Advantages of plant expression systems for the rational design of oral therapeutics

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The journey of therapeutic development from concept to the market is a complex, lengthy and expensive process. Plant expression systems have been envisioned to provide solutions to the later phase of bulk production, by enabling cost effective manufacturing of high amounts of therapeutics. To realize higher expression, several innovative tools have been developed over the past 20 years for various plant organs, especially leaves and seeds. These developments have enabled bulk therapeutic production, but can also make an unparalleled contribution in the early phase of therapeutic discovery and drug optimisation.

Our group has been exploring the prospects of transient expression in *Nicotiana benthamiana* and stable expression in Arabidopsis seeds for early stage exploration of candidate antibodies and antigens. The flexibility of plant expression system has permitted designing formats with various permutations and combinations of protein. At the PBVAB-2015 platform we'll present an example of each of the two expression systems.

As a solution to the recurrent diarrhea causing bacterial infection of F4-bearing enterotoxigenic *Escherichia coli* (ETEC) in piglets; anti-ETEC IgG and IgA antibodies were produced in Arabidopsis. Kilograms of antibody producing Arabidopsis seeds enabled *in vivo* evaluation of oral feed based passive immunisation in a piglet challenge experiment. This led to the evidence, that the IgA isotypes of antibodies are more suitable than IgG in oral prophylaxis of weaned piglet against ETEC.

In *N. benthamiana,* heterologous proteins can be produced in about five days and micrograms of different proteins can be produced in each leaf. Utilizing this benefit together with the recent modular cloning tools, we are investigating a wide combinatorial range of antigen-antibody fusion molecules that can effectively cross the gut barrier and lead to mucosal immunity. The gut barrier is a selectively permeable membrane, were transcytosed antigens can either induce an immune reaction or a tolerogenic reaction. Among parameters, specific targeting to immune cells is important. Our approach aims at modulating the antigen-antibody fusion molecule to fine-tune enhanced uptake, thusly it's anticipated to further the fundamental understanding of mucosal immunity and designing of oral therapeutics.