

Supplementary materials

Synthesis and physicochemical characterization of bovine lactoferrin supersaturated complex with iron (III) ions.

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The amount of iron adsorbed from solution was determined using following equation:

$$q_x = (C_0 - C) \cdot V / m \quad (1)$$

where q_x is the amount of Fe^{3+} adsorbed on bLTF (mg/g), m is the sorbent mass (g), C_0 is the initial concentration of metal ions in aqueous solution (mg/L), C is the concentration of Fe^{3+} in aqueous solution after 24 h of incubation (mg/L) and V is the volume of solution from which sorption occurs (L).

The sorption efficiency was calculated by the equation:

$$E (\%) = 100 \cdot (C_0 - C) / C_0 \quad (2)$$

where E is the sorption effectiveness (expressed in %).

The initial concentration of metal ions in the solution as well as after sorption were calculated as follows:

$$C_x = C_y \cdot k_x \quad (3)$$

where C_x is exact value of the metal concentration in the sample, k_x is a dilution factor, and C_y is a concentration of metal solution derived from direct measurements by ICP-MS.

The metal content in the complex was calculated as follows:

$$q_n = C \cdot k / m \quad (4)$$

where q_n – the content of the metal in the complex, C is the concentration of the metal in the solution, derived from direct measurements by ICP-MS, k is the dilution factor.

The metal desorption was calculated according the formula:

$$X (\%) = 100 - (q_n - q_v) \cdot 100 / q_m \quad (5)$$

where X expressed in the % is the amount of desorbed metal from bLTF, q_m is the metal content in the complex, q_v is the amount of metal desorbed into the buffer.

The distribution coefficient (K_D) for the Fe^{3+} adsorption by bLTF was calculated based on the isotherm data at the equilibrium according to equation:

$$K_D = q_e / C_e \quad (6)$$

where: q_e is the amount of ions adsorbed by protein at equilibrium (mg/g), C_e is the equilibrium concentration of Fe^{3+} in solution (mg/L).

The value of the Gibbs free energy change (ΔG^0) for the silver adsorption by casein was calculated according to the following relationship:

$$\Delta G^0 = -RT \ln K_D \quad (7)$$

where ΔG^0 is the energy of adsorption in kJ/mol, R is the gas constant (8.314 J/mol·K), T is the adsorption absolute temperature in Kelvin (295K) and K_D is the dimensionless distribution coefficient.

Table S. 1. The potential binding sites of Fe³⁺ interaction with bovine lactoferrin (bLTF) calculated with Metal Ion-Binding Site Prediction and Docking Server (MIB)

No.	Binding Residues	Score
1	60D , 92Y , 192Y , 253H	2.648
2	60D , 92Y , 192Y , 253H	2.599
3	395D , 433Y , 526Y , 595H	2.49
4	395D , 433Y , 526Y , 595H	2.387
5	60D , 92Y , 192Y , 253H	2.285
6	395D , 433Y , 526Y , 595H	2.054
7	546D , 550E	1.51
8	546D , 550E	1.484
9	546D , 550E	1.472
10	546D , 550E	1.457
11	546D , 550E	1.456
12	546D , 550E	1.427
13	546D , 550E	1.414
14	546D , 550E	1.399
15	546D , 550E	1.351
16	546D , 550E	1.308
17	546D , 550E	1.3
18	433Y , 526Y	1.275
19	92Y , 192Y	1.186
20	609Q , 613H	1.043
21	115C , 157C , 198C	0.927
22	348C , 358C , 371C , 372A	0.912
23	413E , 595H	0.876
24	9C , 19C , 36C , 37V	0.868
25	157C , 198C	0.812
26	9C , 19C , 36C , 37V	0.808
27	80E , 253H	0.794
28	625C , 630C	0.787
29	625C , 630C	0.761
30	491C , 532C	0.726
31	157C , 173C	0.722
32	157C , 173C	0.712

Table S. 2. Predicted amino acid residues of bLTF that can take part in the Fe³⁺ binding, calculated with MIB

Amino Acid	Score	Number of occurrences
ASP	7.844/7.362/4.378	3
HIS	7.844/7.362/2.956	3
TYR	7.844/7.362	4
GLU	4.378/2.447/2.198	3
GLN	2.956	1
CYS	2.604/2.556/2.424/2.175/1.991/1.98	14
ALA	2.556	1
VAL	2.424	1

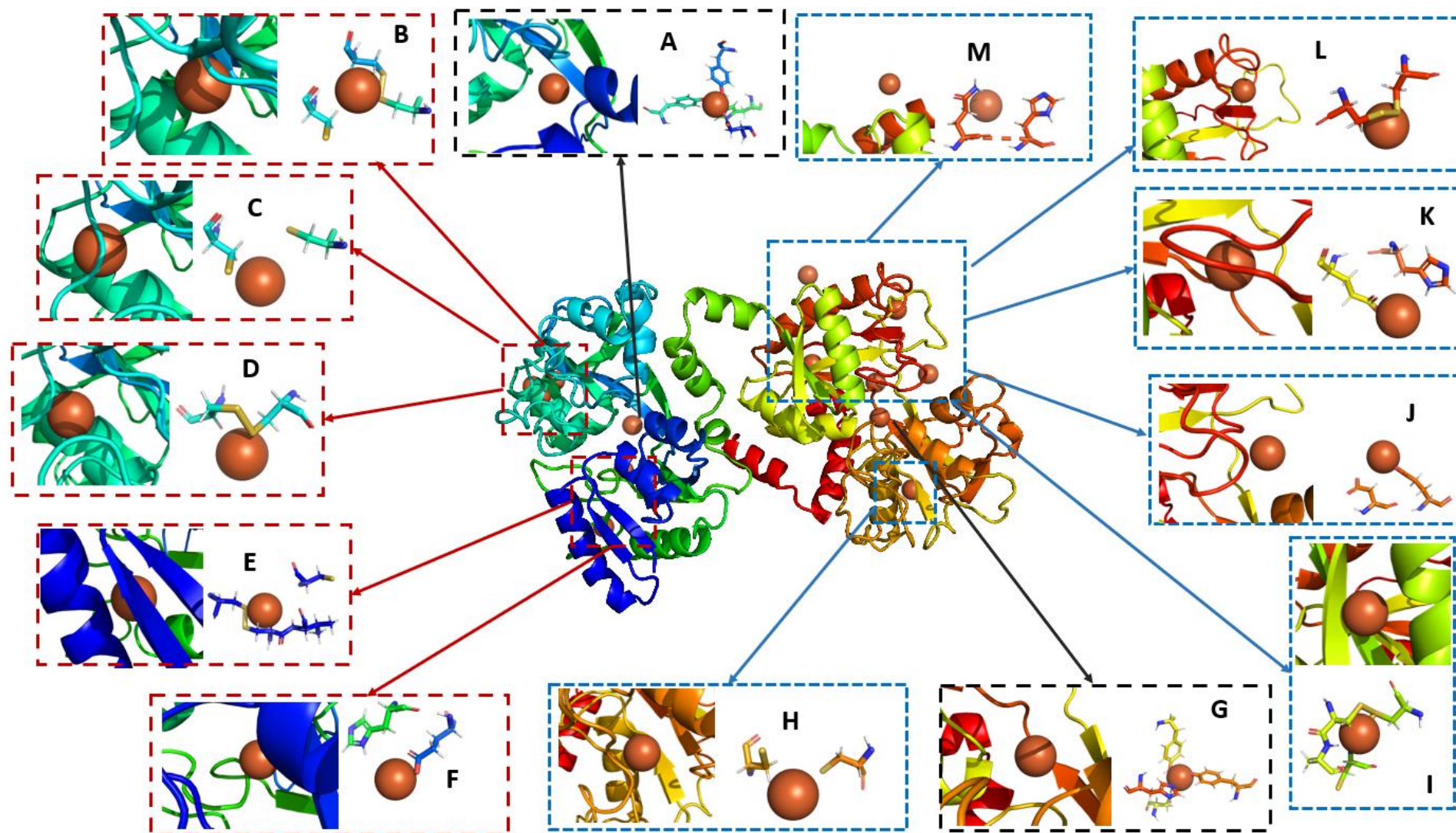
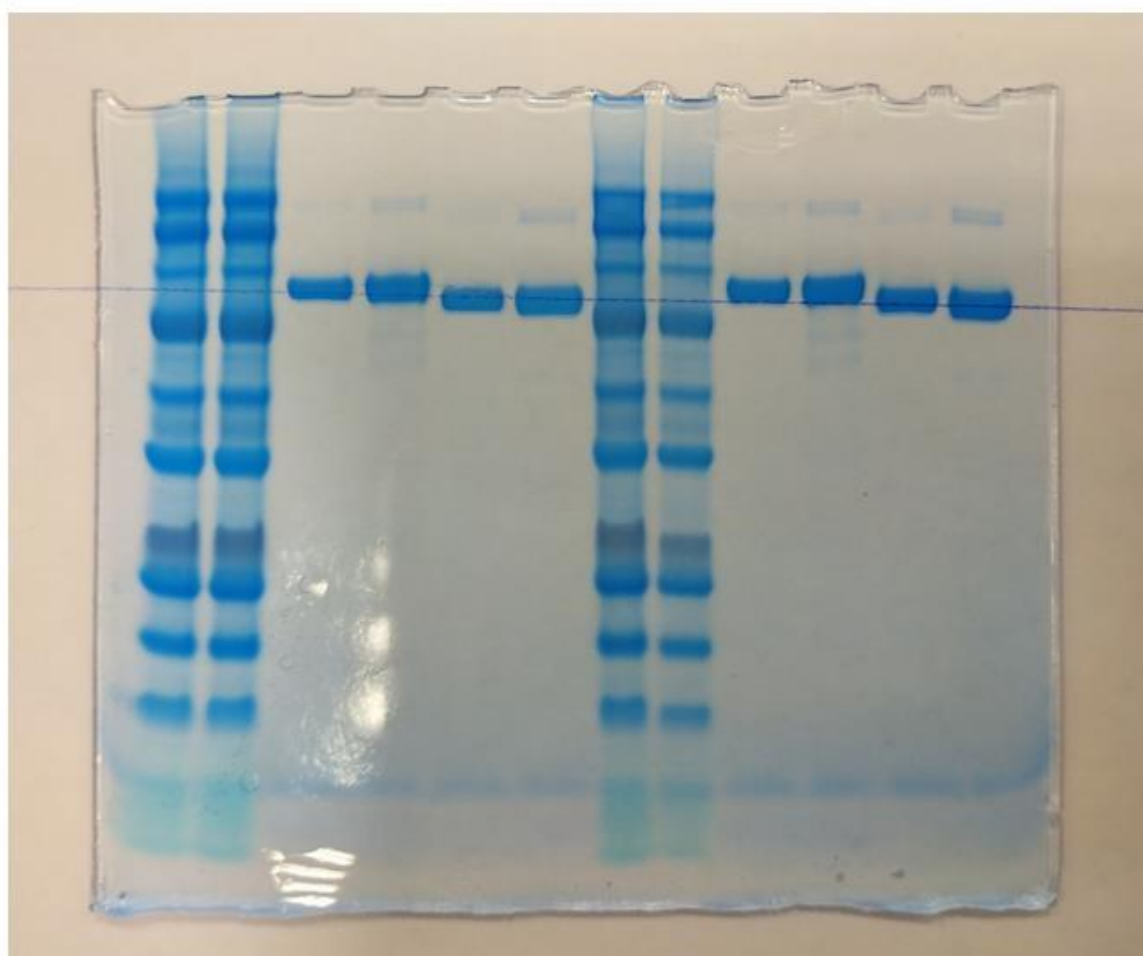


Figure S. 3. Graphical presentation of the potential Fe^{3+} -binding sites (from table S. 1) in bLTF where **A.** site No. 1; **B.** site No. 21; **C.** site No. 25; **D.** site No. 31; **E.** site No. 24; **F.** site No. 27; **G.** site No. 3; **H.** site No. 30; **I.** site No. 22; **J.** site No. 7; **K.** site No. 23; **L.** site No. 28; **M.** site No. 20.



1 2 3 4 5 6 7 8 9 10 11 12

Figure S.4. The full-size (original image) electropherogram of bLTF and Fe-bLTF complex, where 1,2,7,8 – protein mass marker, 3,9 – bLTF in reduced mode, 4,10 – Fe-bLTF in reduced mode, 5,11 – bLTF in non-reduced mode, 6,12 – bLTF in non-reduced mode.