REVIEW ARTICLE

# Multifaceted nanoparticles: emerging mechanisms and therapies in neurodegenerative diseases

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## Abstract

Neurodegenerative diseases are a major global health burden particularly with the increasing ageing population. Hereditary predisposition and environmental risk factors contribute to the heterogeneity of existing pathological phenotypes. Traditional clinical interventions focused on the use of small drugs have often led to failures due to the difficulties of crossing the blood-brain-barrier and reaching brain. In this regard, nanosystems can specifically deliver drugs and improve their bioavailability, overcoming some of the major challenges in neurodegenerative diseases treatment. This review focuses on the use of nanosystems as an encouraging therapeutic approach targeting molecular pathways involved in localized and systematic neurodegenerative diseases. Among this latter, Friedreich's ataxia is an untreatable complex multisystemic disorder and the most spread type of ataxia, that represents a test case to validate the clinical potential of therapeutic strategies based on nanoparticles with pleiotropic effects.

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**Abbreviations:** (AAV) adeno-associated virus; (Aβ) amyloid beta, amyloid β, β-amyloid; (AD) Alzheimer's disease; (AhR) aryl hydrocarbon receptor; (ALS) Amyotrophic Lateral Sclerosis; (ATG7) autophagy related protein 7; (AuNPs) gold nanoparticles; (Au8pXs) gold quantum clusters; (BDNF) brain-derived neurotrophic factor; (BMSCs) bone-marrow mesenchymal stem cells; (Bax) Bcl2 associated X; (C-I, C-II) complex I and complex II of the electron transport chain; (CAT) catalase; (Chol-D6) deuterium-labelled cholesterol; (CNM-Au8) gold nanocrystals; (CNS) central nervous system; (CTRL) control; (Cur@SF NPs) curcumin-loaded silk fibroin nanoparticles; (DBM) dibenzoylmethane; (DIDS) 4,4′-Diisothiocyano-2,2′-stilbenedisulfonic acid; (DRG) dorsal root ganglia; (EAE) experimental autoimmune encephalomyelitis; (ETC) electron-transport chain; (FDA) Food and Drug Administration; ($Fe_2O_3$, $Fe_3O_4$) iron oxide; (Fe-S clusters) iron-sulphur clusters; (FRDA) Friedreich's ataxia; (FXN, iFXN, mFXN) frataxin, intermediate form, mature protein; (GABA) γ-aminobutyric acid; (GFAP) glial fibrillary acid protein; (GLP1) glucagon-like peptide-1; (GSH) glutathione; (GSK3β) glycogen synthase kinase-3 beta; (GSS) glutathione synthetase; (HD) Huntington's disease; (HSPCs) haematopoietic stem and progenitor cell; (Iba-1) ionized calcium-binding adapter molecule 1; (iNOS) inducible nitric oxide synthase; (ITE) 2-(1' H-indole-3'-carbonyl)-thiazole-4-carboxylic acid methyl ester; (JNK) c-Jun N-terminal kinase; (LC3) microtubule-associated proteins 1A/1B light chain 3B; (MFN1/2) mitoferrin 1 and 2; (MOG35-55) myelin oligodendrocyte glycoprotein 35-55; (MPS) mononuclear phagocyte system; (MS) Multiple sclerosis; (NDs) neurodegenerative diseases; (NE) nanoemulsion; (NF-κB) nuclear factor kappa-light-chain-enhancer of activated B cells; (NGF) nerve growth factor; (NPs) nanoparticles; (NRF2) nuclear factor erythroid 2-related factor 2; (PD) Parkinson's disease; (pDNA) plasmid DNA; (PEG) poly(ethylene glycol); (PEI) polyethylenimine; (P(HDCA-co-MePEGCA)) poly(hexadecylcyanoacrylate-co-methoxypolyethyleneglycolcyanoacrylate); (PLGA) poly(lactic-co-glycolic acid); (OL) oligodendrocyte; (RAGE) receptor for advanced glycation end products; (ROS) reactive oxygen species; (SOD) superoxide dismutase; (SPIONs) superparamagnetic iron oxide nanoparticles; (SQSTM1) sequestrome 1; (YG8R) a mouse model of FRDA

# Introduction

Neurodegenerative diseases (NDs) are a heterogeneous group of CNS disorders characterised by chronic and selective neuronal cell death, decreased strength, coordination and mobility, respiratory distress and cognitive deficit. [1] Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), Amyotrophic Lateral Sclerosis (ALS) and Multiple Sclerosis (MS) are the major NDs. [1,2] Although genetic and hereditary predisposition seem to play an important role, especially combined to environmental risk factors, [3] NDs differ in pathophysiology and symptomatology. On the contrary, protein misfolding, aggregation and accumulation of proteins, neuroinflammation, mitochondrial dysfunctions, oxidative stress, dysregulated autophagy, and apoptosis [4] are some of the most important shared biological processes. Most of these processes are also characteristic of Friedreich ataxia (FRDA), a multisystemic autosomal recessive degenerative disorder affecting central and peripheral nervous system, heart, skeletal muscle, and endocrine pancreas. [5,6] With onset before 25 years of age, FRDA affects one in 30,000-50,000 people with prominent neurological manifestations including limb ataxia, spinocerebellar ataxia, dysarthria, muscle weakness of lower limbs, loss of tone and areflexia, alterations of cardiac function and diabetes mellitus. [7] The most severe degenerative processes affect the spinocerebellar and pyramidal tracts as well as the dorsal root ganglia (DRGs) and dentate nuclei of cerebellum, resulting in degeneration of large sensory neurons and neuronal cell death. [8] Cardiac complications and aspiration pneumonia may appear subsequently to neurological symptoms and in most cases are cause of death of patients affected with FRDA. [9,10] Other related complications include coma and stroke. [11]

Most of FRDA cases (96-98%) are caused by a homozygous GAA triplet-repeat expansion mutation in the first intron of frataxin (FXN) gene on chromosome 9. The minority of patients has heterozygous GAA expansions, deletions or point mutations in FXN gene. [5,12] In both cases there is a dramatic reduction of FXN protein expression. Frataxin is a nuclear-encoded mitochondrial protein involved in iron-sulphur (Fe-S) cluster biogenesis, iron homeostasis, maintenance of the redox state, energetic metabolism, purine synthesis and DNA repair. [13,14] FXN is localized to the mitochondrial inner membrane and its deficiency leads to mitochondrial iron accumulation, mitochondrial dysfunction, dysregulated energetic metabolism, increased oxidative stress with generation of mitochondrial reactive oxygen species (ROS) and mitochondrial network dynamic dysregulations. [15] Furthermore, FXN interacts with mitochondrial electron-transport chain (ETC) complexes affecting mitochondrial respiration and energy production. [16]

Recently, life expectancy has increased, but to date there is no cure for FRDA. Therapeutic interventions include treatment with antioxidant compounds to mitigate mitochondrial oxidative stress, β-blockers, and ACE inhibitors to control heart rhythm preventing cardiac failure. [17,18]

The pharmaceuticals targeting mitochondrial dysfunction and metabolism (*Friedreich's Ataxia Research Alliance*, https://curefa.org/index.php) are promising, but none of them have been approved yet by the Food and Drug Administration (FDA).

The poor understanding of FXN related complex pathogenesis mechanisms challenges the development of therapies with long term efficacy and low secondary toxicity, which can cause adverse outcomes. Emerging scientific works suggest the possibility to use nanotechnologies such as nanoparticles and engineered materials to improve the treatment of neurodegenerative diseases. The aim of this review is to discuss the role of nanoparticles and other nanosystems in the treatment of NDs and of FRDA. Data were retrieved mainly from PubMed, from relevant articles published in the last 10 years regarding the use of nanosystems in the management of NDs.

# Nanoparticles and their use as therapeutic strategy

Nanoparticles (NPs) present dimensions between 1 and 100 nanometres. They are synthesized from metals, polymers or carbon compounds and they differ in size, origin, shape and composition. [19] Nanoparticles can be categorized in *organic lipid-based* (micelles, liposomes and nanoemulsions), [20] *polymeric* (polymer micelles and dendrimers), [21] and *inorganic* (carbon nanotubes, nanocrystals, gold nanoparticles, iron oxide nanoparticle) [22] NPs (Fig.1).

Nanoparticles are great candidates for NDs treatment due to their intrinsic properties, namely the high surface to mass ratio, high stability, and both hydrophilic and hydrophobic affinity. They can mimic the lipidic environment of cell membranes, they can encapsulate both hydrophilic and hydrophobic pharmaceuticals [23] and may be functionalized with a variety of molecules for biosensing and drug delivery. [24] Depending on origin and shape, NPs can be flexible, [25] biocompatible [26] and suitable for drug delivery across different compartments, including the blood brain barrier (BBB) [22,27,28].

The morphological and physico-chemical properties of nanoparticles have a strong impact on their cellular internalisation and they can be easily manipulated to achieve active and passive

drug targeting in a controlled fashion and through different routes of administration (oral, transdermal, intravenous, intranasal and pulmonary). [29,30]

The major internalisation pathway is endocytosis (Fig.2), [31] an active transport mechanism. The amount of energy that is required for internalisation is mainly dependent on the size, surface charge and flexibility of the nanosystem, [32] with silica and metal NPs being the ones that require the highest amount of energy. Indeed, lipid nanoparticles are held together by the same hydrophobic interactions that normally occur in cell membranes, hence their fusion and cellular uptake is facilitated compared to silica and metal NPs which have more rigid structures. [31]

Internalisation by passive transport is also possible through intramolecular spaces in the membrane (hydrophobic NPs), protein channels (hydrophilic NPs) and protein carriers (hydrophilic NPs). Mechanisms of NPs uptake have been previously described in detail in [31].

Stimuli-responsive NPs can also be designed to increase internalisation and drug release by modifying the surface of the nanocarrier in order to make it responsive upon a biological stimulus, such as a change in pH, temperature, presence of ROS. [33] pH-sensitive NPs have been mainly used to treat tumours and their microenvironment, as the change in pH is crucial for the development of the lesion and can be exploited for pH-dependent release of drugs. A similar approach is also applicable to the treatment of neurodegenerative diseases, since abnormal ROS production, commonly observed in NDs (FRDA, PD, AD), is linked to pH changes in the brain of NDs patients.

The tunable characteristics of NPs contribute to improve pharmacokinetics compared to conventional drugs and allow to overcome some major problems in the treatment of NDs, such as the difficulty in reaching the target tissue or compartment while also avoiding local and systemic toxicity. Small molecule drugs frequently show poor bioavailability at the desired site of treatment due to hepatic metabolism and clearance. For this reason, the administered dose of drug is higher than the effective dose with consequent accumulation and potentially toxic side effects. Instead, nanosystems can be modified to increase drug bioavailability, reduce the administered dose, and increase retention times. More recently new nanomaterials with further improved characteristics have been developed, such as nanoemulsions and nanocrystals.

Nanoemulsions are nano-sized emulsions, typically in the range of 20-200 nm, in which two immiscible liquids are mixed to form a single phase by means of an emulsifying agent. They differ from standard emulsion due to their reduced size that makes nanoemulsions easier to be

administrated, absorbed, and engrafted. Nanocrystals instead are solid carriers, usually spheres, with amorphous and lipophilic surface, which is often negatively charged. These systems are manufactured for facilitating the delivery of active pharmaceutical ingredients. As a matter of fact, nanoemulsions have already been used for delivering compounds to the CNS, [34,35] demonstrating higher efficacy than intravenous administration of small-molecule drugs. [36,37] Gold nanocrystal suspensions have been tested in preclinical studies in animal models of AD, [38] MS, [39] and PD, [40] showing improvement in neurorepair processes and neuronal resistance. Phase I clinical trials showed that gold nanocrystals (CNM-Au8) treatment is safe and well-tolerated, [41] leading to its approval for phase II clinical trials in patients with early symptoms of amyotrophic lateral sclerosis (ALS) (Table 1). [42]

The most common disadvantages of NP are: 1) the tendency to create aggregates with potential toxicity; 2) the abnormal tissue internalization and excretion from the body; 3) the interaction and adhesion to other cellular components, such as lipids, proteins, and nucleic acids. [43-45] Mostly, these disadvantages are dependent on NPs size, material, and route of administration. The inhalation of nanoparticles may lead to their accumulation in the alveoli, where they can cause oxidative stress–mediated lung inflammation at both acute and chronic stages. Inhalation may also lead to NPs accumulation in the brain through the olfactory bulb, [46] where they can exert neurotoxicity and cause neuronal cell death due to oxidative damage. [47-49] Systemically injected NPs entering the bloodstream to reach distant organs may instead get entrapped into filter organs such as spleen and kidneys, where they accumulate and may determine inflammation by triggering ROS mediated inflammatory pathways. [50,51] To overcome these issues, future optimization processes will aim at improving clearance from the body. For instance, NPs with high targeting capacity can be coated with different molecules depending on the compartment of interest to promote receptor-mediated transcytosis and endocytosis, which also improves drug delivery and residual time at the specific site. [52,53] This strategy is particularly well suited for those pathology characterized by the overexpression of a receptor, whose ligand can be functionalized on the NP surface. [54] Furthermore, “stealth-coating” of NPs with polymers (such as polyethylene glycol and polyzwitterions) and signalling proteins (ex. CD47) prevent opsonization and clearance through the mononuclear phagocyte system (MPS) or complement system, thus improving blood circulation half-life. [55]

Other strategies include using surfactants as stabilizers to avoid the formation of high dimension aggregates and limit unwanted interaction with cellular components, therefore reducing ROS

formation and preventing local toxicity. Such modifications are more applicable to NPs rather than small-molecule drugs due to their versatility in terms of physical-chemical properties, biological functions, and synthetic processes.

## NPs for neuroinflammation and protein aggregation

Neuroinflammation is one of the primary mechanisms triggering neurodegeneration. The neuroinflammatory response in CNS is initiated by microglial cells that activate the production of pro-inflammatory cytokines when they recognize a harmful substance threatening the brain [56] (Fig.3). In addition, neuroinflammation can also be determined by insoluble protein aggregation and dysregulated protein degradation, such as tau, β-amyloid in AD, huntingtin in HD and α-synuclein in PD, that cause the continuous activation of inflammatory cells.

Emerging evidence suggests the potential efficacy of NPs in targeting neuroinflammation and protein aggregation. Phosphatidic acid and cardiolipin immune-polyethylene glycol (PEG), and poly Lactic-*co*-Glycolic Acid (PLGA) liposomes can promote the reduction of neuroinflammation and amyloid β (Aβ) aggregates in AD animal models. [57-60] In this work, lipidic nanocarriers administered intraperitoneally could reach the lymphatic system, and eventually the bloodstream. Here, the liposomes sequestered free Aβ due to their high avidity and affinity, thus shifting the equilibrium and drawing Aβ from the CNS, a phenomenon that has been previously reported and referred to as "sink-effect". [61,62] Moreover, PEG and PLGA are biocompatible polymers that provide a steric barrier for the nanocarriers preventing opsonisation and allowing longer retention times in the blood stream. Polyethylenimine (PEI) loaded NPs have shown similar results, as PEI is a suitable material for improving delivery of different types of molecules. [63] Effective reduction in neuroinflammation and protein aggregates formation has been obtained by using AuNPs as a stabilising surface for chiral peptide inhibitors. In this regard, the enantioselectivity of the inhibitors proved to inhibit Aβ protein aggregation [64] more effectively than free peptide inhibitors and with very low, if any, cytotoxicity. Moreover, AuNPs administration showed significant reduction in Aβ42 fibrillation in vitro, showing shorter, amorphous fibrils. [38] When administered intravenously in mice models, chiral AuNPs were able to exit blood circulation and penetrate the BBB to exert their effect in the brain. After the treatment, AD animal models showed reduced Aβ deposition and significant improvement of spatial learning and memory. Therefore, the use of NPs is

effective in reducing Aβ deposition by increasing the affinity, avidity, and selectivity for free Aβ peptides.

## NPs targeting ROS production and oxidative stress in neurodegenerative diseases

Oxidative stress results from the imbalance of oxidant substances, and the biological system ability to detoxify cells from toxic and dangerous compounds. [65] ROS are produced through different physiological intracellular processes [66] and redox homeostasis is strictly coordinated by the nuclear factor erythroid 2-related factor 2 (Nrf2) which stimulates the antioxidant response through the transcriptional activation of antioxidant genes. Brain has a very low level of antioxidant defences such as superoxide dismutase (SOD1), glutathione peroxidases and catalases, turning into high sensitivity to oxidative damage and ROS overproduction primarily. [67] Moreover, some areas of the brain are rich in iron, which can contribute to ROS overproduction. [68] It is important to notice that ROS production can also result in secondary effects in other tissues, such as lipid peroxidation, DNA damage leading to tissue degeneration and accelerated aging, [69] which might be limited with the use of nanosystems. Emerging preclinical studies investigated potential therapeutic interventions by using antioxidant-loaded nanocarriers. In an in vitro models of PD, polyphenol-loaded nanoliposomes were able to rescue ROS concentration and the expression of genes, involved into antioxidant responses such as catalase (Cat), superoxide dismutase 1 and 2 (SOD1 and SOD2), and glutathione synthetase (GSS), [70] to physiological levels. The protein expression of α-synuclein and phospho-α-synuclein, whose accumulation is typical in Lewy bodies upon ROS damage, was also rescued. Normally, free antioxidants show scarce brain accumulation and may exhaust their antioxidant power before reaching the brain. Antioxidants incorporation into nanosystems could instead effectively increase brain localisation and ROS scavenging activity by protecting them from undesired oxidation from the extracellular environment. In this work, the authors also improved the structure of these nanoliposomes by using brain lipids as a shell, functionalised with an anti-transferrin receptor antibody, to ameliorate BBB penetration and cellular uptake through pinocytosis.

Other successful examples of compounds tested on PD models include vitamin E-loaded resveratrol and naringenin nanoemulsions [36,37] which have been shown to reduce oxidative stress and ROS production more efficiently compared to their free suspensions. This is due to

the synergistic effect of vitamin E, the nanoemulsion incorporation and the intranasal route of administration that ameliorated untargeted brain localisation while also avoiding first pass metabolism. Although the mechanism of action is still unclear, it is likely that ROS reduction also restores dopamine levels in PD models.

Rutin is another molecule with ROS-scavenging activity, which binds to antioxidant enzymes, causing their activation and consequently protecting neurons from neurodegeneration and apoptosis. Rutin also prevents lipid peroxidation and protein and DNA denaturation thus preventing oxidative damage. As disadvantage, this molecule has poor aqueous solubility, requiring the incorporation into nanoemulsions for enhancement of bioavailability at the desired site of action over an extended period in rat models of PD. [71]

Furthermore, in a recent work AuNPs were found effective for mitigating cognitive impairment and oxidative stress in AD animal models given the intrinsic antioxidant power of this gold nanosystem. [72] Collectively these preclinical results underline the benefits of nanosystems on pharmacokinetics and seem promising for clinical translation.

## NPs action on autophagic flux and mitophagy

Autophagy is a multi-stage recycling process required for cellular homeostasis. [73,74] Neuronal autophagy controls the correct balance of anti-inflammatory responses, myelination, modulation of neurotransmission, synaptic pruning, and as neuroprotective mechanism it is involved in removing toxic proteins from neural network. [75,76] Defective autophagy is found in many different NDs, including AD, PD and FRDA, as dysregulation of this mechanism increases neurons susceptibility to neurotoxicity leading to cell death. [77-79] Aggregate-prone proteins such as huntingtin, α-synuclein, misfolded peripheral myelin protein 22 could all be degraded by autophagy. [80] For this reason, the stimulation of autophagy in neurons is a potential therapeutic approach. In vitro studies demonstrated that uncoated and oleic acid-coated NPs are internalized by brain-derived human endothelial cells where they aggregate, thus stimulating the activation of lysosomal proteases and autophagy. [81]

Autophagy is also responsible for general mitochondria quality control and for the removal of dysfunctional mitochondria through engulfment in autophagosomes and degradation in lysosomes. This process of selective autophagy is known as mitophagy and uses the same core autophagy machinery, largely encoded by Autophagy-related Genes. [82] While basal mitophagy is a physiological process that is required for maintaining neuronal health, its upregulation is

often observed in NDs, as demonstrated by the post-mortem analysis of PD patient brains, showing autophagocytosed mitochondria. Furthermore, genetic mutations in mitophagy key proteins, like PINK1 or Parkin, occur in several forms of Parkinson's and Alzheimer's diseases.

Protein aggregation and ROS-mediated neuroinflammation can upregulate mitophagy and dysregulate mitochondrial dynamics, thus promoting mitochondrial fragmentation processes. [83-85] Indeed, mitophagy is also responsible for maintaining the physiological morphology of mitochondria. [86] The accumulation of fragmented mitochondria can block both anterograde and retrograde axonal transport, reducing neurite growth and branching, eventually leading to cell death.

Cerium oxide nanoparticles have been proved efficient in reducing amyloid β-induced mitochondrial fragmentation in a cellular model of AD. [87] These NPs can switch between $Ce^{3+}$ and $Ce^{4+}$ and scavenge ROS and peroxynitrite, a mediator of Aβ aggregates formation and tau protein accumulation. Peroxynitrite can also induce mitochondrial fragmentation, thus cerium oxide nanoparticles could exert a double function which is protective against neuronal cell death.

The use of NPs targeting autophagy in NDs has shown promising results, but it is still controversial. [88] When interacting with subcellular components, NPs can also stimulate autophagy through the upregulation of ROS signalling, which is normally considered a side effect of NPs treatments. [89,90] In vivo studies in wild type animal models demonstrated that the intravenous administration of titanium dioxide NPs caused increased ROS formation and inflammatory responses suggesting a neurotoxic effect. [91,92]

From a therapeutic standpoint, it is important to consider the context of therapeutic intervention: for some patients, the upregulation of autophagic flux might elicit beneficial clearance of aggregates and damaged mitochondria, [93,94] whereas other patients might experience excessive mitochondrial depletion and axo-dendritic shrinkage.

The outcome of autophagy or mitophagy modulation in specific pathological contexts is hardly predictable, and the therapeutic strategy in terms of dosage and administration should be experimentally tested and tailored on the patient in order to avoid dangerous side effects.

## Regulating apoptosis: the neuroprotection strategy

Neuronal apoptosis is a highly conserved mechanism taking place during the development of the nervous system and acts as a regulatory mechanism to maintain tissue homeostasis. [95] Biochemical signals initiating apoptosis include excitotoxicity, glutamatergic hyperactivation, brain-derived neurotrophic factor (BDNF) deficiency, oxidative stress and toxic insults to the brain. [96] However, once the development is complete, the central nervous system has very limited regenerative capacity and abnormal cell death occurring through apoptosis and necrosis is indeed predominant in NDs, though with some differences among the affected neurons. For instance, in PD nigral dopaminergic neurons are the most susceptible to cell death, in HD striatal neurons expressing GABA and enkephalin die more than those expressing GABA and substance P, whereas in ALS motor neurons innervating fast muscles are more affected compared to those innervating slow muscles. [97]

The death of one cell can negatively affect the dynamics of neighbouring cells by secreting proapoptotic factors that can trigger caspase-mediated apoptosis in the neighbours. Therefore, it is crucial to limit the apoptotic damage to ameliorate the disease progress.

Superparamagnetic $Fe_3O_4$ NPs have been used to target apoptosis in PD models. [98] In this work, NPs were coated with oleic acid, loaded with a shRNA against α-synuclein synthesis, and nerve growth factor (NGF) was absorbed to ensure NPs uptake in neurons through NGF-mediated endocytosis. This strategy allowed to specifically target α-synuclein synthesis, whose aggregation in brain leads to apoptosis upregulation in PD affected patients hence to prevent cell death and ameliorate the pathological phenotype. Despite the promising results, clinical translation of iron oxide NPs is controversial because of the potential induction of oxidative stress, iron accumulation and protein aggregation in the brain. [48]

Another approach exploiting NPs is *neuroprotection*, a therapeutic strategy with the aim to defend CNS from both acute and chronic injuries, maintain viability of neuronal progenitor cells and ameliorate neuronal functional recovery. [99] Neuroprotection in NDs aims at regulating apoptosis and autophagy, as well as repairing, and regenerating damaged neuronal cells to change the course of the disease. Emerging evidence supports the hypothesis that natural products, some food-additives, neurotrophic factors, and growth factors provide neuroprotection.

It has been proved that PEG-coated gold nanoparticles loaded with anthocyanin are able to target amyloid β plaques and provide neuroprotection via inhibition of the NF-κB /JNK/GSK3β pathway both in vitro and in vivo in AD models. [100] Anthocyanins have antioxidant and anti-

inflammatory properties that were found neuroprotective against amyloid beta-induced oxidative stress neuroinflammation and neurodegeneration. Indeed, upon intravenous injection of PEG-coated gold nanoparticles loaded with anthocyanin, the expression of markers of activated astrocytes and microglia (GFAP, Iba-1, RAGE), inflammation (p-NF-κB, iNOS) and apoptosis (Bax, cytochrome C, caspase 3) were found reduced in vitro and in vivo. Tau hyperphosphorylation, which is associated with neurodegeneration, was also reduced. Collectively, treatment with anthocyanin-loaded NPs had a strong positive effect on neurons survival rescue in the CA2 and DG regions of hippocampus and cortex of AD mice models. Moreover, these nanoparticles were able to penetrate the BBB and localise in the brain up to 14 days after treatment, due to the PEG coating that increases retention times. Intranasal administration of fibroblast growth factors-conjugated NPs showed neuroprotective effect mitigating AD progression and memory deficits in AD animal models, through the trophic activity of basic fibroblast growth factor on neurons, and the activation of antioxidant pathways that mitigate the neurotoxicity induced by amyloid beta. [101] These NPs were optimised by adding lectins to enhance brain permeability via endocytosis. Similar results were obtained with nicotine-encapsulated PLGA NPs in PD models, [102] as nicotine is neuroprotective against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced parkinsonism, and nanocurcumin in EAE mice (a mouse model of MS) which was able to stimulate neuroprotection and improve myelin repair, due to its anti-inflammatory activity. [103] Moreover, treatment with curcumin-loaded nanoemulsions was found effective for improving motor deficits and antioxidant defences in PD models. [104] Neuroprotection is an encouraging strategy because it allows to stimulate neurons survival also with the employment of natural compounds, which have properties that can be optimised with the use of nanosystems and whose use does not fall under the FDA drug regulations.

## Mitochondrial and metabolic dysfunction in neuroinflammation and degeneration: bioenergetics as novel NDs therapeutic target

Defective mitochondrial metabolism has been observed in NDs, but it has often been considered a consequence of the disease. Recently, nuclear and mitochondrial DNA mutations leading to reduced oxidative metabolism have been found in hereditary forms of NDs, suggesting that metabolic dysfunctions might also be causative of NDs. However, the same mutation leading

to mitochondrial dysfunction can cause very different pathological phenotypes, as compensatory effect arise. [97]

Mitochondria are involved in a network of metabolic pathways and are present in multiple copies within the cell, so that healthy mitochondria can compensate for the defective ones. Defective energetic metabolism and ATP synthesis can originate from dysfunctional ETC complexes and proton pumps in mitochondrial membranes, mistaken diversion of the electron flow to other cellular components leading to ROS formation and oxidative stress, uncoupling, and defective fuelling of the ETC from Krebs' cycle and glycolysis. Although it is difficult to demonstrate the causative role of metabolic defects in NDs, their effect in these diseases is well documented and suggests that bioenergetics can be a new suitable target.

Clean-surfaced, faceted nanocrystals of gold have been used to promote remyelination by enhancing glycolytic metabolism. Indeed, myelination is a process mediated by oligodendrocytes (OLs) that is highly dependent on glycolysis, the primary energetic source of OLs. [39] Compared to bulk gold, which is inert, gold nanocrystals have biological catalytic properties, and they can catalyse the oxidation of nicotinamide adenine dinucleotide hydride (NADH) to NAD+, a critical energetic cofactor and sensor of energy levels. In this work, a suspension of gold nanocrystals (named CNM-Au8) was firstly used to enhance the glycolytic activity in neuronal-glial co-cultures, as confirmed by the increased extracellular lactate and non-mitochondrial ATP production, and then administered to animal models of MS by gavage, showing prominent remyelination and amelioration of movements. These encouraging results have led to the approval of CNM-Au8 in clinical trials (https://clinicaltrials.gov/ct2/results?cond=Neuro-Degenerative+Disease&term=CNM+Au8&cntry=&state=&city=&dist=).

# The case of Friedreich's Ataxia as an example to exploit the pleiotropic effects of nanoparticles

Most of the nanosystems previously discussed have several effects, acting on ROS reduction, autophagy and exerting their function both in the CNS and in distant organs, showing pleiotropic characteristics. In pharmacology, pleiotropy refers to the whole of the effects that a drug can deploy, including the ones for which it was not specifically developed. For neurodegenerative disease treatment, it is of particular importance to find pleiotropic drugs in

order to directly target primary pathways of early pathogenesis while also acting on reversing secondary effects. [105] A good example to test the importance of this concept is FRDA, as it is a multisystem disease affecting central and peripheral nervous system, the musculoskeletal system, the heart, and endocrine pancreas. Together with gait and limb ataxia, FRDA is often associated to cardiomyopathy and diabetes mellitus. Furthermore, in FRDA not all the organs and tissues are affected to the same extent of degeneration and severity of pathologic pathways, [106] despite same genetic background and frataxin expression.

The latter portraits the complexity of FRDA, both in understanding tissue specific biomolecular mechanisms of pathology and in finding suitable and broad-spectrum therapeutic intervention strategies.

The most common strategies targeting the pathological mechanisms driving FRDA onset aim at increasing frataxin expression, since its reduction in FRDA patients causes pathologic oxidative stress and defective mitochondrial metabolism. Indeed, frataxin is involved in several mitochondrial functions, including respiration, iron-sulphur clusters biogenesis, haem synthesis and redox status, [107] as well as mitochondrial dynamics and autophagy. [108] Typically, FXN replacement via gene therapy or protein supplementation is coupled with the use of iron chelators (*deferiprone*) and antioxidants (*vitamin E + coenzyme Q10*). [109] For instance, one of the most widely studied approaches to improve FRDA symptoms, in particular for the amelioration of cardiac functionality, is the administration of adeno-associated virus (AAV) carrying human FXN. [110,111] However, even though a single injection could be effective, AAV-mediated gene therapy is hindered by the stimulation of immune response and difficult virus delivery strategies. More recently, a list of FDA approved drugs has been screened in yeast to find compounds that can increase frataxin levels by posttranslational regulation. [112] The most efficient were dibenzoylmethane (DBM), 4,4′-Diisothiocyano-2,2′-stilbenedisulfonic acid (DIDS), bifonazole, and fipronil. These compounds are likely to act on mitochondrial iron metabolism and ROS formation, though the mechanism of action needs to be fully elucidated. Likewise, the glucagon-like peptide-1 (GLP-1) analogue exenatide, which is already employed in the management of type 2 diabetes mellitus, was found effective in increasing frataxin expression and improving mitochondrial function. [113]

Despite the variety of tested therapeutic approaches, results from clinical trials have been modest. [114] The use of nanoparticles may be the solution to overcome some of the difficulties causing the inefficiency of treatments, including the possibility to act on different systems with a single nanocarrier.

In 2016, J.F Nabhan et al. managed to deliver lipid nanoparticles (LNPs) loaded with FXN mRNA to DRG. [115] Lipid nanoparticles encapsulating FXN mRNA were generated and administered intravenously in adult mice revealing efficient uptake in hepatocytes and correct maturation into the mature form 24 hours post injection. Finally, FXN LNPs were administered intrathecally into the lower lumbar region of the mouse spine and the recombinant human FXN protein was detected at high concentration in DRG.

NPs loaded with curcumin have been effective in alleviating FRDA symptoms in mouse models. [116] Curcumin is known to reduce oxidative stress through Nrf2 activation, and it can also chelate iron, which are both characteristics that can be useful to reduce the symptoms of FRDA, which are often caused by mitochondrial dysfunction, abnormal iron metabolism and increased oxidative stress. However, curcumin is poorly soluble and permeable, therefore it has been loaded in silk fibroin (SF) to generate Cur@SF NPs. The safety of Cur@SF NPs was evaluated in lymphocytes derived from FRDA patients. Results showed no effect on viability, but iron content was reduced in mitochondria because of chelation. Moreover, the activity of antioxidant enzymes (SOD and catalase) increased, along with complexes I and II activity and ATP production. The injection of Cur@SF NPs in mice models of FRDA for one month improves functional performance (strength, coordination, holding times). Furthermore, heart iron, whose accumulation is one of the main causes of myocardial dysfunction in FRDA patients, was significantly reduced.

Recently, a new non-genetic approach for FRDA treatment based on gold quantum clusters ($Au_8$pXs) has shown promising results. Conversely to the other works discussed in this review, Villa and colleagues have exploited the intrinsic characteristics of gold nanostructures ($Au_8$pXs), their excellent ROS scavenger potential, in bone-marrow mesenchymal stem cells (BMSCs) derived from patients with FRDA and in YG8sR mice, which are considered a good animal model of FRDA. [117,118] The YG8sR mice received a single dose of $Au_8$pXs injected in the tail vein without the help of other molecules already used in FRDA treatment. [118] Interestingly, $Au_8$pXs reached many tissues, including cerebellum and cortex, thus demonstrating the capability to cross the BBB, and accumulated in the liver, where they are retained for more than 30 days before being slowly cleared. Of note, the $Au_8$pXs have been proved effective in recovering mitochondrial function, counteracting oxidative stress, and rescuing neuromuscular and cardiac function. [118] Moreover, $Au_8$pX treatment prevented neuronal loss in the cerebellar dentate nuclei and improved neuronal firing properties. [118] Mechanistically, the $Au_8$pX treatment was able to restore the antioxidant pathways, autophagic

flux, mitochondrial dynamics and complex I and II activities while indirectly increasing FXN expression (Fig.4). Taken together, these data encourage the use of $Au_8$pXs as a potential treating option for FRDA patients. The key advantages of this system include the long retention time, that allow a single administration and the possibility to cross the blood brain barrier and reach distant tissues, including muscles and heart, where they can exert their effects on multiple processes, thus showing a pleiotropic effect.

FRDA patients show very different clinical manifestations and onset due to the genetic variability of FXN mutations and protein expression. This heterogeneity will likely strongly affect the efficacy of the treatment and should be considered in view of a clinical trial. In line, in Villa et al., BMSCs were derived from two patients with FRDA carrying different GAA repeats sizes and treated with different $Au_8$pX molarities, 5 μM and 10 μM. [118] Despite both cell lines showed an overall autophagic flux recovery and mitochondrial machinery restoration to physiological levels, BMSCs from the patient carrying the smaller GAA expansion were more vulnerable to FRDA degenerative pathways and more prone to largely benefit from $Au_8$pX treatment. [118] In addition, BMSCs derived from the same patients exhibited different levels of $Au_8$pX activity, in a dose related manner. 5 μM $Au_8$pX administration showed a faster and more effective ROS scavenging activity, despite a slightly lower mitochondrial accumulation. Conversely, treatment with 10 μM $Au_8$pXs primed a robust posttranslational expression of the mature form of FXN, suggesting a multi-target intervention strategy tunable by $Au_8$pX dose. [118]

The analysis of different patients-derived cells to study the transcriptional regulation of FXN expression, ROS production and autophagy upon different dosage of $Au_8$pXs is therefore needed to determine the patient-specific beneficial concentration of gold quantum clusters and facilitate the clinical translation of this treatment.

These examples underline the advantage in the use of nanosystems over conventional small molecule drugs. The pleiotropic effect of nanoparticles would be beneficial for the treatment of other NDs, such as AD and PD because it would allow to treat with a single nanosystem multiple targets, including oxidative stress, autophagy and protein aggregation.

# Conclusions

Neurodegenerative diseases are unfortunately very common, with more than 600 different neurologic disorders, and approximately 50 million people affected (1 in six of the world population). In our modern era, these numbers are expected to increase as population grows and ages. [119] The recent advanced strategies to prevent and manage at least the most common neurological disorders such as AD, PD, HM, MS, and ALS are still not completely effective to counteract the variety of symptoms and to guarantee long-term patient quality of life. New therapies are therefore urgently needed to cover the heterogeneity and temporal complexity of ND and reduce world-wide health burden.

Among the common mechanisms occurring in NDs, none seem to play a primary role in neurodegeneration on its own. Rather, the synergistic action of their complex interactions contributes to the onset and pathogenesis of NDs. So far, the challenge of finding effective pharmacological treatments and resolutive therapies is unsolved. In many cases, addressing and specifically targeting only a unique dysregulated process results into unsatisfactory outcomes, and rather negatively impacts on others biological pathways. The multifactorial nature of NDs requires the development of therapeutic interventions and new compounds targeting simultaneously several mechanisms, risk factors and features shared among NDs spectra. Among others, therapeutic targets include neuronal cell apoptosis and neurodegeneration, as wells as neuroinflammation caused by oxidative stress, protein aggregation, autophagic flux impairment, and mitochondrial dysfunction. However, current therapeutic approaches are limited at mitigating disease progression and preserving surviving neuronal cells and their function, [98-100] whereas strategies that can definitively arrest neurodegeneration and neuroinflammation progression, or even prevent the early pathological degenerative pathways, are still unavailable.

One of the major obstacles in developing efficient treatments is provided by the restrictive nature of BBB, a highly selective semipermeable membrane formed by endothelial cells and surrounding cerebral microcirculation. An intact BBB creates a physical barrier hindering the freely diffusion and passive entrance of cells, particles, and macromolecules, such as drugs, into the brain [120]. While traditional pharmacotherapy may result ineffective to provide efficacy dose for adequate cell repair and treatment, [121] novel strategies based on nanomaterials can be designed to transport drugs across the BBB or to develop self-healing nanosystems.

In comparison to conventional drugs, nanoparticles are therefore good candidates as drug carriers crossing the BBB, leading to a faster rate of drug release and higher bioavailability. Furthermore, NPs can be engineered through various biological reactions, such as receptor–

ligand interaction and antibody-antigen interaction, to conjugate specific biomolecules, which allow targeted delivery of NPs in vivo. Similarly, NPs can cross the blood nerve barrier (BNB), showing the same advantages and site specificity obtained when treating the CNS. In this regard, nerve growth factors conjugated with or encapsulated in nanoparticles have already been used for the treatment of peripheral nerve damage, resulting in efficient reconstruction of axons and promotion of remyelination. [122] However, despite the longer retention times compared to conventional drugs, the relatively frequent injections make it difficult to translate in the clinics. Furthermore, most of the NDs are characterised by stronger CNS pathologic phenotypes, rather than peripheral nerve damage, hence treating peripheral nerves would be beneficial just for some NDs, for example FRDA.

In the framework of the complex genetic and symptomatology, and the multifaceted causative mechanisms characterizing NDs, the largest clinical feasibility will be exerted by nanosystems displaying a wide efficacy in several neurodegenerative pathways.

In addition to carrier properties, the physicochemical characteristics of NPs contribute to their beneficial effects in NDs to dampen neuroinflammation and oxidative stress, while controlling mitochondrial bioenergetics and metabolism.

These processes are particularly jeopardized in Friedreich's Ataxia, due to the pivotal role of frataxin in many mitochondrial functions. Indeed, FRDA is a complex multisystemic disorder, with a wide range of clinical manifestations which also differ among the patients, which makes this disease a good candidate for treatment approaches based on nanosystems. Traditional clinical interventions have often proved to be unsuccessful, whereas nanosystems based therapeutic strategies are showing encouraging results in preclinical studies.

Despite the numerous advantages, the clinical translation of most nanosystems is limited by their intrinsic properties, such as size, shape, stability, and physico-chemical surface features, that affect their adsorption time and biodistribution after administration. There is not a golden route for nanosystem delivery. Comparison studies of pharmacokinetic must be performed in animal models to avoid nanosystem adsorption from filter organs, blood, or body tissues different from the target area, in order to ensure effective dose delivery and treatment efficacy, while preventing their toxic accumulation and aggregation. In line, a clinically relevant application of nanosystem should be founded on controlled biodistribution, reduced toxicity, less frequent administration, and an easy delivery method, aiming at increasing patient benefits and compliance. In this sense, the development of standardised toxicological studies is also

crucial for the improvement of NPs applications. Accordingly, patients suffering from NDs, differ for their genetic background and clinical phenotype, arising the demand for a therapeutic personalized dose and administration strategy.

In summary, NPs have led to promising preclinical results and might represent a huge opportunity and a broader prospect to cure NDs in the near future.

# Competing interests

C.V. and Y.T. are listed as inventors on a patent application (n. IT102019000020724) for Au8-pXs mediated therapy for FRDA. M.M., A.F., D.D.S, P.M., Y.T. and C.V. declare no competing interests.

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# References


1. Hussain R, Zubair H, Pursell S, Shahab M. Neurodegenerative Diseases: Regenerative Mechanisms and Novel Therapeutic Approaches. *Brain Sci*. Sep 15 2018;8(9)doi:10.3390/brainsci8090177

2. Checkoway H, Lundin JI, Kelada SN. Neurodegenerative diseases. *IARC Sci Publ*. 2011;(163):407-19.

3. Kim J, Mook-Jung I. Special issue on neurodegenerative diseases and their therapeutic approaches. *Exp Mol Med*. Mar 13 2015;47:e146. doi:10.1038/emm.2015.13

4. Tan SH, Karri V, Tay NWR, et al. Emerging pathways to neurodegeneration: Dissecting the critical molecular mechanisms in Alzheimer's disease, Parkinson's disease. *Biomed Pharmacother*. Mar 2019;111:765-777. doi:10.1016/j.biopha.2018.12.101

5. Campuzano V, Montermini L, Moltò MD, et al. Friedreich's ataxia: autosomal recessive disease caused by an intronic GAA triplet repeat expansion. *Science*. Mar 08 1996;271(5254):1423-7. doi:10.1126/science.271.5254.1423

6. Koeppen AH. Friedreich's ataxia: pathology, pathogenesis, and molecular genetics. *J Neurol Sci*. Apr 15 2011;303(1-2):1-12. doi:10.1016/j.jns.2011.01.010

7. Dürr A, Cossee M, Agid Y, et al. Clinical and genetic abnormalities in patients with Friedreich's ataxia. *N Engl J Med*. Oct 17 1996;335(16):1169-75. doi:10.1056/NEJM199610173351601

8. Delatycki MB, Bidichandani SI. Friedreich ataxia- pathogenesis and implications for therapies. *Neurobiol Dis*. 12 2019;132:104606. doi:10.1016/j.nbd.2019.104606

9. Cook A, Giunti P. Friedreich's ataxia: clinical features, pathogenesis and management. *Br Med Bull*. Dec 01 2017;124(1):19-30. doi:10.1093/bmb/ldx034

10. Bürk K. Friedreich Ataxia: current status and future prospects. *Cerebellum Ataxias*. 2017;4:4. doi:10.1186/s40673-017-0062-x

11. De Michele G, Perrone F, Filla A, et al. Age of onset, sex, and cardiomyopathy as predictors of disability and survival in Friedreich's disease: a retrospective study on 119 patients. *Neurology*. Nov 1996;47(5):1260-4. doi:10.1212/wnl.47.5.1260

12. Galea CA, Huq A, Lockhart PJ, et al. Compound heterozygous FXN mutations and clinical outcome in friedreich ataxia. *Ann Neurol*. Mar 2016;79(3):485-95. doi:10.1002/ana.24595

13. Prischi F, Konarev PV, Iannuzzi C, et al. Structural bases for the interaction of frataxin with the central components of iron-sulphur cluster assembly. *Nat Commun*. Oct 19 2010;1:95. doi:10.1038/ncomms1097

14. Pandolfo M, Pastore A. The pathogenesis of Friedreich ataxia and the structure and function of frataxin. *J Neurol*. Mar 2009;256 Suppl 1:9-17. doi:10.1007/s00415-009-1003-2

15. González-Cabo P, Palau F. Mitochondrial pathophysiology in Friedreich's ataxia. *J Neurochem*. Aug 2013;126 Suppl 1:53-64. doi:10.1111/jnc.12303

16. González-Cabo P, Vázquez-Manrique RP, García-Gimeno MA, Sanz P, Palau F. Frataxin interacts functionally with mitochondrial electron transport chain proteins. *Hum Mol Genet*. Aug 01 2005;14(15):2091-8. doi:10.1093/hmg/ddi214

17. Mancuso M, Orsucci D, Choub A, Siciliano G. Current and emerging treatment options in the management of Friedreich ataxia. *Neuropsychiatr Dis Treat*. Sep 07 2010;6:491-9. doi:10.2147/ndt.s6916

18. Kosutic J, Zamurovic D. High-dose beta-blocker hypertrophic cardiomyopathy therapy in a patient with Friedreich ataxia. *Pediatr Cardiol*. 2005 Sep-Oct 2005;26(5):727-30. doi:10.1007/s00246-005-0930-7

19. Jeevanandam J, Barhoum A, Chan YS, Dufresne A, Danquah MK. Review on nanoparticles and nanostructured materials: history, sources, toxicity and regulations. *Beilstein J Nanotechnol*. 2018;9:1050-1074. doi:10.3762/bjnano.9.98

20. Sercombe L, Veerati T, Moheimani F, Wu SY, Sood AK, Hua S. Advances and Challenges of Liposome Assisted Drug Delivery. *Front Pharmacol*. 2015;6:286. doi:10.3389/fphar.2015.00286

21. Ahmad Z, Shah A, Siddiq M, Kraatz H-B. Polymeric micelles as drug delivery vehicles. *Rsc Advances*. 2014;4(33):17028-17038.

22. Mitchell MJ, Billingsley MM, Haley RM, Wechsler ME, Peppas NA, Langer R. Engineering precision nanoparticles for drug delivery. *Nat Rev Drug Discov*. 02 2021;20(2):101-124. doi:10.1038/s41573-020-0090-8

23. Aswathanarayan JB, Vittal RR. Nanoemulsions and Their Potential Applications in Food Industry. Review. *Frontiers in Sustainable Food Systems*. 2019-November-13 2019;3doi:10.3389/fsufs.2019.00095

24. Gomez-Gualdron DA, Burgos JC, Yu J, Balbuena PB. Carbon nanotubes: engineering biomedical applications. *Progress in molecular biology and translational science*. 2011;104:175-245.

25. Abbasi E, Aval SF, Akbarzadeh A, et al. Dendrimers: synthesis, applications, and properties. *Nanoscale Res Lett*. 2014;9(1):247. doi:10.1186/1556-276X-9-247

26. Wu W, He Q, Jiang C. Magnetic iron oxide nanoparticles: synthesis and surface functionalization strategies. *Nanoscale Res Lett*. Oct 02 2008;3(11):397-415. doi:10.1007/s11671-008-9174-9

27. Saraiva C, Praça C, Ferreira R, Santos T, Ferreira L, Bernardino L. Nanoparticle-mediated brain drug delivery: Overcoming blood-brain barrier to treat neurodegenerative diseases. *J Control Release*. 08 10 2016;235:34-47. doi:10.1016/j.jconrel.2016.05.044

28. Manzanares D, Ceña V. Endocytosis: The Nanoparticle and Submicron Nanocompounds Gateway into the Cell. *Pharmaceutics*. Apr 17 2020;12(4)doi:10.3390/pharmaceutics12040371

29. Mohanraj V, Chen Y. Nanoparticles-a review. *Tropical journal of pharmaceutical research*. 2006;5(1):561-573.

30. Yildirimer L, Thanh NT, Loizidou M, Seifalian AM. Toxicology and clinical potential of nanoparticles. *Nano Today*. Dec 2011;6(6):585-607. doi:10.1016/j.nantod.2011.10.001

31. Sabourian P, Yazdani G, Ashraf SS, et al. Effect of Physico-Chemical Properties of Nanoparticles on Their Intracellular Uptake. *Int J Mol Sci*. Oct 28 2020;21(21)doi:10.3390/ijms21218019

32. Chen D, Wang J, Wang Y, et al. Promoting Inter-/Intra- Cellular Process of Nanomedicine through its Physicochemical Properties Optimization. *Curr Drug Metab*. 2018;19(1):75-82. doi:10.2174/1389200219666171221122119

33. Price JP, Kruger DF, Saravolatz LD, Whitehouse FW. Evaluation of the insulin jet injector as a potential source of infection. *Am J Infect Control*. Oct 1989;17(5):258-63. doi:10.1016/0196-6553(89)90172-7

34. Teleanu DM, Chircov C, Grumezescu AM, Volceanov A, Teleanu RI. Blood-Brain Delivery Methods Using Nanotechnology. *Pharmaceutics*. Dec 11 2018;10(4)doi:10.3390/pharmaceutics10040269

35. Beltrán-Gracia E, López-Camacho A, Higuera-Ciapara I, Velázquez-Fernández JB, Vallejo-Cardona AA. Nanomedicine review: Clinical developments in liposomal applications. *Cancer Nanotechnology*. 2019;10(1):1-40.

36. Pangeni R, Sharma S, Mustafa G, Ali J, Baboota S. Vitamin E loaded resveratrol nanoemulsion for brain targeting for the treatment of Parkinson's disease by reducing oxidative stress. *Nanotechnology*. Dec 05 2014;25(48):485102. doi:10.1088/0957-4484/25/48/485102

37. Gaba B, Khan T, Haider MF, et al. Vitamin E Loaded Naringenin Nanoemulsion via Intranasal Delivery for the Management of Oxidative Stress in a 6-OHDA Parkinson's Disease Model. *Biomed Res Int*. 2019;2019:2382563. doi:10.1155/2019/2382563

38. Hou K, Zhao J, Wang H, et al. Chiral gold nanoparticles enantioselectively rescue memory deficits in a mouse model of Alzheimer's disease. *Nat Commun*. 09 22 2020;11(1):4790. doi:10.1038/s41467-020-18525-2

39. Robinson AP, Zhang JZ, Titus HE, et al. Nanocatalytic activity of clean-surfaced, faceted nanocrystalline gold enhances remyelination in animal models of multiple sclerosis. *Sci Rep*. 02 11 2020;10(1):1936. doi:10.1038/s41598-020-58709-w

40. Liu Y, Liu W, Xiong S, et al. Highly stabilized nanocrystals delivering Ginkgolide B in protecting against the Parkinson's disease. *Int J Pharm*. Mar 15 2020;577:119053. doi:10.1016/j.ijpharm.2020.119053

41. Marcuzzo S, Isaia D, Bonanno S, et al. FM19G11-Loaded Gold Nanoparticles Enhance the Proliferation and Self-Renewal of Ependymal Stem Progenitor Cells Derived from ALS Mice. *Cells*. 03 23 2019;8(3)doi:10.3390/cells8030279

42. Vucic S, Kiernan MC, Menon P, et al. Study protocol of RESCUE-ALS: A Phase 2,. *BMJ Open*. 01 11 2021;11(1):e041479. doi:10.1136/bmjopen-2020-041479

43. Soni G, Yadav KS. Nanogels as potential nanomedicine carrier for treatment of cancer: A mini review of the state of the art. *Saudi Pharm J*. Mar 2016;24(2):133-9. doi:10.1016/j.jsps.2014.04.001

44. Inglut CT, Sorrin AJ, Kuruppu T, et al. Immunological and Toxicological Considerations for the Design of Liposomes. *Nanomaterials (Basel)*. Jan 22 2020;10(2)doi:10.3390/nano10020190

45. Yang W, Wang L, Mettenbrink EM, DeAngelis PL, Wilhelm S. Nanoparticle Toxicology. *Annu Rev Pharmacol Toxicol*. 01 06 2021;61:269-289. doi:10.1146/annurev-pharmtox-032320-110338

46. Maher BA, Ahmed IA, Karloukovski V, et al. Magnetite pollution nanoparticles in the human brain. *Proc Natl Acad Sci U S A*. 09 27 2016;113(39):10797-801. doi:10.1073/pnas.1605941113

47. Malhotra N, Lee JS, Liman RAD, et al. Potential Toxicity of Iron Oxide Magnetic Nanoparticles: A Review. *Molecules*. Jul 10 2020;25(14)doi:10.3390/molecules25143159

48. Yarjanli Z, Ghaedi K, Esmaeili A, Rahgozar S, Zarrabi A. Iron oxide nanoparticles may damage to the neural tissue through iron accumulation, oxidative stress, and protein aggregation. *BMC Neurosci*. 06 26 2017;18(1):51. doi:10.1186/s12868-017-0369-9

49. Li JJ, Muralikrishnan S, Ng CT, Yung LY, Bay BH. Nanoparticle-induced pulmonary toxicity. *Exp Biol Med (Maywood)*. Sep 2010;235(9):1025-33. doi:10.1258/ebm.2010.010021

50. Nosrati H, Hamzepoor M, Sohrabi M, et al. The potential renal toxicity of silver nanoparticles after repeated oral exposure and its underlying mechanisms. *BMC Nephrol*. 06 18 2021;22(1):228. doi:10.1186/s12882-021-02428-5

51. Kim YS, Kim JS, Cho HS, et al. Twenty-eight-day oral toxicity, genotoxicity, and gender-related tissue distribution of silver nanoparticles in Sprague-Dawley rats. *Inhal Toxicol*. Apr 2008;20(6):575-83. doi:10.1080/08958370701874663

52. Yu S, Xu X, Feng J, Liu M, Hu K. Chitosan and chitosan coating nanoparticles for the treatment of brain disease. *Int J Pharm*. Apr 05 2019;560:282-293. doi:10.1016/j.ijpharm.2019.02.012

53. Reshma VG, Syama S, Sruthi S, Reshma SC, Remya NS, Mohanan PV. Engineered Nanoparticles with Antimicrobial Property. *Curr Drug Metab*. 2017;18(11):1040-1054. doi:10.2174/1389200218666170925122201

54. Agrawal P, Sonali, Singh RP, et al. Bioadhesive micelles of d-α-tocopherol polyethylene glycol succinate 1000: Synergism of chitosan and transferrin in targeted drug

delivery. *Colloids Surf B Biointerfaces*. Apr 01 2017;152:277-288. doi:10.1016/j.colsurfb.2017.01.021

55. Fam SY, Chee CF, Yong CY, Ho KL, Mariatulqabtiah AR, Tan WS. Stealth Coating of Nanoparticles in Drug-Delivery Systems. *Nanomaterials (Basel)*. Apr 20 2020;10(4)doi:10.3390/nano10040787

56. Leng F, Edison P. Neuroinflammation and microglial activation in Alzheimer disease: where do we go from here? *Nat Rev Neurol*. 03 2021;17(3):157-172. doi:10.1038/s41582-020-00435-y

57. Ordóñez-Gutiérrez L, Re F, Bereczki E, et al. Repeated intraperitoneal injections of liposomes containing phosphatidic acid and cardiolipin reduce amyloid-β levels in APP/PS1 transgenic mice. *Nanomedicine*. Feb 2015;11(2):421-30. doi:10.1016/j.nano.2014.09.015

58. Ordóñez-Gutiérrez L, Posado-Fernández A, Ahmadvand D, et al. ImmunoPEGliposome-mediated reduction of blood and brain amyloid levels in a mouse model of Alzheimer's disease is restricted to aged animals. *Biomaterials*. 01 2017;112:141-152. doi:10.1016/j.biomaterials.2016.07.027

59. Jeon SG, Cha MY, Kim JI, et al. Vitamin D-binding protein-loaded PLGA nanoparticles suppress Alzheimer's disease-related pathology in 5XFAD mice. *Nanomedicine*. 04 2019;17:297-307. doi:10.1016/j.nano.2019.02.004

60. Silva-Abreu M, Calpena AC, Andrés-Benito P, et al. PPARγ agonist-loaded PLGA-PEG nanocarriers as a potential treatment for Alzheimer's disease: in vitro and in vivo studies. *Int J Nanomedicine*. 2018;13:5577-5590. doi:10.2147/IJN.S171490

61. DeMattos RB, Bales KR, Cummins DJ, Paul SM, Holtzman DM. Brain to plasma amyloid-beta efflux: a measure of brain amyloid burden in a mouse model of Alzheimer's disease. *Science*. Mar 22 2002;295(5563):2264-7. doi:10.1126/science.1067568

62. DeMattos RB, Bales KR, Cummins DJ, Dodart JC, Paul SM, Holtzman DM. Peripheral anti-A beta antibody alters CNS and plasma A beta clearance and decreases brain A beta burden in a mouse model of Alzheimer's disease. *Proc Natl Acad Sci U S A*. Jul 17 2001;98(15):8850-5. doi:10.1073/pnas.151261398

63. Helmschrodt C, Höbel S, Schöniger S, et al. Polyethylenimine Nanoparticle-Mediated siRNA Delivery to Reduce α-Synuclein Expression in a Model of Parkinson's Disease. *Mol Ther Nucleic Acids*. Dec 15 2017;9:57-68. doi:10.1016/j.omtn.2017.08.013

64. Xiong N, Zhao Y, Dong X, Zheng J, Sun Y. Design of a Molecular Hybrid of Dual Peptide Inhibitors Coupled on AuNPs for Enhanced Inhibition of Amyloid β-Protein Aggregation and Cytotoxicity. *Small*. 04 2017;13(13)doi:10.1002/smll.201601666

65. Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chem Biol Interact*. Mar 10 2006;160(1):1-40. doi:10.1016/j.cbi.2005.12.009

66. Tu BP, Weissman JS. Oxidative protein folding in eukaryotes: mechanisms and consequences. *J Cell Biol*. Feb 02 2004;164(3):341-6. doi:10.1083/jcb.200311055

67. Liu Z, Zhou T, Ziegler AC, Dimitrion P, Zuo L. Oxidative Stress in Neurodegenerative Diseases: From Molecular Mechanisms to Clinical Applications. *Oxid Med Cell Longev*. 2017;2017:2525967. doi:10.1155/2017/2525967

68. Nakamura T, Naguro I, Ichijo H. Iron homeostasis and iron-regulated ROS in cell death, senescence and human diseases. *Biochim Biophys Acta Gen Subj*. 09 2019;1863(9):1398-1409. doi:10.1016/j.bbagen.2019.06.010

69. Fu PP, Xia Q, Sun X, Yu H. Phototoxicity and environmental transformation of polycyclic aromatic hydrocarbons (PAHs)-light-induced reactive oxygen species, lipid peroxidation, and DNA damage. *J Environ Sci Health C Environ Carcinog Ecotoxicol Rev*. Jan 2012;30(1):1-41. doi:10.1080/10590501.2012.653887

70. Marino A, Battaglini M, Desii A, et al. Liposomes loaded with polyphenol-rich grape pomace extracts protect from neurodegeneration in a rotenone-based. *Biomater Sci*. Dec 07 2021;9(24):8171-8188. doi:10.1039/d1bm01202a

71. Sharma S, Rabbani SA, Narang JK, et al. Role of Rutin Nanoemulsion in Ameliorating Oxidative Stress: Pharmacokinetic and Pharmacodynamics Studies. *Chem Phys Lipids*. 05 2020;228:104890. doi:10.1016/j.chemphyslip.2020.104890

72. Dos Santos Tramontin N, da Silva S, Arruda R, et al. Gold Nanoparticles Treatment Reverses Brain Damage in Alzheimer's Disease Model. *Mol Neurobiol*. Feb 2020;57(2):926-936. doi:10.1007/s12035-019-01780-w

73. Williams A, Sarkar S, Cuddon P, et al. Novel targets for Huntington's disease in an mTOR-independent autophagy pathway. *Nat Chem Biol*. May 2008;4(5):295-305. doi:10.1038/nchembio.79

74. Mazure NM, Pouysségur J. Hypoxia-induced autophagy: cell death or cell survival? *Curr Opin Cell Biol*. Apr 2010;22(2):177-80. doi:10.1016/j.ceb.2009.11.015

75. Limanaqi F, Biagioni F, Gambardella S, Ryskalin L, Fornai F. Interdependency Between Autophagy and Synaptic Vesicle Trafficking: Implications for Dopamine Release. *Front Mol Neurosci*. 2018;11:299. doi:10.3389/fnmol.2018.00299

76. Kesidou E, Lagoudaki R, Touloumi O, Poulatsidou K-N, Simeonidou C. Autophagy and neurodegenerative disorders. *Neural regeneration research*. 2013;8(24):2275.

77. Patergnani S, Pinton P. Mitophagy and mitochondrial balance. *Mitochondrial Regulation*. 2015:181-194.

78. Harris H, Rubinsztein DC. Control of autophagy as a therapy for neurodegenerative disease. *Nat Rev Neurol*. Dec 20 2011;8(2):108-17. doi:10.1038/nrneurol.2011.200

79. Bharadwaj PR, Bates KA, Porter T, et al. Latrepirdine: molecular mechanisms underlying potential therapeutic roles in Alzheimer's and other neurodegenerative diseases. *Transl Psychiatry*. Dec 03 2013;3:e332. doi:10.1038/tp.2013.97

80. Chu CT. Mechanisms of selective autophagy and mitophagy: Implications for neurodegenerative diseases. *Neurobiol Dis*. Feb 2019;122:23-34. doi:10.1016/j.nbd.2018.07.015

81. Halamoda Kenzaoui B, Chapuis Bernasconi C, Guney-Ayra S, Juillerat-Jeanneret L. Induction of oxidative stress, lysosome activation and autophagy by nanoparticles in human brain-derived endothelial cells. *Biochem J*. Feb 01 2012;441(3):813-21. doi:10.1042/BJ20111252

82. Hayat M. *Autophagy: Cancer, Other Pathologies, Inflammation, Immunity, Infection, and Aging: Volume 12*. vol 12. Academic Press; 2017.

83. Zhang X, Ren X, Zhang Q, et al. PGC-1α/ERRα-Sirt3 Pathway Regulates DAergic Neuronal Death by Directly Deacetylating SOD2 and ATP Synthase β. *Antioxid Redox Signal*. Feb 20 2016;24(6):312-28. doi:10.1089/ars.2015.6403

84. Hao LY, Giasson BI, Bonini NM. DJ-1 is critical for mitochondrial function and rescues PINK1 loss of function. *Proc Natl Acad Sci U S A*. May 25 2010;107(21):9747-52. doi:10.1073/pnas.0911175107

85. Thomas KJ, McCoy MK, Blackinton J, et al. DJ-1 acts in parallel to the PINK1/parkin pathway to control mitochondrial function and autophagy. *Hum Mol Genet*. Jan 01 2011;20(1):40-50. doi:10.1093/hmg/ddq430

86. Motori E, Puyal J, Toni N, et al. Inflammation-induced alteration of astrocyte mitochondrial dynamics requires autophagy for mitochondrial network maintenance. *Cell Metab*. Dec 03 2013;18(6):844-59. doi:10.1016/j.cmet.2013.11.005

87. Dowding JM, Song W, Bossy K, et al. Cerium oxide nanoparticles protect against Aβ-induced mitochondrial fragmentation and neuronal cell death. *Cell Death Differ*. Oct 2014;21(10):1622-32. doi:10.1038/cdd.2014.72

88. Stern ST, Johnson DN. Role for nanomaterial-autophagy interaction in neurodegenerative disease. *Autophagy*. Nov 2008;4(8):1097-100. doi:10.4161/auto.7142

89. Feng X, Chen A, Zhang Y, Wang J, Shao L, Wei L. Central nervous system toxicity of metallic nanoparticles. *Int J Nanomedicine*. 2015;10:4321-40. doi:10.2147/IJN.S78308

90. He C, Klionsky DJ. Regulation mechanisms and signaling pathways of autophagy. *Annu Rev Genet*. 2009;43:67-93. doi:10.1146/annurev-genet-102808-114910

91. Shrivastava R, Raza S, Yadav A, Kushwaha P, Flora SJ. Effects of sub-acute exposure to TiO2, ZnO and Al2O3 nanoparticles on oxidative stress and histological changes in mouse liver and brain. *Drug Chem Toxicol*. Jul 2014;37(3):336-47. doi:10.3109/01480545.2013.866134

92. Song B, Zhang Y, Liu J, Feng X, Zhou T, Shao L. Unraveling the neurotoxicity of titanium dioxide nanoparticles: focusing on molecular mechanisms. *Beilstein J Nanotechnol*. 2016;7:645-54. doi:10.3762/bjnano.7.57

93. Dagda RK, Cherra SJ, Kulich SM, Tandon A, Park D, Chu CT. Loss of PINK1 function promotes mitophagy through effects on oxidative stress and mitochondrial fission. *J Biol Chem*. May 15 2009;284(20):13843-13855. doi:10.1074/jbc.M808515200

94. Ravikumar B, Vacher C, Berger Z, et al. Inhibition of mTOR induces autophagy and reduces toxicity of polyglutamine expansions in fly and mouse models of Huntington disease. *Nat Genet*. Jun 2004;36(6):585-95. doi:10.1038/ng1362

95. Friedlander RM. Apoptosis and caspases in neurodegenerative diseases. *N Engl J Med*. Apr 03 2003;348(14):1365-75. doi:10.1056/NEJMra022366

96. Kermer P, Liman J, Weishaupt JH, Bähr M. Neuronal apoptosis in neurodegenerative diseases: from basic research to clinical application. *Neurodegener Dis*. 2004;1(1):9-19. doi:10.1159/000076665

97. Area-Gomez E, Guardia-Laguarta C, Schon EA, Przedborski S. Mitochondria, OxPhos, and neurodegeneration: cells are not just running out of gas. *J Clin Invest*. 01 02 2019;129(1):34-45. doi:10.1172/JCI120848

98. Niu S, Zhang LK, Zhang L, et al. Inhibition by Multifunctional Magnetic Nanoparticles Loaded with Alpha-Synuclein RNAi Plasmid in a Parkinson's Disease Model. *Theranostics*. 2017;7(2):344-356. doi:10.7150/thno.16562

99. Lipton SA. Pathologically activated therapeutics for neuroprotection. *Nat Rev Neurosci*. Oct 2007;8(10):803-8. doi:10.1038/nrn2229

100. Kim MJ, Rehman SU, Amin FU, Kim MO. Enhanced neuroprotection of anthocyanin-loaded PEG-gold nanoparticles against Aβ. *Nanomedicine*. Nov 2017;13(8):2533-2544. doi:10.1016/j.nano.2017.06.022

101. Zhang C, Chen J, Feng C, et al. Intranasal nanoparticles of basic fibroblast growth factor for brain delivery to treat Alzheimer's disease. *Int J Pharm*. Jan 30 2014;461(1-2):192-202. doi:10.1016/j.ijpharm.2013.11.049

102. Tiwari MN, Agarwal S, Bhatnagar P, et al. Nicotine-encapsulated poly(lactic-co-glycolic) acid nanoparticles improve neuroprotective efficacy against MPTP-induced parkinsonism. *Free Radic Biol Med*. Dec 2013;65:704-718. doi:10.1016/j.freeradbiomed.2013.07.042

103. Mohajeri M, Sadeghizadeh M, Najafi F, Javan M. Polymerized nano-curcumin attenuates neurological symptoms in EAE model of multiple sclerosis through down regulation of inflammatory and oxidative processes and enhancing neuroprotection and myelin repair. *Neuropharmacology*. Dec 2015;99:156-67. doi:10.1016/j.neuropharm.2015.07.013

104. Ramires Júnior OV, Alves BDS, Barros PAB, et al. Nanoemulsion Improves the Neuroprotective Effects of Curcumin in an Experimental Model of Parkinson's Disease. *Neurotox Res*. Jun 2021;39(3):787-799. doi:10.1007/s12640-021-00362-w

105. Frautschy SA, Cole GM. Why pleiotropic interventions are needed for Alzheimer's disease. *Mol Neurobiol*. Jun 2010;41(2-3):392-409. doi:10.1007/s12035-010-8137-1

106. Chandran V, Gao K, Swarup V, et al. Inducible and reversible phenotypes in a novel mouse model of Friedreich's Ataxia. *Elife*. Dec 19 2017;6doi:10.7554/eLife.30054

107. Santos R, Lefevre S, Sliwa D, Seguin A, Camadro JM, Lesuisse E. Friedreich ataxia: molecular mechanisms, redox considerations, and therapeutic opportunities. *Antioxid Redox Signal*. Sep 01 2010;13(5):651-90. doi:10.1089/ars.2009.3015

108. Edenharter O, Schneuwly S, Navarro JA. Mitofusin-Dependent ER Stress Triggers Glial Dysfunction and Nervous System Degeneration in a. *Front Mol Neurosci*. 2018;11:38. doi:10.3389/fnmol.2018.00038

109. Schmucker S, Puccio H. Understanding the molecular mechanisms of Friedreich's ataxia to develop therapeutic approaches. *Hum Mol Genet*. Apr 15 2010;19(R1):R103-10. doi:10.1093/hmg/ddq165

110. Salami CO, Jackson K, Jose C, et al. Stress-Induced Mouse Model of the Cardiac Manifestations of Friedreich's Ataxia Corrected by AAV-mediated Gene Therapy. *Hum Gene Ther*. 08 2020;31(15-16):819-827. doi:10.1089/hum.2019.363

111. Piguet F, de Montigny C, Vaucamps N, Reutenauer L, Eisenmann A, Puccio H. Rapid and Complete Reversal of Sensory Ataxia by Gene Therapy in a Novel Model of Friedreich Ataxia. *Mol Ther*. 08 01 2018;26(8):1940-1952. doi:10.1016/j.ymthe.2018.05.006

112. Hackett PT, Jia X, Li L, Ward DM. Posttranslational regulation of mitochondrial frataxin and identification of compounds that increase frataxin levels in Friedreich's ataxia. *J Biol Chem*. 06 2022;298(6):101982. doi:10.1016/j.jbc.2022.101982

113. Igoillo-Esteve M, Oliveira AF, Cosentino C, et al. Exenatide induces frataxin expression and improves mitochondrial function in Friedreich ataxia. *JCI Insight*. 01 30 2020;5(2)doi:10.1172/jci.insight.134221

114. Czuba-Wojnilowicz E, Viventi S, Howden SE, et al. Particle-mediated delivery of frataxin plasmid to a human sensory neuronal model of Friedreich's ataxia. *Biomater Sci*. May 07 2020;8(9):2398-2403. doi:10.1039/c9bm01757g

115. Nabhan JF, Wood KM, Rao VP, et al. Intrathecal delivery of frataxin mRNA encapsulated in lipid nanoparticles to dorsal root ganglia as a potential therapeutic for Friedreich's ataxia. *Sci Rep*. Feb 17 2016;6:20019. doi:10.1038/srep20019

116. Xu L, Sun Z, Xing Z, et al. Cur@SF NPs alleviate Friedreich's ataxia in a mouse model through synergistic iron chelation and antioxidation. *J Nanobiotechnology*. Mar 09 2022;20(1):118. doi:10.1186/s12951-022-01333-9

117. Santiago-Gonzalez B, Monguzzi A, Azpiroz JM, et al. Permanent excimer superstructures by supramolecular networking of metal quantum clusters. *Science*. Aug 05 2016;353(6299):571-5. doi:10.1126/science.aaf4924

118. Villa C, Legato M, Umbach A, et al. Treatment with ROS detoxifying gold quantum clusters alleviates the functional decline in a mouse model of Friedreich ataxia. *Sci Transl Med*. 08 18 2021;13(607)doi:10.1126/scitranslmed.abe1633

119. Feigin VL, Vos T, Nichols E, et al. The global burden of neurological disorders: translating evidence into policy. *Lancet Neurol*. 03 2020;19(3):255-265. doi:10.1016/S1474-4422(19)30411-9

120. Profaci CP, Munji RN, Pulido RS, Daneman R. The blood-brain barrier in health and disease: Important unanswered questions. *J Exp Med*. 04 06 2020;217(4)doi:10.1084/jem.20190062

121. Khawli LA, Prabhu S. Drug delivery across the blood-brain barrier. *Mol Pharm*. May 06 2013;10(5):1471-2. doi:10.1021/mp400170b

122. Javed R, Ao Q. Nanoparticles in peripheral nerve regeneration: A mini review. *J Neurorestoratology*. 2022;10:1-12.

123. Klajnert B, Cortijo-Arellano M, Bryszewska M, Cladera J. Influence of heparin and dendrimers on the aggregation of two amyloid peptides related to Alzheimer's and prion diseases. *Biochem Biophys Res Commun*. Jan 13 2006;339(2):577-82. doi:10.1016/j.bbrc.2005.11.053

124. Rekas A, Lo V, Gadd GE, Cappai R, Yun SI. PAMAM dendrimers as potential agents against fibrillation of alpha-synuclein, a Parkinson's disease-related protein. *Macromol Biosci*. Mar 10 2009;9(3):230-8. doi:10.1002/mabi.200800242

125. Igartúa DE, Martinez CS, Del V Alonso S, Prieto MJ. Combined Therapy for Alzheimer's Disease: Tacrine and PAMAM Dendrimers Co-Administration Reduces the Side Effects of the Drug without Modifying its Activity. *AAPS PharmSciTech*. Mar 25 2020;21(3):110. doi:10.1208/s12249-020-01652-w

126. Hollinger KR, Sharma A, Tallon C, et al. Dendrimer-2PMPA selectively blocks upregulated microglial GCPII activity and improves cognition in a mouse model of multiple sclerosis. *Nanotheranostics*. 2022;6(2):126-142. doi:10.7150/ntno.63158

127. Ferrer-Lorente R, Lozano-Cruz T, Fernández-Carasa I, et al. Cationic Carbosilane Dendrimers Prevent Abnormal α-Synuclein Accumulation in Parkinson's Disease Patient-Specific Dopamine Neurons. *Biomacromolecules*. 11 08 2021;22(11):4582-4591. doi:10.1021/acs.biomac.1c00884

128. Xue X, Wang LR, Sato Y, et al. Single-walled carbon nanotubes alleviate autophagic/lysosomal defects in primary glia from a mouse model of Alzheimer's disease. *Nano Lett*. Sep 10 2014;14(9):5110-7. doi:10.1021/nl501839q

129. Guo Q, You H, Yang X, et al. Functional single-walled carbon nanotubes 'CAR' for targeting dopamine delivery into the brain of parkinsonian mice. *Nanoscale*. Aug 03 2017;9(30):10832-10845. doi:10.1039/c7nr02682j

130. Tan JM, Saifullah B, Kura AU, Fakurazi S, Hussein MZ. Incorporation of Levodopa into Biopolymer Coatings Based on Carboxylated Carbon Nanotubes for pH-Dependent Sustained Release Drug Delivery. *Nanomaterials (Basel)*. May 31 2018;8(6)doi:10.3390/nano8060389

131. Glat M, Skaat H, Menkes-Caspi N, Margel S, Stern EA. Age-dependent effects of microglial inhibition in vivo on Alzheimer's disease neuropathology using bioactive-conjugated iron oxide nanoparticles. *J Nanobiotechnology*. Sep 23 2013;11:32. doi:10.1186/1477-3155-11-32

# Figure legends

**Figure 1 NPs classification**. Schematic representation of organic and inorganic NPs.

**Figure 2 Internalisation and target pathways of NPs.** NPs are internalised by different mechanism, including endocytosis mediated by caveolin vesicles, clathrin vesicles, specific receptors along with phagocytosis and pinocytosis. NPs can enter lysosomes before interacting with other cellular components, such as mitochondria. Finally, NPs can exert their effect on ROS production, mitochondrial metabolism, and gene expression.

**Figure 3 Action of NPs on neuroinflammation.** (A-B) After CNS injury, astrocyte and/or microglia cells activate and promote local inflammation. (C) Persistent inflammation leads to stimulation of cytokine/chemokine storm and activation of inflammatory cells (lymphocytes and monocytes). (D) Inflammatory cells (monocytes, neutrophils, T cells) penetrate the BBB and cause neuroinflammation. NPs might mitigate neuroinflammatory response acting on local inflammation by penetrating the BBB. (E) Neuroinflammation, oxidative stress and excitotoxicity affect neuronal survival and enhance apoptosis by stimulating caspases hyperactivation. NPs can reduce neuronal cell death mitigating caspases hyperactivation. (F) Neuronal dysfunction and inflammatory environment stimulate misfolded protein production and protein aggregates (neurofibrillary tangles, amyloid β plaques, Lewy bodies) formation. NPs reduce protein and peptide aggregation and inflammation reducing neuronal toxicity and improving neuronal network and behaviours.

**Figure 4 Gold nanoclusters effect in CNS.** (A) Au8pX are able to migrate across the blood brain barrier acting in different biological pathways (B) such as oxidative stress, autophagy and mitochondrial dynamics. These processes have an indirect effect on frataxin expression.