

1 ***Supplementary Information***

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3 **Membrane insertion exacerbates the  $\alpha$ -Synuclein-Cu(II) dopamine oxidase activity: metallothionein-3**  
4 **targets and silences all  $\alpha$ -Synuclein-Cu(II) complexes**

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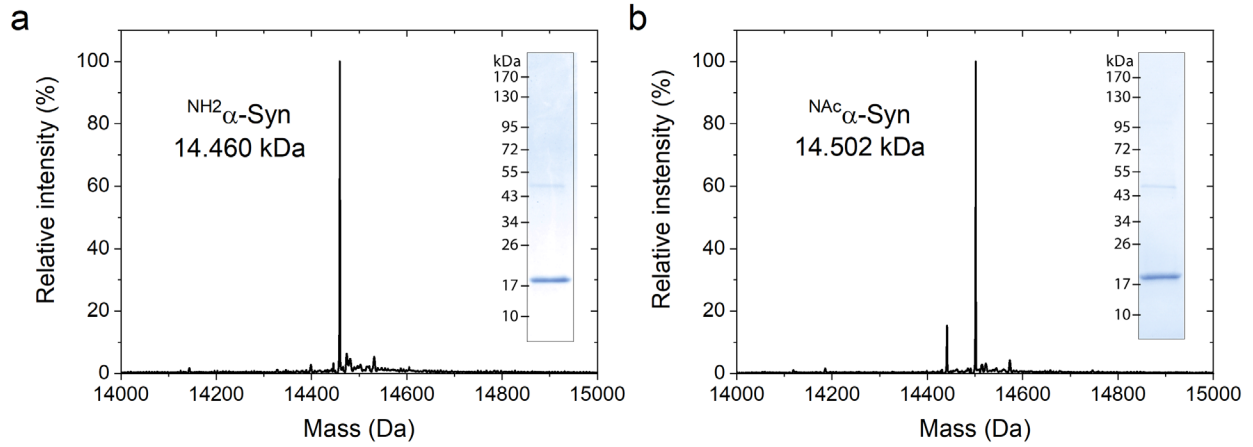
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Supplementary Figures



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43 **Figure S1.** Intact protein mass spectra of purified  $\text{NH}_2\text{-}\alpha\text{-Syn}$  (a) and  $\text{NAc-}\alpha\text{-Syn}$  (b). Insets: SDS-PAGE of the  
44 corresponding purified proteins.

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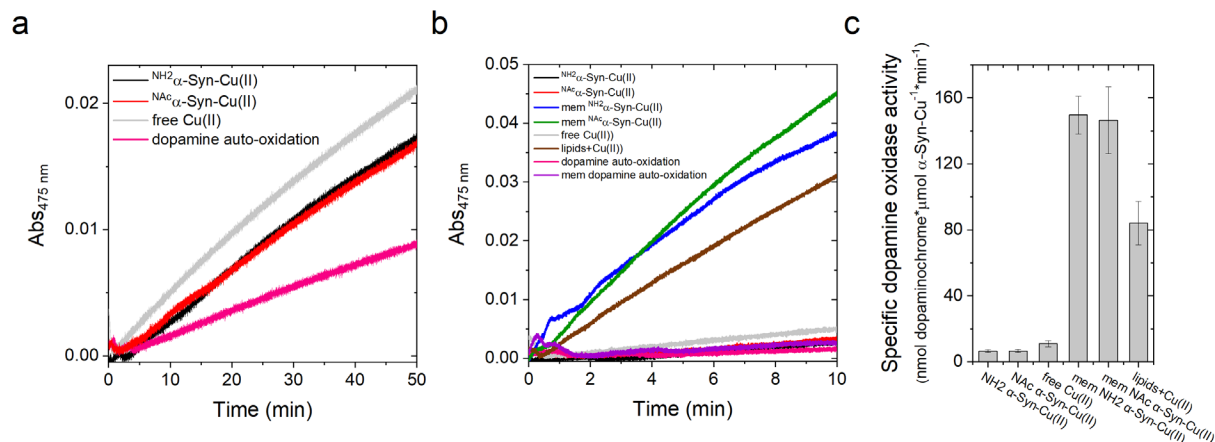
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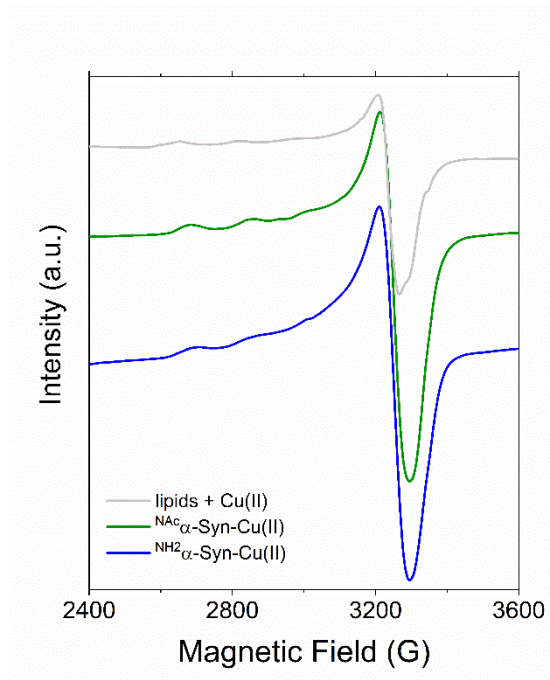
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 74 **Figure S2.** Kinetic traces at 475 nm monitoring dopaminochrome formation upon the reaction of soluble  
 75  $\text{NH}_2\alpha\text{-Syn-Cu(II)}$  (10  $\mu\text{M}$ ; black),  $\text{NAc}\alpha\text{-Syn-Cu(II)}$  (10  $\mu\text{M}$ ; red), and free  $\text{Cu(II)}$  (10  $\mu\text{M}$ ; gray) with dopamine  
 76 (2 mM). Dopamine (2 mM) auto-oxidation is shown in pink. (b) Kinetic traces at 475 nm to monitor  
 77 dopaminochrome formation upon the reaction of soluble and membrane-bound  $\text{NH}_2\alpha\text{-Syn-Cu(II)}$  (10  $\mu\text{M}$ ;  
 78 black and blue, respectively) and  $\text{NAc}\alpha\text{-Syn-Cu(II)}$  (10  $\mu\text{M}$ ; red and green, respectively) with dopamine (2  
 79 mM). The kinetic traces upon reaction of free  $\text{Cu(II)}$  and  $\text{Cu(II)}$  in lipids are shown in gray and brown,  
 80 respectively. Dopamine (2 mM) auto-oxidation in buffer and in the presence of lipids are shown in pink  
 81 and purple, respectively. (c) Specific dopamine oxidase activities determined by quantifying the  
 82 dopaminochrome formed after the reaction of  $\alpha\text{-Syn-Cu(II)}$  (10  $\mu\text{M}$ ) or free  $\text{Cu(II)}$  (10  $\mu\text{M}$ ) with dopamine  
 83 (2mM), for 10 min for soluble forms and 2 min for membrane-bound forms ( $\epsilon_{475}=3,700 \text{ M}^{-1}\text{cm}^{-1}$ ).  
 84 Calculated specific dopamine oxidase activities in  $\text{nmol dopaminochrome} \cdot \mu\text{mol } \alpha\text{-Syn-Cu(II)}^{-1} \cdot \text{min}^{-1}$ :  $\text{NH}_2\alpha\text{-}$   
 85  $\text{Syn-Cu(II)}$ :  $6.5 \pm 0.8$ ;  $\text{NAc}\alpha\text{-Syn-Cu(II)}$ :  $6.6 \pm 0.9$ ; free  $\text{Cu(II)}$ :  $10.9 \pm 1.9$ ; mem  $\text{NH}_2\alpha\text{-Syn-Cu(II)}$ :  $149.6 \pm 11.5$ ;  
 86 mem  $\text{NAc}\alpha\text{-Syn-Cu(II)}$ :  $146.4 \pm 20.2$ ; lipids+ $\text{Cu(II)}$ :  $84.1 \pm 13.2$ .

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91 **Figure S3.** EPR spectra of membrane-bound  $\text{NH}_2\alpha\text{-Syn-Cu(II)}$  (blue),  $\text{NAC}\alpha\text{-Syn-Cu(II)}$  (green), or Cu(II) in lipids  
 92 (gray) (500  $\mu\text{M}$ ) in 20 mM N-ethylmorpholine pH 7.4 and 25% (v/v) glycerol, recorded at 10 K using  $\nu=$   
 93 9.44 GHz, microwave power of 0.77 mW, modulation amplitude of 10.0 G and field sweep of 1200 G.  
 94 Resulting fitting parameters:  $\text{NH}_2\alpha\text{-Syn-Cu(II)}$ :  $g_{\perp} = 2.06$ ,  $g_{\parallel} = 2.28$ ,  $A_{\parallel} = 168$  G;  $\text{NAC}\alpha\text{-Syn-Cu(II)}$ :  $g_{\perp} = 2.06$ ,  
 95  $g_{\parallel} = 2.28$ ,  $A_{\parallel} = 157$  G; lipids- Cu(II):  $g_{\perp} = 2.07$ ,  $g_{\parallel} = 2.33$ ,  $A_{\parallel} = 150$  G.

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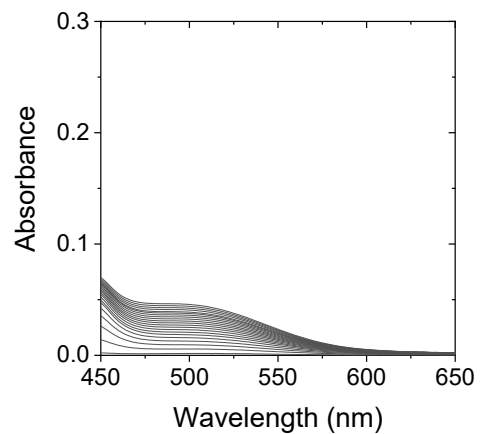
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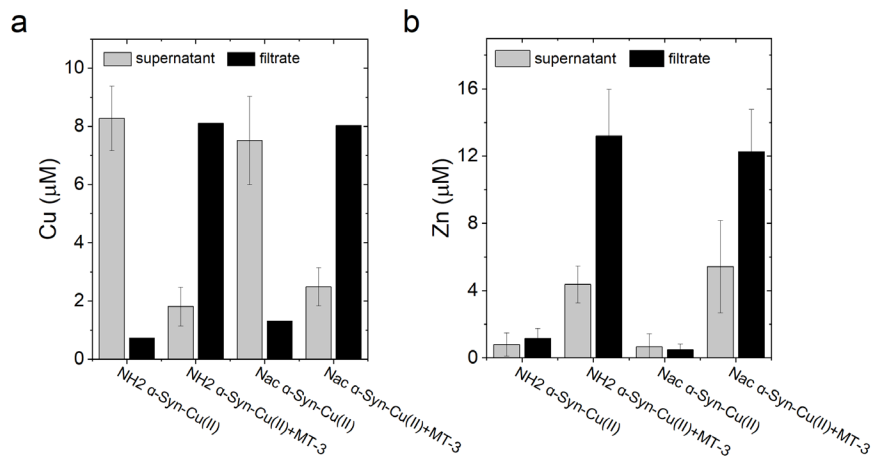
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115 **Figure S4.** Dopamine (1 mM) auto-oxidation in 20 mM N-ethylmorpholine/100 mM NaCl, pH 7.4  
116 determined in the presence MBTH (2 mM, 100 min, 25°C).

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 142 **Figure S5.** ICP-MS copper and zinc quantification after reaction (1 h, 25°C) between membrane-bound α-  
 143 Syn-Cu(II) (10 µM) and Zn<sub>7</sub>MT-3 (2.5 µM), upon separation of membrane-bound α-Syn in the supernatant  
 144 (gray bar) from MT-3 in the filtrate (black bar) using 50-kDa MWCO filters.

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174 **Table S1.** ICP-MS quantification of Cu(II) binding (10  $\mu$ M) to soluble or membrane-bound <sup>NH<sub>2</sub></sup> $\alpha$ -Syn or <sup>NAC</sup> $\alpha$ -  
175 Syn (12  $\mu$ M), using 3-kDa or 50-kDa MWCO filters, respectively, to separate unbound Cu(II) before  
176 analysis.  
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	% of total Cu	
	supernatant	filtrate
<sup>NH<sub>2</sub></sup> $\alpha$ -Syn	99.88 $\pm$ 6.11	0.12 $\pm$ 0.12
<sup>NAC</sup> $\alpha$ -Syn	97.71 $\pm$ 1.69	2.29 $\pm$ 0.36
mem <sup>NH<sub>2</sub></sup> $\alpha$ -Syn	97.26 $\pm$ 1.97	2.74 $\pm$ 0.33
mem <sup>NAC</sup> $\alpha$ -Syn	96.98 $\pm$ 0.81	3.02 $\pm$ 0.35
lipids only	92.49 $\pm$ 5.60	7.51 $\pm$ 7.89

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209 **Table S2.** Retention volumes and peak areas for soluble and membrane-bound <sup>NH2</sup>α-Syn or <sup>NAC</sup>α-Syn (15  
210 μM) after size exclusion chromatography analysis using a Superdex 200 column.  
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	Retention volume (ml)	Area under peak (ml*mAU)
<sup>NH2</sup> α-Syn	13.36	21.83
<sup>NH2</sup> α-Syn + lipids	12.52	2.36
<sup>NAC</sup> α-Syn	13.23	19.54
<sup>NAC</sup> α-Syn + lipids	13.21	1.74

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245 **Table S3.** Michaelis-Menten analysis of the dopamine oxidase activity for membrane-bound <sup>NAc</sup>α-Syn in  
246 20 mM N-ethylmorpholine/100 mM NaCl, pH 7.4 in the presence of increasing H<sub>2</sub>O<sub>2</sub> concentrations.  
247 Dopamine oxidation activity was determined after 20 s (25°C) in the presence of MBTH (2 mM).  
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H <sub>2</sub> O <sub>2</sub> concentration (M)	V <sub>MAX</sub> (nmol quinone*μmol <sup>-1</sup> α-Syn-Cu(II)*min <sup>-1</sup> )	K <sub>M</sub> (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

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276 **Table S4.** Low-temperature luminescence lifetimes of the 425 nm and 575 nm bands recorded on the  
277 reaction products between soluble or membrane-bound <sup>NH<sub>2</sub></sup>α-Syn-Cu(II) or <sup>N<sup>Ac</sup></sup>α-Syn-Cu(II) (10 μM) and  
278 Zn<sub>7</sub>MT-3 (2.5 μM), reacted for 1 h at 25°C.

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	Lifetime (μs)	
	425 nm	575 nm
Cu <sub>4</sub> Zn <sub>4</sub> MT-3	41.5	118.5
<sup>NH<sub>2</sub></sup> α-Syn-Cu(II) + Zn <sub>7</sub> MT-3	41.4	121.3
<sup>N<sup>Ac</sup></sup> α-Syn-Cu(II) + Zn <sub>7</sub> MT-3	43.0	117.5
mem <sup>NH<sub>2</sub></sup> α-Syn-Cu(II) + Zn <sub>7</sub> MT-3	39.8	111.7
mem <sup>N<sup>Ac</sup></sup> α-Syn-Cu(II) + Zn <sub>7</sub> MT-3	40.4	116.8