supplemental Table S1. Data collection and refinement statistics for Zn^{2+}:M-TTR complexes at pH 7.5, 6.5, 5.5, 4.6.

supplemental Table S2. Conservation and coupling among the residues in ZBS in the TTR multiple sequence alignments (MSA).

Column "Conservation" shows the percentage of conservation of each amino acid in the first column for 262 TTR sequences in the MSA from the Protein Families database (Pfam, http://pfam.sanger.ac.uk/) (29). Subsequent numbers show the frequency with which the amino acid at the top of a given column appears in the position indicated when another amino acid appears in the position shown at the beginning of that row (e.g., 11.8% of the sequences that have a cysteine in position 10 have a histidine in position 31, all sequences with a cysteine in position 10 also have a histidine in position 56, and so on). Bold-italic numbers indicate coupling among residues of the same ZBS.

supplemental Fig. S1. MSA analysis of mammalian TTR sequences.
Multiple sequence alignments of mammalian TTR sequences showing increased conservation of residues that form ZBS. Asterisks mark residues that form ZBS.

supplemental Fig. S2. Details of the loop E – α-helix – loop F region.
Close-up views of the loop E – α-helix – loop F region (residues 71–94) of subunit A of Zn^{2+}:M-TTR structures at pH 7.5, 6.5, 5.5 and 4.6 (PDB IDs 3GRG, 3GRB, 3GPS and 3DGD, respectively). This is the same region shown in Fig. 3. Mesh shows electron density from the $2|F_{obs}-F_{calc}|$ omit map contoured at 1σ. The electron densities displayed are limited to within 1.5 Å of the residues. Zn^{2+} ions are shown as gray spheres and water molecules as red spheres. Note the well-defined electron-density map in this region at all pH values.

supplemental Fig. S3. Holo-RBP:TTR interface showing interacting region with and without Zn^{2+}.
(*A*) Zn^{2+}:M-TTR pH 7.5 (red) from PDB ID 3GRG is superimposed on Zn^{2+}-free WT-TTR (green) bound to holo-RBP (orange), showing the disrupted interface of holo-RBP recognition when Zn^{2+} is bound. Zinc-free WT-TTR (green) and holo-RBP (orange) are from PDB ID 1QAB (60). (*B*) Close-up view of the interface region between holo-RBP and TTR. For orientation, in the TTR structure Trp79 on the α-helix is blue and loops 63-67 and 92-98 of holo-RBP are gray. Note that partial unwinding of the α-helix of TTR leads to a loss of complementarity and steric exclusion of holo-RBP.

supplemental Fig. S4. $^{1}H–^{15}N$ TROSY-HSQC spectra of the Zn^{2+}-free (blue) and -bound (red) states of WT-TTR.
WT-TTR $^{2}H/^{15}N$ 100 μM in deuterated Tris-HCl 25 mM, KCl 50 mM, pH 7.5 without $ZnCl_2$ (blue) and in presence of $ZnCl_2$ 400 μM (red). Note changes in position of various resonances due to addition of Zn^{2+}.

supplemental Fig. S5. CSP caused by Zn^{2+} binding in residues involved in ZBS.
The chemical-shift perturbations (CSP) derived from the TROSY-HSQC measurements of fully ^{15}N-^{2}H labeled WT-TTR (100 μM) of residues involved in ZBS were plotted against $ZnCl_2$ concentration.

supplemental Fig. S6. Ramachandran plots
Ramachandran plots of α–helix region (residues 74-82) of all chains of M-TTR:Zn^{2+} complex in various pHs, M-TTR in absence of Zn^{2+} at pH 8.0 and WT-TTR at pHs 5.3, 7.0, 7.5.

supplemental Table S1

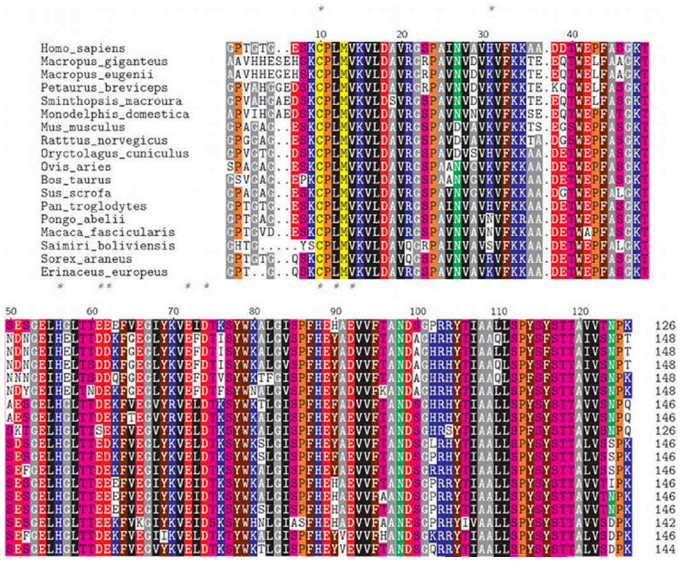
	3GRG (pH7.5)	3GRB (pH6.5)	3GPS (pH5.5)	3DGD (pH4.6)
Data collection				
Space group	$P2_1$	$P2_1$	$P2_1$	$P2_1$
Cell dimensions				
a, *b*, *c* (Å)	47.30, 61.92, 83.32	47.34, 62.19, 83.80	47.26, 61.61, 83.49	46.95, 60.69, 82.98
α, β, γ (°)	90, 90, 90	90, 90, 90	90, 90, 90	90, 90, 90
Resolution (Å)	24.72 - 1.90 (1.94 - 1.90)	26.00 - 1.75 (1.79 - 1.75)	28.92 - 1.78 (1.82 - 1.78)	19.37 - 1.38 (1.42 - 1.38)
R_{sym} or R_{merge}	0.063 (0.394)	0.063 (0.383)	0.061 (0.488)	0.056 (0.774)
I / σI	13.7 (2.7)	21.0 (4.0)	8.1 (3.0)	7.1 (1.0)
Completeness (%)	99.81 % (100 %)	99.47 % (99.16 %)	99.18 % (100 %)	97.2 % (94.7%)
Redundancy	3.7 (3.6)	7.2 (7.0)	5.2 (5.1)	7.1 (7.0)
Refinement				
Resolution (Å)	1.90	1.75	1.78	1.38
No. reflections	41141	46093	43051	92475
R_{work} / R_{free}	19.38 / 25.4	20.6 / 25.9	20.5 / 25.3	15.9 / 20.2
No. atoms				
Protein	3773	3790	3860	4200
Ligand/ion	52	52	60	87
Water	238	327	247	392
B-factors (Å^2)				
Protein	34.58	29.13	34.94	23.17
Zn^{2+} ions	45.29	44.19	46.69	24.64
Water	34.58	41.97	53.32	39.70
R.m.s. deviations				
Bond lengths (Å)	0.018	0.015	0.021	0.030
Bond angles (°)	1.89	1.67	1.90	2.63

Structures were determined using a single crystal at each pH. Values in parentheses are for highest-resolution shell.

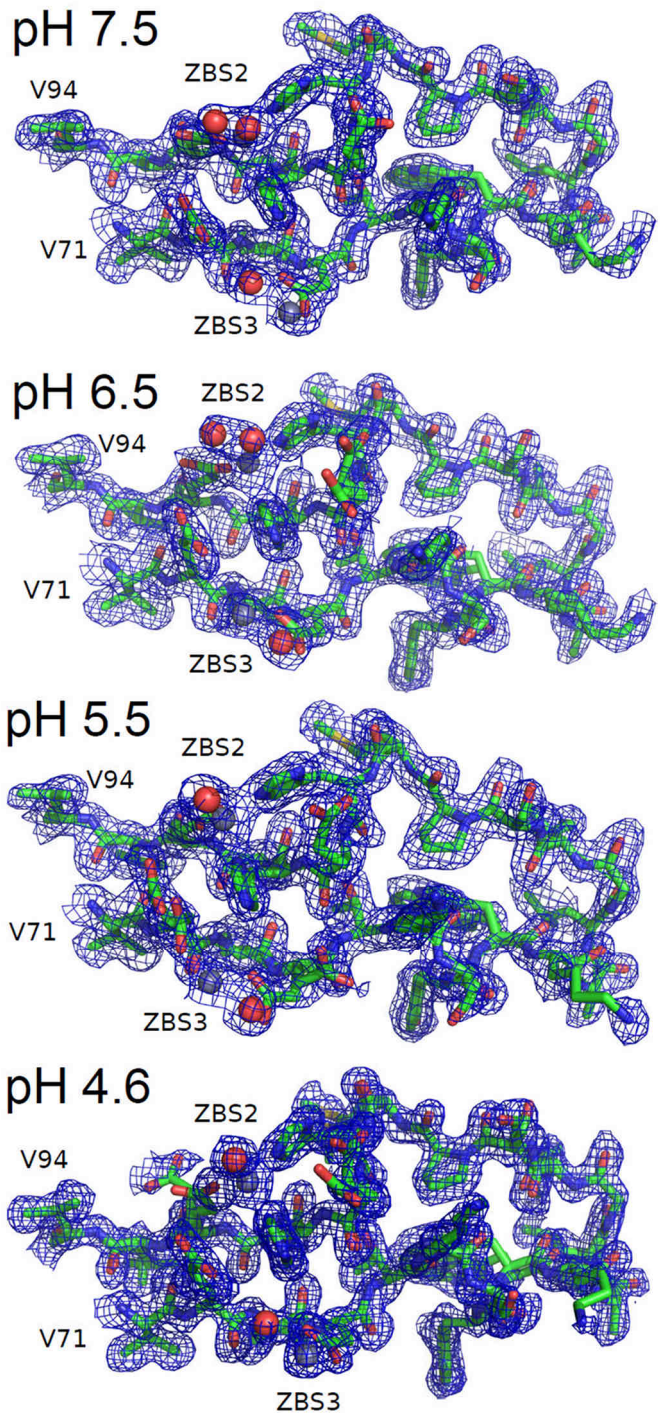
supplemental Table S2

			ZBS1		ZBS2			ZBS3				ZBS4
	Residue and Position	Conservation	C10	H56	H88	H90	E92	H31	E72	D74	E62	E61
ZBS1	C10	12.9%	***X***	***87.2***%	94.4%	75.0%	34.9%	36.4%	58.5%	45.5%	33.8%	40.0%
	H56	14.8%	***100***%	***X***	97.2%	75.0%	34.9%	45.5%	60.4%	47.0%	35.4%	45.0%
ZBS2	H88	13.7%	100%	89.7%	***X***	***75.0***%	***34.9***%	36.4%	58.5%	45.5%	33.8%	40.0%
	H90	3.0%	17.6%	15.4%	***16.7***%	***X***	***14.0***%	27.3%	11.3%	9.1%	9.2%	15.0%
	E92	16.3%	44.1%	38.5%	***41.7***%	***75.0***%	***X***	45.5%	24.5%	25.8%	26.2%	20.0%
ZBS3	H31	4.2%	11.8%	12.8%	11.1%	37.5%	11.6%	***X***	***9.4***%	***7.6***%	6.2%	12.5%
	E72	20.2%	91.2%	82.1%	86.1%	75.0%	30.2%	***45.5***%	***X***	***53.0***%	41.5%	42.5%
	D74	11.8%	88.2%	79.5%	83.3%	75.0%	39.5%	***45.5***%	***66.0***%	***X***	36.9%	47.5%
	E62	24.7%	64.7%	59.0%	61.1%	75.0%	39.5%	36.5%	50.9%	36.4%	***X***	27.5%
ZBS4	E61	15.2%	47.1%	46.2%	44.4%	75.0%	18.6%	45.5%	32.1%	28.8%	***16.9***%	***X***

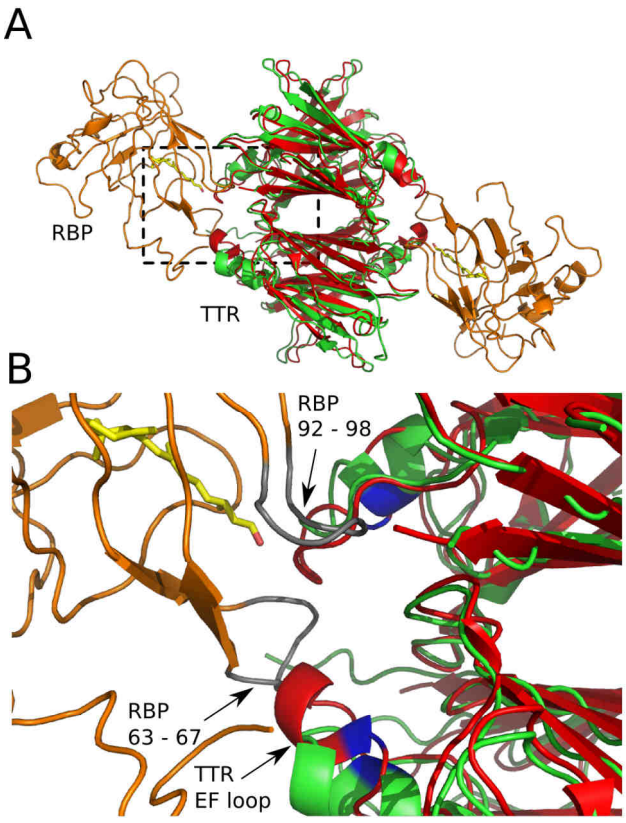
supplemental Fig. S1



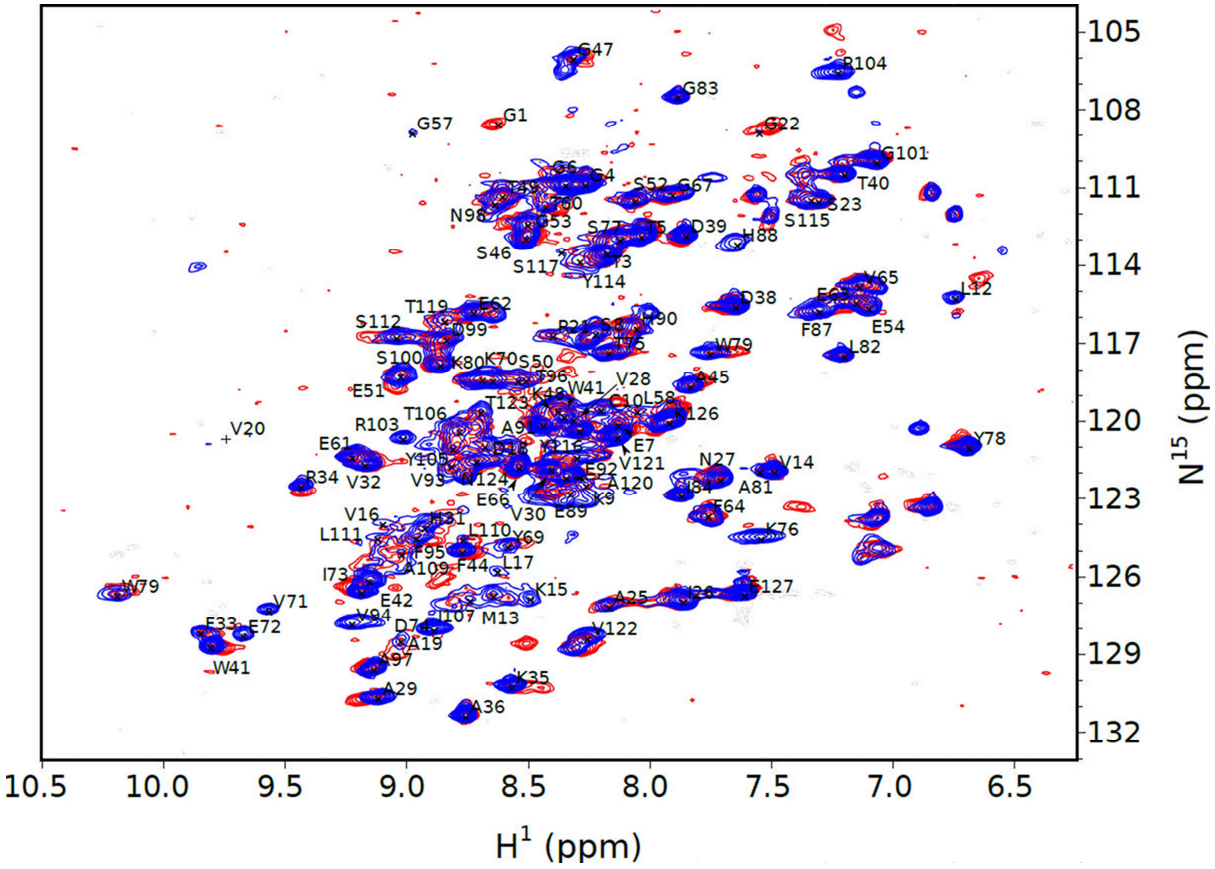
supplemental Fig. S2



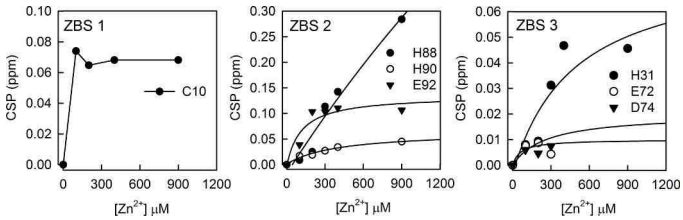
supplemental Fig. S3



supplemental Fig. S4



supplemental Fig. S5.



supplemental Fig. S6