Supplementary materials

Thioaptamers Targeting Dengue Virus Type-2 Envelope Protein Domain III

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TOPO cloning and sequencing: The TOPO cloning kit with the TOPO 2.1 vector and one shot complement cells were obtained from Life technologies and the manufacturer's protocol was followed for cloning the PCR product into the vector. The cloned cells were grown on LB ampicillin and lactose agar plates for 16 hours for blue/white selection. Forty colonies that were white/light blue in color were picked and cultured separately in 2 ml of LB amp each for 12 hours. The plasmids were isolated from the cultures (38 cultures since 2 of them did not grow) using Qiagen mini-prep plasmid isolation kit using the manufacturer's protocol. The isolated plasmids were submitted to Lone Star Labs, Houston for sequencing using M13 forward and reverse primers. The thioaptamer sequences were picked out from both directions to confirm the sequence and corrected for the missing reads from either direction. The random region from the thioaptamer sequences were used for the multiple sequence alignment using the CLUSTAL W program.

Sequence alignment and analysis: CLUSTAL W program, developed by European Bioinformatics Institute (EBI), was used for multiple sequence alignment. The pairwise alignment was set as slow and IUB DNA weight matrix was used. The gap open penalty value was increased to 25 from the default value of 10 to reduce the introduction of gap. A gap extension penalty of 1 was used to minimize introduction of additional gaps. Neighbor joining (NJ) clustering algorithm was used with tree as iteration for each alignment step and number of iterations was set to 10. The job was submitted and the results were viewed using JalView application. The distance tree was calculated using the option of average distance using percentage identity. The numbers in the tree stand for the distance between two sequences and lower number signifies higher similarity among the sequences being compared (Supplementary Fig. 5).

Filter binding assay – data analysis: The image was processed using ImageJ software (the complete procedure is described by Dr. Miller in the following page: http://lukemiller.org/index.php/2010/11/analyzing-gels-and-western-blots-with-image-j/) to obtain the intensities of each dot as areas under the curve. This data was used to calculate the percentage of thioaptamers on each membrane, based on the sum of the corresponding dots on both membranes (total intensity) being 100 percent. A plot of percentage of TA bound to nitrocellulose membrane and the protein concentration was made and the data were fitted using a non-linear regression for single site binding with hill slope (h), using Graphpad Prism software, to calculate the binding constants. The percentage of thioaptamers not bound to the protein, and thus trapped in the nylon membrane, increases with decreasing protein concentration.

$$\mathbf{Y} = \frac{(\mathbf{B_{max} * X^h})}{(\mathbf{K_d{}^h + X^h})} \quad \text{.....equation 1}$$

Also, an analysis of the same set of data using four parameter logistic fit (equation 2) was made to compare the fitting results.

$$\mathbf{Y} = \frac{\mathbf{100}}{(\mathbf{1 + 10^{(logEC50 - X) * h})}} \quad \text{.....equation 2}$$

Electrophoretic Mobility Shift Assay (EMSA): Biotin-labeled thioaptamer DENTA1 was used at a concentration of 1 nM and the DENV-2 EDIII protein was serially two-fold diluted from 10 µM to 78.125 nM to make 9 binding reactions and one with no protein. The reactions were incubated for 15 minutes on ice and loaded on a pre-cast 6% DNA-retardation gel (Invitrogen, Inc). The gel was run at a constant voltage of 80 V for 45 minutes at room temperature. The western blot transfer apparatus was used to electro-transfer the thioaptamers from the gel to nylon membrane. The chemiluminescence detection module (Thermoscientific, Inc) was used to detect the biotin DENTA1 on the nylon membrane as described previously (Supplementary Fig. 6). The free DENTA-1 band in the lane with no protein and the disappearance of the band could be observed with increasing protein concentration. The bands corresponding to the DENTA-1: DENV-2EDIII complex could not be observed clearly. This may be due to the kinetics of binding and dissociation of the thioaptamer and protein during the run leading to diffusion of the protein bound thioaptamer complex along the gel.

Supplementary Figure 1: The sequence of primers and the oligonucleotide library that was used to generate the thioaptamer library

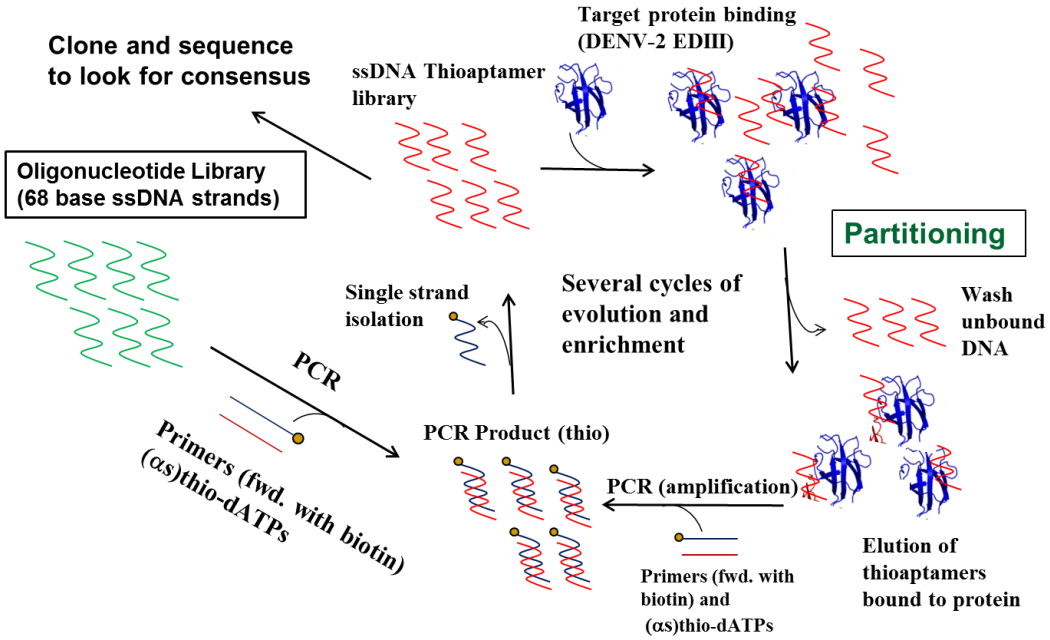
- 5' - CAGATGTCCGTAGACAGTGGCTC [N]24 CCTCGTAGCAGGAGAATGCCG – 3'
- 5' bio-bio-bio-CAGATGTCCGTAGACAGTGGCTC 3' – fwd primer with biotins at 5' end
- 5' CGGCATTCTCCTGCTACGAGG 3' – rev primer

This will make the 5' → 3' strand and the 3' → 5' strands as follows,

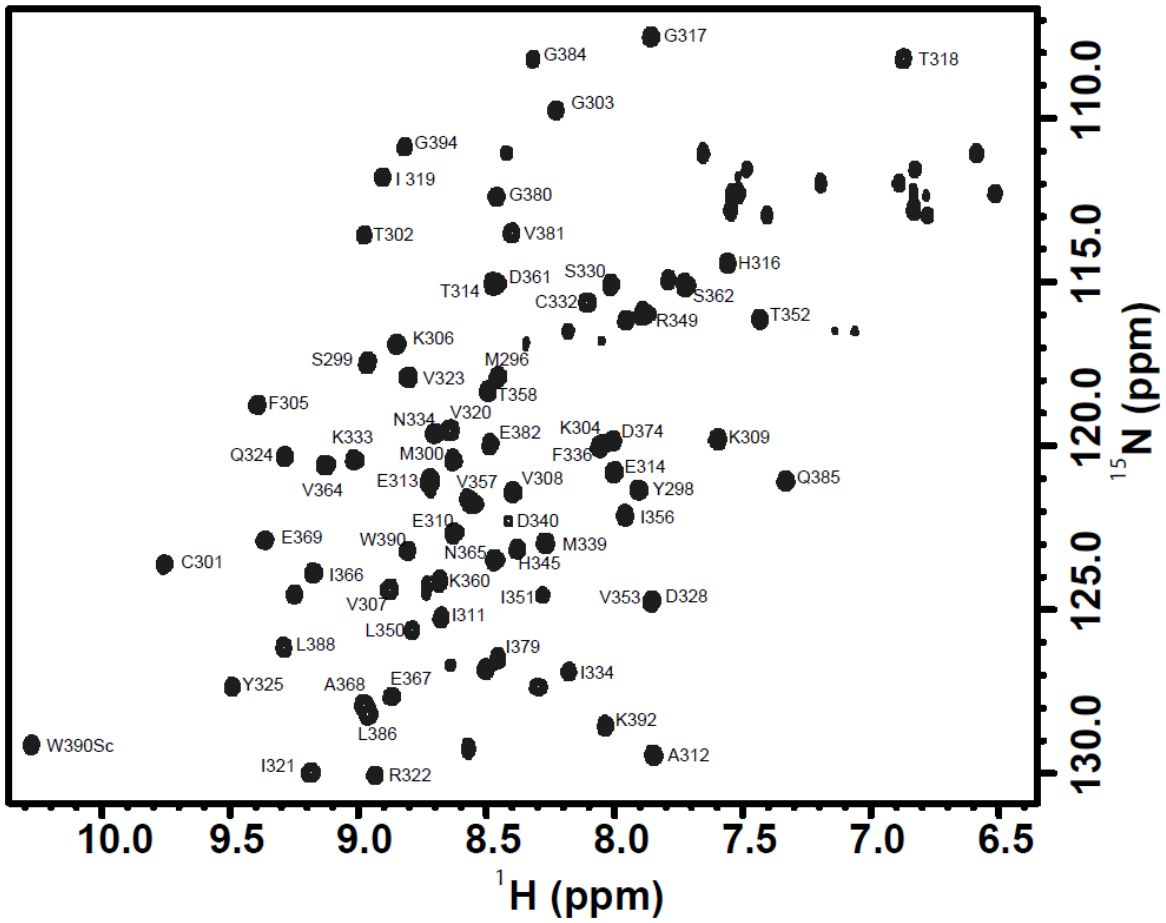
5' Bio-Bio-Bio- CAGATGTCCGTAGACAGTGGCTC [N]24 CCTCGTAGCAGGAGAATGCCG 3'

3' – GTCTACAGGCATCTGTCACCGAG [N]24 GGAGCATCGTCCTCTTACGGC – 5'

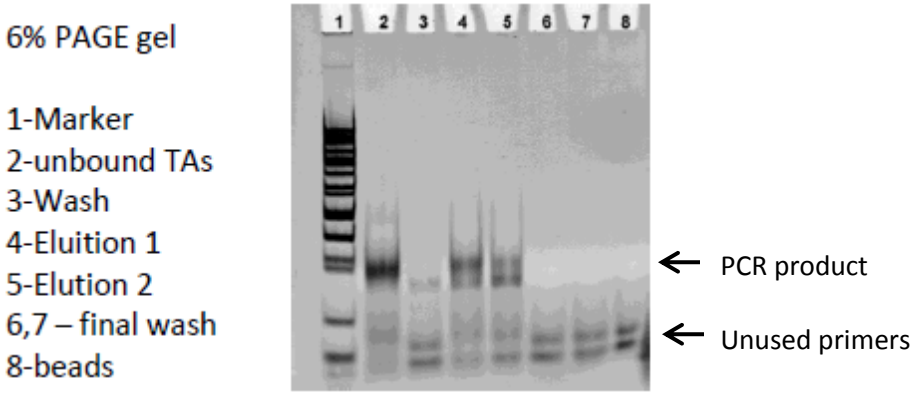
Supplementary Figure 2: The general SELEX process used for selection of thioaptamers is shown. The selection cycle was repeated for 5 iterations before TOPO cloning and sequencing of the enrichedthioaptamer pool.



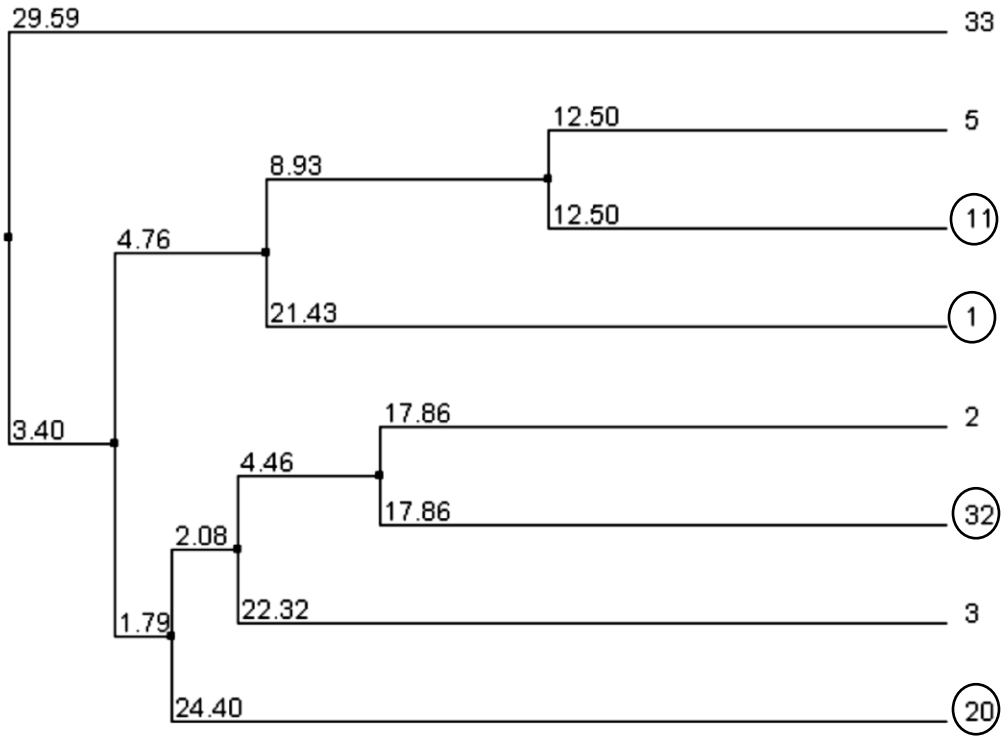
Supplementary Figure 3: NMR $^{1}H,^{15}N$-HSQC of native DENV-2 EDIII protein showing well sepearted chemical shifts in the ^{1}H and ^{15}N dimensions characteristic of well folded protein.



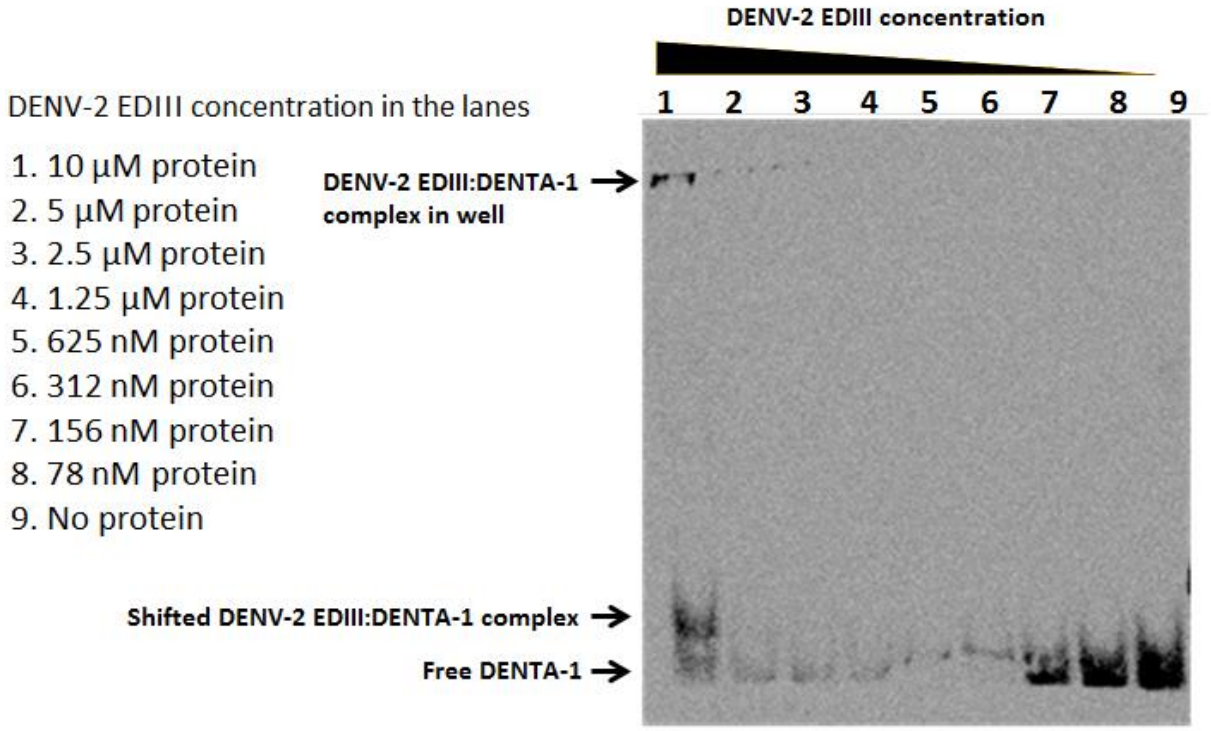
Supplementary Figure 4: PAGE gel showing partitioning efficiency of selection; The thioaptamers eluting at each wash and elution were PCR amplified for same number of cycles. The intensity of PCR product gives the relative amount of thioaptamers that were present in each wash/elution.



Supplementary Figure 5: Phylogenetic tree from the multiple sequence alignment of all the 38 sequences. The distance between the sequences are given by the number at the base of each branch and the repeated sequences are represented once. The sequeces 1, 11, 20 and 32 occurred at least 3 times (circled).



Supplementary Fig. 6: EMSA for biotin-labeled DENTA1 binding to DENV-2 EDIII is observed by chemiluminescence detection of DENTA1. The band for free DENTA1 could be seen when no protein is added (lane 9) and the amount of free DENTA1 decreases with increasing amounts of protein. The shifted band corresponding to the protein-TA complex could be observed in lane 1 with 10 µM protein.



Supplementary Fig. 7: Comparison of epitopes for A) DENTA1 and B) neutralizing monoclonal antibody 1A1D-2. Though the binding sites are adjacent, they do not overlap and may still be able to accommodate the binding of antibody on the virus.