Perspective



# An alternate view of fullerene material's antioxidant activity (NOLF 4 in series:"will nanocarbon onion-like fullerenes (NOLFs) play a decisive role in the future of molecular medicine?")


## Abstract

Although they have exhibited excellent antioxidant effects in both in vitro and in vivo research, a thorough understanding of fullerene material's biological interactions and antioxidant activities remain unsettled science. Pristine fullerenes are electrophilic, and potentially, free radical molecules due to an affinity for electrons when excited. They often behave like electron-deficient alkenes and interact with other molecules through addition and substitution reactions. Structurally, fullerenes possess large, dense, electron clouds and an unusual truncated icosahedral arrangement of carbon atoms that form lower Hückel electron orbital vacancies while maintaining the valence saturation of the outer orbital. Fullerene materials are known to bind with mitochondria, microtubules, enzymes, nucleotides, and other cellular proteins and biomolecules, and this has raised concern among some researchers, but the biocompatibility of fullerene materials, and many of their functionalized derivatives, is now generally accepted. Pristine fullerene materials are often referred to as "super free radical scavengers," but this is an unlikely primary mechanism in biological systems where oxidative stress management is enzymatically responsive to the dynamic quantum criticality of living systems by necessity. Alternately, fullerene material's strong electron affinity is consistent with preventing excess electrons in the ETC by functioning in a capacitor-like electron sink mechanism. There mild electrophilic nature stimulates Nrf-2/ARE pathways producing both an antioxidant response, as well as, mitochondrial biogenesis helping to maintain mitochondrial homeostasis. Finally, bio coronation may allow pristine fullerene materials to scavenge radicals while maintaining dynamic cellular regulation of antioxidant/ROS balance.


**Keywords:** Fullerene materials, perturbative stress, oxidative stress regulation, cell danger response, mitohormesis, para-hormesis, super free radical scavengers, electrophiles, nucleophilic tone, uncoupling proteins, mitochondrial biogenesis

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**Abbreviations:** NOLF, nano onion-like fullerene; ETC, electron transport chain; CDR, cell danger response; ROS, reactive oxygen species; RNS, reactive nitrogen species; ER, endoplasmic reticulum; MitoHormesis, mitochondrial hermes's; Nrf2, nuclear factor, elytroid 2 like 2; KEAP, ketch-like ECH-associated protein 1; ARE, antioxidant response element, EpRE, electrophile-responsive element

## Preface

The following is the fourth in a series of articles on Nano carbon onion-like fullerenes, titled: Will Nanocarbon Onion-Like Fullerenes (NOLFs) Play a Decisive Role in the Future of Molecular Medicine? Which presents a theoretical perspective on fullerene-biological interaction in producing a biological benefit? This fourth article considers fullerene material's excellent antioxidant activity and challenges the "super free radical scavenger" mechanism as the primary contributor to the beneficial biological effects reported in vivo, and presents alternative mechanisms through which fullerene materials may enhance oxidative stress management to produce an antioxidant effect.

## Introduction

Understanding the biological interaction of fullerenes in vivo must necessarily include pursuing an understanding of how fullerene properties mediate mitochondrial and cellular machinery (proteins). Mitochondria play a central role in regulation of cell bioenergetics and biosynthesis[1,2] and the non-energetic roles as regulators of the response to pathologic disturbances altering the cellular energy dynamics[3] apoptosis, intracellular messenger, nuclear gene expression, as well as their manifestation in disease and cancer states.[4] Ordered cellular machinery, such as mitochondria, endoplasmic reticulum, microtubules, and DNA may respond, modify, and adapt, or become chaotic or disordered in response to various stimuli. These stimuli induced disturbances are called perturbations. Mild mitochondrial and cellular perturbations are ubiquitous and stimulate beneficial adaptation to resist better homeostatic stressors, otherwise known as heresies.

The hermetic response is essential for life processes. In the case of mitochondria, this adaptation process is called mitohormesis and helps to maintain the mitochondrial regulatory role in the cell's response to future pathologic free radical stressors.[5,6] Interestingly, mitohormesis has been proposed to underlie the evolution of intelligence and could play a role in maintaining it.[7] However, if perturbations are prolonged or are excessively disruptive, they can lead to sudden or progressive damage to critical cell structures resulting in the decline of the cell if order is not or cannot be restored. Homeostatic and metabolic set point feedback from within the mitochondria acts as a checkpoint before cellular action and to dictate commands or provide signals to change biological outcomes.[1] Damage to mitochondrial respiration enzymes impair normal oxidative phosphorylation (OX/PHOS), resulting in a metabolic shift towards an atypical redox status, disrupted calcium homeostasis, and genomic instability.[8,9] Progressive oxidative stress further damages mitochondrial and cellular structures, DNA, and membranes. Mitochondrial dysfunction is implicated in metabolic, immunologic, and degenerative diseases, as well as cancer and aging.[2]

## Evidence that fullerenes are not free radical scavengers in biological systems

The current consensus is that fullerene materials are often referred to as "super free radical sponges" for their antioxidant effects ranging from inflammation to longevity. Fullerenes reduce oxidative stress, but this antioxidant activity is not necessarily synonymous with free radical scavenging in biological systems. A direct scavenging role of pristine fullerene is a common explanation for many of fullerene's antioxidant activity and resulting benefits in vivo.[10–15] Free radical scavenging activity from in vitro and polymer chemistry application studies[16–18] appears to have heavily influenced perceptions of fullerene material's antioxidant role biological systems. However, potent free radical scavenging evidenced in fullerene polymer and in vitro research falls well short of explaining the many diverse biological effects in vivo. Given the prevailing concept of a free radical scavenger as an antioxidant, it may appear confusing that the free radical scavenging antioxidant activity used in polymer chemistry and food preservation, which is the interplay of phenolic and hydro peroxide-reducing compounds, would not be efficient, nor likely in biological systems where highly regulated and dynamic enzymatic removal of free radicals is the primary antioxidant mechanism.[19] The possible exception is the inhibition of lipid peroxidation and membrane breakdown, where fullerene $C_{60}$ demonstrated a more significant inhibitory effect than vitamin E.[11]

As Forman points out, there are many definitions of word antioxidants. In chemistry, antioxidants are electron donors (nucleophiles.) Free radicals are molecules that are seeking or accepting electrons (electrophiles.) Classically, nucleophile antioxidants reduce or scavenge, electrophilic free radicals to prevent these free radicals from taking electrons from another molecule causing damage, or in many instances, radicalizing it. The nucleophile reduction of the electrophile prevents further propagation of electron stealing, and more importantly, from a biological perspective, propagation of damage and the decrease, or loss of function, to critical cellular structures such as proteins and lipids in membranes, DNA, organelles, and enzymes, including those in the mitochondrial ETC.[19,2]

Pristine fullerene properties also suggest direct free radical scavenging in a biological system is unlikely. Pristine fullerenes behave like electron-deficient alkenes unless modified.[20,21] They are nonpolar dielectric molecules that are described as containing degenerate lower orbitals with unpaired electrons rather than in their outer valence shell. This arrangement contributes to a low reactivity in their native state, which decreases with increasing size. Fullerenes, like other electrophiles, accept electrons but primarily react with other molecules through addition and substitution reactions.[22] Perhaps one of the most persuasive arguments against a potent free radical scavenging role in biological systems is that if left uncontrolled, ROS scavenging would interfere with many aspects of cellular function. The concept that ROS, as a toxic metabolic by-product, fails to consider the emerging importance of ROS in mitochondria's regulatory and sensing role, which includes ROS signaling, oxidative stress management, genetic transcription, mitohormesis, elements of the quantum cell, and structured cell water/hydration. These ROS functions in cellular biochemistry are all necessary for the adaptation and health of the organism itself.[23–25,9] While many fullerene materials appear to produce excellent antioxidant protection in biological systems, nonlinear changes in ROS levels vary for different fullerenes derivatives depending on their chemical structure, and when modified, their surface charge, suggesting the complex nature of fullerene antioxidant effects.[26] It is important to note that fullerenes stimulate Nrf2 signaling in cells resulting in genomic transcription of oxidative stress management and signaling proteins.[27]

Pristine fullerenes and many of their derivatives, behave as mild electrophilic molecules in biological systems. Many known antioxidants are electrophilic or are modified to become electrophilic in vivo. They produce nucleophile tone through the paradoxical oxidative activation of the Nrf2 /KEAP/ARE signaling pathway, which mediates the bio genomic coordination between nuclear and mitochondrial genomes transcribing OXPHOS enzymes and helping to maintain protective oxidoreductases and their nucleophile substrates, rather than through a direct free radical scavenging mechanism.[19,28] Cell-mediated regulation of non-toxic concentrations of enzymatic and other mild electrophiles maintains the appropriate nucleophile tone through a mechanism referred to as '*parahormesis.*' Parahormesis provides a means for boosting antioxidant enzymes, as well as damage removal and repair systems (for proteins, lipids, and DNA), at the optimal levels consistent with good health.[19]

## Oxidative stress management and alternate mechanisms beneficial in biological systems

The oxidative stress theory proposes that ROS, primarily from excess electron build up in the electron carriers, damages mitochondrial membranes, enzymatic proteins of the ETC, and adjacent mitochondrial DNA (mtDNA) found in disease and senescence.[29] Excess electrons in the ETC, especially complexes I, II, and IV, increase ROS production and increase oxidative stress. The uncoupling of OXPHOS from ATP synthase by reducing proton motive differential across the inner mitochondrial membrane helps maintain a low electron density in the ETC, resulting in decreased ROS production and reduction of excess oxidative stress. Chistyakov et al.[30] offered an alternate proposal to free radical scavenging by fullerene $C_{60}$. Since many fullerene materials can translocate across the lipid bilayer[31] they proposed that fullerene $C_{60}$ can decrease ROS production by localizing to the inner mitochondrial membrane. $C_{60}$ then acquires a positive charge by absorbing protons and reduce the proton motive differential across the inner mitochondrial membrane.[30] Unfortunately, while mild uncoupling may have a role in mediating ROS production, uncoupling may exacerbate perturbed homeostasis and has not been shown to increase longevity.[32]

Additionally, unlike individual $C_{60}$, many fullerene molecules, such as NOLFs, are far too large to localize to the inner mitochondrial membrane yet exhibit beneficial, and perhaps offer greater antioxidant cell benefits, than $C_{60}$.[33] Santos et al.[34] reported that $C_{60}$ inhibited uncoupled respiration to State 3 levels, increased State 4 respiration, but reduced the inner membrane electrical potential.[34] These effects on mitochondrial bioenergetics points suggest that fullerene mechanisms in mitochondria are more complicated than simple scavenging since an uncoupling effect can increase State IV levels. The recognized roles of ROS and mitochondria in cells are also changing. While excess ROS creates oxidative stress that is known to damage cellular structures, ROS cannot be considered as merely toxic by-products of OXPHOS energy production. They are highly regulated molecules, acting as second messengers in signaling cascades controlling diverse cell functions. Munroe et al. point out that the mitochondrial antioxidant system does not merely minimize oxidative damage but is instead an integral component of the cell's ROS regulatory system.[9]

There is evidence that the mitochondria may not be the main producer of cellular energy as assumed. ROS production, OXPHOS enzymes, and energy production are not restricted to the mitochondria and that mitochondria may only produce enough ATP for their internal needs[35–37] Morelli (personal communication) proposed that mitochondria's bioenergetics role is to control the production of the bioenergetics machinery. He supports his position by pointing out that the exchange of solutes and polyamines, including ATP and ADP, across the mitochondrial membrane is very difficult [38] and adenine nucleotide transferees (ANT) is involved in mitophagy but does not perform ATP-ADP anti-port function.[39] Alternately, fullerene material's strong electron affinity is consistent with preventing excess electrons in the OX/PHOS enzymes by functioning in a capacitor-like electron sink mechanism.[40] Electrons 'leaking' from enzymes such as ETC complex I-III are primary sources of ROS in the ETC complexes and enzymes enhancing the reduction status of NAD+ and NADP+ pools, as well as, $H_2O_2$ consumption.[9] A capacitor-like electron sink would contribute significantly to maintaining an oxidative state in the ETC. The result would be the decreased production of ROS[32] available to damage to mitochondrial proteins, membranes, mtDNA, as well as other cellular structures that contribute to disease and aging.[41,9,42]

Perhaps, it is more important than the regulation of oxidative stress is mitochondrial biogenesis. Mitochondrial biogenesis is a response to the energy demands of the cell which controls mitochondrial replication, replacement, and mitophagy. Mitochondrial biogenesis is mediated through Nrf-2 signaling pathways and has a much greater effect on mitochondrial related disease and aging than does control of ROS in cells.[43] Mitochondrial DNA transcription and translation involve a regulatory loop between PPAR-Gamma-Coactivator-1α (PGC-1α) and Nrf-2. Fullerene materials activate both antioxidant protection and mitochondrial biogenesis, helping to maintain mitochondrial homeostasis by stimulating Nrf-2 pathways.[44,27]

Naturally occurring fullerene materials have had evolutionary exposure in biological systems. Along with evolved immune defenses, potential exploitation of advantageous fullerene properties is likely to have developed to benefit cell systems. In biological environments, fullerene's are rapidly coated with of corona a proteins, lipids and biomolecules.[45,46] The biocoronation of pristine and derivatives of fullerene material is an evolutionary response that offers an alternative explanation for the free radical scavenging effects of electrophilic fullerenes. Combining electron acceptors with protein or amino electron donors is exploited in polymer chemistry for charge transfer[47] and fullerene-amino derivatives are highly effective radical scavengers.[48]

Fullerene electron acceptors in association with amino, protein, or other molecular electron donors would result from binding with biomolecules and proteins through electrostatic and hydrogen bonding or absorption on to membrane proteins and lipids through electrostatic and dipolar interactions.[49] The free radical scavenging activity of biocoronated fullerene and bimolecular-fullerene complexes regulated by the electromagnetic cellular environment would continue to remain under mitochondrial dynamic control regulating cellular oxidative stress response.

## Conclusion

The concept of ROS as a toxic mitochondrial metabolite in disease and senescence has led to many ROS-lowering proposals that, when tested, failed to show a positive association between antioxidant supplementation and health or longevity benefits. The difficulty in artificially replicating biological systems and fullerene antioxidant observation coming from polymer research, molecular dynamic modeling, as well as in vitro biological studies may not accurately reflect the antioxidant mechanisms of the dynamic oxidative stress management system necessary in living systems. Evidence that pristine fullerene material's antioxidant activity is a result of electron donor scavenging in vivo is also lacking and is inconsistent with fullerene's structure and its known electrophilic electron affinity.[50] The alternative fullerene antioxidant mechanism presented here suggests there is much we have yet to learn regarding the underlying mechanisms of fullerene–biological interaction, which increasingly appears to extend well beyond their antioxidant effect on oxidative stress in cells. Going forward, the future advancement of fullerene material, their functionalization, and their applications in medicine will require a renewed examination of their biologic mechanisms in intact biological systems.

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## Conflicts of interest

Author declares that there is no conflict of interest.

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