

## HINTS FOR THE SUCCESSFUL DISPLAY OF PEPTIDES ON THE COAT PROTEIN OF POTATO VIRUS X FOR VACCINE FORMULATIONS

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A promising application of engineered plant viruses to biopharmaceutical research is aimed to the development of peptide vaccines, utilising chimeric virus particles (CVPs) displaying on their surface, as fusion to the coat protein (CP), peptides of immunological interest (epitopes). The purified CVPs can then be used as carriers for the delivery of immunogenic peptides that can induce specific antibody responses while harmless for human/animal health.

In this work we have exploited a spontaneous Potato Virus X (PVX) mutant that express a truncated, but functional, form of the CP lacking 20 residues (2 to 22) at the N-terminus. Two pPVX201-derived viral expression vectors encoding the *cp* mutant gene have been constructed to fuse, to the 5'-terminus of the *cp* gene, the sequences encoding a panel of peptides (26 in total) varying both in length and amino acid composition. The ability of each chimeric CP to enable virus spreading was evaluated investigating the infection progression induced by each construct *in planta*. From this analysis we were able to define that Tryptophan content and isoelectric point, are two fundamental structural features of the fused peptides that influence CP functions. Depending on the amino acid composition, fusion peptides are able to critically affect both local or systemic movement of PVX. Moreover, we evidenced that the genetic stability and preservation of the correct peptide sequence is heavily influenced by the percent content in serine and threonine of the fused peptide.

Our findings, while describing the effect of additional peptides on virus infectivity (movement), provide new insights into the prediction of peptide sequences that are compatible with the display on PVX and the most favourable conditions to generate infectious CVPs to produce candidate vaccines.