Discovery of High-Affinity Functional Antibodies Specific for CXCR5 and Other Multi-Pass Membrane Proteins

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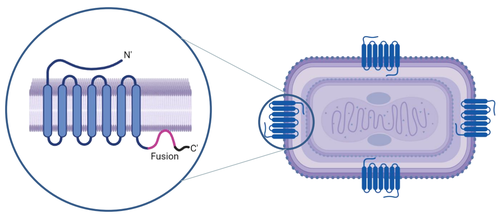
Vaccinex has developed a fusion protein technology to enable the direct incorporation of multi-pass membrane proteins such as GPCRs and ion channels into the membrane of two antigenically distinct poxviruses. The protein of interest is correctly folded and expressed in the cell-derived viral membrane and does not require any detergents or refolding before downstream use. Antigen expressing virus can be readily purified and used for antibody selection using any in vitro display platform where alternating between the two strains eliminates any anti-viral antibodies from being selected. Immunization with the viral strains produces potent antibody responses in mice. Memory B cells can be sorted using antigen virions and then used to make an immunized phage library which has a higher percentage of antigen specific binders than whole spleens, thus enabling antibody selection from a smaller diversity library. This is especially useful for discovering antibodies from a cohort of multiple animals as an alternative to methods such as hybridoma generation. Here we show selection for antibodies against CXCR5 and how this technology can apply to other difficult targets.

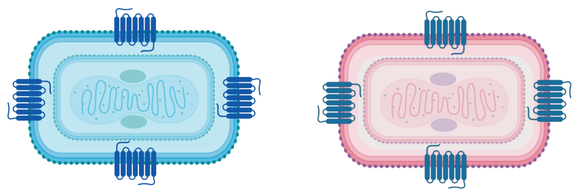
Technology Introduction

Vaccinex fusion protein technology provides for efficient incorporation of multi-pass membrane proteins into the poxvirus membrane. This system ensures native conformations for antibody discovery both *in vitro* and *in vivo*.



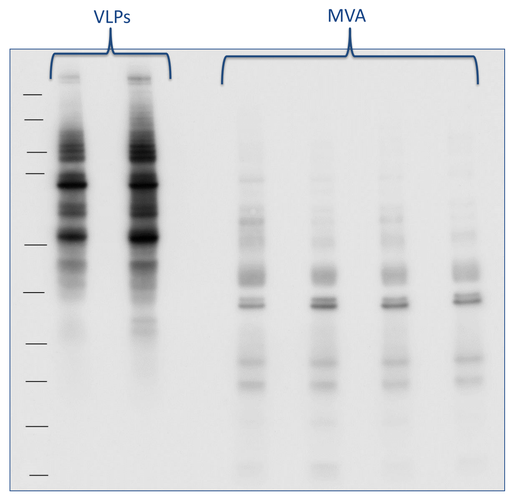
The fusion protein is intra-viral and can be fused with fluorescent proteins like GFP for antigen tracking.

Antigens are produced in two antigenically distinct, highly attenuated BSL1 poxviruses to **eliminate anti-viral** background. Additionally, the poxvirus membrane has limited protein complexity with four known proteins incorporated.



Mammalian Poxvirus (MVA)

Avian Poxvirus (FPV)

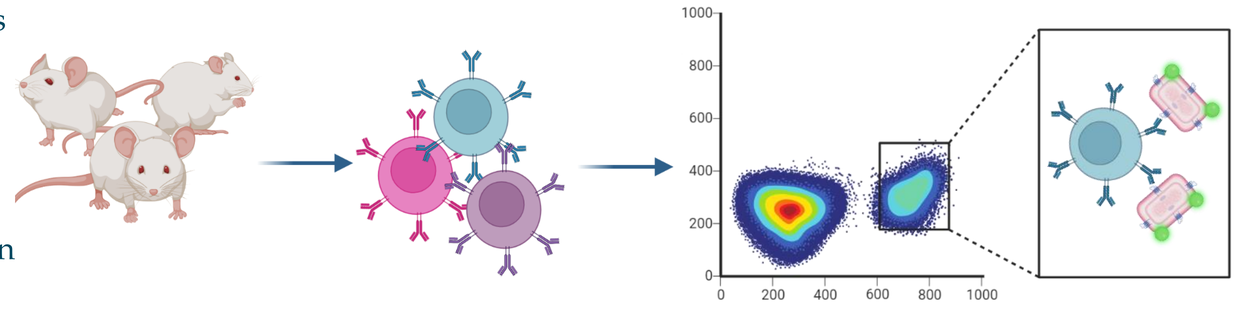


MVA antigen virions contain fewer non-antigen membrane proteins than conventional VLPs.

Western blot of VLPs and MVA virions surface biotinylated and loaded at equal protein concentrations. Detection is with streptavidin-HRP.

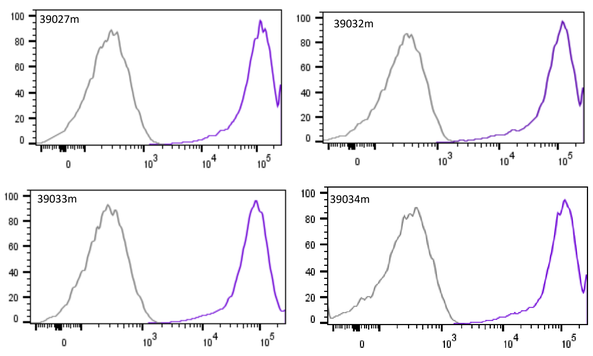
Antibody Discovery Methods

With two antigenically distinct poxvirus strains available, we can immunize animals with one strain and perform downstream applications with the other to avoid anti-virus background. The virions can be coupled to magnetic beads or ELISA plates for phage panning and isolation of antigen specific antibodies. The antigen virions can also be labelled with **biotin** or **fluorescent** beads to allow for **Memory B cell sorting** from mice or other species. Sorting allows for efficient isolation of antigen specific B cells from a pool of multiple mice at one time, increasing the probability of antibody discovery. A phage library generated from the pooled B cells can be quickly generated and panned on the alternative poxvirus followed by analysis as single, secreted IgG clones by flow cytometry for antigen binding and specificity.



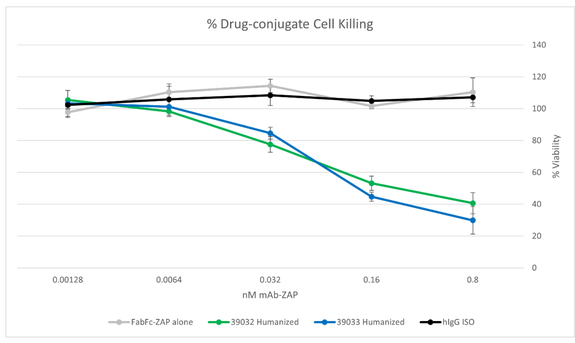
Example: Antibody Discovery for CXCR5

Antibodies against CXCR5 were discovered after panning on CXCR5 virions using an immunized phage library. Clones that were specific by flow cytometry were purified and tested for functional activity in a receptor blocking assay and a migration assay. Several unique antibodies showed functionality in all assays, and two were selected for humanization and testing for internalization on CXCR5+ cells.



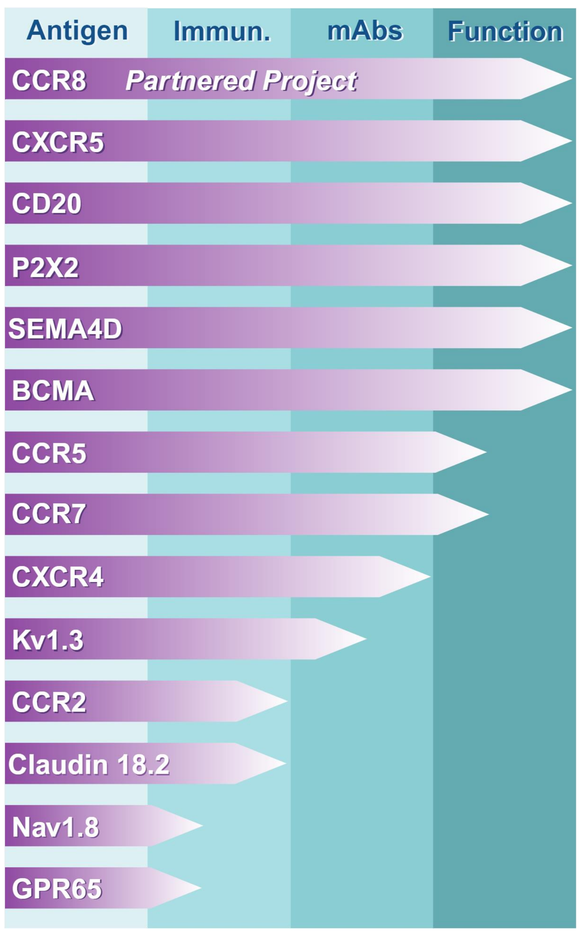
CXCR5+ cells CXCR5- cells

Antibody	Affinity (nM)	% Migration blocking	% Ligand blocking
39027m	0.04	36%	48%
39032m	0.53	44%	74%
39033m	0.34	45%	62%
39034m	0.35	53%	62%



Antibody Discovery Campaign Examples

Antigen	Antigen Type	Unique Antibodies	Unique HCDR3s	Affinity Range	Functional?	Assays
CXCR5	GPCR	266	29	0.04-1.7 nM	Yes	Migration blocking, Internalization
CD20	4-pass	274	77	0.3-50 nM	Yes	Cytotoxicity
P2X2	Ion Channel	161	8	0.4-1.2 nM	Yes	ATP Blocking
SEMA4D	1-pass	241	23	2-50 nM	Yes	Receptor Blocking



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Conclusions

Poxvirus display of complex membrane antigens, including GPCRs, Ion Channels and ECDs in their native conformation provides a versatile tool that can be used both for immunization and selection of strong, specific antibodies. Antigen virions can enhance the efficiency of antibody discovery with B cell sorting from multiple animals which shortens timelines and reduces the costs required to generate larger immunized phage libraries. The use of two antigenically distinct poxviruses with limited membrane diversity facilitates successful antibody selection post-immunization by eliminating any background anti-viral antibodies from the mice. Successful campaigns have been executed for multiple difficult targets including GPCRs and Ion Channels.

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