# Designing antimicrobial peptides as novel anti-infective agents

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## Background.

Due to the growing resistance of pathogens to current antibiotics, there is an **urgent need to develop new agents** with novel mechanisms of action. Antimicrobial peptides (AMPs) seem to be good candidates, even though many times they aren't highly optimized to kill microbes, and their size causes troubles with administration and makes them more expensive to produce. At the moment there are many studies going on to elucidate mechanisms of action and which are the functional structures of AMPs, so it is possible to **improve existing peptides** or **produce new ones** through combinatorial chemistry.

## Antimicrobial peptides: main features.

AMPs are **short cationic amphiphilic** amino acidic chains with **antimicrobial** and/or **immunomodulatory** activities spread throughout almost every living organism.

- ✓ Length: 10 – 50 amino acids
- ✓ Charge: +2 - +9
- ✓ >30% hidrophobic residues

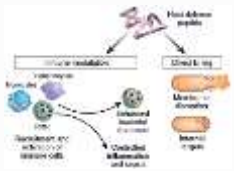

**Figure 1**. Biological role of AMPs.

### Mechanism of action

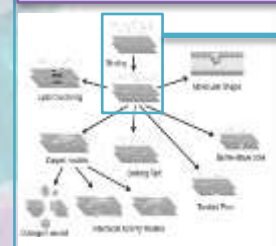

**Figure 2**. Popular models of AMPs mechanism of action.

**Figure 3**. Interaction of AMPs with membranes. Positive charge allows binding to bacterial membranes.

- Induce membrane destabilization
  - **Transmembrane pore models** (barrel-stave and toroidal pore models)
  - **Nonpore models** (carpet model, detergent model, molecular shape models, lipid clustering model, sinking raft model and interfacial activity model)
- Interact with internal targets

### Experimental tools to study the mechanism of action

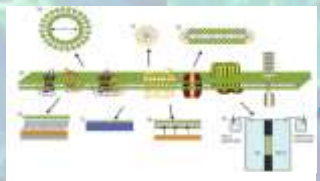

**Figure 4**. Main membrane models. a) cell membrane; b) vesicle; c) micelle; d) bicelle; e) supported lipid monolayer; f) self-assembled lipid monolayer at the air-water interface; g)tethered supported lipid bilayer; h)planar bilayer.

\+ Biophysical techniques

- ✓ **Detection of membrane permeabilization/disruption**
  - Use of spectroscopic probes
- ✓ **Does the peptide operate at the surface or penetrate the membrane?**
  - Tryptophan fluorescence observation, surface area increasing observatoin
- ✓ **What is the conformation of the peptide when bound to the membrane?**
  - Circular dichroism, site-directed spin labeling resonance
- ✓ **Nonspecific leakage *versus* the formation of discrete ion channels or pores**
  - Measurement of single-ion-channel conductance

## Novel improved peptides obtaining.

### Knowledge of main structural features

**Four main structural groups:**
- β-sheet peptides stabilized by S-S
- α-helical peptides
- Structures rich in glycine, proline, tryptophan, arginine or histidine
- Loop peptides with one S-S

**Physicochemical and structural determinants:**
- ✓ Cationicity (+2-+9)
- ✓ Amphippathicity
- ✓ Hidrophobicity (40-60%)
- ✓ Aromatic residues
- ✓ Structural propensity

### Obtaining new peptides

**1. Sequence modification**
Deletion, addition, substitution, truncation.

**2. Minimalist approaches**
*De novo* design based on structural determinants.

**3. Combinatorial libraries**
High throughput approach. Examples: phage display, synthesis on cellulose support.

**4. Template-assisted approach**
Comparison of structural-homologous peptides to find structure-activity relationships. *In silico* aided.

### Peptide evaluation

**A. Bacterial killing activity**
Measure the ability to sterilize a culture of bacteria and/or fungi in rich growth media → **Minimal Inhibitory Concentration**: the lowest concentration in which growth is inhibited.

**B. Selective biological activity**
Measurement of toxicity and litic activity over erythrocytes and mammal cells. → **Therapeutic index**: relationship between lethal dose and effective dose.

### Example: Demegen's P113

Peptide obtained from histatine 5, saliva protein involved in oral cavity's immunologic defense. In phase II of clinical trial.

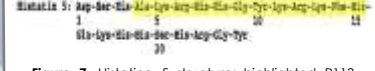

**Figure 7**. Histatine 5 structure; highlighted P113 sequence.

**Finding of the peptide**: observation of the activity of different histatine fragments.

**Administration:**
- ✓ Aerosol

**Uses:**
- ✓ Against candidiasis
- ✓ Against lung infections in cystic fibrosis patients if modified with D-amino acids.

### Carriers

There are many expectations on **synthetic** carriers, but **natural particulates** are also interesting as they are optimized for *in vivo* specific functions.

**A. Pathogen-based strategies**

**Figure 5**. A and B: Bioengineered pathogens for drug delivery. C: virus-mimicking drug carriers.

**B. Cell-based strategies**

**Figure 6**. Bioengineered eukaryotic cells for drug delivery.

### Production

**A. Chemical synthesis**
**Ligation of sequence components**. Allows composition control and overcome genetic limitations, but is expensive. Example: solid-phase synthesis.

**B. Biological synthesis**
**Clone DNA into living organisms so they can produce the peptide**. Production is cheaper, but further purification is needed. Limitation to natural amino acids. Options: bacteria, yeast, animal cells.

## AMPs' future perspectives and challenges.

### Problems to overcome.

- ✖ **Susceptibility to proteolysis**: if administered orally are hydrolyzed by pepsin, trypsin and chymotrypsin; if administered systemically are hydrolyzed by blood proteases or distributed through all the body.
- ✖ **High production cost**: biological synthesis could lower them, but there are many limitations.
- ✖ **Find good carriers**: an interesting field is the one of synthetic carriers that mimic biological carrier's features, but it is important to completely understand the biological mechanisms of the last ones.

### Future goals.

- **Peptides with increased activity**:
  - **Chimeric peptides**: the activity of two peptides in one molecule.
  - **Peptidomimetics** that aren't susceptible to proteolysis (ciclated, PEGligated, non natural amino acids in their sequence…).

## Bibliography.

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