# Reassessing the evidence for the earliest traces of life

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**The isotopic composition of graphite is commonly used as a biomarker in the oldest (>3.5 Gyr ago) highly metamorphosed terrestrial rocks. Earlier studies on isotopic characteristics of graphite occurring in rocks of the approximately 3.8-Gyr-old Isua supracrustal belt (ISB) in southern West Greenland have suggested the presence of a vast microbial ecosystem in the early Archean[1–4]. This interpretation, however, has to be approached with extreme care[5]. Here we show that graphite occurs abundantly in secondary carbonate veins in the ISB that are formed at depth in the crust by injection of hot fluids reacting with older crustal rocks (metasomatism). During these reactions, graphite forms from the disproportionation of Fe(II)-bearing carbonates at high temperature. These metasomatic rocks, which clearly lack biological relevance, were earlier thought to be of sedimentary origin and their graphite association provided the basis for inferences about early life[1–4]. The new observations thus call for a reassessment of previously presented evidence for ancient traces of life in the highly metamorphosed Early Archaean rock record.**

Metasedimentary units, including detrital conglomerates, turbidites, chemically precipitated banded iron formations (BIFs) and metacherts, as well as pillow structures of mafic rocks, indicate the accumulation of ISB rocks in an aquatic environment[6–10]. Carbonate-rich Isua rocks have also originally been interpreted as being part of the sedimentary sucession[10,11]. However, recent studies have disproved this interpretation[6,7,9,12–14]. Metacarbonate deposits and calc-silicate rocks bear signs of having formed by metasomatic reactions during fluid infiltration across contacts between igneous ultramafic rocks and their host rocks[12–14]. It is now widely accepted that most, if not all, carbonate in Isua is metasomatic and not sedimentary in origin[6,7,9].

The light isotopic signatures ($\delta^{13}C \approx -25‰$) of reduced carbon in some of the Isua rocks have been used as evidence for biologic activity in the early Archaean[2–4]. Apart from these few observed light carbon isotope signatures, genetically less indicative bulk carbon isotope ratios (range from $-25$ to $-6‰$) were also obtained, particularly from carbonate-rich ISB rocks[2,15–17]. Ion microprobe analysis of individual graphite particles has yielded $\delta^{13}C$ values from $-18$ to $+2‰$ and has shown isotopically heterogeneous graphite within a single rock specimen[18].

Metamorphically induced isotope exchange between carbonate and graphite in these rocks was thought to have caused the partial loss of a conjectured biogenic light carbon isotope signature of the kind that is typically found in younger, less metamorphosed organic rich sediments in the geological record[2–4]. Furthermore, it has been shown that the original light isotope signature of organic matter is gradually diminished by volatilization during diagenetic and metamorphic alteration to kerogen and ultimately lost on conversion to graphite[15]. No kerogen but only crystalline graphite is found in Isua rocks, complicating the possible biogenic interpretation of graphite in these rocks[15].

Micrometre-size graphite inclusions with a pronounced light $\delta^{13}C$ value (weighted mean $-30 \pm 3‰$; ion microprobe data) were reported to occur in apatite crystals in one sample from the ISB[1]. The graphite was thought to have escaped isotope exchange with the associating carbonates by means of armouring by the host apatite[1], which seems to strengthen the evidence of early life in these rocks. The apatite itself was considered as a potential biomarker[1], in analogy with phosphate precipitates found in organic rich sedimentary deposits[19] such as phosphorites.

The recognition of the secondary, metasomatic origin of carbonate rich rocks in the ISB has highlighted the need for assessment of graphite genesis and its applicability to the interpretation of life at 3.8 Gyr ago. We have for this purpose studied a suite of samples from the ISB including metacarbonates, turbidites, cherts and BIFs (see Methods section for details).

Scanning electron microscope-energy dispersive spectrometer (SEM-EDS) analysis of carbonate phases in the Isua rocks reveals three distinct carbonate cation compositions (Fig. 1). Iron-rich carbonate (MgMn-siderite) was only found in metacarbonate samples from veins occurring as distinct layers in mafic metavolcanic country rock. Compositions with less iron (Fe-dolomite) were found in one metacarbonate layer occurring within metachert (AL17), and in small veinlets within some BIFs and metacherts. Iron-deficient carbonate (calcite) was found in veinlets within BIF, metachert and turbidite sequence samples. These different compositions are likely to reflect the interaction between the country rock and fluid, and explain the dominance of MgMn-siderite in metacarbonate veins within the iron-rich metavolcanic rocks. In field appearance and mineralogy these veins closely resemble magnetite bearing, quartz-poor, highly graphitic bands that were previously considered in ref. 20 to be a carbonate facies BIF, but are now recognized as metasomatic carbonates.

Graphite is relatively abundant (up to approximately 2 wt%) in metacarbonate samples (Fig. 2a), whereas the concentration of reduced carbon in our metasedimentary BIF and metachert samples is below 100 p.p.m.. Micropetrographic analysis reveals that the graphite in metacarbonates occurs closely associated with Fe-bearing carbonate and magnetite or FeMg-amphibole (silicate). The association of graphite with MgMn-siderite and magnetite (Fig. 3a) was observed in all graphitic veins within mafic country rocks, suggesting that graphite and magnetite in these rocks are the

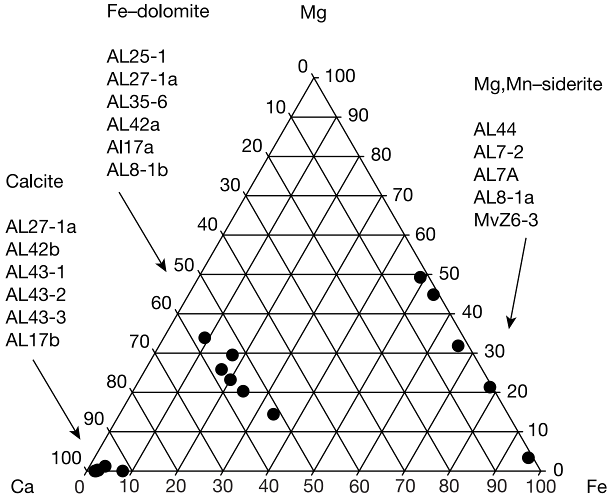

**Figure 1** Carbonate phases can be divided into three distinct populations. These are: calcite ($Fe_{0.02–0.08}Mg_{0.00–0.03}Mn_{0.01–0.04}Ca_{0.89–0.97}CO_3$), Fe–dolomite ($Fe_{0.09–0.36}Mg_{0.14–0.34}Mn_{0.00–0.06}Ca_{0.50–0.57}CO_3$), and MgMn–siderite ($Fe_{0.40–0.85}Mg_{0.03–0.40}Mn_{0.09–0.22}CO_3$). AL8-1, AL17, AL27-1 and AL42 contain carbonate minerals with two different compositions (populations a and b) and support an earlier suggestion of the occurrence of multiple pulses of carbonate metasomatism in the ISB[12]. Not shown are AL13-1, AL15-1B, MvZ6.4D1, MvZ6.4D2, because they do not contain carbonate. Samples MvZ6.4D1 and MvZ6.4D2 are highly graphitic, chlorite and magnetite dominated interlayers within MgMn–siderite-rich metacarbonate veins.

products of thermal disproportionation of the carbonate. Iron is present in reduced (divalent) form in these carbonates, and is oxidized to form magnetite, and the carbonate ion is reduced to graphite. Similar Fe-carbonate dissociation reactions apparently yield graphite in veins within Si-rich metacherts (sample AL17), but the reaction product in the presence of quartz is Fe-silicate rather than magnetite (compare ref. 8). The siderite ($FeCO_3$) disproportionation reaction, yielding graphite and magnetite has been studied in detail[21] at metamorphic $P,T,fO_2$ conditions, and has been suggested earlier as a possible mechanism for graphite formation in the amphibolite facies ($T \approx 550\,°C$; $P \approx 5$ kbar) ISB[17]. Thermal decomposition of pure siderite occurs at temperatures above 450 °C in the reaction:

$$6FeCO_3 \rightarrow 2Fe_3O_4 + 5CO_2 + C$$

The isotopic composition of bulk graphite in the metacarbonates ranges from −12 to −10‰ (Fig. 2b), which is significantly heavier than graphitized kerogen (typically near $\delta^{13}C \approx -25$‰ in Early Archaean rocks[15]). The isotopic composition of the carbonate carbon ranges between $\delta^{13}C$ values of +1 and −9‰ (ref. 22). Most of the graphite and associated Fe-carbonate in metacarbonate samples display a carbon isotopic difference ($\Delta_{graphite-carbonate}$) close to −6‰, in accord with the calculated isotope equilibrium fractionation between the two phases at around 1,500 °C (ref. 23) (assuming $\Delta_{graphite-siderite}$ to be equal to $\Delta_{graphite-calcite}$). This matches the estimated temperature range of metamorphism that these rocks have experienced[24]. We therefore conclude that graphite in these rocks obtained a $\delta^{13}C$ signature between −12 and −10‰ owing to equilibrium isotope fractionation during the thermal dissociation of metasomatic Fe-carbonates. The occurrence of graphitic metacarbonates as distinct veins, the association of graphite with Fe-bearing carbonates and magnetite or FeMg-amphibole, and its isotopic composition of $\delta^{13}C \approx -12$‰ are all consistent with an abiogenic origin of graphite by carbonate reduction.

The earlier study[2] on the isotopic composition of graphite in Isua was focused on carbonate-rich rocks that at the time were believed to have a sedimentary origin. The range of isotopic compositions of reduced carbon ($\delta^{13}C$ from −6 to −25‰) found in ref. 2 is inconsistent with our data that show $\delta^{13}C$ values of graphite in a narrow range between −12 and −10‰ in these rocks. With the current view of the metasomatic origin of carbonate-rich rocks[12–14] and the mechanism of graphite formation in such rocks as shown in this study, the biogenic interpretation given in ref. 2 seems to be open to reassessment.

No distinguishable graphite particles were found in our sedimentary BIF and metachert samples and combustion measurements indicated extremely low (less than 100 p.p.m.) concentrations of reduced carbon. We measured the $\delta^{13}C$ of reduced carbon in these samples and found $\delta^{13}C$ values consistently between −25 and −30‰ (Fig. 2b). In all of these samples the major fraction of reduced carbon combusted at a 450 °C step (shown for AL25 in Fig. 4). Graphite typically combusts at around 700–800 °C, so we conclude that the small amount of isotopically light, reduced carbon is mainly derived from unmetamorphosed recent organic material, which survives acid treatment and cleaning in organic solvents during sample processing. This organic matter is possibly

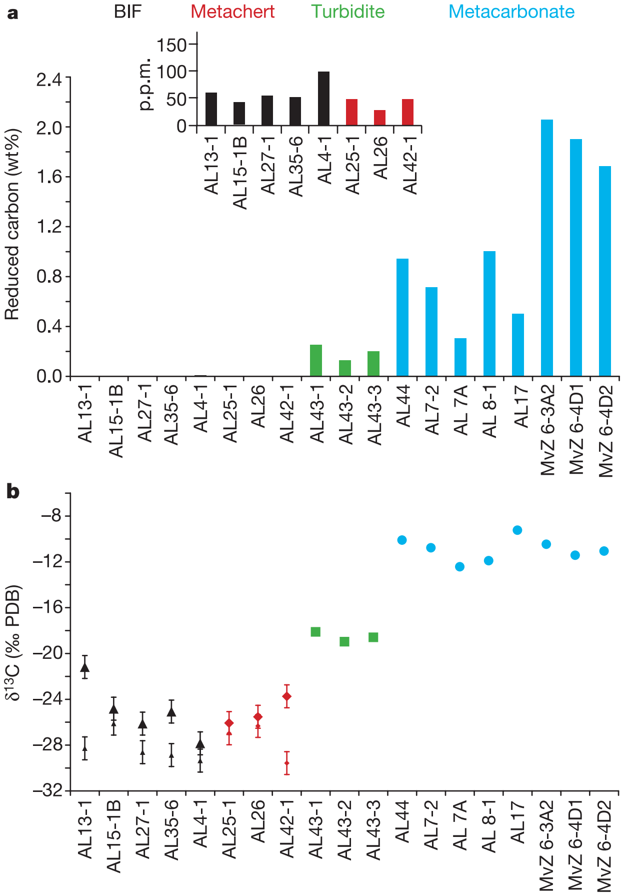

**Figure 2** Abundance and isotope composition of reduced carbon. **a**, Abundances (wt%) of reduced carbon in studied samples. In a separate plot a close-up of low concentrations (carbon content in p.p.m.) in BIFs and metacherts is shown. **b**, Carbon isotope composition of total reduced carbon in BIFs (black triangles), metacherts (red diamonds), turbidites (green squares), metacarbonates (blue circles). Isotopic composition for carbon released at 450 °C is shown for BIFs (small black triangles) and metacherts (small red diamonds).

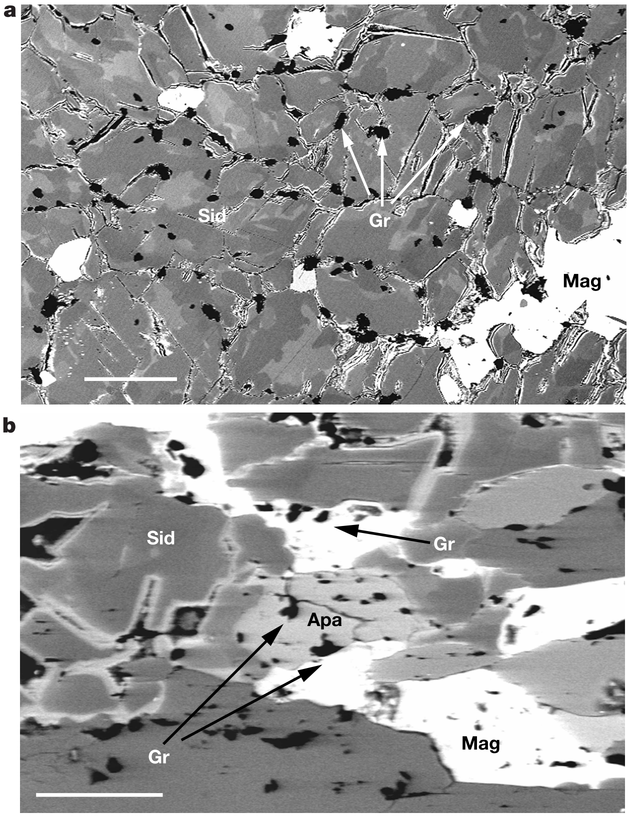

**Figure 3** Backscattered electron (BSE) images of samples. **a**, BSE image of AL44, showing the spatial distribution of graphite (Gr) in relation to MgMn–siderite (Sid) and magnetite (Mag). Scale bar, 200 μm. **b**, BSE image of I-3381, showing an apatite crystal with graphite inclusions (Apa) in relation to magnetite, MgMn-siderite and graphite. Scale bar, 50 μm.

emplaced by percolating ground water[25]. Because almost no carbon phase is found to combust at temperatures above 450 °C (Fig. 4), we conclude that the typical $\delta^{13}\mathrm{C} \approx -28‰$ signature in these graphite-deficient sedimentary rocks is entirely due to recent contamination. We therefore question the palaeobiological significance of earlier single-step combustion experiments on decarbonated bulk rock samples from Isua.

The ion microprobe data on graphite in Isua metacherts and psammitic schists[18] revealed particles with $\delta^{13}\mathrm{C}$ values as light as $-18‰$. These light isotopic compositions cannot be related to recent contamination; they may indeed constitute ancient traces of life. However, the wide range of $\delta^{13}\mathrm{C}$ values and common occurrence of graphite in samples containing carbonate and products of carbonate replacement (amphiboles) calls for further petrographic and isotopic studies and critical evaluation of abiogenic decarbonation processes before a biogenic interpretation of the origin of graphite in these samples[18] can safely be made.

The biogenic interpretation of isotopically light graphite inclusions in apatite crystals in the ISB was based on a single rock sample I-3381 (ref. 1), believed at the time to represent a sedimentary BIF. The recent petrographic analysis using the original thin section of I-3381 has revealed that it contains MgMn–siderite–magnetite–graphite associations and is compositionally akin to Isua metacarbonates[26]. The compositional similarity of I-3381 and AL8-1, as well as the field descriptions, indicate that these two samples were collected from the same metacarbonate outcrop[26]. In all metacarbonate samples, including I-3381, graphite is not restricted to apatite, but occurs as inclusions in most other phases too (Fig. 3b). The petrographic evidence strongly suggests that the origin of graphite in apatite is no different from graphite included in other phases and, regardless of mineral association, is produced epigenetically through thermal disproportionation of ferrous carbonate. This type of graphite consequently appears to be without biological significance and formed in one or several thermal events later than 3.8 Gyr ago. The isotopic systematics of the process responsible for formation of isotopically light graphite (weighted mean $-30 \pm 3‰$) enclosed in apatite crystals in I-3881 (ref. 1) remains to be studied, but our petrographic evidence clearly excludes a primary biogenic origin.

A sample from granulite facies rocks from Akilia island, around 150 km southwest of Isua, has also been reported to contain isotopically light graphite inclusions in apatite crystals[1]. The present study does not include Akilia rocks and our conclusions about the origin of graphite and apatite–graphite association in Isua cannot, without additional petrographic and chemical analyses, be applied to the more extensively metamorphosed rocks from Akilia island, whose protolith and age are currently debated[27].

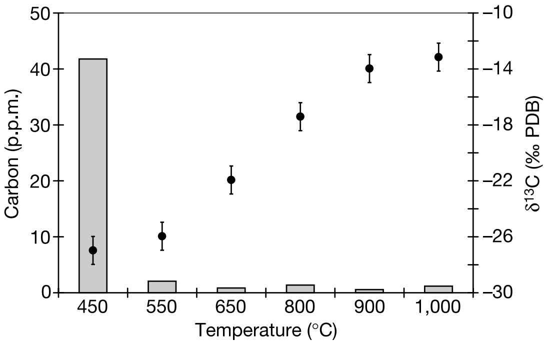

**Figure 4** Stepped-heating combustion data for sample AL25. 1-h steps at 450, 550, 650, 800, 900 and 1,000 °C. Abundance of extracted reduced carbon (p.p.m.) and the isotopic composition ($\delta^{13}\mathrm{C}$) are shown for each temperature step. The reduced carbon component that dominates in this sample is isotopically light ($\delta^{13}\mathrm{C} = -28‰$) and comes off at low temperature.

Significant amounts of graphite (>0.1 wt%) were found in rocks interpreted as graded beds (turbidite), in the western part of the belt[28] (Fig. 2a). The isotopic composition of graphite in three samples from the turbidite sequence is in the present work found to be between $-18$ and $-19‰$ (Fig. 2b), consistent with the original measurements[28]. Graphite in these samples does not coexist with siderite or magnetite; in fact no iron-containing carbonate phases were found in these rocks (Fig. 1), indicating that siderite disproportionation is an unlikely mechanism for graphite production. The original biogenic interpretation of graphite in this formation is therefore not challenged by our observations.

Our study leads to conclusions that require basic modification of some earlier inferences about traces of life in the ancient Isua rocks. A biogenic origin of graphite in carbonate-rich rocks in Isua[1–4] was inferred from the assumption that these rocks had a sedimentary origin. However, recent field and laboratory investigations have shown that most if not all carbonate in Isua is metasomatic in origin. Petrographic and isotopic analyses show that graphite in the metacarbonate rocks, serving as a basis for earlier investigations, is produced abiogenically by disproportionation of ferrous carbonate at high temperature and pressure and at a time later than the formation of the host rock. This type of graphite, including graphite inclusions in apatite, therefore cannot represent 3.8 Gyr-old traces of life. Stepped-temperature combustion accompanied by isotope analysis shows that single-step combustion of bulk rock samples cannot be used for tracing life in graphite-deficient metasediments. ☐

## Methods

Rock samples collected from a zone of relatively low strain[6] in the northeastern part of the ISB include metacarbonates (AL17, AL7-2, AL8-1, AL7A, MvZ6.3A2, MvZ6.4D1, MvZ6.4D2), BIFs (AL4-1, AL27-1, AL13, AL35-6, AL15) and metacherts (AL25, AL26, AL42). From the western part of the belt a metacarbonate sample (AL44) was collected, as well as three samples from a metasedimentary turbidite sequence[28] (AL43-1, AL43-2, AL43-3). We apply the term 'metacarbonate' to cm to m scale carbonate-rich veins, typically cross-cutting the country rock. The metasedimentary samples, described as BIFs and metacherts, also contain small amounts (<5%) of carbonate minerals in small veinlets, but the texture and composition of these rocks have not changed significantly as a result of metasomatism.

Analysis of the cation composition of the carbonate minerals was performed with an EDS in a Cambridge 360 SEM using polished thin sections. Samples were prepared for carbon isotope analysis by decarbonation (4 M HCl solution at 70 °C for 48 h) and cleaning in a 10:1 dichloromethane/ethanol solution. Carbon concentration was determined with a Perkin-Elmer 2400 CHN elemental analyser (graphitic samples), or determined by capacitance manometry from $CO_2$ release during stepped-heating combustion (graphite-deficient samples). Conversion to $CO_2$ was achieved by sealed tube combustion (graphitic samples) or by stepped-heating combustion (graphite-deficient samples). After combustion, $CO_2$ was collected cryogenically (procedure described in ref. 29) and carbon isotopes were measured on a VG PRISM multicollector mass spectrometer. All measurements are Craig-corrected and presented as $\delta^{13}\mathrm{C}$ values ($\delta^{13}\mathrm{C} = ((^{13}\mathrm{C}/^{12}\mathrm{C}_{\mathrm{sample}}/^{13}\mathrm{C}/^{12}\mathrm{C}_{\mathrm{standard}}) - 1) \times 1{,}000$) relative to Pee-Dee Belemnite (PDB). USGS24 and NBS19 were used as standards.

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**Competing interests statement**

The authors declare that they have no competing financial interests.

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# Cultivation of the ubiquitous SAR11 marine bacterioplankton clade

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**The α-proteobacterial lineage that contains SAR11 and related ribosomal RNA gene clones was among the first groups of organisms to be identified when cultivation-independent approaches based on rRNA gene cloning and sequencing were applied to survey microbial diversity in natural ecosystems[1]. This group accounts for 26% of all ribosomal RNA genes that have been identified in sea water and has been found in nearly every pelagic marine bacterioplankton community studied by these methods[2]. The SAR11 clade represents a pervasive problem in microbiology: despite its ubiquity, it has defied cultivation efforts. Genetic evidence suggests that diverse uncultivated microbial taxa dominate most natural ecosystems[3–5], which has prompted widespread efforts to elucidate the geochemical activities of these organisms without the benefit of cultures for study[6,7]. Here we report the isolation of representatives of the SAR11 clade. Eighteen cultures were initially obtained by means of high-throughput procedures for isolating cell cultures through the dilution of natural microbial communities into very low nutrient media. Eleven of these cultures have been successfully passaged and cryopreserved for future study. The volume of these cells, about 0.01 μm$^3$, places them among the smallest free-living cells in culture.**

In an effort to isolate some of the ubiquitous uncultivated Bacteria and Archaea that dominate marine bacterioplankton communities[2], we inoculated fresh Oregon coast seawater samples into microtitre dish wells by dilution, such that on average each well received 22 microbial cells. Media consisted of sterile Oregon coast sea water supplemented with either phosphate (as $KH_2PO_4$) and ammonium (as $NH_4Cl$), or phosphate, ammonium and a defined mixture of organic carbon compounds. The technique of isolating cells by dilution into sterilized natural waters or other dilute media has been used previously[8,9]; it takes advantage of the fact that

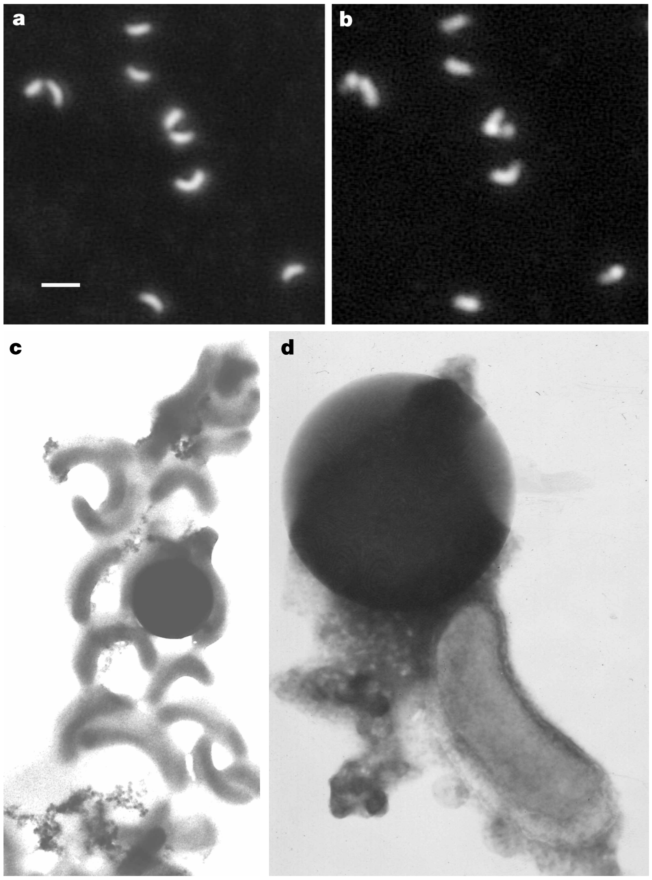

**Figure 1** Photomicrographs of a culture of SAR11 clade isolate HTCC1062. **a**, **b**, Fluorescence images of cells in an identical field of view, stained with the DNA-specific dye DAPI (**a**) and after hybridization with four Cy3-labelled oligonucleotide probes targeting SAR11 cells (**b**). Scale bar (**a**, **b**), 1 μm. **c**, **d**, Transmission electron micrographs of strain HTCC1062. **c**, Shadowed cells with the typical SAR11 clade morphology. **d**, Negatively stained cell. The latex beads in **c** and **d** have a diameter of 0.514 μm.