Table S1. (A) Exchange rates and Protection Factors (PF) measured for amide protons involved in the main chain hydrogen bonds stabilizing the ß-sheets found in DEN4-ED3 (column 1). The numbers given in column 2 and 3 stand for the residue numbers of residues bearing the HN amide groups (donor) and the C=O carbonyl group (acceptor) involved in the hydrogen bond, respectively.

ß-sheet	Donor (HN)	Acceptor (C=0)	k_{ex} (min^{-1})	+/-	PF	+/-	log(PF)	+/-
	19	37	0.00024015	0.000025111	281000	26600	5.4487	0.094662
	22	44						
	25	33						
ß1 - ß2	**27**	31						
	33	25	0.021539	0.0020828	369	32.5	2.567	0.088076
	37	19	0.060076	0.013766	276	51.5	2.4409	0.18659
	44	22						
	32	81						
	34	79	0.000014149	0.000036761	256000	185000	5.4082	0.72266
	36	77	0.0002122	0.000025128	31100	3290	4.4928	0.10579
ß2 - ß7	**77**	36	0.040561	0.0057809	382	47.6	2.5821	0.12461
	79	34	0.0078069	0.00036378	1250	55.7	3.0969	0.04456
	81	32	0.00016631	0.000049409	28100	6440	4.4487	0.22918
	62	82						
	64	80						
ß7 - ß5	**65**	80						
	82	62						
	45	70	0.030191	0.0034243	1700	170	3.2304	0.1
ß3 - ß6	**47**	68	0.0096568	0.00071391	684	47.1	2.8351	0.068859
	70	45						
	50	91						
	52	89	0.0018881	0.000135	9410	628	3.9736	0.066738
ß4 - ß8	**89**	52	0.00024186	0.000036277	41300	5390	4.616	0.13051
	91	50	0.000016164	0.00017437	182000	167000	5.2601	0.91758
	93	48	0.0005641	0.00008017	48800	6070	4.6884	0.12439
	88	103	0.00086799	0.000051457	44800	2510	4.6513	0.056027
	90	101						
ß8 - ß9	**92**	99	0.000010643	0.000082627	318000	282000	5.5024	0.88679
	99	92						
	101	90	0.0016711	0.000080169	6260	287	3.7966	0.045847
	103	88	0.015803	0.001807	4180	429	3.6212	0.10263

Table 2. Sequential and structural similarity among ED3's of four dengue serotypes. [1].

Percent identity/ Cα RMSD (Å)	DEN1-ED3	DEN2-ED3	DEN3-ED3	DEN4-ED3
DEN1-ED3 (PDB: 3IRC)		0.87 Å	0.57 Å	1.01 Å
DEN2-ED3 (PDB: 3UZV)	79%		0.74 Å	0.76 Å
DEN3-ED3 (PDB: 3VTT)	88%	78 %		1.07 Å
DEN4-ED3 (PDB: 3WE1)	71%	77 %	70 %	

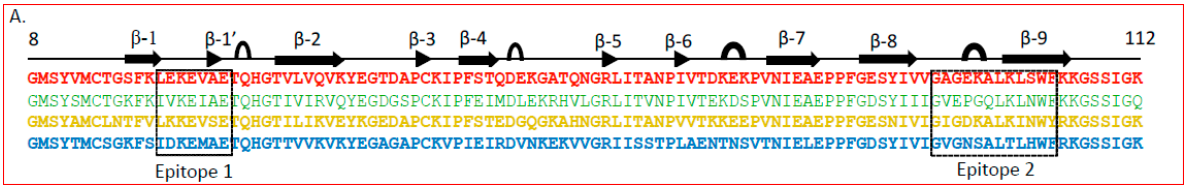


Figure S1. (A) Sequence alignment of four serotypes of DEN ED3. (DEN1-ED3: red, DEN2-ED3: green DEN3-ED3: yellow, DEN4-ED3: blue). The residues are numbered according to Volk et al, 2007 [2] (BMRB code 7087). Both the sequences and structures were retrieved from the PDB-IDs given in the table. The sequences start at Gly 8 and ends at Lys 112 (corresponding to Gly 294 and Lys 398, respectively, in Elahi et al. [3]). The secondary structures indicated on the top are calculated using 3WE1 and DSSP (http://swift.cmbi.ru.nl/gv/dssp/). The epitope regions are those shown in Lisova et al. [1].

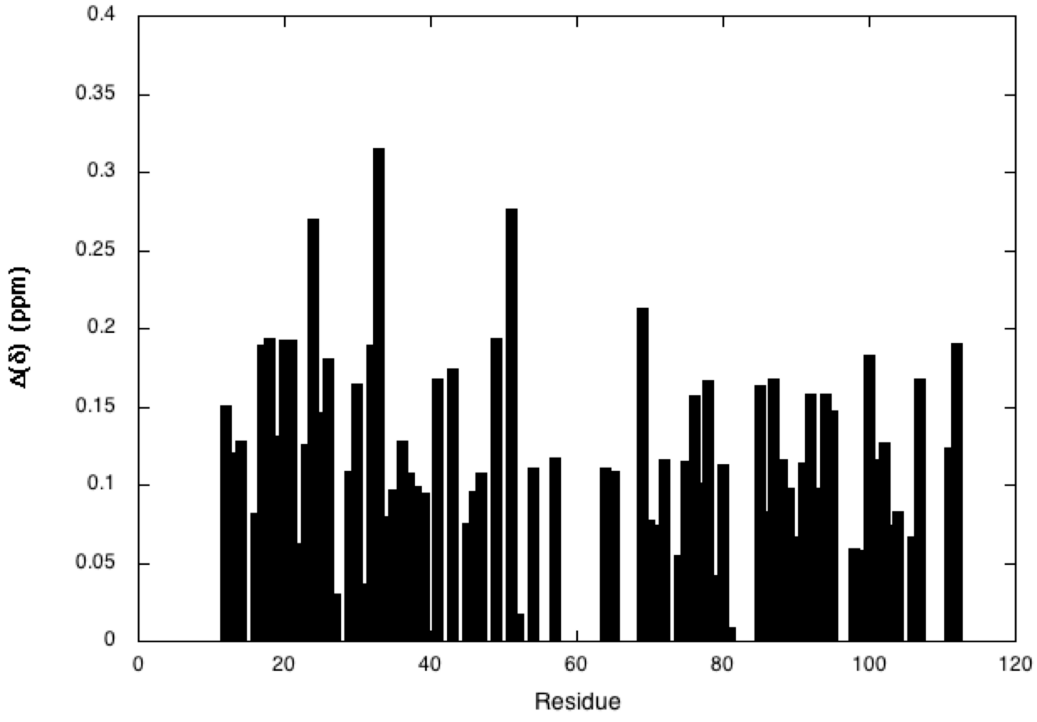


Figure S2. (A) (Right) Average amide chemical-shift variations, calculated as $\Delta\delta = \sqrt{{\Delta\delta_H}^2 + \left(\frac{\Delta\delta_N}{5}\right)^2}$, measured between spectra recorded under the experimental conditions used in the present

denaturation study and under the conditions used for the previously published NMR structural study (25°C, 50mM Tris pH 7.5, 50 mM NaCl) ([1]; BMRB code 7087).

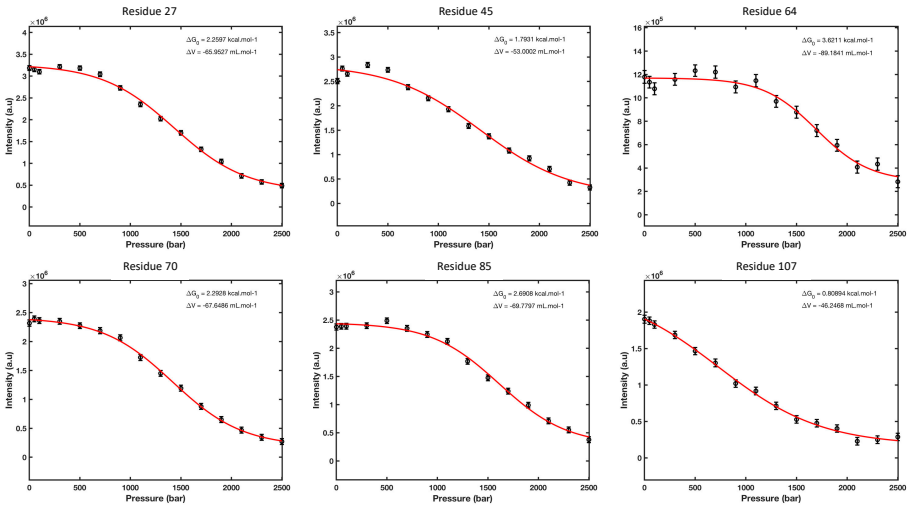


Figure S3. Six representative examples (-90 < ΔV_u^0 < -40 ml/Mol) of fits obtained for the decrease in intensity with pressure of cross-peaks from the corresponding residues (indicated on top of each graph) with Equation 1 (Materials and Methods) implemented on a in-house MATLAB software.

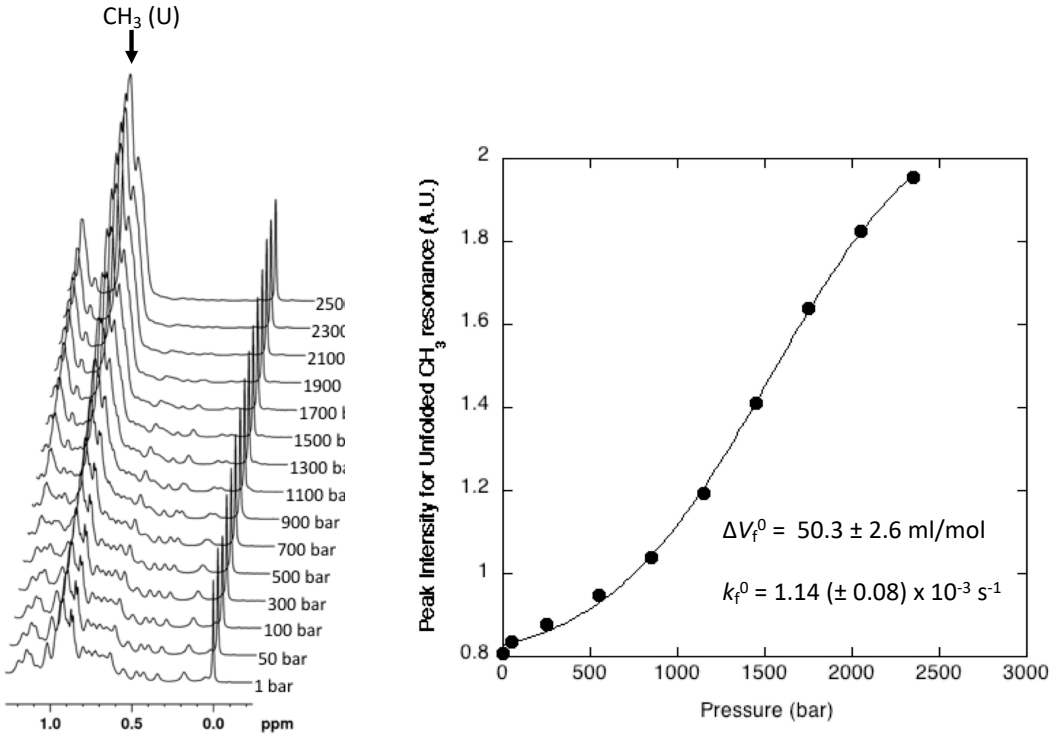


Figure S4. Steady-State experiments followed by High-Pressure 1D Proton NMR. (Left) Stacked plot of the 1D proton NMR spectra recorded on DEN4-ED3 at increasing pressure. The 1D NMR

experiments are recorded once the steady-state is reached, 2 hours after the *P*-Jump. Only the region of the methyl resonances is reported. The arrow indicates the resonance corresponding to unfolded methyl groups (CH_3 (U)), used as probe to monitor the folding/unfolding equilibrium. (Right) Denaturation curves obtained for DEN4-ED3. The curves were fitted with Equation 1 to obtain the values of ΔV_f^0 and ΔG^0 reported in the insert.

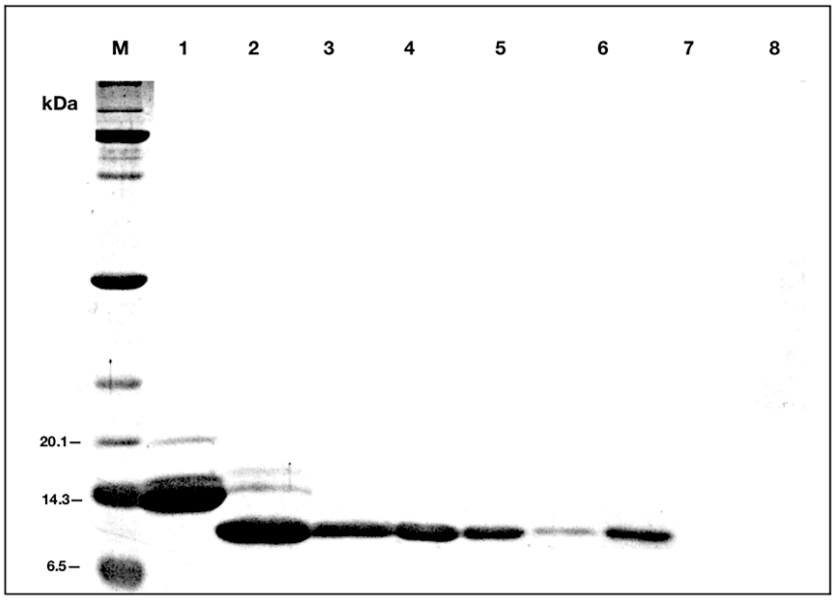
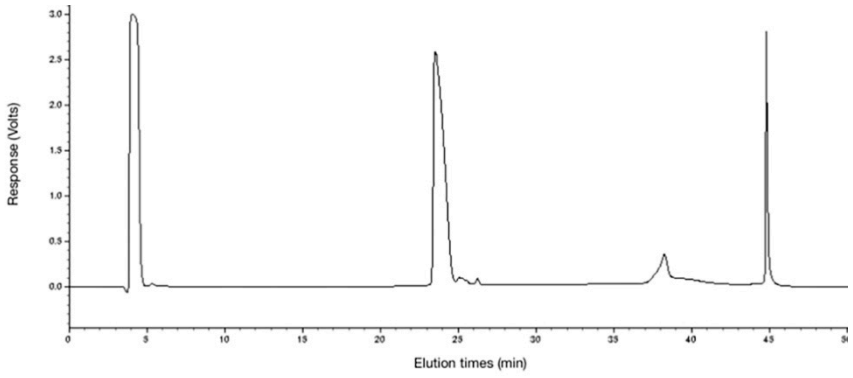


Figure S5. SDS-PAGE analysis of [^{15}N] His-DEN4 ED3 protein before and after thrombin cleavage. **M** represents the molecular weight marker (protein marker, low molecular weight range, FUJIFILM Wako chemicals). Lane-**1**: [^{15}N] His-DEN4 ED3 protein; Lane-**2**: His-tag cleaved [^{15}N] DEN4 ED3 protein; Lanes **3-8** show the His tag purification of ED3 after thrombin cleavage of the His tag. Lanes **3-5** and Lanes **6-8** indicate, respectively, the wash and the elution fractions.

A.



B.

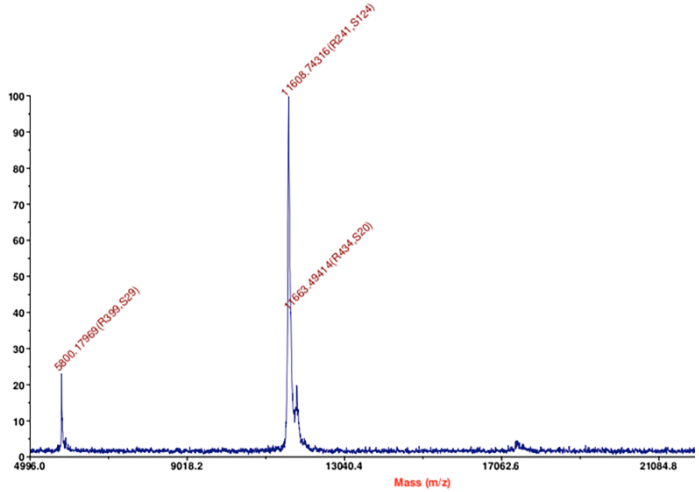


Figure S6. (**A**) Analytical reverse phase HPLC chromatogram of [^{15}N] DEN4 ED3 (**B**) MALDI-TOF spectrum of [^{15}N] DEN4 ED3. The molecular mass was 11608.74 Dalton (Calculated molecular mass=11603.00 Dalton).

References

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