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COMMUNICATION

Light-triggered conversion of non-ionic into ionic surfactants: towards chameleon detergents for 2-D gel electrophoresis†‡

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Caged non-ionic detergents, comprised of polar oligo (ethylene glycol) and non-polar alkyl chains joined by a photocleavable *ortho*-nitrobenzyl sulfonate linker have been synthesized and characterized. The light-triggered transformation of such chameleon surfactant from a charge-neutral into a charged form offers great potential to improve 2-D gel electrophoretic separation of complex protein mixtures.

In recent years, (macro)molecules that respond to external stimuli by characteristic changes of their properties have become increasingly important in so-called "smart" materials. In particular, photoresponsive systems allowing for precise external control over time and location of the non-invasive stimulus have been developed. While the use of photochromic moieties allows for reversibility and therefore true switching processes, many systems relevant to biology,[1] diagnostics,[2] and materials science[3] benefit from the concept of light-triggered, irreversible activation of a protected group or molecule, sometimes referred to as "caged" compounds.[1] Among the most commonly explored photolabile protecting groups has been the *ortho*-nitrobenzyl moiety allowing for the unmasking of various functionalities, such as ethers, esters, phosphates, sulfates *etc.*[4] Upon irradiation, the photocleavage process results in the spatially and temporally defined presentation of these functionalities, allowing for specific attachment[5] and delivery[6] as well as local changes in pH,[7] electrostatic interactions[6,8] and aggregation behavior,[9,10] among others.

Due to the common interest in the often-challenging analysis of less abundant regulatory proteins, such as transcription factors,[11] as well as membrane-bound proteins,[12–14] we have engaged in improving 2-D gel electrophoresis[15–17] (2-D GE) by the use of photoresponsive surfactants. Our chameleon surfactants combine two different sets of properties facilitating the separation of proteins in both dimensions. Specifically, we have targeted non-ionic amphiphiles that solubilize the desired proteins and aid isoelectric focusing in the first dimension, *i.e.* separation based on charge. After the proteins have reached the location of their specific isoelectric point, which is naturally associated with low solubility, irradiation of the gel should trigger the photochemical conversion to an ionic surfactant mimicking sodium dodecyl sulfate (SDS) in the second dimension, *i.e.* separation based on molecular weight, so called SDS-PAGE. Therefore, the detergent would remain associated with the micellar protein-surfactant complex, principally leading to an enhanced separation performance as compared to traditional methods that necessitate detergent exchange.[18–20] Such exchange is usually incomplete leading to formation of a broad distribution of protein-surfactant micelles and therefore loss of resolution, *i.e.* "smearing".[21] Furthermore, the surfactant exchange is associated with significant loss of proteins therefore pushing the concentration of under-represented proteins below the detection limit.[22]

Here, we put forth a concept to overcome these problems by the use of suitable photocaged detergents (Fig. 1). We describe the design and synthesis of such photocaged detergents as well as their photochemistry, aggregation behavior, and protein solubilization ability.

In order to address the problem outline above, we designed amphiphiles based on poly(ethylene glycol) (PEG) and alkyl chains joined *via* a photocleavable *ortho*-nitrobenzyl moiety leading to the release of the corresponding alkyl sulfonate upon irradiation (Fig. 1). Synthesis‡ involved PEGylation of commercially available 3-hydroxy-5-nitrobenzaldehyde **1** to give **4** followed by reduction to yield the corresponding *ortho*-nitrobenzyl alcohol **5**, which was readily coupled with various alkylsulfonyl

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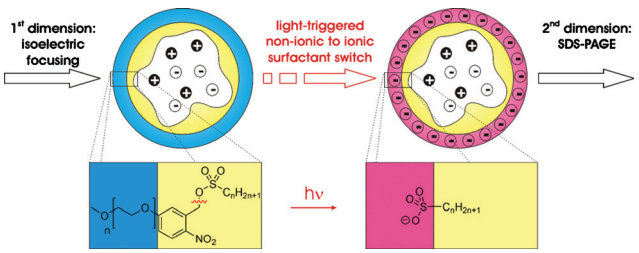
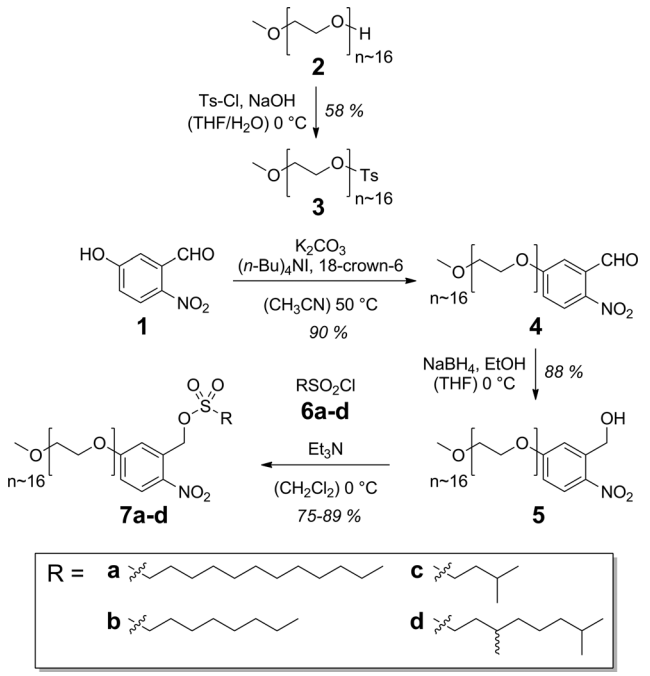


Fig. 1 Improving 2-D gel electrophoresis using photolabile detergents based on amphiphilic *ortho*-nitrobenzylsulfonates.



Scheme 1 Modular synthesis of surfactants **7a–d**.

chlorides to afford the desired detergents **7a–d** (Scheme 1). Note that the PEG-chain was attached to the photoreactive *ortho*-nitrobenzyl moiety *via* an ether linkage in the *para*-position to the nitro group in order to bathochromically shift the absorption maximum and thereby avoiding potentially harmful excitation of (hetero)aromatic amino acid side chain residues, such as tryptophane, tyrosine, phenylalanine, and histidine, in the gel. The synthesis is highly modular by allowing for the introduction of different hydrophobic alkyl side chains in the final step of the sequence.

Variation of both the length and the branching of the alkyl chains, known to affect the packing of the hydrophobic tails in surfactant assemblies such as micelles and bilayers,[23] allowed for convenient tuning of the surfactants' critical micelle concentrations (CMCs),‡ as illustrated in Table 1. Clearly, the formed micelles can be destabilized by shortening the alkyl chain length and increasing the number of methyl branches, *i.e.* **7a** → **7d**. Such control over the surfactants' CMC is absolutely crucial in order to develop and optimize a detergent, which can solubilize the desired proteins and thereby enable their electrophoretic separation. In analogy to other charge-neutral detergents, such as Triton X-100, **7a–d** exhibit rather low CMCs.

Irradiation experiments using selective excitation at 313 nm‡ (for absorption maxima see Table 1) showed rapid transformation of *ortho*-nitrobenzyl sulfonates **7a–d** in water as indicated by the observed changes in the UV/vis absorption spectra (Fig. 2a). Simultaneous HPLC-monitoring confirmed formation of a polar PEG-based fragment, most likely **9** based on MS detection, eluting faster than its parent photocleavable amphiphile **7d** (Fig. 2b). Clearly, irradiation leads to scission of the charge-neutral *ortho*-nitrobenzyl sulfonate surfactant and creates an ionic surfactant in the form of the corresponding dissociated sulfonic acid. The CMCs of the sulfonic acid photoproducts,

Table 1 Absorption maxima and critical micelle concentrations (CMCs) of surfactants **7a–d** and laurylsulfonic acid **8** in water

Compound	**7a**	**7b**	**7c**	**7d**	**8**
λ_{max} (nm)	313	315	320	313	n.d.
CMC (μM)	<10	40	60	75	8000

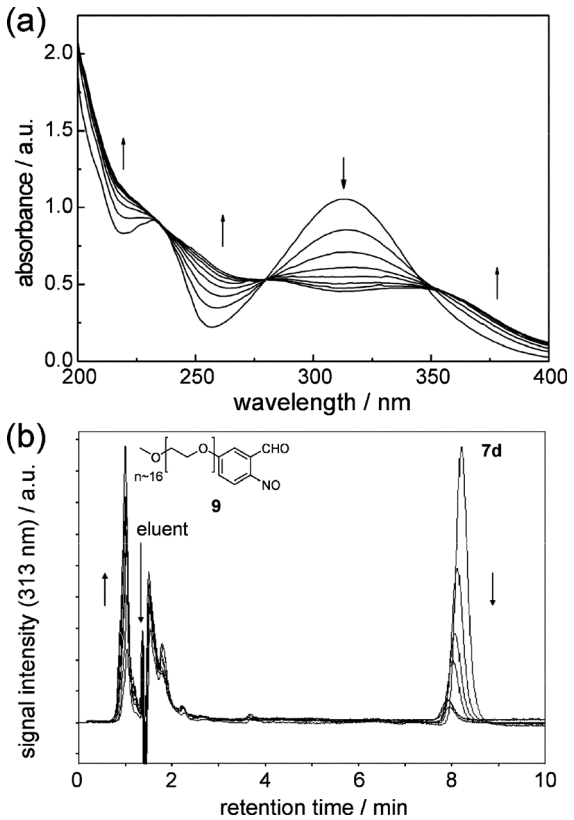


Fig. 2 Irradiation of surfactant **7d** (120 μM in H_2O at 25 °C). (a) UV/vis absorption spectra and (b) HPLC traces, both in time intervals of 90 s.

such as laurylsulfonic acid **8**, and their salts,[24] closely resembling SDS, are typically 2–3 orders of magnitude higher than of their corresponding PEGylated derivatives (Table 1).

To analyze whether our caged detergents can be used to solubilize complex protein mixtures rat dorsal root ganglia were extracted with 2% of detergent **7d** and separated by traditional one-dimensional SDS-PAGE. The separated proteins were then electrophoretically transferred to a nitrocellulose membrane and analyzed by Western blotting for the presence of two different membrane proteins. To determine its solubilization capacity we performed the same experiment in the presence of an equal amount of SDS, the detergent of choice for the extraction of proteins from complex tissue samples. As shown in Fig. 3, β1-integrin as well as the ion channel TRPV4 can be successfully extracted with 3,7-dimethyloctylsulfonic acid suggesting that our caged detergents can not only be used for the solubilization of complex protein mixtures but also for the extraction of proteins that are known to withdraw their detection by classical 2-D GE.[12–14]

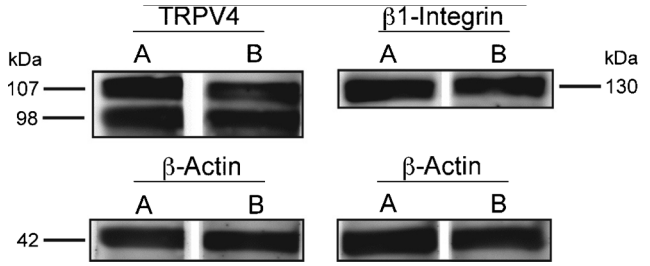


Fig. 3 Solubilization of membrane proteins by uncaged surfactant **7d**. Rat dorsal root ganglia extracted with homogenization buffer containing either SDS (A) or 3,7-dimethyloctylsulfonic acid (B) were analyzed for TRPV4 and β1-integrin immunoreactivity (ir) by Western blotting. To confirm that equal amounts of extracted proteins (40 μg) were compared, blots were also analyzed for β-actin ir. Note that the two bands visualized by TRPV4 ir are due to a glycosylated (upper band) and non-glycosylated (lower band) variant.[25]

Conclusion

In summary, we have proposed a general new concept for improving separation performance of 2-D GE for analysis of complex protein mixtures containing proteins that are notoriously under-represented and difficult to separate. The principle described herein is based on the use of a non-ionic detergent and its *in situ* photochemical conversion to an ionic surfactant. A modular synthesis of photocleavable PEG-based amphiphiles has been developed that allows for convenient control over their CMCs by introducing various alkyl substituents. Future work will be concerned with the use of our caged alkylsulfonate **7d** for the separation of complex protein mixtures in the 2-D GE.

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