Quantitative biology of hydrogen peroxide signaling

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** A R T I C L E   I N F O **

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** A B S T R A C T **

Hydrogen peroxide (H₂O₂) controls signaling pathways in cells by oxidative modulation of the activity of redox sensitive proteins denominated redox switches. Here, quantitative biology concepts are applied to review how H₂O₂ fulfills a key role in information transmission. Equations described lay the foundation of H₂O₂ signaling, giving new insights on H₂O₂ signaling mechanisms, and help to learn new information from common redox signaling experiments. A key characteristic of H₂O₂ signaling is that the ratio between reduction and oxidation of redox switches determines the range of H₂O₂ concentrations to which they respond. Thus, a redox switch with low H₂O₂-dependent oxidability and slow reduction rate responds to the same range of H₂O₂ concentrations as a redox switch with high H₂O₂-dependent oxidability, but that is rapidly reduced. Yet, in the first case the response time is slow while in the second case is rapid. H₂O₂ sensing and transmission of information can be done directly or by complex mechanisms in which oxidation is relayed between proteins before oxidizing the final regulatory redox target. In spite of being a very simple molecule, H₂O₂ has a key role in cellular signaling, with the reliability of the information transmitted depending on the inherent chemical reactivity of redox switches, on the presence of localized H₂O₂ pools, and on the molecular recognition between redox switches and their partners.

1. Introduction

Hydrogen peroxide (H₂O₂) is a non-radical oxidant present in virtually all aerobic organisms. Viewed initially as a detrimental byproduct of oxidative metabolism, today H₂O₂ is recognized to play important roles in cellular physiology [1]. The cellular function of H₂O₂ is supported by coupling of cellular signals with its production. Many enzymatic sources have been identified that produce H₂O₂ directly or produce superoxide radical, which is subsequently dismutated into water and H₂O₂, a process that is accelerated many orders of magnitude by the action of superoxide dismutases. A particularly relevant source of H₂O₂ is NADPH oxidases because their sole function seems to be the tightly-regulated production of superoxide/H₂O₂ [2].

Production of H₂O₂ is balanced by the action of antioxidant enzymatic systems, such as catalase, glutathione peroxidases, and peroxiredoxins, that remove H₂O₂ very rapidly [3,4]. An homeostatic steady-state level of 10⁻⁷ – 10⁻⁸ M results under physiological conditions [5], and changes around this background steady-state level will trigger cellular responses. If these concentration shifts are moderated, transient or localized in space, being a result of for example signaling processes, a physiological stress response – or eustress – is observed [3]. If variations in the H₂O₂ concentration are large, sustained or affect H₂O₂ bulk levels, a pathological stress with deleterious effects for the organism materializes [3]. Thus, oxidative effects are inherently non-linear and biphasic with threshold levels separating the physiological and the pathological domains [6,7]. In addition, eustress and pathological stress can either be oxidative or reductive, depending on whether they are caused by an increase or decrease of H₂O₂ around its background steady-state level.

In this review, quantitative biology concepts are introduced to analyze the transmission of information mediated by H₂O₂ in the oxidative eustress setting.

1.1. H₂O₂ signaling

Signaling pathways are regulated by the reaction of H₂O₂ with proteins harboring redox sensitive moieties, like metal centers or cysteine residues, whose oxidation controls their activity. These proteins denominated redox switches are key players in the regulation of biochemical pathways, including protein phosphatases, kinases or transcription factors [8]. Thus, a change in the concentration of H₂O₂ is matched by a change in the oxidation state of a redox switch, regulating a downstream pathway and transducing the information encoded in the H₂O₂ concentration profile along a signaling cascade.
Chemically, most previously identified redox-controlled switches are thiol proteins [9–11], but metal switches have also been described [12,13]. Thiol switches are proteins with cysteine residues with low pKa that favors their proton dissociation to form a thiolate at physiological pH. Thiolates have a higher reactivity towards H$_2$O$_2$, but the pKa of the cysteine residue is not the only determinant of the reactivity of the thiol protein with H$_2$O$_2$. Rather, stabilization of the transition state between H$_2$O$_2$ and the cysteine residue is critical to achieve high catalytic rates with the protein environment affecting the reactivity of the cysteine group [14]. Thus, reactivity of thiol proteins towards H$_2$O$_2$ spans several orders of magnitude, from the low 20 M$^{-1}$s$^{-1}$ for some protein tyrosine phosphatases, like PTP1B and SHP-2, to the high 10$^4$ M$^{-1}$s$^{-1}$ for peroxiredoxin 2 [15].

The chemical reactivity of redox switches is a potential mechanism underlying specific biological effects caused by different concentrations of H$_2$O$_2$. At low H$_2$O$_2$ concentrations only the most reactive switches will sense H$_2$O$_2$, while less reactive switches will sense H$_2$O$_2$ at high concentrations. As will be described below, such chemical specificity based on the oxidability of the redox switch is just one of several regulatory mechanisms in H$_2$O$_2$ signaling.

2. Quantitative analysis of H$_2$O$_2$ signal processing

2.1. The steady-state approximation

The steady-state concept is central to quantitative analyses in redox biology [16–18]. As a result of continuous formation and elimination, H$_2$O$_2$ settles to a near steady-state given by Eq. 1 in Fig. 1. It is important to test the validity of the steady-state approximation during signaling events when variations of the H$_2$O$_2$ concentration are observed. In other words, does the steady-state approximation hold when H$_2$O$_2$ is not steady? When H$_2$O$_2$ production is increased, for example due to the activation of an NADPH oxidase, the steady-state approximation can be used to calculate the transient dynamics of H$_2$O$_2$ because the very fast elimination of H$_2$O$_2$ by antioxidants systems has a reaction time much quicker than the transient responses formed during signaling events (Fig. 2). Thus, the steady-state approximation is valid even when H$_2$O$_2$ levels change during signaling events.

To make a quantitative analysis of H$_2$O$_2$ signal processing, the simple steady-state scheme of Fig. 1 was extended to include a signaling reaction. The formation of H$_2$O$_2$ is now balanced by two elimination reactions, one being the consumption of H$_2$O$_2$ by antioxidant systems and the other the oxidation of redox switches (Fig. 3). When first-order kinetics are assumed for these elimination processes, H$_2$O$_2$ steady-state is given by Eq. 2 in Fig. 3.

![Image](image1.png)

**Fig. 1.** The steady-state of H$_2$O$_2$. A steady-state is reached when the rates of formation ($v_{\text{formation}}$) are balanced by the rates of elimination ($v_{\text{consumption}}$). The rate of H$_2$O$_2$ elimination is assumed to follow first-order kinetics because in the eustress domain H$_2$O$_2$ does not overload the antioxidant systems. Thus, $v_{\text{consumption}} = k_{\text{consum}} \times [\text{H}_2\text{O}_2]_s$ with $k_{\text{consum}}$ being the pseudo first-order rate constant for the overall consumption of H$_2$O$_2$. The steady-state Eq. 1 is deduced from the equality between the rates of formation and elimination of H$_2$O$_2$.

![Image](image2.png)

**Fig. 2.** Application of the steady-state approximation to H$_2$O$_2$ dynamics during signaling events. To reproduce a transient H$_2$O$_2$ increase, the rate of H$_2$O$_2$ formation was assumed to peak at 5 min and to decay to zero at 20 min as observed in Ref. [19]. Three H$_2$O$_2$ profiles are shown: one was calculated according to steady-state Eq. 1 and two according to simulations reproducing the cell behavior for two values of consumption rate constants $k_{\text{consum}} = 12$ s$^{-1}$ and 1.2 s$^{-1}$. Simulated H$_2$O$_2$ profiles approach that calculated from Eq. 1 when the value of $k_{\text{consum}}$ increases, and for $k_{\text{consum}} = 120$ s$^{-1}$ or higher, simulation curves coincide with the steady-state curve (not shown). This trend is justified by the very fast time scale of the $k_{\text{consum}}$ rate constant. A time scale of 0.06 s is calculated according to the formula $t_{1/2} = \ln(2)/k_{\text{consum}}$, with $\ln(2)$ being the natural logarithm of 2, for a $k_{\text{consum}} = 12$ s$^{-1}$, a lower limit for the value of the $k_{\text{consum}}$ rate constant (see Table 1 below). A $t_{1/2}$ value of 0.06 s is much faster than the time scale associated with the variation of H$_2$O$_2$ formation during signaling events, which is in the minute range, and thus the steady-state approximation is valid. In general, the steady-state approximation is a reasonable assumption when analyzing processes in the minute range or slower because antioxidant systems are usually fast enough.

![Image](image3.png)

**Fig. 3.** The H$_2$O$_2$ steady-state in the presence of signaling. In principle, a signal (Signal in) can modulate either the production or the removal of H$_2$O$_2$, the activation of a NADPH oxidase being a common mechanism. The subsequent change in H$_2$O$_2$ concentration is sensed by a redox switch (Target) that upon oxidation ($v_{\text{signal}}$) transmits information downstream the signaling cascade (Signal out). Similarly to the rate of H$_2$O$_2$ consumption by antioxidant systems, the signaling reaction also follows first-order kinetics, being $v_{\text{signal}} = k_{\text{signal}} \times [\text{H}_2\text{O}_2]_s$ with $k_{\text{signal}}$ referring to the rate constant for the reaction of H$_2$O$_2$ with the redox target. The resulting steady-state H$_2$O$_2$ concentration is given by Eq. 2.
magnitude higher than \( k_{\text{sign}} \) (Table 1), implying that antioxidant reactions vastly outcompete signaling reactions for \( \text{H}_2\text{O}_2 \). Thus, a kinetic bottleneck for \( \text{H}_2\text{O}_2 \) signaling is established \([10,14,15,20]\). If highly efficient antioxidant systems divert more than 99.999% of \( \text{H}_2\text{O}_2 \) from signaling reactions, how are \( \text{H}_2\text{O}_2 \) variations sensed? The rate of signaling is calculated as the product of the rate constant \( k_{\text{sign}} \) by the concentration of \( \text{H}_2\text{O}_2 \) (Fig. 3). So, the rate of the signaling reaction will match the variations of \( \text{H}_2\text{O}_2 \), and the information encoded in the \( \text{H}_2\text{O}_2 \) concentration profile can, in principle, be transmitted downstream the signaling cascade. The key question is whether the information is transferred fast enough when \( V_{\text{signalling}} \) is very slow.

### 2.2. Equations governing \( \text{H}_2\text{O}_2 \) signaling

The issue whether a slow chemical reaction between \( \text{H}_2\text{O}_2 \) and a redox switch ensures timely information transmission during signal processing may be addressed with the help of the minimal mathematical model shown in Fig. 4. This model is formed by the oxidation-reduction cycle of a redox switch, which may be viewed as a switch-on switch-off sequence \([22]\) with the on state – oxidized form of the redox switch – relaying the information encoded in \( \text{H}_2\text{O}_2 \) down the signaling cascade. The solution of this model yields a master equation (Eq. 3 in Fig. 5) describing the time course of the fraction of the redox switch in the reduced form \([23]\). From Eq. 3 two sets of simpler equations are deduced (Fig. 5), namely (i) Eqs. 4 A and 4B describing the effect of input \( \text{H}_2\text{O}_2 \) concentrations on the signaling response, and (ii) Eqs. 5 A and 5B characterizing the time-dependent \( \text{H}_2\text{O}_2 \) signaling properties. The input \( \text{H}_2\text{O}_2 \) concentrations and the response time are two important quantitative measures of redox signaling proposed before \([18]\).

#### 2.3. \( \text{H}_2\text{O}_2 \) dynamic range

The input dynamic range, i.e. the range of \( \text{H}_2\text{O}_2 \) concentrations for which redox switches act as sensors of \( \text{H}_2\text{O}_2 \), depends on the reactivity of the redox switch towards \( \text{H}_2\text{O}_2 \), being inversely proportional to \( k_{\text{target}+\text{H}_2\text{O}_2} \) (Fig. 6A). One source of uncertainty in the plot of Fig. 6A is the \( k_{\text{target}+\text{H}_2\text{O}_2} \) value because, among other factors, \( \text{H}_2\text{O}_2 \) reactivity increases several orders of magnitude upon reaction with phosphate and carbon dioxide yielding peroxymonophosphate \([25]\) and peroxymonocarbonate \([26,27]\), respectively. The reactivity of PTP1B with peroxymonophosphate is 7000-fold higher than with \( \text{H}_2\text{O}_2 \) itself \([25]\), and at pH 7, the presence of carbonate at 25 mM, a physiological level, accelerates the reaction between PTP1B and \( \text{H}_2\text{O}_2 \) from 24 \( \text{M}^{-1} \text{s}^{-1} \) to 202 \( \text{M}^{-1} \text{s}^{-1} \) at 25°C and to 396 \( \text{M}^{-1} \text{s}^{-1} \) at 37°C. The formation of these derivatives is not immediate, taking 5–8 min to reach an equilibrium with \( \text{H}_2\text{O}_2 \) \([25,27]\). Nevertheless, for peroxymonocarbonate, PTP1B accelerates this equilibrium to a few seconds or faster \([26]\). This effect was attributed to oxidation of the active-site cysteine by peroxymonocarbonate possibly formed in the active center of the enzyme \([26]\). Zn(II) complexes and other Lewis acids increase the rate of peroxymonocarbonate formation \([27]\), and one may speculate that Arg221, being present in the active site of PTP1B and being essential for catalysis \([28]\), can act as a Lewis acid catalyzing the formation of peroxymonocarbonate. Therefore, in Fig. 6A the input dynamic range was also calculated for PTP1B in the presence of CO2.

In addition to the reactivity of the redox protein towards \( \text{H}_2\text{O}_2 \), the input dynamic range also depends on the rate of reduction of the redox switch, increasing for high \( k_{\text{switchoff}} \) rate constants, as shown in Fig. 6B. Thus, according to Eqs. 4A and 4B the reduction of the redox switch inhibits the transmission of \( \text{H}_2\text{O}_2 \) signals, as it is observed for the reductions of OxyR by glutaredoxin 1 \([29]\), of Yap1 by thioredoxins 1 and 2 \([30]\), of Pap1 by thioredoxins 1 and 3 \([31,32]\), of PTP1B by redoxin TRP14 and thioredoxin 1 \([33,34]\), of PTEN by thioredoxin 1 \([35]\), and of the NRF2/KEAP1 system by the thioredoxin system \([36]\). The \( k_{\text{switchoff}} \) values for protein phosphatases PTP1B and SHP-2 – \( 2 \times 10^{-3} \text{s}^{-1} \) – \([23]\) are about three orders of magnitude lower than for peroxiredoxins – \( 2 \times 10^{-1} \text{s}^{-1} \) \([36]\). This large difference reflects the value of the rate constant for the reduction of phosphatases – 700 \( \text{M}^{-1} \text{s}^{-1} \) for PTP1B (estimated from \([37]\)) – being much lower than the rate constant for the reduction of peroxiredoxins by thioredoxin – \( 2.1 \times 10^5 \text{M}^{-1} \text{s}^{-1} \) \([38]\), 2.2 \( \times 10^6 \text{M}^{-1} \text{s}^{-1} \) (estimated from \([39]\)) and (2–8) \( \times 10^5 \text{M}^{-1} \text{s}^{-1} \) (estimated from \([40]\)) for peroxiredoxins 2, 3, and 5, respectively. Thus, the input dynamic range for peroxiredoxins is not as low as it could be expected from their high reactivity towards \( \text{H}_2\text{O}_2 \), and may even overlap with that of less reactive proteins. For example, input dynamic ranges for PTP1B and peroxiredoxin 5 are predicted to overlap (Table 2).

Table 2 shows the input dynamic range predicted according to published kinetic data. For \( \text{H}_2\text{O}_2 \) signaling concentrations lower than \( 1 \mu\text{M} \), signaling is probably intermediated by a high reactive protein such as peroxiredoxin 2. For \( \text{H}_2\text{O}_2 \) concentrations higher than \( 1 \mu\text{M} \), mediation of signaling by proteins with different reactivity towards \( \text{H}_2\text{O}_2 \) is feasible, but mediation by a high or by a low reactive protein is not equivalent as the response time is different (see below).

Not considered here is the hyperoxidation of peroxiredoxins, which also mediates transmission of information encoded in \( \text{H}_2\text{O}_2 \) \([41-43]\).


**Master equation:**

\[
\text{Target}_{\text{rd}} = \frac{k_{\text{switch off}}}{k_{\text{switch on}} + k_{\text{target-H2O}_2}} \times [\text{H}_2\text{O}_2] + e^{-(k_{\text{switch off}} + k_{\text{target-H2O}_2})[\text{H}_2\text{O}_2]} \left( \frac{\text{Target}_{\text{rd}}}{k_{\text{switch off}} + k_{\text{target-H2O}_2}} \right)
\]

**Input dynamic range:**

\[
\text{Target}_{\text{rd}} = \frac{k_{\text{target-H2O}_2} \times [\text{H}_2\text{O}_2]}{k_{\text{target-H2O}_2} \times [\text{H}_2\text{O}_2] + k_{\text{switch off}}} = \frac{1}{1 + \frac{k_{\text{switch off}}}{k_{\text{target-H2O}_2} \times [\text{H}_2\text{O}_2]}}
\]

**Response time:**

\[
t_{1/2} = \frac{\ln(2)}{k_{\text{target-H2O}_2} \times [\text{H}_2\text{O}_2] + k_{\text{switch off}}}
\]

Having different input dynamic ranges depending on modified forms of the same protein gives adaptability to signaling systems [44].

2.4. **Response time to H\textsubscript{2}O\textsubscript{2}**

Similarly to the input range, the response time also depends on the reactivity of proteins towards H\textsubscript{2}O\textsubscript{2} and on the value of \(k_{\text{switch off}}\) (Eqs. 5A and 5B). But contrary to the input dynamic range, the response does not depend on the ratio of the rate constant values for these processes, but rather on their sum. Therefore, between two proteins with similar input dynamic ranges, the one with higher reactivity towards H\textsubscript{2}O\textsubscript{2} displays a faster response time for a given H\textsubscript{2}O\textsubscript{2} concentration. For example, PTP1B will respond with a time of approximately 1 s, while peroxiredoxin 5 responds in less than 1 s, even if they have similar input dynamic ranges (Table 2). The response time for PTP1B calculated here is much faster than published estimations [8,26,36] because previous analyses considered only the oxidation of the redox switch, neglecting the impact of its reduction in the acceleration of the response time.

Response time values shown in Table 2 were calculated with Eq. 5B, which hides the influence of H\textsubscript{2}O\textsubscript{2} concentration and \(k_{\text{target-H2O}_2}\). For example, in spite of very different reactivity towards H\textsubscript{2}O\textsubscript{2}, peroxiredoxins 2 and 5 have a similar response time – 0.2 s. Implicit H\textsubscript{2}O\textsubscript{2} concentrations used are, however, different for peroxiredoxins 2 and 5, being those that induce 50% of peroxiredoxin oxidation, as calculated by Eq. 4B. For the same H\textsubscript{2}O\textsubscript{2} concentration, peroxiredoxin 2 has a much faster response time than peroxiredoxin 5, as shown in Fig. 7A.

2.5. **Analyzing typical experiments**

In addition to provide new insights on the mechanisms of H\textsubscript{2}O\textsubscript{2} signaling, the two sets of Eqs. 4 and 5 may be applied to learn new information from typical redox signaling experiments that measure the oxidation time course of redox switches. To this end, three experimental measurements are useful: (i) the fraction of the redox target in the oxidized form, (ii) the response time, and (iii) the H\textsubscript{2}O\textsubscript{2} input dynamic range. The oxidation levels of the redox target and the response time can be estimated from the time course of the oxidation profile of the target under analysis. The measurement of the input dynamic range is more difficult. When H\textsubscript{2}O\textsubscript{2} is added externally, the intracellular concentration of H\textsubscript{2}O\textsubscript{2} is lower than that applied extracellularly, and a gradient across the plasma membrane is established [48]. The magnitude of this gradient is unknown and depends on the cell type and whether peroxiredoxins are active at the external H\textsubscript{2}O\textsubscript{2} concentration applied in the experimental set up. The presence of active peroxiredoxins increases gradients by approximately two orders of magnitude, from values under 10\textsuperscript{48} to values in the 650–1000 range [51,52]. Thus, uncertainties in the values of intracellular H\textsubscript{2}O\textsubscript{2} concentrations impact the determination of the input dynamic range when H\textsubscript{2}O\textsubscript{2} is added extracellularly. Alternatively, if endogenous production of H\textsubscript{2}O\textsubscript{2} is stimulated with a signaling molecule, like a growth factor, the intracellular H\textsubscript{2}O\textsubscript{2} concentration is also unknown. Even if the intracellular H\textsubscript{2}O\textsubscript{2} level is followed with a probe, the conversion of the signal measured to H\textsubscript{2}O\textsubscript{2} concentrations values is usually not done. In spite of these caveats, useful information can still be obtained from the concentration of H\textsubscript{2}O\textsubscript{2} applied in experiments as exemplified below.

Stat3 is inhibited by 5 µM extracellular H\textsubscript{2}O\textsubscript{2} [53], which corresponds to an intracellular concentration in the range of 0.5–0.005 µM if gradients are considered. In spite of this wide range of possible H\textsubscript{2}O\textsubscript{2} concentrations, the involvement of a sensor with reactivity similar to peroxiredoxin 2 can be predicted (Table 2). In addition, the response time observed experimentally is below 60 s, for an extracellular H\textsubscript{2}O\textsubscript{2} concentration of 50 µM [53]. A peroxiredoxin-like sensor is needed to attain such rapid response according to Eqs. 5A and 5B. In fact, peroxiredoxin 2 acts as a sensor, reacting with H\textsubscript{2}O\textsubscript{2} and then relaying the oxidation to form disulfide links between Stat3 monomers [53]. Of note, when oxidation relays are involved response times are slower than those indicated in Table 2 as the oxidation relay step introduces an

Fig. 5. Governing equations of H\textsubscript{2}O\textsubscript{2} signaling. The master equation (Eq. 3) includes the dependence on the sustained H\textsubscript{2}O\textsubscript{2} signaling concentration \([\text{H}_2\text{O}_2]\) attained in the vicinity of the redox switch during the signaling process, as well as the rate constants for oxidation \((k_{\text{target-H2O}_2})\) and reduction \((k_{\text{switch off}})\) of the redox switch, and the fraction of the redox switch in the reduced form at time 0 \((\text{Target}_{\text{rd}}))\). The key features of H\textsubscript{2}O\textsubscript{2} signaling are described by two sets of simpler equations deduced from the master Eq. 3 [8,23]. Eq. 4A is deduced by letting \(t\) tend to infinity and represents the steady-state fraction of the redox switch in the oxidized form \((\text{Target}_{\text{rd}}))\), which is a measure of the amount of information transmitted from H\textsubscript{2}O\textsubscript{2} to the redox target. Calculation of the H\textsubscript{2}O\textsubscript{2} signaling concentration causing a certain steady-state value of target oxidation is done with Eq. 4B, which results from an arrangement of Eq. 4A. The second set of equations (Eqs. 5A and 5B) calculates the response time of the redox switch to H\textsubscript{2}O\textsubscript{2}, giving the time \((t_{1/2})\) needed for oxidizing half of the target present initially, i.e., indicating whether transmission of information proceeds rapid enough. Eq. 5A is deduced by replacing Target\textsubscript{rd} by Target\textsubscript{rd}/2 and \(t\) by \(t_{1/2}\) in Eq. 3 and calculates \(t_{1/2}\) as a function of H\textsubscript{2}O\textsubscript{2} concentration. Eq. 5B is deduced by replacing Eq. 4B in Eq. 5A and calculates \(t_{1/2}\) as a function of the steady-state fraction of the redox switch in the oxidized form and on the value of \(k_{\text{switch off}}\).
**Table 2**

| Redox target | $k_{\text{target} + \text{H}_2\text{O}_2}$ (M⁻¹s⁻¹) | $k_{\text{switchoff}}$ (s⁻¹) | H₂O₂ dynamic range (µM) | Response time to H₂O₂ (s) |
|--------------|---------------------------------|-------------------|-------------------------|--------------------------|
| PTP1B        | 24                              | $2 \times 10^{-3}$ | 9–750                   | 173                      |
| + CO₂        | 396                             | $2 \times 10^{-3}$ | 0.6–45                  | 173                      |
| SHP-2        | 20                              | $2 \times 10^{-3}$ | 11–900                  | 173                      |
| + CO₂        | 167                             | $2 \times 10^{-3}$ | 1.3–108                 | 73                       |
| Prx5         | $3 \times 10^{5}$              | 2                 | 0.7–60                  | 0.2                      |
| Prx2         | $1 \times 10^{7}$              | 2                 | 0.02–1.8                | 0.2                      |

additional delay not considered in the minimal model of Fig. 4.

In another example, a response time of about 4–5 min is estimated from the PTP1B oxidation profile observed when the endogenous production of H₂O₂ is triggered by EGF in A431 cells [54]. This slow response time is compatible with the direct reaction of H₂O₂ with PTP1B (see Table 2). In addition, in this case the H₂O₂ signaling concentration attained in the vicinity of PTP1B can be estimated as rate constants for PTP1B are known: if the level of PTP1B oxidation measured experimentally – approximately 50% [54] – together with $k_{\text{target} + \text{H}_2\text{O}_2} = 396$ M⁻¹s⁻¹ and $k_{\text{switchoff}} = 2 \times 10^{-3}$ s⁻¹, is introduced in equation 4B, a concentration of 5 µM is estimated. By a similar approach, the H₂O₂ concentration attained in the vicinity of SHP-2 during stimulation of Rat-1 cells by PDGF is calculated to be 6 µM, based on the observed SHP-2 oxidation profile [55]. H₂O₂ concentrations in the order of 5–6 µM are much higher than the bulk steady-state H₂O₂ concentration, estimated in the range 0.1–0.01 µM [5], but are still plausible as localized pools of H₂O₂ probably play an important role during signaling [13,36,41,56]. The plausibility of this estimation is reinforced by noting that oxidation profiles of protein phosphatases PTP1B and SHP-2 observed with growth factors are similar to those observed with extracellular H₂O₂ concentrations close to 100 µM [23,55,57]. An extracellular 100 µM H₂O₂ concentration corresponds to an intracellular concentration of 5 µM if an H₂O₂ gradient across the plasma membrane of 20 is established, which is plausible taking into consideration that at this relatively high external H₂O₂ levels peroxiredoxin systems are at least partially inhibited [36,58].

Nonetheless, H₂O₂ concentrations attained during signaling are most probably pathway dependent. As referred above, low H₂O₂ extracellular concentrations, in the order of 5 µM, are in play during the formation of disulfide-linked Stat3 oligomers [53]. On the other hand, the inhibition of protein phosphatase 1 (PP1) probably needs a high dose of H₂O₂ because an associated IC₅₀ of 67 µM was measured in vitro [13]; in fact, this inhibition is probably mediated by localized production of H₂O₂ because colocalization of NOX4 and PP1 was observed and both proteins were identified together in a complex [13].

The previous discussion illustrates how Eqs. 4 and 5 give new insights on the mechanisms of H₂O₂ signaling and how new information is learned from common experimental measures. In addition, other applications for the equations are possible, including for example their fitting to experimental data to determine rate constants [23].

3. Final remarks

The main results of the quantitative biology analysis of H₂O₂ signaling presented here are summarized in the form of Eqs. 4 and 5 and are depicted in Fig. 8. Eqs. 4 and 5 govern the biology of H₂O₂...
Redox switches transmit information along a signaling cascade after being oxidized by H$_2$O$_2$. This oxidation may be direct or, alternatively, indirect when the redox sensor is a high reactive protein, like a peroxiredoxin, that relays the oxidation to a redox switch with low reactivity towards H$_2$O$_2$ (Fig. 9) [4,59,60]. Examples of relay circuits already identified include the original discovery of the Gpx3/Yap1 [61], and subsequently Tpx1/Pap1 [32,62], Tsa1/Stat1 [63], Prxl/Ask1 [64], and Prx2/Stat3 [53]. In the thioredoxin-peroxiredoxin model, thioredoxin, or another protein responsible for the reduction of the redox sensor, acts as a redox relay, mediating the oxidation of a downstream redox switch [60,65].

Independently of the specific mechanism, localized interactions are probably important to attain accurate information transmission [66]. These localized interactions include (1) complexes of NOX with redox switches, favoring switch on of a specific redox sensor, and (2) the interaction of a high reactive sensor, such as a peroxiredoxin, with a target protein, sustaining a specific relay of the oxidative signal. In this second case, the peroxiredoxin will not only relay the oxidative signal downstream but also trap H$_2$O$_2$ [36], avoiding H$_2$O$_2$ diffusion outside the signal locus and, consequently, preventing either unspecific signaling messages or even some form of pathological stress.

In conclusion, the relatively weak oxidation potential of H$_2$O$_2$ is coupled to timely and accurate transmission of information by a combination of chemical switches that balance oxidation and reduction of redox chemical reactions, together with specific protein interactions and localized H$_2$O$_2$ pools.

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