

## LETTERS

# Fossil steroids record the appearance of Demospongiae during the Cryogenian period

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The Neoproterozoic era (1,000–542 Myr ago) was an era of climatic extremes and biological evolutionary developments culminating in the emergence of animals (Metazoa) and new ecosystems<sup>1</sup>. Here we show that abundant sedimentary 24-isopropylcholestanes, the hydrocarbon remains of C<sub>30</sub> sterols produced by marine demosponges, record the presence of Metazoa in the geological record before the end of the Marinoan glaciation (~635 Myr ago). These sterane biomarkers are abundant in all formations of the Huqf Supergroup, South Oman Salt Basin, and, based on a new high-precision geochronology<sup>2</sup>, constitute a continuous 100-Myr-long chemical fossil record of demosponges through the terminal Neoproterozoic and into the Early Cambrian epoch. The demospone steranes occur in strata that underlie the Marinoan cap carbonate (>635 Myr ago). They currently represent the oldest evidence for animals in the fossil record, and are evidence for animals pre-dating the termination of the Marinoan glaciation. This suggests that shallow shelf waters in some late Cryogenian ocean basins (>635 Myr ago) contained dissolved oxygen in concentrations sufficient to support basal metazoan life at least 100 Myr before the rapid diversification of bilaterians during the Cambrian explosion. Biomarker analysis has yet to reveal any convincing evidence for ancient sponges pre-dating the first globally extensive Neoproterozoic glacial episode (the Sturtian, ~713 Myr ago in Oman<sup>2</sup>).

The Neoproterozoic–Cambrian Huqf Supergroup, South Oman Salt Basin (SOSB), is located at the southeastern edge of the Arabian peninsula and comprises the Abu Mahara Group encompassing Sturtian- and Marinoan-equivalent glacial deposits, and the Nafun and Ara Groups<sup>2,3</sup> (Fig. 1). The Abu Mahara Group was deposited in localized rift basins, whereas 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<sup>4</sup>. The Ara Group, which was deposited ~547–540 Myr ago<sup>2</sup>, 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. Well-preserved lipid biomarkers are prevalent in the sedimentary rocks and oils of the Huqf. Previous organic geochemical studies show that SOSB oils, and their precursor source rocks, have a very distinctive molecular and isotopic geochemistry marked by unusual abundances of methylalkanes, steroids and triterpenoids derived from microbiota present at the time of sediment deposition<sup>5,6</sup>.

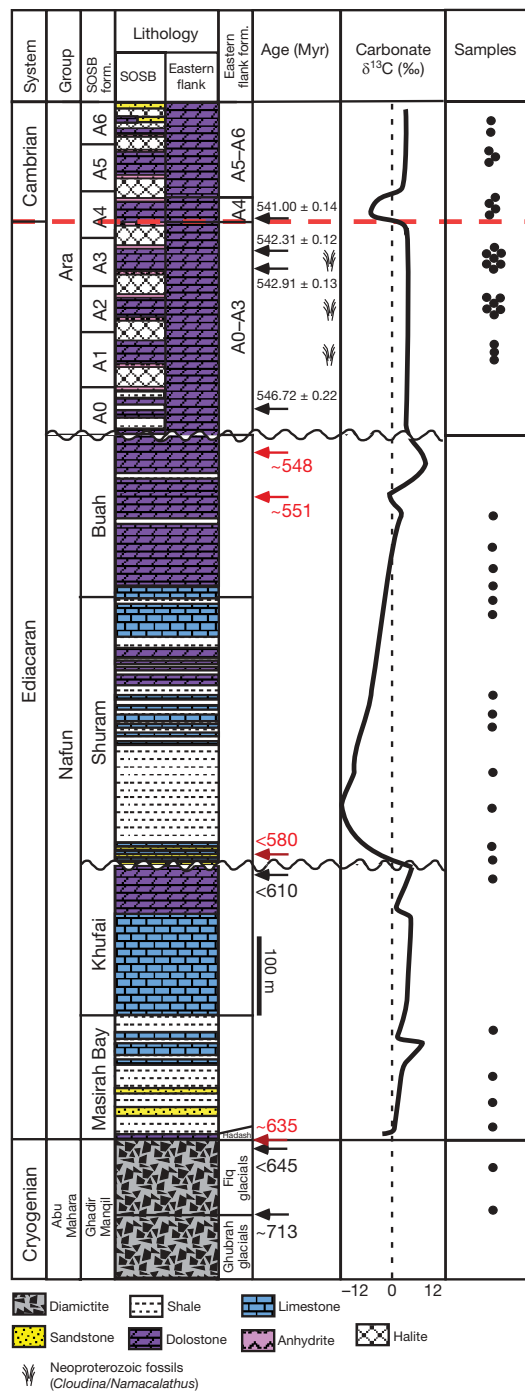
We analysed extractable saturated and aromatic hydrocarbons from 64 sedimentary rock samples, comprising core and cuttings,

from 26 different wells from the petroleum-rich SOSB (Fig. 1). Analyses were carried out via gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) with the high sensitivity, selectivity and accuracy afforded by multiple-reaction-monitoring (MRM) mass spectrometry (see Supplementary Information). To establish the stratigraphic range of specific organic compounds beyond doubt, we isolated kerogens (insoluble, macromolecular organic matter that cannot migrate) from key samples. From these kerogens we generated complementary sets of biomarkers using catalytic hydro-pyrololysis (HyPy). With this technique, covalently bound hydrocarbons are released from the (immobile) kerogen by continuous-flow, temperature-programmed pyrolysis in a stream of high-pressure (15 MPa) H<sub>2</sub> gas and using a molybdenum sulphide catalyst. HyPy is a powerful analytical tool for obtaining high yields of biomarker hydrocarbons with optimal preservation of structure and stereochemistry<sup>7</sup>. Kerogen-bound biomarkers released by HyPy can be unambiguously correlated to a specific stratigraphic interval.

The absolute abundances of extractable C<sub>26</sub>–C<sub>30</sub> steranes (which ranged from ~300 to 13,000 p.p.m. of total saturated hydrocarbons, depending on thermal maturity) and sterane/hopane ratios (0.21–1.50, with an average value of 0.81; Table 1 and Supplementary Table 1) in these Huqf samples are comparable in magnitude to those found in typical Phanerozoic marine organic matter such as the Kimmeridge Clay<sup>8</sup> that sources North Sea petroleum. This contrasts with the trace amounts of regular steranes detected (<1 p.p.m. of total organic carbon) in rock extracts of similar thermal maturity from highly euxinic facies of the 1,640-Myr-old Barney Creek Formation<sup>9</sup>. Eukaryotic microalgae are most probably the principal biological source of steranes in Neoproterozoic–Cambrian sedimentary rocks. Their high absolute concentrations in Huqf sedimentary rocks suggests that marine microbial communities rich in microalgae proliferated in Neoproterozoic oceans.

Accumulation of abundant hopanes and 2-methylhopanes in Huqf sedimentary rocks suggests that bacteria<sup>10</sup> constituted a significant fraction of primary productivity, but the absolute abundance of C<sub>29</sub> steranes and their dominance over C<sub>26</sub>–C<sub>30</sub> steranes (Table 1 and Supplementary Tables 1 and 2) suggests that chlorophyte microalgae were quantitatively important as marine primary producers. This sterane pattern mirrors the C<sub>29</sub> sterol carbon number dominance in many extant chlorophytes<sup>11</sup>. The prominence of C<sub>29</sub> steranes over other steranes is observed in all SOSB formations, including the Cryogenian Ghadir Manquil Formation. High diversity in the structures of the minor SOSB steranes indicates that other groups of microalgae must also have been present, including marine pelagophytes

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**Figure 1 | Stratigraphic column of Huqf Supergroup with representative lithology, biostratigraphy and geochronological constraints.** Stratigraphic distribution of samples in the present study is indicated on the right. Absolute dates in red are from correlation with other dated sections worldwide (Namibia, South China) using comparisons of  $\delta^{13}\text{C}$  carbonate stratigraphic features. Absolute dates in black are from direct U–Pb zircon age measurements on Huqf detrital zircons and ash beds. See Supplementary Information for additional discussion. A typical  $\delta^{13}\text{C}$  carbonate stratigraphic profile is drawn for reference<sup>3</sup>.

and dinoflagellates inferred from 24-*n*-propyl steranes<sup>12</sup> and dinosteranes<sup>11</sup>, respectively. Uncommon steranes detected in these rocks included 27-norcholestanes, 21-norcholestanes, 21-norergostanes and 21-norstigmastanes and a variety of  $\text{C}_{19}$  to  $\text{C}_{20}$  steroids that have had their side-chains excised. Of particular note was the high relative abundance of  $\text{C}_{30}$  steranes with a 24-isopropyl moiety in all formations of the Huqf Supergroup (Fig. 2, Table 1, Supplementary Tables 1 and 2) which signifies demosponge inputs.

**Table 1 | Summary of key biomarker ratios for Huqf rock bitumens and kerogen hydroxylysates**

Formation	Phase	Ster/hop*	% $\text{C}_{29}$ ster†	% $\text{C}_{30}$ ster‡	<i>i</i> - $\text{C}_{30}$ / <i>n</i> - $\text{C}_{30}$ §
Ara carbonates	Bitumen [25]	0.2–1.1 (0.8)	52–75 (69)	1.8–6.7 (2.7)	1.0–1.9 (1.5)
Ara carbonates	Kerogen [8]	0.6–1.0 (0.8)	52–70 (61)	1.9–3.6 (2.8)	0.6–1.4 (0.9)
Thuleilat	Bitumen [5]	0.6–1.3 (0.9)	60–73 (68)	1.9–2.8 (2.5)	1.3–1.6 (1.4)
Thuleilat	Kerogen [2]	1.0–2.5	57–65	2.0–2.5	0.7–1.2
Silicyllite	Bitumen [5]	0.8–1.5 (1.1)	72–76 (74)	1.8–2.4 (2.1)	1.4–2.4 (1.8)
Silicyllite	Kerogen [2]	1.5–2.1	69–75	2.1–2.3	0.7–1.1
U shale	Bitumen [5]	0.8–1.0 (0.9)	60–66 (63)	2.2–2.9 (2.5)	0.8–1.4 (1.1)
U shale	Kerogen [1]	1.2	53	2.7	1.7
Buah	Bitumen [4]	0.7–1.1 (0.9)	64–73 (69)	1.3–1.8 (1.6)	0.6–0.9 (0.7)
Buah	Kerogen [2]	0.9–1.1	63–67	1.3–1.9	0.5–0.7
Shuram	Bitumen [8]	0.6–1.1 (0.8)	65–77 (70)	1.9–2.5 (2.2)	0.8–1.8 (1.2)
Shuram	Kerogen [2]	0.6–1.0	58–60	2.7–4.6	0.7–1.4
Khufai	Bitumen [2]	0.5–0.8	72–73	2.0–3.3	1.3–1.4
Masir. B.	Bitumen [5]	0.5–0.7 (0.7)	58–84 (67)	2.3–13 (4.8)	1.3–16 (4.9)
Masir. B.	Kerogen [2]	0.7–1.2	54–55	3.4–5.6	1.4–1.5
Gh. Manq.	Bitumen [2]	0.4–0.9	56–72	2.7–3.7	0.5–3.3
Gh. Manq.	Kerogen [1]	0.7	66	3.0	1.3

[n] represents number of samples; () are average ratio values for  $n > 2$ ; a more comprehensive biomarker data set is given in Supplementary Tables 1 and 2. Average uncertainties in hopane and sterane biomarker ratios are  $\pm 8\%$  as calculated from multiple analyses of saturated hydrocarbon fractions prepared from an AGSO standard oil ( $n = 30$ ). Masir. B., Masirah Bay; Gh. Manq., Ghadir Manquil.

\* Ratio of ( $\text{C}_{27}$ – $\text{C}_{29}$  steranes)/( $\text{C}_{27} + \text{C}_{29-35}$  hopanes).

† Ratio of  $\text{C}_{29}$  steranes to  $\Sigma\text{C}_{27}$ – $\text{C}_{29}$  steranes.

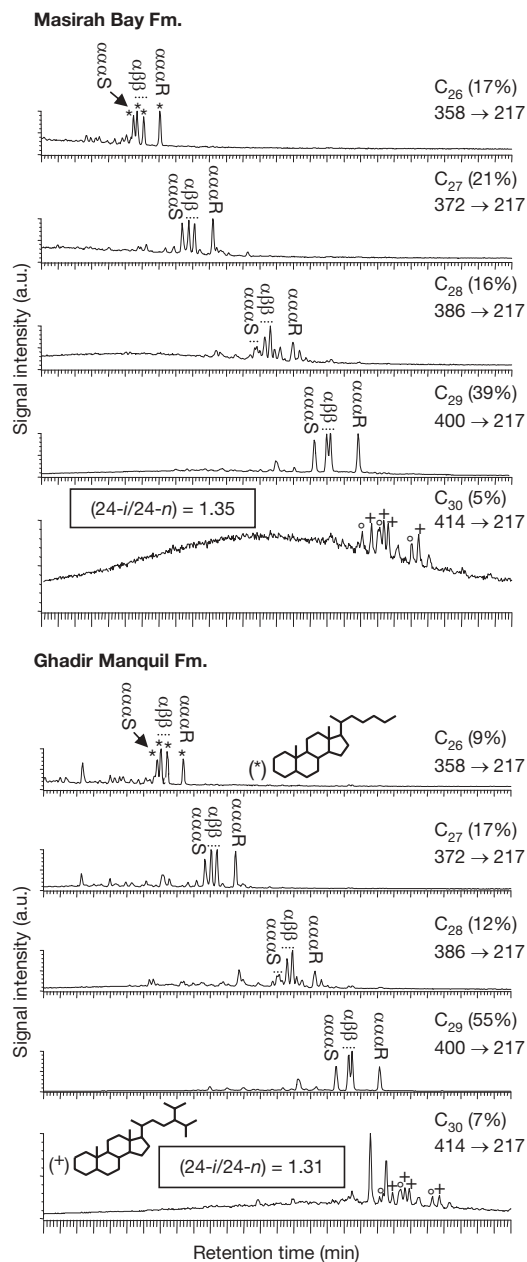
‡ ( $24$ -*n*-propylcholestanes +  $24$ -isopropylcholestanes)/ $\Sigma(\text{C}_{27}$ – $\text{C}_{30}$  steranes).

§  $24$ -*i*-propylcholestanes/ $24$ -*n*-propylcholestanes (using all 4 regular isomers).

|| Formations in the basin centre (Athel basin) which are age equivalent to Ara Group carbonates (see Supplementary Information).

24-Isopropylcholestane is the geologically stable form of 24-isopropylcholesterol and related structures, which are primarily found in certain genera of the Demospongiae<sup>13</sup> and can be biosynthesized *de novo* to function in the sponge cell membrane<sup>14</sup> (Supplementary Information). 24-Isopropylcholestanes were previously shown to be abundant relative to microalgal 24-*n*-propylcholestanes ( $>0.5$ ) in numerous Ediacaran to Early Cambrian oils and calcareous sediments, thus representing anomalously elevated levels of these compounds (Supplementary Information), and on this basis, were proposed as molecular fossils of sponges or their ancestors<sup>15</sup>. Potential precursor sterols were not identified in the choanoflagellate *Monosiga brevicollis*<sup>16</sup>, a representative for the unicellular sister group of animals. The rigorous stratigraphic and geochronologic placement of the SOSB samples in our study constrains the first appearance of sponge biomarkers and suggests that sponges were continuously prevalent in a wide range of Neoproterozoic environments before the known record of other animal fossils, including megascopic animal body fossils  $\sim 575$  Myr ago<sup>17</sup>, trace fossils  $\sim 555$  Myr ago<sup>18</sup> and putative animal embryos  $<632$  to  $>550$  Myr ago<sup>19</sup>. The detection of free and kerogen-bound sponge steranes in sedimentary rocks from the Ghadir Manquil Formation (Fig. 2) of the Huqf Supergroup, found stratigraphically below the Marinoan cap carbonate, suggests a Cryogenian origin of Metazoa. Detrital zircon U–Pb ages of  $\sim 751$  Myr were obtained previously from Ghadir Manquil Formation sediments from SOSB<sup>2</sup>, including from the GM-1 well (Supplementary Fig. 1), so 751 Myr constitutes a maximum age for the Cryogenian sponge biomarkers found in our study. Analysis of a number of pre-Sturtian sediments from other sections worldwide has found no convincing evidence for elevated levels of 24-isopropylcholestanes in rock bitumens (Supplementary Information).

Existing fossil evidence for Ediacaran sponges comes from detection of siliceous spicules derived from hexactinellids in  $\sim 543$ – $549$  Myr sedimentary rocks from Australia<sup>20</sup> and southwestern Mongolia<sup>21</sup>, and from putative siliceous demosponge spicules<sup>22</sup> found alongside preserved sponge tissue and animal embryos<sup>23</sup> in  $<600$ -Myr Doushantuo phosphorites in South China. Molecular phylogenetic classifications using metazoan protein amino acid and nucleic acid sequences usually place the silicisponges, the demosponges and hexactinellids, as the earliest diverging animals<sup>24</sup>. The timing of the sponge



**Figure 2** | MRM GC-MS ion chromatograms of  $C_{26}$ – $C_{30}$  desmethylsteranes released from catalytic hydroxyprolysis of a Masirah Bay Formation (JF-1) and a Ghadir Manquil Formation (GM-1) kerogen. For each sterane carbon number, four diastereoisomers are detected ( $\alpha\alpha\alpha$ 20R,  $\alpha\beta\beta$ 20R,  $\alpha\beta\beta$ 20S,  $\alpha\alpha\alpha$ 20R), indicating a mature geoisomer distribution. Demosponge contributions are evident from abundant 24-isopropylcholestanes ('plus' signs). 24-*n*-propylcholestanes (open circles) are markers of marine pelagophyte algae and this confirms a marine depositional setting for each formation in the SOSB. Stars mark a series of 27-norcholestanes. At right, values in parentheses represent a measure of relative signal intensity for the  $C_{26}$ – $C_{30}$  steranes in acquired MRM chromatograms (though absolute abundances are determined from individual peak areas) and the numbers beneath are the masses (in daltons) of the ion transitions (molecular weight  $\rightarrow$  fragment ion) used in MRM GC-MS in each case. *y* axis, signal intensity; *x* axis, retention time in min (52 to 68 min shown for all traces).

biomarker appearance corresponds well to divergence estimates for the last common ancestor of all living demosponges obtained from molecular clocks<sup>25,26</sup>, and indeed can now be used to more robustly calibrate the molecular clock at the base of the animal tree<sup>1</sup>.

The use of recalcitrant lipid biomarkers offers a promising approach for tracking the earliest sponge contributions to Precambrian sedimentary rocks because outstanding preservation of

soft-body parts, as detected in Doushantuo phosphorites<sup>19,22</sup>, is rare in the geological record. Siliceous sponge spicules are metastable and they can be difficult to isolate and identify unambiguously in clastic sediments. Moreover, several orders of Demospongiae completely lack mineral skeletons. On the other hand, the studies of the lipid compositions of Porifera show a remarkable diversity of distinctive structures with abundance patterns aligned to phylogeny<sup>13,27,28</sup>.

The demosponge biomarker record for the Huqf Supergroup supports the hypothesis that Metazoa first achieved ecological prominence in shallow marine waters of the Cryogenian<sup>1</sup>. It has been proposed that Neoproterozoic sponges and rangeomorphs feeding on reactive dissolved or particulate marine organic matter<sup>29</sup> may have progressively oxygenated their benthic environments as they moved from shallow water into deeper waters<sup>24</sup>. Consistent with this, our data (Table 1 and Supplementary Table 1) show that, on average,  $C_{30}$  steranes comprised 2.7% of total  $C_{27}$ – $C_{30}$  extractable steranes in Huqf samples and 63% of the summed  $C_{30}$  compounds were 24-isopropylcholestanes, suggesting that demosponges must have made a significant contribution to preserved sedimentary organic matter and, therefore, environmental biomass<sup>24</sup>. In contrast, lack of significant sponge steranes in deepwater shales from the Ediacaran Rodda Bed Formation in the Officer basin, Australia<sup>15</sup>, and from the late Cryogenian Aralka Formation (Supplementary Information) suggests that it took longer to colonize deepwater environments. Neoproterozoic sponges would have been at least partly responsible for the ultimate respiration and removal of dissolved organic carbon<sup>24,29</sup>, aiding ventilation of the global ocean and shifts in the modes of carbon and sulphur cycling evident from Ediacaran isotopic and geochemical records<sup>3,30</sup>.

## METHODS SUMMARY

Solvent-rinsed core rock fragments and cuttings were crushed to a fine powder using an alumina ceramic puck mill housed in a SPEX 8510 shatterbox. Rock powders were extracted with a mixture of dichloromethane and methanol (9:1, v/v) using a Dionex Accelerator Solvent Extractor ASE-200 operated under 1,000 p.s.i. at 100 °C. Asphaltenes were precipitated from the resulting organic extracts (bitumens) using *n*-pentane. The maltenes (*n*-pentane solubles) were then fractionated by silica gel adsorption chromatography, eluting successively with hexane, hexane/ $CH_2Cl_2$  (v/v: 4:1) and  $CH_2Cl_2$ / $CH_3OH$  (v/v: 3:1) to yield saturated hydrocarbons, aromatic hydrocarbons and resin fractions, respectively.

Continuous-flow hydroxyprolysis experiments were conducted on 100–2,000 mg of catalyst-loaded pre-extracted sediments or kerogen concentrates as described previously<sup>7</sup>. Hydroxyprolylates were fractionated on silica gel columns, as for rock bitumens.

GC-MS analyses of saturated hydrocarbon fractions were performed on a Micromass AutoSpec Ultima equipped with a HP6890 gas chromatograph and a DB-1MS coated capillary column (60 m  $\times$  0.25 mm i.d., 0.25- $\mu$ m film thickness) using He as carrier gas. Hopane and sterane biomarkers were analysed by MRM GC-MS with a total cycle time of 1.3 s per scan for 26 transitions, including the *m/z* 414 to 217 transition for  $C_{30}$  desmethylsteranes. The GC oven was programmed at 60 °C (2 min), heated to 150 °C at 10 °C min<sup>-1</sup>, further heated to 315 °C at 3 °C min<sup>-1</sup> and held at final temperature for 24 min.

50 ng of deuterated  $C_{29}$  sterane standard [ $d_4$ - $\alpha\alpha\alpha$ -24-ethylcholestan-20R)] was typically added to 1 mg saturates to quantify the polycyclic biomarker content. Yields assume equal mass spectral response factors between analytes. Analytical errors for individual hopanes and steranes concentrations are estimated at  $\pm 30\%$ . Average uncertainties in hopane and sterane biomarker ratios are  $\pm 8\%$  as calculated from multiple analyses of a saturated hydrocarbon fraction from an AGSO standard oil ( $n = 30$ ).

**Full Methods** and any associated references are available in the online version of the paper at [www.nature.com/nature](http://www.nature.com/nature).

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**Supplementary Information** is linked to the online version of the paper at [www.nature.com/nature](http://www.nature.com/nature).

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**Author Contributions** G.D.L. interpreted the data and wrote the manuscript with input from R.E.S., D.A.F., A.S.B. and E.G. G.D.L., E.G., C.S. and A.E.K. acquired the Huqf biomarker data working in the research group of R.E.S., A.S.B. and M.B. screened extant demosponges for their sterol contents. C.E.S. and W.M. made facilities available for HyPy experiments on kerogens and trained C.S. to use the equipment. J.P.G. provided a robust stratigraphic framework for the Huqf Supergroup in the SOSB and with D.A.F. identified key sedimentary rock samples to use in this investigation. S.A.B. and D.J.C. measured important U–Pb ages for ash beds and detrital zircons through the stratigraphy to constrain the age range and distribution of our demosponge biomarkers.

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## METHODS

The outer surfaces of sediment core and larger cuttings fragments were cleaned sequentially by ultrasonication in distilled water, then methanol, then dichloromethane, and finally *n*-hexane for ~20 s per step before extraction. Cleaned core fragments and cuttings were then crushed to a fine powder using an alumina ceramic puck mill housed in a SPEX 8510 shatterbox. Between samples, the puck mill was cleaned by crushing annealed sand three times for 1-min periods each, followed by washing with the same cleaning solvent sequence described above.

Rock powders were extracted with a mixture of dichloromethane and methanol (9:1, v/v) using a Dionex Accelerator Solvent Extractor ASE-200 operated under 1,000 p.s.i. at 100 °C. Asphaltenes were precipitated out from the resulting organic extracts (bitumens) and from the oils using *n*-pentane. In asphaltene-free fractions (maltenes) derived from bitumens, elemental sulphur was removed with activated and solvent-washed copper pellets. The maltenes were then fractionated by silica gel column chromatography eluting successively with hexane, hexane/CH<sub>2</sub>Cl<sub>2</sub> (v/v: 4:1) and CH<sub>2</sub>Cl<sub>2</sub>/CH<sub>3</sub>OH (v/v: 3:1) to yield saturated hydrocarbons, aromatic hydrocarbons and polars/resins (N, S, O compounds), respectively.

Continuous-flow HyPy experiments were performed on 100–2,000 mg of catalyst-loaded pre-extracted sediments or kerogen concentrates as described previously<sup>7</sup>. The isolation of kerogen concentrates was conducted on solvent-extracted rock residues by standard hydrofluoric acid/hydrochloric acid (HF/HCl) extraction procedures. Further treatment of the isolated kerogens involved extraction with dichloromethane by ultrasonication (×3). Extracted sediments and kerogens were initially impregnated with an aqueous methanol solution of ammonium dioxodithiomolybdate, (NH<sub>4</sub>)<sub>2</sub>MoO<sub>2</sub>S<sub>2</sub>, to give a nominal loading of 2 wt% molybdenum. Ammonium dioxodithiomolybdate reductively decomposes *in situ* under HyPy conditions above 250 °C to form a catalytically active molybdenum sulphide (MoS<sub>2</sub>) phase. The catalyst-loaded samples were heated in a stainless steel (316 grade) reactor tube from ambient temperature to 260 °C at 300 °C min<sup>-1</sup> then to 520 °C at 8 °C min<sup>-1</sup>. A hydrogen sweep gas flow of 6 dm<sup>3</sup> min<sup>-1</sup>, measured at ambient temperature and pressure, through the reactor bed ensured that the residence times of volatiles generated was the order of only a few seconds. Products were collected in a silica gel trap cooled with dry ice and the adsorbed pyrolysates were separated into saturates, aromatics and polars using silica gel column chromatography as for rock bitumens. Solvent-extracted, activated copper turnings were added to concentrated solutions of saturated hydrocarbon fractions to remove all traces of elemental sulphur, which is formed from disproportionation of the catalyst during HyPy.

To reduce the levels of background contamination in HyPy, a cleaning run was performed before each sample run whereby the apparatus was heated to 520 °C

using a rapid heating rate (300 °C min<sup>-1</sup>) under high-hydrogen-pressure conditions. Experimental blanks, using annealed silica gel in the reactor tube instead of a kerogen sample, were regularly performed and the products monitored and quantified to ensure that trace organic contamination levels were acceptably low.

For a sub-set of the rock extracts, branched and cyclic saturated hydrocarbons were separated from straight-chain alkanes by treating the saturated hydrocarbon fraction with silicalite molecular sieve. Approximately 5–10 mg of saturated hydrocarbons, dissolved in a minimum volume of *n*-pentane, was placed on a 3 cm bed of activated, crushed silicalite lightly packed into a Pasteur pipette. The silicalite non-adduct (SNA) containing branched and cyclic alkanes was washed through using pentane (4 ml).

A deuterated C<sub>29</sub> sterane standard (d<sub>4</sub>- $\alpha\alpha\alpha$ -24-ethylcholestone (20R), Chiron Laboratories AS) was added to branched/cyclic alkane or total saturate fractions before GC-MS to quantify biomarker peaks, with typically 50 ng internal standard added to a 1 mg aliquot of saturates. In MRM analyses, this standard compound was detected using the *m/z* 404 to 221 transition.

GC-MS analyses on saturated hydrocarbon fractions were carried out on a Micromass AutoSpec Ultima equipped with a HP6890 gas chromatograph (Hewlett Packard) and a DB-1MS coated capillary column (60 m × 0.25 mm i.d., 0.25- $\mu$ m film thickness) using He as carrier gas. The MS source was operated at 250 °C in EI mode at 70-eV ionization energy and with 8,000-V acceleration voltage. Samples were injected in pulsed splitless mode into a Gerstel PTV injector at a constant temperature of 300 °C. For full-scan and selected ion recording (SIR) experiments, the GC oven was programmed at 60 °C (2 min), heated to 315 °C at 4 °C min<sup>-1</sup>, with a final hold time of 35 min. The AutoSpec full-scan duration was 0.8 s plus 0.2 s interscan delay over a mass range of 50 to 600 Da. Hopane and sterane biomarkers were analysed by MRM GC-MS with a total cycle time of 1.3 s per scan for 26 parent-fragment transitions, including the *m/z* 414 to 217 transition for C<sub>30</sub> desmethylsteranes. For MRM, the GC oven was programmed at 60 °C (2 min), heated to 150 °C at 10 °C min<sup>-1</sup>, further heated to 315 °C at 3 °C min<sup>-1</sup> and held at the final temperature for 24 min.

Peak identifications of 24-isopropylcholestanes were confirmed by comparison of retention times with an AGSO oil saturated hydrocarbon standard and with Neoproterozoic oils from Siberia<sup>15</sup> shown previously to contain significant quantities of 24-isopropylcholestanes. Polycyclic biomarkers were quantified assuming equal mass spectral response factors between analytes and the d<sub>4</sub>-C<sub>29</sub>- $\alpha\alpha\alpha$ -ethylcholestone (20R) internal standard. Analytical errors for absolute yields of individual hopanes and steranes are estimated at  $\pm$ 30%. Average uncertainties in hopane and sterane biomarker ratios are  $\pm$ 8% as calculated from multiple analyses of a saturated hydrocarbon fraction prepared from an AGSO standard oil (*n* = 30 MRM analyses).