Venoms are highly complex mixtures of biological compounds ("toxins") which have evolved to serve a range of ecological roles, including the capture of prey and defense against aggressors. In most venoms, proteins and peptides comprise the majority of toxins, and proteomic approaches are therefore central to deciphering the biochemical complexity that underlies the functional diversity of venoms. Mass spectrometry is currently the analytical technique of choice in proteomics. Consequently, developments in mass spectrometry technology—in terms of resolution and sensitivity as well as novel applications—have been one of the major drivers of our understanding of venom biology. These developments have also accelerated the identification and characterization of venom-derived pharmacological tools and lead molecules for development as therapeutics, cosmetics, or agrochemicals. This Special Issue focuses on developments and utilization of proteomic and mass spectrometry approaches that lead to new knowledge on the biology, evolution, or potential application of venoms and toxins.