

Membrane proteins and their involvement in infectious diseases

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Protein-membrane interactions and protein-protein recognition within the membrane milieu are of fundamental importance to fully comprehend a wide range of cellular processes in all organisms. About 40% of all genes in the mammalian genome transcribe for membrane proteins. The paucity in 3D structures for most membrane proteins, the high complexity of the forces involved, and the technical difficulties present challenging obstacles to overcome before fully understanding biological events within membranes. Using a multidisciplinary approach, including cell biology (cells and viruses), biophysics and chemistry, we studied the mode of action of membrane proteins, particularly those involved in infectious diseases. These studies led us to discover and refine mechanisms of action of membrane proteins involved in microbial and viral infections.

Antimicrobial peptides in Innate Immunity: The Underlying Parameters Involved in Target Recognition by Antimicrobial Peptides

Living organisms of all types including plants and humans have been shown to produce a large repertoire of gene-encoded antimicrobial peptides that serve as part of their innate immunity to microbial invasion. They are considered as future antibiotics due to the increasing resistance of bacteria to available antibiotics. We established the *carpet* (Fig. 1) mechanism as an efficient model describing action of antimicrobial peptides. Based on this mechanism we developed a novel repertoire of diastereomeric antimicrobial peptides with potential therapeutic applications.

Viral infection - How do Viral Envelope Proteins Catalyze Viral-Cell Membrane Fusion?

The mechanism by which specific viral envelope proteins catalyze mixing of two membranes (membrane fusion) is still an open question. We focused on gp41 and F, the envelope glycoproteins from HIV (retrovirus) and Sendai virus (paramyxovirus), respectively. We show that: (i) distant viral families share conserved fusion mechanisms, (ii) membrane interaction induces drastic conformational changes in the fusion proteins. These studies led us to propose the *umbrella* (Fig. 2) mechanism for virus-cell fusion. (iii) Synthetic peptides derived from envelope proteins specifically inhibit viral infection at

different stages, making them ideal tools for mechanism studies, as well as promising therapeutic agents.

General Aspects on Protein-Membrane and Protein-Protein Interactions within the Membrane

We studied self- and hetero-assembly of peptides within the membrane milieu. In contrast to recognition in solution, we found that peptide chirality does not affect protein-protein recognition in the membrane, and therefore biological function is preserved. Furthermore, we developed a new assay that allows for the first time in vivo detection of hetero-association between proteins within the membrane milieu. Besides giving us important basic information, these findings serve as new tools for the design of novel compounds to combat infectious diseases.

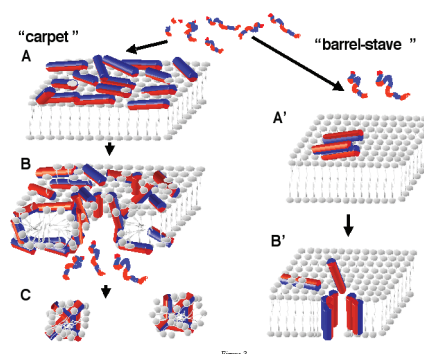


Fig. 1 A cartoon illustrating the barrel-stave (to the right) and the carpet (to the left) models suggested for membrane permeation. In the carpet model the peptides are bound to the surface of the membrane with their hydrophobic surfaces facing the membrane and their hydrophilic surfaces facing the solvent (step A). When a threshold concentration of peptide monomers is reached, the membrane is permeated and transient pores can be formed (step B), a process that can lead also to membrane disintegration (step C). In the barrel-stave model, peptides first assemble on the surface of the membrane (step A'), then insert into the lipid core of the membrane following recruitment of additional monomers (step B') (taken from Shai and Oren, 2001).

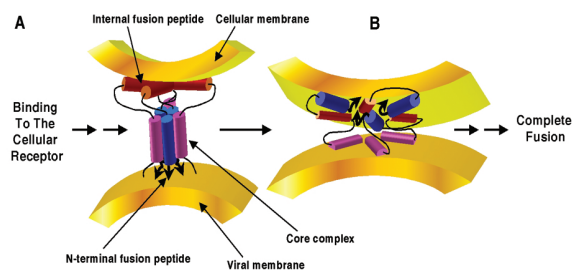


Fig. 2 A model for Paramyxoviridae induced membrane fusion. Binding of the viral surface glycoprotein to the cellular receptor results in the formation of a trimeric core complex (blue and magenta) followed by the insertion of the internal fusion peptides (red) into the target membrane (panel A). Next, the core complex binds to the membranes and deoligomerizes therein (panel B). The N-terminal fusion peptide can then insert into the viral membrane and, together with the internal fusion peptide, facilitate the merging of the membranes (taken from Samuel & Shai, 2001).

Selected Publications

- Ben Efraim, I., Kliger, Y., Hermesh, C. and Shai, Y. (1999) Membrane-induced step in the activation of Sendai virus fusion protein. *J. Mol. Biol.* 285, 609-625.
- Gerber, D. and Shai, Y. (2001) In vivo detection of hetero-association of glycophorin-A and its mutants within the membrane. *J. Biol. Chem.* 276, 31229-31232.
- Ghosh, J.K. and Shai, Y. (1999) Direct Evidence that the N-Terminal Heptad Repeat of Sendai Virus Fusion Protein Participates in Membrane Fusion. *J. Mol. Biol.* 292, 531-546.
- Ghosh, J.K., Peisajovich, S.G. and Shai, Y. (2000) Sendai Virus Internal Fusion Peptide: Structural and Functional Characterization and a Plausible Mode of Viral Entry Inhibition. *Biochemistry* 39, 11581-11592.
- Kliger, Y. and Shai, Y. (2000) Inhibition of HIV-1 Entry Before gp41 Folds Into its Fusion-active Conformation. *J. Mol. Biol.* 295, 163-168.
- Oren, Z. and Shai, Y. (2000) Cyclization of a Cytolytic Amphipathic alpha-Helical Peptide and Its Diastereomer: Effect on Structure, Interaction with Model Membranes, and Biological Function. *Biochemistry* 39, 6103-6114.
- Peisajovich, S.G., Samuel, O. and Shai, Y. (2000) Paramyxovirus F1 Protein Has Two Fusion Peptides: Implications for the Mechanism of Membrane Fusion. *J. Mol. Biol.* 296, 1353-1365.
- Avrahami, D., Oren, Z. and Shai, Y. (2001) Effect of multiple aliphatic amino acids substitutions on the structure, function, and mode of action of diastereomeric membrane active peptides. *Biochemistry* 40, 12591-12603.
- Kliger, Y., Gallo, S.A., Peisajovich, S.G., Munoz-Barroso, I., Avkin, S., Blumenthal, R. and Shai, Y. (2001) Mode of Action of an Antiviral Peptide from HIV-1. Inhibition at a Post-Lipid Mixing Stage. *J. Biol. Chem.* 276, 1391-1397.
- Peisajovich, S.G. and Shai, Y. (2001) SIV Gp41 Binds to Membranes both in the Monomeric and Trimeric States: Consequences for the Neuropathology and Inhibition of HIV Infection. *J. Mol. Biol.* 311, 249-254.
- Pritsker, M., Jones, P., Blumenthal, R. and Shai, Y. (1998) A synthetic all D-amino acid peptide corresponding to the N-terminal sequence of HIV-1 gp41 recognizes the wild-type fusion peptide in the membrane and inhibits HIV-1 envelope glycoprotein-mediated cell fusion. *Proc. Natl. Acad. Sci. USA* 95, 7287-7292.
- Samuel, O. and Shai, Y. (2001) Participation of Two Fusion Peptides in Measles Virus-Induced Membrane Fusion: Emerging Similarity with Other Paramyxoviruses. *Biochemistry* 40, 1340-1349.
- Shai, Y. (2001) Molecular Recognition within the Membrane Milieu: Implications for the Structure and Function of Membrane Proteins [Review]. *J. Membr. Biol.* 182, 91-104.
- Shai, Y. and Oren, Z. (2001) From "carpet" mechanism to de-novo designed diastereomeric cell-selective antimicrobial peptides [Review]. *Peptides* 22, 1629-1641.
- Unger, T., Oren, Z. and Shai, Y. (2001) The effect of cyclization of magainin 2 and melittin analogues on structure, function, and model membrane interactions: implication to their mode of action. *Biochemistry* 40, 6388-6397.

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