


REVIEW

Effects of alpha-synuclein post-translational modifications on metal binding

Nazareno González^{*1}, Trinidad Arcos-López^{†1}, Annekatrin König[‡], Liliana Quintanar[†], Mauricio Menacho Márquez^{* }, Tiago F. Outeiro^{‡§¶ } and Claudio O. Fernández^{***}

^{*}Max Planck Laboratory for Structural Biology, Chemistry and Molecular Biophysics of Rosario (MPLbioR, UNR-MPIbpC), Instituto de Investigaciones para el Descubrimiento de Fármacos de Rosario (IIDEFAR, UNR-CONICET), Universidad Nacional de Rosario, Rosario, Argentina

[†]Department of Chemistry, Center for Research and Advanced Studies (Cinvestav), Mexico City, Mexico

[‡]Department of Experimental Neurodegeneration, Center for Biostructural Imaging of Neurodegeneration, University Medical Center Göttingen, University of Göttingen, Göttingen, Germany

[§]Max Planck Institute for Experimental Medicine, Göttingen, Germany

[¶]Institute of Neuroscience, The Medical School, Newcastle University, Framlington Place, Newcastle Upon Tyne, UK

^{***}Department of NMR-based Structural Biology, Max Planck Institute for Biophysical Chemistry, Göttingen, Germany

Abstract

Parkinson's disease is the second most common neurodegenerative disorder worldwide. Neurodegeneration in this pathology is characterized by the loss of dopaminergic neurons in the substantia nigra, coupled with cytoplasmic inclusions known as Lewy bodies containing α -synuclein. The brain is an organ that concentrates metal ions, and there is emerging evidence that a break-down in metal homeostasis may be a critical factor in a variety of neurodegenerative diseases. α -synuclein has emerged as an important metal-binding protein in the brain, whereas these interactions play an important role in its aggregation and might represent a link between protein aggregation, oxidative damage, and neuronal cell loss. Additionally, α -synuclein undergoes several post-translational

modifications that regulate its structure and physiological function, and may be linked to the aggregation and/or oligomer formation. This review is focused on the interaction of this protein with physiologically relevant metal ions, highlighting the cases where metal-AS interactions profile as key modulators for its structural, aggregation, and membrane-binding properties. The impact of α -synuclein phosphorylation and N-terminal acetylation in the metal-binding properties of the protein are also discussed, underscoring a potential interplay between PTMs and metal ion binding in regulating α -synuclein physiological functions and its role in pathology.

Keywords: metal ions, neurons, Parkinson disease, post-translational modification, protein structure, synuclein.

J. Neurochem. (2019) **150**, 507–521.

[This article is part of the Special Issue "Synuclein".](#)

Received February 28, 2019; revised manuscript received April 11, 2019; accepted May 13, 2019.

Address correspondence and reprint requests to Claudio O. Fernández, Max Planck Laboratory for Structural Biology, Chemistry and Molecular Biophysics of Rosario (MPLbioR, UNR-MPIbpC) and Instituto de Investigaciones para el Descubrimiento de Fármacos de Rosario (IIDEFAR, UNR-CONICET), Universidad Nacional de Rosario, Ocampo y Esmeralda, S2002LRK Rosario, Argentina. E-mail: fernandez@iidefar-conicet.gob.ar and cfernan@gwdg.de

¹These authors contributed equally to this work.

Abbreviations: AcAS, acetylated α -synuclein; AS, α -synuclein; CEST, chemical exchange saturation transfer; CV, cyclic voltammetry; DLB, dementia with lewy bodies; ES-MS, electrospray mass spectrometry; IN-MS, ion mobility-mass spectrometry; ITC, isothermal titration calorimetry; MD, molecular dynamics; MSA, multiple system atrophy; NAC, non-amyloid component region; NMR, nuclear magnetic resonance; PD, Parkinson's disease; PTMs, post-translational modifications; SAXS, X-ray scattering; SUMO, small Ubiquitin-like MODifier; TEM, transmission electron microscopy.

The protein α -synuclein (AS) is a small cytosolic protein of 140 amino acids (~14.5 kDa) that belongs to the synuclein family (together with beta-synuclein and gamma-synuclein), a closely related group of brain-enriched, intrinsically disordered proteins that is expressed in vertebrates (Maroteaux *et al.* 1988). The primary structure of AS can be divided in three characteristic regions: the N-terminal region (residues 1–60) that contains imperfect repeats of the sequence KTKEGV and is involved in lipid binding; the non-amyloid component region (NAC, residues 61–95), which is highly hydrophobic and fibrillogenic; and the C-terminal region (residues 96–140), which is rich in Pro, Asp, and Glu residues that are essential to block rapid AS filament assembly (Fig. 1). Although AS is an intrinsically disordered protein and lacks a defined secondary structure, long-range intramolecular contacts between the C-terminal and the NAC and N-terminal regions have been identified (Bertoncini *et al.* 2005; Fauvet *et al.* 2012; Theillet *et al.* 2016). In the cytosol, AS is proposed to exist as a monomeric protein in which the NAC region tends to be partially shielded from the solvent, induced by transient intramolecular interactions that delay intermolecular aggregation. The protein can adopt an α -helical structure at its N-terminal region when associated with membranes, characterized by the formation of two antiparallel α -helices involving residues 1–37 and 45–92 (Davidson *et al.* 1998; Bussell and Eliezer 2003; Ulmer *et al.* 2005; Borbat *et al.* 2006). α -synuclein is predominantly expressed in pre-synaptic terminals of neurons of the central nervous system, making up to 1% of total soluble protein in the human brain. Physiological roles proposed for this protein include uptake, storage, recycling of neurotransmitter vesicles, and maintenance of dopamine levels (Surguchov *et al.* 1999; Maroteaux *et al.* 1988; Stefanis 2012; Sidhu *et al.* 2004; Yavich *et al.* 2004).

The aggregation of AS is associated to neuropathological disorders such as Parkinson's disease (PD), dementia with Lewy bodies, and multiple system atrophy, collectively referred to as synucleinopathies (Spillantini *et al.* 1997; Volles and Lansbury 2003). Pioneering epidemiological and biochemical studies have linked exposure to heavy metals to the onset of PD (Dexter *et al.* 1991; Gorell *et al.* 1999). Since then, several studies have demonstrated that metal ions can interact with AS, having an important impact in its aggregation (Paik *et al.* 1999; Uversky *et al.* 2001; Natalello *et al.* 2010). Moreover, the homeostasis of essential metal ions, such as Cu and Fe, are affected in Parkinson's disease, as reviewed recently (Garza-Lombó *et al.* 2018). It has also been suggested that Mn exposure could be an environmental factor for the development of this disease (Calne *et al.* 1994); whereas an intricate relation between AS expression and metal ions levels has been revealed (Ducic *et al.* 2015). Using synchrotron-based X-ray fluorescence, it was observed in primary midbrain neurons that over-expression of AS

increases intracellular Mn levels, while decreasing Ca levels. Interestingly, a strong correlation was observed between high concentrations of Mn in neurons with the concentration of other essential metals, such as Cu and Fe (Ducic *et al.* 2015). Moreover, it was demonstrated that Mn promotes the aggregation and the transmission in a prion-like cell-to-cell exosomal manner of AS (Harischandra *et al.* 2019). Overall, these findings have led to the proposal that AS may act as an intracellular store of metal ions in the cell (Peres *et al.* 2016).

While the putative role of AS in metal ion homeostasis remains to be investigated, it is clear that it is a metal-binding protein and that metal ions may play an important role in its aggregation and the etiology of Parkinson's disease. Thus, understanding the nature of AS-metal ion interactions and how they impact in AS properties can provide a link between the accumulation of aggregated proteins, oxidative damage in the brain and neuronal death. The interactions of metal ions with AS and their potential role in Parkinson's disease have been extensively studied in the last two decades, as reviewed in several works (Santnera and Uversky 2010; Miller *et al.* 2012; Leal *et al.* 2012; Viles 2012; Binolfi *et al.* 2012). In this manuscript, an objective review of the most relevant and recently discovered aspects of AS-metal interactions is presented, followed by a discussion on how post-translational modifications may impact these key protein-metal interactions and influence its aggregation and toxicity.

α -synuclein is a metal-binding protein

Metal-anchoring sites for divalent ions in α -synuclein

Early studies showed that metal ions such as Al(III), Cu(II), and Fe(III) can impact the fibrillation rate of AS, possibly by inducing a partially folded state that is more prone to aggregate than the monomeric protein (Fig. 2a) (Paik *et al.* 1999; Uversky *et al.* 2001). Specific effects of metal ions on the nature of AS oligomeric species have also been described: Ca(II) and Co(II) produce high amounts of annular oligomers, whereas Cu(II) and Fe(II) only enhance the population of oligomeric intermediates without modifying the nature of AS oligomers (Lowe *et al.* 2004). However, the metal ion concentrations used in those pioneering studies were in the millimolar range, far greater than those normally occurring in tissues. Subsequent seminal work evaluated the impact of divalent metal ions such as Fe(II), Mn(II), Zn(II), Cu(II), Co(II), and Ni(II) using physiologically relevant micromolar concentrations of these ions (Rasia *et al.* 2005; Binolfi *et al.* 2006). These studies showed that Cu(II) is the only metal ion that accelerates the formation of amyloid fibrils under these conditions. Two-dimensional heteronuclear magnetic resonance (NMR) spectroscopy in combination with mutagenesis studies revealed that metal ions interact with AS at its C-terminal domain, which contains several Asp and Glu residues that strongly attract

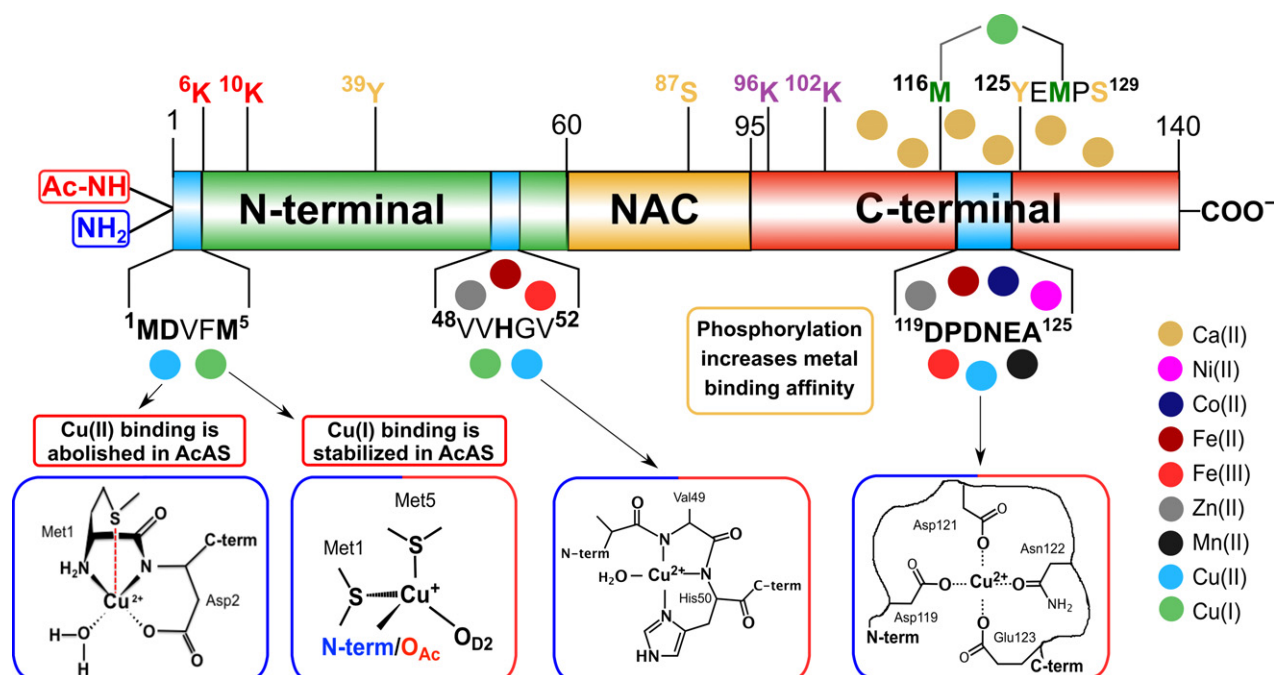


Fig. 1 Metal binding properties and post-translational modifications of AS. AS displays three anchoring sites for metal ion binding: the low affinity, non-specific metal binding site at the acidic DPDNEA segment in the C-terminal (displayed with coloured circles); the His50 site and the first five residues at the N-terminal. His50 can anchor transition-metal ions such as Zn(II), Fe(III), and Fe(II), but it displays higher affinity for Cu(II) and Cu(I) ions. The N-terminus of AS is a high affinity and highly specific site for Cu ions. AS also displays multiple Ca(II) binding sites at

its C-terminal region. The location of AS phosphorylation, sumoylation, and acetylation sites are also shown above: acetylation occurs at the NH₂ terminus (marked in red), Lys6 and Lys10; sumoylation occurs at Lys96 and Lys102 (marked in purple); and phosphorylation occurs at Tyr39, Ser87, Tyr125, and Ser129 (marked in yellow). While acetylation of the NH₂ group of AS abolishes Cu(II) binding, it conserves its Cu(I) binding site, using the acetyl group as a ligand. Finally, phosphorylation at the C-terminal increases metal-binding affinity.

cations and positively charged molecules (Binolfi *et al.* 2006). The ¹¹⁹DPDNEA¹²⁴ sequence at the C-terminal domain was identified as the primary anchoring binding site (site 3) for all divalent metal ions mentioned above (Fig. 1). This non-specific metal-binding site at the C-terminus of AS displays low-binding affinities (conditional $K_D \sim 10^{-6}$ M) (Rasia *et al.* 2005; Binolfi *et al.* 2006), consistent with the electrostatic nature of these AS-metal interactions. On the other hand, the study of Cu(II) binding to AS revealed the presence of two specific binding sites for this metal ion at the N-terminal region of AS: the N-terminal tail encompassing the first six residues ¹MDVFMK⁶ (site 1) and His50 (site 2), the only histidine site in the whole protein (Fig. 1) (Binolfi *et al.* 2006; Binolfi *et al.* 2008). The Cu(II) binding affinity of these two sites are significantly higher than that for the C-terminal site, with conditional K_D values in the nanomolar range, revealing a more physiologically relevant interaction of AS with Cu(II) ions. Overall, these studies demonstrated conclusively that the effect of divalent metal ions on AS fibrillation is determined by their binding properties. Moreover, the strong link between specificity of metal binding to AS and effectiveness in accelerating

filament assembly revealed a hierarchy of AS-metal interactions that might reflect both biological and structural effects.

While the copper-binding properties of AS are discussed below in more detail, it is important to note here that His50 has also been proposed as an anchoring site for weaker interactions with other essential transition-metal ions, such as Zn(II) and Fe(II). An NMR study revealed the presence of two independent, non-interacting Zn(II) sites: one at His50 and another one at the C-terminal Asp121 residue, both sites with similar apparent dissociation constants in the millimolar range. The low affinity that characterizes Zn(II) binding to AS challenges the biological relevance of AS-Zn(II) interactions (Valiente-Gabioud *et al.* 2012). On the other hand, Fe(II) and Fe(III) have also been proposed to interact with AS at the His50 and C-terminal sites with low affinity (Binolfi *et al.* 2006; Bisaglia *et al.* 2009). Isothermal titration calorimetry studies suggested a single binding site for Fe(III) with an apparent $K_D \sim 10^{-5}$ M (Bharathi and Rao 2007), whereas the possibility of two independent binding sites for Fe(III) with similar affinity has also been suggested (Davies *et al.* 2011). A pioneering

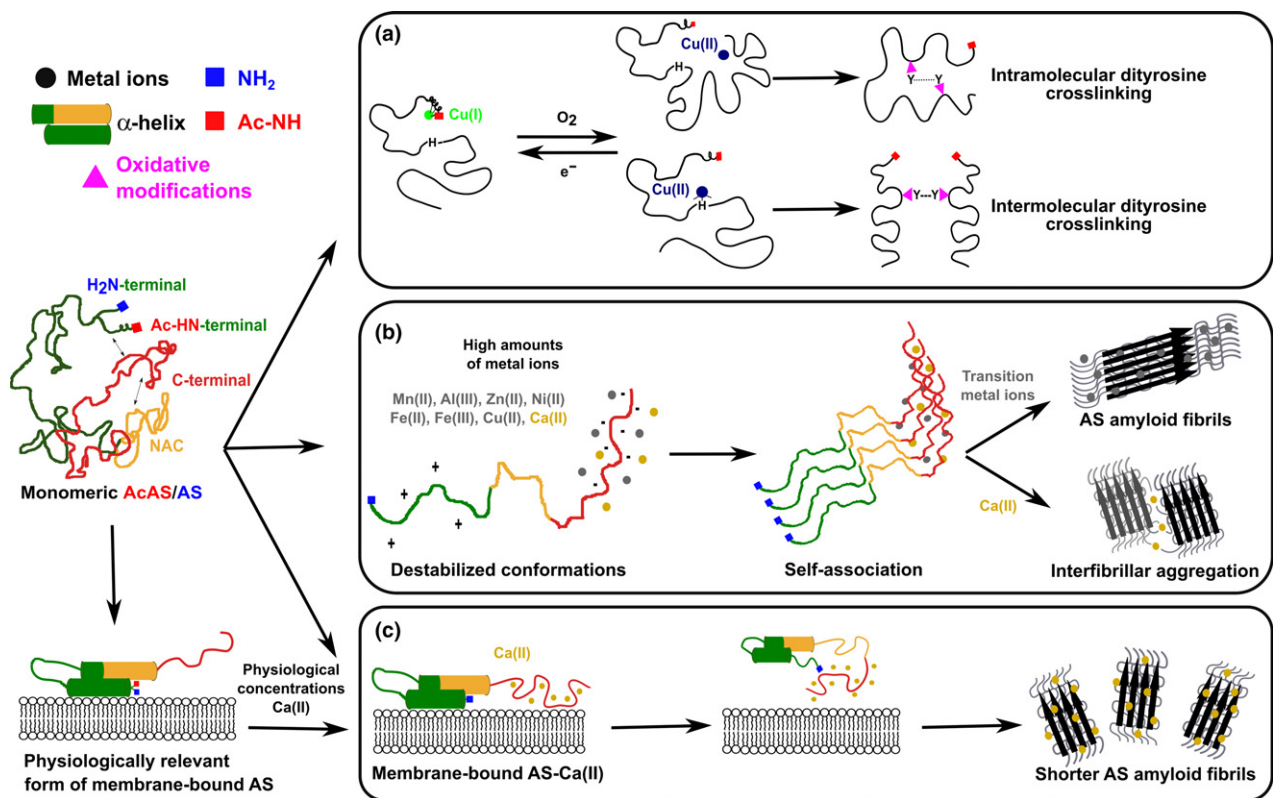


Fig. 2 Molecular mechanisms involved in the enhancement of AS/AcAS aggregation mediated by metal ions. Monomeric AS displays transient long-range intramolecular contacts between the C-terminal and N-terminal regions, whereas an enhancement of α -helix content in the first residues of N-terminal region has been identified in the monomeric AcAS. (a) Cu(II)/Cu(I) redox chemistry of Cu-AcAS can generate aberrant interactions that produce protein aggregates showing both, intramolecular and intermolecular, dityrosine crosslinking. (b) High amounts of metal ions (Mn(II), Al(III), Zn(II), Ni(II), Fe(II), Fe(III), Cu(II), and Ca(II)) interact with the negatively charged C-terminus of AS, leading to destabilization of long-range intramolecular

study using electrospray mass spectrometry (ES-MS), cyclic voltammetry, and fluorescence spectroscopy revealed the formation of an AS-Fe(II) complex with a 1:1 stoichiometry and a reduction potential ~ 0.025 V vs. Ag/AgCl (Peng *et al.* 2010). The ferrous complex can be readily oxidized electrochemically and chemically by O₂ to a putative AS-Fe(III) complex, with H₂O₂ as a co-product. Most recently, the effects of the redox activity of iron complexes with the acetylated α -synuclein (AcAS) on the aggregation properties of the protein were studied (Abeyawardhane *et al.* 2018a). In the absence of O₂, both, Fe(II) and Fe(III)-AcAS complexes polymerized into parallel β -sheet aggregates, whereas under aerobic conditions, the ferrous complex contains a substantially different structure with right-twisted antiparallel β -sheets, displaying elevated toxicity. This seminal study demonstrated that the oxygen reactivity of the AcAS-Fe(II) complex can impact

interactions and promotion of AS self-association. However, transition-metal ions lead to the formation of amyloid fibrils, whereas high amounts of Ca(II) ions promote interfibrillar aggregation. On the other hand, AS and AcAS can adopt an α -helical structure at its N-terminal region when associated with membranes. (C) Ca(II) interactions with AS enhance its association to membranes through the C-terminal. This abnormal membrane association of the acidic tail of AS leads to the formation of short fibrils, showing aggravated bundling. Non-acetylated N-terminal (blue squares), acetylated N-terminal (red squares), metal ions (gray, yellow, blue and green circles), oxidative modifications (pink triangles).

protein conformation and its aggregated structural properties, yielding species that could be relevant to physiological or pathological implications (Abeyawardhane *et al.* 2018a). Unfortunately, while there is a notion that Fe ions may interact with AS at His50 and the C-terminal region, the structural details of these putative Fe binding sites in AS remain to be elucidated.

In summary, AS displays three different anchoring sites for metal ions: the N-terminal tail, His50, and the acidic region in the C-terminal (Fig. 1). While the C-terminal site displays low affinity and non-specific interactions with cations, such as Ca(II), Fe(II), Mn(II), Zn(II), and Cu(II); His50 can anchor metal ions with a taste for imidazole ligands, such as Fe(II), Zn(II), and Cu(II). Of all essential metal ions that AS encounters *in vivo*, the specific interactions of Cu ions with His50 and the N-terminal group are the most physiologically relevant, given the high affinity for copper at these anchoring

sites. Finally, more recent evidence supports an important role for AS in calcium-dependent vesicular trafficking, associated to AS-Ca(II) interactions (Lautenschläger *et al.* 2018). Hence, in the following sections detailed discussions of the interactions of this protein with calcium and copper ions are presented.

Interactions of α -synuclein with calcium

Calcium has a great number of diverse functions in biology and is present as Ca(II) ions. Neuronal Ca(II) homeostasis and signaling regulate multiple neuronal functions, including synaptic transmission, plasticity, and cell survival (Mattson 2007). In healthy neurons, under resting conditions, free cytosolic Ca(II) levels are maintained around 200 nM, and rise to low micromolar concentrations by a mechanism of extracellular Ca(II) influx or Ca(II) release from intracellular stores upon stimulation, whereas its extracellular concentration is ~ 1.4 mM (Schaafsma 1988; Marambaud *et al.* 2009). While resting, intracellular Ca(II) concentrations do not increase, the return time to resting levels after a stimulus is greatly reduced in aged neurons. Nevertheless, dysregulation of Ca(II) homeostasis is observed in a mice model of α -synucleinopathies (Reznichenko *et al.* 2012), whereas a significant amount of Ca(II) has been detected in Lewy bodies of patients with Parkinson's disease (Kimula *et al.* 1983). On the other hand, it has been proposed that over-expression of AS increases mitochondrial and cytosolic Ca(II), causing cell death (Cali *et al.* 2012; Caraveo *et al.* 2014; Angelova *et al.* 2016). Calcium ions can trigger AS aggregation *in vitro*, even at low micromolar concentrations, and thus, it has been proposed that Ca(II) might induce cytoplasmic AS aggregates in cells (Nath *et al.* 2011; Follett *et al.* 2012; Rcom-H'cheo-Gauthier *et al.* 2014). Accordingly, the study of AS-Ca(II) interactions and their role in protein aggregation and Parkinson's disease became an active area of research in the last years (Rcom-H'cheo-Gauthier *et al.* 2016; Zaichick *et al.* 2017). Here we summarized the most important recent findings.

The binding of multiple Ca(II) ions (up to four) to AS (Fig. 1) has been demonstrated using multiple biophysical methods, including ion mobility-mass spectrometry (IM-MS), synchrotron small-angle X-ray scattering and transmission electron microscopy in combination with molecular dynamics (MD) (Han *et al.* 2018). As observed with other divalent metal ions, Ca(II) was shown to induce a structural transition of AS monomers to extended conformations, promoting the exposure of the NAC region and rapid AS fibrillation. The study concluded that Ca(II) ions also trigger non-specific inter-fibrillar aggregation via electrostatic and hydrophobic interactions to produce large AS aggregates (Fig. 2c) (Han *et al.* 2018). Several studies have demonstrated that Ca(II) binds to AS through the C-terminal region (Lowe *et al.* 2004; de Laureto *et al.* 2006); and it interferes with normal AS-membrane interactions. A study employing

site-directed fluorescence labeling of recombinant human AS demonstrated that, in the absence of Ca(II) ions, AS interacts with membranes via its N-terminal domain; whereas the presence of Ca(II) causes an interaction of the C-terminal domain with lipids. This abnormal membrane association of the acidic tail of AS led to the formation of short fibrils that showed aggravated bundling (Fig. 2c) (Tamamizu-Kato *et al.* 2006). Another study used disk-like phospholipid bilayers and small unilamellar vesicles to mimic membranes, found that Ca(II) competes with AS for the binding sites of phospholipids and exerts a negative modulatory effect on the association of AS to membranes, resulting in dissociation of the protein from the membrane surface (Zhang *et al.* 2014). On the other hand, using a microdialysis technique it was proposed that AS binds Ca(II) at the C-terminal region with a $K_D = 2\text{--}300$ μM (Nielsen *et al.* 2001). However, recent NMR and mass spectrometry results suggest that it can bind up to 6-8 calcium ions with a K_D in the range 21 μM (Lautenschläger *et al.* 2018). This dissociation constant lies well within the range of physiological pre-synaptic calcium fluctuations, reaching up to hundreds of micromolar in healthy neurons upon neuronal stimulation. In the same study it was also demonstrated that AS-Ca(II) interaction increases the AS lipid-binding capability. Chemical exchange saturation transfer in solution-state NMR experiments reveal that AS interacts with isolated synaptic vesicles at the N terminus and via its C terminus, which is regulated by the binding of calcium. These findings suggest that under physiological conditions AS might act as a calcium-dependent modulator of vesicle homeostasis at the pre-synaptic terminal, with calcium mediating the localization of AS at the pre-synaptic terminal (Lautenschläger *et al.* 2018). Moreover, it has been proposed also that AS might play a key role in calcium transfer from endoplasmic reticulum to mitochondria and that this function requires the presence of the C-terminal domain of the protein (Cali *et al.* 2012). Indeed, an imbalance in calcium or AS can lead to synaptic vesicle clustering, as seen *ex vivo* and *in vitro*, suggesting that high levels of calcium and/or AS are key elements to neuronal toxicity (Lautenschläger *et al.* 2018). In summary, calcium ions emerge as key modulators of the interaction of AS with synaptic vesicles, underscoring the important role they may play under physiological and cell toxicity conditions.

Interactions of α -synuclein with copper

Copper binding to AS has been studied in detail in the last decades, using a wide range of spectroscopic tools, as reviewed by (Santnera and Uversky 2010; Binolfi *et al.* 2012; Viles 2012). As mentioned above, AS displays three Cu(II) coordination sites with different structural and affinity features (Fig. 1). The C-terminal site $^{119}\text{DPDNEA}^{124}$ (site 3) displays low affinity and low specificity for divalent metal ions, and Cu(II) ions are not the exception. In contrast, the N-terminal region has high specificity for Cu(II) ions,

displaying two anchoring sites: His50 (site 2) and the N-terminal NH₂ group (site 1) (Rasia *et al.* 2005; Binolfi *et al.* 2006; Drew *et al.* 2008; Lee *et al.* 2008; Binolfi *et al.* 2008; Binolfi *et al.* 2010; Ahmad *et al.* 2012). Cu(II) binding to His50 is pH-dependent; at physiological pH a species is stabilized where the metal ion is anchored by the imidazole ring and two deprotonated amide groups to yield a 3N1O coordination mode, with an oxygen atom from a carbonyl group or a water molecule (Fig. 1) (Binolfi *et al.* 2008; Binolfi *et al.* 2010; Valensin *et al.* 2011; Villar-Piqué *et al.* 2017). Finally, the highest affinity site for Cu(II) is located at the N-terminus of AS, where the metal ion is anchored by the NH₂ group, a deprotonated backbone amide, the carboxylate group belonging to Asp2 and a water molecule, yielding a 2N2O equatorial coordination mode (Fig. 1) (Jackson and Lee 2009; Binolfi *et al.* 2010). In addition, the participation of Met1 as an axial ligand in site 1 has been proposed (Rodríguez *et al.* 2016).

As mentioned above, *in vitro* studies indicate that AS binds through its N-terminal region to micelles and phospholipids vesicles resulting in a conformational change from unstructured to α -helical form (Davidson *et al.* 1998; Pfefferkorn and Lee 2010). This phenomenon is relevant for the proposed physiological function of AS, as a regulator of vesicle-cell membrane fusion (Bonini and Giasson 2005; Darios *et al.* 2010; Burré *et al.* 2010). Since Cu coordination to AS is predominantly through the N-terminal and His50, the effect of Cu(II) binding on AS-lipid interaction has been studied. Monitoring changes in the Trp fluorescence properties of an AS F4W variant, it was demonstrated that 1 equivalent of Cu(II) increases the α -helix content of membrane-bound AS by 7%, as compared to the metal free condition. Moreover, the Cu(II) binding affinity for AS is the same or higher in the membrane-bound protein (Lucas and Lee 2011; Dudzik *et al.* 2013). Since AS-membrane association is relevant to the native function of the protein, alterations in cellular copper levels could influence protein conformation and its normal function as it has been shown in a dopaminergic neuroblastoma cellular model where copper exposure increases AS toxicity (Anandhan *et al.* 2015).

On the other hand, copper is a redox-active metal that can promote oxidative damage to proteins. Indeed, the catalytic oxidase activity of the AS-Cu(II) complexes was shown to yield formation of hydroxyl radicals and AS oxidation (Wang *et al.* 2010; Meloni and Vařák 2011). An NMR study demonstrated that the redox cycling of copper bound to AS can generate reactive oxygen species, leading to site-specific metal-catalyzed oxidation on methionine residues under physiologically relevant conditions (Miotto *et al.*, 2014, 2014b). Met1 and Met5 residues can be oxidized rapidly (Met1 faster than Met5) to a sulfoxide species after air exposure of the AS-Cu(I) complexes, whereas Met116 and Met127 remain unaffected (Miotto *et al.*, 2014, 2014b). The sulfoxide species formed at the N-terminal of AS can suffer

elimination of methanesulfonic acid, rendering a species with no thioether moiety (Rodríguez *et al.* 2016). Another consequence of the Cu(I)/O₂ chemistry of the AS-copper complex is the formation of dityrosine crosslinking species (Fig. 2b) (Lucas *et al.* 2010; Tiwari *et al.* 2018). These site-specific Cu-catalyzed oxidation and modifications of AS have been proposed to participate in the mechanism of copper-enhanced AS aggregation. However, while the mechanism of O₂ activation by AS-Cu(I) remains to be elucidated, the structural details of Cu(I) bound to AS have been studied extensively, as discussed below.

The binding of Cu(I) ions to AS has been probed recently by NMR, identifying three anchoring sites with different structural and affinity features: the C-terminal region encompassing residues 116–127, His50, and the first five residues at the N-terminal (Binolfi *et al.* 2011; Miotto *et al.*, 2014, 2014b) (Fig. 1). At the C-terminal region, Met116 and Met127, from the sequence ¹¹⁶MPVDPDNEAYEM¹²⁷, are the anchoring sites for Cu(I), yielding the lowest affinity site for this metal ion in AS (conditional $K_D = 88$ nM). In the N-terminal region, His50 acts as another Cu(I) anchoring site, with a higher affinity for Cu(I) (conditional $K_D = 16$ nM), as compared to the C-terminal site. Finally, the highest affinity Cu(I) binding site involves the first five residues ¹MDVFEM⁵, where Met1 and Met5 are the main metal coordinating groups and contribute greatly to the high affinity for Cu(I) (conditional $K_D = 7$ nM) (Fig. 1) (Miotto *et al.* 2015). The copper concentrations in synaptic vesicles can reach up to 300 μ M (Hopt *et al.* 2003); thus, this N-terminal Cu(I) site would be the most relevant AS-Cu(I) interaction in the cytosol and its study has been of great interest in recent years. X-ray absorption spectroscopy studies with the 1-15 AS fragment proposed that the Cu(I) coordination mode at pH 7.5 would be 2S2N/O, where the sulfur-based ligands are provided by Met1 and Met5 and the NH₂ terminal group could be a nitrogen-based ligand (De Ricco *et al.* 2015). A recent NMR study provides evidence that Asp2 provides one oxygen-based ligand for Cu(I) (Fig. 1) (Gentile *et al.* 2018). Cu(I) coordination to the 1-15 AS fragment has also been studied upon association with membranes, where the formation of 1:2 AS:Cu(I) complex has been proposed, with Cu(I) bridging between two helical peptide chains via Met residues (Dell'Acqua *et al.* 2016). Most interestingly, NMR-based studies have described that Cu(I) binding to the N-terminal region of AS stabilizes local conformations with α -helical secondary structure and restricted mobility (Miotto *et al.* 2015), an event that might be relevant for the association of the protein to membranes and its physiological function in vesicle trafficking.

Post-translational modifications of α -synuclein

α -synuclein can undergo a variety of post-translational modifications (PTMs) including phosphorylation, acetylation, sumoylation, ubiquitination, oxidation, nitration, and

glycation. Thus, several features of AS, including structure, function, aggregation, and membrane binding, can be altered by PTMs (Burré *et al.* 2018). However, the precise physiological relevance of some of the PTMs remains unclear. This section is focused on AS phosphorylation, acetylation, and sumoylation (Fig. 1, top), followed by a discussion of their impact in AS-metal ion binding.

N-terminal acetylation of α -synuclein

Although the physiological function of AS remains to be definitively established, its ability to interact with lipids and synaptic vesicles (Chandra *et al.* 2003) and a possible role in regulating exocytosis (Larsen *et al.* 2006; Nemani *et al.* 2010) suggest that membrane interactions are a key aspect of the function of this protein. As previously described, the N-terminal region of AS is important for membrane recognition and binding (Bisaglia *et al.* 2006; Bodner *et al.* 2009; Bartels *et al.* 2014). α -Synuclein isolated from brain tissue (healthy control individuals or patients with Parkinson's disease or dementia with Lewy bodies), erythrocytes, or mammalian cell lines is ubiquitously acetylated at the N-terminus (Anderson *et al.* 2006; Ohrfelt *et al.* 2011; Bartels *et al.* 2011; Fauvet *et al.* 2012). Acetylation refers to the covalent attachment of an acetyl group (CH₃CO) to the free α -amino group at the N-terminal end of a polypeptide or ϵ -amino group. N-terminal deacetylases are not known yet and, therefore, N-terminal acetylation is considered irreversible.

N-terminal acetylation increases AS helicity at the N-terminus in the free state (Kang *et al.* 2012; Maltsev *et al.* 2012; Dikiy and Eliezer 2014) which increases its affinity to lipid vesicles, known binding targets of AS (Dikiy and Eliezer 2014). Thus, acetylation strongly affects the membrane binding of AS, in a context-dependent manner (Maltsev *et al.* 2012). Acetylated α -synuclein forms fibrils that are morphologically identical to those formed by the non-acetylated variant (Kang *et al.* 2012). However, the growth rate of AcAS fibrils is slower and they are more resistant to aggregation (Kang *et al.* 2012; Bartels *et al.* 2014).

In a recent study, the effect of familial mutations on DOPAL induced oligomerization of AS was investigated (Lima *et al.* 2018). 3,4-dihydroxyphenylacetaldehyde (DOPAL), an aldehyde generated from the enzymatic oxidation of dopamine, causes the formation of AS oligomers. Although AcAS was capable of forming oligomers in the presence of DOPAL, the extent of oligomerization was significantly reduced. However, AcAS carrying the familial mutations A53T, E46K, or H50Q formed larger oligomers than WT AS (Lima *et al.* 2018).

These findings illustrate some of the physiological implications of N-terminal acetylation, and underscores the importance of considering this type of modification when producing recombinant AS in bacteria (Johnson *et al.* 2013).

In addition to N-terminal acetylation, lysine residues can also undergo acetylation. Mass spectrometry analysis of endogenous AS from mice revealed that Lys6 and Lys10 can be acetylated and that acetylation reduces its aggregation *in vitro* and reduces toxicity *in vivo* (de Oliveira *et al.* 2017).

Phosphorylation of α -synuclein

The protein contains numerous putative and confirmed phosphorylation sites: four serine, four tyrosine and ten threonine residues. The most established phosphorylation sites in AS are Ser87 and Ser129 (Fig. 1, top). In brain tissue from healthy individuals only a minority of AS is phosphorylated. In contrast, in brains of patients that suffered from synucleinopathies and in animal models of these diseases, the majority of AS is phosphorylated on Ser129 (Okochi *et al.* 2000; Kahle *et al.* 2000; Fujiwara *et al.* 2002; Takahashi *et al.* 2003; Anderson *et al.* 2006). Currently, we still do not fully understand the role of Ser129 phosphorylation on AS function, but it appears to regulate its structure, membrane binding, aggregation, neurotoxicity and, more recently, subcellular distribution (Fujiwara *et al.* 2002; Anderson *et al.* 2006; Gonçalves and Outeiro 2013; Pinho *et al.* 2019).

Regarding the effect of phosphorylation on AS aggregation, it was initially suggested that phosphorylation on Ser129 and Ser87 inhibit aggregation, but other studies reported opposite observations (Fujiwara *et al.* 2002; Chen and Feany 2005; Anderson *et al.* 2006; Waxman and Giasson 2008; Paleologou *et al.* 2010; Basso *et al.* 2013; Tenreiro *et al.* 2014b). Ser129 is located in the C-terminal region of AS, a region that is involved in protein-protein interactions and where a common binding interface for metal ions is located. Thus, it is not surprising that Ser129 phosphorylation may regulate the interactions with biological relevant ligands such as proteins and metal ions (McFarland *et al.* 2008). Ser129 phosphorylation is thought to be an early molecular alteration in disease, occurring prior to the formation of mature fibrils and inclusions (reviewed in Oueslati 2016; Tenreiro *et al.* 2014a).

It has been shown that this PTM could induce a structurally distinct and functionally more toxic strain of AS (Ma *et al.* 2016). Nevertheless, Ser129 phosphorylation might also play a role in the regulation of normal AS function (Pinho *et al.* 2019).

Phosphorylation on Ser87 decreases the propensity of AS to fibrillize, and was found to be increased in the brains of patients with Alzheimer disease, Lewy body disease or multiple systems atrophy, and in animal models of synucleinopathies (Paleologou *et al.* 2010; Xiong and Yu 2018).

Phosphorylation on tyrosine 39 (pY39) and 125 (pY125) was also detected in human brain tissue (Chen *et al.* 2009; Mahul-Mellier *et al.* 2014). The levels of pY125 seem to diminish during aging and, in contrast to pS129, pY125 decreases oligomer formation and associated toxicity (Chen

et al. 2009). Nevertheless, the role of tyrosine phosphorylation remains unclear, and demands further investigation.

Several kinases were shown to phosphorylate AS *in vitro* and *in vivo*, but it is still unclear what are the most relevant kinases and whether they may be used as targets for therapeutic intervention (Tenreiro *et al.* 2014a). Cell-based studies revealed that Ser87 could be phosphorylated by casein kinase 1 and Dyrk1a (Okochi *et al.* 2000; Kim *et al.* 2006). Phosphorylation of Ser129 can be mediated by casein kinase 1 and 2 (Okochi *et al.* 2000), G-protein-coupled receptors 1, 2, 5, and 6 (Pronin *et al.* 2000) and polo-like kinases (Inglis *et al.* 2009; Mbefo *et al.* 2010). Not much is currently known about phosphatases that would also impact on the levels of phosphorylated AS in the cell, and studies in simple model organisms are starting to provide insight into this aspect of AS biology (Bras *et al.*, 2018).

Sumoylation of α -synuclein

Sumoylation occurs when Small Ubiquitin-like MOdifier (SUMO) proteins are covalently attached to Lys residues by a thioester bond. Three mammalian isoforms members of the ubiquitin-like protein family (SUMO1, 2, and 3) have been described. Sumoylation can alter sub-cellular localization, function and/or solubility of modified proteins. Several SUMO isopeptidases can detach the modifier, rendering a reversible sumoylation as a highly dynamic process (Melchior *et al.* 2003). Typically, SUMO proteins are added to Lys residues within the motif X_hKXD/E, where X_h is a hydrophobic residue and X may be any amino acid. The sequence of AS has a remarkable amount of Lys residues that can be a target for sumoylation (Kim *et al.* 2011). While other PTMs, such as aberrant phosphorylation, could promote Parkinson's disease pathogenesis, sumoylation serves as a protective modification. *In vitro* studies showed that sumoylation reversed the accumulation and fibrillation of AS; while inhibition of sumoylation in a His6-SUMO2 transgenic mouse model resulted in AS inclusion body formation and neurotoxicity (Krumova *et al.* 2011). The protective effect of sumoylation could be because of an enhanced solubility of modified AS, leading to reduced aggregation and decreased toxicity (Krumova *et al.* 2011; Abeywardana and Pratt 2015). *In vivo* and *in cell* studies demonstrated that SUMO conjugation (in yeast and in human cells) occurred at two major sumoylation sites (Lys96 and Lys102) within the ⁹⁵VKKD⁹⁸ and ¹⁰¹GKNE¹⁰⁴ motif (Fig. 1 top) (Dorval and Fraser 2006; Yang *et al.* 2006; Krumova *et al.* 2011; Shahpasandzadeh *et al.* 2014). Studies with synthesized sumoylated AS showed that sumoylation at Lys102 is more efficient in inhibiting aggregation, as compared to the modification at Lys96 (Abeywardana and Pratt 2015). Still, mutations at either Lys residue impair sumoylation and cause increased AS aggregation in living yeast and mammalian cell culture (Krumova *et al.* 2011; Shahpasandzadeh *et al.* 2014). It has also been shown that

sumoylation of AS promotes aggregate clearance by autophagy in yeast (Shahpasandzadeh *et al.* 2014; Popova *et al.* 2015). Conversely, impaired AS ubiquitination and reduced proteasomal degradation has been associated with AS sumoylation (Rott *et al.* 2017). α -synuclein can undergo ubiquitination at Lys96, which causes complete inhibition of fiber formation (Meier *et al.* 2012). Clearly, different PTMs, such as sumoylation and ubiquitination, may compete at a given site and can have differential effects in AS aggregation. On the other hand, the impact of sumoylation of AS in the metal-binding properties of the protein has not been studied, even though the Lys residues involved are in the vicinity of the C-terminal metal-binding site. Thus, further studies are needed to understand the interplay between sumoylation of AS and other PTMs, as well as metal-binding events, to impact aggregation and/or degradation of this protein.

Linking post-translational modifications and metal-binding in α -synuclein

N-terminal acetylation of α -synuclein: Impact on the structure, binding affinity, and aggregation of copper complexes

As reported previously, abundant evidence revealed recently that AS undergoes N-terminal acetylation *in vivo* (Fauvet *et al.* 2012; Theillet *et al.* 2016; Wani *et al.* 2006; Bartels *et al.* 2011; Ohrfelt *et al.* 2011). From several *in vitro* studies focused on the role of acetylation, it was demonstrated that the co-translational modification induces a modest population of α -helical conformation for the first six residues and enhances the lipid-binding properties of the protein, whereas no significant differences were observed in the fibrillation kinetics between acetylated and non-acetylated AS (Kang *et al.* 2012; Maltsev *et al.* 2012). Consistently, N-terminal acetylation of AS abolishes Cu(II) binding at the high-affinity Met1 site (site 1) present in the non-acetylated protein, but maintains low-affinity binding with the His50 (site 2) and Asp121 (site 3) (Fig. 1) (Moriarty *et al.*, 2014b; Mason *et al.* 2016). In other words, N-terminal acetylation abolishes the relevance of the high-affinity site 1 for Cu(II) in AS and preserves the binding at sites 2 and 3. A comparative analysis between the binding features of Cu(II) and Mn(II), a divalent metal ion that binds exclusively to the C-terminal region of AS, revealed that Cu(II) ions distribute differently at the C-terminal binding interface when Met1 is acetylated (Moriarty *et al.*, 2014b). On the other hand, no changes were observed for Cu(II) binding at the His50 site upon N-terminal acetylation of AS. Thus, although Cu(II) binding in the full-length N-terminal acetylated protein can still occur at sites 2 and 3, acetylation of the N-terminus seems to modulate the extent of independence or cooperativity between N- and C-terminal sites. In terms of the amyloid potential of the protein, as mentioned above, no significant differences were observed in the fibrillation kinetics between acetylated and

non-acetylated AS, whereas the fibrillation enhancement observed for AS at an equimolar Cu(II) stoichiometry did not occur with the acetylated variant of the protein (Moriarty *et al.*, 2014b). Indeed, a recent study showed that the conformation of the non-acetylated AS monomer would be strained by macrochelation with Cu(II) at sites 1 and 2, thereby disrupting fibril elongation and promoting amyloid nucleation (Choi *et al.* 2018). According to this study, this interaction leads to the formation of shortened, β -sheet enriched fibrils ($< 0.2 \mu\text{M}$) that are rapidly transmitted and accumulated to neuronal cells, causing neuronal cell death, in sharp contrast to typical AS fibrils (ca $1 \mu\text{M}$). Interestingly, formation of these pathogenic fibrils were not observed for the AcAS-Cu(II) interaction (Moriarty *et al.* 2014b; Mason *et al.* 2016). Overall, these evidences indicate that N-terminal acetylation of AS alters the binding preferences of Cu(II) by redirecting metal binding from site 1 to sites 2 and 3, affecting the morphological and conformational features of the AS amyloid fibrils induced by Cu(II) and its associated neurotoxicity.

Since copper ions are predominantly found in their Cu(I) state in the reducing environment of living cells, characterization of the physiologically relevant Cu(I) complexes became particularly important (Binolfi *et al.* 2011; Miotto *et al.* 2015; Miotto *et al.* 2017; Gentile *et al.* 2018; Abeyawardhane *et al.* 2018b). These studies revealed that Cu(I) binding to the high-affinity Met1-X3-Met5 site is influenced by acetylation at the N-terminus (Fig. 1), whereas the interaction profile of this metal ion at other sites are preserved in the AcAS variant. The conditional affinity for Cu(I) binding at the Met1-X3-Met5 site of AcAS was $^{\circ}K_{d1} = 3.9 \pm 1.0 \text{ nM}$, whereas the value reported for non-acetylated AS was $^{\circ}K_{d1} = 7.8 \pm 1.0 \text{ nM}$ (Gentile *et al.* 2018). In this coordination environment, Met1 and Met5 residues act as the main anchoring moieties for Cu(I) binding to that site, providing S-Cu binding modes, and the N-terminus acetyl group plays a more modest role in terms of Cu(I) binding affinity, acting as a source for the establishment of Cu-O binding modes (Gentile *et al.* 2018). Interestingly, the formation of the high-affinity AcAS-Cu(I) complex at the N-terminal region induced a dramatic impact on protein conformation, leading to stabilized local conformations with α -helical secondary structure and restricted motility (Miotto *et al.* 2015; Miotto *et al.* 2017). These structural changes did not occur when Cu(I) binds to the non-acetylated variant of the protein. Thus, while Met1 and Met5 residues are critical for the binding affinity of the Met1-X3-Met5/Cu(I) complex in AcAS, the N-terminal acetyl group is important in promoting local helical conformations, contributing to the stabilization of these structures by favoring Cu(I) binding. In terms of the redox properties of the complex, acetylation of the N-terminal group was shown to exert a combined effect by stabilizing Cu(I) while significantly destabilizing Cu(II), leading to a significant increase in $\sim 250 \text{ mV}$ of the reduction potential of the site, as compared to non-acetylated

AS (Gentile *et al.* 2018). Overall, these results underscore the key role played by N-terminal acetylation to stabilize the reduced form of the AS-Cu complex in the AcAS variant.

Finally, a recent study demonstrated that Cu(I) binding to the AcAS variant activates O_2 resulting in both intermolecular Y39-Y39 dityrosine crosslinking within the fibrillar core, as well as intramolecular crosslinking within the C-terminal region (Abeyawardhane *et al.* 2018b) (Fig. 2b). The results showed that the dityrosine crosslinking promoted by the Cu(I)/ O_2 reactivity is altered by N-terminal acetylation. Based on the different coordination preferences of Cu(I) versus Cu(II) to the AcAS variant, it was found that the presence of His50 is critical to stabilize the transition from AcAS-Cu(I) to AcAS-Cu(II), a signature of the Cu(I)-promoted oxidation chemistry. Under these conditions, both N-terminal acetylation of AS and His50 facilitate intermolecular crosslinking, reducing parallel β -sheet content and attenuating fibril elongation (Abeyawardhane *et al.* 2018b). In this context, neuronal copper has been proposed to play a functional role in attenuating protein aggregation and fibrillar growth.

Phosphorylation of α -synuclein: Impact on metal coordination to the C-terminal common binding interface

Phosphorylation can have a significant impact on protein conformation at the level of both secondary and tertiary structure since this modification not only alter the charge by the addition of negatively charged phosphate groups, but also may modify hydrogen-bonding patterns and open access to alternative backbone angles (Tholey *et al.* 1999; Andrew *et al.* 2002; Signarvic and DeGrado 2003; Errington and Doig 2005; Bielska and Zondlo 2006). Additionally, phosphorylation may also alter metal ion affinity and specificity, further influencing the folding, structure, and functional properties of protein-metal complexes. Indeed, the importance of metal-phosphoprotein interactions has been highlighted by their role in diverse biological functions as well as potentially pathogenic roles in protein cross-linking and aggregation (Yamamoto *et al.* 2002; Savelieff *et al.* 2013).

As mentioned above, modifying the hydroxyl functionality of a serine, threonine or tyrosine residue into a phosphate group should change its propensity to bind ligands such as metal ions, especially if the residue is positioned among other potential metal-binding residues. In this context, the majority of putative (Y133 and Y136) and disease associated (S129 and Y125) phosphorylation sites in AS occurs in the C-terminal region spanning residues 120-140, which also contains the common interface for the binding of metal ions (Binolfi *et al.* 2006; Castillo-Gonzalez *et al.* 2017; Duce *et al.* 2017). The proximity between phosphorylation and metal-binding sites indicates not only a potential interplay in modulating AS interactions at the C-terminal region, but also the high-degree of flexibility of the C-terminus in the monomeric and aggregated states of the protein suggesting

that phosphorylation within this interface could significantly influence these interactions and contribute to regulate AS function in health and disease.

Based on these considerations a number of studies were performed in order to determine how disease-associated phosphorylation might impact on the specificity and binding affinity of metal ions to the C-terminal region of AS. First, it was reported the effect of phosphorylation on metal binding using synthetic model peptides that correspond to short fragments (119–132) of C-terminal AS, containing two of the four phosphorylation sites identified in the protein, Y125 and S129 (Liu and Franz 2005; Liu and Franz 2007). By using Tb^{3+} as a luminescent probe of metal binding, these studies showed that phosphorylation at Y125 enhanced selectively Tb^{3+} binding compared to non-phosphorylated 119–132 AS or its phosphoserine version pS129, concluding that the type and location of a phosphorylated amino acid influence peptide-metal binding affinity and specificity. In particular, these works demonstrated that the Y125 phosphorylated form of the peptide selectively bound trivalent metal ions such as Fe(III) and Al(III) over the divalent metal ions Cu(II), Zn(II), Ca(II), and Mn(II) (Liu and Franz 2005). Further modifications of the C-terminal peptide by replacing either Asp119 residue or the Asp121 residue with alanine resulted in five- and six-fold drops in the binding affinity. Thus, a coordination environment was proposed in which the phosphoester group on tyrosine provides a metal-binding anchor that is supplemented by carboxylic groups located in its vicinity to establish a multidentate chelator with selectivity for trivalent metal ions (Liu and Franz 2007). Overall, this configuration represented a novel metal-binding site in AS created upon specific phosphorylation.

Another study investigated the binding features of Cu(II), Pb(II), Fe(II), and Fe(III) to longer C-terminal AS fragments spanning the region 107–140 and its mono-phosphorylated forms (pY125 and pS129) (Lu *et al.* 2011). Beyond the region D119–A124, recognized as a common binding interface for divalent metal ions (Binolfi *et al.* 2006), this study demonstrated that the phosphorylation at either Y125 or S129 influences not only the affinity features but also the location of binding sites. The K_d values of Cu(II), Pb(II), and Fe(II) complexes with phosphorylated 107–140 AS were lower than those with the non-phosphorylated peptide, indicating that phosphorylation increases the binding affinities for metal ions. Interestingly, the position of the phosphorylated residues did not show a significant difference in the binding affinities, and similar K_d values of the complexes with Cu(II), Pb(II), and Fe(II) were observed for the two phosphorylated forms pY125 or pS129. Overall, phosphorylation on Y125 and S129 of 107–140 led to the shift of the binding sites of divalent metal ions from the N-terminus to C-terminus, where the phosphorylated amino acids of both pY125 and pS129 were located. Based on the relocation of the binding sites and enhanced affinities of

metal ions to the phosphorylated peptides it was suggested that phosphorylation at the C-terminus might play an important role in regulating metal ion binding, influencing both AS structure and aggregation.

While it is clear that phosphorylation at the C-terminal of AS impacts its metal-binding properties, several discrepancies remain to be investigated. In contrast to the results reported by Liu and Franz for the 119–132 AS fragment (Liu and Franz 2005; Liu and Franz 2007), the work by Lu (Lu *et al.* 2011) revealed that phosphorylation at either Y125 or S129 in the 107–140 AS peptide did not impact its binding affinity to Fe(III). Moreover, the selective binding of trivalent metal ions over divalent metal ions reported by Liu and Franz was not observed in the longer fragment.

Finally, the binding affinities reported for Cu(II) and Fe(II) ions to 107–140 AS (K_d in the 50–200 μ M range) (Lu *et al.* 2011) and for trivalent metal ions to 119–132 AS (K_d in the 10–20 μ M range) (Liu and Franz 2005; Liu and Franz 2007) are not consistent with the previously reported K_d values \sim 1mM for these protein–metal interactions at the C-terminus, as determined by NMR using the full-length protein (Binolfi *et al.* 2006). These discrepancies indicate that conclusions derived from strategies using synthetic model peptides as the sole approach to mimic the effects of metal binding to full-length proteins should be considered with care (i.e., how AS-metal interactions may influence the long-range contacts of the full-length protein remains an open question). Particularly in the case of phosphorylation, studies focused on the full-length protein are clearly needed in order to expand these results toward the understanding of the molecular and structural mechanism underlying the interactions between phosphorylated AS and metal ions, including the identification of novel-binding sites, and to highlight the potential cross-talk between phosphorylation and metal ion binding in regulating AS physiological functions and aggregation properties that are regulated by its C-terminal domain.

Concluding Remarks

α -synuclein profiles as an important metal-binding protein in the brain, displaying three main anchoring sites for metal ions with distinct specificity and affinity features. Among all the AS-metal interactions described thus far, the two most physiologically relevant interactions occur with copper and calcium ions. For Cu ions, His50 and the N-terminal site encompassing the first five residues of AS constitute two independent, highly specific and high affinity anchoring sites. In particular, Cu(I) binding to the N-terminal region of AS stabilizes local conformations with α -helical secondary structure that may be relevant for the association of the protein to membranes and its physiological function in vesicle trafficking. On the other hand, Ca(II) binding at the C-terminal domain of AS exerts a negative modulatory effect on its membrane-binding properties, underscoring the key

role that Ca ions may play as modulators of the interaction of this protein with synaptic vesicles under physiological and cell toxicity conditions.

α -synuclein undergoes a variety of PTMs that may impact its metal-binding properties, although this topic is a relatively unexplored area of research. Acetylation of the N-terminal group stabilizes Cu(I) coordination at this site, while abolishing Cu(II) binding, causing a shift of Cu(II) ions to the His50 site. While N-terminal acetylation of AS impacts significantly its Cu-binding properties, it does not prevent redox cycling of AS-Cu complexes that lead to oxidative modifications, with subsequent effects in the structural features of metal-induced AS amyloid fibrils and their associated neurotoxicity. On the other hand, phosphorylation of the C-terminal domain of AS increases the metal-binding affinity of this region of the protein, although further studies with the full-length AS protein are needed to evaluate the impact of this effect in aggregation and membrane-binding properties.

Recent research findings have established that AS-metal interactions clearly impact the structure, aggregation propensity, and membrane-binding properties of AS, underscoring the relevant role that metal ions may play as modulators of the interaction of AS with synaptic vesicles under physiological and pathological conditions. PTMs of AS, such as acetylation and phosphorylation, can also impact AS-metal interactions, revealing a potential cross-talk between PTMs and metal ion binding in regulating AS physiological functions and its role in pathology. Future research efforts to improve our understanding of the nature of AS-metal ion interactions, their impact in AS structural and membrane-binding properties, and their interplay with PTMs of the protein will surely provide a clear picture of the role of metal ions in AS physiological function and the etiology of Parkinson's disease.

Acknowledgments and conflict of interest disclosure

C.O.F. thanks Universidad Nacional de Rosario (CUA-DAHZ 007/11), ANPCyT- FONCyT (PICT 2014-3704) and the Alexander von Humboldt Foundation (P4507) for financial support. C.O.F. thanks the Max Planck Society (P10390) for support. The authors declare that they have no competing interests. Tiago F. Outeiro edited this Special Issue as a Guest Editor.

References

- Abeyawardhana D. L., Fernandez R. D., Murgas C. J., Heitger D. R., Fomey A. K., Crozier M. K. and Lucas H. R. (2018a) Iron redox chemistry promotes antiparallel oligomerization of alpha-Synuclein. *J. Am. Chem. Soc.* **140**, 5028–5032.
- Abeyawardhana D. L., Fernández R. D., Heitger D. R., Crozier M. K., Wolver J. C. and Lucas H. R. (2018b) Copper induced radical dimerization of alpha-synuclein requires histidine. *J. Am. Chem. Soc.* **140**, 17086–17094.
- Abeywardana T. and Pratt M. R. (2015) Extent of inhibition of α -synuclein aggregation in vitro by SUMOylation is conjugation site- and sumo isoform-selective. *Biochemistry* **54**, 959–961.
- Ahmad A., Burns C. S., Fink A. L. and Uversky V. N. (2012) Peculiarities of copper binding to α -synuclein. *J. Biomol. Struct. Dyn.* **29**, 825–842.
- Anandhan A., Rodriguez-Rocha H., Bohovych I., et al. (2015) Overexpression of alpha-synuclein at non-toxic levels increases dopaminergic cell death induced by copper exposure via modulation of protein degradation pathways. *Neurobiol. Dis.* **81**, 76–92.
- Anderson J. P., Walker D. E., Goldstein J. M., et al. (2006) Phosphorylation of Ser-129 is the dominant pathological modification of alpha-synuclein in familial and sporadic Lewy body disease. *J. Biol. Chem.* **281**, 29739–29752.
- Andrew C. D., Warwicker J., Jones G. R. and Doig A. J. (2002) Effect of phosphorylation on alpha-helix stability as a function of position. *Biochemistry* **41**, 1897–1905.
- Angelova P. R., Ludtmann M. H., Horrocks M. H., et al. (2016) Ca²⁺ is a key factor in alpha-synuclein-induced neurotoxicity. *J. Cell. Sci.* **129**, 1792–1801.
- Bartels T., Choi J. G. and Selkoe D. J. (2011) alpha-Synuclein occurs physiologically as a helically folded tetramer that resists aggregation. *Nature* **477**, 107–110.
- Bartels T., Kim N. C., Luth E. S. and Selkoe D. J. (2014) N-alpha-acetylation of alpha-synuclein increases its helical folding propensity, GM1 binding specificity and resistance to aggregation. *PLoS ONE* **9**, e103727.
- Basso E., Antas P., Marijanovic Z., Goncalves S., Tenreiro S. and Outeiro T. F. (2013) PLK2 modulates alpha-synuclein aggregation in yeast and mammalian cells. *Mol. Neurobiol.* **48**, 854–862.
- Bertoncini C. W., Jung Y. S., Fernández C. O., Hoyer W., Griesinger C., Jovin T. M. and Zweckstetter M. (2005) Release of long-range tertiary interactions potentiates aggregation of natively unstructured alpha-synuclein. *Proc. Natl Acad. Sci. USA* **102**, 1430–5.
- Bharathi R. K. S. (2007) Thermodynamics imprinting reveals differential binding of metals to a-synuclein: Relevance to parkinson's disease. *Biochem. Biophys. Res. Commun.* **359**, 115–120.
- Bielska A. A. and Zondlo N. J. (2006) Hyperphosphorylation of tau induces local polyproline II helix. *Biochemistry* **45**, 5527–5537.
- Binolfi A., Rasia R. M., Bertoncini C. W., et al. (2006) Interaction of alpha-synuclein with divalent metal ions reveals key differences: a link between structure, binding specificity and fibrillation enhancement. *J. Am. Chem. Soc.* **128**, 9893–9901.
- Binolfi A., Lamberto G. R., Duran R., et al. (2008) Site-specific interactions of Cu(II) with r and alfa-synuclein: bridging the molecular gap between metal binding and aggregation. *J. Am. Chem. Soc.* **130**, 11801–11812.
- Binolfi A., Rodriguez E. E., Valensin D., et al. (2010) Bioinorganic chemistry of Parkinson's disease: structural determinants for the copper-mediated amyloid formation of alpha-synuclein. *Inorg Chem.* **49**, 10668–10679.
- Binolfi A., Valiente-Gabioud A. A., Duran R., Zweckstetter M., Griesinger C. and Fernandez C. O. (2011) Exploring the structural details of Cu(I) binding to alpha-synuclein by NMR spectroscopy. *J. Am. Chem. Soc.* **133**, 194–196.
- Binolfi A., Quintanar L., Bertoncini C. W., Griesinger C. and Fernández C. O. (2012) Bioinorganic chemistry of copper coordination to alpha-synuclein: Relevance to Parkinson's disease. *Coord Chem. Rev.* **256**, 2188–2201.
- Bisaglia M., Schievano E., Caporale A., Peggion E. and Mammi S. (2006) The 11-mer repeats of human alpha-synuclein in vesicle interactions and lipid composition discrimination: a cooperative role. *Biopolymers* **84**, 310–316.

- Bisaglia M., Tessari I., Mammi S. and Bubacco L. (2009) Interaction between α -synuclein and metal ions, still looking for a role in the pathogenesis of Parkinson's disease. *Neuromol. Med.* **11**, 239–251.
- Bodner C. R., Dobson C. M. and Bax A. (2009) Multiple tight phospholipid-binding modes of α -synuclein revealed by solution NMR spectroscopy. *J. Mol. Biol.* **390**, 775–790.
- Bonini N. M. and Giasson B. I. (2005) Snaring the function of α -synuclein. *Cell* **123**, 359–373.
- Borbat P., Ramlall T. F., Freed J. H. and Eliezer D. (2006) Inter-helix distances in lysophospholipid micelle-bound α -synuclein from pulsed ESR measurements. *J. Am. Chem. Soc.* **128**, 10004–10005.
- Bras I. C., Tenreiro S., Silva A. M. and Outeiro T. F. (2018) Identification of novel protein phosphatases as modifiers of α -synuclein aggregation in yeast. *FEMS Yeast Res.* **18**(8). <https://doi.org/10.1093/femsyr/foy108>.
- Burré J., Sharma M., Tsetsenis T., Buchman V., Etherton M. R. and Südhof T. C. (2010) α -Synuclein promotes SNARE-complex assembly in vivo and in vitro. *Science* **329**, 1663–1667.
- Burre J., Sharma M. and Südhof T. C. (2018) Cell biology and pathophysiology of α -synuclein. *Cold Spring Harbor Perspectives Med.* **8**(3). <https://doi.org/10.1101/cshperspect.a024091>.
- Bussell R., Jr and Eliezer D. (2003) A structural and functional role for 11-mer repeats in α -synuclein and other exchangeable lipid binding proteins. *J. Mol. Biol.* **329**, 763–778.
- Cali T., Ottolini D., Negro A. and Brini M. (2012) α -Synuclein controls mitochondrial calcium homeostasis by enhancing endoplasmic reticulum-mitochondria interactions. *J. Biol. Chem.* **287**, 17914–17929.
- Calne D. B., Chu N. S., Huang C. C., Lu C. S. and Olanow W. (1994) Manganism and idiopathic parkinsonism: Similarities and differences. *Neurology* **44**, 1583–1586.
- Caraveo G., Auluck P. K., Whitesell L., et al. (2014) Calcineurin determines toxic versus beneficial responses to α -synuclein. *Proc. Natl Acad. Sci. USA* **111**, E3544–3552.
- Castillo-Gonzalez J. A., Loera-Arias M. J., Saucedo-Cardenas O., Montes-de-Oca-Luna R., Garcia-Garcia A. and Rodriguez-Rocha H. (2017) Phosphorylated α -synuclein-copper complex formation in the pathogenesis of Parkinson's disease. *Parkinsons Dis.* **2017**, 9164754.
- Chandra S., Chen X., Rizo J., Jahn R. and Sudhof T. C. (2003) A broken α -helix in folded α -Synuclein. *J. Biol. Chem.* **278**, 15313–15318.
- Chen L. and Feany M. B. (2005) α -Synuclein phosphorylation controls neurotoxicity and inclusion formation in a Drosophila model of Parkinson disease. *Nat. Neurosci.* **8**, 657–663.
- Chen L., Periquet M., Wang X., Negro A., McLean P. J., Hyman B. T. and Feany M. B. (2009) Tyrosine and serine phosphorylation of α -synuclein have opposing effects on neurotoxicity and soluble oligomer formation. *J. Clin. Invest.* **119**, 3257–3265.
- Choi T. S., Lee J., Han J. Y., et al. (2018) Supramolecular modulation of structural polymorphism in pathogenic α -synuclein fibrils using copper(II) coordination. *Angew Chem Int Ed Engl.* **57**, 3099–3103.
- Darios F., Ruy Pérez V., López I., Villanueva J., Gutierrez L. M. and Davletov B. (2010) α -Synuclein sequesters arachidonic acid to modulate SNARE-mediated exocytosis. *EMBO Rep.* **11**, 528–533.
- Davidson W. S., Jonas A., Clayton D. F. and George J. M. (1998) Stabilization of α -synuclein secondary structure upon binding to synthetic membranes. *J. Biol. Chem.* **273**, 9443–9449.
- Davies P., Moualla D. and Brown D. R. (2011) α -Synuclein is a cellular ferredoxinase. *PLoS ONE* **6**, e15814.
- Dell'Acqua S., Pirota V., Monzani E., Camponeschi F., Ricco R. D., Valensin D. and Casella L. (2016) Copper(I) forms a redox-stable 1:2 complex with α -synuclein N-terminal peptide in a membrane-like environment. *Inorg Chem.* **55**, 6100–6106.
- Dexter D. T., Carayon A., Javoy-Agud F., et al. (1991) Alterations in the levels of iron, ferritin and other trace metals in Parkinson's disease and other neurodegenerative diseases affecting the basal ganglia. *Brain* **114**, 1953–1975.
- Dikiy I. and Eliezer D. (2014) N-terminal acetylation stabilizes N-terminal helicity in lipid- and micelle-bound α -synuclein and increases its affinity for physiological membranes. *J. Biol. Chem.* **289**, 3652–3665.
- Dorval V. and Fraser P. E. (2006) Small ubiquitin-like modifier (SUMO) modification of natively unfolded proteins tau and A-synuclein. *J. Biol. Chem.* **281**, 9919–9924.
- Drew S. C., Leong S. L., Pham C. L., et al. (2008) Cu²⁺ binding modes of recombinant α -synuclein - Insights from EPR spectroscopy. *J. Am. Chem. Soc.* **130**, 7766–7773.
- Duce J. A., Wong B. X., Durham H., Devedjian J. C., Smith D. P. and Devos D. (2017) Post translational changes to α -synuclein control iron and dopamine trafficking; a concept for neuron vulnerability in Parkinson's disease. *Mol. Neurodegen.* **12**, 45.
- Ducic T., Carboni E., Lai B., et al. (2015) α -Synuclein regulates neuronal levels of manganese and calcium. *ACS Chem. Neurosci.* **6**, 1769–1779.
- Dudzic C. G., Walter E. D., Abrams B. S., Jurica M. S. and Millhauser G. L. (2013) Coordination of copper to the membrane-bound form of α -synuclein. *Biochemistry* **52**, 53–60.
- Errington N. and Doig A. J. (2005) A phosphoserine-lysine salt bridge within an α -helical peptide, the strongest α -helix side-chain interaction measured to date. *Biochemistry* **44**, 7553–7558.
- Fauvet B., Mbefo M. K., Fares M. B., et al. (2012) α -Synuclein in central nervous system and from erythrocytes, mammalian cells, and Escherichia coli exists predominantly as disordered monomer. *J. Biol. Chem.* **287**, 15345–15364.
- Follett J., Darlow B., Wong M. B., Goodwin J. and Pountney D. L. (2012) Potassium depolarization and raised calcium induces α -synuclein aggregates. *Neurotox Res.* **23**, 378–392.
- Fujiwara H., Hasegawa M., Dohmae N., et al. (2002) α -Synuclein is phosphorylated in synucleinopathy lesions. *Nat. Cell. Biol.* **4**, 160–164.
- Garza-Lombó C., Posadas Y., Quintanar L., Gonsebatt M. E. and Franco R. (2018) Neurotoxicity linked to dysfunctional metal ion homeostasis and xenobiotic metal exposure: redox signaling and oxidative stress. *Antioxid Redox Signal.* **28**, 1669–1703.
- Gentile I., Garro H. A., Delgado Ocaña S., et al. (2018) Interaction of Cu (i) with the Met-X3-Met motif of α -synuclein: binding ligands, affinity and structural features. *Metallomics* **10**, 1383–1389.
- Goncalves S. and Outeiro T. F. (2013) Assessing the subcellular dynamics of α -synuclein using photoactivation microscopy. *Mol. Neurobiol.* **47**, 1081–1092.
- Gorell J. M., Rybicki B. A., Johnson C. C. and Peterson E. L. (1999) Occupational metal exposures and the risk of Parkinson's disease. *Neuroepidemiology* **18**, 303–308.
- Han J. Y., Choi T. S. and Kim H. I. (2018) Molecular role of Ca²⁺ and hard divalent metal cations on accelerated fibrillation and interfibrillar aggregation of α -synuclein. *Sci Rep.* **8**, 1895.
- Harischandra D. S., Rokad D., Neal M. L., et al. (2019) Manganese promotes the aggregation and prion-like cell-to-cell exosomal transmission of α -synuclein. *Sci Signal.* **12**, eaau4543
- Hopt A., Korte S., Fink H., et al. (2003) Methods for studying synaptosomal copper release. *Neurosci Meth.* **128**, 159–172.
- Inglis K. J., Chereau D., Brigham E. F., et al. (2009) Polo-like kinase 2 (PLK2) phosphorylates α -synuclein at serine 129 in central nervous system. *J. Biol. Chem.* **284**, 2598–2602.
- Jackson M. S. and Lee J. C. (2009) Identification of the minimal copper(II)-binding α -synuclein sequence. *Inorg Chem.* **48**, 9303–9307.

- Johnson M., Geeves M. A. and Mulvihill D. P. (2013) Production of amino-terminally acetylated recombinant proteins in *E. coli*. *Methods Mol. Biol.* **981**, 193–200.
- Kahle P. J., Neumann M., Ozmen L., et al. (2000) Subcellular localization of wild-type and Parkinson's disease-associated mutant alpha-synuclein in human and transgenic mouse brain. *J. Neurosci.* **20**, 6365–6373.
- Kang L., Moriarty G. M., Woods L. A., Ashcroft A. E., Radford S. E. and Baum J. (2012) N-terminal acetylation of alpha-synuclein induces increased transient helical propensity and decreased aggregation rates in the intrinsically disordered monomer. *Protein Sci.* **21**, 911–917.
- Kim E. J., Sung J. Y., Lee H. J., et al. (2006) Dyrk1A phosphorylates alpha-synuclein and enhances intracellular inclusion formation. *J. Biol. Chem.* **281**, 33250–33257.
- Kim Y. M., Jang W. H., Quezado M. M., Oh Y., Chung K. C., Junn E. and Mouradian M. M. (2011) Proteasome inhibition induces alpha-synuclein SUMOylation and aggregate formation. *J. Neurol. Sci.* **307**, 157–161.
- Kimura Y., Utsuyama M., Yoshimura M. and Tomonaga M. (1983) Element analysis of Lewy and Adrenal bodies in Parkinson's disease by electron probe microanalysis. *Acta Neuropathol.* **59**, 233–236.
- Krumova P., Meulmeester E., Garrido M., et al. (2011) Sumoylation inhibits A-synuclein aggregation and toxicity. *J. Cell Biol.* **194**, 49–60.
- Larsen K. E., Schmitz Y., Troyer M. D., et al. (2006) Alpha-synuclein overexpression in PC12 and chromaffin cells impairs catecholamine release by interfering with a late step in exocytosis. *J. Neurosci.* **26**, 11915–11922.
- de Lauroto P. P., Tosatto L., Frare E., Marin O., Uversky V. N. and Fontana A. (2006) Conformational properties of the SDS-bound state of alpha-synuclein probed by limited proteolysis: unexpected rigidity of the acidic C-terminal tail. *Biochemistry* **45**, 11523–11531.
- de Oliveira R. M., Vicente Miranda H., Francelle L., et al. (2017) The mechanism of sirtuin 2-mediated exacerbation of alpha-synuclein toxicity in models of Parkinson disease. *PLoS Biol.* **15**, e2000374.
- Lautenschläger J., Stephens A. D., Fusco G., et al. (2018) C-terminal calcium binding of alpha-synuclein modulates synaptic vesicle interaction. *Nat. Commun.* **9**, 712.
- Leal S. S., Botelho H. M. and Gomes C. M. (2012) Metal ions as modulators of protein conformation and misfolding in neurodegeneration. *Coord. Chem. Rev.* **256**, 2253–2270.
- Lee J. C., Gray H. B. and Winkler J. R. (2008) Copper(II) binding to alpha-synuclein, the Parkinson's protein. *J. Am. Chem. Soc.* **130**, 6898–6899.
- Lima V. A. and do Nascimento L. A., Eliezer D. and Follmer C. (2018) Role of Parkinson's disease-linked mutations and N-terminal acetylation on the oligomerization of alpha-synuclein induced by 3,4-Dihydroxyphenylacetaldehyde. *ACS Chem. Neurosci.* **10** (1):690–703. <https://doi.org/10.1021/acchemneuro.8b00498>.
- Liu L. L. and Franz K. J. (2005) Phosphorylation of an alpha-synuclein peptide fragment enhances metal binding. *J. Am. Chem. Soc.* **127**, 9662–9663.
- Liu L. L. and Franz K. J. (2007) Phosphorylation-dependent metal binding by alpha-synuclein peptide fragments. *J. Biol. Inorg. Chem.* **12**, 234–247.
- Lowe R., Pountney D. L., Jensen P. H., Gai W. P. and Voelcker N. H. (2004) Calcium(II) selectively induces alpha-synuclein annular oligomers via interaction with the C-terminal domain. *Protein Sci.* **13**, 3245–3252.
- Lu Y., Prudent M., Fauvet B., Lashuel H. A. and Girault H. H. (2011) Phosphorylation of alpha-synuclein at Y125 and S129 alters its metal binding properties: implications for understanding the role of alpha-synuclein in the pathogenesis of Parkinson's Disease and related disorders. *ACS Chem. Neurosci.* **2**, 667–675.
- Lucas H. R. and Lee J. C. (2011) Copper(II) enhances membrane-bound alpha-synuclein helix formation. *Metallomics* **3**, 280–283.
- Lucas H. R., DeBeer S., Hong M. S. and Lee J. C. (2010) Evidence for copper-dioxygen reactivity during alpha-synuclein fibril formation. *J. Am. Chem. Soc.* **132**, 6636–6637.
- Ma M. R., Hu Z. W., Zhao Y. F., Chen Y. X. and Li Y. M. (2016) Phosphorylation induces distinct alpha-synuclein strain formation. *Sci. Rep.* **6**, 37130.
- Mahul-Mellier A. L., Fauvet B., Gysbers A., et al. (2014) c-Abl phosphorylates alpha-synuclein and regulates its degradation: implication for alpha-synuclein clearance and contribution to the pathogenesis of Parkinson's disease. *Hum. Mol. Genet.* **23**, 2858–2879.
- Maltsev A. S., Ying J. and Bax A. (2012) Impact of N-terminal acetylation of alpha-synuclein on its random coil and lipid binding properties. *Biochemistry* **51**, 5004–5013.
- Marambaud P., Dreses-Werringloer U., and Vingtdeux V. (2009) Calcium signaling in neurodegeneration. *Mol. Neurodegener.* **4**, 6–20.
- Maroteaux L., Campanelli J. T. and Scheller R. H. (1988) Synuclein: a neuron-specific protein localized to the nucleus and presynaptic nerve terminal. *J. Neurosci.* **8**, 2804–2814.
- Mason R. J., Paskins A. R., Dalton C. F. and Smith D. P. (2016) Copper binding and subsequent aggregation of alpha-synuclein are modulated by N-terminal acetylation and ablated by the H50Q missense mutation. *Biochemistry* **55**, 4737–4741.
- Mattson M. P. (2007) Calcium and neurodegeneration. *Aging Cell* **6**, 337–350.
- Mbefo M. K., Paleologou K. E., Boucharaba A., et al. (2010) Phosphorylation of synucleins by members of the Polo-like kinase family. *J. Biol. Chem.* **285**, 2807–2822.
- McFarland M. A., Ellis C. E., Markey S. P. and Nussbaum R. L. (2008) Proteomics analysis identifies phosphorylation-dependent alpha-synuclein protein interactions. *Mol. Cell Proteomics: MCP* **7**, 2123–2137.
- Meier F., Abeywardana T., Dhall A., Marotta N. P., Varkey J., Langen R., Chatterjee C. and Pratt M. R. (2012) Semisynthetic, site-specific ubiquitin modification of alpha-synuclein reveals differential effects on aggregation. *J. Am. Chem. Soc.* **134**, 5468–5471.
- Melchior F., Schergaut M. and Pichler A. (2003) SUMO: ligases, isopeptidases and nuclear pores. *Trends Biochem. Sci.* **28**, 612–618.
- Meloni G. and Vařák M. (2011) Redox activity of alpha-synuclein-Cu is silenced by Zn7-metallothionein-3. *Free Radic Biol Med.* **50**, 1471–1479.
- Miller Y., Ma B. and Nussinov R. (2012) Metal binding sites in amyloid oligomers: Complexes and mechanisms. *Coord. Chem. Rev.* **256**, 2245–2252.
- Miotto M. C., Binolfi A., Zweckstetter M., Griesinger C. and Fernández C. O. (2014) Bioinorganic chemistry of synucleinopathies: Deciphering the binding features of Met motifs and His-50 in AS-Cu(I) interactions. *J. Inorg. Biochem.* **141**, 208–211.
- Miotto M. C., Rodriguez E. E., Valiente-Gabioud A. A., et al. (2014b) Site-specific copper-catalyzed oxidation of alpha-synuclein: tightening the link between metal binding and protein oxidative damage in Parkinson's disease. *Inorg. Chem.* **53**, 4350–4358.
- Miotto M. C., Valiente-Gabioud A. A., Rossetti G., et al. (2015) Copper Binding to the N-Terminally Acetylated, Naturally Occurring Form of Alpha-Synuclein Induces Local Helical Folding. *J. Am. Chem. Soc.* **137**, 6444–6447.
- Miotto M. C., Pavese M. D., Quintanar L., Zweckstetter M., Griesinger C. and Fernandez C. O. (2017) Bioinorganic chemistry of Parkinson's disease: affinity and structural features of Cu(I)

- binding to the full-length beta-synuclein protein. *Inorg Chem.* **56**, 10387–10395.
- Moriarty G. M., Minetti C. A., Remeta D. P. and Baum J. (2014b) A revised picture of the Cu(II)-alpha-synuclein complex: the role of N-terminal acetylation. *Biochemistry* **53**, 2815–2817.
- Natalello A., Benetti F., Doglia S. M., Legname G. and Grandori R. (2010) Compact conformations of α -synuclein induced by alcohols and copper. *Proteins.* **79**(2), 611–21.
- Nath S., Goodwin J., Engelborghs Y. and Pountney D. L. (2011) Raised calcium promotes α -synuclein aggregate formation. *Mol. Cell. Neurosci.* **46**, 516–526.
- Nemani V. M., Lu W., Berge V., et al. (2010) Increased expression of alpha-synuclein reduces neurotransmitter release by inhibiting synaptic vesicle reclustering after endocytosis. *Neuron* **65**, 66–79.
- Nielsen M. S., Vorum H., Lindersson E. and Jensen P. H. (2001) Ca²⁺ binding to α -synuclein regulates ligand binding and oligomerization. *J. Biol. Chem.* **276**, 22680–22684.
- Ohrfelt A., Zetterberg H., Andersson K., et al. (2011) Identification of novel alpha-synuclein isoforms in human brain tissue by using an online nanoLC-ESI-FTICR-MS method. *Neurochem. Res.* **36**, 2029–2042.
- Okochi M., Walter J., Koyama A., et al. (2000) Constitutive phosphorylation of the Parkinson's disease associated alpha-synuclein. *J. Biol. Chem.* **275**, 390–397.
- Oueslati A. (2016) Implication of alpha-synuclein phosphorylation at S129 in synucleinopathies: what have we learned in the last decade? *J. Parkinsons Dis.* **6**, 39–51.
- Paik Seung R., Shin Hyun-Ju, Lee Ju-Hyun, Chang Chung-Soon and Kim J. (1999) Copper(II)-induced self-oligomerization of α -synuclein. *Biochem. J.* **340**, 821–828.
- Paleologou K. E., Oueslati A., Shakked G., et al. (2010) Phosphorylation at S87 is enhanced in synucleinopathies, inhibits alpha-synuclein oligomerization, and influences synuclein-membrane interactions. *J. Neurosci.* **30**, 3184–3198.
- Peng Y., Wang C., Xu H. H., Liu Y.-N. and Zhou F. (2010) Binding of α -synuclein with Fe(III) and with Fe(II) and biological implications of the resultant complexes. *J. Inorg. Biochem.* **104**, 365–370.
- Peres T. V., Parmalee N. L., Martinez-Finley E. J. and Aschner M. (2016) Untangling the manganese- α -synuclein web. *Front. Neurosci.* **10**, 364. <https://doi.org/10.3389/fnins.2016.00364>.
- Pfefferkorn C. M. and Lee J. C. (2010) Tryptophan probes at the α -synuclein and membrane interface. *J. Phys. Chem. B* **114**, 4615–4622.
- Pinho R., Paiva I., Jercic K. G., et al. (2019) Nuclear localization and phosphorylation modulate pathological effects of alpha-synuclein. *Hum. Mol. Genet.* **28**, 31–50.
- Popova B., Kleinknecht A. and Braus G. H. (2015) Posttranslational Modifications and Clearing of Biomolecules. *Biomolecules* **5**, 617–634.
- Pronin A. N., Morris A. J., Surguchov A. and Benovic J. L. (2000) Synucleins are a novel class of substrates for G protein-coupled receptor kinases. *J. Biol. Chem.* **275**, 26515–26522.
- Rasia R. M., Bertoncini C. W., Marsh D., et al. (2005) Structural characterization of copper(II) binding to alpha-synuclein: Insights into the bioinorganic chemistry of Parkinson's disease. *Proc. Natl. Acad. Sci. USA* **102**, 4294–4299.
- Rcom-H'cheo-Gauthier A. N., Goodwin J. and Pountney D. L. (2014) Interactions between Calcium and Alpha-Synuclein in Neurodegeneration. *Biomolecules* **4**, 795–811.
- Rcom-H'cheo-Gauthier A. N., Davis A., Meedeniya A. C. B., and Pountney D. L. (2016) Alpha-synuclein aggregates are excluded from calbindin-D28k-positive neurons in dementia with Lewy bodies and a unilateral rotenone mouse model. *Mol Cell Neurosci.* **77**, 65–75.
- Reznichenko L., Cheng Q., Nizar K., et al. (2012) In vivo alterations in calcium buffering capacity in transgenic mouse model of synucleinopathy. *J. Neurosci.* **32**, 9992–9998.
- De Ricco R., Valensin D., Dell'Acqua S., Casella L., Gaggelli E., Valensin G., Bubacco L. and Mangani S. (2015) Differences in the binding of copper(I) to α - and β -Synuclein. *Inorg Chem.* **54**, 265–272.
- Rodríguez E., Arcos-López T., Trujano-Ortiz L., et al. (2016) Role of N-terminal methionine residues in the redox activity of copper bound to alpha-synuclein. *J. Biol. Inorg Chem.* **21**, 691–702.
- Rott R., Szargel R., Shani V., Hamza H., Savyon M., Elghani F. A., Bandopadhyay R. and Engelender S. (2017) SUMOylation and ubiquitination reciprocally regulate α -synuclein degradation and pathological aggregation. *Proc. Natl. Acad. Sci. USA* **114**, 13176–13181.
- Santner A. and Uversky V. N. (2010) Metalloproteomics and metal toxicology of α -synuclein. *Metallomics* **2**, 378–392.
- Savelieff M. G., Lee S., Liu Y. and Lim M. H. (2013) Untangling amyloid-beta, tau, and metals in Alzheimer's disease. *ACS Chem. Biol.* **8**, 856–865.
- Schaafsma G. (1988) *Calcium in human biology*. ILSI Human Nutrition Reviews, Springer, London.
- Shahpasandzadeh H., Popova B., Kleinknecht A., Fraser P. E. and Outeiro T. F. (2014) Interplay between Sumoylation and Phosphorylation for Protection against. *J. Biol. Chem.* **289**, 31224–31240.
- Sidhu A., Wersinger C. and Vernier P. (2004) α -Synuclein regulation of the dopaminergic transporter: a possible role in the pathogenesis of Parkinson's disease. *FEBS Letters* **565**(1–3), 1–5.
- Signarvic R. S. and DeGrado W. F. (2003) De novo design of a molecular switch: phosphorylation-dependent association of designed peptides. *J. Mol. Biol.* **334**, 1–12.
- Spillantini M. G., Schmidt M. L., Lee V. M., Trojanowski J. Q., Jakes R. and Goedert M. (1997) α -Synuclein in Lewy bodies. *Nature* **388**, 839–840.
- Stefanis L. (2012) α -Synuclein in Parkinson's Disease. *Cold Spring Harb Perspect Med.* **4**, a009399.
- Surguchov A., Surgucheva I., Solessio E. and Baehr W. (1999) Synoretin—A new protein belonging to the synuclein family. *Mol. Cell Neurosci.* **13**, 95–103.
- Takahashi M., Kanuka H., Fujiwara H., Koyama A., Hasegawa M., Miura M. and Iwatsubo T. (2003) Phosphorylation of alpha-synuclein characteristic of synucleinopathy lesions is recapitulated in alpha-synuclein transgenic Drosophila. *Neurosci Lett.* **336**, 155–158.
- Tamamizu-Kato S., Kosaraju M. G., Kato H., Raussens V., Ruyschaert J. M. and Narayanaswami V. (2006) Calcium-triggered membrane interaction of the R-synuclein acidic tail. *Biochemistry* **45**, 10947–10956.
- Tenreiro S., Eckermann K. and Outeiro T. F. (2014a) Protein phosphorylation in neurodegeneration: friend or foe? *Front. Mol. Neurosci.* **7**, 42.
- Tenreiro S., Reimao-Pinto M. M., Antas P., et al. (2014b) Phosphorylation modulates clearance of alpha-synuclein inclusions in a yeast model of Parkinson's disease. *PLoS Genet.* **10**, e1004302.
- Theillet F.-X., Binolfi A., Bekei B., et al. (2016) Structural disorder of monomeric α -synuclein persists in mammalian cells. *Nature*, **530**, 45–50.
- Tholey A., Lindemann A., Kinzel V. and Reed J. (1999) Direct effects of phosphorylation on the preferred backbone conformation of peptides: a nuclear magnetic resonance study. *Biophys J.* **76**, 76–87.
- Tiwari M. K., Leinisch F., Sahin C., Möller I. M., Otzen D. E., Davies M. J. and Bjerrum M. J. (2018) Early events in copper-ion catalyzed oxidation of α -synuclein. *Free Radic. Biol. Med.* **121**, 38–50.

- Ulmer T. S., Bax A., Cole N. B. and Nussbaum R. L. (2005) Structure and dynamics of micelle-bound human alpha-synuclein. *J. Biol. Chem.* **280**, 9595–9603.
- Uversky V. N., Li J. and Fink A. L. (2001) Metal-triggered structural transformations, aggregation, and fibrillation of human alpha-synuclein. A possible molecular link between Parkinson's disease and heavy metal exposure. *J. Biol. Chem.* **276**, 44284–44296.
- Valensin D., Camponeschi F., Luczkowski M., et al. (2011) The role of His-50 of alpha-synuclein in binding Cu(II): pH dependence, speciation, thermodynamics and structure. *Metallomics* **3**, 292–302.
- Valiente-Gabioud A. A., Torres-Monserrat V., Molina-Rubino L., Binolfi A., Griesinger C. and Fernández C. O. (2012) Structural basis behind the interaction of Zn²⁺ with the protein alpha-synuclein and the Aβ peptide: A comparative analysis. *J. Inorg. Biochem.* **117**, 334–341.
- Viles J. H. (2012) Metal ions and amyloid fiber formation in neurodegenerative diseases. Copper, zinc and iron in Alzheimer's, Parkinson's and prion diseases. *Coord. Chem. Rev.* **256**, 2271–2284.
- Villar-Piqué A., Rossetti G., Ventura S., Carloni P., Fernández C. O. and Outeiro T. F. (2017) Copper(II) and the pathological H50Q alpha-synuclein mutant: environment meets genetics. *Commun Integr Biol.* **10**, e1270484.
- Volles M. J. and Lansbury P. T. Jr. (2003) Zeroing in on the pathogenic form of alpha-synuclein and its mechanism of neurotoxicity in Parkinson's disease. *Biochemistry* **42**, 7871–7878.
- Wang C., Liu L., Zhang L., Peng Y. and Zhou F. (2010) Redox reactions of the alpha-synuclein-Cu²⁺ complex and their effects on neuronal cell viability. *Biochemistry* **49**, 8134–8142.
- Wani M. A., Haynes L. D., Kim J., et al. (2006) Familial hypercatabolic hypoproteinemia caused by deficiency of the neonatal Fc receptor, FcRn, due to a mutant beta2-microglobulin gene. *Proc. Natl Acad. Sci. USA* **103**, 5084–5089.
- Waxman E. A. and Giasson B. I. (2008) Specificity and regulation of casein kinase-mediated phosphorylation of alpha-synuclein. *J. Neuropathol. Exp. Neurol.* **67**, 402–416.
- Xiong Y. and Yu J. (2018) Modeling Parkinson's disease in drosophila: what have we learned for dominant traits? *Front Neurol.* **9**, 228.
- Yamamoto A., Shin R. W., Hasegawa K., Naiki H., Sato H., Yoshimasu F. and Kitamoto T. (2002) Iron (III) induces aggregation of hyperphosphorylated tau and its reduction to iron (II) reverses the aggregation: implications in the formation of neurofibrillary tangles of Alzheimer's disease. *J. Neurochem.* **82**, 1137–1147.
- Yang S.-H., Galanis A., Witty J. and Sharrocks A. D. (2006) An extended consensus motif enhances the specificity of substrate modification by SUMO. *EMBO J.* **25**, 5083–5093.
- Yavich L. (2004) Role of Synuclein in Presynaptic Dopamine Recruitment. *Journal of Neuroscience* **24**(49), 11165–11170.
- Zaichick S. V., McGrath K. M. and Caraveo G. (2017) The role of Ca²⁺ signaling in Parkinson's disease. *Dis. Model Mech.* **10**, 519–535.
- Zhang Z., Dai C., Bai J., Xu G., Liu M. and Li C. (2014) Ca²⁺ modulating alpha-synuclein membrane transient interactions revealed by solution NMR spectroscopy. *Biochim Biophys. Acta* **1838**, 853–858.