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Redox Pioneer: Professor Leopold Flohé

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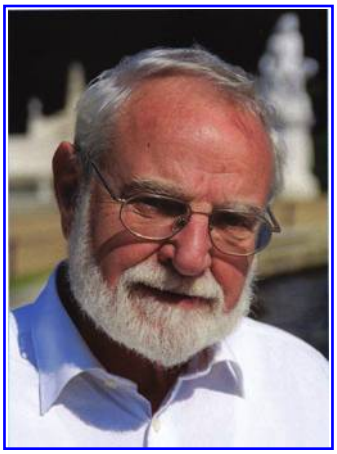

Professor Leopold Flohé

Abstract

Leopold Flohé is recognized here as a Redox Pioneer because has published a article on antioxidant/redox biology, as first author, that has been cited more than 1,000 times, and more than 20 articles have been cited more than 100 times. He obtained the medical doctorate at the Institute of Pharmacology and Toxicology at the University of Tübingen, Germany, in 1968. He held positions in both Academia (Tübingen, Aachen, and Braunschweig, Germany) and industry (Aachen). He is now operating the biotech company MOLISA in Magdeburg, Germany, while teaching as guest professor at the local university. Dr. Flohé is the pioneer who established the selenoprotein nature of glutathione peroxidase (GPx), the first and, for almost 10 years, the only selenoprotein known in animals. His work was pivotal to link the essential trace element selenium to metabolic processes, which led the Food and Drug Administration (FDA) to approve selenium supplementation for humans in 1980, and stimulated selenium biochemistry in general. In recent years, he embarked on investigating how pathogens protect themselves from oxidative killing. His inseminating studies on the thiol-dependent hydroperoxide metabolism of trypanosomatids and mycobacteria defined molecular drug targets, paving the way to new therapeutic strategies for neglected diseases affecting the people of developing countries. *Antioxid. Redox Signal.* 13, 1617–1622.

> I am not the type who falls in love with a molecule just because it has an odd number of electrons, or gets excited about a method that promises the glamour of creativity and novelty. My driving force simply was to make something useful out of available knowledge and technology, and open eyes and serendipities often helped to pick a productive way to success.
>
> —Professor Leopold Flohé

Educational and Professional Training

DR. FLOHÉ obtained his diploma in biochemistry at the Institut für Physiologische Chemie und Biochemie of the University of Tübingen, Germany, in 1967, and the medical doctorate at the Pharmacological Institute of this University in 1968.

Summary of Dr. Flohé's Top Contributions

Dr. Flohé opened the field of selenium biochemistry by identifying this trace element in a mammalian enzyme, glutathione peroxidase, today termed GPx1. His work was pivotal to link the essential element to metabolic processes, which led the FDA to approve selenium supplementation. His studies on GPx1 paved the way for the forthcoming discoveries of the other glutathione peroxidases (GPxs). In recent years, he focused on investigating how pathogens protect themselves from oxidative killing. His inseminating studies on the thiol-dependent hydroperoxide metabolism of trypanosomatids and mycobacteria defined new molecular drug targets.

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Author note: Professor Leopold Flohé is the scientific father of GPx1, the first mammalian selenoenzyme. His pioneering work inspired our studies that led to the identification of GPx4 in 1981. When we finally met, a gratifying friendship and fruitful cooperation expanded from GPx research to the entire area of hydroperoxide physiology.

For a list of frequently cited articles published by Prof. Flohé, *see* Supplemental Tables 1 and 2, available online at www.liebertonline.com/ars.

Background, Development, and Training

Leopold Flohé, Poldi to his friends, was born the year before World War II and suffer from the deprivations that accompanied the war. At that time, when only cheap hobbies were affordable, the boy Poldi became interested in collecting the flowers and weeds he found around his bombed house. He compared them with those systematically described and annotated with their Latin names in a botany book he had saved from the ruins. This way, he became the deep connoisseur of the floral kingdom that still today amazes his colleagues. This experience, together with the biographic landmarks of studies in philosophy, graduation in biochemistry in 1967, and medicine in 1968 at the University of Tübingen, testifies to the epistemologic basis of a love for knowledge, primed by *curiositas naturæ*.

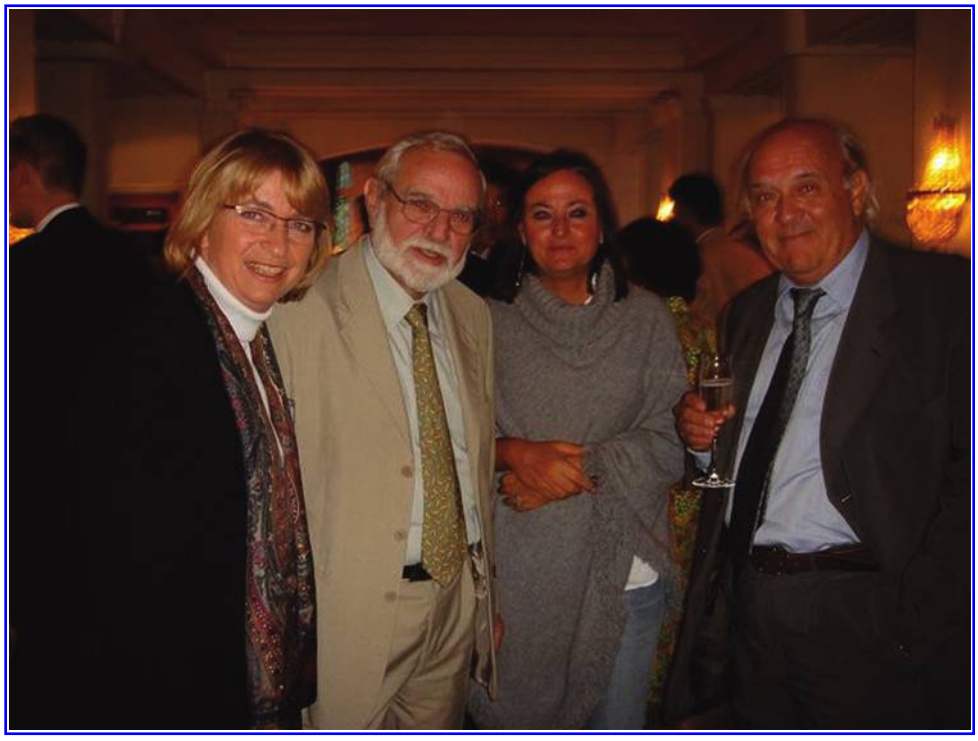
In 1971 Dr. Flohé was granted the *Venia Legendi* for Biochemistry by the University of Tübingen and then served there as Assistant Professor and, from 1979, at the University of Aachen as Professor of Biochemistry. In 1974, he had moved to Aachen to work for the research department of Grünenthal GmbH, from 1976 to 1990 as Vice President for Research and Development, while simultaneously teaching at the University of Aachen. From 1990 to 1995, he was the Scientific Director of Gesellschaft für Biotechnologische Forschung (GBF, now HZI) in Braunschweig, a leading biotechnological institution in Germany, while continuing his academic career at the Technical University of Braunschweig, until obligatory retirement at the age of 65.

Area of Interest in Redox Biology

Glutathione peroxidases as selenoenzymes and as the prototypes of the non-heme peroxidases

Studies on the catalytic mechanism and the peculiar kinetics of GPx1, carried out by Leopold Flohé, preceded the discoveries of the other glutathione peroxidases (GPxs) and of peroxiredoxins (Prxs) that constitute the present scenario of a network of oxidoreductases controlling redox transitions involved in both antioxidant defense and signaling. The discovery that GPx1 contains selenium and that the element is inserted into redox-active proteins as selenocysteine, boosted the innovative field of biochemical selenology, which expanded from the low-molecular-weight compounds contained in plants to bacterial and animal selenoproteins (1). How the early achievements in selenology developed into a now flourishing field of research is the subject of a recent essay written by Leopold Flohé himself (8). Further, the discovery of glutathione peroxidase homologues containing sulfur instead of selenium (CysGPxs) opens new evolutionary horizons. The alternative use of these elements brings into focus the biologic advantage of having selenium rather than sulfur in some of these enzymes (32). Another intriguing aspect of CysGPxs is the switch from glutathione peroxidase to thioredoxin peroxidase activity, which was first described for a GPx of *Plasmodium falciparum* by Dr. Flohé (43) and has increased interest as an important means of thioredoxin-mediated redox regulation.

The creative atmosphere of the "university village" Tübingen, which inspired the young Otto Warburg, a founder of redox biochemistry, as well as other giants of biochemistry, such as Felix Hoppe-Seyler, Friedrich Miescher, and Adolf Butenandt, must have primed the student Leopold Flohé. In his MD thesis, he had disappointed his supervisor with negative results on the supposedly different redox potentials of fetal versus adult hemoglobin and decided to dig deeper into the physiology of red blood cells to determine why red blood cells from newborns turn brown more rapidly than do those of adults. He therefore persuaded his biochemistry supervisor



Leopold Flohé with (*from right*) Regina Brigelius-Flohé, Matilde Maiorino, and Fulvio Ursini at the SFRR Meeting 2006 in Davos

to let him begin a diploma thesis on "Glutathion-Peroxidase," an enzyme described by Gordon Mills in 1957 (35), but, as Poldi learned much later, was declared not to exist at all at a FASEB Meeting in the United States. Apparently only heme peroxidases, which display an unequivocal spectrum, were accepted as catalyzing hydroperoxide processing. Having purified GPx from an enormous amount of bovine blood, Leopold Flohé produced a series of rigorous studies on the enzymology and kinetics of GPx1 (5, 16). Thereby he demonstrated that the enzyme contained neither heme, nor flavin, nor any other known coenzyme but was nevertheless superior to the heme peroxidases in terms of kinetic parameters and specificity. The dogma that hydroperoxides can be processed only by heme-containing enzymes finally had to be revised.

Description of Key Finding 1

Glutathione peroxidase as a selenoenzyme

In 1972, an abstract of the Federation proceedings from Hoekstra's group in Wisconsin reported that, in a model of rat Se deficiency, GPx activity was low, and radioactive selenium, given to Se-deficient rats, co-eluted with GPx1 (39). This observation prompted Leopold Flohé to search for the element in the last tiny aliquot of purified GPx1 left over from kinetic studies. He took advantage of a γ-spectrometer made available to German geologists for analyzing stones brought back from the moon by the Apollo mission. The measurements clearly yielded a precise stoichiometry of four atoms of selenium for one molecule of the tetrameric enzyme, verifying the selenoprotein nature of GPx1 (14). Thus, the moon had its second affair with chemistry, after having lent its Greek name $\Sigma\varepsilon\lambda\eta\nu\eta$ to the element when it was discovered in 1817 by Berzelius.

The connection between selenium and metabolic processes, as established by the groups of Flohé and Hoekstra, had important practical consequences: it enforced a revision of the appreciation of selenium and enabled the FDA to approve Se supplementation for domestic animals in 1979 and for humans in 1981 (36).

During his industrial career at the Grünenthal GmbH in Aachen, Leopold Flohé resumed his GPx research. With Wolfgang A. Günzler and Gert Steffens, he established the first full-length sequence of a selenoprotein, which unequivocally demonstrated that its selenium moiety, selenocysteine, was inserted in the peptide chain as the 21st naturally occurring amino acid (22). This finding was instrumental in the identification of TGA as the universal selenocysteine codon (3, 48).

Description of Key Finding 2

From tetrameric GPx1 to monomeric GPx4

We first met Leopold Flohé in the early 1980s at a Congress in Munich, mumbling in front of our poster, which reported on a monomeric GPx with an antioxidant activity on membranes. His concern was that our monomeric peroxidase could just be a monomeric variant of GPx1. The impossibility of solving the problem just by discussing it, impelled us to investigate. Finally, Poldi's team in Aachen, by partial amino acid and full genomic DNA sequencing, proved that our peroxidase was indeed a distinct protein, now called GPx4 (2, 40). A second mammalian selenoenzyme had come to light.

Poldi's curiosity-driven persistence to answer the question of what the novel gene product might be really good for continued to be fruitful. It led to the paradigm of functional diversity among homologous enzymes that share both active-site and catalytic mechanisms (47). In this context, a major achievement of our ongoing scientific collaboration is the discovery of a functional role of GPx4 that is just the opposite of an antioxidant defense. We demonstrated that, in the final phase of spermatogenesis, GPx4 catalyzes the oxidative protein thiol cross-linking, thereby moonlighting (changing function) into a structural protein of spermatozoa (46). This novel physiologic function not only unraveled the previously enigmatic role of selenium in male fertility; it can also be seen as the prototype of a posttranslational protein modification relevant to cell signaling (10).

Description of Key Finding 3

Hydroperoxide-processing enzymes in pathogens

In 1995, Leopold Flohé decided to leave all of his administrative jobs and to enjoy science full-time. He concentrated his efforts on a multifaceted project of "oxidative stress" that differed from the most fashionable analysis of the defense against ROS in humans. Instead, he pioneered a series of studies on antioxidant defense in pathogens, aiming to find molecular targets for designing new drugs to fight major diseases, such as trypanosomiasis, leishmaniasis, and tuberculosis, which affect the most neglected populations of the planet (15, 37, 45).

Within an amazingly short time, he with his new and young team clarified the hydroperoxide metabolism in trypanosomatids with the identification of two novel enzymes, tryparedoxin and tryparedoxin peroxidase (37). The former proved to be a remote relative of thioredoxin; the latter was homologous to the "thiol-dependent antioxidant protein" of yeast, which had been discovered by Earl Stadtman's team (29) and later was characterized as a thioredoxin peroxidase. These two new peroxidases, together with alkylhydroperoxide reductases (44) and a number of functionally poorly characterized "factors," constituted the youngest peroxidase family, now known as peroxiredoxins (18).

The next challenge was the hydroperoxide metabolism in mycobacteria, which are devoid of GSH and a functional AhpF/AhpC system. Again, antioxidant defense in these organisms was proven to be dominated by redoxin-fueled peroxiredoxins (27).

With these studies, Leopold Flohé provided the basis for the identification, in-depth characterization, and validation of attractive molecular targets, which are now being exploited for drug design at the Molisa GmbH and elsewhere (26, 28).

Other Achievements

Mitochondria as a source of superoxide

The finding of selenium in GPx was achieved a few years after the identification by McCord and Fridovich (33) of the redox activity of another protein of red blood cells containing copper and zinc, erythrocuprein, now known as superoxide dismutase, SOD1. These discoveries brought attention to the fundamental features of the integrated enzymatic system controlling the concentration of "free radicals" and other "reactive oxygen species."

In this field, Dr. Flohé, in collaboration with his students Garriet Loschen and Christoph Richter and the colleagues Angelo Azzi and Britton Chance, authorized classic articles showing for the first time that mitochondria are an important source of superoxide (30, 31). These early publications remained unacknowledged for some time, but have more recently been rediscovered (*e.g.*, in the context of TNF-α-triggered apoptosis (19, 25, 38). Related methodologic articles for the accurate determination of specific activities of SOD and GPx became quotation classics (13, 17).

Beyond "redoxology", a scientific pioneer in transferring science to application

As evident from his employment over the years, Leopold Flohé must also have been a successful manager. While at Grünenthal GmbH, he developed biotechnologic procedures for the production of medications heading toward an integrated treatment of vascular occlusions and reperfusion injury with fibrinolytics (6), SOD (7), and prostacyclin analogues (9). Under his coordination, urokinase/pro-urokinase evolved through the long pathway from basic research (23, 42) to preclinical (4, 21) and clinical development (34) and production in an industrial scale (20).

Also at Grünenthal, he developed Tramadol, a best-selling analgesic (12). Finally, he became intimately involved in the elucidation of the teratogenic (11, 24) and neurotoxic (41) side effects of thalidomide, which had remained a persistent burden of the Grünenthal company.

While tasting a glass of wine and referring to his scientific adventures (8), we remember Poldi saying, "I am not the type who falls in love with a molecule just because it has an odd number of electrons, or gets excited about a method that promises the glamour of creativity and novelty. My driving force simply was to make something useful out of available knowledge and technology, and open eyes and serendipities often helped to pick a productive way to success." This further corroborated our way of viewing him as an illuminist with a romantic trait.

After retirement, Dr. Flohé founded MOLISA GmbH, an innovative company that offers chemical and biologic high-tech services for the discovery and development of novel therapeutics. Since 2008, he has taught as Guest Professor at the Otto-von-Guericke-Universität, Magdeburg, Germany, and tries to coordinate European activities aiming at new therapeutic strategies against protozoal diseases as Chair of the COST Action CM0801, "New Drugs for Neglected Diseases."

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Professor Leopold Flohé owes his broad undergraduate education to a grant of the *Studienstiftung des Deutschen Volkes*. He thanks the *Deutsche Forschungsgemeinschaft* for generous support during his early and late academic period, Grünenthal GmbH for giving him the freedom to spend time and money on academic hobbies, the German Ministry of Research for substantial and ongoing support, the European Union for the chance to chair a COST Action on Drugs for Neglected Diseases, and Klaus-Erich Pollmann, *Otto-von-Guericke-Universität* Magdeburg, for granting him a last academic asylum. His particular thanks go to his teachers at the Tübingen University, Hartmut Ühleke and Friedhelm Schneider, who educated him in critical thinking and professional experimentation; to Rudolf K. Thauer, who persuaded him to embark on a fruitful thesis on oxidative hemoglobin damage; to his first Ph.D. students, Wolfgang A. Günzler, Gerriet Loschen, Rolf Hahn, Klaus-Peter Schwabe, and Albrecht Wendel, whose enthusiasm was pivotal for early successes; to his colleagues at the *Helmholtz Zentrum für Infektionsforschung*, Klaus-Dieter Aumann, Gerry Gross, Hans-Jürgen Hecht, Marisa Montemartini, Hendryk Kalicz, Peter Steiner, and Josef Wissing, who were instrumental in his scientific comeback after 20 years in research administration; to his wife, Regina Brigelius-Flohé, and friends all over the world, Matilde Maiorino, Lester Packer, Rafael Radi, Helmut Sies, and Fulvio Ursini, who kept him busy with their creative ideas; and to his last brilliant Ph.D. students, Heike Budde, Timo Jäger, Everson Nogoceke, and Marcelo Comini, with whom he entered a research field that still keeps him fascinated.

References

1. Birringer M, Pilawa S, and Flohé L. Trends in selenium biochemistry. *Nat Prod Rep* 19: 693–718, 2002.
2. Brigelius-Flohé R, Aumann KD, Blöcker H, Gross G, Kiess M, Klöppel KD, Maiorino M, Roveri A, Schuckelt R, Ursini F, Wingender E, and Flohé L. Phospholipid-hydroperoxide glutathione peroxidase: genomic DNA, cDNA, and deduced amino acid sequence. *J Biol Chem* 269: 7342–7348, 1994.
3. Chambers I, Frampton J, Goldfarb P, Affara N, McBain W, and Harrison PR. The structure of the mouse glutathione peroxidase gene: the selenocysteine in the active site is encoded by the 'termination' codon, TGA. *EMBO J* 5: 1221–1227, 1986.
4. Fincke U, Schneider J, Friderichs E, Giertz H, and Flohé L. Enhanced myocardial salvage by combined treatment with recombinant single-chain urokinase-type plasminogen activator and recombinant human superoxide dismutase in a canine coronary thrombosis model. *Arzneimittelforschung* 38: 138–142, 1988.
5. Flohé L. Glutathione peroxidase: enzymology and biological aspects. *Klin Wochenschr* 49: 669–683, 1971.
6. Flohé L. Single-chain urokinase-type plasminogen activators: new hopes for clot-specific lysis. *Eur Heart J* 6: 905–908, 1985.
7. Flohé L. Superoxide dismutase for therapeutic use: clinical experience, dead ends and hopes. *Mol Cell Biochem* 84: 123–131, 1988.
8. Flohé L. The labour pains of biochemical selenology: the history of selenoprotein biosynthesis. *Biochim Biophys Acta* 1790: 1389–1403, 2009.
9. Flohé L, Böhlke H, Frankus E, Kim SM, Lintz W, Loschen G, Michel G, Müller B, Schneider J, Seipp U, Vollenberg W, and Wilsmann K. Designing prostacyclin analogues. *Arzneimittelforschung* 33: 1240–1248, 1983.
10. Flohé L, Brigelius-Flohé R, Saliou C, Traber MG, and Packer L. Redox regulation of NF-kappa B activation. *Free Radic Biol Med* 22: 1115–1126, 1997.
11. Flohé L, Draeger E, Frankus E, Graudums I, Günzler WA, Helm FC, and Kuutti-Savolainen ER. Studies on the hypothetical relationship of thalidomide-induced embryopathy and collagen biosynthesis. *Arzneimittelforschung* 31: 315–320, 1981.
12. Flohé L and Friderichs E. [Old problems and new aspects in research on analgesia (author's transl)]. *Arzneimittelforschung* 28: 99–106, 1978.
13. Flohé L and Günzler WA. Assays of glutathione peroxidase. *Methods Enzymol* 105: 114–121, 1984.

14. Flohé L, Gunzler WA, and Schock HH. Glutathione peroxidase: a selenoenzyme. *FEBS Lett* 32: 132–134, 1973.
15. Flohé L, Hecht HJ, and Steinert P. Glutathione and trypanothione in parasitic hydroperoxide metabolism. *Free Radic Biol Med* 27: 966–984, 1999.
16. Flohé L, Loschen G, Günzler WA, and Eichele E. Glutathione peroxidase, V. The kinetic mechanism. *Hoppe-Seyler's Z Physiol Chem* 353: 987–999, 1972.
17. Flohé L and Otting F. Superoxide dismutase assays. *Methods Enzymol* 105: 93–104, 1984.
18. Flohé L and R. Harris J. (Eds). *Peroxiredoxin Systems: Structures and Functions.* New York: Springer, 2007, p. 389.
19. Goossens V, Grooten J, De Vos K, and Fiers W. Direct evidence for tumor necrosis factor-induced mitochondrial reactive oxygen intermediates and their involvement in cytotoxicity. *Proc Natl Acad Sci U S A* 92: 8115–8119, 1995.
20. Günzler W and Flohé L. Urinary-type plasminogen activator (uPA). In: *Fibrinolytics and Antifibrinolytics Handbook of Experimental Pharmacology*, edited by Bachmann F. Heidelberg: Springer, 2000, pp. 91–110.
21. Günzler WA, Cramer J, Frankus E, Friderichs E, Giertz H, Hennies HH, Henninger W, Kim SM, Ötting F, Schneider J, Steffens G, Strassburger W, Vollmer A, and Flohé L. Chemical, enzymological and pharmacological equivalence of urokinases isolated from genetically transformed bacteria and human urine. *Arzneimittelforschung* 35: 652–662, 1985.
22. Günzler WA, Steffens GJ, Grossmann A, Kim SM, Otting F, Wendel A, and Flohé L. The amino-acid sequence of bovine glutathione peroxidase. *Hoppe-Seyler's Z Physiol Chem* 365: 195–212, 1984.
23. Günzler WA, Steffens GJ, Otting F, Kim SM, Frankus E, and Flohé L. The primary structure of high molecular mass urokinase from human urine: the complete amino acid sequence of the A chain. *Hoppe-Seyler's Z Physiol Chem* 363: 1155–1165, 1982.
24. Helm FC, Frankus E, Friderichs E, Graudums I, and Flohé L. Comparative teratological investigation of compounds structurally and pharmacologically related to thalidomide. *Arzneimittelforschung* 31: 941–949, 1981.
25. Hennet T, Richter C, and Peterhans E. Tumour necrosis factor-alpha induces superoxide anion generation in mitochondria of L929 cells. *Biochem J* 289: 587–592, 1993.
26. Irigoin F, Cibils L, Comini MA, Wilkinson SR, Flohé L, and Radi R. Insights into the redox biology of *Trypanosoma cruzi*: trypanothione metabolism and oxidant detoxification. *Free Radic Biol Med* 45: 733–742, 2008.
27. Jaeger T, Budde H, Flohé L, Menge U, Singh M, Trujillo M, and Radi R. Multiple thioredoxin-mediated routes to detoxify hydroperoxides in *Mycobacterium tuberculosis*. *Arch. Biochem Biophys* 423: 182–191, 2004.
28. Jaeger T and Flohé L. The thiol-based redox networks of pathogens: unexploited targets in the search for new drugs. *Biofactors* 27: 109–120, 2006.
29. Kim K, Kim IH, Lee KY, Rhee SG, and Stadtman ER. The isolation and purification of a specific "protector" protein which inhibits enzyme inactivation by a thiol/Fe(III)/O2 mixed-function oxidation system. *J Biol Chem* 263: 4704–4711, 1988.
30. Loschen G, Azzi A, Richter C, and Flohé L. Superoxide radicals as precursors of mitochondrial hydrogen peroxide. *FEBS Lett* 42: 68–72, 1974.
31. Loschen G, Flohé L, and Chance B. Respiratory chain linked H(2)O(2) production in pigeon heart mitochondria. *FEBS Lett* 18: 261–264, 1971.
32. Maiorino M, Ursini F, Bosello V, Toppo S, Tosatto SCE, Mauri P, Becker K, Roveri A, Bulato C, Benazzi L, De Palma A, and Flohé L. The thioredoxin specificity of Drosophila GPx: a paradigm for a peroxiredoxin-like mechanism of many glutathione peroxidases. *J Mol Biol* 365: 1033–1046, 2007.
33. McCord JM and Fridovich I. Superoxide dismutase: an enzymic function for erythrocuprein (hemocuprein). *J Biol Chem* 244: 6049–6055, 1969.
34. Meyer J, Bär F, Barth H, Charbonnier B, El Deeb MF, Flohé L, Gülker H, Heikkilä J, Massberg I, Mathey D, Monassier JP, Probst P, Schmitz-Hübner U, Seabra-Gomes R, Strupp G, Uebis R, van der Werf T, Westerhof P, and Windeler J. Randomised double-blind trial of recombinant pro-urokinase against streptokinase in acute myocardial infarction. *Lancet* 22: 863–868, 1989.
35. Mills GC. Hemoglobin catabolism. I. Glutathione peroxidase, an erythrocyte enzyme which protects hemoglobin from oxidative breakdown. *J Biol Chem* 229: 189–197, 1957.
36. National Research Council (Ed), Board on Agricolture, Subcommittee on Selenium. *Selenium in Nutrition*. Washington, DC: National Academy Press, 1983. Revised edition.
37. Nogoceke E, Gommel DU, Kiess M, Kalisz HM, and Flohé L. A unique cascade of oxidoreductases catalyses trypanothione-mediated peroxide metabolism in *Crithidia fasciculata*. *Biol Chem* 378: 827–836, 1997.
38. Poderoso JJ, Carreras MC, Lisdero C, Riobo N, Schopfer F, and Boveris A. Nitric oxide inhibits electron transfer and increases superoxide radical production in rat heart mitochondria and submitochondrial particles. *Arch Biochem Biophys* 328: 8592, 1996.
39. Rotruck JT, Hoekstra WG, Pope AL, Ganther H, Swanson A, and Hafeman D. Relationship of selenium to GSH peroxidase. *Fed Proc* 31, Abstract 691, 1972.
40. Schuckelt R, Brigelius-Flohé R, Maiorino M, Roveri A, Reumkens J, Strassburger W, Ursini F, Wolf B, and Flohé L. Phospholipid hydroperoxide glutathione peroxidase is a selenoenzyme distinct from the classical glutathione peroxidase as evident from cDNA and amino acid sequencing. *Free Radic Res Commun* 14: 343–361, 1991.
41. Schwab BW, Arezzo JC, Paldino AM, Flohé L, Matthiessen T, and Spencer PS. Rabbit sural nerve responses to chronic treatment with thalidomide and supidimide. *Muscle Nerve* 7: 362–368, 1984.
42. Steffens GJ, Günzler WA, Ötting F, Frankus E, and Flohé L. The complete amino acid sequence of low molecular mass urokinase from human urine. *Hoppe Seylers Z Physiol Chem* 363: 1043–1058, 1982.
43. Sztajer H, Gamain B, Aumann KD, Slomianny C, Becker K, Brigelius-Flohé R, and Flohé L. The putative glutathione peroxidase gene of *Plasmodium falciparum* codes for a thioredoxin peroxidase. *J Biol Chem.* 276: 7397–7403, 2001.
44. Tartaglia LA, Storz G, Brodsky MH, Lai A, and Ames BN. Alkyl hydroperoxide reductase from *Salmonella typhimurium*: sequence and homology to thioredoxin reductase and other flavoprotein disulfide oxidoreductases. *J Biol Chem* 265: 10535–10540, 1990.
45. Trujillo M, Mauri P, Benazzi L, Comini M, De Palma A, Flohé L, Radi R, Stehr M, Singh M, Ursini F, and Jaeger T. The mycobacterial thioredoxin peroxidase can act as a one-cysteine peroxiredoxin. *J Biol Chem* 281: 20555–20566, 2006.
46. Ursini F, Heim S, Kiess M, Maiorino M, Roveri A, Wissing J, and Flohé L. Dual function of the selenoprotein

PHGPx during sperm maturation. *Science* 285: 1393–1396, 1999.

47. Ursini F, Maiorino M, Brigelius-Flohé R, Aumann KD, Roveri A, Schomburg D, and Flohé L. Diversity of glutathione peroxidases. *Methods Enzymol* 252: 38–53, 1995.
48. Zinoni F, Birkmann A, Stadtman TC, and Böck A. Nucleotide sequence and expression of the selenocysteine-containing polypeptide of formate dehydrogenase (formate-hydrogen-lyase-linked) from *Escherichia coli*. *Proc Natl Acad Sci U S A* 83: 4650–4654, 1986.

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Abbreviations Used

CysGPx = a glutathione peroxidase containing a Cys residue replacing the catalytic selenocysteine
FDA = Food and Drug Administration
GBF = Gesellschaft für Biotechnologische Forschung
GPx(s) = glutathione peroxidase(s)
GPx1 = glutathione peroxidase 1 (E.C.: 1.11.1.9)
GPx4 = glutathione peroxidase 4 (E.C.: 1.11.1.12)
Prxs = peroxiredoxins
Sec = selenocysteine
SECIS = selenocysteine insertion sequence
SelP = selenoprotein P
SOD = superoxide dismutase