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

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

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20  
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<sup>1,2</sup>. 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<sup>3,4,5</sup>. 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<sup>6</sup>.

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<sup>7,8,9,10</sup>. Microbially mediated diagenetic phenomena on palaeo-redox  
50 fronts offer a potentially powerful alternative record that could extend to continental  
51 settings<sup>11,12</sup>. 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<sup>13,14</sup>. The  
55 bleached colour reflects localised Fe(III) reduction and loss<sup>15</sup>. 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<sup>16,17</sup>.

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<sup>16,17</sup>. 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<sup>13,18,19,20</sup>. These reductants could include H<sub>2</sub> derived  
69 radiolytically from porewater, which could establish a positive-feedback mechanism  
70 for spheroid growth following initial uranium precipitation<sup>20</sup>. 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<sup>21,22,23</sup>.

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<sup>21</sup>; however, most  
80 spheroids lack pyrite altogether<sup>19</sup>. 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<sup>17,19,24</sup>. 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 ( $^{238}\text{U}/^{235}\text{U}$ :  $\delta^{238}\text{U}$ , 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<sup>12,19</sup> to  
96 contain predominantly U(IV). Uranium reduction can occur via many pathways<sup>18,25</sup>,  
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  $\delta^{238}\text{U}$  of –  
103  $0.29 \pm 0.03\%$ , often concentrating the heavier isotope in the reduced product<sup>26,27</sup>.

104

105 Several experimental studies have shown that bacterially reduced and precipitated  
106 uranium is isotopically heavier (i.e. records higher  $\delta^{238}\text{U}$ ) than the dissolved precursor  
107 phase<sup>28,29,30</sup>. 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<sup>31,32</sup>. 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<sup>29,30,33,34</sup>. Consequently, the U-isotopic composition ( $\delta^{238}\text{U}$ ) of reduced  
116 uranium phases in nature is emerging as a new and potentially powerful proxy for  
117 their mode of origin<sup>12,30,31</sup> (**Supplementary Note 1**).

118

119 Here, we report  $\delta^{238}\text{U}$  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  $\delta^{238}\text{U}$  values reflecting  
126 their high-temperature origin<sup>30,34</sup>. We find that reduction spheroids are enriched  
127 towards their cores in uranium characterised by high  $\delta^{238}\text{U}$  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 ( $\delta^{238}\text{U}$ ) 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  $\delta^{238}\text{U}$  values  
137 near the average crustal value of  $-0.29 \pm 0.03\text{‰}$  (ref. 27). In most reduction  
138 spheroids, both uranium concentration and  $\delta^{238}\text{U}$  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  $+0.78\text{‰}$ ;  $n = 4$ ) and isotopically less heavy dark grey core margins ( $+0.07\text{‰}$ ;  $n = 2$ ),  
142 as well as greenish outer halos ( $-0.24\text{‰}$ ;  $n=4$ ). Similarly, the spheroid cores from  
143 Dingwall yielded much heavier values of  $\delta^{238}\text{U}$  (mean  $+0.45\text{‰}$ ;  $n=6$ ) than the halos  
144 ( $+0.04\text{‰}$ ;  $n = 4$ ), the matrix ( $-0.21\text{‰}$ ;  $n = 2$ ), and a nearby bitumen vein ( $-0.16\text{‰}$ ;  $n =$   
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  $0.10\text{‰}$  (**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 ( $\delta^{238}\text{U}$ ) 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<sup>26,35</sup>. Field-based, experimental and geological studies to-date strongly suggest  
155 that these high  $\delta^{238}\text{U}$  values are best explained by bacterial uranium reduction and  
156 precipitation within the spheroids<sup>12,29,30,31,32,33,34</sup>. 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<sup>12</sup>.

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<sup>36</sup>, 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<sup>37</sup>.

169

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

171 The deep biosphere conventionally extends from ~metres depth to several  
172 kilometres<sup>4,38,39</sup>. 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<sup>16,40</sup>; third, some spheroids occur in  
177 hematite-stained igneous basement, hundreds of metres below the uppermost  
178 basement<sup>14</sup>; 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<sup>14,41,42</sup>. 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<sup>22</sup>.

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<sup>24</sup>, 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<sup>24</sup>. 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<sup>44</sup>. 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<sup>45,46</sup>. 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<sup>47</sup>; (2) Devonian red  
229 sandstone at Millport, Great Cumbrae, Scotland; (3) Carboniferous white sandstone at  
230 Heysham, Lancaster, England<sup>24</sup>; (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<sup>48</sup>. The sample from Great Orme's Head  
233 is a copper ore deposit in Carboniferous limestone, North Wales<sup>49</sup>. 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<sup>50</sup>.

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<sub>3</sub> and HF on a hotplate for 24 hours.  
244 Samples were dried and re-digested in concentrated HCl and HNO<sub>3</sub>.

245

### 246 *Isotope analyses*

247 Trace metal concentrations were measured at the Yale Metal Geochemistry Center on  
248 a Thermo-Finnigan Element XR ICP-MS on splits from each digest. The <sup>236</sup>U-<sup>233</sup>U  
249 double spike was added based on uranium concentrations (<sup>238</sup>U/<sup>236</sup>U ~30), prior U  
250 purification via ion exchange methods. The spiked samples were dried and taken up  
251 in 3N HNO<sub>3</sub>. 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<sub>3</sub>  
253 with 50 ppb concentration. Uranium isotopes were measured at the Yale Metal  
254 Geochemistry Center on a Thermo-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 <sup>238</sup>U signal on a 10<sup>11</sup> Ω amplifier.

259

260 Isotopes were measured on Faraday collectors, listed in **Table 1**.  $^{232}\text{Th}$  hydride was  
261 monitored to have a negligible effect on measurement of  $^{233}\text{U}$ . 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  $\delta^{238}\text{U}$  of  $-0.52 \pm 0.08\%$  based  
265 on nine repeats ( $2\sigma$  error = 0.12). Duplicate samples agreed within error.

266

267 Uranium isotope variations of samples and standards are reported as

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

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

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 ( $5\beta$ -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<sup>-1</sup> 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<sup>-1</sup> for 8 min and then 3 °C min<sup>-1</sup>, 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

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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.

317

318

### 319 **References**

320

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478 **Figure legends**

479

480 **Figure 1:** Results of uranium isotope analysis. Error bars represent two standard  
 481 errors.  $\delta^{238}\text{U}$  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.

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497

498 **Table**

499

Isotope	$^{232}\text{Th}$	$^{233}\text{U}$	$^{234}\text{U}$	$^{235}\text{U}$	$^{236}\text{U}$	$^{238}\text{U}$
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

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503