Published in final edited form as:

Methods Mol Biol. 2017 ; 1529: 353–362. doi:10.1007/978-1-4939-6637-0_18.

Probing Oligomerized Conformations of Defensin in the Membrane

Wenxun Gan^{1,2}, Dina Schneidman³, Ning Zhang^{1,2}, Buyong Ma^{4,5}, and Ruth Nussinov^{6,7,8}

¹Research Center of Basic Medical Sciences, Tianjin Medical University, Tianjin, 300060, China.

²Cancer Institute and Hospital, Tianjin Medical University, Tianjin, 300060, China.

³Department of Bioengineering and Therapeutic Sciences, University of California, San Francisco, CA, 94158, USA.

⁴Basic Science Program, Leidos Biomedical Research, Inc., Frederick, MD, 21702, USA.

⁵Cancer and Inflammation Program, National Cancer Institute, Frederick, MD, 21702, USA.

⁶Basic Science Program, Leidos Biomedical Research, Inc., Frederick, MD, 21702, USA.
nussinov@helix.nih.gov.

⁷Cancer and Inflammation Program, National Cancer Institute, Frederick, MD, 21702, USA.
nussinov@helix.nih.gov.

⁸Department of Human Genetics and Molecular Medicine, Sackler School of Medicine, Sackler Institute of Molecular Medicine, Tel Aviv University, Tel Aviv, 69978, Israel.
nussinov@helix.nih.gov.

Abstract

Computational prediction and design of membrane protein–protein interactions facilitate biomedical engineering and biotechnological applications. Due to their antimicrobial activity, human defensins play an important role in the innate immune system. Human defensins are attractive pharmaceutical targets due to their small size, broad activity spectrum, reduced immunogenicity, and resistance to proteolysis. Protein engineering based modification of defensins can improve their pharmaceutical properties. Here we present an approach to computationally probe defensins' oligomerization states in the membrane. First, we develop a novel docking and rescoring algorithm. Then, on the basis of the 3D structure of Sapeicin, an insect defensin, and a model of its antimicrobial ion-channel, we optimize the parameters of our empirical scoring function. Finally, we apply our docking program and scoring function to the hBD-2 (human β -defensin-2) molecule and obtain structures of four possible oligomers. These results can be used in higher level simulations.

Keywords

Molecular docking; Empirical scoring function; Human defensin; Membrane protein; Peptide design; Protein–protein interaction

1 Introduction

Prediction and design of membrane protein–protein interactions have the potential to facilitate biomedical engineering for medical and biotechnological applications [1]. Computational study for weakly stable β -structures in membrane is important to engineer the biophysical properties including oligomerization state [2]. Defensins are crucial to innate immunity. They contribute to the antimicrobial action of granulocytes in the mucosa in the small intestine, in the epithelial host defense in the skin and elsewhere [3, 4]. They have antiviral activity against both enveloped and non-enveloped viruses [5], and they are important in HIV infection [6]. The oligomerization of defensins either forms of ion pores in bacterial membranes or aggregate into positively charged patches which disrupt the integrity of the lipid bilayer [7–9].

Humans express two types of defensins, α and β . Three human β -defensins: h β -defensin-1, -2, and -3, have similar sequences, however, different properties [10]. It has been reported that several molecules can induce or enhance the production of defensins, for example, NOD2/CARD15 [11], TLR2 and TLR4 [12], and IL-12/IL-23/IL-27 [13]. Inducible hBD-2 could play a critical role in the protection of *M. pneumoniae* infection [14]. Human defensins also have complex roles in tumor growth, tumor monitoring, and cancer treatment [15]. hBD-2 exerts its growth suppression effect toward human melanoma cells via downregulation of B-Raf, cyclin D1, and cyclin E expression, upregulation of p21(WAF1) expression and activation of pRB [16]. hBD-2 may also control cell growth via arrest of G1/S transition and pRB activation [17]. Due to their well-established antimicrobial properties, defensins are also being investigated as therapeutics agents, especially as potential source to combat resistant bacteria. Human defensins are also attractive pharmaceutical targets due to their small size, broad activity spectrum, reduced immunogenicity and resistance to proteolysis [10, 18, 19].

Defensins perform their biological functions through three mechanisms: (1) Direct binding and modulation of host cell surface receptors and disruption of intracellular signaling which can inhibit viral replication [20]; (2) an indirect antiviral mechanism, where they function as chemokines to augment and alter adaptive immune responses; and (3) membrane disruption and pore formation [7–9]. The membrane-bound structure and topology of a human α -defensin indicate membrane pores consisting of dimers [21].

The characteristic folds of defensins are β -sheets stabilized with three disulfide bonds (Fig. 1). Their structural features, such as the helical N-terminal domains and oligomerization at the membrane surface, may modulate the efficiency of membrane insertion and selectivity for microbial or host-cell membranes. Both defensin-2 and -3 can interact with membranes as extended β -sheet platforms that present amphipathic helices for insertion into the lipid bilayer [22]. Nonetheless, many questions regarding the antiviral activities of defensins remain. Although significant mechanistic data are known for α -defensins, molecular details for β -defensins inhibition are mostly lacking [5]. The typical β -defensin action mechanism is not yet established, and one of the main challenges for the activation mechanism of the defense is the assembly in the membrane and the mechanism of membrane disruption.

Computational approaches have been employed to explore the dimerization of human β -defensin-2 [23], and to design sequences de novo based on flexible templates [24]. Here we present a computational protocol to probe possible oligomerization states of defensin in the membrane. We evaluate candidate states by a multiple protein–protein docking protocol. We focus on two β -defensins, one is the insect defensin Sapecin and another is human β -defensin-2 (hBD-2). The reason for choosing the two systems are (1) experimental information is available for possible protein–protein interactions and protein–membrane interactions for the insect Sapecin [25](Notes 1 and 2); and (2) human β -defensin-2 (hBD-2) is biologically important. Understanding the mechanism is a necessary first step to design novel antimicrobial peptides.

2 Methods

The system-specific docking protocol uses the following strategies:

2.1 Dock Sapecin Using SymmDock to Test Its Trimeric Assembly

SymmDock is a program to dock proteins and generate protein oligomers in C_n symmetries ($n \geq 2$) [26, 27]. The program can run through a webserver <http://bioinfo3d.cs.tau.ac.il/SymmDock/> or a standalone version. The program can be installed in unix environment by running `./install_SymmDock.pl` from the directory with SymmDock program files.

1. Download experimental pdb structures of Sapecin (1l4v) and defensin-2 (1FD4). Prepare a pdb file with the molecule you want to dock: `unit.pdb` (remove hydrogen atoms if the pdb structure of the protein is obtained by NMR).
2. Create parameter file by running the script: `buildParams.pl n unit.pdb`, where n = the number of symmetric units and `unit.pdb` is the name of the PDB file of one unit. The script will create parameter file named `params.txt`. All the parameters are explained within the parameter file.
3. Create the Connolly surface for the molecule by running the script: `runMSPoint.pl`.
4. Additional input may include potential binding site residues for the molecule, which reduces running time and improve the docking quality. The format of the active site file is as follows: each line includes residue number and chain id for one residue. For example

347 A

348 A

¹-NMR experiments have indicated the likely oligomeric state for Sapecin in the membrane, with Asp4 and Arg23 intermolecular interactions [25]. Our docking and rescoring has identified the two best solutions that have arrangements similar to the conformers suggested from experiment (Fig. 2).

²-In solution, the NMR structure for hBD-2 does not show oligomerization. However, crystal structures of defensins indicate dimerization and higher oligomerization states [31, 32]. A crystal packing pattern of human defensin might also provide information regarding pore formation in the membrane. A pore formed by an octameric assembly could accommodate four water molecules [31]. The question is, though, if the assembly will re-arrange in the membrane. We try to use the parameters developed from docking of Sapecin to investigate the potential oligomerization states of the hBD-2 in the membrane.

The name of the file with the binding site residues is specified in the parameter file. Add or uncomment the activeSiteParams line: *activeSiteParams siteFile.txt 2 0.7*.

Binding site residues can be used in the matching and scoring stage. The integer parameter of activeSiteParams can control the usage of the binding site in the matching stage: (0) don't use, (1) use only for first base point, (2) use for both base points. The last parameter (0.7 here) is for the scoring stage, which specifies the minimal ratio of the active site score in the results. Docking solutions with smaller ratios are discarded.

5. Running the symmetry dock program: *symm_dock.Linux*
`<params_file><output_file>`

The *params_file* is the parameter file “params.txt” that was previously created by “buildParams.pl”. *output_file* is the name of the file that will include the results, which contain the ranking and transformation matrix to create docked pdb structures.

Each line represents one solution, with the following format:

```
# |score | pen. |int. area| as1 | as2 | desolv. | Transformation
1 | 6967 | 2.72 | 1761.00 | 0 | 0 | 461.34 | 2.04 1.07 2.82 34.36 2.80 19.23
```

#—trans number

score—geometric score

pen.—maximal surface penetration of surface points

int. area—buried surface area of the interface

as1—geometric score based only on residues that were given as potential binding site for one side of the interface

as2—geometric score based only on residues that were given as potential binding site for other side of the interface

desolv.—DeLisi desolvation energy [28]

Transformation—transformation matrix to generate oligomer structure: three rotational angles and three translational parameters

6. Generating docked PDB files by running: *transOutput.pl output file n1 n2*.

The output file is the file created by the program earlier. *n1* and *n2* are the numbers of transformations to generate. For example running: “*transOutput.pl output.txt 1 10*” will create PDB files with the first ten transformations. The script generates a file named result.transNumber.pdb, where ‘number’ is the transformation number.

2.2 Rescore the Docking Solution Using DFIRE2 to Evaluate the Protein–Protein Interaction Energy

1. DFIRE2 is a program to calculate protein–protein interactions using knowledge-based functions [29, 30], which is available from <http://sparks-lab.org/>. For each solution generated from Symmetry docking, DFIRE2 energy can be evaluated by running: `DFIRE dfire_pair.lib result.transNumber.pdb`.
2. Refine the scoring function for defensin assembly in the membrane using experimental information as a guide. Normal docking and scoring functions are designed for interactions in aqueous solution or in the crystal complex. In order to reevaluate the docking solutions specifically for defensin in the membrane environment, we re-designed the scoring function to rank the docked defensin oligomer as:

$$E_{\text{membrane}} = \text{binding-energy} * a + \text{desolvation-energy} * b + \text{interface-area} * c$$

Where the parameters a , b , and c are to be optimized from docking of the Sapecin trimer in the membrane to fit experimental observations. The binding energy is calculated with the DFIRE2, and the desolvation energy and the interface area are calculated with the SymmDock. Based on extensive docking of the Sapecin and re-ranking of the solution to fit experimental binding modes, we obtained the optimized parameters: $a = 1$, $b = 0.006$, and $c = 0.003$ (Note 3).

2.3 Docking and Ranking the Human Defensin-2 Oligomers

1. Repeat the symmetry docking procedure using human β -defensin-2 (hBD-2) dimer structure as input to construct hBD2 octamers.
2. Using the optimized scoring function to evaluate the hBD-2 octamer in the membrane. The flowchart of the computational approaches is in Fig. 1. (Notes 4 and 5)

4 Conclusion

Re-parameterizing symmetry dock for membrane environment can provide insight into the oligomerization structures of the membrane damaging antibacterial defensin in membrane. If combined with high level simulations in further optimization of protein structure and sequence, the integrated approach could be a valuable method in computational protein design (Notes 4 and 5).

³.We apply the scoring functions developed from the Sapecin oligomer to probe the oligomerization of hBD-2. The new scoring function clearly helps to identify possible channel forming oligomers. The 20 top ranking octamers have many candidate structures with appropriate channel forming orientations (Table 1 and Fig. 3).

⁴.High level simulations, for example, explicit water molecular dynamics simulations can be performed using the selected hBD-2 oligomers to examine oligomer stability and membrane disruption. Our studies of a similar protein, the pg-1 monomer and dimer, on the membrane surface [33] or in the membrane [34], have shown that MD simulations are a powerful tool to investigate membrane disruption by antimicrobial peptides.

⁵.The outlined approach may not be restricted to symmetric oligomerization. Other protein–protein docking programs can also be used for protein engineering. Such programs include, but not limited to, PatchDock [35] and FireDock [36].

Acknowledgments

The authors are funded in whole or in part with Federal funds from the National Cancer Institute, National Institutes of Health, under contract number HHSN261200800001E. This research was supported (in part) by the Intramural Research Program of the NIH, National Cancer Institute, Center for Cancer Research. This research was funded in part by the US Army Medical Research Acquisition Activity under grant W81XWH-05-1-0002. N.Z. thanks Chinese NFSC grant 30772529 and 973 program grants 2011CB933100 and 2010CB933900.

References

1. Nanda V, Hsieh D, Davis A (2013) Prediction and design of outer membrane protein-protein interactions. *Methods Mol Biol* 1063:183–196. doi:10.1007/978-1-62703-583-5_10 [PubMed: 23975778]
2. Naveed H, Liang J (2014) Weakly stable regions and protein-protein interactions in beta-barrel membrane proteins. *Curr Pharm Des* 20(8):1268–1273 [PubMed: 23713778]
3. Ganz T (2003) Defensins: antimicrobial peptides of innate immunity. *Nat Rev Immunol* 3 (9):710–720 [PubMed: 12949495]
4. Chandrababu KB, Ho B, Yang D (2009) Structure, dynamics, and activity of an all-cysteine mutated human beta defensin-3 peptide analogue. *Biochemistry* 48(26):6052–6061. doi:10.1021/bi900154f [PubMed: 19480463]
5. Wilson SS, Wiens ME, Smith JG (2013) Antiviral mechanisms of human defensins. *J Mol Biol* 425(24):4965–4980. doi:10.1016/j.jmb.2013.09.038 [PubMed: 24095897]
6. Garzino-Demo A (2007) Chemokines and defensins as HIV suppressive factors: an evolving story. *Curr Pharm Des* 13(2):163–172 [PubMed: 17269925]
7. Krishnakumari V, Nagaraj R (2012) Binding of peptides corresponding to the carboxyterminal region of human-beta-defensins-1–3 with model membranes investigated by isothermal titration calorimetry. *Biochim Biophys Acta* 1818(5):1386–1394. doi:10.1016/j.bbmem.2012.02.016 [PubMed: 22386945]
8. Krishnakumari V, Rangaraj N, Nagaraj R (2009) Antifungal activities of human beta defensins HBD-1 to HBD-3 and their C-terminal analogs Phd1 to Phd3. *Antimicrob Agents Chemother* 53(1):256–260. doi:10.1128/AAC.00470-08 [PubMed: 18809937]
9. Brogden KA (2005) Antimicrobial peptides: pore formers or metabolic inhibitors in bacteria? *Nat Rev Microbiol* 3(3):238–250. doi:10.1038/nrmicro1098 [PubMed: 15703760]
10. Spudy B, Sonnichsen FD, Waetzig GH, Grotzinger J, Jung S (2012) Identification of structural traits that increase the antimicrobial activity of a chimeric peptide of human beta-defensins 2 and 3. *Biochem Biophys Res Commun* 427(1):207–211. doi:10.1016/j.bbrc.2012.09.052 [PubMed: 22995312]
11. Voss E, Wehkamp J, Wehkamp K, Stange EF, Schroder JM, Harder J (2006) NOD2/CARD15 mediates induction of the antimicrobial peptide human beta-defensin-2. *J Biol Chem* 281(4):2005–2011. doi:10.1074/jbc.M511044200 [PubMed: 16319062]
12. Gariboldi S, Palazzo M, Zanobbio L, Selleri S, Sommariva M, Sfondrini L, Cavicchini S, Balsari A, Rumio C (2008) Low molecular weight hyaluronic acid increases the self-defense of skin epithelium by induction of beta-defensin 2 via TLR2 and TLR4. *J Immunol* 181 (3):2103–2110 [PubMed: 18641349]
13. Kanda N, Watanabe S (2008) IL-12, IL-23, and IL-27 enhance human beta-defensin-2 production in human keratinocytes. *Eur J Immunol* 38(5):1287–1296. doi:10.1002/eji.200738051 [PubMed: 18389480]
14. Kuwano K, Tanaka N, Shimizu T, Kida Y (2006) Antimicrobial activity of inducible human beta defensin-2 against *Mycoplasma pneumoniae*. *Curr Microbiol* 52(6):435–438. doi:10.1007/s00284-005-0215-7 [PubMed: 16732451]
15. Droin N, Hendra JB, Ducoroy P, Solary E (2009) Human defensins as cancer biomarkers and antitumour molecules. *J Proteomics* 72 (6):918–927. doi:10.1016/j.jprot.2009.01.002 [PubMed: 19186224]

16. Gerashchenko O, Zhuravel E, Skachkova O, Khranovska N, Pushkarev V, Pogrebnoy P, Soldatkina M (2014) Involvement of human betadefensin-2 in regulation of malignant potential of cultured human melanoma cells. *Exp Oncol* 36(1):17–23 [PubMed: 24691279]
17. Zhuravel E, Shestakova T, Efanova O, Yusefovich Y, Lytvin D, Soldatkina M, Pogrebnoy P (2011) Human beta-defensin-2 controls cell cycle in malignant epithelial cells: in vitro study. *Exp Oncol* 33(3):114–120 [PubMed: 21956461]
18. Jarczak J, Kosciuczuk EM, Lisowski P, Strzalkowska N, Jozwik A, Horbanczuk J, Krzyzewski J, Zwierzchowski L, Bagnicka E (2013) Defensins: natural component of human innate immunity. *Hum Immunol* 74(9):1069–1079. doi:10.1016/j.humimm.2013.05.008 [PubMed: 23756165]
19. Bai Y, Liu S, Jiang P, Zhou L, Li J, Tang C, Verma C, Mu Y, Beuerman RW, Pervushin K (2009) Structure-dependent charge density as a determinant of antimicrobial activity of peptide analogues of defensin. *Biochemistry* 48 (30):7229–7239. doi:10.1021/bi900670d [PubMed: 19580334]

Fig. 1. Flowchart for the strategy to investigate the defensin oligomerization in membrane. The ribbon structure in *left corner* highlights three disulfide bonds in human defensin

Fig. 2. Two best conformations with the highest ranking in optimized score function, which fit NMR observation, the *numbers* indicate the ranking from the initial symmetry docking

Fig. 3.

The octamer structures of hBD2 with pore conformation obtained from symmetry docking. The *numbers* indicate the ranking from the initial symmetry docking. The result 1 is the top ranking structure from the initial symmetry docking, and results 103, 216, 172, 74, and 193 are five top ranking structure from our reoptimized score function in membrane. It can be seen that the top re-ranked structures have larger pore sizes

Table 1

Top 20 ranked hBD2 octamers from symmdock with new scoring function

Rank	Score (new ranking)	Result (symmetry dock ranking)
1	14.42336	result.103.pdb
2	13.41008	result.216.pdb
3	12.74822	result.172.pdb
4	12.48742	result.74.pdb
5 *	12.48012	result.193.pdb
6	12.3413	result.97.pdb
7	12.02262	result.170.pdb
8	11.99962	result.251.pdb
9	11.91628	result.151.pdb
10	11.77326	result.133.pdb
11	11.53318	result.9.pdb
12 *	11.4873	result.270.pdb
13	11.33686	result.175.pdb
14	11.27296	result.295.pdb
15	11.23988	result.429.pdb
16	11.14096	result.23.pdb
17	11.09774	result.255.pdb
18 *	11.07944	result.87.pdb
19 *	11.06372	result.124.pdb
20	10.91142	result.298.pdb

* The conformers with are pore-forming octamers