Supporting Information

Binding of monovalent and bivalent ligands by transthyretin causes different short and long distance conformational changes

Alessandra Corazza†,#,‡,*, Guglielmo Verona#, Christopher A. Waudby§,||, P. Patrizia Mangione#,⊥, Ryan Bingham&, Iain Uings&, Diana Canetti#, Paola Nocerino#, Graham W. Taylor#, Mark B Pepys#,¶, John Christodoulou§,||, & Vittorio Bellotti#,⊥,*

Contents:

Methods

Protein expression and purification. A peTM11 plasmid encoding hexahistidine-tagged wild type TTR, provided by Dr Trevor Forsyth, Institut Laue Langevin, Grenoble, France, was transformed into BL21 Star (DE3) cells (ThermoFisherScientific) and plated onto LB-agar plates containing 30 μg/ml kanamicin. A single selected colony was then incubated overnight at 37°C with shaking in LB medium containing 30 µg/ml kanamicin . The culture was adapted to grow in a deuterated background by stepwise addition of Ross medium prepared in $^{2}H_2O$ supplemented with ^{15}N ammonium sulphate and with ^{13}C glucose for 7 steps. The final overnight culture was inoculated into 500 ml of medium, which was then incubated at 37°C. When the A_{600} of the culture reached 0.5, the temperature was reduced to 30°C and protein expression was induced by addition of IPTG at a final concentration 1 mM when the A_{600} reached 0.6. After ~16-18 h, cells were harvested by centrifugation and lysed on ice by sonication. The supernatant after centrifugation at 30min at 11,300 g was loaded onto a HisTrap FF crude nickel affinity chromatography column (GE Healthcare) equilibrated in lysis buffer. After extensive washing with 20 mM Tris-HCl,10 mM imidazole, containing stepwise increasing concentrations of NaCl, 250 mM, 500 mM and 1 M, the column was eluted with 20 mM Tris-HCl, 250 mM NaCl, 250 mM imidazole, pH 8.0. His-tagged TEV protease (Sigma-Aldrich) was added at1% w/w to selectively cleave the hexaHis-tag, which was then removed by affinity chromatography, together with the enzyme. The TTR was then isolated by gel filtration on a Superdex 75 Hi Load 26/60 column (GE Healthcare) equilibrated and eluted with 25 mM Tris-HCl, 100 mM NaCl, pH 8.0. The purity, molecular weight, and the final level of deuteration were determined by SDS-PAGE analysis and mass spectrometry respectively.

NMR spectroscopy. A series of 3D TROSY-based spectra, with ^{2}H decoupling HNCA, HN(CO)CA, HNCACB, HNCO and HN(CA)CO, was acquired to perform the backbone assignment. The TROSY-based experiments HNCA and HN(CO)CA, HNCACB, HNCO and HN(CA)CO had 4096x50x64, 4096x50x96 and 3072x64x64 complex points, respectively. The spectral widths were 18ppm (^{1}H), 27 ppm (^{15}N) and 30 ppm (HNCA and HN(CO)CA), 10 ppm (HNCO and HN(CA)CO) and 70 ppm (HNCACB) in the ^{13}C dimension. The spectra were processed with Topspin 3.5 (Bruker Biospin) or NMRPipe [1] and analysed in Sparky [2].

2048 x 256 complex points were recorded with a sweep width of 15 ppm (^{1}H) and 32 ppm (^{15}N) and 4-8 scans.

Surface Plasmon Resonance. Human wild type TTR isolated from serum (Scipac) was biotinylated according to the method of Alhamadsheh *et al.* [3] using EZ-link sulfo NHS-LC biotin (Thermoscientific) and immobilised in

phosphate buffered saline (PBS), 0.05% Tween20 to achieve 3000-4000 response units of biotinylated TTR bound to the SA-chip surface following the manufacturer's instructions. To block unoccupied sites, biocytin (50 μM) was flowed across the chip (at a flow rate of 50 μl/min, for 1 min). Finally, the chip was washed with Hepes Buffer Saline, pH 7.4 (+ 0.05 % Tween20) running buffer until the SPR signal reached a stable background level.

For all procedures, the running buffer was used at a flow rate of 30 μl/ml. The immobilised TTR surface was exposed to tafamidis at a range of concentrations (0.5 – 0.01 μM) to monitor the association (3 min injection at 30 μl/min) and then running buffer alone to monitor the dissociation phase. Between injections sufficient time was given for the signal to return to pre-injection background. Sensograms were corrected for bulk refractive index effects and analysed external to the Biacore software to enable the analysis of the association and dissociation phases independently.

References:

1. Delaglio, F.; Grzesiek, S.; Vuister, G. W.; Zhu, G.; Pfeifer, J.; Bax, A. NMRPipe: a multidimensional spectral processing system based on UNIX pipes. *J Biomol NMR* **1995,** 6, 277-93.
2. Sparky 3. In San Francisco, 2008.
3. Alhamadsheh, M. M.; Connelly, S.; Cho, A.; Reixach, N.; Powers, E. T.; Pan, D. W.; Wilson, I. A.; Kelly, J. W.; Graef, I. A. Potent kinetic stabilizers that prevent transthyretin-mediated cardiomyocyte proteotoxicity. *Sci Transl Med* **2011,** 3, 97ra81.

Table S1. Temperature coefficients of apo-and holo-TTR with tafamidis and mds84.

	apo-TTR	tafamidis	mds84
RES	**ppb/K**	**ppb/K**	**ppb/K**
G1	***-5.74***		***-5.58***
P2			
T3			***-7.29***
G4	***-4.83***		
T5			
G6	***-5.75***		
E7	-4.32	***-5.06***	***-4.62***
S8		***-5.25***	***-4.55***
K9			
C10			
P11			
L12	-2.01	-1.63	-2.38
M13	-2.22	-3.19	-2.47
V14	3.14	-1.92	2.52
K15	-0.73	-1.42	-1.71
V16	-1.81	-3.27	-2.24
L17	2.49	-1.75	
D18	-1.66	-3.76	***-4.79***
A19	-2.03	-2.23	
V20	-0.59	-0.68	-0.9
R21	***-4.69***	-2.57	***-7.21***
G22		0.11	
S23	-1.04	-2.38	-2.3
P24			
A25	1.31	-0.63	1.05
I26	***-7.89***	***-8.61***	***-7.61***
N27	-0.72	-1.08	-1.25
V28		***-4.96***	
A29	***-5.25***	***-5.36***	***-4.94***
V30	-3.84	***-5.65***	-2.95
H31	-0.23	-2.04	-2.04
V32	-4.47	-4.33	-4.48
F33	-2.36	-2.12	-2.04
R34	-1.88	-1.81	-1.99
K35	-3.84	-3.47	-3.86
A36	***-4.94***	***-4.87***	***-4.94***
A37	-2.96	-2.63	***-8.49***
D38	0.46	0.48	0.28

D39	-1.36	-1.23	-1.41
T40	-2.51	-2.36	-2.33
W41	***-6.46***	***-5.24***	***-5.45***
E42	0.92	1.61	0.91
P43			
F44	-2.49	-2.39	-2.43
A45	0.78	0.45	0.4
S46	***-7.08***	***-6.82***	***-6.95***
G47	0.11	-0.25	-0.12
K48	***-7.57***	***-7.52***	***-7.59***
T49	-2.06	-2.13	-2.04
S50	-3.23	***-5.17***	-2.48
E51	-1.9	-1.56	-1.79
S52	-2.89	-2.73	-1.74
G53	-2.11	-1.74	-0.66
E54	-0.56	-0.84	0.04
L55	***-5.02***	-2.42	-1.64
H56			
G57			
L58	-4.49	***-6.79***	-2.7
T59	0.62	0.79	-0.24
T60	***-4.57***	***-4.57***	-4.38
E61	***-6.84***	***-6.87***	***-6.51***
E62	***-5.29***	***-5.22***	***-4.77***
E63	-0.64	-0.94	-1.1
F64	-1.86	-1.84	-2.29
V65	-3.3	-3.33	-3.52
E66	***-5.35***	***-5.03***	-2.95
G67	-3.1	-3.12	-2.78
I68	-4.24	-4.23	-4.35
Y69	-2.3	-1.99	-1.52
K70	-0.57	-1.11	-0.77
V71	-3.53	-3.02	-2.83
E72	-1.74	-2.03	-1.77
I73	-3.54	-3.58	-2.94
D74	-4.48	-3.8	-3.5
T75	-1.98	-1.8	-1.27
K76	-0.81	-3.29	-2.85
S77	-1.82	-0.63	-1.12
Y78	-0.27	-0.24	-0.38
W79	-2.4	-1.96	-1.78
K80	-2.59	-2.1	-1.84
A81	-2.14	-2.35	-2.4

L82	-0.69	-0.51	-0.69
G83	-1.92	-1.86	-2.03
I84	-1.97	-1.97	-2.07
S85	***-6.09***	***-6.06***	***-6.01***
P86			
F87	-2.08	-2.47	-1.98
H88	-2.24	-2.49	
E89	-3.63	-3.72	-2.73
H90	-1.09	-1.87	-2.05
A91		-1.47	
E92		***-5.56***	
V93	-2.28	-2.28	-3.11
V94	-3.25	-2.94	-4.48
F95		-2.04	
T96	-0.84	***-7.19***	
A97	***-4.84***	***-4.82***	***-4.78***
N98	***-5.27***	***-5.1***	-3.94
D99	***-6.92***	-4.38	-3.66
S100	***-8.07***	***-10.96***	***-11.01***
G101	2.6	3.15	2.68
P102			
R103	***-6.81***	-0.88	***-7.78***
R104	-2.85	-2.59	-2.58
Y105	-2.82	-2.76	-1.48
T106	-2.35	-2.5	-1.48
I107	-2.03	-1.27	-4.14
A108	-1.53	-0.49	-2.29
A109	-0.58	-2.04	-1.55
L110	-1.64	-1.99	
L111	-1.38	-2.52	-1.85
S112	-3.45	-2.8	-1.51
P113			
Y114	***-5.14***	-3.93	
S115	-0.7	1.05	0.38
Y116		0.59	
S117	-3.66	-2.81	
T118	-1.57	-1.46	
T119			
A120		-0.78	0.21
V121		-2.93	
V122	-0.64	-2.45	-0.89
T123	***-5.66***	***-5.5***	***-5.69***
N124	***-7.3***	-0.77	***-6.83***

P125			
K126	-2.58	-2.65	-2.56
E127	-4.16	-3.88	-4

Temperature coefficients values smaller (in bold-italics) or higher than - 4.5 ppb/K indicate the absence or presence, respectively, of a hydrogen bond involving the amide hydrogen of the residue.

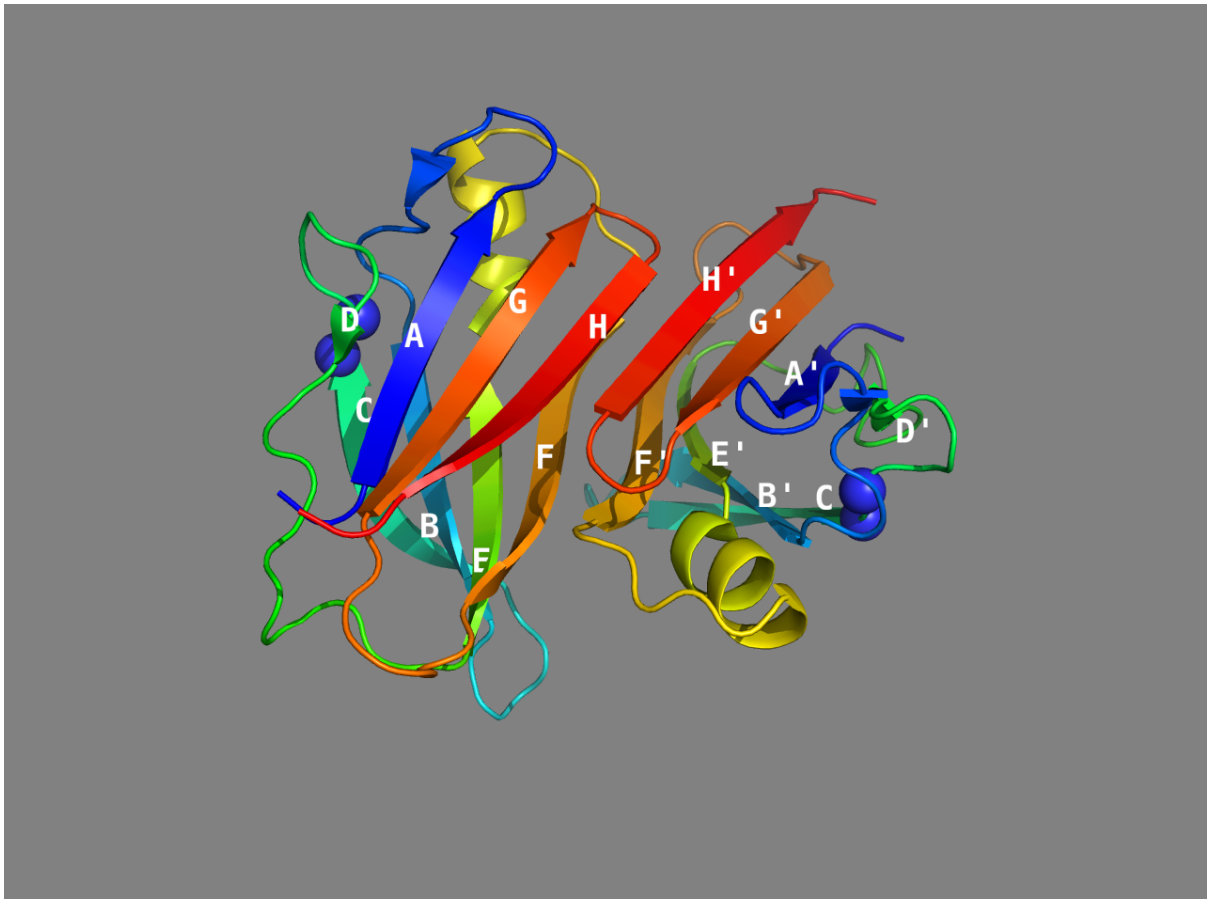

Figure S1. Structure of TTR dimer. The eight strands of the TTR dimer are labelled from A to H (chain A) and A' to H' (chain B). The cleavage site, K48-T49, is represented by spheres.

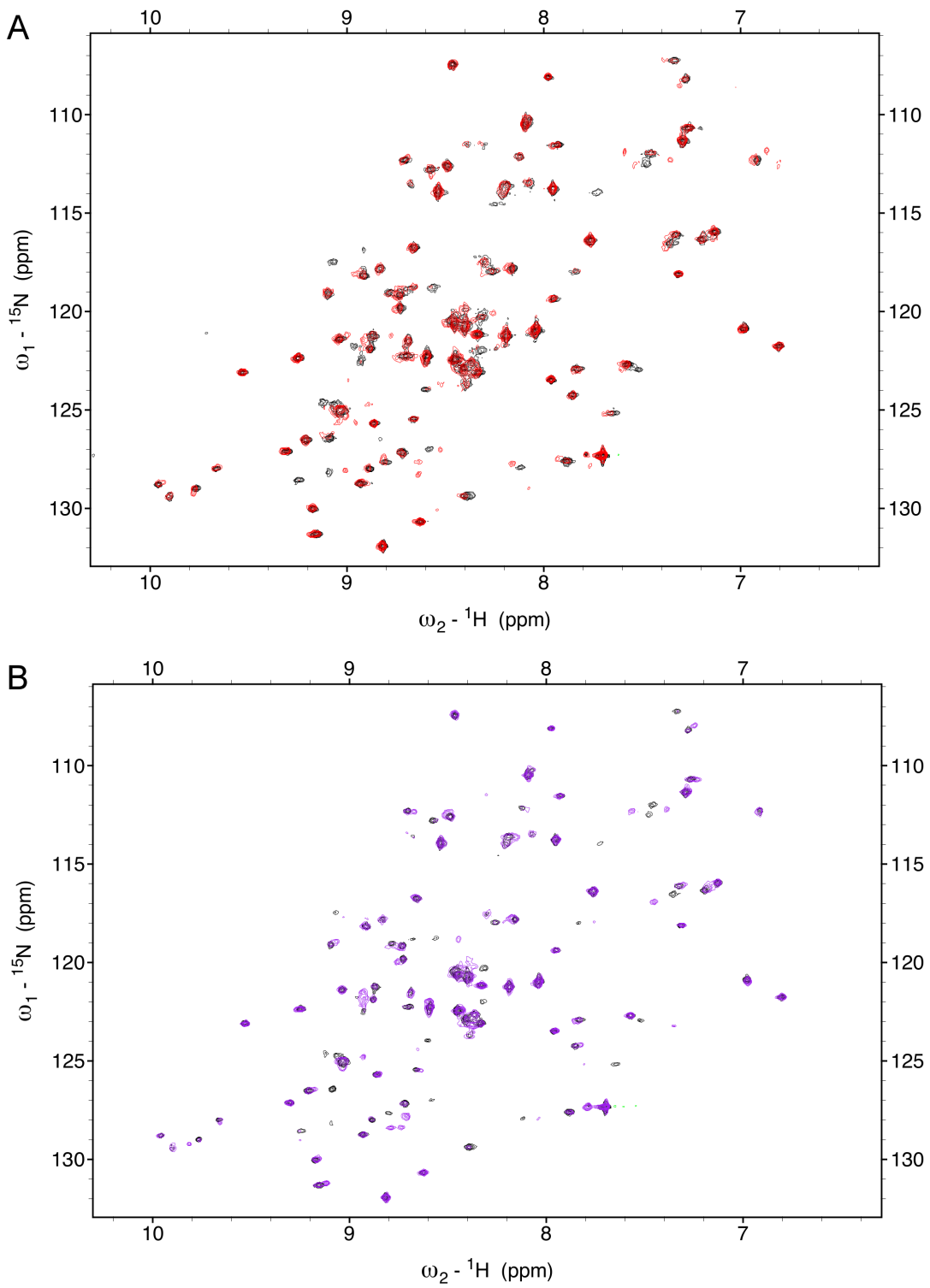

Figure S2. 2D TROSY spectra at sub-stoichiometric ligand:TTR ratios highlight the presence of free and bound peaks. Overlay of the 2D [^{1}H,^{15}N] TROSY spectra of apo-TTR (black) and holo-TTR in the presence of tafamidis (A, red) and mds84 (B, purple) at 0.5:1 ratio. The spectra were acquired at 700MHz.

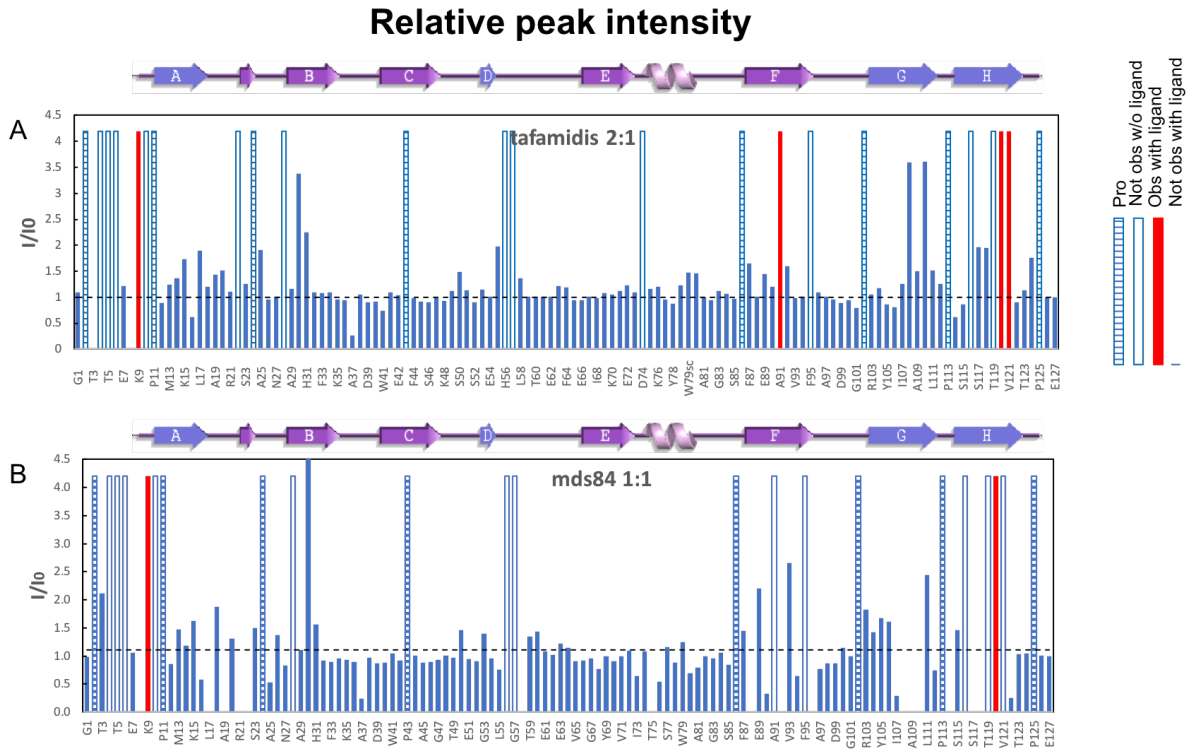

Figure S3. Relative peak intensity of TTR at complete saturation with ligands. (*A*) Peak intensity of TTR in the presence of two-fold molar excess of tafamidis and (*B*) mds84 at equimolar concentration. Values of relative peak intensity of each of the holo-TTR forms with the different ligands are normalized to the corresponding peaks of apo-TTR. Residues not observed in the absence of ligands are shown by empty bars; Pro is shown by striped bars; residues observed only in the presence of ligand are shown by red solid bars. Peaks lost upon ligand binding are shown by empty positions. The horizontal dotted line indicates the average value.

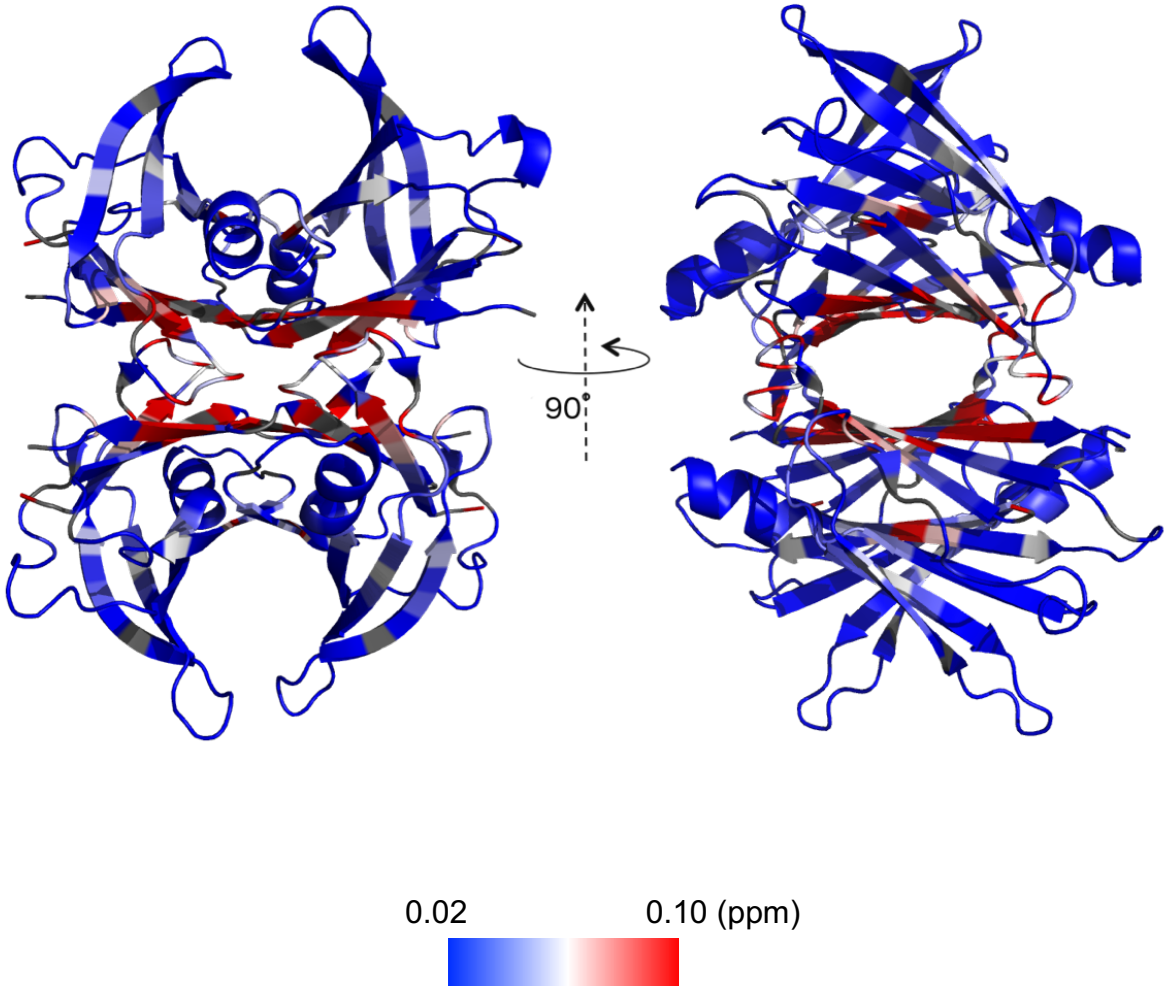

Figure S4. 4,4-dimethyl-4-silapentane-1-sulfonic acid (DSS) is bound in the TTR halogen binding pocket. Chemical shift differences are highlighted on the TTR molecule, shown in two views rotated by 90º. The colour scale gradient is blue-white-red from minimum to maximum combined chemical shift change.

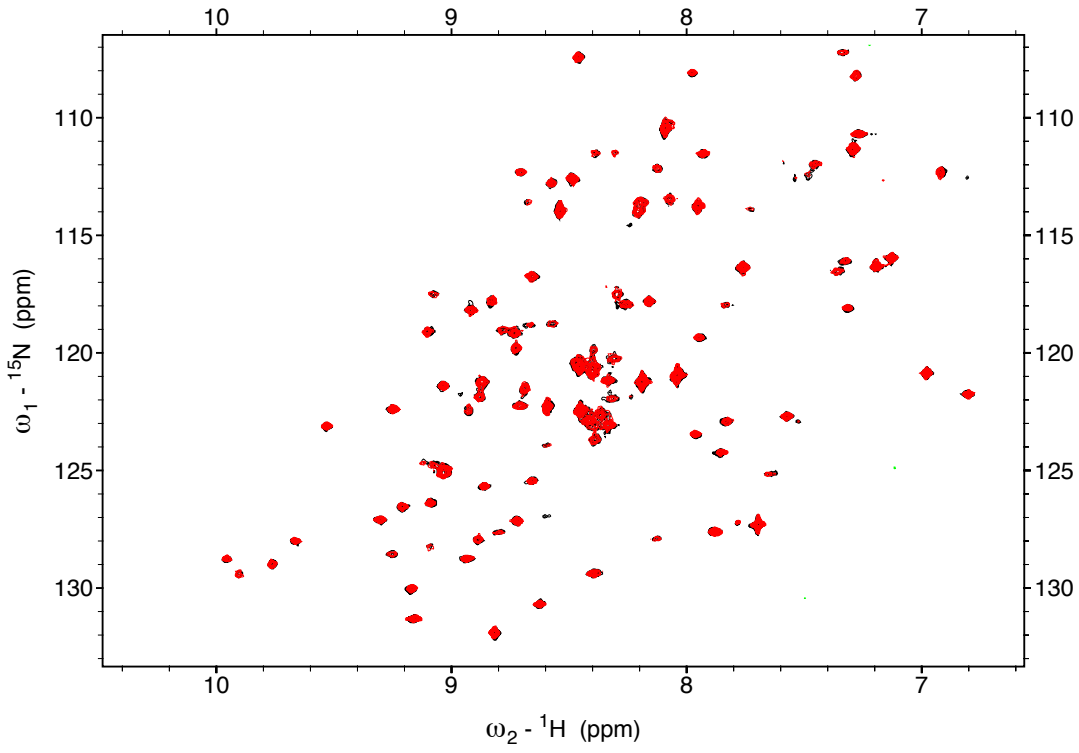

Figure S5. The effect of 1% v/v DMSO is negligible on 2D [^{1}H, ^{15}N] TROSY spectra.

Overlay of 2D TROSY spectra of wt TTR in the absence (black cross peaks) and in the presence (red cross peaks) of 1% v/v DMSO. The spectra were acquired at 310K.

MOVIE LEGENDS

Movie M1. Structural effects of bound mds84 at a 1:1 molar ratio to TTR.

The video shows the CSP variations in a green-light blue-blue gradient colour according to the scale shown in Fig. 2, on the structure of the 1:1 TTR complex with mds84 (PDB: 3IPE).

Movie M2. Structural effects of bound tafamidis at a 1:1 molar ratio to TTR.

The video shows the CSP variations in a yellow-light blue-blue and green-light blue-blue gradient colour according to the scale shown in Fig. 3, on the structure of the 1:1 TTR complex with tafamidis (PDB: 3TCT). The yellow and green chains are those not bound and bound to tafamidis, respectively.

Movie M3. Structural effects of bound tafamidis at a 2:1 molar ratio to TTR.

The video shows the CSP variations in a green-light blue-blue gradient colour according to the scale shown in Fig. 3, on the structure of the 2:1 TTR complex with tafamidis (PDB: 3TCT).