Analytical and Bioanalytical Chemistry

Electronic Supplementary Material

A high-throughput method for the quantification of iron saturation in lactoferrin preparations

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Table S1. Comparison of the absorbance coefficients values for apo- and hololactoferrin based on literature data

	λ [nm]	ε [$M^{-1}cm^{-1}$]	Ref.
apolactoferrin	280	109000	[1]
		98400*	[2]
		101600*	[3]
	279	88500	[4]
	278	107000	[5]
hololactoferrin	280	135000	[1]
		112000*	[6]
	465	2800	[5]
		4640*	[6]
	466	2300	[7]
	470	2300	[8]

* recalculated based on information about A1% or A0.1% for denoted wavelength

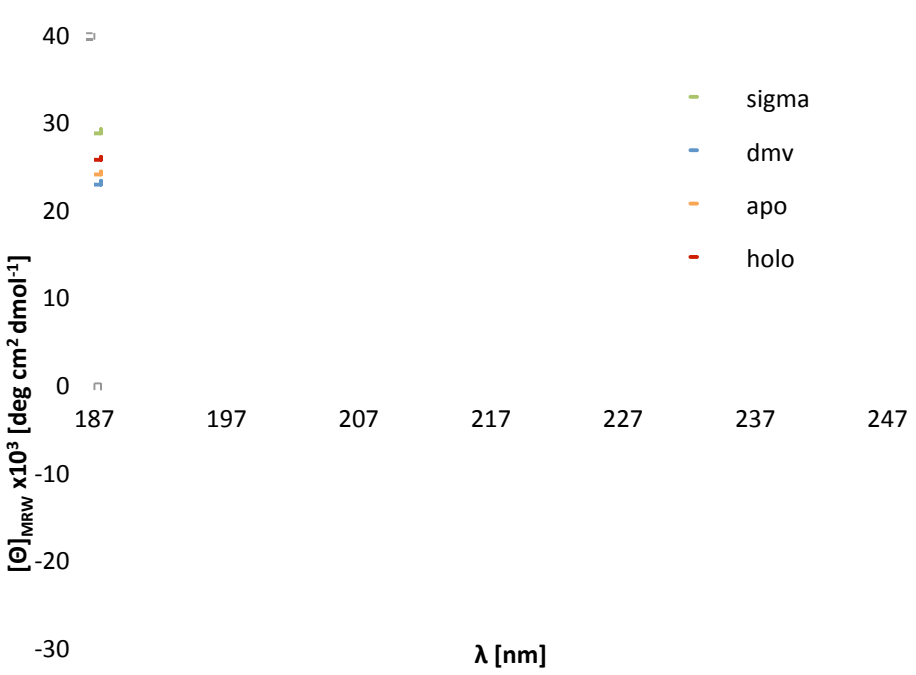

Fig. S1. Circular dichroism spectra of native lactoferrin obtained from Sigma-Aldrich (green) and DMV Int. (blue) companies as well as the apo- (< 2% of iron saturation) and holo-Lf (> 65% iron saturation) produced by us

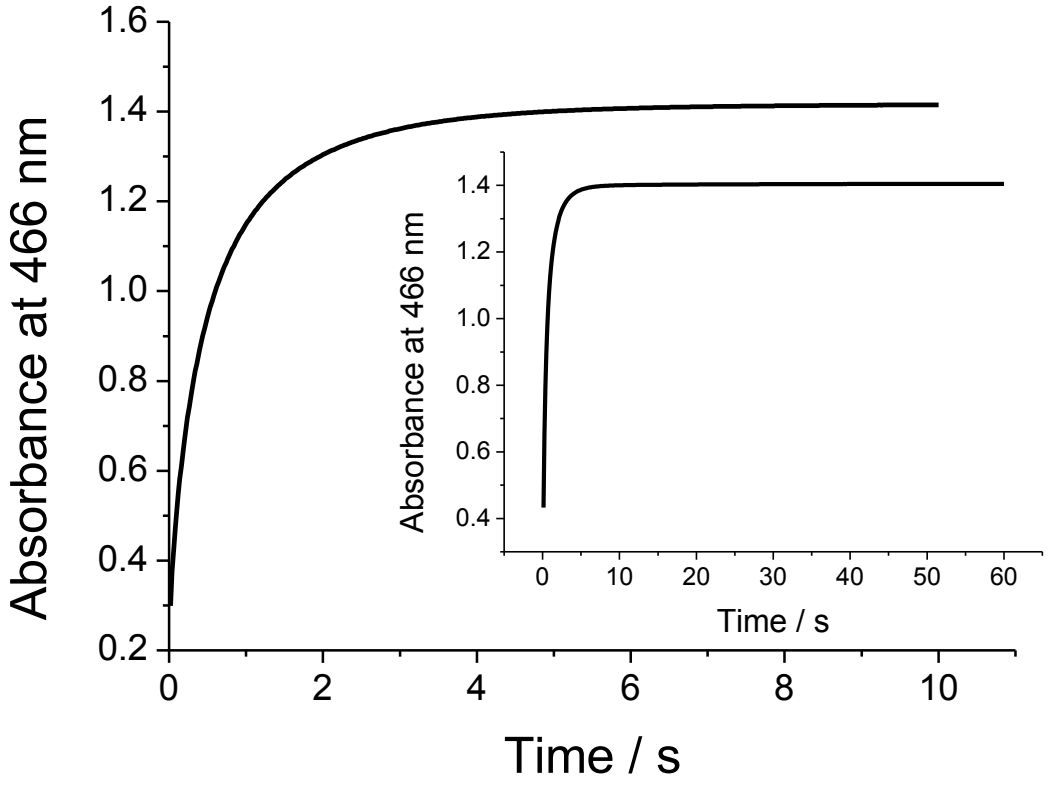

Fig. S2. The increase in the absorbance monitored at 466 nm upon mixing of native lactoferrin (Lf) with a mixture of $Fe(NO_3)_3$ and nitrilotriacetic acid (NTA). Experimental conditions: [Lf] = 0,32 mM, [Fe^{3+}] = 1,28 mM, [NTA] = 1,28 mM , [NaCl] = 150 mM, 50 mM Tris-HCl buffer, pH = 7.4, T = 25 °C

References

1. Brown EM, Parry RM (1974) Spectroscopic study of bovine lactoferrin. Biochemistry (Mosc) 13 (22):4560-4565.
2. Recio I, Visser S (1999) Two ion-exchange chromatographic methods for the isolation of antibacterial peptides from lactoferrin: In situ enzymatic hydrolysis on an ion-exchange membrane. J Chromatogr 831 (2):191-201.
3. Aisen P, Leibman A (1972) Lactoferrin and transferrin: a comparative study. Biochim Biophys Acta - Protein Struct 257 (2):314-323.
4. Aasa R, Malmström BG, Saltman P, Vänngård T (1963) The specific binding of iron(III) and copper(II) to transferrin and conalbumin. Biochim Biophys Acta 75 (0):203-222.
5. Harris WR (1986) Thermodynamics of gallium complexation by human lactoferrin. Biochemistry (Mosc) 25 (4):803-808.
6. Cox TM, Mazurier J, Spik G, Montreul J, Peters TJ (1979) Iron binding proteins and influx of irom across the duodenal brush border: Evidence for specific lactotransferrin receptors in the human intestine. Biochim Biophys Acta - Gen Subjects 588 (1):120-128.
7. Aisen P, Listowsky I (1980) Iron transport and storage proteins. Annu Rev Biochem 49:357-393.
8. Chung TDY, Raymond KN (1993) Lactoferrin: the role of conformational changes in its iron binding and release. J Am Chem Soc 115 (15):6765-6768.