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# $Ca^{2+}$ modulating α-synuclein membrane transient interactions revealed by solution NMR spectroscopy

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ABSTRACT

α-Synuclein is involved in Parkinson's disease and its interaction with cell membrane is crucial to its pathological and physiological functions. Membrane properties, such as curvature and lipid composition, have been shown to affect the interactions by various techniques, but ion effects on α-synuclein membrane interactions remain elusive. $Ca^{2+}$ dynamic fluctuation in neurons plays important roles in the onset of Parkinson's disease and its influx is considered as one of the reasons to cause cell death. Using solution Nuclear Magnetic Resonance (NMR) spectroscopy, here we show that $Ca^{2+}$ can modulate α-synuclein membrane interactions through competitive binding to anionic lipids, resulting in dissociation of α-synuclein from membranes. These results suggest a negative modulatory effect of $Ca^{2+}$ on membrane mediated normal function of α-synuclein, which may provide a clue, to their dysfunction in neurodegenerative disease.



## 1. Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disease among the aging human population, and it is featured with the loss of dopaminergic neurons in the *substantia nigra pars compacta* and the formation of Lewy bodies mainly composed of α-synuclein (α-syn) amyloid fibrils [1,2]. α-Syn is also genetically linked to PD, evidenced by three mutations (A30P, E46K and A53T) that cause familial PD [3–5]. Moreover, transgenic animal models also support the direct link of α-syn and neurodegeneration because overexpression of α-syn leads to similar symptom as that of PD [6,7].

Although the involvement of α-syn in neurodegenerative diseases is well established, the details of its precise biological role remain largely unknown. α-Syn is predominantly expressed in central neurons and abundant (30–60 μM) in presynaptic terminals [8,9]. Recent work highlights its role in maintenance of reserve pools of synaptic vesicles, dopamine neurotransmission, mitochondrial dysfunction, and aging [10–15]. The physiological function of α-syn is hypothesized to involve its interaction with membranes [16]. In vitro studies show that α-syn lacks a defined secondary structure in dilute aqueous solutions. However, the amino-terminal half of α-syn undergoes conformational transitions to α-helical structures upon binding to negatively charged lipid membranes and detergent micelles, while the negatively charged C terminus remains disordered and is proposed to recruit interaction partner to the membranes [17–22].

α-Syn is associated with the cytosolic side of small synaptic vesicles in presynaptic terminals [9]. Membrane bound α-syn is considered to modulate the fusion of synaptic vesicles and the ensuing neurotransmitter release [14,23]. α-Syn membrane binding property is critical to its normal function [24]. However, majority of cellular α-syn is cytosolic, and membrane bound α-syn dissociates from the synaptic vesicle membrane after exocytosis [25,26]. These observations suggest that α-syn undergoes a transition between membrane and cytosolic solutions in cells. This transition between lipid-free and lipid-bound states is likely to modulate the normal function of α-syn. Nevertheless, little is known about the regulating mechanism of α-syn membrane binding and dissociation.

$Ca^{2+}$ is one of the most important signaling entities in neurons since it regulates a series of cellular processes [27,28]. In neurons, free cytosolic $Ca^{2+}$ levels are maintained around 200 nM under resting conditions, and rise to low micromolar concentrations by a mechanism of extracellular $Ca^{2+}$ influx or $Ca^{2+}$ release from intracellular stores upon stimulation [29]. The release of neurotransmitter in neurons is highly related to transient rise in $Ca^{2+}$ levels. Oxidative stress is also linked to $Ca^{2+}$ dysregulation because of the increase in $Ca^{2+}$ influx and intracellular $Ca^{2+}$ release leading to a severe disruption of normal cellular processes [30]. Several recent studies have shown significant roles of $Ca^{2+}$ in normal and pathological functions of α-syn, such as regulating ligand interaction and oligomerization of α-syn [31], accelerating the formation of the potentially more cytotoxic annular oligomers

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[32] and leading to microscopically-visible α-syn aggregates in vivo [33]. Using solution NMR spectroscopy, here we show that $Ca^{2+}$ modulates α-syn membrane interaction and induces a transition of α-syn from lipid-bound to lipid-free state.

NMR spectroscopy is uniquely capable of providing high-resolution, atom-specific information of the structural and dynamic properties of protein in solution. Besides solid NMR, solution NMR has also been used to study the α-syn membrane interaction [34–36]. Here, we focus on the role of $Ca^{2+}$ on modulating α-syn membrane binding by using solution NMR. Nanodiscs containing POPC (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine) and POPA (1-palmitoly-2-oleoyl-sn-glycero-3-phosphate) which are disc-like phospholipid bilayers surrounded by a scaffolding protein and small unilamellar vesicles (SUVs) were applied as membrane mimics, respectively. To understand the effect of $Ca^{2+}$ on the α-syn lipid interactions on an atomic scale, NMR chemical shift mapping was used to detect the chemical environment change of each residue in the protein. Chemical shift perturbations (CSP) that resulted from interaction provide a highly sensitive tool for identification of protein binding sites. The chemical shift and peak intensity were used as an indicator to study structural change and membrane binding of α-syn, in response to $Ca^{2+}$.

## 2. Materials and methods

### 2.1. Preparation of α-syn

A single colony of *Escherichia coli* strain BL21 DE3 Gold harboring the pT7-7 plasmid was used to inoculate 50 mL of ampicillin containing (100 μg/L) M9 minimal media with uniformly labeled $^{15}N$ ammonium sulfate. The culture was shaken at 37 °C until the optical density at 600 nm of a 1 cm path length sample ($OD_{600}$) reached 0.6. α-Syn expression was induced with 0.5 mM IPTG (isopropyl β-D-1-thiogalactopyranoside). The culture was incubated with shaking at 37 °C for 6 h.

α-Syn was purified by boiling the cell homogenate, which precipitates most cellular proteins, leaving α-syn as the major component of the soluble fraction. The boiled cell lysate was precipitated in ammonium sulfate. The pellet was resuspended, and loaded onto a DEAE column in 20 mM Tris, pH 7.7 and eluted in a gradient of 0–500 mM NaCl. Fractions containing α-syn (analyzed by Coomassie-stained SDS-PAGE) were concentrated using a 10-kDa molecular weight cutoff filter to the desired concentration, and loaded onto a Sephacryl™ S-100 HR column (GE Healthcare) and eluted in 20 mM Tris and 200 mM NaCl, pH 7.7. Fractions containing α-syn were combined and lyophilized.

### 2.2. Preparation of ApoA1

The coding sequence of human Apolipoprotein A1 residues 68–267 (ApoA1) amplified by PCR using *Pfu* DNA polymerase was cloned into the *Nde*I/*Bam*HI restriction sites of pET-28a, and bacterial production of ApoA1 in *E. coli* BL21 (DE3) cells was carried out in LB media. Cells harboring *ApoA1* were grown at 37 °C in LB media containing 50 μg/mL kanamycin to an $OD_{600}$ of 0.5, and the temperature was decreased to 25 °C after adding 0.25 mM IPTG to the cell culture for protein expression. Cells were harvested 3 h later, and cell pellet resuspended in buffer A (20 mM Tris–HCl, 500 mM NaCl, pH 8.0) was sonicated for 30 min to lysis the cells. Lysate was centrifuged for 30 min at 30,000 *g*, and the pellet was resuspended in buffer B (20 mM Tris–HCl, 500 mM NaCl, 0.1% Triton, pH 8.0) followed by centrifugation for 30 min at 30,000 *g*. The supernatant was collected and loaded onto His Trap™ FF column (GE Healthcare) preequilibrated with buffer B and eluted in a gradient of 0–500 mM imidazole. Fractions containing ApoA1 were combined and lyophilized.

### 2.3. SUV preparation

POPC (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine) and POPA (1-palmitoly-2-oleoyl-sn-glycero-3-phosphate) were purchased from Avanti Polar Lipids (Alabaster, AL) and used without further purification. SUVs were prepared as described [19].

### 2.4. Nanodisc preparation

ApoA1, POPA, POPC, β-OG (n-octyl-β-D-glucopyranoside) and sodium cholate were mixed in a molar ratio of 1:30:30:120:120. The mixture was vortexed and incubated at room temperature for 30 min without disturbance. The nanodiscs were formed by dialysis to remove the detergent. The nanodiscs were then run on a Sephacryl™ S-200 HR column (GE Healthcare) in 20 mM HEPES, pH 7.0, 100 mM KCl. Fractions containing nanodiscs were concentrated using a 30-kDa molecular weight cutoff filter to the desired concentration.

### 2.5. $^{1}H$–$^{15}N$ HSQC experiments

NMR spectra were acquired at 15 °C on Bruker Avance 600 MHz and 800 MHz spectrometers. Data were analyzed by Sparky software (http://www.cgl.ucsf.edu/home/sparky) [37]. Samples were prepared from lyophilized protein dissolved in buffer containing 20 mM HEPES, 100 mM KCl, 5% $D_2O$, pH 7.0. Two-dimensional $^{1}H$–$^{15}N$ HSQC experiments were used for the $Ca^{2+}$ titration. Chemical

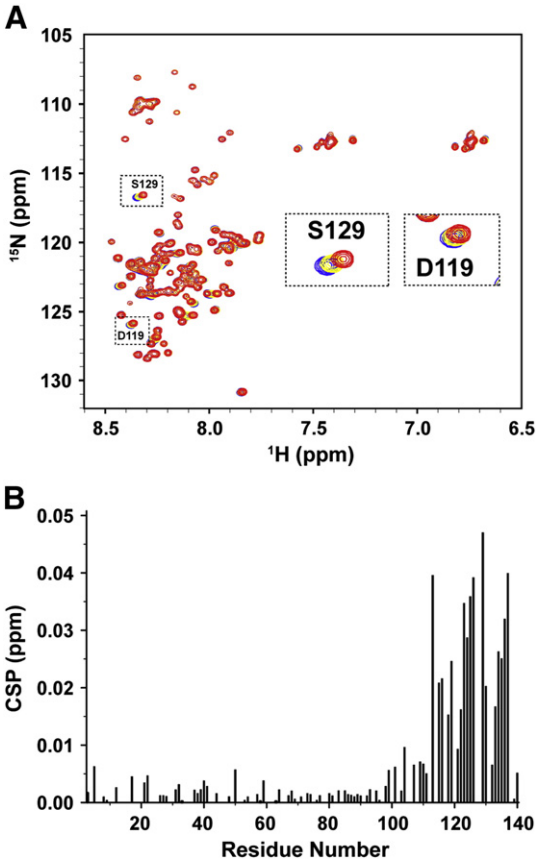


**Fig. 1.** Overlayed $^{1}H$–$^{15}N$ HSQC spectra (A) and chemical shift change (B) of α-syn in the presence of $Ca^{2+}$. (A) Superposition of $^{1}H$–$^{15}N$ HSQC spectra of 0.25 mM $^{15}N$-labeled α-syn in the absence (blue) and presence of 2 mM $Ca^{2+}$ (yellow) or 10 mM $Ca^{2+}$ (red) in 20 mM HEPES, 100 mM NaCl, pH 7.0 at 15 °C. (B) Chemical shift perturbation profiles of α-syn upon addition of 10 mM $Ca^{2+}$, the data are shown according to the equation $(0.04\Delta\delta_N^2 + \Delta\delta_H^2)^{1/2}$, where $\Delta\delta_N$ and $\Delta\delta_H$ represent the change of chemical shift in nitrogen and proton, respectively.

shift assignment is according to BioMagResBank (BMRB entry number 16543 for disordered α-syn).

### 2.6. $^{31}P$ spectrum

The binding of $Ca^{2+}$ to the phosphate group of phospholipids was measured by $^{31}P$ NMR. $^{31}P$ spectra were acquired at 27 °C on a Bruker 600-MHz spectrometer. SUV sample was prepared in buffer containing 20 mM HEPES, 100 mM KCl, 5% $D_2O$, pH 7.0. No proton decoupling was applied during the acquisition of $^{31}P$ spectra.

### 2.7. Circular dichroism spectroscopy

The average secondary structure of α-syn was determined by CD spectroscopy, and CD spectra were recorded on an Applied Photophysics Chirascan instrument. The α-syn:SUV molar ratio was 1:100, and $Ca^{2+}$ was added using a stock solution of $CaCl_2$. Measurements were performed at room temperature in a 1 mm path length quartz cuvette over the range of 190–280 nm, at 1 nm intervals, 1 nm bandwidth and averaging time of 1.0 s. CD spectra of the appropriate buffers were recorded and subtracted from the protein spectra.

## 3. Results

### 3.1. $Ca^{2+}$ binds to the C terminus of α-syn

The overlayed $^{1}H$–$^{15}N$ heteronuclear single-quantum coherence (HSQC) spectra of uniformly $^{15}N$-labeled α-syn in the absence and presence of $Ca^{2+}$ were recorded at 15 °C and pH 7.0 in aqueous buffer. As shown in Fig. 1A, most of the resonances remain intact and only resonances from C-terminal residues shift a little bit, and the chemical shift perturbation in the presence of 10 mM $Ca^{2+}$ is shown in Fig. 1B. Consistent with previous results, $Ca^{2+}$ binds to the C-terminus of α-syn [31]. No broadened or new resonances appear upon adding $Ca^{2+}$, indicating there is no significant conformational change of α-syn and it is still disordered.

### 3.2. $Ca^{2+}$ triggers dissociation of α-syn from membrane surface

α-Syn has been shown to bind to acidic phospholipids and undergoes a conformational change to a highly helical structure. Membrane binding of α-syn is predicted to mediate its biological role in neurons [14,24]. To reveal $Ca^{2+}$ effect on α-syn membrane interactions, nanodiscs that consisted of POPA/POPC (1:1 in molar ratio) were used as the membrane mimics and $^{1}H$–$^{15}N$ HSQC spectra were used to monitor the interaction. Bax group has demonstrated that solution NMR spectroscopy can be employed to quantify the equilibrium of α-syn in free and lipid-bound state in a site-specific manner [35,36]. Consistent with their report, some signals are attenuated considerably or even missing when adding nanodiscs (Fig. 2A and B). When 1 mM $Ca^{2+}$ is added into the α-syn and membrane nanodisc solution, previously disappeared signals reappear and weak signals become stronger (Fig. 2C). This observation suggests that $Ca^{2+}$ increases lipid-free state α-syn. Site-specific signal attenuation profile in the presence of membrane nanodiscs with $Ca^{2+}$ was shown in Fig. 2D. Signals from N-terminal residues were largely attenuated in the presence of nanodiscs, while signals from C-terminal residues exhibit relatively small changes. Once $Ca^{2+}$ is added, signals from N-terminal residues show substantial increase, suggesting more α-syn in lipid-free state in the presence of $Ca^{2+}$. The nanodiscs used in our study consist of POPA/POPC (1:1 in molar ratio), and the lipid: protein ratio is 100:1. Residues whose $^{1}H$–$^{15}N$ cross-peaks are significantly over-lapped were eliminated from further analysis.

Our results clearly show that $Ca^{2+}$ diminishes α-syn membrane interaction, contradicting with a previous study which suggests that $Ca^{2+}$ promotes α-syn membrane association because $Ca^{2+}$ neutralizes the negative charged acidic tail of α-syn [38]. Since we used nanodiscs which have a tabular surface as membrane mimics, additional

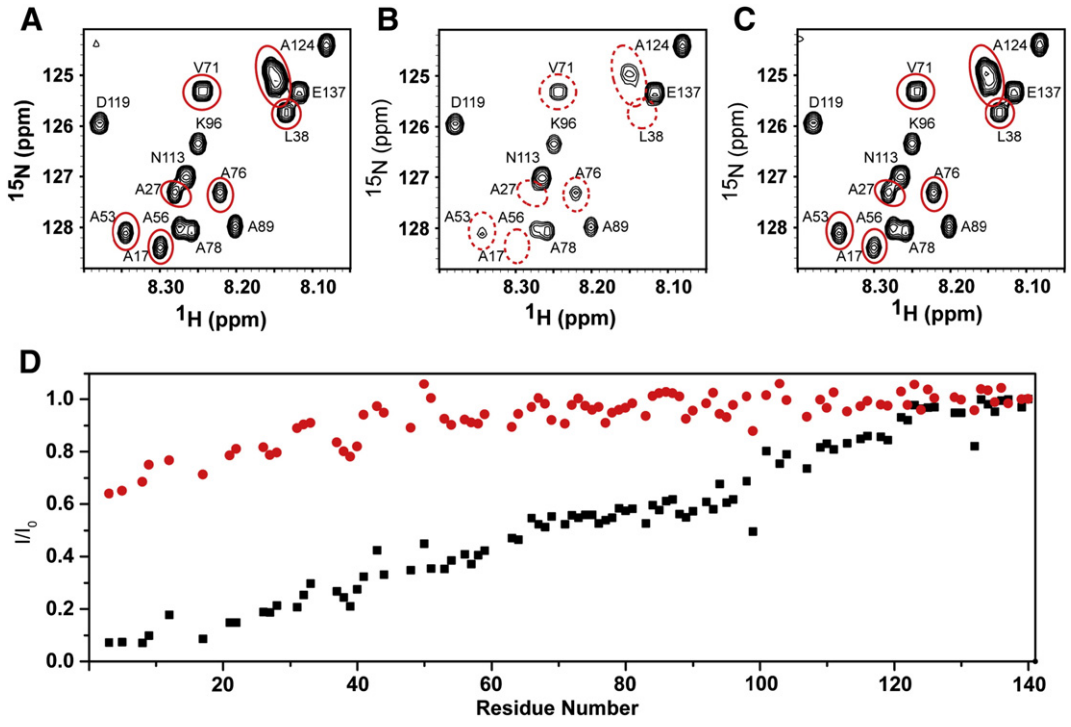


**Fig. 2.** Signal attenuation observed in the $^{1}H$–$^{15}N$ HSQC spectrum of α-syn showing the effect of $Ca^{2+}$ on the α-syn membrane interaction. (A–C) Part of the $^{1}H$–$^{15}N$ HSQC spectra of 0.125 mM $^{15}N$-labeled α-syn in the absence (A) and presence of 12 mM POPC/POPA (1:1 in molar ratio) nanodiscs (B) or 12 mM POPC/POPA (1:1 in molar ratio) nanodiscs and 1 mM $Ca^{2+}$ (C). Cross-peaks that broadened or disappeared upon addition of nanodiscs are around by red circles. (D) Fractional signal attenuation in the HSQC spectra of B (black squares) and C (red solid circles) relative to lipid-free spectra is shown as a function of residue number.

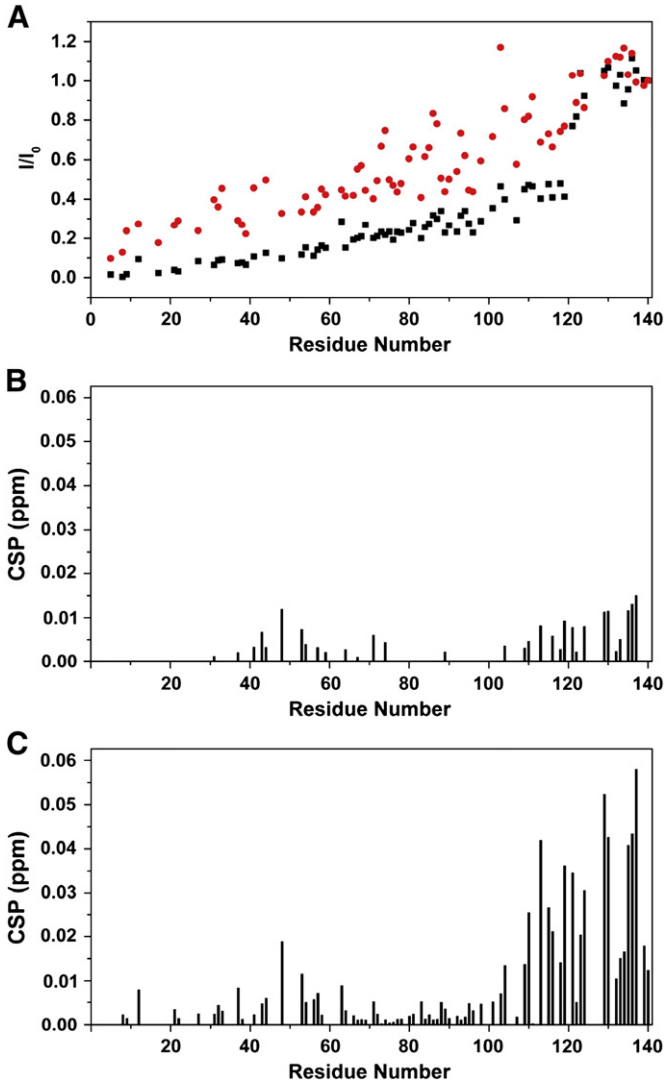


**Fig. 3.** Disrupting role of $Ca^{2+}$ on the interaction of α-syn with SUVs. (A) Fractional signal attenuation in the HSQC spectra of 0.25 mM α-syn in the presence of 24 mM POPC/POPA (1:1 in molar ratio) SUVs (black squares) or 24 mM POPC/POPA (1:1 in molar ratio) SUVs and 4 mM $Ca^{2+}$ (red solid circles) relative to lipid-free spectra is shown as a function of residue number. (B–C) Chemical shift perturbation profiles of α-syn upon addition of 4 mM (B) and 8 mM (C) $Ca^{2+}$, the data are shown according to the equation $(0.04\Delta\delta_N^2 + \Delta\delta_H^2)^{1/2}$,where $\Delta\delta_N$ and $\Delta\delta_H$ represent the change of chemical shift in nitrogen and proton, respectively.

experiments were carried out in order to investigate if the membrane morphology affects the function of $Ca^{2+}$ on α-syn membrane binding. First, we use the same lipid composition of membrane nanodiscs and SUVs in the study. The similar signal attenuation pattern in membrane nanodiscs and SUVs suggests that α-syn has the same binding mode in membrane nanodiscs and SUVs (Fig. 3). This result indicates that the disrupting role of $Ca^{2+}$ was insensitive to the morphology of membrane mimics. Titration of $Ca^{2+}$ caused substantial chemical shift change of α-syn C-terminal region in the presence of $Ca^{2+}$ at 4 mM or higher concentration, and shift pattern was similar to that as shown in Fig. 1. α-Syn also shows the tendency of decrease in helical content in the presence of varying concentrations of $Ca^{2+}$ by CD spectroscopy (Fig. S1), but it is not as pronounced as that revealed by NMR spectroscopy.

### *3.3. $Ca^{2+}$ competes with α-syn for the binding sites of phospholipids according to its charge*

In order to investigate the mechanism by which $Ca^{2+}$ regulates the α-syn membrane interaction, we used $^{31}P$ NMR spectroscopy to probe the change in the chemical environment of phospholipid in response to $Ca^{2+}$ addition [39,40] (Fig. 4). SUVs that consisted of POPA/POPC (1:1 in molar ratio) were used as the membrane mimics. As expected, $Ca^{2+}$ binding induced substantial chemical shift change of the phosphorus signal of POPA, other than that of zwitterionic POPC (Fig. 4A). Same directional shift of the phosphorus signal was observed as a result of α-syn binding (Fig. 4B), indicating that both $Ca^{2+}$ and α-syn are bound to the same phosphate region in the lipid. The broadening of $^{31}P$ resonance in the presence of $Ca^{2+}$ and α-syn may due to binding induced by dynamic change of the head group [41,42].

In addition, we replaced $Ca^{2+}$ by $Mg^{2+}$, another divalent cation, to further investigate whether the role of $Ca^{2+}$ in α-syn membrane binding is primarily contributed by its charge. A similar result was observed, which indicated that $Mg^{2+}$ could also cause α-syn to disassociate from the membrane (Fig. 5).

## 4. Discussion

In dopaminergic neurons, α-syn partitions between a disordered cytosolic state and helical phospholipid-bound state. Studies suggest that membrane bound α-syn has a high aggregation propensity, since membrane binding facilitates a local increase in α-syn concentration [43,44], while $Ca^{2+}$ is reported to trigger C-terminus membrane binding and induce aggregation of α-syn [38]. In contrast to their speculations of $Ca^{2+}$ function on α-syn membrane binding, our results

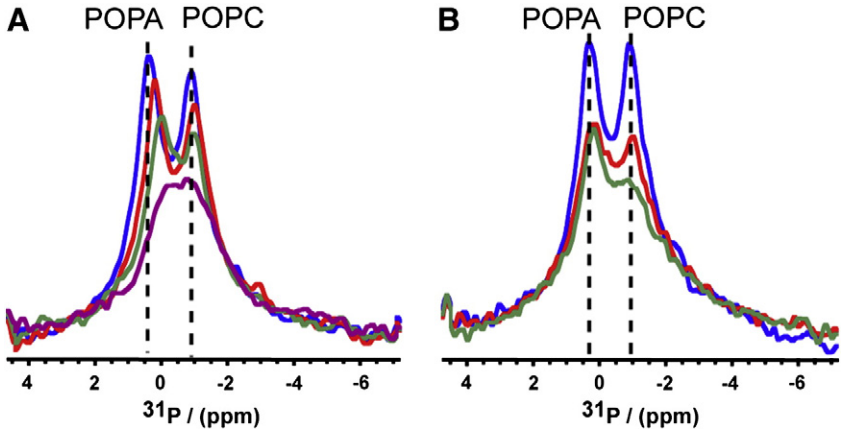


**Fig. 4.** $Ca^{2+}$ bound to the phosphate group of anionic phospholipids. One-dimensional $^{31}P$ NMR spectra of POPC/POPA (1:1 in molar ratio) SUVs induced by the binding of $Ca^{2+}$ with different molar ratios to phospholipids, 0 (blue), 0.2 (red), 0.4 (green) and 0.8 (purple) (A) or α-syn with different molar ratios to phospholipids, 0 (blue), 0.01 (red) and 0.02 (green) (B). The SUVs used in this study consist of POPC/POPA (1:1 in molar ratio), and the concentration is 6 mM.

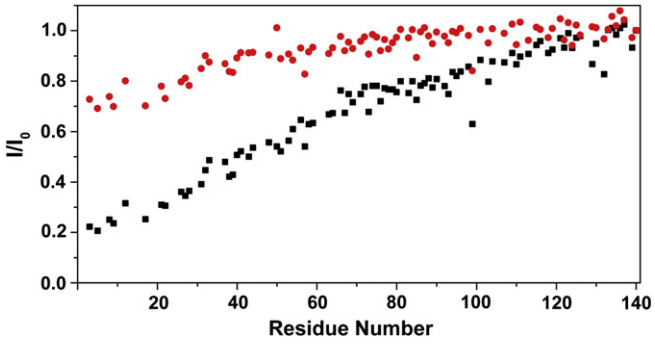


**Fig. 5.** Signal attenuation observed in the $^{1}H$–$^{15}N$ HSQC spectrum of α-syn showing the effect of $Mg^{2+}$ on the α-syn membrane interaction. Fractional signal attenuation in the HSQC spectra of 0.125 mM α-syn in the presence of 12 mM POPC/POPA (1:1 in molar ratio) nanodiscs (black squares) or 12 mM POPC/POPA (1:1 in molar ratio) nanodiscs and 1 mM $Mg^{2+}$ (red solid circles) relative to lipid-free spectra is shown as a function of residue number.

suggested that $Ca^{2+}$ competes with α-syn for the binding sites of phospholipids and exerts a negative modulatory effect on α-syn membrane interaction, resulting in dissociation of α-syn from the membrane surface (Fig. 6). Rapid and transient rise in $Ca^{2+}$ levels is one of the most prominent events in postsynaptic neurons; this local increase in $Ca^{2+}$ concentrations results in a number of short-term and long-term synapse-specific alterations [29]. The results of fluorescence and CD experiments by incubating of $Ca^{2+}$ with POPC/POPA/α-syn complexes for 16 h showed that $Ca^{2+}$ triggered α-syn membrane association of the acidic tail as a potential mechanism leading to α-syn aggregation [38]. Here, we studied the short-term effects of $Ca^{2+}$ on α-syn membrane binding in membrane mimics by estimating the equilibrium profile of α-syn in membrane bound and membrane free state, and the results suggested that $Ca^{2+}$ induced dissociation of α-syn from membrane surface, resulting in increased lipid free state α-syn.

In nanodiscs, 1 mM $Ca^{2+}$ did not bind to α-syn since little chemical shift change was observed (Fig. 2). Increased $Ca^{2+}$ concentration resulted in more α-syn in lipid-free state and substantial chemical shift changes of α-syn C-terminal region were observed when $Ca^{2+}$ concentration rises to 4 mM (Fig. S2). Study in SUVs showed similar results; under conditions of low $Ca^{2+}$ concentration (4 mM or lower), addition of $Ca^{2+}$ changes the attenuation profile but causes subtle chemical shift changes, while at higher $Ca^{2+}$ concentration, titration of $Ca^{2+}$ causes substantial chemical shift change of α-syn C-terminal region. α-Syn membrane affinity was also estimated in this study [36], and the result showed that the Kd.t in nanodiscs with 1 mM $Ca^{2+}$ is 20–30 times to that without $Ca^{2+}$, indicating lower affinity of α-syn to membrane surface in the presence of $Ca^{2+}$. Taken together with the competitive binding of $Ca^{2+}$ with α-syn to phospholipids, we speculated that $Ca^{2+}$ firstly binds to membrane at low concentration and disrupts α-syn membrane interaction, and after the binding sites in the membrane are saturated, $Ca^{2+}$ will then bind to the C-terminus of α-syn as the same pattern as in aqueous buffer. Besides $Ca^{2+}$, $Mg^{2+}$ also caused disassociation of α-syn from membrane surface, but titration of $Na^{+}$ had little effect on the attenuation profile of α-syn in nanodiscs and even the charge quantity of $Na^{+}$ was as the same as that of $Ca^{2+}$ or $Mg^{2+}$ (Fig. S3). A previous study showed that the interaction of $Na^{+}$ to membrane is much weaker than that of $Ca^{2+}$ and $Mg^{2+}$ [45]. We speculated that $Ca^{2+}$ disrupts α-syn membrane binding mainly according to its charge, but whether the metal ions have the disrupting effect or not is related to its binding affinity to membranes.

A recent study indicated that $Ca^{2+}$ disrupts $CD3_{CD}$–membrane interaction, and has a positive feedback effect on amplifying and sustaining CD3 phosphorylation in T-cell [46]. Studies on amyloid peptides which are the major constituents of amyloid deposits in various amyloid diseases showed that amyloid-beta and IAPP are membrane disruptive via channel formation and by detergent-like mechanism, and that ions like zinc and calcium are shown to block these membrane disruptions [47,48]. It has been suggested that the mechanism of toxicity in PD involves direct, disruptive interactions between α-syn and cellular membranes [49]. The predominant changes in aging neurons include increased $Ca^{2+}$ release from intracellular stores and increased extracellular $Ca^{2+}$ influx [28]. The resulting changes in neuronal $Ca^{2+}$ concentration may lead to a transition of α-syn from lipid-bound state to lipid-free state in presynaptic terminals. Thus, membrane mediated normal function of α-syn may be regulated by $Ca^{2+}$ dynamics, which provides a clue, to their dysfunction in aging neurons.

Nanodiscs are of suitable size for solution NMR analyses, while SUV causes significant line-broadening of NMR peaks originating from lipid-binding. Recent studies indicated that lipid bilayer curvature plays a role on membrane–amyloid interactions, and the binding affinity of α-syn with membrane also has been shown to be sensitive to membrane curvature [50–54]. When bound to the nanodisc bilayer, α-syn showed a peak $ET_{eff}$ (the efficiency of transfer) close to that measured using vesicles in FRET (Förster Resonance Energy Transfer) experiments, suggesting that nanodiscs induce an extended helix conformation in α-syn [55]. In this study, binding affinity of α-syn to nanodiscs and SUVs was estimated, respectively. Although α-syn has a higher affinity for SUVs than nanodiscs of the same composition because of differences in membrane morphology and curvature, the interaction pattern of $Ca^{2+}$ on α-syn membrane interaction was similar in these two systems.

In conclusion, we showed that $Ca^{2+}$ binds to anionic lipid and weakens the α-syn membrane interaction, resulting in dissociation of α-syn from membrane surface. This result gives a new insight of $Ca^{2+}$ role on α-syn membrane interaction. As α-syn is a curvature-sensing protein, we used both nanodiscs and SUVs as membrane mimics in this study. Similar results were observed in these two systems, indicating that nanodiscs may be a good membrane mimics for solution NMR study of transient protein membrane interactions.

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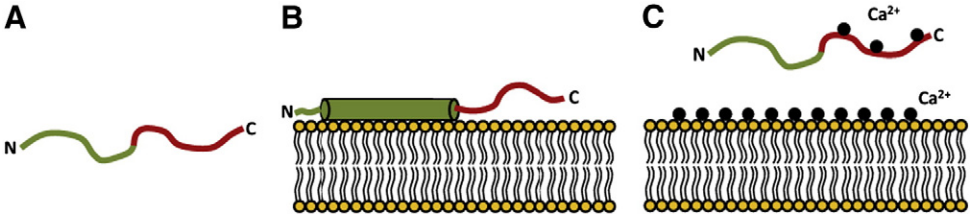


**Fig. 6.** Schematic representation of the proposed effects of $Ca^{2+}$ on α-syn membrane interaction. (A) α-Syn behaves as an unstructured protein in dilute aqueous solution. (B) The amino-terminal half (shown in green) of α-syn undergoes conformational transitions to α-helical structures upon binding to negatively charged lipid membranes, while the negatively charged C terminus (shown in red) remains disordered. (C) $Ca^{2+}$ (shown as black spots) induces dissociation of α-syn from membrane surface.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bbamem.2013.11.016.

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