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# A Trans-Kingdom Antimicrobial Peptide Targeting Cystic Fibrosis Pathogens

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**Abbreviations:** DOPC: 1,2-dioleoyl-sn-glycero-3-phosphocholine; DOPG: 1,2-dioleoylsn-glycero-3-phospho-(1′-rac-glycerol); DOPE: 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine; DPC: n-dodecyl phosphatidylcholine; LPS: Lipopolysaccharide; LUV: Large Unilamellar Vesicles; SDS: Sodium Dodecyl Sulphate; TFE: 2,2,2-trifluoroethanol

## Introduction

More than 90% of lung infections in cystic fibrosis (CF) patients are caused by *Pseudomonas aeruginosa* [1]; further CF pathogens include clinical isolates of *Burkholderia cepacia*, *Staphylococcus aureus* and *Stenotrophomonas maltophilia*, a newly emerging pathogen [2]. Current therapies are targeted at reducing obstruction, inflammation, or infection, but pathogenic bacteria easily develop resistance to conventional antibiotics [3]. Such molecules affect vital microbial functions through recognition and interaction with specific targets involved in metabolic reactions within cells. The susceptibility of these target molecules to mutations makes it easy for the microbes to become resistant to antibiotics. This strongly encourages the quest of novel antimicrobials especially for the treatment of chronic infections.

### Antimicrobial peptides in the treatment of cystic fibrosis

Antimicrobial peptides are a key component of the innate immune system in multicellular eukaryotes acting as the first line of defense against infectious agents [1,4]. In particular, Cationic Antimicrobial Peptides (CAMPs) are small positively charged peptides with an amphipathic structure, active against Gram-positive and Gram-negative bacteria, fungi, as well as protozoa. Cationicity is an important feature that favors the attraction and binding of CAMPs to the microbial cell surface composed of negatively charged components, such as lipopolysaccharides (LPS) present in the outer membrane of Gram-negative or lipoteichoic acids in the cell wall of Gram-positive ones [5] while the amphipathic nature of the CAMPs fosters their insertion into the membrane [3].

The chemico-physical features of these peptides make them extremely attractive candidates for development as therapeutics for CF. Indeed they are usually multifunctional exhibiting not only fast acting and broad-spectrum bactericidal properties but also anti-inflammatory and immunomodulatory activities [6,7]. Some endogenous cationic antimicrobial peptides are secreted by lung epithelial cells; however their concentration in the lung is quite low and the high-salt environment created on the apical side of CF epithelial cells impair their effectiveness [1] as observed for human cathelicidin AMP LL37, histidine-richpeptide P-113, indolicidins, gramicidins, bactenecins, and magainins, all known to be salt sensitive [8]. Therefore, a promising therapeutic approach would be to exogenously apply cationic antimicrobial peptides with combined antimicrobial and anti-inflammatory activity.

Over the last decades, efforts have been addressed to identify novel antimicrobial peptides from diverse sources [9].

### An innovative approach to identify a cryptic and valuable peptide: the story of VLL-28

Schmidtchen et al. demonstrated that heparin-binding motifs of endogenous mammalian proteins exhibit antimicrobial activity [10]. They suggested that the regular spacing of cationic residues in heparin binding peptides generates amphipathic/cationic structures closely resembling those of typical CAMPs. Starting from their work and based on our expertise on thermophilic proteins/enzymes [11-17], we hypothesized that several anionic-polymers-binding proteins could be used as sources of CAMPs, including nucleic acid binding proteins such as transcriptional factors or proteins involved in stabilization or repair of DNA and RNA. With this purpose we have employed a new *in silico* method for the identification of potential cryptic CAMPs (funded by FFC grant #20/2014, Pane et al. manuscript submitted) based on the correlation between antimicrobial activity and charge/hydrophobicity. By employing this method a potential CAMP has been identified in the primary structure of Stf76 protein [16], a transcription factor encoded by pSSVx, a hybrid plasmid–virus from the archaeon *Sulfolobus islandicus* [17,18]. The selected 28-residue peptide, encompassing Val-37 and Arg-64, was named VLL-28. It has a net charge of +8 at pH 7 and 43% of hydrophobic residues; hence, it closely resembles CAMPs in length, charge and hydrophobic residues content [19]. Stf76 solution structure was recently resolved by Nuclear Magnetic Resonance (NMR) spectroscopy and residues involved in the interaction with DNA were identified [16]. Very interestingly the main group of residues involved in DNA binding is in the region 37-64. The characterization of VLL-28 confirmed that it possesses all the typical features of CAMPs. First of all, VLL-28 turned out to be a broad-spectrum antimicrobial peptide, being active on Gram+ and Gram- bacteria including some clinically relevant strains in CF, such as *P. aeruginosa* and *S. aureus*. Secondly, VLL-28 shows several relevant chemical physical properties. In particular, CD studies demonstrated that VLL-28 is unstructured in solution, whereas it acquires a well-defined secondary structure in the presence of membrane mimetics, like TFE, DPC and SDS. In addition both the leakage and fusogenic assays showed that the peptide interacts and selectively damages the DOPC/DOPG (4/1) or DOPE/DOPG (4/1) LUVs, which mimic bacterial membranes likely inducing the formation of a pore. Finally, we found that VLL-28 retains the ability to bind nucleic acids even if, differently from the parent protein, it displays a preferential binding to ssDNA and RNA and the propensity to form

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unspecific high molecular weight aggregates upon interaction with nucleic acids. In agreement with these findings, in *E. coli* cells VLL-28 was found to be not entirely located in the membrane but also in the cytoplasm though at lower concentration. Altogether, these results suggest that antimicrobial properties of this peptide reside in a multi-layered killing mechanism involving both membrane damages and nucleic acid binding as demonstrated for buforinII [20].

VLL-28 is the first CAMP isolated from the archaeal kingdom being active against bacterial pathogens. Indeed, the updated antimicrobial peptide database (APD, http://aps.unmc.edu/AP/) includes only four AMPs from archaea, all halocins (or microhalicin) from halophilic microorganisms targeting archaeal species. VLL-28 derives from a thermophilic transcription factor encoded by a virus infecting *Sulfolobus* spp. which thrives under harsh conditions. Interestingly, thermophilic proteins are usually more stable to extreme chemico-physical conditions such as pH, temperature, denaturing agents, salt concentration and moreover the protein thermostability is often associated with increased proteolytic resistance [21,22]. Because of its thermophilic nature, it is expected that VLL-28 could represent an optimal scaffold to get variants with improved resistance to proteases thus overcoming one of the major bottlenecks of the employment of CAMPs for CF treatment. VLL-28 primary structure could be modified in terms of length and amino acid composition, to increase its protease stability, without affecting its activity. In particular the insertion of specific chemical modifications such as i) an all-hydrocarbon bridge within the peptide backbone connecting a couple of unnatural α,α−δ substituted amino acids forming a peptide macrocycle [23] or ii) D-aminoacids and/or modified amide bounds, could render VLL-28 less susceptible to protease cleavage [24]. All these studies are currently underway.

In addition to a broad bactericidal activity, VLL-28 is also endowed with significant anti-Candida activity [19], an advantage, since those pathogens can be present in the CF lungs and other antibiotics used in CF therapy often lack useful Gram-positive and fungal coverage [1] (Figure 1).

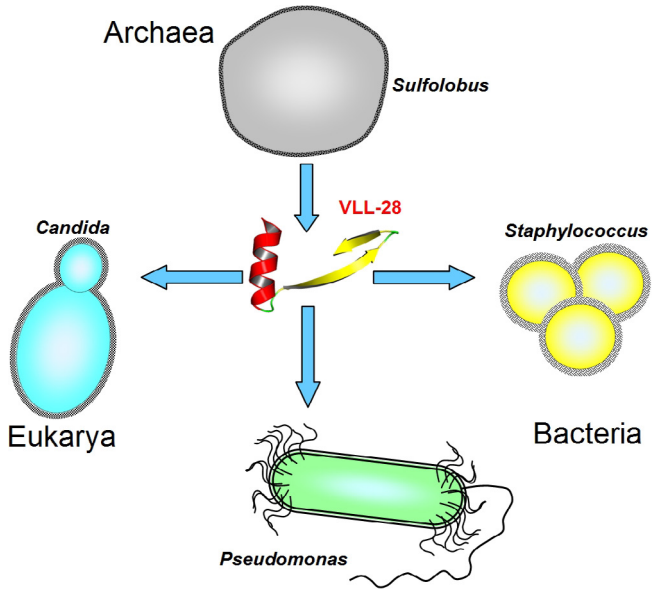


**Figure 1:** Schematic representation of the antimicrobial activity of VLL-28 peptide.

## Conclusion

Being VLL-28 a potent antimicrobial and antifungal agent, it represents a promising "lead compound" for future development of novel drugs to be exploited in therapeutic treatment of CF disease. Since the resistance to proteases is a prerequisite for the treatment of CF, innovative approaches voted to the achievement of such goal well suit to this demanding task. We aim at obtaining a panel of protease-resistant VLL-28 derivative peptides with anti-microbial activity against different relevant clinical strains for CF to be used in alternative to and/or in combination with conventional antibiotics.

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