East European Journal of Physics. **2.** 584-593 (2026)
DOI:10.26565/2312-4334-2026-2-65 ISSN 2312-4334

A MOLECULAR DOCKING STUDY OF AMYLOID-POLYSACCHARIDE COMPOSITES: II. INTERACTIONS WITH BIOLOGICALLY ACTIVE PROTEINS AND POLYPHENOLS

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Received March 1, 2026; revised April 13, 2026; accepted May 11, 2026

Amyloid fibrils, structurally unique protein aggregates, are increasingly emerging as a novel type of proteinaceous nanomaterial with an expanding range of applications. One example of a biomedical application of amyloid-based nanomaterials is the fabrication of biocompatible hydrogel adhesives for wound healing. The present study was undertaken to evaluate the possibility of utilizing the lysozyme amyloid fibrils integrated with polysaccharide chitosan as a polymeric matrix for incorporation the agents with pronounced wound healing capabilities such as polyphenols and biologically active proteins lactoferrin and conalbumin. Using the molecular docking technique the binding affinities, amino acid composition of the binding sites and possible competitive interactions between polyphenols have been characterized in the two-, three- and four-component systems. Polyphenolic compounds were found to display an ability to associate with bioactive proteins, with the highest binding affinities being revealed for curcumin enol, quercetin and sesamin. In the three- and four-component systems the binding sites for polyphenols are either localized exclusively on lactoferrin or conalbumin or encompass amino acid residues of both fibrillar lysozyme and bioactive proteins. Combinations of polyphenols that can compete with each other for binding sites have been identified. These findings provide a basis for the development of novel amyloid-based nanoformulations with wound-healing properties.
Keywords: *Lysozyme amyloid fibrils; Chitosan; Polyphenols; Lactoferrin; Conalbumin; Binding sites; Binding affinity; Molecular docking*
PACS: 87.14.C++c, 87.16.Dg

Amyloid fibrils, highly ordered protein assemblies distinguished by the presence of β-structured core region are currently regarded as a prospective type of biocompatible, biodegradable and structurally stable proteinaceous nanomaterials [1, 2]. A rapidly expanding area of their potential applications includes removal of heavy metals and other contaminants from water [3], sensing of various substances such as glucose [4], heavy metals [5], nitrogen dioxide [6] etc., production of bioplastic for food packaging [7], fabrication of conductive nanowires [8], cell scaffolding [9], drug delivery [10], to name only a few. Growing evidence indicates that amyloid–based materials have strong pro-regenerative potential and can accelerate wound repair [11, 12]. In particular, the hydrogel derived from lysozyme amyloid fibrils was used to prepare injectable adhesives with improved anti-swelling and antibacterial capabilities to stimulate wound closure and hemostasis [13, 14]. An advantageous feature of fibrillar lysozyme is its intrinsic antibacterial activity [15] that can be increased in the composite hydrogels from amyloid fibrils and other biopolymers with wound healing properties. In this regard, much attention has been given to chitosan, a natural polymer with antimicrobial, mucoadhesive, and anti-inflammatory activities that are favorable for wound treatment [16, 17]. Further loading of composite hydrogels with various therapeutic agents enables the creation of more efficient nanosystems for wound healing applications.

In the previous paper in this series, we obtained molecular docking predictions of the binding sites for six polyphenolic compounds from different classes in the binary system of fibrillar lysozyme–chitosan [18]. The aim of the present work was to extend the above studies by introducing additional therapeutic components, such as the functional proteins lactoferrin and conalbumin (ovotransferrin), which, like polyphenols, can promote wound repair through multiple mechanisms [19-23]. Specifically, polyphenols are known to reduce oxidative stress, modulate inflammatory responses, and enhance tissue regeneration [23], lactoferrin displays immuno-modulating activities, promotes tissue granulation, reepithelization, and synthesis of the elements of the extracellular matrix [19-21], conalbumin has antioxidant, anti-inflammatory, and immuno-stimulating properties [22, 24].

METHODS

The structures of polyphenols under study drawn in MarvinSketch software, v.18.10, ChemAxon were then optimized with Avogadro 1.1.0 software using the Universal Force Field21. The structures of bovine lactoferrin (PDB ID 1BLF) and conalbumin from chicken egg white (PDB ID 8FEI) were taken from the Protein Data Bank. The structure of the 12-mer chitosan (CS) was derived from PolySac3DB, a database of polysaccharide 3D structures (http://polysac3db.cermav.cnrs.fr). The Avogadro 1.1.0 software was utilized to protonate the chitosan molecule and optimize its geometry. The docking of the proteins and polyphenols to amyloid fibrils was conducted using the web-based server HDOCK which combines template-based and free docking [25]. The most energetically favorable docking complexes were visualized using VMD.

Cite as: V. Trusova, U. Malovytsia, O. Zhytniakivska, G. Gorbenko, East Eur. J. Phys. 2, 584 (2026), https://doi.org/10.26565/2312-4334-2026-2-65

RESULTS AND DISCUSSION

The examined systems contain two components of bioactive polymeric matrix, viz. the lysozyme amyloid fibrils and chitosan, and two therapeutic agents representing different classes of polyphenols (curcumin (enol and keto froms), gallic acid, salicylic acid, quercetin, resveratrol, sesamin) and functional proteins (lactoferrin and conalbumin). The molecular docking approach was employed to uncover the possible binding preferences of these components. Shown in Fig. 1 are the most energetically favorable complexes between lactoferrin and the examined polyphenolic compounds.

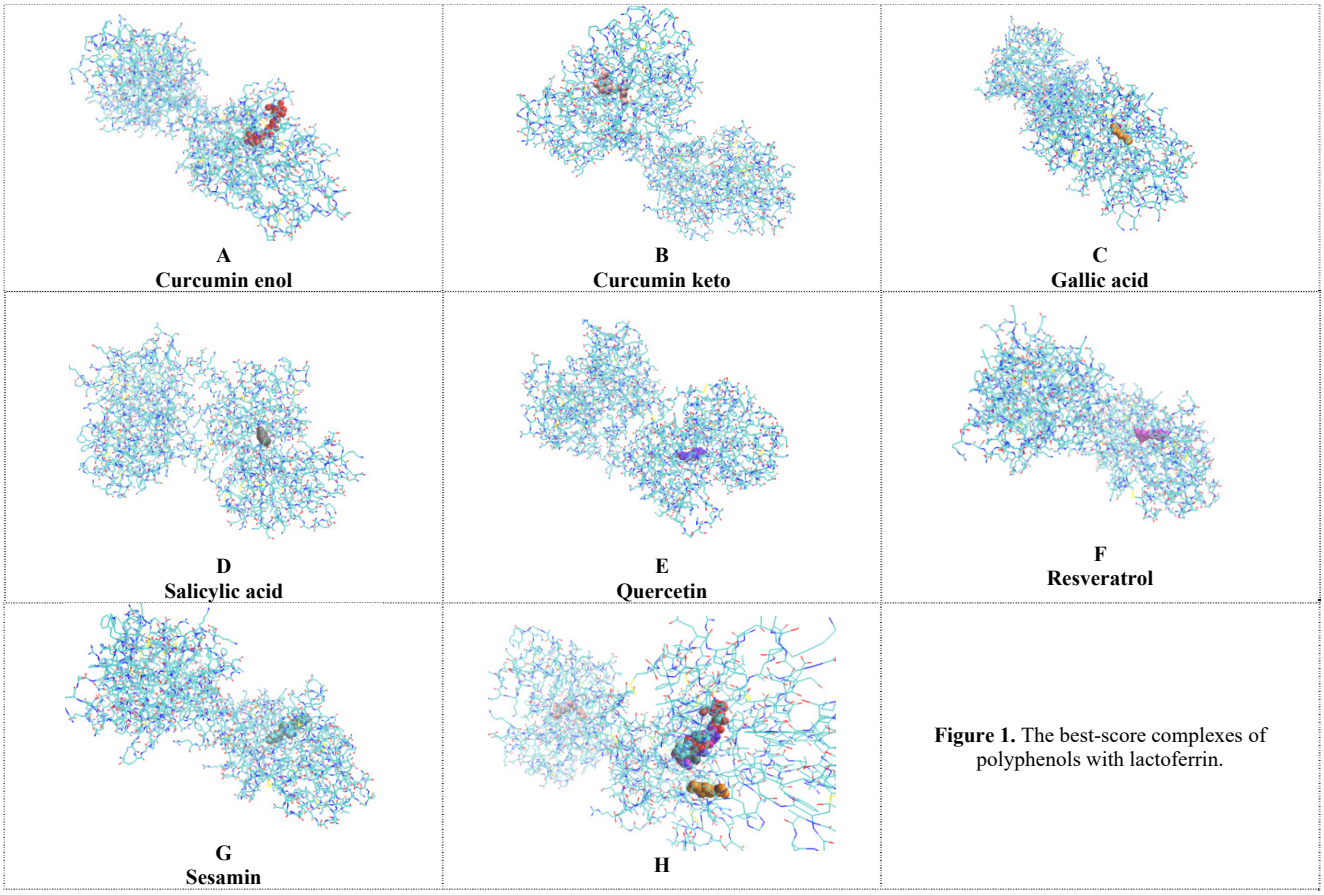

Figure 1. The best-score complexes of polyphenols with lactoferrin.

The comparative depiction of the docking poses (Fig. 1, H) indicates that the binding sites for most polyphenols are close to each other, whereas the curcumin keto and gallic acid occupy remote sites. In the case of conalbumin (Fig. 2), two predominant types of the binding sites are observed – the sites of the first type accommodate curcumin keto, gallic and salicylic acids, quercetin, resveratrol, and sesamin reside on the sites of the second type, while curcumin enol is located at the remote site with completely different interface residues (Table 1).

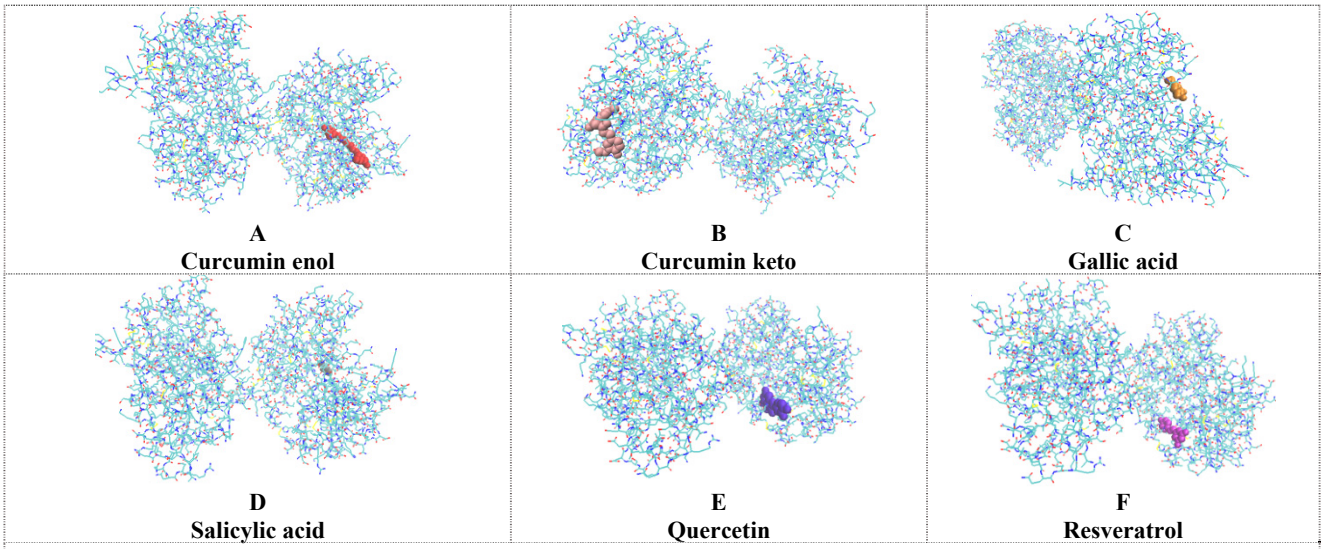

Figure 2. The best-score complexes of polyphenols with conalbumin
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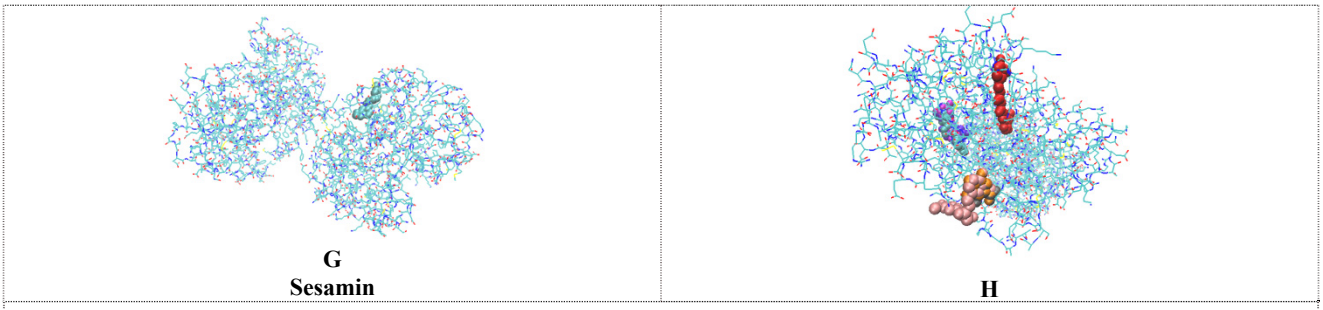


G
Sesamin

H

Figure 2. The best-score complexes of polyphenols with conalbumin (*continuation*)

Table 1. The interface residues in the complexes of polyphenols with lactoferrin and conalbumin

Polyphenol	Lactoferrin	Conalbumin
Curcumin enol	VAL_{350A} PRO_{352A} GLU_{353A} GLU_{354A} ARG_{463A} ASP_{508A} ASP_{509A} SER_{519A} LYS_{520A} GLU_{521A} LYS_{522A} TYR_{523A} TYR_{524A} GLY_{525A} TYR_{526A} THR_{527A} GLY_{528A} ARG_{531A} HIS_{595A} LYS_{637A} ASN_{638A} LEU_{639A} LEU_{640A} ASN_{642A}	VAL_{350A} GLU_{354A} THR_{377A} ALA_{393A} LEU_{394A} ARG_{460A} ALA_{515A} SER_{516A} SER_{517A} HIS_{518A} GLU_{519A} LYS_{520A} PHE_{522A} GLY_{523A} TYR_{524A} THR_{525A} GLY_{526A} ARG_{529A} TRP_{557A} HIS_{592A} ASN_{632A} LYS_{633A} ASP_{634A}
Curcumin keto	ILE_{11A} GLU_{15A} PHE_{41A} ALA_{42A} THR_{58A} LEU_{59A} ASP_{60A} MET_{63A} LEU_{119A} GLY_{120A} ARG_{121A} CYS_{160A} CYS_{183A} SER_{185A} PHE_{190A} GLY_{191A} TYR_{192A} SER_{193A} GLY_{194A} HIS_{253A} GLN_{295A} ARG_{296A} ASP_{297A} LEU_{298A}	ARG_{414A} TYR_{415A} ASP_{416A} ARG_{427A} PRO_{428A} ALA_{429A} SER_{430A} TYR_{431A} PHE_{432A} HIS_{542A} TYR_{581A} ARG_{582A} GLU_{588A} LEU_{640A}
Gallic acid	GLU_{413A} ASN_{414A} ARG_{415A} TYR_{433A} TYR_{526A} LYS_{544A} ASP_{546A} PRO_{593A} ASN_{594A} HIS_{595A} ASN_{642A} ASN_{644A} THR_{645A}	GLU_{413A} ARG_{414A} TYR_{415A} ASP_{416A} ARG_{427A} PRO_{428A} ALA_{429A} SER_{430A} TYR_{431A} PHE_{432A} HIS_{542A} TYR_{581A} ARG_{582A} LEU_{640A}
Salicylic acid	GLU_{354A} ASN_{393A} LEU_{394A} GLU_{413A} ARG_{463A} TYR_{524A} TYR_{526A} HIS_{595A} LYS_{637A} ASN_{638A} LEU_{639A} LEU_{640A} PHE_{641A} ASN_{642A}	GLU_{413A} ARG_{414A} TYR_{415A} ASP_{416A} ARG_{427A} PRO_{428A} ALA_{429A} SER_{430A} TYR_{431A} PHE_{432A} HIS_{542A} ARG_{582A}
Quercetin	GLU_{354A} ASN_{393A} LEU_{394A} ASP_{395A} GLU_{413A} ARG_{463A} ASN_{518A} SER_{519A} GLU_{521A} TYR_{524A} GLY_{525A} TYR_{526A} THR_{527A} GLY_{528A} HIS_{595A} LYS_{637A} ASN_{638A} LEU_{639A} LEU_{640A} PHE_{641A} ASN_{642A}	VAL_{458A} GLY_{459A} TRP_{464A} CYS_{478A} ASN_{479A} PHE_{480A} ASP_{481A} SER_{492A} PRO_{493A} LEU_{668A} LYS_{669A} CYS_{671A} ASN_{672A} SER_{674A}
Resveratrol	GLU_{354A} ASN_{393A} LEU_{394A} GLU_{413A} ARG_{463A} SER_{519A} TYR_{524A} GLY_{525A} TYR_{526A} THR_{527A} GLY_{528A} HIS_{595A} LYS_{637A} ASN_{638A} LEU_{639A} LEU_{640A} PHE_{641A} ASN_{642A}	VAL_{458A} GLY_{459A} TRP_{464A} CYS_{478A} ASN_{479A} PHE_{480A} ASP_{481A} SER_{492A} PRO_{493A} SER_{496A} LEU_{498A} ILE_{665A} LEU_{668A} LYS_{669A} CYS_{671A} ASN_{672A}
Sesamin	GLU_{353A} GLU_{354A} THR_{377A} ASN_{393A} LEU_{394A} ASP_{395A} TYR_{398A} ARG_{463A} ASN_{518A} SER_{519A} GLU_{521A} LYS_{522A} TYR_{523A} TYR_{524A} GLY_{525A} TYR_{526A} THR_{527A} GLY_{528A} HIS_{595A} LYS_{637A} ASN_{638A} LEU_{639A} ASN_{642A}	TYR_{400A} VAL_{458A} TRP_{464A} VAL_{465A} CYS_{478A} ASN_{479A} PHE_{480A} ASP_{481A} LEU_{498A} ILE_{665A} LEU_{668A} LYS_{669A} CYS_{671A} ASN_{672A} PRO_{673A} LEU_{677A}

These results are suggestive of the possibility that the use of certain polyphenol combinations may lead to competitive interactions between these compounds for the binding sites on lactoferrin or conalbumin.

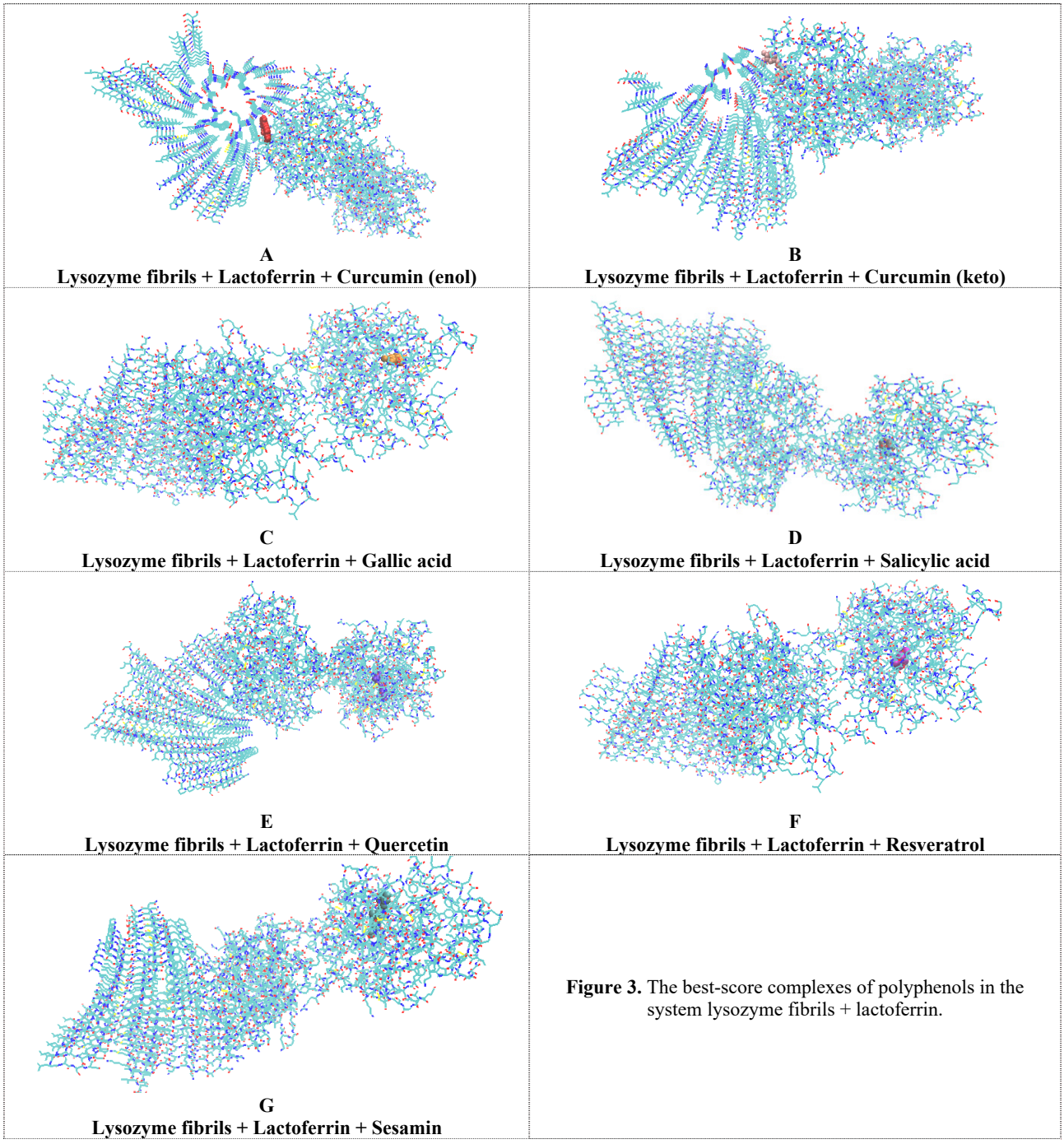
The analysis of the best score values (Table 2) showed that quercetin forms the strongest complexes with lactoferrin, while curcumin (enol) - with conalbumin, with the binding affinities increasing in the order: salicylic acid < gallic acid < resveratrol < curcumin keto < curcumin enol < sesamin < quercetin for lactoferrin, and in the order salicylic acid < gallic acid < resveratrol < curcumin keto < quercetin < sesamin < curcumin enol for conalbumin. Notably, gallic and salicylic acids displayed the lowest affinity for both proteins. Another observation noteworthy is that polyphenols appeared to have slightly different (curcumin enol) or higher affinities for lactoferrin and conalbumin compared to those for the lysozyme fibrils [18].

Table 2. The best score values for the complexes of polyphenols with lactoferrin, conalbumin and chitosan

Polyphenol	Lactoferrin	Conalbumin	Chitosan
Curcumin enol	-177.30 (0.633)	-181.39 (0.652)	-18.64 (0.067)
Curcumin keto	-162.58 (0.563)	-151.14 (0.506)	-17.23 (0.066)
Gallic acid	-128.36 (0.394)	-117.76 (0.344)	-16.58 (0.065)
Salicylic acid	-107.48 (0.299)	-98.36 (0.263)	-13.78 (0.062)
Quercetin	-204.36 (0.748)	-169.24 (0.595)	-21.45 (0.071)
Resveratrol	-154.94 (0.525)	-126.49 (0.385)	-14.78 (0.063)
Sesamin	-187.76 (0.680)	-172.04 (0.608)	-17.94 (0.067)

*The confidence scores for binding probability are given in parentheses, with the values > 0.7 indicating a high probability, 0.5-0.7 moderate probability, and < 0.5 - low probability.

Accordingly, in the systems lysozyme fibrils + proteins the binding sites for polyphenols predominantly encompass the amino acid residues of lactoferrin or conalbumin (Figs. 3, 4), although in some cases (marked in grey in Table 3) the amino acids of fibrillar lysozyme also contribute to stabilization of the complexes being formed.



A
Lysozyme fibrils + Lactoferrin + Curcumin (enol)

B
Lysozyme fibrils + Lactoferrin + Curcumin (keto)

C
Lysozyme fibrils + Lactoferrin + Gallic acid

D
Lysozyme fibrils + Lactoferrin + Salicylic acid

E
Lysozyme fibrils + Lactoferrin + Quercetin

F
Lysozyme fibrils + Lactoferrin + Resveratrol

G
Lysozyme fibrils + Lactoferrin + Sesamin

Figure 3. The best-score complexes of polyphenols in the system lysozyme fibrils + lactoferrin.

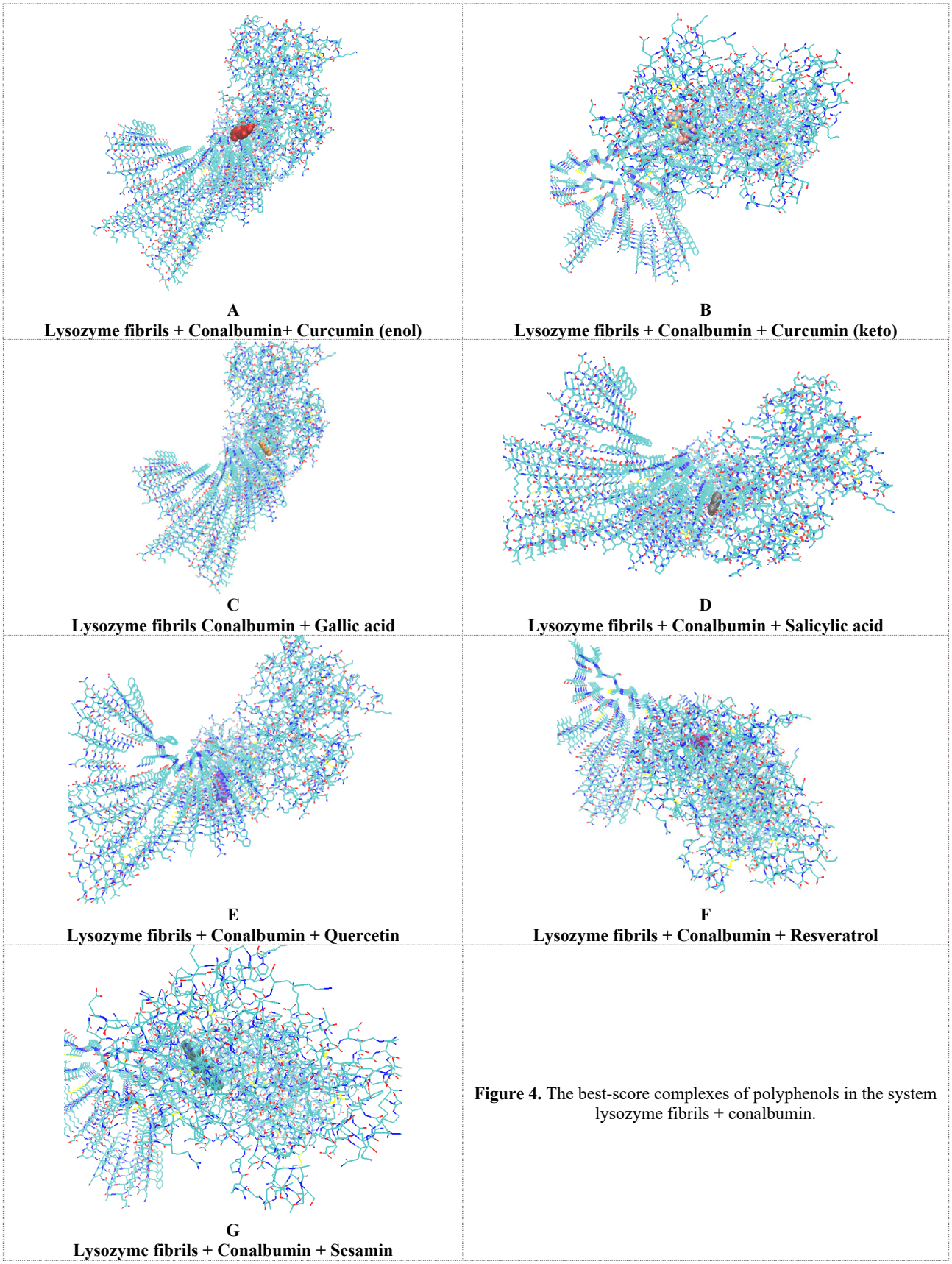
In the lactoferrin-containing systems, only curcumin enol and curcumin keto were found to form contacts with both lactoferrin and lysozyme fibrils, whereas the binding sites for other polyphenols are localized exclusively on the protein molecule (Fig. 3, Table 3).

Table 3. The interface residues in the complexes of polyphenols with the components of the systems lysozyme fibrils + lactoferrin / conalbumin in the absence and presence of chitosan

	Lactoferrin	**Conalbumin**	**Lactoferrin**	**Conalbumin**
	Lysozyme fibrils		**Lysozyme fibrils + chitosan**	
Curcumin enol	PHE_{34C} GLU_{35C} PHE_{34E} GLU_{35E} SER_{36E} PHE_{34G} GLU_{35G} SER_{36G} PHE_{34I}	TRP_{63A} GLY_{26C} TRP_{63C} GLY_{26E} TRP_{63E} GLY_{26G} TRP_{63G} GLY_{26I} TRP_{63I}	PHE_{34A} PHE_{34C} GLU_{35C} SER_{36C} PHE_{34E} GLU_{35E} SER_{36E} PHE_{34G} GLU_{35G}	VAL_{350A} GLU_{354A} THR_{377A} ALA_{393A} ARG_{460A} ALA_{515A}

	Lactoferrin	Conalbumin	Lactoferrin	Conalbumin
	Lysozyme fibrils		**Lysozyme fibrils + chitosan**	
	GLU_{35I} SER_{36I} GLN_{13A} PRO_{14A} PHE_{17A} LYS_{18A} ARG_{21A} GLY_{175A} GLU_{176A} ASN_{179A} SER_{185A} ARG_{186A} PRO_{188A} PRO_{292A} PRO_{293A} GLN_{295A}	VAL_{81A} SER_{88A} GLN_{271A} SER_{272A} ASP_{273A} PHE_{274A} GLY_{275A} VAL_{276A} ASP_{277A} THR_{278A} PHE_{285A} ILE_{305A} MET_{306A} LYS_{308A} GLY_{685A} LYS_{686A}	SER_{36G} PHE_{34I} SER_{36I} GLN_{13A} PRO_{14A} PHE_{17A} ARG_{21A} LYS_{174A} ARG_{186A} PRO_{188A} PRO_{292A} PRO_{293A} GLN_{295A}	SER_{516A} SER_{517A} HIS_{518A} GLU_{519A} LYS_{520A} PHE_{522A} GLY_{523A} TYR_{524A} THR_{525A} GLY_{526A} ARG_{529A} TRP_{557A} HIS_{592A} ASN_{632A} LYS_{633A} ASP_{634A}
Curcumin keto	PHE_{38A} ASN_{39A} PHE_{38C} ASN_{39C} PHE_{38E} GLU_{216A} LYS_{280A} ASN_{281A} GLN_{287A} PHE_{289A} GLY_{290A} SER_{291A} ARG_{296A} ASP_{302A} SER_{303A}	ARG_{61A} ASP_{66A} PRO_{70A} GLY_{71A} SER_{72A} ASP_{66C} VAL_{458A} TRP_{464A} CYS_{478A} ASN_{479A} PHE_{480A} ASP_{481A} SER_{492A} PRO_{493A} LEU_{498A} LEU_{68A} LYS_{669A} CYS_{671A} ASN_{672A}	SER_{36G} ASN_{37G} PHE_{38G} SER_{36I} ASN_{37I} PHE_{38I} ASN_{39I} SER_{93A} LYS_{197A} GLN_{200A} ASP_{201A} ASN_{217A} LEU_{218A} ASP_{223A} GLN_{226A} TYR_{227A} PRO_{292A} PRO_{293A} GLY_{294A} ARG_{296A}	TRP_{63E} CYS_{64E} ASN_{65E} TRP_{63G} CYS_{64G} ASN_{65G} GLY_{26I} TRP_{63I} ASN_{65I} PHE_{432A} ARG_{582A} GLU_{583A} ASN_{585A} ALA_{587A} GLU_{588A} LYS_{660A}
Gallic acid	GLU_{413A} ASN_{414A} ARG_{415A} TYR_{433A} TYR_{526A} LYS_{544A} ASP_{546A} PRO_{593A} ASN_{594A} HIS_{595A} ASN_{642A} ASN_{644A} THR_{645A}	TRP_{63A} CYS_{64A} ASN_{65A} TRP_{63C} CYS_{64C} ASN_{65C} TRP_{264A} LYS_{308A} ARG_{309A} VAL_{310A} PRO_{311A} SER_{312A} GLN_{678A} MET_{679A} PHE_{682A}	SER_{36G} ASN_{37G} PHE_{38G} SER_{36I} PHE_{38I} SER_{193A} GLY_{194A} PHE_{196A} LYS_{197A} GLN_{200A} ASN_{217A} PRO_{293A} GLY_{294A} ARG_{296A}	TRP_{28I} CYS_{30I} SER_{60I} TRP_{62I} TRP_{445A} GLY_{469A} LEU_{470A} ILE_{471A} ASN_{473A} ARG_{474A} THR_{571A} ASP_{572A}
Salicylic acid	GLU_{354A} ASN_{393A} LEU_{394A} GLU_{413A} ARG_{463A} TYR_{524A} TYR_{526A} HIS_{595A} ASN_{638A} LEU_{639A} LEU_{640A} PHE_{641A} ASN_{642A}	TRP_{63A} CYS_{64A} ASN_{65A} TRP_{63C} CYS_{64C} ASN_{65C} TRP_{63E} TRP_{264A} LYS_{308A} ARG_{309A} VAL_{310A} PRO_{311A}	PHE_{38G} PHE_{38I} SER_{193A} GLY_{194A} PHE_{196A} LYS_{197A} GLN_{200A} ASN_{217A} PRO_{293A} GLY_{294A} ARG_{296A}	TRP_{28I} CYS_{30I} SER_{60I} TRP_{62I} TRP_{445A} GLY_{469A} LEU_{470A} ILE_{471A} ASN_{473A} ARG_{474A} THR_{571A}
Quercetin	GLU_{354A} ASN_{393A} LEU_{394A} ASP_{395A} GLU_{413A} ARG_{463A} SER_{519A} TYR_{524A} GLY_{525A} TYR_{526A} THR_{527A} GLY_{528A} HIS_{595A} LYS_{637A} ASN_{638A} LEU_{639A} LEU_{640A} PHE_{641A} ASN_{642A}	VAL_{458A} GLY_{459A} TRP_{464A} CYS_{478A} ASN_{479A} PHE_{480A} ASP_{481A} SER_{492A} PRO_{493A} LEU_{668A} LYS_{669A} CYS_{671A} ASN_{672A} SER_{674A}	GLU_{354A} ASN_{393A} LEU_{394A} ASP_{395A} GLU_{413A} ARG_{463A} SER_{519A} GLU_{521A} TYR_{524A} GLY_{525A} TYR_{526A} THR_{527A} GLY_{528A} HIS_{595A} LYS_{637A} ASN_{638A} LEU_{639A} LEU_{640A} PHE_{641A} ASN_{642A}	TRP_{28I} ILE_{58I} ASN_{59I} SER_{60I} ARG_{61I} TRP_{62I} SER_{72I} ASN_{74I} TRP_{445A} LEU_{470A} ASN_{473A} ARG_{474A} THR_{571A} ASP_{572A}
Resveratrol	GLU_{354A} ASN_{393A} LEU_{394A} GLU_{413A} ARG_{463A} SER_{519A} GLU_{521A} TYR_{524A} GLY_{525A} TYR_{526A} THR_{527A} GLY_{528A} HIS_{595A} LYS_{637A} ASN_{638A} LEU_{639A} LEU_{640A} PHE_{641A} ASN_{642A}	ASN_{59A} SER_{60A} ARG_{61A} GLY_{71A} SER_{72A} ASN_{59C} VAL_{458A} TRP_{464A} CYS_{478A} ASN_{479A} PHE_{480A} ASP_{481A} LEU_{498A} CYS_{671A} ASN_{672A}	GLU_{354A} ASN_{393A} LEU_{394A} GLU_{413A} ARG_{463A} SER_{519A} TYR_{524A} GLY_{525A} TYR_{526A} THR_{527A} GLY_{528A} HIS_{595A} LYS_{637A} ASN_{638A} LEU_{639A} LEU_{640A} PHE_{641A} ASN_{642A}	GLU_{35C} LYS_{33E} GLU_{35E} ARG_{45E} LYS_{33G} GLU_{35G} ARG_{45G} ASP_{52G} LYS_{33I} GLU_{35I} ARG_{45I} ASP_{52I} CS_7 CS_8 CS_9 CS_{10}
Sesamin	GLU_{353A} GLU_{354A} ASN_{393A} LEU_{394A} ASP_{395A} TYR_{398A} ARG_{463A} ASN_{518A} SER_{519A} GLU_{521A} LYS_{522A} TYR_{523A} TYR_{524A} GLY_{525A} TYR_{526A} THR_{527A} GLY_{528A} ARG_{531A} HIS_{595A} THR_{636A} LYS_{637A} ASN_{638A} LEU_{639A} PHE_{641A} ASN_{642A}	TYR_{400A} VAL_{458A} TRP_{464A} VAL_{465A} CYS_{478A} ASN_{479A} PHE_{480A} ASP_{481A} LEU_{498A} ILE_{665A} LEU_{668A} LYS_{669A} CYS_{671A} ASN_{672A} PRO_{673A} LEU_{677A}	GLU_{35A} PHE_{34C} GLU_{35C} SER_{36C} PHE_{34E} GLU_{35E} SER_{36E} PHE_{34G} GLU_{35G} SER_{36G} PHE_{34I} GLU_{35I} SER_{36I} PHE_{17A} ARG_{21A} PRO_{292A} PRO_{293A} GLY_{294A} GLN_{295A}	LYS_{33E} PHE_{34E} GLU_{35E} ARG_{45E} LYS_{33G} PHE_{34G} GLU_{35G} ARG_{45G} LYS_{33I} PHE_{34I} GLU_{35I} ARG_{45I} ASP_{52I} CS_7 CS_8 CS_9 CS_{10} CS_{11}

At the same time, in the conalbumin-containing systems, quercetin and sesamin reside on the protein molecule, while the other polyphenolic compounds form contacts with both conalbumin and lysozyme fibrils (Fig. 4, Table 3).



A
Lysozyme fibrils + Conalbumin+ Curcumin (enol)

B
Lysozyme fibrils + Conalbumin + Curcumin (keto)

C
Lysozyme fibrils Conalbumin + Gallic acid

D
Lysozyme fibrils + Conalbumin + Salicylic acid

E
Lysozyme fibrils + Conalbumin + Quercetin

F
Lysozyme fibrils + Conalbumin + Resveratrol

G
Lysozyme fibrils + Conalbumin + Sesamin

Figure 4. The best-score complexes of polyphenols in the system lysozyme fibrils + conalbumin.

The addition of chitosan to the system lysozyme fibrils + lactoferrin / conalbumin resulted in the association of polyphenols with completely different or slightly changed binding sites (Figs. 5, 6, Table 3). As judged from Table 3, the

smallest changes in the presence of chitosan were observed in the lactoferrin-containing systems for quercetin (the appearance of GLU_{521A} in the binding site) and resveratrol (the disappearance of GLU_{521A} in the binding site). Likewise, the analysis of the composition of binding sites showed that in the ternary systems with lactoferrin there exists a possibility of competitive interactions between salicylic acid, quercetin, resveratrol and sesamin; while in the systems with conalbumin gallic acid can compete with salicylic acid, quercetin – with resveratrol and sesamin. In the quaternary systems with lactoferrin the competition may occur between curcumin keto, gallic and salicylic acids, quercetin and resveratrol, curcumin enol and sesamin, while in the systems with conalbumin the competitive ligands are represented by quercetin, gallic and salicylic acids, resveratrol and sesamin. Obviously, the possibility of competitive interactions must be taken into account while incorporating the mixtures of different polyphenols into a polymeric matrix.

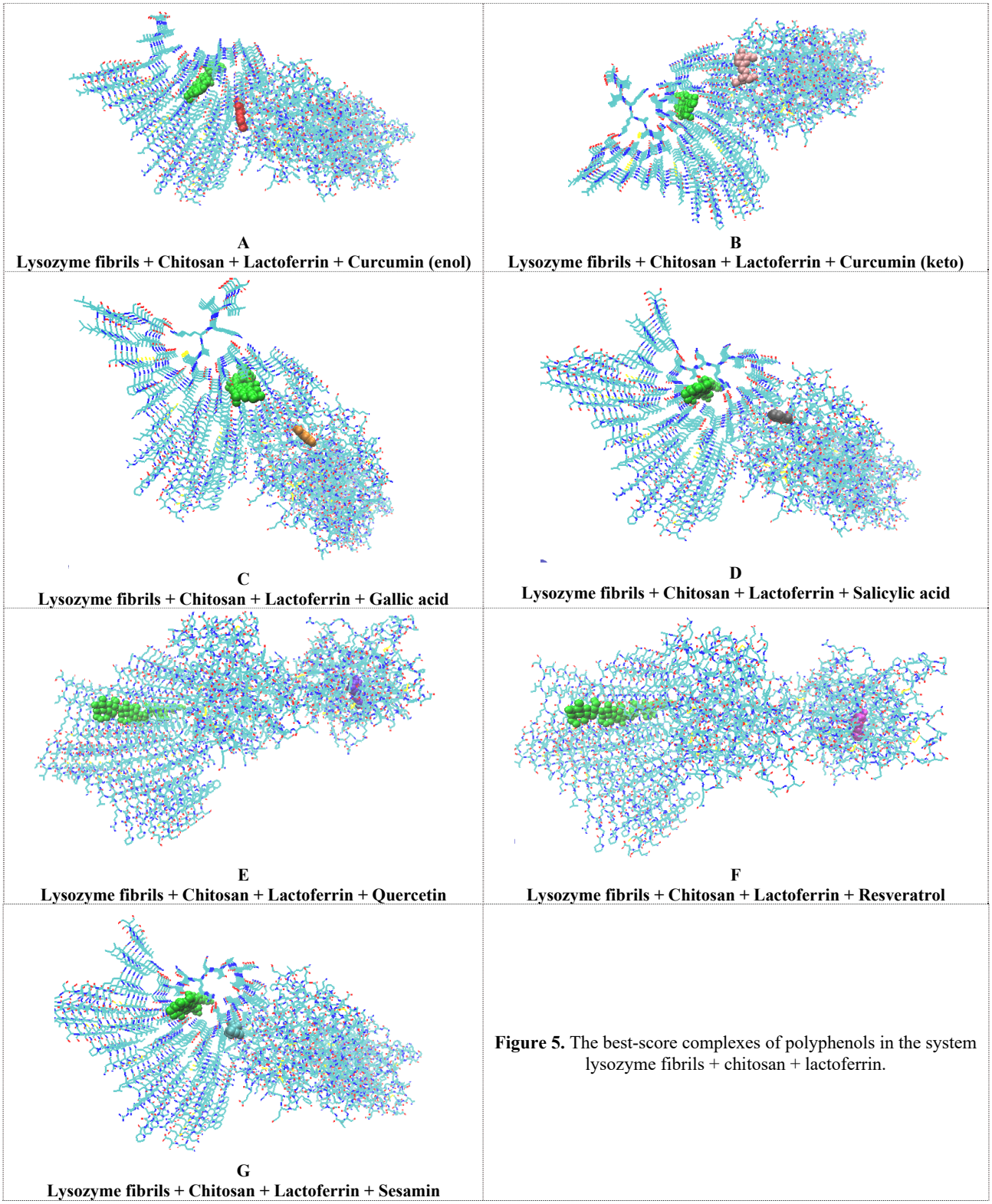


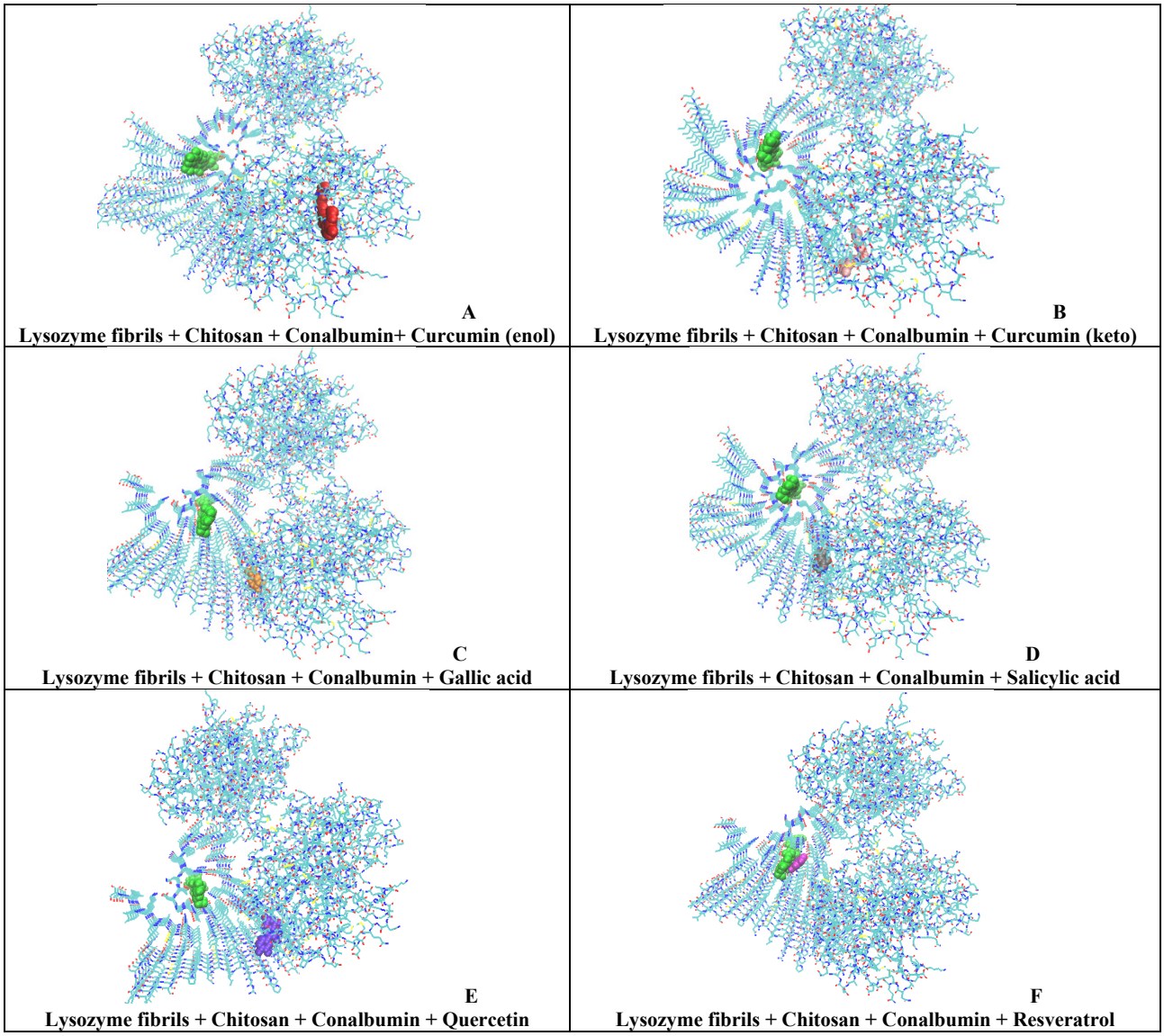
Figure 5. The best-score complexes of polyphenols in the system lysozyme fibrils + chitosan + lactoferrin.

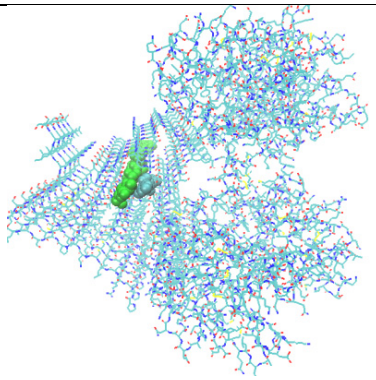
As seen from Table 4, in most cases, chitosan did not exert influence on the binding affinities of polyphenols – the changes in the best score values were less than 10%. The most pronounced increases in the best score values were observed in the systems with lactoferrin / conalbumin and curcumin enol, by 14% and 10%, respectively. On the contrary, the binding affinities of the gallic and salicylic acids showed a decrease (~12%) in the systems with chitosan and conalbumin (Table 4).

Table 4. The best score values for polyphenol binding with the components of the systems lysozyme fibrils + lactoferrin / conalbumin, in the absence and presence of chitosan

Polyphenol	Lysozyme fibrils + Lactoferrin	Lysozyme fibrils + Chitosan + Lactoferrin	Lysozyme fibrils +Conalbumin	Lysozyme fibrils +Chitosan + Conalbumin
	-333.24 (0.975)	-335.82 (0.976)	-280.81 (0.932)	-295.49 (0.948)
Curcumin enol	-179.21 (0.642)	-204.77 (0.749)	-185.38 (0.669)	-183.19 (0.660)
Curcumin keto	-169.33 (0.596)	-177.26 (0.633)	-167.26 (0.586)	-183.75 (0.663)
Gallic acid	-127.27 (0.388)	-128.33 (0.393)	-141.75 (0.459)	-124.36 (0.375)
Salicylic acid	-106.88 (0.297)	-113.55 (0.325)	-117.21 (0.342)	-102.86 (0.280)
Quercetin	-205.28 (0.751)	-203.43 (0.744)	-171.98 (0.608)	-173.77 (0.617)
Resveratrol	-154.12 (0.521)	-154.71 (0.524)	-132.80 (0.415)	-135.29 (0.427)
Sesamin	-187.74 (0.680)	-197.86 (0.723)	-173.6 (0.616)	-185.91 (0.672)

Interestingly, in the systems with conalbumin resveratrol and sesamin displayed the ability to form contacts with chitosan, so that the binding sites for these polyphenols contain only amino acid residues of lysozyme fibrils and monomeric subunits of chitosan (Table 3), while in the absence of polysaccharide resveratrol form contacts only with conalbumin, while sesamin associate with both the protein and fibril (Fig. 6, F, G). In the presence of chitosan the binding affinity of resveratrol remained practically unchanged, while that of sesamin slightly increased (Table 4).





G
Lysozyme fibrils + Chitosan + Conalbumin + Sesamin

Figure 6. The best-score complexes of polyphenols in the system lysozyme fibrils +chitosan + conalbumin

Another noteworthy observation is that the association of the examined polyphenols with chitosan per se is very weak; the best docking scores range from -13 to -21 (Table 2). However, as shown in our previous work [18], when chitosan resides in the grooves of lysozyme fibrils, most polyphenolic compounds tend to form contacts with both the amyloid fibrils and the polysaccharide, and binding affinities are much higher than those for free chitosan. At the same time, in the systems complemented by lactoferrin or conalbumin, polyphenols exhibit more complex binding behavior, so that only resveratrol and sesamin interact exclusively with fibrillar lysozyme and chitosan in the presence of conalbumin.

CONCLUSIONS

In summary, the present study demonstrated the possibility of creating nanosystems composed of four biologically active components: lysozyme amyloid fibrils, chitosan as the polymeric matrix, and lactoferrin or conalbumin and polyphenols as therapeutic agents. The molecular docking technique provided insights into the behavior of these components in binary, ternary, and quaternary systems. The main results are as follows: i) the binding affinity of polyphenols for functional proteins increases in the order salicylic acid < gallic acid < resveratrol < curcumin keto < curcumin enol < sesamin < quercetin for lactoferrin, and in the order salicylic acid < gallic acid < resveratrol < curcumin keto < quercetin < sesamin < curcumin enol for conalbumin, suggesting that quercetin, sesamin and curcumin enol form the strongest complexes with the proteins; ii) among 24 examined ternary and quaternary combinations of various components in 10 systems polyphenols prefer to associate with lactoferrin or conalbumin, while in the remaining systems they form contacts with both fibrillar lysozyme and bioactive proteins; iii) in the ternary and quaternary systems polyphenols can compete for the binding sites; iv) in the presence of conalbumin in quaternary systems resveratrol and sesamin showed the binding preferences for the lysozyme fibrils and chitosan. These findings can serve as a basis for the rational design and fabrication of novel nanocomposites for biomedical applications, particularly in wound healing.

Acknowledgements

This work was supported by the Ministry of Education and Science of Ukraine (the project “Design of novel biocompatible nanocomposites and improvement of ultrasound-based diagnostic methods for medical applications” No. 0126U001036).

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ДОСЛІДЖЕННЯ АМІЛОЇД – ПОЛІСАХАРИДНИХ КОМПОЗИТІВ МЕТОДОМ МОЛЕКУЛЯРНОГО ДОКІНГУ: І. ВЗАЄМОДІЇ З БІОЛОГІЧНО АКТИВНИМИ БІЛКАМИ ТА ПОЛІФЕНОЛАМИ

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Амілоїдні фібрили — білкові агрегати з унікальною структурою — є новим типом наноматеріалів білкової природи з широким спектром застосувань. Одним із прикладів біомедичного застосування амілоїдних наноматеріалів є отримання біосумісних гідрогелевих покриттів для загоєння ран. Дане дослідження було спрямоване на оцінку можливості використання амілоїдних фібрил лізоциму, інтегрованих з полісахаридом хітозаном, в якості полімерного матриксу для інкорпорації агентів з вираженими ранозагоювальними властивостями, такими як поліфеноли та біологічно активні білки лактоферин та кональбумін. За допомогою методу молекулярного докінгу були визначені такі характеристики, як афінність, амінокислотний склад сайтів зв'язування та можливі конкурентні взаємодії між поліфенолами в двох-, трьох- та чотирьохкомпонентних системах. Виявлена здатність поліфенольних сполук до асоціації з біоактивними білками, при цьому найвища афінність спостерігалась для куркуміну в єнольній формі, кверцетину та сесаміну. У трьох- та чотирьохкомпонентних системах сайти зв'язування поліфенолів локалізувались або виключно на молекулах лактоферину чи кональбуміну, або ж містили амінокислотні залишки і фібрилярного лізоциму, і біоактивних білків. Встановлено, у яких комбінаціях поліфеноли можуть конкурувати між собою за сайти зв'язування. Отримані результати створюють основу для дизайну нових амілоїдних нанокомпозитів з ранозагоювальними властивостями.

Ключові слова: *амілоїдні фібрили лізоциму; хітозан; поліфеноли; лактоферин; кональбумін; сайти зв'язування; спорідненість зв'язування; молекулярний докінг*