UNIVERSITÀ DEGLI STUDI DI PADOVA

DIPARTIMENTO DI BIOLOGIA

Corso di Laurea in Biotecnologie

ELABORATO DI LAUREA

Nanobodies against LRRK2: a new tool for Parkinson's research

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ANNO ACCADEMICO 2023/2024

Abstract

Parkinson's disease is a common neurodegenerative disorder characterized by the progressive loss of dopaminergic neurons, which causes motor and non-motor symptoms. Several studies highlighted the key role of leucine-rich repeat kinase 2 (LRRK2), an enzyme involved in various cellular functions. Importantly, mutations in its gene result in abnormal kinase activity, leading to Parkinson's pathogenesis. Therefore, a strategy to modulate its activity is strongly pursued by research, and various inhibitors and interactors have already been discovered. In particular, nanobodies (Nbs) are versatile compounds which bind to LRRK2 generating multiple effects. This project aims to explore the potential of Nbs as a new tool to study the kinase in BV2 microglia cells in distinct treatment conditions. After Nbs transfection, two compounds, MLi-2 and chloroquine, have been used to trigger the relocation of cytoplasmic LRRK2 into different subcellular structures. Through immunocytochemistry and confocal microscopy, co-localization between Nbs and the kinase has been analyzed. As a result, the experiments showed that Nbs could represent a valid strategy to track LRRK2, although some unexpected outcomes occurred, probably due to aspecific interactions or background noise in microscope acquisitions. However, the project highlights the potential of Nbs even for the development of targeted therapies for Parkinson's disease.

Contents

1. Introduction

1. 1 Parkinson's disease

Parkinson's disease (PD) is the second most common neurodegenerative disorder after Alzheimer's disease (AD), affecting roughly 1-2% of the global population over the age of 60 and up to 5% over the age of 85 (Usmani et al., 2021). It is characterized by the progressive loss of dopaminergic neurons in the *Substantia Nigra pars compacta* (SNpc), which leads to the reduction of the amount of dopamine in the midbrain. This neurotransmitter is crucial for movement control: that is why its decrease is responsible for the key clinical features of PD, such as bradykinesia (slowness of movement), resting tremors, postural instability and rigidity. It can also include non-motor symptoms like cognitive dysfunction, mood fluctuations and sleep issues (Iannotta and Greggio, 2021).

As well as other diseases, PD is a synucleinopathy, meaning that neurons often show Lewy bodies, which are cytoplasmatic inclusions mainly consisting of aggregated and misfolded alpha-synuclein, a protein encoded by *SNCA* gene (Russo et al., 2022).

Another important aspect to define PD deals with neuroinflammation, a condition displayed as hyperactivated microglia, which provides immune responses in case of brain injury and is related to the oxidative stress that leads to neurons degeneration (Wallings and Tansey, 2019).

In relation to the aetiology of PD, it is widely acknowledged that it is a complex disorder where genetic and environmental factors or lifestyle can play a role. This is particularly relevant to sporadic (or idiopathic) PD, which represents most of the cases, while around 5-10% belong to familial PD (Iannotta and Greggio, 2021). Currently, several genes involved in the disease have been discovered, including the aforementioned *SNCA* gene, but the one that has been the focus of extensive research since its discovery in 2004 is certainly the *LRRK2* gene on chromosome 12q12 (Park8 locus), whose protein product is leucine-rich repeat kinase 2 (Taymans et al., 2023). Various studies highlighted important point mutations in this enzyme as the most common genetic source of autosomal dominant PD: this is the reason behind the great interest in targeting LRRK2, with the aim of precisely understanding its function and developing therapies.

1. 2 Leucine-rich repeat kinase 2 (LRRK2)

LRRK2 is a large 2527-amino acid and 286 kDa multidomain protein belonging to the ROCO family (Taymans et al., 2023). Its structure consists of a set of domains that have different roles in both the catalytic activity and interactions with other proteins. The GTPase function is carried out by the Ras-Of-Complex (ROC) coupled to the C-terminal of the ROC (COR) domain, which is essential for activation by specifically mediating protein homodimerization and, in association with the serine-threonine kinase domain (KIN), forms the catalytic core. LRRK2 contains four other interaction domains: the leucine-rich repeat domain (LRR), the armadillo repeat domain (ARM), the ankyrin repeat domain (ANK), and the WD40 repeat domain, which can be found in the C-terminus (Iannotta and Greggio, 2021). LRRK2 is expressed in a ubiquitous manner throughout the body, but especially in kidney, lung and brain cells (in particular, neurons, astrocytes and microglia) (Russo et al., 2022). It is ordinarily kept in an inactive state and appears as a J-shaped monomer with the binding of LRR with KIN domain. When dimerization occurs, LRRK2 can cover a range of cell-type specific functions, regarding phagocytosis, inflammatory events, synaptic vesicle trafficking and cytoskeletal dynamics. This is due to the fact that LRRK2, being a kinase, phosphorylates specific substrates, which include a subset of Rab GTPases, small proteins regulating secretory and endocytic pathways as well as motility along cytoskeleton in neurons (Alessi and Pfeffer, 2024). The kinase can also provide autophosphorylation, which occurs at position S1292: this represents an efficient readout of the enzyme activity.

Although the LRRK2 regulatory mechanism has not yet been fully clarified, research discovered that one of the most common pathogenic mutations of the *LRRK2* gene in humans is the G2019S, which results in hyperactivation of the KIN domain through a *gain of function* mechanism. This has been associated with molecular phenotypes of inherited and idiopathic PD, leading to impairment in physiological cell functions. This also results in an increased propensity by kinase to form filaments around microtubules (MTs), interfering with intracellular transport (Iannotta and Greggio, 2021).

Since mutations related to PD enhance kinase's activity, inhibition of this enzyme is actively being pursued as a potential disease-modifying PD treatment.

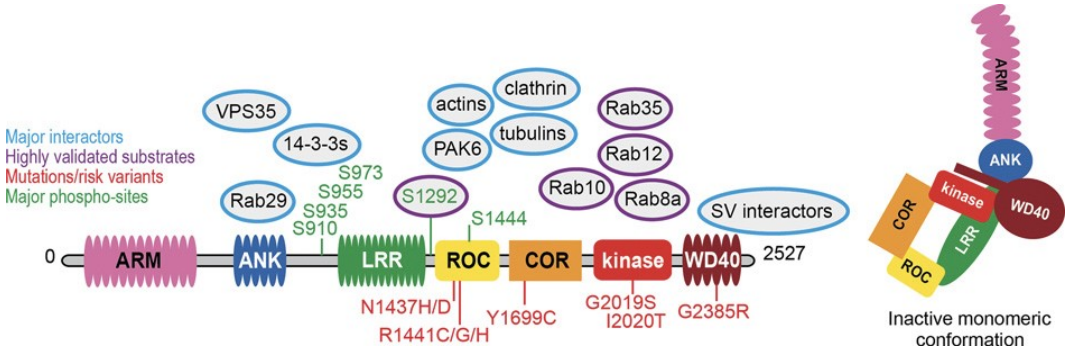


Figure 1.1: Schematic representation of LRRK2 structure with the seven different protein domains. Pathogenic and risk variants such as G2019S are written in red, phosphorylation sites like S1292 are labeled in green, principal interactors are circled in cyan and highly validated substrates in purple (left). J-shaped monomeric conformation of LRRK2 (right).

1. 3 LRRK2 inhibitors

There are two categories of LRRK2 inhibitors.

Type I inhibitors, such as MLi-2, are able to trigger the kinase relocation to MTs by blocking the ATP-binding pocket of the KIN domain in a closed conformation. In this way, a disease-associated phenotype is mimed, which is very interesting and useful for carrying out studies about the enzyme.

Instead, type II inhibitors, for example GZD-824, act by keeping LRRK2 in an open form. As they occupy a site immediately adjacent to the point where ATP binds, they prevent the protein from this association (Alessi and Pfeffer, 2024).

Inhibitors belonging to the first class account for most of the compounds employed to study LRRK2, while those of the second category are poorly exploited. Considering that toxic side effects due to the implementation of only one type of inhibitor have been demonstrated, a promising strategy to avoid numerous problems is precisely promoting the application of a broader range of compounds to target LRRK2 functions in an alternative way (Singh et al., 2022).

1. 4 Nanobodies

ATP-competitive kinase inhibitors are not the only successful approach developed to specifically bind a certain conformation of LRRK2 for drug discovery: a new tool is represented by nanobodies (Nbs), of which a wide variety has been identified and characterized. They are small and stable single-domain fragments derived from

camelid heavy chain-only antibodies and have the peculiar capacity of recognizing hidden spots that conventional antibodies cannot reach (Soliman et al., 2020). As their site on the enzyme is topographically separated from the ATP-binding pocket, they bind to LRRK2 through a different mechanism from type I and II inhibitors.
Nbs are very versatile and there are many different kinds, which can generate multiple effects on the functionality of the kinase, by modifying its activity or not: in this way, the potential for their application in research is improved.
In particular, some Nbs can increase LRRK2 autophosphorylation, as well as the amount of LRRK2-mediated Rab10 phosphorylation. Instead, other Nbs, such as Nb28, are neutral, leaving KIN activity unaffected, apart from the generation of phosphorylated LRRK2 (Singh et al., 2022).

2. Aims of the project

This project aims to investigate Nbs as a new tool to study LRRK2 in BV2 microglia cells: since they specifically bind to the kinase, what proves to be interesting to verify is whether both the enzyme and the binding Nb colocalize. As LRRK2 is normally diffused in the cytosol, two compounds have been used to modulate its subcellular location and visualize the co-localization with Nbs tagged with GFP:

1) MLi-2, a type I inhibitor which triggers LRRK2 association to MTs;
2) Chloroquine (CQ), autophagy inhibitor that induces LRRK2 association to lysosomes.

To pursue this goal and visualize the effects of the treatments, we performed immunocytochemistry followed by image acquisition, expecting to observe evidence of diffused cytoplasmic LRRK2 relocation respectively into filaments (MTs) or small puncta (lysosomes).
Moreover, co-localization between LRRK2 and Nbs has been examined.

3. Materials and Methods

3. 1 Cell lines

3. 1. 1 BV2 microglia cells

The immortalized BV2 microglial cell line is derived from C57/BL6 murine neonatal microglia, which represents brain's innate immune system, and it is commonly exploited in neurodegeneration studies as an alternative to primary microglia (Henn, 2009). This type of cells is of great interest in this project due to its ability of naturally express LRRK2, so there is no kinase transfection needed, as opposed to HEK cells.

Cells have been thawed and seeded in a T75 flask, in a culture medium composed of DMEM (Dulbecco's Modified Eagle Medium) high glucose, FBS (fetal bovine serum) 10% and 1% Penicillin/Streptomycin. Cells were passaged when 80% confluency was reached. Cells were detached from the flask with Trypsin, counted with a Luna II automated cell counter, and seeded in a 24-well plate at a density of $2*10^5$ cells per well. Finally, cells were allowed to seed overnight and treated the following day.

3. 1. 2 HEK293T cells

The cells belonging to the immortalized HEK293T line, which stands for Human Embryonic Kidney, are isolated from the kidney of a human embryo. They are among the most widely used cells in research because of their elevated growth rate. Unlike BV2 cells, they do not express endogenous LRRK2, but the DNA of the kinase can be easily delivered to cells, thanks to their good propensity to transfection.

In the first experiment of the project, this type of cells acts as a positive control to verify the procedure applied to BV2 cells.

3. 2 Transfection methods

3. 2. 1 HEK cells: Lipofectamine 2000 method

Transfection in HEK cells was performed with Lipofectamine 2000 (Thermo Fisher Scientific). It is based on a reagent composed of cationic lipid subunits which can

interact with each other forming liposomes in an aqueous environment. Liposomes are a kind of vesicles that have the ability to incorporate compounds such as DNA and transfer them to cells without damage by fusing with the plasma membrane.
In the first experiment, both LRRK2-encoding DNA and Nbs-encoding DNA were delivered to HEK cells through the same transfection mix.

3. 2. 2 BV2 cells: Magnetofection Technology

As microglial cells are reluctant to classic transfection methods, a specific protocol for the BV2 cell line was used: Glial-Mag™ (OZ Biosciences). It consists of a formulation of magnetic nanoparticles that ensures high transfection efficiency for this type of cells.
The culture was plated the day before the application of this method, as recommended by the protocol. The transfection mix was prepared combining the DNA solution (Nbs-encoding DNA or peGFP DNA) with the Glial-Mag magnetic nanoparticles reagent, in order to form complexes. The mixture was spread dropwise onto the cells in a uniform distribution and then Glial-Boost was added to enhance transfection efficiency. After a 30 minutes incubation on the kit-specific magnetic plate, the cell culture was placed under standard conditions (37°C in a CO_2 incubator) for 2 hours. At the end of this procedure, a medium change was performed.

3. 3 Cell treatments

For each condition analyzed in both experiments, half of the transfected cells has been treated with the compound under investigation, while the other half has been provided with vehicle control. The vehicle is the solvent in which the substance delivered to the cells is dissolved, therefore it consists in the negative control as the compound of interest is not present.
In the first experiment, a treatment with the type I LRRK2 inhibitor MLi-2 was performed at a concentration of 200 nM for 90 minutes. As previously reported, MLi-2 induces the kinase relocation to MTs in both HEK and BV2 cells.

In the second experiment, chloroquine was used to trigger LRRK2 relocation to lysosomes in BV2 cells. Treatment was performed with 50 uM of chloroquine for 3 hours.

After treatment, cells were immediately fixed with 4% paraformaldehyde (PFA) under a chemical hood for 20 minutes, followed by 3 washes with PBS.

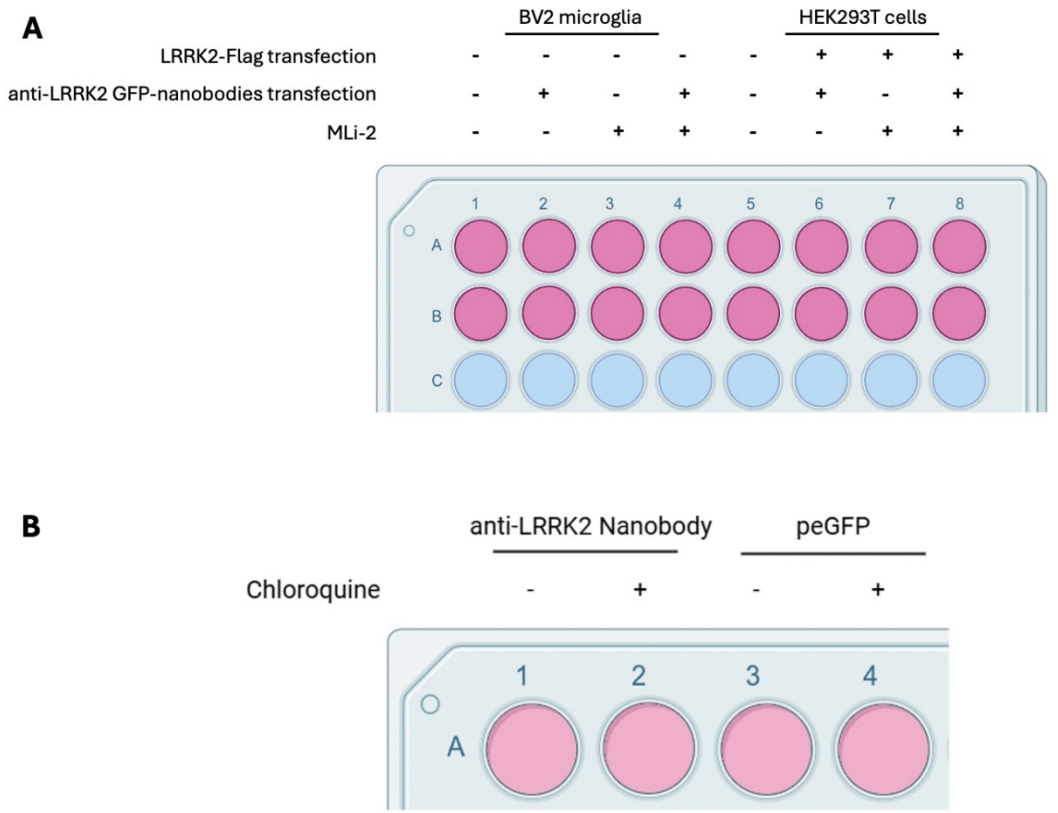


Figure 3.1: Schematic representation of the experimental conditions. **(A)** In the first experiment both BV2 and HEK cells have been transfected with GFP-tagged Nbs, while only HEK line has received LRRK2-Flag. Treatment with the type I LRRK2 inhibitor MLi-2 has been carried out in half of the wells. In this way, all the possible conditions could be explored: Nbs^{-}/MLi-2^{-}, Nbs^{+}/MLi-2^{-}, Nbs^{-}/MLi-2^{+}, Nbs^{+}/MLi-2^{+}. **(B)** The second experiment involved only BV2 cells: Nbs have been introduced in half of them, while peGFP has been delivered in the other half. Chloroquine has been used to treat half of the cells in both conditions.

3. 4 Immunocytochemistry

Immunocytochemistry was used to assess the co-localization of LRRK2 with Nbs in HEK cells, as well as the relocation of LRRK2 and the Nbs in response to treatments. It is based on the utilization of a primary antibody directed to the protein of interest, followed by a fluorophore-conjugated secondary antibody that binds the primary.

3. 4. 1 Permeabilization and blocking

The first step of this method was represented by cell permeabilization: it involved creating holes in the plasma membrane to facilitate the entrance of the antibodies.
Cells were permeabilized with 300 uL of 0.1% Triton X-100 per well for 20 minutes.
Then, cells were incubated with blocking buffer, composed of FBS 5% in PBS. Every well received 360-400 uL of the solution and was left for 1 hour at room temperature. This aimed at reducing background noise, because aspecific sites inside the cells were bound by the proteins contained in the buffer instead of the antibodies that had to be employed: in this way, only specific sites were kept free for antibodies.

3. 4. 2 Primary antibody

A rabbit antibody (abcam ab6046) has been utilized at 1:300 dilution in all the cells to target tubulin, which is the main component of MTs, while a mouse antibody (Sigma F1804) has been employed only against LRRK2-Flag in both HEK and BV2 transfected cells at 1:200 dilution, as endogenous LRRK2 produced by microglial cells does not bear a Flag reachable by antibodies.
The cells were incubated with primary antibodies overnight in a humified chamber at 4°C.

3. 4. 3 Secondary antibody

Cells were rinsed 3 times with 100 uL PBS to remove the unbound primary antibody. The compounds employed in this passage are specie-specific, according to the antibodies which have been utilized formerly: anti-rabbit-alexafluor 568

antibodies have been delivered in all the cells to primary tubulin-targeted ones, while anti-mouse-alexafluor 405 have bound to anti-LRRK2-Flag antibodies.
In this step, a 30 uL drop containing antibodies dissolved in blocking buffer was placed in contact with every chamber slide for 1 hour at room temperature. The excess residues have been removed by 3 washings with PBS.

3. 4. 4 DAPI nuclear staining

Nuclear staining has been performed using DAPI, which stands for 4'6-diamidino-2-phenylindole. It specifically labels DNA by strongly binding to adenine-thymine clusters: in this way, it forms fluorescent complexes which can be visualized as blue spots with microscope (Chazotte, 2011). After incubation and washed, cells were incubated with 1:10000 DAPI in PBS for 5 minutes at room temperature, followed by three washes with PBS. Finally, coverslips were mounted using mowiol as mounting agent.

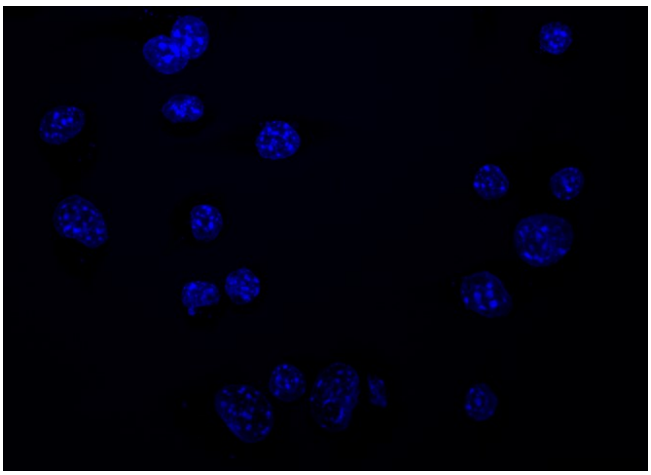


Figure 3.2: Microscope image of BV cells nuclei stained with DAPI.

3. 5 Image Acquisition

Images were acquired with a Leica SP5 confocal microscope.
The output for each acquisition is split into three channels, which can be distinguished by colour:

- Red is for tubulin (MTs).
- Blue is for the non-endogenous LRRK2-Flag in MLi-2 experiment and for nuclei of BV2 cells in the chloroquine experiment. It is important to note that in this case anti-Flag antibodies have been utilized, so of course only

LRRK2 bearing the Flag could be visualized, while endogenous kinase in BV2 cells remained unhighlighted.

- Green is for GFP-tagged Nbs and, in the second experiment, for GFP encoded by the specific plasmid.

3. 6 Data Analysis

All the images have been analyzed through ImageJ Fiji program. Two functions of this software have been exploited for the diverse purposes of both experiments:

1) JACoP (Just Another Colocalisation Plugin) provides Pearson's coefficient, which is a number between -1 and +1 representing co-localization. It has been used for an estimate of LRRK2 and anti-LRRK2 Nbs co-localization in HEK cells and for calculating anti-LRRK2 Nbs and MTs co-localization in treated HEK and BV2 cells.
2) Cell Counter is a tool that helps with the counting of distinct types of cells. In particular, in the second experiment it has been useful for quantifying how many BV2 cells showed diffused cytoplasmic distribution of GFP and in how many the relocation of anti-LRRK2 GFP-tagged Nbs into small puncta (lysosomes) has been enhanced.

4. Results and Discussion

4. 1 LRRK2, Nbs and tubulin co-localization in response to MLi-2 treatment

In the MLi-2 experiment, we aimed to examine two crucial aspects:

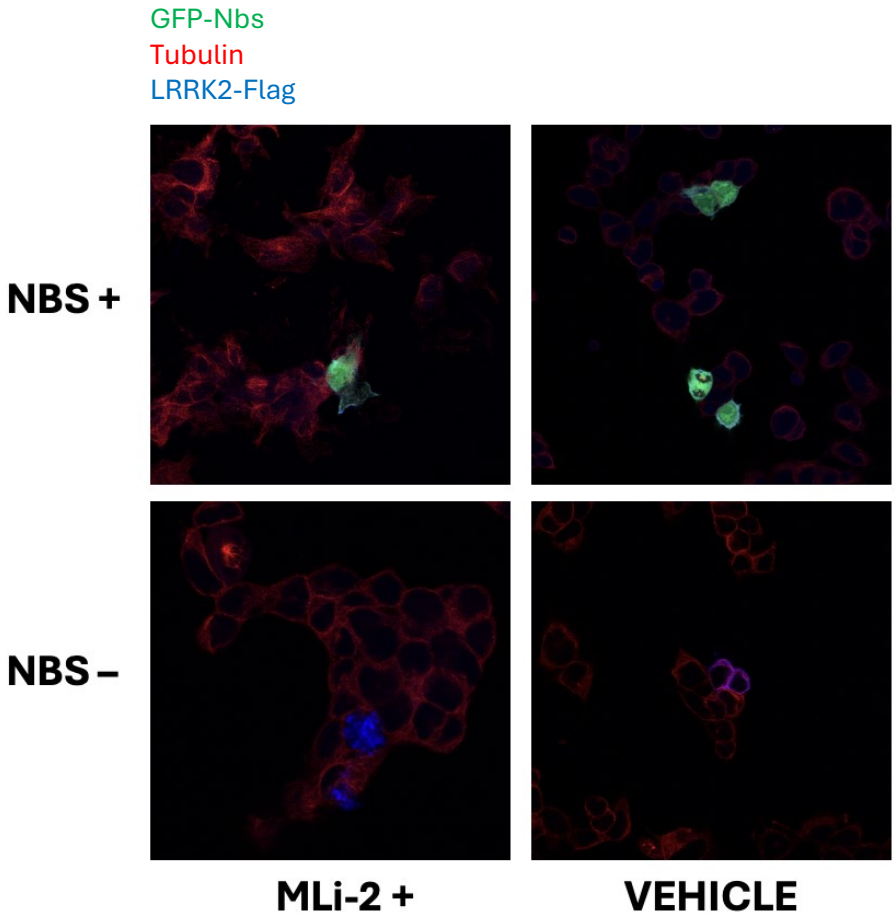
1) Co-localization between LRRK2-Flag (blue) and Nbs (green) in HEK cells;
2) Co-localization between Nbs (green) and MTs (red) in HEK and BV2 treated cells.

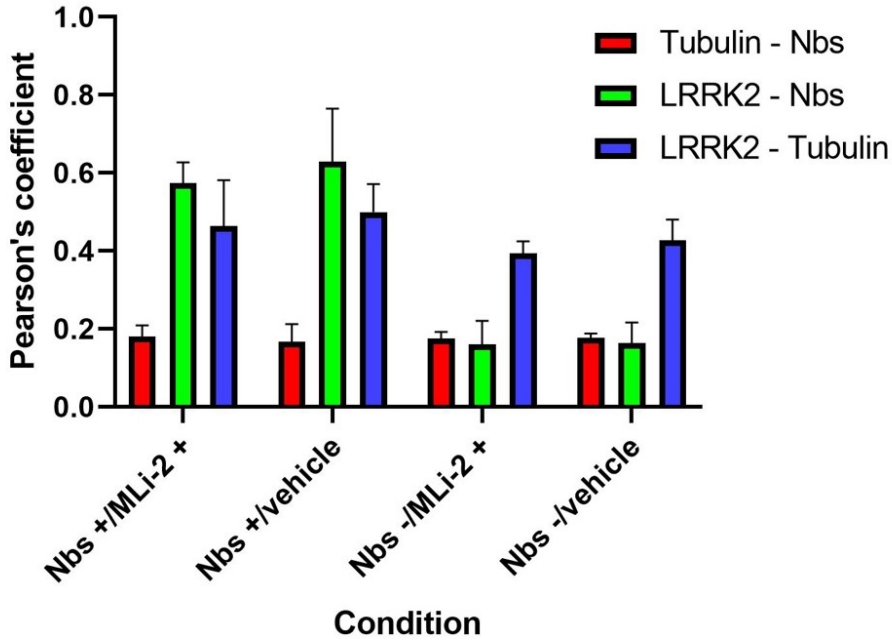
As for the transfection procedure, it is important to note that LRRK2-Flag was delivered only to HEK cells, as they do not express the kinase naturally. However, among the four HEK wells corresponding to the four conditions studied, one well remained without LRRK2-Flag as background control.

The treatment with MLi-2 involved two wells of each type of cells: one with Nbs and one without them. As for HEK cells, this concerned only those that were transfected even with LRRK2-Flag: in this way, one well per cell type could act as a negative control (Nbs^{-}/$MLi\text{-}2^{-}$).

Immunocytochemistry was performed using antibodies with the specific purpose of highlighting MTs in all cells and the LRRK2-Flag in HEK cell line. After image acquisition, data were analyzed through JAcoP tool by ImageJ Fiji in order to measure co-localization with Pearson's coefficient. Results are presented in the following graphs, which partially confirm our expectations.

4. 1. 1 HEK293T cells images and graph



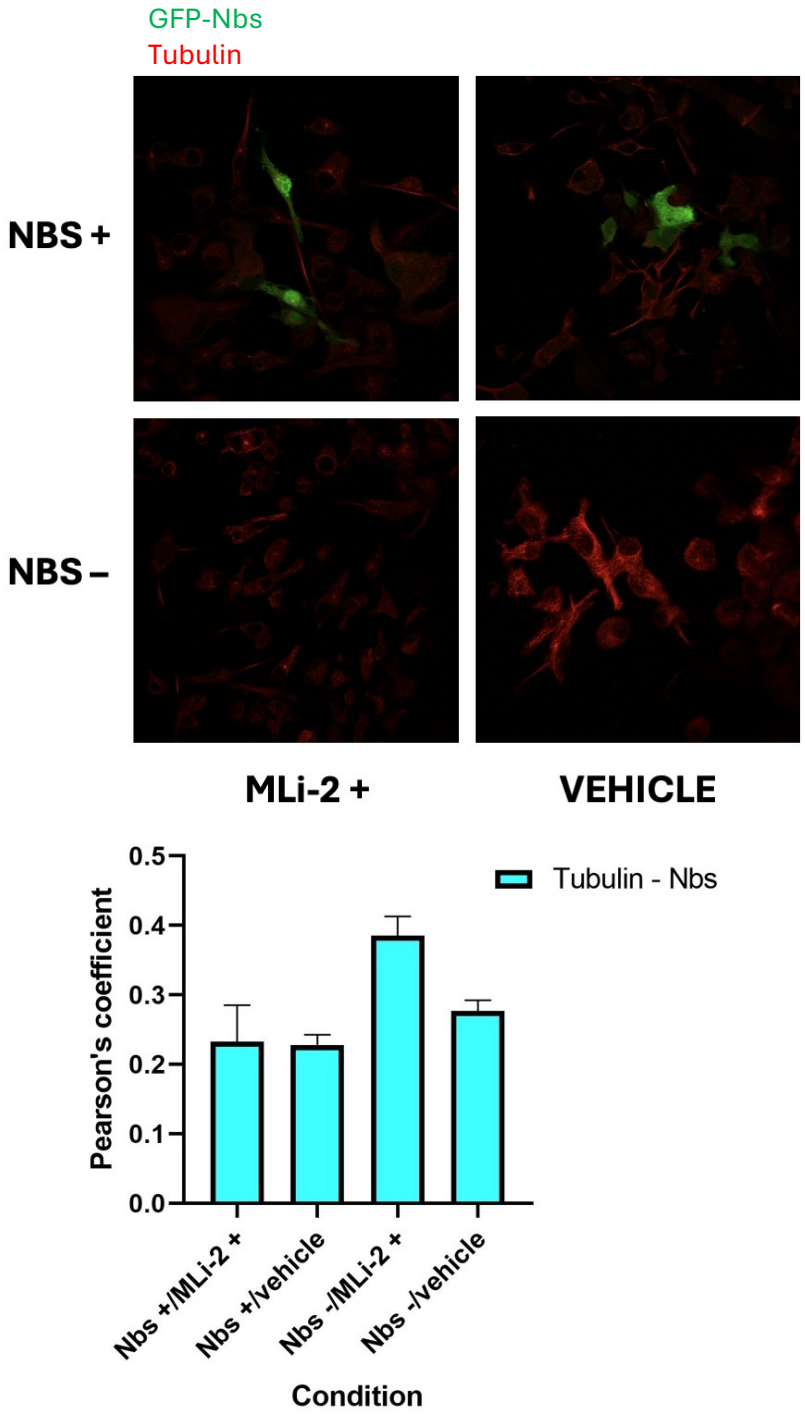


The information concerning HEK cells is divided into 3 data groups, highlighted with different colours in the legend. In each of them co-localization between different elements is investigated.

Good co-localization between LRRK2-Flag and Nbs in Nbs^+ conditions probably demonstrates that they bound in a correct way and suggests that the transfection procedure has been successful. Actually, GFP signal is present even when Nbs were not employed (in Nbs^- conditions): this could be due to the interfering background noise that we partially tried to subtract through a specific function within the software.

Moreover, we expected to observe both Nbs and LRRK2 association to MTs (tubulin) only in MLi-2 treated cells. Instead, relatively high Pearson's coefficient values can be noticed across all conditions for the co-localization of LRRK2 and tubulin, including those without treatment, while the tubulin - Nbs data group shows low values even when treatment was performed. Therefore, this graph shows that Nbs did not fully reflect the responses of LRRK2 to MLi-2 treatment.

4. 1. 2 BV2 microglia cells images and graph



As microglial cells already produce LRRK2, we introduced only Nbs in this type of cells. For this reason, only one data group is reported, which refers to co-localization between Nbs and tubulin.

Resulting values are quite unexpected: in fact, in Nbs^{-} the co-localization should have been near to 0, as Nbs were not present. Instead, the bars corresponding to these conditions represent an even higher measure than the others. As well as the

situation observed in HEK cells, this result could suggest the presence of aspecific signal, probably due to background noise that did not let us measure the outputs of the images in an effective way.

Furthermore, MLi-2 treatment triggers the association of LRRK2 (and therefore Nbs) with MTs; thus, Pearson's coefficient values in treated cells should have been much higher than in vehicle control. Actually, we did not examine LRRK2 directly, as the endogenous kinase does not bear the Flag. Therefore, we could hypothesize that maybe Nbs did not bind properly to LRRK2, or that treatment did not yield to the expected effects.

4. 2 LRRK2 relocation into lysosomes in response to CQ treatment

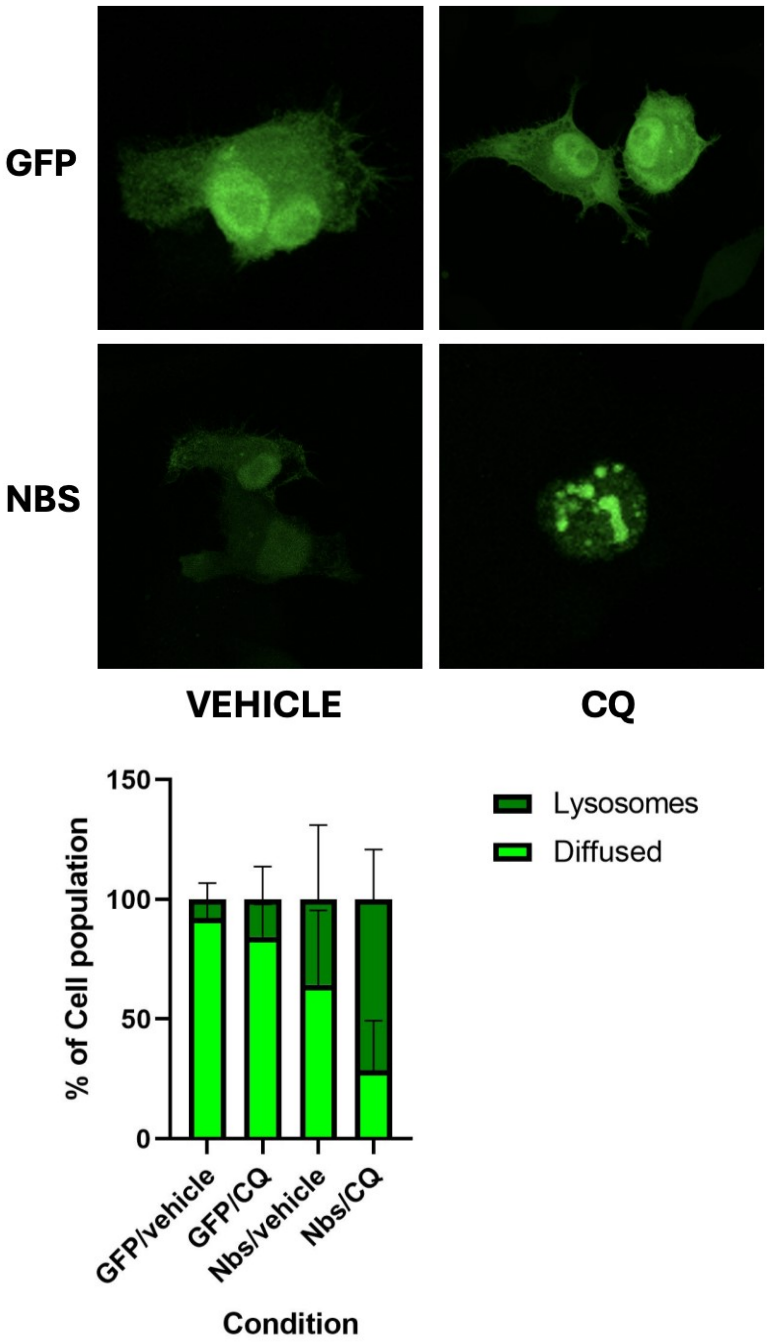
In the experiment involving chloroquine, we expected to see different cell states depending on the conditions of each well. In particular, in BV2 cells that were transfected with anti-LRRK2 Nbs, the purpose was to verify whether we could observe Nbs relocating to small puncta, represented by lysosomes, when treatment occurred. In untreated cells, instead, we predicted to find a diffused cytoplasmic GFP signal.

Nbs have been introduced into half of the BV2 cells, while the other half acted as a negative control by receiving peGFP (the plasmid encoding Green Fluorescent Protein). Even peGFP-transfected BV2 cells, which did not have Nbs, should have exhibited a uniform GFP localization regardless of treatment with chloroquine.

Furthermore, although the BV2 cell line already expresses LRRK2, an attempt has been made to deliver the LRRK2-Flag encoding DNA with the purpose of visualising it through immunocytochemistry. Although inserting LRRK2-Flag into microglial cell line would have been useful for tracking the kinase in those cells through specific antibodies, the procedure has not been successful.

Instead, DAPI nuclear staining was performed. In this way, within all the cells involved in this experiment the nuclei have been marked. This passage was useful to identify and counting microglial cells, as they often present an irregular shape.

As for immunocytochemistry, GFP-tagged Nbs and free GFP encoded by peGFP did not need additional antibodies to be observed, as the protein naturally emits a green fluorescence when excited with a wavelength of 395 nm.



The graph shows the percentage of transfected cell population that exhibited a diffused cytoplasmatic localization (light green) or a relocation into lysosomes (dark green) in the four studied conditions.

The numbers confirm our expectations pretty well. In fact, in peGFP-transfected cells a major diffused GFP signal is present, even with chloroquine treatment.

The same result can be noticed in the vehicle control condition, although the percentage value is a little lower.
As predicted, chloroquine treatment in Nbs-transfected cells induces relocation into small puncta for the majority of the cells.
The variability shown by these results could suggest that the transfection procedure or chloroquine treatment probably had different effects on the cells.

5. Conclusions

In conclusion, this project showed interesting aspects of LRRK2 interactions with Nbs in different cell lines and conditions, demonstrating that they could represent a valid tool for LRRK2 research. However, as we came across various challenges, some aspects should be taken into consideration.
Among the two experiments that have been carried out, only the second one produced results that were in line with expectations, while the other showed mostly unpredicted values. As reported before, the central issue was probably represented by background noise in images: in fact, the JAcoP tool seemed not to distinguish it from the real signal, considering both equally relevant.
In future studies, aspecific interactions between proteins could be prevented by optimizing protocols and experimental conditions. Also, an extra experiment could be performed using free GFP instead of GFP-tagged Nbs, with the purpose of comparing co-localization results. Otherwise, another software for image analysis could be tried for better estimates of measures.
Moreover, various treatment compounds could be employed, as well as more specific antibodies for immunocytochemistry.
As a future perspective, it would be interesting to explore all the possibilities that Nbs could offer: in fact, they exist in numerous kinds and could be used to carry out similar experiments. Nbs could even represent a useful tool to find a smart way to control LRRK2 activity. In this way, strategies to ease scientific research could be developed, and, hopefully, even potential therapies for the treatment of Parkinson’s disease.

Bibliography

Alessi, D.R., Pfeffer, S.R., 2024. Leucine-Rich Repeat Kinases. Annual Review of Biochemistry 93, 261–287. https://doi.org/10.1146/annurev-biochem-030122-051144

Chazotte, B., 2011. Labeling Nuclear DNA Using DAPI. Cold Spring Harb Protoc 2011, pdb.prot5556. https://doi.org/10.1101/pdb.prot5556

Henn, A., 2009. The suitability of BV2 cells as alternative model system for primary microglia cultures or for animal experiments examining brain inflammation. ALTEX 83–94. https://doi.org/10.14573/altex.2009.2.83

Iannotta, L., Greggio, E., 2021. LRRK2 signaling in neurodegeneration: two decades of progress. Essays in Biochemistry 65, 859–872. https://doi.org/10.1042/EBC20210013

Russo, I., Bubacco, L., Greggio, E., 2022. LRRK2 as a target for modulating immune system responses. Neurobiology of Disease 169, 105724. https://doi.org/10.1016/j.nbd.2022.105724

Singh, R.K., Soliman, A., Guaitoli, G., Störmer, E., Von Zweydorf, F., Dal Maso, T., Oun, A., Van Rillaer, L., Schmidt, S.H., Chatterjee, D., David, J.A., Pardon, E., Schwartz, T.U., Knapp, S., Kennedy, E.J., Steyaert, J., Herberg, F.W., Kortholt, A., Gloeckner, C.J., Versées, W., 2022. Nanobodies as allosteric modulators of Parkinson's disease–associated LRRK2. Proc. Natl. Acad. Sci. U.S.A. 119, e2112712119. https://doi.org/10.1073/pnas.2112712119

Soliman, A., Cankara, F.N., Kortholt, A., 2020. Allosteric inhibition of LRRK2, where are we now. Biochemical Society Transactions 48, 2185–2194. https://doi.org/10.1042/BST20200424

Taymans, J.-M., Fell, M., Greenamyre, T., Hirst, W.D., Mamais, A., Padmanabhan, S., Peter, I., Rideout, H., Thaler, A., 2023. Perspective on the current state of the LRRK2 field. npj Parkinsons Dis. 9, 104. https://doi.org/10.1038/s41531-023-00544-7

Usmani, A., Shavarebi, F., Hiniker, A., 2021. The Cell Biology of LRRK2 in Parkinson's Disease. Molecular and Cellular Biology 41, e00660-20. https://doi.org/10.1128/MCB.00660-20

Wallings, R.L., Tansey, M.G., 2019. LRRK2 regulation of immune-pathways and inflammatory disease. Biochemical Society Transactions 47, 1581–1595. https://doi.org/10.1042/BST20180463