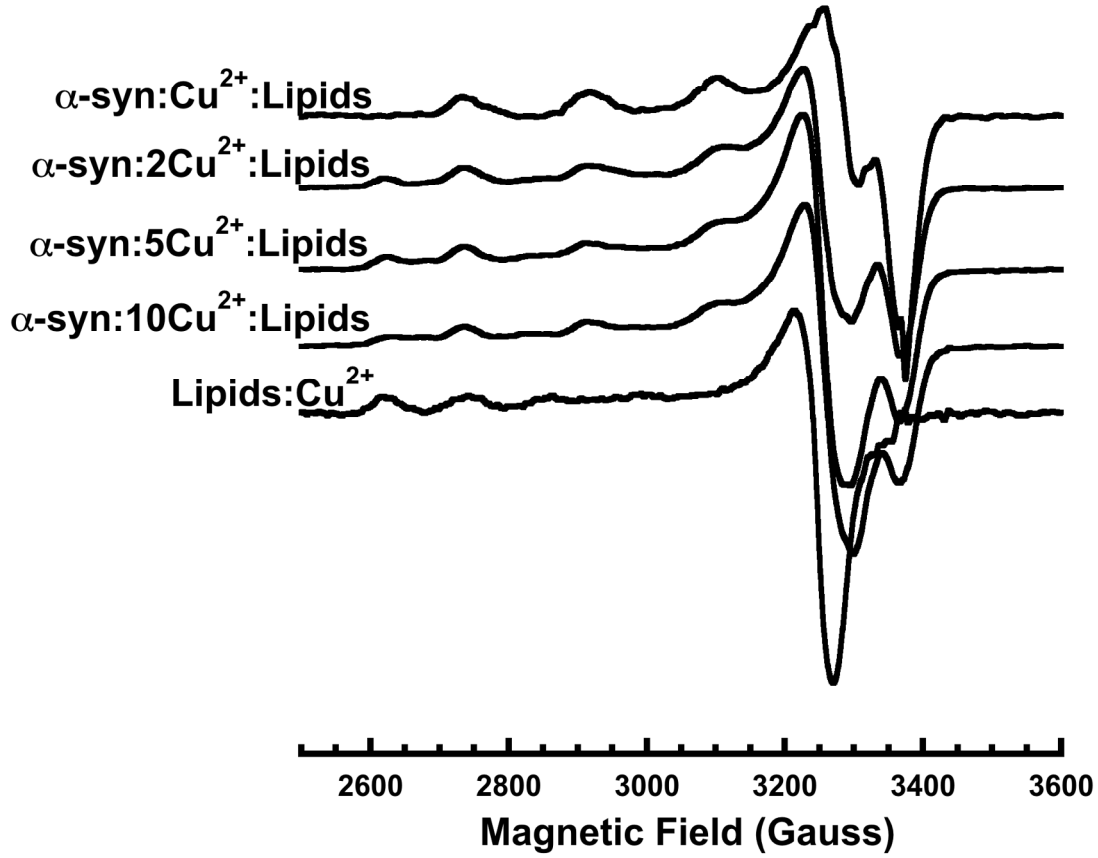
Copper Coordination to the Membrane Bound Form of α-Synuclein

Supplemental Figures

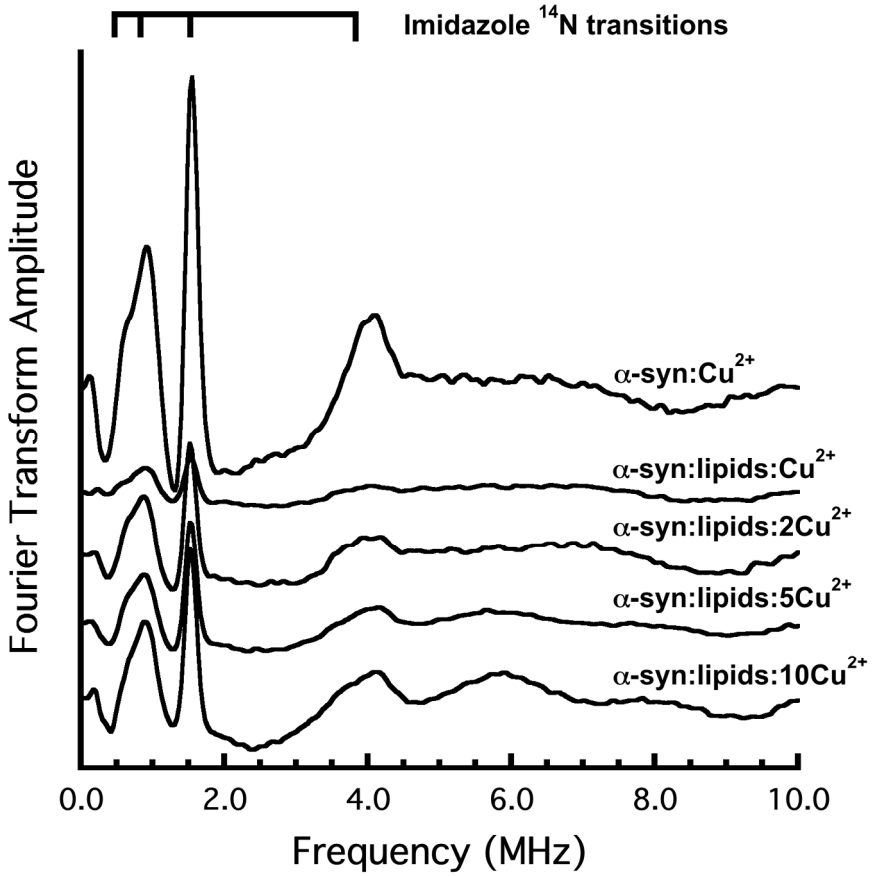
Christopher G. Dudzik, Eric D. Walter, Benjamin S. Abrams, Melissa S. Jurica and Glenn L. Millhauser*

Figure S1



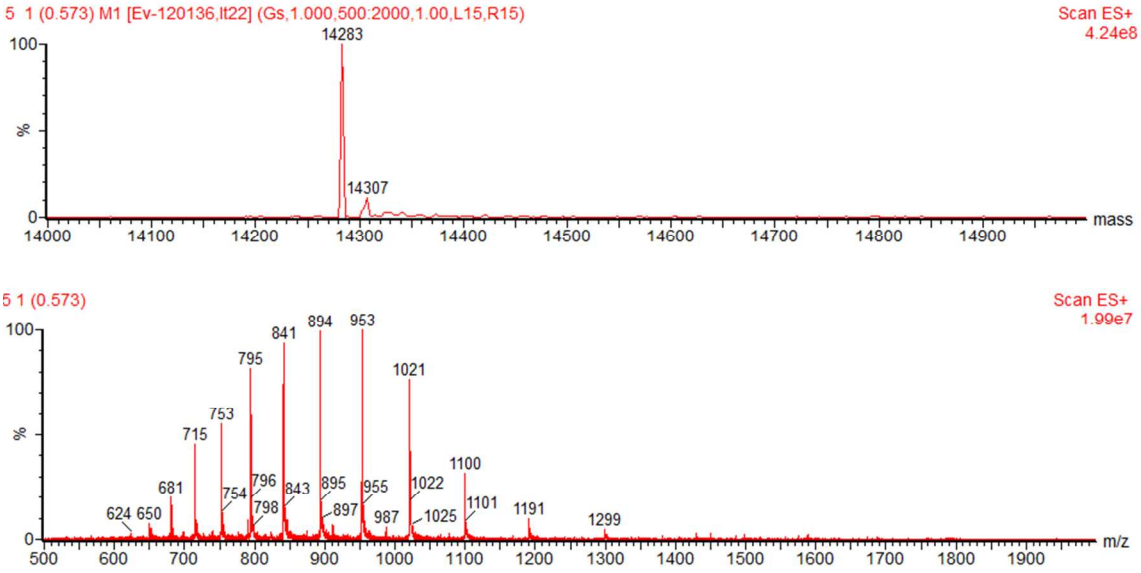
Supplemental Figure 1. X-Band EPR spectra of membrane-bound α-syn (50μM α-syn) as a function of added Cu^{2+}. Copper is increased at a fixed α-syn and lipid composition. With greater than 1.0 equivalent of copper, the spectra exhibit features of a superposition of membrane bound α-syn and lipid copper complexes, consistent with a single membrane-bound α-syn copper binding site with affinity sufficient to outcompete the lipids.

Figure S2



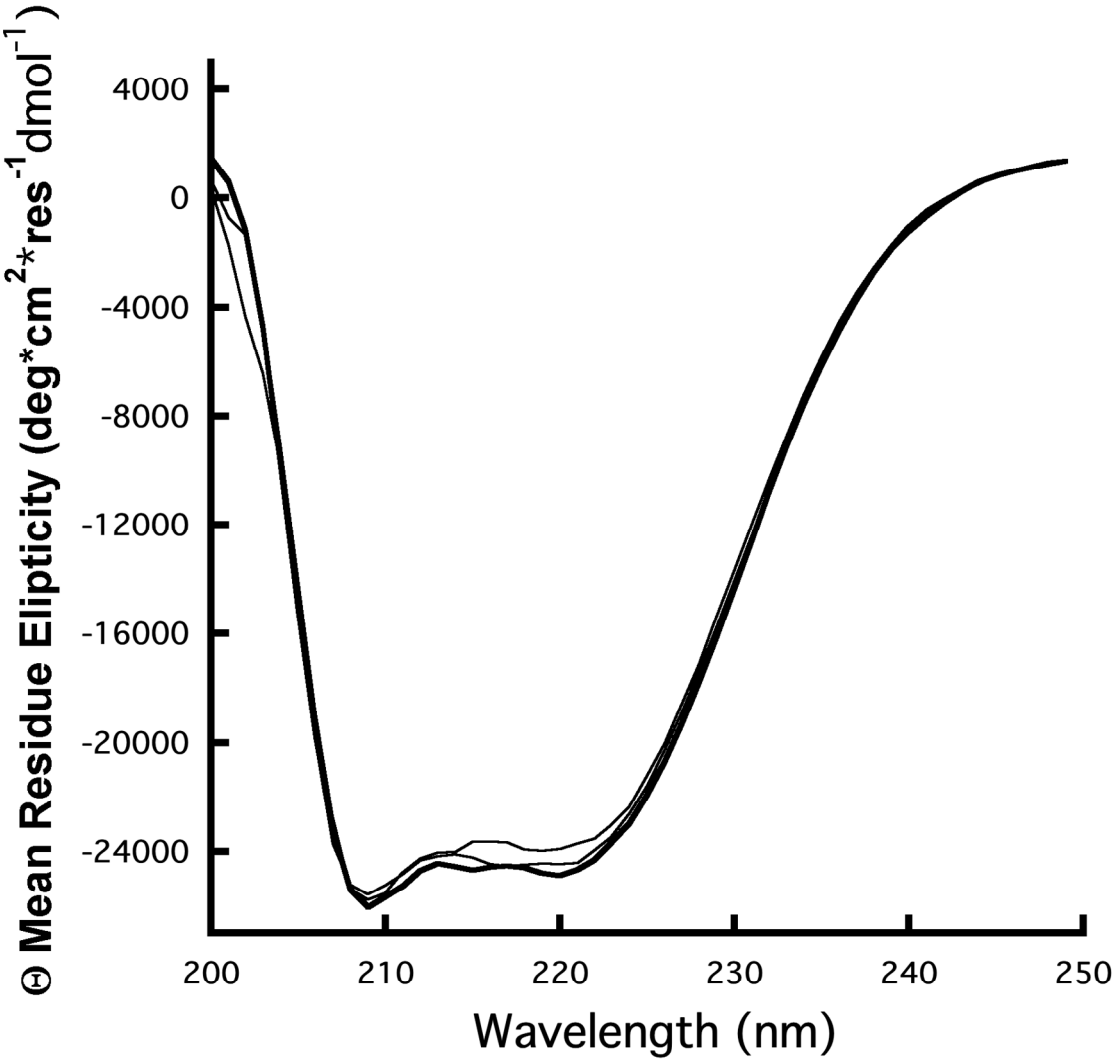
Supplemental Figure 2. Three-pulse ESEEM spectra of α-syn (50μM α-syn, 15mM lipids) with up to 10 equivalents of Cu^{2+}, both in solution and in the presence of lipid SUV. The spectra of the samples in solution and in the presence of SUV with greater than 1 equivalent of Cu^{2+} reveal the expected quadrupolar transitions associated with the imidazole remote nitrogen and demonstrate coordination by His50 whereas the sample with stoichiometric concentrations of α-syn and Cu^{2+} in the presence of SUV do not. While the ^{14}N quadrupolar features of the samples containing lipid SUV with 2, 5 and 10 equivalents of copper suggest a Cu^{2+}-imidizole interaction, these peaks saturate only at copper concentrations well in excess of 250 μM – 500 μM copper, suggesting a very low affinity interaction.

Figure S3



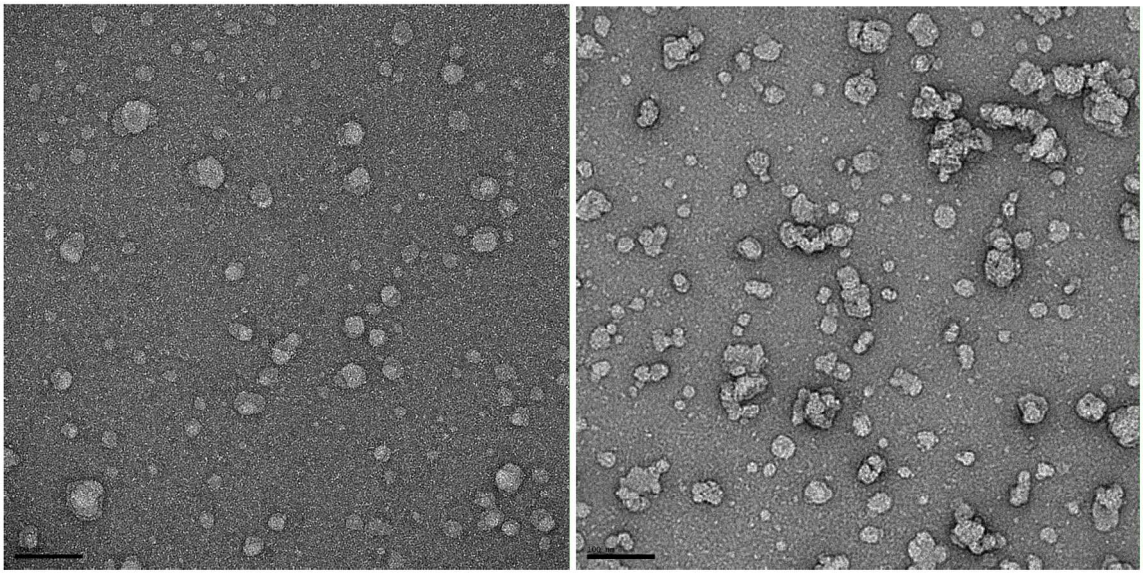
Supplementary Figure 3: Mass spectrometry data for α-syn N-trunc mutant. Deconvolution (above) reveals the expected mass of 14283 as the major species (>90%) of the sample. Wild-type α-syn has a mass of 14456.

Figure S4



Supplementary Figure 4: CD spectra of wild-type α-syn, α-syn N-trunc, and α-syn H50A.

Figure S5



Supplementary Figure 5: Transmission Electron Microscopy images of 70%POPC:30%POPG SUVs both without α-syn (left) and with α-syn (right, lipid:α-syn ratio 300:1). The black bar at lower left is equivalent to 100nM. Most SUVs are in the range 20 – 50 nm, with no indication of tubes or other unusual structures. Samples for electron microscopy were prepared by spotting 3µl onto glow-discharged carbon-coated copper grids followed by staining with 2% (w/v) uranyl acetate solution. Samples were analyzed using a JEOL 1230 microscope operating at 120kV.