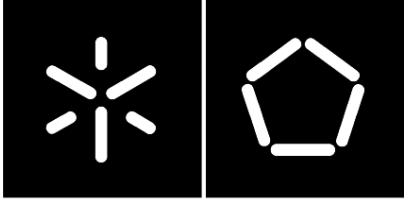
Universidade do Minho

Vinício Miguel Pereira Oliveira

Analysis of output properties of the Peroxiredoxin / Thioredoxin / Thioredoxin Reductase system

January 2015



Universidade do Minho

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Analysis of output properties of the Peroxiredoxin / Thioredoxin / Thioredoxin reductase system

Master's Thesis

Master's in Bioinformatics

Work under the orientation of:

Professor Armindo Salvador

Professor Isabel Rocha

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Análise das propriedades de output do sistema Peroxiredoxina / Tioredoxina / Tioredoxina Reductase

Resumo

Desde os anos 90 o peróxido de hidrogénio (H2O2) tem vindo a ser reconhecido como um segundo mensageiro. No entanto o modo de transdução dos seus sinais ainda não é conhecido. A Peroxiredoxina II (Prx II) é uma proteína com uma vasta distribuição por todo o organismo. Esta proteína é muito reativa com H2O2 sendo que o reduz eficientemente através do gasto de equivalentes redutores transferidos pela Tioredoxina I (Trx I). Uma análise às várias interações deste sistema demonstrou indícios da participação deste na regulação de vários processos celulares. De forma a clarificar o modo como estes processos regulam os sinais de H2O2, o nosso objetivo foi caracterizar as propriedades input – output no estado estacionário do sistema Peroxiredoxina/ Tioredoxina/ Tioredoxina Reductase (Prx/Trx/TrxR) através da definição de um modelo matemático capaz de mimicar o comportamento do sistema. Esta análise demonstrou que o modelo contém cinco regimes funcionais distintos. Estes regimes foram avaliados de acordo com uma série de critérios de performance que consistem em i) maiores valores possíveis para os ganhos logarítmicos (alterações na concentração de uma variável dependente em relação a alterações na concentração de uma variável independente) dos outputs em relação a alterações na concentração de H2O2 e ii) máxima robustez do sistema, ou seja, o mínimo valor possível para a sensibilidade dos outputs em relação a alterações nos parâmetros estruturais do sistema. Esta análise demonstrou que sistemas que operam dentro do Regime 1 têm a melhor performance. Além disso também divulgamos o seguinte princípio de design: a redução de H2O2 pela forma reduzida de Prx II deverá ser um dos passos mais lentos do sistema. Finalmente, as características do Regime 1, relativas às alterações nas concentrações dos outputs em relação a alterações nas concentrações de H2O2, mostraram estar em concordância com as diferentes funções sugeridas para cada output pelos dados experimentais.

Analysis of output properties of the Peroxiredoxin / Thioredoxin / Thioredoxin Reductase system

Summary

Hydrogen peroxide (H2O2) has been widely accepted to play a role as a second messenger. However the way this signaling is transduced is still poorly understood. Peroxiredoxin II (Prx II) is a ubiquitous protein with a very high reactivity with H2O2, efficiently reducing it through the expense of reducing equivalents from Thioredoxin I (Trx I). Analysis of the various interactions of this system showed evidence for a role in the regulation of several cellular processes. In order to clarify how this system helps regulate these processes in response to H2O2, we aimed to characterize the steady – state input – output properties of the Peroxiredoxin/ Thioredoxin/ Thioredoxin reductase (Prx/Trx/TrxR) system by setting a mathematical model that mimics the behavior of this system. This analysis performed showed five distinct functional regimes. We evaluated each regime against a set of performance criteria consisting of i) high logarithmic gains (changes in a dependent variable concentration with respect to changes in an independent variable concentration) of the outputs regarding changes in H2O2 concentrations and ii) high robustness of the system, which is the lowest sensitivity possible from the outputs to changes in structural parameters. From this analysis we showed that systems operating in Regime 1 have the best performance. Furthermore we uncovered the following design principle: the reduction of H2O2 by reduced Prx II must be one of the slowest steps of the system. Finally, characteristics of Regime 1 regarding changes in the outputs with respect to changes in H2O2 concentrations showed to be in accord with the roles suggested for each output by the experimental data.

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Abbreviations List

Akt, Protein kinase B

AP-1, Activator protein 1

AR, Androgen receptor

ASK1, Apoptosis signal – regulating kinase 1

ATP, Adenosine triphosphate

c-Abl, Abelson murine leukemia viral oncogene homolog 1

Cdk5, Cyclin dependent kinase 5

Cys, Cysteine

ERp46, Endoplasmatic reticulum resident protein 46

GSTpi, Glutathione S transferase pi

HO·, Hydroxil radical

JNK, c-Jun NH2 – terminal kinase

kdpx, rate constant for the condensation of peroxiredoxin II

kop1, rate constant for the sulfinilation of peroxiredoxin II

kopx, rate constant for the reduction of hydrogen peroxide

krpx, rate constant for the reduction of peroxiredoxin II

krsx, rate constant for the reduction of sulfinic peroxiredoxin II

krtx, rate constant for the reduction of thioredoxin I

MST1, Mammalian STE20 – like kinase 1

NADPH, Nicotinamide adenine dinucleotide phosphate

NF-kB, Nuclear factor kappa B

NOX, NADPH oxidase enzyme

$O_2^{\cdot -}$, Superoxide

Pap 1, AP-1 like transcription factor

PARP, Poly ADP ribose polymerase

PD, Parkinson's Disease

PDGF, Platelet derived growth factor

PDGFR, Platelet derived growth factor receptor

PDGFR-β, Platelet derived growth factor receptor beta

Prx I, Peroxiredoxin I

Prx II, Peroxiredoxin II

Prx/Trx/TrxR, Peroxiredoxin/Thioredoxin/Thioredoxin reductase system

$PrxSO_2H$, Sulfinic form of peroxiredoxin

$PrxSO_3H$, Sulfonic form of peroxiredoxin

PrxSOH, Sulfenic form of peroxiredoxin

PrxT, Total peroxiredoxin

PTEN, Phosphatase and tensin homolog

PTPases, Protein tyrosine phosphatases

Ref-1, Redox factor 1

ROS, Reactive oxygen species

Srx, Sulfiredoxin

STAT3, Signal transducer and activator of transcription 3

TNF-α, Tumor necrosis factor alpha

Tpx, Thioredoxin peroxidase

Trx I, Thioredoxin I

TrxR, Thioredoxin reductase

TrxSH, Reduced form of thioredoxin

TrxSS, Oxidized thioredoxin

TrxT, Total thioredoxin

TSA, Thiol specific antioxidant protein

Txl, Thioredoxin – like protein

TXNIP, Thioredoxin interacting protein

Vdpx, Peroxiredoxin II condensation flux

VEGFR2, Vascular endothelial growth factor receptor 2

Vop1, Peroxiredoxin II sulfinilation flux

Vopx, Hydrogen peroxide reduction flux

Vrpx, Peroxiredoxin II reduction flux

Vrsx, Sulfinic peroxiredoxin II reduction flux

Vrtx, Thioredoxin reduction flux

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1 Introduction

Reactive oxygen species (ROS) are a group of molecules described as toxic for the cells. These molecules can be Superoxide ($O_2^{\cdot-}$), hydroxyl radical (HO·) and hydrogen peroxide (H_2O_2). High concentrations of these species lead to incidence of tumors and other severe injuries.[1] H_2O_2 has been reported to work as a "fertilizer" for cancer cells, which produce even more H_2O_2 in order to maintain a favorable environment.[2] Many other factors lead to H_2O_2 production, such as radiation[1], inflammation and aging. In addition hydrogen peroxide itself can exacerbate inflammation and aging by causing protein and DNA damage.[2]

Despite its toxicity at high doses, in low amounts hydrogen peroxide acts as a second messenger in signal transduction. From all the ROS, hydrogen peroxide is the most suitable target to study the involvement of these species in signaling. $O_2^{\cdot-}$ has a very low half life time which makes it very difficult to track. In addition once inside the cell it is rapidly converted into H_2O_2. In addition there is still no evidence for in vivo targets for $O_2^{\cdot-}$.[3] In the case of HO·, it is a species that reacts with many organic molecules. This lack of specificity thus makes it unsuitable as a messenger.

1.1 Peroxiredoxin / Thioredoxin / Thioredoxin reductase system

Hydrogen peroxide and other ROS in high concentrations are a peril to the cells. They can be produced in the cell, for instance in mitochondria or in peroxisomes.[4] Since at high concentrations they lead to oxidative stress, cells need mechanisms to protect themselves. They have specific groups of proteins that promote that protection. Among these, Peroxiredoxins have a very high specificity for hydrogen peroxide.[5] These proteins are widely distributed among all living organisms, being in some the only hydrogen peroxide specific antioxidant.[6] They were discovered in 1988 and described as a Thiol-specific antioxidant protein (TSA). Their activity on reducing hydrogen peroxide

is exerted by thiols.[7] In a first step the peroxidatic cysteine of the active site of reduced Prx (Prx-SH) reacts with H_2O_2 which leads to oxidization to the sulfenic form (Prx-SOH).[8] The active site of the most widespread peroxiredoxins contains a second Cys denoted by "resolving cysteine". In a second step of the catalytic site the sulfenic acid condensates with the resolving cysteine forming a disulfide bridge.[5]

Sulfenic acids are very reactive and may react with a second H_2O_2 molecule, being oxidized to the sulfinic form (Prx-SO_2H), a process usually called "overoxidation".[8] The sulfinic acid can also react with a third H_2O_2 and be "hyperoxidized" to a sulfonic species (Prx-SO_3H).[9] These reactions are only relevant at very high H_2O_2 concentrations.

The activity of peroxiredoxin is maintained by reduction of the disulfide by thioredoxin.[10]

Thioredoxin is a disulfide reductase protein, which has a role as peroxiredoxin recycler. This recycling is obtained through the transference of the disulfide of the peroxiredoxin to thioredoxin. At this stage thioredoxin is reduced back by Thioredoxin reductase with the expense of reducing equivalents from NADPH.[11]

All these steps together make the Peroxiredoxin / Thioredoxin / Thioredoxin reductase antioxidant system (figure 1).

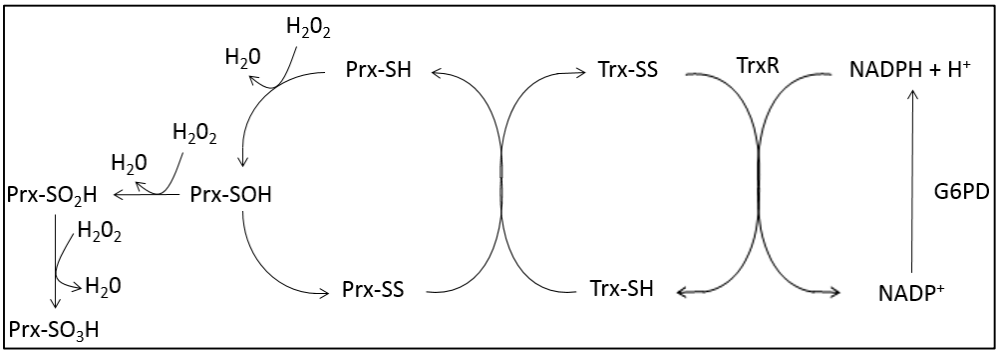

Figure 1 Prx /Trx /TrxR system as adapted from Wu et al Trx1 regulation of Prx I[12] and Adimora et al model of hydrogen peroxide elimination by Jurkat cells.[13] As shown in the model the activity of Prx is dependent on Trx so it can be regenerated and activated. For each complete cycle, the conditions will allow the oxidation of the sulfenic form to the sulfinic and sulfonic species. This only happens under certain conditions, i.e. Prx regeneration is so fast that it won't be able to accumulate dimers and due to high levels of hydrogen peroxide overoxidation occurs. The activity of Trx is itself regulated by TrxR and NADPH.

1.2 Hydrogen peroxide and signaling

Many studies over the last years have been made on the ambiguous role of hydrogen peroxide. Before the 1990's H_2O_2 was mostly viewed as dangerous to the cell, promoting oxidative stress. But first studies in the 70's, had already showed that addition of hydrogen peroxide would mimic the activity of insulin,[14] thus hinting at a possible signaling role. It was by the 90's that a rise on studies on the possible signaling role of peroxide was emerging. This concept gained wider acceptance once it was observed that small concentrations of H_2O_2, both exogenous and endogenous, activated the transcription factor NF-kB.[15,16] A final catalyst to the importance of studying ROS as signaling molecules was the finding that they were involved in cell growth and proliferation. Studies demonstrated that propagation of growth factor signaling needed H_2O_2. In these experiments stimulation with growth factors led to increase of intracellular levels of H_2O_2.[17]

Relating to hydrogen peroxide signaling, a family of ROS generating enzymes, the Nox (NADPH oxidase) family turned to be of great interest.[18] The various isoforms of these enzymes have been found to be located in various compartments in a same cell.[19] This suggests a role in distinct signaling pathways. Also this distribution goes along with the fact that H_2O_2 has a wide and time specific distribution along the cell. The regulated production and degradation of H_2O_2 plus its compartmentalization make it a very suitable second messenger.

Recently a role of H_2O_2 in signaling was clarified, showing that induced hydrogen peroxide production led to temporary inactivation of Prx I. This leads to an amplification of the signal given the fact that H_2O_2 is free to inactivate tyrosine phosphatases. This study has shown that Nox enzymes not only produce peroxide as they facilitate the inactivation of Prx I. This action occurs through phosphorylation by tyrosine kinase during the signaling (figure 2).

In order to guarantee cell safety, this inactivation only happens to the proteins localized in the plasma membrane, near the sites of H_2O_2 production. Cytosolic Prxs are not inactivated, even

though cytosolic Prx I is also prone to phosphorylation. In addition Prx II wasn't shown to be phosphorylated during the signaling.[20]

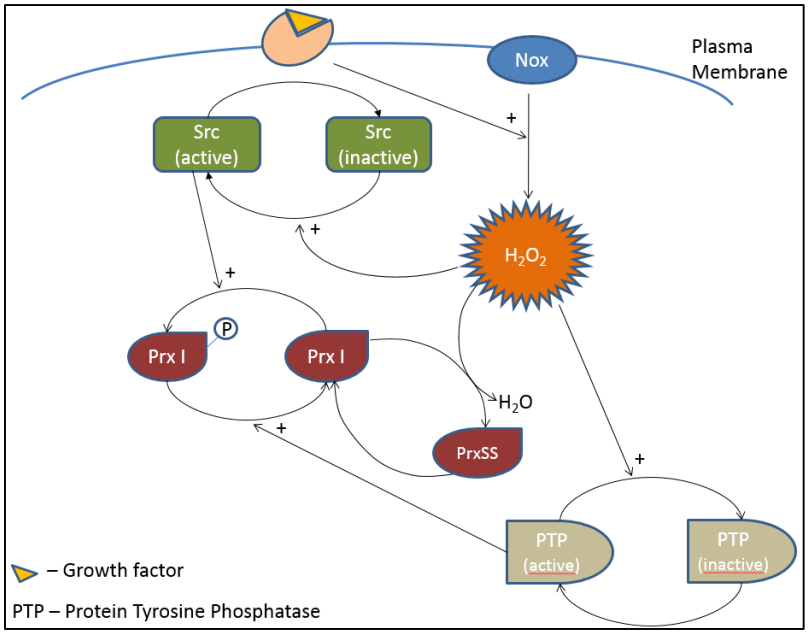

Figure 2 Model of hydrogen peroxide accumulation near the plasma membrane adapted from Woo et al.[20] Growth factors induce H_2O_2 production by Nox enzymes. This facilitates the activation of Src kinase family that inactivates Prx I. H_2O_2 inactivates protein phosphatases continuing the signaling cascade.

1.3 Peroxiredoxins in signaling

In recent years peroxiredoxins were shown to play a role in signal transduction through interactions with several different proteins, and in various oxidation states. These observations imply a shift from the classical definition of these proteins as antioxidant defenses to that of redox sensors. This conceptual shift started in the 90's. Below are described some of these interactions.

One interesting interaction described is with the androgen receptor (AR). AR plays an important role in development and progression of prostate cancer.[21] It was shown that association of Prx I to AR leads to increase of the latter protein's binding activity. Stimulatory effects produced by

hypoxia/reoxygenation were increased by peroxiredoxin. In case of hypoxia and unregulated oxygenation there is an acidic shift of Prx I's Cys52 to its sulfinic or sulfonic form. This brings us to the interesting fact that antioxidant activity is unnecessary for this interaction. Another observation on the regulation by Prx I is that it was shown to increase ligand-stimulated activity of AR. Thus the association of these two proteins is not restricted to just one stimulus.[22]

Hypoxia and reoxygenation in tumors is thought to be responsible for a "forced" natural selection. This condition leads to the selection of cancer cells with greater abilities to survive. This study clearly brings the notion of a physiologic function of Prx in the nucleus.[22] The most important observation was the role for Prx independent of its antioxidant activity.

Peroxiredoxin can also play a role in regulating protein kinases. For instance Prx I interacts with c-Jun NH_2-terminal kinase (JNK). JNK is involved in regulation of cell proliferation, cell death and DNA repair as well by inducing AP-1 (activator protein 1) transcription factor activity.[23] In order to be activated JNK needs to be released from a complex with the glutathione S-transferase pi (GSTpi). The role of Prx in this case is to prevent the dissociation of this complex by strengthening/stabilizing it. Lately this ends in suppression of PARP (Poly (ADP-ribose) polymerase) cleavage.[24] In addition, as shown in the previous interaction, this regulation is independent of Prx antioxidant activity. Studies in lung cancer cells showed that the peroxidatic Cys is oxidized to the sulfinic/sulfonic forms. Those redox – inactive forms were as effective in stabilizing JNK as the active one.[24]

Some more recent research showed that Prx I associates with PTEN (phosphatase and tensin homolog). PTEN is a lipid phosphatase with a major role as phosphatidylinositol – 3- phosphatase. This interaction leads to a negative regulation of Akt (protein kinase B). Thus, PTEN is seen as a tumor suppressor protein.[25]

Under high levels of hydrogen peroxide PTEN is oxidized becoming inactive. Ultimately this leads to an increase in Akt activity. Normally under oxidative stress (~25μM H_2O_2)[26] Prx binds to PTEN protecting its lipid phosphatase activity. Under strong oxidative stress (~500 μM H_2O_2)[26] there's a decrease in the binding between the two proteins leading to a hindrance of PTEN activity. Some studies showed that Prx I binds PTEN in an equimolar ratio. Thus, an excess of Prx I doesn't increase PTEN activity. This interaction occurs between the catalytic site of Prx (Cys 51 and Cys172) and the C2 domain of PTEN. It is possible that Cys 51 regulates the binding/disruption

of the Prx-PTEN complex. Being the peroxidatic cysteine it may respond to increases in H_2O_2 levels. In addition it was shown that the peroxidase-inactive C52S Prx mutant stays bound in the complex even with increasing levels of hydrogen peroxide. Excessive levels of Trx lead to the rapid reduction of Prx, always leaving it prone to oxidation, thus reducing its binding ability to PTEN.[26]

Once again it is seen another role for peroxiredoxin beyond the antioxidant one. In this case an interaction with a phosphatase, where even thioredoxin, another antioxidant protein, may play a role. The fact that the peroxidase activity may contribute to regulate this interaction is interesting. It suggests that depending on the levels of hydrogen peroxide Prx may either complex with PTEN or respond to a higher oxidative stress by scavenging H_2O_2. This study clearly points to a possible redox signaling system.

A fact that may consolidate this hypothesis is the chaperone function described for Prx. It was shown that under heat or oxidative stress, Prx shifts to a high molecular weight form. This change leads to a switch on the activity of peroxiredoxin. At a lower molecular weight there is predominantly peroxidase activity, whereas the highest molecular weight complex exhibits mostly chaperone activity with very little peroxidase activity.[27,28] Finally, the chaperone activity of Prx was shown to enhance resistance to heat shock and to cell death.[27,28]

The peroxidatic cysteine was shown to be important to determine the function of the protein, acting as a "H_2O_2 sensor".[27,28] The fact that the notion of Prx as a hydrogen peroxide sensor is referred as important to this particular function consolidates the hypothesis of giving an even more special role to this protein. Given the fact that chaperone activity is important to signaling, evidence relating Prx to redox signaling becomes stronger with the notion of this sensor function.

1.3.1 Peroxiredoxin II in signaling

This work focuses on Prx II. This has a very wide distribution along the organism and it is very abundant in various cell types. For instance, it is the third most abundant protein in human erythrocyte.[29]

A model of Prx II's role in hydrogen peroxide metabolism and sensing in human erythrocyte was developed in our group.[30] This model suggests that the peroxidase activity of Prx II in these cells is subject to a strong but readily reversible inhibition by a hitherto unidentified inhibitor. This computational analysis also suggested that the main role of Prx II in human erythrocytes would be in signaling.[30]

Prx II has a small number of known interactions, but all with relevant physiological context. There are connections to vascular development, cancer and even neurodegenerative diseases.[31–33]

One of these interactions is with cyclin dependent kinase 5 (Cdk5) in Parkinson's disease (PD) models. It was shown that in mice models treated with the MPTP neurotoxin, Prx II underwent phosphorylation by Cdk5, at Threonine89. Thus it would lose its peroxidase activity leading to neuronal cell death.[32] Additionally it was observed in human PD patients increased levels of Prx II, pointing to a neuro-protective role.[32]

In relation to cancer and vascular development, Prx II has two interesting indirect interactions. The first one is with vascular endothelial growth factor receptor 2 (VEGFR2). It was observed that Prx II co-localizes with this receptor in caveolae, protecting it from oxidative inactivation. This protective role was exerted either by eliminating surrounding H_2O_2 or by reducing back oxidized VEGFR2. This result is by itself of great interest, because caveolae structures are related to signaling. During this study it was also observed that Prx II knockout mice were healthy. However in pathogenic tissues Prx II plays an important role. The antiangiogenic effect of knocking out Prx II leads to a reduced tumor growth for instance. This has brought not only a new insight on redox regulation by Prx II as an antiangiogenic target in cancer treatment.[31]

In a similar way, Prx II influences the proliferation and migration of smooth muscle cells in injured arteries. In this study it was shown that Prx II was a specific and main regulator of platelet derived growth factor receptor (PDGFR). [33]

Mouse embryonic fibroblast and NIH3T3 cells ablated of Prx II showed increased production of H_2O_2 upon PDGF stimulation. Hydrogen peroxide in its turn increases phosphorylation and consequent activation of PDGFR. Re-introduced Prx II reduced the PDGF-induced proliferation in these cell lines. Furthermore it was observed that stimulation with PDGF led to recruitment of Prx

II to PDGFR-β. This suggested that Prx II regulates PDGFR activity through maintenance of the reduced state of protein tyrosine phosphatases in the surrounding environment of PDGFR.[33]

This mechanism was also observed in human aortic vascular smooth muscle cells, where Prx II selectively suppressed PDGFR phosphorylation. Finally it was shown that neointimal layers were thicker in injured carotid arteries in Prx II knockout mice than in wild type. Thus, these results suggest a Prx II regulation of H_2O_2 signaling in vascular remodeling.[33]

Recently one of the very few direct protein interaction partners was found. It was observed that Prx II and ERp46 indeed associate in Jurkat and endothelial cells. More interestingly is that this is one of the first partners that require specifically hyperoxidized Prx II, which is known for its decameric structure.[34]

This association also requires ERp46 to contain intramolecular disulfides. In this case the most probable candidates are the C-terminal Cys381 and Cys388. Even though this interaction was shown to be non-covalent, a disulfide exchange between oxidized dimeric Prx II and reduced ERp46 was shown to occur. Thus an initial step of this non covalent interaction could require a disulfide bond between both proteins.[34]

Studies on the consequences of this particular interaction still remain to be done. However given the distribution and roles of both proteins, a role in redox signaling may be a possible outcome. It was shown that ERp46 is not restricted to the ER but is also present in the cytosol and in the plasma membrane. It even was proposed that it plays a role in various functions such as inhibition of NOX2 activation in lipid rafts, by direct association induced by atorvastatin[35], and modulation of adiponectin receptor AdipoR1 activity in the plasma membrane.[36]

Since Prx II was also shown to localize in plasma membrane associated structures, such as caveolae[31] and is mainly present in cytoplasm, it is plausible to suggest a possible role in redox signaling.

1.4 Thioredoxin in signaling

The vision that thioredoxin (Trx) works as an oxidant sensor and plays a role in regulation of redox signaling has been emerging. Besides its role in the maintenance of Prx reduced form, Trx has many other interactions with hundreds of other proteins. Many of those interactions are a part of different pathways with important physiological consequences. Most of Trx's functions are related to proliferation and apoptosis.[37] These interactions point to a Trx regulatory role in terms of modulation of the redox signal.

Interaction between Trx and ASK-1 (apoptosis signal-regulating kinase 1)[38] is of great relevance. This was one of the first described interactions related to a possible redox signaling role.

This interaction is redox dependent, which means that it is only possible to occur when Trx is in its reduced form. In this case either of Trx1 active site cysteines binds to ASK-1 Cys250 to form an intermolecular disulfide.[39] In the case of Trx2 the binding occurs between Trx2 Cys90 and ASK-1 Cys30.[40] Association of Trx with ASK-1 induces then ubiquitination and degradation of this protein leading to the inactivation of its kinase-mediated signal transduction activity. [39] In the presence of hydrogen peroxide, thioredoxin will be oxidized and the binding with ASK-1 disrupted.[39] This leads to the activation of ASK-1 activity. This kind of interaction, as referred, was a first look at Trx and redox signaling. In this first case we have a known redox agent, H_2O_2, involved in a signaling pathway regulated by Trx.

A similar and very recent described interaction occurs between Trx and mammalian STE20 – like kinase-1 (MST1).[41]

MST1 is a serine/threonine kinase with a ubiquitous distribution.[42] This protein mediates an oxidative stress – induced cell death signaling pathway.[43,44]

Interaction between Trx and MST1 only occurs under reducing conditions, where Trx active site cysteines are needed. Also the SARAH domain (residues 431-487) of MST1 is crucial for this interaction.[41] This association leads to inhibition of MST1 kinase activity. Again presence of hydrogen peroxide will inhibit this interaction. In this case the action of thioredoxin reductase (TrxR) is of great importance. TrxR will reduce the oxidized Trx returning its capability to associate to

MST1. Tumor necrosis factor alpha (TNF-α) promotes ROS production, thus enhancing MST1 activity. TNF-α is then responsible for the release of MST1 from the Trx-MST1 complex.

Beyond kinases activity, Trx is known to regulate other types of processes. NF-kB (nuclear factor kappa B) is a transcription factor that is involved in cellular response to certain stimuli. It binds to DNA and promotes the expression of many genes depending on the stimulus.[45] Interestingly interaction of Trx with NF-kB increases the DNA binding activity as does a stimulus from ROS. It is through the reduction of a disulfide bond that Trx exerts its activity on NF-kB.[46]

Interactions at the transcription factors level doesn't resume only to NF-kB. Other example of regulation of transcriptional activity is the interaction of Trx with Ref-1 (redox factor 1). Ref-1 is a molecule that stimulates AP-1(activator protein 1) DNA binding.[47] Association is done by the formation of a covalent heterodimer between the active site of Trx and Ref-1. This interaction leads to an increase in the binding activity of AP-1.[47] This interaction is another example of the involvement of thioredoxin in the regulation of transcription factors. This shows that either in a direct or indirect way there is a redox regulation of these proteins. These examples of interactions not only give us insight on the versatility of thioredoxin as it does in redox signaling

Trx was also shown to regulate PTEN activity. This interaction occurs in an opposite way of the one between Prx and this same phosphatase. Thus, association of Trx active site and PTEN C2 domain leads to inhibition of its phosphatase activity. This leads to activation of Akt kinase activity leading to inhibition of apoptosis. Not only is Trx showed to be involved in tumorigenesis as it is also included in a signaling pathway leading to this specific physiological consequence.[48]

The role given to Trx was that of an antioxidant, which by disulfide reduction would clear the system of reactive oxygen species. As we may see interactions of thioredoxin show an involvement in many pathways. Kinase activity, transcription factors and many other processes have in thioredoxin a redox regulator. These processes are also prone to the action of hydrogen peroxide. All this evidence brings us closer to the implication of thioredoxin in redox signaling.

1.5 Prx/Trx/TrxR system in redox signaling

The Prx/Trx/TrxR system is one of a set of antioxidant defenses present in almost every organism. However more can be pointed to this system beyond the given definition. A factor that promotes this is the high reactivity and specificity for hydrogen peroxide added to its ubiquitous distribution. H_2O_2 is becoming more and more envisioned as a signalling molecule rather than a threat to the cells. Throughout the years it has been described that at lower concentrations it leads to signalling effects. Advances in studies showed that there really is a redox regulation of many signalling pathways with relevant physiological consequences. The entire mechanism on how this signalling works is not quite well understood.

The same way as hydrogen peroxide, there has been a development in the understanding of the roles of peroxiredoxin and thioredoxin. Both of these proteins, as pointed before, have a very ubiquitous distribution. Prx is the protein with the higher reactivity and specificity for hydrogen peroxide. Hydrogen peroxide is not very reactive at low concentrations having also signaling effects, with the most probable signaling sensor being peroxiredoxin. Some relevant interactions of Prx and Trx place them in a regulatory role in some signaling pathways. Not only do they have this role as they have opposite effects in most of the cases (figure 3 A and B). This last observation is consistent with the observation that Prx and Trx work as a system in regulating some cellular processes.

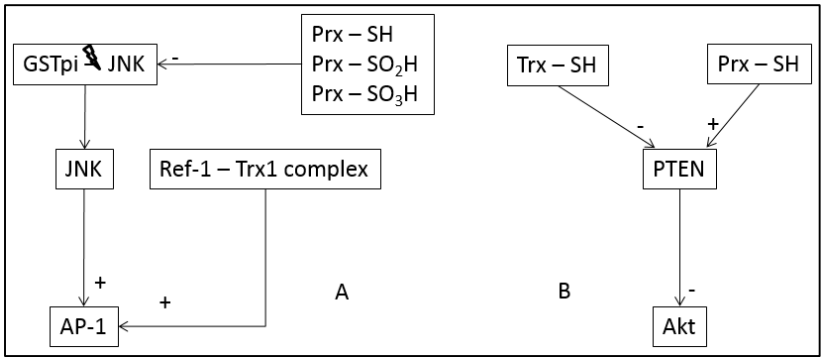

Figure 3 We present two schemes to show how the same protein interacts with Prx and Trx **A)** Redox regulation of AP-1 transcriptional activity. View of the different redox regulation by Trx and Prx. As described, Prx inhibits release of JNK from complex with GSTpi. Thus the consequent AP-1 activation by JNK is inhibited. Trx by forming a complex with Ref-1 stimulates activation of AP-1. **B)** Redox regulation of the lipid phosphatase PTEN. As described PTEN activity is induced by Prx and leads to inactivation of Akt. In an opposite effect Trx inhibits PTEN activity, thus leading to activation of Akt.

Another very interesting case is ASK1. As referred it is known to interact with Trx1. A recent study brought some interesting insights on the redox regulation of this protein. It was shown that it not only interacts with Prx I, as the latter would work as the H_2O_2 signal transducer. Thus oxidized Prx I would interact with ASK1 activating it through transference of disulfide.[49] In its turn, as described, Trx1 would reduce back ASK1 inactivating it. Integration of both interactions shows a possible role for redox modulation of signaling for some proteins as is the case of ASK1 (figure 4).

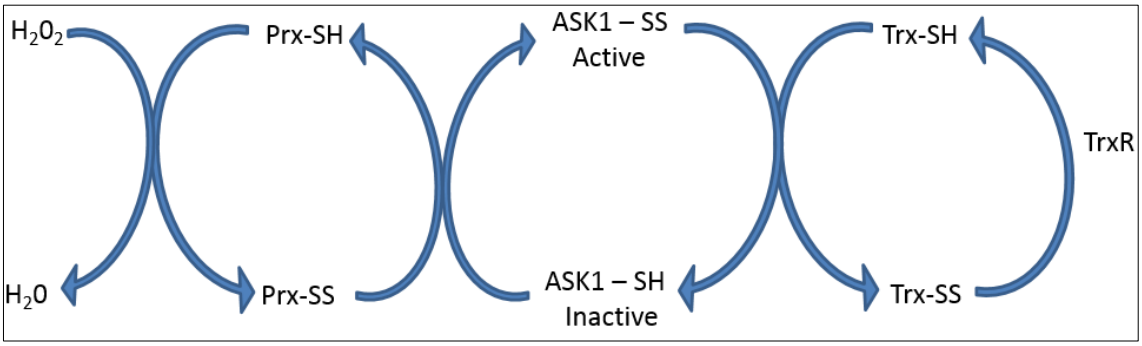

Figure 4 Simplified model of ASK1 redox regulation adapted from Jarvis et al. This model proposes a H_2O_2 signal transduction through disulfide transference from Prx I to target proteins. Trx I readily reduces these proteins back inactivating them.

On a more recent study it was shown one more possible mechanism for the promotion of H_2O_2 signaling. In this case it involves the whole Prx system making this study of great interest. Particularly it is shown how a peroxiredoxin (thioredoxin peroxidase Tpx) can amplify the hydrogen peroxide signal by leading to oxidation of thioredoxin (thioredoxin-like protein Txl1 or Trx3). Loss of Trx increases Pap 1 activity under normal conditions, which also means, and was shown that Trx is a repressor of Pap1 expression. Inactivation of Pap1 is obtained by reduction of this protein by thioredoxin which inhibits oxidation and activation induced by H_2O_2. Finally it was observed that oxidation of Trx is promoted by Prx resolving cysteine. This oxidation allows the amplification of the H_2O_2 signal transduction.[50]

1.6 Prx/Trx/TrxR system, a cell's "fire alarm system"

A detection system is seen as a "reader" of the surrounding environment, providing new information about its status[51]. Thus, the main function of such system is to inform an antifire agent (either human or mechanical) of the probability of an unwanted fire[51]. Further ahead, the agent, in its place is capable of executing a determined set of actions according to the information. These actions can be: "investigation of the presence and attributes of the fire; fighting the fire; escaping; notifying others; help others escape"[51]. All of these actions are either for human and/or mechanical execution and should be designed in order to minimize the losses.

When trespassing to the biological processes world, one can find many analogies with all kinds of manmade systems. Such as the observation of a system like the Prx/Trx/TrxR being the biological analog of a sophisticated fire alarm system, a "Hydrogen Peroxide Alarm System".

Taking the characteristics of both systems together, we have: a) Prx system works in order to protect the cell from oxidative stress minimizing losses by executing certain actions; b) The system as a whole works by updating information of the redox state of the cell and responding to it; c) actions can be read as the modulation of the H_2O_2 signal, which dictates the context of the cell.

The biological H_2O_2 alarm system should be constituted and function very similarly as its fire analog. In a biological context such system should:

- Fight H_2O_2 increasing levels (regulation of defense mechanisms signaling cascades);
- Notify the cell (other cellular components in a way similar to the previous action);
- "Help other cells escape" (through apoptosis in cases of heavy loads of oxidative stress). This last point means that the goal is maintaining the neighboring cells' integrity.

All of these actions are clearly designed in order to minimize damage and losses, as such system should do.

Analyzing now the different components of the system and their roles, it is easy to observe the similar aspects with components from real fire systems.

Sensor device/ initiating device: In the case of fire systems, such devices take many forms in order to detect changes in various parameters[52]. Some detectors, or sensors, are designed to respond in a way that best fit the variation detected. Peroxiredoxin plays that role perfectly, as it will respond to changes in levels of H_2O_2. Depending on the state of the system and the concentration of peroxide the responses will vary. On a first response, the first cycle, the one without overoxidation act more as a sensor/sprinkler device, clearing H_2O_2 and passing the message by protecting inactivation of different proteins. In this case the cell will grow and proliferate normally in response to the low levels of H_2O_2.

Notification appliances: In fire systems, the most common are audible and visible signals that deliver instructions to the occupants. There is also the existence of trained personnel giving real time instructions.[52] In the case of Prx we have two distinct alarms.

Prx II are very sensitive to overoxidation. In cases when the cell is facing such state, it is observable the formation of Prx-SO2H. Normally these forms undergo oligomerization and form decameric structures[34]. These structures can even form filaments in the cytosol and near the plasma membrane[53]. Such behavior and localization suggest a role in warning the cell of a possible oxidative stress and the prediction of depletion of reducing equivalents. It is also related to the triggering of cellular defense systems. So in this case, $PrxSO_2H$ is analogous to a warning alarm, giving survival instructions to the cell.

With high increases in H_2O_2 the cell loses its capability to reset the system. In these conditions occurs an accumulation of PrxSS, because of the unavailability of reduced Trx to reduce it back. There is the possibility for the disulfide species of Prx function as an analogous to a self-destruction alarm for these conditions (high oxidative stress and depletion of reducing equivalents).

Seen, for instance, in the interaction of Prx I with ASK1[49]. Increase of this alarm leads to apoptosis, sparing the neighboring cells.

Under low quantities of H_2O_2 these species won't accumulate. There is still a response to H2O2 from PrxSOH and PrxSS, the action of this system's switch readily reverses it, which makes these interactions short events under these conditions.

Switch mechanism: Normally a circuit only functions while the switch is closed, or "ON".

In the present biological context the system is powered by the reducing equivalents. Thus this system will keep functioning, while there is availability of reducing equivalents. In this case, the reduced Trx I functions in a way analogous to a switch. While there is availability of the power supply, the switch will be "ON" allowing the system to work properly. The function of this switch is thus maintained by TrxR, which assures the availability of reducing equivalents and consequently TrxSH. in cases of oxidative stress the supply will be depleted hindering the maintenance of the switch and consequently turning the system down. Thus there will be accumulation of PrxSS, which will produce H_2O_2 – induced responses leading to apoptosis signaling.

The function of this switch seems to be versatile, since while it is available, not only it powers the whole system, as it readily shuts down the responses from PrxSOH and PrxSS to H_2O_2, making it also working in an analogous way as a clock alarm switch.

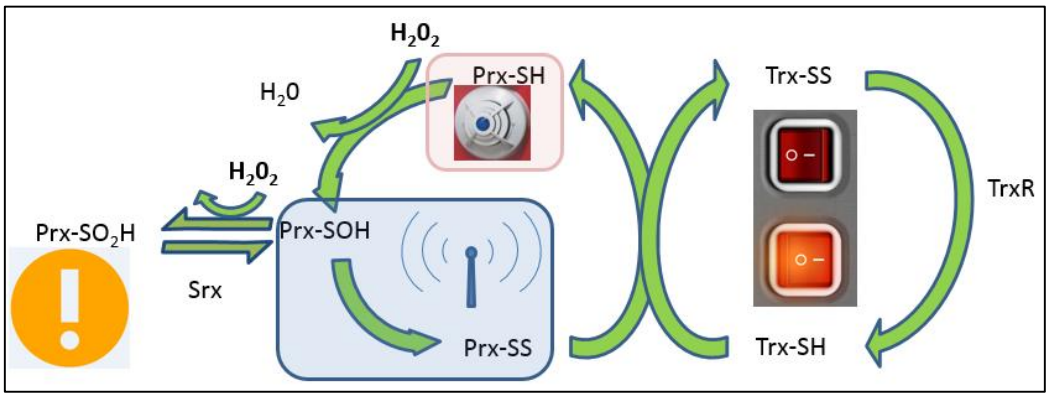

Figure 5 Working hypothesis for the signaling role of the Prx/Trx/TrxR system according to gathered information. Reduced Prx senses H_2O_2 and the systems responds by transmitting the signal through PrxSOH and PrxSS, which under low quantities of H_2O_2

will be reversed by Trx I. Additionally there is the response of $PrxSO_2H$ which responds to the presence of H_2O_2 by warning the cell of a possible dangerous event.

2 Problem and objective

Over the last years there has been an advance in the characterization of the functions of hydrogen peroxide. These developments led to the definition of H_2O_2 as a signaling molecule. The targets of further researches are the mechanisms underlying this signal transduction.

Parallel to the advances in the functions of H_2O_2 were also the development of the insight on the roles of peroxiredoxin and thioredoxin. These two proteins have been shown to regulate several signaling pathways suggesting that the Prx/Trx/TrxR system plays an important role in the H_2O_2 signal transduction. However, the mode how this system regulates cellular processes is still poorly understood.

We aim to clarify this problem by:

1. Cataloguing the known interactions of the various redox forms of peroxiredoxin and thioredoxin with other proteins that participate in the regulation of cellular processes.
2. Characterizing the steady state input-output characteristics of the Prx/Trx/TrxR system with regards to the interactions catalogued in point 1.
3. Use these results to help understand how the Prx/Trx/TrxR system helps coordinating distinct cellular processes and find the design principles that warrant effective operation.

3 Methodology

3.1 Biochemical systems analysis

An organism can be seen as a complex network of reactions. For instance we can think of the metabolic map. It represents all the reactions and interactions that occur in a given organism. As we can see many reactions depend on others and they all connect along the way. Thus it is observable that they look like a system of reactions that work together in order to bring life. A system is, thus defined as a set of interactions of different components, each with its individual properties changing according to the state of the system as a whole.

A given system can be represented in different levels of complexity. Thus it may be analysed in various ways depending on the focus and the study in case.

Our aim is to analyse the characteristics of the different outputs from the Prx/Trx/TrxR system. Thus we rely on quantitative information, which best characterizes how reactions occur. For this kind of complexity a kinetic approach is preferable. This kind of approach uses quantitative information allowing a mathematical and computational analysis of our system which will then give information on its behaviour with changes in relevant variables.

3.1.1 Kinetic modelling

To begin with the setup of the model we gathered the most relevant processes in literature. The mechanisms of the peroxiredoxin system were already described in the Introduction. Additionally, we identify the main reactions and rate constants for the present model, with some assumptions in order to simplify it. As seen in table I, the majority of rate constants have been determined, in exception for the reduction of $PrxSO_2H$ and reduction of TrxSH.

Sulfiredoxin (Srx) catalyses the reduction of $PrxSO_2H$ back to PrxSOH, requiring ATP and Trx.[54] It is a highly inefficient enzyme, having a k_{cat} = 0.18min^{-1} ref. [54]. For simplicity we assume that the reduction of $PrxSO_2H$ follows pseudo-first-order kinetics, with rate constant, krsx (Table I). A very recent study showed that in mice red blood cells a substantial fraction of $PrxSO_2H$ is degraded by proteases.[55] However we neglected this process.

The reduction of TrxSS back to TrxSH is catalysed by TrxR. The later recycles Trx with NADPH through a ping-pong mechanism.[56] We assume that the enzyme is with NADPH and always very far from saturation with TrxSS as occurs in human erythrocytes. Under these conditions this reaction follows pseudo-first-order kinetics, with rate constant krtx (Table I).

All other processes were considered to adhere to mass action kinetics. The remaining rate constants were obtained from literature or estimated in ref. [30]. Mechanisms for these processes are described in the Introduction.

Table I Steps of the Prx/Trx/TrxR system and the respective rate constants, considered in the present kinetic model for Prx II and Trx I. In this model krsx and krtx are pseudo-first-order rate constants for the reduction of the sulfinic species and reduction of thioredoxin, respectively.

Reactions	Rate constants
$PrxSH + H_2O_2 \rightarrow PrxSOH + H_2O$	Prx oxidation[57], $kopx = 10^8 M^{-1}s^{-1}$
$PrxSOH \rightarrow PrxSS$	Prx condensation[58], $kdpx = 1.7s^{-1}$
$PrxSS + TrxSH \rightarrow PrxSH + TrxSS$	Prx reduction / Trx oxidation[57], $krpx = 2.1\ x\ 10^5 M^{-1}s^{-1}$
$TrxSS \rightarrow TrxSH$	Trx reduction[30], $krtx = 5.5s^{-1}$
$PrxSOH + H_2O_2 \rightarrow PrxSO_2H + H_2O$	Prx sulfinylation[58], $kop1 = 1.2\ x\ 10^4 M^{-1}s^{-1}$

$PrxSO_2H \rightarrow PrxSOH$	Reduction of Prx sulfinic form[30], $krsx = 10^{-4}s^{-1}$

3.1.1.1 Graph representation

The first step for the analysis of a system is its representation. Graphs have been used to represent network systems in various areas, for instance informatics or even electronic circuits. For biochemical systems this type of representation has also been used. Particularly, directed graphs are used in case of biochemical reactions. This allows a better visualization of the interactions between the different components of a system and how material and information flow.

The setup of the graphical representation needs to take in account the relevant interactions and processes for the system in analysis. These are gathered in table I and represented in Figure 6.

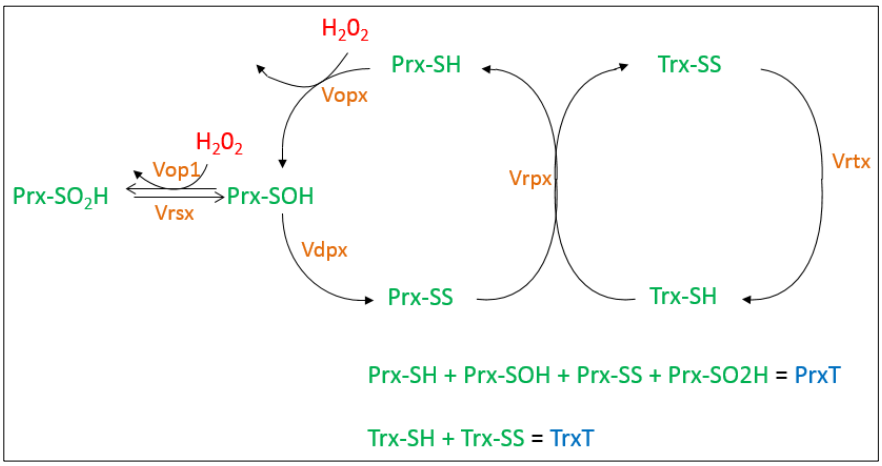

Figure 6 Graphical representation of the Prx/Trx/TrxR system. There are two different sets of variables: 1: State variables: Red – Independent variables, Green – Dependent variables, Blue – Aggregated variables; 2: Flux variables: Orange.

3.1.1.2 Variables

Normally a system is composed of two groups of variables: state variables and flux variables.

State variables correspond to everything that can be characterized in terms of concentration or other physical quantities, e.g. inputs, outputs and temperature. State variables are thus any quantities whose values change and influence certain processes.

State variables can also be divided in other three types: independent, dependent and aggregated variables:

- Independent variables can be defined as fixed, they are set values independent of other variables. In the present model, we have only one independent variable, H_2O_2.

- Dependent variables are all the variables with values that depend on the processes of the system and the independent variables. Our model has six dependent variables: PrxSH, PrxSOH, PrxSS, $PrxSO_2H$, TrxSH and TrxSS.

- Aggregated variables are the sum of certain variables. They also represent mass conservation equations. The two equations present in figure 5 are the mass conservation equations for our system. We have PrxT and TrxT corresponding to total peroxiredoxin and total thioredoxin respectively.

Flux variables are the rates of given processes, and are related to state variables through rate laws.

A rate law can be defined as a function of all the state variables that influence a given process. In other words defining a flux as a function of concentrations. For the model in figure 5 we have 6 flux variables. Each flux can be defined by the respective concentrations and rate constants:

- $Vopx = kopx\ PrxSH\ \mathrm{H_2O_2}$
- $Vdpx = kdpx\ PrxSOH$
- $Vrpx = krpx\ PrxSS\ TrxSH$
- $Vop1 = kop1\ PrxSOH\ \mathrm{H_2O_2}$
- $Vrtx = krtx\ TrxSS$
- $Vrsx = krsx\ PrxSO_2H$

3.1.1.3 Computational implementation

The model has been set as a system of algebraic-differential equations according to Kirchoff's node equation or Mass balance equation (equations 1-6). This describes the behavior of the dependent variables of the system. It states that the difference between what enters and what leaves the pool, must accumulate in the pool.

$$\frac{dPrxSH}{dt} = krpx\ PrxSS\ TrxSH - kopx\ PrxSH\ \mathrm{H_2O_2} \quad (1)$$

$$\frac{dPrxSOH}{dt} = kopx\ PrxSH\ \mathrm{H_2O_2} + krsx\ PrxSO_2H \\ -kdpx\ PrxSOH - kop1\ PrxSOH\ \mathrm{H_2O_2} \quad (2)$$

$$\frac{dPrxSO_2H}{dt} = kop1\ PrxSOH\ \mathrm{H_2O_2} - krsx\ PrxSO_2H \quad (3)$$

$$\frac{dTrxSS}{dt} = krpx\ PrxSS\ TrxSH - krtx\ TrxSS \quad (4)$$

$$PrxT = PrxSH + PrxSOH + PrxSO_2H + PrxSS \quad (5)$$

$$TrxT = TrxSH + TrxSS \quad (6)$$

3.1.1.4 Logarithmic gains

The logarithmic gain characterizes approximately the percent change in a dependent variable with a 1% change in an independent variable with other independent variables and parameters held constant. This factor is used to describe the extent to which a signal is amplified or attenuated during its propagation. The logarithmic gain of a dependent variable X with changes in an independent variable Y is given by:

$$L(X,Y) = \frac{\partial \log(X)}{\partial \log(Y)} = \frac{\partial X}{\partial Y}\frac{Y}{X} \quad (7)$$

3.1.1.5 Sensitivities

The parameter sensitivities characterize the behavior of a system in response to changes in its parameters. These factors relate then the outputs and the system's parameters, which are useful to determine the robustness of a system to changes in external factors, such as temperature for instance. Specifically the rate constant sensitivities characterize the change of a dependent variable in going from one steady state to another as a result of a change in a rate constant parameter. The sensitivity of a dependent variable X for changes in a parameter k is given by:

$$S(X,k) = \frac{\partial \log(X)}{\partial \log(k)} = \frac{\partial X}{\partial k}\,\frac{k}{X} \tag{8}$$

The robustness of a dependent variable is given by the sum of the absolute values of the sensitivities of that variable to all the structural parameters of the system:

$$\sum |S(X,k_i)| \tag{9}$$

The model and respective computational analysis have been implemented in Mathematica™ 9.0.1[59].

3.1.2 Design-space approach

The design space approach is the core method of this study. This approach consists in the establishment of a design space that relates genotypes and phenotypes, which is the understanding

of how an organism responds to changes in the environment. Ultimately this clarifies the function and the fitness of a system in a given context.

Parameters are used as representation of the genotype, since its digital representation is still an obstacle. This representation involves a dimensional compression by scaling all variables and parameters. This process reduces the number of parameters by combining them to create new dimensionless ones.

Through steady-state analysis it is possible to gather some information on how systems respond to changes in inputs and external factors considering they are in a state of equilibrium.

A system in steady state has no accumulation of mass or energy. In Kirchoff's node equation since the difference between flux in and flux out corresponds to the accumulation in the pool, means that in steady state it is zero. Thus, time derivatives are equal to zero.

Equations 1-6 are transformed and we obtain the dimensionless steady state system as seen in equations 7-12.

$$\rho\ prxss\ trxsh = \alpha\ prxsh \tag{10}$$

$$\alpha\ prxsh + \sigma\ prxsooh = prxsoh + \alpha\ \eta\ prxsoh \tag{11}$$

$$\alpha\ \eta\ prxsoh = \sigma\ prxsooh \tag{12}$$

$$\rho\ \gamma\ prxss\ trxsh = \delta\ trxss \tag{13}$$

$$prxss + prxsh + prxsoh + prxsooh = 1 \tag{14}$$

$$trxss + trxsh = 1 \tag{15}$$

Where the six new parameters are:

$$\alpha = \frac{kopx\ \mathrm{H_2O_2}}{kdpx} \quad \rho = \frac{krpx\ \mathrm{TrxT}}{kdpx} \quad \sigma = \frac{krsx}{kdpx} \quad \delta = \frac{krtx}{kdpx} \quad \gamma = \frac{PrxT}{TrxT} \quad \eta = \frac{kop1}{kopx}$$

And the normalized variables:

$$prxsh = \frac{PrxSH}{PrxT} \quad prxss = \frac{PrxSS}{PrxT} \quad prxsoh = \frac{PrxSOH}{PrxT} \quad prxsooh = \frac{PrxSO_2H}{PrxT}$$

$$trxsh = \frac{TrxSH}{TrxT} \quad trxss = \frac{TrxSS}{TrxT}$$

Each side of the steady state equations 7 – 12 is a sum of several terms. Each term is potentially dominant depending on the conditions, which are defined by a set of inequalities. Since each term can be dominant there are as many solutions as there are combinations of dominant terms. Because of all the different solutions a design space is partitioned in different regions of different phenotypes. Thus each solution can be taken as a phenotype respective to a given region. However, not all the potential solutions are valid and in order to be validated, each solution is tested against the respective inequalities. The inequalities and solution of each region define its boundaries in the design space. The local behavior of the system within each region is characterized by the Logarithmic Gains and Sensitivities.

These characteristics (Logarithmic Gains and Sensitivities) are compared against a set of quantitative criteria in order to evaluate the fitness of each region respecting to the role in H_2O_2 signal transduction, Table II.

Thus, the criteria defined to evaluate the performance of each region are set regarding the two functions of this system, defense and signaling:

Criteria 1: In terms of defensive function this system should focus on eliminating H_2O_2. Thus the H_2O_2 reduction flux should have the maximum logarithmic gain with respect to changes in H_2O_2 concentration.

Criteria 2 – 5: Each signaling output of this system (PrxSS, $PrxSO_2H$, PrxSOH and TrxSS) should respond to changes in H_2O_2 concentration. These values should be the maximum possible since they correspond to signal amplification. In this case these criteria can be evaluated by the maximum value of the logarithmic gain of each output with respect to changes H_2O_2 concentration.

Criteria 6 – 13: Overall this system should not be sensitive to changes in their structural parameters. Any arbitrary change shouldn't lead to a response of the system that could lead to the

incorrect activation of signaling pathways. The sum of the sensitivities of each different output in respect to changes in the system's parameters should then be the minimum possible. It is important that some key components of this system are also robust to changes in parameters. In this case PrxSH, TrxSH are two important components that maintain the system able to sense H_2O_2 and consequently exert protection and signal transduction. The reduction flux of Trx is also important, given the fact that it maintains TrxSH activity.

Ultimately the region with the best performance will give the design principles under which the system should operate in order to perform a role in signaling.

Table II Performance criteria for evaluation of the fitness of the Prx/Trx/TrxR system in a role in signaling with respect to the analyzed interactions and proposed working hypothesis. Function in H_2O_2 elimination is not disregarded, since it is also related to the regulation of cellular processes. Criteria 1 – 5 are with respect to logarithmic gains, which are preferred the highest values possible. Criteria 6 – 13 are with respect to the robustness of the system, which are preferred the values closest to 0, meaning they are not sensitive to parameters.

Criteria	Definition	Expression
1	High gain of hydrogen peroxide reduction flux with respect to changes in H_2O_2 concentration	$\text{Maximize } L(\text{Vopx}, H_2O_2)$
2	High gain of PrxSS concentration with respect to changes in H_2O_2 concentration	$\text{Maximize } L(\text{PrxSS}, H_2O_2)$
3	High gain of $PrxSO_2H$ concentration with respect to changes in H_2O_2 concentration	$\text{Maximize } L(PrxSO_2H, H_2O_2)$
4	High gain of PrxSOH concentration with respect to changes in H_2O_2 concentration	$\text{Maximize } L(\text{PrxSOH}, H_2O_2)$
5	High gain in TrxSS concentration with respect to changes in H_2O_2 concentration	$\text{Maximize } L(\text{TrxSS}, H_2O_2)$
6	High robustness of H_2O_2 consumption flux to changes in parameters	$\text{Minimize } \sum_i \lvert S(\text{Vopx}, p_i)\rvert$
7	High robustness of PrxSS to changes in parameters	$\text{Minimize } \sum_i \lvert S(\text{PrxSS}, p_i)\rvert$
8	High robustness of PrxSO2H to changes in parameters	$\text{Minimize } \sum_i \lvert S(\text{PrxSO2H}, p_i)\rvert$
9	High robustness of PrxSOH to changes in parameters	$\text{Minimize } \sum_i \lvert S(\text{PrxSOH}, p_i)\rvert$

10	High robustness of PrxSH to changes in parameters	$\text{Minimize} \sum_i \lvert S(\text{PrxSH}, p_i)\rvert$
11	High robustness of TrxSH to changes in parameters	$\text{Minimize} \sum_i \lvert S(\text{TrxSH}, p_i)\rvert$
12	High robustness of TrxSS to changes in parameters	$\text{Minimize} \sum_i \lvert S(\text{TrxSS}, p_i)\rvert$
13	High robustness of thioredoxin reduction flux to changes in parameters	$\text{Minimize} \sum_i \lvert S(V_{rtx}, p_i)\rvert$

4 Results

Our first step in order to analyze the Prx/Trx/TrxR system was to assemble a small database with interactions of Prx I, Prx II and Trx I. Table III represents some relevant interactions gathered in the literature, their conditions and consequences. The conditions show which form of Prx and Trx interact with their respective partners and the mechanism of those interactions. The physiological consequences will show what results from each given interaction. With this information it is possible to relate different forms of Prx and Trx with different outcomes.

Since Prx I is structurally similar to Prx II and has more described interactions, it is presented in order to give some insight of what may be happening with Prx II. The later has few known interactions and almost all are indirect ones, meaning that it participates in the regulation of cellular processes but only through protection from oxidation. However, recently Prx II had a shift in focus and promising studies on interaction partners for this protein are increasing.

Trx I has many interesting interactions described, adding to the fact that it also interacts with both Prx I and Prx II.

Some interesting interactions are the one between Prx I and ASK1 and also the one with c-Abl. Discovering and describing the interaction with ASK1, brought some insight on how apoptosis signaling can be transduced. This interaction has a disulfide transference induced by H_2O_2 which is proposed by the authors that this occurs between PrxSS and ASK-SH. This signal occurs only under conditions where Trx I is unavailable, either by depletion or inhibition. Unavailability of Trx I would prevent reduction of ASK1 and PrxSS, thus ensuring the disulfide transfer and consequent activation of ASK1.[49] PrxSOH can also be a candidate for this disulfide exchange, since the

condensation ($PrxSOH \rightarrow PrxSS$) is one of the slowest steps in the Prx/Trx/TrxR system. PrxSOH participates itself in disulfide exchanges, for instance its condensation reaction that leads to the formation of a disulfide (PrxSS). The fact that Trx I is a direct inhibitor of ASK1[38] fits this role of transduction of a H_2O_2-induced signaling by the Prx/Trx/TrxR system.

The ability of Prx I to exert pro-survival interactions, such has the one with AR, even increased in the sulfinic form, suits the role proposed for this oligomeric structures. Since the latter were shown to be related with cell cycle arrest in response to H_2O_2. This leads to another interesting interaction presented in Table III. Prx I was shown to interact with Abelson murine leukemia viral oncogene homolog 1 (c-Abl) inhibiting its kinase activity, promoting cell survival. Despite being a poorly understood interaction, it was observed that Prx I peroxidase activity is not required for this interaction.[60] Thus, leading to the possibility for a participation of the higher molecular weight in this interaction.

For the case of Prx II, as stated before, it has few known interactions and the ones described can be seen as indirect, since some depend on the peroxidase activity and H_2O_2 reduction from Prx II. As shown in Table III, in some cases, there is co-localization in specific cellular compartments known to be related with signaling. Additionally in some interactions Prx II is specifically recruited near other proteins in response to H_2O_2.

Similarities with Prx I start to appear in cases where Prx II interacts with transcriptions factors, such as JNK. It was shown a similar behavior as the one between Prx I and JNK, where in the cytosol Prx II attenuates activation of JNK, but can also increase cancer cells survival against DNA damage through JNK pathway. The latter only occurring with a nuclear localization of both proteins.[61] In this particular interaction, the sulfinic form of Prx I was shown to be as effective in hindering activation of JNK as the reduced form. Given the similarities between Prx I and II, the same is possible to be occurring with the latter.

One of the most interesting results is the interaction with ERp46. This particular interaction is the first described partner for sulfinic Prx II. However the mechanism is not well understood, the same occurring for the consequences of this interaction.

Taking a look at the interaction partners of Trx I, there are two interesting observations. The first one is that almost all the interaction partners are related to inhibition of apoptosis and only the reduced form of Trx I (Trx-$(SH)_2$) has known interactions described. Moreover some of these partners are also described for Prx I and Prx II. It is reasonable to believe in the possibility of a relation between the other partners and Prx I and II.

The second observation is the existence of an inhibitor for Trx I. Thioredoxin Interacting Protein (TXNIP) is indeed an interesting protein to study, since it can regulate all the H_2O_2 signal transduction system, by direct inhibition of the reductase activity of Trx I.[62]

Overall these results point us towards the possible outputs of this system. According to what was obtained the most immediate is $PrxSO_2H$. For part of some interactions PrxSOH and PrxSS are other two outputs of this system since they may interact with some proteins through disulfide exchange. In the case of Prx II and some of the signaling regulation it exerts in some cases in response to H_2O_2 it should be expected an increase in the rate of reduction of H_2O_2 which is the response expected to correspond to those interactions.

Table III Resume of some relevant interactions with the different forms of Prx I, Prx II and Trx I and their respective physiological consequences.

Protein	Interaction Partner	Conditions for interaction	Physiological consequence
Prx I	PTEN	Cys51 regulates H_2O_2 induced disruption of Prx-PTEN complex. A heterodimer is formed between the N and C terminus of Prx and the C2 domain of PTEN. Reduced Prx (PrxSH) is needed but the peroxidatic activity is not.[26]	Prx I protects the lipid phosphatase activity of PTEN by hindering the latter's oxidation by H_2O_2[26]
Prx I	AR	Interaction of Prx I and AR occurs with reduced (PrxSH) and overoxidized ($PrxSO_2H$) forms. Thus, the peroxidatic activity is not important.[22]	Interaction between both these proteins increases AR transactivation, which increases cell survival.[22]
Prx I	ASK1	Interaction occurs through disulfide exchange induced by H_2O_2. This exchange occurs between PrxSS and ASK1.[49]	This interaction leads to activation of ASK1 kinase activity. Leading to phosphorylation and activation of JNK kinase pathway.[49]

Prx I	Sty1	Interaction occurs through disulfide exchange induced by H_2O_2. This exchange occurs between PrxSOH and Sty1.[63]	Activation of Sty1 transcriptional activity occurs. Leading to expression of oxidative stress resistance.[63]
Prx I	Pap1	Interaction occurs through disulfide exchange induced by H_2O_2. This exchange occurs between PrxSS and Pap1. Lack of TrxSH leads to a lower level of activation of this protein.[50,64]	This interaction also leads to activation of Pap1 transcription factor activity. The latter leads to expression of oxidative stress resistance.[50,64]
Prx I	(c-Abl)	This interactions occurs specifically with the c-Abl SH3 domain. Again antioxidant activity is irrelevant for this interaction.[60]	c-Abl tyrosine kinase activity is inhibited as result of this interaction.[60]

Protein	**Interaction Partner**	**Conditions for interaction**	**Physiological consequence**
Prx II	VEGFR2	This interaction occurs only with the co-localization of both proteins in caveolae structures. Peroxidatic activity is important in order to protect VEGFR2 from inactivation.[31]	VEGFR2 is inactivated by H_2O_2. This can be either protected or reversed by action of Prx II, which will reduce either H_2O_2 or oxidized VEGFR2 back.[31]
Prx II	Cdk5	Neurons treated with a neurotoxin lead to phosphorylation of threonine 89 of Prx II by Cdk5.[32]	This interaction leads to inhibition of Prx II and consequent increase in oxidative stress in neuronal cells.[32]
Prx II	PDGFR	Upon PDGF stimulation, Prx II is recruited to PDGFR and relieves PTPases from inactivation. Thus peroxidase active Prx II is required.[33]	Prx II negatively regulates PDGF-induced cell proliferation.[33]
Prx II	ERp46	One of the few direct interactions of Prx II. The interacting form of Prx II is the sulfinic ($PrxSO_2H$) in a decameric conformation.[34]	This interaction has been recently described and is poorly understood in terms of consequences. Interestingly ERp46 is related to redox functions such as inhibition of NOX2.[34]

Prx II	JNK	This interaction is independent of the peroxidase activity of Prx II but very little is known about the mechanisms of this interaction besides the similarities with Prx I.[61]	This interaction shows two different outcomes. If both proteins are located in the cytoplasm there is an attenuation of JNK activity. However, a nuclear localization leads to increased cell survival through JNK pathway in cancer cells.[61]
Trx I	TXNIP	Interaction occurs between reduced Trx I active site Cys 32 and TXNIP Cys 63 and 247.There is a formation of a mixed disulfide.[62]	Interaction with TXNIP leads to inhibition of Trx I disulfide reductase activity.[62]
Trx I	ASK1	Disulfide formation between reduced Trx I Cys 32 or 35 and ASK1 N-terminal Cys 250.[39]	Association of Trx I with ASK1 leads to inhibition of the kinase activity of the latter. This interaction also induces ASK1 ubiquitination and degradation. Ultimately inhibiting apoptosis.[39]

Protein	**Interaction Partner**	**Conditions for interaction**	**Physiological consequence**
Trx I	MST1	Trx I active site Cys 32 and 35 are required for this interaction. Interaction occurs with the SARAH domain of MST1 (residues 431 – 487).[41]	Trx I inhibits MST1 kinase activity by blocking its homodimerization.[41]
Trx I	PTEN	Interaction occurs between Trx I Cys 32 and PTEN Cys 212 involving the formation of a disulfide between both.[48]	Trx I inhibits lipid phosphatase activity of PTEN and consequently leads to activation of the protein kinase B (Akt) which is an inhibitor of apoptosis.[48]
Trx I	Ref-1	Trx I forms a heterodimer with Ref-1. This interaction requires reduced Trx I and involves Cys 32 and 35 from Trx I active site.[65]	The formation of this complex leads to activation of AP-1 transcriptional activity.[65]

In order to obtain more information on the properties of the Prx/Trx/TrxR system, we performed the design space approach. After solving for steady state and validating the different solutions against the inequalities, there was a group of five functionally distinct regimes with valid

steady state solutions, Table IV. Each set of solutions for each regime corresponds to its respective phenotype, as defined in the design space approach.

Table IV Steady state solutions for the five distinct regimes. These solutions characterize the phenotype of each Regime.

Variable \ Regime	1	2	3	4	8
PrxSH	$PrxT$	$\frac{kdpx\ PrxT}{H_2O_2\ kopx}$	$\frac{kdpx\ krsx\ PrxT}{{H_2O_2}^2\ kop1\ kopx}$	$\frac{krpx\ PrxT\ TrxT}{H_2O_2\ kopx}$	$\frac{krtx\ TrxT}{H_2O_2\ kopx}$
PrxSOH	$\frac{H_2O_2\ kopx\ PrxT}{kdpx}$	$PrxT$	$\frac{krsx\ PrxT}{H_2O_2\ kop1}$	$\frac{krpx\ PrxT\ TrxT}{kdpx}$	$\frac{krtx\ TrxT}{kdpx}$
$PrxSO_2H$	$\frac{{H_2O_2}^2\ kop1\ kopx\ PrxT}{kdpx\ krsx}$	$\frac{H_2O_2\ kop1\ PrxT}{krsx}$	$PrxT$	$\frac{H_2O_2\ kop1\ krpx\ PrxT\ TrxT}{kdpx\ krsx}$	$\frac{H_2O_2\ kop1\ krtx\ TrxT}{kdpx\ krsx}$
PrxSS	$\frac{H_2O_2\ kopx\ PrxT}{krpx\ TrxT}$	$\frac{kdpx\ PrxT}{krpx\ TrxT}$	$\frac{kdpx\ krsx\ PrxT}{H_2O_2\ kop1\ krpx\ TrxT}$	$PrxT$	$PrxT$
TrxSH	$TrxT$	$TrxT$	$TrxT$	$TrxT$	$\frac{krtx\ TrxT}{krpx\ PrxT}$
TrxSS	$\frac{H_2O_2\ kopx\ PrxT}{krtx}$	$\frac{kdpx\ PrxT}{krtx}$	$\frac{kdpx\ krsx\ PrxT}{H_2O_2\ kop1\ krtx}$	$\frac{krpx\ PrxT\ TrxT}{krtx}$	$TrxT$

In order to obtain the characteristics of the different components of this system we analyzed all the different sensitivities and logarithmic gains of each variable with respect to changes in parameters and in H_2O_2 concentration, Table V. These properties are thus characteristic of each phenotype and can be compared against a set of performance criteria in order to analyze the local fitness of each Regime with respect to a role in signaling.

Table V Gains and sensitivities of the various variables of the system to changes in parameters and hydrogen peroxide. Vopx refers to the flux for reduction of H_2O_2, and Vrtx the flux for the reduction of Trx by TrxR.

Regime 1									
	Sensitivities								Log gains
	kopx	krpx	kop1	krsx	kdpx	krtx	PrxT	TrxT	H_2O_2
PrxSH	0	0	0	0	0	0	1	0	0
PrxSOH	1	0	0	0	-1	0	1	0	1
$PrxSO_2H$	1	0	1	-1	-1	0	1	0	2
PrxSS	1	-1	0	0	0	0	1	-1	1
TrxSH	0	0	0	0	0	0	0	1	0
TrxSS	1	0	0	0	0	-1	1	0	

Vopx	1	0	0	0	0	0	1	0	1
Vrtx	1	0	0	0	0	0	1	0	1
									1

Regime 2

	Sensitivities								Log gains
	kopx	krpx	kop1	krsx	kdpx	krtx	PrxT	TrxT	H_2O_2
PrxSH	-1	0	0	0	1	0	1	0	-1
PrxSOH	0	0	0	0	0	0	1	0	0
$PrxSO_2H$	0	0	1	-1	0	0	1	0	1
PrxSS	0	-1	0	0	1	0	1	-1	0
TrxSH	0	0	0	0	0	0	0	1	0
TrxSS	0	0	0	0	1	-1	1	0	0
Vopx	0	0	0	0	1	0	1	0	0
Vrtx	0	0	0	0	1	0	1	0	0

Regime 3

	Sensitivities								Log gains
	kopx	krpx	kop1	krsx	kdpx	krtx	PrxT	TrxT	H_2O_2
PrxSH	-1	0	-1	1	1	0	1	0	-2
PrxSOH	0	0	-1	1	0	0	1	0	-1
$PrxSO_2H$	0	0	0	0	0	0	1	0	0
PrxSS	0	-1	-1	1	1	0	1	-1	-1
TrxSH	0	0	0	0	0	0	0	1	0
TrxSS	0	0	-1	1	1	-1	1	0	-1
Vopx	0	0	-1	1	1	0	1	0	-1
Vrtx	0	0	-1	1	1	0	1	0	-1

Regime 4

	Sensitivities								Log gains
	kopx	krpx	kop1	krsx	kdpx	krtx	PrxT	TrxT	H_2O_2
PrxSH	-1	1	0	0	0	0	1	1	-1
PrxSOH	0	1	0	0	-1	0	1	1	0
$PrxSO_2H$	0	1	1	-1	-1	0	1	1	1
PrxSS	0	0	0	0	0	0	1	0	0
TrxSH	0	0	0	0	0	0	0	1	0
TrxSS	0	1	0	0	0	-1	1	1	0
Vopx	0	1	0	0	0	0	1	1	

Vrtx	0	1	0	0	0	0	1	1	0 0
Regime 8									
	Sensitivities								Log gains
	kopx	krpx	kop1	krsx	kdpx	krtx	PrxT	TrxT	H_2O_2
PrxSH	-1	0	0	0	0	1	0	1	-1
PrxSOH	0	0	0	0	-1	1	0	1	0
$PrxSO_2H$	0	0	1	-1	-1	1	0	1	1
PrxSS	0	0	0	0	0	0	1	0	0
TrxSH	0	-1	0	0	0	1	-1	1	0
TrxSS	0	0	0	0	0	0	0	1	0
Vopx	0	0	0	0	0	1	0	1	0
Vrtx	0	0	0	0	0	1	0	1	0

By quantitatively evaluating the criteria in Table II with the values from Table V, which are characteristic of each different Regime we are able to predict the Regime with the best performance. As stated we are addressing the role of this system in H_2O_2 signal transduction without disregarding its role in H_2O_2 elimination. Quantitative evaluation of the performances is resumed in Table VI.

Addressing first the function in H_2O_2 elimination we expect the highest logarithmic gain for the reduction flux of H_2O_2 with respect to changes in H_2O_2 concentration. Regime 1 shows the best performance having a logarithmic gain of 1. This means that in this regime with an increase in H_2O_2 we have also increase in the reduction flux. Regimes 2, 4 and 8 perform worse, having no gain in the reduction flux. The worst case of all is Regime 3, which has a logarithmic gain of -1. The latter means that with increase in H_2O_2 there is decrease of the reduction flux of H_2O_2 meaning that Regime 3 is not suited for this function.

Addressing the function in H_2O_2 signal transduction we evaluate the logarithmic gains of the four outputs: PrxSS, PrxSO2H, PrxSOH and TrxSH. The latter is analyzed through gains in TrxSS, which are expected to be in the same proportion as for the sulfenic and disulfide forms.

Again it is expected that the outputs are sensitive to the input, having a logarithmic gain equal to or higher than 1.

For the first output shown in Table VI, PrxSS, Regime 1 shows the best performance having a Log Gain equal to 1. In this case the changes occurring are in the same direction, which is: with an increase of 1% in H_2O_2 there is an increase of 1% in PrxSS. The remaining Regimes are not fit for a function in signaling in respect to this output. Regimes 2, 4 and 8 show no gain with changes in H_2O_2 and Regime 3 shows a Log gain equal to -1, meaning there will be a decrease in PrxSS with increase in H_2O_2.

There is a substantial gain of $PrxSO_2H$ with changes in H_2O_2 concentration in Regime 1. The sulfinic form of Prx has a logarithmic gain of 2, meaning there is amplification of the original signal. Regimes 2, 4 and 8 also show positive logarithmic gains, in this case equal to 1. Even though these are good gains, Regime 1 still has a better performance in this case. The one presenting the worst performance is Regime 3, which for the sulfinic form of Prx II there is no gain with changes in H_2O_2.

For the sulfenic form of Prx II, the best performing Regime is once again Regime 1, where it has a logarithmic gain of 1 with changes in H_2O_2 concentration. Similarly to PrxSS, Regimes 2, 4 and 8 are the follow ups in terms of performance showing no gain in PrxSOH with changes in H_2O_2. Finally Regime 3 shows the worst result for a gain with increase in H_2O_2 being then the worst performing Regime. This Regime has a logarithmic gain of -1, which means there is decrease of 1% in the concentration of PrxSOH with increase of 1% in the concentration of H_2O_2.

Finally for gains in TrxSS concentration with respect to changes in H_2O_2 concentration Regime 1 has the best results. In this regime TrxSS has an increase in 1% with 1% increase in H_2O_2 concentration. The remaining Regimes present the same results as in the previous case, having Regimes 2, 4 and 8 no responses to changes in H_2O_2 concentration and Regime 3 performing worse with a negative logarithmic gain.

Through the analysis of the logarithmic gains, Regime 1 presents the best performance in terms of functionality, both for H_2O_2 elimination and H_2O_2 signal transduction.

In terms of Robustness, a system is said to be Robust if it has no sensitivity to changes in its structural parameters. A system should always be robust, meaning that the sum of the sensitivities of a variable to changes in the structural parameters should be the closest to 0 as possible. For the present system in addition to the different outputs it is suited that some key components, namely PrxSH, TrxSH and Vrtx, should be robust to changes in parameters in order to guarantee the good functioning of this system.

Analyzing the robustness of the reduction flux of H_2O_2, Regimes 1, 2 and 8 all perform equally good, having each a sum of sensitivities of 1. Regimes 3 and 4 in their turn are very sensitive to changes in the structural parameters having the worst performances.

In the case of Criterion 7, the sensitivities of PrxSS to changes in parameters, Regimes 4 and 8 are the best performing. Following up are Regimes 1 and 2 that present a much lower robustness. Regime 3 is the one with the worst performance being very sensitive to parameters with a sensitivity sum of 6.

For the robustness of $PrxSO_2H$, in an opposite way to the previous criteria, has the best performance within Regime 3, being very robust to changes in parameters. Regime 2 shows some robustness, while the remaining Regimes show a total influence over $PrxSO_2H$ of 5 and even 6 meaning they are very sensitive to changes in parameters.

In respect to PrxSOH, the Regime with the best performance is Regime 2. Within the latter PrxSOH is very robust to changes in parameters. Designs within the remaining Regimes 1, 3 and 8 have the same value for the total influence of the parameters, being somewhat sensitive. Regime 4 has the worst performance for this criterion being the Regime which presents the highest sensitivity to changes in parameters.

Finally in terms of the Robustness of TrxSS to changes in parameters the Regime with the highest Robustness is Regime 8. Regimes 1 and 2 present the same sensitivity being less robust than Regime 8. Finally Regimes 3 and 4 are the most sensitive of all having a total influence of the parameters over TrxSS of 5 and 4 respectively.

As stated before there are some components beyond the system's outputs which should also be robust to changes in parameters in order to maintain the well-functioning of this system.

Starting by PrxSH, designs within Regime 1 are the most robust with a total influence of 1. Regimes 2 and 8 present some sensitivity to parameters being the second best performing Regimes. The worst robustness is found in Regimes 3 and 4 which have a total influence of 5 and 4, respectively, meaning that PrxSH will have larger changes in concentration with changes in parameters within these two Regimes.

Analysis of the robustness of TrxSH, shows that Regimes 1, 2, 3 and 4 are very robust, thus have the best performances. In the case of Regime 8, it shows some sensitivity to changes in parameters being for this criterion the worst performing Regime.

Finally for the reduction flux of Trx I, the most robust designs are within Regimes 1, 2, and 8. All three show a good robustness to changes in the structural parameters. On an opposite way, Regime 3 performs poorly in this criterion, being very sensitive to parameters, while Regime 4 is somewhat sensitive.

This analysis shows that even though not all the outputs of this system are robust in Regime 1, as it would be preferred, they all have the best performances within this Regime in terms of logarithmic gains. Additionally this Regime has the best performance for the robustness of other key components of this system. Finally the remaining Regimes don't show, by far, to be suited for a function in elimination of H_2O_2 neither a function in signal transduction, given their logarithmic gains results.

Table VI Performance evaluation results of each regime regarding a function in signal transduction and elimination of H_2O_2. In terms of Robustness directly related parameters to each variable are being taken in account given the fact the latter are expected to be sensitive to the former.

Criteria		Regime				
		1	***2***	***3***	***4***	***8***
H_2O_2 **scavenging**	**1)** $Maximize\ L(Vopx, H_2O_2)$	**1**	0	– 1	0	0
	2) $Maximize\ L(PrxSS, H_2O_2)$	**1**	0	– 1	0	0

H_2O_2 as signaling molecule	**3)** $Maximize\ L(PrxSO_2H, H_2O_2)$	**2**	1	0	1	1
	4) $Maximize\ L(PrxSOH, H_2O_2)$	**1**	0	– 1	0	0
	5) $Maximize\ L(TrxSS, H_2O_2)$	**1**	0	– 1	0	0
Robustness	**6)** $Minimize\ \sum_i \lvert S(Vopx, p_i)\rvert$	**2**	**2**	4	3	**2**
	7) $Minimize\ \sum_i \lvert S(PrxSS, p_i)\rvert$	4	4	6	**1**	**1**
	8) $Minimize\ \sum_i \lvert S(PrxSO_2H, p_i)\rvert$	5	3	**1**	6	5
	9) $Minimize\ \sum_i \lvert S(PrxSOH, p_i)\rvert$	3	**1**	3	4	3
	10) $Minimize\ \sum_i \lvert S(TrxSS, p_i)\rvert$	3	3	5	4	**1**
	11) $Minimize\ \sum_i \lvert S(PrxSH, p_i)\rvert$	**1**	3	5	4	3
	12) $Minimize\ \sum_i \lvert S(TrxSH, p_i)\rvert$	**1**	**1**	**1**	**1**	3
	13) $Minimize\ \sum_i \lvert S(V_{rtx}, p_i)\rvert$	**2**	**2**	4	3	**2**

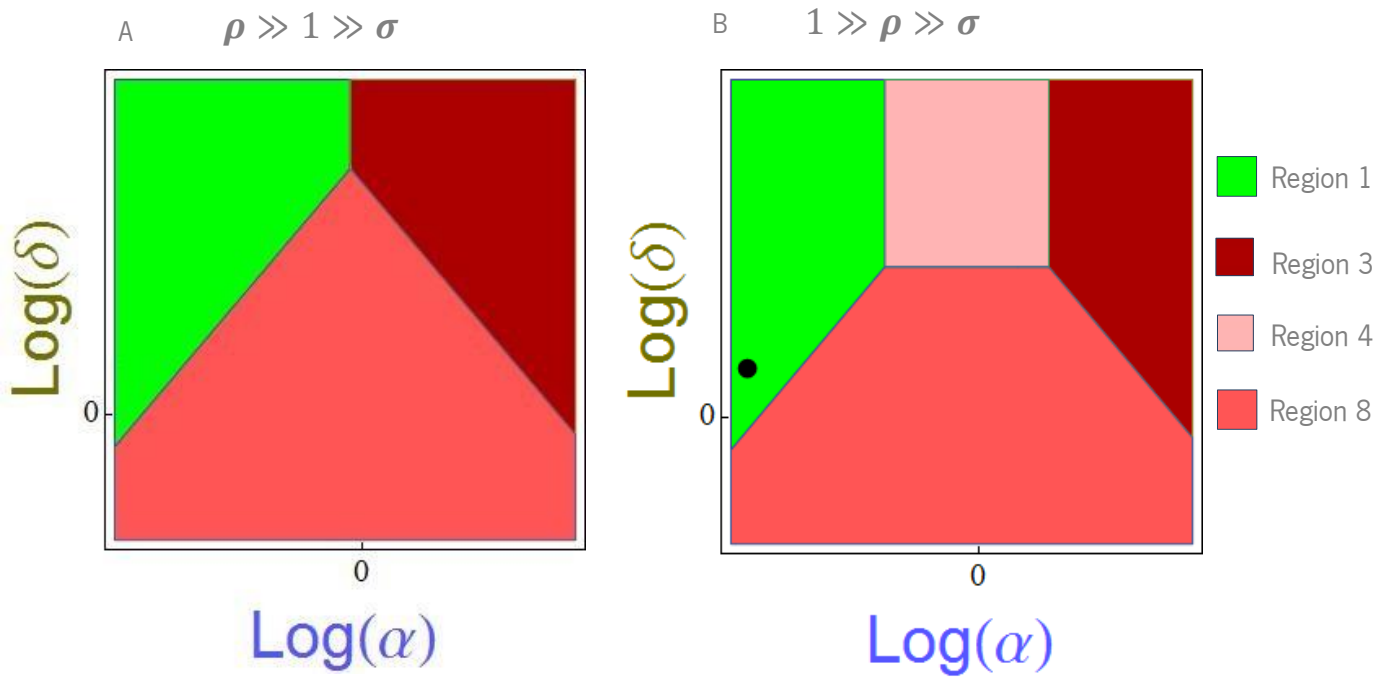

Figure 7 Design space of the Prx/Trx/TrxR system with the five distinct functional regimes 1,2,3,4, and 8 as labeled in each panel. The representations are with respect to the system presented in Figure 6. Panel A represents the conditions where the maximum rate of reduction of PrxSS is greater than the maximum rate of condensation of PrxSOH: $\boldsymbol{\rho \gg 1}$ and the latter is greater than the maximum rate for $PrxSO_2H$ reduction by sulfiredoxin: $\boldsymbol{1 \gg \sigma}$. Panel B represents the conditions where the maximal rate for condensation of PrxSOH is greater than the maximal rate of PrxSS reduction. The latter is also greater than the maximal rate of $PrxSO_2H$ reduction. The x – axis, alpha, represents the normalized apparent rate constant of the reduction of H_2O_2. The y – axis, delta, represents the normalized rate constant for the reduction of Trx I by TrxR. In panel B it is shown the normal operating point for erythrocytes (black dot).

We mapped the various regions into the parameters space, focusing on the interplay between the parameters α and δ, the normalized apparent rate constant for H_2O_2 reduction by reduced Prx II and the normalized rate constant for reduction of Trx I by TrxR, respectively, in order to obtain the design principles that best suit the addressed functions, Figure 7.

Thus, from the analysis of the criteria evaluation results and the different regions in the parameters space, we predicted that systems within Regime 1 have the best performance in terms of H_2O_2 elimination and H_2O_2 signal transduction:

$$\alpha < \mathrm{Min}[1, \rho, \frac{\delta}{\gamma}, \sqrt{\frac{\sigma}{\eta}}, \frac{1}{\eta}] \quad (16)$$

$$\frac{H_2O_2\ \mathrm{kopx}}{\mathrm{kdpx}} < \mathrm{Min}[1, \frac{\mathrm{krpx\ TrxT}}{\mathrm{kdpx}}, \frac{\mathrm{krtx\ TrxT}}{\mathrm{kdpx\ PrxT}}, \sqrt{\frac{\mathrm{kopx\ krsx}}{\mathrm{kdpx\ kop1}}}, \frac{\mathrm{kopx}}{\mathrm{kop1}}] \quad (17)$$

Therefore, we have found the following Design Principle (equation 17):

- The normalized apparent rate constant for H_2O_2 reduction by reduced Prx II (α) must be less than the minimum of:
    - 1;
    - The normalized rate constant for the reduction of PrxSS(ρ);
    - The ratio $(\frac{\delta}{\gamma})$ between the normalized rate constant for the reduction of TrxSS (δ) and the ratio between total Prx II and total Trx I (γ);
    - The square root of the ratio $(\frac{\sigma}{\eta})$ between the normalized rate constant for the reduction of $PrxSO_2H$ (σ) and the ratio between the rate constant for the sulfinilation of Prx II and the rate constant for the H_2O_2 reduction (η);
    - The ratio between the rate constant for the H_2O_2 reduction and the rate constant for the sulfinilation of Prx II $(\frac{1}{\eta})$.

5 Discussion

In this work we addressed the Prx/Trx/TrxR system and whether its properties fit a role in the transduction of H_2O_2 signals.

H_2O_2 was only described as being toxic and deleterious to the cells, but since the last two decades it has been widely accepted as a second messenger because of its characteristics at lower quantities. However the way it regulates downstream events is still not understood.

Additionally, over the years, many interactions between the antioxidant Prx/Trx/TrxR system and various proteins related to the regulation of several cellular processes have been described. Consequently, given the high reactivity of this system for H_2O_2, its role has been under question.

By inventorying this system's interactions and analyzing its properties through the design space approach we aimed to clarify if and how this system helps regulate cellular processes through transduction of H_2O_2 signaling.

The analysis on the interactions showed that the Prx/Trx/TrxR system participates in defense and in the transduction of H_2O_2 signals in a way analogous to a fire alarm system. This analysis showed that the possible outputs are the sulfinic, sulfenic and disulfide forms of Prx II. TrxSH regulates the transduction by PrxSS and PrxSOH by reducing their target proteins disulfides or reducing disulfide Prx directly. Additionally the peroxidase activity is important for the regulation of several other relevant processes.

The design space approach showed that this system can function in five qualitatively distinct steady state regimes. These five regimes are thus characterized by their steady state solutions (Table IV) and their logarithmic gains and sensitivities (Table V).

In order to find the Regime with the best fitness for a role in signaling, a set of performance indices was defined. From results from Table VI, it is visible the best suitability of systems within Regime 1 to play both functions of scavenging H_2O_2 and transduction of H_2O_2 signaling. This Regime is the only of all five Regimes that shows logarithmic gains in concentrations of the various outputs with respect to changes in H_2O_2 concentrations equal to or higher than 1. The remaining Regimes show no response or even negative logarithmic gains. Analyzing Table V, focusing on the

results obtained for Regime 1, we assess the differences of the various outputs of this system within this Regime. The sulfenic and disulfide forms of Prx II have both a logarithmic gain of 1 with respect to changes in H_2O_2 concentration. This means that 1% increase in H_2O_2 leads to 1% increase in each of these outputs, thus they linearly increase with H_2O_2. In terms of robustness they present similar values, being PrxSOH slightly more robust than PrxSS. PrxT, total concentration of peroxiredoxin, TrxT, total concentration of thioredoxin and krtx, which represents the enzyme Thioredoxin Reductase are the parameters with most relevance overall to the system. Addressing sensitivities to these parameters it is visible that PrxSS and PrxSOH have a positive sensitivity to changes in PrxT. This means that this protein is prone to oxidation with an increase in PrxT. Additionally PrxSS is also sensitive to TrxT. In this case, increases in TrxT lead to a decrease in PrxSS, relating this output to regulation by Trx I. The latter result is also supported by the properties of TrxSS within this Regime. TrxSS has a logarithmic gain of 1 and is even sensitive to changes in PrxT. This can be related to the reduction and regulation function of thioredoxin, which has a linear increase with both H_2O_2 and PrxT. These three outputs overall have the properties that fit a transduction relay where linear increases in both PrxSS and PrxSOH are regulated by TrxSH, translated here in the logarithmic gain in TrxSS. The latter, in its turn, has a negative sensitivity to changes in krtx, thus the role of TrxR is also important for the functioning of this system since it is responsible to reduce TrxSS back to TrxSH. Furthermore, systems within Regime 1 are characterized by being fully reduced. In Table IV we see that PrxSH and TrxSH account for the majority of the respective proteins quantities. This leads to the lack of responses of both forms to very small changes in H_2O_2, which is seen in Table V. Furthermore they are only sensitive to the parameters that represent the respective total quantities of each protein. The sulfinic form of Prx II has the highest logarithmic gain of all the outputs regarding changes in H_2O_2 concentration. This result suggests a major relevance of this form of Prx II in this system, since it is the only one with amplification of the original signal from H_2O_2, given by a logarithmic gain greater than 1. On the other hand, $PrxSO_2H$ is the most sensitive output to changes in the structural parameters in systems within this regime. However from the three parameters referred, this form is only sensitive to the total concentration of Prx II. If there is any regulation on the amplification of the signals by

$PrxSO_2H$, according to the results it would rest on krsx, which is known to slowly reduce $PrxSO_2H$ back to PrxSOH. The lack of a more effective regulator supports the relevance of this form of Prx II, which is further supported by its logarithmic gain.

With the prediction that systems within Regime 1 are the ones that best perform a function in H_2O_2 scavenging and transduction of H_2O_2 signals, we found the relationships (Design Principles) that define this region in the Design Space (equation 17):

$$\frac{H_2O_2\ \mathrm{kopx}}{\mathrm{kdpx}} < \mathrm{Min}[1, \frac{\mathrm{krpx\ TrxT}}{\mathrm{kdpx}}, \frac{\mathrm{krtx\ TrxT}}{\mathrm{kdpx\ PrxT}}, \sqrt{\frac{\mathrm{kopx\ krsx}}{\mathrm{kdpx\ kop1}}}, \frac{\mathrm{kopx}}{\mathrm{kop1}}]$$

In biological terms, this means that the reduction of H_2O_2 by Prx II must be one of the slowest processes of the system, in exception for the condensation of PrxSOH to PrxSS, which is the only process not represented in the Design Principle as a constraint. In order for Regime 1 to exist the condition: $H_2O_2 < \frac{\mathrm{kdpx}}{kop1}$ should be satisfied, implying that the rate constant for the sulfinilation of Prx II must be lower than the rate constant for the condensation of Prx II. When kop1 becomes greater than kdpx the system will operate in another region of the Design Space.

From the analysis of some interactions it is observed the importance of $PrxSO_2H$, PrxSS and PrxSOH in signaling. For instance, for the sulfinic Prx II it was observed that in response to H_2O_2 occurs formation of filaments of oligomeric structures of this form which leads to cell cycle arrest.[53] In the case of sulfenic and disulfide Prx, it was observed for Prx I that this form plays an important role in the transference of disulfides in H_2O_2 – induced activation of ASK1[49], Pap1[50,64] and Sty1[63]. Given the structural similarity between Prx I and II it was predicted that Prx II would share this mechanism in some of its interactions. Indeed, very recently it was described the interaction between Prx II and STAT3 where the mechanism is proposed to be the same as in Sty1 for instance.[66] The participation of Trx I is also observed in both cases, where it reduces the targets, opposing the effects of the interaction with Prx I and II respectively.

These interactions suggest that the characteristics of the Prx/Trx/TrxR system occur in vivo.

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