



**Figure S5.** CDH catalyses the production of 5-methyl-2-cyclohexen-1-one. <sup>1</sup>H NMR spectra of 26 nM CDH (red; top trace) and 26 μM cold-denatured CDH (blue; bottom trace) incubated with 1 mM 3-methylcyclohexanone for 1 hour at 25°C. Inset shows detail of feature at c. 7.15 ppm, which corresponds to the proton highlighted in the structure of 5-methyl-2-cyclohexen-1-one. This feature is entirely absent in the cold-denatured spectrum. It has the characteristic ddd ( $J$  (Hz) = 9.93, 5.83, 2.96) shape expected for the 5-methyl isomer and would be absent in the case that CDH catalysed the production of the 3-methyl isomer.