Substance P (SP) and Neurokinin A (NKA) neuropeptides, belonging to the Tachykinin (TK) family are involved in the regulation of a large number of physiological processes by interacting as agonists with different GPCR subtypes of Neurokinins. The characterization of the active peptide conformations of the Tachykinins is essential for the elucidation of the mechanism of their action and for the design of drugs. To address the molecular basis for the recognition of the TKs by the receptor and the factors modulating neuropeptide active conformations, we studied peptide secondary structures by CD and FTIR spectroscopies in aqueous solutions and membrane-mimic systems. The analysis of the spectral data reveals that TKs adopt different conformations, as the dominant peptide conformation switches from flexible polyproline II in aqueous solutions and DMPC liposomes to α helical or β sheet structure in the presence of the negatively charged micelles and DMPG vesicles. To assess the mode of the interaction of the TKs with the membrane we performed measurements of the Trp fluorescence, the fluorescence quenching by brominated lipids and the surface electrostatic potential. We observed apparent differences between the two peptides with respect to the mode of interaction with the membrane. While the C-terminal segment of SP binds and inserts into lipid bilayer, NKA binds, but most likely stays on the membrane surface. Finally, by using different biophysical diagnostic methods we found that SP and NKA have ability to form fibrils in vitro.