Supporting Information

General method for synthesizing the disulfide linked peptides.
Peptides were obtained by solid-phase peptide synthesis, and purified by HPLC. The concentration of peptide was determined by measuring the tyrosine absorbance at 280 nm. Peptide was diluted to 10 μM with 1 mM ammonium acetate buffer (pH = 7) containing 5% DMSO. The resulting solution was stirred in the dark at room temperature for 24 h. Purification by reverse-phase semi-preparative HPLC was carried out by use of an HP 1100 Series HPLC apparatus with detection at 220 nm. A linear gradient of 10-35% acetonitrile/water (0.1% TFA) over 25 min was used with a flow rate of 5 mL/min. Separation was achieved with a C18 Vydac column (5 μm, 250 mm length, 10 mm I.D.).

Michaelis-Menten kinetics.
To quantify glycosidase reactivity, pNP-glycoside substrates were incubated with 5 μM metallopeptide, 1 mM ascorbate and 1 mM H_2O_2 in 50 mM sodium phosphate buffer (pH 7.4) at 37°C and the change of absorbance at 405 nm was monitored. Initial velocities were measured at varying concentrations of substrates to determine the Michaelis-Menten parameters.

Isothermal titration calorimetry.
Titration of L-fucose to peptides was performed at 37°C by use of a MicroCal isothermal titration calorimeter. A degassed solution of 1 mM CuGGH-tOL-NH_2 in 40 mM sodium phosphate buffer (pH 7.4) was prepared and loaded into the calorimeter cell. A solution of 15 mM L-fucose in the same buffer was titrated into the sample in 10 μL aliquots over a period of 24 s at 4.5 min intervals. Similar conditions were used to titrate 15 mM L-fucose to 2 mM Cu-free GGH-tOL-NH_2. Data were collected automatically and subsequently analyzed with an Origin Software package provided by MicroCal. Control experiments, following titration of titrant into buffer, or buffer into titrant, were also performed and used to subtract background heat changes.

Surface plasmon resonance.
All surface plasmon resonance measurements were made at a flow rate of 30 μL/min by use of a BiaCore T100 instrument (GE Healthcare) at 25°C. An α-L-fucose-$O(CH_2)_3NHCO(CH_2)_5NH$-biotin (GlycoTech) was immobilized to a streptavidin sensor chip Series S SA (GE Healthcare). Varying concentrations of metallopeptides in 10 mM HEPES saline (pH 7.4) were injected over the immobilized fucose for 2 min. Following injection, the analytes were allowed to dissociate from the sensor for 3 min, followed by the regeneration of the sensor surface by use of 15 μL 10 mM HCl. The dissociation constant (K_D) was calculated from the responses obtained at equilibrium.

LC-ESI-MS Analysis.
A solution containing 25 μM H-trisaccharide was incubated with 50 μM metallopeptides, 1 mM ascorbate and 1 mM H_2O_2 at 37°C for 0-120 min. A 10 μL volume of the resulting solution was diluted with 90 μL acetonitrile before injection to an LC-MS instrument. Liquid chromatography was performed by use of an Agilent 1200 Series LC system equipped with a Sepax HP-amino column (3 μM, 1.0 × 100 mm). The mobile phase

consisted of 80% acetonitrile and 20% 50 mM ammonium acetate in water (isocratic). The flow rate was 0.1 mL/min, and the temperature of the column oven was 25 °C. The LC system was directly connected to a Brücker MicroTOF mass spectrometer without further stream splitting. The ESI-MS spectra were acquired under positive ion mode. The temperature of the drying gas (N_2) was 180 °C, at a gas flow rate of 4 L/min and a nebulizing pressure of 0.4 Bar.

FACS (Fluorescence Activated Cell Sorting) Analysis.

Human leukemia cells Jurkat were cultured in serum-free AIM V medium (Life Technologies) in an atmosphere containing 5% CO_2 at 37°C. Human O-type red blood cells were purchased from Innovative Research, Inc. To remove anticoagulants, human O-type erythrocytes were rinsed three times with PBS and resuspended in PBS. The tested copper complexes were added to 1×10^6 erythrocytes in PBS buffer, followed by the addition of 200 μM ascorbate. Cells were incubated at 37°C for 4 hours, with a shaking speed of 250 rpm. After reaction, cells were rinsed by PBS. After fixation by 2% paraformaldehyde, cells were rinsed by PBS and blocked by 1% BSA (bovine serum albumin) for 30 min. Cells were subsequently again rinsed three times with PBS, then resuspended in PBS. Cell number was counted by use of a hemocytometer. Approximately 1×10^5 cells were stained by 0.1 μg FITC-labelled blood group H2 antibody BRIC231 (Santa Cruz Biotechnology) in PBS for 30 min. Cells were rinsed with 0.1% BSA and resuspended in 0.1% BSA, and then analyzed by use of a BD LSRII flow cytometer. Autofluorescence of cells was measured from the corresponding unstained samples. Jurkat cells, a leukemia cell line that does not express H2-antigen,[1] were used as a negative control to this antibody, and normalized as 0%.

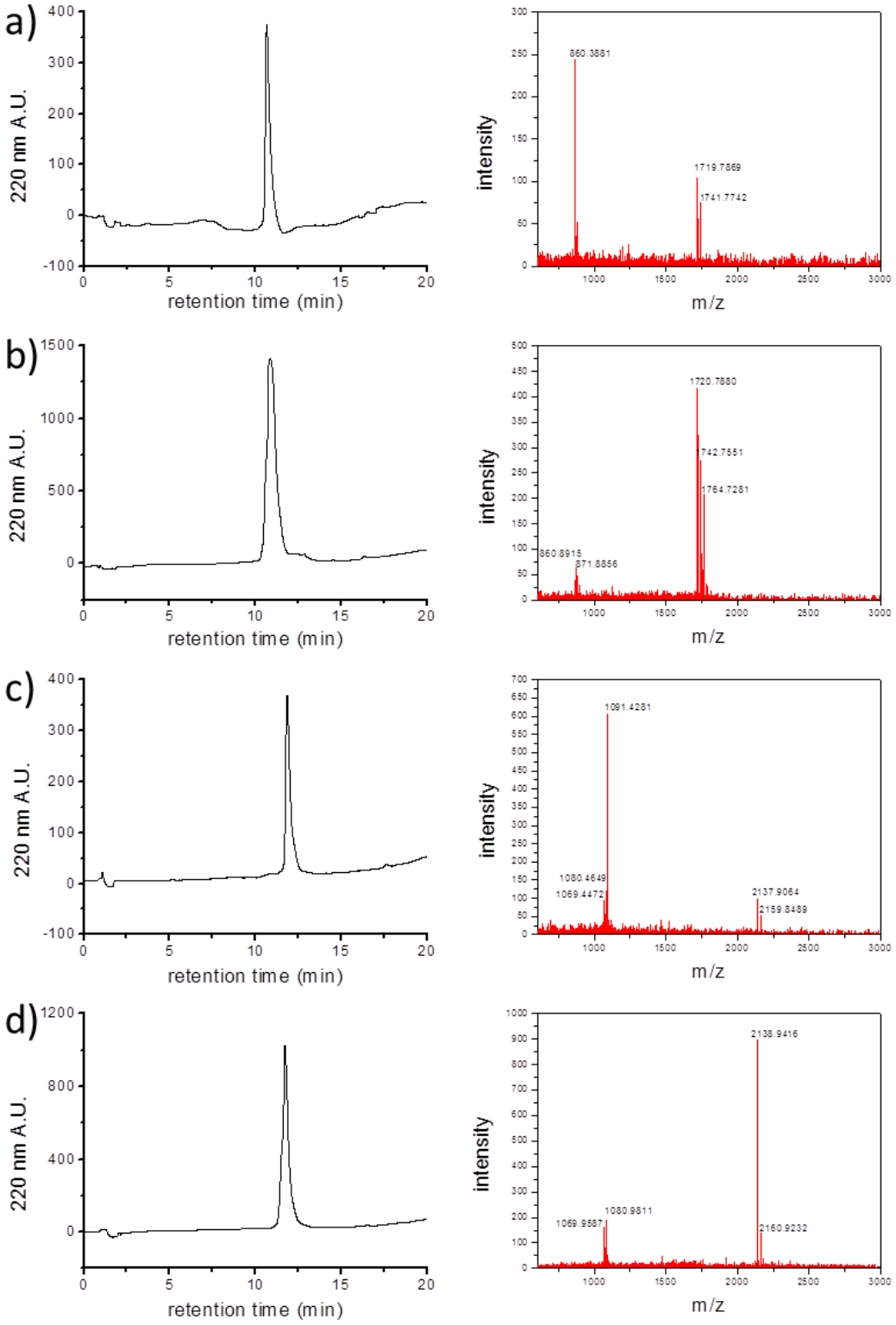

Figure S1. HPLC profiles (left) and HRMS profiles (right) for peptides. HPLC chromatograms were obtained by use of a SunFire C18 column (5 µm, 150 mm length, 4.6 mm I.D.). A linear gradient of 0-40% acetonitrile/water (0.1% TFA) was applied over 20 min at a flow rate of 1

mL/min. HRMS (ESI): (a) calculated m/z for $[GGH\text{-}tOL\text{-}NH_2+H]^+$ 1719.8206, found 1719.7869; (b) calculated m/z for $[GGH\text{-}tOL\text{-}OH+H]^+$ 1720.8046, found 1720.7880; (c) calculated m/z for $[GGH\text{-}OL\text{-}NH_2+H]^+$ 2138.0059, found 2137.9064; (d) calculated m/z for $[GGH\text{-}OL\text{-}OH+H]^+$ 2138.9899, found 2138.9416.

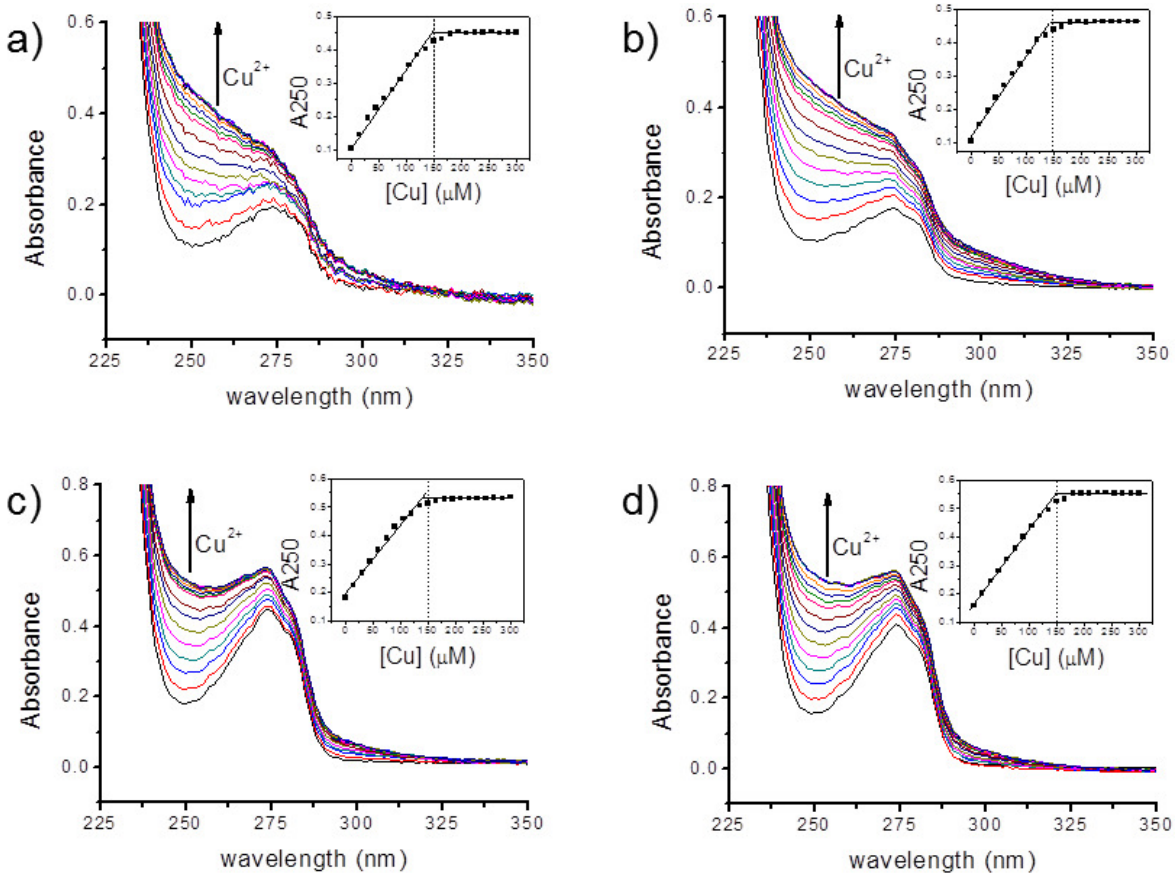

Figure S2. Cu titration into peptides monitored by UV absorbance. The indicated amount of $CuCl_2$ was added to a solution containing 150 μM of each peptide (calculated from the tyrosine absorbance at 280 nm) in 100 mM sodium phosphate buffer (pH 7.4). (a) GGH-tOL-NH_2, (b) GGH-tOL-OH, (c) GGH-OL-NH_2, (d) GGH-OL-OH. Insert: change of absorbance at 250 nm following the addition of Cu(II).

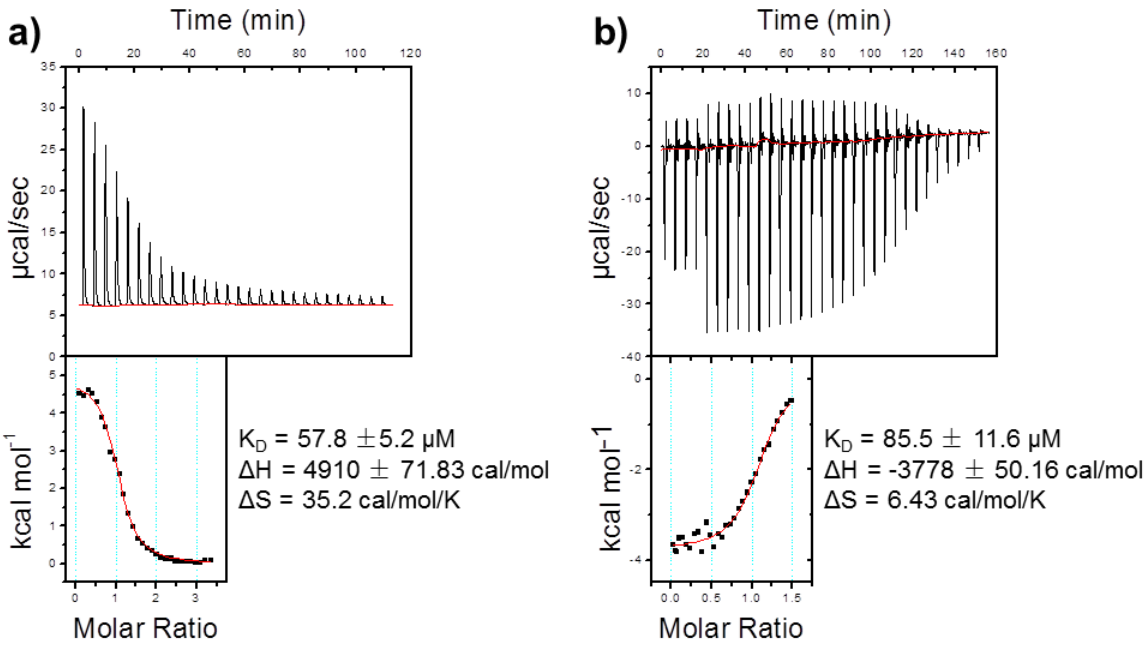

Figure S3. ITC study of the interaction between peptides and L-fucose. CuGGH-tOL-NH_2 (a), and GGH-tOL-NH_2 (b).

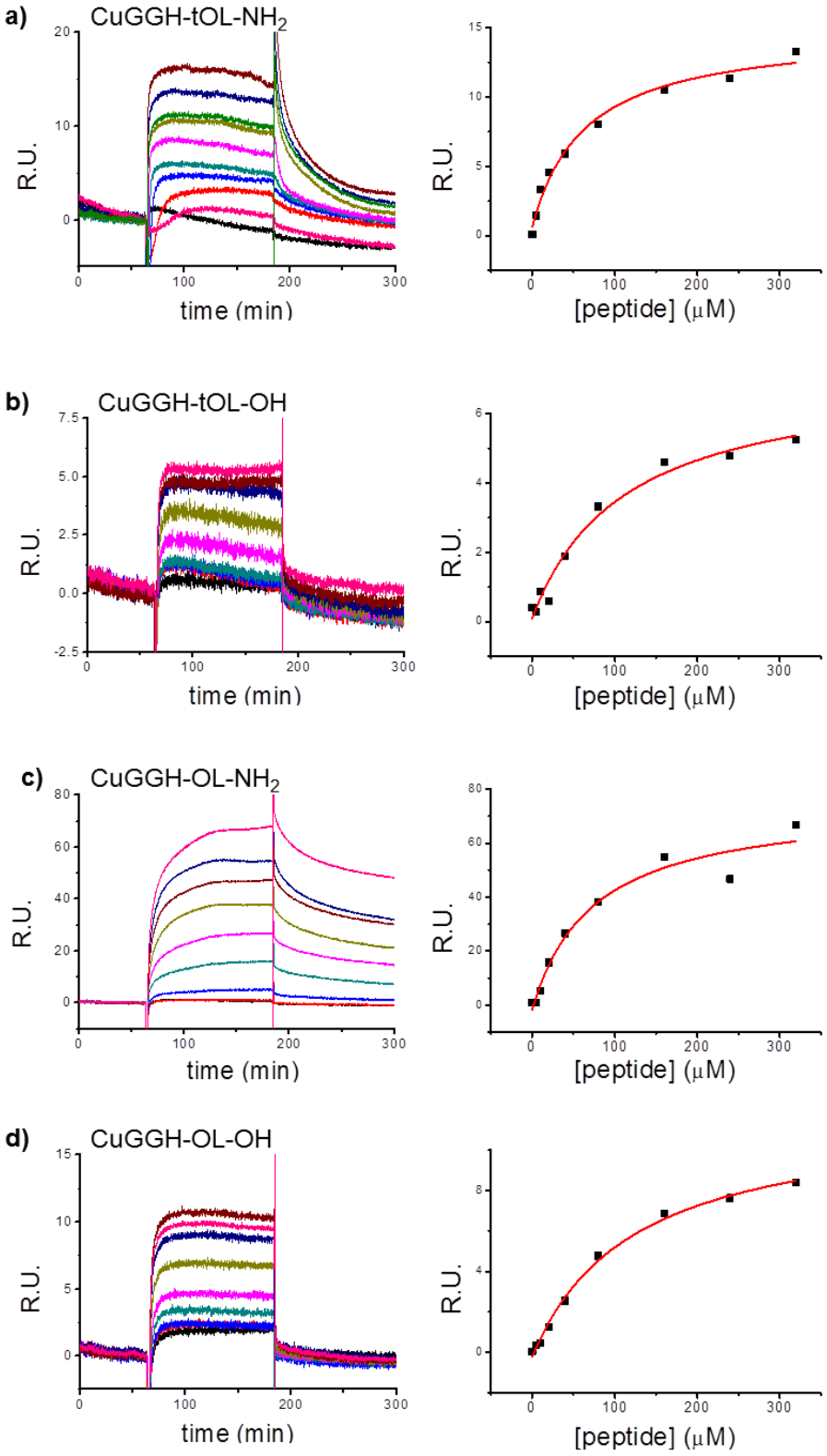

Figure S4. SPR sensorgrams showing the binding of metallopeptides to α-L-fucose. Dissociation constants K_D were calculated from the responses at equilibrium. Experiments were performed in triplicate.

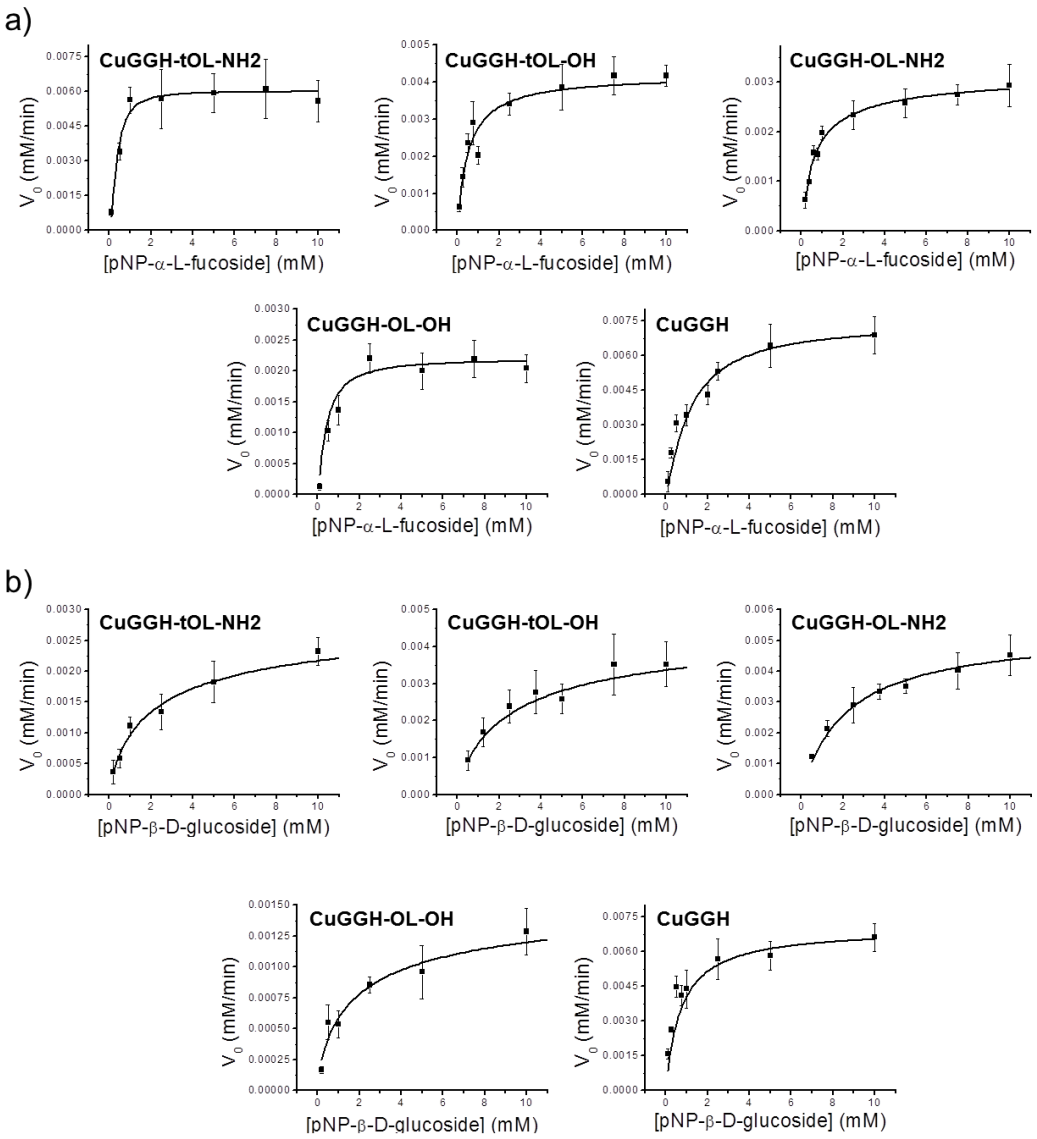

Figure S5. Michaelis–Menten kinetics of glycosidase reactivity. Initial velocity was measured by incubating the substrate, pNP-α-L-fucoside (a), or pNP-β-D-glucoside (b), with 5 μM metallopeptide, 1 mM ascorbate and 1 mM H_2O_2 in 50 mM sodium phosphate buffer (pH 7.4) at 37 °C.

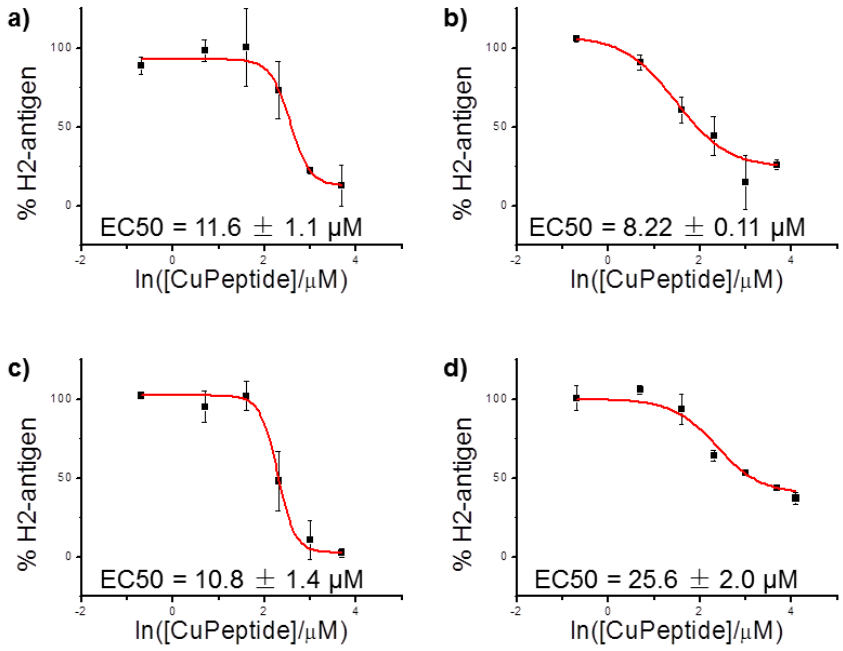

Figure S6. H2-antigen removal profile from human O-type erythrocytes. Human erythrocytes were treated with various concentrations of metallopeptides CuGGH-tOL-OH (a), CuGGH-OL-NH_2 (b), CuGGH-OL-OH (c), and CuGGH (d) in the presence of 200 μM ascorbate. The amount of H2-antigen of human erythrocytes was evaluated by FACS. The fluorescence of control erythrocytes is normalized to 100%, and Jurkat is normalized as 0%. Background from autofluorescence has been subtracted.

Reference.

[1] Y. Cao, A. Merling, U. Karsten, R. Schwartz-Albiez, *Glycobiology* **2001**, *11*, 677-683.