APPLICATION NOTE

SALIPRO BIOTECH

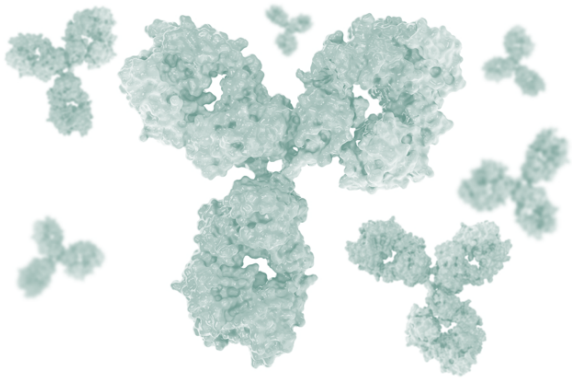
Monoclonal antibodies against native multispanning membrane proteins with Salipro®

Abstract

The majority of small-molecule drugs target multi-spanning membrane proteins. However, it's been difficult to generate functional monoclonal antibodies (mAbs) against these targets. Only few mAbs have been approved for this important class of drug targets that involves GPCRs, Ion Channels and Transporters.

The key to make functional mAbs is to use pure, stable, homogeneous antigen for immunization, mAb selection/sorting and for in vitro HTS characterization.

The Salipro® technology enables all of the above for fragile membrane protein targets. In addition, Salipro® represents a validated approach for membrane protein epitope mapping by cryo-EM. Working with Salipro® allows to unlock entirely novel opportunities in drug discovery and antibody development.



Salipro Biotech collaborates with Top20 pharma company

In this study, an Ion Channel (IC) was reconstituted into Salipro® particles (Salipro-IC) according to previously published protocols (see references). Usually, membrane proteins are inherently unstable and difficult to purify in a detergent environment. Furthermore, the presence of detergent may complicate immunisation and impair subsequent steps in the mAb discovery workflow.

To address this problem, we developed the proprietary Salipro® technology that allows the reconstitution of membrane proteins into a lipid nano-membrane environment (Frauenfeld et al., 2016, Lyons et al., 2017, Chien et al., 2017, Flayhan et al. 2018, Kintzer et al., 2018, Nguyen et al., 2018; Kanonenberg et al., 2019).

FIGURE 1: Reconstitution of membrane proteins into Salipro® particles - Antigen generation.

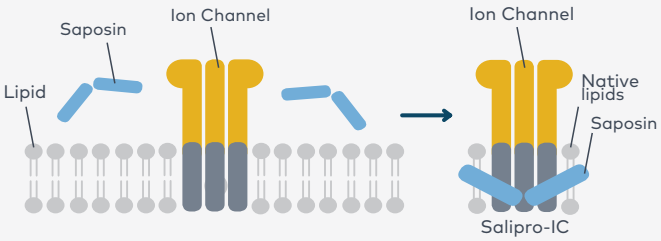
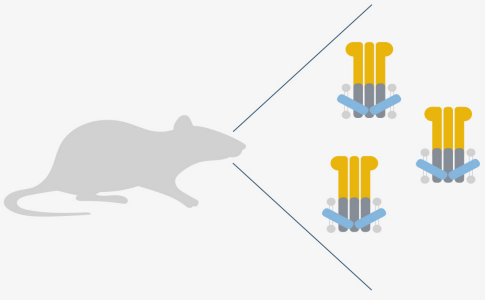


FIGURE 2: Adjuvant-free Immunisation. An ion channel was reconstituted into Salipro® particles (Salipro®-IC) and used for subcutaneous (SC) injection without adjuvants.



The Salipro® technology allows to stabilise, analyse and visualise membrane proteins in a native lipid environment (Figure 1). Membrane proteins embedded in the Salipro® system show increased thermostability and increased half-life while preserving functionality of the drug target (Frauenfeld et al., 2016, Chien et al., 2017, Flayhan et al., 2018, Kanonenberg et al., 2019).

The Salipro® technology can be applied to membrane proteins of all classes from a wide range of cell types and is now frequently used in academic and industry laboratories worldwide.

Results

Here, we demonstrate how multi-spanning membrane proteins in lipid Salipro® particles can be used for the generation and development of membrane protein specific mAbs.
To evaluate the potential of the Salipro® technology in mAb discovery, mice were immunized according to the scheme in Figures 2 and 3, without adjuvants. One group of mice received Salipro-IC as antigen, while a control group received Salipro lipid-only particles that did not contain the Ion Channel. Animals were bled prior to immunization and one week after the third immunization dose. Sera were analyzed using Salipro-IC coated ELISA plates and a strong Ion Channel specific IgG response could be demonstrated post immunization (Figure 4).

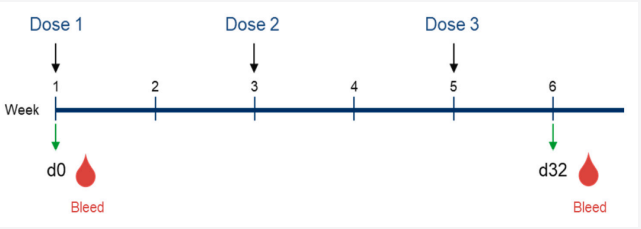
B cells were harvested from the spleen of Salipro-IC immunized mice and FACS was used to sort for activated and class switched B cells.

Salipro-IC particles were then used as full-length Ion Channel probe to sort single B cells that specifically bound to the native membrane protein.

Here, biotin labelling at the Salipro scaffold protein allowed formation of streptavidin fluorescent Salipro-IC particles (Salipro-IC/SA-PE, Figure 5). Moreover, the scaffold protein of empty Salipro particles was covalently labeled with Alexa Fluor 647 (empty Salipro-AF647) and used as a control to distinguish between Ion Channel and scaffold protein B cell interaction (Figure 5). Live and class-switched B cells were first incubated with 20 nM empty Salipro-AF647 particles, followed by the addition of 2 nM Salipro-IC/SA-PE particles.

FACS analysis revealed that the Ion Channel in the Salipro-IC/SA-PE particles bound specifically to a population of the B cells, enabling sorting of Ion Channel specific B cells (Figure 6).

FIGURE 3: Immunisation via SC injection of Salipro®-IC. Empty (lipid-only) Salipro® particles were used as immunization control.



	Host	n/group	Immunogen
Mice	WT A/J	3	Salipro-IC
Mice	WT A/J	3	Salipro lipid-only

FIGURE 4: IgG serology was analyzed using Salipro-IC coated ELISA plates

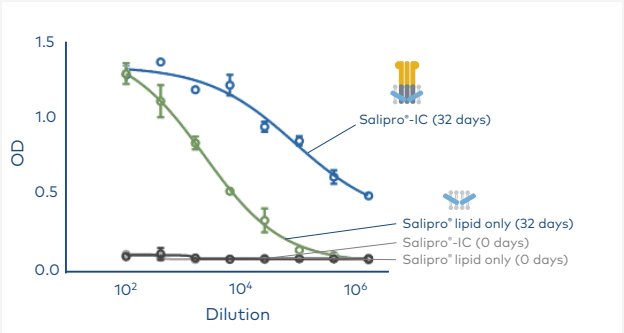


FIGURE 5: Fluorescent labelling of the Salipro® scaffolds, resulting in Salipro-AF647 for empty particles and Salipro-IC/SA-PE for particles containing the Ion Channel.

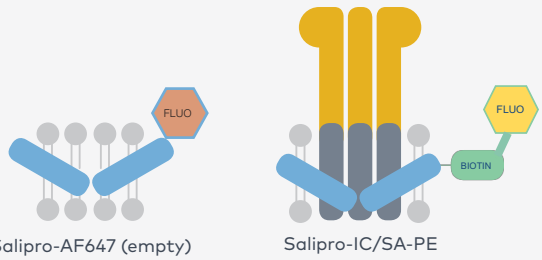
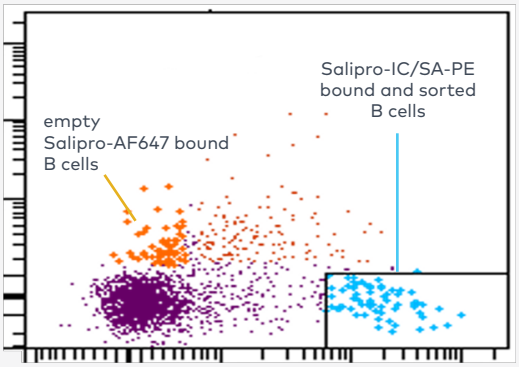


FIGURE 6: Single B-cell sorting, clear separation of cell populations via FACS.



Furthermore, single sorted B cells were seeded into cell culture wells. Initially 1065 wells were cultured, and detectable levels of IgG were seen in 345 of the wells (Figure 7), confirming antigen specfic single cell sorting of antibody producing and live B cells as source for subsequent mAb cloning steps. IgG expression levels from the putative mAb clones are shown in table 1.

This study demonstrates how the Salipro® technology enables mAb generation and discovery using purified and native membrane proteins. The mAb discovery workflow for multi-spanning membrane proteins (GPCRs, Ion Channels, Transporters) becomes as straight-forward as when working with soluble proteins.

Summary

The Salipro® technology makes it possible to work with membrane proteins as pure, soluble, and native nano-membrane entities without the presence of disruptive detergents. As shown in this study, fluorescently labelled Salipro® particles enable single B cell sorting using a purified and native multi-spanning membrane protein. 345 Ion Channel specific mAb leads could be generated using Salipro® in combination with standard immunization and single B cell sorting methodology.

Membrane proteins in Salipro® can be used as immunization antigen, as fluorescent bait (or probe) to sort antigen specific B cells and for in vitro HTS experiments using the full-length and functional membrane protein at all stages. In addition, Salipro® represents a validated approach for epitope mapping in combination with cryo-EM (Figure 8). In summary, Salipro® unlocks novel opportunities in mAb discovery.

References

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FIGURE 7: Sorted B cells produce IgGs in culture. 345 out of 1065 wells expressed detectable levels of IgG (32.4%)

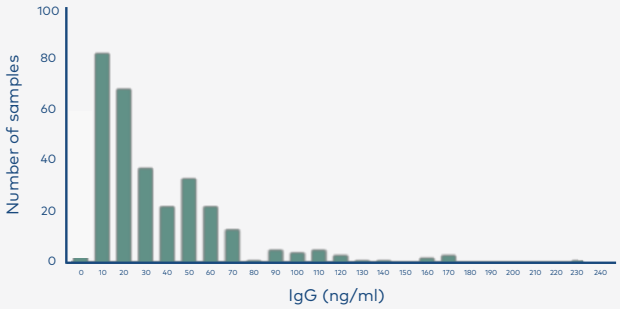
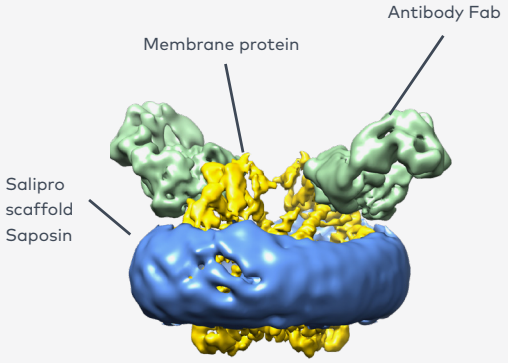


TABLE 1: . IgG quantification from 345 Ion Channel specific mAb lead cultures.

IgG quant	n	% of IgG positive	% of total
< 1 ng/ml	4	1.2	0.3
> 1 ng/ml < 10 ng/ml	82	23.8	7.7
> 10 ng/ml < 20 ng/ml	69	20	6.5
> 20 ng/ml < 50 ng/ml	116	33.6	10.9
> 50 ng/ml < 100 ng/ml	57	16.5	5.4
> 100 ng/ml	18	5.2	1,7

FIGURE 8: Epitope mapping with Salipro® and cryoEM. Structure of a membrane protein (orange) embedded in a lipid Salipro® environment (blue), with two Fab fragments bound (green) (Kintzer et al., 2018)



Antibody generation against a full-length membrane protein within a native lipid environment:

"Salipro®- it works like a soluble protein"