An autocatalytic organic reaction network based on cross-catalysis

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Here we report a simple autocatalytic organic reaction network based on the redox chemistry of quinones and reactive oxygen species. Autocatalysis arises from the cross-activation between the H₂O₂-catalyzed deprotection of a pro-benzoquinone arylboronic ester probe and the benzoquinone-catalyzed H₂O₂ production through redox cycling with ascorbate in an aerated buffered solution.

Autocatalysis, a process in which the chemical product accelerates its own production,¹⁻⁴ is encountered in both natural and synthetic systems, with relevance in the chemistry of life (prebiotic chemistry,⁵ homochirality,⁶ ...), and information processing (molecular amplification,⁷ responsive materials,^{3,8} oscillating systems,^{5b} ...). Examples of synthetic systems have been so far largely fueled by inorganic (and mostly redox) autocatalytic reactions, but further developments are hampered by the complexity of their mechanism and their narrow chemical diversity as compared to small organic molecules-based reactions.³ Typical examples of organic autocatalysis are the popular and mechanistically more simple acid autocatalysis⁹ or autocatalysis based on template replication.³ In parallel, autocatalytic organic reaction networks emerge as a promising playground to rationally design new autocatalytic reactions.³ An illustrative example is the dendrimer chain reaction (DCR), a type of branched-chain reaction developed in the groups of Shabat, Philipps or Anslyn over the last decade.^{7,10,11} Briefly, the concept relies on the simultaneous release of multiple equivalents of the trigger from the dendritic disintegration of a molecular reservoir probe. Another example of an autocatalytic organic reaction network was provided by Whitesides and his colleagues taking advantage of self-propagating thiolate-disulfide exchange in view to build networks showing bistability or oscillations in a continuous-stirred tank reactor.^{5b} An elegant variation in which the propagation involves a Meldrum acid derivative was lately proposed by Anslyn for the detection of VX nerve agent.¹² Alternatively, autocatalytic reactions wherein an organic ligand activates the metal-based catalyst of its own production have also been proposed.^{13,14} Finally, photoactivated autocatalytic reaction schemes were recently described.^{15,16} A simple autocatalytic set that so far has only been marginally explored is by cross-catalysis.^{2,17,18}

Here, we propose such an autocatalytic reaction scheme based on the redox chemistry of reactive oxygen species (ROS) and quinones (Scheme 1). Conceptually, the autocatalytic behavior

of this chemical network arises from the cross-activation of two catalytic loops,^{19,20} each producing the catalyst of the other, *i.e.* H₂O₂ catalyzes the production of a quinone through Loop 1 while the quinone catalyzes the production of H₂O₂ through Loop 2. The system relies on a properly designed arylboronic ester probe that serves as a substrate reservoir in Loop 1 in order to release

Scheme 1. Cross-catalytic reaction scheme illustrated for the arylboronic probes P₁ and P₂, and the reducing agent ascorbate (Asch⁻). In this scheme, H₂O₂ and 2,6-dimethyl-para-benzoquinone (DMBQ) play the role of catalyst (*i.e.*, Cat₁ and Cat₂) in loops 1 and 2, respectively, while DHA is the two-electron oxidized form of ascorbate, *i.e.* the dehydroascorbate. Dashed arrows does not represent reactions but are used to evidence the cross-catalytic relationship.

quinone that subsequently redox cycle in the presence of a reducing agent (in the present work, ascorbate or Asch⁻) and dissolved molecular oxygen (Loop 2). Ideally, to fully take advantage of the high amplification rates and wide dynamic response offered by such an autocatalytic approach, the starting components must possess a high level of stability to avoid self-start of the system.¹⁹ In a previous report,²⁰ we were confronted with the instability of our H₂O₂-sensitive probes built around a redox cycling naphthohydroquinone core masked by a boronic ester function (the molecular structure of these probes are recalled in Scheme S1 of SI). In the search for a stable and reactive probe, we speculated that more stable probes could be obtained from benzoquinone derivatives directly masked by a boronic ester function. A rapid screening of the redox cycling properties of a series of benzoquinones in the presence of Asch⁻ and dissolved molecular oxygen showed that dimethyl substituted benzoquinones, such as the 2,6-dimethyl-para-benzoquinone (DMBQ), are good hits as redox cyclers (Figure S1). Therefore, we decided to design the new boronic probes P₁ and P₂ (Scheme 1) by masking the DMBQ with a boronic ester group either in position 1 or 4.

We first investigated the H_2O_2 -catalyzed deprotection of these probes (Loop 1). This reaction is a two-step process (Scheme S2) starting from an H_2O_2 -driven deprotection step which converts the probe into the hydroquinone DMBQH₂ (oxidative cleavage of the C-B bond), followed then by an autoxidation step eventually leading to the concomitant release of DMBQ and H_2O_2 , the latter being therefore regenerated. The reaction kinetics between H_2O_2 and the reservoir probe P_1 in an air-saturated Tris buffer (0.1 M pH 8.5) containing 10 μM EDTA (Tris-EDTA buffer) was monitored by UV-vis spectroscopy through the appearance of the quinone at 257 nm (Figure S2). Reaction progresses for different amount of the catalyst (probe to H_2O_2 ratios ranging from 0.1 to 4 equivalents) are shown in Figure 1A. In the absence of H_2O_2 (Figure S2-A and brown curve in Figure 1A), P_1 slowly decomposes into the quinone after more than 3 hrs in solution. Even if not ideal, this probe possesses a better stability than the naphthoquinone-based probes we had previously investigated (Figure S3).²⁰ Under substoichiometric conditions, the maximal apparent conversion of the probe into DMBQ far exceeds the initial H_2O_2 concentration, confirming thus the catalytic nature of the process. For all curves a maximum is observed, due to a lack of long term stability of the released quinone in the slightly basic buffered aqueous media and in the presence of H_2O_2 (Figure S5). In consequence, the maximal apparent conversion is always lower than 100%. Loop 1 was also characterized for P_2 (Figures 1B and S4). With this probe, there is no discernible apparition of the benzoquinone in the absence of H_2O_2 even after more than 10 hrs, indicating the greater intrinsic stability of P_2 relative to P_1 in this buffer. This higher stability is however at the expense of a slower H_2O_2 reactivity than P_1 , leading to lower probe conversion yields due to the greater competition with the quinone degradation pathway. The difference of reactivity between the two probes is necessarily due to different H_2O_2 deprotection rates, since the two processes share the same autoxidation step (the intermediate DMBQH₂ is produced in both cases). Most likely, steric hindrance from the two methyl substituents during the nucleophilic attack of deprotonated form of H_2O_2 kinetically disadvantages P_2 over P_1 . Interestingly, for both probes, but

Figure 1. Kinetics of the H_2O_2 -mediated probe conversion into DMBQ (Loop 1) recorded at 257 nm during the reaction of 50 μM of (A) P_1 or (B) P_2 with different concentrations of H_2O_2 (see the legend on the graphs) in an air-saturated Tris-EDTA buffer (the absorbance was converted into % probe conversion using $\epsilon_{\text{DMBQ}} = 21000 \text{ M}^{-1}\cdot\text{cm}^{-1}$).

especially for P_2 , a clear latency was observed before the quinone starts to accumulate in Figure 1. At first sight, it can be assumed that this latency is linked to the transient accumulation of DMBQH₂ if deprotection is fast relative to autoxidation. Another possibility is related to the autoxidation process itself. In general, autoxidation of an hydroquinone (QH_2) occurs through a complex free-radical chain mechanism, with semiquinone and superoxide as chain-carrying species.²¹ It has been shown that in most cases, the autoxidation of QH_2 is mainly driven by the kinetics and thermodynamics of the comproportionation reaction step, which, experimentally, can thus lead to the appearance of an autoinduction phase in the kinetics.²¹ Examination of the autoxidation of DMBQ under our selected experimental conditions is presented in Figure S6 and S7. At the highest concentration tested (50 μM), the shape of the kinetic trace is in agreement with a pseudo-first-order kinetic (O_2 is instantaneously replenished from the gas phase and thus may be considered as constant during the reaction progress) characterized by a rate constant of 0.0035 s^{-1} , which corresponds to an apparent second order autoxidation rate constant of $k_{\text{ox,app}} = 14 \text{ M}^{-1}\cdot\text{s}^{-1}$. This value agrees with the data reported by Roginsky *et al.* under physiological conditions,²¹ and confirms the higher autoxidability of DMBQH₂ when the pH is increased. It is also worth noting that, as expected from its higher formal potential, the autoxidation of DMBQH₂ is slower than that of 1,4-naphthohydroquinone (1,4-NQH₂) for which a rate of $k_{\text{ox,app}} = 3300 \text{ M}^{-1}\cdot\text{s}^{-1}$ was previously determined electrochemically.²⁰ The slow autoxidation rate of DMBQ was confirmed by cyclic voltammetry, where voltammograms were virtually unchanged whether they were recorded in the presence or absence of O_2 (see Figure S8). For the lowest concentrations of DMBQH₂ in Figure S7, a clear sigmoidal shape is observed, attesting of the autoinductive nature of the autoxidation. Thus, consideration of the whole complexity of the mechanism is required to account for the latencies observed in Figure 1. For that purpose, numerical simulations of the overall H_2O_2 -mediated deprotection process (Loop 1) were successfully achieved (see SI for details and Figures S9-S11). Simulations permitted assessing the deprotection rate constants, k_{dep} , which were evaluated to be 17 and $1.2 \text{ M}^{-1}\cdot\text{s}^{-1}$ for P_1 and P_2 , respectively, confirming a ten-fold disadvantage for the sterically hindered probe. These values compare well with that previously determined for our arylboronic probe involving a self-immolative spacer ($3 \text{ M}^{-1}\cdot\text{s}^{-1}$),²⁰ but also for other arylboronic probes.²² However, a main difference is the stability of the probes P_1 and P_2 that is largely improved (8×10^{-7} and $< 10^{-8} \text{ s}^{-1}$ for P_1 and P_2 , respectively) compared to our previous self-immolative probe (*i.e.*, 10^{-5} s^{-1}).

Next we evaluated the propensity of the quinone to efficiently redox cycle (Loop 2), as depicted in scheme S3. The time-course of the redox cycling reaction was monitored spectrophotometrically from the absorbance decrease of AsCH^+ at 262 nm as a function of different AsCH^+ concentrations and catalytic amounts of DMBQ in an air-saturated Tris-EDTA buffer. Whatever the concentrations, a systematic exponential decrease of absorbance was observed (Figure 2A and S12), a

behavior that agrees with a redox cycling rate controlled by the reduction step (see SI). The experimental kinetic curves in Figures 2 and S12 were all fitted to a single exponential decay from which k_{obs} values were extracted. Plotting k_{obs} as a function of DMBQ concentration shows a linear trend (Figure S13), from the slope of which the apparent rate constant value characterizing the rate-limiting reduction step (*i.e.* $k_{red,app}$) can be extracted.

Figure 2 (A) Redox cycling kinetics (Loop 2) involving DMBQ at different concentrations (see the legend on the graph) and AsCH^- at a fixed concentration (50 μM) in an air-saturated Tris-EDTA buffer. On the ordinate, $[\text{AsCH}^-]$ was obtained from the absorbance at 262 nm (specific to the ascorbate) and using $\epsilon_{\text{AsCH}^-} = 11933 \text{ M}^{-1}\cdot\text{cm}^{-1}$. (B) Apparent rate constant $k_{red,app}$ extracted from the redox cycling kinetics of DMBQ and 1,4-NQ.

A non-constant value was thus obtained (Figure 2B), indicating a dependency on the ascorbate concentration. Such non trivial behavior was already reported for quinones belonging to the benzoquinone family,²³ but also for the 1,4-NQ in our previous report.²⁰ Compared with the 1,4-NQ, the redox cycling of DMBQ in the presence of ascorbate is more than one order of magnitude faster. This latter observation agrees with what was previously reported by Roginski *et al.* at pH 7.4.²⁴ The reason for this difference is that the rate determining step of the complex multi-step redox cycling mechanism (the complete mechanism is reported in the SI) is the single-electron transfer from ascorbate to the quinone (reaction S10), which, at a given pH, is favored for quinone having the highest Q/Q^- standard potential (*e.g.*, $E^{\circ}_{\text{Q/Q}^-}$ of -80 and -140 mV for DMBQ and 1,4-NQ, respectively). In addition, the high apparent redox cycling rate determined for DMBQ suggests that the main pathway for redox cycling only marginally involves the slowly-oxidizing species DMBQH_2 . A first consequence is that the apparent rate constant $k_{red,app}$ strongly depends on the reducing conditions, with a tendency to decrease as the concentration of the reducing agent is increased (Figure 2B). Such trend has been perfectly reproduced through numerical simulation (see SI and figure S14-S16), meaning that it can be rationalized through the given set of reactions. Most likely, this apparent inhibition is the consequence of a favored accumulation of the poorly reactive DMBQH_2 resulting from the competition between the reduction of the semiquinone (reaction S11) and its reoxidation by O_2 (reaction S2). Another important consequence of the poor reactivity of DMBQH_2 is that a redox cycle involving an obligatory two-electron donor becomes particularly slow or even completely prohibited. This was verified experimentally by substituting ascorbate with the reducing enzyme/substrate couple diaphorase/NADH (Figure

S17) or by an electrochemical reduction of the quinone (which is a “mandatory” two-electron process in aqueous media, Figure S8). Therefore, only reductants behaving as single-electron donors (such as ascorbate) are recommended to induce efficient redox cycling of the selected benzoquinone. The counterpart of this type of reducing agent is a greater propensity to have a cross-reactivity with O_2 , thus leading to potentially greater nonspecific amplification in the autocatalytic system. Note also that recording the electrochemical response of a given quinone in aerated conditions is not necessarily sufficient to predict its redox cycling properties as previously proposed.²⁴ It could rather provide information on whether the QH_2 form is sufficiently reactive for the two-electron redox cycling process to be kinetically relevant for a given reducing agent.

Having examined the catalytic role of both H_2O_2 and DMBQ in Loop 1 and 2, respectively, it is now time to demonstrate that these two molecules (here we will focus mainly on H_2O_2) can trigger an autocatalytic process when the reservoir probe (P_1 or P_2) and the reducing ascorbate agent are simultaneously mixed in an air-saturated Tris-EDTA buffer (*i.e.*, when the two catalytic processes can be coupled through cross-activation). As shown in Figure 3A and B, for solutions initially containing 50 μM AsCH^- , 2.5 μM P_1 or P_2 , and different triggering amounts of H_2O_2 , ascorbate depletion no longer follows the classical exponential decay, but rather presents an S-shaped kinetic trace, witnessing an autocatalytic process. As a further evidence of the autocatalysis, half-reaction time ($t_{1/2}$) scales linearly with the log of the H_2O_2 concentration over two orders of magnitude (Figure 3D).^{4,19} Kinetics of the autocatalytic processes are in line with the H_2O_2 probe reactivities since the reaction is much faster for the most reactive probe P_1 (the slope value is -7.2 min/log) than P_2 (slope value is -25.8 min/log). The value of the slope ratio, *i.e.* around 3.6, may be rationalized in the framework of a simple cross-catalytic model.^{18,19} As demonstrated in ref 19, the time response of a cross-catalytic system is inversely proportional to the geometric mean between the catalytic rate of each loop. In the present case, as the two systems share the same Loop 2, only the difference in the rate of Loop 1 matters. In consequence, given the similar experimental conditions, the time response ratio should therefore only be set by the square root of the deprotection rate constant ratio, *i.e.* $\sqrt{\frac{k_{dep,P_1}}{k_{dep,P_2}}} = \sqrt{\frac{17}{1.2}} \sim 3.8$ a value

that successfully accounts for the experimental observation. In addition, numerical simulation of the whole system showed excellent agreement with experience (Figures S18 to S20). The rate of the autocatalytic process is also shown to depend on both the probe and AsCH^- concentrations, as evidenced with P_2 in Figure 3C, where response times have been largely decreased for the higher starting concentrations (slope value is now -3.8 min/log, corresponding to a 6.9-fold decrease). Finally, as expected from the relative instability of the starting components, self-start of the reaction is also observed in the absence of H_2O_2 . This is due to the instability of the coreactants (*i.e.*, instability of the probe and/or autoxidation of ascorbate). We have shown that one of the probes is much

more stable than the other; the similar H_2O_2 threshold concentration for which the autocatalytic response no longer coincides with the nonspecific response would therefore suggest that, in both cases, the ascorbate autoxidation is the main source of nonspecific background amplification. Improvement of the system by extension of the dynamic concentration range and linearity over more than two decades would therefore require a reducing agent capable of fast single-electron transfer, while being much more stable and less cross-reactive with O_2 . Finally, a similar autocatalytic behavior is observed when the system is triggered by DMBQ instead of H_2O_2 (Figures S21 and S22), illustrating the advantageous properties of an autocatalytic system based on two cross-catalysts. However, the DMBQ concentrations required to initiate the reaction are hundred-fold lower than with H_2O_2 . The reason is simply due to the kinetic asymmetry between the two loops (Loop 2 is faster than Loop 1).¹⁹ Note that the autocatalysis could be also indirectly started from an upstream H_2O_2 - or quinone-producing reaction in order to build chemical reaction networks with increased complexity and extended functionalities.

Figure 3 Reaction kinetics of the autocatalytic ascorbate oxidation monitored at 262 nm in an air-saturated Tris-EDTA buffer in the presence of (A) 2.5 μM P₁ + 50 μM AschH, (B) 2.5 μM P₂ + 50 μM AschH, (C) 50 μM P₂ + 500 μM AschH, and different concentrations of H_2O_2 (see the legend on the graphs). (D) Semi-logarithmic plot of the half-reaction time recovered from the graphs in A to C as a function of the initial H_2O_2 concentration.

To conclude, the present work demonstrates, with a clear mechanistic understanding of the reactions involved, the feasibility of building a simple molecular autocatalytic reaction based on two cross-activated catalytic loops, necessitating only an H_2O_2 -sensitive probe and a reducing agent in an aerated aqueous medium. The two autocatalysts (*i.e.*, H_2O_2 and the redox cycling quinone) can trigger the autocatalytic ascorbate oxidation/boronic probe deprotection and accumulate exponentially. This simple molecular amplification system may be easily used to build innovative and sensitive chemosensing strategies for analysis or imaging. Alternatively, the selective and autocatalytic release of quinone and H_2O_2 opens the route for developing new quinone- or ROS

generator-based drugs.^{25,26} Finally, it may prove useful in the understanding of complex dynamic behavior occurring in natural systems.²⁷

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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 stearothermophilus* (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 μ M EDTA (Promega). The buffer was filtered over a 0.2 μ m 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 μ L 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 μ L 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 $25^{\circ}\text{C} \pm 1^{\circ}\text{C}$.

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

A

B

Scheme S2. Reaction mechanisms assumed the H₂O₂-catalyzed deprotection of (A) P₁ and (B) P₂. Note that the boronic esters are rapidly hydrolyzed into their boronic acid counterparts once dissolved in an aqueous buffer.

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 diluted 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 P_1 and S_2

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) S_2 or (solid lines) P_1 . 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. H_2O_2 . 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 P₂

Figure S4. (A) UV-vis spectra of a freshly prepared dilute solution of P₂ (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. H₂O₂ 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 H₂O₂ (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: DMBQH2 autoxidation

Figure S6. (A) UV-vis spectra of 50 μM DMBQ (blue line) and 50 μM DMBQH₂ (black line) in an aerated 0.1 M Tris-EDTA buffer (pH 8.5). The spectra in red show the evolution of the DMBQH₂ 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 ($\text{Abs} = \text{Abs}_0 \times [1 - \exp(-k_{\text{obs}}t)]$) with $k_{\text{obs}} = 3.5 \times 10^{-3} \text{ s}^{-1}$. Optical path length: 1 cm.

3.7. Figure S7: Concentration dependence of the DMBQH2 autoxidation kinetics

Figure S7. Autoxidation kinetics of DMBQH₂ 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 ($\epsilon_{\text{DMBQ}} = 21000 \text{ M}^{-1} \cdot \text{cm}^{-1}$).

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 P₁

Figure S10. (A) Experimental kinetic plots (monitored at 257 nm) recorded during the deprotection of $50 \mu\text{M}$ P₁ by different concentrations of H_2O_2 (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 ($\epsilon_{\text{DMBQ}} = 21000 \text{ M}^{-1}\cdot\text{cm}^{-1}$). (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 P₂

Figure S11. (A) Experimental kinetic plots (monitored at 257 nm) recorded during the deprotection of $50 \mu\text{M}$ P₂ by different concentrations of H_2O_2 (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 ($\epsilon_{\text{DMBQ}} = 21000 \text{ M}^{-1}\cdot\text{cm}^{-1}$). (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.

3.12. 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. (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. (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 $k_{red,app}$ with the ascorbate concentration

Figure S16. (black squares) Experimental plot of the rate constant $k_{red,app}$ (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 O₂ replenishment or (green triangles) S0 to S7 + S10 to S14 without O₂ replenishment. The rate constants are listed in Table S3, entry 7.

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

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

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 $t_{1/2}$ (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 $t_{1/2}$ (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 P_2 triggered by H_2O_2

Figure S19. (Left, top) Autocatalytic kinetic traces recorded at 262 nm during the reaction of 2.5 μM P_2 with 50 μM ascorbate and different triggering concentrations of H_2O_2 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 H_2O_2 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 H_2O_2 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 P_2 with 0.5 mM ascorbate and different concentrations of H_2O_2 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 H_2O_2 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 H_2O_2 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 DMBQH₂ 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^{•-}) and superoxide anion are involved as chain propagating species as follows:^{1, 2}

Autoxidation chain reaction starts from the single-electron transfer between O₂ and QH₂ (initiation or reaction S0), that is known to be quite slow under near-neutral conditions because spin-restricted.³ However, this initiation allows the production of the chain propagating species Q^{•-} and O₂^{•-}, 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 QH₂ since small impurities of Q allow to drastically change the response and hide the phenomena.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_{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 *k₀*, no value was found in the literature because of the difficulty to determine it independently. For our simulations, we have thus considered *k₀* 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:

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 H₂O₂. 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.

		Rate constant	Simulation entry			
			1	2	3	4
DMBQH ₂ autoxidation reactions		$k_{ox} (M^{-1} \cdot s^{-1})$	14			
	Initiation	$k_0 (M^{-1} \cdot s^{-1})$		0.00001	0.00001	0.00001
	Comproportionation /dismutation Q ^{•-}	$k_{co,1} ; k_{dis,-1} (M^{-1} \cdot s^{-1})$		50 ; 1.15×10^8 [4]	50 ; 1.15×10^8	50 ; 2×10^8
	Q ^{•-} /O ₂	$k_2 ; k_{-2} (M^{-1} \cdot s^{-1})$		1×10^7 ; 2.2×10^8 [2]	1×10^7 ; 2.2×10^8	2×10^8 ; 8×10^8
	QH ₂ / O ₂ ^{•-}	$k_3 (M^{-1} \cdot s^{-1})$		1×10^5 [2]	5×10^5	1×10^5
	Superoxide dismutation	$k_4 (M^{-1} \cdot s^{-1})$		10^4 [5]	10^4	10^4
	Chain termination	$k_5 (M^{-1} \cdot s^{-1})$		1×10^8 [2]	5×10^8	1×10^8
Side reactions	Self-degradation	$k_6 (M^{-1} \cdot s^{-1})$	0.8	0.8	0.8	0.8
	Peroxidation	$k_7 (M^{-1} \cdot s^{-1})$	0.1	0.1	0.1	0.1

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 10 μM, 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.

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.

		Rate constant	Simulation entry	
			5	6
DMBQH ₂ autoxidation reactions		$k_{ox} (M^{-1} \cdot s^{-1})$		
	Initiation	$k_0 (M^{-1} \cdot s^{-1})$	0.00001	0.00001
	Comproportionation /dismutation Q ^{•-}	$k_{co,1} ; k_{dis,-1} (M^{-1} \cdot s^{-1})$	50 ; 2 × 10 ⁸	50 ; 2 × 10 ⁸
	Q ^{•-} /O ₂	$k_2 ; k_{-2} (M^{-1} \cdot s^{-1})$	2 × 10 ⁸ ; 8 × 10 ⁸	2 × 10 ⁸ ; 8 × 10 ⁸
	QH ₂ / O ₂ ^{•-}	$k_3 (M^{-1} \cdot s^{-1})$	1 × 10 ⁵	1 × 10 ⁵
	Superoxide dismutation	$k_4 (M^{-1} \cdot s^{-1})$	10 ⁴	10 ⁴
	Chain termination	$k_5 (M^{-1} \cdot s^{-1})$	1 × 10 ⁸	1 × 10 ⁸
Side reactions	Self-degradation	$k_6 (M^{-1} \cdot s^{-1})$	0.8	0.8
	Peroxidation	$k_7 (M^{-1} \cdot s^{-1})$	0.1	0.1
Probe reactivity	Deprotection	$k_{dep} (M^{-1} \cdot s^{-1})$	17	1.2
	Instability	$k_{inst} (s^{-1})$	8 × 10 ⁻⁷	10 ⁻⁸
O ₂ renewal		$k_{15} ; k_{-15} (s^{-1})$	0.025 ; 100	0.025 ; 100

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 cyclers, and O₂ 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:

$$-\frac{d[Asc]}{dt} = \frac{[DMBQ]_0}{\frac{1}{k_{ox}[O_2]_0} + \frac{1}{k_{red}[Asc]}} = k_{redox}[DMBQ]_0$$

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

Which, after integration, leads to

$$\frac{[\text{Asc}]_0 - [\text{Asc}]}{k_{ox} [\text{O}_2]_0} + \frac{1}{k_{red}} \ln \frac{[\text{Asc}]_0}{[\text{Asc}]} = [\text{DMBQ}]_0 t \quad (\text{eq. S1})$$

From there, two limiting situations can be encountered, leading to two very different kinetic responses. On the one hand, if the autoxidation rate of DMBQH₂ 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).

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

On the other hand, if the autoxidation rate of DMBQH₂ 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).

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

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:

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

Plotting k_{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 $k_{red,app}$ that can be reported as a function of the ascorbate concentration (graph B in Figure S13). The plot shows that $k_{red,app}$ 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).

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.

		Rate constant	Simulation entry
			7
DMBQH ₂ autoxidation reactions		$k_{ox} (\text{M}^{-1} \cdot \text{s}^{-1})$	
	Initiation	$k_0 (\text{M}^{-1} \cdot \text{s}^{-1})$	0.00001
	Comproportionation /dismutation Q ^{•-}	$k_{co,1} ; k_{dis,-1} (\text{M}^{-1} \cdot \text{s}^{-1})$	50 ; 2 × 10 ⁸
	Q ^{•-} /O ₂	$k_2 ; k_{-2} (\text{M}^{-1} \cdot \text{s}^{-1})$	2 × 10 ⁸ ; 8 × 10 ⁸
	QH ₂ /O ₂ ^{•-}	$k_3 (\text{M}^{-1} \cdot \text{s}^{-1})$	1 × 10 ⁵
	Superoxide dismutation	$k_4 (\text{M}^{-1} \cdot \text{s}^{-1})$	10 ⁴
	Chain termination	$k_5 (\text{M}^{-1} \cdot \text{s}^{-1})$	1 × 10 ⁸
Side reactions	Self-degradation	$k_6 (\text{M}^{-1} \cdot \text{s}^{-1})$	0.8
	Peroxidation	$k_7 (\text{M}^{-1} \cdot \text{s}^{-1})$	0.1
Ascorbate reduction reactions	Quinone reduction	$k_{10} ; k_{-10} (\text{M}^{-1} \cdot \text{s}^{-1})$	4000 ; 1 × 10 ⁸
	Semiquinone reduction	$k_{11} ; k_{-11} (\text{M}^{-1} \cdot \text{s}^{-1})$	4.5 × 10 ⁵ ; 2.2 × 10 ⁴
	Superoxide reduction	$k_{12} (\text{M}^{-1} \cdot \text{s}^{-1})$	5 × 10 ⁴
	Ascorbyl dismutation	$k_{13} (\text{M}^{-1} \cdot \text{s}^{-1})$	1 × 10 ⁶
	Ascorbate autoxidation	$k_{14} (\text{M}^{-1} \cdot \text{s}^{-1})$	0.32
O ₂ renewal		$k_{15} , k_{-15} (\text{s}^{-1})$	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,⁹ this value of the apparent rate

constant is expected to be lower than k_{10} . The value was adjusted to $4000 \text{ M}^{-1}\cdot\text{s}^{-1}$ 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 $k_{-10} = 2.1 \times 10^8 \text{ M}^{-1}\cdot\text{s}^{-1}$ for.⁴ This value was slightly adjusted to $10^8 \text{ M}^{-1}\cdot\text{s}^{-1}$ 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_{11}) 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 ($k_{red,app}$) 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 $10^5 \text{ M}^{-1}\cdot\text{s}^{-1}$.⁴ To obtain the best fits, k_{11} was adjusted to $4.5 \times 10^5 \text{ M}^{-1}\cdot\text{s}^{-1}$ 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:

$$\Delta E_{11} = E_{Q^{\bullet}/Q^{2-}}^{0'} - E_{Asc^{\bullet}/AscH^-}^{0'} = RT/F \ln K_{11}$$

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

▪ 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.*, $5 \times 10^4 \text{ M}^{-1}\cdot\text{s}^{-1}$) 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.*, $10^6 \text{ M}^{-1}\cdot\text{s}^{-1}$) was also recovered from the literature.⁹ The high driving force between the one-electron reduction potentials $E^0(\text{DHA}/\text{Asc}^{\bullet})$ and $E^0(\text{Asc}^{\bullet}/\text{AscH}^-)$ (-174 mV and +282 mV, respectively) makes the reverse reaction very slow (estimated at $0.7 \text{ M}^{-1}\cdot\text{s}^{-1}$ 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.^{10, 11} A bimolecular rate constant of $0.32 \text{ M}^{-1}\cdot\text{s}^{-1}$ 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 $k_{red,app}$ 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 through 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).

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^{•-}, 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 H₂O₂ 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.

		Rate constant	Simulation entry	
			8	9
DMBQH ₂ autoxidation reactions		$k_{ox} (M^{-1} \cdot s^{-1})$		
	Initiation	$k_0 (M^{-1} \cdot s^{-1})$	0.00001	0.00001
	Comproportionation /dismutation Q ^{•-}	$k_{co,1} ; k_{dis,-1} (M^{-1} \cdot s^{-1})$	50 ; 2 × 10 ⁸	50 ; 2 × 10 ⁸
	Q ^{•-} /O ₂	$k_2 ; k_{-2} (M^{-1} \cdot s^{-1})$	2 × 10 ⁸ ; 8 × 10 ⁸	2 × 10 ⁸ ; 8 × 10 ⁸
	QH ₂ / O ₂ ^{•-}	$k_3 (M^{-1} \cdot s^{-1})$	1 × 10 ⁵	1 × 10 ⁵
	Superoxide dismutation	$k_4 (M^{-1} \cdot s^{-1})$	10 ⁴	10 ⁴
	Chain termination	$k_5 (M^{-1} \cdot s^{-1})$	1 × 10 ⁸	1 × 10 ⁸
Side reactions	Self-degradation	$k_6 (M^{-1} \cdot s^{-1})$	0.8	0.8
	Peroxidation	$k_7 (M^{-1} \cdot s^{-1})$	0.1	0.1
Probe reactivity	Deprotection	$k_{dep} (M^{-1} \cdot s^{-1})$	17	17
	Instability	$k_{inst} (s^{-1})$	8 × 10 ⁻⁷	8 × 10 ⁻⁷
Ascorbate reduction reactions	Quinone reduction	$k_{10} ; k_{-10} (M^{-1} \cdot s^{-1})$	4000 ; 1 × 10 ⁸	4000 ; 1 × 10 ⁸
	Semiquinone reduction	$k_{11} ; k_{-11} (M^{-1} \cdot s^{-1})$	4.5 × 10 ⁵ ; 2.2 × 10 ⁴	4.5 × 10 ⁵ ; 2.2 × 10 ⁴
	Superoxide reduction	$k_{12} (M^{-1} \cdot s^{-1})$	5 × 10 ⁴	5 × 10 ⁴
	Ascorbyl	$k_{13} (M^{-1} \cdot s^{-1})$	1 × 10 ⁶	1 × 10 ⁶

	dismutation			
	Ascorbate autoxidation	$k_{14} \text{ (M}^{-1}\cdot\text{s}^{-1}\text{)}$	0.32	0.32
O ₂ renewal		$k_{15} ; k_{-15} \text{ (s}^{-1}\text{)}$	0.025 ; 100	0.025 ; 100

5. References

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