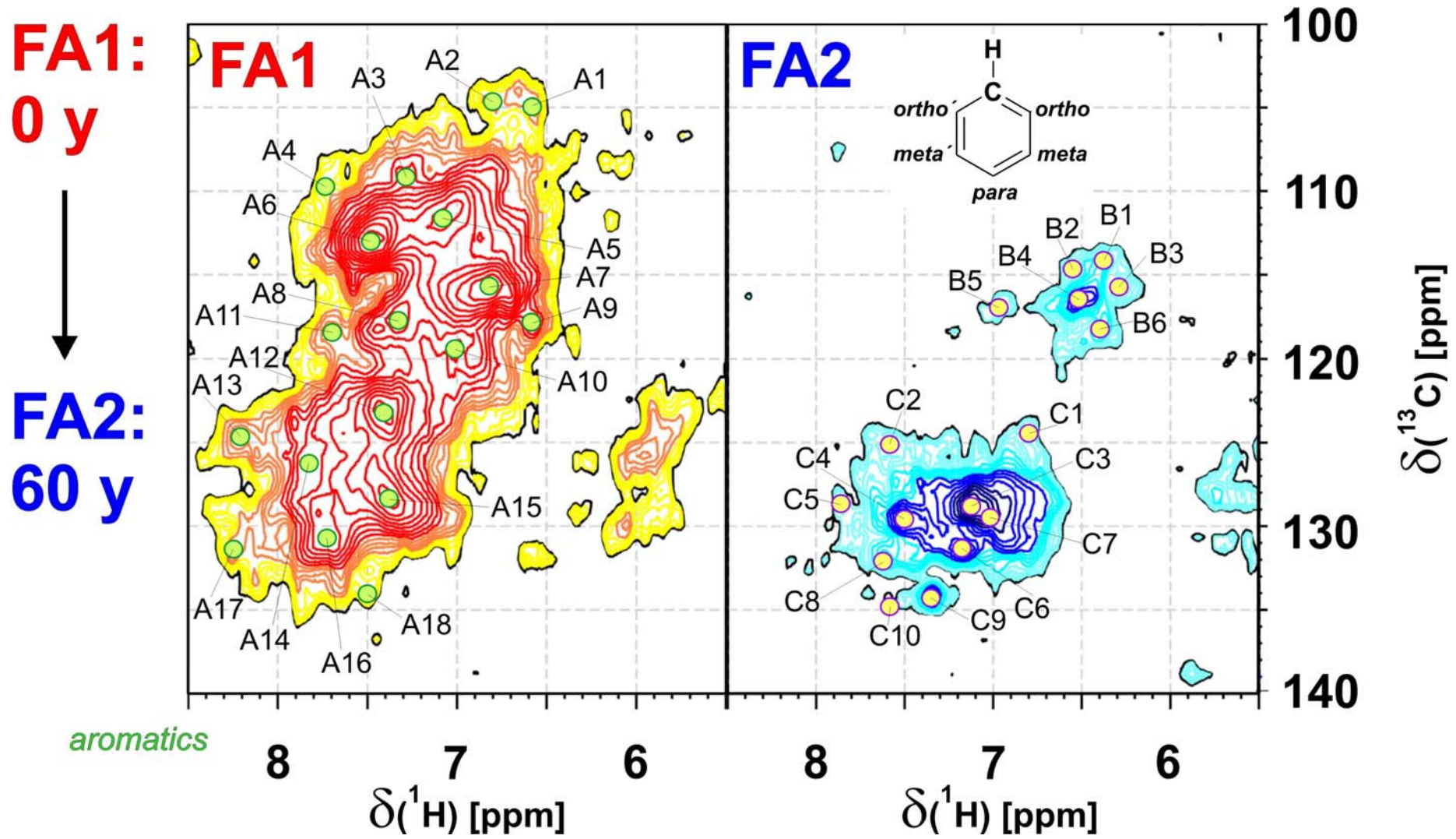
occurrence of mass spectral peaks relates with
counts of chemically relevant structures



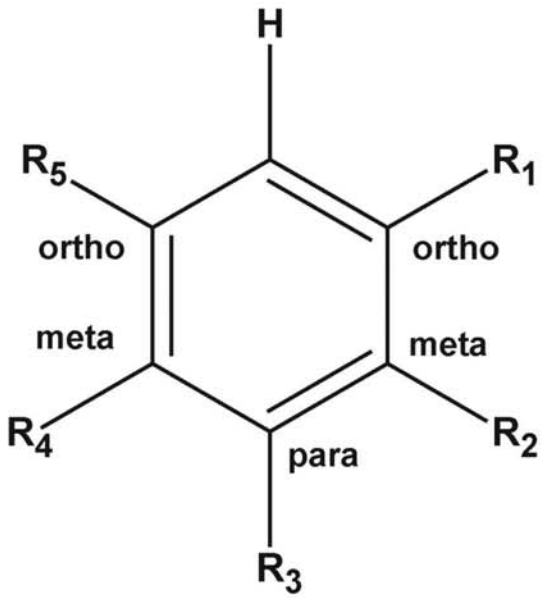


evolution of groundwater during 60 years

SPARIA analysis (**S**ubstitution **P**atterns in **A**romatic **R**ings by **I**ncrement **A**nalysis)



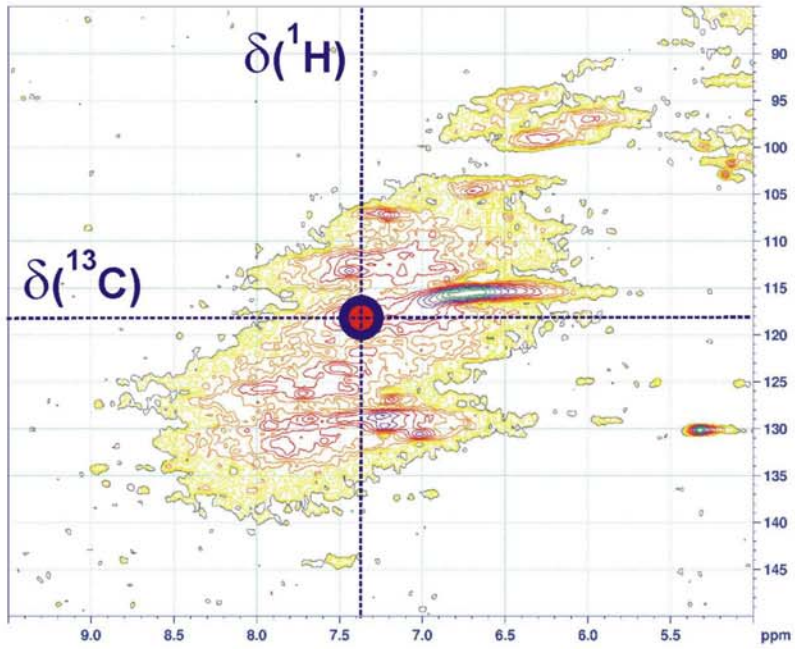
Prediction of substitution patterns in aromatic rings by increment analysis (SPARIA)



forward mode

inverse mode

$^{1}H, ^{13}C$ HSQC NMR of Suwannee River NOM



neutral	Ar-CO-X	Ar-O-X
-H	$-COOCH_3$	$-OCH_3$
$-C_2H_5$	$-COC_2H_5$	-OH
$-CH=CH_2$	-COOH	

8 substituents in 5 positions = 32768 combinations