Supplementary Materials: A New Folding Kinetic Mechanism for Human Transthyretin and the Influence of the Amyloidogenic V30M Mutation

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Figure S1. Characterization of the TTR native species. Size-exclusion chromatograms of WT-TTR and V30M-TTR in native conditions (20 mM sodium phosphate buffer, 150 mM sodium chloride, pH 7.0) and 25 °C. The single peak with an elution volume of approximately 22 mL and a molecular weight of nearly 55 kDa corresponds to the native tetrameric form of TTR.

Figure S2. Fluorescence spectra of WT-TTR and V30M-TTR at intermediate urea concentrations. Fluorescence spectra of 1.0 µM WT-TTR and V30M-TTR obtained upon dialysis of fully-unfolded protein samples against 1.0 M and 2.0 M urea (in 20 mM sodium phosphate buffer, 150 mM sodium chloride, pH 7.0), evidencing differences in emission maxima (with excitation wavelength of 290 nm). While at 1.0 M urea, both WT- and V30M-TTR show emission maxima close to 340 nm (characteristic of native tetramers), which is not the case at 2.0 M urea, where only WT-TTR exhibits the emission spectrum characteristic of native tetrameric TTR.
Figure S3. Fits of TTR refolding traces using a single-step model. Fluorescence intensity decays (upper panels) and weighted residuals (lower panels) obtained for WT- and V30M-TTR at different urea concentrations, pH 7.0 and 25 °C, at a 1.0 μM protein concentration. Intrinsic fluorescence was monitored at 380 nm, with an excitation wavelength of 290 nm. Data were fitted to a single-step mechanism ($U \rightarrow T$), with no intermediate species, considering first-order (left panels) and second-order (right panels) reactions. In both cases, the fits agree poorly with the experimental data, indicating that the process is likely to occur through a more complex mechanism, involving the presence of intermediate species.
Figure S4. Dependence of the kinetic constants on TTR concentration. Apparent rate constants $k_{app1}$ (circles) and $k_{app2}$ (squares) at different TTR protein concentrations were obtained using a mechanism with two first-order consecutive steps (A) and a first-order step followed by a second-order step (B), as was used in the proposed mechanism in the present paper.
Figure S5. Size-exclusion chromatogram of WT-TTR at 0.4 M urea. Chromatogram of tetrameric WT-TTR loaded to a size-exclusion chromatography (SEC) column conveniently equilibrated with 0.4 M urea, 20 mM sodium phosphate buffer, 150 mM sodium chloride, pH 7.0, run at a flow rate of 0.4 mL/min, 25 °C. The single peak observed elutes at a volume of approximately 22 mL (corresponding to ~55 kDa, a molecular weight characteristic of native tetrameric TTR), as observed in the absence of urea.