Supplementary Information

Membrane insertion exacerbates the α-Synuclein-Cu(II) dopamine oxidase activity: metallothionein-3 targets and silences all α-Synuclein-Cu(II) complexes

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Supplementary Figures

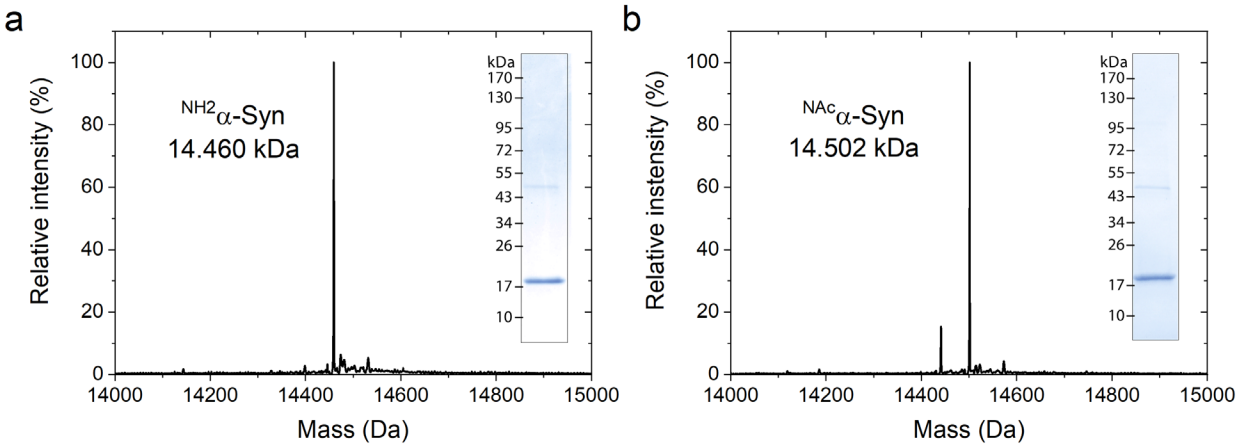

Figure S1. Intact protein mass spectra of purified ^{NH2}α-Syn (a) and ^{NAc}α-Syn (b). Insets: SDS-PAGE of the corresponding purified proteins.

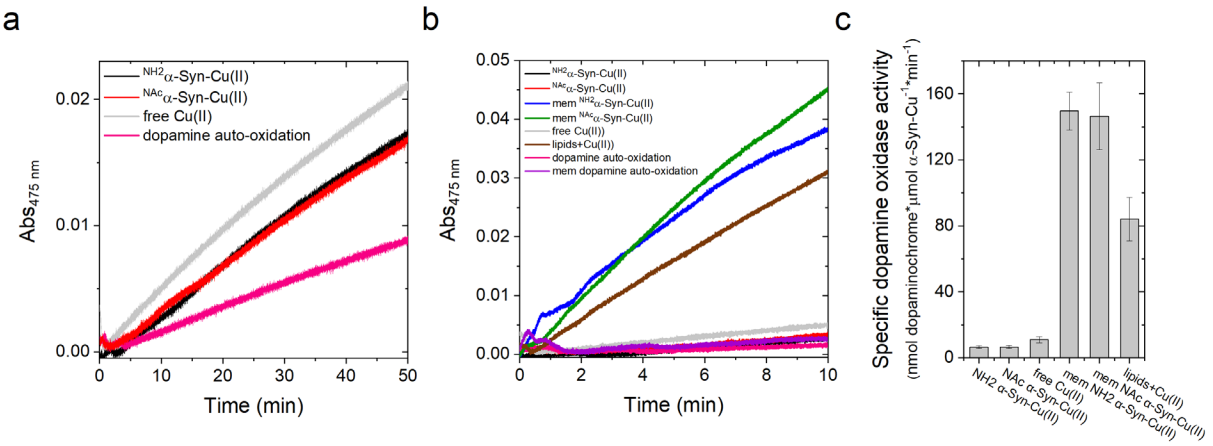

Figure S2. Kinetic traces at 475 nm monitoring dopaminochrome formation upon the reaction of soluble ^{NH2}α-Syn-Cu(II) (10 µM; black), ^{NAc}α-Syn-Cu(II) (10 µM; red), and free Cu(II) (10 µM; gray) with dopamine (2 mM). Dopamine (2 mM) auto-oxidation is shown in pink. (b) Kinetics traces at 475 nm to monitor dopaminochrome formation upon the reaction of soluble and membrane-bound ^{NH2}α-Syn-Cu(II) (10 µM; black and blue, respectively) and ^{NAc}α-Syn-Cu(II) (10 µM; red and green, respectivey) with dopamine (2 mM). The kinetic traces upon reaction of free Cu(II) and Cu(II) in lipids are shown in gray and brown, respectively. Dopamine (2 mM) auto-oxidation in buffer and in the presence of lipids are shown in pink and purple, respectively. (c) Specific dopamine oxidase activities determined by quantifying the dopaminochrome formed after the reaction of α-Syn-Cu(II) (10 µM) or free Cu(II) (10 µM) with dopamine (2mM), for 10 min for soluble forms and 2 min for membrane-bound forms (ε_{475}=3,700 $M^{-1}cm^{-1}$) . Calculated specific dopamine oxidase activities in nmol dopaminochrome*µmol α-Syn-Cu(II)$^{-1}$*min^{-1}: ^{NH2}α-Syn-Cu(II): 6.5 ± 0.8; ^{NAc}α-Syn-Cu(II): 6.6 ± 0.9; free Cu(II): 10.9 ± 1.9; mem ^{NH2}α-Syn-Cu(II): 149.6 ± 11.5; mem ^{NAc}α-Syn-Cu(II): 146.4 ± 20.2; lipids+Cu(II): 84.1 ± 13.2.

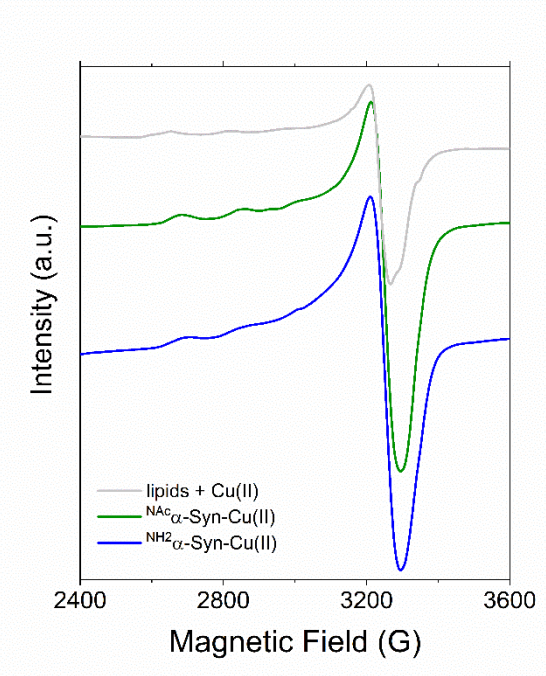

Figure S3. EPR spectra of membrane-bound ^{NH2}α-Syn-Cu(II) (blue), ^{NAc}α-Syn-Cu(II) (green), or Cu(II) in lipids (gray) (500 µM) in 20 mM N-ethylmorpholine pH 7.4 and 25% (v/v) glycerol, recorded at 10 K using υ= 9.44 GHz, microwave power of 0.77 mW, modulation amplitude of 10.0 G and field sweep of 1200 G. Resulting fiiting parameters: ^{NH2}α-Syn-Cu(II): $g\perp$ = 2.06 , $g_{||}$= 2.28, $A_{||}$= 168 G; ^{NAc}α-Syn-Cu(II); $g\perp$ = 2.06 , $g_{||}$= 2.28, $A_{||}$= 157 G; lipids- Cu(II): $g\perp$ = 2.07 , $g_{||}$= 2.33, $A_{||}$= 150 G.

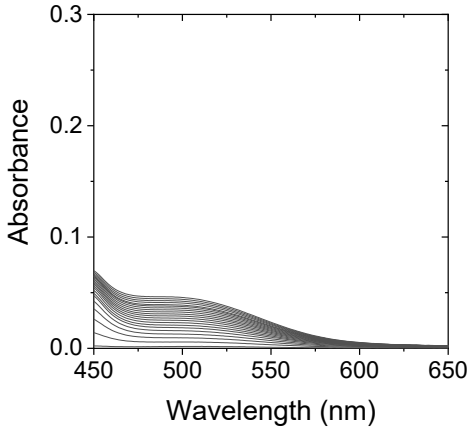

Figure S4. Dopamine (1 mM) auto-oxidation in 20 mM N-ethylmorpholine/100 mM NaCl, pH 7.4 determined in the presence MBTH (2 mM, 100 min, 25°C).

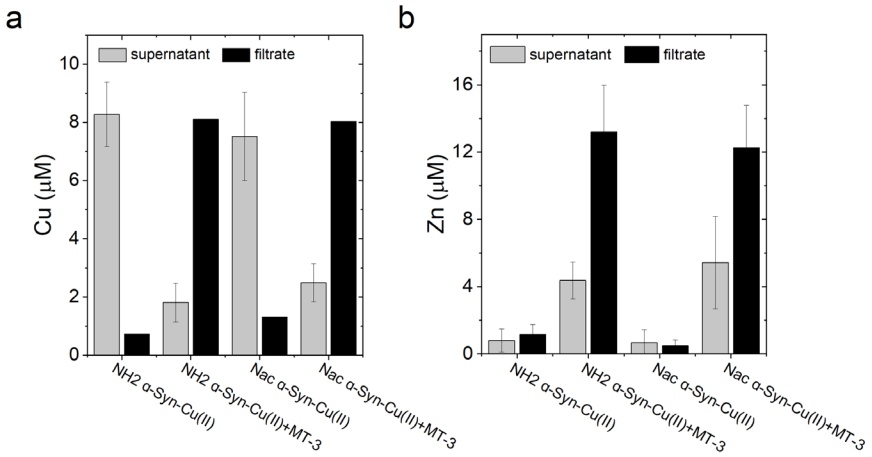

Figure S5. ICP-MS copper and zinc quantification after reaction (1 h, 25°C) between membrane-bound α-Syn-Cu(II) (10 μM) and Zn_7MT-3 (2.5 μM), upon separation of membrane-bound α-Syn in the supernatant (gray bar) from MT-3 in the filtrate (black bar) using 50-kDa MWCO filters.

Table S1. ICP-MS quantification of Cu(II) binding (10 µM) to soluble or membrane-bound ^{NH2}α-Syn or ^{NAc}α-Syn (12 µM), using 3-kDa or 50-kDa MWCO filters, respectively, to separate unbound Cu(II) before analysis.

	% of total Cu	
	supernatant	**filtrate**
^{NH2}α-Syn	99.88 ± 6.11	0.12 ± 0.12
^{NAc}α-Syn	97.71 ± 1.69	2.29 ± 0.36
mem ^{NH2}α-Syn	97.26 ± 1.97	2.74 ± 0.33
mem ^{NAc}α-Syn	96.98 ± 0.81	3.02 ± 0.35
lipids only	92.49 ± 5.60	7.51 ± 7.89

Table S2. Retention volumes and peak areas for soluble and membrane-bound ^{NH2}α-Syn or ^{NAc}α-Syn (15 μM) after size exclusion chromatography analysis using a Superdex 200 column.

	Retention volume (ml)	Area under peak (ml*mAU)
^{NH2}α-Syn	13.36	21.83
^{NH2}α-Syn + lipids	12.52	2.36
^{NAc}α-Syn	13.23	19.54
^{NAc}α-Syn + lipids	13.21	1.74

Table S3. Michaelis-Menten analysis of the dopamine oxidase activity for membrane-bound ^{NAc}α-Syn in 20 mM N-ethylmorpholine/100 mM NaCl, pH 7.4 in the presence of increasing H_2O_2 concentrations. Dopamine oxidation activity was determined after 20 s (25°C) in the presence of MBTH (2 mM).

H_2O_2 concentration (M)	V_{MAX} (nmol quinone*µmol^{-1} α-Syn-Cu(II)*min^{-1})	K_M (mM)
0.00	1281.6 ± 63.6	0.29 ± 0.05
0.05	1551.6 ± 128.5	0.10 ± 0.02
0.10	1880.2 ± 104.5	0.11 ± 0.01
0.25	1873.3 ± 44.3	0.05 ± 0.00
0.50	1970.2 ± 104.2	0.06 ± 0.01
1.00	2044.78 ± 39.56	0.12 ± 0.01

Table S4. Low-temperature luminescence lifetimes of the 425 nm and 575 nm bands recorded on the reaction products between soluble or membrane-bound ^{NH2}α-Syn-Cu(II) or ^{NAc}α-Syn-Cu(II) (10 μM) and Zn_7MT-3 (2.5 μM), reacted for 1 h at 25°C.

	Lifetime (μs)	
	425 nm	**575 nm**
Cu_4Zn_4MT-3	41.5	118.5
^{NH2}α-Syn-Cu(II) + Zn_7MT-3	41.4	121.3
^{NAc}α-Syn-Cu(II) + Zn_7MT-3	43.0	117.5
mem ^{NH2}α-Syn-Cu(II) + Zn_7MT-3	39.8	111.7
mem ^{NAc}α-Syn-Cu(II) + Zn_7MT-3	40.4	116.8