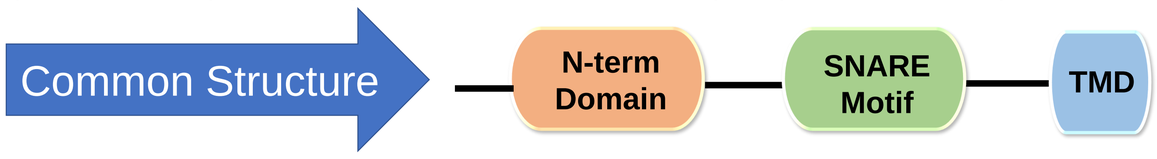
‘SNARE PROTEINS’-MAXIMIZING PROTEIN EXPRESSION IN MAMMALIAN CELLS

Angita Shrestha, Michael Mullin, Alex Zwetsloot & Maja Firczuk

Introduction

SNAREs (soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors) are an evolutionary conserved family, **regulating all the intracellular trafficking pathways by catalysing vesicle fusion to the cognate target membranes**. Some of the best characterised SNARE proteins include Syntaxin1, Snap23/Snap25 (Synaptosome-associated protein of 23 kDa/25 kDa), Vamp2/8 (Vesicle associated membrane protein 2/8). Although SNARE proteins vary in size, they have similar structure: N terminal domain, SNARE motif (α-helix domain) and a single transmembrane domain (TMD). Some SNARE proteins lack TMD.



Mammalian cells play a key role in the production of protein biopharmaceuticals. However, secretion can be a limiting factor for the productivity of these mammalian cell lines. Over the years, significant efforts have been made to develop strategies for producing large amounts of recombinant proteins from mammalian cells. One such strategy involves effective reprogramming of the exocytic pathway in the secretory vesicle fusion machinery. In this context, the co-expression of SNARE proteins has shown to improve the production of secreted recombinant proteins in a variety of mammalian cells (*Peng et al,2010*).

The Protein and Cellular Sciences (PCS) department at GSK is involved in the production of range of biopharmaceutical proteins in mammalian cells. The use of SNAREs holds great promise to overcome bottlenecks in the manufacture of proteins, increasing the success rate of reagent generation in the department.

Location of SNAREs in the cell

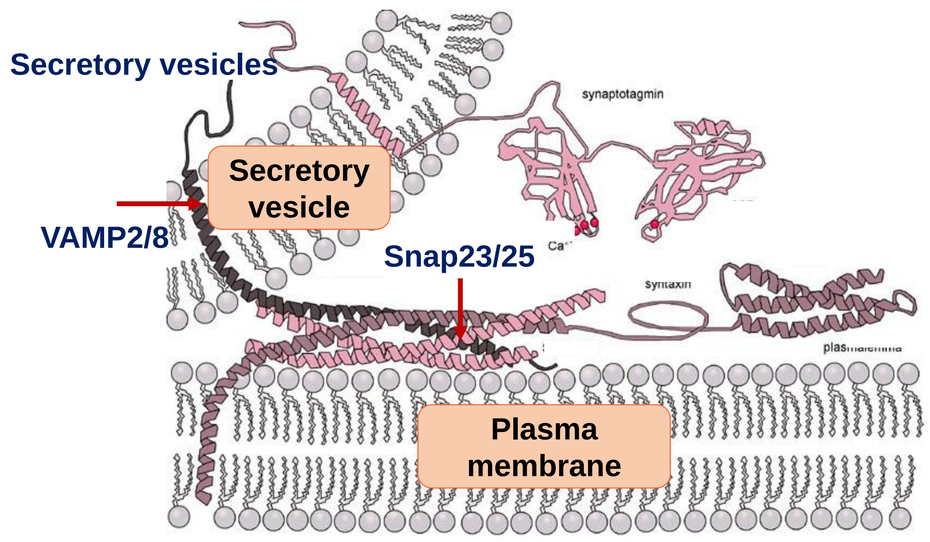
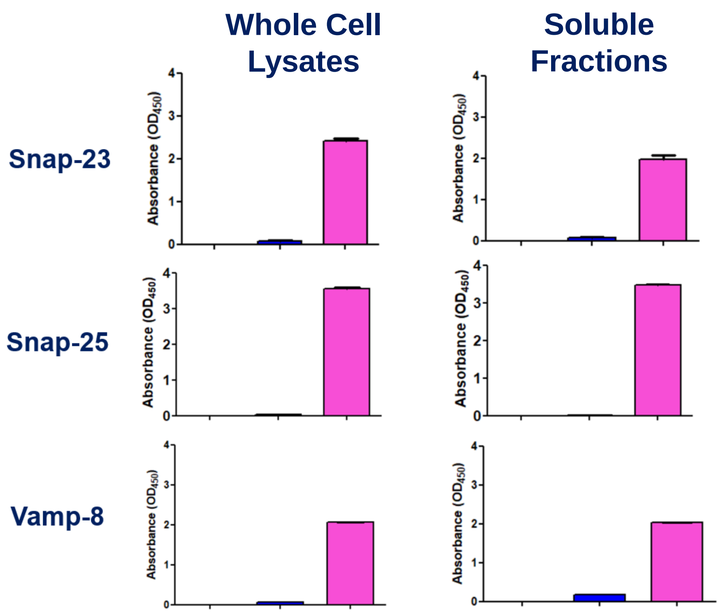
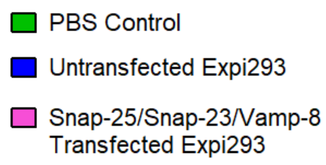


Figure 1: Cellular location of SNAREs. Vamp2/8 is primarily located at vesicular membrane whereas Snap-25 and Snap-23 are anchored to the cytosolic face of membranes via palmitoyl side chains covalently bound to cysteines in the SNARE motif. *Figure adapted from Jahn et al, 2006.*

Expression of SNAREs in Expi293 cells

Expi293 cells were transfected with either Snap-23, Snap-25 or Vamp-8 and their expression was analyzed using ELISA. These expression profiles were compared to the untransfected control Expi293 cells which ubiquitously express low amounts of SNAREs.

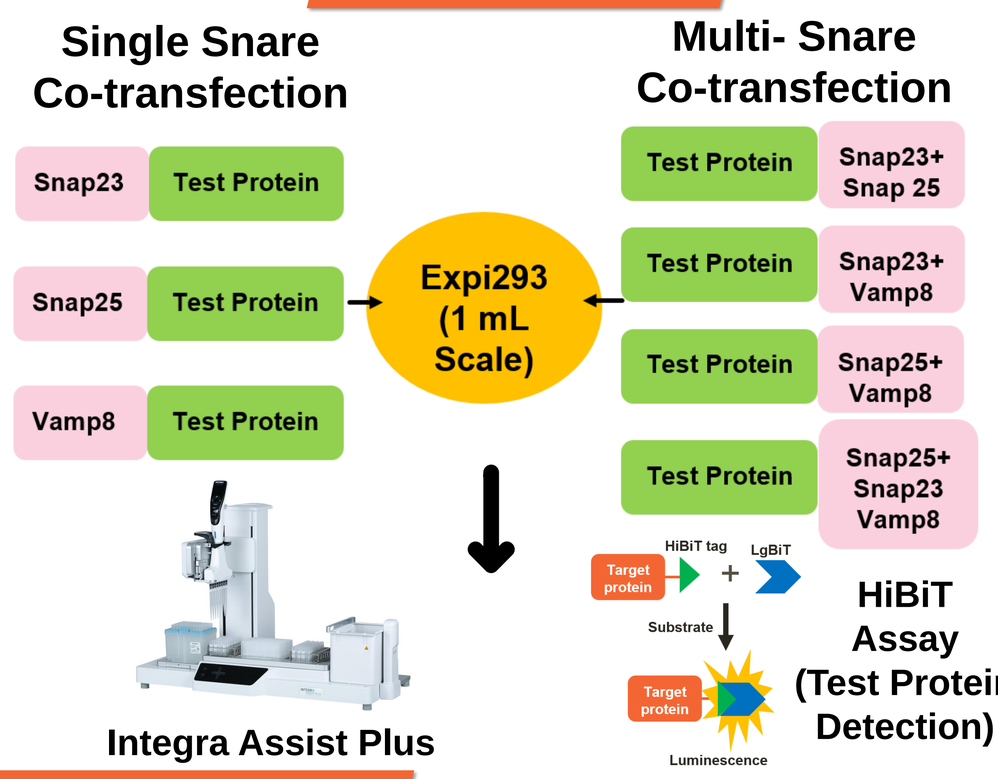




Results:

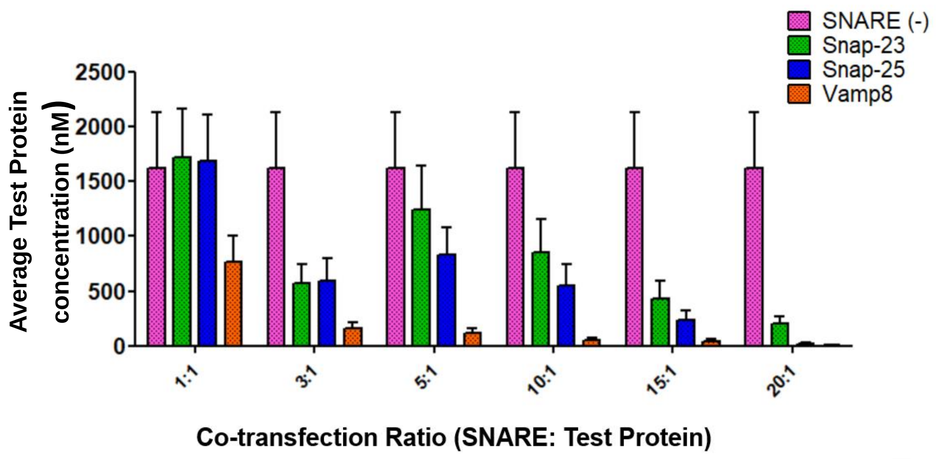
- Snap-23, Snap-25 and Vamp-8 were detected in transfected Expi293 cells using ELISA.
- The expression of Snap-25 was higher than Snap-23 and Vamp-8.
- Snap-23 and Vamp-8 were also detected in very low amounts in untransfected Expi293 cells, suggesting that these SNAREs are ubiquitously expressed in Expi293 cells.

Methods



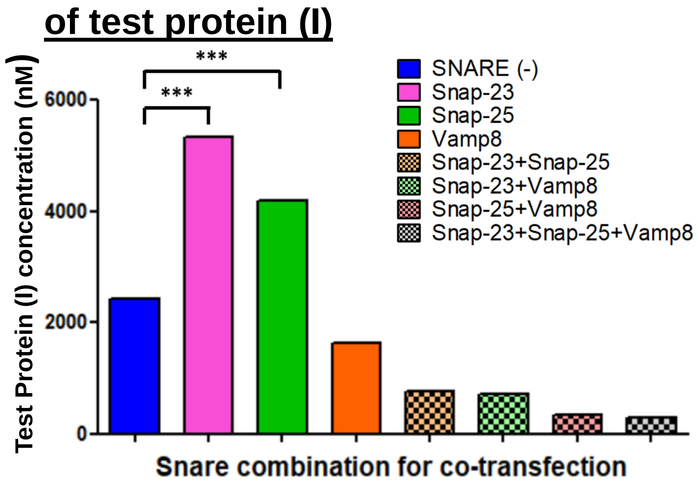
Protein Co-Expression Study

a) Analysis of Co-Transfection Ratio



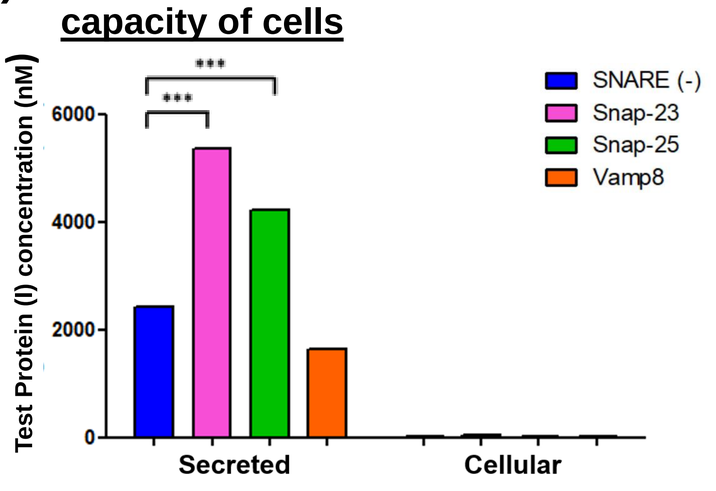
- Expi293 cells were co-transfected with different ratios of SNARE expressing plasmid to test protein plasmid, and the expression of the test protein was analyzed. Data are presented as mean of 11 test protein (A-K) concentration. Overall, 1:1 (SNARE: Test Protein) ratio gave results comparable to SNARE (-) control. Increasing the amount of SNAREs during co-transfection decreased the test protein expression.

b) Effect of SNAREs on the expression of test protein (I)



- Test protein (I) was co-transfected with either single SNARE plasmid or multiple SNARE plasmids (1:1). The control test protein was expressed without SNAREs. The expression supernatant was harvested, and the test protein expression was analyzed using HiBiT assay. Single SNARE co-transfection with Snap-23 and Snap-25 improved the expression of test protein compared to the control. *Data are analyzed by one-way ANOVA followed by Tukey’s multiple comparison test. Statistical significance is shown with asterisks.***p<0.001*

c) Effect of SNAREs on the secretory capacity of cells



- The amount of test protein (I) secreted or left in the transfected cells (cellular) was examined by analyzing the supernatant and cell lysates harvested from the cells co-transfected with and without SNAREs. In both situations, >95% of test protein was secreted out of the cells. *Data are analyzed by one-way ANOVA followed by Tukey’s multiple comparison test. Statistical significance is shown with asterisks.***p<0.001*

Conclusion

- In this study, we explored the use of different SNAREs for improving the yield of secreted proteins. Eleven test proteins (A-K) were co-expressed with different combinations of SNAREs. Out of the 11-test proteins analyzed, 7 of them showed higher protein yield when co-expressed with one or more combinations of SNAREs (see summary table).
- The total amount of test protein (secreted + cellular) produced by Snap-23 and Snap-25 overexpressing cells were higher than the control SNARE(-) cells. However, >95% of the test protein was secreted out of the cells in both SNARE (+) and SNARE (-) situations. This suggests that the increase in the test protein expression could be due to a global boost in overall protein production rather than just increase in the cells’ secretion capacity.

Summary Table

Protein	Snap25	Snap23	Vamp8	Snap25+ Snap23	Snap25+ Vamp8	Snap23+ Vamp8	Snap25+Snap23 + Vamp8
A	✓	✓	✗	✓	✓	✓	✗
B	✓	✓	✗	✓	✗	✓	✗
C	✓	✗	✓	✗	✗	✗	✗
D	✗	✗	✗	✗	✗	✗	✗
E	✗	✗	✗	✗	✗	✗	✗
F	✗	✗	✓	✗	✗	✗	✗
G	✓	✗	✗	✗	✗	✗	✗
H	✗	✗	✗	✗	✗	✗	✗
I	✓	✓	✗	✗	✗	✗	✗
J	✗	✓	✗	✗	✗	✗	✗
K	✗	✗	✗	✗	✗	✗	✗

✓ Expression higher than the control ✗ Expression similar/lower than the control

References

1. *Jahn R, Scheller RH. SNAREs–engines for membrane fusion. Nat Rev Mol Cell Biol. 2006;7:631*
2. *Peng RW, Abellan E, Fussenegger M. Differential effect of exocytic SNAREs on the production of recombinant proteins in mammalian cells. Biotechnol Bioeng. 2011;108:611.*