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 membrane proteins, which can be classified into three major groups: (i) water-soluble peptides and proteins that undergo substantial conformational changes to allow them to interact with and insert into the membrane (e.g. peptide and protein toxins), (ii) integral membrane proteins that fold into active forms within the membrane (e.g. receptors, ion channels), and (iii) proteins containing both integral parts and water/membrane soluble regions (e.g. envelope proteins of viruses). The paucity in 3D structures for 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, which includes peptide chemistry (peptide synthesis and chemical ligation), molecular (protein expression) and cell biology (cells, bacteria and viruses), biophysics, and in vovo studies with animal models, we studied the three types of membrane proteins, focusing on those involved in infectious diseases. The distinct advantage in studying this variety of biological systems lies in our ability to extract general rules that underline most types of interactions involved between proteins and membranes. These studies led us to discover and refine mechanisms of action of membrane proteins involved in infectious diseases; namely, microbial and viral infections. A summary of our achievements is given below:

**Antimicrobial peptides in Innate Immunity: The Underlying Parameters Involved in Target Recognition by Antimicrobial Peptides** - Living organisms of all types produce a large repertoire of gene-encoded cationic antimicrobial peptides (AMPs) (termed also host-defense peptides) that serve as part of the innate immunity. Since 1991 we have established the "carpet" mechanism as a model describing an efficient membrane permeation process used by many antimicrobial peptides, as compared to the "barrel-stave" mechanism used by peptide ion channels. Most importantly, we disproved accepted dogmas on the role of a specific structure, sequence or chirality in biological function. Furthermore, we showed that peptide assembly in solution and particularly in membranes is a crucial parameter controlling target cell specificity. The success of our model is also reflected by our ability to develop a novel family of cell selective antimicrobial diastereomeric peptides based on predictions not possible by other models. This new family seems to have the highest potential for future therapeutics, urgently needed due to increasing resistance of bacteria to available antibiotics. Indeed, they presented the first example of antimicrobial peptides that were active against bacterial infection when injected intravenously. Furthermore, based on existing evidence that antimicrobial peptides can also kill cancer cells in vitro, we moved forward and de-novo designed a diastereomeric peptide with high selectivity toward cancer cells and determined its mode of action. Most importantly, the peptide was highly potent in animal models of primary and metastatic tumors when injected intratumor or intravenously (the first reported study). In contrast, the same peptide, but composed of all L-amino acids, was highly active only in vitro and could not discriminate between tumor and non-tumor cells.

**Viral infections** - 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 leading to their participation in the actual membrane fusion event. These studies led us to propose the "umbrella" mechanism for virus-cell fusion. (iii) Synthetic peptides derived from regions within viral envelope proteins specifically interact with the parental envelope proteins at different stages of the fusion process, making them ideal tools for characterizing the different steps of the fusion process, as well as promising therapeutic agents. Our finding that peptide chirality is not required for recognition in the cell
membrane allowed us to synthesize new enantiomeric peptide inhibitors that specifically block infection by their parental viruses. Furthermore, our finding that the actual membrane fusion process does not depend on peptide chirality assists in clarifying fundamental questions regarding the mechanism by which proteins can catalyze mixing of two opposing membranes.

A recent breakthrough is our finding that HIV can evade the immune response by using its fusion peptide (FP) which recognizes and targets T-cell receptor molecules and interferes with antigen-specific T-cell activation. Utilizing the same strategy of HIV, we demonstrated that the FP provides a novel peptide with a potential to cure autoimmune diseases.

**General Aspects of Protein-Membrane and Protein-Protein Interactions within the Membrane Milieu** – Within the systems described, we studied self- and hetero-assembly of peptides with different structures when bound on the surface or inserted into the membrane, and the role of lipid charge on these interactions. Interestingly, although it is generally accepted that the chiral nature of most biologically relevant macromolecules restricts specific interactions to "chiral partners", we found that the lipid bilayer environment allows the interaction of two transmembrane (TM) domains with opposite chiralities, or even between an all L-amino acid TM and its diastereomer. Furthermore, we developed a new assay that allows for the in vivo detection of hetero-association between proteins within the membrane milieu. Hence, we are able to extend our experimental results to in vivo systems adding important insights. Besides giving us important basic information, these findings serve as new tools for the design of novel compounds to combat infectious diseases by interfering with the functional assembly of membrane proteins.

**Selected publications**


**Fig. 1** The barrel-stave and the carpet models suggested for membrane permeation. In the carpet model the peptides interact only with the lipid head groups, whereas in the barrel-stave model peptides insert into the lipid core. The destruction of a cancer cell is shown as an example.

**Fig. 2** A cartoon illustrating the three major steps in HIV Cell entry and interfering with the immune response of T-cells.


Acknowledgements
The Harold S. and Harriet B. Brady Professorial Chair in Cancer Research. Israel Science Foundation; USA-Israel Binational Agricultural Research and Development Fund (BARD); UK-Israel Foundation; Pasteur-Weizmann; The Minerva Foundation; Weizman-Yale; European Community; The Prostate Cancer (former CapCure) Foundation; The Israel Cancer Association (ICA); The Israel Cancer Research Foundation (ICRF), UK prostate Cancer Foundation; The Estate of Julius and Hanna Rosen, The Dr. Joseph Cohn Minerva Center for Biomembrane Research