SUPPLEMENT

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1. IR spectroscopy of lactoferrin

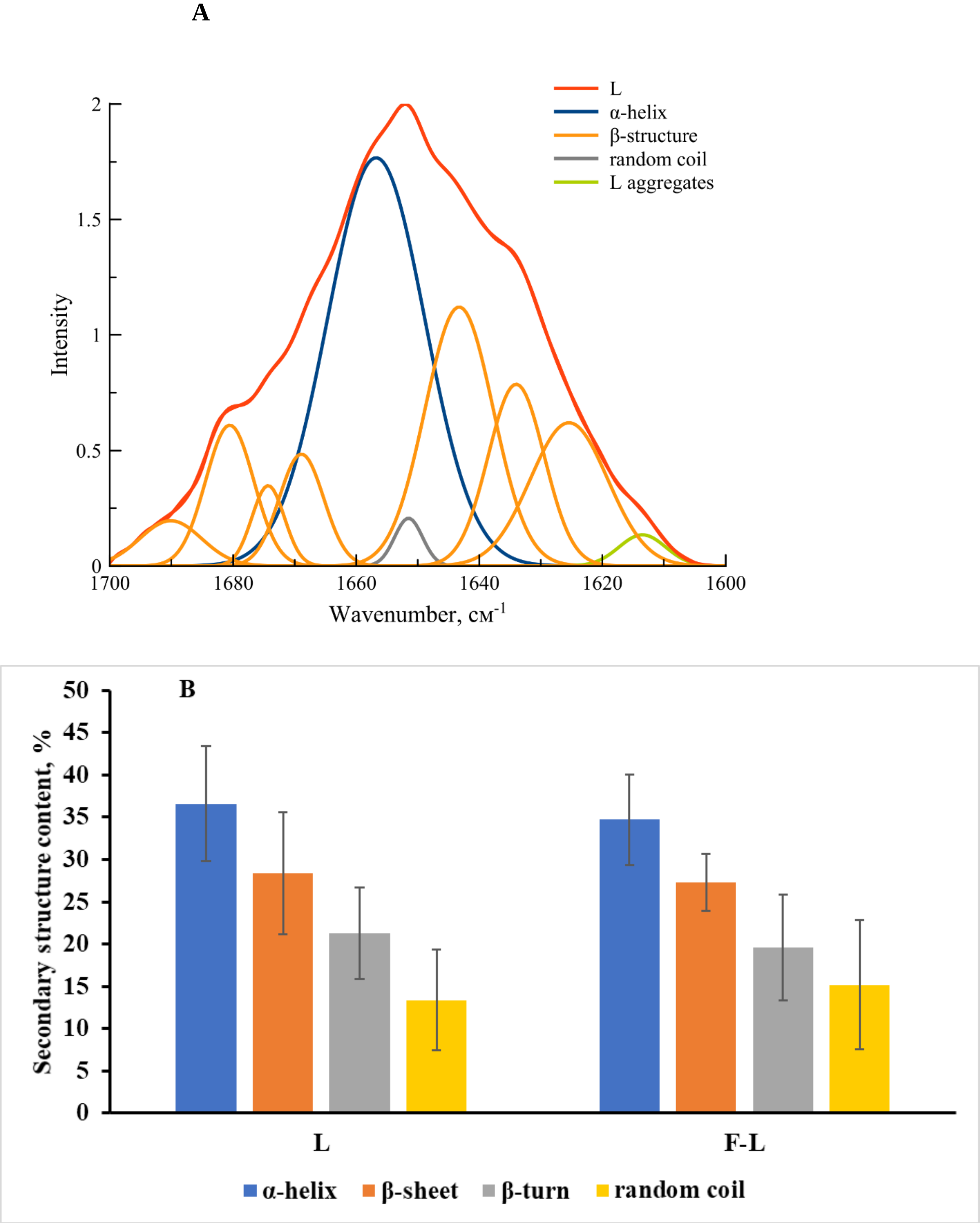

Figure S1. A, B. Example of deconvolution of amide I band of FT-IR spectrum of lactoferrin (A) and characteristics of the secondary structure of lactoferrin complexed with fucoidan (B) at 2.5 mg mL-1 lactoferrin in 0.05 M tris-HCl buffer.

Amide I band of FT-IR spectrum of lactoferrin 1660-1700 cm^{-1} was analyzed using deconvolution according the algorithm described in [Yang H.]. For this purpos the FT-IR spectra underwent correction of baseline and flattering, and the spectrum region corresponding to 1600-1700 sm^{-1} was approximated by Gauss function with use of Levenberg-Macquardt algorithm. The initial peaks were detected and aligned to the secondary structures was performed according to [Yang H.]. The summary area of peaks corresponding to α-helixes, β-structures, random structures was taken as 100%. Maximum of the α-helix component is the range of 1653-1656 cm^{-1}, random structures – 1647-1651 cm^{-1}, β-turns – 1665-1668 cm^{-1}, 1674-1677 cm^{-1}, 1679-1686 cm^{-1}, β-sheets 1623-1624 cm^{-1}, 1633-1639 cm^{-1}, 1641-1643 cm^{-1}.

Thus, the secondary structure of lactoferrin molecule consisted of α-helixes 36 %, β-sheets 30 %, β-turns 20% and random structures 14%. Formation of polyelectrolyte complex with fucoidan F-L after incubation of lactoferrin with fucoidan for 30 min (1:1 w/w) did not result in protein unfolding.

**Yang H. et al. Obtaining information about protein secondary structures in aqueous solution using Fourier transform IR spectroscopy //Nature protocols. – 2015. – Т. 10. – №. 3. – С. 382-396.*

2. Tryptophan fluorescence of lactoferrin

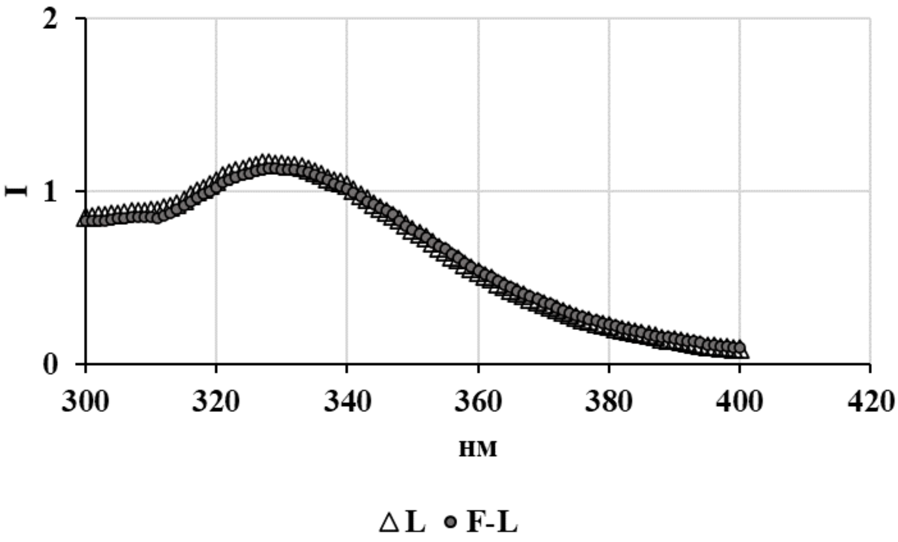

Figure S2. Spectra of tryptophan fluorescence (excitation wavelength 280 nm) of lactoferrin (L) and fucoidan-lactoferrin complex (F-L) in tris-HCI buffer, pH 7.4. Average of 7 measurements is presented.

3. <u>ABTS-radical scavenging activity</u> of fucoidan (F), lactoferrin (L) and F-L complex in 50 mM tris-HCl buffer at each component's concentration 0.5 mg mL^{-1}.

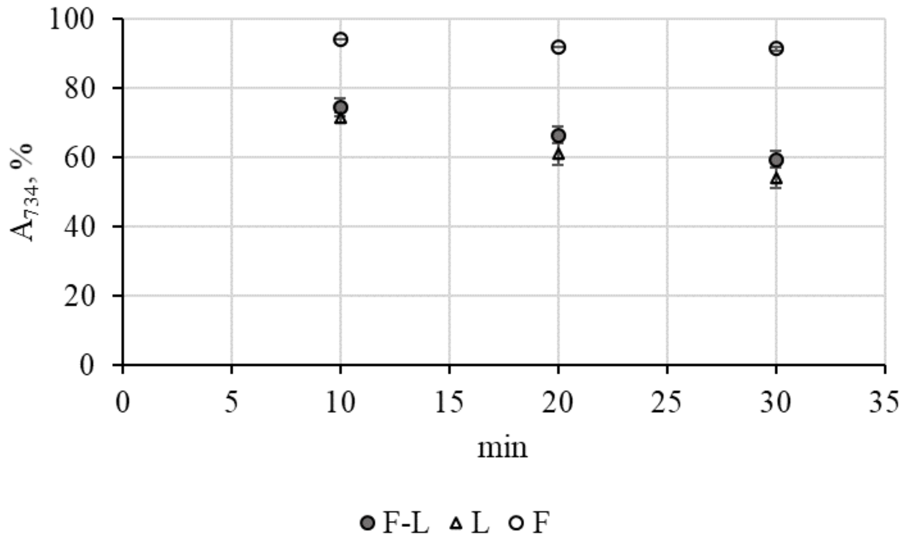

Figure S3. Dynamics of decline in ABTS radical absorbance at 734 nm (A734) in 50 mM tris-HCl buffer (pH 7.4) in presence of 0.5 mg mL^{-1} fucoidan (F) and lactoferrin (L) separately or in complex F-L.

There was no significant difference among lactoferrin and F-L complex according to the data of four independent experiments.

4. Anticoagulant activity: effects of lactoferrin on fucoidan anticoagulant properties according to the assay of thrombin and prothrombin time.

For the prothrombin time (PTT) evaluation, fucoidan, lactoferrin or their complex were added to 100 μL of platelet-poor plasma, incubated for 2 min at 37 ^{o}C. Then 200 μL of thromboplastin-calcium mixture which was previously heated at 37 ^{o}C was added and time which it took plasma to clot was measured with use of coagulometer SOLAR CGL 2110 (Belarus).

For thrombin time (TT) evaluation the prothrombin assay kit “Thromboplastin” (“Renam”, Russia) was used. Fucoidan, lactoferrin or their complex were added to 150 μL of platelet-poor plasma, incubated for 2 min at 37 ^{o}C, followed by addition of 150 μL of working solution of Thrombin-reagent and measured time for plasma clotting with use of coagulometer SOLAR CGL 2110 (Belarus).

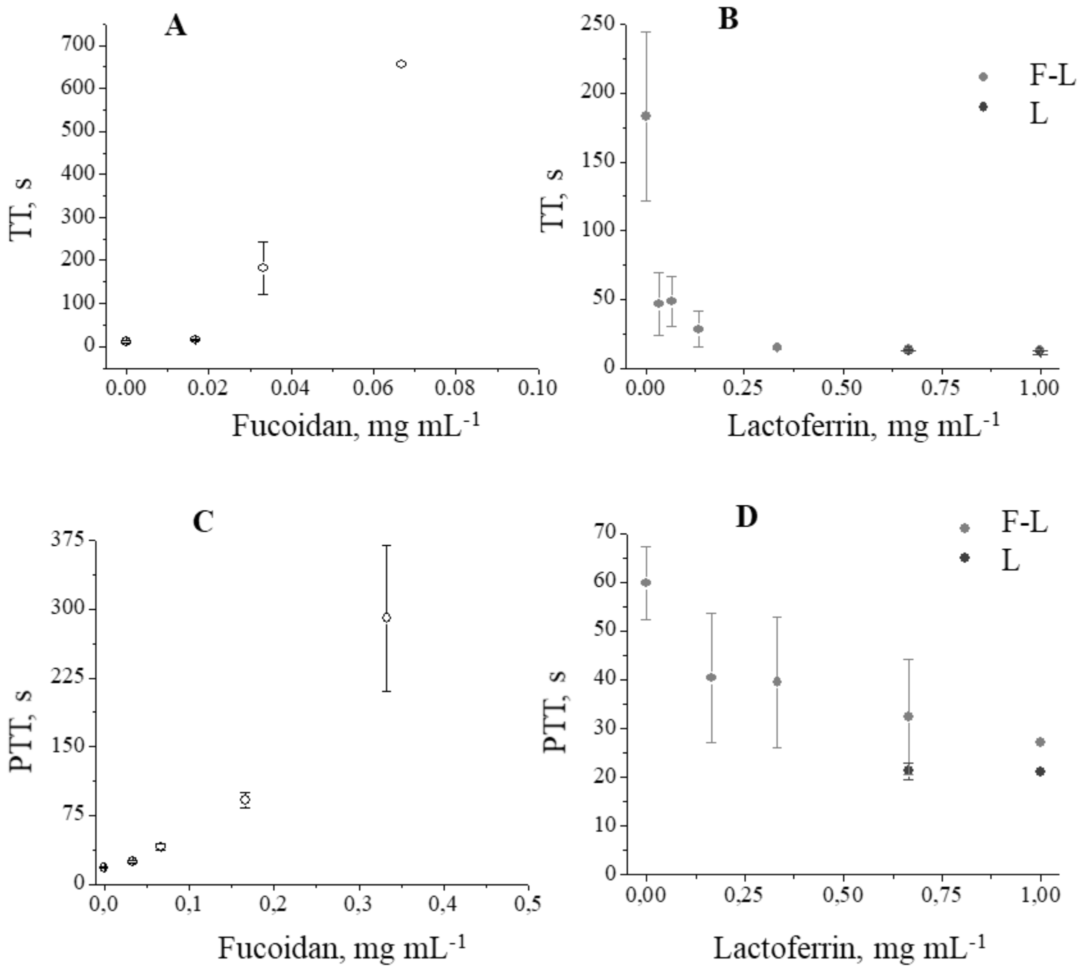

Figure **S4.** Dependence of thrombin time (TT): A – on concentration of fucoidan (F), B – on concentration of lactoferrin (L) added separately or in complex with 0.033 mg mL^{-1} fucoidan (F-L) and dependence of prothrombin time (PTT): C - on concentration of fucoidan (F), D - on concentration of lactoferrin (L) added separately or in complex with 0.167 mg mL^{-1} fucoidan (F-L).