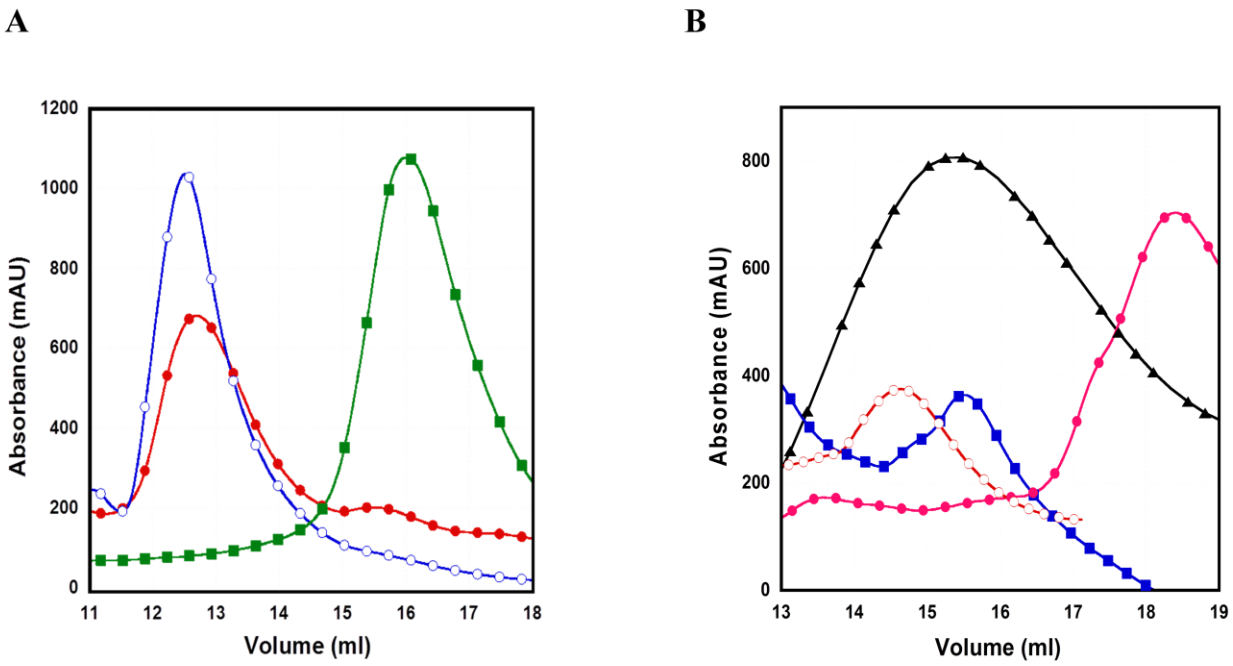
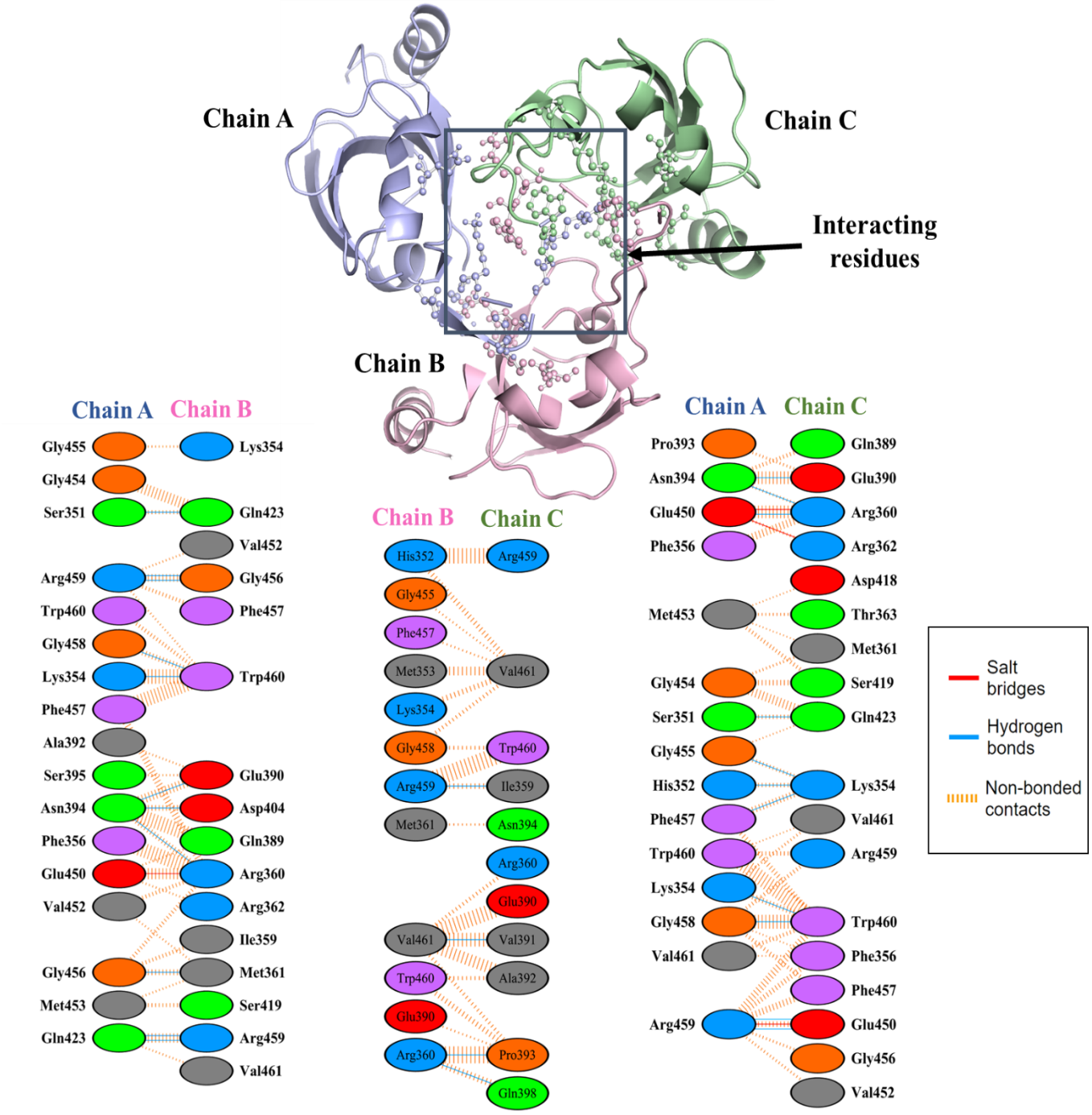
Supplementary information

Supplementary Figure S1:



Supplementary Fig S1. Representative elution profiles of HtrA3 and some of its variants using Superdex 200 gel-filtration column. A) Plot of absorbance at 215 nm as function of elution volume for mature HtrA3 (solid circles), HtrA3 S305 (open circles) and HtrA3 PDZ (solid squares). **B)** Plot of absorbance at 215 nm as function of elution volume for HtrA3 F142D (solid triangles), HtrA3 F142A (open circles), HtrA3 F255D (solid squares) and HtrA3 N-SPD (solid circles).

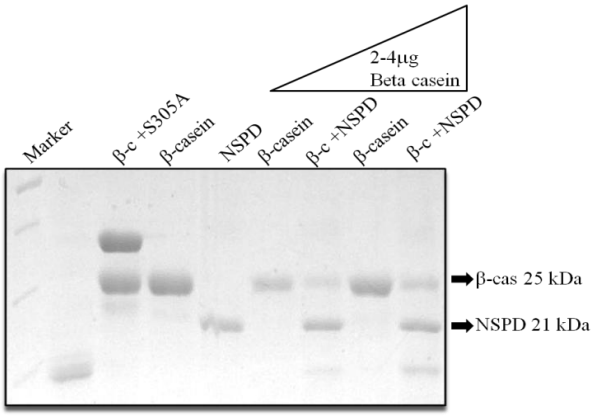
Supplementary Figure S2:



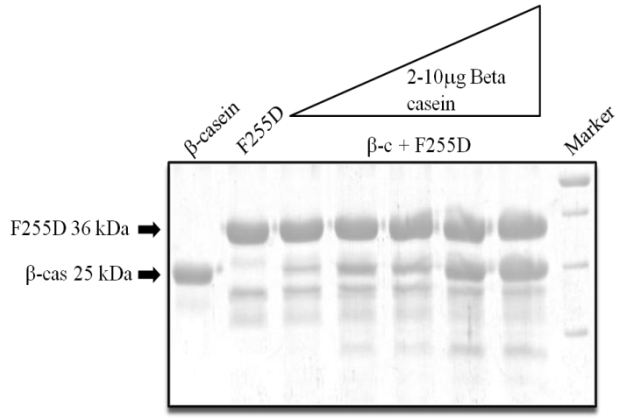
Supplementary Figure S2: Cartoon representation of HtrA3 PDZ trimer assembly along with the interacting residue details generated from PDBsum.

Supplementary Figure S3:

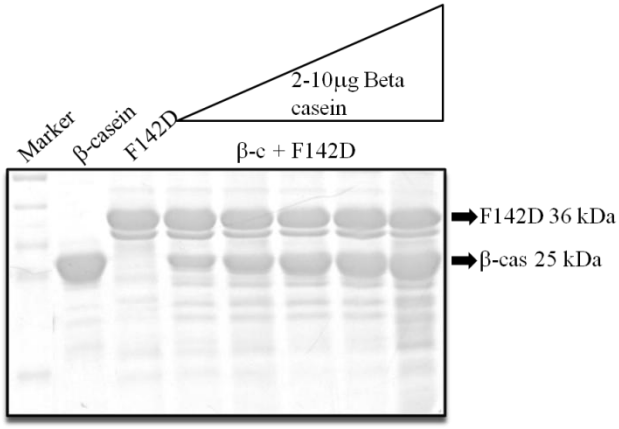
A



B

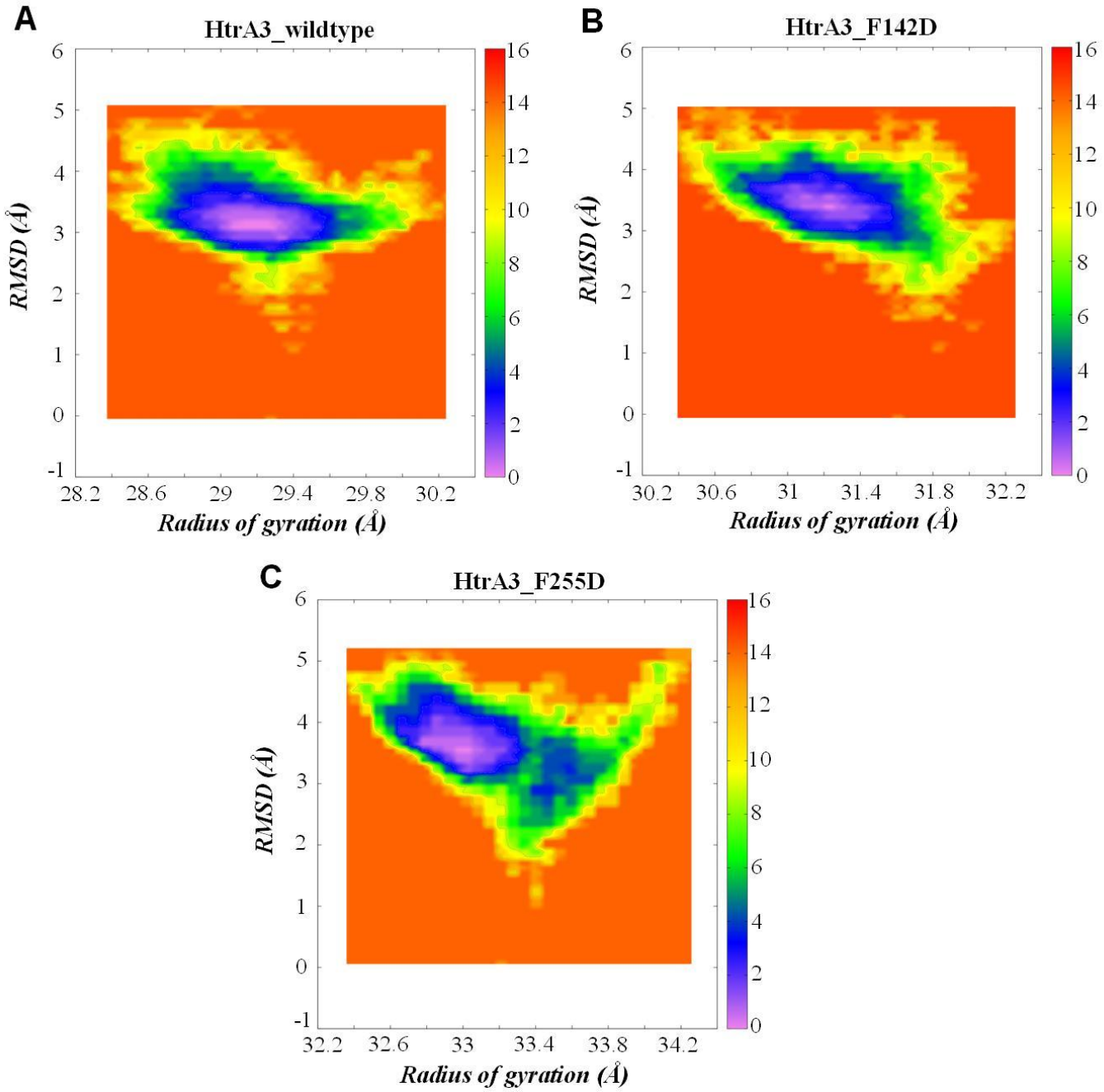


C



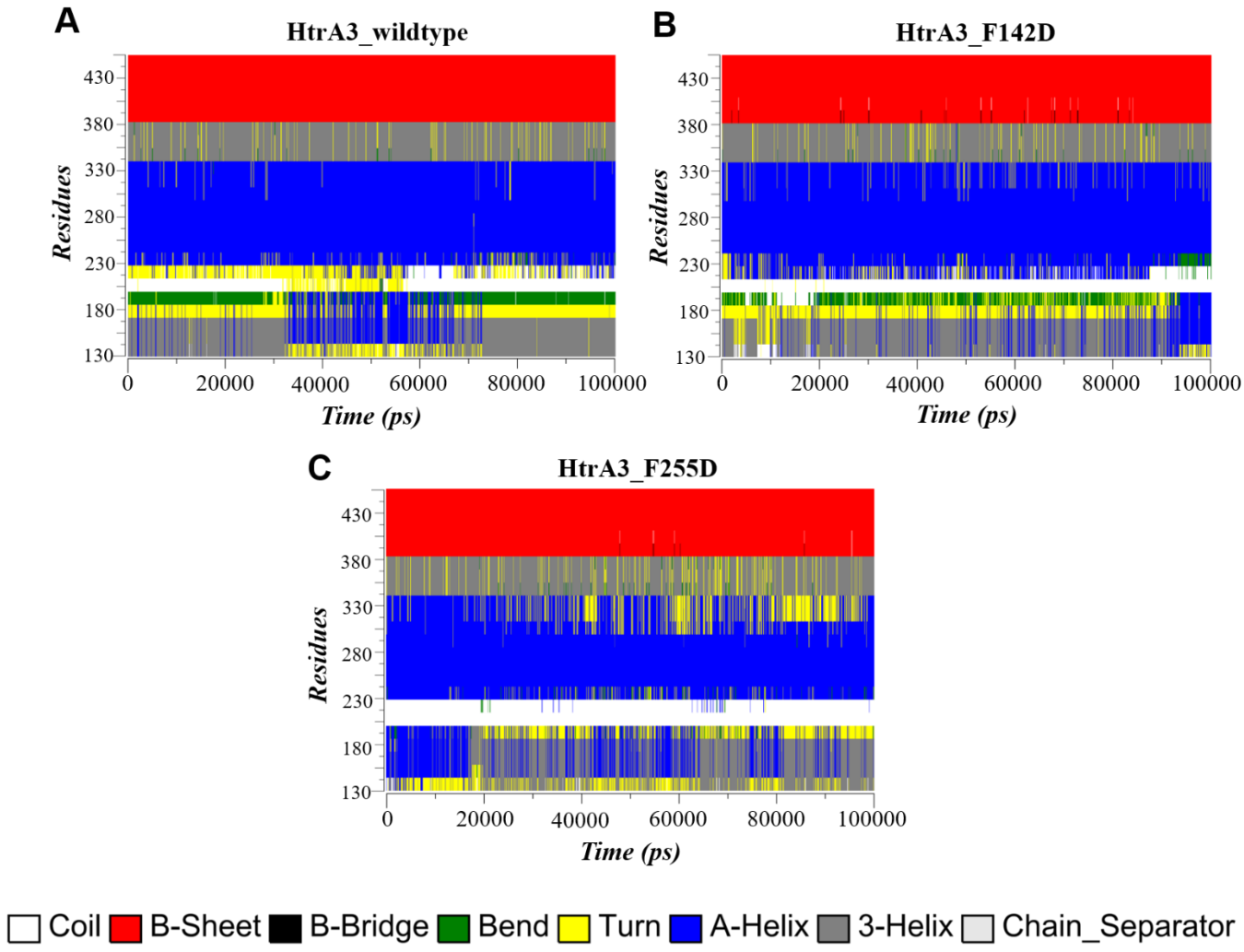
Supplementary Fig. S3: Proteolytic activity of HtrA3 and some of its variants with β-casein (β-c) as substrate. Representative images of β-casein cleavage assays (2-10µg) with (**A**) HtrA3 N-SPD, (**B**) HtrA3 F255D and (**C**) HtrA3 F255D. All reactions were incubated at 37°C for 2 hr

Supplementary Figure S4:



Supplementary Figure S4: Free energy landscape of **A)** HtrA3 wildtype (mature), **B)** HtrA3 F142D and **C)** HtrA3 F255D representing a cross-relation plot between RMSD and Radius of gyration of different conformations (generated during MDS run) where each conformation is coloured in rainbow spectrum from violet (the lowest energy) to red (the highest energy) on the basis of its Gibb's free energy value.

Supplementary Figure S5:



Supplementary Figure S5: Graphical representation of the evolution of secondary structures of **A)** HtrA3 wildtype (mature), **B)** HtrA3 F142D and **C)** HtrA3 F255D with respect to time (at picosecond time-scale) during molecular dynamics simulation. Here, secondary structures such as coils, beta sheets, beta bridges, loop bends, beta turns, alpha helices, 3_{10} helices and chain separators are represented by white, red, black, green, yellow, blue, deep grey and light grey colours respectively.