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Docking studies of carbohydrate ligands against native and mutant surfactant protein-D from Lung Alveolar type II cells

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Abstract

Pulmonary surfactant protein-D is a water soluble protein belongs to c-type mammalian lectin of collectins super family, have significant role in antimicrobial host-defense mechanism by binding of carbohydrate ligands with its carbohydrate recognition domain. Previous studies explains the contributions of Phe335 to ligand recognition by SP-D and showed site specific substitution of Leu for Phe335 decreased affinities for maltoside and maltotriose without altering affinity for maltose or glucose. However, substitution of Tyr or Trp restored affinity. Taking this into consideration, in our computational model an attempt has been made to study the importance of Phe335 position towards affinity of binding with ligands using Molegro Virtual Docker (MVD). F335G showed high dock score with p-nitrophenyl-alpha-D-maltoside (-96.8654 kcal/mol), p-nitrophenyl-beta-D-maltoside (-91.1205kcal/mol) and maltotriose (-81.8233kcal/mol). Further, virtual screening of 62 various mannose derived carbohydrate ligands were carried out and the top three compounds were selected based on the binding affinity with SP-D. It was observed that Arg343 residue interactions predominated in most of the cases. Moreover, Glu321, Asn323, Pro319, Glu329, Asn341 and Asp342 residue interactions were also observed.

Keywords: surfactant protein, binding affinity, docking.

INTRODUCTION

Lungs among all vertebrate groups differ in structure, function and embryological origin but have few common characteristics such as internal, fluid lined, gas holding structures that inflate and deflate cyclically. Because of these common characteristics all the lungs face potential problems related to the surface tension of the fluid and attack from pathogens, allergens and pollutants [1].

Pulmonary surfactant is a mixture of lipids (majority are phospholipids) and proteins (surfactant proteins) which are responsible in reducing the surface tension of the alveoli [2]. The surfactant is synthesized and secreted by the alveolar type-II cells, which is composed of 90% lipids (80% phospholipids and 10% neutral lipids) and 10% surfactant proteins (SP-A, SP-B, SP-C and SP-D). Phospholipids are classified as 80% Phosphatidylcholine (PC) lipids which constitute Dipalmitoylphosphatidylcholine (DPPC) and 10% Phosphatidylglycerol (PG) lipids [3]. Similarly, the surfactant associated proteins are classified as either hydrophilic (SP-A and SP-D) or hydrophobic surfactant proteins (SP-C and SP-B) [4]

The structures of SP-A and SP-D indicate that they are primarily involved in host-defense mechanisms where the carbohydrate recognition domains (CRDs) multivalently bind carbohydrate-coated surfaces on target cells, and the collagenous stalks may elicit effector functions through binding to cell-surface receptors [5]. Both SP-A and SP-D bind to the lipopolysaccharides (LPS) of certain bacteria and specifically bind to and activate alveolar macrophages [6-10]. The binding of LPS to SP-D has been shown to be mediated by the CRD [9, 11].

Literature reports suggest that SP-A and SP-D act in the first line immune defense of the lung, by binding to pathogens and promoting phagocytosis.[12] Moreover, Phe335 position in SP-D played critical role in ligand recognition when compared to SP-A, revealing a critically important role for SP-D, in particular, in the control of lung inflammation.[13]

SP-D is a collagenous C-type lectin that belongs to the collectin family member and has a vital role in the host-defense mechanism against the microbes, in regulating the immune responses in lungs [12], other tissues and also in regulating the cell surface expression of the alveolar macrophage β_2-integrins [14]. SP-D consists of 4 structural domains which includes a N-terminal domain, collagenous domain, hydrophobhic coiled-coil neck region and carbohydrate recognition domain[15].

In this paper, we report functional analysis on binding of various carbohydrates at the CRD region of SP-D. The main emphasis of the work is to state the importance of Phe335, an active site residue which confers stability of ligand binding within CRD. Owing to this important feature, a mutational study was conducted to selectively represent the stability, orientation and non-bonded contacts of carbohydrate moieties with SP-D.

RESULTS AND DISCUSSION

Consistency in results was obtained when the compounds reported in literature [13] were docked with the SP-D protein 2GGU using MVD (Table 2) software. It was reported earlier that a high affinity of binding existed between maltotriose and CRD of SP-D and similar observations were made in our computational study (I_{50}: 0.94 vs -70.505 kcal/mol dock score). Maltotriose was found to be more potent than maltose, but less potent than *p*-NP maltoside (-70.505 vs -77.424 kcal/mol). The increase in binding affinity from glucose to maltose and maltotriose can be attributed to the strong ligand interactions made by the end groups of maltotriose with amino acid residues of CRD region of SP-D (Figure 2).

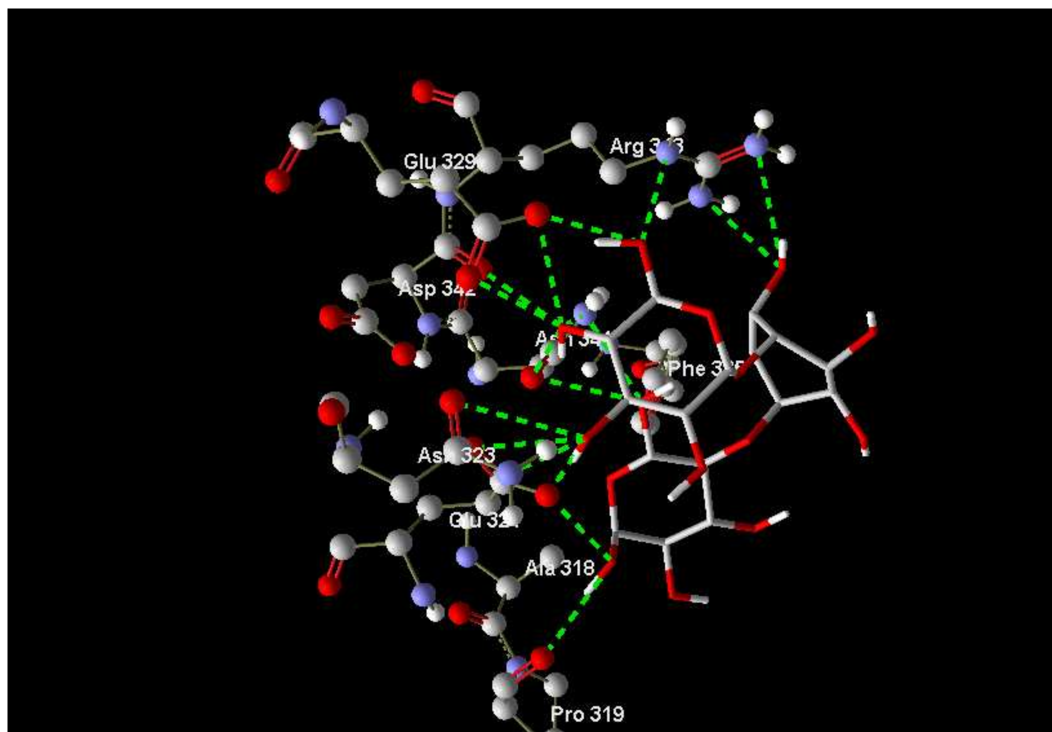

Figure 2: Image showing H-bond interactions of maltotriose (2GGU bound ligand) with CRD region of SP-D. H-bonds are shown as dotted lines. Maltotriose represented as sticks and the amino acid residues as ball and stick formats

Table 2: Correlation between experimental values and computational dock scores as obtained with compounds selected from literature

CRD competitor*	I_{50}	Dock score (kcal/mol)	H-Bond Interactions
Glucose	3.5	-36.13	Glu333, Arg343, Glu329, Asp342, Glu321, Asn323, Asp325, Asn341
Maltose	2.3	-47.63	Asn337, Thr336, Glu333, Arg343, Arg349
Maltotriose	0.94	-70.50	Glu321, Asn323, Glu329, Asn341, Asp342, Glu329, Pro319, Arg343
p-Nitrophenyl-α- D-maltoside	0.32	-77.42	Thr336, Ala290, Arg343, Asn337, Pro319, Glu321
p-Nitrophenyl- α- D-maltoside	3.0	-38.04	Ala290, Thr336, Asn341, Glu333, Arg343

**from ref. 13*

Screening Studies

Following such correlation, a virtual screening study was performed with 62 various carbohydrate ligands extracted from sweetdb. Using default parameters of Molegro virtual docker software, the top three compounds were selected based on the binding affinity and moldock scores with SP-D (Table 3). It was observed that Arg343 residue interactions predominated in most of the cases. Moreover, Glu321, Asn323, Pro319, Glu329, Asn341 and Asp342 residue interactions were also observed. Interestingly, the third best ligand (Linucs ID 384), Thr336 and Asn337 interactions suggest that the ligand orientation and geometry was different than the remaining.

Table 3: Top 3 carbohydrates of Screened compounds

Linucs ID	Name of the compound	Moldock score (kcal/mol)	No. of hydrogen bonds	Interacting atom residues
568	a-D-Manp-(1-2)-a-D-Manp-(1-3)-Ser	-97.92	13	OE1 Glu321 OD1 Asn323 OE1 Glu329 OD1 Asn341 O Asp342 OD1 Asp342 ND2 **Arg343** (3) O Gly320 OE2 Glu321 O Pro319 (2)
26	a-D-Manp-(1-2)-a-D-Manp-(1-2)-a-D-Manp-(1-2)-D-Man	-97.44	17	ND2 Asn337(2) O Pro319 OE2 Glu321 NH1 **Arg343**(2) NH2 Arg343 OE2 Glu333 ND2 Asn341 OE2 Glu329 OE1 Glu321 OD1 Asn323 OE1 Glu329 OD1 Asn341 O Asp342 OE2 Glu321 ND2 Asn323
384	b-D-GlcpNAc-(1-6)-a-D-Manp-(1-1)-Methyl	-80.14	8	N Thr336(2) OG1 Thr336(2) N Asn337 ND2 Asn337 OE2 Glu333 NH1 **Arg343**

These three compounds were subjected to docking against the mutated proteins of 2GGU and results were tabulated in Table-3, where it was evident that the ligand with Linucs ID 384 showed high affinity with F335P (-86.0327kcal/mol) when compared with native protein.

Mutational Studies

Binding affinity ranges between SP-D and CRD competitors such as glucose, maltose, maltotriose, *p*-nitrophenyl-alpha-D-maltoside and p-nitrophenyl-beta-D-maltoside with native and mutated proteins are -58.18 to -81.82 kcal/mol, -77.42 to -96.86 kcal/mol, -71.39 to -91.12 kcal/mol, -23.98 to -39.38 kcal/mol and -28.32 to -56.29 kcal/mol respectively (Table 4).

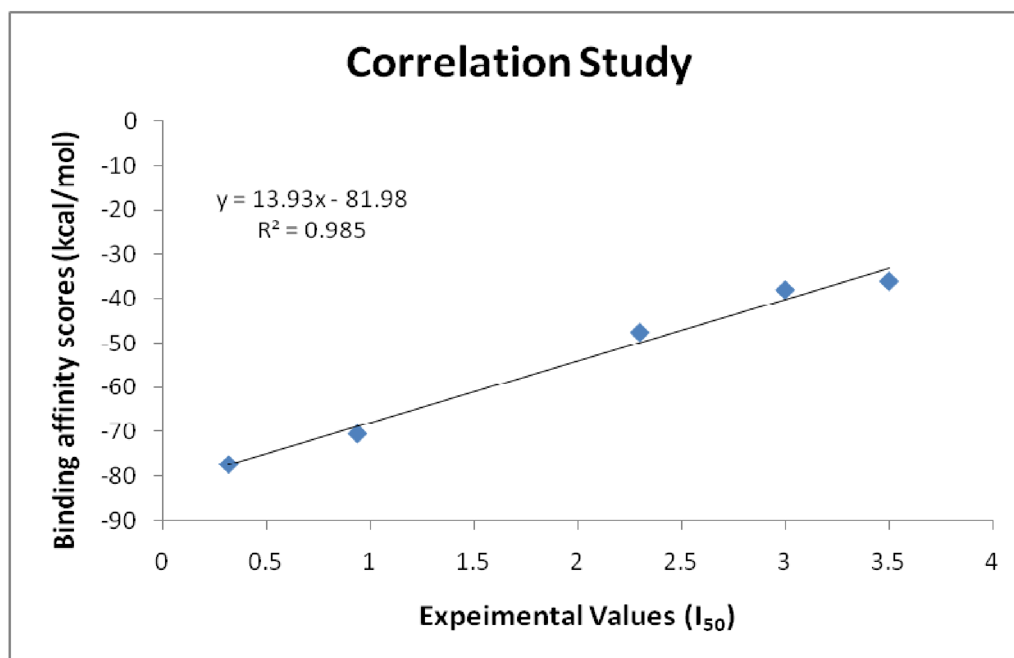

Figure 3: Correlation graph between experimentally derived data and computational dock scores of ligands given in Table 2

It was convincing experimentally that Phe335 contributes to the ligand recognition and our computational studies are valid where the correlation between experimental ans computational derived dock scores was 0.985 (Figure 3). And hence a screening analysis was performed to distinguish the dependency of amino acid residues towards ligand binding. Moreover, the next part of the study concerning the chance of mutation by the remaining residues other than Phe335 was to recognize the best possible ligands against SP-D.

The protein that was mutated at position 335 with Leucine (F335L) had less binding affinity to all the ligands and substitution of Tyrosine or Tryptophan (F335Y or F335W) for leucine restored the binding affinity. It was found that F335G mutation has high binding affinity with all the carbohydrates except with the glucose, where the affinity is slightly decreased (Table 4). It was also found that F335I mutation has moderate to high affinity with maltose.

Table 4: Binding affinity scores of docked complexes of native protein and mutant proteins with the five carbohydrate ligand molecules. It is calculated in the form of kcal/mol

Position of residue and its mutation	Glucose	Maltose	maltotriose	p-nitrophenyl-α -D-maltoside	p-nitrophenyl-β-D-maltoside
F335*	-36.13	-47.63	-70.50	-77.42	-83.51
F335L*	-34.80	-41.07	-58.18	-70.53	-79.43
F335Y*	-36.76	-51.49	-70.81	-78.42	-78.64
F335W*	-35.01	-44.75	-72.93	-74.35	-86.17

F335A	-36.73	-50.08	-68.45	-74.27	-71.82
F335R	-23.97	-40.86	-75.54	-84.44	-76.74
F335M	-36.52	-28.31	-62.46	-86.43	-71.39
F335K	-24.93	-48.04	-73.74	-84.17	-72.18
F335C	**-39.38**	-51.75	-74.38	-77.23	-78.65
F335P	-33.55	-49.66	-74.86	-78.26	-77.20
F335S	-28.54	-50.76	-75.92	-75.61	-87.82
F335E	-38.35	-46.12	-69.77	-84.34	-82.28
F335H	-28.48	-40.78	-62.62	-73.38	-73.77
F335Q	-35.22	-53.67	-72.37	-77.69	-72.49
F335T	-35.18	-51.62	-77.28	-74.72	-85.72
F335N	-30.34	-55.63	-75.88	-78.98	-73.86
F335D	-37.31	-53.76	-72.15	-76.78	-90.33
F335I	-35.73	**-56.28**	-64.53	-72.94	-72.73
F335V	-34.69	-50.13	-72.99	-77.84	-85.17
F335G	-34.57	**-54.28**	**-81.82**	**-96.86**	**-91.12**

** indicates experimental results which are computationally valid. The energy values are expressed as MolDock scores (kcal/mol)*

Similar mutational study was carried out with the best compounds from carbohydrate screening study. The range of binding affinities of top three screened carbohydrate ligands such as 1) a-D-Manp-(1-2)-a-D-Manp-(1-2)-a-D-Manp-(1-2)-D-Man, 2) b-D-GlcpNAc-(1-6)-a-D-Manp-(1-1)-Methyl, and 3) a-D-Manp-(1-2)-a-D-Manp-(1-3)-Ser with native and mutated proteins are-41.0808 to -97.448 kcal/mol, -62.2377 to -83.1886 kcal/mol and -48.5094 to -97.922 kcal/mol respectively (Table 5).

Table 5: Mutational analysis of top three screened ligands. The energy values are expressed as MolDock scores (kcal/mol)

Position of residue and its mutation	a-D-Manp-(1-2)-a-D-Manp-(1-2)-a-D-Manp-(1-2)-D-Man	b-D-GlcpNAc-(1-6)-a-D-Manp-(1-1)-Methyl	a-D-Manp-(1-2)-a-D-Manp-(1-3)-Ser
F335	**-97.44**	-80.14	**-97.92**
F335L	-51.38	-67.87	-55.26
F335Y	-67.31	-61.95	-58.46
F335W	-68.79	-66.28	-58.42
F335A	-52.71	-68.65	-54.95
F335R	-54.90	-55.84	**-48.50**
F335M	**-41.08**	-66.16	-51.99
F335K	-45.62	-75.41	-56.03
F335C	-55.74	-64.74	-50.81
F335P	-63.93	**-86.03**	-62.88

F335S	-77.45	-66.49	-73.52
F335E	-76.14	-69.78	-62.74
F335H	-58.05	-66.24	-57.12
F335Q	-84.26	-81.74	-61.76
F335T	-75.16	**-62.23**	-59.47
F335N	-55.32	-64.37	-63.83
F335D	-79.15	-69.91	-63.99
F335I	-61.17	-66.47	-68.85
F335V	-57.76	-77.66	58.25
F335G	**-72.52**	**-83.18**	**-64.67**

MATERIALS AND METHODS

Receptor X-ray Structure

The three-dimensional crystal structure coordinates of SP-D in complex with maltotriose (PDB code: 2GGU) [13] in its trimeric form was selected as the receptor model in this study. The PDB (Protein Data Bank) structure was obtained from the RCSB (Research Collaboratory for Structural Bioinformatics) database (http://www.rcsb.org).

Mutational Study

It has been reported by Crouch et. al. on contributions of Phe335 to ligand recognition by SP-D [13] site directed substitution of Leu for Phe335 decreased affinities for maltoside and maltotriose without altering affinity for maltose or glucose. However, substitution of Tyr or Trp restored affinity. Taking this into consideration, a conventional mutational study was carried out to study the affinity of binding to glucose, maltose, maltotriose etc. Phe335 position was allowed to mutate with all other amino acids and subsequent affinity data was collected from dock runs using Molegro Virtual Docker (MVD).

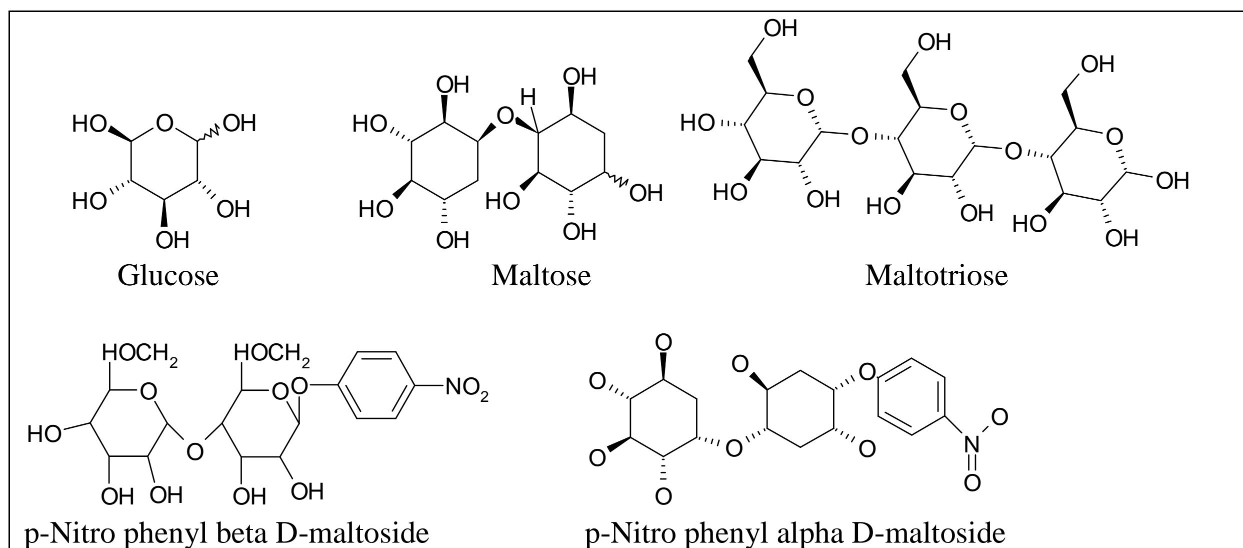

Figure 1: Chemical compounds used for docking correlation with experimental data from ref. 13

Ligands for Docking

The ligands chosen for the study is obtained from [13] such as glucose, maltose, maltotriose, p-nitrophenyl-alpha-D-maltoside, *p*-nitrophenyl-beta-D-maltoside (Figure 1) and nearly 62 compounds of mannose family were screened from SWEET database (Table-1). All chemical structures were drawn using ISIS Draw 2.3 software (www.mdli.com) and are converted to 3D mol2 files using ProDrg2 server (http://davapc1.bioch.dundee.ac.uk/prodrg/).

Table 1: List of carbohydrates with their Linucs Ids

S. No	Linucs Id	Name of the carbohydrate
1	21	a-D-Manp-(1-[2)-a-D-Manp-(1]n-2)-D-Man
2	22	a-D-Manp-(1-3)-a-D-Manp-(1-4)-D-GlcNAc
3	23	a-D-Manp-(1-3)-a-D-Manp-(1-2)-D-Man
4	24	a-D-Manp-(1-6)-a-D-Manp-(1-6)-D-Man
5	25	a-D-Manp-(1-3)-a-D-Manp-(1-2)-a-D-Manp-(1-2)-D-Man-ol
6	26	a-D-Manp-(1-2)-a-D-Manp-(1-2)-a-D-Manp-(1-2)-D-Man
7	27	a-D-Manp-(1-3)-a-D-Manp-(1-2)-a-D-Manp-(1-2)-D-Man
8	30	a-D-Manp-(1-3)-a-D-Manp-(1-2)-a-D-Manp-(1-2)-a-D-Manp-(1-2)-D-Man
9	83	a-D-Manp-(1-4)-a-L-Rhap-(1-9)-9-hydroxy-Nonanoate-(1-1)-Methyl
10	87	a-D-Galp-(1-2)-a-D-Manp-(1-4)-a-L-Rhap-(1-9)-9-hydroxy-Nonanoate-(1-1)-Methyl
11	116	a-L-Rhap-(1-6)+ | D-Glc | a-D-Manp-(1-2)+
12	120	a-D-Galp-(1-6)-a-D-Manp-(1-4)-b-D-Manp
13	121	a-D-Galp-(1-6)-a-D-Manp-(1-4)-b-D-Manp-(1-4)-b-D-Manp
14	79	a-D-Manp-(1-4)+ | a-D-GalpA-(1-2)-a-D-Manp-(1- | Repeat-3)-b-D-Galp-(1-3)+
15	81	a-D-Manp-(1-6)+ | D-Man | a-D-Manp-(1-3)+
16	218	b-D-Galp-(1-4)-b-D-GlcpNAc-(1-2)-a-D-Manp-(1-6)+ | b-D-Manp-(1-4)-D-GlcNAc | b-D-Galp-(1-4)-b-D-GlcpNAc-(1-2)-a-D-Manp-(1-3)+
17	226	a-D-Manp-(1-3)-b-D-Manp-(1-4)-D-GlcNAc
18	231	a-D-Manp-(1-6)-b-D-Manp-(1-4)-b-D-GlcpNAc-(1-4)-b-D-GlcpNAc-(1-4)-Asn
19	232	a-L-Fucp-(1-6)+ | b-D-GlcpNAc-(1-4)-Asn | a-D-Manp-(1-6)-b-D-Manp-(1-4)-b-D-GlcpNAc-(1-4)+
20	233	b-D-GlcpNAc-(1-2)-a-D-Manp-(1-6)+ | b-D-Manp-(1-4)-D-GlcNAc | b-D-GlcpNAc-(1-2)-a-D-Manp-(1-3)+
21	244	b-D-Galp-(1-4)-b-D-GlcpNAc-(1-2)-a-D-Manp-(1-3)-b-D-Manp-(1-4)-D-GlcNAc
22	245	b-D-Galp-(1-4)-b-D-GlcpNAc-(1-2)-a-D-Manp-(1-6)-b-D-Manp-(1-4)-D-GlcNAc
23	248	a-D-Neup5Ac-(2-6)-b-D-Galp-(1-4)-b-D-GlcpNAc-(1-2)-a-D-Manp-(1-3)-b-D-Manp-(1-4)-D-GlcNAc
24	249	a-D-Neup5Ac-(2-6)-b-D-Galp-(1-4)-b-D-GlcpNAc-(1-2)-a-D-Manp-(1-6)-b-D-Manp-(1-4)-D-GlcNAc
25	261	a-D-Neup5Ac-(2-3)-b-D-Galp-(1-4)-b-D-GlcpNAc-(1-2)-a-D-Manp-(1-3)-b-D-Manp-(1-4)-D-GlcNAc
26	290	a-D-Manp-(1-2)-a-D-Manp-(1-3)-b-D-Manp-(1-4)-D-GlcNAc
27	291	a-D-Manp-(1-2)-a-D-Manp-(1-2)-a-D-Manp-(1-3)-b-D-Manp-(1-4)-D-GlcNAc
28	310	a-D-Manp-(1-1)-a-D-GlcpN
29	318	a-D-Manp-(1-1)-b-D-GlcpN
30	333	a-D-Manp-(1-2)-a-D-Manp-(1-1)-Methyl
31	334	a-D-Manp-(1-2)-a-D-Manp-(1-1)-Methyl
32	336	a-D-Manp-(1-6)-a-D-Manp-(1-1)-Methyl

33	384	b-D-GlcpNAc-(1-6)-a-D-Manp-(1-1)-Methyl
34	386	b-D-Galp-(1-4)-b-D-GlcpNAc-(1-2)-a-D-Manp
35	393	a-D-Manp-(1-4)-b-L-Rhap-(1-3)-D-Gal
36	394	a-D-Manp-(1-4)-b-L-Rhap-(1-3)-D-Gal
37	417	a-D-Galp-(1-2)-a-D-Manp-(1-9)-9-hydroxy-Nonanoate-(1-1)-Methyl
38	418	a-D-Manp-(1-4)-a-L-Rhap-(1-3)-a-D-Galp-(1-1)-Phenyl
39	443	a-D-Manp-(1-2)-a-D-Manp
40	450	a-D-Manp-(1-2)-a-D-Manp-(1-2)-a-D-Manp
41	453	a-D-Manp-(1-2)-a-D-Manp-(1-2)-a-D-Manp-(1-2)-a-D-Manp
42	454	a-D-Manp-(1-2)-a-D-Manp-(1-2)-a-D-Manp-(1-2)-a-D-Manp-(1-2)-a-D-Manp-(1-2)-a-D-Manp
43	478	a-D-Manp-(1-4)-L-Rha
44	559	a-D-Manp-(1-3)-Ser
45	568	a-D-Manp-(1-2)-a-D-Manp-(1-3)-Ser
46	569	a-D-Manp-(1-2)-a-D-Manp-(1-3)-Thr
47	839	repeat-3)-b-D-Manp-(1-2)-a-D-Manp-(1-2)-a-D-Manp-(1-
48	841	repeat-3)-a-D-Manp-(1-3)-a-D-Manp-(1-2)-a-D-Manp-(1-2)-a-D-Manp-(1-
49	842	repeat-3)-a-D-Manp-(1-3)-a-D-Manp-(1-2)-a-D-Manp-(1-2)-a-D-Manp-(1-2)-a-D-Manp-(1-
50	844	repeat-2)-a-D-Manp-(1-4)-a-L-Rhap-(1-3)-a-D-Galp6Ac-(1-
51	899	a-D-Manp-(1-4)-a-D-Glcp-(1-4)-D-Man
52	900	a-D-Manp-(1-4)-a-D-Manp-(1-4)-D-Glc
53	901	a-D-Manp-(1-4)-a-D-Manp-(1-4)-D-Man
54	936	a-D-Manp-(1-2)-a-D-Manp-(1-2)-D-Man-ol
55	937	a-D-Manp-(1-2)-a-D-Manp-(1-2)-a-D-Manp-(1-2)-D-Man-ol
56	946	a-D-Manp-(1-[3)-a-D-Manp-(1]n-3)-D-Man
57	1053	a-D-Manp-(1-2)-a-D-Manp-(1-6)-D-Man
58	1054	a-D-Manp-(1-2)-a-D-Manp-(1-6)-a-D-Manp-(1-6)-D-Man
59	1055	a-D-Manp-(1-2)-a-D-Manp-(1-2)-a-D-Manp-(1-6)-D-Man
60	1056	a-D-Manp-(1-2)-a-D-Manp-(1-2)-a-D-Manp-(1-2)-a-D-Manp-(1-6)-D-Man
61	1057	a-D-Manp-(1-2)-a-D-Manp-(1-2)-a-D-Manp-(1-2)-a-D-Manp-(1-2)-D-Man
62	1058	a-D-Glcp-(1-6)-a-D-Manp-(1-2)-a-D-Manp-(1-2)-a-D-Manp-(1-2)-D-Man

CONCLUSION

The docking results confirmed the correlation between experimental activities of CRD competitors and computationally derived dock scores. Considering the clearance of microbial pathogens by SP-D, various carbohydrate ligands extracted from sweetdb resulted in three best compounds displaying the affinities of ligands with SP-D. Finally, mutational analysis revealed the importance of various mutations in accordance to Phe335. F335G showed high dock score with *p*-nitrophenyl-alpha-D-maltoside (-96.8654 kcal/mol), p-nitrophenyl-beta-D-maltoside (-91.1205kcal/mol) and maltotriose (-81.8233kcal/mol). This study clearly states the importance of computational tools in analyzing mutational aspects of surfactant protein-D and further analysis and comparison with the remaining surfactants would highlight the features necessary for binding with collectin members.

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