Although within the scientific community at large Bruce Merrifield is and will remain celebrated for the pivotal discovery and dedicated crafting of solid phase peptide synthesis methodology, there are also other areas where his unique scientific acumen was applied to with distinctive success. Among them, those of glucagon antagonists and antimicrobial peptides are arguably the better known. Over my 25 year-long relationship with Bruce, I had occasion to work with him in both areas, but it is to the latter I will devote these brief reminiscences, as our collaboration in this fast expanding research field reached from its very dawn till relatively recently.

2006, the year of Bruce’s passing, marked the silver jubilee of antimicrobial peptide research, officially initiated with the publication of the seminal paper on the cecropins by Boman (see Figure 1) and coworkers.1 Hans had befriended Bruce during his postdoc (1958–60) in Fritz Lipmann’s group at Rockefeller, and his logical enthusiasm over the finding of a family of eukaryotic, gene-encoded peptides with remarkably potent antimicrobial activities must have been contagious for Bruce, who started to collaborate with Hans on the cecropins in late 1981. On joining Bruce’s lab in January 1982, the very first project given to me was to synthesize the putative 37-residue sequence of cecropin A, to confirm some structural assignations tentatively proposed for the C-terminal region. A previous synthetic attempt2 had turned out an active compound, albeit nonidentical with the natural peptide. Building on that result and benefiting from several improvements emanating at the time from Bruce and Jimmy Tam’s joint research—not least the very useful high-low HF cleavage method3—I completed my assignment in two-and-a-half months, which at the time was regarded a reasonable time for a relative novice charged with the fully manual synthesis of a 37-residue, tritium-labeled peptide. Our synthetic product was completely identical with the native peptide by microbiological as well as biochemical (enzyme digest + HPLC) criteria, and we were able to confirm the hitherto uncertain structure of the C-terminal end by means of plasma desorption MS, a forerunner of modern MALDI-TOF MS. In addition, our report4 discussed the relevance of the Trp-2 residue for antimicrobial activity and thus set the basis for further analogue work.5 In retrospect, that initial 2-year stint in the cecropin project appears as a decisive period, where Bruce’s gracious support and encouragement became the beacon blazing away the often usual angst of a nascent scientific career. A thorough and constructive draft reviewer if ever I saw one, Bruce returned each one of my versions generously endowed with pencil scribbles which, readily deciphered, brought gradual improvement to my struggling manuscripts. To Bruce I owe a great deal of any abilities I may have achieved as a (non-native) writer of English scientific texts. Those early 1980s cecropin manuscripts, first examples of the substantial contribution synthetic peptides were to have in the development of the antimicrobial peptide field, were followed by other papers on the activity of cecropins on model membranes6 and on cecropin D and analogues,7 resulting from the work of Jürgen Fink, a German postdoc at Bruce’s lab during 1986–87. By that time I had moved back to the University of Barcelona, but my relationship with Bruce remained active through extended summer stays every year, and he suggested that we undertake the synthesis of the recently reported prepro forms of cecropin A and B,8 whose relatively short size (64 and 61 amino acids, respectively) made their synthesis a feasible target. Working alongside Dr. Zong-Qu Li (a visitor from the University of Wuhan, in China), we prepared both precursors and their putative downstream products in suitably labeled forms that allowed to study their posttranslational processing in considerable detail.9

By then, other laboratories, mainly Lehrer’s at UCLA,10 Shunji Natori’s in Tokyo11–13 and Michael Zasloff in Phila-
delphia, had discovered similar antimicrobial activities in biological systems other than Boman’s Cecropia moth, and in several cases applied synthetic peptide chemistry to explore mechanisms of action and to develop analogues with improved properties. The field of antimicrobial peptide research was taking off to become one of the most dynamic areas of peptide-related research over the next decades.

One remarkable result of Bruce and Jürgen’s work was the finding that cecropin A-(1-11) D-(12-37), a hybrid peptide combining the N-terminus of cecropin A with the C-terminus of cecropin D, was about 50 times more active than cecropin D itself. This confirmed Bruce and Hans Boman’s long-held view that a hydrophilic, strongly basic N-terminal segment and a hydrophobic C-terminus were the main requisites for antibacterial activity, in tune with the hypothesis earlier advanced by the Tosteson and Tosteson for the membrane activity of the bee venom toxin melittin. The sequence hybridation concept was to prove one of the most useful tools in the medicinal chemistry of antimicrobial peptides, leading to the development of what indeed became a new class of artificial peptide antibiotics with improved properties. A particularly fruitful hybridation strategy was that between cecropin A and melittin, first tested at Bruce’s lab by David Wade and myself in the late 1980s, then actively pursued at many other laboratories including my own. The cecropin A-melittin (CA-M) hybrids were not only equipotent in antimicrobial activity with the parent peptides but largely devoid of their undesirable cytotoxic effects on eukaryotic cells, thereby broadening their therapeutic index. Equally rewarding was the finding by Josep Ubach, then my graduate student at the University of Barcelona, that one could effect drastic (about 2/3) size reduction on...
CA-M peptides without substantial loss in antibiotic performance.19 Downsized hybrids such as CA(1-8)M(1-18) or CA(1-7)M(5-9) by way of their ready synthetic accessibility and good antimicrobial activity, have since been adopted as models in numerous studies on mechanism of action,20–26 as well as explored for prospective therapeutic application to a number of targets.27–33 Bruce’s lab contributions to the field continued steadily well into the last decade,34–37 mostly through the efforts of Drs. Padmaja Juvvadi, Ken Rotondi, and Satyanarayana Vunnam, and—last but not least—of Libby (Mrs.) Merrifield herself, who in the early 90s had decided that her long-silent biologist skills must be put again to use. Bruce himself beautifully summed up the story in his review of the field at the first informal scientific discussions (e.g., at lunch at the Rockefeller cafeteria). Peptides and proteins, made up multiple homochiral building blocks, provided unique opportunities to explore the role of chirality in biological recognition. In particular, the first evidences on the mechanism of action of antimicrobial peptides starting to emerge in the mid 80s were strongly suggestive of a relatively unusual situation in biochemistry, where a chiral effector bound a nonchiral (membrane) target. If this was so, then reversing the chirality of the peptide should have no adverse effect on its antibiotic activity and, moreover, protect it from proteolysis. The different antimicrobial peptides being developed in Bruce’s lab in the mid-late 80s, particularly the cecropin A-hybrids, were an ideal platform to test these hypotheses. In his influential 1990 PNAS article17 (Bruce’s third most cited article), the above hypothesis was conclusively demonstrated, thus opening up a fertile line of thought for the de novo design of peptide antibacterials. In particular, the D-enantiomer principle was successfully applied to the original set of CA-M hybrids.38 But Bruce’s curiosity about the role of topology in antimicrobial peptides was not yet satisfied. Taking a cue from the pioneering work of Prelog and Gerlach39 and Shemyakin et al.40 on enniatin and other cyclic peptides, as well as from the retroenantio/retroinverso concept introduced by Goodman et al.41 Bruce’s (and our) laboratory again used the CA-M hybrids to dissect the specific contributions of chirality, sequence, and peptide bond direction to antimicrobial activity. The pattern emerging from the study42 was slightly more complex (i.e., microorganism-dependent) than originally anticipated, but it was nonetheless clear that peptide chirality played virtually no role, whereas either sequence or amide bond direction were critical against most bacterial targets, and for a few strains both features were required. Shortly afterward, Bruce and Drs. Vunnam and Juvvadi applied a parallel approach to melittin, showing that the (undesirable) cytotoxic effects could be neatly set apart from the microbialidal activity in the retroenantio analogues.36

Bruce’s latest contributions to the antimicrobial peptide field are from 1999.43,44 By that time, the field had undergone extraordinary growth, with active structures regularly being unveiled in practically all types of biological systems. At the time of this writing (December 2007), almost 800 natural sequences have been catalogued,45 while the number of analogues, de novo replicas and other related structures regularly reported in peptide journals, as well as in microbiological or biotechnological publications, is truly difficult to estimate and highlights the decisive impact that synthetic peptides have had on this research area. In any fair review of the field, the relevance of Bruce’s 25-year long input can’t be overlooked, particularly how his early work and further sustained contributions helped set the pace for the spectacular blossoming that has ensued.

REFERENCES