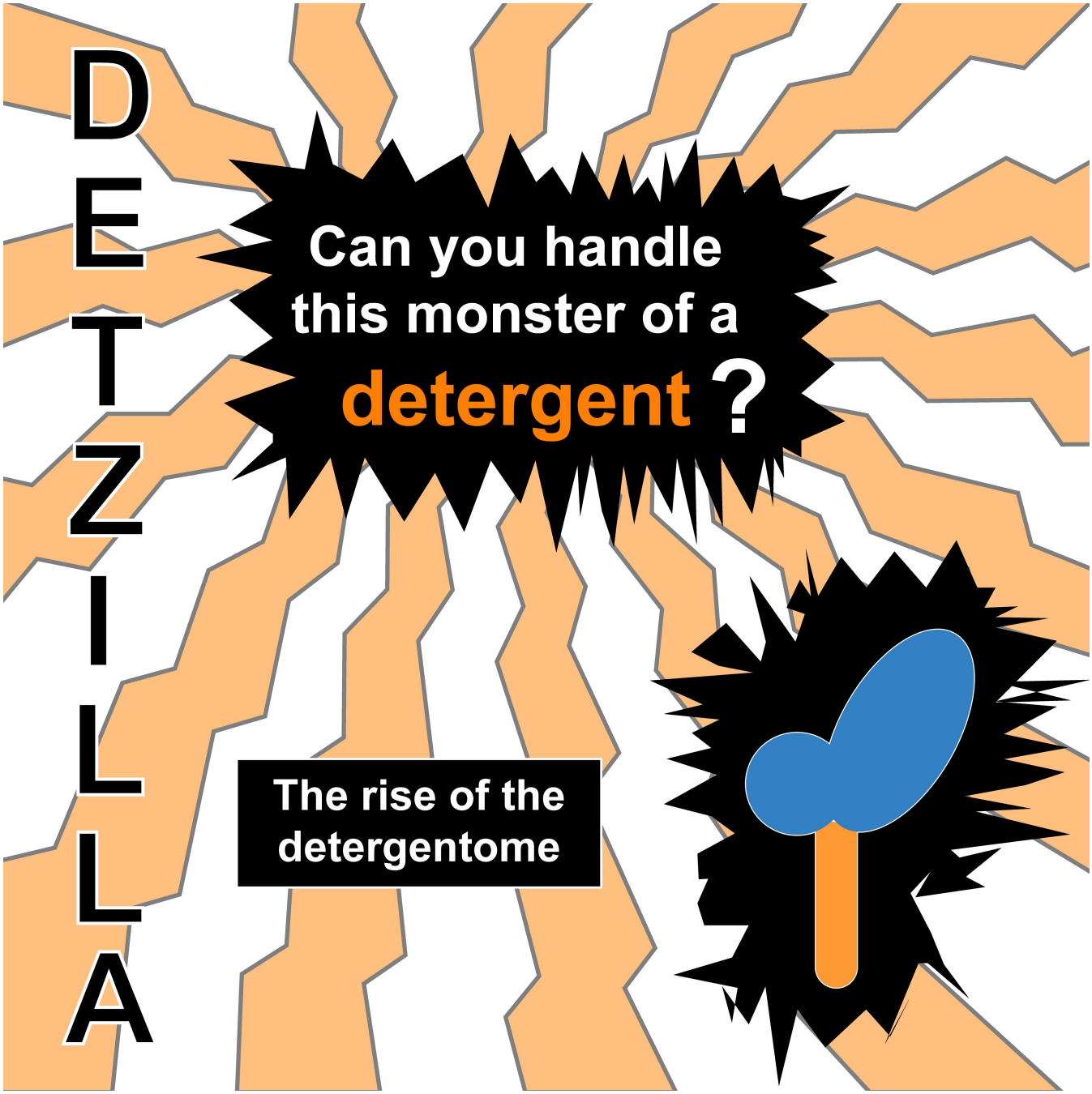
ChemPlusChem
Review
doi.org/10.1002/cplu.202300386
Chemistry Europe
European Chemical Societies Publishing
www.chempluschem.org

Trends in the Diversification of the Detergentome

Virginia Wycisk,*[a] Marc-Christian Wagner,[a] and Leonhard H. Urner*[a]



Detergents are amphiphilic molecules that serve as enabling steps for today's world applications. The increasing diversity of the detergentome is key to applications enabled by detergent science. Regardless of the application, the optimal design of detergents is determined empirically, which leads to failed preparations, and raising costs. To facilitate project planning, here we review synthesis strategies that drive the diversification of the detergentome. Synthesis strategies relevant for industrial and academic applications include linear, modular, combinatorial, bio-based, and metric-assisted detergent synthesis. Scopes and limitations of individual synthesis strategies in context with industrial product development and academic research are discussed. Furthermore, when designing detergents, the selection of molecular building blocks, i.e., head, linker, tail, is as important as the employed synthesis strategy. To facilitate the design of safe-to-use and tailor-made detergents, we provide an overview of established head, linker, and tail groups and highlight selected scopes and limitations for applications. It becomes apparent that most recent contributions to the increasing chemical diversity of detergent building blocks originate from the development of detergents for membrane protein studies. The overview of synthesis strategies and molecular blocks will bring us closer to the ability to predictably design and synthesize optimal detergents for challenging future applications.

1. Introduction

Detergents mainly contain a polar head group and a non-polar tail which are held together by a linker (Figure 1). Due to their amphiphilic properties, detergents are surface active molecules and therefore commonly described as surfactants. Soap is another word often used although the terms "synthetic detergents" or just "detergents" are becoming increasingly common. In 2022, the term "detergentome" was coined, which is used as synonym for the entirety of all detergents that exists at a certain time point.[2] Detergents enable today's world applications and are frequently used in households,[3] medical products,[4] food processing,[5] and academic research products, like for solubilization of hydrophobic compounds,[6] biophysical analysis of intact membrane proteins,[7] proteomics,[8] and microfluidics[9] (Figure 1a).

The amphiphilic properties of detergents are essential to their unique utility profiles. Driven by the hydrophobic effect, detergents spontaneously form aggregates above a certain concentration threshold, which is known as critical aggregation concentration (cac).[10] Alternatively, the term critical micelle concentration (cmc) is established in literature, which is the minimal concentration of a detergent required to form micelles.[10] The abbreviation cac is more widely applicable since it covers all detergent aggregate morphologies, while cmc can only be used specifically for micelle-forming detergents.[8b] Detergent aggregates in water consist of a polar outer shell filled with detergent head groups and a non-polar inner core filled with detergent tail groups. The region between detergent head and tail is called palisade region and plays a role in solubilizing amphiphilic guest molecules.[6b] The symmetry, polydispersity, and packing order of detergent aggregates is difficult to generalize.[11] However, due to their amphiphilic character, detergent aggregates can encapsulate and solubilize water-insoluble compounds, including dyes,[6b] drugs,[12] membrane proteins,[7] and carbon nanotubes.[6a] Both the cac and cmc of detergents are common measures for aggregate stability and dynamics.[13] Detergent aggregates are in dynamic equilibrium with monomers and smaller oligomers.[10] The lower the cac or cmc, the higher the aggregation tendency and the more

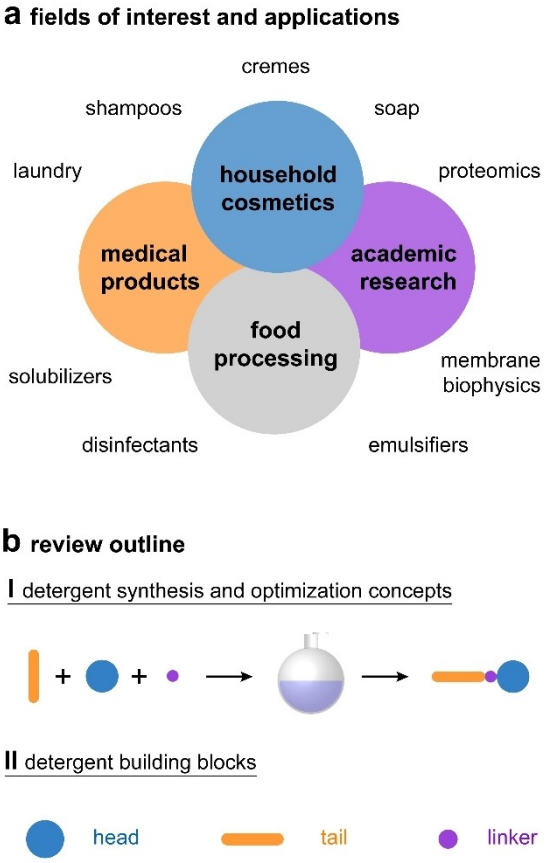


Figure 1. Overview of research fields in detergent science and review outline. a) Schematic showing research field in detergent science and individual applications in which detergents play a role. b) Overview of main topics to be addressed in this review, i.e., detergent synthesis and optimization concepts and detergent building blocks.

[a] *Dr. V. Wycisk, M.-C. Wagner, Dr. L. H. Urner*
TU Dortmund University
Department of Chemistry and Chemical Biology
Otto-Hahn-Str. 6
44227 Dortmund (Germany)
E-mail: virginia.wycisk@tu-dortmund.de
leonhard.urner@tu-dortmund.de

resistant detergent aggregates can be against dilution.[13] Due to the dynamic nature of detergent aggregates, co-localization of aggregates and encapsulated guest molecules cannot be guaranteed.[12–13] The dynamic nature of detergent aggregates can limit their utility for applications in which this co-localization is important, such as membrane protein purification and *in vivo* drug delivery.[12–13] A jackscrew to reduce aggregation dynamics is to reduce the cac or cmc. In contrast, precise tuning of cac or cmc may be less relevant for applications in which excess of detergent material is used, such as in the cases of household applications, i.e., laundry, shampoos, soap formulations (Figure 1a). Regardless of the application, the molecular structures of detergents are key to the ability to precisely tune their properties. We expect an overview of detergent synthesis strategies and building blocks will serve as a pool of inspiration for the design of new detergents.

The aim behind this review is to facilitate the expansion of the detergentome that is a necessary key enabling step for new applications. Detergent science is a multibillion-dollar business and detergent optimization procedures can enable the obtainment of profitable product formulations for individual applications (Figure 1a). Aggregation properties of detergents depend on different parameters, like molecular structure, temperature, pH, salt concentration, additives, solvent, etc.[3b,14] Therefore, it can be difficult to deduce molecular level design guidelines.[14a] Detergents are commonly optimized by empirical screening, which leads to failed preparations, and raising costs.[15] To facilitate scientific exchange on detergent synthesis and related structure-property studies, herein, we provide an overview on established detergent synthesis concepts and building blocks, including head, linker, and tail groups (Figure 1b).

2. Results and discussion

2.1. Synthesis and optimization concepts

2.1.1. Linear and biosynthesis

The field of synthetic detergent chemistry recently surpassed its 100th anniversary and different synthesis and optimization concepts are available to diversify the detergentome.[16] With the phrase "diversification of the detergentome" we aim to describe the observation that increasingly new detergent structures are entering the literature that differ from previously established detergent structures. The diversification of the detergentome offers new possibilities for structure-property studies and already contributed to new innovations in various research disciplines, including cleaning applications,[3a,17] membrane protein research,[2b,7,14a,18] proteomics,[8] solubilization,[6,19] microfluidics,[9] and the formulation chemistry in food and pharma industry.[20]

In the first part of this work, we review detergent synthesis and optimization concepts that serve as enabling step for structure-property studies and the development of detergent formulation for new applications (Figure 2). We anticipate that the systematic overview of detergent synthesis and optimization concepts will facilitate the work for both established- and new researchers entering the field as well as for researchers working together with detergent designers on multidisciplinary projects.

The utilized synthesis concept for detergents available in mass markets, like detergents for handwashing or laundry applications, are held as simple as possible, such as in the case of linear synthesis (Figure 2). For example, the detergent sodium dodecyl sulfate (SDS) is synthesized in one step through the sulfation of fatty alcohols.[21] In contrast to many detergent

Dr. Virginia Wycisk received her doctoral degree in chemistry from the Freie UniversitätBerlin (Germany). After focusing on switchable molecules for high-resolution microscopy at the University of Oxford (United Kingdom) as a postdoctoral fellow, she extended her research field to detergent science. She is currently working as a senior Postdoc in the independent research group of Dr. Leonhard H. Urner. Her current research interest is focused on the application-oriented synthesis of new detergents to promote sustainability in detergent research.

Marc-Christian Wagner studied German philology and musicology in Saarbrücken in 2016 and shifted to Chemical Biology at Technische Universität Dortmund where he currently works with Dr. Leonhard Urner. Marc-Christian's research interest focuses on the synthesis and characterization of detergents for medically relevant applications. He maintains a strong dedication to his musicality by playing the violin and piano in his personal free time.

Leonhard H. Urner is an independent group leader at TU Dortmund University. He is interested in all aspects of detergent science and nanotechnology with relevance to medical research and sustainability. He received scientific awards from the German Chemical Society, SEPAWA e.V., and was recently appointed as a young colleague of North Rhine-Westphalian's Academy of Sciences, Humanities and the Arts. His research group is financially supported by the Ministry of Culture and Science of the State of North Rhine-Westphalia (NRW return program) and the Fonds der Chemischen Industrie.

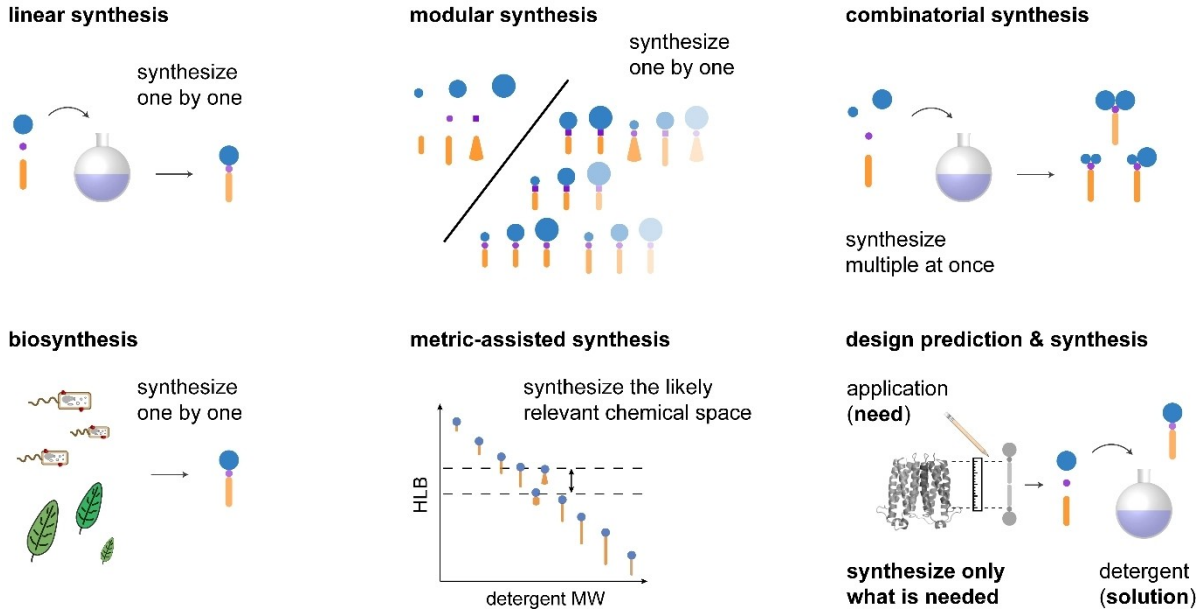


Figure 2. Detergent synthesis and optimization concepts. Schematic visualization of an overview of synthesis and optimization concepts that are used to find new detergents for individual applications. Detergents are synthesized one by one using linear synthesis, modular synthesis, and biosynthesis. Combinatorial synthesis enables the preparation of multiple detergents at once. Metric-assisted detergent synthesis is used to narrow down the chemical space in which it is likely to find new detergents with suitable properties. Detergent design prediction can reduce detergent synthesis to the solution that is needed for the benefit of project and environmental resources.

batches produced for structure-property studies in academia, homogeneity is not necessarily a key requirement for industrial applications. Detergents are commonly sold in the form of "detergent-containing" products and product heterogeneity depends on the composition of utilized starting materials. Krister Holmberg reported that SDS with technical grade can contain 98% SDS and 2% dodecanol.[22] The properties of such a blend vary with its composition.[3b,22] The concept of blends is used to optimize properties of detergent formulations.[3b] Every detergent has its own pros and cons for a given application. To combine favourable properties of different detergents, mixtures can be prepared. For example, in laundry detergent formulations, anionic and non-ionic detergents are commonly mixed. On the one hand, anionic detergents are used to solubilize soil, but they are prone to precipitate in the presence of doubly charged cations. On the other hand, non-ionic detergents are soluble in the presence of doubly charged cations but not as efficient in solubilizing soil as anionic detergents. Mixing both non-ionic and anionic detergents can lead to blends that exhibit beneficial properties of both individual detergents while neglecting their disadvantages.[3b] From a synthetic view point, properties of detergent mixtures are easier to tune than those of individual detergents, since no extra synthesis is required.[3b]

Complementary to chemical synthesis and mixing, detergents can be obtained efficiently from biosynthesis through upstream or downstream processing (Figure 2). In upstream processing, products are isolated from culture medium and prepared to the final form for intended applications. In downstream processing, cells are separated and lysed to purify the product. Both approaches have pros and cons for individual production scales, however, downstream processing has been mentioned to be an economic bottleneck for manufacturing.[23] According to current market outlooks, the global detergent market value is estimated to be around 120 billion USD and will grow in the years ahead. Biosynthetic detergents are expected to be the main driver for future market growth. The manufacturing costs of biosynthetic detergents are inversely proportional to the market volume.[24] The production of more expensive biosynthetic detergents with large market volumes of 100–20,000 m^3, like sophorolipids or rhamnolipids, typically costs 2–5 $/kg. This is about 30% more than the prize to pay for chemical detergents. The production of more expensive biosynthetic detergents with smaller market volumes, such as in the cases of the antibiotic detergents surfactin or iturin, costs as much as 14 million $/kg. Expensive starting materials and low product yields are currently the main cause for high production costs of biosynthetic detergents. For more details on strategies that are under evaluation to reduce production costs for biosynthetic detergents we refer to the book BIOSURFACTANTS.[24] Apart from production costs, establishing the synthesis of biosurfactants offers many advantages. Once established, complex detergent molecules may be obtainable from readily available starting materials, like biomass, nutrients, and water. Furthermore, biosynthetic detergents offer the opportunity to obtain chemical products based on renewable material cycles. Interestingly, the anticipated role of biosynthetic detergents in future market growth can align with measures against climate change, some of which underline the need for more climate-neutral, production cycles in the chemical industry.[25]

2.1.2. Modular and combinatorial synthesis

In contrast to detergent science in industry, the focus in academic research projects is commonly put on knowledge gain through structure-property relation studies and for the benefit of applications. Academic research projects funded by third parties are increasingly urged to produce outputs that meet global demands.[26] Project resources are naturally limited. In other words, if the project aim is to find suitable detergents to enable a defined application for which previously established detergents do not work, then the first aim can be the synthesis of a detergent library for application-oriented screenings. Detergent synthesis involves planning, synthesis, purification, and characterization, all of which can be time-consuming and require a significant portion of the project budget. We expect that a transparent communication of synthesis and optimization concepts will facilitate scientists to reduce project costs related to detergent synthesis.

To facilitate detergent synthesis, we review not only synthesis concepts with industrial relevance but also concepts that recently facilitated the diversification of the detergentome in academic research, including modular synthesis and combinatorial synthesis (Figure 2).[2b,14a,27] Both synthesis approaches can be used to rapidly synthesize systematically constructed detergent libraries for structure-property studies. To understand how structural elements of detergents affect experimental outcomes in structure-property studies, detergent libraries are ideally constructed in a way that when two detergents are compared only one structural parameter changes at the time. Important for planning the optimization of detergent libraries through modular synthesis is the fact that detergents can be divided into molecular blocks, i.e., head group, linker, tail.[14a] In this regard, the advent of click chemistry[28] had a lasting impact on the detergent field. Size and functionality of molecular blocks can be tuned and readily combined to obtain detergents in high yields from readily available starting materials and by following simple product isolation procedures. When these conditions are met, the time required for the synthesis of detergent libraries can be reduced. While most detergents are principally constructed in a modular way, at least two further conditions are required to enable a successful translation of modular synthesis, i.e., availability of starting materials and established synthesis protocols.

An established example that builds on modularity, applicability of click chemistry, availability of starting materials, and established synthesis protocols are oligoglycerol detergents (Figure 3).[14a,27a,29] The head group and linker chemistry of oligoglycerol detergents has been established to scale not only the relative size, polarity, and number of functional groups of the oligoglycerol head but also to systematically combine different generations of dendritic oligoglycerol head groups and hydrophobic tails via different linkages.[27a,30] Oligoglycerol detergents have been used in numerous structure-property studies which contributed to a better understanding about supramolecular properties of amphiphiles.[27b] Furthermore, underlying studies also enabled the discovery of innovations in context with solubilization of hydrophobic substances,[6a,27a,31] protein-resistant surface modifications,[32] gene transfection,[33] staining agents for optical microscopy,[34] microfluidics,[9] mass spectrometry analysis of soluble proteins,[35] membrane protein purification,[14a,15,30,35b,36] the mass spectrometry analysis of protein-ligand interactions directly from physiologically relevant buffers,[37] top-down characterization of intact membrane protein complexes following native mass spectrometry.[38] Seen from a broader perspective, modular synthesis concepts have not only been harnessed for oligoglycerol detergents but also for the synthesis of amphiphilic molecules more widely, including polymers,[39] Janus glycodendrimers,[40] self-labelled amphicalixarenes,[41] dendritic amphiphiles,[27b] peptides,[42] lipid nanocarriers.[43]

Despite recent advances in the modular synthesis of detergents, one time-limiting aspect remains unchanged compared to linear synthesis sequences. Detergents are synthesized one by one and synthesis effort scales with the size of the detergent library. To accelerate the synthesis of structurally diversified detergent libraries, a combinatorial synthesis was recently developed by Urner and co-workers.[2b] The authors used methallyl dichloride and equimolar mixtures of head group precursors to generate tree detergent head groups in one step (Figure 4). Subsequently, the three detergent head groups can either be functionalized in one pot to a trimeric detergent mixture or separated and converted into three detergents separately (Figure 4). The combinatorial detergent synthesis developed by Urner *et al.* enables a gradual tuning of detergent properties, such as polarity and molecular shape, with unprecedented resolution and gives access to asymmetric hybrid detergents and detergent mixtures in high yields.[2b] Moreover, Zhao *et al.* and Yang *et al.* established further synthesis strategies to facilitate the expansion of the detergentome, i.e., two-dimensional, clickable detergent libraries and Ugi-mediated detergent assembly reactions, respectively (Figure 4).[27c,d] We anticipate the synthesis methods provided by Zhao *et al.*,[27d] Yang *et al.*,[27c] and Urner *et al.*[2b] will serve as enabling steps that transform the structural diversification and optimization of detergents for challenging future applications.

2.1.3. Metric-assisted synthesis

Despite ongoing successes in finding synthesis concepts for new and established detergents, we expect the ability to predict the design of detergents for individual applications is yet an unmet but highly desirable aim in the field. Detergent design predictions could allow one to reduce the empirical detergent optimization part in projects while focusing only on the detergent solution that is needed, thus reducing project costs (Figure 5).[3b] Computational design approaches and metric-assisted synthesis approaches have constantly been evaluated over the past years regarding their capabilities to reach this overarching aim.[3b,15,44] While computational tools can provide guidance in the design of new chemical products and for selecting promising ingredients for product formulations, a metric-assisted approach based on the hydrophilic-lipophilic balance (HLB) has attracted attention in various research fields.

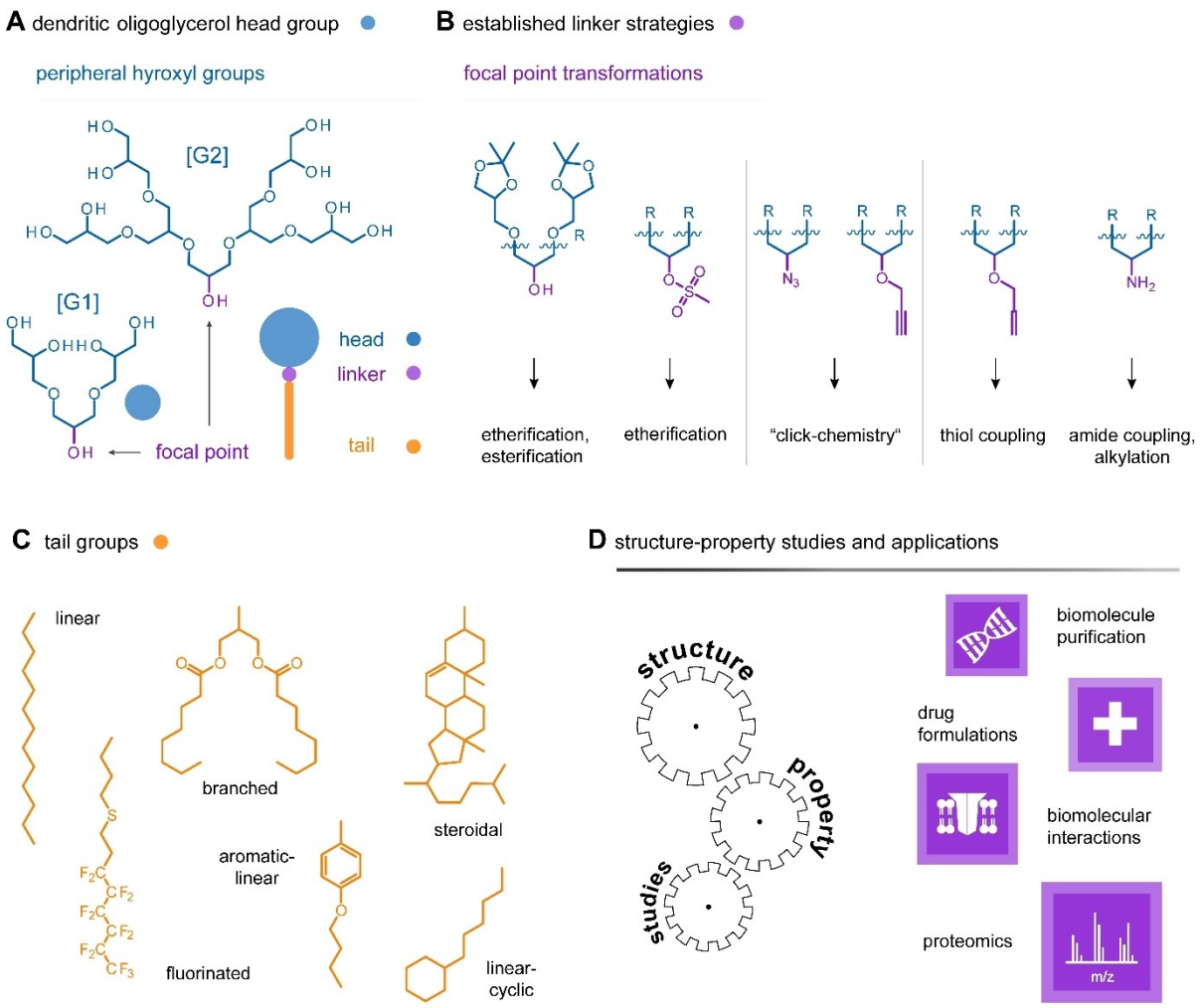


Figure 3. Modular synthesis of dendritic oligoglycerol detergents (OGDs). A–C) Overview of the modular structure of dendritic OGDs, including established head groups, linker strategies, and tail groups. D) Modular synthesis of OGDs and the repertoire of building blocks facilitate structure-property studies for the benefit of applications.

The HLB concept introduced by Griffin in 1949 describes the overall polarity of a detergent as the relative molecular weight of the hydrophobic detergent tail in the form of HLB numbers.[14c] HLB numbers typically range from zero to twenty. The lower the HLB number, the lower the overall polarity of the detergent. The idea to correlate HLB values of detergents with their utility for applications to facilitate the optimization of detergent formulations has been established before, for example, in food and cosmetic industries.[45] The idea to correlate the utility of detergents with HLB numbers has recently also been more established in context with membrane protein purification and crystallization.[2b,15,46]

Membrane proteins are biomolecules that are anchored in cell membranes. They fulfil vital functions and are targets for more than 50% of current drugs on the market.[47] Membrane proteins are commonly isolated from cell membranes with non-ionic detergents to study their structures and interactions with small molecules, like drugs, nucleotides, or lipids.[18] Like with other applications, suitable detergents in membrane protein research are identified by empirical screening, leading to failed preparations, and thereby raising costs.[15] Finding new detergents can be a laborious process that involves not only empirical screening but also the synthesis of detergent libraries.[15] Based on the assumption that optimal protein purification outcomes correlate with certain detergent HLB numbers,[2,46b,48] Urner and co-workers developed an HLB-assisted concept to enable a more streamlined optimization of detergents within five steps (Figure 5A):[15] First, a chemical space is defined around an initial detergent structure. For example, in the case of [G1] OGDs, 11 detergent structures with varying hydrophobic tails and HLB numbers were identified from which four structures were selected based on suitable physicochemical properties, including solubility and amphiphilicity (Figure 5A). Second, the selected detergents are synthesized and applied to membrane protein purification. Third, membrane protein purification outcomes are compared with HLB numbers to identify the HLB range that correlates to desired protein purification outcomes. In the case of [G1] OGDs, the optimal HLB range was found to range from 11.2 to 12.6 (Figure 5A). Fourth, synthesis strategies other than lengthening

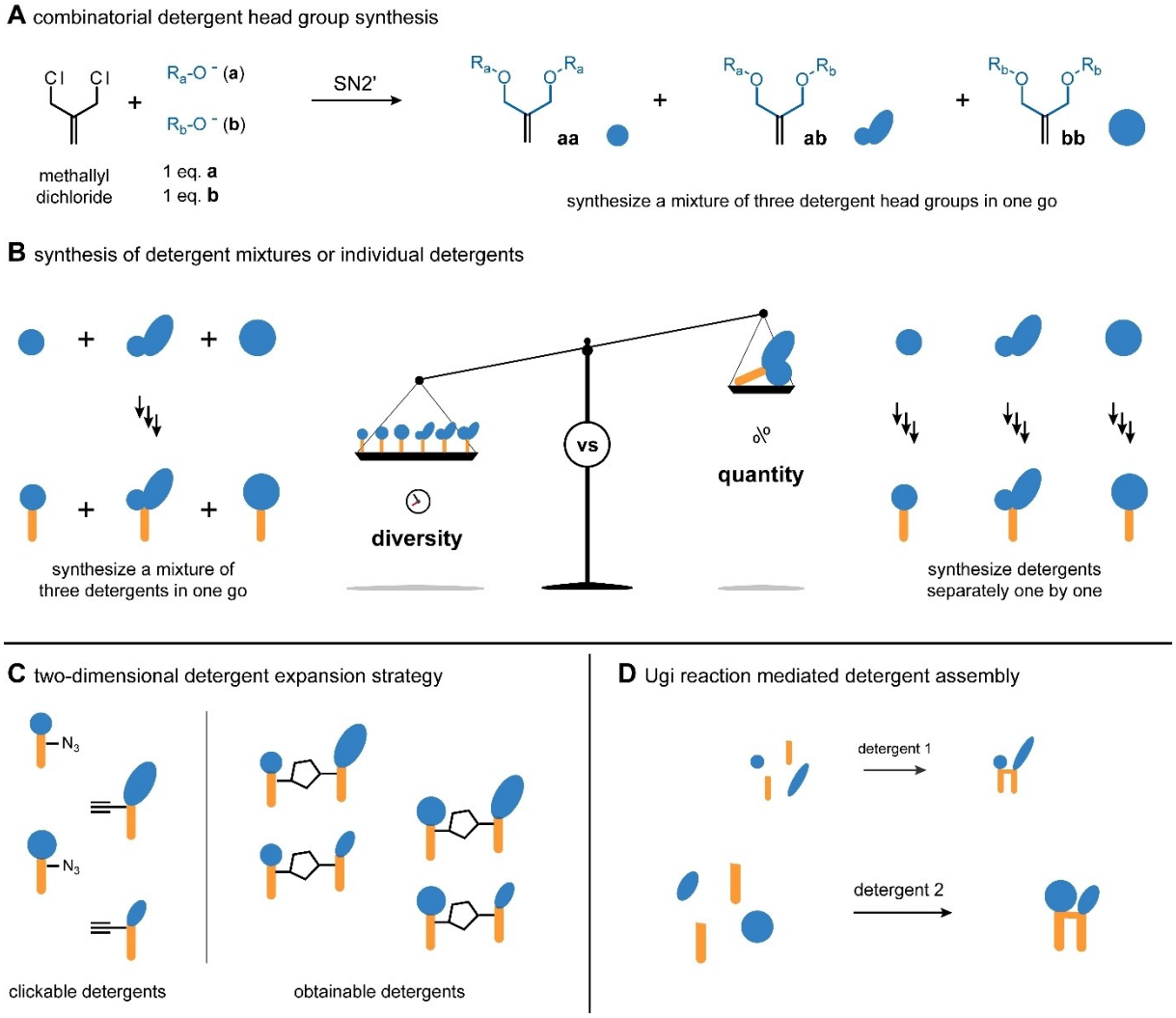


Figure 4. Overview of recent synthesis strategies for diversified detergent libraries. A) Combinatorial synthesis based on methallyl dichloride is used to obtain three detergent head groups in one step. B) Subsequently, detergent head groups can be functionalized to trimeric detergent mixtures or separated and functionalized to three individual detergents. C) Two-dimensional detergent expansion strategy based on clickable detergents enables the systematic fusion of different detergents into hybrid detergents. D) Ugi-mediated detergent assembly of suitable building blocks enables the fusion of four building blocks into hybrid detergents in one step. Figure 4A–B were taken from Ref. [2b] and modified with permission from the authors. Figure 4B is reproduced from Ref. [2b]; Copyright (CC BY 3.0), with modifications and permission from the authors.

or shortening the hydrophobic tail in detergents are used to shift HLB numbers of detergents into the HLB range that correlates to desired protein purification outcomes (Figure 5A–B). Fifth, the utility of HLB-assisted detergent for protein purification is evaluated and new, suitable detergents can be found.

The results obtained from the HLB-assisted detergent optimization by Urner and co-workers led to qualitative and detergent class-specific design guidelines that can enable a streamlined optimization of detergents for applications like protein purification (Figure 6).[15] In membrane protein purification, it is important to establish detergents either for the extraction of large protein quantities from biological membranes or for the solubilization of proteins upon detergent exchange and in the absence of membranes.[15,18] For both applications, optimal HLB ranges were identified based on literature analysis in which suitable detergents are likely to be found (Figure 6). Both HLB ranges were condensed into qualitative HLB guidelines that serve now as guidance for the identification of new detergents that likely enable the extraction and/or solubilization of membrane proteins:[15]

(1) Non-ionic detergents with HLB values between 11 and 14 likely enable the extraction and affinity purification of large protein quantities from membranes (Figure 6).
(2) Non-ionic detergents with HLB values between 11 and 18 likely enable the solubilization of membrane proteins upon detergent exchange (Figure 6).
(3) Modifying the hydrophobic tail within a detergent class to shift HLB values towards a detergent-class-specific, optimal HLB window, i.e., 11.2 to 12.6 in the case of [G1] OGDs, can lead to the obtainment of new detergents. Given that the empirically determined and detergent-class-specific, opti-

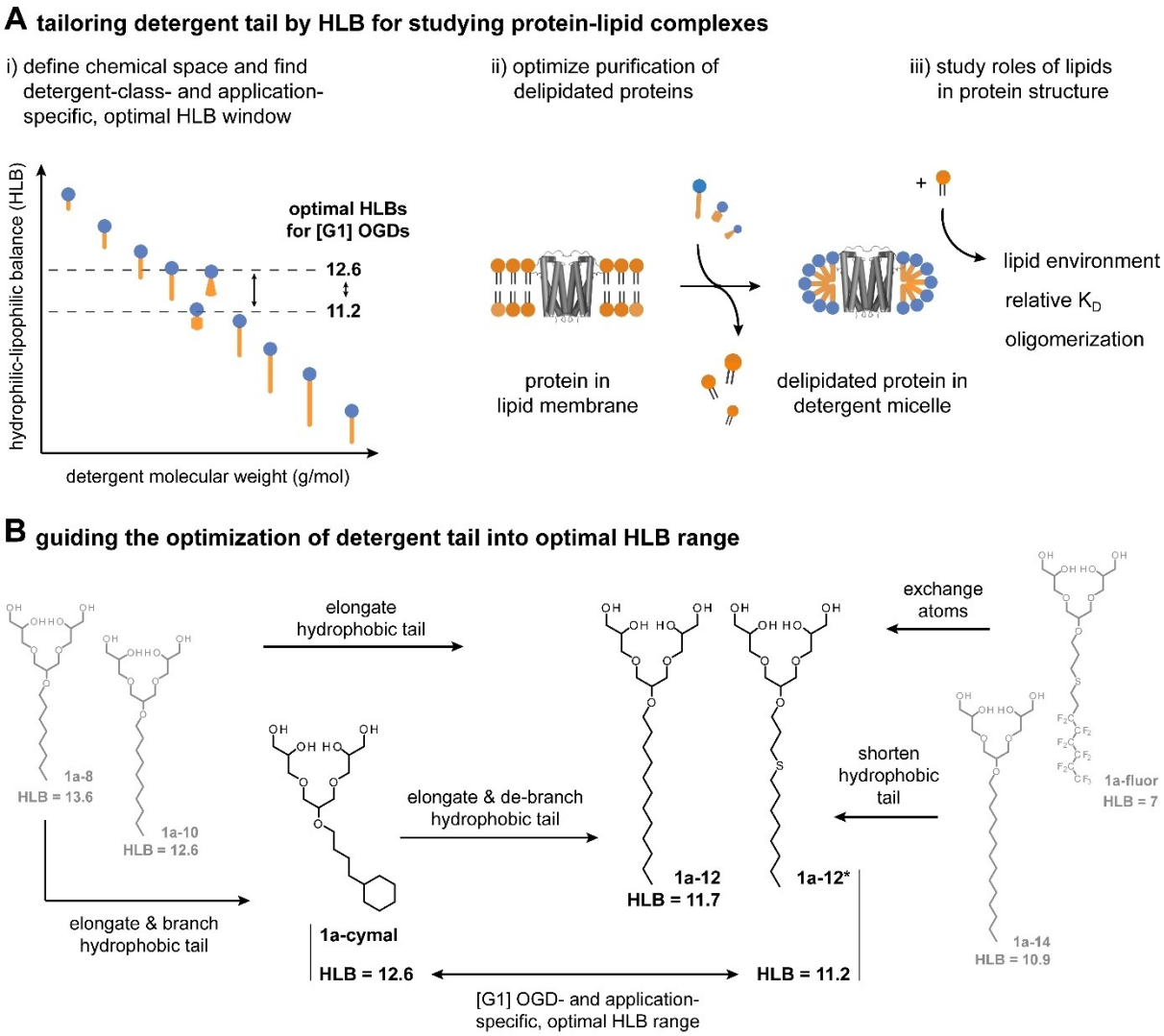


Figure 5. Overview of metric-assisted detergent optimization for protein purification. A) Overview of steps involved in metric-assisted optimization of [G1] OGDs in context with membrane protein purification and delipidation (i–iii). B) Overview of design strategies to bring the HLB values of [G1] OGDs into the [G1] OGD- and application-specific, optimal HLB range, i.e., 11.2–12.6. [G1] OGDs within the optimal HLB range are likely suitable for the purification of delipidated bacterial membrane proteins for in vitro membrane studies. Figure 5A was taken from Ref. [15] and modified with permission from the authors. Figure 5A is reproduced from Ref. [15]; Copyright (CC BY 4.0), with modifications and permission from the authors.

mal HLB windows overlaps with the HLB window defined in (1) above, i.e., between 11 and 14, then new detergents are likely to enable the purification of large protein quantities from biological membranes (Figure 6). Given that the empirically determined and detergent-class-specific, optimal HLB windows overlaps with the HLB window defined in (2) above, i.e., between 11 and 18, then new detergents are likely to solubilize proteins upon detergent exchange (Figure 6).

Available synthesis strategies to increase or decrease HLB values of detergents include lengthening, shortening, branching, debranching of the hydrophobic tail and atom exchange (Table 1). The utility of all these synthesis strategies for individual applications has been established before in numerous case studies (Figure 5B) (Table 1).[15] The HLB-assisted detergent optimization established by Urner and co-workers enables to rationalize the selection of these design strategies

Table 1. Overview of hydrophobic tail modifications accessible through organic synthesis and their effect on HLB numbers of detergents.

hydrophobic tail modification	effect on detergent HLB
increase number of methylene groups	decrease
substitute carbon for heteroatom (N, O, S)	decrease
substitute hydrogen for halogens (F, Cl, I)	decrease
elongate & branch by adding rings, e.g., cyclohexane	decrease
reduce number of methylene groups	increase
substitute heteroatom (N, O, S) for carbon	increase
substitute halogens (F, Cl, I) for hydrogen	increase
shorten & de-branch by removing rings, e.g., cyclohexane	increase

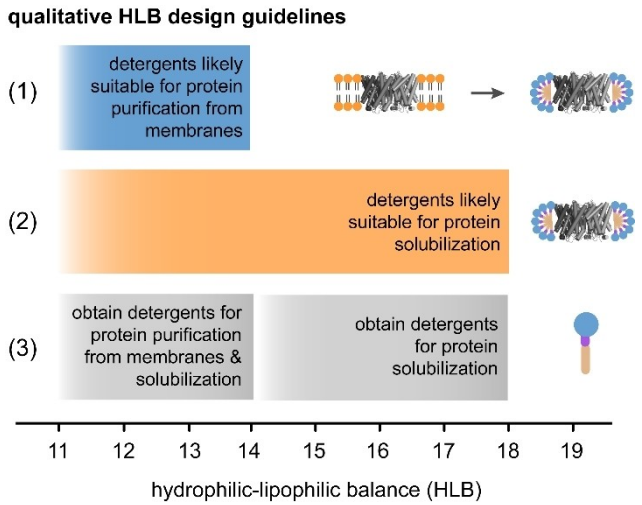


Figure 6. Qualitative HLB guidelines for detergent optimization in protein purification. (1) Non-ionic detergents with HLB values between 11 and 14 are likely suitable for extraction and affinity purification of large protein quantities. (2) Non-ionic detergents with HLB values between 11 and 18 are likely suitable for protein solubilization. (3) Shifting HLBs of non-ionic detergents into the ranges defined in (1) and (2) enables likely the production of detergents that are suitable for protein purification from membranes and/or protein solubilization. Image and figure caption were taken from Ref. [+] with permission from the authors. Figure 6 is reproduced from Ref. [15]; Copyright (CC BY 4.0), with permission from the authors.

since the impact of each strategy, i.e., increasing or decreasing the detergent HLB number, is well understood (Table 1). The HLB design guidelines proposed by Urner and co-workers are of qualitative nature since HLB numbers do not give specific details on detergent molecular structure which is a determining factor for experimental outcomes. Furthermore, certain modifications, such as fluorination, affect not only hydrophobicity but also fluorophilicity of compounds.[49] Since HLB numbers are calculated from the molecular weight of detergents and detergent tails,[14c] differences in fluorophilicity are not captured by this approach.[15] However, correlating detergent HLB numbers and utility for applications has not only proven to be a useful strategy for membrane protein research but also for food and pharma industry[45b,50] and indexing cell surface properties of bacteria.[51] We anticipate the overview of hydrophobic tail modifications summarized in this work to be interesting for detergent designers working across different disciplines (Table 1). In addition, case studies suggest putting the molecular shape of detergents into context via the packing parameter concept or the concept of curvophilicity are also interesting starting points for detergent optimization.[2b,19b] The packing parameter concept is used to estimate aggregate morphologies from molecular parameters, i.e., head group area, length of the tail, and volume of the tail.[14d] Curvophilicity is commonly used to describe qualitatively how likely a detergent will form curved aggregates, for example, in comparison to curvophobic molecules, like phospholipids.[19b] Despite the HLB and packing parameter, further parameters for correlation metrics are available, including the hydrophilic-lipophilic difference,[52] basicity of functional groups,[35] membrane/water partition coefficients,[53] and pKa values.[54] Metric-assisted design approaches continue to be important tools for the optimization of detergents for applications in various disciplines. We expect that computational- and metric-assisted detergent optimization approaches bring us closer to the ability to predict the design of suitable detergents for individual applications.

2.2. Detergent head groups

2.2.1. Standard head groups

While reviewing the general scope and utilities of different approaches to obtain new detergents it became apparent that the strategy that is used to fuse molecular blocks into detergents is just as important for the design of detergents as the selection of molecular blocks, i.e., head, linker, tail. Since the molecular structure of detergents is the primary driver for experimental outcomes, here we also review established and recent developments of head group, linker, and tail structures, all of which serve as enabling steps for structure-property studies and applications.

In mass market products, like soaps and shampoos, detergents form the core of product formulations.[55] Detergent formulations are tailored to outbalance parameters like stability, solubilization capabilities, wetting properties, foaming, degradability, eco-friendliness, antimicrobial properties, and biocompatibility.[22] While linear hydrocarbon chains are most common in mass market detergents, significant differences are observed when comparing head group structures (Figure 7). Established head groups for detergents in mass market products include sulfates, sulfonates, laureth sulfates, carboxylates, sarcosines, amino acids, quarternary ammonium ions, Esterquats, N-amine oxides, fos-choline, N-alkyl sulfonates, oligoglycerol, mono- or diethanol amides, oligoethylene glycols, and carbohydrates (Figure 7). The list of head groups presented in Figure 7 is a representative overview that may not cover all possible head groups.

The natural life cycle of detergents includes synthesis, application, and disposal. Among anionic head groups, phosphates are also possible head groups or additives. Phosphates are causing environmental problems as nutrient pollution in aquatic ecosystems and promote extensive algae growth.[16] The latter aspects led to first laws restricting the addition of phosphates in detergents in various regions of the world.[16] Furthermore, quaternary ammonium detergents are also of environmental relevance. The historic background of quaternary ammonium detergents has recently been summarized by Hora *et al.*[17b] Due to their antimicrobial properties, quaternary ammonium detergents are active ingredients in disinfectants for use to deactivate microbes and viruses, including SARS-CoV-2.[17b] Quaternary ammonium detergents are considered to degrade relatively slow by hydrolysis, photolysis, or microbial activity; thus leading to environmental persistence and potential long-term effects for flora, fauna, and microbiota. Chemical incorporation of ester bonds in-between hydrophobic tails and hydrophilic head groups of quaternary ammonium detergents have been introduced to obtain Esterquats, which have been

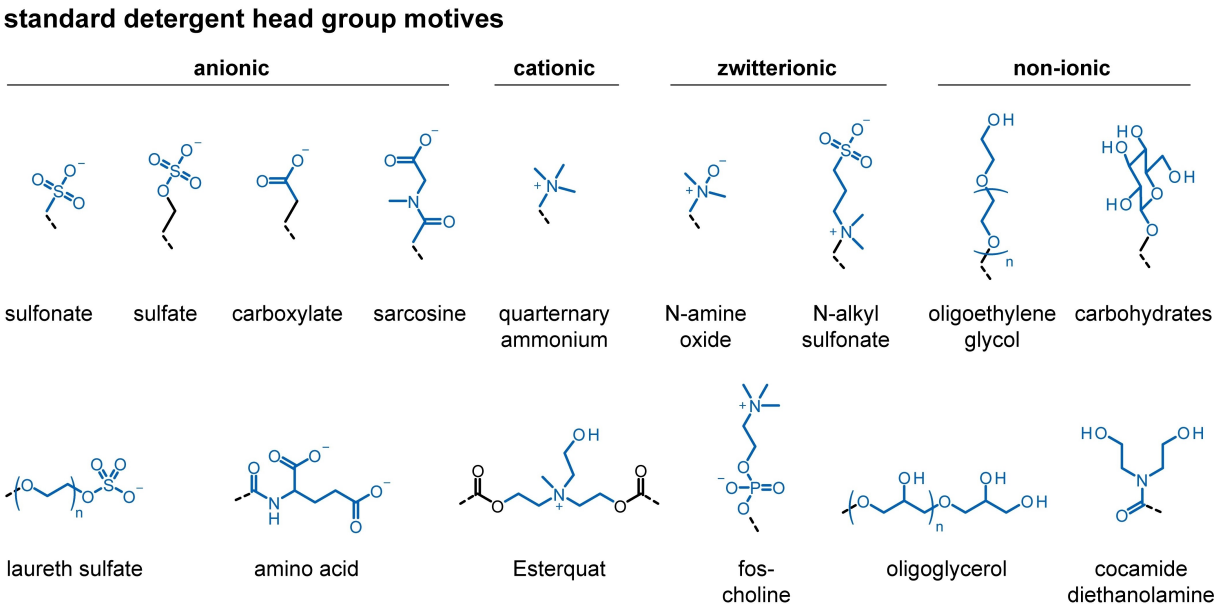


Figure 7. Overview of structures of established detergent head groups in mass markets. Anionic detergents can contain sulfates, sulfonates, carboxylates, or amino acids. Cationic detergents can contain quaternary ammonium head groups. Zwitterionic can contain N-amine oxide, fos-choline, and N-alkyl sulfonate. Non-ionic detergents can contain oligoglycerol, oligoethylene glycols, or carbohydrates.

proven beneficial for enhanced degradability (Figure 7).[56] While sales and environmental pollution with quaternary ammonium detergents have risen during the SARS-CoV-2 pandemic, Hora *et al.* proposed to focus future efforts on monitoring their presence and concentrations in engineered and environmental systems, and to implement technologies to remediate their presence, if necessary.[17b] We expect this approach also to be important for other detergent classes. For example, anionic surfactants contribute to 60% of world surfactant production, thus leading to a constant flux of these compounds into ecosystems.[57] However, their level of toxicity and behaviour in environmental compartments has not yet been fully understood.

2.2.2. Biosynthetic head groups

Biosynthetic detergents are expected to drive future market growth. Conveniently, organisms can synthesize rather complex molecular structures with ease that would be comparable difficult to synthesize for chemists. Herein, we highlight commonly known biosynthetic detergents, including rhamnolipids, sophorolipids, saponins, trehalose lipids, and sodium lauryl glucose (Figure 8). Head groups of biosynthetic detergents are obtained from non-polar tails in one go from biological samples. Therefore, we did not delineate the structure of biosynthetic head groups from non-polar tails in our review of common biosynthetic detergents.

Rhamnolipids were first reported by Bergstrom *et al.* in 1946 as "oily glycolipid" that consists of rhamnose sugar that are linked via a-1,2-glycosidic bonds to mixtures of saturated and unsaturated ß-hydroxy fatty acid chains which themselves are held together by ester bonds.[58] The biosynthesis is well understood.[59]

For example, the bacterium *P. aeruginosa* synthesizes the rhamnolipid head group from GDP-mannose, a substrate of the deoxy sugar pathway.[60] The head group is then conjugated to fatty acids that originate from the general fatty acid biosynthetic pathway. The length and saturation of the fatty acid chains can be externally controlled by means of growth conditions and hydrocarbon chains used for feeding bacteria.[61] Bacteria simultaneously produce rhamnolipids together with other lipids. Obtainable rhamnolipid fractions are naturally heterogenous, thus leading to elaborate purification processes given that individual rhamnolipid species need to be characterized.[62] The exact physiochemical properties and functions of individual rhamnolipid species are not well understood.[63] However, the general utility of the amphiphilic properties that originate from the combination of rhamnose head groups and fatty acid chains has been evaluated in context with different applications, including emulsification, solubilisation, surface wetting, inhibition of bacterial and fungi growth, disruption of bacterial biofilms, use as anticancer agents, environmental remediation, and plant protection.[63–64]

Another popular class of biosynthetic detergents are sophorolipids, which are isolated from the non-pathogenic yeast species *Candida sp.*[65] The structure of sophorolipids commonly consists of polar β-1,2-D-glucopyranosyl-D-glucopyranose headgroup, and non-polar C16 or C18 fatty acid chains. The carbohydrate in sophorolipids can be mono- or diacetylated and exists as a cyclic, lactonized form or a non-cyclic form that contains a free carboxylic acid (Figure 8). The biosynthesis of sophorolipids includes three steps, i.e., i) the oxidation of oleic acid by the cytochrome P450 monooxygenase to the ω-hydroxy-fatty-acid, ii) glycosylation with UDP-glucose, mediated

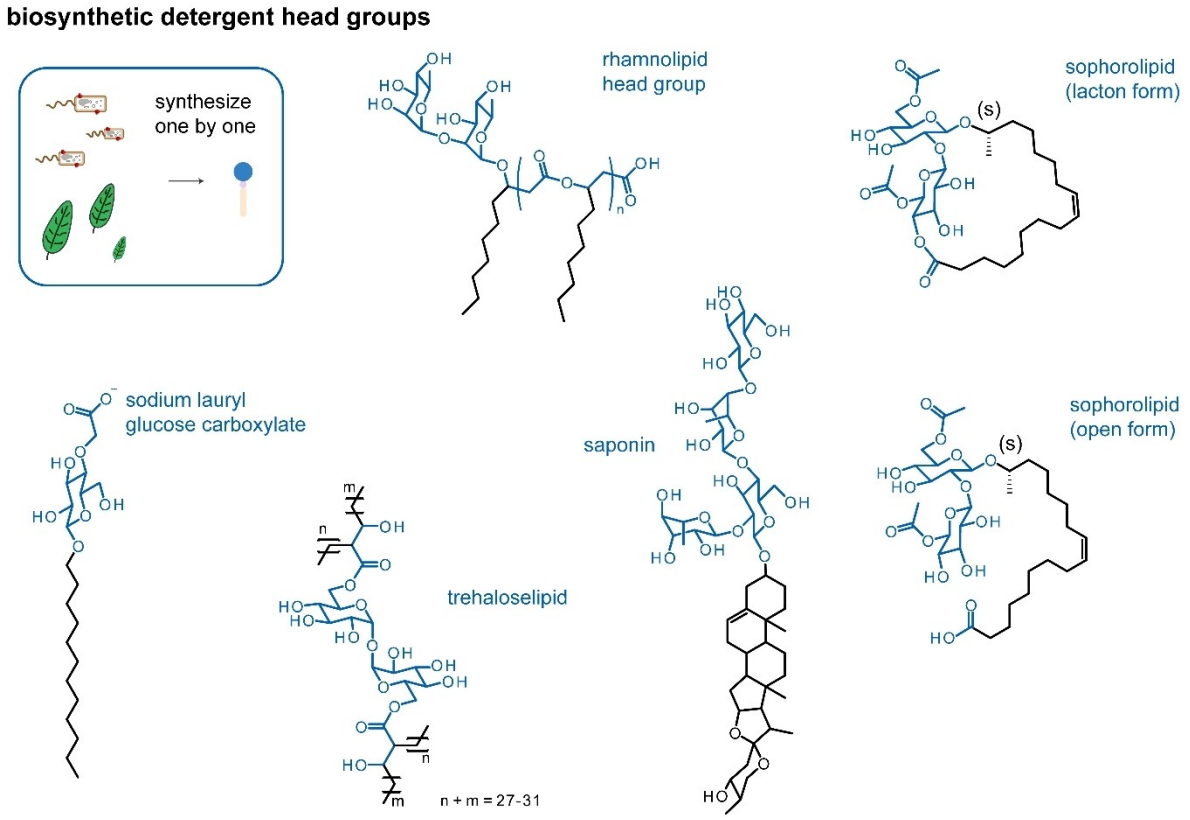


Figure 8. Overview of biosynthetically obtainable detergent head groups. Non-ionic head groups are obtainable from trehaloselipids, saponin detergents and sophorololipid. Mixed anionic/non-ionic detergent head groups are obtainable from rhamnolipids and sodium lauryl glucose carboxylate.

by the glycosyltransferases I and II which yields a non-acetylated acidic sophorolipid, and iii) acetylation of the monosaccharides with Acetyl-CoA and acetyltransferases and/or lactonization via lactonesterases.[65] Non-acetylated sophorolipids can be used as a precursor for the preparation of head-tail detergents, bolaamphiphiles, and charged amino acid derivatives.[66] Delbeke *et al.* reported that the modification of di-acetylated sophorolipid lactones can also be used as a strategy to obtain new amphiphilic architectures.[66b] The pH responsive character of sophorolipids affects its ability to complex cations, like copper,[67] interfacial properties, and aggregation properties.[68] Like rhamnolipids, sophorolipids are commonly described as biodegradable, low toxic, and have been highlighted in context with applications, such as the development of antimicrobials and anticancer agents and the ability to harness their amphiphilic properties for emulsification, solubilization, and remediation purposes.[69]

Another important class of biosynthetic detergents are saponins. The term saponin origins from the word sapo (Latin: soap) and describes a diverse class of glycosides that are naturally occurring as secondary metabolites in plants and lower marine animals, like sea cucumbers. Structurally, saponins usually consist of a non-polar steroidal triterpenoid system, and a water-soluble carbohydrate head group, consisting of D-glucose, D-galactose, D-glucuronic acid, D-xylose, L-arabinose, L-rhamnose, and D-fructose (Figure 8). Saponins are extracted from organisms by means of maceration extraction using methanol or ethanol, reflux, and Soxhlet extraction via heating and cooling or a subsequent combination of both. Complementary, green extraction technologies like ultrasound- or microwave-assisted extractions have also been reported.[70] The biosynthesis starts with the build-up of polar steroid of triterpenoid system from the common precursor 2,3-oxidosqualene, which is converted into the fused ring systems by means of oxidosqualene cyclases. Prior to glycosylation, the saponin backbone can be modified, thus leading to the structurally diverse chemical space of saponins.[71] Common modifications are the introduction of hydroxyl-, keto-, aldehyde, and carboxy moieties at various position, mediated via the prominent monooxygenase class P450.[71] Subsequent biosynthetic glycosylation of the non-polar tail is conducted by the family of UDP-glycosyltransferases. In summary, the synthesis of saponins is comprehensive. For more details, we refer to the article from Yang *et al.*[72] Saponins have been established as stabilizer, emulsifier, and as additive to intensify foam in shampoos.[73] Stanimirova *et al.* have also shown the potential of saponins of Q. saponaria as adjuvants to vaccines, which induce the production of cytokines like interleukins and interferons in animal system, therefore stimulating immune responses.[74] Like rhamnolipids and sophorolipids, saponins have also been reported to exhibit anti-inflammatory antimicrobial, anticancer, antifungal, and cytotoxic properties.[75] Furthermore, the non-polar tail of saponins can be absorbed into lipid membranes which can alter membrane composition and fluidity. The latter

aspect may also play a role in defining its utility for membrane protein purification.[76] For example, saponins and lipids can form nanodiscs that enable the investigation of proteins by nuclear magnetic resonance spectroscopy.[77]

Other established bio-based detergent classes include trehalose lipids and sodium lauryl glyoxylate. Trehalose lipids consists of a di-glucose head group whose hydroxy groups are esterified, as seen by many naturally occurring trehalose lipids synthesized by *M. tuberculosis*, with the most prominent being 6,6'-dimycolate (Figure 8).[78] Early on, organic synthesis of the disaccharide was found to be quite difficult, because yields of the desired α,α-trehalose via Koenigs-Knorr glycosylation were abysmal.[79] Newer studies conducted by Pratt *et al.* described the formation of the 1,1-α,α-glycosidic bond by an intramolecular aglycone delivery with complete stereoselectivity and in excellent yields.[80] Furthermore, enzymatic synthesis in bacteria has been described via three different pathways. De Smet *et al.* propose detailed mechanisms obtained by their assays using mycobacteria.[81] The variability of different syntheses underlines the importance of the molecule in the organisms. Trehalose lipids may act as energy reserve, cell wall component in form of glycolipids, as a pathogenetic factor of bacteria for tuberculosis, or as signaling molecule in trehalose metabolism which has been linked to glucose transport and glycolysis.[82] The current bottleneck for a translation into wider markets relates to the fact that trehalose lipids are notoriously difficult to be obtained in pure form since they commonly co-purify with other compounds from biological samples.[82] More broadly, the alkylation of trehalose can lead to detergents, some of which turned out to enable the purification of intact membrane proteins, including G protein-coupled receptors (GPCRs).[83]

In contrast, sodium lauryl glucose carboxylate consists of a polar, carboxylate-containing glucose unit and a non-polar C12 carbon tail (Figure 8). Like SDS, glucose carboxylate detergents belong to the class of anionic detergents and are obtainable from coconut oil, glucose, or dodecyl α-D-glucosides.[55,84] Sodium lauryl glucose carboxylates are used as an alternative for alkyl ether sulfates, which are commonly found in shampoos. General characteristics include good water solubility, reduced sensitivity to hard water, good foaming properties, and satisfying skin mildness.[85] Taken together, the field of bio-based detergents is a well-established and continuously emerging field. In addition to the biosynthetic detergents discussed above, plipastatin, fengycin, emulsan, alasan, iturin A, mannosylerythritol lipids, lichenysin, viscosin, flavolipids, cellobiose lipids, serrawettin W1, phospho-/sulfo-/polyol lipids, and streptofactin also count as established candidates in the field. For further information on bio-based detergents, other than those discussed here, we refer to reviews of Markande *et al.* and Rahman *et al.*[86]

2.2.3. Saccharide head groups

In addition to cosmetic or household detergent products, detergents are relevant for membrane protein studies.[87] Non-ionic carbohydrate-based detergents turned out to be beneficial for studying intact membrane protein structures. The latter aspect is relevant for drug discovery since intact protein structures serve as tools for drug optimization.[7b,18,20b] While most membrane protein crystal structures were solved in n-dodecyl-ß-D-maltoside (DDM),[88] the fusion of two DDM monomers into lauryl maltose neopentyl glycol (LMNG) turned out to be a breakthrough.[89] LMNG can enable structural studies not only on stabile membrane proteins but also on exceptionally fragile membrane proteins with high relevance for drug discovery, such as GPCRs or ion channels.[7b,37,90] Until today, different carbohydrate-based head group architectures have entered the detergentome, including dendronic triglycoside head groups,[91] monosaccharide-corded glycoside head groups,[92] and mannitol-based head groups.[93] The repertoire of carbohydrate head groups enables systematic structural variations for structure-property studies, including changes in the number of mono- and disaccharide head groups, connectivity, and branching (Figure 9). While it is not only the head group structure that determines experimental outcomes, certain head groups in combination with dedicated linker and tail structures can deliver detergents that turned out to be exceptionally beneficial for the investigation of membrane protein structure and function (Figure 9). For more details on the development of most suitable carbohydrate-based detergents for membrane protein studies we refer to the review of Lee *et al.*[7b]

2.2.4. Hybrid head groups

As discussed above, the fact that every detergent has individual pros and cons for applications can be compromised by the production of detergent blends.[3b,22] Non-covalent mixing of detergents requires no extra synthesis and properties of blends may be gradually tuned by varying mixing ratios.[3b] The properties of detergent blends depend not only on detergent mixing ratios but also on other factors, including pH, temperature, salt, solvent, etc.[3b,14] Consequently, structure-property studies become notoriously difficult, and blends are optimized by empirical screening.[3b] Another strategy to harness beneficial properties of different detergents is to fuse their head groups covalently into hybrid detergents (Figure 10). Early studies in this direction were performed by Catanoiu *et al.* on hybrid detergents containing the non-ionic head groups triethylene glycol and inositol (Figure 10).[14b] The authors concluded that properties of hybrid detergents depend on the way the two head groups are attached to the hydrophobic tail and that the comparatively low solubility of the inositol parent detergent is considerably enhanced when being combined covalently with triethylene glycol (Figure 10). The paper published by Catanoiu *et al.* was the first of its kind where the role of head group structure in hybrid carbohydrate/oligoethylene oxide detergents has been studied systematically.[14b]

In recent years, the field of non-ionic hybrid detergents has been flourishing. Urner and co-workers synthesized another hybrid detergent by combining the head groups of tetraethylene glycol monooctyl ether (C8E4) and DDM through a

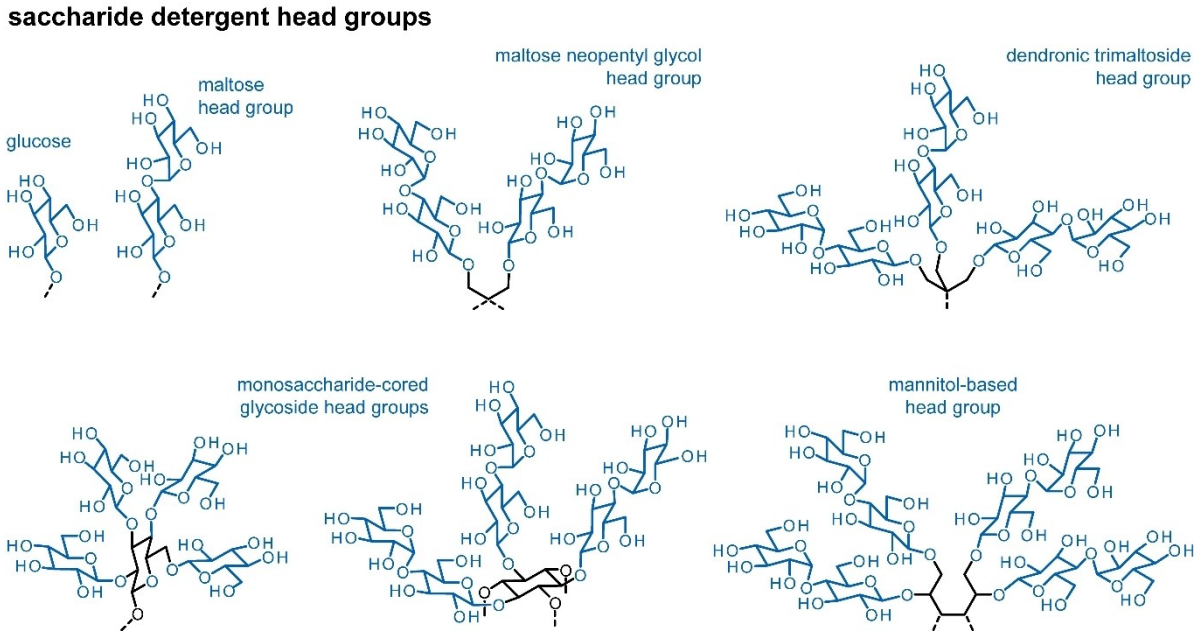


Figure 9. Overview of synthetic saccharide detergent head groups. Changing the branching, connectivity, and number of saccharide units in detergent head groups has led to the obtainment of new saccharide detergents for applications in membrane protein research.

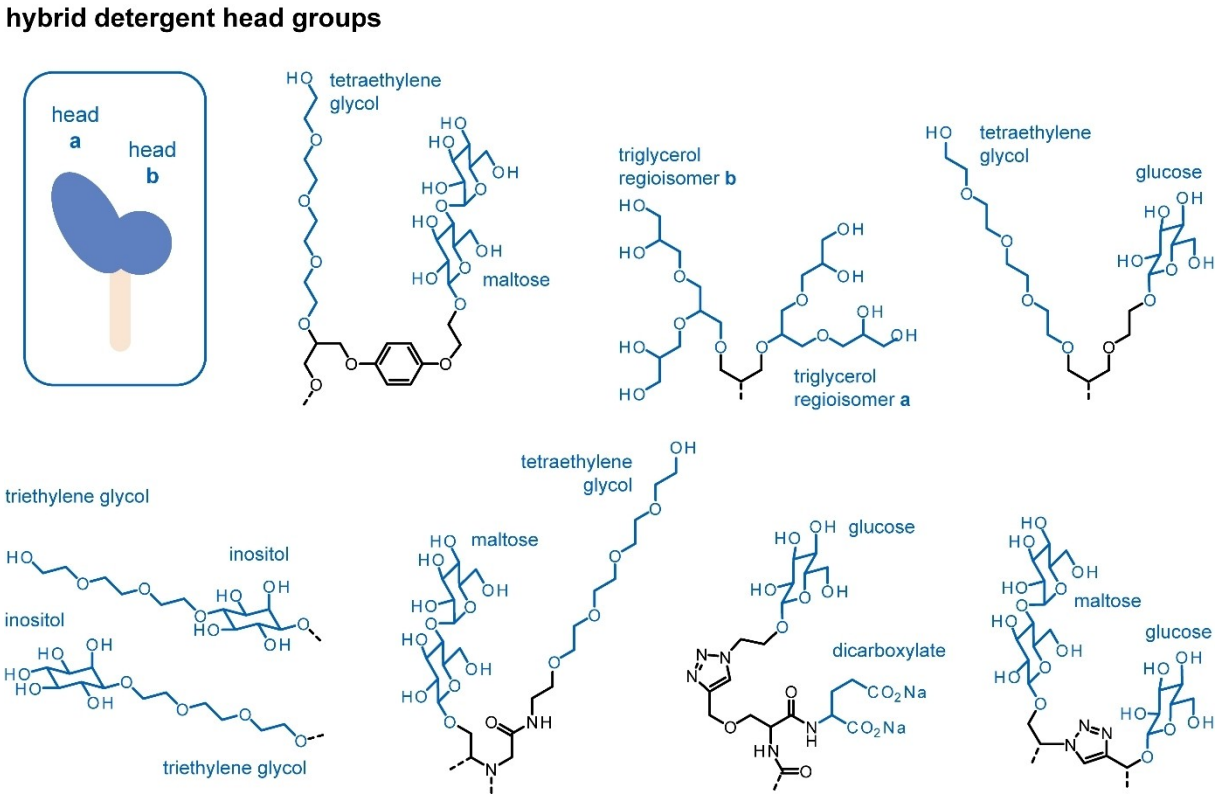


Figure 10. Overview of hybrid detergent head group structures. Established head group combinations include polyethylene glycols, saccharides, triglycerol regioisomers or dicarboxylate. Combining head groups of different detergents can lead to the obtainment of hybrid detergents whose properties can be superior compared to those of individual detergents.

hydroquinone linker (Figure 10).[2a] The idea was to combine favorable properties of both detergents for membrane protein purification and native mass spectrometry analysis. C8E4 is considered as harsh detergent in solution, which can lead to protein denaturation and failing purifications.[94] However, given a membrane protein is sufficiently stable in C8E4, this detergent

can enable the facile native mass spectrometry analysis.[94–95] On the contrary, DDM is considered as mild detergent in solution, which can stabilize proteins in solution.[7a,d] However, DDM is less compatible to native mass spectrometry compared to C8E4 which can hamper experiments.[14a] The head groups of C8E4 and DDM are determining structural motifs for protein purification and mass spectrometry outcomes.[2a,94a] Results obtained from Urner and co-workers indicate that fusing head groups of DDM and C8E4 can lead to a hybrid detergent that exhibits the beneficial properties of individual detergents while neglecting some of their disadvantages.[2a] For example, the C8E4-DDM hybrid detergent was able to stabilize the membrane protein AqpZ in the absence of membranes better than C8E4, had milder delipidating properties than C8E4, and was more compatible to mass spectrometry than DDM.[2a] However, the DDM–C8E4 hybrid detergent is not as efficient in releasing proteins from membranes as DDM and its synthesis is lengthy, suggesting a need for improvements.[2a]

In light of these scientific achievements, a combinatorial synthesis has been developed by Urner and co-workers to facilitate the synthetic access to the chemical space of non-ionic hybrid detergents (Figure 10).[2b] This development originated from the initial observation that the reaction of two acetal-protected, triglycerol regioisomers (**a**, **b**) with methallyl dichloride resulted in a mixture of three second-generation, acetal-protected triglycerol detergent head group regioisomers (**aa**, **ab**, **bb**).[30] The asymmetric head group was identified by NMR analysis of trimeric head group mixtures by means of reference compounds and was identified as main product (**ab**) in combinatorial synthesis (Figure 10).[30] Later, this approach was successfully employed to fuse other head group combinations, like glycerol and tetraethylene glycol, glucose and tetraethylene glycol, and ethylene glycol and tetraethylene glycol (Figure 10).[2b] The ability to readily fuse different non-ionic detergent head groups into hybrid detergents can be used to gradually scale molecular properties of detergents with unprecedented resolution, including overall polarity and shape.[2b] Furthermore, the concept of designing hybrid detergents can be used to obtain detergents with low cac values.[2] The latter aspect will facilitate the design of detergents for studying the delipidation and function of membrane proteins.[2,96] In addition to non-ionic hybrid detergents, also first mixed, ionic/non-ionic hybrid detergents were introduced based on a dicarboxylate anionc in combination with different carbohydrate head groupswith superior properties for membrane protein studies (Figure 10).[97]

The potential of designing hybrid detergents is now established for individual applications in membrane protein purification and analysis. Moreover, alternative approaches were developed to further facilitate the synthetic access to the chemical space of non-ionic hybrid detergents. Zhao *et al.* developed a two-dimensional expansion strategy to merge two monomeric detergents into hybrid detergents via copper-catalyzed click chemistry.[27d] Following this strategy, the head groups and tails of mono- and disaccharide detergents, like n-octyl-ß-D-maltoside (OG) and DDM, can be readily fused into asymmetric hybrids by means of a triazole ring (Figure 10). In addition to asymmetric detergents, the authors also demonstrated the utility of this approach to obtain novel symmetric hybrid detergents. Furthermore, Zhao *et al.* showed that this approach can be used to obtain new detergents for the structural analysis of membrane proteins through cryogenic electron microscopy or nuclear magnetic resonance spectroscopy.[27d] An alternative strategy for the synthesis of hybrid detergents has been established based on the Ugi reaction. The Ugi reaction is a multicomponent reaction involving a ketone/aldehyde, an amine, and a carboxylic acid, which form a bis-amide once an isocyanide is added.[98] Yang *et al.* translated this concept to detergent science by carefully designing the functionality of molecular blocks, i.e., head, linker, tail, which will form a combination of different detergent head groups through a dipeptide linker once they are mixed accordingly (Figure 10).[27c] The utility of making new detergents via the two-dimensional expansion strategy or Ugi reaction was demonstrated in the context with membrane proteins that are notoriously difficult to study but which are important for drug discovery research, like GPCRs.[99] While the utility of hybrid detergents in context with membrane protein research is now established and synthesis methods can provide facile access to their chemical space, future studies will evaluate their potential in detergent science and disciplines beyond membrane protein research.

2.3. Tail groups

2.3.1. Linear and branched tails

While reviewing current trends in the diversification of the detergentome, we realized that an increasing number of non-polar tails has been entering the literature over the past years. Only the combination of polar head and non-polar tail will give detergents their unique amphiphilic character that serves as enabling step for applications. The number of new tail structures entering literature has been flourishing and detergent designers interfacing detergent science and membrane protein research made a significant contribution (Figure 11–15). Our aim is to provide an overview of available tail structures, which can serve as a source for exchange and inspiration for the broader detergent science community beyond the boundaries of individual applications and product formulations.

Detergent tails that are commonly used in mass markets include linear alkyl tails, such as in the cases of SDS and TWEEN-20, substituted aromatic tails, such as in the case of Triton X-100, fluorinated chains, such as in the cases of perfluoroalkyl substances, and cholesterol, such as in the case of CHAPS. For every detergent tail a sub-research field has been evolved over the past years, which we will briefly discuss in the following paragraphs (Figure 11–15).

Linear alkyl chains are among the most widely used detergent tails (Figure 11). Typically, chain lengths of 12 or 18 carbon atoms are used and the optimal chain length depends on the head group and application.[14a] Introduction of hetero atoms, such as disulfide bonds, at different positions of the alkyl

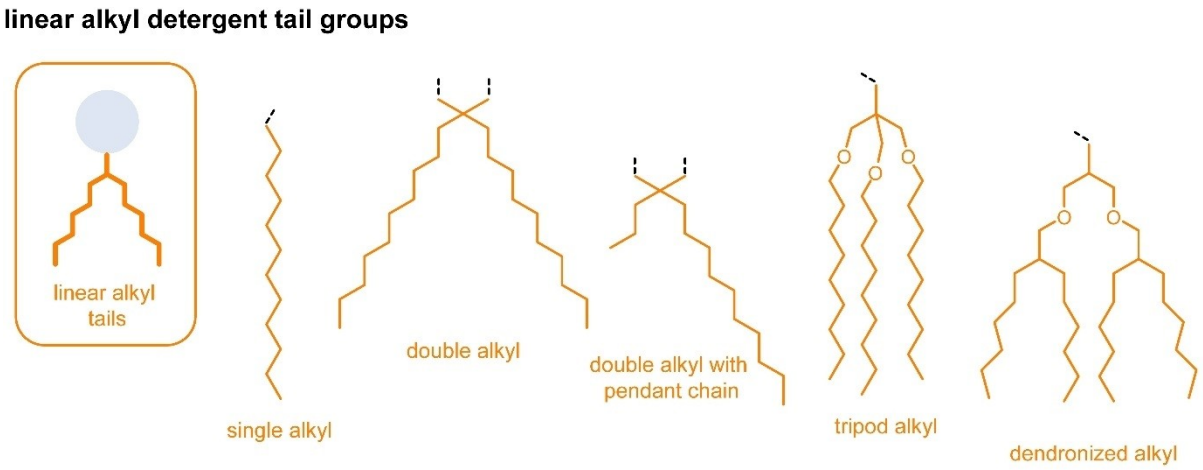


Figure 11. Overview of linear and branched tail groups. Established tail groups include single and double alkyl tails, asymmetric pendant chains, tripod and dendronized alkyl chains.

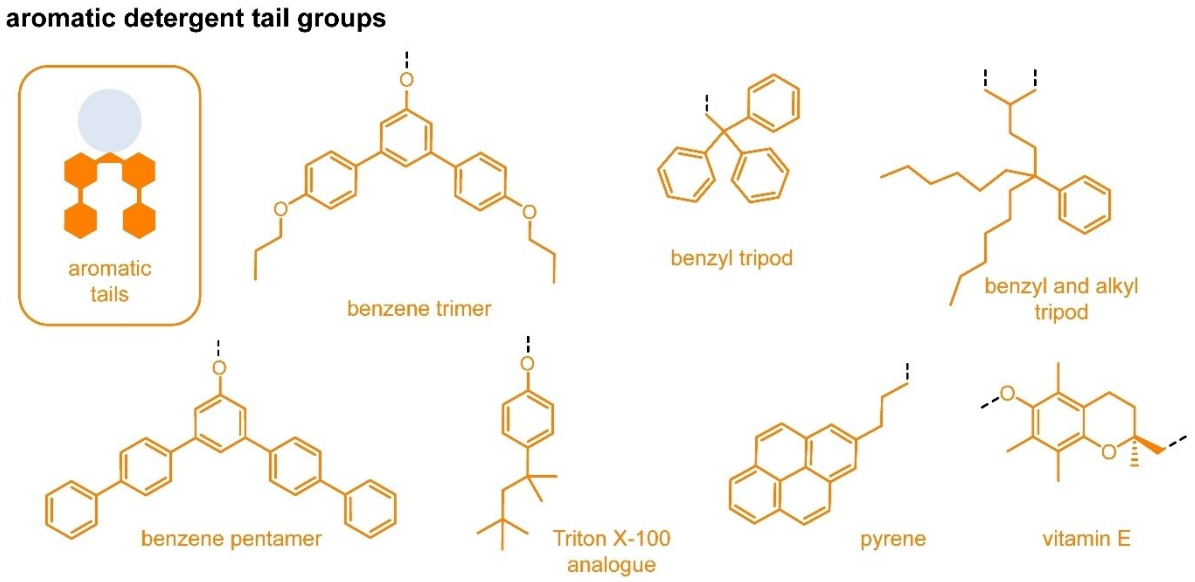


Figure 12. Overview of aromatic detergent tail groups. Established aromatic tail groups include benzene trimers, benzyl tripods, benzyl and alkyl tripods, benzene pentamers, mono-alkylated benzene, such as in the cases of Triton X-100 and pyrene.

chains can change aggregation concentration and improve the solubilization of membrane proteins.[100]

Increasing the bulkiness of linear tail groups can be achieved by introducing branch points. Branched detergent tails include double tail, tripod tail, and dendronized tails (Figure 11). Double tails are expected to mimic the hydrophobic environment of lipid membranes better than single tails, which can be beneficial for stabilizing the structure of intact membrane proteins.[14a] Furthermore, double alkyl tails are preferred in cases where the hydrophobic chain has to compensate a large hydrophilic group and very long single alkyl chains are not favored. However, branched alkyl chains were reported to decrease the rate of biodegradation.[101] While detergents with single alkyl chains are readily biodegradable, the implementation of branching points can cause a reduction of the rate of biodegradation.[101] Detergents with branched alkyl chains may not be the first choice for the design of mass market products whose disposal routes contribute to environmental pollution, like hand soap or shampoos.

In context with membrane protein purification, the Chae group reported a number of double tails with chain lengths ranging from one to 14 carbon atoms (Figure 11).[89,102a–c] The authors found that double tails with 11–14 carbon atoms can be superior for membrane protein stabilization compared to reference detergents such as DDM.[92b,93] Double tail architectures were also designed with different chain lengths. The synthesis typically starts with the preparation of the longer alkyl chain followed by the attachment of a shorter group, which is

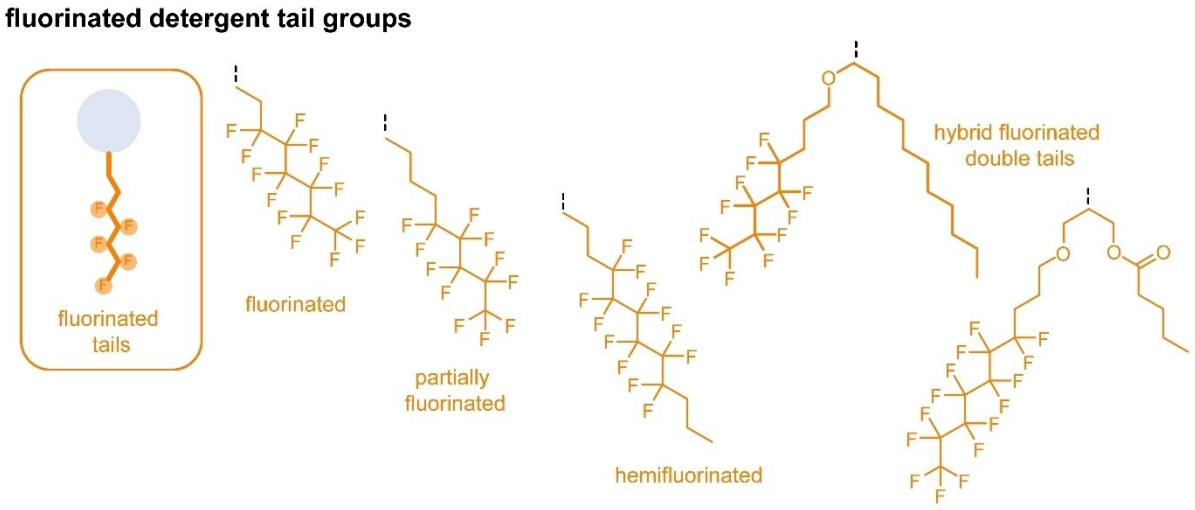


Figure 13. Overview of fluorinated detergent tail groups. Established fluorinated tail groups include fully fluorinated tails, partially fluorinated tails, fluorinated/-non-fluorinated double tails.

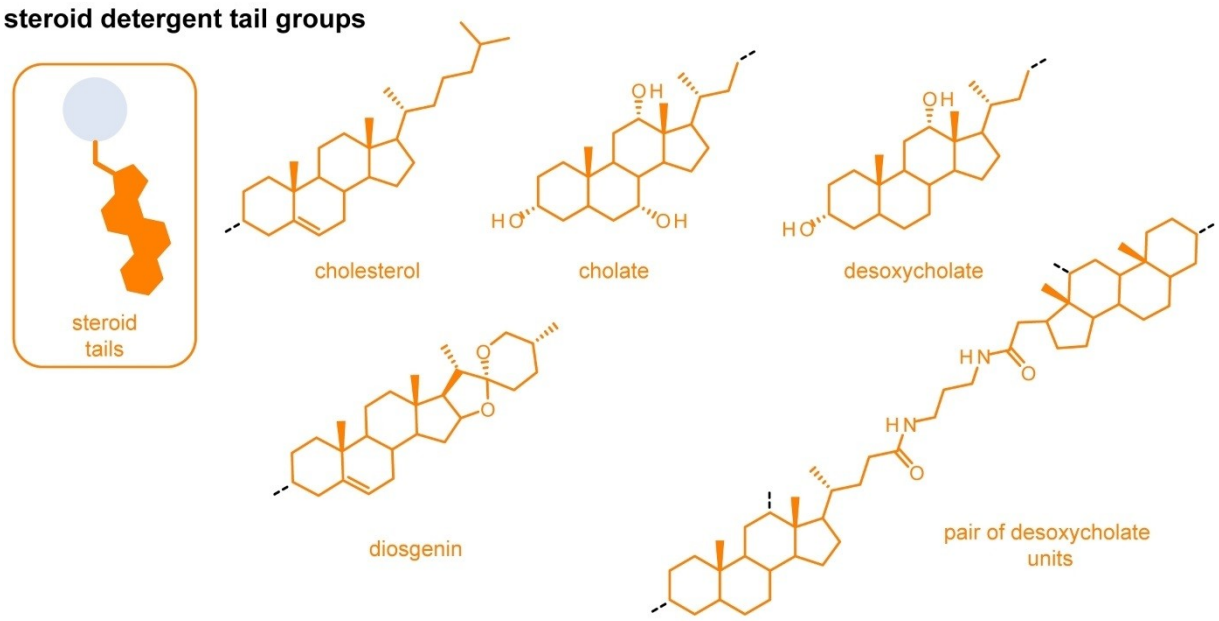


Figure 14. Overview of steroid-based detergent tail groups. Established structures are related to cholesterol, cholate, desoxycholate or diosgenin.

commonly described as "pendant chain" (Figure 11).[103] Detergents with asymmetric double tails can enhance protein stability compared to commercially available detergents especially when the pendant chain is significantly shorter than the main alkyl tail.[104] Further methylation of the linear alkyl tails was also reported but did not reveal increased membrane protein stability compared to detergents with linear tails.[105] Tripod and dendronic alkyl tails have also been proven to stabilize membrane proteins and even retain their initial activity (Figure 11).[91,106]

In addition to linear and branched tails, an increasing number of aromatic tail groups has been reported (Figure 12). The idea behind the implementation of aromatic tail groups is to modulate the size, dynamic, and encapsulation efficiency of detergent aggregates due to the presence of additional aromatic-aromatic interactions (Figure 12).[27a,31a,107] Benzene trimers and pentamers have been synthesized and tend to form smaller micelles compared to DDM (Figure 12). In membrane protein analysis, detergents with polyaromatic tails could maintain the stability of native protein structures over long-term better than commercial detergents with linear tails, such as DDM.[107–108] Tripod groups consisting of three benzene rings are commercially available and can be easily functionalized with polar groups to obtain detergents (Figure 12).[109] Tripod tails bearing one phenyl ring and two alkyl chains are synthesized via a multi-step approach.[110] Both tripod synthesis approaches

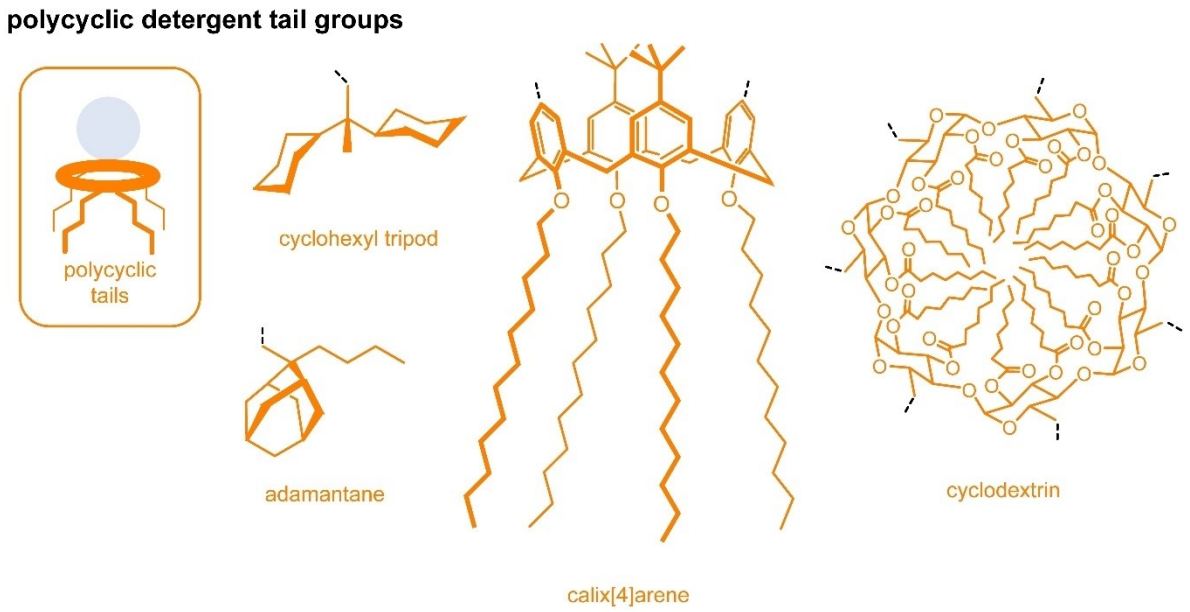


Figure 15. Overview of common polycyclic detergent tail groups. Established examples include detergents containing cyclohexyl tripod tails, adamantane, calix[4]arene, and cyclodextrin.

can deliver detergents that efficiently stabilize membrane proteins that are sensitive to detergent-induced denaturation. The Chae group also reported detergents with benzyl and alkyl tripod with further substitutions in *para*-position of the phenyl ring.[111] These detergents led to more efficient solubilization of a membrane protein complex than the standard detergent DDM. The probably most commonly used detergent with an alkylated benzene ring is Triton X-100, which can efficiently solubilize proteins (Figure 12).[112] Fluorescent polycyclic tail groups, like pyrene, enable an optical readout, however, related detergents have also been reported to have comparatively high cmc values.[113] Pyrene groups are also used as a hydrophobic anchor group to attach_amphiphilic molecular systems to surfaces through hydrophobic and electrostatic interaction (Figure 12).[114] Furthermore, a common molecule found in cell membranes is vitamin E and has also been used as a hydrophobic tail in detergents (Figure 12). Detergents consisting of vitamin E tail groups revealed enhanced stability of membrane proteins compared to standard detergent DDM.[115] Taken together, recent additions to the repertory of aromatic tail groups have been studied to improve the utility of detergents for membrane protein purification and for the supramolecular functionalization of surfaces (Figure 12). Future studies may show how the use of aromatic tail groups can improve the utility of detergents for applications beyond the once mentioned here.

A unique sub-class of non-polar tails are fluorinated tails, which are widely applied in industrial and customer products due to their resistance to grease, oil and water (Figure 13). Moreover, per- and polyfluoroalkyl substances (PFAS) consist of stable C–F bonds and are therefore extremely resistant to thermal, chemical or biological degradation.[116] Due to their environmental persistence and bio-accumulative properties, they are a growing concern in the context of environmental pollution and human health.[117] Furthermore, and in contrast to linear hydrocarbon and aromatic tails, fluorinated tails are not miscible with aliphatic hydrocarbon chains. In membrane protein research, this background led to the idea that detergents with fluorinated tails might interfere less with protein-lipid interactions compared to non-fluorinated detergents, which can enable an enhanced stabilization of functional membrane proteins.[118] In summary, fluorinated detergent tails have been successfully used to extract,[119] solubilize,[119b,120] stabilize[119b,120–121] and refold membrane proteins[120c,122] (Figure 13). Due to the low miscibility of fluorinated tails with hydrocarbon tails, proteins can be difficult to transfer into fluorinated detergent micelles. To overcome this bottleneck, detergents with hemifluorinated tails bearing a hydrogenated tip have been developed, which can stabilize encapsulated proteins in solution for more than 2.5 months (Figure 13).[121b,122] Partially fluorinated tails can show better stabilization efficiency of membrane proteins compared to DDM although the fluorinated content in the hydrophobic chains tends to form rod-like and poorly defined micelles (Figure 13).[120b] The latter aspect may be considered as unusual because the formation of globular and defined micelles is considered as a key requirement for applications in membrane protein biochemistry.[120a] Another strategy to increase the miscibility with hydrocarbon tails is to fuse fluorinated and non-fluorinated tails into hybrid double tails.[119a,121a] In this regard, the Durand group designed two hybrid double tails: i) a long fluorinated chain with a long alkyl chain (10 carbon atoms) and ii) a long fluorinated chain with a short alkyl chain (4 carbon atoms) (Figure 13).[119a,121a] Detergents with a short aliphatic pendant chain have been

shown to stabilize membrane proteins for 3 months whereas hybrid double tails containing long alkyl chains were suffering from poor water solubility. Moreover, these detergents were reported to form mixed micelles with common detergents and demonstrated increased extraction yields from biological membranes.[119a] More lately, fluorinated detergents were shown to be able to stabilize lipid nanodiscs.[123] Zwitter-ionic and fluorinated detergents were shown to solubilize and stabilize membrane proteins.[124] Furthermore, fluorinated glycerol detergents are suitable for the preparation of highly stable water-in-fluorinated oil emulsions for microfluidics applications.[9a] Despite their environmental persistence and growing skepticism regarding their impact on human health and environment,[116,125] fluorinated detergents form an integral part within a growing research direction whose potential for future applications is under investigation.

In addition to linear, branched, aromatic, and fluorinated tails, steroid-based detergents are particularly interesting because they are structurally very similar to cholesterol, a molecule found in eukaryotic cell membranes (Figure 14). Analogue structures are found in bacterial membranes and plants, i.e., hopanoids.[126] Mammalian liver cells produce bile salts, which are steroid-based molecules also known as digestive detergents as their micelles can readily absorb lipids.[127] Bile salts have been extensively studied to enhance transepithelial permeability for different marker molecules and drugs.[128] Furthermore, bile salts are widely known and used as bile soap (gall soap) in laundry applications. In line with physiological roles of bile salts in fat digestion and transport, detergents bearing a cholesterol tail group can be used to modulate membrane permeability and to extract proteins from membranes.[7d,14a,128a] Steroid structures are illustrated in Figure 14 and include cholesterol, cholates, desoxycholates and diosgenins. Detergents containing steroid tails are generally well established in membrane protein research. For example, the detergent Chobimalt consists of a polar branched tetrasaccharide head and a non-polar cholesterol (Figure 14). Chobimalt can form mixed micelles with DDM that can extract and stabilize the human kappa opioid receptor type 1 (hKOR1).[129]

Diosgenin analogues lack the hydroxy groups of cholic acid and consist of an additional spiroketal ring while maintaining the native structure of membrane proteins in detergent solutions (Figure 14).[130] Interestingly, a pair of two desoxycholate units has been linked via amide bonds and revealed efficient protein stabilization over 20 days (Figure 14).[131] Compared to conventional detergents, these so-called tandem facial amphiphiles display a separation of the hydrophilic and hydrophobic groups along the long axis of the molecule.[132] Furthermore, the Zhang group has synthesized a facial amphiphile based on cholate where the steroid core contains polar groups on one side and non-polar groups on the other side.[133] These detergents can also be used for membrane protein stabilization and crystallization. The probably most widely used steroid-based detergents are CHAPS and CHAPSO (Figure 14). Both CHAPS and CHAPSO are sulfobetaine derivatives which are derived from cholic acid.[134] Furthermore, doping detergent micelles with steroid-based additives is a common strategy to enhance protein stability, in particular when working with more fragile proteins, like GPCRs.[135]

Apart from linear, branched, aromatic, fluorinated, and steroid-based tails, also detergents containing larger polycyclic structures have been established, including cyclohexyl tripod, adamantane, and calix[4]arene (Figure 15). Like other tripods discussed above, tripod amphiphiles with two cyclohexyl rings and a methyl group can also be used to solubilize and stabilize membrane proteins (Figure 15).[136] Another common polycyclic scaffold is adamantane (Figure 15). Originally it was obtained from fractional distillation of petroleum. Due to its high melting and boiling points it was assumed to be structurally similar to diamond and called adamantane.[137] The implementation of adamantane groups into the hydrophobic tail of detergents can be employed to improve the efficiency of membrane protein solubilization (Figure 15).[138] Furthermore, adamantane has been employed as a hydrophobic backbone for trimeric cationic surfactants.[139] More broadly, adamantane is known for its unique supramolecular host-guest chemistry in conjunction with cyclodextrins.[140] For example, capping the end of the hydrophobic backbones of azobenzene-based oligoglycerol detergents with adamantane enabled the supramolecular functionalization of cyclodextrin-coated gold surfaces with photo-switchable polarity.[32b] Alkylated cyclodextrin molecules have been shown to encapsulate guest molecules in different domains of their aggregate structures (Figure 15).[141] A more exotic polycyclic motive in detergent science is calix[4]arene. Detergents made from calix[4]arene consists of four bridged phenol rings and four aliphatic chains (Figure 15). Calix[4]arene detergents have been found to form shape-persistent micelles containing exactly seven or twelve monomers depending on the linker between head and tail, respectively.[41,142] Calixarene-based detergents can be used in membrane protein purification[143] and to support membrane protein crystallization.[46a] Polycyclic detergent tails represent a valuable extension to the repertoire of existing tails and are useful for specialized applications.

2.4. Linker groups

2.4.1. Standard linker groups

Polarity and molecular shape of detergents are determining factors in applications.[2b,14d] Both parameters are mainly regulated by the structure of the polar head and non-polar tail. The functional group that connects head and tail covalently is called the linker. Tuning the chemistry of the linker can also be used to fine tune polarity, shape, and supramolecular properties of detergents.[144] Furthermore, the chemistry of the linker has been harnessed to enable remote control over polarity, shape, and amphiphilicity in response to external stimuli, such as pH, redox, light, and enzymatic activity (Figure 16).[27b,145] The most common class of linkers are covalent linkers, including thioether,[9b,15] ether,[30,102b,103,146] ester,[147,148] amine,[149] amide,[30,146b,150] or phenyl groups[30,151] (Figure 16). Detergents bearing ether linkers were reported to be more effective in

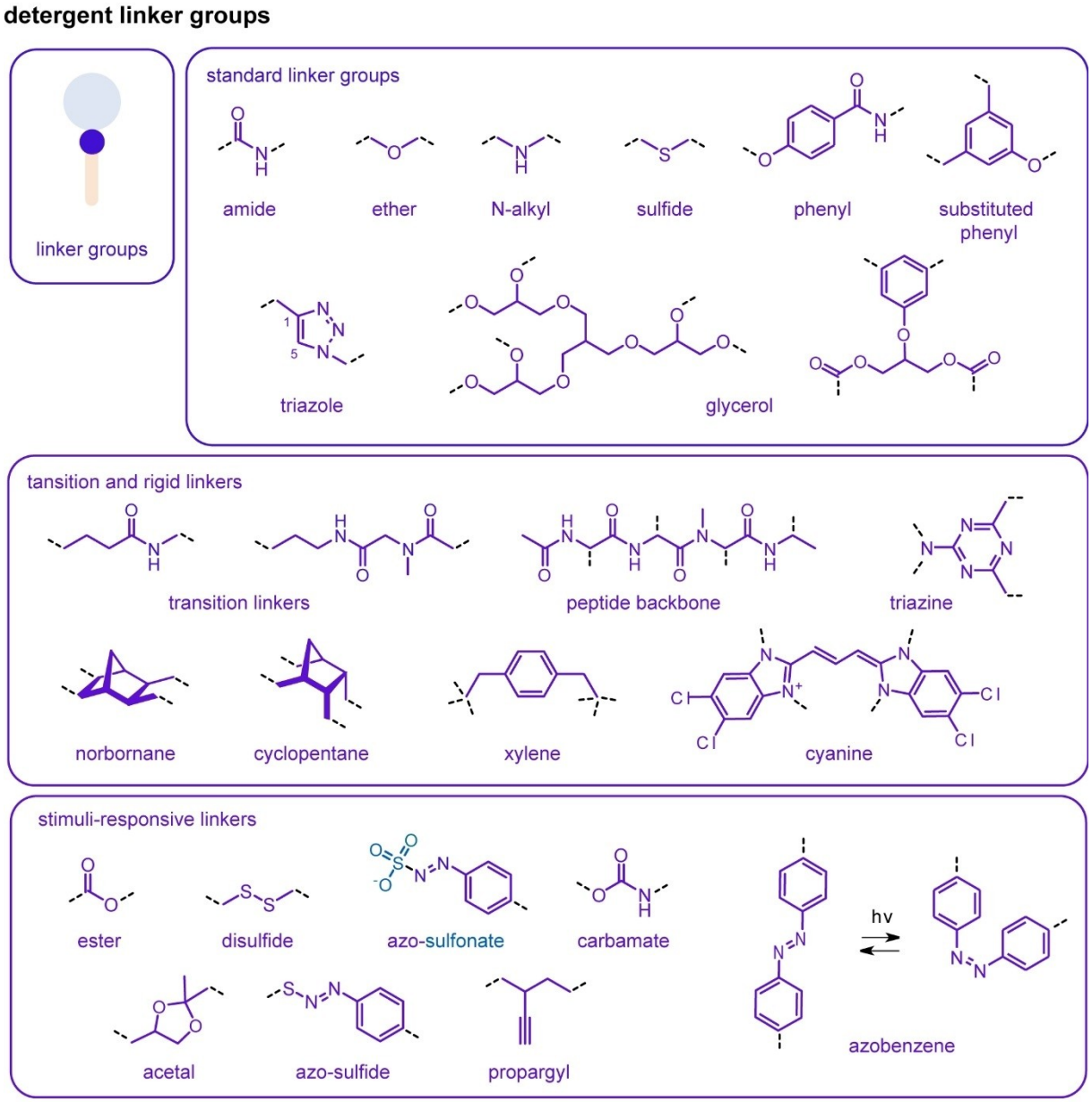


Figure 16. Overview of detergent linker groups. Established categories include standard linker groups, transition and rigid linkers, stimuli-responsive linkers. Standard linkers consist of common functional groups such as amides, ethers, flexible glycerols or phenols. Transition and rigid linkers include alkyl amides, peptide backbones, rigid cyclopentanes or triazines. Stimuli-responsive linkers are labile to irradiation of light (azo-sulfonate, azo-sulfide), pH (esters, carbamates, acetals), redox chemistry (disulfide) or transition metals (propargyl). Other categories include azobenzene as photo-switchable linkers.

membrane protein stabilization than analogue detergents with amide groups.[146b,152] Moreover, a connection between head and tail via phenyl groups can be an advantage when aromatic interactions between aggregates and guest molecules are favored.[2a,153] Triazoles are another popular class of linkers, which are commonly obtained by copper-catalyzed click reactions (Figure 16).[29,154] Interestingly, 1,2,3-triazoles are chemically similar to amide bonds. Like amide bonds, they have a hydrogen-bonding acceptor side, i.e., at C(1)-N position, and a hydrogen bond donor side, i.e., at C(5) position (Figure 16).[155] Therefore 1,2,3-triazoles can be considered as amide bond mimetics.[155a,156] The implementation of triazole into amphiphilic molecules can affect aromaticity, rigidity, and solubility,[144e] increase biostability,[156] alter supramolecular properties,[144c,155b] affect antimicrobial and anticancer properties,[157] and can change viscosity.[158]

2.4.2. Transition and rigid linkers

Recently, Tao *et al.* introduced the concept of transition linkers to gradually tune the polarity between polar head and non-polar tail (Figure 16).[144d] Transition linkers enable the synthesis of micelles with gradual polarity changes that have low cmc

values. Moreover, peptide backbones have been shown to stabilize integral membrane proteins. β-Strand peptides with 8 residues have organized into a well-ordered secondary nanostructure which resembled the thickness of membranes.[159] Lipopeptide detergents were designed by the Privé group and self-assembled into a cylindrical micelle.[160] They are reported to have non-denaturing properties, can disperse phospholipid membranes, and have promising potential for the structural analysis of membrane proteins.

In addition to polarity and shape, the stiffness of detergents is another determining factor for aggregation and solubilization properties. The effect of molecular rigidity on supramolecular properties of amphiphilic molecules has been well-established in the field of polymers and ethylene glycol-containing amphiphilic dendrons, which revealed that structural rigidity decreases water solubility.[161] In the cases of polymers and smaller bolaamphiphilic detergents, decreased conformational flexibility has also been found to lower water-solubility.[144e,161b] Closer analysis of the link between molecular rigidity, water solubility, and supramolecular properties led to the explanation that the water solubility of a rigid detergent can be interpreted as a cac.[162] According to Israelachvili *et al.* aggregates of infinite size can be formed above the cac thus leading to phase separation which becomes observable by eye in the form of reduced water solubility.[162] In addition to water solubility, rigid building blocks linking the head and tail can determine the shape and morphology of aggregates. For example, a cyanine dye has been used as a core with hydrophilic and hydrophobic groups attached to each side of the indolenine nitrogen atoms to provide an extended planar π-system that drives the formation of sheet-like or tubular supramolecular structures (Figure 16).[163]

The concept and utility of detergent rigidity has been brought further into membrane protein research by the Chae group. Different case studies confirmed that the stability of membrane proteins in detergent micelles is sensitive to the rigidity of the detergent micelle surrounding the protein. Chemically, fine tuning of detergent rigidity has been addressed by tuning the flexibility of the linker between rigid saccharide head groups and more flexible tails. A well-established example is the triazine linker.[149,164] The chemical structure of triazine provides three positions to be used for the covalent attachment of head and tail groups (Figure 16). The rigid and planar structure of triazine dictates the position of hydrophilic and hydrophobic group which in turn influences the shape of the detergent molecule and protein purification outcomes.[164b] Other approaches to modulate rigidity of detergents are based on the incorporation of a norbornane[165] and more flexible cyclopentane rings (Figure 16).[166] Compared to the norbornane, the cyclopentane ring is more flexible which was interpreted to be beneficial for detergents in use for protein stabilization.[166] Another publication reports a xylene group linking two maltose-based detergent molecules and showed enhanced stability of membrane proteins compared to reference detergents, like DDM.[157c] Furthermore, glycerol-based linkers have been reported to link several detergents to create a flexible core that can change the detergent conformation according to the shape of membrane proteins (Figure 16).[168] Taken together, rigidity is an important parameter when designing detergents for individual applications.[143a,144e,149,161,168] The flexibility of detergents also determines their foldability which opens new directions in the design of tailor-made detergents for membrane protein research.[169]

2.4.3. Stimuli-responsive linkers

As such, native supramolecular systems are of rather limited use when a controlled assembly and disassembly is crucial for the intended application.[170] A common route to circumvent this problem is to introduce moieties which modulate the physicochemical properties of the detergents in response to external stimuli, including cleavable linkers and switchable linkers (Figure 16). Cleavable linkers are functional groups that can be degraded by environment changes, e.g., through pH changes, enzymes, reduction, or irradiation with light. Ester groups are a prominent example for cleavable linkers, which can be induced via low pH values or esterases (Figure 16).[171] Disulfide bonds are linkers that can be cleaved by reducing agents, like glutathione which is also found in cells, thus enabling a redox-triggered micelle degradation and release of encapsulated drugs (Figure 16).[147b] Other disulfide detergents reported by Xue *et al.* revealed good extraction yields and efficient stabilization of membrane proteins comparable to commercially available detergents.[172] Complete cleavage was achieved with mild reducing agents based on phosphines and was compatible with several membrane proteins. Carbamate groups are widely applied in drugs for medicinal chemistry due to their metabolic stability and their ability to pass cellular membranes (Figure 16).[173] Moreover, carbamates are stable in neutral pH but they are prone to degrade under acid-catalyzed hydrolysis.[174] Carbamate groups have been used as linkers between head and tail groups and have the potential to tune the degradability of a detergent (Figure 16).[14a,175] ProteaseMAX and MaSDeS are commercially available detergents with build-in carbamate linkers and were used for in-gel protein digestion and for degradation in MS analysis, respectively.[176] Furthermore, a carbon atom bound to two oxygen atoms is called an acetal group and is commonly used as a protecting group for carbonyls and diols (Figure 16). Acetals are susceptible to acid-catalyzed hydrolysis and stable under basic, oxidative and reductive conditions.[177] Implementing acetal protecting groups into detergent backbones, such as in the case of commercially available RapiGestSF, has proven useful for enhancing membrane protein solubility and accelerating in-solution detergent and protein digestion for proteomics applications.[178] Another concept published by Liu et al. reported propargyl or allyl groups close to the ether linkage to introduce a point for palladium-catalyzed cleavage through biorthogonal decaging mechanisms.[179] The implementation of photo-switchable linkers into detergents, like azobenzene, presents another well-established modality to control the formation, polarity, and packing of aggregates.[6a,31b,35b,145b] Azobenzene can adopt two isomeric states, i.e., *cis* and *trans* (Figure 16). Both isomers are

transferable into one another by irradiation with light, which causes measurable changes in molecular shape and polarity.[180] Azobenzene-based detergents can show photo-switchable polarity and shape,[35b] aggregation behaviour,[170] water-solubility,[144e] and solubilization properties.[6a,31b] More lately, it was found that azobenzene isomers can differ in terms of basicity, which offers the opportunity to design detergents with photo-switchable protonation sides for membrane protein mass spectrometry.[35b] For more information about azobenzene and its scientific relevance we refer to other reviews.[181] The concept of harnessing the photo-responsive character of azobenzene for remote control has been brought further to obtain a photo-cleavable variant of the detergent SDS.[182] The detergent called Azo has been developed to overcome limitation of SDS in proteomics applications, including its incompatibility with mass spectrometry (Figure 16).[182] The Azo detergent contains a sulfonate group connected to the azo double bond and alkylated benzene (Figure 16). Exposing Azo-containing samples to ultraviolet light induces detergent degradation, which has been used to improve sample compatibility to mass spectrometry-based proteomics experiments.[182] In another case study, a maltose head has been linked to a non-polar tail through an azo-sulfide linker (Figure 16).[183] The azo group either linked to a sulfonate or sulfide can be cleaved *via* irradiation with ultraviolet light to expel nitrogen gas, which results in complete breakdown of the detergent nanocarrier. Whether they change polarity, are cleavable or dictate the geometry of the molecules and supramolecular aggregates, linkers between polar and non-polar groups play an important role in the development of tailor-made detergents for individual applications.

3. Conclusions

In summary, we reviewed the general scope and utilities of different synthesis strategies for the ability to obtain detergents, including linear-, bio-, modular-, combinatorial-, and metric-assisted detergent synthesis. Short, linear synthesis sequences are desirable if availability of large detergent quantities is a key requirement, like in the cases of detergent products for mass markets. Even though biosynthetic detergents are currently more expensive than chemical detergents in terms of production costs, biosynthetic detergents are expected to significantly contribute to future market growth. Furthermore, substituting chemical detergents for biosynthetic detergents can support climate-neutral production cycles. Modular chemistry and combinatorial chemistry are key enabling steps to accelerate the diversification of the detergentome for the benefit of structure-property studies. Metric-assisted approaches but also computational approaches can be useful to narrow down the chemical space of the detergentome to a chemical space that is likely going to contain suitable detergents or mixtures thereof for applications. Reference data and empirical screenings that produce high data quality outputs are needed for these approaches to work properly. Once established, we anticipate that computational and metric-assisted approaches can be used to significantly reduce the time and costs required for detergent optimizations. Finally, we provided an overview of common head, tail, and linker groups, and highlighted utilities of selected building blocks for individual applications. We anticipate both the overview of synthesis strategies and the overview of building blocks that shape the detergentome provided serve as a pool for inspiration and scientific exchange that facilitates the optimization of detergents for challenging future applications.

Acknowledgements

The Ministry of Culture and Science of the German State of North Rhine-Westphalia is gratefully acknowledged for providing financial support through a NRW return program grant. Open Access funding was enabled and organized by Project DEAL. Open Access funding enabled and organized by Projekt DEAL.

Conflict of Interests

The authors declare no conflict of interest.

Keywords: amphiphiles · detergentome · detergents · lipids · membrane proteins

[1] D. H. Everett, *Pure Appl. Chem.* **1972**, *31*, 577–638.
[2] a) L. H. Urner, I. Liko, K. Pagel, R. Haag, C. V. Robinson, *BBA-Biomembranes* **2022**, *1864*, 183958; b) L. H. Urner, A. Ariamajd, A. Weikum, *Chem. Sci.* **2022**, *13*, 10299–10307.
[3] a) D. Bajpai, V. K. Tyagi, *J. Oleo Sci.* **2007**, *56*, 327–340; b) K. C. Cheng, Z. S. Khoo, N. W. Lo, W. J. Tan, N. G. Chemmangattuvalappil, *Heliyon* **2020**, *6*, e03861.
[4] a) *JAMA* **1944**, *126*, 1152–1153; b) K. Harkin, A. Dee, *Int. J. Technol. Assess. Health Care* **2018**, *34*, 92–93.
[5] a) M. Nitschke, S. S. e Silva, *Crit. Rev. Food Sci. Nutr.* **2018**, *58*, 631–638; b) L. Delhalle, B. Taminiau, S. Fastrez, A. Fall, M. Ballesteros, S. Burteau, G. Daube, *Front. Microbiol.* **2020**, *11*.
[6] a) C. Kördel, A. Setaro, P. Blümmel, C. S. Popeney, S. Reich, R. Haag, *Nanoscale* **2012**, *4*, 3029–3031; b) A. R. Tehrani-Bagha, K. Holmberg, *Materials* **2013**, *6*, 580–608.
[7] a) M. le Maire, P. Champeil, J. V. Möller, *Biochim. Biophys. Acta* **2000**, *1508*, 86–111; b) H. J. Lee, H. S. Lee, T. Youn, B. Byrne, P. S. Chae, *Chem* **2022**, *8*, 980–1013; c) N. T. Johansen, F. G. Tidemand, M. C. Pedersen, L. Arleth, *Biochimie* **2023**, *205*, 3–26; d) G. G. Prive', *Methods* **2007**, *41*, 388–397.
[8] a) A. E. Speers, C. C. Wu, *Chem. Rev.* **2007**, *107*, 3687–3714; b) J.-S. Behnke, L. H. Urner, *Anal. Bioanal. Chem.* **2023**, *415*, 3897–3909.
[9] a) M. S. Chowdhury, W. Zheng, S. Kumari, J. Heyman, X. Zhang, P. Dey, D. A. Weitz, R. Haag, *Nat. Commun.* **2019**, *10*, 1–10; b) M. S. Chowdhury, X. Zhang, L. Amini, P. Dey, A. K. Singh, A. Faghani, M. Schmueck-Henneresse, R. Haag, *Nano-Micro Lett.* **2021**, *13*, 147.
[10] A. Helenius, K. Simons, *Biochim. Biophys. Acta* **1975**, *415*, 29–79.
[11] a) S. Fujii, Y. Sanada, T. Nishimura, I. Akiba, K. Sakurai, *Langmuir* **2012**, *28*, 3092–3101; b) M. T. Ivanović, M. R. Hermann, M. Wójcik, J. Pérez, J. S. Hub, *J. Phys. Chem. Lett.* **2020**, *11*, 945–951.
[12] P. H. Stahl, in *The Practice of Medicinal Chemistry (Second Edition)* (Ed.: C. G. Wermuth), Academic Press, London, **2003**, pp. 631–648.
[13] Y. Lu, E. Zhang, J. Yang, Z. Cao, *Nano Res.* **2018**, *11*, 4985–4998.
[14] a) L. H. Urner, I. Liko, H.-Y. Yen, K. K. Hoi, J. R. Bolla, J. Gault, F. G. Almeida, M.-P. Schweder, D. Shutin, S. Ehrmann, R. Haag, C. V. Robinson, K. Pagel, *Nat. Commun.* **2020**, *11*, 564; b) G. Catanoiu, D. Blunk, C. Stubenrauch, *J. Colloid Interface Sci.* **2012**, *371*, 82–88; c) W. C. Griffin, *J. Soc. Cosmet. Chem.* **1949**, *1*, 311–326; d) J. N. Israelachvili, D. J. Mitchell, B. W. Ninham, *J. Chem. Soc. Faraday Trans. 2* **1976**, *72*, 1525–1568.

[15] L. H. Urner, F. Junge, F. Fiorentino, T. J. El-Baba, D. Shutin, G. Nölte, R. Haag, C. V. Robinson, *Chem. Eur. J.* **2023**, *29*, e202300159.
[16] A. C. Kogawa, B. G. Cernic, L. G. Domingos do Couto, H. R. N. Salgado, *Saudi Pharm. J.* **2017**, *25*, 934–938.
[17] a) H. G. Hauthal, *Detergent Polymers, Other Ingredients, Basics, Tests, Assessment of Sustainability und Environmental Evaluation* **2007**, *44*, 45–61; b) P. I. Hora, S. G. Pati, P. J. McNamara, W. A. Arnold, *Environ. Sci. Technol. Lett.* **2020**, *7*, 622–631.
[18] L. H. Urner, *Curr. Opin. Chem. Biol.* **2022**, *69*, 102157.
[19] a) J. W. McBain, R. C. Merrill Jr., J. R. Vinograd, *J. Am. Chem. Soc.* **1941**, *63*, 670–676; b) D. Lichtenberg, H. Ahyayauch, F. M. Goñi, *Biophys. J.* **2013**, *105*, 289–299.
[20] a) N. Natalie, A. Michael, A. Rogers, *in Encyclopedia of Food Chemistry: Surfactants*, (Eds.: L. Melton, F. Shahidi, P. Varelis), Academic Press, **2016**, pp. 2276–2282; b) D. Milic, D. B. Veprintsev, *Front. Pharmacol.* **2015**, *6*, 1–24.
[21] K. Takei, K. Tsuto, S. Miyamoto, J. Wakatsuki, *J. Am. Oil Chem. Soc.* **1985**, *62*, 341–347.
[22] K. Holmberg, in *Ullmann's Encyclopedia of Industrial Chemistry*, **2019**, pp. 1–56.
[23] K. Invally, A. Sancheti, L.-K. Ju, *Food Bioprod. Process.* **2019**, *114*, 122–131.
[24] N. Kosaric, F. V. Sukan, in *Biosurfactants*, CRC Press, Boca Raton, **2014**.
[25] a) J. Kranz, M. Schwichow, P. Breitenmoser, K. Niebert, *Sustainability* **2022**, *14*, 4194; b) A. Uhl, A. Schmidt, C. Jensch, D. Köster, J. Strube, *Processes* **2022**, *10*, 1289.
[26] K. Wowk, L. McKinney, F. Muller-Karger, R. Moll, S. Avery, E. Escobar-Briones, D. Yoskowitz, R. McLaughlin, *Palgrave Commun.* **2017**, *3*, 35.
[27] a) M. Wyszogrodzka, R. Haag, *Chem. Eur. J.* **2008**, *14*, 9202–9214; b) B. N. S. Thota, L. H. Urner, R. Haag, *Chem. Rev.* **2015**, *116*, 2079–2102; c) M. Yang, W. Luo, W. Zhang, H. Wang, D. Xue, Y. Wu, S. Zhao, F. Zhao, X. Zheng, H. Tao, *Chem. Asian J.* **2022**, *17*, e202200372; d) F. Zhao, Z. Zhu, L. Xie, F. Luo, H. Wang, Y. Qiu, W. Luo, F. Zhou, D. Xue, Z. Zhang, T. Hua, D. Wu, Z.-J. Liu, Z. Le, H. Tao, *Chem. Eur. J.* **2022**, *28*, e202201388.
[28] H. C. Kolb, M. G. Finn, K. B. Sharpless, *Angew. Chem. Int. Ed.* **2001**, *40*, 2004–2021.
[29] B. Trappmann, K. Ludwig, M. R. Radowski, A. Shukla, A. Mohr, H. Rehage, C. Böttcher, R. Haag, *J. Am. Chem. Soc.* **2010**, *132*, 11119–11124.
[30] L. H. Urner, K. Goltsche, M. Selent, I. Liko, M.-P. Schweder, C. V. Robinson, K. Pagel, R. Haag, *Chem. Eur. J.* **2021**, *27*, 2537–2542.
[31] a) A. Richter, A. Wiedekind, M. Krause, T. Kissel, R. Haag, C. Olbrich, *Eur. J. Pharm. Sci.* **2010**, *40*, 48–55; b) C. Kördel, C. S. Popeney, R. Haag, *Chem. Commun.* **2011**, *47*, 6584–6586.
[32] a) M. Wyszogrodzka, R. Haag, *Biomacromolecules* **2009**, *10*, 1043–1054; b) O. Nachtigall, C. Kördel, L. H. Urner, R. Haag, *Angew. Chem. Int. Ed.* **2014**, *53*, 9669–9673.
[33] S. Malhotra, H. Bauer, A. Tschiche, A. M. Staedtler, A. Mohr, M. Calderón, V. S. Parmar, L. Hoeke, S. Sharbati, R. Einspanier, R. Haag, *Biomacromolecules* **2012**, *13*, 3087–3098.
[34] a) T. Heek, C. Fasting, C. Rest, X. Zhang, F. Würthner, R. Haag, *Chem. Commun.* **2010**, *46*, 1884–1886; b) T. Heek, J. Nikolaus, R. Schwarzer, C. Fasting, P. Welker, K. Licha, A. Herrmann, R. Haag, *Bioconjugate Chem.* **2013**, *24*, 153–158; c) K. Huth, T. Heek, K. Achazi, C. Kühne, L. H. Urner, K. Pagel, J. Dernedde, R. Haag, *Chem. Eur. J.* **2017**, *23*, 4849–4862.
[35] a) L. H. Urner, Y. B. Maier, R. Haag, K. Pagel, *J. Am. Soc. Mass Spectrom.* **2019**, *30*, 174–180; b) L. H. Urner, M. Schulze, Y. B. Maier, W. Hoffmann, S. Warnke, I. Liko, K. Folmert, C. Manz, C. V. Robinson, R. Haag, K. Pagel, *Chem. Sci.* **2020**, *11*, 3538–3546.
[36] L. H. Urner, E. Mohammadifar, K. Ludwig, D. Shutin, F. Fiorentino, I. Liko, F. G. Almeida, D. Kutifa, R. Haag, C. V. Robinson, *ACS Appl. Polym. Mater.* **2021**, *3*, 5903–5911.
[37] M. T. Agasid, L. Sørensen, L. H. Urner, J. Yan, C. V. Robinson, *J. Am. Chem. Soc.* **2021**, *143*, 4085–4089.
[38] C. A. Lutomski, T. J. El-Baba, J. D. Hinkle, I. Liko, J. L. Bennett, N. V. Kalmankar, A. Dolan, C. Kirschbaum, K. Greis, L. H. Urner, P. Kapoor, H.-Y. Yen, K. Pagel, C. Mullen, J. E. P. Syka, C. V. Robinson, *Angew. Chem. Int. Ed.* **2023**, e202305694.
[39] a) J.-F. Lutz, H. Schlaad, *Polymer* **2008**, *49*, 817–824; b) A. Sunder, R. Mülhaupt, R. Haag, H. Frey, *Adv. Mater.* **2000**, *12*, 235–239; c) Y. Gu, B. Liu, Q. Liu, Y. Hang, L. Wang, J. L. Brash, G. Chen, H. Chen, *ACS Appl. Mater. Interfaces* **2019**, *11*, 47720–47729.
[40] V. Percec, P. Leowanawat, H.-J. Sun, O. Kulikov, C. D. Nusbaum, T. M. Tran, A. Bertin, D. A. Wilson, M. Peterca, S. Zhang, N. P. Kamat, K. Vargo, D. Moock, E. D. Johnston, D. A. Hammer, D. J. Pochan, Y. Chen, Y. M. Chabre, T. C. Shiao, M. Bergeron-Brlek, S. André, R. Roy, H.-J. Gabius, P. A. Heiney, *J. Am. Chem. Soc.* **2013**, *135*, 9055–9077.
[41] M. S. Becherer, B. Schade, C. Böttcher, A. Hirsch, *Chem. Eur. J.* **2009**, *15*, 1637–1648.
[42] Z. Zheng, L. Yuan, J.-J. Hu, F. Xia, X. Lou, *Chem. Eur. J.* **2022**, *29*, e202203225.
[43] R. can der Meel, S. Chen, J. Zaifman, J. A. Kulkarni, X. R. S. Zhang, Y. K. Tam, M. B. Bally, R. M. Schiffelers, M. A. Ciufolini, P. R. Cullis, Y. Y. C. Tam, *Small* **2021**, *17*, 2103025.
[44] a) X. Pan, L. Sun, G. Sun, P. Rong, Y. Lu, J. Li, Y. Liu, C. Zhang, Z. Song, *Int. J. Food Eng.* **2020**, *16*; b) I. Rezić, *Monatsh. Chem.* **2011**, *142*, 1219–1225.
[45] a) T. Joseph Lin, in *Cosmetic Science and Technology* (Eds.: K. Sakamoto, R. Y. Lochhead, H. I. Maibach, Y. Yamashita), Elsevier, Amsterdam, **2017**, pp. 657–670; b) T. Vilgis, *Rep. Prog. Phys.* **2015**, *78*, 124602.
[46] a) J. Breibeck, A. Rompel, *BBA – General Subject* **2019**, *1863*, 437–455; b) J. N. Umbreit, J. L. Strominger, *PNAS* **1973**, *70*, 2997–3001.
[47] a) M. Rask-Andersen, M. S. Almén, H. B. Schiöth, *Nat. Rev. Drug Discovery* **2011**, *10*, 579–590; b) A. S. Hauser, M. M. Attwood, M. Rask-Andersen, H. B. Schiöth, D. E. Gloriam, *Nat. Rev. Drug Discovery* **2017**, *16*, 829–842.
[48] D. R. Storm, S. O. Field, J. Ryan, *J. Supramol. Struct.* **1976**, *4*, 221–231.
[49] T. Hasegawa, *Chem. Rec.* **2017**, *17*, 903–917.
[50] S. Alam, M. S. Algahtani, M. Zaki Ahmad, J. Ahmad, *Cosmetics* **2020**, *7*, 1–12.
[51] J. E. Zajic, W. Seffens, *in Biotechnology for the Oils and Fats Industry*, ed. C. Ratledge, P. Dawson, J. Rattray, American Oil Chemists' Society, USA, 1984, ch. 1921, pp. 1241–1256.
[52] J.-L. Salager, N. Marquez, A. Graciaa, J. Lachaise, *Langmuir* **2000**, *16*, 5534–5539.
[53] H. Heerklotz, J. Seelig, *Biophys. J.* **2000**, *78*, 2435–2440.
[54] I. A. Belousova, I. V. Kapitanov, A. E. Shumeiko, M. K. Turovskaya, T. M. Prokop'eva, A. F. Popov, *Theor. Exp. Chem.* **2008**, *44*, 93–100.
[55] P. A. Cornwell, *Int. J. Cosmet. Sci.* **2018**, *40*, 16–30.
[56] S. Mishra, V. K. Tyagi, *J. Oleo Sci.* **2007**, *56*, 269–276.
[57] J. R. G. Asio, J. S. Garcia, C. Antonatos, J. B. Sevilla-Nastor, L. C. Trinidad, *Emerg. Contam.* **2023**, *9*, 100205.
[58] S. Bergstrom, H. Theorell, H. Davide, *Arch. Biochem.* **1946**, *10*, 165–166.
[59] M.-F. Giraud, J. H. Naismith, *Curr. Opin. Struct. Biol.* **2000**, *10*, 687–696.
[60] K. Chiku, K. Tsunemi, M. Yamamoto, M. Ohnishi-Kameyama, M. Yoshida, T. Ishii, F. Taguchi, M. Iwaki, Y. Ichinose, H. Ono, *Biosci. Biotechnol. Biochem.* **2013**, *77*, 505–510.
[61] U. A. Ochsner, T. Hembach, A. Fiechter, in *Downstream Processing Biosurfactants Carotenoids*, Springer Berlin Heidelberg, Berlin, Heidelberg, **1996**, pp. 89–118.
[62] H. Chong, Q. Li, *Microb. Cell Fact.* **2017**, *16*, 137.
[63] A. M. Abdel-Mawgoud, F. Lépine, E. Déziel, *Appl. Microbiol. Biotechnol.* **2010**, *86*, 1323–1336.
[64] a) S. A. Adu, M. S. Twigg, P. J. Naughton, R. Marchant, I. M. Banat, *Pharmaceutica* **2022**, *14*, 360; b) E. Haba, A. Pinazo, O. Jauregui, M. J. Espuny, M. R. Infante, A. Manresa, *Biotechnol. Bioeng.* **2003**, *81*, 316–322; c) J. Crouzet, A. Arguelles-Arias, S. Dhondt-Cordelier, S. Cordelier, J. Pršić, G. Hoff, F. Mazeyrat-Gourbeyre, F. Baillieul, C. Clément, M. Ongena, S. Dorey, *Front. Bioeng. Biotechnol.* **2020**, *8*; d) M. Rosenberg, S. Kjelleberg, in *Advances in Microbial Ecology* (Ed.: K. C. Marshall), Springer US, Boston, MA, **1986**, pp. 353–393; e) S. Itoh, T. Suzuki, *Agr. Biol. Chem. Tokyo* **1972**, *36*, 2233–2235.
[65] I. N. A. Van Bogaert, J. Zhang, W. Soetaert, *Process Biochem.* **2011**, *46*, 821–833.
[66] a) M. Pala, J. Everaert, A. Ollivier, R. Raeymaekers, K. Quataert, S. L. K. W. Roelants, W. Soetaert, C. V. Stevens, *ACS Sustainable Chem. Eng.* **2022**, *10*, 12234–12244; b) E. I. P. Delbeke, M. Movsisyan, K. M. Van Geem, C. V. Stevens, *Green Chem.* **2016**, *18*, 76–104; c) Y. Peng, F. Totsingan, M. A. R. Meier, M. Steinmann, F. Wurm, A. Koh, R. A. Gross, *Eur. J. Lipid Sci. Technol.* **2015**, *117*, 217–228.
[67] P. Dhar, H. Havskjold, M. Thornhill, S. Roelants, W. Soetaert, H. R. Kota, I. Chernyshova, *J. Colloid Interface Sci.* **2021**, *585*, 386–399.
[68] A. A. Ba, J. Everaert, A. Poirier, P. Le Griel, W. Soetaert, S. L. K. W. Roelants, D. Hermida-Merino, C. V. Stevens, N. Baccile, *Green Chem.* **2020**, *22*, 8323–8336.
[69] a) H. Liu, H. Chen, G. Huang, Y. Yu, R. Li, S. Chen, *Chin. J. Chem. Eng.* **2023**, *59*, 270–278; b) D. W. G. Develter, L. M. L. Lauryssen, *Eur. J. Lipid Sci. Technol.* **2010**, *112*, 628–638; c) V. K. Gaur, R. K. Regar, N. Dhiman, K. Gautam, J. K. Srivastava, S. Patnaik, M. Kamthan, N. Manickam,

Bioresour. Technol. **2019**, *285*, 121314; d) S. Pal, N. Chatterjee, A. K. Das, D. J. McClements, P. Dhar, *Adv. Colloid Interface Sci.* **2023**, *313*, 102856.
[70] E. Moghimipour, S. Handali, *Annu. Res. Rev. Biol.* **2014**, *5*, 207–220.
[71] J. M. Augustin, V. Kuzina, S. B. Andersen, S. Bak, *Phytochemistry* **2011**, *72*, 435–457.
[72] Y. Yang, S. Laval, B. Yu, in *Adv. Carbohydr. Chem. Biochem., Vol. 71* (Ed.: D. Horton), Academic Press, **2014**, pp. 137–226.
[73] Ö. Güçlü-Üstündağ, G. Mazza, *Crit. Rev. Food Sci. Nutr.* **2007**, *47*, 231–258.
[74] R. Stanimirova, K. Marinova, S. Tcholakova, N. D. Denkov, S. Stoyanov, E. Pelan, *Langmuir* **2011**, *27*, 12486–12498.
[75] a) J.-Y. Kim, K.-W. Park, K.-D. Moon, M.-K. Lee, J. Choi, S.-T. Yee, K.-H. Shim, K.-I. Seo, *Food Chem. Toxicol.* **2008**, *46*, 3753–3758; b) M. Thakur, M. F. Melzig, H. Fuchs, A. Weng, *Botanics: Targets and Therapy* **2011**, *1*, 19–29.
[76] G. F. Kersten, T. Teerlink, H. J. Derks, A. J. Verkleij, T. L. van Wezel, D. J. Crommelin, E. C. Beuvery, *Infect. Immun.* **1988**, *56*, 432–438.
[77] S. D. McCalpin, T. Ravula, A. Ramamoorthy, *J. Phys. Chem. Lett.* **2022**, *13*, 1705–1712.
[78] A. Franzetti, I. Gandolfi, G. Bestetti, T. J. P. Smyth, I. M. Banat, *Eur. J. Lipid Sci. Technol.* **2010**, *112*, 617–627.
[79] G. J. F. Chittenden, *Carbohydr. Res.* **1969**, *9*, 323–326.
[80] M. R. Pratt, C. D. Leigh, C. R. Bertozzi, *Org. Lett.* **2003**, *5*, 3185–3188.
[81] K. A. L. De Smet, A. Weston, I. N. Brown, D. B. Young, B. D. Robertson, *Microbiology* **2000**, *146*, 199–208.
[82] A. D. Elbein, Y. T. Pan, I. Pastuszak, D. Carroll, *Glycobiology* **2003**, *13*, 17R–27R.
[83] H. Tao, Y. Fu, A. Thompson, S. C. Lee, N. Mahoney, R. C. Stevens, Q. Zhang, *Langmuir* **2012**, *28*, 11173–11181.
[84] S. Balamurugan, P. Lakshmanan, L. Piramuthu, D. Geetha, *Vietnam J. Chem.* **2023**, *61*, 135–146.
[85] a) Y. Takagi, M. Shimizu, Y. Morokuma, M. Miyaki, A. Kiba, K. Matsuo, K. Isoda, H. Mizutani, *Int. J. Cosmet. Sci.* **2014**, *36*, 305–311; b) A. O. Barel, M. Paye, H. I. Maibach, in *Handbook of Cosmetic Science and Technology* 3rd ed., CRC Press, New York, **2010**.
[86] a) A. R. Markande, D. Patel, S. Varjani, *Bioresour. Technol.* **2021**, *330*, 124963; b) P. K. S. M. Rahman, E. Gakpe, *Bio/Technology* **2008**, *7*, 360–370.
[87] M. Hajbabaie, M. R. Sabour, G. Dezvareh, H. Nematollahi, A. M. Hatami, *PREPRINT (Version 1) available at Research Square* **2022**.
[88] A. Stetsenko, A. Guskov, *Crystals* **2017**, *7*, 1–16.
[89] P. S. Chae, S. G. F. Rasmussen, R. R. Rana, K. Gotfryd, R. Chandra, M. A. Goren, A. C. Kruse, S. Nurva, C. J. Loland, Y. Pierre, D. Drew, J.-L. Popot, D. Picot, B. G. Fox, L. Guan, U. Gether, B. Byrne, B. Kobilka, S. H. Gellman, *Nat. Methods.* **2010**, *7*, 1003–1008.
[90] C. Breyton, W. Javed, A. Vermot, C.-A. Arnaud, C. Hajjar, J. Dupuy, I. Petit-Hartlein, A. Le Roy, A. Martel, M. Thépaut, C. Orelle, J.-M. Jault, F. Fieschi, L. Porcar, C. Ebel, *BBA-Biomembranes* **2019**, *1861*, 939–957.
[91] A. Sadaf, Y. Du, C. Santillan, J. S. Mortensen, I. Molist, A. P. Seven, P. Hariharan, G. Skiniotis, C. J. Loland, B. K. Kobilka, L. Guan, B. Byrne, P. S. Chae, *Chem. Sci.* **2017**, *8*, 8315–8324.
[92] a) A. Sadaf, M. Ramos, J. S. Mortensen, Y. Du, H. E. Bae, C. F. Munk, P. Hariharan, B. Byrne, B. K. Kobilka, C. J. Loland, L. Guan, P. S. Chae, *ACS Chem. Biol.* **2019**, *14*, 1717–1726; b) M. Ehsan, Y. Du, N. J. Scull, E. Tikhonova, J. Tarrasch, J. S. Mortensen, C. J. Loland, G. Skiniotis, L. Guan, B. Byrne, B. K. Kobilka, P. S. Chae, *J. Am. Chem. Soc.* **2016**, *138*, 3789–3796.
[93] H. Hussain, Y. Du, N. J. Scull, J. S. Mortensen, J. Tarrasch, H. E. Bae, C. J. Loland, B. Byrne, B. K. Kobilka, P. S. Chae, *Chem. Eur. J.* **2016**, *22*, 7068–7073.
[94] a) E. Reading, I. Liko, T. M. Allison, J. L. P. Benesch, A. Laganowsky, C. V. Robinson, *Angew. Chem. Int. Ed.* **2015**, *54*, 4577–4581; b) I. Liko, J. T. S. Hopper, T. M. Allison, J. L. P. Benesch, C. V. Robinson, *J. Am. Soc. Mass Spectrom.* **2016**, *27*, 1099–1104.
[95] A. Laganowsky, E. Reading, T. M. Allison, M. B. Ulmschneider, M. T. Degiacomi, A. J. Baldwin, C. V. Robinson, *Nature* **2014**, *510*, 172–175.
[96] H. Ilgü, J.-M. Jeckelmann, M. S. Gachet, R. Boggavarapu, Z. Ucurum, J. Gertsch, D. Fotiadis, *Biophys. J.* **2014**, *106*, 1660–1670.
[97] K.-A. Nguyen, M. Peuchmaur, S. Magnard, R. Haudecoeur, C. Boyère, S. Mounien, I. Benammar, V. Zampieri, S. Igonet, V. Chaptal, A. Jawhari, A. Boumendjel, P. Falson, *Angew. Chem. Int. Ed.* **2018**, *57*, 2948–2952.
[98] S. E. Denmark, Y. Fan, *J. Org. Chem.* **2005**, *70*, 9667–9676.
[99] K. Sriram, P. A. Insel, *Mol. Pharmacol.* **2018**, *93*, 251–258.
[100] D. Xue, T. Xu, H. Wang, M. Wu, Y. Yuan, W. Wang, Q. Tan, F. Zhao, F. Zhou, T. Hu, Z. Jiang, Z.-J. Liu, S. Zhao, D. Liu, K. Wüthrich, H. Tao, *Chem. Eur. J.* **2019**, *25*, 11635–11640.
[101] M. Stjerndahl, C. G. van Ginkel, K. Holmberg, *J. Surfactants Deterg.* **2003**, *6*, 319–324.
[102] a) M. Ehsan, S. Katsube, C. Cecchetti, Y. Du, J. S. Mortensen, H. Wang, A. Nygaard, L. Ghani, C. J. Loland, B. K. Kobilka, B. Byrne, L. Guan, P. S. Chae, *ACS Chem. Biol.* **2020**, *15*, 1697–1707; b) P. S. Chae, R. R. Rana, K. Gotfryd, S. G. F. Rasmussen, A. C. Kruse, K. H. Cho, S. Capaldi, E. Carlsson, B. Kobilka, C. J. Loland, U. Gether, S. Banerjee, B. Byrne, J. K. Lee, S. H. Gellman, *Chem. Commun.* **2013**, *49*, 2287–2289; c) K. H. Cho, H. E. Bae, M. Das, S. H. Gellman, P. S. Chae, *Chem. Asian J.* **2014**, *9*, 632–638; d) W.-X. Hong, K. A. Baker, X. Ma, R. C. Stevens, M. Yeager, Q. Zhang, *Langmuir* **2010**, *26*, 8690–8696.
[103] H. E. Bae, Y. Du, P. Hariharan, J. S. Mortensen, K. K. Kumar, B. Ha, M. Das, H. S. Lee, C. J. Loland, L. Guan, B. K. Kobilka, P. S. Chae, *Chem. Sci.* **2019**, *10*, 1107–1116.
[104] H. E. Bae, C. Cecchetti, Y. Du, S. Katsube, J. S. Mortensen, W. Huang, S. Rehan, H. J. Lee, C. J. Loland, L. Guan, B. K. Kobilka, B. Byrne, P. S. Chae, *Acta Biomater.* **2020**, *112*, 250–261.
[105] H. Hussain, T. Helton, Y. Du, J. S. Mortensen, P. Hariharan, M. Ehsan, B. Byrne, C. J. Loland, B. K. Kobilka, L. Guan, P. S. Chae, *Analyst* **2018**, *143*, 5702–5710.
[106] H. E. Bae, J. S. Mortensen, O. Ribeiro, Y. Du, M. Ehsan, B. K. Kobilka, C. J. Loland, B. Byrne, P. S. Chae, *Chem. Commun.* **2016**, *52*, 12104–12107.
[107] M. Ehsan, Y. Du, J. S. Mortensen, P. Hariharan, Q. Qu, L. Ghani, M. Das, A. Grethen, B. Byrne, G. Skiniotis, S. Keller, C. J. Loland, L. Guan, B. K. Kobilka, P. S. Chae, *Chem. Eur. J.* **2019**, *25*, 11545–11554.
[108] M. Ehsan, A. Kumar, J. S. Mortensen, Y. Du, P. Hariharan, K. K. Kumar, B. Ha, B. Byrne, L. Guan, B. K. Kobilka, C. J. Loland, P. S. Chae, *Chem. Asian J.* **2019**, *14*, 1926–1931.
[109] M. Das, H. E. Bae, H. J. Lee, J. Go, P. S. Chae, *Anal. Methods* **2015**, *7*, 5808–5813.
[110] P. S. Chae, A. C. Kruse, K. Gotfryd, R. R. Rana, K. H. Cho, S. G. F. Rasmussen, H. E. Bae, R. Chandra, U. Gether, L. Guan, B. K. Kobilka, C. J. Loland, B. Byrne, S. H. Gellman, *Chem. Eur. J.* **2013**, *19*, 15645–15651.
[111] P. S. Chae, K. H. Cho, M. J. Wander, H. E. Bae, S. H. Gellman, P. D. Laible, *Biochim. Biophys. Acta* **2014**, *1838*, 278–286.
[112] P. S. Chae, M. J. Wander, K. H. Cho, P. D. Laible, S. H. Gellman, *Mol. Syst. Biol.* **2013**, *9*, 626–629.
[113] C. S. Popeney, A. Setaro, R.-C. Mutihac, P. Blümmel, B. Trappmann, J. Vonneman, S. Reich, R. Haag, *ChemPhysChem* **2012**, *13*, 203–211.
[114] a) C. A. Hunter, J. K. M. Sanders, *J. Am. Chem. Soc.* **1990**, *112*, 5525–5534; b) O. Nachtigall, R. Lomoth, C. Dahlstrand, A. Lundstedt, A. Gogoll, M. J. Webb, H. Grennberg, *Eur. J. Org. Chem.* **2014**, *2014*, 966–972.
[115] M. Ehsan, Y. Du, I. Molist, A. B. Seven, P. Hariharan, J. S. Mortensen, L. Ghani, C. J. Loland, G. Skiniotis, L. Guan, B. Byrne, B. K. Kobilka, P. S. Chae, *Org. Biomol. Chem.* **2018**, *16*, 2489–2498.
[116] E. Panieri, K. Baralic, D. Djukic-Cosic, A. Buha Djordjevic, L. Saso, *Toxics* **2022**, *10*, 44.
[117] M. Bonato, F. Corrà, M. Bellio, L. Guidolin, L. Tallandini, P. Irato, G. Santovito, *Int. J. Environ. Res. Public Health.* **2020**, *17*, 8020.
[118] E. Chabaud, P. Barthélémy, N. Mora, J.-L. Popot, B. Pucci, *Biochimie* **1998**, *80*, 515–530.
[119] a) C. Bonnet, P. Guillet, S. Igonet, A. Meister, A. Marconnet, S. Keller, A. Jawhari, G. Durand, *J. Org. Chem.* **2019**, *84*, 10606–10614; b) M. Wehbie, K. K. Onyia, F. Mahler, A. Le Roy, A. Deletraz, I. Bouchemal, C. Vargas, J. O. Babalola, C. Breyton, C. Ebel, S. Keller, G. Durand, *Langmuir* **2021**, *37*, 2111–2122.
[120] a) M. Abla, G. Durand, C. Breyton, S. Raynal, C. Ebel, B. Pucci, *J. Fluorine Chem.* **2012**, *134*, 63–71; b) A. Polidori, S. Raynal, L.-A. Barret, M. Dahani, C. Barrot-Ivolot, C. Jungas, E. Frotscher, S. Keller, C. Ebel, C. Breyton, F. Bonneté, *New J. Chem.* **2016**, *40*, 5364–5378; c) E. Frotscher, B. Danielczak, C. Vargas, A. Meister, G. Durand, S. Keller, *Angew. Chem. Int. Ed.* **2015**, *54*, 5069–5073.
[121] a) F. Legrand, C. Breyton, P. Guillet, C. Ebel, G. Durand, *J. Org. Chem.* **2016**, *81*, 681–688; b) M. Abla, S. Unger, S. Keller, F. Bonneté, C. Ebel, B. Pucci, C. Breyton, G. Durand, *J. Colloid Interface Sci.* **2015**, *445*, 127–136.
[122] K.-H. Park, C. Berrier, F. Lebaupain, B. Pucci, J.-L. Popot, A. Ghazi, F. Zito, *Biochem. J.* **2007**, *403*, 183–187.
[123] F. Mahler, A. Meister, C. Vargas, G. Durand, S. Keller, *Small* **2021**, *17*, 2103603.

[124] M. Soulié, A. Deletraz, M. Wehbie, F. Mahler, I. Bouchemal, A. Le Roy, I. Petit-Härtlein, S. Keller, A. Meister, E. Pebay-Peyroula, C. Breyton, C. Ebel, G. Durand, *Biochimie* **2023**, *205*, 40–52.
[125] E. S. Baker, D. R. U. Knappe, *Anal. Bioanal. Chem.* **2022**, *414*, 1187–1188.
[126] B. J. Belin, N. Busset, E. Giraud, A. Molinaro, A. Silipo, D. K. Newman, *Nat. Rev. Microbiol.* **2018**, *16*, 304–315.
[127] a) A. F. Hofmann, D. M. Small, *Annu. Rev. Med.* **1967**, *18*, 333–376; b) B. Staels, V. A. Fonseca, *Diabetes Care* **2009**, *32*, S237-S245; c) J. Y. L. Chiang, *J. Lipid Res.* **2009**, *50*, 1955–1966.
[128] a) Y. Kumar, R. Yadav, A. Bhatia, *Future Virol.* **2020**, *15*, 779–782; b) E. Moghimipour, A. Ameri, S. Handali, *Molecules* **2015**, *20*, 14451–14473.
[129] S. C. Howell, R. Mittal, L. Huang, B. Travis, R. M. Breyer, C. R. Sanders, *Biochem.* **2010**, *49*, 9572–9583.
[130] a) M. Ehsan, M. Das, V. Stern, Y. Du, J. S. Mortensen, P. Hariharan, B. Byrne, C. J. Loland, B. K. Kobilka, L. Guan, P. S. Chae, *ChemBioChem* **2018**, *19*, 1433–1443; b) P. S. Chae, S. G. F. Rasmussen, R. R. Rana, K. Gotfryd, A. C. Kruse, A. Manglik, K. H. Cho, S. Nurva, U. Gether, L. Guan, C. J. Loland, B. Byrne, B. K. Kobilka, S. H. Gellman, *Chem. Eur. J.* **2012**, *18*, 9485–9490.
[131] P. S. Chae, K. Gotfryd, J. Pacyna, L. J. W. Miercke, S. G. F. Rasmussen, R. A. Robbins, R. R. Rana, C. J. Loland, B. Kobilka, R. Stroud, B. Byrne, U. Gether, S. H. Gellman, *J. Am. Chem. Soc.* **2010**, *132*, 16750–16752.
[132] Y. Cheng, D. M. Ho, C. R. Gottlieb, D. Kahne, M. A. Bruck, *J. Am. Chem. Soc.* **1992**, *114*, 7319–7320.
[133] a) S. C. Lee, B. C. Bennett, W.-X. Hong, Y. Fu, K. A. Baker, J. Marcoux, C. V. Robinson, A. B. Ward, J. R. Halpert, R. C. Stevens, C. D. Stout, M. J. Yeager, Q. Zhang, *PNAS* **2013**, *110*, E1203-E1211; b) Q. Zhang, X. Ma, A. Ward, W.-X. Hong, V.-P. Jaakola, R. C. Stevens, M. G. Finn, G. Chang, *Angew. Chem. Int. Ed.* **2007**, *46*, 7023–7025.
[134] L. M. Hjelmeland, *PNAS* **1980**, *77*, 6368–6370.
[135] D. Cornut, M. Soulié, A. Moreno, G. N. M. Boussambe, M. Damian, S. Igonet, P. Guillet, J.-L. Banères, G. Durand, *Biochimie* **2023**, *205*, 27–39.
[136] P. S. Chae, H. E. Bae, M. Ehsan, H. Hussain, J. W. Kim, *Org. Biomol. Chem.* **2014**, *12*, 8480–8487.
[137] R. C. Fort, P. v R Schleyer, *Chem. Rev.* **1964**, *64*, 277–300.
[138] P. S. Chae, H. E. Bae, M. Das, *Chem. Commun.* **2014**, *50*, 12300–12303.
[139] H. Zhu, J. Guo, C. Yang, S. Liu, Y. Cui, X. Zhong, *Synth. Commun.* **2013**, *43*, 1161–1167.
[140] B. L. May, P. Clements, J. Tsanaktsidis, C. J. Easton, S. F. Lincoln, *J. Chem. Soc. Perkin Trans. 1* **2000**, 463–469.
[141] A. C. Rodrigo, S. Malhotra, C. Böttcher, M. Adeli, R. Haag, *RSC Adv.* **2014**, *4*, 61656–61659.
[142] M. Kellermann, W. Bauer, A. Hirsch, B. Schade, K. Ludwig, C. Böttcher, *Angew. Chem. Int. Ed.* **2004**, *43*, 2959–2962.
[143] a) H. Hussain, Y. Du, E. Tikhonova, J. S. Mortensen, O. Ribeiro, C. Santillan, M. Das, M. Ehsan, C. J. Loland, L. Guan, B. K. Kobilka, B. Byrne, P. S. Chae, *Chem. Eur. J.* **2017**, *23*, 6724–6729; b) J. Dauvergne, E. M. Desuzinges, C. Faugier, S. Igonet, M. Soulié, E. Grousson, D. Cornut, F. Bonneté, G. Durand, E. Dejean, A. Jawhari, *ChemistrySelect* **2019**, *4*, 5535–5539.
[144] a) M. Srujan, V. Chandrashekhar, R. C. Reddy, R. Prabhakar, B. Sreedhar, A. Chaudhuri, *Biomaterials* **2011**, *32*, 5231–5240; b) M. L. Hupfer, M. Kaufmann, L. Rousille, J. Preiß, D. Weiß, K. Hinrichs, V. Deckert, B. Dietzek, R. Beckert, M. Presselt, *Langmuir* **2019**, *35*, 2561–2570; c) A. K. Singh, B. Schade, M. Rosati, R. Rashmi, V. Dichiarante, G. Cavallo, P. Metrangolo, R. Haag, *Macromol. Biosci.* **2022**, *22*, 2200108; d) W. Luo, M. Yang, Y. Zhao, H. Wang, X. Yang, W. Zhang, F. Thao, S. Zhao, H. Tao, *Chem. Eur. J.* **2022**, *28*, e202202242; e) L. H. Urner, B. Schade, M. Schulze, K. Folmert, R. Haag, K. Pagel, *ChemPhysChem* **2019**, *20*, 1690–1697.
[145] a) S. Chen, R. Costil, F. K.-C. Leung, B. L. Feringa, *Angew. Chem. Int. Ed.* **2021**, *60*, 11604–11627; b) S. Hecht, *Small* **2005**, *1*, 26–29.
[146] a) M. Ehsan, H. Wang, S. Katsube, C. F. Munk, Y. Du, T. Youn, S. Yoon, B. Byrne, C. J. Loland, L. Guan, B. K. Kobilka, P. S. Chae, *ChemBioChem* **2022**, *23*, e202200027; b) A. Sadaf, J. S. Mortensen, S. Capaldi, E. Tikhonova, P. Hariharan, O. Ribeiro, C. J. Loland, L. Guan, B. Byrne, P. S. Chae, *Chem. Sci.* **2016**, *7*, 1933–1939.
[147] a) A. Mittal, A. K. Singh, A. Kumar, Parmanand, K. Achazi, R. Haag, S. K. Sharma, *Polym. Adv. Technol.* **2020**, *31*, 1208–1217; b) Rashmi, A. K. Singh, K. Achazi, S. Ehrmann, C. Böttcher, R. Haag, S. K. Sharma, *Polym. Chem.* **2020**, *11*, 6772–6782.
[148] Rashmi, F. Zabihi, A. K. Singh, K. Achazi, B. Schade, S. Hedtrich, R. Haag, S. K. Sharma, *Int. J. Pharm.* **2020**, *580*, 119212.
[149] L. Ghani, S. Kim, H. Wang, H. S. Lee, J. S. Mortensen, D. Katsube, Y. Du, A. Sadaf, W. Ahmed, B. Byrne, L. Guan, C. J. Loland, B. K. Kobilka, W. Im, P. S. Chae, *Chem. Eur. J.* **2022**, *28*, e202200116.
[150] a) K. H. Cho, B. Byrne, P. S. Chae, *ChemBioChem* **2013**, *14*, 452–455; b) T. Guitton-Spassky, F. Junge, A. K. Singh, B. Schade, K. Achazi, M. Maglione, S. Sigrist, R. Rashmi, R. Haag, *Nanoscale* **2023**, *15*, 7781–7791.
[151] M. Ehsan, H. Wang, C. Cecchetti, J. S. Mortensen, Y. Du, P. Hariharan, A. Nygaard, H. J. Lee, L. Ghani, L. Guan, C. J. Loland, B. Byrne, B. K. Kobilka, P. S. Chae, *ACS Chem. Biol.* **2021**, *16*, 1779–1790.
[152] M. Das, Y. Du, J. S. Mortensen, P. Hariharan, H. S. Lee, B. Byrne, C. J. Loland, L. Guan, B. K. Kobilka, P. S. Chae, *ChemBioChem* **2018**, *19*, 2225–2232.
[153] A. Setaro, C. S. Popeney, B. Trappmann, V. Datsyuk, R. Haag, S. Reich, *Chem. Phys. Lett.* **2010**, *493*, 147–150.
[154] S. Prasad, K. Achazi, B. Schade, R. Haag, S. K. Sharma, *Macromol. Biosci.* **2018**, *18*, 1800019.
[155] a) N. Agouram, E. M. El Hadrami, A. Bentama, *Molecules* **2021**, *26*, 2937; b) V. Haridas, S. Sahu, P. P. P. Kumar, A. R. Sapala, *RSC Adv.* **2012**, *2*, 12594–12605.
[156] L.-M. Rečnik, W. Kandioller, T. L. Mindt, *Molecules* **2020**, *25*, 3576.
[157] a) Z. Xu, S.-J. Zhao, Y. Liu, *Eur. J. Med. Chem.* **2019**, *183*, 111700; b) A. K. Kabi, S. Sravani, R. Gujjarappa, A. Garg, N. Vodnala, U. Tyagi, D. Kaldhi, V. Singh, S. Gupta, C. C. Malakar, in *Nanostructured Biomaterials: Basic Structures and Applications* (Ed.: B. P. Swain), Springer Singapore, Singapore, **2022**, pp. 401–423; c) M. R. E. S. Aly, H. A. Saad, M. A. M. Mohamed, *Biorg. Med. Chem. Lett.* **2015**, *25*, 2824–2830.
[158] a) L.-G. Qiu, A.-J. Xie, Y.-H. Shen, *Colloid Polym. Sci.* **2005**, *283*, 1343–1348; b) V. D. Sharma, M. A. Ilies, *Med. Res. Rev.* **2014**, *34*, 1–44.
[159] H. Tao, S. C. Lee, A. Moeller, R. S. Roy, F. Y. Siu, J. Zimmermann, R. C. Stevens, C. S. Potter, B. Carragher, Q. Zhang, *Nat. Methods.* **2013**, *10*, 759–761.
[160] C.-L. McGregor, L. Chen, N. C. Pomroy, P. Hwang, S. Go, A. Chakrabartty, G. G. Privé, *Nat. Biotechnol.* **2003**, *21*, 171–176.
[161] a) J. M. Fuller, K. R. Raghupathi, R. R. Ramireddy, A. V. Subrahmanyam, V. Yesilyurt, S. Thayumanavan, *J. Am. Chem. Soc.* **2013**, *135*, 8947–8954; b) M. Ballauff, *Angew. Chem. Int. Ed.* **1989**, *28*, 253–267.
[162] J. N. Israelachvili, in *Intermolecular and Surface Forces (Third Edition)* (Ed.: J. N. Israelachvili), Academic Press, San Diego, **2011**, pp. 503–534.
[163] B. Schade, A. K. Singh, V. Wycisk, J. L. Cuellar-Camacho, H. von Berlepsch, R. Haag, C. Böttcher, *Chem. Eur. J.* **2020**, *26*, 6919–6934.
[164] a) L. Ghani, X. Zhang, C. F. Munk, P. Hariharan, B. Lan, H. S. Yun, B. Byrne, L. Guan, C. J. Loland, X. Liu, P. S. Chae, *Bioconjugate Chem.* **2023**, *34*, 739–747; b) L. Ghani, C. F. Munk, X. Zhang, S. Katsube, Y. Du, C. Cecchetti, W. Huang, H. E. Bae, S. Saouros, M. Ehsan, L. Guan, X. Liu, C. J. Loland, B. K. Kobilka, B. Byrne, P. S. Chae, *J. Am. Chem. Soc.* **2019**, *141*, 19677–19687.
[165] M. Das, Y. Du, O. Ribeiro, P. Hariharan, J. S. Mortensen, D. Patra, G. Skiniotis, C. J. Loland, L. Guan, B. K. Kobilka, B. Byrne, P. S. Chae, *J. Am. Chem. Soc.* **2017**, *139*, 3072–3081.
[166] M. Das, F. Mahler, P. Hariharan, H. Wang, Y. Du, J. S. Mortensen, E. P. Patallo, L. Ghani, D. Glück, H. J. Lee, B. Byrne, C. J. Loland, L. Guan, B. K. Kobilka, S. Keller, P. S. Chae, *J. Am. Chem. Soc.* **2020**, *142*, 21382–21392.
[167] K. H. Cho, Y. Du, N. J. Scull, P. Hariharan, K. Gotfryd, C. J. Loland, L. Guan, B. Byrne, B. K. Kobilka, P. S. Chae, *ACS Chem. Biol.* **2015**, *21*.
[168] A. Sadaf, S. Kim, H. E. Bae, H. Wang, A. Nygaard, Y. Uegaki, Y. Du, C. F. Munk, S. Katsube, J. Bae, C. W. Choi, H.-J. Choi, B. Byrne, S. H. Gellman, L. Guan, C. J. Loland, B. K. Kobilka, W. Im, P. S. Chae, *Acta Biomater.* **2021**, *128*, 393–407.
[169] T. Youn, S. Yoon, B. Byrne, P. S. Chae, *ChemBioChem* **2022**, *23*, e2022002.
[170] L. H. Urner, B. N. S. Thota, O. Nachtigall, S. Warnke, G. von Helden, R. Haag, K. Pagel, *Chem. Commun.* **2015**, *51*, 8801–8804.
[171] a) D. Verma, Rashmi, K. Achazi, A. Kumar Singh, B. Schade, R. Haag, S. K. Sharma, *Polym. Adv. Technol.* **2022**, *33*, 2601–2609; b) S. Prasad, K. Achazi, C. Böttcher, R. Haag, S. K. Sharma, *RSC Adv.* **2017**, *7*, 22121–22132.
[172] D. Xue, J. Wang, X. Song, W. Wang, T. Hu, L. Ye, Y. Liu, Q. Zhou, F. Zhou, Z.-X. Jiang, Z.-J. Liu, H. Tao, *Langmuir* **2019**, *35*, 4319–4327.
[173] a) A. K. Ghosh, M. Brindisi, *J. Med. Chem.* **2015**, *58*, 2895–2940; b) A. Matošević, A. Bosak, *Archives of Industrial Hygiene and Toxicology* **2020**, *71*, 285–299.
[174] a) D. Liu, J. Hu, W. Qiao, Z. Li, S. Zhang, L. Cheng, *Biorg. Med. Chem. Lett.* **2005**, *15*, 3147–3150; b) D. Liu, i. Hu, W. Qiao, Z. Li, S. Zhan, L. Cheng, *Lipids* **2005**, *40*, 839–848.

[175] A. A. Williams, E. W. Sugandhi, R. V. Macri, J. O. Falkinham III, R. D. Gandour, *J. Antimicrob. Chemother.* **2007**, *59*, 451–458.
[176] Y.-H. Chang, Z. R. Gregorich, A. J. Chen, L. Hwang, H. Guner, D. Yu, J. Zhang, Y. Ge, *J. Proteome Res.* **2015**, *14*, 1587–1599.
[177] T. W. G. P. G. M. Wuts, in *Protective Groups in Organic Synthesis*, **1999**, pp. 293–368.
[178] a) Y.-Q. Yu, M. Gilar, P. J. Lee, E. S. P. Bouvier, J. C. Gebler, *Anal. Chem.* **2003**, *75*, 6023–6028; b) S. M. Moore, S. M. Hess, J. W. Jorgenson, *J. Proteome Res.* **2016**, *15*, 1243–1252; c) Y.-Q. Yu, M. Gilar, J. C. Gebler, *Rapid Commun. Mass Spectrom.* **2004**, *18*, 711–715.
[179] L. Liu, Z. Zhu, F. Zhou, D. Xue, T. Hu, W. Luo, Y. Qiu, D. Wu, F. Zhao, Z. Le, H. Tao, *ACS Omega* **2021**, *6*, 21087–21093.
[180] G. S. Hartley, *Nature* **1937**, *140*, 281–281.
[181] a) A. A. Beharry, G. A. Woolley, *Chem. Soc. Rev.* **2011**, *40*, 4422–4437; b) M. Di Martino, L. Sessa, M. Di Matteo, B. Panunzi, S. Piotto, S. Concilio, *Molecules* **2022**, *27*, 5643; c) Z. Mahimwalla, K. G. Yager, J.-i. Mamiya, A. Shishido, A. Priimagi, C. J. Barrett, *Polym. Bull.* **2012**, *69*, 967–1006; d) F. A. Jerca, V. V. Jerca, R. Hoogenboom, *Nat. Chem. Rev.* **2022**, *6*, 51–69.
[182] K. A. Brown, B. Chen, T. M. Guardado-Alvarez, Z. Lin, L. Hwang, S. Ayaz-Guner, S. Jin, Y. Ge, *Nat. Methods* **2019**, *16*, 417–420.
[183] K. A. Brown, M. K. Gugger, D. S. Roberts, D. Moreno, P. S. Chae, Y. Ge, S. Jin, *Langmuir* **2023**, *39*, 1465–1473.

Manuscript received: July 24, 2023
Revised manuscript received: September 4, 2023
Accepted manuscript online: September 5, 2023
Version of record online: September 28, 2023