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Supplementary Materials for

High-throughput mapping of CoA metabolites by SAMDI-MS to optimize the cell-free biosynthesis of HMG-CoA

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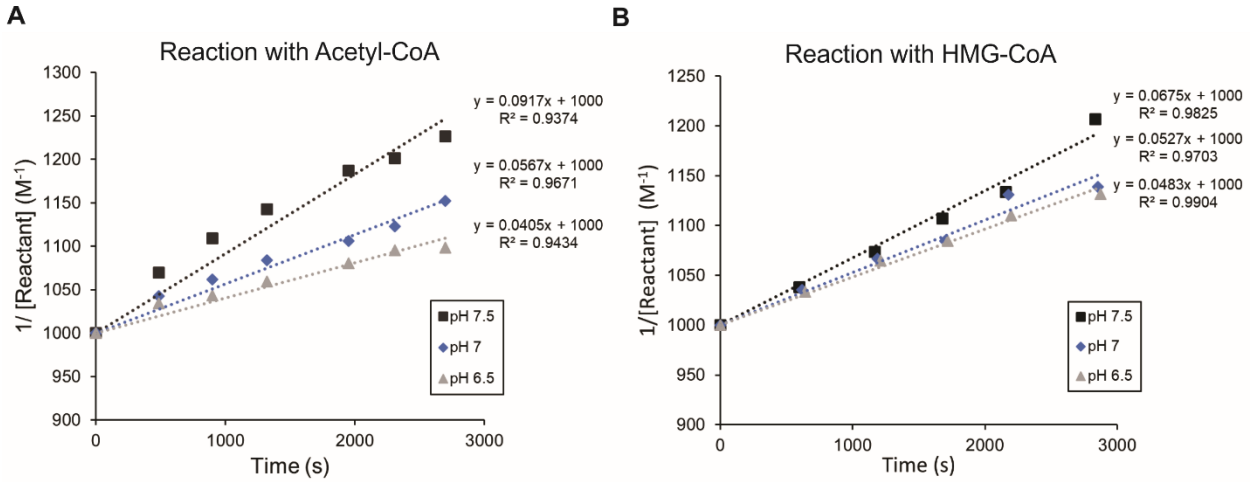

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	2nd Order Rate Constant (M^{-1} s^{-1})	
pH	Acetyl-CoA	HMG-CoA
7.5	0.092	0.068
7.0	0.057	0.053
6.5	0.041	0.048

Fig. S1. Second-order kinetics for the reaction of CoA metabolites with the peptide. The peptide, of sequence CAK(Me_3)SA, was reacted with pure Acetyl-CoA and HMG-CoA (purchased from Sigma Aldrich). For each reaction, 1 mM of peptide was mixed with 1mM of CoA metabolite in buffer (100 mM BisTris, 10 mM Phosphate at the pH values listed). The reactions were performed at a 200 uL scale and incubated at 42°C. Reaction timepoints were obtained by sampling 10 µL of reaction mixture at desired times and spotting it, in triplicate, onto 384 spot array plates presenting a 20% maleimide SAM. The reaction progress at each timepoint was determined by performing SAMDI and analyzing the areas under the curve for the peaks corresponding to the unreacted peptide and ligated product. The yield was then fitted according to a standard second order rate plot for both (**A**) Acetyl-CoA and (**B**) HMG-CoA. The plots adopt linear relationships, where slope gives the second order rate constants of the reactions. (**C**) The table summarizes the experimentally determined rate constants across pH conditions.

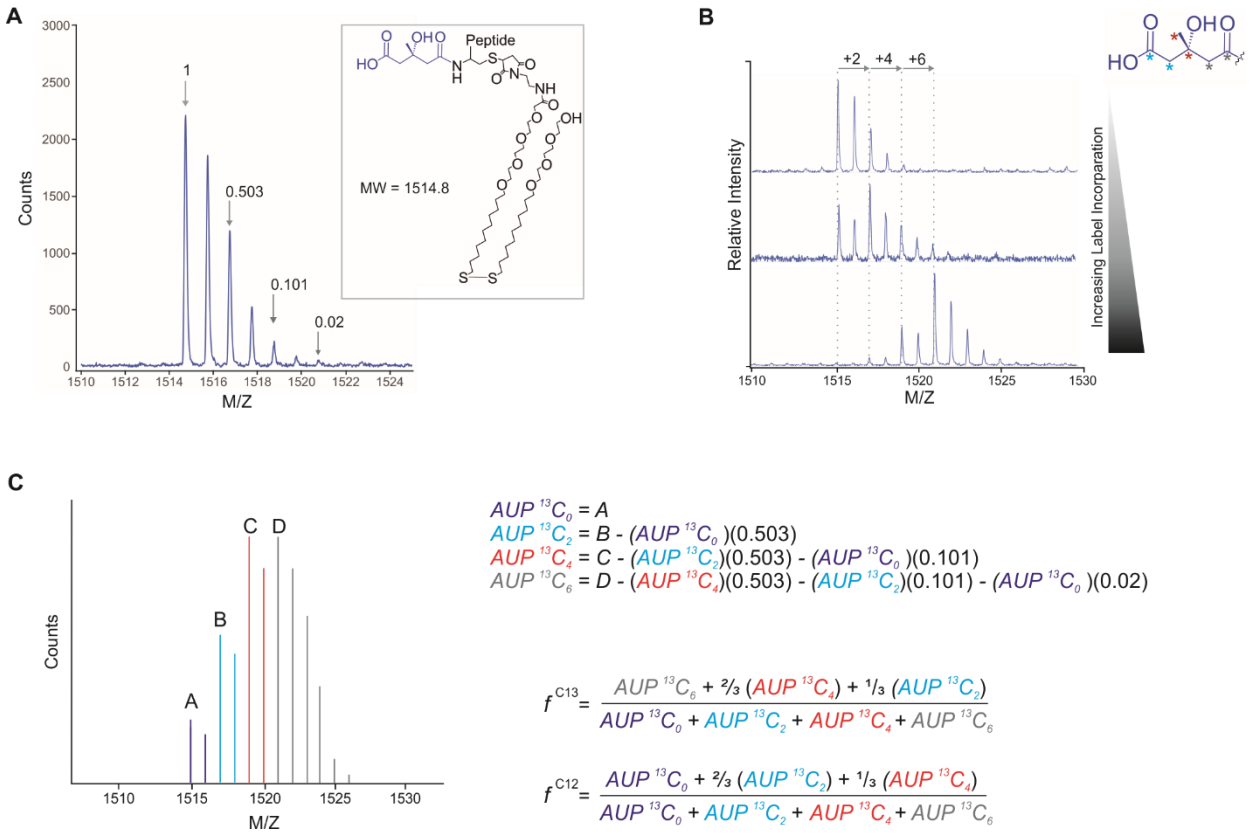

Fig. S2. Description of spectral analysis. (A) In SAMDI mass spectra, the entire monolayer is desorbed by the laser of the mass spectrometer and detected as a disulfide to a molecule of the tri(ethylene)glycol background. For the HMG adduct, this observed product has a M/Z of just under 1515 mass units. The natural isotopic splitting pattern can be calculated or determined experimentally, giving the relative area of each isotopic peak. (**B**) When the cell-free reaction is provided isotopically labeled $^{13}C_6$ glucose, the incorporation of this label is observed as a peak splitting pattern of +2, +4, and +6 mass units, relative to the unlabeled species. This is because a single 6-carbon molecule of HMG-CoA is synthesized from 3x Ac-CoA units containing 2 carbons that are either labeled or unlabeled, depending on the source of the Ac-CoA. The distribution of these possible HMG labeling states depends on the relative concentrations of unlabeled versus $^{13}C_2$ -labeled Ac-CoA in the cell-free reaction. (**C**) The four label states (0, +2, +4, or +6) can each be separately quantified from the convoluted spectra by accounting for the overlap from the natural isotopic splitting of the peaks in front of it. From the integrated area under the peak (AUP) of the four peaks A, B, C, and D, the AUP for each of the labeling states was calculated as shown. The deconvoluted AUP for each of the labeling states was then used to determine the fraction of ^{13}C label, f^{C13}, in the observed HMG product; this was done by weighting the AUP for each labeled state by the relative number of labeled carbons in it, then dividing by the sum of all four states. The fraction of unlabeled carbon in the HMG product, f^{C12} can be calculated from these values as well using a similar equation.

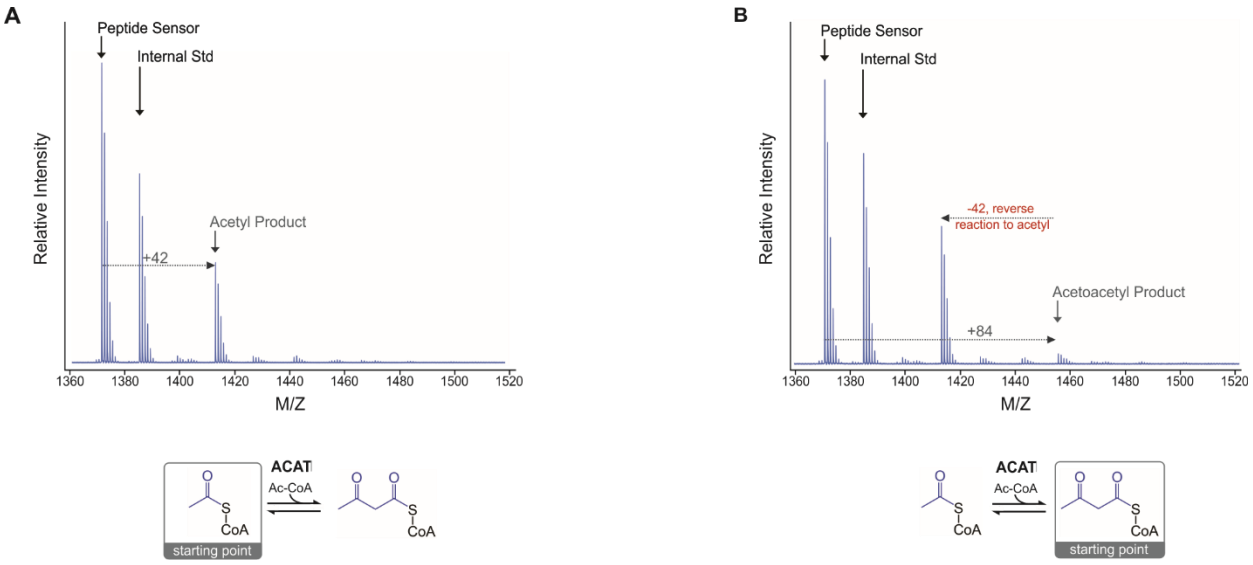

Fig. S3. The pathway intermediate, AA-CoA, is not observed. To explore why we do not observe the AA-CoA pathway intermediate, we incubated 1 mM of Ac-CoA or AA-CoA in cell-free lysate overexpressing the ACAT enzyme for 2 hours. The cell free reactions were given 1 mM each of CoA, NAD^+, and ATP, but no glucose was provided. (**A**) When this cell-free reaction was incubated with Ac-CoA, this acetyl species is the primary species we capture and detect. (**B**) Conversely, when the cell-free lysate is incubated with AA-CoA, a relatively small amount of acetoacetyl species is detected and the acetyl species is by far the primary product. The ACAT enzyme is reversible and these results suggests that it strongly favors the reverse reaction. In the reactions with the full pathway, the small quantities of AA-CoA produced are likely rapidly converted to HMG-CoA, and so we do not observe any significant accumulation of AA-CoA.

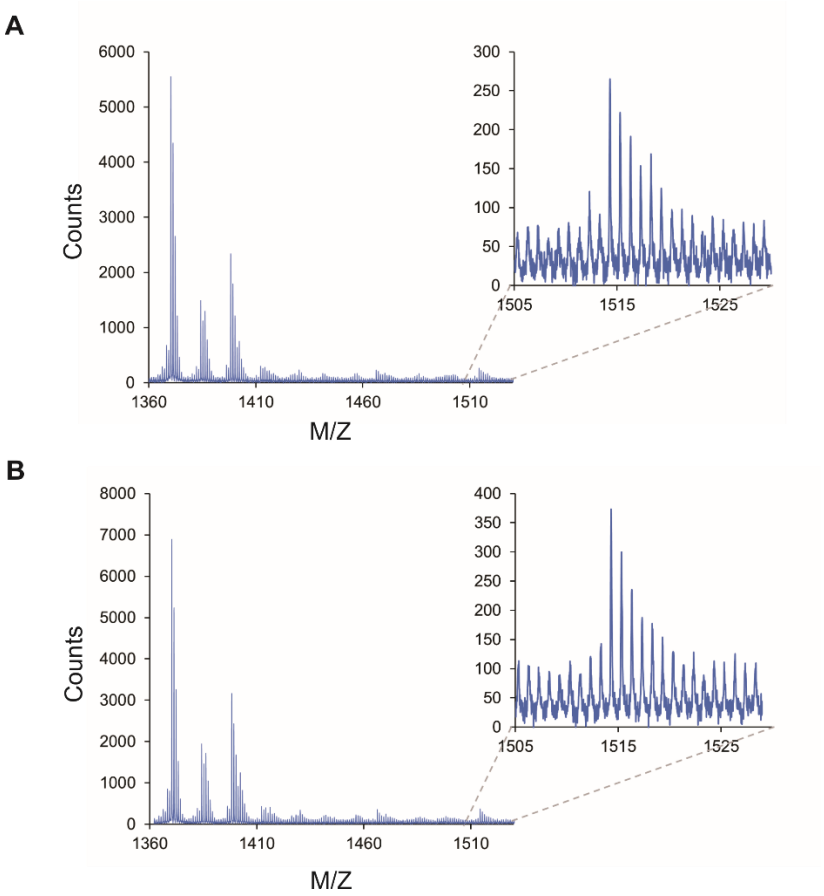

Fig. S4. Limit of detection for HMG-CoA. In order to explore the limit of detection, reactions were performed using known concentrations of HMG-CoA under conditions identical to those used in the large-scale, cell free reaction arrays (0.9 mM peptide and 0.1 mM internal standard, in a BisTris and phosphate buffer system at pH 6.5, incubated at 42° C for 3 hours). Shown here are two spectra corresponding to reactions with HMG-CoA concentrations of (**A)** 10 µM and (**B)** 5 µM. The peak corresponding to the peptide reactant appears at 1371 mass units and the peak corresponding to the captured HMG metabolite appears at 1515 mass units. As shown, the HMG ligated product is detectable at 5 µM. We also attempted a reaction with 1 µM of HMG-CoA, but at this concentration, the ligation product was no longer discernable.