Poly-Alanine-Independent Conformational Conversion of the Nuclear poly(A) Binding Protein 1 (PABPN1)

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SUPPLEMENTAL DATA

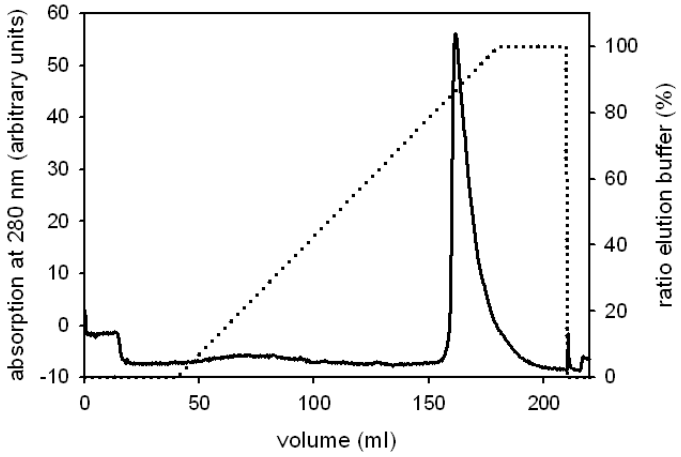

FIGURE S1. Elution peak of PABPN1 from Source 15S column. Solid line, absorption at 280 nm; dotted line, KCl gradient in % (from 150 mM to 500 mM).

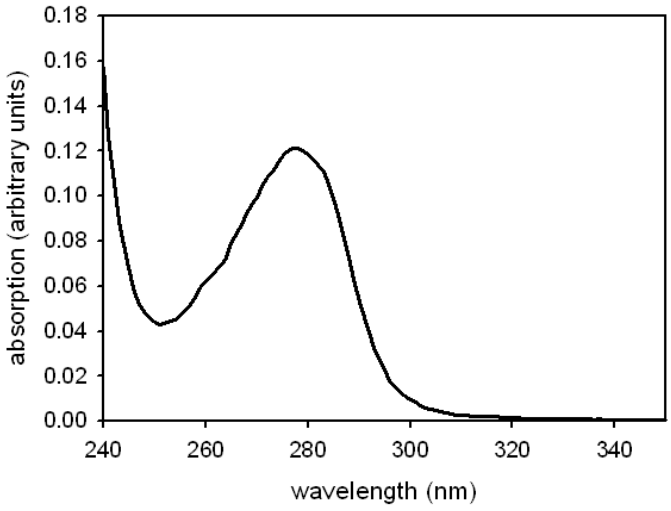

FIGURE S2. Typical UV spectrum of PABPN1.

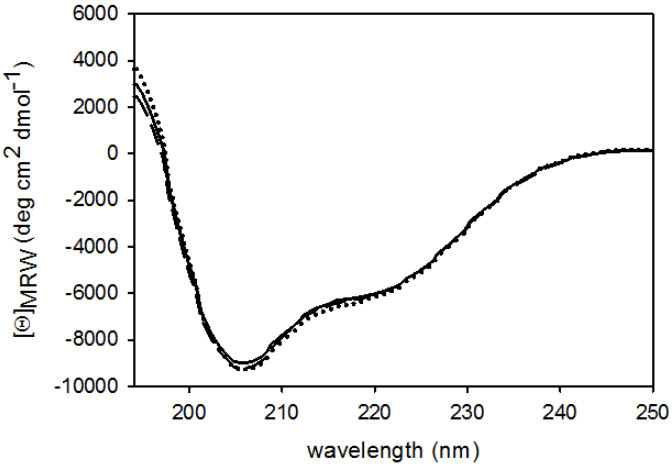

FIGURE S3. Far UV CD spectra of monomeric full-length PABPN1 variants. WT-PABPN1 (solid line), ΔAla-PABPN1 (dotted line) and (+7)Ala-PABPN1 (dashed line).

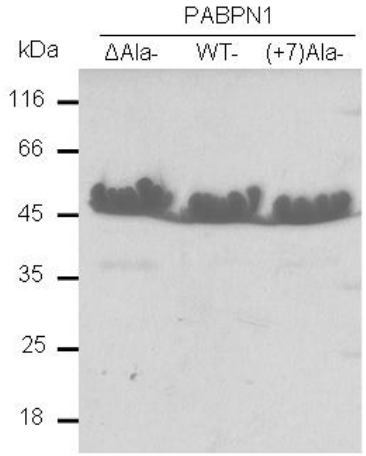

FIGURE S4. Coomassie-stained gel of purified full-length PABPN1 variants. The unusual migration on SDS-PAGE at ca. 50 kDa has previously been reported (4,8).

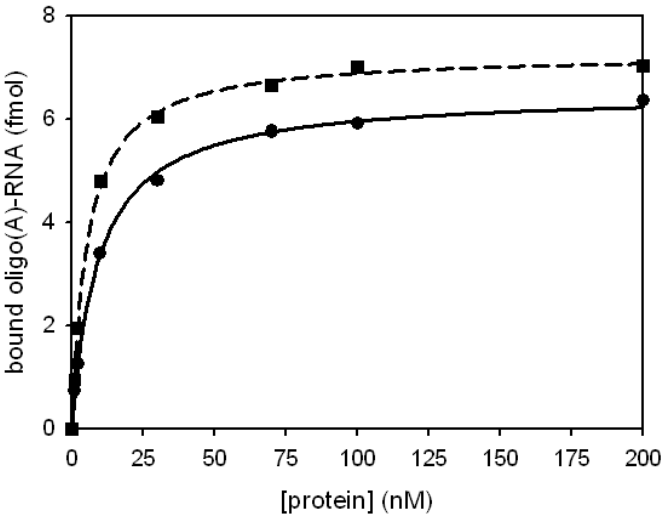

FIGURE S5. Filter binding assay to confirm the native structure of monomeric SUMO-ΔN114 (squares) and as a control of monomeric WT-PABPN1 (circles). Evaluation was performed according to (4).

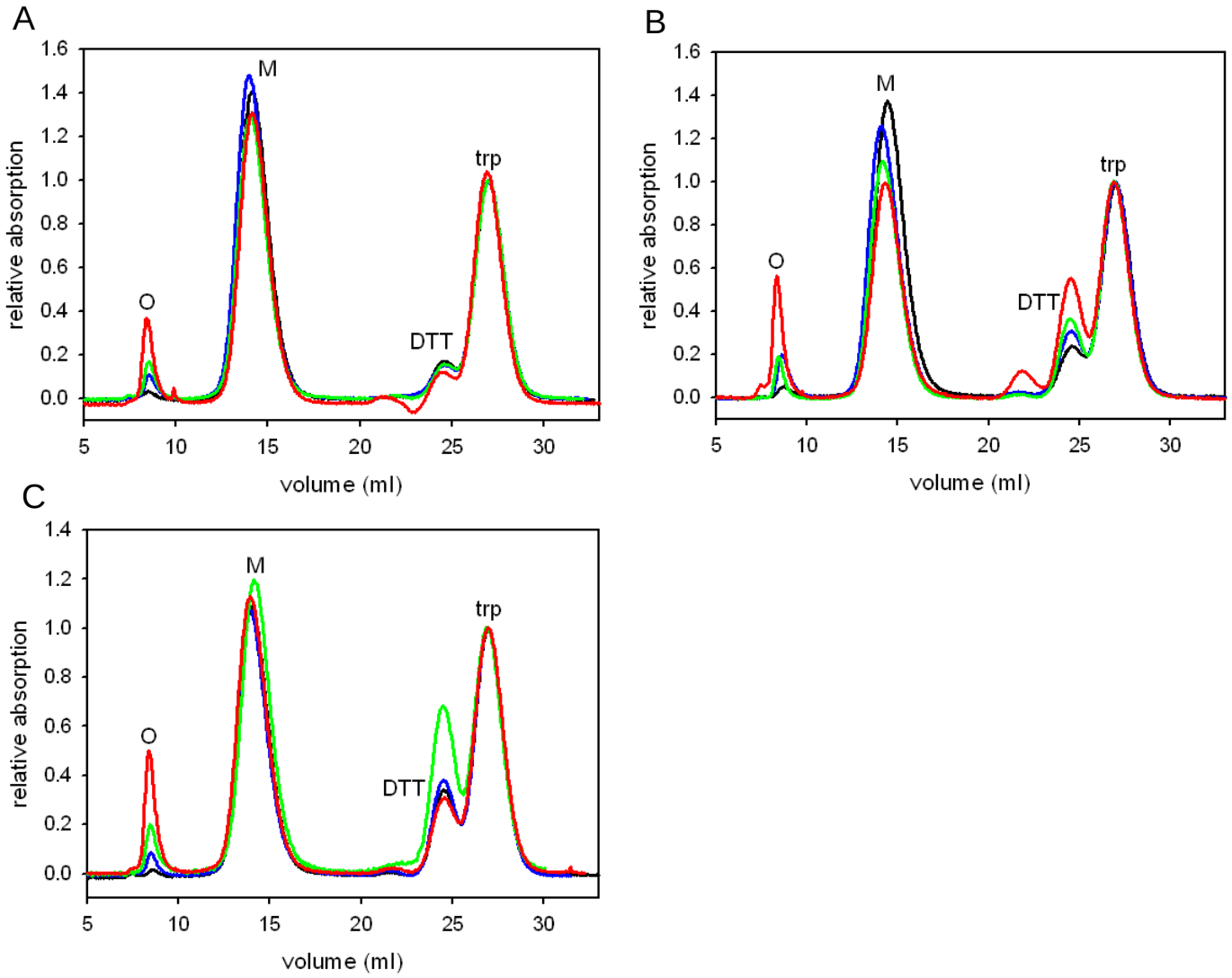

FIGURE S6. Time-dependent size exclusion chromatography of the fibrillation samples of WT-PABPN1 (A), ΔAla-PABPN1 (B) and (+7)Ala-PABPN1 (C). Samples were incubated at 60 µM in 50 mM Tris/HCl pH 7.9, 1.5 M KCl, 1 mM EDTA, 1 mM DTT, 10% (v/v) glycerol at 20°C. After 15 min (black lines), 12 h (blue lines), 24 h (green) and 48 h (red), aliquots were loaded on a Superdex S200 column. The chromatograms were normalized to the absorption of the added tryptophan standard (trp). The chromatograms show monomeric protein (M), oligomeric species (O) and a peak corresponding to DTT (DTT).

A

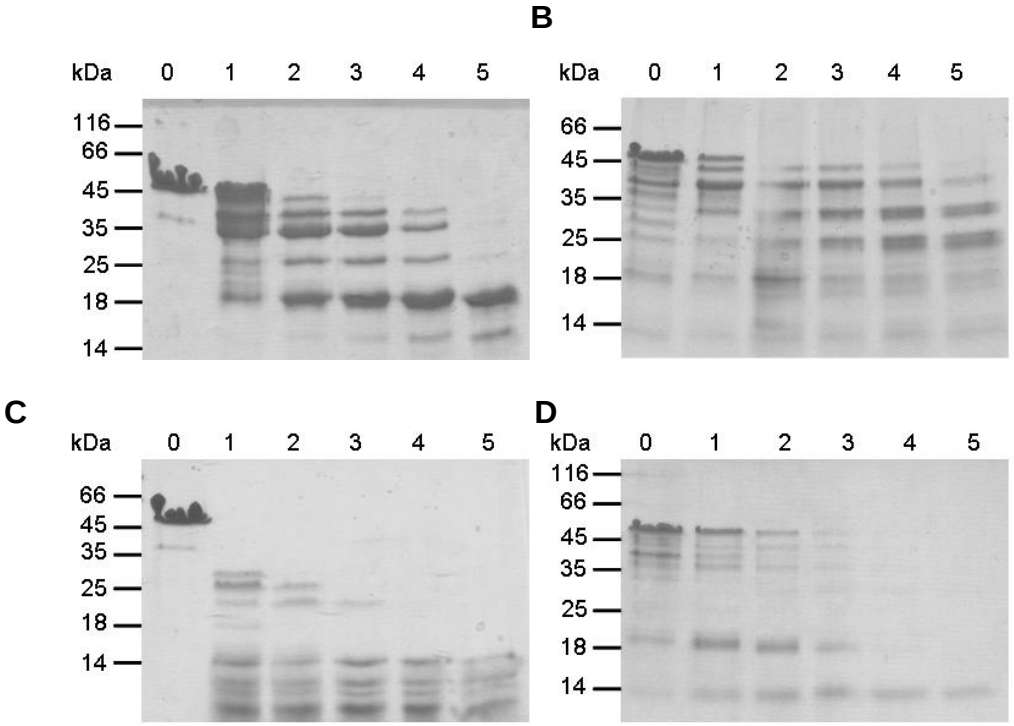

FIGURE S7. Limited proteolysis of ΔAla-PABPN1. Time-dependent proteolysis of monomeric (A, C) and fibrillar ΔAla-PABPN1 (C, D) after 10 min (1), 30 min (2), 1 h (3), 2 h (4) and 3 h (5). For control, protein before the addition of protease is shown in lane 0. (A, B) Limited proteolysis with trypsin at a mass ratio of 1:600. (C, D) Limited proteolysis with proteinase K at a mass ratio of 1:1000.

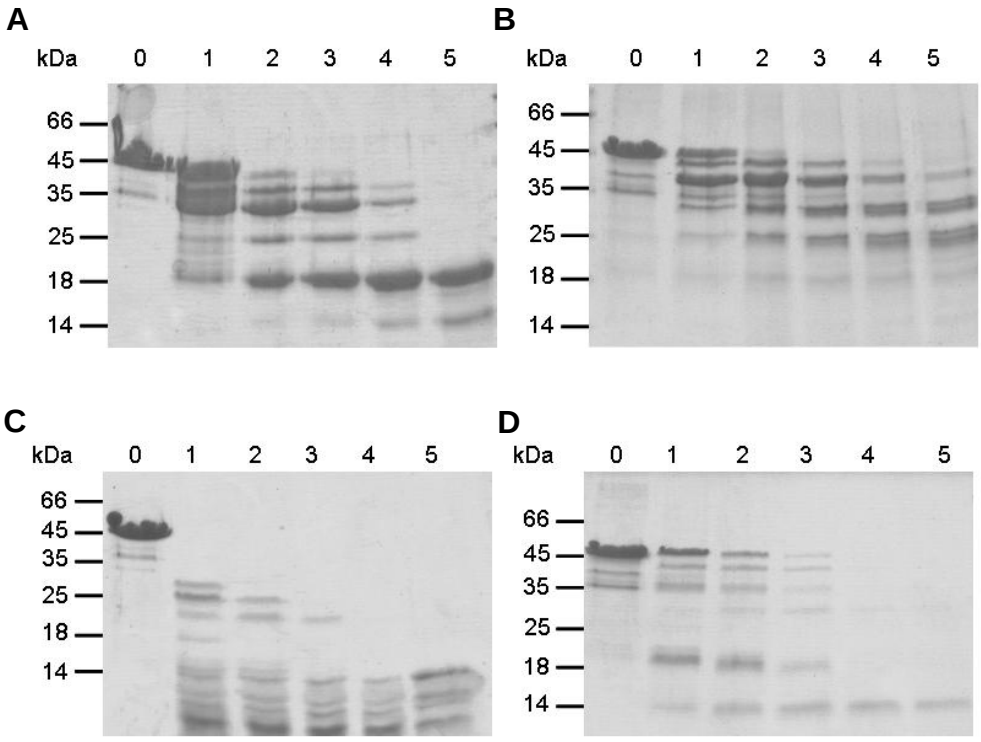

FIGURE S8. Limited proteolysis of (+7)-Ala-PABPN1. Time-dependent proteolysis of monomeric (A, C) and fibrillar (C, D) protein after 10 min (1), 30 min (2), 1 h (3), 2 h (4) and 3 h (5). For control, protein before the addition of protease is shown in lane 0. (A, B) Limited proteolysis with trypsin at a mass ratio of 1:600. (C, D) Limited proteolysis with proteinase K at a mass ratio of 1:1000.