Supplemental Material for

THE CRYSTAL STRUCTURE OF IRON-FREE HUMAN SERUM TRANSFERRIN PROVIDES INSIGHT INTO INTER-LOBE COMMUNICATION AND RECEPTOR BINDING

Jeremy Wally[1], Peter J. Halbrooks[2], Clemens Vonrhein[3], Mark A. Rould[4], Stephen J. Everse[2], Anne B. Mason[2] and Susan K. Buchanan[1]

From [1]National Institute of Diabetes & Digestive & Kidney Diseases, National Institutes of Health, 50 South Drive, Bethesda, MD 20892 USA, [2]Department of Biochemistry, University of Vermont, College of Medicine, 89 Beaumont Avenue, Burlington, VT 05405 USA, [3]Global Phasing Ltd., Sheraton House, Castle Park, Cambridge, CB3 0AX, UK, and [4]Department of Molecular Physiology and Biophysics, University of Vermont, College of Medicine, 89 Beaumont Avenue, Burlington, VT 05405 USA

Supplemental Table 1. Observed polar contacts between citrate molecules and apo-hTF.

Citrate		Apo-hTF-Gly			
Molecule	Atom	Molecule	Residue	Atom	Distance (Å)
9201	O4	B[a]	H350	N	2.82
			H349	N	3.15
	O7			N	2.61
9202	O1	A	-	-	-
	O2		T457	N	2.46
	O3		H585	NE2	2.94
	O4		R632	NH1	2.62
9203[b]	O4	A	Y517	N	2.43
			T518	N	2.42
9204	O1	B	R632	NH1	3.13
	O4		T457	N	2.32
	O5		-	-	-
9205[b]	O1	B	Y517	N	2.36
	O2		T518	N	2.88
			Y517	N	3.10
			T518	N	3.10
	O5		Y517	N	2.88
9206	O1	B	G127	N	3.09
	O2		A126	N	2.80
	O5		S125	N	2.81
9207[b]	O1	A	K296	NZ	2.85
	O4		R124	NH1	3.15
	O5		K206	NZ	2.66
	O6				2.61

[a] Citrate 9201 forms a crystal contact between the C-lobes of symmetry related molecules.

[b] Citrates 9203, 9205 and 9207 are not present in the apo-hTF-NG structure.

Supplemental Fig. 1: Topology map of the secondary structure of apo-hTF. The subdomains are colored N1- red, N2- blue, C1- green, and C2- yellow. Secondary structural elements are labeled by amino acid and by designation based upon analysis of the structure using PROCHECK (1). The linker region between the lobes of apo-hTF is shown as a dashed line to illustrate the connectivity between the lobes. The figure was made using TopDraw (2).

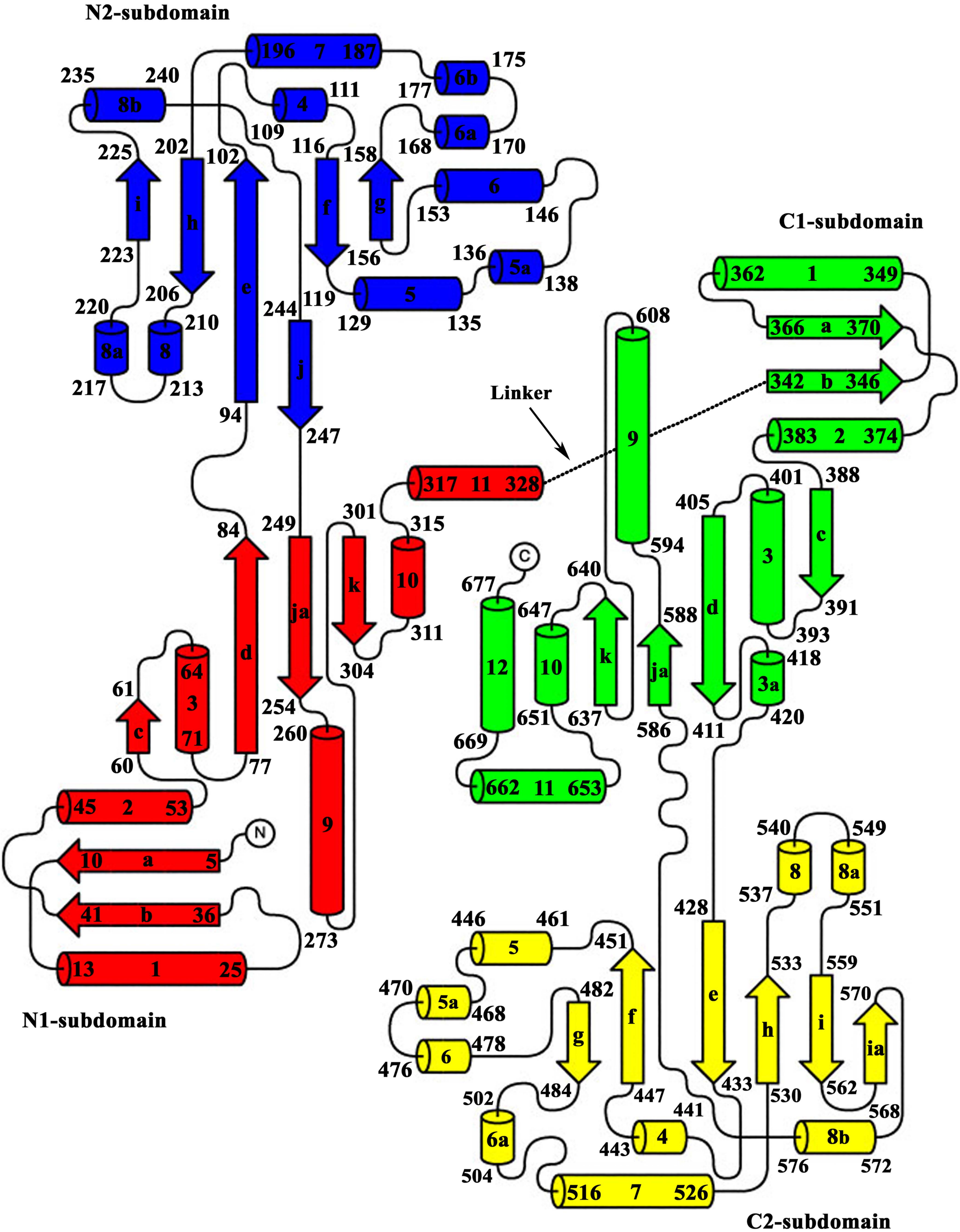
Supplemental Fig. 2: Crystal structure of the two molecules of apo hTF in the asymmetric unit showing the interaction between the N-lobes of the molecules. Both molecules are colored by B factor (from lowest to highest, blue → green → red). The seven citrate molecules and four glycerol molecules observed in the structure are shown in stick representation. The figure was made in PYMOL (3).

Supplemental Fig. 3: Superposition of the four apo-hTF C-lobe structures. The lobes are colored apo-hTF-Gly molecule A red, apo-hTF-Gly molecule B blue, apo-hTF-NG molecule A green, and apo-hTF-NG molecule B orange. Domain rotations of 51.0, 48.7, 50.3 and 48.0° respectively relative to the closed pig Tf structure are required to bring the subdomains into register (4). The superposition was made using SPDBV (5) and figure was made in PYMOL (3).

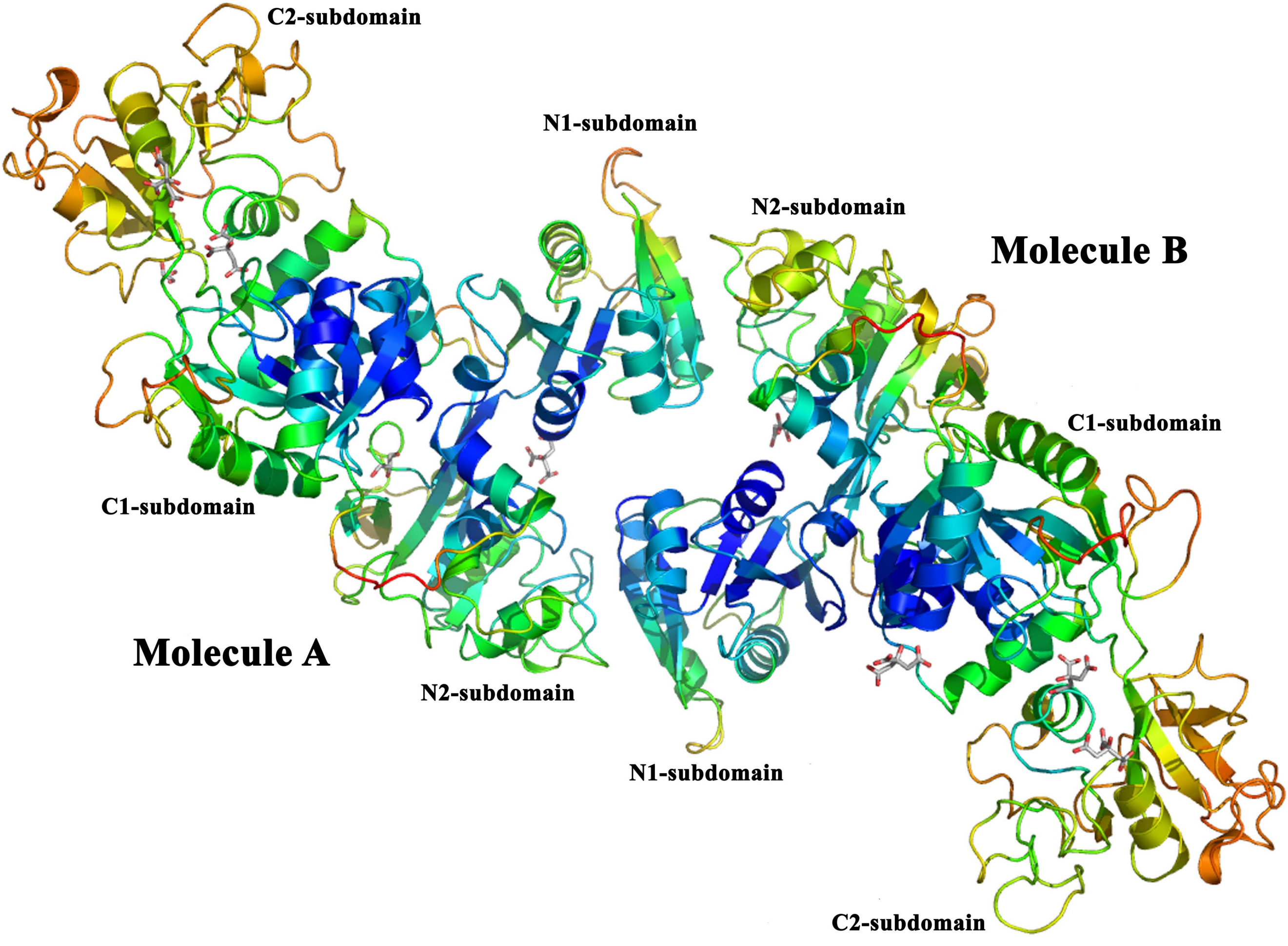
Supplemental Fig. 4**:** Orientation of the bridging citrate molecules present in the iron-binding clefts of the N-lobe of apo-hTF. In the N-lobe of apo-hTF-Gly molecule A, citrate 9207 bridges the side chains of the dilysine trigger residues (Lys206 and Lys296) as well as Arg124 (normally bound to the synergistic carbonate anion). The corresponding citrate in apo-hTF-NG is absent in the structure, and the corresponding citrates (9206) in molecule B of both structures is non-bridging.

(1) Laskowski RA, MacArthur MW, Moss DS and Thornton JM (1993). *J. Appl. Cryst.*, **26**, 283-291.
(2) Bond, CS. (2003) *Bioinformatics* **19**, 311-312.
(3) Delano, WL. (2002) The PyMOL Molecular Graphics System. DeLano Scientific, San Carlos, CA, USA.
(4) Hayward S, Kitao, A, Berendsen HJC (1997) *Proteins, Structure, Function and Genetics*, 27, 425-437.
(5) Guex N and Peitsch MC (1997). *Electrophoresis* **18**, 2714-2723.

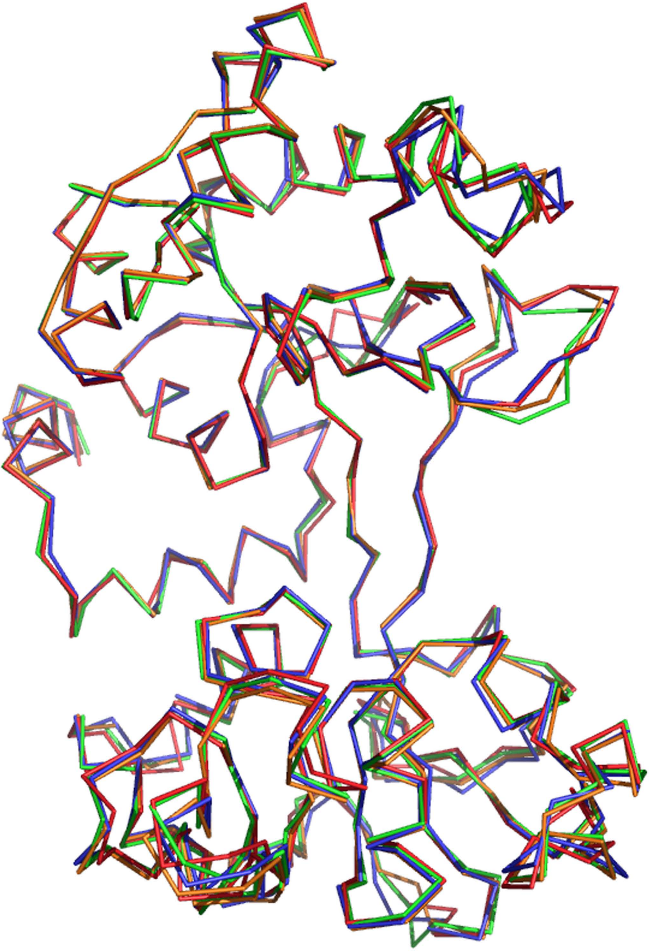
Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4