

Calcium Binds to Transthyretin with Low Affinity

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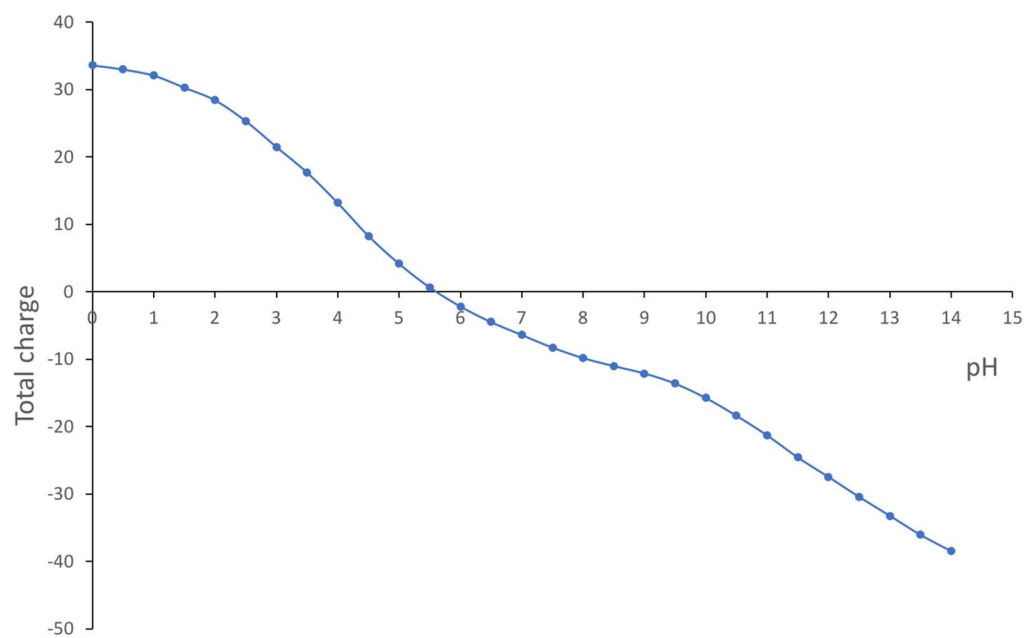


Figure S1. TTR charge as a function of pH.

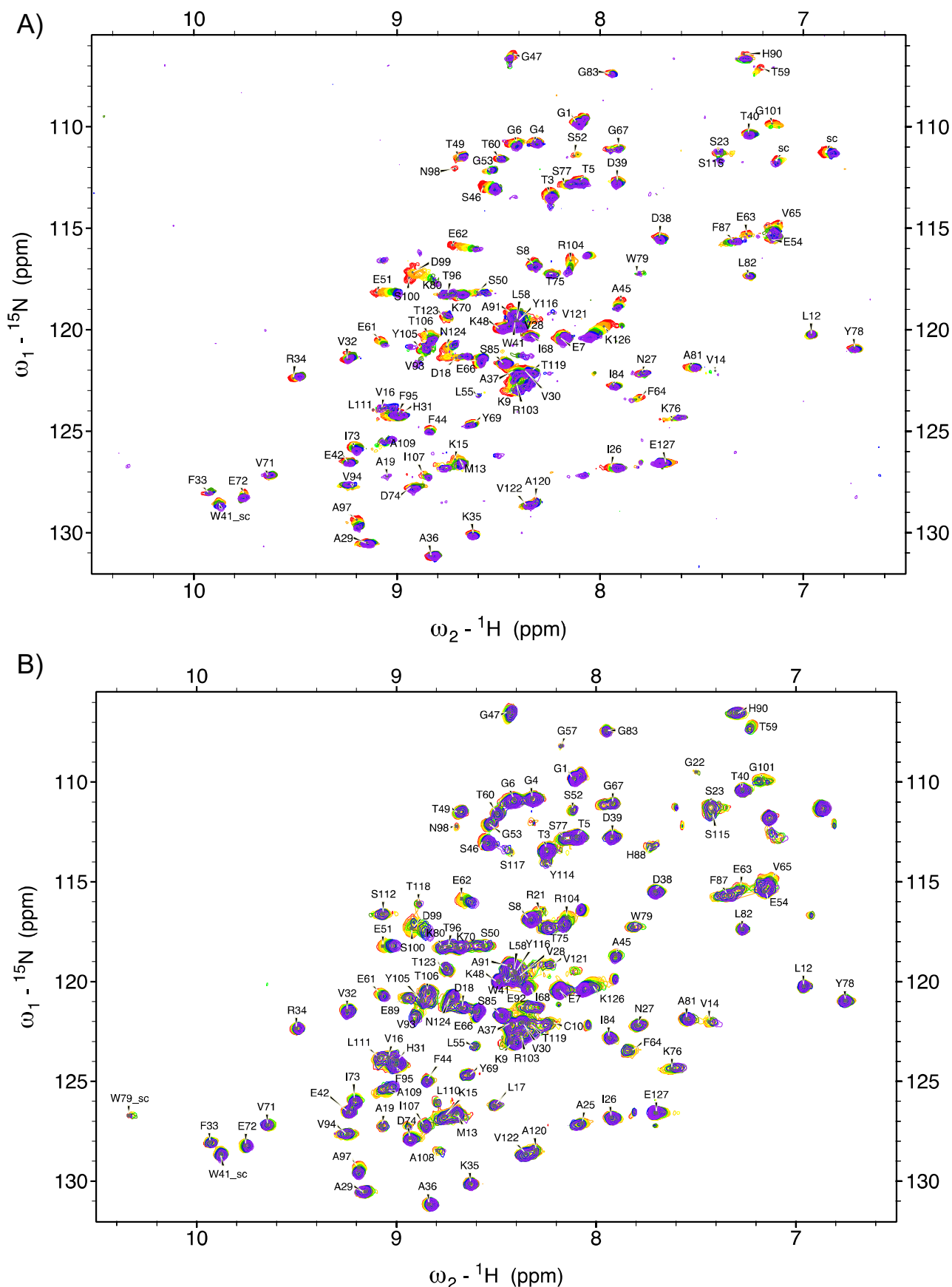


Figure S2. Overlay of 2D [^1H , ^{15}N] TROSY spectra of TTR acquired at 700 MHz recorded at increasing calcium concentration at pH 7.4 without (A) and with 154 mM NaCl (B). The color code for panel A is: 0mM red, 5 mM orange, 10mM yellow, 20mM green, 40mM blue, 60mM purple. The color code for panel B is: 0 mM red, 1.3 mM orange, 4.6 mM gold, 7.9 mM yellow, 14.5 mM green, 24.3 mM chartreuse green, 37.3 mM blue and 62.7 mM purple.

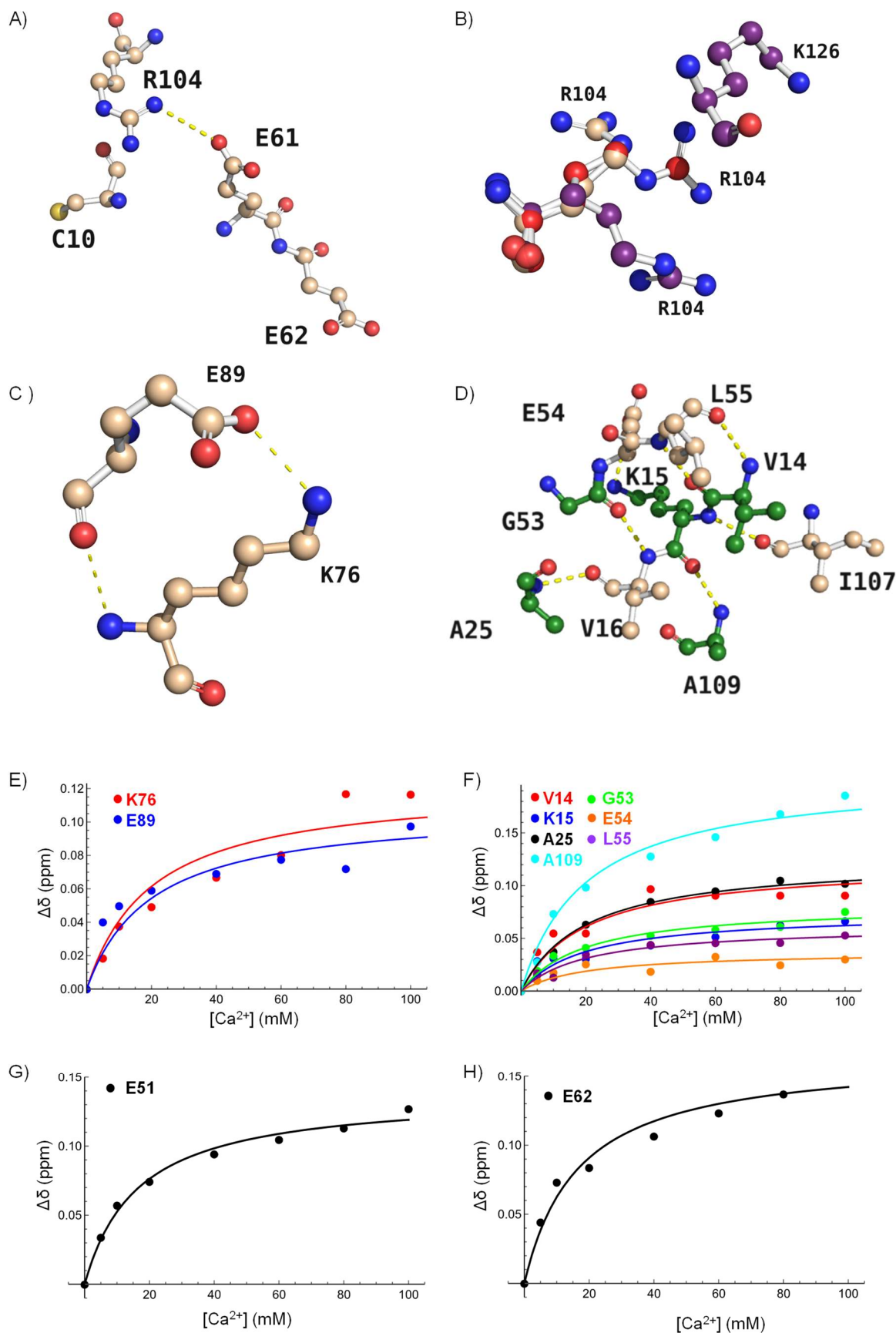


Figure S3. (A) C10, R104 and E61 residues taken from 5CN3 TTR structure. (B) Position of R104 side chain with respect to K126 in 5CN3 (beige), 1TTA (purple) and 4N85 (red) structures. (C) H bonds between E89 and K76 in 5CN3 structure. (D) H bond network in 5CN3 structure connecting significantly shifting residues in presence of calcium (coloured in green). (E-H) Chemical shift perturbation as a function of Ca^{2+} concentration recorded at pH 6.5 and fitted with equation [1] (see main text) for residues with $\Delta\delta \geq$ average $+2\sigma$ belonging to putative sites 3-6.

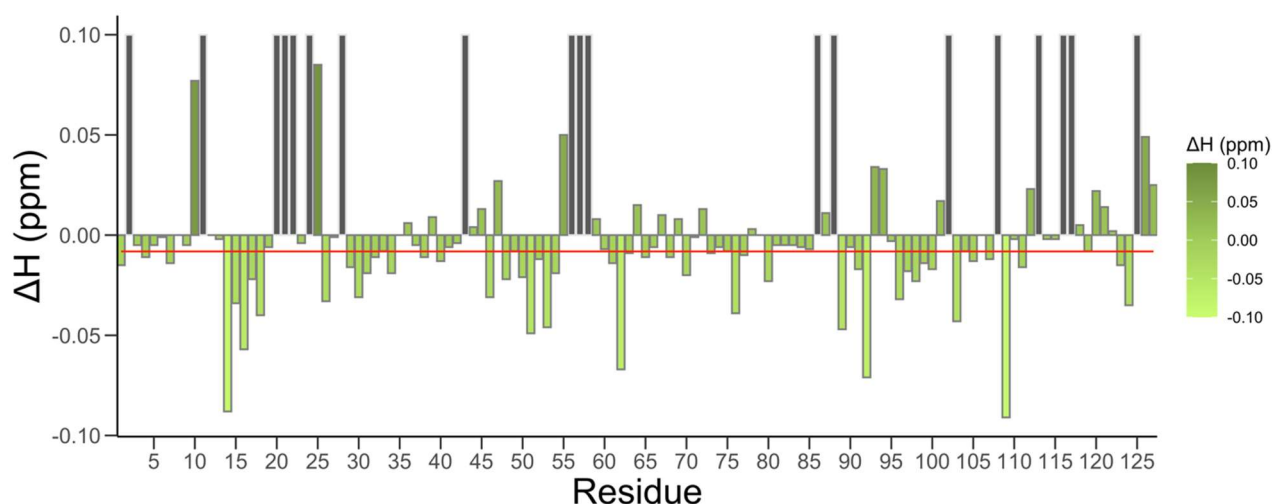


Figure S4. Bar plot of TTR H^N chemical shift variation with 150 mM NaCl. Gray bars indicate prolines and residues that could not be followed during titration. The H^N chemical shift variation observed in the presence of NaCl is depicted in a light-dark green gradient. The red line corresponds to the average value.

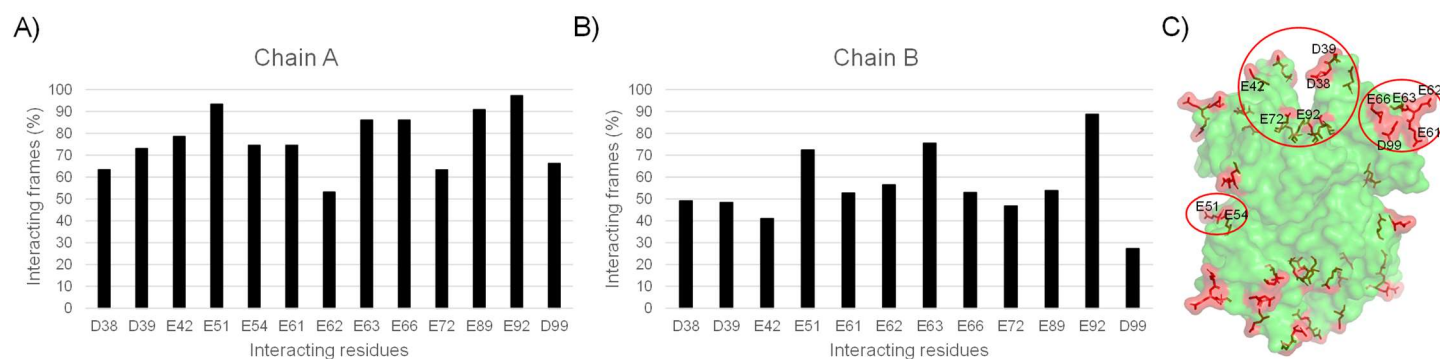


Figure S5. Persistency of electrostatic interactions between Ca^{2+} and TTR residues obtained from 15 ns MD simulations for protein chain A (A) and chain B (B). (C) The interacting residues are coloured in red on the protein structure (pdb: 5cn3) and the three interacting patches are highlighted by red circles.