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Supplementary Information

Exploring Artificial Metalloglycosidases as Selective Catalysts for the Recognition and Degradation of the sLex Tetrasaccharide

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EXPERIMENTAL METHODS

Materials

All materials used were of reagent grade or higher purity standards. $CuCl_2$ was the copper salt used in all assays and obtained from Sigma Aldrich. Rink amide MBHA resin was obtained from Protein Technologies and 9-fluorenylmethoxycarbonyl (Fmoc)-amino acids were obtained from Aapptec or VWR. All *para*-nitrophenyl conjugated carbohydrates were obtained from Sigma Aldrich. Biotin-PAA-sLe^x, biotin-PAA-sLe^a, biotin-PAA-Le^x, biotin-PAA-Le^a, biotin-PAA-Le^b, and biotin-PAA-Le^y, were obtained from GlycoNZ, (formerly known as GlycoTech). All peptides were purified over a Phenomenex C18 semi-preparative column with an HP 1050 HPLC. LC-MS assays were performed on a Bruker Elute SP HPLC with Bruker Impact II QqTOF Mass Spectrometer equipped with a HyperCarb LC-column (4.6 x 100 mm) with 5-micron particle size. 3'-Sialyl-Lewis^x tetrasaccharide was obtained from CarboSynth. α2-3,6,8

Neuraminidase (Sialidase) was obtained from New England Biolabs (NEB). Cell culture reagents were obtained from Gibco and ThermoFisher. Alexa Fluor® 488 Mouse Anti-Human CD15s (CSLEX) was obtained from BD Bioscience and Fisher Scientific.

Peptide Synthesis and Purification

Peptides were synthesized by traditional Fmoc solid-phase peptide synthesis (SPPS) methodologies. Following synthesis, peptides were deprotected, cleaved, and purified via RP-HPLC. The active peptide contains a disulfide bridge, which allows for carbohydrate binding and was synthesized using traditional methods with DMSO.[33]

Surface Plasmon Resonance

SPR studies were performed using a BIAcore T200 device (Cytiva, formerly GE Healthcare life sciences) at 25°C. The binding assay was conducted in PBS buffer, which contained 20 mM phosphate (pH 7.4), 137 mM NaCl and 2.7mM KCl, plus 0.05% Tween. All-biotinylated-PAA-carbohydrate Lewis antigens were first captured on the surface of an S series SA sensorchip at a density ranging from 350-791 RU. Next, two-fold series of peptide dilutions were then injected at a rate of 30 mL/min over surfaces using multiple cycle kinetics. Each data point was repeated at least twice. Regeneration of the surfaces were done by injection of 2M NaCl for 60s at 30uL/min. Data were fitted using steady state analysis by BIAevaluation software (version 3.1).

Catalyst Characterization via *para*-Nitrophenyl Assay

Copper derivatives of peptides that show the capacity to bind carbohydrate antigen via SPR were then further evaluated by use of an absorbance assay where 5 μM metallopeptide was incubated with a derivatized-sugar that contained a *p*-nitrophenyl moiety conjugated to the anomeric carbon. The reaction mixture was incubated in 50 mM phosphate buffer at pH 7.4 at 37 °C, along with ascorbate and hydrogen peroxide to facilitate metal-mediated redox

chemistry. Liberation of *para*-nitrophenolate anion results in absorbance at 405 nm, which is an indication of successful hydrolysis and is easily monitored via UV-Vis spectroscopy. Each experiment was performed in triplicate.

LC-MS Degradation of sLe^x Tetrasaccharide

All LC-MS experiments were performed on a Bruker Elute SP HPLC with Bruker Impact II QqTOF Mass Spectrometer equipped with a 96-well autosampler. The ESI-MS spectra were acquired in positive mode. Dry gas source (nitrogen) was 3.0 l/min at 220 °C. A binary solvent system was used for liquid chromatography whereby A was 99 % d-H_2O, 0.9 % acetonitrile, and 0.1 % formic acid, and B was 90 % acetonitrile, 9.9 % d-H_2O, and 0.1 % formic acid. All solvents used for LC-assays were of LC-MS grade. All reactions were performed in 20 mM ammonium carbonate buffer, pH 8.8 at 37 °C with 2 mM ascorbic acid and 2 mM hydrogen peroxide. 50 μM Cu-peptide was incubated with either 100 μM or 150 μM sLe^x tetrasaccharide. Reaction aliquots were taken every 15 min and diluted in chilled water and further analyzed by LC methodologies. A 15 μL aliquot was then taken from each time point and injected onto a HyperCarb LC-column (4.6 x 100 mm) with 5-micron particle size.[34] Subsequent reactant and products were separated out via a linear 4.5 % gradient of A to B and then subsequently washed with 100 % B for 2 min to further clean the column. For additional experiments to see if catalysts could be further stimulated for activity, an additional 1 mM aliquot of ascorbic acid and hydrogen peroxide was added every 45 min. Both reactant and products were quantified through the extraction of the base peak current (BPC) using MZmine.[35]

Removal of sLe^x Antigen from HL-60 Cells

HL60 cells were donated by Dr. Lapalombella from the Comprehensive Cancer Center at The Ohio State University. HL60 cells were cultured in a complete medium comprised of 80 % Iscove's Modified Dulbecco's media and 20 % fetal-bovine serum (FBS) which were obtained

from Gibco. Cells were passaged three times prior to use in cell assays. Cells were centrifuged at 100 g for 5 min and subsequently counted by a hemocytometer. Cells were resuspended in PBS buffer, pH 7.4. A sample of 2.5 x 10^5 cells were treated with 25 μM Cu-peptide, Cu-GGH, or sialidase enzyme as a positive control. Untreated cells were used as a negative control. All cells were incubated with or without 500 μM ascorbate filtered through a 0.22 μm filter for 4 h at 37 °C with a rotation speed of 125 RPM. After treatment, cells were washed 3x and fixed for 30 min by 2 % paraformaldehyde. Cells were washed 3x and blocked by 1 % bovine serum albumin (BSA) for 30 min, cells were washed with PBS buffer with 0.1 % BSA and stained with 5 μL of CSLEX1 antibody from BD Biosciences. Cells were rinsed 3x again and resuspended in 0.1 % BSA and counted on an Aria III flow cytometer equipped with a laser with an excitation of 488 nm. Positive and negative cells were gated, and 2 x 10^4 cells were counted. Cells which were not treated, nor stained, were background subtracted for cellular autofluorescence. Each experiment was performed in triplicate and the error bars represent the standard deviation.

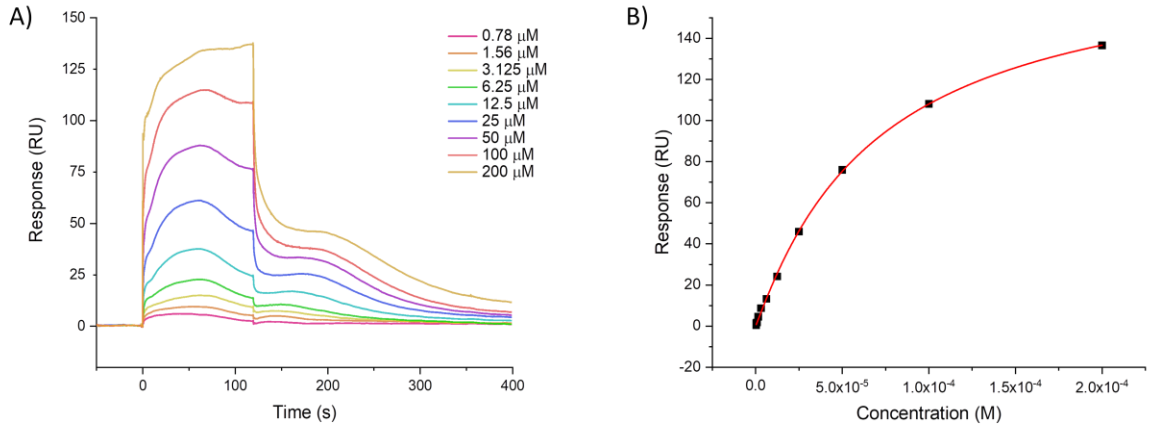

Figure S1. Binding curves for ATCUN-t-OL peptide against biotinylated-sLe^x. A) SPR sensorgram and B) Response units were plotted against peptide concentration. Affinity was calculated using the fitting tool of BiaEvaluation software.

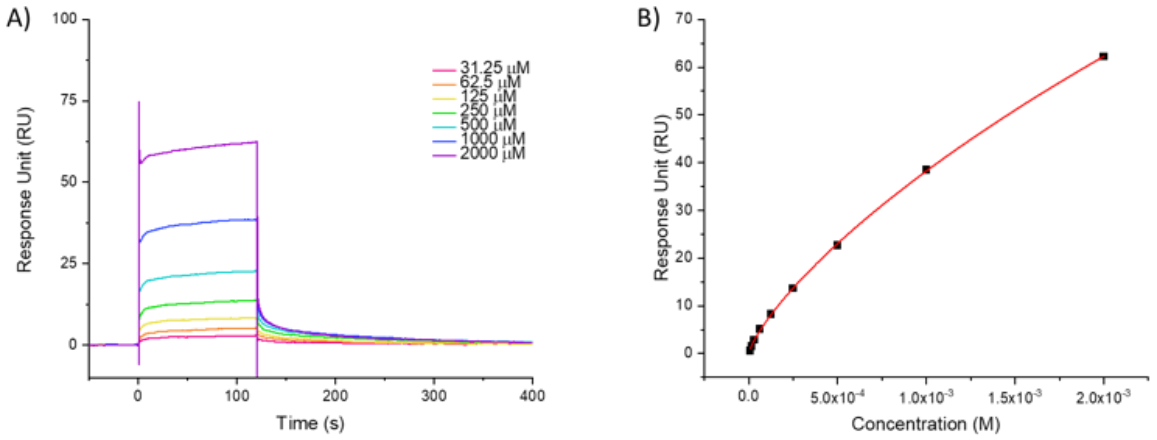

Figure S2. Binding curves for ATCUN-t-OL peptide against biotinylated-sLe[a]. A) SPR sensorgram and B) Response units were plotted against peptide concentration. Affinity was calculated using the fitting tool of BiaEvaluation software.

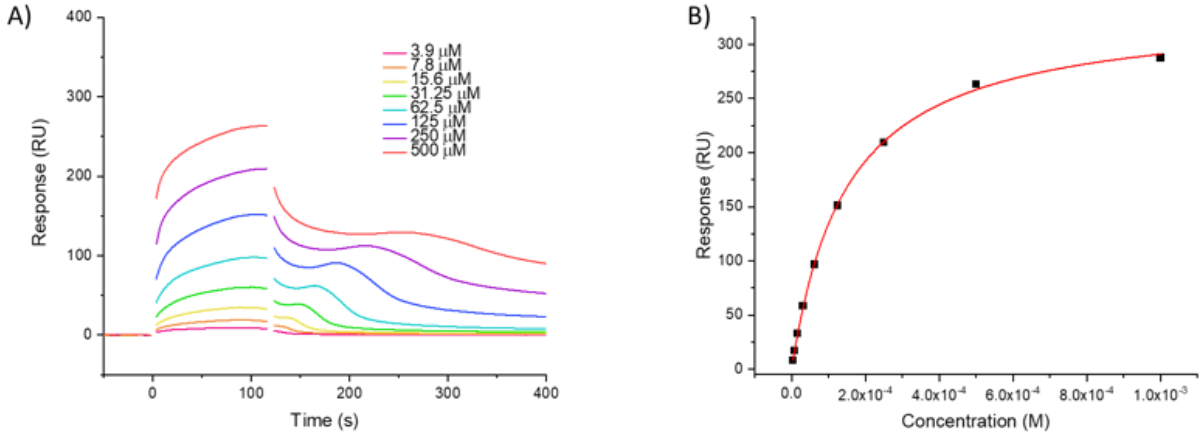

Figure S3. Binding curves for ATCUN-t-OL peptide against biotinylated-Le[a]. A) SPR sensorgram and B) Response units were plotted against peptide concentration. Affinity was calculated using the fitting tool of BiaEvaluation software.

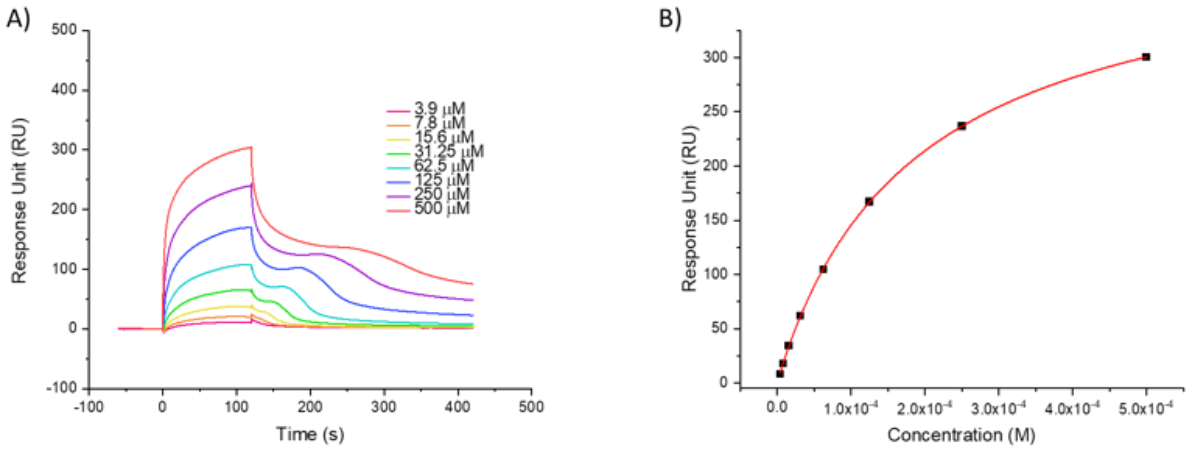

Figure S4. Binding curves for ATCUN-t-OL peptide against biotinylated-Le^{y}. A) SPR sensorgram and B) Response units were plotted against peptide concentration. Affinity was calculated using the fitting tool of BiaEvaluation software.

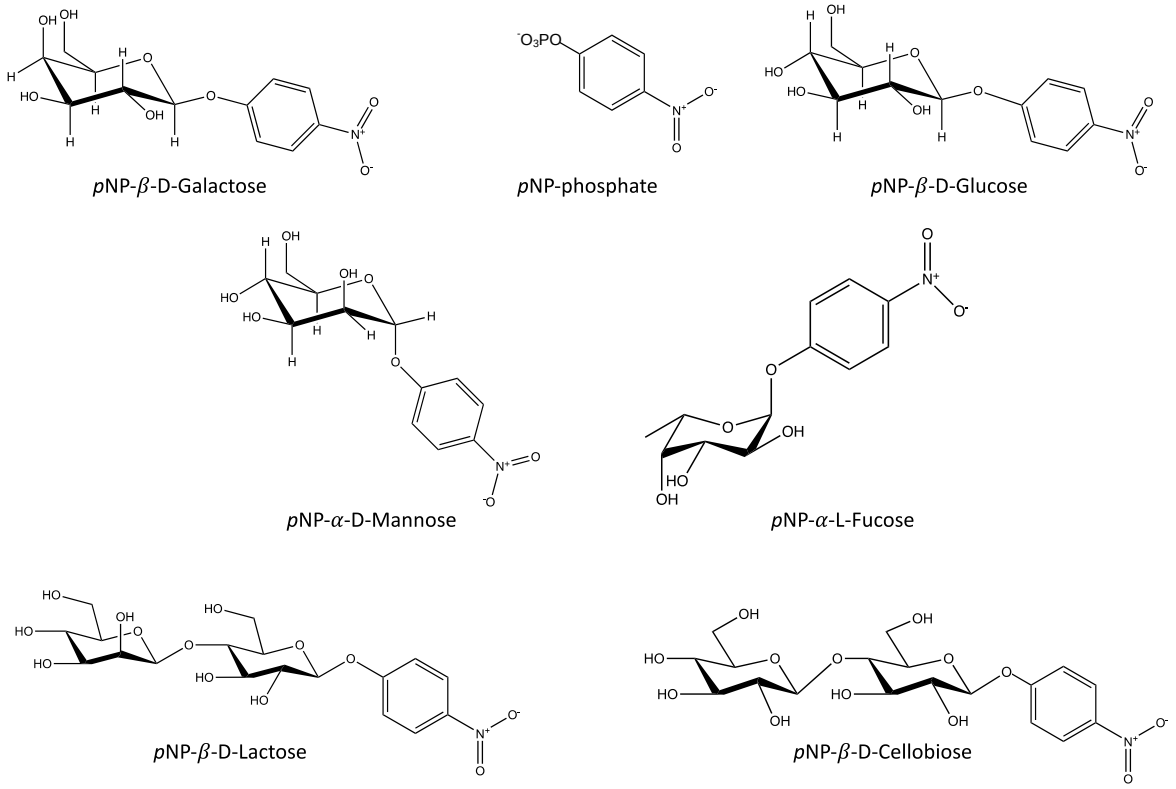

Figure S5. Para-nitrophenyl conjugated carbohydrates used for kinetic assays.

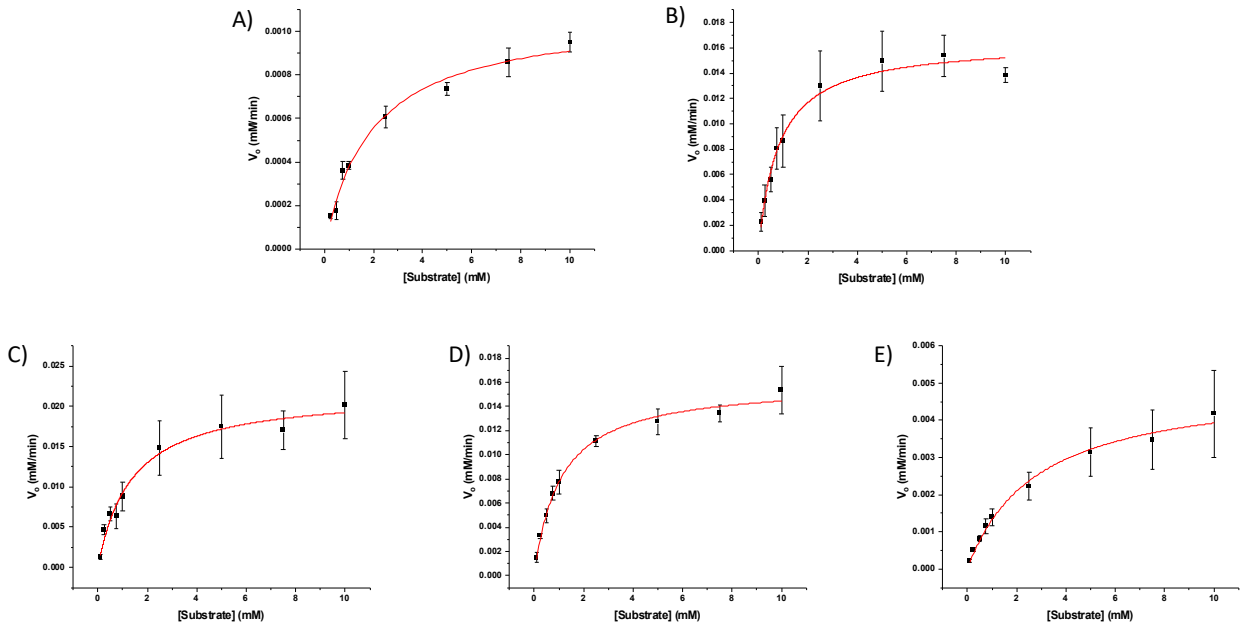

Figure S6. Compiled para-nitrophenyl assay data for ATCUN-t-OL peptide. A) pNP-phosphate, B) pNP-beta-galactose, C) pNP-alpha-mannose, D) pNP-beta-lactose, E) pNP-beta-cellulose. All assays were performed in 50 mM sodium phosphate buffer, pH 7.4, with 1 mM ascorbic acid, 1 mM hydrogen peroxide, and 5 μM Cu-catalyst.

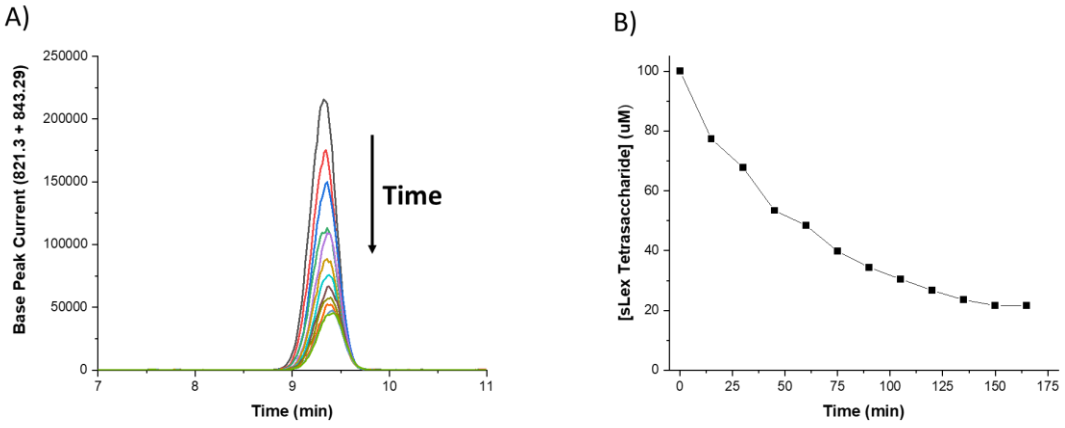

Figure S7. Base Peak Current (BPC) for sLe^x Degradation A) Sialidase reaction BPC chromatograms for 100 μM sLex tetrasaccharide. B) Reaction progress over time. 100 μM for sLe^x tetrasaccharide, 200 U sialidase, 50 mM ammonium acetate buffer, pH 5.5.

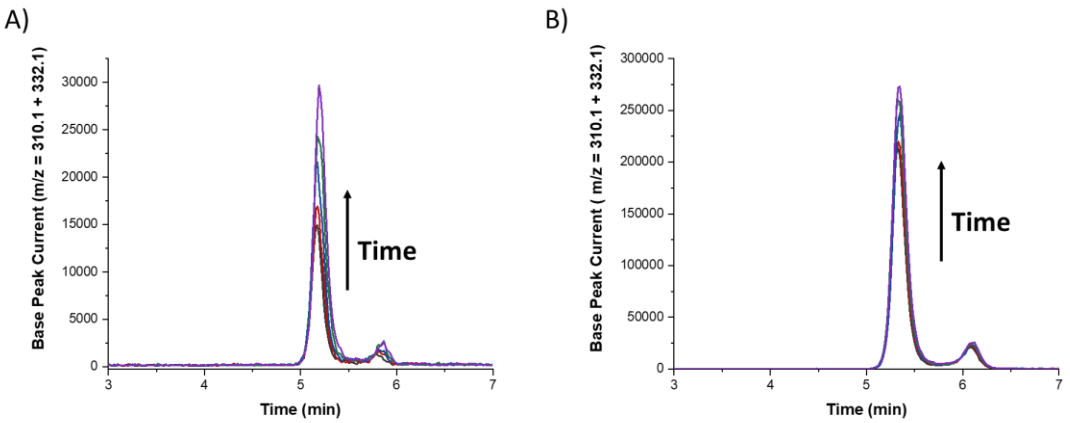

Figure S8. Base Peak Current (BPC) for Sialic Acid Product Detection. A) BPC results for sialic acid over 60 minutes for a 50 μM reaction of Cu-ATCUN-t-OL-2 with 100 μM sLe^x tetrasaccharide, B) BPC results for sialic acid over 60 minutes for a 200 enzyme U reaction of sialidase enzyme with 100 μM sLe^x tetrasaccharide.

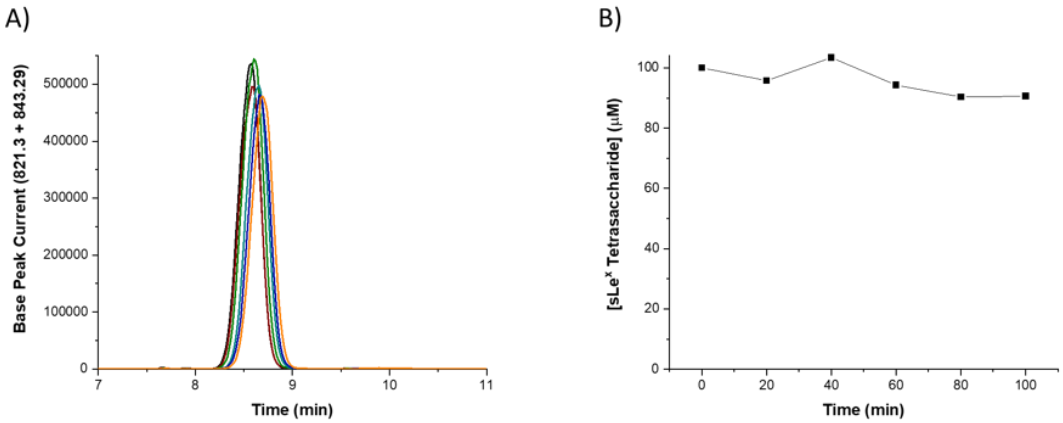

Figure S9. Base Peak Current (BPC) for sLe^x Degradation A) Control reaction BPC chromatograms for 100 μM sLe^x tetrasaccharide. B) Reaction progress over time. 100 μM for sLe^x tetrasaccharide, no copper catalyst, 2 mM ascorbic acid, 2 mM hydrogen peroxide, 20 mM ammonium carbonate buffer, pH = 8.8.

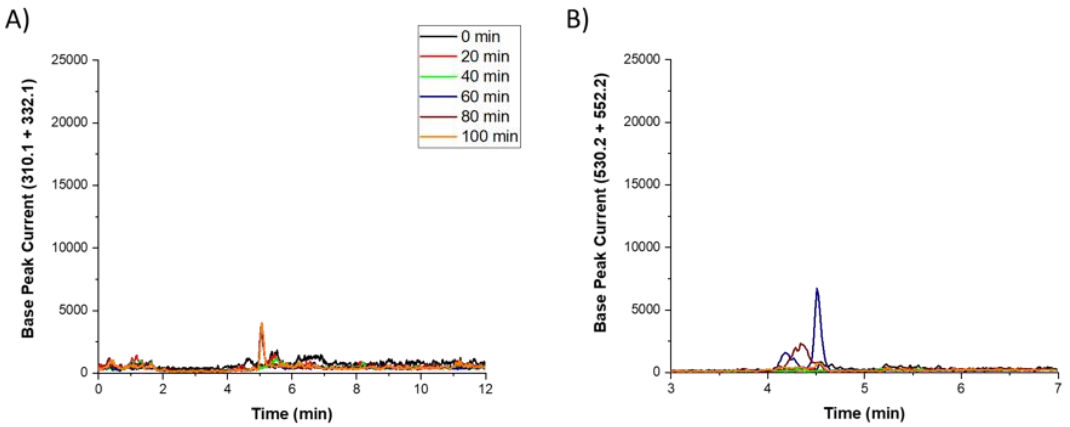

Figure S10. Base Peak Current (BPC) for sialic acid and Le^x detection in a control reaction A) BPC for sialic acid from 0 to 100 min. B) BPC for Le^x from 0 to 100 min.

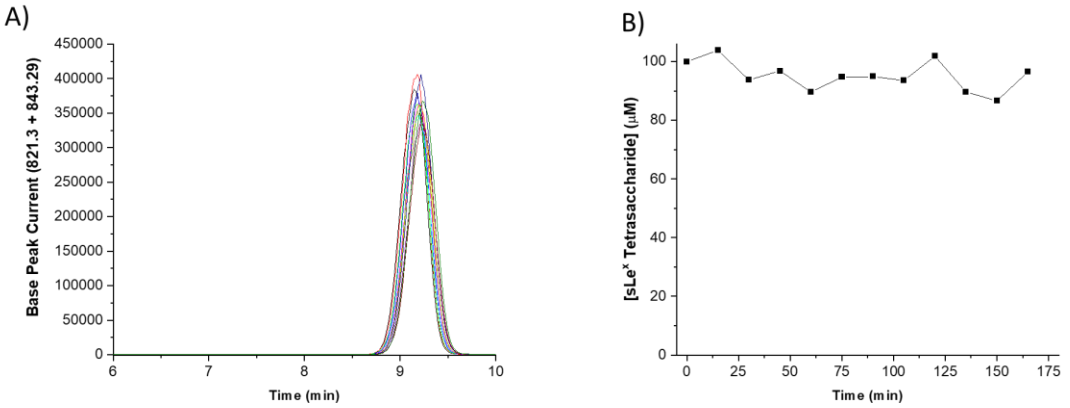

Figure S11. Base Peak Current (BPC) for sLe^x Degradation A) 50 μM Cu-GGH tripeptide reaction BPC chromatograms for 100 μM sLe^x tetrasaccharide. B) Reaction progress over time. 100 μM for sLe^x tetrasaccharide, 50 μM Cu-GGH, 2 mM ascorbic acid, 2 mM hydrogen peroxide, 20 mM ammonium carbonate buffer, pH = 8.8.

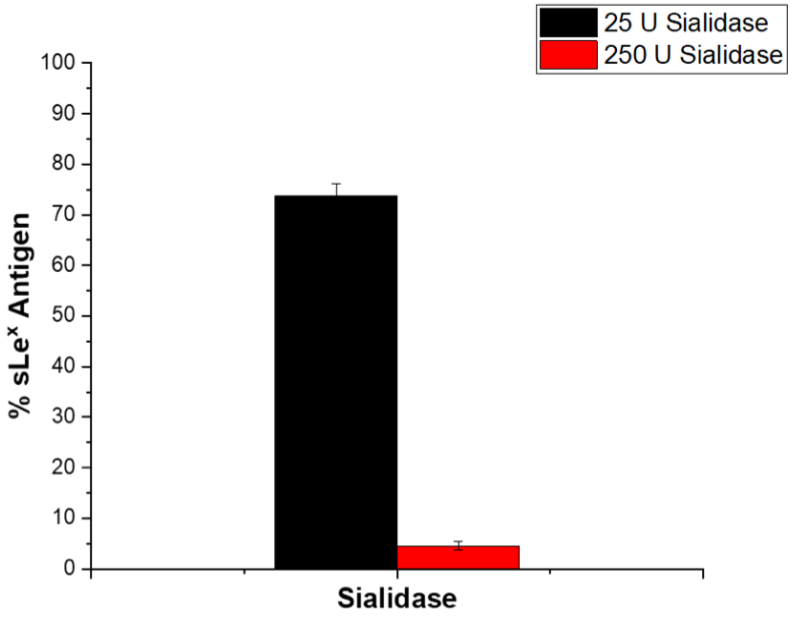

Figure S12. sLe^x Antigen Removal from HL-60 Cells by α2-3,6,8 Sialidase. sLe^x antigen concentration dependence for sialidase enzyme 25 U (black) or 250 U (red).