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The non-Newtonian behavior of detergents during concentration is increased by macromolecules, in *trans*, and results in their over-concentration

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ABSTRACT

Concentration of pure membrane proteins in detergent solution results in detergent concentration, albeit in unknown amounts. This phenomenon is observed in every lab working on membrane proteins, but has seldom been investigated. In this study, we explored the behavior of detergents mixed with membrane proteins during the step of sample concentration using centrifugal devices. We show that detergent over-concentrate with the presence of polymers, typically membrane or soluble proteins but also polysaccharides. The over-concentration of detergents depends on centrifugal force applied to the device. With the use of a specific dye, we observed the formation of a mesh on the concentrator device. Importantly, reducing the centrifugal speed allows to reduce the concentration of detergents when mixed to macromolecules, as tested with 3 different membrane proteins. All together, these results highlight the non-Newtonian behavior of detergents and provides a solid framework to investigators to improve drastically biochemical and structural studies of membrane proteins.

1. Introduction

Detergents are essential tools to handle membrane proteins. Their use is widely spread from membrane proteins extraction, purification, biochemical investigations and structural determination [1–5]. Hundreds of detergents are commercially available and new ones are being designed with the goal to gain in membrane protein stability [6,7].

Despite this wide range of detergents available, they still remain sometimes difficult to use for many investigators, as detergents are hardly detectable. Thus, their impact on membrane proteins remained unforeseeable, despite a clear effect on protein function, or crystallization for example [8]. In order to track them, specific techniques have been developed like the use of radioactivity [9–12], thin layer chromatography [13], or more recently MALDI-

ToF [14]. With the later we were able to track and quantify detergents during purification of various membrane proteins, thereby bringing light on their behavior around their cognate protein. This allowed for a web server to be created to represent detergent belts around membrane proteins [15]. Overall, these tools allow rationalization of their use.

Here, we focus on understanding the behavior of detergents during the crucial step of sample concentration. Indeed, many techniques require a high protein concentration that are typically achieved using concentration devices in microfuges, which segregate smaller or greater particles than a given molecular weight cutoff. This is particularly the case for structural approaches such as X-ray crystallography and cryo-EM that require high protein concentration. However, this concentration step results in detergent concentration [14,16], in usually unknown proportions, and thus impacts experiments for downstream studies and leads to unproductive results in crystallization or cryo-EM grid preparation. In addition, for harsh detergents, a high concentration might denature the protein. Using our method of detergent quantification with MALDI-ToF, we quantified detergent along the concentration

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process, allowing to understand how it behaves and how to prevent its accumulation in the concentration compartment.

2. Material and methods

2.1. Detergent quantification by MALDI-ToF

All detergents were measured and quantified by MALDI-ToF mass spectrometry, except for Cholate, as described in Ref. [14]. Briefly, detergents are mixed with their deuterated cognate in known amount, and 1 μL of the mixture is added to 9 μL of matrix solution which is adapted to the type of detergent. 1 μL of the final solution is laid on the MALDI target. Measures were carried out with a Mass Spectrometer equipped with a nitrogen UV laser ($\lambda = 337 \text{ nm}$, 3 ns pulse) and an accelerating voltage of 5 kV. Data were obtained by collecting 300 laser shots and spectra were recorded in a positive reflectron mode. UDM (undecyl-beta-D-maltopyranoside) was used to quantify DDM (dodecyl-beta-D-maltopyranoside).

2.2. Cholate quantification

A solution of 2 mg/mL of sodium cholate (Sigma, C6445) is prepared to realize a standard curve. In black 96 well microplates, a standard curve of 0,1,3,5 and 7 μg of sodium cholate are deposited in a well, completed with buffer containing 20 mM Hepes-Na pH 7.5, 100 mM NaCl until 10 μL . For the sample, 2 or 4 μL of protein were mixed with the same buffer until 10 μL . In each well, 190 μL of concentrated sulfuric acid were added and samples were incubated for 10 min under agitation at room temperature. The absorbance is read at 389 nm on the spectrophotometer SAFAS FLX-Xenius. Special precautions must be taken to handle concentrated sulfuric acid, and waste must be carefully collected. The use of a clear lid above the 96 well plate is recommended, even during reading. Used concentrated, sulfuric acid is not as reactive as the diluted one, and its use is nevertheless very easy to a cautious experimentalist.

2.3. Use of Rhodamine-PE to visualize detergent concentration

RhodaminePE (1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl), Avanti Polar Lipids, 810157) in 100% chloroform is evaporated with a SpeedVac (SPD SpeedVac, rotor RH64-11, ThermoScientific) for 15 min at room temperature. The pellet of RhodaminePE is suspended in 20 mM HEPES-Na pH 7.5, 100 mM NaCl, 0.035% DDM, to a final concentration of 500 μM . This solution is then diluted to 5 μM of RhodaminePE to a buffer containing Ovalbumin (Ovalbumin 1 mM is solubilized in 20 mM HEPES-Na pH7.5, 100 mM NaCl, 0.035% DDM) and concentrated at 20 $^{\circ}\text{C}$ as described below. 15 ml of solution were concentrated.

2.4. BmrA purification

BmrA was expressed and purified as described in Ref. [17]. Briefly, BmrA is overexpressed in C43(DE3) ΔAcrB for 5 h at 22 $^{\circ}\text{C}$. Then, cultures are centrifuged and bacterial pellet resuspended in 50 mM Tris-HCl pH 8.0. Bacteria are lysed by 3 passages at 18,000 psi with a microfluidizer (Microfluidics IDEX Corp). Lyzed bacterial suspension is first centrifuged 15 min at 15,000 $\times\text{g}$, 4 $^{\circ}\text{C}$ to discard unbroken cells, then membranes are collected by centrifugation for 1 h, 4 $^{\circ}\text{C}$ at 180,000 $\times\text{g}$. Membranes are resuspended in 50 mM Tris-HCl pH 8.0 (50 mL buffer corresponding to 4 L of culture), and centrifuged again with identical parameters. Membranes are suspended in 20 mM Tris-HCl pH 8.0, 0.3 M sucrose, 1 mM EDTA (in 5 mL total) and stored in liquid nitrogen.

Membranes are solubilized at 5 mg/mL in 20 mM Tris-HCl pH 8.0, 100 mM NaCl, 15% glycerol, 4.5% (v/v) Triton X-100 and 1X CLAPA antiprotease, under gentle agitation at 4 $^{\circ}\text{C}$. Solubilized membranes are centrifuged at 100,000 $\times\text{g}$, 4 $^{\circ}\text{C}$ for 40 min. The supernatant is loaded onto a pre-equilibrated Ni²⁺-NTA column, unbound material is washed with 20 mM HEPES-NaOH pH 8.0, 100 mM NaCl, 20 mM imidazole, 1.3 mM DDM, 1 mM sodium cholate. BmrA is eluted from the column using the same buffer with 200 mM imidazole. The fractions of BmrA are pooled, diluted 10 times in 20 mM HEPES-NaOH pH 8.0, 100 mM NaCl, 1.3 mM DDM (0.035% weight/volume), 1 mM sodium (0.03% weight/volume) cholate and loaded again onto the same resin, preequilibrated without imidazole, for a second affinity chromatography. The pool of BmrA is concentrated on a 50-kDa cutoff Amicon (Millipore) device at 1000 $\times\text{g}$, 4 $^{\circ}\text{C}$, and injected onto a Superdex 200 (GE healthcare) using a mobile phase 20 mM HEPES-NaOH pH 7.5, 100 mM NaCl, 0.7 mM DDM and 0.7 mM sodium cholate.

2.5. AcrB purification

Five milliliters of AcrB membranes prepared as described in Ref. [18] were solubilized in 40 mL buffer (10% glycerol, 50 mM Tris-HCl, 10 mM imidazole, 2% DDM) for 1 h under agitation at 4 $^{\circ}\text{C}$, followed by centrifugation at 150,000 $\times\text{g}$ for 1 h at 4 $^{\circ}\text{C}$. Supernatant was collected and incubated 20 min with loose Ni-NTA resin (pre-washed with 20 mM Tris-HCl pH 7.0, 0.2% DDM, 10% glycerol, 10 mM imidazole) and applied on a gravity column. The resin was washed with the same buffer followed by a more stringent wash with 50 mM imidazole. AcrB was eluted with 20 mM Tris-HCl pH 7.0, 0.02% DDM, 10% glycerol, 200 mM imidazole, concentrated at 2000 $\times\text{g}$ on a 50-kDa cutoff Amicon (Millipore) device. Concentrated protein was diluted 40 times to 20 mL final volume in 10 mM Hepes-NaOH pH 7.0, 0.02% DDM (0.4 μM final AcrB concentration), divided in 2 for concentration experiment at 5000 $\times\text{g}$ or 500 $\times\text{g}$.

2.6. LacY expression and purification

E. coli lactose permease (LacY) mutant C154G was expressed and purified as described in Refs. [14,19]. Briefly, transformed XL1-Blue bacteria were cultured in 4 1-L culture batches in 5 L flasks of LB media supplemented with ampicillin (100 $\mu\text{g}/\text{mL}$ final), at 37 $^{\circ}\text{C}$ until OD_{600nm} reached 0.6, and induced with IPTG for 4 h. Cells were harvested, suspended in 80 mL of 20 mM HEPES-NaOH pH 7.5, 100 mM NaCl, 0.1 mM PMSF and left overnight at 4 $^{\circ}\text{C}$ under gentle agitation. Cells were lysed by 3 passages at 18,000 psi with a microfluidizer (Microfluidics IDEX Corp), unbroken cells were discarded by centrifugation 15 min at 15,000 $\times\text{g}$, 4 $^{\circ}\text{C}$ and membranes harvested by centrifugation of the supernatant for 1 h, 4 $^{\circ}\text{C}$ at 180,000 $\times\text{g}$. Dry membranes were stored at -20 $^{\circ}\text{C}$.

Dry membranes were suspended in 20 mL buffer A (50 mM sodium phosphate, 500 mM NaCl, 5% glycerol, 5 mM imidazole, 0.035% DDM) using a glass homogenizer, incubated for 1 h at 4 $^{\circ}\text{C}$ under agitation with 2% DDM final. Solubilized membranes were spun 20 min at 4 $^{\circ}\text{C}$ at 40,000 $\times\text{g}$, and supernatant loaded onto a pre-equilibrated Nickel column. Weakly bound contaminants were discarded using 20% buffer B (50 mM sodium phosphate, 500 mM NaCl, 5% glycerol, 250 mM imidazole, 0.035% DDM) and LacY eluted with 100% step of buffer B. The elution peak was collected, concentrated on a 50-kDa cutoff Amicon (Millipore) device at 5000 $\times\text{g}$, 4 $^{\circ}\text{C}$, and loaded on a Superdex 200 (GE Healthcare) with a mobile phase of 20 mM HEPES-NaOH pH 7.5, 100 mM NaCl, 0.02% DDM.

Concentration experiment was conducted at 0.1 mg/ml final (2.3 μM) in 20 mM HEPES-NaOH pH 7.5, 100 mM NaCl, 0.02% DDM. Twenty five milliliters of purified LacY were divided in 2

concentrators and concentrated at 5000×g or 500×g, and DDM measured as described above and below.

2.7. Concentration experiments

All concentrations were carried out on a Multifuge X1R (Heraeus) equipped with a HighConic II rotor (ThermoScientific). Concentrator devices were all made of regenerated cellulose, containing 15 mL volume above the concentrating membrane, with a 50-kDa cutoff (Amicon Ultra-15 device). All devices were placed such that the membranes were parallel to the rotation axis with the membranes placed vertically, contrary to what is recommended by the manufacturer, to reduce protein aggregation on the membrane. All concentration experiments were carried out at fixed temperature (20 °C or 4 °C), at fixed centrifugal forces (500 or 5000×g), for specified time interval. Samples were homogenized by gently inverting the tube 3 times in-between each centrifugal run if more than one run was needed to fully concentrate the sample. Some experiments were carried out without mixing, as stated in the text. All experiments were carried out to reach the dead volume of the concentrator or close to it. Experiments were stopped when the retained volume felt appropriate, and this volume measured (Table 1). Note that every experiment differs in retained volume, and is the reason why the detergent amounts are reported in quantities and not in concentrations. The buffer use in every experiment is 50 mM Hepes-Na pH 7.5, 100 mM NaCl. It is important to not use Hepes-free acid, as it prevents crystallization of the MALDI matrix and thus proper measurement.

Dextran (80 kDa, Sigma-Aldrich ref: 00892), Ovalbumine (ThermoFischer ref: 23209), BSA (fraction V, Euromedex ref: 04-100-812-C) and lysozyme (Sigma-aldrich ref: L6876).

3. Results

3.1. DDM over-concentrate with macromolecules in a concentration-dependent manner

It has been shown in several articles that detergents over-concentrate during the concentration step [8,9,12,14,16]. The reason for this phenomenon however remained unclear, ranging from direct interactions between micelles and detergent belts, non-specific interactions with the protein, or other effects. To investigate this phenomenon, detergent behavior was evaluated during the concentration step in varying conditions for the most used detergent, the dodecyl-maltopyranoside (DDM) (Fig. 1A) [1,2]. The negative control of solely DDM without macromolecule results in an absence of DDM over-concentration, all the DDM molecules go through the filter and end up in the flow-through fraction, as expected (Fig. 1B). Indeed, even though the concentrator used display a 50-kDa cutoff, and the DDM micelle being formed from 80 to 110 monomers has an expected mass around 50-kDa, it is stated by the manufacturer that the pores made by regenerated cellulose open during centrifugation and let objects twice the mass of the molecular cutoff pass through. On the contrary, the addition of proteins to the same DDM solution results in over-concentration of DDM in the retained fraction. This behavior is observed in a concentration-dependent manner, as DDM over-concentrates with increasing protein concentration. Of note, for BSA or Ovalbumin, the effect is observed even at modest protein concentration, while for Lysozyme a higher protein concentration is needed to observe an impact on detergent concentration. Lysozyme being a smaller protein of 14 kDa, it goes through the concentrator pores (50-kDa) and doesn't concentrate at low concentrations. After a certain lysozyme concentration (100 μM), the protein is concentrated

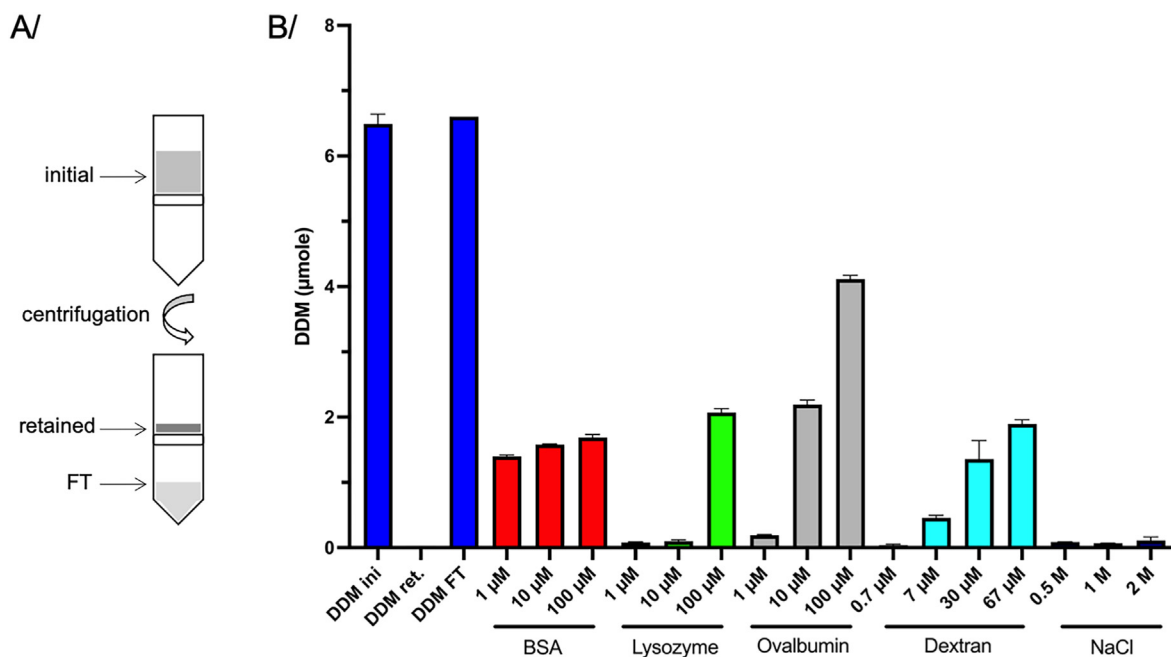


Fig. 1. Detergent over-concentration with macromolecules. A/Overall scheme of the experiment, where a detergent-containing solution is placed on a concentrator, centrifuged, resulting in retained and flow-through (FT) fractions. B/DDM quantification during concentration experiments, at 5000×g. Dark blue: quantification when no macromolecule is added to a DDM solution at 2x Critical Micelle Concentration (CMC) (0.03% (w/v), 0.6 mM), for initial, retained and FT fractions. Red: concentration of DDM in the presence of increasing quantity of BSA. Green: concentration of DDM in the presence of increasing quantity of Lysozyme. Grey: concentration of DDM in the presence of increasing quantity of Ovalbumin. Cyan: concentration of DDM in the presence of increasing quantity of Dextran (80 kDa). Black: concentration of DDM in the presence of increasing quantity of sodium chloride. Error bars show the standard deviation, the experiment being done in triplicate. All experiments were carried out in the same buffer base (50 mM Hepes-Na pH 7.5, 100 mM NaCl), and concentration was stopped when it reached the dead volume of the concentrator. Note that this volume varies with each experiments, retained volumes are reported in Table 1.

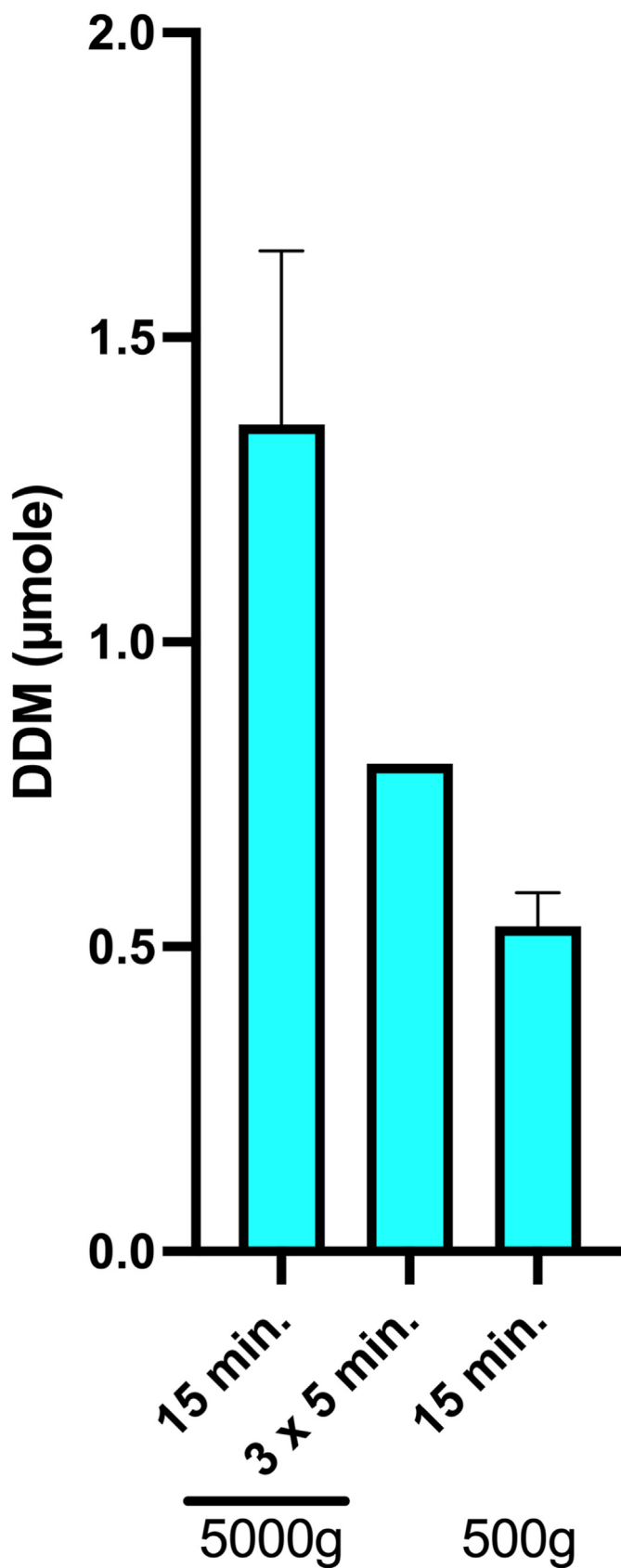


Fig. 2. Influence of centrifugal force on detergent over-concentration. A DDM solution (0.03% (w/v), 0.6 mM) with dextran was concentrated for 15 min at 5000×g, or 3 times 5 min at 5000×g with mixing in-between, or one time at 500×g. Error bars show the standard deviation, experiments reproduced 3 times. The volume reduction factor is almost the same in all 3 experiments (STable 1).

enough to create an effect on the detergent and results on its over-concentration. To evaluate the impact of non-protein type polymers, Dextran was also tested and shows the same pattern of DDM over-concentration with increasing Dextran concentration. Sodium chloride however, has no influence, even at high concentration, refuting an effect of charges on the phenomenon.

These results exemplify the impact of macromolecules, but not necessarily proteins, on DDM over-concentration, in a concentration-dependent manner. It has been demonstrated that BSA can bind a few lipid monomers [12] (up to 20 monomers), so some detergent binding is expected, albeit with limited influence. Detergent binding has however not been detected on Lysozyme or Ovalbumin, neither on Dextran, ruling out the direct interaction of DDM with macromolecules in the context of over-concentration. The over-concentration of DDM is thus happening *in trans*.

3.2. DDM over-concentration depends on centrifugal force

To further evaluate the influence of macromolecules on DDM over-concentration, the same condition as in Fig. 1B (30 µM Dextran) was concentrated at different centrifugal speed (Fig. 2). Maximum DDM amount was found in the concentrated fraction when the device was spun 15 min at maximum speed of 5000×g. Interestingly, 3 runs of 5 min done at the same speed, but with mixing in-between, reduces the amount of DDM in the concentrated fraction. A further decrease in centrifugal force to 500×g diminishes even more the DDM quantity. The volume reduction factor is the same in all three experiments, starting from the same initial volume (15 ml) and ending in almost the same dead volume (STable 1), stating that the effect on DDM concentration is not linked to experimental differences but is resulting from the different force applied. There is thus an effect on centrifugal forces on the accumulation of DDM in this context.

3.3. Over-concentration varies with the micelle size

We thus investigated the impact of the type of detergent on the over-concentration phenomena (Fig. 3). In addition to DDM, another detergent with 12 carbon tail was investigated, while carrying a zwitterionic head group, FosCholine 12 (FC12). FC12 has a smaller aggregation number than DDM, and results in a micelle of around 40 kDa. Cholate, another charged detergent, was also included in the study. Cholate has a very small aggregation number (less than 10 monomers) and carries a different structure compared to the classical one of DDM. For each detergent, an over-concentration is being observed in the presence of Ovalbumin. The amount of detergent kept in the retained fraction of the concentrator varies from one detergent to the other, and is in general greater for charged detergents. For Cholate, 2 concentrations were tested as the over-concentration was not anticipated to be so large for the lower concentration (0.08%). The larger concentration of 0.8% results in a much greater retention of Cholate in the presence of Ovalbumin. The mixture of DDM and Cholate was tested since it was successfully implemented for structure resolution of several proteins [17,20]. It was previously reported that the mixed DDM/Cholate micelle is smaller than the DDM micelle so it is surprising to observe more detergent retained than DDM or Cholate alone. It is probably due to the fact that the micelle is smaller [17], but it also results in more objects in solution, creating a crowding effect (cf. below). The addition of Ovalbumin results in the over-concentration of both detergents.

3.4. Visualization of a detergent gradient clogging the concentrating device

In order to visualize how detergents over-concentrate, a

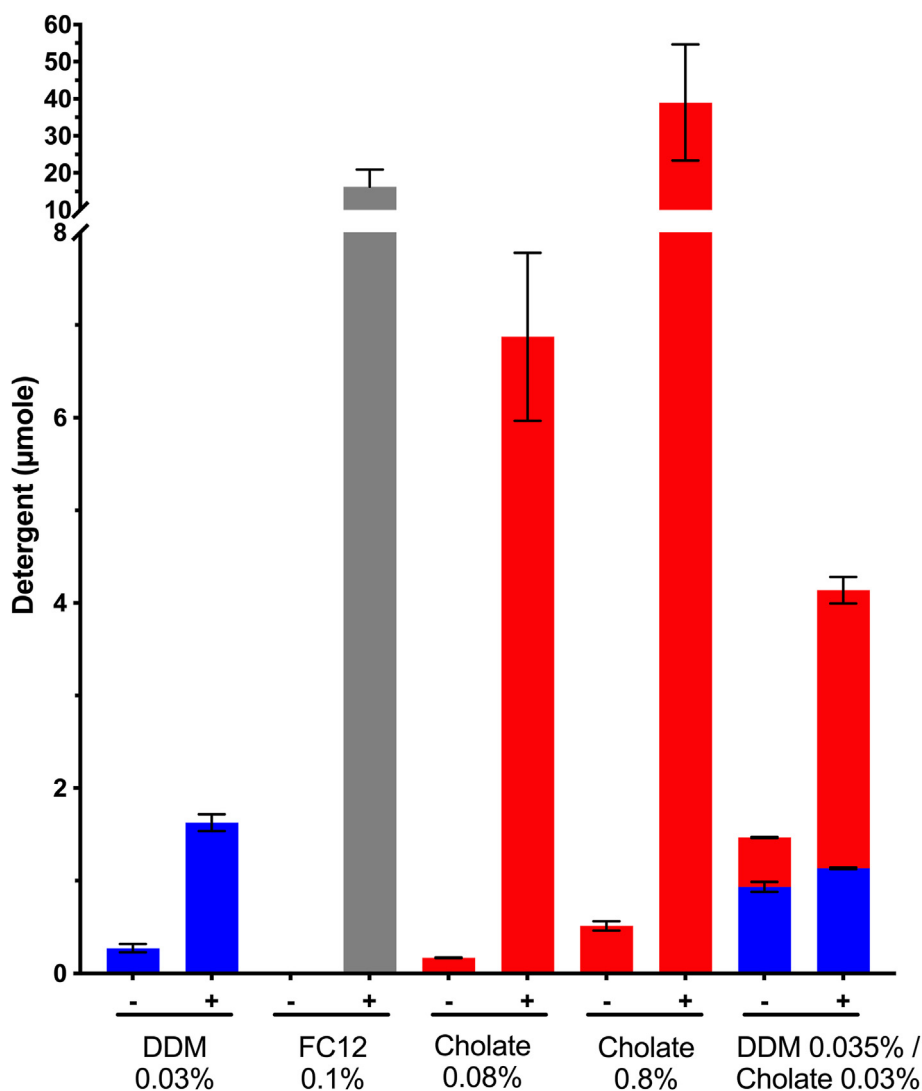


Fig. 3. Impact of the micelle size on the concentration of detergents. A solution with detergent and with (+) or without (-) Ovalbumin (1 mM) was concentrated at 5000×g until the void volume or until the solution does not pass through the filter anymore. Dark blue represents DDM, grey is for FosCholine 12 and red for sodium cholate. DDM at 0.03%(w/v) (0.6 mM), FC12 at 0.1%(w/v) (2.8 mM), Cholate 0.08%(w/v) (1.9 mM), Cholate 0.8%(w/v) (19 mM), and DDM/Cholate mixture at 0.035%(w/v) (0.7 mM) DDM/0.03%(w/v) (0.7 mM) Cholate. Error bars show the standard deviation, measures were done in triplicate and conducted in the same buffer (50 mM Hepes-Na pH 7.5, 100 mM NaCl).

hydrophobic dye was added to the DDM solution. The dye, Rhodamine-PE (Rho-PE), is composed of a phosphatidyl ethanolamine moiety that insert into DDM micelles, and a rhodamine moiety that confers absorbance at 570 nm. Rho-PE was added in small quantity in order to perturb as little as possible the DDM micellar properties.

A batch solution of DDM solution with Ovalbumin was complemented with Rho-PE, divided in concentrators and concentrated by 5-min. increments without mixing with the goal to maximize the observation (Fig. 4). For each centrifugation time, a sample was collected at given depth and quantified using absorbance (Fig. 4). With time, a darker patch forms towards the bottom of the concentrator filter. Quantification shows that Rho-PE absorbance increases equally. A maximum absorbance is reached after 30 min, also coinciding with the fact that further concentration only decreases volume by minimal amounts. This experiment reveals the creation of a concentrated patch at the bottom of the concentrator after initial concentration, that grows upwards as time goes, thereby creating a mesh preventing further macromolecule to go through.

3.5. Impact on membrane protein concentration

With the knowledge of detergent behavior influenced by macromolecules, this behavior was evaluated in the presence of membrane proteins. Three membrane proteins were investigated, the multidrug transporter BmrA from *B. subtilis* [17,21], Lactose permease from *E. coli* (LacY) [19,22], and the Acridone resistance protein from *E. coli* (AcrB) [18]. These proteins were chosen as they have all been subjected to structural studies by X-ray crystallography or cryoEM, which require high protein concentrations and might thus experience increased detergent concentration. Additionally, it was previously observed for BmrA and LacY that, after concentration of a pool after a nickel column followed by separation on a size exclusion chromatography, the quantity of detergent over-concentrated was higher than if the same concentration was performed without protein [14]. It was already an indication of detergent over-concentration triggered by membrane proteins.

BmrA was purified with the detergent mixture DDM and Cholate that yields smaller micelles as well as smaller detergent belts around BmrA [17]. Concentration at 500×g results in less detergent

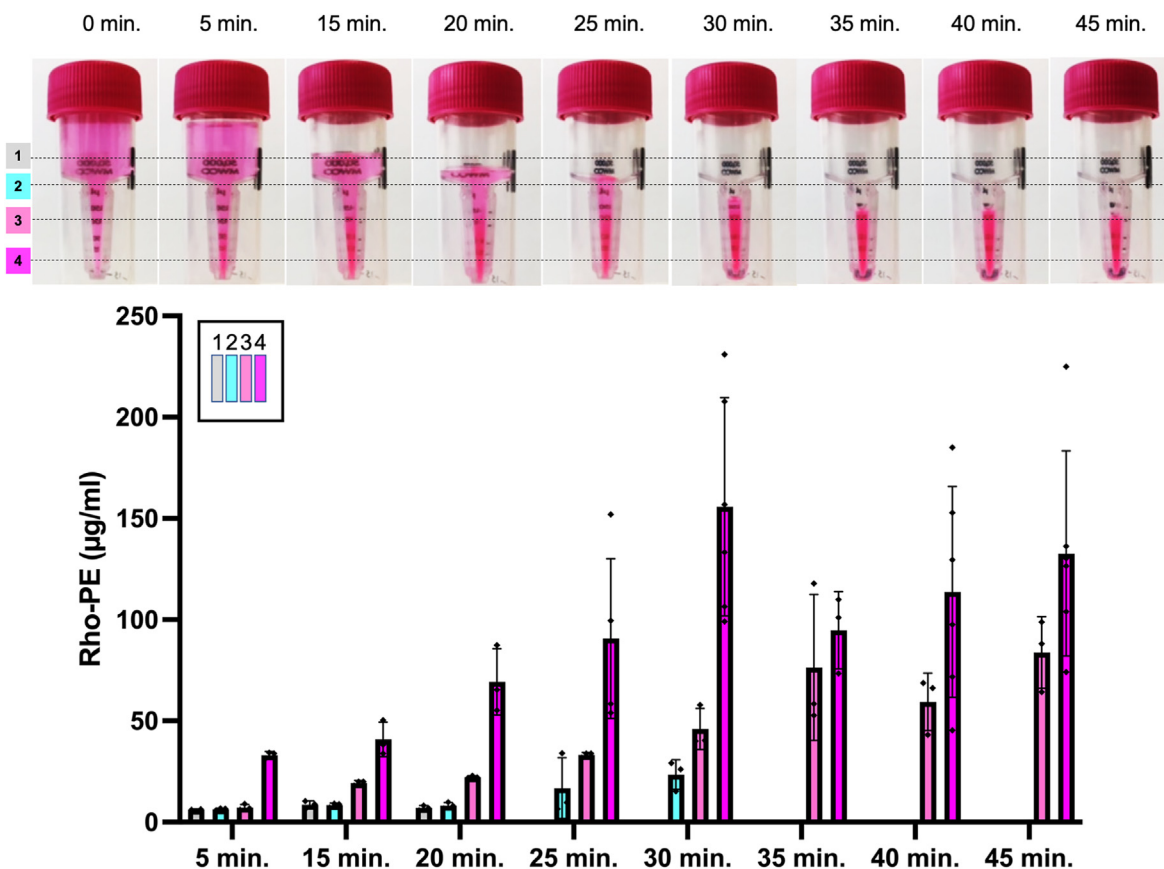


Fig. 4. Visualization of the detergent gradient on a concentrating device. A solution of DDM (0.035%(w/v) (0.7 mM) mixed with Rhodamine-PE (Rho-PE, dye) and Ovalbumin (1 mM) was concentrated by 5-min. increments at 5000 \times g, without mixing in-between each run. A photo was taken at each step, with a representative experiment shown on top. Rho-PE was quantified by spectrophotometry by pipetting a sample at different depth positions along the concentrator (levels 1, 2, 3 or 4). Rho-PE quantification is shown below for each time point and depth level colored according to the legend (square). Each data point is represented. The histogram corresponds to the mean with standard deviation. The experiments were conducted in triplicates or sextuplicates, photo experiments conducted in decaplicates showing always the same pattern.

retained, and more detergent passing through the filter compared to the higher speed concentration at 5000 \times g (Fig. 5). This pattern is observed for both DDM and Cholate independently. Together, these effects add up to more detergent being retained when the highest speed is used. Of note, the BmrA concentration used for this experiment is 4 μ M, much less than protein concentration used in previous figures, which reflects the fact that detergent over-concentration is always present yet subtle enough to stay in disguise. LacY and AcrB were both purified in DDM only, and subjected to differential speed centrifugation before detergent quantification. For LacY, a lot more detergent passes through the concentrator at 500 \times g compared to 5000 \times g, reflecting the fact that less detergent is retained, as suggested by quantification in the concentrated fraction. Similar effects are observed for AcrB. For both LacY and AcrB, the concentrations used in the experiment (2.3 μ M and 0.4 μ M respectively) highlights the fact that the detergent over-concentration phenomenon is a hidden phenomenon in membrane proteins biochemical analyses.

4. Discussion

Detergent over-concentration is a real problem for membrane protein investigations, as it blurs signals and is concentrated in usually unknown amounts. In some cases, a high amount of detergent leads to protein denaturation. To shed light on this phenomenon, we have conducted the present study, resulting in the major finding that DDM gets over-concentrated by

macromolecules in solution, in a concentration-dependent manner. This behavior is also observed with non-protein polymers such as dextran, but not with salt, revealing that it happens *in trans*, *i.e.*, with no direct interaction with the polymer. It could have been hypothesized some kind of interaction between detergent micelles and detergent belts surrounding membrane proteins, but the data presented on soluble proteins and Dextran refute this claim. We could also modulate this phenomenon by playing with the centrifugal forces, reducing the over-concentration by decreasing the centrifugal speed. Centrifugal forces are described as shear forces, which directly points to behavior of non-Newtonian fluids. Furthermore, it has previously been shown that detergents can behave as non-Newtonian fluids in specific conditions [23–25]. Here, we observe that macromolecules enhance the described non-Newtonian behavior of detergents as observed during centrifugation. It is this property that results in detergent over-concentration. In support of this, it has been shown in colloidal suspensions that shear thickening arises, at the molecular level, from particle clustering [26]. It is tempting to speculate that a similar phenomenon happens with detergent micelles and macromolecules in suspension in the concentrator. This increased viscosity is also observed on the concentrator when Rho-PE is added to bring color to detergent micelles. Indeed, the formation of a brighter spot at the bottom of the concentrator right at the beginning of the concentration experiment denotes the formation of a mesh, precluding passage of further detergent through the concentrator pores. It results in the mesh growing as

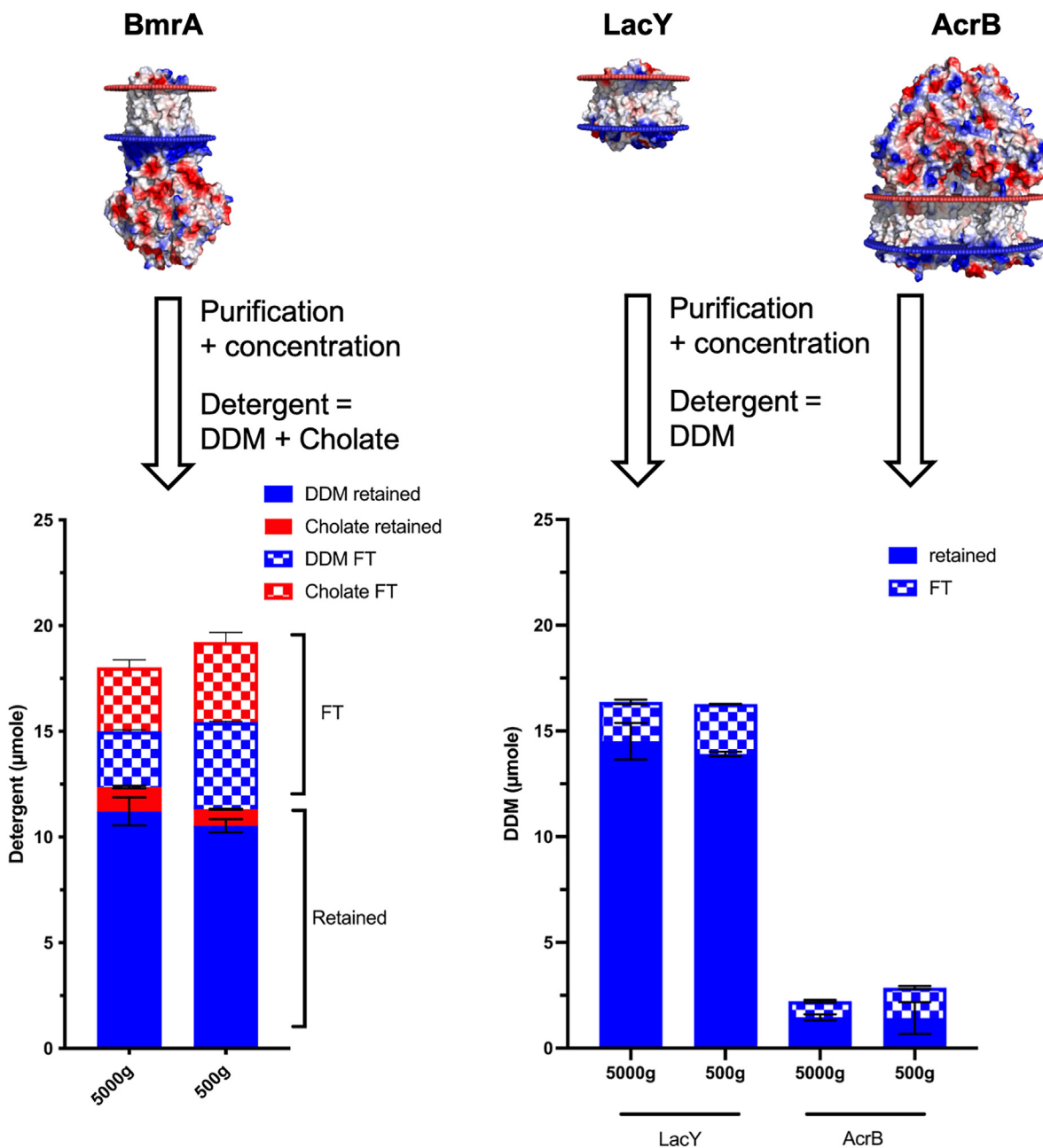


Fig. 5. Detergent over-concentration in presence of membrane proteins. Three membrane proteins were purified and subjected to centrifugation at different speed (500 or 5000×g) followed by detergent quantification. For each protein, the retained detergent is represented as solid color, and the flow-through fraction with white squares. DDM is colored in blue, and Cholate in red. BmrA was purified in the mixture of DDM and cholate, LacY and AcrB were purified in DDM.

more time is spent concentrating, thereby clogging the whole device. This direct visualization of a phenomenon is also reminiscent of the observation that more time spent concentrating a membrane protein does not correlate with more liquid passing through the concentrator. Now, our investigations shed light on a well-known fact in many labs across the world but never explained.

Interestingly, the non-Newtonian behavior of detergents can be modulated with centrifugal force and concentration. For example, mixing the solution in-between two centrifugation steps decreases the local macromolecule concentration and reduces the accumulation of detergent. Also, the total amount of detergent remaining after the process can be modulated depending on the application. If

fewer detergent concentration is needed, a decreased centrifugal force can be applied. On the contrary, if stability of the membrane protein is required, a higher detergent concentration is privileged to help stabilize the protein, reached by applying higher centrifugal forces, thereby providing a mean to biochemists to control and modulate their sample.

This strategy was adopted to solve the X-ray and cryo-EM structures of BmrA [6,17]. It has been previously shown that controlling the amount of detergent favors crystallization of the ADP/ATP exchanger [8]. We hypothesized the same for BmrA as crystals were initially not of good quality. We decreased the centrifugal force to 500×g to decrease detergent concentration and grow good crystals for structure solving. This sample was also of good quality

to be imaged by cryo-EM and yielded also a structure by this approach.

Altogether, the results presented here reveal the non-Newtonian behavior of detergent when in solution with macromolecules. With this knowledge in hands, investigators are also provided with means to control it and identify it to better handle their samples.

Author contributions

VC and PF conceptualized the work. AG, VZ, SM, EPB and VC gathered and collected data. VC, PF, VZ and AG analyzed data. VC wrote the manuscript with contributions from all authors.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.biochi.2022.09.004>.

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