Supporting Information

Fundamental Kinetic and Selectivity Properties of the Anti-Aging, Antioxidant Active Ingredient EUK-134

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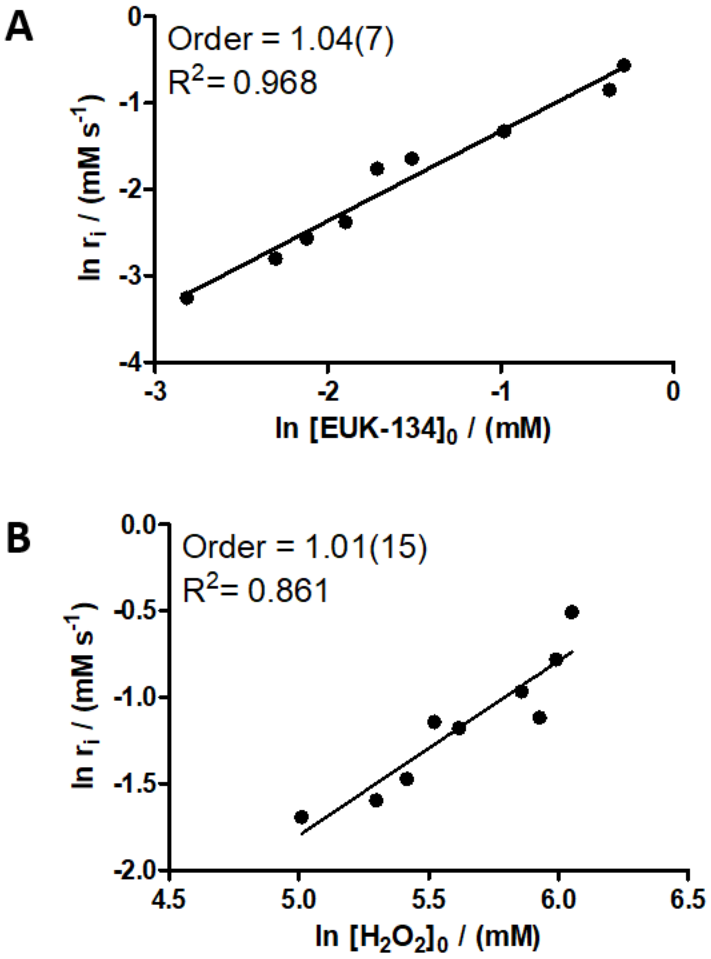

Figure S1. (A) Order with respect to EUK-134. [H_2O_2] was held constant at 150 mM. (y = 1.04x - 0.290). (B) Plots used to determine the order of the reaction with respect to H_2O_2. [EUK-134] was held constant at 0.2475 mM. (y = 1.01x - 6.84). For both: [Trizma] = 50 mM, pH = 8.1.

Table S1. Initial rates obtained for EUK-134 at a range of concentrations and [H_2O_2] at 150 mM, resulting in an order of 1.04 with respect to EUK-134. Conditions are detailed in Figure S1.

[EUK-134] (mM)	**r_i (mM s^{-1})**	**ln[EUK-134] (mM)**	**ln(r_i) (mM s^{-1})**
0.06	0.039	-2.81	-3.25
0.10	0.061	-2.30	-2.80
0.12	0.077	-2.12	-2.57
0.15	0.093	-1.90	-2.38
0.18	0.17	-1.71	-1.76
0.22	0.19	-1.51	-1.64
0.38	0.27	-0.98	-1.33
0.69	0.43	-0.37	-0.85
0.75	0.57	-0.29	-0.57

Table S2. Initial rates obtained for EUK-134 at 0.2475 mM and H_2O_2 varied, resulting in an order of 1.01 with respect to EUK-134. Conditions are detailed in Figure S1.

[H_2O_2] (mM)	**r_i (mM s^{-1})**	**ln[H_2O_2] (mM)**	**ln(r_i) (mM s^{-1})**
150	0.18	5.01	-1.69
200	0.20	5.30	-1.60
225	0.23	5.42	-1.47
275	0.32	5.52	-1.14
250	0.31	5.62	-1.18
375	0.38	5.86	-0.97
350	0.33	5.93	-1.12
400	0.46	5.99	-0.78
425	0.60	6.05	-0.51

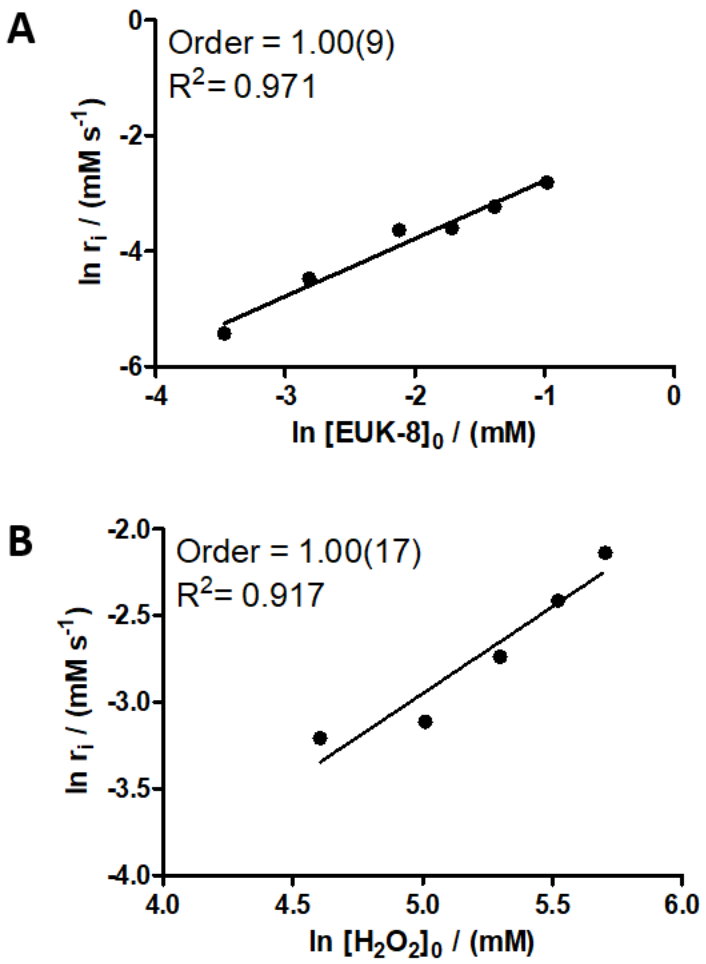

Figure S2. (A) Order with respect to EUK-8. [H_2O_2] was held constant at 150 mM. (y = 1.00x - 1.78) (B) Plots used to determine the order of the reaction with respect to H_2O_2. [EUK-8] was held constant at 0.2475 mM. (y = 1.0022x - 7.9612). For both: [Trizma] = 50 mM, pH = 8.1.

Table S3. Initial rates obtained for EUK-8 at a range of concentrations and [H_2O_2] at 150 mM, resulting in an order of 1.00 with respect to EUK-8. Conditions are detailed in Figure S2.

[EUK-8] (mM)	**r_i (mM s^{-1})**	**ln[EUK-8] (mM)**	**ln(r_i) (mM s^{-1})**
0.031	0.0044	-3.47	-5.43
0.060	0.011	-2.81	-4.48
0.120	0.021	-2.12	-3.85
0.180	0.026	-1.71	-3.65
0.250	0.039	-1.39	-3.23
0.375	0.062	-0.98	-2.79

Table S4. Initial rates obtained for EUK-8 at 0.2475 mM and H_2O_2 varied, resulting in an order of 1.00 with respect to EUK-8. Conditions are detailed in Figure S2.

[H_2O_2] (mM)	**r_i (mM s^{-1})**	**ln[H_2O_2] (mM)**	**ln(r_i) (mM s^{-1})**
100	0.040	4.61	-3.21
150	0.044	5.01	-3.11
200	0.065	5.30	-2.74
250	0.089	5.52	-2.41
300	0.12	5.70	-2.14

Table S5. EUK-134 catalase activity after multiple aliquots. [H_2O_2] = 150 mM, [Trizma] = 50 mM, pH = 8.1.

EUK-134	**1st aliquot H_2O_2**		
	TON	**TOF (s^{-1})**	**R_i (mM s^{-1})**
T1	27.44	0.18	0.61
T2	19.49	0.13	0.39
T3	25.08	0.17	0.45
Average	24.00	0.16	0.48
Standard Deviation	4.08	0.03	0.11
	2nd aliquot H_2O_2		
T1	30.84	0.21	0.44
T2	31.45	0.21	0.42
T3	29.30	0.20	0.47
Average	30.53	0.20	0.45
Standard Deviation	1.11	0.01	0.02
	3rd aliquot H_2O_2		
T1	20.62	0.10	0.19
T2	21.06	0.11	0.29
T3	20.56	0.10	0.30
Average	20.75	0.104	0.26
Standard Deviation	0.28	0.001	0.06

Table S6. EUK-8 catalase activity after multiple aliquots. [H_2O_2] = 150 mM, [Trizma] = 50 mM, pH = 8.1.

EUK-8			
Trial	**TON**	**TOF (s^{-1})**	**Rate (mM s^{-1})**
1	18.75	0.13	0.36
2	11.14	0.10	0.25
3	11.36	0.08	0.44
4	16.34	0.11	0.38
5	18.97	0.13	0.44
6	16.32	0.11	0.42
Average	15.48	0.11	0.42
Standard Deviation	3.47	0.018	0.03

Table S7. Selectivity of EUK-134 for catalase vs peroxidase activity. Catalase assay conditions: 20 µM EUK-R, 20 mM H_2O_2 (PBS, pH 7.4); Peroxidase assay conditions: 20 µM EUK-R, 5 µM H_2O_2, 100 µM ABTS (PBS, pH 7.4).

Trial	TON Catalase	TON Peroxidase	TOF Catalase	TOF Peroxidase	C/P
1	112.39	0.443	1.56×10^{-2}	6.15×10^{-5}	2.54×10^{2}
2	145.41	0.518	2.02×10^{-2}	7.19×10^{-5}	2.81×10^{2}
3	120.30	0.430	1.67×10^{-2}	5.97×10^{-5}	2.80×10^{2}
Average	126.03	0.46	0.018	6.44×10^{-5}	272
Standard Deviation	17.24	0.05	0.002	6.6×10^{-6}	15

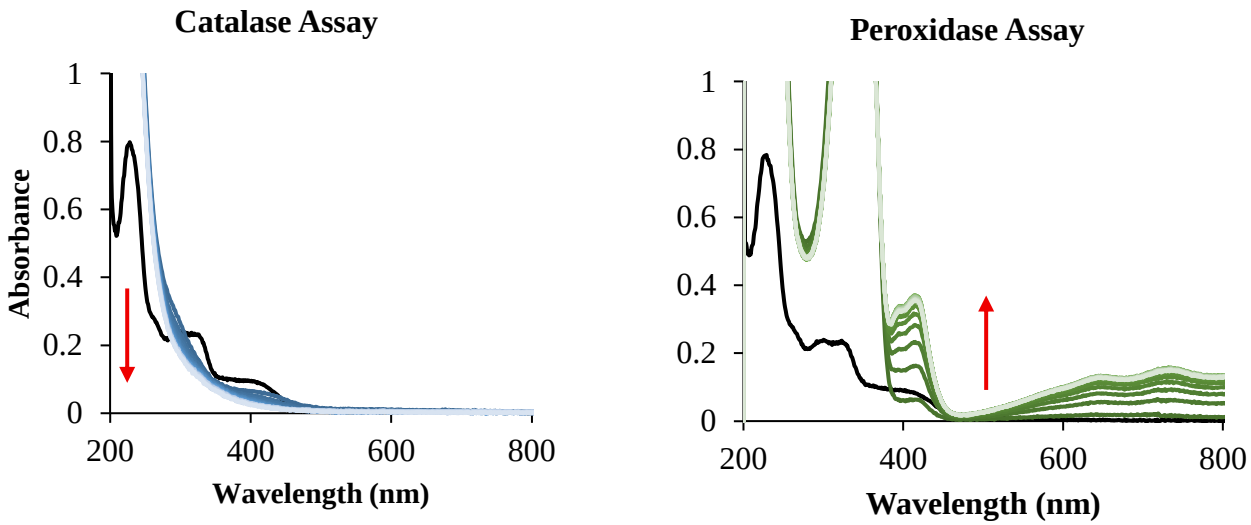

Figure S3. Example of UV-vis spectra from (Left) Catalase assay: 20 µM EUK-134 upon addition of 20 mM H_2O_2 and (right) Peroxidase assay: 20 mM EUK-134, 100 mM ABTS, 5 mM H_2O_2. For both: in phosphate buffer solution, pH 7.4.

Experimental Methods

Quantifying Catalase Activity for Efficiency and Robustness Studies. Molecular oxygen evolution from the decomposition of H_2O_2 was measured via an O_2 microsensor probe (UniSense, Denmark) placed in a hermetically sealed 15 mL reactor. In each experiment, 1.5 mL of a 1.5 mM catalyst solution in 50 mM tris(hydroxymethyl)aminomethane (Tris) buffer (pH 8.1)

was added to the reactor. For calibration, room pressure was set at 159 mmHg then the cell was flushed with N_2 for 30 s until pressure reached 0 mV. Subsequently, 0.5 mL of 150 mM H_2O_2 was introduced, and measurements were conducted at 298 K. The reaction was recorded ΔP_{O_2} (mmHg) vs. time (seconds) until complete saturation occurred (V_{max}). The data were collected at 0.2 s intervals and the baseline-O_2 concentration, which was calculated from readings obtained 30 s before the initiation of the reaction was subtracted from all initial values.

Using the ideal gas law, ΔP_{O_2} was used to calculate the number of O_2 moles L^{-1} produced from the headspace of the reactor (13 mL) via equation **1**:

$$PV = nRT \quad \textbf{(1)}$$

P = pressure (atm), R = 0.08206 atm L $mol^{-1}K^{-1}$, n = millimoles (mM), T = 298 K and V = 0.013 L.

The TON was calculated for 1.5 mM EUK-134 (the total solution volume after H_2O_2 addition was 2 mL) via equation 2 for the total reaction time:

$$TON = \frac{moles\ of\ O_2\ produced}{moles\ of\ Catalyst} \quad \textbf{(2)}$$

The TOF was determined via equation **3**:

$$TOF(s^{-1}) = \frac{\text{TON}}{reaction\ time\ (s)} \quad \textbf{(3)}$$

To determine whether complex decomposition resulted from reactions with H_2O_2 or pH fluctuations, the pH was monitored before and after the addition of H_2O_2. The tris buffer (pH 8.0) remained stable throughout the experiments, confirming that decomposition was driven by H_2O_2 interactions rather than environmental conditions The robustness was evaluated by adding a single

aliquot of H_2O_2 (150 mM per aliquot) after oxygen evolution had plateaued from the previous H_2O_2 aliquot addition in the same closed cell.

Kinetics: Determining the reaction order with respect to the catalyst. To determine the reaction order with respect to the catalyst, experiments were conducted where the concentration of 150 mM H_2O_2 was held constant, and the concentration of the catalyst was varied in 50 mM Trisma buffer at 298 K with an O_2 microsensor probe (UniSense, Denmark) to obtain equation **4**, which was derived from equation (**1)**:

$$r_i = k_{obs}[Catalyst]^n \quad \textbf{(4)}$$

This setup analyzes the effect of the catalyst concentration on the r_i. In each experiment, the pressure from the evolution of O_2 was monitored over time, measured in mmHg, and converted to mM s^{-1} of O_2 produced. The r_i was obtained by systematically calculating the slope over different time intervals after H_2O_2 injection (90-150 s, 150-210 s, 170-230 s, etc.) to identify the steepest slope, which is representative of the maximum rate at the initial point of the reaction (Equation **5**):

$$r_i = \frac{\Delta[O_2]}{\Delta t} \quad \textbf{(5)}$$

The slope of the natural log of the initial rates represents the order with respect to the catalyst (n) in equation **1** (S1A & S2A)**.** Equation (**4)** was rearranged to obtain equation (**6)**, which is a linear relationship between the initial rates (mM s^{-1}) and the catalyst concentrations (mM) with the slope representing k_{obs} (s^{-1}) in **Figures** 3a & 4a:

$$k_{obs} = \frac{r_i}{[Catalyst]} \quad \textbf{(6)}$$

To obtain the overall 2nd order constant kinetic constant (k) in equation **1**, k_{obs} was divided by the constant concentration of 150 mM H_2O_2 used in this series of experiments (Equation **7**):

$$k = \frac{k_{obs}}{[H_2O_2]} \quad \textbf{(7)}$$

Determining the reaction order with respect to H_2O_2. In a complementary set of experiments, the concentration of H_2O_2 varied while the concentration of catalyst was held constant. The initial rates of the reaction were again measured by monitoring the amount of oxygen produced. A plot of the initial rates (r_i) versus H_2O_2 concentration was used to determine k_{obs}, the reaction order with respect to H_2O_2 (*m)* and the overall 2nd order kinetic constant (*k*) (**Figure** 3b, 4b, S1B, S2B).

Selectivity Experimental Methods

Peroxidase Activity. This assay was adapted from previously reported methods by Doctrow *et al.* and Lu *et al.*, which inspired the design of our peroxidase activity measurements.[1, 28] The peroxidase activity of the catalysts was determined by monitoring the H_2O_2-dependent oxidation of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) to its radical cation ($ABTS^{\bullet+}$) at 414 nm via UV-Vis spectrophotometry (200-900 nm). While Doctrow *et al.* monitored $ABTS^{\bullet+}$ formation at 714 nm, we opted to monitor the absorbance at 414 nm, which was based on the work of Cano *et. al.*, who experimentally determined the molar extinction coefficient to be ε_{414} = 31,100 M^{-1} cm^{-1}.[1, 32] Although there is a minor overlap with the catalyst absorbance at this wavelength, appropriate controls and background subtraction were used to accurately quantify the formation of $ABTS^{\bullet+}$. The reaction was initiated by adding 5 μM H_2O_2 to a solution of phosphate-buffered saline (PBS, pH 7.4) containing 100 μM ABTS and 20 μM of the catalyst for a total volume of 3 mL in a quartz cuvette at 298 K. The increase in absorbance at 414 nm was monitored every 5 min over a 2-h period, indicating the formation of $ABTS^{\bullet+}$. The molar extinction coefficient (ε_{414} = 31,100 M^{-1} cm^{-1}) was used to calculate the concentration of $ABTS^{\bullet+}$

during the reaction via Beer's Law (A = εcl).[32] The controls included PBS (blank), H_2O_2 alone, ABTS alone, and the catalyst alone under identical conditions. The TON for peroxidase activity was calculated via equation **8**:

$$\boldsymbol{TON_{Peroxidase}} = \frac{moles\ of\ ABTS^{\cdot+}\ Produced}{moles\ of\ Catalyst} \qquad (\mathbf{8})$$

The TOF was calculated via equation **9**:

$$\boldsymbol{TOF_{Peroxidase}(s^{-1})} = \frac{TON_{Peroxidase}}{reaction\ time\ (s)} \qquad (\mathbf{9})$$

Catalase Activity. The catalase activity of the synthesized catalysts was assessed by monitoring the disproportionation of H_2O_2 into water and oxygen via UV-vis spectrophotometry (200–900 nm range). The catalyst (20 μM) was added to a phosphate-buffered saline solution (PBS, pH 7.4) and the reaction was initiated by the addition of H_2O_2 (20 mM) for a total volume of 3 mL in a quartz cuvette at 298 K. The reaction was monitored every 5 min over a 2-h period by tracking the decrease in absorbance at 240 nm, which is characteristic of H_2O_2. The cuvette was inverted between each scan to ensure negligible oxygen interference. The concentration of H_2O_2 was determined via Beer's law (A = εcl) with a molar absorptivity of $\varepsilon_{240} = 43.6\ M^{-1}\ cm^{-1}$.[28] The TON of H_2O_2 consumption was calculated via equation **10,** and the TOF was calculated via equation **11:**

$$\boldsymbol{TON_{Catalase}} = \frac{moles\ of\ H_2O_2\ consumed}{moles\ of\ Catalyst} \qquad (\mathbf{10})$$

$$\boldsymbol{TOF_{Catalase}(s^{-1})} = \frac{TON_{Catalase}}{reaction\ time\ (s)} \qquad (\mathbf{11})$$

The controls included PBS (blank), H_2O_2 alone, and the catalyst alone. The selectivity was calculated via equation **12**:

$$\boldsymbol{Selectivity\ (C/P)} = \frac{Catalase_{TOF(s^{-1})}}{Peroxidase_{TOF(s^{-1})}} \qquad (\mathbf{12})$$