

## Supplementary figures

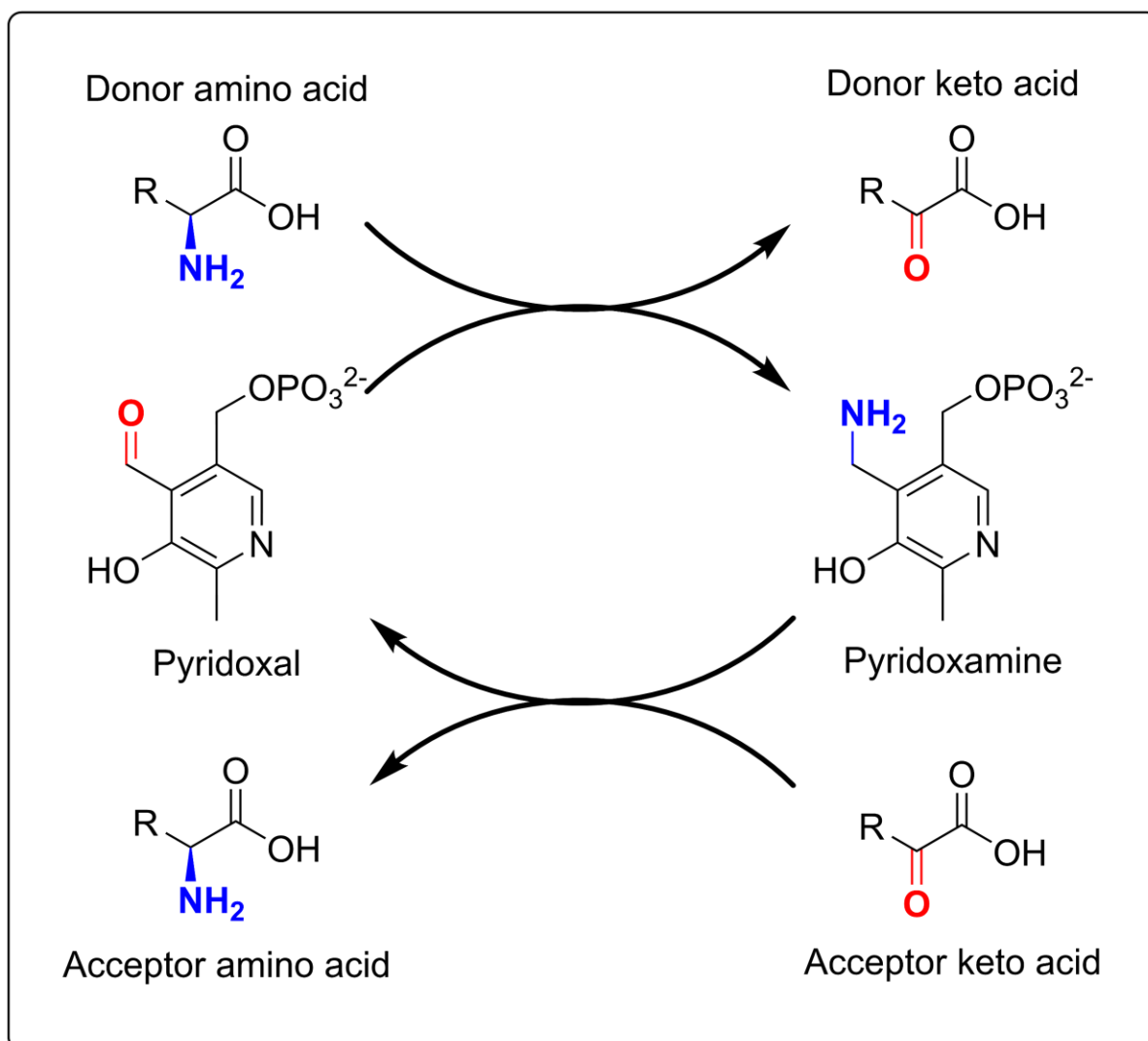
# Prebiotic Synthesis of Aspartate Using Life's Metabolism as a Guide

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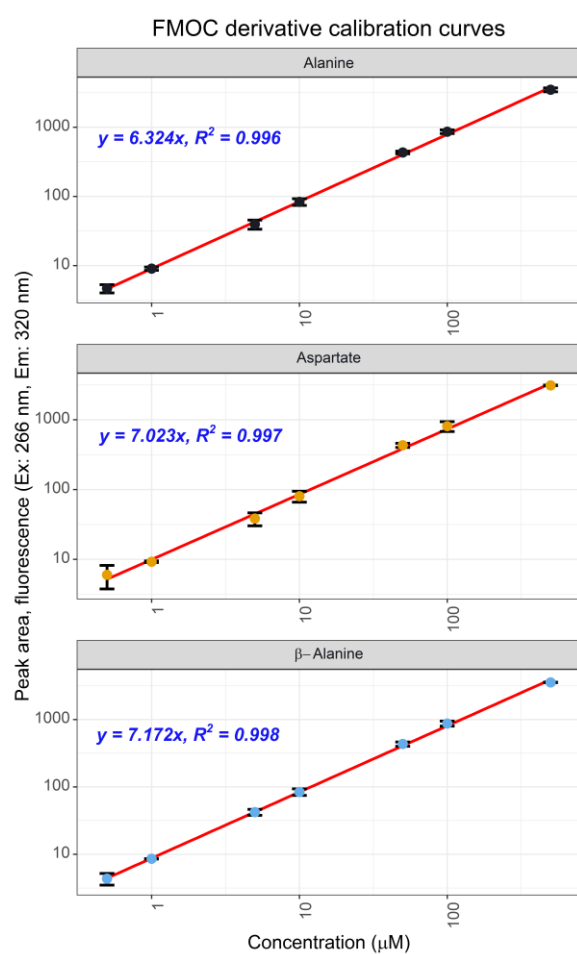
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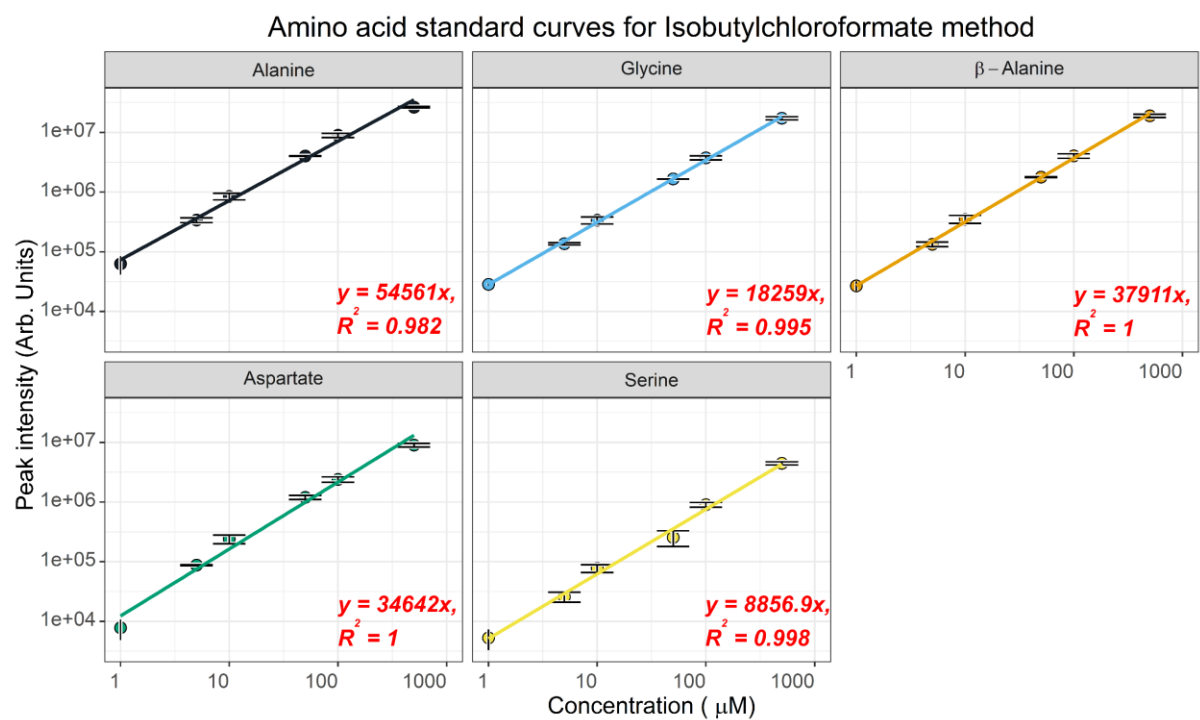
Key words: protometabolism, origins of life, aspartate, oxaloacetate, pyridoxal, metabolism



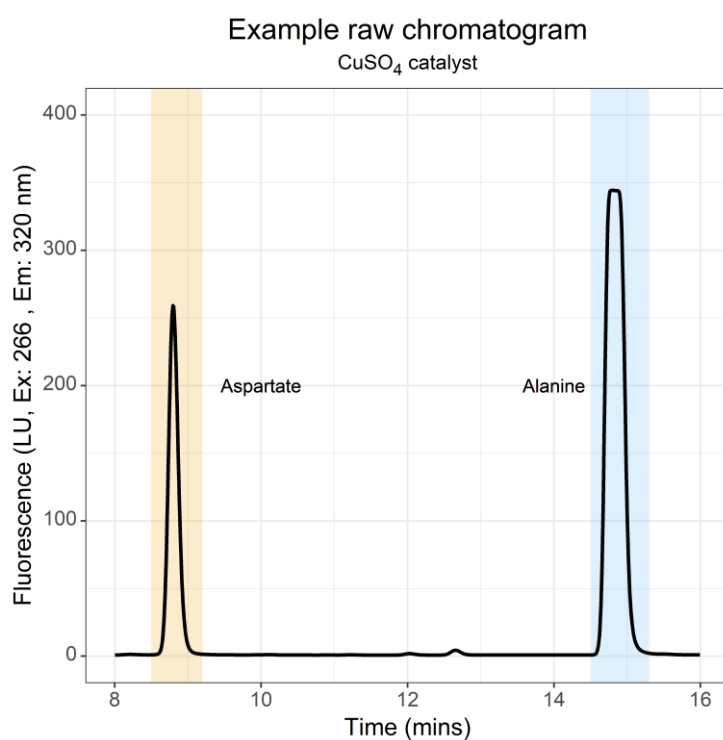
**Figure S1:** Transamination reaction cycle of amino acids with pyridoxal-5-phosphate that are observed in modern metabolism. Amino groups have been coloured blue and  $\alpha$ -keto groups in red to illustrate the reaction process taking place.



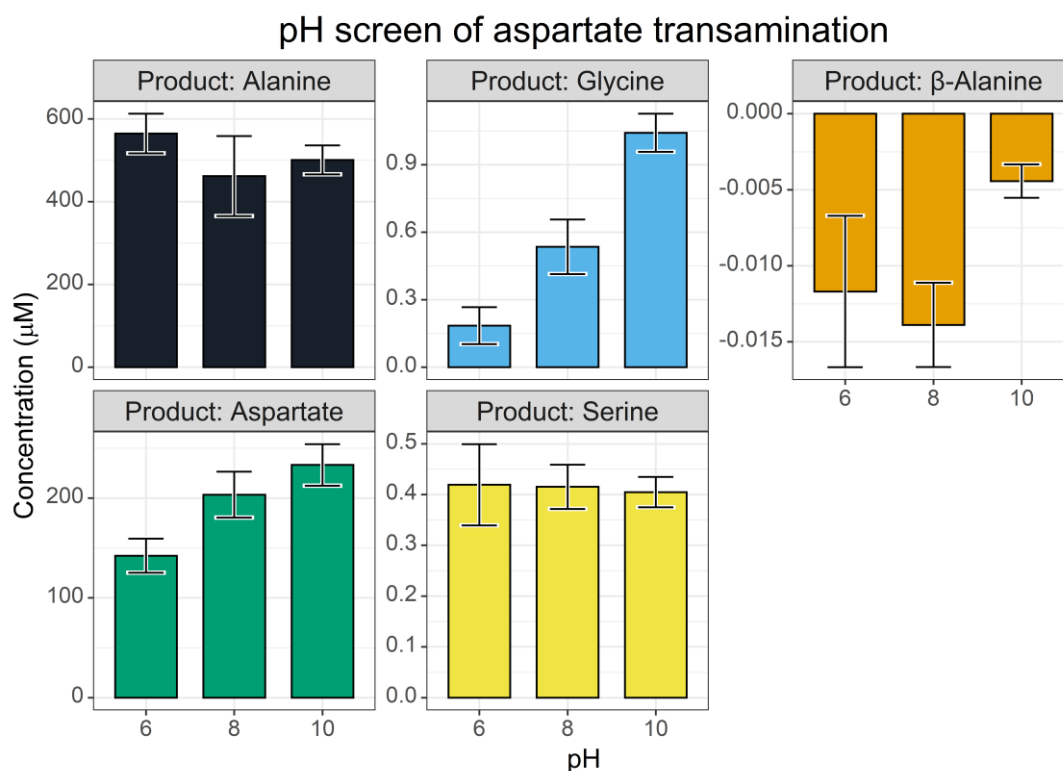
**Figure S2.** FMOC-amino acid calibration curves.  $N = 3 \pm \text{SD}$ .



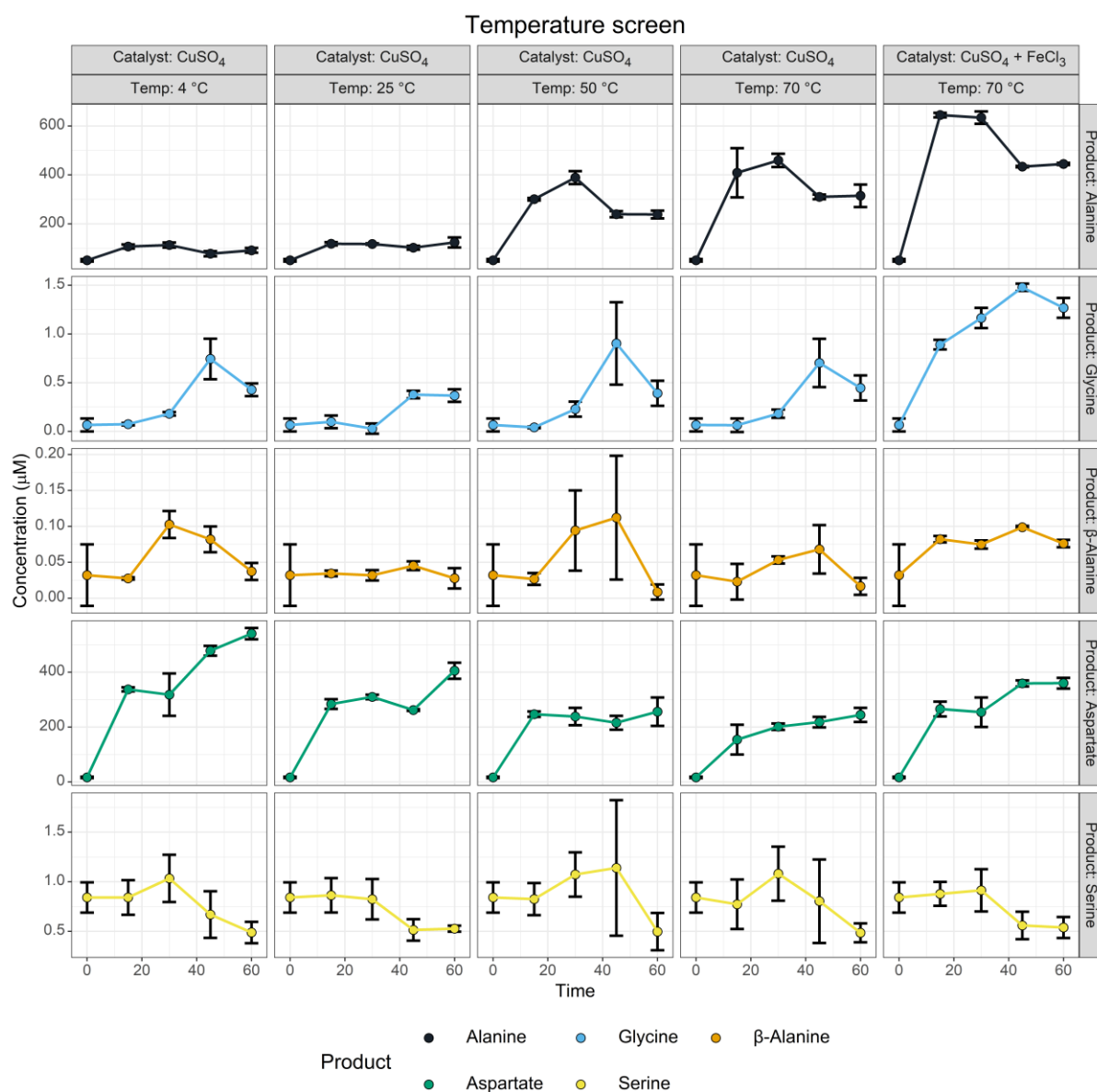
**Figure S3.** iBuCF-amino acid calibration curves for five amino acids.  $N = 3 \pm \text{SD}$ .



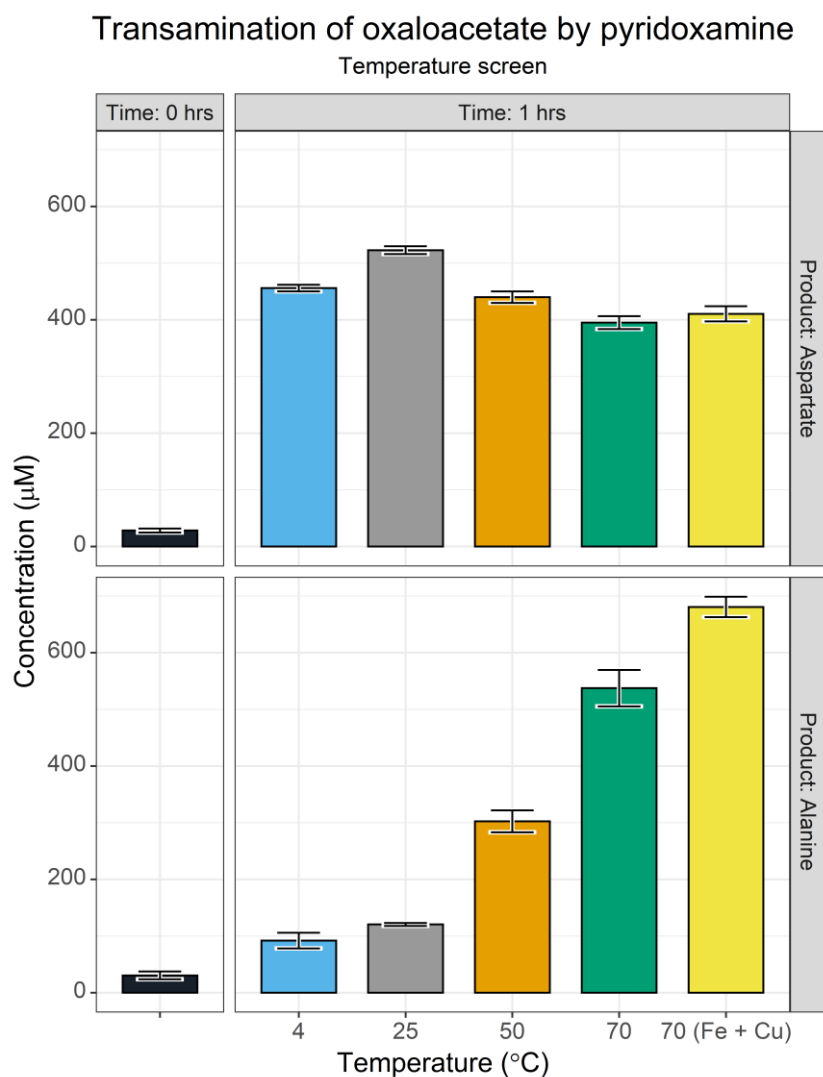
**Figure S4.** An example HPLC chromatogram from the cation screen (see section 3.1.1). The orange bar shows the aspartate-FMOC peak, the blue bar shows the alanine-FMOC peak. The squared peak shape of the alanine peak compared to the aspartate peak indicates likely detector saturation.



**Figure S5.** GC-MS verification for pH screen. Yields of selected amino acids were determined at the 1-hr time point for three different pH values via the iBuCF GC-MS method. Reactions were conducted with 10 mM pyridoxamine, 10 mM oxaloacetate in 50 mM ammonium bicarbonate with 1 mM  $\text{CuSO}_4$  at 70 °C. Concentrations have been normalised relative to a water control.  $N = 3 \pm \text{SD}$ .

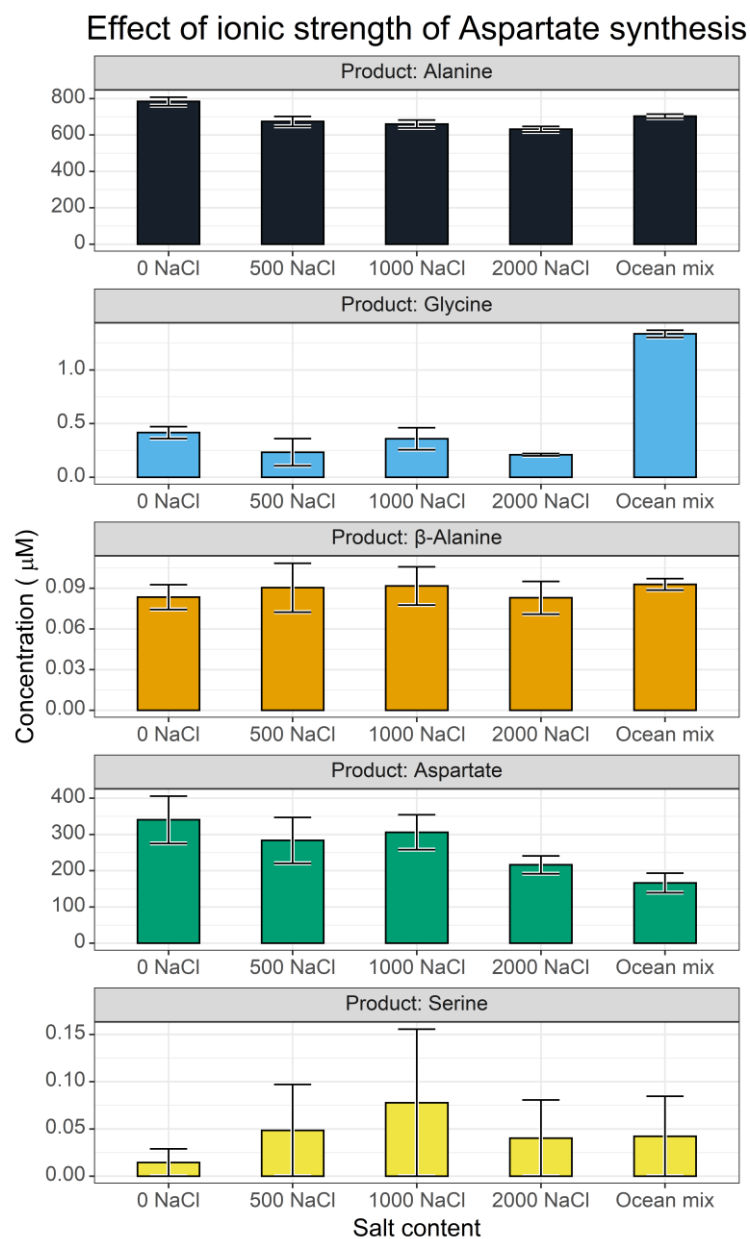


**Figure S6.** Temperature screen in expanded format. Yields of amino acids determined during a 1-hr reaction across five different temperature conditions via the iBuCF GC-MS method. Data are identical to those in Figure 6 but have been expanded to show the changes in amino acids with low yields.  $N = 3 \pm \text{SD}$ .

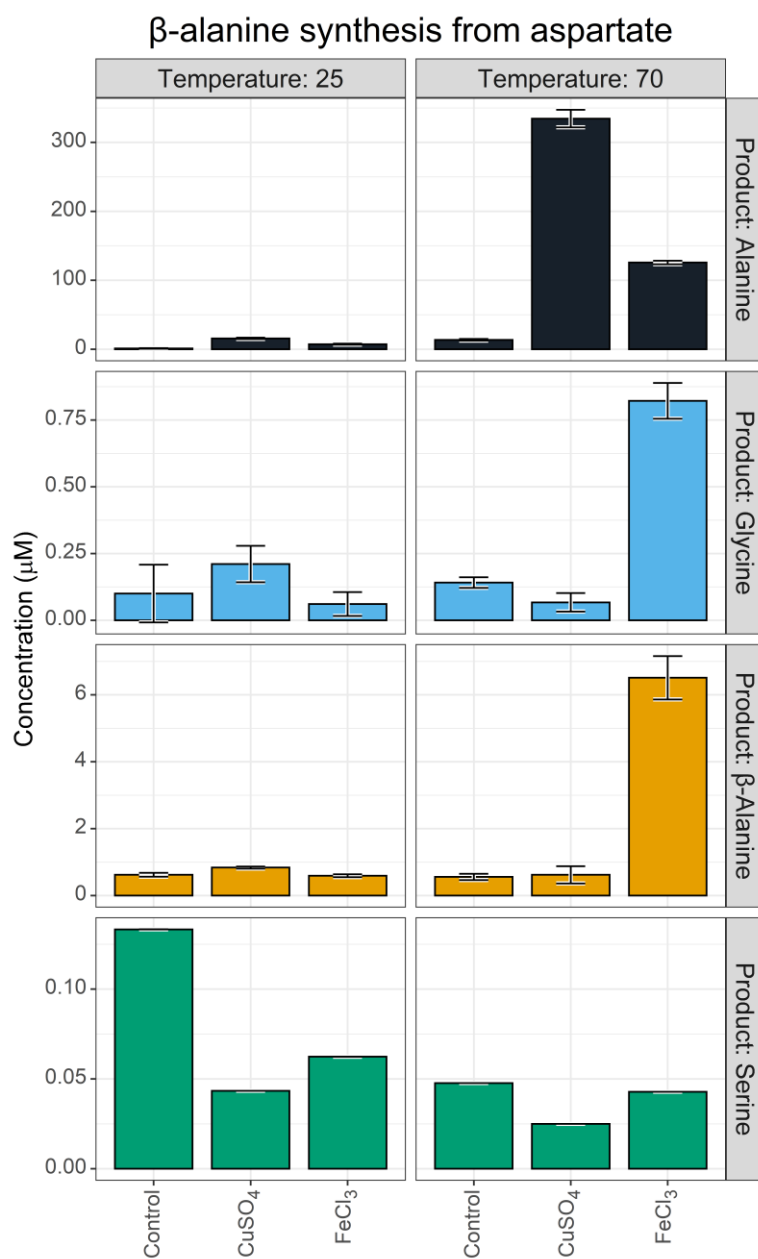


**Figure S7.** HPLC data for the temperature screen. Yields of aspartate and alanine determined during via the FMOc-HPLC method. Reactions were conducted with 10 mM pyridoxamine, 10 mM oxaloacetate in 50 mM ammonium bicarbonate (pH 7.8) with 1 mM  $\text{CuSO}_4$  (with and without 1 mM  $\text{FeCl}_3$ ) as required.  $T = 0$  is common to all temperature screens.  $N = 3 \pm \text{SD}$ .





**Figure S8.** GC-MS verification of the ionic strength screen. Yields of selected amino acids after a 3-hour reaction as determined by the iBuCF GC-MS method. Reactions were conducted with 5 mM pyridoxamine, 5 mM oxaloacetate in 50 mM ammonium bicarbonate (pH 7.8) at 70 °C with 1 mM  $\text{CuSO}_4$ , and the appropriate salt mixture as required.  $N = 3 \pm \text{SD}$ .



**Figure S9.** GC-MS verification of  $\beta$ -alanine synthesis from aspartate. Yields of selected amino acids after a 3-hour reaction as determined by the iBuCF GC-MS method. Reactions were conducted with 10 mM aspartate, 10 mM pyridoxal in 50 mM ammonium bicarbonate (pH 7.8) with 1 mM metal catalyst.  $N = 3 \pm \text{SD}$ .