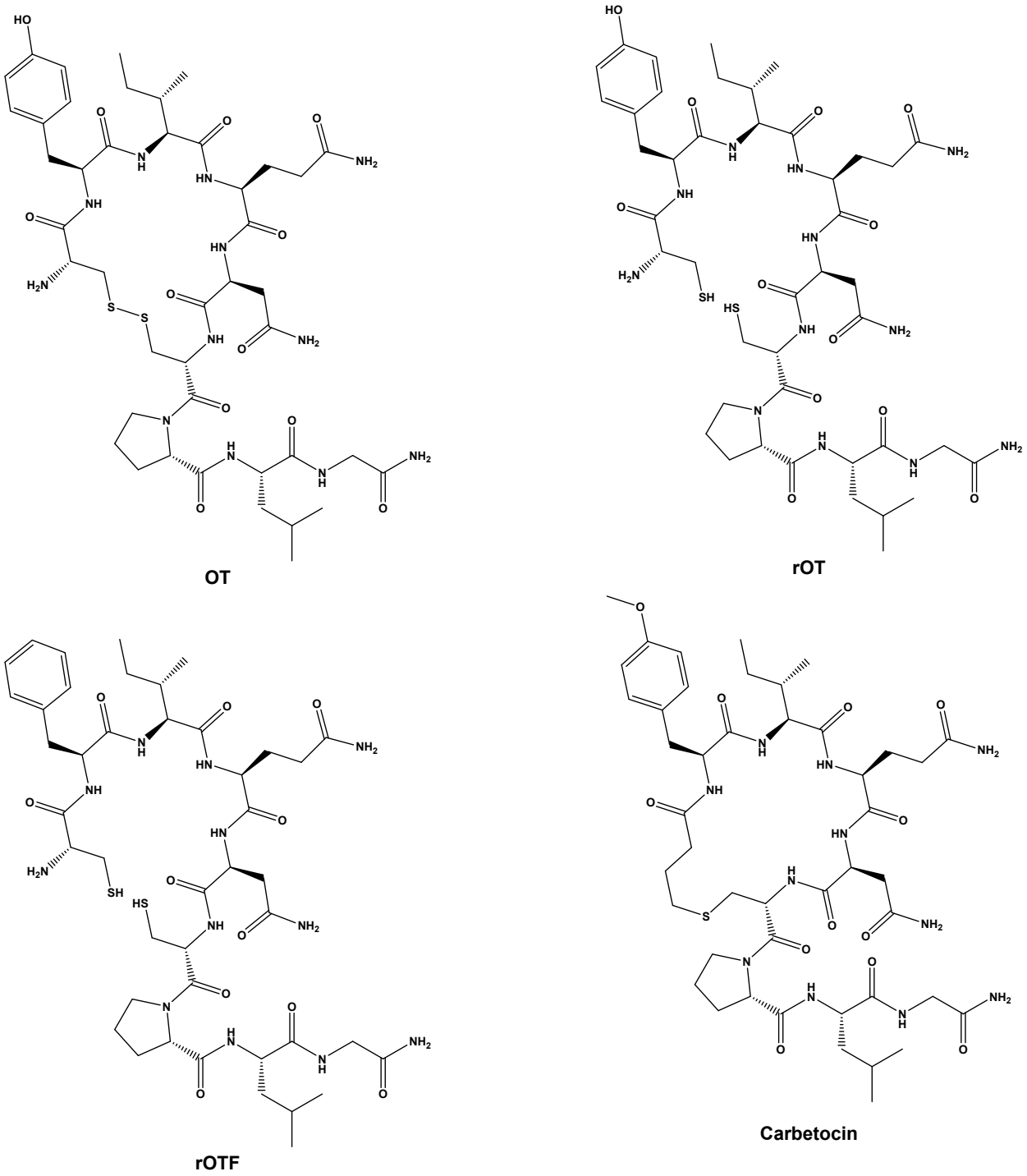

Figure S1. Chemical structures of oxidized oxytocin (oxOT), reduced oxytocin (rOT), Y2F-oxytocin (rOTF), and carbetocin.

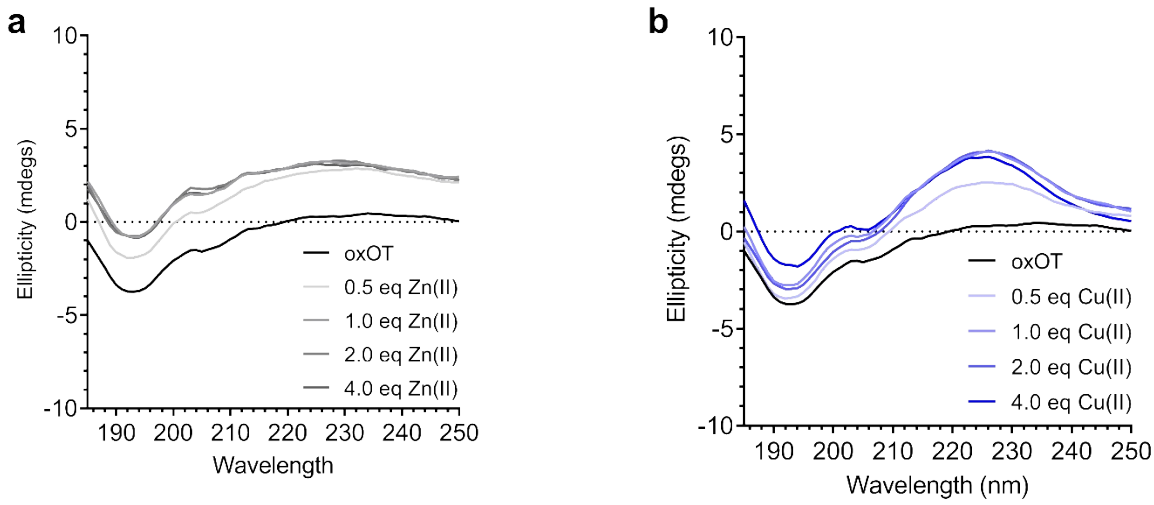

Figure S2. Titration of A) Zn(II) and B) Cu(II) into 40 μM oxytocin. Spectra averaged over eight scans and collected with 1 mm path length.

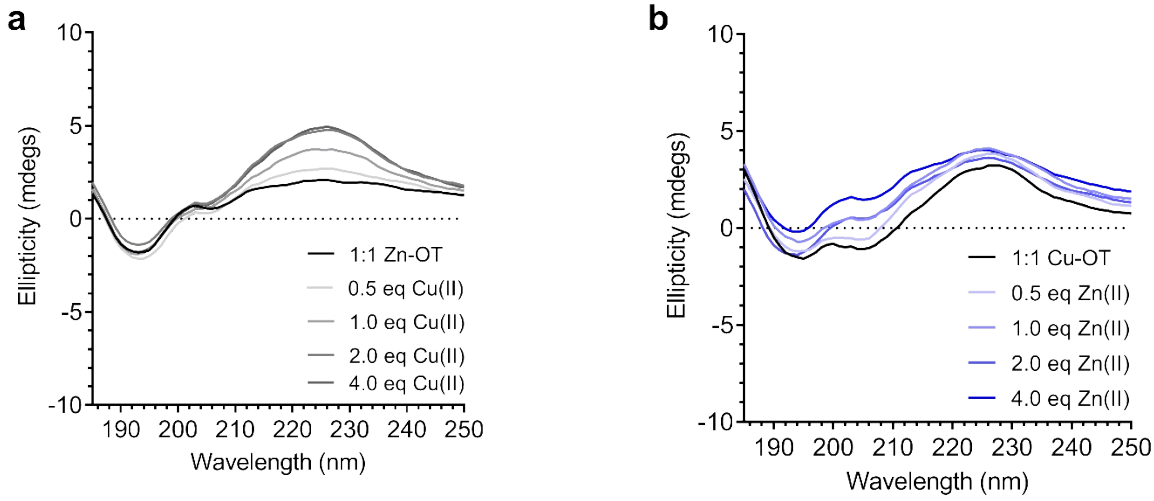

Figure S3. Circular dichroism spectra of the competition between Cu(II) and Zn(II) for oxytocin binding. A) Cu(II) titration into the preformed Zn(II)-oxOT complex (1:1 Zn(II) to oxOT), and B) Zn(II) titration into the preformed Cu(II)-oxOT complex (1:1 Cu(II) to oxOT). Spectra averaged over eight scans and collected with 40 μM oxOT and 1 mm path length. The increase in the band at 229 nm from Cu(II) titrated into Zn(II)-oxOT and the increase in the band at 205 nm from Zn(II) titrated into Cu(II)-oxOT indicates binding competition between the two metal ions for oxOT.

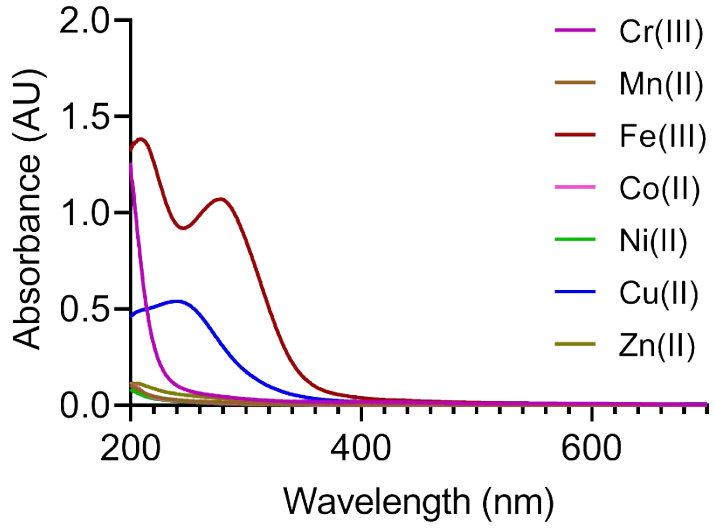

Figure S4. Electronic absorption spectra of 200 μM metal salts ($CrCl_3$, $MnCl_2$, $FeCl_3$, $CoCl_2$, $NiCl_2$, $CuCl_2$, $ZnCl_2$) in 15 mM phosphate buffer pH 7.4.

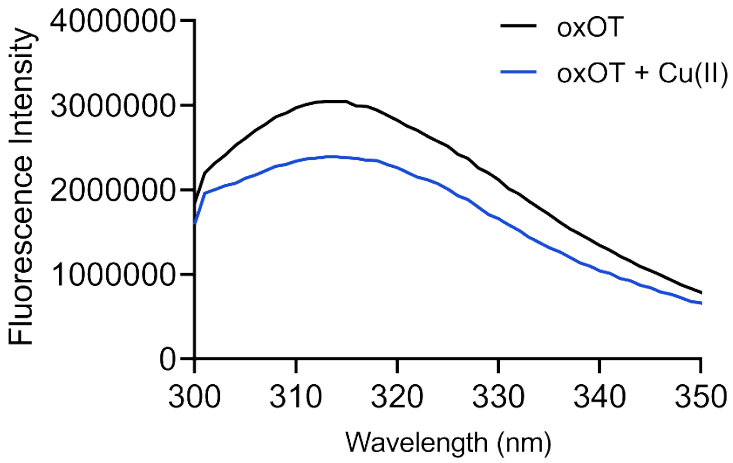

Figure S5. Emission spectra of oxOT and Cu(II) added to 30 μM oxOT. Excitation wavelength of 275 nm was used. Cu(II) quenches fluorescence but does not alter the wavelength of maximum emission suggesting that Cu(II) does not directly interact with the tyrosine ring system.

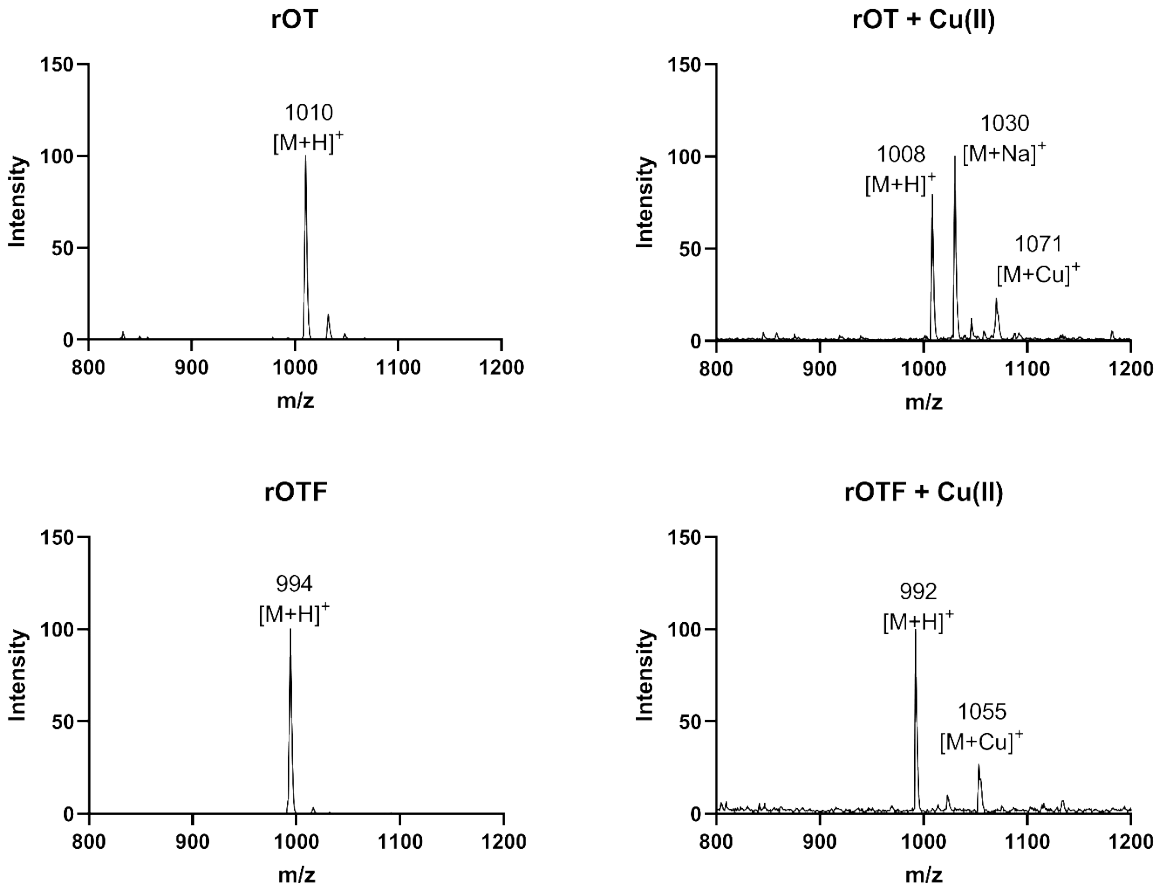

Figure S6. Mass spectra of 50 μM rOT and rOTF before and after addition of Cu(II) (1 equivalent) reveal a loss of two protons upon Cu(II) addition consistent with formation of disulfide bridge. The presence of copper-peptide complexes for both peptides is also observed. Samples were prepared in 50/50 acetonitrile/water and run via direct injection using 50/50 acetonitrile water with no acid additives.

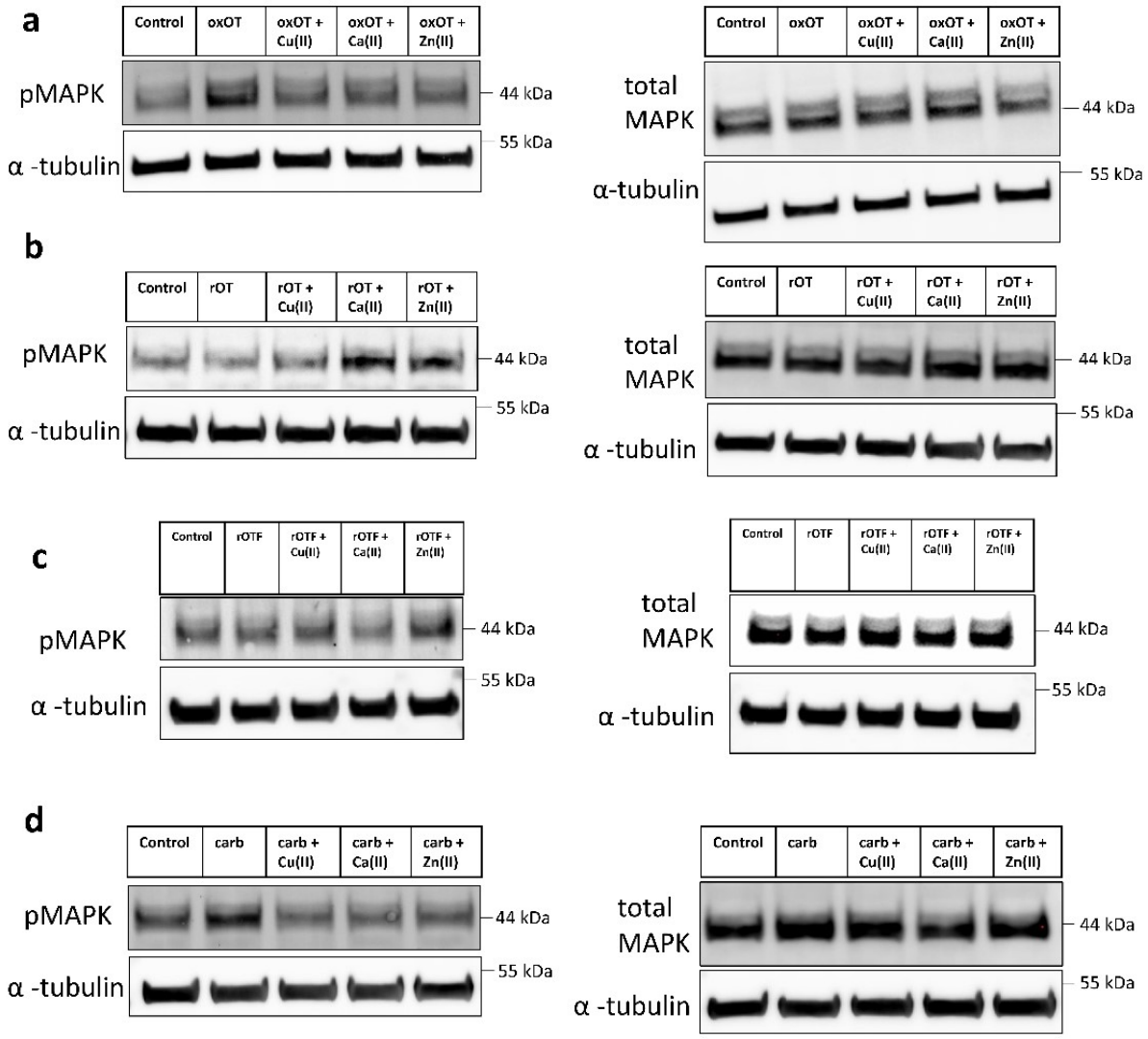

Figure S7. Representative MAPK blots for oxOT (a), rOT (b), rOTF (c), and carbetocin (d).

Materials and Methods

Chemicals and Reagents. All chemicals and reagents were purchased from Fisher Scientific, Spectrum Chemicals or Sigma-Aldrich Oxidized, unless otherwise noted. Oxytocin (OT) was purchased through Fisher from Alfa Aesar. Dimethylformamide (DMF), 4-methylpiperidine, N,N,N',N'-tetramthyl-O-(1Hbenzotriazol-1-yl)uronium hexafluorophosphate (HBTU), N,N-diisopropylethylamine (DIEA), dichloromethane (DCM), 2,2,2-trifloroacetic acid (TFA), diethyl ether, acetonitrile, methanol, formic acid (FA), $CrCl_3 \cdot 6H_2O$, $MnCl_2$, $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$, $CoCl_2$, $NiCl_2$, $CuCl_2$, NaOH, HCl, tris(2-carboxyethyl)phosphine (TCEP) resin, and ethylenediaminetetraacetic acid (EDTA), were purchased from ThermoFisher. $ZnCl_2$ and $FeCl_3$ were purchased from Sigma-Aldrich. 5,5'-Dithiobis(2-nitrobenzoic acid) (DTNB) and glutathione (GSH) were purchased from ThermoFisher but manufactured by Acros Organics. Piperidine was purchased from Spectrum Chemicals. Wang resin preloaded with Fmoc-Gly-OH and Fmoc-protected amino acids were purchased from ThermoFisher but manufactured by ChemImplex.

Buffered solutions and metal salt solutions were made using Direct-Q 3 deionized water (>18 MΩ, Millipore).

Solid-phase peptide synthesis and RP-HPLC purification of linear oxytocin and oxytocin analogs. Human oxytocin (sequence CYIQNCPLG) and an analog (OTF; sequence CFIQNCPLG) were synthesized via Fmoc-based solid-phase peptide synthesis (SPPS) method. For a 0.2 mmol synthesis, Wang resin preloaded with Fmoc-Gly-OH was swelled for 2 hours in 5x DMF. Resin was washed 5 times with 2x resin volume of DMF. The Fmoc-Gly-OH residue was deprotected with 20% piperidine in DMF or 25% 4-methylpiperidine in DMF by shaking for 1 minute then repeating for 10 minutes. The deprotected resin was washed 10x with 2x resin volume DMF. Amino acids (4.0 equivalents) and HBTU (3.9 equivalents) were dissolved in minimal DMF and DIEA (10.0 equivalents) was added. Washed resin was then suspended in amino acid/HBTU/DIEA solution and shaken at room temperature for 40 minutes. The resin was again washed 10x with 2x resin volume DMF. This process was repeated from the deprotection step for each amino acid addition. After addition of final amino acid, the resin was washed 10x with 2x resin volume DMF, then washed 10x with 2x resin volume DCM and allowed to dry overnight. To cleave peptide from resin, resin was saturated with 95:5 TFA:water solution and allowed to shake for 1-4 hours. The solution was separated from resin, and crude product was precipitated in chilled diethyl ether and the suspension was centrifuged at 3900 rpm for 10 minutes at 4°C. The pellet was washed thrice with chilled diethyl ether followed by centrifugation after each wash. The pellet was dried under a stream of nitrogen overnight.

Purification was performed with RP-HPLC on an Agilent Technologies 1260 Infinity II HPLC with coupled Agilent Technologies 1260 Infinity II UV-Vis detection system. Purification of crude OT was performed using an Agilent Zorbax SB-C3 column (9.4 x 250 mm) at a flow rate of 3.75 mL/min using a gradient of water with 0.1% FA (Solvent A) and acetonitrile with 0.1% FA (Solvent B). The column was equilibrated and crude OTF loaded onto the column with 10% Solvent B held constant for 5 minutes. Solvent B increased from 10% to 20% from 5-6 minutes, then from 20% to 80% from 6-26 minutes. Solvent B was decreased back to 10% from 26-27 minutes then held constant at 10% from 27-30 minutes. OTF elutes off column at 41% Solvent B. Fractions containing OTF were confirmed by electrospray ionization mass spectrometry (ESI-MS) using an Agilent Technologies 1260 Infinity II coupled with an Agilent Technologies InfinityLab LC/MSD, dried, and stored at -20°C.

Oxidation and Reduction of Oxytocin. OT was purchased through ThermoFisher from Alfa Aesar. The concentration of reduced and oxidized OT were determined by DTNB assay in 15 mM phosphate, pH 7.4 using the molar extinction coefficient of 5-thionitrobenzoic acid, which absorbs strongly at 412 nm (ε=14,150 M^{-1} cm^{-1}). It was found that ≤10% of OT (Alfa Aesar) was reduced, i.e. ≥90% of the thiols are oxidized in disulfide bonds.

UV-Visible spectroscopy. All measurements were recorded on either a Shimadzu UV-1800 or Shimadzu UV-1900 at room temperature using Starna Cells quartz cuvettes with a 1 cm path length.
To determine actual concentrations of oxidized and reduced OT or OTF in the sample, a DTNB assay was performed. Absorbance of dilutions of known concentration of GSH and DTNB in 15 mM phosphate buffer pH 7.4 were measured at 412 nm yielding a linear graph. Absorbance of 10 μM OT or OTF and DTNB in 15 mM phosphate buffer pH 7.4 was measured at 412 nm and the concentration of free thiols in the OT or OTF solution could be extrapolated from the linear graph.

For metal titrations into apo-peptide (OT oxidized, OT reduced, OTF, Carbetocin), peptide was dissolved to 25 μM in 15 mM phosphate buffer pH 7.4. All metal and EDTA solutions were dissolved in Milli-Q water. Varying equivalents (12.5-125 μM) of metal salts were added to peptide, allowed to equilibrate at room temperature for 5 minutes, and measured with water as a spectral reference.

Following data collection, buffer spectrum was subtracted from all spectra and all spectra were normalized to account for dilution.

Circular dichroism spectroscopy. All measurements were recorded on a Chirascan Circular Dichroism spectrophotometer (Applied Photophysics) at room temperature using Starna Cells quartz cuvettes with a 1 mm path length. For metal titrations into apo-peptide, peptide was dissolved to 40 μM in 15 mM phosphate buffer pH 7.4. All metal salts were dissolved in Milli-Q water. Varying equivalents (20-200 μM) of metal salts were added to peptide, allowed to equilibrate at room temperature for 5 minutes, and measured. For each metal addition, 8 scans were averaged with a bandwidth of 0.5 nm. Following data collection, buffer spectrum was subtracted from all spectra.

For metal competition studies apo-OT was dissolved to 40 μM in 15 mM phosphate buffer pH 7.4. All metal salts were dissolved in Milli-Q water. OT was incubated with 1.0 equivalents (40 μM) $CuCl_2$ or $ZnCl_2$ for 10 minutes at room temperature. Then varying equivalents (20-200 μM) of competing metal salts were added, allowed to equilibrate for 5 minutes at room temperature, and measured. For each metal addition, 8 scans were averaged with a bandwidth of 0.5 nm. Following data collection, buffer spectrum was subtracted from all spectra.

Fluorescence spectroscopy. All measurements were recorded on a SpectraMax i3x Multi-Mode Microplate Reader (Molecular Devices) at room temperature using Corning half-area, black-wall, clear-bottom 96-well plates. Peptide was dissolved to 30 μM in 15 mM phosphate buffer pH 7.4. For the measurement of a copper oxOT complex, copper was titrated into oxOT for a 1:1 complex and this was incubated for 5 minutes prior to recording the emission spectra. Excitation at 275 nm was used and emission was recorded from 300 to 350 nm.

Plasmid Amplification and Purification. GFP tagged human oxytocin receptor open reading frame clone was purchased from Origene and transformed into *E. coli*. Amplified plasmid was extracted and purified using a QIAGEN plasmid maxi prep kit. Purified plasmid was then sequenced (GENEWIZ).

Cell Culture. HEK293T cells were maintained in Dulbecco's modified medium (DMEM) supplemented with 10% fetal bovine serum (FBS) (Gibco) 1x penicillin-streptomycin (Corning), 2 mM L-glutamine (Gibco), 1 mM sodium pyruvate (Gibco) at 37 ℃ and 5% CO_2.

Cell Transfection and Stimulation Conditions.HEK293T cells were plated and grown to 60-70% confluence before transfection with GFP tagged human oxytocin receptor clone (Origene) using Attractene transfection agent (QIAGEN). 12 hours post-transfection cells were examined under a fluorescent microscope for GFP expression to confirm successful transfection. Media was then replaced and cells were stimulated 12 hours later.

For cell stimulations with metal bound peptide, peptide hormones and metal chloride salts (1:1 molar ratio) were incubated at 37 °C for 10 minutes in DPBS and then added to plated cells at a final concentration of 1 μM for 5 minutes before lysing.

Western Blot Analysis and Densitometry

HEK293T cells were transfected and stimulated as previously described and then lysed at 5 minutes in RIPA buffer (150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, 50 mM Tris pH 7.4) with EDTA free protease inhibitor (ThermoFisher) and phosphatase inhibitor (Sigma). Lysates were put one ice for 15 minutes before being vortexed and centrifuged at 15000 x G at 4 °C. Protein was quantified after freezing the lysates by BCA assay (Invitrogen). Samples were then made using 10 μg of protein with PBS (Gibco), sample buffer (Invitrogen), and 2-mercaptoethanol (BioRad). Samples were loaded into a 4-12% bis-tris 12 well gel (Invitrogen) and run for one hour at 100 V and then was transferred on to a PVDF

membrane using a Trans-Blot Turbo Transfer System (BioRad). The membranes were blocked for one hour in 5% BSA in TBST buffer. Membranes were then incubated overnight at 4 °C with primary antibodies and washed three times the following day with TBST buffer before incubating secondary antibodies for one hour. Membranes were imaged on a Chemidoc MP Imager (BioRad). Primary antibodies used were p44/42 MAPK (Erk1/2) (1:2,000 Cell Signaling Technologies), Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (1:1,000 Cell Signaling Technologies), and tubulin.

For densitometry, two separate gels were run and stained for either total MAPK and tubulin or phosphorylated MAPK and tubulin. Fluorescence of the bands was quantified using Image Lab software (Biorad). First the ratio of tubulin bands to total MAPK bands was calculated from one gel and then the ratio of phosphorylated MAPK to tubulin was calculated from a second gel. The ratio of tubulin normalized phosphorylated MAPK to tubulin normalized total MAPK was then calculated.