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pH gradient mitigation in the leaf cell secretory pathway – One goal, several effects

Plant expression systems are used for the production of complex secreted proteins. However, recombinant proteins face considerable biochemical challenges along the secretory pathway, including proteolysis and pH variation between organelles. Partial neutralization of the Golgi lumen pH by ectopic expression of Influenza virus M2 proton channel is a useful way to stabilize acid-labile recombinant proteins and peptides in leaf cells [1]. Transient co-expression assays with fusion protein hybrids differentially susceptible to proteolytic breakdown allowed detecting a significant alteration of host protease activities upon M2 channel expression. We showed that recombinant protein susceptibility to proteolysis in the plant cell secretory pathway is pH-dependent [2]. We also assessed the 'off-target' effects of M2 channel expression in agroinfiltrated *Nicotiana benthamian*a leaves. An iTRAQ proteomics analysis revealed a cell-wide impact of M2 on the leaf proteome, to alter the steady-state levels of proteins not only in the secretory pathway but also in other cellular compartments. Our data pointed to a defence response-attenuating effect of M2 upon agroinfiltration [3]. We report the potential of the influenza M2 ion channel as a novel tool to neutralize the pH in acidic subcellular compartments and to increase the yield of secreted recombinant proteins in plants.

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[2] Jutras, P. V., Goulet, M.-C., Lavoie, P.-O., D'Aoust, M.-A., Sainsbury, F., and Michaud, D. (2018) Recombinant protein susceptibility to proteolysis in the plant cell secretory pathway is pH-dependent. *Plant Biotechnol. J.*, 1–11.

[3] Jutras, P. V., Sainsbury, F., Goulet, M.-C., Lavoie, P.-O., Tardif, R., Hamel, L.-P., D'Aoust, M.-A., and Michaud, D. (2018) pH gradient mitigation in the leaf cell secretory pathway alters the defense response of *Nicotiana benthamiana* to agroinfiltration. *BioRxiv*. https://doi.org/10.1101/431767