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Supporting information for article:

Identification of the critical linker residues conferring differences in the compactness of DENV-4 NS5 from *Dengue virus* serotypes 1–3

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Supplementary figure legend

Figure S1: Small angle X-ray scattering pattern (\circ) for the DENV-4 linker mutants (A) -S264H, (B) -K271T, (C) -K271T/D273N, (D) -T269P/K271T/D273N, (E) -S264H/T269P/K271T/D273N, (F) -S266N/T267A and (G) $-\Delta S_{266}T_{267}$ mutants in concentration depended manner. (*Inset*) Guinier plots show linearity for all the mutants, indicating no aggregation.

Figure S2: *Ab initio* envelopes superimposed onto the BUNCH models (cartoon representation) and shape of wild type NS5 (*grey*) for the mutants (A) -S264H, (B) -K271T, (C) -K271T/D273N, (D) -T269P/K271T/D273N, (E) -S264H/T269P/K271T/D273N, (F) -S266N/T267A and (G) $-\Delta S_{266}T_{267}$. The RdRp is colored *magenta* and the methyltransferase in *blue*. Regions not resolved in the crystal structures are presented as dots.

Figure S3: Porod-Debye plot (\circ) and fit line ($—$; *red*) for (A) DENV-4 NS5 and its mutants (B) -K271T/D273N, (C) -K271T, (D) -S266N/T267A and (E) $-\Delta S_{266}T_{267}$. Compared to DENV-4 NS5 (Porod-exponent (P) of 3.8), the mutant K271T/D273N has the similar P-value of 3.8, while mutants K271T, S266N/T267A and $\Delta S_{266}T_{267}$ revealed a slightly smaller P-value of 3.7, 3.6 and 3.6, respectively.

Figure S4: Comparison between the R_g distributions (pool, *grey*; ensemble, *line*) shows a non-random flexibility as confirmed by R_{flex} for the DENV-4 linker mutants in the flexibility characterization based on EOM 2.0 analysis.

Figure S5: Comparison of the zoom in profiles of size exclusion chromatography from K271T/D273N (—; *blue*), S266N/T267A (—; *magenta*) and $\Delta S_{266}T_{267}$ (—; *cyan*). The arrows indicate the peak maxima of the proteins. The DENV-4 NS5 S266N/T267A and $\Delta S_{266}T_{267}$ mutants eluted out earlier than the mutant K271T/D273N, indicating an overall dimension difference among the mutants.

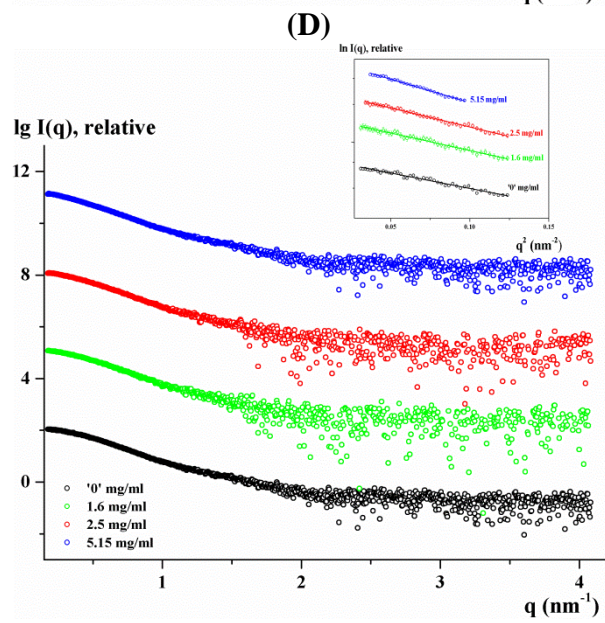
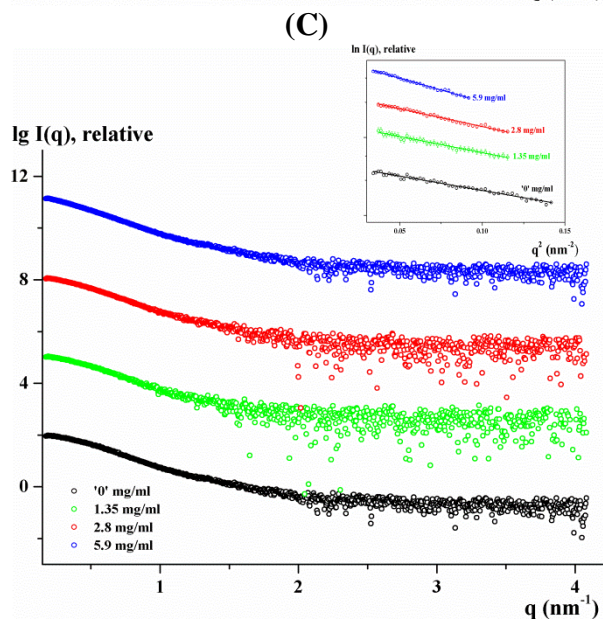
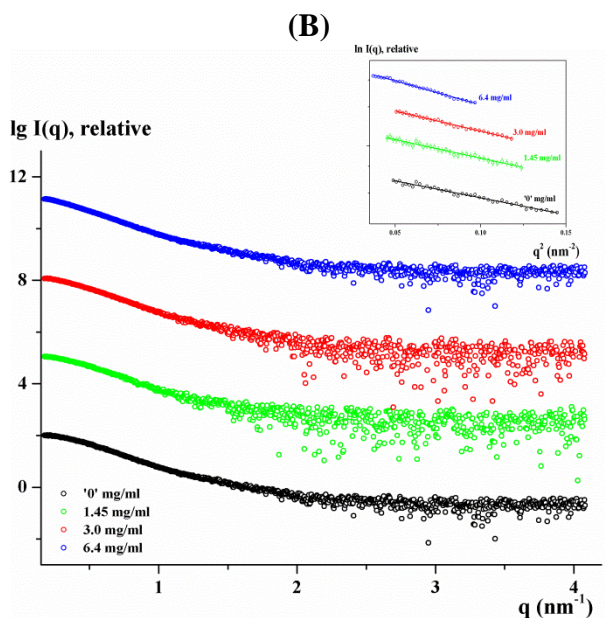
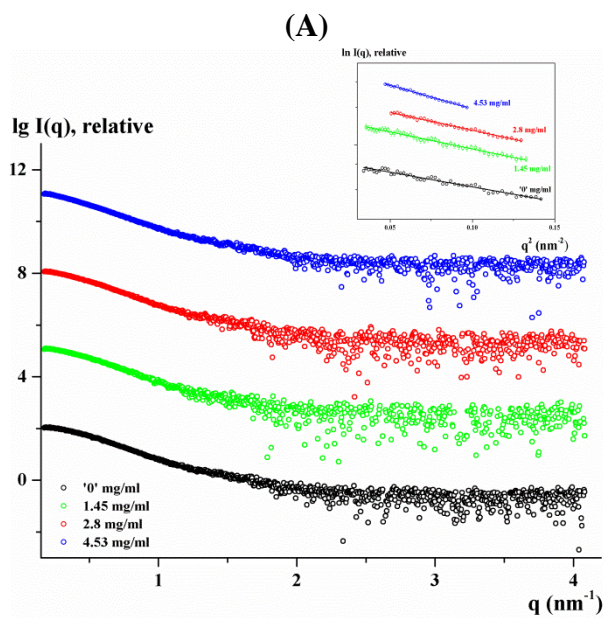


Figure S1 A-D

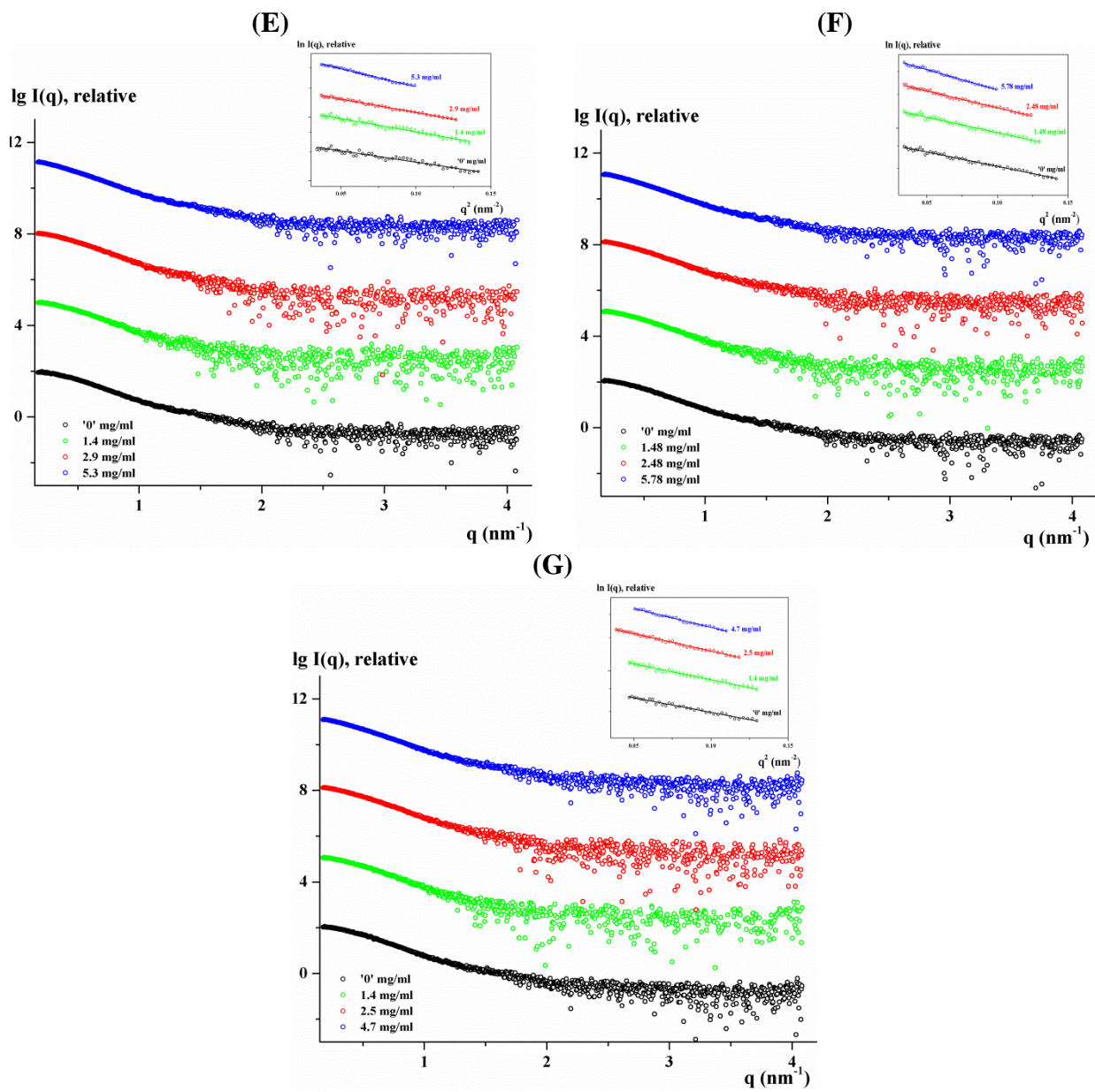


Figure S1 E-G

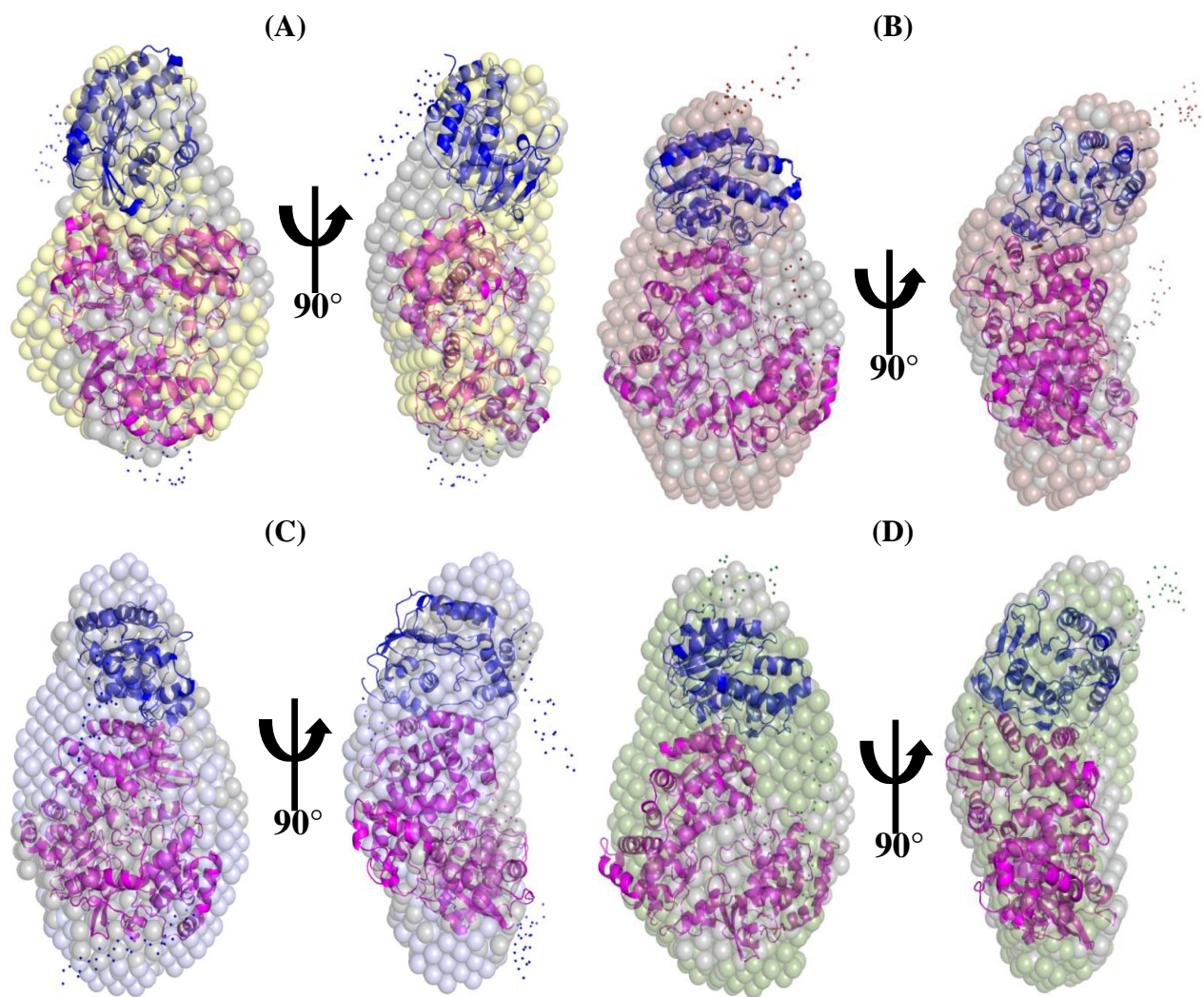


Figure S2 A-D

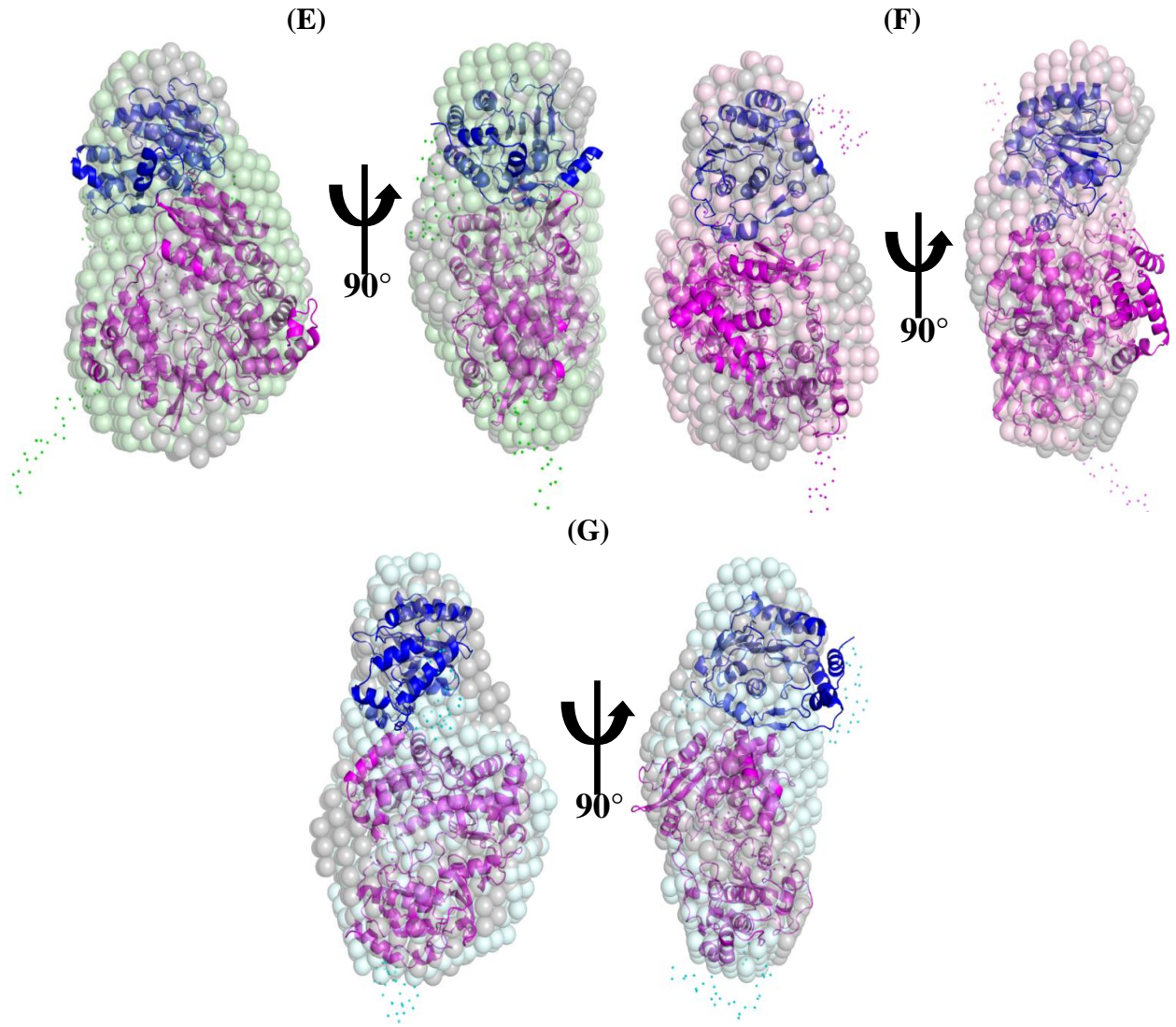


Figure S2 E-G

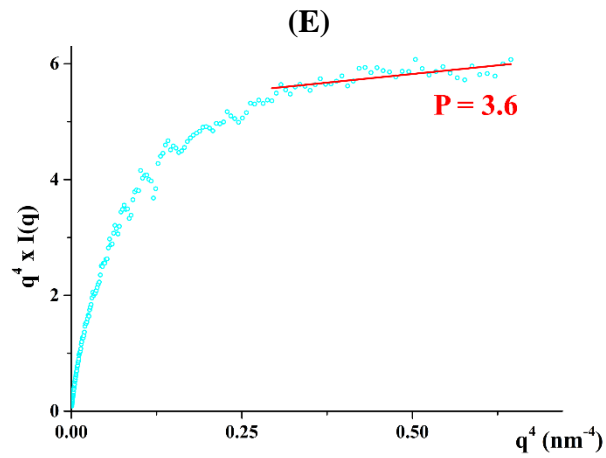
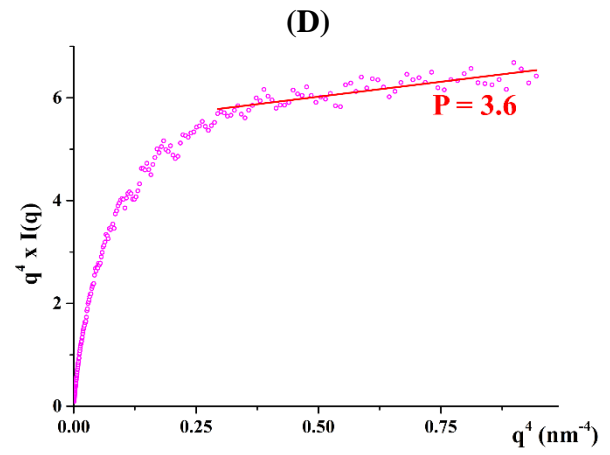
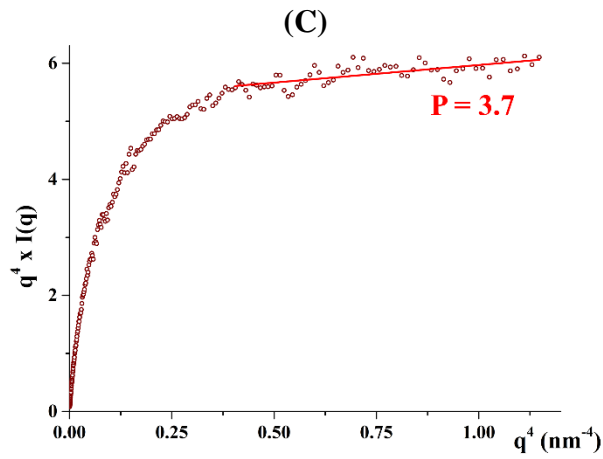
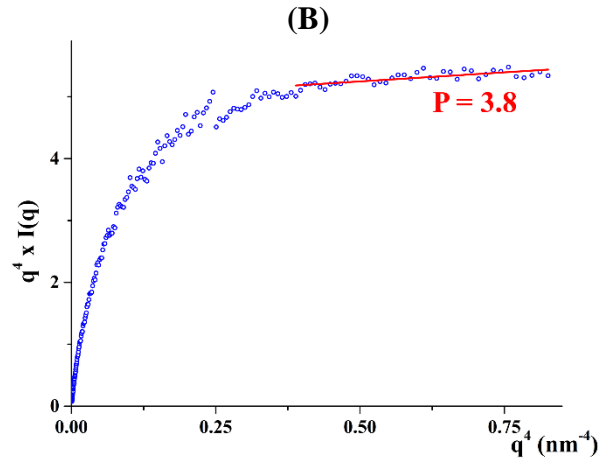
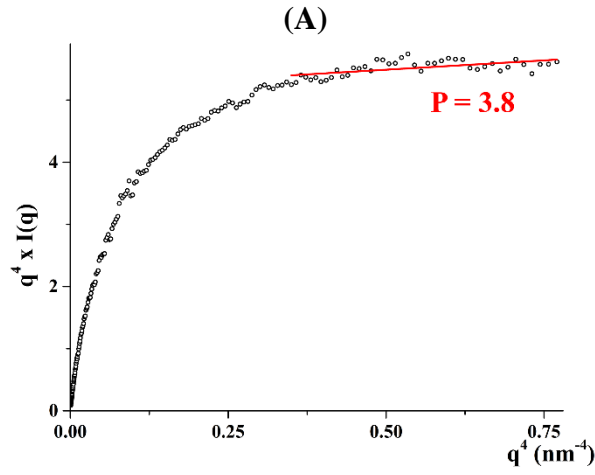


Figure S3

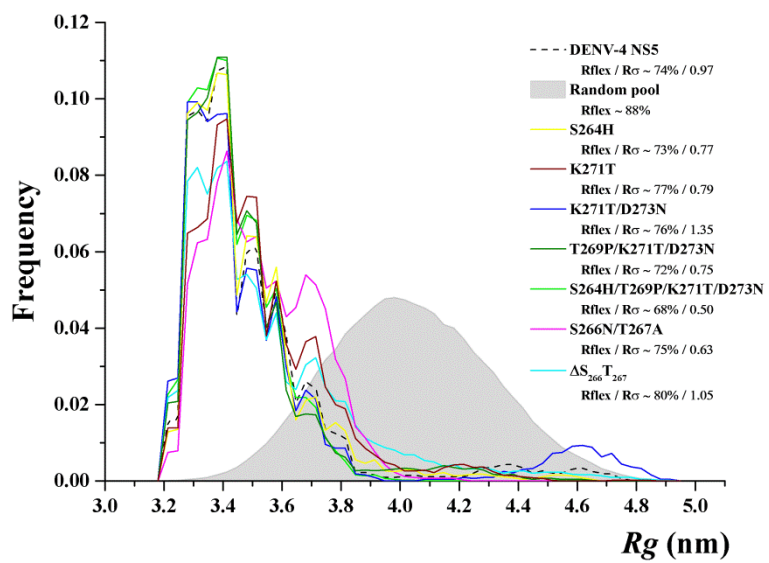


Figure S4

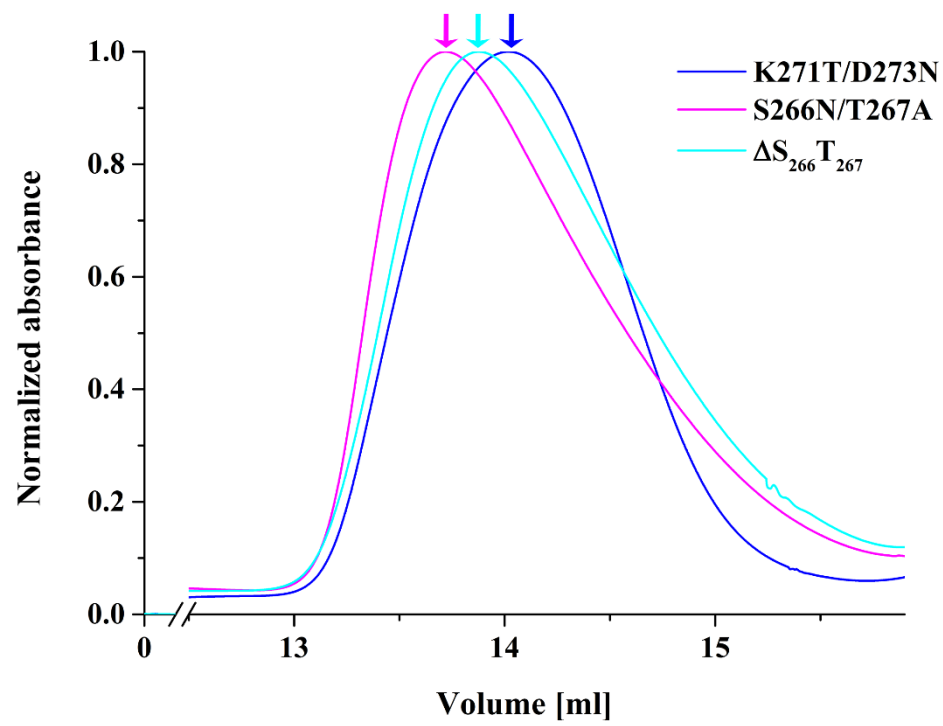


Figure S5