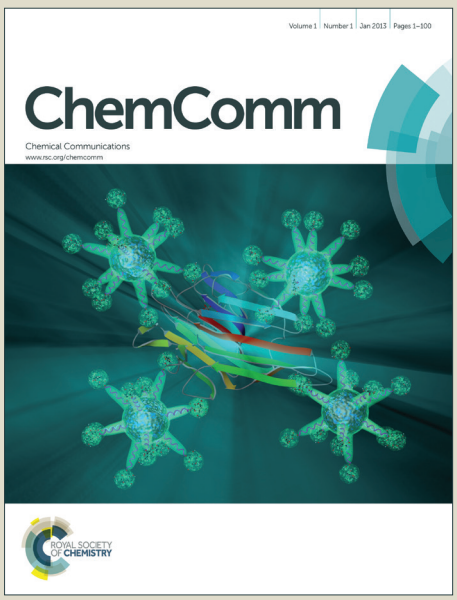
ChemComm

Accepted Manuscript



ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/chemcomm

Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

Catalytic Antioxidant Therapy by Metallodrugs: Lessons from Metallocorroles

Adi Haber and Zeev Gross*

Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX
DOI: 10.1039/b000000x

Significance: The fundamental science behind the "antioxidant therapy", i.e., the importance of eliminating oxidizing species that may damage vital biomolecules and induce biological malfunctions, is well established. Nevertheless, quite disappointing results were reported in several recent meta-analyses that addressed the effects of vitamins and other antioxidant supplements on the many diseases that are associated with oxidative events. This emphasizes the importance of elucidating factors that might be responsible for the large gap between the hypothesis and practice. ***Recent Advances***: Classical "antioxidant therapy" deals with stoichiometric antioxidants whose role is sacrificial, i.e., they are consumed on a one-to-one basis by being oxidized instead of vital biomolecules. On the other hand, *catalytic antioxidants* detoxify the reactive species without being permanently oxidized, and one molecule may hence disarm numerous oxidants. The benefits of catalytic antioxidants in pure chemical systems and in animal models of many diseases are quite established and have been summarized in several reviews. ***Critical Issues***: The main aim of this article is to provide a perspective on the utility of metal-based catalytic antioxidants, with focus on those chelated by corroles, for disease prevention or treatment. Particular emphasis is on the often-ignored fact that redox-based therapeutics is potentially harmful because it may actually induce rather than decrease oxidative stress. ***Future Directions***: Investigations aimed at identifying the factors that increase the antioxidant versus pro-oxidant potency of synthetic metal complexes are crucial for the optimal design of redox-based drug candidates that may be used for combating the numerous diseases that are affected by oxidative stress.

Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

Oxidative stress and disease

Reactive oxygen/nitrogen species (ROS/RNS) are important signalling molecules, that when kept at low levels are essential for the survival of organisms and their health.[1] These oxidant molecules are involved in the defence mechanism of the body against invaders as well as in the regulation of the proliferative response.[2] Oxidative stress occurs when the amount of ROS/RNS rises above a threshold level. This may result from their overproduction (e.g., inflammation) or due to a deficiency in the availability/activity of antioxidants. Regardless of the reason, oxidative stress leads to damage to various cellular components and induces many biological malfunctions and diseases.[3]

Antioxidant enzymes are major components of the body's defence system against oxidative stress. However, the activity of such enzymes decreases with age and under pathological conditions,[4] and they may even display pro-oxidant activity under certain circumstances.[5] An example of the latter is the superoxide dismutases (SOD) enzyme family that catalyzes the disproportionation of $O_2^{\cdot -}$ to O_2 and H_2O_2.[6] It has been shown that when SOD1 (Cu/Zn SOD) undergoes oxidative modifications it not only loses its desired potency, but also gains significant pro-oxidative activity due to the formation of copper-bound hydroxyl or peroxycarbonate radicals.[7] Oxidatively modified SOD1 promotes the development of Alzheimer's and Parkinson's diseases, as well as of amyotrophic lateral sclerosis (ALS).[8] A potent reagent for the oxidation of SOD1 is H_2O_2, the product of the SOD-catalyzed reaction.[9] The conclusion is that when the action of SOD1 is not coupled with that of a H_2O_2 decomposition enzyme– catalases, glutathione peroxidase, or peroxiredoxins (CAT, GPx, or Prx, respectively)– it may actually induce damage rather than protect the cells against oxidative stress.

Antioxidant-rich diet and appropriate food supplements are often recommended for helping the body fight off oxidative stress. Recent meta-analyses of human trials with vitamins and food additives were however quite disappointing.[10] Among the reasons for these discouraging results are the limited bioavailability of dietary antioxidants, the fact that they very often do not react faster with ROS/RNS than the vital biomolecules they need to protect,[11] and that their reaction products may actually augment rather than prevent oxidation.[12] In addition, the mode of action of the nutritional antioxidants is sacrificial, i.e., they are consumed in a *stoichiometric* ratio and may hence not be available when needed. We, for example, have shown that as much as 250 mol% punicalagin– the most active ingredient of pomegranate juice– is needed to reduce the half-life of protonated peroxynitrite (HOONO) by 50%.[13] It is hence clear that new antioxidant approaches are needed for combating the many diseases associated with oxidative stress.

Catalytic antioxidants: benefits and classification

The above shortcomings of *stoichiometric* antioxidants (**SAO**) may be overcome by using *catalytic* antioxidants. The latter term is often used for all cases in which one single molecule of catalyst induces the detoxification of numerous ROS/RNS molecules. We, however, wish to emphasize that the catalytic antioxidants should be further classified by how they perform their catalytic action: independent catalytic antioxidants (**ICA**) and (cofactor)-dependent catalytic antioxidants (**DCA**) (**Fig. 1**). The term **ICA** implies that the decomposition of the ROS/RNS proceeds by the catalyst without the need of any additional compounds. SOD and CAT are representative members of the **ICA** class: the low-valent metal ion present in these enzymes (Cu^{+1} in SOD and Mn^{+3} in SOD) reduces $O_2^{\cdot -}$ and H_2O_2, respectively, and the thus formed high-valent metal ion oxidizes a second molecule of the toxin. Altogether, a catalytic cycle is completed without the involvement of any co-reductant (**Fig. 1a**). On the other hand, the term **DCA** refers to catalytic antioxidants that require the aid of other cofactors for completing their full catalytic cycle. This class includes antioxidant enzymes from the glutathione peroxidase (GPx) and Peroxiredoxin (Prx) families: both reduce H_2O_2 to H_2O, but require glutathione and thioredoxin, respectively, as cofactors (**Fig. 1b**).[14] The latter are **SAO**'s that are oxidized during the process and activity is regained by specific enzymes that reduce their oxidized form.[15] One must however keep in mind that levels of glutathione and similar cofactors needed for the proper action of **DCA** enzymes are largely depleted under severe oxidative stress.[16]

Therapies that rely on over-expression of antioxidant enzymes in cell culture and *in vivo* have indeed provided protection against deleterious effects in some oxidative stress models.[17] However, external supply of these enzymes to animals had mixed success, as the large size of proteins leads to low cell permeability, short circulating half-life, and antigenicity.[18] To overcome many of these limitations, an increasing number of (relatively) low molecular-weight *synthetic catalytic antioxidants* has been developed. Their beneficial effects have been demonstrated in quite an impressively large number of *in vivo* model systems.[19] Nevertheless, one aspect that is too often ignored is that these catalytic antioxidants are transition-metal-containing complexes that have also the potential of acting as pro-oxidants. Special attention must hence be devoted for achieving the desired balance between these conflicting aspects (*vide infra*).

Controlling pro- vs. antioxidant activity: taking lessons from nature

The interactions between heme-containing enzymes and ROS/RNS are of prime importance for a variety of different purposes, including signal transduction, respiration, catalytic oxidation reactions and toxin detoxification. The heme enzymes

all have a common prosthetic group composed of iron chelated by a porphyrin macrocycle that occupies the four equatorial positions of the metal ion. The particular role that these enzymes perform is dictated by delicate differences in the structure of their active site: the oxidation state of the metal ion (+2 or +3), the number of substitution-inert axial ligands (0, 1 or 2), the identity of the axial ligand(s), the architecture of the active site, and the involvement of co-factors.[20] Heme enzymes with either pro- or antioxidant activity (activating or neutralizing small oxidant molecules, respectively) share in common the first stage of the catalytic cycle: they form reactive reaction intermediates that feature metal-oxygen bonds. What defines the role of the particular enzyme is how these intermediates react with other molecules present in the medium.

In the case of pro-oxidant enzymes, such as cytochrome P450 (P450) and peroxidases, the high valent metal intermediates formed by the reaction with ROS/RNS oxidize organic molecules, an activity of prime importance for both biosynthesis (of steroids and hormones, for example) and biodegradation (metabolism of foreign compounds such as drugs).[21] On the other hand, the H_2O_2-*oxidized* reaction intermediate of CAT is *reduced* by another equivalent of H_2O_2. The outcome is that CAT is a true **ICA**: it catalytically decomposes toxic H_2O_2 into harmless oxygen and water without the need of any co-factor.[22] In fact, all peroxidases may catalyze the CAT-characteristic reaction in the absence of their natural targets, and this is actually a mechanism for protecting the enzymes from being harmed by excess H_2O_2.[23] The main difference between CAT and the peroxidases is that the very small binding pocket of CAT does not allow access of any substrates other than H_2O_2, which shuts down its pro-oxidative capability and accentuates its anti-oxidative role. Phrased differently, the heme enzymes that utilize H_2O_2 for substrate oxidation have also some anti-oxidative activity, but this is usually just a "side effect" of the desired oxidation reaction. Controlling the pro- vs. anti-oxidant activity of synthetic and hence non protein-conjugated metal complexes is a very demanding task in terms of proper design of desired action.

The development of synthetic metal-porphyrin complexes with pro- and antioxidant activity

Great efforts have been invested over the years in understanding the mechanism of action of heme enzymes. Based on the acquired knowledge, inorganic chemists aimed to design synthetic macrocylic complexes that will display either pro- or antioxidant activity. The more than 30 years of research in this field have been reviewed quite frequently [usually separately for pro- and antioxidant activity[24,25]], and only a small selection of important milestones is provided here.

Pro-oxidant activity

The first successful model system for oxidation catalysis was introduced by Groves et al. in 1979, in which a simple synthetic metalloporphyrin was used together with iodosylbenzene for catalyzing P450-like reactions.[26] Largely improved catalytic activity was later achieved by incorporating electron-withdrawing groups on the β-pyrrole carbon atoms and the *ortho*-positions of the *meso*-phenyl groups (**Fig. 2**).[24a, 27] However the vast majority of reported reactions still rely on primary oxidants that produce large amounts of waste (such as iodobenzene from iodosylbenzene) and not on O_2 or H_2O_2 where water is the sole co-product. A similar reservation concerns the large achievements in the utilization of synthetic heme enzyme models for asymmetric catalysis, for which Groves et al. reported the first case in 1983: a 50% enantiomeric excess in an epoxidation reaction catalyzed by a chiral iron porphyrin.[28] Numerous porphyrin molecules have later been designed with particular structural motifs in order to yield specific positioning of the substrate relative to the active metal-oxo species.[29] This approach is reminiscent of P450 enzymes that employ particular protein moieties within the binding pocket for placing the substrate in the desired manner relative to the prosthetic group. The much more elegant and nature-like approach of using proteins for inducing chirality has been much less investigated.[30] We have described one such case, by taking advantage of the exceptionally large affinity of amphipolar metallocorroles to serum albumins. The bioconjugated was used as catalyst for the asymmetric synthesis of the chiral sulfoxide-containing drug, R-modafinil (Nuvigil), with H_2O_2 as oxidant.[31]

SOD mimics

The first attempts to develop synthetic catalytic antioxidants have focused on eliminating the primary oxidant in living systems, $O_2^{\cdot -}$. Pasternack et al. were the first to introduce metalloporphyrins as SOD mimics in 1979, revealing them to be **ICA** and to have a rate constant of $3 \cdot 10^7$ $M^{-1}s^{-1}$ for $O_2^{\cdot -}$ dismutation by the iron(III) complex of a porphyrin substituted with positively charged *para*-methylpyridinium groups on its *meso* positions, FeTM-4-PyP.[32] The SOD activity displayed by analogous iron(III) and manganese(III) porphyrins were quite similar.[33] Contemporary research in the field, conducted mainly by the group of Batinic-Haberle, is however dedicated to the manganese complexes.[25a] This practice was adopted because the iron complexes appeared to be cytotoxic under conditions that the manganese porphyrins displayed cellular protection against $O_2^{\cdot -}$.[34] This phenomenon might be related to the tendency of iron porphyrins to lose their metal ion at low pH, thus inducing harmful pro-oxidative chemistry (Fenton chemistry).[33b, 35] The structures of the most investigated catalytic antioxidants are depicted in **Fig. 2**, and their mode of action and catalytic rates for decomposition of the most important ROS/RNS are listed in **Table 1** and **Table 2**, respectively.

The application of the first manganese-based SOD mimic MnTM-4-PyP in a cellular environment revealed two severe limitations: its SOD activity was reduced relative to that displayed *in vitro* and it turned out to be cytotoxic.[36] Both aspects were attributed to intercalation of that compound into DNA. This problem was resolved by using the *meta* and *ortho* isomers, MnTM-3-PyP and MnTM-2-PyP, respectively, wherein the aryl groups form larger dihedral angles with the porphyrin plane and thus prevent intercalation.[36] While MnTM-3-PyP displayed SOD activity comparable to that of MnTM-4-PyP, the rate constant disclosed for MnTM-2-PyP was 10-fold higher (**Table 2**), a phenomenon termed "the *ortho* effect".[36] The increased activity of MnTM-2-PyP was attributed to the close proximity of the positive charges to the metal center, a feature that greatly affects the M(III)/M(II) redox potential and also supplies electrostatic facilitation for reaction with the negatively charged $O_2^{\cdot -}$.[37]

Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

Table 1. The mode of action of enzymatic and synthetic catalytic antioxidants for decomposition of ROS/RNS.

	$O_2^{\cdot-}$	$ONOO^-$	H_2O_2	comments	references
SOD	**ICA**	-	-	Damaged by H_2O_2	9, 38
CAT	-	-	**ICA**		25b
GPx	-	-	**DCA**		25b
Prx	-	**DCA**	**DCA**		25b
Iron(III) porphyrins	**ICA**	**ICA**	-	Damaged by H_2O_2	32, 39
Manganese(III) porphyrins	**ICA**	**DCA**	-	Damaged by H_2O_2	36, 40
Iron(III) corroles	**ICA**	**ICA**	**ICA**		41
Manganese(III) corroles	**ICA**	**ICA**	**ICA**		41

Table 2. Catalytic rate constants ($M^{-1}s^{-1}$) for the decomposition of ROS/RNS by metalloporphyrins and metallocorroles.*

		Charge[d]	$O_2^{\cdot-}$	$ONOO^-$	H_2O_2	references
Enzymes			$2.3\cdot10^9$ (SOD)	$0.8\text{-}7\cdot10^7$ (GPx, Prx)	$0.2\text{-}1.8\cdot10^8$ (CAT, GPx, Prx)	42
Iron(III) porphyrins	FeTM-4-PyP	+4	$3\cdot10^7$	$2.2\cdot10^6$	a	32, 39
	FeTPPS	-4	$6\cdot10^5$	$8.6\cdot10^5$	a	33a, 43
Manganese(III) porphyrins*	MnTM-4-PyP	+4	$3.8\cdot10^6$	$4.3\cdot10^6$	20, b	36, 40
	MnTM-3-PyP	+4	$4.1\cdot10^6$	$3.8\cdot10^6$	b	36, 40a
	MnTM-2-PyP	+4	$6.0\cdot10^7$	$1.9\cdot10^7$	b	36, 40a
	MnTE-2-PyP	+4	$5.7\cdot10^7$	$3.4\cdot10^7$	c	34, 44
	MnTnHex-2-PyP	+4	$3.0\cdot10^7$	$1.3\cdot10^7$	a	7, 44-45
	MnTnBuOE-2-PyP	+4	$6.8\cdot10^7$	$3.4\cdot10^7$	ND	46
Iron(III) corroles	**1-Fe**	-2	$1.7\cdot10^6$	$3.1\cdot10^6$	6400	41
Manganese(III) corroles	**1-Mn**	-2	$4.8\cdot10^5$	$8.6\cdot10^4$	5.6	41
	2-Mn	+3	$8.7\cdot10^5$	ND	ND	41a
	3-Mn	+2	$2.2\cdot10^6$	ND	ND	41a
	4-Mn	+2	$1.8\cdot10^6$	$4.0\cdot10^5$	ND	41a, 47

* Note that for decomposition of peroxynitrite by manganese porphyrins the cited values are for the first half of the catalytic cycle, which is not completed in the absence of **SAO**. It becomes k_{cat} for very reducing **SAO**'s, such as ascorbate.

a = no data, extensively bleached

b = the half-life times for oxidative degradation are: 30, 28, and 105 s for MnTM-4-PyP, MnTM-3-PyP, and MnTM-2-PyP, respectively [36].

c = a value of 2 min^{-1} has been reported [48].

d = The number of charges due to the water-solubilizing substituents.

ND = no data.

Nevertheless, MnTM-3-PyP turned out to be equally potent as a $O_2^{\cdot-}$ scavenger in cellular systems because of its 10-fold higher lipophilicity (and hence higher bioavailability) relative to MnTM-2-PyP.[49] Increasing the lipophilicity by introducing longer alkyl chains on the pyridinium-moieties had minor effects on SOD activity,[50] as may be exemplified by very similar rate constants of MnTM-2-PyP and its ethyl and hexyl analogs, MnTE-2-PyP and MnTnHex-2-PyP, respectively (**Table 2**). However, increased lipophylicity had a major influence on their efficiency against oxidative-stress induced injuries,[51] emphasizing again the importance of bioavailability for obtaining optimal biological activity.

The best performing SOD scavengers were disclosed by the group of Batinic-Haberle in 2008: analogs of MnTM-3-PyP and MnTM-4-PyP, but brominated at all eight β-pyrrole positions. These synthetic complexes displayed $O_2^{\cdot-}$ dismutation rate constants approaching that of native SOD: $4.7\cdot10^8$ $M^{-1}s^{-1}$ for the *para* isomer and $7.1\cdot10^8$ $M^{-1}s^{-1}$ for the *meta* isomer, compared to ~10^9 $M^{-1}s^{-1}$ for the enzyme.[52] However, the increase in electron-deficiency of these brominated complexes led to the stabilization of the manganese ion in its +2 oxidation state, which consequently reduced the metal/ligand stability to a level that was deduced to be inappropriate for *in vivo* applications.

Porphyrins with negatively charged substituents were also investigated, but they display lower SOD activities than the pyridinium-substituted derivatives.[34, 37b, 44, 53] The lower catalytic rates (**Table 2**) disclosed for porphyrins such as FeTPPS may be attributed mainly to the absence of strong electron-withdrawing groups, leading to a less positive M(III)/M(II) redox potential and lower thermodynamic driving force for the oxidation of $O_2^{\cdot-}$.[37b] A recent investigation by us revealed another reason for not investing into such complexes: the cellular uptake of FeTPPS is

extremely low and its pro-oxidant activity is very high (*vide infra*).[54]

Peroxynitrite decomposition catalysts

The reaction between $O_2^{\cdot-}$ and nitric oxide as to form peroxynitrite is so fast (diffusion controlled) that it kinetically competes with the normal pathways for elimination of the reagents.[55] This issue becomes more pronounced under oxidative stress, since SOD activity declines and the concentration of $O_2^{\cdot-}$ consequently increases. The deleterious effects of peroxynitrite (*in its protonated and/or CO_2-trapped form*) in living systems were recognized at the beginning of the 1990's.[56] The ability of GPx and Prx to act as peroxynitrite reductases was identified much later than their CAT-like activity.[42b, 55] The mechanisms of action of these two enzymes towards H_2O_2 and peroxynitrite are practically identical. It is according to the **DCA** mechanism depicted in **Fig. 1b**, with only one difference: the first step involves transformation of the enzyme to its oxidized state by peroxynitrite instead of H_2O_2.

The first synthetic metalloporhyrin to display peroxynitrite decomposition activity was FeTM-4-PyP, disclosed by M. K. Stern and colleagues in 1996.[39b] The group of J. T. Groves has introduced the manganese(III) complex of the same porphyrin, MnTM-4-PyP for the same purpose.[57] One important realization was that while iron(III) porphyrins are **ICA**, their manganese counterparts are **DCA**: the presence of co-reductants is mandatory for turning their action catalytic (*vide infra*). As such co-reductants are naturally present in the body one may understand why manganese(III) porphyrins attenuate damage attributed to peroxynitrite much more in animal studies than predicted by their non-catalytic *in vitro* activity.[58] Like in the case of SOD activity, an *ortho* effect was disclosed for the rate of reaction by which peroxynitrite oxidizes Mn(III) porphyrins (**Table 2**).[40a]

Limited CAT activity

The discovery of metalloporphyrins as SOD mimics was accompanied by an important observation – FeTM-4-PyP was bleached during the reaction and it consequently lost most of its $O_2^{\cdot-}$ scavenging activity.[32] This phenomenon was prevented by the addition of small amounts of CAT, but not by the addition of SOD, indicating that the H_2O_2 formed during the catalytic decomposition of $O_2^{\cdot-}$ was responsible for the bleaching of the catalyst. Later work from several groups has revealed that all other investigated water soluble iron(III) porphyrins also undergo extensive oxidative degradation in the presence of H_2O_2.[35, 59] The manganese(III) porphyrins were shown to be more stable in this respect,[33b, 34] thus enabling the determination of a catalytic rate constant for H_2O_2 decomposition by MnTM-4-PyP: 20 $M^{-1}s^{-1}$.[40b] This value is however based on *initial rates*, as the half-life for oxidative degradation of this compound and its *meta* and *ortho* analogs are 30, 28, and 105 s, respectively.[36] The total catalytic turnover number for MnTM-4-PyP is hence still below 30 and the catalytic rate constant are much too low for being considered as a true CAT analog.[40b]

It is also worth mentioning that manganese salen complexes have been introduced as **ICA** that display both SOD and CAT activity, with the latter feature more pronounced than for porphyrins. In a most recent publication, Higuchi and coworkers have connected an acid/base moiety onto manganese salens for increasing their CAT activity.[60] Consistent with their hypothesis, significant changes in the relative CAT/SOD activities were obtained upon the introduction of the acid/base moiety. The main problems associated with metallosalens were however not resolved. Each of the examined complexes was bleached within 2 min from the start of the reaction and oxygen evolution stopped due to catalyst decomposition. The total turnover numbers hence remained at the level of 1-15. Most important in the context of this perspective article, the complexes with higher catalase-like activity displayed also increased peroxidase-like activity.

Brief summary

A possible impression of the analysis provided so far is that pro-oxidant activity is necessarily harmful and undesired for treating diseases affected by oxidative stress. This conclusion is certainly not true in the cases where the compounds act as **DCA**'s, a process during which they oxidize **SAO**'s (Figure 1b). What is more, recent research has uncovered cellular redox biology that may actually benefit from metalloporphyrin catalyzed oxidations such as that of protein-derived thiols. These and related aspects are out of the scope of this focused perspective article, but the interested reader is referred to a recently published excellent assembly of reviews (Forum issue, *Antioxidants & Redox Signalling* **2014**, 20(15)). There are also many reviews that summarize the beneficial effects of porphyrin-based catalytic antioxidants in animal-based disease models.[38, 61] The largest advances are with the manganese(III) complex of the imidazolium-substituted porphyrin compound (AEOL10150), currently developed as a countermeasure to acute radiation exposure.[62] Administration of this compound to rats demonstrated a significant protective effect to the lungs from injury caused due to the cascade of events that follow irradiation, including the production of ROS.

Corroles as ROS/RNS Decomposition Catalysts

Intensive investigations of corroles began only in 1999, when simple and efficient methods for the synthesis of triarylcorroles were gradually discovered and explored.[63] Corrole-metal complexes (metallocorroles) as ROS/RNS decomposition catalysts were first disclosed in 2006,[41b] coinciding with publications on synthetically availability water-soluble corroles.[64] Structures of corrole complexes that were investigated as catalytic antioxidants are depicted in **Fig. 2**.

Corroles share the aromaticity of porphyrins, but possess one carbon less in the basic skeleton. This causes some important changes relative to porphyrins: a) lower symmetry of the molecule; b) a tri-anionic (rather than di-anionic for porphyrins) equatorial coordination plane; and c) a smaller core size. The first aspect allowed for highly selective electrophilic substitution on the skeleton and the design of water-soluble derivatives with unique amphipolarity due to the presence of polar substituents on only one pole of the otherwise lipophilic molecule (**Fig. 3**). The resulting compounds are much more lipophilic than most porphyrins utilized as catalytic antioxidants, with a $logP_{o/w}$ of 0.14 and 0.57 for the non-metallated bis-sulfonated corrole and its iron(III) complex, respectively. The two latter features are

responsible for several advantages of transition metal complexes of corroles with regard to their utility as catalytic antioxidants in biological systems: a) they do not undergo demetallation under biologically relevant conditions; b) they are very powerful reductants; c) oxo-metal complexes are surprisingly stable in the corrole-provided coordination environment; and d) they are very weak pro-oxidants toward substrates, as well as towards themselves (less bleaching); and d) The relationship between the chemical and structural phenomena is that the combination of a tri-anionic and relatively small metal-coordination core makes corroles very strong σ donors.[65] Binding of the metal to the corrole N atoms is hence more covalent and of higher affinity, so that there is no release of the metal under neutral or low pH. Moreover, the d orbitals of the metal are pushed to a much higher energy level (> 1 V relative to porphyrins, **Fig. 4**), which increases the reducing power of the metal(III) state and stabilizes the higher metal oxidation states.[65-66] Taken together, high valent metal-oxo corrole intermediates are weaker oxidants and less prone to oxidative degradation. Examples that illustrate the above are the isolation of chromium and manganese corroles in +5 and even +6 oxidation states, as well as the low reactivity of (O)Cr(V) and (O)Mn(V) corroles towards organic substrates.[67] Recent work has shown that even the $[(O)Fe(IV)]^{2+}$ moiety may be isolated for triazacorroles (corrolazines).[68] The reducing power may be appreciated by the activation of O_2 by trivalent iron and manganese corroles [which are inert in almost all other coordination environments],[69] as well as the inaccessibility of divalent oxidation states of these derivatives under biologically relevant conditions.[70]

SOD activity

Considering the low oxidative power of SOD mimics as an advantage is actually a conceptual change, since the work with metalloporphyrins proved that is important to turn them into stronger oxidants that would be able to oxidize $O_2^{\cdot-}$. The fundamental chemistry behind this difference is the mechanism by which metallocorroles detoxify superoxide.[41a] Native SOD and the metalloporphyrin-based SOD-mimics dismutate $O_2^{\cdot-}$ by redox cycling between the divalent and trivalent states of the metal (**Fig. 5**). These compounds are **ICA**: the first $O_2^{\cdot-}$ is oxidized by the M^{+3} state to form dioxygen, and then a second $O_2^{\cdot-}$ molecule is reduced by M^{+2} to H_2O_2 (without the support of a **SAO**). Since the first step was elucidated to be rate limiting, electron-withdrawing groups were introduced on the porphyrin macrocycle for inducing a positive shift of the M^{+2}/M^{+3} redox potential and thereby increasing SOD activity.[34, 37b] Metallocorroles can however not be reduced to form M^{+2} complexes at reasonable potentials,[70] and catalysis actually starts by the reduction rather than oxidation of $O_2^{\cdot-}$. The contrast with metalloporphyrins and native SOD is quite remarkable: $O_2^{\cdot-}$ is reduced by Cu^{+1} in Cu/Zn-SOD, by Mn^{+2} in Mn-SOD and manganese porphyrin-based SOD mimics, but by Mn^{+3} and Fe^{+3} in metallocorroles.

Fast kinetics experiments have been used to deduce that this step is rate limiting for iron(III) corroles, followed by much faster oxidation of $O_2^{\cdot-}$ by the high valent metallocorrole (formally Fe^{+4}).[41a] This conclusion was later validated by introducing 5,15-bis-pyridinium corroles with C10-aryl groups that differ in their electron-donating power.[71] The efficiency for eliminating superoxide by the corresponding manganese(III) complexes did indeed increase when the C10-substituent was more electron-donating, up to a limit in which oxidation of superoxide became slower than its reduction. The earlier described *ortho*-effect found for metalloporphyrins affected metallocorroles in two opposing directions (**Table 2**): the favorable electrostatic facilitation is diminished by the undesired shift in redox potential. The tris-*ortho*-pyridinium isomer (**2-Mn**) displayed the smallest rate constant for $O_2^{\cdot-}$ dismutation, because its M^{+3}/M^{+4} redox potential is more positive than that of the bis-pyridinium analogs **3-Mn** and **4-Mn**.[41a] The two latter compounds display practically identical rate constants because **3-Mn** has a kinetic *ortho* effect but **4-Mn** has a less positive redox potential. It is important to mention that the redox potentials and catalytic rates for ROS/RNS decomposition by the iron(III) and manganese(III) complexes of the negatively-charged bis-sulfonated corrole (**1-Mn** and **1-Fe**, respectively, **Fig. 2**) are of the same order of magnitude as those for metal complexes of pyridinium-substituted metallocorroles (**Table 2**).[41a, 71] So while negatively charged porphyrins were greatly abandoned as catalytic antioxidants (particularly so as SOD mimics), the bis-sulfonated corroles with their two negative charges are actually the most investigated within that family.

Peroxynitrite decomposition

In the case of peroxynitrite decomposition catalysis, the plausible reaction schemes for both corroles and porphyrins involve identical high valent reaction intermediates (**Fig. 6**). The iron(III) complexes of both are **ICA**, i.e., they catalyze isomerization of peroxynitrite to nitrate without "external" help. Investigations of iron porphyrins revealed the formation of an (O)Fe(IV) species and nitrogen dioxide ($\cdot NO_2$), which then rapidly combine as to form iron(III) and nitrate (NO_3^-).[39, 72] Similar homolytic cleavage of the O-O bond of peroxynitrite, with a very short-lived iron(IV) corrole intermediate, has been identified as the most crucial step in the catalytic cycle of the iron(III) corrole (83 and yet unpublished work). The observable reaction intermediate in catalysis by manganese(III) porphyrins is (O)Mn(IV), but the reaction has shown to start by *heterolytic* cleavage of peroxynitrite to form (O)Mn(V) and nitrite (NO_2^-). This is followed by a superfast in-cage redox reaction between the two that leads to (O)Mn(IV) and $\cdot NO_2$.[73] Importantly, the (O)Mn(IV) species is not recycled back to the initial +3 oxidation state fast enough,[57] and may cause oxidative damage to biomolecules, e.g. DNA strand scission.[73] Fast reduction of the (O)Mn(IV) porphyrin back to the resting Mn(III) state occurs only when catalysis is performed in the presence of co-reductants (artificial or biological). The conclusions are that: a) manganese(III) porphyrins act as a **DCA** (rather than **ICA**) of peroxynitrite; and b) that their utility strongly depends on the availability of co-reductants.[57] The naturally present non-catalytic antioxidants do not react fast enough with peroxynitrite itself, e.g. 88 $M^{-1}s^{-1}$ for ascorbate and 580 $M^{-1}s^{-1}$ for glutathione, and thus do not protect vital biomolecules from this toxin. They do however come into effect in combination with complexes such as MnTM-4-PyP. The reaction of the corresponding (O)Mn(IV) species with ascorbate has a rate constant of $> 3 \cdot 10^7$ $M^{-1}s^{-1}$, which leads to the situation where the initial reaction of Mn(III) with peroxynitrite becomes rate limiting and $k_{ox} = k_{cat}$ (**Fig. 7**, top right).[57] The rate constant

for the reaction of glutathione with the (O)Mn(IV) species is smaller than k_{ox}, but formation of the (O)Mn(IV) may still be expected to be the rate limiting step at the high concentrations (2 mM) that are typical of biological milieus.[40a]

Despite of the similarity in the reaction mechanism of peroxynitrite with the manganese(III) complexes of porphyrins and corroles, the latter act as **ICA** due to a unique disproportionation mechanism (**Fig. 7**, bottom right).[41b, 71] These conclusions were reached by observing that manganese(III) corroles decompose peroxynitrite even without any reducing additive (with up to 1900 catalytic turnovers) and that the reaction product was nitrite and not nitrate (as for iron(III) porphyrins and corroles). Other important observations are that there is no change in the catalyst oxidation state during catalysis at physiologically relevant pH, that the reaction of independently prepared (O)Mn(V) corrole is extremely fast, and the accumulation of an (O)Mn(V) intermediate in catalysis performed at high pH. The catalytic cycle deduced from these investigations consists of *heterolytic* cleavage of the peroxynitrite's O-O bond, which leads to formation of nitrite and an (O)Mn(V) intermediate in the first and rate determining step. In contrast with catalysis by manganese(III) porphyrin, the (O)Mn(V) intermediate is much more long-lived and does not undergo the fast redox reaction with nitrite. Instead, it reacts with another peroxynitrite molecule as to form molecular oxygen and another equivalent of nitrite.

The important consequences of the catalytic cycles depicted in Figure 7, in particular the fact that only two-electron pathways are involved in catalysis by manganese(III) corroles, were first demonstrated in purely chemical systems. The main results obtained regarding the reactions of peroxynitrite with different substrates were that while both the iron(III) and the manganese(III) corroles efficiently prevented oxidation, the nitration of tyrosine and fluorescein were only prevented by the manganese(III) corrole.[13] The latter result is fully consistent with the oxidative nitration mechanism by which phenols are nitrated by peroxynitrite:[74] its homolytic decomposition products (produced by either spontaneous or metal-assisted pathways) induce one electron oxidation of phenol, which is trapped by $\cdot NO_2$.[56b] The formation of powerful oxidants and $\cdot NO_2$ is circumvented only in the catalytic cycle of the manganese(III) corroles and that is the reason for their efficacy in preventing nitration. The relevance of these findings were later confirmed in cellular investigations, which revealed that manganese(III) corroles are superior of the other corrole- and porphyrin-based peroxynitrite decomposition catalysts for the prevention of intracellular nitration and consequential death of the insulin-producing beta-cells.[75] It is also important to point out that excessive amounts of nitrotyrosine in patients are well-established biomarkers for the involvement of peroxynitrite in the diseases under study.[56a]

CAT activity

Literature review of synthetic metalloporphyrins reveals that they are surprisingly poor catalysts for the decomposition of H_2O_2, because most of them are extensively bleached.[76] One plausible reason is that they cleave the O-O bond of H_2O_2 in a homolytic fashion, leading to the formation of (O)M(IV) + $\cdot$OH (M = Fe or Mn) and oxidative degradation of the catalyst by the hydroxyl radical. Another possibility is heterolytic cleavage to $[(O)M(V)]^+$ (**Fig. 7**, top) and decomposition of this hyperactive species via self-oxidation as reported for many non-sterically hindered derivatives.[77] Nevertheless, there are also reports that describe isolation of the product from heterolytic cleavage of H_2O_2 by manganese(III) porphyrins.[78] This and other $[(O)Mn(V)]^+$ intermediates were found to be stable enough for spectroscopic characterization at low temperatures and their reactivity profile have been determined. These studies revealed a large reactivity towards a range of organic and inorganic substrates, *but not for oxidation of* H_2O_2.[79] The necessary conclusion is that the manganese porphyrins may be predicted to act as pro- rather than antioxidants with regard to H_2O_2. According to this analysis, metalloporphyrins could still display antioxidant performance of the **DCA** type, if used in combination with **SAO** that recycle the reactive intermediates. While we have not found in the literature any study that looked into the combined effect of metalloporphyrins and **SAO** on the decomposition of H_2O_2, there are reports of using metalloporpyrins together with H_2O_2 for catalytic oxidation of hydrocarbons.[77] In any case, the presence of reducing agent in cells could well be the reason that synthetic metalloporphyrins that perform poorly in pure chemical systems still display some CAT-like activity in protecting against H_2O_2-induced cellular damage.[40b, 80] An appreciation of how delicate the predicted action of **DCA** may be deduced from a recent study by Batinic-Haberle and coworkers, who concluded that "MnTE-2-PyP^{5+} may act both as an anti- and pro-oxidant; in the latter case it can increase the levels of reactive oxygen species."[81]

The main superiority of **1-Fe** relative to other synthetic metal complex is its excellent CAT-like activity. A study that looked into H_2O_2 decomposition by FeTPPS, **1-Fe**, and **1-Mn** showed that FeTPPS was completely bleached, **1-Fe** acted very fast and efficiently (turnover frequency > 120 s^{-1}), and that catalysis by **1-Mn** is very slow at neutral pH.[41c] Another investigation has disclosed that the reaction of (independently prepared) (O)Mn(V) with H_2O_2 is very fast,[82] which suggests that its formation is the rate determining step (**Fig. 7,** bottom). More in depth examinations that were performed for **1-Fe** revealed the formation of the μ-oxo compound **1-Fe**-O-**1-Fe**, which is likely to be a less active catalyst than **1-Fe**.[41c] Preventing the formation of this species by supplying axial ligands was indeed advantageous and may account for the very high potency of **1-Fe** in preventing H_2O_2-mediate damage in biological systems, where amino acid residues that may be involved in axial ligation are present. Consistent with that hypothesis, the efficiency of **1-Mn** to decompose H_2O_2 was largely increased in the presence of proteins.[82] In the context of this perspective article it is crucial to keep in mind that no matter how efficient an SOD (natural or synthetic mimic) would be, it will still lead to the formation of H_2O_2 and other toxic species (such as HOCl and the hydroxyl radical) if not coupled to CAT or a CAT-mimic. **1-Fe** is highly beneficial in this respect because it displays both SOD and CAT activity. Using synthetic molecules that display SOD activity but are devoid of any CAT activity was in fact reported to kill cells, and used advantageously in the context of cancer treatment.[83]

Investigations that addressed the potential therapeutic utility of metallocorroles

In depth biological investigations of corroles commenced with the aim of using the non-redox active but highly fluorescent gallium(III) complex of the bis-sulfonated corrole (**1-Ga**) for optical imaging of cancerous cells and tumors. The results obtained with **1-Ga** and a breast cancer-targeted cell penetration protein (HerPBK10) revealed spontaneous formation of a very tight conjugate. This bioconjugate (Her-Ga) exhibited excellent targeting properties to heregulin-positive cancer cells. The surprise was that Her-Ga also displayed dark toxicity towards the cancerous cells, which raised the interest in checking its applicability in tumor-implanted mice.[84] As little as 0.008 mg/kg of Her-Ga was sufficient for complete inhibition of tumor growth *in vivo*, and even some shrinkage of the tumor was displayed with this dosage.[85] What is more, the same 0.008 mg/kg dose reduced tumor size by about 60% even when non-bioconjugated **1-Ga** was used. Mechanistic investigations have disclosed that Her-Ga disrupts the mitochondrial potential, leading to the formation of $O_2^{\cdot -}$ and thus acting as a pro-oxidant.[86] A most recent investigation revealed that the toxicity may further be enhanced by the production of singlet oxygen when light irradiation is added.[87] Additional work revealed that **1-Ga** displayed cytotoxicity also in the absence of the specific carrier protein towards breast, melanoma, and ovarian cancerous cells primarily by arresting DNA replication.[88] On the other hand and consistent with its antioxidant activity, **1-Fe** did not display any cytotoxicity towards cancerous cells.

Most work in the field of corroles as catalytic antioxidants has been dedicated to bis-sulfonated corrole-metal complexes, and specifically to the iron complex **1-Fe**, whose beneficial effects are summarized in Figure 8. One reason for this practice is that the redox potentials and catalytic rate constants for ROS/RNS decomposition of this negatively-charged metallocorrole are of the same magnitude as those of pyridinium-substituted analogs (**Table 2**). Additionally, the synthetic up-scaling of the bis-sulfonated compounds is very facile. A major concern regarding these compounds was that they might not penetrate the cell membrane due to their negative charge. To address this issue, cells were incubated with **1-Ga** (typically 20 μM in the medium for 30 min) and examined by fluorescent confocal microscopy. This disclosed that for SH-SY5Y human neuroblastoma cells,[89] insulinoma RIN-m cells,[75b] and J774.A1 macrophage cells,[90] the compound is internalized and accumulates in the cytoplasm but remains excluded from the nucleus. The very strong non-covalent binding of these amphipolar compounds to various serum proteins is apparently responsible for the ability of these negatively charged compounds to penetrate cell membranes, via an endocytosis pathway.[84] This includes binding to HSA and transferrin, for which the K_d values were determined to be ≤ 1nM and 10 nM, respectively.[91] The compounds were found to bind to lipoproteins as well, with about 40 and 10 molecules remaining bound to LDL and HDL, respectively, even after extensive dialysis of the bioconjugates.[13]

Large preference for binding to lipoproteins, and specifically HDL, was deduced by HPLC separation of full human serum pre-treated with various metal complexes of the bis-sulfonated corrole. These inspections revealed that 60% eluted together with the HDL fraction in the cases of **1-Mn** and **1-Ga**.[92] For the lead compound **1-Fe**, the preference for HDL binding was 85%, with the other 15% being associated with LDL. This finding is quite important since these two lipoproteins are the main cholesterol carriers [note that while there is more cholesterol in the LDL fraction, the molarity of HDL is 7-times larger]. The general selectivity of the three amphipolar corroles for binding to HDL and LDL suggests strong interactions with the lipidic envelop of the lipoproteins. Consistent with this hypothesis, FeTPPS with its symmetrically distributed negative charges did not display that phenomenon.[92] The larger affinity (relative to **1-Ga** and **1-Mn**) of **1-Fe** to HDL points towards some specific coordination interaction as an additional mode of binding. The additional binding mode for **1-Fe**/HDL was identified by spectroscopy as coordination of two proximal histidine moieties to the iron atom, although the identity of the HDL-associated protein(s) that contribute(s) these residues is still unknown. Nevertheless, it is important to emphasize that the preference for HDL binding was also evident in serum taken from mice to which **1-Fe** was applied by *ip* injection.[90]

Metallocorroles in models of neurodegenerative diseases

1-Fe and the pyridinium-substituted manganese(III) corroles **2-Mn** and **4-Mn** were examined in cellular models of neurodegenerative diseases.[75a] Human neuroblastoma cells (SH-SY5Y) and mice motor neuron-neuroblastoma fusion cells (NSC-34) were pre-incubated with 20 μM of the analyzed compound, followed by the induction of oxidative stress. With 700 μM SIN-1 (a compound that forms peroxynitrite *in situ*) as toxin, all examined metallocorroles increased cell survival by a factor of 3 to 4 and they were also highly effective for preventing intracellular nitration. **2-Mn** and **4-Mn** provided roughly the same amount of protection also when used at a concentration of 5 μM, at which **1-Fe** had no beneficial effect. The better performance of the manganese corroles in this experimental system, taken together with the evidence for the importance of preventing nitration, is consistent with the earlier mentioned mode of action on peroxynitrite. Only manganese corroles are true **ICA** against nitration by it, since their catalytic cycle is devoid of NO_2. In parallel analysis of the porphyrin FeTPPS revealed that this compound was much less potent than all three corrole-metal complexes. Very different results were obtained when oxidative stress was induced by the addition of H_2O_2 (200 μM) for 24 h. **2-Mn** and **4-Mn** had practically no effect on cellular viability even at a concentration of 20 μM, consistent with their very slow mode of action against this toxin. On the other hand, **1-Fe**, the only catalytic antioxidant to date to display significant CAT-like activity, significantly increased cell survival at concentrations as low as 1 μM. For treatment with 20 μM of **1-Fe**, a 2-fold increase was observed for the SH-SY5Y cells and a 7-fold elevation for the NSC-34 cells. **1-Fe** even displayed neurorescue activity, as it provided the same level of cellular protection even when added 30 min after the addition of H_2O_2. Further examinations were conducted by treatment of the cells for 24 h with 6-hydroxydopamine (6-OHDA, 40 μM), a parkinsonism-related neurotoxin that induces the formation of a variety of ROS/RNS by the cells. All three metallocorroles significantly increased cell survival, but the pyridinium-substituted complexes were more efficient. The three compounds also displayed neurorescue, but in this case **1-Fe** was more efficient providing significant cellular protection even when

added as much as 3 h after the addition of the toxin. This shift in the relative potencies of the compounds implies the involvement of different ROS/RNS at different times after the addition of the indirect toxin, 6-OHDA.

The bis-sulfonated metallocorroles **1-Fe**, **1-Mn** and **1-Ga** were further investigated in neuronal precursor retinal ganglion cells (RGC-5), both in their undifferentiated immature state and as differentiated cells that display characteristics similar to mature neurons.[93] Possible neuroprotective activity was examined in cells that were deprived of serum for 48 h, a treatment that greatly reduces cell viability and involves the production of $O_2^{\cdot-}$. Incubation of **1-Fe** or **1-Mn** at concentrations of 10 or 100 nM during the serum deprivation (but in the presence of 1% BSA) resulted in 30-50% cell rescue, whereas the non-redox active **1-Ga** had no effect. In addition, the transition metal complexes were shown to completely suppress $O_2^{\cdot-}$ production in undifferentiated and partially in differentiated RGC-5 cells. The lower efficiency in the latter case may be attributed to the fact that the drug used for differentiation (staurosporine) causes some cellular apoptosis. The results obtained with **1-Fe** and **1-Mn** were comparable to those obtained with polyethylene glycol-conjugated SOD (30 U/mL), which was also less efficient in the differentiated cells.

In vivo real-time confocal imaging was used to examine the effect of **1-Fe**, **1-Mn** and **1-Ga** on $O_2^{\cdot-}$ production in individual rat retinal ganglion cells following optic nerve transection. The corroles were applied by intravitreal (to the eye) injection at a concentration of 100 nM three days after nerve transection and were analyzed the following day. **1-Fe** and **1-Mn**, but not **1-Ga**, significantly reduced the number of $O_2^{\cdot-}$-positive cells in the retina.[93] In a follow up study, where **1-Fe** was administered one day after nerve transection and the analysis performed four days later, the treatment was much less effective.[94] It has been shown that the $O_2^{\cdot-}$ burst in RGC occurs 3 - 5 days after the axonal damage. It is reasonable to speculate that the levels of **1-Fe** in the RGC decreased over time, accounting for the low activity displayed when the compound was applied on the first day following transection.

Metallocorroles in models of diabetes

The cytoprotective activity of **1-Fe**, **1-Mn** and **4-Mn** has been explored in a cellular model of diabetes using insulinoma RIN-m cells.[75b] Cellular protection was observed when the RIN-m cells were pre-incubated with the metallocorroles and then exposed to SIN-1 (800 μM). **4-Mn** was the most potent, increasing cell survival 6-fold at a concentration of 5 μM and higher. 20 μM of **1-Fe** were needed to achieve the same level of protection, while **1-Mn** at this concentration increased cell viability only by a factor of 3. The three compounds also attenuated SIN-1 induced intracellular protein nitration with the same relative efficiencies: 90% for **4-Mn**, 50% for **1-Fe**, and 25% for **1-Mn** for treatment with 20 μM of each. The superiority of **4-Mn** points towards larger cellular uptake of the positively charged derivative, and probably also to a preferred decomposition mode of peroxynitrite (**Fig. 6**). Supporting evidence for the latter hypothesis has been obtained by the comparison between **4-Mn** and a porphyrin-based structural analog that is also substituted by two *para*-pyridinium groups at the 5,15 *meso*-positions. The latter reduced nitration by only 35%.

In vivo investigations were only performed with **1-Fe**, which was examined for its efficacy against the development of diabetes complications.[95] Rats received a single *ip* injection of streptozotocin (STZ, 50 mg/kg), which turned them diabetic within 3 days. Oral treatment with 20 mg/kg/day of **1-Fe** for a period of 7 weeks began only 2 week after the induction of diabetes, at which the fasting glucose levels were already very high (> 500 mg/dL). Treatment with **1-Fe** significantly attenuated the development of cataract, decreasing both the severity of the condition and its occurrences; and the negative effect of diabetes on the kidneys was almost completely cancelled out. In addition, serum cholesterol and triglyceride levels were lower in the **1-Fe**-treated group relative to the control. A positive control was also applied in these studies (α-lipoic acid. 50 mg/kg/day), which further emphasized the superior effects of **1-Fe**. The positive control displayed comparable advantages regarding kidney function, but much lower benefits on cataract incidents and severity. It also had no positive impact on body weight and lipid profile, and highly elevated the serum level of the hepatic enzyme alkaline phosphatase.

Metallocorroles in cellular and animal models of atherosclerosis

The cholesterol carrying lipoproteins LDL ("bad cholesterol") and HDL ("good cholesterol") play major roles in cholesterol homeostasis and the development of atherosclerosis, which eventually lead to cardiovascular diseases.[96] Oxidized LDL (oxLDL) is well and long known to be highly atherogenic,[97] while it has much more recently been discovered that the intrinsically antiatherogenic HDL becomes dysfunctional and proinflammatory upon its oxidation.[98] The selective binding of sulfonated metallocorroles to HDL and LDL, together with the catalytic potency of the manganese and iron complexes, suggested that the latter might be of significant value in the ongoing search for therapies that may prevent cardiovascular diseases. The first relevant examinations focused on the effects on lipoproteins under induced oxidative stress.[13] Investigations on the ability of the metallocorroles to prevent $CuSO_4$–induced oxidative damage to LDL revealed the following: **1-Ga** had no effect, consistent with it being non-redox active; **1-Fe** completely prevented LDL oxidation; and **1-Mn** acted as a pro-oxidant. The conflicting behaviour of **1-Mn**, which displayed antioxidant activity in purely chemical systems and in cell cultures, may be explained by the different environments: only toxin present in the chemical system (**ICA** activity), the presence of recyclable **SAO** within the cells (**DCA** activity), and encapsulation by a target-rich lipoprotein (pro-oxidant activity). Later research focused on the ability of **1-Fe** to protect HDL; and in that case not just from oxidation but also from consequential deleterious effects.[92] Oxidative damage to HDL was induced by a variety of methods, which included $CuSO_4$ and the peroxynitrite-releasing SIN-1 molecule. The results revealed protection against these toxins by **1-Fe** and preservation of the following anti-atherogenic activities of HDL that are impaired due to oxidation: cholesterol efflux, anti-apoptotic capability, and antioxidant activity. The **1-Fe**/HDL conjugates were even more potent antioxidants than native HDL, indicating that **1-Fe** boosts the natural antioxidant activity of HDL. Interestingly **1-Fe** also reduced the pro-atherogenicity of already oxidized LDL (oxLDL), as revealed by decreased

oxidative damage caused to macrophages by **1-Fe**/oxLDL conjugates relative to oxLDL alone.

Cellular penetration of **1-Fe** into macrophages and its possible cytoprotective effects were the focus of a separate investigation that also included three different iron porphyrins: the pyridinum-substituted FeTM-4-PyP, FeTPPS with its four symmetrically distributed sulfonate moieties, and the amphipolar hemin with two carboxylate head groups.[54] The methodology for determining cellular uptake relied on the pro-oxidative effects of the iron complexes, i.e., their ability to catalyze oxidation of luminol by H_2O_2. One outcome of this study was that much lower concentrations of FeTPPS relative to **1-Fe** could be identified by this method. This shows that the peroxidase-like activity of FeTPPS is much larger than that of **1-Fe**, and vice versa, that the potency of **1-Fe** as **ICA** of H_2O_2 is much larger than that of FeTPPS. Both FeTM-4-PyP and hemin were found to accumulate in the cells to high levels, but this was shown to be associated with pronounced cytotoxicity induced by these complexes. Regarding the other two complexes, the intracellular levels of **1-Fe** were found to be 5-fold higher than those determined for FeTPPS, a phenomenon attributed to the amphipolar nature of the former. This structural characteristic enables very strong binding to lipoproteins,[92] which apparently serve as shuttles for transporting the negatively charged molecule into the cells. The uptake of **1-Fe** by the macrophages was quantified ($2.4{\cdot}10^8$ molecules/cell) and shown to be 2 to 3 orders of magnitude larger than intracellular concentrations of native SOD reported in the literature. The conclusion was that out of the four examined iron(III) complexes, only **1-Fe** displayed reasonable cellular uptake together with no cytotoxicity.[54] What is more, basal cell viability even increased due to treatment by **1-Fe**, which served to indicate that it rescued against the high oxidative stress that is characteristic of macrophages.

Examination of the cytoprotective potential against several toxins added to the macrophages was performed for the two non-toxic compounds, FeTPPS and **1-Fe**. The identical results obtained with and without FeTPPS clearly indicated that its intracellular concentration is too small to come into effect. On the other hand, **1-Fe** was found to provide protection against externally applied oxidative stress: doubling the amount of cells that survived treatment with H_2O_2 or SIN-1. The quite small intracellular fraction of **1-Fe** also saved the cells from damage induced by compounds that are not ROS/RNS themselves, but rather induce cellular production of such toxins. This includes a two times elevation in cell survival of cells exposed to oxLDL for 24 h or to a combination of lipopolysaccharides and interferon-γ (INFγ) for 48 h. These features were shared by the manganese analog, **1-Mn**, despite of its pro-oxidant activity in non-cellular lipoprotein oxidation studies.[90] This again highlights the mode of action dependence of metal complexes on reaction conditions: **1-Mn** may display both pro- and anti-oxidant activity, which depends on the amounts of **SAO** present in the system.

The *in vivo* efficacy of **1-Fe**, **1-Mn**, and **1-Ga** has been addressed in apolipoprotein E deficient mice, a most established murine model for the development of atherosclerosis.[13] 12 weeks old mice were orally treated for 10 weeks with 10 mg/kg/day of the various compounds, and the following results were disclosed: **1-Ga** displayed no beneficial effects on atherosclerosis development, **1-Mn** displayed moderate efficiency, while **1-Fe** was highly potent. The higher efficiency of **1-Fe** relative to **1-Mn** is consistent with the significantly larger catalytic rates disclosed for the former regarding the decomposition of all major biologically relevant ROS/RNS (**Table 2**). The 60% attenuation of atherosclerosis by **1-Fe** exceeded the best results obtained in the same animal model by the most potent dietary antioxidants, such as red wine and pomegranate juice. An additional phenomenon identified in that study, which is of relevance not only to atherosclerosis, is the reduction of total blood cholesterol levels in mice treated with **1-Fe** relative to control mice. This beneficial outcome was also displayed in **1-Fe** treated diabetic rats.[95]

In a follow up publication the cholesterol reduction effect was shown to be unrelated to the antioxidant properties of **1-Fe**.[99] It turned out that both **1-Fe** and its non-redox active analog **1-Ga** act as allosteric inhibitors of the enzyme that catalyzes the rate determining step of cholesterol biosynthesis, HMG-CoA reductase (HMGCR). **1-Mn** was much less effective in this respect, indicating the importance of the identity of the chelated ion for binding to the enzyme. The symmetrical porphyrin analog FeTPPS had no effect on HMGCR activity, pointing again at the amphipolarity of the corrole compounds as a pivotal factor. Indeed this new and exciting property of **1-Fe** opens new horizons and requires further investigation.

Safety, toxicity and cell/organ accumulation aspects of 1-Fe

Any efforts of proceeding from drug discovery to drug development must address toxicity issues. The concern of potential cytotoxicity of the transition metal complexes was addressed by 48 h incubation of macrophage cells (J774.A1) with the sulfonated metallocorroles. Cellular viability decreased only slightly with as much as 100 μM of **1-Fe** or **1-Mn**, while for the same incubation time **1-Ga** displayed an IC_{50} of 10 μM.[90] Incubation of the cells with a more reasonable 20 μM concentration of **1-Fe** for 24 h increased rather than decreased cellular viability by up to 150% relative to control cells, indicating that this compound actually protects the cells from the basal oxidative stress present in the cultured macrophages [54]. The luminol-based detection method was used for revealing the time course for internalization pattern of **1-Fe** into the cells. It was found that **1-Fe** continues to enter the cells during a 24 h incubation period, and also that the process is reversible for cells incubated in corrole-free medium.[90] For 2 h incubation, saturation was achieved with 100 μM of the compound in the culture media, at which the amount of intracellular **1-Fe** reached $6{\cdot}10^8$ molecules/cell.[54] This method could not be applied on **1-Mn** since this compound did not effectively catalyze the luminol oxidation reaction, and its cellular uptake could thus not be followed. Using fluorescence microscopy, the analogous compound **1-Ga** was found to accumulate in the cytoplasm and to be excluded from the nucleus.[90] One may assume this holds for **1-Fe** as well, but this hypothesis has not been proved yet.

A much more focused safety and toxicity study was recently reported for **1-Fe**,[95] which started by examining potential hERG inhibition by the compound. The hERG potassium channel is involved in coordinating the hearts beating, and is considered the main target for adverse drug-induced cardiac effects. Inhibition of the activity of this channel may lead to sudden death, and thus

drugs that inhibit the hERG channel will never reach the clinic. Drug candidates that display IC_{50}>10 μM are generally considered not to be cardiotoxic; and **1-Fe** passed this test as it displayed an IC_{50} of 22.7 μM. **1-Fe** was also evaluated regarding potential mutagenicity by the AMES test, and has shown to have no mutagenic potential in both its "native form" and its "metabolized form" (i.e. following its treatment with liver extract). General toxicology examinations in rats revealed that **1-Fe** was overall well tolerated at 30 and 100 mg/kg/day (oral gavage for 7 days), while mild adverse effects were observed only for treatment with 300 mg/kg/day and specifically in one rat. This dose is more than 10-fold higher than the doses of **1-Fe** that were used for therapeutic purposes.

Regulatory examinations that focus on pharmacokinetic and bio-distribution were not yet reported, so we are left to speculate that the strong interactions of **1-Fe** with serum lipoproteins will most likely affect the distribution of the compound between various tissues and organs *in vivo*. Progress for following organ distribution was reported for **1-Ga**, the fluorescent analog of **1-Fe**, by collecting specific organ from mice *ip* injected with 10 mg/kg **1-Ga**.[89] Fluoresce imaging revealed that the compound accumulated in the kidneys, liver, lungs, heart, and pancreas. It also reached the brain blood vessels, but apparently did not cross the blood brain barrier. This kind of information is of prime importance for deciding on which of the numerous diseases that are affected by oxidative stress the metallocorroles could display an optimal effect. Such an approach has been used for the anticancer effect of **1-Ga**, which has displayed preferred accumulation in breast cancer tumors when conjugated to a specific target-delivering protein.[85]

Conclusions and Future outlook

There may be no doubt about the large potential of metal-based catalytic oxidants as drug candidates for the numerous diseases where oxidative stress is heavily involved. It is hence quite surprising to realize that only one compound has apparently proceeded beyond pre-clinical trials so far.[100] Of the many possible reasons that come into account, we have emphasized the concern that the most commonly used iron and manganese complexes display both pro-oxidant and anti-oxidant properties. This may be illustrated by MnTM-4-PyP and MnTM-2-PyP by analyzing data from independent publications.[79, 101] MnTM-2-PyP reacts about 10 times faster than MnTM-4-PyP with peroxynitrite, but an investigation of the corresponding oxo-metal complexes revealed that (O)MnTM-2-PyP reacts 3 orders of magnitudes slower than (O)MnTM-4-PyP with a variety of organic and inorganic substrates. This clearly shows that the anti- vs. pro-oxidant activity of MnTM-2-PyP, but not of MnTM-4-PyP, is favourable for utilization as a catalytic antioxidant. Unfortunately, there are almost no reports that focused on the anti- vs. pro-oxidant potency of particular complexes in the same publication. We also note that despite of the large advances with imidazolium-substituted metalloporphyrins,[100] they still perform as poor as other porphyrins and salen complexes for the *catalytic* decomposition of H_2O_2.[48] On the other hand, there are recent reports where such complexes were used as pro-oxidant catalysts for oxidation of organic substrates by H_2O_2.[102] Even for the only complexes that decompose H_2O_2 without suffering from extensive bleaching, i.e., the iron corroles and manganese corrolazines,[41c, 103] detailed information about the selectivity towards H_2O_2 relative to other substrates is still incomplete. Gaining control about these two opposing features requires a great knowledge of their chemistry and realization that their dominancy might differ significantly in pure chemical environment relative to biological media (e.g., as displayed for **1-Mn**). This strongly depends on factors like association to proteins and the local concentration of naturally present reducing agents. Furthermore, broad range activity against the various ROS is needed for effectively avoiding oxidative damage. Compounds that display excellent $O_2^{\cdot-}$ dismutation activity, but are ineffective for decomposing the thus produced H_2O_2, are actually pro-oxidants. They promote the transformation of the mild $O_2^{\cdot-}$ toxin to the more damage-inducing H_2O_2. One recent report has in fact disclosed that the potent SOD mimics MnTE-2-PyP and MnTnHex-2-PyP actually reduce cellular viability under high $O_2^{\cdot-}$ concentrations, in cancer cells where such an effect is beneficial.[33b] An in depth analysis of the results obtained for MnTnHex-2-PyP and its iron analog in purely chemical, cellular, and *in vivo* investigations serves as an excellent demonstration of how the factors discussed in this assay come into play.[33b]

Despite of the fact that porphyrins are the most effective agents for both catalytic antioxidant therapy and photodynamic therapy (PDT),[38, 61, 104] issues of cellular uptake, modes of intracellular penetration, and the distribution within cells are much less studied for catalytic antioxidants than for PDT agents. The reason is that the main tool for those purposes is optical imaging, for which the photophysical properties of most PDT-relevant porphyrins (metal-free or chelates with Zn, Al or Pd) are ideal. On the contrary, these techniques cannot be utilized for the non-emitting manganese and iron complexes of neither porphyrins nor corroles. Method development efforts for cellular, *ex vivo* and *in vivo* determination of these complexes are hence highly desired and intensively explored.[33b, 54, 105] Toxicity studies, which are often considered as of low *scientific* interest, are of prime importance for drug development efforts. Fortunately, such information has started to appear for some of the discussed metalloporphyrins and the first animal trials that addressed potential toxicity issues of **1-Fe** were recently published.[95, 105b, 106] We look forward reading more about those issues for many other catalytic antioxidants.

Another aspect that is emphasized in this article is the distinction between **SAO**, **DCA**, and **ICA**, and how these different antioxidant classes act to neutralize ROS. It is important to realize that some **SAO** might be almost as damaging as ROS, and that the efficacy of **DCA** strongly depends on how reducing the environment is. Examples that testify for the limitations of **SAO** are the failure and even damage of antioxidant therapies by dietary supplements. One must also keep in mind quite a few variables that affect the performance of **DCA** in both advantageous and unfavourable fashions that were outlined in earlier paragraphs. We conclude by expressing our trust that the issues highlighted in this perspective article will raise the attention to both the practical and the scientific aspects that are required for advancing catalytic antioxidants towards clinical trials.

Acknowledgements

The KAMIN project by the Israel Ministry of Trading is acknowledged for supporting the ongoing pre-clinical investigations of corrole-based catalytic antioxidants.

Notes and references

[a] *Schulich Faculty of Chemistry, Technion – Israel Institute of Technology, Technion City, Haifa 32000, Israel. E-mail: adihaber@tx.technion.ac.il; Fax: 972 4829 5703; Tel: 972 4829 5977*
[b] *Schulich Faculty of Chemistry, Technion – Israel Institute of Technology, Technion City, Haifa 32000, Israel. E-mail: chr10zg@tx.technion.ac.il; Fax: 972 4829 5703; Tel: 972 4829 3954*
* Corresponding author, E-mail: chr10zg@tx.technion.ac.il

1(a) Y. J. Suzuki, H. J. Forman and A. Sevanian, *Free Radical Biol. Med.*, 1997, **22**, 269; (b) T. Finkel, *Curr. Opin. Cell Biol.*, 2003, **15**, 247.

2 T. Finkel and N. J. Holbrook, *Nature*, 2000, **408**, 239.

3(a) S. Man Son, M. Whalin, D. Harrison, W. Taylor and K. Griendling, *Current Diabetes Reports*, 2004, **4**, 247; (b) T. Finkel and N. J. Holbrook, *Nature*, 2000, **408**, 239; (c) R. Stocker and J. F. Keaney, *J. Thromb. Haem.*, 2005, **3**, 1825; (d) P. I. Moreira, S. L. Siedlak, G. Aliev, X. Zhu, A. D. Cash, M. A. Smith and G. Perry, *J. Neural Transmission*, 2005, **112**, 921.

4 S.-H. Lee, J.-I. Choi, S.-J. Heo, M.-H. Park, P.-J. Park, B.-T. Jeon, S.-K. Kim, J.-S. Han and Y.-J. Jeon, *Food Science and Biotechnology*, 2012, **21**, 239.

5 *Nat Rev Drug Discov*, 2009, **8**, 600.

6 J. M. McCord and M. A. Edeas, *Biomed. Pharmacother.*, 2005, **59**, 139.

7 J. S. Valentine, P. A. Doucette and S. Zittin Potter, *Annu. Rev. Biochem*, 2005, **74**, 563.

8 E. Kabashi, P. N. Valdmanis, P. Dion and G. A. Rouleau, *Ann. Neurol.*, 2007, **62**, 553.

9 S. A. Ezzi, M. Urushitani and J.-P. Julien, *J. Neurochem.*, 2007, **102**, 170.

10(a) S. R. Steinhubl, *Am. J. Cardiol.*, 2008, **101**, 14D; (b) G. Bjelakovic, D. Nikolova, L. L. Gluud, R. G. Simonetti and C. Gluud, *JAMA, J. Am. Med. Assoc.* , 2007, **297**, 842.

11(a) P. Burn, *Nat Rev Drug Discov*, 2010, **9**, 187; (b) C. Ortega-Camarillo, A. M. Guzmán-Grenfell, R. García-Macedo, A. M. Rosales-Torres, A. Ávalos-Rodríguez, G. Durán-Reyes, R. Medina-Navarro, M. Cruz, M. Díaz-Flores and J. Kumate, *Mol. Cell. Biochem.*, 2006, **281**, 163.

12(a) B. Holst and G. Williamson, *Curr. Opin. Biotechnol.*, 2008, **19**, 73; (b) A. Decker, *Nutr. Rev.*, 1997, **55**, 396; (c) I. M. C. M. Rietjens, M. G. Boersma, L. d. Haan, B. Spenkelink, H. M. Awad, N. H. P. Cnubben, J. J. van Zanden, H. v. d. Woude, G. M. Alink and J. H. Koeman, *Environ. Toxicol. Pharmacol.*, 2002, **11**, 321.

13 A. Haber, A. Mahammed, B. Fuhrman, N. Volkova, R. Coleman, T. Hayek, M. Aviram and Z. Gross, *Angew. Chem., Int. Ed.* , 2008, **47**, 7896.

14 S. Toppo, L. Flohé, F. Ursini, S. Vanin and M. Maiorino, *Biochimica et Biophysica Acta (BBA) - General Subjects*, 2009, **1790**, 1486.

15 J. Fujii and Y. Ikeda, *Redox Report*, 2002, **7**, 123.

16 H. L. Martin and P. Teismann, *The FASEB Journal*, 2009, **23**, 3263.

17(a) G. Li, Y. Chen, J. T. Saari and Y. J. Kang, *American Journal of Physiology - Heart and Circulatory Physiology*, 1997, **273**, H1090; (b) Y. S. Ho, R. Vincent, M. S. Dey, J. W. Slot and J. D. Crapo, *Am. J. Respir. Cell Mol. Biol.*, 1998, **18**, 538.

18(a) S. G. Shaffer, D. H. O'Neill and D. W. Thibeault, *J. Pediatr.*, 1987, **110**, 942; (b) S. G. Simonson, K. E. Welty-Wolf, Y. C. T. Huang, D. E. Taylor, S. P. Kantrow, M. S. Carraway, J. D. Crapo and C. A. Piantadosi, *J. Appl. Physiol.*, 1997, **83**, 550.

19 B. J. Day, *Drug Discovery Today*, 2004, **9**, 557.

20(a) J. Dawson, *Science*, 1988, **240**, 433; (b) T. L. Poulos, *J Biol Inorg Chem*, 1996, **1**, 356; (c) H. Li and T. L. Poulos, *Structure*, 1994, **2**, 461.

21 P. R. Ortiz de Montellano, *Cytochrome P450: Structure, Mechanism, and Biochemistry, Second Edition.*, Plenum, New York, N. Y., 1995.

22 P. Nicholls, *Arch. Biochem. Biophys.*, 2012, **525**, 95.

23 J. Vlasits, C. Jakopitsch, M. Bernroitner, M. Zamocky, P. G. Furtmüller and C. Obinger, *Arch. Biochem. Biophys.*, 2010, **500**, 74.

24(a) D. Mansuy, *Comptes Rendus Chimie*, 2007, **10**, 392; (b) W. Nam, *Acc. Chem. Res.*, 2007, **40**, 522.

25(a) I. Batinic-Haberle, Z. Rajic, A. Tovmasyan, J. S. Reboucas, X. Ye, K. W. Leong, M. W. Dewhirst, Z. Vujaskovic, L. Benov and I. Spasojevic, *Free Radical Biol. Med.*, 2011, **51**, 1035; (b) B. J. Day, *Biochem. Pharmacol.*, 2009, **77**, 285.

26 J. T. Groves, T. E. Nemo and R. S. Myers, *J. Am. Chem. Soc.*, 1979, **101**, 1032.

27 M. Grinstaff, M. Hill, J. Labinger and H. Gray, *Science*, 1994, **264**, 1311.

28 J. T. Groves and R. S. Myers, *Journal of the American Chemical Society*, 1983, **105**, 5791.

29 G. Simonneaux and P. Le Maux, *Coord. Chem. Rev.*, 2002, **228**, 43.

30(a) M. T. Reetz, *Angewandte Chemie International Edition*, 2011, **50**, 138; (b) M. Creus and T. R. Ward, *Organic & Biomolecular Chemistry*, 2007, **5**, 1835.

31 I. Nigel-Etinger, A. Mahammed and Z. Gross, *Catalysis Science & Technology*, 2011, **1**, 578.

32 R. F. Pasternack and B. Halliwell, *J. Am. Chem. Soc.*, 1979, **101**, 1026.

33(a) R. F. Pasternack, A. Banth, J. M. Pasternack and C. S. Johnson, *J. Inorg. Biochem.*, 1981, **15**, 261; (b) J. B. Aitken, E. L. Shearer, N. M. Giles, B. Lai, S. Vogt, J. S. Reboucas, I. Batinic-Haberle, P. A. Lay and G. I. Giles, *Inorg. Chem.*, 2013, **52**, 4121.

34 I. Batinić-Haberle, I. Spasojević, P. Hambright, L. Benov, A. L. Crumbliss and I. Fridovich, *Inorg. Chem.*, 1999, **38**, 4011.

35 M. F. Zipplies, W. A. Lee and T. C. Bruice, *J. Am. Chem. Soc.*, 1986, **108**, 4433.

36 I. Batinic-Haberle, L. Benov, I. Spasojevic and I. Fridovich, *J. Biol. Chem.*, 1998, **273**, 24521.

37(a) J. S. Reboucas, I. Spasojevic, D. H. Tjahjono, A. Richaud, F. Mendez, L. Benov and I. Batinic-Haberle, *Dalton Transactions*, 2008, **0**, 1233; (b) J. S. Rebouças, G. DeFreitas-Silva, I. Spasojević, Y. M. Idemori, L. Benov and I. Batinić-Haberle, *Free Radical Biol. Med.*, 2008, **45**, 201.

38 S. Miriyala, I. Spasojevic, A. Tovmasyan, D. Salvemini, Z. Vujaskovic, D. St. Clair and I. Batinic-Haberle, *Biochim. Biophys. Acta (BBA) - Molecular Basis of Disease*, 2012, **1822**, 794.

39(a) J. Lee, J. A. Hunt and J. T. Groves, *J. Am. Chem. Soc.*, 1998, **120**, 7493; (b) M. K. Stern, M. P. Jensen and K. Kramer, *J. Am. Chem. Soc.*, 1996, **118**, 8735.

40(a) G. Ferrer-Sueta, I. Batinić-Haberle, I. Spasojević, I. Fridovich and R. Radi, *Chem. Res. Toxicol.*, 1999, **12**, 442; (b) B. J. Day, I. Fridovich and J. D. Crapo, *Arch. Biochem. Biophys.*, 1997, **347**, 256.

41(a) M. Eckshtain, I. Zilbermann, A. Mahammed, I. Saltsman, Z. Okun, E. Maimon, H. Cohen, D. Meyerstein and Z. Gross, *Dalton Transactions*, 2009, 7879; (b) A. Mahammed and Z. Gross, *Angew. Chem. Int. Ed.*, 2006, **45**, 6544; (c) A. Mahammed and Z. Gross, *Chem. Comm.*, 2010, **46**, 7040.

42(a) K. Briviba, R. Kissner, W. H. Koppenol and H. Sies, *Chem. Res. Toxicol.*, 1998, **11**, 1398; (b) M. Dubuisson, D. Vander Stricht, A. Clippe, F. Etienne, T. Nauser, R. Kissner, W. H. Koppenol, J.-F. Rees and B. Knoops, *FEBS Lett.*, 2004, **571**, 161; (c) L. Flohé, G. Loschen, W. A. Günzler and E. Eichele, *Hoppe-Seyler's Z. Physiol. Chem.*, 1972, **353**, 987; (d) D. Klug, J. Rabani and I. Fridovich, *J. Biol. Chem.*, 1972, **247**, 4839; (e) B. Chance and N. Oshino, *Biochem J.*, 1971, **122**, 225.

43 M. P. Jensen and D. P. Riley, *Inorg. Chem.*, 2002, **41**, 4788.

44 I. Batinić-Haberle, J. S. Rebouças and I. Spasojević, *Antioxidants & Redox Signaling*, 2010, **13**, 877.

45 I. Batinic-Haberle, I. Spasojevic, R. D. Stevens, P. Hambright and I. Fridovich, *J. Chem. Soc., Dalton Trans.*, 2002, 2689.

46 T. Weitner and I. Batinic-Haberle, *ADMET & DMPK*, 2014, **2**, 185.

47 Z. Gershman, I. Goldberg and Z. Gross, *Angewandte Chemie International Edition*, 2007, **46**, 4320.

48 R. Kachadourian, C. A. Johnson, E. Min, I. Spasojevic and B. J. Day, *Biochem. Pharmacol.*, 2004, **67**, 77.

49 I. Kos, L. Benov, I. Spasojevic , J. S. Reboucas and I. Batinic-Haberle, *J. Med. Chem.*, 2009, **52**, 7868.

50 I. Kos, J. S. Rebouças, G. DeFreitas-Silva, D. Salvemini, Z. Vujaskovic, M. W. Dewhirst, I. Spasojević and I. Batinić-Haberle, *Free Radical Biol. Med.*, 2009, **47**, 72.

51(a) A. Okado-Matsumoto, I. Batinić-Haberle and I. Fridovich, *Free Radical Biol. Med.*, 2004, **37**, 401; (b) I. Batinić-Haberle, M. M. Ndengele, S. Cuzzocrea, J. S. Rebouças, I. Spasojević and D. Salvemini, *Free Radical Biol. Med.*, 2009, **46**, 212; (c) Z. Rajic, A. Tovmasyan, I. Spasojevic, H. Sheng, M. Lu, A. M. Li, E. B. Gralla, D. S. Warner, L. Benov and I. Batinic-Haberle, *Free Radical Biol. Med.*, 2012, **52**, 1828.

52 G. DeFreitas-Silva, J. S. Reboucas, I. Spasojevic, L. Benov, Y. M. Idemori and I. Batinic-Haberle, *Arch. Biochem. Biophys.*, 2008, **477**, 105.

53(a) R. F. Pasternack and W. R. Skowronek, *J. Inorg. Biochem.*, 1979, **11**, 261; (b) D. P. Riley, *Chem. Rev.*, 1999, **99**, 2573; (c) F. C. Friedel, D. Lieb and I. Ivanović-Burmazović, *J. Inorg. Biochem.*, 2012, **109**, 26.

54 A. Haber, M. Aviram and Z. Gross, *Inorg. Chem.*, 2012, **51**, 28.

55 G. Ferrer-Sueta and R. Radi, *ACS Chemical Biology*, 2009, **4**, 161.

56(a) P. Pacher, J. S. Beckman and L. Liaudet, *Physiol. Rev.*, 2007, **87**, 315; (b) C. Szabo, H. Ischiropoulos and R. Radi, *Nat. Rev. Drug. Discov.*, 2007, **6**, 662.

57 J. Lee, J. A. Hunt and J. T. Groves, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 2913.

58(a) T.-J. Wu, N. H. Khoo, F. Zhou, B. J. Day and D. A. Parks, *Free Radical Res.*, 2007, **41**, 127; (b) J. P. Crow, N. Y. Calingasan, J. Chen, J. L. Hill and M. F. Beal, *Ann. Neurol.*, 2005, **58**, 258.

59(a) K. Murata, R. Panicucci, E. Gopinath and T. C. Bruice, *J. Am. Chem. Soc.*, 1990, **112**, 6072; (b) T. G. Traylor and F. Xu, *J. Am. Chem. Soc.*, 1990, **112**, 178; (c) V. Lepentsiotis, R. van Eldik, F. F. Prinsloo and J. J. Pienaar, *J. Chem. Soc., Dalton Trans.*, 1999, 2759.

60 Y. Noritake, N. Umezawa, N. Kato and T. Higuchi, *Inorg. Chem.*, 2013, **52**, 3653.

61(a) I. Batinic-Haberle, I. Spasojevic, H. M. Tse, A. Tovmasyan, Z. Rajic, D. K. St. Clair, Z. Vujaskovic, M. W. Dewhirst and J. D. Piganelli, *Amino Acids*, 2012, **42**, 95; (b) A. Tovmasyan, H. Sheng, T. Weitner, A. Arulpragasam, M. Lu, D. S. Warner, Z. Vujaskovic, I. Spasojevic and I. Batinic-Haberle, *Medical principles and practice : international journal of the Kuwait University, Health Science Centre*, 2013, **22**, 103; (c) I. Batinic-Haberle, Z. Rajic, A. Tovmasyan, X. Ye, K. W. Leong, M. W. Dewhirst, Z. Vujaskovic, L. Benov and I. Spasojevic, *Free Radic. Biol. Med.*, 2011, **51**, 1035.

62(a) Z. N. Rabbani, F. K. Salahuddin, P. Yarmolenko, I. Batinic-Haberle, B. A. Thrasher, B. Gauter-Fleckenstein, M. W. Dewhirst, M. S. Anscher and Z. Vujaskovic, *Free Radical Res.*, 2007, **41**, 1273; (b) Z. N. Rabbani, I. Batinic-Haberle, M. S. Anscher, J. Huang, B. J. Day, E. Alexander, M. W. Dewhirst and Z. Vujaskovic, *International Journal of Radiation Oncology*Biology*Physics*, 2007, **67**, 573.

63(a) Z. Gross, N. Galili and I. Saltsman, *Angew. Chem., Int. Ed.* , 1999, **38**, 1427; (b) D. T. Gryko, *Chem. Com.*, 2000, **22**, 2243; (c) D. T. Gryko and K. Jadach, *J. Org. Chem.*, 2001, **66**, 4267; (d) D. T. Gryko and B. Koszarna, *Org. Biomol. Chem.* , 2003, **1**, 350.

64(a) I. Saltsman, A. Mahammed, I. Goldberg, E. Tkachenko, M. Botoshansky and Z. Gross, *J. Am. Chem. Soc.*, 2002, **124**, 7411; (b) A. Mahammed, I. Goldberg and Z. Gross, *Org. Lett.*, 2001, **3**, 3443.

65 Z. Gross and H. B. Gray, *Commen. Inorg. Chem.*, 2006, **27**, 61

66(a) A. Kumar, I. Goldberg, M. Botoshansky, Y. Buchman and Z. Gross, *J. Am. Chem. Soc.*, 2010, **132**, 15233; (b) R. K. Hocking, S. D. George, Z. Gross, F. A. Walker, K. O.

Hodgson, B. Hedman and E. I. Solomon, *Inorganic Chemistry*, 2009, **48**, 1678.

67(a) R. Zhang, D. N. Harischandra and M. Newcomb, *Chemistry – A European Journal*, 2005, **11**, 5713; (b) H.-Y. Liu, F. Yam, Y.-T. Xie, X.-Y. Li and C. K. Chang, *Journal of the American Chemical Society*, 2009, **131**, 12890; (c) R. S. Czernuszewicz, V. Mody, A. Czader, M. Gałęzowski and D. T. Gryko, *Journal of the American Chemical Society*, 2009, **131**, 14214; (d) H. Zhao, K. Pierloot, E. H. G. Langner, J. C. Swarts, J. Conradie and A. Ghosh, *Inorganic Chemistry*, 2012, **51**, 4002; (e) A. E. Meier-Callahan, H. B. Gray and Z. Gross, *Inorganic Chemistry*, 2000, **39**, 3605; (f) G. Golubkov and Z. Gross, *Journal of the American Chemical Society*, 2005, **127**, 3258; (g) *Angewandte Chemie International Edition*, 2003, **42**, 4507; (h) R. A. Eikey, S. I. Khan and M. M. Abu-Omar, *Angewandte Chemie International Edition*, 2002, **41**, 3591.

68(a) A. J. McGown, W. D. Kerber, H. Fujii and D. P. Goldberg, *Journal of the American Chemical Society*, 2009, **131**, 8040; (b) K. Cho, P. Leeladee, A. J. McGown, S. DeBeer and D. P. Goldberg, *Journal of the American Chemical Society*, 2012, **134**, 7392.

69(a) L. Simkhovich, I. Goldberg and Z. Gross, *Inorg. Chem.*, 2002, **41**, 5433; (b) I. Aviv-Harel and Z. Gross, *Chem. Eur. J.*, 2009, **15**, 8382.

70 J. Shen, M. El Ojaimi, M. Chkounda, C. P. Gros, J.-M. Barbe, J. Shao, R. Guilard and K. M. Kadish, *Inorganic Chemistry*, 2008, **47**, 7717.

71 Z. Okun and Z. Gross, *Inorg. Chem.*, 2012, **51**, 8083.

72 J. Lee, J. A. Hunt and J. T. Groves, *Bioinorg. Med. Chem. Lett.* , 1997, **7**, 2913.

73 J. T. Groves and S. S. Marla, *J. Am. Chem. Soc.*, 1995, **117**, 9578.

74 H. Gunaydin and K. N. Houk, *Chem. Res. Toxicol.*, 2009, **22**, 894.

75(a) L. Kupershmidt, Z. Okun, T. Amit, S. Mandel, I. Saltsman, A. Mahammed, O. Bar-Am, Z. Gross and M. B. H. Youdim, *J. Neurochem.*, 2010, **113**, 363; (b) Z. Okun, L. Kupershmidt, T. Amit, S. Mandel, O. Bar-Am, M. B. H. Youdim and Z. Gross, *ACS Chem. Biol.*, 2009, **4**, 910.

76 A. Mahammed and Z. Gross, *Catal. Sci. Technol.*, 2011, **1**, 535.

77 R. A. Sheldon, *Metalloporphyrins in Catalytic Oxidations*, Marcel Dekker, New York, 1994.

78(a) W. Nam, I. Kim, M. H. Lim, H. J. Choi, J. S. Lee and H. G. Jang, *Chemistry – A European Journal*, 2002, **8**, 2067; (b) N. Jin, D. e. E. Lahaye and J. T. Groves, *Inorganic Chemistry*, 2010, **49**, 11516.

79 N. Jin and J. T. Groves, *Journal of the American Chemical Society*, 1999, **121**, 2923.

80 J. Milano and B. J. Day, *Nucleic Acids Res.*, 2000, **28**, 968.

81 M. C. Jaramillo, M. M. Briehl, J. D. Crapo, I. Batinic-Haberle and M. E. Tome, *Free Radical Biol. Med.*, 2012, **52**, 1272.

82 A. Mahammed and Z. Gross, *J. Am. Chem. Soc.*, 2005, **127**, 2883.

83 N. Kasugai, T. Murase, T. Ohse, S. Nagaoka, H. Kawakami and S. Kubota, *J. Inorg. Biochem.*, 2002, **91**, 349.

84 H. Agadjanian, J. J. Weaver, A. Mahammed, A. Rentsendorj, S. Bass, J. Kim, I. J. Dmochowski, R. Margalit, H. B. Gray, Z. Gross and L. K. Medina-Kauwe, *Pharm. Res.*, 2006, **23**, 367.

85 H. Agadjanian, J. Ma, A. Rentsendorj, V. Valluripalli, J. Y. Hwang, A. Mahammed, D. L. Farkas, H. B. Gray, Z. Gross and L. K. Medina-Kauwe, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **106**, 6105.

86 J. Y. Hwang, J. Lubow, D. Chu, J. Ma, H. Agadjanian, J. Sims, H. B. Gray, Z. Gross, D. L. Farkas and L. K. Medina-Kauwe, *Mol. Pharm.*, 2011, **8**, 2233.

87 J. Y. Hwang, D. J. Lubow, D. Chu, J. Sims, F. Alonso-Valenteen, H. B. Gray, Z. Gross, D. L. Farkas and L. K. Medina-Kauwe, *J. Controlled Release*, 2012, **163**, 368.

88 P. Lim, A. Mahammed, Z. Okun, I. Saltsman, Z. Gross, H. B. Gray and J. Termini, *Chem. Res. Toxicol.*, 2011, **25**, 400.

89 Z. Okun, L. Kupershmidt, M. B. H. Youdim and Z. Gross, *Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry*, 2011, **11**, 380.

90 A. Haber, *Metallocorroles for attenuation of atherosclerosis*, Springer-Verlag Berlin-Heidelberg, 2012.

91(a) A. Haber, H. Agadjanian, L. K. Medina-Kauwe and Z. Gross, *J. Inorg. Biochem.* , 2008, **102**, 446; (b) A. Mahammed, H. B. Gray, J. J. Weaver, K. Sorasaenee and Z. Gross, *Bioconjug. Chem.*, 2004, **15**, 738.

92 A. Haber, M. Aviram and Z. Gross, *Chem. Sci.*, 2011, **2**, 295.

93 A. Kanamori, M.-M. Catrinescu, A. Mahammed, Z. Gross and L. A. Levin, *J. Neurochem.*, 2010, **114**, 488.

94 M.-M. Catrinescu, W. Chan, A. Mahammed, Z. Gross and L. A. Levin, *Exp. Eye Res.*, 2012, **97**, 31.

95 A. Haber, I. Angel, A. Mahammed and Z. Gross, *J. Diabetes Complicat.*, 2013, DOI: 10.1016/j.jdiacomp.2013.02.005.

96 M. Aviram and M. Rosenblat, in *Redox Genome Interactions in Health and Disease.* , eds. J. Fuchs, M. Podda and L. Packer, Marcel Dekker, NY, 2004, pp. 557 – 590

97(a) M. Aviram and B. Fuhrman, *Mol. Cell. Biochem.*, 1998, **188**, 149; (b) I. Maor, T. Hayek, R. Coleman and M. Aviram, *Arterioscler. Thromb. Vasc. Biol.*, 1997, **17**, 2995.

98(a) M. J. Chapman, *Pharmacol. Ther.*, 2006, **111**, 893; (b) G. Ferretti, T. Bacchetti, A. Nȕgre-Salvayre, R. Salvayre, N. Dousset and G. Curatola, *Atherosclerosis*, 2006, **184**, 1.

99 S. DeWeerdt, *Nature*, 2012, **485**, S4.

100 T. McGovern, B. J. Day, C. W. White, W. S. Powell and J. G. Martin, *Free Radical Biol. Med.*, 2011, **50**, 602.

101 F. De Angelis, N. Jin, R. Car and J. T. Groves, *Inorganic Chemistry*, 2006, **45**, 4268.

102(a) H. Srour, P. L. Maux and G. Simonneaux, *Inorganic Chemistry*, 2012, **51**, 5850; (b) R. D. Paula, M. M. Q. Simões, M. G. P. M. S. Neves and J. A. S. Cavaleiro, *Catal. Commun.*, 2008, **10**, 57; (c) R. De Paula, M. M. Q. Simões, M. G. P. M. S. Neves and J. A. S. Cavaleiro, *J. Mol. Catal. A: Chem.*, 2011, **345**, 1.

103 D. E. Lansky, A. A. Narducci Sarjeant and D. P. Goldberg, *Angewandte Chemie International Edition*, 2006, **45**, 8214.

104(a) M. Ethirajan, Y. Chen, P. Joshi and R. K. Pandey, *Chem. Soc. Rev.*, 2011, **40**, 340; (b) L. B. Josefsen and R. W. Boyle, *Theranostics*, 2012, **2**, 916.

105(a) I. Spasojevic, Y. Chen, T. J. Noel, P. Fan, L. Zhang, J. S. Rebouȕas, D. K. St. Clair and I. Batinic-Haberle, *Free Radical Biol. Med.*, 2008, **45**, 943; (b) T. Weitner, I. Kos, H. Sheng,

A. Tovmasyan, J. S. Reboucas, P. Fan, D. S. Warner, Z. Vujaskovic, I. Batinic-Haberle and I. Spasojevic, *Free Radical Biol. Med.*, 2013, **58**, 73; (c) V. Mouraviev, T. N. Venkatraman, A. Tovmasyan, M. Kimura, M. Tsivian, V. Mouravieva, T. J. Polascik, H. Wang, T. J. Amrhein, I. Batinic-Haberle and C. Lascola, *J. Endourol.*, 2012, **26**, 1420; (d) T. Lee, X.-a. Zhang, S. Dhar, H. Faas, S. J. Lippard and A. Jasanoff, *Chem. Biol.*, 2010, **17**, 665.

106 S. C. Gad, D. W. Sullivan, Jr., J. D. Crapo and C. B. Spainhour, *Int J Toxicol*, 2013, **32**, 274.

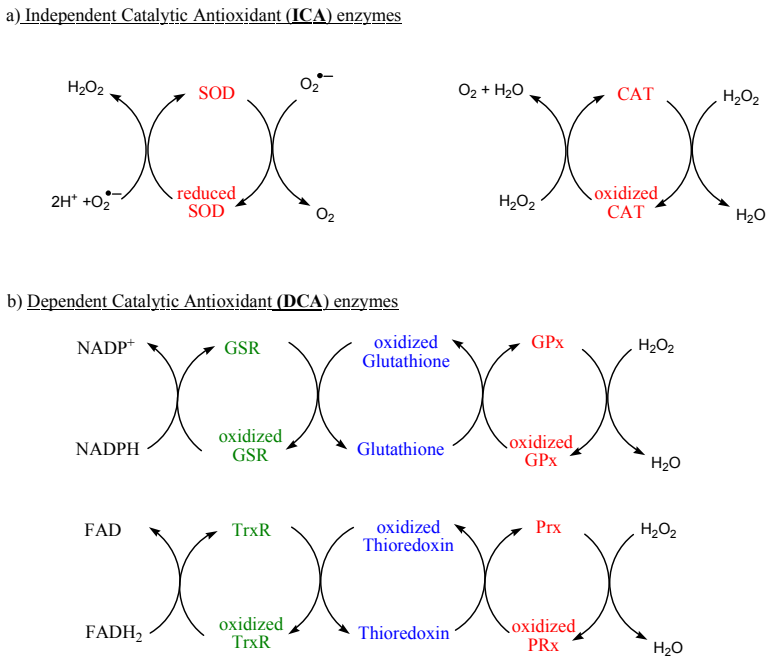


Fig. 1 The mechanism of: (a) ICA enzymes exemplified by the catalytic cycles of CAT and SOD, whereby the corresponding ROS are catalytically decomposed without the involvement of any other cofactor; and (b) DCA enzymes, exemplified by the catalytic cycles of GPx and Prx, that require the aid of a SAO, glutathione and thioredoxin, respectively. The SAO are then recycled by the corresponding enzymes, glutathione reductase (GSR) and thioredoxin reductase (TrxR), which are themselves recycled by other SAO.

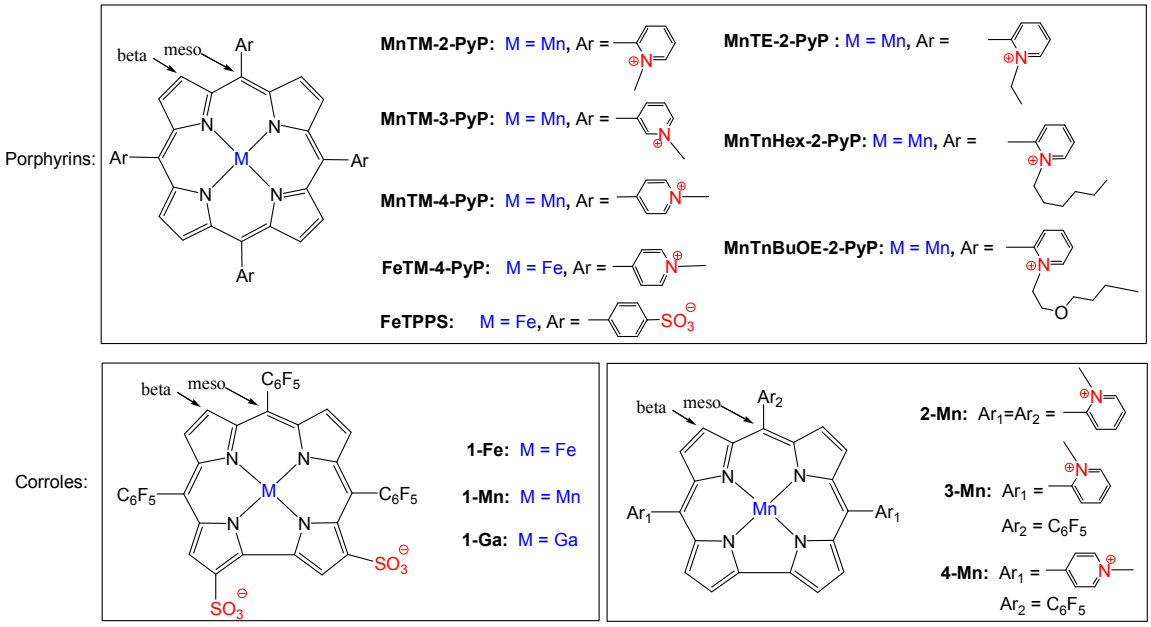


Fig. 2 Chemical structures of the most investigated porphyrin- and corrole-based catalytic antioxidants. The β-pyrrole and *meso* positions are marked.

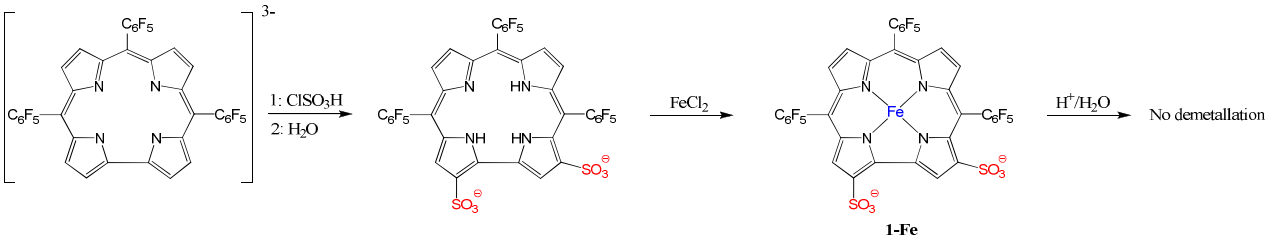


Fig. 3 Facile synthesis of amphipolar metallocorroles. The corrole is non-symmetrical, allowing for selective substitution to produce amphipolar derivatives such as the bis-sulfonated corrole. The iron ion is tightly bound within this strong tri-anionic ligand, thus iron(III) corroles are not demetallated under acidic conditions.

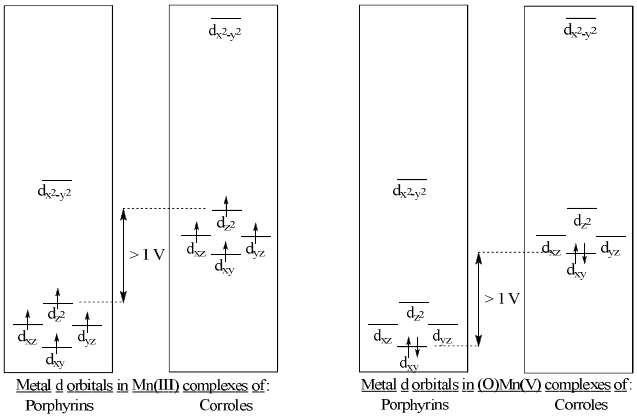


Fig. 4 The relative energy of metal d-orbitals in metalloporphyrins and metallocorroles. The corrole is a very strong σ donor, elevating the metal d orbitals of metallocorroles by more than 1 V relative to those of metalloporphyrins. The consequences are demonstrated for: a) Mn(III) (high spin d^4, left panel), as to show that metallocorroles are much stronger reducing agents because their electrons are in very high energy d orbitals; and b) (O)Mn(V) (low spin d^2, right panel), for illustrating that corrole chelation stabilizes this high oxidation state with regard to reduction by easily oxidized substrates.

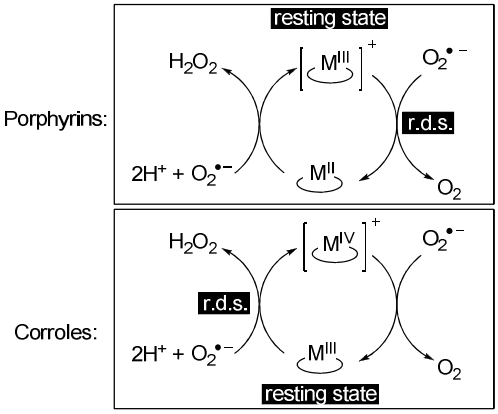


Fig. 5 Mechanisms for superoxide radical decomposition by porphyrin and corrole metal complexes, with M = Mn or Fe. The net reaction outcome is identical (formation of H_2O_2 and O_2) and the resting state is +3 in both cases. Differences are that: a) the rate-limiting step is $O_2^{\cdot-}$ oxidation for metalloporphyrins and $O_2^{\cdot-}$ reduction for metallocorroles; and b) during catalysis, the metalloporphyrins shuttle between +2/+3 and the metallocorroles between +3/+4 oxidation states.

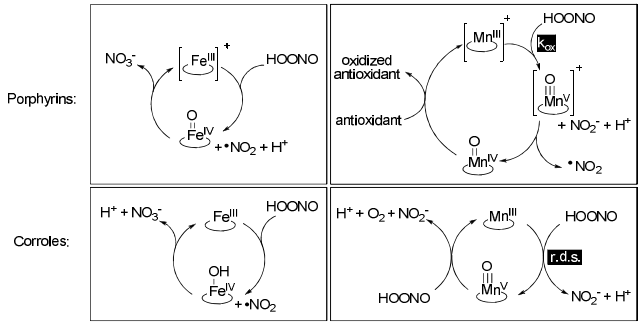


Fig. 6 Catalytic cycles for peroxynitrite decomposition by porphyrin and corrole metal complexes: The iron(III) porphyrins and corroles share the same mechanism of peroxynitrite isomerization. Both manganese(III) complexes form a (O)Mn(V) intermediate, which in the case of porphyrins reacts immediately with co-formed nitrite as to lead to NO_2 and a long-lived (O)Mn(IV) species that is inert to peroxynitrite. A full catalytic cycle is completed *only* in the presence of SAO. The (O)Mn(V) corrole intermediates are not reduced by nitrite, are much more long-lived, do react with another peroxynitrite molecule, and complete a full catalytic cycle even in the absence of any co-reductant. The net outcome is disproportionation of peroxynitrite to oxygen and nitrite. Note that the Mn-oxygen bond order is 3 for Mn(V) and 2.5 for Mn(IV).

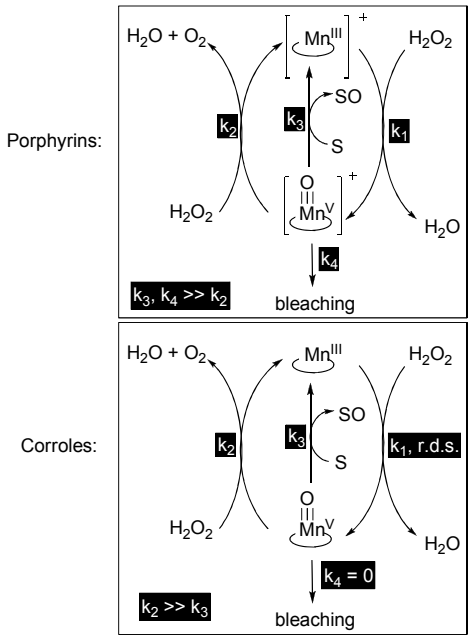


Fig. 7 Plausible mechanism for catalytic hydrogen peroxide decomposition by porphyrin and corrole manganese(III) complexes. The first step is similar and leads to the formation of a (O)Mn(V) intermediate in both cases. Bleaching and/or oxidation of a substrate are the main routes of reaction for the porphyrin-chelated intermediate, whereas (O)Mn(V) corroles react preferably with a second H_2O_2 molecule and are not bleached. S = substrate and SO = oxidized substrate, where S could be either a non-catalytic antioxidant (leading to antioxidant activity) or a vital biomolecule (resulting in pro-oxidative activity). Note that the Mn-oxygen bond order is 3 for Mn(V), as indicated in the drawing.

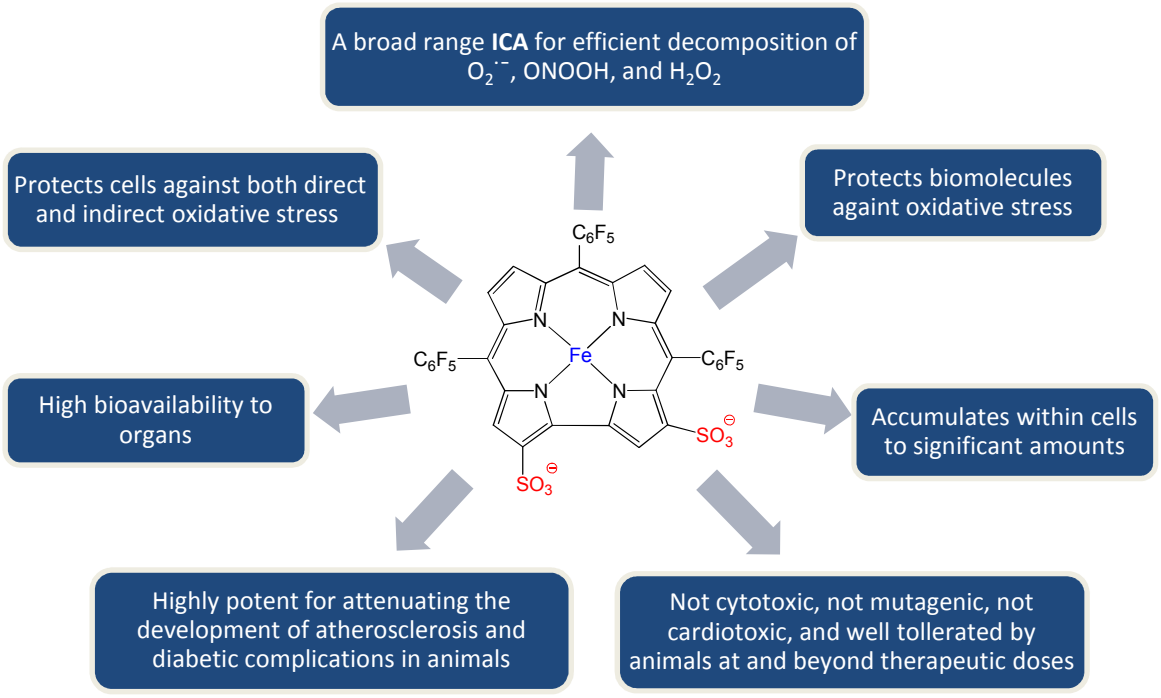


Figure 8. The beneficial effects of **1-Fe**, the most investigated metallocorrole regarding medicinal applications.