

Supplementary Material for “Assessing the iron delivery efficacy of transferrin in clinical samples by native electrospray ionization mass spectrometry” by Jake W. Pawlowski, Noelle Kellicker, Cedric E. Bobst and Igor A. Kaltashov

Figure S1. Carbonate (left) and Oxalate (right) coordinated Fe in the N-lobe of Tf.

Figure S2. Calculated mass shifts between holo- and apo-Tf for the +21 and +20 charge states. Theoretical mass shift is for two Fe^{3+} and CO_3^{2-} is 229.7Da.

Figure S3. Shown is the +21 charge state of aTf. Dashed lines represent the calculated mass shift for either the addition of a hexose due to glycation (162.1 Da) as well as the addition or absence of a sialic acid (291.2 Da).

Figure S4. Calculated mass is based on the amino acid sequence of Tf and the mass of two fully sialylated biantennary glycan chains. This figure highlights the importance of experimentally determining the mass of aTf in order for the correct synergistic anion and metal composition to be assigned.

Figure S5. Deconvoluted spectra of recombinant Tf reconstituted with iron using carbonate and oxalate as synergistic anions subjected to SEC fractionation (red) and albumin depletion on a BDR column (blue). Peak area's were integrated and there was no significant change in the percent area for any of the three synergistic anion peaks.

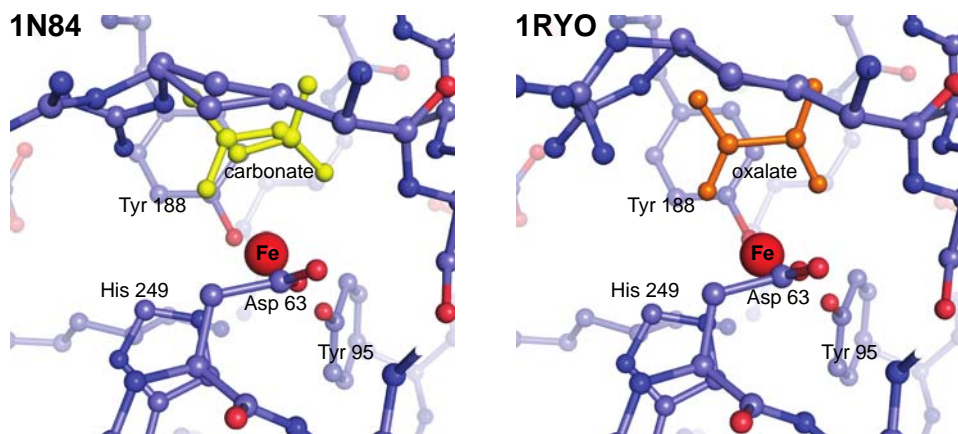


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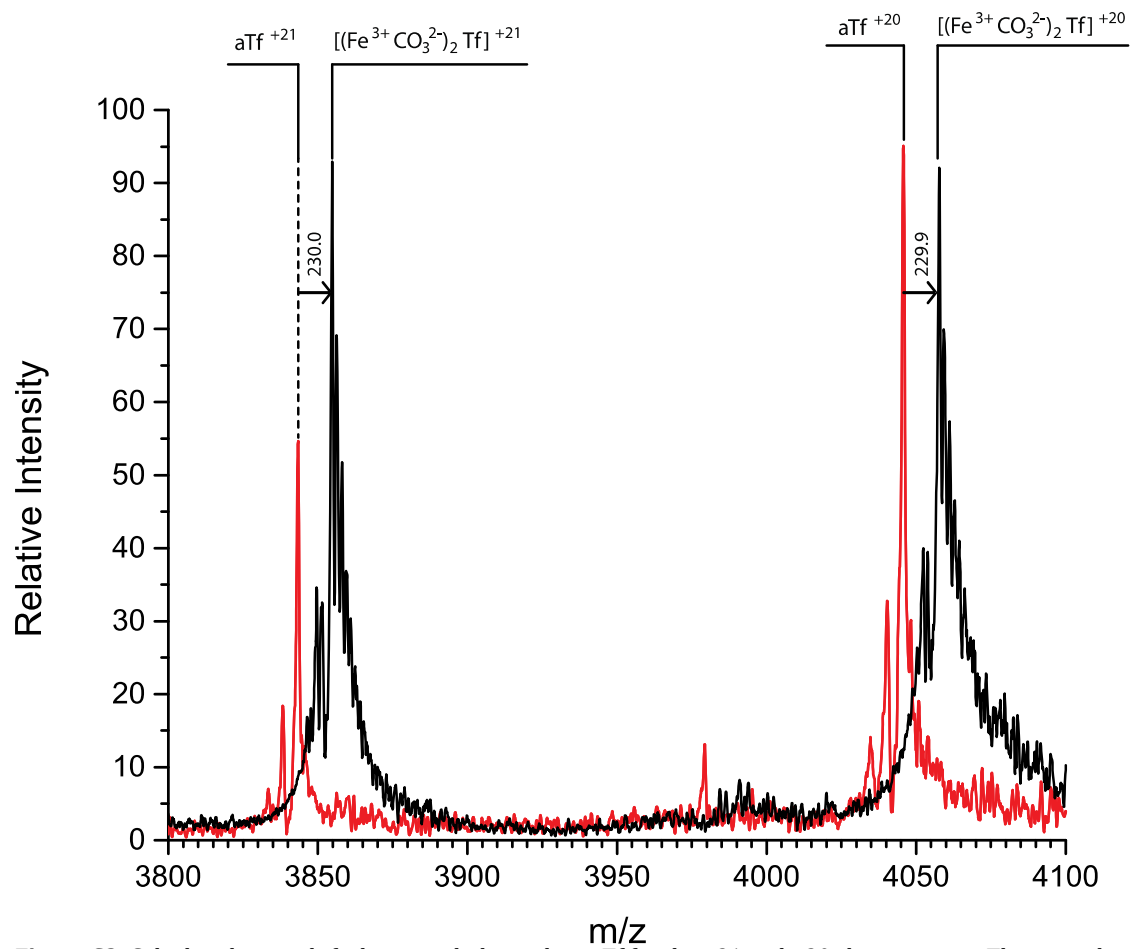


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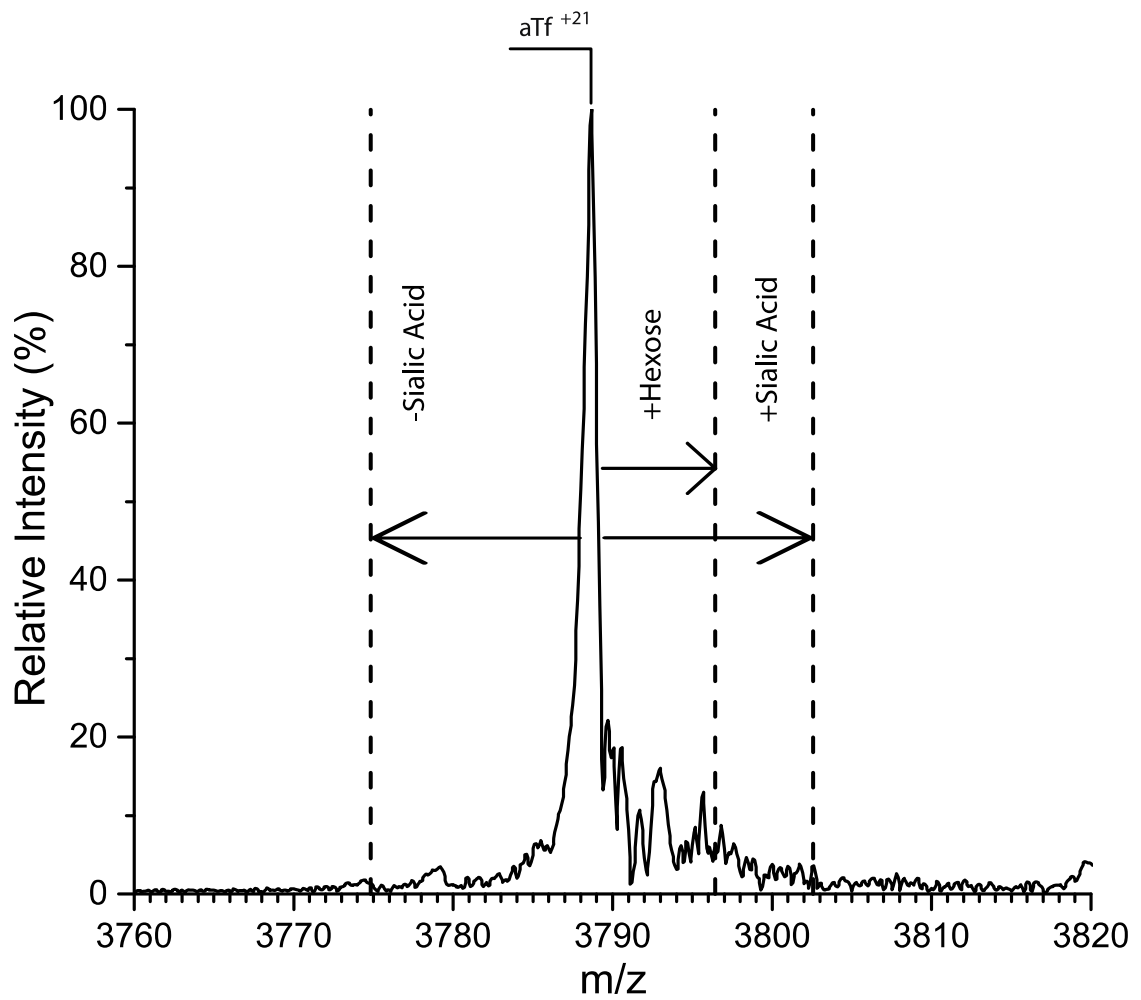


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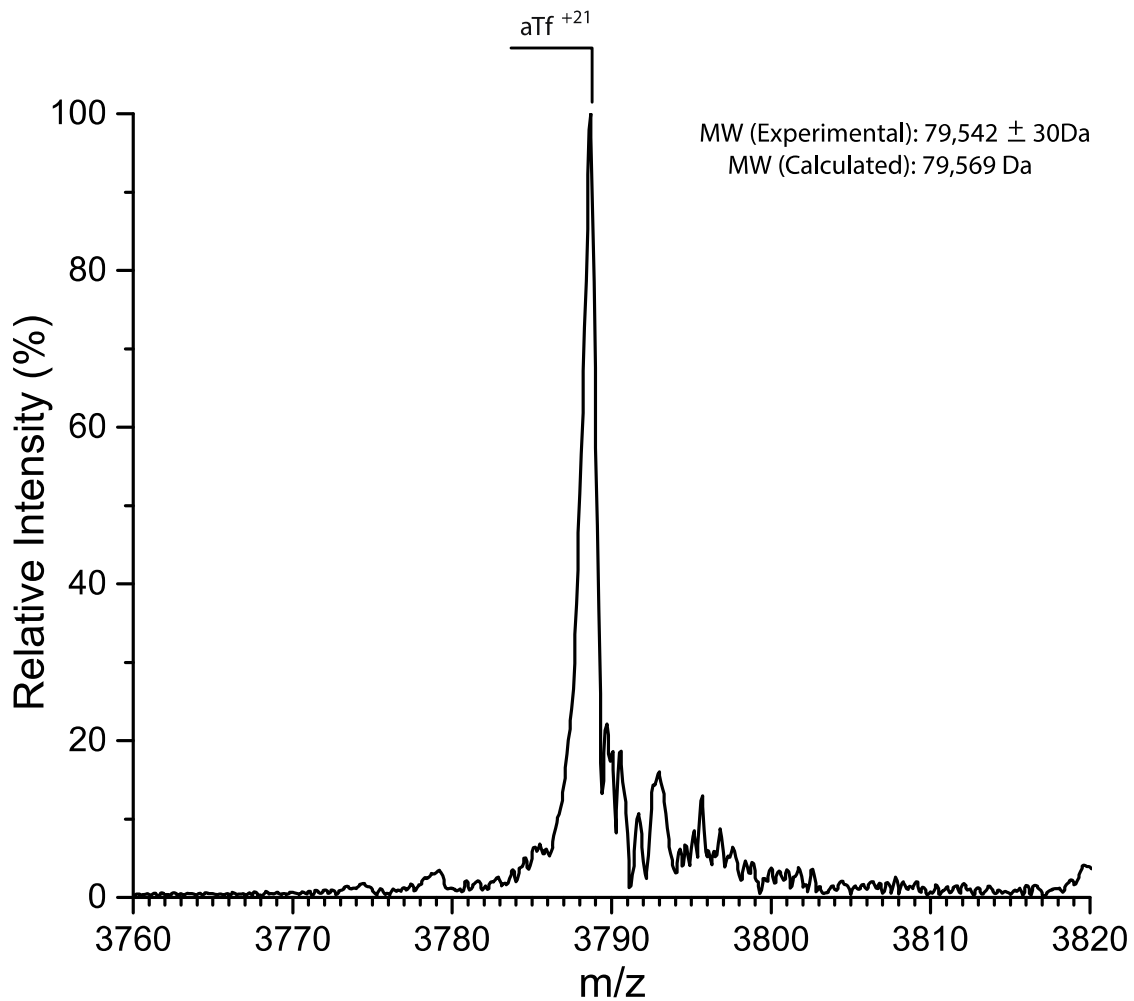


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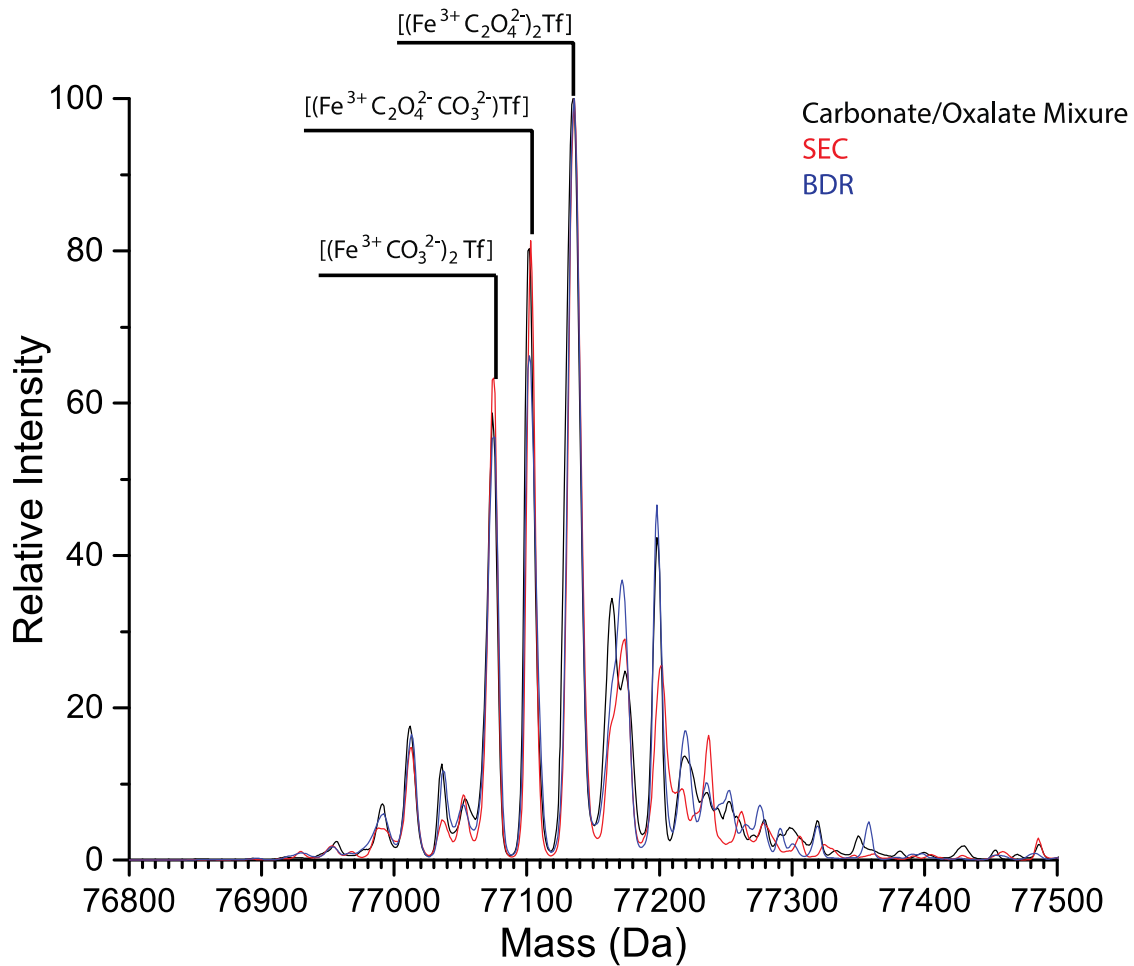


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Synergistic Anion Composition	% Area		
	Orig. Mix	SEC	BDR
2 CO_3^{2-}	21.5%	23.8%	24.0%
$CO_3^{2-} / C_2O_4^{2-}$	28.5%	27.1%	27.9%
2 $C_2O_4^{2-}$	50.0%	49.1%	48.0%