CHAPTER 1

Peroxynitrite: The Basics

WILLEM H. KOPPENOL*[a]

[a]Institute of Inorganic Chemistry, Department of Chemistry and Applied Biosciences, Swiss Federal Institute of Technology, Zürich 8093, Switzerland
*E-mail: koppenol@inorg.chem.ethz.ch

1.1 History

1.1.1 Before 1990

In this section, I will focus on the early discovery of peroxynitrite, its ability to nitrate aromatic compounds, its sensitivity to carbon dioxide and early determination with permanganate.

More than a 100 years ago, Baeyer and Villiger[1] proposed that a "Nitrosopersäure" (ROONO) was formed as an intermediate in reactions of nitrite, ethyl nitrite, and amyl nitrite (R^1ONO) with hydrogen peroxide and ethyl hydroperoxide (R^2OOH). They came to the conclusion that an adduct between R^1ONO and R^2OOH was formed that yielded R^1OH and R^2ONO_2. Had a direct oxidation of the nitrite taken place, R^1ONO_2 and R^2OH would have been the products. In the case of the reaction between nitrous acid and hydrogen peroxide, nitrate (NO_3^-) is ultimately formed and the accompanying formula shows an adduct between peroxynitrous acid and water.[1] Although they did not provide direct evidence for the structure of peroxynitrous acid, we may credit them with the discovery of this reactive species. In 1907, Raschig[2] prepared a solution of bromide in hydrogen peroxide and one of bromide in nitrous acid. Both solutions stayed clear, but upon mixing, dibromine was formed as

RSC Detection Science Series No. 7
Peroxynitrite Detection in Biological Media: Challenges and Advances
Edited by Serban F. Peteu, Sabine Szunerits, and Mekki Bayachou

Published by the Royal Society of Chemistry, www.rsc.org

deduced from the reddish-brown color and the smell. He assumed that two hydrogen peroxide molecules reacted with one nitrous acid and proposed the formula HNO_4 and the name "Übersalpetersäure"; apparently he was not aware of the publication of Baeyer and Villiger.[1] In 1922, Trifonow[3,4] explored the properties of the short-lived product of the reaction between nitrous acid and hydrogen peroxide for analytical purposes. He concluded that "naszente Persalpetersäure" (nitric acid *in statu nascendi*) is capable of oxidizing aniline and nitrating aromatic compounds. He proposed using these reactions for the detection of nitrite and aromatic compounds due to the intensively colored products. In 1929, Gleu and Roell[5] reported that the reaction of azide with ozone results in a deep orange–red solution that smells of hypochlorite and sometimes nitrogen dioxide. Although stable in alkaline solution, the color disappears rapidly upon neutralization by addition of hydrogen carbonate, or by lowering of the pH. In spite of considerable efforts, they were unable to isolate the new unstable compound, but their experiments allowed them to exclude hydrogen peroxide as the oxidant, and they concluded that they were dealing with peroxynitrous acid. Gleu and Hubold described in their paper of 1935 a simple synthesis of peroxynitrite from hydrogen peroxide and nitrite at low pH: one mixes nitrite and hydrogen peroxide, adds acid followed by base within 2 s. If done correctly, the deep yellow color of peroxynitrite is observed.[6] Use of a quenched-flow reactor improves the yield.[7] Kortüm and Finckh[8] showed in 1941 that the absorption maximum of peroxynitrite anion ($ONOO^-$) in the UV ultraviolet range is close to that of NO_3^-, but the intensity is about 15 times higher and the band is much broader. The initiation of the polymerization of methyl acrylate and the hydroxylation and nitration of aromatic compounds by peroxynitrous acid, reported in 1952 by Halfpenny and Robinson,[9] was rationalized in terms of homolysis of the O–O bond in ONOOH. However, in 1954, Anbar and Taube[10] studied the reactions of peroxynitrite labeled with two O^{18}, and found doubly labeled NO_3^- as a product of intramolecular rearrangement of peroxynitrite and, in the presence of an excess of unlabeled nitrite, singly labeled nitrite and nitrate as products of O^{18} transfer from peroxynitrite to nitrite, a result that is not easily explained by homolysis. The first kinetics study of peroxynitrous acid involving its isomerization to NO_3^- appeared in 1962 [11] and the second in 1969.[12] The latter gives a pK_a of 6.6 and a rate of isomerization of 0.10 s^{-1}, obtained at a temperature of 1 °C and an ionic strength of 0.5 M. It also mentioned that peroxynitrite vanishes quickly in the presence of carbonate or borate. Formation of peroxynitrite from nitrate in solution by ultraviolet light was demonstrated in 1964.[13] The same study also showed that permanganate oxidized peroxynitrite.[13] Hughes and Nicklin reported in 1968 the extinction coefficient of peroxynitrite at 302 nm of 1670 ± 50 M^{-1} cm^{-1},[14] which is within the error of that obtained by Bohle and coworkers in 1994 with pure tetramethylammonium peroxynitrite, 1705 ± 10 M^{-1} cm^{-1}.[15] Blough and Zafiriou made a very important observation in 1985 of a yellow color after mixing a mostly anaerobic alkaline solution of superoxide with that of nitrogen monoxide. They concluded that superoxide and nitrogen monoxide react to form peroxynitrite.[16]

As nitrogen monoxide was identified in 1987 as an "endothelium-derived relaxing factor",[17,18] and SOD extends the life of nitrogen monoxide,[19] the finding of Blough and Zafiriou could be relevant to physiology!

We see that a few properties and reactions that have been discovered can be used to quantitate peroxynitrite: its yellow color, its ability to nitrate aromatic compounds and the reduction of purple permanganate to green manganate. An excellent review on the "older" chemistry of peroxynitrite by Edwards and Plumb appeared in 1993.[20] It also discusses the role of peroxynitrite in atmospheric chemistry.

From 1901 until 1990, *ca.* 40 papers on peroxynitrite appeared. Since then, the number of publications has sharply increased to well over 12 000 as a recent search (November 2014) on the Web of Science showed.

1.1.2 1990 and Later

Up to 1990, oxidative injury to tissues was explained with a modification of the Haber–Weiss cycle that included catalysis by iron.[21] In essence, the presence of iron(II) and hydrogen peroxide was postulated; injury was caused by the Fenton reaction,[22] which, at neutral pH, yields the hydroxyl radical[23] or a higher oxidation state of iron,[24] as reviewed by Koppenol and Bounds.[25] However, Beckman *et al.*[26] pointed out that "generation of strong oxidants by the iron-catalyzed Haber–Weiss reaction is not an entirely satisfactory explanation for superoxide dismutase (SOD)-inhibitable injury *in vivo*." Since SOD protects, superoxide must be damaging itself—which it is not[27]—or react to form a more reactive species. Instead of a reaction between some undefined iron(III) complex and superoxide, Beckman *et al.*,[26] referring to the work of Blough and Zafiriou,[16] proposed that superoxide reacts very quickly with nitrogen monoxide to form peroxynitrite. As the reaction in question is a radical–radical reaction, this assumption is quite reasonable. Thus, formation of peroxynitrous acid is kinetically far more feasible than the "iron-catalyzed Haber–Weiss reaction".[26] Beckman *et al.* also described some scavenger studies to find out whether peroxynitrous acid underwent homolysis to produce nitrogen dioxide and hydroxyl radicals. They concluded that "a potent oxidant similar to $HO^{\bullet}$ in reactivity" was formed.[26] Furthermore, they pointed out that peroxynitrous acid, being long-lived, can diffuse over several cell diameters. For that reason it is a more selective and toxic oxidant than the hydroxyl radical. It is of importance that it also nitrates aromatic compounds. The question of whether nitrogen monoxide and superoxide production under pathological conditions could be high enough to generate peroxynitrite was posed,[26] and we now know that this is so.

First, what are the basics of peroxynitrite chemistry? When it became clear in the early 1990s that peroxynitrite is biologically relevant, we prepared an overview of its thermodynamic and kinetic properties.[28] Much of what we published is still relevant, but some important details have changed. Nevertheless, the paper is still being cited and has now garnered over 1000 citations. Peroxynitrous acid isomerizes with a rate constant of 1.1 s^{-1}. Earlier

and slightly higher rate constants have been reviewed.[29] The pK_a of peroxynitrous acid is 6.5 to 6.8, depending on the ionic strength and temperature.[30] The peroxynitrite anion is fairly stable, but at pH values equal to the pK_a and higher, there is decomposition to nitrite and dioxygen, a reaction that proceeds *via* an adduct between peroxynitrite and peroxynitrous acid.[29,31,32] The spectrum of the anion has a broad maximum at 302 nm (ε = 1705 ± 10 M^{-1} cm^{-1}).[15] Peroxynitrous acid is a powerful one- and two-electron oxidizing agent; the calculated one- and two-electrode potentials are, respectively: $E^{\circ\prime}$(ONOOH, $H^+/NO_2^{\bullet}$, H_2O) = +1.6 ± 0.1 V and $E^{\circ\prime}$(ONOOH, H^+/NO_2^-, H_2O) = +1.3 ± 0.1 V, both at pH 7.[30] These electrode potentials, as all others, are given relative to the normal hydrogen electrode. Experimentally, a one-electron electrode potential over *ca.* +1 V[33] was measured by cyclic voltammetry in a cell in which alkaline peroxynitrite was repeatedly mixed with acid to generate the short-lived peroxynitrous acid ($t_{1/2}$ = 0.63 s at 25 °C). The lack of pH dependence indicates that a transient $HOONO^{\bullet -}$ was formed. It is clear that peroxynitrous acid is a potent one-electron oxidant. The notion that 30% of peroxynitrous acid undergoes homolysis to nitrogen dioxide and the hydroxyl radical is widespread in the literature.[34] We reviewed all evidence, pro and contra, extensively with the conclusion that, if any, homolysis is limited to at most 5%.[35] It may be appropriate to illustrate this point with an example. Above, decomposition to nitrite and dioxygen was mentioned. One can, in principle, explain these products by two homolyses:[36–38]

$$ONOOH \rightarrow NO_2^{\bullet} + HO^{\bullet} \quad (1.1)$$

$$ONOO^- \rightarrow NO^{\bullet} + O_2^{\bullet -} \quad (1.2)$$

followed by:

$$NO^{\bullet} + NO_2^{\bullet} \rightarrow N_2O_3\,(+\,H_2O) \rightarrow 2NO_2^- + 2H^+ \quad (1.3)$$

$$HO^{\bullet} + H^+ + O_2^{\bullet -} \rightarrow H_2O + O_2 \quad (1.4)$$

If this is so, then the *rate* of nitrite and dioxygen formation is limited by the slowest process, which is the reaction in eqn (1.2), that is, it will not depend on the concentration of peroxynitrite.[38] The rate constant of the reaction in eqn (1.2), as determined by the reduction of permanganate by superoxide, is 0.020 s^{-1}.[39] Furthermore, the *relative yield* of nitrite and dioxygen should also not depend on the peroxynitrite concentration. However, experimentally, we found that both the rate and the relative yield increase with the peroxynitrite concentration.[31,32,40] Furthermore, we showed that peroxynitrate is an intermediate:[32]

$$ONOOH + ONOO^- \rightarrow O_2NOO^- + NO_2^- + H^+ \quad (1.5)$$

which itself decomposes:

$$O_2NOO^- \rightarrow NO_2^- + O_2 \quad (1.6)$$

Thus, we concluded that peroxynitrous acid behaves like other peracids.[35]

Biochemically far more important is the reaction with carbon dioxide. In 1993, Radi's group rediscovered that peroxynitrite is not stable in the

presence of hydrogen carbonate.[41] Two years later, Lymar and Hurst[42] found that the peroxynitrite anion reacts rapidly with dissolved carbon dioxide, which leads to $ONOOCO_2^-$, nitrosoperoxycarbonate or 1-carboxylato-2-nitrosodioxidane. This compound itself, or its homolysis products, nitrogen dioxide and trioxidocarbonate(·−)[43,44] are strongly oxidizing: $E^\circ(NO_2^\bullet/NO_2^-)$ = +1.04 V and $E^\circ(CO_3^{\bullet-}/CO_3^{2-})$ = +1.57 V.[45] Although the extent of homolysis is generally assumed to be *ca.* 30%, we estimated it, using nitrogen monoxide as a scavenger of nitrogen dioxide and trioxidocarbonate(·−), at *ca.* 4%.[46] Thus, peroxynitrite, itself an oxidant with an electrode potential greater than +1 V, may cause the formation of equally strong, or even stronger, oxidants. Reduction of peroxynitrite on a microelectrode with modified surfaces has been used to detect it in biological samples.[47,48] In 2001, Amatore and coworkers showed that it is possible to oxidize peroxynitrite at a potential of +0.5 V,[49] quite close to the potential of +0.44 V estimated in 1992.[28] This method has been used to detect and quantify peroxynitrite in single fibroblasts[49] and macrophages.[50] Polymerized hemin on a carbon electrode has also been used to oxidize, and thereby, detect peroxynitrite.[51] Chemiluminescence has been used to detect peroxynitrite as well. This technique is sensitive, but in the case of dichlorodihydrofluorescein and dihydrorhodamine, the reaction is zero-order in the indicator molecule,[52] which detracts from its usefulness. Detection based on fluorescence is often not specific for a particular oxidant, and great care should be taken in the interpretation of results.[53,54] A direct reaction has been established for weakly fluorescent boronates that become strongly fluorescent after reaction with peroxynitrite.[55,56] As these compounds also react with hydrogen peroxide, there is again the issue of specificity. It is not important that the reaction of boronate with peroxynitrite is much faster than with hydrogen peroxide; it is the product of the rate constant and concentration that counts. In the following chapters, much more will be said about these techniques.

Tetramethylammonium peroxynitrite crystallizes in the *cis*-conformation[57] and Raman spectroscopy studies indicate that this is also the conformation in solution.[58]

1.2 Peroxynitrite *In Vivo*

Can peroxynitrite be formed *in vivo*? Under most conditions, superoxide is disposed of by Cu,Zn-SOD, present in the cytosol at a concentration of *ca.* 10 μM. Is that enough to prevent the formation of peroxynitrite? Let us assume that the concentration of nitrogen monoxide is *ca.* 10 nM. If we compare the products of the rate constant of superoxide with Cu,Zn-SOD and with nitrogen monoxide ($k_{SOD} = 2 \times 10^9$ M^{-1} s^{-1},[59,60] $k_{NO^\bullet} = 1.6 \times 10^{10}$ M^{-1} s^{-1},[61] respectively), and the concentrations just mentioned, we get 2×10^4 s^{-1} and 1.6×10^2 s^{-1}, respectively. Thus only 1% of superoxide ends up as peroxynitrite. However, near activated macrophages the concentration of nitrogen monoxide may be in the micromolar region, say 10 μM, and in that case *ca.* 90% of all superoxide is converted to peroxynitrite. This calculation is simplistic, but it illustrates why the SOD concentration has to be 5–10 μM. There

is another reason why SOD is essential. Although superoxide reacts quite quickly with its protonated form, this reaction is second order in superoxide: the initial phase of the reaction is very fast but the end stage proceeds very slowly, and a concentration of zero superoxide is "never" reached. It is the reaction with SOD, which is first order in both superoxide and in proteins, that finishes off superoxide.

We have now established that formation of peroxynitrite *in vivo* is kinetically feasible. Evidence for its formation in activated macrophages was detected by its ability to nitrate tyrosine 108 of Cu,Zn-SOD.[62,63] At that time it was also shown that peroxynitrous acid oxidized thiols[64] and lipids.[65] Furthermore, peroxynitrous acid not only nitrates tyrosine, it also hydroxylates it.[66] In atherosclerotic tissue, nitrotyrosine was detected by immunohistochemistry,[67] which is an important observation as it shows that peroxynitrite plays a role in that disease. Antibodies against nitrotyrosine are now commercially available. Furthermore, nitrotyrosine can be detected by high-performance liquid chromatography.[66] Interestingly, the nitration of tyrosine is a reaction that is zero order in tyrosine, and the yield is low,[66] and only somewhat higher in the presence of carbon dioxide. By contrast, tryptophan is nitrated in a bimolecular reaction, but with a low rate constant.[68,69] To the best of my knowledge, no immunological detection method for nitrated tryptophan has been developed.

As shown in a review I co-authored with Beckman,[70] the bad properties of nitrogen monoxide are nearly all those of peroxynitrite. In the title of that review, we refer to nitrogen monoxide, superoxide and peroxynitrite as the good, the bad and the ugly. Even before endothelium-derived relaxing factor was identified as nitrogen monoxide, it was known that superoxide removed it.[19] However, it is not just the removal of a signaling molecule that is important, the reaction product is a powerful oxidizing and nitrating agent. While formation of peroxynitrous acid is thus harmful under normal conditions, it is used by activated macrophages to remove microorganisms and other inflammatory insults. For the same reason, activated neutrophils make another inorganic compound, bleach or oxidochlorate(1−).[71] Thus, anywhere inflammation occurs, one may expect to find oxidized, nitrated and chlorinated biomolecules. Unsurprisingly, there is a large number of diseases where formation of peroxynitrite has been established.[72]

1.3 Challenges to the Detection of Peroxynitrite

For the development of a sensor it is necessary to test it with pure peroxynitrite. As mentioned above, peroxynitrite can be synthesized by mixing hydrogen peroxide with nitrous acid, followed by rapid quenching with base.[6,7] Oxygenation of hydroxylamine,[73] ozonization of an azide solution[5,74] and treating solid potassium superoxide with nitrogen monoxide[75] have also been used. None of these syntheses results in the preparation of a pure product: common contaminants are the decay products nitrite and nitrate, and, dependent on the synthesis, remaining reactants, nitrite, hydroxylamine,

azide and hydrogen peroxide. *In vivo* one may encounter hydrogen peroxide and nitrite. Only biomimetic synthesis in ammonia reported by Bohle and coworkers[15,76] results in a pure preparation of tetramethylammonium peroxynitrite. Contaminants are important because they may interfere with the detection of peroxynitrite; their presence requires control experiments.

Detection of the "footprint" of peroxynitrite, nitrated tyrosine, is well-established. Although useful for diagnostic purposes, this method cannot be used for time-resolved detection in cells or tissues. The remaining techniques are fluorescence and electrochemistry. Sensitivity and selectivity are important issues. The steady-state concentration will be very low, presumably in the nanomolar region. As one would like to determine the target of peroxynitrite in cells or tissues, detection should not disturb that concentration, that is, not more than 10% of the peroxynitrite should react with the reporter molecule or with the electrode. The issue of selectivity has been brought up for methods based on fluorescence,[53] but it applies equally to electrochemical detections. The importance of reliable methods to detect peroxynitrite in tissues and biological samples has recently been stressed by Chen *et al.*[77]

1.4 Nomenclature

For reasons unknown, many working in the field of radical research use the abbreviations ROS and RNS, which stand for "reactive oxygen species" and "reactive nitrogen species", respectively. Generally, superoxide, the hydroxyl radical, singlet dioxygen and hydrogen peroxide are "ROS", and nitrogen monoxide, nitrogen dioxide and peroxynitrite belong to "RNS". However, as already discussed, superoxide and nitrogen monoxide are not reactive, and neither is hydrogen peroxide. These acronyms are thus very misleading. In 2013, a commentary in the *Biophysical Journal* contained the sentence: "Superoxide can be quite damaging because it has an extremely high affinity for electrons, ripping them away from nearby proteins, lipids, and nucleic acids *via* oxidation."[78] In the 30 year old compilation of rate constants for reactions of superoxide by Bielski and coworkers[27] one finds that superoxide does not react with amino acids. It behaves as a mild reductant, while its hydronated form may act as an oxidant. By addressing these species collectively as ROS and RNS one ignores the efforts of many chemists that have characterized the different thermodynamic and kinetic properties of these molecules. The use of these acronyms has also been lamented in a recent editorial in *Free Radical Biology and Medicine*.[54] Furthermore, these species have their own names that should be used: for $O_2^{\bullet -}$ one can still use the venerable name superoxide; the systematic name is dioxide(·1−). For other species named in this chapter the names are, with the allowed one in italics: O_2, dioxygen; $HO_2^{\bullet}$, hydrogen dioxide or hydridodioxygen(·); H_2O_2, *hydrogen peroxide*, dihydridodioxygen or dioxidane; $HO^{\bullet}$, *hydroxyl radical*, hydridooxygen(·) or oxidanyl; $NO^{\bullet}$, nitrogen monoxide or oxidonitrogen(·); $NO_2^{\bullet}$, nitrogen dioxide or dioxidonitrogen(·); $ONOO^-$, *peroxynitrite* or (dioxido)oxidonitrate(1−); ONOOH, *peroxynitrous acid* or (hydridodioxido)oxidonitrogen; $ONOOH^{\bullet -}$,

(hydridodioxido)oxidonitrate(·1−); O_2NOO^- *peroxynitrate* or (dioxido)dioxidonitrate(1−); $CO_3^{\bullet -}$, trioxidocarbonate(1·−); $ONOOCO_2^-$, nitrosoperoxycarbonate or 1-carboxylato-2-nitrosodioxidane.[79,80] The reader will have noticed that the systematic names are based on the selection of a central atom and that the attached atoms are named as negatively charged groups (even if they are not). Furthermore, one can often use names based on -ane nomenclature: methane for CH_4, oxidane for H_2O, azane for NH_3, *etc.* The advantage of the new nomenclature recommendations is that the name tells you what the chemical composition is. Names such as nitric oxide or nitrous oxide do not do that; use of that nomenclature leads to the name "pernitric oxide" for $NO_2^{\bullet}$, which nobody uses, and there would be no name for $NO_3^{\bullet}$.

Acknowledgements

Research on peroxynitrite has been supported by the Swiss Federal Institute of Technology and the Swiss National Research Foundation.

References

1. A. Baeyer and V. Villiger, *Ber. Dtsch. Chem. Ges.*, 1901, **34**, 755–762.
2. F. Raschig, *Ber. Dtsch. Chem. Ges.*, 1907, **40**, 4580–4588.
3. I. Trifonow, *Z. Anorg. Allg. Chem.*, 1922, **124**, 123–135.
4. I. Trifonow, *Z. Anorg. Allg. Chem.*, 1922, **124**, 136–139.
5. K. Gleu and E. Roell, *Z. Anorg. Allg. Chem.*, 1929, **179**, 233–266.
6. K. Gleu and R. Hubold, *Z. Anorg. Allg. Chem.*, 1935, **223**, 305–317.
7. J. W. Reed, H. H. Ho and W. L. Jolly, *J. Am. Chem. Soc.*, 1974, **96**, 1248–1249.
8. G. Kortüm and B. Finckh, *Z. Phys. Chem.*, 1941, **48B**, 32–49.
9. E. Halfpenny and P. L. Robinson, *J. Chem. Soc.*, 1952, 928–938.
10. M. Anbar and H. Taube, *J. Am. Chem. Soc.*, 1954, **76**, 6243–6247.
11. J. D. Ray, *J. Inorg. Nucl. Chem.*, 1962, **24**, 1159–1162.
12. W. G. Keith and R. E. Powell, *J. Chem. Soc. A*, 1969, 90.
13. H. M. Papée and G. L. Petriconi, *Nature*, 1964, **204**, 142–144.
14. M. N. Hughes and H. G. Nicklin, *J. Chem. Soc. A*, 1968, 450–452.
15. D. S. Bohle, B. Hansert, S. C. Paulson and B. D. Smith, *J. Am. Chem. Soc.*, 1994, **116**, 7423–7424.
16. N. V. Blough and O. C. Zafiriou, *Inorg. Chem.*, 1985, **24**, 3502–3504.
17. L. J. Ignarro, J. M. Buga, K. S. Wood, R. S. Byrns and G. Chaudhuri, *Proc. Natl. Acad. Sci. U. S. A.*, 1987, **84**, 9265–9269.
18. R. M. J. Palmer, A. G. Ferrige and S. Moncada, *Nature*, 1987, **327**, 524–526.
19. R. J. Gryglewski, R. M. J. Palmer and S. Moncada, *Nature*, 1986, **320**, 454–456.
20. J. O. Edwards and R. C. Plumb, *Prog. Inorg. Chem.*, 1993, **41**, 599–635.
21. W. H. Koppenol, *Redox Rep.*, 2001, **6**, 229–234.
22. W. H. Koppenol, *Free Radical Biol. Med.*, 1993, **15**, 645–651.
23. Z. Maskos, J. D. Rush and W. H. Koppenol, *Free Radical Biol. Med.*, 1990, **8**, 153–162.

24. H. Bataineh, O. Pestovsky and A. Bakac, *Chem. Sci.*, 2012, **3**, 1594–1599.
25. W. H. Koppenol and P. L. Bounds, Redox-active metals: Iron and copper, in *Principles of Free Radical Biomedicine*, ed. K. Pantopoulos and H. M. Schipper, Nova Science Publishers, Inc., Hauppage, NY, 2011, pp. 91–111.
26. J. S. Beckman, T. W. Beckman, J. Chen, P. A. Marshall and B. A. Freeman, *Proc. Natl. Acad. Sci. U. S. A.*, 1990, **87**, 1620–1624.
27. B. H. J. Bielski, D. E. Cabelli, R. L. Arudi and A. B. Ross, *J. Phys. Chem. Ref. Data*, 1985, **14**, 1041–1100.
28. W. H. Koppenol, J. J. Moreno, W. A. Pryor, H. Ischiropoulos and J. S. Beckman, *Chem. Res. Toxicol.*, 1992, **5**, 834–842.
29. C. Molina, R. Kissner and W. H. Koppenol, *Dalton Trans.*, 2013, **42**, 9898–9905.
30. W. H. Koppenol and R. Kissner, *Chem. Res. Toxicol.*, 1998, **11**, 87–90.
31. R. Kissner and W. H. Koppenol, *J. Am. Chem. Soc.*, 2002, **124**, 234–239.
32. D. Gupta, B. Harish, R. Kissner and W. H. Koppenol, *Dalton Trans.*, 2009, 5730–5736.
33. C. Kurz, X. Zeng, S. Hannemann, R. Kissner and W. H. Koppenol, *J. Phys. Chem. A*, 2005, **109**, 965–969.
34. S. Goldstein and G. Merényi, *Methods Enzymol.*, 2008, **436**, 49–61.
35. W. H. Koppenol, P. L. Bounds, T. Nauser, R. Kissner and H. Ruegger, *Dalton Trans.*, 2012, **41**, 13779–13787.
36. J. W. Coddington, J. K. Hurst and S. V. Lymar, *J. Am. Chem. Soc.*, 1999, **121**, 2438–2443.
37. S. V. Lymar, R. F. Khairutdinov and J. K. Hurst, *Inorg. Chem.*, 2003, **42**, 5259–5266.
38. M. Kirsch, H.-G. Korth, A. Wensing, R. Sustmann and H. de Groot, *Arch. Biochem. Biophys.*, 2003, **418**, 133–150.
39. M. Sturzbecher, R. Kissner, T. Nauser and W. H. Koppenol, *Inorg. Chem.*, 2007, **46**, 10655–10658.
40. R. Kissner, T. Nauser, P. Bugnon, P. G. Lye and W. H. Koppenol, *Chem. Res. Toxicol.*, 1997, **10**, 1285–1292.
41. A. Denicola, B. A. Freeman, M. Trujillo and R. Radi, *Arch. Biochem. Biophys.*, 1996, **333**, 49–58.
42. S. V. Lymar and J. K. Hurst, *J. Am. Chem. Soc.*, 1995, **117**, 8867–8868.
43. R. Meli, T. Nauser and W. H. Koppenol, *Helv. Chim. Acta*, 1999, **82**, 722–725.
44. M. G. Bonini, R. Radi, G. Ferrer-Sueta, A. M. D. Ferreira and O. Augusto, *J. Biol. Chem.*, 1999, **274**, 10802–10806.
45. D. A. Armstrong, R. E. Huie, S. V. Lymar, W. H. Koppenol, G. Merényi, P. Neta, D. M. Stanbury, S. Steenken and P. Wardman, *BioInorg. React. Mech.*, 2013, **9**, 59–61.
46. R. Meli, T. Nauser, P. Latal and W. H. Koppenol, *J. Biol. Inorg. Chem.*, 2002, **7**, 31–36.
47. J. Xue, X. Y. Ying, J. S. Chen, Y. H. Xian, L. T. Jin and J. Jin, *Anal. Chem.*, 2000, **72**, 5313–5321.
48. W. C. A. Koh, J. I. Son, E. S. Choe and Y. B. Shim, *Anal. Chem.*, 2010, **82**, 10075–10082.

49. C. Amatore, S. Arbault, D. Bruce, P. de Oliveira, M. Erard and M. Vuillaume, *Chem.–Eur. J.*, 2001, **7**, 4171–4179.
50. C. Amatore, S. Arbault, C. Bouton, K. Coffi, J.-C. Drapier, H. Ghandour and Y. Tong, *ChemBioChem*, 2006, **7**, 653–661.
51. S. F. Peteu, T. Bose and M. Bayachou, *Anal. Chim. Acta*, 2013, **780**, 81–88.
52. J. Glebska and W. H. Koppenol, *Free Radical Biol. Med.*, 2003, **35**, 676–682.
53. B. Kalyanaraman, V. Darley-Usmar, K. J. A. Davies, P. A. Dennery, H. J. Forman, M. B. Grisham, G. E. Mann, K. Moore, L. J. Roberts II and H. Ischiropoulos, *Free Radical Biol. Med.*, 2012, **52**, 1–6.
54. H. J. Forman, O. Augusto, R. Brigelius-Flohe, P. A. Dennery, B. Kalyanaraman, H. Ischiropoulos, G. E. Mann, R. Radi, L. J. Roberts II, J. Vina and K. J. A. Davies, *Free Radical Biol. Med.*, 2015, **78**, 233–235.
55. A. Sikora, J. Zielonka, M. Lopez, J. Joseph and B. Kalyanaraman, *Free Radical Biol. Med.*, 2009, **47**, 1401–1407.
56. J. Zielonka, A. Sikora, M. Hardy, J. Joseph, B. P. Dranka and B. Kalyanaraman, *Chem. Res. Toxicol.*, 2012, **25**, 1793–1799.
57. M. Wörle, P. Latal, R. Kissner, R. Nesper and W. H. Koppenol, *Chem. Res. Toxicol.*, 1999, **12**, 305–307.
58. J.-H. M. Tsai, J. G. Harrison, J. C. Martin, T. P. Hamilton, M. van der Woerd, M. J. Jablonsky and J. S. Beckman, *J. Am. Chem. Soc.*, 1994, **116**, 4115–4116.
59. D. Klug, J. Rabani and I. Fridovich, *J. Biol. Chem.*, 1972, **247**, 4839–4842.
60. E. M. Fielden, P. B. Roberts, R. C. Bray, D. J. Lowe, G. N. Mautner, G. Rotilio and L. Calabrese, *Biochem. J.*, 1974, **139**, 49–60.
61. T. Nauser and W. H. Koppenol, *J. Phys. Chem. A*, 2002, **106**, 4084–4086.
62. H. Ischiropoulos, L. Zhu and J. S. Beckman, *Arch. Biochem. Biophys.*, 1992, **298**, 446–451.
63. C. D. Smith, M. Carson, M. van der Woerd, J. Chen, H. Ischiropoulos and J. S. Beckman, *Arch. Biochem. Biophys.*, 1992, **299**, 350–355.
64. R. Radi, J. S. Beckman, K. M. Bush and B. A. Freeman, *J. Biol. Chem.*, 1991, **266**, 4244–4250.
65. R. Radi, J. S. Beckman, K. M. Bush and B. A. Freeman, *Arch. Biochem. Biophys.*, 1991, **288**, 481–487.
66. M. S. Ramezanian, S. Padmaja and W. H. Koppenol, *Chem. Res. Toxicol.*, 1996, **9**, 232–240.
67. J. S. Beckman, Y. Z. Ye, P. G. Anderson, J. Chen, M. A. Accavitti, M. M. Tarpey and C. R. White, *Biol. Chem. Hoppe-Seyler*, 1994, **375**, 81–88.
68. S. Padmaja, M. S. Ramezanian, P. L. Bounds and W. H. Koppenol, *Redox Rep.*, 1996, **2**, 173–177.
69. B. Alvarez, H. Rubbo, M. Kirk, S. Barnes, B. A. Freeman and R. Radi, *Chem. Res. Toxicol.*, 1996, **9**, 390–396.
70. J. S. Beckman and W. H. Koppenol, *Am. J. Physiol. Cell Physiol.*, 1996, **271**, C1424–C1437.
71. J. K. Hurst and S. V. Lymar, *Acc. Chem. Res.*, 1999, **32**, 520–528.
72. P. Pacher, J. S. Beckman and L. Liaudet, *Physiol. Rev.*, 2007, **87**, 315–424.
73. M. N. Hughes and H. G. Nicklin, *J. Chem. Soc. A*, 1971, 164–168.

74. W. A. Pryor, R. Cueto, X. Jin, W. H. Koppenol, M. Ngu-Schwemlein, G. L. Squadrito, P. L. Uppu and R. M. Uppu, *Free Radical Biol. Med.*, 1995, **18**, 75–83.
75. W. H. Koppenol, R. Kissner and J. S. Beckman, *Methods Enzymol.*, 1996, **269**, 296–302.
76. D. S. Bohle, P. A. Glassbrenner and B. Hansert, *Methods Enzymol.*, 1996, **269**, 302–311.
77. X. Chen, H. Chen, R. Deng and J. Shen, *Biomed. J.*, 2014, **37**, 120–126.
78. J. J. Saucerman, *Biophys. J.*, 2013, **105**, 1287–1288.
79. W. H. Koppenol, *Pure Appl. Chem.*, 2000, **72**, 437–446.
80. N. G. Connelly, T. Damhus, R. M. Hartshorn and A. T. Hutton, *Nomenclature of Inorganic Chemistry. IUPAC Recommendations 2005*, Royal Society of Chemistry, Cambridge, 2005.