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Review

Beyond Bulk Oxidative Stress: Why Antioxidant Therapies Fail and Redox Biomarkers Disappoint

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Abstract

Reactive oxygen species (ROS), reactive nitrogen species (RNS) and reactive sulphur species (RSS) constitute a coupled molecular language whose marks, the reversible post-translational modifications of cysteine and tyrosine residues, are formed, interconverted and reversed within subcellular microdomains characterised by their own geometry and kinetics. Two decades of broad-spectrum antioxidant clinical trials using vitamin E, beta-carotene, and N-acetylcysteine, amongst other compounds, have produced predominantly neutral and in some settings harmful results, although the outcomes vary appreciably with the compound, the disease, the dose and the trial design, and several of the most studied polyphenols, curcumin, resveratrol and quercetin, display a broadly comparable pattern, compounded in their case by low oral bioavailability of the parent aglycone, which limits the concentration reaching the target tissue. The redox biomarkers available in clinical practice, plasma GSH/GSSG, urinary 8-oxo-dG, and serum MDA, show disappointing individual prognostic value. The present work argues that both failures are consistent with a shared mechanistic explanation: they treat the redox language as undifferentiated noise when it is, in fact, spatially structured information. While other contributing factors, including analytical variability, clinical heterogeneity, and cohort design, are acknowledged, this mechanistic convergence offers a parsimonious interpretive framework. We analyse the failure of global antioxidants from the logic of the microdomain, suggesting that non-specific scavenging suppresses eustress and distress with comparable efficacy, and the failure of conventional redox biomarkers from the loss of spatial and temporal resolution that occurs when signals from multiple compartments are collapsed into a systemic index. Building upon this dual analysis, we propose the principles of an alternative strategy: pharmacological interventions acting on specific microdomain levers (NOS recoupling, restoration of mitochondrial -SSH tone, and Prx/Trx system support) and a precision redox diagnostic framework that replaces global indices with compartment-resolved biomarker panels. The objective is not to silence the redox language, but to learn to read it and, when necessary, to correct its grammar with precision.

Keywords: redox language; ROS-RNS-RSS microdomains; antioxidant failure; precision redox biomarkers; eustress; distress; persulphidation; Prx/Trx; post-translational modifications; precision redox medicine

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1. Introduction

Cells speak a language that for decades we mistook for noise. Reactive oxygen species (ROS), reactive nitrogen species (RNS) and reactive sulphur species (RSS) were initially described as toxic by-products of aerobic metabolism, agents of damage to DNA, lipids and proteins [1,2], but research over the past two decades has revealed a radically more sophisticated reality: they constitute a coupled molecular language whose grammar unfolds within subcellular microdomains with their own geometry and kinetics, writing, editing and erasing post-translational modifications (PTMs) with spatiotemporal precision [3,4]. Because this vocabulary is borrowed from chromatin biology, its chemical meaning here should be stated at the outset. Writing denotes the formation of a reversible covalent modification on a susceptible cysteine or tyrosine residue, editing denotes the enzyme-catalysed or thiol-exchange-mediated interconversion between such modifications, and erasing denotes the return of the modified residue to its free thiol state. No dedicated and universal enzyme class equivalent to chromatin writers and erasers is implied, and the boundaries of the analogy are set out explicitly in Section 2.2. A glossary of key terms used throughout this review is provided in Appendix A. The question is no longer how many reactive species exist, but what message they are transmitting and whether that message is being correctly read.

This paradigm shift was neither immediate nor linear. For decades, the model of non-specific oxidative damage predominated, formalised by Sies in 1985 as an imbalance between oxidant production and antioxidant capacity. The conceptual turn began with the experimental demonstration that certain growth factors require transient H_2O_2 generation for signalling: Sundaresan et al., 1995, showed that PDGF signalling in vascular smooth muscle cells depends on H_2O_2 [5]. The proposal of H_2O_2 as a second messenger was formalised by Reth, 2002 [6]. The molecular mechanism explaining how such a reactive molecule achieves signal specificity was articulated by D'Autréaux and Toledano, 2007 [7], and by Winterbourn, 2008, who demonstrated that specificity emerges from kinetic constraints rather than from the chemistry of the oxidant [8]. The role of peroxiredoxins as compartment-specific sensors was established by Rhee's group [9,10], culminating in the demonstration that Prx phosphorylation allows local H_2O_2 accumulation for mitotic signalling [11]. The integration of all this knowledge into the concept of "oxidative eustress", physiological signalling by H_2O_2 at low concentrations, qualitatively distinct from pathological distress, was formulated by Sies, 2017 [12], and consolidated in the landmark reviews of the field, Sies and Jones, 2020 [13]; Sies et al., 2022 [4]; and Rhee, 2006 [14]. This historical trajectory is relevant to the present work because it defines precisely where current therapeutic and diagnostic tools fail: they were designed under the paradigm of non-specific damage, and they continue to operate under it.

This conceptual transformation carries an uncomfortable translational consequence: two of the pillars upon which clinical redox medicine has been built, therapeutic antioxidants and oxidative stress biomarkers, are conceptually misaligned with a spatially structured information system, not merely insufficiently powerful [4]. This inadequacy is neither trivial nor remediable through minor technical adjustments: it stems from a premise, increasingly challenged, that reactive species are homogeneous entities whose mere abundance determines damage, a premise under which the logical strategies are to reduce their global concentration and to measure their accumulated footprint. Microdomain biology suggests that this premise is insufficient; what matters is identity, subcellular localisation, duration and the kinetic competition established with the editing systems of the compartment in which reactive species act [15]. Changing the premise compels a change in tools.

The present work develops this argument in two parts. The first (Section 2) analyses the systematic failure of global antioxidants from the mechanics of the microdomain, including both classical broad-spectrum antioxidants and polyphenols, whose failure adds an additional layer: the bioavailability barrier that prevents reaching the microdomain before the problem of non-specificity even arises. The second (Section 3) analyses the prognostic failure of conventional redox biomarkers: we argue that collapsing signals from multiple compartments into a systemic index erases precisely the information that contains real diagnostic value. Building upon this dual diagnosis, Section 4 proposes the principles of an alternative strategy coherent with the nature of the redox language: precision pharmacological interventions targeting specific microdomain levers and a diagnostic framework that restores the lost spatial and temporal resolution. This analysis is deliberately narrower than the general conceptual framework of ROS-RNS-RSS microdomains introduced in our companion review [3]; here, we focus specifically on the clinical and translational consequences of that framework for antioxidant therapy and redox diagnostics.

Figure 1 illustrates the core paradigm shift introduced in Section 1: reactive oxygen, nitrogen and sulphur species do not act as diffuse noise but as a spatially encoded molecular language within subcellular microdomains. The cutaway cell shows four distinct compartments, mitochondrial matrix (Complex I/III), perinuclear region, endoplasmic reticulum and plasma-membrane NOS, each generating localised H_2O_2 (yellow), $NO^{\bullet}$ (blue) and H_2S (green) signals within nanometre radii. On a single cysteine residue, the kinetic competition between sulphenylation (-SOH, red), S-nitrosylation (-SNO, blue) and persulphidation (-SSH, green) is depicted, together with compartment-specific editors (Prx/Trx, GSNOR, and CBS/CSE). Green arrows denote adaptive eustress signalling (e.g., NRF2-mediated biogenesis), while red arrows denote pathological distress. The figure illustrates the proposed reason why global interventions may underperform, namely, that they do not take account of the geometry and kinetics that confer specificity (Sies et al., 2022, Winterbourn, 2008) [4,8].

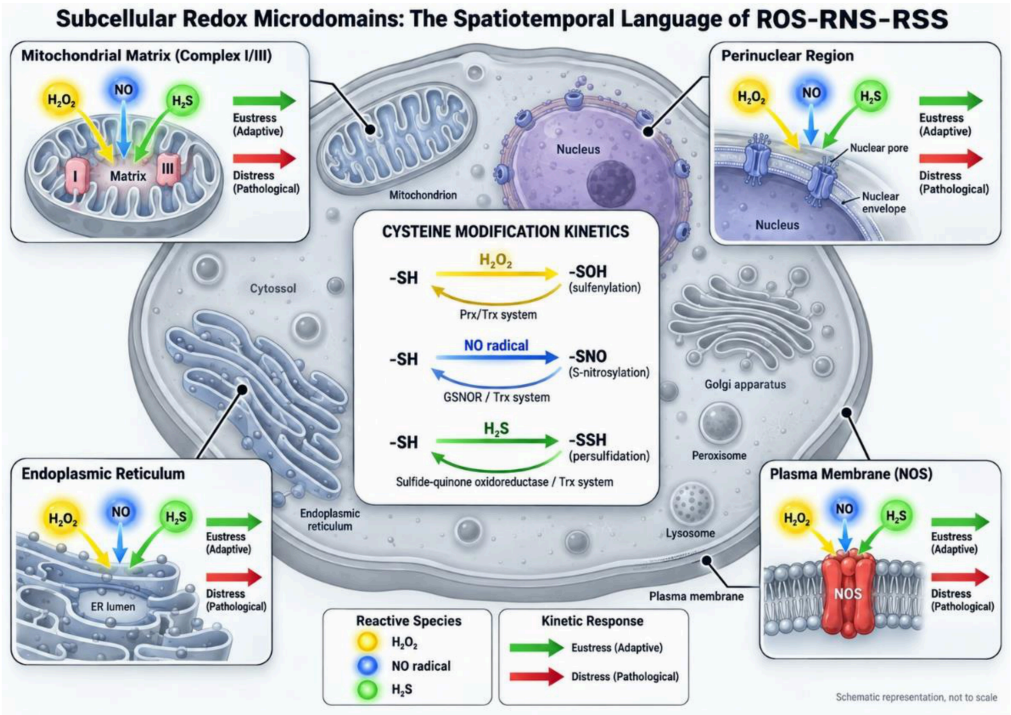


Figure 1. Geometry and kinetics define redox signalling specificity within subcellular microdomains.

Literature Search Strategy

This article is a narrative rather than a systematic review, and it does not claim the exhaustiveness that the latter format requires, but the search procedure is reported here so that readers can judge the basis on which the evidence was assembled. PubMed and MEDLINE, Scopus, and the Web of Science Core Collection were searched from database inception to 31 May 2026, with a final verification search performed on 5 June 2026, and ClinicalTrials.gov was searched over the same period for registered interventional studies of the compounds and therapeutic levers discussed here.

The search combined four groups of terms. The first covered reactive species and their compartmentalisation, including oxidative stress, reactive oxygen species, reactive nitrogen species, reactive sulphur species, hydrogen sulphide, microdomain, compartmentalisation and subcellular localisation. The second covered reversible cysteine chemistry and its enzymology, including sulphenylation, S-nitrosylation, persulphidation, peroxiredoxin, thioredoxin, sulphiredoxin, GSNOR, NADPH oxidase and nitric oxide synthase uncoupling. The third covered interventions, including antioxidant, vitamin E, beta-carotene, N-acetylcysteine, polyphenol, curcumin, resveratrol, quercetin, AP39, setanaxib, GKT137831 and MitoQ. The fourth covered measurement, including redox biomarker, 8-oxo-dG, malondialdehyde, isoprostane, 3-nitrotyrosine, redox proteomics and proximity labelling. These groups were combined with each other and with the names of the two pilot indications examined in Section 4.3.

Records were eligible if they were published in English and reported original experimental, clinical or epidemiological data, or if they constituted authoritative reviews, consensus statements or methodological guidelines issued by recognised bodies in the field. Priority was given to randomised controlled trials, prospective cohort studies, mechanistic work performed in human tissue and landmark conceptual contributions, with older publications retained where they established a concept that remains in current use. Records were excluded if they were conference abstracts without accompanying data, preprints subsequently superseded by a peer-reviewed version, or studies whose redox measurements were reported without any description of pre-analytical handling. Titles and abstracts were screened by one author, the retained full texts were reviewed and agreed by at least two authors, and disagreements were resolved by discussion. Reference lists of the included reviews were hand-searched for sources that the electronic strategy had not retrieved. Because selection involved this element of expert judgement rather than a pre-registered protocol, the resulting evidence base should be read as a considered but deliberately non-exhaustive synthesis.

2. Problem 1, the Persistent Failure of Global Antioxidants

2.1. The Clinical Evidence

The disappointing performance of antioxidants in large-scale clinical trials is one of the more reproducible findings of modern clinical research and one whose interpretation remains contested. The HOPE trial (Heart Outcomes Prevention Evaluation) enrolled 9541 patients at high cardiovascular risk and randomised them to 400 IU/day of natural-source vitamin E or placebo in a double-blind, 2 × 2 factorial design with a median follow-up of 4.5 years; the rate of the composite primary endpoint (cardiovascular death, myocardial infarction, or stroke) was virtually identical between groups (16.2% vs. 15.5%, relative risk: 1.05, $p = 0.33$), and the HOPE-TOO extension, extending follow-up to a median of 7.0 years, confirmed that vitamin E was associated with a significantly higher rate of heart failure across the HOPE cohort (RR: 1.13, $p = 0.03$) [16,17]. The ATBC trial (Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study) in smokers not only failed to reduce lung cancer but increased its incidence in the beta-carotene arm [18]. Meta-

analyses of vitamin E and C supplementation in cardiovascular and neurodegenerative disease have consistently produced neutral or marginally negative results [19,20]. N-acetylcysteine, the most widely used glutathione precursor in clinical practice, has shown inconsistent and largely non-disease-modifying results across several neurodegenerative and ischaemia–reperfusion contexts, a pattern documented in most detail for Parkinson's disease, discussed immediately below [21,22]. It is worth noting that the mechanistic rationale for NAC in neurodegeneration is not without foundation: intravenous administration elevates brain glutathione levels measurably in Parkinson's disease patients, and open-label studies (n = 65) have reported an approximately 13% improvement in UPDRS motor scores after three months of combined intravenous and oral treatment, with a 4–9% increase in dopamine transporter signal on DaTscan imaging [21,22]. However, no powered randomised controlled trial has yet confirmed these signals, and the systematic review of the evidence concludes that the field requires substantially larger, biomarker-anchored multicentre studies to determine whether the pharmacokinetic constraints of NAC delivery can be overcome before a clinical verdict on efficacy can be rendered [22]. This distinction is important: the failure so far is not evidence of conceptual irrelevance of the glutathione pathway, but of the difficulty of delivering a global precursor to the specific mitochondrial microdomain where the deficit resides.

2.2. How the Logic of the Microdomain May Account for the Failure

To understand why, it is necessary to recall the functional architecture of the redoxome. Reactive species are not distributed homogeneously in the cytosol: they are generated from localised sources (Complexes I and III of the respiratory chain, endosomal NOX, coupled NOS at the plasma membrane, and xanthine oxidase), they act on substrates within radii of nanometres to a few tens of nanometres, and their lifetime is constrained by compartment-specific systems that consume them or that maintain the local reducing environment, among them mitochondrial Prx; cytosolic GPx; and, for S-nitrosothiol homeostasis, GSNOR. The result is that the mitochondrial microdomain, the perinuclear microdomain and the endoplasmic reticulum microdomain of the same cell can simultaneously exhibit radically different redox states [23]. Read from the perspective of microdomain redox biology, these results are less surprising than they first appear. It should be emphasised, however, that compartment non-selectivity is one candidate explanation among several rather than a sufficient account on its own. Population selection, baseline redox status, dose, formulation, pharmacokinetics, timing of the intervention relative to disease course, choice of endpoint, concomitant therapy and the pro-oxidant behaviour that several of these compounds display under particular conditions are all plausible and potentially interacting contributors and they deserve to be weighed as competing hypotheses rather than recorded as secondary caveats. The framework developed below is therefore offered as a mechanistic hypothesis that future trials would need to discriminate from these alternatives, not as an established cause of the observed results.

Within each microdomain, the biological outcome is determined by the kinetic competition among three principal reversible cysteine PTMs of local proteins: sulphenylation (-SOH, ROS writing), S-nitrosylation (-SNO, RNS writing) and persulphidation (-SSH, RSS writing), alongside tyrosine nitration as a related but distinct RNS mark. Which of the three cysteine modifications "wins" on a given residue, and what functional consequences follow, depends on several factors acting together. The first is the local concentration of each reactive species. The second is the intrinsic reactivity of the residue itself, which is governed by its thiol pKa, its solvent accessibility, the electrostatic and hydrophobic character of its immediate environment, its proximity to metal centres or to enzymes capable of catalysing the modification, and any mark already present on that residue. The third is

the activity of the systems that limit the lifetime of each mark, which act with markedly different degrees of directness. The Prx/Trx system consumes peroxides and sustains the thioredoxin-dependent reducing environment through which many, though by no means all, protein sulphenic acids are resolved, and it is not a direct eraser acting on every target protein. GSNOR metabolises S-nitrosoglutathione and thereby shifts the transnitrosation equilibria that govern protein S-nitrosothiol levels, rather than acting as a universal protein denitrosylase. The CBS, CSE and 3-MST pathway supplies the sulphide and persulphide species from which protein persulphidation arises, rather than catalysing the modification of individual targets. The fourth is the kinetics of the reverse reactions, which determine how long a given mark persists once formed, and the fifth is the spatial geometry of the microdomain itself [24].

A systemic antioxidant that scavenges H_2O_2 with equal avidity across all compartments acts with complete indifference to this architecture. It suppresses the mitochondrial eustress signalling that activates mitochondrial biogenesis via NRF2. It cancels the perinuclear H_2O_2 signal that regulates cell proliferation. It interferes with the oxidant burst initiated by the phagocytic NADPH oxidase, NOX2, which generates superoxide that is then converted, spontaneously or through superoxide dismutase, into the H_2O_2 used to eliminate pathogens. And simultaneously it may or may not reduce H_2O_2 in the specific pathological microdomain, depending on whether it reaches that particular compartment at a sufficient concentration [13].

One plausible net result in a clinical trial is a partial cancellation of opposing effects that yields a neutral outcome. In settings such as the ATBC trial, disruption of eustress signals that control proliferation in epithelia exposed to carcinogens offers one candidate explanation, among others, for the increase in oncological risk that was observed.

Figure 2 sets out a hypothesised mechanism for the clinical results obtained with broad-spectrum antioxidants and documented in Section 2. The left panel depicts a non-specific scavenger such as vitamin E indiscriminately quenching H_2O_2 across mitochondrial, perinuclear and phagosomal microdomains, simultaneously extinguishing beneficial eustress signals (NRF2 mitochondrial biogenesis, epithelial proliferation, and immune killing-shown crossed out) and pathological distress. NAC follows a related logic despite acting indirectly, as a cysteine precursor that raises glutathione availability without compartment selectivity, rather than as a direct, kinetically competitive scavenger of H_2O_2. The right panel contrasts the precision approach: a targeted lever (e.g., AP39 or NOS recoupling) acts exclusively in the diseased microdomain, preserving physiological eustress while raising the local distress threshold. Insets reference neutral or harmful trial outcomes (HOPE, ATBC, and NAC meta-analyses). The schematic illustrates the proposition that insufficient potency may not be the limiting factor and that systemic scavenging can cancel opposing redox messages within the same cell. It is an illustrative model of a hypothesised mechanism and not a direct representation of the trial evidence.

2.3. The Concept of the Distress Threshold and Its Therapeutic Implication

The distinction between redox eustress and distress is not quantitative but contextual: it depends on whether the intensity and duration of the reactive signal exceeds local editing capacity. The same level of H_2O_2 that in a cardiomyocyte with functional Prx3 and Trx2 represents an adaptive eustress signal becomes, in a cardiomyocyte with hyperoxidised and inactive Prx3, the beginning of the collapse towards peroxynitrite and irreversible PTMs [25]. Two caveats should be stated explicitly here, because these terms are considerably easier to define than to apply. The boundary between eustress and distress is not a fixed threshold. It is enzyme-specific, since the susceptibility of a given cysteine to oxidation and the reserve capacity of the local editing system differ from one protein and one compartment

to the next, and it is strongly context-dependent, since the same signal amplitude may be adaptive in a cell whose repair capacity is intact and injurious in one whose reserve is already exhausted. In practice, the boundary can frequently be located only retrospectively, once the functional outcome is known. We therefore use eustress and distress as heuristic descriptors of two regions of a continuum and not as operationally defined categories separated by an agreed quantitative cut-off. The framework developed below does not depend on such a cut-off existing.

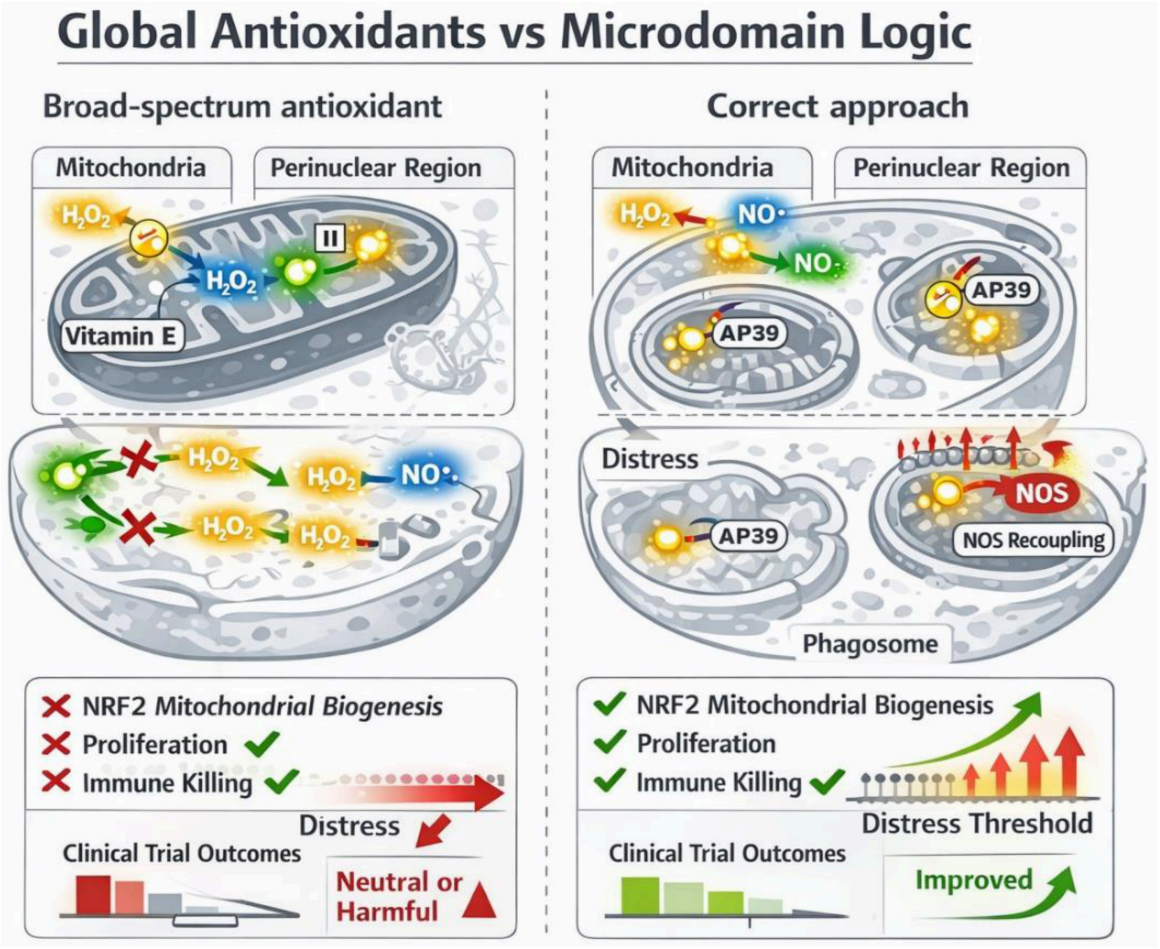


Figure 2. Hypothesised mechanism: non-specific scavenging may suppress both eustress and distress. Illustrative model only and not a direct representation of the evidence from the vitamin E, β-carotene and NAC trials (HOPE and ATBC).

This has a fundamental therapeutic implication: the correct target is not the reactive species but the control threshold of the microdomain. Raising that threshold, by restoring the editing capacity of the Prx/Trx system, recoupling uncoupled NOS, and replenishing mitochondrial -SSH tone via slow H_2S donors, allows the microdomain to tolerate eustress signalling without collapsing into pathological distress [26]. This logic underpins the precision pharmacological interventions described in Section 4.

2.4. Why Endogenous Natural Antioxidants Do Not Fail: Compartment Specificity

A frequent argument in defence of exogenous antioxidants is that endogenous antioxidant systems also "scavenge" reactive species. The answer lies in architecture: mitochondrial superoxide dismutase (SOD2) acts exclusively in the mitochondrial matrix; Prx3 resides predominantly in that same matrix, where it acts as one of the principal mitochondrial peroxide sinks; and glutaredoxin-2 isoforms operate across both the matrix and

the intermembrane space. This compartmentalisation is pronounced, though never fully impermeable to metabolic exchange, whereas systemic exogenous antioxidants largely lack this specificity [27].

2.5. Polyphenols: When Failure Occurs Before Reaching the Microdomain

Polyphenols, resveratrol, quercetin, curcumin, epigallocatechin-3-gallate (EGCG), luteolin, and apigenin represent the most widely consumed group of natural antioxidants worldwide, both as dietary supplements and through functional foods. Their in vitro antioxidant capacity is in many cases superior to that of vitamin E or beta-carotene, and the cellular and animal models demonstrating protective effects on markers of oxidative damage number in the thousands. Nevertheless, clinical trials with purified polyphenols have frequently produced neutral or inconsistent results in cardiovascular, neurodegenerative, metabolic and oncological disease, a pattern that resembles, without being identical to, that of their lipophilic predecessors. This generalisation should be applied with care, since polyphenols are a chemically and pharmacokinetically heterogeneous group, and outcomes vary appreciably between individual compounds, formulations, doses and indications [28]. A critical analysis of 293 curcumin clinical trials registered on ClinicalTrials.gov and PubMed confirms this pattern: despite the growing volume of registered studies, the vast majority remain in Phase I or Phase II, and those addressing efficacy in malignant or inflammatory disease fail to achieve the plasma concentrations demonstrated to be biologically active in preclinical models [29]. A methodological study of 171 systematic reviews on oral curcumin found that relevant subgroup or sensitivity analyses stratifying by bioavailability formulation were reported in the methods and results of only 3.5% and 6.4% of reviews, respectively, meaning that the clinical evidence base is profoundly confounded by the simultaneous testing of compounds with radically different pharmacokinetic profiles under the same "curcumin" designation [30].

The mechanism of failure is, however, qualitatively different from that of vitamin E or beta-carotene and merits separate analysis. In the case of vitamin E, the problem is conceptual: it reaches tissues but acts with compartment non-specificity, suppressing eustress and distress indistinguishably. In the case of polyphenols, there is an earlier and more elementary barrier: most of the administered compound never reaches the target tissue at a biologically relevant concentration. The problem is not where they act, it is that they do not arrive [31].

2.5.1. The Bioavailability Barrier: Solubility, Absorption and First-Pass Metabolism

Most therapeutically interesting polyphenols are highly hydrophobic, poorly soluble molecules that struggle to reach a biologically relevant plasma concentration after oral administration. Curcumin, the most studied case, has a water solubility of only about 11 ng/mL at physiological pH, and Phase I trials confirm that oral doses of up to 12 g/day remain safe yet still fail to raise plasma levels above the nanomolar range, owing to poor absorption, rapid glucuronidation and sulphation, and extensive first-pass metabolism [32]. Resveratrol is somewhat more soluble but undergoes the same first-pass fate: administration of 25 mg to healthy volunteers yields a maximum free-form plasma concentration of around 4 ng/mL, with bioavailability of the active compound below 1% of the administered dose [33]. Quercetin follows a similar pattern, being extensively converted to glucuronide and sulphate conjugates that retain partial antioxidant activity, such as inhibition of NADPH oxidase-derived superoxide, but lack the direct vasorelaxant potency of the parent aglycone [34]. Low systemic availability of the parent aglycone should not, however, be equated with biological inactivity. Conjugated metabolites circulate at appreciable concentrations, some are deconjugated locally within tissues under inflammatory

conditions, tissue distribution is uneven rather than uniform, and effects exerted within the intestinal lumen or on the gut microbiota require no systemic absorption at all. The claim advanced here is therefore the narrower one, that the concentrations reaching intracellular microdomains are generally far below those used to demonstrate direct antioxidant activity in vitro.

The consequence is a fundamental disconnect between the micromolar concentrations (1–50 μM) used in vitro to demonstrate antioxidant activity and the concentrations any of these compounds actually reach in human plasma or, still less, within the mitochondrial microdomain, a disconnect that clinical trials faithfully reproduce even though the nutritional supplementation literature has tended to minimise it [35]. More than twenty-five bioavailability-enhancing curcumin formulations have been tested clinically precisely because solubility, permeability, pH instability and hepatic metabolism compound simultaneously, with the effect that the existing evidence base conflates compounds whose pharmacokinetic profiles differ by orders of magnitude under a single biochemical name [36].

2.5.2. The Microbiome as an Unexpected Transformer: Active Metabolites of Colonic Origin

A further complication is that much of the biological activity attributed to polyphenols in animal and human studies derives not from the parent compound but from metabolites generated by the intestinal microbiome, short-chain phenolic acids and urolithins with distinct absorption and activity profiles [37]. Resveratrol, for instance, is colonically converted to dihydroresveratrol, which acts differently on the NRF2 and sirtuin pathways [38,39]. This metabolic heterogeneity is one of the principal reasons why the response to a given polyphenol varies substantially between individuals, a source of variability that clinical trials in this field rarely stratify for [40].

2.5.3. Towards Microdomain-Directed Delivery: Nanoformulations and Targeting Conjugates

The logical solution converges with the vitamin E lesson: what is needed is not a more potent antioxidant but a delivery system that reaches the correct microdomain at sufficient concentrations. Nanoformulations, solid lipid nanoparticles, liposomes, cyclodextrins, nanoemulsions and PLGA polymeric nanoparticles have raised curcumin bioavailability ten- to one-hundred-fold in animal models, with encouraging early human pharmacokinetic data [41].

Yet increasing systemic bioavailability without directing it to the pathological microdomain merely relocates the non-specificity problem from the subcellular to the tissue level. The necessary further step is conjugation with subcellular targeting ligands, the mitochondria-targeting cation triphenylphosphonium (TPP^+), already validated in the mitochondria-directed ubiquinone MitoQ [42], or cell-penetrating peptides for endosomal and reticular delivery. Curcumin-TPP^+ and quercetin-TPP^+ conjugates accumulate selectively in mitochondria with active membrane potential and, in principle, could exert antioxidant activity at substantially lower concentrations than the free compound, although conjugate-specific potency data for these two compounds remain to be established by dedicated pharmacological studies. Polyphenol failure therefore has two layers: a bioavailability barrier addressable with formulation technology and, once that is resolved, a compartment non-specificity barrier addressable only with subcellular targeting. Most improved curcumin formulations in clinical development address only the first, which may raise biological activity without producing robust clinical efficacy [36].

3. Problem 2, the Predictive Blindness of Current Redox Biomarkers

3.1. Current Clinical Biomarkers and Their Limitations

The oxidative stress biomarkers available in routine clinical practice share a structural characteristic: they measure the outcome of processes occurring in multiple cellular and tissue compartments, integrated over time, in a systemic sample (plasma, urine, and erythrocytes). The most widely used are the GSH/GSSG ratio in erythrocytes, 8-oxo-7,8-dihydroguanine (8-oxo-dG) in urine or plasma, serum malondialdehyde (MDA) measured as TBARS, plasma 3-nitrotyrosine (3-NT) as a marker of protein nitration, and urinary isoprostanes as surrogates of in vivo lipid peroxidation [43].

Each of these biomarkers has well-established mechanistic value in cellular and animal models and shows statistically significant differences between patient groups and healthy controls in virtually all published studies. The problem is not that they do not reflect oxidative stress: it is that they do not reflect the state of the microdomain relevant to the pathology in the individual patient [44,45].

3.2. The Problem of Lost Spatial Resolution

Consider the most illustrative clinical scenario. Two patients with heart failure with preserved ejection fraction (HFpEF) present identical levels of plasma 3-nitrotyrosine. In the first patient, the elevated level reflects protein nitration in the mitochondrial microdomain of the cardiomyocyte, where uncoupled NOS produces peroxynitrite that nitrates mitochondrial aconitase and impairs oxidative metabolism [46]. In the second patient, the same level predominantly reflects nitration in circulating macrophages activated during a systemic subclinical inflammatory process without a direct cardiac correlate. These two patients have radically different prognoses and would respond in opposite ways to an intervention directed at the cardiac microdomain, yet their plasma biomarkers are indistinguishable [43].

Figure 3 visualises the fundamental limitation analysed in Section 3: conventional biomarkers (plasma GSH/GSSG, urinary 8-oxo-dG, and serum MDA) collapse signals from multiple subcellular compartments into a single, uninformative average. The upper half shows four microdomains (mitochondria, nucleus, ER, and macrophage) emitting distinct, reversible PTMs (Prx3-SO_2H, 3-NT, and -SSH) as coloured kinetic waves. The lower half depicts plasma receiving all contributions as one averaged bar, with fading arrows indicating dilution and loss of spatial identity; irreversible footprints (sulphonation and stable nitration) further erase temporal resolution. A contrasting "precision panel" (Prx3-SO_2H + ADMA/SDMA + thiosulphate) restores compartment specificity. This offers one explanation of the prognostic AUC-ROC values of approximately 0.55 to 0.65 reported for these markers in cardiovascular cohorts, based on the reasoning that a compartment-specific signal is averaged away when it is expressed as a systemic index.

The underlying mechanism is straightforward: plasma receives redox contributions from all cells in all tissues, weighted by their metabolite turnover rate and their permeability to oxidation products. The result is a weighted average that substantially degrades the spatial resolution information that carries diagnostic value [47]. It should be noted that analytical noise, pre-analytical variability, and clinical heterogeneity of cohorts further compound this informational loss, though the spatial averaging problem constitutes the principal structural limitation.

3.3. The Problem of Lost Temporal Resolution

The second lost dimension is temporal. Reversible PTMs, sulphenylation (-SOH), S-nitrosylation (-SNO), and persulphidation (-SSH), have half-lives of minutes to a few hours in the context of active editing systems. What is measured in plasma is fundamentally the residue of irreversible processes (sulphonation to -SO_2H/-SO_3H, stable tyrosine

nitration, and completed lipid peroxidation) or of long-lived pools that have lost their kinetic connection to the current state of the microdomain [48]. A biomarker that measures accumulated damage over the preceding days or weeks has little value for guiding an intervention whose objective is the state of the microdomain at the present moment.

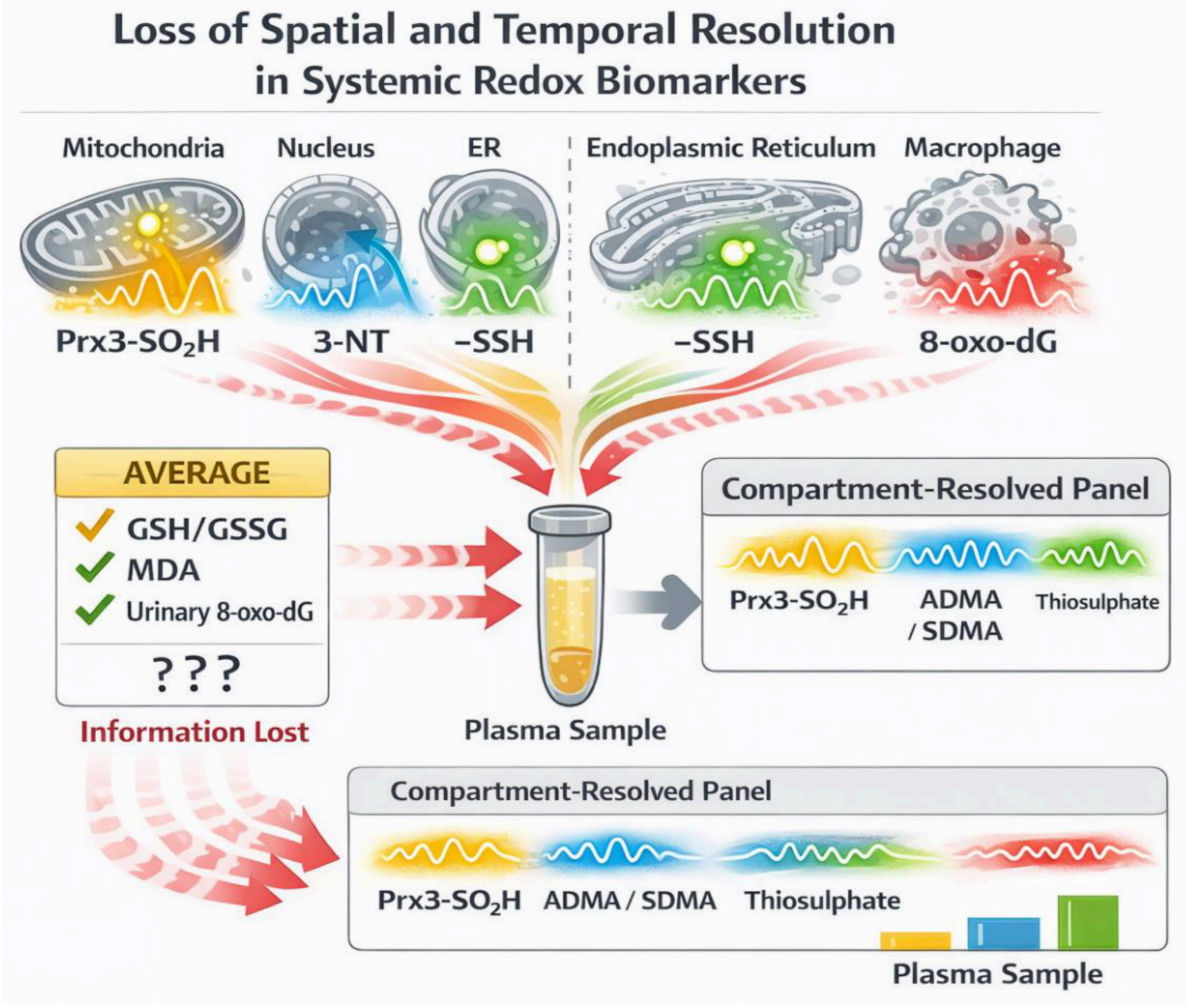


Figure 3. Systemic indices can collapse compartment-specific signals into a low-resolution average, helping to explain AUC-ROC values of only 0.55–0.65.

3.4. The Problem of Pre-Analytical Variability

To the conceptual limitations, a systematically underestimated technical problem is added: the redox lability of biological samples. GSH spontaneously oxidises to GSSG in unprocessed blood within minutes [49], protein mixed disulphides redistribute during processing, and 8-oxo-dG can be artefactually generated during DNA extraction through guanine oxidation [50]. Pre-analytical variations due to time to centrifugation, storage temperature and the number of freeze–thaw cycles are comparable in magnitude to the differences between pathological groups in many studies. This introduces a background noise that further degrades the already low individual prognostic value of these markers [51].

3.5. Evidence of Prognostic Failure in Large Cohorts

The clinical consequence of these limitations is documentable in the cohort literature. In prospective cohorts of adults followed for incident cardiovascular events, typically a composite endpoint of myocardial infarction, stroke or cardiovascular death assessed over horizons of three to ten years, single systemic markers of lipid peroxidation and

oxidative DNA damage, namely plasma or urinary F2-isoprostanes, serum MDA measured as TBARS and urinary or plasma 8-oxo-dG, discriminate between individuals who will and will not experience an event with AUC-ROC values that, based on the broader biomarker literature, generally show only modest discriminative performance and rarely exceed an AUC-ROC of 0.65 when each marker is entered singly into a logistic or Cox proportional hazards model and whose independent contribution would be expected to attenuate once conventional clinical predictors such as CRP, troponin and BNP are included in the same model [52]. This range should be read as an approximate span across heterogeneous cohorts, assay platforms and event adjudication criteria, and not as a single pooled estimate, since no harmonised meta-analysis of discrimination for these markers is currently available. The mechanistic reason for this attrition is precisely that described in Section 3.2: the cardiovascular contribution to systemic MDA and isoprostane levels is diluted by hepatic, inflammatory, and metabolic sources, such that the marker no longer reflects the state of the coronary endothelial or cardiomyocyte microdomain with any spatial fidelity. In Alzheimer's disease, oxidative DNA damage markers such as 8-oxo-dG are significantly elevated in nuclear and mitochondrial DNA extracted from vulnerable brain regions in both mild cognitive impairment and late-stage disease compared with age-matched controls, yet the overlap between distributions in tissue-based studies is wide enough that these markers have not translated into a validated, individually predictive in vivo biomarker, rendering them of limited use for precisely the early detection window where intervention would be most valuable. In ALS, oxidative markers such as 8-oxo-dG and lipid peroxidation products have shown prognostic value only as part of composite panels combined with neurofilament and iron-status markers, not as independent single biomarkers [53]. The pattern is consistent, and the mechanistic explanation is as described: there is no signal to predict with tools that cannot visualise the microdomain [54].

4. Towards an Alternative Strategy: Pharmacological Precision and Compartment-Resolved Diagnosis

The dual diagnosis presented in Sections 2 and 3, conceptually inadequate global antioxidants and systemic redox biomarkers without spatiotemporal resolution, defines by exclusion the requirements of a viable alternative strategy. In this section we articulate the principles of that alternative across two complementary planes: the therapeutic and the diagnostic.

4.1. Principles of Precision Redox Pharmacology

If the correct target is the microdomain's control threshold rather than circulating reactive species, interventions must act on editing capacity, the source of reactive species, and RSS tone. Four levers follow this logic, at markedly different stages of translational readiness, from Phase 2 clinical data to purely preclinical evidence [55].

Support for the mitochondrial Prx/Trx system. Hyperoxidised Prx3 (-SO_2H) loses its guardian function in the mitochondrial microdomain; Srxn1 can reactivate it in an ATP-dependent step, though its expression often falls in disease [56]. Boosting Srxn1 or maintaining reduced Trx2, for example, via NRF2 activators such as sulforaphane, which induce sulfiredoxin expression and reduce peroxiredoxin hyperoxidation, raises the distress threshold without suppressing eustress, although these activators are not themselves organelle-selective and their net effect still depends on reaching the mitochondrial pool preferentially [57].

Recoupling of endothelial NOS. Uncoupled NOS, oxidised at its BH4 cofactor or arginine site, generates superoxide instead of NO, simultaneously destroying NO/cGMP signalling [58]. Restoring coupling via BH4 supplementation, L-arginine or enhancement of

GTPCH1 expression and activity, which preserves the BH4/BH2 ratio, corrects the source itself, in the correct compartment, rather than scavenging its products [59].

Restoration of mitochondrial -SSH tone via slow H_2S donors. Persulphidation (-SSH) of mitochondrial cysteines, demonstrated most directly for Complex I subunits, protects against irreversible oxidation and preserves eustress-range signalling [60,61]. The tone of this modification is itself enzymatically buffered, since rhodanese-type sulphurtransferases limit the accumulation of free sulfane sulphur and thereby prevent the disulphide stress that would otherwise follow unrestrained sulphide oxidation, a control principle first resolved in bacteria [62]. Endogenous H_2S has a very short half-life, so mitochondria-targeted slow-release donors such as AP39 (an H_2S-donating moiety linked to the lipophilic TPP^+ cation) restore -SSH tone with better pharmacokinetics and lower systemic toxicity than free H_2S. In anaesthetised rats subjected to myocardial ischaemia–reperfusion (30 min ischaemia, 120 min reperfusion), AP39 at 1 $\mu mol \cdot kg^{-1}$ reduced infarct size by approximately 40% relative to vehicle, with direct mitochondrial measurements confirming attenuated ROS generation and inhibited permeability transition pore opening, independent of the cytosolic RISK pathway [63]. In a mouse model of cardiac arrest and resuscitation, the AP39 dose needed to markedly improve neurological outcome and survival was three orders of magnitude lower than for the inorganic donor Na_2S, with preserved mitochondrial integrity and reduced cortical oxidative stress [64]. In a rat transient middle cerebral artery occlusion model, AP39 given 10 min after reperfusion significantly reduced infarct volume at 24 h and 7 days, with reduced neuroinflammation and preserved BDNF/NGF signalling [65]. Together these results indicate that TPP^+-mediated compartmentalisation is a mechanistic requirement, not merely a delivery convenience, for H_2S donors to act safely at the correct microdomain.

Selective inhibition of NOX in the inflammatory microdomain. NADPH oxidases (NOX) are compartment-specific superoxide sources with distinct physiological roles: phagocytic NOX2, endoplasmic-reticulum NOX4, and vascular/mitogenic NOX1 and NOX5 [66]. Selective inhibitors, GKT137831/setanaxib for NOX1/4, and next-generation NOX2 inhibitors, reduce $O_2^{\bullet -}$ production in the pathological microdomain without touching physiological sources elsewhere, a principle that has already produced the first positive clinical results in fibrotic disease. In a mouse bile-duct-ligation model of liver fibrosis, GKT137831 significantly attenuated ROS production, hepatocyte apoptosis and histological fibrosis, while genetic NOX4 deletion reproduced the same protection, establishing causal specificity [67]. Clinically, setanaxib advanced to a Phase 2 randomised trial in primary biliary cholangitis (NCT03226067, $n = 111$), where the twice-daily treatment significantly reduced serum alkaline phosphatase by week 24 ($p = 0.002$ vs. placebo) and improved fatigue scores in patients with moderate-to-severe baseline fatigue [68]. A subsequent Phase 2b/3 trial, TRANSFORM (NCT05014672), tested higher doses (1200 and 1600 mg/day) in PBC patients with elevated liver stiffness already on standard care [69]. In July 2024, the sponsor reported topline results showing statistically significant reductions in alkaline phosphatase of 19% in the 1600 mg/day arm and 14% in the 1200 mg/day arm relative to placebo at week 24, alongside a positive trend in liver stiffness; these figures are sponsor-communicated topline data [70] and have not yet appeared in a peer-reviewed publication.

4.2. Precision Redox Diagnostic Framework

In parallel with therapeutic interventions, precision redox medicine requires replacing current systemic indices with a diagnostic framework that restores the lost spatial and temporal resolution. We propose four principles to guide the design of next-generation redox diagnostic panels. These principles span a spectrum of translational readiness: some elements are already accessible in clinical or near-clinical practice, whereas others remain

primarily translational research tools or prospective exploratory targets. This distinction is made explicit within each principle below.

Principle 1, Compartment specificity. Biomarkers must be selected on the basis of their predominant origin in the compartment that is pathologically relevant for each indication. For mitochondrial cardiac pathology: Prx3-SO_2H in circulating cells as a surrogate for systemic mitochondrial stress and the NAD^+/NADH ratio in plasma or the thiosulphate/sulphate ratio as indicators of mitochondrial sulphenic sulphur flux. For endothelial pathology: the L-arginine/ADMA ratio as a marker of L-arginine availability for endothelial NOS and nitrates/nitrites as a reflection of NO bioactivity in the vascular microdomain.

Principle 2, Capture of reversible PTMs, not only irreversible damage. Diagnostic value lies in the current functional state of the microdomain, not in accumulated damage. Redox proteomic techniques based on dimedone-based chemical probes for the capture and quantification of -SOH [71], isobaric iodoTMT reagents or SNOSID for -SNO capture and site-mapping [72], and tag-switch methods with diazide-based probes for direct -SSH quantification [73] allow measurement of the current PTM distribution in proteins from primary cells of the tissue of interest. Although their implementation in routine clinical practice still requires technological development, their use in translational research cohorts is already feasible.

Principle 3, Multiparametric panels with rigorous pre-analytical controls. No single redox biomarker has sufficient resolution to characterise the state of a microdomain. Panels must combine at least one marker of editing capacity (Prx3 activity or reduced/oxidised Trx2 ratio), one source marker (3-NT for NOS signalling or GEH for active NOX), and one RSS tone marker (thiosulphate, simplified persulphidoproteomics). Standardisation of pre-analytical protocols, maximum time to processing, anticoagulant, temperature, and artefactual oxidation inhibitors is a non-negotiable requirement for these panels to produce reproducible data across laboratories [15].

Principle 4, Dynamic biomarkers for guiding intervention. The greatest value of a precision redox diagnostic framework is not point-in-time diagnosis but monitoring of the response to intervention. A panel anchored to pharmacodynamic biomarkers specific to the therapeutic lever employed, Prx3 hyperoxidation to monitor Srxn1/Trx2 support, the GSNO/3-NT ratio to monitor NOS recoupling, or thiosulphate to monitor restoration of -SSH tone, is proposed here as such a framework, allowing verification of whether the intervention is reaching the correct microdomain and whether the distress threshold is being effectively raised. Without a pharmacodynamic anchor of this kind, redox clinical trials remain blind to the biology they seek to modify [44].

A framework of this kind is useful only if it can eventually leave the research laboratory, and the distance between a research-grade assay and a routine clinical test deserves to be stated plainly rather than assumed away. Reversible PTM proteomics, persulphide quantification with diazide probes and Prx3 hyperoxidation assays currently depend on mass spectrometry platforms, bespoke reagents and analyst expertise that few hospital laboratories possess, with turnaround times measured in days rather than in the hours that most clinical decisions allow. None of the proposed markers yet has an internationally agreed reference method, a certified reference material or an external quality assessment scheme, and all three are prerequisites for laboratory accreditation under ISO 15189 [74] and for regulatory qualification as a companion diagnostic. Cost per sample currently sits one to two orders of magnitude above that of a plasma GSH/GSSG or MDA determination, and reimbursement will reasonably depend on demonstrated incremental clinical value rather than on mechanistic plausibility alone.

A realistic translational path therefore runs through a staged sequence. The first stage is analytical validation and pre-analytical harmonisation of one or two anchor markers per

indication within dedicated multicentre cohorts, with agreed sample handling, stability data and interlaboratory reproducibility. The second is simplification of those assays into targeted higher-throughput formats, such as multiple reaction monitoring panels or validated immunoassays, that a clinical laboratory could reasonably run. The third is prospective evaluation of clinical utility within trials that actually use the marker to select patients or to titrate therapy, since analytical performance alone has never been sufficient to establish clinical value. Until these steps have been completed, the panels proposed here are best regarded as research tools with a defined development pathway, rather than as diagnostics awaiting adoption.

Figure 4 summarises the alternative strategy proposed in Section 4.1. Each panel corresponds to one pharmacological lever: (1) mitochondrial Prx/Trx support via NRF2/Srxn1 activators (sulforaphane), restoring hyperoxidised Prx3; (2) endothelial NOS recoupling (BH4 or L-arginine supply); (3) mitochondrial -SSH tone restoration with targeted slow H_2S donors (AP39-TPP$^+$), protecting Complex I; and (4) selective NOX1/4 inhibition (setanaxib). Before/after states illustrate raised distress thresholds without loss of eustress. Insets link the levers to pilot indications: HFpEF (PKGα oxidation reversal) and Parkinson's disease (DJ-1/α-synuclein protection). The diagram, positioned before Table 1, demonstrates that the correct therapeutic target is not bulk reactive species but the control threshold of the relevant microdomain, which is the conceptual reversal proposed here rather than a claim of full clinical validation.

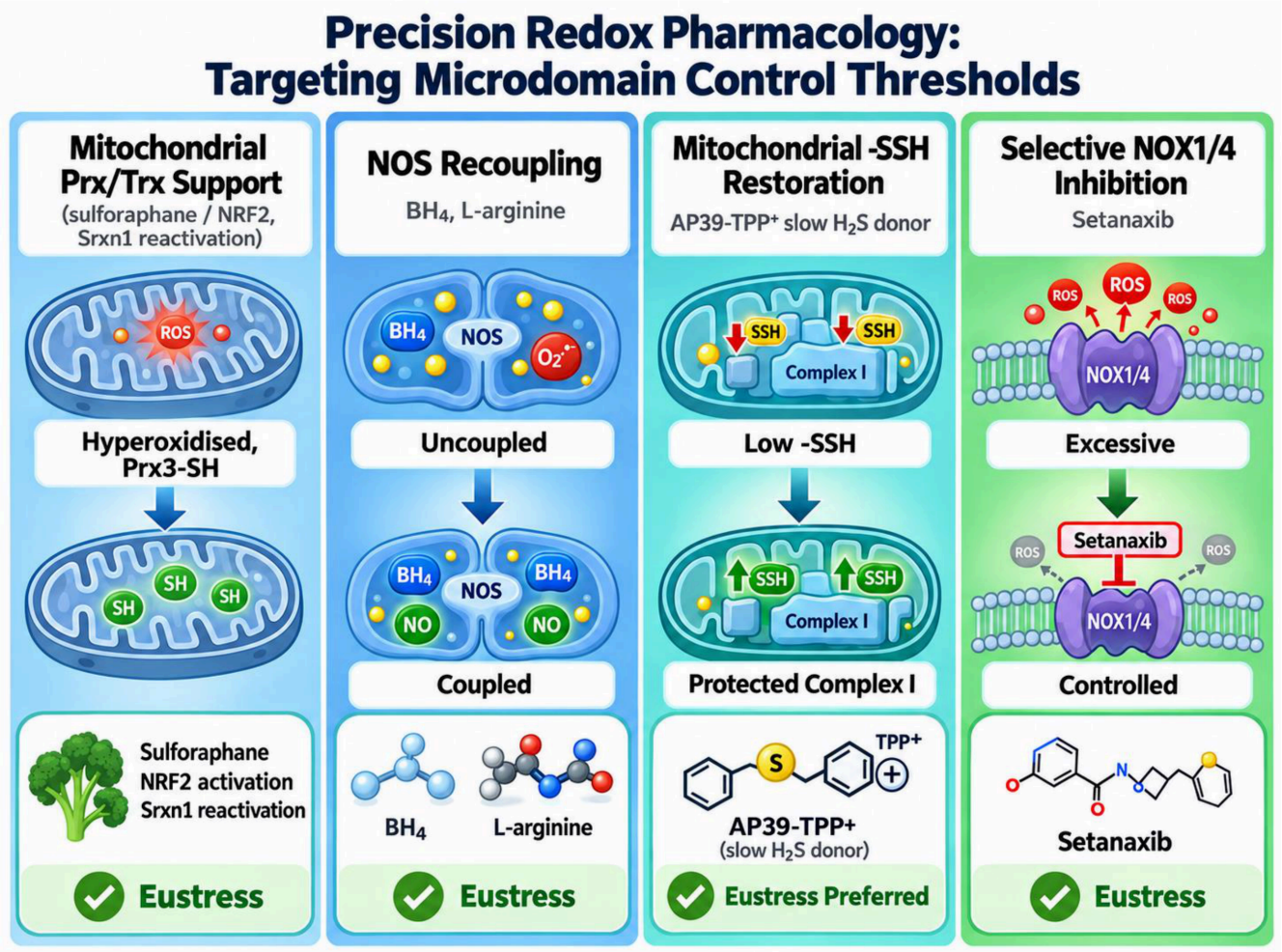


Figure 4. Pharmacological levers that act on specific microdomain control systems instead of bulk scavenging (see also Table 1).

Table 1. Comparison between the conventional redox paradigm and the proposed precision framework.

Dimension	Conventional Paradigm	Proposed Precision Framework	Current Stage of Development
Unit of analysis	Systemic oxidative stress	Subcellular microdomain state	Established concept, translational implementation still pending
Primary biomarker	GSH/GSSG, MDA, 8-oxo-dG (plasma/urine)	Prx3-SO_2H, GSNOR, thiosulphate, -SSH/-SNO proteomics	Conventional biomarkers, routine clinical use. Proposed biomarkers, research-grade assays
Spatial resolution	None (tissue/systemic average)	Compartment-specific (mitochondria, endosome, nucleus)	Feasible in translational cohorts, not yet standardised for clinical use
Temporal resolution	Accumulated damage (days–weeks)	Current functional state (hours)	Analytically demonstrated, pre-analytical protocols not yet harmonised across laboratories
Therapeutic target	Reactive species (scavenge them)	Microdomain control threshold (raise it)	Conceptual reversal argued throughout Sections 2–4 of this work
Intervention	Broad-spectrum antioxidant	Specific lever: Prx/Trx, NOS recoupling, H_2S-SSH, selective NOX	Heterogeneous by lever: setanaxib, positive Phase 2b (PBC); AP39, preclinical only; NOS recoupling and Srxn1 support, preclinical/early translational
Monitoring	Systemic biomarker (no pharmacodynamic anchor)	Dynamic panel anchored to the intervened lever	Proposed only, no validated pharmacodynamic panel yet in clinical use
Individual prediction	AUC-ROC 0.55–0.65	Not established, hypothesis to validate in prospective cohorts	Conventional, established but poor discrimination. Proposed, unvalidated hypothesis awaiting prospective cohorts

Table 1 condenses the reconceptualisation argued across Sections 2–4 into a single side-by-side comparison. Each row contrasts one dimension, spanning the unit of analysis, the primary biomarker, spatial and temporal resolution, the therapeutic target, the type of intervention, and the monitoring strategy, between the conventional systemic paradigm and the proposed microdomain framework. Read together, the rows summarise why global antioxidants and systemic biomarkers underperform and what a compartment-resolved alternative would look like in practice.

4.3. Pilot Indications: Why HFpEF and Parkinson's Disease Are the Most Immediately Translatable Candidates

The precision framework proposed in Sections 4.1 and 4.2 is not equally applicable to all redox pathologies simultaneously. Two indications exceptionally meet the requirements necessary to be the first to benefit from a microdomain strategy: they have a well-characterised subcellular redox pathophysiology, an identifiable microdomain biomarker, and a specific therapeutic lever already in clinical development. Those two indications are heart failure with preserved ejection fraction (HFpEF) [75] and Parkinson's disease [76,77].

In HFpEF, the microdomain redox mechanism is well established and is that described in Section 4.1: endothelial NOS uncoupled by BH4 depletion, a direct consequence of the systemic inflammatory environment generated by the typical comorbidities of this disease, produces superoxide instead of NO in the coronary endothelial microdomain [78]. The superoxide reacts with residual NO to form peroxynitrite, which nitrates key cardiomyocyte proteins: protein kinase G (PKG) becomes oxidised and inactive, unable to phosphorylate

titin and maintain diastolic compliance, and the endoplasmic reticulum unfolded protein response collapses through S-nitrosylation of IRE1α [75].

Left ventricular biopsies from HFpEF patients show significantly higher 3-nitrotyrosine content than controls, with reduced PKG activity and elevated passive cardiomyocyte stiffness [79]. This is precisely the type of compartment signal that conventional systemic biomarkers cannot capture: plasma nitrotyrosine reflects contributions from multiple tissues and cannot distinguish pathological nitration in cardiomyocytes from physiological nitration in circulating macrophages [80]. The candidate microdomain biomarker for HFpEF is the L-arginine/ADMA ratio as a proxy for L-arginine availability for endothelial NOS [81], combined with nitrates/nitrites as a reflection of NO bioactivity in the vascular microdomain [82].

The therapeutic lever already has clinical proof of concept: empagliflozin improves endothelial and cardiomyocyte function in human HFpEF samples through reduction of pro-inflammatory-oxidative pathways and direct reversal of PKGα oxidative dimerisation, restoring the kinase to its monomeric active form and recovering its capacity to phosphorylate titin and reduce passive cardiomyocyte stiffness [83]. This finding is mechanistically important beyond its clinical implications: it demonstrates that the pathological outcome (diastolic stiffness) is causally connected to a specific redox post-translational modification (PKGα disulphide dimerisation at Cys42) in a defined subcellular compartment (cardiomyocyte cytosol/sarcomere interface) and that reversing that modification produces a measurable functional improvement. It thereby validates the central thesis of the microdomain framework: that the appropriate target is not the circulating reactive species but the specific PTM in the compartment where it produces pathological consequences.

In Parkinson's disease, mitochondrial microdomain redox dysfunction in the substantia nigra is the most reproducible pathophysiological finding of the disease. Complex I deficiency, initially reported by Schapira and colleagues in a 1989 letter [84] and confirmed in a full post-mortem study in 1990 [85], with a reduction of approximately 37% in NADH-ubiquinone reductase activity, anatomically specific to the substantia nigra, generates an elevated and chronic superoxide flux in the mitochondrial microdomain of dopaminergic neurones. This superoxide, in the presence of iron accumulated in the substantia nigra of Parkinson's patients [86], generates hydroxyl radicals via the Fenton reaction, initiating the lipid peroxidation cascade that precedes and predicts neuronal loss. Two protein redox sensors document the chronology of this microdomain damage: DJ-1, whose oxidation of Cys106 to cysteinesulphinic acid under oxidative stress directs the protein to the mitochondria as a neuroprotective mechanism [87], and α-synuclein, whose selective nitration on tyrosine residues by peroxynitrite is present in the Lewy bodies of Parkinson's patients and promotes fibril formation [88]. The translational relevance of DJ-1 as a compartment-resolved biomarker is supported by the finding that oxidised DJ-1 interacts with the 20S proteasome in erythrocytes of early-stage Parkinson's disease patients and that this interaction is detectable in peripheral blood at an early clinical stage of disease, offering a window for compartment-resolved redox diagnosis in an accessible tissue [89]. Crucially, GSH depletion in the substantia nigra precedes Complex I dysfunction and iron accumulation, already present in presymptomatic incidental Lewy body disease, identifying the loss of mitochondrial editing capacity as the initiating event in the redox collapse [90]. This sequence, editor failure first, followed by oxidant accumulation and finally irreversible PTMs in key proteins such as α-synuclein and aconitase, is precisely the pattern of progressive microdomain distress that the proposed diagnostic framework is designed to detect at the earliest possible stage and that the therapeutic framework targets by restoring editing capacity before the collapse becomes irreversible. The most directly aligned therapeutic lever is restoration of mitochondrial -SSH tone via slow H_2S donors,

given that persulphidation of Complex I cysteines, demonstrated most directly for the NDUFB7 subunit in cardiomyocytes, protects against irreversible oxidation and preserves Complex I activity [60,61].

The selection of these two indications as pilot candidates is not arbitrary. Both share three properties that make them testable with currently available tools: a microdomain pathophysiology documented in human tissue, a measurable compartment biomarker in accessible samples (L-arginine/ADMA ratio and nitrates/nitrites for HFpEF, erythrocyte oxidised DJ-1 and nitrated α-synuclein for Parkinson's) [81,82,88], and a therapeutic lever in active clinical development. The first precision redox clinical trial in these indications, designed with the microdomain concept as the unit of analysis and compartment-resolved pharmacodynamic biomarkers as the primary pharmacological engagement endpoint, would constitute the proof-of-principle demonstration of the proposed framework.

5. Discussion

5.1. A Reinterpretation of the Failed Trials

The accumulated evidence of therapeutic failures with antioxidants must not be read as evidence that oxidative stress is irrelevant to the diseases studied. Read from the microdomain framework, that evidence is compatible with the opposite reading, namely, that redox signalling is sufficiently finely regulated that perturbing it with non-specific tools may yield no net benefit and, in certain settings, net harm. The negative trials with vitamin E, beta-carotene and N-acetylcysteine do not refute the redox hypothesis; they are at least consistent with it, interpreted through a more sophisticated mechanistic lens [13].

This reinterpretation has important implications for research policy. One implication, which should itself be tested rather than assumed, is that further trials of broad-spectrum antioxidants at different doses or in different populations may add limited information unless they are accompanied by a conceptual redesign. What appears necessary is a generation of trials built around the microdomain as the unit of analysis, with compartment-resolved pharmacodynamic biomarkers and interventions directed at specific levers. This is a hypothesis about trial design, and its validation will require the prospective data that such trials would themselves generate [72].

This reframing also has implications beyond individual trial design. If compartment-resolved biomarkers are to guide patient selection and dose titration for microdomain-directed therapies, they will need to be developed and validated as companion diagnostics in parallel with the drugs themselves, rather than added retrospectively once a candidate reaches late-phase trials. This has direct consequences for how the pharmaceutical industry and regulatory agencies plan precision redox programmes, since companion diagnostic development, analytical validation and regulatory qualification typically require lead times comparable to those of the therapeutic candidate itself, and building them as an afterthought is a recognised cause of delay in other precision medicine fields.

5.2. Limitations of the Proposed Framework

The diagnostic and pharmacological framework proposed in this work has real limitations that are worth making explicit. First, the compartment-resolved biomarkers proposed, especially reversible PTM proteomics, are not yet available in routine clinical practice and require specialised analytical infrastructure. Their large-scale implementation in multicentre clinical trials requires a pre-analytical standardisation and analytical validation effort that is ongoing but not yet completed.

Second, the relationship between mitochondrial microdomain biomarkers measured in circulating cells (leucocytes and platelets) and the state of the microdomain in the target tissue (cardiomyocyte, neurone, and hepatocyte) assumes a degree of correlation that is

documented for some disease–biomarker combinations but not systematically validated. Target-tissue biopsy remains the gold standard for direct microdomain characterisation, with the risks and accessibility limitations this entails [72].

Third, the proposed pharmacological interventions, mitochondria-directed H_2S donors, selective NOX inhibitors, and Srxn1 activators, are at different stages of preclinical and clinical development. None has yet demonstrated modification of clinical events in Phase III trials. The proposed framework is a map of the right direction, not a clinical protocol ready for implementation.

Fourth, the proposed levers carry risks that must be weighed against their conceptual advantages and that are not yet fully characterised. Compartment-directed delivery reduces but does not eliminate off-target action in microdomains other than the intended one, since organelle targeting via the TPP^+ cation, for instance, is not absolute and residual accumulation in non-target mitochondria across tissues remains possible. The long-term mitochondrial safety of TPP^+-conjugated compounds such as AP39 has only been assessed over short experimental windows, and chronic accumulation of lipophilic cations in the mitochondrial matrix is a recognised concern for this chemical class. In addition, interindividual heterogeneity in the baseline expression and activity of redox editors, Prx3, Srxn1, Trx2 and NOX isoforms among them, is likely to be substantial and is not yet mapped, which means that a lever calibrated against average editor capacity may be insufficiently active in some patients and excessive in others.

Fifth, the framework as presented treats each microdomain largely in isolation, whereas mitochondrial, endothelial and nuclear microdomains are metabolically and spatially interconnected, so that an intervention raising the distress threshold in one compartment could, in principle, shift the redox burden towards an adjacent one rather than resolving it. Whether such compensatory shifts occur, and at what magnitude, is an open empirical question that compartment-resolved longitudinal biomarker panels, rather than single-timepoint measurements, would be needed to answer.

Challenges of Clinical Implementation

Beyond the scientific limitations above, translating this framework into routine practice faces three practical obstacles worth stating explicitly. The first is cost: compartment-resolved redox proteomics and multiparametric dynamic panels are markedly more expensive per sample than a plasma GSH/GSSG or MDA assay, and healthcare systems will reasonably ask for demonstrated incremental clinical value before reimbursing them. The second is tissue access: several of the proposed biomarkers are best measured in the target tissue itself, yet cardiomyocyte or neuronal biopsy is rarely justifiable outside specific clinical circumstances, so progress depends on validating circulating surrogates, leucocytes, platelets or extracellular vesicles, against tissue truth in dedicated cohorts. The third concerns regulation: compartment-targeted nanoformulations and lipophilic cation conjugates sit at the intersection of pharmaceutical and nanomedicine regulatory pathways, which typically require additional characterisation of biodistribution, clearance and long-term tissue accumulation relative to conventional small molecules, and this adds development time that should be anticipated rather than treated as a late-stage surprise. Beyond this near-term translational path, a longer-term research agenda for the field is outlined in Box 1, which considers how artificial intelligence could extend this diagnostic framework.

Box 1. Outlook: the role of artificial intelligence in the next generation of redox biomarkers.

The precision diagnostic framework proposed here generates high-dimensionality multiparametric panels whose interpretation exceeds conventional clinical algorithms, exactly the kind of problem, with tens of variables and non-linear interactions between editors and sources, for which deep learning is mathematically well suited. AI applied to redox biology has, however, followed a trajectory disconnected from the microdomain framework: powerful tools exist but have not been articulated as an integrated system for this problem [91]. What follows should therefore be read as a structured research agenda rather than an established framework; the individual components exist and are validated in their own domains, but their coordinated deployment as a precision redox diagnostic architecture has not yet been demonstrated in prospective cohorts.

A first layer already exists in computational prediction of cysteine redox PTMs: DeepNitro predicts Tyr nitration (AUC 0.65), Trp nitration (AUC 0.80) and Cys S-nitrosylation (AUC 0.70) [92]; DeepCSO predicts S-sulphenylation via LSTM networks (AUC 0.82–0.85) [93]; and pCysMod simultaneously predicts five cysteine modifications, S-nitrosylation, S-palmitoylation, S-sulphenylation, S-sulphidation and S-sulphinylation, with an AUC of 0.79–0.88 [94], all trained on the CysModDB database of over 70,000 verified sites [95]. A second, tissue-level layer is the OxiMouse redox proteomic atlas, which mapped roughly 34,000 cysteine sites across ten mouse tissues during ageing, showing that redox regulation is tissue-specific and temporally dynamic, precisely the high-dimensional data that ML models are suited to interpret [96].

A second application is patient stratification by oxidative signature. ML on oxidative biomarker panels already has proof of principle: an SVM trained on plasma thiols, oxidised LDL, glutathione peroxidase, zinc and the Cu/Zn ratio predicted pulmonary injury severity in hospitalised COVID-19 patients [97], and models built on oxidative stress-related genes (DUSP1, PRDX6, and S100A8) diagnosed diabetic nephropathy with an AUC of 0.946 [98]. These results share the same structural limitation the present framework identifies: training on systemic rather than compartment-specific biomarkers reintroduces the background noise that degrades conventional biomarkers, such that retraining on compartment-resolved panels, as proposed here, remains the necessary, untaken step, consistent with the broader promise of machine learning in clinical medicine [99].

The most ambitious application, and the genuine methodological gap this work identifies, is a graph neural network (GNN) trained on the functional topology of the redoxome, the nodes being proteins with reactive cysteines and the edges being kinetic competition or redox-equivalent transfer between editors and sources. Feasibility is already established in adjacent biochemistry: a GNN trained on the Reactome network, which includes core redox pathways, classified 51 tissue types and recovered pathological reactions in psoriasis [100]. Extending this to the redoxome specifically, with microdomain topology as the structural dimension and OxiMouse or iodoTMT PTM proteomics as training data, is the concrete proposal advanced here. The experimental tool to generate microdomain-resolved training data, PL-OxICAT, which combines redox proteomics with proximity labelling to resolve cysteine oxidation within defined subcellular compartments at a resolution of a few tens of nanometres, already exists [101]. Microdomain data generated with PL-OxICAT, structured as a compartment-resolved redox graph and modelled with GNNs, could then predict microdomain redox signature and guide selection of the optimal therapeutic lever.

6. Conclusions

The historical failure of broad-spectrum antioxidants does not invalidate the redox hypothesis itself, but rather points to a conceptual limitation in applying wide-band tools to an information system with a fine spatial structure and high compartmentalisation. In the same way, the limited predictive capacity of conventional redox biomarkers does not reflect an absence of relevant biological signals, but rather the irreversible loss of spatial and temporal resolution that occurs when multiple subcellular compartments are collapsed into a single systemic index.

The conceptual alternative proposed here does not require technologies that do not yet exist, but rather a change in the unit of analysis. Replacing the concept of "systemic oxidative stress" with that of "subcellular microdomain state" as the object of measurement and as the target of intervention opens a radically different pharmacological and diagnostic space. The tools with the correct conceptual profile to operate within that space already exist at various stages of development, including mitochondria-directed H_2S donors, selective NOX isoform inhibitors, Srxn1/Prx3 pathway activators, and reversible post-translational modification proteomics. The primary challenge may be at least as much epistemologi-

cal as technological. The hypothesis advanced here is that recognising the redox system as operating with the logic of a spatially structured code, rather than simple accumulation, opens a more productive therapeutic and diagnostic space than has hitherto been explored. Whether this reframing translates into improved clinical outcomes remains to be demonstrated in prospective trials designed with this logic as their organising principle.

This reframing carries direct implications for the design of clinical trials, for the selection of therapeutic efficacy biomarkers, and for the interpretation of omics data in inflammatory, metabolic, and neurodegenerative contexts. A trial that intervenes on the redox system without stratifying by the state of the relevant microdomains in the target tissue incorporates a source of structural variability that no increase in sample size can correct.

The ultimate objective is not to silence the redox language, but to learn to read it with the resolution that its complexity demands, to intervene with surgical precision in the dialect that is being misexpressed, and to verify that the message has been correctly amended.

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Appendix A. Glossary of Key Terms

For readers less familiar with redox biochemistry: a microdomain is a subcellular compartment (e.g., the mitochondrial matrix or the endothelial cytosol) in which reactive species are generated, act and are neutralised within a confined volume, so that their local concentration differs substantially from the average measured in plasma or whole-tissue lysates. Redox eustress denotes physiological, low-amplitude signalling by reactive species, whereas redox distress denotes pathological, high-amplitude or sustained signalling that overwhelms local repair capacity. A post-translational modification (PTM) is a reversible chemical mark added to a cysteine or tyrosine residue of a protein; sulphenylation (-SOH), S-nitrosylation (-SNO) and persulphidation (-SSH) are the three principal examples discussed here, and each can be written, read and erased by specific enzymes in a manner analogous to an epigenetic mark. Prx3-SO_2H refers to the hyperoxidised, catalytically inactive form of peroxiredoxin 3, and Srxn1 (sulphiredoxin 1) is the enzyme that reverses this inactivation. These terms are defined again at first use in the main text.

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