

SUPPLEMENTARY MATERIAL

The immunoturbidimetric assay quantitatively assesses whether tafamidis stabilizes the transthyretin (TTR) tetramer fold against urea-denaturation (Fig. S1) [1]. Urea, a chaotropic agent, is routinely used when assessing protein structure and folding. It interferes with the non-covalent forces that mediate and stabilize a proteins' three-dimensional structure (i.e., hydrogen bonds and hydrophobic interactions), reducing the energy barrier for protein unfolding. In urea, TTR tetramers are very stable and relatively resistant to denaturation whereas TTR monomers rapidly and irreversibly unfold, which prevents monomers from reassembling into TTR tetramers. Thus, tetramer dissociation becomes the sole determinant of TTR tetramer concentration, and decreases in tetramer concentration over time provide a direct measure of tetrameric dissociation. The immunoturbidity assay measures TTR tetramer in plasma. In this method, urea is added and after 48 hours to allow tetramer dissociation, glutaraldehyde is added and a TTR antibody is used to quantify the TTR protein. The glutaraldehyde, a cross-linking agent, heavily modifies the unfolded monomers such that they are no longer recognized by the TTR antibody. Therefore, TTR immunoturbidity specifically measures the remaining concentration of TTR tetramers. Taken together, the percentage decrease in TTR immunoturbidity after 48 hours of incubation in the presence of urea (the Fraction of Initial, FOI), provides a measure of urea-induced TTR tetramer dissociation. FOI is a quickly-measured, reliable output amenable to automation.

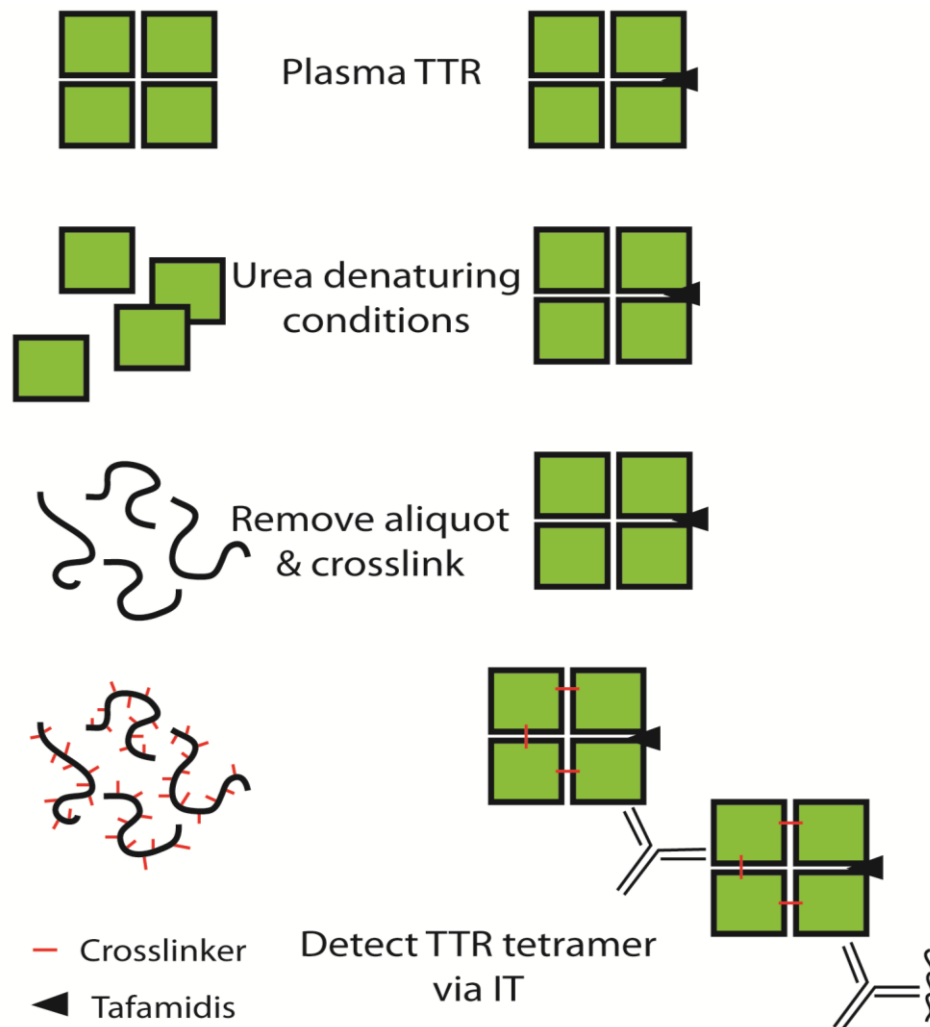
To evaluate the effect of tafamidis in the clinical setting, tetramer dissociation is measured in plasma samples taken before the first dose of tafamidis (FOI baseline) and after dosing (FOI dosed) and 'percent TTR stabilization' is calculated as:

$$\frac{FOI \text{ dosed} - FOI \text{ baseline}}{FOI \text{ baseline}} \times 100$$

In experiments where tafamidis is added ex vivo, tafamidis ('FOI dosed') or vehicle ('FOI baseline') is added 15 minutes prior to the urea incubation. A value of 0% TTR stabilization indicates that tafamidis had no impact on dissociation, whereas 100% indicates a 2-fold increase and 200% a 3-fold increase in the amount of TTR tetramer remaining after 48 hours of incubation in urea.

The immunoturbidimetric assay determines whether a human plasma sample is considered TTR "stabilized" or "non-stabilized". Data from placebo-treated healthy volunteers participating in a Phase 1 study evaluating single and multiple doses of tafamidis were analyzed to assess random variation in FOI and demonstrated that values of percentage TTR stabilization above 32% are unlikely to occur in patients who do not receive TTR stabilizing therapy. Hence, a cut-off value of 32% was chosen above which tafamidis is considered to have stabilized the TTR tetramer fold.

Fig. S1 Overview of the transthyretin (TTR) stabilization immunoturbidity (IT) assay. In the presence of urea, TTR monomer, in equilibrium with TTR tetramer, quickly denatures to unfolded monomer, which is irreversible in high urea concentrations, preventing the monomers from reassembling into TTR tetramers whereby tetramer dissociation becomes the sole determinant of TTR tetramer concentration. After incubation in urea, samples are cross-linked, quenched, and TTR levels measured using the IT assay.



REFERENCES

1. Bulawa CE, Connelly S, Devit M, Wang L, Weigel C, Fleming JA, Packman J, Powers ET, Wiseman RL, Foss TR, Wilson IA, Kelly JW, Labaudinière R. Tafamidis, a potent and selective transthyretin kinetic stabilizer that inhibits the amyloid cascade. *Proc Natl Acad Sci USA*. 2012;109:9629-34.