

# Evaluating ELP fusions for the expression of antibodies in transgenic tobacco plants

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Plants are promising vehicles for the expression of recombinant proteins with regard to medical and veterinary purposes. Since the first report of a recombinant antibody derived from transgenic plants, various antibody derivatives have been produced in different crop species using several expression systems. In order to enhance the accumulation of two anti-HIV neutralising antibodies (2F5 & 2G12) in tobacco leaves and seeds, synthetic repeats of elastin-like polypeptides (ELP) were C-terminally fused to both antibody chains.

Constructs for ubiquitous and seed-specific expression of light and heavy chains with/without fusion of both anti-HIV antibodies were used for the transformation of tobacco *via* the Agrobacterium-mediated gene transfer. Stable transformed plants expressing the non-assembled chains with/without the synthetic protein in the ER were selected. The accumulation of light or heavy chain ELP fusion proteins in leaves and seeds was increased compared to the corresponding chains without ELP. Transgenic tobacco lines with single chromosome insertions were crossed to obtain the complete anti-HIV antibodies. The successful assembly of both chains as fusion proteins was verified in tobacco leaves as well as in seeds. The ELP fusion does not influence the assembly of the full-length antibodies. An enhancement of the expression level of the assembled antibodies was confirmed for the tobacco-expressed 2F5 antibody and the corresponding ELP fusions.

The recombinant 2F5 antibody derivatives were affinity purified and used for antigen binding analysis with the surface plasmon resonance technology (Biacore). Binding parameters to a therapeutic peptide derived from gp41, Fuzeon<sup>®</sup>, similar to the animal cell produced 2F5 were obtained. In a first experiment the virus neutralisation capacity of the plant-expressed mAb was determined showing a 9-fold lower HIV neutralisation capacity compared to the standard antibody. Finally, the N-glycan profile of the four plant-expressed 2F5 antibodies was investigated by LC-MS. The main N-glycans of these recombinant antibodies were non-immunogenic high-mannose type N-glycans.