

# Hybrid Molecular/Enzymatic Catalytic Cascade for Complete Electro-oxidation of Glycerol Using a Promiscuous NAD-dependent Formate Dehydrogenase from *Candida boidinii*

*Sofiene Abdellaoui,<sup>a</sup> Madelaine Seow Chavez,<sup>b</sup> Ivana Matanovic,<sup>b,c</sup>  
Andrew R. Stephens,<sup>a</sup> Plamen Atanassov<sup>b</sup> and Shelley D. Minteer<sup>a\*</sup>*

*<sup>a</sup>Departments of Chemistry and Materials Science and Engineering, 315 S 1400 E Room 2020,  
Salt Lake City, UT 84112, USA.*

*<sup>b</sup>The Department of Chemical and Biological Engineering, Center for Micro-Engineered  
Materials (CMEM), University of New Mexico, Albuquerque, NM 87131, USA.*

*<sup>c</sup>Theoretical Division, Los Alamos National Laboratory, Los Alamos, NM 87545, USA*

*E-mail: [minteer@chem.utah.edu](mailto:minteer@chem.utah.edu)*

*Tel : +1(801) 587-8325*

## Materials and methods

### Chemicals

4-amino-TEMPO (free radical), glycerol, sodium mesoxalate monohydrate, sodium oxalate, sodium formate, glycerol- $^{13}\text{C}_3$ , sodium hydroxide and formate dehydrogenase (5.0-15.0 U  $\text{mg}^{-1}$ , E.C. 1.2.1.2) were purchased from Sigma Aldrich.  $\beta$ -Nicotinamide Adenine Dinucleotide ( $\text{NAD}^+$  and NADH) was purchased Research Products Research Corp.

### Enzymatic assays

The enzymatic assays were performed by UV-Vis spectrometry using the Synergy HTX Multi-Mode Reader (BioTek). Enzyme (1 U/mL as final concentration) was added to mixtures containing substrate (formate or mesoxalate) in 100 mM phosphate buffer pH 8.0 in a volume of 200  $\mu\text{L}$ . After 2 minutes, the reactions were quenched with 0.8 mL of  $\text{K}_2\text{HPO}_4$  (pH 9.5) followed by the addition of 5 mM  $\text{NAD}^+$ . The levels of produced formic acid were established in a coupled assay with the addition of 1 U of formate dehydrogenase and the absorbance at 340 nm ( $\epsilon = 6220 \text{ M}^{-1} \text{ cm}^{-1}$ ) was recorded. The formate concentration was quantified by the Michaelis-Menten equation containing  $K_m$  and  $V_m$  of the formate dehydrogenase obtained in the same condition of this essay.

### Electrochemical measurements

Electrochemical experiments were performed using a CH Instruments 611E potentiostat with a standard three electrode cell. Cyclic voltammetry (CV) experiments were performed using a saturated calomel electrode (SCE) reference electrode, a Pt mesh counter electrode, and a 3 mm glassy carbon as a working electrode. CVs were run at  $10 \text{ mV s}^{-1}$  and  $25^\circ \text{C}$  unless otherwise noted. The complete cascade oxidation of  $^{13}\text{C}$ -labeled glycerol was performed using a SCE reference electrode, a Pt mesh auxiliary electrode, and a 1 cm x 1 cm Toray carbon paper as a working electrode. The potential for the glycerol experiments was held at 0.80 V vs SCE at  $25^\circ \text{C}$ .

### $^{13}\text{C}$ -NMR

$^{13}\text{C}$ -NMR analysis was performed on a 400 MHz NMR (Varian Inova 400). Samples were analyzed using  $\text{D}_2\text{O}$  at  $25^\circ \text{C}$ .

## **GC with Thermal Conductivity Detector (GC/TCD)**

Enzymatic reaction was carried out in 500  $\mu$ l reaction volumes in 2 ml GC vials at room temperature under argon atmosphere for 1 h with 1 U/mL cbFDH in the presence of 100 mM substrate (formate or mesoxalate) and 5 mM NAD<sup>+</sup>, in 100 mM phosphate buffer at pH 8.0. Headspace analysis was carried out by manual injection into the GC with a gas-tight syringe. A Trace™ 1310 GC equipped with Carboxen®-1010 PLOT column (30m X 0.32mm) coupled with a TCD was used to detect CO<sub>2</sub>. The column temperature was programmed as follows: 35 °C hold for 2 min then a ramp to 250 °C at 24 °C/min. The injector temperature was 230 °C (10:1 split) and the TCD temperature was set at 230 °C. The carrier gas was helium at a flow rate of 1 mL/min.

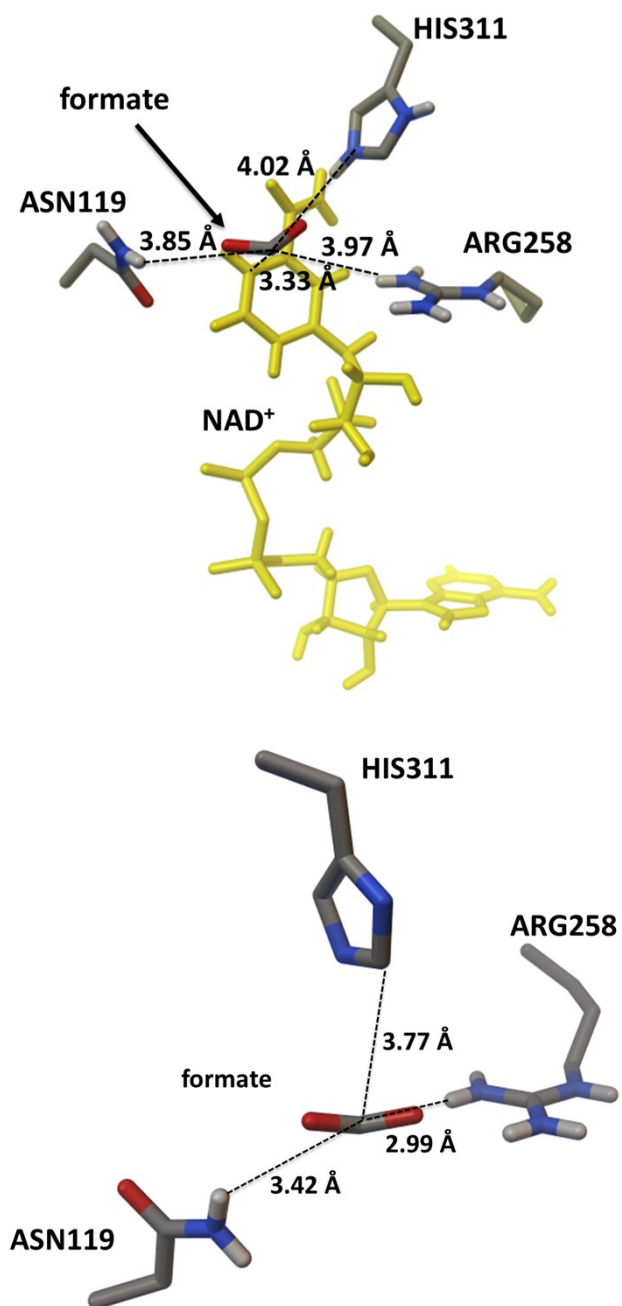
## **Catalytic glycerol cascade**

The 10 hour glycerol oxidation cascade was performed in a sealed container using 8 mM <sup>13</sup>C<sub>3</sub>-glycerol, 5 mM TEMPO-NH<sub>2</sub>, in presence and in absence of 1 U/mL cbFDH with 100 mM phosphate buffer, pH 8.0, at 25° C. The oxidation cascade was performed at a potential of 0.8 V vs SCE for 10 hours. Prior to the experiment, the buffer solution was purged with N<sub>2</sub> to help remove excess CO<sub>2</sub> in the reaction solution. A small canister of NaOH pellets (~100 mg) was suspended above the solution so that the NaOH was exposed to the atmosphere in the headspace of the reaction container without leaking into the solution. The NaOH was used to capture <sup>13</sup>C-enriched CO<sub>2</sub> through the formation of Na<sub>2</sub>CO<sub>3</sub> + H<sub>2</sub>O, which was detected by <sup>13</sup>C-NMR (D<sub>2</sub>O).

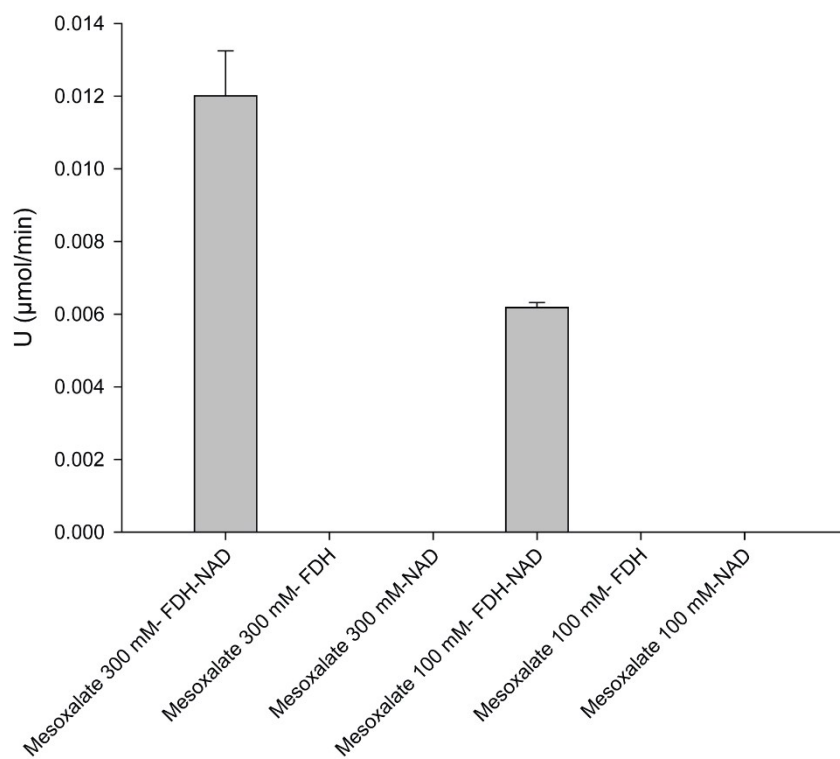
## **Docking simulations**

Molecular docking simulations of formate anion and mesoxalate dianion to cbFDH were performed using AutoDock Vina software. Box of size 16x16x16 Å around HIS311, ASN119, and ARG258 amino acids was set as a search space with HIS311, ASN119, ARG258 set as flexible. Simulations of holo-cbFDH were performed in the presence of NAD<sup>+</sup>, which was treated as rigid. For each case the docking simulations were done a few dozen times with increasing search box size and exhaustiveness of search in order to test the reliability of the results. As each simulation provides up to nine plausible binding positions with a decreasing binding energy, the most frequent model with the lowest binding energy was chosen as the binding site of the substrate and is shown on Figures S1 and 1. The binding models were further visualized and analyzed using AutoDockTools.

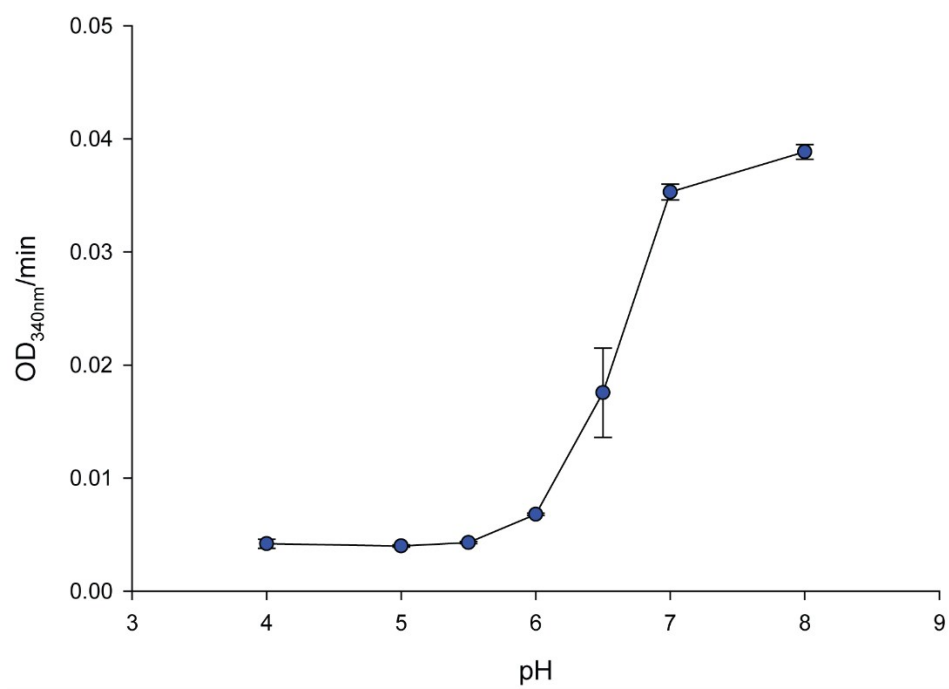
## Supplemental figures



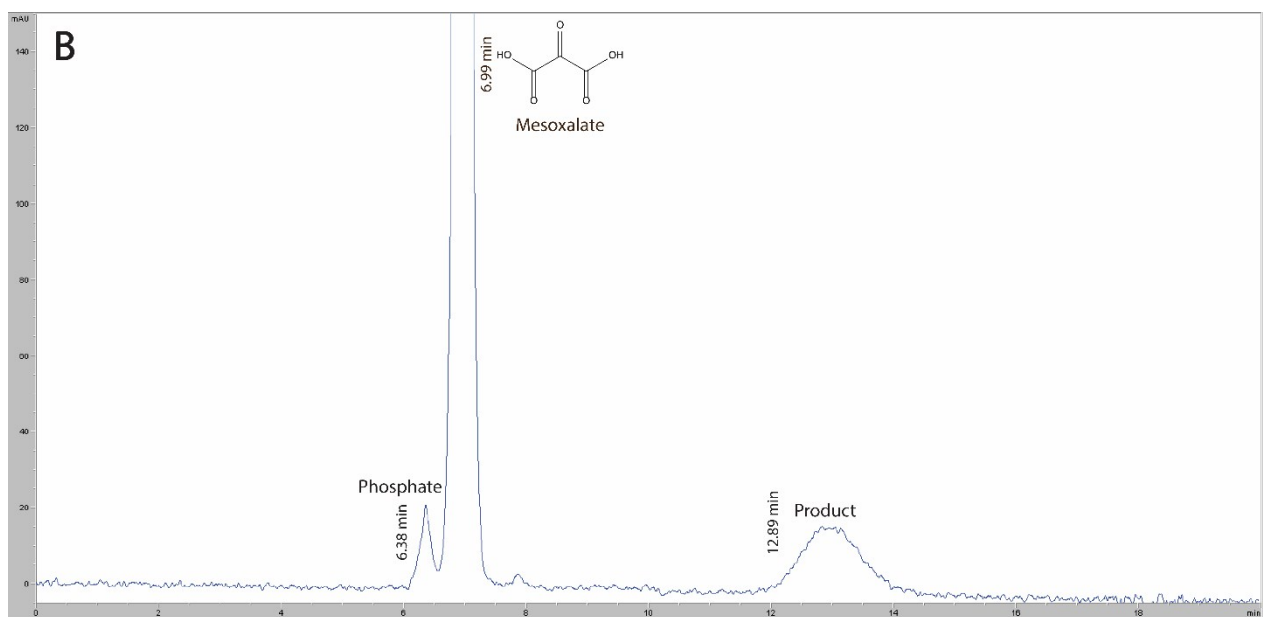
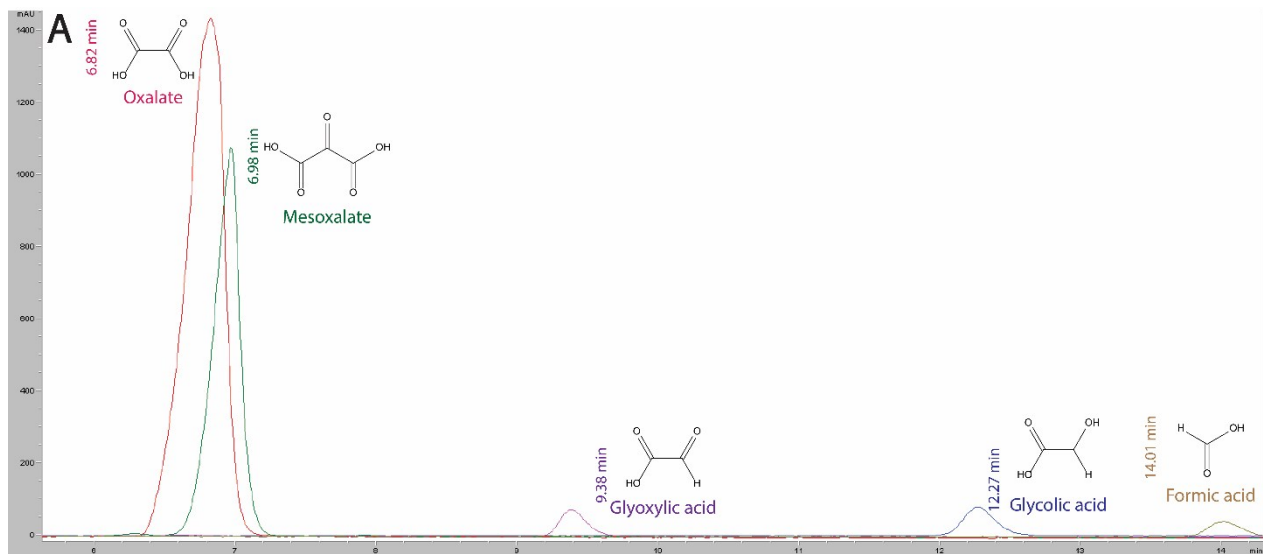
**Fig. S1** Binding of formate to holo (up) and apo (bottom) form of CbFDH as obtained using AutoDock Vina. The binding models were analyzed using AutoDockTools.<sup>1</sup>



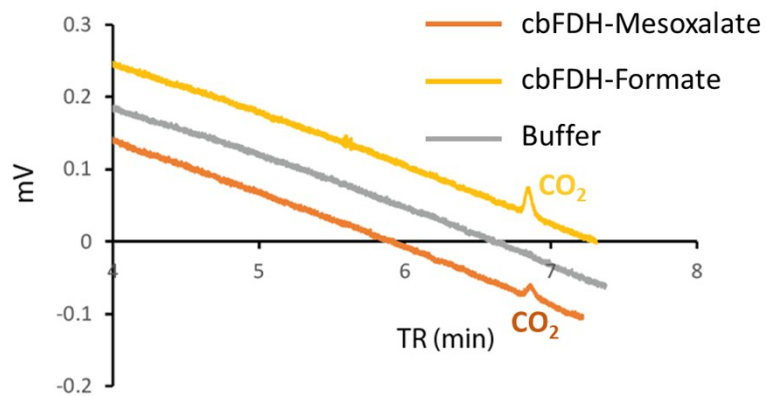
**Fig. S2** Activity controls of cbFDH (1 U/mL) with mesoxalate performed at 25 °C in 0.1 M potassium phosphate buffer, pH 8.0 with 5 mM NAD<sup>+</sup>. All initial velocities were determined in triplicate. Error bars correspond to one standard deviation.



**Fig. S3** Activity of cbFDH (1 U/mL) at pH with mesoxalate performed at 25 °C in 0.1 M citrate-phosphate buffer, with 100 mM mesoxalate and 5 mM NAD<sup>+</sup>. All initial velocities were determined in triplicate. Error bars correspond to one standard deviation.

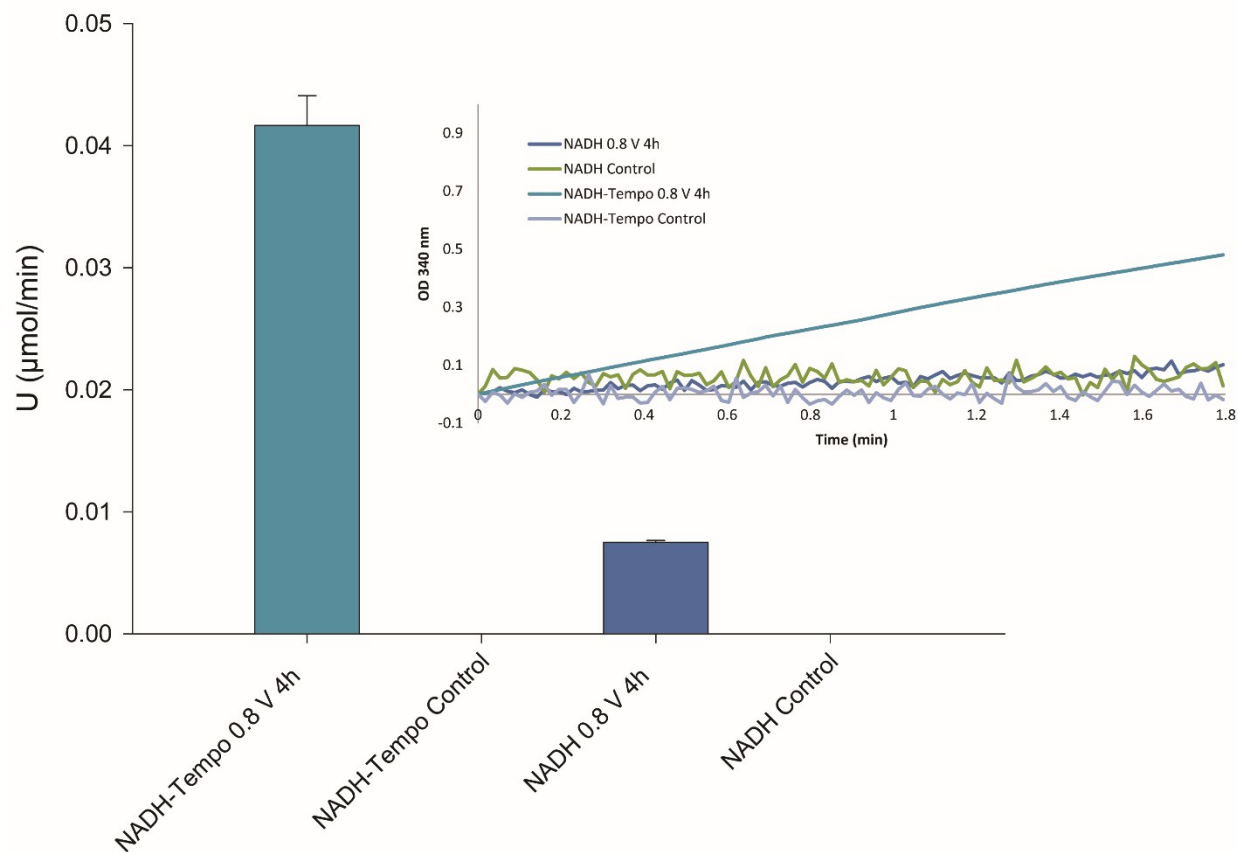


**Fig. S4** HPLC chromatogram of standards at pH 0.8 (A) and sample after reaction with cbFDH (1 U/mL) in the presence of 100mM mesoxalate and 5 mM NAD<sup>+</sup> for 10h at 25 °C in 0.1 M potassium phosphate buffer, pH 8.0.

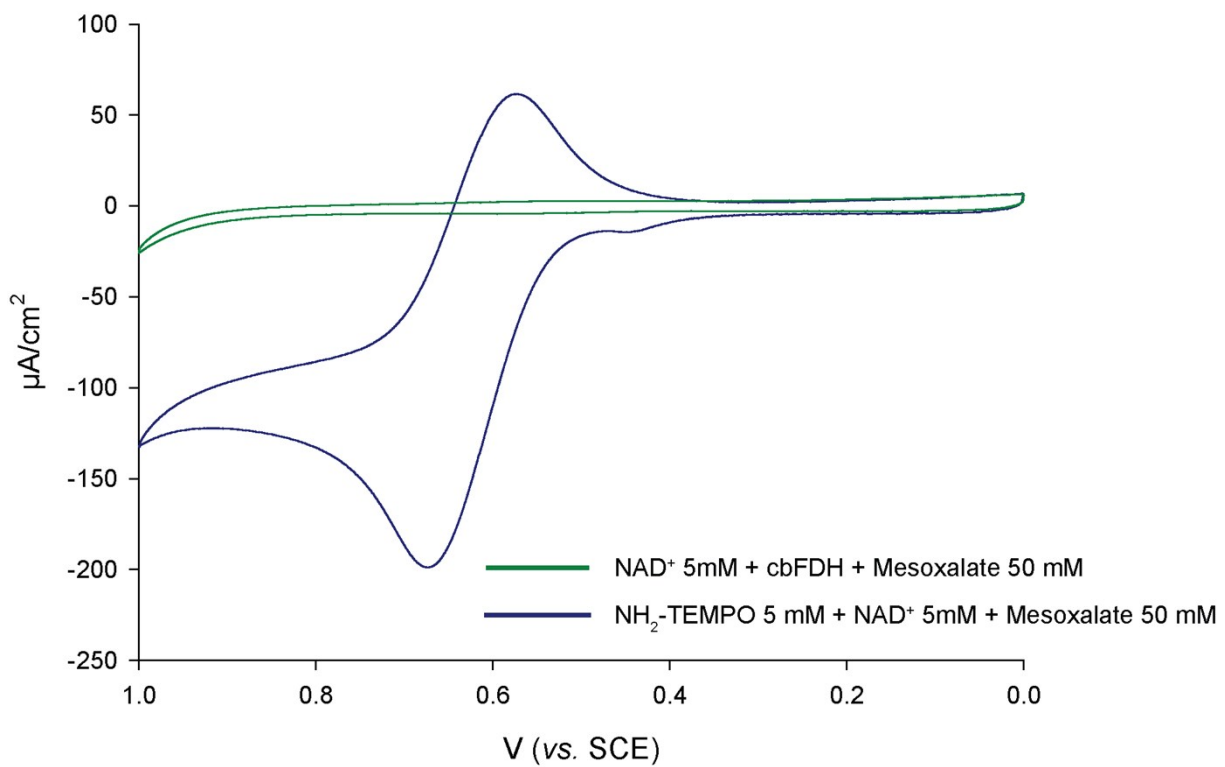


**Fig. S5** GC/TCD chromatograms monitoring the reaction of cbFDH (1 U/mL) with 100 mM formate or mesoxalate in the presence 5 mM NAD<sup>+</sup> in 0.1 M potassium phosphate buffer, pH 8.0, in argon (Ar) atmosphere. Control was performed with buffer saturated with Ar.

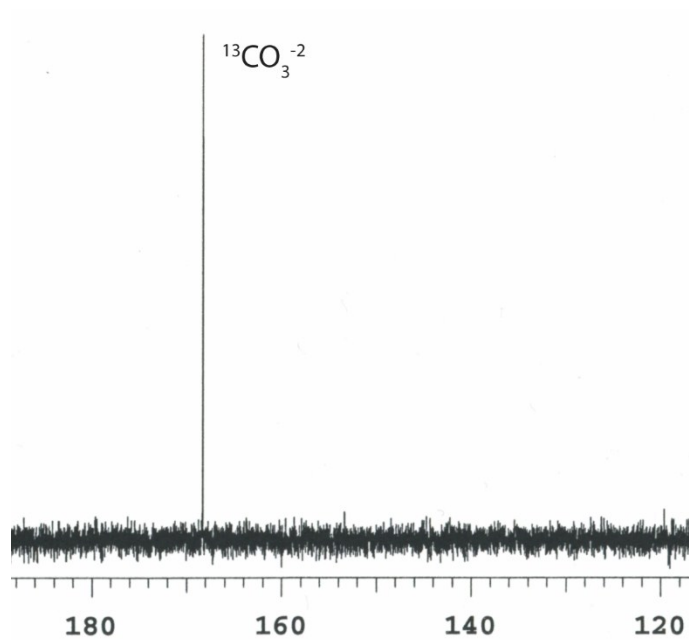




**Fig. S6.** Activity of cFDH (1 U/mL) with 300 mM formate and  $\text{NAD}^+$  produced from the electro-oxidation at 0.8 V (vs. SCE) of 5 mM NADH either directly on the electrode surface or in the presence of 5 mM of  $\text{NH}_2$ -TEMPO at pH 8.0 during 4h and 25 °C. The activity of cbFDH was monitored at 340 nm as shown in the inset. Controls were performed before electro-oxidation. The electro-oxidation was performed by chronoamperometry with a glassy carbon electrode (3 mm diameter), platinumium mesh counter electrode, and SCE reference electrode.



**Fig. S7.** Representative cyclic voltammograms with 50 mM mesoxalate and 5 mM  $\text{NAD}^+$  in presence of cbFDH or  $\text{NH}_2$ -TEMPO. All experiments were performed with a glassy carbon electrode (3 mm diameter), platinum mesh counter electrode, and SCE reference electrode in 100 mM phosphate buffer pH 8.0 at 10 mV/s and 25 °C.



**Fig. S8**  $^{13}\text{C}$ -NMR spectrum of  $^{13}\text{CO}_2$  trapped in the form of  $\text{Na}_2^{13}\text{CO}_3$  from  $^{13}\text{C}$ -labeled glycerol after 10 h of oxidation by 5 mM  $\text{NH}_2$ -TEMPO combined with cbFDH (1 U/mL) at 0.8 V (vs. SCE) in in 0.1 M phosphate buffer, with 5 mM  $\text{NAD}^+$ .

#### References

1. O. Trott and A. J. Olson, *Journal of Computational Chemistry*, 2010, **31**, 455-461.