## Supporting Information

# Structure-based mutagenesis reveals critical residues in the transferrin receptor participating in the mechanism of pH-induced iron release from human serum transferrin

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## Supplementary Data

### Equation for A→B Model Including Initial Decay

The time dependence of the fluorescence intensity *F(t)* is given by the following equation:

$$F(t) = F_A e^{-k_1 t} + F_B(1 - e^{-k_1 t}) + F_D e^{-k_d t}$$

This is based on an equation derived in the Supplemental Data section of Byrne, S. L., Chasteen, N. D., Steere, A. N., and Mason, A. B. (2010) The unique kinetics of iron-release from transferrin: The role of receptor, lobe-lobe interactions and salt at endosomal pH, *J. Mol. Biol. 396*, 130-140, [1] where $F_A$ and $F_B$ are the molar fluorescence intensity constants of species A and B. Here, an additional term accounting for the initial decay in fluorescence is included where $k_d$ is the rate constant for the initial decay and $F_D$ is the corresponding intensity constant.

Origin formula:

y=FA*exp(-k1*x) + FB*(1-exp(-k1*x)) + FD*exp(-kd *x)+ y0

The half-life ($t_{1/2}$) was calculated by the following equation:

$t_{1/2} = \ln(2)/k_d$.

**Supporting Information Table 1.**
Recombinant production of sTFR mutants.

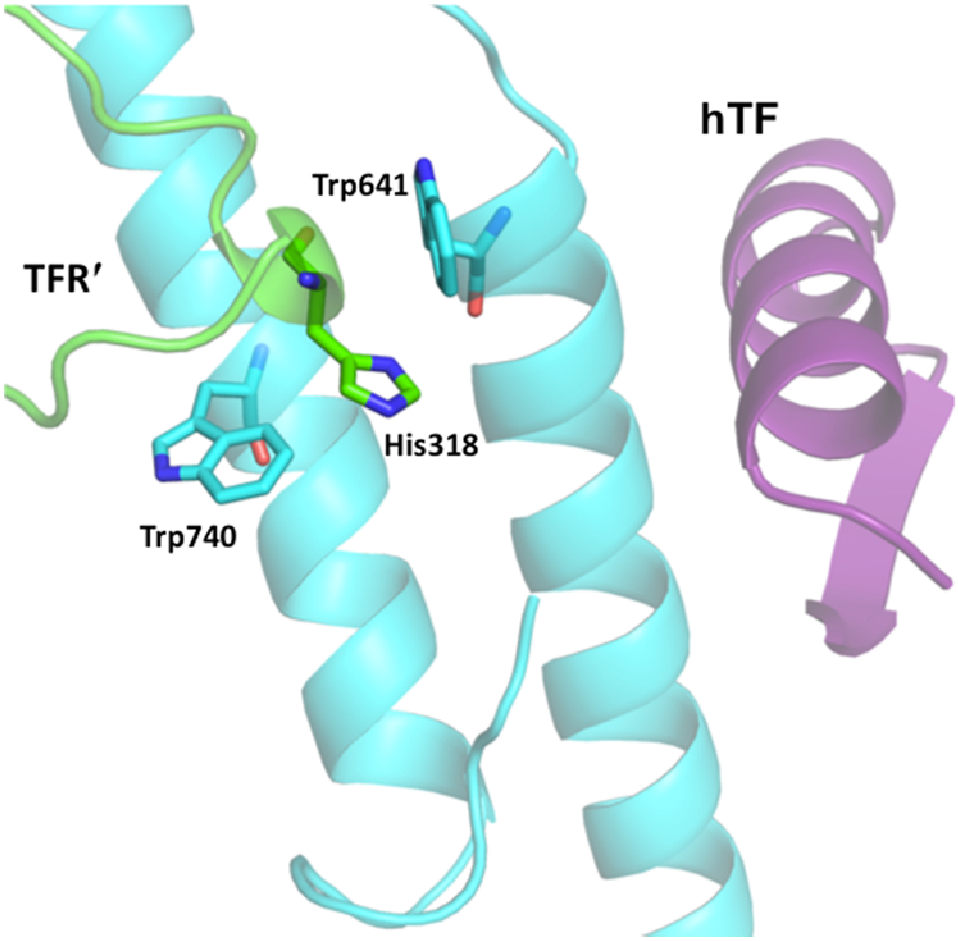
| Construct | mg/L |
|---|---|
| sTFR[a] | 30-40 |
| H318A sTFR | 37 |
| [E465A,E468A] sTFR | <1.0 |
| [H475A,H684A] sTFR | 15 |
| Δ757-760 sTFR | 30 |

[a] From reference 2.

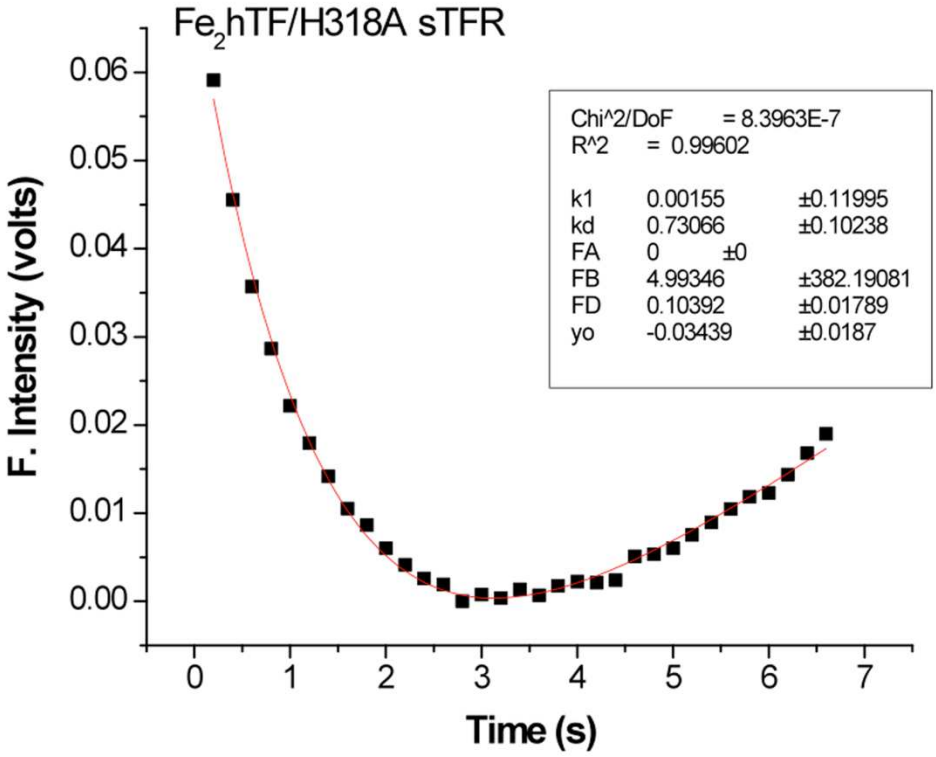
**Supporting Information Table 2.** Oligonucleotide primers (forward) used to prepare sTFR mutants. The substituted bases resulting in mutation are shown in bold.

| Mutant | Oligonucleotide primer |
|---|---|
| H318A | 5'- CTGGATTCCCTTCCTTCAAT**GC**CACTCAGTTTCCACCATCT-3' |
| [E465A,E468A] | 5'- GGTTGGTGCGACTG**C**ATGGCTAG**C**GGGATACCTTTCGT-3' |
| H475A | 5'- GAGGGATACCTTTCGTCCCTG**GC**TTTAAAGGCTTTCACTTATAT-3' |
| H684A | 5'- GTGTCATGAGAGTGGAGTAT**GC**CTTCCTCTCTCCCTAC-3' |
| Δ757-760 | 5'-CTGGTGACGTTTGGGACATTTAAATGTGATACCCATAGCT-3' |

**Supporting Information Figure 1.** TFR-TFR′-C1intersection prepared from the $Fe_N$hTF/sTFR crystal structure (PDB ID: 3S9L).[3] His318 of one TFR monomer (TFR′, green) is within 3.6 Å and 4.1 Å of two tryptophan residues in the helical domain of the other TFR monomer (TFR, cyan, Trp641 and Trp740, respectively).



**Supporting Information Figure 2.** Analysis of the initial quench in tryptophan fluorescence of the $Fe_2$hTF/H318A sTFR complex to the A → B model which also includes the initial decay term. Again, all data were corrected to zero at the fluorescent minimum before fitting. Because the fluorescent increase following the minimum affects the fit, an equal number of data points on each side of the fluorescent minimum were included in the fitting process.



## REFERENCES


1. Byrne, S. L., Chasteen, N. D., Steere, A. N. & Mason, A. B. (2010). The unique kinetics of iron-release from transferrin: The role of receptor, lobe-lobe interactions and salt at endosomal pH. *J. Mol. Biol. 396*, 130-140.
2. Byrne, S. L., Leverence, R., Klein, J. S., Giannetti, A. M., Smith, V. C., MacGillivray, R. T., Kaltashov, I. A. & Mason, A. B. (2006). Effect of glycosylation on the function of a soluble, recombinant form of the transferrin receptor. *Biochemistry 45*, 6663-6673.
3. Eckenroth, B. E., Steere, A. N., Chasteen, N. D., Everse, S. J. & Mason, A. B. (2011). How the binding of human transferrin primes the transferrin receptor potentiating iron release at endosomal pH. *Proc. Natl. Acad. Sci. U. S. A. 108*, 13089-13094.