Shell matrix diagenesis

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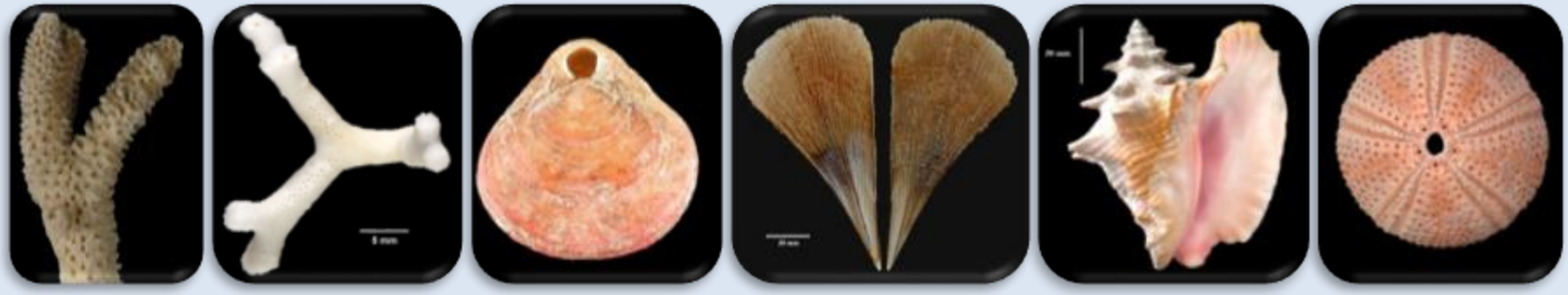
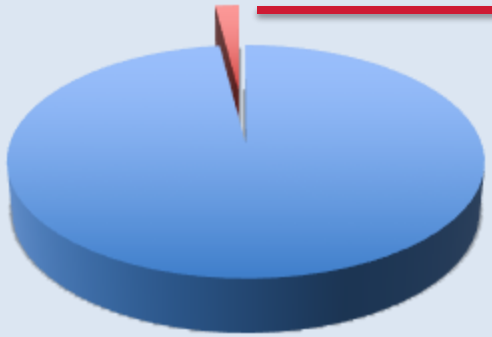
Calcifying matrix in metazoans (non vertebrates)

Cnidaria Bryozoa Brachiopoda Mollusca Echinodermata



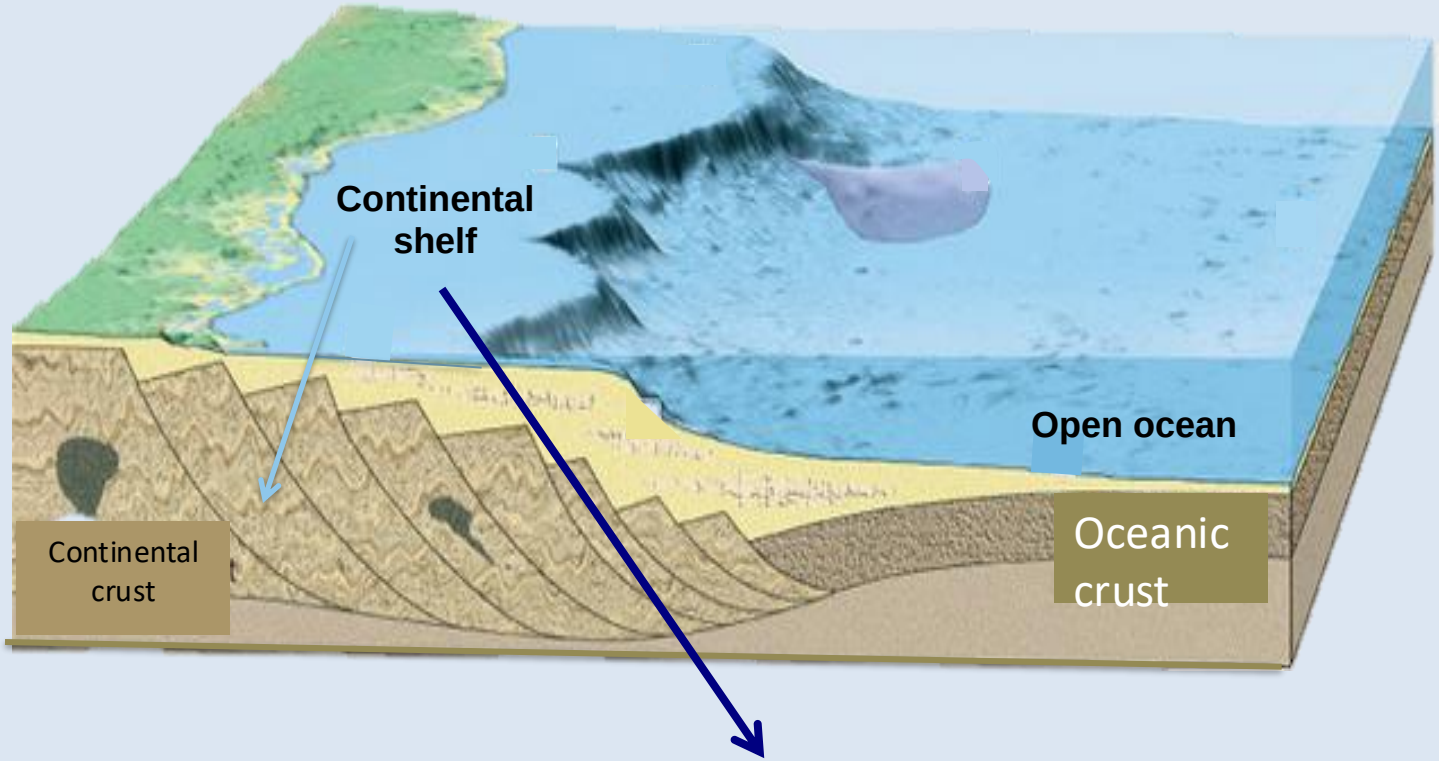
Decalcification

$CaCO_3$
+ Minor & Trace Elements
Mg, Sr...



ORGANICS 0.05 to 1-2 wt-% of the skeletal tissue

CaCO3 biomineralization & global carbonate cycle



Biogenic $CaCO_3$ in neritic environments: about $2.5.10^9$ T/yr

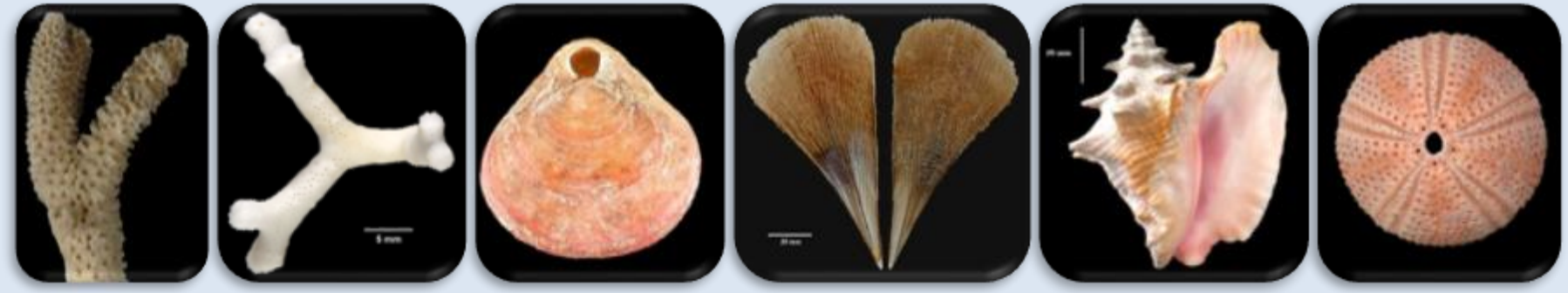
From Milliman, 1993; Wollast, 1993; Langer et al., 1997

Neritic environments

- **Benthic**
- **Aragonite & (Mg) calcite**
- **Corals, foraminif., molluscs, algae**

Estimated prod.: about $2,5 . 10^9$ T/yr (corals: $0,9 . 10^9$ T/yr)

Cnidaria Bryozoa Brachiopoda Mollusca Echinodermata



0.05 to 1-2 wt-% of the skeletal tissue

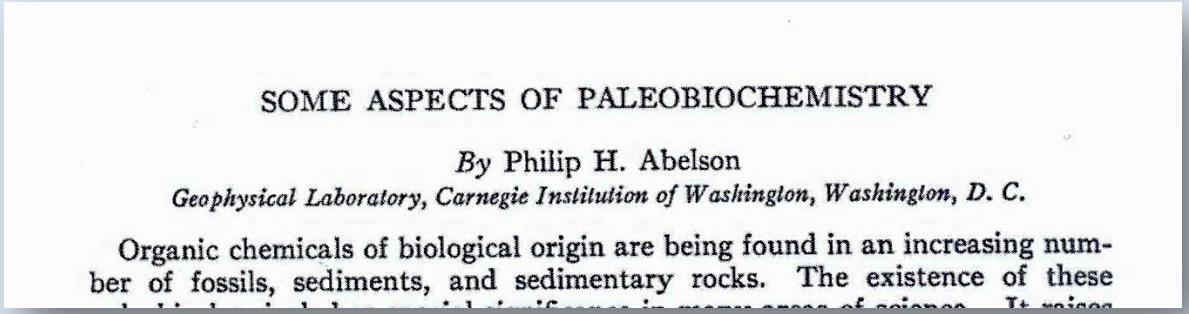
1.2 . 10^6 T/yr < calcifying matrix < 50 . 10^6 T/yr

in neritic environments

Important source of biomolecules in the fossil record

How do these molecules fossilize ?

- Abelson, 1954. Amino acids in fossils. Carn. Inst. Washington Yearb., 53, 97-108.



SOME ASPECTS OF PALEOBIOCHEMISTRY

By Philip H. Abelson

Geophysical Laboratory, Carnegie Institution of Washington, Washington, D. C.

Organic chemicals of biological origin are being found in an increasing number of fossils, sediments, and sedimentary rocks. The existence of these

Paleobiochemistry / Molecular Paleontology

2 approaches

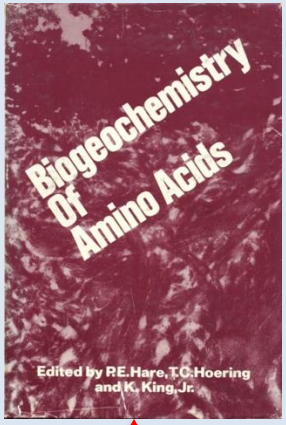
Work on fossils

→ **Matrix extraction and analysis**

Artificial diagenesis experiments

→ **Heated samples, matrix extraction & analysis**

The 'Golden Age' of fossil proteins analysis



Abelson

Vallentyne
Grégoire

Mitterer
Weiner
Westbroek

Collins
Muyzer

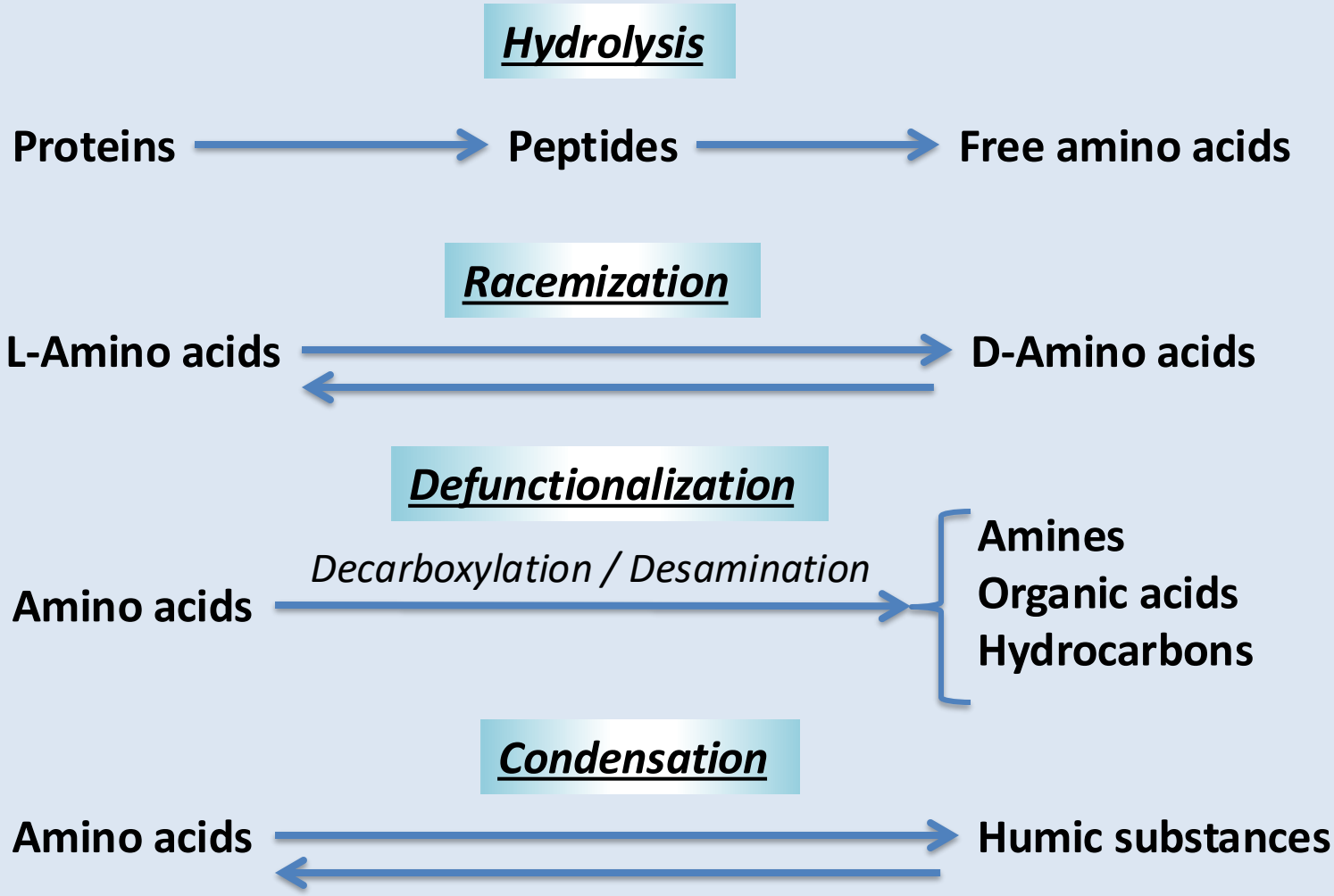
1950 1960 1970 1980 1990 2000 2010

PCR

The 'Golden Age' of fossil DNA analysis

Research on fossil proteins in biominerals overshadowed by that on fossil DNA

Degradation pathway of skeletal matrix proteins



Adapted from Mitterer 1985; Hare & Hoering, 1977

Hydrolysis of skeletal matrix proteins

1. Stability of peptidic bonds

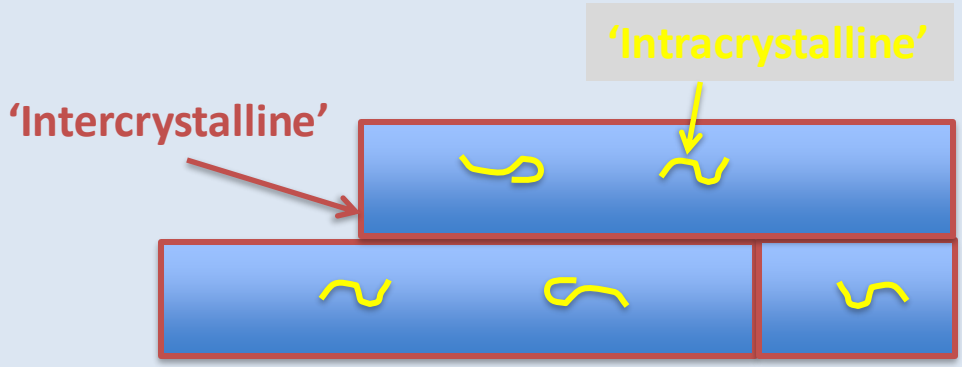
2. 3D structure of the protein

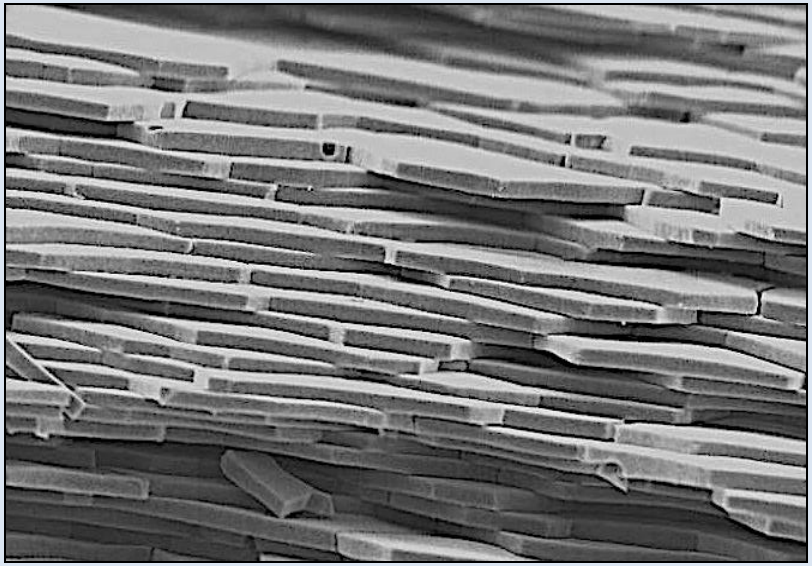
3. PTMs *(glycos. phosphor.)*

4. Localization in the biomineral

5. Water: interstitial/linked

6. CaCO3 polymorph: calcite vs. aragonite





Nacre *Nucula* sp., Lutetian

Example 1: thermal stability of nacre proteins
Artificial diagenesis experiments

P. margaritifera

Nacre only !!
1st & 2nd Bleaching treatments

3rd bleach

Heated at 100° C during 10 days (dry conditions)

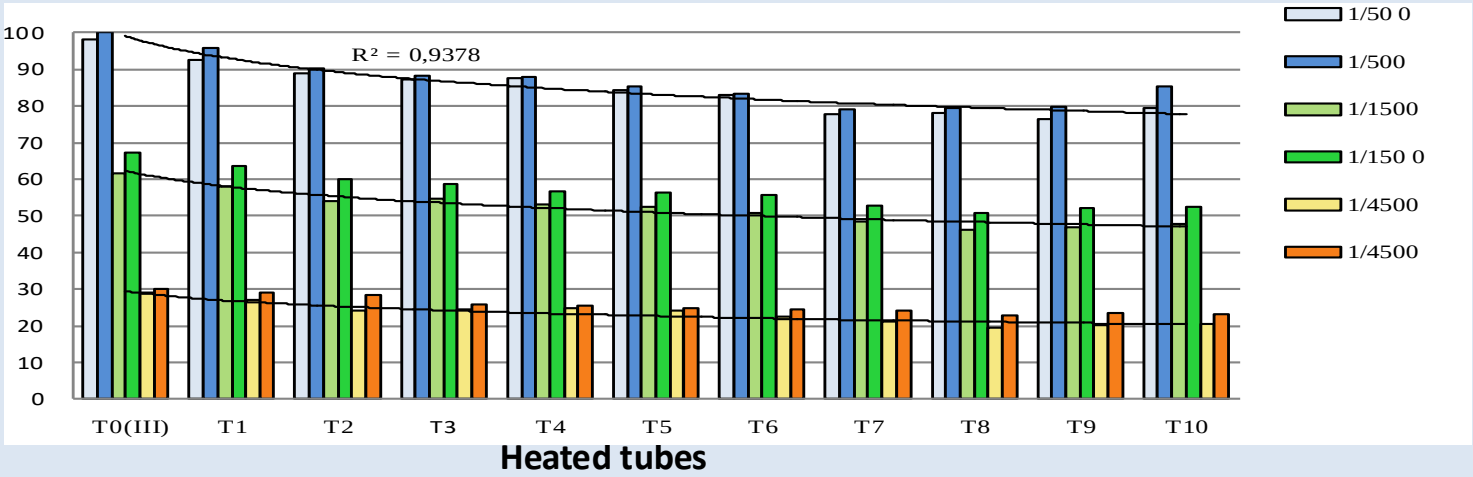
- Daily sampling: T1, T2… T5… T10

- Matrix extraction
- Matrix quantification, ELISA, proteomics

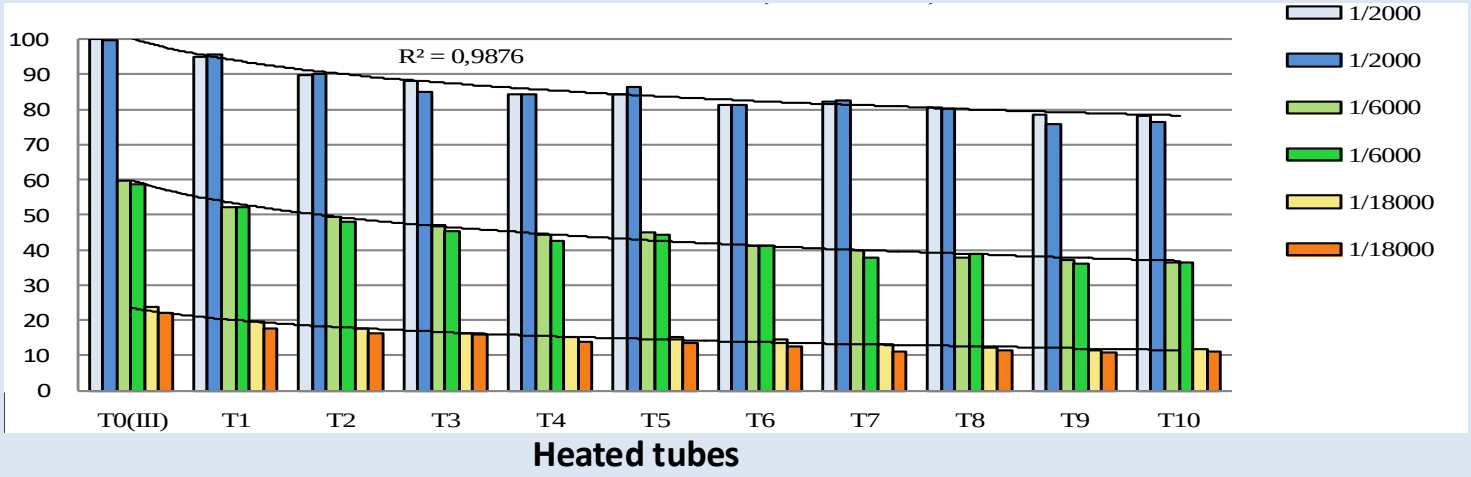
Example 1

Immunological tests on the ‘intracrystalline’ matrix

Antibody A:
α–ASM *Pmarg*



Antibody B:
α–LS-AIM *Pmarg*



After 10 days at 100°C, loss of 20% reactivity

Example 1

T0	T5 (120 hours)	T10 (240 hours)
Nacrein A1 (13)	Nacrein A1 (7)	Nacrein A1 (4)
Nacrein B3 (11)	Nacrein B3 (6)	Nacrein B3 (6)
Nacrein B4 (11)	Nacrein B4 (5)	Nacrein B4 (5)
Nacrein C5 (12)	-	-
PIF (11)	PIF (3)	PIF (3)
Gly-Rich protein 2 (3)	-	-
Shematrin 8 (2)	Shematrin 8 (2)	Shematrin-like (1)
Perlin (3)	-	-
Asn-rich protein (1)	-	-
USP 7 (1)	USP 7 (1)	USP 7 (1)
USP 11 (1)	-	-
USP 12 (1)	-	-

(Parker et al., 2015)

*** Persistence of some shell proteins**

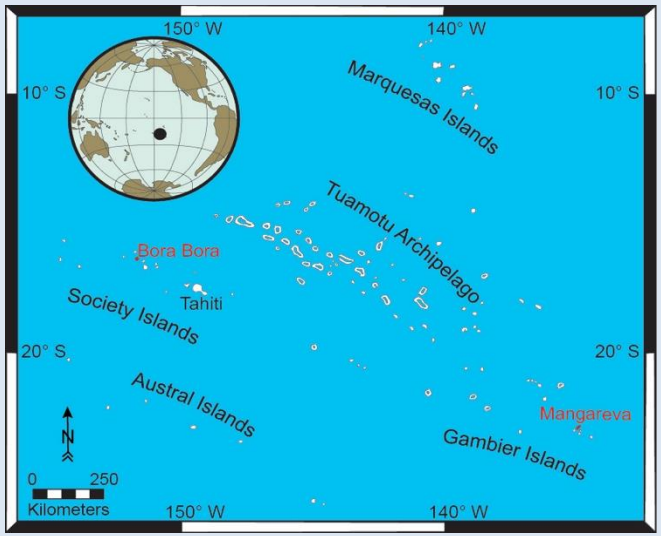
*** Apparent degradation of others: differential degradation**

*** Diagenesis: -> Disappearance of some proteins**

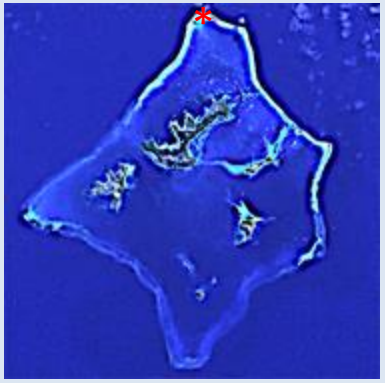
-> Decrease of the number of identified peptides

Example 2: sub-fossil *Tridacna of French Polynesia*

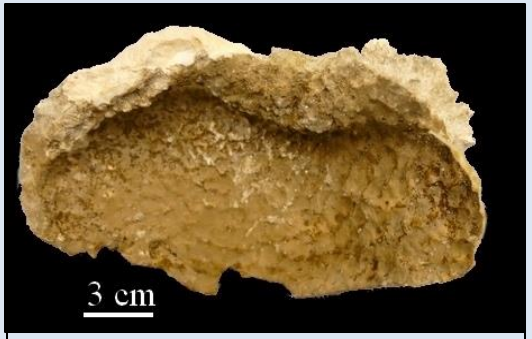
(Collaboration: Takeshi Takeuchi, OIST + the Univ. Geneva, Master work of A. Chmiel)



Mangareva



GAM-48: Collected alive



GAM-14: 3320 +/- 30 BP

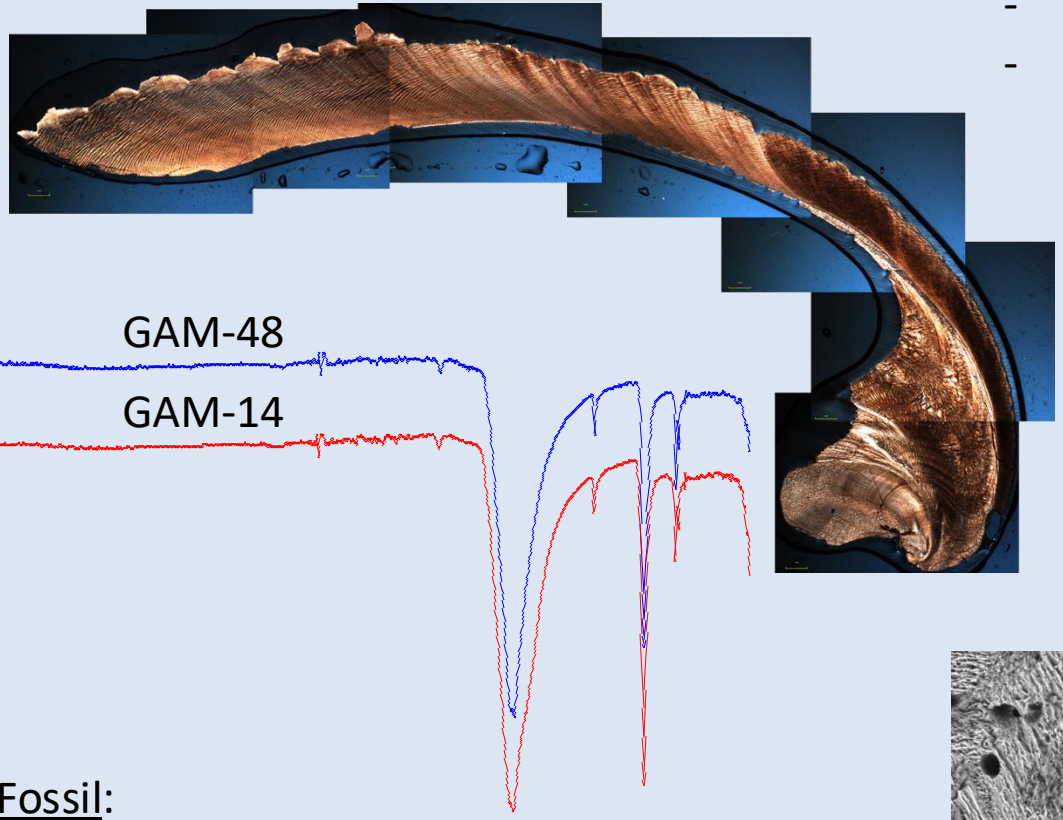
Diagenetic context:

- **Reef flat**
- **Rich in percolating H_2O**
- **Not favourable for good preservation of aragonite**

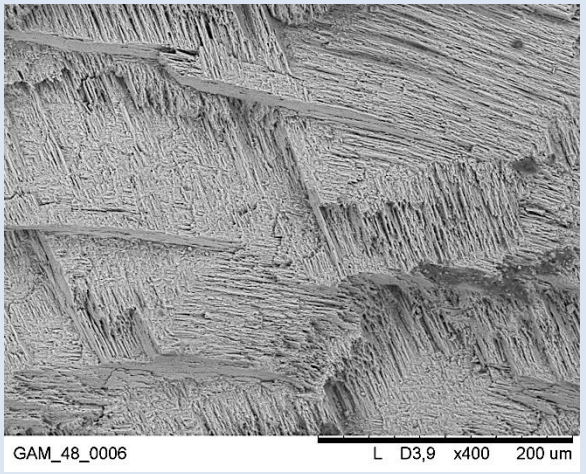
Is there still a biochemical signal ?

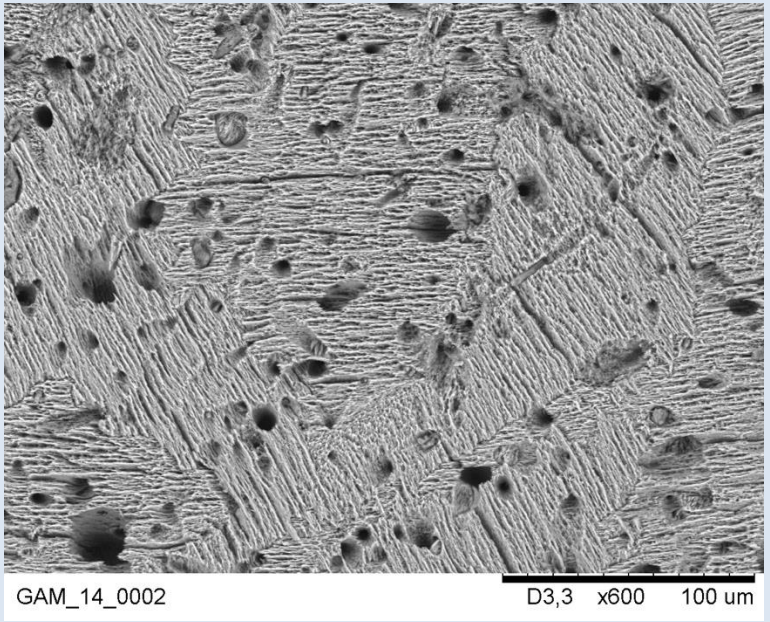
***Control on the mineral phase*:**

- Thin sections	- Cathodoluminescence	- XRF
- SEM	- Epifluorescence	- FT-IR



- Fully aragonitic
- Crossed-lamellar & complex crossed lamellar microstructures



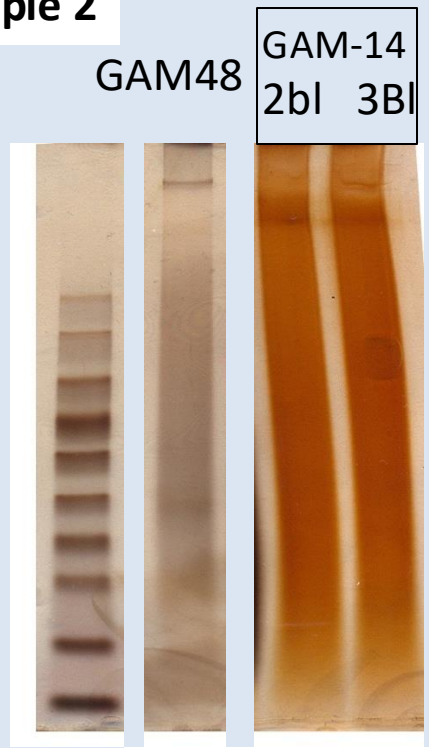


<u>Fossil</u>:

- No recrystallization into calcite.

- Important alterations & perforations in the outemost and innermost layers of the shell

Example 2



- 'Fossil' sample: 2 & 3 thorough bleaching steps: 2bl, 3bl

➔ get the most protected proteins

- Extraction and analysis on 1D gels
- Proteomics

Reference: transcriptome from *Tridacna crocea* *(Dr. Takeshi TAKEUCHI, Pr. Nori SATOH, OIST)*

	GAM-48	GAM-14, 2 bl	GAM-14, 3bl
Nb protein hits	134	40	32
Nb proteins hits identified by more than 2 peptides	46	4	7
Nb proteins hits identified by 1 peptides	88	36	25

FRESH SUB-FOSSIL

Example 2

LIVING: GAM-48	SUB-FOSSIL: GAM-14-2 & GAM-14-3

TRINITY_DN232224_c0_g1_i1|m.393634

MRGIAVFAVLLLAVAANAQGPPTRTKKGKRPGDNPDIGLPSGKDIPDGRR
FAPPAAPRAAPVPPARAPAAAAPAPAPAGYRKALPPAAARPPVLPPAAGR
PAGGIPPTRTRKGKRPAGRMGPAYRVAPPAPLAPKNPPMFGNPWAFAPAP
AAPVAPAVSCEASCGYFWAPVCSVYGNTYDNDCRLGCSGEPYACEGQCPC
EEPKPAPAAASPMLGLFGSSSCGCGYHYDPVCTDDGDEVMNECLALCDGK
TIACSSHCPC

Protein sequence coverage: 25%

MRGIAVFAVLLLAVAANAQGPPTRTKKGKRPGDNPDIGLPSGKDIPDGRR
FAPPAAPRAAPVPPARAPAAAAPAPAPAGYRKALPPAAARPPVLPPAAGR
PAGGIPPTRTRKGKRPAGRMGPAYRVAPPAPLAPKNPPMFGNPWAFAPAP
AAPVAPAVSCEASCGYFWAPVCSVYGNTYDNDCRLGCSGEPYACEGQCPC
EEPKPAPAAASPMLGLFGSSSCGCGYHYDPVCTDDGDEVMNECLALCDGK
TIACSSHCPC

Protein sequence coverage: 15%

Pro: 19.5; Ala: 17.4; Gly: 10.4 pI: 8.78

TRINITY_DN230191_c0_g1_i1|m.402396

GQWEFDGSGAGGFGGQFPLG GLGLANWKYNAKTGQWEFDGFGAGGA
GSQFTGSGNWKWNATSGHWQFVGAGGQGKGQSWWTSNMKGAALLK
KLKALLQAQAMARWNEWQKTTKSGFVGRQRPSHIVQTIRGKMGRNEIGG
AKLAILGGKNRAKPMVQPKTRSPPNSNAN

Protein sequence coverage: 30%

GQWEFDGSGAGGFGGQFPLGGLGLANWKYNAKTGQWEFDGFGAGGA
GSQFTGSGNWKWNATSGHWQFVGAGGQGKGQSWWTSNMKGAALLK
KLKALLQAQAMARWNEWQKTTKSGFVGRQRPSHIVQTIRGKMGRNEIGG
AKLAILGGKNRAKPMVQPKTRSPPNSNAN

Protein sequence coverage: 11%

Gly: 18.8; Ala: 10.6; Lys: 9.4 pI: 11.45

Example 2

LIVING: GAM-48

SUB-FOSSIL: GAM-14-2 & GAM-14-3

TRINITY_DN253411_c2_g2_i3|m.459507

ETMNKVILIVFSGLLAVQLVSAQSHTTWAAAQVPGLGRMTPPTTDYPEYMLHMAVG
EIMRAPTENKAAYAAAKVYNPVMDMSDKVQQALEDRVLQLRHPPGTPYYRRKLDFD
VMQLVIGAYYKTLNISAPQQLGSFYGPPPANHWAGASQPVGPPARQPGPLPPAGPPA
GPAMGPPTSIRRGFRPRAQGIYSPFEPTPWELDRAVQDIHMARTEKQAVKAAAGVHR
IGLDLADIVVNALEEKIARLRRPNWTGFRPPPIPRGLNVHGLVRHAFYEIQRIAQAKAAA
DAAAAAAAAAAKAKKTPPPPTPRAGSKIPTTLPPTPPPKPYKKPRQPSKPNPPPSPKKTK
PPKRDFMTDFIQNRRKQRQPPPAPKLFKQAQITRPPFVQPVRRQTLNPFPTQPSVPPY
FEPTKITRPPYVQPQRRDPVDPFFSQPSYRPKPQVEPFRPPPNVPRPPKINWKKKAAAK
PVPTSVSLTEKGPTSISAASSNSNKSPVSYETIPSQNSAKPAFMKIPKPNVPPAPFRSEPP
KPKSLFPKGNSGRPSDIPKAVLSSNKGKGKRPSSKTVEPLFQSTETTPSPEEQQLFNRYP
GLFENKARMRVANLAQHRLETVGPSAEISKPPLKGPNPQPPKVSNKKMPVSKPPQQA
AVKVAPKIIKPSKVWSPLGNLGSNINEILKFSIDGPSESVPIPTAAPLTTKAPTTTTKKPTT
TTPRKQEKVKPIRKTKVKRRRKVVSKAKSKSFAIKLAKKPEKPKKPQGADKLTQLHKLL
EGISPSQLQTLVDLIKAKANEGKPKPLPKPEPLPKPKPIFAPPPPPGSEHKGPPREFRSQ
MQSSRSGPYRPNSDYGPPPDNRGPPPDWARGPGGRPRGPPGPPGPGGPGGPGMR
GGPDLSNPQIARLIRVMKQGGHPKNNFLSGRTPGSSAAAAGGEAPEAGEGPTGLLGN
PLMMSMLMNRGGQGGGGGIASLLGGGAAGGANPLAALMPGGAGGAGGGEGGM
NPAMLAAIMGGGQGGGGGGMGALGGGYGGMLAGLGL

Protein sequence coverage: 38%

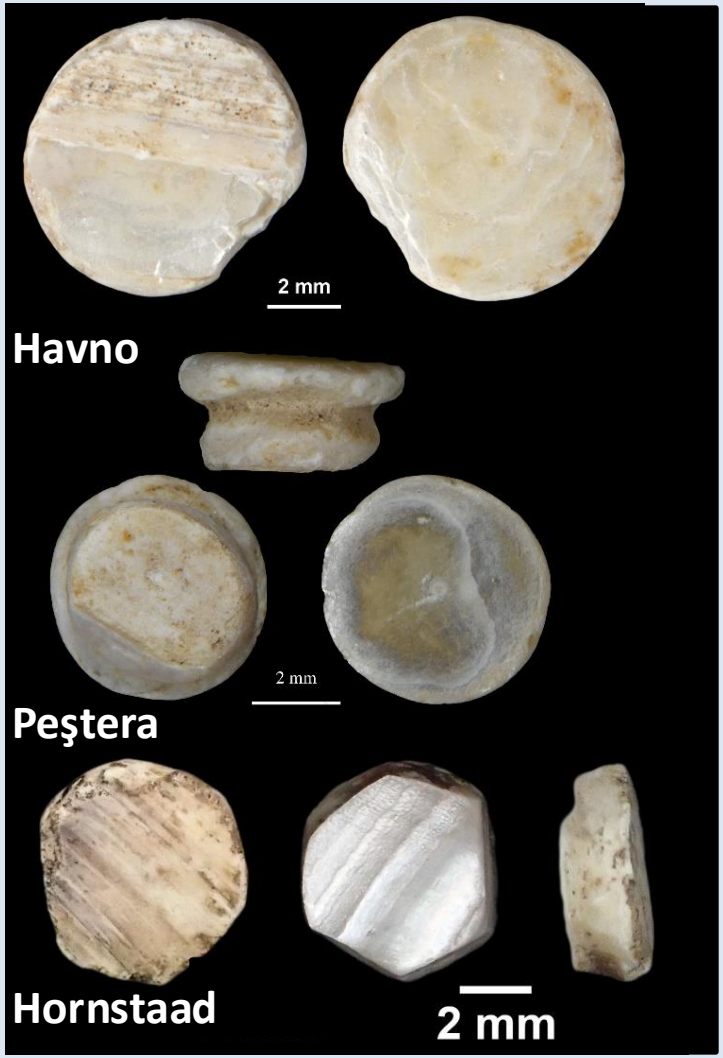
ETMNKVILIVFSGLLAVQLVSAQSHTTWAAAQVPGLGRMTPPTTDYPEYMLHMAVG
EIMRAPTENKAAYAAAKVYNPVMDMSDKVQQALEDRVLQLRHPPGTPYYRRKLDFD
VMQLVIGAYYKTLNISAPQQLGSFYGPPPANHWAGASQPVGPPARQPGPLPPAGPPA
GPAMGPPTSIRRGFRPRAQGIYSPFEPTPWELDRAVQDIHMARTEKQAVKAAAGVHR
IGLDLADIVVNALEEKIARLRRPNWTGFRPPPIPRGLNVHGLVRHAFYEIQRIAQAKAAA
DAAAAAAAAAKAKKTPPPPTPRAGSKIPTTLPPTPPPKPYKKPRQPSKPNPPPSPKKTK
PPKRDFMTDFIQNRRKQRQPPPAPKLFKQAQITRPPFVQPVRRQTLNPFPTQPSVPPY
FEPTKITRPPYVQPQRRDPVDPFFSQPSYRPKPQVEPFRPPPNVPRPPKINWKKKAAAK
PVPTSVSLTEKGPTSISAASSNSNKSPVSYETIPSQNSAKPAFMKIPKPNVPPAPFRSEPP
KPKSLFPKGNSGRPSDIPKAVLSSNKGKGKRPSSKTVEPLFQSTETTPSPEEQQLFNRYP
GLFENKARMRVANLAQHRLETVGPSAEISKPPLKGPNPQPPKVSNKKMPVSKPPQQA
AVKVAPKIIKPSKVWSPLGNLGSNINEILKFSIDGPSESVPIPTAAPLTTKAPTTTTKKPTT
TTPRKQEKVKPIRKTKVKRRRKVVSKAKSKSFAIKLAKKPEKPKKPQGADKLTQLHKLL
EGISPSQLQTLVDLIKAKANEGKPKPLPKPEPLPKPKPIFAPPPPPGSEHKGPPREFRSQ
MQSSRSGPYRPNSDYGPPPDNRGPPPDWARGPGGRPRGPPGPPGPGGPGGPGMR
GGPDLSNPQIARLIRVMKQGGHPKNNFLSGRTPGSSAAAAGGEAPEAGEGPTGLLGN
PLMMSMLMNRGGQGGGGGIASLLGGGAAGGANPLAALMPGGAGGAGGGEGGM
NPAMLAAIMGGGQGGGGGGMGALGGGYGGMLAGLGL

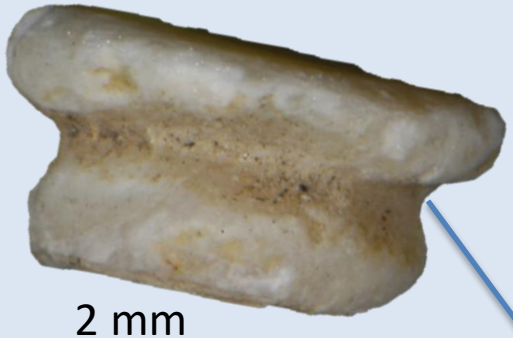
Protein sequence coverage: 4%

Pro: 17.2; Gly: 10.1; Ala: 9.6 pI: 10.63

➔ **Follow the diagenetic behavior of one protein species**

Example 3: archaeological samples





Double button pearl: « Doppelknöpfe »

Groove

- Complex process of fabrication
- Inserted in pierced leather clothes

« Pearlies »

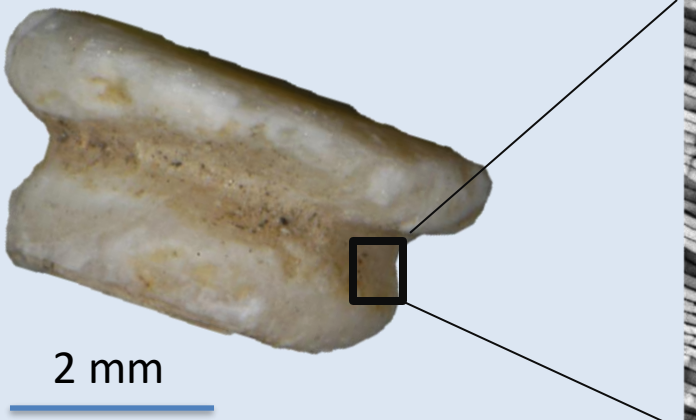
Example 3: archaeological samples

Archaeological sites

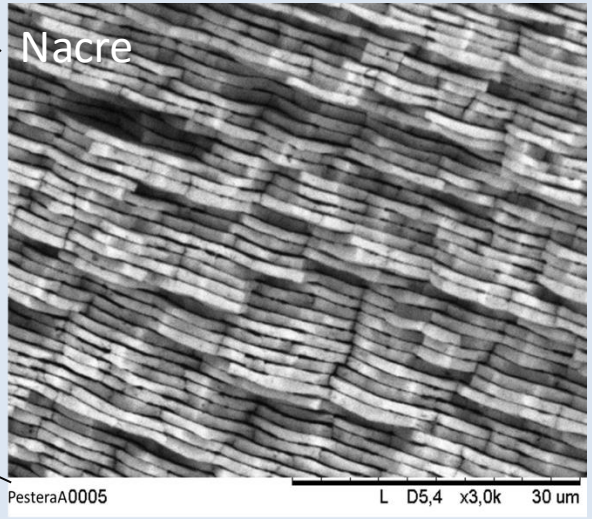
- **Havnø (Dk)**: Ertebølle Hunter-gatherers-fishers (*4420 - 3590 BC*)
- **Hornstaad (Sw)**: Neolithic (*3918 - 3902 BC*)
- **Peştera Ungurească (Ro)**: Late Neolithic / beginning Bronze Age

Marine origin? Long-distance exchange of raw materials ?

Example 3: archaeological samples



Scanning Electron Microscopy



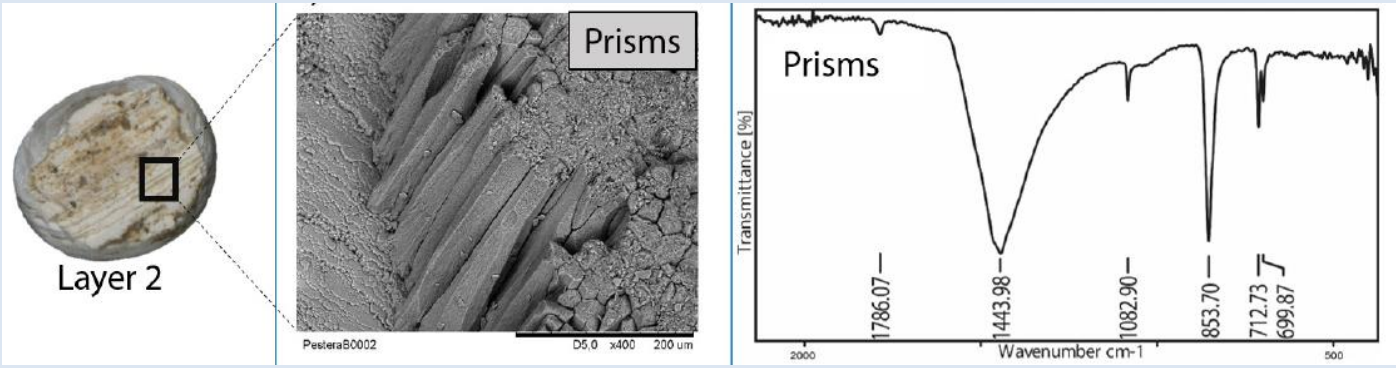
Nacre in bivalves

Paleotaxodonta: marine
Cryptodonta
Pteriomorpha: marine
Paleoheterodonta: fw
Heterodonta
Anomalodesmata: marine

Typical « brickwall » bivalve nacre

Clues:

1



2 Stable isotope geochemistry: $\delta^{18}O$ and $\delta^{13}C$: negative (suggests a freshwater / estuarine origin)

Example 3: archaeological samples

Micro-extraction & proteomics

Proteins in Unionoid shells & beads	Hyriopsis cumingii (Triangle sail mussel)
• Hic74	Unknown function
• Hic52	Unknown function
• Silkmapin	Unknown function

Hic74: Ala/Gly–rich
pI: 4.8; A: 30.8%; G: 25.6%; S: 10.6%

Hic52: Gly/Gln-rich
pI: 10.2; G: 28%; Q: 12%.

Silkmapin: Gly-rich
pI: 6.9; G: 33%.

Freshwater mussel !!
Unionidae

Example 3: archaeological samples

* Exploitation of local freshwater shell resources for making button-pearls.

* No use of marine nacreous shells.

* Ornaments = prestige, concept not associated to the rarity of the resource; what made the button-pearls rare was the effort made to fabricate them.

* No necessity of long-distance trading exchange of raw material but…

* ... Propagation of the know-how to craft double button pearls.

* Powerful use of proteomics in archeology…

In summary...

- Persistence of some shell proteins after long heating (10d, 100° C)
- Differential degradation pattern
- Diagenesis seen by proteomics:
  - Disappearance of some proteins
  - Decrease of the number of identified peptides

Time to revisit diagenesis of Skeletal Matrix Proteins by proteomics

Possibility to track the diagenetic behavior of one given protein

Well-suited for archaeological samples