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Stability of Purple Membranes from *Halobacterium salinarum* toward Surfactants: Inkjet Printing of a Retinal Protein

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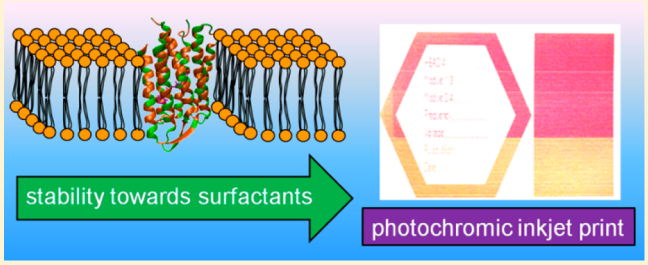
Supporting Information

ABSTRACT: Inkjet printing is a versatile technique widely applied in biological microarray technology. Because of its photochemical and photophysical properties, bacteriorhodopsin (BR) from *Halobacterium salinarum* holds promise for applications in nanotechnology, and inkjet printing would simplify the transfer of BR to suitable substrates. Surfactants are essential parts of inkjet formulations tuning viscosity, rheology, and spreading behavior of the solution. However, many surfactants destabilize the structure of proteins and often cause denaturation accompanied by a complete loss of function. Inkjet printing of membrane proteins is particularly challenging and special care must be taken in the choice of the surfactant. For BR, the situation is complicated by the fact that the structural integrity of BR depends on its native membrane environment, the so-called purple membrane (PM). PM contains 10 lipid molecules per BR monomer and is very sensitive toward surfactants. In this work, we identified surfactants suitable for inkjet formulations containing PM. Initially, we screened a variety of technically relevant surfactants for compatibility with PM using the UV−vis absorption of the retinal chromophore as a natural probe. Promising candidates were selected, and their impact on the structure of PM and BR was analyzed using UV−vis spectroscopy, CD spectroscopy, and small-angle X-ray scattering (SAXS). We identified two surfactants compatible with PM and suitable for inkjet formulations. An inkjet formulation containing PM as dye component was developed. We demonstrate that the photochromic properties of BR are maintained upon inkjet printing.



1. INTRODUCTION

Bacteriorhodopsin (BR) is an integral membrane protein from *Halobacterium salinarum*. In its native environment, BR forms two-dimensional crystals, the so-called purple membranes (PM) containing BR and lipids only.[1−3] BR is the prototype of seven transmembrane helix proteins and shares close homologies with other retinal proteins.[4−11] Structure and function of BR have been extensively studied giving a detailed picture of light-dependent vectorial proton transport across the membrane.[12−17] As long as BR is embedded inside PM, BR is astonishingly stable against thermal and chemical stress. BR is an efficient photochemical switch with great promise for technological applications.[18−22] Initial work was focused on holographic applications of BR-D96N, a mutant with enhanced photochromic properties.[20] Furthermore, BR has an extremely high cross-section for two-photon absorption[23] making it an interesting material for two-photon data storage relevant for applications of BR in security features.[24−27]

Inkjet printing is increasingly used in biological microarray technology and is particularly useful for accurate transfer of proteins to solid substrates.[28−32] The application of BR as a photochromic compound in security features requires the preparation of printing inks, which preserve the functional and structural integrity of BR. Moreover a long shelf life of the PM print is required. However, such inks usually contain surfactants to facilitate a stable coating of substrates. This is of particular concern for membrane proteins like BR, which critically depend on an undisturbed membrane environment. Interaction of BR and PM with surfactants has been predominantly studied in the context of solubilization for structural analysis,[33−42] and it is known that some detergents severely change the crystalline assembly of PM.[43] However, virtually no information is available about surfactants not solubilizing BR. The motivation of our experiments was 2-fold. First, we wanted to identify surfactants not solubilizing BR and thus preserving the native structure of PM. Second, we were interested in the physical mechanisms determining the impact of a particular surfactant on the structure of PM and BR.

We tested numerous surfactants in view of their influence on the long-term stability of BR in inkjet formulations. Initially, we screened 24 technically relevant surfactants for compatibility with suspended PM using the UV−vis absorption of the retinal chromophore as a natural probe. In a pretest, five potentially suitable surfactants were selected. Subsequently, the temper-

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ature-dependent interaction of these surfactants with PM was analyzed in detail using CD spectroscopy and small-angle X-ray scattering (SAXS) yielding information about BR trimer interactions (CD_{VIS} spectroscopy), preservation of secondary structure elements (CD_{UV}), and the crystallinity of PM (SAXS analysis). An inkjet formulation including PM was developed, which preserved the photochromic properties of BR before and after printing.

2. MATERIALS AND METHODS

2.1. Materials. In the experiments, we used PM comprising the variant BR-D96N, where aspartic acid in position 96 is replaced for asparagine. PM was supplied by the Oesterhelt group at the Max-Planck-Institute of Biochemistry (Martinsried, Germany) as a crude material. PM preparations were purified by standard procedures[44] and finally isolated from sucrose density gradients.

Chemicals were purchased from Acros Organics (Geel, Belgium), Sigma/Aldrich/Fluka (Taufkirchen, Germany), and QED Bioscience Inc. (San Diego) and used as received.

For surfactant screening, 1% solutions in water of commercially available surfactants were used. Plastic single use micro cuvettes with an optical path length of 1 cm were filled with a 1% solution of surfactant, then the required amount of PM suspension (0.1%) was added by a micropipet and the solution thoroughly mixed.

2.2. Optical Spectroscopy. UV−vis spectra were measured using an UVIKON 922 spectrometer (Kontron) or a LAMBDA 35 (Perkin-Elmer) at RT and 50 °C.

CD spectroscopy was done on a JASCO J-810 spectrometer (Jasco Corp.) in 1 cm quartz cuvettes. Each spectrum was recorded at least twice at a temperature of 20 °C ± 0.2 °C, except another temperature is explicitly mentioned. The spectral range for high concentrated samples was 290−700 nm, and after dilution 1:40 with water, the samples were analyzed in the range from 190 to 300 nm.

2.3. Small Angle X-ray Scattering (SAXS). Surfactants were dissolved in distilled water and PM was added. These PM suspensions were deposited on a thin plastic substrate and slowly dried overnight. PM multilayers were analyzed with a SAXS apparatus comprising a Phillips PW1830 X-ray generator (Cu Kα, Ni filter) and a KKK-type Kratky camera (Anton Paar, Graz, Austria) with position sensitive detector and a sample−detector distance of 200 mm.

2.4. Inkjet Printing. Photochromic inkjet inks were prepared starting from 50 g of PM in suspension (OD ≈ 90). Fifteen grams of glycerol, 1.5 mL of 5% Zonyl FSN 100, and 2 mL of 30% Antifoam YA-30 were added. The photochromic properties of BR were adjusted by optional addition of arginine, betaine, or sodium acetate. Inkjet inks containing PM were printed at 60 °C processing temperature with a piezo print head SpectraNova PH 256/80 AAA (Nova/Spectra, Lebanon, NH) mounted on a CNC platform (Isel AG, Germany).

3. RESULTS AND DISCUSSION

Figure 1 illustrates stepwise denaturation of PM due to surfactant action. Intermediate states accompanied by a loss of function are indicated by a change in color compared to the initial purple state.

We prescreened 24 surfactants in suspensions containing 0.1% BR and 1% surfactant (w/w) using the UV−vis absorption of the retinal chromophore as an indicator for BR stability. A full list of all surfactants tested can be found in the Supporting Information (Table S1). Five surfactants successfully passed the pretest (Table 1), and the structural integrity of BR in the presence of these surfactants was analyzed using a variety of analytical methods, which are listed in Table 2.

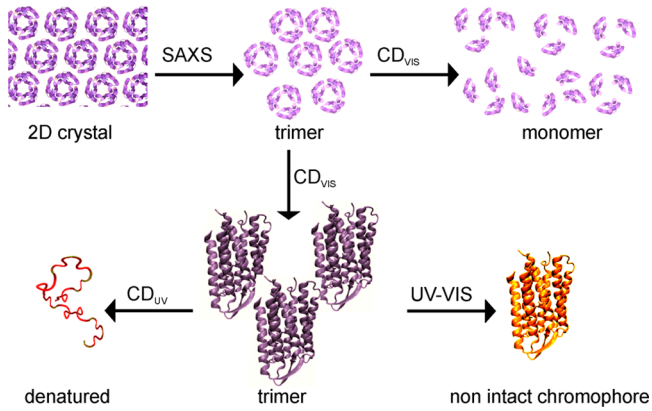

Figure 1. Stepwise denaturation of PM by surfactants. First, the lipid−protein interaction is weakened by intercalation of detergent molecules accompanied by a loss of the hexagonal lattice. Second, the interaction between BRs within the trimers is distorted causing monomerization. Finally, the tertiary structure of BR is lost, and the retinal chromophore is irreversibly destroyed.

Table 1. Surfactants That Passed the Initial Screening at Room Temperature for 50 h

trade name[a]	molecular weight	classification	HLB[b]	PM stability at RT for 50 h
BRIJ35	1200	nonionic	16.9	+
Pluronic F68	8400	nonionic	>24	+
Surfynol 485	<1800	nonionic	17	+
Tetronic 1307	18600	amphoter	>24	+
Zonyl FSN 100	≈ 950	nonionic	n.a.	+

[a]Same compound available with other trade names. [b]HLB = hydrophilic lipophilic balance; n.a. = not applicable.

It turned out that, within a particular class of surfactants, those with a higher molecular weight (MW) were less detrimental to PM than the homologous compounds with a

Table 2. Methods Employed to Determine Structural Motifs of PM Related to Its Long-Term Stability

structural motif	analytical method[a]	figure of merit
secondary structure	CD_{UV}	about 74% α-helical conformation
		no random coil contribution
integrity of the retinal chromophore	UV−vis	maximal absorption at 570 nm unchanged
		absence of characteristic absorption at 380 nm indicating free retinal
trimeric assembly of BR	CD_{VIS}	biphasic CD spectrum: (+) 540 nm/(−) 600 nm
crystallinity of PM	SAXS	characteristic Bragg peaks

[a]CD_{UV}/CD_{VIS} = circular dichroism in the UV/VIS; UV−vis = absorption spectrum in the UV and VIS; SAXS = small angle X-ray scattering.

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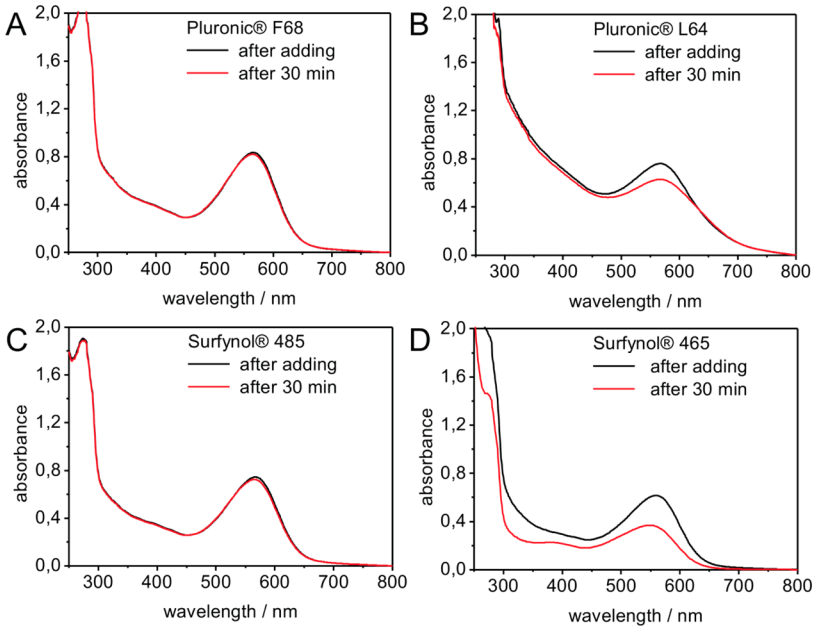

Figure 2. Dependence of the PM stability on the molecular weight of the surfactant. (A,C) High molecular weight of surfactants cause no spectral changes in the UV−vis spectra of BR in the presence of a 10-fold (w/w) excess of surfactant. (B,D) With low molecular weight surfactants spectral changes appear within 30 min of exposure. Black lines: UV−vis spectrum taken immediately after addition of the surfactant. Red lines: absorption spectra after 30 min at room temperature.

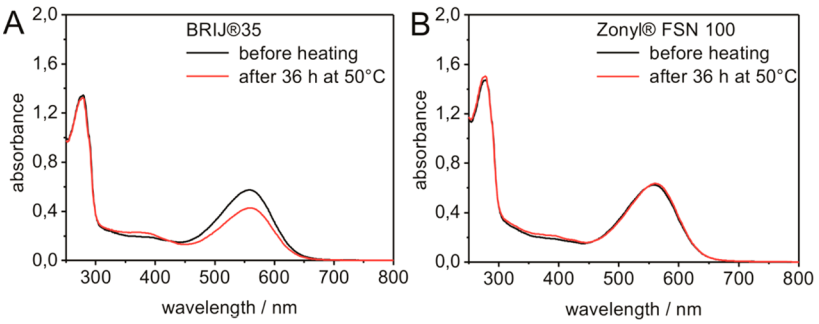

Figure 3. Temperature-dependent stability of PM in the presence of a 10-fold (w/w) excess of surfactant. Black lines: UV−vis spectra of PM immediately after mixing with the surfactant. Red lines: UV−vis spectra of PM after 36 h at 50 °C. (A) PM mixed with BRIJ 35. The absorption band of free retinal is visible at 380 nm indicating denaturation of BR. (B) PM mixed with Zonyl FSN 100; no observable difference.

Table 3. Analysis of Surfactants That Passed the Initial Screening[a]

detection method	UV−vis		CD_{UV}	CD_{VIS}		
figure of merit	absorption maximum at 570 nm unchanged		α-helix motif	biphasic CD spectrum (+) 540 nm/ (-) 600 nm		ST in the range of 25−50 mN/m
conditions	RT 50 h	50 °C 36 h	50 °C 48 h	50 °C 48 h	rev. 70 °C	ST 0.1% [mN/m]
BRIJ35	+	−	+	o	o	44
Pluronic F68	+	+	+	+	+	50
Surfynol 485	+	−	+	o	o	51
Tetronic 1307	+	−	n.a.	n.a.	n.a.	44
Zonyl FSN 100	+	+	+	+	+	21

[a]ST = surface tension; n.a. = not applicable; o = not analyzed due to instability of the retinal chromophore at 50 °C.

lower MW. These effects were virtually independent of the chemical nature of the surfactant. As an example, Figure 2 shows UV−vis spectra of PM suspensions containing Pluronic F68 (MW 8400), Pluronic L64 (MW 2900), Surfynol 485 (MW < 1800), and Surfynol 465 (MW < 850), respectively (Figure 2). Surfactants with high MW possibly will intercalate PM less efficiently and are thus less detrimental to PM.

3.1. Temperature-Dependent UV−Vis Spectroscopy: Stability of the Retinal Chromophore. We analyzed the sensitivity of PM toward selected surfactants (Table 1) at elevated temperatures. From a technical point of view, stability of printing inks toward elevated temperatures is required as processing temperatures usually exceed 50 °C. Furthermore, interactions of surfactants with PM are exaggerated at elevated

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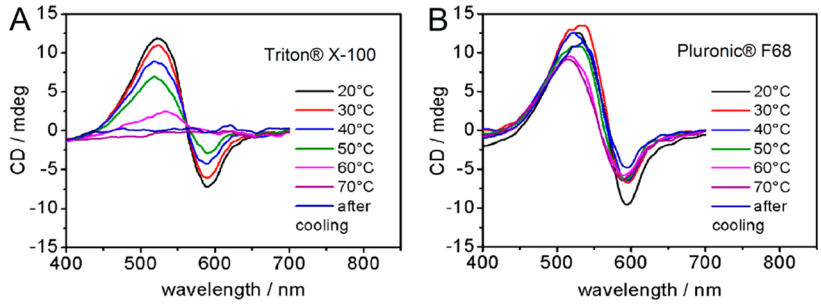

Figure 4. Regular trimeric arrangement of BR molecules in the presence of 10-fold excess of surfactant. Shown are temperature-dependent CD_{VIS} spectra (A) 0.1% PM in 1% TritonX-100 and (B) 0.1% PM in 1% Pluronic F68. For Pluronic F68, a reversible and, for Triton X-100, an irreversible behavior is observed.

temperatures making possible detrimental effects particularly noticeable.

Figure 3 shows temperature dependent UV−vis spectra of PM in the presence of a 10-fold excess of surfactant. After 36 h of heating at 50 °C, some detergents irreversibly deteriorate PM (see also table 3). As an example, a UV−vis spectrum of a PM suspension containing BRIJ 35 is shown in Figure 3a. This is in contrast to, e.g., Zonyl FSN 100 (Figure 3b), which shows no temperature-dependent interactions with PM (Figure 3b). UV−vis spectroscopy was complemented by CD_{VIS} spectroscopic analysis of BR. No change in BR secondary structure was observed upon incubation of PM in suspension of selected surfactants (Supporting Information Figure S3).

3.2. Preservation of Trimeric Assembly of BR and Crystallinity of PM. Figure 4 shows temperature-dependent CD_{VIS} spectra of PM in the presence of surfactants. Initially, PM gives rise to a biphasic CD spectrum indicative of the regular trimeric assembly of BR in PM.[45] The CD spectra demonstrate that the trimeric assembly of BR in PM is preserved in suspensions containing Pluronic F68 (Figure 4b) heated to 70 °C, whereas Triton X-100, a surfactant known to solubilize BR,[34] leads to irreversible loss of the trimeric assembly of BR in PM (Figure 4a) after incubation at 70 °C. Likewise, biphasic CD_{VIS} spectra were observed in PM suspensions containing Zonyl FSN 100 (see Table 3). Figure 5 shows SAXS profiles of PM prepared with Zonyl FSN 100. It

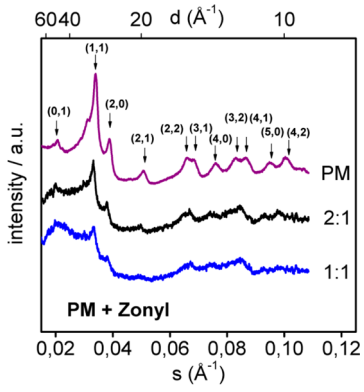

Figure 5. Preservation of the hexagonal lattice of PM in the presence of high excess of surfactant. Small-angle X-ray scattering (SAXS) of PM treated with the surfactant Zonyl FSN 100 in various ratios. Shown SAXS profiles of PM films without surfactant (top) and PM films prepared with surfactants in a ratio of 2:1 (center) and 1:1 (bottom). All films show regular Bragg diffraction indicating preservation of the two-dimensional crystal lattice.

is demonstrated that the crystalline lattice is well preserved in suspensions containing Zonyl FSN 100. In conclusion, Pluronic F68 and Zonyl FSN 100 passed the second screening and are thus suitable surfactants for inkjet formulations containing PM (Table 3).

3.3. PM Inkjet Formulation: Preservation of Photochromic Properties. As Zonyl FSN 100 efficiently reduces surface tension (Table 3), we developed a PM inkjet formulation containing Zonyl FSN 100 as surfactant. Figure 6

Figure 6. Image of a photochromic inkjet print recorded immediately after illumination. The upper part of the print was illuminated with blue light using a filter with a transmission maximum at 410 nm (accumulation of purple B-state), whereas the lower part was illuminated with red light using a filter with a transmission cut-on at 550 nm (accumulation of yellow M-state).

shows an inkjet print revealing photochromic properties. The long-term stability of photochromism was analyzed using a custom-made apparatus (see Supporting Information Figure S4). PM inkjet prints were continuously switched between the purple B-state and the yellow M-state, and the photochromic properties were analyzed. In our experiments, PM inkjet prints preserved their photochromic properties over more than 45 000 switching cycles, which is comparable to the stability of native PM.[46]

4. CONCLUSIONS

From the point of view of physical chemistry, inkjet formulations are complex mixtures, and the sensitivity of proteins toward particular ingredients or processing conditions is difficult to predict. The stability of proteins in inkjet formulations depends on a variety of physical parameters.[47] However, the impact of surfactants is of particular importance

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for inkjet printing of integral membrane proteins within their native membrane. Therefore, we have analyzed the interaction of PM with selected surfactants using UV−vis spectroscopy, CD spectroscopy, and SAXS as physical methods. We have identified two surfactants compatible with PM and thus suitable for inkjet formulations. For the first time, we have demonstrated that inkjet printing of an integral membrane protein within its native membrane is possible without loss of function. The surfactants ZonylFSN 100 and PluoronicF68 preserve the structural and optical properties of PM. We conclude that surfactants suitable for inkjet printing of membrane proteins belong to the class of nonionic high MW surfactants.

■ ASSOCIATED CONTENT

Supporting Information

Surfactants tested in a pretest; chemical structure of surfactants, which passed the pretest; CD-UV spectroscopy of BR in the presence of different surfactants; custom-made system for testing the photochromic properties of PM inkjet prints. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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