Bean peptides have higher *in silico* binding affinities than ezetimibe for the N-terminal domain of cholesterol receptor Niemann-Pick C1 Like-1

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Abstract

Niemann-Pick C1 like-1 (NPC1L1) mediates cholesterol absorption at the apical membrane of enterocytes through a yet unknown mechanism. Bean, pea, and lentil proteins are naturally hydrolyzed during digestion to produce peptides. The potential for pulse peptides to have high binding affinities for NPC1L1 has not been determined. In this study, *in silico* binding affinities and interactions were determined between the N-terminal domain of NPC1L1 and 14 pulse peptides (5≥ amino acids) derived through pepsin-pancreatin digestion. Peptides were docked in triplicate to the N-terminal domain using docking program AutoDock Vina, and results were compared to those of ezetimibe, a prescribed NPC1L1 inhibitor. Three black bean peptides (-7.2 to -7.0 kcal/mol) and the cowpea bean dipeptide Lys-Asp (-7.0 kcal/mol) had higher binding affinities than ezetimibe (-6.6 kcal/mol) for the N-terminal domain of NPC1L1. Lentil and pea peptides studied did not have high binding affinities. The common bean peptide Tyr-Ala-Ala-Ala-Thr (-7.2 kcal/mol), which can be produced from black or navy bean proteins, had the highest binding affinity. Ezetimibe and peptides with high binding affinities for the N-terminal domain are expected to interact at different locations of the N-terminal domain. All high affinity black bean peptides are expected to have van der Waals interactions with SER130, PHE136, and LEU236 and a conventional hydrogen bond with GLU238 of NPC1L1. Due to their high affinity for the N-terminal domain of NPC1L1, black and cowpea bean peptides produced in the digestive track have the potential to disrupt interactions between NPC1L1 and membrane proteins that lead to cholesterol absorption.

Keywords: NPC1L1, bean peptides, cholesterol absorption, black beans, AutoDock Vina

1. Introduction

Cholesterol is necessary in the human body to regulate cell membrane fluidity and to produce steroids, vitamin D, and bile acids. While all human cells have the capability of producing cholesterol, the liver is the main site of endogenous blood serum cholesterol production. Cholesterol is produced from acetyl-CoA, which is converted into mevalonic acid early in cholesterol biosynthesis ([1]). The production of mevalonic acid by HMG-CoA reductase is the first committed step in cholesterol biosynthesis. Mevalonic acid is converted to squalene and later on to cholesterol. Humans can produce cholesterol endogenously or they can acquire it from their diet. The body maintains cholesterol homeostasis by increasing the expression of HMG-CoA reductase and proteins that aid in cholesterol absorption when the absorption of cholesterol from foods is low.

Dietary cholesterol in the digestive track forms micelles with other lipids and bile acids, and is then absorbed into enterocytes, where it is later transferred to the liver in chylomicrons through portal vein circulation ([2]). In the liver, free cholesterol is esterified to fatty acids and is packaged into low density lipoprotein (LDL) micelles which distribute cholesterol to cells throughout the body. Excess cholesterol in the body is returned to the liver by high density lipoprotein (HDL) micelles. Cholesterol cannot be fully metabolized by the body, rather, it is converted into bile salts or transferred directly to the gallbladder to be released upon digestion in order to be excreted with undigested chyme or aid in the absorption of dietary cholesterol and lipids ([1-2]).

Excess cholesterol in blood serum is associated with an increased risk of heart disease ([3]), the current leading cause of death in the United States ([4]). Therefore,

maintaining proper cholesterol levels in the blood is essential for good health. Excess serum cholesterol levels can be reduced by three main biochemical mechanisms: inhibiting endogenous cholesterol production by inhibiting HMG-CoA reductase with statins, increasing the amount of cholesterol and bile acids that are excreted by stopping the reabsorption of cholesterol in the liver, and inhibiting the absorption of dietary cholesterol in the intestine ([5]).

Niemann-Pick C1 Like-1 (NPC1L1) is a transmembrane receptor present in the liver and small intestine that mediates cholesterol absorption through a presently unknown mechanism. Intestinal NPC1L1 aids in the absorption of dietary cholesterol from food, while hepatic NPC1L1 aids in the reabsorption of cholesterol bound for the gallbladder ([6]). NPC1L1 has an N-terminal domain that binds cholesterol, multiple extracellular and intracellular loops, and a C-terminal tail that contains an internalization motif ([7-8]). Only the structure of the N-terminal domain is known, and this domain has a gated cavity that binds a single molecule of cholesterol ([7]).

Originally, NPC1L1 was thought to mediate cholesterol absorption by binding free cholesterol at its N-terminal domain which would lead to NPC1L1 translocating from the apical membrane to the cytoplasm of enterocytes ([9]), but a recent study suggests that neither cholesterol binding to the N-terminal domain nor translocation of NPC1L1 is necessary for NPC1L1 to mediate cholesterol absorption ([10]). The interaction between NPC1L1 and flotillins, which form cholesterol rich membrane microdomains, has been proposed as a necessary step in NPC1L1 mediated cholesterol absorption ([11-12]), so dietary cholesterol absorption might be mediated by the formation of microdomains rather than cholesterol binding to NPC1L1 or NPC1L1 internalization. While the

formation of microdomains was originally proposed as an intermediate step in NPC1L1 internalization that would eventually lead to cholesterol absorption, the formation of the microdomain itself might be the cause of cholesterol absorption.

Ezetimibe (Pubchem CID: 150311) is a β -lactam compound that binds to extracellular loop C of intestinal NPC1L1 to inhibit NPC1L1 and block the absorption of dietary cholesterol ([13]). Ezetimibe is predicted to block configurational changes necessary for NPC1L1 to interact with flotillins and form cholesterol rich membrane microdomains ([11]). Ezetimibe is commonly prescribed alongside a statin to reduce the amount of cholesterol that is absorbed into the body and produced endogenously ([8]), but cholesterol blood serum levels are reduced by ezetimibe and statins through two separate mechanisms. Ezetimibe alone is capable of significantly reduce serum cholesterol levels by inhibiting NPC1L1 ([14]).

Consumption of pulses, such as beans, peas, and lentils, is associated with improved cardiovascular health and lower blood serum cholesterol levels ([15-16]). Pulses are an inexpensive food source available worldwide rich in fiber and protein. The addition of certain pulse proteins to high cholesterol and saturated fat diets has been shown to reduce blood cholesterol levels in animal studies ([17-18]), but mechanisms that can lead pulse proteins to reduce the absorption of dietary cholesterol have not been detailed. In the human digestive system, peptides are generated from pulse proteins hydrolyzed by pepsin in the stomach and pancreatic proteases, such as trypsin and chymotrypsin, in the small intestine ([19]).

In this study, the potential for pulse peptides produced from common bean, cowpea bean, lentil, and pea proteins in the digestive system to inhibit the interactions

between NPC1L1 and flotillins by strongly binding to NPC1L1 was investigated using computational dockings. Peptide bioavailability in the blood is not an issue when determining if peptides will bind to intestinal NPC1L1 as peptides can directly interact with intestinal NPC1L1 without having to enter the bloodstream. The aim of this study was to determine the potential for small pulse peptides produced naturally in the digestive track to interact more strongly than ezetimibe with the N-terminal domain of NPC1L1, the only structurally characterized domain of NPC1L1, using computational docking program AutoDock Vina.

2. Materials and methods

2.1 Selection of peptide sequences

Peptide sequences with five or less amino acids from pulse proteins produced through pepsin-pancreatin hydrolysis after being applied heat were selected from the literature (**Table 1**) ([20-24]). Common bean (*Phaseolus vulgaris*), cowpea bean (*Vigna unguiculata*), lentil (*Lens culinaris*), and pea (*Pisum sativum* var. Bajka) peptide sequences were used in this study. Common bean peptide sequences came from three varieties: black, great northern, and navy. Peptide sequences generated from proteins hydrolyzed by exogenous human enzymes, such as alcalase from bacteria or bromelain from pineapples, were not studied.

2.2 Preparation of Ligand and NPC1L1 Structural Files

Peptide structural files were created using BIOVIA Discovery Studio (DS4.5, Dassault Systèmes BIOVIA, Discovery Studio Modeling Environment, Release 4.5, San

Diego. 2015). The structure of ezetimibe was obtained from www.chemicalbook.com. The peptide and ezetimibe structures were protonated as if they were in aqueous solution at pH 7.0. Ligand structures were applied the “Clean Geometry” function twice using DS4.5 to optimize their geometry and minimize their starting energy. Ligand structural files were typed with the CHARMM36 force field using DS4.5. The nitrogen atom in ezetimibe was not automatically typed by the CHARMM36 force field, so it was manually typed as a non-hydrogen bonding nitrogen (N) since its participation in hydrogen bonding has not been detailed. Ligand files were opened in AutoDock Tools (version 1.5.6) ([25]), where Gasteiger partial charges were automatically added. The root of each structure was detected, and all rotatable bonds were left as rotatable.

The crystallographic structure of the N-terminal domain of NPC1L1 (3QNT) was downloaded from the RCSB Protein Data Bank. Since only the structure of the N-terminal domain of NPC1L1 is known, it is the only NPC1L1 structure that can be used to perform docking experiments. The protein was protonated as if it was in aqueous solution at pH 7.0. The two N-Acetyl-D-glucosamine post-translational modifications on ASN54 and ASN138 present in the crystal structure were kept in the structural file. The N-terminal domain file was opened in AutoDock Tools, and the structure of the N-terminal domain was saved as a rigid PDBQT receptor file. Using AutoDock Tools, search space dimensions (X= 42, Y= 64, Z= 44) and center point (X= -14.012, Y= -23.549, Z= -11.688) were determined on a one angstrom scale for the N-terminal domain. The search space encompassed the whole exterior of the N-terminal domain since it does not have a well-defined external active site.

2.3 Docking and analysis of binding

Search space dimensions, center point, and exhaustiveness (350) were all detailed in a configuration file. The configuration file created was used to run AutoDock Vina three times per ligand, generating three repetition runs (repruns) per ligand ([26]). The binding affinity reported for each ligand is the highest binding affinity (lowest binding energy) of the three repruns for that ligand, so that the energy of the most stable complex between the ligand and the NPC1L1 N-terminal domain is not underestimated. Molecular mass, hydrophobicity as determined by the Wimley-White scale, net charge at pH 7.0, and isoelectric point for each peptide were determined using PepDraw (www.pepdraw.com, 2015). Chemical structures were drawn using ChemDraw Professional 15.1 (PerkinElmer Informatics).

Ligand interactions with the N-terminal domain were analyzed using DS4.5. Only the stable conformation with the lowest binding energy for each reprun was analyzed. Stable conformations were those without donor-donor, acceptor-acceptor, and positive-positive repulsion interactions. The interactions reported for each ligand were present in all three repruns for that ligand. Ligands without consistent interactions between their repruns had their results omitted from the main study, but are available as supplementary information, since it is unknown whether those ligands have two modes of interaction or AutoDock Vina failed to compute the correct conformation for one of the repruns. When all the conformations obtained from each reprun interacted with the same amino acid of the N-terminal domain, but with different types of interactions, only the weakest interaction type is reported for that amino acid (van der Waals < alkyl

hydrophobic < π < charged = hydrogen bond < salt bridge). The weakest interaction type is reported so that the strength of each interaction is not overestimated.

3. Results

From the literature search, 15 peptide sequences were selected to be studied: 6 from common bean varieties, 5 from cowpea bean, 2 from lentil, and 2 from pea. For common bean peptide sequences, 3 came from black beans (1 shared with navy beans), 2 came from navy beans (1 shared with black beans), and 2 came from great northern beans. Black and navy bean proteins are both capable of producing the peptide Tyr-Ala-Ala-Ala-Thr. The lentil peptide sequence Lys-Leu-Arg-Thr was unable to generate a stable conformation for each reprim, so it was not analyzed.

Three black bean peptides (-7.2 to -7.0 kcal/mol), including Tyr-Ala-Ala-Ala-Thr which can also be produced by navy bean proteins, and the cowpea dipeptide Lys-Asp (-7.0 kcal/mol) have higher *in silico* binding affinities (lower binding energy) than ezetimibe (-6.6 kcal/mol) for the N-terminal domain of NPC1L1 (**Table 2**). A terminal aryl amino acid is present in all the black bean peptides studied with a high binding affinity for the N-terminal domain (**Figure 1**). Studied lentil and pea peptides did not have higher binding affinities than ezetimibe for the N-terminal domain.

Peptides with a higher binding affinity than ezetimibe are predicted to interact with the N-terminal domain in a location different from where ezetimibe is expected to interact (**Figure 2A**). The high binding affinity black bean peptides Tyr-Ala-Ala-Ala-Thr, Glu-Arg-Ala-Phe, and Phe-Ala-Thr-Gly-Thr are all expected to have van der Waals interactions with SER130, PHE136, and LEU236 and a conventional hydrogen bond

with GLU238 of NPC1L1 (**Table 3, Figure 2B**). The low binding affinity peptides Asp-Leu-Lys-Leu-Pro from peas and Val-Asn-Arg-Leu-Met from lentils are expected to interact with the N-terminal domain in the same location as ezetimibe. None of the stable conformations analyzed interacted with the N-Acetyl-D-glucosamine modifications present in the structure of the N-terminal domain.

The peptides Tyr-Ala-Gly-Gly-Ser, Ser-Gly-Ala-Gly-Tyr, Leu-Asp-Ser-Leu-Thr, Gly-Cys-Thr-Leu-Asn, and Met-Arg-Asp-Leu-Lys had stable conformations for each of their reprints (**Supplementary Table S1**), but no consistent interactions with the N-terminal domain between all three of their reprints. The peptides Tyr-Ala-Gly-Gly-Ser, Ser-Gly-Ala-Gly-Tyr, and Met-Arg-Asp-Leu-Lys had similar interactions in 2 of their 3 reprints (**Supplementary Table S2**).

4. Discussion

Bean, pea, and lentil peptides produced through simulated gastrointestinal digestion were docked to the N-terminal of NPC1L1 to determine if they would have a higher binding affinity than ezetimibe for the N-terminal domain of NPC1L1. Peptides were docked in triplicate, and the results of all three dockings for the same peptide were compared for precision. The bean peptides Tyr-Ala-Ala-Ala-Thr, Glu-Arg-Ala-Phe, Lys-Asp, and Phe-Ala-Thr-Gly-Thr are predicted to inhibit interactions between NPC1L1 and flotillins, which lead to dietary cholesterol absorption, because they have higher *in silico* binding affinities than ezetimibe for the N-terminal domain of NPC1L1. Consumption of bean proteins has not been shown to impair the biological activity of statins and ezetimibe, so there is currently no evidence to suggest that individuals cannot consume

black and cowpea bean proteins to produce the high affinity peptides and take ezetimibe and statins.

The high affinity black bean peptides Tyr-Ala-Ala-Ala-Thr, Glu-Arg-Ala-Phe, and Phe-Ala-Thr-Gly-Thr have been shown to be potential inhibitors of angiotensin converting enzyme (ACE), a regulator of blood pressure ([23]). This gives black bean peptides the potential to be bioactive in multiple areas of the body, but inhibition of proteins, such as ACE, by dietary peptides is limited by the absorption of the peptide into the bloodstream, which is usually low due to further hydrolysis of the peptide by proteases before entering the bloodstream. In contrast, the potential decrease in dietary cholesterol absorption caused by the high affinity peptides presented here is a more realistic model because the peptides would not have to enter the bloodstream to interact with intestinal NPC1L1. The high affinity cowpea bean dipeptide Lys-Asp has antioxidant capabilities ([22]), but this is the first study to suggest this peptide might have a role in dietary cholesterol absorption. Consumption of cowpea bean proteins has been shown to reduce serum cholesterol levels in humans with moderate hypercholesterolemia ([27]).

While this is the first study to dock peptides to NPC1L1, others have docked small synthetic compounds to the N-terminal domain of NPC1L1 (PDB: 3QNT). A thieno[2,3-d]pyrimidine compound and various β -lactam compounds are predicted to interact at the same location (cavity 1) of the N-terminal domain of NPC1L1 as the cowpea dipeptide Lys-Asp (**Figure 2C**) ([28-29]). The cowpea bean dipeptide Lys-Asp, the thieno[2,3-d]pyrimidine compound, and the experimental β -lactam compounds are all predicted to interact with LEU99, SER102, LEU103, HIS124, and THR128 of

NPC1L1. SER102 and LEU103 of NPC1L1 are involved in a gating mechanism that exposes the cholesterol binding pocket of the N-terminal domain of NPC1L1 to free cholesterol molecules in the intestine ([7]). Therefore, molecules such as Lys-Asp binding to SER102, LEU103, and other amino acids involved in exposing the cholesterol pocket of the N-terminal domain are predicted to block cholesterol from binding to the N-terminal domain of NPC1L1. The role of cholesterol binding to the cholesterol pocket of the N-terminal domain on the absorption of dietary cholesterol is still unclear ([10,12]). Ezetimibe in our study did not bind to cavity 1, as was predicted in previous studies. This was probably due to previous dockings being localized to cavity 1 instead of encompassing the whole domain, as was done in this study.

Huo et al. ([30]) docked ezetimibe and 38 compounds from herbs used in traditional Chinese medicine to cavity 1 of the N-terminal domain after determining that cavity 1 was the preferred ligand binding location of several test compounds. Ezetimibe in that study and the cowpea dipeptide Lys-Asp in this study both had interactions with SER53, SER102, LEU213, ALA214, and PRO215 of NPC1L1. Along with SER102, LEU213, ALA214, and PRO215 are involved in the gating mechanism of the cholesterol binding pocket of the N-terminal domain of NPC1L1 ([7]). Huo et al. localized their docking experiments to cavity 1 of the N-terminal domain, hence the difference in results for ezetimibe between our and that study. Every ligand that has docked to cavity 1 of the N-terminal domain of NPC1L1 is predicted to interact with SER102 of NPC1L1.

In conclusion, cavity 1 of the N-terminal domain appears to be important in the binding of small ligands to the domain even though it is not the active site of ezetimibe ([13]). Multiple compounds, including small black and cowpea bean peptides, are

expected to have higher binding affinities than ezetimibe for the N-terminal domain of NPC1L1. Future research should focus on determining whether the high affinity black and cowpea bean peptides presented here reduce dietary cholesterol absorption *in vivo*, since those bean peptides are predicted to block interactions that lead to dietary cholesterol absorption. Bean peptides with a high binding affinity for the N-terminal domain of NPC1L1 have the potential to bind to NPC1L1 and block interactions between NPC1L1 and membrane proteins that lead to dietary cholesterol absorption.

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Figure Legends

Figure 1. Chemical structure of ezetimibe and bean peptides with a high binding affinity for the N-terminal domain of NPC1L1.

Figure 2. Expected binding location and conformation of each peptide with a high binding affinity for the N-terminal domain of NPC1L1. Tyr-Ala-Ala-Ala-Thr = purple, Glu-Arg-Ala-Phe = yellow, Lys-Asp = orange, Phe-Ala-Thr-Gly-Thr = light blue, ezetimibe = green, and N-acetyl glucosamine modified Asn residues = grey. Peptides with a high binding affinity for the N-terminal domain are expected to bind to the N-terminal domain in a separate location from ezetimibe (A). Tyr-Ala-Ala-Ala-Thr, Glu-Arg-Ala-Phe, and Phe-Ala-Thr-Gly-Thr are all expected to bind to similar residues of the N-terminal domain, whose general location is labeled (B). The solvent surface of NPC1L1 goes from neutral (white) to hydrophilic (blue), with increasing saturation of the blue color indicating more hydrophilic areas. Residues of the N-terminal domain which are predicted to interact with Lys-Asp and other small compounds that have been previously docked to this area are part of a cavity (C).

Table 1. Heat processing and pepsin-pancreatin hydrolysis parameters used to generate studied peptide sequences

Protein Source	Heat applied (Temperature, Time)	Pepsin hydrolysis (Temperature, Time, pH)	Pancreatin hydrolysis (Temperature, Time, pH)
Common bean ^a	(35°C, 1 h)	(37°C, 2 h, 2.0)	(37°C, 2 h, 7.5)
Cowpea bean ^b	(25°C, 2 h)	(37°C, 2 h, 2.0)	(37°C, 2 h, 7.0)
Cowpea bean ^c	(35°C, 1 h)	(37°C, 2 h, 2.0)	(37°C, 2 h, 7.5)
Lentil ^d	(100°C, 10 min) then (25°C, 1h)	(37°C, 2 h, 2.5)	(37°C, 1 h, 7.0)
Pea ^e	(100°C, 10 min) then (25°C, 1h)	(37°C, 2 h, 2.5)	(37°C, 1 h, 7.0)

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Table 2. Binding affinity for the N-terminal domain of NPC1L1 and molecular properties of ligands

Ligand	Source ^a	Binding Affinity (kcal/mol)	Rotatable Bonds	Mass (Da)	Hydrophobicity (Kcal/mol)	Net Charge at pH 7.0	Isoelectric Point
Tyr-Ala-Ala-Ala-Thr	Black & Navy	-7.2	15	495.23	8.94	0	5.29
Glu-Arg-Ala-Phe	Black	-7.0	17	521.26	12.13	0	6.72
Lys-Asp	Cowpea	-7.0	11	261.13	14.34	0	6.44
Phe-Ala-Thr-Gly-Thr	Black	-7.0	16	495.23	8.34	0	5.32
Ezetimibe	Synthetic	-6.6	8	409.43	-	0	-
Phe-Thr-Thr-Gln	Great Northern	-6.5	17	495.23	7.46	0	5.38
Asp-Leu-Lys-Leu-Pro	Pea	-6.5	20	584.35	11.98	0	6.77
Lys-Gly-Ala-Arg	Cowpea	-6.2	17	430.26	14.16	2	11.52
Val-Asn-Arg-Leu-Met	Lentil	-6.2	22	631.35	8.18	1	11.11
Leu-Asn-Leu	Cowpea	-5.9	12	358.22	6.25	0	5.58

a. Black, navy, and Great Northern are all common bean varieties. Cowpea refers to cowpea beans, which are not common beans.

Table 3. Expected interactions between ligands and the N-terminal domain of NPC1L1

Ligand	Interacting Amino Acid of NPC1L1 (Average Distance in Å) ^a					
	van der Waals	Alkyl Hydrophobic	π	Conventional Hydrogen Bond	Unconventional Carbon Hydrogen Bond	Electrostatic
Tyr-Ala-Ala-Ala-Thr	ARG80, LEU81, THR83, SER130, PRO131, ASN132, LEU135, PHE136, PRO225, VAL229, ILE233, GLN234, LEU236	-	π - alkyl ALA228 (5.37), PRO235 (4.60)	GLN161 (3.26), PRO235 (3.18), ASN237 (3.51/3.99), GLU238 (4.54)	HIS126 (5.72), ASN237 (3.95), GLU238 (3.88)	-
Glu-Arg-Ala-Phe	SER130, LEU135, PHE136, PRO235, LEU236, ASN237	-	-	ASN132 (4.44), GLN234 (3.62), GLU238 (4.81)	HIS126 (5.07)	Charged GLU238 (4.78)
Lys-Asp	PHE35, GLU38, SER53, ASN54, SER56, GLN95, LEU99, SER102, LEU103, ASN127, VAL153, LEU213, ALA214, PRO215, LEU216, ILE218	-	π - cation HIS124 (5.60)	SER98 (3.68), THR128 (3.66), TYR156 (5.95)	VAL55 (3.92)	-
Phe-Ala-Thr-Gly-Thr	LEU81, THR83, ASN122, HIS126, SER130, PRO131, PHE136, GLN234, PRO235, LEU236, ASN237, GLN247	-	π - alkyl LEU135 (6.22)	ARG80 (6.58), ASN132 (5.10), GLU238 (4.26)	GLY239 (3.54), VAL240 (4.89), ALA241 (3.68)	Charged GLU238 (4.26)
Ezetimibe	GLN167, SER171, SER173, VAL240, CYS243, ASN244, CYS259	-	π - alkyl ARG174 (6.00), ARG242 (4.77), ALA260 (4.25) π - anion ASP170 (4.87)	ASP170 (4.82), ARG174 (5.24), ALA260 (3.44), ALA261 (4.12)	-	-
Phe-Thr-Thr-Gln	PHE205	-	-	ASN211 (5.35)	-	-
Asp-Leu-Lys-Leu-Pro	GLN167, TYR169, SER171, SER173, LEU183, SER194, ALA195, VAL240, ALA241, ASN244	VAL185 (5.61), ARG242 (6.32), CYS243 (5.40), ALA260 (4.01), ALA261(4.68)	-	ASP170(3.89), ARG174 (4.53), CYS197 (4.78), ALA199 (4.28), ARG242 (3.98)	ASN198 (3.53)	Charged GLU166 (4.70) Salt Bridge ASP170 (4.19)
Lys-Gly-Ala-Arg	ARG80, THR83, THR87, GLN88, HIS126, SER130, PRO131, LEU135, PHE136, PRO235, LEU236	-	-	LEU81 (3.39), ASN132 (3.69), GLN234 (4.45), ASN237 (3.91), GLU238 (4.55)	-	Charged GLU238 (5.89)

Val-Asn-Arg-Leu-Met	GLN167, TYR169, SER171, VAL185, SER194, ASN198, ALA199, VAL240, ALA241, ASN244, ALA261	CYS243 (4.64), ALA260 (4.00)	-	GLU166 (4.01), ASP170 (3.96/4.12), SER173 (3.25), ARG174 (4.66), CYS197 (4.79)	ARG242 (4.51)	Charged GLU166 (5.83) Salt Bridge ASP170 (4.53)
Leu-Asn-Leu	CYS189, GLY190, VAL191, TYR192, GLY207, THR209, ASN211, ASP217, THR219, PHE220	ARG201 (4.85)	π-alkyl PHE205 (4.48)	ASN204 (3.88), ILE218 (5.14)	ASP208 (3.25)	Charged ASP208 (4.33)

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- a. When a ligand interacts more than once with an amino acid in NPC1L1 using the same type of interaction, the average distance for each interaction in this scenario is separated by a slash.

A **C** **B**