ANALYSIS OF MOLECULAR BIOMARKERS COVALENTLY BOUND WITHIN NEOPROTEROZOIC SEDIMENTARY KEROGEN

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Abstract— Catalytic hydropyrolysis (HyPy) is a powerful analytical technique for fragmenting macromolecular organic matter, such as kerogen (insoluble sedimentary organic matter), and releasing covalently-bound molecular constituents including branched and cyclic biomarker hydrocarbons. Here we illustrate our molecular approach to paleobiology with lipid biomarker data collected from rock bitumens and kerogens hosted within sedimentary units of the Neoproterozoic Huqf Supergroup, South Oman Salt Basin, Sultanate of Oman. We emphasize that parallel analyses of *free* and *bound* biomarker pools affords more confidence that we have correctly identified syngenetic compounds. One enigmatic class of compounds that is prominent in many late Proterozoic and Cambrian sedimentary rocks and oils, including from the Huqf Supergroup, is a series of C_{14}-C_{30} mid-chain methylalkanes which were originally denoted *X-peaks*. Despite their abundance in the Precambrian rock record, little is known about the organisms responsible for their biosynthesis. Here we propose a possible origin of X-peak methylalkanes from colorless sulfur bacteria (a very heterogeneous group of chemolithotrophic γ-proteobacteria). In modern marine settings, these bacteria are abundant mat formers wherever a sedimentary sulfide-rich horizon intersects the seafloor producing a steep geochemical redox gradient. This condition may have been met more commonly on shallow marine shelves in late Neoproterozoic basins and these benthic mats may have acted as environmental buffers consuming hydrogen sulfide. If our hypothesis is correct, proliferation of sulfide-oxidizing benthic microbial mats, commencing in the late Cryogenian in South Oman Salt Basin, implies unique and specific benthic conditions during the evolution of the earliest metazoans.

INTRODUCTION

Utility of Lipid Biomarkers

Lipid biomarkers are fossil biochemicals detected in the geological record whose basic skeletal features have been sufficiently well-preserved to allow unambiguous links to known, contemporary natural product precursors. Biomarkers can yield valuable information pertaining to the source organisms, the thermal maturity of the host organic matter and the palaeoenvironmental conditions (of redox, salinity, etc.) in the water column and at the sediment surface which prevailed during deposition (for a recent review see Brocks and Summons, 2003). Hydrocarbon skeletons of lipids have been utilized as molecular fossils to track evolutionary and environmental change in the Precambrian (e.g. Summons et al., 1988a, 1988b, 1999; Summons and Walter, 1990; Pratt et al., 1991; Logan et al., 1997, 1999, 2001; Peng et al., 1998; Brocks et al., 1999, 2003, 2005; Moldowan et al., 2001; Li et al., 2003; Dutkiewicz et al., 2004; Olcott et al., 2005; McKirdy et al., 2006; George et al., 2007; Marshall et al., 2007; Sherman et al., 2007), providing unique insights in this regard.

The most useful biomarker molecules are those that are thermodynamically-stable, with a limited number of well-defined biological sources and which are relatively straightforward to analyze. Certain lipid compound classes fit all these criteria, and polycyclic terpenoids or highly branched hydrocarbon skeletons, such as hopanoids or steroids, are extremely resistant to degradation. Hopanes, steranes and other hydrocarbon biomarkers may survive hundreds of millions of years of burial in sedimentary rocks and in their petroleum products and can even be detected in some late Archean sedimentary rocks (2700 Ma, such as report-

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ed in Brocks et al., 1999; Sherman et al., 2007). Other biochemicals (carbohydrates, proteins, nucleic acids) generally do not survive for long in the environment (less than a few Ma) in unambiguously recognizable form although their aromatic derivatives (dibenzofurans, benzocarbazoles, etc) are stable.

Molecular biomarker studies of Precambrian rocks can be regularly compromised by the high thermal maturity of the preserved organic matter and the possibility of organic contamination from petroleum-derived fluids, such as core drilling fluids or from organic pollutants in outcrop environments, or modern additives which can be incorporated during sample processing and storage (Grosjean and Logan, 2007). Extreme care must be taken in selecting appropriate sediments and cross-checking analytical protocols used to extract and detect biomarkers.

Although specific biological sources have been identified for many lipids in environmental samples, the gaps in our knowledge are large. Lipid biomarker applications interpretations are often based on a few key lipid types. Thus, it is important to develop new techniques to expand our current lipid inventory for modern taxa to try and recognize and better constrain the source(s) of any distinctive core hydrocarbon skeletons preserved in the ancient sedimentary record.

Bound Biomarker Approaches for Precambrian Sedimentary Organic Matter

Conventional approaches for studying ancient biomarkers make extensive use of the hydrocarbons (alkanes and aromatic compounds) and simple functionalized compounds (alcohols, carboxylic acids, etc. preserved at low thermal maturity only, a poor prospect for many Precambrian sedimentary rocks) in the extractable material obtained from rocks (termed *bitumen*) using common organic solvents such as dichloromethane or solvent mixtures. Often ignored in organic geochemical investigations are the significant quantities of well-preserved biomarker structures that are covalently bound within the highly cross-linked and insoluble macromolecular network (or *kerogen*) which often comprises the bulk of the total organic matter (typically over 90% w/w) in pre-oil window maturity sedimentary rocks. The insoluble and polymeric nature of kerogen, however, makes it difficult to characterize directly by conventional analytical techniques without first fragmenting it into smaller constituents using either chemical or thermal degradation techniques.

Using the technique of catalytic hydropyrolysis (or *HyPy*, Love et al., 1995) to fragment kerogen we can release biomarkers from Precambrian organic matter with minimal change to structure and stereochemistry, thereby preserving their most diagnostic features. HyPy uses high pressure hydrogen gas (typically 15 MPa) and a molybdenum sulfide catalyst to provide a potent reaction medium and the treatment is performed in continuous flow mode to minimize residence times of products to a few seconds. For highly cross-linked kerogen, particularly in thermally mature sediments where weak covalent linkages such as ester and sulfide have already been cleaved at low maturity, then pyrolysis techniques appear better suited to perform fragmentation rather than chemolysis to cleave the dominant C-O and C-C cross-linkages. This is because mass transfer through geopolymer networks is poor with bulky chemical reagents and not all reactive sites in the macromolecule can be accessed, typically resulting in low yields of soluble products from kerogens (see Farrimond et al., 2003). The most promising chemolysis approach for fragmenting mature kerogen is probably ruthenium tetroxide oxidation, since this reagent attacks both aromatic rings and ether bonds and hence achieves a reasonable conversion of kerogen to soluble products (e.g. Peng et al., 1998). HyPy is a much more rapid approach, however, and releases the bulk of analyzable products in simple hydrocarbon form which can be analysed in great detail by both conventional GC-MS and MRM-GC-MS techniques and compared directly with soluble biomarker distributions. The potency of the reaction medium in HyPy, using high pressure hydrogen gas and a molybdenum sulphide catalyst, allows us to generate genuine molecular constituents from even the most thermally mature and recalcitrant Precambrian kerogens (Brocks et al., 2003; Marshall et al., 2007), and this hasn't been demonstrated convincingly for any other analytical pyrolysis technique to date.

Another major advantage of the kerogen-bound components released by HyPy is that these *bound* constituents are much less susceptible to contamination than extractable hydrocarbons since they are linked within an immobile solid matrix deposited synchro-

nously with the host sedimentary rock. Being covalently-bound, the biomarkers are also immobile in rocks and therefore more assuredly genuine. Kerogen is formed rapidly in geologic time, with formation commencing in the water column and the macromolecular organic matter being largely in place over a timescale of hundreds to thousands of years of subsurface burial after deposition (Farrimond et al., 2003). The most common organic contaminants pervading into ancient sedimentary rocks are soluble and mobile molecular species (e.g. in-situ migrated petroleum or refined petroleum-derived additives introduced during sample collection and storage and product work-up). The ability to exhaustively remove soluble organic contaminants from sediments using solvent treatment while typically leaving no problematic residue has been demonstrated previously for oil-window mature sediment cores contaminated with oil-based drilling muds (e.g. Murray et al., 1998). Eliminating all traces of contamination from overmature samples may also require an initial low temperature HyPy treatment (terminating around 340°C) to drive off strongly adsorbed molecules not accessible to solvent extraction (Brocks et al., 2003; Marshall et al., 2007) prior to subsequent high temperature HyPy (to 520°C).

Examples of HyPy molecular geochemical applications performed to date include generation of genuine hydrocarbon cleavage products from a variety of Phanerozoic kerogens, coals and macromolecular petroleum fractions (Love et al., 1995, 1996, 1997, 1998; Bishop et al., 1998; Murray et al., 1998; Farrimond et al., 2003; Russell et al., 2004; Bowden et al., 2006; Lockhart et al., 2008); oil-window-mature Neoproterozoic kerogens from Huqf Supergroup, South Oman Salt Basin (Love et al., 2005a, 2006); highly mature 2.7 and 3.4 Ga Archean kerogens from Pilbara Craton, Australia (Brocks et al., 2003; Marshall et al., 2007); abiotic insoluble organic matter in carbonaceous chondrite meteorites (Sephton et al., 2004, 2005); and for releasing the core hydrocarbon skeletons of functionalized lipids and aliphatic biopolymers from cells of cultured microorganisms (Love et al., 2005b). Despite the obviously attractive capabilities of HyPy for analyzing covalently-bound biomarkers, the technique has not been widely used due to the specialist nature of the equipment required to perform this high pressure treatment safely and routinely.

By using sophisticated and sensitive metastable reaction monitoring (MRM)-GC-MS for detection of key biomarkers in hydropyrolysates, some of our recent work has helped identify potential biosignatures for microbial life in early Archean oceans (Marshall et al., 2007), as well as specific steroid markers which mark the earliest appearance of basal animals (sponges) in the Neoproterozoic (Love et al., 2005a, 2006). Huqf rocks and oils from South Oman contain the least thermally altered Neoproterozoic organic matter used to date for molecular biomarker work (Grantham et al., 1988; Höld et al., 1999; Grosjean et al., in press) and as such preserve a wealth of biomarker information.

In this paper, we further illustrate our molecular approach and demonstrate the detail of molecular biomarker information preserved within Neoproterozoic kerogens isolated from sedimentary rocks from the Huqf Supergroup, South Oman Salt Basin, Sultanate of Oman. Parallel analyses of separate *free* and *bound* biomarker pools affords more confidence that we are looking at genuine ancient biomarkers in our analyses and can help identify contaminant inputs. We also speculate on the biological origins of an homologous series of mid-chain methylalkanes known as *X-peaks*, which are ubiquitous in all sedimentary rocks and oils analyzed from Huqf Strata from South Oman (Grosjean et al., in press). Despite their abundance and presumed importance in numerous late Proterozoic-Early Cambrian sedimentary environments, we currently do not know the organisms responsible for their biosynthesis. By looking at the paleoenvironmental context of their depositional setting and their co-occurrence with other hydrocarbons in the geologic record, however, then we can derive a hypothesis about possible source biota.

MATERIALS AND METHODS

Sediments

The Huqf Supergroup, South Oman Salt Basin (SOSB), provides one of the best preserved, most continuous sections of late Neoproterozoic through Early Cambrian strata (ca. 713 – 540 Ma; see McCarron, 2000; Grotzinger et al., 2002; Amthor et al., 2003, 2005; Fike et al., 2006; Allen, 2007; Bowring et al.,

2007). The Huqf Supergroup is divided into the Abu Mahara, the Nafun, and the Ara Groups. The Abu Mahara consists of Sturtian- (~713 Ma) and Marinoan- (~635 Ma) equivalent glacial deposits deposited in localized rift basins (Bowring et al., 2007). The Nafun Group records two shallowing-upward siliciclastic-carbonate sequences (Masirah Bay Formation – Khufai Formation; Shuram Formation – Buah Formation) deposited in a regionally extensive sag basin (McCarron, 2000). The Ara Group (~ 547–540 Ma; Bowring et al., 2007) consists of a series of carbonate-evaporite sequences (A0-A6) within the SOSB preserved solely in the subsurface. The Ara Group contains the Ediacaran-Cambrian boundary at the base of the fourth (A4) carbonate unit (Amthor et al., 2003). All formations in the SOSB contain abundant biomarkers, including 24-isopropylcholestanes produced from demosponges which in the late Cryogenian Abu Mahara Gp. currently constitute the oldest fossil evidence for metazoa (Love et al., 2005a, 2006). In this paper, we illustrate our general biomarker approach using molecular data obtained for an Ara Group carbonate rock sample (OMR229, A1C carbonate stringer, Minassa-1 well core, depth = 3399.83 m), a Nafun Group marl sample (sample OMR002, Buah Fm., Amal-SE-1 well cuttings, depth = 2345-2375 m), and a lime mudstone from the (late Cryogenian) Ghadir Manquil Fm, Abu Mahara Group (OMR190, GM-1 well core, depth = 2420-2426 m).

Analytical Techniques

All catalytic hydropyrolysis (HyPy) experiments on kerogens and pre-extracted sedimentary rocks were performed at University of Nottingham using high pressure stainless steel apparatus in the Snape laboratories. Experimental details for HyPy procedure are given elsewhere (Love et al., 1995, 2005a; Marshall et al., 2007). All molecular analyses of hydrocarbons from rock bitumens and hydropyrolysates were performed on a Micromass Autospec Ultima instrument in Prof. Roger Summons labs at MIT, with the analytical parameters used being similar to those reported previously for full scan GC-MS and metastable reaction monitoring (MRM)-GC-MS analyses (Grosjean et al., in press). Analyte separations in GC-MS were routinely performed on a DB-1MS coated capillary column (60 m x 0.25 mm i.d., 0.25 μm film thickness) using He as carrier gas.

RESULTS AND DISCUSSION

Comparison of Free and Kerogen-bound Saturated Hydrocarbon Profiles

Total ion current chromatograms (Fig. 1) of total saturated hydrocarbons generally show a similar carbon number pattern and range of *n*-alkanes and mid-chain methylalkanes (X-peaks, see below) for both the free alkane fractions of rock bitumens and for the kerogen hydropyrolysates. One consistent observable difference between free and bound molecular patterns for sediments of all ages and preserved up to high thermal maturity (late-oil window), for reasons not entirely understood, is that branched and polycyclic alkanes are proportionally less abundant in the kerogen pyrolysates compared with the corresponding bitumen extract. Close inspection of Fig. 1 reveals that pristane (Pr), phytane (Ph), X-peaks (x), hopanes and steranes are all present in lower abundance relative to the dominant *n*-alkanes in the kerogen hydropyrosate in comparison with the corresponding solvent extract for this Ara Gp. carbonate rock. Possible explanations are that resistant polymethylenic biopolymers, such as algaenans from microalgae (Gelin et al., 1999), made some contribution to the kerogen phase and/or that the greater steric bulk of branched and cyclic lipids leads to a preferential incorporation of functionalized lipids containing linear alkyl chains into the kerogen structure during diagenesis. The lower relative amounts of X-peaks in kerogen hydropyrolysates supports the view of Höld et al. (1999) that the precursor biogenic structures for X-peaks are most probably free functionalized lipids rather than core hydrocarbon constituents of a refractory biopolymer (see below).

Confirmation of Binding of Biomarkers into Kerogen

One option to confirm that the HyPy molecular products were predominantly generated from cleaving covalent bonds and releasing bound species, and are not just residual trapped hydrocarbons which escaped solvent extraction, is to perform deuterium labelling using pure high pressure deuterium gas instead of hydrogen as the reagent gas in HyPy. When covalent bonds are cleaved then generally one or more D atoms (Farrimond et al., 2003) are added to the product molecule at the binding site (with each fragment becoming one atomic mass unit heavier for every D atom incorporat-

ed compared with non-labelled molecules). This approach has even be applied to Precambrian kerogens (Brocks et al., 2003) although deuterium pyrolysis is a very expensive treatment and deuterium is a much less reactive reagent gas than hydrogen resulting in lower conversions of kerogen to soluble products.

An alternative means of achieving this confirmation can be derived from comparisons of the relative abundances of specific biomarker isomers captured by the solvent-extractable and kerogen-bound fractions. Such an approach relies on the empirical relationship already demonstrated from HyPy experiments on many ancient kerogens that the kerogen-bound hopane and sterane biomarkers released by HyPy exhibit a slightly less mature isomeric distribution than the corresponding free (extractable) biomarker hydrocarbons (e.g. Love et al., 1995, 1996; Bishop et al. 1998; Murray et al., 1998; Bowden et al., 2006) and this offset can be preserved to fairly high thermal maturity levels (late-oil window maturity, Lockhart et al., 2008). This is probably because the kerogen-bound biomarker pool is immobile and protected against alteration of structural and stereochemical features during thermal maturation reactions since this pool is sequestered by binding within a high molecular weight macromolecular matrix.

Furthermore, certain key rearranged isomers found in rock bitumens (such as diasteranes and neohopanes) should be absent or extremely low in abundance in kerogen products since these secondary transformation products are formed as hydrocarbons from the earliest stages of diagenesis. Possessing no

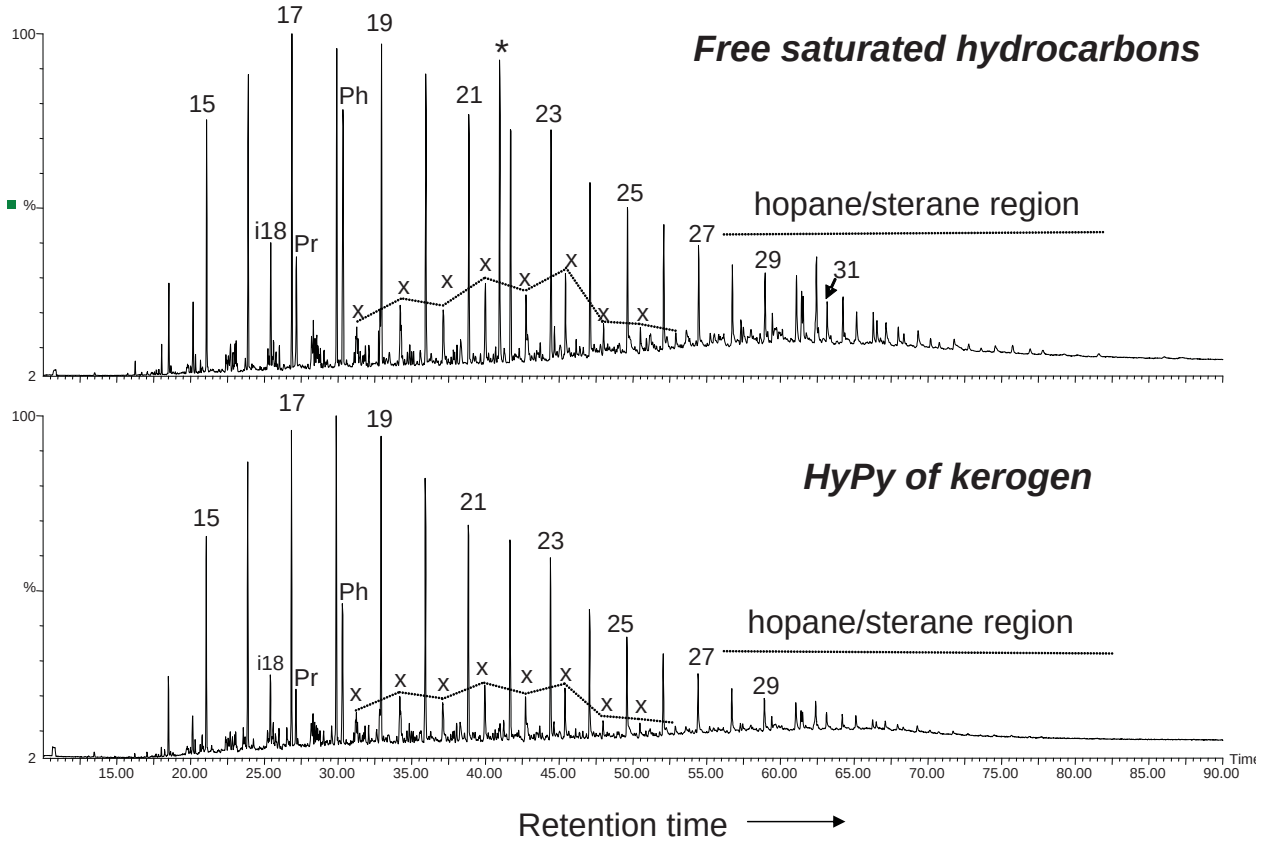

Figure 1—Total ion current (TIC) chromatograms comparing the distributions of saturated hydrocarbons released by solvent extraction (top) in comparison with those generated from HyPy of the corresponding kerogen from an Ara Group carbonate rock (OMR 229). Numbers refer to carbon chain lengths of *n*-alkanes. X indicate a homologous series of C_{19}-C_{26+} mid-chain methylalkanes known as *X-peaks* exhibiting a slight even over odd carbon number preference. X-peaks elute immediately before *n*-alkanes for compounds with equivalent number of carbon atoms. * = internal branched alkane standard (3-methylheneicosane).

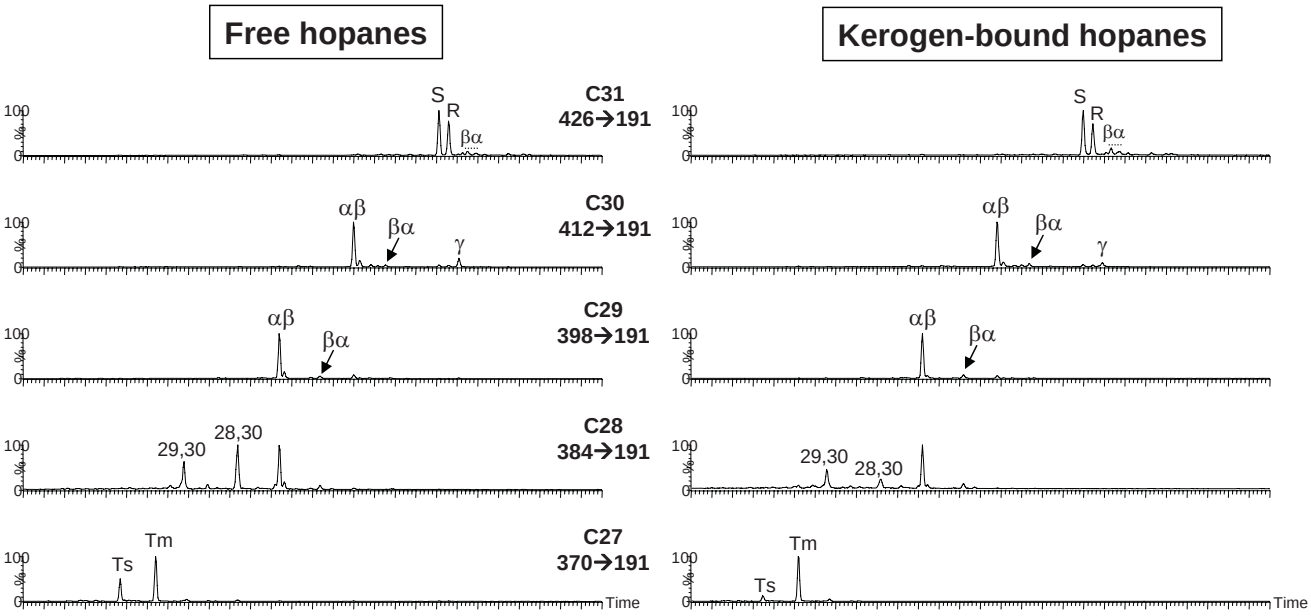

Figure 2—MRM-GC-MS (M^+ —> 191) chromatograms showing C_{27}-C_{31} hopanes for the free (extractable) hydrocarbons and kerogen HyPy products from a Buah Formation marl sample (OMR002). Note the proportionally higher amounts of rearranged hopanes, especially C_{27} Ts (18α,21β-22,29,30-trisnorneohopane) and 28,30-bisnorhopane in the free hydrocarbon extract compared with the corresponding kerogen HyPy products. Free hydrocarbon fractions contain slightly less moretanes (βα isomers) relative to the more thermodynamically stable αβ hopanes when compared with kerogen products. In this example, C_{29} %(βα/αβ+βα) = 6.0% for free hydrocarbons and 9.5% for kerogen (±1.0%). So overall, bound biomarkers should exhibit a slightly *less mature* isomeric profile than for the corresponding free molecules, and this offset should be preserved up to high thermal maturity (late-oil window maturity; Lockhart et al., 2008).

reactive functional groups for binding, then these rearranged hydrocarbons rarely become linked into kerogen. Typical compositional differences between free and kerogen-bound hopanes are illustrated for an uncontaminated Nafun group sedimentary rock in Fig. 2. Deviation from this commonly recognized free versus bound pattern is extremely useful for flagging contaminant hydrocarbons in rock bitumens and for checking whether the HyPy products have indeed been generated predominantly from covalent bond cleavage (and are not just residual bitumen which escaped extraction). So, the hopane and sterane isomer distributions for free versus bound organics are not expected to be identical and hence we can apply a useful and routine self-consistency check since these compounds are ubiquitous and abundant polycyclic biomarkers in virtually all Phanerozoic and most Precambrian sedimentary rocks.

X-peaks: Possible Biomarkers for Non-photosynthetic Sulfur Bacteria?

X-peaks are an unusual series of C_{14}-C_{30} mid-chain monomethyl-branched alkanes, but with greatest abundance in the C_{20}-C_{26} range, which have been detected before in Huqf oils and sedimentary rocks (Klomp et al., 1986; Höld et al., 1999, Grosjean et al., in press) as well as in other late Proterozoic-Early Cambrian samples. These include heavy petroleum from Pakistan (Grantham et al., 1988), source rocks and oils from the East European (Russian) Platform (Bazhenova and Arefiev, 1996), and oils from the East Siberian Platform (Fowler and Douglas, 1987). Despite their abundance in ancient sedimentary rocks and oils, little is known about the organisms responsible for their biosynthesis.

The position of methyl branching in the X-peaks has been determined via observation of the mass of the

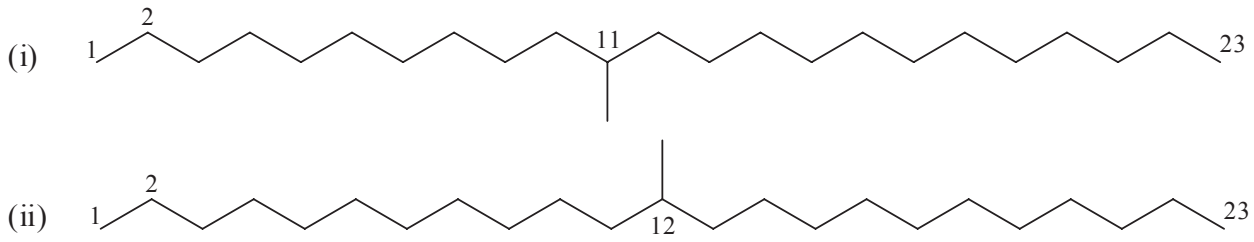

Figure 3—Molecular structure of (i) 11-methyltricosane and (ii) 12-methyltricosane, prominent C_{24} constituents of the "X-peak" mid-chain methylalkane homologous series. Many X-peaks alkanes, such as these, have a methyl branch 12 or 13 carbon atoms from one end of the linear chain, especially for the C_{20}-C_{26} members of the series.

major fragment ions produced during mass spectrometry. For compounds with fewer than 16 carbon atoms, alkylation occurs predominantly at the iso (C-2) or anteiso (C-3) position. With increasing carbon number, the proportion of 2- and 3-methyl alkanes decreases, and the locus of branching occurs towards the centre of carbon chains, with mid-chain preference achieved (Figure 3) for compounds in the range C_{22} to C_{26} (Höld et al., 1999).

To date, no reports of these same methyalkane skeletons in lipids of any extant organisms have been published and the source of these alkanes remains unknown (hence the term of *X-peaks*). These X-peak compounds, containing a single methyl branch, are not to be confused with quaternary-branched alkanes (containing 2 ethyl substituents at the quaternary carbon site, also known as BACQS) which have recently been shown to be hydrocarbon contaminants from polyethylene sample bags (Grosjean and Logan, 2007). BACQS were previously interpreted as biogenic hydrocarbon skeletons produced by unknown but ubiquitous modern and ancient microbes (Kenig et al., 2003).

One explanation proposed for the carbon number pattern of branching in the X-peaks is that they derived from a $C_{28(+)}$ precursor compound which contained a C_{24} hydrocarbon chain with a methyl group attached at either the C-12 or C-13 position, possibly bonded to a polar head group, such as a sugar unit (Höld et al., 1999). This would give rise to two series of compounds, both contributing to the overall X-peaks; the "12-methyl" series and the "13-methyl" series, which can be monitored via the *m/z* = 182 and *m/z* = 196 mass fragmentograms, respectively. Cleavage of a C_{24} hydrocarbon tail from a polar group could explain both the abundance of X-C_{24} and the tendency towards mid-chain branching but does not entirely explain the carbon number patterns found for the whole series though. Polyfunctionalized lipid precursors (Höld et al., 1999) are therefore a possibilty and are generally less well documented in lipid surveys of extant taxa than simple fatty acids and alcohols.

Our molecular data shows that a slight even-over-odd predominance (EOP) of X-peak carbon numbers is evident in gas chromatograms obtained for both free bitumens and kerogen hydropyrolysates (Fig. 1) indicating that, in comparison to the *n*-alkanes which show no discernible carbon number preference, there is probably a narrow spectrum of biological sources for these compounds. The magnitude of the original EOP for the precursor lipids will have been significantly reduced by thermal cracking reactions during long term catagenesis. The carbon number patterns of X-peaks are hence more consistent with being derived from a homologous series of parent lipids of differing chain lengths obtained by progressively adding acetate units that extend the chain by two carbon atoms while maintaining the positions of methyl branching relative to one end of the molecule. This is the normal mode of chain extension for acetogenic lipids biosynthesized by organisms (e.g. Birch, 1967).

Although here we don't present a definitive match between X-peaks and core lipid skeletons in extant taxa, taken together, several lines of evidence suggest that the X-peaks hydrocarbons preserved in Huqf strata were sourced from non-photosynthetic bacteria with a sulfur-oxidizing catabolic metabolism (encompassing a phylogenetically heterogeneous group

of γ-proteobacteria), or at least from some group of bacteria which thrived around the chemocline. Four independent lines of evidence include:-

i) methyl-branched alkyl lipids are often biosynthesized by bacteria in the form of hydrocarbons and fatty acids; such as those produced by cyanobacteria (Gelpi et al., 1970; Robinson and Eglinton, 1990), by uncharacterized symbiotic bacteria in extant sponges (Thiel et al., 1999) and by certain sulfate-reducing bacteria such as *Desulfobacter* sp. (Dowling et al., 1986); as well as in the form of alkyl glycerol ether lipids, such as those biosynthesized by bacteria (possibly sulfate-reducers) in marine anaerobic methane-oxidizing communities (Hinrichs et al., 2000),

ii) compound-specific delta ^{13}C analyses of X-peaks from three Huqf sediment bitumens and one Huqf oil (Höld et al., 1999) showed that these were generally depleted in ^{13}C by about 1‰ relative to the average value for individual *n*-alkanes, although the magnitude of this depletion can be significantly higher (4‰ or higher for one rock bitumen) when compared with the longer-chain *n*-alkanes (*n*-C_{21+}, which are probably mainly algal-derived). Thus, a biogenic source for X-peaks may be chemoautotrophic bacteria, fixing carbon via the Calvin cycle, but residing at a greater depth in waters with dissolved inorganic carbon (DIC) routinely ^{13}C-depleted relative to surface seawater (Hayes et al., 1999),

iii) samples in which X-peaks were most abundant, particularly in horizons within the Thuleilat and Silicylite Formations (around the Ediacaran-Cambrian boundary) of the restricted Athel sub-Basin within the SOSB (Amthor et al., 2005) but also in Ara Gp. carbonate rocks (Amthor et al., 2003; Fike et al., 2006; Bowring et al., 2007), contain high levels of 28,30-bisnorhopane, a C_{28} hopane compound. The high occurrence of 28,30-bisnorhopane in other well studied organic-rich Phanerozoic sedimentary sequences, such as in the Kimmeridge Clay Formation (Jurassic, UK) and the Monterey Formation (Miocene, USA) suggests this feature is frequently associated with organic matter deposited in stratified marine environments, particularly with sulfidic bottom waters or sedimentary pore-waters (Moldowan et al., 1984; Schoell et al., 1992),

iv) the absence or generally very low abundances of aromatic hydrocarbon biomarkers (such as C_{15}-C_{22} aryl isoprenoids with the 2,3,6-trimethyl substitution pattern and the C_{40} compound, isorenieratane) for Chlorobiacaea (green sulfur bacteria) in most Huqf Formation rocks in South Oman is surprising given that many Huqf-sourced oils and bitumens are sulfur-rich (Höld et al., 1999). This suggests that free hydrogen sulfide and other reactive inorganic sulfide species (such as HS- and polysulfides) did not pervade into the photic zone and sulfidic conditions were most probably restricted to either the bottom layers of microbial mats on the seafloor or within sedimentary pore waters in the sub-surface. One explanation for this is that colorless sulfur bacteria, perhaps as members of benthic microbial mat communities, could have oxidized hydrogen sulfide (and related inorganic sulfide species) and acted as an environmental buffer against sulfide toxicity for benthic communities.

Mid-chain monomethylalkanes were first proposed as possible markers for benthic sulfide-oxidizing bacteria by Logan et al. (1999). They noted anomalously high abundances of an alkane homologous series present in Late Ediacaran rock extracts from the Centralian Superbasin which co-eluted with X-peaks. This series was particularly prominent in benthic mat facies strata, and this was one line of evidence which pointed to a source of these alkanes from benthic bacterial mats. Sulfur isotopic evidence for ^{34}S-depleted pyrite preserved in the fossilized mat facies suggested that a possible source was colorless sulfur bacteria. Unfortunately, the diagnostic *immature* carbon number pattern highlighted for the alkane series prominent in their benthic mat facies samples is inconsistent with these compounds being syngenetic with ancient and mature host sedimentary rocks. Our kerogen HyPy experiments (Fig. 1) confirm that our X-peak methyl-

alkanes are definitely a series of syngenetic Neoproterozoic biomarkers. The very slight EOP of carbon numbers observed for X peaks in Fig 1. contrasts with the strikingly strong carbon predominance evident in the chromatograms published by Logan et al. (1999) for their designated *X-peak* series for the Wallara-1 mat facies sample. Each member of their series is only represented every 2 carbon atoms rather than having a full homologous series with each carbon number represented. Such a distribution is indicative of modern contamination and is similar to the carbon number patterns reported for contaminant alkylcyclopentanes, alkylcyclohexanes and BACQs series in polyethylene sample bags (Grosjean and Logan, 2007). So this unusual series of alkanes reported by Logan et al., (1999) cannot be considered genuine Ediacaran biomarkers.

One reason that the X-peaks may have survived so well in Huqf Supergroup is possibly due to the low level of acidic clay-catalyzed diagenetic and catagenetic rearrangements which occurred as evidenced by the low abundances of rearranged steranes (diasteranes) and hopanes (neohopanes) in South Oman sedimentary rocks and oils. While this is expected for carbonate rocks (due to acid-base buffering during diagenesis), hydrocarbons from clastic samples present in the basin generally show only low levels of rearranged steranes and hopanes as well. In reactions with acidic clay minerals, the methyl group can migrate along the linear chain producing a complex cluster of numerous methylalkane structural isomers with no prominent X-peaks (Kissin, 1986). This isomerization process may have masked the input of X-peaks amongst other monomethylalkane isomers in Neoproterozoic shales from other marine basins (Summons et al., 1988b) and also in older Meso- and Paleoproterozoic shales (Summons et al., 1988a, 1990; Pratt et al., 1991).

Fig 4. illustrates how the X-peak series is significantly more pronounced over the other methylal-

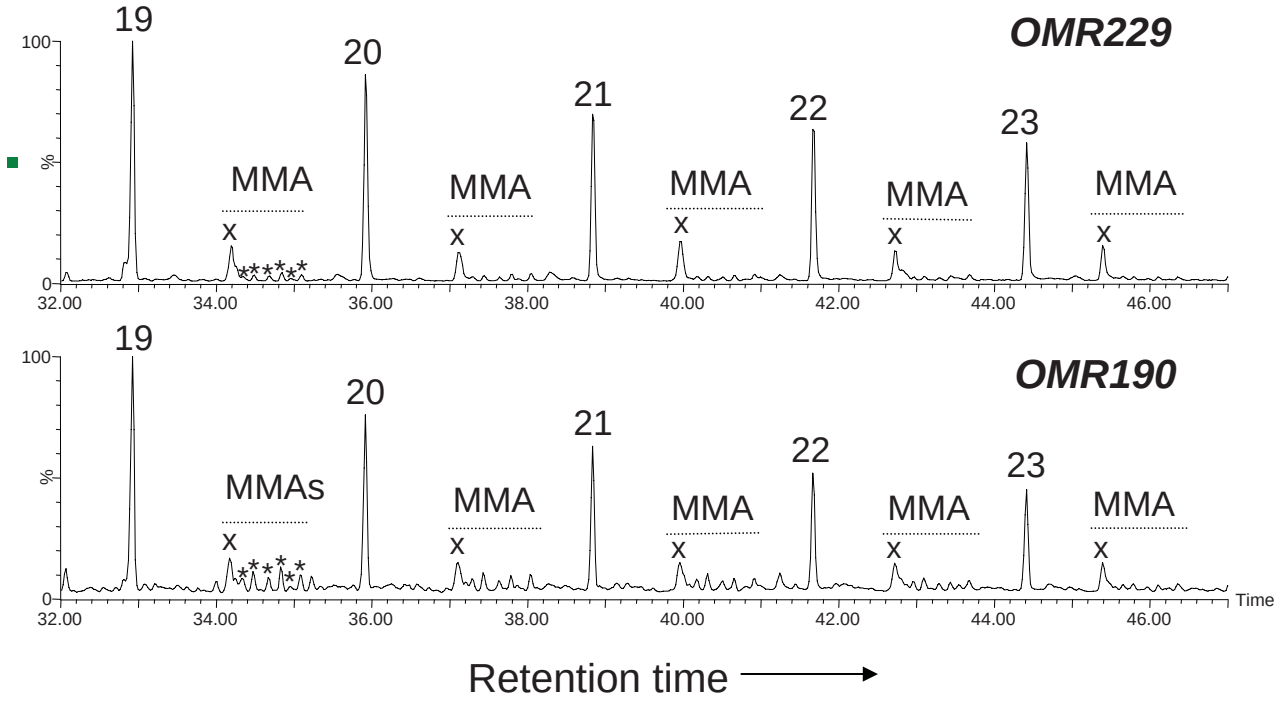

Figure 4—Total ion current (TIC) chromatograms comparing the distributions of C_{19}-C_{24} saturated hydrocarbons generated by HyPy from kerogens isolated from an Ara Group carbonate rock (OMR229) and an Abu Mahara Group lime mudstone sample (OMR190). Numbers refer to carbon chain lengths of *n*-alkanes. X indicate the C_{20}-C_{24} X-peak homologous series of mid-chain (mono)methylalkanes. Other monomethylalkane (MMA) structural isomers elute immediately after X peaks (and some mid-chain compounds co-elute with X-peaks). *C_{20} MMAs are highlighted. Note the lower abundance of the MMA series relative to X-peak compounds in the kerogen products from the carbonate rock sample.

kane isomers in kerogen hydropyrolysates from an Ara carbonate rock sample in comparison with a lime mudstone from the Abu Mahara Group. Both rocks contain sedimentary organic matter of similar thermal maturity but the latter sample contains higher proportions of clastic minerals than the carbonate rock. This isomerization effect will be further exacerbated at increasingly higher thermal maturity levels. A recognized characteristic of alkane profiles in chromatograms from other Paleoproterozoic to Neoproterozoic age sedimentary rocks and their petroleum products, in comparison with Phanerozoic marine sedimentary organic matter, is the higher abundance of partially-resolved clusters of methylalkanes relative to *n*-alkanes (Summons et al., 1988a, 1988b, 1990; Pratt et al., 1991; Logan et al., 1997). Although diverse bacteria can directly biosynthesize other methylalkane skeletons, such as isoalkanes (2-methylalkanes) and anteisoalkanes (3-methylalkanes), it is possible that isomerized X-peaks have contributed strongly to the abundant methylalkane clusters preserved in these older strata, especially for rocks of 1.4 Ga and older (Scott et al., 2008). In Proterozoic marine basins older than South Oman Salt Basin, colorless sulfur bacteria would have predominantly occupied a niche around the chemocline in marine waters rather than as benthic mats on the seafloor.

Future work will target extant benthic microbial mats and microbial cultures for lipid screening using both standard extraction/chemical methods and HyPy (Love et al., 2005b) to determine whether or not the X-peak hydrocarbon skeletons are core constituents of lipids produced by non-photosynthetic sulfur-oxidizing bacteria or other microorganisms. Analysis of the fatty acid portion of membrane phospholipids extracted from *Beggiatoa* mats from the Gulf of Mexico produced mainly just saturated and unsaturated linear C_{16} and C_{18} fatty acids (Zhang et al., 2005). No fatty acids chains longer than C_{22} were reported and only low relative amounts of methyl-branched fatty acids were generally detected, including low amounts (0.21 mole%) of 10-methylhexadecanoic acid derived from sulfate-reducing bacteria (Dowling et al., 1986) in the mat. Of course, free fatty acids are just one of a number of possible lipid compound classes that may contain the X-peak hydrocarbon skeleton. So, initial analyses of modern microbial biomass have thus far failed to identify any lipids with core skeletons corresponding closely to X-peaks.

The closest link thus far obtained with simple functionalized lipids are with C_{14}-C_{25} mid-chain branched alkanoic acids found in select modern demosponges (Thiel et al., 1999), which are more likely biosynthesized by unspecified bacterial symbionts present within sponge tissue rather than by the host. The exact carbon number patterns and positions of methyl-substituents in these sponge lipids are not consistent, however, with these being the source of X-peaks found in Huqf sedimentary rocks and oils. Similar arguments hold against cyanobacteria and sulfate reducing bacteria as prospective sources, and these also generally produce methyl-branched lipids with less than 22 carbon atoms in the core hydrocarbon skeletons (Gelpi et al., 1970; Robinson and Eglinton, 1990; Dowling et al., 1986; Hinrichs et al., 2000). In addition, there is no observed correlation between the abundance of 24-isopropylcholestanes (a proxy for demosponge input, McCaffrey et al., 1994) and X-peak/*n*-alkane ratios in Huqf samples. It seems unlikely that sponge and associated symbiont biomass production was so prolific during deposition of the Ara and Athel Gp. strata in the Neoproterozoic South Salt Basin as to explain the high abundances of X-peaks relative to *n*-alkanes (derived from mixed sources of microalgal and bacterial primary producers).

While it is possible that the biological sources of the Proterozoic and Cambrian X-peaks are now extinct (as proposed by Fowler and Douglas, 1987), screening efforts with extant cultures and microbial mats are only in their infancy. A prominent series of sulfur-bound C_{18}-C_{32} mid-chain monomethyl alkanes (reported as 9-methylalkanes, though it is possible that 12- and 13-methylalkanes were also constituents as these three series of structural isomers co-elute) from an organic sulfur-rich limestone of the Upper Jurassic Calcaires en Pacquette Formation (France) is suggestive that the source organisms were prevalent and synthesizing these (or structurally similar) lipids in stratified sulfidic marine environments in the Phanerozoic (Van Kaam-Peters and Sinninghe Damsté, 1997). Abundant and diverse methylalkane series, including mid-chain methylalkanes, have also been found in extracts from extant microbial mats from hypersaline and hydrothermal environments (Shiea et al., 1990; Kenig et al.,

1995). As for Precambrian shales, isomerization reactions involving clay minerals may have transformed X-peak series into a full series of all possible methylalkane isomers in Phanerozoic black shales and other clastic sedimentary rocks, thus muting the preserved X-peak signal.

Constraining the Temporal Record of Colorless Sulfur Bacteria

Canfield and Teske (1996) proposed that non-photosynthetic sulfide-oxidizing bacteria evolved between 0.64 and 1.05 Ga and that this explained a temporal shift in the sulfur isotopic composition of sedimentary sulfides (mainly pyrite) in Neoproterozoic sedimentary rocks of this age. They argued that this evolutionary event was likely triggered by a rise in atmospheric oxygen (estimated as reaching 5-18% PAL) which allowed shallow benthic environments on the continental shelf to become oxygenated and also coincided with the first appearance of animals. Hayes et al. (1999) also showed that unusually large offsets (ε>32‰) existed between the carbon isotopic compositions for carbonate and the co-occurring sedimentary organic matter during the late Proterozoic at 752, 740-732 and 623-600 Ma and this was explained as reflecting enhanced biogenic contributions of chemosynthetic bacteria at this time. Bailey et al. (2007) also proposed that at least a subset of the microfossils interpreted as fossilized animal embryos in the Edicaran Doushantuo Fm. in South China may in fact be remnants of giant colorless sulfur bacteria, although this alternative assignment has been contested (Yin et al., 2007).

Biomarker data from Huqf strata record the earliest appearance of basal animals in the Neoproterzoic (Love et al., 2005a, 2006) after the Sturtian glaciation (*ca*. 713 Ma, Bowring et al., 2007) and so support the idea of oxic marine waters being present at least on the shallowest shelves of certain ocean basins at this time. Rather than defining the divergence ages of certain groups of colorless sulfur bacteria, this timing most likely coincides with an ecological transition related to the change in the fundamental redox structure of Neoproterozoic oceans in the aftermath of low latitude glaciation (Hurtgen et al., 2006). After the Sturtian glaciation in South Oman Salt Basin, chemosynthetic sulfide-oxidizing bacteria were no longer confined predominantly to the water column but became widespread and abundant microbes in benthic marine shelf environments where steep opposing gradients of sulfide and oxygen intersected the seafloor. This special benthic redox boundary layer condition would have provided a unique niche for colorless sulfide-oxidizing bacteria to thrive in microbial mats as suggested by Logan et al. (1999) for the Late Ediacaran; but in our hypothesis this condition was met significantly earlier on the shallow shelves in certain late Cryogenian ocean basins.

An earlier radiation of colorless sulfur bacteria prior to the Neoproterozoic can be inferred, however, from multiple sulfur isotope records from the Meso- and Paleoproterozoic (Johnston et al., 2005, 2006, in press) and even from Archean sedimentary rocks (Kaufman et al., 2007; Phillippot et al., 2007). These geochemical records provide evidence for an oxidative sulfur cycle in existence involving sulfur compound-disproportionation, which requires sulfur intermediate chemical species produced by sulfide oxidation from either phototrophic or non-phototrophic bacteria, well before the Neoproterozoic.

Oxygenation of oceans in the Edicaran was apparently a long and protracted process from geochemical and stable isotopic records compiled (Fike et al., 2006; Canfield et al., 2007; McFadden et al., 2008; Scott et al., 2008; Shen et al., 2008) and at least three distinct progressive oxidative stages can be identified before the Ediacaran-Cambrian boundary in the South Oman Salt Basin (Fike et al., 2006). We observed from our biomarker data that X-peaks are particularly prominent in the Ara and Athel Gp. strata (Fig. 1; also see Grosjean et al., in press) which span the Late Ediacaran to Early Cambrian (*ca*. 548-540 Ma). This timing is coincident with stage III of Ediacaran progressive oxygenation defined by Fike et al. (2006) from $\delta^{34}S$ sulfur isotope patterns of sedimentary pyrite versus carbonate-associated sulfate. In contrast, while X-peaks could still be discerned in chromatograms for the underlying Nafun Group strata (ca. 635-550 Ma; Bowring et al., 2007), their relative abundance was generally much lower than in Ara and Athel rocks and oils (Grosjean et al., in press). Only in the Shuram Formation., stratigraphically above the negative minimum value of $\delta^{13}C$ of carbonate but still within the previously recognized $\delta^{13}C$ isotope *Shuram Excursion* for carbonate (Fike et al., 2006) have prominent X-peaks been noted in Na-

fun Group rocks to date. The Shuram carbon isotopic excursion has been explained as an oxidation event (Fike et al., 2006) involving the remineralization and removal of a vast reservoir of dissolved or particulate marine organic matter (Rothman et al., 2003). So it is possible then that X-peak abundances relative to *n*-alkanes can define progressive oxygenation of ocean basins and their highest abundances in Ara and Athel Gp rocks and oils (Grosjean et al., in press) may reflect steep geochemical gradients at the water-sediment interface as marine waters became more oxic, providing a stable niche for colorless sulfur bacteria to flourish in benthic mats. The Late Ediacaran-Early Cambrian generally appears to be the period in which the highest abundance of X-peaks are detected in the sedimentary record, in rocks and oils from South Oman (Höld et al., 1999; Grosjean et al.,in press), the Russian Platform (Bazhenova and Arefiev, 1996), Eastern Siberia (Fowler and Douglas, 1987) and Pakistan (Grantham et al., 1988). This is consistent with our hypothesis that colorless sulfur bacteria, which oxidize sulfide, are a possible source of these compounds.

Benthic microbial mats, containing colorless sulfur bacteria, could also have provided a cohesive sedimentary surface substrate for Ediacaran benthic fauna colonizing the seafloor and aided the long-term preservation of trace and body fossils (Droser et al., 2002). It has also been proposed that olenid trilobites found in Late Cambrian to early Ordovician sedimentary rocks contained chemoautotrophic symbionts, particularly colorless sulfur bacteria, to cope with sulfide-rich seafloor conditions (Fortey, 2000). The compatibility of this later host animal and symbiotic bacterial association may have roots in the specific benthic conditions present during the evolution of the earliest metazoans when sulfide-oxidizing bacterial mats may have been widespread in shallow marine environments.

CONCLUSIONS

The detailed analysis of the pool of biomarker hydrocarbon skeletons covalently-bound within sedimentary kerogen (insoluble macromolecular organic matter) can be achieved rapidly and with excellent reproducibility using a combination of catalytic hydropyrolysis (HyPy) and gas chromatography-mass spectrometry (GC-MS) techniques, particularly metastable reaction monitoring (MRM)-GC-MS. The HyPy analyses are usually conducted in parallel with conventional biomarker analysis, which use the free hydrocarbons found in solvent-extractable rock bitumens. Close monitoring of the bound biomarker profiles helps us to identify any anomalous and suspicious biomarker features found in free hydrocarbons which may be possible contaminants. In this way, this combination of separate *free* plus *bound* biomarker pools affords much more confidence that we are looking at genuine syngenetic biomarkers in our analyses.

While biogenic sources of many lipid compound classes have been identified, not all the main homologous series of alkane compounds detected in Precambrian sediments have been successfully assigned to contemporary lipid precursor(s). A prime example of this is for the so-called *X-peaks*. These are a series of C_{14}-C_{30} mid-chain methylalkanes which are detectable (and sometimes highly abundant) in numerous late Neoproterozic-Early Cambrian sediments and oils, including in all the formations from the Huqf Supergroup in the South Oman Salt Basin. A number of independent lines of compelling evidence suggest that the source organisms for the X-peak hydrocarbon skeletons may be colorless sulfur bacteria (a heterogeneous group of γ-proteobacteria which can oxidize hydrogen sulfide and other sulfur species), or at least a group of bacteria which thrive around the oxic/anoxic redox interface in marine environments. These colorless sulfur bacteria would have been important in maintaining extremely low free hydrogen sulfide concentrations in shallow benthic marine environments in the Neoproterozoic and helped nurture early animal life. Establishing a link between the X-peak methylalkanes prominent in Neoroterozic and Early Cambrian sedimentary rocks and oils with lipid precursor(s) in extant microorganisms will help test existing hypotheses about biogeochemical cycling and the timing of oxygenation of ocean basins during this important period of climatic change and biological evolutionary developments (Canfield and Teske, 1996; Hayes et al., 1999; Fike et al., 2006; Canfield et al., 2007; McFadden et al., 2008; Scott et al., 2008; Shen et al., 2008).

The enigma of the X-peak methylalkanes highlights our need to better characterize and compile a more complete inventory of lipid structures in extant microbial cultures and microbial mats in order to be able to more accurately interpret the ancient molecular

biomarker record. Progress can be made through rapid screening methods, such as HyPy of whole cells (Love et al., 2005b), used in conjunction with other modern analytical techniques, such as high performance liquid chromatography (especially HPLC-MS), which allow us to directly probe the structure of complex, intact lipids.

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