

## SUPPORTING INFORMATION

# Identification of the Oxygen Activation Site in Monomeric Sarcosine Oxidase: Role of Lys265 in Catalysis†

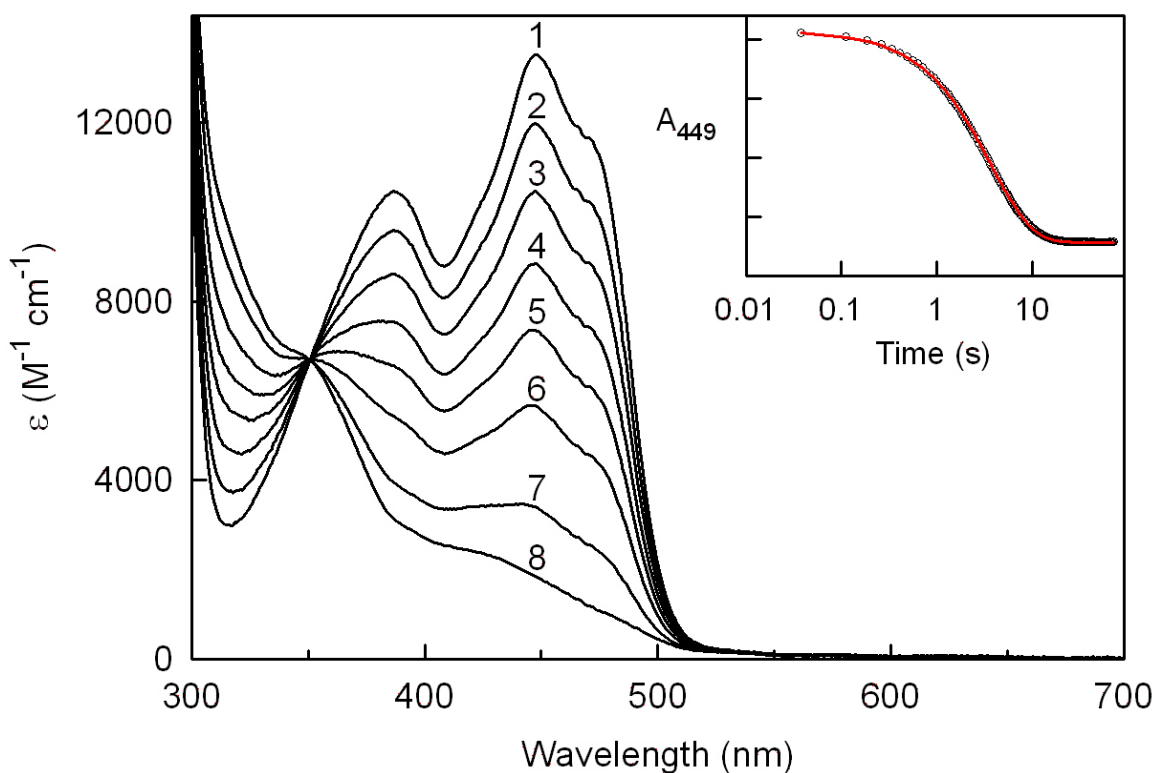
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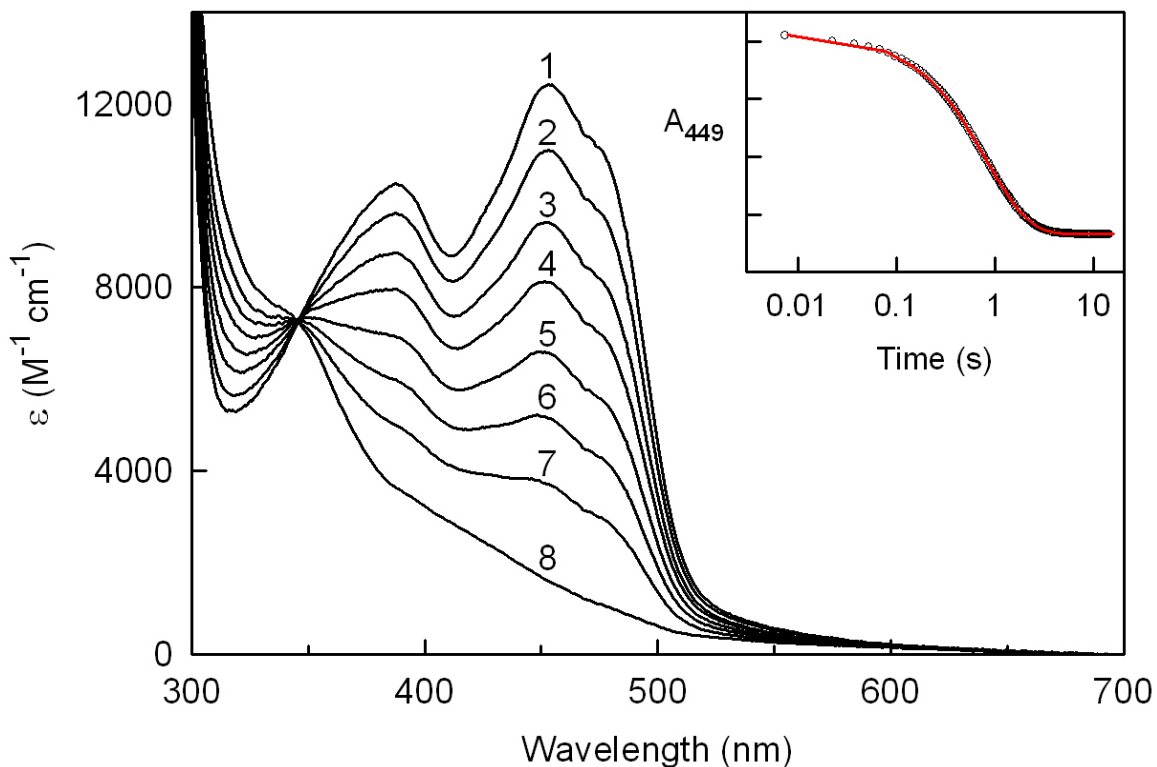
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Running Title: Oxygen Activation Site in Monomeric Sarcosine Oxidase



**Figure S1** Anaerobic reaction of Lys265Met with unlabeled 1.0 mM sarcosine monitored by stopped-flow diode array spectroscopy. Curves 1 to 8 were obtained from 37.5 ms to 34.95 s after mixing the enzyme with 1.0 mM sarcosine in 100 mM potassium phosphate buffer, pH 8.0, containing 50 mM glucose and glucose oxidase (14.7 U/mL) at 25 °C. The inset shows a plot of the observed absorbance decrease at 449 nm. The red line was obtained by fitting a single exponential equation ( $y = Ae^{-kt} + B$ ) to the data (open circles).



**Figure S2** Anaerobic reaction of Lys265Met with 140 mM deuteriosarcosine monitored by stopped-flow diode array spectroscopy. Curves 1 to 8 were obtained from 7.49 ms to 14.25 s after mixing the enzyme with 140 mM [N-methyl-D<sub>3</sub>]-sarcosine in 100 mM potassium phosphate buffer, pH 8.0, containing 50 mM glucose and glucose oxidase (14.7 U/mL) at 25 °C. The inset shows a plot of the observed absorbance decrease at 449 nm. The red line was obtained by fitting a single exponential equation ( $y = Ae^{-kt} + B$ ) to the data (open circles).