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Reduction spheroids preserve a uranium isotope record of the ancient deep continental biosphere

1 **Reduction Spheroids Preserve a Uranium Isotope Record of the Ancient Deep**
2 **Continental Biosphere**
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21

22 **Life on Earth extends to several kilometres below the land surface and seafloor.**

23 **This deep biosphere is second only to plants in its total biomass, is metabolically**

24 **active and diverse, and is likely to have played critical roles over geological time**

25 **in the evolution of microbial diversity, diagenetic processes, and biogeochemical**

26 **cycles. However, these roles are obscured by a paucity of fossil and geochemical**

27 **evidence. Here, we apply the recently developed uranium-isotope proxy for**

28 **biological uranium reduction to reduction spheroids in continental rocks (red**

29 **beds). Although these common palaeo-redox features have previously been**

30 **suggested to reflect deep bacterial activity, unequivocal evidence for biogenicity**

31 **has been lacking. Our analyses reveal that the uranium present in reduction**

32 **spheroids is isotopically heavy, which is most parsimoniously explained as a**

33 **signal of ancient bacterial uranium reduction, revealing a compelling record of**

34 **Earth's deep biosphere.**

35

36   The subsurface represents a vast habitat containing up to a fifth of Earth's current

37   total biomass including diverse bacteria, archaea and fungi[1,2]. In recent decades,

38   accelerating exploration of the deep biosphere has revealed new microbial groups,

39   new ecological niches, and new modes of microbe–mineral interaction[3,4,5]. However,

40   a full understanding of the limits of the deep biosphere, its contribution to total

41   planetary biomass, and its biogeochemical significance requires the detection and

42   analysis of robust deep biosignatures in the rock record. Such biosignatures could also

43   refine search images for past or present life on Mars, whose surface has long been

44   uninhabitable but may conceal a warm, wet interior[6].

45

46   It is difficult to confirm that possible traces of the ancient deep biosphere are truly

47   biogenic, post-burial in origin, and pre-modern. Few reported cellular or molecular

48   fossils pass all three tests unequivocally, and most candidates represent subseafloor

49   environments[7,8,9,10]. Microbially mediated diagenetic phenomena on palaeo-redox

50   fronts offer a potentially powerful alternative record that could extend to continental

51   settings[11,12]. Of longstanding interest in this connection are reduction spheroids,

52   which are very common mm–dm-scale bleached spots found most commonly (but not

53   exclusively) in Proterozoic–Phanerozoic red beds, i.e., sedimentary rocks deposited in

54   oxidizing terrestrial environments and rich in early diagenetic hematite[13,14]. The

55   bleached colour reflects localised Fe(III) reduction and loss[15]. Many examples contain

56   small, dark, central "cores" where redox-sensitive elements including uranium,

57   vanadium, and nickel are highly concentrated, and organic matter may also be

58   present[16,17].

59

60   The mechanisms that produce reduction spheroids in the subsurface have hitherto

61   been unclear. They are sometimes attributed to the oxidation of organic-rich cores,

62   but the cores are usually darkened not by organic matter but by opaque metalliferous

63   minerals. Although organic carbon is present in some reduction spheroid cores and

64   may have stimulated their formation, organic carbon present prior to reduction is

65   typically too scarce to have reduced the surrounding halos[16,17]. It has thus been

66   proposed instead that spheroids form around localized chemolithotrophic microbial

67   populations, which would catalyse the oxidation of mobile reductants supplied

68   through groundwater[13,18,19,20]. These reductants could include H_2 derived

69   radiolytically from porewater, which could establish a positive-feedback mechanism

70   for spheroid growth following initial uranium precipitation[20]. Confirmation of a biotic

71   mode of origin would distinguish these common geological features as perhaps the

72   most widely accessible, recognisable and distinctive traces of the ancient deep

73   biosphere. It would also add weight to previous suggestions that reduction spheroids

74   could be a target for astrobiological sampling on Mars, where iron reduction is

75   regarded as a plausible metabolic strategy for past or present life[21,22,23].

76

77   Direct evidence for the biogenicity of reduction spheroids has hitherto been lacking or

78   equivocal. Authigenic pyrite present in some spheroids has a sulphur isotope

79   composition consistent with but not diagnostic of a bacterial origin[21]; however, most

80   spheroids lack pyrite altogether[19]. Molecular biomarkers can be extracted from the

81   organic matter commonly associated with reduction spheroids (e.g., **Supplementary**

82   **Figure 1**), but are likely to pre-date the origin of the spheroids themselves, so cannot

83   shed light on their biogenicity. This organic matter is also damaged and isotopically

84   modified by exposure to ionising radiation commonly emitted by uranium in

85 reduction spheroids[17,19,24]. Hence, determination of reduction spheroid biogenicity is

86 non-trivial and necessitates the analysis of authigenic phases demonstrably associated

87 with spheroid formation.

88

89 Here, we focus on a new low-temperature palaeo-redox proxy, the isotopic

90 composition of uranium (^{238}U/^{235}U: δ^{238}U, in standard delta notation, relative to the

91 CRM-112a standard; **Equation 1**). Uranium enrichment appears to be a universal

92 feature of reduction spheroids, occurring both in the cores and in the halos as a result

93 of the highly localised reduction of soluble U(VI) to insoluble U(IV) (ref. 19). Thus,

94 uranium phases (both mineralized and non-mineralized) in reduction spheroids and

95 analogous low-temperature redox-front uranium deposits have been shown[12,19] to

96 contain predominantly U(IV). Uranium reduction can occur via many pathways[18,25],

97 both abiotic (coupled to the oxidation of various aqueous, mineral, and organic

98 species) and biotic (i.e., enzymatic catalysis by chemolithotrophic microorganisms

99 capable of facultatively utilising U(VI) as an electron acceptor, including iron- and

100 sulphate-reducers). The uranium isotope system is controlled by low-temperature

101 redox reactions that significantly fractionate the uranium isotope composition

102 preserved in environmental samples away from a crustal (high-T) average δ^{238}U of −

103 0.29 ± 0.03‰, often concentrating the heavier isotope in the reduced product[26,27].

104

105 Several experimental studies have shown that bacterially reduced and precipitated

106 uranium is isotopically heavier (i.e. records higher δ^{238}U) than the dissolved precursor

107 phase[28,29,30]. Bhattacharyya et al. (ref. 12) recently determined a bacterial origin for

108 isotopically heavy authigenic uranium phases in roll front ore deposits, which

109 resemble reduction spheroids inasmuch as they are mineralised paleo-redox fronts

110   formed at low temperatures in subsurface aquifers. Field studies confirm that modern

111   groundwaters inoculated with metal reducing bacteria become isotopically lighter in

112   uranium as the heavier isotope is preferentially precipitated[31,32]. Experimental studies

113   so far have shown that, by contrast, abiotically reduced uranium either remains

114   unfractionated or is isotopically lighter, regardless of the reductant

115   responsible[29,30,33,34]. Consequently, the U-isotopic composition ($\delta^{238}U$) of reduced

116   uranium phases in nature is emerging as a new and potentially powerful proxy for

117   their mode of origin[12,30,31] (**Supplementary Note 1**).

118

119   Here, we report $\delta^{238}U$ analyses of the dark cores, bleached halos, and surrounding

120   matrix of reduction spheroids collected from continental red beds in outcrop,

121   primarily at Dingwall in northern Scotland and Budleigh Salterton in southwest

122   England, sites where spheroids are both especially uraniferous, and can be linked to

123   unusually well constrained formation depths from their geological context. Spheroids

124   from other localities of diverse ages were analysed for comparison, as were

125   uraniferous hydrothermal veins expected to yield near-crustal $\delta^{238}U$ values reflecting

126   their high-temperature origin[30,34]. We find that reduction spheroids are enriched

127   towards their cores in uranium characterised by high $\delta^{238}U$ values. This result is most

128   parsimoniously explained as a signal of ancient bacterial U(VI) reduction, implying

129   that the spheroids themselves are most likely bacterial in origin.

130

131   **Results and Discussion**

132   *Uranium isotope values*

133   The cores of reduction spheroids have uniformly higher uranium concentrations and

134   heavier uranium isotope compositions ($\delta^{238}U$) compared to the host rock in all

135   samples (**Supplementary Table 1**; **Figure 1)**. All hydrothermal vein samples and

136   most of the red-bed matrix from the reduction-spheroid localities yielded $\delta^{238}U$ values

137   near the average crustal value of -0.29 ± 0.03‰ (ref. 27). In most reduction

138   spheroids, both uranium concentration and $\delta^{238}U$ increased from the matrix through

139   the halo into the core. At Budleigh Salterton, the large size of the spheroids made it

140   possible to discriminate between isotopically heavier black inner cores (mean

141   +0.78‰; n = 4) and isotopically less heavy dark grey core margins (+0.07‰; n = 2),

142   as well as greenish outer halos (-0.24‰; n=4). Similarly, the spheroid cores from

143   Dingwall yielded much heavier values of $\delta^{238}U$ (mean +0.45‰; n=6) than the halos

144   (+0.04‰; n = 4), the matrix (-0.21‰; n = 2), and a nearby bitumen vein (-0.16‰; n =

145   1). Spheroid cores from the other localities, where palaeodepth was less well

146   constrained, also recorded values heavier than the crustal range, and all were heavier

147   than their respective matrices by at least 0.10‰ (**Supplementary Table 1**).

148

149   ***Reduction spheroid biogenicity***

150   The reduction spheroids analysed here are enriched in uranium and show increasingly

151   heavy isotopic compositions ($\delta^{238}U$) towards their reduced cores. In some cases, the

152   matrix to core interval of reduction spheroids expresses U isotope variation

153   approximating almost the entire natural range of low-temperature systems on

154   Earth[26,35]. Field-based, experimental and geological studies to-date strongly suggest

155   that these high $\delta^{238}U$ values are best explained by bacterial uranium reduction and

156   precipitation within the spheroids[12,29,30,31,32,33,34]. In particular, given the

157   environmental similarity between reduction spheroids and roll-front ore deposits, our

158   interpretation receives support from the recent measurement of isotopically heavy

159   biogenic uranium phases associated with the latter[12].

160

161 Since Fe(III) and U(VI) reduction are carried out by the same groups of

162 microorganisms using the same reductants, and occur coextensively and concurrently

163 in modern aquifers[36], our results strongly imply that the reduction and dissolution of

164 ferric iron responsible for the presence of the bleached spheroids themselves was also

165 bacterially mediated. We infer that reduction spheroids, which are both spatially and

166 temporally widespread, represent an important record of the geological history of the

167 deep biosphere, which was potentially Earth's largest reservoir of biomass prior to the

168 proliferation of land plants[37].

169

170 ***A record of the ancient deep biosphere***

171 The deep biosphere conventionally extends from ~metres depth to several

172 kilometres[4,38,39]. There is clear evidence that many—perhaps most—reduction

173 spheroids form at the deeper end of this range. In brief: first, halos are commonly

174 spherical, whereas shallow non-nodular features would be flattened by compaction;

175 second, radiometric ages of authigenic minerals concentrated within some spheroids

176 are $>10^7$ years younger than the host rock[16,40]; third, some spheroids occur in

177 hematite-stained igneous basement, hundreds of metres below the uppermost

178 basement[14]; fourth, at many localities, the distribution of spheroids was clearly

179 influenced by pre-existing faults, fractures, cataclastic zones and cleavages younger

180 than the host rock[14,41,42]. Our findings evince a clear signal of bacterial uranium

181 reduction in spheroids demonstrably formed at multi-km depth, including one locality

182 (Dingwall) where they appear to be related to the early-stage biodegradation of

183 hydrocarbons, and a weaker but consistent signal at all other localities. We conclude

184 that reduction spheroids represent an important and widespread archive of the deep

185   continental biosphere, present through much of Earth's geological record. This

186   finding lends weight to the suggestion that reduction spheroids be targeted for

187   analysis and sample return were they to be discovered on Mars[22].

188

189

190   **Methods**

191   *Sample localities*

192   The Dingwall spheroids are hosted by red mudstones of the middle Devonian

193   Millbuie Sandstone Group, which forms part of a thick continental succession (the

194   Old Red Sandstone). As previously described by ref. 24, the cores are black, spherical

195   nodules a few mm across, composed of solid hydrocarbons with uranium present as

196   microscopic inclusions of uraninite and other minerals (impure xenotime and possibly

197   brannerite). These cores occupy green-grey non-nodular halos that can extend for

198   several centimetres, and occur through a stratigraphic thickness of 10 m (**Figure 2**).

199   The post-compaction origin of the Dingwall spheroids is confirmed by (1) the

200   spherical shape of the cores; (2) the lack of compaction drapes over them; and, (3) the

201   presence of solid hydrocarbon residues (bitumen) within the cores that clearly derive

202   from source rocks in the underlying kilometre of stratigraphy[24], which must have been

203   deeply buried in order to reach thermal maturity and generate hydrocarbons (i.e.,

204   about 3 km assuming a normal geothermal gradient). Migration occurred while the

205   succession was still deeply buried, as demonstrated by the presence of bitumen-

206   bearing quartz veins through a stratigraphic thickness of ~10 m in the conglomerate

207   directly underlying the spheroid-hosting mudstone[24]. These veins, which yield fluid-

208   inclusion temperatures close to 100 °C (ref. 43)— equivalent to ~3–4 km depth

209   assuming a normal geothermal gradient—occur within 5 m of the spheroids

210   themselves. We therefore infer that the Dingwall spheroids formed at depths of

211   several kilometres and may be genetically related to hydrocarbon migration and

212   (bio)degradation.

213

214   The reduction spheroids at Budleigh Salterton are hosted by red mudstone in the latest

215   Permian Littleham Mudstone Formation, a ~200-m thick unit within the New Red

216   Sandstone Supergroup, a laterally and stratigraphically extensive Permo-Triassic

217   continental succession[44]. The specimens analysed here are spherical pale green

218   nodules with diffuse black centres; uranium is present as fine-grained coffinite and is

219   not associated with organic matter[45,46]. Cross-cutting relationships described by ref.

220   38 show that these spheroids formed penecontemporaneously with sheet-like copper

221   nodules, which themselves replaced an earlier generation of crack-seal calcite veins

222   generated by overpressure during compaction dewatering. These relationships suggest

223   the Budleigh Salterton reduction spheroids formed relatively early, at depths of up to

224   around 1–2 km (ref. 46).

225

226   In addition to Budleigh Salterton and Dingwall, reduction spheroids were collected

227   from the field at four other localities: (1) the Mesoproterozoic (~1.4 Ga) red beds of

228   the Sibley Group of Ontario, sampled at a road cut near Nipigon[47]; (2) Devonian red

229   sandstone at Millport, Great Cumbrae, Scotland; (3) Carboniferous white sandstone at

230   Heysham, Lancaster, England[24]; (4) Triassic red siltstone at Hartlepool, Co. Durham,

231   England. The sample from Ord Burn was collected from a hydrothermal vein in

232   Caledonian granite, Sutherlandshire, Scotland[48]. The sample from Great Orme's Head

233   is a copper ore deposit in Carboniferous limestone, North Wales[49]. The sample from

234   Laxey is a hydrothermal vein-hosted lead-zinc ore deposit in Lower Palaeozoic slates

235   above Caledonian granite, Isle of Man[50].

236

237   ***Sample preparation***

238   Samples were cut, cleaned and crushed in an agate mill, or micro-drilled with a

239   tungsten carbide drill bit (previously tested to not contaminate U isotope analysis, and

240   cleaned between samples) to target the specific components of reduction spheroids,

241   host rocks and other samples (**Figure 3**). Approximately 0.1-0.4 g of each powdered

242   and homogenised sample were ashed in a 100 °C oven for 24 hours. Samples were

243   digested in a 3:1 mixture of concentrated HNO_3 and HF on a hotplate for 24 hours.

244   Samples were dried and re-digested in concentrated HCl and HNO_3.

245

246   ***Isotope analyses***

247   Trace metal concentrations were measured at the Yale Metal Geochemistry Center on

248   a Themo-Finnigan Element XR ICP-MS on splits from each digest. The ^{236}U-^{233}U

249   double spike was added based on uranium concentrations ($^{238}U/^{236}U$ ~30), prior U

250   purification via ion exchange methods. The spiked samples were dried and taken up

251   in 3N HNO_3. The U was then purified using the UTEVA column chemistry method

252   (after ref. 51; see methods of ref. 39, 52). Purified U was dissolved in 0.75 N HNO_3

253   with 50 ppb concentration. Uranium isotopes were measured at the Yale Metal

254   Geochemistry Center on a Themo-Finnigan Neptune Plus Multi-Collector ICP-MS at

255   low mass resolution using a Jet sampler cone and a standard skimmer cone. Sample

256   were introduced through an Elemental Scientific μFlow PFA nebulizer at ~50 μL/min

257   via an Elemental Scientific Apex IR. A 50 ppb sample solution yielded 32–40 volts of

258   ^{238}U signal on a 10^{11} Ω amplifier.

259

260   Isotopes were measured on Faraday collectors, listed in **Table 1**. ^{232}Th hydride was

261   monitored to have a negligible effect on measurement of ^{233}U. Measurements

262   consisted of five blocks, each block 10 cycles, each cycle 4.19 s. Blank U level was

263   less than 50 pg. External reproducibility was assessed using full protocol duplicates of

264   the geostandard NOD-A-1, which yielded an average δ238U of -0.52 +/-0.08‰ based

265   on nine repeats (2σ error = 0.12). Duplicate samples agreed within error.

266

267   Uranium isotope variations of samples and standards are reported as

268   δ^{238}U$_{CRM\ 112a}$, which is defined as:

269    $$\delta^{238}U = \left(\left[\frac{\left(\frac{^{238}U}{^{235}U}\right)_{sample}}{\left(\frac{^{238}U}{^{235}U}\right)_{CRM-112a}} \right] - 1 \right) \times 1000 \text{ ‰ (Equation 1)}$$

270

271   ***Biomarker analyses***

272   Quantitative biomarker data (**Supplementary Note 2**) were obtained by gas

273   chromatography-mass spectrometry (GC-MS) from the spheroids from Dingwall as

274   follows. Core samples were prepared by rinsing twice with distilled water and again

275   with dichloromethane (DCM), and ultrasonicated with DCM and methanol. All

276   glassware was thoroughly cleaned with a 93:7 mixture of DCM/MeOH. Crushed

277   samples were weighed, recorded, transferred into pre-extracted thimbles, dried with a

278   rotary evaporator, and separated into aliphatic, aromatic and polar fractions via silica

279   column chromatography using hexane, hexane/DCM in the ratio 3:1 and DCM/MeOH

280   respectively. Prior to GC-MS analysis, an internal standard (5β-Cholane, Agilent

281   Technologies) was added to the saturated fraction before injection into the GC-MS

282   machine, and subsequent biomarker identification. This was done using an Agilent

283 6890N gas chromatograph fitted with a J&W DB-5 phase 50 m MSD and a quadruple

284 mass spectrometer operating in SIM mode (dwell time 0.1 s per ion and ionisation

285 energy 70eV). Samples were injected manually using a split/splitless injector

286 operating in splitless mode (purge 40 ml min-1 for 2 min). The temperature

287 programme for the GC oven was 80–295 °C, holding at 80 °C for 2 min, rising to 10

288 °C min^{-1} for 8 min and then 3 °C min^{-1}, and finally holding the maximum temperature

289 for 10 min. Data were obtained by comparing with the response of the internal

290 standard.

291

292 **Data availability statement**

293 All data generated during and/or analysed during this study are available from the

294 corresponding author upon reasonable request.

295

307

308 **Author contributions**

309 **SM** conceived the study and wrote the manuscript. **SM, JP** and **AvSH** prepared the

310 samples. **AvSH** conducted the isotopic analyses and wrote the methods section. **JP**

311   contributed the samples and supporting locality information. **SB** conducted the GC-

312   MS analysis. All authors contributed to the design of the study and edited the

313   manuscript.

314

315   **Competing interests statement**

316   The Authors declare no competing interests.

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319   **References**
320
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478 **Figure legends**
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480 **Figure 1**: Results of uranium isotope analysis. Error bars represent two standard
481 errors. $\delta^{238}U$ values are shown from reduction spheroid innermost cores (black), core
482 margins (grey), bleached halos (cyan) and surrounding red-bed matrix (magenta).
483 Boxes indicate physically contiguous samples. **a:** Results from reduction spheroids of
484 constrained formation depth. **b:** Results from other reduction spheroids. **c:** Results
485 from three hydrothermal veins.
486

487 **Figure 2**: Schematic view of geological context of Dingwall samples. Key to
488 symbols: stipples = igneous basement; parallel lines = mudrock; dots = sandstone;
489 open circles = conglomerate; bulls' eyes = reduction spheroids. Bitumen is present at
490 the cores of the reduction spheroids and in the local fractures. Adapted from ref. 24.
491

492 **Figure 3**: Example of a freshly exposed reduction spheroid. This spheroid shows a
493 distinctive dark grey core, a bleached halo and a red-brown matrix flecked with
494 smaller reduction spots. The coin is ~21 mm across. Devonian red sandstone,
495 Millport, Great Cumbrae, Ayrshire, Scotland.
496

497

498 **Table**
499

Isotope	^{232}Th	^{233}U	^{234}U	^{235}U	^{236}U	^{238}U
Cup	L3	L2	L1	C	H1	H3
Amplifier	10^{11}	10^{11}	10^{12}	10^{11}	10^{11}	10^{11}

500

501 **Table 1.** Neptune Faraday Detector Setup

502
503