



Differential Transthyretin Binding, Kinetic Stability and Additive Ex Vivo Stabilization by AG10 Compared to Tafamidis

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Background

- Transthyretin (TTR) amyloidosis (ATTR) is a progressive, fatal disease wherein deposition of either mutant or wild-type TTR amyloid can cause severe organ damage and dysfunction.
- ATTR cardiomyopathy (ATTR-CM) results in a high burden of morbidity and mortality from progressive heart failure with few therapeutic options.
- Formation of TTR amyloid is initiated by dissociation of destabilized tetrameric TTR into its constituent monomers and subsequent misfolding, aggregation, and tissue deposition as amyloid fibrils.
- AG10, an investigational molecule, is a highly selective and potent stabilizer of TTR that mimics the T119M rescue mutation and has been studied in Phase 1 and 2 clinical studies.^{1,2}

Hypothesis

- AG10 displays differential TTR binding, kinetic stability, and a higher degree of stabilization compared to other TTR stabilizers.
- In vitro, AG10 achieves near-complete stabilization of TTR at clinical concentrations.

Materials & Methods

- Commercially available tafamidis (TAF) was used in this study. Purified human TTR was purchased from Athens Research and AlexoTech. Pooled human serum and plasma was from Innovative Research.
- Thermodynamic stability (K_d) of TTR interaction was determined by microscale thermophoresis (MST) using a Nanotemper Monolith NT.115 instrument with red maleimide labelled TTR. Data was processed using PALMIST software.
- Kinetic stability was assessed by surface plasmon resonance (SPR) using a GE Biacore T200 instrument. Purified TTR was immobilized on CM5 chips via NHS/EDC coupling. Data was processed using GE T200 Evaluation software.
- The ability of each stabilizer to prevent accelerated tetramer dissociation over 72 hrs at pH 3.8 alone or in combination was measured by Western Blots¹. Tetrameric TTR bands were quantified using Li-Cor Image Studio software.
- The binding site occupancy of TTR in serum was measured by fluorescent probe exclusion assay (FPE) according to an established method³. Target occupancy was calculated from progress curves at 1 hr.
- Therapeutic concentrations associated with administration of 80 mg tafamidis meglumine (trough = 16 μ M and peak = 26 μ M) were estimated from publicly available FDA documents.^{4,5}

TTR Binding Affinity of AG10 and Tafamidis by Microscale Thermophoresis

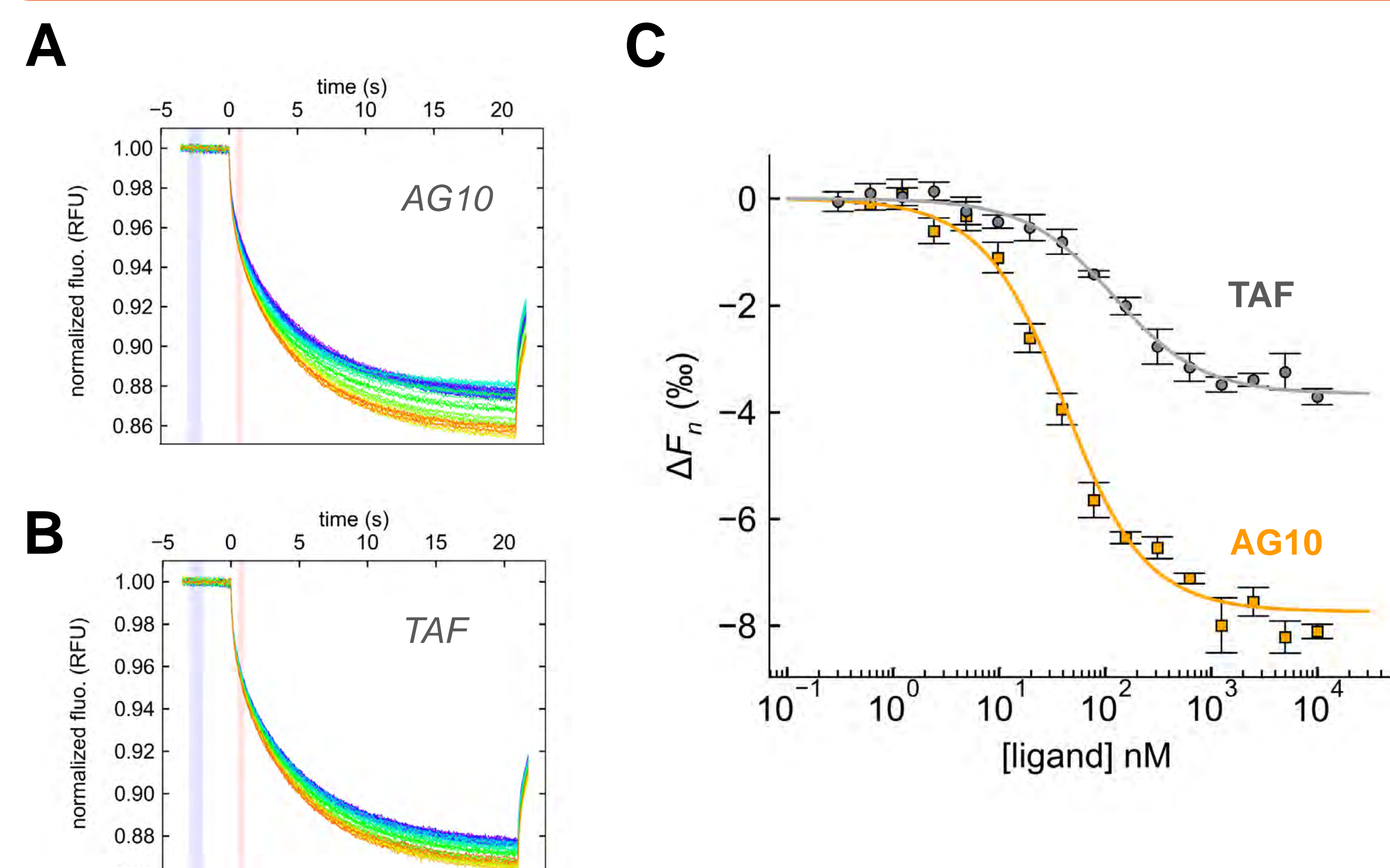


Figure 1. Microscale thermophoresis (MST) quantitation of TTR binding affinity. Mean and standard error shown. Averaged MST traces of fluorescently labeled purified human TTR titrated with:

- A) AG10** $K_d = 32 \pm 11$ nM, $n=4$
B) TAF $K_d = 110 \pm 20$ nM, $n=4$
C) Normalized average fluorescence dose response curves

TTR Binding Kinetics of AG10 and Tafamidis by Surface Plasmon Resonance

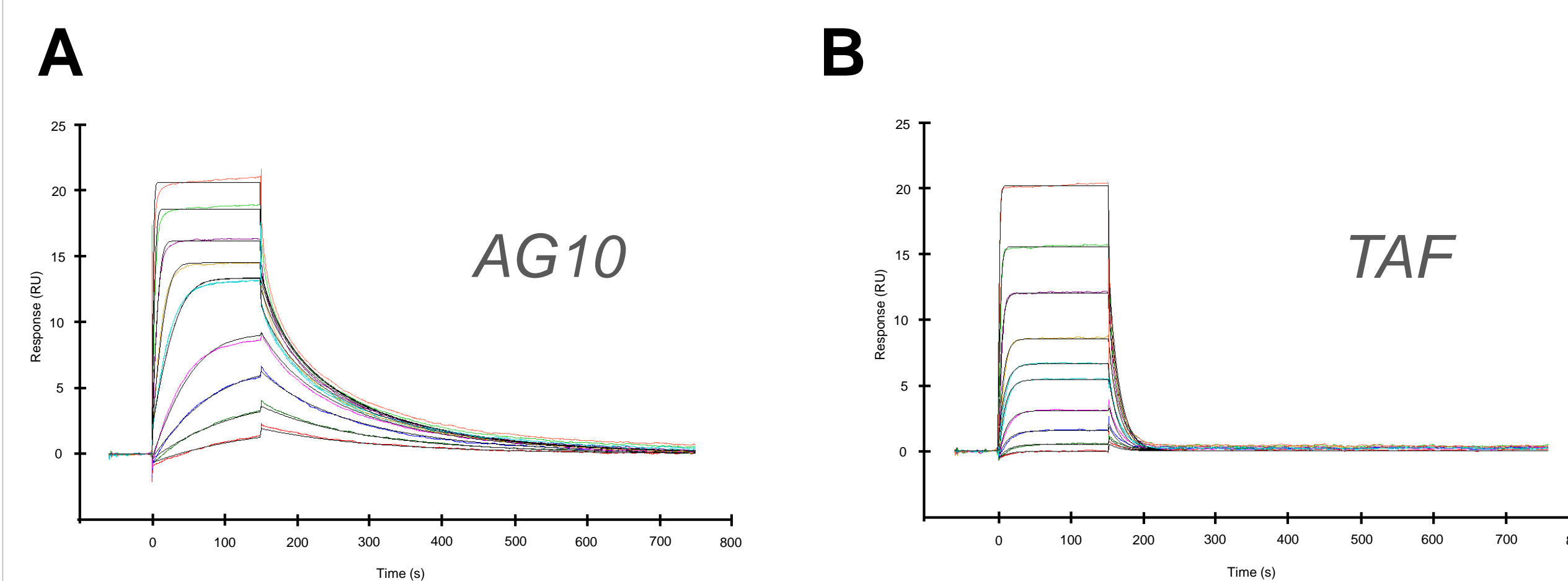


Figure 2. Surface plasmon resonance (SPR) characterization of TTR binding kinetics. Representative SPR sensorgrams from an immobilized purified human TTR surface probed with either AG10 or TAF. Mean and standard deviation of residence times (τ) and K_d were measured as:

- A) AG10:** $\tau = 50 \pm 4$ s $K_d = 16 \pm 2$ nM, $n=4$
B) TAF: $\tau = 12 \pm 3$ s $K_d = 120 \pm 30$ nM, $n=4$

Stabilization of TTR by AG10 and Tafamidis (Western Blot Assay)

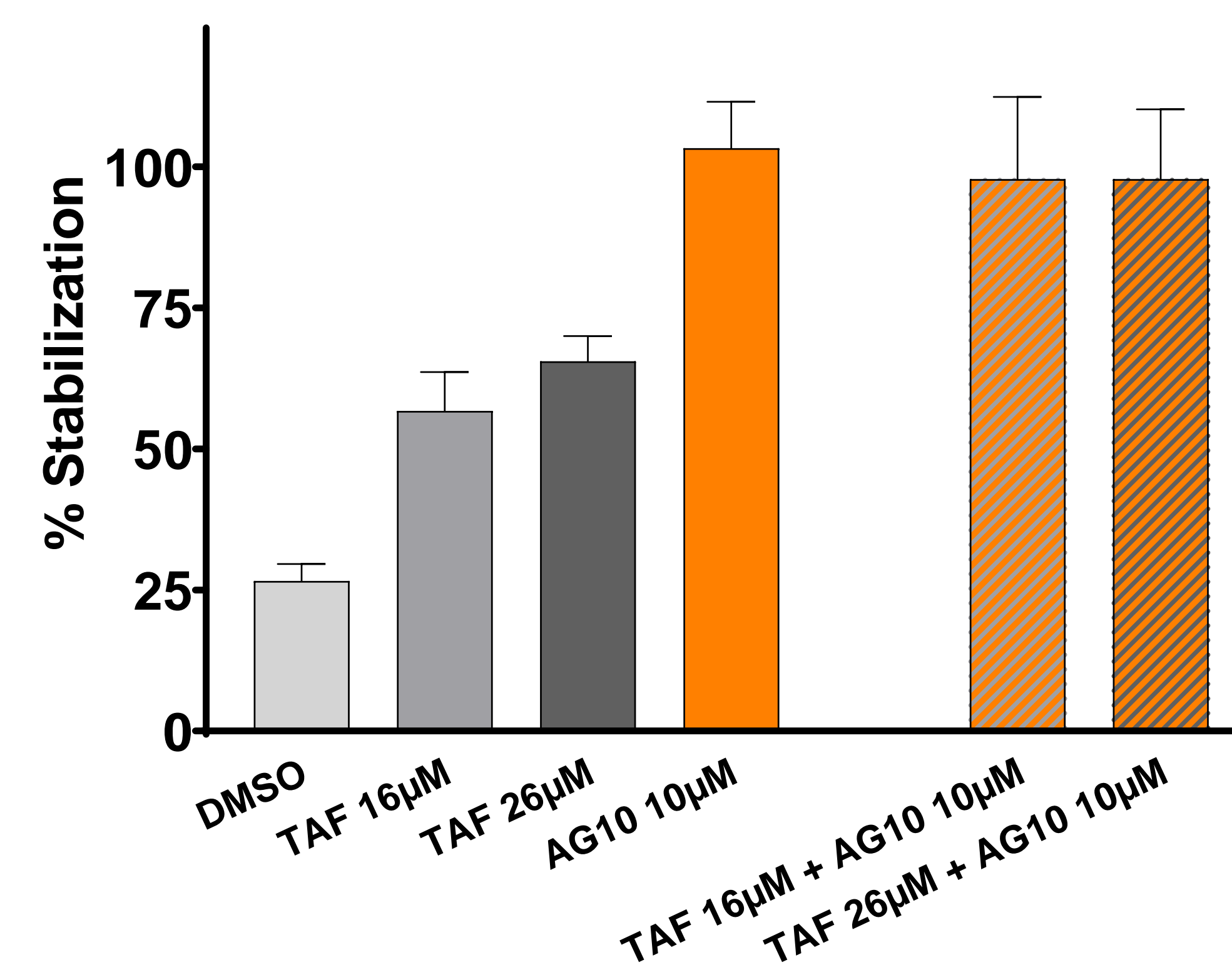


Figure 3. Western Blot quantitation of tetrameric TTR in plasma samples incubated with stabilizers. Each bar represents the percentage of tetrameric TTR remaining after 72 hr incubation at pH 3.8, $n = 3$ independent experiments. DMSO = control. Mean and standard deviation shown.

TTR Target Site Occupancy by FPE Assay

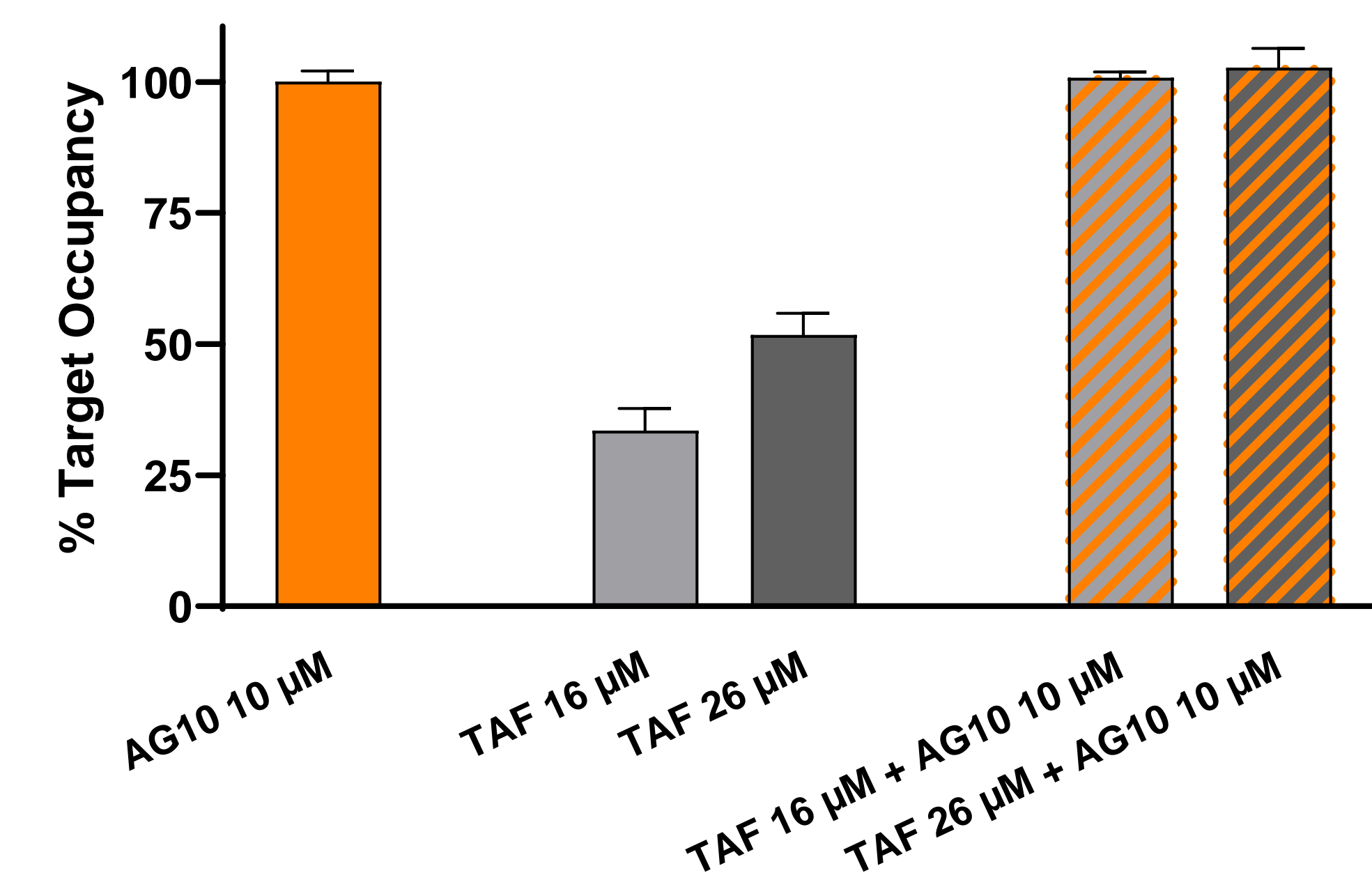


Figure 4. FPE characterization of TTR binding site occupancy in serum incubated with stabilizer, $n = 12$ for each condition tested. Target occupancy with mean and standard deviation shown.

Biophysical and Pharmacodynamic Properties of AG10 and Tafamidis

	Biophysical properties		
	MST K_d (nM) [†]	SPR K_d (nM) [*]	SPR residence time (s) [*]
AG10	32 ± 11	16 ± 2	50 ± 4
TAF	110 ± 20	120 ± 30	12 ± 3

	PD assay properties	
	WB Stabilization (%) [*]	FPE Target Occupancy (%) [*]
10 μ M AG10	103 ± 8	100 ± 2
16 μ M TAF	57 ± 7	34 ± 4
26 μ M TAF	66 ± 5	52 ± 4
16 μ M TAF + 10 μ M AG10	98 ± 15	101 ± 1
26 μ M TAF + 10 μ M AG10	98 ± 12	103 ± 4

[†] Mean ± standard error. ^{*} Mean ± standard deviation.

Summary

- The affinity of AG10 for purified TTR, as measured by MST, is 3X greater than that of tafamidis. Kinetic stability by SPR reveals over 4X longer residence time for AG10 bound to TTR as compared to tafamidis.
- When evaluated at therapeutic plasma concentrations, in vitro incubation of tafamidis alone does not completely stabilize tetrameric TTR.
- In vitro addition of AG10 to tafamidis results in complete stabilization of tetrameric TTR.
- Therapeutic concentrations of tafamidis in serum do not fully occupy the TTR binding site. Addition of AG10 to tafamidis treated samples results in complete binding site occupancy.

Conclusions

- The extended residence time of AG10 compared to tafamidis results in improved TTR binding site occupancy and stabilization.
- In vitro, AG10 completely stabilized TTR in plasma samples with or without therapeutic concentrations of tafamidis.
- These findings support further development of AG10 as a disease-modifying treatment for patients with ATTR cardiomyopathy

References

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