Generarea si investigarea unor noi peptide antimicrobiene, cu dimensiune redusa. Corelarea structurii peptidelor cu functia lor (BIOPEP)/Rational design and generation of synthetic, short antimicrobial peptides. Linking structure to function

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Major motivations for focusing our project on **a**nti**m**icrobial **p**eptides (AMP's)

- Antibiotic resistance is now one of the most pressing global healthcare problems facing society.
- After half a century of almost complete control over microbial infections, the past decade has brought a worldwide resurgence of infectious diseases due to the evolution of antibiotic-resistant strains at an alarming rate
- Although hospital-acquired, or nosocomial, infections in the United States have gradually declined, approximately 70% of them are resistant to at least one antibiotic, and the trend is increasing.
- For example, of the approximately two million people who acquired nosocomial infections in 2009 in U.S. hospitals, ~ 99 000 died from the infection.
- AMPs have provided a new approach to developing antibiotics because they play a central role in the innate immunity system of many organisms. In some primitive organisms that lack a humoral response, AMPs and a few enzymes provide the main defense against bacteria, yeast, fungus, and even viruses.

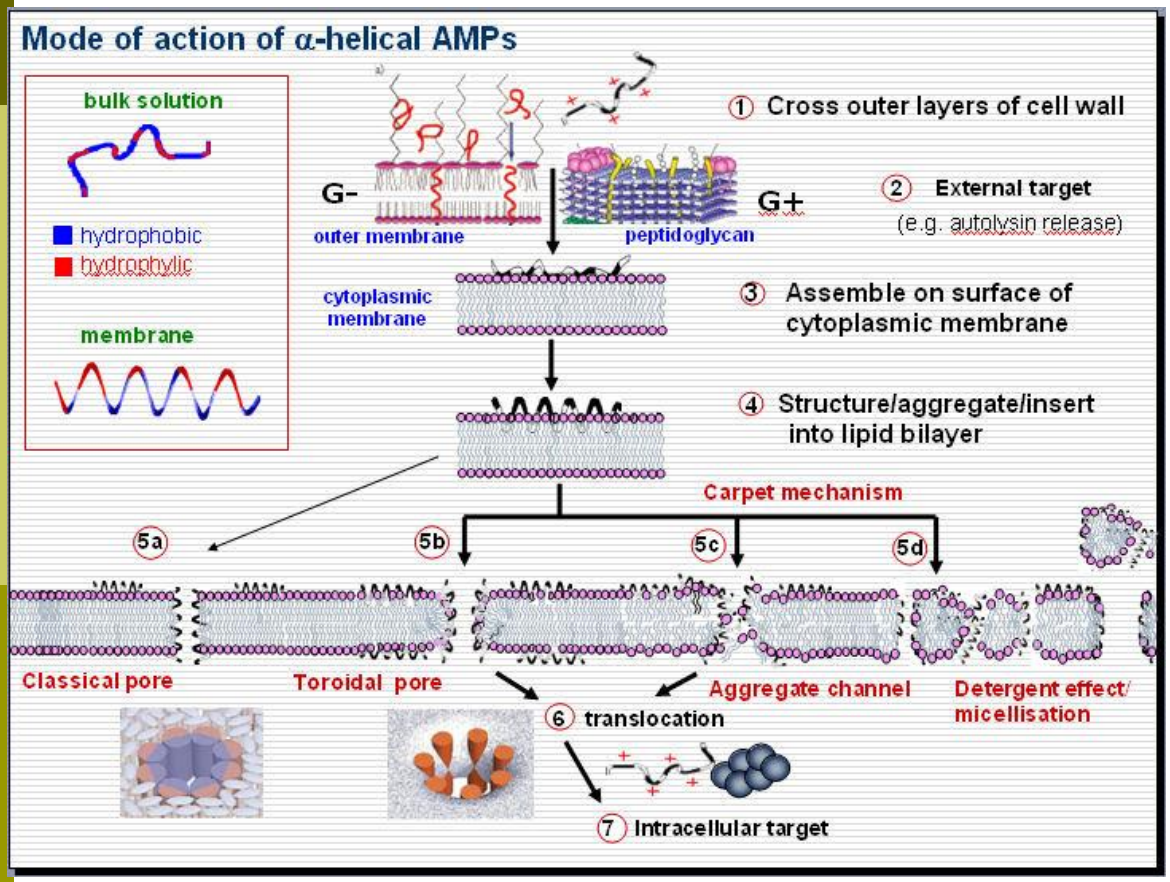
Some introductory info: what are AMP's ?

➢Naturally-occurring AMPs are basic peptides composed of 12 – 50 amino acids that are distributed throughout all kingdoms of life, ribozomally or non-ribozomally synthesized.

➢AMPs make up one class of a growing number of membrane-active peptides that include anticancer and antiviral peptides, cell-penetrating peptides, viral fusion peptides, and venom peptides. **For all these classes of peptides, interactions between the membrane lipid bilayer and the peptide are central to their biological functions.**

➢AMPs display extensive sequence heterogeneity; however they do share a number of common characteristics, including a **net positive charge of ≥ +2** (with +4 to +6 being most common), ~ **50 – 70 % hydrophobic amino acids**, and a propensity to fold into **amphipathic conformations** in the presence of membranes.

Action mechanisms of AMPs on various cells



- The morphology of Gram-negative and –positive bacteria treated with cationic antimicrobial peptides as examined by electron microscopy indicates that the cells are killed due to disruption of the cytoplasmic membrane.

- Membrane defects were observed even at low concentration at which the peptides were not bactericidal. However, no lesions in the bacterial cell surface were discernible.

- The inner membrane of Gram-negative bacteria is composed of anionic lipids like phosphatidyl glycerol (PG) and cardiolipin which would favor the association of cationic peptides.

N. Sitaram, R. Nagaraj, Biochimica et Biophysica Acta 1462 (1999) 29-54

Actual context and limitations of AMP-based investigations

- Clinically tested antimicrobial peptide drugs were derived from natural peptides by what is essentially a trial-and-error approach, and their production cost is prohibitive, as compared to conventional antibiotics.

- Understandably, there is an urgent need to design novel AMPs which are as potent and selective as possible, while being short and structurally simple.

- The major hurdle to achieve this desiderate of rational design AMPs lies in our current reduced by our lack of understanding of the detailed mechanism of short AMP action, which determine the major barriers for converting them into drugs:

  the high cost of production on a large scale
  toxicity to host cells
  susceptibility to proteolytic degradation.

Our goal:

- *We will undertake rational design, synthesis, and testing of a series of short cationic peptides we envision proteolityc and salt resistant, based on an elementary amphipathic templates of up to 11 amino acid residues, searching for the minimum number of aminoacids and optimal architecture able to confer the peptide optimal lytic activity and specificity against various pathogens.*

- *We wish identify optimal tagging architectures of the peptides with hydrophobic aminoacid stretches, as a powerful approach to achieve selective peptide adsorption, as well as salt resistance to bacterial membranes.*

The main questions we address

- *Which is the minimal length for de-novo designed and synthesized peptides, which endow them with lytic activity and specificity against selected pathoges ?*
- *Which are minimum structural requirements for synthetic short peptides, with potential to turn them into potent and less complex, i.e., less expensive, AMPs ?*
- *Which are the needed primary sequence order and topology arrangement of Trp residues for optimal peptide anchoring to the membrane, and how this influences peptide salt resistance, antimicrobial activity, membrane affinity and translocation ?*
- *Which are the best candidates and action mechanisms of de novo synthesized short peptides, for the generation of a new class of antimicrobial with improved lytic properties and pathogenic specificity ?*
- *Do such short, synthetic AMPs translocate across bilayers, and is membrane translocation required for activity?*

BIOPEP - Management structure

Project Coordinator **(CO)** is Dr. Tudor Luchian, head of the 'Laboratory of Molecular Biophysics and Medical Physics', at the 'Alexandru I. Cuza' University in Iasi (UAIC).

Partner's coordinators:

- The National Institute for Research and Development of Isotopic and Molecular Technologies (**P1**), **Dr. Ioan Turcu**

- Horia Hulubei National Institute of Physics and Nuclear Engineering (IFIN-HH) (**P2**), **Dr. Mihai Radu**

- Babes-Bolyai University (**P3**), **Dr. Ion Grosu**

Project's layout

The project itself revolves around three main topics:

Design and synthetis of short AMP peptides (Topic 1)

Revealing basic molecular information of AMP-membrane interaction (Topic 2)

Short AMP activity on natural membrane interfaces (Topic 3)

Means to an end

- We will undertake **synthesis**, followed by **in vitro and in vivo testing** of a series of short cationic peptides, based on an elementary amphipathic templates of **up to 11 amino acid residues**, searching for **the minimum number of aminoacids and optimal architecture**, able to confer the peptide optimal **lytic activity, salt resistance and specificity against various pathogens, in cost-effective way**.

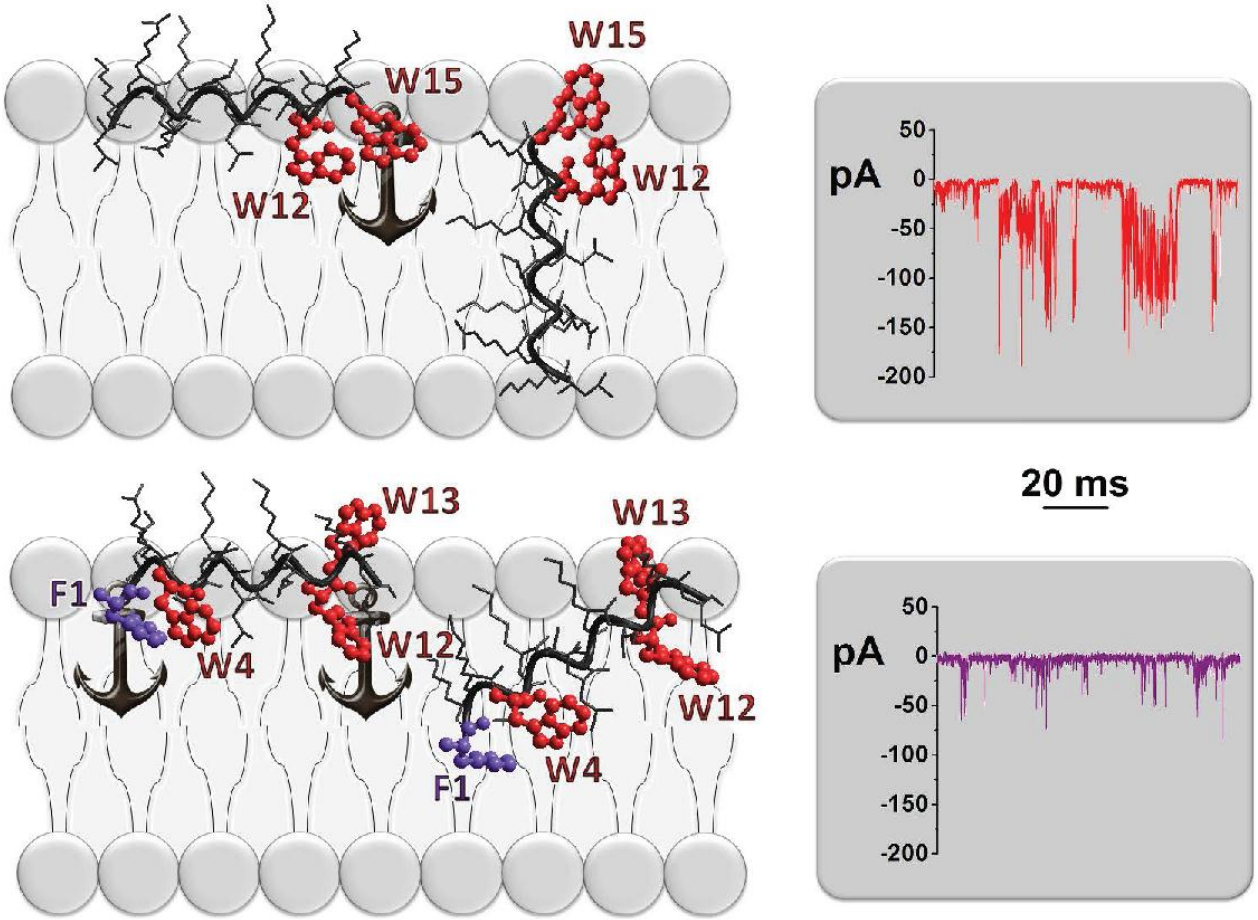
- Apart from their biological activities, other aspects will to be considered when developing these peptides for systemic use, including **in vivo stability, resistance to salt and proteolysis**.

- The **synthetic nature** of these peptides is expected to **alleviate the risk of carrying biological pathogens**, as it happen to natural antibacterial peptides isolated from organisms. The structural simplicity may also offer technological advantages for mass production and purification.

- We expect that based on their **structural simplicity**, such peptides will be **cheaper** and in therefore readily available for further development for systemic use.

Expected impacts following project's implementation

- One of the potential scientific impact of this project, is to provide a better understanding regarding the rational design of such peptides, and shed light into their structure-function relationship.
- Therefore, another feature of this projects lies in its major technological impact, by: (i) creating novel protocols, which will better assist many areas of molecular pharmacology and (ii) large-scale design and implementation of novel protocols regarding antimicrobial peptide design, construction and testing.
- With respect to the economical impact of this project, we are set to identify potential partners form the pharma industries, which would endeavor to put in practice some of our results regarding molecular pharmacology, as to better serve Romanian market from an innovative research experience acquired in related research.
- Besides the scientific goals, training and education will be also key results of the project.
- All in all, we aim at providing practical benefits of the tax payer: (i) structure-activity relationships of such short antimicrobial peptides, salt resistant, will be proposed (ii) several methods for improving their specificity of the peptides will be tested (iii) novel solutions will be advanced in the on-going fight against conventionally-resistant pathogens.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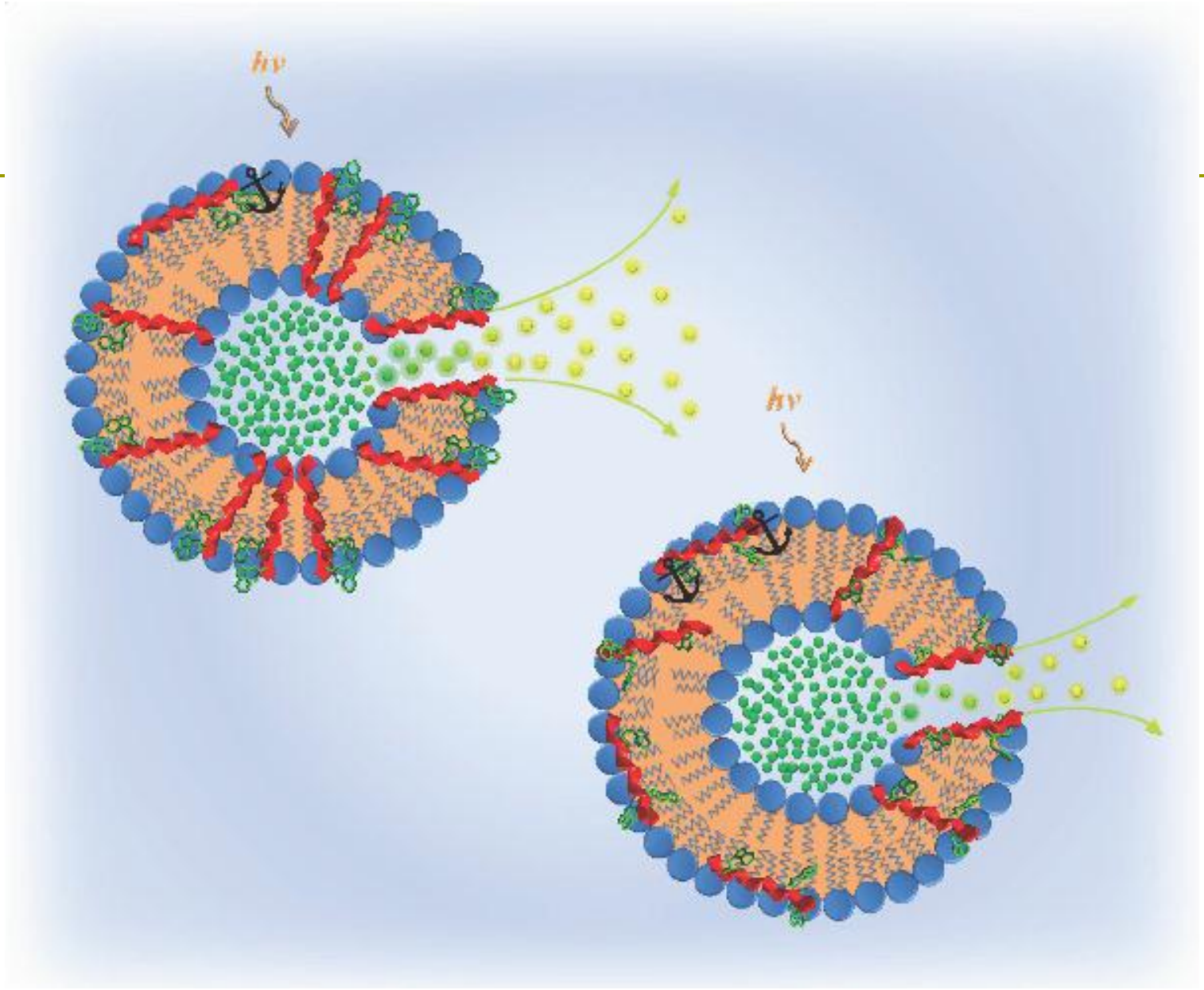
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