EXPRES^{2}ION

BIOTECHNOLOGIES

EXPRES^{2}ION BIOTECHNOLOGIES

- Founded in 2010 in Copenhagen, Denmark
- A CRO spin-off from Affitech A/S
- Proprietary technology platform for production of proteins based on *Drosophila* S2 insect cells
- > 10 years experience with S2 cells from research to GMP production for human clinical trials

OUR FOCUS

- 100% focus on Drosophila S2 cells
- Vector and cell line development
- Upstream process development
- Small-scale Protein production
- Process Transfer to CMOs for GMP production

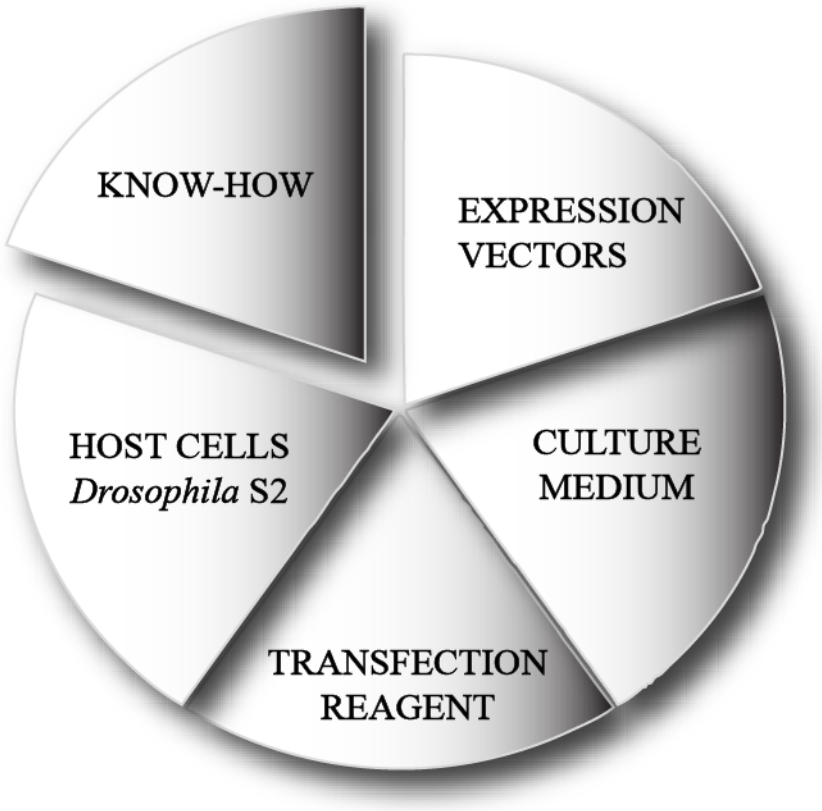
AIM

- Introduce the *Drosophila* S2 system as a complement to the Baculovirus system

- Make the S2 system available to academic centers for free

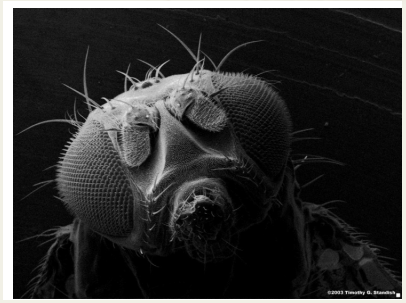
A COMPLETE PROTEIN EXPRESSION SYSTEM

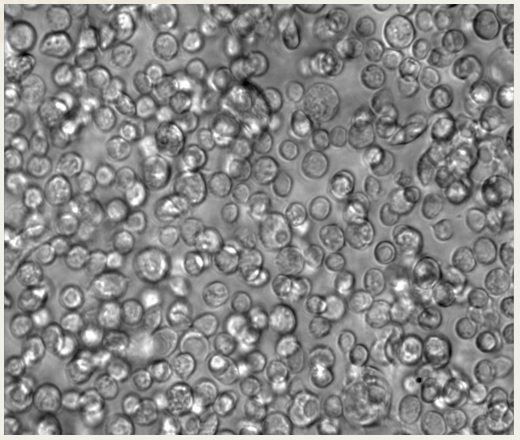
- >10 years experience with *Drosophila* S2
- Vaccine clinical trials up to Phase II



DROSOPHILA S2 INSECT CELLS

- Derived from the late stages of *Drosophila melanogaster* (fruit fly) embryos (Schneider, 1972)
- Doubling times of 18-24 hrs (both serum-containing and serum-free medium)
- Cell size: 9-10 µm in diameter
- Grow well between 5 and 140E6 cells/ml in suspension
- Grow at 23°C to 27°C
- Very robust





LITERATURE EXAMPLES OF S2 EXPRESSED PROTEINS

	Family	S2 Produced
Secreted	Anti-angiogenic	Endostatin, Canstatin
	Cytokines & growth factors	VEGF, IL5, IL12, EPO, RANKL, TNF, lymphotactin
	Enzymes	Sea raven type II antifreeze protein
	Plasma proteins	Human transferrin
	Monoclonal antibodies	IgG, IgM, scFv, Fc, chimeras & Fab fragments
Viral	Viral Proteins	JEV, rabies virus, West Nile virus, Influenza, Dengue fever
	Virus like particles	JEV glycoprot (E), HIV, Rotavirus
Complex surface	Receptors	CD23*, EGFR*, Her2*, ADAM33*, glucagon rec., MHC class II I-Ed
	Integrins & related receptors	αIIbβ3, suPAR
	GPCRs	D1 and D2 dopamine receptors, human mu opioid receptor, IL-5 receptor α, chemokine CxCR3, CXCL11 receptors

*soluble versions

FURTHER EXAMPLES OF S2 EXPRESSED PROTEINS

Protein class	Protein name	Source	Reference	Comments
GPCR[a]	M_1 muscarinic acetylcholine receptor	*Drosophila*	Millar et al. (1995) Yagodin et al. (1999)	Robust receptor expression; ~2.5 pmol/mg Imaging of intracellular Ca^{2+}
GPCR	M_2 muscarinic receptor	Pig	Graziano et al. (1998)	Robust receptor expression; ~5 pmol/mg
GPCR	$D2_L$ dopamine receptor	Rat	Schetz et al. (2003)	Robust receptor expression; ~10 pmol/mg. Stereoselective drug binding is similar to that in mammalian cells.
GPCR	D_1 dopamine receptor	Rat	Schetz et al. (2003)	Robust receptor expression; ~8.5 pmol/mg. Stereoselective drug binding is similar to that in mammalian cells.
GPCR	Glucagon receptor	Human	Graziano et al. (1998)	Ultra-high expression; ~100 pmol/mg. Anisotropy of fluorescent ligands.
GPCR	Tachykinin receptor (STKR)	*Stomoxy calcitrans*	Torfs et al. (2000)	Positively coupled to phospholipase C and adenylyl cyclase
			Torfs et al. (2001)	Receptor expression; ~0.15 pmol/million cells. Imaging of intracellular Ca^{2+}
GPCR	Octopamine receptor (OMAB)	*Drosophila*	Van Poyer et al. (2001)	Positively coupled to adenylyl cyclase and activated by the phentolamines: tyramine and octopamine
GPCR	Leucokinin receptor	*Drosophila*	Radford et al. (2002)	Immunocytochemistry and western blotting
GPCR	Inositol (1,4,5)-triphosphate receptor (IP_3R)	*Drosophila*	Pollock et al. (2003)	Ultra-high protein expression: ~400 mg/liter
GPCR	Interleukin 5 (IL-5) receptor	Human	Johanson et al. (1995)	Robust receptor expression; 17 mg/liter. The peptide ligand, IL-5, is also expressed at ~22 mg/liter.
GPCR	Chemokine(CXCR3)[b] receptor	Human	Hensbergen et al. (2001)	Expression of CXCR3 receptors is similar to that in mammalian cells
GPCR	Chemokine(CXCL11)[c] receptor	Human/ murine	Hensbergen et al. (2001)	Expression of CXCL11 receptors is similar to that in mammalian cells
Ion channel	γ-Aminobutyric acid receptor ($GABA_C$)	*Drosophila*	Millar et al. (1994)	Robust expression; ~3 pmol/mg Whole cell patch clamp
Ion channel	γ-Aminobutyric acid receptor (GABA)	*Drosophila*	Grolleau et al. (2000)	Single-channel recordings.
Ion channel	Nicotinic acetylcholine receptor	*Drosophila*	Lansdell et al. (1997)	Robust receptor expression; ~6.5 pmol/mg
Ion channel	Chloride channel	*Drosophila*	Asmild at al. (2000)	Endogeneous channels. Whole cell patch clamp recording gives an estimate of 0.02 channels/μM^2.
Ion channel	Voltage-dependent sodium channel	*Drosophila*	Meadows et al. (2002)	Patch clamp recordings
Ion channel	Transient receptor potential–like (TRPL)	*Drosophila*	Goel et al. (2001)	Immunoprecipitation and immunoblotting. Imaging of intracellular Ca^{2+}. Comparison of expression with *Sf*9 cells.
Enzyme	Chloramphenicol 3-*O*-acetyltransferase	Bacterial	Di Nocera and Dawid (1983)	Effective reporter protein
Enzyme	Galactokinase	Bacterial	Johansen et al. (1989)	Robust expression; ~3 mg/liter. Effective reporter protein.
Enzyme	Dihydrofolate reductase	Bacterial	Van der Straten et al. (1987)	Confers resistance to methotrexate
Enzyme	Hygromycin B phosphotransferase	Bacterial	Van der Straten et al. (1987)	Confers resistance to hygromycin B
Enzyme	Neomycin	Bacterial	Van der Straten et al. (1987)	Confers resistance to G418
Enzyme	Glucosidase II	*Drosophila*	Ziak et al. (2001)	Endogenous enzyme and only one isoform expressed
Peptide	Neurotensin	Human	Friry et al. (2002)	Levels of mRNA transcribed similar to that in mammalian cells
Antibody	IgG_1 monoclonal antibody	Human	Kirkpatrick et al. (1995a)	BiP-mediated assembly of both heavy and light chains
Oncogene protein	H-ras	Human	Johansen et al. (1989)	H-ras is growth-inhibitory, thus an inducible promoter is required
Cytoskeletal protein	Cadherin	*Drosophila*	Dubreuil et al. (1999)	Western blotting
Cytoskeletal protein	Neuroglian	*Drosophila*	Dubreuil et al. (1999)	Western blotting
Glycoprotein	HIV–gp120[d]	AIDS virus	Culp et al. (1991)	Robust protein expression; ~35 mg/liter. Glycosylation is limited to simple core sugars.
Glycoprotein	Erythropoeitin (EPO)	*Drosophila*	Lee et al. (2000)	Robust protein expression is; ~12mg/liter
Apoptotic protein	Presenilin	*Drosophila*	Ye and Fortini (1999)	Western blotting
Apoptotic protein	Inhibitor of apoptosis (IAP1)	*Drosophila*	Muro et al. (2002)	Comparison of expression with that in *Sf*9 cells

[a]GPCR: G protein–coupled receptor.
[b]The alternative nomenclature for the chemokine (CXCR3) receptor is interferon inducible protein-10 (IP-10).
[c]The alternative nomenclature for the chemokine (CXCL11) receptor is interferon inducible protein-9 (IP-9).
[d]HIV: Human immunodeficiency virus.

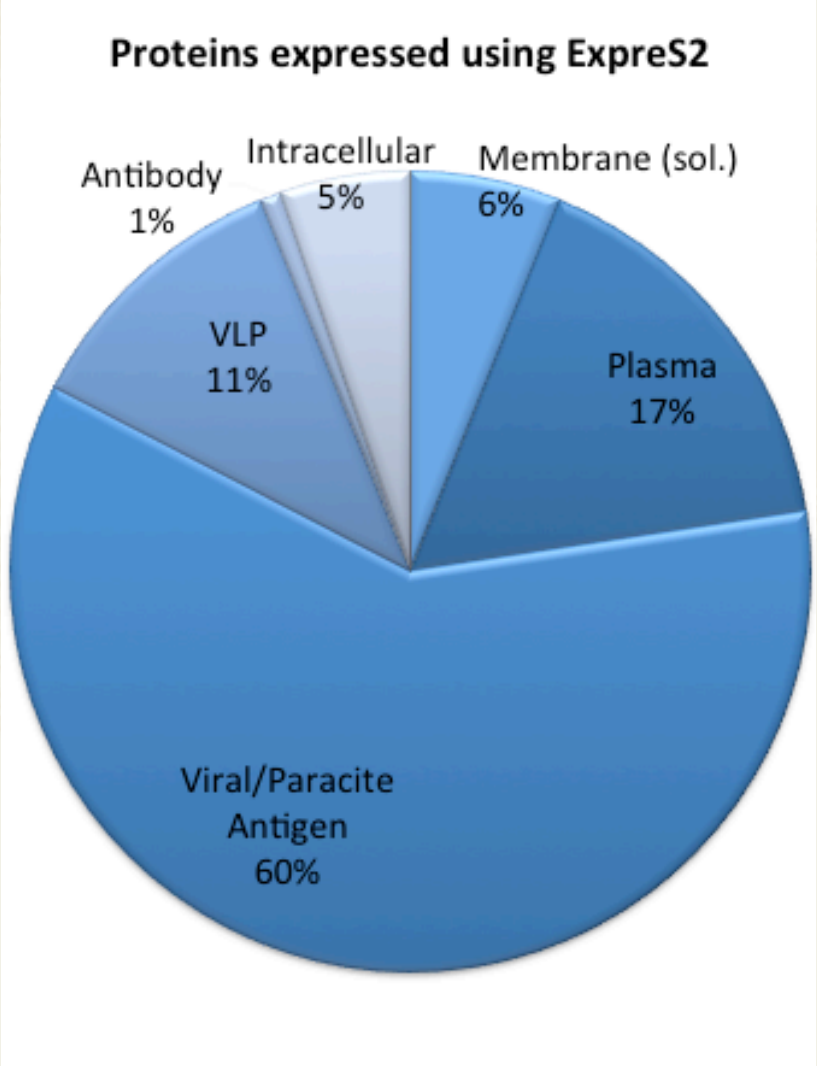
*"Following drug selection, a polyclonal S2 cell line can be induced to express on the order of 2 to 100 pmol/mg membrane protein for G-coupled protein receptors, 4000 to 100,000 sites/cell for other membrane receptors and 3 to 35 mg/liter for soluble and secreted proteins." **

*** Protein Expression in the Drosophila Schneider 2 Cell System,** Current Protocols in Neuroscience**,** John A. Schetz, Eswar P.N. Shankar, 2004

Proteins expressed by ExpreS^{2}ion

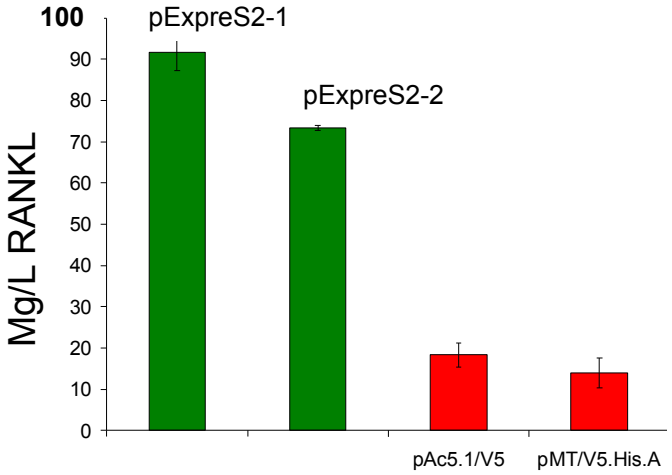
Protein Class
Secreted Binding Proteins
Biomarkers
Cytokines
Differentiation Factors
Growth factors
HDACs
IgGs
Kinases
Malaria antigens
Receptors *
Membrane proteins *
Metal binding proteins
Methyltransferases
Plasma Proteins
Proteases
Secreted Signaling Molecules
Viral antigens

*** Soluble version**

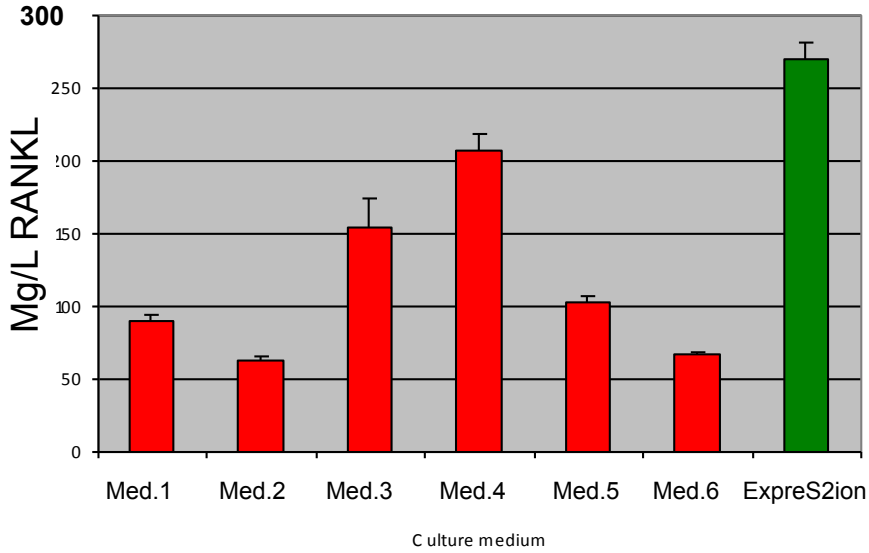


IMPROVED YIELDS

Vector development

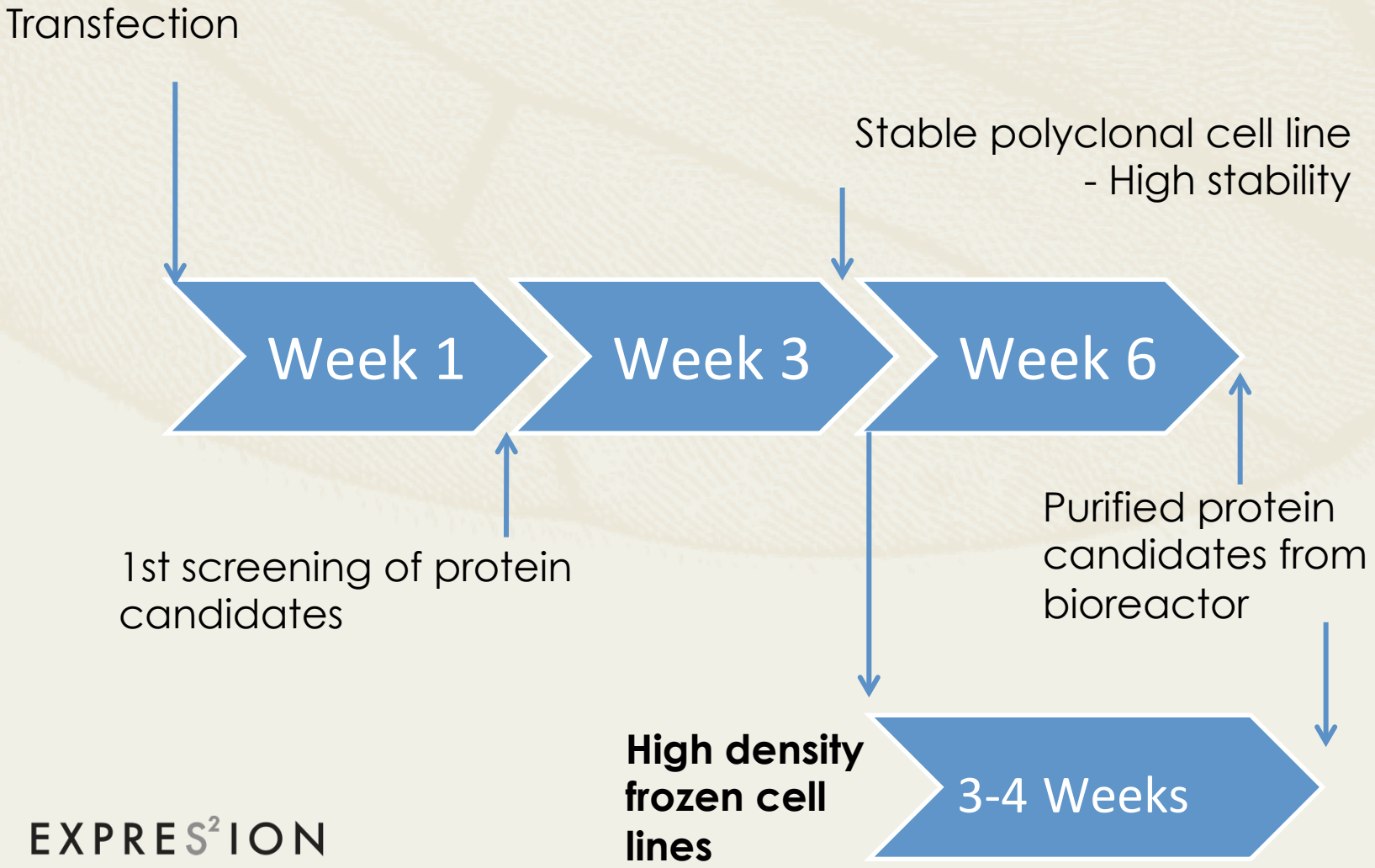


Medium development



*Adaptation to individual media were performed

QUICK TO PROTEIN AND STABLE PROCESS



BENEFITS of S2 SYSTEM

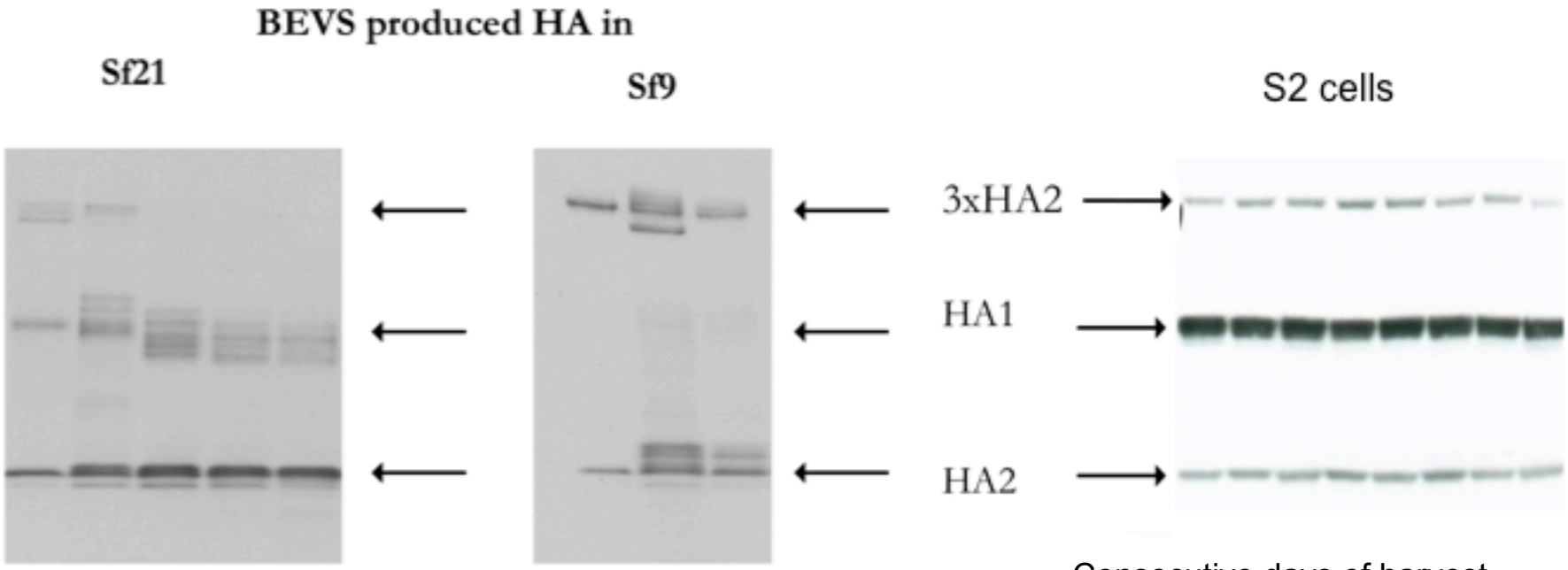
Compared to Baculovirus insect cell systems

- Non-viral, Non-lytic
- More homogeneous product
- High reproducibility and consistency between batches
- Potential for Higher yield
- Long-term stability of frozen cell stocks

Compared to other S2 systems

- Increased yield
- Protocols for large-scale production
- Including Perfusion processes

More homogeneous product



Effect of different harvest time points

Consecutive days of harvest
from a bioreactor running perfusion

BEVS: Baculovirus Expression Vector System

High-throughput protein expression in *Drosophila* S2 cells

- Robust cells and increased expression allows growth, transfection and expression testing in 1ml, 96 well format

- Fast scale-up for selected proteins for structure and functional studies

Take-home messages

- Complementary system to Baculovirus for structure and functional studies
- Opportunity to further improve hit-rate for complex proteins
- Potential yield and consistency advantages for secreted proteins

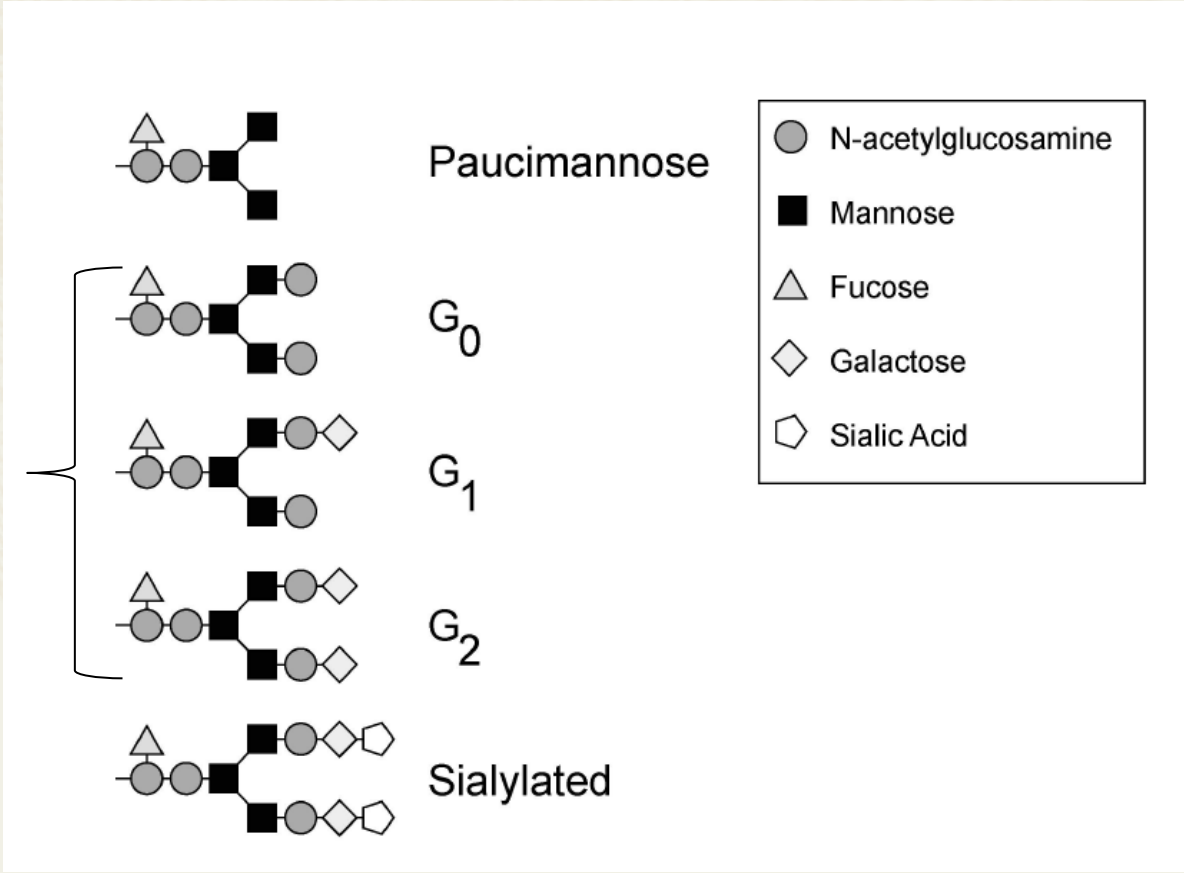
Questions?

GLYCOSYLATION

Insect (S2)

CHO

Human



N-glycosylations found in RANKL based on MALDI-TOF analysis

0 N-glycosylation **+1 N-glycosylation** **+2 N-Glycosylation**