

Folding mechanisms steer amyloid fibrils formation propensity of highly homologous proteins

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Supplementary Information

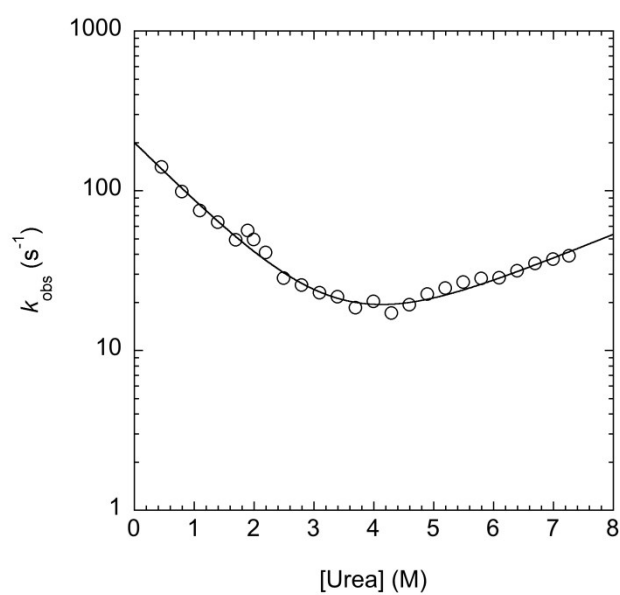


FIGURE S11 – Chevron plot of M14₅₂₋₁₅₁ in Urea

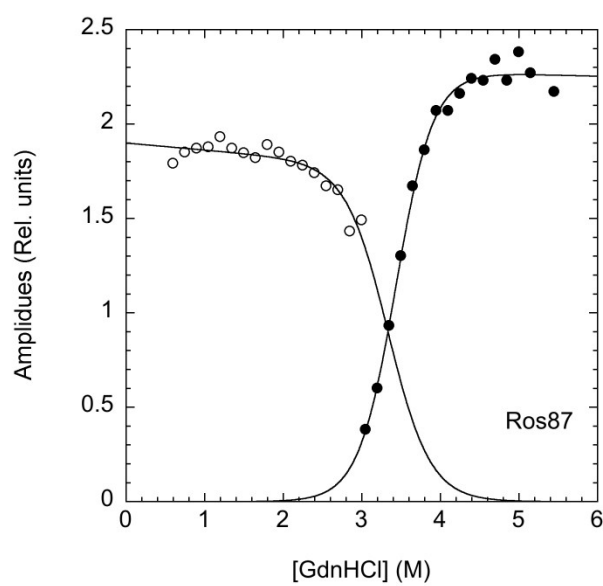


FIGURE S12 – Folding and unfolding kinetic transitions of Ros87. Open and filled circles refer to the amplitudes of the kinetic traces observed in the kinetic refolding and unfolding experiments respectively.

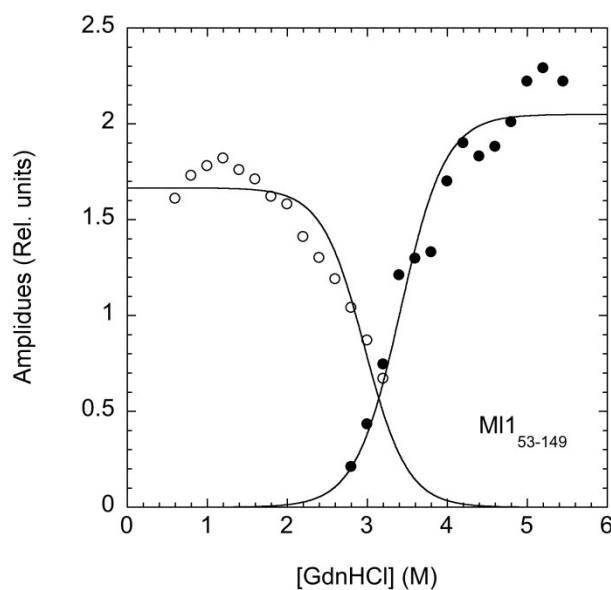


FIGURE SI3 – Folding and unfolding kinetic transitions of Ml1₅₃₋₁₄₉

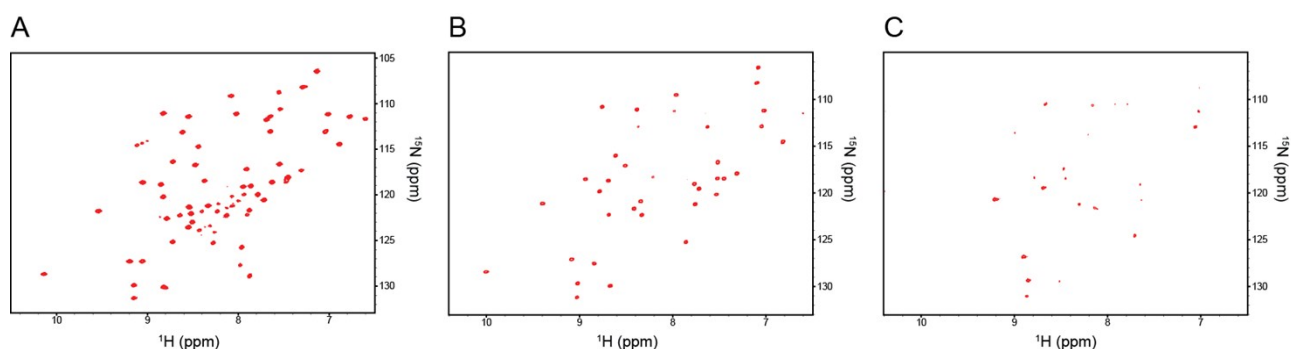


FIGURE SI4 – Unfolding of Ml1₅₃₋₁₄₉ by NMR: ¹H-¹⁵N-HSQC of the protein at 298K (A), 323K (B) and at 353K (C). NMR samples contained 0.8 mM ¹⁵N- Ml1₅₃₋₁₄₉, 20 mM phosphate buffer, 0.2 M NaCl, 4 mM TCEP, pH 6.8, and 90% H₂O/10% ²H₂O. The experiments were acquired on a Varian Unity INOVA 500 MHz spectrometer. Temperature-induced chemical shift perturbations have been monitored in a series of ¹H-¹⁵N HSQC spectra acquired in a range from 278 to 353 K at regular intervals of temperature. The resonances exhibit a continuous chemical shift variation indicating a fast protein folding process and some of them still preserved a good spectral dispersion at 353 K. The presence of visible cross-peaks up to 353 K is in agreement with the mechanism of folding described by the other techniques.

MI1₅₃₋₁₄₉ 1~~~SAAKPE~ALEPA VPIRKSVT**PD YIIC**LDGKK FKSLKRHLST AKWHLPADYP MVAPNYAAAR SALAKTMGLG RKPKEPEART RKKAAA~⁹⁷
MI4₅₂₋₁₅₁ 1GRPAENP~~VLTPA VNPKKSVF**PD YIVS**LEDGRK FKSMKRHLGL TKWDLPRDYP MVAPNYAATR SALAKASGLG RKAAPVKKAP AKRKAKA¹⁰⁰
Ros87 1~~~AVNVEKQKPA VSVRKSVQDD **HIVC**LECGGS FKSLKRHLTT EKWDLFVDYP MVAPAYAEAR SRLAKEMGLG QRRKANR~~~⁸⁷

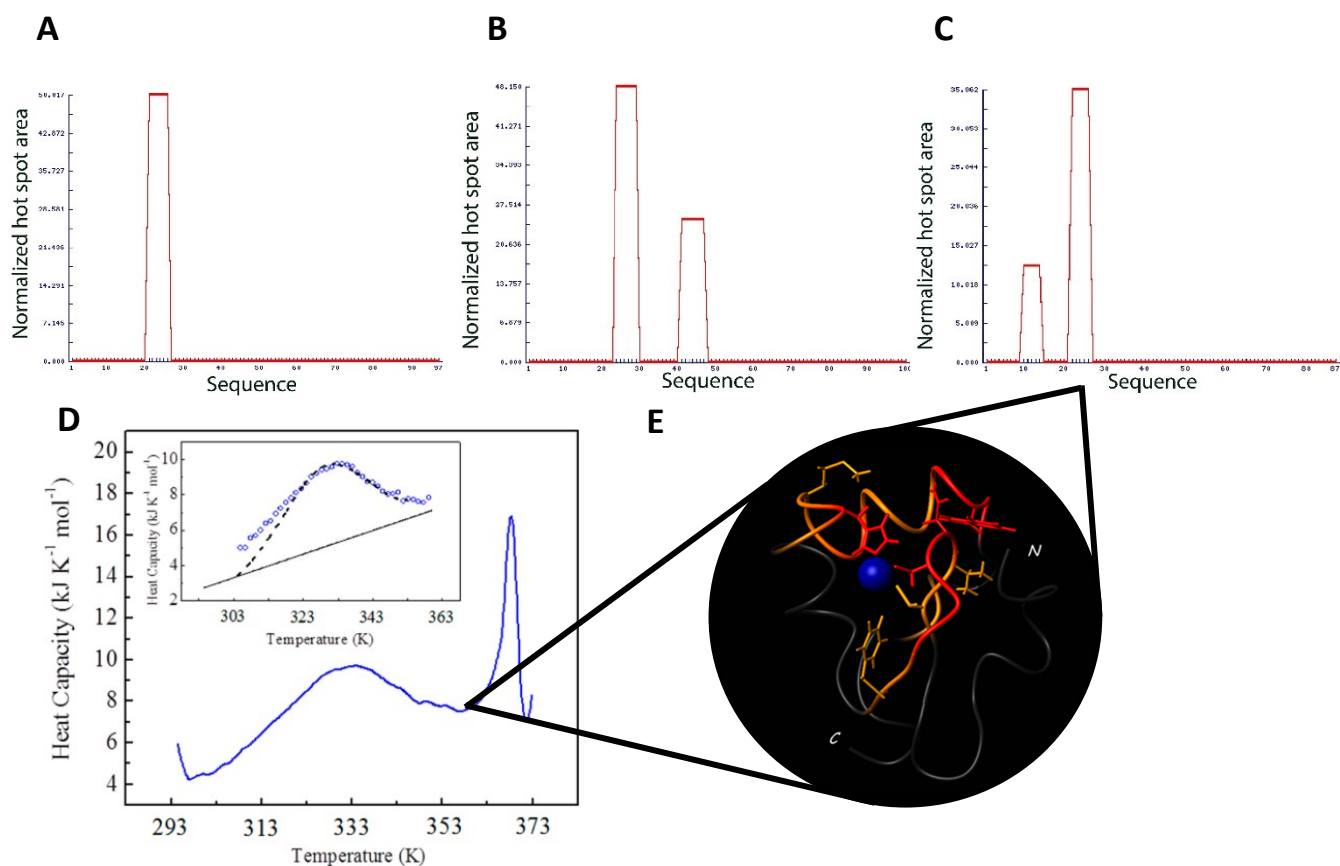


FIGURE SI5 - Alignment of the three proteins: the amino acid stretches with high propensity to aggregate are in red; AGGRESCAN results for Ros87 (C), MI4₅₂₋₁₅₁ (B) and MI1₅₃₋₁₄₉ (A); thermal unfolding of Ros87 followed by DSC (D); Ros87 zinc containing intermediate present at 353K (Palmieri *et al.*, JACS 2013).

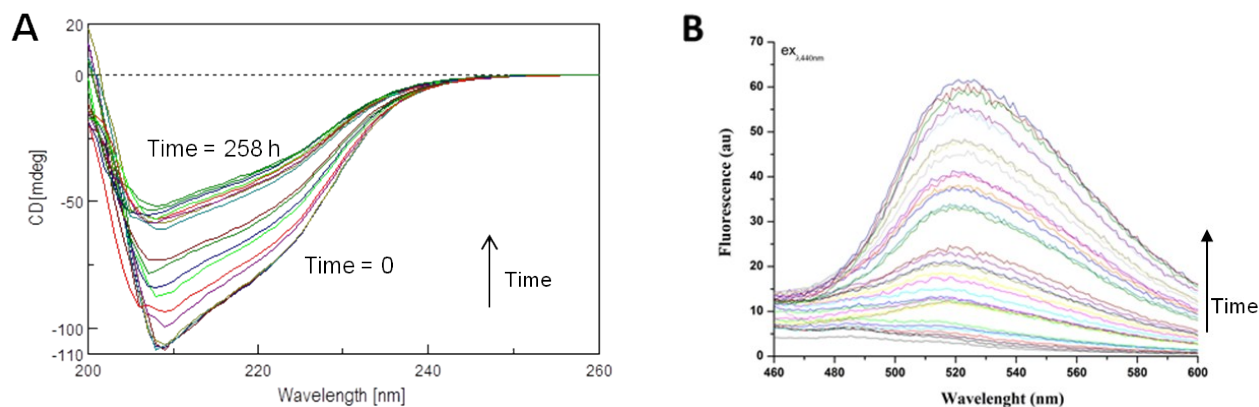


Figure SI6 - (A) CD spectra of Ros87 incubated at 298K recorded every 12 hours for 10 days. (B) ThT fluorescence assay of Ros87 incubated at 298K: spectra were recorded every 12 hours.

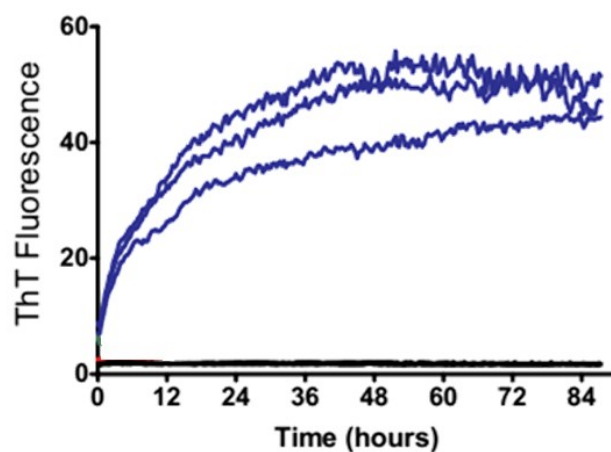


FIGURE SI7 – Aggregation behavior of unfolded Ros87 in acidic conditions (pH=3) at 298K followed by Thioflavin T fluorescence, recorded as a triplicate.

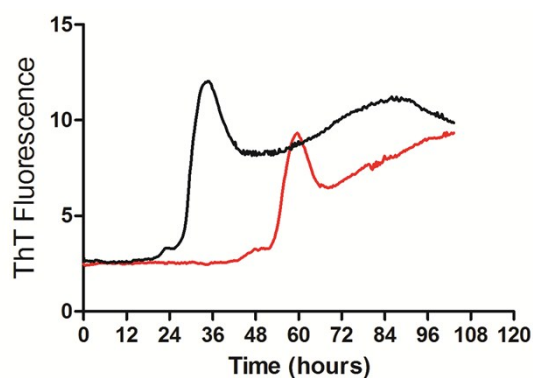


FIGURE SI8 - Aggregation behavior of Ros87 (black) and MI4₅₂₋₁₅₁ (red) at 300 μ M and 298K followed by Thioflavin T fluorescence.

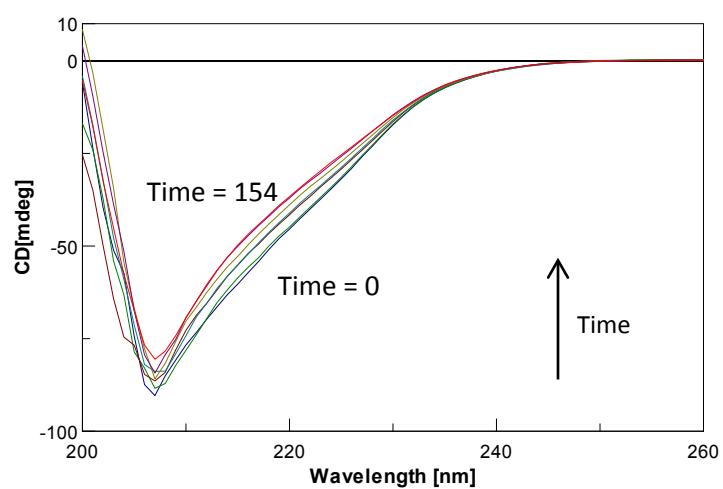


FIGURE SI9 - CD spectra of MI4₅₂₋₁₅₁ incubated at 288K recorded every 24 hours for 154 hours.

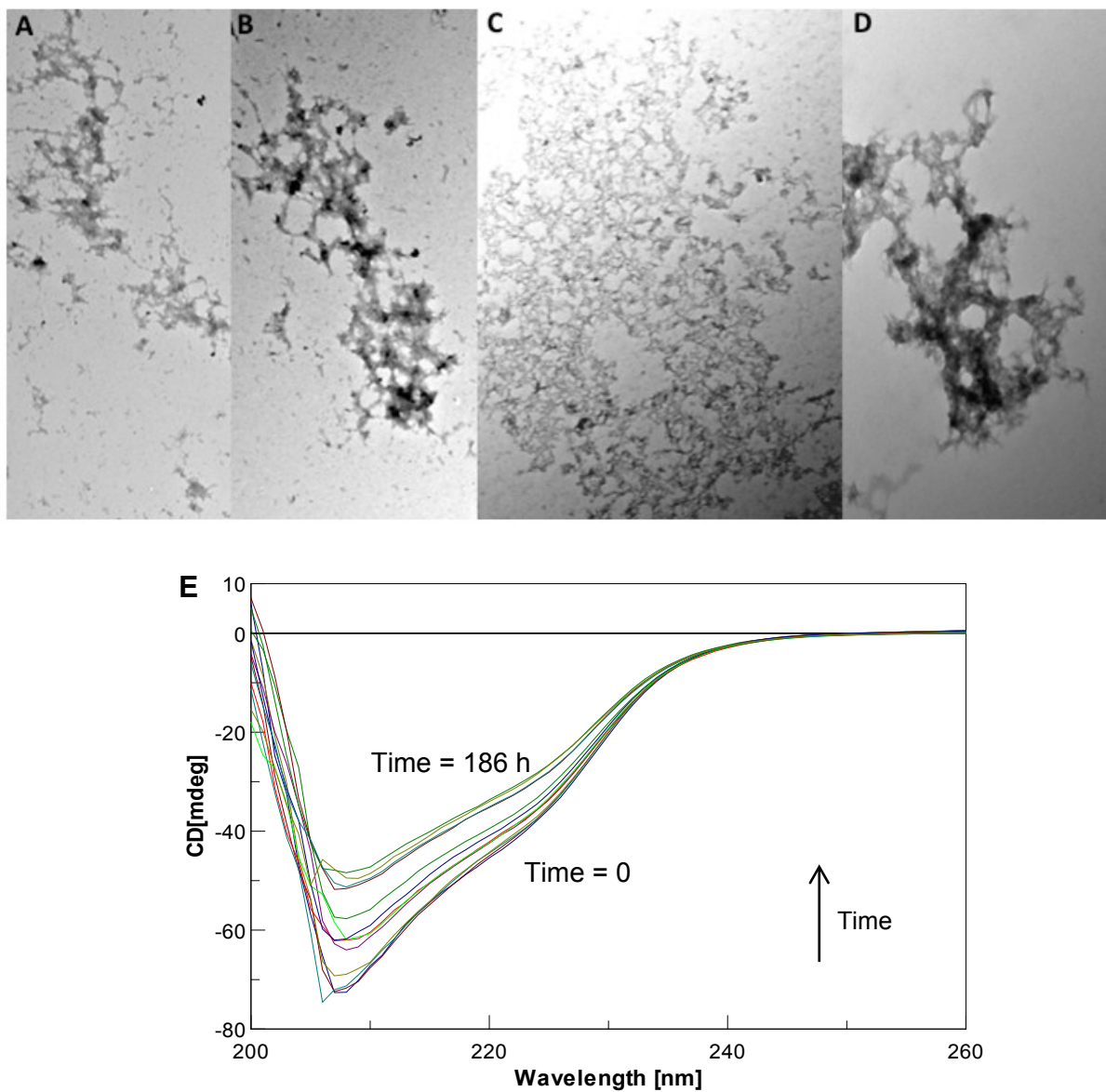


Figure SI10 - Aged M11₅₃₋₁₄₉: amorphous aggregates (panels A-D) - CD spectra of M11₅₃₋₁₄₉ incubated at 298K recorded every 12 hours for 186 days (panel E).

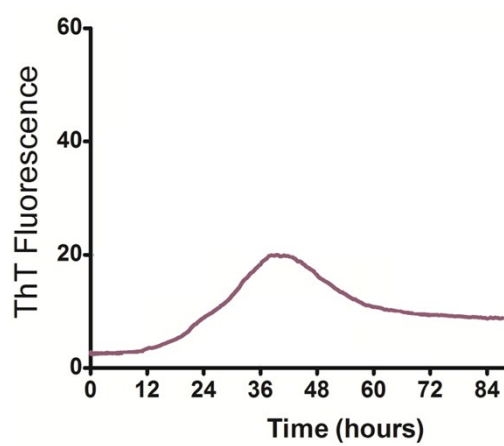


FIGURE SI11 - Aggregation behavior of M11₅₃₋₁₄₉ at 300 μ M and 298K followed by Thioflavin T fluorescence.