

Fig. A: Purification and mass characterization of HpRF and HpDML1 (A) 0.1% SDS – 10% PAGE of purified recombinant HpRF - (His)₆. M: molecular mass standards. Lane 1: Purified HpRF- (His)₆ protein. (B) 0.1% SDS – 10% PAGE of purified recombinant HpDML1 - (His)₆. M: molecular mass standards. Lane 1: Purified HpDML1- (His)₆ protein. (C) Mass spectra of purified HpRF - (His)₆. Three sharp peaks corresponding to molecular mass of HpRF – (His)₆ (1) 26.5 kDa (monocharged species) (2) 13.27 kDa (dicharged species) and (3) 8.85 kDa (tricharged species) are obtained (D) Mass spectra of purified HpDML1. A sharp peak corresponding to molecular mass of HpDML1 – (His)₆ (1) 9.589 kDa is obtained. (E) Circular dichroism spectra of HpDprA, HpRF and HpDML1. Full length HpDprA, HpRF or HpDML1 (100 µg/ ml) were used to record the spectra in 1X PBS buffer. The spectra were recorded in the wavelength range 190 - 300 nm in a Jasco J- 500A spectropolarimeter. A subset of spectra for wavelength range 200 nm to 300 nm is shown here. All measurements were recorded at 25°C. Spectra presented are representative of three independent experiments.

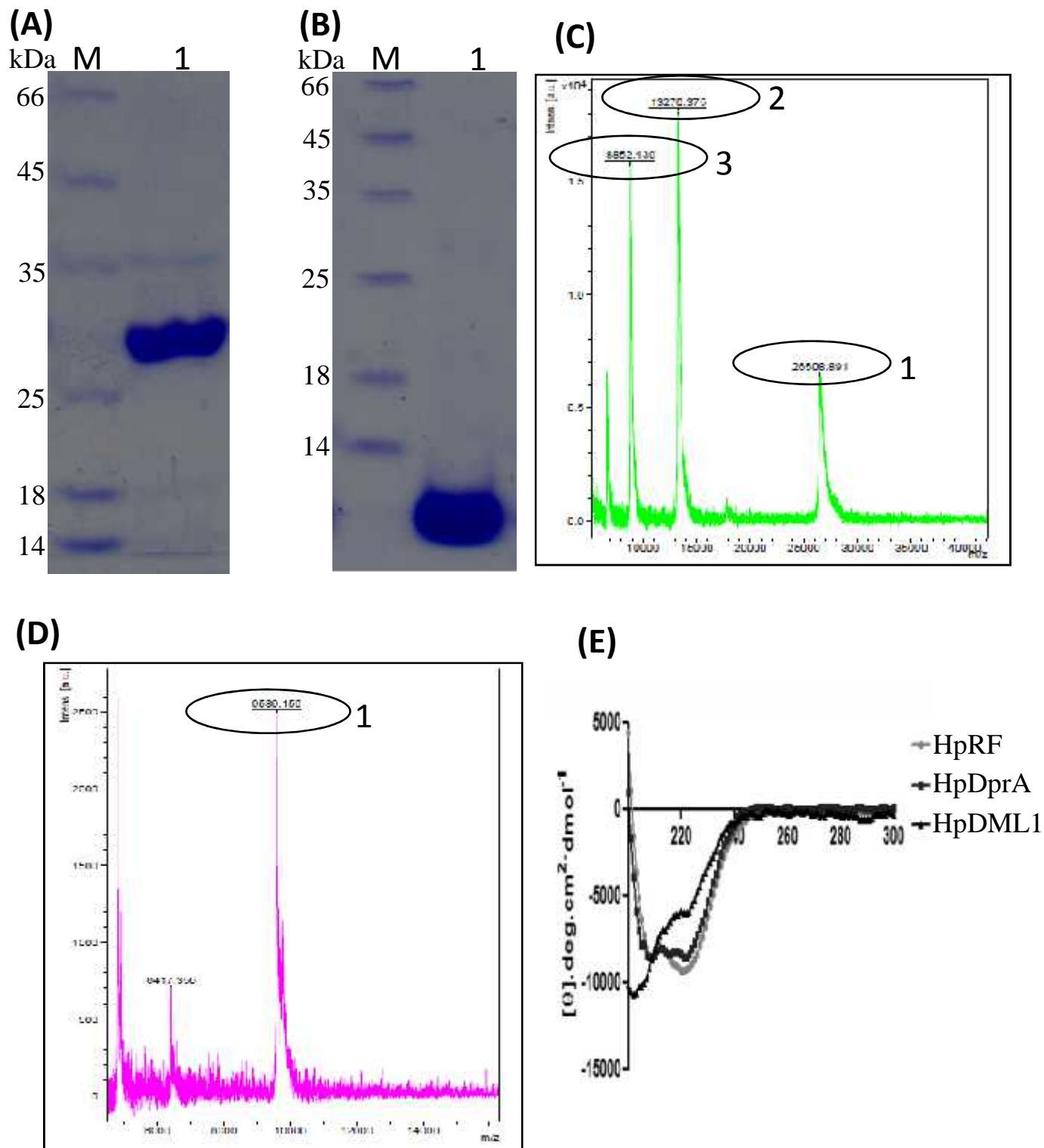


Fig. 1

Fig. B: Crosslinking of HpDprA, HpRF and HpDML1 using gluteraldehyde. 5 μ M of HpDprA (A) or 6 μ M HpRF (B) was incubated with (lanes 2 to 5: (left to right) 0.01, 0.05, 0.1, 0.5%) gluteraldehyde at 4°C for 10 minutes. (C) 140 μ M of HpDML1 was incubated with (lanes 2 to 5: (left to right) 0.1, 0.5, 1, 2%) gluteraldehyde at 4°C for 30 minutes. Lane 1 is protein alone. Lane 6 shows standard protein marker. 1.5 μ g of protein was loaded per well. Reaction mixtures were analyzed by SDS-PAGE and visualized by silver staining.

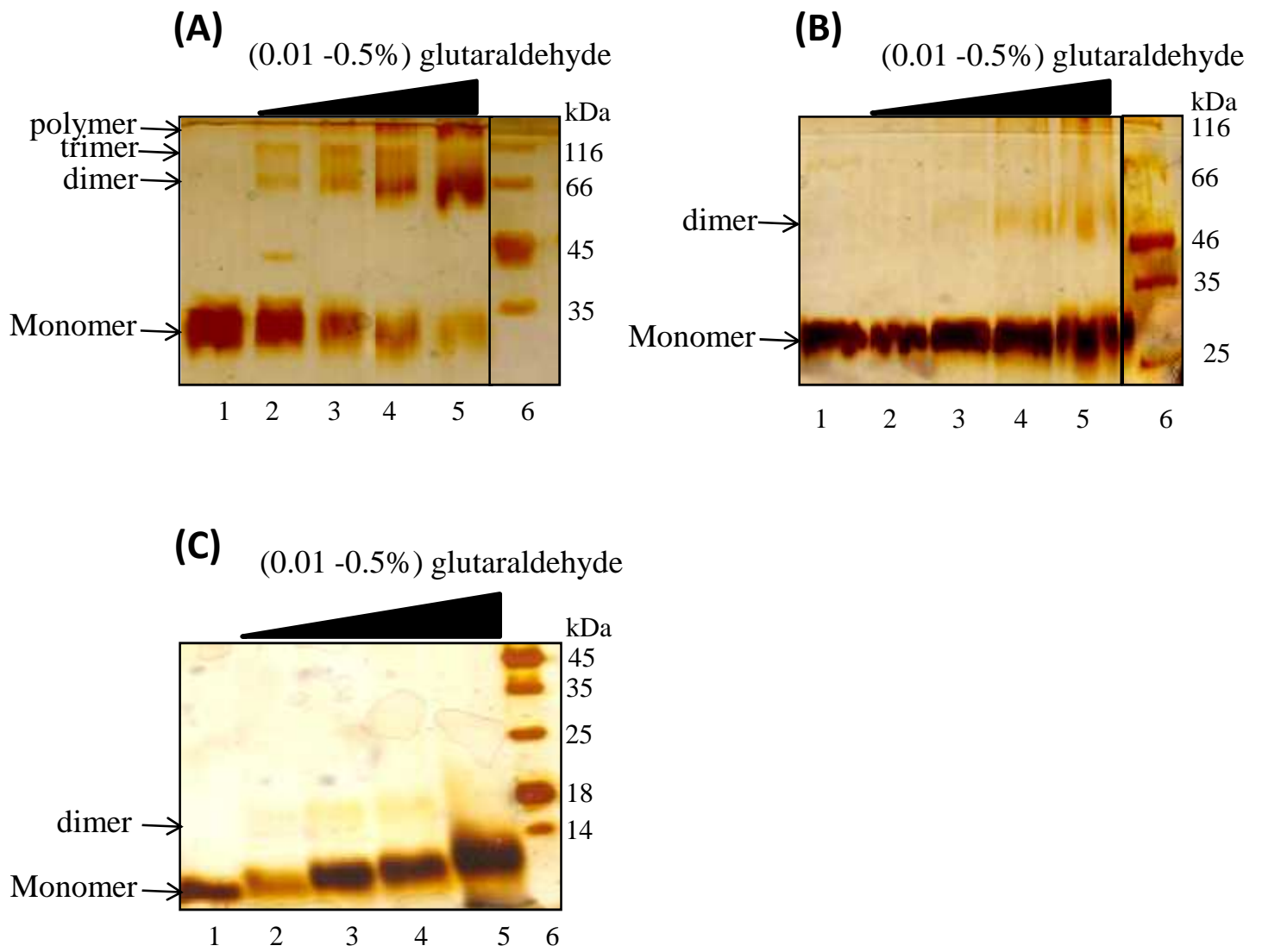
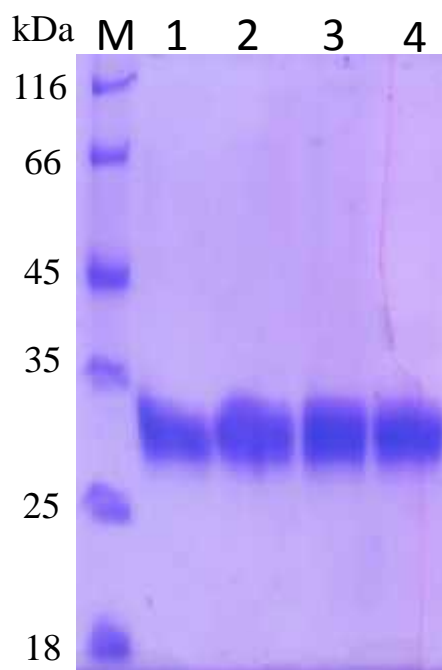


Fig. 2

Fig. C: Purification and CD spectroscopy of HpDprAR_{48A/R49A} and HpDprAR_{48A/R49A/K133A}
(A) 0.1% SDS – 10% PAGE analysis of purified recombinant wtDprA and mutants. M: molecular mass standards. Lane1: wild type, lane2: HpDprAR_{48A/R49A}, lane3: HpDprAR_{48A/R49A/K133A} (B) An overlay of Far – UV circular dichroism spectra of HpDprA, double and triple mutants. The spectra were taken in phosphate buffered saline in a wavelength range 190 -300 nm. A subset of spectra from 200 to 250 nm wavelength range is shown here. The observed two minima at 222 nm and 209 nm are characteristic of α – helix spectrum.

(A)



(B)

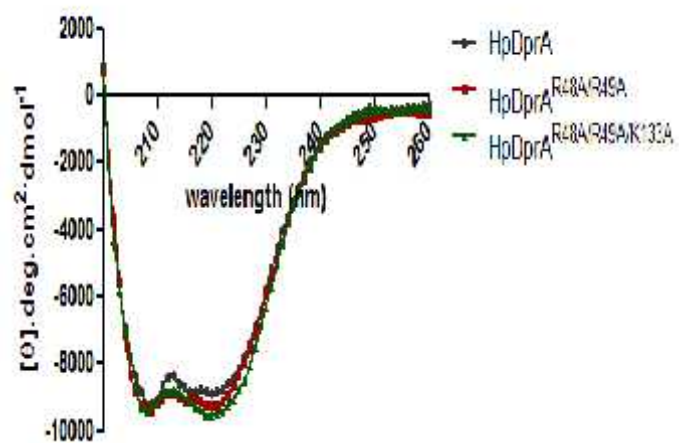


Fig. 3