Published in final edited form as:

Chembiochem. 2014 October 13; 15(15): 2225–2231. doi:10.1002/cbic.201402231.

Piloting the Membranolytic Activities of Peptides with a Self-organizing Map

Yen-Chu Lin^a, Dr. Jan A. Hiss^a, Dr. Petra Schneider^a, Peter Thelesklaf^b, Yi Fan Lim^a, Max Pillong^a, Dr. Fabian M. Koehler^a, Prof. Dr. Petra S. Dittrich^a, Prof. Dr. Cornelia Halin^a, Prof. Dr. Silja Wessler^b, and Prof. Dr. Gisbert Schneider^{*,a}

^aSwiss Federal Institute of Technology (ETH), Department of Chemistry and Applied Biosciences, Vladimir-Prelog-Weg 4, 8093 Zürich (Switzerland) ^bParis-Lodron University, Department of Molecular Biology, Division of Microbiology, Billroth Strasse 11, 5020 Salzburg (Austria)

Abstract

Antimicrobial peptides (AMPs) show remarkable selectivity toward lipid membranes and possess promising antibiotic potential. Their modes of action are diverse and not fully understood, and innovative peptide design strategies are needed to generate AMPs with improved properties. We present a de novo peptide design approach that resulted in new AMPs possessing low-nanomolar membranolytic activities. Thermal analysis revealed an entropy-driven mechanism of action. The study demonstrates sustained potential of advanced computational methods for designing peptides with the desired activity.

Keywords

drug discovery; machine learning; membrane interaction; peptide design; vesicles

Peptides can interact with lipid membranes, leading to adsorption or integration of the peptide into the lipid bilayer, and even membrane disruption.[1] Membranolytic peptides, in particular synthetic antimicrobial peptides (AMPs), might be of particular use in a combined antimicrobial strategy together with non-peptide antibiotics.[2–4] There is emerging evidence for lipid membrane components and microdomains playing a seminal role in the epidemiology of human diseases, including cancer cell growth and metastasis, immunomodulation, and infection by pathogenic microorganisms.[5] In this context a cell's plasma membrane represents an ideal drug target for innovative bioactive agents that destroy life-threatening cellular pathogens. Still, steering the target selectivity of membraneinteracting peptides remains a key question for the rational design of amino acid sequences with improved properties.

Computational methods help in generating (quantitative) sequence- and structure-activity relationship [(Q)SAR] models for automated peptide design.[6–9] Empirical modeling approaches, statistical QSAR methods in particular, have been employed to obtain new

AMPs and other membrane-interacting peptides. Jensen et al., for example, recently presented the application of principal component analysis (PCA)/partial least squares projection to latent structures (PLS) to finding peptides with activity toward antibiotic-resistant *Pseudomonas aeruginosa*, [10,11] whereas Maccari et al. used random forest models. [12]

Two decades ago, we introduced the concept of artificial neural networks for QSAR modeling in combination with evolutionary algorithms for ab initio sequence generation and optimization. [13, 14] This approach has found broad application ever since. [15–18] Recently Fjell et al. employed conventional multilayer feed-forward networks for AMP design. [2, 19–20] In this context one may distinguish between supervised and unsupervised neural network learning schemes. [21] Whereas supervised methods generate predictive models by minimizing an error function that requires peptide activities for regression or class labels for classification, unsupervised methods are based on the basic principle of chemical similarity without the explicit need for activity data. These strictly similarity-based computational techniques group peptides according to a similarity metric. The obtained clusters may be analyzed for common features or the identification of peptides for activity testing. A particular advantage of unsupervised over supervised learning is its applicability to small sets of reference data.

Here we present a straightforward unsupervised machinelearning approach for generating peptides with desired membrane activities in a strictly sequence-oriented fashion. Our computational method requires at least one template sequence as a reference. We investigated the membranolytic effects and membrane selectivities of two such templates: protonectin (ILGTILGLLKGL_{NH₂}) [22, 23] and decoralin (SLLSLIRKLIT_{NH₂}). [24] two cationic AMPs from wasp venom. By computational design and residue permutation we generated variations of the template sequences and analyzed their effects with the aid of model lipid vesicles as membrane surrogates, surface plasmon resonance (SPR), isothermal titration calorimetry (ITC), circular dichroism (CD) spectroscopic measurements, and bacterial growth assays. Using a computational model of amino acid sequence space we were readily able to identify peptides with significantly improved membranolytic and membrane-selective activities, as well as remarkable antibacterial effects. Our results reveal critical sequence features for membranolytic activity and selectivity, and motivate unsupervised machine-learning techniques for efficient peptide design.

We first investigated protonectin and decoralin and derivative sequences for their ability to induce membrane leakage or rupturing. Large unilamellar vesicles (LUVs) served as a surrogate for bacterial and eukaryotic cell membranes. They allowed us to study the direct membrane preferences of the peptides without disturbance by other membrane-bound macromolecules. We prepared two sizes of LUVs (target diameters of 0.1 or 1 μm) with lipid compositions of 1) 70% POPE and 30% POPG (this lipid mixture is referred to from now on as “PEPG”), or 2) 100 % POPC. The effective size distributions of the LUVs were determined by dynamic light scattering and showed 143 ± 2 nm for the 0.1 μm preparations (polydispersity = 0.07 ± 0.01) and 278 ± 5 nm (polydispersity = 0.20 ± 0.01) for the 1 μm preparations (values given as means $\pm$ standard errors). There was no statistically significant difference in size distribution between different batches of lipid vesicles and lipid

compositions. We tested three different concentrations of each peptide (1, 10, 100 μM). Because many peptides exhibited membranolytic activity at a concentration of 100 μM (Table 1) we decided only to consider activities at concentrations $\leq 10 \mu\text{M}$. Overall, protonectin and decoralin showed stronger membranolytic activity on POPC vesicles than on PEPG vesicles, and both peptides exhibited greater activity when amidated at their C termini than their counterparts with free C termini (Table 1); this is in accordance with previous studies on cationic helical AMPs.[25] Deletion of either C-terminal or N-terminal amino acids abolished or strongly reduced the membranolytic activities of the two natural AMPs. In the case of decoralin, deletion of the N terminus had a much stronger effect on the activity on POPC vesicles than deletion of the C terminus. We also observed modified membranolytic activities when we conservatively replaced C-terminal residues (Table 1; peptides P3, P4, D3, D4). The C-terminal sections of several AMPs—from the marine AMP pardaxin, for example—have actually been shown to be particularly critical for membrane preference and insertion.[26] Some of these C-terminal regions adopt α -helical conformations only in the presence of negatively charged lipids.[27, 28] Apparently, subtle differences of hydrophobicity and the positioning of charged residues critically affect membrane insertion.[29]

To investigate the influence of potentially conserved residue patterns, we synthesized and tested 20 randomly permuted derivatives of the protonectin and decoralin templates: 45% (9 out of 20) of the permuted sequences exhibited sustained activity in the vesicle rupture assay but showed different membrane selectivity from the appropriate mother peptide. We also synthesized 15 peptides with balanced amino acid frequency (5% chance for each residue); none of these possessed membranolytic activity. It is noteworthy that the majority of the permuted peptides exhibited deviating activity against Gram-positive and Gram-negative pathogens relative to the templates (Table 1) and presented diverse hemolytic potential. These results indicate that the mechanisms leading to actual antimicrobial or hemolytic activity are more complex than lipid vesicle rupture; this is in line with a recent study by Wimley and coworkers.[30] Importantly, our analysis suggests that membrane interaction may be anticipated for a peptide with an appropriate overall amino acid composition, and this confirms earlier reports.[31, 32] Residue permutation of naturally occurring membrane-interacting peptides may thus be considered a viable strategy for probing the relevance of residue patterns and structural integrity for general membrane activity. Keep in mind that the differences in membrane lipid compositions, between, for example, Gram-positive and Gram-negative bacteria and eukaryotic cell lines,[33] will most likely not be captured by this simplifying concept.

Using CD spectroscopy we estimated the secondary structure contents of the peptides in water and in 50% trifluoroethanol (TFE). We observed a relationship between the TFE-inducible helix content (coil-to-helix transition) and the peptides' activities in vesicle and bacterial growth assays (Table 1). For protonectin and its derivatives with activity against *Escherichia coli* and *Staphylococcus aureus* the average inducible helix content was threefold for active peptides and 2.4-fold for inactive ones. This trend is also prominent for decoralin and derivatives, (4.6-fold vs. 3.5-fold). With only 0.9-fold inducible helix content, sequence PS5 lacked direct membranolytic activity, as might be expected, but still showed antibacterial activity (Table 1); this might be attributed to a different mechanism or cellular

target. From the results of our CD measurements we conclude that membrane-rupturing activity of a peptide seems to be more likely with a higher inducible helix content.[34] This finding is in agreement with previous studies,[35] in particular with work by Bechinger and co-workers, who used solid-state NMR spectroscopy to observe helix formation and orientation of some AMPs upon contact with lipid bilayers.[36] Other biophysical studies confirmed differential interaction of amphipathic AMPs with zwitterionic versus anionic lipids.[37] Using deep-UV Raman spectroscopy Cooley and co-workers provided the first evidence of lipid-dependent secondary structure (helix) formation and aggregation of ME1 peptide in the presence of micelles.[38] Recently, Torrent et al. observed interdependencies between aggregation-promoting, amyloid-like properties and antimicrobial activity, with positively charged residues playing a function-determining feature of AMPs.[39] Pursuing this hypothesis, we tested the influence of computed peptide amphiphilicity, expressed as the hydrophobic moment (μ_H) of an energy-minimized helical structure, with measured inducible helix content (Table 1). Notably, we did not observe strong correlation ($r^2 = 0.13$). This finding puts into perspective the usefulness of the total hydrophobic moment as a decisive molecular descriptor for modeling quantitative structure–activity relationships for AMPs,[40] and would warrant future descriptor development.

Motivated by the high number of membranolytic peptides that were successfully obtained from permuted protonectin and decoralin sequences, we computationally generated 10000 11-mer peptides with a residue frequency that mimics the amino acid compositions of the template AMPs (Figure 1). The overall residue bias was the only design constraint; we did not apply additional design patterns (e.g., positional preferences) during ab initio sequence generation. For further computational analysis, we represented all 11-mers from our study (sequences presented in Table 1) and the virtually generated sequences as real-valued vectors by a cross-correlation pharmacophore feature approach (PepCATS descriptor) that we had previously successfully employed for classifying membrane-targeting peptides[41] and designing MHC-I binding peptides.[17] We chose this molecular representation because it exhaustively captures residue neighborhoods, which was shown to be important for the activities of some AMPs with direct membrane lytic activity.[42, 43] Briefly, this descriptor method results in a sequence-length-independent numerical molecular representation of the pharmacophoric feature distribution (hydrogen bonding, lipophilic, aromatic, charged interaction potential) along the amino acid sequence.[44] It captures all pairwise residue correlation patterns along the peptide sequence.

We then employed the unsupervised self-organizing map (SOM)[45] algorithm for clustering the peptides according to these properties, and obtained a two-dimensional map for visual inspection (Figure 1). The peptide density was approximately equally distributed over the map without revealing empty areas devoid of sequence data (Figure 1 A). Each cluster may be regarded as a local feature model of peptide sequence space. We observed a distinct area on the SOM, containing only membrane-active sequences from the training set (Figure 1 B). The pharmacophoric features along these sequences apparently share a common pattern. This observation is a direct consequence of the molecular representation (PepCATS descriptor), which turned out to be suitable for an approximate distinction between active and inactive peptides. The most densely populated “activity island” (cluster

11/8) contained a total of 47 peptides including decoralin and its derivatives D3, D4, and DS3. From this set we selected, synthesized, and tested eight of the computer-generated sequences (Figure 1 C; peptides labeled SOM1–SOM8). Because the sequence generation was guided by the amino acid compositions of the template AMPs, there is an expected prevalence of isoleucine, leucine, and positively charged residues in these peptides. It is noteworthy that the selected designer peptides completely lack negatively charged amino acids, a trait that emerged as a feature during SOM training. We specifically chose the peptide closest to the cluster centroid (SOM1), as well as peptides from the vicinity of the known actives.

We observed medium membranolytic effects for one of the automatically designed peptide sequences (SOM8) and strong ones for two others (SOM5, SOM6), with SOM6 exhibiting low nanomolar activity ($EC_{50} = 59 \pm 1$ nM for PEPG; 7 ± 2 nM for POPC vesicles, Table 2, Figure S1 in the Supporting Information). The designer peptides SOM5 and SOM6 each possess a tenfold inducible helix content, as determined by CD spectroscopy; this correlates with their strong membranolytic activity on vesicles and exceeds the values measured for the natural templates decoralin (6.1-fold) and protonectin (2.8-fold). In bacterial growth assays, SOM5 and SOM6 exhibited strong bacteriostatic effects against *E. coli* and *S. aureus* (Figure S1). Notably, whereas SOM6 showed mild hemolytic activity against mouse red blood cells [minimal hemolytic concentration (MHC) ~ 10 μ M], SOM5 lacked hemolytic activity up to a concentration of 100 μ M (Table 1).

We used SPR to assess the direct interaction between peptides (protonectin, decoralin, DS2, SOM6) and lipid membranes. The sensorgrams reveal that signal intensity (peptide binding) increases as a function of the peptides' concentrations (Figure S2). Evidently, all four tested peptides directly interacted with the membrane as a prerequisite for their activity seen in the vesicle rupture assay. The membrane binding measured for protonectin and decoralin reached similar levels on immobilized PEPG and POPC vesicles, thus implying nonselective activity of these two AMPs toward zwitterionic and negatively charged membranes. The sensorgrams for SOM6 suggest slow "off" rates for this peptide (Figure S2D); these are not observable for protonectin and decoralin (Figure S2 A, B). Decoralin derivative DS2 features intermediate behavior (Figure S2 C). To exclude artifacts caused by improper vesicle immobilization we probed the membrane integrity in the SPR experiments by non-contact AFM to visualize the surface structure of vesiclecoated SPR chips.[46] The resulting image shows that the sensor surface bears intact LUVs with only isolated patches of immobilized planar bilayers (Figure 2). One may thus conclude that both SPR and the vesicle rupture assay probe peptide interaction with intact vesicles. The AFM measurement is a strong argument for allowing direct comparison of results between the two techniques.

We then investigated the thermodynamics of peptide–vesicle interactions by ITC. For this purpose we followed an established experimental procedure with 10 μ M vesicles as injectant and 100 μ M peptide solution in the measuring chamber of the calorimeter.[47] Results of a preliminary study with varying concentrations of decoralin (5, 50, 100 μ M) confirmed this setting to ensure a satisfactory signal-to-noise ratio. For protonectin and decoralin we observed concentration-dependent decreases in heat rates per injection (Figure 3 A, B), in perfect agreement with earlier ITC results obtained for selected cationic helical AMPs.[17]

Their K_D values confirm their preference for POPC vesicles observed in the vesicle assay (Table 3). The ITC traces show that the measured absolute heat rates of cationic helical AMPs tend to be very small ($0.3\text{--}4\text{ }\mu\text{J s}^{-1}$, Figure 3 A, B) or undetectable (Figure 3 C, D). The measured binding enthalpy differences (ΔH) and derived entropy changes ($\Delta T \Delta S$) clearly indicate entropically driven interactions between these peptides and the lipid vesicles. The ΔH values for DS2 and SOM6 are virtually zero (Figure 3 C, D), so it is safe to conclude that these peptides are entropy-driven membrane ligands. For now, it remains unknown whether or not the observed differences reflect different modes of action. One may speculate whether the pronounced membrane activity of SOM6 might be due to its N-terminal twin-lysine motif, obtained by chance during random peptide generation. Futaki and co-workers recently demonstrated that substituting lysine by arginine in the model AMP [KLAKLAK]₂ significantly increases membrane permeation.[48] The mutated sequence [RLARLAR]₂ no longer possessed membranolytic activity but did efficiently translocate across the membrane into the cytoplasm. In fact, the lower “off” rate of SOM6 (Figure S2 D) relative to protonectin and decoralin (Figure S2 A and B) is in agreement with the known effect of arginine/lysine substitutions on the membrane residence times of cell-penetrating peptides.[49] Diminished membrane translocation of lysine-rich AMPs has been attributed to weaker ionic interaction of the ammonium (lysine) than the guanidinium (arginine) side chain with the polar lipid head groups.[50]

Supervised machine-learning requires a large set of training examples to develop a reliable QSAR model. In fact, many AMPs are readily available from public databases,[2, 51, 52] but unless their targets and modes of action are annotated and individual models are generated for each subclass[53] there is a risk of poor generalization capacity of the trained model. In this study we have validated the unsupervised, data-driven SOM technique for de novo peptide design, taking cationic AMPs with direct membranolytic activity as an example. The SOM algorithm works with large and small sets of training data and has already been applied to amino acid sequence analysis.[54–56] The results of our proof-of-concept study demonstrate that eleven active sequences were sufficient for SOM training. The “activity island” found in the SOM model inherently defines the model’s applicability domain, and this turned out to be decisive for prioritizing and selecting peptides with AMP-like properties. In principle, the method also works with a single known active template, because the SOM algorithm identifies local clusters of sequences that share mutual features with the template without modeling an explicit mathematical QSAR function. Picking candidate peptides from these local applicability domains represents a valuable complementary approach to fully fledged supervised model building, specifically in the absence of quantitative training data.[57, 58] This computational approach is broadly applicable to sequence-based peptide design, without requiring large training data sets and running the risk of working with over-trained, non-predictive QSAR models.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We thank Sarah Haller, Anna M. Perna, and Maria Iolyeva for technical assistance. We thank Phil Hill (University of Nottingham, UK) for the GFP-expressing *S. aureus* strain. ETH Zürich and the Swiss National Science Foundation (SNF, grants nos. 205321-134783 and 206021-133768) supported this research study financially.

Abbreviations

PEPG	mixture of 70% POPE + 30% POPG
POPC	1-palmitoyl-2-oleoyl- <i>sn</i> -glycero-3-phosphocholine
POPE	1-palmitoyl-2-oleoyl- <i>sn</i> -glycero-3-phosphoethanolamine
POPG	1-palmitoyl-2-oleoyl- <i>sn</i> -glycero-3-phospho-(1'- <i>rac</i> -glycerol)

References

- [1]. Pasupuleti M, Schmidtchen A, Malmsten M. Crit Rev Biotechnol. 2012; 32:143. [PubMed: 22074402]
- [2]. Fjell CD, Hiss JA, Hancock RE, Schneider G. Nat Rev Drug Discovery. 2011; 11:37. [PubMed: 22173434]
- [3]. Vila-Farrés X, Giralt E, Vila J. Curr Med Chem. 2012; 19:6188. [PubMed: 22978329]
- [4]. Anantharaman A, Rizvi MS, Sahal D. Antimicrob Agents Chemother. 2010; 54:1693. [PubMed: 20176897]
- [5]. Van Meer G, Voelker DR, Feigenson GW. Nat Rev Mol Cell Biol. 2008; 9:112. [PubMed: 18216768]
- [6]. Zhou P, Wang C, Ren Y, Yang C, Tian F. Curr Med Chem. 2013; 20:1985. [PubMed: 23317161]
- [7]. Kliger Y. Biopolymers. 2010; 94:701. [PubMed: 20564036]
- [8]. Hiss JA, Hartenfeller M, Schneider G. Curr Pharm Des. 2010; 16:1656. [PubMed: 20222857]
- [9]. Audie J, Swanson J. Chem Biol Drug Des. 2013; 81:50. [PubMed: 23066895]
- [10]. Jenssen H, Fjell CD, Cherkasov A, Hancock RE. J Pept Sci. 2008; 14:110. [PubMed: 17847019]
- [11]. Jenssen H, Lejon T, Hilpert K, Fjell CD, Cherkasov A, Hancock RE. Chem Biol Drug Des. 2007; 70:1342.
- [12]. Maccari G, Di Luca M, Nifosí R, Cardarelli F, Signore G, Boccardi C, Bifone A. PLoS Comput Biol. 2013; 9:e1003212. [PubMed: 24039565]
- [13]. Schneider G, Schuchhardt J, Wrede P. Comput Appl Biosci. 1994; 10:635. [PubMed: 7704662]
- [14]. Schneider G, Schrödl W, Wallukat G, Müller J, Nissen E, Rösnspeck W, Wrede P, Kunze R. Proc Natl Acad Sci USA. 1998; 95:12179. [PubMed: 9770460]
- [15]. Schneider G, Lin Y-C, Koch CP, Pillong M, Perna AM, Reutlinger M, Hiss JA. Chimia. 2013; 67:859. [PubMed: 24594327]
- [16]. Hiss, JA, Schneider, G. De Novo Molecular Design. Schneider, G, editor Wiley-VCH; Weinheim: 2013. 441
- [17]. Koch CP, Perna AM, Weissmüller S, Bauer S, Pillong M, Baleeiro RB, Reutlinger M, Folkers G, Walden P, Wrede P, Hiss JA, et al. ACS Chem Biol. 2013; 8:1876. [PubMed: 23772559]
- [18]. Hiss JA, Reutlinger M, Koch CP, Perna AM, Schneider P, Rodrigues T, Haller S, Folkers G, Weber L, Baleeiro RB, Walden P, et al. Future Med Chem. 2014; 6:267. [PubMed: 24575965]
- [19]. Fjell CD, Jenssen H, Cheung WA, Hancock RE, Cherkasov A. Chem Biol Drug Des. 2011; 77:48. [PubMed: 20942839]
- [20]. Fjell CD, Jenssen H, Hilpert K, Cheung WA, Panté N, Hancock RE, Cherkasov A. J Med Chem. 2009; 52:2006. [PubMed: 19296598]
- [21]. Duda, RO, Hart, PE, Stork, DG. Pattern Classification. Wiley; New York: 2001.

- [22]. Dohtsu K, Okumura K, Hagiwara K, Palma MS, Nakajima T. *Nat Toxins*. 1993; 1:271. [PubMed: 7513243]
- [23]. Baptista-Saidemberg NB, Saidemberg DM, Palma MS. *J Proteomics*. 2011; 74:2123. [PubMed: 21693203]
- [24]. Konno K, Rangel M, Oliveira JS, Dos Santos Cabrera MP, Fontana R, Hirata Y, Hide I, Nakata Y, Mori K, Kawano M, Fuchino H, et al. *Peptides*. 2007; 28:2320. [PubMed: 17981364]
- [25]. Dennison SR, Phoenix DA. *Biochemistry*. 2011; 50:1514. [PubMed: 21241054]
- [26]. Oren Z, Shai YA. *Eur J Biochem*. 1996; 237:303. [PubMed: 8620888]
- [27]. Hancock RE, Rozek A. *FEMS Microbiol Lett*. 2002; 206:143. [PubMed: 11814654]
- [28]. Papo N, Shai Y. *Peptides*. 2003; 24:1693. [PubMed: 15019200]
- [29]. Glukhov E, Burrows LL, Deber CM. *Biopolymers*. 2008; 89:360. [PubMed: 18186149]
- [30]. He J, Krauson AJ, Wimley WC. *Biopolymers*. 2014; 102:1. [PubMed: 23893525]
- [31]. Hilpert K, Elliott MR, Volkmer-Engert R, Henklein P, Donini O, Zhou Q, Winkler DF, Hancock RE. *Chem Biol*. 2006; 13:1101. [PubMed: 17052614]
- [32]. Schmitt MA, Weisblum B, Gellman SH. *J Am Chem Soc*. 2007; 129:417. [PubMed: 17212422]
- [33]. Epand RM, Epand RF. *J Pept Sci*. 2011; 17:298. [PubMed: 21480436]
- [34]. Wang K, Dang W, Yan J, Chen R, Liu X, Yan W, Zhang B, Xie J, Zhang J, Wang R. *Antimicrob Agents Chemother*. 2013; 57:4632. [PubMed: 23836163]
- [35]. Wieprecht T, Beyermann M, Seelig J. *Biophys Chem*. 2002; 96:191. [PubMed: 12034440]
- [36]. Aisenbrey C, Pendem N, Guichard G, Bechinger B. *Org Biomol Chem*. 2012; 10:1440. [PubMed: 22218372]
- [37]. Alves ID, Goasdoué N, Correia I, Aubry S, Galanth C, Sagan S, Lavielle S, Chassaing G. *Biochim Biophys Acta Gen Subj*. 2008; 1780:948.
- [38]. Halsey CM, Xiong J, Oshokoya OO, Johnson JA, Shinde S, Beatty JT, Ghirlanda G, Ji Ji RD, Cooley JW. *ChemBioChem*. 2011; 12:2125. [PubMed: 21796753]
- [39]. Torrent M, Valle J, Nogués MV, Boix E, Andreu D. *Angew Chem Int*. 2011; 50:10686.
- [40]. Jenssen H. *Expert Opin Drug Discovery*. 2011; 6:171.
- [41]. Schneider, G, Broger, C. *From Symbiosis to Eukaryotism—Endocytobiology*. Wagner, E, Normann, J, Greppin, H, Hackstein, JHP, Herrmann, RG, Kowallik, KV, Schenk, HEA, Seckbach, J, editors Vol. VII. Geneva University Press; Geneva: 1999. 589–602.
- [42]. Koeppe RE II, Hatchett J, Jude AR, Providence LL, Andersen OS, Greathouse DV. *Biochemistry*. 2000; 39:2235. [PubMed: 10694389]
- [43]. Michalek M, Vincent B, Podschun R, Grötzinger J, Bechinger B, Jung S. *Antimicrob Agents Chemother*. 2013; 57:2955. [PubMed: 23587944]
- [44]. Reutlinger M, Koch CP, Reker D, Todoroff N, Schneider P, Rodrigues T, Schneider G. *Mol Inf*. 2013; 32:133.
- [45]. Kohonen T. *Biol Cybern*. 1982; 43:59.
- [46]. Minget-Leclercq MP, Deleu M, Brasseur R, Dufrêne YF. *Nat Protoc*. 2008; 3:1654. [PubMed: 18833202]
- [47]. Abraham T, Lewis RN, Hodges RS, McElhaney RN. *Biochemistry*. 2005; 44:2103. [PubMed: 15697236]
- [48]. Nakase I, Okumura S, Katayama S, Hirose H, Pujals S, Yamaguchi H, Arakawa S, Shimizu S, Futaki S. *Chem Commun*. 2012; 48:11097.
- [49]. Futaki S. *Biopolymers*. 2006; 84:241. [PubMed: 16333858]
- [50]. Su Y, Doherty T, Waring AJ, Ruchala P, Hong M. *Biochemistry*. 2009; 48:4587. [PubMed: 19364134]
- [51]. Wang G, Li X, Wang Z. *Nucleic Acids Res*. 2009; 37:D933. [PubMed: 18957441]
- [52]. Wang G. *Protein Pept Lett*. 2007; 14:57. [PubMed: 17266652]
- [53]. Gogoladze G, Grigolava M, Vishnepolsky B, Chubinidze M, Duroux P, Lefranc MP, Pirtskhalava M. *FEMS Microbiol Lett*. 2014; 357:63. [PubMed: 24888447]
- [54]. Schneider G, Wrede P. *Prog Biophys Mol Biol*. 1998; 70:175. [PubMed: 9830312]

- [55]. Schneider G, Fechner U. *Proteomics*. 2004; 4:1571. [PubMed: 15174127]
- [56]. Yan A. *Comb Chem High Throughput Screening*. 2006; 9:473.
- [57]. Schneider G, Tanrikulu Y, Schneider P. *Future Med Chem*. 2009; 1:213. [PubMed: 21426077]
- [58]. Reker D, Rodrigues T, Schneider P, Schneider G. *Proc Natl Acad Sci USA*. 2014; 111:4067. [PubMed: 24591595]

Figure 1.

Two-dimensional self-organizing map with 20×15 clusters (Voronoi fields). A) Overall peptide density in each cluster (left), and residue frequency used for peptide design (right). B) Location of the eight synthesized membrane-rupturing peptides [SOM1–SOM8, cluster (11/8)], which also contains the most active designer peptide (SOM6) among other active training peptides. White fields represent empty clusters.

Figure 2.

A) AFM topographic image of an HPA SPR sensor chip with immobilized POPC vesicles.

B) Magnified 3D phase image of a selected region of the sensor surface [upper left quadrant

in (A)]. C) Cross section measurement along the white line shown in (A).

Figure 3.

ITC measurements of peptide-vesicle ($\varnothing = 0.1 \mu m$) interactions for A) protonectin, B) decoralin, and the designed peptides C) DS2 and D) SOM6. Vesicles (10 mM) were titrated to 100 μM peptide solution. The first injections were omitted from data analysis (circled data points in A and B).

Table 1

Assay results and properties of protonectin, decoralin, their variations (prefix P, D), permuted sequences (PS, DS), and the de novo designed peptides (SOM). Bacterial growth inhibition was performed with 100 $\mu\text{g mL}^{-1}$ peptide.

ID	Sequence	Vesicle Ø [µm]	Peptide concentration in vesicle (LUV) assay						MHC [µM]	E. coli (GFP)	S. aureus (GFP)	µH	Fold- inducible helix content
			100 µM		10 µM		1 µM						
			PEPG	POPC	PEPG	POPC	PEPG	POPC					
protonectin _{NH₂}	ILGTILGLLKGL _{NH₂}	0.1	+++ [a]	+++ [a]	—	+++ [a]	—	—	10	—	+ [c]	0.24	2.8
		1	+++ [a]	+++ [a]	+ [c]	+++ [a]	—	—					
protonectin _{OH}	ILGTILGLLKGL _{OH}	0.1	+ [c]	+++ [a]	—	—	—	—	inactive	—	—	0.24	2.2
		1	+ [c]	+++ [a]	—	—	—	—					
P1	ILGTILGLLKG-[d]	0.1	—	+++ [a]	—	—	—	—	100	—	—	0.26	3.2
		1	—	+++ [a]	—	—	—	—					
P2	-LGTLGLLKGL[d]	0.1	—	++ [b]	—	—	—	—	100	—	—	0.24	2.8
		1	—	+++ [a]	—	—	—	—					
P3	ILGTILGLLKGI	0.1	+++ [a]	+++ [a]	—	+++ [a]	—	—	100	—	+ [c]	0.24	3.1
		1	+++ [a]	+++ [a]	—	+++ [a]	—	—					
P4	ILGTILGLLKSL	0.1	+++ [a]	+++ [a]	++ [b]	+++ [a]	—	+ [c]	10	—	+ [c]	0.29	3.9
		1	+++ [a]	+++ [a]	+++ [a]	+++ [a]	—	+ [c]					
PS1	LGGILLTLKGI	0.1	+++ [a]	+++ [a]	—	+++ [a]	—	—	100	—	+ [c]	0.29	3.3
		1	+++ [a]	+++ [a]	—	+++ [a]	—	—					
PS2	LGLILGLGTKLI	0.1	+ [c]	+ [c]	—	—	—	—	100	—	—	0.29	2.4
		1	+ [c]	+ [c]	—	—	—	—					
PS3	GKGLTILGLILL	0.1	+++ [a]	+++ [a]	+++ [a]	+ [c]	++ [b]	—	10	+ [c]	+ [c]	0.29	3.3
		1	+++ [a]	+++ [a]	+++ [a]	+ [c]	+ [c]	—					
PS4	ILLGGKLLTGLI	0.1	—	—	—	—	—	—	100	—	—	0.28	1.7
		1	—	—	—	—	—	—					
PS5	TLILGKLIGILL	0.1	—	—	—	—	—	—	inactive	+ [c]	+ [c]	0.23	0.9
		1	—	—	—	—	—	—					
PS6	KITLLGGLLLIG	0.1	+++ [a]	+ [c]	+++ [a]	—	—	—	10	+ [c]	+ [c]	0.30	2.1
		1	+++ [a]	+ [c]	+++ [a]	—	—	—					

ID	Sequence	Vesicle Ø [µm]	Peptide concentration in vesicle (LUV) assay						MHC [µM]	<i>E. coli</i> (GFP)	<i>S. aureus</i> (GFP)	µH	Fold-inducible helix content
			100 µM		10 µM		1 µM						
			PEPG	POPC	PEPG	POPC	PEPG	POPC					
PS7	ITGLLLLGIGLK	0.1	+++ [a]	+ [c]	+++ [a]	—	—	—	10	+ [c]	+ [c]	0.05	3.1
		1	+++ [a]	+ [c]	+++ [a]	-	—	—					
PS8	LILGTLIGGKLL	0.1	—	—	—	—	—	—	100	—	—	0.21	2.1
		1	—	—	—	—	—	—					
PS9	TGIGLILGLLLK	0.1	+++ [a]	+++ [a]	+++ [a]	+++ [a]	++ [b]	—	10	+ [c]	—	0.07	4.1
		1	+++ [a]	+++ [a]	+++ [a]	+++ [a]	+ [c]	—					
PS10	KGILGLLLTGIL	0.1	+++ [a]	+++ [a]	+++ [a]	+++ [a]	—	—	10	+ [c]	+ [c]	0.24	3.4
		1	+++ [a]	+++ [a]	+++ [a]	+++ [a]	—	—					
decoralin _{NH₂}	SLLSLIRKLIT _{NH₂}	0.1	+++ [a]	+++ [a]	+++ [a]	+++ [a]	—	+++ [a]	10	+ [c]	+ [c]	0.45	6.2
		1	+++ [a]	+++ [a]	+++ [a]	+++ [a]	—	+++ [a]					
decoralin _{OH}	SLLSLIRKLIT _{OH}	0.1	+++ [a]	+++ [a]	—	++ [b]	—	—	100	—	+ [c]	0.45	3.6
		1	+++ [a]	+++ [a]	—	++ [b]	—	—					
D1	SLLSLIRKLI ^[d]	0.1	+++ [a]	+++ [a]	+ [c]	+++ [a]	—	++ [b]	10	+ [c]	+ [c]	0.54	4.2
		1	+++ [a]	+++ [a]	+ [c]	+++ [a]	—	++ [b]					
D2	-LLSLIRKLIT ^[d]	0.1	—	+++ [a]	—	+ [c]	—	—	100	—	+ [c]	0.51	4.4
		1	—	+++ [a]	—	+ [c]	—	—					
D3	SLLSLIRKLIS	0.1	+++ [a]	+++ [a]	+ [c]	+++ [a]	—	++ [b]	10	+ [c]	+ [c]	0.45	4.7
		1	+++ [a]	+++ [a]	+	+++ [a]	—	+++ [a]					
D4	SLLSLIRKLY _T	0.1	+++ [a]	+++ [a]	—	+++ [a]	—	+ [c]	100	+ [c]	+ [c]	0.45	4.4
		1	+++ [a]	+++ [a]	—	+++ [a]	—	+ [c]					
DS1	LLLSKIRSLIT	0.1	—	—	—	—	—	—	100	—	—	0.32	3.6
		1	—	—	—	—	—	—					
DS2	SILLRISLTK	0.1	+++ [a]	+ [c]	—	—	—	—	100	+ [c]	+ [c]	0.29	4.8
		1	+++ [a]	+ [c]	+ [c]	—	—	—					
DS3	ILSSLIKRLIT	0.1	++ [b]	+++ [a]	—	+ [c]	—	—	100	—	+ [c]	0.53	5.6
		1	+++ [a]	+++ [a]	—	+ [c]	—	—					
DS4	ILRSTKSLIL	0.1	—	—	—	—	—	—	100	—	—	0.38	1.6

ID	Sequence	Vesicle Ø [µm]	Peptide concentration in vesicle (LUV) assay						MHC [µM]	<i>E. coli</i> (GFP)	<i>S. aureus</i> (GFP)	µ _H	Fold-inducible helix content
			100 µM		10 µM		1 µM						
			PEPG	POPC	PEPG	POPC	PEPG	POPC					
DS5	ISTRKLLSILL	1	—	—	—	—	—	—	10	+ [c]	+ [c]	0.52	4.0
		0.1	+++ [a]	+++ [a]	—	++ [b]	—	—					
		1	+++ [a]	+++ [a]	+ [c]	+++ [a]	—	—					
DS6	TLILRLSSKLI	0.1	—	—	—	—	—	—	inactive	—	—	0.47	3.1
		1	—	—	—	—	—	—					
DS7	LILLSLKSTIR	0.1	—	—	—	—	—	—	inactive	+ [c]	+ [c]	0.28	2.1
		1	—	—	—	—	—	—					
DS8	LLSLILRTIKS	0.1	—	++ [b]	—	—	—	—	100	—	+ [c]	0.51	4.7
		1	—	+++ [a]	—	—	—	—					
		0.1	—	—	—	—	—	—					
DS9	LLIRSTIKSLL	1	—	—	—	—	—	—	inactive	—	—	0.34	2.2
		0.1	—	—	—	—	—	—					
DS10	SLIRKTSILL	0.1	—	—	—	—	—	—	inactive	—	—	0.34	2.2
		1	—	—	—	—	—	—					
SOM8	RPVINLACKLW	0.1	+	+++ [a]	—	+	—	—	100	n.d.	n.d.	0.47	1.0
		1	+	+++ [a]	—	-	—	—					
SOM5	YLLTIILRLKM	0.1	++ [b]	+++ [a]	+++ [a]	+++ [a]	+++ [a]	+++ [a]	100	+ [c]	+ [c]	0.44	10.0
		1	++ [b]	++ [b]	+++ [a]	+++ [a]	+++ [a]	+++ [a]					
SOM6	KKLLCLIINWL	0.1	+++ [a]	+++ [a]	+++ [a]	+++ [a]	+++ [a]	+++ [a]	10	+ [c]	+ [c]	0.40	11.0
		1	+++ [a]	+++ [a]	+++ [a]	+++ [a]	+++ [a]	+++ [a]					

MHC: minimal hemolytic concentration. GFP: green fluorescent protein. n.d.: not determined.

[a] Peptide-induced LUV lytic activity > 75%

[b]_{50–75%}

[c]_{25–50%} relative to triton treatment.

[d] Dash denotes terminal deletion. “+” in the bacterial growth inhibition assays represent peptides that were active at 100 µg mL⁻¹ concentration.

Table 2

Membranolytic activities of the templates and of the computationally designed peptides, measured in a vesicle rupture assay (mean $\pm$ s.d.; $n = 3$). Lipid vesicles $\varnothing = 0.1 \mu\text{m}$.

ID	EC ₅₀ [μM]	
	PEPG	POPC
protonectin _{NH₂}	6.8 $\pm$ 1.3	2.0 $\pm$ 1.3
decoralin _{NH₂}	7.4 $\pm$ 1.4	1.0 $\pm$ 1.5
DS2	17 $\pm$ 1.2	inactive
SOM6	0.059 $\pm$ 0.001	0.007 $\pm$ 0.002

Table 3

Thermodynamic and kinetic values for the interaction of protonectin and decoralin with lipid vesicles ($\varnothing = 0.1 \mu\text{m}$) obtained by isothermal titration calorimetry.

Peptide + lipid	H [kJ mol ⁻¹]	$-T \Delta S$ [kJ mol ⁻¹]	K_A [mol ⁻¹ × 10 ³]	K_D [μM]	n
protonectin + PEPG	19 ± 19	-43 ± 17	27 ± 28	88 ± 70	2 ± 1
protonectin + POPC	4 ± 2	-32 ± 2	100 ± 145	20 ± 24	3 ± 1
decoralin + PEPG	4 ± 3	-28 ± 1	15 ± 10	98 ± 69	3 ± 1
decoralin + POPC	1 ± 0	-32 ± 1	380 ± 123	3 ± 1	4 ± 1

$T \Delta S$ given for 298 K; n is the stoichiometric factor.