RESEARCH ARTICLE

The PDB database is a rich source of alpha-helical anti-microbial peptides to combat disease causing pathogens

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Abstract

The therapeutic potential of α -helical anti-microbial peptides (AH-AMP) to combat pathogens is fast gaining prominence. Based on recently published open access software for characterizing α -helical peptides (PAGAL), we elucidate a search methodology (SCALPEL) that leverages the massive structural data pre-existing in the PDB database to obtain AH-AMPs belonging to the host proteome. We provide *in vitro* validation of SCALPEL on plant pathogens (*Xylella fastidiosa*, *Xanthomonas arboricola* and *Liberibacter crescens*) by identifying AH-AMPs that mirror the function and properties of cecropin B, a well-studied AH-AMP. The identified peptides include a linear AH-AMP present within the existing structure of phosphoenolpyruvate carboxylase (PPC20), and an AH-AMP mimicking the properties of the two α -helices of cecropin B from chitinase (CHIT125). The minimum inhibitory concentration of these peptides are comparable to that of cecropin B, while anionic peptides used as control failed to show any inhibitory effect on these pathogens. Substitute therapies in place of conventional chemotherapies using membrane permeabilizing peptides like these might also prove effective to target cancer cells. The use of native structures from the same organism largely ensures that administration of such peptides will be better tolerated and not elicit an adverse immune response. We suggest a similar approach to target Ebola epitopes, enumerated using PAGAL recently, by selecting suitable peptides from the human proteome, especially in wake of recent reports of cationic amphiphiles inhibiting virus entry and infection.

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Introduction

The abundance of alpha helical (AH) structures present within proteins bears testimony to their relevance in determining functionality¹. AHs are key components in protein-protein interaction interfaces², DNA binding motifs³, proteins that permeate biological membranes⁴, and anti-microbial peptides (AMP)^{5,6}. Not surprisingly, these AHs are the targets for antibody binding^{7,8} and therapeutic agents⁹. These therapies in turn use AH peptides against both viral^{10–12} and bacterial pathogens¹³.

Some AHs have unique characteristics, which are strongly correlated to their significance in the function of a protein⁷. For example, hydrophobic residues aligned on one surface (characterized by a hydrophobic moment¹⁴), is critical for virus entry into host cells¹⁵, and in the permeabilizing abilities of AH-AMPs¹⁶. Often, AHs have cationic residues on the opposite side of the hydrophobic surface, which helps them target bacterial membranes^{17,18}. We have previously implemented known methods¹⁹ of evaluating these properties, and provided this as open source software (PAGAL)²⁰. PAGAL was used to characterize the proteome of the Ebola virus⁷, and to correlate the binding of the Ebola protein VP24²¹ to human karyopherin²² with the immune suppression and pathogenicity mechanisms of Ebola and Marburg viruses²³.

Plant pathogens, like *Xylella fastidiosa* (Xf)²⁴, *Xanthomonas arboricola* (Xa)²⁵ and *Liberibacter crescens* (Lc)²⁶ are a source of serious concern for economic²⁷ and humanitarian reasons²⁸. Specifically, we have been involved in developing novel strategies to counter the Pierce's disease causing Xf, having previously designed a chimeric protein with anti-microbial properties that provides grapevines with enhanced resistance against Xf²⁹. Cecropin B (CECB) is the lytic component of this chimeric protein^{30,31}. However, the non-nativeness of CECB raises concerns regarding its viability in practical applications³².

In an effort to replace CECB with an equivalent peptide from the grapevine/citrus genome, we present a design methodology to select AH-AMPs from any given genome - Search characteristic **alpha helical** peptides in the PDB database and locate it in the genome (**SCALPEL**). CECB consist of two AHs, joined by a small loop. The N-terminal AH is cationic and hydrophobic, while the C-terminal AH consists of primarily hydrophobic residues. Characterizing all available AHs from plant proteins in the PDB database allowed us to identify a peptide with a large hydrophobic moment and a high proportion of positively charged residues, present in both grapevine and citrus (our organisms of interest), mirroring the linear cationic CECB N-terminal AH. One such match was a twenty residue long AH from phosphoenolpyruvate carboxylase in sunflower³³. The sequence of this peptide was used to find homologous peptides in the grapevine and citrus genome (PPC20). Subsequently, we used the SCALPEL algorithm to detect two contiguous AHs connected with a loop, mirroring the properties of CECB in a chitinase (CHITI25) from *Nicotiana tabacum* (PDBid:3ALG)³⁴. Subsequently, we demonstrate through bioassay experiments that PPC20 from the grapevine and citrus genome, and CHITI25 from the tobacco genome, inhibit Xf, Xa and Lc growth. The minimum inhibitory concentration of these peptides are comparable to that of CECB, while anionic peptides used as controls failed to show any

inhibitory effect with these pathogens. Further, we observed variation in the susceptibility of the pathogens to these peptides.

Materials and methods

In silico

The PDB database was queried for the keyword 'plants', and proteins with the exact same sequences were removed. This resulted in a set of ~2000 proteins (see list.plants.txt in [Dataset 1](#)). These proteins were analyzed using DSSP³⁵ to identify the AHs, and AHs with the same sequence were removed. This resulted in ~6000 AHs (see ALPHAHELICES.zip in [Dataset 1](#)). PAGAL was applied to this set of AHs (see RawDataHelix.txt in [Dataset 1](#)). This data was refined to obtain peptides with different characteristics. We also computed the set of all pairs of AHs that are connected with a short (less than five residues) loop (see HTH in [Dataset 1](#)). This set is used to extract a pair of AHs, such that one of them is cationic with a large hydrophobic moment, while the other comprises mostly of hydrophobic residues. The PAGAL algorithm has been detailed previously²⁰. Briefly, the Edmundson wheel is computed by considering a wheel with centre (0,0), radius 5, first residue coordinate (0,5) and advancing each subsequent residue by 100 degrees on the circle, as 3.6 turns of the helix makes one full circle. We compute the hydrophobic moment by connecting the center to the coordinate of the residue and give it a magnitude obtained from the hydrophobic scale (in our case, this scale is obtained from Jones *et al.*¹⁹). These vectors are then added to obtain the final hydrophobic moment. The color coding for the Edmundson wheel is as follows: all hydrophobic residues are colored red, while hydrophilic residues are colored in blue: dark blue for positively charged residues, medium blue for negatively charged residues and light blue for amides. All protein structures were rendered by PyMol (<http://www.pymol.org/>). The sequence alignment was done using ClustalW³⁶. The alignment images were generated using Seaview³⁷. Protein structures have been superimposed using MUSTANG³⁸.

In vitro

Synthesized chemical peptides were obtained from GenScript USA, Inc. The protein molecular weight was calculated per peptide then diluted to 2000 μ M or 3000 μ M stock solutions with phosphate buffered saline. Stock solutions were stored in -20°C and thawed on ice before use.

Using the stock solutions, we made dilute solutions of 300 μ M, 250 μ M, 200 μ M, 150 μ M, 100 μ M, 75 μ M, 50 μ M, 30 μ M, 25 μ M, and 10 μ M to a final volume of 100 μ l of phosphate buffered saline. Dilute peptide solutions were stored in -20°C and thawed on ice before use.

Xylella fastidiosa 3A2 (PD3)³⁹, *Xanthomonas arboricola* 417 (TYS)⁴⁰, and *Liberibacter crescens* BT-1 (BM7)⁴¹ media were prepared and autoclaved at 121°C for 15–30 minutes, then cooled and poured into 100 × 15mm sterile petri dishes. Kanamycin (50 μ g/ml) was added to PD3.

Bacteria were inoculated and allowed to grow in liquid medium at 28°C: Xf (5 days), Xa (3 days), and Lc (3 days) to reach the exponential phase. The inoculum was diluted to a working OD of 0.5 (1 × 10⁷ cells/ml). 10 μ l of the OD 0.5 was plated with 90 μ l of liquid

media and spread on the pre-made agar plates to create a confluent lawn of bacteria. The bacteria were given an hour to set at room temperature. 10 μ l of each peptide concentration was spotted onto a plate of agar preseeded with a layer of bacterium. After spotting the plates were incubated at 28°C for 2 to 10 days till zones of clearance were clearly visible and the plates were scored for the minimum inhibitory concentration (MIC) as that beyond which no visible clearance was observed. Data presented is in triplicate, and were identical.

Results

Dataset 1. Data used for SCALPEL search methodology to identify plant alpha helical - antimicrobial peptides in the PDB database

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list.plants.txt: list of PDB IDs resulting from querying the PDB database with the keyword 'plant'. ALPHAHHELICES.zip: DSSP analysis of proteins listed in list.plants.txt to identify alpha helices. RawDataHelix.txt: PAGAL analysis of alpha helices listed in ALPHAHHELICES.zip. HTH: Set of all pairs of alpha helices connected with a short (<five residues) loop.

Existing AH-AMPs: the positive controls

Cecropin B (CECB) was used as a positive control, as it is known to target membrane surfaces and creates pores in the bacterial outer membrane^{30,31}. CECB consists of an cationic amphipathic N-Terminal with a large hydrophobic moment (Figure 1a), and a C-Terminal comprising mostly of hydrophobic residues, which consequently has a low hydrophobic moment, (Figure 1b) joined by a short loop. Another positive control was a linear AH-AMP consisting of the residues 2-22 of the N-Terminal in CECB (CBNT21) (Figure 1a). The sequences of these are shown in Table 1.

SCALPEL: Identifying native AH-AMP peptides from the host proteome

Linear AH-AMPs. In order to choose a peptide mimicking CBNT21 (cationic, amphipathic, large hydrophobic moment), we directed our search to 'locate a small peptide with a large hydrophobic moment and a high proportion of positively charged residues' on the raw data computed using PAGAL (See RawDataHelix.txt in Dataset 1). A small peptide is essential for quick and cost effective iterations. Table 2 shows the best matching AHs. Next, we used the sequence of these AHs to search the grapevine and citrus genomes, choosing only those that are present in both genomes. This allowed us to locate

Figure 1. Edmundson wheel for AHs in the known AMPs that were used as control. The color coding for the Edmundson wheel is as follows: all hydrophobic residues are colored red, while hydrophilic residues are colored in blue: dark blue for positively charged residues, medium blue for negatively charged residues and light blue for amides. The hydrophobic moment arrow is not to scale. (a) N-terminal of Cecropin B (CECB) shows its amphipathic nature, with one side being cationic and the other side hydrophobic. (b) C-terminal of CECB consists of mostly hydrophobic residues, and thus has a low hydrophobic moment. (c) Edmundson wheel for PPC20. (d) Edmundson wheel for 3ALGA.α4, which corresponds to the C-terminal of CECB and comprises mostly of hydrophobic residues (low hydrophobic moment). (e) Edmundson wheel for 3ALGA.α5, which corresponds to the cationic, N-terminal of CECB with a large hydrophobic moment.

Table 1. Sequences of peptides used in this study. CO: control peptides SC: SCALPEL generated peptides.

CO	CECB	KWKVFKKIEKMGRNIRNGIVKAGPAIAVLGEAKAL full length CECB from <i>Hyalophora cecropia</i> (silk moth)
CO	CBNT21	WKVFKKIEKMGRNIRNGIVKA N-terminal CECB (minus the first lysine)
SC	PPC20	TIWKGVPKFLRRVDTALKNI Linear cationic AH-AMP from phosphoenolpyruvate carboxylase (PDBid:3ZGBA)
SC	CHITI25	TAYGIMARQPNRSKSFIDSSIRLAR CECB like AH-AMP from chitinase <i>Nicotiana tobaccum</i> (PDBid:3ALGA)
SC	ISS15	TLDELELFTDAVERW Linear anionic peptide from isoprene synthase from gray poplar (PDBid:3N0FA)

Table 2. Identifying AHs with cationic properties from plant proteins with known structures. All AHs in plant proteins are analyzed using PAGAL, and the data is pruned for AHs with a high proportion of positive residues, and finally sorted based on their hydrophobic moment. The first match is present in both grapevine and citrus (PDBid:3ZGBA.α11, which is a phosphoenolpyruvate carboxylase from sunflower). We ignored a small π AH in the beginning of this peptide comprising four residues. This peptide has been named PPC20. HM: Hydrophobic moment, RPNR: Relative proportion of positive residues among charged residues, Len: length of the α , NCH: number of charged residues.

PDB.α	Len	HM	RPNR	NCH
3ZGBA.α11 (PPC20)	24	12.6	0.8	8
4HWIA.α10	17	12.3	0.9	9
4BXHB.α11	23	12.3	0.8	8
2J376.α1	18	10.5	0.9	8
3J61R.α4	21	10.4	0.9	10
3J60G.α3	44	10.2	0.8	22
1W07A.α4	21	9.9	0.8	10
2WWBM.α1	17	9.5	0.9	8
1B8GA.α17	27	7.3	0.9	11
3J61L.α1	19	7.2	1	9

an AH from phosphoenolpyruvate carboxylase from sunflower, a key enzyme in the C4-photosynthetic carbon cycle which enhances solar conversion efficiency (PDBid:3ZGBA.α11)³³. **Figure 2a** shows the specific AH located within the protein structure, marked in green and blue. Although DSSP marks the whole peptide stretch as one AH, we chose the AH in blue due to the presence of a small π helix preceding that. We named this peptide PPC20 (**Figure 2, Table 1**). This peptide is fully conserved (100% identity in the 20 residues) in both grapevine (Accession id:XP_002285441) and citrus (Accession id:AGS12489.1). **Figure 2b,c** shows the Pymol rendered AH surfaces of PPC20. The Asp259 stands out as a negative residue in an otherwise positive surface (**Figure 2c**). Since previous studies have noted dramatic transitions with a single mutation on the polar face, it would be interesting to find the effect of mutating Asp259 to a cationic residue⁴².

Non-linear AH-AMPs consisting of two AHs. Next, we located two AHs within chitinase from *Nicotiana tobaccum* (PDBid:3ALGA.α4

and 3ALGA.α5)³⁴ connected by a short random coil such that one of the AHs is cationic and hydrophobic, while the other AH is comprised mostly of hydrophobic, uncharged residues (CHITI25, **Figure 3a, Table 1**). This peptide mimics the complete CECB protein (**Figure 3b**). While the properties of the AHs in CHITI25 is reversed from that of CECB, the order in which these AHs occur is not important for functionality. The multiple sequence alignment of CHITI25 from grapevine, citrus and tobacco is shown in **Figure 3c**. CHITI25 from tobacco is the most cationic (five), followed by citrus (four) and grapevine (three). Thus, it is possible that the antimicrobial properties of CHITI25 from grapevine would be lower than CHITI25 from tobacco. These peptides can be subjected to mutations to enhance their natural antimicrobial properties in such a scenario⁴³.

Negative control - an anionic AH-AMP. We also located an anionic AH-AMP using a similar strategy - a 13 residue peptide present within the structure of isoprene synthase from gray poplar

Figure 2. Peptide PPC20 from phosphoenolpyruvate carboxylase in sunflower (PDBid:3ZGBA.α11). (a) 3ZGBA.α11 is marked in green and blue. We ignore the π AH, and also the small AH preceding it (marked in green). PPC20 is marked in blue. (b) Hydrophobic surface of PPC20. (c) Charged surface of PPC20. Asp259 stands out as a negative residue in an otherwise positive surface.

Figure 3. Peptide CHITI25 from chitinase in tobacco (PDBid:3ALGA). (a) PDBid:3ALGA.α4 in green, loop in magenta and 3ALGA.α5 in blue. (b) Superimposing CECB (PDBid:2IGRA) in red with CHITI25 in green using MUSTANG³⁸. Note, that the order of the AHs are reversed. (c) Multiple sequence alignment of CHITI25 from grapevine (CHITIVit), citrus (CHITICit) and tobacco (CHITITob). CHITITob is the more cationic than CHITIVit or CHITICit.

(PDBid:3N0FA.α18)⁴⁴. We also used phosphate buffered saline as a negative control. We have extended this helix on both terminals by including one adjacent residue from both terminals to obtain ISS15 (Table 1).

In vitro results

We have validated our peptides using plating assays (Table 3, Figure 4). CECB, the well-established AH-AMP, is the most potent among all the peptides tested, having minimum inhibitory concentrations of

Table 3. Minimum Inhibitory Concentration of peptides tested (μM). It can be seen that CECB is the most efficient among all the peptides for all three pathogens, while the anionic ISS15 does not show any effect even at higher concentrations. However, while CHITI25 is almost as effective as CECB for *Xf*, it fails to inhibit *Lc* growth. Also, *Xa* is much more susceptible to these peptides compared to the other two pathogens. Finally, the anionic ISS15 has no effect on these pathogens. Data is in triplicate, and were identical.

	Bacteria	CECB	CBNT21	PPC20	CHITI25	ISS15
γ Proteobacteria	<i>Xylella fastidiosa</i> 3A2 (<i>Xf</i>)	100	200	150	100	>300
	<i>Xanthomonas arboricola</i> 417 (<i>Xa</i>)	25	25	50	150	>300
α Proteobacteria	<i>Liberibacter crescens</i> BT-1 (<i>Lc</i>)	100	200	200	>300	>300

Figure 4. *In vitro* validation of SCALPEL methodology. Plating assay to determine minimum inhibitory concentration (MIC) of SCALPEL identified peptides for *Xanthomonas arboricola*. Counter-clockwise: 300μM, 250μM, 200μM, 150μM, 100μM 75μM, 50μM, 30μM, 25μM, 10μM, PBS. CECB: MIC 25, CBNT2: MIC 10, PPC20: MIC 50, CHITI25: MIC 150, ISS15: MIC >300.

between 25 μ M (for *Xa*) to 100 μ M (for *Xf* and *Lc*). This shows the variations in susceptibilities of different organisms. Understanding this differential susceptibility would require a deeper understanding of the underlying mechanism by which these AH-AMPs work⁴⁵, as well as the difference in the membrane composition of these gram-negative pathogens⁴⁶. Mostly, CBNT21 has a slightly lower potency, indicating a role for the C-terminal AH in CECB, which comprises of mostly hydrophobic residues for *Xf* and *Lc*. This results corroborates a plausible mechanism suggested by others in which the anionic membranes of bacteria is targeted by the cationic N-terminal, and followed by the insertion of the C-terminal AH into the hydrophobic membrane creating a pore. PPC20 and CHIT125 have comparable potencies with CECB and CBNT21, although *Lc* appears to be resistant to CHIT125. Finally, the anionic peptide used as a negative control shows no effect on these pathogens.

Discussion

The repertoire of defense proteins available to an organism is being constantly reshaped through genomic changes that confer resistance to pathogens. Genetic approaches aim at achieving the same goal of enhancing immunity through rational design of peptides^{13,47}, which are then incorporated into the genome^{29,31,48}. Also, it is important to ensure that these non-endogenous genomic fragments have minimal effect on humans for their commercial viability³². Identifying peptides from the same genome helps allay these concerns to a significant extent. The key innovation of the current work is the ability to identify peptides with specific properties (cationic AHs with a hydrophobic surface, linear or otherwise) from the genome of any organism of interest. Such peptides also present less likelihood of eliciting an adverse immune response from the host.

Alternate methods

Alternate computational methods for finding such new AMPs based on known AMPs could be of two kinds, although neither method is as effective in obtaining our results. Firstly, a sequence search using BLAST can be done to find a corresponding peptide in the genome, say for cecropin B. However, a BLAST of the cecropin sequence does not give any significant matches in the grapevine or citrus genomes, and is a dead end. In principle, what we need is a peptide with cecropin B like properties - and that information is not encoded in the linear sequence, but in the Edmundson wheel of the AH. The second method for such a search is to find structural homology in the PDB database through a tool like DALILITE⁴⁹. However, AHs are almost indistinguishable structurally, and the results will give rise to many redundancies. Thus, there are no existing methods tailored to incorporate the quantifiable properties of AHs in the search. We, for the first time, have proposed such a method in SCALPEL.

Computer-assisted design strategies have also been applied in designing *de novo* AMPs^{50,51}. Other hand curated comprehensive databases for 'for storing, classifying, searching, predicting, and designing potent peptides against pathogenic bacteria, viruses, fungi, parasites, and cancer cells'⁵² do not enjoy the automation and vastness of available data elucidated in the SCALPEL methodology.

Limitations and future directions

There are several caveats to our study. We are yet to ascertain the hemolytic nature of the identified peptides, and will be performing these experiments in the near future. In fact, the selective cytotoxicity against human cancer cells, might be used as a substitute therapy in place of conventional chemotherapy^{53,54}. Although, we have not measured the lipid permeabilizing abilities of our peptides, a recent study has found that potency in permeabilizing bacteria-like lipid vesicles does not correlate with significant improvements in anti-microbial activity, rendering such measurements redundant⁵⁵. The electrostatic context of a peptide is known to have a significant bearing on its propensity to adopt an AH structure. The ability to predict the folding of peptides requires significant computational power and modelling expertise⁵⁶. Peptides often remain in random coil conformations, and achieve helical structures only by interacting with anionic membrane models⁵⁷. It is also possible to measure peptide helicity through circular dichroism spectroscopy⁵⁸. However, our results have been all positive based on selected choices of peptides arising from our search results, and suggest a high likelihood of getting anti-microbial activity from these peptides. Additionally, we may have to resort to other innovative techniques that have been previously adopted to overcome thermodynamic instability or proteolytic susceptibility⁵⁹⁻⁶².

Conclusion

To summarize, we establish the presence of a large number of AH-AMPs 'hidden' in the universal proteome. We have designed a methodology to extract such peptides from the PDB database - the 'Big Data' center in proteomics. We demonstrate our results on well known plant pathogens - *Xf*, *Xa* and *Lc*. The feasibility of using such peptides in cancer therapies is also strong⁵⁴. The ability to choose a peptide from the host itself is an invaluable asset, since nativeness of the peptide allays fears of eliciting a negative immune response upon administration. The problem of antibiotic resistance is also increasing focus on peptide based therapies^{9,63}, since it is 'an enigma that bacteria have not developed highly effective cationic AMP-resistance mechanisms'⁶⁴. Lastly, in face of the current Ebola outbreak^{65,66}, we strongly suggest the possibility of developing peptides derived from the human genome to target viral epitopes, such as those enumerated for the Ebola virus recently⁷. A recent study has reported the inhibition of the Ebola virus entry and infection by several cationic amphiphiles⁶⁷, suggesting the SCALPEL generated cationic peptides with the aid of cell penetrating peptides⁶⁸ could achieve similar results.

Data availability

F1000Research: Dataset 1. Data used for SCALPEL search methodology to identify plant alpha helical - antimicrobial peptides in the PDB database, [10.5256/f1000research.5802.d39823](https://doi.org/10.5256/f1000research.5802.d39823)⁶⁹

Author contributions

SC wrote the computer programs. MP performed the *in vitro* experiments. All authors analyzed the data, and contributed equally to the writing and subsequent refinement of the manuscript.

Competing interests

No competing interests were disclosed.

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References

- Azzarito V, Long K, Murphy NS, *et al.*: Inhibition of α -helix-mediated protein-protein interactions using designed molecules. *Nat Chem.* 2013; 5(3): 161–173. [PubMed Abstract](#) | [Publisher Full Text](#)
- Lee JH, Zhang Q, Jo S, *et al.*: Novel pyrrolopyrimidine-based α -helix mimetics: cell-permeable inhibitors of protein-protein interactions. *J Am Chem Soc.* 2011; 133(4): 676–679. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Landschulz WH, Johnson PF, McKnight SL: The leucine zipper: a hypothetical structure common to a new class of DNA binding proteins. *Science.* 1988; 240(4860): 1759–1764. [PubMed Abstract](#) | [Publisher Full Text](#)
- Dathe M, Wieprecht T: Structural features of helical antimicrobial peptides: their potential to modulate activity on model membranes and biological cells. *Biochim Biophys Acta.* 1999; 1462(1–2): 71–87. [PubMed Abstract](#) | [Publisher Full Text](#)
- Wang G: Structures of human host defense cathelicidin LL-37 and its smallest antimicrobial peptide KR-12 in lipid micelles. *J Biol Chem.* 2008; 283(47): 32637–32643. [PubMed Abstract](#) | [Publisher Full Text](#)
- Wang G: Human antimicrobial peptides and proteins. *Pharmaceuticals (Basel).* 2014; 7(5): 545–594. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Chakraborty S, Rao B, Asgeirsson B, *et al.*: Characterizing alpha helical properties of Ebola viral proteins as potential targets for inhibition of alpha-helix mediated protein-protein interactions [v1; ref status: approved with reservations 1, <http://f1000r.es/4lg>]. *F1000Res.* 2014; 3: 251. [Publisher Full Text](#)
- Lee JE, Fusco ML, Hessel AJ, *et al.*: Structure of the Ebola virus glycoprotein bound to an antibody from a human survivor. *Nature.* 2008; 454(7201): 177–182. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Hancock RE, Chapple DS: Peptide antibiotics. *Antimicrob Agents Chemother.* 1999; 43(6): 1317–1323. [PubMed Abstract](#) | [Free Full Text](#)
- Judice JK, Tom JY, Huang W, *et al.*: Inhibition of HIV type 1 infectivity by constrained α -helical peptides: implications for the viral fusion mechanism. *Proc Natl Acad Sci U S A.* 1997; 94(25): 13426–13430. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Champagne K, Shishido A, Root MJ: Interactions of HIV-1 inhibitory peptide T20 with the gp41 N-HR coiled coil. *J Biol Chem.* 2009; 284(6): 3619–3627. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Hong W, Li T, Song Y, *et al.*: Inhibitory activity and mechanism of two scorpion venom peptides against herpes simplex virus type 1. *Antiviral Res.* 2014; 102: 1–10. [PubMed Abstract](#) | [Publisher Full Text](#)
- Zeitler B, Herrera Diaz A, Dangel A, *et al.*: De-novo design of antimicrobial peptides for plant protection. *PLoS One.* 2013; 8(8): e71687. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Eisenberg D, Weiss RM, Terwilliger TC: The helical hydrophobic moment: a measure of the amphiphilicity of a helix. *Nature.* 1982; 299(5881): 371–374. [PubMed Abstract](#) | [Publisher Full Text](#)
- Badani H, Garry RF, Wimley WC: Peptide entry inhibitors of enveloped viruses: the importance of interfacial hydrophobicity. *Biochim Biophys Acta.* 2014; 1838(9): 2180–2197. [PubMed Abstract](#) | [Publisher Full Text](#)
- Chen Y, Guarnieri MT, Vasil AI, *et al.*: Role of peptide hydrophobicity in the mechanism of action of α -helical antimicrobial peptides. *Antimicrob Agents Chemother.* 2007; 51(4): 1398–1406. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Brogden KA: Antimicrobial peptides: pore formers or metabolic inhibitors in bacteria? *Nat Rev Microbiol.* 2005; 3(3): 238–250. [PubMed Abstract](#) | [Publisher Full Text](#)
- Huang Y, Huang J, Chen Y: Alpha-helical cationic antimicrobial peptides: relationships of structure and function. *Protein Cell.* 2010; 1(2): 143–152. [PubMed Abstract](#) | [Publisher Full Text](#)
- Jones MK, Anantharamaiah GM, Segrest JP: Computer programs to identify and classify amphipathic alpha helical domains. *J Lipid Res.* 1992; 33(2): 287–296. [PubMed Abstract](#)
- Chakraborty S, Rao B, Dandekar A: PAGAL - Properties and corresponding graphics of alpha helical structures in proteins. [v2; ref status: indexed, <http://f1000r.es/4e7>] *F1000Res.* 2014; 3: 206. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Zhang AP, Abelson DM, Bornholdt ZA, *et al.*: The ebolavirus VP24 interferon antagonist: know your enemy. *Virulence.* 2012; 3(5): 440–5. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Xu W, Edwards MR, Borek DM, *et al.*: Ebola virus VP24 targets a unique NLS binding site on karyopherin alpha 5 to selectively compete with nuclear import of phosphorylated STAT1. *Cell Host Microbe.* 2014; 16(2): 187–200. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Chakraborty S, Rao B, Asgeirsson B, *et al.*: Correlating the ability of VP24 protein from Ebola and Marburg viruses to bind human karyopherin to their immune suppression mechanism and pathogenicity using computational methods [v1; ref status: approved with reservations 1, <http://f1000r.es/4o3>]. *F1000Res.* 2014; 3: 265. [Publisher Full Text](#)
- Hopkins D, Purcell A: *Xylella fastidiosa*: cause of Pierce's disease of grapevine and other emergent diseases. *Plant Disease.* 2002; 86: 1056–1066. [Publisher Full Text](#)
- Ryan RP, Vorholter FJ, Potnis N, *et al.*: Pathogenomics of *Xanthomonas*: understanding bacterium-plant interactions. *Nat Rev Microbiol.* 2011; 9(5): 344–355. [PubMed Abstract](#) | [Publisher Full Text](#)
- Leonard MT, Fagen JR, Davis-Richardson AG, *et al.*: Complete genome sequence of *Liberibacter crescens* BT-1. *Stand Genomic Sci.* 2012; 7(2): 271–283. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Alston JM, Fuller KB, Kaplan JD, *et al.*: Assessing the returns to R&D on perennial crops: the costs and benefits of Pierce's disease research in the California winegrape industry. *Aust J Agric Resour Econ.* 2014. [Publisher Full Text](#)
- Strange RN, Scott PR: Plant disease: a threat to global food security. *Annu Rev Phytopathol.* 2005; 43: 83–116. [PubMed Abstract](#) | [Publisher Full Text](#)
- Dandekar AM, Gouran H, Ibanez AM, *et al.*: An engineered innate immune defense protects grapevines from Pierce disease. *Proc Natl Acad Sci U S A.* 2012; 109(10): 3721–3725. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Moore AJ, Beazley WD, Bibby MC, *et al.*: Antimicrobial activity of cecropins. *J Antimicrob Chemother.* 1996; 37(6): 1077–1089. [PubMed Abstract](#) | [Publisher Full Text](#)
- Sharma A, Sharma R, Imamura M, *et al.*: Transgenic expression of cecropin B, an antibacterial peptide from *Bombyx mori*, confers enhanced resistance to bacterial leaf blight in rice. *FEBS Lett.* 2000; 484(1): 7–11. [PubMed Abstract](#) | [Publisher Full Text](#)
- Shelton AM, Zhao JZ, Roush RT: Economic, ecological, food safety, and social consequences of the deployment of Bt transgenic plants. *Annu Rev Entomol.* 2002; 47: 845–881. [PubMed Abstract](#) | [Publisher Full Text](#)
- Paulus JK, Schlieper D, Groth G: Greater efficiency of photosynthetic carbon fixation due to single amino-acid substitution. *Nat Commun.* 2013; 4: 1518. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Ohnuma T, Numata T, Osawa T, *et al.*: Crystal structure and mode of action of a class V chitinase from *Nicotiana tabacum*. *Plant Mol Biol.* 2011; 75(3): 291–304. [PubMed Abstract](#) | [Publisher Full Text](#)
- Joosten RP, te Beek TA, Krieger E, *et al.*: A series of PDB related databases for everyday needs. *Nucleic Acids Res.* 2011; 39(Database issue): D411–419. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

36. Larkin MA, Blackshields G, Brown NP, *et al.*: **Clustal W and Clustal X version 2.0.** *Bioinformatics*. 2007; **23**(21): 2947–2948.
[PubMed Abstract](#) | [Publisher Full Text](#)
37. Gouy M, Guindon S, Gascuel O: **SeaView version 4: A multiplatform graphical user interface for sequence alignment and phylogenetic tree building.** *Mol Biol Evol*. 2010; **27**(2): 221–224.
[PubMed Abstract](#) | [Publisher Full Text](#)
38. Konagurthu AS, Whisstock JC, Stuckey PJ, *et al.*: **MUSTANG: a multiple structural alignment algorithm.** *Proteins*. 2006; **64**(3): 559–574.
[PubMed Abstract](#) | [Publisher Full Text](#)
39. Ionescu M, Zaini PA, Baccari C, *et al.*: ***Xylella fastidiosa* outer membrane vesicles modulate plant colonization by blocking attachment to surfaces.** *Proc Natl Acad Sci U S A*. 2014; **111**(37): E3910–E3918.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
40. Lindow S, Olson W, Buchner R: **Colonization of Dormant Walnut Buds by *Xanthomonas arboricola* pv. *juglandis* Is Predictive of Subsequent Disease.** *Phytopathology*. 2014; **104**(11): 1163–1174.
[PubMed Abstract](#) | [Publisher Full Text](#)
41. Fagen JR, Leonard MT, Coyle JF, *et al.*: ***Liberibacter crescens* gen. nov., sp. nov., the first cultured member of the genus *Liberibacter*.** *Int J Syst Evol Microbiol*. 2014; **64**(Pt 7): 2461–6.
[PubMed Abstract](#) | [Publisher Full Text](#)
42. Jiang Z, Vasil AI, Hale JD, *et al.*: **Effects of net charge and the number of positively charged residues on the biological activity of amphipathic alpha-helical cationic antimicrobial peptides.** *Biopolymers*. 2008; **90**(3): 369–383.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
43. Wang G, Hanke ML, Mishra B, *et al.*: **Transformation of human cathelicidin LL-37 into selective, stable, and potent antimicrobial compounds.** *ACS Chem Biol*. 2014; **9**(9): 1997–2002.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
44. K'oksal M, Zimmer I, Schnitzler JP, *et al.*: **Structure of isoprene synthase illuminates the chemical mechanism of teragram atmospheric carbon emission.** *J Mol Biol*. 2010; **402**(2): 363–373.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
45. Shai Y: **Mechanism of the binding, insertion and destabilization of phospholipid bilayer membranes by alpha-helical antimicrobial and cell non-selective membrane-lytic peptides.** *Biochim Biophys Acta*. 1999; **1462**(1–2): 55–70.
[PubMed Abstract](#) | [Publisher Full Text](#)
46. Koebnik R, Locher KP, Van Gelder P: **Structure and function of bacterial outer membrane proteins: barrels in a nutshell.** *Mol Microbiol*. 2000; **37**(2): 239–253.
[PubMed Abstract](#) | [Publisher Full Text](#)
47. Hancock RE, Sahl HG: **Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies.** *Nat Biotechnol*. 2006; **24**(12): 1551–1557.
[PubMed Abstract](#) | [Publisher Full Text](#)
48. Gray D, Li Z, Hopkins D, *et al.*: **Transgenic grapevines resistant to Pierce's disease.** *HortScience*. 2005; **40**(4): 1104–1105.
[Reference Source](#)
49. Holm L, Kaariainen S, Rosenstrom P, *et al.*: **Searching protein structure databases with DaliLite v.3.** *Bioinformatics*. 2008; **24**(23): 2780–2781.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
50. Freer V, Ho B, Ding JL: **De novo design of potent antimicrobial peptides.** *Antimicrob Agents Chemother*. 2004; **48**(9): 3349–3357.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
51. Fjell CD, Hiss JA, Hancock RE, *et al.*: **Designing antimicrobial peptides: form follows function.** *Nat Rev Drug Discov*. 2011; **11**(1): 37–51.
[PubMed Abstract](#) | [Publisher Full Text](#)
52. Wang G: **Database-Guided Discovery of Potent Peptides to Combat HIV-1 or Superbugs.** *Pharmaceuticals (Basel)*. 2013; **6**(6): 728–758.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
53. Mader JS, Hoskin DW: **Cationic antimicrobial peptides as novel cytotoxic agents for cancer treatment.** *Expert Opin Investig Drugs*. 2006; **15**(8): 933–46.
[PubMed Abstract](#) | [Publisher Full Text](#)
54. Douglas S, Hoskin DW, Hilchie AL: **Assessment of antimicrobial (host defense) peptides as anti-cancer agents.** *Methods Mol Biol*. 2014; **1088**: 159–170.
[PubMed Abstract](#) | [Publisher Full Text](#)
55. He J, Krauson AJ, Wimley WC: **Toward the de novo design of antimicrobial peptides: Lack of correlation between peptide permeabilization of lipid vesicles and antimicrobial, cytolytic, or cytotoxic activity in living cells.** *Biopolymers*. 2014; **102**(1): 1–6.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
56. Piana S, Klepeis JL, Shaw DE: **Assessing the accuracy of physical models used in protein-folding simulations: quantitative evidence from long molecular dynamics simulations.** *Curr Opin Struct Biol*. 2014; **24**: 98–105.
[PubMed Abstract](#) | [Publisher Full Text](#)
57. Mishra B, Epand RF, Epand RM, *et al.*: **Structural location determines functional roles of the basic amino acids of KR-12, the smallest antimicrobial peptide from human cathelicidin LL-37.** *RSC Adv*. 2013; **3**(42): 19560–19571.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
58. Huang YB, He LY, Jiang HY, *et al.*: **Role of helicity on the anticancer mechanism of action of cationic-helical peptides.** *Int J Mol Sci*. 2012; **13**(6): 6849–6862.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
59. Chapman RN, Dimartino G, Arora PS: **A highly stable short alpha-helix constrained by a main-chain hydrogen-bond surrogate.** *J Am Chem Soc*. 2004; **126**(39): 12252–12253.
[PubMed Abstract](#) | [Publisher Full Text](#)
60. Bird GH, Madani N, Perry AF, *et al.*: **Hydrocarbon double-stapling remedies the proteolytic instability of a lengthy peptide therapeutic.** *Proc Natl Acad Sci U S A*. 2010; **107**(32): 14093–14098.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
61. Bird GH, Boyapalle S, Wong T, *et al.*: **Mucosal delivery of a double-stapled RSV peptide prevents nasopulmonary infection.** *J Clin Invest*. 2014; **124**(5): 2113–2124.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
62. Harrison RS, Shepherd NE, Hoang HN, *et al.*: **Downsizing human, bacterial, and viral proteins to short water-stable alpha helices that maintain biological potency.** *Proc Natl Acad Sci U S A*. 2010; **107**(26): 11686–11691.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
63. Oyston PC, Fox MA, Richards SJ, *et al.*: **Novel peptide therapeutics for treatment of infections.** *J Med Microbiol*. 2009; **58**(Pt 8): 977–987.
[PubMed Abstract](#) | [Publisher Full Text](#)
64. Peschel A, Sahl HG: **The co-evolution of host cationic antimicrobial peptides and microbial resistance.** *Nat Rev Microbiol*. 2006; **4**(7): 529–536.
[PubMed Abstract](#) | [Publisher Full Text](#)
65. Piot P: **The F1000Research: Ebola article collection [v1; ref status: not peer reviewed, <http://f1000r.es/4otj>].** *F1000Res*. 2014; **3**: 269.
[Publisher Full Text](#)
66. Piot P: **Ebola's perfect storm.** *Science*. 2014; **345**(6202): 1221.
[PubMed Abstract](#) | [Publisher Full Text](#)
67. Shoemaker CJ, Schornberg KL, Delos SE, *et al.*: **Multiple cationic amphiphiles induce a Niemann-Pick C phenotype and inhibit Ebola virus entry and infection.** *PLoS One*. 2013; **8**(2): e56265.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
68. Montrose K, Yang Y, Sun X, *et al.*: **Xentry, a new class of cell-penetrating peptide uniquely equipped for delivery of drugs.** *Sci Rep*. 2013; **3**: 1661.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
69. Chakraborty S, Phu M, Rao BJ, *et al.*: **Dataset 1 in: The PDB database is a rich source of α -helical anti-microbial peptides to combat disease causing pathogens.** *F1000Research*. 2014.
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Jean-Marc Berjeaud

Laboratory of Ecological and Biological Interactions, University of Poitiers, Poitiers, France

Title and Abstract:

The Title of the article, "The PDB database is a rich source of alpha-helical anti-microbial peptides to combat disease causing pathogens", is appropriate for the content of the article. However because the detected peptides were solely tested toward plant pathogens the term of "plant" in the title could be considered. The abstract represent rather well the work presented in the article except the two last sentences which are expectations of the authors but were not studied in this work. Particularly the sentence : "The use of native..." assert that peptide structure extracted from native proteins will be without adverse effect to the host but it has to be proved in my opinion.

Article content:

The paper describes the use of a software and an *in silico* method developed by the authors to screen the protein database (PDB) to find new antimicrobial peptides on the basis of the secondary structures of these peptides. The method is innovative and very interesting. Moreover the authors proved the efficacy of their method as they synthesized peptides from portions of protein sequences presenting secondary structures resembling cecropin B and demonstrated the antimicrobial activity of these new peptides.

However I did not understand why they used an anionic peptide as the negative control. Indeed it is well known that the global positive charge of the peptides is required for their initial stacking on the membrane of target cells. Thus it is predictable that any anionic peptide will be inactive.

Conclusions:

The main problem concerns the conclusions of the article. Indeed there are not sufficient experimental evidences in the article to assert that the alpha-helical peptides with antimicrobial activity have a strong potency to act toward cancer cells. In my experience I tested several alpha-helical peptides which were solely antimicrobial. Thus I strongly suggest to moderate this conclusions part of the manuscript.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 04 Jun 2015

Sandeep Chakraborty, Tata Institute of Fundamental Research, India

Dear Dr Berjeaud,
We would like to thank you for taking the time to review this paper. Please find our responses below.

Title and Abstract: The Title of the article, "The PDB database is a rich source of alpha-helical anti-microbial peptides to combat disease causing pathogens", is appropriate for the content of the article. However because the detected peptides were solely tested toward plant pathogens the term of "plant" in the title could be considered.

We are in the process of testing these peptides on other pathogens. Furthermore, considering that the mechanism of the inhibitory effect of these peptides is independent of the pathogen host, we are quite confident of replicating our results on other 'non-plant' pathogens.

The abstract represent rather well the work presented in the article except the two last sentences which are expectations of the authors but were not studied in this work. Particularly the sentence : "The use of native" assert that peptide structure extracted from native proteins will be without adverse effect to the host but it has to be proved in my opinion.

We believe this is an important hypothesis, although unproven, which differentiates SCALPEL from other methods of identifying anti-microbial peptides. However, we have modified the statement in the abstract to make this less of an assertion, and more of a hypothesis.

Article content: The paper describes the use of a software and an in silico method developed by the authors to screen the protein database (PDB) to find new antimicrobial peptides on the basis of the secondary structures of these peptides. The method is innovative and very interesting. Moreover the authors proved the efficacy of their method as they synthesized peptides from portions of protein sequences presenting secondary structures resembling cecropin B and demonstrated the antimicrobial activity of these new peptides.

We appreciate the positive comments.

However I did not understand why they used an anionic peptide as the negative control. Indeed it is well known that the global positive charge of the peptides is required for their initial stacking on the membrane of target cells. Thus it is predictable that any anionic peptide will be inactive.

We have used this as a negative control. If our experimental setup in the process of adding the peptides had any undesired conditions which was inhibiting the pathogens, this anionic peptide would show positive results. So, this is slightly different from a null negative control, as it involves adding a peptide.

Conclusions: The main problem concerns the conclusions of the article. Indeed there are not sufficient experimental evidences in the article to assert that the alpha-helical peptides with antimicrobial activity have a strong potency to act toward cancer cells. In my experience I tested several alpha-helical peptides which were solely antimicrobial. Thus I strongly suggest to moderate this conclusions part of the manuscript.

We appreciate your concern regarding the lack of confirmatory evidence of AH peptides as anti-cancer therapeutics. However, this continues to be an active front in research, and we hope that SCALPEL will provide further avenues for testing this hypothesis. We have cited two recent papers- <http://www.ncbi.nlm.nih.gov/pubmed/25270878> and <http://www.ncbi.nlm.nih.gov/pubmed/24101917> in this context. Also, we have modified the text to reflect the lack of conclusiveness in such studies. Once again, we are thankful for your insightful comments, and hope to have addressed your concerns.

Thanking you,
Sincerely,
Sandeep Chakraborty
Plant Sciences Department,
University of California, Davis, CA 95616, USA.

Competing Interests: No competing interests were disclosed.

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