

Supporting Information

for

Copper(II) enhances membrane-bound

α -synuclein helix formation

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Materials and Methods.

Copper(II) sulfate pentahydrate ($\text{Cu}^{\text{II}}\text{SO}_4 \cdot 5 \text{H}_2\text{O}$; Sigma-Aldrich 99.999 %) was stored in a dessicator. Deionized water for anaerobic copper stock solutions and pH 7.0 buffer solutions containing 20 mM 3-{N-morpholino}propanesulfonic acid (MOPS; SigmaUltra > 99.5 %) and 100 mM sodium chloride (NaCl; Sigma 99.5 %) were sterile filtered (0.22 μm) and stored under an inert atmosphere (10 % H_2 , 90% N_2) within a glovebox (Coy Laboratory Products; Grass Lake, Michigan) following thorough deoxygenation *via* standard Schlenk techniques. Synthetic phospholipids, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphate (POPA) and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC), in chloroform were purchased from Avanti Polar Lipids (Alabaster, AL) and used as received. Equimolar POPA:POPC vesicles were prepared using a Branson 415 Sonifier.

Absorption data were collected on a Cary 300 Bio or Agilent 8453 spectrophotometer. Tryptophan fluorescence was measured using a Horiba Jobin Yvon Fluorolog-3 spectrofluorimeter ($\lambda_{\text{ex}} = 295 \text{ nm}$, $\lambda_{\text{obs}} = 300 - 500 \text{ nm}$, slit widths = 2 nm). Protein samples for fluorescence experiments were prepared in the glovebox using deoxygenated buffer solution (20 mM MOPS, 100 mM NaCl, pH 7.0) and removed from the glovebox in 1 cm screw-capped quartz cuvettes. Data were analyzed as previously published using the program IGOR 6.01 (Wavemetrics).¹ Circular dichroism (CD) spectra were collected on a Jasco J-715 spectropolarimeter using a 0.1 cm quartz cuvette ($\lambda_{\text{obs}} = 200 - 400 \text{ nm}$; bandwidth = 1 – 2 nm; scan rate = 200 nm/min; accumulation = 5). The mean residue ellipticity, $[\Theta]$ ($\text{deg cm}^2 \text{ dmol}^{-1}$), was calculated according to the equation $[\Theta] = (100\theta)/(cIN)$, where θ is the measured ellipticity (mdeg), c is the sample concentration (mM), l is the path length (cm), and N is the number of amino acids. Percent α -helicity was calculated using the equation $([\Theta]_{222\text{nm}} - 3000)/(-39,000 \times 100)$. Reference values for $[\Theta]_{222\text{nm}} = 0$ and $-36,000$ ($\text{deg cm}^2 \text{ dmol}^{-1}$) were used for 0% and 100% helicity, respectively.²

Recombinant Protein Expression and Purification. Protein (wild-type and F4W) was expressed and purified as previously described.^{1, 3} Sample homogeneity was verified by SDS-PAGE gel analyses visualized by silver-staining methods (Phastsystem, Amersham Biosciences). All purified proteins were concentrated using a Millipore Amicon Concentrator (MWCO 3k) and stored at $-80 \text{ }^\circ\text{C}$. Prior to experiments, all protein samples were exchanged into the appropriate buffer (20 mM MOPS, 100 mM NaCl, pH 7.0) using gel filtration chromatography (PD-10 column, GE Healthcare) and filtered through Millipore Microcon YM-100 (MWCO 100k) spin filter units to remove any oligomeric material. Protein concentrations were determined using a value for molar absorptivity estimated on the basis of amino-acid content: $\epsilon_{280 \text{ nm}} = 5,120 \text{ M}^{-1} \text{ cm}^{-1}$ (wild-type) and $\epsilon_{280 \text{ nm}} = 10,810 \text{ M}^{-1} \text{ cm}^{-1}$ (F4W).

Preparation of Anaerobic Copper Solutions. Copper(II) stock solutions were prepared in the glovebox by dissolution of $\sim 1 \text{ g}$ of $\text{Cu}^{\text{II}}\text{SO}_4 \cdot 5 \text{H}_2\text{O}$ in $\sim 10 \text{ mL}$ deoxygenated deionized water. The sample concentration was calculated based on the known molar absorptivity ($\epsilon = 11 \text{ M}^{-1} \text{ cm}^{-1}$) of the *d-d* transitions at 833 nm using a 1 mm cuvette.

This concentrated solution was then adjusted for preparation of serially diluted copper(II) stocks ranging in concentrations from 3.75 μ M to 3.75 mM. Each vial was then fitted with a rubber septum and sealed with a crimper for removal from the glovebox. Copper(II) additions to protein samples were conducted on the benchtop using a Hamilton 25 μ L syringe. Samples were equilibrated for 5 min by stirring before data collection.

Preparation of Liposomes. Synthetic membranes were prepared using a 1:1 molar ratio of POPA and POPC, similar to our previous report but with slight modifications.⁴ Chloroform was removed through evaporation under a nitrogen stream for 15 min resulting in an even layer of lipids. The lipids were further dried by vacuum desiccation for 45 min to ensure complete removal of organic solvent. Dehydrated samples were resuspended in 20 mM MOPS, 100 mM NaCl buffer, pH 7.0, to a final concentration of 5 mg/mL. Complete dissolution was achieved by ultrasonication (50% duty cycle, microtip limit) in a water bath for 45 minutes. Vesicle solutions were then brought into the glovebox and diluted with deoxygenated buffer to a final concentration of 2.5 mg/mL and equilibrated overnight (~14 h) at 34 °C. Following equilibration, vesicles were uncapped and opened to the glovebox atmosphere for 30 mins in order to confirm complete removal of O₂. An average vesicle hydrodynamic radius of 50 nm was measured using a Wyatt DynaPro Nanostar dynamic light scattering instrument.

References

1. J. C. Lee, H. B. Gray and J. R. Winkler, *J. Am. Chem. Soc.*, 2008, **130**, 6898-6899.
2. Y. H. Chen, J. T. Yang and K. H. Chau, *Biochemistry*, 1974, **13**, 3350-3359.
3. J. C. Lee, R. Langen, P. A. Hummel, H. B. Gray and J. R. Winkler, *Proc. Natl. Acad. Sci. U. S. A.*, 2004, **101**, 16466-16471.
4. C. M. Pfefferkorn and J. C. Lee, *J. Phys. Chem. B*, 2010, **114**, 4615-4622.