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Short Communication

Dioxygen reactivity of a biomimetic [4Fe-4S] compound exhibits [4Fe-4S] to [2Fe-2S] cluster conversion

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ABSTRACT

Fumarate and nitrate reductase (FNR) is a gene regulatory protein that controls anaerobic to aerobic respiration in *Escherichia coli*, for which O_2 serves as a control switch to induce a protein structural change by converting [4Fe-4S] cofactors to [2Fe-2S] clusters. Although biomimetic models can aid in understanding the complex functions of their protein counterparts, the inherent sensitivity of discrete [Fe-S] molecules to aerobic conditions poses a unique challenge to mimic the O_2-sensing capability of FNR. Herein, we report unprecedented biomimetic O_2 reactivity of a discrete [4Fe-4S] complex, $[Fe_4S_4(SPh^F)_4]^{2-}$ (**1**) where SPh^F is 4-fluorothiophenolate, in which the reaction of **1** with $O_{2(g)}$ in the presence of thiolate produces its [2Fe-2S] analogue, $[Fe_2S_2(SPh^F)_4]^{2-}$ (**2**), at room temperature. The cluster conversion of **1** to **2** can also be achieved by employing disulfide as an oxidant under the same reaction conditions. The [4Fe-4S] to [2Fe-2S] cluster conversion by O_2 was found to be significantly faster than that by disulfide, while the reaction with disulfide produced higher yields of **2**.

Iron-sulfur ([Fe-S]) cofactors are ubiquitous in biology and are known for several functions, one of which is the ability to change its nuclearity in response to the environment [1,2]. In *Escherichia coli,* conversion of [4Fe-4S] to [2Fe-2S] is utilized for gene regulation by fumarate and nitrate reductase (FNR). FNR is an O_2 sensor and a transcriptional regulator acting as the control switch from anaerobic to aerobic metabolism. Conversion of the [4Fe-4S] into a [2Fe-2S] cluster by O_2 induces a protein conformational change from a DNA-binding dimer into two non-DNA binding monomers [1–4]. After FNR is released from DNA, >300 genes encoding for aerobic metabolism are able to be expressed [5,6]. Upon further O_2 exposure, [2Fe-2S] has been shown to decompose leaving apoFNR which can import a newly biosynthesized [4Fe-4S] cluster [7,8]. However, it has been recognized that a direct repair from [2Fe-2S]-FNR to [4Fe-4S]-FNR is also possible. While the exact nature of the [2Fe-2S]-FNR remains unknown, biochemical and spectroscopic studies suggest that all four, or three, of the original bridging sulfides from [4Fe-4S] retain in the form of [2Fe-2S]-bound cysteine persulfides, and the [4Fe-4S] cluster can be repaired from [2Fe-2S] cluster in the presence of Fe^{2+} and dithiothreitol (DTT) as a reductant, without adding additional sulfide (Scheme 1) [9–11].

Synthetic modeling of FNR's cluster conversion by O_2 is a difficult task because most [Fe-S] clusters decompose in aerobic conditions [13]. Outside of a protein environment, [4Fe-4S] to [2Fe-2S] cluster conversion has been shown only twice. The first example came from Holm and coworkers using an outer sphere oxidant, ferricenium, to convert model cluster $[Fe_4S_4Cl_4]^{2-}$ to $[Fe_2S_2Cl_2]^{2-}$ (Scheme 2A) [14]. More recently, Tatsumi and coworkers converted the highly oxidized all ferric [4Fe-4S] amide bound cluster to [2Fe-2S] in the presence of pyridine as a ligand source (Scheme 2B) [15]. While both models achieved cluster conversion, they lack biologically relevant oxidants and cluster ligands. Herein, we report [4Fe-4S] to [2Fe-2S] cluster conversion with a thiolate ligated [4Fe-4S] model complex using O_2 or disulfide as oxidants (Scheme 2C).

One of the differences between [Fe-S] containing proteins and small molecule [Fe-S] clusters is the presence and absence of the protected coordination environment for the [Fe-S] core. In FNR, the same cysteine residues harboring a [4Fe-4S] cluster are re-used to hold [2Fe-2S] cluster after the O_2 reaction (Scheme 1), which is difficult to imitate with simple model complexes. We thought providing extra thiolate ligands available for [2Fe-2S], a presumed product from [4Fe-4S]/O_2, may help overcome this challenge. Accordingly, we investigated on the effect of external thiolate in O_2 reactivity of [4Fe-4S] with a known [16] model cluster, $[Et_4N]_2[Fe_4S_4(SPh^F)_4]$ ($[Et_4N]_2$•**1**), where SPh^F is 4-fluorothiophenolate by UV–Vis spectroscopy. In the absence of thiolate, the **1**/O_2 reaction resulted in decrease of the characteristic absorption band of **1** at 450 nm followed by the production of an insoluble black

Abbreviations: DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene; DTT, dithiothreitol; [Fe-S], iron sulfur cluster; FNR, fumarate and nitrate reductase

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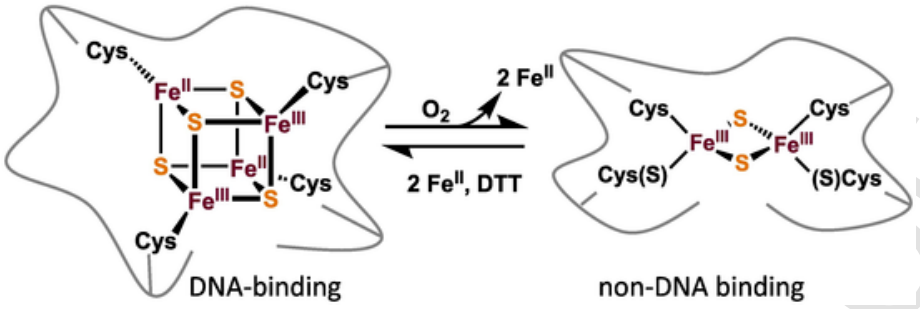
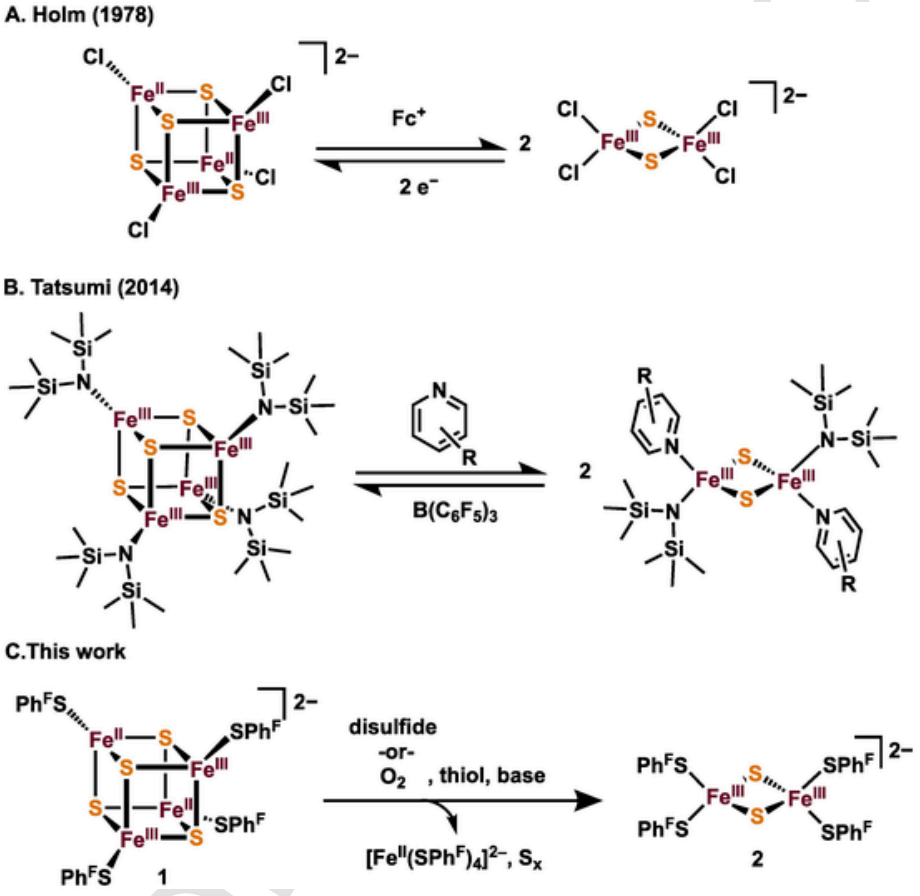


Scheme 1. [4Fe-4S] to [2Fe-2S] cluster transformation by O_2 in FNR [12].



Scheme 2. Synthetic cluster conversions.

precipitate, FeS, suggesting complete decomposition of **1** (Fig. 1A). However, when **1** was reacted with O_2 in the presence of excess (~50 equivalents) thiol and base, 4-fluorothiophenol and 1,8-diazabicyclo [5.4.0]undec-7-ene (DBU), the solution noticeably turned from brown to purple indicating [2Fe-2S] cluster formation. Within 10 min of O_2 exposure, the charge transfer band at ~450 nm from **1** shifted to ~480 nm characteristic of $[Fe_2S_2(SPh^F)_4]^{2-}$ (**2**) (Fig. 1B) [17].

To further characterize the products obtained from **1**/O_2 in the presence of thiolate, ^{1}H NMR spectroscopy was employed following a reaction in a synthetic scale. After combining $[Et_4N]_2$•**1** (0.013 mmol, 1 equiv) with $HSPh^F$ (10 equiv) and DBU (10 equiv) in acetonitrile, excess $O_{2(g)}$ was bubbled through the reaction mixture and allowed to react for 30 s at room temperature before removing all volatiles. Consistent with UV–Vis monitoring, the NMR spectrum of the crude product showed $[Fe_2S_2(SPh^F)_4]^{2-}$ (**2**) as the major product displaying well-resolved *m*-H and *o*-H resonances of the -SPh^F ligand (Fig. 2). The peak integration of the *m*-H resonance at 8.9 ppm relative to the tetraethylammonium counter cation indicates that **1** was converted to **2** in ~124% yield (0.016 mmol, 1.2 equiv), indicating the protonated DBU must serve as an additional counter cation in solution. The NMR spectrum also shows minor paramagnetic Fe-containing side products with signals at 36.3 ppm and 22.7 ppm which correspond to the *m*-H signals of a linear [3Fe-4S] cluster, $[Fe_3S_4(SPh^F)_4]^{3-}$, and a mononuclear Fe compound, $[Fe(SPh^F)_4]^{2-}$ (**3**), respectively (Fig. S1). Recrystallization of the crude products from acetonitrile and diethyl ether led to the isolation of $[Et_4N]_2$•**2** as a dark purplish black powder in 94% yield (0.013 mmol, 0.9 equiv) [18]. In biological studies with FNR, ~60% conversion of [4Fe-4S] to [2Fe-2S] was reported upon O_2 exposure [3]. Our results show that the biomimetic cluster conversion by O_2 is possible with a small synthetic model. However, the synthetic system requires extra thiolate to stabilize the resulting [2Fe-2S] cluster, different from the protein where the cysteine residues are pre-positioned to harbor a [2Fe-2S] cluster after the O_2 reaction.

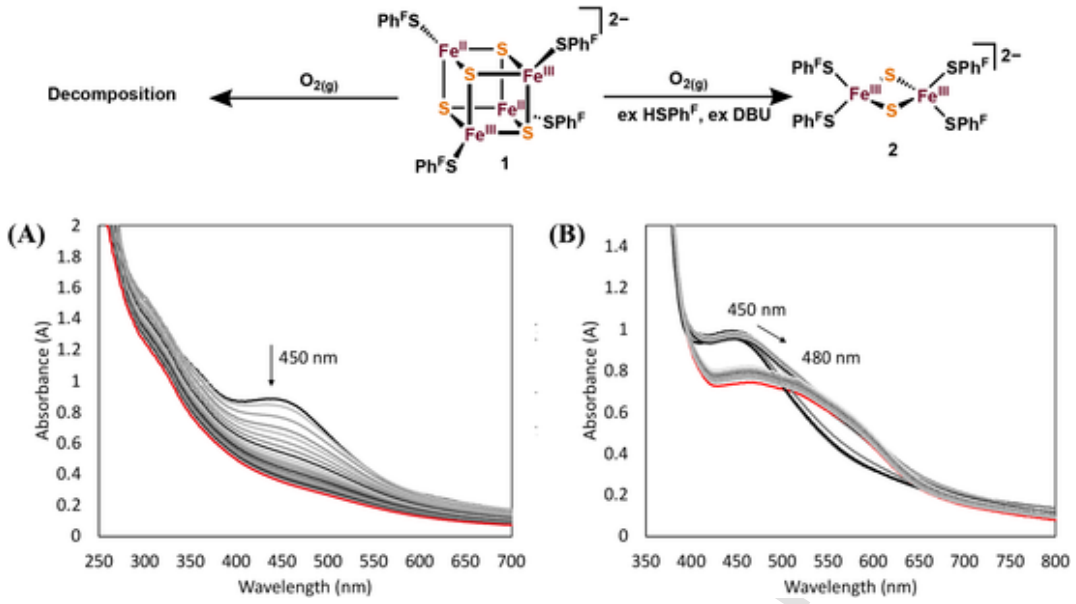


Fig. 1. UV–Vis spectra of $[Fe_4S_4(SPh^F)_4]^{2-}$ (**1**) upon exposure to O_2 in absence (A) and presence (B) of excess (50 equiv) of thiol and base at room temperature in MeCN over 10 min.

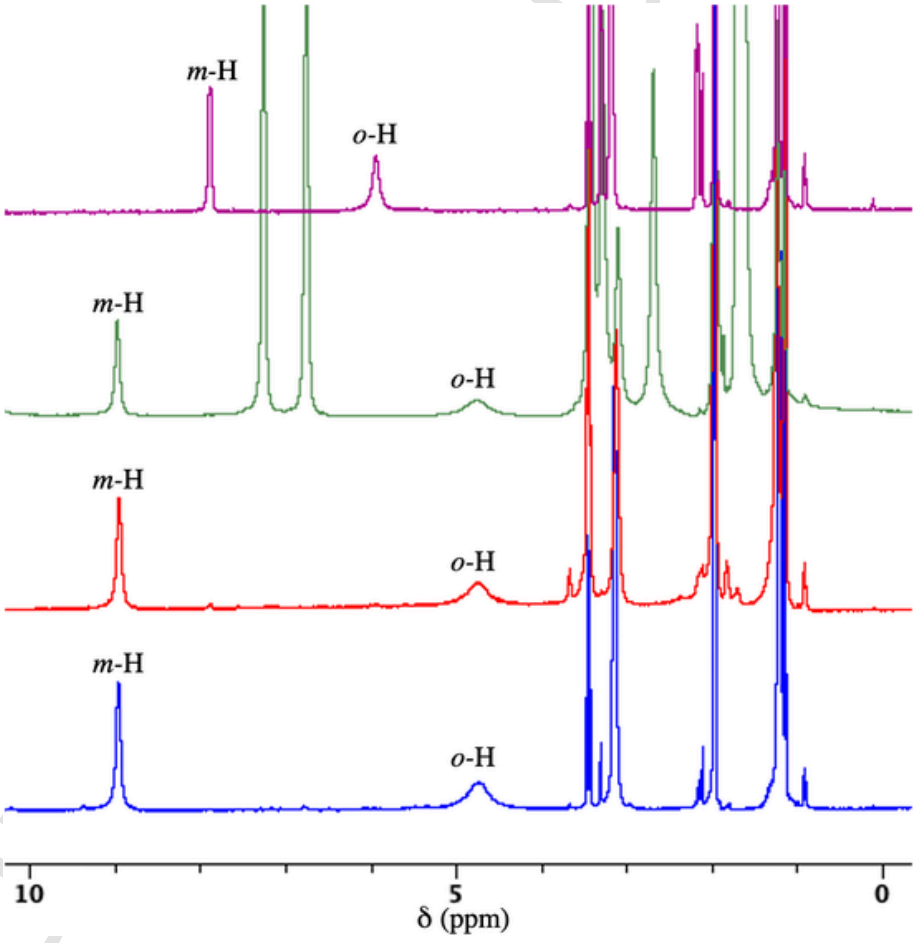


Fig. 2. ^{1}H NMR (CD_3CN, rt., 400 MHz) of $[Fe_4S_4(SPh^F)_4]^{2-}$ (**1**) (purple, top); the in situ reaction product from **1**/O_2/thiol/base (green, upper middle), where peaks with * are from external thiolate; the purified reaction product from **1**/O_2/thiol/base (red, lower middle); authentic $[Fe_2S_2(SPh^F)_4]^{2-}$ (**2**) (blue, bottom). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The reaction between $[Fe_4S_4(SPh^F)_4]^{2-}$ (**1**) and O_2 is expected to produce reduced oxygen species such as superoxide (O_2^-), H_2O_2, or water. Although we were not able to detect such species from our reactions, we examined the reactivity of **1** with H_2O_2 to gain further chemical insights. We found that the overall reactivity of **1** with H_2O_2 was analogous to what was observed with **1**/O_2. In the absence of thiolate, H_2O_2 gradually decomposed **1** to an insoluble black precipitate. However, in the presence of $HSPh^F$ (10 equiv) and DBU (10 equiv), the reaction between **1** and H_2O_2 (1 equiv) produced the same reaction products as those from **1**/O_2, in which $[Fe_4S_4(SPh^F)_4]^{2-}$ (**2**) was observed as the main product along with $[Fe(SPh^F)_4]^{2-}$ (**3**) and $[Fe_3S_4(SPh^F)_4]^{3-}$ as side products (Fig. S4).

Given the cluster conversion seen with O_2 only when extra thiol and base are present, we next examined whether the cluster conversion was the result from direct reaction between **1** and O_2 or from the reaction between **1** and a secondary oxidation product such as disulfide derived from thiol/O_2. Disulfide is a biologically prevalent oxidant whose concentration depends on the cellular compartments and oxidative conditions [19]. Since the breakdown of a [4Fe-4S] cluster into [2Fe-2S] clusters requires a *net* two-electron oxidation ($[Fe_4S_4]^{2+} \rightarrow 2\ [Fe_2S_2]^{2+} + 2\ e^-$), we first examined the reaction between **1** and 1–50 equiv. of $(SPh^F)_2$ over several days. The results showed that disulfide alone was unreactive towards **1** [20]. However, similar to **1**/O_2 reactivity, the presence of thiolate changes the reactivity pattern and enables disulfide to become a sufficient oxidant to induce a cluster conversion. After combining **1** (0.024 mmol, 1 equiv) with disulfide (1 equiv), thiol (2 equiv), and DBU (4 equiv) for 48 h in MeCN at room temperature, we observed all of **1** was consumed with a concomitant formation of the corresponding [2Fe-2S] cluster, $[Fe_2S_2(SPh^F)_4]^{2-}$ (**2**) in 40% yield (0.019 mmol, 0.8 equiv) by ^{1}H NMR spectroscopy, where the yield was calculated based on a hypothetical stoichiometric reaction following Eq. (1). Upon recrystallization of the product mixture from acetonitrile and diethyl ether at −35 °C, $[Et_4N]_2$•**2** was isolated in 35% yield (0.017 mol, 0.7 equiv).

$$\left[Fe_4S_4\left(SPh^F\right)_4\right]^{2-}\ (\mathbf{1}) + 2\,{}^F PhS^- + \left(SPh^F\right)_2 \rightarrow 2\left[Fe_2S_2\left(SPh^F\right)_4\right]^{2-}\ (\mathbf{2}) \quad (1)$$

In attempts to improve the overall [2Fe-2S] cluster yield, we increased the equivalents of thiol and disulfide to four and two respectively, which roughly doubled the amount of compound **2** produced (~75%) (Table 1, entry 2). Interestingly, a noticeable amount (0.8 equiv) of an unexpected Fe^{II}-containing byproduct, $[Fe(SPh^F)_4]^{2-}$ (**3**), was observed upon increasing the amounts of thiolate and disulfide. The formation of **3** suggests that the conversion of **1** into **2** by disulfide cannot be accomplished by a single step as is written in Eq. (1) because Fe^{II} cannot be generated if direct oxidation of **1** by disulfide took place. Since the bridging sulfide (S^{2-}) was the only other remaining redox-accessible entity in the reaction mixture, we next investigated on the fate of the bridging sulfide by carrying out the reaction of **1** (0.012 mmol, 1 equiv) with $HSPh^F$ (4 equiv), DBU (5 equiv), and $(SPh^F)_2$ (2 equiv) in the presence of 4 equiv. of sulfur atom acceptor, PPh_3, to sequester and quantify the amount of elemental sulfur (S_x) produced. ^{31}P NMR analysis showed that the reaction generated 1.5 equiv. (0.018 mmol) of S_x which was trapped as triphenylphosphinesulfide, S = PPh_3. Additionally, we were able to observe significant amounts of **3** (0.02 mmol, 1.8 equiv) and reduced amounts of **2** (0.004 mmol, 0.3 equiv) by ^{1}H NMR spectroscopy. Based on these data we propose that the conversion of [4Fe-4S] to [2Fe-2S] by disulfide first proceed following Eq. (2) in which the redox reaction takes place with the bridging sulfide to yield the first equivalent of **2**, along with elemental sulfur (S_x) and **3**. In the absence of PPh_3, the latter two products, **3** and S_x, can further react to produce the second equivalent of **2** following Eq. (3) [21–23]. Combining these two steps, Eqs. (2) and (3), would result in a net [4Fe-4S] to [2Fe-2S] cluster conversion as we initially hypothesized in Eq. (1).

$$\left[Fe_4S_4\left(SPh^F\right)_4\right]^{2-}\ (\mathbf{1}) + 2\left(SPh^F\right)_2 + 4\,{}^F PhS^- \rightarrow \left[Fe_2S_2\left(SPh^F\right)_4\right]^{2-}\ (\mathbf{2}) + 2\left[Fe\left(SPh^F\right)_4\right]^{2-}\ (\mathbf{3}) + 2\,S_x \quad (2)$$

$$\left[Fe\left(SPh^F\right)_4\right]^{2-}\ (\mathbf{3}) + 2\,S_x \rightarrow \left[Fe_2S_2\left(SPh^F\right)_4\right]^{2-}\ (\mathbf{2}) + \left(SPh^F\right)_2 + 2\,{}^F PhS^- \quad (3)$$

The current studies show that both O_2 and disulfide are potent oxidants to induce a biomimetic [4Fe-4S] to [2Fe-2S] conversion for which the presence of extra thiolate plays an important role. However, drastically different reaction kinetics (30 s vs 48 h) suggest that the cluster conversion by O_2 and disulfide must operate by a different mechanism. O_2 can directly react with a [4Fe-4S] cluster with or without thiolate but the external thiolate can aid the formation of [2Fe-2S] cluster for a synthetic compound. In contrast, the lack of reactivity of the [4Fe-4S] cluster with thiolate or disulfide alone (Table 1, entries 3&4) indicates cluster conversion can only be achieved in combination of thiolate and disulfide. We conjecture that a transient binding of thiolate to **1**, although unfavorable, may be needed to shift the redox potential of **1** to initiate the oxidative cluster conversion by disulfide. Although the [4Fe-4S] to [2Fe-2S] cluster conversion by disulfide is slow, this reaction may imply how solvent exposed [4Fe-4S] clusters can be disrupted by the cellular thiol homeostasis (e.g., the ratio between reduced and oxidized glutathione, GSH: GSSG) resulted from the oxidative environment [24,25]. To the best of our knowledge, this work is the first example of synthetic [4Fe-4S] cluster conversion to [2Fe-2S] using O_2. This study also shows how a mild oxidant, such as disulfide, can induce cluster conversion in the presence of thiolate.

Table 1
Conversion of $[Fe_4S_4(SPh^F)_4]^{2-}$ (**1**) to $[Fe_2S_2(SPh^F)_4]^{2-}$ (**2**) in the presence of disulfide, thiol, and base.

Entry	*m*$HSPh^F$	*n*$(PhFS)_2$	$[Fe_2S_2(SR)_4]^{2-}$ (**2**)
			yield[a]
1	2	1	40%
2	4	2	75%
3	–	2	0%
4	4	–	0%

[a] Based on ^{1}H NMR spectroscopy. Generation of 2 equiv. of **2** is considered 100% following Eq. (1).

Author statement

K.M.O. performed experiments, analyzed data, and drafted the paper. R.L.L and Z.Z. performed experiments and analyzed data. E.K provided direction and oversight, and edited the paper.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jinorgbio.2022.111714.

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