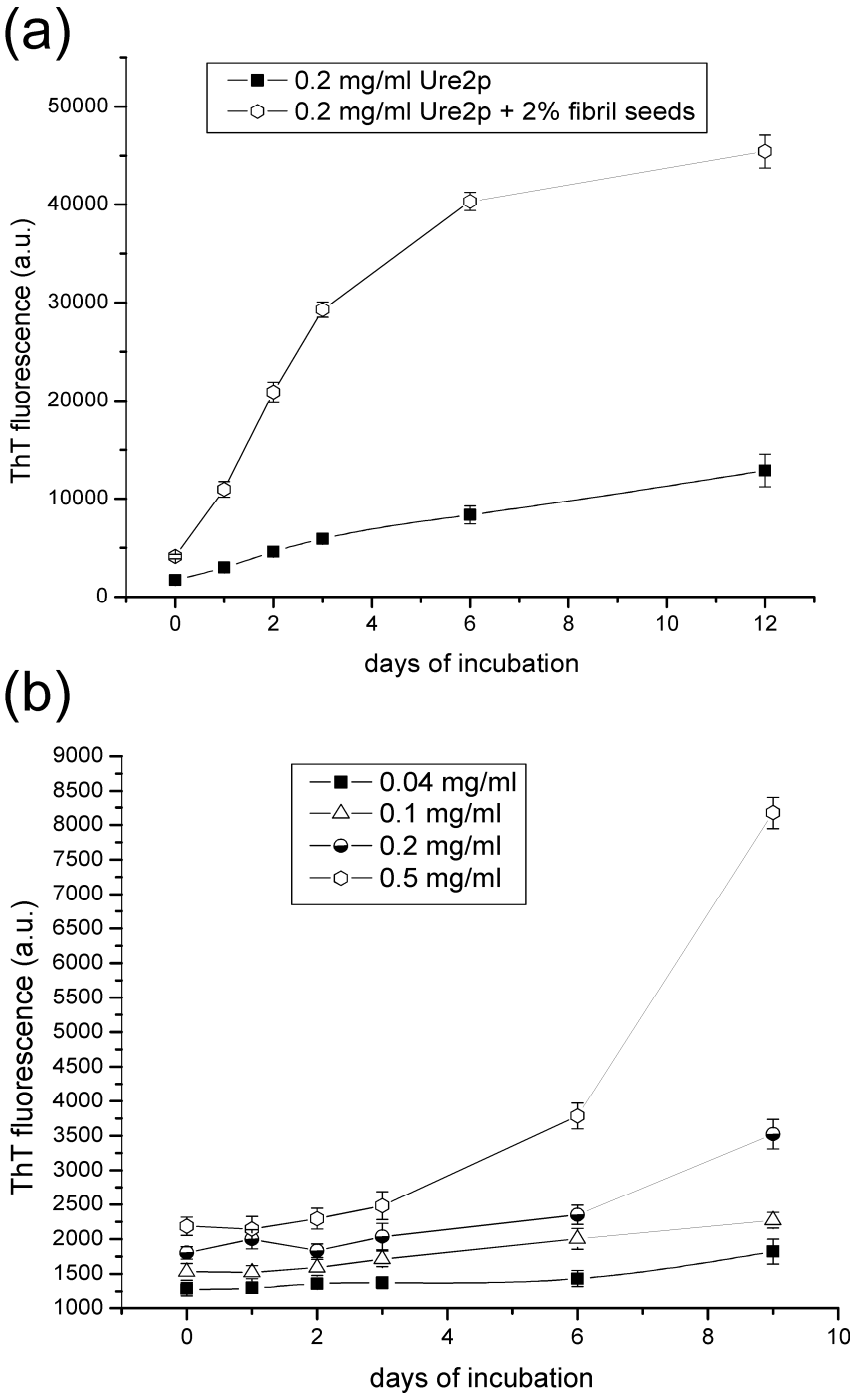


Figure S1: (a) Comparison of spontaneous (filled squares) and seeded (open circles) fibril growth by full-length Ure2p in assembly buffer A, monitored by Thioflavin T fluorescence. (b) Dependence of spontaneous Ure2p fibril growth on protein concentration in assembly buffer A.

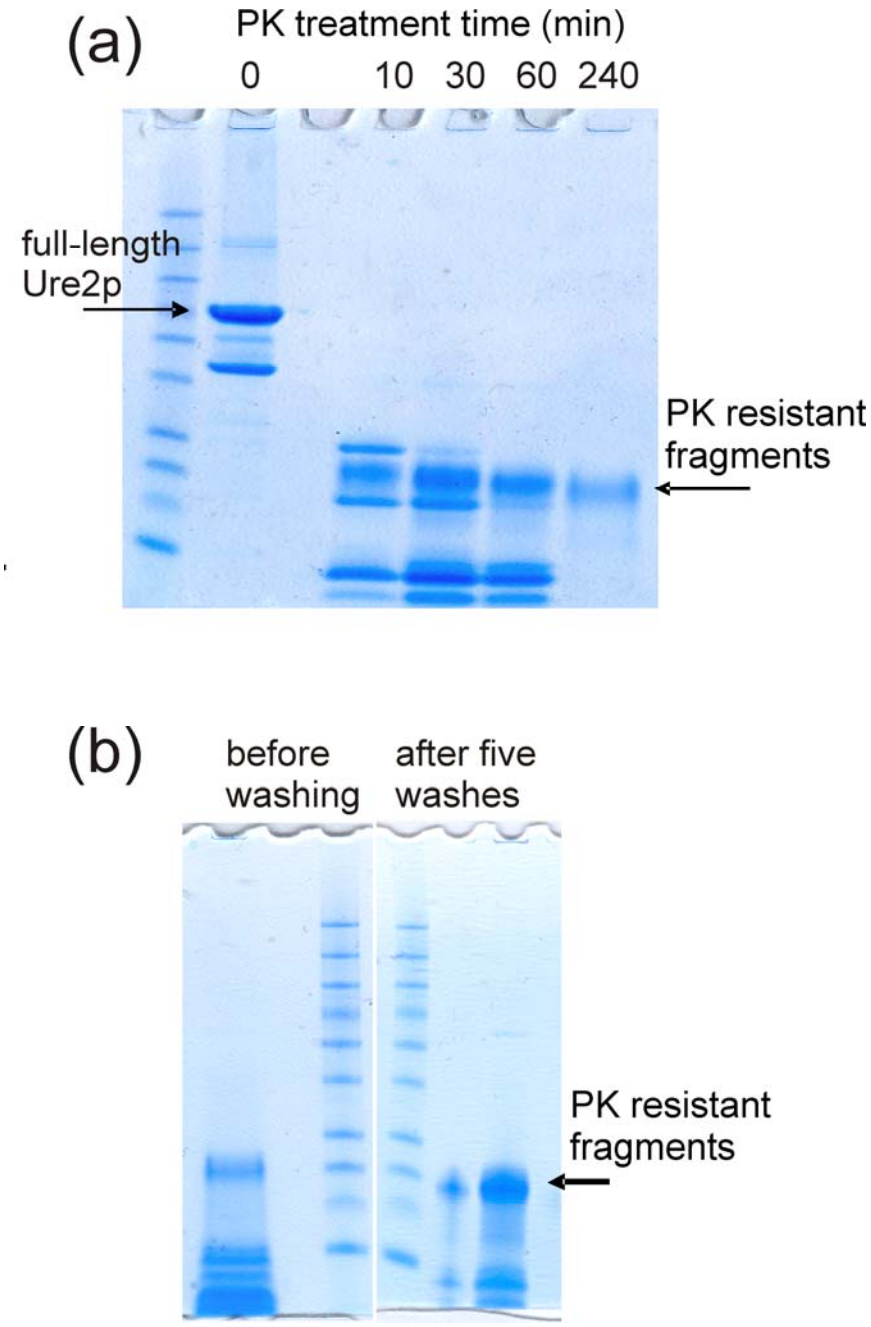


Figure S2: (a) Proteinase K (PK) digestion of full-length Ure2p fibrils. 2 mg of spontaneously formed fibrils (10 mg/ml) were treated with 0.01 mg/ml PK for various times. Digestion products were run on SDS-PAGE, followed by Coomassie staining. (b) PK digestion of U-Ile-EX-Ure2p fibrils. 40 mg of fibrils were treated with 0.01 mg/ml PK for 4 h. The insoluble material was collected by centrifugation at 20,000 X *g* for 30 min at 4° C and washed five times by resuspension in $H_2O$ and subsequent centrifugation. Aliquots were taken and applied on SDS-PAGE, followed by Coomassie staining to confirm the efficiency of digestion.

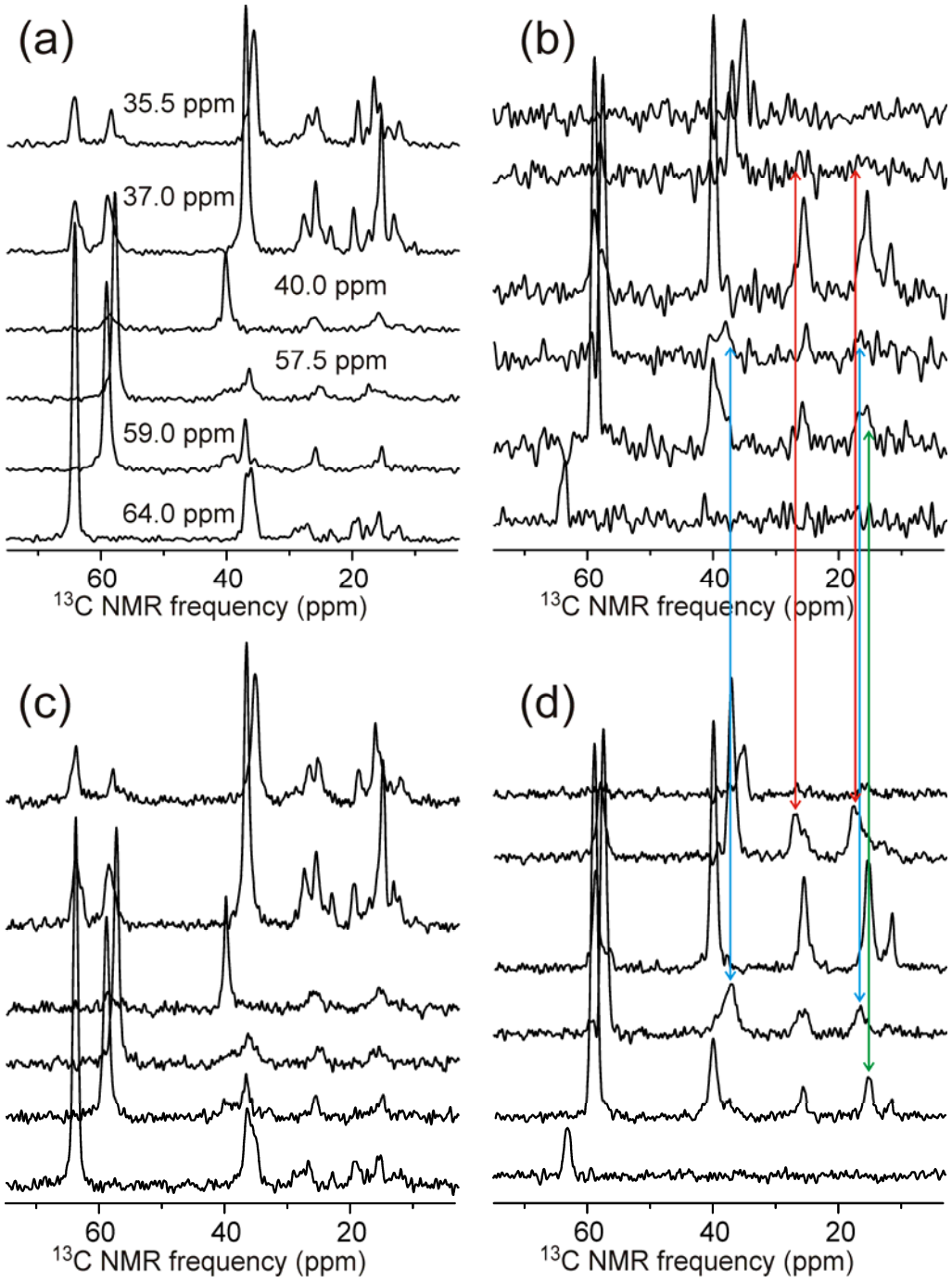


Figure S3: 1D slices from 2D $^{13}C$-$^{13}C$ NMR spectra of U-Ile-EX-Ure2p (a), PK-U-Ile-EX-Ure2p (b), U-Ile-SP-Ure2p (c), and PK-U-Ile-SP-Ure2p (d) fibrils. Slices were extracted from the 2D spectra in Figs. 2 and 4, at the $^{13}C$ chemical shifts shown in (a). Colored vertical lines indicate some of the spectral features in (d) that are absent in (b).

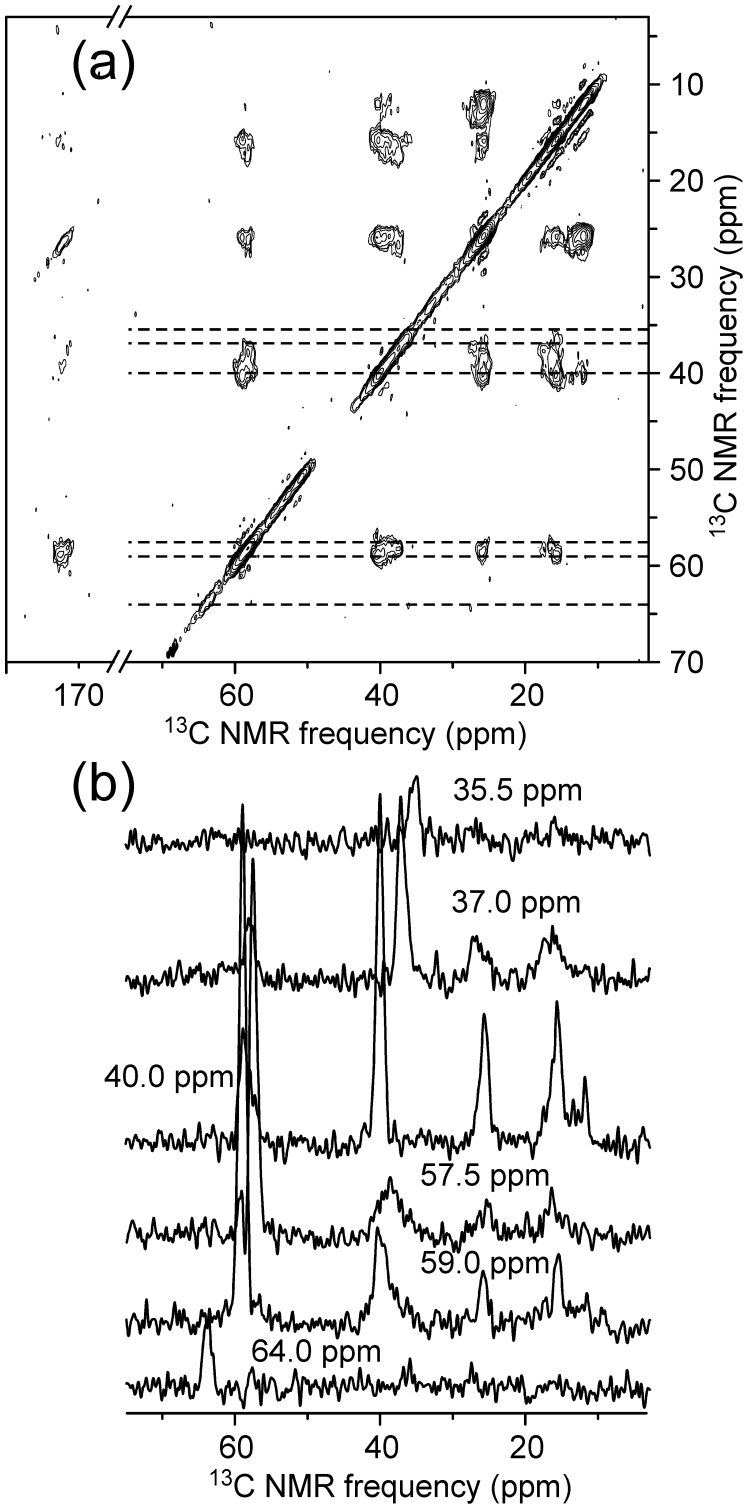


Figure S4: (a) 2D $^{13}$C-$^{13}$C NMR spectrum of U-Ile-SP-Ure2p$_{1\text{-}89}$ fibrils. (b) 1D slices at the indicated $^{13}$C chemical shifts, which correspond to the dashed lines in the 2D spectrum.

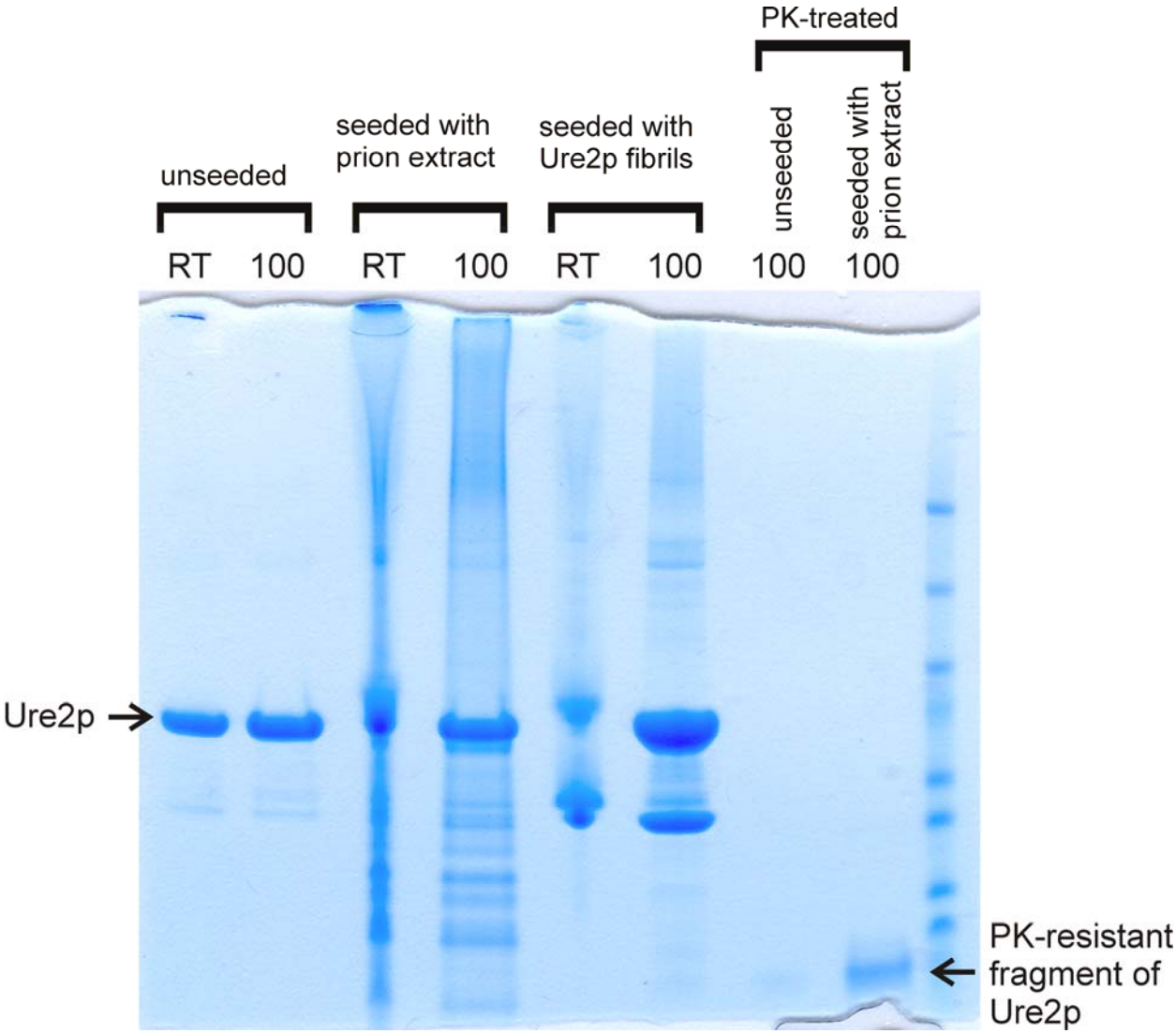


Figure S5: Demonstration by SDS-PAGE that addition of seeds during Ure2p fibril formation accelerates the appearance of SDS- and PK-resistant aggregates. 0.2 mg/ml of Ure2p was incubated without rotation in buffer A for three days at room temperature either alone or with 2% Ure2p seeds (purified from [URE3] lysates or recombinant fibrils). Aliquots were treated with the SDS-PAGE sample buffer containing 2% SDS for 5 min either at room temperature (RT) or at 100°C (100). SDS treatment did not fully dissolve the seeded (but not unseeded) Ure2p aggregates, confirming their amyloid nature. Additionally, 50 µl aliquots were treated with 0.01 mg/ml PK overnight at 37° C, spun down at 13000 X *g* for 10 min, and loaded on the SDS-PAGE gel, which was stained with Coomassie blue.