

1

2 **Title:** Uranium Isotope Fractionation by Abiotic Reductive Precipitation

3

4

5 **Authors:** Shaun T. Brown^{1,2*}†, Anirban Basu^{1,2,3}†, Xin Ding¹‡, John N. Christensen¹, Donald J.
6 DePaolo^{1,2}

7 **Affiliations:**

8 ¹Energy Geosciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720.

9 ²Department of Earth and Planetary Science, University of California, Berkeley, CA 94720.

10 ³Department of Earth Sciences, Royal Holloway, University of London, Egham, Surrey, TW20
11 0EX.

12 *Correspondence to: stbrown@lbl.gov.

13 † Joint first authors who contributed equally to this manuscript.

14 ‡CAS Key Laboratory of Crust-Mantle Materials and Environments, School of Earth and Space
15 Sciences, University of Science and Technology of China, Hefei 230026, China

16

17

18 **Abstract:** Significant uranium (U) isotope fractionation has been observed for the first time
19 during abiotic reduction of aqueous U, counter to the expectation that uranium isotopes are only
20 fractionated by bio-associated enzymatic reduction. In our experiments, aqueous U is removed
21 from solution by reductive precipitation onto the surfaces of synthetic iron monosulfide. The
22 magnitude of uranium isotopic fractionation increases with decreasing aqueous U removal rate
23 and with increasing amounts of neutrally charged aqueous Ca-U-CO₃ species. Our discovery
24 means that abiotic U isotope fractionation likely occurs in any reducing environment with
25 aqueous Ca ≥1mM, and that the magnitude of isotopic fractionation changes in response to
26 changes in aqueous major ion concentrations that effect U speciation. Our results have
27 implications for the study of anoxia in the ancient oceans and other environments.

28

29 **Significance Statement:** We use an experimental approach to demonstrate that ²³⁸U/²³⁵U is
30 fractionated by abiotic reduction onto the surface of abiotic synthetic iron monosulfide,
31 contradicting the findings of earlier studies. We further demonstrate that for abiotic reduction
32 reactions the aqueous U speciation and removal rate control the extent of isotopic fractionation.
33 U isotopic fractionation is observed when the primary aqueous species are Ca-UO₂-CO₃,
34 suggesting that aqueous U speciation affects the relative forward and backward reaction rates
35 and by extrapolation the relative roles of nuclear field shift effect (NFS) and mass dependent
36 kinetic fractionation.

37 **\body**

38 **Introduction:** Uranium isotope fractionation in the environment was historically assumed to be
39 negligible; however, variations in ²³⁸U/²³⁵U of up to 9‰ have been documented in both
40 experimental and natural settings (1-7). The discovery of variable ²³⁸U/²³⁵U has affected
41 geochronology and cosmochemistry, changing the methodology for high precision ages and
42 proving the existence of the short-lived isotope ²⁴⁷Cm (4, 8, 9). Variations in ²³⁸U/²³⁵U are also
43 used for investigating biological U cycling on Earth and other planets (10) oxidation of the
44 Earth's atmosphere (11), and nuclear forensics (12). Stylo and coauthors (10) recently concluded
45 that microbial U reduction produced a unique U isotopic signature compared to abiotic U
46 reduction. The finding of unique isotopic effects by microbial reduction warrant further
47 investigation in order to develop the quantitative use of ²³⁸U/²³⁵U in environmental chemistry.

48 Although the standard mass dependent isotope fractionation (MDF) effects for uranium
49 isotopes are expected to be small (13, 14) and favor the lighter isotopes in the product phases,
50 there are additional effects due to nuclear size and shape (nuclear field shift effect; NFS) that
51 preferentially partition the ²³⁸U isotope into the lowest possible electron density configuration at
52 the nucleus (15, 16). For example, in isotope exchange experiments where U of two oxidation
53 states such as U(III) and U(IV) are allowed to equilibrate, the U(III) has higher ²³⁸U/²³⁵U by
54 2.3‰ based on experimental results and theoretical calculations (15-17). This observed isotopic
55 fractionation is presumably the sum of MDF and NFS effects. Studies aimed at replicating
56 inorganic U isotope fractionation during reductive precipitation of U(VI) to U(IV), which most
57 likely are not equilibrium experiments, have found either no isotope fractionation or
58 fractionation that results in the reduced product U(IV) phase having lower ²³⁸U/²³⁵U, contrary to
59 equilibrium NFS predictions (3, 10, 18) but potentially consistent with non-equilibrium MDF.
60 Although there is some confusion (or uncertainty) about the issue in the literature, it is likely that

61 NFS effects are most strongly expressed in equilibrium isotopic partitioning, whereas MDF
62 effects are likely to apply to both equilibrium and non-equilibrium (kinetically-controlled)
63 processes. Fundamental in interpreting the observed $^{238}\text{U}/^{235}\text{U}$ isotopic fractionation is
64 understanding the chemical reactions that result in U(VI) reduction including the extent to which
65 lab experiments and natural systems depart from equilibrium.

66 The study of chemical factors affecting U reduction in the environment is of considerable
67 prior interest (19). With respect to abiotic reduction, the role of mineral surfaces (20), kinetic and
68 steric effects inhibiting U reduction (21), the role for single- or two electron transfer and
69 accompanied U(V) disproportionation, and the effects of aqueous U speciation (22-24) are
70 thought to significantly affect the rate and efficiency of reduction. Our study focuses specifically
71 on U speciation, because it is known to affect the efficiency of aqueous U(VI) removal from
72 solution (25, 26).

73 A key issue in evaluating the consistency of the isotope exchange experiments described
74 above is the speciation of aqueous uranium. The isotopic exchange experiments (15, 17) were
75 conducted at low pH (<5) where the primary U(VI) and U(IV) species are both aqueous, and
76 isotopic equilibrium between aqueous U(VI) and U(IV) is relatively fast (27). At higher pH, in
77 the range of 6-8, and in typical aqueous solution compositions relevant to most Earth surface
78 processes, aqueous U(VI) is primarily a mixture of multiple hydrated U-CO₃ and U-M-CO₃
79 molecules (where M is an alkaline Earth metal such as Ca or Mg) (28, 29). For example, in the
80 absence of dissolved calcium the primary uranyl carbonate complex is $\text{UO}_2(\text{CO}_3)_2^{2-}$ whereas in
81 the presence of $\geq 1\text{mM}$ calcium the primary species is the neutrally charged $\text{Ca}_2\text{UO}_2(\text{CO}_3)_3^0$ and
82 the second most abundant species is $\text{CaUO}_2(\text{CO}_3)_3^{2-}$.

83 Aqueous U(VI) speciation also influences the rate of U(VI) reduction and possibly the
84 reaction pathways (30). Previous experimental studies observed rapid U removal, consistent with
85 sorption to the mineral surface or rapid U(VI) reduction (3, 10, 18). While these studies tested
86 multiple reductants and variable aqueous fluid compositions (e.g. 0-100 mM HCO_3^-), one
87 consistent feature of the experiments was rapid removal of aqueous U(VI). What was not
88 investigated is the effect of high concentrations aqueous Ca^{2+} , which increase the proportion of
89 neutral ternary calcium-uranyl-carbonato complexes that are known to be more difficult to sorb
90 or reduce by microbial processes (25, 26, 31). Since Ca-uranyl-carbonato complexes are nearly
91 ubiquitous in circumneutral pH waters (e.g. 32, 33) the effects of aqueous U speciation on
92 isotopic fractionation is likely to be important in most Earth surface environments (34, 35).

93 **Approach:** To evaluate the role of aqueous U speciation on the isotopic fractionation
94 between U(VI) and U(IV) we designed a series of abiotic U(VI) reduction experiments with
95 synthesized mackinawite (FeS) crystals under anoxic conditions. FeS is a well-established U
96 reductant under a variety of laboratory and environmental conditions (e.g. 10, 36). FeS was
97 added to pre-equilibrated U(VI)- NaHCO_3 - CaCl_2 solutions (pH 7.1) that were continuously
98 mixed for the duration of the experiments. The experimental solutions were prepared with
99 different initial CaCl_2 concentrations (0-2 mM) to vary the ratio of negatively charged to neutral
100 aqueous Ca-uranyl-carbonato complexes. All experiments had identical FeS and HCO_3^-
101 concentrations (0.7mM and 6mM respectively).

102 The post-experiment FeS particles were characterized by scanning electron microscope-
103 energy dispersive spectroscopy (SEM; Fig S1). The Fe:S ratio is ~ 1.7 , consistent with other
104 synthetic FeS (37). Uranium is observed coating the FeS particles and also elsewhere in the

105 beaker (Fig S1). The U is not associated with significant amounts of Ca or CO₃ in any of the
106 studied run products. The ²³⁸U/²³⁵U data are reported in standard stable isotope delta notation (1-
107 3) where (²³⁸U/²³⁵U)₀ is the measured ²³⁸U/²³⁵U at the start of each experiment.

108 **Results:** Aqueous U speciation for each experiment was calculated using Geochemists
109 Workbench with a modified version of the LLNL V8 R6 "combined" database, incorporating
110 updated formation constants for ternary and carbonate uranium species (19, 28) (Tables S1 and
111 S2). All experiments are undersaturated in CaCO₃ polymorphs and the U(VI) minerals schoepite
112 and meta-schoepite. The sums of all negatively charged and neutrally charged aqueous U(VI)
113 species were tabulated for each experiment and are reported in Table 1 as the percent of neutral
114 uranium species (U_{neutral}). For the range of 0-2 mM calcium, calculated U_{neutral} varies from 3 to
115 72% (Fig S1). Reduction is thermodynamically favorable in each experiment, with the Gibbs
116 free energy (ΔG) of the half-reaction ranging from -3.7 to -17.5 kJ mol⁻¹.

117 U(VI) concentrations decreased with time during all experiments, regardless of the
118 solution composition (Fig 1; Fig S2; Table 1). Each experiment shows a linear correlation
119 between ln[U] and time ($r^2 \geq 0.92$), consistent with pseudo-first order kinetics with rate constants
120 (k) given by the slope of the ln(U)-time relationship in each experiment (Fig S2). U(VI) removal
121 half-lives ($t_{1/2} = \ln(2)/k$) range from 2.3 hours in the Ca-free experiment to 12.6 hours in the 2.0
122 mM Ca experiment, with a correlation coefficient between $t_{1/2}$ and calcium concentration of
123 $r^2 = 0.98$.

124 The $\delta^{238}\text{U}$ of the aqueous U(VI) remaining in solution varies from 0‰ at the start of the
125 experiments to as low as -2.29‰ after 98% U(VI) removal (Fig 2; Table 1). The U isotope
126 fractionation factor (α) for each experiment was determined by linear least squares regression
127 methods (38) and varies from 1.00023 to 1.00084 (Fig 2). These α 's can also be expressed as
128 enrichment factors (ϵ) where $\epsilon = 1000\ln(\alpha)$. All of the α 's obtained from our experiments are
129 distinct from previously published abiotic U(VI) reduction experiments where $\alpha \leq 1.0000$ (3, 10,
130 18), but overlap with microbial reduction experiments (6, 10, 39). The magnitude of U isotope
131 fractionation is strongly correlated with the calculated U_{neutral} ($r^2 = 0.998$; Fig 3).

132 **Discussion:**

133 **U removal mechanism:** The first order rates derived from Fig 1 are consistent with a
134 single characteristic U removal mechanism in each experiment. If more than one removal
135 mechanism (e.g. sorption and reduction) with distinct rates removed substantial amounts of
136 aqueous U, a single rate constant is unlikely to fit time-series. We conclude that the change in U
137 removal rate constants in Fig 1 is not due to changing proportions of reduction and sorption but
138 due to a change in the rate of a single removal mechanism. This is further supported by the
139 isotopic results where each experiment produces a single fractionation factor that fits all $\delta^{238}\text{U}$
140 from both early and late time (Fig S1). Changing proportions of sorption and reduction during
141 the course of an experiment would result in different ϵ values characteristic to these mechanisms,
142 which will make it impossible for a single ϵ to describe all data from an experiment. In addition,
143 experimental studies of U sorption onto quartz and ferrihydrite confirm that varying Ca
144 concentrations do not affect sorption rates (25), which leads us to conclude that the observed
145 relationship between the U removal rate constant and U speciation is a change in the U reduction
146 rate.

147 Earlier studies also report that in experiments with similarly synthesized FeS the final U
148 product is U(IV) that is consistent with uraninite (10, 40-42). In those studies the experiments

149 have fluid with no dissolved Ca, and the rates of U removal are likely much faster than in our
150 experiments since the experiments produced quantitative removal of aqueous U within several
151 minutes. Despite these differences, the final reaction product in the presence of FeS is reduction
152 to U(IV). Stylo et al (10) conducted U(VI) reduction experiments with a similarly synthesized
153 FeS where sorption initially removes U(VI) from solution rapidly, but the final solid phase is
154 99% UO_2 and hence must be uraninite. Furthermore, other recent studies with FeS as a reductant
155 demonstrate by spectroscopic methods that U reduction to U(IV) and not sorption or U(VI)
156 precipitation is the ultimate U product (40, 42)

157 The interpretation that sorption is insignificant in our experiments does not mean there is
158 no sorption of aqueous U(VI), but rather that sorption must be reversible and not rate
159 determining. The point of zero charge (PZC) condition for disordered mackinwaite is at pH 7.5,
160 meaning that at the experiment pH of 7.1 the FeS surface charge is positive and still allows for
161 the sorption of negatively charged U-CO_3 molecules (37). The inferred small fraction of U
162 sorption in our experiments is different from similar studies of U(VI) reduction by FeS (e.g. 10).
163 This difference might be due to several factors including: (i) the lower starting U concentration
164 in our experiments reduces the sorption rate, (ii) the higher HCO_3^- concentration produces
165 aqueous U-CO_3 molecules less favorable for sorption, and (iii) the higher pH of our experiments
166 compared to (10) results in a smaller departure from PZC, which reduces the sorption rate. For
167 example, the proton balance on the FeS surface is at least 50% lower in our experiments
168 compared to those of (10).

169

170 **U isotope fractionation mechanism:** The $^{238}\text{U}/^{235}\text{U}$ isotope ratio shifts of aqueous U(VI)
171 in our experiments require that U(VI) reduction is one of the mechanisms of U removal from
172 solution, and that there is isotopic exchange between U(VI) and U(IV). The only known
173 mechanism that induces U isotope fractionation at both the magnitude and direction observed in
174 our experiments is the NFS and this can only occur in the presence of U(VI) and U(IV) at
175 circumneutral pH. Since the reductant in the experiments is a solid and the experiments have a
176 net transfer of aqueous U(VI) to solid U(IV) we infer that there must be isotopic exchange
177 between the aqueous U(VI) and U(IV), possibly involving the mineral surfaces (Fig S3-S5).
178 Thus, the isotopic fractionation can be manifested in the aqueous U(VI) pool via reversible weak
179 sorption, likely to be the case in our experiments. An additional pathway for reduction is
180 dissolution of FeS with U reduction by aqueous S^{-2} , but this mechanism is unlikely to be the
181 primary pathway because the solubility of FeS is relatively low, and the kinetics of dissolution
182 are slower than the observed U removal rates (43).

183 The magnitude of $^{238}\text{U}/^{235}\text{U}$ fractionation in the 2.0 mM Ca experiment is similar to the
184 magnitude of $^{238}\text{U}/^{235}\text{U}$ fractionation in microbial U reduction experiments (6, 10, 39). Using the
185 correlation between α and U_{neutral} the predicted abiotic α at 100% neutral species is $\alpha=1.00105$
186 (± 0.00005) which is statistically indistinguishable from the oxidation experiment value of
187 1.00110 (± 0.00020) in acidic media (44). This result suggests that as the rate of U removal is
188 slowed, the $^{238}\text{U}/^{235}\text{U}$ fractionation approaches the theoretical equilibrium values even in
189 circumneutral pH solutions and with abiotic reductive precipitation. Our results establish U
190 isotope ratios as a robust proxy for U(VI) reduction, however, based on this interpretation the
191 observation of $^{238}\text{U}/^{235}\text{U}$ fractionation in natural systems cannot be viewed as a fingerprint of
192 microbial U reduction as proposed by (10).

193 Our experimental results are in apparent conflict with those of earlier studies of U isotope
194 fractionation by abiotic reductants (3, 10, 18). The earlier studies found either no isotope
195 fractionation or $\alpha < 1.0000$. The primary difference between our experiments and earlier
196 experiments is the rate at which U is removed from solution (Fig 4). The timescale of the
197 zerovalent zinc and iron experiments were 3 and 1 hours respectively, and while the FeS
198 experiments of Stylo were up to 150 hours, U removal from solution was nearly instantaneous
199 via adsorption. Figure 4 illustrates α as a function of the aqueous U(VI) half-life for our
200 experimental results and for the prior studies (3, 10, 18, 44). We only include the FeS
201 experiments from (10) because U concentration and isotopic compositions can be described with
202 a distillation model while the other solid phase reductant experiments cannot. It is clear from Fig
203 4 that the degree of U isotope fractionation is related to the overall rate of U removal from
204 solution regardless of whether the initial removal step is primarily sorption or reduction. This
205 observation suggests that the rate of U removal in earlier experiments (3, 10, 18) interfered with,
206 and possibly inhibited isotopic equilibrium during reduction (as hypothesized (3, 10, 18)). In
207 order to observe NFS U isotopic fractionation in the residual aqueous U(VI) the net removal rate
208 from solution must be slow enough to allow for U isotopic equilibrium between U(VI) and
209 U(IV). Rapid removal of aqueous U inhibits subsequent isotopic equilibrium during reduction
210 between aqueous U and reduced U. The inhibition of isotopic equilibrium between sorbed U(VI)
211 and sorbed U(IV) is evident because the experiments of (10) had U sorbed to the mineral surface
212 for three times longer than our longest experiment but still did not obtain the expected U isotope
213 equilibrium between U(VI) and U(IV).

214 The U oxidation experiments of (44) further support the conclusion that the half-life of
215 the aqueous U reactant is important in controlling the magnitude of isotope fractionation during
216 abiotic redox reactions. In aqueous U oxidation experiments of (44) the half-life of oxidizing
217 U(IV) is approximately 36 hours and these experiments yield $\alpha = 1.0011$. In spite of the different
218 experimental approach the relationship between the U(IV) half-life and isotopic fractionation is
219 consistent with the relationship determined from our experiments (Fig 4). We can also
220 hypothesize, based on the $t_{1/2}$ - α relationship, that the necessary aqueous U half-life to achieve the
221 predicted NFS fractionation of 1.3‰ at 300K (16) is approximately 65 hours.

222 The isotope fractionation that is expressed in the experiments on the left side of Fig 4,
223 especially the 5 mM FeS experiment, require a fractionation mechanism that is different from the
224 predicted NFS. The inferred α of less than 1.0000 and the fast removal rates suggest that kinetic
225 mass dependent fractionation may be important at faster U removal rates. One possible
226 mechanism for kinetic MDF of U could be related to the desolvation and/or decarbonation of
227 aqueous U species. Molecular dynamics simulations have been used to demonstrate the
228 importance of desolvation in the fractionation of isotopes for other cations in fluid mineral
229 systems (e.g. 45). Using the desolvation-fractionation relationship from (45) (~0.5‰ for each
230 percent mass difference between two isotopes) the desolvation-driven fractionation for U would
231 be $\alpha = 0.99935$ compared to the maximum observed $\alpha = 0.9996 \pm 0.0001$ (Fig 4; (10)). In general,
232 experimentally observed isotopic fractionation factors related to desolvation are partially
233 attenuated compared to the simulated values, likely due to competing reaction steps (45).

234 Assuming that the observed $^{238}\text{U}/^{235}\text{U}$ fractionation at fast removal (Fig 4) is primarily
235 controlled by kinetic effects we can construct a model that interprets all of the experimental
236 results as a balance between equilibrium isotope fractionation and kinetic isotope fractionation.
237 The dashed gray curve in Fig 4 is calculated using model parameters as described by DePaolo

238 (46), namely, the relative forward and backward reaction rates and the limiting pure kinetic and
239 pure equilibrium fractionation factors. Details of the modeling are provided in the SI. The model
240 predicts that at fast rates U isotope fractionation will be dominated by kinetic effects with a
241 $\alpha < 1.0000$. In contrast, as the rate of U removal slows the observed isotopic fractionation
242 transitions to the equilibrium value, which is primarily a result of the NFS effect.

243 One possible way to confirm the competition between mass dependent kinetic effects and
244 the NFS effect with U isotope fractionation would be simultaneous high precision measurements
245 of both $^{238}\text{U}/^{234}\text{U}$ and $^{238}\text{U}/^{235}\text{U}$, as previously proposed by (3). Since the NFS effect is
246 disproportionately large for the ^{238}U - ^{235}U isotope pair compared to the ^{238}U - ^{234}U pair the mass
247 difference normalized fractionation of $^{238}\text{U}/^{235}\text{U}$ should increase relative to $^{238}\text{U}/^{234}\text{U}$ as the
248 contribution of the NFS effect to the net isotope fractionation increases (15, 16).

249

250 **Environmental and Geologic Implications** The speciation-dependent model for U
251 isotope fractionation has implications for interpreting $\delta^{238}\text{U}$ in the environment and the geologic
252 record, as illustrated by the U isotopic record for the Black Sea. The Black Sea has been an
253 important natural laboratory for studying element and isotopic distributions in anoxic water in
254 order to make inferences about past periods of widespread anoxia in the global oceans (47). A
255 recent study of the Black Sea water column found that the effective U isotopic fractionation is
256 between ($\epsilon^{238}\text{U}$) 0.63‰ and 0.84‰ depending on the applied fractionation model (48). In this
257 study the deviation between the observed U isotope fractionation and the theoretical NFS value
258 of $\sim 1.3\%$ is explained by diffusion-limited reduction in the sediments and back-diffusion of pore
259 water U to the water column. Despite high concentrations of U(VI) reductants in the water
260 column, any abiotic U removal in the water column was ruled out in previous studies. We
261 calculated the U speciation using updated formation constants, species (28), and prior published
262 water column compositions for the Black Sea. The calculated fraction U_{neutral} is 60% in modern
263 Black Sea water, and the corresponding fractionation according to our model would be
264 $\epsilon^{238}\text{U} = 0.7\%$. The remarkably close agreement between field studies and our speciation-
265 dependent fractionation model suggests that the aqueous speciation of U(VI) must be taken into
266 account to determine the appropriate isotopic fractionation factor to interpret U isotope
267 observations from the environment. In the case of the oceans, if the fraction of U_{neutral} has
268 changed in the over geologic time, as one might predict from changes in Ca/Mg, $p\text{CO}_2$ and pH,
269 the isotopic fractionation factor during marine U reduction may have also changed and should be
270 accounted for in models of paleo-anoxia. Changes in speciation, however, are not likely to affect
271 the U fractionation over short (< 1 Myr) timescales since the residence time for Ca in the oceans
272 is > 1 Myr (49).

273 Several studies have used $\delta^{238}\text{U}$ to quantify the reduction of U(VI) in groundwater related
274 to U mining and environmental contamination (5, 7, 50). In most studies the inferred U isotopic
275 fractionation factor is significantly smaller than ($\epsilon^{238}\text{U}$) 1.3‰, the theoretical NFS value. While
276 the magnitude of U isotope fractionation is consistent with U reduction, quantifying the amount
277 of reductive U precipitation compared to adsorption or other non-fractionating mechanisms is
278 still a challenge. Applying our speciation-fractionation model to the groundwater at the Smith
279 Ranch-Highlands U mine (7), we calculate an effective $\epsilon^{238}\text{U}$ 0.7‰, similar to the value 0.78‰
280 inferred from variations in the $\delta^{238}\text{U}$ of site groundwater (7). The agreement between the
281 observed and calculated $\epsilon^{238}\text{U}$ suggests that at this particular site the removal of aqueous U(VI)

282 from groundwater is primarily by reduction and that adsorption and hydrodynamic dispersion
283 effects are small.

284 **Materials and Methods:**

285 Experimental Methods.

286 All reagents and experiments were prepared inside a Coy Laboratory Products vinyl
287 anaerobic chamber with an atmosphere of approximately 2.5% H₂-2.5% CO₂-95% N₂. 18.2 MΩ
288 water was degassed using industrial grade N₂ and 2-micron pore sized diffusers for 1 hour per
289 liter of water. All remaining reagents were prepared from the degassed water.

291 FeS (mackinawite) synthesis

292 FeS was synthesized by dissolving 18.37 g FeCl₂ powder and 11.73 g Na₂S powder in
293 separate bottles containing 200 ml Milli-Q water (51). The solution was mixed by hand shaking
294 several times over three days until the particles started to settle to the bottom of the container.
295 The supernatant was poured off along with any suspended particles. The mixture was then re-
296 suspended in DI water a total of 3 times to rinse away excess aqueous Fe and S. The final
297 mixture was transferred to a serum bottle and sealed with butyl septa. Aliquots from this bottle
298 were suspended in DI water in a syringe and then pressed through a 0.2 μm syringe filter to
299 remove particles smaller than 200 nm. The remaining particles from the filter were suspended in
300 DI water and transferred to a clean serum bottle for storage. Well-mixed aliquots from this bottle
301 were sampled with a 1 ml syringe and used for the uranium reduction experiments. Multiple 1 ml
302 aliquots from this vial were sampled and weighed to determine the concentration of FeS in the
303 stock solution. Multiple measurements yielded a mean concentration of 6 g/L. The purpose of
304 selecting particles larger than 0.2 micron in diameter was to allow the complete separation of
305 reactant and product at each sampling interval. FeS particles larger than 0.2 microns are likely
306 aggregated smaller particles and likely have an effective surface area much higher than the
307 nominal particle size suggests.

308 Uranium reduction experiments

309 U reduction experiments were performed inside the same anaerobic chamber. All aqueous
310 reagents were made with oxygen-free DI water and all containers were equilibrated in the
311 anaerobic chamber for >48 hours to remove adsorbed oxygen. Experimental solutions containing
312 a 40 ml solution of CaCl₂-NaHCO₃-U were made in 50 ml centrifuge tubes. First CaCl₂ and
313 NaHCO₃ solutions were diluted and mixed for 24 hours to visually check for carbonate mineral
314 precipitation. Next ~80 micrograms of U (as UO₂(CO₃)₂) was added to the vials and mixed for
315 24 hours to equilibrate the U-CaCl₂-NaHCO₃ solution. Mixed solutions were sampled prior to
316 the addition of FeS and are labeled as time 3 mg of FeS was added to the experiments and the
317 vials were continuously mixed using an end-over-end rotator. One ml samples were collected
318 from the experiments on time intervals designed to sample across 0-90% of the U removal time.
319 At each sampling interval the solution pH was measured. The pH of each experiment was 7.1 for
320 the entire duration.

322 Uranium Purification

323 Experimental solutions were spiked with IRMM 3636a ²³³U-²³⁶U double spike such that
324 ²³⁸U/²³⁶U ≈20. Solutions were dried down and subsequently dissolved in 3M HNO₃. Uranium
325 purification was carried out using UTEVA resin in ~200 ul Teflon columns with HNO₃ and HCl
326 as the eluants following published protocols (6).

327 Mass Spectrometry

328 Purified uranium was analyzed on the Thermo Fisher NeptunePlus at Lawrence Berkeley
329 National Laboratory. Solutions containing ~50 ng/g total U were introduced to the mass
330 spectrometer using an ESI Apex fitted with an ACM module and a 100 µl/min nebulizer. The
331 isotopic composition of U was quantified by static multi collection and referenced to the
332 international standard Certified Reference Material CRM112a. Spike subtraction and U
333 concentration calculations were completed using a single step sample-spike deconvolution
334 calculation (SI). The published isotopic compositions are normalized to the time zero sample
335 from each experiment. Typical daily uncertainty on the $^{238}\text{U}/^{235}\text{U}$ standard is <0.1 and based on
336 6-12 measurements per session.

337 Scanning Electron Microscopy

338 A post-experiment filtrate of a single experiment was imaged with SEM and analyzed for
339 elemental composition using EDS. The filtrate was not rinsed to avoid dissolving or oxidizing
340 solid phases. As shown in Figs S3 and S4, plates of Fe-sulfide ranged up to ~100 microns in
341 extent (compare S and Fe EDS maps). Also included in the filtrate are particles of NaCl and
342 CaO, as indicated by the EDS elemental maps, likely formed with the drying of the filter from
343 the experimental solution. Some FeS grains are associated with U, and usually the U is
344 correlated with O, suggesting precipitation of uranium oxide. In the lower left of Fig. S4 is a
345 grain of uranium oxide that is not associated with FeS, however in most instances U is directly
346 associated with FeS. U concentrations on FeS grains were found to be up to ~20 weight percent
347 (Fig. S5).

348

349

350 **Acknowledgments:** A.B. and S.T.B. designed the experiments; X.D., J.N.C. and S.T.B.
351 conducted the experiments and analytical work, and all authors analyzed the data and
352 contributed to writing the manuscript. We benefitted from the constructive comments of
353 Francois Tissot on an earlier draft and two anonymous reviewers. The project was
354 initiated with support from the Laboratory Directed Research and Development (LDRD)
355 program of Berkeley Lab, and completed with support from the Director, Office of
356 Science, of the U.S. Department of Energy under Contract No. DE-AC02-05CH11231.

357

358 **references**

- 359 1. Andersen MB, Stirling CH, Weyer S (2017) Uranium isotope fractionation. *Reviews in Mineralogy and*
360 *Geochemistry* doi:10.2138/rmg.2017.82.19.
- 361 2. Weyer S, Anbar AD, Gerdes A, Gordon GW (2008) Natural fractionation of $^{238}\text{U}/^{235}\text{U}$. *Geochimica et*
362 *Cosmochimica Acta* 72:345–359.
- 363 3. Stirling CH, Andersen MB, Potter E-K, Halliday AN (2007) Low-temperature isotopic fractionation of
364 uranium. *Earth and Planetary Science Letters* 264(1-2):208–225.
- 365 4. Hiess J, Condon DJ, McLean N, Noble SR (2012) $^{238}\text{U}/^{235}\text{U}$ Systematics in Terrestrial Uranium-Bearing
366 Minerals. *Science* 335(6076):1610–1614.
- 367 5. Murphy MJ, Stirling CH, Kaltenbach A (2014) Fractionation of $^{238}\text{U}/^{235}\text{U}$ by reduction during low
368 temperature uranium mineralisation processes. *Earth and Planetary Science Letters* 388:306–317.

- 369 6. Basu A, Sanford RA, Johnson TM, Lundstrom CC, Löffler FE (2014) Uranium isotopic fractionation factors
370 during U(VI) reduction by bacterial isolates. *Geochimica et Cosmochimica Acta* 136:100–113.
- 371 7. Brown ST, et al. (2016) Isotopic evidence for reductive immobilization of uranium across a roll-front
372 mineral deposit. *Environmental Science & Technology* 50(12):6189–6198.
- 373 8. Tissot F, Dauphas N, Grove TL (2017) Distinct $^{238}\text{U}/^{235}\text{U}$ ratios and REE patterns in plutonic and
374 volcanic angrites: Geochronologic implications and evidence for U isotope fractionation during magmatic
375 processes *Geochimica et Cosmochimica Acta* 213. doi:10.1016/j.gca.2017.06.045.
- 376 9. Tissot FLH, Dauphas N, Grossman L (2016) Origin of uranium isotope variations in early solar nebula
377 condensates. *Science Advances* 2:e:1501400.
- 378 10. Stylo M, et al. (2015) Uranium isotopes fingerprint biotic reduction. *Proceedings of the National Academy
379 of Sciences*:201421841–6.
- 380 11. Reinhard CT, Planavsky NJ, Olson SL, Lyons TW, Erwin DH (2016) Earth's oxygen cycle and the
381 evolution of animal life. *Proceedings of the National Academy of Sciences* 113(32):8933–8938.
- 382 12. Christensen JN, Dresel PE, Conrad ME (2010) Isotopic Tracking of Hanford 300 Area Derived Uranium in
383 the Columbia River. *Environmental Science & Technology* 44(23):8855–8862.
- 384 13. Urey HC (1947) The thermodynamic properties of isotopic substances. *Journal of the Chemical Society
385 (Resumed)*. doi:10.1039/jr9470000562.
- 386 14. Bigeleisen J, Mayer MG (1947) Calculation of equilibrium constants for isotopic exchange reactions. *J
387 Chem Phys*. doi:10.1063/1.1746492.
- 388 15. Nomura M, Higuchi N, Fujii Y (1996) Mass dependence of uranium isotope effects in the U (IV)-U (VI)
389 exchange reaction. *Journal of the American Chemical Society* 118(38):9127–9130.
- 390 16. Bigeleisen J (1996) *Nuclear Size and Shape Effects in Chemical Reactions. Isotope Chemistry of the Heavy
391 Elements* (American Chemical Society).
- 392 17. Dujardin T, Lonchamp G (1990) Review of the French Chemex process.
- 393 18. Rademacher LK, et al. (2006) Experimentally determined uranium isotope Fractionation during reduction of
394 hexavalent U by bacteria and zero valent iron. *Environmental Science & Technology* 40(22):6943–6948.
- 395 19. Maher K, Bargar JR, Brown GE Jr (2012) Environmental speciation of actinides. *Inorg Chem* 52:3510–
396 3532.
- 397 20. Liger E, Charlet L, Van Cappellen P (1999) Surface catalysis of uranium(VI) reduction by iron(II).
398 *Geochimica et Cosmochimica Acta* 63(19-20):2939–2955.
- 399 21. Stewart BD, Neiss J, Fendorf S (2007) Quantifying Constraints Imposed by Calcium and Iron on Bacterial
400 Reduction of Uranium(VI). *Journal of Environment Quality* 36(2):363–10.
- 401 22. Ilton ES, et al. (2010) Influence of Dynamical Conditions on the Reduction of U VI at the
402 Magnetite–Solution Interface. *Environmental Science & Technology* 44(1):170–176.
- 403 23. Singer DM, et al. (2012) U(VI) Sorption and Reduction Kinetics on the Magnetite (111) Surface.
404 *Environmental Science & Technology* 46(7):3821–3830.

- 405 24. Yuan K, Renock D, Ewing RC, Becker U (2015) Uranium reduction on magnetite: Probing for pentavalent
406 uranium using electrochemical methods. *Geochimica et Cosmochimica Acta* 156(C):194–206.
- 407 25. Fox PM, Davis JA, Zachara JM (2006) The effect of calcium on aqueous uranium (VI) speciation and
408 adsorption to ferrihydrite and quartz. *Geochimica et Cosmochimica Acta*. doi:10.1016/j.gca.2005.11.027.
- 409 26. Brooks SC, et al. (2003) Inhibition of Bacterial U(VI) Reduction by Calcium. *Environmental Science &*
410 *Technology* 37(9):1850–1858.
- 411 27. Rona E (1950) Exchange Reactions of Uranium Ions in Solution. *J Am Chem Soc* 72(10):4339–4343.
- 412 28. Dong W, Brooks SC (2006) Determination of the Formation Constants of Ternary Complexes of Uranyl and
413 Carbonate with Alkaline Earth Metals (Mg 2+, Ca 2+, Sr 2+, and Ba 2+) Using Anion Exchange Method.
414 *Environmental Science & Technology* 40(15):4689–4695.
- 415 29. Endrizzi F, Rao L (2014) Chemical Speciation of Uranium(VI) in Marine Environments: Complexation of
416 Calcium and Magnesium Ions with [(UO₂)(CO₃)₃]⁴⁻ and the Effect on the Extraction of Uranium from
417 Seawater. *Chem Eur J* 20(44):14499–14506.
- 418 30. Behrends T, Van Cappellen P (2005) Competition between enzymatic and abiotic reduction of uranium (VI)
419 under iron reducing conditions. *Chemical Geology*. doi:10.1016/j.chemgeo.2005.04.007.
- 420 31. Ulrich K-U, Veeramani H, Bernier-Latmani R, Giammar DE (2011) Speciation-Dependent Kinetics of
421 Uranium(VI) Bioreduction. *Geomicrobiology Journal* 28(5-6):396–409.
- 422 32. Lee J-Y, Yun J-I (2013) Formation of ternary CaUO₂(CO₃)₃²⁻ and Ca₂UO₂(CO₃)₃(aq) complexes under
423 neutral to weakly alkaline conditions. *Dalton Trans* 42(27):9862–8.
- 424 33. Bernhard, G., Geipel, G., Reich, T., Brendler, V., Amayri, S., & Nitsche, H. (2001). Uranyl (VI)
425 carbonate complex formation: Validation of the Ca₂UO₂(CO₃)₃(aq) species. *Radiochimica*
426 *Acta*, 89(8), 511-518.
- 427
428 34. Chen X, Romaniello SJ, Herrmann AD, Wasylenki LE, Anbar AD (2016) Uranium isotope fractionation
429 during coprecipitation with aragonite and calcite. *Geochimica et Cosmochimica Acta* 188(C):189–207.
- 430 35. Chen X, Romaniello SJ, Anbar AD (2017) Uranium isotope fractionation induced by aqueous speciation:
431 Implications for U isotopes in marine CaCO₃ as a paleoredox proxy. *Geochimica et Cosmochimica Acta*
432 215:162–172.
- 433 36. Han Y-S, Gallegos TJ, Demond AH, Hayes KF (2011) FeS-coated sand for removal of arsenic(III) under
434 anaerobic conditions in permeable reactive barriers. *Water Research* 45(2):593–604.
- 435 37. Wolthers M, Charlet L, van Der Linde PR, Rickard D, van Der Weijden CH (2005) Surface chemistry of
436 disordered mackinawite (FeS). *Geochimica et Cosmochimica Acta* 69(14):3469–3481.
- 437 38. Scott KM, Lu X, Cavanaugh CM, Liu JS (2004) Optimal methods for estimating kinetic isotope effects from
438 different forms of the Rayleigh distillation equation. *Geochimica et Cosmochimica Acta* 68(3):433–442.
- 439 39. Stirling CH, Andersen MB, Warthmann R (2015) Isotope fractionation of ²³⁸U and ²³⁵U during
440 biologically-mediated uranium reduction. *Geochimica et Cosmochimica Acta*.
441 doi:10.1016/j.gca.2015.03.017.
- 442 40. Hyun SP, Davis JA, Sun K, Hayes KF (2012) Uranium(VI) Reduction by Iron(II) Monosulfide
443 Mackinawite. *Environmental Science & Technology* 46(6):3369–3376.

- 444 41. Hua B, Deng B (2008) Reductive immobilization of uranium (VI) by amorphous iron sulfide.
445 *Environmental Science and Technology* 42:8703–8708.
- 446 42. Veeramani H, et al. (2013) Abiotic reductive immobilization of U (VI) by biogenic mackinawite.
447 *Environmental Science and Technology* 47:2361–2369.
- 448 43. Rickard D (2006) The solubility of FeS. *Geochimica et Cosmochimica Acta* 70(23):5779–5789.
- 449 44. Wang X, Johnson TM, Lundstrom CC (2015) Isotope fractionation during oxidation of tetravalent uranium
450 by dissolved oxygen. *Geochimica et Cosmochimica Acta* 150:160–170.
- 451 45. Hofmann AE, Bourg IC, DePaolo DJ (2012) Ion desolvation as a mechanism for kinetic isotope
452 fractionation in aqueous systems. *Proceedings of the National Academy of Sciences* 109(46):18689–18694.
- 453 46. DePaolo DJ (2011) Surface kinetic model for isotopic and trace element fractionation during precipitation of
454 calcite from aqueous solutions. *Geochimica et Cosmochimica Acta* 75(4):1039–1056.
- 455 47. Arnold GL, Anbar AD, Barling J, Lyons TW (2004) Molybdenum isotope evidence for widespread anoxia
456 in mid-proterozoic oceans. *Science* 304(5667):87–90.
- 457 48. Rolison JM, Stirling CH, Middag R, Rijkenberg MJA (2017) Uranium stable isotope fractionation in the
458 Black Sea: Modern calibration of the $^{238}\text{U}/^{235}\text{U}$ paleo-redox proxy. *Geochimica et Cosmochimica Acta*
459 203:69–88.
- 460 49. Broecker WS, Peng TH (1982) Tracers in the Sea.
- 461 50. Basu A, et al. (2015) Isotopic and geochemical tracers for U (VI) reduction and U mobility at an in situ
462 recovery U mine. *Environmental Science & Technology* 49(10):5939–5947.
- 463 51. Butler EC, Hayes KF (1998) Effects of Solution Composition and pH on the Reductive Dechlorination of
464 Hexachloroethane by Iron Sulfide. *Environmental Science & Technology* 32(9):1276–1284.

465 **Figure Legends**

466 **Fig. 1. (A)** Dissolved U concentrations in the solution phase as a function of experiment time showing a decrease in
467 the rate of U removal with increasing Ca concentration. **(B)** Linearized U concentration-time relationship used to
468 determine the rate constant (k) in inverse hours (h^{-1}). The rate constants change by a factor of ~ 5 over the
469 investigated [Ca] range of 0-2 mM.

470
471 **Fig. 2.** $\delta^{238}\text{U}$ of dissolved uranium in the solution for each experiment as a function of remaining aqueous U
472 fraction. Curves in each panel are the best-fit logarithmic function. Uncertainty in $\delta^{238}\text{U}$ and [U] are smaller than the
473 symbol sizes. Fractionation factors (α) and isotope enrichment factors (ϵ) corresponding to the best fit curves are
474 given for each experiment.

475
476 **Fig. 3.** Relationship between neutrally charged U species fraction in solution and U isotope fractionation factor.
477 Uncertainties in the thermodynamic speciation models are estimated to be 5% and the calculated uncertainty of
478 $\epsilon^{238}\text{U}$ is $\sim 5\%$ (1σ) for all experiments. The observed correlation between U_{neutral} and $\epsilon^{238}\text{U}$ suggests that at high
479 fractions of U_{neutral} , $\epsilon^{238}\text{U}$ approaches the range of theoretical NFS isotope enrichment factors (~ 1.0 - 1.3%).

480
481 **Fig 4.** The relationship between the half-life of aqueous U and the isotopic fractionation factor in our experiments
482 and literature sources (3, 10, 18). Symbols for this study are the same as Fig 1 and all literature experiments are
483 labeled. The $t_{1/2}$ incorporates all mechanisms that remove U from solution. In the case of the (10) experiments we
484 inferred that $t_{1/2}$ must be ≤ 15 minutes based on the quantitative removal of aqueous U(VI) before their first reported
485 analysis (30 minutes). The $t_{1/2}$ for (3, 18) were estimated from the reported concentration-time relationships. Curve
486 fitting is to the present work, the 5mM FeS reduction experiment by (10) ($\alpha=0.9996$), and the U(IV) oxidation
487 experiment by (44). A model curve (dashed gray line) reasonably fits the data and assumes that U reduction in the

488 literature experiments is largely reflective of kinetic MDF effects and not NFS, whereas the experiments presented
489 in this present work are intermediate between the two effects. See the SI for a more detailed description of the
490 model.
491

492