

## REVIEW

# PrP meets alpha-synuclein: Molecular mechanisms and implications for disease

Tuane C. R. G. Vieira<sup>1</sup> | Caroline A. Barros<sup>1</sup> | Renato Domingues<sup>2</sup> |  
Tiago Fleming Outeiro<sup>2,3,4,5</sup>  

<sup>1</sup>Institute of Medical Biochemistry Leopoldo de Meis and National Institute of Science and Technology for Structural Biology and Bioimaging, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

<sup>2</sup>Department of Experimental Neurodegeneration, Center for Biostructural Imaging of Neurodegeneration, University Medical Center Göttingen, Göttingen, Germany

<sup>3</sup>Max Planck Institute for Multidisciplinary Sciences, Göttingen, Germany

<sup>4</sup>Faculty of Medical Sciences, Translational and Clinical Research Institute, Newcastle University, Newcastle Upon Tyne, UK

<sup>5</sup>Scientific Employee with an Honorary Contract at Deutsches Zentrum für Neurodegenerative Erkrankungen (DZNE), Göttingen, Germany

## Correspondence

Tuane C. R. G. Vieira, Institute of Medical Biochemistry Leopoldo de Meis and National Institute of Science and Technology for Structural Biology and Bioimaging, Federal University of Rio de Janeiro, Rio de Janeiro, RJ 21941-902, Brazil.

Email: [tuane@bioqmed.ufrj.br](mailto:tuane@bioqmed.ufrj.br)

Tiago Fleming Outeiro, Department of Experimental Neurodegeneration, University Medical Center Göttingen, Waldweg 33, 37073 Göttingen, Germany. Email: [touteir@gwdg.de](mailto:touteir@gwdg.de)

## Funding information

Deutsche Forschungsgemeinschaft, Grant/Award Number: SFB1286 (B8) and EXC 2067/1-390729940; Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ); Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)

## Abstract

The discovery of prions has challenged dogmas and has revolutionized our understanding of protein-misfolding diseases. The concept of self-propagation via protein conformational changes, originally discovered for the prion protein (PrP), also applies to other proteins that exhibit similar behavior, such as alpha-synuclein (aSyn), a central player in Parkinson's disease and in other synucleinopathies. aSyn pathology appears to spread from one cell to another during disease progression, and involves the misfolding and aggregation of aSyn. How the transfer of aSyn between cells occurs is still being studied, but one important hypothesis involves receptor-mediated transport. Interestingly, recent studies indicate that the cellular prion protein (PrP<sup>C</sup>) may play a crucial role in this process. PrP<sup>C</sup> has been shown to act as a receptor/sensor for protein aggregates in different neurodegenerative disorders, including Alzheimer's disease and amyotrophic lateral sclerosis. Here, we provide a comprehensive overview of the current state of knowledge regarding the interaction between aSyn and PrP<sup>C</sup> and discuss its role in synucleinopathies. We examine the properties of PrP and aSyn, including their structure, function, and aggregation. Additionally, we discuss the current understanding of PrP<sup>C</sup>'s role as a receptor/sensor for aSyn aggregates and identify remaining unanswered questions in this area of research. Ultimately, we posit that exploring the interaction between aSyn and PrP<sup>C</sup> may offer potential treatment options for synucleinopathies.

**Abbreviations:** aSyn, alpha-synuclein; CJD, Creutzfeldt-Jakob disease; HSPG, heparan sulfate proteoglycans; Lag3, lymphocyte-activation gene 3; NKA, Na<sup>+</sup>/K<sup>+</sup>-ATPase; NMDAR, N-methyl-D-aspartate receptors; PD, Parkinson's disease; PrP, prion protein; TSEs, transmissible spongiform encephalopathies.

This article is part of the special issue "Protein aggregation in aging and neurodegeneration".

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial-NoDerivs](https://creativecommons.org/licenses/by-nc-nd/4.0/) License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2023 The Authors. *Journal of Neurochemistry* published by John Wiley & Sons Ltd on behalf of International Society for Neurochemistry.

## KEYWORDS

alpha-synuclein, neurodegeneration, Parkinson's disease, prion, prion diseases, PrP

## 1 | INTRODUCTION

### 1.1 | Prions and the expansion of the prion concept

The term “prion” refers to a protein responsible for causing various neurodegenerative diseases known as transmissible spongiform encephalopathies (TSEs), such as Creutzfeldt-Jakob in humans and mad cow disease in bovine cattle. Stanley Prusiner first coined the term in 1982, and the discovery of prions challenged the prevailing scientific understanding of infectious diseases, which had previously held that viruses, bacteria, or fungi were the only agents to cause infections (Prusiner, 1982).

Named after scrapie, the TSE affecting sheep, the infectious form of the prion protein (PrP<sup>Sc</sup>) was hypothesized to be derived from a normal cellular protein called PrP<sup>C</sup>. In 1986, researchers discovered the gene encoding the PrP<sup>C</sup> protein. The gene was named *PRNP* and was found to be highly conserved across species (Basler et al., 1986). Over the years, it was also discovered that PrP<sup>C</sup> was involved in various cellular processes, including cell adhesion, signal transduction, and neuroprotection (Legname, 2017; Wulf et al., 2017), but the functions of PrP<sup>C</sup> are still not fully understood. Different mouse strains in which the PrP gene is ablated were generated to try to reveal its functions, but the phenotypes observed are still controversial (Marín-Moreno et al., 2020; Steele et al., 2007).

The capacity of PrP<sup>Sc</sup> to act as an infectious agent depends on the presence of PrP<sup>C</sup>, which gets converted into the PrP<sup>Sc</sup> form (Prusiner et al., 1990). This self-propagating ability is connected to a change in protein conformation that leads to the formation of aggregates that act as a template that induces the conversion of normal cellular proteins into the misfolded conformation. This concept was later extended to several other proteins related to non-infectious diseases, such as other neurodegenerative diseases and cancer (Eraña et al., 2017; Scialò et al., 2019; Silva et al., 2013). For this, the terms “prionoid” or “prion-like” have been introduced and are now used for proteins that present a replicative cycle based on conformational remodeling. Since these other diseases seem to lack the infectious nature of TSEs, it is important to consider the use of terms that distinguish them to avoid confusion in the general public.

Identifying the prion-like behavior of proteins other than PrP suggests that the mechanisms of protein misfolding and aggregation that underlie prion diseases may be more widespread than previously thought. This also suggests that understanding the molecular underpinnings of protein aggregation and conversion may lead to the development of therapeutic strategies that may be more generally applicable (or adaptable) for slowing or halting the progression of various devastating disorders for which we still lack effective therapies.

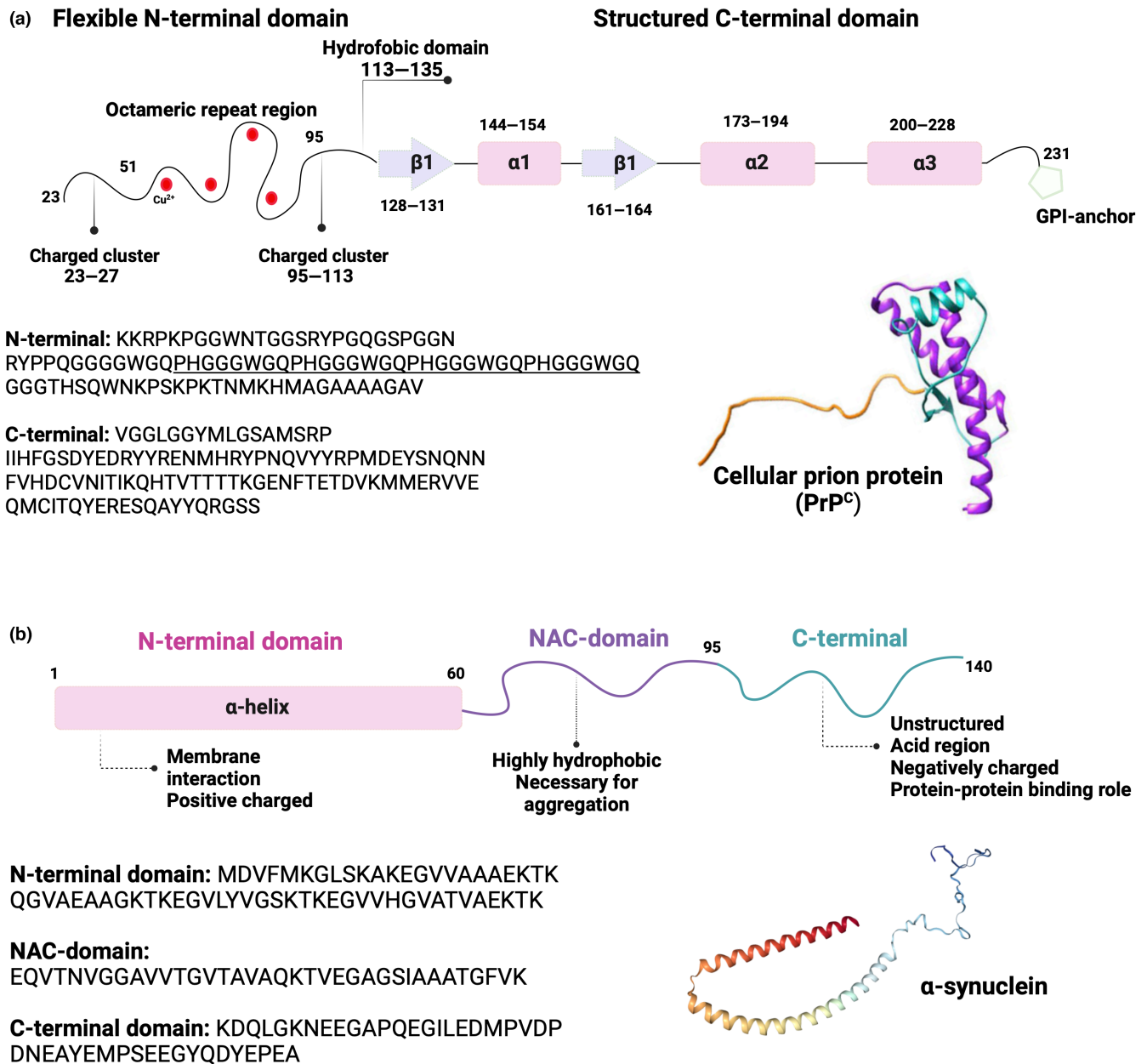
### 1.2 | PrP structure, function, and aggregation

PrP<sup>C</sup> is a glycoprotein found on the surface of cells in various organs and tissues, including the central nervous system (CNS) and peripheral nervous system (Bendheim et al., 1992). PrP<sup>C</sup> is anchored to the cell membrane through glycosylphosphatidylinositol, primarily in lipid rafts. After it reaches the extracellular membrane, PrP<sup>C</sup> can travel back to the cell interior for either recycling or degradation, through clathrin-dependent and caveolin pathways (Peters et al., 2003; Shyng et al., 1994).

PrP<sup>C</sup> is composed of two structural domains: a flexible N-terminal domain and a globular C-terminal domain (Figure 1a; Donne et al., 1997; Riek et al., 1997). The N-terminal domain spans from amino acids 23 to 124 and consists of several distinct regions. These include two positively charged clusters known as CC1 and CC2, an octarepeat region (most mammalian PrP proteins contain five or six repeats of a peptide with 8 or 9 amino acid residues with the sequence P(Q/H)GGG(G/-)WGQ), and a hydrophobic domain (Figure 1a; Donne et al., 1997; Riek et al., 1997). The C-terminal domain consists of three  $\alpha$ -helices and two antiparallel  $\beta$ -sheets, spanning from amino acids 125 to 230. It also has a disulfide bridge connecting cysteine 179 and cysteine 214. In addition, oligosaccharides can attach to asparagine 181 and asparagine 197 residues, resulting in mono-glycosylation or di-glycosylation (Haraguchi et al., 1989).

Interestingly, PrP mutations linked to familial forms of prion diseases are mostly located in the C-terminal domain, where they increase the propensity of the protein to aggregate (Eghiaian et al., 2004; Wopfner et al., 1999).

As stated earlier, the physiological functions of PrP<sup>C</sup> are still elusive, but it seems to play a role in regulating sleep, as evidenced by studies in PrP<sup>-/-</sup> Zrchl mice which display disrupted circadian rhythms and more fragmented sleep (Tobler et al., 1996). Consistently, the interaction of PrP<sup>C</sup> with monoaminergic receptors was considered essential for modulating melatonin synthesis and depressive-like behavior (Roguski & Gill, 2017). Another study, using a transgenic mouse model expressing the mouse homolog of the PrP variant linked to familial Creutzfeldt-Jakob disease (CJD; D178N/V129), reported anomalies in sleep and in electroencephalogram recordings, together with functional impairments in memory and motor abilities (Dossena et al., 2008). Sleep deprivation is associated with decreased levels of both PrP<sup>C</sup> and mGluR1, and with an increase in the levels of A $\beta$  peptides, suggesting that lack of sleep may have a detrimental impact on neuronal plasticity (da Luz et al., 2020). These data suggest that PrP<sup>C</sup> interacts with various receptors and molecules that modulate sleep, mood, memory, and neuronal plasticity and that alterations in PrP<sup>C</sup> expression or function may lead to sleep disturbances and cognitive impairments.



**FIGURE 1** Amino acid sequence, domains, and structure of cellular prion protein (PrP<sup>C</sup>) and alpha-synuclein (aSyn). (a) The mature PrP<sup>C</sup> at the cell surface has a sequence of 209 amino acids (UNIPROT code P04156 depicted) divided into N- and C-terminal domains. The unstructured N-terminal domain has charged and hydrophobic amino acid clusters and an octapeptide repeat region (underlined) that interacts with different molecules. The globular C-terminal domain comprises three  $\alpha$ -helices and a small antiparallel  $\beta$ -sheet. (b) aSyn (UNIPROT code P37840 depicted) is an intrinsically disordered protein composed of 140 amino acids, and its structure is divided into three domains: the N-terminal, non-amyloid component (NAC), and C-terminal domain. The N-terminal region is positively charged and gains an  $\alpha$ -helix coiled structure after interaction with lipid membranes. This  $\alpha$ -helix coiled structure extends to the NAC domain. The NAC domain is highly hydrophobic and involved in fiber formation, and the C-terminal is negatively charged and shows a random coil structure. Created with BioRender.com.

Additional research has demonstrated that PrP<sup>C</sup> plays a significant role in supporting neuronal growth during developmental stages and in adult neurogenesis (Brown et al., 1997; Dupiereux et al., 2008; Kim et al., 2004; Nuvolone et al., 2013; Rangel et al., 2009; Steele et al., 2006; Weise et al., 2004). In fact, the neuroprotective function of PrP<sup>C</sup> was connected to its interaction with copper ions and N-methyl-D-aspartate receptors (NMDAR), regulating NMDAR

nitrosylation and activity by reducing the influx of calcium ions into neurons (Gasperini et al., 2015; Stys et al., 2012). Strikingly, calcium ion dysregulation is a critical factor in various neurodegenerative processes, so the regulation of this process by PrP<sup>C</sup> can be seen as neuroprotective (Zündorf & Reiser, 2011).

The binding of PrP<sup>C</sup> to neural cell adhesion molecule (NCAM) promotes cell adhesion, particularly in neurons, contributing to the

establishment and maintenance of neuronal networks (Santucci et al., 2005; Schmitt-Ulms et al., 2001; Slapšak et al., 2016). NCAM is a transmembrane glycoprotein involved in cell adhesion and signal transduction in the nervous system, playing a crucial role in neural development, synaptic plasticity, and neuronal migration (Ditlevsen et al., 2008). The interaction with PrP<sup>C</sup> enhances NCAM-mediated signaling pathways, including activating intracellular kinases such as Fyn, which are important regulators of synaptic plasticity (Santucci et al., 2005).

The interaction between PrP and its various partners is not only crucial for its many physiological functions but also it plays a role in the development of prion diseases. In prion diseases, PrP is converted from the normal cellular form (PrP<sup>C</sup>) into the misfolded, aggregated form PrP<sup>Sc</sup>, which can then propagate and spread throughout the body. This conversion is thought to occur through a templating mechanism, in which PrP<sup>Sc</sup> acts as a template that induces the misfolding of PrP<sup>C</sup> (Bueler et al., 1993).

Alongside with the templating mechanism, the pathogenicity of PrP is influenced by its interaction with other partners, such as nucleic acids, lipids, and polysaccharides, all of which can enhance its propagation and spread (Alves Conceição et al., 2022; Silva et al., 2022; Supattapone, 2014).

Thus, the ability of PrP to interact with multiple partners constitutes a “double-edged sword.” While these interactions are important for its normal physiological functions, they can also contribute to its pathogenic role in prion diseases. A greater understanding of these interactions is necessary to enable the development of therapeutic strategies for treating these devastating diseases.

### 1.3 | PrP<sup>C</sup>: A troublesome receptor at the cell surface

Recent studies have revealed that PrP<sup>C</sup> may act as a receptor/sensor for different protein aggregates, modulating different neuropathologies both in terms of neuronal toxicity and transmission of pathogenic aggregates to adjacent regions (Resenberger et al., 2011; Scialò & Legname, 2020). This discovery has important implications for understanding the development of neurodegenerative disorders and provides novel possibilities for treatment.

The interaction between PrP<sup>C</sup> and amyloid- $\beta$  (A $\beta$ ) peptides, which are central to the pathology of Alzheimer's disease (AD), is an example of how PrP<sup>C</sup> may act as a troublesome receptor. Although PrP<sup>C</sup> is not the only receptor for A $\beta$  at the cell surface (Benilova et al., 2012; De Strooper & Karran, 2016), the interaction between PrP<sup>C</sup> and A $\beta$  enhances the toxicity of A $\beta$ , exacerbating neuronal damage and promoting the formation of amyloid plaques. This interaction appears oligomeric-specific and occurs via the CC region of PrP<sup>C</sup> (amino acids 23–27 and 94–110; Freir et al., 2011). The mechanism of toxicity seems to involve Fyn kinase, a member of the Src family of kinases (SFKs), that has been associated with diverse functions in the CNS including myelination and synaptic transmission. Dysfunction of Fyn kinase is associated with neurodegenerative

diseases (Guglietti et al., 2021). Fyn kinase phosphorylates NMDAR, increasing calcium influx, and initiating long-term potentiation (LTP; Trepanier et al., 2012). However, sustained NMDAR phosphorylation leads to excitotoxicity (Um et al., 2012). The A $\beta$  oligomer-PrP<sup>C</sup> interaction leads to phosphorylation of the Fyn kinase via the metabotropic glutamate receptor 5 (mGluR5) and subsequent phosphorylation of the NMDAR2B, leading to synaptic dysfunction in the hippocampus, LTP deficits, and destabilization of the dendritic spines. This phosphorylation cascade has also been associated with increased hyperphosphorylated Tau levels, affecting the progression of AD (Salazar & Strittmatter, 2017; Um et al., 2012).

Recent research revealed that AD brains contain different types and levels of PrP<sup>C</sup> isoforms (reviewed in Zhang et al., 2019). This profile changes with the progression and severity of the disease, providing a potential tool for diagnosing and evaluating AD. Additionally, PrP<sup>C</sup> has been detected in amyloid plaques in AD (Schwarze-Eicker et al., 2005; Takahashi et al., 2011).

The function of PrP<sup>C</sup> in AD is still unclear, but reduced PrP<sup>C</sup> expression in the hippocampus is linked to aging and an increased risk of AD, suggesting a protective role in pathology (Whitehouse et al., 2010). Some studies focused on measuring PrP<sup>C</sup> levels in different AD Braak stages and found that the levels increase in the initial stages of the pathology but decrease in later stages, leading to neuronal death (Vergara et al., 2015). Differences in glycosylated forms of PrP<sup>C</sup> have also been reported (Saijo et al., 2011). Nevertheless, other studies found no significant differences in PrP<sup>C</sup> expression between healthy individuals and those with AD (Abu Rumeileh et al., 2017; Dohler et al., 2014).

PrP<sup>C</sup> was shown to also interact with Tau aggregates and promote their internalization, thereby facilitating the propagation of tau pathology (Corbett et al., 2020; De Cecco et al., 2020). Likewise, PrP<sup>C</sup> was shown to interact with TDP-43, a protein that affects neurons in amyotrophic lateral sclerosis and in frontotemporal lobar degeneration, increasing the uptake of TDP-43 fibrils and modulating toxicity (Scialò et al., 2021). PrP<sup>C</sup> has also been implicated in the pathogenesis of Parkinson's disease (PD) as it was shown to interact with alpha-synuclein (aSyn), a major component of Lewy body inclusions in the brains of PD patients, leading to increased cellular toxicity and the spread of pathological aggregates (Ferreira et al., 2017).

The interaction between PrP<sup>C</sup> and various protein aggregates or with aggregation-prone proteins adds a layer of complexity to prion disease pathogenesis and may influence the amplification and spread of prion pathology.

### 1.4 | a-Synuclein structure, function, and aggregation

aSyn is an intracellular 140 amino acid protein abundantly expressed in neurons (Murphy et al., 2000). In its soluble state, aSyn is an intrinsically disordered protein, but it can acquire structured segments when bound to membranes (Eliezer et al., 2001; Park et al., 2002). This structural flexibility may explain its multiple interacting

partners and the variety of functions it has been associated with (Brás et al., 2020).

aSyn can be divided in three distinct domains according to its primary sequence: (i) the N-terminal domain (1–60) contains a multi-repeated imperfect consensus hexameric sequence (KTKEGV) that make it amphipathic and positively charged, adopting alpha-helical conformation when interacting with membranes (Burré et al., 2010; Davidson et al., 1998; Jo et al., 2000); (ii) the Non-Amyloid Component Domain (61–95) is a central hydrophobic region, essential for aSyn aggregation, and contains additional KTKEGV repeats; (iii) the C-terminal domain (96–140) contains a stretch of negatively charged residues enabling the protein to interact with various small molecules and proteins such as vesicle-associated membrane protein 2 and Synaptobrevin-2, two regulators of synaptic exocytosis and endocytosis, respectively (Figure 1b; Burré et al., 2010; Sun et al., 2019).

Experiments using aSyn knock-out (KO)/knock-down, or overexpression have been conducted to gain insight into its physiological function. Most notably, aSyn plays a regulatory role in neurotransmitter release, synaptic function, and plasticity (Abeliovich et al., 2000; Brás et al., 2020; Burré, 2015; Burré et al., 2010; Domingues et al., 2022; Marques & Outeiro, 2012). However, aSyn can also be detected in the nucleus, where it is assumed to be involved in transcriptional regulation and regulation of histone acetylation (Goers et al., 2003; Kontopoulos et al., 2006; Koss et al., 2022; Pinho et al., 2019). In the presence of lipid vesicles, aSyn is found in a folded, membrane-bound state (Liu et al., 2021), where the N-terminal region connects with the central region, maintaining the C-terminal unstructured (Jao et al., 2008; Runfola et al., 2020).

aSyn-KO mice do not display major phenotypes or abnormalities but, upon closer examination, alterations in synaptic vesicle modifications, decreased striatal dopamine and neurotransmission acceleration, and inhibition of the soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNARE) complex assembly have been observed (Burré et al., 2010; Butler et al., 2017). In addition, neurons depleted of aSyn have impaired synaptic vesicle mobilization (Cabin et al., 2002), and reduced synaptic vesicle pool reserves (Murphy et al., 2000).

On the other hand, overexpression of aSyn in mice and in primary neurons decreases vesicle release and recycling after endocytosis, leading to mislocalization of SNARE proteins and sequestration of aSyn aggregates on SNARE proteins, such as synaptobrevin-2 causing a blockade on vesicle docking (Choi et al., 2013; Garcia-Reitböck et al., 2010; Nemani et al., 2010). An excess of aSyn also reduces dopamine reuptake, via dopamine active transporter, and dopamine production via tyrosine hydroxylase, causing impairment in neurotransmitter transport (Hansen et al., 2013; Masliah et al., 2000). In animal models, overexpression of aSyn results in redistribution of synaptic vesicles to locations further from the active zone and, in hippocampal neurons, it can induce the loss of synaptic proteins and enlarged vacant vesicles, leading to abnormalities in vesicle priming, fusion, and docking (Janezic et al., 2013).

aSyn aggregation into insoluble structures known as Lewy bodies and Lewy neurites is a pathognomonic pathological hallmark

of PD. While the role of aSyn aggregates is still controversial, the prevailing theory is that some types of aSyn-containing aggregates might be neurotoxic, contributing to neuronal dysfunction and, eventually, death.

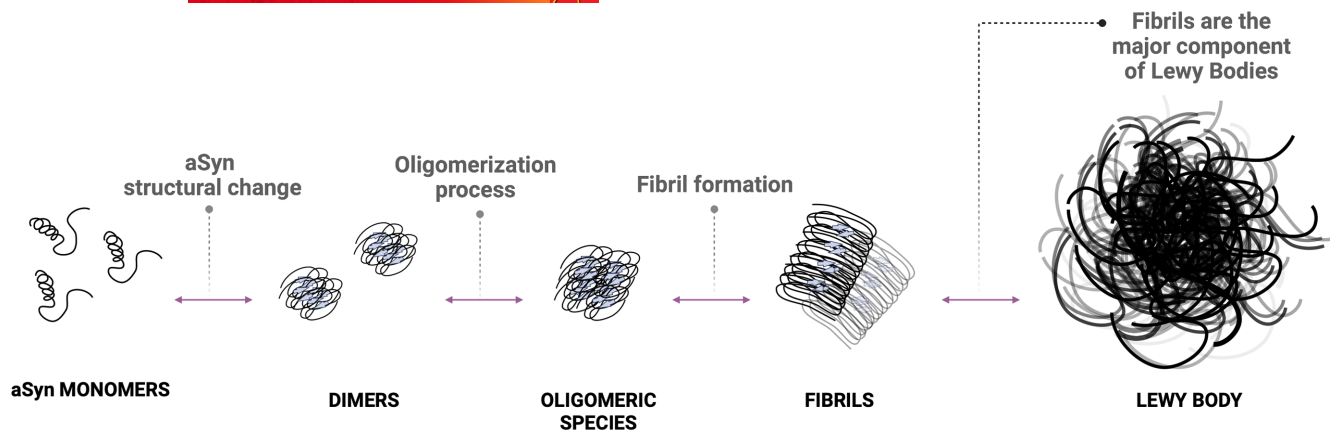
Synucleinopathies are a group of neurodegenerative diseases characterized by the progressive accumulation of aSyn-containing protein aggregates in different cells of the nervous tissue, such as neuronal and glial cells. Synucleinopathies include PD, multiple system atrophy, and dementia with Lewy bodies (Brás et al., 2020). Recent studies using cryo-electron microscopy suggest that the arrangement of aSyn in fibrillar material extracted from brain tissue from individuals who had a synucleinopathy may be different (Guerrero-Ferreira et al., 2018, 2019; Li, Ge, et al., 2018; Li, Zhao, et al., 2018), but additional studies on this topic are still necessary.

During the early stages of fibrillization, aSyn undergoes partial folding into a pre-molten globule-like conformation (Uversky et al., 2001). The precise molecular factors involved in this conformational change are still unclear, but likely include protein concentration imbalances, mutations, posttranslational modifications (PTMs), and environmental factors such as changes in pH, salt concentration, inflammation, or the presence of chemical factors such as polyamines (Antony et al., 2003; Fujiwara et al., 2019; Guzzo et al., 2021; Manzanza et al., 2021; Sandal et al., 2008; Wu et al., 2009). The oligomerization process likely begins with the formation of dimers, followed by the formation of soluble and non-fibrillar oligomeric species with different morphologies, such as annular, chain-like, or spherical structures. Upon reaching a critical concentration, some of these oligomers may convert into protofilaments, protofibrils, and other high-molecular-weight species that can be amorphous or amyloid-like aggregates (Figure 2; Hijaz & Volpicelli-Daley, 2020).

Under certain conditions, spherical oligomers may convert into ring-like structures, which can permeabilize membranes in *in vitro* studies, by forming pore-like structures that affect membrane potential and ion distribution (Kim et al., 2009; Lashuel et al., 2002). However, such pore-forming structures have never been observed in biological systems (cells or brain tissue). Current evidence suggests that aSyn oligomers and protofibrils, rather than larger insoluble aggregates, may be the most cytotoxic species (Casella et al., 2021; Outeiro et al., 2008; Tanaka et al., 2004; Winner et al., 2011). Consistently, several studies aimed at stabilizing aSyn oligomers report increased cytotoxicity (Fusco et al., 2017; Ingelsson, 2016), but this is also a matter of intense debate in the field.

## 1.5 | Synucleinopathies as prion-like diseases

The analogy between prion diseases and synucleinopathies is mainly because of the apparent spreading of protein pathology from cell to cell, region to region, and even organ to organ (Holmqvist et al., 2014). Braak et al. (2003) hypothesized that the topography of synucleinopathies is related to the severity of the clinical symptoms, and that disease progression could be separated into distinct stages. In PD, the



**FIGURE 2** Schematic representation of alpha-synuclein (aSyn) in Lewy body formation. Monomeric aSyn undergoes structural changes that increase the probability of forming dimers, oligomers, and other aggregated species. Once formed, these aggregated species can further form amyloid fibers that are the major component of Lewy bodies. Created with BioRender.com.

first stage is characterized by olfactory deficits, constipation, and sleep disturbances, which reflect the involvement of the olfactory bulb, enteric system, and caudal brainstem, respectively. In the subsequent stages, motor symptoms may correspond to a time when aSyn pathology has reached the substantia nigra. At the final stages, the pathology reaches the neocortex, resulting in psychiatric symptoms and cognitive decline (Braak et al., 2003; Fullard et al., 2017).

The exact mechanisms underlying the transfer of aSyn aggregates are still being actively investigated. These aggregates can then be released and taken up by neighboring cells through various processes that include, but are not necessarily limited to, direct diffusion of aSyn through the cellular membrane, endoplasmic reticulum-Golgi-dependent exocytosis, aSyn secretion in extracellular vesicles, tunneling nanotubes, or receptor-mediated endocytosis (Figure 3; Danzer et al., 2012; Emmanouilidou et al., 2010).

Once internalized by recipient cells, aSyn aggregates may induce the misfolding and aggregation of intracellular aSyn, thus perpetuating the cycle of aggregate formation (Masuda-Suzukake et al., 2013; Paumier et al., 2015). Experiments using aSyn KO mice showed that endogenous aSyn is essential for the spreading of pathology, a process that does not require aSyn toxicity (Dening et al., 2022). Consequently, transmitting aSyn aggregates between cells may contribute to the progression of neurodegeneration observed in PD and related disorders.

Interestingly, experiments using proteinase K aimed at decreasing the abundance of cell surface proteins have revealed a correlation between reduced levels of aSyn internalization and protease treatment, suggesting cell surface proteins contribute to the spreading of pathology (Lee et al., 2008).

One of these cell-surface proteins is lymphocyte-activation gene 3 (LAG3), an immunoglobulin family member expressed in neurons, microglia, and immune cells (Anderson et al., 2016; Liu et al., 2018). LAG3 can bind to recombinantly produced mouse aSyn pre-formed fibrils (PFFs) via its D1 domain, colocalizing with multiple Rab proteins and endosomal GTPases implicated in the internalization of extracellular aSyn (Mao et al., 2016; Figure 4a). However, the involvement of LAG3 in the spreading of aSyn pathology is controversial, as some

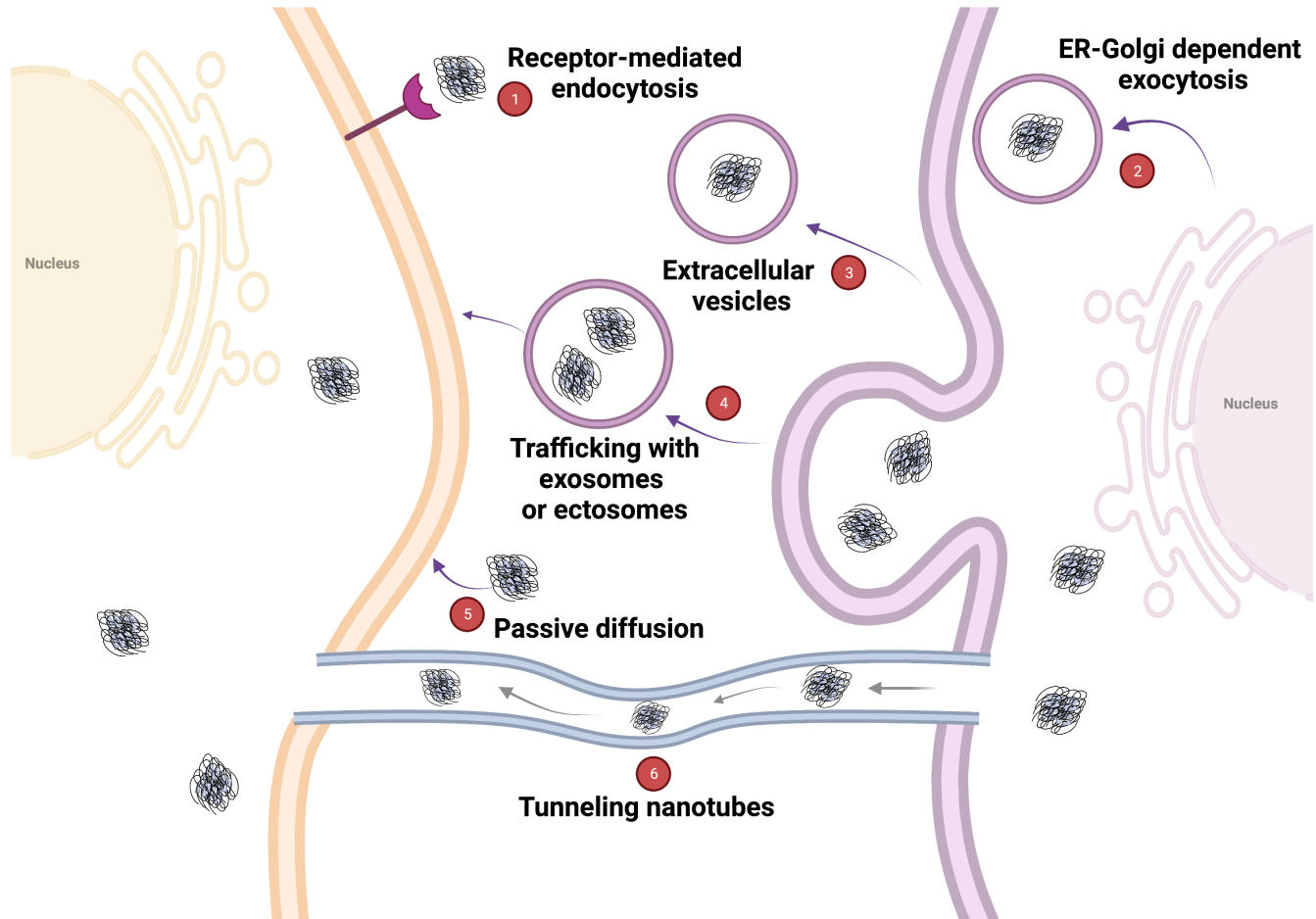
studies presented conflicting findings regarding the levels of expression of LAG-3, which seem to be negligible in both mouse and human neurons (Emmenegger et al., 2021). Nevertheless, while it remains possible that an interaction between certain aSyn aggregated species and LAG-3 may occur in disease settings, this does not appear to be the only relevant receptor to be considered.

The  $\text{Na}^+/\text{K}^+$ -ATPase (NKA) is a membrane protein that utilizes ATP to import two potassium ions and export three sodium ions. NKA is composed of three subunits, and the  $\alpha 3$  subunit is linked to several neurodegenerative diseases (Geering, 2008; Ohnishi et al., 2015; Petrushanko et al., 2016; Ruegsegger et al., 2016; Shrivastava et al., 2015), and plays a crucial role in diverse cellular processes, including neuronal activity (Clausen et al., 2017). The  $\alpha 3$  subunit interacts with  $\text{A}\beta$ , superoxide dismutase 1, and aSyn, and is hypothesized to regulate the endocytosis of these proteins into neuronal cells (Ohnishi et al., 2015; Ruegsegger et al., 2016; Shrivastava et al., 2015). This interaction leads to the entrapment of  $\alpha 3$ -NKA within clusters of aSyn, resulting in a decline in the efficiency of sodium export following an action potential and dysregulation of the neuronal refractory period (Figure 4b).  $\alpha 3$ -NKA was identified, along with neurexin 1 $\alpha$  and 2 $\alpha$ , using proteomic analyses, highlighting its potential involvement in synapse function (Shrivastava et al., 2015).

## 1.6 | Interactions between aSyn and PrP

PrP and aSyn have been found to interact and influence each other's behavior (De Cecco & Legname, 2018). In particular, PrP<sup>C</sup> has been shown to regulate the internalization of aSyn and to act as a mediator of neuronal damage caused by aSyn oligomers (Aulić et al., 2017; Corbett et al., 2020; Ferreira et al., 2017). Interestingly, PrP<sup>Sc</sup> infection increases aSyn phosphorylation at serine 129, a PTM considered a marker of LB pathology (Chen et al., 2021).

Soluble aSyn aggregates were suggested to interact with PrP at the cell surface (Figure 5a), and this was connected to toxicity and to



**FIGURE 3** Possible mechanisms of alpha-synuclein (aSyn) spread between cells. aSyn transmission between two cells is hypothesized to occur through several mechanisms. (1) Receptor-mediated endocytosis may transport aSyn aggregates into the cell. Other possible mechanisms include (2) Golgi-mediated exocytosis and (3 and 4) extracellular vesicles, exosomes, and ectosomes that can fuse with the membrane of another cell. Aggregates can be internalized into neighboring cells (5) by passive diffusion and (6) move through tunneling nanotubes. Created with BioRender.com.

impairment of synaptic plasticity (Corbett et al., 2020), but the role of PrP<sup>C</sup> in the pathology of PD, remains uncertain.

Based on existing literature, the interaction between PrP and aSyn is likely dependent on the type of aSyn species (monomeric, oligomeric, fibrillar, etc.).

Using solid-phase assays, fibrillar and monomeric aSyn showed no or weak affinity to full-length PrP<sup>C</sup> (PrP 23–231; Corbett et al., 2020). In contrast, soluble aSyn aggregates are bound with high affinity to PrP 23–231 (in the nanomolar range; Corbett et al., 2020). However, it is important to recognize that these differences may also be as a result of the nature of the assay, since aggregated proteins tend to be “sticky,” exhibiting a greater propensity to adhere and display binding in assays performed on solid surfaces when compared to soluble species (Corbett et al., 2020). While a study using surface plasmon resonance reported no interaction between PrP<sup>C</sup> and aSyn oligomers (La Vitola et al., 2019), another demonstrated the interaction between full-length PrP and aSyn oligomers and monomers, although with higher affinity for oligomers (Thom et al., 2021).

In these solid-phase assays, the interaction with soluble aSyn aggregates occurred mainly through the N-terminus of PrP since the affinity was 8-times weaker with PrP 91–231, and no binding was detected with PrP 119–231 (Corbett et al., 2020). These data suggest that the binding site of these aSyn species resides mainly between residues 23 and 90 of PrP. However, using a more physiological assay, we have previously reported that PrP<sup>C</sup> residues from 93 to 109 are involved in the interaction of aSyn oligomeric species with PrP in rat hippocampal brain slices, as we could block the effects of aSyn using the PrP antibody 6D11 (targeting the epitope region 93–109 of PrP<sup>C</sup>; Ferreira et al., 2017).

It was also shown that monomeric aSyn and PrP interact *in vitro*, leading to the formation of liquid droplets that are highly dynamic, thermo-responsive, and reversible through the interaction of the N-terminal region of PrP<sup>C</sup> with the C-terminal region of aSyn. In addition, aSyn-PrP condensates appear more prone to transitioning to a solid fibrillar state (Agarwal et al., 2022).

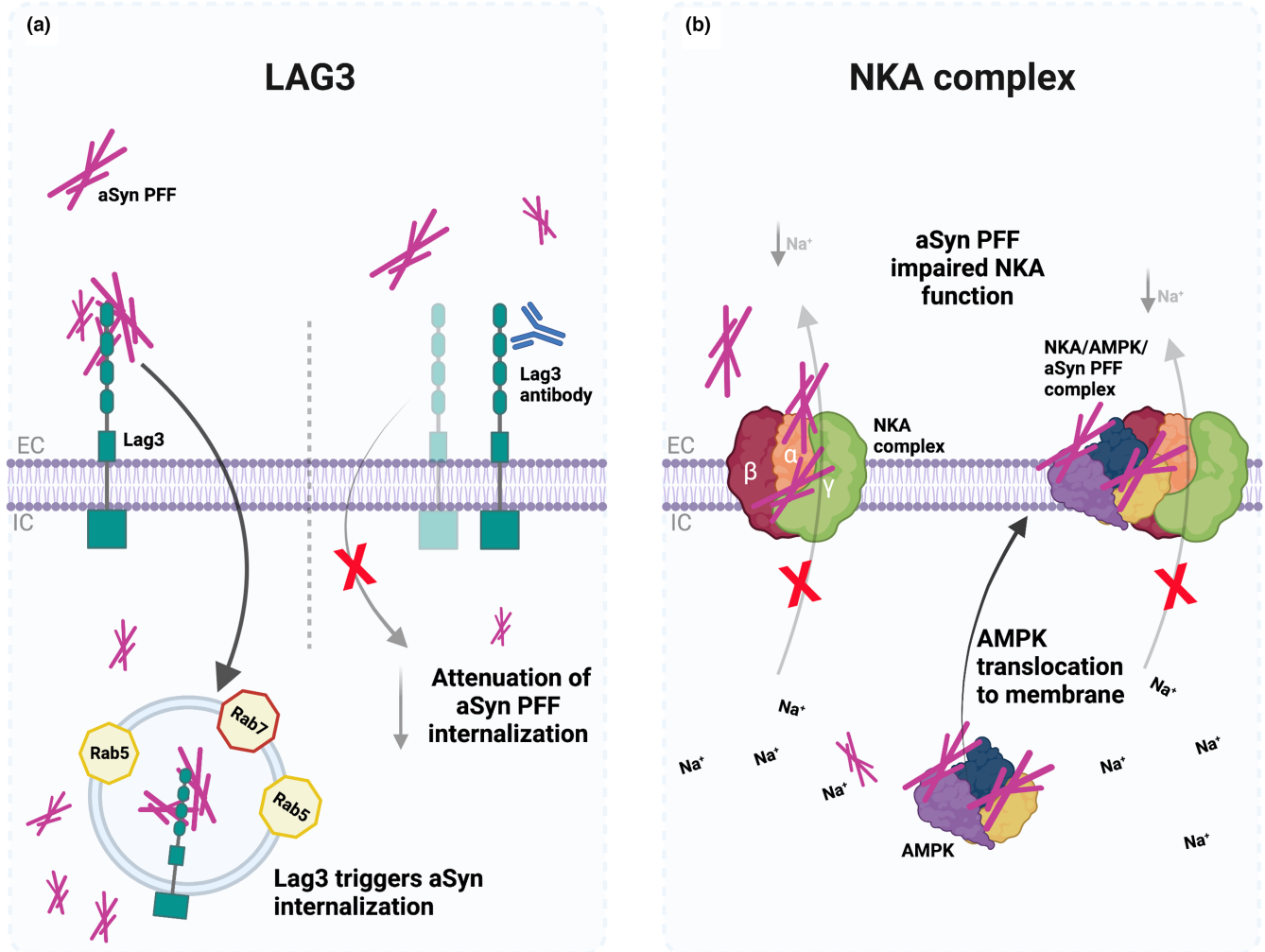
The controversy in the findings described here suggests that differences in the experimental systems need to be further dissected,

by conducting systematic studies using different methods and different aSyn species.

### 1.7 | The role of PrP on aSyn toxicity

As reported for Aβ oligomers, the connection between PrP<sup>C</sup> and NMDAR can be unbalanced by aSyn aggregates. The interaction of

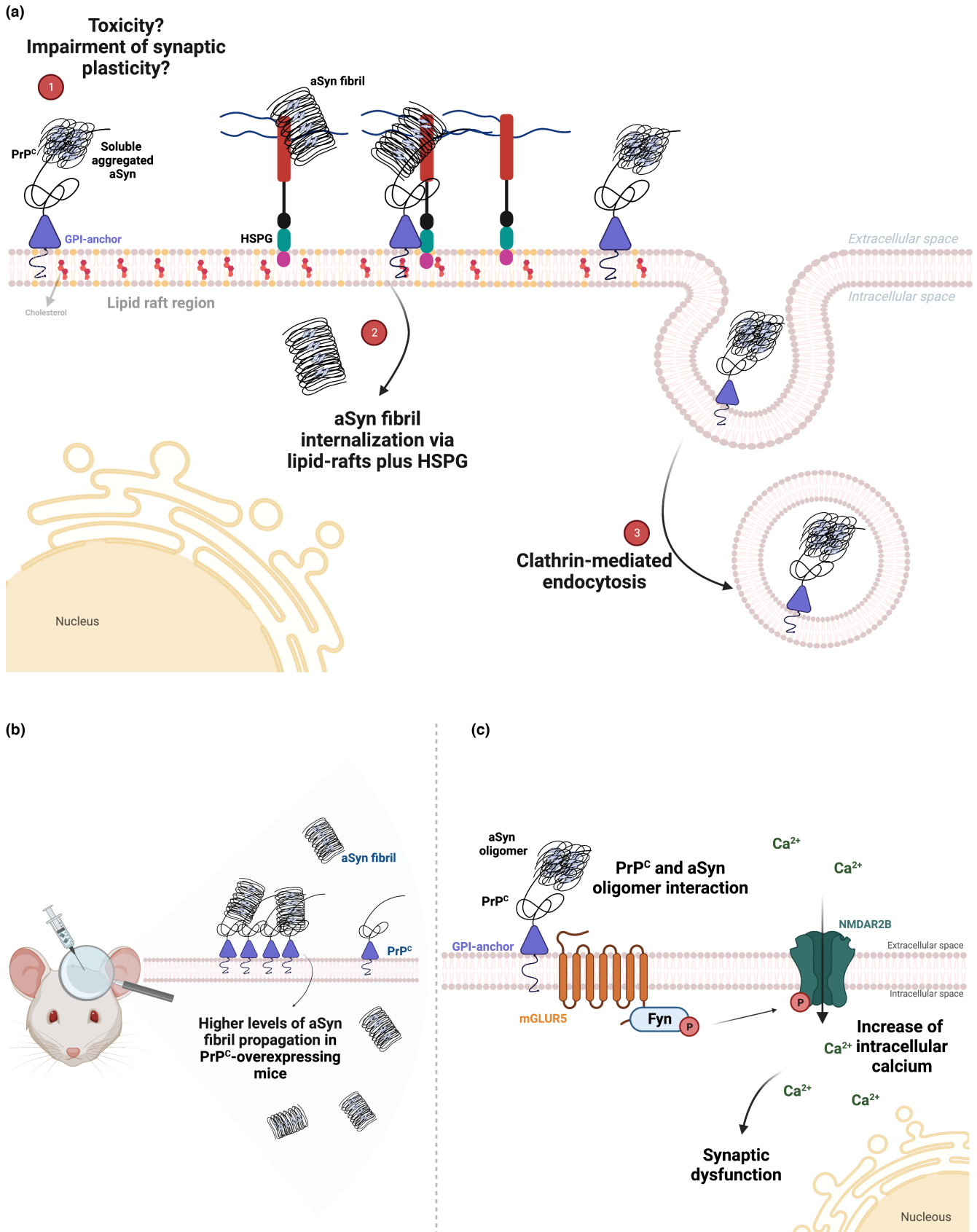
PrP<sup>C</sup> with oligomeric aSyn leads to Fyn kinase-mediated phosphorylation of mGluR5, activating the NMDAR2B and, ultimately, leading to an increase in intracellular calcium that causes synaptic dysfunction, including LTP impairment (Figure 5c; Ferreira et al., 2017). In addition, this interaction has been hypothesized to promote the formation of cofilin/actin rods by rearranging the cytoskeleton and affecting actin dynamics, blocking axonal transport (Brás et al., 2018; Silva et al., 2021).



**FIGURE 4** Lymphocyte-activation gene 3 (Lag3) and Na<sup>+</sup>/K<sup>+</sup>-ATPase (NKA) are involved in the internalization of alpha-synuclein (aSyn) preformed fibers (PFF). (a) Lag3 can directly interact with aSyn PFF, promoting their internalization across the cell membrane. Rab5 and Rab7 participate in this process. The absence of Lag3, or the use of Lag3 antibodies, attenuates the propagation of aSyn PFF. (b) The presence of aSyn aggregates impairs NKA function by interacting with the extracellular segments of α3-NKA. Another possibility is that the interaction of aSyn PFF with AMPK may contribute to the translocation of AMPK to the cell membrane and promote interaction with NKA. Thus, NKA function may also be impaired by the formation of the NKA/AMPK/PFF complex. Created with BioRender.com.

**FIGURE 5** Cellular prion protein (PrP<sup>C</sup>)-mediated internalization of alpha-synuclein (aSyn). (a) (1) The interaction between PrP<sup>C</sup> and aggregated aSyn may contribute to toxicity and impairment of synaptic plasticity. (2) Heparan sulfate proteoglycans (HSPG) are involved with internalizing aSyn and PrP<sup>Sc</sup> aggregates. One hypothesis is that the presence of PrP<sup>C</sup> in HSPG-rich regions is involved with the internalization of aSyn fibrils. (3) The internalization of PrP-aSyn aggregates must take place via clathrin-mediated endocytosis. (b) Although PrP<sup>C</sup> is not exclusive for spreading aSyn aggregates, its overexpression increases fibril spreading in mouse and cell models. (c) One mechanism for PrP<sup>C</sup>-mediated cytotoxicity is the activation of NMDAR2B receptors. Interaction of PrP<sup>C</sup> with oligomeric aSyn promotes the formation of PrP<sup>C</sup>-mGluR5-Fyn cluster, activating NMDAR2B and promoting changes in intracellular calcium homeostasis. Dysregulation of calcium levels in cells is critical for neurodegeneration and cell death. Created with BioRender.com.





As mentioned earlier, PrP<sup>c</sup> is a known partner of NKAs, thereby promoting ion uptake (Williams et al., 2021). Consistently, NKA inhibition by cardiac glycosides reduces both NKA and PrP<sup>c</sup> levels

(Mehravian et al., 2022) suggesting the interdependence of these molecules. Since interfering with NKA activity can lead to calcium signaling dysregulation and, ultimately, to neuronal death (Kinoshita

et al., 2022), it is possible that the interaction between aSyn and PrP<sup>C</sup> may disturb the interaction of NKAs with PrP<sup>C</sup>, thereby exacerbating aSyn toxicity. Thus, we consider that modulating these interactions should be further investigated as a possible strategy for therapeutic intervention.

Nevertheless, contrasting results were also reported, questioning the importance of PrP<sup>C</sup> for aSyn toxicity. In this case, primary hippocampal neurons from PrP WT or from PrP KO mice (PrP<sup>-/-</sup> Zrch1) were shown to be equally susceptible to aSyn oligomers, as both mice had equal memory impairment and neuroinflammatory responses, suggesting that aSyn toxicity was independent of PrP<sup>C</sup> (La Vitola et al., 2019). However, it is not possible to rule out that the role of PrP<sup>C</sup> as a receptor/sensor for aSyn may vary depending on the type of aSyn species, the brain region, and other as-of-yet unidentified factors.

## 1.8 | The role of PrP on the spreading of aSyn pathology

The precise mechanism(s) involved in the spreading of aSyn pathology is/are still elusive. aSyn can transfer between cells via passive and active mechanisms. Endocytic pathways are responsible for the internalization of most aSyn, especially aggregated aSyn (Neupane et al., 2023). The receptor-mediated internalization through PrP<sup>C</sup> is an active mechanism and is clathrin-mediated (Thom et al., 2021; Figure 5a).

PrP<sup>C</sup> interacts with heparan sulfate proteoglycans (HSPG), another co-receptor for various protein aggregates at the cell surface, and HSPG facilitates PrP<sup>Sc</sup> propagation (Vieira & Silva, 2016). Since HSPG is essential for mediating the internalization of aSyn fibrils via a lipid-raft-dependent route (Hudák et al., 2019), the presence of PrP<sup>C</sup> in HSPG-rich regions may facilitate aSyn internalization (Figure 5a). However, the extent to which the interaction between these partners can mediate and modulate aSyn internalization still needs further investigation.

While deletion of *PRNP* in mice (PrP<sup>-/-</sup> Zrch1) does block the spreading of aSyn, overexpression of PrP<sup>C</sup> (Tga20) enhances the spreading of aSyn pathology in a model using stereotactic injections of aSyn PFFs (Urrea et al., 2018; Figure 5a, b).

In primary cortical neurons (from WT and PrP<sup>-/-</sup> Zrch1 mice) and in SH-SY5Y cells (WT and overexpressing PrP<sup>C</sup> using the pCIneoPRNP vector), the PrP<sup>C</sup> was found to increase the uptake of aSyn monomers and oligomers without triggering cytotoxicity. Nevertheless, the same study showed that PrP<sup>C</sup> plays a role in the behavioral alterations and in the shorter lifespans observed in mice with aSyn pathology (Thy1-Syn, a transgenic line that displays a more rapidly progressive  $\alpha$ -synucleinopathy phenotype; Thom et al., 2021). Ablation of *PRNP* in Thy1-Syn PrP0/0 mice (a cross between Thy1-Syn and PrP<sup>-/-</sup> Zrch1 mice) ameliorates the behavioral deficits in Thy1-Syn mice (Thom et al., 2021). These data reinforce the hypothesis that PrP<sup>C</sup> contributes to aSyn internalization and disease progression, possibly by acting as a receptor at the cell surface.

## 1.9 | Outlook: Implications for neurodegeneration

The discovery of cellular prion protein as a receptor (or sensor) for protein aggregates has provided valuable insight into the mechanisms underlying neurodegenerative diseases, including synucleinopathies. Nevertheless, the relationship between PrP and aSyn is likely complex and may not be equally relevant for different synucleinopathies, since different aSyn fibrillar species seem to occur in different synucleinopathies, as mentioned earlier. Additionally, since different pools of aSyn species may be present at different stages of disease, the relevance of the interaction may also vary during disease progression. In earlier stages, when soluble oligomeric species may be dominant, the interaction may lead to neuronal dysfunction, whereas at later stages it may be more relevant for the spreading of pathology. All of this will need to be considered for the design of effective therapeutic strategies.

An additional issue is that PrP is probably not the only player involved in the uptake and spreading of aSyn. To solve this, we must develop more specific models where the contribution of different pathways can be isolated and then introduced in a stepwise manner in order to establish causality/synergistic effects.

Nevertheless, the possible contribution of the aSyn-PrP interaction to the onset and disease progression in synucleinopathies makes it an enticing therapeutic target, using a variety of possible approaches such as immunotherapy, anti-sense oligonucleotides, or small molecules.

### AUTHOR CONTRIBUTIONS

**Tuane C. R. G. Vieira:** Conceptualization; funding acquisition; supervision; writing – original draft; writing – review and editing. **Caroline A. Barros:** Writing – original draft. **Renato Domingues:** Writing – original draft. **Tiago Fleming Outeiro:** Conceptualization; funding acquisition; project administration; supervision; writing – original draft; writing – review and editing.

### ACKNOWLEDGMENTS

T.F.O. is supported by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) under Germany's Excellence Strategy—EXC 2067/1-390729940, and by SFB1286 (B8). T.C.R.G.V. is supported by Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) and by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). Open Access funding enabled and organized by Projekt DEAL.

### CONFLICT OF INTEREST STATEMENT

Tiago F. Outeiro is an Editor with the *Journal of Neurochemistry*, and the Editor of the current special issue, but played no role in the peer review of this manuscript.

### PEER REVIEW

The peer review history for this article is available at <https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/jnc.15992>.

**DATA AVAILABILITY STATEMENT**

Not applicable.

**ORCID**Tiago Fleming Outeiro  <https://orcid.org/0000-0003-1679-1727>**TWITTER**Tiago Fleming Outeiro  touteiro**REFERENCES**

- Abeliovich, A., Schmitz, Y., Fariñas, I., Choi-Lundberg, D., Ho, W. H., Castillo, P. E., Shinsky, N., Verdugo, J. M. G., Armanini, M., Ryan, A., Hynes, M., Phillips, H., Sulzer, D., & Rosenthal, A. (2000). Mice lacking alpha-synuclein display functional deficits in the nigrostriatal dopamine system. *Neuron*, *25*, 239–252.
- Abu Rumeileh, S., Lattanzio, F., Stanzani Maserati, M., Rizzi, R., Capellari, S., & Parchi, P. (2017). Diagnostic accuracy of a combined analysis of cerebrospinal fluid t-PrP, t-tau, p-tau, and A $\beta$ 42 in the differential diagnosis of Creutzfeldt-Jakob disease from Alzheimer's disease with emphasis on atypical disease variants. *Journal of Alzheimer's Disease*, *55*, 1471–1480.
- Agarwal, A., Arora, L., Rai, S. K., Avni, A., & Mukhopadhyay, S. (2022). Spatiotemporal modulations in heterotypic condensates of prion and  $\alpha$ -synuclein control phase transitions and amyloid conversion. *Nature Communications*, *13*, 1154.
- Alves Conceição, C., Assis de Lemos, G., Barros, C. A., & Vieira, T. C. R. G. (2022). What is the role of lipids in prion conversion and disease? *Frontiers in Molecular Neuroscience*, *15*, 1032541.
- Anderson, A. C., Joller, N., & Kuchroo, V. K. (2016). Lag-3, Tim-3, and TIGIT: Co-inhibitory receptors with specialized functions in immune regulation. *Immunity*, *44*, 989–1004.
- Antony, T., Hoyer, W., Cherny, D., Heim, G., Jovin, T. M., & Subramaniam, V. (2003). Cellular polyamines promote the aggregation of alpha-synuclein. *The Journal of Biological Chemistry*, *278*, 3235–3240.
- Aulić, S., Masperone, L., Narkiewicz, J., Isopi, E., Bistaffa, E., Ambrosetti, E., Pastore, B., de Cecco, E., Scaini, D., Zago, P., Moda, F., Tagliavini, F., & Legname, G. (2017).  $\alpha$ -Synuclein amyloids hijack prion protein to gain cell entry, facilitate cell-to-cell spreading and block prion replication. *Scientific Reports*, *7*, 10050.
- Basler, K., Oesch, B., Scott, M., Westaway, D., Walchli, M., Groth, D. F., McKinley, M. P., Prusiner, S. B., & Weissmann, C. (1986). Scrapie and cellular PrP isoforms are encoded by the same chromosomal gene. *Cell*, *46*, 417–428.
- Bendheim, P. E., Brown, H. R., Rudelli, R. D., Scala, L. J., Goller, N. L., Wen, G. Y., Kasczak, R. J., Cashman, N. R., & Bolton, D. C. (1992). Nearly ubiquitous tissue distribution of the scrapie agent precursor protein. *Neurology*, *42*, 149–156.
- Benilova, I., Karran, E., & De Strooper, B. (2012). The toxic A $\beta$  oligomer and Alzheimer's disease: An emperor in need of clothes. *Nature Neuroscience*, *15*, 349–357.
- Braak, H., Del Tredici, K., Rüb, U., de Vos, R. A., Jansen Steur, E. N., & Braak, E. (2003). Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiology of Aging*, *24*, 197–211.
- Brás, I. C., Dominguez-Meijide, A., Gerhardt, E., Koss, D., Lázaro, D. F., Santos, P. I., Vasili, E., Xylaki, M., & Outeiro, T. F. (2020). Synucleinopathies: Where we are and where we need to go. *Journal of Neurochemistry*, *153*, 433–454.
- Brás, I. C., Lopes, L. V., & Outeiro, T. F. (2018). Sensing  $\alpha$ -synuclein from the outside via the prion protein: Implications for neurodegeneration. *Movement Disorders*, *33*, 1675–1684.
- Brown, D. R., Schulz-Schaeffer, W. J., Schmidt, B., & Kretschmar, H. A. (1997). Prion protein-deficient cells show altered response to oxidative stress due to decreased SOD-1 activity. *Experimental Neurology*, *146*, 104–112.
- Bueler, H., Aguzzi, A., Sailer, A., Greiner, R. A., Autenried, P., Aguet, M., & Weissmann, C. (1993). Mice devoid of PrP are resistant to scrapie. *Cell*, *73*, 1339–1347.
- Burré, J. (2015). The synaptic function of  $\alpha$ -synuclein. *Journal of Parkinson's Disease*, *5*, 699–713.
- Burré, J., Sharma, M., Tsetsenis, T., Buchman, V., Etherton, M. R., & Südhof, T. C. (2010). Alpha-synuclein promotes SNARE-complex assembly in vivo and in vitro. *Science*, *329*, 1663–1667.
- Butler, B., Sambo, D., & Khoshbouei, H. (2017). Alpha-synuclein modulates dopamine neurotransmission. *Journal of Chemical Neuroanatomy*, *83–84*, 41–49.
- Cabin, D. E., Shimazu, K., Murphy, D., Cole, N. B., Gottschalk, W., McIlwain, K. L., Orrison, B., Chen, A., Ellis, C. E., Paylor, R., Lu, B., & Nussbaum, R. L. (2002). Synaptic vesicle depletion correlates with attenuated synaptic responses to prolonged repetitive stimulation in mice lacking alpha-synuclein. *The Journal of Neuroscience*, *22*, 8797–8807.
- Cascella, R., Chen, S. W., Bigi, A., Camino, J. D., Xu, C. K., Dobson, C. M., Chiti, F., Cremades, N., & Cecchi, C. (2021). The release of toxic oligomers from  $\alpha$ -synuclein fibrils induces dysfunction in neuronal cells. *Nature Communications*, *12*, 1814.
- Chen, D. D., Gao, L. P., Wu, Y. Z., Chen, J., Hu, C., Xiao, K., Chen, C., Shi, Q., & Dong, X. P. (2021). Accumulation of prion and abnormal prion protein induces hyperphosphorylation of  $\alpha$ -synuclein in the brain tissues from prion diseases and in the cultured cells. *ACS Chemical Neuroscience*, *12*, 3838–3854.
- Choi, B. K., Choi, M. G., Kim, J. Y., Yang, Y., Lai, Y., Kweon, D. H., Lee, N. K., & Shin, Y. K. (2013). Large  $\alpha$ -synuclein oligomers inhibit neuronal SNARE-mediated vesicle docking. *Proceedings of the National Academy of Sciences of the United States of America*, *110*, 4087–4092.
- Clausen, M. V., Hilbers, F., & Poulsen, H. (2017). The structure and function of the Na,K-ATPase isoforms in health and disease. *Frontiers in Physiology*, *8*, 371.
- Corbett, G. T., Wang, Z., Hong, W., Colom-Cadena, M., Rose, J., Liao, M., Asfaw, A., Hall, T. C., Ding, L., DeSousa, A., Frosch, M. P., Collinge, J., Harris, D. A., Perkinson, M. S., Spires-Jones, T. L., Young-Pearse, T. L., Billinton, A., & Walsh, D. M. (2020). PrP is a central player in toxicity mediated by soluble aggregates of neurodegeneration-causing proteins. *Acta Neuropathologica*, *139*, 503–526.
- da Luz, M. H. M., Pino, J. M. V., Santos, T. G., Antunes, H. K. M., Martins, V. R., de Souza, A. A. L., Torquato, R. J. S., & Lee, K. S. (2020). Sleep deprivation regulates availability of PrP C and A $\beta$  peptides which can impair interaction between PrP C and laminin and neuronal plasticity. *Journal of Neurochemistry*, *153*, 377–389.
- Danzer, K. M., Kranich, L. R., Ruf, W. P., Cagsal-Getkin, O., Winslow, A. R., Zhu, L., Vanderburg, C. R., & McLean, P. J. (2012). Exosomal cell-to-cell transmission of alpha synuclein oligomers. *Molecular Neurodegeneration*, *7*, 42.
- Davidson, W. S., Jonas, A., Clayton, D. F., & George, J. M. (1998). Stabilization of alpha-synuclein secondary structure upon binding to synthetic membranes. *The Journal of Biological Chemistry*, *273*, 9443–9449.
- De Cecco, E., Celauro, L., Vanni, S., Grandolfo, M., Bistaffa, E., Moda, F., Aguzzi, A., & Legname, G. (2020). The uptake of tau amyloid fibrils is facilitated by the cellular prion protein and hampers prion propagation in cultured cells. *Journal of Neurochemistry*, *155*, 577–591.
- De Cecco, E., & Legname, G. (2018). The role of the prion protein in the internalization of  $\alpha$ -synuclein amyloids. *Prion*, *12*, 23–27.
- De Strooper, B., & Karran, E. (2016). The cellular phase of Alzheimer's disease. *Cell*, *164*, 603–615.
- Dening, Y., Straßl, T., Ruf, V., Dirscherl, P., Chovsepian, A., Stievenard, A., Khairnar, A., Schmidt, F., Giesert, F., Herms, J., Levin, J., Dieterich, M., Falkai, P., Weisenhorn, D. V., Wurst, W., Giese, A., & Pan-Montojo, F. (2022). Toxicity of extracellular alpha-synuclein is independent of intracellular alpha-synuclein. *Scientific Reports*, *12*, 21951.

- Ditlevsen, D. K., Povlsen, G. K., Berezin, V., & Bock, E. (2008). NCAM-induced intracellular signaling revisited. *Journal of Neuroscience Research*, *86*, 727–743.
- Dohler, F., Sepulveda-Falla, D., Krasemann, S., Altmepfen, H., Schlüter, H., Hildebrand, D., Zerr, I., Matschke, J., & Glatzel, M. (2014). High molecular mass assemblies of amyloid- $\beta$  oligomers bind prion protein in patients with Alzheimer's disease. *Brain*, *137*, 873–886.
- Domingues, R., Sant'Anna, R., da Fonseca, A. C. C., Robbs, B. K., Foguel, D., & Outeiro, T. F. (2022). Extracellular alpha-synuclein: Sensors, receptors, and responses. *Neurobiology of Disease*, *168*, 105696.
- Donne, D. G., Viles, J. H., Groth, D., Mehlhorn, I., James, T. L., Cohen, F. E., Prusiner, S. B., Wright, P. E., & Dyson, H. J. (1997). Structure of the recombinant full-length hamster prion protein PrP(29-231): The N terminus is highly flexible. *Proceedings of the National Academy of Sciences of the United States of America*, *94*, 13452–13457.
- Dossena, S., Imeri, L., Mangieri, M., Garofoli, A., Ferrari, L., Senatore, A., Restelli, E., Balducci, C., Fiordaliso, F., Salio, M., Bianchi, S., Fioriti, L., Morbin, M., Pincherle, A., Marcon, G., Villani, F., Carli, M., Tagliavini, F., Forloni, G., & Chiesa, R. (2008). Mutant prion protein expression causes motor and memory deficits and abnormal sleep patterns in a transgenic mouse model. *Neuron*, *60*, 598–609.
- Dupiereux, I., Falisse-Poirrier, N., Zorzi, W., Watt, N. T., Thellin, O., Zorzi, D., Pierard, O., Hooper, N. M., Heinen, E., & Elmoualij, B. (2008). Protective effect of prion protein via the N-terminal region in mediating a protective effect on paraquat-induced oxidative injury in neuronal cells. *Journal of Neuroscience Research*, *86*, 653–659.
- Eghaian, F., Grosclaude, J., Lesceu, S., Debey, P., Doublet, B., Treguer, E., Rezaei, H., & Knossow, M. (2004). Insight into the PrP<sup>C</sup>  $\rightarrow$  PrP<sup>Sc</sup> conversion from the structures of antibody-bound ovine prion scrapie-susceptibility variants. *Proceedings of the National Academy of Sciences of the United States of America*, *101*, 10254–10259.
- Eliezer, D., Kutluay, E., Bussell, R., & Browne, G. (2001). Conformational properties of alpha-synuclein in its free and lipid-associated states. *Journal of Molecular Biology*, *307*, 1061–1073.
- Emmanouilidou, E., Melachroinou, K., Roumeliotis, T., Garbis, S. D., Ntzouni, M., Margaritis, L. H., Stefanis, L., & Vekrellis, K. (2010). Cell-produced alpha-synuclein is secreted in a calcium-dependent manner by exosomes and impacts neuronal survival. *The Journal of Neuroscience*, *30*, 6838–6851.
- Emmenegger, M., De Cecco, E., Hruska-Plochan, M., Eninger, T., Schneider, M. M., Barth, M., Tantardini, E., de Rossi, P., Bacioglu, M., Langston, R. G., Kaganovich, A., Bengoa-Vergniory, N., Gonzalez-Guerra, A., Avar, M., Heinzer, D., Reimann, R., Häslér, L. M., Herling, T. W., Matharu, N. S., ... Aguzzi, A. (2021). LAG3 is not expressed in human and murine neurons and does not modulate  $\alpha$ -synucleinopathies. *EMBO Molecular Medicine*, *13*, e14745.
- Eraña, H., Venegas, V., Moreno, J., & Castilla, J. (2017). Prion-like disorders and transmissible spongiform encephalopathies: An overview of the mechanistic features that are shared by the various disease-related misfolded proteins. *Biochemical and Biophysical Research Communications*, *483*, 1125–1136.
- Ferreira, D. G., Temido-Ferreira, M., Vicente Miranda, H., Batalha, V. L., Coelho, J. E., Szegö, É. M., Marques-Morgado, I., Vaz, S. H., Rhee, J. S., Schmitz, M., Zerr, I., Lopes, L. V., & Outeiro, T. F. (2017). A-synuclein interacts with PrP<sup>C</sup> to induce cognitive impairment through mGluR5 and NMDAR2B. *Nature Neuroscience*, *20*, 1569–1579.
- Freir, D. B., Nicoll, A. J., Klyubin, I., Panico, S., Mc Donald, J. M., Risse, E., Asante, E. A., Farrow, M. A., Sessions, R. B., Saibil, H. R., Clarke, A. R., Rowan, M. J., Walsh, D. M., & Collinge, J. (2011). Interaction between prion protein and toxic amyloid  $\beta$  assemblies can be therapeutically targeted at multiple sites. *Nature Communications*, *2*, 336.
- Fujiwara, S., Kono, F., Matsuo, T., Sugimoto, Y., Matsumoto, T., Narita, A., & Shibata, K. (2019). Dynamic properties of human  $\alpha$ -synuclein related to propensity to amyloid fibril formation. *Journal of Molecular Biology*, *431*, 3229–3245.
- Fullard, M. E., Morley, J. F., & Duda, J. E. (2017). Olfactory dysfunction as an early biomarker in Parkinson's disease. *Neuroscience Bulletin*, *33*, 515–525.
- Fusco, G., Chen, S. W., Williamson, P. T. F., Cascella, R., Perni, M., Jarvis, J. A., Cecchi, C., Vendruscolo, M., Chiti, F., Cremades, N., Ying, L., Dobson, C. M., & de Simone, A. (2017). Structural basis of membrane disruption and cellular toxicity by  $\alpha$ -synuclein oligomers. *Science*, *358*, 1440–1443.
- García-Reitböck, P., Aichtchik, O., Bellucci, A., Iovino, M., Ballini, C., Fineberg, E., Ghetti, B., Della Corte, L., Spano, P. F., Tofaris, G. K., Goedert, M., & Spillantini, M. G. (2010). SNARE protein redistribution and synaptic failure in a transgenic mouse model of Parkinson's disease. *Brain*, *133*, 2032–2044.
- Gasperini, L., Meneghetti, E., Pastore, B., Benetti, F., & Legname, G. (2015). Prion protein and copper cooperatively protect neurons by modulating NMDA receptor through S-nitrosylation. *Antioxidants & Redox Signaling*, *22*, 772–784.
- Geering, K. (2008). Functional roles of Na,K-ATPase subunits. *Current Opinion in Nephrology and Hypertension*, *17*, 526–532.
- Goers, J., Manning-Bog, A. B., McCormack, A. L., Millett, I. S., Doniach, S., Di Monte, D. A., Uversky, V. N., & Fink, A. L. (2003). Nuclear localization of alpha-synuclein and its interaction with histones. *Biochemistry*, *42*, 8465–8471.
- Guerrero-Ferreira, R., Taylor, N. M., Arteni, A. A., Kumari, P., Mona, D., Ringler, P., Britschgi, M., Lauer, M. E., Makky, A., Verasdonck, J., Riek, R., Melki, R., Meier, B. H., Böckmann, A., Bousset, L., & Stahlberg, H. (2019). Two new polymorphic structures of human full-length alpha-synuclein fibrils solved by cryo-electron microscopy. *eLife*, *8*, e48907.
- Guerrero-Ferreira, R., Taylor, N. M., Mona, D., Ringler, P., Lauer, M. E., Riek, R., Britschgi, M., & Stahlberg, H. (2018). Cryo-EM structure of alpha-synuclein fibrils. *eLife*, *7*, e36402.
- Guglietti, B., Sivasankar, S., Mustafa, S., Corrigan, F., & Collins-Praino, L. E. (2021). Fyn kinase activity and its role in neurodegenerative disease pathology: A potential universal target? *Molecular Neurobiology*, *58*, 5986–6005.
- Guzzo, A., Delarue, P., Rojas, A., Nicolai, A., Maisuradze, G. G., & Senet, P. (2021). Missense mutations modify the conformational ensemble of the. *Frontiers in Molecular Biosciences*, *8*, 786123.
- Hansen, C., Björklund, T., Petit, G. H., Lundblad, M., Murmu, R. P., Brundin, P., & Li, J. Y. (2013). A novel  $\alpha$ -synuclein-GFP mouse model displays progressive motor impairment, olfactory dysfunction and accumulation of  $\alpha$ -synuclein-GFP. *Neurobiology of Disease*, *56*, 145–155.
- Haraguchi, T., Fisher, S., Olofsson, S., Endo, T., Groth, D., Tarentino, A., Borchelt, D. R., Teplow, D., Hood, L., Burlingame, A., Lycke, E., Kobata, A., & Prusiner, S. B. (1989). Asparagine-linked glycosylation of the scrapie and cellular prion proteins. *Archives of Biochemistry and Biophysics*, *274*, 1–13.
- Hijaz, B. A., & Volpicelli-Daley, L. A. (2020). Initiation and propagation of  $\alpha$ -synuclein aggregation in the nervous system. *Molecular Neurodegeneration*, *15*, 19.
- Holmqvist, S., Chutna, O., Bousset, L., Aldrin-Kirk, P., Li, W., Björklund, T., Wang, Z. Y., Roybon, L., Melki, R., & Li, J. Y. (2014). Direct evidence of Parkinson pathology spread from the gastrointestinal tract to the brain in rats. *Acta Neuropathologica*, *128*, 805–820.
- Hudák, A., Kusz, E., Domonkos, I., Jósavay, K., Kodamullil, A. T., Szilák, L., Hofmann-Apitius, M., & Letoha, T. (2019). Contribution of syndecans to cellular uptake and fibrillation of  $\alpha$ -synuclein and tau. *Scientific Reports*, *9*, 16543.
- Ingelsson, M. (2016). Alpha-synuclein oligomers-neurotoxic molecules in Parkinson's disease and other Lewy body disorders. *Frontiers in Neuroscience*, *10*, 408.
- Janežic, S., Threlfell, S., Dodson, P. D., Dowie, M. J., Taylor, T. N., Potgieter, D., Parkkinen, L., Senior, S. L., Anwar, S., Ryan, B., Deltheil, T., Kosillo, P., Cioroch, M., Wagner, K., Ansorge, O., Bannerman, D. M., Bolam, J. P., Magill, P. J., Cragg, S. J., & Wade-Martins, R. (2013). Deficits in dopaminergic transmission precede



- neuron loss and dysfunction in a new Parkinson model. *Proceedings of the National Academy of Sciences of the United States of America*, 110, E4016–E4025.
- Jao, C. C., Hegde, B. G., Chen, J., Haworth, I. S., & Langen, R. (2008). Structure of membrane-bound alpha-synuclein from site-directed spin labeling and computational refinement. *Proceedings of the National Academy of Sciences of the United States of America*, 105, 19666–19671.
- Jo, E., McLaurin, J., Yip, C. M., St George-Hyslop, P., & Fraser, P. E. (2000). Alpha-synuclein membrane interactions and lipid specificity. *The Journal of Biological Chemistry*, 275, 34328–34334.
- Kim, B. H., Lee, H. G., Choi, J. K., Kim, J. I., Choi, E. K., Carp, R. I., & Kim, Y. S. (2004). The cellular prion protein (PrP<sup>C</sup>) prevents apoptotic neuronal cell death and mitochondrial dysfunction induced by serum deprivation. *Molecular Brain Research*, 124, 40–50.
- Kim, H. Y., Cho, M. K., Kumar, A., Maier, E., Siebenhaar, C., Becker, S., Fernandez, C. O., Lashuel, H. A., Benz, R., Lange, A., & Zweckstetter, M. (2009). Structural properties of pore-forming oligomers of alpha-synuclein. *Journal of the American Chemical Society*, 131, 17482–17489.
- Kinoshita, P. F., Orellana, A. M. M., Nakao, V. W., de Souza Port's, N. M., Quintas, L. E. M., Kawamoto, E. M., & Scavone, C. (2022). The Janus face of ouabain in Na<sup>+</sup>/K<sup>+</sup>-ATPase and calcium signalling in neurons. *British Journal of Pharmacology*, 179, 1512–1524.
- Kontopoulos, E., Parvin, J. D., & Feany, M. B. (2006). Alpha-synuclein acts in the nucleus to inhibit histone acetylation and promote neurotoxicity. *Human Molecular Genetics*, 15, 3012–3023.
- Koss, D. J., Erskine, D., Porter, A., Palmoski, P., Menon, H., Todd, O. G. J., Leite, M., Attems, J., & Outeiro, T. F. (2022). Nuclear alpha-synuclein is present in the human brain and is modified in dementia with Lewy bodies. *Acta Neuropathologica Communications*, 10, 98.
- La Vitola, P., Beeg, M., Balducci, C., Santamaria, G., Restelli, E., Colombo, L., Caldinelli, L., Pollegioni, L., Gobbi, M., Chiesa, R., & Forloni, G. (2019). Cellular prion protein neither binds to alpha-synuclein oligomers nor mediates their detrimental effects. *Brain*, 142, 249–254.
- Lashuel, H. A., Petre, B. M., Wall, J., Simon, M., Nowak, R. J., Walz, T., & Lansbury, P. T. (2002). Alpha-synuclein, especially the Parkinson's disease-associated mutants, forms pore-like annular and tubular protofibrils. *Journal of Molecular Biology*, 322, 1089–1102.
- Lee, H. J., Suk, J. E., Bae, E. J., Lee, J. H., Paik, S. R., & Lee, S. J. (2008). Assembly-dependent endocytosis and clearance of extracellular alpha-synuclein. *The International Journal of Biochemistry & Cell Biology*, 40, 1835–1849.
- Legname, G. (2017). Elucidating the function of the prion protein. *PLoS Pathogens*, 13, e1006458.
- Li, B., Ge, P., Murray, K. A., Sheth, P., Zhang, M., Nair, G., Sawaya, M. R., Shin, W. S., Boyer, D. R., Ye, S., Eisenberg, D. S., Zhou, Z. H., & Jiang, L. (2018). Cryo-EM of full-length alpha-synuclein reveals fibril polymorphs with a common structural kernel. *Nature Communications*, 9, 3609.
- Li, Y., Zhao, C., Luo, F., Liu, Z., Gui, X., Luo, Z., Zhang, X., Li, D., Liu, C., & Li, X. (2018). Amyloid fibril structure of alpha-synuclein determined by cryo-electron microscopy. *Cell Research*, 28, 897–903.
- Liu, C., Zhao, Y., Xi, H., Jiang, J., Yu, Y., & Dong, W. (2021). The membrane interaction of alpha-synuclein. *Frontiers in Cellular Neuroscience*, 15, 633727.
- Liu, Y., Sorce, S., Nuvolone, M., Domange, J., & Aguzzi, A. (2018). Lymphocyte activation gene 3 (Lag3) expression is increased in prion infections but does not modify disease progression. *Scientific Reports*, 8, 14600.
- Manzanza, N. O., Sedlackova, L., & Kalaria, R. N. (2021). Alpha-synuclein post-translational modifications: Implications for pathogenesis of Lewy body disorders. *Frontiers in Aging Neuroscience*, 13, 690293.
- Mao, X., Ou, M. T., Karuppagounder, S. S., Kam, T. I., Yin, X., Xiong, Y., Ge, P., Umanah, G. E., Brahmachari, S., Shin, J. H., Kang, H. C., Zhang, J., Xu, J., Chen, R., Park, H., Andrabi, S. A., Kang, S. U., Gonçalves, R. A., Liang, Y., ... Dawson, T. M. (2016). Pathological alpha-synuclein transmission initiated by binding lymphocyte-activation gene 3. *Science*, 353, aah3374.
- Marin-Moreno, A., Espinosa, J. C., & Torres, J. M. (2020). Transgenic mouse models for the study of prion diseases. *Progress in Molecular Biology and Translational Science*, 175, 147–177.
- Marques, O., & Outeiro, T. F. (2012). Alpha-synuclein: From secretion to dysfunction and death. *Cell Death & Disease*, 3, e350.
- Masliyah, E., Rockenstein, E., Veinbergs, I., Mallory, M., Hashimoto, M., Takeda, A., Sagara, Y., Sisk, A., & Mucke, L. (2000). Dopaminergic loss and inclusion body formation in alpha-synuclein mice: Implications for neurodegenerative disorders. *Science*, 287, 1265–1269.
- Masuda-Suzukake, M., Nonaka, T., Hosokawa, M., Oikawa, T., Arai, T., Akiyama, H., Mann, D. M., & Hasegawa, M. (2013). Prion-like spreading of pathological alpha-synuclein in brain. *Brain*, 136, 1128–1138.
- Mehrabian, M., Wang, X., Eid, S., Yan, B. Q., Grinberg, M., Siegner, M., Sackmann, C., Sulman, M., Zhao, W., Williams, D., & Schmitt-Ulms, G. (2022). Cardiac glycoside-mediated turnover of Na, K-ATPases as a rational approach to reducing cell surface levels of the cellular prion protein. *PLoS One*, 17, e0270915.
- Murphy, D. D., Rueter, S. M., Trojanowski, J. Q., & Lee, V. M. (2000). Synucleins are developmentally expressed, and alpha-synuclein regulates the size of the presynaptic vesicular pool in primary hippocampal neurons. *The Journal of Neuroscience*, 20, 3214–3220.
- Nemani, V. M., Lu, W., Berge, V., Nakamura, K., Onoa, B., Lee, M. K., Chaudhry, F. A., Nicoll, R. A., & Edwards, R. H. (2010). Increased expression of alpha-synuclein reduces neurotransmitter release by inhibiting synaptic vesicle recluster after endocytosis. *Neuron*, 65, 66–79.
- Neupane, S., De Cecco, E., & Aguzzi, A. (2023). The hidden cell-to-cell trail of alpha-synuclein aggregates. *Journal of Molecular Biology*, 435, 167930.
- Nuvolone, M., Kana, V., Hutter, G., Sakata, D., Mortin-Toth, S. M., Russo, G., Danska, J. S., & Aguzzi, A. (2013). SIRP $\alpha$  polymorphisms, but not the prion protein, control phagocytosis of apoptotic cells. *The Journal of Experimental Medicine*, 210, 2539–2552.
- Ohnishi, T., Yanazawa, M., Sasahara, T., Kitamura, Y., Hiroaki, H., Fukazawa, Y., Kii, I., Nishiyama, T., Kakita, A., Takeda, H., Takeuchi, A., Arai, Y., Ito, A., Komura, H., Hirao, H., Satomura, K., Inoue, M., Muramatsu, S. I., Matsui, K., ... Hoshi, M. (2015). Na, K-ATPase  $\alpha$ 3 is a death target of Alzheimer patient amyloid- $\beta$  assembly. *Proceedings of the National Academy of Sciences of the United States of America*, 112, E4465–E4474.
- Outeiro, T. F., Putcha, P., Tetzlaff, J. E., Spoelgen, R., Koker, M., Carvalho, F., Hyman, B. T., & McLean, P. J. (2008). Formation of toxic oligomeric alpha-synuclein species in living cells. *PLoS One*, 3, e1867.
- Park, S. M., Jung, H. Y., Kim, T. D., Park, J. H., Yang, C. H., & Kim, J. (2002). Distinct roles of the N-terminal-binding domain and the C-terminal-solubilizing domain of alpha-synuclein, a molecular chaperone. *The Journal of Biological Chemistry*, 277, 28512–28520.
- Paumier, K. L., Luk, K. C., Manfredsson, F. P., Kanaan, N. M., Lipton, J. W., Collier, T. J., Steece-Collier, K., Kemp, C. J., Celano, S., Schulz, E., Sandoval, I. M., Fleming, S., Dirr, E., Polinski, N. K., Trojanowski, J. Q., Lee, V. M., & Sortwell, C. E. (2015). Intrastriatal injection of pre-formed mouse alpha-synuclein fibrils into rats triggers alpha-synuclein pathology and bilateral nigrostriatal degeneration. *Neurobiology of Disease*, 82, 185–199.
- Peters, P. J., Mironov, A., Jr., Peretz, D., van Donselaar, E., Leclerc, E., Erpel, S., DeArmond, S. J., Burton, D. R., Williamson, R. A., Vey, M., & Prusiner, S. B. (2003). Trafficking of prion proteins through a caveolae-mediated endosomal pathway. *The Journal of Cell Biology*, 162, 703–717.
- Petrushanko, I. Y., Mitkevich, V. A., Anashkina, A. A., Adzhubei, A. A., Burnysheva, K. M., Lakunina, V. A., Kamanina, Y. V., Dergousova, E. A., Lopina, O. D., Ogunshola, O. O., Bogdanova, A. Y., & Makarov, A. A. (2016). Direct interaction of beta-amyloid with Na,K-ATPase as a putative regulator of the enzyme function. *Scientific Reports*, 6, 27738.



- Pinho, R., Paiva, I., Jercic, K. G., Fonseca-Ornelas, L., Gerhardt, E., Fahlbusch, C., Garcia-Esparcia, P., Kerimoglu, C., Pavlou, M. A. S., Villar-Piqué, A., Szegő, É., da Fonseca, T. L., Odoardi, F., Soeroes, S., Rego, A. C., Fischle, W., Schwamborn, J. C., Meyer, T., Kügler, S., ... Outeiro, T. F. (2019). Nuclear localization and phosphorylation modulate pathological effects of alpha-synuclein. *Human Molecular Genetics*, 28, 31–50.
- Prusiner, S. B. (1982). Novel proteinaceous infectious particles cause scrapie. *Science*, 216, 136–144.
- Prusiner, S. B., Scott, M., Foster, D., Pan, K. M., Groth, D., Mirenda, C., Torchia, M., Yang, S. L., Serban, D., Carlson, G. A., Hoppe, P. C., Westaway, D., & DeArmond, S. J. (1990). Transgenic studies implicate interactions between homologous PrP isoforms in scrapie prion replication. *Cell*, 63, 673–686.
- Rangel, A., Madroñal, N., Gruart, A., Gavín, R., Llorens, F., Sumoy, L., Torres, J. M., Delgado-García, J. M., & Del Río, J. A. (2009). Regulation of GABA(a) and glutamate receptor expression, synaptic facilitation and long-term potentiation in the hippocampus of prion mutant mice. *PLoS One*, 4, e7592.
- Resenberger, U. K., Harmeier, A., Woerner, A. C., Goodman, J. L., Müller, V., Krishnan, R., Vabulas, R. M., Kretschmar, H. A., Lindquist, S., Hartl, F. U., Multhaup, G., Winklhofer, K. F., & Tatzelt, J. (2011). The cellular prion protein mediates neurotoxic signalling of  $\beta$ -sheet-rich conformers independent of prion replication. *The EMBO Journal*, 30, 2057–2070.
- Riek, R., Hornemann, S., Wider, G., Glockshuber, R., & Wuthrich, K. (1997). NMR characterization of the full-length recombinant murine prion protein, mPrP(23–231). *FEBS Letters*, 413, 282–288.
- Roguski, A., & Gill, A. C. (2017). The role of the mammalian prion protein in the control of sleep. *Pathogens*, 6, 58.
- Ruegsegger, C., Maharjan, N., Goswami, A., Filézac de L'Etang, A., Weis, J., Troost, D., Heller, M., Gut, H., & Saxena, S. (2016). Aberrant association of misfolded SOD1 with  $\text{Na}^+/\text{K}^+$ ATPase- $\alpha 3$  impairs its activity and contributes to motor neuron vulnerability in ALS. *Acta Neuropathologica*, 131, 427–451.
- Runfola, M., De Simone, A., Vendruscolo, M., Dobson, C. M., & Fusco, G. (2020). The N-terminal acetylation of  $\alpha$ -synuclein changes the affinity for lipid membranes but not the structural properties of the bound state. *Scientific Reports*, 10, 204.
- Saijo, E., Scheff, S. W., & Telling, G. C. (2011). Unaltered prion protein expression in Alzheimer disease patients. *Prion*, 5, 109–116.
- Salazar, S. V., & Strittmatter, S. M. (2017). Cellular prion protein as a receptor for amyloid- $\beta$  oligomers in Alzheimer's disease. *Biochemical and Biophysical Research Communications*, 483, 1143–1147.
- Sandal, M., Valle, F., Tessari, I., Mammi, S., Bergantino, E., Musiani, F., Brucale, M., Bubacco, L., & Samori, B. (2008). Conformational equilibria in monomeric alpha-synuclein at the single-molecule level. *PLoS Biology*, 6, e6.
- Santucci, A., Sytnyk, V., Leshchynska, I., & Schachner, M. (2005). Prion protein recruits its neuronal receptor NCAM to lipid rafts to activate p59fyn and to enhance neurite outgrowth. *The Journal of Cell Biology*, 169, 341–354.
- Schmitt-Ulms, G., Legname, G., Baldwin, M. A., Ball, H. L., Bradon, N., Bosque, P. J., Crossin, K. L., Edelman, G. M., DeArmond, S. J., Cohen, F. E., & Prusiner, S. B. (2001). Binding of neural cell adhesion molecules (N-CAMs) to the cellular prion protein. *Journal of Molecular Biology*, 314, 1209–1225.
- Schwarze-Eicker, K., Keyvani, K., Görtz, N., Westaway, D., Sachser, N., & Paulus, W. (2005). Prion protein (PrP<sup>Sc</sup>) promotes beta-amyloid plaque formation. *Neurobiology of Aging*, 26, 1177–1182.
- Scialò, C., Celauro, L., Zatonni, M., Tran, T. H., Bistaffa, E., Moda, F., Kammerer, R., Buratti, E., & Legname, G. (2021). The cellular prion protein increases the uptake and toxicity of TDP-43 fibrils. *Viruses*, 13, 1625.
- Scialò, C., De Cecco, E., Manganotti, P., & Legname, G. (2019). Prion and prion-like protein strains: Deciphering the molecular basis of heterogeneity in neurodegeneration. *Viruses*, 11, 261.
- Scialò, C., & Legname, G. (2020). The role of the cellular prion protein in the uptake and toxic signaling of pathological neurodegenerative aggregates. *Progress in Molecular Biology and Translational Science*, 175, 297–323.
- Shrivastava, A. N., Redeker, V., Fritz, N., Pieri, L., Almeida, L. G., Spolidoro, M., Liebmann, T., Bousset, L., Renner, M., Léna, C., Aperia, A., Melki, R., & Triller, A. (2015).  $\alpha$ -Synuclein assemblies sequester neuronal  $\alpha 3$ - $\text{Na}^+/\text{K}^+$ -ATPase and impair  $\text{Na}^+$  gradient. *The EMBO Journal*, 34, 2408–2423.
- Shyng, S. L., Heuser, J. E., & Harris, D. A. (1994). A glycolipid-anchored prion protein is endocytosed via clathrin-coated pits. *The Journal of Cell Biology*, 125, 1239–1250.
- Silva, J. L., Rangel, L. P., Costa, D. C., Cordeiro, Y., & De Moura Gallo, C. V. (2013). Expanding the prion concept to cancer biology: Dominant-negative effect of aggregates of mutant p53 tumour suppressor. *Bioscience Reports*, 33, e00054.
- Silva, J. L., Vieira, T. C., Cordeiro, Y., & de Oliveira, G. A. P. (2022). Nucleic acid actions on abnormal protein aggregation, phase transitions and phase separation. *Current Opinion in Structural Biology*, 73, 102346.
- Silva, M. O., Santejo, M., Babcock, I., Magalhães, A., Minamide, L. S., Castillo, E., Gerhardt, E., Fahlbusch, C., Swanson, R. A., Outeiro, T. F., Bamburg, J. R., & Liz, M. A. (2021). Cofilin pathology is a new player on  $\alpha$ -synuclein-induced spine impairment in models of hippocampal synucleinopathy. *bioRxiv*. <https://doi.org/10.1101/2021.02.02.425931>
- Slapšak, U., Salzano, G., Amin, L., Abskharon, R. N. N., Ilc, G., Zupančič, B., Biljan, I., Plavec, J., Giachin, G., & Legname, G. (2016). The N terminus of the prion protein mediates functional interactions with the neuronal cell adhesion molecule (NCAM) fibronectin domain. *The Journal of Biological Chemistry*, 291, 21857–21868.
- Steele, A. D., Emsley, J. G., Ozdinler, P. H., Lindquist, S., & Macklis, J. D. (2006). Prion protein (PrP<sup>Sc</sup>) positively regulates neural precursor proliferation during developmental and adult mammalian neurogenesis. *Proceedings of the National Academy of Sciences of the United States of America*, 103, 3416–3421.
- Steele, A. D., Lindquist, S., & Aguzzi, A. (2007). The prion protein knockout mouse: A phenotype under challenge. *Prion*, 1, 83–93.
- Stys, P. K., You, H., & Zamponi, G. W. (2012). Copper-dependent regulation of NMDA receptors by cellular prion protein: Implications for neurodegenerative disorders. *The Journal of Physiology*, 590, 1357–1368.
- Sun, J., Wang, L., Bao, H., Premi, S., Das, U., Chapman, E. R., & Roy, S. (2019). Functional cooperation of  $\alpha$ -synuclein and VAMP2 in synaptic vesicle recycling. *Proceedings of the National Academy of Sciences of the United States of America*, 116, 11113–11115. <https://doi.org/10.1073/pnas.1903049116>.
- Supattapone, S. (2014). Synthesis of high titer infectious prions with cofactor molecules. *The Journal of Biological Chemistry*, 289, 19850–19854.
- Takahashi, R. H., Tobiume, M., Sato, Y., Sata, T., Gouras, G. K., & Takahashi, H. (2011). Accumulation of cellular prion protein within dystrophic neurites of amyloid plaques in the Alzheimer's disease brain. *Neuropathology*, 31, 208–214.
- Tanaka, M., Kim, Y. M., Lee, G., Junn, E., Iwatsubo, T., & Mouradian, M. M. (2004). Aggregates formed by alpha-synuclein and synphilin-1 are cytoprotective. *The Journal of Biological Chemistry*, 279, 4625–4631.
- Thom, T., Schmitz, M., Fischer, A. L., Correia, A., Correia, S., Llorens, F., Pique, A. V., Möbius, W., Domingues, R., Zafar, S., Stoops, E., Silva, C. J., Fischer, A., Outeiro, T. F., & Zerr, I. (2021). Cellular prion protein mediates  $\alpha$ -synuclein uptake, localization, and toxicity in vitro and in vivo. *Movement Disorders*, 37, 39–51.
- Tobler, I., Gaus, S. E., Deboer, T., Achermann, P., Fischer, M., Rulicke, T., Moser, M., Oesch, B., McBride, P. A., & Manson, J. C. (1996). Altered circadian activity rhythms and sleep in mice devoid of prion protein. *Nature*, 380, 639–642.
- Trepanier, C. H., Jackson, M. F., & MacDonald, J. F. (2012). Regulation of NMDA receptors by the tyrosine kinase Fyn. *The FEBS Journal*, 279, 12–19.



- Um, J. W., Nygaard, H. B., Heiss, J. K., Kostylev, M. A., Stagi, M., Vortmeyer, A., Wisniewski, T., Gunther, E. C., & Strittmatter, S. M. (2012). Alzheimer amyloid- $\beta$  oligomer bound to postsynaptic prion protein activates Fyn to impair neurons. *Nature Neuroscience*, *15*, 1227–1235.
- Urrea, L., Segura-Feliu, M., Masuda-Suzukake, M., Hervera, A., Pedraz, L., García Aznar, J. M., Vila, M., Samitier, J., Torrents, E., Ferrer, I., Gavín, R., Hagesawa, M., & del Río, J. A. (2018). Involvement of cellular prion protein in  $\alpha$ -synuclein transport in neurons. *Molecular Neurobiology*, *55*, 1847–1860.
- Uversky, V. N., Lee, H. J., Li, J., Fink, A. L., & Lee, S. J. (2001). Stabilization of partially folded conformation during alpha-synuclein oligomerization in both purified and cytosolic preparations. *The Journal of Biological Chemistry*, *276*, 43495–43498.
- Vergara, C., Ordóñez-Gutiérrez, L., Wandosell, F., Ferrer, I., del Río, J. A., & Gavín, R. (2015). Role of PrP<sup>C</sup> expression in tau protein levels and phosphorylation in Alzheimer's disease evolution. *Molecular Neurobiology*, *51*, 1206–1220.
- Vieira, T., & Silva, J. (2016). Glycosaminoglycans in prion and prion-like diseases. In G. G. Giuseppe Legname (Ed.), *The prion phenomena in neurodegenerative diseases* (Vol. 1, pp. 67–87). Nova Science Pub Inc.
- Weise, J., Crome, O., Sandau, R., Schulz-Schaeffer, W., Bähr, M., & Zerr, I. (2004). Upregulation of cellular prion protein (PrP<sup>C</sup>) after focal cerebral ischemia and influence of lesion severity. *Neuroscience Letters*, *372*, 146–150.
- Whitehouse, I. J., Jackson, C., Turner, A. J., & Hooper, N. M. (2010). Prion protein is reduced in aging and in sporadic but not in familial Alzheimer's disease. *Journal of Alzheimer's Disease*, *22*, 1023–1031.
- Williams, D., Mehrabian, M., Arshad, H., Eid, S., Sackmann, C., Zhao, W., Wang, X., Ghodrati, F., Verkuyl, C. E., Watts, J. C., & Schmitt-Ulms, G. (2021). The cellular prion protein interacts with and promotes the activity of Na,K-ATPases. *PLoS One*, *16*, e0258682.
- Winner, B., Jappelli, R., Maji, S. K., Desplats, P. A., Boyer, L., Aigner, S., Hetzer, C., Loher, T., Vilar, M., Campioni, S., Tzitzilonis, C., Soragni, A., Jessberger, S., Mira, H., Consiglio, A., Pham, E., Masliah, E., Gage, F. H., & Riek, R. (2011). In vivo demonstration that alpha-synuclein oligomers are toxic. *Proceedings of the National Academy of Sciences of the United States of America*, *108*, 4194–4199.
- Wopfner, F., Weidenhofer, G., Schneider, R., von Brunn, A., Gilch, S., Schwarz, T. F., Werner, T., & Schatzl, H. M. (1999). Analysis of 27 mammalian and 9 avian PrPs reveals high conservation of flexible regions of the prion protein. *Journal of Molecular Biology*, *289*, 1163–1178.
- Wu, K. P., Weinstock, D. S., Narayanan, C., Levy, R. M., & Baum, J. (2009). Structural reorganization of alpha-synuclein at low pH observed by NMR and REMD simulations. *Journal of Molecular Biology*, *391*, 784–796.
- Wulf, M. A., Senatore, A., & Aguzzi, A. (2017). The biological function of the cellular prion protein: An update. *BMC Biology*, *15*, 34.
- Zhang, Y., Zhao, Y., Zhang, L., Yu, W., Wang, Y., & Chang, W. (2019). Cellular prion protein as a receptor of toxic amyloid- $\beta$ 42 oligomers is important for Alzheimer's disease. *Frontiers in Cellular Neuroscience*, *13*, 339.
- Zündorf, G., & Reiser, G. (2011). Calcium dysregulation and homeostasis of neural calcium in the molecular mechanisms of neurodegenerative diseases provide multiple targets for neuroprotection. *Antioxidants & Redox Signaling*, *14*, 1275–1288.

**How to cite this article:** Vieira, T. C. R. G., Barros, C. A., Domingues, R., & Outeiro, T. F. (2023). PrP meets alpha-synuclein: Molecular mechanisms and implications for disease. *Journal of Neurochemistry*, *00*, 1–15. <https://doi.org/10.1111/jnc.15992>