In vitro neutralisation of Zika virus by an engineered protein targeting the viral envelope fusion loop

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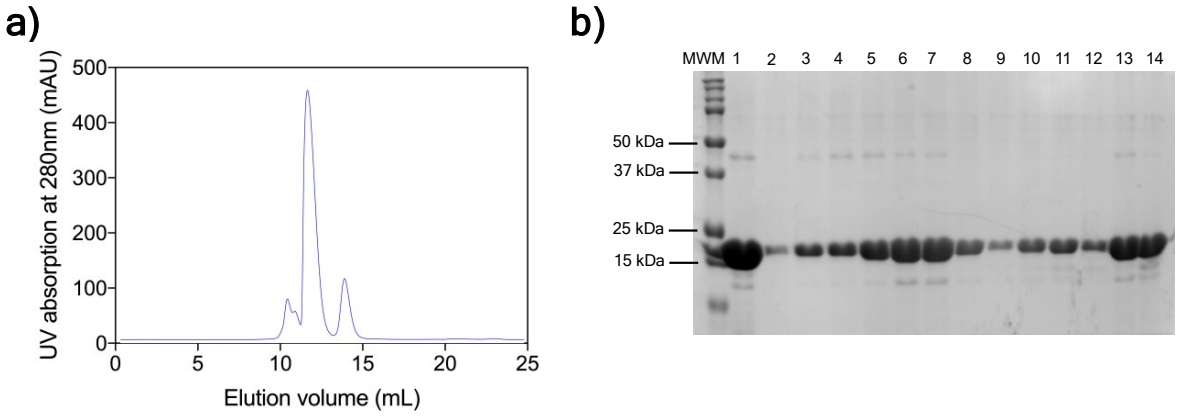
SUPPLEMENTARY INFORMATION

ADDITIONAL METHODS

Thermal unfolding of the ZIKV Env and ZVPA3 protein complex by circular dichroism

The ZIKV Envelope and ZVPA3 proteins were diluted at the 1:3.75 ZVPA3:$E_{(Dimer)}$ ratio in 1X PBS buffer and immediately used for the assay. The ZIKV Envelope protein alone was used as control. Absorption spectra from 200 to 260 nm were acquired varing the temperature from 20 to 100 ºC with a Jasco J-1100 spectropolarimeter coupled to a Peltier device. Spectra were acquired at 1 ºC intervals using a quartz cuvette of 0.1 cm path length and three accumulations. Ellipticity at 222 nm was normalized, plotted against temperature, and fit by regression analysis using Graphpad Prism v.7. to determine the melting temperature (T_m) of the complex and the Env protein.



Related to Method section: Production of ZVPA3

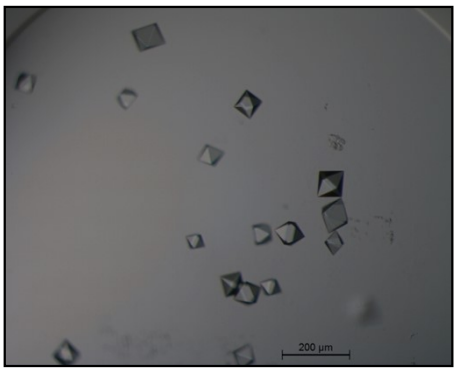
Supporting Figure S1. ZVPA3 production. (A) SEC chromatogram of ZVPA3 after affinity chromatography purification. UV absorbance at 280 nm (mAU) was monitored over time, and protein fractions were collected. (B) 15% SDS-PAGE of SEC eluted fractions showing a predominant band in all lanes around 20 kDa. The molecular weight marker (MWM) used was the PageRuler^{TM} Plus Prestained Protein Ladder (ThermoFisher Scientific).

a)

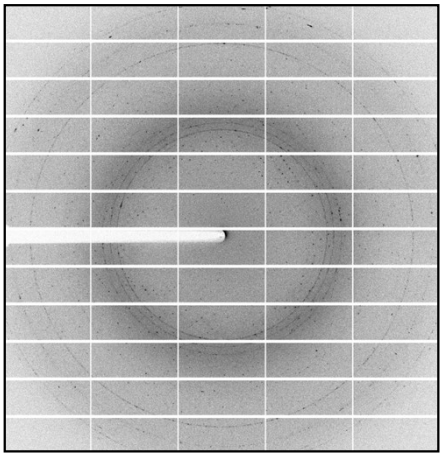
PDB entry		7ZFM
Data Collection	Isotropic	Anisotropic
Spacegroup	$P\ 4_32_12$	$P\ 4_32_12$
Cell Parameters		
a, *b*, *c* (Å)	81.62 81.62 113.28	81.62 81.62 113.28
α, β, γ (°)	90.0 90.0 90.0	90.0 90.0 90.0
Resolution (Å)	66.2-5.0 (1.88-1.85)	66.3-5.0 (1.80-1.71)
R_{merge} (%)	4.4 (78.2)	4.5 (109.5)
R_{meas} (%)	4.6 (83.5)	4.7 (118.0)
R_{pim} (%)	1.2 (28.5)	1.3 (41.0)
$CC_{(1/2)}$ (%)	100 (80.5)	100 (58.0)
Mean(I)/σ(I)	31.3 (2.2)	28.4 (1.5)
Wilson B-factor	32.8	32.8
Completeness (spherical)	100 (100)	88.6 (31.0)
Completeness (ellipsoidal)	-	95.2 (54.9)
Number of Unique Reflections	33666	37153
Multiplicity	13.5 (8.1)	12.4 (7.6)

Refinement		
Resolution (Å)	-	51.4 - 1.71
Number of Reflections	-	37150
R_{work} (%)	-	17.6
R_{free}(%)	-	21.1
No. atoms	-	3048
Protein	-	2618
Ligand/ion	-	135
Water	-	295
R.m.s. deviations		
Bond lengths (Å)	-	0.01
Bond angles (°)	-	1.75
Ramachandran plot(%)		
Favored	-	96.3
Outliers	-	0.31
Rotamer outliers (%)	-	1.03
MolProbity score	-	1.29
Clashscore	-	2.02
Mean B-factor (Å^2)	-	40.28
Macromolecules	-	37.08
Ligands	-	80.63
Solvent	-	50.15

b)

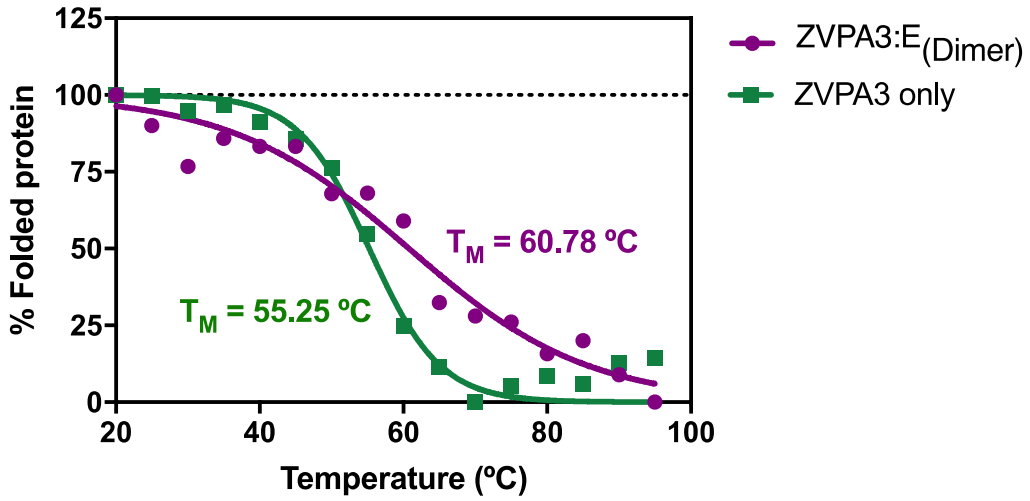


c)



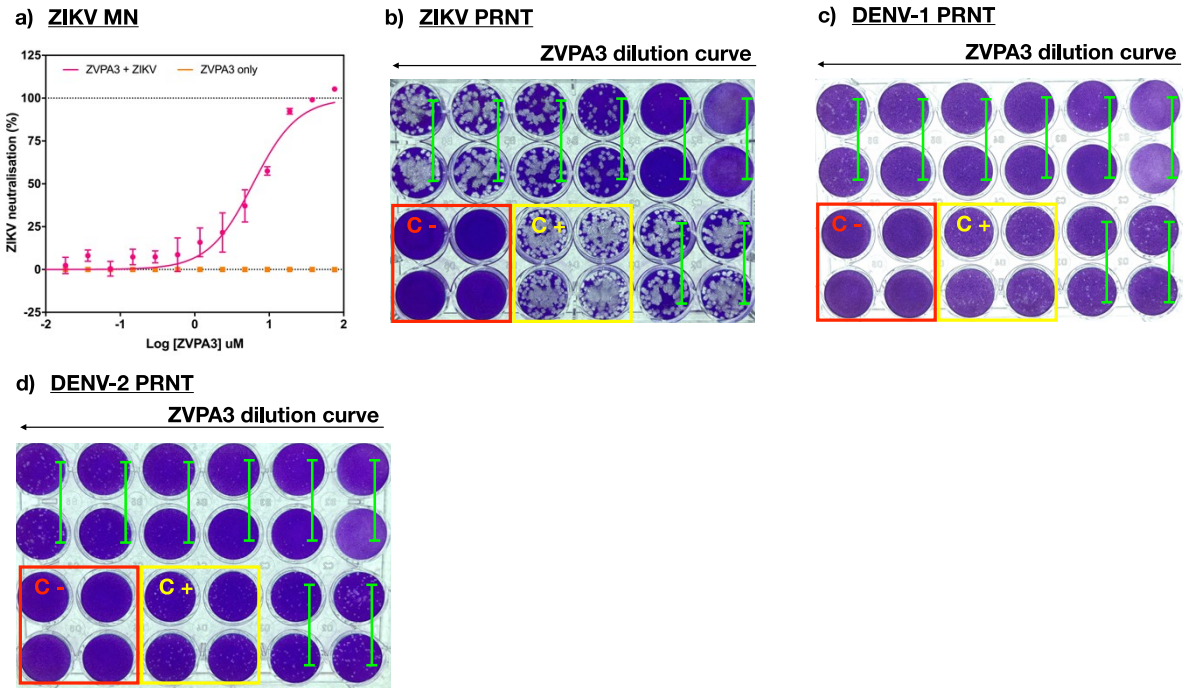
Related to Method section: Crystallization, data collection and structure determination

Supporting Figure S2. (A) Data Collection and refinement statistics of the ZVPA3 protein. Statistics for the highest-resolution shell are shown in parentheses. 5% of reflections were used to calculate R_{free}. (B) ZVPA3 crystals and (C) X-ray diffraction pattern.



Related to the Additional Methods section: Thermal unfolding of the ZIKV Env and ZVPA3 protein complex by circular dichroism.

Supporting Figure S3. Thermal stability of the ZIKV Env and ZVPA3 protein complex. The melting profile of the ZIKV Env – ZVPA3 complex was determined through CD experiments (in purple) and compared to that obtained for the ZVPA3 protein alone (in green). The respective melting temperatures are shown in the figure.



Related to Method section: Flavivirus-neutralising activity determination through plaque-reduction neutralization test (PRNT) and microneutralization (MN) test

Supporting Figure S4. Assessment of flavivirus neutralization *in vitro*. (A) Assessment of ZIKV (pink curve) neutralisation activity by microneutralization test. The assay was performed in triplicates and the data is shown as the median plus standard deviation. The ZVPA3 protein was used as a control (orange curve) and shows no unspecific binding with the antibodies used to develop the assay. Dotted lines at 0 and 100% are shown for clarity. PRNT plates showing neutralization against ZIKV (B), DENV-1 (C) and DENV-2 (D) are also shown. Highlighted in red and yellow are the negative and positive control wells, respectively. The dilution curve of ZVPA3 is represented in green in each plate.