



Neuroprotective transition metal dichalcogenide nanoflower treatments alleviate pathological cell stress associated with Parkinson's disease



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Parkinson's disease (PD) is triggered by irreversible degeneration of dopaminergic neurons in the midbrain, hypothalamus, and thalamus. Although the molecular etiology of pathology remains unclear, progressive aggregation of alpha-synuclein (α -syn) and mitochondrial dysfunction are two mechanisms implicated in neuronal degeneration. The current study investigates the neuroprotective properties of molybdenum disulfide (MoS_2) and molybdenum diselenide (MoSe_2) nanoflowers (NFs) in neurons and astrocytes exposed to α -syn aggregates. It was found that MoS_2 and MoSe_2 transcriptionally downregulated α -syn-induced unfolded protein response, and upregulated autophagy and exocytosis of α -syn fibrils. TMD NFs alleviated α -syn-induced damage of cell mitochondria, simultaneously altering metabolic adaptation, with a decrease in ROS levels observed. Neuroprotective properties were further explored in *Caenorhabditis elegans* that overexpress α -syn. Nematodes experienced a drastic reduction in the amount of aggregated α -syn which resulted in a significant increase in *C. elegans* lifespan. These findings indicate that MoS_2 or MoSe_2 NFs could be used as novel therapeutic to decelerate the progression of PD.

Parkinson's disease (PD) is the fastest increasing neurological disorder, with an estimated count of 25.2 million diagnoses worldwide by 2050¹. In the United States alone, there are approximately 60,000 cases of PD diagnosed annually, accounting for a share of the medical market worth at least \$52 billion². PD is characterized by the progressive degeneration of dopaminergic neurons in the midbrain, hypothalamus, and thalamus³. This gives rise to numerous PD symptoms, including bradykinesia, postural and rest tremors, rigidity of the muscles, and postural instability^{4,5}, albeit symptoms are heterogeneous between patients⁶. The etiology of PD has yet to be completely understood, however, a growing body of evidence suggests that the onset and propagation of neurodegeneration are triggered by the abrupt aggregation of alpha synuclein (α -syn) and progressive mitochondrial dysfunction^{7–9}.

α -Syn is a low molecular weight protein consisting of 140 amino acid residues that is encoded by the chromosome 4 SNCA gene¹⁰. Although the exact physiological function of α -syn remains unclear, it is known that α -syn

facilitates trafficking of presynaptic vesicles releasing neurotransmitters into the synaptic cleft^{11–14}. α -Syn is primarily localized in the membranes of presynaptic terminals of dopaminergic neurons¹⁵, although it is also present in many organelles, such as the mitochondria¹⁶, the endoplasmic reticulum¹⁷, Golgi apparatus^{18,19}, and nuclei²⁰. Although cytosolic α -syn is an intrinsically disordered protein, it folds into an α -helical structure upon embedding into the lipid membranes^{21–23}. Under pathological conditions, α -syn aggregates into β -sheet-rich oligomers and fibrils^{24,25}. This conformational transition of α -syn is implicated with the onset and progression of PD^{26,27}. Along with the accumulation of toxic protein aggregates, PD pathology heavily involves mitochondrial dysfunction^{28–31}. Under pathological conditions, mitochondria produce an excessive amount of reactive oxygen species (ROS), experience a detrimental depolarization of membrane potential, and a decrease in the production of ATP³². Mitochondrial dysfunction further exacerbates cell malfunction, which results in the degeneration of both neurons and astrocytes as observed in PD.

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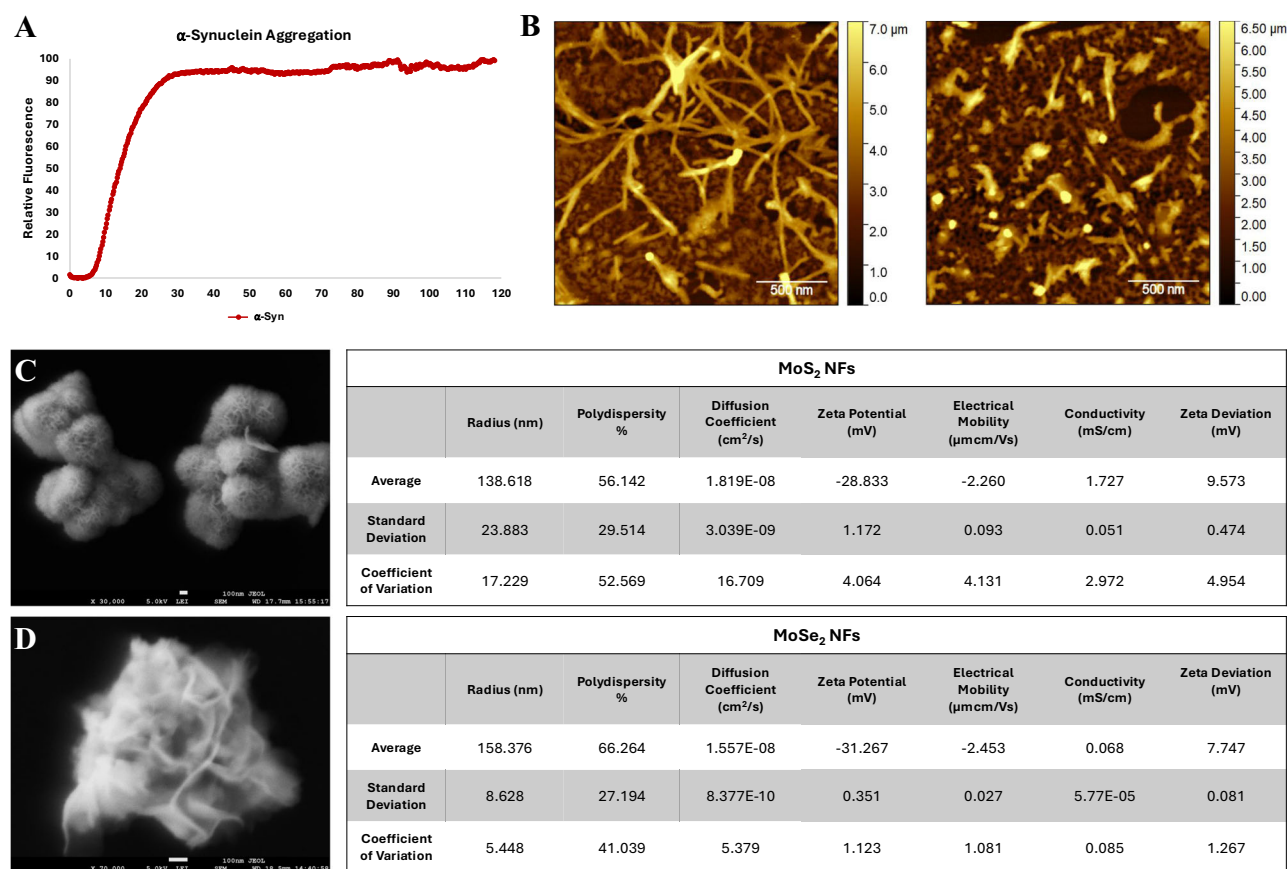


Fig. 1 | Characterization of protein aggregates and TMD nanoflowers. A ThT aggregation kinetics of expressed and purified α -syn from *E. coli* to be used downstream as an in vitro PD model. **B** AFM images of mature, aggregated α -syn fibrils prior to sonication (left) and post sonication (right). Oligomers and smaller α -syn aggregates are known to be more cytotoxic, and all aggregates were sonicated prior to

administration into neuronal cells. **C** Representative SEM image of MoS₂ NFs taken at $\times 30,000$ magnification (left) and a table of physical characterization utilizing DLS and zeta potential. **D** Representative SEM image of MoSe₂ NF taken at $\times 70,000$ magnification (left) and a table of physical characterization utilizing DLS and zeta potential. Table categories and means of measurement in bold.

Decades of research have investigated the efficacy of therapeutic approaches to treat PD, primarily focused on inhibiting amyloid fibril formation, reducing amyloid burden in the brain, and alleviating mitochondrial dysfunction. For instance, numerous attempts have been made to inhibit α -syn aggregation using small molecular candidates³³, natural polyvinyls³⁴, polyphenols^{35–37}, antioxidants^{38,39}, and protein chaperones⁴⁰. Several research groups explored the possibility of utilizing antibodies, such as Cinpanemab⁴¹ and Prasinezumab⁴², to reduce the burden of cytotoxic α -syn aggregates in the brain⁴³. Finally, therapeutic properties of Coenzyme Q₁₀³⁸ and Idebenone^{44,45} in alleviation of mitochondrial dysfunction were explored. However, these strategies did not reach expected neuroprotection allowing only to alleviate PD symptoms, resulting in the absence of a neuroprotective therapeutic for PD patients⁴⁶. These limitations catalyzed the search for novel neuroprotective therapeutic approaches that can be used to decelerate the progression of PD.

Transition metal dichalcogenides (TMD) are a class of nanomaterials with unique optical, catalytic, and biological properties.^{47–49} TMDs are comprised of a transition metal atom, such as molybdenum (Mo) or tungsten (W), coordinated with two chalcogenide atoms of sulfur (S) or selenium (Se).⁵⁰ Hydrothermal synthesis enables self-assembly of metal and chalcogenide atoms into nanoflakes that subsequently organize into nanoflowers (NFs), supramolecular aggregates with high surface area to volume ratio. TMDs, including molybdenum disulfide (MoS₂) NFs, exhibit unique catalase, superoxide dismutase, and peroxidase properties⁵¹. Furthermore, it has been shown that both MoS₂ and molybdenum diselenide (MoSe₂) NFs facilitated mitochondrial biogenesis and reduced levels of ROS in neurons and astrocytes.⁵² These and other biological properties of TMD

NFs open new avenues for the utilization of these nanomaterials as therapies for cancer,⁵³ inflammation,⁵⁴ and Alzheimer's disease (AD)⁵⁵ treatment. However, therapeutic potential of TMD NFs in PD remains unclear.

This study investigates neuroprotective properties of MoS₂ and MoSe₂ NFs. The results show that both TMD NFs rescued neurons and astrocytes from toxic effects of α -syn fibrils. Elucidation of molecular mechanisms of the observed neuroprotection activity implicate that TMD NFs transcriptionally activate exocytosis and autophagy of toxic protein aggregates. This leads to the suppression of α -syn-induced unfolded protein response (UPR) of the endoplasmic reticulum (ER), and, as a result, a decrease in ROS levels in both neurons and mitochondria. Furthermore, TMD NFs improve mitochondrial health simultaneously facilitating upregulation of mitochondrial biogenesis proteins. These results demonstrate the therapeutic potential of TMD NFs as a neuroprotective drug candidate capable of decelerating PD.

Results

Generation and characterization of pathogenic α -Syn and TMD NF Treatments

To model PD in vitro, α -syn was expressed and aggregated into fibrillar species that were later administered to cell cultures. Aggregation of α -syn showed characteristic lag time of 8.42 ± 0.72 h and a half-time of 13.92 ± 1.38 h (Fig. 1), which is consistent with previous literature from the Kurouski^{56,57} and other research groups^{58,59}. Atomic force microscopy (AFM) revealed the presence of long, mature α -syn fibrils after 5 days of protein aggregation. After sonication, AFM shows much smaller aggregation species (Fig. 1) that were then administered to cells.

MoS₂ and MoSe₂ NFs were synthesized using a hydrothermal synthesis protocol as previously described³². Scanning electron microscopy (SEM) revealed the characteristic NF morphology (Fig. 1). Using dynamic light scattering, the radius, polydispersity, and diffusion coefficient for both nanomaterials was determined. For MoS₂ NFs, the measurements were determined to be 138.618 ± 23.883 nm, 56.142 ± 29.514%, and 1.819 E-08 ± 3.039 E-09 cm²/s respectively. For MoSe₂ NFs, these measurements were determined to be 158.376 ± 8.628 nm, 66.264 ± 27.194%, and 1.557 E-08 ± 8.377 E-10 cm²/s, respectively (Fig. 1). Utilization of Malvern Zetasizer Nano ZS revealed zeta potential, electrical mobility, conductivity, and zeta deviation of synthesized TMD NFs. For MoS₂ NFs, the measurements were determined to be -28.833 ± 1.172 mV, -2.260 ± 0.093 μmcm/Vs, 1.727 ± 0.051 mS/cm, and 9.573 ± 0.474 mV, respectively. For MoSe₂ NFs, these measurements were determined to be -31.267 ± 0.351 mV, -2.453 ± 0.027 μmcm/Vs, 0.068 ± 5.77E-05 mS/cm, and 7.747 ± 0.081 mV, respectively (Fig. 1).

TMD NF treatments alleviate α-syn-induced mitochondrial impairment and boost mitochondrial biogenesis in neurons and astrocytes

Endocytosis of α-syn aggregates by neurons and astrocytes triggers a cascade of pathological processes that results in a depolarization of mitochondrial membranes, and consequently, impaired ATP biosynthesis, which ultimately leads to a cell death^{60–63}. Expanding upon this, JC-1 assay was used to determine the extent to which TMD NFs could reverse α-syn-induced mitochondrial membrane potential. An uptake of α-syn fibrils induced significant mitochondrial damage in both neurons and astrocytes. Compared to the control, N27 neurons experienced a 257% increase, while DI astrocytes a 327% increase in mitochondrial damage upon exposure to α-syn aggregates. Similarly to N27 neurons, α-syn fibrils triggered a 251% increase in the mitochondrial damage in CTX astrocytes (Fig. 2).

The subsequent (12 h) treatment of these neurons with different concentrations of MoS₂ NFs resulted in 15.8–55.4% decreases in the magnitude of α-syn-induced mitochondrial damage. DI and CTX astrocytes treated with MoS₂ NFs also experienced a strong dose-dependent therapeutic relief, which resulted in 31.5–62.7% (DI) and 31.9–49.7% (CTX) decrease in the mitochondrial impairment. Similar effects were observed for MoSe₂ NFs in both neurons and astrocytes. N27 neurons exposed to α-syn fibrils and subsequently (12 h) treated with MoSe₂ NFs exhibited 33.5–64.0% reduction in the mitochondrial impairment. In DI and CTX astrocytes exposed to α-syn fibrils, MoSe₂ NFs caused a strong concentration-dependent reduction in mitochondrial damage ranging from 24.9 to 63.7%. These results indicate that both MoSe₂ and MoS₂ NFs efficiently rescue cell mitochondrial from cytotoxic effects caused by α-syn aggregates.

It is important to determine whether TMD NFs only rescue cell mitochondria or also facilitate the synthesis of novel mitochondria, a process known as mitochondrial biogenesis^{64–66}. To answer this question, changes in the expression of two mitochondrial proteins, succinate dehydrogenase subunit A (SDH-A) and cytochrome c oxidase subunit I (COX-I) were analyzed. ELISA revealed that exposition of neurons and astrocytes to α-syn fibrils caused a strong downregulation in the expression of both SDH-A and COX-I (Fig. 2). N27 neurons experienced only a small, 1.34% and 1.39% decrease in the expression of SDH-A and COX-I protein levels, respectively. However, a substantially higher magnitude (4.32% for SDH-A and 5.60% for COX-I) of downregulation of mitochondrial biogenesis was observed in DI, as well as in CTX astrocytes (7.48% for SDH-A and 7.82% for COX-I). These results indicate that endocytosis of α-syn aggregates strongly suppresses mitochondrial biogenesis in both neurons and astrocytes.

A dose-dependent upregulation in mitochondrial biogenesis was observed in N27 neurons after administration of MoS₂ and MoSe₂ NF. Treatments with both MoS₂ and MoSe₂ only experienced slight alterations in mitochondrial biogenesis at the lowest doses, however, did experience much greater effects at the highest concentration. As the concentration of

MoS₂ NFs increased from 0.1 to 1.0 mg/mL, SDH-A was upregulated from 0.51 to 5.49%. At the same concentrations of MoS₂, COX-I was upregulated from 1.57 to 12.35%. When treated with MoSe₂ NFs, the expression of SDH-A was upregulated from 7.63 to 13.11%, while COX-I expression was upregulated from 5.38 to 8.39%. Similarly to neurons, DI astrocytes underwent a dose-dependent upregulation of mitochondrial biogenesis upon receiving TMD NF subsequently to α-syn aggregates. As the concentration of MoS₂ NFs increased from 0.1 to 1.0 mg/mL, an upregulation of SDH-A from 1.40% to 3.74% was observed. COX-I upregulation was found to increase from 2.76% to 8.60% in the cells exposed to 0.1–1.0 mg/mL of MoS₂ NFs. When treated with MoSe₂ NFs, SDH-A experienced an upregulation from 5.68 to 12.43%, while COX-I expression was alleviated from 4.16% to 7.22%. CTX astrocytes treated with MoS₂ NFs had little to no change in the levels of SDH-A expression. At the highest concentration of MoS₂ NFs, only a 1.37% increase in SDH-A expression was observed. At the same time, a significant upregulation (1.81–7.43%) of COX-I expression was observed in CTX astrocytes exposed to MoS₂ NFs. CTX astrocytes treated with MoSe₂ NFs showed a more prominent dose-dependent response for both mitochondrial proteins. The expression of SDH-A was elevated by 6.44%, 10.80%, and 13.02%. The COX-I protein experienced an increase in the expression from 5.09 to 8.03% as the concentration of MoSe₂ NFs increased. These results indicate that MoS₂ and MoSe₂ NFs not only protect mitochondria from α-syn aggregates but also upregulate key mitochondrial proteins in both neurons and astrocytes.

TMD NF treated cells ability to respond to mitochondrial stress

To understand how PD cells treated with TMD NFs would be able to respond to mitochondrial stress, a Seahorse XF Mito Tox assay was conducted. This assay reveals changes in the oxygen consumption rate (OCR), extracellular acidification rate (ECAR), ATP production, and energy map as a response to α-syn aggregates alone and α-syn aggregates with the subsequent cell treatment with TMD NFs (Fig. 3).

All cell types exhibited a decrease in basal OCR as a response to α-syn aggregates, which indicates toxic nature of α-syn fibrils to neurons and astrocytes (Fig. 3G). We found that subsequent administration of MoSe₂ NFs to DI astrocytes increased OCR, while this effect was not observed for MoS₂ NFs. The opposite effect was observed on N27 cells. Specifically, administration of MoS₂ NFs increased OCR, while MoSe₂ NFs had no effect on N27 cells. Finally, no increase in OCR was observed as a result of MoS₂ or MoSe₂ NFs treatment. These results indicate that MoS₂ and MoSe₂ had different effects on N27, DI, and CTX cells. These findings also indicate that TMD NFs do not rescue cells by the direct change in OCR, but rather activate alternative mechanisms in the cells, as discussed below. ATP analyses confirmed that α-syn fibrils caused strong mitochondrial impairment in N27 and DI cells, while substantially weaker effects were observed in CTX. Administration of TMD NFs did not result in the immediate increase in ATP levels in all cell types. Since ATP production and OCR are interconnected, these results are consistent with OCR findings discussed above.

ECAR analysis directly measures changes in extracellular pH as a result of the increase in the rate of glycolysis. A decrease in ECAR levels was observed only for N27 as a result of α-syn aggregates exposure, while no change in ECAR levels was observed for DI and CTX. Administration of TMD NFs increased ECAR in all cell types. These TMD NFs shifted cell metabolism towards glycolysis.

Cellular energy maps were generated by plotting OCR against ECAR for each experimental group for N27 neurons (Fig. 3J), DI astrocytes (Fig. 3K), and CTX astrocytes (Fig. 3L). N27, DI, and CTX exposed to α-syn aggregates shifted towards glycolytic and quiescent states, which indicates strong mitochondrial impairment. Cells that received the subsequent MoS₂ and MoSe₂ treatment exhibited a greater shift towards glycolysis. This indicates TMD NFs engage a sophisticated cascade of metabolic pathways aiming to rescue neurons and astrocytes from the cytotoxic effects of α-syn aggregates. It should be noted that control group receiving neither α-syn aggregates nor TMD NFs shifted towards an energetic state.

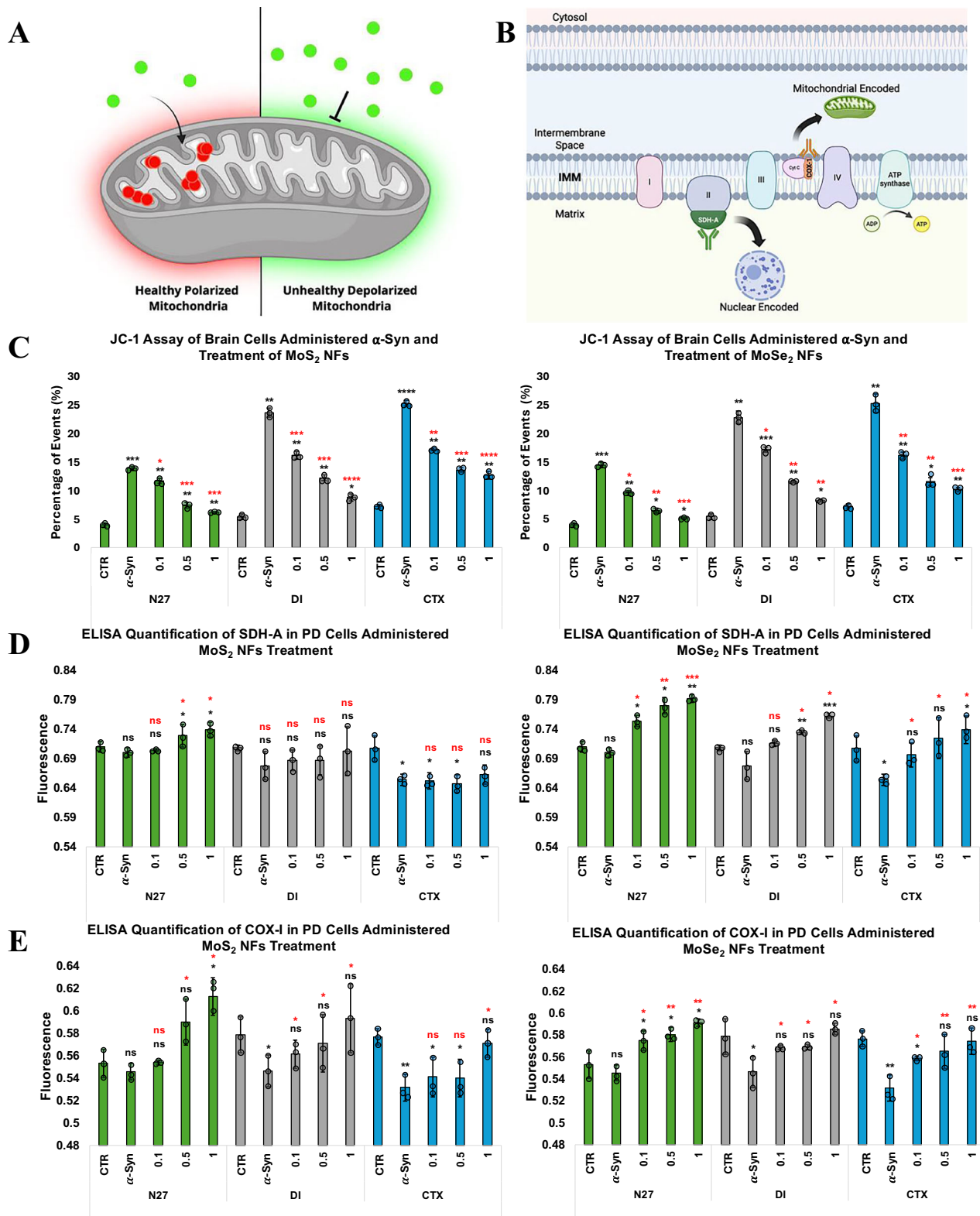


Figure 2

TMD NF treatments alleviate α -Syn-induced ROS and reverse cellular stress granule formation

Internalization of aggregated α -syn by neuronal cells results in an increase in ROS^{67,68} and translation of stress granule proteins from the nucleus to the cytosol⁶⁹. Flow cytometry-based ROS assay was used to determine the extent to which TMD NFs altered ROS levels in neurons and astrocytes. An

exposition of N27 neurons, DI, and CTX astrocytes to α -syn aggregates caused a significant increase in the ROS levels. Specifically, N27 neurons experienced a 213% increase, while DI and CTX astrocytes had 132% and 59.3% increase in ROS, respectively (Fig. 4). A subsequent (12 h) treatment of neurons and astrocytes exposed to α -syn aggregates with MoS₂ NFs resulted in a dose-dependent decrease in the cellular ROS levels. N27

Fig. 2 | TMD NF effects on mitochondrial health in PD cells. **A** Experimental diagram depicting the mechanism in which JC-1 cell dye interacts with mitochondrial membranes that is subsequently measured and quantified using a flow cytometer. **B** Illustration of ELISA-measured mitochondrial proteins of the electron transport chain quantified for mitochondrial biogenesis. **C** JC-1 mitochondrial damage assay conducted on N27 neurons (green), DI astrocytes (gray), and CTX astrocytes (blue) treated with MoS₂ NFs [left] and MoSe₂ NFs [right]. Mitochondrial biogenesis ELISA quantifications of nuclear encoded SDH-A (**D**) and mitochondrially encoded COX-I (**E**) when treated with MoS₂ NFs [left] and MoSe₂ NFs [right] in N27 neurons (green), DI astrocytes (gray), and CTX astrocytes (blue). Experimental groups of cells receiving no α -syn or NF treatment (CTR), cells only exposed

to α -syn (α -syn), and cells exposed to α -syn and subsequent NF treatments of 0.1 mg/mL (0.1), 0.5 mg/mL (0.5), or 1.0 mg/mL (1). Two-way ANOVA and Student's *t* test was utilized to analyze statistical significance of reported results, with * *P* < 0.05, ** *P* < 0.005, *** *P* < 0.0005, and NS not significant. Black significance markings relate to comparisons made to control cells that did not receive α -syn or NF treatments. Red significance markings relate to comparisons made to PD-induced control cells that were exposed to α -syn but no TMD NF treatments. All JC-1 and ELISA experimentation conducted on *n* = 3 independent biological replicates. All histograms detail the calculated mean of measurements with respective standard deviation. All diagrams created in BioRender. Mitchell, C. (2026) <https://BioRender.com/xrc9n2q>.

neurons exposed to the lowest concentration of MoS₂ NFs (0.1 mg/mL) experienced a moderate (13.4%) ROS reduction. Higher concentrations of MoS₂ NFs (0.5 mg/mL and 1.0 mg/mL) caused a substantially higher (24.8% and 43.8%, respectively) decrease in the ROS levels. Similar results were observed for DI astrocytes. The lowest concentration of MoS₂ NFs resulted in 7.6% reduction, while administration of 0.5 mg/mL and 1.0 mg/mL MoS₂ NFs led to the significantly larger decrease (31.5% and 41.8%, respectively) in ROS levels. Similar magnitudes of MoS₂ NFs-triggered decrease in ROS levels in CTX astrocytes were also observed. Specifically, a decrease in ROS to 4.9%, 23.6%, and 36.1% was observed in the cells exposed to 0.1, 0.5, and 1.0 mg/mL MoS₂ NFs.

A much larger magnitude in the suppression of ROS levels was observed in neurons and astrocytes exposed to MoSe₂ NF. Specifically, N27 neurons exposed to 0.1 mg/mL MoSe₂ NFs experienced a 22.5% reduction in ROS levels. At 0.5 and 1.0 mg/mL, the reduction of ROS in 63.0% and 71.6% were recorded. A similar magnitude in the reduction of ROS was observed in DI astrocytes exposed to MoSe₂ NFs. The lowest concentration of NFs resulted in 8.9% reduction of ROS. At the same time, medium and high (0.5 and 1.0 mg/mL) concentrations of MoSe₂ NFs caused a suppression of ROS levels on 57.6% and 77.8%, respectively. A graduate decrease in ROS levels (11.1%, 60.3%, and 74.3%) in CTX astrocytes was also observed with a graduate increase in the concentration of MoS₂ NFs (0.1, 0.5, and 1.0 mg/mL). It should be noted that for all cell types, the highest concentration of NFs reduced ROS levels to the baseline observed in the cells experienced no α -syn. These results show that both MoSe₂ and MoS₂ NFs drastically reduce α -syn-induced ROS levels in neurons and astrocytes.

Cells can form, maintain, and dissociate dynamic condensates known as stress granules⁷⁰. These condensates are comprised of RNA and RNA-binding proteins. Stress granules regulate multiple cellular processes focused on the minimization of cellular stress^{70,71}. In order to probe the effects TMD NF therapeutics have on cellular stress mechanisms, immunofluorescent tracking of T-cell intracellular antigen 1-related (TIA-R) protein was conducted. TIA-R is a nucleic acid binding protein localized inside the nucleus under normal functioning conditions but vacates to the cytosol upon activation via cellular stress in pathological environments. To ensure that MoS₂ and MoSe₂ NFs caused no cellular stress, neurons and astrocytes were treated with TMD NFs. Immunofluorescent staining of these cells revealed confined localization of TIA-R in the nuclei, indicating that TMD NF alone did not induce any stress in neurons and astrocytes (Fig. 4). Immunofluorescent staining revealed the appearance of stress granules in the cytosol of neurons and astrocytes exposed to α -syn aggregates. From all analyzed cells, neurons had the most drastic response to α -syn aggregates with nearly complete evacuation of TIA-R from the nuclei. CTX astrocytes were also observed to form high levels of stress granules in the cytosol. DI astrocytes, however, did not respond with the same degree of magnitude, forming fewer stress granules in response to α -syn aggregates.

All cell types treated with MoS₂ and MoSe₂ NFs experienced a significant reduction in stress granule formation. It was found that TMD NFs administration resulted in the re-localization of TIA-R back to the nuclei, with very few fluorescent signals still present in the cytosol. Cells receiving MoSe₂ NFs treatments experienced even greater stress relief compared to their MoS₂ NFs counterparts. Thus, TIA-R imaging confirms that TMD NFs do provide neuroprotection via regulating stress response, but more in-

depth investigation is needed to better determine the complete molecular mechanistic machinery.

Elucidation of neuroprotective mechanisms of TMD NF

Accumulation of α -syn aggregates in neuronal cells triggers the unfolded protein response (UPR)^{72,73} in the endoplasmic reticulum (ER)^{74,75}, reduction of exocytosis⁷⁶, and increased mitophagy^{62,77}, (Fig. 5). Using quantitative polymerase chain reaction (qPCR), changes in the transcriptional regulation and expression of genes involved in UPR, exocytosis, and mitophagy were determined in neurons and astrocytes exposed to α -syn fibrils followed by TMD NFs treatment, as well as α -syn fibrils alone (control), in order to investigate and explore NFs' effects on cellular environments.

Impregnation of aggregated α -syn into neuronal cells significantly increases the UPR via upregulation of key genes, including protein kinase R-like endoplasmic reticulum kinase (*PERK*), activating transcription factor 6 (*ATF6*), C/EBP homologous protein (*CHOP*), and X-box binding protein 1 (*XBPI*). Administration of both MoS₂ and MoSe₂ NF alleviated the pathogenic burden of aggregated α -syn in N27 neurons on the level of genetic regulation, (Fig. 5). Specifically, MoS₂ NFs downregulated all four UPR genes after 18 h of TMD NFs administration. *ATF6* experienced a 62% reduction, while *XBPI* underwent a 95% downregulation in the cells exposed to α -syn fibrils followed by MoS₂ NFs treatment. *PERK* and *CHOP* each experienced major downregulation in such cells, with 90% and 78% reductions, respectively. Administration of MoSe₂ NFs resulted in downregulation in all four chosen UPR genes at the 18 h timepoint. *ATF6* experienced a 25% reduction, having the least impact. The other UPR genes, however, had very strong downregulations with 94%, 94%, and 80% reductions for *XBPI*, *PERK*, and *CHOP*, respectively.

In neurons, accumulation of aggregated proteins also first triggers downregulation of autophagy genes, microtubule-associated protein 1 light chain 3 beta (*LC3B*) and sequestosome 1 (*p62*). This initial suppression is followed by upregulation of these genes as neurons degrade and autophagy amyloid aggregates. MoS₂ NFs upregulated both *LC3B* and *p62* after 3 h and 6 h of treatment, indicating their innate ability to advance cell autophagy and quickly mitigate amyloid-induced stress in the cells. *LC3B* was found to be subsequently downregulated at the 18 h, while *p62* maintained upregulated. The exocytosis response was upregulated at the later timepoints, indicating a TMD-facilitated ability to remove α -syn fibrils. MoSe₂ NFs triggered upregulation of *p62* 2.22-fold at the 3 h timepoint and then tapers off for the remaining timepoints as the presence of aggregates decreases.

Cluster of differentiation 81 and 63 (*CD81* and *CD63*) are exosomal markers that can be used to quantify changes in the cell exocytosis activity. Both *CD81* and *CD63* were strongly downregulated in the cells exposed to α -syn fibrils alone. In the neurons exposed to α -syn fibrils followed by TMD NFs treatment, *CD81* was unchanged in the two early time points and then experienced a downregulation at the 9 h timepoint. From that point forward, there was a great upregulation, hinting at the cell's ability to exocytose increasing. *CD63* was initially upregulated at the 3 h timepoint and remained upregulated. More research will be conducted to identify and quantify the contents of the exosomes to provide more insight into cellular response. In the cells exposed to MoSe₂ NFs, *CD81* was downregulated at the early timepoints, then experienced an upregulation up to 2.57x at 18 h. *CD63* was upregulated much faster, with upregulation occurring at the 9 h,

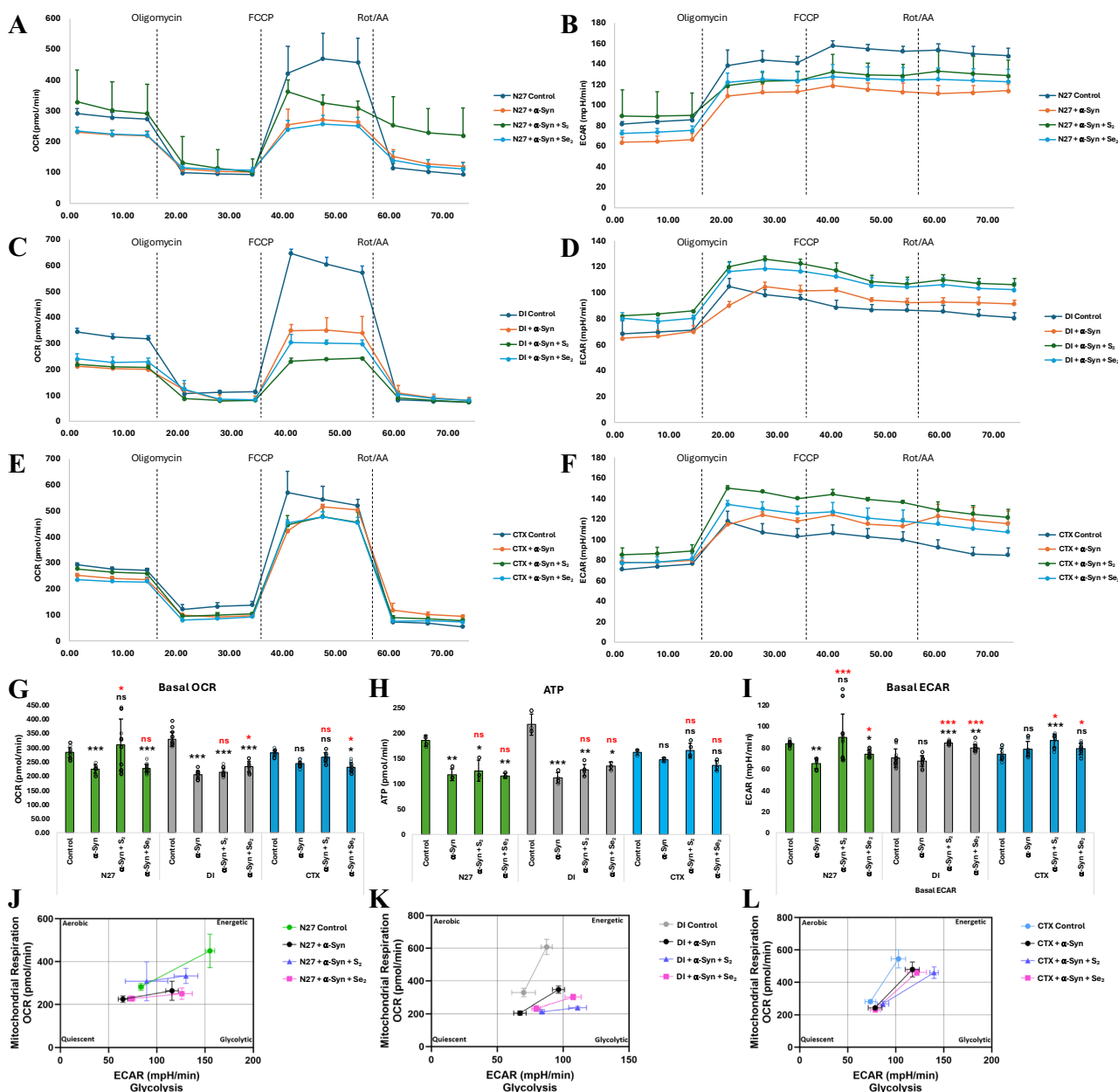


Fig. 3 | Seahorse XF Mito Tox stress test profiles. Oxygen consumption rate (OCR) maps for N27 neurons (A), DI astrocytes (C), and CTX astrocytes (E) treated with TMD NFs. Extracellular acidification rate (ECAR) maps for N27 neurons (B), DI astrocytes (D), and CTX astrocytes (F) treated with TMD NFs. Histograms reporting basal OCR (G), basal ECAR (H), and ATP production (I) for N27 neurons (green), DI astrocytes (gray), and CTX astrocytes (blue). Two-way ANOVA and Student's *t* test were utilized to analyze statistical significance of reported results, with * *P* < 0.05,

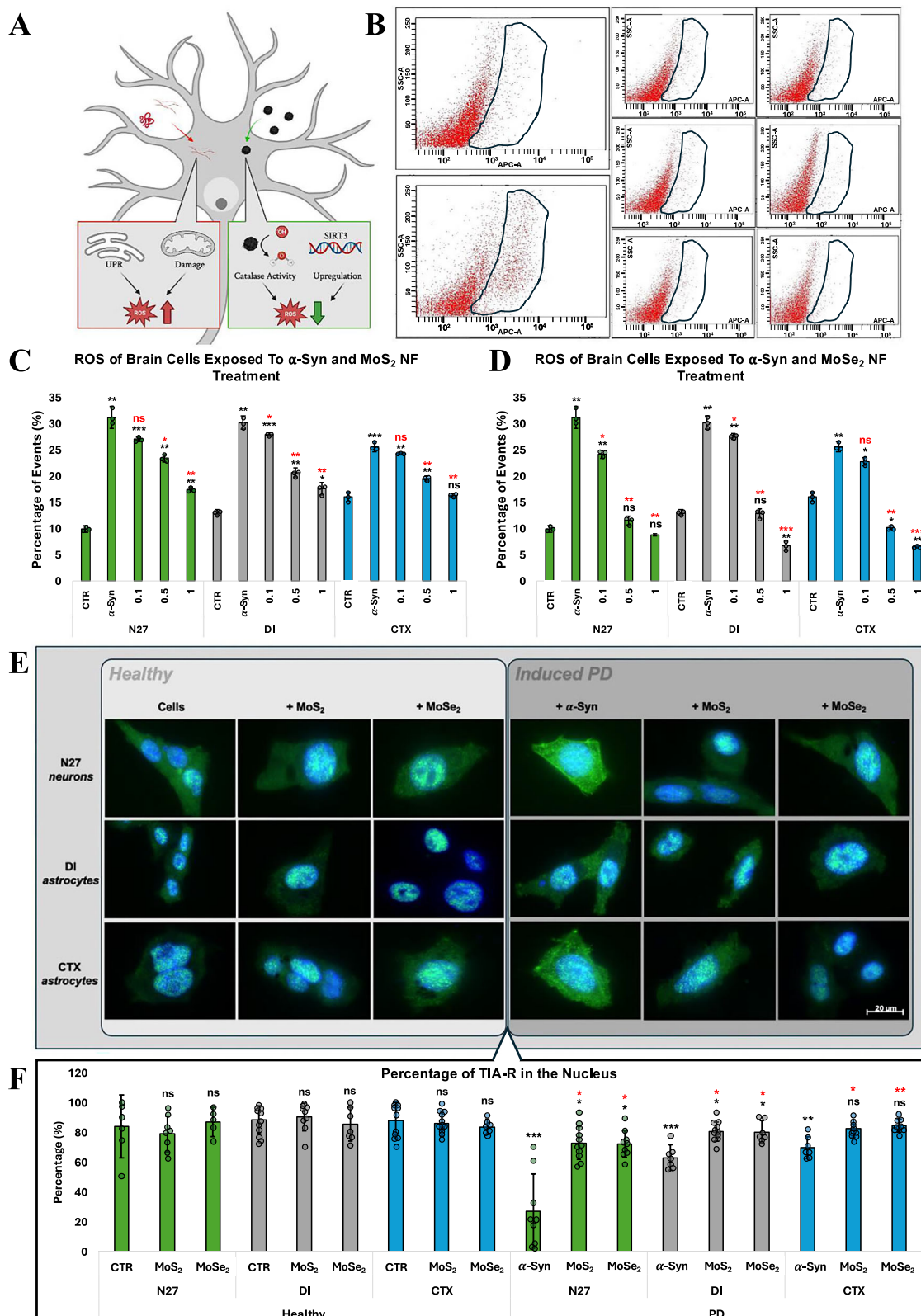
** *P* < 0.005, *** *P* < 0.001, and NS- not significant. Black significance markings relate to comparisons made to the respective control group receiving no α -syn and no NFs, while red significance markings relate to comparisons made to the respective PD group receiving no NFs. XF Cell Energy Phenotype Profiles for N27 neurons (J), DI astrocytes (K), and CTX astrocytes (L) for all experimental groups. All Seahorse experimentation conducted on *n* = 6 independent biological replicates. All reported data represents the mean value of measurements with respective standard deviation.

12 h, and 18 h timepoints, with a maximum upregulation of 1.74x at the 18 h timepoint.

As was discussed above, amyloid aggregates damage cell mitochondria, which in turn, results in the upregulation of mitophagy. Two major players involved in mitophagy are the PTEN-induced putative kinase 1 (*PINK1*) and parkin (*PRKN*) proteins. qPCR revealed that neurons exposed to α -syn fibrils alone experienced strong upregulation of both *PINK1* and *PRKN*. MoS_2 NFs strongly affected the regulatory elements of the mitophagy pathway. At the 9 h, 12 h, and 18 h timepoints, both *PINK1* and *PRKN* were significantly downregulated, indicating the cells decreased need for mitophagy due to the presence of healthier mitochondria. Administration of $MoSe_2$ NF also led to downregulation of mitophagy. At the 18 h timepoint,

PINK1 experienced a 74% reduction. The *PRKN* gene experienced a reduction at the 6 h, 9 h, 12 h, and 18 h timepoints, culminating at an 89% reduction for the 18 h timepoint. Summarizing, both MoS_2 and $MoSe_2$ NF were able to downregulate the UPR and mitophagy responses, while upregulating autophagy and exocytosis pathways in a neuroprotective manner.

Results reported here utilized qPCR analysis reveal important insights into the effects TMD NFs have on cell pathway signaling in the context of treatment in a PD model system. It is important to note that further protein-level investigation utilizing Western blotting and proteomics is vital to understand distinct protein expression in cellular, *C. elegans*, and murine models and are topics of vigorous on-going follow up studies.



Elucidation of neuroprotective effects of TMD NFs in vivo

C. elegans are a well-characterized and established multicellular organism utilized for a multitude of experiments, including drug screenings^{78–80}, understanding mechanisms of neurodegenerative diseases^{81–83}, and fundamental biology^{84,85}. To probe the neuroprotective effects that TMD NFs, *C. elegans* that overexpress the α -syn protein were utilized. The overexpressed

α -syn was tagged with green fluorescent protein allowing amyloid aggregates to form puncta and be tracked using fluorescent microscopy. MoSe₂ or MoSe₂ NFs were supplemented in the nematodes' diet. The quantification of fluorescent puncta can be used to track the rate of α -syn accumulation in nematodes. Additionally, *C. elegans* lifespan was analyzed for further determination of TMD NF neuroprotective properties.

Fig. 4 | TMD NFs alleviation of pathological cell stress. **A** Schematic depicting the generation of ROS by cytotoxic aggregates and depletion of ROS via TMD NFs. Schematic created in BioRender. Mitchell, C. (2026) <https://BioRender.com/yy6862e>. **B** Flow cytometry graphs depicting each cell measured during the experiment. Depicts control N27 neurons (top left) and induced-PD N27 cells receiving α -syn (bottom left). MoS₂ (middle column) and MoSe₂ (right column) NF treated N27 cells at 0.1 mg/mL (top row), 0.5 mg/mL (middle row), and 1.0 mg/mL (bottom row). Histograms of ROS assay conducted on N27 neurons (green), DI astrocytes (gray), and CTX astrocytes (blue) treated with MoS₂ NFs (**C**) and MoSe₂ NFs (**D**). Experimental groups of cells receiving no α -syn or NF treatment (CTR), cells only exposed to α -syn (α -Syn), and cells exposed to α -syn and subsequent NF treatments of 0.1 mg/mL (0.1), 0.5 mg/mL (0.5), or 1.0 mg/mL (1). Two-way ANOVA and Student's *t*-test were utilized to analyze statistical significance of reported results, with * $P < 0.05$, ** $P < 0.005$, *** $P < 0.0005$, and NS not significant. Black significance markings relate to comparisons made to control cells that did not receive α -syn or NF treatments. Red significance markings relate to comparisons made to PD-induced control cells that received α -syn but no TMD NF treatments. ROS experimentation was conducted on $n = 3$ independent biological replicates.

E Immunofluorescent imaging of the stress granule protein TIA-R in healthy [light gray box] N27 neurons (top row), DI astrocytes (middle row), and CTX astrocytes (bottom row) when treated with no NFs (first column), MoS₂ NFs (second column), or MoSe₂ NFs (third column). Images of induced PD [dark gray box] N27 neurons (top row), DI astrocytes (middle row), and CTX astrocytes (bottom row) when treated with no NFs (fourth column), MoS₂ NFs (fifth column), or MoSe₂ NFs (sixth column). Cell images taken at 400X magnification. **F** Histograms of calculated TIR-R stress granule protein puncta as the percentage present in the nucleus per the entire cell for N27 neurons (green), DI astrocytes (gray), and CTX astrocytes (blue). Two-way ANOVA and Student's *t* test were utilized to analyze statistical significance of reported results, with * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and NS – not significant. Black significance markings relate to comparisons made to control cells that did not receive α -syn or NF treatments. Red significance markings relate to comparisons made to PD-induced control cells that received α -syn but no TMD NF treatments. Quantification of stress granules was conducted on at least $n = 5$ independent biological replicates. All histograms represent the mean value of measurements with respective standard deviation.

C. elegans receiving MoS₂ NFs in their diet exhibited a slight increase in life expectancy compared to the control group. The control nematodes had a calculated survival expectancy ($p = 50\%$) of 18.5 days, (Fig. 6). *C. elegans* supplied with 0.1 mg/mL MoS₂ NFs in their diet did not experience a shift in the $p = 50\%$ (18.5 days). While nematodes receiving 0.5 mg/mL supplementation of the same nanostructures had an increase in $p = 50\%$ of one day (19.5 days), the nematodes receiving the highest concentration of MoS₂ NFs (1.0 mg/mL) demonstrated an extension of $p = 50\%$ of two days (20.5 days).

Upon investigation of the quantity of puncta present in each group of worms on Day 5, Day 10, and Day 15, a clear dose-dependent reduction in the accumulation rate of α -syn was clear, (Fig. 6). On Day 5, control nematodes had an average of 20.8 counted puncta (20.8 ± 6.34). As the concentration of MoS₂ increased, there was a reduction of 9.62%, 17.31%, and 36.54% when administered 0.1 mg/mL (18.8 ± 3.96), 0.5 mg/mL (17.2 ± 4.21), and 1.0 mg/mL (13.2 ± 2.95), respectively. On Day 10, the control group had an average of 22.8 puncta present (22.8 ± 4.21). A dose-dependent reduction of 21.05%, 28.95%, and 31.58% was observed for the 0.1 mg/mL (18.0 ± 2.74), 0.5 mg/mL (16.2 ± 2.28), and 1.0 mg/mL (15.6 ± 3.21) groups, respectively. The greatest reductions in puncta quantity were observed on Day 15. The control group had 22.4 ± 4.16 puncta count. When administered 0.1 mg/mL MoS₂, there was a 43.75% reduction in the puncta count (12.6 ± 3.85). Nematodes supplemented with 0.5 mg/mL had a reduction of 52.68% (10.6 ± 2.41) in puncta present. The greatest reduction was observed in nematodes receiving the highest concentration (1.0 mg/mL) of MoS₂ NFs, with puncta count reduced by 58.93% (9.2 ± 3.11). Although exact molecular mechanisms of MoS₂ NF triggered amyloid reduction remains unclear, these results help explain the dose-dependent increase in life expectancy of *C. elegans*.

MoSe₂ NFs exhibited a significant increase in life expectancy when administered to *C. elegans*. Nematodes supplied with 0.1 mg/mL and 0.5 mg/mL both experienced an increase in $p = 50\%$ of three days (21.5 days). This represents a 16.22% increase in life expectancy. The nematodes receiving the highest dosage of MoSe₂ (1.0 mg/mL) experienced the strongest magnitude of extension in life expectancy. This group demonstrated a seven-day extension in life expectancy, with a $p = 50\%$ value of 25.5 days.

Analysis of fluorescent puncta in the nematodes receiving MoSe₂ NF treatments revealed a significant reduction in amyloid burden. On Day 5, a clear dose-dependent reduction in puncta was observed. As the MoSe₂ NF concentrations increased from 0.1 to 1.0 mg/mL, there were reductions of 21.15%, 38.46%, 46.15% calculated (16.4 ± 2.07 , 12.8 ± 3.56 , 11.2 ± 2.77), respectively. At the next timepoint, Day 10, *C. elegans* that received MoSe₂ NFs continued to exhibit a drastic decrease in the number of puncta. There were decreases of 32.46%, 49.12%, and 45.61% as the concentration of NFs increased from 0.1 mg/mL (15.4 ± 4.34) to 0.5 mg/mL (11.6 ± 2.07) and 1.0 mg/mL (12.4 ± 5.03). Day 15 revealed the most drastic reduction of

puncta in nematodes administered MoSe₂ NF treatment. Upon receiving 0.1 mg/mL MoSe₂ NFs, there was a 50.0% reduction in the counted puncta (11.2 ± 3.11). The nematodes treated with 0.5 mg/mL MoSe₂ experienced a 50.89% reduction in aggregates (11.0 ± 2.45). The highest magnitude of reduction was found in *C. elegans* exposed to 1.0 mg/mL MoSe₂ NFs, with a 67.86% decrease in the puncta present at Day 15 (7.2 ± 1.92). These results revealed that MoSe₂ and MoS₂ NFs exert similar therapeutic effects, however, MoSe₂ enacts significantly higher magnitudes of neuroprotective properties in *C. elegans*.

Discussion

The onset and progression of PD is driven by the pathological aggregation of α -syn and propagation of cytotoxic aggregates from cell to cell^{24,86,87}. Upon endocytosis, α -syn aggregates damage mitochondria^{88,89} and ER⁹⁰ which results in increased ROS⁹¹, stress granule formation⁹², and reduced autophagy⁹³, ultimately leading to cell death⁹⁴. Expanding upon this, NF ability to reverse or dampen key pathological processes and alleviate cell stress was investigated.

JC-1 assay revealed that TMD NFs alleviate α -syn-triggered depolarization of cell mitochondrial. The Seahorse XF Mito Tox assay revealed that TMD NFs were able to shift neuronal cells into a glycolytic state. This glycolytic shift is hypothesized to aid the PD cells in overcoming the pathogenic effects of α -syn aggregates. However, this beneficial shift is not able to alleviate the pathogenic stress to the level of the control neuronal cells. Furthermore, ELISA-based analysis of the expression of COX-I and SDH-A proteins indicated that both MoS₂ or MoSe₂ NFs induced mitochondrial biogenesis in neurons and astrocytes to a certain degree. Previously, our group demonstrated that TMD NFs-induced mitochondrial biogenesis was activated via upregulation of the expression of peroxisome proliferator-activated receptor-gamma coactivator (*PGC-1 α*), which in turn, upregulated nuclear respiratory factor (*NRFs*), the key player in mitochondrial biogenesis⁵². It is important to note that upregulation of *PGC-1 α* was linked to the downregulation of steroid receptor coactivator (*SRC-3*) and general control non-depressible 5 (*GCN5*) factors that sense energy excess in the cell. Summarizing, the results of the current study indicate that TMD NFs reduce α -syn-triggered mitochondrial damage simultaneously facilitating mitochondrial biogenesis in neurons and astrocytes. These conclusions are further supported by downregulation of mitophagy in the cells sequentially exposed to α -syn fibrils and TMD NFs, as fewer mitochondria are tagged for recycling.

Although the full molecular mechanism of TMD NFs-suppressed ROS levels in cells is yet to be determined, previously reported findings showed that MoS₂ or MoSe₂ NFs exhibited catalase, peroxidase, and superoxide dismutase activities⁵¹. To show the direct reduction activity of TMD NFs, an in vitro methyl orange reduction assay was conducted (Fig. S1). Direct quantification on the rate of reduction shows a dose-dependent correlation

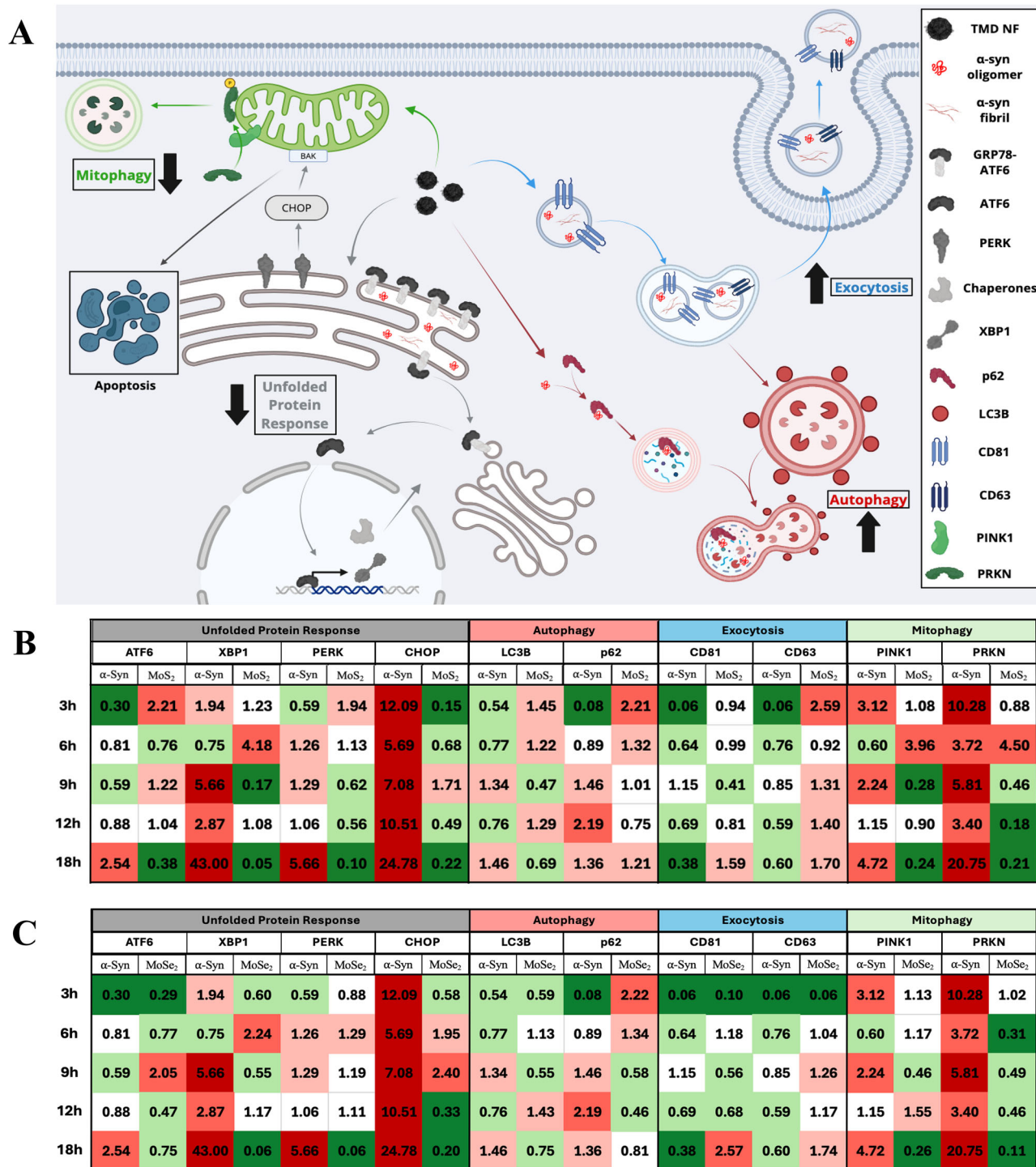


Fig. 5 | Mechanistic Investigation Behind TMD NF Neuroprotective Abilities.

A Schematic of known cellular pathways negatively affected by introduction of aggregated α -syn, with the four major cascades color coded: unfolded protein response (gray), autophagy (red), exocytosis (blue), and mitophagy (green). Schematic created in BioRender. Mitchell, C. (2026) <https://BioRender.com/ii4cy0j>. Heat

map of calculated changes in gene expression via qPCR for N27 neurons treated with MoS₂ NFs (B) or MoSe₂ NFs (C) at 3 h, 6 h, 9 h, 12 h, and 18 h post NF treatment and compared to control cells. The $2^{-\Delta\Delta C_T}$ values are shaded as: 0.01–0.40 (dark green), 0.41–0.80 (light green), 0.81–1.20 (white), 1.21–2.0 (light red), 2.1–5.0 (bright red), 5.0+ (maroon).

for both MoS₂ and MoSe₂ NFs. Therefore, TMD NFs are capable of degrading free radicals and remove hydrogen peroxide from the cells, minimizing detrimental effect of such toxic species on both neurons and astrocytes. However, it is hypothesized that the neuroprotective properties of TMD NFs do not exclusively rely on direct ROS reduction, and that TMD NFs activate other cellular pathways. It has been previously shown that an upregulation of *PGC-1 α* activated the expression of steroid receptor

coactivator-3 (*SIRT-3*) and estrogen-related receptor alpha (*ERR α*) that suppress ROS levels in cells⁹⁵. Currently reported experimental results demonstrate that MoS₂ or MoSe₂ NFs also downregulate the UPR of ER, which further decreased the magnitude of ROS in both neurons and astrocytes. This evidence is further supported by re-localization of stress granule protein to the nuclei in the cells sequentially exposed to α -syn fibrils and TMD NFs. qPCR-based analyses of *LC3B*, *p62*, *CD81*, and *CD63*,

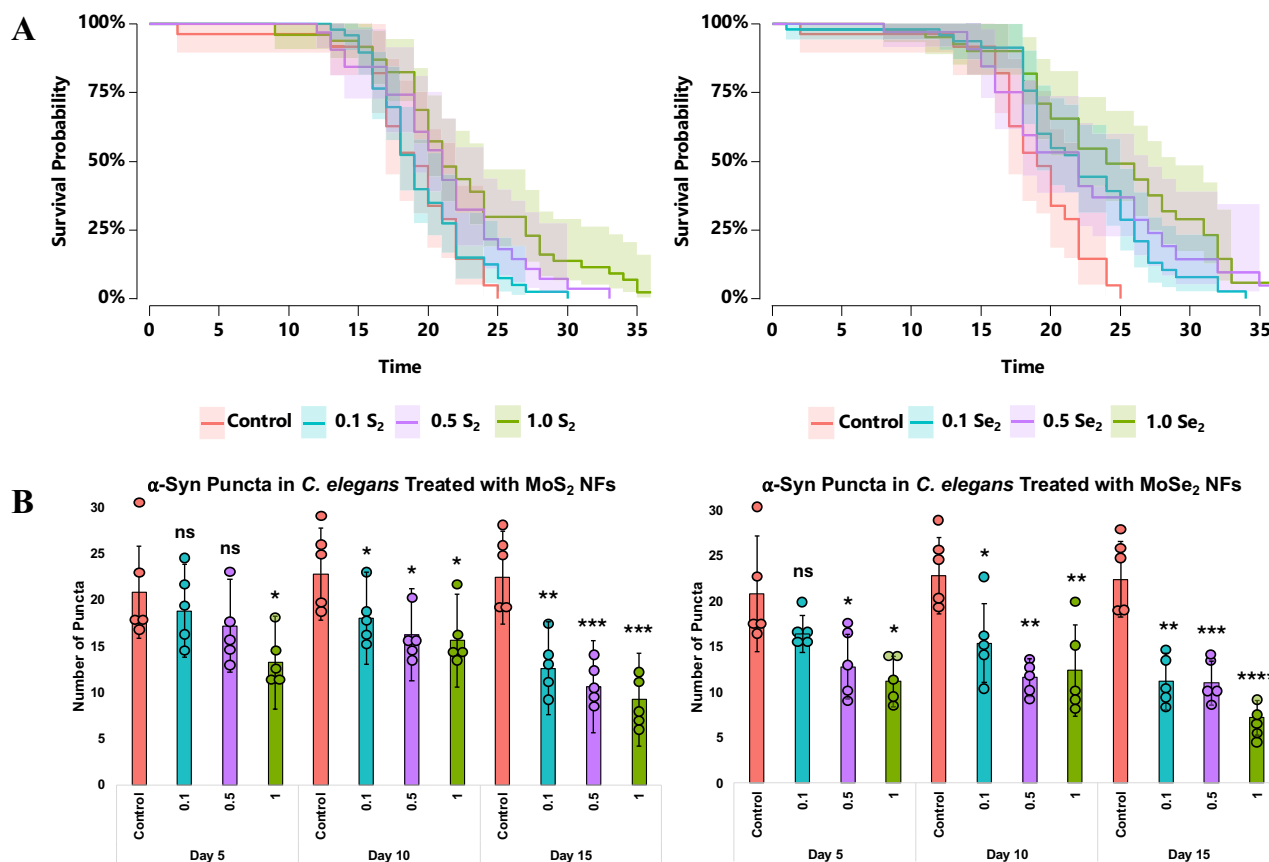


Fig. 6 | TMD NF administration into an in vivo PD model increases lifespan.

A Calculated Kaplan-Meier life expectancy curves of PD *C. elegans* when administered MoS₂ NFs [left] or MoSe₂ NFs [right] at 0.1 mg/mL (blue), 0.5 mg/mL (purple), 1.0 mg/mL (green) or 0 mg/mL (red) concentrations, with respective 95% confidence intervals. **B** Image analyzed fluorescent α-syn aggregates in the pharynx of PD *C. elegans* when treated with MoS₂ NFs [left] or MoSe₂ NFs [right] after 5, 10, or 15 days of treatment. Experimental groups of PD nematodes receiving no NF treatment (control), or administered NF treatments of 0.1 mg/mL (0.1), 0.5 mg/mL

(0.5), or 1.0 mg/mL (1). Log-Rank (Mantel-Haenszel), two-way ANOVA, Kruskal-Wallis test, and Student's *t* test were utilized to analyze statistical significance of reported results, with * *P* < 0.05, ** *P* < 0.005, *** *P* < 0.0005, **** *P* < 0.00005 and NS – not significant. Significance markings coincide with comparisons made to each respective timepoint's control nematodes that did not receive any NF treatment. Quantification of fluorescent puncta was conducted utilizing *N* = 5 independent organismal samples. Histograms represent mean value of measured puncta with respective standard error across specimens.

suggest that a decrease in the cell ROS levels could be linked to TMD NFs-facilitated autophagy and exocytosis of toxic amyloid aggregates by neurons and astrocytes.

Utilization of *C. elegans* as a model living system confirmed observed in vitro neuroprotective properties of TMD NFs. Fluorescence imaging revealed a suppression of puncta formations in the nematodes exposed to both MoS₂ or MoSe₂ NFs. As expected, such amyloid-depleted nematodes exhibited much longer lifespan compared to the control group of *C. elegans*. It was hypothesized that TMD NFs could directly inhibit α-syn overexpressed by *C. elegans* used in the current study. To test this hypothesis, thioflavin T kinetic assay was used. In vitro aggregation of α-syn in the presence of different concentrations of MoS₂ or MoSe₂ NFs did not reveal any inhibition properties of TMD NFs (Fig. S2). These results indicate that autophagy and other molecular mechanisms are responsible for the observed reduction of puncta formations in the nematodes exposed to both MoS₂ or MoSe₂ NFs, rather than direct aggregation inhibition of α-syn by TMD NFs.

It is important to note that both in vivo and in vitro assays used in the current study revealed dissimilar magnitude in the neuroprotective activity of MoS₂ or MoSe₂ NFs present at the same concentrations. Specifically, MoSe₂ NF exhibited a much more potent neuroprotective ability than MoS₂ NFs. Such difference could be linked to the different chemical structures of the nanomaterials, as well as their size and shape. Detailed elucidation of the

relationship between neuroprotection activity, chemical and physical properties of TMD NFs is the subject of a separate study.

This study focuses on the alleviation of PD pathology in cellular culture and simplistic *C. elegans* systems. While the detailed results reveal TMD NFs' ability to alleviate pathological changes, there are limitations that need to be considered. Neither model system employs a blood-brain barrier and allows for free uptake of NFs. Neither model system has mobile, adaptive immune systems that could be affected by uptake of NFs. However, TMD NFs have proven the initial potential of treating pathogenic PD by reduction of ROS, revitalization of damaged mitochondria, ameliorating transcriptional dysregulation of the UPR, autophagy, exocytosis, and mitophagy pathways, alleviation of amyloid burden, and extension of lifespan, as well as provide similar neuroprotective properties in healthy neuronal cells (Fig. S3)⁵², as previously published. The ability of NFs to cross the BBB, as well as their effects on immune cells, are the current subjects of separate, rigorous research.

In summary, experimental findings reported in the current study demonstrate that MoS₂ or MoSe₂ NFs were capable of reducing the cytotoxic effects of α-syn fibrils in both neurons and astrocytes. The mechanism of neuroprotective activity includes suppression of α-syn-induced UPR in the ER, and TMD NFs-facilitated autophagy and exocytosis of α-syn fibrils. In parallel, MoS₂ or MoSe₂ NFs diminished α-syn-induced damage of cell mitochondria while simultaneously stimulating mitochondrial biogenesis via activation of *PGC-1α*. This resulted in a suppression of mitophagy and a

drastic reduction in ROS levels. Utilization of *C. elegans* that overexpressed α -syn confirmed in vitro observed dose-dependent effect of TMD NFs. Such nematodes experienced a drastic reduction in the α -syn aggregation which resulted in a significant increase in *C. elegans* lifespan. Cumulative results suggest TMD NFs are potential neuroprotective therapeutics for PD, with further in-depth studies needed.

Methods

Synthesis of TMD NFs

The synthesis of MoS₂ and MoSe₂ NFs was conducted using protocols previously described by Mitchell et al.⁵². Fabricated TMD NFs were washed with MilliQ water and ethanol, desiccated, and stored as dry powders at room temperature. Scanning electron microscopy (SEM) at the Material Characterization Facility at Texas A&M University was performed to confirm expected morphological parameters of the nanostructures [RRID: SCR_022202]. NFs were prepared in deionized (DI) water and deposited on TEM grids before being allowed to dry for imaging.

For size, polydispersity, and diffusion coefficient measurements, dynamic light scattering (DLS) readings were conducted using Wyatt DynaPro NanoStar in a quartz cuvette. NFs were prepared in DI water at a 1.0 mg/mL concentration and sonicated prior to DLS measurement. The solution of NFs was allowed to sit for one minute before measurements were taken. Measurements were gated in the 100 nm to 1000 nm range, and reported.

To determine zeta potential and other physical properties, readings were conducted utilizing a Malvern Zetasizer Nano ZS. NFs were prepared in DI water at a 1.0 mg/mL concentration and sonicated prior to measurement. 1 mL of solution was placed in a specialized zeta potential cuvette and measured. Use of the Texas A&M University Laboratory for Synthetic-Biologic Interactions (RRID: SCR_022287) is acknowledged and appreciated for zeta potential measurements.

Cell cultures

Rat dopaminergic midbrain N27 neurons were purchased from MilliporeSigma (Burlington, MA, USA: cat. no. SCC048). Neurons were grown in RPMI 1640 cell medium (Thermo Fisher Scientific) supplemented with 10% heat-inactivated fetal bovine serum (Invitrogen). Rat CTX TNA2 and DI TNC1 astrocyte cells were purchased from American Type Culture Collection (Manassas, VA, USA: cat. nos. CRL-2006 and CRL-2005) and cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (Invitrogen). All cell types were cultured in T-75 flasks (Thermo Fisher Scientific) and incubated at 37 °C in a 5% CO₂ environment. All cell types were utilized prior to the 10th passage to ensure reliability and reproducibility of the results.

Expression and purification of α -syn

To express α -syn, the pET21a- α -synuclein plasmid was transformed into BL21 *Escherichia coli* (*E. coli*) strain Rosetta. The transformed bacteria were incubated at 37 °C in Luria broth (LB) media until reaching OD₆₀₀ of 0.8–1.2. Next, cells were exposed to 1 mM of IPTG to induce expression of α -syn. Induced bacteria were incubated for additional 4–5 h under the same experimental conditions. Bacteria were collected by centrifugation at 8000 rpm for 10 min. Bacterial pellets were resuspended in Tris lysis buffer (10 mM EDTA, 150 mM NaCl, 50 mM Tris HCl, pH 7.4) and placed in a water bath pre-heated to 78 °C for 30 min. Next, bacterial suspensions were centrifuged at 16,000 rpm for 40 min to pellet cellular debris that were discarded. Supernatant was collected and treated with streptomycin sulfate (10% solution, 136 μ L/mL) in glacial acetic acid (228 μ L/mL) followed by centrifugation at 16,000 rpm for 20 min to precipitate bacterial proteins, lipids, and nucleic acids. α -Syn present in the supernatant was precipitated by saturated ammonium sulfate ((NH₄)₂SO₄). The pellet was then resuspended and washed with ammonium acetate (100 mM NH₄(CH₃COO)) and absolute ethanol. Harvested α -syn was resuspended in phosphate buffer solution (PBS). Protein samples were concentrated using centrifugation in Amicon Ultra 10 kDa protein filters (Merck Millipore).

Prior to size exclusion chromatography (SEC), protein samples were centrifuged at 14,000 $\times$ g for 30 min to remove aggregates and impurities. For each SEC cycle, 500 μ L of concentrated protein was syringe injected into an AKTA pure FPLC system (GE Healthcare) equipped with a gel-filtration column (Superdex 200 10/300) that was maintained at 4 °C. Proteins were eluted isocratically using PBS, pH 7.4 at a flow rate of 0.5 mL/min. Collected α -syn was reconcentrated in Amicon Ultra 10 kDa protein filters (Merck Millipore) with a final concentration of 400 μ M.

α -syn aggregation

A 500 μ L solution of 400 μ M α -syn was aggregated in conical 1.5 mL microcentrifuge tubes at 37 °C at 510 rpm for 5 days. Fibril formation was confirmed using infrared (IR) spectroscopy and atomic force microscopy (AFM). Mature fibrils were stored at –20 °C until further utilization.

α -syn-induced cytotoxicity

To model cytotoxic effects of α -syn fibrils, α -syn aggregates were added to neurons and astrocytes. For this, neurons and astrocytes were kept overnight at 37 °C in 5% CO₂ to reach ~90% confluency. Fibrils were water sonicated for 10 min prior to administration to cells to induce fragmentation into smaller, more toxic structures. Sonicated α -syn was administered to cells via addition to the media at a final concentration of 40 μ M. Cells were allowed to incubate for 12 h with the aggregates before the addition of TMD NFs.

TMD NFs treatments

After 12 h induction with α -syn, 10% (10 μ L) of the media was removed from each sample. A 2 mg/mL stock of MoS₂ or MoSe₂ NFs was resuspended in PBS. The 2 mg/mL stock was probe sonicated at 70% power for 30 s to completely solubilize the NFs. The 2 mg/mL stock was then serially diluted into the three tested concentrations: 1.0 mg/mL, 0.5 mg/mL, and 0.1 mg/mL. Equivalent volumes of the tested concentrations of NFs were administered to cells (10 μ L). Control cells received equivalent volumes of PBS (10 μ L). The final volume of NFs for each sample is 10% solution consistent across all cell types and prepared concentrations of NFs. For cellular experiments, this results in additions of 1 μ g, 5 μ g, or 10 μ g delivered to each well labeled 0.1 mg/mL, 0.5 mg/mL, or 1.0 mg/mL, respectively. This culminates to final concentrations of 0.01 μ g/ μ L, 0.05 μ g/ μ L, and 0.10 μ g/ μ L, respectively. All labels denoted in this reporting are made in the stock solution concentrations of 0.1 mg/mL, 0.5 mg/mL, or 1.0 mg/mL for ease of identification.

Flow cytometry assays

To analyze ROS levels, cells were stained with CellROX Deep Red Reagent (Thermo Fisher Scientific). To investigate the direct health of mitochondria, cells were stained with MitoProbe JC-1 (Thermo Fisher Scientific). Each experiment was run in triplicate ($n = 3$) to ensure statistical power. The dyed cells were analyzed using an LSRII BD flow cytometer (BD). Data analysis was conducted using LSRII software by first setting an experimental gate around alive cells using the FSC-A/SSC-A graph to remove debris from the analysis based on healthy control cells. Experimental analysis was the conducted setting corresponding gates in the correct fluorescent channel to measure either ROS or JC-1 compared to negative and positive controls.

Seahorse XF Mito Tox assay

Cells' ability to respond to stress was conducted using Agilent Seahorse XF Mito Tox assay. Cells were seeded into specialized Seahorse 96-well cell culture plates at 20,000 cells per well. After adherence overnight, sonicated α -syn fibrils were added to the cells at a final concentration of 40 μ M. After 12 h, 10% solution of 1.0 mg/mL NFs were added to the wells. After 12 h incubation, the Seahorse XF Mito Tox assay was conducted as instructed by Agilent Seahorse XF Cell Mito Stress Test Kit User Manual. Specialized Seahorse DMEM was prepared and applied to each of the wells. To prepare the sensor cartridge, drugs were loaded into each port of each well: 25 μ L of 13.5 μ M oligomycin in Port A, 25 μ L of 20 μ M FCCP in Port B, and 25 μ L of

Table 1 | Sequence of forward and reversed primers used for qPCR

Gene	Forward Primer	Reversed Primer
<i>ATF6</i>	5' ACA AGA CCG AAG ATG TCC ATT GTG 3'	5' GAT CCT GGT GTC CAT GAC CTG A 3'
<i>LC3B</i>	5' TTC TTC CTC CTG GTG AAT GG 3'	5' CTG GGA GGC ATA GAC CAT GT 3'
<i>P62</i>	5' AGA AGT GGA CCC ATC CAC AG 3'	5' AGA AAC CCA TGG ACA GCA TC 3'
<i>CD81</i>	5' GTC CCT CAA GCA GCA ACT CA 3'	5' TCA TCT CGA AGA TCA TAA TGA CAG C 3'
<i>PERK</i>	5' AGG ATA CGT GTC CCG ATA CC 3'	5' TCT CTG GTG GTG TTT CCA GC 3'
<i>XBP1</i>	5' CTG GTT GCT GAA GAG GAG G 3'	5' CAT GGG GAG ATG TTC TGG AG 3'
<i>CD63</i>	5' AGA GCA TCA AGA AGC GTC GG 3'	5' CTG CTA CGC CAA TGG CAA TC 3'
<i>CHOP</i>	5' ACT CTC CAG ATT CCA GTC AG 3'	5' GCC GTT CAT TCT CTT CAG C 3'
<i>PINK1</i>	5' TAT GAA GCC ACC ATG CCC AC 3'	5' CTG CTC CCT TTG AGA CGA CA 3'
<i>PRKN</i>	5' CCA GAG GTC CAG CAG CTA AA 3'	5' CCT TCG CAG GTG ACT TTC CT 3'
<i>GAPDH</i>	5' CAC CCT GTT GCT CTA GCC ATA TTC 3'	5' GAC ATC AAG AAG GTG GTG AAG CAG 3'

1.0 μ M rotenone and antimycin A in Port C. Seahorse XFe96 Analyzer was used to run the experiment. Data was saved and analyzed using Agilent Seahorse Analytics.

Enzyme-linked immunosorbent assay (ELISA)

Changes in mitochondrial biogenesis were analyzed using the MitoBio-genesis In-Cell ELISA Colorimetric Kit (Abcam). First, cells were fixed in the plate using BD Cytofix Fixation Buffer (BD Biosciences). Cells were permeabilized, blocked, and incubated with primary antibodies. Cells were washed and administered secondary antibodies. After washing, cells were treated with AP development solution. The absorbance ($\lambda = 405$ nm) of each well was measured using a Tecan plate reader (Tecan Life Sciences) to quantify expression of succinate dehydrogenase subunit A (SDH-A). The media was removed, and the cells were administered horseradish peroxidase (HRP) development solution. Absorbance ($\lambda = 600$ nm) was measured using a Tecan plate reader (Tecan Life Sciences) to quantify expression of cytochrome c oxidase subunit I (COX-I). Each experimental condition was run in triplicate ($n = 3$) to ensure statistical power.

Quantitative polymerase chain reaction (qPCR)

All primers were designed based on rat gene sequences documented in the NCBI database and developed using the SnapGene custom oligonucleotide PCR primer generation software, Table 1. All primers were fabricated by Integrated DNA Technologies (IDT). RNA was extracted from the harvested cell pellet using a GeneJET RNA Purification Kit (Thermo Scientific). RNA concentration was measured using a NanoDrop One instrument. Next, RNA was converted to complementary DNA (cDNA) using Super-Script II Reverse Transcriptase (Invitrogen) with random primers (Invitrogen). Synthesized cDNA, constructed primers, and Luna Universal qPCR Master Mix (New England Biolabs) were mixed and placed in a QuantStudio 7 Flex Real-Time PCR System (Thermo Scientific). Changes in gene expression levels were calculated using the comparative C_T method ($2^{-\Delta\Delta C_T}$). GAPDH was used as a housekeeping gene to calculate $2^{-\Delta\Delta C_T}$. Each qPCR reaction was run in triplicate ($n = 3$) to ensure statistical power.

Stress granule imaging

After washing with PBS, cells were fixed with 100 μ L BD Cytofix™ Fixation Buffer (BD Biosciences). Next, cells membranes were permeabilized with Triton-X and blocked with bovine serum albumin (BSA) to limit unspecific protein binding. Administration of 100 μ L T-cell intracellular antigen 1-related protein (TIA-R) primary antibodies (Cell Signaling Technologies, Danvers, MA: TIAR (D32D3) XP(R) Rabbit mAb, cat. no. 8509 T, lot 2) was conducted at 1:1000 dilution at 4 °C overnight to ensure complete binding. Treated cells were washed to remove unbound fluorescent antibodies. Cells were treated with 100 μ L goat anti-rabbit Alexa Fluor 488 conjugated secondary antibodies (Invitrogen: Goat Anti-Rabbit IgG (H + L) Alexa

Fluor™ Plus 488, cat. No. A32731TR, lot WK341761) at 1:500 dilution at room temperature for 1 h protected from light. Cells were subsequently washed and covered in PBS. Cells were imaged using an EVOS M5000 microscope (Thermo Fisher Scientific) to track the presence of induced stress granules. Images were analyzed using the ImageJ “Analyze Particles” software to quantify the number of fluorescent puncta in the nucleus and cytoplasm of each cellular condition and reported.

C. elegans assays

Transgenic fluorescently tagged alpha synuclein strain NL5901, with genotype pkIs2386 [unc-54p::alphasynuclein::YFP + unc-119(+)], was purchased from University of Minnesota Caenorhabditis Genetic Center (CGC). *C. elegans* were kept on nematode growth medium (NGM) plates seeded with OP50 *E. coli* and at 20 °C until reaching an egg-producing age. To ensure accurate life span tracking, age synchronization was conducted by collecting all worms and eggs and bleaching the solution to remove all adult worms, according to Sutphin & Kaerberlein's protocol⁹⁶. Three days after synchronization, nematodes were transferred onto experimental NGM plates doped with fluorodeoxyuridine (FUDR) to prevent eggs from hatching. A total of ten nematodes ($N = 10$) were transferred to each experimental plate. The plates were seeded with *E. coli* supplemented with the different tested concentrations of TMD NFs. NF supplementation was conducted by mixing concentrated 2% stocks in PBS with 10x concentrated OP50 *E. coli* at a 1:1 ratio before plating, quickly drying, and UV irradiating for a final concentration of 1% TMD NFs. This results in a final delivered NF value of 0.1 μ g, 0.5 μ g, or 1.0 μ g per plate for the 0.1 mg/mL, 0.5 mg/mL, and 1.0 mg/mL conditions, respectively. All labels denoted in this reporting are made in the stock solution concentrations of 0.1 mg/mL, 0.5 mg/mL, or 1.0 mg/mL for ease of identification. Each experimental group was done in quintuplicate, with five plates each. Daily counts of the number of alive and dead nematodes were conducted to generate a Kaplan-Meier survival curve and calculate statistical analyses. Nematodes were censored when the total number of worms (alive + dead) was less than the number of plated worms. Censors represent missing worms that possibly climbed up the sides of the petri dish and dried out, or tunneled through the agar and were stuck.

Fluorescent Imaging of C. elegans

Fluorescent imaging of endogenous α -syn puncta was conducted via an EVOS M5000 microscope (Thermo Fisher Scientific). Individual nematodes were placed in 5 μ L of 50 mM sodium azide (NaN_3) for immobilization. The nematodes were imaged in the green fluorescent protein (GFP) light channel. Five nematodes ($N = 5$) were imaged for each experimental condition at each given time point. Images were analyzed using ImageJ software to quantify the number of fluorescent puncta present in each worm. The average number of puncta across the five nematodes was recorded. Fluorescent threshold was set between 76 and 255 in ImageJ prior to analyzing puncta using the “Analyze Particles” feature.

Reduction of methyl orange in vitro

To quantify direct ROS catalysis by TMD NFs, an in vitro assay for the reduction of methyl orange was conducted according to Arif (2025)⁹⁷. A solution of 3.22 μM methyl orange (Thermo Fisher Scientific, CAS 547-58-0) was made in MQ Water. A 6 mg/mL solution of sodium borohydride was also generated in MQ Water. TMD NF stock solutions were made in MQ Water. For each NF and concentration, 5 mL of methyl orange solution was placed in a 15 mL Falcon tube. 500 μL of the corresponding NF solution was added. 4 μL of the sodium borohydride solution was added. The solution was mixed and placed in ice with the lid loosely closed to allow for gas escape. The reaction was allowed to continue for 10 min. After 10 min, tubes were centrifuged at 4250 $\times g$ for 5 min to pellet NFs. 2 mL of each solution was placed in a quartz cuvette, and the absorbance was read at $\lambda = 645 \text{ nm}$ utilizing the M4 UV-Vis Spectrophotometer (VWR) and recorded.

Data analysis and statistics

ROS and JC-1 analysis via flow cytometry was calculated relative to negative and positive controls using LSR II software. COX-I and SDH-A quantification via fluorescent ELISA was completed with Tecan plate reader. Calculated values were subsequently analyzed using two-way ANOVA followed by Kruskal-Wallis and Dunn's *post hoc* tests to report significant differences across experimental conditions.

Quantification of the relative expression of key genes was calculated using the established comparative C_T method ($2^{-\Delta\Delta C_T}$). Real-time PCR data was processed in Microsoft Excel for Microsoft 365 (Microsoft Corporation, USA). Average $\Delta\Delta C_T$ values were reported as a heat map across experimental conditions and across time points.

In vivo data was analyzed by Kaplan-Meier survival curve, reporting 95% confidence interval (CI), followed by two-way ANOVA, log-rank (Mantel-Haenszel), Kruskal-Wallis, and Dunn's *post hoc* tests to report significant differences in life expectancy across the experimental conditions. Aggregated $\alpha\text{-syn}$ quantification via fluorescent imaging was analyzed with ImageJ. Calculated values were analyzed using two-way ANOVA followed by Kruskal-Wallis and Dunn's *post hoc* tests.

No data points were excluded from any of the described experiments in this manuscript, and no tests for outliers were conducted. Statistical analyses were conducted in the JASP software (JASP Team, 2026). Detailed statistical analysis available in Supplemental Information, Table S1.

Data availability

All datasets generated and analyzed here within this study are publicly available in Zenodo public repository, with access available at <https://doi.org/10.5281/zenodo.22014807>. Source data for Fig. 1 labeled "Aggregation-Alpha_Synuclein", Fig. 2 labeled "TMD_JC1" and "TMD_ELISA", Fig. 3 labeled "TMD_Seahorse", Fig. 4 labeled "TMD_ROS" and "TMD_Stress Granules", Fig. 5 labeled "TMD_qPCR", and Fig. 6 labeled "TMD_CEle-gans". Supplemental information source data for S1 labeled "TMD_Methyl Orange", S2 labeled "TMD_NF Kinetics" and "TMD_Fluorescence", and S3 labeled "TMD_Health Controls".

Received: 4 November 2025; Accepted: 8 September 2026;

Published online: 26 September 2026

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Acknowledgements

The authors would like to acknowledge the Material Characterization Facility at Texas A&M University [RRID: SCR_022202] and the Texas A&M University Laboratory for Synthetic-Biologic Interactions [RRID: SCR_022287] for experimental help. The authors thank the Texas A&M University academic institutional license [CC BY 4.0] for providing access to BioRender. Figures 2, 4, and 5 were partially generated with BioRender.com (<https://BioRender.com/xrc9n2q>, <https://BioRender.com/yy6862e>, and <https://BioRender.com/ii4cy0j>, respectively).

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Funding

We are grateful to the National Institute of Health for the financial support provided (R35GM142869).

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1038/s42003-026-10992-5>.

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Peer review information *Communications Biology* thanks Lijun Dai, Bo Liang and the other, anonymous, reviewer(s) for their contribution to the peer review of this work. Primary Handling Editors: Dr Ophelia Bu and Dr Ibrahim Javed. A peer review file is available.

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