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Generation of membrane proteins in polymer-based lipoparticles as flow cytometry antigens

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ABSTRACT

A major obstacle in the study of membrane proteins is the challenge in generating purified targets in a stable and native conformation. Potentially destabilizing detergents are often used to extract targets out of their native membranes. One approach to overcome these destabilization issues is to present targets in a lipid bilayer environment. While numerous lipid bilayer presentation approaches such as Nanodiscs and liposomes have been used through the years, most still require a detergent purified protein intermediate. Styrene-maleic acid lipoparticles (SMALPs) have been developed to directly solubilize membranes without the use of detergents and present membrane proteins in a native lipid bilayer environment. These discoidal lipid bilayer particles have been evaluated on several membrane protein classes (*e.g.* ion channels, transporters) and applications (*e.g.* spectroscopy, electron microscopy). Here, cannabinoid receptor 1 (CB1), a class A G-protein coupled receptor, was incorporated into a SMALP (CB1-SMALP) and shown to retain its native fold through binding studies with a CB1-specific conformational antibody. The binding studies were conducted in yeast and mammalian display formats using fluorescence-activated cell sorting (FACS). Compared to detergent preparations, CB1-SMALP also exhibited a marked increase in thermostability. This study provides the framework for a platform to generate full-length membrane protein screening antigens for flow cytometry-based studies.

1. Introduction

Integral membrane proteins play significant roles in a wide variety of essential cellular processes including vital functions as receptors, transporters, and channels [1]. Additionally, they represent an important class of drug targets with ~60% of approved drugs [2] and nearly all large molecule therapies targeting this class. Typically, purified membrane proteins for *in vitro* studies are prepared using detergents to extract targets out of their native lipid membranes and facilitate downstream purification [3]. However, detergents are poor membrane mimetics which can lead to destabilization and loss of function of the target membrane protein [4]. One of the approaches to overcome the destabilization issues is to reformulate membrane proteins in a stabilizing lipid bilayer environment. Presentation of membrane proteins in such an environment has been achieved in several platforms including Nanodiscs [5], liposomes [6], and bicelles [7], with varying degrees of success and utility. However, most of these platforms still require detergent purified protein as an intermediate step.

Recently, styrene-maleic acid copolymers (SMAs) have been used to generate nanometer-sized particles to present membrane proteins in a native lipid bilayer environment [8]. These SMA co-polymers are composed of repeating units of styrene and maleic acid building blocks, that in the presence of lipids, form planar disc-like lipid bilayer particles called SMA lipoparticles (SMALPs) [9]. Compared to other lipid bilayer presentation approaches, SMALPs have the distinct advantage of being able to directly solubilize lipid membranes circumventing the need for potentially destabilizing detergents. Furthermore, these particles retain endogenous lipids which could be useful in maintaining stability and/or function. SMALPs have been shown to efficiently present a diverse set of membrane proteins (of different classes) in their native conformation [10]. They have shown particular utility in numerous functional studies including enzymatic activity, spectroscopic characterization, and substrate binding [10].

To expand the toolkit of applications compatible with this technology, we present the use of SMALPs as a platform to generate full-length membrane protein antigens for flow cytometry-based applications, such as fluorescence-activated cell sorting (FACS). In this study, we prepared the human cannabinoid receptor 1 (CB1), a class A G-protein coupled receptor involved in neurotransmitter release [11], in a SMALP (CB1-SMALP) and showed that it is in its native conformation through binding studies with a CB1-specific conformational antibody (CB1 Ab). Moreover, incorporation into SMALPs imparts additional

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stability to the target compared to typical detergent preparations. The binding studies of CB1-SMALP to the CB1 antibody were performed in two display formats, yeast and mammalian display, and showed only CB1-specific binding. This work outlines a broadly applicable direct solubilization and lipid bilayer presentation approach to generate full-length membrane proteins antigens for flow cytometry-based studies.

2. Materials and methods

2.1. Materials

The SMA copolymer used for preparing SMALPs had a styrene to maleic acid anhydride ratio of 2:1 and a weight-average molecular weight of 7.5 kDa (SMA2000, Cray Valley). Before use, the SMA copolymer was hydrolyzed following previously disclosed protocols [12]. The *n*-dodecyl-β-D-maltoside (DDM) used for detergent purification was procured from Anatrace.

2.2. Methods

2.2.1. Construct design and protein expression

A Vκ1 signal peptide followed by a caspase-cleavable FLAG tag was introduced to the N-terminus of the pFastBac vector (Invitrogen) containing CB1 1-472 amino acid backbone followed by a C-terminus caspase-cleavable TagGFP2 and 8xHis tag. Virus was prepared using the Bac-to-Bac baculovirus expression system (Gibco BRL). Viral stocks were made and amplified in *Sf9* insect cells. Protein production was carried out in *Sf9* cells (Expression Systems) in shake flasks with 2.5% virus addition at 27 °C for 48 h.

The *Escherichia coli* production of a chimeric KcsA-Kv1.3 with an N-terminal 6xHis tag has been previously described [13]. For both CB1 and KcsA-Kv1.3, cells were harvested by centrifugation and the cell pellets were collected.

2.2.2. SMA/Detergent solubilization and protein purification

The method for preparing SMALPs was modified from the published protocol by Lee et al. [12]. Cell paste (overexpressing CB1 or KcsA-Kv1.3) was resuspended in buffer A (50 mM Tris pH 8.0 and 500 mM NaCl) with protease inhibitors. The cells were lysed using a tip sonicator for 2 min at 30 s on/off intervals. SMA solubilization was done on either whole cells or isolated membranes. For SMALPs prepared through a membrane isolation intermediate, the lysed cells were subjected to centrifugation at 9000*g* for 45 min. The supernatant was collected and ultracentrifuged at 100,000*g* for 1 h to pellet the membranes. The isolated membrane pellet was then dounce homogenized in buffer A until a confluent suspension was achieved.

Lysed whole cells or isolated membranes were solubilized by titrating 2.5% (w/v) SMA copolymer in buffer A (SMA stock solution) until the turbid suspension visibly clarified. This typically required approximately 10-fold w/v of SMA stock solution compared to the starting material. The clarified solution was rotated overnight at 4 °C. Solubilization of whole cells required ~4x more SMA stock solution volume compared to isolated membranes. Insoluble fractions were removed by ultracentrifugation for 1 hr at 100,000 *g*. Next, the supernatant was collected and imidazole was added to a final concentration of 10 mM. Pre-equilibrated (in buffer A) Ni-NTA resin (Thermo Fisher) was then added to the supernatant and rotated overnight at 4 °C. The resin was allowed to settle into an empty column and underwent sequential washes with 20 column volumes (CV) buffer A, 10 CV buffer A + 50 mM imidazole, and 10 CV buffer A. Bound proteins were eluted with buffer A + 300 mM imidazole. The imidazole was removed by dialysis against buffer B (50 mM Tris pH 7.5 and 150 mM NaCl) overnight at 4 °C using a Slide-a-Lyzer cassette (MWCO 3000, Thermo Fisher). Samples were concentrated to the desired concentration using Vivaspin spin concentrators with the appropriate MWCO.

CB1 detergent purification (for control experiments) was done in a similar process to the SMALP purification with a few marked differences. Solubilization was done with 8.5 mM DDM (50x CMC) for 2 h and all succeeding buffers contained 0.5 mM DDM. Binding to the Ni-NTA resin was done in 4 h instead of overnight.

2.2.3. T_m measurement

Purified CB1 in either SMALP or DDM was incubated at a set temperature between 20 and 100 °C for 5 min using a thermocycler. Samples were spun at 20,000 g to remove precipitate. Supernatants were chromatographed on an Agilent Infinity HPLC with a fluorescence detector (emission detection at 488-nm) with a Tosoh G4000SWXL column (1 mL/min) for fluorescence size exclusion chromatography (FSEC). The area under the peak chromatogram for each temperature was measured for each sample and plotted using Graphpad Prism. T_m was calculated based on the inflection point of the plotted areas.

2.2.4. Dynamic light scattering

Dynamic light scattering (DLS) experiments were performed on a Nano Zetasizer (Malvern Instruments) equipped with a He-Ne laser. CB1-SMALP samples were sterile-filtered (0.2 um) and transferred into 40 μL disposable microcuvettes for DLS measurements (15 scans, 10 s each).

2.2.5. Surface plasmon resonance

Surface plasmon resonance (SPR) binding studies were conducted using a Biacore T200 system (GE Healthcare). A Biacore CM5 chip was used to create an anti-CB1 antibody (CB1 Ab) functionalized surface. Specifically, CB1 Ab was introduced onto the 1-ethyl-3-(3-dimethylaminopropylcarbodi-imide (EDC)/N-hydroxysuccinimide activated carboxy surface for 8 mins, followed by injection of 1 M ethanolamine for 7 mins to inactivate the remaining free carboxyl groups. The binding response of CB1- or KcsA-Kv1.3-SMALP to the immobilized CB1 Ab was measured after a 20 min incubation at a flow rate of 10 μL/min.

2.2.6. Yeast display of CB1 Fab

The CB1 Fab heavy and light chain sequences were inserted into two separate plasmids by transformation into a *Saccharomyces cerevisiae* yeast strain auxotrophic for uracil and leucine. In this Fab yeast display system, the heavy chain is fused to the C-terminal domain of alpha agglutinin (on the yeast cell wall surface), and a galactose-inducible promoter controls expression of both chains. After transformation, the yeast cells were propagated at 30 °C in synthetic complete media lacking tryptophan, uracil and leucine containing 2% w/v dextrose (SCD –WUL). To induce Fab display, the fully grown SCD –WUL yeast culture was diluted 10-fold into SCGR –WUL (same as SCD –WUL except substituting 2% w/v galactose and 2% w/v raffinose for dextrose) and incubated at 20 °C for 48–72 h [14]. Prior to input into binding assays, the induced yeast cells were harvested by centrifugation and washed thoroughly with phosphate-buffered saline supplemented with 5 mg/mL bovine serum albumin.

2.2.7. Yeast display binding experiments

Yeast displaying specific Fabs were aliquoted at 1 x 10^6 cells/well and washed in 1x PBS (no Mg^{2+} no Ca^{2+}) with 0.5% BSA (PBS-BSA). Antigens (in SMALP or DDM) were added at a concentration of 500 nM and incubated at 25 °C for 1 h. The cells were washed with 1x PBS-BSA and incubated with Dylight 650-conjugated anti-Fab (Fisher scientific HAB2111) at 5 μg/mL for 30 min to detect surface display. The cells were then washed thrice with 1x PBS-BSA and resuspended in the same buffer. The plate was then read on an LSR II flow cytometer (BD Biosciences) and for emission at 488-nm and 650-nm wavelengths. For all FACS plots shown, quadrant thresholds were defined using double-negative binding experiments [binding of uninduced yeast to buffer alone (no antigen)]. Therefore, quadrant 1 (Q1, *top left*) is positive for antigen binding and negative for antibody display. Quadrant 2 (Q2, *top right*) is positive for both antibody display and antibody binding.

Conversely, quadrant 3 (Q3, *bottom left*) is negative for both antibody display and antigen binding. Lastly, quadrant 4 (Q4, *bottom right*) is positive for antibody display and negative for antigen binding.

2.2.8. Mammalian display CB1 Ab

For CB1 Ab and an irrelevant control antibody, the V_H and V_L sequences were cloned into a bicistronic acceptor plasmid containing their respective heavy and light chain constant domains, a hygromycin resistance gene, and *Lox*P recombination sequences for stable cell line integration (Innovative Targeting Solutions). The plasmid allowed for surface expression of the respective IgGs through fusion of the heavy chain to the transmembrane domain of platelet-derived growth factor receptor (PDGFR). Cells from a modified HEK293 cell line (Innovative Targeting Solutions) were transfected with the plasmid and a Cre recombinase expression vector (10:1 DNA ratio) using the transfection reagent polyethylenimine (PEI) in Opti-MEM media (Gibco). Transfection was carried out for 48 h and a pool of stable hygromycin resistant cells were selected (100 μg/mL) and used for binding studies.

2.2.9. Mammalian display binding experiments

Cell lines were aliquoted at 0.5–1 × 10^6 cells/well and washed in 1x PBS (no Mg^{2+} no Ca^{2+}) + 2% FBS. The CB1-SMALPs were added at a concentration of 500 nM and incubated on ice for 1 h. The cells were washed twice with 1 × PBS + 2% FBS and then incubated with Dylight 650-conjugated anti-Fab antibody (Fisher Scientific HAB2111) at 5 μg/mL for 15–30 mins to detect expression. The cells were then washed twice with 1 × PBS + 2% FBS and resuspended in the same buffer. The plate was read on the LSR II flow cytometer (BD Biosciences) for emission at 488-nm and 650-nm wavelengths.

3. Results and discussion

3.1. Membrane protein SMALP formation and characterization

To facilitate purification, biophysical characterization, and to enable FACS-based binding experiments, human CB1 receptor was engineered with an N-terminal FLAG tag and C-terminal tandem GFP and 8xHis tags. This construct was expressed in *Sf9* insect cells followed by solubilization and purification optimization based on published protocols (see Methods for more detail). Two methods for SMA solubilization, utilizing either isolated membranes or whole cells, were screened for optimal solubilization efficiency. Both purifications were done by Ni^{2+}-affinity chromatography in the batch method. SDS-PAGE analysis of the eluates showed comparable purity of the CB1 receptor with the whole cell solubilization method being marginally more pure (Fig. 1A). Two distinct bands at ~70 kDa (expected molecular weight) and ~80 kDa were observed in the gels and have both been confirmed by Western blot analyses to be full-length CB1 receptor (data not shown). Currently, we have no evidence that different glycosylation states of CB1 account for the observed anomalous differential migration in SDS-PAGE gels. Possible explanations for this observation are incomplete unfolding in SDS and/or differential SDS solvation, both of which have been noted in many membrane proteins [15]. Compared to a typical DDM detergent purification (Fig. 1A), the SMA purification resulted in lower CB1 receptor yield but higher purity. Based on these results, the ensuing CB1-SMALP samples in this paper were prepared using the whole cell solubilization method.

Taking advantage of the fluorescence from the GFP fused to CB1, we used a modified fluorescence size exclusion chromatography (FSEC) protocol to determine the melting temperature (T_m) of CB1 in either SMALP or DDM to gain insight into the stability of each preparation [16]. Briefly, samples were subjected to increasing heat stress before FSEC. The area under each FSEC profile was measured and the T_m was then calculated based on the inflection point of these measurements (Fig. 1B). In this assay, CB1-SMALP had a significantly higher T_m of 72.4 °C compared to 63.6 °C for CB1 prepared in DDM (Fig. 1B). Further characterization of CB1-SMALP by dynamic light scattering (DLS) analysis indicated a hydrodynamic diameter of 15 nm, slightly larger than previously reported for other targets (12 nm) [8,17] (Fig. 1C).

3.2. SPR binding experiments

Maintaining native fold is necessary to demonstrate successful incorporation of a membrane protein target into a SMALP. To this end, we utilized an in-house CB1-specific conformational antibody (CB1 Ab) in binding studies to validate native conformation. Surface plasmon resonance (SPR) was employed for conducting the initial antibody binding experiments [18]. Preliminary studies with CB1-SMALP immobilized on the SPR surface and CB1 Ab as the analyte showed no CB1-specific binding (data not shown). However, inverting the screening format with CB1 Ab immobilized on the surface and the CB1-SMALP as the analyte showed a binding response (Fig. 2). For testing the specificity of this interaction, a control membrane protein in SMALP (ion channel KcsA-Kv1.3 in SMALP) was introduced to the CB1 Ab-immobilized surface, which exhibited some non-specific binding (~35%). We hypothesize that the inherent negative charge of the SMA polymer could facilitate non-specific binding of large molecule analytes. Nevertheless, because a portion of the SPR signal was still CB1-specific, the data suggests that the CB1 in SMALP was in its native conformation. While a binary assessment of binding was possible in these experiments, the high non-specific binding (~35%) would make determinations of kinetic binding properties difficult. Further optimization of experimental parameters may ameliorate some of the non-specific binding issues associated with SMALPs. For example, reducing the density of immobilized moieties may decrease the contribution of electrostatic patches on the SPR surface which in turn could reduce non-specific binding. Alternatively, the development of zwitterionic SMA polymers [19] may circumvent the issue by counterbalancing the negative charge.

3.3. Display binding experiments

Surface display technologies are powerful protein engineering tools because of their ability to screen a large number of protein sequence permutations [20]. Here we applied two display approaches, yeast and mammalian, to demonstrate native conformation binding of the SMALP reagents as well as compatibility of these reagents on flow cytometry-based applications.

Yeast display binding experiments were conducted using flow cytometry with the Fab portion of the CB1 Ab (CB1 Fab) displayed on the yeast surface and different membrane proteins antigens. The fused GFP on CB1 provided the fluorescence binding signal and bypassed the need for exogenous antigen labeling. Induction of Fab surface display was monitored using a Fab-specific antibody conjugated with Dylight 650. As expected, binding was not observed with CB1 antigens (SMALP or DDM) incubated with uninduced yeast cells (Fig. 3, *first row*). Membrane proteins prepared in detergent are notoriously unstable so binding experiments were first done with freshly prepared antigens. For both CB1-DDM and CB1-SMALP, binding was observed with induced yeast cells, indicating native conformation for the CB1-SMALP preparation (Fig. 3, *second row*). To assess the stability of the antigens, they were subjected to a freeze-thaw cycle before binding to yeast-displaying Fab. After a single freeze-thaw of the CB1-DDM antigen, the percentage of yeast cells that maintained binding to the antigen decreased from 24.2% to 13.1%. In contrast, CB1-SMALP treated identically had a minimal decrease in binding (18.6% to 17.8%) (Fig. 3, *third row*). To further evaluate the stability of the CB1 preparations, the antigens were stressed with 15 consecutive freeze-thaw cycles and CB1-SMALP still retained binding to yeast displaying CB1 Fab while the CB1-DDM sample did not show binding (Fig. 3, *fourth row*). This information combined with the increase in T_m mentioned earlier highlight the increased stability imparted by incorporation into SMALPs. Considering

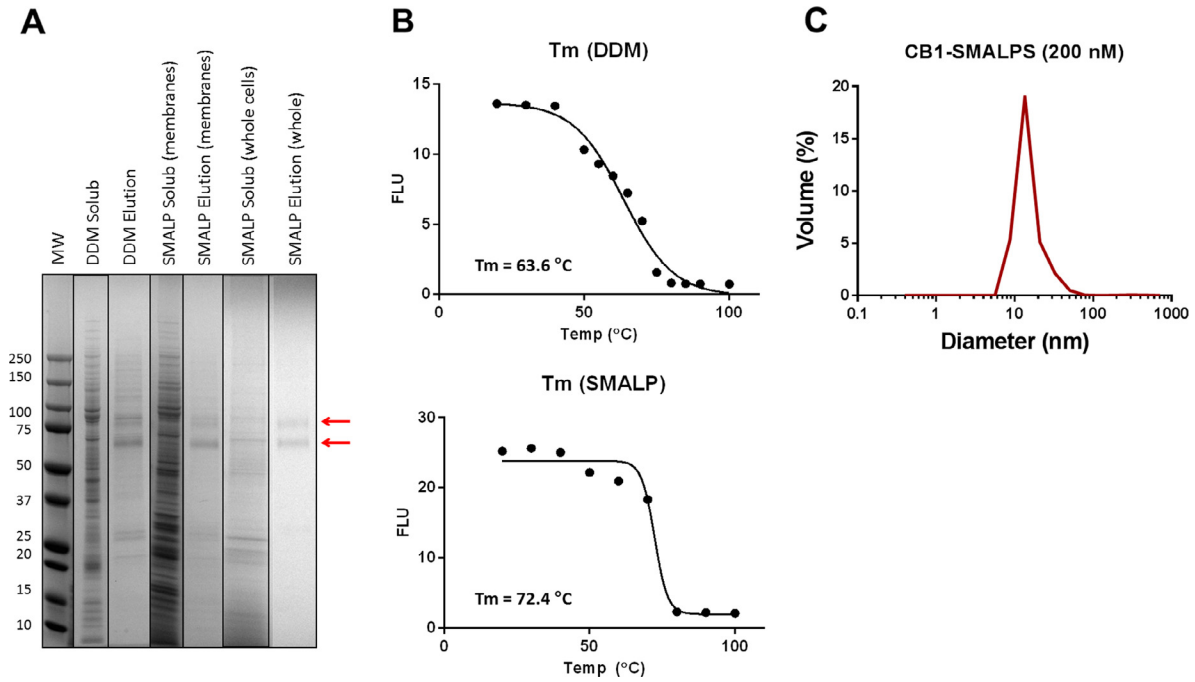

Fig. 1. Purification and characterization of CB1-SMALP. (A) SDS-PAGE analysis of CB1 in detergent and SMALP (whole cell and isolated membrane preparation) reflected the solubilization efficiency and purity of each preparation. Two bands were attributed to CB1 (red arrows). (B) T_m determination of CB1-DDM and CB1 SMALP based on analytical FSEC after heat stress indicated increased stability of the SMALP preparation. (C) Volume-weighted size distributions of CB1-SMALP obtained for dynamic light scattering (DLS) showed an average diameter of ~15 nm (see Fig. S1 of the Supplementary Materials for intensity-weighted size distributions). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

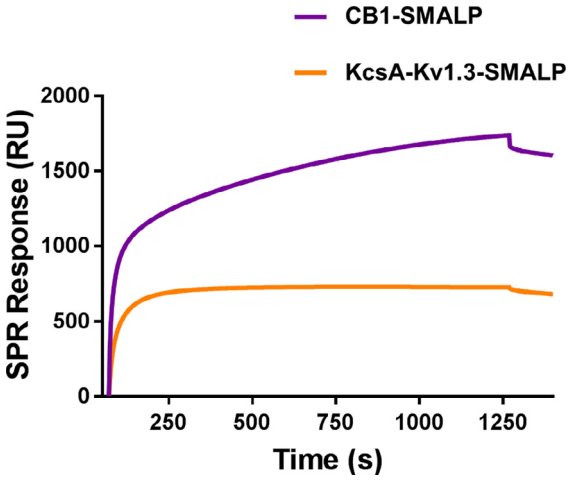

Fig. 2. SPR binding experiment. Overlaid SPR sensograms of CB1- (purple) and KcsA-Kv1.3-SMALPs (orange) flowed over immobilized CB1 Ab. Although a CB1-specific binding signal was observed for CB1-SMALP, a portion of the signal was attributed to non-specific binding as illustrated by the binding of KcsA-Kv1.3-SMALP. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the non-specific binding issues with SMALPs and SPR, CB1-SMALP antigens were also screened against yeast cells displaying an irrelevant Fab. No binding was observed with the CB1-SMALP antigen (Fig. 3, *last row*). The non-specific binding issue observed in SPR was likely not an inherent property of SMALPs but may be a result of incompatibility with the (surface-based) assay format.

For the mammalian display binding experiments, antibodies were displayed on the surface of cells as whole IgGs. CB1-SMALP binding was screened against a cell line engineered with CB1 Ab displayed on the surface. As a negative control, an irrelevant membrane protein antibody was also engineered in a separate cell line to screen for non-specific binding in this assay. Binding to CB1-SMALP was monitored on both cell lines and only observed in the cells displaying CB1 Ab, again indicating native conformation of CB1 in the SMALP and no observable non-specific binding (Fig. 4).

Considering the diversity and challenging nature of studies requiring full-length purified membrane proteins, a single “one size fits all” solution to prepare targets has yet to be achieved. While detergent-based purifications are still the most common approach for generating reagents, detergents are poor approximations of the native lipid bilayer environment and are known to disrupt/destabilize targets. Consequently, screening and selection of the appropriate detergent is required for each step of the reagent generation process (*i.e.* solubilization, stability, specific application). The appropriate detergent needs to be present above the critical micelle concentration (CMC) in downstream processes to maintain structure and function. Furthermore, detergent purified membrane proteins need to be verified that they have maintained their native state.

The two lipid bilayer approaches that are most comparable to SMALPs are liposomes and Nanodiscs. Liposomes are spherical unilamellar vesicles formed from lipids and can incorporate membrane protein targets into their lipid bilayer (proteoliposomes) [21]. These have particular utility when the application requires compartmentalization such as with transporter assays [22]. However, orientation (inward vs. outward) of membrane proteins in these particles is difficult to control and effectively, about half the target is presented the opposite orientation. They have also been useful in generating membrane protein immunogens, likely due to their size (~100 nm) helping to elicit an immune response [23]. Nanodiscs are discoidal lipoparticles formed using a truncated version of apolipoprotein A1 called membrane scaffold protein (MSP) at their periphery [24]. The specific MSP construct used to form the Nanodisc determines the size of the particle and varies from 9.8 to 12.8 nm [25]. Nanodiscs have been used in numerous applications including NMR, Resonance Raman, functional studies, cryo-EM, and SPR [26]. With SPR, they do not have the same non-specific binding issues we observed with SMALPs, likely due to the net neutral

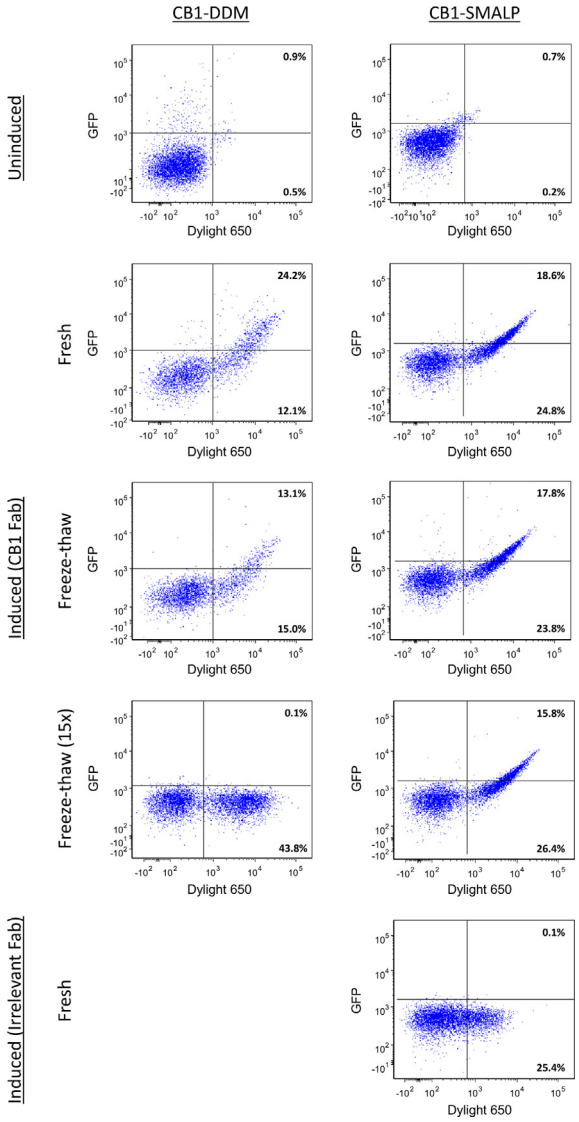

Fig. 3. Yeast display binding experiments. Binding experiments of CB1-DDM and CB1-SMALP to yeast displaying anti-CB1 or irrelevant Fab. The x-axis shows display of the Fab as monitored by Dylight 650 fluorescence and the y-axis shows binding of the antigen to the yeast through GFP fluorescence. (*Top row*) Uninduced cells did not bind to either preparation. (*Second row*) Freshly prepared CB1 in DDM and SMALP bound to yeast displaying the CB1 Fab indicating native conformation. (*Third row*) Decrease in binding of the DDM preparation compared to SMALP after a single freeze-thaw cycle. (*Fourth row*) Loss of binding of CB1-DDM after 15 freeze-thaw cycles; CB1-SMALP still retained binding to the yeast displayed CB1 Fab. (*Last row*) No binding observed for the CB1-SMALP to yeast displaying an irrelevant Fab. Relative Fab display levels in these experiments are available in Fig. S3 of the Supplementary Materials.

charge of the Nanodisc particle. A stable Nanodisc preparation also requires target-dependent optimization of multiple factors including ratios between target, lipids, and MSPs. The primary drawback for both liposomes and Nanodiscs is the requirement of detergent-purified membrane protein before formation of the respective particles. Additionally, both approaches require the screening and addition of exogenous lipids.

The polymer-based SMALP has been broadly used in presenting membrane protein targets in a native lipid bilayer environment bypassing the need for detergents and exogenous lipids. Several of these membrane proteins in SMALPs have been reported to be in their native conformation using a variety of applications [10]. For example, the ABC transporter MRP1 in SMALP has been shown to bind the ligand estrone sulfate in radiolabeled binding experiments [27]. In the case of the GPCR adenosine A_{2A} receptor ($A_{2A}R$), not only was it reported as functional in a SMALP but it also had increased thermostability compared to detergent preparations [28]. Our data with CB1-SMALPs illustrates yet another membrane protein amenable to SMALPs and mirrors many of these reports of increased membrane protein stability.

The use of SMALPs is not limited to the functional reconstitution of membrane proteins. Applications have broadened into structural studies such as the crystal structure of *H. walsbyi* bacteriorhodopsin determined using SMALPs and lipidic cubic phase (LCP) [29]. This was the first reported crystal structure of a membrane protein in LCP without the aid of detergents. More recently, the novel cryo-electron microscopy (cryo-EM) structure of alternative complex III (ACIII) with 48 transmembrane α-helices was determined using SMALPs [30]. A more unique application of SMALPs was the characterization of the local lipid environment around the *E. coli* expressed potassium channel KcsA [31]. Biochemical analysis of the lipids in KcsA-SMALP revealed it was enriched with anionic lipids.

The display technologies shown here can be used to screen libraries of proteins for such applications as modulating protein-ligand affinity, increasing protein stability, and altering enzyme activity [20]. In some of these applications, it may be necessary to generate screening reagents. Case in point, display technologies can be used to affinity mature antibodies to target membrane proteins [32]. The simplest antigen generation strategy is to generate the soluble extracellular domain (ECD) of a target. However, this may not be applicable in all cases and a full-length membrane protein reagent may be required. We have shown here that SMALPs can be used as an accessible platform to generate such reagents. Assuming the target is functional in the expression system, generating membrane protein reagents in SMALPs is particularly advantageous because maintaining a target in a lipid bilayer throughout purification increases the likelihood that the target is in its native state when functional assays and/or tool molecules (*i.e.* ligands, conformational antibodies) are not available.

4. Conclusion

We have shown that CB1 can be incorporated into a SMALP, retains its native conformation, and has increased stability. In the process of confirming the native conformation of CB1 in SMALP, we have also demonstrated SMALP's compatibility with flow cytometry and some of its drawbacks when applied to surface-based methods like SPR. As demonstrated here, SMALP is a straightforward method for generating functional membrane protein screening reagents. Development of a robust, broadly applicable detergent-free direct solubilization and lipid bilayer presentation approach such as this could expand the list of prosecutable membrane protein targets such as those not amenable to detergent solubilization.

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Declarations of interest

None.

Data availability

The raw/processed data required to reproduce these findings cannot be shared at this time due to legal reasons.

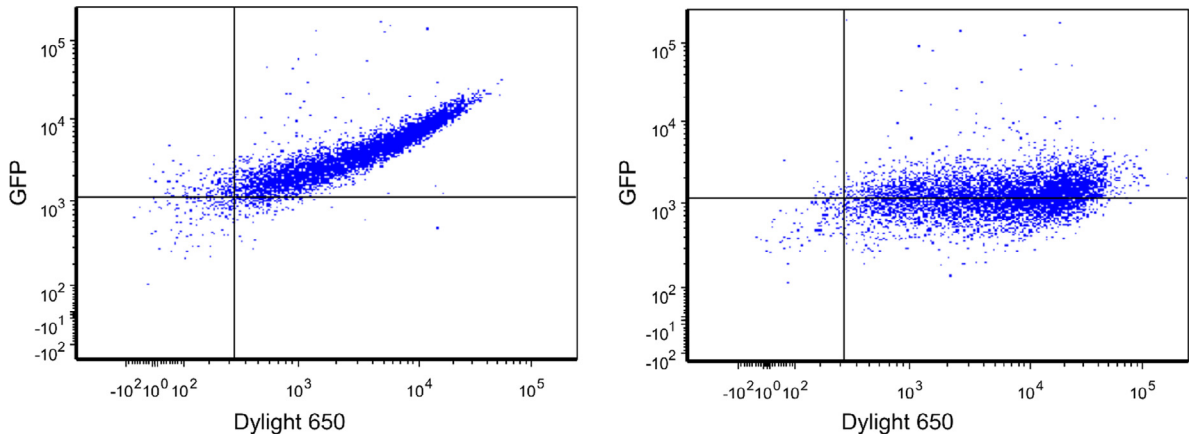

Fig. 4. Mammalian display binding experiments. Mammalian cells were engineered to display either CB1 Ab or an irrelevant Ab. Antibody surface display (x-axis) and binding (y-axis) were monitored by appropriate fluorescence signals, Dylight 650 and GFP, respectively. CB1-SMALP bound to cells displaying CB1 Ab (*left*) but not to those displaying the irrelevant Ab (*right*). Relative Ab display levels in these experiments are available in Fig. S4 of the Supplementary Materials.

Author contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.eurpolymj.2018.10.017.

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