Supporting information

Small Molecules Reaction Networks That Model ROS Dynamic of the Rhizosphere

Supporting Web Enhanced Objects:

Time-lapse photos of front propagation at 300 × time speed (1s is 5 minutes):
Lapse_Clip1
Time-lapse photos of pattern formation at 60x time speed (1s is 1 minute):
Lapse_Clip2 Pattern Formation with 0.1 mM MB, 0.1 mM DMBQ, 2 mM NaBH4
Lapse_Clip3 Pattern Formation with 0.05 mM MB, 0.1 mM DMBQ, 2 mM NaBH4 (starting from anoxic solution).

Materials and Methods

All chemicals were purchased from Sigma-Aldrich and used without further purification. Deionized water and buffers used in the experiments were treated with Chelex resin to remove transition metal ions. 10 mM stock solution of DMBQ in ACN and 10 mM MB in water were prepared weekly and stored at room temperature. 0.1 M stock solution of $NaBH_4$ in 1 mM NaOH in purified water was prepared daily. Basic pH slowed decomposition of $NaBH_4$ to hydrogen and borates, but the NaOH concentration was too low to affect the pH of the buffered solutions used in the experiments. All reactions were performed in Britton-Robinson buffers (0.02M CH_3COOH, 0.02M H_3PO_4, 0.02M H_3BO_3) adjusted to pH ranging from 5 to 9. Typical reactions in the presence of both DMBQ and MB, ROS measurements, and reaction-diffusion studies were performed in a Britton-Robinson buffer pH 8.5. Each experiment was performed at least 3 times, and data used for data fitting consists of averaged values from 3 different experiments.

Kinetic experiments

Kinetics were measured at 300 and 600 nm in UV-transparent Coring Acrylic 96-well plate with BIO-TEK Synergy HT Plate reader at 30s intervals. The plate was shaken for 2s between each measurement to ensure efficient oxygen transport thorough the solution.

For measurement of pH dependent H_2DMBQ oxidation, reduced H_2DMBQ stock solutions were prepared by adding small amount of solid $NaBH_4$ to a 1 mM stock solution of DMBQ in water. After the yellow colour disappeared, the pH of the solution was brought to 3 with 1M HCl. At low pH the excess $NaBH_4$ was hydrolysed to hydrogen. After gas evolution ceased, the pH was readjusted to 3 to supress spontaneous DMBQ oxidation and the solution was used immediately. Stock solutions were diluted 20 times and the low [HCl] concentration present in the solution did not change the buffered pH values more than 0.01 pH units (verified with pH meter).

The DMBQ-catalysed reduction of MB was measured in an Agilent Technology CARY 100 UV-vis spectrophotometer. For anoxic measurements aliquots of DMBQ and MB stock solutions were added to 3mL of Ar-purged buffer (Oxygen concentration below 0.1 mg/L, measured with an O_2 sensor) in a custom-made UV cell with septum under Ar flow. To start the reaction, 0.03 mL of an 0.1M stock solution of $NaBH_4$ was added with a glass syringe through the septum and the solution was mixed by hand. For the measurement of oxygen consumption, the reaction was performed in the air-saturated solution in a closed cell without stirring, to prevent the reabsorption of oxygen from the air.

Oxidation reactions in the presence of both redox compounds (MB and DMBQ) were usually performed at pH 8.5. Aliquots of both compounds in their oxidized forms were mixed with the buffer and the reaction was initiated by the addition of the excess of the reducing agent to solution. Typical final concentrations were 0.05 mM of DMBQ, 0.05mM MB and 1 mM of $NaBH_4$.

H_2O_2 concentrations were measured using Thermo Fisher Scientific Amplex® Red Enzyme assay kit. Measurements were done after the oxidation reactions for both MB and DMBQ were complete (1h after the addition of $NaBH_4$ to the solution). Concentrations of H_2O_2 were calculated from corresponding calibration curves.

Oxygen measurements

Oxygen measurements were made at 20 s intervals with Mettler Toledo Oxygen Sensor in 10 mL samples under constant mechanical stirring. In a typical experiment, 0.1 mL aliquot of 0.1M $NaBH_4$ was added to the solution 60s after the measurement started. At least 3 measurements were made for each experimental condition and the data from the measurements were averaged for further analysis.

Data fitting

Kinetic constants were found using COPASI software[1] to fit the data to the proposed reaction mechanisms Fitting was performed using Evolutionary Programming methods. For simplicity, the proton concentration was maintained fixed as 3.1×10^{-9} M (pH 8.5) and protons and water were not included in mass balance equations. The superoxide dismutation constant was calculated from the pH

dependence equation proposed by Bielski and Allen.[2] Oxygen transport was modelled as an equilibrium between gas phase and solution, as proposed by Olsen et al.[3] Reactions between semiquinone radicals were considered to be diffusion-controlled. H_2DMBQ oxidation mechanism and constants were found based on values from Roginsky et al.[4] Since the rate of spontaneous reaction between H_2DMBQ and oxygen was 10^{10} orders of magnitude lower than DMBQ catalysed reaction rates and had no effect on overall curve fitting performance, it was excluded from the multicomponent reaction mechanism present on Figure 2b, to reduce overall number of parameters and improve overall quality of fitting. Proposed LMB oxidation mechanism was based on mechanisms previously proposed by Zhang et al.[5] and Katafias and Koter[6]; rate constants were found using values from Katafias and Koter[6] as an initial approximation. Reduction of DMBQ by $NaBH_4$ was calculated from a separate measurement as a pseudo first order reaction constant. DMBQ-related parameters were maintained constant for the fitting of the reaction in the presence of MB and DMBQ.

Reaction-diffusion experiments

Travelling wave experiments were performed in 1 mm layer of 1 % agarose gel in the presence of buffer, 1mM DMBQ and 1mM MB. To prevent oxygen transport, gels were covered with a plastic glass lid. Reactions were started when a small sample of $NaBH_4$ (approximately 0.1 mm in diameter) were put on top of the gel. Images were collected using an iPhone 6s camera with Lapse It 2.5 Pro App at 60 s intervals and

rendered at 5 frames/s (1s equals to 5 min real time). Distances were calculated using image analysis with ImageJ software.

Stationary patterns

To observe travelling wave patterns, the 0.1 M $NaBH_4$ solution was added as a thin layer (1mm) to 10 mL of buffer, MB, and DMBQ solution of mentioned in text concentrations in 11 cm Petri dish and briefly mixed by hand. For line pattern formation, 10 mL of buffer and corresponding concentrations of MB, DMBQ and $NaBH_4$ were mixed in a closed 20 mL vial and the resulting colorless solution was poured into the 11 cm Petri dish open to the air. The dish was placed on top of a light box equipped with white LED. Images were collected using the iPhone 6s camera with Lapse It 2.5 Pro App at 10 s intervals and rendered at 6 frames/s (1s equals to 1 min real time). Experiments were repeated in 1% agarose gel, where no patter formation was observed, and in 50 mL solution, which formed a 5mm layer of liquid in the Petri dish.

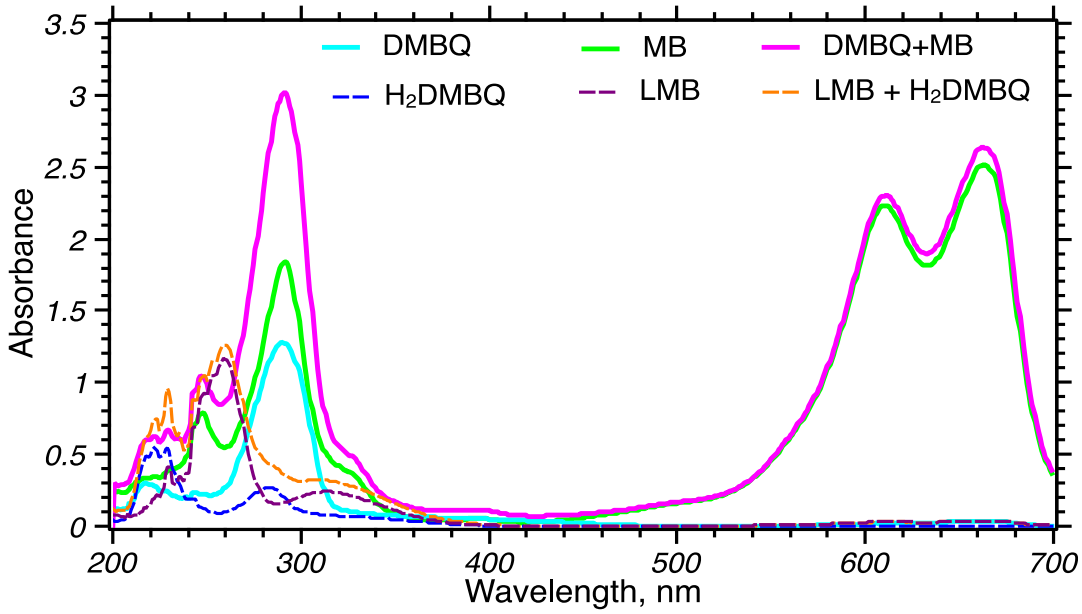


Figure S1. UV-vis spectrum of oxidized and reduced forms of DMBQ and MB.

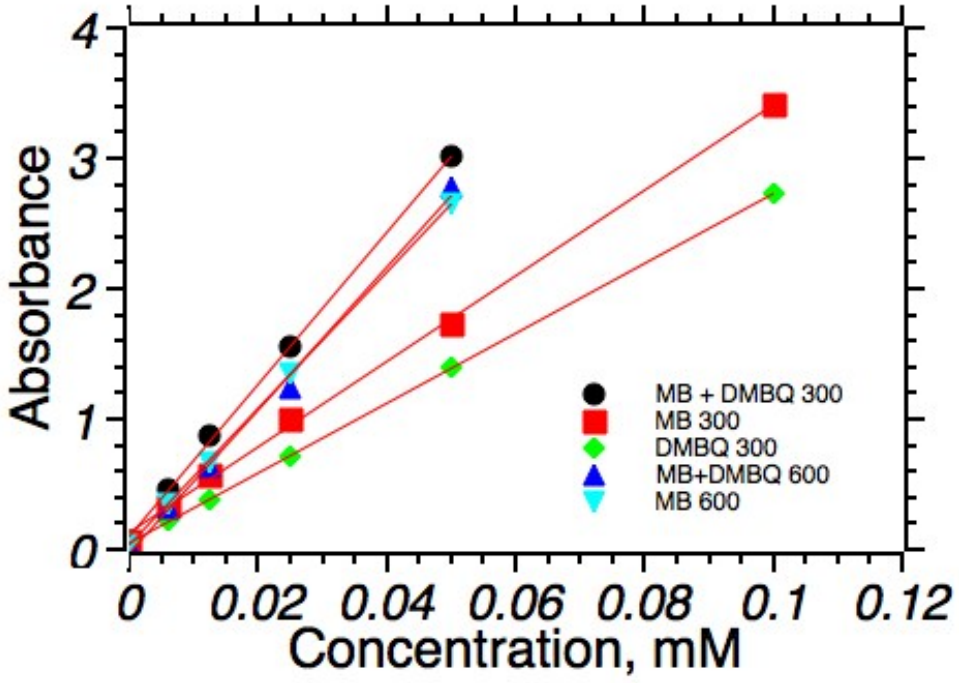


Figure S2. Calibration curves for MB and DMBQ at different wavelength.

Calibration equations:

$MB_{300} = 0.12 + 33000*[MB]$ (1)
$DMBQ_{300} = 0.05 + 28770*[DMBQ]$ (2)
$MB_{600} = 0.029 + 52465*[MB]$ (3)

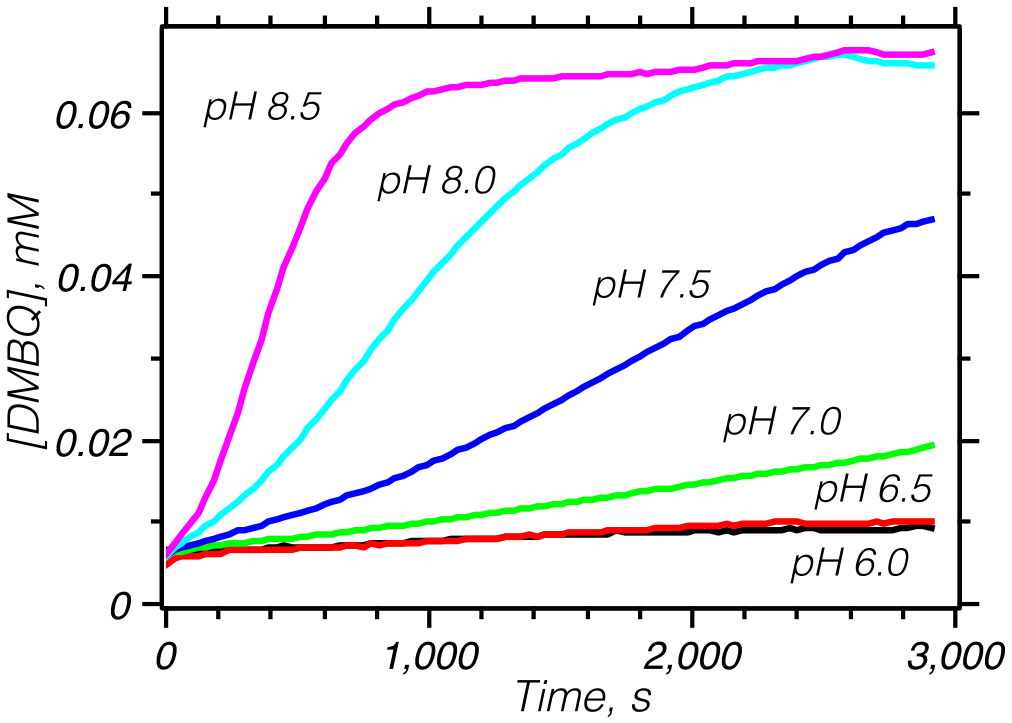


Figure S3. pH dependent oxidation of H_2DMBQ in BR buffer.

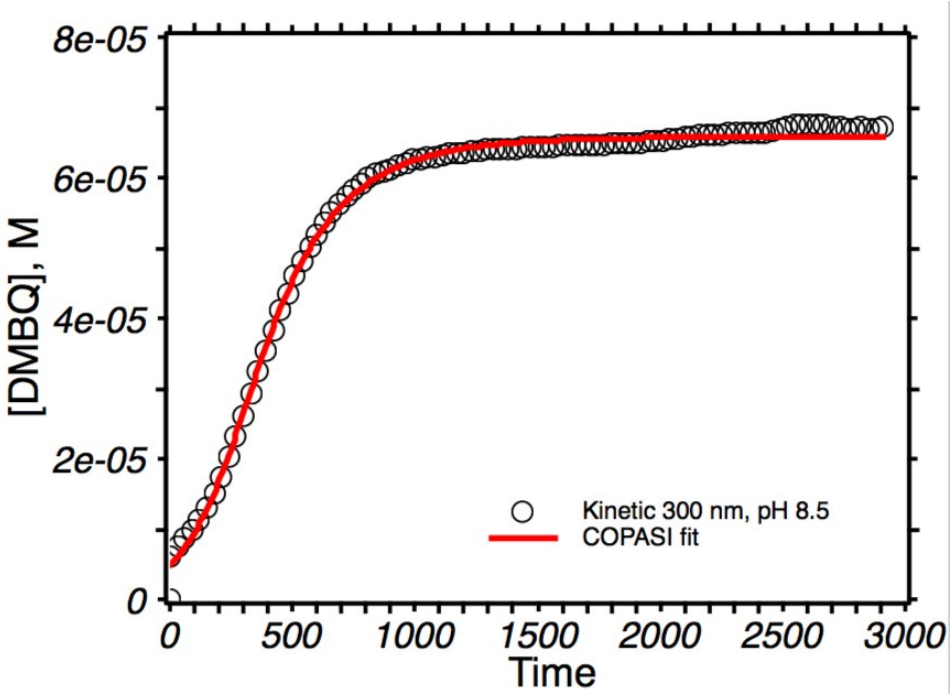
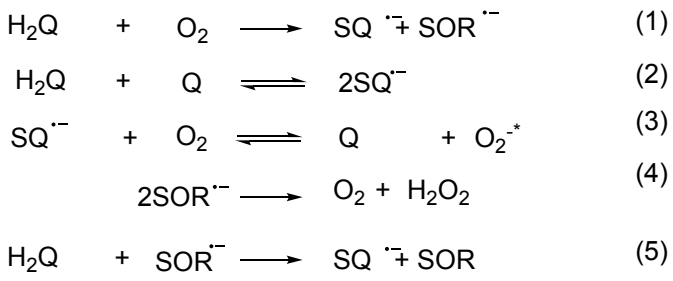


Figure S4. DMBQ data points and COPASI fitting at pH 8.5 according to the mechanism proposed at Scheme 1b.



Scheme S1. H_2DMBQ oxidation reaction.

$$\frac{d([H2Q]\cdot V_{compartment\_1})}{dt} = -V_{compartment\_1}\cdot(k1_{(reaction\_5)}\cdot[H2Q]\cdot[SOR]) - V_{compartment\_1}\cdot(k1_{(reaction\_1)}\cdot[H2Q]\cdot[O2]) - V_{compartment\_1}\cdot((k1_{(reaction\_2)}\cdot[BQ]\cdot[H2Q] - k2_{(reaction\_2)}\cdot[SQ]\cdot[SQ]))$$

$$\frac{d([SQ]\cdot V_{compartment\_1})}{dt} = +V_{compartment\_1}\cdot(k1_{(reaction\_5)}\cdot[H2Q]\cdot[SOR]) - V_{compartment\_1}\cdot((k1_{(reaction\_4)}\cdot[SQ]\cdot[O2] - k2_{(reaction\_4)}\cdot[SOR]\cdot[BQ])) + V_{compartment\_1}\cdot(k1_{(reaction\_1)}\cdot[H2Q]\cdot[O2]) + 2\cdot V_{compartment\_1}\cdot((k1_{(reaction\_2)}\cdot[BQ]\cdot[H2Q] - k2_{(reaction\_2)}\cdot[SQ]\cdot[SQ]))$$

$$\frac{d([BQ]\cdot V_{compartment\_1})}{dt} = +V_{compartment\_1}\cdot((k1_{(reaction\_4)}\cdot[SQ]\cdot[O2] - k2_{(reaction\_4)}\cdot[SOR]\cdot[BQ])) - V_{compartment\_1}\cdot((k1_{(reaction\_2)}\cdot[BQ]\cdot[H2Q] - k2_{(reaction\_2)}\cdot[SQ]\cdot[SQ]))$$

$$\frac{d([O2]\cdot V_{compartment\_1})}{dt} = +V_{compartment\_1}\cdot(k1_{(reaction\_3)}\cdot[SOR]\cdot[SOR]) - V_{compartment\_1}\cdot((k1_{(reaction\_4)}\cdot[SQ]\cdot[O2] - k2_{(reaction\_4)}\cdot[SOR]\cdot[BQ])) - V_{compartment\_1}\cdot(k1_{(reaction\_1)}\cdot[H2Q]\cdot[O2])$$

$$\frac{d([H2O2]\cdot V_{compartment\_1})}{dt} = +V_{compartment\_1}\cdot(k1_{(reaction\_3)}\cdot[SOR]\cdot[SOR]) + V_{compartment\_1}\cdot(k1_{(reaction\_5)}\cdot[H2Q]\cdot[SOR])$$

$$\frac{d([SOR]\cdot V_{compartment\_1})}{dt} = -2\cdot V_{compartment\_1}\cdot(k1_{(reaction\_3)}\cdot[SOR]\cdot[SOR]) - V_{compartment\_1}\cdot(k1_{(reaction\_5)}\cdot[H2Q]\cdot[SOR]) + V_{compartment\_1}\cdot((k1_{(reaction\_4)}\cdot[SQ]\cdot[O2] - k2_{(reaction\_4)}\cdot[SOR]\cdot[BQ])) + V_{compartment\_1}\cdot(k1_{(reaction\_1)}\cdot[H2Q]\cdot[O2])$$

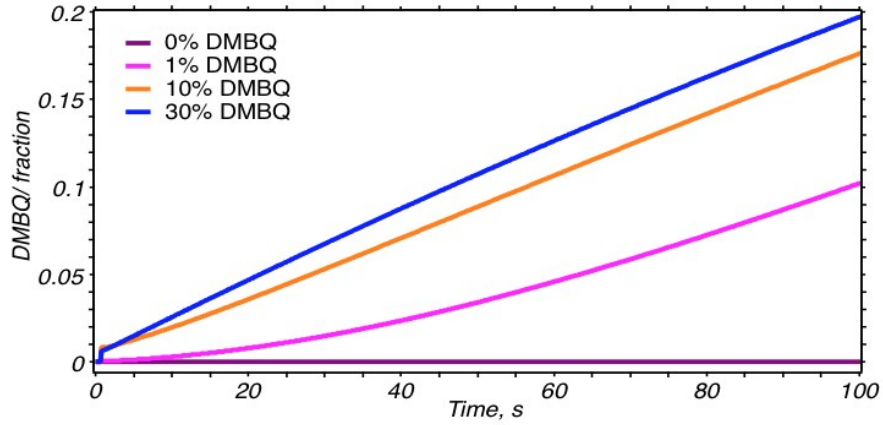
Scheme S2. List of ODE used to fit the data.

Table S1. H_2DMBQ oxidation parameters at pH 8.5 according to the mechanism at Scheme S1. (Note that the protons are excluded from mass balance equations).

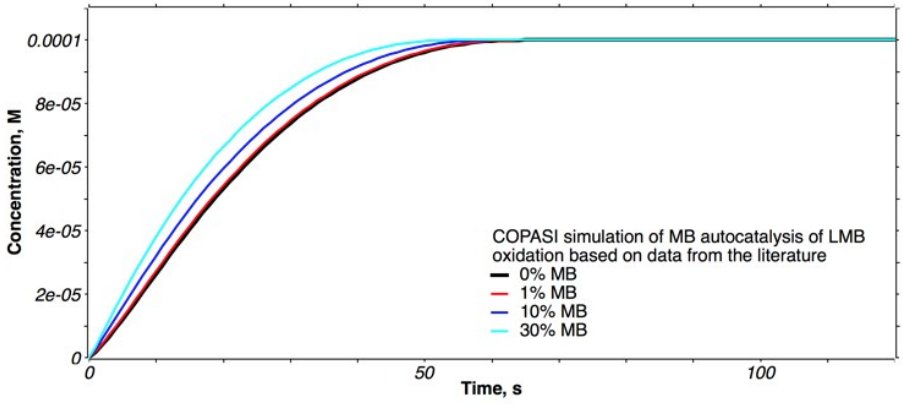
Reaction (see Scheme 1b)	Rate
k_1	1.1×10^{-7}
k_2	$(1.11\pm 0.01)\times 10^{3}$
k_{-2}	1×10^{8}
k_3	$(9.9 \pm 0.3)\times 10^{7}$
k_{-3}	5.0×10^{6} (fixed, see [4])
k_4	1.8×10^{4}
k_5	2.0×10^{-6}

Note: reactions 1 and 5 were excluded from the model of reaction in the presence of both MB and DMBQ, because their do not contribute to the overall shape of the fitting curve.

a)



b)



c)

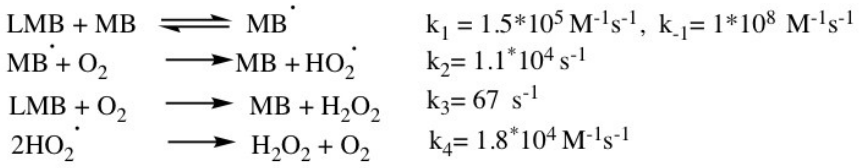


Figure S5. (a) COPASI simulation of autocatalytic activation of H_2DMBQ oxidation at different concentrations of DMBQ added to solution, using values from Table S1. (b) COPASI simulation of autocatalytic activation of LMB oxidation at different concentrations of MB using data from the literature.[6] (c) Reaction mechanism with constants adjusted to pH 8.5 from Katafias et al.[6]

DMBQ Chemistry

$NaBH_4 + Q \longrightarrow borates + H_2Q$ (1)

$H_2Q + Q \rightleftharpoons 2SQ^{\bullet -} + 2H^+$ (2)

$SQ^{\bullet -} + O_2 \rightleftharpoons Q + O_2^{-*}$ (3)

MB Chemistry

$MB + LMB \rightleftharpoons 2MB^{\bullet} + 2H^+$ (4)

$MB^{\bullet} + O_2 \rightleftharpoons MB + O_2^{-*}$ (5)

Hydride Transfer

$MB + H_2Q \rightleftharpoons LMB + Q$ (6)

ROS Chemistry

$2\ O_2^{\bullet -} + 2H^+ \rightleftharpoons 2HO_2 \longrightarrow O_2 + H_2O_2$ (7)

$O_{2(g)} \rightleftharpoons O_2$ (8)

Reductant decomposition

$NaBH_4 \longrightarrow borates + H_2$ (9)

Scheme S3. MB and DMBQ co-oxidation reactions.

$$\frac{d([RED]\cdot V_{compartment\_1})}{dt} = -V_{compartment\_1}\cdot(k1_{(reaction\_8)}\cdot[RED]) - V_{compartment\_1}\cdot(k1_{(reaction\_1)}\cdot[RED]\cdot[Q])$$

$$\frac{d([RED\_0]\cdot V_{compartment\_1})}{dt} = +V_{compartment\_1}\cdot(k1_{(reaction\_8)}\cdot[RED]) + V_{compartment\_1}\cdot(k1_{(reaction\_1)}\cdot[RED]\cdot[Q])$$

$$\frac{d([Q]\cdot V_{compartment\_1})}{dt} = +V_{compartment\_1}\cdot((k1_{(reaction\_3)}\cdot[SQ]\cdot[O2] - k2_{(reaction\_3)}\cdot[SOR]\cdot[Q])) - V_{compartment\_1}\cdot((k1_{(reaction\_2)}\cdot[H2Q]\cdot[Q] - k2_{(reaction\_2)}\cdot[SQ]\cdot[SQ])) + V_{compartment\_1}\cdot((k1_{(reaction\_6)}\cdot[H2Q]\cdot[MB] - k2_{(reaction\_6)}\cdot[Q]\cdot[LMB])) - V_{compartment\_1}\cdot(k1_{(reaction\_1)}\cdot[RED]\cdot[Q])$$

$$\frac{d([H2Q]\cdot V_{compartment\_1})}{dt} = -V_{compartment\_1}\cdot((k1_{(reaction\_2)}\cdot[H2Q]\cdot[Q] - k2_{(reaction\_2)}\cdot[SQ]\cdot[SQ])) - V_{compartment\_1}\cdot((k1_{(reaction\_6)}\cdot[H2Q]\cdot[MB] - k2_{(reaction\_6)}\cdot[Q]\cdot[LMB])) + V_{compartment\_1}\cdot(k1_{(reaction\_1)}\cdot[RED]\cdot[Q])$$

$$\frac{d([MB]\cdot V_{compartment\_1})}{dt} = +V_{compartment\_1}\cdot((k1_{(reaction\_5)}\cdot["MB*"]\cdot[O2] - k2_{(reaction\_5)}\cdot[MB]\cdot[SOR])) - V_{compartment\_1}\cdot((k1_{(reaction\_4)}\cdot[LMB]\cdot[MB] - k2_{(reaction\_4)}\cdot["MB*"]\cdot["MB*"])) - V_{compartment\_1}\cdot((k1_{(reaction\_6)}\cdot[H2Q]\cdot[MB] - k2_{(reaction\_6)}\cdot[Q]\cdot[LMB]))$$

$$\frac{d([LMB]\cdot V_{compartment\_1})}{dt} = -V_{compartment\_1}\cdot((k1_{(reaction\_4)}\cdot[LMB]\cdot[MB] - k2_{(reaction\_4)}\cdot["MB*"]\cdot["MB*"])) + V_{compartment\_1}\cdot((k1_{(reaction\_6)}\cdot[H2Q]\cdot[MB] - k2_{(reaction\_6)}\cdot[Q]\cdot[LMB]))$$

$$\frac{d([O2]\cdot V_{compartment\_1})}{dt} = -V_{compartment\_1}\cdot((k1_{(reaction\_5)}\cdot["MB*"]\cdot[O2] - k2_{(reaction\_5)}\cdot[MB]\cdot[SOR])) + V_{compartment\_1}\cdot(k1_{(reaction\_7)}\cdot[SOR]\cdot[SOR]) + V_{compartment\_1}\cdot((k1_{(reaction\_9)}\cdot[O2g] - k2_{(reaction\_9)}\cdot[O2])) - V_{compartment\_1}\cdot((k1_{(reaction\_3)}\cdot[SQ]\cdot[O2] - k2_{(reaction\_3)}\cdot[SOR]\cdot[Q]))$$

$$\frac{d([SQ]\cdot V_{compartment\_1})}{dt} = -V_{compartment\_1}\cdot((k1_{(reaction\_3)}\cdot[SQ]\cdot[O2] - k2_{(reaction\_3)}\cdot[SOR]\cdot[Q])) + 2\cdot V_{compartment\_1}\cdot((k1_{(reaction\_2)}\cdot[H2Q]\cdot[Q] - k2_{(reaction\_2)}\cdot[SQ]\cdot[SQ]))$$

$$\frac{d(["MB*"]\cdot V_{compartment\_1})}{dt} = -V_{compartment\_1}\cdot((k1_{(reaction\_5)}\cdot["MB*"]\cdot[O2] - k2_{(reaction\_5)}\cdot[MB]\cdot[SOR])) + 2\cdot V_{compartment\_1}\cdot((k1_{(reaction\_4)}\cdot[LMB]\cdot[MB] - k2_{(reaction\_4)}\cdot["MB*"]\cdot["MB*"]))$$

$$\frac{d([SOR]\cdot V_{compartment\_1})}{dt} = +V_{compartment\_1}\cdot((k1_{(reaction\_5)}\cdot["MB*"]\cdot[O2] - k2_{(reaction\_5)}\cdot[MB]\cdot[SOR])) - 2\cdot V_{compartment\_1}\cdot(k1_{(reaction\_7)}\cdot[SOR]\cdot[SOR]) + V_{compartment\_1}\cdot((k1_{(reaction\_3)}\cdot[SQ]\cdot[O2] - k2_{(reaction\_3)}\cdot[SOR]\cdot[Q]))$$

$$\frac{d([H2O2]\cdot V_{compartment\_1})}{dt} = +V_{compartment\_1}\cdot(k1_{(reaction\_7)}\cdot[SOR]\cdot[SOR])$$

Scheme S4. List of ODE used to fit data of oxidation of mixture of H_2DMBQ and LMB proposed in Scheme 1 (main text).

Table S2. Reaction constants for fitting to data Figure 3 according to the proposed mechanism (Note that the protons are excluded from mass balance equations).

Reaction		k, $M^{-1}s^{-1}$	
		pH 8.5	pH 7.4*
$NaBH_4 + Q \longrightarrow$ borates + H_2Q	k_1	$(3.0\pm1.3)\times10^4$	
$H_2Q + Q \rightleftharpoons 2SQ$	k_2	$(1.1\pm0.01)\times10^3$	$(8.0\pm2.0)\times10^{2}$ [a]
	k_{-2}	1×10^8	$(1.50\pm0.02)\times10^{7}$ [a]
$SQ + O_2 \rightleftharpoons Q + SOR$	k_3	1×10^8	
	k_{-3}	$(2.5\pm0.01)\times10^6$	
$MB + LMB \rightleftharpoons MB^*$	k_4	$(6.47\pm0.56)\times10^3$	1.2×10^{3} [b]
	k_{-4}	1.0×10^8	1×10^{8} [b]
$MB^{\cdot} + O_2 \longrightarrow MB + SOR$	k_5	6×10^4	1.1×10^{4} [b]
$MB + H2Q \rightleftharpoons LMB + Q$	k_6	$(2.6\pm0.3)\times10^3$	
	k_{-6}	$(12.5\pm10)\times10^3$	
$2SOR \longrightarrow O_2 + H_2O_2$	k_7	1.8×10^4	2×10^{5} [c]
$NaBH_4 \longrightarrow$ borates + H_2	k_8	$(3.0\pm1.3)\times10^4$	
$O_{2(g)} \rightleftharpoons O_2$	k_9	0.025±0.003	
	k_{-9}	0.001	

** Literature [a] from [4] [b] from [6] [c]Ref. [2]

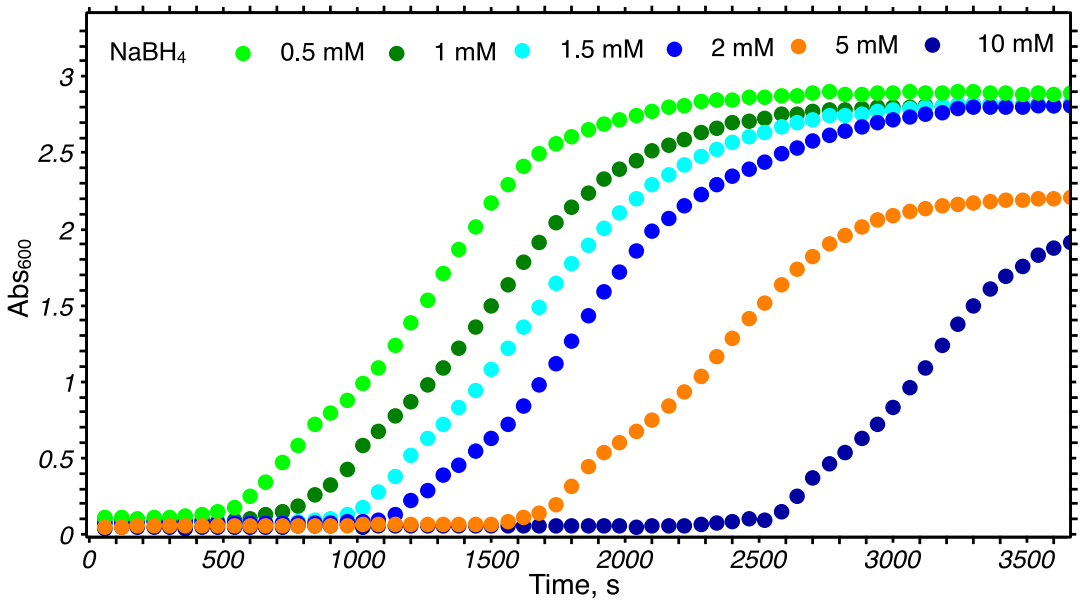


Figure S6. $NaBH_4$ dependent switch between oxidized and reduced states of MB and DMBQ oxidations.

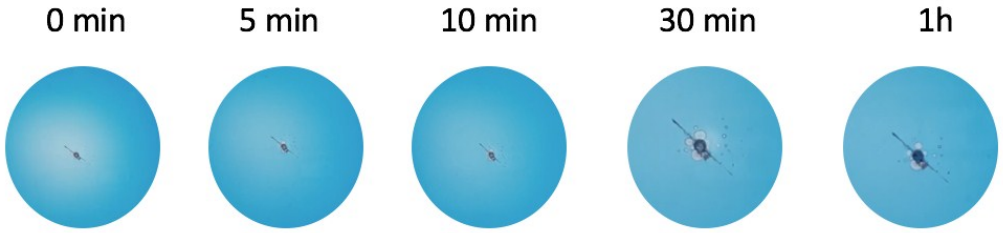


Figure S7. Control experiment for traveling wave in the absence of DMBQ. No reduction is observed over 1h period. Increase of the area of the application point can be explained by gradual degradation of the agarose gel due to spontaneous decomposition of $NaBH_4$ accompanied by the formation of hydrogen gas bubbles.

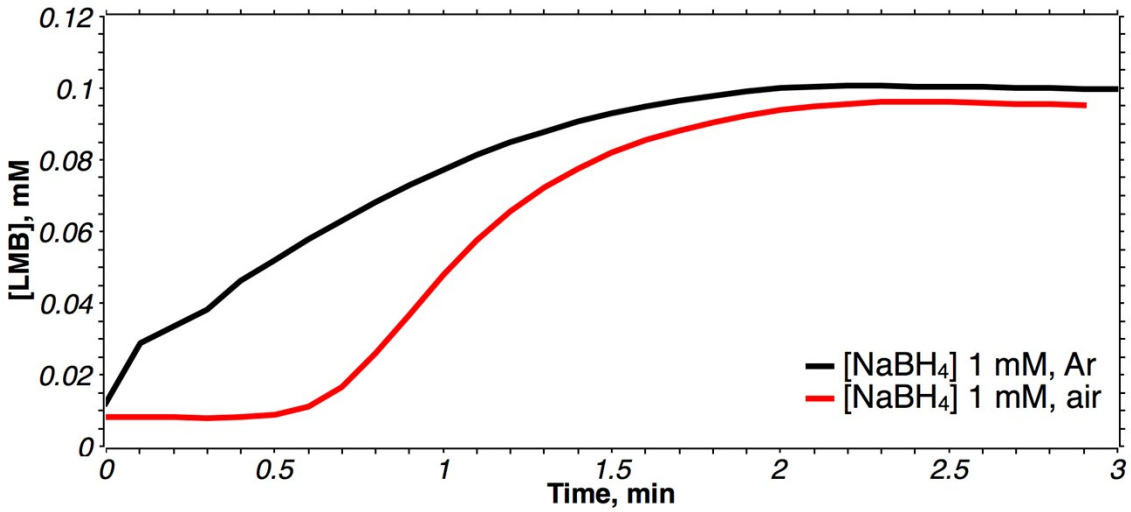


Figure S8. a) LMB formation in solution under argon and in the presence of oxygen from the air (0.225mM [O_2]). In the presence of oxygen sharp change between oxidation and reduction state is observed due to autocatalytic H_2DMBQ oxidation and oxygen removal. This autocatalytic reaction is responsible for signal generation in thin layer of agarose gel.

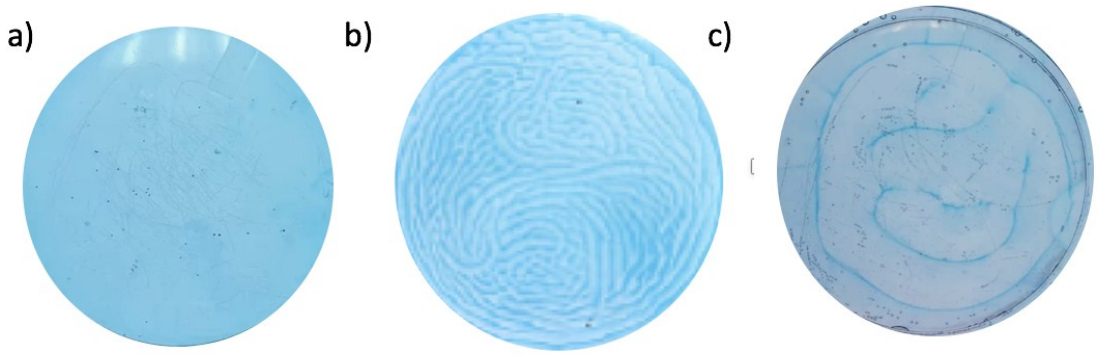
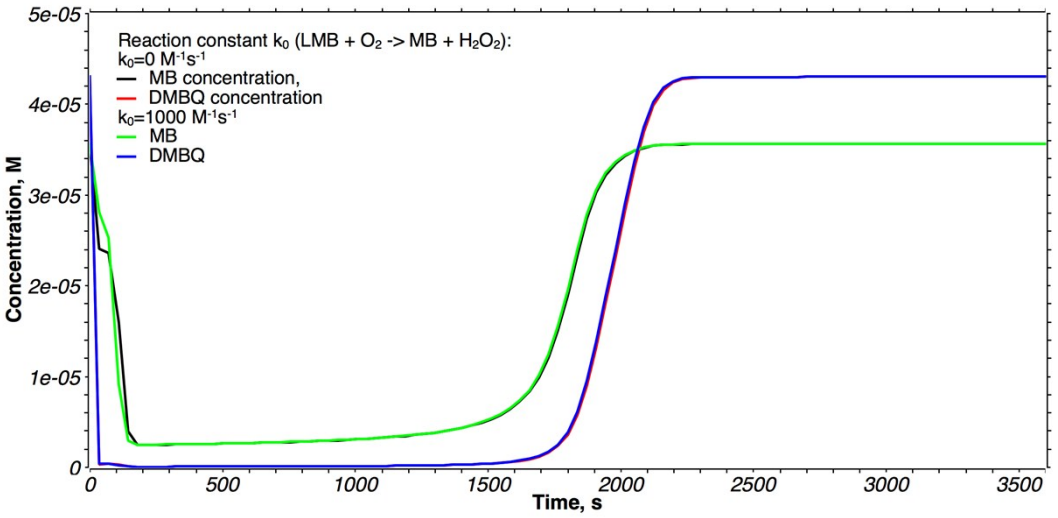


Figure S9. Formation of spatial patterns due to Raleigh-Bernard instability. a) Uniform darkening of the surface without pattern formation is observed in 1% agarose gel, where convection is arrested; b) thin spacing between lines is observed in 1mm deep liquid layer on a surface; c) broad spacing is observed in 5mm deep liquid layer on a surface.

a)



b)

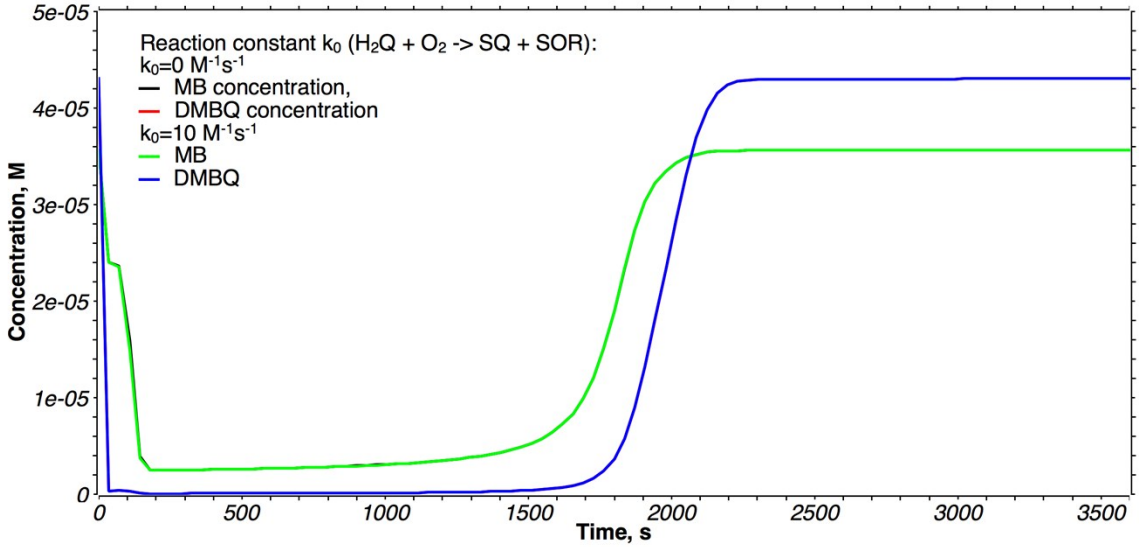
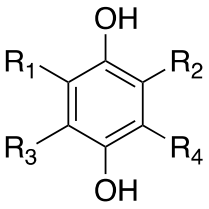
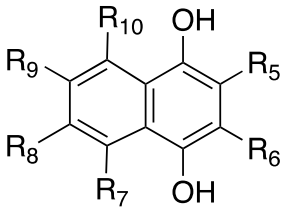


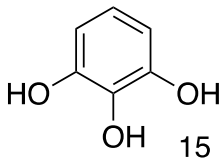
Figure S10. Rationale of using simplified reaction mechanism. Effect of spontaneous uncatalyzed oxidation on overall shape for (a) LMB and (b) H_2DMBQ oxidation leads to overlapped lines.





5: $R_1 = R_2 = MeO$; $R_3 = R_4 = H$
6: $R_1 = MeO$; $R_2 = R_3 = R_4 = H$
7: $R_1 = Me$; $R_2 = R_4 = MeO$; $R_4 = H$
8: $R_1 = R_3 = Me$; $R_2 = R_4 = H$
9: $R_1 = R_2 = R_3 = R_4 = H$
10: $R_1 = R_4 = OH$; $R_2 = R_3 = H$
11: $R_1 = R_2 = CH_3$; $R_3 = R_4 = H$
12: $R_1 = R_4 = MeO$; $R_2 = R_3 = H$

13: $R_5 = R_6 = R_7 = R_8 = R_9 = R_{10} = H$
14: $R_5 = OH$; $R_6 = R_7 = R_8 = R_9 = R_{10} = H$



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Scheme S5. Quinones tested as catalysts for MB reduction in the presence of $NaBH_4$.

Table S3. Quinone catalysis on MB reduction at different pH in the presence of 10mM of $NaBH_4$. +++ reduction rate under 1 min, ++ reduction rate under 10 min, + reduction rate under 30 min, - no reaction.

	8.5	8.0	7.5	7.0	6.5
5	+++	+++	+++	+++	++
6	++	+	+	-	-
7	+++	+++	+++	+++	++
8	++	++	+	+	-
9	-	-	-	-	-
10	-	+	+	++	++
11	++	+	-	-	-
12	+++	+++	+++	+++	+
13	+++	+++	+++	+++	+++
14	-	+	+	++	++
15	-	-	-	+	+

Supporting information references

1 S. Hoops, S. Sahle, R. Gauges, C. Lee, J. Pahle, N. Simus, M. Singhal, L. Xu, P. Mendes and U. Kummer, COPASI—a complex pathway simulator, *Bioinformatics*, 2006, **22**, 3067–3074.

2 B. H. J. Bielski and A. O. Allen, Mechanism of the disproportionation of superoxide radicals, *J. Phys. Chem.*, 1977, **81**, 1048–1050.

3 L. Olsen, M. J. Hauser and U. Kummer, Mechanism of protection of peroxidase activity by oscillatory dynamics, *Eur. J. Biochem.*, 2003, **270**, 2796–2804.

4 V. A. Roginsky, L. M. Pisarenko and C. Michel, The kinetics and thermodynamics of quinone – semiquinone – hydroquinone systems under physiological conditions, *J. Chem. Soc., Perkin Trans.*, 1999, 871–876.

5 Y.-X. Zhang and R. J. Field, Simplification of a Mechanism of the Methylene Blue-HS-O2 CSTR Oscillator: A Homogeneous Oscillatory Mechanism with Nonlinearities but No Autocatalysis, *J. Phys. Chem.*, 1991, **95**, 723–727.

6 A. Katafias and S. Koter, Autooxidation of leuco-Methylene Blue – the Role of the Methylene Blue Radicals and Reactive Oxygen Species, *Pol. J. Chem.*, 2009, **Vol. 83**, **n**, 1139–1146.