Supporting Information

High Temperature SELMA: Evolution of DNA-Supported Oligomannose Clusters Which Are Tightly Recognized by HIV bnAb 2G12

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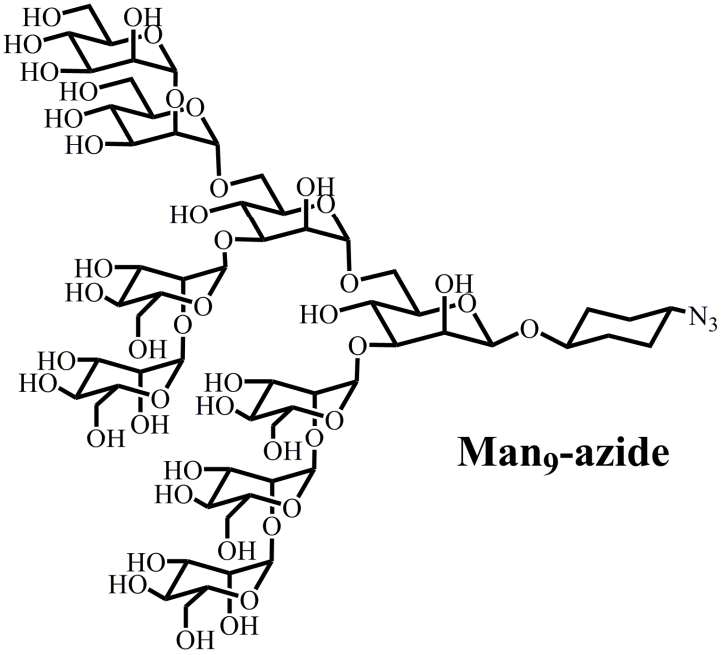
Materials

All synthetic oligos were purchased from Integrated DNA Technologies. A complete list of oligos and primers for SELMA is in SI Table 1. Vent polymerase, Vent(exo) polymerase, Bst polymerase, T4 polynucleotide kinase, Exonuclease I, Taq polymerase and hydrophilic streptavidin magnetic beads were purchased from New England Biolabs. Desalting columns were prepared using Sephadex G-50 superfine resin which was purchased from GE Healthcare. Antibody 2G12 was purchased from Polymun Scientific. Protein A Dynabeads and a TOPO-TA cloning kit were purchased from Invitrogen. ATP (γ-32P) was purchased from Perkin Elmer.

All reagents, buffers and buffer components were purchased from National Diagnostics, Sigma-Aldrich, Acros Organics, New England Biolabs, or Fisher and used without further purification. Nitrocellulose membranes (0.45µm) were purchased from Biorad. PVDF membranes (0.45µm, immobilon-FL) were purchased from Millipore. Water was purified with a Milli-Q Ultrapure water purification system. Prepared buffers were sterilized by filtration through 0.22 µm syringe filters obtained from Millipore.

Man_9-azide was prepared according to literature[1,2].

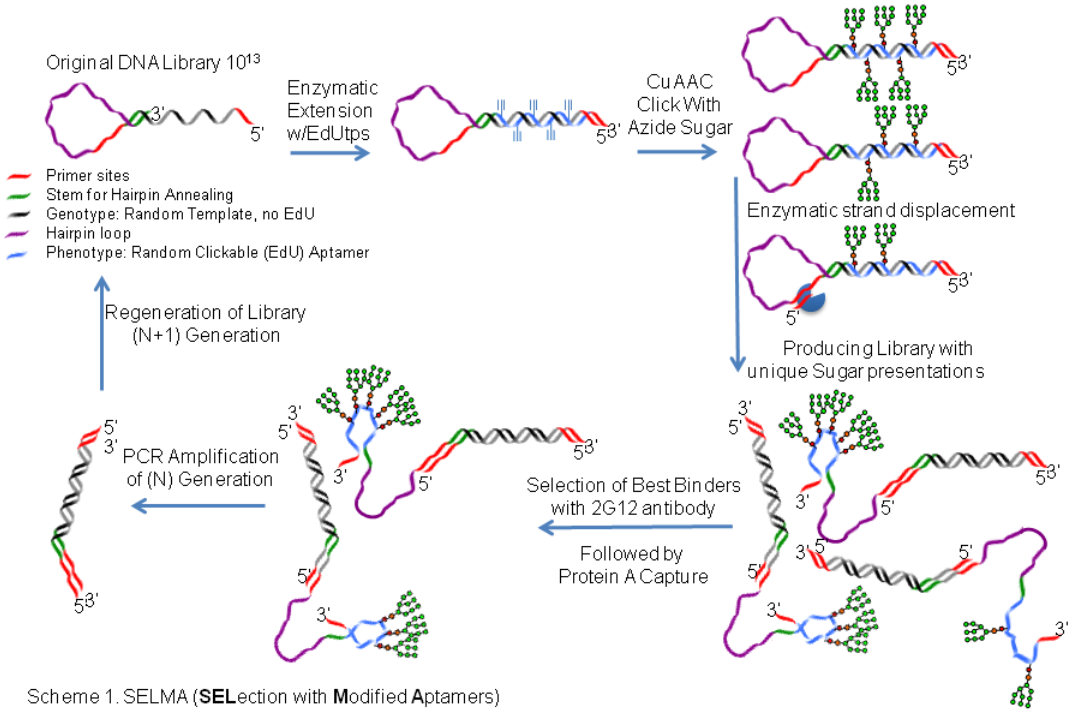


Man_9-azide

SI Table 1: DNA oligonucleotides

Oligonucleotide	Sequence
Hairpin library	5′CTTGTCGTCTCCTGTGTGCTTNNNNNNNNNNNNNNNNNNNNNNNNNCCCGTACCCGTTAAAACTCCACCTCATAACCGCA Blue=aptamerrev binding region Red=stem region Green=loop region underlined=aptamerfor binding region N_{25}=**(N1:15%/28%/29%/28%:A/G/C/T)**Randomized region
Regeneration primer	5′biotin/CCC GTA CCC GAATAT AAA ATA AAA ATATAA AAT ATA AAATTG CGGTTATGA GGT GGA GTT
Aptamerfor	5′TGCGGTTATGAGGTGGAGTT
Aptamerfor-biotin	5′biotin/TGCGGTTATGAGGTGGAGTT
Aptamerrev	5′CTTGTCGTCTCCTGTGTGCTT
Aptamerrev-biotin	5′biotin/CTTGTCGTCTCCTGTGTGCTT
Stem Primer	5′CGGGTACGGG
Clone 1 Template	5′CTTGTCGTCTCCTGTGTGCTTTATCCGTAGGTTGCACCGTGGGTCTCCCGTACCCG
Clone 2 Template	5′CTTGTCGTCTCCTGTGTGCTTTATCCGTAGGTTGCACTGTGGGTCTCCCGTACCCG
Clone 3 Template	5′CTTGTCGTCTCCTGTGTGCTTTATCCGTAGGTTGCACCGTGGGTCTCCCGTACCCG
Clone 4 Template	5′CTTGTCGTCTCCTGTGTGCTTTATCCGTAGGTTGCACCGTGGGTGTCCCGTACCCG
Clone 5 Template	5′CTTGTCGTCTCCTGTGTGCTTTATCCGTAGGTTGCATTGTGGGTCTCCCGTACCCG
Clone 6 Template	5′CTTGTCGTCTCCTGTGTGCTTTATCCGTAGGTTGCACCGTGCGTCTCCCGTACCCG
Clone 7 Template	5′CTTGTCGTCTCCTGTGTGCTTTATCCGTAGGTTGCACCATGGGTCTCCCGTACCCG
Clone 8 Template	5′CTTGTCGTCTCCTGTGTGCTTTATCCGTAGGTTTCACCTGGGACTCCCGTACCCG
Clone 9 Template	5′CTTGTCGTCTCCTGTGTGCTTTATCCGTAGGTTACACCGTGGGTCTCCCGTACCCG
Clone 10 Template	5′CTTGTCGTCTCCTGTGTGCTTTATCCGTAGGTTGCACCGAGGGTCTCCCGTACCCG
Clone 11 Template	5′CTTGTCGTCTCCTGTGTGCTTTATCCGTAGGTTGCACCGTGGGTCACCCGTACCCG
Clone 12 Template	5′CTTGTCGTCTCCTGTGTGCTTTATCCGTAGGTTGCACTATGGTCTCCCGTACCCG
Clone 13 Template	5′CTTGTCGTCTCCTGTGTGCTTTATCCGTAGGTTACACCGTGGATCTCCCGTACCCG
Clone 14 Template	5′CTTGTCGTCTCCTGTGTGCTTAGGTATCGTCACGAACGAACGGCGCCCCGTACCCG
Clone 15 Template	5′CTTGTCGTCTCCTGTGTGCTTCAGTTTGCTAGAGTTGGAGTAAGGTCCCGTACCCG
Clone 16 Template	5′CTTGTCGTCTCCTGTGTGCTTTTCGGTGGGTCTACGCGGTCCTTATCCCGTACCCG
Clone 17 Template	5′CTTGTCGTCTCCTGTGTGCTTCGTAATGTGTGTGTGCTGCTTGGTTCCCGTACCCG
Clone 1_M1(Edu to C)	5′CTTGTCGTCTCCTGTGTGCTTTATCCGTAGGTTGCGCCGTGGGTCTCCCGTACCCG
Clone 1_M2(Edu to C)	5′CTTGTCGTCTCCTGTGTGCTTTATCCGTGGGTTGCACCGTGGGTCTCCCGTACCCG
Clone 1_M3(Edu to C)	5′CTTGTCGTCTCCTGTGTGCTTTGTCCGTAGGTTGCACCGTGGGTCTCCCGTACCCG
Stem Primer Long	5′CGGGTACGGGAGACCCACGGTGCA
Template 1	5′GCACCGTGGGTCTCCCGTACCCGAAAAAA/3Biotin
Template 2	5′GTAGGTTGCACCGTGGGTCTCCCGTACCCGAAAAAA /3Biotin

Selection method: SeLMA Overview



Scheme 1. SELMA (**SEL**ection with **M**odified **A**ptamers)

SELMA at 37° C

In a slight deviation from our previous efforts,[1,2] the first generation library was produced from a synthetic library devoid of the hairpin loop. This modification was implemented for synthetic ease and cost efficiency. New primers were used (sequences are located in SI Table 1). The library was ordered from IDT-DNA to contain 15% A in the template strand random region, yielding 15% EdU in the (+)-sense strand of the library.

First generation library synthesis

Thermopol buffer (1X final concentration), synthetic library (100pmol), library regeneration primer (120 pmol), dNTPs (200 µM each final concentration), 4 U of Vent polymerase and H_2O was added to a final volume of 100 µl in a PCR tube. The reaction was heated to 95° C for 20 seconds, cooled to 64° C for 30 seconds followed by 2 minutes at 72° C. The annealing and elongation steps were repeated 3 times to afford the desired dsDNA product. 30 U of Exonuclease I was added and the reaction was incubated at 37° C for 30 minutes. 4 M NaCl was added to a final concentration of 500 mM and EDTA was added to a final concentration of 5 mM. The product was then incubated with streptavidin magnetic beads for 30 minutes with intermittent mixing. The beads were washed twice with wash buffer (20 mM Tris pH 8.0, 500 mM NaCl) followed by the addition of 40 µl 100 mM NaOH for 4 minutes to elute the unbiotinylated strand. The supernatant was immediately mixed with 4 µl of 1 M HCl followed by 1 µl of 1 M Tris pH 8. The unbiotinylated starting library containing the hairpin loop was then used without further purification in selection.

Glycosylation of the library using click chemistry was performed as in reference 2, with slight modification. The 31 µl reaction mixture containing EdU-extended hairpin, THPTA ligand (0.9 mM final), $CuSO_4$ (0.8 mM final), and Man_9 azide (2.7 mM) were

combined into a capless 0.5-mL microfuge tube. 15 µL of freshly-dissolved 250 mM sodium ascorbate was placed into a second capless microfuge tube. 5 µl H_2O, 1.25 µl THPTA (10mM) and 1.2 µl (35mM) Man_9 azide were placed in a third capless tube. The three tubes were placed in a 25 ml pear-shaped flask with side arm, and flushed with argon for 2 hours. Under efflux of argon, micropipettors were inserted into the flask to transfer 1 µl sodium ascorbate to the tube containing the DNA, THPTA, $CuSO_4$ and Man_9-Azide. After one hour, an additional 0.5 µl sodium ascorbate was transferred, followed by the additional solution of THPTA and Man_9-azide, and the reaction was allowed to proceed for another hour after which it was buffer-exchanged twice and strand-displaced as described previously.

Strand displacement was also performed slightly differently from reference 2, at 65° C using Bst 2.0 WarmStart, followed by a folding step of 70° C for 2 minutes and slow cooling to room temperature at a rate of 0.3 °/second.

All 2G12 selections were performed as in reference 2, with the following modifications. 2G12 incubation was done for one hour at 37 ° C. Recoveries were performed using 1.5 mg protein A dynabeads on a rotator at 37 °C. For all rounds, beads were washed with 100 µl and 150 µl 2G12 binding buffer (20 mM Tris pH 7.5, 150 mM NaCl, 2 mM $MgSO_4$) which was pre-warmed to 37° C. The beads were resuspended with 30 µl elution buffer (20 mM Tris pH 8, 50 mM NaCl, 1.5 mg/ml BSA, 5% Tween-20) and placed in a boiling water bath for 2 minutes. The beads were magnetically separated and the supernatant was used in a 230 µl PCR reaction premix (minus polymerase) containing primer 1 and primer 2. 30 µl of the premix was aliquoted to 3 tubes and used in a pilot PCR reaction in which tubes were removed at various PCR cycle numbers. It is important to avoid excessive cycling as this can lead to unwanted side reactions. The pilot PCR reactions were run on agarose and the optimum PCR cycle number was empirically determined. Polymerase was added to the remaining 200 µl reaction, and PCR was run at the estimated optimal number.

Regeneration of the library was performed as previously described, however the 80 ° C step after the second ExoI incubation was omitted.

Note: After round 4, there was a significant build-up of a high molecular weight artifact. 10 µl of recovery PCR product was run on a 10% acrylamide gel and the band of desired size (80 bp) was excised. It was washed for 10 minutes with 1 ml buffer (20 mM Tris pH 9) and then ground with a pipette tip and mixed with 200 µl of buffer (20 mM Tris pH 9). The tube containing the gel slurry was placed in a boiling water bath for 10 minutes and 10 µl of the supernatant was used in a 230 µl PCR premix and pilot PCR as described previously, and the optimized PCR was used in library regeneration as described. No further artifacts were observed in subsequent rounds of selection.

SI Table 2: [2G12] and library enrichment by round

Selection round	2G12 concentration used (nM)	Optimal PCR cycles for recovery
1	100	22
2	100	15
3	50	16
4	50	15
5	50	12
6	5	15
7	5	13
8	5	14
9	5	13

Note: In rounds 2, 4, 6 and 8 the library was counterselected against protein A magnetic beads by incubation with 0.75 mg beads for 30 minutes and then the supernatant was used in positive selection for binding to 2G12.

Cloning of selected library

After 7 and 9 rounds of library generation/selection and amplification of the selected mannose-DNA from round 7, 2 µl of the amplification PCR product was used in a 100 µl amplification reaction using Vent(exo) polymerase according to the same parameters as used previously, except primer aptamerfor was used instead of primer aptamerfor-biotin. 5 U Taq polymerase was added to the PCR product and the reaction was incubated for 30 minutes at 72° C to ensure optimal incorporation of overhanging adenosine nucleotides at the 3' ends of both strands. A TOPO TA cloning kit was then used to clone the library according to manufacturer's instructions. 70 colonies were picked into LB broth and the plasmid isolated and sequenced:

Selected Clones

Sequence identifiers:

Random Region (T's in the random sequence correspond to positions at which Man_9 moieties are located when the clone is prepared for binding assays)

37for (primer)

37rev (primer)

Uncolored text is the stem region

Of the colonies sequenced, several clones were observed multiple times. In all 37 new sequences were observed

Clone 1 was found in 14 colonies

Clone 2 was found in 4 colonies

Clone 10 was found in 2 colonies

Sequences of clones studied in binding assays, (+) strand, 5'->3':

Clone 1
TGCGGTTATGAGGTGGAGTTTTAACGGTACGGGAGACCCACGGTGCAACCTACGGATAAAGCACACAGGAGACGACAAG

Clone 2
TGCGGTTATGAGGTGGAGTTTTAACGGGTACGGGAGACCCACAGTGCAACCTACGGATAAAGCACACAGGAGACGACAAG

Clone 3
TGCGGTTATGAGGTGGAGTTTTAACGGGTACGGGAGACCCCCGGTGCAACCTACGGATAAAGCACACAGGAGACGACAAG

Clone 4
TGCGGTTATGAGGTGGAGTTATAACGGGTACGAGACACCCACGGTGCAACCTACGGATAAAGCACACAGGAGACGACAAG

Clone 5
TGCGGTTATGAGGTGGAGTTTTAACGGGTATGGGAGACCCACAATGCAACCTACGGATAAAGCACACAGGAGACGACAAG

Clone 6
TGCGGTTATGAGGTGGAGTTTTAACGGGCACAGGAGACGCACGGTGCAACCTACGGATAAAGCACACAGGAGACGACAAG

Clone 7
TGCGGTTATGAGGTGGAGTTTTAACGGATACGGAAGACCCATGGTGCAACCTACGGATAAAGCACACAGGAGACGACAAG

Clone 8
TGCGGTTATGAGGTGGAGTTTTAACGGGTAAGGGAGTCCCAGGTGAAACCTACGGATAAAGCACACAGGAGACGACAAG

Clone 9
TGCGGTTATGAGGTGGAGTTTTAACGGGTACGGGAGACCCACGGTGTAACCTACGGATAAAGCACACAGGAGACGACAAG

Clone 10
TGCGGTTATGAGGTGGAGTTTTAACGGGTACGGG**AGACCCTCGGTGCAACCTACGGATA**AAGCACACAGGAGACGACAAG

Clone 11
TGCGGTTATGAGGTGGAGTTCTAACGGGTACAGG**TGACCCACGGTGCAACCTACGGATAA**AGCACACAGGAGACGACAAG

Clone 12
TGCGGTTATGAGGTGGAGTTTTAACGGGTACGGG**AGACCATAGTGCAACCTACGGATA**AAGCACACAGGAGACGACAAG

Clone 13
TGCGGTTATGAGGTGGAGTTTTAACGGGTACGGG**AGATCCACGGTGTAACCTACGGATA**AAGCACACAGGAGACGACAAG

Clone 14
TGCGGTTATGAGGTGGAGTTATAACAGGTACGGAGCGCCGTTCGTTCGTGACGATACCTAAGCACACAGGAGACGACAAG

Clone 15
TGCGGTTATGAGGTGGAGTTTTAACTGGTAGGGACCTTACTCCAACTCTAGCAAACTGAAGCACACAGGAGACGACAAG

Clone 16
TGCGGTTATGAGGTGGAGTTTCAAAGAGTAAGGG**ATAAGGACCGCGTAGACCCACCGAA**AAGCACACAGGAGACGACAAG

Clone 17
TGCGGTTATGAGGTGGAGTTTTAACGGGTACGAGAACCAAGCAGCACACACACATTACGAAGCACACAGGAGACGACAAG

Sequences of clones not studied in binding assays, (+) strand, 5'->3':

Clone A
TGCGGTTATGAGGTGGAGTTAAATGGATAAGGGTGAATGTGTCTGAATCATAGTATAGAAGCACACAGGAGACGACAAG

Clone B
TGCGGTTATGAGGTGGAGTTTAAACGCGTACGGGAGACCCACGGTGCGACCTACGGATAAAGCACACAGGAGACGACAAG

Clone C
TGCGGTTATGAGGTGGAGTTTTAACGGATACGGGCATGCGGTGACTCAATGTGAATCATAAGCACACAGGAGACGACAAG

Clone D
TGCGGTTATGAGGTGGAGTTTTAACGGGTAGAGGATATGGTGTGTCGTGCACATCCACAAAGCACACAGGAGACGACAAG

Clone E
TGCGGTTATGAGGTGGAGTTTTAACGGGTACGGGAGACCCACGGTGCAACTTACGGATAAAGCACACAGGAGACGACAAG

Clone F
TGCGGTTATGAGGTGGAGTTCTAACTGGTACGGGTGAATGTGTCTGAATCATAGTACAGAAGCACACAGGAGACGACAAG

Clone G
TGCGGTTATGAGGTGGAGTTATAACGGGTACGGATGTCACGCAATGATAATATCTGAGTAAGCACACAGGAGACGACAAG

Clone H
TGCGGTTATGAGGTGGAGTTTTAACGTGTACGGGTGAATGTGTCTGAGTCATAGTACAGAAGCACACAGGAGACGACAAG

Clone I
TGCGGTTATGAGGTGGAGTTTCAACGGGTACAGGAGACCCACGGTGCAACCTACGGGTAAAGCACACAGGAGACGACAAG
Clone J
TGCGGTTATGAGGTGGAGTTTCAACGGGTACGGGAGACCCACAGTGCAACCTACGGATAAAGCACACAGGAGACGACAAG
Clone K
TGCGGTTATGAGGTGGAGTTTAAACGGGTACGGGAGACCCACAGTGCAACCTACGGATAAAGCACACAGGAGACGACAAG
Clone L
TGCGGTTATGAGGTGGAGTTTTAACGGGTACGGGAGACCCACTGTGCAATCTACGGATAAAGCACACAGGAGACGACAAG
Clone M
TGCGGTTATGAGGTGGAGTTTTAATTGGTACGGGAGACCCACGGTGCAACATACGGATAAAGCACACAGGAGACGACAAG
Clone N
TGCGGTTATGAGGTGGAGTTTTAACGGGTACAGGAGACCCTCGGTGCAACCTACGGATAAAGCACACAGGAGACGACAAG
Clone O
TGCGGTTATGAGGTGGAGTTTTAACGTGTACGGGAGACCCACAGTGCAACCTACGGATAAAGCACACAGTAGACGACAAG
Clone P
TGCGGTTATGAGGTGGAGTTTTAACGGGTACGGGAGACCCACTGTGCAACCTACGGATAAAGCACACAGGAGACGACAAG
Clone Q
TGCGGTTATGAGGTGGAGTTTTAACGGATACGGGAGACTCACGGTGCAACCTACGGATAAAGCACACAGGAGACGACAAG
Clone R
TGCGGTTATGATGTGGAGTTTTAACCGCAACGGGAGACCCACGGNGCAACCTACGGATAAAGCACACAGGAGACGACAAG
Clone S
TGCGGTTATGAGGTGGAGTTTTAACGGGTACGAGAGACCCACGGTGCAACATACGGATAAAGCACACAGGAGACGACAAG
Clone T
TGCGGTTATGAGGTGGAGTTTTAACAGCTACGAGAGACCCACTGTGCAACCTACGGATAAAGCACACAGGAGACGACAAG

Sequence Alignments

CLUSTAL W (1.81) multiple sequence alignment of clones with tight binding to 2g12

```
* - single, fully conserved residue
Clone_13        AGATCCACGGTGTAACCTACGGATA
Clone_9         AGACCCACGGTGTAACCTACGGATA
Clone_8         AGTCCCA-GGTGAAACCTACGGATA
Clone_7         AGACCCATGGTGCAACCTACGGATA
Clone_1         AGACCCACGGTGCAACCTACGGATA
Clone_11        TGACCCACGGTGCAACCTACGGATA
Clone_3         AGACCCCCGGTGCAACCTACGGATA
Clone_4         ACACCCACGGTGCAACCTACGGATA
Clone_5         AGACCCACAATGCAACCTACGGATA
Clone_2         AGACCCACAGTGCAACCTACGGATA
Clone_12        AGAC-CATAGTGCAACCTACGGATA
Clone_6         AGACGCACGGTGCAACCTACGGATA
Clone_10        AGACCCTCGGTGCAACCTACGGATA
                     *    ** ************
```

CLUSTAL W (1.81) multiple sequence alignment of clones with any detectable binding to 2g12

```
* - single, fully conserved residue
Clone_5         -------AGACCCACAATGCAACCTACGGATA
Clone_2         -------AGACCCACAGTGCAACCTACGGATA
Clone_10        -------AGACCCTCGGTGCAACCTACGGATA
Clone_3         -------AGACCCCCGGTGCAACCTACGGATA
Clone_6         -------AGACGCACGGTGCAACCTACGGATA
Clone_4         -------ACACCCACGGTGCAACCTACGGATA
Clone_1         -------AGACCCACGGTGCAACCTACGGATA
Clone_13        -------AGATCCACGGTGTAACCTACGGATA
Clone_9         -------AGACCCACGGTGTAACCTACGGATA
Clone_11        -------TGACCCACGGTGCAACCTACGGATA
Clone_7         -------AGACCCATGGTGCAACCTACGGATA
Clone_8         --------AGTCCCAGGTGAAACCTACGGATA
Clone_12        --------AGACCATAGTGCAACCTACGGATA
Clone_14        GCGCCGTTCGTTCGTGACGATACCT-------
                        *     *  ****
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Preparation of selected clones and mutants for filter binding assay

For binding studies, the template synthetic oligos for each clone were obtained from IDT. According to our previous procedure, each clone (100pmol) was prepared by polymerase extension of a primer against the synthetic template (using EdUTP in place of dTTP), then glycosylated using vacuum degassing method and purified via urea PAGE.[2] Normally, the click reaction was ~90 % complete, as visualized by PAGE, and in cases where it was incomplete, the material was desalted and the reaction was repeated. The glycosylated and purified ssDNA was then radioactively phosphorylated using polynucleotide kinase and ATP (γ-^{32}P) according to manufacturer's instructions. The desalted radiolabeled glycosylated aptamer was then used in the filter binding assay described below.
For synthesis of mutants having EdU to T mutations, see pg. **S15**

Filter binding
Binding Buffer (20 mM Tris HCl pH 7.5, 150 mM NaCl, 4 mM $MgSO_4$, 50 ug/mL BSA) was prepared freshly and filtered through 0.2μM syringe filter.
2G12 serial dilution was prepared in quadruplet. 2G12 dilutions of 500nM, 125nM, 31.25nM, 7.81nM, 1.95nM, 0.49nM, 0.12nM, and 0.03 nM were used in the filter binding assays.

Sufficient radiolabeled DNA (enough to produce an adequate radiogram after overnight exposure, generally 50-100fmol) was added to 180 μl binding buffer/BSA. The solution was heated to 70° C for 5 minutes and allowed to cool to room temperature. Then, 5 μL of the radiolabeled and diluted aptamer was added to a 50 μL aliquot of the antibody. For each dilution, the experiment was repeated in quadruplicate. After binding for 1 hr, the solution was then filtered through a nitrocellulose/PVDF sandwich and the radioactivity in each membrane quantified by exposure to a phosphor screen followed by phosphor imaging. The data were then fit to $F_{bound}=(F_{max}[2G12])/(K_d+[2G12])$. The results are tabulated in **SI Table 3**.

Note: Nitrocellulose was exposed to 0.4 M NaOH for 10 minutes, washed extensively with H_2O, and then soaked in binding buffer prior to the filter binding assay. PVDF was soaked in methanol prior to extensive washing with H_2O and soaking in binding buffer prior to the filter binding assay.

Filter Binding Results:

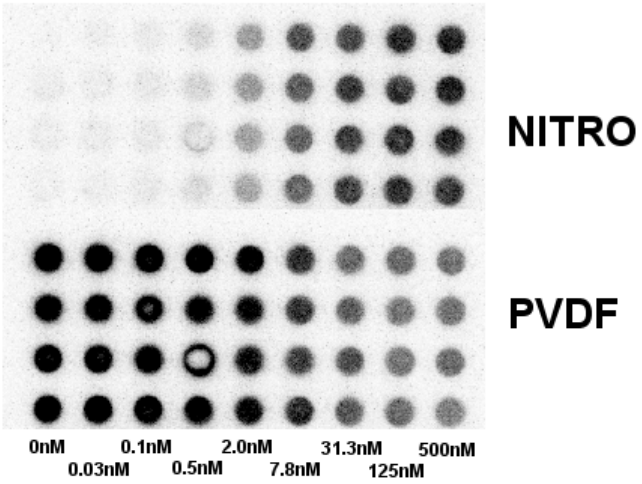
SI Table 3: Nitrocelluose Filter Binding Results

Clone ID	Glycans	Sequence	Kd (nM)[a]	Fb_{max}[a]
1	3	AGACCCACGGSGCAACCSACGGASA	3.1±0.1	57.9±0.5
2	3	AGACCCACAGSGCAACCSACGGASA	1.7±0.2	60.9±1.3
3	3	AGACCCCCGGSGCAACCSACGGASA	2.3±0.4	30.0±1.0
4	3	ACACCCACGGSGCAACCSACGGASA	6.9±1.1	40.9±1.3
5	3	AGACCCACAASGCAACCSACGGASA	5.9±1.2	54.9±2.2
6	3	AGACGCACGGSGCAACCSACGGASA	3.4±0.3	59.6±1.1
7	4	SGACCCACGGSGCAACCSACGGASA	11.9±2.5	46.9±2.2
8	4	GAGSCCCAGGSGAAACCSACGGASA	8.3±1.0	67.4±1.7
9	4	AGACCCSCGGSGCAACCSACGGASA	4.3±0.4	64.8±1.1
10	4	AGACCCASGGSGCAACCSACGGASA	6.1±0.9	67.6±2.1
11	4	AGACCASAGSGCAACCSACGGASA	3.7±0.4	50.9±1.0
12	4	AGACCCACGGSGSAACCSACGGASA	9.4±1.7	59.9±2.4
13	5	AGASCCACGGSGSAACCSACGGASA	15.9±2.1	52.3±1.0
14	7	GCGCCGSSCGSSCGSGACGASACCS	>500nm	ND[b]
15	6	ACCSSACSCCAACSCSAGCAAACSG	NB[c]	ND[b]
16	2	ASAAGGACCGCGSAGACCCACCGAA	NB[c]	ND[b]
17	2	AACCAAGCAGCACACACACASSACG	NB[c]	ND[b]
1-M1(UtoC)	2	AGACCCACGGCGCAACCSACGGASA	NB[c]	ND[b]
1-M2(UtoC)	2	AGACCCACGGSGCAACCCACGGASA	NB[c]	ND[b]
1-M3(UtoC)	2	AGACCCACGGSGCAACCSACGGACA	NB[c]	ND[b]
1-M(UtoT)	2	AGACCCACGGTGCAACCSACGGASA	NB[c]	ND[b]
1-M2(UtoT)	2	AGACCCACGGSGCAACCTACGGASA	NB[c]	ND[b]
1-M3(UtoT)	2	AGACCCACGGSGCAACCSACGGATA	NB[c]	ND[b]

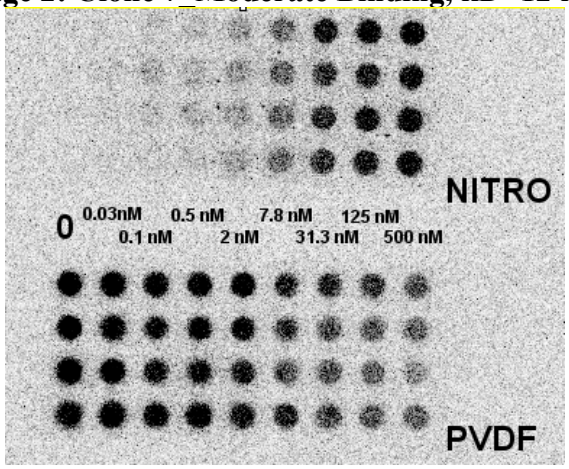
Table 1. Sequences and binding of clones obtained from selections performed at 37 °C.
[a]Binding constant and Fb_{max} determined by Nitrocellulose/PVDF filter binding assay.
[b]Not determined. [c] No binding was found.

Representative Blots from Filter Binding Assay:

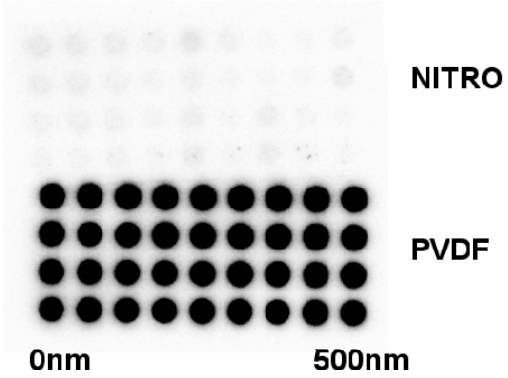
SI Image 1: Clone 2_Good Binding, kD=1.7nM



SI Image 2: Clone 7_Moderate Binding, kD=12 nM

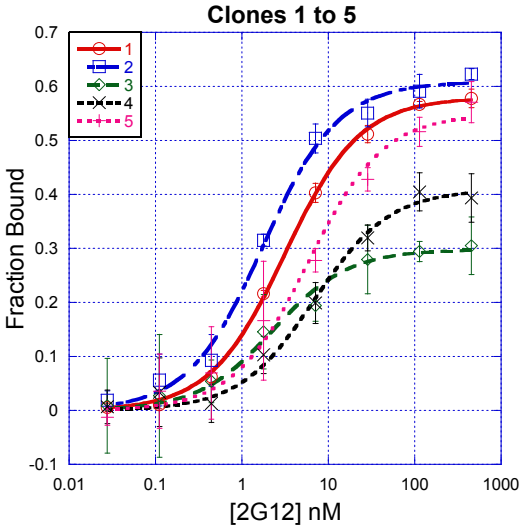


SI Image 3: Clone 1 Mutant 3, 2 glycans (Mutation EdU to C)_No Binding

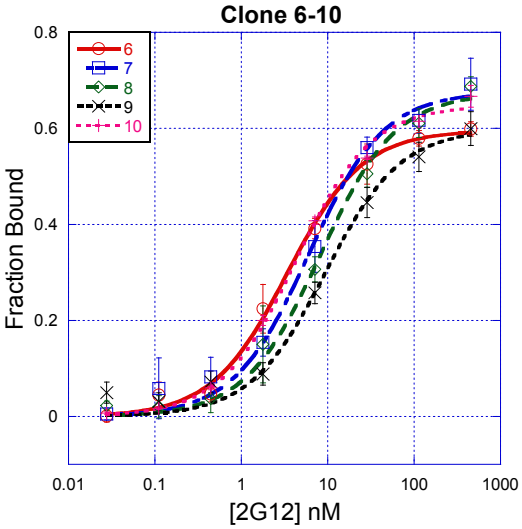


Binding Curves

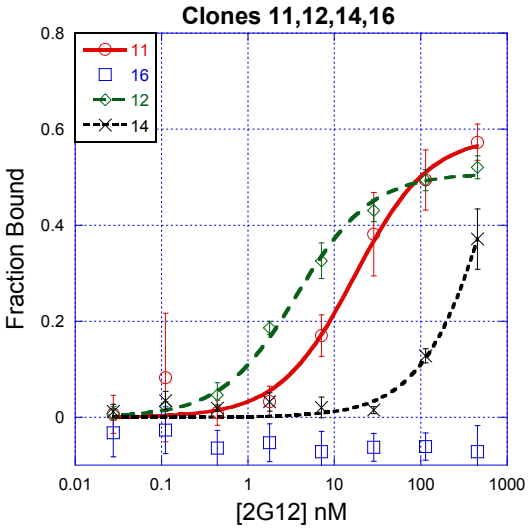
SI Figure 1



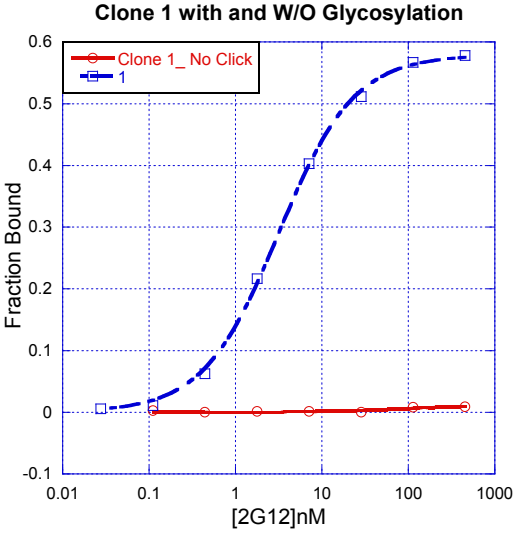
SI Figure 2



SI Figure 3

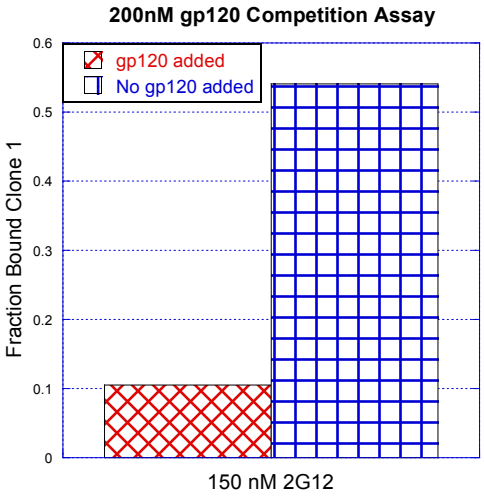


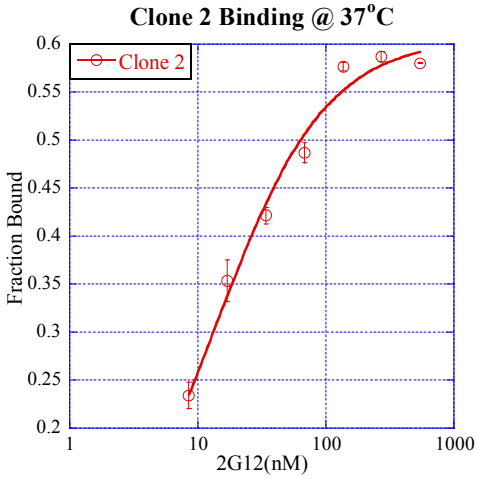
SI Figure 4



SI Figure 5

SI Figure 6





BLItz (Biolayer Interferometry) Analysis of 2G12-Clone 1 Binding Kinetics

We examined the binding of clone 1 to 2G12 in real time via biolayer interferometry (BLI) using a ForteBio BLItz instrument. Clone 1, modified with a 5'-$(A)_5$ spacer and biotin tag, was immobilized on a streptavidin sensor exposure of the sensor to a 20 nM aptamer solution for 300 seconds at a shaking rate of 2200rpm, yielding an average response of 0.25 nm. 2G12 was associated to the surface at once at each of several concentrations, followed by dissociation in blank binding buffer (20 mM Tris pH 7.5, 150 mM NaCl, 4 mM $MgSO_4$, 0.20 mg/ml BSA, 0.02 % Tween-20). The data has been referenced against aptamer loading and nonspecific 2G12/biosensor interaction. For each sensor and at each concentration, background binding of the sensor to 2G12 was recorded without any immobilized aptamer, followed by dissociation of the nonspecifically bound 2G12. These reference runs were then subtracted from data of 2G12 association/dissociation to aptamer-loaded sensor.

Clone 1 was synthesized on a 400pmol scale from Template 1-5T and Biotin tagged stem primer-5A, as seen below:

Step 1: Elongation with BST DNA polymerase and EdUTP mixed bases.

Starting Materials:

```
5'biotin/AAAAACGGGTACGGG
         TTTTTGCCCATGCCCTCTGGGTGCCACGTTGGATGCCTATTTCGTGTGTCCTCTGCTGTTC
```

Product:

```
5'biotin/AAAAACGGGTACGGGAGACCCACGGUGCAACCUACGGAUAAAGCACACAGGAGACGACAAG
         TTTTTGCCCATGCCCTCTGGGTGCCACGTTGGATGCCTATTTCGTGTGTCCTCTGCTGTTC
```

Step 2: Click reaction

Product:

```
5'biotin/AAAAACGGGTACGGGAGACCCACGGSGCAACCSACGGASAAAGCACACAGGAGACGACAAG
         TTTTTGCCCATGCCCTCTGGGTGCCACGTTGGATGCCTATTTCGTGTGTCCTCTGCTGTTC
```

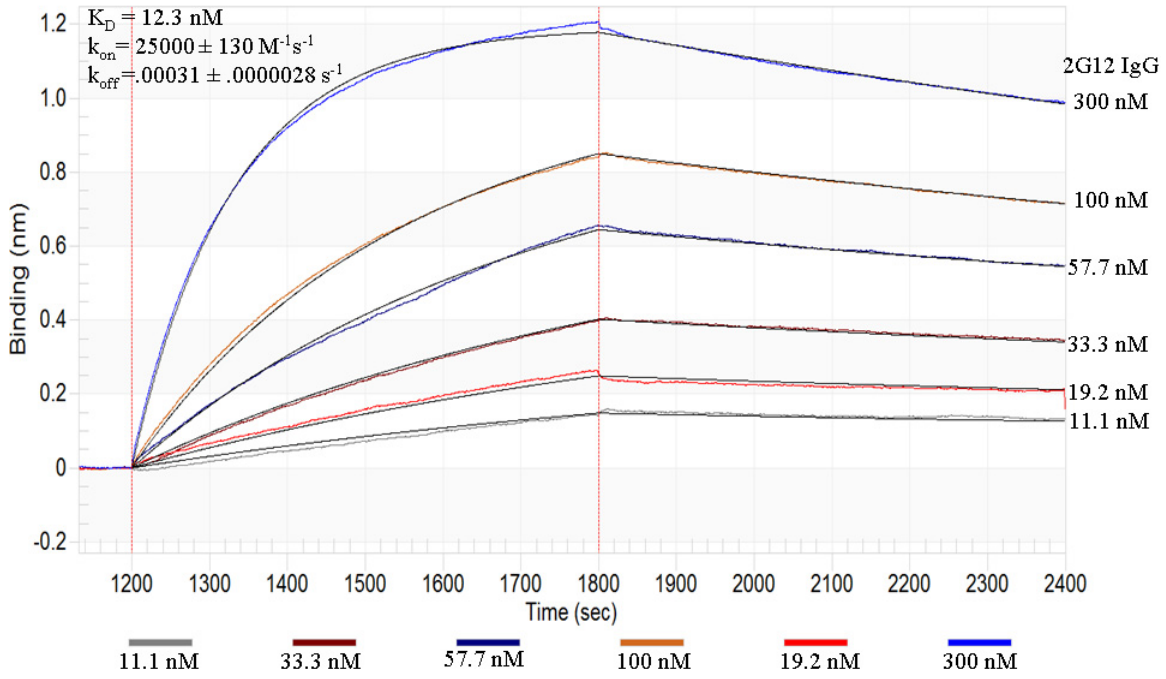
Isolated product following urea PAGE purification:

```
5'biotin/AAAAACGGGTACGGGAGACCCACGGSGCAACCSACGGASAAAGCACACAGGAGACGACAAG
```

SI Table 4: BLItz™ Method

Step	Type	Duration(s)	Position
1	Initial Baseline	600	Tube
2	Aptamer Loading	300	Drop (1μM pure aptamer)
3	Custom Wash	1200	Tube
4	Baseline	600	Tube
5	Association of 2G12	600	Tube
6	Dissociation of 2G12	600	Tube

SI Figure 7: BLItz™ Data: binding of wt2G12 to Clone 1 w/5'A_5Tail

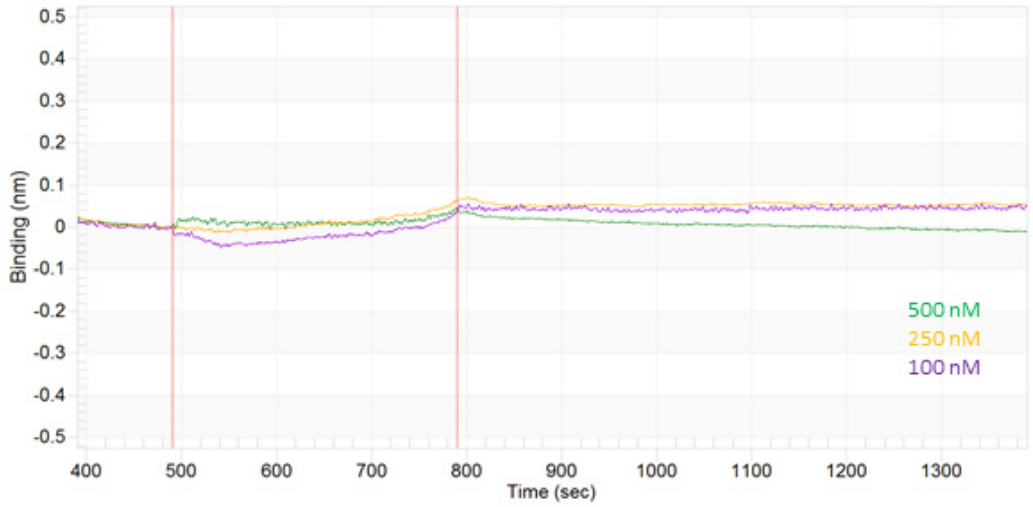


SI Table 5: Globally fit data to 1:1 binding model.

Conc. (nM)	KD (M)	ka (1/Ms)	ka Error	kd (1/s)	kd Error	Rmax	Rmax Error	R equilibrium
300	1.23E-08	2.50E+04	1.26E+02	3.08E-04	2.85E-06	1.24	0.002	1.195
100	1.23E-08	2.50E+04	1.26E+02	3.08E-04	2.85E-06	1.23	0.006	1.092
57.73	1.23E-08	2.50E+04	1.26E+02	3.08E-04	2.85E-06	1.30	0.009	1.072
33.33	1.23E-08	2.50E+04	1.26E+02	3.08E-04	2.85E-06	1.22	0.010	0.894
19.25	1.23E-08	2.50E+04	1.26E+02	3.08E-04	2.85E-06	1.21	0.012	0.740
11.11	1.23E-08	2.50E+04	1.26E+02	3.08E-04	2.85E-06	1.19	0.014	0.566

The above variation in Rmax values results from the small variation in loading between different SA biosensor tips. The reported error is the calculated standard error of the curve fit.

SI Figure 8: BLItz™ Data: no binding of mutant 2G12 I19R to Clone 1 w/5'A_5Tail



Synthesis of Mutants of EdU to C or T

Mutants EdU to C were prepared in the same manner as all other clones for binding studies, except that the template oligos were ordered with a G instead of an A at each desired carbohydrate deletion location.

Oligos Ordered 5'->3'

M1 (C)

CTTGTCGTCTCCTGTGTGCTTTATCCGTAGGTTGCGCCGTGGGTCTCCCGTACCCG

M2 (C)

CTTGTCGTCTCCTGTGTGCTTTATCCGTGGGTTGCACCGTGGGTCTCCCGTACCCG

M3 (C)

CTTGTCGTCTCCTGTGTGCTTTGTCCGTAGGTTGCACCGTGGGTCTCCCGTACCCG

Mutants EdU to T were prepared using the following methods:

Oligos Ordered 5'->3'

Stem Primer

CGGGTACGGG

Stem Primer Long

CGGGTACGGGAGACCCACGGTGCA

Template 1

GCACCGTGGGTCTCCCGTACCCGAAAAAA/3Biotin

Template 2

GTAGGTTGCACCGTGGGTCTCCCGTACCCGAAAAAA /3Biotin

Clone 1 Full Template

CTTGTCGTCTCCTGTGTGCTTTATCCGTAGGTTGCACCGTGGGTCTCCCGTACCCG

Synthesis of Clone 1 M1(T) EdU to T Mutant

Polymerase Reaction

Reagent	V µL
H_2O	41.5
Thermo Pol buffer 10x	10
Stem Long Primer 10µM	25
Clone 1 Template 10µM	20
EdU/dA/dC/dGTP Mixed bases 10mM each	2
BST DNA Polymerase	1.5

Water, Thermo Pol Buffer, Long Stem primer, and Clone 1 Template were combined into a PCR tube and heated to 95 °C for 30 seconds. After cooling to 4 °C, EdU/dA/dC/dGTP mix was added, followed by BST DNA polymerase. The mixture was heated at 60 °C for 5 minutes.

Starting Materials

CGGGTACGGGAGACCCACGGTGCA
GCCCATGCCCTCTGGGTGCCACGTTGGATGCCTATTTCGTGTGTCCTCTGCTGTTC

Product of Polymerase Reaction

CGGGTACGGGAGACCCACGGTGCAACCUACGGAUAAAGCACACAGGAGACGACAAG
GCCCATGCCCTCTGGGTGCCACGTTGGATGCCTATTTCGTGTGTCCTCTGCTGTTC

Following polymerase extension, the product was desalted through a 1.5 mL Sephadex G-50 column. Fractions containing product were concentrated under reduced pressure in vacuum centrifuge. Product was reconstituted in H_2O for the click reaction.

Click Reaction

Reagent	V(µL)	Final
Reconstituted extension reaction	34	
THPTA (10 mM)	6	1.2 mM
$CuSO_4$ (10 mM)	5	1.0 mM
Man_9Azide (35 mM)	3	2.1 mM
Sodium Ascorbate (250 mM)	2	10 mM
Total Reaction	50	

Product of Click Reaction

CGGGTACGGGAGACCCACGGTGCAACCSACGGASAAAGCACACAGGAGACGACAAG
GCCCATGCCCTCTGGGTGCCACGTTGGATGCCTATTTCGTGTGTCCTCTGCTGTTC

Synthesis of Clone 1 M2(T) EdU to T Mutant

Polymerase Reaction #1

Reagent	V µL

H_2O	41.5
Thermo Pol buffer 10x	10
Stem Primer 10μM	25
Template 3 10μM	20
EduTP Mixed bases 10mM each	2
BST DNA Polymerase	1.5

Water, Thermo Pol Buffer, Stem primer, and Template 3 were combined into a PCR tube and heated to 95 °C for 2 minutes. After cooling to 4 °C, EdU/dA/dC/dGTP mix was added, followed by BST DNA polymerase. The reaction mixture was cycled 5 times between 45 °C (2min) and 60 °C (2min).

Step 1: Starting Materials

```
                5'CGGGTACGGG
3'biotin/AAAAAAGCCCATGCCCTCTGGGTGCCACG
```

Step 1: Product

```
                5'CGGGTACGGGAGACCCACGGUGC
3'biotin/AAAAAAGCCCATGCCCTCTGGGTGCCACG
```

To the reaction was added 12.5 μL of NaCl (12.5μL) and 1 μL EDTA (500mM). The reaction was added to 1mg Streptavidin magnetic beads and mixed by rotation for 30 minutes. The beads were washed 4 times with wash buffer (20mM Tris pH7.5, 500mM NaCl.) 30 μL of Elution Buffer was added (20mMTris pH7.5 150mM NaCl) and the beads were heated to 95 °C for 1 minute. The supernatant was removed and saved. This process was repeated a second time to ensure optimal recovery.

Step 1: Isolated Product

```
                5'CGGGTACGGGAGACCCACGGUGC
```

Polymerase Reaction #2

Reagent	V μL
DNA/H_2O	70
Thermo Pol buffer 10x	10
Template 2 10μM	20
DNTP Mixed bases 10mM each	2
BST DNA Polymerase	1.5

Recovered product from step 1, Thermo Pol Buffer, and Template 2 were combined into a PCR tube and heated to 95 °C for 2 minutes. After cooling to 4 °C, dT/dA/dC/dGTP mix was added, followed by BST DNA polymerase.The reaction mixture was cycled 5 times between 45 °C (2min) and 60 °C (2min).

Step 2:Starting Materials

```
                5'CGGGTACGGGAGACCCACGGUGC
3'biotin/AAAAAAGCCCATGCCCTCTGGGTGCCACGTTGGATG
```

Step 2: Product of Polymerase Reaction

```
                5'CGGGTACGGGAGACCCACGGUGCAACCTAC
3'biotin/AAAAAAGCCCATGCCCTCTGGGTGCCACGTTGGATG
```

The biotinylated template was removed by streptavidin magnetic bead treatment as in Step 1.

Step 2: Isolated Product

5′CGGGTACGGGAGACCCACGGUGCAACCTAC

Polymerase Reaction #3

Reagent	V µL
DNA/H_2O	70
Thermo Pol buffer 10x	10
Clone 1 Template 10µM	20
EdUTP Mixed bases 10mM each	2
BST DNA Polymerase	1.5

Recovered product from step 2, Thermo Pol Buffer, and Clone 1 Template were combined into a PCR tube and heated to 95 ^{o}C for 2 minutes. After cooling to 4 ^{o}C, EdU/dA/dC/dGTP mix was added, followed by BST DNA polymerase. The mixture was heated at 60 ^{o}C for 5 minutes.

Step 3:Starting Materials

5′CGGGTACGGGAGACCCACGGUGCAACCTAC
GCCCATGCCCTCTGGGTGCCACGTTGGATGCCTATTTCGTGTGTCCTCTGCTGTTC

Step 3: Product of Polymerase Reaction

5′CGGGTACGGGAGACCCACGGUGCAACCTACGGTUAAAGCACACAGGAGACGACAAG
GCCCATGCCCTCTGGGTGCCACGTTGGATGCCTATTTCGTGTGTCCTCTGCTGTTC

Following polymerase extension, the product was desalted through a 1.5 mL Sephadex G-50 column. Fractions containing product were concentrated under reduced pressure in vacuum centrifuge. Product was reconstituted in H_2O for the click reaction.

Click Reaction

Reagent	V(µL)	Final
Reconstituted extension reaction	34	
THPTA (10 mM)	6	1.2 mM
$CuSO_4$ (10 mM)	5	1.0 mM
Man_9Azide (35 mM)	3	2.1 mM
Sodium Ascorbate (250 mM)	2	10 mM
Total Reaction	50	

Product of Click Reaction

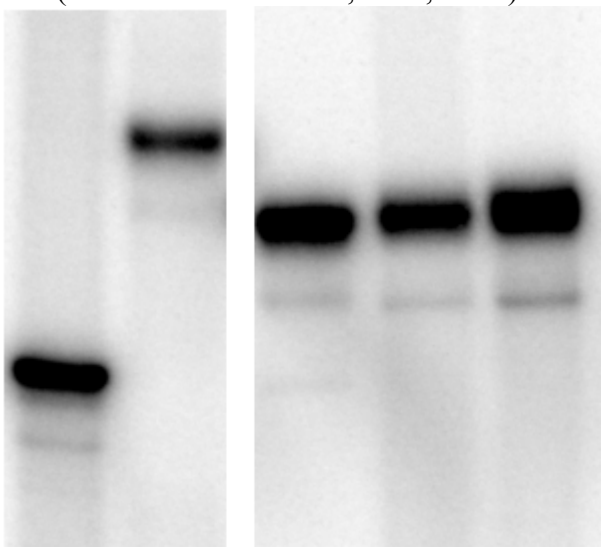
5′CGGGTACGGGAGACCCACGGSGCAACCTACGGTSAAAGCACACAGGAGACGACAAG
GCCCATGCCCTCTGGGTGCCACGTTGGATGCCTATTTCGTGTGTCCTCTGCTGTTC

Synthesis of Clone 1 M3(T) EdU to T Mutant

M3(T) was synthesized in an identical fashion to M2(T), except that TTP was used in the first two extensions, and EdUTP was used in the third.

Preparative denaturing PAGE purification of Mutants
As previously described[2], all glycosylated clones required PAGE purification to achieve high quality binding results. All purifications were done on 10% Urea PAGE (20cmx20cmx1.5mm, 22W, 1hr).

SI Gel 1: Radiolabeled, glycosylated and purified Clone 1 with mutants in 10 % Urea Gel (20cmx20cmx0.75mm, 30W, 1.5hr). Overnight Exposure to phosphorimaging plate.

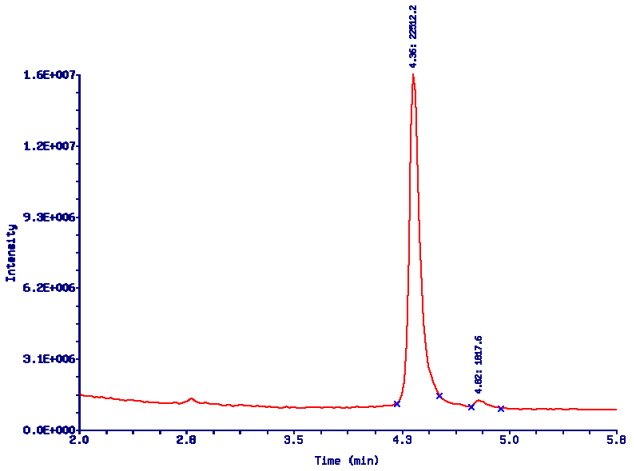


Lane 1: 56 nt template w/o glycosylation_Clone 1 Template. **Lane 2**: 3 glycan control_Clone 1 glycosylated and purified. **Lane 3**: Clone 1 M1 (T) glycosylated and purified. **Lane 4**: Clone 1 M2 (T) glycosylated and purified. **Lane 5**: Clone 1 M3 (T) glycosylated and purified.

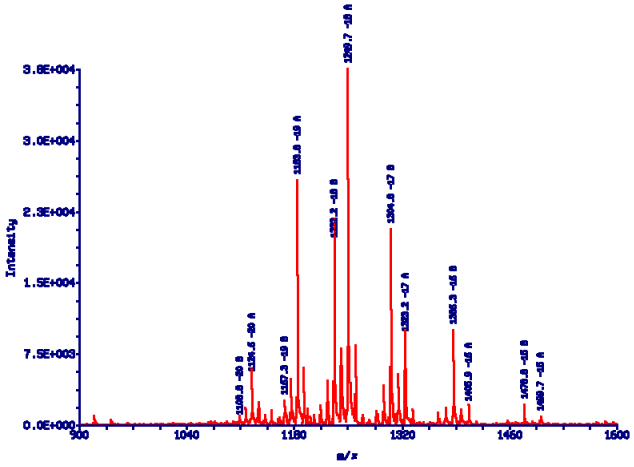
LC/MS Analysis of Mutants
Method: 260 nm 2x50mm Clarity MS C18 2.6u 5% B @ 0min, 10%B @ 1 min, 25% B @ 5 min, 60^{o}C, A=1%HFIPA/0.1%DIEA, B=65%ACN/water/0.075%HFIPA/0.0375% DIEA
LC/MS analysis performed by Novatia, LLC.

GlycoDNA	Calculated Mass	Experimental Mass
Clone 1	22198.0	22198.2
Clone 1 M1(T)	20588.4	20587.7
Clone 1 M2(T)	20588.4	20584.7
Clone 1 M3(T)	20588.4	20588.9

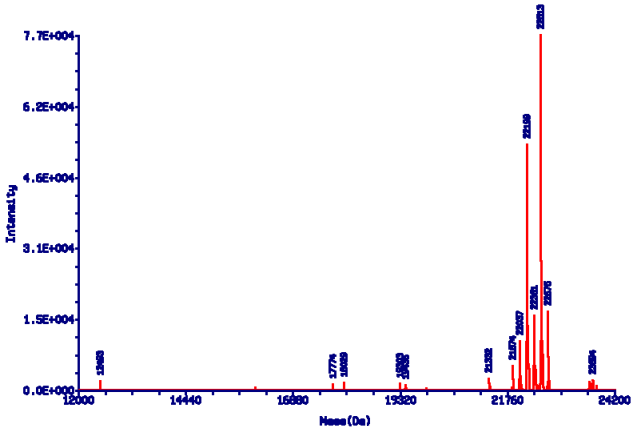
SI Figure 8: LC/MS - Clone 1



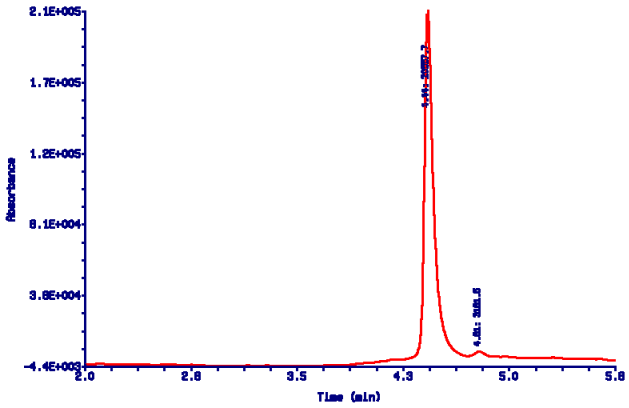
SI Figure 9: MS - Clone 1



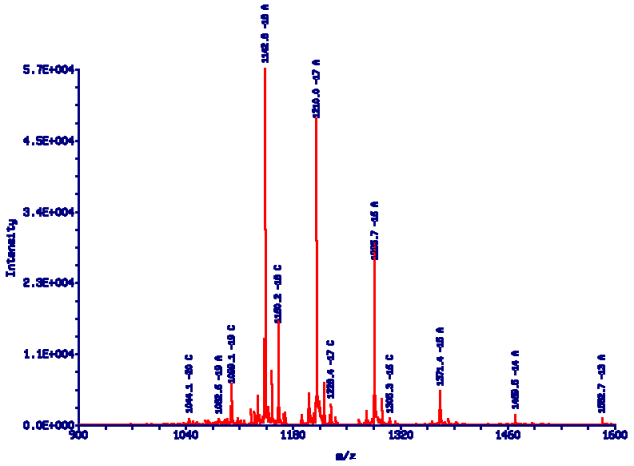
SI Figure 10: Deconvoluted MS - Clone 1



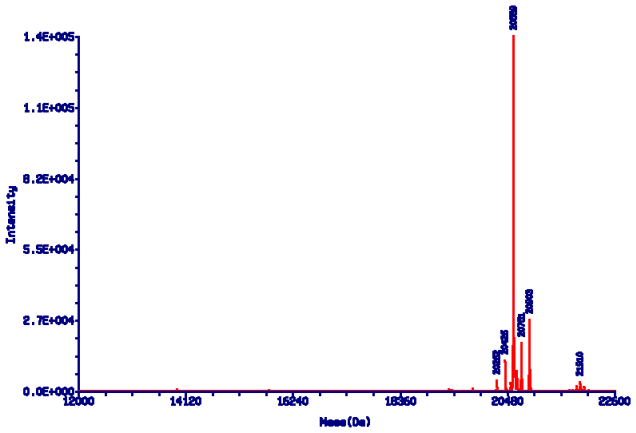
SI Figure 11: LC/MS - Clone 1 M1 (T)



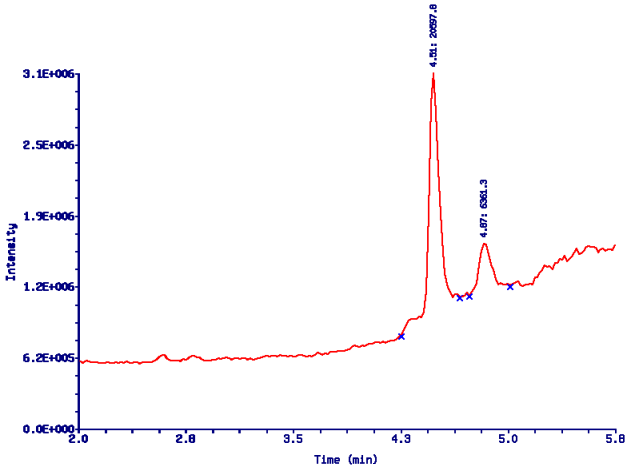
SI Figure 12: MS - Clone 1 M1 (T)



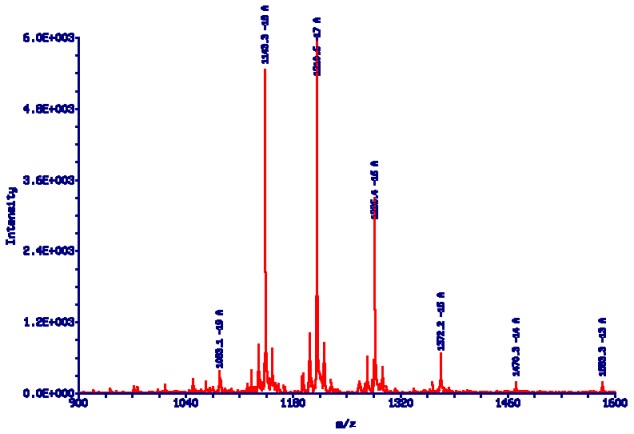
SI Figure 13: Deconvoluted MS - Clone 1 M1 (T)



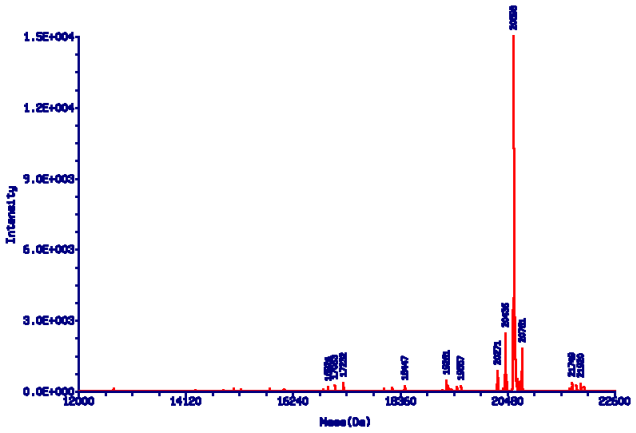
SI Figure 14: LC/MS - Clone 1 M2 (T)



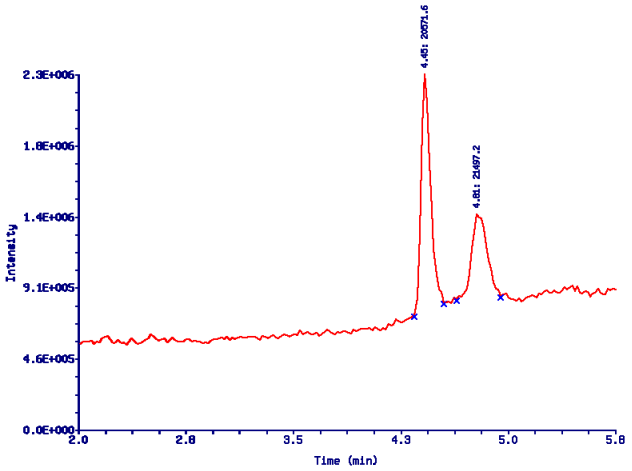
SI Figure 15: MS - Clone 1 M2 (T)



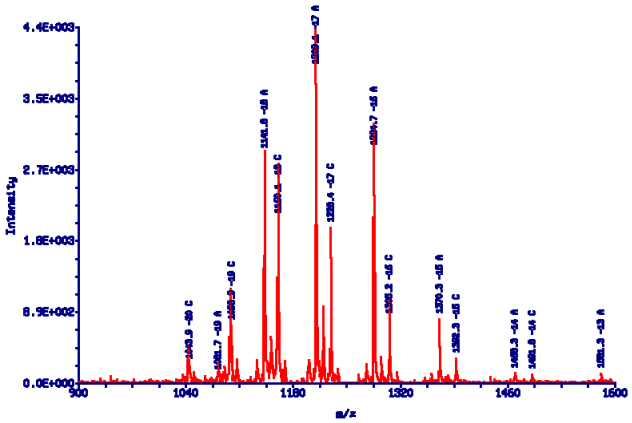
SI Figure 16: Deconvoluted MS - Clone 1 M2 (T)



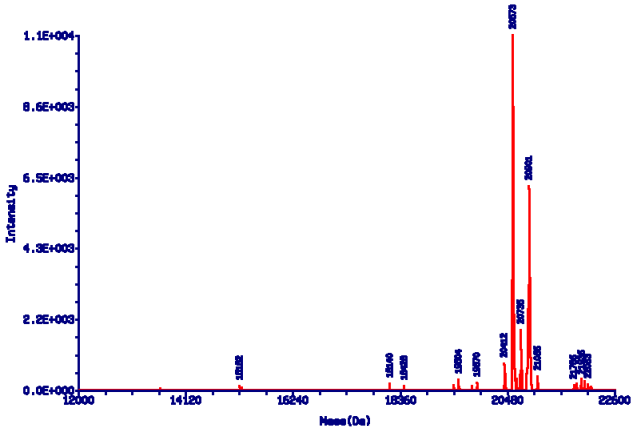
SI Figure 17: LC/MS - Clone 1 M3 (T)



SI Figure 18: MS - Clone 1 M3 (T)



SI Figure 19: Deconvoluted MS - Clone 1 M3 (T)



References

(1) MacPherson, I. S.; Temme, J. S.; Habeshian, S.; Felczak, K.; Pankiewicz, K.; Hedstrom, L.; Krauss, I. J. *Angew. Chem.-Int. Edit.* **2011**, *50*, 11238-11242.

(2) Temme, J. S.; Dryzga, M. G.; MacPherson, I. S.; Krauss, I. J. *Chem. Eur. J.* **2013**, *19*, 17291-17295.