Supporting Information

An autocatalytic organic reaction network based on cross-catalysis

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1. Experimental Section

1.1. Reagents and Materials

Trizma®Base, minimum (99.9%) titration, ethylene diamine tetra acetic (99.999%), sodium hydroxide (99%), dimethylsulfoxide (99.7%), N,N-dimethylformamide (99%), were purchased from Sigma Aldrich/Merk. The P1 pinacol ester (>97%), 2,6-diMethyl-4- (4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenol), DMBQ (> 98%, 2,6-dimethylcyclohexa-2,5-diene-1,4-dione), 2,5-diMe-1,4-BQ (> 98%, p-xyloquinone, 2,5-dimethylcyclohexa-2,5-diene-1,4-dione), DMBQH2 (> 98%, 2,6-dimethylbenzene-1,4-diol), and NADH (β-NAD disodium salt hydrate, reduced form) were purchased from TCI company. The P2 pinacol ester (3,5-dimethyl-4(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenol) was bought from fluorochem. The 1,4-naphthoquinone (97%) was obtained from Janssen. Hydrogen peroxide for analysis was purchased from Acros Organics. The diaphorase from Bacillus steaothermophilus (E.C. 1.6 99.) was purchased from Nipro (Japan). In order to get rid of residual traces of the free prosthetic group of the enzyme (i.e., FMN), a purification step by centrifugation over a 10 kDa Microcon® membrane was performed, followed by 3 washes with a PBS buffer (pH 7.5) and centrifugation (11,000 x g, 30 min, 4°C), with a final redispersion of the enzyme in the PBS buffer. The specific activity of the enzyme was determined by UV-Visible spectroscopy by monitoring at 600 nm the discoloration of the 2,6-dichlorophenolindophenol indicator in the presence of NADH. For long term storage, the enzyme solution was aliquoted and stored at -20°C.

1.2. Instruments

Standard spectrophotometric characterizations were carried out with a Cary 60 UV-vis spectrophotometer (Agilent Technologies) and a quartz cuvette of 1-cm optical path-length (Hellma Analytics). For high-throughput kinetic experiments, a Spark™ multimode microplate reader (Tecan, Switzerland) with standard Corning® 96-well UV transparent, clear flat bottom microplates (Thermo Fisher) were used.

The electrochemical experiments of cyclic voltammetry were performed with an Autolab potentiostat (EcoChemie) interfaced to a PC system with a GPSE version 4.4 software. A standard three electrode cell equipped with a glassy carbon disk electrode, a saturated calomel electrode, and a platinum wire as working, reference, and counter electrode, respectively, was used.

1.3. Procedures

All experiments were performed in a Tris-EDTA buffer of pH 8.5 prepared from a 0.1 M Tris buffer at pH 8.5 to which were added 10 µM EDTA (Promega). The buffer was filtered over a 0.2 µm polypropylene membrane (Pall). The mother solutions of each of the boronic ester probes were prepared in pure DMSO (Fluka) at a concentration of 50 mM, and then diluted to the desired concentration in the Tris-EDTA buffer. Mother solutions of hydrogen peroxide (for analysis, 35 wt. % solution in water, stabilized, Acros organics) and NADH were daily prepared and diluted in Milli-Q water at the appropriate concentrations and then diluted to the desired concentrations in the Tris-EDTA buffer.

For high-throughput kinetic experiments, the reactions were initiated by injecting 100 µL of a solution of either probe (investigation of Loop 1), ascorbate (investigation of Loop 2) or probe + ascorbate (investigation of autocatalysis) at a given concentration to 100 µL of solutions at varied concentrations of either H2O2 (investigation of Loop 1 and autocatalysis) or DMBQ (investigation of Loop 2 and autocatalysis) previously distributed into the wells of the microplate. The plate was then rapidly
introduced into the microplate reader and shaken for 5 s before reading by UV-vis spectrophotometry every 10 s. All the experiments performed on the microplates were done in triplicate and achieved in an air-conditioned room where the temperature was maintained at 25°C ± 1°C.
2. Additional Schemes

2.1. Scheme S1: Naphthoquinone-based probes $S_1$ and $S_2$

![Scheme S1. Probe $S_1$ and $S_2$.]

2.2. Scheme S2: Two-step representation of Loop 1

![Scheme S2. Reaction mechanisms assumed the H$_2$O$_2$-catalyzed deprotection of (A) $P_1$ and (B) $P_2$. Note that the boronic esters are rapidly hydrolyzed into their boronic acid counterparts once dissolved in an aqueous buffer.]

A

B
2.3. Scheme S3: Two-step representation of Loop 2

Scheme S3. Simplified reaction scheme of the redox cycling with DMBQ.
3. Additional Figures

3.1. Figure S1: Screening of redox cyclers

Figure S1. Screening of the redox cycling properties of different quinones (the molecular structure of each of the quinones tested is shown above). The curves on the graph are the kinetics traces monitored at 262 nm for each quinone (1 µM) in the presence of ascorbate (200 µM) in a Tris-EDTA buffer (pH 8.5). Optical path length: 0.6 cm.
3.2. Figure S2: Spectrophotometric characterization of the reactivity and stability of P1

**Figure S2.** (A) UV-vis spectra of a freshly prepared dilute solution of P1 (50 µM) as a function of time. The starting spectrum is highlighted in black. All the subsequent spectra in red were recorded every 10 min over 10 h. The final spectrum is in blue. (B) Same experience as in A but after adding 1 equiv. H₂O₂ to the probe solution. The starting spectrum is highlighted in black. All the subsequent spectra in blue were recorded every 10 min over 10 h. The final spectrum is in red. (C) Evolution of the UV-vis spectrum of a 10 µM solution of DMBQ. The starting spectrum is highlighted in black and the subsequent spectra in red were recorded every 1 min over 1 h. The final spectrum is in blue. (D) UV-vis kinetic traces at 257 nm recovered from graphs A and B. All experiments were performed at 25°C in an air-saturated Tris-EDTA buffer (0.1 M, pH 8.5). Optical path length: 1 cm.
3.3. **Figure S3: Comparison of the reactivity of P₁ and S₂**

**Figure S3.** UV-vis kinetic traces recorded at 262 nm or 257 nm (wavelengths specific of 1,4-NQ or DMBQ, respectively) for solutions containing 50 µM of (dashed lines) S₂ or (solid lines) P₁. The black lines correspond to the probe alone in solution, while the red lines correspond to the probe solutions in the presence of 1 equiv. H₂O₂. All experiments were performed at 25°C in an air-saturated Tris-EDTA buffer at pH 8.5. Optical path length: 1 cm.
3.4. Figure S4: Spectrophotometric characterization of the reactivity and stability of P$_2$

**Figure S4.** (A) UV-vis spectra of a freshly prepared dilute solution of P$_2$ (50 µM) as a function of time. The starting spectrum is highlighted in black. All subsequent spectra in red were recorded every 10 min over 10 h. The final spectrum is in blue. (B) Same experience as in A but after adding 1 equiv. H$_2$O$_2$ to the probe solution. The starting spectrum is highlighted in black. All subsequent spectra in blue were recorded every 10 min over 10 h. The final spectrum is in red. (C) UV-vis kinetic traces at 257 nm recovered from graphs A and B. All experiments were performed at 25°C in an air-saturated Tris-EDTA buffer (0.1 M, pH 8.5). Optical path length: 1 cm.
3.5. Figure S5: DMBQ stability

Figure S5. Time-course absorbance change recorded at 257 nm for solutions of 50 µM DMBQ mixed with different concentrations of H$_2$O$_2$ (1.5-150 µM). The experiments were performed at 25°C in an air-saturated Tris-EDTA buffer (0.1 M, pH 8.5). Optical path length: 0.6 cm.
3.6. Figure S6: DMBQH$_2$ autoxidation

**Figure S6.** (A) UV-vis spectra of 50 µM DMBQ (blue line) and 50 µM DMBQH$_2$ (black line) in an aerated 0.1 M Tris-EDTA buffer (pH 8.5). The spectra in red show the evolution of the DMBQH$_2$ solution with time (recorded every 30 s, total time: 20 min). (B) (black dots) Absorbance change at 257 nm recovered from the spectra in A. The dashed red curve represents the best fit of an exponential law to the experimental data (Abs = Abs$_0$ x [1 - exp(-k$_{obs}$t)]) with k$_{obs}$ = 3.5 x 10$^{-3}$ s$^{-1}$). Optical path length: 1 cm.

3.7. Figure S7: Concentration dependence of the DMBQH$_2$ autoxidation kinetics

**Figure S7.** Autoxidation kinetics of DMBQH$_2$ followed by UV-visible spectroscopy at 257 nm at different initial concentrations (see the legend on the graph) in an air-saturated Tris-EDTA buffer (0.1 M, pH 8.5). The DMBQ concentration on the ordinate was determined from the recorded absorbance value and the extinction coefficient of DMBQ (Ɛ$_{DMBQ}$ = 21000 M$^{-1}$·cm$^{-1}$).
3.8. Figure S8: Electrochemistry of DMBQ

Figure S8. Cyclic voltammetric responses of DMBQ (50 µM) in (A) a deaerated or (B) air-saturated Tris-EDTA buffer (0.1 M, pH 8.5).

3.9. Figure S9: Simulations of DMBQH₂ autoxidation

Figure S9. Comparison of the (dashed) theoretical and (solid) experimental kinetics curves obtained for the autoxidation of DMBQH₂ at different concentrations (black: 5 µM; red: 10 µM; blue: 30 µM; and green: 50 µM) in an air-saturated Tris-EDTA buffer (pH 8.5). The theoretical kinetic curves were obtained by simulation of the autoxidation reaction scheme reported in paragraph “simulations” (set of reactions S0 to S7) and using the freeware COPASI and the rate constants gathered in entries 1 to 4 in Table S1 (see also the legend on the graph which indicates the corresponding entry).
3.10. **Figure S10: Simulations of Loop 1 for P<sub>1</sub>**

**Figure S10.** (A) Experimental kinetic plots (monitored at 257 nm) recorded during the deprotection of 50 µM P<sub>1</sub> by different concentrations of H<sub>2</sub>O<sub>2</sub> (see the legend on the graph) in an air-saturated Tris-EDTA buffer (pH 8.5). The DMBQ concentration on the ordinate was determined from the recorded absorbance value and the extinction coefficient of DMBQ ($\varepsilon_{\text{DMBQ}} = 21000$ M<sup>-1</sup>·cm<sup>-1</sup>). (B) Theoretical kinetic curves obtained from simulation of the catalytic deprotection reaction scheme reported in paragraph "simulations" (set of reactions S0 to S9) and using the freeware COPASI and the rate constants gathered in Table S2, entry 5.

3.11. **Figure S11: Simulations of Loop 1 for P<sub>2</sub>**

**Figure S11.** (A) Experimental kinetic plots (monitored at 257 nm) recorded during the deprotection of 50 µM P<sub>2</sub> by different concentrations of H<sub>2</sub>O<sub>2</sub> (see the legend on the graph) in an air-saturated Tris-EDTA buffer (pH 8.5). The DMBQ concentration on the ordinate was determined from the recorded absorbance value and the extinction coefficient of DMBQ ($\varepsilon_{\text{DMBQ}} = 21000$ M<sup>-1</sup>·cm<sup>-1</sup>). (B) Theoretical kinetic curves obtained from simulation of the catalytic deprotection reaction scheme reported in paragraph "simulations" (set of reactions S0 to S9) and using the freeware COPASI and the rate constants gathered in Table S2, entry 6.
Figure S12: Redox cycling experiments

Figure S12. Time-course absorbance change recorded at 262 nm (corresponding to the monitoring of ascorbate concentration) during the redox cycling of DMBQ at different concentrations (reported on the top of each graph) and also as a function of different ascorbate concentrations (see the legend on the graphs) in an air-saturated Tris-EDTA buffer (0.1 M, pH 8.5). The dashed black curves are the nonlinear regression fits of a monoexponential decay function to the experimental plots from which the value of $k_{obs}$ was extracted. Each experimental curve is the average of triplicates.
3.13. Figure S13: Variation of $k_{obs}$ with DMBQ concentration

![Figure S13](image)

**Figure S13.** (A) Plot of the observed rate constants $k_{obs}$ as a function of DMBQ concentration. (B) Plot of the apparent second order rate constant $k_{red,app}$ as a function of the ascorbate concentration.

3.14. Figures S14 and S15: Simulations of the redox cycling Loop 2

![Figure S14](image)

**Figure S14.** (A) Experimental kinetic traces (monitored by UV-visible spectroscopy at 262 nm) recorded during the redox cycling of DMBQ at different concentrations (see the legend of the graphs) in the presence of 50 µM ascorbate in an air-saturated Tris-EDTA buffer (pH 8.5). (B) Theoretical kinetics traces obtained by simulation of the redox cycling reaction reported in paragraph “simulations” (set of reactions S0 to S7 + S10 to S15) and using the freeware COPASI and the rate constants listed in Table S3, entry 7.
Figure S15. (Left) Experimental kinetic traces (monitored by UV-visible spectroscopy at 262 nm) recorded during the redox cycling of DMBQ at different concentrations of DMBQ and ascorbate (see the legend on the graphs) in an air-saturated Tris-EDTA buffer (pH 8.5). (Right) Theoretical kinetics obtained by simulation of the redox cycling reaction reported in paragraph “simulations” (set of reactions S0 to S7 + S10 to S15) and using the freeware COPASI and the rate constants listed in Table S3, entry 7.
3.15. **Figure S16**: Simulations of the dependence of the apparent rate $k_{\text{red,app}}$ with the ascorbate concentration

![Graph showing the dependence of $k_{\text{red,app}}$ on ascorbate concentration](image)

**Figure S16**. (black squares) Experimental plot of the rate constant $k_{\text{red,app}}$ (same data than in Figure S13) as a function of ascorbate concentration. Theoretical plots obtained by simulations using the freeware COPASI from the set of reactions (red circles) S0 to S7 + S10 to S15 with $O_2$ replenishment or (green triangles) S0 to S7 + S10 to S14 without $O_2$ replenishment. The rate constants are listed in Table S3, entry 7.

3.16. **Figure S17**: Redox cycling with the diaphorase/NADH couple

![Graph showing time-course absorbance change](image)

**Figure S17**. Time-course absorbance change recorded at 340 nm (corresponding to the monitoring of NADH concentration) during the redox cycling of different DMBQ concentrations (see the legend on graph) in the presence of diaphorase (10 nM) /NADH (250 μM) as reducing agent in an air-saturated Tris-EDTA buffer at pH 8.5. Optical path length: 0.6 cm.
3.17. Figure S18: Simulations of autocatalysis with P₁ triggered by H₂O₂

![Experimental and Simulated Kinetic Traces](image)

**Figure S18.** (Left, top) Autocatalytic kinetic traces recorded at 262 nm during the reaction of 2.5 μM P₁ with 50 μM ascorbate and different triggering concentrations of H₂O₂ in an air-saturated Tris-EDTA buffer (pH 8.5). (Left, bottom) Semi-logarithmic calibration curve obtained from the plot of t₁/₂ (recovered from the crossing of the horizontal line on the left top graph, which thus corresponds to the time for which the absorbance is decrease half the maximal value) as a function of H₂O₂ concentration. (Right, top) Theoretical autocatalytic kinetics obtained by simulation of the autocatalytic reaction scheme reported in the paragraph "simulations" (set of reactions S₀ to S₁₅) and using the freeware COPASI. (Right, bottom) Theoretical semi-logarithmic calibration curve obtained from the plot of t₁/₂ (recovered from the crossing of the horizontal line on the right top graph) as a function of H₂O₂ concentration. The rate constants values used for the simulations are listed in Table S₄, entry 8.
3.18. **Figures S19 and S20: Simulations of autocatalysis with P2 triggered by H2O2**

**Figure S19.** (Left, top) Autocatalytic kinetic traces recorded at 262 nm during the reaction of 2.5 μM P2 with 50 μM ascorbate and different triggering concentrations of H2O2 in an air-saturated Tris-EDTA buffer (pH 8.5). (Left, bottom) Experimental semi-logarithmic calibration curve obtained from the plot of threshold times \( t_{1/2} \) as a function of H2O2 concentration. (Right, top) Theoretical autocatalytic kinetics obtained by simulation of the autocatalytic reaction scheme reported in the paragraph “simulations” (set of reactions S0 to S15) and using the freeware COPASI. (Right, bottom) Theoretical semi-logarithmic calibration curve obtained from the plot of \( t_{1/2} \) as a function of H2O2 concentration. The rate constants values used for the simulations are listed in Table S4, entry 9.
Figure S20. (Left, top) Autocatalytic kinetic traces recorded at 262 nm during the reaction of 50 μM P1 with 0.5 mM ascorbate and different concentrations of H2O2 in an air-saturated Tris-EDTA buffer (pH 8.5). (Left, bottom) Experimental semi-logarithmic calibration curve obtained from the plot of threshold times $t_{1/2}$ as a function of H2O2 concentration. (Right, top) Theoretical autocatalytic kinetics obtained by simulation of the autocatalytic reaction scheme reported in the paragraph “simulations” (set of reactions S0 to S15) and using the freeware COPASI. (Right, bottom) Theoretical semi-logarithmic calibration curve obtained from the plot of $t_{1/2}$ as a function of H2O2 concentration. The rate constants values used for the simulations are listed in Table S4, entry 9.
3.19. **Figures S21 and S22: Autocatalysis with P₁ or P₂ triggered by DMBQ**

**Figure S21.** (A) Autocatalytic kinetic traces recorded at 262 nm during the reaction of 2.5 μM P₁ with 50 μM ascorbate and different concentrations of DMBQ (ranging from 0 to 1000 nM) in an air-saturated Tris-EDTA buffer (pH 8.5). (B) Semi-logarithmic calibration curve obtained from the plot of $t_{1/2}$ as a function of DMBQ concentration.

**Figure S22.** (A) Autocatalytic kinetic traces recorded at 262 nm during the reaction of 2.5 μM P₂ with 50 μM ascorbate and different concentrations of DMBQ (ranging from 0 to 1000 nM) in an air-saturated Tris-EDTA buffer (pH 8.5). (B) Semi-logarithmic calibration curve obtained from the plot of $t_{1/2}$ as a function of DMBQ concentration.
4. Simulations

Numerical simulations were performed using the freeware COPASI (http://copasi.org).

4.1. Simulation of the DMBQH$_2$ autoxidation

It is well established that the autoxidation mechanism of hydroquinones is much more complex than a simple bimolecular reaction. It is indeed an autoinductive radical chain mechanism, in which the semiquinone (Q$^\cdot$) and superoxide anion are involved as chain propagating species as follows:\textsuperscript{1,2}

\begin{align*}
\text{Initiation} & : \quad \text{QH}_2 + \text{O}_2 \xrightleftharpoons[k_{-1}^{\text{dis}}]{k_1} \text{Q}^\cdot + 2 \text{H}^+ + \text{O}_2^{2-} & (S0) \\
\text{Comproportionation/dismutation} & : \quad \text{QH}_2 + \text{Q} \xrightleftharpoons[k_{-1}^{\text{dis}}]{k_1} 2 \text{Q}^\cdot + 2 \text{H}^+ & (S1) \\
\text{Semiquinone autoxidation} & : \quad \text{Q}^\cdot + \text{O}_2 \xrightleftharpoons[k_2]{k_1} \text{Q} + \text{O}_2^{2-} & (S2) \\
\text{Hydroquinone superoxidation} & : \quad \text{QH}_2 + \text{O}_2^{2-} \xrightarrow{k_1} \text{Q}^\cdot + \text{H}_2\text{O}_2 & (S3) \\
\text{Superoxide dismutation} & : \quad 2 \text{O}_2^{2-} \xrightarrow{k_{\text{dis}}} \text{O}_2 + \text{H}_2\text{O}_2 & (S4) \\
\text{Cross-dismutation} & : \quad \text{Q}^\cdot + \text{O}_2^{2-} + 2 \text{H}^+ \xrightarrow{k_2} \text{Q} + \text{H}_2\text{O}_2 & (S5)
\end{align*}

Autoxidation chain reaction starts from the single-electron transfer between O$_2$ and QH$_2$ (initiation or reaction S0), that is known to be quite slow under near-neutral conditions because spin-restricted.\textsuperscript{3} However, this initiation allows the production of the chain propagating species Q$^\cdot$ and O$_2^{2-}$, which participates in a set of faster reactions, involving either the semiquinone (reactions S1 and S2), the anion superoxide (reaction S3 and S4) or both (reaction S5). The rate acceleration of this mechanism originates from the product Q being the catalyst of its own formation through the comproportionation reaction S1. Actually, this may or may not be observed for a given quinone depending on the conditions and, most importantly, on the purity of QH$_2$ since small impurities of Q allow to drastically change the response and hide the phenomena.\textsuperscript{2} The simulations were carried out by taking into account reactions S0 to S5 and different set of kinetic rate constants (entry 2 to 4 in Table S1). For those of reactions S0 to S5 that are pH-dependent, we used rate constants at an effective pH (here pH 8.5). For the sake of comparison, we also simulated a simple bimolecular reaction with a constant $k_{\text{ox}}$ of 14 M$^{-1}$·s$^{-1}$ (entry 1).

To start, we collected data available in the literature for the different reactions (entry 2), mostly from ref 2 where it was either measured or calculated under physiological conditions. Concerning $k_0$, no value was found in the literature because of the difficulty to determine it independently. For our simulations, we have thus considered $k_0$ as always sufficiently small that it has no significant influence on the simulations. A value of 0.00001 M$^{-1}$·s$^{-1}$ was thus systematically used. In addition, several side reactions leading to the degradation of DMBQ can occur, as observed in Figure S5. We simulated the quinone degradation through the following set of two different reactions:

\begin{align*}
\text{Quinone self-degradation} & : \quad 2\text{Q} \xrightleftharpoons[k_2]{k_1} \text{P} & (S6) \\
\text{Quinone peroxidation} & : \quad \text{Q} + \text{H}_2\text{O}_2 \xrightarrow{k_1} \text{P'} & (S7)
\end{align*}

Which indeed correspond to “self-degradation” (reaction S6) accounting for the reactivity in the buffer alone, and a “peroxidation” (reaction S7) accounting for the reactivity in the presence of H$_2$O$_2$. No
detailed studies have been done on the real chemical transformations occurring here so these reactions are just phenomenologically relevant.

**Table S1. Rate constants values used for the different autoxidation kinetic simulations of DMBQH₂.**

<table>
<thead>
<tr>
<th>DMBQH₂ autoxidation reactions</th>
<th>Simulation entry</th>
<th>Rate constant</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initiation</td>
<td>$k_0$ (M⁻¹s⁻¹)</td>
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<td>0.00001</td>
<td>0.00001</td>
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<td></td>
</tr>
<tr>
<td>Comproportionation/dismutation</td>
<td>$k_{\text{ox}}$ : $k_{\text{red}}$</td>
<td>50 ; $1.15 \times 10^4$ [4]</td>
<td>50 ; $1.15 \times 10^8$</td>
<td>50 ; $2 \times 10^8$</td>
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<td></td>
</tr>
<tr>
<td>Q⁻/O₂</td>
<td>$k_2$ (M⁻¹s⁻¹)</td>
<td>$1 \times 10^7$ ; $2.2 \times 10^4$ [2]</td>
<td>$1 \times 10^7$ ; $2.2 \times 10^8$</td>
<td>$2 \times 10^7$ ; $8 \times 10^8$</td>
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</tr>
<tr>
<td>QH⁻/ O₂⁻</td>
<td>$k_5$ (M⁻¹s⁻¹)</td>
<td>$1 \times 10^5$ [2]</td>
<td>$5 \times 10^5$</td>
<td>$1 \times 10^5$</td>
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<tr>
<td>Superoxide dismutation</td>
<td>$k_4$ (M⁻¹s⁻¹)</td>
<td>$10^6$ [5]</td>
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<tr>
<td>Chain termination</td>
<td>$k_5$ (M⁻¹s⁻¹)</td>
<td>$1 \times 10^5$ [2]</td>
<td>$5 \times 10^8$</td>
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</tr>
<tr>
<td>Side reactions</td>
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<td>$k_6$ (M⁻¹s⁻¹)</td>
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<tr>
<td>Peroxidation</td>
<td>$k_7$ (M⁻¹s⁻¹)</td>
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</table>

Figure S9 presents the simulations associated to entry 1 to 4. Simulations from entry 1 is not satisfying, especially for low concentrations for which no lag is observed. This confirms the utility of considering the autoxidation process in all its complexity. Results from entry 2 (complete mechanism with values from the literature) are able to account for the self-acceleration of the process. However, it is clear that the kinetic constants have to be adjusted since the simulated kinetic curves are too slow compared to the experimental ones. Entry 3 differs from entry 2 only for reactions S3 and S5 that are accelerated, leading to the overall acceleration of the process. Except for the 10µM, this leads in general to quite good simulations compared to experimental data. In entry 4, only reaction S2 has been accelerated in both forward and backward directions along with an increase of the equilibrium constant. This last set of kinetic constants (entry 4) is the best fit of our experimental data and therefore it was selected for the simulations of loops 1 and 2.

### 4.2. Simulation of the catalytic probe deprotection (Loop 1)

The simulations were carried out by taking into account reactions S0 to S7 for the autoxidation process together with the two following reactions accounting for both the deprotection step (Reaction S8) and probe instability (Reaction S9), the latter leading to the non-specific release of hydroquinone.

Deprotection: $\text{pro-QH}_2 + \text{H}_2\text{O}_2 \xrightarrow{k_{\text{prot}}} \text{QH}_2$

(8)

Instability: $\text{pro-QH}_2 \xrightarrow{k_{\text{inst}}} \text{QH}_2$

(9)

The simulations using the rate constants tabulated in Table S2 are presented in Figures S10 and S11.
### Table S2. Rate constant values used for the simulation of Loop 1.

<table>
<thead>
<tr>
<th>Simulation entry</th>
<th>Rate constant</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( k_\text{ox} ) (M(^{-1}) s(^{-1}))</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initiation</td>
<td>( k_0 ) (M(^{-1}) s(^{-1}))</td>
<td>0.00001</td>
<td>0.00001</td>
</tr>
<tr>
<td>DMBQH(_2) autoxidation reactions</td>
<td>( k_\text{ox,1} : k_\text{dis,1} ) (M(^{-1}) s(^{-1}))</td>
<td>50 ; (2 \times 10^8)</td>
<td>50 ; (2 \times 10^8)</td>
</tr>
<tr>
<td>Q(^{-})/O(_2)</td>
<td>( k_2 : k_2 ) (M(^{-1}) s(^{-1}))</td>
<td>(2 \times 10^8); (8 \times 10^8)</td>
<td>(2 \times 10^8); (8 \times 10^8)</td>
</tr>
<tr>
<td>QH(_2)/O(_2)^(-)</td>
<td>( k_3 ) (M(^{-1}) s(^{-1}))</td>
<td>(1 \times 10^5)</td>
<td>(1 \times 10^5)</td>
</tr>
<tr>
<td>Superoxide dismutation</td>
<td>( k_4 ) (M(^{-1}) s(^{-1}))</td>
<td>(10^4)</td>
<td>(10^4)</td>
</tr>
<tr>
<td>Chain termination</td>
<td>( k_5 ) (M(^{-1}) s(^{-1}))</td>
<td>(1 \times 10^8)</td>
<td>(1 \times 10^8)</td>
</tr>
<tr>
<td>Side reactions</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Self-degradation</td>
<td>( k_6 ) (M(^{-1}) s(^{-1}))</td>
<td>0.8</td>
<td>0.8</td>
</tr>
<tr>
<td>Peroxidation</td>
<td>( k_7 ) (M(^{-1}) s(^{-1}))</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Probe reactivity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deprotection</td>
<td>( k_\text{dep} ) (M(^{-1}) s(^{-1}))</td>
<td>17</td>
<td>1.2</td>
</tr>
<tr>
<td>Instability</td>
<td>( k_\text{inst} ) (s(^{-1}))</td>
<td>(8 \times 10^{-7})</td>
<td>(10^{-8})</td>
</tr>
<tr>
<td>O(_2) renewal</td>
<td>( k_{15} : k_{15} ) (s(^{-1}))</td>
<td>0.025 ; 100</td>
<td>0.025 ; 100</td>
</tr>
</tbody>
</table>

### 4.3. Simulation of the redox cycling reaction (Loop 2).

If we first hypothesize that Loop 2 can be formally approximated by the simplified redox cycling reaction depicted in Scheme S3, under only two basic assumptions (i.e., a steady-state is rapidly reached for both forms of the redox cycler, and O\(_2\) concentration in solution remains approximately constant due to its continuous and rapid renewal from the air surrounding the open microwells), it can be established that the variation of ascorbate concentration in solution with time is governed by the following rate equation:

\[
-\frac{d [\text{Asc}]}{dt} = \frac{[\text{DMBQ}]_0}{k_\text{ox} [O_2]_0 + k_\text{red} [\text{Asc}]} = k_\text{redox} [\text{DMBQ}]_0
\]

with 
\[
\frac{1}{k_\text{redox}} = \frac{1}{k_\text{ox} [O_2]_0} + \frac{1}{k_\text{red} [\text{Asc}]}
\]

Which, after integration, leads to

\[
\frac{[\text{Asc}]_0 - [\text{Asc}]}{k_\text{ox} [O_2]_0} + \frac{1}{k_\text{red} [\text{Asc}]} \ln \frac{[\text{Asc}]_0}{[\text{Asc}]} = [\text{DMBQ}]_0 t
\]  
(eq. S1)

From there, two limiting situations can be encountered, leading to two very different kinetic responses. On the one hand, if the autoxidation rate of DMBQH\(_2\) is much slower than the reduction
rate of DMBQ by ascorbate, the concentration of the latter is expected to be linearly consumed until full depletion (control by the oxidation step with zeroth order on ascorbate, eq. S2).

Control by the oxidation step: \[ [\text{Asc}] = [\text{Asc}]_0 - k_{\text{ox}} [\text{O}_2]_0 [\text{DMBQ}]_0 t \] (eq. S2)

On the other hand, if the autoxidation rate of DMBQH\(_2\) is much faster than the reduction rate of DMBQ by ascorbate, an exponential decay of its concentration is predicted (control by the reduction step with first order on ascorbate, eq. S3).

Control by the reduction step: \[ [\text{Asc}] = [\text{Asc}]_0 \exp(-k_{\text{red}} [\text{DMBQ}]_0 t) \] (eq. S3)

As shown in Figure S12, the time-course of the redox cycling reaction was monitored spectrophotometrically through the absorbance decrease of ascorbate at 262 nm as a function of different DMBQ and Asch\(^-\) concentrations in an air-saturated Tris-EDTA buffer (pH 8.5). A systematic exponential decrease of absorbance was observed until almost complete extinction of absorbance, a behaviour being thus in line with a redox cycling process that is rate-controlled by the reduction reaction (conditions predicted by the equation S3). Experimental kinetic plots in Figure S12 were then each fitted to a single exponential decay (dotted curves on the graphs of Figure S12) with the following equation:

Single exponential decay: \[ \text{Abs} = \text{Abs}_0 \exp(-k_{\text{obs}} t) \] (eq. S4)

Plotting \(k_{\text{obs}}\) as a function of \([\text{DMBQ}]_0\) shows a quasi-linearity for each ascorbate concentration (graph A in Figure S13). The slope of the linear regressions on the left graph allows the determination of an apparent second order rate constant \(k_{\text{red,app}}\) that can be reported as a function of the ascorbate concentration (graph B in Figure S13). The plot shows that \(k_{\text{red,app}}\) is not constant, even indicating a clear tendency to decrease as the ascorbate concentration is increased, a behaviour that was also previously observed in the case of 1,4-NQ and which suggests a more complicated redox cycling mechanism than the simple one proposed above.\(^6,7,8\)

The quinone-based catalytic oxidation of ascorbate in aerobic conditions has been again thoroughly studied by Roginsky.\(^9\) On top of reactions S1 to S5, a complementary set of equations is required to correctly describe it. Several species in the system may undergo single-electron reduction by ascorbate (Asch\(^-\)): the quinone (reaction S10), the semiquinone (reaction S11), and the superoxide anion (reaction S12). These single electron-transfer reactions result in the release of ascorbyl radical anion (Asc\(^-\)), the latter being capable to dismutate into dehydroascorbate (DHA) and ascorbate (reaction S13). In addition, ascorbate is also susceptible to autoxidation (reaction S14).

1. Quinone reduction
   \[ Q + \text{AscH}^- \overset{k_{\text{ox}}}{\underset{k_{\text{red}}}{\rightleftharpoons}} Q^- + H^+ + \text{Asc}^- \] (S10)

2. Semiquinone reduction
   \[ Q^- + \text{AscH}^- + H^+ \overset{k_{\text{ox}}}{\underset{k_{\text{red}}}{\rightleftharpoons}} \text{QH}_2^- + \text{Asc}^- \] (S11)

3. Superoxide reduction
   \[ \text{O}_2^- + \text{AscH}^- + H^+ \overset{k_{\text{ox}}}{\underset{k_{\text{red}}}{\rightleftharpoons}} \text{H}_2\text{O}_2^- + \text{Asc}^- \] (S12)
Simulations have been performed with the rate constants of entry 7 in the table S3 after several rounds of iterative adjustments as described below:

**Table S3. Rate constant values used for the redox cycling kinetic simulations.**

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Rate constant</th>
<th>Simulation entry</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ascorbyl dismutation</strong></td>
<td>2 Asc⁺⁺ → Asc⁻⁻ + DHA</td>
<td>(S13)</td>
</tr>
<tr>
<td><strong>Ascorbate autoxidation</strong></td>
<td>O₂ + Asc⁻⁻ → O₂⁺⁺ + Asc⁺⁺ + H⁺</td>
<td>(S14)</td>
</tr>
</tbody>
</table>

- **DMBQH₂ autoxidation reactions**
  - Initiation: \( k_0 (M^{-1} \cdot s^{-1}) \) 0.00001
  - Comproportionation/dismutation: \( k_{iso} : k_{dbiso} (M^{-1} \cdot s^{-1}) \) 50 ; \( 2 \times 10^8 \)
  - \( Q^+/O_2 \): \( k_2 : k_2 (M^{-1} \cdot s^{-1}) \) 2 × 10⁴ ; \( 8 \times 10^9 \)
  - \( QH_2/O_2^+ \): \( k_3 (M^{-1} \cdot s^{-1}) \) 1 × 10⁵
  - Superoxide dismutation: \( k_4 (M^{-1} \cdot s^{-1}) \) 1 × 10⁴
  - Chain termination: \( k_5 (M^{-1} \cdot s^{-1}) \) 1 × 10⁵

- **Side reactions**
  - Self-degradation: \( k_6 (M^{-1} \cdot s^{-1}) \) 0.8
  - Peroxidation: \( k_7 (M^{-1} \cdot s^{-1}) \) 0.1

- **Ascorbate reduction reactions**
  - Quinone reduction: \( k_{90} : k_{90} (M^{-1} \cdot s^{-1}) \) 4000 ; \( 1 \times 10^8 \)
  - Semiquinone reduction: \( k_{11} : k_{11} (M^{-1} \cdot s^{-1}) \) 4.5 × 10^3 ; \( 2.2 \times 10^4 \)
  - Superoxide reduction: \( k_{12} (M^{-1} \cdot s^{-1}) \) 5 × 10⁴
  - Ascorbyl dismutation: \( k_{13} (M^{-1} \cdot s^{-1}) \) 1 × 10⁶
  - Ascorbate autoxidation: \( k_{14} (M^{-1} \cdot s^{-1}) \) 0.32

- **O₂ renewal**
  - \( k_{15} , k_{15} (s^{-1}) \) 0.025 ; 100

**Reaction S10:**
The forward reaction rate between the ascorbate and the quinone is particularly important since it controls the redox cycling. The determined apparent rate constant \( k_{red,app} \) has values ranging between 700 and 1600 M⁻¹·s⁻¹, with an average value around 1150 M⁻¹·s⁻¹. According to Roginsky,⁶ this value of the apparent rate constant is expected to be lower than \( k_{10} \). The value was adjusted to 4000 M⁻¹·s⁻¹ in the entry 7 of table S3 in order to obtain the best fit. For the reverse reaction involving the two radicals, Roginsky measured by pulse radiolysis at pH 7.4 in 50 mM phosphate buffer a value of \( k_{30} = 2.1 \times 10^8 \) M⁻¹·s⁻¹ for.⁴ This value was slightly adjusted to \( 10^8 \) M⁻¹·s⁻¹ in the entry 7 of table S3, a value that is in line with its expected tendency to decrease with pH.

**Reaction S11:**
The kinetic constant of the outer-sphere one-electron transfer reaction S11 (i.e., \( k_{13} \)) is important because this reaction transforms the highly reactive autoxidation chain propagating semiquinone
into the poorly reactive QH₂. Typically, this reaction explains the tendency of the apparent redox cycling rate constant \( (k_{\text{red,app}}) \) to decrease with the ascorbate concentration increase as observed experimentally (Figure 2B). Again, Roginsky measured it by pulse radiolysis at pH 7.4 in 50 mM phosphate buffer and reported for \( k_{11} \) a value of \( 10^5 \text{ M}^{-1}\text{s}^{-1} \). To obtain the best fits, \( k_{11} \) was adjusted to \( 4.5 \times 10^4 \text{ M}^{-1}\text{s}^{-1} \) in the entry 7 of Table S3, a value that is in contradiction with the expected tendency to increase with pH. Since the reaction is reversible, an equilibrium constant \( K_{11} \) can be determined from the reduction potentials of the Q⁻/Q₂⁻ couple (+363 mV) and the Asc⁻/AscH⁻ couple (+282 mV), through the following relationship:

\[
\Delta E_{11} = E^{0'}_{\text{Q}^-/\text{Q}_2^-} - E^{0'}_{\text{Asc}^-/\text{AscH}^-} = \frac{RT}{F} \ln K_{11}
\]

A value of 22.5 was estimated, which then allows to calculate \( k_{-11} = 2 \times 10^4 \text{ M}^{-1}\text{s}^{-1} \).

- **Reaction S12:**
  This reaction allows to terminate the autoxidation chain propagation through reduction of the superoxide. However, its effect too slow down the autoxidation reaction is less significant than reaction S11 since it does not lead to the poorly reactive intermediate QH₂. The value of \( k_{12} \) at pH 7 (i.e., \( 5 \times 10^6 \text{ M}^{-1}\text{s}^{-1} \)) was recovered from the literature and used in the simulation without further adjustment.⁹

- **Reaction S13:**
  The value of \( k_{13} \) at pH 7 (i.e., \( 10^6 \text{ M}^{-1}\text{s}^{-1} \)) was also recovered from the literature.⁹ The high driving force between the one-electron reduction potentials \( E^{0'}(\text{DHA/Asc}^-) \) and \( E^{0'}(\text{Asc}^-/\text{AscH}^-) \) (-174 mV and +282 mV, respectively) makes the reverse reaction very slow (estimated at \( 0.7 \text{ M}^{-1}\text{s}^{-1} \) at pH 7) and justify that reaction S13 can be considered as irreversible.

- **Reaction S14:**
  Ascorbate was reported to autoxidize very slowly in the absence of a catalyst.⁹¹¹ A bimolecular rate constant of \( 0.32 \text{ M}^{-1}\text{s}^{-1} \) was experimentally evaluated by monitoring the slow decrease of ascorbate absorbance in the Tris-EDTA aerated buffer.

Figures S14 and S15 illustrate the good adequation between the simulated and experimental kinetics. Previously, the dependence of \( k_{\text{red,app}} \) with the ascorbate concentration was extracted from the experimental data (see Figure 2B). A similar procedure can be applied to the simulated data, leading to the results presented in Figure S16. Once again it shows clearly the validity of our kinetic model.

Figure S16 also aims at illustrating the importance of the fast renewal of O₂ within the solution of the open microwell to maintain approximately constant its concentration during redox cycling reaction. In terms of simulation, the renewal of O₂ was considered though the equilibrium reaction 15 which mimics an O₂ reservoir through the species A (that aims at accounting for the gas phase exchanging O₂ at the liquid/air interface).

\[
\text{Regeneration of O}_2: \quad A \xrightleftharpoons[k_{11}]{k_{15}} \text{O}_2 \quad (S15)
\]

Results of simulations with the kinetic set of entry 7, with and without considering this O₂ renewal, are compared (Figure S16). It clearly reveals that O₂ renewal plays an important role for the redox cycling reaction.
4.4. Simulation of the autocatalysis

Simulation of the autocatalytic process requires to consider all the above reactions and associated kinetic rate constants (Reactions S0 to S15). We therefore performed the numerical simulations of the kinetics of AscH\textsubscript{1}, using the rate constant listed in entries 8 and 9 in Table S4. The simulations are reported in Figures S18 to S20 for comparison to the experimental data. The shape of the simulated kinetic traces is as expected, characteristic of an auto-catalytic reaction on account of:

- a lag phase which depends on the initial H\textsubscript{2}O\textsubscript{2} concentration and during which the ascorbate concentration remains approximately constant;

- an exponential phase which characterizes the accelerated decrease of the ascorbate concentration;

- a final phase that level-off as the ascorbate is almost completely consumed.

Table S4. Rate constant values used for the autocatalysis kinetic simulations.

<table>
<thead>
<tr>
<th>DMBQH\textsubscript{2} autoxidation reactions</th>
<th>Simulation entry</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rate constant</td>
<td>8</td>
</tr>
<tr>
<td>(k_{\text{ox}}) (M\textsuperscript{-1} · s\textsuperscript{-1})</td>
<td>0.00001</td>
</tr>
<tr>
<td>Initiation</td>
<td>(k_{\text{0}}) (M\textsuperscript{-1} · s\textsuperscript{-1})</td>
</tr>
<tr>
<td>Comproportionation/dismutation Q\textsuperscript{-}</td>
<td>(k_{1} ; k_{2}) (M\textsuperscript{-1} · s\textsuperscript{-1})</td>
</tr>
<tr>
<td>Q\textsuperscript{-}/O\textsubscript{2}</td>
<td>(k_{3}) (M\textsuperscript{-1} · s\textsuperscript{-1})</td>
</tr>
<tr>
<td>Superoxide dismutation</td>
<td>(k_{4}) (M\textsuperscript{-1} · s\textsuperscript{-1})</td>
</tr>
<tr>
<td>Chain termination</td>
<td>(k_{5}) (M\textsuperscript{-1} · s\textsuperscript{-1})</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Side reactions</th>
<th>Simulation entry</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rate constant</td>
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</tr>
<tr>
<td>Self-degradation</td>
<td>(k_{6}) (M\textsuperscript{-1} · s\textsuperscript{-1})</td>
</tr>
<tr>
<td>Peroxidation</td>
<td>(k_{7}) (M\textsuperscript{-1} · s\textsuperscript{-1})</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Probe reactivity</th>
<th>Simulation entry</th>
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</thead>
<tbody>
<tr>
<td>Rate constant</td>
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</tr>
<tr>
<td>Deprotection</td>
<td>(k_{\text{dep}}) (M\textsuperscript{-1} · s\textsuperscript{-1})</td>
</tr>
<tr>
<td>Instability</td>
<td>(k_{\text{inst}}) (s\textsuperscript{-1})</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ascorbate reduction reactions</th>
<th>Simulation entry</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rate constant</td>
<td>8</td>
</tr>
<tr>
<td>Quinone reduction</td>
<td>(k_{10} ; k_{10}) (M\textsuperscript{-1} · s\textsuperscript{-1})</td>
</tr>
<tr>
<td>Semiquinone reduction</td>
<td>(k_{11} ; k_{11}) (M\textsuperscript{-1} · s\textsuperscript{-1})</td>
</tr>
<tr>
<td>Superoxide reduction</td>
<td>(k_{12}) (M\textsuperscript{-1} · s\textsuperscript{-1})</td>
</tr>
<tr>
<td>Ascorbyl dismutation</td>
<td>(k_{13}) (M\textsuperscript{-1} · s\textsuperscript{-1})</td>
</tr>
<tr>
<td>Ascorbate autoxidation</td>
<td>(k_{14}) (M\textsuperscript{-1} · s\textsuperscript{-1})</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>O\textsubscript{2} renewal</th>
<th>Simulation entry</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rate constant</td>
<td>8</td>
</tr>
<tr>
<td>(k_{15} ; k_{15}) (s\textsuperscript{-1})</td>
<td>0.025 ; 100</td>
</tr>
</tbody>
</table>
5. References

6. V. A. Roginsky, G. Bruchelt, H. B. Stegmann, Fully reversible redox cycling of 2,6-dimethoxy-1,4-benzoquinone induced by ascorbate, Biochemistry (Mosc.), 1998, 63, 200-206.