

1. BIOGRAPHICAL SKETCH

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NAME: **Nicoletta Plotegher**

ORCID: <https://orcid.org/0000-0001-7421-8705>

POSITION TITLE: **Assistant Professor**

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
University of Trento, Italy	BSc/MSc	2004-2009	Biophysics
University of Padova, Italy	Ph.D.	2010-2013	Biosciences and Biotechnology
University of California, Irvine, USA	Junior Specialist	2012	Biophysics
University of Pavia and University of Padova, Italy	Post-doctoral Fellow	2013-2015	Biochemistry
University College London, UK	Marie Curie Post-doctoral Fellow	2015-2018	Cell Physiology and pathophysiology
University of Padova, Italy	Veronesi Post-doctoral Fellow	2018-2019	Cell Physiology and pathophysiology
University of Padova, Italy	Senior Post-doctoral Fellow	2019-2022	Cell Physiology and pathophysiology

A. Personal Statement

My main research interests are centered on the molecular mechanisms that govern neuronal homeostasis and the pathways that are deregulated in neurodegeneration. Since interdisciplinarity is our best chance to unravel the complexity of brain physiology, I use different biophysical, bioimaging and biochemical methods, applied to in vitro systems, cells and animal models.

My PhD research (Dept. of Biology, Univ. of Padova) was mainly focused on the characterization of the aggregation of alpha-synuclein, a small protein involved in synaptic vesicles function, and its contribution to neurons death in Parkinson's disease (PD). To study alpha-synuclein oligomerization in live cells and correlate this process with cellular damage, I took advantage of the advanced fluorescence microscopy techniques I learned as Visiting Scientist at the University of California (USA) in the lab of Prof. Enrico Gratton (funded through the Aldo Gini Fellowship to spend up to 1-year abroad during the PhD). This approach allowed to characterize alpha-synuclein oligomerization in live cells using fluorescence correlation spectroscopy and fluorescence lifetime imaging. I also studied the possibility of using chaperone proteins to interfere with the formation of toxic oligomers, with a special focus on 14-3-3 proteins. All these studies were integrated in my PhD thesis which received the "Angelo Marai award" for the best PhD thesis on neurodegenerative diseases in Italy in 2014. During my first postdoc, I became interested in small molecules able to promote alpha-synuclein aggregation. I found that DOPAL, a highly reactive dopamine catabolite, is able to covalently modify alpha-synuclein, causing alterations in the synaptic vesicles pool and inducing the formation of oligomers able to damage lipid membranes. Building upon this robust expertise on alpha-synuclein, I am continuing to investigate the patho-physiological consequences of alpha-synuclein aggregation in brain-relevant cell types.

The growing interest for pathways able to influence alpha-synuclein aggregation brought me to a fascinating player: the lysosome. Lysosomal defects are a key feature of neurodegenerative diseases, suggesting that lysosomal function is important not only to prevent alpha-synuclein aggregation process, but also for neuronal health. Moved by the enthusiasm of better understanding the consequence of defective lysosomes on neuronal physiology, I applied for funding to join Prof. Duchen's lab at University College London (UK), one of the world-leading laboratories in cell bioenergetics and calcium signaling studied using advanced and functional live imaging. This was possible thanks to a Marie Skłodowska-Curie Individual Fellowship I obtained in 2015 to study the pathophysiology of neurons from a murine model in which the lysosomal enzyme GBA was depleted and

alpha-synuclein was aggregated. I found that these deficiencies were associated with a reduced capacity of neurons to handle calcium influx induced by physiological glutamate stimulation and increased energy demands during neuronal activity, which led to increased neuronal damage. Interestingly, the interplay among lysosomal impairment, alpha-synuclein accumulation and altered calcium signaling is key in many different neurodegenerative diseases. Following our new findings, the Editor of Trends in Neurosciences invited us to submit an Opinion paper about the excitotoxicity damage induced by physiological glutamate to neurons when mitochondrial bioenergetics is compromised.

In 2018 I obtained a postdoctoral fellowship from Fondazione Veronesi to move to Italy and dissect the interplay between GBA function and the PD-kinase LRRK2. The project was based on the previous observation we made about the depletion of GBA upon knock out of LRRK2 in mice brains. To understand the relationship between GBA and LRRK2 I put together a network of people working on these enzymes and we found a positive correlation between GBA hydrolytic activity and LRRK2 kinase activity in different PD-relevant models. Our study highlights how GBA function is relevant for the pathophysiological mechanisms of genetic forms of PD. In this frame, I am co-supervisor of a PhD student exploring the link between GBA and another PD-protein, DJ-1, in lysosomal function.

PD-GBA mutations impact on GBA enzymatic activity and stability, altering neuronal physiology. Thus, any molecule able to reactivate or stabilize GBA would represent a promising therapeutic target to preserve neurons health. Based on this idea, I teamed up with Prof. Wim Versees (University of Bruxelles, Belgium) to produce nanobodies able to ameliorate GBA function (the project was first funded by the Michael J. Fox Foundation in 2019 and funding was renewed in 2021). We produced and characterized 20 nanobodies, among which we shortlisted a subset of promising molecules; we characterized them in vitro and in cell models to understand their impact on lysosomal GBA activity and described their interaction with the enzyme via structural biology. We are now testing their impact on GBA mutants in patient derived models, focusing not only in PD but also considering Gaucher disease patients, which carry homozygous mutations in the *GBA* gene (funded by Telethon Foundation).

The lipid β -glucosylcholesterol, one of the products of GBA and of the non-lysosomal glucocerebrosidase GBA2, is altered in different neurodegenerative diseases and other glycosterols, as β -glucosyl- β -sitosterol, are associated with a neurological disease comprising amyotrophic lateral sclerosis (ALS) and parkinsonism with dementia. The impact of altered glycosterols homeostasis in neurons is unknown because their physiological function is still unclear. I hypothesize that they may impact on different membrane receptor by changing membrane properties or by direct interactions. To unravel this aspect, I am exploring different approaches. One possibility I work on is feeding mice and zebrafish with the plant-derived glycosterols to partially recapitulate ALS and/or PD phenotypes and perform morphological, physiological and transcriptional studies (in collaboration with Prof. Dalla Valle and Prof. Cagnin, Dept. Biology, Univ. of Padova). In parallel, my collaborator Prof. Scarso (Dept. Mol. Sciences and Nanosystems, Univ. Ca' Foscari) synthesized glycosterols functionalized with beads and fluorophores, I use to identify protein interactors by affinity purification and mass spectrometry and to image the lipid localization by fluorescence microscopy. To further investigate glycosterol role in neuronal function, we deplete β -glucosylcholesterol by inhibiting GBA2 in cerebellar neurons and are investigating the physiological and morphological features of mature cerebellar neurons by confocal and Ca^{2+} imaging. In fact, GBA2 seems crucial to preserve healthy nerve cells since its mutations cause Hereditary Spastic Paraplegia and inhibition of GBA2 leads to reduced β -glucosylcholesterol levels (project funded by the Spastic Paraplegia Foundation in 2021).

Building on this diverse and strong background that allowed me to investigate different pathways that can contribute to neuronal dyshomeostasis and neurodegeneration, I have started my lab with the goal of better understanding what are the molecular mechanisms that drive neuron dysfunction and death in neurodevelopmental and neurodegenerative diseases.

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2022-	Assistant Professor, University of Padova, Italy
2019-2022	Senior Postdoctoral Fellow, Department of Biology, University of Padova, Italy
2018-2019	Veronesi Postdoctoral Fellow, Department of Biology, University of Padova, Italy
2015-2018	Marie Curie Postdoctoral Fellow, Cell and Developmental Biology Department, University College London, UK
2013-2015	Postdoctoral Fellow, University of Pavia and University of Padova, Italy
2010-2013	Graduate Student, PhD School in Biosciences, University of Padova, Italy

Honors

- **Telethon grant 2023-2025:** “GBA1-derived Nanobodies as a novel therapeutic strategy in neuropathic Gaucher disease”
- MJFF grant 2021-2023: “GBA1 stabilization and activation through nanobodies and derived peptide mimetics in neuronal models”
- **MJFF grant 2019-2021:** “GBA1 stabilization through nanobodies: a versatile tool to improve wild type and mutant enzyme function”
- **HSP grant 2021-2022:** “Unraveling the role of glucocerebrosidases and glucosyl-cholesterol in Hereditary Spastic Paraplegia”

C. Contributions to Science

(1) We have investigated how GBA1 defects impact cellular and neuronal function and started developing novel therapeutic approaches for GBA1-related diseases. The focus of this theme of research in our lab is studying the impact of defects in the lysosomal enzyme glucocerebrosidase (GCase) on cellular and neuronal function, especially on organelles. We were able to show that GCase defects impact not only on lysosomal function, but also affects calcium signaling. We have now developed nanobodies against GCase in order to stabilize and/or activate the enzyme to finally develop novel therapeutic strategies against Parkinson's and Gaucher disease.

- Dal Maso T., Sinisgalli C., Zilio G., Tessari I., Pardon E., Steyaert J., Ballet S., Greggio E., Versées W.* and **Plotegher N.*** (2025) *Developing nanobodies as allosteric molecular chaperones of glucocerebrosidase function.* Nature Communications 16(4890):1-9.
- Kedariti M., Frattini E., Baden P., Cogo S., Civiero L., Ziviani E., Aureli M., Kaganovich A., Cookson M. R., Stefanis L., Surface M., Deleidi M., Di Fonzo A., Alcalay R. N., Rideout H., Greggio E., **Plotegher N.** (2022) *LRRK2 kinase activity regulates GCase level and enzymatic activity differently depending on cell type in Parkinson's disease.* NPJ Parkinsons Disease 8(1):92.
- **Plotegher N.**, Filadi R., Pizzo P., Duchen M. (2021) *Excitotoxicity revisited: mitochondria on the verge of a nervous breakdown* Trends in Neurosciences 44(5):342-351. Featured on the cover in April 2021.
- **Plotegher N.**, Perocheau D., Ferrazza R., Massaro G., Bhosale G., Zambon F., Rahim A. A., Guella G., Waddington S. N., Szabadkai G., Duchen M. R. (2020) *Impaired cellular bioenergetics caused by GBA1 depletion sensitises neurons to calcium overload.* Cell Death and Differentiation 27(5):1588-160.
- **Plotegher N.** and Duchen M. R. (2017) *Mitochondrial dysfunction and neurodegeneration in lysosomal storage disorders.* Trends in Molecular Medicine 23(2):116-134.

(2) We have shown that dopamine catabolism is crucial in determining the vulnerability of certain types of neurons. In these works, we investigated how the dopamine catabolite DOPAL is able to impact on alpha-synuclein homeostasis and more general we showed and characterize how DOPAL causes impaired proteostasis in neuronal cells, affecting neuronal function.

- Masato A., **Plotegher N.**, Thor A., Adams S., Sandre M., Cogo S., De Lazzari F., Fontana C. M., Martinez P. A., Strong R., Bellucci A., Bisaglia M., Greggio E., Dalla Valle L., Boassa D., Bubacco L. (2023) *DOPAL initiates α Synuclein-mediated impaired proteostasis in neuronal projections leading to enhanced vulnerability in Parkinson's disease.* NPJ Parkinsons Disease 9(1):42.
- Masato A., **Plotegher N.**, Boassa D., Bubacco L. (2019) *Impaired dopamine metabolism in Parkinson's Disease pathogenesis.* Molecular Neurodegeneration 14(1):35.
- **Plotegher N.**, Berti G., Ferrari E., Tessari I., Zanetti M., Lunelli L., Greggio E., Bisaglia M., Veronesi M., Girotto S., Dalla Serra M., Perego C., Casella L., Bubacco L. (2017) *DOPAL derived alpha-synuclein oligomers impair synaptic vesicles physiological function.* Scientific Reports 7:40699.

3) We investigated the key role of ceramides in certain forms of Parkinson's disease. We demonstrated that ceramides unbalance is associated to Parkinson's disease, either in cellular and animal models, suggesting that targeting ceramide metabolic pathway may be a valuable therapeutic approach for the disease.

- Mingione A., Pivari F., **Plotegher N.**, Dei Cas M., Zulueta A., Bocci T., Trinchera M., Albi E., Maglione V., Caretti A., Bubacco L., Paroni R., Bottai D., Ghidoni R., Signorelli P. (2021) *Inhibition of Ceramide Synthesis Reduces α -Synuclein Proteinopathy in a Cellular Model of Parkinson's Disease* International Journal of Molecular Sciences 22(12):6469. PMID: PMC8234676

- **Plotegher N.**, Bubacco L., Greggio E., Civiero L. (2019) *Ceramides in Parkinson's disease: from recent evidence to new hypotheses*. *Frontiers in Neuroscience* 13:330.
- Ferrazza R., Cogo S., Bubacco L., Greggio E., Guella G., Civiero L., **Plotegher N.** (2016) *LRRK2 deficiency impacts on ceramide metabolism in brain*. *Biochemical and Biophysical Research Communications* 478(3):1141-6.

4) We investigated alpha-synuclein aggregation in a variety of cellular models. The contribution of my research in this frame is linked to the discovery of inhibitors of alpha-synuclein aggregation (such as 14-3-3 proteins), or to the development of novel methods to study the oligomerization process in live cells, by exploiting advanced fluorescence imaging methods (such as N&B or fluorescence lifetime), or photoresponsive peptides able to trigger alpha-synuclein aggregation in a controlled manner.

- Marafon G., Crisma M., Masato A., **Plotegher N.**, Bubacco L., Moretto A. (2021) *Photoresponsive Prion-Mimic Foldamer to Induce Controlled Protein Aggregation* *Angew Chem Int Ed Engl.* 60(10):5173-5178.
- **Plotegher N.**, Kumar D., Tessari I., Brucale M., Munari F., Tosatto L., Belluzzi E., Greggio E., Bisaglia M., Capaldi S., Aioanei D., Mammi S., Monaco H.L., Samorì B. and Bubacco L. (2014) *The chaperone like protein 14-3-3 eta interacts with human alpha-synuclein aggregation intermediates rerouting the amyloidogenic pathway and reducing alpha-synuclein cellular toxicity*. *Human Molecular Genetics* 23(21):5615-29.
- **Plotegher N.**, Stringari C., Jahid S., Veronesi M., Girotto S., Gratton E., Bubacco L. (2015) *NADH fluorescence lifetime is an endogenous reporter of alpha-synuclein aggregation in live cells*. *FASEB Journal* 29(6):2484-94.
- **Plotegher N.**, Gratton E., Bubacco L. (2014) *Number and Brightness analysis of alpha-synuclein oligomerization and the associated mitochondrial morphology alterations in live cells*. *Biochimica and Biophysica Acta, General Subjects* 1840(6):2014-2024.

5) We studied the impact of alpha-synuclein fibrils on non-neuronal cell models, such as monocytes, astrocytes or microglia. We showed that these aggregates can trigger inflammatory responses that have very specific features and that glial cells intervene to remove the alpha-synuclein fibrils to protect brain cells from different type of damages.

- Codolo G., **Plotegher N.**, Pozzobon T., Tessari I., Bubacco L., de Bernard M. (2013) *Triggering of inflammasome by aggregated α -Synuclein, an inflammatory response in Parkinson's disease*. *Plos One*, 8(1): e55375.
- Streubel-Gallasch L., Giusti V., Sandre M., Tessari I., **Plotegher N.**, Giusto E., Masato A., Iovino L., Battisti I., Arrigoni G., Greggio E., Tremblay M., Bubacco L., Erlandsson A., Civiero L. (2020) *Parkinson's disease-associated LRRK2 interferes with astrocyte-mediated alpha-synuclein clearance* *Molecular Neurobiology* 58(7):3119-3140.
- Russo I., Berti G., **Plotegher N.**, Bernardo G., Filograna R., Bubacco L., Greggio E. (2015) *Leucine-rich repeat kinase 2 positively regulates inflammation and down-regulates NF- κ B p50 signaling in cultured microglia cells*. *J Neuroinflammation* 12(1):230.

My complete bibliography: <https://pubmed.ncbi.nlm.nih.gov/?term=nicoletta+plotegher&sort=date>