Physiological temperatures decrease dimerization of dengue and Zika recombinant envelope proteins
Supporting Information

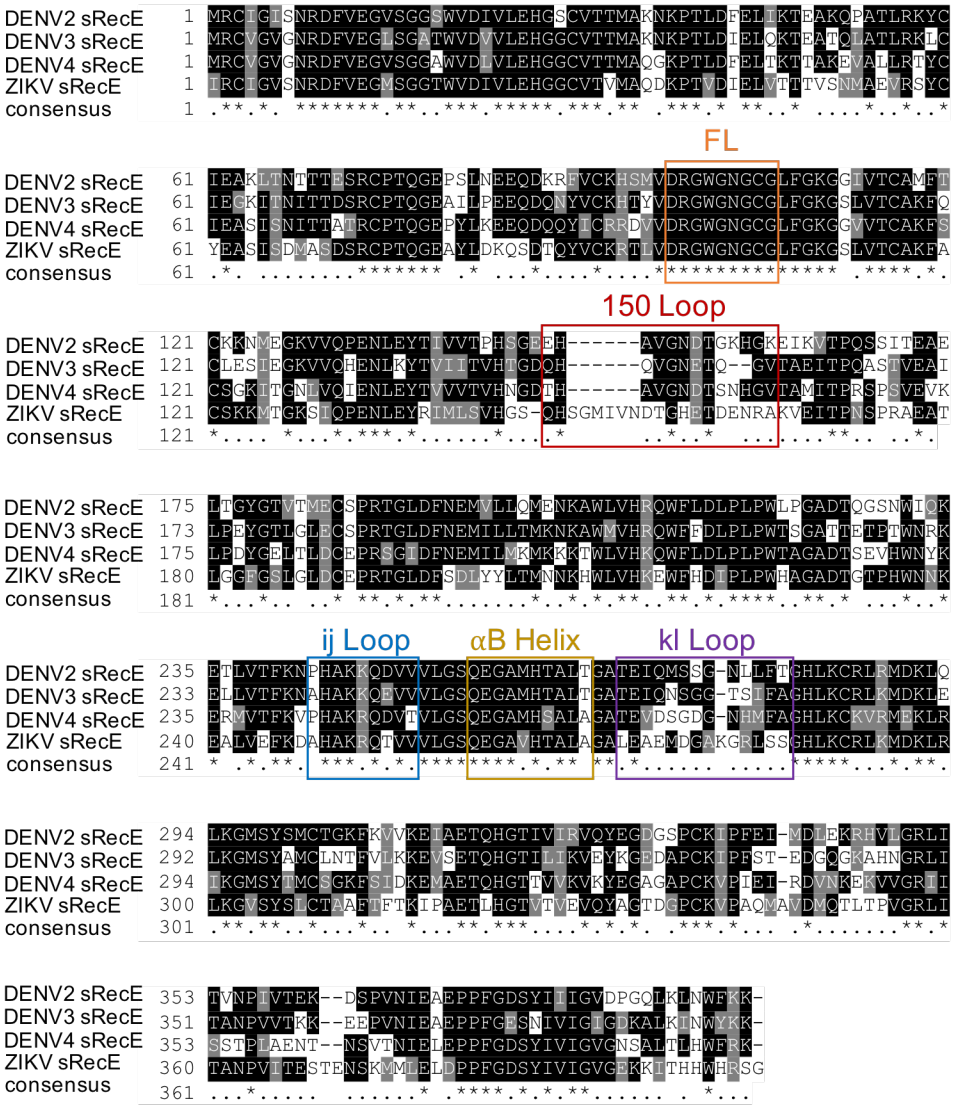


Figure S1. Amino Acid (AA) Sequence Alignment of DENV2-4 & ZIKV sRecE
TCoffee/BoxShade AA sequence alignment of DENV2-4 & ZIKV sRecE used in this study. Common motifs such as the fusion loop (FL, orange), 150 glyan loop (red) and the ij loop (blue), αB helix (gold) kl loop (purple) motifs present at the sRecE dimer interface as observed in crystal structures (PDB: 1OAN (DENV2), 1UZG (DENV3), 3UAJ (DENV4), 5LBV (ZIKV)).

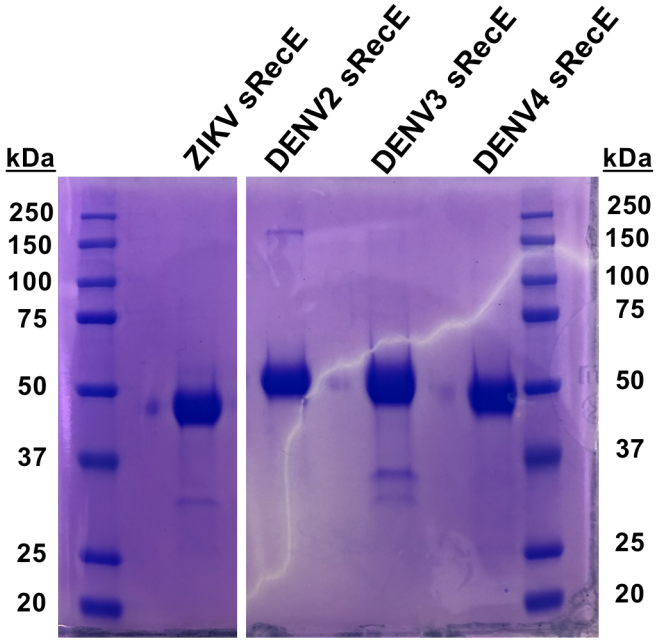


Figure S2. SDS-PAGE analysis of purified DENV2-4 & ZIKV sRecE
8 µg of DENV2-4 & ZIKV sRecE were reduced with β-mercaptoethanol and boiled for 5min at 95˚C. Samples were then loaded onto a Bio-Rad Mini-PROTEAN Any kD TGX Stain-Free gel and stained using commassie brilliant blue stain. Expected MW for DENV2, DENV3, DENV4 and ZIKV sRecE are, 45.1 kDa, 43.1 kDa, 44.5 kDa and 45.1 kDa, respectively.

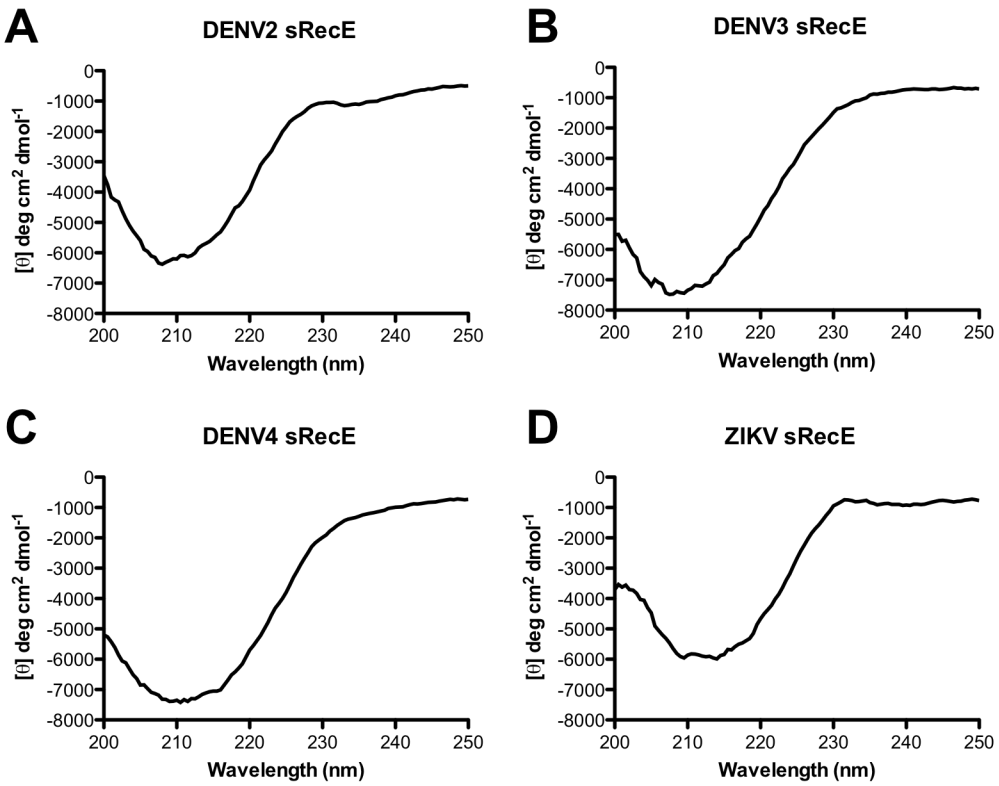


Figure S3. ***Far-UV CD spectral scans of DENV2-4 & ZIKV sRecE***
CD spectral scans of DENV2 (A), DENV3 (B), DENV4 (C) and ZIKV (D) sRecE from 210-250nm with the mean residue ellipticity [θ] plotted on the y-axis. Folded DENV2-4 & ZIKV sRecE was observed with a lowered mean residue ellipticity at 213nm, representative anti-parallel β-sheet absorption (see methods for data collection details). Data reported in panels A-D was collected at 25μM for DENV2 & DENV4 sRecE, 20μM for ZIKV sRecE and 3.125μM for DENV3 sRecE.

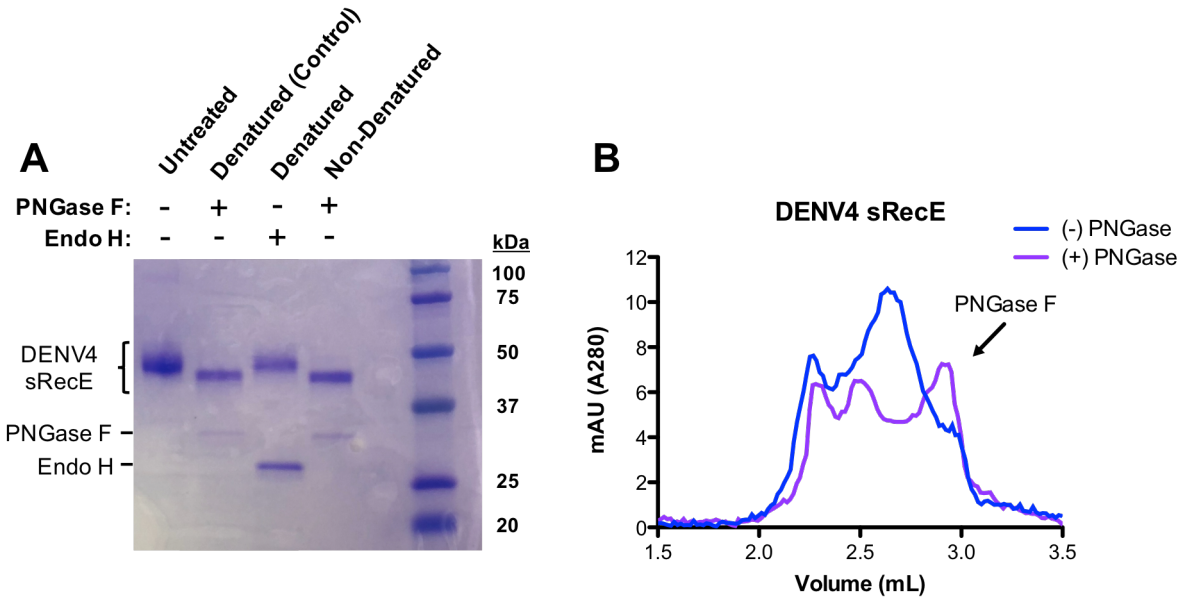


Figure S4. ***Analysis of PNGase F and Endo H Treated DENV4 sRecE***
A) SDS-PAGE analysis of PNGase F and Endo H treated DENV4 sRecE. 10µg of denatured DENV4 sRecE was treated with PNGase F or Endo H, from NEB, at 37˚C for 1hr and 40µg of non-denatured DENV4 sRecE with PNGase F for 20hrs at 37˚C. 1µg of DENV4 sRecE from each reaction was reduced with β-mercaptoethanol and boiled for 5min at 95˚C, then loaded onto a Bio-Rad Mini-PROTEAN Any kD TGX Stain-Free gel and stained using commassie brilliant blue stain for SDS-PAGE analysis. Deglycosylation of non-denatured DENV4 sRecE was observed via increased protein mobility in PNGase F treated DENV4 sRecE as compared to untreated DENV4 sRecE (A). Previously, Endo H enzymatic digests have been used to assess the degree of DENV1-4 and DENV2 sRecE glycan processing (Hacker et. al. Journal of General Virology (2009), 90, 2097–2106). DENV4 sRecE mobility was not affected post Endo H treatment, indicating DENV4 sRecE contains mature, processed N-linked glycans. (A). B) SEC analysis of non-denatured, PNGase F treated DENV4 sRecE. 20µg of untreated or PNGase F treated, non-denatured DENV4 sRecE was loaded and ran on a Superdex 200 Increase 3.2/300 g/L column at 18˚C using 1x PBS pH 7.4 at a flow rate of 0.1 mL/min. DENV4 sRecE treated with or without PNGase F eluted as two peaks, similar to the elution profile observed in the DENV4 sRecE SEC-MALS experiments (Figure 1B). PNGaseF and Endo H reactions were performed following the PNGase F and Endo H NEB kit protocols.

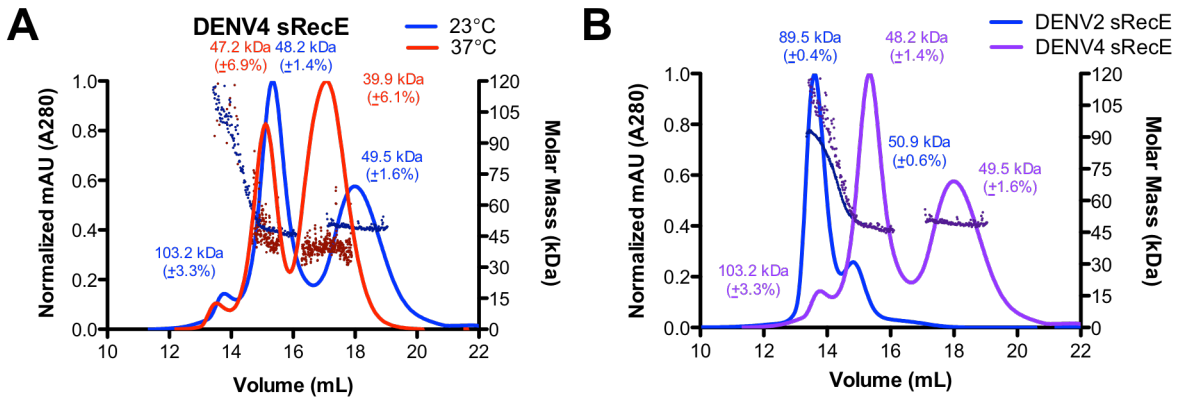


Figure S5. ***SEC-MALS Analysis of DENV2 & DENV4 sRecE***
A) DENV4 sRecE SEC-MALS experiments at 23˚C and 37˚C at 55µM plotted with absorbance at 280nm on the left y-axis and calculated molar mass plotted right y-axis. MALS calculated molar mass indicates that both major peaks at 23˚C and 37˚C correspond to a molar mass consistent with the monomer (44.5kDa), while the minor peak at 23˚C has a molar mass consistent with a dimer. B) Overlay of DENV2 and DENV4 sRecE SEC UV trace with calculated molar mass measured at 23˚C. Despite DENV4 sRecE being predominantly monomeric, a gradual transition in molar mass between the first and second eluting protein fractions is observed for both DENV2 and DENV4 sRecE, indicating monomer and dimer are both present and in exchange during the experiment.

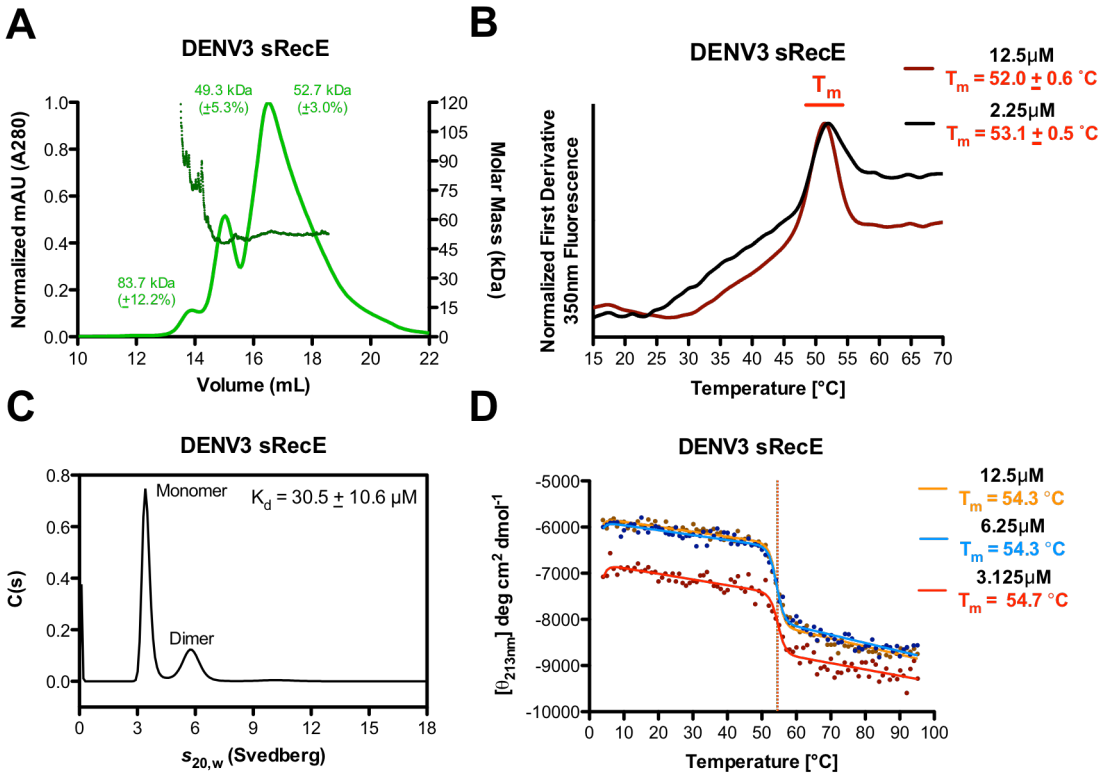
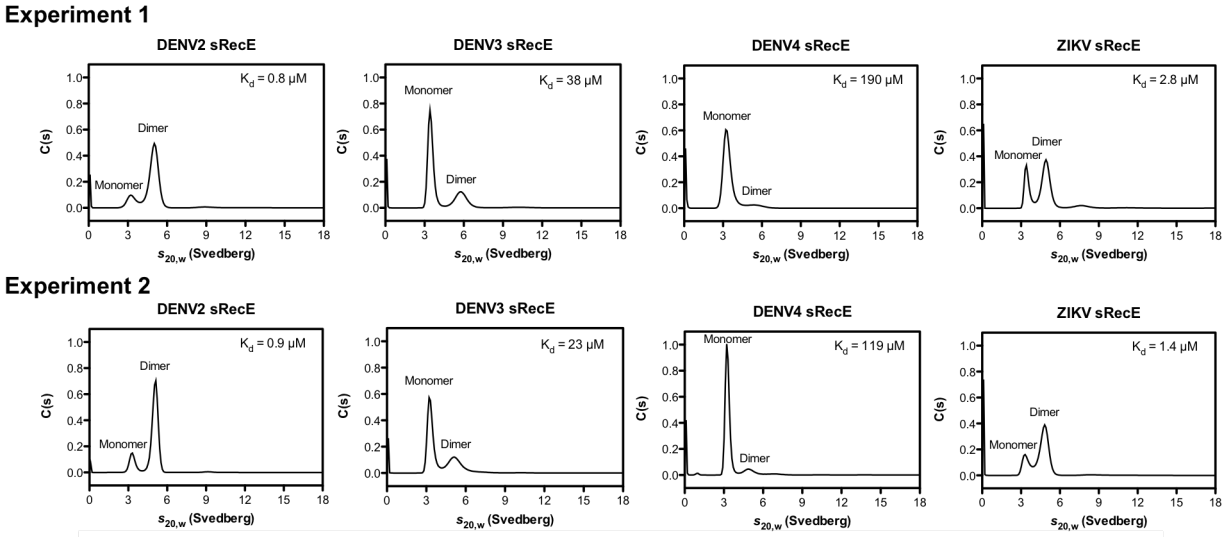


Figure S6. ***Characterization of DENV3 sRecE Oligomerization and Thermostability***
A) SEC UV traces of DENV3 sRecE at 58μM at room temperature (23˚C) with MALS calculated molecular mass plotted on the right y-axis. Similar to DENV4 sRecE (Figure 1B), the DENV3 sRecE elution profile contains 3 peaks, with a minor peak MW calculated to be 83.7 kDa, representing a small dimer fraction, followed by two consecutive major peaks with observed MWs at 49.3 kDa and 52.7 kDa, which are near the expected MW of the monomer, 43.1 kDa. As observed with DENV2 and DENV4, a gradual transition in the MALS calculated molar mass between the first two eluting peaks indicates the monomer and dimer fractions are in exchange. In contrast to DENV4 sRecE, the monomer protein, represented by the last elution peak, is the predominant species. The Superdex 10/300 gL column was ran using 1x PBS pH 7.4 (see methods for exact composition). B) nanoDSF thermal melts measured at 350nm from 15-70˚C, reveal a similar transition at both 12.5μM and 2.25μM with a calculated T_m of ~52-53˚C. T_m values reported at mean ± standard deviation of two independent experiments. C) Sedimentation velocity AUC experiment at 9μM indicate DENV3 sRecE in solution is predominantly monomer with an extrapolated K_d of 30.5 ± 10.6μM. See Figure S7 for details. D) Far-UV CD thermal melt from 15-95˚C of DENV3 sRecE at 3.125μM-12.5μM monitored at 213nm. All three concentrations produced a T_m of 54.3-54.7˚C, consistent the nanoDSF measured T_m of ~52-53˚C.



Protein	DENV2 sRecE	DENV3 sRecE	DENV4 sRecE	ZIKV sRecE
K_d (µM)	0.9 ± 0.1	30.5 ± 10.6	154.5 ± 50.2	2.1 ± 1

Figure S7. ***Measuring sRecE Homodimer Affinity via AUC Sedimentation Velocity***
Replicates of sedimentation velocity AUC experiments for DENV2-4 and ZIKV sRecE (Experiment 1 & 2) at 21˚C at 9 µM. Extrapolated K_d values for each replicate experiment are reported in the graph insets for each sRecE. The table shows the mean ± standard deviation sRecE K_d from Experiment 1 and 2 which are reported in the body of the text in Figure 2 and for DENV3 sRecE in Figure S6C.

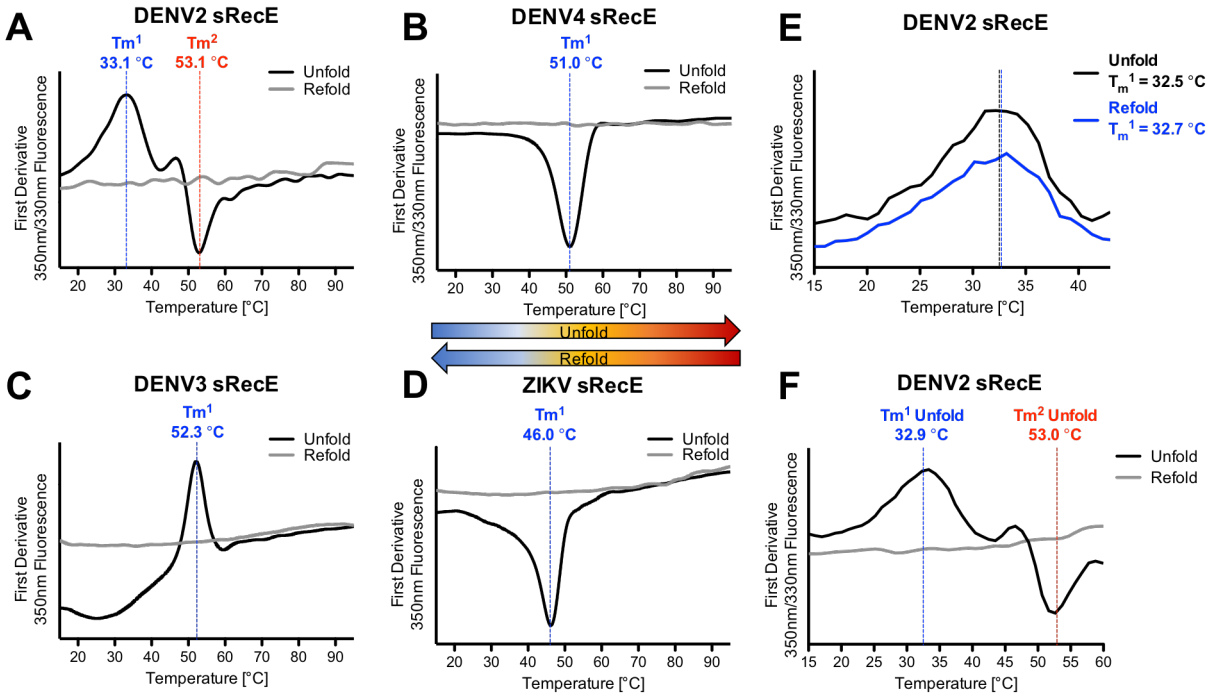
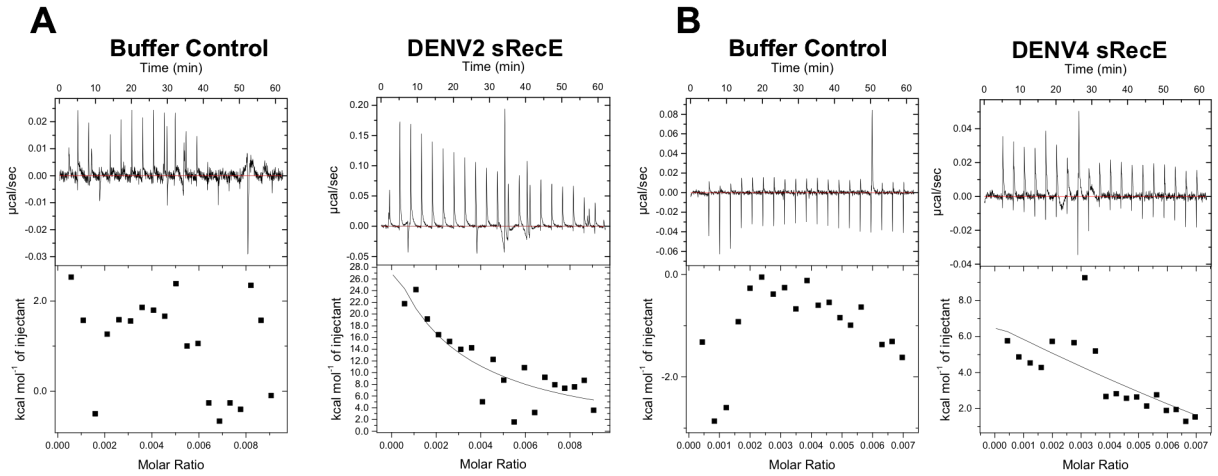


Figure S8. ***nanoDSF 15-95deg (Unfold/Refold) of DENV2-4 & ZIKV sRecE***
The ability of sRecE to refold were assessed by monitoring the fluorescence ratio 350/330nm or 350nm upon heating from 15 to 95˚C and cooling down to 15˚C (A-D). The first derivative plot of ratio 350nm/330nm for DENV2 sRecE (A) produced two midpoint transitions at T_m^1 = 33.1˚C and T_m^2 = 53.1˚C. Only one transition was observed for thermal denaturation measured DENV4 (B) DENV3 (C), DENV4 (B) and ZIKV (D) sRecE with measured T_m values of 51.0˚C, 52.3˚C and 46.0˚C, respectively. Irreversible unfolding was observed for all sRecE heated to 95˚C (A-D). The DENV2 sRecE T_m^1 transition was reversible, as similar T_m values were observed when the protein was heated to 43˚C and then cooled down to 15˚C (E). However, heating of DENV2 sRecE beyond T_m^2 to 60˚C caused irreversible unfolding, indicative DENV2 sRecE monomer unfolding (F). Data reported in panels A-F) was collected at 2.25µM for DENV2, DENV4 & ZIKV sRecE and 12.5µM for DENV3 sRecE.



	Method	Dissociation Constant (K_d)	Enthalpy ($\Delta H°_{bind}$)	Entropy ($T\Delta S°_{bind}$)	Gibbs Free Energy ($\Delta G°_{bind}$)
Experiment 1 (See Figure 5)	DENV2 sRecE ITC (37°C)	13.0 ± 8.0 µM	-72.3 ± 7.5 kcal/mol	-65.4 ± 7.5 kcal/mol	-6.9 ± 0.4 kcal/mol
Experiment 2 (Figure S9A)	DENV2 sRecE ITC (37°C)	18.4 ± 18.0 µM	-85.2 ± 21.1 kcal/mol	-78.4 ± 21.1 kcal/mol	-6.7 ± 0.6 kcal/mol
Experiment 1 (Figure S9B)	DENV4 sRecE ITC (23°C)	No dissociation observed	No dissociation observed	No dissociation observed	No dissociation observed

Figure S9. ***Dissociation ITC of DENV2 sRecE & DENV4 sRecE***
Replicate ITC isotherm of DENV2 sRecE (A) dissociation at 37˚C at 54 µM, and ITC isotherm of DENV4 sRecE (B) dissociation at 23˚C at 41.5 µM with buffer titration controls. Table shows DENV2 sRecE K_d and ΔH° values obtained from the homodimer dissociation model fit to the integrated isotherm peaks, for each independent experiment corresponding to Experiment 1 (Figure 5) and Experiment 2 (A), with values reported as mean $\pm$ std. error of the fit. ΔS° and ΔG° values are not obtained from the homodimer dissociation model and were calculated using the K_d and ΔH° values from the fitted model and Equations [3] and [4] (see Methods). ΔS° and ΔG° values reported as mean $\pm$ propagated std. error. The DENV2 sRecE dissociation ITC mean $\pm$ std. deviation K_d, ΔH°, ΔS° and ΔG° values reported in Figure 5 and in the body of the text were obtained from DENV2 sRecE Experiment 1 and 2.

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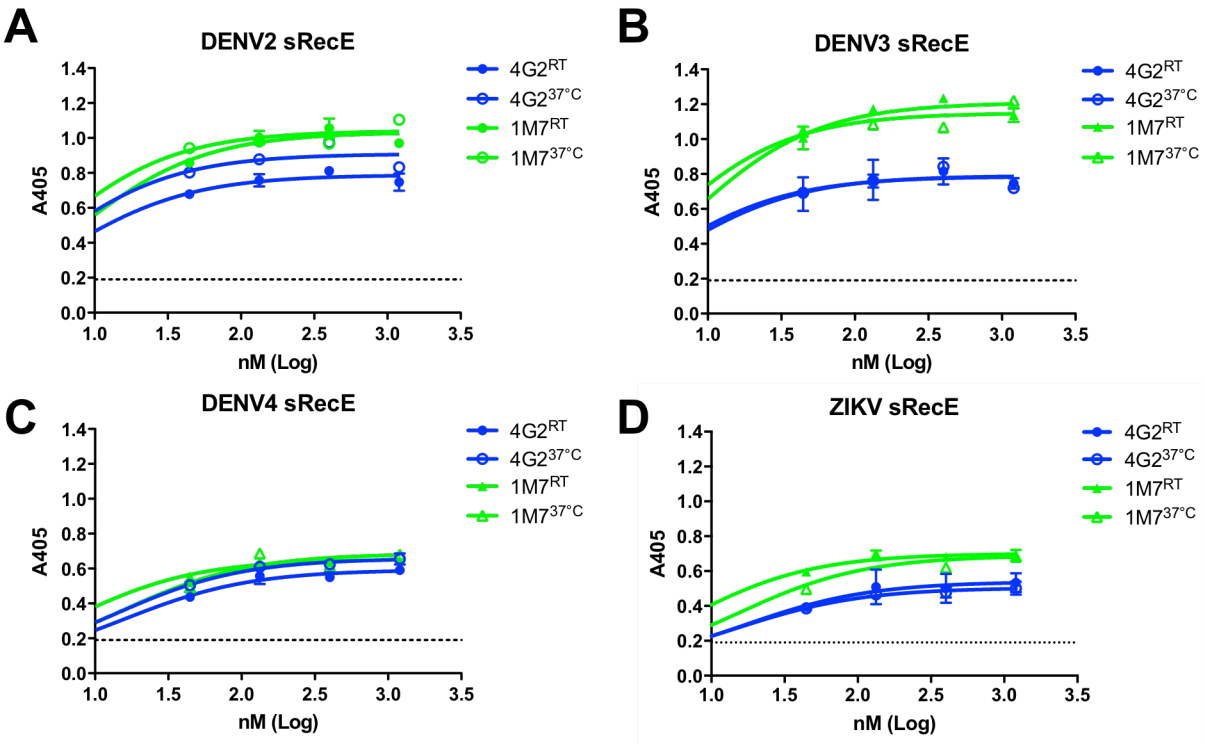

Figure S10. ***Monomer Fusion Loop Epitope (FLE) Ab Binding to sRecE at Room Temperature (23˚C) and Physiological Temperature (37˚C)***

The effect of temperature on FLE Ab binding to DENV2 (A), DENV3 (B), DENV4 (C) & ZIKV (D) sRecE. sRecE were incubated in solution with either a conformational (4G2) or linear (1M7) FLE Ab at 23˚C or 37˚C for 1hr. The sRecE-bound Ab complex was immobilized via the sRecE C-terminal 6x His-tag and Ab was labeled using an anti-Mouse-AP conjugated secondary Ab. Measurement of AP substrate turnover at A405 was plotted on the y-axis with sRecE concentration on the x-axis. Similar 4G2 and 1M7 FLE binding to DENV2-4 & ZIKV sRecE (A-D) was observed at both 23˚C and 37˚C. Values reported as mean $\pm$ standard deviation.