



## Review article

## Quantitative biology of hydrogen peroxide signaling

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## ABSTRACT

Hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) 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  $\text{H}_2\text{O}_2$  fulfills a key role in information transmission. Equations described lay the foundation of  $\text{H}_2\text{O}_2$  signaling, give new insights on  $\text{H}_2\text{O}_2$  signaling mechanisms, and help to learn new information from common redox signaling experiments. A key characteristic of  $\text{H}_2\text{O}_2$  signaling is that the ratio between reduction and oxidation of redox switches determines the range of  $\text{H}_2\text{O}_2$  concentrations to which they respond. Thus, a redox switch with low  $\text{H}_2\text{O}_2$ -dependent oxidability and slow reduction rate responds to the same range of  $\text{H}_2\text{O}_2$  concentrations as a redox switch with high  $\text{H}_2\text{O}_2$ -dependent oxidability, but that is rapidly reduced. Yet, in the first case the response time is slow while in the second case is rapid.  $\text{H}_2\text{O}_2$  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,  $\text{H}_2\text{O}_2$  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  $\text{H}_2\text{O}_2$  pools, and on the molecular recognition between redox switches and their partners.

## 1. Introduction

Hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) is a non-radical oxidant present in virtually all aerobic organisms. Viewed initially as a detrimental byproduct of oxidative metabolism, today  $\text{H}_2\text{O}_2$  is recognized to play important roles in cellular physiology [1]. The cellular function of  $\text{H}_2\text{O}_2$  is supported by coupling of cellular signals with its production. Many enzymatic sources have been identified that produce  $\text{H}_2\text{O}_2$  directly or produce superoxide radical, which is subsequently dismutated into water and  $\text{H}_2\text{O}_2$ , a process that is accelerated many orders of magnitude by the action of superoxide dismutases. A particularly relevant source of  $\text{H}_2\text{O}_2$  is NADPH oxidases because their sole function seems to be the tightly-regulated production of superoxide/ $\text{H}_2\text{O}_2$  [2].

Production of  $\text{H}_2\text{O}_2$  is balanced by the action of antioxidant enzymatic systems, such as catalase, glutathione peroxidases, and peroxiredoxins, that remove  $\text{H}_2\text{O}_2$  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  $\text{H}_2\text{O}_2$  concentration are large, sustained or affect

$\text{H}_2\text{O}_2$  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  $\text{H}_2\text{O}_2$  around its background steady-state level.

In this review, quantitative biology concepts are introduced to analyze the transmission of information mediated by  $\text{H}_2\text{O}_2$  in the oxidative eustress setting.

1.1.  $\text{H}_2\text{O}_2$  signaling

Signaling pathways are regulated by the reaction of  $\text{H}_2\text{O}_2$  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  $\text{H}_2\text{O}_2$  is matched by a change in the oxidation state of a redox switch, regulating a downstream pathway and transducing the information encoded in the  $\text{H}_2\text{O}_2$  concentration profile along a signaling cascade.

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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  $\text{H}_2\text{O}_2$ , but the pKa of the cysteine residue is not the only determinant of the reactivity of the thiol protein with  $\text{H}_2\text{O}_2$ . Rather, stabilization of the transition state between  $\text{H}_2\text{O}_2$  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  $\text{H}_2\text{O}_2$  spans several orders of magnitude, from the low  $20 \text{ M}^{-1}\text{s}^{-1}$  for some protein tyrosine phosphatases, like PTP1B and SHP-2, to the high  $10^7 \text{ M}^{-1}\text{s}^{-1}$  for peroxiredoxin 2 [15].

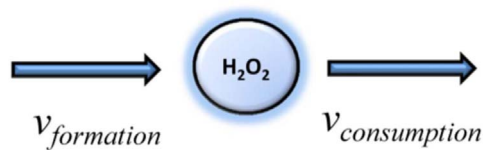
The chemical reactivity of redox switches is a potential mechanism underlying specific biological effects caused by different concentrations of  $\text{H}_2\text{O}_2$ . At low  $\text{H}_2\text{O}_2$  concentrations only the most reactive switches will sense  $\text{H}_2\text{O}_2$ , while less reactive switches will sense  $\text{H}_2\text{O}_2$  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  $\text{H}_2\text{O}_2$  signaling.

## 2. Quantitative analysis of $\text{H}_2\text{O}_2$ 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,  $\text{H}_2\text{O}_2$  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  $\text{H}_2\text{O}_2$  concentration are observed. In other words, does the steady-state approximation hold when  $\text{H}_2\text{O}_2$  is not steady? When  $\text{H}_2\text{O}_2$  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  $\text{H}_2\text{O}_2$  because the very fast elimination of  $\text{H}_2\text{O}_2$  by antioxidant 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  $\text{H}_2\text{O}_2$  levels change during signaling events.

To make a quantitative analysis of  $\text{H}_2\text{O}_2$  signal processing, the simple steady-state scheme of Fig. 1 was extended to include a signaling reaction. The formation of  $\text{H}_2\text{O}_2$  is now balanced by two elimination reactions, one being the consumption of  $\text{H}_2\text{O}_2$  by antioxidant systems



Steady-state:

$$\begin{aligned} v_{\text{formation}} &= v_{\text{consumption}} \\ v_{\text{formation}} &= k_{\text{cons}} \times [\text{H}_2\text{O}_2]_{\text{ss}} \\ [\text{H}_2\text{O}_2]_{\text{ss}} &= \frac{v_{\text{formation}}}{k_{\text{cons}}} \end{aligned} \quad (1)$$

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

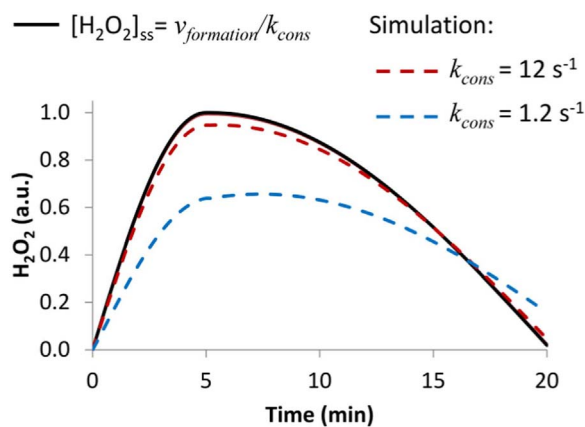


Fig. 2. Application of the steady-state approximation to  $\text{H}_2\text{O}_2$  dynamics during signaling events. To reproduce a transient  $\text{H}_2\text{O}_2$  increase, the rate of  $\text{H}_2\text{O}_2$  formation was assumed to peak at 5 min and to decay to zero at 20 min as observed in Ref. [19]. Three  $\text{H}_2\text{O}_2$  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 –  $1.2 \text{ s}^{-1}$  and  $12 \text{ s}^{-1}$ . Simulated  $\text{H}_2\text{O}_2$  profiles approach that calculated from Eq. 1 when the value of  $k_{\text{cons}}$  increases, and for  $k_{\text{cons}} = 120 \text{ s}^{-1}$  or higher, simulation curves coincide with the steady-state curve (not shown). This trend is justified by the very fast time scale of the  $k_{\text{cons}}$  rate constant. A time scale of 0.06 s is calculated according to the formula  $t_{1/2} = \ln(2)/k_{\text{cons}}$ , with  $\ln(2)$  being the natural logarithm of 2, for a  $k_{\text{cons}} = 12 \text{ s}^{-1}$ , a lower limit for the value of the  $k_{\text{cons}}$  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  $\text{H}_2\text{O}_2$  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.

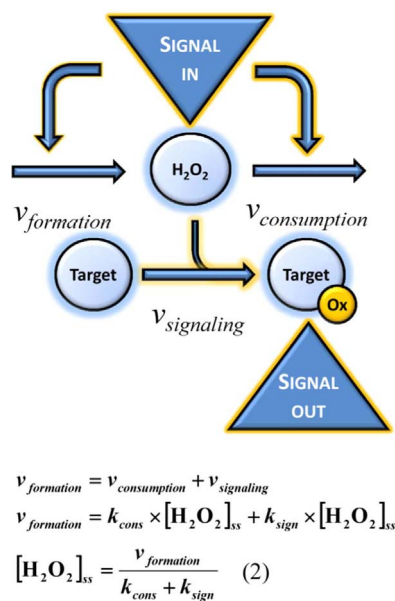


Fig. 3. The  $\text{H}_2\text{O}_2$  steady-state in the presence of signaling. In principle, a signal (Signal in) can modulate either the production or the removal of  $\text{H}_2\text{O}_2$ , the activation of a NADPH oxidase being a common mechanism. The subsequent change in  $\text{H}_2\text{O}_2$  concentration is sensed by a redox switch (Target) that upon oxidation ( $v_{\text{signaling}}$ ) transmits information downstream the signaling cascade (Signal out). Similarly to the rate of  $\text{H}_2\text{O}_2$  consumption by antioxidant systems, the signaling reaction also follows first-order kinetics, being  $v_{\text{signaling}} = k_{\text{sign}} \times [\text{H}_2\text{O}_2]$  with  $k_{\text{sign}}$  referring to the rate constant for the reaction of  $\text{H}_2\text{O}_2$  with the redox target. The resulting steady-state  $\text{H}_2\text{O}_2$  concentration is given by Eq. 2.

and the other the oxidation of redox switches (Fig. 3). When first-order kinetics are assumed for these elimination processes,  $\text{H}_2\text{O}_2$  steady-state is given by Eq. 2 in Fig. 3.

Eq. 2 shows the relative magnitude of  $k_{\text{cons}}$  and  $k_{\text{sign}}$  only is needed to predict whether signaling processes affect directly the  $\text{H}_2\text{O}_2$  steady-state. According to published data,  $k_{\text{cons}}$  is five to six orders of

**Table 1**

Competition between enzymatic antioxidants and redox switches for  $\text{H}_2\text{O}_2$ .  $k_{\text{cons}}$  and  $k_{\text{sign}}$  are pseudo first-order rate constants calculated as the product between  $k_{\text{target}+\text{H}_2\text{O}_2}$ , the chemical rate constant between  $\text{H}_2\text{O}_2$  and its target protein, and the concentration of the target protein, either an enzymatic antioxidant or a redox switch. For protein concentrations and rate constant values see [8,15]. The rather high value for catalase concentration refers to the peroxisome [21].

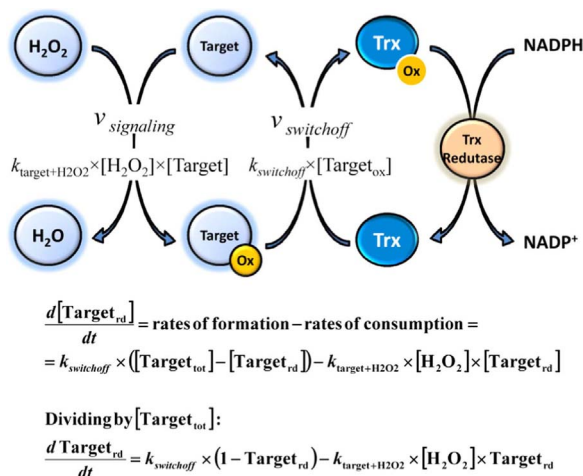
	$k_{\text{target}+\text{H}_2\text{O}_2}$ ( $\text{M}^{-1}\text{s}^{-1}$ )	[protein] ( $\mu\text{M}$ )	$k_{\text{target}+\text{H}_2\text{O}_2} \times \text{protein}$ ( $\text{s}^{-1}$ )
<b>Antioxidant</b>			$k_{\text{cons}}$
Prx	$10^5$ – $10^7$	10	1–100
GPx	$6 \times 10^7$	0.2–10	12–600
Catalase	$10^7$	$10^3$	$10^4$
<b>Redox switch</b>			$k_{\text{sign}}$
PTP1B	24	0.01	$2.4 \times 10^{-7}$
SHP-2	20	0.01	$2.0 \times 10^{-7}$
Keap1	140	1	$1.4 \times 10^{-4}$

Prx – Peroxiredoxin; GPx – Glutathione peroxidase 1.

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  $v_{\text{signaling}}$  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



**Fig. 4.** A minimal model of  $\text{H}_2\text{O}_2$  signaling mediated by redox switches. A redox switch (Target) is oxidized by  $\text{H}_2\text{O}_2$  and then reduced by, for example, a member of the thioredoxin family (Trx). The rate of target oxidation is equaled to the rate of the chemical reaction between  $\text{H}_2\text{O}_2$  and the reduced form of the target ( $k_{\text{target}+\text{H}_2\text{O}_2} \times [\text{H}_2\text{O}_2] \times [\text{Target}_{\text{rd}}]$ ), and the rate of reduction is set to  $k_{\text{switchoff}} \times [\text{Target}_{\text{ox}}]$ , with  $k_{\text{switchoff}}$  being a pseudo first-order rate constant. The differential equation is built, simplified by dividing by the total concentration of target  $[\text{Target}_{\text{tot}}]$ , and solved with a software like Maxima [24]. The resulting solution describes the time course of the target in terms of its molar fraction in the reduced state ( $\text{Target}_{\text{rd}}$ ), thus avoiding the utilization of absolute concentrations, a measure that is difficult to measure experimentally.

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  $25^\circ\text{C}$  and to  $396 \text{ M}^{-1}\text{s}^{-1}$  at  $37^\circ\text{C}$ . 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 equilibration 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  $\text{CO}_2$ .

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 [34], and of the NRF2/KEAP1 system by the thioredoxin system [35].  $k_{\text{switchoff}}$  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 –  $2 \text{ s}^{-1}$  [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],  $2.2 \times 10^5 \text{ M}^{-1}\text{s}^{-1}$  (estimated from [39]) and  $(2\text{--}8) \times 10^5 \text{ M}^{-1}\text{s}^{-1}$  (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:**

$$\text{Target}_{\text{rd}}|_t = \frac{k_{\text{switchoff}}}{k_{\text{switchoff}} + k_{\text{target+H2O2}} \times [\text{H}_2\text{O}_2]} + e^{-(k_{\text{switchoff}} + k_{\text{target+H2O2}} \times [\text{H}_2\text{O}_2])t} \left( \text{Target}_{\text{rd}}|_0 - \frac{k_{\text{switchoff}}}{k_{\text{switchoff}} + k_{\text{target+H2O2}} \times [\text{H}_2\text{O}_2]} \right) \quad (3)$$

**Input dynamic range:**

$$\text{Target}_{\text{ox}} = \frac{k_{\text{target+H2O2}} \times [\text{H}_2\text{O}_2]}{k_{\text{target+H2O2}} \times [\text{H}_2\text{O}_2] + k_{\text{switchoff}}} = \frac{1}{1 + \frac{k_{\text{switchoff}}}{k_{\text{target+H2O2}} \times [\text{H}_2\text{O}_2]}} \quad (4A)$$

$$[\text{H}_2\text{O}_2] = \frac{\text{Target}_{\text{ox}}}{1 - \text{Target}_{\text{ox}}} \frac{k_{\text{switchoff}}}{k_{\text{target+H2O2}}} \quad (4B)$$

**Response time:**

$$t_{1/2} = \frac{\ln(2)}{k_{\text{target+H2O2}} \times [\text{H}_2\text{O}_2] + k_{\text{switchoff}}} \quad (5A)$$

$$t_{1/2} = \frac{\ln(2) \times (1 - \text{Target}_{\text{ox}})}{k_{\text{switchoff}}} \quad (5B)$$

**Fig. 5.** Governing equations of H<sub>2</sub>O<sub>2</sub> signaling. The master equation (Eq. 3) includes the dependence on the sustained H<sub>2</sub>O<sub>2</sub> signaling concentration [H<sub>2</sub>O<sub>2</sub>] attained in the vicinity of the redox switch during the signaling process, as well as the rate constants for oxidation ( $k_{\text{target+H2O2}}$ ) and reduction ( $k_{\text{switchoff}}$ ) of the redox switch, and the fraction of the redox switch in the reduced form at time 0 ( $\text{Target}_{\text{rd}}|_0$ ). The key features of H<sub>2</sub>O<sub>2</sub> 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 infinite and represents the steady-state fraction of the redox switch in the oxidized form ( $\text{Target}_{\text{ox}}$ ), which is a measure of the amount of information transmitted from H<sub>2</sub>O<sub>2</sub> to the redox target. Calculation of the H<sub>2</sub>O<sub>2</sub> 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<sub>2</sub>O<sub>2</sub>, 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  $\text{Target}_{\text{rd}}|_t$  by  $\text{Target}_{\text{rd}}|_0/2$  and  $t$  by  $t_{1/2}$  in Eq. 3 and calculates  $t_{1/2}$  as a function of H<sub>2</sub>O<sub>2</sub> 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  $k_{\text{switchoff}}$ .

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<sub>2</sub>O<sub>2</sub>

Similarly to the input range, the response time also depends on the reactivity of proteins towards H<sub>2</sub>O<sub>2</sub> and on the value of  $k_{\text{switchoff}}$  (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<sub>2</sub>O<sub>2</sub> displays a faster response time for a given H<sub>2</sub>O<sub>2</sub> concentration. For example, PTP1B will respond with a time of approximately 3 min, 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. 5 B, which hides the influence of H<sub>2</sub>O<sub>2</sub> concentration and  $k_{\text{target+H2O2}}$ . For example, in spite of very different reactivity towards H<sub>2</sub>O<sub>2</sub>, peroxiredoxins 2 and 5 have a similar response time – 0.2 s. Implicit H<sub>2</sub>O<sub>2</sub> 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<sub>2</sub>O<sub>2</sub> concentration, peroxiredoxin 2 has a much faster response time than peroxiredoxin 5, as shown in Fig. 7A.

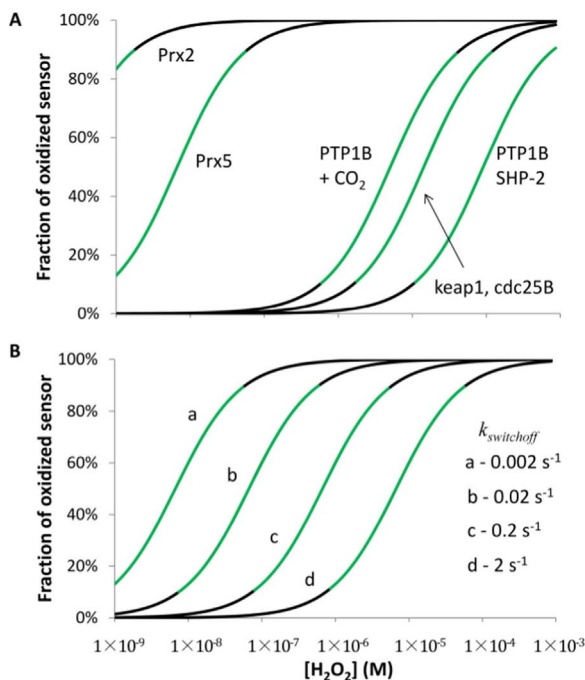
## 2.5. Analyzing typical experiments

In addition to provide new insights on the mechanisms of H<sub>2</sub>O<sub>2</sub> 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 experi-

mental measurements are useful: (i) the fraction of the redox target in the oxidized form, (ii) the response time, and (iii) the H<sub>2</sub>O<sub>2</sub> 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<sub>2</sub>O<sub>2</sub> is added externally, the intracellular concentration of H<sub>2</sub>O<sub>2</sub> 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<sub>2</sub>O<sub>2</sub> concentration applied in the experimental set up.** The presence of active peroxiredoxins increases gradients by approximately two orders of magnitude, from values under 10 [48–50] to values in the 650–1000 range [51,52]. Thus, **uncertainties in the values of intracellular H<sub>2</sub>O<sub>2</sub> concentrations impact the determination of the input dynamic range when H<sub>2</sub>O<sub>2</sub> is added extracellularly.** Alternatively, if endogenous production of H<sub>2</sub>O<sub>2</sub> is stimulated with a signaling molecule, like a growth factor, the intracellular H<sub>2</sub>O<sub>2</sub> concentration is also unknown. Even if the intracellular H<sub>2</sub>O<sub>2</sub> level is followed with a probe, the conversion of the signal measured to H<sub>2</sub>O<sub>2</sub> concentrations values is usually not done. In spite of these caveats, useful information can still be obtained from the concentration of H<sub>2</sub>O<sub>2</sub> applied in experiments as exemplified below.

Stat3 is inhibited by 5  $\mu\text{M}$  extracellular H<sub>2</sub>O<sub>2</sub> [53], which corresponds to an intracellular concentration in the range of 0.5–0.005  $\mu\text{M}$  if gradients are considered. In spite of this wide range of possible H<sub>2</sub>O<sub>2</sub> 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<sub>2</sub>O<sub>2</sub> concentration of 50  $\mu\text{M}$  [53]. A peroxiredoxin-like sensor is needed to attain such rapid response according to Eqs. 5 A and 5B. In fact, peroxiredoxin 2 acts as a sensor, reacting with H<sub>2</sub>O<sub>2</sub> 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



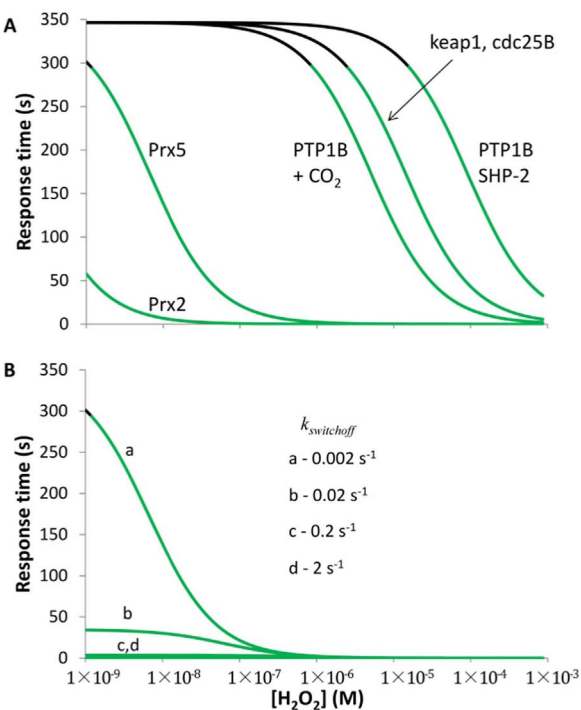


**Fig. 6.**  $H_2O_2$  input dynamic range for redox switches. Plots of Eq. 4A show the range of  $H_2O_2$  concentrations to which thiol proteins with different reactivity respond. The following  $k_{target+H_2O_2}$  values were used: peroxiredoxin 2 (Prx2) –  $1 \times 10^7 \text{ M}^{-1}\text{s}^{-1}$ ; peroxiredoxin 5 (Prx5) –  $3 \times 10^5 \text{ M}^{-1}\text{s}^{-1}$ ; Kelch-like ECH-associated protein 1 (Keap1) –  $140 \text{ M}^{-1}\text{s}^{-1}$ ; cell division cycle 25B (cdc25B) –  $140 \text{ M}^{-1}\text{s}^{-1}$ ; protein tyrosine phosphatase 1B (PTP1B) –  $24 \text{ M}^{-1}\text{s}^{-1}$ ; PTP1B in presence of bicarbonate (PTP1B +  $CO_2$ ) –  $396 \text{ M}^{-1}\text{s}^{-1}$ ; and, src-homology 2 containing tyrosine phosphatase (SHP-2) –  $20 \text{ M}^{-1}\text{s}^{-1}$ . In (A)  $k_{switchoff}$  value was  $2 \times 10^{-3} \text{ s}^{-1}$ , while in (B) the influence of a range of  $k_{switchoff}$  values is shown for peroxiredoxin 5. Input  $H_2O_2$  concentrations sustaining information transmission by redox switches are shown in green and were defined as the input eliciting a 10–90% oxidation of the protein. Below 10% oxidation, redox switch response is considered too weak to transmit efficiently the  $H_2O_2$  signal, while above 90% oxidation, the response is near saturated to further increase in the  $H_2O_2$  concentration.

**Table 2**  
 $H_2O_2$  dynamic range and response time for thiol proteins. Calculation of  $H_2O_2$  dynamic range was done with Eq. 4B, assuming 10% and 90% of target oxidation, respectively for the lower and upper limit. Calculation of the response time to  $H_2O_2$  was done with Eq. 5B, assuming 50% of target oxidation. For PTP1B and SHP-2 data for the reactivity obtained in the presence of  $CO_2$  [26] is also shown.

Redox target	$k_{target+H_2O_2}$ ( $M^{-1}s^{-1}$ )	$k_{switchoff}$ ( $s^{-1}$ )	$H_2O_2$ dynamic range ( $\mu M$ )	Response time to $H_2O_2$ (s)
PTP1B	24	$2 \times 10^{-3}$	9–750	173
+ $CO_2$	396	$2 \times 10^{-3}$	0.6–45	173
SHP-2	20	$2 \times 10^{-3}$	11–900	173
+ $CO_2$	167	$2 \times 10^{-3}$	1.3–108	173
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  $H_2O_2$  is triggered by EGF in A431 cells [54]. This slow response time is compatible with the direct reaction of  $H_2O_2$  with PTP1B (see Table 2). In addition, in this case the  $H_2O_2$  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  $k_{target+H_2O_2} = 396 \text{ M}^{-1}\text{s}^{-1}$  and  $k_{switchoff} = 2 \times 10^{-3} \text{ s}^{-1}$ , is introduced in equation 4B, a concentration of  $5 \mu M$  is estimated. By a similar approach, the  $H_2O_2$  concentration attained in the vicinity of SHP-2 during stimulation of Rat-1 cells by PDGF is calculated to be  $6 \mu M$ , based on the observed SHP-2 oxidation profile [55].  $H_2O_2$  concentra-



**Fig. 7.** Response time of redox switches to  $H_2O_2$ . Plots of Eq. 5A show the time needed to reach 50% of the overall response of sensors to  $H_2O_2$ . Values of rate constants are the same as those used in Fig. 6. In (A)  $k_{switchoff}$  value was  $2 \times 10^{-3} \text{ s}^{-1}$ , while in (B) the influence of a range of  $k_{switchoff}$  values is shown for peroxiredoxin 5. Input  $H_2O_2$  concentrations supporting rapid information transmission by redox switches are shown in green and were defined as the input eliciting responses faster than 10 min. Nonetheless, some processes may be compatible with longer responses times to  $H_2O_2$ , including for example apoptosis [45] or adaptation pathways [46,47].

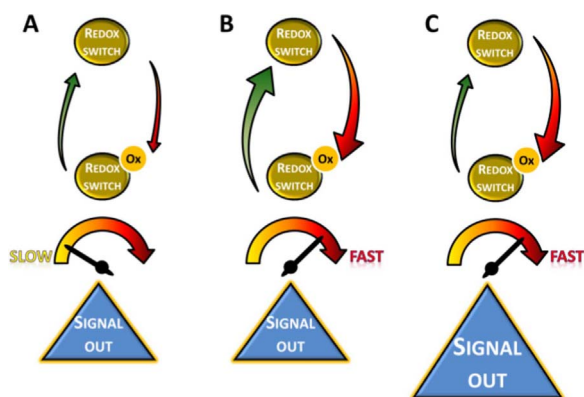
tions in the order of 5–6  $\mu M$  are much higher than the bulk steady-state  $H_2O_2$  concentration, estimated in the range 0.1–0.01  $\mu M$  [5], but are still plausible as localized pools of  $H_2O_2$  probably play an important role during signaling [13,36,41,56]. The plausibility of this estimation 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  $H_2O_2$  concentrations close to 100  $\mu M$  [23,55,57]. An extracellular 100  $\mu M$   $H_2O_2$  concentration corresponds to an intracellular concentration of 5  $\mu M$  if an  $H_2O_2$  gradient across the plasma membrane of 20 is established, which is plausible taking into consideration that at this relatively high external  $H_2O_2$  levels peroxiredoxin systems are at least partially inhibited [36,58].

Nonetheless,  $H_2O_2$  concentrations attained during signaling are most probably pathway dependent. As referred above, low  $H_2O_2$  extracellular concentrations, in the order of 5  $\mu M$ , 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  $H_2O_2$  because an associated  $IC_{50}$  of 67  $\mu M$  was measured in vitro [13]; in fact, this inhibition is probably mediated by localized production of  $H_2O_2$  because colocalization of NOX4 and PP1 was observed and both proteins were identified together in a complex [13].

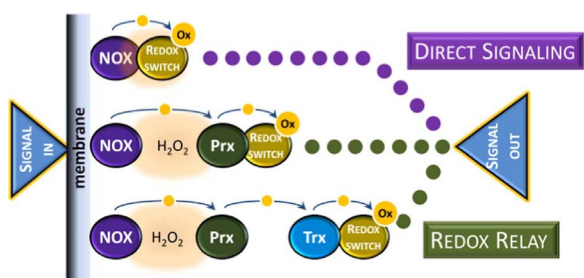
The previous discussion illustrates how Eqs. 4 and 5 give new insights on the mechanisms of  $H_2O_2$  signaling and how new information is learned from common experimental measures. In addition, other applications for the equations are possible, including for example their fitting to experimental data to determine rate constants [23].

### 3. Final remarks

The main results of the quantitative biology analysis of  $H_2O_2$  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  $H_2O_2$



**Fig. 8. Redox signaling properties.** Redox characteristics of the redox switch define the properties of the transmission of information encoded in  $H_2O_2$ . When the ratio between oxidation and reduction is similar the same information output is observed (panels A and B), but the redox switch with higher redox turnover has a faster response (panel B). If oxidation dominates over reduction, signal transduction is favored by displacing the redox switch to the oxidized form (Panel C).



**Fig. 9.** Mechanisms of  $\text{H}_2\text{O}_2$  signaling. In the direct signaling pathway,  $\text{H}_2\text{O}_2$  reacts directly with a redox switch. In the redox relay mechanism, the sensor of  $\text{H}_2\text{O}_2$ , a high-reactive protein such as a peroxiredoxin, relays the oxidation to a redox switch. The redox relay mechanism can be made more complex by the intervention of additional intermediates, as exemplified in the thioredoxin-peroxiredoxin model in which the protein responsible for the reduction of the redox sensor acts as a redox relay.

signaling and provide a quantitative framework with predictive power. In addition, common experimental measurements, like response time and oxidation profile of redox switches, may be analyzed with these equations, giving hints on the mechanisms of signal processing underlying experimental observations. Although the analysis was focused on thiol switches, these equations also apply to other types of redox switches.

The input dynamic range, i.e., the  $\text{H}_2\text{O}_2$  concentration range to which redox switches respond, depends not only on their  $\text{H}_2\text{O}_2$ -induced oxidability but also on their rate of reduction (Fig. 8). Eq. 4 indicates that the ratio between the kinetics constants of these two processes defines the sensitivity of the redox switch to  $\text{H}_2\text{O}_2$ . Peroxiredoxins and protein phosphatases respond in ranges of  $\text{H}_2\text{O}_2$  concentration that are not as far apart as it could be predicted based solely in their very large different reactivity towards  $\text{H}_2\text{O}_2$ , because peroxiredoxins are reduced faster than protein phosphatases (Table 2). The response time also depends on the rates of oxidation and reduction of the redox switch given by Eq. 5. In this case, it is not the ratio between oxidation and reduction that determines the response time, but their effects add up to increase the rate of response.

Redox switches transmit information along a signaling cascade after being oxidized by  $\text{H}_2\text{O}_2$ . 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  $\text{H}_2\text{O}_2$  (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/Sty1 [63], Prx1/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  $\text{H}_2\text{O}_2$  [36], avoiding  $\text{H}_2\text{O}_2$  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  $\text{H}_2\text{O}_2$  is coupled to timely and accurate transmission of information by a combination of chemical reactions that balance oxidation and reduction of redox switches, together with specific protein interactions and localized  $\text{H}_2\text{O}_2$  pools.

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## References

- [1] D.P. Jones, H. Sies, The redox code, *Antioxid. Redox Signal.* 23 (2015) 734–746, <http://dx.doi.org/10.1089/ars.2015.6247>.
- [2] R.P. Brandes, N. Weissmann, K. Schröder, Nox family NADPH oxidases: molecular mechanisms of activation, *Free Radic. Biol. Med.* 76 (2014) 208–226, <http://dx.doi.org/10.1016/j.freeradbiomed.2014.07.046>.
- [3] H. Sies, Hydrogen peroxide as a central redox signaling molecule in physiological oxidative stress: oxidative eustress, *Redox Biol.* 11 (2017) 613–619, <http://dx.doi.org/10.1016/j.redox.2016.12.035>.
- [4] L. Flohé, The impact of thiol peroxidases on redox regulation, *Free Radic. Res.* 50 (2016) 126–142, <http://dx.doi.org/10.3109/10715762.2015.1046858>.
- [5] B. Chance, H. Sies, A. Boveris, Hydroperoxide metabolism in mammalian organs, *Physiol. Rev.* 59 (1979) 527–605.
- [6] N. Urban, D. Tsisipatis, F. Hausig, K. Kreuzer, K. Erler, V. Stein, M. Ristow, H. Steinbrenner, L.-O. Klotz, Non-linear impact of glutathione depletion on C. elegans life span and stress resistance, *Redox Biol.* 11 (2017) 502–515, <http://dx.doi.org/10.1016/j.redox.2016.12.003>.
- [7] C. Espinosa-Diez, V. Miguel, D. Mennerich, T. Kietzmann, P. Sánchez-Pérez, S. Cadenas, S. Lamas, Antioxidant responses and cellular adjustments to oxidative stress, *Redox Biol.* 6 183–197. doi:<http://dx.doi.org/10.1016/j.redox.2015.07.008>, 2015.
- [8] H.S. Marinho, C. Real, L. Cyrne, H. Soares, F. Antunes, Hydrogen peroxide sensing, signaling and regulation of transcription factors, *Redox Biol.* 2 (2014) 535–562, <http://dx.doi.org/10.1016/j.redox.2014.02.006>.
- [9] N. Brandes, S. Schmitt, U. Jakob, Thiol-based redox switches in eukaryotic proteins, *Antioxid. Redox Signal.* 11 (2009) 997, <http://dx.doi.org/10.1089/ars.2008.2285>.
- [10] R. Brigelius-Flohé, L. Flohé, Basic principles and emerging concepts in the redox control of transcription factors and antioxidants, *Redox Signal.* 15 (2011) 2335–2381, <http://dx.doi.org/10.1089/ars.2010.3534>.
- [11] Y.-M. Go, D.P. Jones, The redox proteome, *J. Biol. Chem.* 288 (2013) 26512–26520, <http://dx.doi.org/10.1074/jbc.R113.464131>.
- [12] J.-W. Lee, J.D. Helmann, The PerR transcription factor senses H2O2 by metal-catalysed histidine oxidation, *Nature* 440 (2006) 363–367, <http://dx.doi.org/10.1038/nature04537>.
- [13] C.X. Santos, A.D. Hafstad, M. Beretta, M. Zhang, C. Molenaar, J. Kopec, D. Fotinou, T.V. Murray, A.M. Cobb, D. Martin, M.Z. Silva, N. Anilkumar, K. Schröder, C.M. Shanahan, A.C. Brewer, R.P. Brandes, E. Blanc, M. Parsons, V. Belousov, R. Cammack, R.C. Hider, R.A. Steiner, A.M. Shah, Targeted redox inhibition of protein phosphatase 1 by Nox4 regulates eIF2 $\alpha$ -mediated stress signaling, *EMBO J.* 35 (2016) 319–334, <http://dx.doi.org/10.15252/emboj.201592394>.
- [14] G. Ferrer-Sueta, B. Manta, H. Botti, R. Radi, M. Trujillo, A. Denicola, Factors affecting protein thiol reactivity and specificity in peroxide reduction, *Chem. Res. Toxicol.* 24 (2011) 434–450, <http://dx.doi.org/10.1021/tx100413v>.
- [15] C.C. Winterbourn, M.B. Hampton, Thiol chemistry and specificity in redox signaling, *Free Radic. Biol. Med.* 45 (2008) 549–561, <http://dx.doi.org/10.1016/j.freeradbiomed.2008.05.004>.
- [16] A. Boveris, E. Cadenas, Cellular sources and steady-state levels of reactive oxygen species, in: L.B. Clerch, D.J. Massaro (Eds.), *Oxyg. Gene Expr. Cell. Funct.* Marcel Dekker, New York, 1997, pp. 1–25.
- [17] G.R. Buettner, B.A. Wagner, V.G.J. Rodgers, Quantitative Redox Biology: an approach to understanding the role of reactive species in defining the cellular redox environment, *Cell Biochem. Biophys.* 67 (2013), <http://dx.doi.org/10.1007/s12013-011-9320-3>.

- [18] C.S. Pillay, B.D. Eagling, S.R.E. Driscoll, J.M. Rohwer, Quantitative measures for redox signaling, *Free Radic. Biol. Med.* 96 (2016) 290–303, <http://dx.doi.org/10.1016/j.freeradbiomed.2016.04.199>.
- [19] Y.S. Bae, S.W. Kang, M.S. Seo, I.C. Baines, E. Tekle, P.B. Chock, S.G. Rhee, Epidermal Growth Factor (EGF)-induced generation of hydrogen peroxide role in egf receptor-mediated tyrosine phosphorylation, *J. Biol. Chem.* 272 (1997) 217–221, <http://dx.doi.org/10.1074/jbc.272.1.217>.
- [20] H.J. Forman, M. Maiorino, F. Ursini, Signaling functions of reactive oxygen species, *Biochem. (Mosc.)* 49 (2010) 835–842, <http://dx.doi.org/10.1021/bi9020378>.
- [21] N. Oshino, B. Chance, H. Sies, The properties of the secondary catalase-peroxide complex (compound II) in the hemoglobin-free perfused rat liver, *Arch. Biochem. Biophys.* 159 (1973) 704–711.
- [22] E.-M. Hanschmann, J.R. Godoy, C. Berndt, C. Hudemann, C.H. Lillig, Thioredoxins, peroxiredoxins, and peroxiredoxins—molecular mechanisms and health significance: from cofactors to antioxidants to redox signaling, *Antioxid. Redox Signal.* 19 (2013) 1539–1605, <http://dx.doi.org/10.1089/ars.2012.4599>.
- [23] P.M. Brito, F. Antunes, Estimation of kinetic parameters related to biochemical interactions between hydrogen peroxide and signal transduction proteins, *Cell. Biochem.* 2 (2014) 82, <http://dx.doi.org/10.3389/fchem.2014.00082>.
- [24] Maxima, Maxima, a Computer Algebra System., n.d. <<http://maxima.sourceforge.net/>>.
- [25] J. LaButti, G. Chowdhury, T.J. Reilly, K.S. Gates, Redox regulation of protein tyrosine phosphatase 1B (PTP1B) by peroxymonophosphate (=O3POOH), *J. Am. Chem. Soc.* 129 (2007) 5320, <http://dx.doi.org/10.1021/ja070194j>.
- [26] H. Zhou, H. Singh, Z.D. Parsons, S.M. Lewis, S. Bhattacharya, D.R. Seiner, J.N. LaButti, T.J. Reilly, J.J. Tanner, K.S. Gates, The biological buffer bicarbonate/CO<sub>2</sub> potentiates H<sub>2</sub>O<sub>2</sub>-mediated inactivation of protein tyrosine phosphatases, *J. Am. Chem. Soc.* 133 (2011) 15803–15805, <http://dx.doi.org/10.1021/ja2077137>.
- [27] D.F. Trindade, G. Cerchiaro, O. Augusto, A role for peroxymonocarbonate in the stimulation of biotinyl peroxidation by the bicarbonate/carbon dioxide pair, *Chem. Res. Toxicol.* 19 (2006) 1475–1482, <http://dx.doi.org/10.1021/tx060146x>.
- [28] Z.-Y. Zhang, Protein tyrosine phosphatases: structure and function, substrate specificity, and inhibitor development, *Annu. Rev. Pharmacol. Toxicol.* 42 (2002) 209–234, <http://dx.doi.org/10.1146/annurev.pharmtox.42.083001.144616>.
- [29] M. Zheng, Activation of the OxyR transcription factor by reversible disulfide bond formation, *Science* 279 (1998) 1718–1722, <http://dx.doi.org/10.1126/science.279.5357.1718>.
- [30] A. Delaunay, A.-D. Isnard, M.B. Toledano, H2O2 sensing through oxidation of the Yap1 transcription factor, *EMBO J.* 19 (2000) 5157–5166, <http://dx.doi.org/10.1093/emboj/19.19.5157>.
- [31] J.D. Brown, A.M. Day, S.R. Taylor, L.E. Tomalin, B.A. Morgan, E.A. Veal, A. Peroxiredoxin, Promotes H2O2 signaling and oxidative stress resistance by oxidizing a thioredoxin family protein, *Cell Rep.* 5 (2013) 1425–1435, <http://dx.doi.org/10.1016/j.celrep.2013.10.036>.
- [32] I.A. Calvo, S. Boronat, A. Domènech, S. García-Santamarina, J. Ayté, E. Hidalgo, Dissection of a redox relay: H2O2-dependent activation of the transcription factor Pap1 through the peroxidatic Tpx1-thioredoxin cycle, *Cell Rep.* 5 (2013) 1413–1424, <http://dx.doi.org/10.1016/j.celrep.2013.11.027>.
- [33] M. Dagnell, J. Frijhoff, I. Pader, M. Augsten, B. Boivin, J. Xu, P.K. Mandal, N.K. Tonks, C. Hellberg, M. Conrad, E.S.J. Arner, A. Ostman, Selective activation of oxidized PTP1B by the thioredoxin system modulates PDGF- $\gamma$  Receptor tyrosine kinase signaling, *Proc. Natl. Acad. Sci. USA* 110 (2013) 13398–13403, <http://dx.doi.org/10.1073/pnas.1302891110>.
- [34] U. Schwertassek, A. Haque, N. Krishnan, R. Greiner, L. Weingarten, T.P. Dick, N.K. Tonks, Reactivation of oxidized PTP1B and PTEN by thioredoxin 1, *FEBS J.* 281 (2014) 3545–3558, <http://dx.doi.org/10.1111/febs.12898>.
- [35] M. Cebula, E.E. Schmidt, E.S.J. Arner, TrxR1 as a potent regulator of the Nrf2-Keap1 response system, *Antioxid. Redox Signal.* 23 (2015) 823–853, <http://dx.doi.org/10.1089/ars.2015.6378>.
- [36] R.D.M. Travasso, F.S. dos Aidos, A. Bayani, P. Abranches, A. Salvador, Localized redox relays as a privileged mode of cytoplasmic hydrogen peroxide signaling, *Redox Biol.* 12 (2017) 223–245, <http://dx.doi.org/10.1016/j.redox.2017.01.003>.
- [37] Z.D. Parsons, K.S. Gates, Thiol-dependent recovery of catalytic activity from oxidized protein tyrosine phosphatases, *Biochemistry* 52 (2013) 6412–6423, <http://dx.doi.org/10.1021/bi400451m>.
- [38] B. Manta, M. Hugo, C. Ortiz, G. Ferrer-Sueta, M. Trujillo, A. Denicola, The peroxidase and peroxynitrite reductase activity of human erythrocyte peroxiredoxin 2, *Arch. Biochem. Biophys.* 484 (2009) 146–154, <http://dx.doi.org/10.1016/j.abb.2008.11.017>.
- [39] E.-M. Hanschmann, M.E. Lönn, L.D. Schütte, M. Funke, J.R. Godoy, S. Eitner, C. Hudemann, C.H. Lillig, Both thioredoxin 2 and glutaredoxin 2 contribute to the reduction of the mitochondrial 2-Cys peroxiredoxin Prx3, *J. Biol. Chem.* 285 (2010) 40699–40705, <http://dx.doi.org/10.1074/jbc.M110.185827>.
- [40] M.S. Seo, S.W. Kang, K. Kim, I.C. Baines, T.H. Lee, S.G. Rhee, Identification of a new type of mammalian peroxiredoxin that forms an intramolecular disulfide as a reaction intermediate, *J. Biol. Chem.* 275 (2000) 20346–20354, <http://dx.doi.org/10.1074/jbc.M001943200>.
- [41] P.A. Karplus, A primer on peroxiredoxin biochemistry, *Free Radic. Biol. Med.* 80 (2015) 183–190, <http://dx.doi.org/10.1016/j.freeradbiomed.2014.10.009>.
- [42] S.G. Rhee, H.A. Woo, I.S. Kil, S.H. Bae, Peroxiredoxin functions as a peroxidase and a regulator and sensor of local peroxides, *J. Biol. Chem.* 287 (2012) 4403–4410, <http://dx.doi.org/10.1074/jbc.R111.283432>.
- [43] A.V. Peskin, N. Dickerhof, R.A. Poynton, L.N. Paton, P.E. Pace, M.B. Hampton, C.C. Winterbourn, Hyperoxidation of peroxiredoxins 2 and 3: rate constants for the reactions of the sulfenic acid of the peroxidatic cysteine, *J. Biol. Chem.* 288 (2013) 14170–14177, <http://dx.doi.org/10.1074/jbc.M113.460881>.
- [44] N. Brenner, T. Friedlander, Adaptive response and enlargement of dynamic range, *Math. Biosci. Eng.* 8 (2011) 515–528, <http://dx.doi.org/10.3934/mbe.2011.8.515>.
- [45] F. Antunes, E. Cadenas, U.T. Brunk, Apoptosis induced by exposure to a low steady-state concentration of H2O2 is a consequence of lysosomal rupture, *Biochem. J.* 356 (2001) 549–555.
- [46] J.M. Davies, C.V. Lowry, K.J. Davies, Transient adaptation to oxidative stress in yeast, *Arch. Biochem. Biophys.* 317 (1995) 1–6, <http://dx.doi.org/10.1006/abbi.1995.1128>.
- [47] A.G. Wiese, R.E. Pacifici, K.J. Davies, Transient adaptation of oxidative stress in mammalian cells, *Arch. Biochem. Biophys.* 318 (1995) 231–240.
- [48] F. Antunes, E. Cadenas, Estimation of H2O2 gradients across biomembranes, *FEBS Lett.* 475 (2000) 121–126.
- [49] V. Oliveira-Marques, L. Cyrne, H.S. Marinho, F. Antunes, A quantitative study of NF- $\kappa$ B activation by H2O2: relevance in inflammation and synergy with TNF- $\alpha$ , *J. Immunol.* 178 (2007) 3893–3902.
- [50] V. Oliveira-Marques, T. Silva, F. Cunha, G. Covas, H.S. Marinho, F. Antunes, L. Cyrne, A quantitative study of the cell-type specific modulation of c-Rel by hydrogen peroxide and TNF- $\alpha$ , *Redox Biol.* 1 (2013) 347–352, <http://dx.doi.org/10.1016/j.redox.2013.05.004>.
- [51] N.J. Adimora, D.P. Jones, M.L. Kemp, A model of redox kinetics implicates the thiol proteome in cellular hydrogen peroxide responses, *Antioxid. Redox Signal.* 13 (2010) 731–743, <http://dx.doi.org/10.1089/ars.2009.2968>.
- [52] B.K. Huang, H.D. Sikes, Quantifying intracellular hydrogen peroxide perturbations in terms of concentration, *Redox Biol.* 2 (2014) 955–962, <http://dx.doi.org/10.1016/j.redox.2014.08.001>.
- [53] M.C. Sobotta, W. Liou, S. Stöcker, D. Talwar, M. Oehler, T. Ruppert, A.N.D. Scharf, T.P. Dick, Peroxiredoxin-2 and STAT3 form a redox relay for H2O2 signaling, *Nat. Chem. Biol.* 11 (2015) 64–70, <http://dx.doi.org/10.1038/nchembio.1695>.
- [54] S.R. Lee, K.S. Kwon, S.R. Kim, S.G. Rhee, Reversible inactivation of protein-tyrosine phosphatase 1B in A431 cells stimulated with epidermal growth factor, *J. Biol. Chem.* 273 (1998) 15366–15372.
- [55] T.-C. Meng, T. Fukada, N.K. Tonks, Reversible oxidation and inactivation of protein tyrosine phosphatases in vivo, *Mol. Cell.* 9 (2002) 387–399, [http://dx.doi.org/10.1016/S1097-2765\(02\)00445-8](http://dx.doi.org/10.1016/S1097-2765(02)00445-8).
- [56] D.E. Heppner, Y.M.W. Janssen-Heininger, A. van der Vliet, The role of sulfenic acids in cellular redox signaling: Reconciling chemical kinetics and molecular detection strategies, *Arch. Biochem. Biophys.* 616 (2017) 40–46, <http://dx.doi.org/10.1016/j.abb.2017.01.008>.
- [57] A. Rinna, M. Torres, H.J. Forman, Stimulation of the alveolar macrophage respiratory burst by ADP causes selective glutathionylation of protein tyrosine phosphatase 1B, *Free Radic. Biol. Med.* 41 (2006) 86–91, <http://dx.doi.org/10.1016/j.freeradbiomed.2006.03.010>.
- [58] M.C. Sobotta, A.G. Barata, U. Schmidt, S. Mueller, G. Millonig, T.P. Dick, Exposing cells to H2O2: a quantitative comparison between continuous low-dose and one-time high-dose treatments, *Free Radic. Biol. Med.* 60 (2013) 325–335, <http://dx.doi.org/10.1016/j.freeradbiomed.2013.02.017>.
- [59] D.E. Fomenko, A. Koc, N. Agisheva, M. Jacobsen, A. Kaya, M. Malinouski, J.C. Rutherford, K.-L. Siu, D.-Y. Jin, D.R. Winge, V.N. Gladyshev, Thiol peroxidases mediate specific genome-wide regulation of gene expression in response to hydrogen peroxide, *Proc. Natl. Acad. Sci. USA* 108 (2011) 2729–2734, <http://dx.doi.org/10.1073/pnas.1010721108>.
- [60] L.E.S. Netto, F. Antunes, The roles of peroxiredoxin and thioredoxin in hydrogen peroxide sensing and in signal transduction, *Mol. Cells* 39 (2016) 65–71, <http://dx.doi.org/10.14348/molcells.2016.2349>.
- [61] A. Delaunay, D. Pflieger, M.B. Barrault, J. Vinh, M.B. Toledano, A thiol peroxidase is an H2O2 receptor and redox-transducer in gene activation, *Cell* 111 (2002) 471–481.
- [62] A.P. Vivancos, E.A. Castillo, B. Biteau, C. Nicot, J. Ayté, M.B. Toledano, E. Hidalgo, A cysteine-sulfenic acid in peroxiredoxin regulates H2O2-sensing by the antioxidant Pap1 pathway, *Proc. Natl. Acad. Sci. USA* 102 (2005) 8875–8880, <http://dx.doi.org/10.1073/pnas.0503251102>.
- [63] E.A. Veal, V.J. Findlay, A.M. Day, S.M. Bozonet, J.M. Evans, J. Quinn, B.A. Morgan, A 2-Cys peroxiredoxin regulates peroxide-induced oxidation and activation of a stress-activated MAP kinase, *Mol. Cell.* 15 (2004) 129–139, <http://dx.doi.org/10.1016/j.molcel.2004.06.021>.
- [64] R.M. Jarvis, S.M. Hughes, E.C. Ledgerwood, Peroxiredoxin 1 functions as a signal peroxidase to receive, transduce, and transmit peroxide signals in mammalian cells, *Free Radic. Biol. Med.* 53 (2012) 1522–1530, <http://dx.doi.org/10.1016/j.freeradbiomed.2012.08.001>.
- [65] L.E.S. Netto, M.A. de Oliveira, C.A. Tairum, J.F. da Silva Neto, Conferring specificity in redox pathways by enzymatic thiol/disulfide exchange reactions, *Free Radic. Res.* 50 (2016) 206–245, <http://dx.doi.org/10.3109/10715762.2015.1120864>.
- [66] C.C. Winterbourn, Are free radicals involved in thiol-based redox signaling? *Free Radic. Biol. Med.* 80 (2015) 164–170, <http://dx.doi.org/10.1016/j.freeradbiomed.2014.08.017>.