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One-Pot Production of Fluorescent Surface-Labeled Polymeric Nanoparticles via Miniemulsion Polymerization with Bodipy Surfmers

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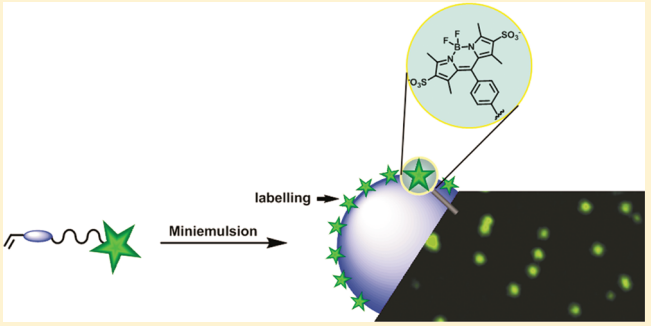
ABSTRACT: New molecules combining the functionalities of surface activity, polymerizability, and fluorescent properties within one molecule that could be seen as a fluorescent surfmer (*surf*actant and mono*mer*) were successfully synthesized. A long hydrocarbon tail capped with a methacrylamide unit was anchored to a mono- or double-sulfonated Bodipy core. Time-resolved fluorescence measurements in aqueous solutions of the dyes enable one to trace the micelle formation and to find strong polarity dependence of fluorescence properties of the dyes. By using these molecules, polystyrene nanoparticles with fluorescent interfaces were synthesized via miniemulsion polymerization. The surfmers can be used alone or as surfactant additional to sodium dodecyl sulfate that provides stable emulsions and dispersions with a wide range (100−250 nm) of realizable particle sizes after polymerization. All synthesized nanoparticles showed bright fluorescence and can be easily investigated with conventional fluorescent confocal microscopy. Only the fluorescence of particles with double-sulfonated surfmer used is strongly sensitive to fluorescent quenchers as sodium iodide or methyl viologene dissolved in the continuous phase. In contrast, the fluorescence of nanoparticles labeled with the monosulfonated surfmer is only weakly quenched that might be explained by specific orientation of the Bodipy core at particle/water interface.



INTRODUCTION

Fluorescence is a powerful tool to gain further insight into a widespread field of material interactions; beneficial is the noninvasive and nondestructive nature of the measurements for the sample. In recent years there have been an abundance of applications in the interdisciplinary field between chemistry, physics, and medicine. For this reason a large variety of fluorescent labels and probes were realized. One promising class of stable fluorophors is the dipyrromethene boron difluoride (Bodipy) derivatives due to their outstanding properties, such as high quantum yields, excellent thermal-, photo-, and chemical-stability, as well as good resistance toward aggregation, and additionally a lot of sides are accessible for chemical modification.[1,2] They were first synthesized by Treibs and Kreuzer in 1968.[3] Bodipy's show narrow absorption and emission bands; this is especially interesting for applications where more than one dye has to be employed and superposition has to be prevented, which is often the case especially when dealing with cells and cellular uptake. For the application in biological systems water-soluble dyes are necessary. The first water-soluble Bodipy was introduced by Wories et al.[4] by sulfonation of the 2- and 6-position of the Bodipy core; they were able to show that this Bodipy was nontoxic in rats. Only a few more water-soluble Bodipy derivatives were realized ever since, bearing carboxylic acids,[5−7] phosphonic acids,[8] oligo(ethylene glycol)s,[9−12] sulfobetains,[13] and sulfonated peptides.[14] Furthermore, a small number of amphiphilic molecules were synthesized to gain insight into lipid and membrane systems and reactions or dynamics in associated systems.[15−18] Additionally, sensor systems are becoming the focus of interest to trace ions[19−21] or pH[22] values for example in cells.

In this area often nanoparticle systems come into play, as they have several advantages. The first one is the possibility to have several dyes in one particle for ratiometric labeling; another point is the problem that several dye classes tend to be cytotoxic in their free form.[23] Additionally, fluorescent nanoparticles show a much higher fluorescent signal than fluorescent molecules that are used for labeling.[24] As the dye is entrapped in the particle, the local concentration is much higher, so that the absolute amount of dye needed for detection is reduced. A major problem in the field of particle systems is the mobility of the dye; often the dyes are physically entrapped in the polymeric bulk, either by encapsulation in the synthesis of the particle[25] or by swelling and soaking in of a dye solution into the particle.[26,27] Depending on the hydrophilicity of the

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dye and the swellability of the polymeric material, leaching of the dye could be pronounced and reach up to 50% within 48 h.[28]

Another approach is to covalently attach the label to the particle, either by copolymerizing a dye with a polymerizable function[27] or by covalently attaching a dye to a functionalized particle surface.[29] The latter has the advantage that the dye is in direct contact to the surrounding media, and neither interaction with the polymeric bulk material that might change slightly the photophysical behavior of the dye nor aggregation of the dye within the polymerization of the monomer could occur. Additionally, in the case where sensor systems are monitored, the analyte has no need to diffuse into the particle, which prevents a potential reduction of the analyte concentration at the interaction side. Disadvantageous is the need for coupling reagents to covalently attach the dye to the surface and the removal of the same afterward as well as a limited control of the post functionalization efficiency.

A versatile tool for the fabrication of functionalized nanoparticles by (co)polymerization or for the encapsulation of a great variety of substances is the miniemulsion (co)-polymerization.[30,31] In difference to the conventional emulsion polymerization, no diffusion step is involved in the polymerization by the addition of an (ultra)hydrophobe to the oil phase, which results in an osmotic pressure inside the droplets that is counteracting the Laplace pressure and thus suppressing the net diffusion. Also, the particle nucleation mechanism is different: while in emulsion polymerization homogeneous and micellar nucleation is predominant,[32,33] miniemulsion polymerization shows droplet nucleation.[30,31,34] Conversely, it means that the monomer droplet shows compositional and morphological similarity with the latter polymer particle to some extent and could thus be seen as a nanoreactor, where the initial composition of the dispersed phase is preserved in the particles.[30] This is especially of high interest when a hydrophobic dye should be incorporated or copolymerized; due to the low water solubility of the fluorescent dye, it is impossible to use a batch emulsion process where the monomer needs to diffuse to the polymerization side.[25]

Nevertheless, all heterophase copolymerizations with functional comonomers have the drawback that not only the surface of the particles is functionalized, but depending on the hydrophilicity of the comonomer, it might be also buried inside the particles[35] or form hairy structures on the particle surface[25,36] or even water-soluble homopolymers,[35−38] thus complicating a prediction of the functionalization density.

An alternative to overcome the aforementioned drawbacks is the use of a polymerizable surfactant. Polymerizable surfactants, also known as surfmers,[39] which is an acronym of surfactant and monomer, are a type of substances that can make the employment of a "normal" surfactant superfluous and lead to a direct and exclusive functionalization of the particle surface when used in miniemulsion polymerization, as herein the interphase between the continuous and dispersed phase is maintained throughout the polymerization. In batch emulsion polymerization highly reactive surfmers are likely to be polymerized in the early stages of the polymerization and become buried inside the growing particles leading to a destabilization of the dispersion or forming homopolymers causing bridging flocculation.[40,41] Therefore, the miniemulsion process in combination with surfmers was already discussed in the 1990s,[42,43] but only little was published[44] apart from the past few years, when more papers emerged in the topic.[45−49]

Herein the synthesis and application of two new surfmers are presented that are bearing, to the best of our knowledge, for the first time a Bodipy label in the headgroup, as an additional surfactant or sole surfactant in miniemulsion polymerization, for a fluorescent decoration of nanoparticle surfaces. The proof of copolymerization was conducted by HPLC measurements and the location of the surfmer at the surface of the particles by variation in fluorescent lifetime in the presence of a fluorescent quencher.

EXPERIMENTAL PART

Materials. The following reagents were purchased from commercial suppliers and used without further purification: hydrazine hydrate (100%, Acros), 4-hydroxybenzaldehyde (≥98%, Merck), 2,4-dimethylpyrrole (97%, Acros), boron trifluoride diethyl etherate (minimum 46.5% BF_3, Alfa Aesar), triethylamine (≥99.5%, Fluka), 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ, 98% Alfa Aesar), hydrobromic acid (47%, VWR tech grade), phenothiazine (98+%, Alfa Aesar), chlorosulfonic acid (99%, Aldrich), methyl viologen dichloride (98%, Sigma-Aldrich), sodium hydrogen carbonate powder (99.5%, WTL Laborbedarf), sodium iodide (99.5%, Sigma-Aldrich), sodium sulfate (anhydrous puriss., Sigma-Aldrich), 11-bromo-1-undecanol (97%, Alfa Aesar), methacryloyl chloride (97%, Alfa Aesar), sodium carbonate (99.5%, Deutero), phthalimide potassium salt 98% (Merck), 2,6 di-*tert*-butyl-*p*-cresol (Fisher Scientific), 2,2′-azobis(2-methylbutyronitrile) (V59, Wako), sodium iodide for analysis (Merck), potassium carbonate (anhydrous, 99%, Alfa Aesar), trifluoroacetic acid (99.5+%, Alfa Aesar), and magnesium sulfate extra pure anhydrous (Merck). Dry solvents (with water content smaller than 30 ppm) were purchased from Acros. All other solvents were p.a. or HPLC grade and purchased from different suppliers. Deuterated solvents for NMR measurements were supplied by Aldrich. Styrene (St) and methyl methacrylate (MMA) were purchased from Merck as synthesis grade and were distilled under reduced pressure and stored at 4 °C under argon.

Methods. Column chromatography was carried out using silica gel (Kieselgel 60, particle size 0.040−0.063 mm, 230−400 mesh) purchased from Fluka. For thin-layer chromatography (TLC) aluminum plates precoated with 0.2 mm silica gel 60 labeled with a fluorescents indicator were used (Alugram Sil G/UV 0.2 mm silica gel with fluorescent indicator, Machery-Nagel, Düren, Germany).

^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance spectrometers with 250 or 300 MHz. For ^{1}H and ^{13}C NMR spectra tetramethylsilane was used as an external standard.

ESI mass spectra were obtained from a Q-ToF Ultima 3 spectrometer with LockSpray interface from Micromass (Waters). Samples were injected with a concentration of ∼1 mg/mL in methanol.

Surface tension was determined by the DuNoüy ring methode, using a DuNoüy ring with a diameter of 10 mm and a wire thickness of 0.3 mm, at 20 °C with a DCAT 21 device (Dataphysics, Filderstadt, Germany), on a sample volume of ∼2 mL. All values presented were averaged over ten repetitions of push−pull cycles.

HPLC measurements were performed on an Agilent Technologies 1200 Series device, employing a C4 column of Marcherey-Nagel and a solvent gradient THF/H_2O/0.1% TFA from (0/40/60) to (10/100/0).

The average hydrodynamic diameter D_H of the particles and the paticle size distribution were measured by dynamic light scattering (DLS) using a Nanophox PCCS (Sympatec GmbH, Clausthal-Zellerfeld, Germany) at an scattering angle of 90° and a temperature of 25 °C. Dispersions were diluted to ∼0.1 wt % with distilled water. The measurement parameters were set to a count rate of 200 kcps, measuring time was 100 s for each run, and three repetitions were conducted. The raw data were plotted in Origin software (OriginLab), and a gauss fit was done.

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The ζ-potential measurements were performed in 10^{-3} mol/L KCl solutions on a Malvern Nano-Z device at 25 °C; therefore, latexes were diluted to a solid content of 0.02%.

Scanning electron microscopy (SEM) images were taken with a Gemini 1530 (Carl Zeiss AG, Oberkochen, Germany). The samples were prepared by drop casting of 0.01 wt % dispersions on silicon wafers.

Confocal laser scanning microscopy (CLSM) was performed with the TCS SP5X (Leica Mikrosysteme Vertrieb GmbH, Wetzlar) microscope. Steady-state fluorescence spectra were measured with a Spex FluoroLog 3 spectrofluorometer. Decays of fluorescence were recorded with the time correlation single photon counting (TCSPC) technique (FluoTime 200, PicoQuant GmbH). A cuvette (thickness 10 or 1 mm) with a solution/diluted dispersion was excited by a supercontinuum pulsed laser SC450-2-PP (10 MHz, ~1 mW/nm, pulse duration ~10 ps) (Fianium, Inc.). Output radiation of the laser was passed through a 4F monochromator[50] to select the needed wavelength. Typically, the radiation with a bandwidth of 10−15 nm was used. Right angle geometry of detection was chosen for fluorescence collection. Glan-Thompson polarizers (for excitation and detection) were arranged under magic angle condition. An additional long-pass filter Brightline 519/LP (Semrock Inc.) was placed in front of a Sciencetech Model 9030 monochromator for better eliminating scattered light. A counting photomultiplier PMA 165 (PicoQuant GmbH) was used as detector. The fluorescence lifetime (τ) for a single-exponential model or average lifetime ($\overline{\tau}$) for a multiexponential model was estimated with the software FluoFit (PicoQuant GmbH) in agreement with eqs 1−3:

$$I(t) = \int_{-\infty}^{t} IRF(t') \sum_{i=1}^{n} A_i e^{-(t-t')/\tau_i} \, dt' \quad (1)$$

$$\overline{\tau} = \sum_{i=1}^{n} f_i \tau_i \quad (2)$$

$$f_i = \frac{A_i \tau_i}{\sum_{i=1}^{n} A_i \tau_i} \quad (3)$$

It should be noted that in a subsequent part of this document symbol τ is always attributed to a single-exponential decay, whereas $\overline{\tau}$ to a multiexponential one.

The quantum yield (QY) of fluorescence was measured with the fluorescence standard rhodamine 6G in ethanol (QY = 0.95[51]), in agreement with the widely used method.[52]

Synthesis. All synthesis steps were carried out under water- and air-free conditions, as inert gas argon was used.

*2-(11-Hydroxyundecyl)isoindole-1,3-dione (Compound **2**).* The compound was synthesized according to Pérez et al.[53] with major modifications in the work-up. 11-Bromo-1-undecanol (25.00 g, 99.5 mmol) and potassium phthalimide (20.9 g, 112.8 mmol) were dissolved in dimethylformamide (250 mL) and stirred at 80 °C for 16 h. Afterward, the reaction mixture was allowed to cool to room temperature and was diluted with EtOAc (250 mL). The organic layer was washed with equivalent volumes of water several times; the combined aqueous phases were extracted twice with EtOAc (150 mL) each. The organic layer was washed with brine twice and dried over magnesium sulfate, the solvent was evaporated under reduced pressure, and the crude product was recrystallized from methanol. The desired compound was obtained as a white crystalline powder in 83% yield (26.2 g, 82.5 mmol): R_f = 0.37 (hexane/Et_2O 1:3). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 7.84 (dd, J = 3.0, 5.5 Hz, 2H, H_{Ar}), 7.70 (dd, J = 3.0, 5.5 Hz, 2H, H_{Ar}), 3.65 (m, 4H, $-CH_2O$, $-CH_2N$), 1.77−1.47 (m, 5H, $-CH_2-CH_2N$, $-CH_2-CH_2O$, $-OH$), 1.45−1.15 (m, 14H, $-CH_2-$). ^{13}C NMR (63 MHz, $CDCl_3$): δ = 168.6 (−C═O), 133.9 (C_{Ar}), 132.3 (C_{Ar}), 123.3 (C_{Ar}), 63.2 ($-CH_2OH$), 38.2 ($-CH_2N$), 32.9 ($-CH_2-$), 29.6 ($-CH_2-$), 29.5 ($-CH_2-$), 29.5 ($-CH_2-$), 29.5 ($-CH_2-$), 29.2 ($-CH_2-$), 28.7 ($-CH_2-$), 26.9 ($-CH_2-$), 25.8 ($-CH_2-$). LRMS (FD$^+$-MS m/z): calcd for $C_{19}H_{27}NO_3$ $[M]^-$ = 316.2; found 315.0.

*11-Aminoundecan-1-ol[54] (Compound **3**).* A solution of **2** (10.03 g, 31.6 mmol) in ethanol (185 mL) was cooled to 0 °C, and hydrazine hydrate (6.2 mL, 127.7 mmol) was added slowly. After stirring for 15 min at 0 °C the reaction was refluxed for 3 h. The work-up was done according to Fletcher et al.[54] Afterward, the reaction was allowed to cool to room temperature, diluted with 4 M HCl (200 mL), and filtered, and the filtrate was washed with DCM (100 mL) before being basified to pH 14 with concentrated NaOH solution. The water phase was extracted five times with CH_2Cl_2 (100 mL), the extracts were combined and dried over Na_2SO_4, and the solvent was evaporated to give the desired compound in 92% yield (5.42 g, 28.9 mmol) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.58 (t, J = 6.6 Hz, 2H, $-CH_2OH$), 2.65 (t, J = 6.8 Hz, 2H, $-CH_2NH_2$), 1.72 (s, 3H, $-NH_2$, $-OH$), 1.60−1.47 (m, 2H, $-CH_2-CH_2OH$), 1.47−1.10 (m, 16H, $-CH_2-$). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 62.8 ($-CH_2OH$), 42.3 ($-CH_2NH_2$), 33.8 ($-CH_2-CH_2NH_2$), 33.0 ($-CH_2-CH_2OH$), 29.7 ($-CH_2-$), 29.6 ($-CH_2-$), 29.6 ($-CH_2-$), 29.5 ($-CH_2-$), 27.0 ($-CH_2-$), 25.9 ($-CH_2-$). LRMS (ESI-MS m/z): calcd for $C_{11}H_{26}NO^+$ $[M + H]^+$ = 188.19; found 188.22.

*11-Bromoundecan-1-ammonium Bromide[55,56] (Compound **4**).* A suspension of **3** (10.00 g, 53.4 mmol) in aqueous HBr (concentrated, 40 mL) was stirred at 100 °C overnight, resulting in a dark brown solution. Cooling to room temperature caused the precipitation of the crude product as a dark gray solid that was recrystallized from acetone. The product was obtained in 83% yield (14.78 g, 44.6 mmol) as a pale gray solide. ^{1}H NMR (300 MHz, DMSO): δ = 7.80 (bs, 3H, $-NH_3$), 3.51 (t, J = 6.7 Hz, 2H, $-CH_2Br$), 2.74 (dd, J = 6.1 Hz, 14.5 Hz, 2H, $-CH_2-NH_3$), 1.86−1.67 (m, 2H, $-CH_2-$), 1.64−1.44 (m, 2H, $-CH_2-$), 1.42−1.13 (m, 14H, $-CH_2-$). ^{13}C NMR (75 MHz, DMSO): δ = 38.7 ($-CH_2NH_3$), 35.2 ($-CH_2Br$), 32.2 ($-CH_2-CH_2NH_3$), 28.8 ($-CH_2-$), 28.8 ($-CH_2-$), 28.7 ($-CH_2-$), 28.5 ($-CH_2-$), 28.1 ($-CH_2-$), 27.5 ($-CH_2-$), 26.8 ($-CH_2-$), 25.8 ($-CH_2-$). LRMS (ESI-MS m/z): calcd for $C_{11}H_{25}BrN^+$ $[M]^+$ = 250.12; found 250.14.

*N-(11-Hydroxyundecyl)methacrylamide (Compound **5**).* In a two-phase system, consisting of DCM (10 mL) as bottom layer and a solution of Na_2CO_3 (0.87 g, 8.25 mmol) in water (6 mL), 11-bromoundecan-1-ammonium bromide (1.00 g, 3.02 mmol) was dissolved, and the reaction mixture was brought to 0 °C. Subesquently, methacryloyl chloride (0.38 mL, 3.89 mmol) was slowly added, and the reaction was stirred for 30 min at 0 °C and afterward for 4 h at room temperature. Before separating the layers, water (10 mL) and DCM (10 mL) were added. The aqueous phase was transferred into alkaline milieu with saturated Na_2CO_3 solution and extracted twice with DCM. The organic layers were combined, laced with BHT as inhibitor, and washed with saturated Na_2CO_3 solution and water until the pH was neutral. The organic phase was dried over Na_2SO_4, and subsequently the solvent was evaporated. The crude product was purified by column chromatography on silica gel (petrol ether/diethyl ether 4:1, followed by pure diethyl ether) to give compound **5** in 81% (0.78 g, 2.45 mmol) yield, as polymerization inhibitor a small amount of phenothiazine was added. R_f = 0.68 (Et_2O). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.90 (s, 1H, NH), 5.65 (d, J = 0.8 Hz, 1H, CH=), 5.34−5.22 (m, 1H, CH=), 3.38 (t, J = 6.9 Hz, 2H, $-CH_2Br$), 3.28 (m, 2H, $-CH_2NH$), 1.94 (m, 3H, $-CH_3$), 1.90−1.74 (m, 2H, $-CH_2-$), 1.61−1.46 (m, 2H, $-CH_2-$), 1.40 (m, 2H, $-CH_2-$), 1.25 (s, 12H, $-CH_2-$). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 168.52 (−N−C═O), 140.3 (C_q=), 119.2 (=CH_2), 39.8 ($-CH_2N$), 34.1 ($-CH_2Br$), 32.9 ($-CH_2-$), 29.6 ($-CH_2-$), 29.5 ($-CH_2-$), 29.5 ($-CH_2-$), 29.4 ($-CH_2-$), 29.3 ($-CH_2-$), 28.8 ($-CH_2-$), 28.2 ($-CH_2-$), 27.0 ($-CH_2-$), 18.8 ($-CH_3$). LRMS (FD-MS m/z): calcd for $C_{15}H_{28}BrNO$ = 317.14; found 317.8 $[M]^-$.

All further reaction steps were carried out under water-free conditions and under striked exclusion of light by covering the equipment with aluminum foil.

*Compound **8**.* Bodipy **8** was synthesized according to the procedure reported by Liu et al.[57] To a solution of 4-hydroxybenzaldehyde (1.12 g, 9.13 mmol) and 2,4-dimethylpyrrole (2.00 mL, 19.8 mmol) in THF (300 mL) a few drops of TFA were added, and the reaction was stirred overnight. Complete consumption of the aldehyde was verified by

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TLC. Subsequently, DDQ (2.05 g, 9.0 mmol) dissolved in THF (330 mL) was added dropwise within 40 min, and the reaction was stirred at ambient temperature for 4 h; meanwhile, the color changed from orange to red to nearly black. After the addition of dry triethylamine (54 mL, 388.5 mmol) and 45 min of stirring, $BF_3{\cdot}Et_2O$ (54 mL, 588 mmol) was added slowly under ice bath cooling. The reaction was stirred overnight at room temperature, the conversion was checked by TLC, and the mixture was filtered through a pad of Celite. The filtrate was washed with brine twice and dried over Na_2SO_4, and the volatile substances evaporated under reduced pressure. The crude product was redissolved in DCM (300 mL) and washed twice with 0.5 M $NaHCO_3$ solution and subsequently several times with water, dried over Na_2SO_4, and concentrated to dryness. Compound **8** was received as a cherry-red solid in 52% yield (1.60 g, 4.7 mmol) after column chromatography on silica gel, using petrol ether/EtOAc (v/v 2:1) as the eluent: R_f = 0.5 (PET/EtOAc 2:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.12 (d, J = 8.6 Hz, 2H, CH_{Ar}), 6.94 (d, J = 8.6 Hz, 2H, CH_{Ar}), 5.98 (s, 2H, CH_{pyrrol}), 2.55 (s, 6H, pyrrol−CH_3), 1.44 (s, 6H, pyrrol−CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 156.4 (C_{Ar}), 155.4 (C_{Ar}), 143.3 (C_{Ar}), 141.9 (C_{Ar}), 132.0 (C_{Ar}), 129.5 (C_{Ar}), 127.3 (C_{Ar}), 121.3 (C_{Ar}), 116.3 (C_{Ar}), 14.7 (pyrrol-CH_3). LRMS (FD-MS m/z): calcd for $C_{19}H_{18}BF_2N_2O^-$ = 339.15; found 339.3 $[M - H]^-$.

Compound ***9***. Potassium carbonate (0.22 g, 1.58 mmol) was dried under vacuum for 15 min under slight warming. Subsequently, DMF (70 mL) and **8** (0.45 g, 1.32 mmol) were added; through the resulting solution argon was bubbled for 30 min. Afterward, **5** (0.42 g, 1.32 mmol) was added as well as small amounts of phenothiazine and sodium iodide. The reaction solution was stirred for 24 h at 100 °C; after cooling to room temperature, the reaction solution was poured into brine (200 mL), and the aqueous phase was extracted with EtOAc several times. The combined organic fractions were washed with brine twice and dried over Na_2SO_4. The crude product was purified by column chromatography on silica gel, using a gradient of petrol ether/EtOAc 4:1 (v:v) to 2:1. The product was obtained as a gluing dark red solid, in 44% yield: R_f = 0.26 (PET/EtAc 2:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.16 (d, J = 8.7 Hz, 2H, CH_{Ar}), 7.00 (d, J = 8.7 Hz, 2H, CH_{Ar}), 5.98 (s, 2H, CH_{pyrrol}), 5.79 (s, 1H, N*H*), 5.70−5.63 (m, 1H, CH_2=), 5.35−5.25 (m, 1H, C*H*=), 4.01 (t, J = 6.6 Hz, 2H, CH_2O), 3.40−3.23 (m, 2H, CH_2NH), 2.56 (s, 6H, pyrrol−CH_3), 1.97 (m, 3H, CH_3), 1.87−1.73 (m, 2H, O−CH_2−CH_2), 1.38 (m, 22H, pyrrol-CH_3, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 168.6 (*C*(=O)−N), 159.9 (C_{Ar}), 155.4 (C_{Ar}), 143.5 (C_{Ar}), 142.2 (C_{Ar}), 140.5 (C_q=), 132.1 (C_{Ar}), 129.3 (C_{Ar}), 127.0 (C_{Ar}), 121.3 (C_{Ar}), 119.3 (CH_2=), 115.3 (C_{Ar}), 68.4 (−CH_2−O), 39.9 (−CH_2N), 29.8 (−CH_2−), 29.7 (−CH_2−), 29.7 (−CH_2−), 29.5 (−CH_2−), 29.5 (−CH_2−), 27.2 (−CH_2−), 26.3 (−CH_2−), 18.9 (CH_3−C=CH_2), 14.8 (pyrrol-CH_3). FD: LRMS (FD-MS m/z): calcd for $C_{34}H_{46}BF_2N_3O_2$ = 577.37; found $[M^+]$ = 577.2.

Compounds ***10a*** *and* ***10b***. In a small Schlenk tube Bodipy **9** (0.35 g, 0.60 mmol) was dissolved in dry DCM (14 mL), and some crystals of BHT were added as a radical scavenger. In parallel, chlorosulfonic acid (0.16 mL, 2.4 mmol) was diluted with DCM (10 mL).

Monosulfonation. Subsequently, the reaction flask was immersed in a ice/salt mixture and cooled to −25 °C; the diluted chlorosulfonic acid (2.62 mL, 0.63 mmol) was added dropwise over a period of 15 min. The reaction was stirred at −25 °C for 30 min and afterward allowed to warm to RT, stirring further for 1 h. During that time the sulfonated product starts to precipitate; by addition of methanol (15 mL) the reaction mixture becomes homogeneous, and the proton was replaced by a sodium ion. Therefore, $NaHCO_3$ (0.11 g, 1.24 mmol) was added and the reaction stirred for 20 min.

The solvents were removed under reduced pressure, and the received solid was purified by column chromatography on silica gel. To elute the product, a gradient of EtOAc/MeOH 16:1 (v:v) to 5:1 (v:v) was used. The title compound **10a** was obtained as a red powder, in 50% yield (0.21 g, 0.30 mmol): R_f = 0.38 (EtOAc/MeOH 5:1). ^{1}H NMR (300 MHz, MeOD): δ = 7.27−7.15 (m, 2H, CH_{Ar}), 7.14−7.05 (m, 2H, CH_{Ar}), 6.15 (s, 1H, CH_{pyrrol}), 5.69−5.60 (m, 1H, C*H*=), 5.34 (m, 1H, C*H*=), 4.05 (t, J = 6.4 Hz, 2H, −CH_2−O), 3.22 (t, J = 7.1 Hz, 2H, −CH_2−N), 2.75 (s, 3H, pyrrol−CH_3), 2.52 (s, 3H, pyrrol−CH_3), 1.93 (m, 3H, CH_3−C=), 1.87−1.76 (m, 2H, −CH_2−), 1.72 (s, 3H, pyrrol−CH_3), 1.62−1.43 (m, 7H, pyrrol−CH_3, −CH_2−), 1.44−1.20 (m, 14H, −CH_2−). ^{13}C NMR: (75 MHz, MeOD): δ = 171.3 (*C*=ONH), 161.7 ($C_{HeteroAr}$−SO_3), 159.5 (C_{Ar}O), 153.5, 146.8 (C_{Ar}), 145.3 (C_{Ar}), 141.5 (C_{Ar}), 141.3 (C_{Ar}), 141.3 (C_q=), 134.4 (C_{Ar}), 130.9 (C_{Ar}), 130.5 (C_{Ar}), 127.8 (C_{Ar}), 123.5 (C_{Ar}), 120.1 (CH_2=), 116.5 (C_{Ar}), 69.2 (−CH_2O−), 40.7 (−CH_2N−), 30.9 (−CH_2−), 30.7 (−CH_2−), 30.6 (−CH_2−), 30.6 (−CH_2−), 30.5 (−CH_2−), 30.4 (−CH_2−), 30.3 (−CH_2−), 28.0 (−CH_2−), 27.1 (−CH_2−), 18.8 (CH_3−C=), 15.1 (pyrrol−CH_3), 14.7 (pyrrol−CH_3), 14.1 (pyrrol−CH_3), 13.3 (pyrrol−CH_3). LRMS (ESI-MS m/z): calcd for $C_{34}H_{45}BF_2N_3NaO_5S$ $[M + 2Na^+]^+$ = 702.29; found 702.30.

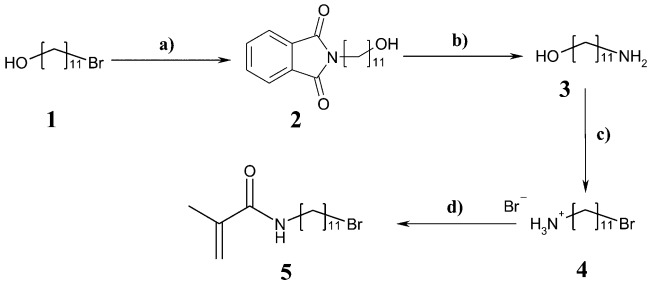
Double Sulfonation. The double-sulfonated compound could be obtained in the same manner as the monosulfonated one; only the concentration of the chlorosulfonic acid dilution and the amount of $NaHCO_3$ have to be doubled: R_f = 0.5 (EtOAc/MeOH 2:1). ^{1}H NMR (250 MHz, MeOD): δ = 7.98 (s, 1H, *C*=ON*H*), 7.22 (d, J = 8.8 Hz, 2H, CH_{Ar}), 7.12 (d, J = 8.8 Hz, 2H, CH_{Ar}), 5.71−5.60 (m, 1H, =CH_{trans}), 5.40−5.29 (m, 1H, =CH_{cis}), 4.07 (t, J = 6.3 Hz, 2H, −CH_2−O), 3.27−3.12 (m, 2H, −CH_2−NH), 2.78 (s, 6H, pyrrol−CH_3), 1.93 (m, 3H, CH_3−C=), 1.88−1.76 (m, 2H, CH_2−CH_2−O), 1.73 (s, 6H, pyrrol−CH_3), 1.64−1.20 (m, 16H, −CH_2−). ^{13}C NMR (63 MHz, MeOD): δ = 171.2 (*C*=ONH), 161.8 (C_{Ar}), 156.4 (C_{Ar}), 147.2 (C_{Ar}), 143.5 (C_{Ar}), 141.5 (C_q=), 135.7 (C_{Ar}), 132.1 (C_{Ar}), 130.5 (C_{Ar}), 127.5 (C_{Ar}), 120.2 (CH_2=), 116.7 (C_{Ar}), 69.2 (CH_2−O), 40.7 (CH_2−NH), 30.6 (−CH_2−), 30.6 (−CH_2−), 30.5 (−CH_2−), 30.4 (−CH_2−), 30.4 (−CH_2−), 30.3 (−CH_2−), 28.0 (−CH_2−), 27.1 (−CH_2−), 18.8 (CH_3−C=), 14.4 (pyrrol−CH_3), 13.64 (pyrrol−CH_3). ESI: LRMS (ESI-MS m/z): calcd for $C_{34}H_{45}BF_2N_3NaO_5S$ $[M + Na^+]$ = 758.25; found 758.27.

RESULTS AND DISCUSSION

Synthesis of Dyes. In the design of the synthetic route to the surfmer, it was taken into account that Bodipy dyes are extremely robust to thermal and chemical[2] treatment; nevertheless, the purification of Bodipy dyes is rather tedious. For this reason it was decided to use a convergent synthesis strategy, where the Bodipy core is preformed and connected to the hydrophobic tail that contains already the polymerizable group in the last step, before the formation of the ionic headgroup of the surfmer.

The synthesis of the hydrophobic tail with the polymerizable group is shown in Scheme 1. The starting point of the synthesis is the commercially available 11-bromo-1-undecanol (**1**), which is converted to 11-amino-1-undecanol[54] in a Gabriel synthesis, employing the Ing−Mansk[58] variation. By treatment of compound **3**[55,56] with boiling hydrobromic acid, the alcohol group is converted to bromine; additionally, the amine is

Scheme 1. Synthesis of the Hydrophobic Tail Containing the Polymerizable Unit[a]



[a]Reagents and conditions: (a) phthalimide potassium salt, DMF, 80 °C, 16 h; (b) hydrazine monohydrate, EtOH, 15 min, 0 C, 3 h reflux; (c) HBr (concentrated), 100 °C, overnight; (d) methacryloyl chloride, H_2O, DCM, Na_2CO_3, 30 min 0 °C, 4 h, RT.

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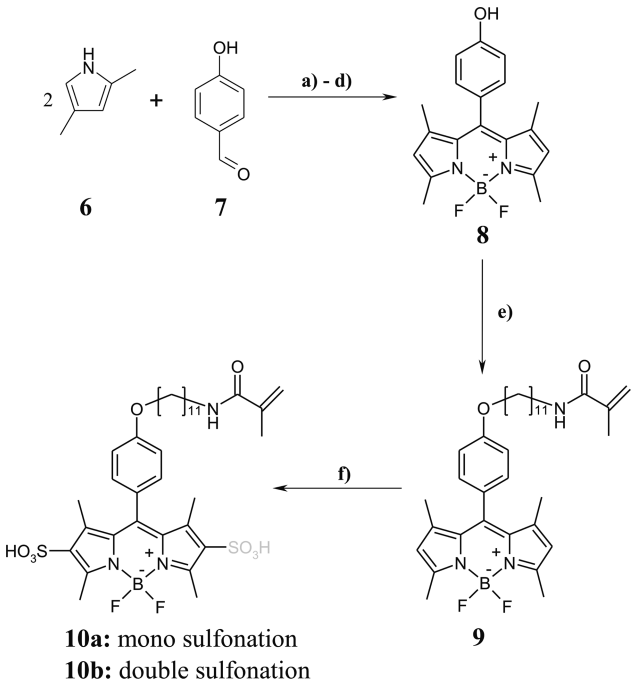
protonated, so that a quaternization reaction is prevented. The introduction of the polymerizable group to the hydrophobic tail is realized under Schotten–Baumann conditions.

The amide linkage between the polymerizable group and the alkyl spacer seems to be beneficial as methacrylamides are less likely to hydrolyze or polymerize under various synthetic conditions.

The Bodipy core **8** with a phenolic moiety in the *meso* position was synthesized according to a procedure reported earlier by Liu et al.[57] The acidic phenolic OH group at the Bodipy core and the nucleophilic substitutable bromine at the polymerizable alkyl unit allow one to link the Bodipy dye with the hydrophobic, polymerizable unit in a convergent synthesis step by Williamson ether formation. The synthesis is shown in Scheme 2. The Williamson ether formation has proven its

Scheme 2. Synthesis Route of Mono- or Double-Sulfonated Bodipy Surfmer[a]



[a]Reagents and conditions: (a) TFA, THF, RT, overnight; (b) DDQ, THF, RT, 4 h; (c) triethylamine, RT, 45 min; (d) $BF_3{\cdot}Et_2O$, 30 min, 0 °C, RT overnight; (e) **5**, K_2CO_3, NaI, phenothiazine, DMF, 100 °C, 24 h.

applicability to Bodipy's containing a phenolic moiety in the *meso* position for incorporation into more complex structures before.[59,60]

In the final step, the hydrophilic headgroup was created by either mono- or double-sulfonation of the Bodipy core in the 2- or 2,6-position with chlorosulfonic acid. The substitution of the hydrogens in the 2- and 6-position has been already used to create a couple of water-soluble Bodipy derivatives without any surface activity.[4,61−63]

Miniemulsion Copolymerization. Dye-labeled nanoparticles were synthesized via free radical polymerization in miniemulsion (all ratios of the chemical components are presented in Table 1) using the hydrophobic initiator V59. The compounds **10a** and **10b** show the interfacial activity even at such low amounts as used for the miniemulsion polymerizations. The values of surface tension drop to γ = 48.5 mN/m for **10a** and γ = 48.0 mN/m for **10b** (solutions were prepared by addition of 1.5 or 1.3 mg of dyes to 2.4 g of water as used for the miniemulsion polymerization of **PS2** and **PS3**). The polymerization leads to a stable dispersion with an average particles diameter of 250 nm (measured by DLS) and rather high negative ξ-potential (after performed dialysis). As can be seen from the data in Table 1, the dyes **10a** and **10b** can effectively stabilize nanoparticles after the miniemulsion process. In order to reduce the particle size the surfmers **10a** or **10b** were used in combination with sodium dodecyl sulfate as anionic surfactant. In fact, copolymerization of the dyes in presence of SDS leads to a considerable reduction of particle size (down to 100 nm, as it is shown in Table 1). Additionally, miniemulsion polymerizations were carried out with the nonsurface active polymerizable dye **9** as a control experiment. In this experiment dye **9** was dissolved in the organic phase prior to emulsification.

Table 1. Synthesis and Characterization of Polystyrene Nanoparticles

sample	surfactant/ amount, mg	dye/ amount, mg	ξ-potential, mV	D_H,[a] nm	% solid content
PS1[b]	SDS/72	**9**/4.5	−64.3	102 ± 17	20.9
PS2[c]		**10a**/1.5	−58.2	210 ± 29	9.5
PS3[c]		**10b**/1.3	−70.0	251 ± 28	9.6
PS4[b]	SDS/72	**10a**/8.8	−68.0	103 ± 17	21.4
PS5[b]	SDS/72	**10b**/8.4	−54.9	98 ± 14	20.8

[a]Hydrodynamic diameter estimated by DLS. Solid content was determined gravimetrically. Amount of components in miniemulsion polymerization. [b]24 g of water, 6 g of styrene, 250 mg of hexadecane, and 100 mg of initiator V59; amounts of SDS and dyes are displaced in the table. [c]2.4 g of water, 300 mg of styrene, 34 mg of hexadecane, and 14 mg of V59.

To proof the copolymerization of surfmers, the dispersions were freeze-dried; subsequently, the polymer was dissolved in THF and subjected to HPLC analysis. Only traces of the free surfmer are detectable in polymeric samples, meaning that most of the surfmer is incorporated to the polymeric backbone.

Photophysical Properties of Dyes in Solutions. Table 2 summarizes the spectroscopic properties of the synthesized dyes. Absorption, steady-state fluorescence spectra and decays of fluorescence in different solvents are presented in Figures 1a and 1b. Compound **9** is insoluble in water and well soluble only in organic solvents. Absorption (λ_{max} = 503 nm) and emission maxima (λ_{max} = 514 nm) are as expected for dyes possessing the similar Bodipy core. The molar extinction coefficient is relatively large (8.8×10^4 L/(mol cm)). The decay of fluorescence recovered from time-resolved measurements (Figure 1b) was fitted by a single-exponential function and

Table 2. Photophysical Characteristics of Synthesized Dyes

dye	solvent	λ_{max} of abs, nm/molar extinction, L/(mol cm)	λ_{max} of fluorescence, nm	fluorescence lifetime, ns	quantum yield, %
9	toluene	503/ 8.8×10^4	514	3.2[a]	69
10a	water	493/ 4.7×10^4	510	1.4[b]	6
10b	water	497/ 6.7×10^4	510	0.29[b]	∼1

[a]τ. [b]$\bar{\tau}$.

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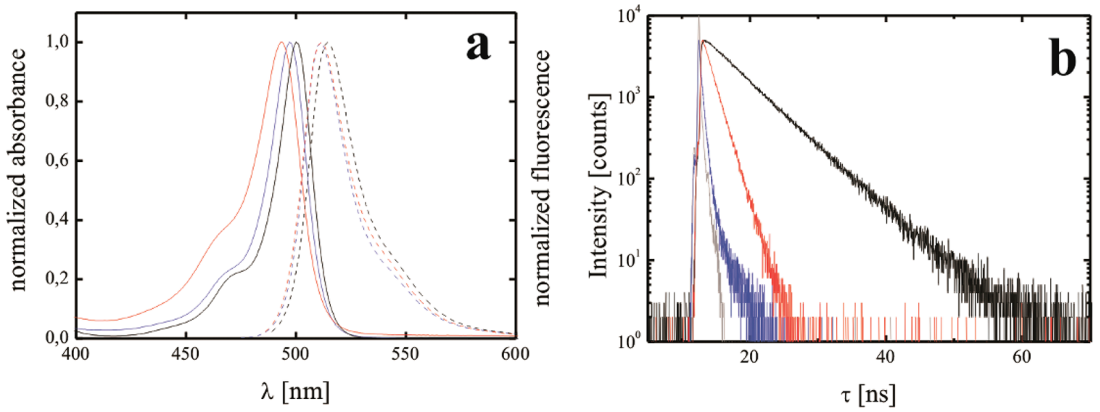

Figure 1. (a) Normalized fluorescence (dashed lines) and absorption (solid lines) spectra of dye **9** in toluene (black lines); dye **10a** in H_2O (red lines); dye **10b** in H_2O (blue lines). Fluorescence spectra are recorded at concentration 1×10^{-6} mol/L; absorption at 5×10^{-6} mol/L. (b) Decays of fluorescence: dye **9** in toluene (black lines), dye **10a** in H_2O (red lines), dye **10b** in H_2O (blue lines); the instrument response function of the setup (IRF) (gray line).

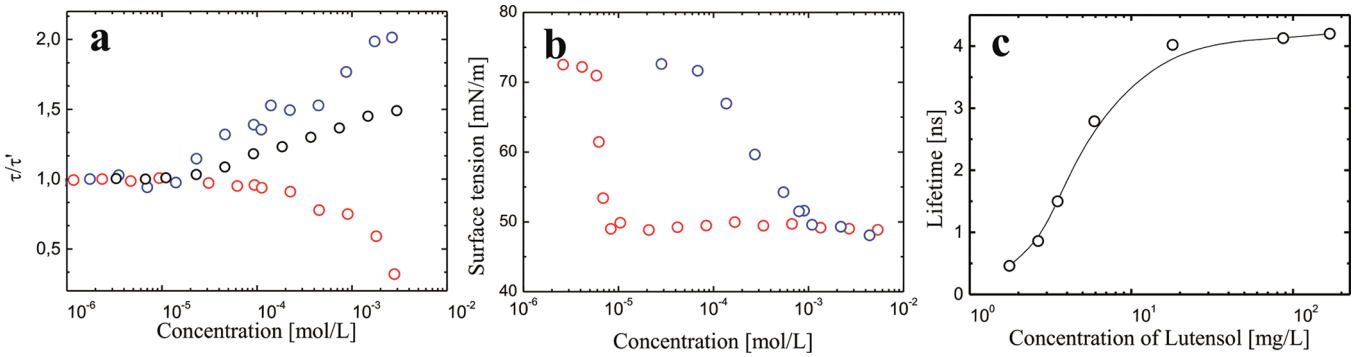

Figure 2. (a) Concentration dependence of the normalized lifetime in case of water solutions of **10a** (red circles) and **10b** (blue circles) and toluene solution of **9** (black circles). τ' is the lifetime measured at a concentration $c = 10^{-6}$ mol/L: 1.4 ns (**10a**), 0.29 ns (**10b**), and 3.2 ns (**9**). (b) Concentration dependence of surface tension in case of water solutions of **10a** (red circles) and **10b** (blue circles). (c) Lifetime of water solution **10b** ($c = 1 \times 10^{-5}$ mol/L) at different amounts of Lutensol AT50 added. Solid red line is a guide to the eye only.

the fluorescence lifetime estimated as $\tau = 3.2$ ns. The QY is moderately high and is estimated as 69%. In contrast to compound **9**, dyes **10a** and **10b** are well soluble in water. Dye **10a** is additionally partially soluble in nonpolar organic solvents as toluene and styrene. Absorption spectra in water have smaller molar extinction coefficients and are hypsochromically shifted with respect to spectra of dye **9** in toluene. It was found that an aqueous environment provides significant altering of the fluorescence properties. The QY of the fluorescence is dropping down with increasing number of sulfo groups. Thus, fluorescence of **10a** in aqueous solution shows a double-exponential decay with an average lifetime of $\overline{\tau} = 1.4$ ns and the QY of 6%. The double-sulfonated dye in water demonstrates only weak fluorescence (QY ~ 1%) and extremely short lifetime of $\overline{\tau} = 0.29$ ns.

In general, the modification of the Bodipy core with sulfo groups does not change the fluorescent properties in the same way as in our experiments. Fluorescence lifetime and quantum yield of sulfonated and nonsulfonated derivatives show unimportant differences.[1] Therefore, in order to obtain additional information about intermolecular interactions in solutions of dye **9**, **10a**, and **10b**, the concentration dependence of the fluorescence lifetime was tested. In Figure 2a, the dependence of normalized lifetime (normalization factor τ' is the lifetime measured at a concentration $c = 10^{-6}$ mol/L when negligible effect of intermolecular interaction or association are expected) on concentration of solutions. In the experiment an increase of the lifetime is observed for dye **9** in toluene at concentrations higher than 5×10^{-5} mol/L. This effect can be ascribed as reabsorption of emitted photons in solutions with high optical density and subsequent secondary emission that increases the apparent lifetime of the excited state.[64] Thus, the experiment shows a response of the lifetime on the setup geometry (mainly the length of the optical pathways for absorbed and emitted photons) when concentrated solutions are investigated. Additionally, it was supposed that any other reason (nether self-quenching or other cooperative phenomena) can be responsible for lifetime changing in toluene solution of hydrophobic compound **9**. In contrast, the solution of monosulfonated dye **10a** in water shows a decreasing lifetime with increasing dye concentration. We believe that such behavior reflects the process of micelle formation, and thus, the cmc (critical micelle concentration) can be very approximately placed in the diapason of the concentration range 10^{-5}–10^{-4} mol/L. In fact, micelle formation leads to shortening of the interchromophore distance and amplifies the process of self-quenching. For a more precise estimation of cmc, we investigated the concentration dependence of the surface tension. The results shown in Figure 2b indicate that the value of cmc for surfmer **10a** can be estimated as 9×10^{-6} mol/L.

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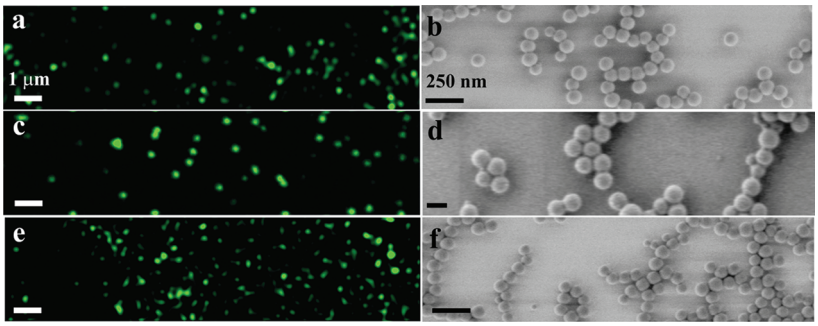

Figure 3. (a, c, e) CLSM micrographs of the particles adsorbed onto the surface of microscope slides. (b, d, f) Corresponding SEM micrographs. (a, b) Dispersion **PS1**; (c, d) dispersion **PS2**; (e, f) dispersion **PS4**. Scale bar is 1 μm for all CLSM images and 250 nm for all SEM images.

Experiments with solutions of compound **10b** demonstrate a very strong influence of solvent polarity on the fluorescent properties. For instance, the lifetime of **10b** measured in less polar solvents is significantly longer: τ = 2.97 ns in ethanol and τ = 4.31 ns in a mixture of DMF/chloroform (1/1). It is very likely that the ultrashort fluorescence lifetime in water reflects the response of an excited state on very polar media. Solutions with concentrations down to 1×10^{-7} mol/L were investigated, and no changes in lifetime were detected. Accordingly to this fact, the lifetime shortening due to association of the dye molecule can be excluded. On the other hand, the increase of the dye concentration gives a stronger raise of normalized lifetime as compared to dye **9**. It should be noted that micelle formation can make possibly both association of molecules and changing polarity of media in proximity of dyes. The media becomes more hydrophobic. The cmc value for surfmer **10b** was estimated from surface tension measurements as 8×10^{-4} mol/L (Figure 2b). Therefore, it can be hypothesized that the increase of lifetime in the case of **10b** also might be attributed to micelle formation.

To prove this idea, the following experiment was performed. Interestingly, the addition of small amounts of the nonionic surfactant Lutensol AT50 to an aqueous solution of dye **10b** recovers its fluorescent properties (the dependence between the Lutensol AT50 concentration and the fluorescence lifetime of **10b** is presented in Figure 2b). The fluorescence lifetime was estimated as τ = 4.02 ns in a solution containing 1×10^{-5} mol/L of dye **10b** and 18 mg/L (7.3×10^{-6} mol/L) of Lutensol AT50. This result represents a strong hydrophobic interaction between **10b** and the nonionic surfactant and suggests that micelle formation can lead to a lifetime increase.

An explanation for the different behavior of **10a** and **10b** in the micellar solution might be derived from the difference in the charge density around the Bodipy core, thus influencing the packing density and preventing stacking in the case of **10b**.

Photophysical Properties of the Dyes Copolymerized via Miniemulsion Process. Miniemulsion copolymerization of the hydrophobic precursor **9** (dispersion **PS1**) did not modify strongly its spectral characteristics; however, the fluorescence lifetime becomes significantly longer (τ = 5.5 ns). The obtained result indicates the spectral properties of the dye incorporated covalently within the polymeric nanoparticles. The fluorescence lifetime of dyes **10a** (dispersion **PS2**) and **10b** (dispersion **PS3**) are modified in the same way ($\overline{\tau}_{PS2}$ = 1.3 ns and $\overline{\tau}_{PS3}$ = 0.73 ns) and show a double-exponential behavior. The dispersions were tested with different concentrations of the dispersed phase (from almost transparent solution at solid content 0.001% to slightly milky one at solid content 0.1%) and found no influence of scattering effects on the fluorescent lifetime. Considering intermediate values of the lifetime (ranging between a very short lifetime in water and a rather long lifetime within the polymeric nanoparticles), the existence of preferentially surface labeling of nanoparticles can be assumed when fluorescence surfmers **10a** and **10b** are used. The multiexponential behavior of the lifetime might indicate a distribution of the dye in the outer layer of the polymeric particles (some molecules settle down closer to the particles center than others) and the presence of gradient of polymer hydration within the outer layer. Copolymerization of the dyes **10a** and **10b** in the presence of SDS leads to a further increase of the lifetime (up to $\overline{\tau}$ = 3.6 ns for **10a** (dispersion **PS4**) and $\overline{\tau}$ = 3.9 ns for **10b** (dispersion **PS5**).

There is a direct correlation between the position of the dye (water phase, water/polymer interface, or polymeric matrix) and its lifetime:

$$\overline{\tau}_{10b}^{water} < \overline{\tau}_{10b}^{PS3} < \overline{\tau}_{10b}^{PS5} < \tau_{9}^{PS1}$$

The lifetime is importantly longer for molecules located near the nanoparticle interface than in water but shorter in respect to the dye distributed inside a solid polymeric matrix. The fact that the lifetime in case of dispersion **PS3** is shorter than in the case of dispersion **PS5** allows one to assume a more hydrophobic character of the environment in proximity of the fluorophore molecules when SDS is used as additional surfactant. Thus, SDS might in some degree shield the contacts between dyes **10a**/**10b** and water molecules that will be confirmed additionally by quenching tests.

In whole, extension of fluorescence lifetime in all described dispersions and very likely increased QY provide bright fluorescent nanoparticles easily resolved by CLSM (Figure 3). Figures 3a, 3c, and 3e display the CLSM images of nanoparticles adsorbed on top of microscope slides. Also, micrographs in Figures 3b, 3d, and 3f show the particles morphology as detected by SEM microscopy.

To prove the fact of preferable localization of the surfmers in the surface layer of the nanopraticles, quenching experiments were performed with fine dialyzed dispersions. Initially, the iodine anion (I^-) was used which is a well-known quencher due to the heavy atom effect. Increasing amounts of NaI dissolved in the continuous phase lowered the lifetime and steady-state intensity. The fluorescence lifetime is dropping down in the range from $\overline{\tau}$ = 0.73 ns (without a quencher) to $\overline{\tau}$ = 0.42 ns at 0.5 M concentration of NaI in case of **PS3** and from $\overline{\tau}$ = 3.9 ns to $\overline{\tau}$ = 2.05 ns in case of **PS5**. This experiment likely reflects the process of dynamic quenching, and therefore, experimental

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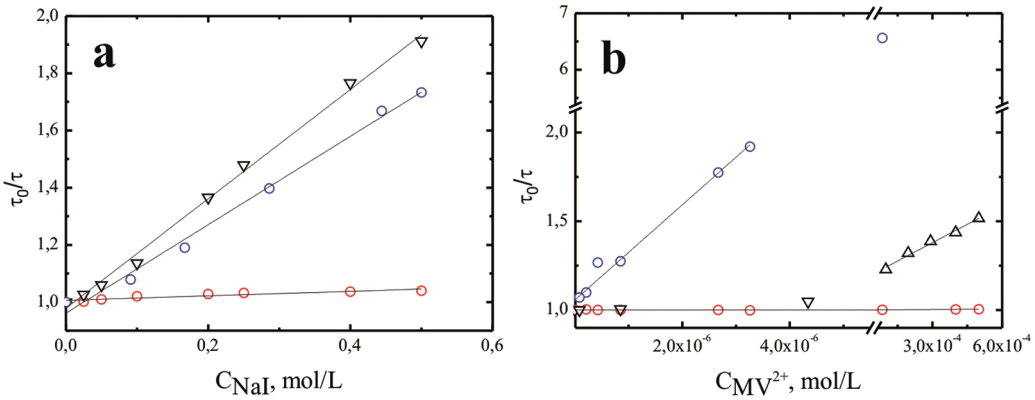


Figure 4. Quenching of fluorescence in case of dispersions **PS1** (red circles), **PS3** (blue circles), and **PS5** (black triangles). The black lines reflect the result of fitting according to eq 4. Quenchers: sodium iodide (a) and methyl viologen dichloride (b).

results can be fitted in agreement with the Stern–Volmer equation:

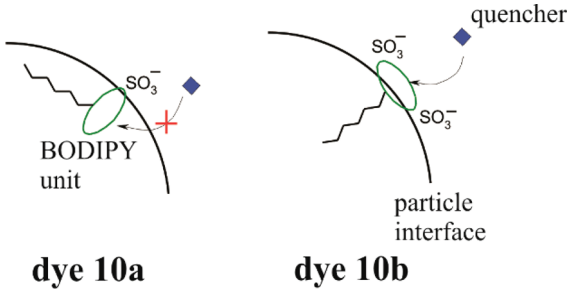
$$\frac{\tau_0}{\tau} = 1 + k_q\tau_0[Q] = 1 + K_D[Q] \quad (4)$$

The equation provides values of the Stern–Volmer quenching constant K_D = 1.55 L/mol (dispersion **PS3**) and K_D = 1.91 L/mol (dispersion **PS5**). Numbers of bimolecular quenching constant, calculated in agreement with eq 4, were estimated as $k_q = 2.06 \times 10^9$ L/(mol s) (dispersion **PS3**) and $k_q = 4.87 \times 10^8$ L/(mol s) (dispersion **PS5**). Such relatively low values of the constants can be explained by two reasons. First, polymeric nanoparticles have much slower diffusion rates in comparison to molecules in solutions. Second, the surfaces of the nanoparticles are negatively charged (Table 1) that leads to an additional electrostatic repulsion between nanoparticles and the negatively charged quencher I^-. Additionally, a shielding effect of SDS, as noted earlier, might be responsible for a lower value of the bimolecular quenching constant in the case of dispersion **PS5**.

In further experiments, the quenching effect of methyl viologen (MV^{2+}) dichloride (Figure 3b) was investigated. Since the quencher is positively charged, it can interact more efficiently with the dyes and quench them. Additionally, a superposition of the dynamic and static quenching in case of MV^{2+} provides very high quenching efficiency in micelles,[65] solutions,[66] and in proximity of an interface.[67] In the experiments the fluorescence lifetime was changing from $\bar{\tau}$ = 0.73 ns (without a quencher) to $\bar{\tau}$ = 0.38 ns at 3.3×10^{-6} mol/L concentration of MV^{2+} in the case of **PS3** and from $\bar{\tau}$ = 3.9 ns to $\bar{\tau}$ = 2.56 ns at 5×10^{-4} M of MV^{2+} in the case of **PS5**. Thus, abnormally high values of the Stern–Volmer quenching constant were observed for dispersion **PS3** $K_D = 2.7 \times 10^5$ L/mol, which judges about impact of static mechanism additionally to collision quenching. For dispersion **PS5** the value K_D = 688 L/mol was calculated. The important detail was obtained by investigation of quenching of dispersion **PS1**. No traces of interaction were detected between the quencher and the dye even at high concentration of quencher NaI (Figure 3a) or methyl viologen dichloride (Figure 3b). Thus, the dye was effectively protected by the polymeric matrix. This clear contrast in quenching behavior of dispersions **PS1** (from one side) and **PS3** and **PS5** (from another side) makes it possible to conclude that the fluorescent surfmer molecules **10b** are primarily located at the surface if used as comonomers in miniemulsion polymerization.

Only a very weak quenching effect was observed for dispersions **PS2** and **PS4** even in aqueous solutions of MV^{2+} with concentrations up to 10^{-3} mol/L. Since dye **10a** is soluble in water as well as in organic solvents, miniemulsion copolymerization results in a distribution of the dye between the surface and volume of the particle. Moreover, the orientation of the monosufonated Bodipy unit at the particles interface might differ from double-sulfonated one, as shown in Scheme 3. Thus, direct interaction of a quencher and the π-electronic system of the dye **10a** might be restricted.

Scheme 3. Probable Orientation of Dyes 10a and 10b on the Surface of Nanoparticles after Miniemulsion Polymerization



CONCLUSIONS

In the present work, new fluorescence surfmers with outstanding properties were synthesized. The new molecules joint within their chemical structure different abilities: to be polymerizable, to emit photons, and to show interfacial activity. The miniemulsion polymerization performed with participation of the new surfmers is a direct one-pot procedure for the production of nanoparticles selectively fluorescence-labeled at the interface. Without additional synthesis and purification steps aqueous dispersions of hydrophobic nanoparticles were obtained showing a strong fluorescent response to substances dissolved in the continuous phase. In the case of the double-sulfonated surfmer a strong quenching of particles fluorescence was observed by sodium iodide or methyl viologen dissolved in water media. Thus, these nanoparticles can be interesting for sensing applications based on fluorescence quenching or fluorescence resonance energy transfer effects. Additionally, a strong influence of polarity on the fluorescence lifetime was found for the investigated surfmers. Therefore, processes

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changing interfacial polarity can be investigated by using the newly synthesized nanoparticles. For instance, absorption of synthetic or natural macromolecules onto particles interface or particles association/aggregation might be quantified. These issues are currently examined in our laboratory.

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Notes

The authors declare no competing financial interest.

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