Supporting Information

Concerted Deoligomerization and Nonfunctional Reassembly of the Hexameric Proteasomal ATPase Mpa upon Chemical and Thermal Perturbation

Pushpkant Sahu[a], Samapti Mondal[a], Bincy Lukose[b], Thalappil Pradeep[a,c], Hema Chandra Kotamarthi*[a]

Authors affiliations

[a]*Department of Chemistry, Indian Institute of Technology Madras, Chennai – 600036, India.*

**E-mail: hemachandra@iitm.ac.in*

[b]*Department of Biotechnology, Indian Institute of Technology Madras, Chennai – 600036, India.*

[c]*International Centre for Clean Water, IIT Madras Research Park, Chennai, 600113, India*

Table of contents:

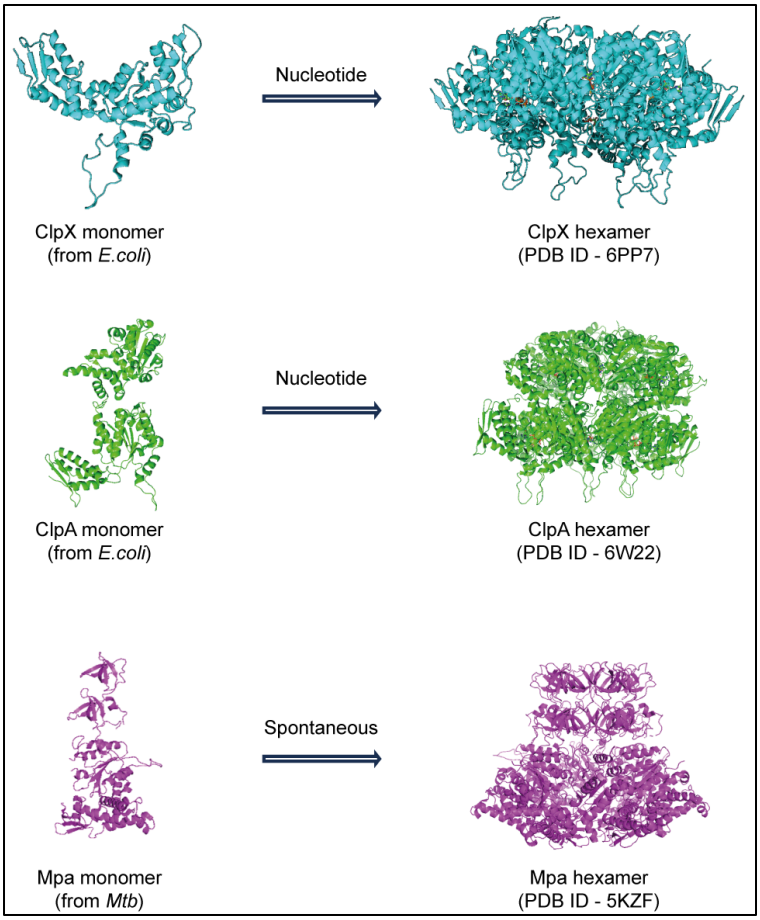

Figure S1. Spontaneous formation of Mpa homo-hexamers. ClpX and ClpA form hexamers in the presence of nucleotide, whereas Mpa forms hexamers spontaneously due to the presence of an Interdomain.

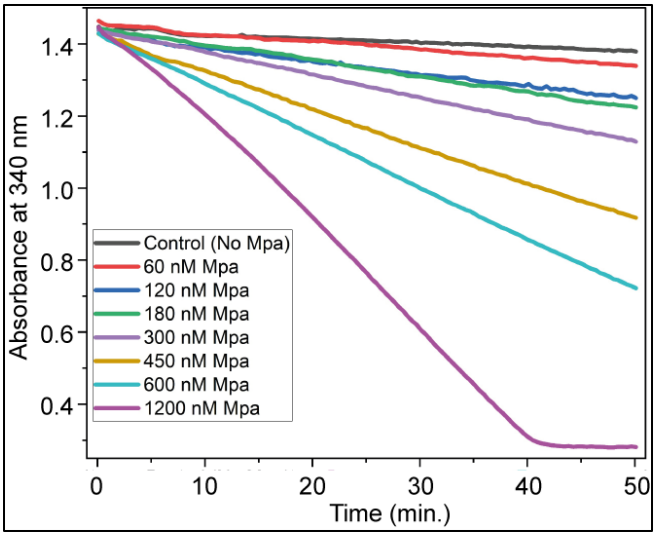

Figure S2 . ATPase activity of Mpa. ATPase rate at different Mpa concentrations is determined using NADH coupled assay monitoring the change in NADH absorbance at 340 nm.

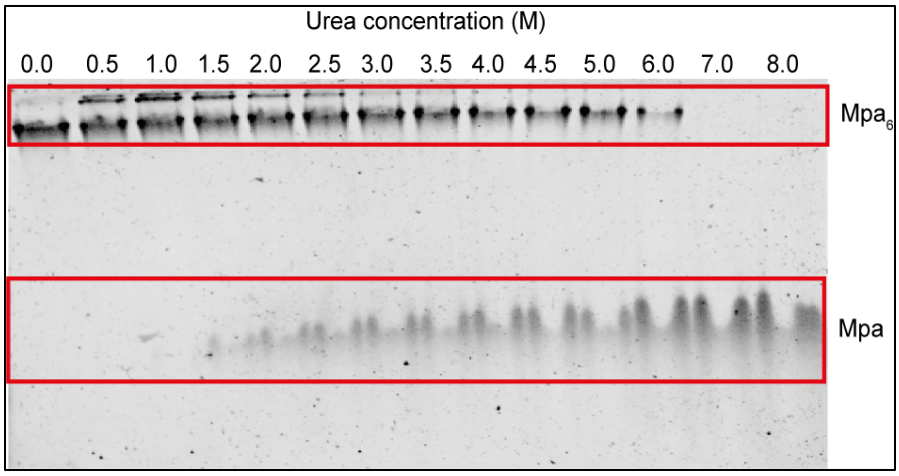

Figure S3. **Stability of Mpa hexamer with varying urea concentrations:** Native PAGE (7.5%) analysis of Mpa at different urea concentrations.

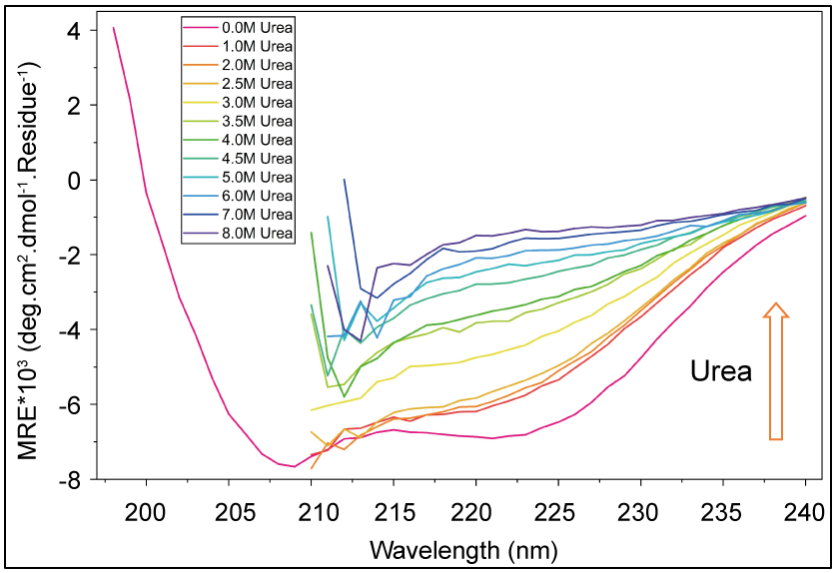

Figure S4. Far-UV CD spectra of Mpa with varying urea concentrations

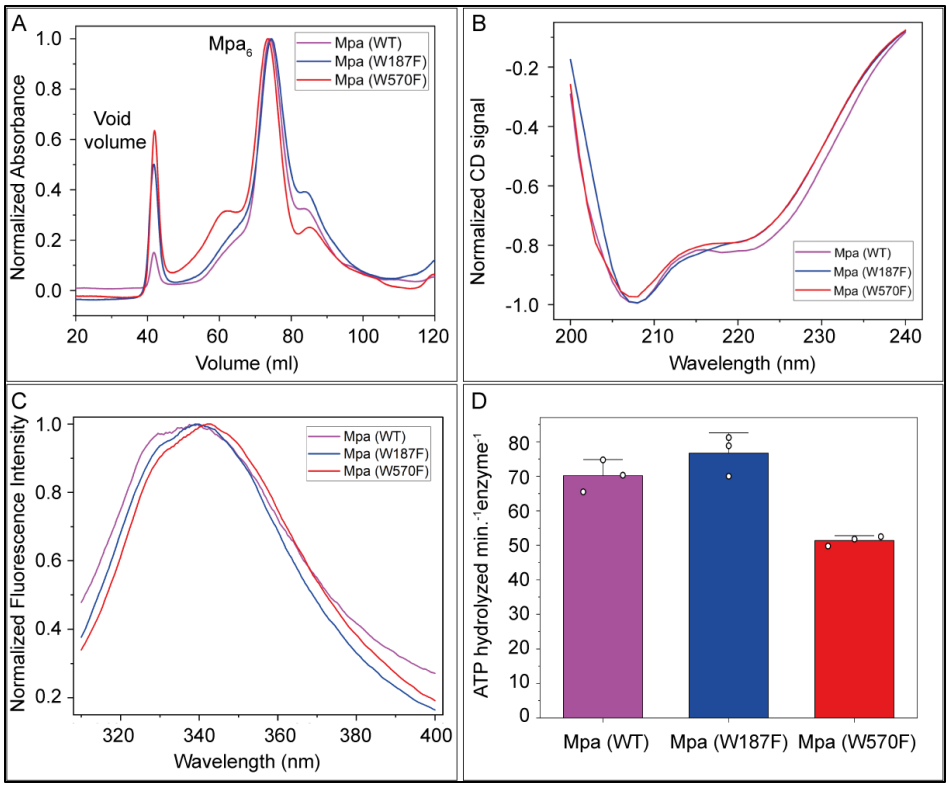

Figure S5. Size and structural comparison of Mpa and its mutants (A) SEC chromatogram of Mpa and its mutants showing a similar elution profile, suggesting that the Mpa mutant also forms a hexamer like the wild type. (B) Normalized far-UV CD spectra indicate similar secondary structures between the Mpa mutants and wild type. (C) Normalized tryptophan fluorescence spectra (excitation at 295 nm) showing a ~3 nm red shift for the W570F mutant, likely due to more solvent exposure of W187. (D) ATPase activity shows an approximately 30% decrease (~0.7-fold) for the W570F mutant, possibly due to conformational changes in the Walker B motif (ATP hydrolysis motif). Data are presented as mean ± SD (n = 3). Individual data points are shown in white circles.

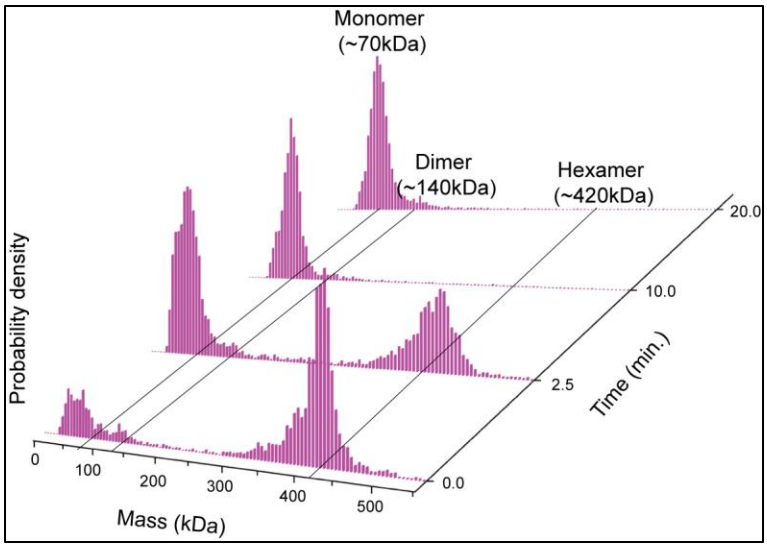

Figure S6. Variation in Mass distribution with time for Mpa in 8 M urea measured by single-particle mass photometry

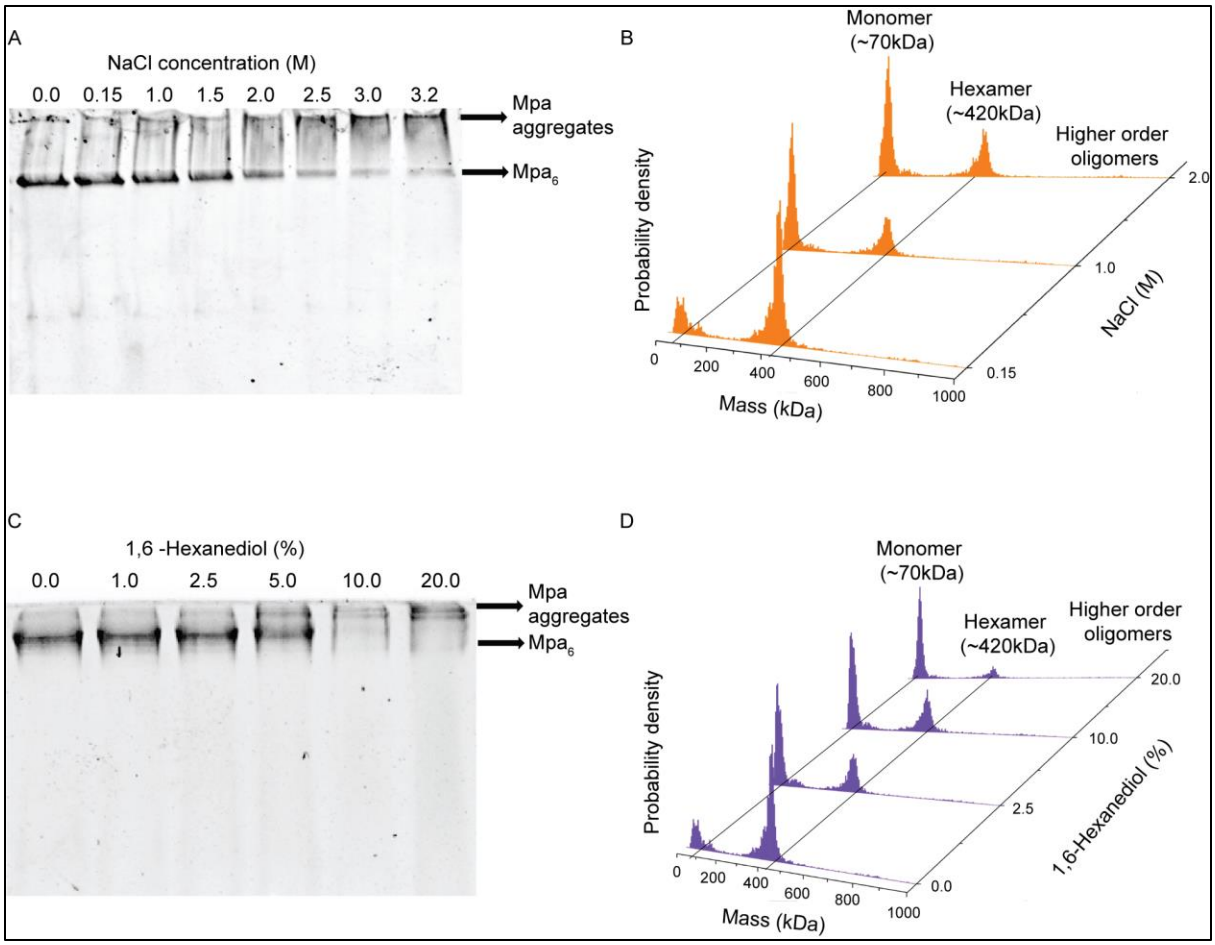

Figure S7. **Stability of Mpa hexamer with varying NaCl and 1,6-hexanediol concentrations:** (A) Native PAGE (7.5%) analysis and (B) mass distributions analysis by single particle mass photometry of Mpa at varying salt (NaCl) concentrations. (C) Native PAGE (7.5%) analysis and (D) mass distributions analysis by single particle mass photometry of Mpa at varying 1,6-hexanediol concentrations.

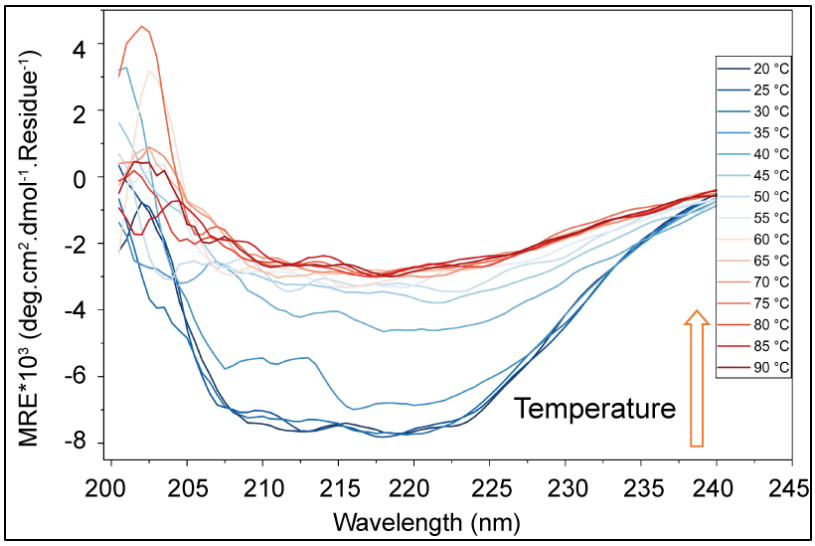

Figure S8. **Far-UV CD spectra of Mpa with varying temperatures.**

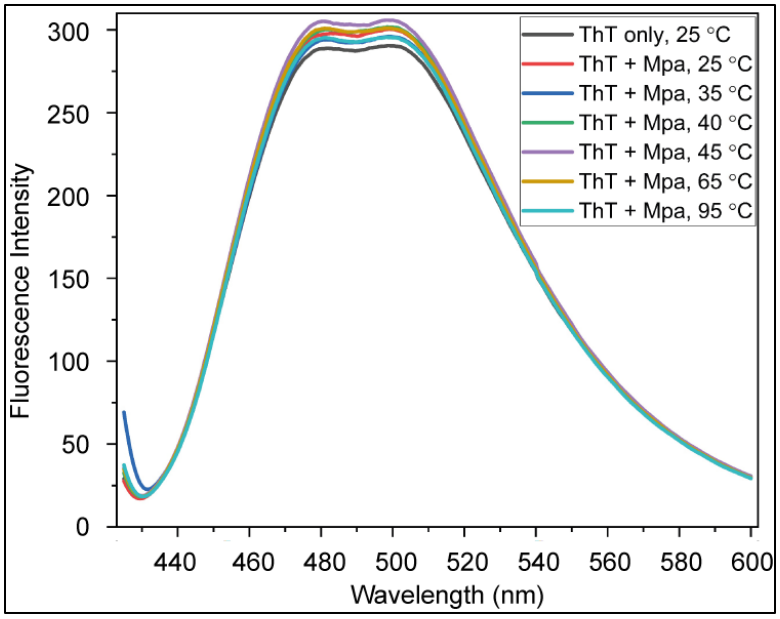

Figure S9. **Thioflavin T (ThT) binding assay for Mpa aggregates:** ThT fluorescence of Mpa was recorded from 425 nm to 600 nm (excited at 412 nm) at different temperatures. The insignificant change in ThT fluorescence properties indicate that Mpa aggregates are nonamyloid in nature.

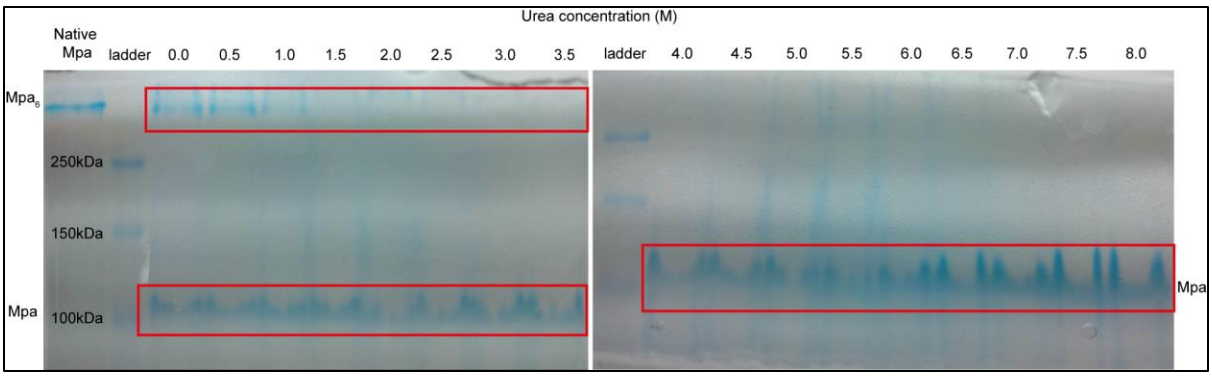

Figure S10. **Refolding analysis of chemically denatured Mpa:** Native PAGE (7.5%) analysis for Mpa refolding at different urea concentrations.

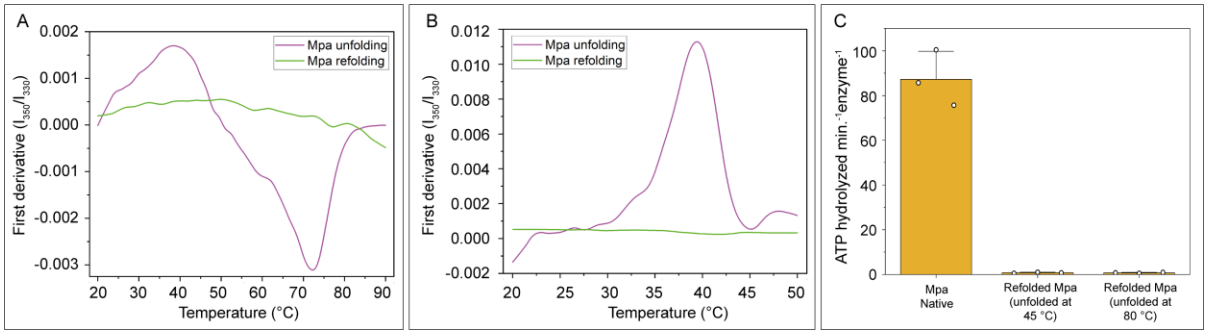

Figure S11. Refolding analysis of thermally denatured Mpa: (A, B) First derivative of the tryptophan fluorescence emission intensity ratio I_{350}/I_{330} (excited at 280 nm) during thermal unfolding and refolding, measured by nano-DSF. (A) Thermal unfolding up to 90 °C; (B) unfolding up to 50 °C followed by cooling to 20 °C to assess refolding. The data indicate that Mpa undergoes irreversible unfolding. (C) ATPase activity of native and thermally refolded Mpa (previously unfolded at 45 °C and 80 °C), showing loss of enzymatic activity upon thermal treatment. Data are presented as mean ± SD (n = 3). Individual data points are shown in white circles.