
Preface

In the late 1980s and early 1990s, it became evident that the old dogma concerning the permeability properties of the cell membrane to proteins and peptides was not valid in several new and important cases. First, in 1988, two independent research groups demonstrated the shuttling properties for an HIV tat trans-activator protein (1, 2). Secondly, in 1991, the group of Alain Prochiantz reported (3) on cellular internalization of the homeodomain of Antennapedia (a *Drosophila* homeoprotein), followed in 1994 by the discovery of a short peptide, pAntp(43–58) or penetratin, which was necessary and sufficient for this translocation (4). Today, hundreds of such short peptides are known, and they are defined as cell-penetrating peptides (CPPs) (5) though a few research groups call them as protein transduction domains (PTDs, reflecting their protein origin sometimes), Trojan peptides, model amphipathic peptides (MAPs), or membrane translocating sequences (MTS).

In general, CPPs are still difficult to define exactly due to some uncertainties in characterizing their translocating mechanisms. However, today's understanding is that CPPs *are relatively short peptides, 5–40 aa, with the ability to gain access to the cell interior by means of different mechanisms, mainly including endocytosis, and with the capacity to promote the intracellular delivery of covalently or noncovalently conjugated bioactive cargoes* (6).

This handbook is divided into five parts, summarizing the most important areas of CPP research. Introductory Part I briefly presents the historical background of CPP studies, the classifications of the available CPPs, and summarizes the possibilities to predict them. An overview of penetratin studies is also included due to the importance of this CPP for the whole field.

Since this handbook is mainly the update of existing CPP methods, in the situation where the mechanisms of CPP uptake are still not totally clarified, the Part II deals with the methods for testing CPP mechanisms. The structure of CPPs and their interactions with phospholipid membranes are important factors in their functioning and, hence, the methods to study these are an essential part of this handbook. Manipulations of the kinetics and thermodynamics of CPP uptake are one set of important tools to study CPP mechanisms. Approaches for the testing of endocytotic pathways of CPP uptake are also described, among these fluorescent and electron microscopy together with functional splice correction assay and toxicity methods. Different CPP uptake experiments are compared and it is becoming clear that it is often best to apply several methods in a complementary manner in order to most comprehensively evaluate CPP uptake mechanisms due to the complexity of these processes.

Part III presents a representative and brief summary of methods that attempt to use the unique properties of CPPs to study biochemical intracellular mechanisms of interaction and signal transduction. Of special interest is the mimicry of proteins by short peptides in their sequences. Several examples of such protein mimicry are available and the methods for these are presented here, starting with an overview chapter of the field. I believe that this is one of the most exciting CPP applications, possibly becoming an

important therapeutic approach for interfering with intracellular protein–protein interactions. This goal is certainly achievable only when we will learn to unite the CPP and protein mimicking properties of these peptides. I hope the selected chapters will serve to stimulate work toward this goal.

Part IV summarizes the quickly growing field that applies CPPs to improve the delivery of the oligonucleotides involved in gene modulation, particularly for gene silencing by antisense or siRNA oligonucleotides. The application of splice correcting oligonucleotides is the modern antisense strategy where several different chemically modified oligonucleotides serve as efficient splice redirectors. It is hoped that this approach may lead to novel gene therapies, I am especially pleased to present some siRNA delivery strategies using CPPs. It seems that we are not far from siRNA therapies that harness CPPs to improve and precisely target delivery in vivo based upon the efficient in vitro applications already available today. In parallel, the methods for transfection of plasmids by CPPs or their chemically modified analogs are developing efficiently and quickly.

Part V is another important part of this handbook, discussing ideas for turning CPP-based strategies into drugs. Tumor-selective targeting with flexible CPP technologies has been fueling the CPP research for years, and now the first fruits of these studies have become available. Additional organ-selective delivery strategies are also described, demonstrating that the combination of CPPs with novel nanoparticles and polymer systems is an efficient method for drug delivery. This point is underscored by the contributions of authors based at pharma companies that have contributed their ideas about CPP applications in drug development to this handbook.

In summary, the short history of research of CPPs has clearly demonstrated that CPPs have helped us to expand beyond several long held dogmas. This presents us with superb opportunities to study many intracellular mechanisms in new ways and promote the future development of novel drug

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