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.

Man₉-azide was prepared according to literature¹².
# SI Table 1: DNA oligonucleotides

| Oligonucleotide       | Sequence                                                                 |
|-----------------------|---------------------------------------------------------------------------|
| Hairpin library       | **5’** [CTTGTCGTCTCCTGTGTGCTT]**NNNNNNNNNNNNNNNNNNNNNNNNNCCCTA**<br>**CCCGTTAAACTCCACCTCTCTAACCACCA**<br>Blue=aptamerrev binding region<br>Red=stem region<br>Green=loop region<br>underlined=aptamerfor binding region<br>N_{25}=(N1:15%/28%/28%/28%:A/G/C/T) Randomized region |
| **Aptamerfor**         | 5’TGGGTTATGAGGTGAGTT                                                      |
| **Aptamerfor-biotin**  | 5’biotin/TGGGTTATGAGGTGAGTT                                               |
| **Aptamerrev**         | 5’CTTGTCGTCTCCTGTGTGCTT                                                   |
| **Aptamerrev-biotin**  | 5’biotin/CTTGTCGTCTCCTGTGTGCTT                                            |
| **Stem Primer**        | 5’CGGATACCCG                                                             |
| **Clone 1 Template**   | 5’CTTGTCGTCTCCTGTGTGCTTATTCGCTAGGTGGACGGGTCTTCCCTACCCCG                  |
| **Clone 2 Template**   | 5’CTTGTCGTCTCCTGTGTGCTTATTCGCTAGGTGGACGGGTCTTCCCTACCCCG                  |
| **Clone 3 Template**   | 5’CTTGTCGTCTCCTGTGTGCTTATTCGCTAGGTGGACGGGTCTTCCCTACCCCG                  |
| **Clone 4 Template**   | 5’CTTGTCGTCTCCTGTGTGCTTATTCGCTAGGTGGACGGGTCTTCCCTACCCCG                  |
| **Clone 5 Template**   | 5’CTTGTCGTCTCCTGTGTGCTTATTCGCTAGGTGGACGGGTCTTCCCTACCCCG                  |
| **Clone 6 Template**   | 5’CTTGTCGTCTCCTGTGTGCTTATTCGCTAGGTGGACGGGTCTTCCCTACCCCG                  |
| **Clone 7 Template**   | 5’CTTGTCGTCTCCTGTGTGCTTATTCGCTAGGTGGACGGGTCTTCCCTACCCCG                  |
| **Clone 8 Template**   | 5’CTTGTCGTCTCCTGTGTGCTTATTCGCTAGGTGGACGGGTCTTCCCTACCCCG                  |
| **Clone 9 Template**   | 5’CTTGTCGTCTCCTGTGTGCTTATTCGCTAGGTGGACGGGTCTTCCCTACCCCG                  |
| **Clone 10 Template**  | 5’CTTGTCGTCTCCTGTGTGCTTATTCGCTAGGTGGACGGGTCTTCCCTACCCCG                  |
| **Clone 11 Template**  | 5’CTTGTCGTCTCCTGTGTGCTTATTCGCTAGGTGGACGGGTCTTCCCTACCCCG                  |
| **Clone 12 Template**  | 5’CTTGTCGTCTCCTGTGTGCTTATTCGCTAGGTGGACGGGTCTTCCCTACCCCG                  |
| **Clone 13 Template**  | 5’CTTGTCGTCTCCTGTGTGCTTATTCGCTAGGTGGACGGGTCTTCCCTACCCCG                  |
| **Clone 14 Template**  | 5’CTTGTCGTCTCCTGTGTGCTTATTCGCTAGGTGGACGGGTCTTCCCTACCCCG                  |
| **Clone 15 Template**  | 5’CTTGTCGTCTCCTGTGTGCTTATTCGCTAGGTGGACGGGTCTTCCCTACCCCG                  |
| **Clone 16 Template**  | 5’CTTGTCGTCTCCTGTGTGCTTATTCGCTAGGTGGACGGGTCTTCCCTACCCCG                  |
| **Clone 17 Template**  | 5’CTTGTCGTCTCCTGTGTGCTTATTCGCTAGGTGGACGGGTCTTCCCTACCCCG                  |
| **Clone 1_M1(Edu to C)** | 5’TGGGTTATGAGGTGAGTGCATATCCGTGGGTCTTCCCGTACCCG                             |
| **Clone 1_M2(Edu to C)** | 5’TGGGTTATGAGGTGAGTGCATATCCGTGGGTCTTCCCGTACCCG                             |
| **Clone 1_M3(Edu to C)** | 5’TGGGTTATGAGGTGAGTGCATATCCGTGGGTCTTCCCGTACCCG                             |
| **Stem Primer Long**   | 5’CGGATACCCG                                                             |
| **Template 1**         | 5’GCACCGGTGGGTCTCCCGTACCGCAANAAAAA/3Biotin                               |
| **Template 2**         | 5’GTAGGTTGGACCGGTGGGTCTCCCGTACCGCAANAAAAA /3Biotin                       |
**Selection method: SeLMA Overview**

![](image)

**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 H₂O 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), CuSO₄ (0.8 mM final), and Man₉ 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 H₂O, 1.25 μL THPTA (10mM) and 1.2 μL (35mM) Man₉ 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, CuSO₄ and Man₉-Azide. After one hour, an additional 0.5 μL sodium ascorbate was transferred, followed by the additional solution of THPTA and Man₉-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 MgSO₄) 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 Man9 moieties are located when the clone is prepared for binding assays)

**37fod** (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

Clone 11
TGCGGTTATGAGGTGGAGTTCTAACGGGTACAGG

Clone 12
TGCGGTTATGAGGTGGAGTTTTAACGGGTACGGG

Clone 13
TGCGGTTATGAGGTGGAGTTTTAACGGGTACGAGAACCAAGCAGCACACACATTACGAAG

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

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
TCACGGGTACAGGAGACCCACGGTGCAACCTACGGATAAGACACAGGAGACGACAAG
Clone J
TCAACGGGTACAGGAGACCCACGGTGCAACCTACGGATAAGACACAGGAGACGACAAG
Clone K
TAAACGGGTACAGGAGACCCACGGTGCAACCTACGGATAAGACACAGGAGACGACAAG
Clone L
TTAACGGGTACAGGAGACCCACGGTGCAACCTACGGATAAGACACAGGAGACGACAAG
Clone M
TTAACGGGTACAGGAGACCCACGGTGCAACCTACGGATAAGACACAGGAGACGACAAG
Clone N
TTAACGGGTACAGGAGACCCACGGTGCAACCTACGGATAAGACACAGGAGACGACAAG
Clone O
TTAACGGGTACAGGAGACCCACGGTGCAACCTACGGATAAGACACAGGAGACGACAAG
Clone P
TTAACGGGTACAGGAGACCCACGGTGCAACCTACGGATAAGACACAGGAGACGACAAG
Clone Q
TTAACGGGTACAGGAGACCCACGGTGCAACCTACGGATAAGACACAGGAGACGACAAG

**Sequence Alignments**

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

| Clone     | Sequence                                      |
|-----------|-----------------------------------------------|
| Clone_1   | AGATCCACGGTGTAACCTACGGATA                     |
| Clone_2   | AGACCCACGGTGTAACCTACGGATA                     |
| Clone_3   | AGACCCACGGTGTAACCTACGGATA                     |
| Clone_4   | AGACCCACGGTGTAACCTACGGATA                     |
| Clone_5   | AGACCCACGGTGTAACCTACGGATA                     |
| Clone_6   | AGACCCACGGTGTAACCTACGGATA                     |
| Clone_7   | AGACCCACGGTGTAACCTACGGATA                     |
| Clone_8   | AGACCCACGGTGTAACCTACGGATA                     |
| Clone_9   | AGACCCACGGTGTAACCTACGGATA                     |
| Clone_10  | AGACCCACGGTGTAACCTACGGATA                     |

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

| Clone     | Sequence                                      |
|-----------|-----------------------------------------------|
| Clone_1   | AGATCCACGGTGTAACCTACGGATA                     |
| Clone_2   | AGACCCACGGTGTAACCTACGGATA                     |
| Clone_3   | AGACCCACGGTGTAACCTACGGATA                     |
| Clone_4   | AGACCCACGGTGTAACCTACGGATA                     |
| Clone_5   | AGACCCACGGTGTAACCTACGGATA                     |
| Clone_6   | AGACCCACGGTGTAACCTACGGATA                     |
| Clone_7   | AGACCCACGGTGTAACCTACGGATA                     |
| Clone_8   | AGACCCACGGTGTAACCTACGGATA                     |
| Clone_9   | AGACCCACGGTGTAACCTACGGATA                     |
| Clone_10  | AGACCCACGGTGTAACCTACGGATA                     |

* - single, fully conserved residue

*    ** ************
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. 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-32P) 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 MgSO₄, 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 $F_{\text{bound}}=(F_{\text{max}}[2\text{G12}])/(K_d+[2\text{G12}])$. The results are tabulated in SI Table 3.

Note: Nitrocellulose was exposed to 0.4 M NaOH for 10 minutes, washed extensively with H₂O, and then soaked in binding buffer prior to the filter binding assay. PVDF was soaked in methanol prior to extensive washing with H₂O and soaking in binding buffer prior to the filter binding assay.
Filter Binding Results:

**SI Table 3: Nitrocellulose Filter Binding Results**

| Clone ID | Glycans | Sequence | Kd (nM)<sup>a</sup> | Fb<sub>max</sub><sup>a</sup> |
|----------|---------|----------|----------------------|---------------------|
| 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       | AGACCCACGGSGCAACCSACGGASA | 6.9±1.1 | 40.9±1.3 |
| 5        | 3       | AGACCCACAGSGCAACCSACGGASA | 5.9±1.2 | 54.9±2.2 |
| 6        | 3       | AGACGGACGGSGCAACCSACGGASA | 3.4±0.3 | 59.6±1.1 |
| 7        | 4       | SGACCCACGGSGCAACCSACGGASA | 11.9±2.5 | 46.9±2.2 |
| 8        | 4       | GAAGACCGSSGAAACCSACGGASA | 8.3±1.0 | 67.4±1.7 |
| 9        | 4       | AGACCCCGGGSGCAACCSACGGASA | 4.3±0.4 | 64.8±1.1 |
| 10       | 4       | AGACCCACGGSGCAACCSACGGASA | 6.1±0.9 | 67.6±2.1 |
| 11       | 4       | AGACCAASGGSGCAACCSACGGASA | 3.7±0.4 | 50.9±1.0 |
| 12       | 4       | AGACCCACGGGSAACCSACGGASA | 9.4±1.7 | 59.9±2.4 |
| 13       | 5       | AGACCCACGGSGAAACCSACGGASA | 15.9±2.1 | 52.3±1.0 |
| 14       | 7       | GCGCCCGSGSGSGGACGASAACS | >500nm | ND<sup>b</sup> |
| 15       | 6       | ACCSSACSCAAACSCSAGCAAACSG | NB<sup>c</sup> | ND<sup>b</sup> |
| 16       | 2       | ASAAGGACCGGSGACACCCACGA | NB<sup>c</sup> | ND<sup>b</sup> |
| 17       | 2       | ACAAAGGACGACAAACACACCACAG | NB<sup>c</sup> | ND<sup>b</sup> |
| 1-M1(UtoC) | 2   | AGACCCACGGSACAACCSACGGASA | NB<sup>c</sup> | ND<sup>b</sup> |
| 1-M2(UtoC) | 2   | AGACCCACGGSACAACCSACGGASA | NB<sup>c</sup> | ND<sup>b</sup> |
| 1-M3(UtoC) | 2   | AGACCCACGGSACAACCSACGGASA | NB<sup>c</sup> | ND<sup>b</sup> |
| 1-M(UtoT) | 2    | AGACCCACGGSACAACCSACGGASA | NB<sup>c</sup> | ND<sup>b</sup> |
| 1-M2(UtoT) | 2   | AGACCCACGGSACAACCSACGGASA | NB<sup>c</sup> | ND<sup>b</sup> |
| 1-M3(UtoT) | 2   | AGACCCACGGSACAACCSACGGASA | NB<sup>c</sup> | ND<sup>b</sup> |

Table 1. Sequences and binding of clones obtained from selections performed at 37 °C.

<sup>a</sup>Binding constant and Fb<sub>max</sub> determined by Nitrocellulose/PVDF filter binding assay.

<sup>b</sup>Not determined. <sup>c</sup>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
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
TTTTGCCCATGCCCCTCTGGGTGCCACGTGATGCCCTATTTCGTGCTGTCTCTGCTGTC

**Product:**

5’biotin/AAAAACGGGTACGGGAGACCCACGGUGCAACCUACGGAUAAAGCACACAGGAGACGACAAG
TTTTGCCCATGCCCCTCTGGGTGCCACGTGATGCCCTATTTCGTGCTGTCTCTGCTGTC

**Step 2: Click reaction**

**Product:**

5’biotin/AAAAACGGGTACGGGAGACCCACGGSGCAACSCACGGASAAAGCACACAGGAGACGACAAG
TTTTGCCCATGCCCCTCTGGGTGCCACGTGATGCCCTATTTCGTGCTGTCTCTGCTGTC

Isolated product following urea PAGE purification:

5’biotin/AAAAACGGGTACGGGAGACCCACGGSGCAACSCACGGASAAAGCACACAGGAGACGACAAG
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’A₅Tail

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’A₅Tail**

![Graph showing binding data](image)

**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)**
  - CTTGTGTCGTCTCCTGTGCTTTATCCGTAGGTTGCGGGTGGGTCTCCCGTACCCG
- **M2 (C)**
  - CTTGTGTCGTCTCCTGTGCTTTATCCGTGGGTTGCACCGTGGGTCTCCCGTACCCG
- **M3 (C)**
  - CTTGTGTCGTCTCCTGTGCTTTATCCGTGGGTTGCACCGTGGGTCTCCCGTACCCG

Mutants EdU to T were prepared using the following methods:

**Oligos Ordered  5’->3’**

- **Stem Primer**
  - CGGGTACGGG
- **Stem Primer Long**
  - CGGGTACGGGAGACCCACGGTGCA

**Template 1**

- GCACCGGTGGGTCTCCCGTACCGGAAAAAA/3Biotin

**Template 2**

- GTAGGTTGACCGTGGGTCTCCCGTACCGGAAAAAA /3Biotin

**Clone 1 Full Template**

- CTTGTGTCGTCTCCTGTGCTTTATCCGTAGGTTGACCGTGGGTCTCCCGTACCGG
**Synthesis of Clone 1 M1(T) EdU to T Mutant**

**Polymerase Reaction**

| Reagent                                      | V µL |
|----------------------------------------------|------|
| H₂O                                          | 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 H₂O for the click reaction.

**Click Reaction**

| Reagent                                      | V(µL) | Final          |
|----------------------------------------------|-------|----------------|
| Reconstituted extension reaction             | 34    |                |
| THPTA (10 mM)                                | 6     | 1.2 mM         |
| CuSO₄ (10 mM)                                | 5     | 1.0 mM         |
| ManoAzide (35 mM)                            | 3     | 2.1 mM         |
| Sodium Ascorbate (250 mM)                    | 2     | 10 mM          |
| Total Reaction                               | 50    |                |

**Product of Click Reaction**

CGGGTACGGGAGACCCACGGTGCAACCUACGGAUAAAGCACACAGGAGACGACAAG
GCCCATGCCCTCTGGGTGCCACGTTGGATGCCTATTTCGTGTGTCCTCTGCTGTTC

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

**Polymerase Reaction #1**

| Reagent | V µL |
|---------|------|
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/AAAAAGCCCATGCCCTCTGGGTGCCACG

Step 1: Product
5’ CGGGTACGGGAGACCCACGGUGC
3’ biotin/AAAAAGCCCATGCCCTCTGGGTGCCACG

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/H2O                  | 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/AAAAAGCCCATGCCCTCTGGGTGCCACGTTGGATG

Step 2: Product of Polymerase Reaction
5’ CGGGTACGGGAGACCCACGGUGC
3’ biotin/AAAAAGCCCATGCCCTCTGGGTGCCACGTTGGATG
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/H2O                      | 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 °C for 2 minutes. 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.

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 H2O for the click reaction.

**Click Reaction**

| Reagent                           | V(µL) | Final |
|-----------------------------------|-------|-------|
| Reconstituted extension reaction  | 34    |       |
| THPTA (10 mM)                     | 6     | 1.2 mM|
| CuSO₄ (10 mM)                     | 5     | 1.0 mM|
| Man₉Azide (35 mM)                 | 3     | 2.1 mM|
| Sodium Ascorbate (250 mM)         | 2     | 10 mM |
| Total Reaction                    | 50    |       |

Product of Click Reaction
5’CGGGTACGGGAGACCCACGGUGCAACCTACGGTUAAAGCACACAGGAGACGACAAG
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   | Description                                |
|--------|--------------------------------------------|
| 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°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 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)

![LC/MS - Clone 1 M3 (T)](image17)

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

![MS - Clone 1 M3 (T)](image18)

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

![Deconvoluted MS - Clone 1 M3 (T)](image19)
References

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