Supplementary Figures

Supplementary Fig. 1: Structure of Cu^{II}(gtsm) and Zn^{II}(gtsp).

Supplementary Fig. 2: Expression levels of *MT1* **(A),** *MT2* **(B)** or *MT3* **(C)** mRNA in rat PC12 cells treated with 1μM CuII(gtsm) for 5 hr were quantified by qRT-PCR. Target gene expression levels were normalized to *GAPDH* expression levels, and are expressed as fold activation compared to cells treated with DMSO. Data are pooled from 3 independent experiments performed in triplicate. p=0.05, by Student's t test.

Supplementary Fig. 3: Sucrose density gradient separation of synaptophysin and chromogranin C in PC12 cells. Differentiated cells were fractionated by sucrose density ultracentrifugation and the fractions examined for synaptophysin and chromogranin C by western blot. Fractions corresponding to the density of synaptic like microvesicles (SLMV) and large dense core vesicles (LDCV) are indicated. Fractions 8-19 were also examined for levels and localization of common organelle markers including ER (calnexin), Golgi (GM130) and the plasma membrane (Na^+K^+ATPase a1).

Supplementary Figure 4: Sucrose density gradient separation of synaptophysin, SNAP-25 and syntaxin-1 in PC12 cells treated with gtsmH_2 alone or CHX alone (synaptophysin only). Differentiated cells were fractionated by sucrose density ultracentrifugation and the fractions 14-20 examined for synaptophysin, SNAP-25 and syntaxin-1 by western blot (**A**). PC12 cells were treated with 1 μM Zn^{II}(gtsp) and cell lysates were examined for expression of synaptophysin by western blot (**B**).

Supplementary Fig. 5: No increase in synaptophysin or chromogranin C was observed in fraction one of sucrose density gradient separation of Cu^{II}(gtsm)-treated PC12 cells. PC12 cells were treated for 5 hr with DMSO or 1 μM Cu^{II}(gtsm) and fractionated by sucrose density ultracentrifugation. The fractions were examined for synaptophysin and chromogranin C by western blot. Fraction one corresponds to cytosolic proteins and potentially proteins released from damaged vesicles and organelles. Fraction 13

(synaptophysin) and Fraction 16 (chromogranin C) are shown for comparison to fraction one.

Supplementary Fig. 6: $CuCl_2$ does not increase cell death. PC12 cells were treated for 5 hr with 10, 100 or 1,000 μM $CuCl_2$ and cell death was measured using the LDH assay.

Supplementary Fig. 7: Density gradient separation of vesicular (synaptophysin) and sub-cellular organelle proteins from PC12 cells treated with Cu^{II}(gtsm) or control treatments. Cultures were treated with 1 μM Cu^{II}(gtsm) and 1 or 1,000 μM $CuCl_2$ for 5 hr and cells were fractionated by sucrose gradient ultracentrifugation. Fractions were analyzed by western blot for synaptophysin or calnexin (ER marker as a control) and blots of fractions 8-13 were analyzed by densitometry for relative protein expression. The graphs and images are representative of two-five separate western blots. **A:** Cu^{II}(gtsm)-treated, representative densitometry for synaptophysin; **B:** Cu^{II}(gtsm)-treated, representative densitometry of immunoblot for calnexin; **C:** $CuCl_2$-treated, representative densitometry of immunoblot for synaptophysin; **D:** Zn^{II}(gtsp)-treated, representative densitometry of immunoblot for synaptophysin.

Supplementary Fig. 8: Schematic indicating proposed effects of excess Cu on the secretory vesicle pathway. After entering and accumulating in the cell, Cu induces synaptophysin to translocate from SLMVs to LDCVs by an unknown mechanism involving V-ATPase. V-ATPase could also potentially affect vesicle maturation (dotted line). ATP7a is known to translocate from Golgi to membranes and transport Cu into unidentified vesicles (which could be LDCVs in some cell types). Cu remains sequestrated in the LDCVs as excess Cu prevents Ca^{2+} influx and exocytosis.