## Electronic Supplementary Information: Determination of biocatalytic parameters of a copper radical oxidase using real-time reaction progress monitoring

Stephanie M. Forget<sup>1,2,</sup> Roderick (Fan) Xia<sup>1,2</sup>, Jason E. Hein<sup>1\*</sup>, and Harry Brumer<sup>1,2\*</sup>

(1) Department of Chemistry, University of British Columbia, Vancouver, British Columbia V6T 1Z1, Canada;

(2) Michael Smith Laboratories, University of British Columbia, Vancouver, British Columbia V6T 1Z4, Canad

\*H. Brumer (hbrumer@msl.ubc.ca) and J.E. Hein (Jhein@chem.ubc.ca)



**Figure S1.** Alternate methods of introducing  $O_2$  into reaction; addition of 6 mM (red circles) or 1.5 mM (black squares)  $H_2O_2$ ; bubbling with air (blue triangles); manual agitation with micropipette (purple triangles).



Figure S2. Michaelis-Menten curves obtained for AlcOx substrates (A) BnOH; (B) CinOH



**Figure S3.** Competition experiment between AlcOx substrates BnOH (blue triangles) and CinOH (black squares). Oxidation products are PhCHO (pink triangles) and cinnamaldehyde (red circles). Despite the lack of HRP in the reaction, complete conversion was obtained nonetheless, due to a high *Cgr*AlcOx loading compared with other reactions in this study.



**Figure S4.** The effect of pH on AlcOx reaction profiles for 1 mM BnOH with 1.2  $\mu$ M catalase and 15 mM H<sub>2</sub>O, and 17.8  $\mu$ M HRP (50 mM NaP<sub>i</sub> buffer).



**Figure S5.** Reaction profiles curves at various AlcOx concentrations (**A**) raw data (**B**) time normalized data with rate order set to 1. Reaction conditions: 5mM BnOH, 1.2  $\mu$ M Cat, 17.8  $\mu$ M HRP, 9.5-36 nM AlcOx, 8 mM H<sub>2</sub>O<sub>2</sub>, 50 mM NaP<sub>i</sub>, pH 7.



**Figure S6.**  $H_2O_2$  doing experiment at (**A**) 5 mM BnOH with addition of 15 mM  $H_2O_2$  at 120 minutes; (**B**) New reaction initiated at 2.7 mM BnOH compared to reaction progress obtained after spiking (yellow trace is time and concentration shifted from (**A**) for comparison).



**Figure S7.** Reaction profiles curves at various BnOH concentrations (**A**) raw data, (**B**) time normalized data with rate order set to 0.5, (**C**) pairwise VTNA analysis showing rate orders approaching saturation as the

concentration of substrate increases. Reaction conditions: 1-10 mM BnOH, 1.2  $\mu$ M Cat, 17.8  $\mu$ M HRP, 9.5 nM AlcOx, 8 mM H<sub>2</sub>O<sub>2</sub>, 50 mM NaP<sub>i</sub>, pH 7.



Figure S8. Small molecule HRP substrates tested as activator (A) acetylsalicylic acid; (B) phenol; (C) guaicol; (D) pyruvic acid.



**Figure S9.** Reaction progress curves for 1 mM BnOH with 9.5 nM AlcOx, 1.2  $\mu$ M Cat, 15 mM H<sub>2</sub>O<sub>2</sub> with (**A**) various K<sub>3</sub>Fe(CN)<sub>6</sub> concentrations (1-10 mM) and/or 17.8  $\Box$ M HRP as indicated in the legend; (**B**) Optimal product conversions are obtained when both H<sub>2</sub>O<sub>2</sub> and Cat are included in the assays with K<sub>3</sub>Fe(CN)<sub>6</sub>.



**Figure S10.** Reaction progress for BnOH oxidation by AlcOx obtained at various concentrations of  $K_3Fe(CN)_6$  for VTNA (**A**) raw data; (**B**) transformed data, (**C**) pairwise VTNA analysis. Reaction conditions: 1-20 mM  $K_3Fe(CN)_6$ , 5 mM BnOH, 1.2  $\mu$ M Cat, 9.5 nM AlcOx, 8 mM  $H_2O_2$ , 50 mM NaP<sub>i</sub>, pH 7.



**Figure S11.** Effect of butyraldehyde addition on reaction progress on the oxidation of 0.5 mM BnOH under the indicated conditions. The red curve shows the effect of the addition of 0.5 mM butyraldehyde at 13 minutes.



**Figure S12.** Irreversible inhibition experiment with various concentrations of butyraldehyde and various incubation times, as indicated.



**Figure S13.** Initial rate kinetics using HRP-ABTS assay for 1 mM BnOH oxidation indubated with butyraldehyde at the indicated concentrations: 0 mM (\_\_\_\_\_); 0.28 mM (\_\_\_\_\_); 1.4 mM (\_\_\_\_\_); 2.8 mM (\_\_\_\_\_); 4.2 mM (\_\_\_\_\_); 5.6 mM (\_\_\_\_\_); 8.4 mM (\_\_\_\_\_). B. Slopes extracted from the curves in panel A from 0 to 0.5 minutes (\_\_\_\_) and from 3.5 to 4 minutes (\_\_\_\_); all slopes were converted into s<sup>-1</sup> and were plotted against the concentration of butyraldehyde.