Antigen Virions for Antibody Discovery Against Transmembrane Proteins

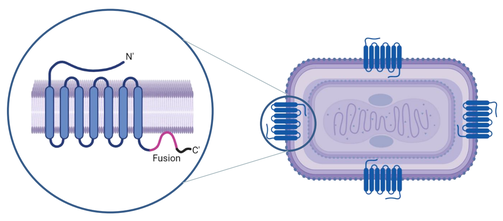
E. Smith, M. Scrivens, L. Mueller, L. Balch, S. Shi, R. Kirk, M. Gil-Moore, E. Gersz, J. Mayer, A. Howell, H. Bussler, E. Evans, M. Zauderer

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 the two antigenically distinct poxviruses: Modified Vaccinia Ankara (MVA) and Fowlpox Virus (FPV). The protein of interest is correctly folded and expressed in the mammalian 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, and immunization with the viral strains produces potent antibody responses in mice. Phage libraries from either naïve or immunized mice can be generated and panned on antigen virions where alternating between the two strains reduces the selection of anti-viral antibodies. Here we give examples of successful antibody discovery campaigns against the GPCRs CCR8 and CXCR5, the transmembrane protein Claudin18.2, and the Ion channel P2X7.

ActivMAb Technology

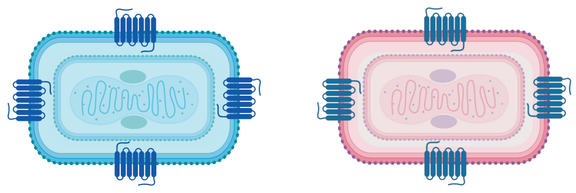
Technology Introduction

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*.

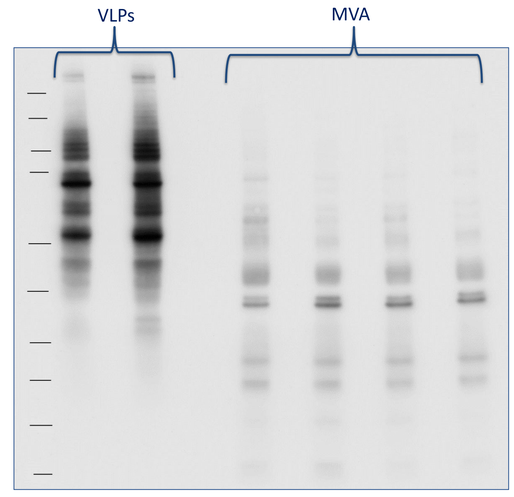


The fusion protein is intra-viral and can be fused with fluorescent proteins like GFP for antigen tracking.

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)

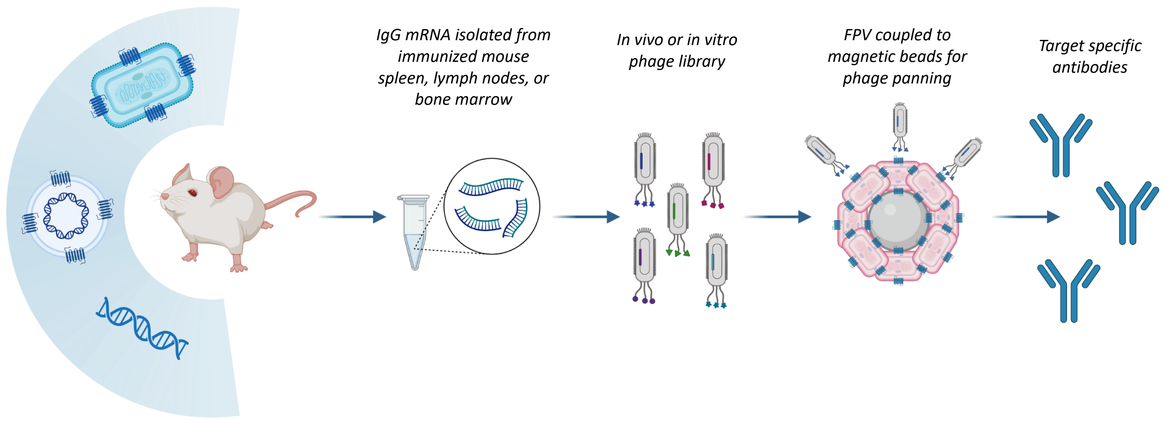


MVA antigen virions contain fewer non-antigen membrane proteins than conventional VLPs.

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

Antibody Discovery Methods

Antigen virions are easily used for *in vitro* panning with phage libraries using traditional methods such as magnetic beads or ELISA plates. For *in vivo* applications, antigen virions can be used as an immunogen alone or in combination with other methods such as mRNA, DNA or cells to generate potent immune responses. The antigen virions can also be labelled with **biotin** using FSL-biotin which inserts into the poxviral membrane to avoid any modification of the protein antigen.



In vitro Phage Panning for CCR8

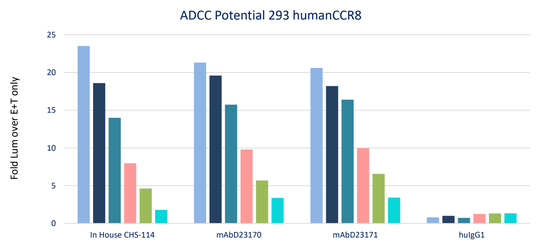
A naïve Fab phage library with an approximate diversity of $2x10^{10}$ clones was used to pan for 4 rounds on Human CCR8 antigen virions. Unique, specific clones were purified and tested for human and cyno binding, affinity and ADCC functionality as compared to a control antibody.

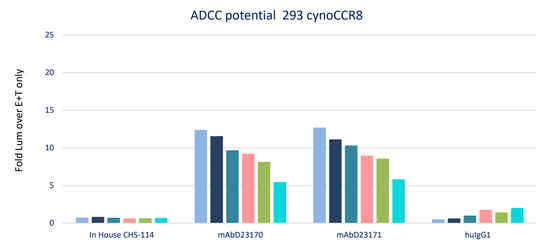
Antibodies were high affinity for Human CCR8, an some had high affinity for Cyno CCR8.

nM Affinity		
	HuCCR8	CynoCCR8
mAbD23028	1.02	0.58
mAbD23170	1.29	0.77
mAbD23171	0.32	0.3
mAbD23164	0.32	8.53

Antibodies showed no off-target binding to PBMCs and strong ADCC killing activity of target cells.

Off-target Binding to PBMCs (Fold over Isotype Control)		
	mAbD23170	mAbD23171
Monocytes	1.09	1.10
CD4+	1.05	1.00
CD25+	0.99	0.98
CD3- Lymphocytes	1.05	0.98

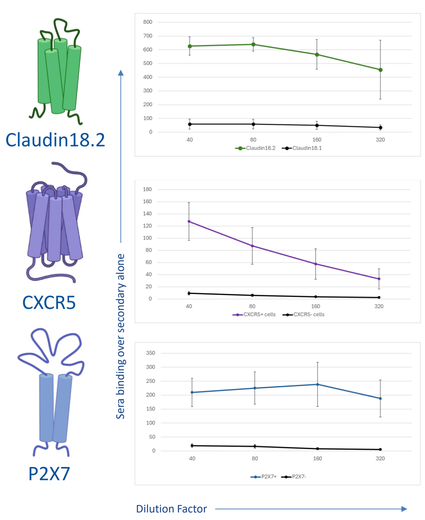




Example *In vivo* Antibody Discovery Campaign Examples

Balb/c or C57Bl/6 mice were immunized with MVA antigen virions expressing human Claudin 18.2, CXCR5 or P2X7 every 14 days for up to 3 immunizations. Sera was tested for antigen specific antibodies by flow cytometry and spleens from the mice with the highest titer were used to generate phage libraries. After panning on FPV antigen virions, unique specific antibodies against all three targets were sequenced, purified and tested for affinity and functionality.

Examples of sera titers from MVA immunized mice

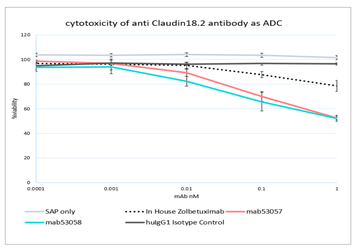


Claudin 18.2	
% Homology with Mouse	88.9%
Unique clones	53 (85.5%)
Unique HCDR3s	17 (27.4%)

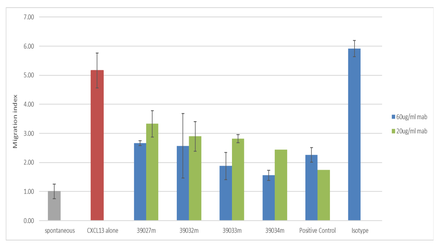
CXCR5	
% Homology with Mouse	83.2%
Unique clones	124 (70%)
Unique HCDR3s	25 (10%)

P2X7	
% Homology with Mouse	80.8%
Unique clones	124 (83.8%)
Unique HCDR3s	16 (10.8%)

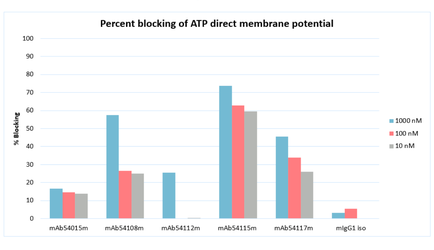
Antibodies show ADC activity



Antibodies inhibit cell migration



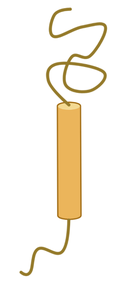
Antibodies show blocking of ATP induced membrane potential



Antigen Virions

GLP1R
FZD4
FZD7
GPR65
GPRC5D

CCR2
CCR5
CCR7
CCR8
CXCR4
CXCR5

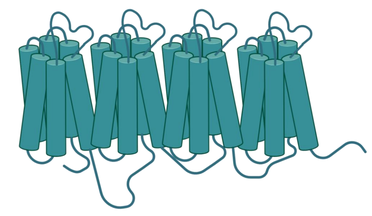


Her2
Cov2-RBD
BCMA

Kv1.3

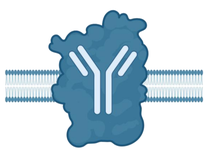
CD20
Claudin 18.2
XPRI
Claudin 6
Claudin 9

P2X2
P2X7



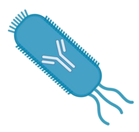
Nav1.8
Nav1.7

Summary of Antigen Virion Capabilities

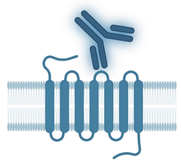


- Retains native conformation and orientation
- No purification required
- Specific viral membrane complexity
- Cleaner than VLPs

- Potent immunogens
- No adjuvant required
- Ready to inject aliquots



- Well suited for phage display
- Two antigenically distinct viruses for alternating selection
- Biosafety level 1 for lab use



- Functional antibody discovery
- Compatible with bead coupling or ELISA
- Biotinylation service available
- Customized for your project needs

SCAN ME

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