Antioxidants and Nanotechnology: Promises and Limits of Potentially Disruptive Approaches in the Treatment of Central Nervous System Diseases

Antioxidants and nanotechnology: Promises and limits of potentially disruptive approaches in the treatment of central nervous system diseases

Chiara Martinelli†, Carlotta Pucci*†, Matteo Battaglini*†, Attilio Marino, and Gianni Ciofani**

Dr. Chiara Martinelli, Dr. Carlotta Pucci, Matteo Battaglini, Prof. Gianni Ciofani
Istituto Italiano di Tecnologia, Smart Bio-Interfaces, Viale Rinaldo Piaggio 34, 56025 Pontedera, Pisa, Italy
Email: chiara.martinelli@iit.it, carlotta.pucci@iit.it, gianni.ciofani@iit.it

Matteo Battaglini
Scuola Superiore Sant'Anna, The Biorobotics Institute, Viale Rinaldo Piaggio 34, 56025 Pontedera, Pisa, Italy

**Corresponding authors*
†These authors contributed equally to this work

Keywords: antioxidants; nanomaterials; drug delivery; central nervous system disorders.

Abstract

Many central nervous system (CNS) diseases are still incurable and only symptomatic treatments are available. Oxidative stress is suggested to be a common hallmark, being able to cause and exacerbate the neuronal cell dysfunctions at the basis of these pathologies, such as mitochondrial impairments, accumulation of misfolded proteins, cell membrane damages, and apoptosis induction. Several antioxidant compounds have been tested as potential countermeasures for CNS disorders, but their efficacy has been often hindered by the loss of antioxidant properties due to enzymatic degradation, low bioavailability, poor water solubility, and insufficient blood-brain barrier crossing efficiency. To overcome the limitations of antioxidant molecules, exploitation of nanostructures, either for their delivery or with inherent antioxidant properties, has been proposed. In this review, after a brief discussion concerning the role of the blood-brain barrier in the CNS and the involvement of oxidative stress in some neurodegenerative diseases, the most interesting researches concerning the use of nano-antioxidants will be introduced and discussed, focusing on the synthesis procedures, functionalization strategies, *in vitro* and *in vivo* tests, and on recent clinical trials.

1. Introduction

Reactive oxygen species (ROS) are normally produced as byproducts of our physiological metabolism, in particular of the aerobic respiration carried out by mitochondria.[1] Physiologically, the production and removal of ROS need to be maintained in a precise balance, in order to get suitable signaling necessary for cell growth and correct metabolism.[2] When ROS are not properly scavenged by means of intracellular enzymes or endogenous antioxidant molecules, they start accumulating and damaging biological macromolecules, such as lipids, proteins, and DNA. In this case, the imbalance towards ROS species accumulation is often referred to as oxidative stress. Indeed, oxidative impairment of mitochondrial DNA and proteins induces further ROS production and reduces ATP synthesis.[3] These effects become detrimental in the case of damaged and aged mitochondria.[4]

The ability of mitochondria to dysregulate calcium homeostasis, which contributes to regular neuronal activity, has been shown to be involved in the onset of many neurodegenerative diseases.[5–9] The mechanism underlying this process relies on hyperactivation of N-methyl-D-aspartate (NMDA)-type receptors, that increase intraneuronal calcium concentration and stimulate opening of the mitochondrial transition pore (MTP), leading to release of pro-apoptotic proteins.[10] NF-E2-related factor 2 (Nrf2) transcription factor has been proven to be implicated in the antioxidant response, by activating free radical scavengers and antioxidant enzymes. Its down-regulation correlates with neurodegenerative diseases (NDs), while its overexpression presents many beneficial and protective effects.[11] Due to the above-mentioned reasons, it is evident that the brain is extremely sensitive to oxidative stress.[2] Many central nervous system (CNS) diseases are characterized by accumulation of ROS, which induce severe damages to the brain tissues and irreversible neurodegeneration.[12,13]

In the latest decades, the consumption of antioxidant molecules in the daily diet has demonstrated to show positive effects in relieving symptoms in patients affected by NDs.[14] Many efforts have been devoted to investigating the properties of both natural and synthetic compounds, and many researches have shown their efficacy on *in vitro* and *in vivo* models of human disorders.

Despite the great promises and the potentiality of these molecules, their application in medicine is still limited because of their low bioavailability and poor solubility in water. Moreover, they easily undergo degradation, dramatically reducing their antioxidant capacity.[15] Another great difficulty encountered, in general, by therapies of NDs is the need to cross the blood-brain barrier (BBB), the natural defense system that isolates and protects the CNS from possible dangerous agents. In order to overcome all these issues, nanomedicine has been proposed as an innovative tool to deliver antioxidants to the brain. Due to their physicochemical characteristics, nanoparticles are able to encapsulate a significant amount of drug, protecting it from degradation, and increasing its bioavailability, stability, and pharmacokinetic properties, while reducing possible toxic side effects.[16] Nanoparticles can be easily functionalized with specific ligands that favor the crossing of the BBB and/or that target particular cells or organelles.[17] For instance, being the mitochondria the organelles where ROS are produced in larger amounts, they represent one of the most coveted target site of antioxidant therapies.[18] The development of nanomedicine and the possibility to design *ad hoc* nanocarriers for targeted delivery of antioxidants to the brain have paved the way for innovative therapeutic solutions.

Many different systems, both inorganic and organic, have been developed and tested for this purpose. Polymeric nanoparticles, for instance, are being exploited for the treatment of many diseases, such as cancer, due to their biocompatibility and structural stability, and several polymers have been already approved by the Food and Drug Administration (FDA).[19] Lipid-based nanoparticles, such as liposomes and solid lipid nanoparticles, also offer a versatile

platform for the fabrication of nano-delivery systems. In particular, liposomes are well-recognized nanoparticles for medical applications, due to their low toxicity and to the possibility to encapsulate both hydrophobic and hydrophilic drugs.[16] For this reason, they could be exploited also for antioxidant therapy. Finally, several kinds of inorganic nanoparticles, such as cerium oxide nanoparticles, are being tested not only as possible delivery tools, but also for their inherent antioxidant activity.[20]

In this work, firstly, a description of the most common NDs and some examples of neurological pathologies sharing mitochondrial dysfunctions and excessive ROS production will be provided. Then, a detailed overview of different nanomaterials, of their main fabrication procedures, and of their applications as efficient and versatile delivery systems will be provided, underlining their multiple advantages and the few limitations that need to be overcome before further development in the clinical context. Moreover, the main strategies for active targeting of these nano-systems to the brain and, in particular, to mitochondria, will be also introduced. Finally, an overview of the clinical trials will be provided showing the importance of additional investigations to confirm the disruptive potential of nano-antioxidants for treating CNS diseases.

2. Reactive oxygen species and oxidative stress

With the term ROS, small reactive molecules, such as superoxide anions (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical ($^{\bullet}OH$), are identified.[21] ROS play a pivotal role in the maintenance of cellular functions and are involved in several important processes, including energy production and metabolism, autophagy, apoptosis, and immune responses.[22] Under physiological conditions, ROS are mainly generated by mitochondria through the reduction of molecular O_2[15] and their levels are maintained under control by several defense mechanisms, including endogenous antioxidant molecules (glutathione, cysteine, vitamins, selenoprotein) and antioxidant enzymes (catalase, superoxide dismutase, glutathione peroxidase).[23] Oxidative stress occurs when these defense mechanisms are overwhelmed and are no longer

able to control the ROS physiological levels, leading to serious impairment of cellular functions.[23] Indeed ROS, being extremely reactive molecules, are able to interact with several intracellular macromolecules, compromising their proper structure and functions. One of the main damages caused by oxidative stress is due to lipid peroxidation: ROS reacting with polyunsaturated fatty acids (PUFA) present in cell membranes generate peroxyl radicals that can subsequently react with other PUFA, generating lipid peroxides. Lipid peroxides are more unstable than PUFA and can easily break into radicals by a chain reaction that can seriously compromise the integrity of cell membranes.[24] Other important targets of ROS are proteins, nucleic acids, and mitochondria.[22] The brain, and in particular neurons, can be severly impaired by the accumulation of ROS. This is mainly due to two reasons: i) while accounting for only 2% of the total body weight, the brain is a very energy and oxygen demanding organ, being responsible for the consumption of approximately 25% of all glucose and 20% of oxygen present in the human body, thus representing fertile soil for the formation of high amounts of ROS[25]; ii) neurons are extremely sensitive to ROS-induced damages, due to the high concentrations of metals involved in redox reactions, high levels of PUFA, and relatively low expression of antioxidant defense systems.[26] Taking into account the high sensitivity of CNS and neurons to ROS, it is not surprising that a high level of oxidative stress has been observed in several brain disorders, including Alzheimer's disease, Parkinson's disease, brain stroke, multiple sclerosis, and many other pathologies, collectively indicated as neurodegenerative disorders (NDs).[22,27] In the following paragraphs, we will discuss how some of the most common CNS disorders are linked to oxidative stress, giving a rationale for the exploitation of nano-antioxidants in the treatment of neurological diseases.

3. Neurological diseases associated to oxidative stress

3.1 Alzheimer's disease

In 2018, it has been estimated that the total amount of people worldwide suffering from dementia accounted for more than 50 million, and two-thirds were referred to Alzheimer's

disease patients.[28] The number of people suffering from dementia is expected to triple by 2050, with over 152 million cases expected and an economic and social burden jumping from 1 trillion US$ in 2018 to the expected 2 trillion US$ by 2030.[28]

Alzheimer's disease is characterized by a progressive loss of cognitive abilities and by a significant reduction of brain volume. The main hallmark of Alzheimer's disease is the presence of the so-called “senile plaques”, extracellular accumulation of the peptide amyloid-β (Aβ) in the gray matter of the brain[29] (**Figure 1**). Another hallmark of the disease is the presence of an intracellular accumulation of the hyperphosphorylated Tau protein.[30] Alzheimer's disease has been also associated with a high level of oxidative stress and impairment of mitochondrial activities.[26] The initial cause leading to the development of Alzheimer's disease is still unknown, and several theories have been proposed during the years, based on the most common features of the disease. The main model proposed to explain Alzheimer's disease development is the amyloid cascade hypothesis, in which the enzymatic cleavage of the type I membrane protein amyloid precursor protein (APP) is identified as the root of the pathology.[31] Following the amyloid cascade hypothesis, the accumulation of Aβ should be the key factor causing Alzheimer's disease: it has been widely demonstrated how Aβ accumulation can provoke mitochondrial impairment and oxidative stress, leading to neuronal cell death.[32] This hypothesis has generated many controversies during the years, and has been recently doubted.[33]

In 2004, Swerdlow *et al.* proposed the innovative hypothesis of the mitochondrial cascade to explain the cause of sporadic Alzheimer's disease.[34,35] Following this theory, the impairment of mitochondrial functions would not be a consequence of the accumulation of Aβ, but the failure of the energetic mitochondrial activity leading to a shift in the metabolism of APP and to an accumulation of Aβ with consequent neuronal cell death.[34,35] In both hypotheses, what emerges is that Alzheimer's disease is associated with oxidative stress: however, it is not clear whether it is a result of mitochondrial imbalance, is generated by Aβ accumulation, or

whether the high production of ROS is *per se* the starting point in the cascade of events causing Alzheimer's disease [34]. It has been demonstrated that high levels of ROS might induce mitochondrial damage in neuronal cells and that oxidative stress might stimulate the production of Aβ.[32] So, as commented by some authors in the literature,[26] these three key elements of Alzheimer's disease (ROS, mitochondrial impairments, and Aβ production and accumulation) are linked in a vicious loop, where Aβ might lead to mitochondrial dysfunctions that in turn might generate oxidative stress, further mitochondrial damage, and higher levels of Aβ; Alzheimer's disease has also been moreover associated with compromised cellular antioxidant defense systems.[26] Currently, all the treatments used for Alzheimer's disease are symptomatic and are not able to revert neurodegeneration.[36]

3.2 Parkinson's disease

Parkinson's disease is the most diffuse movement disorder and the second most common neurodegenerative pathology after Alzheimer's disease. It has been calculated that Parkinson's disease affects between 0.1 and 0.2% of the population at any given time.[37] While the most famous feature commonly associated with Parkinson's disease is the resting tremor, there are other movement impairments associated, including rigidity, bradykinesia, and postural instability.[38] The movement disorders present in Parkinson's disease patients are usually linked to the loss of the dopaminergic neurons of the *substantia nigra*.[38] The vast majority of Parkinson's disease cases are idiopathic and strongly associated with age, while only a small amount have been associated with genetic factors (5-10% of the cases).[38] From a histological point of view, the most common hallmark associated with Parkinson's disease is the presence of the so-called Lewy's bodies (LB).[39,40] LB are inclusions composed of aggregated proteins widely spread in the CNS of Parkinson's disease patients. The major molecular component of LB is the protein α-synuclein, widely expressed in the CNS, representing 1% of the total cytosolic protein content.[39] The aggregation of α-synuclein and the formation of LB has been strongly correlated with neuronal death (**Figure 2**).[39] The causes that lead to α-synuclein

aggregation in insoluble fibrils are still largely unknown, nevertheless several mechanism and cellular pathways have been linked to Parkinson's disease pathogenesis, as described in the following.

Dopamine has been linked to the aggregation of α-synuclein, and this might explain why dopaminergic neurons are one of the main targets of neurodegeneration in Parkinson's disease. Dopamine is, in fact, able to undergo auto-oxidation, generating H_2O_2 and causing oxidative stress that might cause cellular damages leading to Parkinson's disease pathogenesis.[41]

Mitochondrial dysfunctions have been linked with Parkinson's disease. In particular, it has been largely shown how the inhibition of the mitochondrial electron transport chain complex I caused by toxic compounds like 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and rotenone might induce the production of high levels of ROS, leading to Parkinson's disease-like symptoms *in vivo*.[42]

Oxidative stress seems to be a major hallmark of Parkinson's disease. Similarly to what we described concerning Alzheimer's disease, it is not clear whether oxidative stress is a cause of Parkinson's disease or rather a consequence of other cellular impairments. For example, we discussed the role of mitochondrial dysfunction in Parkinson's disease pathogenesis, but mithocondria are not only producer of ROS, yet also one of the main targets of oxidative stress-induced damage.[43] It has been demonstrated that α-synuclein aggregation is affected by elevated ROS levels.[43] and even the cellular clearance pathways like the ubiquitin-proteasome system (UPS) can be impaired by high levels of ROS.[44] The correlation between oxidative stress and Parkinson's disease has been enhanced by the observation that chronic exposure to pro-oxidative compounds like paraquat and 6-OHDA can induce Parkinson-like symptoms.[45]

Even if the real causing mechanisms of Parkinson's disease are still under investigation, it seems that oxidative stress plays a central role in the neurodegeneration associated with the disease, being able to cause most of the cellular impairments typical of Parkinson's disease

that, in turn, can lead to the generation of even more ROS in a self-reinforcing cycle.[42,43,46] Similarly to what we previously mentioned about Alzheimer's disease, the low level of antioxidant enzymatic activity like superoxide dismutase (SOD) or glutathione peroxidase (GPx) observed in Parkinson's disease patients seems to confirm the link between oxidative stress and the disease.[43] Currently, there are no treatments able to revert the neurodegeneration of the dopaminergic neurons, and the gold standard in Parkinson's disease treatment is still the use of 3,4-dihydroxy-L-phenylalanine (L-DOPA).[47] L-DOPA is highly effective in counteracting the motor symptoms of the early stage of the disease, however the chronic treatment with L-DOPA has been correlated with the development of dyskinesia.[47] Several antioxidant based treatments have been tested during the years, including vitamin E, melatonin, coenzyme Q_{10}, and creatine. However, the results obtained have usually been modest: the reason for the small effects of antioxidant based therapies might be the low local brain concentration of these molecules, the insufficient increment in antioxidant capacities of neurons compared to the high level of oxidative stress typical of Parkinson's disease, or the imple fact that, despite these molecules may be able to reduce oxidative stress, they are incapable of reverting neurodegeneration.[48]

3.3 Ischemic stroke

Nowadays, stroke is one of the leading causes of death worldwide.[49–52] Blood vessels occlusion, due to fatty acid deposits, induces high blood pressure and consequent stroke occurrence. When blood, oxygen, and nutrients cannot efficiently reach the brain, an acute and localized neurological deficit follows,[53] and neurons undergo death and necrosis. Ischemic stroke is the most common pathology (approx. 85% cases) and usually it involves only a part of the brain.[54] Accumulation of ROS plays a central role both in the onset of the pathology [55] and in the restoration of normal blood flow to the ischemic tissue. During this phase, called ischemia/reperfusion injury, ROS levels further rise, damaging interested tissues and causing mitochondrial impairment, inflammation, and consequent cellular apoptosis and

necrosis.[56] Mitochondria are the main sources of ROS production after ischemia/reperfusion injury, because of an increase of the mitochondria membrane potential. Opening of mitochondrial permeability transition pore (mPTP) leads to Ca^{2+} fluxes into mitochondria with subsequent swelling and possible ATP deprivation, leading to cell necrosis; NADPH oxidase is also an important source of ROS in ischemia/reperfusion injury.[57]

As stated above, the presence of excessive ROS levels induces damages to lipids, and lipid peroxidation provokes membrane breakage and dysregulation of transmembrane ion gradients with subsequent cell death [58]. In particular, neurons undergo continuous activation at the synapse.[59]

Actually, no treatments for ischemic stroke have been approved in the USA so far, except for thrombus removal by mechanical procedures or by intravenous injection of tissue-type plasminogen activator (tPA). This enzyme is able to activate plasmin, which contributes to dissolve the obstructing thrombus. However, this practice is effective only if the injection occurs in the first 3 hours after the ischemic event[60] and, unfortunately, produces further damages to the starved neurons.[61]

Nowadays, despite the many struggles and efforts put in basic research, suitable therapies for ROS-induced brain disease are not yet accepted in clinical settings. Successful possibilities come from the introduction of neuroprotective agents, such as brain-derived neurotrophic factor (BDNF), which demonstrated to be efficient upon direct intracerebroventricular administration.[62] After conjugation to poly(ethylene glycol) (PEG) and to anti-transferrin receptor antibody, it correctly localizes into the brain of rats with transient forebrain ischemia, showing potential therapeutic efficacy.[63]

Neuroendothelial breakage occurs up to 12 hours after reperfusion injury, so after ROS have already damaged cells and tissues.[64] It is evident that the best therapeutic strategy is required to be able of crossing the BBB before breakdown, in order to prevent ROS production upon reperfusion.

3.4 Other central nervous system disorders

In this section, we will describe some further neurodegenerative conditions characterized by accumulation of high levels of ROS and by mitochondrial dysfunctions. Patients typically suffer from critical neurological impairments leading to a fatal outcome, due to the absence of efficient therapies.

Huntington's disease (HD) is an inherited progressive neurodegenerative disorder caused by an autosomal dominant mutation in the huntingtin gene and characterized by progressive motor and cognitive injury and multiple psychiatric symptoms.[65,66] Oxidative stress, metabolic dysregulations, and mitochondrial dysfunctions are some of the key events playing a role in its pathogenesis. The mutated gene encodes for a protein that undergoes N-ter cleavage and successive oligomerization, causing aggregates formation that, due to their insolubility, deposit into neurons.[67] This causes impairment of normal cellular functions and increased ROS levels, ending up with neuronal death.[68] The major motor impairment is triggered by the problematic control of involuntary movements and is the typical manifestation of the disease (for this reason also named "chorea"). Oxidative stress plays an important role in HD pathogenesis, and markers of oxidative damage have been found in plasma, brain, lymphoblasts and cerebrospinal fluids.[69,70] Tunez *et al.* reported increased oxidative stress, less efficient antioxidant systems and high DNA fragmentation.[71] Moreover, it has been found that mitochondria become very sensitive to stress induced by Ca^{2+} and ROS in mutant striatal neurons.[72]

Currently, there are no available treatments for this pathology and the only possibility is supporting patients in relieving the symptoms. Many studies have demonstrated the beneficial effects of antioxidant molecules in *in vitro* models of HD. Ascorbic acid, α-tocopherol and idebenone showed great potential in reducing or preventing the oxidative damage in neurons.[73,74] Melatonin has been also used as antioxidant treatment, since it is effective in reducing increased lipid peroxidation and in restoring renal glutathione content.[75]

Interestingly, coenzyme Q_{10} showed high redox potential in murine models of HD, capacity to protect neurons in a dose-dependent manner, and contributed to reduce the volume of striatal lesions,[76] attenuating brain atrophy and improving motor performance in transgenic models.[77,78]

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease that involves degeneration of upper and lower motor neurons in the primary motor cortex, brainstem, and spinal cord, with involvement of skeletal muscles, which become atrophic, and a fatal outcome within 2-5 years from the initial diagnosis.[79] Genetic mutations in the SOD1 gene are at the basis of the hereditary ALS. Aggregates of SOD1 aberrantly accumulate on the outer membrane and matrix of mitochondria, increasing ROS responsible for mitochondrial abnormalities and neuronal death.[80,81]

ALS can be sporadic, in the majority of cases, and familial, caused by hereditary genetic mutations.[82] It can be caused by many different environmental factors, but a prominent role is imputable to oxidative stress,[83] induced by exposure to different exogenous risk factors (e.g., heavy metals, pesticides, electromagnetic fields).[84–86] Common cellular anomalies include loss of mitochondrial membrane potential, changes in electron transport, formation of aggregates, altered Ca^{2+} homeostasis and excitotoxicity.[87,88]

Currently, there are no therapies for ALS, and only two drugs have been clinically approved. The first one is riluzole, a drug targeting excitotoxicity,[89] the second one is edavarone, an antioxidant agent able to scavenge lipid peroxides and hydroxyl radicals.[90] However, both these therapies only provide a mitigation of the symptoms.[91] Regarding other antioxidants for ALS treatment, AEOL 10150 is the most promising. This molecule is able to eliminate ROS, and has been already evaluated in a clinical trial.[92]

Multiple sclerosis (MS) is a chronic disease of the central nervous system that presents a series of neurological dysfunctions leading to disability. It is characterized by problems to the propagation of nerve impulses, due to damages at the myelin sheath that protects axons.

Moreover, it can be defined as an "autoimmune disease", because some evidence have been reported that in this pathology the immune system recognizes myelin as a foreign body attacking its own cells.[93] Many factors can contribute to MS development, from genetic to complex environmental issues (e.g., high-fat/carbohydrate diet).[94,95] Interestingly, MS patients display reduced levels of some antioxidant molecules in the blood[96] and higher quantities of lipid peroxidation products in cerebrospinal fluid[97] with respect to healthy people. Activated microglia and macrophages are involved in ROS increment and consequent degeneration of myelin and neurons.[98,99]

Unfortunately, no therapies are available for MS and the only possibility is currently treating symptoms and preventing relapse. Innovative strategies are aimed at targeting both the immune system and neurodegeneration.[100] Improvement of antioxidant therapies will be useful to reduce the risk of MS onset and inhibit its irreversible progression; [101] however, although some clinical trials have been already performed with antioxidant molecules in MS patients, further studies are required in order to determine the efficacy of combined administration of antioxidant supplements and conventional drugs.[102,103]

Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) is a neurodegenerative disorder, phenotypically characterized by walking difficulties and gait unsteadiness symptoms. The gene mutated and responsible for the pathology encodes for sacsin (SACS), a protein involved in mitochondrial network organization.[104] It has been reported that ARSACS cells show bioenergetic and mitochondrial impairment, denoted by reduced respiratory chain activities and low mitochondrial ATP synthesis.[104] Sacsin is involved in interconnecting the mitochondrial network and contributes to their proper localization in neurons,[105] and lack of sacsin induces a hyper fused mitochondrial phenotype and a balloon-like morphology.[105] ARSACS is characterized by degeneration of the spinal cord and progressive damage of the peripheral nerves with consequent retinal changes and cognitive impairment. This disease evolves from infancy to adult age, when patients perform

uncoordinated movements (ataxia) and perceive weak legs, up to complete degeneration with lack of bladder and intestinal control. This pathology shares many features with the most common autosomal recessive cerebellar ataxia, Friedreich's ataxia (FA); indeed, FA is the leading condition in differential diagnosis of ARSACS. Nowadays, no treatments are described for this kind of diseases and it is commonly suggested a psychological therapy, in order to correct the heavy cognitive issues.[106]

In the case of FA, possible therapeutic strategies are focusing on mitochondrial disfunction, by the administration of iron-chelating agents, that could prevent its accumulation and induced oxidative damage.[107,108] Antioxidant molecules including Vincerinone®, or coenzyme Q_{10} and its synthetic analog idebenone, have shown preliminary promising results. However, large clinical trials are currently missing.[109–111] The development of *ad hoc* nanomaterials in the near future will be the key for enhancing the low bioavailability of these molecules and the need for mitochondrial targeting.

Brain cancer development and progression have been linked with the presence of ROS: it has in fact been shown that high levels of ROS can mediate genetic mutation or alteration to growth factors and membrane receptors, leading to uncontrolled cell growth and proliferation.[112] This notwithstanding, pro-oxidative compounds, rather than antioxidant-based strategies, are usually used as possible therapy in cancer: this is mainly due to the fact that the high metabolism of cancer cells makes them more sensitive to ROS-induced damages with respect to healthy cells.[112] However, the ability of antioxidant compounds, like several members of the flavonoids family, to reduce inflammation and oxidative stress of healthy CNS cells while reducing cancer cell proliferation and viability has been reported.[113]

Traumatic brain injury (TBI), as the name suggests, is a disorder caused by traumatic events that usually lead to physical damage like tearing of neurons or disruption of blood vessels.[114] Usually this first primary physical damage is accompanied by inflammation, accumulation of ROS, BBB disruption, and mitochondrial dysfunction, causing the so-named secondary injury,

that can lead to the damage of healthy tissue not directly affected by the first traumatic event.[115] The damages caused by the secondary injury can induce neuronal cell death that, in turn, can cause further release of inflammatory factors and ROS, leading to progressive neurodegeneration.[114, 115] Several antioxidant compounds like curcumin, melatonin, and lipoic acid have shown great promise in the treatment of TBI.[116-118]

4. Blood-brain barrier: the main obstacle to the treatment of neurodegenerative diseases

The brain is protected and surrounded by three main barriers: the arachnoid barrier, the blood-cerebrospinal fluid barrier and the blood-brain barrier (BBB).[119,120] The BBB, in particular, is a continuous membrane enveloping the brain and separating the blood flow from the brain environment. The basic component of the BBB is the neurovascular unit (NVU), constituted by the endothelial cells of brain capillaries and by brain astrocytes that are in direct contact with brain blood vessels, thanks to cellular extensions called astrocytic endfeet and to pericytes (**Figure 3**).[121] Other cells contribute to the formation and maintenance of the BBB, including neurons, oligodendrocytes, microglia, and mast cells.[119] The main characteristic of the BBB is the presence of tight junctions, which are cell-to-cell contact structures mainly constituted by occludin and claudin proteins,[120] and the biological function of which is blocking the paracellular transport of most molecules.[121] Other molecular components that form or maintain tight junctions include VE-cadherin, *zonula occludens* proteins (ZO-1, ZO-2, and ZO-3), junctional adhesion molecules (JAM-A, JAM-B, and JAM-C), and cingulin.[121]

The evolutionary meaning of the BBB is to provide protection and strict control of the brain environment: the BBB, in fact, acts as a selective barrier granting the passage of only selected molecules through a complex system of transport mechanisms and enzymes able to metabolize substances in transit. Some small molecules (under 500 Da) are able to pass the BBB through passive diffusion, but most molecules, including nutrients and brain waste metabolites, need facilitative or even energy-dependent active transport systems to enter and exit the brain environment.[122] Some facilitative transport systems include solute carriers

(SLC), that grant the passage of small nutrients like glucose (such as glucose transporter-1, GLUT-1), or aminoacids carriers (such as large neutral aminoacid transporter 1, LAT1), while large macromolecules are transported through active transport systems like the receptor-mediated transcytosis (one major example is the transferrin receptor, TfR, mediated transport system) or the adsorptive-mediated transcytosis (AMT).[121] Another class of transport proteins present in the BBB is the group of ABC efflux transporter (ATP binding cassette), that actively efflux possible toxic compounds from brain capillary endothelium preventing their crossing into the CNS (one major example is the P-glycoprotein, Pgp).[121]

During the embryonic development, the brain endothelial cells gain a highly polarized structure, in particular in terms of differential expression of transport proteins on abluminal and luminal cell membrane. Some of the aforementioned transport systems, such as GLUT-1 and LAT1, are expressed on both sides of the brain endothelial cell membrane, granting a bi-directional transport of substances to and from the CNS.[119] On the other hand, some active transport systems, like TfR-mediated transport, are found only on luminal membranes facilitating the transport from the blood to the brain. Finally, other ATP-dependent transporters are localized on the abluminal cell membrane (one example is the excitatory amino acid transporter 1, EAAT1)[119]. Brain endothelial cells also express a large variety of ion transporters to maintain intracellular and extracellular pH and ion concentration: also in this case, it has been observed a differential expression of the transporters. As an example, the sodium pump (for the active influx of sodium and efflux of potassium) is localized on the abluminal cell membrane, while the sodium-hydrogen pump is present in the luminal side.[123] Finally, brain endothelium is also able to metabolize and inactivate possible toxic compounds thanks to a collection of enzymes, like cytochrome P450.[119]

While the complex organization of the BBB is a vital necessity in order to grant a strict control of the brain environment, on the other side it represents one of the main obstacles for the delivery of drugs and therapeutic compounds to the brain. In fact, it has been calculated

that almost no large therapeutic molecule, and only 2% of small-molecule drugs are able to reach the brain.[122] This is due to several factors, including the inability of most drugs to cross the brain endothelium, their chemical composition or molecular dimension, and the enzymatic inactivation and efflux operated by cytochrome P450 and ABC efflux transporters, respectively.[122,124] To overcome these limitations, one possible strategy is to bypass the transport across BBB by administering drugs trough trans-cranial or trans-nasal delivery.[119] Another strategy relies on the temporary weakening of the brain endothelium barrier properties, either by opening the tight junctions (by means of hyperosmotic solutions, chemical stimuli or focused ultrasounds) or by inhibiting the efflux pump of the endothelium [119]. The in-depth discussion of these strategies is beyond the scope of this work and, for a more detailed description, we strongly suggest to check the work of Furtado *et al.*;[119] here, we will mainly focus on the use of nanostructures as valid tools to overcome BBB and to treat CNS diseases, and several BBB targeting strategies based on nanomaterials will be discussed in detail.

It is very important to stress out that, while BBB might be an obstacle in the treatment of CNS pathologies, the involvement of the brain endothelium in neurodegenerative diseases goes far beyond that of a selective barrier. Indeed, it has been shown that several pathological conditions including Alzheimer's disease, Parkinson's disease, multiple sclerosis, epilepsy, stroke and brain tumors might lead to BBB inflammation and breakdown.[121] Currently, there is no perfect and "to go" strategy to overcome the BBB, and each method should be evaluated on the basis of the patient's clinical history, taking into account the condition of the patient's brain endothelium.

5. Organic antioxidants as possible therapeutic agents in neurodegenerative diseases

Many neurodegenerative diseases are caused by excessive ROS production, which induces substantial damages to biological structures, especially to neurons.[12,13] These cells are very

sensitive to oxidative stress, due to their low antioxidant defense systems, high consumption of oxygen, and presence of polyunsaturated fatty acids in their membranes.[125]

Due to the prominent role of oxidative stress in neurodegenerative diseases, a possible therapeutic intervention can be based on the consumption of supplementary or natural antioxidants for relieving the symptoms of these pathologies.[14] It has to be considered that antioxidants are not cell-specific molecules, therefore they often display low efficiency as therapeutic agents for NDs.[126] Creation of targeted delivery systems for antioxidants could improve their availability at the proper location, without altering healthy cells.[127]

During the recent years, antioxidant nanomaterials have gained recognition for their multiple advantages, including their ability to protect molecules, increasing bioavailability, improving pharmacokinetic properties, and even, in some cases, for their intrinsic antioxidant properties, that can be exploited for scavenging free radicals. The possibility to design multifunctional tools integrating diagnostic and therapeutic properties has given nanomedicine the premises for its application to human diseases.[16]

Organic antioxidants have raised great importance as possible therapeutic agents and can be divided into endogenous and exogenous molecules.[128]

5.1 Endogenous organic antioxidants

Our body principally exploits endogenous antioxidant enzymes (SOD, catalase, glutathione peroxidase), macromolecules, and small molecules for maintaining the correct ROS balance.[23] Nevertheless, their intracellular levels are not always enough to counteract excessive ROS production, and cells are not able to synthetize larger quantities upon oxidative damage.[129]

Delivery of SOD, mediated by nanoparticles, has been reported in many studies. Mesoporous silica nanoparticles were designed for SOD delivery; moreover, functionalization with human immunodeficiency virus 1 (HIV) transactivator protein (TAT) favored transmembrane delivery of the enzyme with efficient ROS scavenging.[130] Poly(D,L-lactide-co-glycolide)

acid (PLGA) nanoparticles (NPs) were used to encapsulate SOD and deliver it to human neurons upon oxidative damage, displaying a dose-dependent protective activity. NPs were able to stabilize the enzyme and demonstrated better efficacy respect to SOD alone or PEG-SOD [131]. A micellar system containing SOD conjugated to oxidation-sensitive amphiphilic polysulfide/PEG block copolymers efficiently neutralized superoxide by converting it into hydrogen peroxide, successively removed by the polysulfides.[132]

Endogenous macromolecules, such as bilirubin, albumin and ferritin, and small molecules, such as vitamins, ascorbic acid and glutathione can also act as antioxidants.[133] Nevertheless, they work at very strict redox and pH conditions and, if taken at high doses, they can lead to detrimental consequences to our health. The introduction of endogenous antioxidant small molecules, such as ascorbic acid and vitamin E, has been proved to be effective in blocking the symptoms connected to NDs *in vivo*.[134] It has been demonstrated that sodium-vitamin C co-transporters (SVCT) are essential for preserving the right amount of ascorbic acid in the plasma and into the cerebrospinal fluid. Following the onset of ischemic stroke, its absorption rises for neutralizing ROS.[135]

Mu *et al.* synthetized a carbogenic nanozyme starting from lysine and ascorbic acid with increased antioxidant activity with respect to plain ascorbic acid; this nanoplatform presents multienzyme mimetic activity, reducing peroxide and glutathione disulfide in a thrauma-injured murine model *in vivo* (**Figure 4**).[136]

Concerning Alzheimer's disease, assumption of vitamin E has been associated to a reduced risk of developing this pathology,[137] while glutathione has been found to be related to Parkinson's disease and Alzheimer's disease pathogenesis.[138,139] Reduction in GSH levels in mitochondria correlates to oxidative damage[140] and its decrease in the frontal cortex has been found in patients with diagnosed Alzheimer's disease.[141] Vitamin D deficiency has recently been demonstrated a possible factor leading to abnormal neurological development,[142] and in Parkinson's disease brain, loss of dopaminergic neurons has been correlated to low levels of

vitamin D in serum.[143] Similarly, vitamin B deficiencies have been correlated to neurodegenerative diseases onset.[144]

Coenzyme Q_{10} is a vitamin analogue able to act as intermediate during mitochondrial respiration and it has been shown having also intrinsic antioxidant properties.[145] Administration of CoQ_{10} has many beneficial effects in patient's affected by NDs, however its bioavailability is very low. Development of micelles, nanostructured lipid carriers and polymeric nanoparticles has provided the required therapeutic efficacy.[146–149]

5.2 Exogenous organic antioxidants

Regarding exogenous agents, increasing recognition is coming to synthetic compounds and to phytochemicals present in fruits and vegetables with antioxidant, anti-inflammatory, and protective properties.[150] Many efforts have been devoted to demonstrate their therapeutic efficacy for neurodegenerative disorders, even though results have been sometimes contradictory. This can be linked to the multiple mechanisms leading to ROS formation, hence administration of a single antioxidant could not be sufficient to counteract oxidative stress in a pathological context.[151]

Polyphenols are natural antioxidants produced as secondary metabolites in fruits, vegetables, tea, wine, and juices. They provide neuroprotective effects, due to their ability to interact with transition metals and endogenous enzymes, influencing many cellular pathways.[152] The most common phenols are flavonoids, such as flavonols, flavones, and anthocyanidins. Quercetin, found in apples, tea, and onions, and catechin are the most common polyphenols together with the non-flavoid resveratrol, commonly found in red grapes.[153,154]

Interestingly, anthocyanins have been successfully applied to amyotrophic lateral sclerosis (ALS) therapy,[155] while resveratrol has shown protective effects in Alzheimer's disease patients.[156] Catechins, extracted from green tea leaves, displayed very strong antioxidant properties[157] and their administration reduced Aβ production/aggregation *in vitro* and *in vivo* (**Figure 5**). [158]

Finally, curcumin, a non-flavonoid polyphenol derived from turmeric, has demonstrated potent anti-oxidative capacity and anti-amyloidogenic effects. Upon injection into mice models of Alzheimer's disease, curcumin crossed the BBB, bound plaques, and was also able to reduce amyloid levels preventing fibrils and oligomer formation.[159] In a mouse model of focal cerebral ischemia, it has been demonstrated that Nrf2 and HO-1 expression was enhanced by curcumin that protected brain from damage.[160]

The antioxidant properties of phenolic molecules can be attributed to the capability of phenol groups to convert H_2O_2 into water.[161,162] However, these molecules can be easily oxidized; therefore, they need to be encapsulated into nanocarriers, such as nanoemulsions, liposomes, and solid lipid nanostructures.[163,164] Phenolic compounds have been also conjugated with polymeric components, in order to obtain a more controlled and tunable release.[165] Finally, a new strategy has been elaborated by polymerizing phenols into a polymer chain with ROS-responsive linkers.[166]

6. Inorganic nano-antioxidants for biomedical applications

6.1 Cerium oxide nanoparticles

Cerium oxide nanoparticles (nanoceria) have been vastly investigated due to their antioxidant properties, and have shown great promise for biomedical applications. In nanoceria, cerium is present in two valence states on the surface of cerium oxide nanoparticles (Ce^{3+}/Ce^{4+}), and the continuous redox shift between these two states is at the basis of their ROS scavenging ability.[167] As described in the work of Celardo *et al*,[168] cerium oxide nanoparticles undergo a self-regenerating cycle of redox reactions from Ce^{3+} to Ce^{4+} and back from Ce^{4+} to Ce^{3+}, while eliminating ROS like superoxide and hydrogen peroxide (**Figure 6**).

Cerium oxide nanoparticles present several advantages over other antioxidant compounds:

1) usually, antioxidant molecules present in a biological environment, like vitamins, need constant replenishment either through diet or constant administration. Nanoceria, on the other hand, thanks to their self-regenerating cycle, could act as a ROS scavenger

potentially endlessly with a single administration;

2) while antioxidant enzymes like SOD or catalase are able to eliminate only specific types of ROS, cerium oxide nanoparticles are able to scavenge a vast range of ROS, including superoxides, hydrogen peroxides, hydroxyl radicals, and even reactive nitrogen species like nitric oxide radicals;[168–170]
3) since the antioxidant ability of cerium oxide nanoparticles are strictly related to the Ce^{3+}/Ce^{4+} ratio on the surface of the nanostructures; this can be easily tuned by playing with the synthesis parameters.[171]

These properties have attracted the attention of the research community, and cerium oxide nanoparticles have been tested both *in vitro* and *in vivo* as a potential treatment for pathological conditions related to oxidative stress, including neurodegenerative diseases. Our group has been investigating the activity of nanoceria on neuronal cells in several works, showing that cerium oxide nanoparticles are able to counteract the damage induced by a pro-oxidative insult on different kinds of neuronal cells, like differentiated PC-12 and SH-SY5Y.[172–174] Moreover, we were also able to demonstrate that cerium oxide nanoparticles are capable of stimulating neuronal differentiation, increasing neurite length and numbers, and dopamine secretion levels;[172–174] moreover, we showed how nanoceria affects the gene expression of neuronal cells, down-regulating the expression of genes involved in inflammation and up-regulating genes involved in cellular defense mechanism and neuroprotection.[175] Nanoceria antioxidant effects have been evaluated also using *in vitro* models of neurodegenerative diseases. D'Angelo *et al.*[176] showed as nanoceria present the ability to prevent Aβ-induced damage in an Alzheimer's disease *in vitro* model, affecting transduction pathways involved in neuronal death and neuroprotection. The same group confirmed these results in 2012 by demonstrating that antibody-conjugated PEGylated nanoceria, targeting Aβ aggregates, were able to prevent neuronal cell death in an Alzheimer's disease *in vitro* model.[177] Cerium oxide nanoparticles have shown also the ability to prevent

Aß-induced mitochondrial damage *in vitro*, preventing neuronal cell death.[178] Pinna *et al.* showed that cerium oxide nanoparticles are able to protect PC12 cells in manganese-induced Parkinson's disease *in vitro* models, by reducing cell death levels and modulating dopamine production.[179] An extremely interesting result has been obtained by Song *et al.*, that demonstrated as cerium oxide nanoparticles with organic coating were able to stimulate cellular autophagic clearance pathways in a non-toxic way.[180] As commented by the authors, these results show that nanoceria could be used to enhance the clearance and degradation of toxic aggregates present in several pathological conditions, including neurodegenerative diseases.

In recent years, nanoceria have been efficiency evaluated also on *in vivo* and *ex vivo* models. In the work of Ganesana and colleagues, the real-time antioxidant activity of nanoceria was measured using an electrochemical biosensor based on cytochrome C; in particular, it was calculated that the specific cerium oxide nanoparticles used in this work (around 15 nm diameter) exhibit a SOD-like activity equivalent to 527 U of SOD for 1 µg/ml of nanoceria, being able to reduce superoxide levels in a mice brain slice.[181] Another interesting work by Estevez *et al.* showed how cerium oxide nanoparticles are able to reduce cell death levels up to 50% in an *ex vivo* model of ischemia based on mouse hippocampal brain slices.[182] *In vivo* studies have demonstrated that nanoceria could be used as a protective agent against several forms of neuronal damage, including cerebral ischemia/reperfusion injury,[183] lead-induced hippocampal damage,[184] diabetic neurophaty,[185] and cognitive impairments caused by hypoxia.[186] Yan *et al.* developed a nanozyme based on Pt/CeO_2 with efficient catalytic activity for the treatment of neurotrauma, and *in vivo* studies demonstrated that this material can improve the healing and reduce neuroinflammation. [187] *In vivo* studies proved that nanoceria might be further exploited in the treatment of autoimmune diseases like multiple sclerosis and amyotrophic lateral sclerosis. Heckman *et al.* demonstrated that custom cerium oxide nanoparticles (2.9 nm in diameter) stabilized with citrate/EDTA were able to reduce

oxidative stress and ameliorate symptoms and motor impairments in murine models of MS (experimental autoimmune encephalomyelitis animal model, EAE). Interestingly, the authors showed that nanoceria had beneficial effects when administrated both in a preventative and in a therapeutic regimen, suggesting that they might have both protective and therapeutic effects.[188] The same group tested nanoceria in a murine model of ALS showing that the administration of cerium oxide nanoparticles could prevent muscle loss and increase the life expectancy of the animals.[189] Also Eitan *et al*. obtained good results using *in vivo* model of MS, showing that a combined treatment with lenalidomide and nanoceria could reduce the disease symptoms, white matter damage, and inflammatory response in EAE animals.[190] Nanoceria showed promising results also for the treatment of *in vivo* models of Alzheimer's disease. In fact, in the work of Kwon *et al.*, Tri-phenyl-phosphonium $(TPP)^+$-functionalized cerium oxide nanoparticles were administrated to 5XFAD transgenic Alzheimer's disease mouse models; thanks to the functionalization, nanoceria specifically targeted mitochondria, ameliorating neuronal cells loss, limiting mitochondrial morphological damage, and mitigating reactive glycolysis. Interestingly, the authors did not observe any reduction in Aβ plaques deposition.[191]

Lastly, *in vivo* tests showed the beneficial effect of nanoceria in Parkinson's disease models. In another work by Kwon *et al.*, three different kinds of cerium oxide nanoparticles (3 nm diameter plain particles,TPP^+-functionalized particles, and clusters of 300 nm in diameter) were used to study the effect of extracellular, intracellular, and mitochondrial targeting particles in a MPTP-induced Parkinson's disease mouse model.[192] The authors showed that both intracellular and mitochondrial targeting cerium oxide nanoparticles were able to elicit a neuroprotective effect by reducing ROS levels, inflammatory response, tyrosine hydroxylase (TH), and lipid peroxidation. Nanoceria clusters localized in extracellular space; although they were able to scavenge ROS and reduce neuroinflammation, they did not show any effect against TH or lipid peroxidation.[192] Hegazy *et al*. demonstrated that the treatment with 0.5

mg/kg of cerium oxide nanoparticles in 6-OHDA-induced rats Parkinson's disease models was able to increase dopamine striatal concentration, reduce neuronal ROS and apoptosis levels, and improve motor functions.[193] Kim *et al.* reported that cerium oxide nanoparticles efficiently accumulated at the site of ischemic stroke, thanks to BBB breakage caused by ischemia, and were able to reduce ROS accumulation into damaged tissues upon stroke induction. Treated animals showed reduced apoptosis and smaller pathological areas as compared to control animals.[194]

Despite the good results obtained using cerium oxide nanoparticles, major concerns have been raised concerning their use. Several articles have reported no protective or even toxic effects of nanoceria both *in vitro*[195] and *in vivo*.[196,197] As commented by several authors in the literature, many parameters, including agglomeration, size, preparation conditions, surface charge, pH, salinity of dispersion media and *in vivo* route of administration can affect nanoceria efficiency and biocompatibility.[198,199] Another concern has been raised by the fact that cerium oxide nanoparticles seem to accumulate in mammals body without being completely expelled even after a long period of time. For example, Yokel *et al.* showed that after the administration of 30 nm diameter nanoceria in rats, it was possible to found nanostructures in spleen, liver and bone marrow even after 90 days. Moreover, the authors observed very little decrement in nanoceria concentration over the 90 days period.[200] Thus, despite many promising results, nanoceria still need further investigations before any clinical application is considered; in particular it is of pivotal importance to deeply understand the biodistribution, the possible adverse effects, and how these are affected by nanoparticle features (size, coatings, charge and surface characteristics, etc.) in order to eventually develop a highly standardized and optimized nano-formulation exploitable in human healthcare.

Cerium oxide nanoparticles are synthesized by two kinds of approaches: the chemical methods and the green-based methods.[201]

The chemical approaches are currently the most used, but they imply the use of organic solvents, high temperatures and pressure, and external additives. The solution-precipitation method is the most popular and most convenient. Usually, cerium nitrate hexahydrate is chosen as a precursor from which $Ce(OH)_4$ precipitates in the presence of bases, such as aqueous ammonia or NaOH, and surfactants or stabilizers. CeO_2 nanoparticles are then obtained by dehydration and heating of the hydroxide.[202] The hydrothermal method is another synthetic route in aqueous dispersion in which the reaction is carried out inside an autoclave.[202] Other less popular chemical approaches to prepare ceria nanoparticles are, for instance, the solvothermal method, ball milling, thermal decomposition, microwave-assisted method, oxidation.[202,203]

More recently, green-oriented and cost-effective synthetic approaches have been also studied. These alternative procedures include plant-mediated synthesis, fungus-mediated synthesis, nutrient-mediated synthesis, and biopolymer-mediated synthesis.[203] For example, Patil *et al.* synthesized CeO_2 nanoparticles in presence of pectin, extracted from Indian red pomelo fruit peels, that acts as a reducing agent during the reaction.[204,205] The obtained cerium oxide nanoparticles have a diameter ≤ 40 nm, with good antioxidant activity.[204]

6.2 Selenium nanoparticles

Selenium (Se) is an essential element that plays vital roles in the human body. It is present in several proteins including GPx, thioredoxin reductase (TrxR), and selenoproteins.[196] Although Se is involved in several ROS scavenging reaction and its supplementation has been correlated to enhanced antioxidant functions,[207] it has been widely demonstrated that high doses of Se can be pro-oxidative and even toxic.[208] The main problem in the use of Se as an antioxidant supplement is that the difference between therapeutic and toxic concentration is very narrow.[208] On the other hand, nanoparticles made based on selenium (SeNPs) have shown to have similar or even higher beneficial effect compared to Se-based compounds, but with lower toxicity.[208] Several authors have reported interesting results concerning the use of

SeNPs as a therapeutic agent for neurodegenerative diseases.[209,210] Several works demonstrated that SeNPs are able to reduce Aβ aggregation and prevent Aβ-induced toxicity in PC12 cells.[211–213] In another work, SeNPs were able to reduce neuronal death, ROS levels, and behavioral disorders in a *C. Elegans in vivo* model of Huntington Disease, by reducing also the aggregation of huntingtin protein.[209] In 2018, Amani *et al.* reported the use of SeNPs as a countermeasure for ischemic stroke.[210] In this work, PEGylated SeNPs functionalized with an anti-transferrin receptor monoclonal antibody (OX26-PEG-SeNPs) were tested as a neuroprotective agent both *in vitro* and *in vivo*, using a murine model of ischemic stroke. OX26-PEG-SeNPs showed enhanced targeted transport in the brain, and were able to reduce brain edema and hippocampal neuronal cell loss. With further molecular analysis, the authors showed that OX26-PEG-SeNPs affect several cellular pathways including inflammation, antioxidant defense, autophagy, and apoptosis-related factors. In order to test the biocompatibility of OX26-PEG-SeNPs organ biodistribution and histological analyses were performed by the authors, showing no toxic effect even after 28 days from the intraperitoneal administration.[210] Even though the literature on the use of SeNPs in neurodegenerative diseases treatment is somewhat limited when compared to cerium oxide nanoparticles, the results presented in these works are promising enough to justify further *in vivo* testing for brain disorders.

6.3 Other antioxidant inorganic nanomaterials

Other inorganic nanomaterials have been tested as possible antioxidants for the treatment of ROS-induced disorders. Manganese oxide nanoparticles, such as MnO_2 and Mn_3O_4 NPs, have shown interesting abilities as antioxidants, being able to mimic catalase activity by scavenging H_2O_2 and generating oxygen as a byproduct. Tootoonchi *et al.* demonstrated that MnO_2 NPs can be exploited to elicit cytoprotective effects upon murine β-cell insulinoma. Singh *et al.* have shown in two different works that Mn_3O_4 nanoflowers, mimicking the action of three different antioxidant enzymes (CAT, SOD, and GPx), were able to prevent oxidative

damage in cells without affecting the physiological antioxidant cellular functions, and were able to show protective effects in an *in vitro* model of Parkinson's disease based on MPTP-treated SHSY-5Y.[214,215]

Platinum-based nanomaterials are another group of nanoparticles that have shown interesting results as nano-antioxidants, being able to replicate CAT and SOD activity.[216] In the works of Takamiya *et al.*, Pt nanoparticles were used as a countermeasure to prevent and ameliorate the damages induced by ischemic stroke, preserving NVU structure and neurological functions in a mouse model of cerebral infarction.[217,218]

Mu *et al.* instead developed a trimetallic (triM) nanozyme with multi-enzyme-mimetic activity as efficient scavenger of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in brain thraumatic injuries (**Figure 7**).[219]

Yttrium oxide nanoparticles (Y_2O_3) protective antioxidant effects were reported in HT-22 mouse hippocampal neuronal cells, in a rat model of lead-induced neuronal damage, and in an *in vivo* model of photo degeneration. However, Y_2O_3 ability to ameliorate neurodegenerative disorders in animal models still needs to be tested.[184,220, 221]

Recently, an interesting work proposed the use of Pd hydride (PdH) nanoparticles as a possible treatment of Alzheimer's disease. The results obtained by Zhang *et al.* were quite remarkable, showing that PdH were able to scavenge $^{\bullet}OH$, suppress Aβ over-production, reverse synaptic deficit, ameliorate mitochondrial dysfunction, and even reduce cognitive impairments in Alzheimer's disease mice models.[222] The authors theorized that the results obtained were due not only to PdH nanoparticles' antioxidant ability, yet also to their capacity to generate hydrogen molecule (H_2) as a by-product of $^{\bullet}OH$ scavenging. This interesting work could lay the basis for the future exploitation of PdH in Alzheimer's disease and other neurodegenerative diseases characterized by high levels of oxidative stress.

Lastly, some carbon-based nanomaterials including fullerene, graphene, carbon nanotubes and carbon clusters have been studied as antioxidants and as potential countermeasures for some

CNS disorders.[223–229] Even if these nanostructures are carbon-based and, under a strictly chemical point of view, organic, we prefer to briefly mention them at the end of this paragraph, as they are synthesis products.

Fullerene nanoparticles (in particular spherical structures with 30 carbon double bonds, C_{60}) have shown high antioxidant efficacy with very low cytotoxicity levels even *in vivo*.[226] One of the most interesting abilities of fullerene is its interaction with Aβ: Several works have reported that fullerene is able to prevent and counteract the aggregation of Aβ, demonstrating that it could be an ideal candidate for the treatment of Alzheimer's disease.[223–225] C_{60} has also been investigated as a potential treatment of Parkinson's disease: polyhydroxylated fullerene derivatives $C_{60}(OH)_{24}$ have shown the remarkable ability to prevent MPP^+ induced cell death in SK-N-MC neuroblastoma cells, being able to rescue cell viability and reduce ROS levels and ROS-induced damages.[230]

Even if the antioxidant ability of carbon nanotubes has been reported, works featuring carbon antioxidant as intrinsically antioxidant nanomaterials are extremely limited.[217] Two dimensional carbon-based nanomaterials have also shown antioxidant ability, like in the case of the study from Qiu *et al.*, where the ROS scavenging capacity of graphene was investigated using electron paramagnetic resonance spectroscopy (EPR), and it was reported that graphene oxide was able to scavenge both OH^- and O^{2-} radicals.[222] Bitner *et al.* reported the use of carbon nanoparticles as countermeasure for cerebrovascular dysfunctions related to traumatic brain injury (TBI): poly(ethylene glycol)-functionalized hydrophilic carbon clusters (PEG-HCCs) were administered to a mild TBI/hypotension/resuscitation rat model showing that these nanostructures were able to restore cerebral blood flow, while normalizing O_2^- and nitric oxide levels.[228]

7. Nanovectors for antioxidant targeting and delivery

Antioxidant therapy for central nervous system disorders is subjected to a series of problems related to the low bioavailability, poor solubility in water, and fast degradation of the majority

of the conventional antioxidants.[15] To overcome these issues, biocompatible nanoparticles can be used as delivery systems for these molecules. Owing to their peculiar physicochemical properties, such as large surface-to-volume ratio and small sizes (between 1-1000 nm), nanoparticles behave very differently from the bulk materials. Nanoparticles are able to encapsulate a significant amount of drug, preventing its degradation, improving its bioavailability with low impact on the immune system, delivering it to the target site, and often enhancing its therapeutic efficacy.[16] The versatility of nanomaterials makes them easily adaptable for their final purpose. More importantly, the possibility to introduce specific functional groups on their surface that enable their targeting towards desired tissues paves the way for a more sophisticated and precise treatment.[17] Several nanosystems have been already approved by the FDA, especially for cancer medicine.[16] The application of nanomaterials in antioxidant therapy is more recent, but there have been already some promising results with different kinds of nanosystems (**Figure 8**).

In this section, an overview of the main nanomaterials used for antioxidant therapy will be provided, as well as a description of the main preparation techniques and the current strategies for active targeting.

7.1 Polymeric nanovectors

Polymeric nanoparticles are solid nanoparticles whose shape could be either a nanosphere or a nanocapsule, depending on the preparation method.[17,231] The antioxidant molecule is generally loaded in the core or covalently bound to the polymer that forms the particle, acting the nanomaterials mainly as a drug delivery system. In some particular cases, such as polydopamine, the polymer itself has inherent antioxidant activity.[15] Polymer nanoparticles offer high structural integrity and stability, long shelf life, and they can also be prepared with materials that are responsive to particular stimuli for a controlled release of their cargo.[19],

The matrix of polymeric nanoparticles is usually made of synthetic biocompatible and biodegradable polymers, such as poly(D,L-lactide-co-glycolide) acid (PLGA), poly(lactide) acid (PLA), poly(methyl methacrylate) (PMMA), and poly(ε-caprolactone) (PCL).

PLGA nanoparticles have been extensively used in nanomedicine since their *in vivo* biodegradation is well known, and they have been already accepted by the FDA for drug delivery purposes.[232] In the context of antioxidant therapy for CNS diseases, curcumin-loaded PLGA nanoparticles have been proved to possess higher neuroprotective activity than the free drug, preventing the phosphorylation of Akt and Tau proteins caused by oxidative stress in SK-N-SH cells.[233] The authors also demonstrated the fundamental role played by the polymeric matrix in the release kinetics of the drug, in the nanoparticles uptake, and in the expression of some relevant neuroprotective- and antioxidant-associated genes.[233]

Nanocurm™, a formulation of curcumin loaded into NVA622 polymeric nanoparticles (derived from the polymerization of N-isopropylacrylamide, vinylpyrrolidone and acrylic acid), was demonstrated to be able to significantly decrease the proliferation and growth of different brain tumor cell lines, inducing G2/M arrest and apoptosis.[234]

Yun *et al.* prepared poly(butyl cyanoacrylate) (PBCA) or poly(D,L-lactide-co-glycolide (PLGA) nanoparticles encapsulating SOD, with targeting moieties directed towards *N*-methyl-D-aspartate (NMDA) receptor 1. These nanomaterials efficiently protected neurons *in vitro* and *in vivo*.[235]

An interesting option for stroke treatment came from local delivery of native superoxide dismutase (SOD) loaded into in biodegradable poly(D,L-lactide co-glycolide) nanoparticles, which provided a sustained protective effect, with a 65% reduction in infarct volume[236].

Polymeric nanoparticles have been synthesized, modified with glycopeptides (g7), and loaded with cholesterol, the levels of which are very low in HD mice. After intraperitoneal injection, these nanoparticles were able to reach neurons, contributing to cognitive dysfunctions recovery.[237] Recently, shrinkable nanoparticles made of PEG-poly(ε-caprolactone) carrying

therapeutic molecules were designed to be responsive to thrombin or matrix metalloproteinase-9, abundant at the site of ischemic stroke, and targeted delivery was obtained by conjugating them to specific overexpressed proteins (**Figure 9**).[238]

In addition to synthetic polymers, natural polymers like chitosan, alginate or gelatin are used to prepare nanocarriers.[17] Chitosan nanoparticles have been used to encapsulate polyphenols extracted from *Ilex paraguariensis*, also known as yerba mate, and the resulting system showed good release profile and antioxidant activity.[239]

Antioxidant molecules can also be attached directly to the polymer chains by covalent bonds. The conjugation reactions are usually esterifications or acylations, mediated by coupling agents like dicyclohexyl carbodiimide, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide or N-hydroxysuccinimide esters.[240] For example, tannic acid has been covalently attached to poly(metacrylic acid) to form tannic acid-decorated nanoparticles, the antioxidant activity of which is higher than that of the antioxidant alone.[241]

Polymeric nanoparticles can be synthesized with several techniques. The two main procedures are the emulsion-solvent evaporation process and the nanoprecipitation technique (or solvent diffusion method).[242] In the emulsion-solvent evaporation process, the polymer is dissolved in an organic solvent that is immiscible with water and then mixed with an aqueous solution containing a surfactant or a stabilizer. Then, the emulsion is homogenized by sonication, and the organic solvent is removed under reduced pressure. In the nanoprecipitation technique, instead, the polymer is initially dissolved into an organic solvent that is completely miscible with water. Upon addition of water, the polymer will be no longer soluble in the new water/solvent mixture, and it will start precipitating into nanoparticles. The solvent is then removed by either evaporation or dialysis. Compared to the other procedure, nanoprecipitation requires less toxic solvents, like dimethyl sulfoxide or acetone. Both these two techniques are very sensitive to preparation parameters, like the mixing order, the mixing time, or the temperature.[17,243]. Other preparation protocols involve the polymerization of

monomers in an emulsion where the polymer directly precipitates into nanoparticles, and, for more hydrophilic polymers, gelation or coacervation techniques.[244]

Finally, as already mentioned earlier, there is another category of polymeric nanoparticles where the repetitive units of the polymers (or one portion of a copolymer) are antioxidant molecules *per se*; therefore, the polymer nanostructure itself owns inherent antioxidant activity. For example, melanin-like nanoparticles are synthesized by polymerization, through chemical oxidation, of dopamine.[245,246] Due to the presence of functional groups such as catechol, melanin acts as a scavenger against reactive oxygen and nitrogen species. Liu *et al.* demonstrated that PEG-melanin-like nanoparticles (PEGMeNPs) have neuroprotective and anti-inflammatory activity *in vitro* on Neuro 2A cells, without affecting cell viability and mitochondrial function **(Figure 10)**.[246] These nanoparticles were also able to decrease the infarct area of ischemic brain on *in vivo* rat models of ischemic stroke.[246]

Kang *et al.* synthesized poly(vanillin oxalate) nanoparticles that are able, in the presence of H_2O_2, to generate CO_2 bubbles, exploited for enhanced ultrasound imaging, and to release vanillin, a well-known antioxidant molecule. These multifunctional nanoparticles have proven to be effective both *in vitro* and *in vivo* for hepatic ischemia/reperfusion injury, but they could be promising for other kinds of ischemic injuries, in particular at brain level.[162]

Stimuli-responsive antioxidant nanomaterials have been developed in order to be able to work upon oxidative stress, for example in the presence of hydrogen peroxide. Nanoparticles incorporating copolyoxalate have been designed able to activate in presence of H_2O_2 and degrade it into safe compounds *in vivo* (**Figure 11**).[166]

7.2 Lipid-based nanovectors

Lipid-based nanoparticles include systems made of lipids and/or phospholipids. They can be classified depending on their structure. Liposomes are composed of a mixture of amphiphilic phospholipids, lipids and cholesterol organized into one or more lipid bilayer enclosing an aqueous core. Due to this peculiar morphology, liposomes are able to encapsulate hydrophilic

drugs in the aqueous compartment and hydrophobic drugs in the lipid portion.[16],[247] Liposomes were the first nanoparticles to be approved by FDA for the treatment of cancer.[248] Within the field of antioxidant therapy, liposomes have been used to deliver several kinds of antioxidant drugs and enzymes.[249] For example, quercetin-loaded liposomes could have potential applications in the treatment of Alzheimer's disease, because they successfully inhibit acetylcholinesterase activity in hippocampus and improve memory deficits caused by the administration of AF64A, a cholinergic neurotoxin, in rats.[250] Curcumin-modified liposomes were shown to prevent Aβ formation in Alzheimer's disease and, due to their high affinity for amyloid deposit, they could also be used for diagnostic purposes.[251]

As already mentioned, antioxidant phytochemicals have gained important recognition as neuroprotective agents. Due to their poor solubility, rapid clearance, and low stability, they have been often encapsulated into lipidic carriers. Unilamellar liposomes have been developed to carry quercetin to rat brain model of ischemia/reperfusion injury; this treatment preserved the activity of antioxidant enzymes and inhibited edema formation.[252] Further, Sinha *et al.* demonstrated that it is possible to prevent oxidative damage upon ischemia induction by supplying ascorbic acid and α-tocopherol encapsulated or intercalated in unilamellar liposomes a few hours prior to the ischemic insult.[253]

Wiley *et al.*, designed lipopolysaccharide (LPS) modified liposomes targeting TLR4 receptor on the microglia in SOD-1 mutant mice and loaded them with minocycline demonstrating preferential targeting of microglia cells and a delay in disease progression.[254] Interestingly, the administration of Cu, Zn SOD liposomes favored the delivery of the antioxidant enzyme to the brain, enhancing its protective activity in ischemia/reperfusion injury.[255,256]

One of the techniques exploited to prepare liposomes consists in the initial formation of a thin film of lipids by evaporation of organic solvent from a lipid/solvent solution; the film is then dispersed in an aqueous medium, triggering the formation of large multilamellar vesicles. A subsequent step of sonication, extrusion or homogenization is necessary to obtain small

unilamellar vesicles with a good monodispersity. Otherwise, liposomes can be formed by the "reverse-phase evaporation method", in which a certain amount of water is added to a solution of phospholipids dissolved into an organic solvent, forming a water-in-oil emulsion, and thereafter the organic solvent is removed under reduced pressure, resulting into the formation of liposomes.[257]

Another typology of lipid-based nanoparticles is represented by solid lipid nanoparticles (SLNs), whose matrix is composed of lipids that are solid at body temperature. SLNs are mainly used to encapsulate hydrophobic drugs due to the nature of the matrix.[258] However, the high crystallinity of the lipid core can give rise to low drug loading efficiency and/or inefficient drug release. For this reason, one or more lipids that are liquid at body temperature are often included in the formulation. These systems are called nanostructured lipid carriers (NLCs), and their inner structure and the release profile can be modulated by varying the amount of liquid lipid in the particle.[259] NLCs have been proposed to encapsulate sesamol, a component of sesame seeds and sesame oil with antioxidant activity, improving its pharmacological profile and reducing oxidative stress, ischemia/reperfusion-induced neurobehavioral deficits, and cellular damage *in vivo*, where the free drug failed (**Figure 12**). [260]

Rat models of ischemic/reperfusion injury were fed with solid lipid nanoparticles loaded with curcumin and exhibited improvement in cognitive functions, increased levels of endogenous antioxidant enzymes, enhanced activity of mitochondria, and concomitant decrease of lipid peroxidation.[261] In a very promising work aimed at targeting mitochondria, curcumin was encapsulated in solid lipid nanoparticles. Upon administration to HD rats, mitochondrial activity was recovered and endogenous antioxidant enzymes efficiency was restored.[262]

With respect to the majority of other kinds of nanovectors, the preparation of SLN and NLCs does not require the use of toxic organic solvents. The two principal fabrication methods are the high-pressure homogenization (HPH) and the microemulsion technique.[263] HPH involves

breaking up primary large lipidic aggregates into sub-micron particles by using a high-pressure homogenizer. Here, the particles are pushed at high pressure (5-50 MPa) through a nozzle of a few microns. The high shear stress breaks the initially large particles into smaller objects, with sizes in the nanoscale.[258] Prior to the injection into the homogenizer, the lipids are melted at a temperature 5-10°C higher than their melting points, and an aqueous surfactant solution, warmed at the same temperature, is added. The drug can be either melted directly with the lipids (hot homogenization) or added to the melted lipids and cooled down to room temperature (cold homogenization) before adding water. After the homogenization, the nanoemulsion is let cool down at room or lower temperature. Ultrasonication is often used as an alternative to the HPH to reduce aggregates size. In the microemulsion method, the lipids are melted and mixed under stirring with a small amount of a surfactant solution at the same temperature. Thereafter, a sufficiently high volume of water at 2-3°C is added to solidify the initial lipid droplets.[263,264]

7.3 Other organic nanovectors

Besides polymeric and lipid-based nanoparticles, other organic nanomaterials have been used for drug delivery purposes and, in particular, for the treatment of CNS disorders.

Dendrimers are synthetic three-dimensional branched macromolecules with a well-defined symmetric structure.[265] They have a radial distribution consisting of an inner core, from where the "branches" grow through chemical reactions. Dendrimers are indeed interesting for biomedical applications because of their low polydispersity in terms of size and molecular weight, and the easy control of the surface functionalization.[265] Hydroxyl-terminated polyamidoamine dendrimers carrying the antioxidant N-acetyl cysteine were able to reduce oxidative stress level in healthy BV2 murine microglia, more efficiently than plain N-acetyl cysteine.[266]

Polymersomes are structural analogs of liposomes, but there are made of amphipathic block copolymers instead of lipids. A block copolymer is a polymer containing two portions with

different features; usually, one block is hydrophilic and the other is hydrophobic, imparting amphiphilic properties to the whole macromolecule. In polymersomes, the block copolymers are distributed in bilayers, where the hydrophilic part is oriented toward the aqueous solvent, whereas the lipophilic block is composing the layer.[17] The physicochemical properties of polymersomes can be easily modulated by carefully choosing the right blocks, their molecular weight, and the ratio between the two blocks. Poly(ethylen-glycol)-b-poly(D,L-lactic-co-glycolic) acid polymersomes loaded with curcumin and functionalized with specific peptides targeting the BBB, for instance, have been shown to ameliorate cognitive dysfunction induced by $A\beta_{1-42}$ in mice.[267]

Finally, micelles are another group of nanoparticles made of amphiphilic polymers or lipids with a spherical shape composed of a hydrophobic core and a hydrophilic shell.[17] Deng *et al.* efficiently loaded thymol, a phenol extracted from *Thymus vulgaris L.* and *Origanum vulgare L.* with antioxidant properties, in Tween 80 micelles, demonstrating that the encapsulated compound has higher antioxidant activity with respect to the plain one in aqueous environment, where thymol is normally insoluble.[268] An interesting study demonstrated that insulin-d-α-tocopherol succinate (INVITE)-loaded-curcumin micelles improved the efficacy of mesenchymal stromal cells (MSCs), able to enhance neuronal protection and replace dead motor neurons of the spinal cord. This innovative approach was shown to present great promise for ALS treatment.[269]

7.4 Inorganic nanovectors

Besides inorganic nanoparticles that possess an inherent antioxidant activity, inorganic nanosystems can be also exploited as passive carriers for drug delivery purposes. For example, silica (SiO_2) nanoparticles, due to their peculiar mesoporous structure, ensure high loading capacity and homogeneous distribution of drugs. Moreover, the strong covalent Si-O bonds make these nanoparticles very resistant to degradation. SiO_2 have been proven to be biocompatible, with low toxicity.[270]

Also iron oxide nanoparticles could be used as antioxidants delivery systems because of their biocompatibility and, in particular, their tunable magnetic properties. For example, superparamagnetic iron oxide nanoparticles (SPIONs) interact with external alternated magnetic fields and, therefore, they can be used both for hyperthermia and as contrast agents in magnetic resonance imaging (MRI).[16] Moreover, the application of an external magnetic field can be exploited to favor the crossing of the BBB of SPIONs, as recently demonstrated.[271] Iron oxide nanoparticles functionalized with catalase were shown to be able to convert ROS into O_2 mitigating hypoxia effects in tumor microenvironment. The catalse activity in these particles was mantained even in hypoxic conditions, and resulted three times more stable with resepct to the free catalase.[272] In another example, SOD was complexed with both mesoporous silica[273] and iron oxide[274] nanoparticles, giving a great reduction of necrotic brain tissue.

PLA-coated mesoporous silica nanoparticles, functionalized with a ligand of the low-density lipoprotein receptor, were efficiently loaded with resveratrol, and, being PLA degraded in presence of ROS, the system ensured a stimuli-responsive release of the antioxidant. Moreover, these nanoparticles were shown to favor the passage through an *in vitro* BBB model and to reduce oxidative stress of rat microglia.[275]

In a very recent work, Amanzadeh *et al.* fabricated quercetin-conjugated SPIONs and demonstrated that these nanoparticles were able to improve learning and memory of intact Wistar rats, more efficiently than the drug alone (**Figure 13**).[276] This result is really promising, especially for a potential treatment of neurodegenerative diseases.

Inorganic nanoparticles can be synthesized in different ways depending on the starting materials and on the desired final product. Mesoporous SiO_2 nanoparticles are usually synthetized by a sol-gel procedure using tetraethyl orthosilicate as a precursor and surfactants as template agents.[277] Iron oxide nanoparticles are usually prepared by precipitation of ferrous or ferric salts in aqueous media. Hydrothermal synthesis is a general procedure that

can be used for a large range of materials; it involves the crystallization of the substance from aqueous solutions at high temperature and pressure. Other procedures include microemulsions, polyol process, decomposition in organic media, and pyrolysis.[278]

7.5 Active targeting in antioxidant therapy for central nervous system disorders

Crossing the BBB is one of the major obstacles for the treatment of central nervous system disorders. Nanotechnology has given a great improvement in this sense in the recent years. As a simple example, it has been demonstrated that small lipid nanoparticles help drugs penetrating the BBB more efficiently.[279] This kind of passive targeting, however, is difficult to be controlled and can induce to aspecific accumulation in different sites.[280] Targeted drug delivery, on the other hand, aims at being the nanoparticles selectively uptaken by the interested tissues.[281] Active targeting exploits the strong interactions between a ligand and a specific receptor that is overexpressed on the desired target site.[281]

To foster receptor-mediated endocytosis of endothelial cells of the BBB, nanoparticles should be thus functionalized with ligands that bind to specific receptors overexpressed on endothelial cells, like transferrin receptors, insulin receptors, low-density lipoprotein receptors.[18] The conjugation of ligands on the surface of the nanoparticles is simple and straightforward, and several antibodies and proteins have been already used for this purpose. However, since this kind of ligands are unstable and can cause reactions from the immune system, peptides and aptamers are often preferred.[16] Cell-penetrating peptides, for instance, are known to efficiently cross the BBB, and they have been conjugated to different kind of nanoparticles.[282]

In order to increase the efficacy of the treatment with antioxidants, the drug should be ideally localized mainly around the site of action. Mitochondria are the preferred target in antioxidant therapy, because they are the organelles where ROS are produced at higher extent.[18] Moreover, as already mentioned, oxidative stress is often linked to mitochondrial dysfunction.[283] There are several strategies to target mitochondria. One of these is the

exploitation of the electric potential gradient across the inner mitochondrial membrane by using lipophilic cations as targeting agents.[284] TPP^+ is one of the most used cations to target mitochondria, and several drug-TPP^+ conjugates have been already patented.[285] MitoQ and MitoVit E are examples of these kind of complexes, where TPP^+ is linked to quinone and vitamin E, respectively, and they have been shown to accumulate inside mitochondria.[286] The aforementioned hydroxyl-terminated polyamidoamine dendrimers conjugated to N-acetyl cysteine were functionalized with TPP^+, and they accumulate in mitochondria at a higher extent with respect to non-functionalized dendrimers (**Figure 14**).[287]

Short mitochondria-targeting peptides are also exploited. These can be natural aminoacidic sequences or synthetic peptides where both hydrophobic and cationic amino acids are present.[285,287] For example, Kang *et al.* designed a new cell-penetrating peptide with a specific mitochondria-targeting sequence (MTS), that is a precursor of mitochondrial proteins, efficiently recognized by the translocation machinery at the mitochondrial membrane.[288] MTS peptides internalization in mitochondria is mediated by the translocase of the outer membrane and by the translocase of the inner membrane complexes.[285] The same group conjugated the synthetic MTS peptide to a metallothionein, a small protein with antioxidant properties, and the resulting complex was successful in restoring the activity of mitochondria and in reducing ROS production.[289,290]

8. Clinical trials

As already stressed out before, the fundamental role ROS play in many different neurodegenerative pathologies and neurological diseases open new perspectives for antioxidant therapy. Just a few antioxidant drugs, such as idebenone, edaravone, and dimethyl fumarate, have been already approved for use in humans to treat neurological diseases, while many other drugs are still currently under preclinical and clinical evaluation. In this section, more relevant clinical trials regarding antioxidant compounds / nanocarriers to treat different pathologies of the central nervous system will be discussed in detail.

Concerning Alzheimer's disease, 24 of the 46 started clinical trials involving antioxidants have been completed.[291] Many different antioxidants, such as alpha-lipoic acid and omega-3 fatty acids, have been already tested with promising results [292,293] Recent clinical trials investigated the effects of resveratrol, a natural antioxidant polyphenol, in slowing the progression of Alzheimer's disease. This polyphenol, which is highly expressed in the grape skin, is well tolerated by patients up to 5 g/day,[294] and therefore was orally administered at high-doses (500-2000 mg/day) in mild to moderate Alzheimer's disease patients. Resveratrol was found, together with its metabolites, in cerebrospinal fluid, indicating the CNS availability of this polyphenol.[295-297]. The levels of the matrix metallopeptidase 9 (MMP9) marker in cerebrospinal fluid and in plasma blood resulted remarkably lower in resveratrol-treated patients with respect to the placebo-treated patients;[297] MMP9 plays a key role in neurodegeneration and neuro-inflammation regulating BBB permeability;[298-301] the decrease of its levels in cerebrospinal fluid may indicate that resveratrol limits brain permeability, the infiltration of leukocytes, and other inflammatory agents. Furthermore, resveratrol modulated neuroinflammation and induced adaptive immunity by increasing macrophage-derived chemokine (MDC), interleukin (IL)-4, and fibroblast growth factor (FGF)-2.[297] However, patients treated with these doses of resveratrol were subjected to a greater brain volume loss compared to the placebo group.[295,297]. For this reason, in another clinical trial, lower doses of resveratrol were tested; an oral pharmacological preparation consisting of 5 mg of resveratrol, 5 g of glucose and 5 g of malate[302] was administered to patients with Alzheimer's disease to study its efficacy in slowing the progression of the pathology.[303] Results showed that the preparation was safe and well tolerated by patients; moreover, after 12 months, all the scores related to memory, attention, reasoning, language, orientation, and praxis showed less deterioration in the treated with respect to the control group. However, the score differences between groups were not statistically significant, and larger studies will be required to evaluate the beneficial effects of resveratrol in Alzheimer's disease patients.

Another natural compound with antioxidant properties used for the treatment of Alzheimer's disease patients is epigallocatechin-gallate, the major catechin in green tea.[304] This catechin has been also successfully exploited to improve cognitive performances and decelerate the Alzheimer's disease-like progression in Down's syndrome patients;[305] specifically, at 12 months from the beginning of the trial, participants treated with epigallocatechin-gallate and cognitive training had significantly higher scores in visual recognition, memory, inhibitory control, and adaptive behavior.[306]

Vitamin E[307] and oral curcumin (Curcumin C3 Complex®[308]) have also been tested in clinical trials for the treatment of Alzheimer's disease symptoms.[309,310] Unfortunately, in both cases, no evident clinical efficacy was reported, probably due to the limited bioavailability of these compound, as already pointed out in the previous sections. It this clear, in these cases, how the encapsulation in nanocarriers, such as liposomes or lipid nanoparticles, could dramatically improve the efficacy, the targeting ability, and the antioxidant efficacy of these kinds of molecules.[311] However, the great majority of nanocarriers (e.g., liposomes) for brain diseases that are being currently tested in clinical trials are applied for the treatment of cancer, or, rarely, of meningitis, and not for the therapy of neurodegenerative conditions.[312]

With respect to studies involving Alzheimer's disease patients, a relatively low number of clinical trials on antioxidants for the treatment of Parkinson's disease has been completed. Resveratrol has been used only in 2 clinical trials involving Parkinson's disease patients,[313] while no trials have been found using catechins. 4 of the 64 completed clinical trials exploited glutathione (*via* intranasal administration) as natural antioxidant agent in Parkinson's disease patients.[314] Most of the results obtained by the clinical trials exploiting resveratrol and glutathione have not yet been published, nor have been reported on repository sites. Only results from a double-blind placebo-controlled phase IIb study with intranasal glutathione were recently published.[315,316] In this study, higher Unified Patkinson's disease Rating Scale (UPDRS) and UPDRS motor subscores were observed over baseline, but these improvements

in the treatment group were not statistically higher than those ones achieved by the placebo group; placebo-related improvements resulted more robust than those observed in previous Parkinson's disease studies. Appropriately-powered longer-duration studies on a larger cohort of participants will be required to further investigate glutathione effects *versus* placebo in Parkinson's disease patients.

Interesting results have been observed with N-acetylcysteine (NAC).[317,318] In a phase 1 clinical trial,[318] a single 150 mg/kg NAC intravenous infusion was administered to patients with Parkinson's disease and Gaucher disease and in healthy controls;[319] reduced-to-oxidized glutathione ratios were measured in blood, and glutathione levels in the brain were monitored, for the first time, by using a 7 T magnetic resonance spectroscopy (MRS); NAC infusion induced a significant increase of blood glutathione redox ratios in all the groups. This enhancement was followed by an increment of glutathione concentrations in brain in all subjects. In another clinical trial,[320,321] repeated oral NAC administrations were able to significantly increase different peripheral antioxidant parameters (catalase and glutathione redox ratio) with respect to baseline; however, brain glutathione levels resulted unchanged, probably due to the low oral NAC bioavailability. An overview of N-acetylcysteine effects in neurodegenerative diseases has been recently reported by Tardiolo *et al.*[321]

An effective antioxidant nanoformulation is nanocurcumin (curcumin encapsulated in nanomicelles; SinaCurcumin®), whose immunomodulatory activity has been exploited for the treatment of multiple sclerosis (MS).[102] In a recent clinical trial,[322,323] MS patients received capsules of nanocurcumin (curcumin encapsulated in polymeric nanoparticles; NanoCurc™) daily for 6 months or a placebo, as control; real-time PCR was performed on blood samples to detect the gene expression levels of miRNAs, miRNA-dependent targets, transcription factors and pro-inflammatory cytokines. ELISA assays were then carried out to analyze cytokine secretion. Interestingly, MS patients treated with nanocurcumin were characterized by significantly lower expression levels of inflammatory miRNAs (miR-145, miR-132, and miR-

16), STAT1, NF-κB and AP-1. Such results indicated that nanocurcumin may be able to inhibit neuroinflammation in MS patients. Other antioxidants that have been used in clinical trials for counteracting the degenerative effects of MS include natural antioxidants (ginkgo, biloba, vitamin E/selenium, essential fatty acids;[324,325]), inosine,[326] NAC,[327] and melatonin.[328,329]

9. Conclusions

Studies discussed in this review display remarkable data concerning the use of nano-antioxidants as potential treatment of several CNS pathologies. Intrinsically antioxidant nanomaterials such as nanoceria or other inorganic nanostructures seem to be able to elicit a strong ROS scavenging activity, overcoming the efficiency of commonly used substances and being able to ameliorate several dysfunctions associated with CNS disorders like oxidative stress, mitochondrial impairments, and even cognitive problems in animal models.

Organic antioxidants as well represent a valid tool to treat central nervous system diseases, and, particularly, neurodegenerative pathologies. The encapsulation of these compounds in nanocarriers like lipid-based or polymeric nanostructures seems to be able to overcome the limitations connected to the use of plain drugs, such as low brain targeting efficiency, low solubility, bioavailability, and biocompatibility. Owing to these properties, antioxidant-loaded nanocarriers may be able to reduce toxic side effects, and to control their cargo release and biological responses.

Despite these interesting results, nano-antioxidant based strategies for the treatment of CNS need further investigations and optimization before being exploitable in clinical applications. Further in-depth studies involving the test of nano-antioxidants in animal models of various brain diseases are of pivotal necessity in order to fully understand the efficiency and the possible adverse effects of these materials. However, generally, antioxidants represent a valid tool to treat central nervous system diseases, and, particularly, neurodegenerative pathologies,

and an increasing number of clinical trials exploiting antioxidant-loaded smart nanocarriers is envisaged in the next future.

Acknowledgments
This research is partially supported by the *Fondazione CaRiPLo*, grant nr. 2018-0156, and by the Italian Ministry of Health, grant RF-2016-02361610.

Received: ((will be filled in by the editorial staff))
Revised: ((will be filled in by the editorial staff))
Published online: ((will be filled in by the editorial staff))

References

[1] S. N. Vaishnavi, A. G. Vlassenko, M. M. Rundle, A. Z. Snyder, M. A. Mintun, M. E. Raichle, *Proc. Natl. Acad. Sci. U. S. A.* **2010**, *107(41)*, 17757-17762.

[2] M. Valko, D. Leibfritz, J. Moncol, M. T. D. Cronin, M. Mazur, J. Telser, *Int. J. Biochem. Cell. Biol.* **2007**, *39(1)*, 44-84.

[3] K. A. Jellinger, *J. Neural Transm. Suppl.*, **2003**, *(65)*, 101-44.

[4] D. Nicholls, *Sci. Aging Knowledge Environ.* **2002**, *2002(31)*, pe12.

[5] S. Smaili, H. Hirata, R. Ureshino, P. T. Monteforte, A. P. Morales, M. L. Muler, J. Terashima, K. Oseki, T. R. Rosenstock, G. S. Lopes, C. Bincoletto, *An. Acad. Bras. Cienc.* **2009**, *81(3)*, 467-475.

[6] R. H. Swerdlow, S. M. Khan, *Exp. Neurol.* **2009**, *218(2)*, 308-15.

[7] R. Banerjee, A. A. Starkov, M. F. Beal, B. Thomas, *Biochim. Biophys. Acta.* **2009**, *1792(7)*, 651-63.

[8] S. Almeida, A. B. Sarmento-Ribeiro, C. Januário, A. C. Rego, C. R. Oliveira, *Biochem. Biophys. Res. Commun.* **2008**, *374(4)*, 599-603.

[9] S. Petri, M. Kiaei, M. Damiano, A. Hiller, E. Wille, G. Manfredi, N. Y. Calingasan, H. H. Szeto, M. F. Beal, *J. Neurochem.* **2006**, *98(4)*, 1141-1148.

[10] J. C. Martinou, D. R. Green, *Nat. Rev. Mol. Cell. Biol.* **2001**, *2(1)*, 63-67.

[11] H. E. de Vries, M. Witte, D. Hondius, A. J. M. Rozemuller, B. Drukarch, J. Hoozemans, J. van Horssen, *Free Radic. Bio. Med.* **2008**, *45(10)*, 1375-83.

[12] L. Zuo, T. Zhou, B. K. Pannell, A. C. Ziegler, T. M. Best, *Acta Physiol. (Oxf).* **2015**, *214(3)*, 329-348.

[13] D. A. Patten, M. Germain, M. A. Kelly, R. S. Slack, *J. Alzheimers Dis.* **2010**, *20 Suppl 2*, S357-67.

[14] P. P. Zandi, J. C. Anthony, A. S. Khachaturian, S. V. Stone, D. Gustafson, J. A. T.

Tschanz, M. C. Norton, K. A. Welsh-Bohmer, J. C. S. Breitner, *Arch. Neurol.* **2004**, *61(1)*, 82-8.

[15] Y. Liu, J. Shi, *Nano Today*. **2019**, *27*, 146-177.

[16] C. Martinelli, C. Pucci, G. Ciofani, *APL Bioeng.* **2019**, *3(1)*, 011502.

[17] J. Nicolas, S. Mura, D. Brambilla, N. MacKiewicz, P. Couvreur, *Chem. Soc. Rev.* **2013**, *42(3)*, 1147-235.

[18] H. Gao, Z. Pang, X. Jiang, *Pharm. Res.* **2013**, *30(10)*, 2485-98.

[19] K. Hadinoto, A. Sundaresan, W. S. Cheow, *Eur. J. Pharm. Biopharm.* **2013**, *85(3 Pt A)*, 427-43.

[20] A. Burns, W. T. Self, in *Smart Nanoparticles for Biomedicine*, (Ed. G. Ciofani), Elsevier, **2018**, Ch. 11.

[21] C. Tapeinos, A. Pandit, *Adv. Mater.* **2016**, *28(27)*, 5334.

[22] G. Pizzino, N. Irrera, M. Cucinotta, G. Pallio, F. Mannino, V. Arcoraci, F. Squadrito, D. Altavilla, A. Bitto, *Oxid. Med. Cell. Longev.* **2017**, *2017*, 8416763.

[23] V. Lobo, A. Patil, A. Phatak, N. Chandra, *Pharmacogn. Rev.* **2010**, *4(8)*, 118-26.

[24] S. Kwiecien, K. Jasnos, M. Magierowski, Z. Sliwowski, R. Pajdo, P. Jagiellonian, *J. Physiol. Pharmacol.* **2014**, *65(5)*, 613-22.

[25] M. Bélanger, I. Allaman, P. J. Magistretti, *Cell. Metab.* **2011**, *14(6)*, 724-38.

[26] J. Wojsiat, K. M. Zoltowska, K. Laskowska-kaszub, U. Wojda, *Oxid. Med. Cell. Longev.* **2018**, *2018*, 6435861.

[27] I. Dalle-donne, R. Rossi, R. Colombo, D. Giustarini, A. Milzani, *Clin. Chem.* **2006**, *52(4)*, 601-23.

[28] World Alzheimer Report 2018: The state of the art of dementia research: New frontiers, can be found under https://www.alz.co.uk/news/world-alzheimer-report-2018-state-of-art-of-dementia-research-new-frontiers, accessed: November, 2019.

[29] P. Cras, M. Kawai, D. Loweryf, P. Gonzalez-dewhit-t, B. Greenbergt, G. Perry, *Proc.*

Natl. Acad. Sci. U. S. A. **1991**, *88(17)*, 7552-6.

[30] I. Grundke-Iqbal, K. Iqbal, Y. Tung, M. Quinlan, H. M. Wisniewski, L. I. Bindert, *Proc. Natl. Acad. Sci. U. S. A.* **1986**, *83(13)*, 4913-7.

[31] V. W. Chow, M. P. Mattson, P. C. Wong, and M. Gleichmann, *Neuromolecular Med.* **2010**, *12(1)*, 1-12.

[32] L. Pagani, A. Eckert, *Int. J. Alzheimers Dis.* **2011**, *2011*, 925050.

[33] K. Herrup, *Nat. Neurosci.* **2015**, 18(6), 794-9.

[34] R. H. Swerdlow, *Neurotox. Res.* **2012**, *22(3)*, 182–194.

[35] R. H. Swerdlow, S. M. Khan, *Med. Hypotheses.* **2004**, *63(1)*, 8-20.

[36] K. G. Yiannopoulou, S. G. Papageorgiou, *Ther. Adv. Neurol. Disord.* **2013**, *6(1)*, 19-33.

[37] O.B. Tysnes, A. Storstein, *J. Neural Transm. (Vienna).* **2017**, *124(8)*, 901-905.

[38] P. M. Antony, N. J. Diederich, R. Kruger, R. Balling, *FEBS J.* **2013**, *280(23)*, 5981-93.

[39] W. S. Kim, K. Kågedal, G. M. Halliday, *Alzheimers Res. Ther.* **2014**, *6(5)*, 73.

[40] K. Wakabayashi, K. Tanji, F. Mori, H. Takahashi, *Neuropathology.* **2007**, *27(5)*, 494-506.

[41] S. B. Berman, T. G. Hastings, *J. Neurochem.* **1999**, *73(3)*, 1127-37.

[42] J. D. Guo, X. Zhao, Y. Li, G. R. Li, X. L. Liu, *Int. J. Mol. Med.* **2018**, *41(4)*, 1817-1825.

[43] B. G. Trist, D. J. Hare, K. L. Double, *Cell. Chem. Biol.* **2018**, *25(7)*, 807-816.

[44] R. Betarbet, T. B. Sherer, J. T. Greenamyre, *Exp. Neurol.* **2005**, *191 Suppl 1*, S17-27.

[45] J. Bové, D. Prou, C. Perier, S. Przedborski, *NeuroRx.* **2005**, *2(3)*, 484-94.

[46] S. Manoharan, G. J. Guillemin, R. S. Abiramasundari, M. M. Essa, M. Akbar, M. D. Akbar, *Oxid. Med. Cell. Longev.* **2016**, *2016*, 8590578.

[47] E. L. Lane, *Eur. J. Neurosci.* **2019**, *49(3)*, 384-398.

[48] R. Filograna, M. Beltramini, L. Bubacco, M. Bisaglia, *Curr. Neuropharmacol.* **2016**, *14(3)*, 260-71.

[49] T. Finkel, N. J. Holbrook, *Nature.* **2000**, *408(6809)*, 239-47.

[50] R. A. Floyd, K. Hensley, *Neurobiol. Aging.* **2002**, *23(5)*, 795-807.

[51] A. D. Lopez, C. D. Mathers, M. Ezzati, D. T. Jamison, C. J. Murray, *Lancet.* **2006**, *367(9524)*, 1747-57.

[52] R. L. Sacco, S. E. Kasner, J. P. Broderick, L. R. Caplan, J. J. Connors, A. Culebras, M. S. V. Elkind, M. G. George, A. D. Hamdan, R. T. Higashida, B. L. Hoh , L. S. Janis , C. S. Kase , D. O. Kleindorfer , J.-M. Lee , M. E. Moseley , E. D. Peterson , T. N. Turan , A. L. Valderrama , and H. V. Vinters, *Stroke.* **2013**, *44(7)*, 2064-89.

[53] P. Deb, S. Sharma, K. M. Hassan, *Pathophysiology.* **2010**, *17(3)*, 197-218.

[54] R. A. G. Patel, C. J. White, *Vasc. Med.* **2011**, *16(1)*, 19-28.

[55] L. B. Goldstein, R. Adams, M. J. Alberts, L. J. Appel, L. M. Brass, C. D. Bushnell, A. Culebras, T. J. DeGraba, P. B. Gorelick, J. R. Guyton, R. G. Hart , G. Howard , M. K.-Hayes, J.V. (Ian) Nixon , and R. L. Sacco, *Stroke.* **2006**, *37(6)*, 1583-633.

[56] S. Cadenas, *Free Radic. Biol. Med.* **2018**, *117*, 76-89.

[57] L. Lin, X. Wang, Z. Yu, *Biochem. Pharmacol.* **2016**, *5(4)*, 213.

[58] A. P. Halestrap, *Biochem. Soc. Trans.*, **2006**, *34(Pt 2)*, 232-7.

[59] U. Dirnagl, C. Iadecola, M. A. Moskowitz, *Trends Neurosci.* **1999**, *22(9)*, 391-7.

[60] G. A. Donnan, H. Ma, S. Schwarz, D. Georgiadis, W. Hacke, *Adv. Neurol.* **2003**, *92*, 347-59.

[61] X. Wang, T. Mori, T. Sumii, E. H. Lo, *Stroke.* **2002**, *33(7)*, 1882-8.

[62] D. Wu, W. M. Pardridge, *Proc. Natl. Acad. Sci. U. S. A.* **1999**, *96(1)*, 254-9.

[63] Y. Zhang, W. M. Pardridge, *Brain Res.* **2001**, *889(1-2)*, 49-56.

[64] G. Y. Yang, A. Lorris Betz, *Stroke.* **1994**, *25(8)*, 1658-64; discussion 1664-5.

[65] A. Kumar, R. R. Ratan, *J. Huntingtons Dis.* **2016**, *5(3)*, 217-237.

[66] J. P. G. Vonsattel, M. Difiglia, *J. Neuropathol. Exp. Neurol.* **1998**, *57(5)*, 369-84.

[67] C. Soto, *Nat. Rev. Neurosci.* **2003**, *4(1)* 49-60.

[68] C. Carmo, L. Naia, C. Lopes, A. C. Rego, *Adv. Exp. Med. Biol.* **2018**, *1049*, 59-83.

[69] S. E. Browne, M. F. Beal, *Antioxid. Redox Signal.* **2006**, *8(11-12)*, 2061-73.

[70] N. Klepac, M. Relja, R. Klepac, S. Hećimović, T. Babić, V. Trkulja, *J. Neurol.* **2007**, *254(12)*, 1676-83.

[71] I. Túnez, F. Sánchez-López, E. Agüera, R. Fernández-Bolaños, F. M. Sánchez, I. Tasset-Cuevas, *J. Med. Chem.* **2011**, *54(15)* 5602-6.

[72] D. Lim, L. Fedrizzi, M. Tartari, C. Zuccato, E. Cattaneo, M. Brini, E. Carafoli, *J. Biol. Chem.* **2008**, *283(9)*, 5780-9.

[73] M. Dorota Majewska, J. A. Bell, *Neuroreport.* **1990**, *1(3-4)*, 194-6.

[74] M. Miyamoto, T. H. Murphy, R. L. Schnaar, J. T. Coyle, *J. Pharmacol. Exp. Ther.* **1989**, *250 (3)*, 1132-1140.

[75] I. Túnez, P. Montilla, M. D. C. Muñoz, M. Feijóo, M. Salcedo, *J. Pineal Res.* **2004**, *37(4)*, 252-6.

[76] M. F. Beal, D. R. Henshaw, B. G. Jenkins, B. R. Rosen, J. B. Schulz, *Ann. Neurol.* **1994**, *36(6)*, 882-8.

[77] L. Yang, N. Y. Calingasan, E. J. Wille, K. Cormier, K. Smith, R. J. Ferrante, M. Flint Beal, *J. Neurochem.* **2009**, *109(5)*, 1427-39.

[78] R. J. Ferrante, O. A. Andreassen, A. Dedeoglu, K. L. Ferrante, B. G. Jenkins, S. M. Hersch, M. F. Beal, *J. Neurosci.* **2002**, *22(5)*, 1592-9.

[79] L. C. Wijesekera, P. Nigel Leigh, *Orphanet J. Rare Dis.* **2009**, *4*, 3.

[80] L. I. Bruijn, M. K. Houseweart, S. Kato, K. L. Anderson, S. D. Anderson, E. Ohama, A. G. Reaume, R. W. Scott, D. W. Cleveland, *Science*. **1998**, *281(5384)*, 1851-4.

[81] C. Vijayvergiya, M. F. Beal, J. Buck, G. Manfredi, *J. Neurosci.* **2005**, *25(10)*, 2463-70.

[82] S. Morgan, R. W. Orrell, *Br. Med. Bull.* **2016**, *119(1)*, 87-98.

[83] E. P. Simpson, A. A. Yen, S. H. Appel, *Curr. Opin. Rheumatol.* **2003**, *15(6)*, 730-6.

[84] N. Ercal, H. Gurer-Orhan, N. Aykin-Burns, *Curr. Top. Med. Chem.* **2005**, *1(6)*, 529-39.

[85] S. Mostafalou, M. Abdollahi, *Toxicology.* **2018**, *409*, 44-52.

[86] M. Simkó, M. O. Mattsson, *J. Cell. Biochem.* **2004**, *93(1)*, 83-92.

[87] H. Kawamata, G. Manfredi, *Mech. Ageing Dev.* **2010**, *131(7-8)*, 517-26.

[88] M. L. Tradewell, L. A. Cooper, S. Minotti, H. D. Durham, *Neurobiol. Dis.* **2011**, *42(3)*, 265-75.

[89] B. Oskarsson, T. F. Gendron, N. P. Staff, *Mayo Clin. Proc.* **2018**, *93(11)*, 1617-1628.

[90] M. K. Jaiswal, *Med. Res. Rev.* **2019**, *39(2)*, 733-748.

[91] J. Dorst, A. C. Ludolph, A. Huebers, *Ther. Adv. Neurol. Disord.* **2017**, *11*, 1756285617734734.

[92] S. Zoccolella, A. Santamato, P. Lamberti, *Neuropsychiatr. Dis. Treat.* **2009,** *5*, 577–595.

[93] H. F. McFarland, R. Martin, *Nat. Immunol.* **2007**, *8(9)*, 913-9.

[94] T. Olsson, L. F. Barcellos, L. Alfredsson, *Nat. Rev. Neurol.* **2016**, *13(1)*, 25-36.

[95] M. J. Bagur, M. A. Murcia, A. M. Jiménez-Monreal, J. A. Tur, M. M. Bibiloni, G. L. Alonso, M. Martínez-Tomé, *Adv. Nutr.* **2017**, *8(3)*, 463-472.

[96] H. T. Besler, S. Omoğlu, Z. Oku, *Nutr. Neurosci.* **2002**, *5(3)*, 215-20.

[97] M. I. S. Hunter, B. C. Nlemadim, D. L. W. Davidson, *Neurochem. Res.* **1985**, *10(12)*, 1645-52.

[98] R. Dringen, *Antioxid. Redox Signal.* **2005**, *7(9-10)*, 1223-33.

[99] J. Van Horssen, M. E. Witte, G. Schreibelt, H. E. de Vries, *Biochim. Biophys. Acta.* **2011**, *1812(2)*, 141-50.

[100] R. S. Lopez-Diego, H. L. Weiner, *Nat. Rev. Drug Discov.* **2008**, *7(11)*, 909-25.

[101] Y. Gilgun-Sherki, E. Melamed, D. Offen, *J. Neurol.* **2004**, *251(3)*, 261-8.

[102] S. Dolati, M. Ahmadi, L. Aghebti-Maleki, A. Nikmaram, F. Marofi, R. Rikhtegar, H. Ayromlou, M. Yousefi, *Pharmacol. Reports.* **2018**, *70(6)*, 1158-1167.

[103] M. Khalili, S. Eghtesadi, A. Mirshafiey, G. Eskandari, M. Sanoobar, M. A. Sahraian, A.

Motevalian, A. Norouzi, S. Moftakhar, A. Azimi, *Nutr. Neurosci.* **2014**, *17(1)*, 16-20.

[104] C. Criscuolo, C. Procaccini, M. C. Meschini, A. Cianflone, R. Carbone, S. Doccini, D. Devos, C. Nesti, I. Vuillaume, M. Pellegrino, A. Filla, G. De Michele, G. Matarese, F. M. Santorelli, *J. Neurol.* **2015**, *262(12)*, 2755-63.

[105] M. Girard, R. Larivière, D. A. Parfitt, E. C. Deane, R. Gaudet, N. Nossova, F. Blondeau, G. Prenosil, E. G. M. Vermeulen, M. R. Duchen, A. Richter, E. A. Shoubridge, K. Gehring, R. A. McKinney, B. Brais, J. P. Chapple, and P. S. McPherson, *Proc. Natl. Acad. Sci. U. S. A.* **2012**, *109(5)*, 1661-6.

[106] Y. Bouhlal, R. Amouri, G. El Euch-Fayeche, F. Hentati, *Parkinsonism Relat. Disord.* **2011**, *17(6)*, 418-22.

[107] S. L. Perlman, in *J. Child. Neurol.* **2012**, *27(9)*, 1217-22.

[108] C. J. Strawser, K. A. Schadt, D. R. Lynch, *Expert Rev. Neurother.* **2014**, *14(8)*, 949-57.

[109] M. H. Parkinson, J. B. Schulz, P. Giunti, *J. Neurochem.* **2013**, *126, Suppl 1*, 125-41.

[110] T. V. Aranca, T. M. Jones, J. D. Shaw, J. S. Staffetti, T. Ashizawa, S. H. Kuo, B. L. Fogel, G. R. Wilmot, S. L. Perlman, C. U. Onyike, S. H. Ying & T. A. Zesiewicz, *Neurodegener. Dis. Manag.* **2016**, *6(1)*, 49-65.

[111] T. Meier, G. Buyse, *J. Neurol.* **2009**, *256, Suppl 1*, 25-30.

[112] M. Rinaldi, M. Caffo, L. Minutoli, H. Marini, R.V. Abbritti, F. Squadrito, V. Trichilo, A. Valenti, V. Barresi, D. Altavilla, M. Passalacqua, G. Caruso, *Int. J. Mol. Sci.* **2016**, *17(6)*, pii: E984.

[113] M. Vidak, D. Rozman, R. Komel, *Molecules.* **2015**, *20*, 19406.

[114] V.N. Bharadwaj, D.T. Nguyen, V.D. Kodibagkar, S.E. Stabenfeldt, *Adv. Healthc. Mater.* **2018**, *7(1)*.

[115] B.A. Bony, F.M. Kievit, *Pharmaceutics.* **2019**, *11(9)*, 473.

[116] S. Sharma, Y. Zhuang, Z. Ying, A. Wu, F. Gomez-Pinilla, *Neuroscience.* **2009**, *161(4)*, 1037-1044.

[117] N. Osier, E. McGreevy, L. Pham, A. Puccio, D. Ren, Y.P. Conley, S. Alexander, C.E. Dixon, *Int. J. Mol. Sci.* **2018**, *19*, 1539.

[118] E.D. Hall, R.A. Vaishnav, A.G. Mustafa, *Neurotherapeutics.* **2010**, *7(1)*, 51-61.

[119] D. Furtado, M. Björnmalm, S. Ayton, A. I. Bush, K. Kempe, *Adv. Mater.* **2018**, *30(46)*, e1801362.

[120] N. J. Abbott, A. Friedman, *Epilepsia.* **2012**, *53, Suppl 6*, 1-6.

[121] N. J. Abbott, A. A. K. Patabendige, D. E. M. Dolman, S. R. Yusof, D. J. Begley, *Neurobiol. Dis.* **2010**, *37(1)*, 13-25.

[122] W. M. Pardridge, *NeuroRx.* **2005**, *2(1)*, 3-14.

[123] B. V. Zlokovic, *Neuron.* **2008**, *57(2)*, 178-201.

[124] J. A. G. Agúndez, F. J. Jiménez-jiménez, H. Alonso-navarro, *Front. Cell. Neurosci.* **2014**, *8*, 335.

[125] A. C. Rego, C. R. Oliveira, *Neurochem. Res.* **2003**, *28(10)*, 1563-74.

[126] N. V. Margaritelis, *Pharmacol. Res.* **2016**, *111*, 126-132.

[127] V. V. Shuvaev, S. Tliba, J. Pick, E. Arguiri, M. Christofidou-Solomidou, S. M. Albelda, V. R. Muzykantov, *J. Control. Release.* **2011**, *149(3)*, 236-41.

[128] M. Carocho, I. C. F. R. Ferreira, *Food Chem. Toxicol.* **2013**, *51*, 15-25.

[129] B. Poljsak, D. Šuput, I. Milisav, *Oxid. Med. Cell. Longev.* **2013**, *2013*, 956792.

[130] Y. P. Chen, C. T. Chen, Y. Hung, C. M. Chou, T. P. Liu, M. R. Liang, C. T. Chen, C. Y. Mou, *J. Am. Chem. Soc.* **2013**, *135(4)*, 1516-23.

[131] M. K. Reddy, L. Wu, W. Kou, A. Ghorpade, V. Labhasetwar, *Appl. Biochem. Biotechnol.* **2008**, *151(2-3)*, 565-77.

[132] P. Hu, N. Tirelli, *Bioconjug. Chem.* **2012**, *23(3)*, 438-49.

[133] R. A. Jacob, *Subcell. Biochem.* **1996,** *25*, 1-16.

[134] E. Head, J. Rofina, S. Zicker, *Vet. Clin. North Am. Small Anim. Pract.* **2008**, *38(1)*, 167-78.

[135] B. Gess, S. Sevimli, J. K. Strecker, P. Young, W. R. Schäbitz, *PLoS One.* **2011**, *6(2)*, e17139.

[136] X. Mu, H. He, J. Wang, W. Long, Q. Li, H. Liu, Y. Gao, L. Ouyang, Q. Ren, S. Sun, J. Wang, J. Yang, Q. Liu, Y. Sun, C. Liu, X.D. Zhang, W. Hu, *Nano Lett.* **2019**, *19(7)*, 4527-4534.

[137] J. A. Joseph, B. Shukitt-Hale, N. A. Denisova, D. Bielinski, A. Martin, J. J. McEwen, P. C. Bickford, *J. Neurosci.* **1999**, *19(18)*, 8114-21.

[138] L. Volicer, P. B. Crino, *Neurobiol. Aging.* **1990**, *11(5)*, 567-71.

[139] P. Damier, E. C. Hirsch, P. Zhang, Y. Agid, F. Javoy-Agid, *Neuroscience.* **1993**, *52(1)*, 1-6.

[140] M. Perluigi, F. Di Domenico, A. Giorgi, M. E. Schininà, R. Coccia, C. Cini, F. Bellia, M. T. Cambria, C. Cornelius, D. A. Butterfield, V. Calabrese, *J. Neurosci. Res.* **2010**, *88(16)*, 3498-507.

[141] P. K. Mandal, M. Tripathi, S. Sugunan, *Biochem. Biophys. Res. Commun.* **2012**, *417(1)*, 43-8.

[142] G. Mpandzou, E. Aït Ben Haddou, W. Regragui, A. Benomar, M. Yahyaoui, *Rev. Neurol. (Paris).* **2016**, *172(2)*, 109-22.

[143] J. Wang, D. Yang, Y. Yu, G. Shao, Q. Wang, *Nutrients.* **2016**, *8(3)*, 142.

[144] G. Pietro Sechi, E. Sechi, C. Fois, N. Kumar, *Nutr. Rev.* **2016**, *74(5)*, 281-300.

[145] F. L. Crane, *J. Am. Coll. Nutr.* **2001**, *20(6)*, 591-8.

[146] Y. Uekaji, M. Onishi, D. Nakata, K. Terao, A. Paananen, R. Partanen, H. Yoshii, *J. Phys. Chem. B.* **2014**, *118(39)*, 11480-6.

[147] S. Kumar, R. Rao, A. Kumar, S. Mahant, S. Nanda, *Curr. Drug Deliv.* **2016**, *13(8)*, 1184-1204.

[148] N. K. Swarnakar, A. K. Jain, R. P. Singh, C. Godugu, M. Das, S. Jain, *Biomaterials.* **2011**, *32(28)*, 6860-74.

[149] B. K. Nanjwade, V. T. Kadam, F. V. Manvi, *J. Biomed. Nanotechnol.* **2013**, *9(3)*, 450-60.

[150] N. A. Mazo, V. Echeverria, R. Cabezas, M. Avila-Rodriguez, V. V. Tarasov, N. S. Yarla, G. Aliev, G. E. Barreto, *Curr. Pharm. Des.* **2017**, *23(28)*, 4180-4188.

[151] M. P. Murphy, *Free Radic. Biol. Med.* **2014**, *66*, 20-3.

[152] M. E. Obrenovich, N. G. Nair, A. Beyaz, G. Aliev, V. P. Reddy, *Rejuvenation Res.* **2010**, *13(6)*, 631-43.

[153] K. Wolfe, X. Wu, R. H. Liu, *J. Agric. Food Chem.* **2003**, *51(3)*, 609-14.

[154] D. Albani, L. Polito, A. Signorini, G. Forloni, *Biofactors.* **2010**, *36(5)*, 370-6.

[155] A. N. Winter, E. K. Ross, H. M. Wilkins, T. R. Stankiewicz, T. Wallace, K. Miller, D. A. Linseman, *Nutr. Neurosci.* **2018**, *21(6)*, 414-426.

[156] B. Bellaver, D. G. Souza, D. O. Souza, A. Quincozes-Santos, *Toxicol. In Vitro.* **2014**, *28(4)*, 479-84.

[157] K. S. Panickar, M. M. Polansky, R. A. Anderson, *Nutr. Neurosci.* **2009**, *12(3)*, 105-13.

[158] K. Rezai-Zadeh, D. Shytle, N. Sun, T. Mori, H. Hou, D. Jeanniton, J. Ehrhart, K. Townsend, J. Zeng, D. Morgan, J. Hardy, T. Town and J. Tan, *J. Neurosci.* **2005**, *25(38)*, 8807-14.

[159] F. Yang, G. P. Lim, A. N. Begum, O. J. Ubeda, M. R. Simmons, S. S. Ambegaokar, P. Chen, R. Kayed, C. G. Glabe, S. A. Frautschy, G. M. Cole, *J. Biol. Chem.* **2005**, *280(7)*, 5892-901.

[160] C. Yang, X. Zhang, H. Fan, Y. Liu, *Brain Res.* **2009**, *1282*, 133-41.

[161] S. Park, J. Yoon, S. Bae, M. Park, C. Kang, Q. Ke, D. Lee, P. M. Kang, *Biomaterials.* **2014**, *35(22)*, 5944-53.

[162] C. Kang, W. Cho, M. Park, J. Kim, S. Park, D. Shin, C. Song, D. Lee, *Biomaterials.* **2016**, *85*, 195-203.

[163] N. de O. F. Pinto, T. H. S. Rodrigues, R. de C. A. Pereira, L. M. A. e. Silva, C. A.

Cáceres, H. M. C. de Azeredo, C. R. Muniz, E. S. de Brito, K. M. Canuto, *Ind. Crops Prod.* **2016**, *86*, 279-288.

[164] Z. Fang, B. Bhandari, *Trends Food Sci. Technol.* **2010**, *21(10)*, 510-523.

[165] E. Ko, D. Jeong, J. Kim, S. Park, G. Khang, D. Lee, *Biomaterials.* **2014**, *35(12)*, 3895-902.

[166] D. Lee, S. Bae, Q. Ke, J. Lee, B. Song, S. A. Karumanchi, G. Khang, H. S. Choi, P. M. Kang, *J. Control. Release.* **2013**, *172(3)*, 1102-10.

[167] A. Y. Estevez, J. S. Erlichman, *Nanomedicine.* **2014**, *9(10)*, 1437-40.

[168] I. Celardo, J. Z. Pedersen, L. Ghibelli, *Nanoscale.* **2011**, *3(4)*, 1411-20.

[169] Y. Xue, Q. Luan, D. Yang, X. Yao, K. Zhou, *J. Phys. Chem. C* **2011**, *115(11)*, 4433-4438.

[170] J. M. Dowding, T. Dosani, A. Kumar, S. Seal, W. T. Self, *Chem. Commun.* **2012**, *48(40)*, 4896-8.

[171] A. Gupta, S. Das, C. J. Neal, S. Seal, *J. Mater. Chem. B.* **2016**, *4*, 3195-3202.

[172] M. Battaglini, C. Tapeinos, I. Cavaliere, A. Marino, A. Ancona, N. Garino, V. Cauda, F. Palazon, D. Debellis, G. Ciofani, *ACS Biomater. Sci. Eng.* **2019**, *5*, 670.

[173] G. Ciofani, G. G. Genchi, I. Liakos, V. Cappello, M. Gemmi, A. Athanassiou, B. Mazzolai, V. Mattoli, *Pharm. Res.* **2013**, *30(8)*, 2133-45.

[174] A. Marino, C. Tonda-Turo, D. De Pasquale, F. Ruini, G. Genchi, S. Nitti, V. Cappello, M. Gemmi, V. Mattoli, G. Ciardelli, G. Ciofani, *Biochim. Biophys. Acta Gen. Subj.* **2017**, *1861(2)*, 386-395.

[175] G. Ciofani, G. G. Genchi, B. Mazzolai, V. Mattoli, *Biochim. Biophys. Acta Gen. Subj.* **2014**, *1840(1)*, 495-506.

[176] B. D'Angelo, S. Santucci, E. Benedetti, S. Di Loreto, R. Phani, S. Falone, F. Amicarelli, M. Ceru, A. Cimini, *Curr. Nanosci.* **2009**, *5*, 167.

[177] A. Cimini, B. Dangelo, S. Das, R. Gentile, E. Benedetti, V. Singh, A. M. Monaco, S.

Santucci, S. Seal, *Acta Biomater.* **2012**, *8(6)*, 2056-67.

[178] J. M. Dowding, W. Song, K. Bossy, A. Karakoti, A. Kumar, A. Kim, B. Bossy, S. Seal, M. H. Ellisman, G. Perkins, W. T. Self, E. Bossy-Wetzel, *Cell Death Differ.* **2014**, *21(10)*, 1622-32.

[179] A. Pinna, L. Malfatti, G. Galleri, R. Manetti, S. Cossu, G. Rocchitta, R. Migheli, P.A. Serra, P. Innocenzi, *RSC Adv.* **2015,** *5*, 20432-20439.

[180] W. Song, S. Soo Lee, M. Savini, L. Popp, V. L. Colvin, L. Segatori, *ACS Nano.* **2014**, *8(10)*, 10328-10342.

[181] M. Ganesana, J. S. Erlichman, S. Andreescu, *Free Radic. Biol. Med.* **2012**, *53(12)*, 2240-9.

[182] A. Y. Estevez, S. Pritchard, K. Harper, J. W. Aston, A. Lynch, J. J. Lucky, J. S. Ludington, P. Chatani, W. P. Mosenthal, J. C. Leiter, S. Andreescu, J. S. Erlichman, *Free Radic. Biol. Med.* **2011**, *51(6)*, 1155-63.

[183] T. Zhang, C. Y. Li, J. J. Jia, J. S. Chi, D. Zhou, J. Z. Li, X. M. Liu, J. Zhang, L. Yi, *Curr. Med. Sci.* **2018**, *38(1)*, 144-152.

[184] A. Hosseini, A. M. Sharifi, M. Abdollahi, R. Najafi, M. Baeeri, S. Rayegan, J. Cheshmehnour, S. Hassani, Z. Bayrami, M. Safa, *Biol. Trace Elem. Res.* **2015**, *164(1)*, 80-9.

[185] R. Najafi, A. Hosseini, H. Ghaznavi, S. Mehrzadi, A. M. Sharifi, *Brain Res. Bull.* **2017**, *131*, 117-122.

[186] A. Arya, A. Gangwar, S. K. Singh, M. Roy, M. Das, N. K. Sethy, K. Bhargava, *Int. J. Nanomedicine.* **2016**, *11*, 1159-73.

[187] R. Yan, S. Sun, J. Yang, W. Long, J. Wang, X. Mu, Q. Li, W. Hao, S. Zhang, H. Liu, Y. Gao, L. Ouyang, J. Chen, S. Liu, X.D. Zhang, D. Ming, *ACS Nano.* **2019**, *13(10)*, 11552-11560.

[188] K. L. Heckman, W. Decoteau, A. Estevez, K. J. Reed, W. Costanzo, D. Sanford, J. C.

Leiter, J. Clauss, K. Knapp, C. Gomez, P. Mullen, E. Rathbun, K. Prime, J. Marini, J. Patchefsky, A. S. Patchefsky, R. K. Hailstone, J. S. Erlichman, *ACS Nano.* **2013**, *7(12),* 10582-96.

[189] W. Decoteau, K. L. Heckman, A. Y. Estevez, K. J. Reed, W. Costanzo, D. Sandford, P. Studlack, J. Clauss, E. Nichols, J. Lipps, M. Parker, B. Hays-Erlichman, J. C. Leiter, J. S. Erlichman, *Nanomedicine.* **2016**, *12(8)*, 2311-2320.

[190] E. Eitan, E. R. Hutchison, N. H. Greig, D.Tweedie, H. Celik, S. Ghosh, K. W. Fishbein, R. G. Spencer, C. Y. Sasaki, P. Ghosh, S. Das, S. Chigurapati, J. Raymick, S. Sarkar, S. Chigurupati, S. Seal, M. P. Mattson, *Exp. Neurol.* **2016,** *280,* 121.

[191] H. J. Kwon, M. Y. Cha, D. Kim, D. K. Kim, M. Soh, K. Shin, T. Hyeon, I. Mook-Jung, *ACS Nano.* **2016**, *10(2),* 2860-70.

[192] H. J. Kwon, D. Kim, K. Seo, Y. G. Kim, S. I. Han, T. Kang, M. Soh, T. Hyeon, *Angew. Chem. Int. Ed.* **2018**, *57(30),* 9408-9412.

[193] M. A. Hegazy, H. M. Maklad, D. M. Samy, D. A. Abdelmonsif, B. M. El Sabaa, F. Y. Elnozahy, *Neurochem. Int.* **2017**, *108*, 361-371.

[194] C. K. Kim, T. Kim, I.-Y. Choi, M. Soh, D. Kim, Y.-J. Kim, H. Jang, H.-S. Yang, J. Y. Kim, H.-K. Park, S. P. Park, S. Park, T. Yu, B. W. Yoon, S. H. Lee, T. Hyeon, *Angew. Chem. Int. Ed.* **2012**, *51(44),* 11039-43.

[195] W. Lin, Y. W. Huang, X. D. Zhou, Y. Ma, *Int. J. Toxicol.* **2006**, *25(6)*, 451-7.

[196] A. Nemmar, P. Yuvaraju, S. Beegam, M. A. Fahim, B. H. Ali, *Oxid. Med. Cell. Longev.* **2017**, *2017*, 9639035.

[197] M. Kumari, S. Indu, S. Srinivas, K. Kamal, P. Grover, *Mutat. Res. Genet. Toxicol. Environ. Mutagen.* **2014**, *775-776*, 7-19.

[198] J. Gagnon, K. M. Fromm, *Eur. J. Inorg. Chem.* **2015**, *27*, 4510-4517.

[199] S. Patil, A. Sandberg, E. Heckert, W. Self, S. Seal, *Biomaterials.* **2007**, *28(31)*, 4600-7.

[200] R. A. Yokel, T. C. Au, R. Macphail, S. S. Hardas, D. A. Butterfield, R. Sultana, M.

Goodman, M. T. Tseng, M. Dan, H. Haghnazar, J. M. Unrine, U. M. Graham, P. Wu, E. A. Grulke, *Toxicol. Sci.* **2012**, *127(1)*, 256-68.

[201] S. Kargozar, F. Baino, S. J. Hoseini, S. Hamzehlou, M. Darroudi, J. Verdi, L. Hasanzadeh, H. W. Kim, M. Mozafari, *Nanomedicine.* **2018**, *13(23)*, 3051-3069.

[202] N. Thakur, P. Manna, J. Das, *J. Nanobiotechnology.* **2019**, *17(1)*, 84.

[203] A. Dhall, W. Self, *Antioxidants.* **2018**, *7(8)*, pii: E97.

[204] S. N. Patil, J. S. Paradeshi, P. B. Chaudhari, S. J. Mishra, B. L. Chaudhari, *Appl. Biochem. Biotechnol.* **2016**, *180(4)*, 638-654.

[205] M. K. Zahran, H. B. Ahmed, M. H. El-Rafie, *Carbohydr. Polym.* **2014**, *111*, 971-8.

[206] S. Esmaeili, R. S. Fazelifard, S. Ahmadzadeh, M. Shokouhi, *KAUMS J. (FEYZ)* **2013**, *16(7)*, 779-780.

[207] N. Wang, H. Y. Tan, S. Li, Y. Xu, W. Guo, Y. Feng, *Oxid. Med. Cell. Longev.* **2017**, *2017*, 7478523.

[208] H. Wang, J. Zhang, H. Yu, *Free Radic. Biol. Med.* **2007**, *42(10)*, 1524-33.

[209] W. Cong, R. Bai, Y.-F. Li, L. Wang, C. Chen, *ACS Appl. Mater. Interfaces.* **2019**, *11(38)*, 34725-34735.

[210] H. Amani, R. Habibey, F. Shokri, S. J. Hajmiresmail, O. Akhavan, A. Mashaghi, H. Pazoki-Toroudi, *Sci. Rep.* **2019**, *9(1)*, 6044.

[211] L. Yang, W. Wang, J. Chen, N. Wang, G. Zheng, *J. Biomed. Mater. Res. A.* **2018**, *106(12)*, 3034-3041.

[212] L. Yang, N. Wang, G. Zheng, *Nanoscale Res. Lett.* **2018**, *13(1)*, 303.

[213] D. Sun, W. Zhang, Q. Yu, X. Chen, M. Xu, Y. Zhou, J. Liu, *J. Colloid Interface Sci.* **2017**, *505*, 1001-1010.

[214] N. Singh, M. A. Savanur, S. Srivastava, P. D. Silva, G. Mugesh, *Angew. Chem. Int. Ed.* **2017**, *56(45)*, 14267-14271.

[215] N. Singh, A. Savanur, S. Srivastava, *Nanoscale.* **2019**, *11(9)*, 3855-3863.

[216] T. Hamasaki, T. Kashiwagi, T. Imada, N. Nakamichi, S. Aramaki, K. Toh, S. Morisawa, H. Shimakoshi, Y. Hisaeda, S. Shirahata, *Langmuir.* **2008**, *24(14),* 7354-64.

[217] M. Takamiya, Y. Miyamoto, T. Yamashita, K. Deguchi, Y. Ohta, K. Abe, *Neuroscience.* **2012**, *221,* 47-55.

[218] M. Takamiya, Y. Miyamoto, T. Yamashita, K. Deguchi, Y. Ohta, Y. Ikeda, T. Matsuura, K. Abe, *J. Neurosci. Res.* **2011**, *89(7),* 1125-33.

[219] X. Mu, J. Wang, Y. Li, F. Xu, W. Long, L. Ouyang, H. Liu, Y. Jing, J. Wang, H. Dai, Q. Liu, Y. Sun, C. Liu, X.D. Zhang, *ACS Nano.* **2019**, *13(2),* 1870-1884.

[220] D. Schubert, R. Dargusch, J. Raitano, S. W. Chan, *Biochem. Biophys. Res. Commun.* **2006**, *342(1),* 86-91.

[221] R.N. Mitra, M.J. Merwin, Z. Han, S.M. Conley, M.R. Al-Ubaidi, M.I. Naash, *Free Radic. Biol. Med.* **2014**, *75,* 140-8.

[222] L. Zhang, P. Zhao, C. Yue, Z. Jin, Q. Liu, X. Du, Q. He, *Biomaterials.* **2019**, *197,* 393-404.

[223] Y. Qiu, Z. Wang, A. C. E. Owens, I. Kulaots, Y. Chen, A. B. Kane, R. H. Hurt, *Nanoscale.* **2014**, *6(20),* 11744-55.

[224] J. E. Kim, M. Lee, *Biochem. Biophys. Res. Commun.* **2003**, *303(2),* 576-9.

[225] L. Xie, Y. Luo, D. Lin, W. Xi, X. Yang, G. Wei, *Nanoscale.* **2014**, *6(16),* 9752-62.

[226] A. G. Bobylev, L. G. Marsagishvili, Z. A. Podlubnaya, *Biophysics (Oxf).* **2010**, *55(5),* 699-702.

[227] N. Gharbi, M. Pressac, M. Hadchouel, H. Szwarc, S. R. Wilson, F. Moussa, *Nano Lett.* **2005**, *5(12),* 2578-2585.

[228] A. Galano, *Nanoscale.* **2010**, *2(3),* 373-80.

[229] B. R. Bitner, D. C. Marcano, J. M. Berlin, R. H. Fabian, L. Cherian, J. C. Culver, M. E. Dickinson, C. S. Robertson, R. G. Pautler, T. A. Kent, J.M. Tour, *ACS Nano.* **2012**, *6(9),* 8007-14.

[230] X. Cai, H. Jia, Z. Liu, B. Hou, C. Luo, Z. Feng, W. Li, J. Liu, *J. Neurosci. Res.* **2008**, *86(16)*, 3622-34.

[231] M. Sharma, in *Applications of Targeted Nano Drugs and Delivery Systems*, (Eds. S. S. Mohapatra, S. Ranjan, N. Dasgupta, R.K. Mishra, S. Thomas), Elsevier, **2019**, Ch.18.

[232] F. Danhier, E. Ansorena, J. M. Silva, R. Coco, A. Le Breton, V. Préat, *J. Control. Release.* **2012**, *161(2)*, 505-22.

[233] G. Djiokeng Paka, S. Doggui, A. Zaghmi, R. Safar, L. Dao, A. Reisch, A. Klymchenko, V. G. Roullin, O. Joubert, C. Ramassamy, *Mol. Pharm.* **2016**, *13(2)*, 391-403.

[234] K. J. Lim, S. Bisht, E. E. Bar, A. Maitra, C. G. Eberhart, *Cancer Biol. Ther.* **2011**, *11(5)*, 464-73.

[235] X. Yun, V. D. Maximov, J. Yu, H. Zhu, A. A. Vertegel, M. S. Kindy, *J. Cereb. Blood Flow Metab.* **2013**, *33(4)*, 583-92.

[236] M. K. Reddy, V. Labhasetwar, *FASEB J.* **2009**, *23(5)*, 1384-95.

[237] M. Valenza, J. Y. Chen, E. Di Paolo, B. Ruozi, D. Belletti, C. Ferrari Bardile, V. Leoni, C. Caccia, E. Brilli, S. Di Donato, M. M. Boido, A. Vercelli, M. A. Vandelli, F. Forni, C. Cepeda, M. S. Levine, G. Tosi, E. Cattaneo, *EMBO Mol. Med.* **2015**, *7(12)*, 1547-64.

[238] X. Guo, G. Deng, J. Liu, P. Zou, F. Du, F. Liu, A. T. Chen, R. Hu, M. Li, S. Zhang, Z. Tang, L. Han, J. Liu, K.N. Sheth, Q. Chen, X. Gou, J. Zhou, *ACS Nano.* **2018**, *12(8)*, 8723-8732.

[239] R. Harris, E. Lecumberri, I. Mateos-Aparicio, M. Mengíbar, A. Heras, *Carbohydr. Polym.* **2011**, *84*, 803.

[240] M. F. Seifu, L. K. Nath, *Polym. Technol. Mater.* **2019**, *58(2)*, 158-171.

[241] N. Sahiner, S. B. Sengel, *Colloids Surfaces A Physicochem. Eng. Asp.* **2016**, *508*, 30.

[242] R. Sinha, G. J. Kim, S. Nie, D. M. Shin, *Mol. Cancer Ther.* **2006**, *5(8)*, 1909-17.

[243] C. Pucci, F. Cousin, F. Dole, J. P. Chapel, C. Schatz, *Langmuir.* **2018**, *34(7)*, 2531-2542.

[244] H. K. S. Yadav, A. A. Almokdad, S. I. M. Shaluf, M. S. Debe, in *Nanocarriers for Drug Delivery: Nanoscience and Nanotechnology in Drug Delivery*, (Eds. S. Mohapatra, S. Ranjan, N. Dasgupta, R. Kumar , S. Thomas) Elsevier, **2019,** Ch. 17.

[245] K. Y. Ju, Y. Lee, S. Lee, S. B. Park, J. K. Lee, *Biomacromolecules.* **2011**, *12(3),* 625-32.

[246] Y. Liu, K. Ai, X. Ji, D. Askhatova, R. Du, L. Lu, J. Shi, *J. Am. Chem. Soc.* **2017**, *139(2),* 856-862.

[247] M. Gulati, M. Grover, S. Singh, M. Singh, *Int. J. Pharm.* **1998**, *165,* 129-168.

[248] Y. Barenholz, *J. Control. Release.* **2012**, *160(2),* 117-34.

[249] Z. E. Suntres, *J. Toxicol.* **2011**, *2011,* 152474.

[250] T. Tong-Un, S. Muchimapura, W. Phachonpai, J. Wattanathorn, *Am. J. Neurosci.* **2010**, *1*, 21.

[251] S. Mourtas, A. N. Lazar, E. Markoutsa, C. Duyckaerts, S. G. Antimisiaris, *Eur. J. Med. Chem.* **2014**, *80,* 175-83.

[252] S. Sarkar, N. Das, *Mech. Ageing Dev.* **2006**, *127(4),* 391-7.

[253] J. Sinha, N. Das, M. K. Basu, *Biomed. Pharmacother.* **2001**, *55(5),* 264-71.

[254] N. J. Wiley, A. B. Madhankumar, R. M. Mitchell, E. B. Neely, E. Rizk, G. L. Douds, Z. Simmons, J. R. Connor, *Adv. Nanoparticles.* **2012**, *1(3),* 44-53.

[255] S. Imaizumi, V. Woolworth, R. A. Fishman, P. H. Chan, *Stroke.* **1990**, *21(9),* 1312-7.

[256] S. Imaizumi, V. Woolworth, H. Kinouchi, S. F. Chen, R. A. Fishman, P. H. Chan, *Acta Neurochir. Suppl. (Wien).* **1990**, *51*, 236-8.

[257] A. Akbarzadeh, R. Rezaei-Sadabady, S. Davaran, S. W. Joo, N. Zarghami, Y. Hanifehpour, M. Samiei, M. Kouhi, K. Nejati-Koshki, *Nanoscale Res. Lett.* **2013**, *8(1),* 102.

[258] K. Mäder, W. Mehnert, *Adv. Drug Deliv. Rev.* **2001**, *47(2-3),* 165-96.

[259] F. Tamjidi, M. Shahedi, J. Varshosaz, A. Nasirpour, *Innov. Food Sci. Emerg. Technol.*

2013, *19*, 29-43.

[260] P. Hassanzadeh, F. Atyabi, R. Dinarvand, A. R. Dehpour, M. Azhdarzadeh, M. Dinarvand, *Daru.* **2017**, *25(1)*, 25.

[261] V. Kakkar, S. K. Muppu, K. Chopra, I. P. Kaur, *Eur. J. Pharm. Biopharm.* **2013**, *85(3 Pt A)*, 339-45.

[262] R. Sandhir, A. Yadav, A. Mehrotra, A. Sunkaria, A. Singh, S. Sharma, *Neuromolecular Med.* **2014**, *16(1)*, 106-18.

[263] P. Ganesan, D. Narayanasamy, *Sustain. Chem. Pharm.* **2017**, *6*, 37-56.

[264] W. Mehnert, K. Mader, *Adv. Drug Deliv. Rev.* **2001**, *47(2-3)*, 165-196.

[265] L. P. Wu, M. Ficker, J. B. Christensen, P. N. Trohopoulos, S. M. Moghimi, *Bioconjug. Chem.* **2015**, *26(7)*, 1198-211.

[266] A. Sharma, K. Liaw, R. Sharma, Z. Zhang, S. Kannan, R. M. Kannan, *Theranostics.* **2018**, *8(20)*, 5529-5547.

[267] T. T. Jia, Z. G. Sun, Y. Lu, J. Gao, H. Zou, F. Y. Xie, G. Q. Zhang, H. Xu, D. X. Sun, Y. Yu, Y. Zhong, *Int. J. Nanomedicine.* **2016**, *11*, 3765-75.

[268] L. L. Deng, M. Taxipalati, F. Que, H. Zhang, *Sci. Rep.* **2016**, *6*, 38160.

[269] G. Tripodo, T. Chlapanidas, S. Perteghella, B. Vigani, D. Mandracchia, A. Trapani, M. Galuzzi, M. C. Tosca, B. Antonioli, P. Gaetani, M. Marazzi, M.L. Torre, *Colloids Surfaces B Biointerfaces.* **2015**, *125*, 300-8.

[270] C. Bharti, N. Gulati, U. Nagaich, A. Pal, *Int. J. Pharm. Investig.* **2015**, *5(3)*, 124-33.

[271] A. Grillone, M. Battaglini, S. Moscato, L. Mattii, C. De Julián Fernández, A. Scarpellini, M. Giorgi, E. Sinibaldi, G. Ciofani, *Nanomedicine.* **2019**, *14(6)*, 727-752.

[272] T. Y. Yen, Z. R. Stephen, G. Lin, Q. Mu, M. Jeon, S. Untoro, P. Welsh, M. Zhang, *Adv. Healthc. Mater.* **2019**, *8(20)*, e1900826.

[273] J. Ambati, A. M. Lopez, D. Cochran, P. Wattamwar, K. Bean, T. D. Dziubla, S. E. Rankin, *Acta Biomater.* **2012**, *8(6)*, 2096-103.

[274] M. Chorny, E. Hood, R. J. Levy, V. R. Muzykantov, *J. Control. Release.* **2010**, *146(1),* 144-51.

[275] Y. Shen, B. Cao, N. R. Snyder, K. M. Woeppel, J. R. Eles, X. T. Cui, *J. Nanobiotechnology.* **2018**, *16(1),* 13.

[276] E. Amanzadeh, A. Esmaeili, R. E. N. Abadi, N. Kazemipour, Z. Pahlevanneshan, S. Beheshti, *Sci. Rep.* **2019**, *9(1),* 6876.

[277] A. Lodha, M. Lodha, A. Patel, J. Chaudhuri, J. Dalal, M. Edwards, D. Douroumis, in *J. Pharm. Bioallied Sci.* **2012**, *4 (Suppl 1),* S92-4.

[278] G. Salas, R. Costo, M. del P. Morales, in *Nanobiotechnology: Inorganic Nanoparticles vs Organic Nanoparticles*, (Eds: J.M. de la Fuente, V. Grazu), Elsevier, **2012**, Ch. 2.

[279] M. Saeedi, M. Eslamifar, K. Khezri, S. M. Dizaj, *Biomed. Pharmacother.* **2019**, *111*, 666-675.

[280] S. Barua, S. Mitragotri, *Nano Today.* **2014**, *9(2),* 223-243.

[281] S. Xu, B. Z. Olenyuk, C. T. Okamoto, S. F. Hamm-Alvarez, *Adv. Drug Deliv. Rev.* **2013**, *65(1),* 121-38.

[282] M. Malhotra, S. Prakash, *Curr. Nanosci.* **2011**, 7(1), 81-93.

[283] A. H. Bhat, K. B. Dar, S. Anees, M. A. Zargar, A. Masood, M. A. Sofi, S. A. Ganie, *Biomed. Pharmacother.* **2015**, *74,* 101-10.

[284] S. S. Sheu, D. Nauduri, M. W. Anders, *Biochim. Biophys. Acta*. **2006**, *1762(2),* 256-65.

[285] J. Zielonka, J. Joseph, A. Sikora, M. Hardy, O. Ouari, J. Vasquez-Vivar, G. Cheng, M. Lopez, B. Kalyanaraman, *Chem. Rev.* **2017**, *117(15),* 10043-10120.

[286] G. Hughes, M. P. Murphy, E. C. Ledgerwood, *Biochem. J.* **2005**, *389(Pt 1),* 83-9

[287] M. C. Frantz, P. Wipf, *Environ. Mol. Mutagen.* **2010**, *51(5),* 462-75.

[288] G. Schatz, *J. Biol. Chem.* **1996**, *271(50),* 31763-6.

[289] B. Ruttkay-Nedecky, L. Nejdl, J. Gumulec, O. Zitka, M. Masarik, T. Eckschlager, M. Stiborova, V. Adam, R. Kizek, *Int. J. Mol. Sci.* **2013**, *14(3),* 6044-66.

[290] Y. C. Kang, M. Son, S. Kang, S. Im, Y. Piao, K. S. Lim, M. Y. Song, K. S. Park, Y. H. Kim, Y. K. Pak, *Exp. Mol. Med.* **2018**, *50(8)*, 105.

[291] "U.S. National Library of Medicine," can be found under https://clinicaltrials.gov/ct2/results?cond=Alzheimer&term=antioxidant&cntry=&state=&city=&dist=, accessed: November, 2019.

[292] L. Shinto, J. Quinn, T. Montine, H. H. Dodge, W. Woodward, S. Baldauf-Wagner, D. Waichunas, L. Bumgarner, D. Bourdette, L. Silbert, J. Kaye, *J. Alzheimers Dis.* **2014**, *38(1)*, 111-20.

[293] "U.S. National Library of Medicine," can be found under https://clinicaltrials.gov/ct2/results?cond=&term=NCT00090402&cntry=&state=&city=&dist=, accessed: November, 2019.

[294] K. R. Patel, E. Scott, V. A. Brown, A. J. Gescher, W. P. Steward, K. Brown, *Ann. N. Y. Acad. Sci.* **2011**, *1215*, 161-9.

[295] R. S. Turner, R. G. Thomas, S. Craft, C. H. Van Dyck, J. Mintzer, B. A. Reynolds, J. B. Brewer, R. A. Rissman, R. Raman, P. S. Aisen, *Neurology.* **2015**, *85(16)*, 1383-91.

[296] "U.S. National Library of Medicine," can be found under https://clinicaltrials.gov/ct2/results?cond=&term=NCT01504854&cntry=&state=&city=&dist=, accessed: November, 2019.

[297] C. Moussa, M. Hebron, X. Huang, J. Ahn, R. A. Rissman, P. S. Aisen, R. S. Turner, *J. Neuroinflammation.* **2017**, *14(1)*, 1.

[298] B. Vafadari, A. Salamian, L. Kaczmarek, *J. Neurochem.* **2016**, *139 (Suppl 2)*, 91–114.

[299] M. Verslegers, K. Lemmens, I. Van Hove, L. Moons, *Prog. Neurobiol.* **2013**, *105*, 60–78.

[300] A. Reijerkerk, G. Kooij, S.M. van der Pol, S. Khazen, C.D. Dijkstra, H.E. de Vries, *FASEB J.* **2006**, *20*, 2550–2552.

[301] E. Candelario-Jalil, Y. Yang, G.A. Rosenberg, *Neuroscience.* **2009**, *158*, 983–994.

[302] "U.S. National Library of Medicine," can be found under https://clinicaltrials.gov/ct2/results?cond=&term=NCT00678431&cntry=&state=&city=&dist=, accessed: November, 2019.

[303] C. W. Zhu, H. Grossman, J. Neugroschl, S. Parker, A. Burden, X. Luo, M. Sano, *Alzheimerss Dement. (N.Y.).* **2018**, 4, 609-616.

[304] "U.S. National Library of Medicine," can be found under https://clinicaltrials.gov/ct2/show/NCT00951834, accessed: November, 2019.

[305] "U.S. National Library of Medicine," can be found under https://clinicaltrials.gov/ct2/results?cond=&term=NCT01699711&cntry=&state=&city=&dist=, accessed: November, 2019.

[306] R. de la Torre, S. de Sola, G. Hernandez, M. Farré, J. Pujol, J. Rodriguez, J. M. Espadaler, K. Langohr, A. Cuenca-Royo, A. Principe, L. Xicota, N. Janel, S. Catuara-Solarz, G. Sanchez-Benavides, H. Bléhaut, I. Dueñas-Espín, L. Del Hoyo, B. Benejam, L. Blanco-Hinojo, S. Videla, M. Fitó, J.M. Delabar, M. Dierssen; TESDAD study group, *Lancet Neurol.* **2016**, *15(8)*, 801-810.

[307] "U.S. National Library of Medicine," can be found under https://clinicaltrials.gov/ct2/results?cond=&term=NCT00040378&cntry=&state=&city=&dist=, accessed: November, 2019.

[308] "U.S. National Library of Medicine," can be found under https://clinicaltrials.gov/ct2/results?cond=&term=NCT00099710&cntry=&state=&city=&dist=, accessed: November, 2019.

[309] R. J. Kryscio, E. L. Abner, A. Caban-Holt, M. Lovell, P. Goodman, A. K. Darke, M. Yee, J. Crowley, F. A. Schmitt, *JAMA Neurol.* **2017**, *74(5)*, 567-573.

[310] J. M. Ringman, S. A. Frautschy, E. Teng, A. N. Begum, J. Bardens, M. Beigi, K. H. Gylys, V. Badmaev, D. D. Heath, L. G. Apostolova, V. Porter, Z. Vanek, G.A. Marshall, G. Hellemann, C. Sugar, D.L. Masterman, T.J. Montine, J.L. Cummings,

G.M. Cole, *Alzheimers Res. Ther.* **2012**, *4(5)*, 43.

[311] X. Dong, *Theranostics.* **2018**, *8(6)*, 1481-1493.

[312] "U.S. National Library of Medicine," can be found under https://clinicaltrials.gov/ct2/results?cond=brain&term=liposomes&cntry=&state=&city=&dist=, accessed: November, 2019.

[313] "U.S. National Library of Medicine," can be found under https://clinicaltrials.gov/ct2/results?cond=&term=NCT03095092&cntry=&state=&city=&dist=, accessed: November, 2019.

[314] "U.S. National Library of Medicine," can be found under https://clinicaltrials.gov/ct2/results?cond=&term=NCT01398748&cntry=&state=&city=&dist=, accessed: November, 2019.

[315] "U.S. National Library of Medicine," can be found under https://clinicaltrials.gov/ct2/results?cond=&term=NCT02424708&cntry=&state=&city=&dist=, accessed: November, 2019.

[316] M. J. Holmay, M. Terpstra, L. D. Coles, U. Mishra, M. Ahlskog, G. Öz, J. C. Cloyd, P. J. Tuite, *Clin. Neuropharmacol.* **2013**, *36(4)*, 103-6.

[317] "U.S. National Library of Science," can be found under https://clinicaltrials.gov/ct2/results?cond=&term=NCT01470027&cntry=&state=&city=&dist=, accessed: November, 2019.

[318] "U.S. National Library of Medicine," can be found under https://clinicaltrials.gov/ct2/results?cond=&term=NCT01427517&cntry=&state=&city=&dist=, accessed: November, 2019.

[319] L. D. Coles, P. J. Tuite, G. Öz, U. R. Mishra, R. V. Kartha, K. M. Sullivan, J. C. Cloyd, M. Terpstra, *J. Clin. Pharmacol.* **2018**, *58(2)*, 158-167.

[320] "U.S. National Library of Medicine," can be found under https://clinicaltrials.gov/ct2/results?cond=&term=NCT02212678&cntry=&state=&city

=&dist=, accessed: November, 2019.

[321] G. Tardiolo, P. Bramanti, E. Mazzon, *Molecules.* **2018**, *23(12)*, pii: E3305.

[322] T. Squillaro, A. Cimini, G. Peluso, A. Giordano, M. A. B. Melone, *Biochem. Pharmacol.* **2018**, *154*, 303-317.

[323] "U.S. National Library of Medicine," can be found under https://clinicaltrials.gov/ct2/results?cond=&term=NCT03150966&cntry=&state=&city=&dist=, accessed: November, 2019.

[324] "U.S. National Library of Medicine," can be found under https://clinicaltrials.gov/ct2/results?cond=&term=NCT00997438&cntry=&state=&city=&dist=, accessed: November, 2019.

[325] "U.S. National Library of Medicine", can be found under https://clinicaltrials.gov/ct2/show/NCT00010842, accessed: November, 2019.

[326] "U.S. National Library of Medicine," can be found under https://clinicaltrials.gov/ct2/results?cond=&term=NCT00067327&cntry=&state=&city=&dist=, accessed: November, 2019.

[327] "U.S. National Library of Medicine," can be found under https://clinicaltrials.gov/ct2/results?cond=&term=NCT03032601&cntry=&state=&city=&dist=, accessed: November, 2019.

[328] "U. S National Library of Medicine," can be found under https://clinicaltrials.gov/ct2/results?cond=&term=NCT01279876&cntry=&state=&city=&dist=, accessed: November, 2019.

[329] "U.S. National Library of Medicine," can be found under https://clinicaltrials.gov/ct2/results?cond=&term=NCT02463318&cntry=&state=&city=&dist=, accessed: November, 2019.

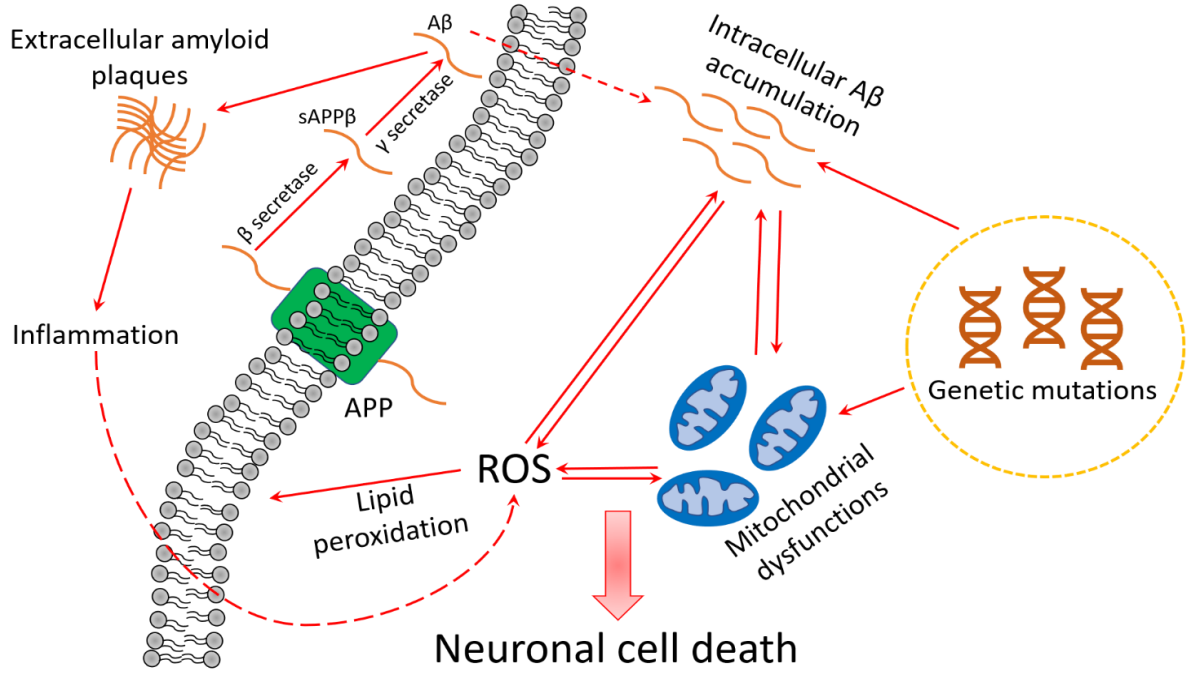


Figure 1. Main hallmarks of Alzheimer's disease and their interconnections. ROS, mitochondrial dysfunction, and accumulation of Aβ aggregates are the most common key points of Alzheimer's disease and all of them can cause or be caused by the others thorugh a positive feedback loop. Genetic mutations in genes involved in Aβ metabolism or mitochondrial functions are also linked to the development of Alzheimer's disease. At the end, the impairments caused by these hallmarks lead to the death of neuronal cells.

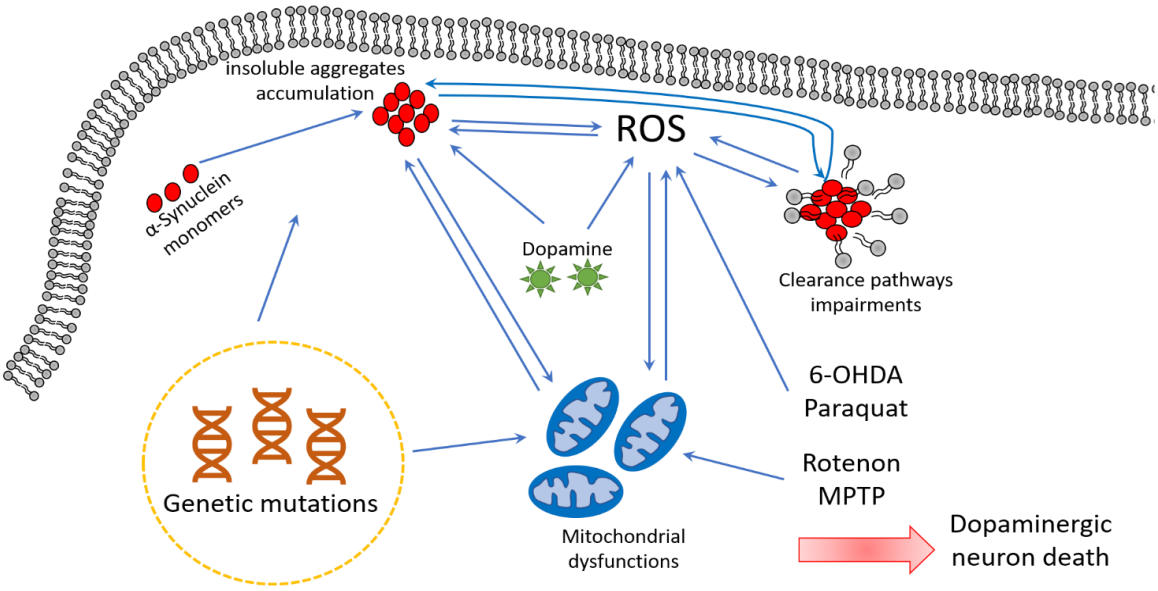


Figure 2. Main hallmarks of Parkinson's disease and their interconnections. The accumulation of insoluble aggregated of α-synuclein is one of the main hallmarks of Parkinson's disease. These aggregates can lead to a high level of ROS, and to impairments in cellular clearance pathways or mitochondrial dysfunctions that, in turn, can exacerbate the accumulation of α-synuclein. ROS, mitochondrial dysfunctions, and impairments in clearance pathways are also interconnected into a self-reinforcing loop. Other factors can contribute to the development of Parkinson's disease like genetic mutations, dopamine oxidation, and exposure to toxin. The final outcome is the degeneration of the dopaminergic neurons.

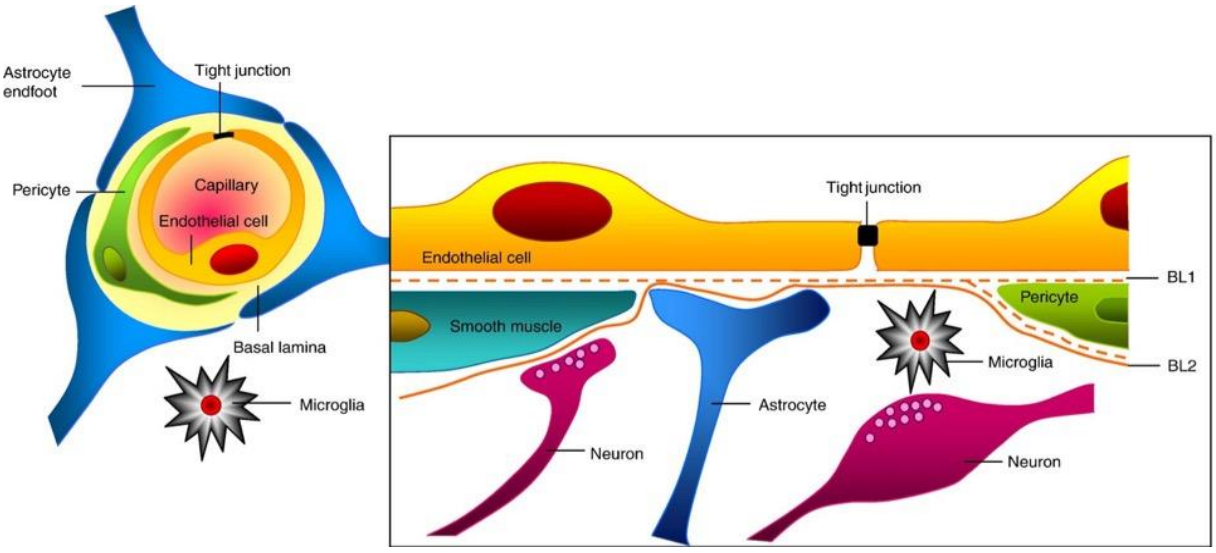


Figure 3. The structure of the blood-brain barrier and of the neurovascular unit, mainly composed by brain capillaries endothelial cells, perycites, astrocytic endfeet and microglia. The characteristic of the brain endothelial cells is the presence of tight junction that act as a barrier for paracellular transport. Reproduced with permission. [121] Copyright 2010, Elsevier.

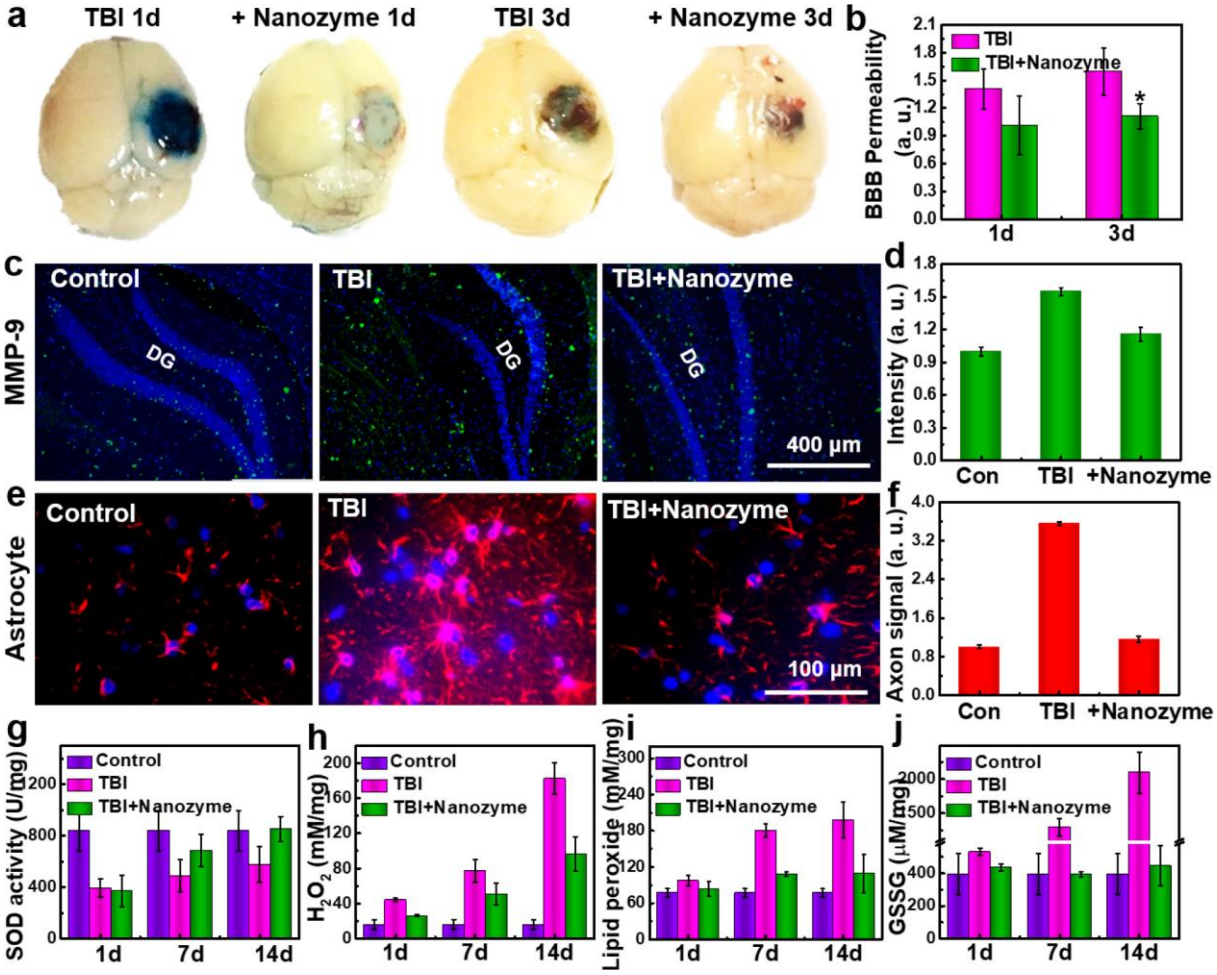


Figure 4. *In vivo* administration of carbogenic nanozyme to mice with traumatic brain injury. (a) optical images of mice brains showing the BBB permeability by Evans blue staining (the higher the blue intensity, the higher the BBB permeability); (b) analysis of the BBB permeability assay showing the decrease of the permeability after treatment with nanozymes; (c, d) MMP-9 level in hippocampus and (e, f) astrocytes activation level decrement following nanozyme injection; (g) SOD activity, (h) H_2O_2 concentration, (i) lipid peroxidation, and (j) glutathione disulfide concentration after nanozyme treatment. Reproduced with permission. [136] Copyright 2019, American Chemical Society.

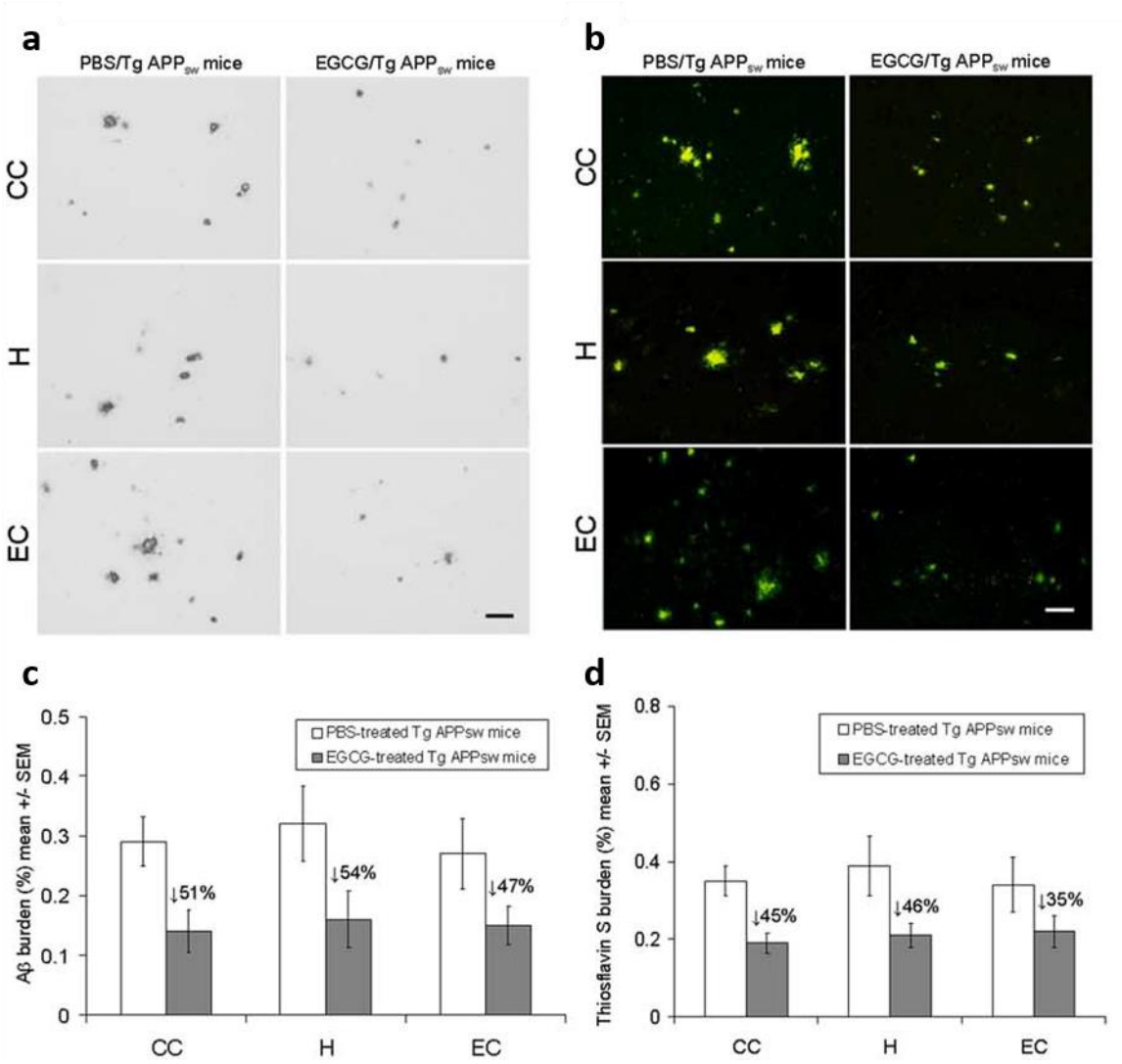


Figure 5. Transgenic mice model of Alzheimer's disease (Tg APPsw) examined upon peripheral injection of green tea epigallocatechin-3-gallate (EGCG, left) or vehicle (PBS, right). (a) Mouse brain coronal paraffin sections stained with anti-human Aβ antibody (4G8). CC, cingulate cortex; H, hippocampus; EC, entorhinal cortex. (b) Percentages of 4G8-immunoreactive Aβ plaques (mean ±1 SEM) and indication of their reduction in each brain region. (c) Mouse brain sections stained with thioflavin S. (d) Percentages of thioflavin S plaques (mean ±1 SEM) and indication of their reduction in each brain region. Scale bar corresponds to 50 µm. Reproduced with permission. [158] Copyright 2005, Society for Neuroscience.

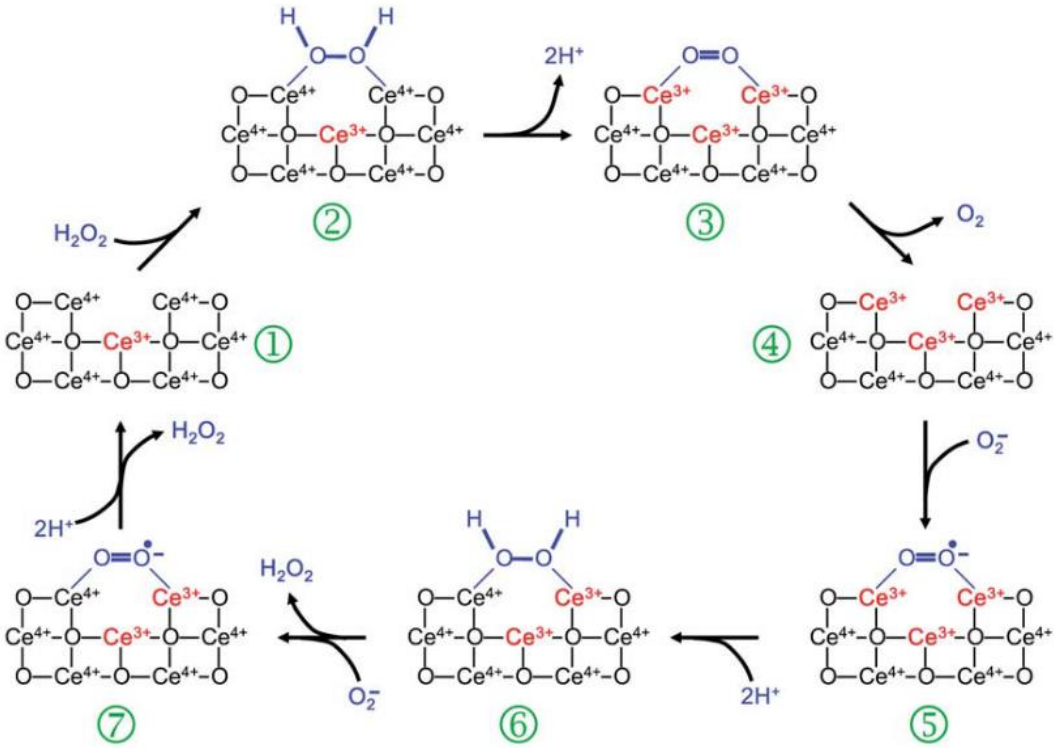


Figure 6. The suggested model for the self-regenerating redox cycle of nanoceria. Ce^{4+} present on the surface of the nanoparticles are able to bind and scavange H_2O_2 generating oxygen, hydrogen, and shifting to Ce^{3+} (reaction 1-4). Ce^{3+} can now bind and eliminate O^{2-} generating H_2O_2 and reverting to the initial Ce^{4+} (reactions 5-7). Reproduced with permission. [168] Copyright 2011, Royal Society of Chemistry.

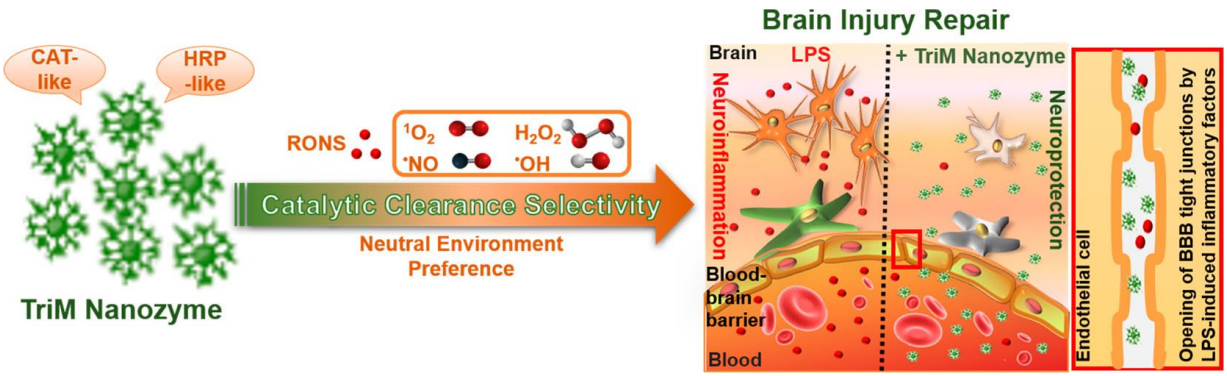


Figure 7. Trimetallic nanozymes (TriM Nanozyme) scavenging properties againsts ROS and RNS, with higher activity in neutral environment, and their action in repairing brain injury. Reproduced with permission. [219] Copyright 2019, American Chemical Society.

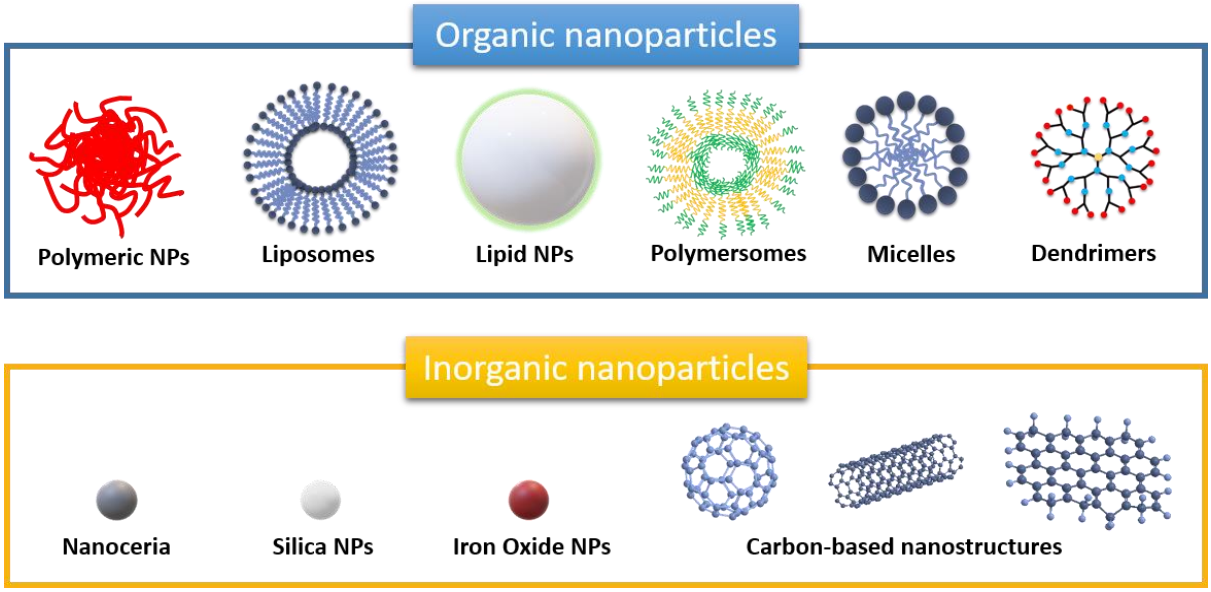


Figure 8. Different classes of nanosystems exploited in the antioxidant therapy for central nervous disorders.

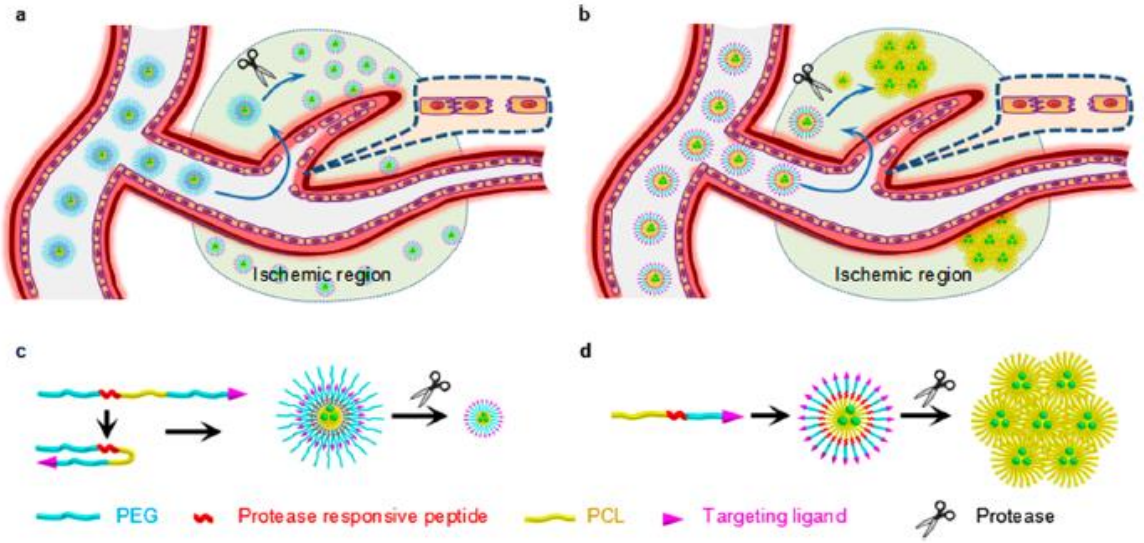


Figure 9. Schematic diagrams of (a, c) shrinkable and (b, d) expandable PEG-poly(ε-caprolactone) nanoparticles responsive to thrombin or matrix metalloproteinase-9, abundant at the site of ischemic stroke. Reproduced with permission. [238] Copyright 2018, American Chemical Society.

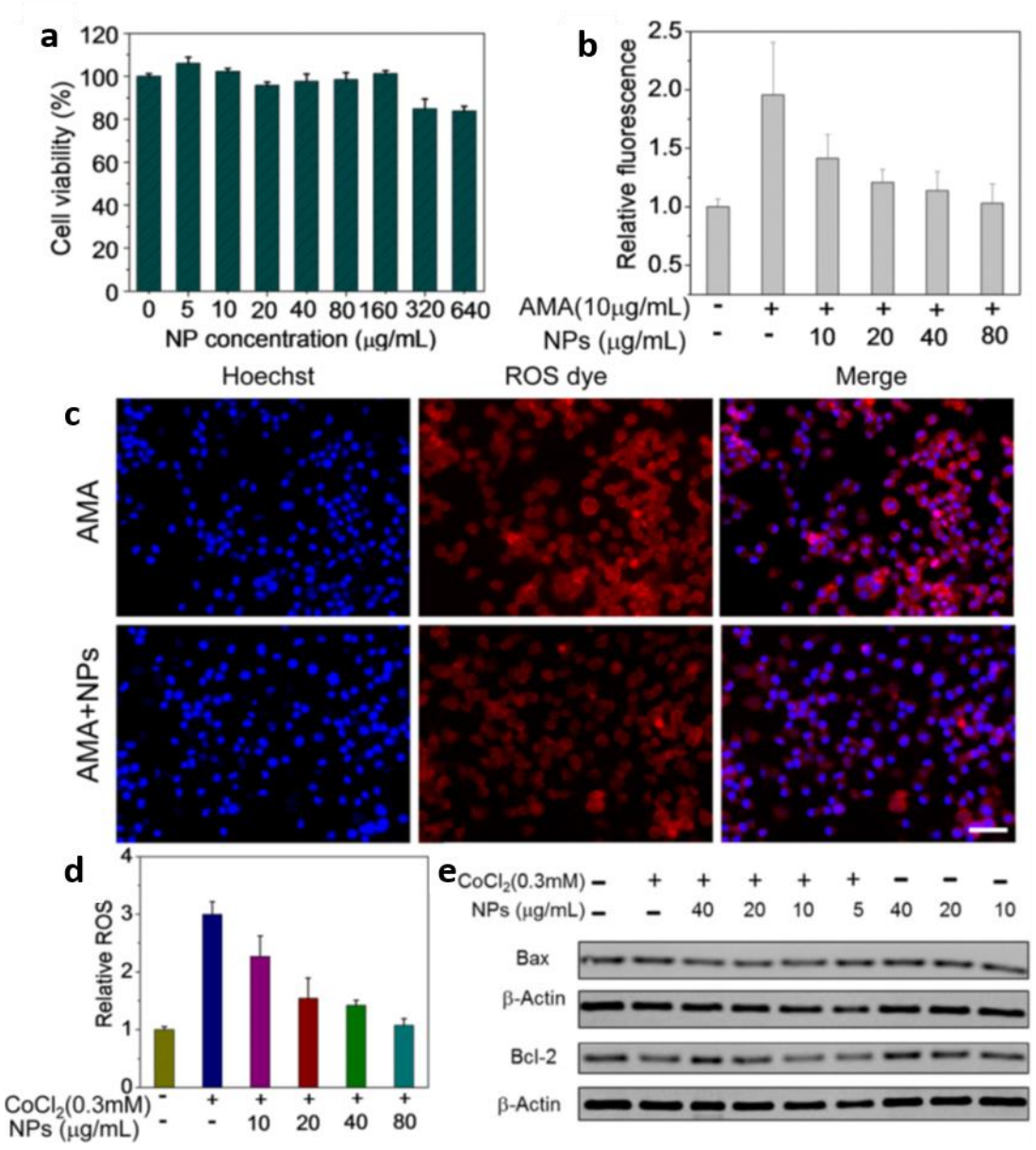


Figure 10. (a) Cytotoxicity, (b) intracellular $O_2^{\bullet-}$ scavenging activity, and (c) confocal fluorescence images of $O_2^{\bullet-}$ levels in Neuro 2A cells treated with melanin-like nanoparticles. (d) Protective effective of melanin-like nanoparticles against ROS in Neuro 2A cells under $CoCl_2$-induced hypoxic conditions. (e) Expression of Bax and Bcl-2 in $CoCl_2$-stimulated Neuro 2A cells with *vs.* without PEGMeNPs, as well as in cells treated only with PEG-MeNPs. Reproduced with permission. [246] Copyright 2017, American Chemical Society.

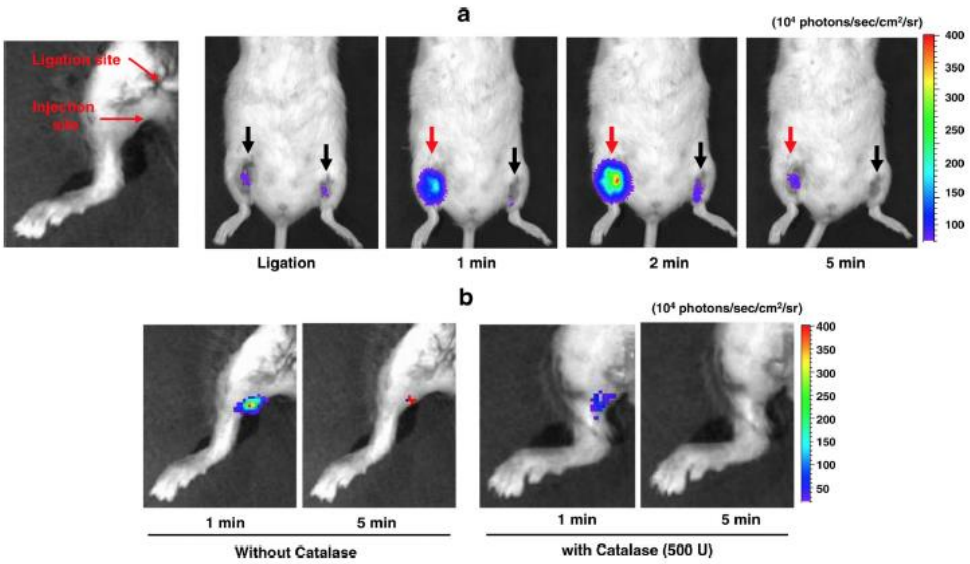


Figure 11. (a) *In vivo* imaging of H_2O_2 in hind limbs of mice treated with rubrene-loaded HPOX nanoparticles (HPOX/Rb) upon ischemia induction and direct injection of nanoparticles distal to the ligation sites. Red arrow indicates reperfusion at different time points (right hind limb), black arrow indicates ligated left hind limb (ischemia), (b) with or without the H_2O_2 degrading enzyme catalase. Reproduced with permission. [166] Copyright 2013, Elsevier.

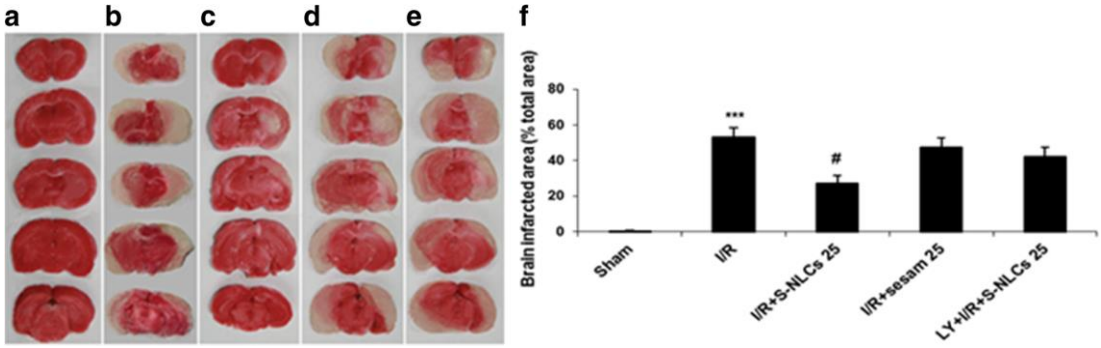


Figure 12. Mice brain coronal sections stained with triphenyltetrazolium chloride (TTC): (a) sham; (b) ischemia/reperfusion (I/R), in which the infarcted area is shown in white; (c) I/R + nanostructured lipid carriers (S-NLCs, containing 25 mg/kg of sesamol); d) I/R + 25 mg/kg sesamol; (e) I/R + LY294002 (25 µg/µl) + S-NLCs (25 mg/kg). (f) shows the percentage of the infarcted area, demonstrating as the treatment with S-NLCs is effective in reducing the damage. Reproduced under the terms of the CCA 4.0 International Licence. [260] Copyright 2017, Springer.

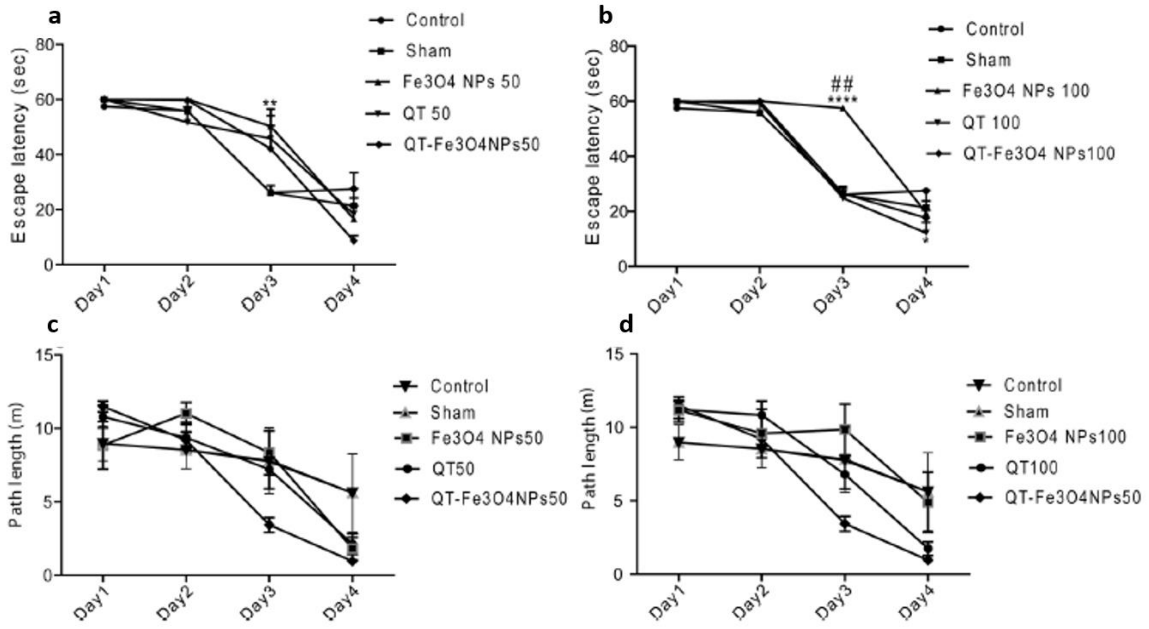


Figure 13. Morris water maze test performed in rats treated with quercetin (QT), superparamagnetic iron oxide nanoparticles (Fe_3O_4 NPs, SPIONs), quercetin-conjugated SPIONs (QT- Fe_3O_4 NPs) at different concentrations (50 and 100 mg/kg) and compared to controls and sham (vehicle of quercetin). (a, b) represent the mean escape latency and (c, d) the path length during all training days. Reproduced under the terms of the CCA 4.0 International Licence. [276] Copyright 2019, Nature Publishing Group.

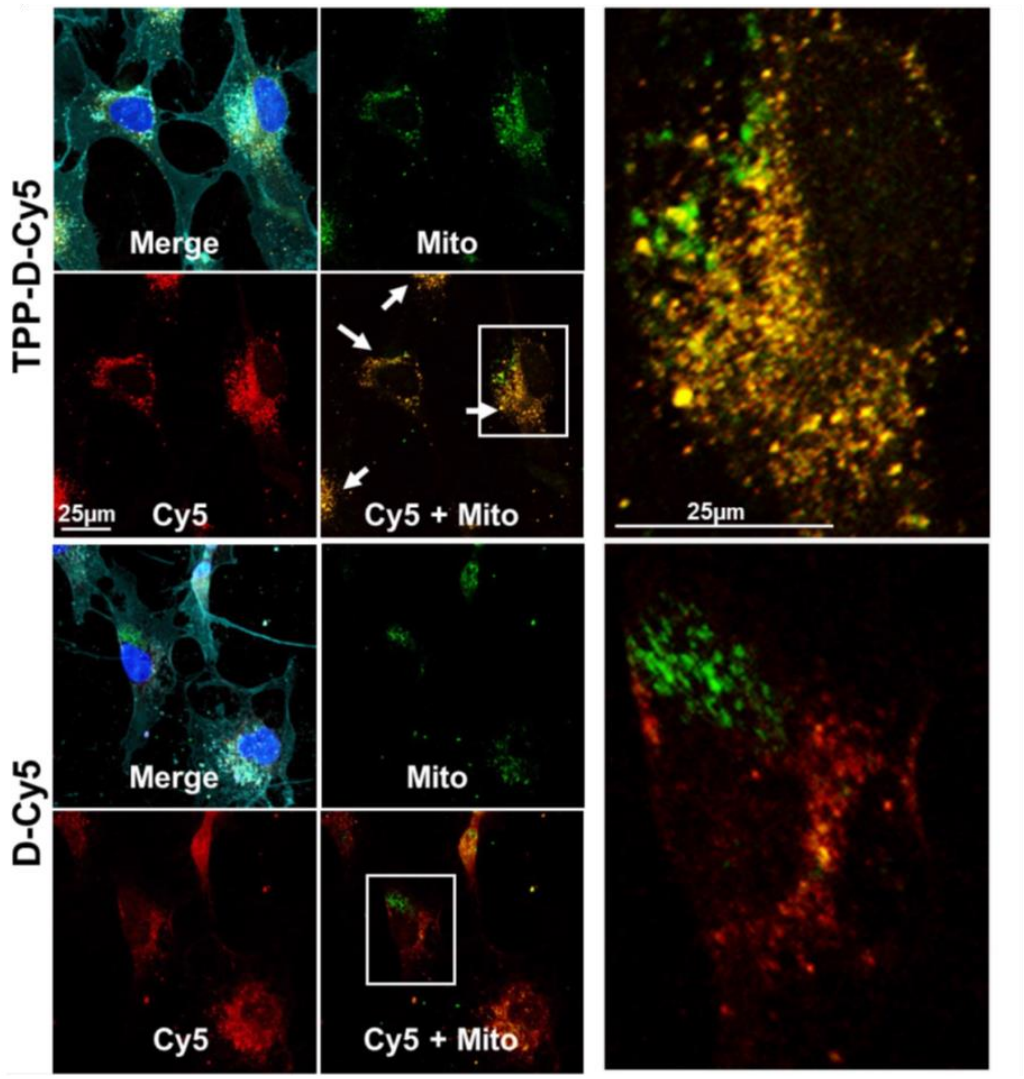


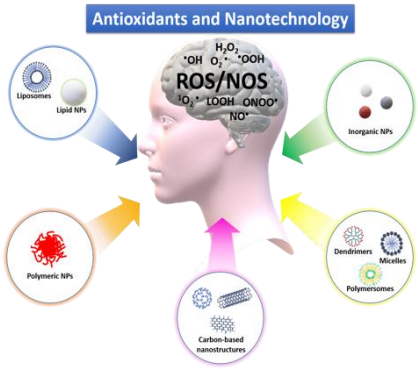
Figure 14. Confocal images showing the colocalization of TPP^+-dendrimers (TPP-D-Cy5, in red) with mitochondria (green), with respect to the non-functionalized dedrimers (D-Cy5). Colocalization is evidenced by the yellow signal resulting from the overlapping of TPP^+-dendrimers and mitochondria signals. Reproduced under the terms of the CCA 4.0 International Licence. [287] Copyright 2018, Ivyspring.

Nanotechnological approaches for antioxidant therapy of central nervous system diseases improve the bioavailability and the delivery of antioxidant molecules, prevent their degradation, and facilitate blood-brain barrier crossing and targeting to the desired sites within the brain.

Keyword: Nano-antioxidants

Chiara Martinelli†, Carlotta Pucci*†, Matteo Battaglini*†, Attilio Marino, and Gianni Ciofani**

Antioxidants and nanotechnology: Promises and limits of potentially disruptive approaches in the treatment of central nervous system diseases

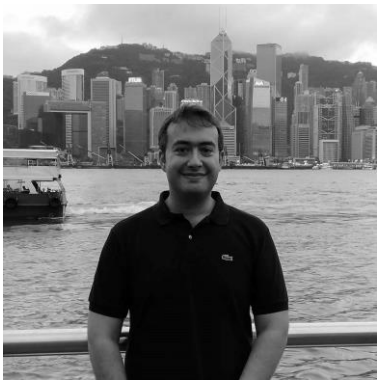


Chiara Martinelli received her Ph.D. in Medical Nanotechnology from the European School of Molecular Medicine in Milan, Italy. Currently, she is working as a postdoctoral fellow in the Smart Bio-Interfaces Research Line of the Italian Institute of Technology in Pontedera, Pisa, Italy. She develops antioxidant nanovectors for space medicine applications and for the treatment of central nervous system diseases. Her research interests focus on multifunctional nanomaterials, drug delivery, biological interactions, and cellular responses to microgravity.

Carlotta Pucci received her Ph.D. in Chemical Sciences from the University of Rome "La Sapienza" (Italy) working on the synthesis of liposomes and on the study of their interactions with biomacromolecules. She is currently a postdoctoral fellow in the Smart Bio-Interfaces Research Line of the Italian Institute of Technology in Pontedera, Pisa, Italy. Her activities deal with the fabrication and characterization of different kinds of biocompatible and multifunctional nanomaterials for the treatment of central nervous system diseases.

Matteo Battaglini received a B.Sc. degree in biotechnology in 2014 and a M.Sc. degree in industrial and molecular biotechnology in 2015 at the University of Pisa (Italy). He is currently a third-year Ph.D. candidate in Biorobotics at the *Scuola Superiore Sant'Anna* (Pisa, Italy), working in the Smart Bio-Interfaces Research Line at the Italian Institute of Technology. His research interests include the design, fabrication, and test of smart nanomaterials for human healthcare, and the development of innovative *in vitro* models of complex biological structures.

Attilio Marino received his Ph.D. in Biorobotics (*Scuola Superiore Sant'Anna*, Pisa, Italy). Post-doctoral research of Dr. Marino was sponsored by *Fondazione Umberto Veronesi* and, currently, by the European Research Council at the Italian Institute of Technology, Smart Bio-Interfaces Research Line. His main research interests are in the fields of nanomaterials for biomedical applications, 3D nanostructured scaffolds for tissue engineering, bio-hybrid systems for the modeling of blood-brain barrier, and biology in altered gravity conditions.



Gianni Ciofani, Ph.D., is Research Director at the Italian Institute of Technology, where he is Principal Investigator of the Smart Bio-Interfaces Research Line (Pontedera, Italy). His main research interests are in the field of smart nanomaterials for nanomedicine, bio/non-bio interactions, and biology in altered gravity conditions. He is coordinator or unit leader of many grants/projects, and in 2016 he was awarded a European Research Council (ERC) Starting Grant.