Supporting Information for:
Fluorotryptophan incorporation modulates the structure and stability of transthyretin in a site-specific manner

Xun Sun, H. Jane Dyson, Peter E. Wright*

Affiliations:
[1]Department of Integrative Structural and Computational Biology and Skaggs Institute of Chemical Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037, USA

* Correspondence to Peter E. Wright wright@scripps.edu

Table S1 Molecular weight of TTR labeled with 5FW/6FW measured by ESI-TOF-MS

Construct	Calculated molecular weight for fully labeled (Da)	Measured molecular weight (Da)	Incompletely labeled [a]
WT-5FW	13928.6	13926.8	N.A.
W41Y-5FW	13887.6	13885.6	N.A.
WT-6FW	13928.6	13927.7	7%
W41Y-6FW	13887.6	13885.6	N.A.

[a] Incomplete labeling calculated as the relative abundance of incompletely labeled species as compared to the sum of the abundance of fully, singly (if applicable) and non-labeled species found in the respective mass spectrum. The ionization efficiency was assumed to be the same for labeled and non-labeled TTR species under our experimental condition. N.A. means mass not observed.

Table S2 Longitudinal relaxation time constant (T_1) and linewidth for WT-TTR with fluorinated Trp at 14.1 T and 298 K

Construct	W41 T_1 (s)[a]	W41 line width (Hz)[b]	W79 T_1 (s)	W79 line width (Hz)
WT-5FW	1.22 ± 0.04	102 ± 1	1.20 ± 0.06	141 ± 2
WT-6FW	1.17 ± 0.10	139 ± 2	1.14 ± 0.05	146 ± 3
Unfolded WT-5FW	0.76 ±0.02	31 ± 1	0.72 ± 0.03	31 ± 1
Unfolded WT-6FW	0.71 ± 0.02	32 ± 1	0.78 ± 0.04	33 ± 1

[a] Uncertainties calculated as one standard deviation from 50 bootstrapped datasets.

[b] Line width includes a 10-Hz broadening factor from the exponential apodization function.

Table S3 Translational diffusion coefficients measured by diffusion ordered NMR spectroscopy at 21.2 T and 298 K

Construct	Concentration (μM)	Translational diffusion coefficient ($\times 10^{-7}$ cm^2/s)[a]
WT	1000	4.8 ± 0.1
WT	750	4.9 ± 0.1
WT	500	4.8 ± 0.1
WT	250	4.8 ± 0.1
WT	100	5.1 ± 0.2
WT-5FW	100	4.8 ± 0.2
WT-6FW	100	4.8 ± 0.1
W41Y	100	4.7 ± 0.2
W41Y-6FW	100	4.8 ± 0.2

[a] Uncertainties propagated as standard error from the linear regression.

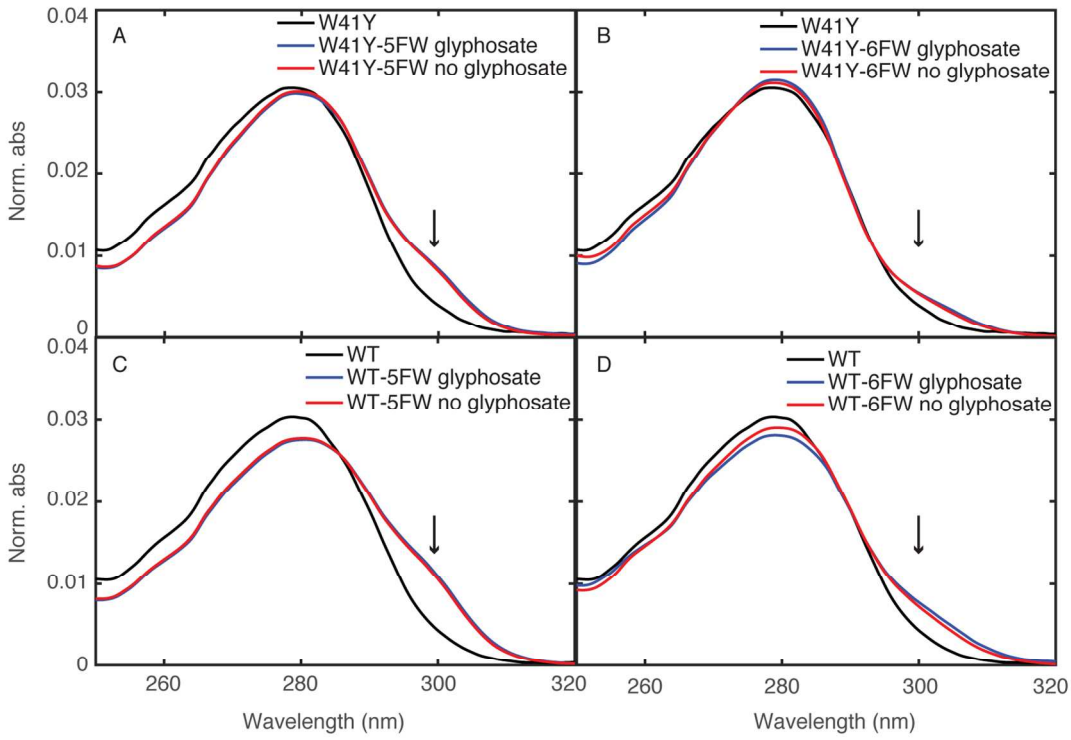

Figure S1. Comparison of UV absorbance spectra for W41Y and WT-TTR labeled with 5FW or 6FW. The inclusion of 1 mg/L glyphosate as the inhibitor for aromatic amino acid biosynthesis during expression is not needed to achieve high incorporation yield for labeled TTR. The absorbance side band at around 300 nm denoting the incorporation of fluorinated Trp is labeled by arrow. In all cases, the absorbance spectra with and without glyphosate are highly similar. Spectra are normalized by the area under the curve for easy view.

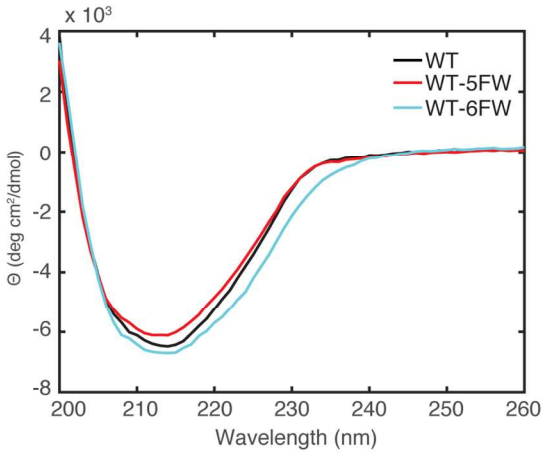

Figure S2. Comparison of CD spectra for TTR variants. Incorporation of 5FW or 6FW to WT-TTR does not significantly change the secondary structures of labeled WT-TTR as compared to unlabeled WT-TTR.

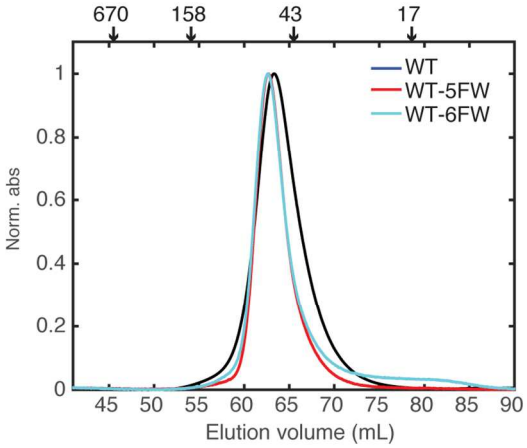

Figure S3. Comparison of Superdex 75 size-exclusion elution profiles of WT, WT-5FW and WT-6FW. The absorbance at 280 nm is normalized by the peak height around 62 mL, which corresponds to the molecular weight of a tetrameric TTR (55.5 kDa). The molecular weight calibration (kDa) is labeled on the top for reference.

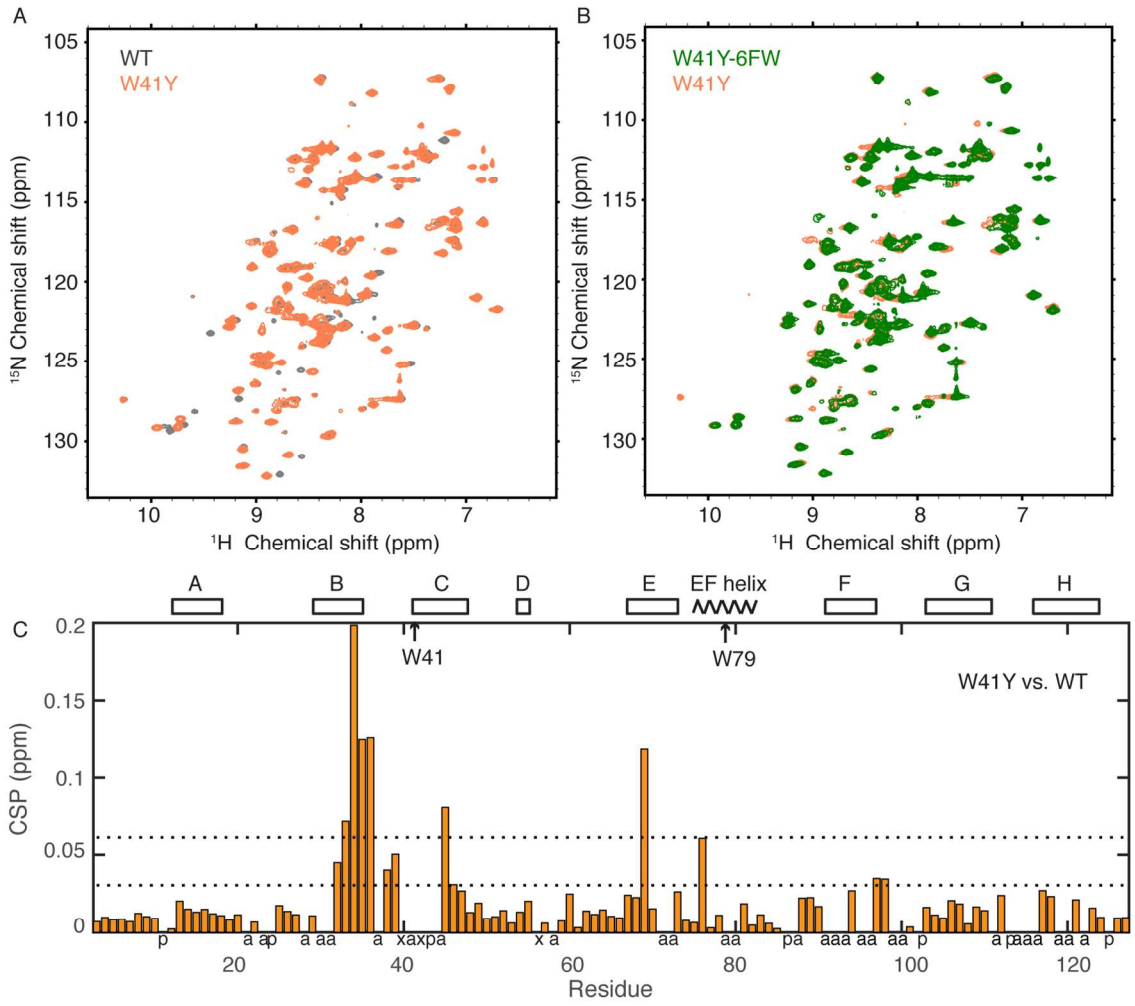

Figure S4. Backbone amide chemical shifts of W41Y and W41Y-6FW measured by HSQC. (A, B) Overlaid [^{1}H-^{15}N]-HSQC spectra of WT (A) and W41Y-6FW (B) with W41Y collected at 298 K and 900 MHz. (C) Weighted average backbone amide CSP comparison for W41Y with WT. The dashed lines denote one or two standard deviations. Letter a denotes ambiguous CSP, x for missing peaks and p for proline residues. Two Trp residues at 41 and 79 and secondary structures are labeled on top of figure.

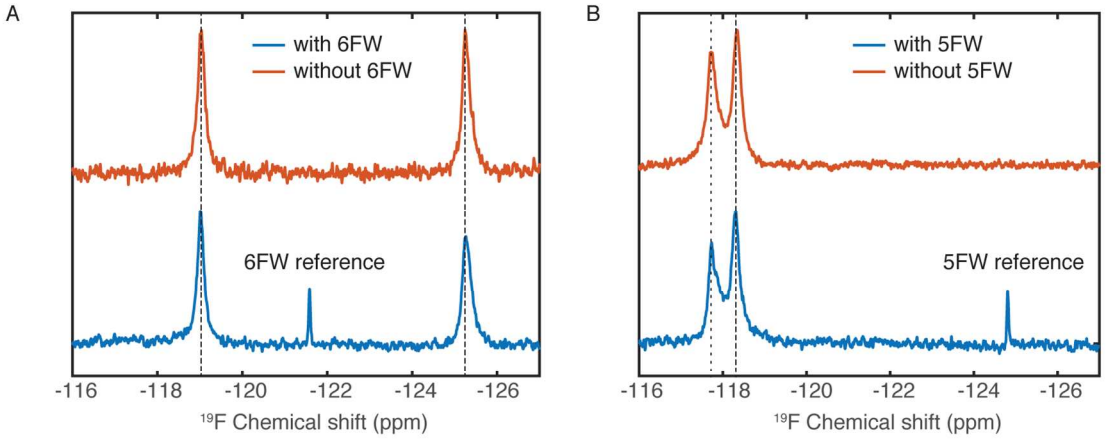

Figure S5. Comparison of ^{19}F-NMR spectra of WT-5FW (A) and WT-6FW (B) with and without the internal reference. 6FW and 5FW were used as reference for WT-5FW and WT-6FW, respectively. In both cases (each spectrum was from a different protein prep), the presence of the internal standard does not alter the chemical shifts of either W41 or W79, as indicated by the dashed vertical lines. The intensity is normalized to the highest peak and each base line is offset purposely to show the individual spectrum. All spectra were recorded at 298 K with 100-200 μM TTR (monomer concentration).

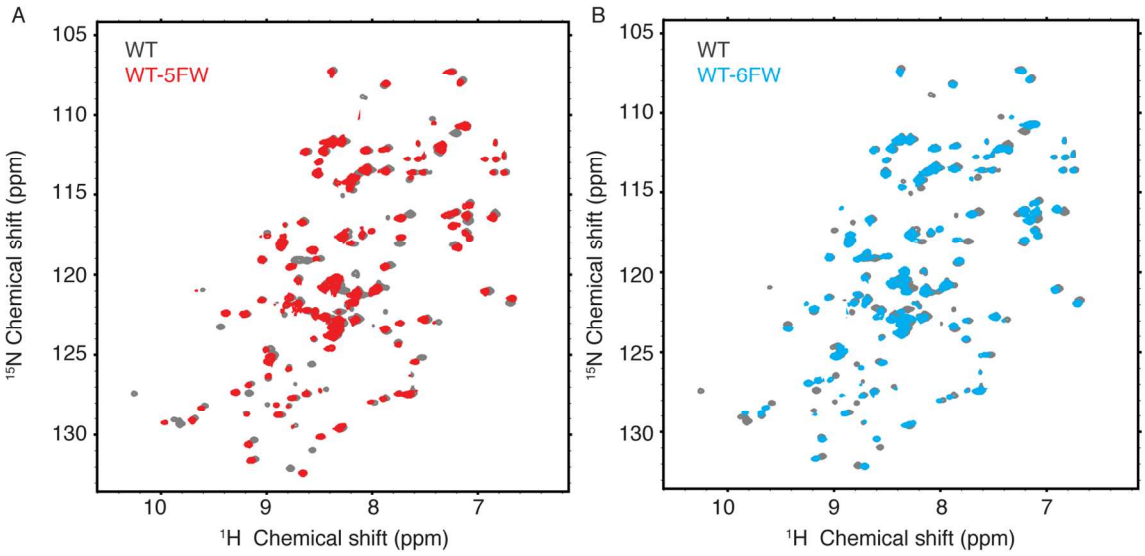

Figure S6. Backbone amide chemical shift comparisons of WT-5FW and WT-6FW. (A, B) Overlaid [^{1}H-^{15}N]-HSQC spectra of WT-5FW (A) and WT-6FW (B) with WT-TTR collected at 298 K and 900 MHz.

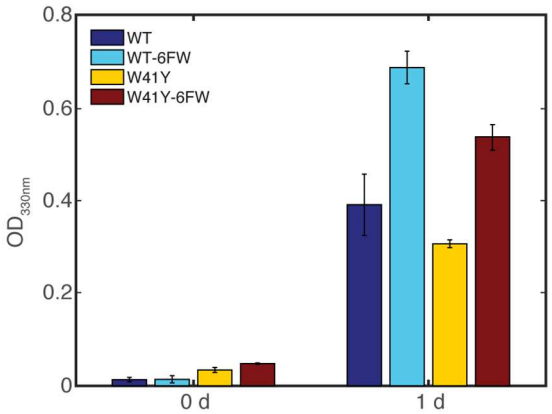

Figure S7. Aggregation comparison for TTR mutants, monitored by optical density at 330 nm (OD_{330}) at 310 K for 1 day. W41Y is not aggregation-prone as compared to WT. The 6FW-labeling increases the aggregation propensity for WT and W41Y, respectively.