Supplementary information

Thermal Degradation of Thaumatin at Low pH and Its Prevention Using Alkyl Gallates

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S1) Synthesis of BEMPO-3 spin probe.

Synthesis of ethyl 2-bromohexanoate

2-Bromohexanoic acid (30 mmol, 5.85 g) was added into a round bottom flask filled with ethanol (250 mL) under constant stirring. Concentrated sulfuric acid (5 mmol, 272 µL) was added subsequently and the mixture was refluxed overnight. Extra solvent was evaporated under vacuum and the concentrated mixture was dissolved in ethyl acetate. The ethyl acetate solution was washed by saturated sodium bicarbonate solution three times, and it was further dried by anhydrous sodium sulfate. The final product was acquired after ethyl acetate was removed under vacuum and it was used in next step reaction without further purification.

1H NMR (500 MHz, Chloroform-*d*) δ 4.30-4.17 (m, 3H), 2.13-2.04 (m, 1H), 2.04-1.94 (m, 1H), 1.49-1.22 (m, 7H), 0.92 (t, *J*=7.1 Hz, 3H). 13C NMR (126 MHz, Chloroform-*d*) δ 169.91, 61.86, 46.16, 34.62, 29.38, 21.98, 13.95, 13.80.

Synthesis of ethyl 2-nitrohexanoate

The procedure was described in a previous publication.[1]

^{1}H NMR (500 MHz, Chloroform-*d*) δ 5.10 (dd, *J* = 9.5, 5.4 Hz, 1H), 4.29 (q, *J* = 7.3 Hz, 2H), 2.35 – 2.23 (m, 1H), 2.20 – 2.09 (m, 1H), 1.45 – 1.34 (m, 4H), 1.32 (t, *J* = 7.2 Hz, 3H), 0.93 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 164.65, 88.19, 62.92, 29.95, 27.66, 21.92, 13.88, 13.61.

Synthesis of Ethyl 2-butyl-4-formyl-2-nitropentanoate

The procedure was described in a previous publication.[1]

^{1}H NMR (500 MHz, Chloroform-*d*) δ 9.57 (d, *J* = 7.2 Hz, 1H), 4.31 – 4.16 (m, 2H), 2.85 (m, 1H), 2.65 – 2.44 (m, 1H), 2.32 – 2.03 (m, 3H), 1.47 – 1.21 (m, 7H), 1.17 (m, 3H), 0.92 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 201.77, 201.59, 166.72, 95.31, 95.15, 62.91, 62.89, 42.07, 41.84, 35.47, 34.20, 33.78, 33.73, 25.62, 25.55, 22.51, 22.44, 15.39, 15.37, 13.81, 13.77, 13.69, 13.68.

Synthesis of 2-butyl-2-(ethoxycarbonyl)-4-methyl-3,4-dihydro-2*H*-pyrrole 1-oxide

The procedure was described in a previous publication.[1]

^{1}H NMR (500 MHz, Chloroform-*d*) δ 6.72-6.66 (m, 1H), 4.14 – 3.96 (m, 2H), 3.11 – 2.73 (m, 1H), 2.47-2.27 (m, 1H), 2.13 – 1.46 (m, 6H), 1.27 – 0.98 (m, 11H), 0.77-0.65 (m, 3H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 170.05, 169.66, 140.07, 139.62, 82.53, 82.44, 61.85, 61.84, 37.00, 36.58, 33.63, 33.07, 32.53, 32.02, 24.91, 24.64, 22.55, 22.47, 18.91, 18.28, 13.97, 13.79, 13.73, 13.72, 13.70.

S2) EPR with DMPO & Guanidine Hydrochloride

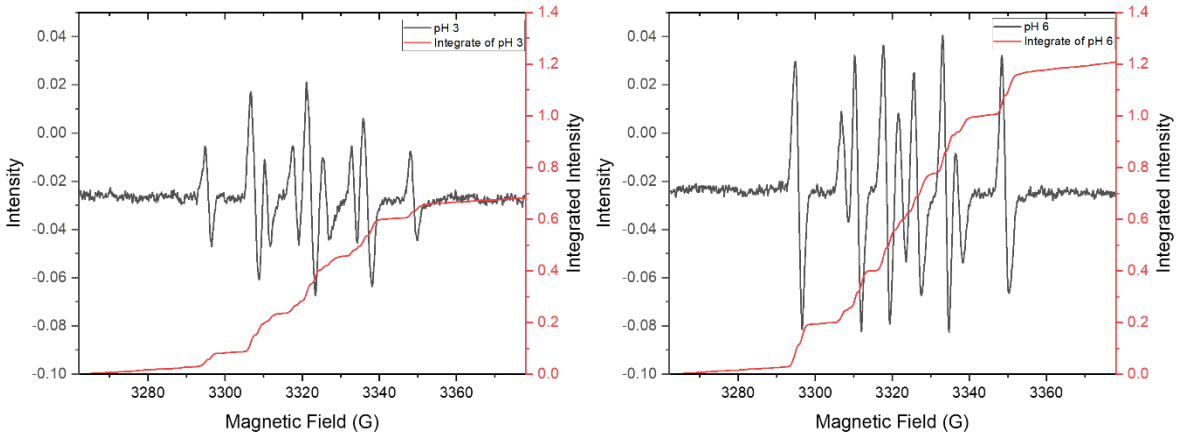

Figure S2: Thaumatin solution (1 mg/mL) in 6 M Guanidine HCL with 5 mM DMPO at pH 3 and pH 6. The solution was heated at 80°C for 1 hour and then the EPR spectra was obtained immediately. The measurement was taken with 40 replicates. The spectra and area under the curve were expressed.

S3) Emulsion Oxidation Test

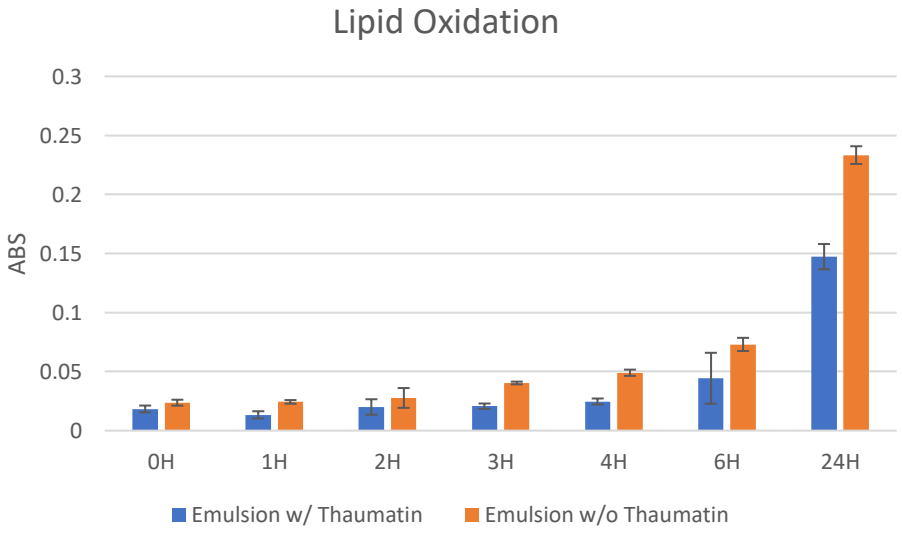

Figure S3: Oxidizable 10% emulsion (20% linoleic acid + 80% medium chain triglyceride) was incubated at 80°C. Measurement was taken to assess the extent of oxidation over time. Control sample was made to volume with Milli-Q water, while treatment sample has thaumatin stock solution (2 mg/mL) included to the final concentration of (1 mg/mL).

S4) C11 BIODIPY Data and Control

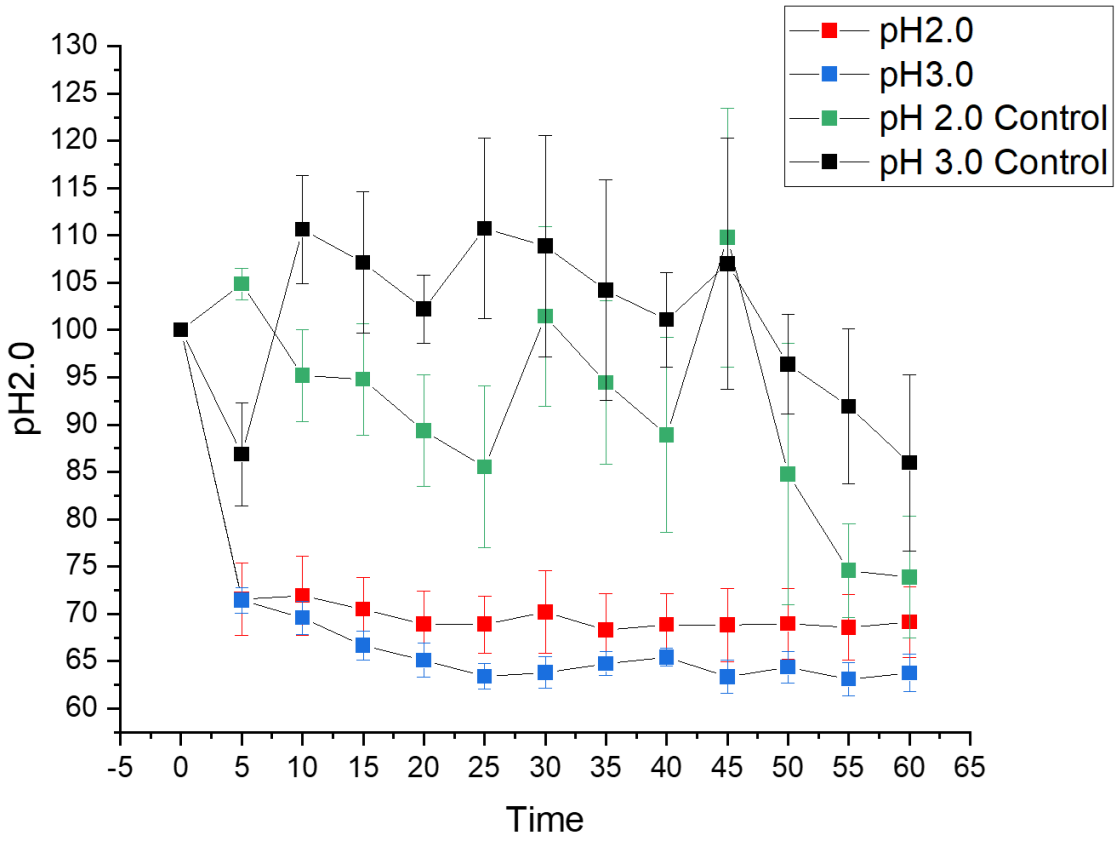

Figure S4: C11 BIODIPY 581/591 red fluorescence (591 nm) decay over time in thaumatin solution heated at 80 °C. The interaction between pH and time was not statistically significant ($F_{4,155} = 1.621$, $p = 0.093$). However, the main effects of pH ($F_{4,155} = 76.61$, $p < 0.0001$) and time ($F_{4,155} = 134.93$, $p < 0.0001$) were statistically significant. Control was C11 BIODIPY in MQ water alone.

Reference

1. Stolze, K.; Udilova, N.; Rosenau, T.; Hofinger, A.; Nohl, H., Spin adduct formation from lipophilic EMPO-derived spin traps with various oxygen- and carbon-centered radicals. *Biochemical pharmacology* **2005,** *69* (2), 297-305.