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A novel cell permeability assay for macromolecules

Yensi Alejandra Flores Bueso
University College Cork https://orcid.org/0000-0002-2118-2195

Sidney Peter Walker
University College Cork National University of Ireland

Mark Tangney (m.tangney@ucc.ie)
https://orcid.org/0000-0002-6314-1260

Methodology article

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Abstract

Background

Many cell permeabilisation methods to mediate internalisation of various molecules to mammalian or bacterial cells have been developed. However, no size-specific permeability assay suitable for all cells exists.

Results

We report for the first time, use of intrinsically biotinylated cell components as the target for reporter molecules for assessing permeabilisation. Due to its well-described biotin binding activity, we developed an assay using Streptavidin (SAv) as a molecular weight marker for assessing eukaryotic and prokaryotic cell internalisation, using flow cytometry as a readout. This concept was tested here in the development of host DNA depletion strategies for microbiome analysis of formalin-fixed samples. This strategy requires differential cell permeabilisation, where mammalian cells but not bacterial cells are permeabilised, and are subsequently treated with a nuclease. Here, the internalisation of a SAv-conjugate was used as a reference for nucleases of similar dimensions. With this assay, it was possible to demonstrate that FF does not generate pores which allow the introduction of 60 KDa molecules in both mammalian and bacterial membranes/envelopes. Among surfactants tested, Saponin showed the best selectivity for mammalian cell permeabilisation, which, when coupled with Benzonase nuclease, provided the best results for host DNA depletion, representing a new host depletion strategy for formalin fixed samples.

Conclusion

The assay presented provides researchers with a sensitive and accessible tool for discerning membrane/cell envelop permeability for different size macromolecules.

Background

The role that different macromolecules play within the cellular milieu is routinely studied with *in vitro* techniques that involve their *ex vitro* modification (labelling) and subsequent cellular internalisation [1]. Since the membrane of live mammalian cells is virtually impermeable against polar and charged molecules with a molecular weight (MW) larger than ~ 118 Da [2, 3], and the outer membrane of Gram-negative (G-) bacteria is only permeable to hydrophilic molecules smaller than ~ 600 Da [4], the internalisation of biomolecules must be artificially induced. For *in vitro* studies, this is achieved by permeabilising the cell membrane/envelope [5]. A plethora of permeabilisation methods have been developed for mammalian cells [6], and to a lesser extent for bacteria [7], including solvents, detergents, toxins, and enzymes [8, 9]. Routinely used permeabilisation agents include solvents (Alcohol, acetone),

detergents (Triton X-100, Saponin), toxins (crotalicidin, streptolysin-O), and enzymes (Lysozyme, Proteinases) and even hydrochloric acid [5, 8, 9]. However, no single method has yet been found suitable for every cell type or biomolecule and the evaluation of their efficacy is established empirically, in a case-by-case basis, which is laborious and often irreproducible. This can be partially attributed to the restricted methods available, which are only able to assess membrane permeability for small molecule membrane impermeable dyes, such as SITOX, YO-PRO, PI (< 1 KDa) and 7-AAD (1.3 KDa) [10, 11]. Methods for examining cell permeability to large molecules are not standard. Thus, we hypothesised that an accessible method enabling a comprehensive assessment of cell permeabilisation, in terms of molecular weight (MW) cut-offs and applicable to all cell types, could facilitate cell permeabilisation assessment.

Such a method could be enabled by exploitation of intrinsic cellular motifs as targets for reporter molecules of a set size. Analogous assays for examining cell permeability to small molecules utilise cellular DNA as the target for reporters (DNA-binding small molecule dyes such as Propidium Iodide and 7-AAD (7-amino-actinomycin D)). For examination of macromolecules, we here exploited biotin as the target, an intrinsic and essential co-factor for many enzymes in all domains of life, from prokaryotes [12] to eukaryotes [13]. Streptavidin (SAv), strongly binds biotin in one of the strongest ($K_d \sim 10^{-15}$ M), highly specific, and rapid interactions observed in nature [14], and has been exploited for many purposes [15]. SAv is a globular tetramer, with a MW of ~ 52 KDa dimension of 5 nm and each monomer is able to interact with a biotin molecule [16]. With this information, we developed a new assay, using flow cytometry as a readout. Here, the detection of naturally biotinylated intracellular proteins by SAv serves as a MW marker for cell internalisation. This assay can be easily adapted for research on different biomolecules in eukaryotic or prokaryotic cells and is scalable to high-throughput settings.

This concept was tested here for the development of a host depletion strategy in FF samples for microbiome analysis. The *in vitro* study of cells often requires their fixation [17]. Formaldehyde is the most widely adopted fixative, since it preserves the overall cellular structure, although partially permeabilising cellular membranes/envelopes [18, 19]. For microbial analysis, FFPE archives could provide access to an unprecedented number of samples [20]. A key consideration however, similar to non-fixed samples, is that a high ratio of host to bacterial DNA impairs effective microbial profiling [21]. Thus, several host depletion strategies with different levels of success have been published for non-fixed samples [22]. However, to date this has not been investigated for FF cells. Here we aimed at studying the permeabilisation state of FF bacterial and mammalian membranes/envelopes and assess the permeabilisation efficacy of non-ionic surfactants for FF cells, as required for host depletion strategies [23]. A SAv-conjugate with similar dimensions to DNAse was used as a marker for cell internalisation, allowing a clear assessment of the surfactants' permeabilisation efficacy. The assay was validated by assessing nuclease activity with qPCR and flow cytometry.

Results

Study Overview

The aim of this study was to develop an accessible and universal assay for assessing cell permeabilisation for *in vitro* analysis, such that it is applicable to any cell type (eukaryotic or prokaryotic), and for multiple macromolecules. To achieve this, we hypothesised that: *1)* permeabilisation could be defined in terms of a universal macromolecule size feature, such as Molecular Weight (MW). *2)* An intrinsic cellular factor could serve as an internalisation marker for molecules of different MW.

To test these hypotheses we sought an easily accessible internalisation marker and found Streptavidin (SAv) as an ideal candidate. As such, we used SAv to design an easily accessible assay that only requires labelling of cells with the SAv-conjugate of choice, after permeabilisation. Labelled cells can be analysed by flow cytometry (as performed here) or any by other means compatible SAv and available to researchers. See sFigure 1a for the workflow of this assay. In this setting, the SAv-conjugate binds cell intrinsic biotin only if the cell membrane/envelope is permeable to it. Thus indicating whether the permeabilisation method used is effective for molecules with similar MW. This approach was tested here in bacterial (*E. coli*) and mammalian (4T1) cells, with 4 different permeabilisation agents and 2 different SAv conjugates. In addition, the assay was validated with a functional experiment assessing the internalisation and activity of a nuclease with dimensions similar to those of the SAv conjugate tested, as expanded below.

SAv allows the assessment of membrane permeabilisation

Streptavidin-Cy5 served as the cell internalisation marker for Benzonase nuclease, a dimeric nuclease, with a MW of 60 KDa [24], each monomer corresponding to the dimensions of DNAse I – a compact monomers with a MW of ~ 30 KDa and dimensions of 4.6 × 4 × 3.5 nm [25]. Benzonase exhibited the highest levels of activity of 6 DNAses examined (sFigure 2b). The permeabilisation capability of FF and 4 non-ionic detergents for 60 KDa molecules was assessed in 4T1 and *E. coli* cells following the workflow in sFigure 1a. Here, impermeabilised 4T1 cells showed significantly less SAv-Cy5 fluorescence than those exposed to detergents (Fig. 1a) and only *E. coli* cells (Fig. 1b) treated with Triton-X were permeabilised, as evidenced by a 363X increase in fluorescence ($p < 0.001$). This indicates that fixation does not permeabilise cells to large molecules. Among the detergents tested, Saponin displayed the highest membrane selectivity capacity for SAv, with a 186X ($p < 0.001$) increase in fluorescence for 4T1 cells and no significant fluorescence change for *E. coli* ($p > 0.05$). This held true for *E. coli* cells exposed to higher Saponin concentrations (sFigure 2a). When a larger molecule was examined – 360 KDa SAv-PE – internalisation was much lower (2%-25%) than that observed for SAv-Cy5, as expected, although patterns of detergent efficacy varied, with internalisation only detectable for Digitonin (10.8%) and Saponin (25%) (sFigure 3).

Validation of the permeabilisation strategy by nuclease activity

Nuclease activity in permeabilised cells was tested by measuring the fluorescence emitted by a cell permeable, double-stranded DNA intercalating dye (CytoPhase Violet), after treatment with a permeabilisation (P+) agent and Benzonase (sFigure 1b). A reduction in CytoPhase signal is indicative of a reduction in DNA content, and thus higher nuclease activity. Results in Fig. 2 reflect those in Fig. 1.

Permeabilised cells exhibited lower CytoPhase signal. For 4T1 cells, the most significant CytoPhase signal reduction (30.8%, $p < 0.001$) was observed for cells permeabilised with Saponin. Conversely, for *E. coli* Saponin did not lead to any significant decrease in fluorescence (4.5% decrease, $p > 0.05$), while treatment with Triton-X (P + DNAse + control), showed a 43.7% ($p < 0.001$) decrease. It was also noticeable that harvesting or pre-treatments did not significantly affect the integrity of the E. coli cells envelope, as impermeabilised cells exposed to Benzonase did not show a significant decrease in CytoPhase signal. These results were verified by qPCR, in a mixed FF cell population, with 1×10^7 *E. coli* and 1×10^6 4T1 cells, where cells exposed to the Host DNA depletion (HD) strategy were harvested and DNA purified. Eluted DNA was analysed by qPCR. As seen in sFigure 2c, for 4T1 cell, the quantity of genomes retrieved after HD were reduced by a log-fold ($p < 0.01$). On the other hand, HD treatment allowed for a higher (truer) representation of bacterial DNA, which exhibited a 3X ($p < 0.01$) increase in the number of genomes recovered. Altogether, these results validate the permeabilisation assessment strategy and confirm that Saponin shows the best cell selective permeabilisation capacity.

Discussion

In this study, we developed a universal assay to evaluate cell permeabilisation of biomolecules that can reduce the case-by-case evaluation and optimisation of permeabilisation strategies. Since cell internalisation is directly proportional to molecule size [2], a universal evaluation of permeabilisation can be achieved by establishing MW cut-offs, where MW internalisation markers indicate the permeabilisation efficiency expected for biomolecules of similar size. For this purpose a MW marker should fulfil two key criteria: 1) Universality - that is applicable to all cell types, and 2) Size modulation, that the size of the marker can be adjusted to different sizes investigated. Fitting these criteria is the Biotin-SAv interaction, since Biotin is an intrinsic cell co-factor present across all domains of life [12, 13], and thus allows the evaluation of permeabilisation treatments in a wide variety of cells, as disparate as the Gram-negative *E. coli* and 4T1 murine cancer cells examined here. This is exemplified in Fig. 1, where Saponin and Tween-20 proved effective at permeabilising 4T1 cells but not *E. coli*. In addition, different forms of SAv (monomeric, dimeric) [16], coupled with the plethora of available SAv conjugates of different MW, provide for size modulation, as demonstrated in Figs. 1 and s2 for SAv-Cy5 (60KDa) and SAv-PE (360 KDa).

By implementing a MW internalisation marker, the proposed approach defines the efficiency at which biomolecules of different sizes can be internalised into the cells tested. As shown in Figs. 1 and s2, the 4T1 permeabilisation efficiency of treatment with Saponin was < 25% for the 360 KDa size molecule, and > 70% for the 60 KDa molecule for 4T1 cells. This serves to report on permeabilisation efficiency based on reporter molecule size. Reporter molecule size may be an important overlooked factor that contributes to the controversial efficiencies reported for available permeabilisation protocols, which has forced researchers to optimise protocols for each cell type and biomolecule studied, as reflected by the plethora of biomolecule and cell type specific permeabilisation methods publication [7, 26, 27].

To reduce the need for this case-by-case approach, the assay developed in this study provides an accessible universal platform applicable for multiple cell types and biomolecules. Here, the MW-cut off

for a permeabilisation treatment in a cell type is established by using markers with the MW of interest. All biomolecules with MW equal or below that of the marker subjected to the specified treatment will exhibit the same degree of permeability as the marker, without the need of optimisation. This was demonstrated and validated here, since SAv-Cy5 could correctly predict whether a nuclease of comparable dimensions could gain entry to the targeted cells (Figs. 1 & 2).

This protocol was validated in the evaluation of host depletion strategies in FF samples for microbiome analysis. Here, structural differences between mammalian membranes and G- bacterial envelopes inform the choice of permeabilisation agent [28]. While both, the mammalian and the G- bacterial outer membrane (OM) are mostly composed by phospholipids [29, 30], the mammalian bilayer also contains variable contents of cholesterol [29, 31] and the OM of G- bacteria contains a tightly packed lipopolysaccharide (LPS) structure that protects it against surfactants [32, 33]. This favours the use of non-ionic detergents [28], which exclusively target cholesterol (Saponin and Digitonin) [34, 35]. Different levels of success have been reported for host depletion strategies in non-fixed (NF) samples [22], but not for FF samples. While the underlying principles guiding permeabilisation agent of choice proposed are valid for NF samples, these do not always apply to FF samples [19, 36]. This was investigated here, and it is clear from the results of this study that formalin fixation does not induce pores allowing the entrance of 60 KDa molecules in both mammalian and G- bacterial cells. This highlights the need for bacterial lysis strategies in the processing FF samples for microbial analyses.

It was also proven here that whilst all permeabilisation agents tested induce pores in mammalian cells, Saponin had the highest mammalian cell selective permeabilisation capability. This was confirmed by HD experiments, where a marked reduction in DNA quantity was observed after treatment with Saponin + Benzonase for host (4T1) cells only (Figs. 2 & 3). These results are supported by recently-published evidence on non-fixed samples, for Saponin [23, 37] and Benzonase [22, 36]. This information can provide foundational knowledge for the development of host depletion strategies for formalin-fixed, paraffin-embedded (FFPE) tissues, and assist in unlocking the potential of FFPE samples, which could provide researchers with unprecedented access to samples.

Conclusions

Here is presented an accessible and universal assay for assessing cell permeabilisation for macromolecules, applicable for multiple biomolecules and cell types (Prokaryotes and Eukaryotes). The universality of this assay was achieved by defining permeabilisation in terms of MW-cut offs and by defining a MW marker that is intrinsic to all types of cells. Given the nature of the analyses and the uniformity of the marker used across cell types, this assay is well suited for scaling to high-throughput experiments, allowing in-parallel permeability assessment. Furthermore, while not explored in this study, the protocol presented could be adapted for the study of live cells or cells fixed with other fixation strategies.

Material And Methods

Cell culture. *Mus musculus* mammary gland cancer cells (4T1) were grown at 37^{o}C 5% CO2, in Roswell Park Memorial Institute (RPMI) media supplemented with 10% v/v Foetal Bovine Serum, 100 U/mL penicillin and 100 µg/mL of streptomycin (ThermoFisher). The cells were harvested with 0.5 ml/10 cm^{2} trypsin by centrifugation at 180 x g, washed with Phosphate Buffer Saline (PBS), and counted with a NucleoCounter® NC-100™ (chemometect, Copenhagen) following manufacturer's instructions. The cells were fixed in 40 ml of 4% w/v buffered formalin for 24 h at room temperature (RT).

Bacterial growth conditions. *Escherichia. coli* K12 MG1655 carrying a P16Lux plasmid [38] was grown aerobically at 37^{o}C to an OD_{600} of 0.8 in Luria-Bertani (LB) medium supplemented with 300 µg/ml Erythromycin and harvested by centrifugation at 3000 x g, for 10 min at 4^{o}C, suspended to a 2X concentration in 4% w/v buffered formalin for 24 h at RT.

Counting fixed bacterial cells. Bacterial cell suspensions were counted following the instructions of the bacterial counting kit (Invitrogen). In brief, after fixation, a 10% aliquot was taken from this suspension and serially diluted (100X) with filtered sterilised 0.15M NaCl solution to obtain a cell density of approximately 1×10^{6} cells in 989 µl of NaCl. Bacterial cells were stained with 1 µl of SytoBC and 10 µl (1×10^{6}) of counting beads were added to the suspension. Cells were counted in an LSR II Flow Cytometer (BD Biosciences, NJ, USA). The acquisition trigger was set to side scatter and set to 800.

Membrane permeabilisation assay. After fixation, 8×10^{7} 4T1 cells were harvested at 180 x g for 10 min, washed once with 20 ml of Tris Buffer Saline (TBS) (50 mM Tris, 150 mM NaCl, pH 7.6), and suspended to a final density of 2.5×10^{6} cells per ml in TBS. Similarly, 5×10^{9} *E. coli* cells, were harvested at 300 x g for 10 min, washed once with 20 ml of TBS and suspended to a final density of 2.5×10^{7} cell per ml in TBS. 500 µl of the cell suspensions were aliquoted into 1.5 ml tubes and treated with a permeabilisation agent. Permeabilisation agents tested were: Triton X-100 (0.1% v/v), Tween-20 0.2% v/v, Saponin (0.1% w/v), Digitonin (0.5 µg/ml). All were acquired form Sigma-Aldrich. Concentrations used were as described for several protocols [6].

The cells were permeabilised for 25 min, at 25^{o}C, shaking at 500 rpm. Permeabilised cells were washed once with TBS (centrifugation speeds as above) and blocked on ice with TBS + 1% w/v Bovine Serum Albumin (BSA) for 30 min. Blocked cells were exposed to 0.75 µg of Cyanine-5 (Cy5) or Phycoerythrin (PE) labelled Streptavidin (SAv-Cy5, MW = 60 KDa or SAv-PE, MW = 360 KDa) (Biolegend, CA, USA) for 30 min at 25^{o}C, shaking at 280 rpm. Cells were washed with 1 ml of 0.15 M NaCl solution and resuspended in 350 µl of the same solution for analysis. Bacterial cells were also labelled with 1 µl of SytoBC (Invitrogen) for 5 min and analysed by flow cytometry in a BD LSRII. 4T1 Cells were identified and gated based on their Forward/Side scatter and *E. coli* cells were detected using the 488-1 (Fluorescein isothiocyanate - FITC), 525/50 filter for SytoBC and gated using the side scatter. Cy5 positive cells were detected with the red 670/14 filter. PE positive cells were detected with the yellow/green 780/60 filter. For each experimental replicate, 3 × 10,000 events were recorded for 4T1 cells and 3 × 100,000 for bacteria.

DNAse screening. A screen for selecting the DNAse that had highest activity in depleting DNA in a reaction buffer containing Saponin. DNAses tested: Recombinant DNAse I [1-2U, 1 µl] (Sigma-Aldrich), Turbo DNAse [2U, 1 µl] (Thermo-Fisher), Molysis DNase [2 µl] (Molzym GmbH & Co, Bremen, Germany), RQ1 DNAse [20U, 20 µl] (Promega), Benzonase [75 U, 0.3 µl] (Sigma-Aldrich). 5×10^6 4T1 cells, FF for 48 h were treated with 0.2% w/v Saponin and the DNAse tested. Reactions were set in reaction buffers provided or suggested by supplier for 20 min at 37^{o}C. The reaction was stopped by either: the addition of Ethylenediaminetetraacetic acid (EDTA) for Benzonase, the supplied reaction Stop Buffer, or by incubating at 75^{o}C (DNAse I). After which, cells were subject to DNA purification with the QIAamp DNA Mini Kit (QIAGEN). DNA yield was measured with Qubit™ dsDNA HS Assay Kit (Invitrogen). All reactions were performed in triplicate. A no-DNAse control was included. This was incubated under the same conditions with buffer supplied for DNAse I, but without the nuclease.

Saponin Titration. Different w/v saponin concentration (0.1%, 0.25%, 0.5%, 1%) were tested in 1×10^6 *E. coli cells* that were fixed, washed, permeabilised, blocked and imaged as described for membrane permeabilisation assay.

DNA depletion assay. Cells were fixed, washed and permeabilised as described for the membrane permeabilisation assay. 2.5×10^5 4T1 or 2.5×10^6 *E. coli* cells were permeabilised, blocked with 500 µl of 1% BSA in TBS + $MgCl_2$ (20 mM Tris-HCL, 20 mM NaCl, 2 mM MgCl2, pH 8) for 30 min on ice. Blocked cells were treated with 1.5 µl (≥ 375 units) of Benzonase nuclease (Sigma-Aldrich) for 30 min at 37^{o}C, shaking at 360 rpm. Treatment was stopped by the addition of 100 mM EDTA. The cells were washed once with TBS and suspended in 0.15M NaCl, where they were stained with 10 µM CytoPhase Violet (Biolegend) for 1 h at RT, shaking at 200 rpm in the dark. Bacterial cells were labelled with 100 µM of BacLight red (Invitrogen) for 15 min at RT, shaking at 200 rpm and analysed by flow cytometry. 4T1 cells were identified and gated based on their Forward/Side scatter and *E. coli* cells were detected using the 561 laser (Yellow/Green) 660/20 filter for BacLight red and gated using the side scatter. CytoPhase + cells were detected with the 355 (UV) laser and 450/50 filter.

Confirmation of host depletion (HD) strategy. The efficacy of the combined treatment was verified by qPCR in DNA purified from a mixed cell suspension, consisting of 1×10^7 *E. coli* cells and 1×10^4 4T1 cells. Cells were incubated for 30 min at 37^{o}C, shaking at 360 rpm in TBS or the optimised HD buffer (0.2% Saponin, in TBS + $MgCl_2$ (20 mM Tris-HCL, 20 mM NaCl, 2 mM $MgCl_2$), pH 8) with or without 500 U of Benzonase. The treated cells were then processed for DNA purification following instructions of the QIAamp DNA FFPE Tissue Kit (QIAGEN) and the purified DNA analysed by qPCR.

Quantitative PCR (qPCR). Reactions were prepared using LUNA Universal qPCR master mix (NEB, USA) and 0.25 µM of each primer (Table 1). The thermal profile included a 1 min at 95^{o}C initial denaturation, followed by 40 cycles of denaturation at 95^{o}C x 10 sec, annealing for 15 sec at the temperature specified by NEB's annealing temperature (Ta) calculator for Hot Start Taq, followed by 20–40 sec of extension at 68 ^{o}C. For each assay, a 5-point standard curve was made from $\log_{10}$ dilutions of gene blocks

corresponding to species-specific genetic regions (Table 1), using an initial concentration of 10^7 copies. Primers and gene-blocks were acquired from IDT (Coralville, USA). Efficiency between 95% – 105% and R-square values > 0.995 were deemed as acceptable. All samples were run in triplicate.

Table 1
Primers used for qPCR analysis

Strain/Cell line	Gene/ Accession No	Primer/Probe sequence	F/R	Product size (bp)
E coli MG1655 [CP032667]	IS5-like element IS5 family transposase AYG17556.1 [CP032667: 230175–231191]	5'GCC GAA CTG TCG CTT GAT GA	F	217
		5'ATT TGT CTC AGC CGA TGC CG	R	
4T1 cells [ATCC® CRL-539™] *Mus musculus* [10090]	BetaActin AC144818.4 [NC000071.6: 73696 – 73082]	5'GAT TAC TGC TCT GGC TCC TAG	F	147
		5'GAC TCA TCG TAC TCC TGC TTG	R	

Statistical analyses. Flow cytometry data was exported and analysed in FlowJo (BD, UK) and raw data exported to R. All statistical testing and visualisation was performed in the R environment (v3.6.3). Tests of means performed using paired samples T-Test, and visualisation performed using GGplot2 package (v3.2.1) within the R environment.

Abbreviations

BSA: Bovine Serum Albumin

Cy5: Cyanine 5

DNAse+: DNAse positive

coli: Escherichia coli

FBS: Foetal Bovine Serum

FF: Formalin fixed

FFPE: Formalin Fixed Paraffin Embedded

FITC Fluorescein isothiocyanate

HD: Host DNA Depletion

KDa: Kilodalton

$MgCl_2$: Magnesium chloride

NaCl: Sodium chloride

NF: Non-fixed

OD_{600}: Optical density at 600 nanometres

P+: Permeabilisation positive

PBS: Phosphate-buffered saline

PE: Phycoerythrin

qPCR: Quantitative Polymerase Chain Reaction

RT Room temperature (25^{o}C)

SAv: Streptavidin

Ta: Annealing temperature

TBS: Tris-buffered saline

Tris-HCl: Trisaminomethane hydrochloride

Declarations

Ethics approval and consent to participate: Not Applicable

Consent for publication: Not Applicable

Availability of data and materials: All data generated or analysed during this study are included in this published article. However, raw flow cytometry data is available from the corresponding author on reasonable request

Competing interests: The authors declare that they have no competing interests

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Authors' contributions: YFB & MT conceived and designed the study. YFB executed laboratory work. SPW & YFB performed data analyses. YFB & MT interpreted results and wrote the manuscript. All authors read and approved the final manuscript.

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Figures

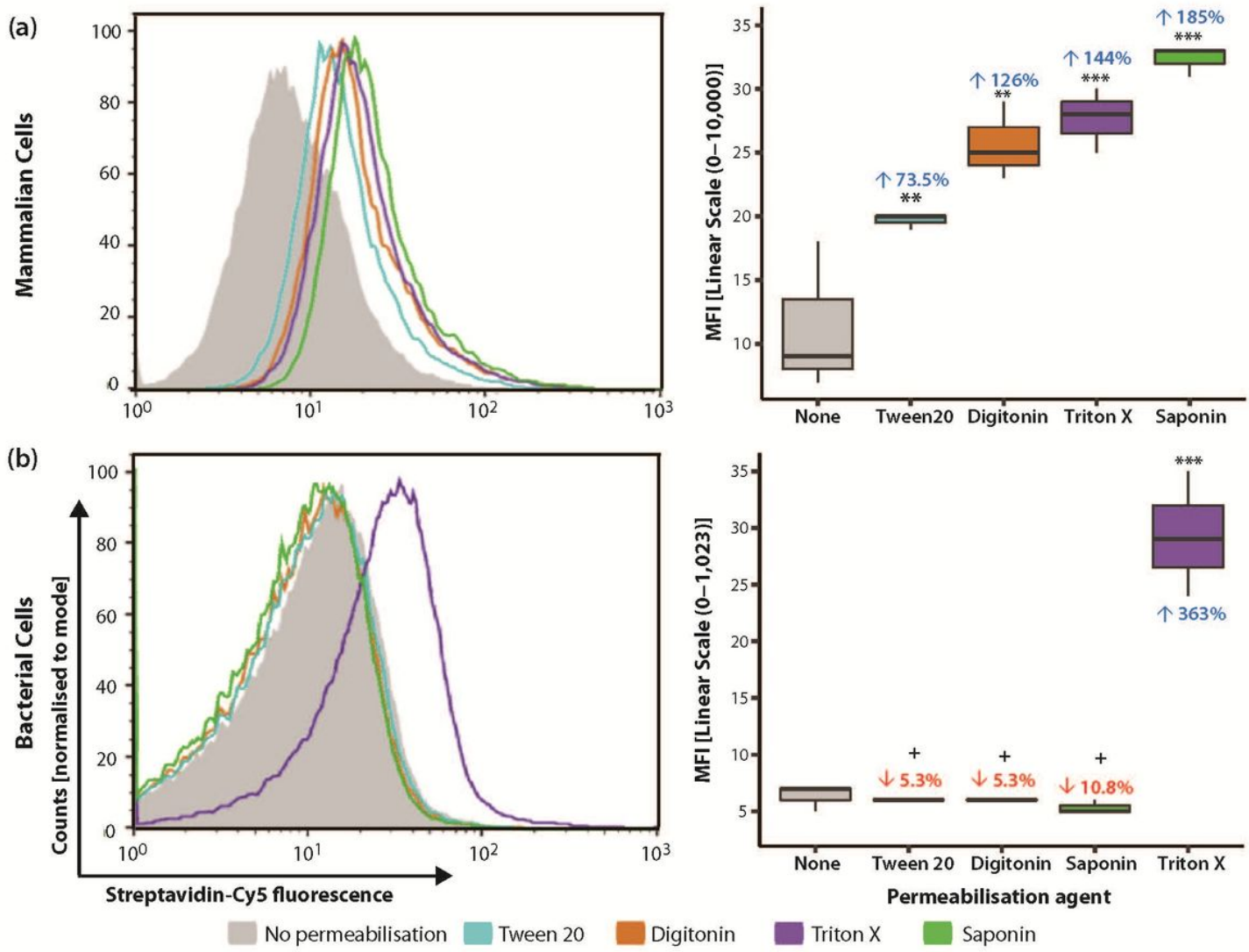

Figure 1

Membrane permeabilisation. Cell permeabilisation is measured by the internalisation of SAv-Cy5 for (a) 4T1 cells and (b) E. coli. (Left) Histograms showing Cy5+ maximum fluorescence intensity (n = ⍰ 6). (Right) Box plot showing median fluorescence intensity. Deviation (%) from impermeabilised shown above each box. In all cases p = +<0.1, * < 0.05, ** < 0.01, *** < 0.001 and n = 6.

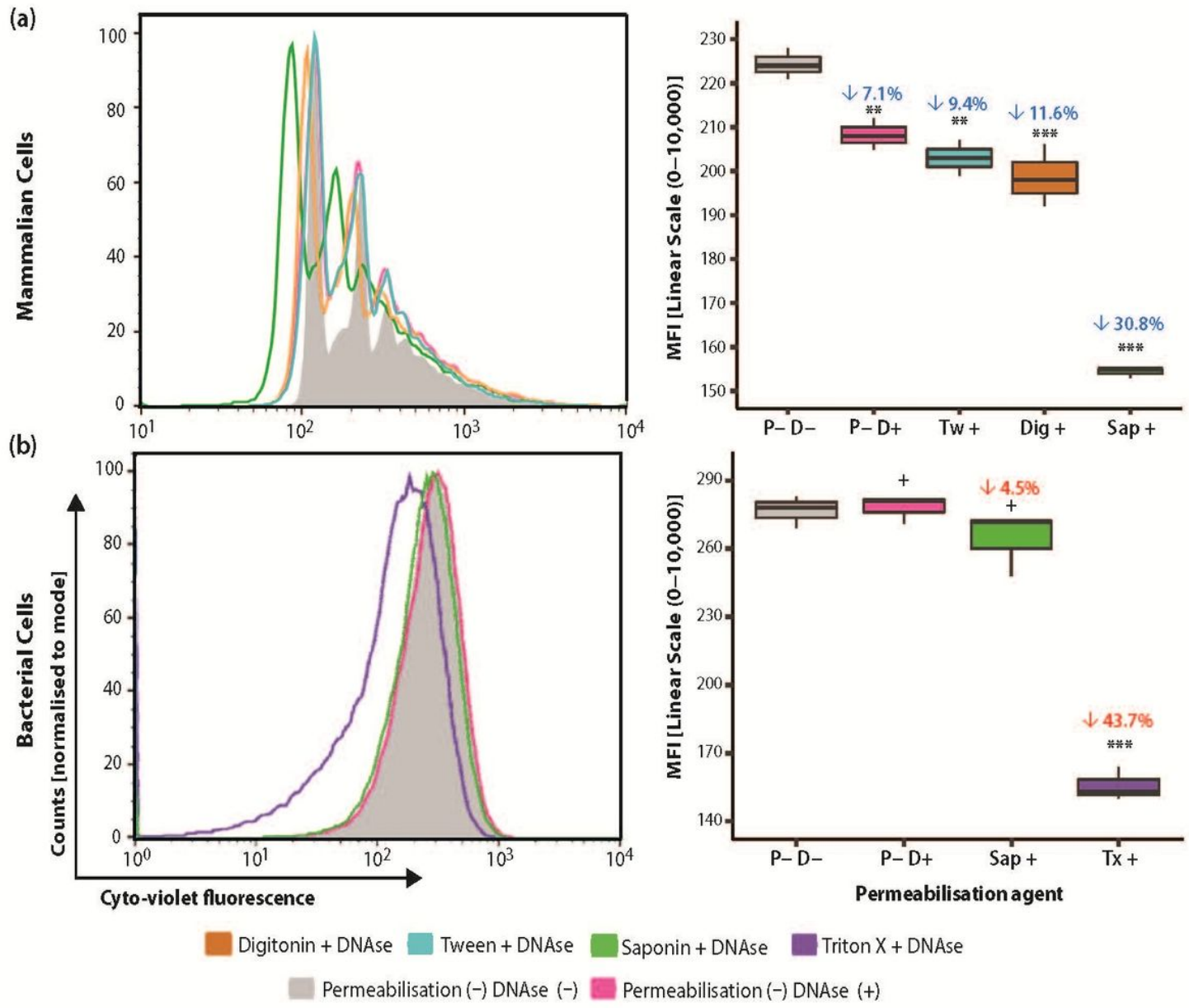

Figure 2

DNA depletion. DNA depletion is measured here by a reduction in fluorescence of the double-stranded DNA intercalating dye CytoPhase, measured for (a) 4T1 cells and (b) E. coli. (Left) Histograms showing the maximum fluorescence intensity for CytoPhase+ cells. (Right) Box plot showing median fluorescence intensity.. Figure Deviation (%) from impermeabilised DNAse- shown above each box. In all cases p = + <0.1, * < 0.05, ** < 0.01, *** < 0.001 and n = 6.

Supplementary Files

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- sFigure3SAvPEassessmentofpermeabilisation.pptx

- sFigure2Optimisationandconfirmationofhostdepletion.pdf
- sFigure1experimentalapproach.pptx
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