

# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

185,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index  
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?  
Contact [book.department@intechopen.com](mailto:book.department@intechopen.com)

Numbers displayed above are based on latest data collected.  
For more information visit [www.intechopen.com](http://www.intechopen.com)



# Inhibitors and Activators of SOD, GSH-Px, and CAT

Xianyong Ma, Dun Deng and Weidong Chen

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/65936>

## Abstract

Reactive oxygen species (ROS) is harmful to our health, and SOD, CAT, and GPX are the major antioxidant enzymes that defend us from effects of ROS. In medicine, food, and dairy industries, antioxidant enzymes often surround complex environments. For better utilization of these enzymes, the inhibitors (including competitive inhibitors and noncompetitive inhibitors) and activators of SOD, CAT, and GPX are described in detail in this chapter. Also, the structure and catalytic mechanism of these antioxidants are summarized.

**Keywords:** reactive oxygen species, superoxide dismutase, catalase, glutathione peroxidase

## 1. Introduction

Reactive oxygen species (ROS) such as superoxide ( $O_2^{\cdot -}$ ), hydrogen peroxide ( $H_2O_2$ ), and hydroxyl radical (OH) are highly reactive oxidant species, which are by-products of intracellular metabolic processes, causing macromolecular damage. Reactive oxygen species are harmful to health and cause damage to crucial substances such as DNA, RNA, proteins, and lipids. Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) are three major antioxidant enzymes in animals. Superoxide dismutase (SOD), the first defense line of against oxygen-derived free radicals, catalyzes the dismutation of the superoxide anion ( $O_2^{\cdot -}$ ) into hydrogen peroxide ( $H_2O_2$ ), which is then transformed into  $H_2O$  and  $O_2$  by catalase. Glutathione peroxidase (GSH-PX or GPX) is a selenoprotein, which also protects the organism from oxidative damage; the main biochemical function of GPX is to reduce lipidic or nonlipidic hydroperoxides as well as  $H_2O_2$  through oxidization of glutathione [1]. Although both enzymes can reduce  $H_2O_2$ , there are some differences between CAT and GPX. CAT, one of the enzyme with highest

turnover rates, is the main enzyme involved in reduction of  $\text{H}_2\text{O}_2$  via the Fenton reaction. This enzyme is almost exclusively expressed in peroxisomes [2]. GPX is specific for its hydrogen donor, but nonspecific for  $\text{H}_2\text{O}_2$ , and it degrades  $\text{H}_2\text{O}_2$  using reduced glutathione in a powerful manner [3].

From the perspective of protection, improving antioxidant activity would be helpful for organisms to survive under various stresses, but from another respect, the antioxidant activity should be inhibited. Some anticancer agents, such as xenobiotics and radiation, act by producing ROS to kill tumor cells. Cells with high levels of antioxidant enzymes are resistant to these anticancer agents. Therefore, the levels of cellular antioxidant enzymes will influence the sensitivity of tumor cells to anticancer therapies [4]. Thus, more detailed information about an activator and inhibitor of SOD, CAT, and GPX should be known, for better utilization of these enzymes.

## 2. SODs

### 2.1. The structure and function of SOD

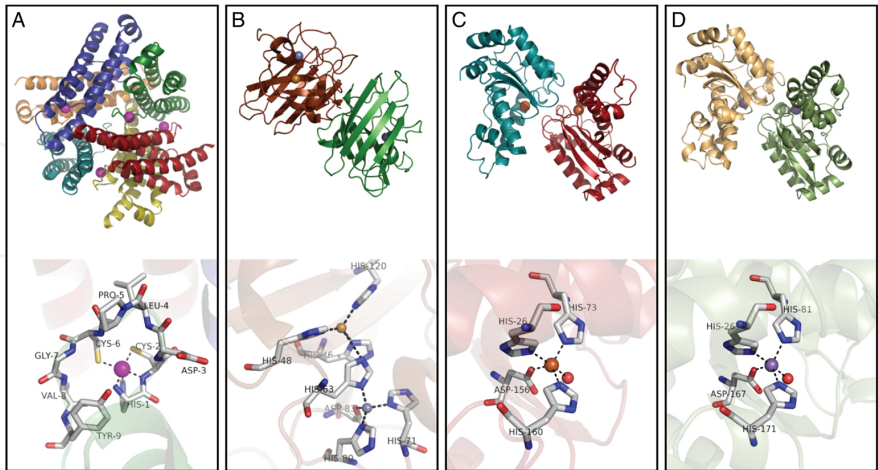
Superoxide dismutases (SODs, EC 1.15.1.1) are ubiquitous and one important class of antioxidant metalloenzymes against the harmful effects of superoxide free radicals. The main function of SODs was to decompose superoxide radicals into molecular oxygen and hydrogen peroxide inside cells, which reaction is as follows:



Based on the functional metal cofactors located at the active sites, four distinct classes of SODs have been found (**Figure 1**). SODs of Class I specifically require manganese or iron ion for catalytic activity (MnSOD and FeSOD) and enzymes that function with either of the two metal ions so-called cambialistic SODs. Class II is copper- and zinc-dependent enzymes (CuZn-SODs). Members of the two classes are found in both prokaryotes and eukaryotes. Nickel-containing SODs are mainly identified from marine actinomycetes and cyanobacteria [5, 6]. Enzymatic reactions of SODs depending on different metals indicate that SODs are developed by cells to offset the harmful effects of reactive oxygen species and match its surroundings.

Due to their antioxidative effects, SODs exhibited dramatic potential in medicine, cosmetic, food, agriculture, and chemistry industry. For example, considerable clinical experiments have shown that SODs could prevent oncogenesis and reduce the cytotoxic effects of anticancer drugs. Recently, SODs have found to prevent many diverse diseases such as cardiovascular diseases, diabetes, asthma, infertility, neurological disorders, and transplant rejection. SODs have also been successfully utilized as a major component in cosmetics for skin protection. In animal husbandry, SODs are considered to be one kind of strong antioxidative enzymes, which can reduce the oxidative stress of animal and prevent the oxidation of animal products, improve the quality of animal products such as meat, egg, or milk. In order to make better use

of SODs, their characteristics, the most suitable environment and influencing factors, etc., should be known especially the information of inhibitor and activator.



**Figure 1.** A comparison of the enzyme structures and active sites for the four SODs, (a) streptomyces coelicolor NiSOD (PDB, 1t6u), (b) human Cu/ZnSOD (PDB, 1pu0), (c) *E. coli* FeSOD (PDB, 1isa), and (d) MnSOD (PDB, 1vew). (adapted from reference [7]).

2.2. Inhibitors of SODs

2.2.1. Different inhibitors on sensitivity of SODs

Cu/Zn-SODs, Fe-SODs, and Mn-SODs are sensitive to different inhibitors, and we could distinguish these metal-specific SODs based on different inhibitory reactions (**Table 1**). The activity of Cu/Zn-SODs was inhibited by KCN, but Fe-SODs and Mn-SODs were not sensitive to KCN. Whereas, completely different phenomenon was observed when incubated in chloroform-ethanol (1:3, V/V) component solvent, as a result Fe-SODs and Mn-SODs activity were almost lost, while Cu/Zn-SOD was not sensitive to chloroform-ethanol. Fe-SODs and Mn-SODs are highly homologous and exhibit structural similarity, it is assumed that they originate from the same ancestry [8]. Fe-SODs and Mn-SODs can be distinguished by their different sensitivities to H<sub>2</sub>O<sub>2</sub>, because Mn-SOD activity was not sensitive to H<sub>2</sub>O<sub>2</sub>. In addition, NaN<sub>3</sub> also is used to detect the type of MnSOD, when the SOD was inhibited by neither KCN nor H<sub>2</sub>O<sub>2</sub> [9].

| SODs      | Inhibitors |                    | H <sub>2</sub> O <sub>2</sub> | NaN <sub>3</sub> |
|-----------|------------|--------------------|-------------------------------|------------------|
|           | KCN        | Chloroform-ethanol |                               |                  |
| Cu/Zn-SOD | +          | -                  | +                             | +                |
| Fe-SOD    | -          | +                  | +                             | +                |
| Mn-SOD    | -          | +                  | -                             | +                |

“+” indicate reaction and “-” indicate no reaction.

**Table 1.** Effect of inhibitors on different cofactors of SODs activity.

### 2.2.2. Effects of metal ions on SOD activity

$\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Fe}^{2+}$ , and  $\text{Mn}^{2+}$  are cofactors of superoxide dismutase, and they are vital to enzymatic activity. But heterogeneous expressed SODs were often existed in terms of apoenzyme or combined other metals present in a culture medium [10], thus might result in partial or total loss of activity. So that reconstituted metal ion is necessary to recovery activity. Apoenzyme was prepared by a metal removal procedure according reference [11] and simplified as follow:

1. Purified SOD was dialyzed against denature buffer: 20 mM 8-hydroxyquinoline, 2.5 mM guanidinium chloride, 5 mM Tris, 0.1 mM EDTA, pH 3.8, at 4°C for 18 h.
2. Purified SOD was dialyzed against 5 mM Tris buffer (pH 7.8), containing 1 mM metal ions for 18 h.
3. Excess metal ions were removed by dialysis against 5 mM Tris buffer (pH 7.8).

To be attention, only specific metal ions combined to SOD, the activity could be recovered. But for Fe-SOD and Mn-SOD, because they share highly homologous, Fe-substituted Mn-SODs also are active, but the activity is lower than Mn-reconstituted SOD.

SODs are metalloenzyme, and they will be inhibited by chelators [12, 13], such as EDTA and cuprizone (a copper-specific chelator). They should be avoided losing metal cofactors caused by chelators in the experiment. But a Mn-SOD from *Mycobacterium* sp. JC1 DSM 3803 was not sensitive to 10 mM EDTA [8], which may be due to the highly tightness of metal cofactors binding to enzymes.

$\text{Co}^{2+}$ ,  $\text{Hg}^{2+}$ ,  $\text{K}^{+}$  and,  $\text{Al}^{3+}$  and other metal ions also show their inhibition effects on SOD activity. A Mn-SOD from deep-sea thermophile *Geobacillus* sp. EPT3 was activated only by  $\text{Mn}^{2+}$  among nine tested metal ions [12]. A Cu-/Zn-SOD from black soybean was also activated only by  $\text{Cu}^{2+}$  [13] and a similar result was shown in the report of Liu et al. [14]. But there are a few exceptions, for example, a manganese-containing superoxide dismutase was activated by  $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$ , and  $\text{Al}^{3+}$ .

### 2.2.3. Singlet molecular oxygen inactivation of superoxide dismutase

High reactive singlet molecular oxygen ( $^1\text{O}_2$ ) is one kind of short-lived intermediate from oxidation reaction which oxidizes a variety of biological molecules easily, including lipids, nucleic acids, and proteins, and it also promotes deleterious processes such as lipid peroxidation, membrane damage, and cell death [15]. The biochemical production of singlet oxygen has been proposed to contribute to the destructive effects on a number of biological processes [16]. *In vivo*, singlet molecular oxygen is produced under normal and pathophysiological conditions. It is known to be particularly reaction with histidine, which often located at the active sites of SODs, so singlet molecular oxygen may result in prevention of the activities of those enzymes including CATs and GPXs. *In vitro*, singlet molecular oxygen could be produced by photoactivated dyes, such as methylene blue or rose bengal [17], so inactivation of SODs, CATs, and GPXs should be avoid in application.

## 2.3. Different activator on activity of SODs

### 2.3.1. *Effect of carbohydrates on activities of superoxide dismutase*

Carbohydrates, such as maltose, sucrose, lactose, trehalose, glucose, D-fructose, D-trehalose, D-xylose, and so on could stabilize an enzyme structure. For example, trehalose plays a strong promotive effect on superoxide dismutase [18]. Trehalose is a kind of polyol compound with many hydroxyl groups, which has strong hydration ability and can change the free energy to the favorable direction in solution. The multihydroxyl structure of trehalose can connected with both the surface of the enzyme protein and the external water through hydrogen bonding, so that the structure of the enzyme is stable, and the enzyme activity was protected [19].

### 2.3.2. *Polyethylene glycol modified SOD to improve its efficiency*

Polyethylene glycols (PEGs) are considered to be a safety and nonimmunogenic materials. They also have multihydroxyl compounds and can be activated by many activators, such as cyanuric, dicyclohexylcarbodiimide, N-hydroxysuccinimide, and 1,1'-carbonyldiimidazole, then activated polyethylene glycol was conjugated with the  $\epsilon$ -NH<sub>2</sub> group of SOD. PEG conjugated with SOD not only enhance the stability of the enzyme, but also avoid enzymatic immunogenicity. For example, Beckman et al. [20] reported that a superoxide dismutase conjugated with polyethylene glycol greatly increased endothelial the cell oxidant resistance and half-life of the enzyme.

### 2.3.3. *Cyclodextrin modified SOD to enhance its stability*

Cyclodextrins (CD), cyclic oligosaccharides containing six ( $\alpha$ -CD), seven ( $\beta$ -CD) or eight ( $\gamma$ -CD)  $\alpha$ -1-4-linked D-glucopyranose units have been used to stabilize enzymes in order to increase their activities and favor immobilization. On the one hand, superoxide dismutase modified by  $\beta$ -cyclodextrin could improve its performance. A superoxide dismutase was glycosylated by cyclodextrin-branched carboxymethylcellulose and its plasma half-life time was prolonged from 4.8 min to 7.2 h, its anti-inflammatory activity also increased by 2.2 times [21]. On the other hand, cyclodextrin and its derivative could synthesize SOD mimics. Puglisi et al. [22] reported a 6A,6B-Dideoxy-6A,6B-di[(N-salicylidene)amino]- $\beta$ -cyclodextrin conjugated with a manganese(III) complex showed a SOD-like activity and a good solubility that favor its application.

## 3. CAT

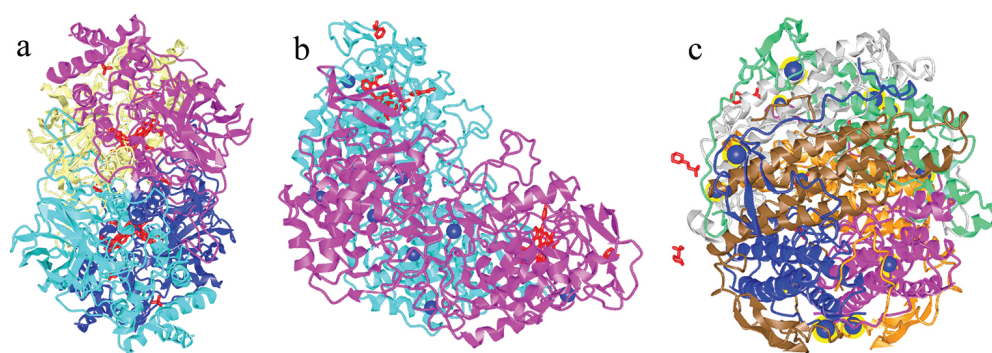
### 3.1. The structure and function of CAT

CAT(EC 1.11.1.6) catalyzes the decomposition of hydrogen peroxide to water and oxygen, widely exists in animals, plants, and microorganisms. Its uniqueness lies in the enzymatic prosthetic group(ferriprotoporphyrin IX) that could catalyze the same reaction as the holoenzyme.





According to the significant catalytic activity, CATs can be divided into three distinct subgroups: typical catalases, a typical catalases and catalase-peroxidases [23]. Two subgroups, typical catalases and catalase-peroxidases, contain heme, but the third group has no heme, namely manganese catalases. Most of catalases belong to typical catalases, except catalases in the domain of Archaea. Although there are differences in the primary structure among these typical catalases, but the three-dimensional structure appears well conserved. Most of these hydroperoxidases are homotetramers with four prosthetic heme groups (**Figure 2(a)**, PDB, 1E93) [24].



**Figure 2.** The three-dimensional structure of three types of catalases. (a) a typical catalase (depleted in iron) from *Proteus mirabilis*, (b) a catalase-peroxidase from *Synechococcus elongatus* PCC7942, (c) a manganese catalase from *Lactobacillus plantarum*. Color scheme: blue ball—Fe or Mn, red—heme group, others colors indicate different subunit.

Catalase-peroxidases may originate from ancestral, a relatively large monomeric unit comprising more than 700 amino acids, indicating that they probably from duplication of an ancestral gene [25]. And catalase-peroxidases show much higher sequence homology with heme peroxidases than with typical catalases. Recently, a three-dimensional structure of catalase-peroxidases has been obtained and is shown in **Figure 2(b)** (PDB, 3WXO) [26].

There is little structural information about manganese catalases up to now. Subunits with molecular weights around 30 kDa are recognized as tetramers or hexamers, and are remarkably stable at high temperatures [23, 27]. A crystal structure of a manganese catalase from *Lactobacillus plantarum* at 1.33 Å resolution is established (**Figure 2(c)**, PDB, 1O9I) [28].

### 3.2. Inhibitor of catalase

#### 3.2.1. Noncompetitive inhibitor

##### 3.2.1.1. Sodium azide, amine, and cyanide

Sodium azide ( $\text{NaN}_3$ ), amine, and cyanide are nonspecific inhibitors of CAT. Catalase-peroxidases are very sensitive to  $\text{NaN}_3$ , a lower concentration of  $\text{NaN}_3$  could lead to the enzyme

lose its activity by 50%. The inhibitory efficiency order was sodium azide>amine>cyanide>3-amino-1,2,4-triazole(**Table 2**) [29]. A similar result was found in the other typical monofunctional catalases [30, 31].

| Inhibitors             | Inhibitor concentration (mM) required for 50% inhibition |            |
|------------------------|--|------------|
|                        | Catalase   | Peroxidase |
| 3-Amino-1,2,4-triazole | 6  | 30         |
| Hydroxylamine          | 0.02   | 1.5        |
| Sodium azide           | 0.025  | 0.15       |
| Potassium cyanide      | 1  | 1          |

**Table 2.** Effect of inhibitors on the catalase and peroxidase activity of the catalase-peroxidase of *Archaeoglobus fulgidus* [29].

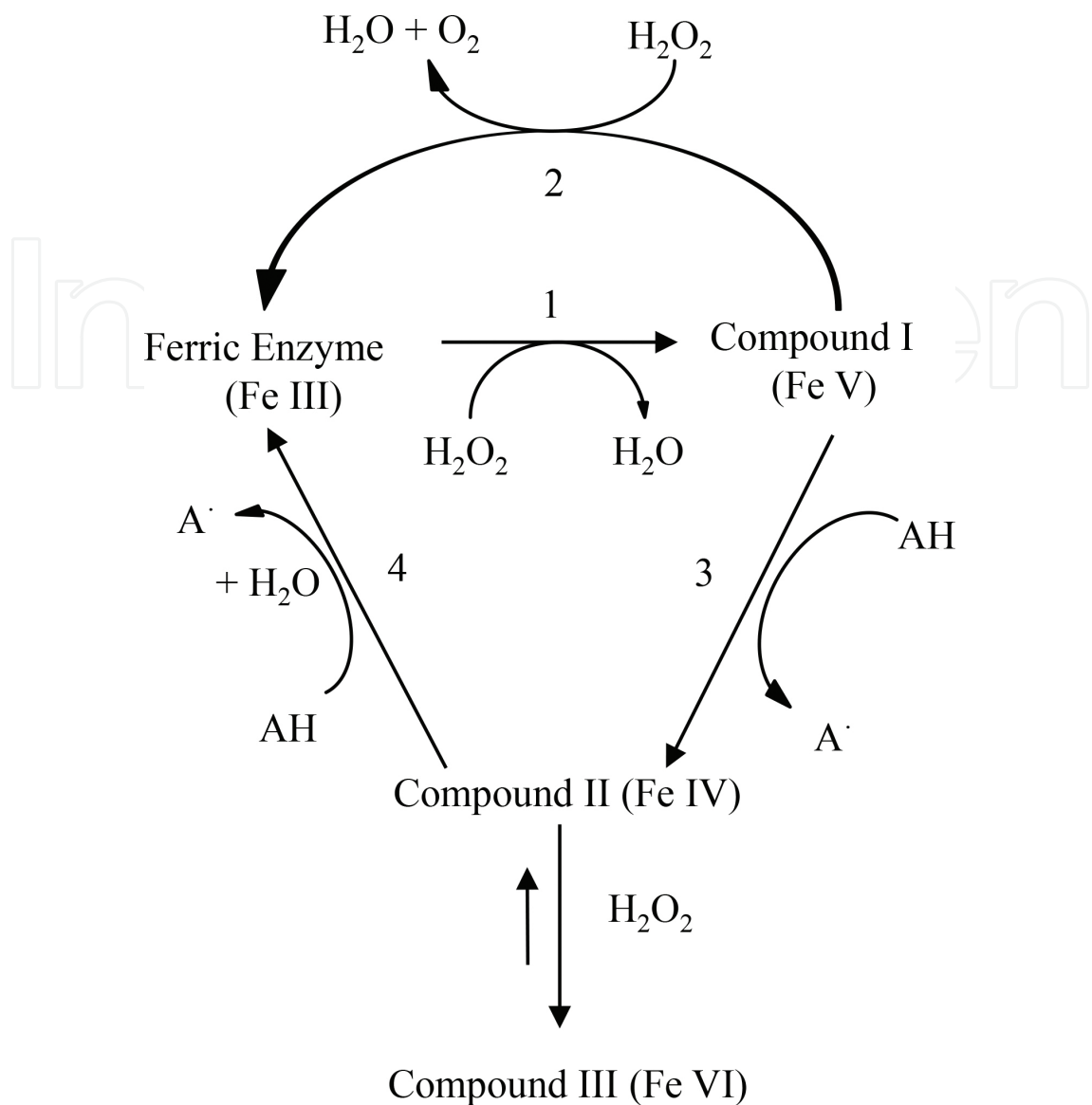
#### 3.2.1.2. 3-Amino-1,2,4-triazole

3-Amino-1,2,4-triazole (aminotriazole, ATZ) as a noncompetitive catalase-specific inhibitor is used to study on physiological changes in organisms [32]. Aminotriazole could combine catalase-H<sub>2</sub>O<sub>2</sub> compound I, thus results in loss of enzymatic activity. In alcohol-induced liver injury, catalase plays a dual role. On the one hand, catalase could scavenge hydrogen peroxide originated from alcohol to water, but on the other hand, catalase decomposes alcohol that might be harmful to liver, some research studies [33] show that catalase is inhibited by ATZ, which attenuated alcohol-induced acute liver injury.

#### 3.2.1.3. Salicylic acid

Salicylic acid acts as an electron donor for the peroxidative cycle of catalase, it is a noncompetitive inhibitor of catalases. It is interesting to note that different CAT salicylic acid exhibits different inhibitory property. CAT1 and CAT2 are two isoenzymes from maize (*Zea mays* L.). The Lineweaver-Burk plot of SA inhibition of CAT1 and CAT2 shows that CAT1 is noncompetitive manner, while CAT2 is inhibited in a competitive manner [34]. SA has a dual function on catalase, which means SA can both inhibit and activate its activity. Durner and Klessig [35] examined the effects of SA on the formation of the various redox states or reaction intermediates of catalase (**Figure 3**). The absorption spectrum of compounds I, II and III was different, thus various redox states or reaction intermediates of catalase can be distinguished spectroscopically by their absorption spectra in the Soret (near UV) region. Through the difference at the absorption spectra of intermediates, SA was confirmed acting as a one-electron donor that siphons compound I from the extremely fast catalytic cycle into the relatively slow peroxidative cycle (~1000 times slower) by promoting the formation of compound II [36, 37].





**Figure 3.** The reaction cycles of catalase [35].

3.2.2. Competitive inhibitor

3.2.2.1. Metal ions

Catalase mainly used in industrial sectors such as textiles, pulp, and paper, their work environment often with high concentration of metal ions. Previous studies have elaborated that catalase can be inhibited by certain metal ions (including  $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$ , and  $\text{Ag}^+$ ), a process depends on the metal, concentration, the tissue, and species [38]. Lee et al. [39] compared several divalent metal ions on catalase-peroxidase (KatG) activity, only the manganese ion revealed some inhibitory effects on the recombinant KatG activity, and EDTA could relieve partly inhibited activity. This implies that manganese may competitively bind to near the heme group and be involved in the enzyme reaction.

#### 3.2.2.2. *p*-Hydroxybenzoic acid is a competitive inhibitor of catalases from maize

Phenolic compounds, such as salicylic acid, aspirin, benzoic acid, o-coumaric acid, and *p*-hydroxybenzoic acid play a role in the induction of abiotic stress resistance. But only *p*-hydroxybenzoic acid showed the inhibitory effect on two catalases from maize in a competitive manner, the other compounds were in noncompetitive manner. Weak inhibition by *p*-hydroxybenzoic acid was also found in both isozymes, only 15 and 9% activity was inhibited, respectively [34].

### 3.3. Activator of catalases

Metformin is a commonly used antidiabetic drug with AMP-activated protein kinase (AMPK)-dependent hypoglycemic activities. A recent study [40] shows that metformin can significantly enhance the activity of catalase. Although metformin bound to CAT by interacting with hydrogen bonds..., metformin did not affect the expression level of catalase, just affecting its activities, such as Lys449, Val450, and Glu455 residues in murine CAT. The preliminary study indicated that metformin might be a new drug to alleviate oxidative injury and enhance the defense ability of antioxidants.

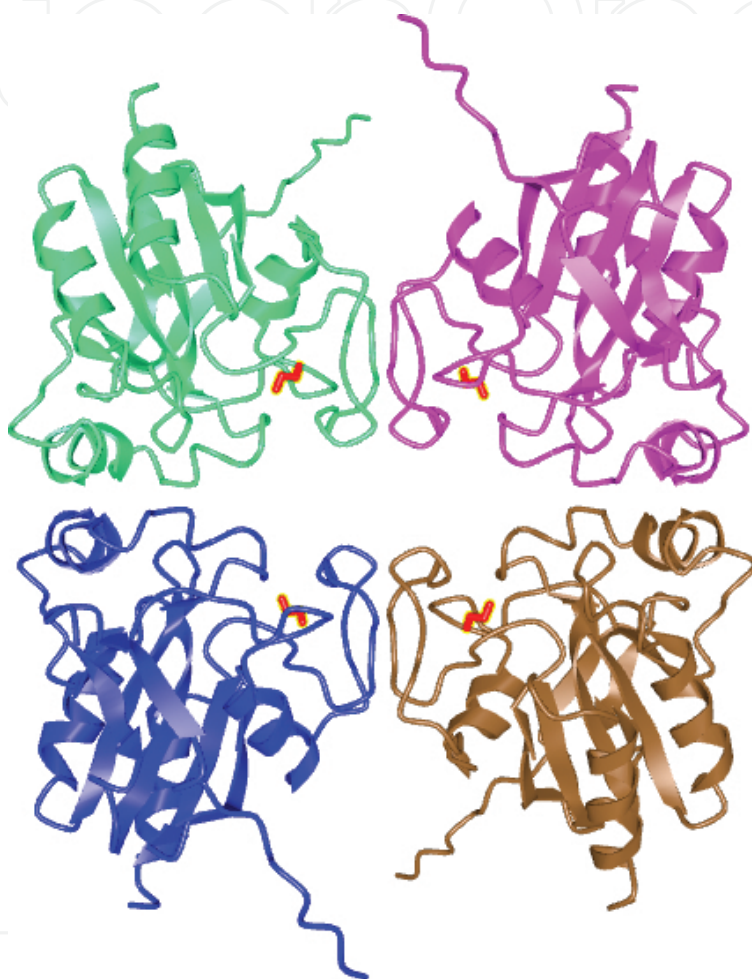
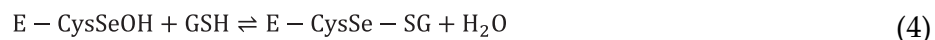
## 4. GPX

### 4.1. The structure and function of GPX

GPX is an important selenium-containing enzyme which protects cells from lipid peroxide damage and H<sub>2</sub>O<sub>2</sub>. GSH-Px widely existed in the body, there are eight family members: GPX1 is the most abundant selenoperoxidase and is ubiquitously expressed in almost all tissues; GPX2 expression is most prominent in the gastrointestinal tract cytoplasm; GPX3 is greatest expressed in the kidney, and also in various tissues, and is secreted into extracellular fluids as a glycoprotein; GPX4 is the only GPX enzyme that reduces phospholipid hydroperoxides, different with other members, it is not a tetramer, but a monomer; GPX6 was identified as a selenoprotein in the human genome by homology search. GPX1-4 and GPX6 are selenium-containing protein, but GPX5 does not contain selenocysteine or Se in active site [41, 42]. More recently, GPX7 (NPGPx) and GPX8 were discovered, but detailed information about these two kinds of enzymes is little up to now [43].

Molecular weight of GSH-PX in human red blood cell was 95,000 Da and that in bovine red blood cell was 83,000 Da, and they are all tetrameric selenoenzyme. A typical crystal structure of human glutathione peroxidase (2I3Y) is shown in **Figure 4**.

GSH-PX contains one selenocysteine per subunit and selenocysteine plays an important role in catalyst degradation of lipid peroxide, a widely accepted mechanism was proposed as follow:



**Figure 4.** Three-dimensional structure of human glutathione peroxidase.

#### 4.2. The effect of selenium on GPX activity

There are approximate 25 selenoproteins in humans, and selenium is an essential cofactor for these proteins, including the glutathione peroxidases. There is a complex relationship among utilization of selenium, GPX activity, and methylation. GPX synthesis utilizes selenium via selenocysteine and homocysteine is derived from *S*-adenosylhomocysteine, and the latter was formed as a result of methylation reactions including methylation of selenium [44]. From the view of selenium forms, the inorganic forms (such as sodium selenate and sodium selenite) of

selenium were more effective on increasing the GPX activity compared with selenomethionine [44]. To be attention, different members of GPXs response differently to selenium deficiency, a phenomenon called the “hierarchy of selenoproteins.” This means that upon selenium deprivation, some proteins decline fast, whereas others remain synthesized until selenium becomes severely deficient [45]. The expression of GPX1 mRNA, protein, and its activity in tissues is more sensitive than other selenoperoxidases or selenoproteins [46].

### 4.3. The effect of vitamin E on GPXs activity

DL-Alpha-tocopherol (vitamin E) also is an antioxidant, but it is different from selenium acting on GPX directly, vitamin E plays its antioxidant function through combining free radical, named “chain-breaking reaction.” Vitamin E was considered to be the first line of defense against lipid peroxidation and free oxygen radicals that might suppress the enzymes, such as GPX. It seems that the sensitivity of GPXs was various. In vitamin E-deficient rat brain microsomes, phospholipid hydroperoxide glutathione peroxidase activity was significantly decreased but GPX activity was not affected. And in liver homogenate, phospholipid hydroperoxide glutathione peroxidase activity was approximately 20 times lower than that of GPX [47].

### 4.4. Inhibitors of GPXs

#### 4.4.1. Competitive inhibitors

##### 4.4.1.1. Misonidazole

GPX can combine to an electrophilic compound that might result in loss of its activity. More and more evidence shows that upregulation of the GPX system may serve to protect cancer cells from oxidative stress caused by anticancer drugs, thus block GPX that may help to treat cancer disease. A number of inhibitors of GPXs have been reported to use as therapeutics, such as thiol-containing inhibitors that bind covalently to a selenium atom in the active site [48], nonthiol inhibitors misonidazole [49]. However, thiols tend to combine ubiquitous multivalent metal ions and are easily oxidized, thus leading to nonspecific interactions with proteins. Recently, acylhydrazones have been reported as potential inhibitors of bovine glutathione peroxidase [50]. These inhibitors overcome the disadvantages of thiol-containing inhibitors, but the efficiency needs to be further improved.

##### 4.4.1.2. Penicillamine and its analogues

D-Penicillamine is a drug to chelate metals in tissue and promotes its excretion in the urine. D-Penicillamine hydrochloride could competitively inhibit GSH-PX, that means the concentration of hydrogen peroxide and reduced glutathione were inversely proportion [51].

L-penicillamine hydantoin is an analogue of glutathione, but the acting configuration is different from D-penicillamine hydrochloride. After treated with L-penicillamine hydantoin, GPX activity was inhibited whatever the peroxide ( $H_2O_2$ , *tert*-butyl hydroperoxide or cumene

hydroperoxide) used as substrate of the reaction. In the presence of 100  $\mu\text{M}$  L-penicillamine hydantoin, the enzyme reactions catalyzed by glutathione peroxidase were inhibited, but neither glutathione transferases, nor glutathione reductase were affected by L-penicillamine hydantoin [52].

#### 4.4.2. Noncompetitive inhibitors

##### 4.4.2.1. DL-Buthionine-[S, R]-sulfoximine

DL-Buthionine-[S, R]-sulfoximine (BSO) can be used as an inhibitor to estimate the scavenging efficiency of  $\text{H}_2\text{O}_2$  after GPX inhibition. The inhibitory effect did not act on GPX directly, but through suppress the synthesis of GSH by inhibiting  $\gamma$ -glutamylcysteine synthetase, that cause glutathione decreased sharply in many tissues, especially kidney, liver, and pancreas [53]. BSO has an obvious inhibitory effect, for example in human fibroblasts cells, after 500  $\mu\text{M}$  BSO treated, the GSH levels decreased to  $154.0 \pm 16.9$  nmol/mg protein from  $418.4 \pm 13.1$  nmol/mg protein [54].

##### 4.4.2.2. Gold(I) thioglucose

Gold(I) thioglucose in the presence excess of glutathione (GSH) leads to strong and reversible inhibition of selenium-GPXs. Gold(I) could competitively combine in reduced form of selenocysteine in active sites, and gold(I) forms a dead-end complex with glutathione peroxidase resulting in suppression of GPXs. So glutathione peroxidase could be a target of gold drugs that used in the treatment of disease caused by excessive activity of GPXs, such as rheumatoid arthritis [55].

### 4.5. Activators of GPXs

To our knowledge, most literature studies on enhancing GPXs activity were about how to regulate expression of GPXs, study on the activator by acting the enzyme directly was few and most of them are GPX-mimetic compounds.

#### 4.5.1. Enhance activity of GPX mimetics

For some GPX mimetics, its activity can be enhanced by electron-donating. naphthalene *peri*-diselenide mimetics was increased by electron-donating methoxy substituents, while a further 100-fold increase was observed with the corresponding ditelluride. This was attributed to the ability of the methoxy group to stabilize the increasing positive charge at the selenium atom during the rate-determining step of the catalytic cycle, which involves the oxidation of Se(II) to Se(IV), thus improved their catalytic activity to levels comparable with their aliphatic counterparts [56]. Others report that 6-bromo-substituted diselenides also enhanced its activity by threefold [57]. Another strategy is to change the aqueous solubility of the mimetics. Diaryl selenides containing *o*-hydroxymethylene substituents function as peroxide-destroying mimetics of the antioxidant selenoenzyme glutathione peroxidase. Several selenide analogues

were attached to polyethylene glycol (PEG) oligomers greatly improved aqueous solubility and catalytic activity (10–100 folds) [58].

## Fund support

This work was supported by grants from the Key project of Natural Science Foundation of Guangdong Province (2014A030311010), Guangdong Province Modern Agro-industry Technology Research System (2016LM1080)

## Author details

Xianyong Ma<sup>1,2,3\*</sup>, Dun Deng<sup>1,2,3</sup> and Weidong Chen

\*Address all correspondence to: 407986619@qq.com

1 Institute of Animal Science, Guangdong Academy of Agricultural Sciences, Guangzhou, China

2 Key Laboratory of Animal Nutrition and Feed Science in South China, Ministry of Agriculture, Guangzhou, China

3 Guangdong Key Laboratory of Animal Breeding and Nutrition, Guangzhou, China

## References

- [1] Kaynar H, Meral M, Turhan H, Keles M, Celik G, Akcay F. Glutathione peroxidase, glutathione-S-transferase, catalase, xanthine oxidase, Cu–Zn superoxide dismutase activities, total glutathione, nitric oxide, and malondialdehyde levels in erythrocytes of patients with small cell and non-small cell lung cancer. *Cancer Letters*. 2005;227: 133–139.
- [2] Chance B, Sies H, Boveris A. Hydroperoxide metabolism in mammalian organs. *Physiological Reviews*. 1979;59: 527–605.
- [3] Dorval J, Hontela A. Role of glutathione redox cycle and catalase in defense against oxidative stress induced by endosulfan in adrenocortical cells of rainbow trout (*Oncorhynchus mykiss*). *Toxicology and Applied Pharmacology*. 2003;192: 191–200.
- [4] Zhong W, Yan T, Lim R, Oberley LW. Expression of superoxide dismutases, catalase, and glutathione peroxidase in glioma cells. *Free Radical Biology and Medicine*. 1999;27: 1334–1345.



- [5] Eitinger T. In vivo production of active nickel superoxide dismutase from *Prochlorococcus marinus* MIT9313 is dependent on its cognate peptidase. *Journal of Bacteriology*. 2004;186: 7821–7825.
- [6] Bryngelson PA, Arobo SE, Pinkham JL, Cabelli DE, Maroney MJ. Expression, reconstitution, and mutation of recombinant *Streptomycescoelicolor* NiSOD. *Journal of the American Chemical Society*. 2004;126: 460–461.
- [7] Abreu IA, Cabelli DE. Superoxide dismutases—a review of the metal-associated mechanistic variations. *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics*. 2010;1804: 263–274.
- [8] Nam JS, Yoon JH, Lee HI, Kim SW, Ro YT. Molecular cloning, purification, and characterization of a superoxide dismutase from a fast-growing *Mycobacterium* sp. Strain JC1 DSM 3803. *The Journal of Microbiology*. 2011;49: 399–406.
- [9] Mayer BK, Falkinham JO. Superoxide dismutase activity of *Mycobacterium avium*, *M. intracellulare*, and *M. scrofulaceum*. *Infection and Immunity*. 1986;53: 631–635.
- [10] Whittaker MM, Whittaker JW. Thermally triggered metal binding by recombinant *Thermus thermophilus* manganese superoxide dismutase, expressed as the apo-enzyme. *Journal of Biological Chemistry*. 1999;274: 34751–34757.
- [11] Kirby T, Blum J, Kahane I, Fridovich I. Distinguishing between Mn-containing and Fe-containing superoxide dismutases in crude extracts of cells. *Archives of Biochemistry and Biophysics*. 1980;201: 551–555.
- [12] Zhu YB, Wang GH, Ni H, Xiao AF, Cai HN. Cloning and characterization of a new manganese superoxide dismutase from deep-sea thermophile *Geobacillus* sp. EPT3. *World Journal of Microbiology & Biotechnology*. 2014;30: 1347–1357.
- [13] Wang S, Shao B, Liu S, Ye X, Rao P. Purification and characterization of Cu, Zn-superoxide dismutase from black soybean. *Food Research International*. 2012;47: 374–379.
- [14] Liu J, Wang J, Yin M, Zhu H, Lu J, Cui Z. Purification and characterization of superoxide dismutase from garlic. *Food and Bioproducts Processing*. 2011;89: 294–299.
- [15] Halliwell B, Gutteridge JMC. *Free Radicals in Biology and Medicine*. Oxford University Press. 1999.
- [16] Kanofsky JR. Singlet oxygen production by biological systems. *Chemico-Biological Interactions*. 1989;70: 1–28.
- [17] Kim SY, Kwon OJ, Park J-W. Inactivation of catalase and superoxide dismutase by singlet oxygen derived from photoactivated dye. *Biochimie*. 2001;83: 437–444.

- [18] Xueshan Y, Xiaopu Y, Wenli F. Effect of carbohydrates on activities of superoxide dismutase extracted from sheep blood. *Food Science*. 2011;32: 100–103.
- [19] Sola-Penna M, Meyer-Fernandes JR. Stabilization against thermal inactivation promoted by sugars on enzyme structure and function: why is trehalose more effective than other sugars? *Archives of Biochemistry and Biophysics*. 1998;360: 10–14.
- [20] Beckman JS, Minor RL, Jr., White CW, Repine JE, Rosen GM, Freeman BA. Superoxide dismutase and catalase conjugated to polyethylene glycol increases endothelial enzyme activity and oxidant resistance. *Journal of Biological Chemistry*. 1988;263: 6884–6892.
- [21] Valdivia A, Pérez Y, Ramírez H, Cao R, Villalonga R. Improved pharmacological properties for superoxide dismutase modified with  $\beta$ -cyclodextrin–carboxymethylcellulose polymer. *Biotechnology Letters*. 2006;28: 1465–1470.
- [22] Puglisi A, Tabbi` G, Vecchio G. Bioconjugates of cyclodextrins of manganese salen-type ligand with superoxide dismutase activity. *Journal of Inorganic Biochemistry*. 2004;98: 969–976.
- [23] Zamocky M, Koller F. Understanding the structure and function of catalases: clues from molecular evolution and in vitro mutagenesis. *Progress in Biophysics and Molecular biology*. 1999;72: 19–66.
- [24] Andreoletti P, Sainz G, Jaquinod M, Gagnon J, Jouve HM. High-resolution structure and biochemical properties of a recombinant *Proteus mirabilis* catalase depleted in iron. *Proteins: Structure, Function, and Bioinformatics*. 2003;50: 261–271.
- [25] Welinder KG. Bacterial catalase-peroxidases are gene duplicated members of the plant peroxidase superfamily. *Biochimica et Biophysica Acta*. 1991;1080: 215–220.
- [26] Kamachi S, Hirabayashi K, Tamoi M, Shigeoka S, Tada T, Wada K. The crystal structure of isoniazid-bound KatG catalase-peroxidase from *Synechococcus elongatus* PCC7942. *FEBS Journal*. 2015;282: 54–64.
- [27] Allgood GS, Perry JJ. Characterization of a manganese-containing catalase from the obligate thermophile *Thermoleophilum album*. *Journal of Bacteriology*. 1986;168: 563–567.
- [28] Whittaker MM, Barynin VV, Igarashi T, Whittaker JW. Outer sphere mutagenesis of *Lactobacillus plantarum* manganese catalase disrupts the cluster core. Mechanistic implications. *European Journal of Biochemistry*. 2003;270: 1102–1116.
- [29] Kengen SW, Bikker FJ, Hagen WR, de Vos WM, van der Oost J. Characterization of a catalase-peroxidase from the hyperthermophilic archaeon *Archaeoglobus fulgidus*. *Extremophiles*. 2001;5: 323–332.
- [30] Lee DH, Oh DC, Oh YS, Malinverni JC, Kukor JJ, Kahng HY. Cloning and characterization of monofunctional catalase from photosynthetic bacterium

- Rhodospirillum rubrum* S1. Journal of Microbiology and Biotechnology. 2007;17: 1460–1468.
- [31] Shima S, Netrusov A, Sordel M, Wicke M, Hartmann CG, Thauer KR. Purification, characterization, and primary structure of a monofunctional catalase from *Methanosarcina barkeri*. Archives of Microbiology. 1999;171: 317–323.
- [32] Ueda M, Kinoshita H, Yoshida T, Kamasawa N, Osumi M, Tanaka A. Effect of catalase-specific inhibitor 3-amino-1,2,4-triazole on yeast peroxisomal catalase in vivo. FEMS Microbiology Letters. 2003;219: 93–98.
- [33] Ai Q, Ge P, Dai J, Liang TC, Yang Q, Lin L, Zhang L. The catalase inhibitor aminotriazole alleviates acute alcoholic liver injury. Sheng Li Xue Bao. 2015;67: 97–102.
- [34] Horváth E, Szalai G, Pál M, Páldi E, Janda T. Differences between the catalase isozymes of maize (*Zea mays* L.) in respect of inhibition by various phenolic compounds. Acta Biologica Szegediensis. 2002;46: 33–34.
- [35] Durner J, Klessig DF. Salicylic acid is a modulator of tobacco and mammalian catalases. Journal of Biological Chemistry. 1996;271: 28492–28501.
- [36] Havir EA, McHale NA. Biochemical and developmental characterization of multiple forms of catalase in tobacco leaves. Plant Physiology. 1987;84: 450–455.
- [37] Zamocky M, Herzog C, Nykyri LM, Koller F. Site-directed mutagenesis of the lower parts of the major substrate channel of yeast catalase A leads to highly increased peroxidatic activity. FEBS Letters. 1995;367: 241–245.
- [38] Atli G, Alptekin O, Tukul S, Canli M. Response of catalase activity to Ag<sup>+</sup>, Cd<sup>2+</sup>, Cr<sup>6+</sup>, Cu<sup>2+</sup> and Zn<sup>2+</sup> in five tissues of freshwater fish *Oreochromis niloticus*. Comparative Biochemistry and Physiology: Toxicology & Pharmacology. 2006;143: 218–224.
- [39] Lee HI, Yoon JH, Nam JS, Kim YM, Ro YT. Cloning, expression and characterization of the catalase-peroxidase (KatG) gene from a fast-growing *Mycobacterium* sp. strain JC1 DSM 3803. Journal of Biochemistry. 2010;147: 511–522.
- [40] Dai J, Liu M, Ai Q, Lin L, Wu K, Deng X, et al. Involvement of catalase in the protective benefits of metformin in mice with oxidative liver injury. Chemico-Biological Interactions. 2014;216: 34–42.
- [41] Lei XG, Cheng WH, McClung JP. Metabolic regulation and function of glutathione peroxidase-1. Annual Review of Nutrition. 2007;27: 41–61.
- [42] Kryukov GV, Castellano S, Novoselov SV, Lobanov AV, Zehtab O, Guigo R, Gladyshev VN. Characterization of mammalian selenoproteomes. Science. 2003;300: 1439–1443.
- [43] Toppo S, Vanin S, Bosello V, Tosatto SC. Evolutionary and structural insights into the multifaceted glutathione peroxidase (Gpx) superfamily. Antioxidants and Redox Signaling. 2008;10: 1501–1514.

- [44] Bermingham EN, Hesketh JE, Sinclair BR, Koolaard JP, Roy NC. Selenium-enriched foods are more effective at increasing glutathione peroxidase (GPx) activity compared with selenomethionine: a meta-analysis. *Nutrients*. 2014;6: 4002–4031.
- [45] Hofmann B, Hecht HJ, Flohe L. Peroxiredoxins. *Biological Chemistry*. 2002;383: 347–364.
- [46] Bermano G, Nicol F, Dyer JA, Sunde RA, Beckett GJ, Arthur JR, Hesketh JE. Tissue-specific regulation of selenoenzyme gene expression during selenium deficiency in rats. *Biochemical Journal*. 1995;311 (Pt 2): 425–430.
- [47] Bourre J-M, Dumont O, Clément M, Dinh L, Droy-Lefaix M-T, Christen Y. Vitamin E deficiency has different effects on brain and liver phospholipid hydroperoxide glutathione peroxidase activities in the rat. *Neuroscience Letters*. 2000;286: 87–90.
- [48] Chaudiere J, Wilhelmsen EC, Tappel AL. Mechanism of selenium-glutathione peroxidase and its inhibition by mercaptocarboxylic acids and other mercaptans. *Journal of Biological Chemistry*. 1984;259: 1043–1050.
- [49] Kumar KS, Weiss JF. Inhibition of glutathione peroxidase and glutathione transferase in mouse liver by misonidazole. *Biochemical Pharmacology*. 1986;35: 3143–3146.
- [50] Wilde F, Lemmerhirt H, Emmrich T, Bednarski PJ, Link A. Microwave-assisted synthesis and evaluation of acylhydrazones as potential inhibitors of bovine glutathione peroxidase. *Molecular Diversity*. 2014;18: 307–322.
- [51] Mercurio SD, Combs GF, Jr. Drug-induced changes in selenium-dependent glutathione peroxidase activity in the chick. *Journal of Nutrition*. 1985;115: 1459–1470.
- [52] Mestdagh N, Poupaert J, Hénichart J-P, Vamecq J. Effect of l-penicillamine hydantoin, an analogue of glutathione, on rat liver glutathione peroxidase, reductase and transferase reactions. *Biochemical Pharmacology*. 1992;43: 1529–1537.
- [53] Griffith OW, Meister A. Glutathione: interorgan translocation, turnover, and metabolism. *Proceedings of the National Academy of Sciences of the United States of America*. 1979;76: 5606–5610.
- [54] Masaki H, Okano Y, Sakurai H. Differential role of catalase and glutathione peroxidase in cultured human fibroblasts under exposure of H<sub>2</sub>O<sub>2</sub> or ultraviolet B light. *Archives for Dermatological Research. Archiv für Dermatologische Forschung*. 1998;290: 113–118.
- [55] Chaudiere J, L. Tappel A. Interaction of gold(I) with the active site of selenium-glutathione peroxidase. *Journal of Inorganic Biochemistry*. 1984;20: 313–325.
- [56] Press DJ, Back TG. Enhanced glutathione peroxidase activity of conformationally restricted naphthalene peri-dichalcogenides. *Organic Letters*. 2011;13: 4104–4107.

- [57] Singh VP, Poon J-f, Butcher RJ, Lu X, Mestres G, Ott MK, Engman L. Effect of a bromo substituent on the glutathione peroxidase activity of a pyridoxine-like diselenide. *The Journal of Organic Chemistry*. 2015;80: 7385–7395.
- [58] McNeil NMR, Press DJ, Mayder DM, Garnica P, Doyle LM, Back TG. Enhanced glutathione peroxidase activity of water-soluble and polyethylene glycol-supported selenides, related spirodioxyselenuranes, and pincer selenuranes. *The Journal of Organic Chemistry*. 2016;81: 7884–7897.