

1 Metals enable a non-enzymatic acetyl CoA pathway

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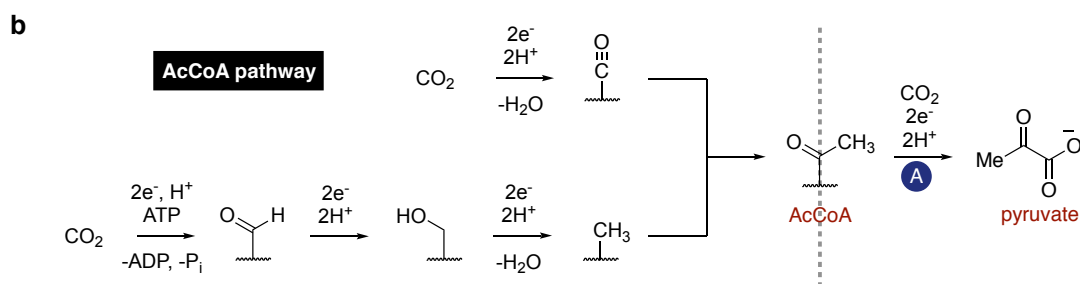
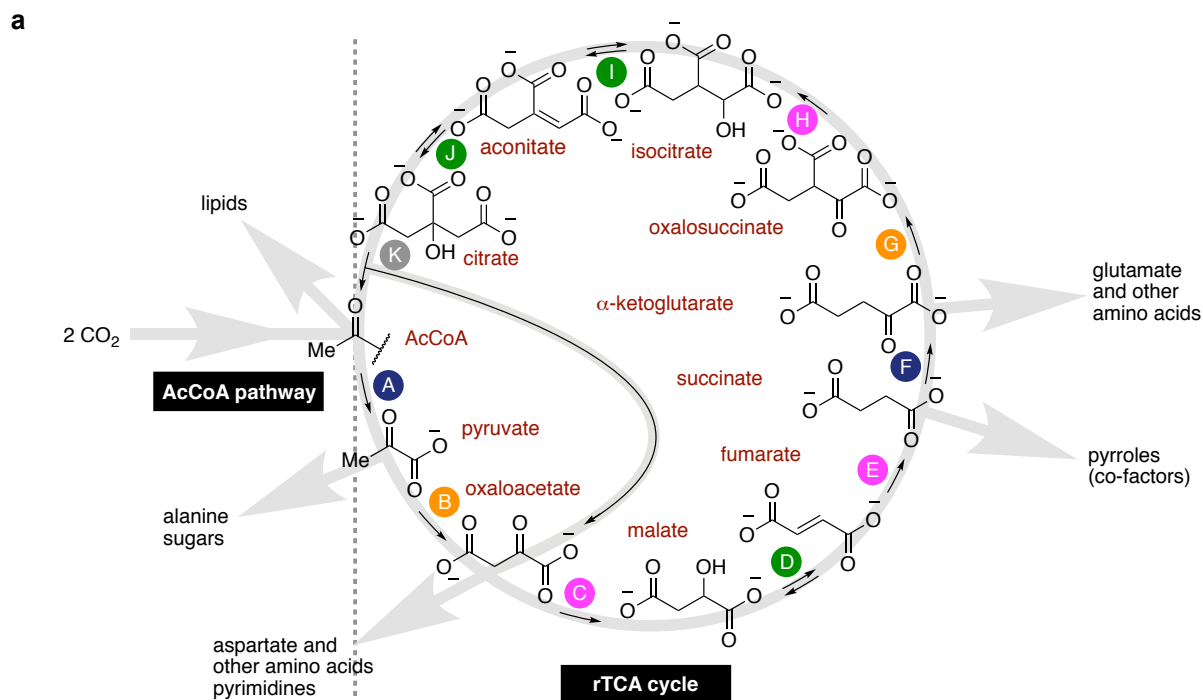
6 **The evolutionary origins of carbon fixation, the biological conversion of CO₂ to metabolites, remain**
7 **unclear. Phylogenetics indicates that the AcCoA pathway, the reductive fixation of CO₂ to acetyl and**
8 **pyruvate, was the key biosynthetic route used by the Last Universal Common Ancestor (LUCA) to build**
9 **its biochemistry. However, debate exists over whether CO₂ fixation is a relatively late invention of pre-**
10 **LUCA evolution or whether it dates back to prebiotic chemistry. Here we show that zero-valent forms**
11 **of the transition metals known to act as co-factors in the AcCoA pathway (Fe⁰, Ni⁰, Co⁰) fix CO₂ on their**
12 **surface in a manner closely resembling the biological pathway, producing acetate and pyruvate in near**
13 **mM concentrations following cleavage from the surface. The reaction is robust over a wide range of**
14 **temperatures and pressures with acetate and pyruvate constituting the major products in solution at 1**
15 **bar of CO₂ and 30 °C. The discovered conditions also promote 7 of the 11 steps of the rTCA cycle and**
16 **amino acid synthesis, providing a stunning direct connection between simple inorganic chemistry and**
17 **ancient CO₂-fixation pathways. The results strongly support the notion that CO₂-fixation pathways are**
18 **an outgrowth of spontaneous geochemistry.**

19 From the very earliest stages of the transition from chemistry to biochemistry at the origin of life, synthetic
20 pathways must have operated to build molecular complexity from simple starting materials.^{1,2} Most efforts
21 to understand synthetic prebiotic chemistry have focused on synthesizing biologically relevant compounds
22 through de novo chemical routes that bear minimal resemblance to their biosynthesis.³ An alternative and
23 more parsimonious possibility is that some metabolic pathways are “chemical fossils” of primitive chemis-
24 try that operated before the emergence of enzymes, RNA or cells.^{4,5} These extant anabolic pathways
25 might have originated as chemical paths of least resistance to relieve pent-up redox gradients between
26 the reduced iron that formed the early Earth’s bulk and its comparatively oxidized CO₂-rich atmosphere
27 and oceans.⁶ The anabolic systems of greatest interest in this regard are two CO₂-fixing pathways used

1 by autotrophic organisms^{7,8}: the reductive AcCoA pathway⁹ (also known as the Wood-Ljungdahl pathway),
2 which converts CO₂ to acetyl CoA and then to pyruvate, and the reductive tricarboxylic acid cycle¹⁰ (rTCA
3 cycle; also known as the reverse Krebs cycle).^{11,12} Phylogenetic studies indeed point to the presence of
4 the AcCoA pathway in the last universal common ancestor.¹³ An ancestral pathway consisting of the Ac-
5 CoA pathway and an incomplete form of the rTCA cycle is proposed to have been operative in prebiotic
6 chemistry and consequently in early life.¹⁴ Other studies suggest these two pathways were once unified
7 in their complete forms as part of a larger network that embodies stabilized network autocatalysis, a fea-
8 ture that could serve to amplify its constituent chemicals (Figure 1a).^{15,16} While these conceptions of prebi-
9 otic chemistry provide strong explanatory power for why the structure of metabolism is as it is, supporting
10 chemical evidence is critically lacking.^{17,18,19} In this context, our laboratory is engaged in systematic efforts
11 to uncover non-enzymatic chemistry related to these biological CO₂-fixation pathways. We recently re-
12 ported that all of the reduction and (de)hydration reactions of the rTCA cycle can be driven in sequence
13 under a common set of conditions by the combination of Zn²⁺, Cr³⁺ and Fe⁰.²⁰ In this paper, we turn our
14 attention to the search for simple chemical reagents for the first two C-C bond-forming events within a
15 hybrid AcCoA pathway/rTCA cycle; the reductive fixation of two molecules of CO₂ to generate an acetyl
16 group and further reductive carboxylation to form pyruvate. These metabolic reactions rely heavily on
17 catalysis by metalloenzymes and co-factors utilizing Fe, Ni, Co, Mo or W,^{21,22,23,24} but the mechanistic
18 steps they enable - reductions, dehydrations and migratory insertions – are also encountered in classical
19 transition metal catalysis (Figure 1b).

20 **Results and discussion**

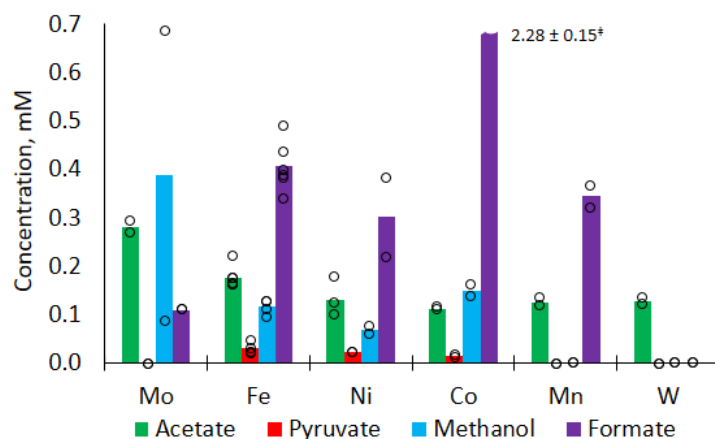
21 Anticipating a link between the role of metallic cofactors in extant microbial metabolism and in prebiotic
22 chemistry,^{25,26,27} we set out to investigate whether simple zero-valent forms of the metals involved in the
23 reductive AcCoA pathway and in pyruvate biosynthesis might promote C-C bond formation from CO₂ in
24 water. We initially screened reactions of 1 mmol of Fe, Co, Ni, Mn, Mo and W powders (for specifications
25 see Table S1) in a 1 M KCl solution in deionized H₂O by heating to 100 °C under 35 bar CO₂ pressure for
26 16 h. The reaction mixtures were then treated with KOH to precipitate hydroxides, which were removed
27 by centrifugation prior to analysis by ¹H NMR and GC-MS (Figures S1 – S8). Quantification was achieved
28 by comparison to a calibration curve prepared from authentic samples (Figure S9). To our surprise, all of



1

2 **Figure 1** (a) Hypothetical proto-anabolic network consisting of the AcCoA pathway and the rTCA cycle, showing
 3 the role of its intermediates as universal biosynthetic precursors. (b) Mechanistic outline of the AcCoA pathway
 4 and the first reductive carboxylation (step A) of the rTCA cycle, giving pyruvate. For clarity, organic and metallic
 5 co-factors are depicted as a squiggly line.

6



7

8 **Figure 2** Carbon fixation by metals under mild, hydrothermal conditions: 100 °C, 35 bar CO₂, 1 M KCl in H₂O,
 9 pH = 7 (except for Mo, where the initial unbuffered pH was 2), 16 h (see SI for experimental and analytical
 10 details, as well as error analysis). Bar chart shows mean values of at least two independent runs.

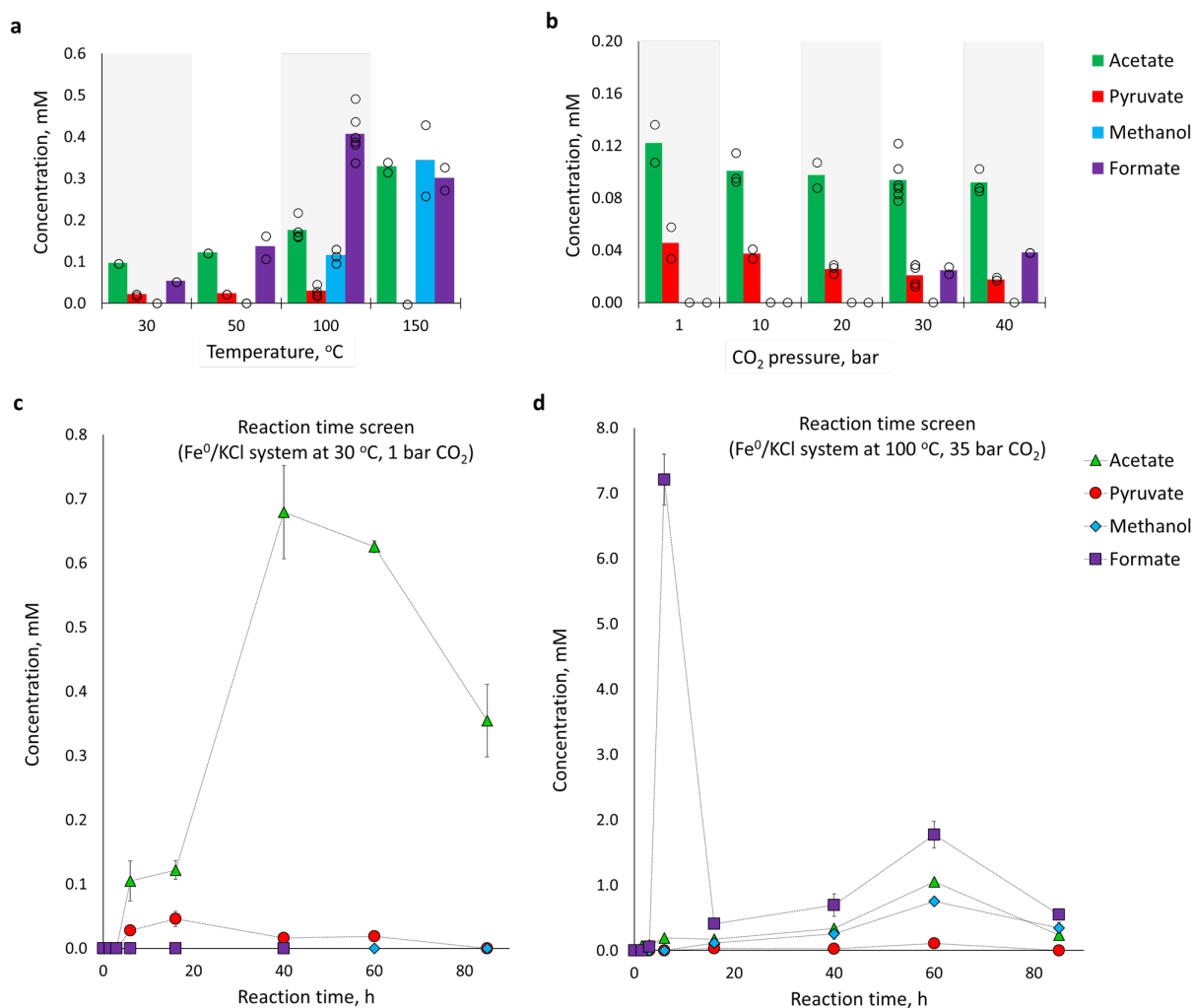
11 [‡] Formate concentrations: 2.13 mM, 2.44 mM; reported error corresponds to mean standard deviation.

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2 these metals were found to promote the formation of acetate in up to 0.28 ± 0.01 mM concentration, with
3 considerable amounts of pyruvate observed in the cases of iron (0.03 ± 0.01 mM), nickel (0.02 ± 0.00 mM)
4 and cobalt (0.01 ± 0.00 mM) (Figure 2). Substantial quantities of formate (up to 2.3 ± 0.2 mM in the case
5 of cobalt) and methanol (up to 0.39 ± 0.00 mM in the case of molybdenum) were also found in almost all
6 cases. Control experiments in the absence of metal powders or in the absence of CO₂ did not produce
7 detectable quantities of carbon fixation products (Figures S10 and S11).

8 In light of iron's position as the premier Earth-abundant metal²⁸ and its demonstrated ability to drive non-
9 enzymatic reduction reactions of the rTCA cycle,²⁰ we elected to study iron-mediated CO₂ fixation in more
10 detail by evaluating the influence of temperature, pressure, time, pH, salt identity and salt concentration
11 on the reaction outcome. First, we studied the effect of temperature over the range 30-150 °C under typical
12 conditions (35 bar CO₂, unbuffered 1 M KCl solution in deionized H₂O, 16 h) (Figure 3a, see also Figure
13 S12 and Table S2). At the lower end of the temperature range, acetate is the major product in solution,
14 with pyruvate and formate formed in slightly smaller quantities. Increasing the reaction temperature to 100
15 °C results in the appearance of methanol among the other products. At 150 °C, pyruvate is no longer
16 observed, presumably due to thermal decomposition. The reaction is robust to CO₂ pressure over the
17 investigated range 1-40 atm at 30 °C, with acetate and pyruvate being the major products at lower pres-
18 sures (Figure 3b, see also Figure S13 and Table S3). The reaction progress was monitored at different
19 times under two representative sets of conditions. At 30 °C and 1 bar CO₂ acetate and pyruvate are the
20 major products, with the former reaching nearly 0.7 mM after 40 h before decreasing in concentration
21 (Figure 3c). At 100 °C and 35 bar CO₂ the initial buildup of formate is rapid, increasing to 7.2 ± 0.4 mM
22 after 6 h before decreasing sharply as acetate and pyruvate begin to appear (Figure 3d, see also Figure
23 S14 and Table S4). After 60 h, acetate and pyruvate reach maximal concentrations of 1.09 ± 0.00 mM and
24 0.11 ± 0.01 mM, respectively. Stopping the reaction at this time reveals that no Fe⁰ visibly remains, though
25 only $\approx 1\%$ of the available electrons from Fe⁰ were channeled towards the described C1-C3 products in
26 solution (assuming Fe²⁺ is the terminal product, see Table S5), indicating that Fe⁰ predominantly reacts to
27 form gaseous products under these conditions.²⁹ By 85 h, the concentrations of all products in solution
28 decrease, and ethanol is detected in the reaction mixtures for the first time (Figure S15). The disappear-
29 ance of the Fe⁰ "fuel" necessary to maintain the reaction in a far-from-equilibrium steady state presumably

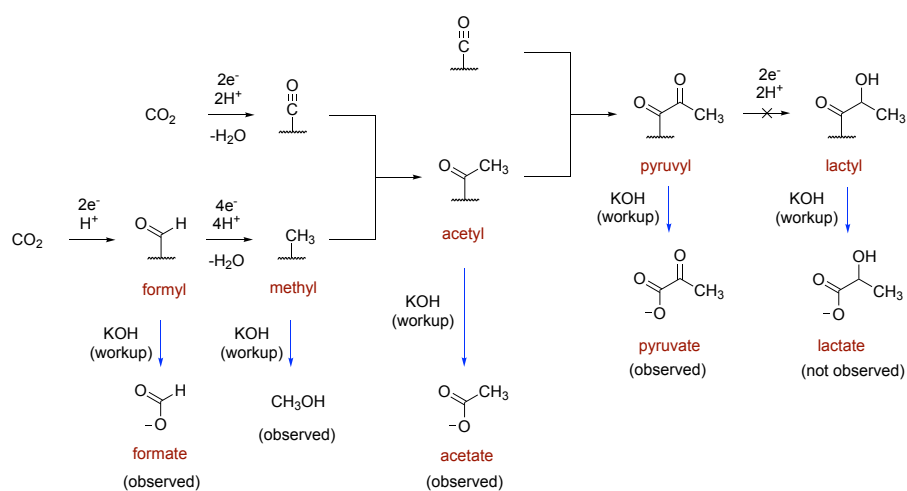
1 causes the thermal decomposition of C2 and C3 products to outcompete their generation.³⁰ Neither chang-
2 ing the initial unbuffered pH nor swapping the K⁺ electrolyte for other biologically relevant inorganic cations
3 (Na⁺, Mg²⁺ and Ca²⁺) had significant influence on carbon fixation (Figures S16 – S26 and Tables S6 –
4 S17). However, the salt concentration had a more significant effect, with a drop of acetate and pyruvate
5 yields by over 20% in the absence of KCl (Figure S27 and Table S18).



6
7 **Figure 3** Effect of temperature, pressure and reaction time on iron-promoted CO₂ fixation in aqueous solution.
8 **a** Effect of temperature (35 bar CO₂; 16 h). **b** Effect of CO₂ pressure (30 °C; 16 h). **c** Reaction progress over
9 time (30 °C; 1 bar CO₂). **d** Reaction progress over time (100 °C; 35 bar CO₂). All reactions are 1 mmol Fe in 1
10 mL of a 1 M KCl solution. In **a** and **b** bar charts show mean values of at least two independent runs. In **c** and **d**
11 error bars correspond to the mean average deviation from at least two independent runs. Lines connecting the
12 data points do not represent a model fit.

13
14 Several additional experimental observations helped to gain insight into the mechanism of the reaction.
15 First, in the absence of basic workup with KOH prior to NMR analysis, no C1 – C3 carbon fixation products
16 were observed in solution (Figure S29). Second, introduction of formate, methanol or acetate into the
17 reactor under typical reaction conditions did not result in their conversion to higher C2 or C3 products

1 (Figure S30). Thus, formate, methanol and acetate, free in solution, do not appear to be intermediates in
 2 the reaction. Third, lactate, the product of parasitic reduction of pyruvate, was never observed in any of
 3 our experiments, despite the fact that it is readily detected upon exposure of an aqueous pyruvate solution
 4 to metallic Fe (Figure S30d). Interestingly, Fe-mediated carbon fixation reactions performed with CO in-
 5 stead of CO₂ at 30 °C produced only tiny amounts of acetate and no detected pyruvate (Figure S31 and
 6 Table S19). On the basis of these observations and the reaction's kinetic profile, we propose a preliminary
 7 mechanism whereby carbon fixation occurs on the surface of the reduced metal to produce surface-bound
 8 species. Initial reduction of CO₂ occurs to generate surface-bound carbon monoxide and formyl groups.
 9 Further reductions of the formyl group with expulsion of water leads to a surface-bound methyl group.
 10 Chain growth via migratory insertion of carbon monoxide into the methyl group produces an acetyl species,
 11 which itself can undergo further migratory insertion of CO to furnish a surface-bound pyruvyl species. The
 12 resistance of the pyruvyl species to reduction by Fe⁰ may be rationalized by a diminished reactivity of the
 13 surface-bound species compared to pyruvate in solution. Basic workup at the end of the reaction with
 14 KOH is therefore required to cleave the surface-bound formyl, methyl, acetyl or pyruvyl species to furnish
 15 formate, methanol, acetate or pyruvate in solution, respectively (Figure 4). The kinetic profile observed in
 16 Figure 3c may therefore be interpreted as an initial saturation of the Fe surface with formyl and CO groups,
 17 followed by subsequent reactions of those groups to produce acetyl and, eventually, pyruvyl groups. Fur-
 18 ther study is required to distinguish between the intermediacy of surface-bound metal carboxylates and
 19 surface acyl metal species. Regardless, the proposed mechanistic picture is reminiscent of the enzymatic
 20 mechanisms of the reductive AcCoA pathway and the first step of the rTCA cycle.²⁷



22 **Figure 4** Plausible mechanism for carbon fixation on the surface of Fe⁰ and the detection of formate, methanol,
 23 acetate and pyruvate in aqueous solution following hydrolysis with KOH. The depicted surface bound structures
 24 may represent a surface-bound carboxylate or an acyl metal species.

1 Finally, we set out to test the mutual compatibility of the carbon fixation conditions with other steps of
2 the rTCA cycle. In our previous work, Fe^0 , Zn^{2+} and Cr^{3+} was found to promote the three-step oxaloacetate-
3 to-succinate sequence and the three-step oxalosuccinate-to-citrate sequence (Figure 1a, steps C-E and
4 steps H-J), however, strongly acidic conditions were required (1 M HCl in H_2O).²⁰ When oxaloacetate was
5 heated for 16 h in the presence of Fe powder and Cr^{3+} at 140 °C under 35 bar of CO_2 , an appreciable
6 amount of succinate was formed alongside the carbon fixation products, as evidenced by NMR and GC-
7 MS (Figures S32 and S33). An analogous experiment carried out with *in situ* generated oxalosuccinate
8 (heated at 140 °C for 16 h under 35 bar of CO_2 , in the presence of Fe powder and Cr^{3+}) resulted in the
9 formation of citrate (detected by GC-MS, Figure S34). Although both of these reaction sequences are less
10 selective under the mildly acidic conditions afforded by CO_2 -saturated water than in 1 M HCl, they none-
11 theless suggest the potential compatibility of the carbon fixation conditions shown here with the non-en-
12 zymatic promotion of rTCA cycle reaction sequences. Lastly, the introduction of hydrazine into the other-
13 wise identical Fe-rich conditions allowed for the non-enzymatic synthesis of the amino acid alanine from
14 pyruvate (Figure S35).²⁰

15 **Conclusions**

16 We have shown that zero-valent forms of metals used by metalloenzymes to catalyze biological carbon-
17 fixing pathways are able to promote CO_2 fixation resembling the AcCoA pathway and the first step of the
18 rTCA cycle in an experimentally trivial manner that is robust to changes in temperature, pressure, salts
19 and pH. Acetate is produced as the major C2 product for all metals studied, whereas Fe^0 , Ni^0 and Co^0
20 produce pyruvate in up to ~0.1 mM concentrations. The reaction operates even at 30 °C and 1 atm CO_2
21 pressure and is highly selective for acetate and pyruvate under these conditions, offering a striking exper-
22 imental parallel between simple chemistry and chemoautotrophic CO_2 -fixation. Indeed, CO_2 -fixing organ-
23 isms using Fe^0 corrosion as their sole source of electrons still exist on the Earth today.^{31,32} Control and time
24 course experiments support a mechanism where CO_2 fixation occurs on the surface to produce surface-
25 bound intermediates, notably reminiscent of some aspects of Wächtershäuser's initial theory of surface
26 metabolism.⁴ The temperature limits and critical need to liberate surface-bound species with KOH prior to
27 analysis may explain why pyruvate was not observed in previous studies on the reaction of CO_2 with
28 Fe^0 .^{29,33} It is also more geochemically plausible than hydrothermal syntheses relying on other C1 feed-
29 stocks, such as the synthesis of activated acetate from CO and CH_3SH ,^{34,35} the low yielding synthesis of
30 pyruvate (0.07%) in neat formic acid at high temperature and high pressure (250 °C, 2000 bar),³⁶ or using

1 highly reducing (-1.1 V) electrochemistry on greigite electrodes.³⁷ Iron is the most abundant transition
2 metal on Earth and in meteorites.^{28,38} Fe⁰ is produced in small amounts by serpentinization,³⁹ is found in
3 the Earth's crust as telluric iron,⁴⁰ is produced transiently in the mantle,⁴¹ and is the major component of
4 the Earth's core. Thus, numerous plausible geological scenarios might be imagined in which Fe⁰ and other
5 reduced metals could have been continuously produced and consumed by proto-metabolism on the early
6 Earth. The Fe⁰-promoted carbon fixation conditions are mutually compatible with other non-enzymatic
7 transformations, describing a reaction network equivalent to the entire reductive AcCoA pathway, 7 of the
8 11 reactions of the rTCA cycle and amino acid synthesis. The observation that surface-bound pyruvate is
9 not reduced to lactate in the presence of Fe⁰ - though not yet fully understood - opens up new mechanistic
10 possibilities for how further non-enzymatic anabolic reactions might be promoted in the face of potential
11 parasitic off-cycle reactions.⁴² The observed reactivity represents a direct parallel between prebiotic chem-
12 istry and the CO₂-fixing pathways used by primitive autotrophic life, strongly supporting the hypothesis
13 that metabolism originated as prebiotic geochemistry.

14 **Data availability.**

15 The authors declare that the data supporting the findings of this study are available within the paper's Supple-
16 mentary Information files.

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22 tance with NMR experiments.

23 **Additional information**

24 Supplementary information is available for this paper.

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26 **Competing financial interests**

27 The authors declare no competing financial interests.

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