ADVANCED FUNCTIONAL MATERIALS
www.afm-journal.de

Bioconjugation in Materials Science

Christin Bednarek, Ute Schepers, Franziska Thomas,* and Stefan Bräse**

With the advent of bioconjugation chemistry in the last two decades, highlighted by the Nobel Prize 2022, the quest for possible novel applications has been greatly intensified, broadening the prospects of these mostly simple, specific, and high-yield reactions. The advancement of bioconjugation methods is anticipated to expand the scope of bioinstructive and bioadaptive materials science in the future. This perspective article will discuss the reactions developed in this research area over the last 10 years for coupling various biological entities such as polysaccharides, oligonucleotides, peptides, and proteins. Building on this, the impact of bioconjugation reactions in materials science and 3D printing, including their challenges and requirements is shown. Established procedures for modifying molecular structures such as Covalent and Metal Organic Frameworks (COF/MOF) or hybrid materials for biomedical applications and the scope for future research and optimization will be presented.

1. Introduction

Bioconjugation of materials was originally developed by biochemists and has been studied for decades exclusively for biochemical and biomedical applications. At that time, the most common methods of bioconjugation to materials were coupling via a crosslinking agent, such as a bifunctional linker, or enzyme-mediated conjugation with, e.g., proteases or glycosyltransferases. However, very easy-to-use bioorthogonal bioconjugation reactions,[1] such as click chemistry or light-induced reactions, have enormously expanded the applications and the utility of biofunctionalized materials in many emerging areas of materials science, e.g., 3D printing, tissue engineering, sensor design and even the biocatalyzed production of chemical building blocks. In the broadest sense, the term "bioconjugation" refers to a collection of chemical, often but not inevitably bioorthogonal,[1] strategies for ligating two molecules, one of which usually represents a biomolecule, such as a protein, a peptide, an oligosaccharide, or an oligonucleotide. The resulting constructs combine the properties and characteristics of both starting materials (**Scheme 1**). Ideally, a bioconjugation reaction is highly biocompatible, proceeds fast, and is selective without side reactions. The power of these techniques enables us to address new research areas, e.g., expanding our understanding of biological pathways by profiling biomolecules and cellular events, developing potential medical (imaging) agents, or creating new hybrid materials for materials sciences. Beyond that, there is also the possibility of using bioconjugation approaches for 3D printing.

In this perspective article, we focus exclusively on reactions in aqueous media with special emphasis on photo-bioconjugation and methods suitable for light-induced 3D printing. Knowing the functionalities required for a particular bioconjugation, the simplicity and other advantages of the reaction can be easily transferred to materials science. The molecular scaffolds that can be considered for biofunctionalization are very diverse, ranging from classical inorganic nanomaterials to framework structures such as COF or MOF, polymers and surfaces optimized for cell adhesion, materials for medical applications, (semi)synthetic biomaterials and biopolymers such as synthetic peptides,[2,3,4] proteins, glycans resembling extracellular matrix components or nucleic acids.[5] Using biocompatible reactions, it is possible to interfere with biological materials during the bioconjugation process, as is the case with 3D bioprinting in the presence of cells.

C. Bednarek, S. Bräse
Karlsruhe Institute of Technology (KIT)
Institute of Organic Chemistry (IOC)
Fritz-Haber-Weg 6, 76131 Karlsruhe, Germany
E-mail: braese@kit.edu

C. Bednarek, U. Schepers, F. Thomas, S. Bräse
3DMM2O–Cluster of Excellence (EXC-2082/1–390761711)
KIT, 76131 Karlsruhe, Germany
E-mail: ute.schepers@kit.edu; franziska.thomas@oci.uni-heidelberg.de

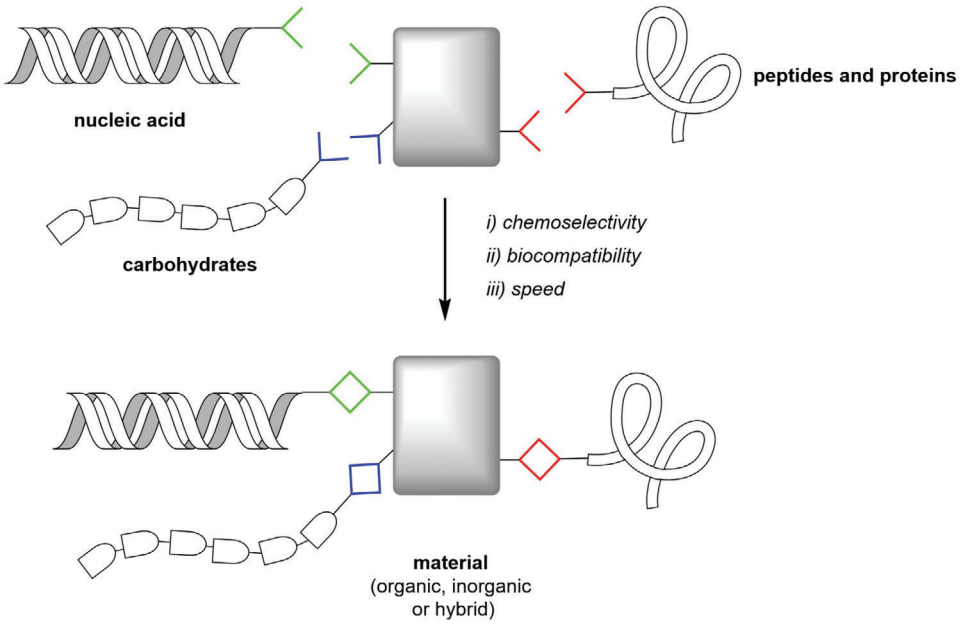
U. Schepers
Karlsruhe Institute of Technology (KIT)
Institute of Functional Interfaces (IFG)
Hermann-von-Helmholtz-Platz 1, 76344 Eggenstein-Leopoldshafen, Germany

F. Thomas
Heidelberg University
Institute of Organic Chemistry (OCI)
Im Neuenheimer Feld 270, 69120 Heidelberg, Germany

S. Bräse
Karlsruhe Institute of Technology (KIT)
Institute of Biological and Chemical Systems–Functional Molecular Systems (IBCS-FMS)
Hermann-von-Helmholtz-Platz 1, 76344 Eggenstein-Leopoldshafen, Germany

The ORCID identification number(s) for the author(s) of this article can be found under https://doi.org/10.1002/adfm.202303613

DOI: 10.1002/adfm.202303613



Scheme 1. Schematic illustration of biofunctionalization of materials by bioconjugation.

2. Challenges (and Opportunities)

Due to heterogeneity, the conjugation of biomolecules to an artificial material is more challenging than bioconjugation to dissolved components. One challenge is the selectivity of the reaction when more than one functionality is present in the biomolecule, e.g., OH groups in carbohydrates. In addition, many materials are inherently hydrophobic, unlike the biomolecules with which they are reacted. This requires special considerations in the bioconjugation techniques since they are usually carried out in aqueous media. Also, certain biomolecules – proteins or nucleic acids – adopt a three-dimensional structure crucial for their function. Immobilization of a material, especially if it is a hydrophobic surface, can lead to its denaturation and loss of biological function. A particular challenge, but also an opportunity, is using light-induced bioconjugation methods. These offer high spatial and temporal control but can be harmful to biomolecules due to the high intensity of the light sources and the fact that most of these bioconjugations are radical reactions.

In recent decades, many different bioconjugation methods have been used to couple biologically active components, most of which are also derived from natural sources, onto materials. Bioconjugates include peptides (e.g., RGD peptides), proteins, antibodies, enzymes, lectins, carbohydrates, extracellular components, lipids, nucleic acids, and small metabolic molecules.[6] They all require a different bioconjugation strategy.

Smaller or larger peptides and proteins are less stable concerning epimerization or denaturation but offer ample bioconjugation opportunities depending on the alpha carbon side chain. Natural amino acids contain carboxylates, aliphatic and phenolic hydroxyl groups, primary amino groups, thiols, thioethers, or sometimes selenols found in some bacteria. The inherent nucleophilicity of thiols or selenols makes cysteine or selenocysteine appealing targets for chemoselective bioconjugation because they are site-specific. Besides the natural amino acids, artificial amino acids with functional groups such as azides, tetrazines or alkynes or alkenes, halides, or maleimides can be introduced by either modifying the genetic code in protein expression[7] or by chemical or semisynthetic means. However, the risk of denaturing the defined three-dimensional structure of proteins and peptides when immobilized on a material remains, and the lack of methods for the structural characterization of on-surface proteins is a significant problem.

Unlike peptides, unprotected linear, cyclic, and origami-type nucleic acids are negatively charged and very polar and have fewer addressable groups for direct functionalization. However, chemical oligonucleotide synthesis is very efficient, and introducing modified nucleotides or modifications of the terminibearing azide, alkene, or alkyne functions is highly feasible.[8] The highly selective base pairing can then be exploited to, e.g., bind larger nucleotides. Other obstacles that need to be considered are the stability of nucleic acids and the possible loss of three-dimensional structure when conjugated into a material (vide supra). Stability depends on the type of nucleic acid: While DNA has good stability under many conditions, RNA is very sensitive to acids and bases, and ribonucleases are ubiquitous. To increase stability chemically, nucleic acid analogs such as peptide or locked nucleic acids could be considered.[9]

The bioconjugation of natural and artificial monomeric, oligomeric, and polymeric carbohydrates lacks chemoselectivity and efficiency.[10] The variety of functional groups that can be addressed in bioconjugation reactions is small. In addition to the obligatory hydroxyl groups, which are sometimes well-discriminated, *N*-acetyl groups and carboxylates can be found. The stereochemistry also has a decisive influence on the reactivity of the functional groups. For example, an equatorial OH group reacts faster in a Michael addition than an axial OH group. However, in the last decade, Carolyn Bertozzi and colleagues

have shown in detail that the functionalization of carbohydrate monomers with azide and alkyne functional groups does not affect the metabolization of the precursor carbohydrates to the oligosaccharide chain.[11] Azide/alkyne functionalized oligosaccharides are presented at the cell surface for biorthogonal bioconjugation. These oligosaccharides can even be harvested for further bioconjugation to materials.[12] The synthesis of oligosaccharides has improved significantly in the last decade with the advent of efficient chemical and enzymatic methods, making such modified carbohydrates readily available.[13]

Many challenges and opportunities can be seen in the next 5 to 10 years. In the supplementary information, most of the recent bioconjugation methods are listed. In this regard, we look very positively to a new chapter of the interaction between materials sciences and life sciences.

3. Bioconjugation Methods

This chapter provides a condensed overview of the main bioconjugation methods used in both materials design and biological applications. First, thermal bioconjugation reactions are reviewed from the perspective of long-established methods and recent trends. Light-driven bioconjugations, which are particularly interesting to materials scientists because they allow spatiotemporal control of polymerization or functionalization, are then discussed. Since most of the light-driven bioconjugations have been developed from the corresponding thermally-driven reactions, only the first subchapter contains more detailed mechanistic considerations, as these largely apply to the corresponding light-driven reactions in the second subchapter.

3.1. Thermal bioconjugation reactions

3.1.1. Thiol-Mediated Bioconjugation

Thiols are very good nucleophiles and therefore react readily with good reaction kinetics in nucleophilic substitutions and additions.[14] Considering the low abundance of the thiol-containing amino acid cysteine in natural proteins (1.9%) and the absence of thiols in nucleic acids and carbohydrates, thiol-mediated bioconjugation methods appear very attractive for the biofunctionalization of materials and biomolecules.[14] Over the years, several chemical methods with high chemoselectivity have been developed: Native chemical ligation – the reaction of a thioester fragment with a peptide or protein bearing an N-terminal cysteine residue, producing a native peptide bond in the condensation product[15] – or substitution to alkyl halides or perfluoroarenes. [16] Although limited by the low stability of thiol-succinimide adducts to hydrolysis or thiol exchange, the Michael addition of thiols to maleimides (**1**) is the most widely used because of the excellent reaction kinetics and easy availability of maleimide reagents.[17]

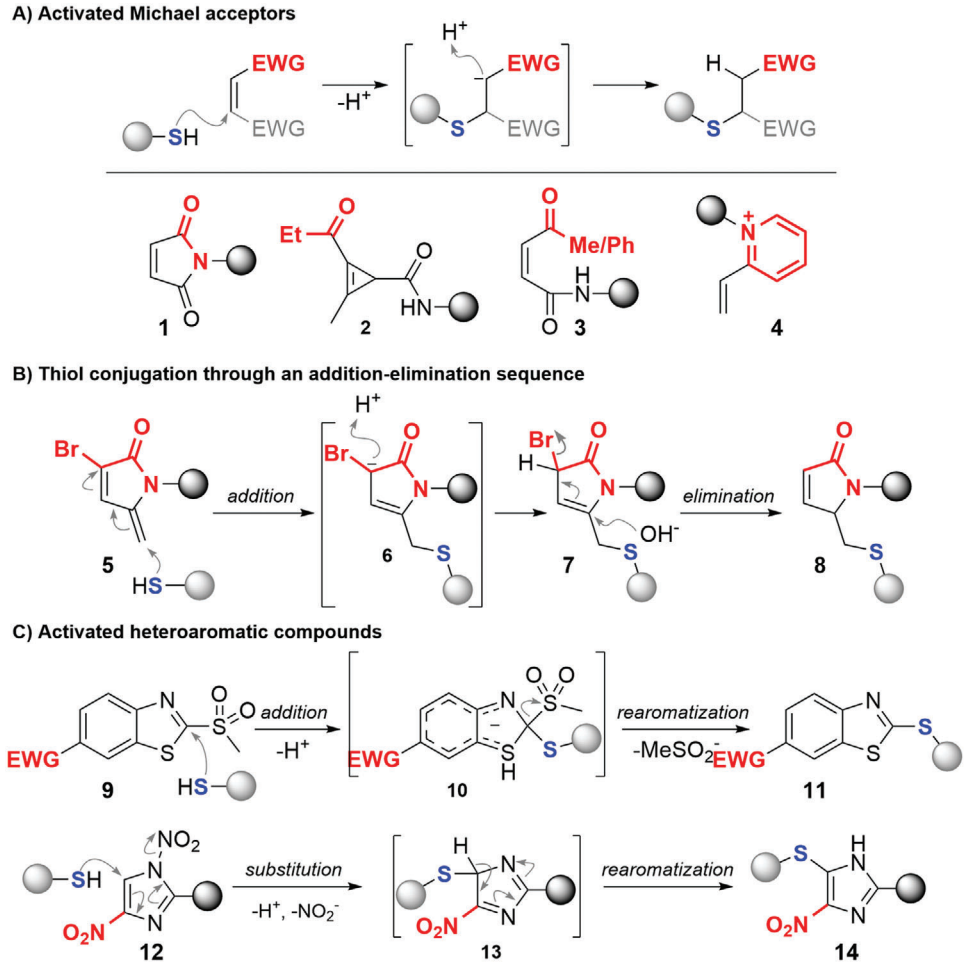
In recent years, several reagents have been developed that retain the chemoselectivity combined with the favorable reaction kinetics of maleimide-thiol chemistry but provide stable conjugation products.[18] Many of these methods are still based on Michael addition but on Michael acceptors that release strain after the addition reaction, e.g., cyclopropenyl ketone **2**,[19] and/or exhibit high electrophilicity due to electron-withdrawing groups such as additional carbonyl groups or pyridinium residues that stabilize the negatively charged addition intermediate (**Scheme 2**A).[20] Interestingly, maleimides are activated Michael acceptors; however, linear representatives such as carbonyl acrylamide **3** show similar reactivity but provide stable conjugation products. Another way to activate the Michael acceptor is to introduce a leaving group that allows addition-elimination sequences (Scheme 2B). One representative is the 3-bromo-5-methylene-pyrrolone reagent **5**, which enables bioconjugation in a 1,6-thio Michael addition.[21] The anionic intermediate **6** is stabilized by both the carbonyl and the bromine. The subsequent S_N2 prime reaction with hydroxide yields the monofunctionalized conjugate **8**, which can be converted to dual-functionalized conjugates by a second, albeit much slower, Michael addition.[22]

In addition to activated Michael acceptors, activated heteroaromatic compounds such as **9** or **12** have been used for fast bioconjugation, although not yet in the context of the biofunctionalization of materials. Activation occurs through electron-withdrawing substituents that lead to electron deficiency of the aromatic system, allowing the addition of the thiol compound to form an anionic σ-complex (Scheme 2C). A leaving group, for example, the sulfone in **9** or the 4-nitro group in **12,** enables rapid rearomatization, the driving force of the reaction.[23] Further strategies of fast thiol-based bioconjugation include using hypervalent iodine or α-cation activated reagents.[24] For a detailed description of the progress in this field, we refer to the excellent reviews by Ochtrop and Hackenberger or Chen and Gao.[18]

3.1.2. Oxime ligation and Staudinger Ligation

Oxime ligation is one of the longest-established biorthogonal ligation methods.[25] Briefly, it involves highly chemoselective oxime formation from the condensation of aldehydes or ketones **15** and alkoxy amines **16**, with water being the only side product (**Scheme 3**A). The conjugation reaction is relatively slow at physiological pH but accelerated at pH 4 to 6 or by adding aromatic amines, forming a reactive Schiff base as an intermediate.[26] Compared to imines, oximes are more hydrolytically stable but still show reversibility under physiological and mildly acidic conditions.[27] For some applications, this may be desirable, especially to induce environment responsiveness or self-healing properties, as shown for oxime-crosslinked polyethylene glycol (PEG)-based and acrylamide-based hydrogels (vide infra).[28]

Staudinger ligation is probably one of the most widely used bioconjugation methods with various reported applications in protein synthesis, protein or nucleic acid labeling, and biofunctionalization in vitro, in vivo, and in rodent models.[5g] Chemically, Staudinger ligation is a phosphine-azide-based transformation between an azide fragment (**21**) and another fragment with an ortho-triarylphosphine-substituted benzoic acid methyl ester (**20**, Scheme 3B).[11] As with many other ligation methods, a capture step occurs first, followed by a rearrangement. The azide fragment is reacted with the phosphine moiety to form an iminophosphorane intermediate **22**, which subsequently converts to the amide ligation product **24** after



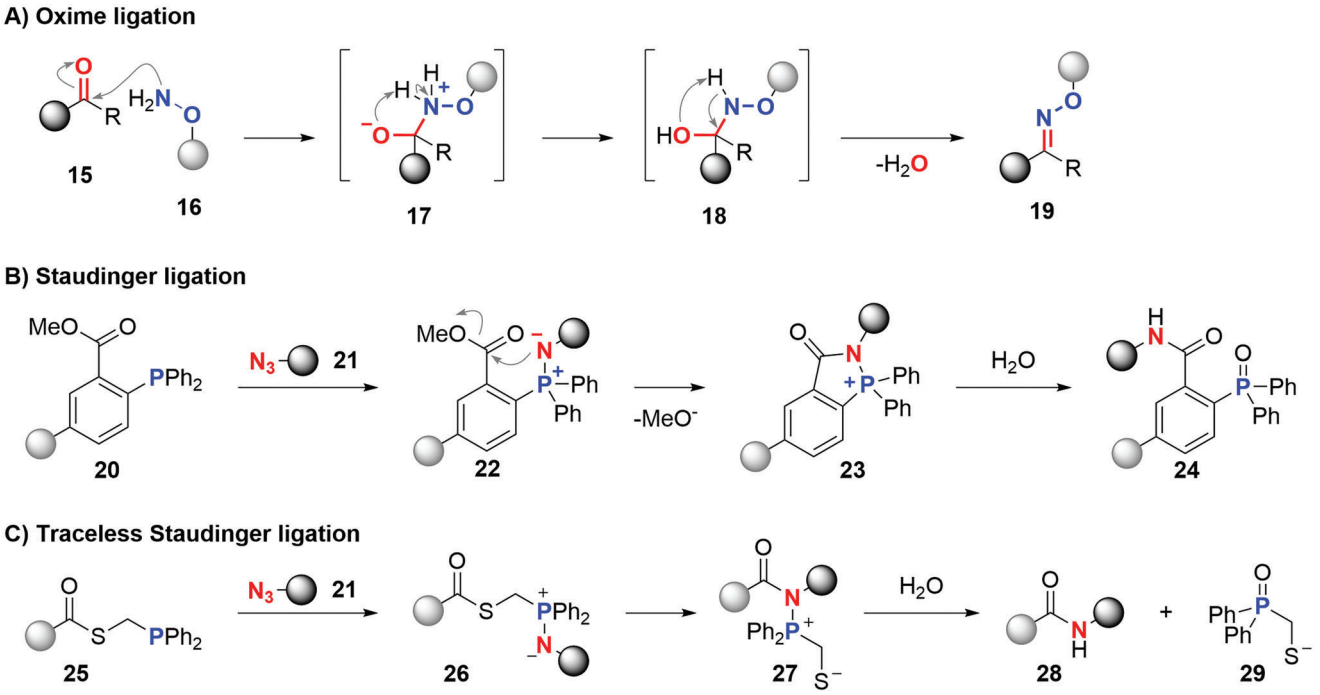
Scheme 2. A–C) Thiol-based bioconjugation strategies.

O-to-*N* acyl transfer and aqueous work-up. An advancement of the Staudinger ligation is the so-called "traceless" variant, which was developed to allow the formation of ligation products with native peptide bonds, which is particularly interesting for peptide and protein synthesis (Scheme 3C).[29] The difference to the conventional method is that the phosphine moiety is introduced as an auxiliary such as the phosphinomethylthioate in **25**, which can be easily hydrolytically cleaved after the rearrangement step.[29c] The azide and phosphine units are biocompatible, and the azide is among the smallest biorthogonal functional groups.[30] Nevertheless, the relatively poor reaction kinetics and the oxygen sensitivity of the phosphines are major limitations of this reaction and may be the reason why the Staudinger ligation has hardly been used for material synthesis and functionalization.[31] One of the rare recent examples includes a click-release strategy on gold nanoparticles.[32]

3.1.3. Cycloadditions

Cycloadditions have been widely used to functionalize biomolecules and materials. The Nobel Prize-winning azide-alkyne cycloadditions and tetrazine ligation are probably the most prominent among them. However, we would also like to highlight the classical Diels-Alder reaction and sydnone cycloadditions, the latter of which have only recently been established in the field of bioconjugation.

The copper(I)-catalyzed Huisgen 1,3-dipolar cycloaddition of azides and terminal alkynes (CuAAC) was independently developed by the groups of Sharpless and Meldal (**Scheme 4**A).[33] The alkyne **30** and azide moieties **21** exclusively form the 1,4-disubstituted triazole **31.** In addition, the product is very small and minimally interferes with the structures to which it is bound. Therefore, CuAAC has been widely applied in chemical biology with proteins, nucleic acids, and carbohydrates, as well as in materials design and functionalization.[5c-f,34] The complex mechanism of CuAAC is still under investigation, with new aspects discussed in the last decade. The most accepted mechanism was presented by Himo et al. and involved a pathway via binuclear copper acetylide intermediates.[35] Although CuAAC is always considered user-friendly and widely applicable, the reaction conditions often must be optimized for a specific purpose. This is reflected in the variety of catalysts and catalytic systems used in CuAAC, ranging from simple copper salts and copper(I) complexes to copper rods and complex heterogeneous systems such as metal-organic frameworks, copper-binding



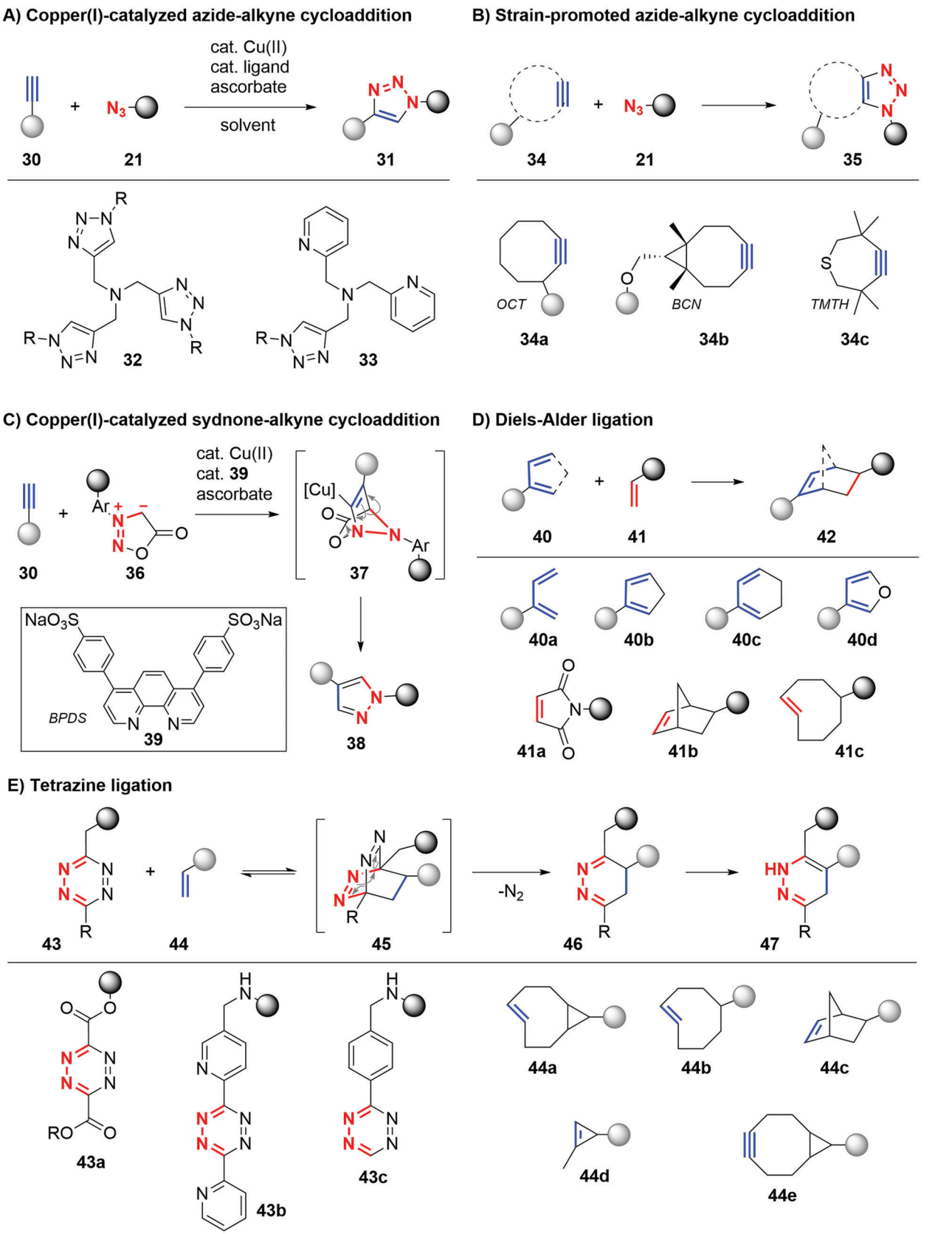
Scheme 3. A–C) Bioconjugation by oxime ligation and Staudinger ligation.

cellulose, and other nanostructured materials.[34b,36] One significant limitation of CuAAC is the copper-induced formation of reactive oxygen species (ROS), which can lead to various undesired side reactions.[37] Self-sacrificing ligands that scavenge ROS, such as **32** and **33**, have been designed to overcome this problem. Additionally, they stabilize the copper(I) species, thus accelerating the reaction.[34b,35b,38]

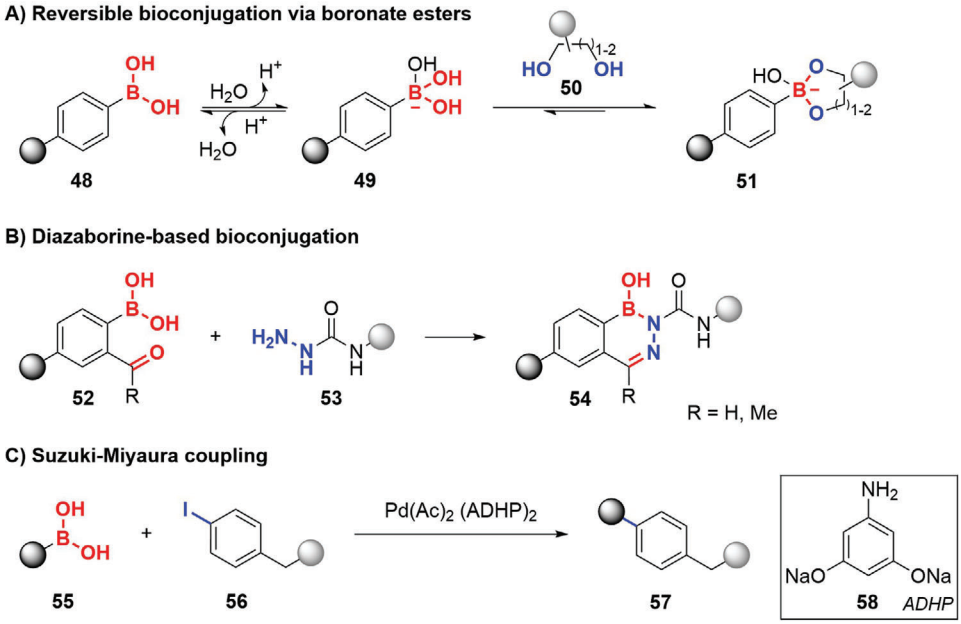
However, limitations remain, such as copper-induced toxicity to cells due to the strong binding of copper to certain proteins and thiol-containing molecules and copper-induced oxidative stress.[39] This led to the development of copper-free azide-alkyne cycloaddition on strained alkynes (SPAAC – strain-promoted azide-alkyne cycloaddition, Scheme 4B), which is slower compared to CuAAC but fast enough to be used in complex systems such as cells.[40] The reaction rates of SPAAC depend largely on the strain of the alkyne (**34**) used.[41] Unsubstituted cyclooctynes (**34a**) react significantly slower than bicyclo[2.1.0]nonyne (**34b**) or dibenzocyclooctynes, and these, in turn, react slower than more strained cycloheptynes such as **34c**.[42] Although azides have been most commonly used in these strain-promoted 1,3-dipolar cycloadditions for bioconjugation, other 1,3-dipoles such as nitrones, diazo compounds, or nitrile oxides react at higher rates and, in some cases, allow for different substitution patterns.[43]

Sydnones are other alternative 1,3-dipoles extensively studied in 1,3-cycloadditions in recent years.[44] The sydnone-based cycloaddition is a two-step reaction sequence involving a [2+3] cycloaddition followed by a retro-Diels-Alder reaction to give a heterocyclic conjugation product and, in the case of **36**, carbon dioxide.[45] However, the classical Huisgen cycloadditions on alkynes require higher temperatures and show only moderate reaction rates. In contrast, copper(I)-phenanthroline complexes catalyze the reaction efficiently and, similar to CuAAC, selectively afford the 1,4-disubstituted pyrazole cycloadduct **38** (Scheme 4C).[46] Although requiring higher amounts of copper(I) compared to CuAAC, copper(I)-catalyzed sydnone-alkyne cycloaddition (CuSAC) proceeds under mild reaction conditions with high yield and excellent regioselectivity and is very tolerant to functional groups.[47] Azaiminosydnones were also studied in CuSAC, with the predominant formation of 2,5-disubstituted triazoles under mild conditions.[48]

In addition to [2+3] cycloadditions, Diels-Alder reactions (DAR) have been applied for bioconjugation.[49] In DAR with standard electron demand, an electron-rich diene reacts with an electron-poor dienophile to form a six-membered cyclic adduct in a concerted manner (Scheme 4D).[50] The small size of the reactive groups, high degree of biorthogonality, predictability of regio- and stereoselectivity, and fast reaction rates in aqueous solvents due to hydrogen bonding to the activated complex and hydrophobic effects make DAR extremely attractive for the functionalization of biomolecules and materials.[51] The diene component (**40**) can be linear or cyclic (e.g., **40a–d**). Electron-deficient dienophiles such as maleimides **41a** and strained alkenes (**41b,c**) offer moderate to good reaction rates under mild reaction conditions. A classic diene-dienophile pair is represented by furan as the diene and maleimide as the dienophile.[52] Since DAR is known to give very high yields and literally requires no purification, it has found widespread application in materials synthesis and functionalization.[52–53] However, one aspect that should be considered is the reversibility of the reaction. As oxime ligation discussed previously, this can be exploited for developing degradable or self-healing materials[52,54] and for drug delivery or biomolecule formulation, e.g., through reversible-addition-fragmentation chain-tranfer (RAFT) polymers.[5h] In a recent report, biofunctionalized 3D-printed thermogelling hydrogels



Scheme 4. A–E) Bioconjugations based on cycloaddition reactions.



Scheme 5. A–C) Bioconjugations based on boronic acids.

are reported to gain shape fidelity through DAR-mediated crosslinking.[55]

In contrast to DAR, tetrazine ligation provides robust linkage with excellent rates.[49] Tetrazine ligation, the reaction between a 1,2,4,5-tetrazine (**43**) and an alkene (**44**), whose mechanism is best described by a reaction cascade consisting of a DAR with inverse electron demand followed by a rapid retro-DAR after the release of nitrogen (Scheme 4E). In the final step, 1,3-prototropic isomerization gives 1,4-dihydro-pyridazine **47**.[56] Unlike DAR, tetrazine ligation is irreversible. The rates of tetrazine ligation are extremely fast, up to 10^5 M^{-1} s^{-1}, especially with strained electron-rich dienes such as bicyclo[6.1.0]nonene **44a** and tetrazines with additional electron-withdrawing substituents (e.g., **43a**), thus allowing efficient bioconjugation at low concentration of the reactants.[57] However, for some applications, it is advantageous to have slower rates. Tetrazine ligation allows rates to be varied by many orders of magnitude by simply changing the nature and pattern of substitution of the reactive groups.[58] Another advantage is the high chemoselectivity, which makes the reaction orthogonal to other conjugation reactions.[59] Together, tetrazine ligation is an extremely powerful chemical biology and nanotechnology tool.[58c]

3.1.4. Bioconjugation with Boronic Acids

Boronic acids have a unique reactivity due to the unoccupied p-orbital in the boron center, which makes them strong Lewis acids.[60] In an aqueous solution, boronic acids are therefore in equilibrium with the anionic hydroxy boronates (**49**),[61] both of which readily form esters with 1,2- and 1,3-diols (**50**),[62] which can be used to bind biologically relevant diols such as carbohydrates or dopamine (**Scheme 5**A).[63] However, this process is reversible but can be controlled by adjustment of the pK_a value of the boronic acid.[62b] The corresponding boronate esters **51**, which are more stable than boronic esters, are preferentially formed at the pK_a value or above.[64] Using boronic acids with electron-withdrawing groups (EWGs) can decrease the pK_a value, resulting in more stable boronate esters.

The formation of boronic and boronate esters is highly reversible. It has been used to design dynamic materials, such as dynamic, near-floating, self-healing hydrogels or responsive hydrogels that can be degraded to carbohydrates under physiological or acidic conditions.[65] Combinations of crosslinks, e.g., a combination of oxime crosslinks and boronate esters or alginate calcium(II) crosslinks and boronate esters, were used to impart shape memory to materials, with the boronate esters providing the dynamic properties to the system.

In addition to reversible conjugations, boronic acids can also be used for irreversible bioorthogonal transformations. Diazaborines (**54**) are irreversibly formed from 2-formylbenzeneboronic acids or 2-acetylbenzene boronic acids (**52**) and semicarbazides (**53**) in excellent to good yields and under physiological conditions (Scheme 5B).[66] Boronic acids are versatile reagents for forming C-C bonds in organic chemistry. Suzuki-Miyaura cross-coupling (Scheme 5C) and Chan-Lam coupling have been used for bioconjugation at such mild conditions as pH 8 and 37 °C or even room temperature and may be of interest for materials synthesis in the future.[67]

3.2. Light-Induced Bioconjugation Reactions

Thermal bioorthogonal click reactions and bioconjugation methods proceed spontaneously under the conditions for which they are optimized. However, for some applications, particularly in materials development and synthesis, it is desirable to control the bioconjugation reaction spatially and temporally to adapt material properties or functionalization density. Light-induced

bioconjugation provides this control while retaining the advantage of bioorthogonality or at least high chemoselectivity. Typically, light-induced bioconjugation involves the formation or deprotection of a reactive intermediate by light irradiation or the light-induced activation of the catalyst required for a particular bioconjugation.[68] This subchapter presents an overview of the most important light-induced bioconjugation methods, including strategies for using light to trigger classical click reactions.

3.2.1. Light-Induced Thiol-mediated Bioconjugation

The most important light-induced reaction involving thiols is the thiol-ene (thiol-yne) click reaction (TEC), a radical reaction of a thiyl radical **60** that adds to an alkene **41** or alkyne with anti-Markovnikov selectivity to form a carbon radical **61** (**Scheme 6**A).[69] In a chain reaction, **61** can abstract hydrogen from another thiol molecule (**59**) to form the thioether bond. This efficient click chemistry allows perfect spatiotemporal control and has found wide application in material synthesis, surface modification, and polymer coating.[70] In bioconjugation, it is important to note that TEC can lead to side reactions, especially with oxidation-sensitive residues.[71] In addition, the reaction must proceed under mild, ideally physiological, conditions that require good water solubility and stability of the photoinitiators, of which **63** and **64** or the recently introduced **65** have proven very suitable.[72] All these photoinitiators work in the visible light spectrum. TEC has been successfully used in the bioconjugation of peptides and carbohydrates, both in chemical biology and in the biofunctionalization of materials.[72a,73]

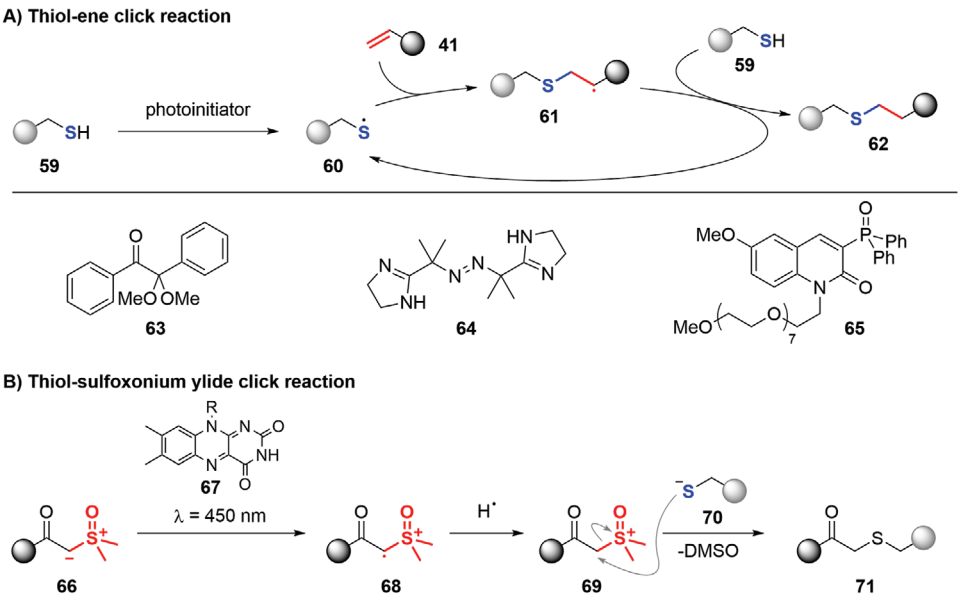
An interesting alternative light-induced bioconjugation involving thiols is a recently reported thiol-sulfoxonium ylide photo click reaction in which thioether conjugation products (**71**) are formed, with dimethyl sulfoxide being the only by-product (Scheme 6B).[74] Sulfoxoniumylides **66** are stable and water-soluble, and the corresponding click reaction is initiated with a riboflavin-based photocatalyst **67** at 450 nm and otherwise physiological conditions. The authors were able to demonstrate various modifications on partly complex peptides.

3.2.2. Light-induced oxime and Staudinger ligation

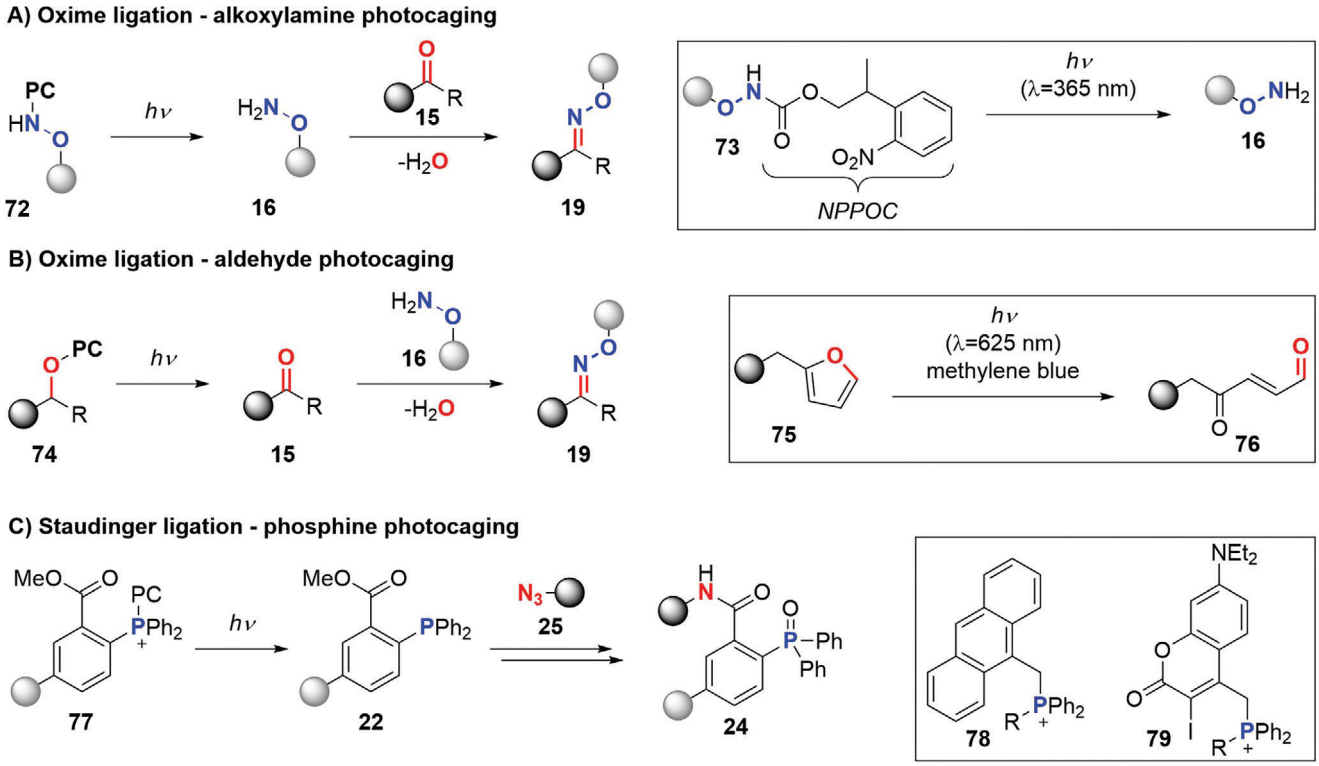
To achieve spatiotemporal control over material properties in oxime and Staudinger ligation, photocaging (PC) or light-induced in situ formation of the active functional groups was used (**Scheme 7**).[68] For example, in oxime ligation, Farahani et al. used photocaged alkoxyl amines **73** that were unmasked in near-UV light, allowing local crosslinking with high precision even in the presence of living cells (Scheme 7A).[75] Similarly, photocaging of the aldehyde component **15** was demonstrated with the photocage removed at 370 nm.[76] A more recent approach used furanyl compound **75**, which upon photooxidation with the photosensitizer methylene blue and red light that lies within the optical window of biological tissue, yields an unsaturated 1,4-diketone **76** that theoretically enables in vivo polymerization (Scheme 7B).[77] In Staudinger and "traceless" Staudinger ligation, photocaged phosphine reagents were explored that would, in perspective, provide spatiotemporal control over the reaction (Scheme 7C). Of note here are the efforts of the Lam group, which used 9-anthracenylmethyl phosphonium salts (**78**) cleaved in blue light.[78] In addition, Kalayci et al. presented a light-sensitive coumarin group that was successfully used to mask phosphine (**79**). This photocage was shown to be removed under even milder green light.[79]

3.2.3. Light-induced Cycloadditions

Like the conventional cycloadditions used for bioconjugation, the light-induced cycloadditions are also based on either [2+3] cycloadditions or DAR. The tetrazole photo-click reaction is



Scheme 6. A,B) Light-induced thiol-mediated bioconjugation.

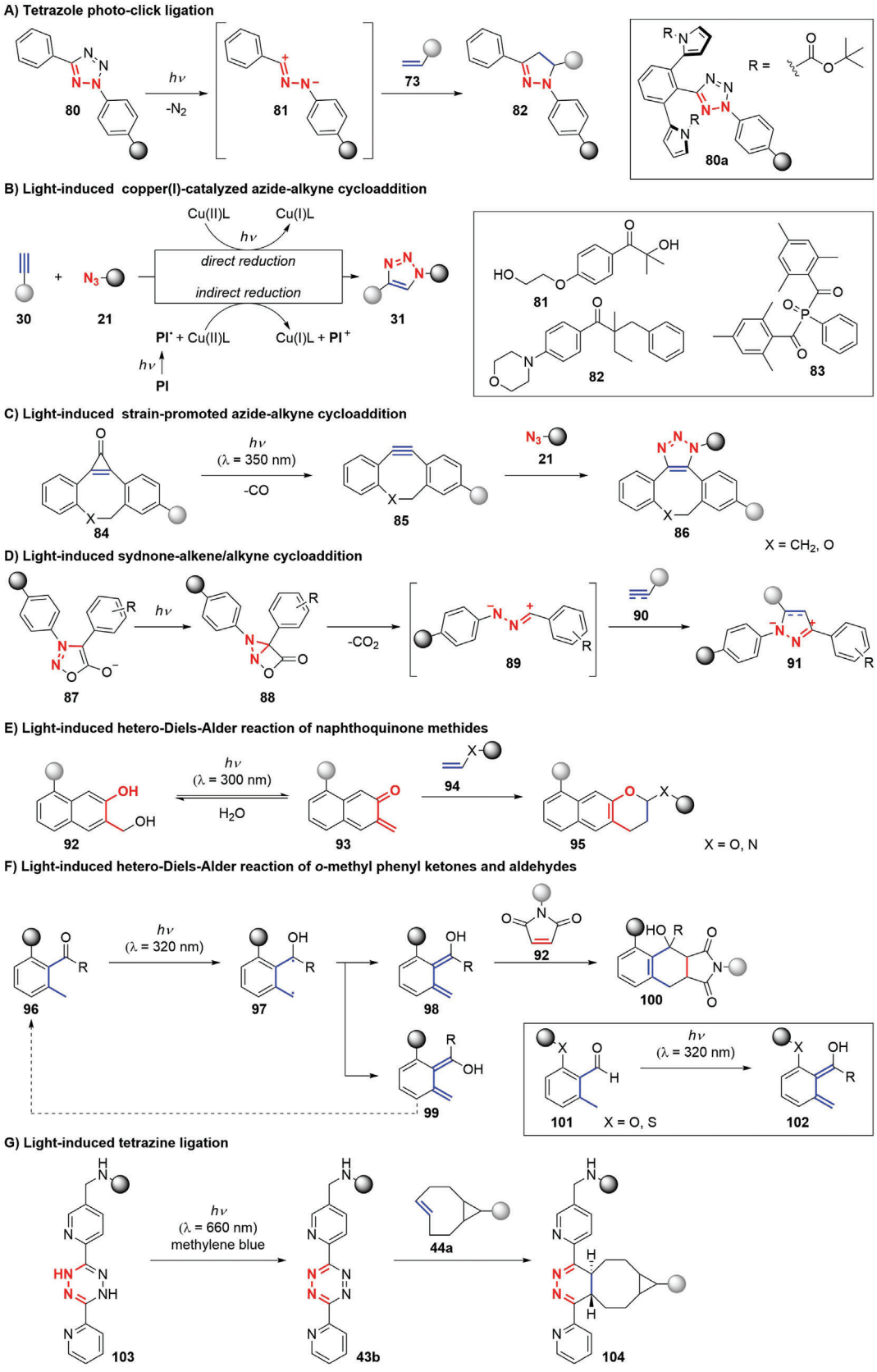


Scheme 7. A–C) Light-induced oxime and Staudinger ligation.

probably the most important and best-studied light-induced cycloaddition (NITEC – nitrile imine mediated tetrazole-ene cycloadditions).[68] However, we will also highlight the developments of the light-initiated CuAAC, SPAAC, CuSAC, or DAR variants.

The light-induced [2+3] cycloaddition of nitrilimines, which are formed in situ from 2,5-diaryltetrazoles **80**, and alkenes, was first described in the early 1960s by Huisgen, who observed the formation of pyrazoline conjugation products after irradiation of a reaction mixture of 2,5-diphenyl tetrazole and methyl crotonate with a mercury lamp.[80] The mechanism, widely accepted then and to this day, involves a light-induced ring rupture and cleavage of nitrogen to form a nitrilimine **81**, which is a reactive 1,3-dipole, and subsequent [2+3]cycloaddition with an alkene (**73**) but also alkyne or quinone dipolarophile (**Scheme 8**A).[81] Nitrilimines are less stable in aqueous solution than other 1,3-dipoles, such as azides, but the corresponding tetrazole precursors show excellent stability in water. This reaction is thus very suitable as a bioconjugation method.[82] The nature of the 2,5-diaryltetrazole has a significant influence on the reaction rate. Electron-donating groups on the aryl substituents accelerate the rate, as does the use of strained 2,5-diaryltetrazoles.[83] Improved selectivity of the reaction is achieved by using sterically shielded tetrazoles such as **80a** that promote the cycloaddition and not the competing nucleophilic addition.[84] A major drawback of the NITEC compared to other light-induced bioconjugations is its photoinitiation in UV light, which is harmful to cells. The wavelength of the photoactivation can be tuned by changing the substituents at the aryl moieties at the tetrazole. Amino groups or stilbene residues and naphthyl, coumaryl, or pyrene substituents allowed photoactivation in visible light.[85] Oligothiophene-based tetrazoles activated by a laser at 405 nm also showed improved biocompatibility.[85b,86] Generally, NITEC was applied in the bioconjugation of proteins,[82,87] nucleic acids[5c,88] and carbohydrates,[89] but due to its excellent spatiotemporal control, has also been recognized as a powerful technique to synthesize and biofunctionalize materials.[90] NITEC has been applied in synthesizing polymer networks and micropatterning nanoparticles.[91] Recent applications also include the design of sensory microparticles using NITEC. For instance, microparticles were functionalized with fluorescent pyrazoline–peroxyoxalate to be chemiluminescent upon exposure to hydrogen peroxide.[92]

Light-driven CuAAC is realized by photolysis of Cu(II) to the catalytically active species Cu(I). This can be achieved directly after UV excitation of the copper ligand via ligand-metal charge transfer[93] or indirectly by a photoinitiator forming a reducing radical species in the excited state (Scheme 8B).[94] Light-driven CuAAC with a photoinitiator is usually faster and allows tuning of the wavelength for photoexcitation, which can range from the UV to visible light depending on the photoinitiator used. Typical photoinitiators are shown in Scheme 8B.[95] The main advantage of light-driven CuAAC is the excellent temporal control of the reaction because Cu(I) species are very sensitive to air and can therefore be rapidly oxidized to inactive Cu(II). Since this process is reversible, the reaction can be restarted at anytime.[96] The excellent reaction rates and the versatility of the reaction make it attractive for a wide range of applications, especially in the field of materials design and functionalization.[97] However, the commonly used photoinitiators often lack solubility in aqueous



Scheme 8. A–G) Bioconjugations based on light-induced cycloadditions.

media and/or can cause cytotoxicity, limiting the application of this reaction in cellular environments. Therefore, the use of naturally-occurring, biocompatible photoinitiators such as pyruvate, which acts as both a photoinitiator and ROS scavenger, is of interest, with the limitation that photoinitiation occurs under UV light.[98]

Strained alkynes, as used for SPAAC, are readily masked as photosensitive cyclopropenones, for example, cyclopropenone **84**, which decomposes into carbon monoxide and the reactive alkyne species upon irradiation with blue light (Scheme 8C).[99] This method has been used for labeling, surface immobilizing biomolecules, and functionalizing hydrogels and nanoparticles.[99–100] Also, dicyclopropenones have been utilized as crosslinkers in chemical biology and should have potential in materials synthesis.[101] To enhance the biocompatibility of the reaction, it was shown that the alkyne could be generated from cyclopropenones by nonresonant two- and three-photon excitation using near-infrared radiation.[102]

The light-induced bioconjugation of diarylsydnones, alkenes, or alkynes does not require a metal catalyst to form 1,3-dipole **89**.[103] UV irradiation of diarylsydnones such as **87** is assumed to lead initially to photoisomerization to bicyclic compound **88**, from which, similarly to the tetrazole photo-click ligation, the reactive intermediate **89** is generated to undergo [2+3] cycloaddition with the dienophile (Scheme 8D).[104] Diaryl sydnones react preferentially via the light-induced reaction pathway over the thermal cycloaddition pathway to form 1,3-pyrazoles **91**. The reaction rate can be increased if strained alkynes are used, resulting in complete conversion within a few minutes.[103a]

Reactive heterodienes can be formed from aromatic phenols or carbonyl compounds such as **92** and **96** upon light irradiation. For example, irradiation with UV light generates the reactive *o*-quinone methide **93** from 3-(hydroxymethyl)-2-naphthol **92**, which undergoes rapid DAR with electron-rich vinyl ethers or enamines (**94**, Scheme 8E).[105] Disadvantages of this reaction are the limited hydrolytic stability of the cycloadducts, especially the *N,O*-acetyl cycloadducts from the reaction of **93** and enamines, which already hydrolyze under physiological conditions.[105b] In addition, the heterodiene intermediate can also react with thiols in a Michael addition, which is reversible under light irradiation.[106] More extensively studied and particularly widely used in the fields of materials design, functionalization, and direct laser writing (DLW) is light-induced DAR with *o*-tolyl ketones or aldehydes **96**, from which *o*-quinodimethanes **98** are formed in situ as reactive heterodienes (Scheme 8F).[107] Light irradiation generates a biradical that rearranges to form *E*- or *Z*-enols (**98** or **99**), with only the *E*-enols **98** participating in DAR with electron-deficient dienophiles. The *Z*-enols are converted into the starting material in a[1,5] sigmatropic rearrangement.[108] To promote the formation of the desired *E*-enol, *o*-tolyl aldehydes have been explored that have a hydrogen bond acceptor in the ortho-position of the aldehyde moiety in **101** that stabilizes the *E*-conformer of the reactive intermediate **102**, thus accelerating the reaction rate (Scheme 8F).[109] Of particular interest in this context are *o*-quinodimethane thioethers, which allow photoinitiation in visible light and are thus suitable for biological applications.[110] In addition to light-induced DAR, light-induced tetrazine ligations have been investigated using photocaged strained alkynes such as **84**, which are also widely used in light-induced SPAAC (vide supra) or photocaged cyclopropenes.[111] Another approach involves the photochemical oxidation of dihydrotetrazine **103** to the reactive tetrazine compound **43b** using methylene blue as the photosensitizer (Scheme 8G).[112] This variant of light-induced tetrazine ligation has been applied to the biofunctionalization of glass surfaces and polymer crosslinking to form hydrogels.[113] Recently, photocaged tetrazines have allowed spatiotemporal control of this rapid bioconjugation method in living cells.[114]

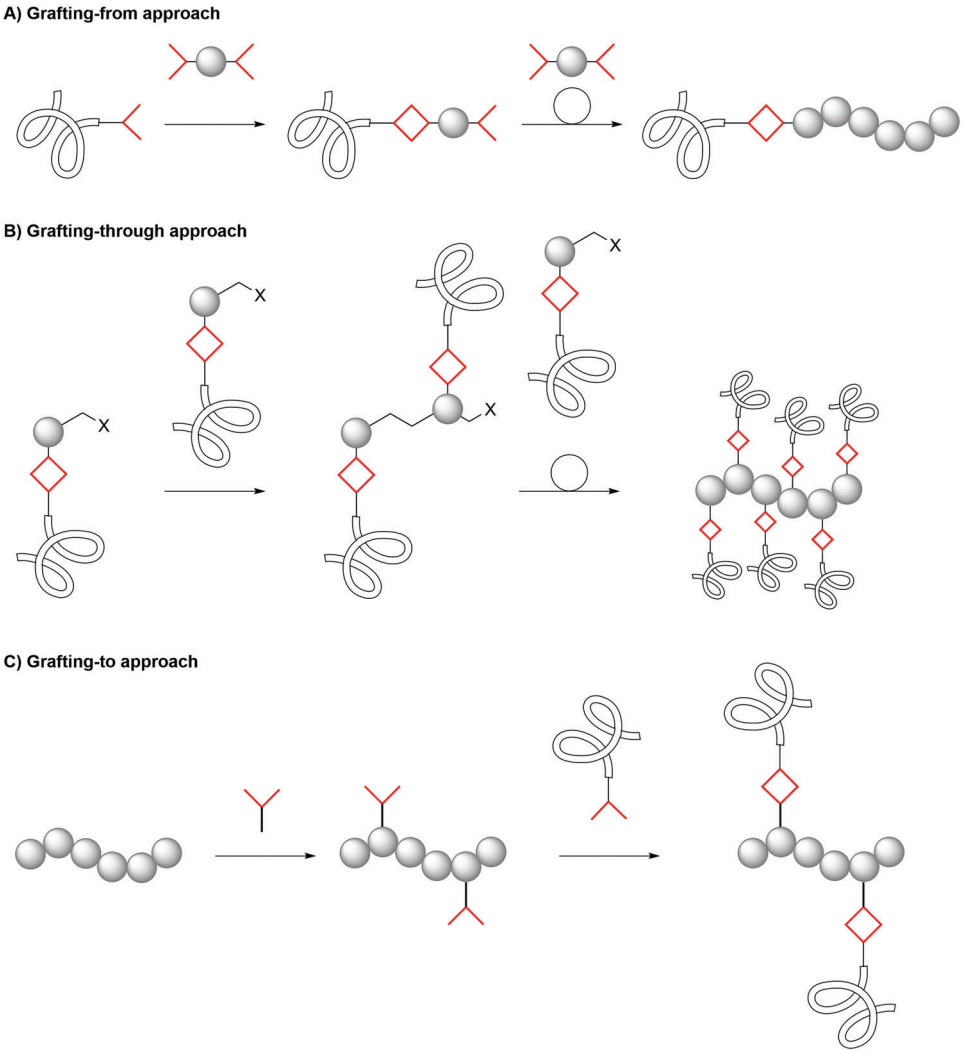
4. Applications

As described above, conjugating all kinds of synthetic and natural polymers with biomolecules to biohybrid materials opens a chemical space with interesting novel functions for various applications. Due to the rapid development of methods to understand the structure of proteins, even those that are AI-based (alpha fold), and an enormous increase of bioconjugation methods, including the routes of light-induced bioconjugation as described, material bioconjugates will not only increase the number of materials that can be used for biological applications but broaden the scope of the applications in biological chemistry. Applications vary from energy storage by the use of redox-active polymers, bioimaging,[115] drug delivery,[116] sensors,[117] diagnostics,[118] composites[119] up to theranostic applications,[120] and multimodal contrast agents[121] and recently for tissue engineering.

5. Biofunctionalization and Materials

To obtain biofunctionalized materials, grafting-from, grafting-through, or grafting-to strategies, i.e., pre- or postfunctionalization, can be applied (**Scheme 9**).[122] Grafting-from and grafting-through strategies, in which the biomolecule is involved in the polymerization, offer many advantages, especially in synthesis, as hydrophobic material scaffolds with hydrophilic modifications can be assembled directly with the biomolecule, avoiding the often tedious optimization of reaction conditions during postfunctionalization or diffusion problems.[8a,123] Typical grafting-from techniques are atom transfer radical polymerization (ATRP) or RAFT polymerization, where the biomolecule is either part of the initiator or attached to the chain transfer reagent. Grafting-through methods include all strategies that use monomers modified with biomolecules that can be obtained, for example, by solid-phase synthesis methods. This has been demonstrated for chain-growth polymerization (CGP), ring-opening polymerization (ROP), and ring-opening metathesis polymerization (ROMP).

Despite recent advances in prefunctionalization strategies, grafting methods remain the most commonly used approach to functionalize materials, although determination of the best functionalization conditions can often be challenging. The development of powerful bioconjugation methods allows very precise chemoselective modification, avoiding side reactions that could affect polymer properties. In particular, the flexibility of the modification chemistry, especially the biomolecules, is very advantageous, allowing multiple functionalization and patterning. In addition, not all materials can be subjected to the grafting-from or grafting-through methods, as the polymerization conditions are too harsh and would damage or completely destroy the



Scheme 9. A–C) Schematic representation of common approaches to functionalizing materials.

biomolecule. This is the case, for example, with pyrolysis-derived polymers. Compared to prefunctionalization, postfunctionalization usually requires additional reaction steps. First, the material is prefunctionalized with a reactive group, enabling subsequent biofunctionalization (Scheme 9). Second, a spacer is often attached before the desired biomolecule is added via bioconjugation. A detailed overview of advances in bioconjugation and representative examples of light-induced grafting-to syntheses of biofunctionalized materials are given in the following chapters.

6. Bioconjugation in 3D Printing

As bioconjugation is becoming more and more prominent in biomaterials development and bioengineering by 3D bioprinting, the variety of materials is manifold, ranging from inorganic to organic and hybrid materials and even hybrid biopolymers.[124] Bioconjugation even shapes and structures materials resulting in adaptive biomaterials (for review, see[125]). The interaction of biological and abiological (e.g., inorganic or polymeric) species causes a novel and unique interface. Both starting materials can be tuned to control the final interfacial structure for a specific purpose or application like catalysis, optics, sensing, etc.[126] Especially additive manufacturing by light-induced bioconjugation enables a plethora of biomaterials and hybrid materials with unique properties, including a high-resolution structuring that is beyond the function of the building blocks themselves. The following chapters give a brief overview of some selected recent applications and challenges for a selected class of materials. This overview is by far only limited as the number of light-induced bioconjugation approaches for biomaterials, even in 3D printing, is constantly increasing, and more is yet to come.

Even though light-induced bioconjugation by photoclick reactions usually does not need harsh chemistries, the molecular space of bioconjugated precursor molecules suitable for 3D additive manufacturing by light-induced processes is still not fully investigated, therefore still limiting the number of biological applications. Traditionally, these light-induced printing processes mainly use photopolymers such as methacrylates and acrylates, requiring vast amounts of radicals for polymerization. Recent breakthrough also in click photochemistry

enabled a much broader choice of available biomaterials, making these techniques more versatile, provided that they can generate solid materials from liquid or viscous precursors. 3D printing techniques by using light-induced bioconjugation such as stereolithography (SLA), digital light processing (DLP),[127] DLW,[128] volumetric printing (VP),[129] computed axial lithography (CAL),[130] continuous liquid interface production (CLIP),[131] rapid-scanning multifocus multiphoton 3D laser printing (MFMP-3DP),[132] femtosecond projection two-photon lithography (FP-TPL),[133] and light-sheet 3D printing[129,134] enables relatively fast and controlled construction of various architectures and simple light sources.[135] Recently, even some of those technologies, as mentioned above, overcame the slow printing rates.[134,136] Using a wide variety of biological polymers such as peptides/proteins, nucleic acids, and carbohydrates in combination with polymer chemistry-related innovations, these 3D printing technologies have been used in microfluidics, dentistry, biomedical devices, tissue engineering, and biosensors. Some of these technologies can be even used for printing or writing of inorganic materials.[137] Nonetheless, with some of the printing technologies such as DLW and DLP, there remain challenges that have to be overcome to allow for a broader use in bioconjugation and tissue printing.[138]

According to Fick's laws of diffusion, with an average diffusion coefficient of common free radicals $\approx 1 \times 10^{-5}$ cm^{-2} s^{-1} in both polar and nonpolar solvents,[139] and an average free-radical lifetime of ≈10 ms, this diffusion is ≈10 µm.[140] As a result, printing objects smaller than 10 µm is almost excluded due to this adverse effect of molecular diffusion, regardless of the quality of the optics. To overcome this, the exposure time in a single printing cycle is typically between 10^1 s and 10^2 s.[127]

Reaction speed in optical 3D printing is highly important especially when it comes to biological applications in the presence of cells.

The 3D printing process using DLW or DLP is also significantly influenced by the viscosity of bioconjugated precursor materials. When the precursors have a higher viscosity, the printing resolution can be improved due to the slowed-down molecular diffusion and shape preservation of the printed objects in their initial localization.[131b] However, this higher viscosity can pose challenges in exchanging the resin or the hydrogel precursors between printing different structures.[130a,141] Additionally, some photoclick reactions, such as the tetrazine-click reaction, may generate gaseous by-products like N_2 that scatter light and form voids by gas bubbles in the materials, affecting the quality and efficiency of the printing process. Therefore, it is crucial to consider both the viscosity of the precursor and the by-products produced to ensure optimal 3D printing results.

A big issue is still the very limited amount of biofunctionalized materials that can be used. Most polymeric materials, called photoresists, are functionalized with methacrylates or acrylates using ATRP for photopolymerization and require vast amounts of radicals for polymerization.[130a] In classic photolithography, there are different types of photoinitiators. A photoinitiator absorbs the light and produces a radical, which results in photopolymerization and cationic photoinitiators, which are photo-acid generators that produce cations by light exposure (for review, see[138]). There are already many photoinitiators that are water compatible and absorb even visible light.[142] In two-photon polymerization as applied in DLW, however, the situation is much more complicated as, to our best knowledge, the photoinitiators used for this technology are based on radical formation by light exposure[138,143] with a few exceptions reported recently.[144] Both the photoinitiator and the biomaterial monomers have to be transparent at the laser wavelength to prevent the absorption of the laser beam at the surface of the volume and the biomaterial monomer also has to be transparent at the two-photon absorption wavelength ($\lambda/2$).[144] The photoinitiator needs a high radical quantum yield and highly-active radical species generated. Benzophenone and its derivatives, as well as lithium phenyl (2,4,6-trimethylbenzoyl) phosphinate (LAP), are the free radical photoinitiators that are most frequently utilized.[138,145] Most of the materials that are used in DLW or two-photon photoionization (2PPi) printing are therefore employing radical polymerization such as methacrylates/acrylates or vinyl ethers. While DLW is well suited for post-printing bioconjugation to materials and subsequent interaction with living cells, the above properties are unfavorable for additive manufacturing in the presence of living cells, as cells react to the high number of radicals with nonspecific DNA double-strand breaks and lipid peroxidation. Recently, thiol-ene chemistry and radical-free Diels-Alder photoclick reactions were discovered to work in biocompatible materials with specific rheological, biological, mechanical, and chemical properties that can be used for 3D (or 4D) bioprinting application even in presence of cells.[146] 3D light-based printing technologies with higher printing speed and resolution must be developed along with the corresponding bioconjugated precursors.

7. Photochemical 2D and 3D Printing for Precision Tissue and Material Engineering

During the last decade, photochemical reactions have been increasingly used in the field of soft tissue engineering and biofabrication.[147] The materials used here are mainly soft materials such as hydrogels, biological polymers, and materials that mimic the physicochemical properties of tissues. Due to their capability to bind large amounts of water, hydrogels can store nutrients and all kinds of growth factors, metabolites, etc., to allow for the survival of embedded cells or organisms and they can be used to create complex 3D structures with specific biological properties. In hydrogels, bioconjugation and light can be used to define the spatiotemporal biophysical[148] and biochemical properties in many biological applications. A vast variety of materials are bioconjugated via methacrolyl moieties. Even biomolecules, such as DNA strands, can be conjugated into precursor materials to develop smart materials such as the photo-curable DNA hydrogel called D-gel (**Figure 1**).[149] The gel's shape can be dynamically controlled through DNA hybridization-induced double crosslinking, making it faster than traditional DNA gels.[150] Modular macroscopic structures with programmable reconfiguration and directional movement, like the D-gel palm, can be fabricated using programmable self-assembly, giving rise to its use in soft robotics and smart devices.

Besides the above-mentioned ATRF reactions, the most frequent applications of light-induced bioconjugations in hydrogels are photoclick reactions. Even though these "photoclick" bioconjugations include fast reaction times, high yields, and mild

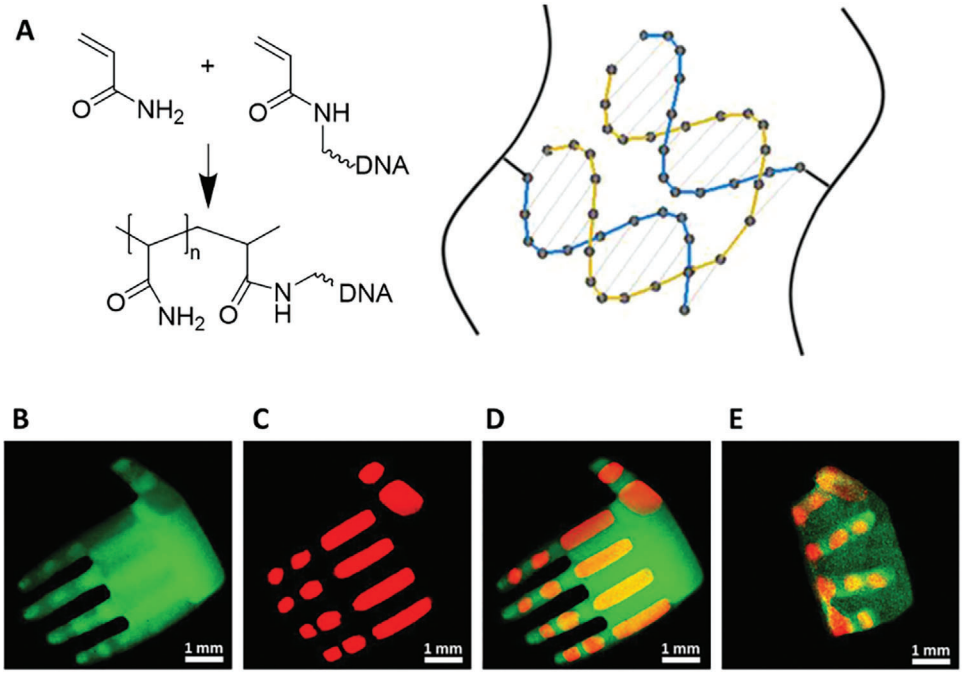


Figure 1. The polymerization reaction for synthesizing a D-gel. A) A scheme showing the formation of a DNA crosslinker. Fluorescent images of the hydrogel palm. B) Green: nonresponsive poly acrylamide (PAAm) gel. C) Red: D-gel. D) Overlay of (A) and (B). E), A closed first structure formed by adding the trigger strand B1. Adapted with permission.[149] Copyright 2019, Mary Ann Liebert, Inc., publisher.

reaction conditions, each has its own advantages and limitations when it comes to bioconjugation in the presence of living cells.[151] The most frequently used photoclick reactions in terms of bioconjugation in presence of living cells are photo-mediated azide-alkyne cycloadditions, other 1,3-dipolar cycloadditions, Diels-Alder and inverse-electron-demand Diels-Alder additions (IEDA), radical-alternating addition, chain-transfer additions and nucleophilic additions (e.g., thiol-ene and thiol-Michael) in addition to ATRP (for review see[151]). A few years ago, bio-orthogonal photoclick chemistries have become increasingly important in biomaterials research due to their ability to create intricate hydrogel materials that offer dynamic, cell-instructive microenvironments. By using a multifunctional PEG-tetrazine macromer with a dinorbornene peptide, IEDA reaction was

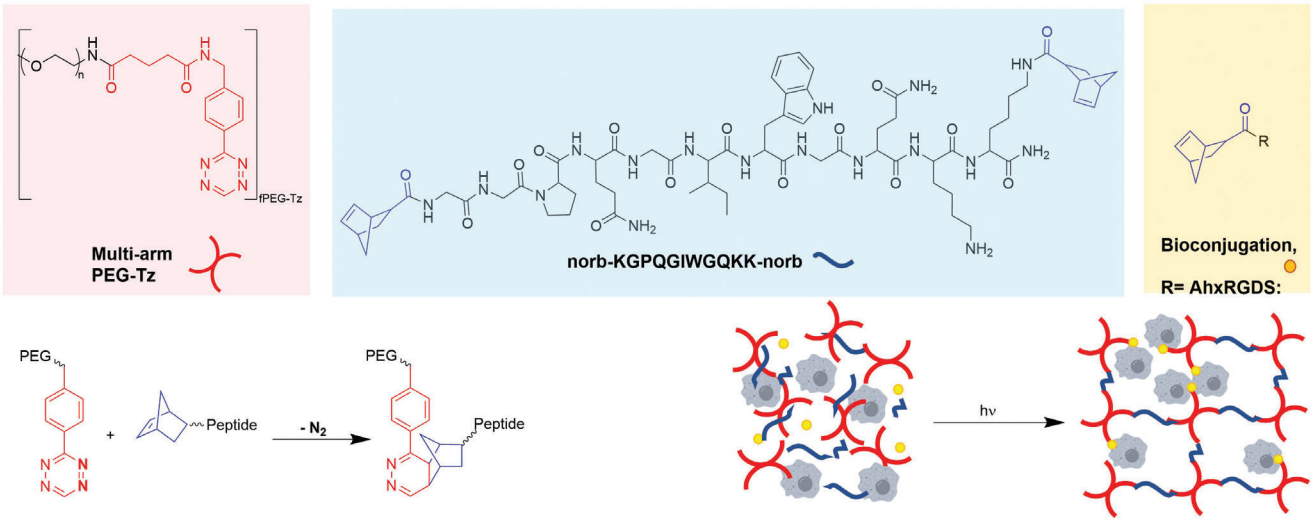
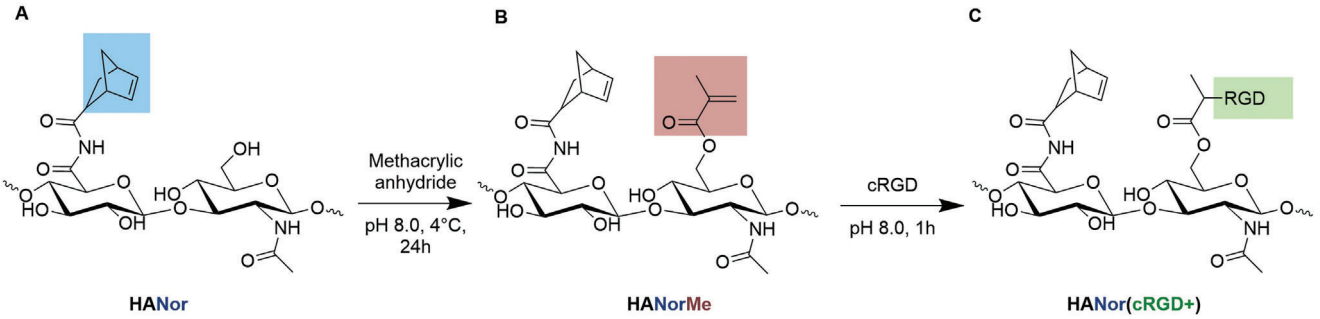
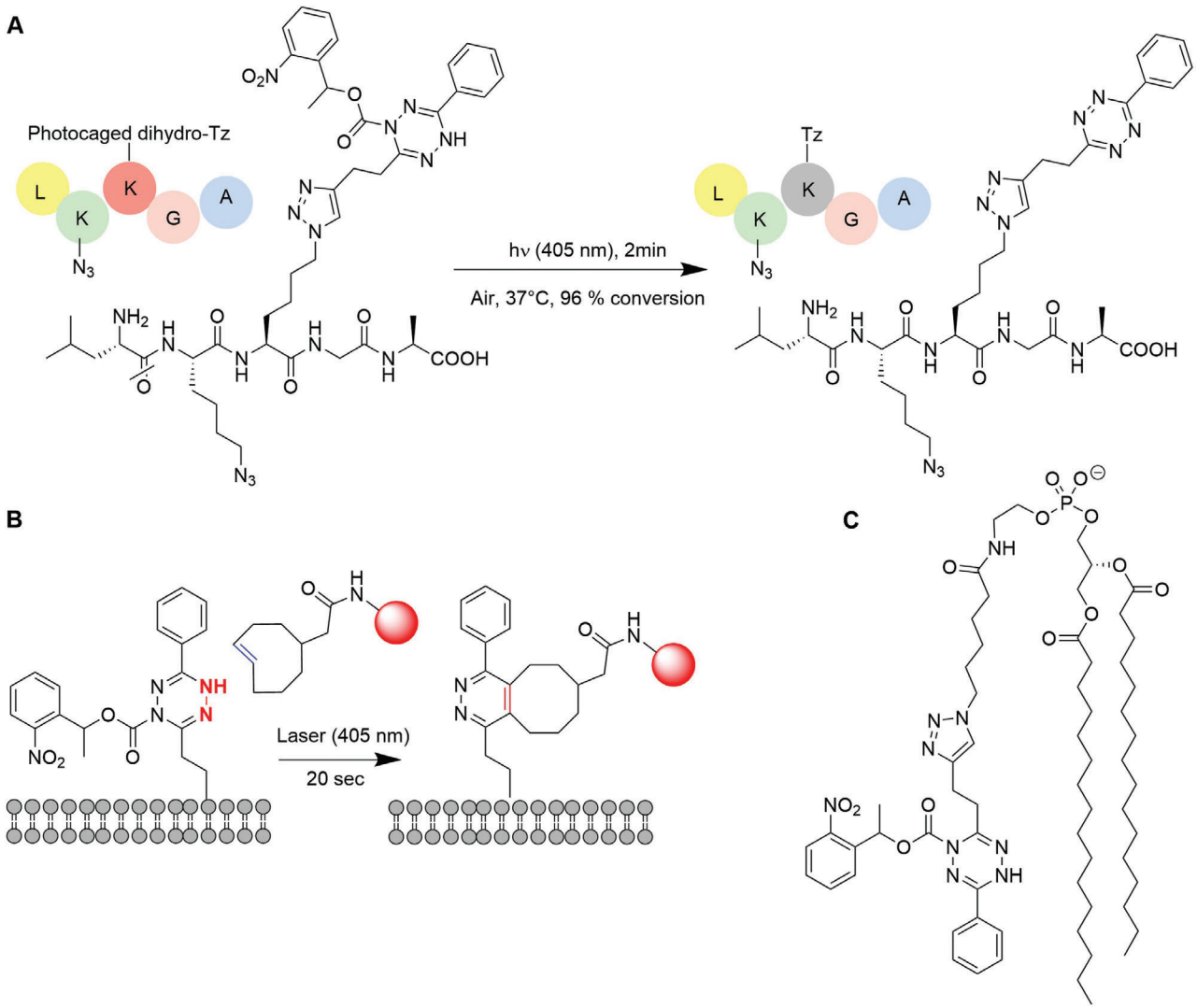


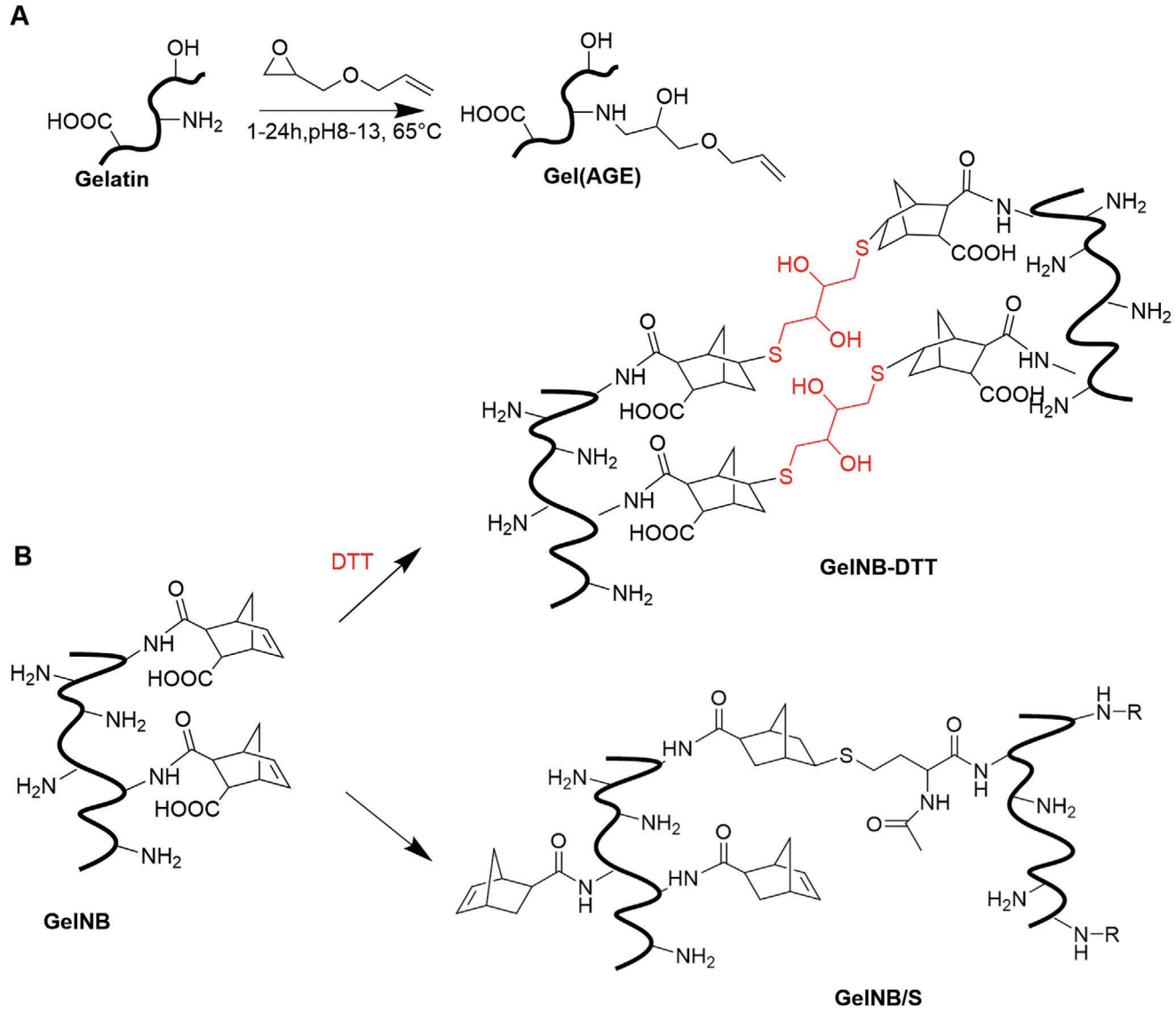
Figure 2. Hydrogel formation using the tetrazine-norbornene click chemistry. Adapted with permission.[152] Copyright 2013, American Chemical Society. Red: multifunctional PEG-Tetrazine (PEG-Tz); Blue: cell-degradable dinorbornene crosslinker peptide; Yellow: mono-norbornenyl functionalized peptides (for cell encapsulation and protein patterning experiments). Bottom: reaction of PEG-Tz and norbornene-functionalized peptide resulting in the cycloaddition product.



Scheme 10. Functionalization of HANor (hyaluronic-acid norbonene) macromers with thiolated peptides. A-B) hydroxyl groups in HANor macromers are converted to HANorMe macromers. B-C) thiolated RGD (arginylglycylaspartic acid) adhesive peptide (cRGD) is added to create peptide-functionalized HANor(cRGD+) macromers. Adapted with permission.[154] Copyright 2022, Wiley-VCH GmbH.



Scheme 11. Modulation of the lipid bilayers with tetrazines. Adapted with permission.[114] Copyright 2022, Springer Nature. A) Light-triggered formation of tetrazine peptide, where a photocaged dihydrotetrazine amino acid was introduced during Fmoc solid-phase peptide synthesis. B) When a photoprotected dihydrotetrazine is uncaged using light, it forms tetrazine that reacts quickly with dienophiles, including trans-cyclooctene attached to fluorescent probes. A cartoon demonstrates the use of photocaged dihydrotetrazine-diacylphospholipid for live-cell photoactivation of tetrazine ligation on cellular membranes. C) Structure of the modified phospholipid.



Scheme 12. A) Synthesis of GelAGE. Reaction with thiols such as DTT results in crosslinked hydrogels. B) Gelatin-norbonene (GelNB): Crosslink with DTT and gelantin-thiolate (GelS)[142,146a,158]

discovered as a novel crosslinking chemistry that enables the formation of cell-laden hydrogels by light-induced processes within minutes (**Figure 2**).[148,152]

The high cytocompatibility of the polymerization resulted in excellent postencapsulation viability of human mesenchymal stem cells. Furthermore, the specificity of the tetrazine-norbornene reaction facilitated the sequential modification of the network via thiol-ene photochemistry. The accessibility of the tetrazine molecule in comparison to other bioorthogonal click reagents was subsequently investigated as a new tool for developing cell-instructive hydrogels of all kinds (i.e., alginates,[153] hyaluronic acids (HA),[154] and gelatins[155]), etc. for tissue engineering applications (**Scheme 10**).

The rapid kinetics of bioorthogonal cycloaddition reactions between tetrazines and strained dienophiles make them useful for labeling proteins, lipids, and glycans.[152] However, controlling this chemistry in the presence of living mammalian cells with spatial and temporal precision remains challenging as the gelation time (several minutes up to an hour) is still too slow to prevent cells from sedimentation, especially in 3D bioprinting. A recent study by Liu et al.[114] introduces a versatile approach to the light-activated formation of tetrazines from photocaged dihydrotetrazines, allowing live-cell spatiotemporal control of rapid bioorthogonal cycloaddition with dienophiles. Photocaged dihydrotetrazines are stable and enable early-stage incorporation of bioorthogonal handles into biomolecules. They also allow nontoxic light to trigger tetrazine ligations on living mammalian cells, as demonstrated by tagging reactive phospholipids with fluorophores to modify HeLa cell membranes with single-cell spatial resolution (**Scheme 11**).[114]

Another remarkable feature of several recent studies is the photocontrol of the CuAAC reaction. This has also been used

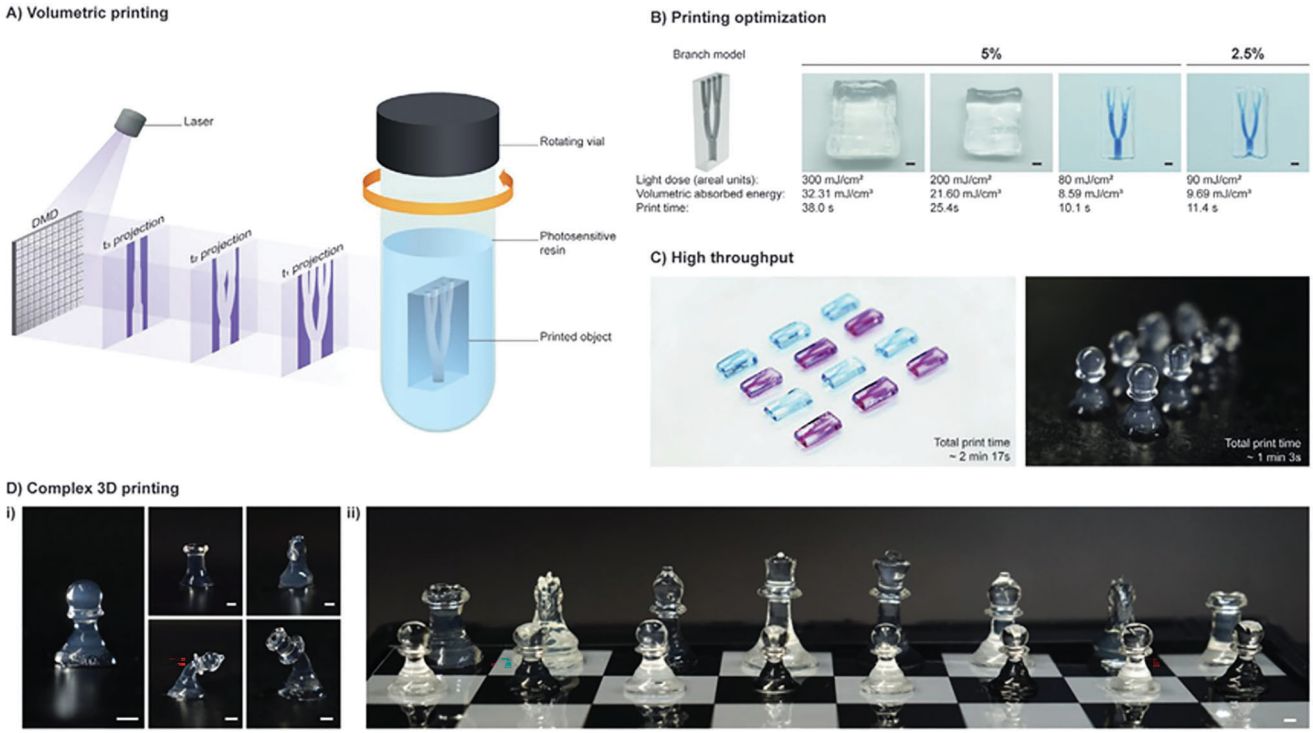


Figure 3. A–D) Volumetric printing of GelNB and DTT. Adapted with permission.[146a] Copyright 2021, Wiley-VCH GmbH.

for array patterning. For example, the photochemical activation of an azido-phenol linked to DNA resulted in bioconjugation to an amino-functionalized glass slide.[156]

Although it has been proven that photoclick tetrazine-norbornene reactions are several orders of magnitude faster, there are few applications for using light-induced 3D bioprinting. Whereby 3D bioprinting by light-induced technologies using thiol-ene chemistries for the additive manufacturing of complex biological or biomimetic cellular constructs is becoming one of the main bioconjugation types used so far, as the conditions are relatively mild.

Although thiol-ene chemistry has made progress, it is still not widely used in peptide and biomaterial science as well as in 3D bioprinting compared to the analogous Michael addition through an ionic mechanism when the alkene (i.e maleimide-moiety) is part of a Michael acceptor system (for review see[72a]). Thiol-ene photo click chemistry is well-suited for peptide bioconjugations due to its ability to selectively react with a thiol and alkene even in the presence of mixtures of nucleophiles and electrophiles. However, the molecular space of bioconjugated precursor molecules for 3D additive manufacturing using light-induced processes still needs more research. For this reason the number of biological applications is still limited. Recent breakthroughs also in click photochemistry allow for a much wider range of available biomaterials, making these techniques more versatile, provided they can produce solid materials from liquid or viscous precursors. Step-growth crosslinking enables reducing the degree of substitution, retaining biopolymer properties. Oxygen insensitivity and selective reaction between thiol and –ene groups allow for superior control over crosslinking, resulting in homogeneous network formation with reduced shrinkage and stress, overcoming methacryloyl functionalized gelatin's (GelMA) non-biodegradable chains and network defects.[72a]

The use of secondary thiol-ene systems in DLP printing is preferred due to their better thermal storage stability, reactivity, and mechanical properties.[157] Allylated gelatin (GelAGE) and dithiothreitol (DTT) have been used as a thiol-ene clickable bio-ink for biofabrication resulting in a fully degradable network with tunable properties, which is a significant improvement over traditional GelMA (**Scheme 12**).[158]

Photoclick thiol-ene reactions have been used to functionalize gelatin and collagen-based materials for all kinds of 3D bioprinting, including 2PPi-based DLW. Most of the light-assisted bioprinting techniques, including high-definition two-photon stereolithography as recently demonstrated by Dobos et al.[159] enables a better preservation of the bioactive and mechanical properties of gelatin by largely leaving RGD motifs unmodified thereby limiting the influence that functionalization can have on physical gelation.[160]

Rizzo et al.[146a] recently discussed volumetric printing (VP), a light-mediated technique for 3D printing that can create complex, low-defect objects within seconds. To create very efficient bioconjugated materials, they used thiol-ene click reactions with optimized GelNB for volumetric bioprinting, which shows superior performance in physicochemical and biocompatibility aspects compared to gelatin (meth-)acryloyl precursors (**Figure 3**). The thiol-norbornene reaction produces the fastest VP reported to date, making it more suitable for cell encapsulation. The GelNB approach enables the generation of cellular free-form constructs with excellent cell viability and tissue maturation potential, with properties that can be fine-tuned over a broad stiffness range (≈40 Pa to ≈15 kPa) by varying the polymer content, thiol–ene

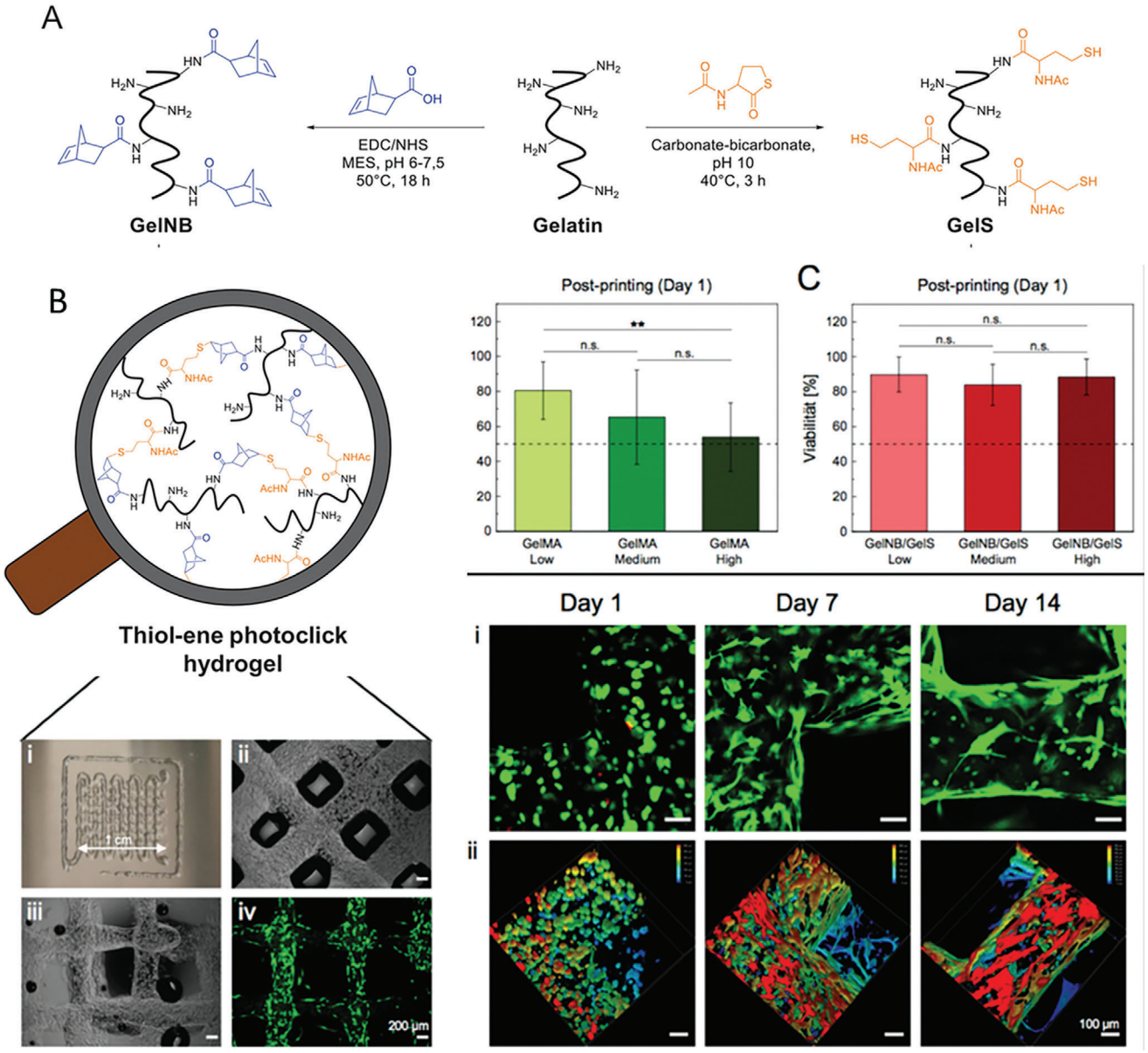


Figure 4. A) Synthesis of GelNB and GelS from gelatin precursor. B) 3D bioprinting of a GelNB/GelS hydrogel grid (1 cm × 1 cm) with four layers on a glass slide was performed. C) Cell viability analysis of 3D bioprinted neonatal human dermal fibroblasts (NHDF) was conducted using GelMA and GelNB/GelS with different degrees of crosslinking- Viability was analyzed by the percentage of live cells over the total cell count, and statistical analysis was performed using one-way ANOVA (Analysis of Variance). Live/dead staining with calcein-AM (green, live cells) and propidium iodide (red, dead cells) of 3D bioprinted NHDF in the GelNB/GelS (Medium) bioink was performed at 1, 7, and 14 days postprinting. Distribution of NHDF within the hydrogel along the *z*-axis was analyzed using *z*-stacks of 300 µm. Adapted with permission.[142] Copyright 2021, Wiley-VCH GmbH.

ratio, and thiolated crosslinker. VP has potential applications in high-throughput bioprinting, soft robotics, and regenerative medicine.

Norbornene-functionalized gelatin hydrogels have also been studied and compared to different thiol crosslinkers. Crosslinker-free hydrogel system based on norbornene (GelNB) and thiol-functionalized gelatin (GelS) has been developed, which showed increased differentiation of adipose-derived stem cells, mesenchymal stem cells, and other primary cells scaffolds[142,161] The photocrosslinking by intramolecular crosslinking is inhibited as the biofunctionalization is on different gelatin molecules. GelNB/GelS hydrogels (**Figure 4**) were successfully adapted to generate highly form-stable cartilage materials from mesenchymal and adipose stem cells in mice. The GelNB/GelS system can influence the differentiation fate of ASCs by adapting its mechanical properties. The softer hydrogel (GelNB/GelS-low) better supports dipogenic differentiation, while the harder hydrogel (GelNB/GelS-high) promotes osteogenic differentiation.[162]

Rizzo et al.[163] have recently adapted photoclick thiol-ene reactions to a new radical-free photocrosslinking strategy for

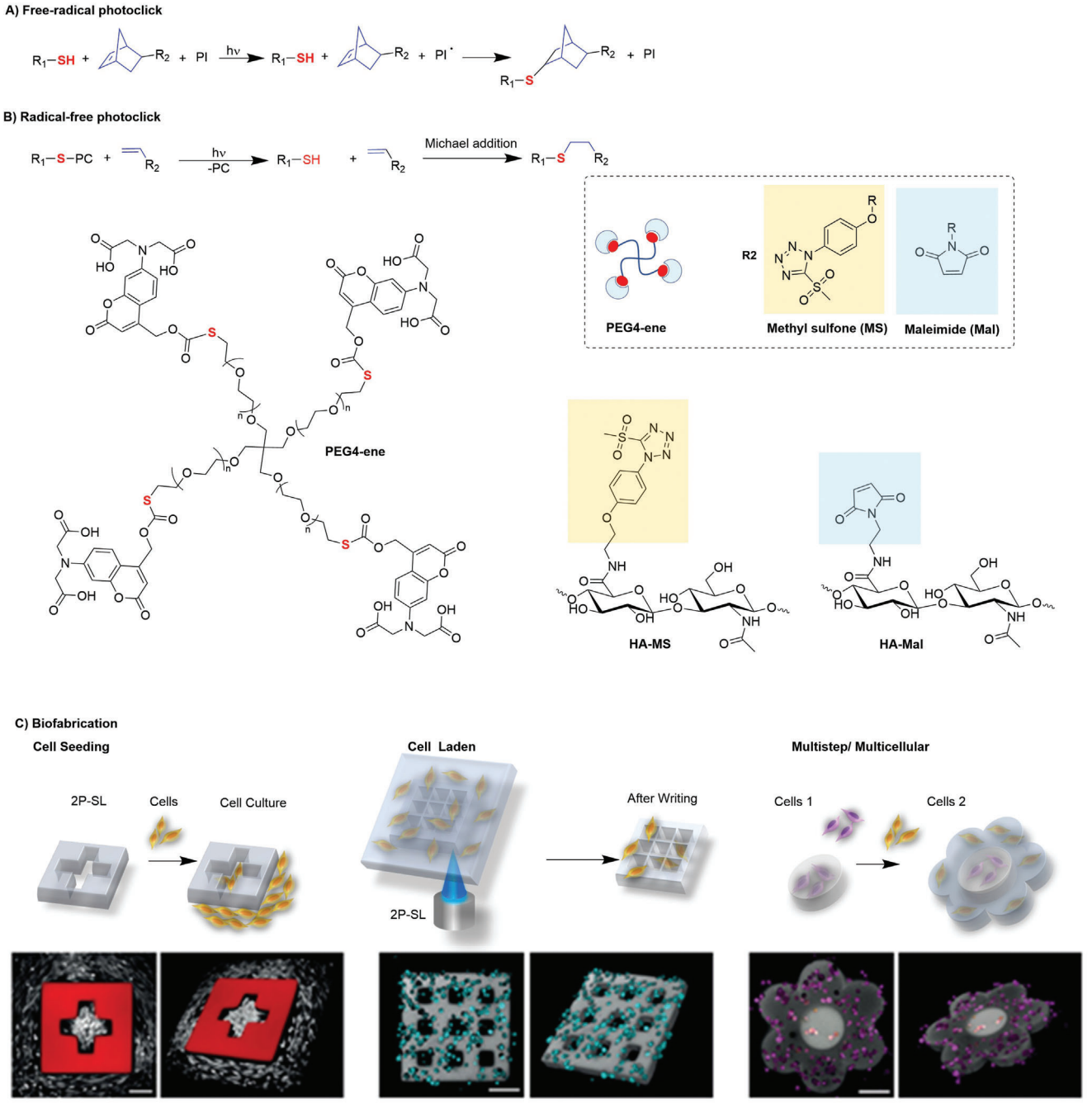
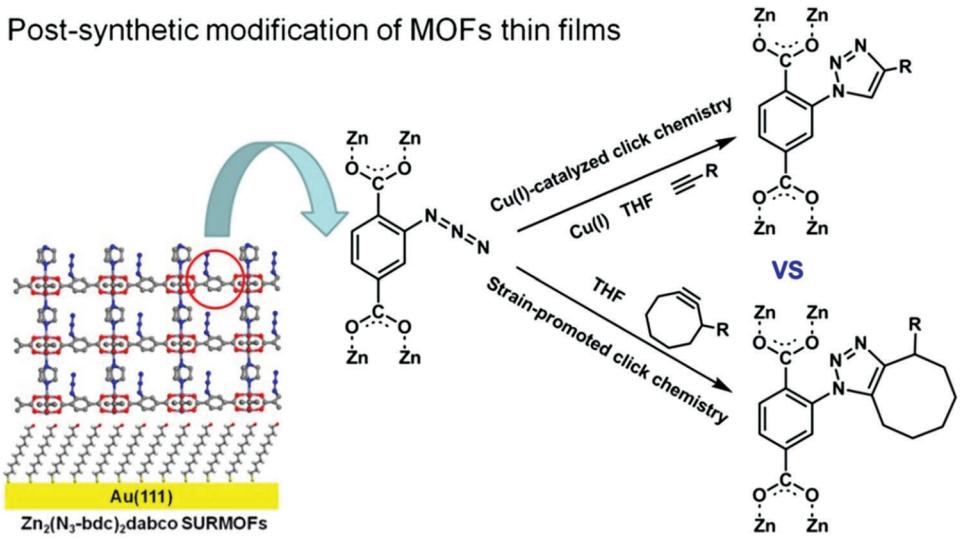


Figure 5. A) Thiol-norbornene step-growth chemistry is illustrated as an example of free-radical photo crosslinking. Upon light absorption, the photoinitiator radicals drive the formation of a crosslinked network. B) In the case of the free-radical (RF) approach upon light absorption, the photocages (PC) are removed in a radical-free fashion. This exposes thiols (red) to Michael addition with -ene functionalized polymers, forming a crosslinked hydrogel. Chemical structure of the crosslinker PEG4SPC. PEG4SH is caged with PC via the formation of a thiocarbonate bond. Upon one- or two-photon excitation, PC is removed, and PEG4SH is re-formed. The chemical structure of hyaluronic acid methyl sulfone (HA-MS) and hyaluronic acid maleimide (HA-Mal) (left) and photorheology (right) showed improved performances with the use of high molecular weight HA bearing -ene groups. C) 2PPi bioprinting cell seeding after writing or SL, writing from cell-laden hydrogels, and writing multifunctional cell-laden hydrogels. Adapted with permission.[163] Copyright 2021, Wiley-VCH GmbH.



Scheme 13. Surface-attached MOFs (SURMOFs) click chemistry. Adapted with permission. [172] Copyright 2015, American Chemical Society.

light-based biofabrication. As stated above, current photoresists in these techniques rely on photoinitiators that produce radical-initiating species, raising concerns about potential cytotoxicity when cells are encapsulated in the bio-ink. Alternatively, a photo-Michael addition can be applied, in which a coumarin-based hydrophilic group blocks a thiol-functionalized photocrosslinker, which is released by light and then reacts to form a hydrogel. The photocrosslinking is stable, does not upregulate ROS-associated genes, and was successfully used for high-resolution two-photon stereolithography (2P-SL) with low polymer concentration in the presence of several cell types, paving the way for radical-free light-based bioprinting (**Figure 5**).

8. Bioconjugated (in)Organic Materials in Additive Manufacturing

The past decade has witnessed rapid advancements in nanoparticle and surface modification technologies, especially in bioconjugated nanoparticles for medical and biological applications and surface sensors.[164] In particular, inorganic nanoparticles (NPs), including iron oxide NPs, gold NPs, quantum dots (QDs), and rare earth NPs, i.e., from gadolinium as well as silica NPs,[165] which possess intrinsic properties of the biological conjugate. Due to the vast amount of recent reviews[166] that exist for bioconjugates on NPs and surfaces, we will focus on their application in additive manufacturing processes. onic acid maleimide (HA-Mal) (left) and photorheology (right) showed improved performances with the use of high molecular weight HA bearing -ene groups.[144]

8.1. Graphene, Carbon Nanotubes and Dots, and Fullerenes

Graphene sheets and versatile graphene oxide (GO)[167] are suitable for bioconjugation. In particular, azide functionalizations can be installed easily and are a common platform for click chemistry – copper or strain-promoted.

Single-walled carbon nanotube (SWNT)[168] and carbon dots[168–169] can combine several advantageous properties of conventional semiconductor-based QDs (namely, size- and wavelength-dependent luminescence emission, resistance to photobleaching, facile bioconjugation) without being encumbered with intrinsic toxicity. Carbon nanotubes and carbon dots[169] can be produced inexpensively and on a large scale with several variants, including doping. There are several surface chemistries available, including polar functional groups. Boron-nitride nanotubes are similar to SWNT and can be modified, e,g, with polydopamine.[170]

8.2. Metal-Organic Frameworks

Biomaterials are often used in medical applications, such as implants, prosthetics, and drug delivery systems, where they are often composed of various materials, such as metals, ceramics, polymers, and composites. Inorganic hybrid biomaterials can also be designed for biosensors and bioelectronics. They are inherently designed to interact with biological systems to achieve a desired effect by attaching biomolecules, such as proteins, peptides, and nucleic acids, to the material. Besides, nanoparticulate systems that have been extensively used for bioconjugation are MOFs which lately got much attention regarding their use as biomaterials.[5b] MOFs are self-assembled structures composed of metal ions (nodes) and organic linkers. As MOFs contain a well-defined network of pores, they have a wide range of applications, such as gas storage, catalysis, and drug delivery. The biofunctionalization of the linker can already be introduced in the assembly process. Depending on the pore size of the MOF and the biopolymers, 2D (surface array biofunctionalization) and 3D assemblies are possible.[5a] However, some bioconjugation methods and/or biopolymers are metal sensitive (e.g., thiols, alkyne, and copper) – in this case, covalent frameworks or gels originated from MOF structures with subsequent leaching of the metal[171] are alternatives (**Scheme 13**).[171]

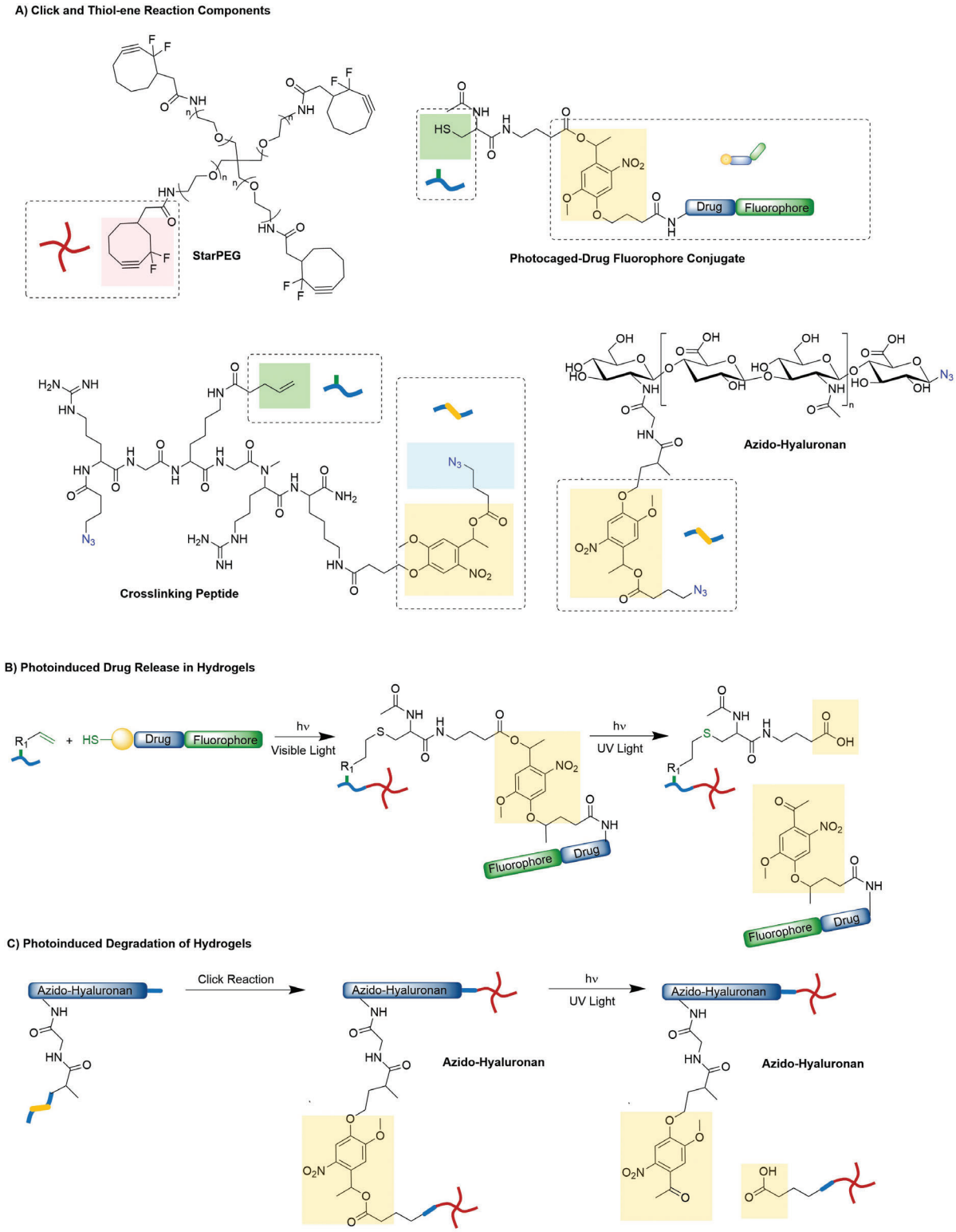


Figure 6. Mechanism of photo-controllable hydrogels for photoactivated drug release or photopatterning using o-NB moieties. (A) Click-functionalized macromolecular precursors (PEG-tetraDIFO3 and bis(azide)-functionalized polypeptides or HA oligosaccharides form a 3D hydrogel structure by polymerization via the SPAAC reaction. In the presence of visible light ($\lambda = 490–650$ nm or 860 nm), thiol-containing drugs/prodrugs biomolecules containing an o-NB group are added by a thiol-ene photoclick reaction. The o-NB group within either the backbone of the polymer network (C) or at the drug/prodrug (B) is photocleaved by UV-light exposure ($\lambda = 365$ nm or 740 nm (two-photon)) that results in degradation of the network (C) or drug release (B). Adapted with permission.[179] Copyright 2011, Springer Nature.

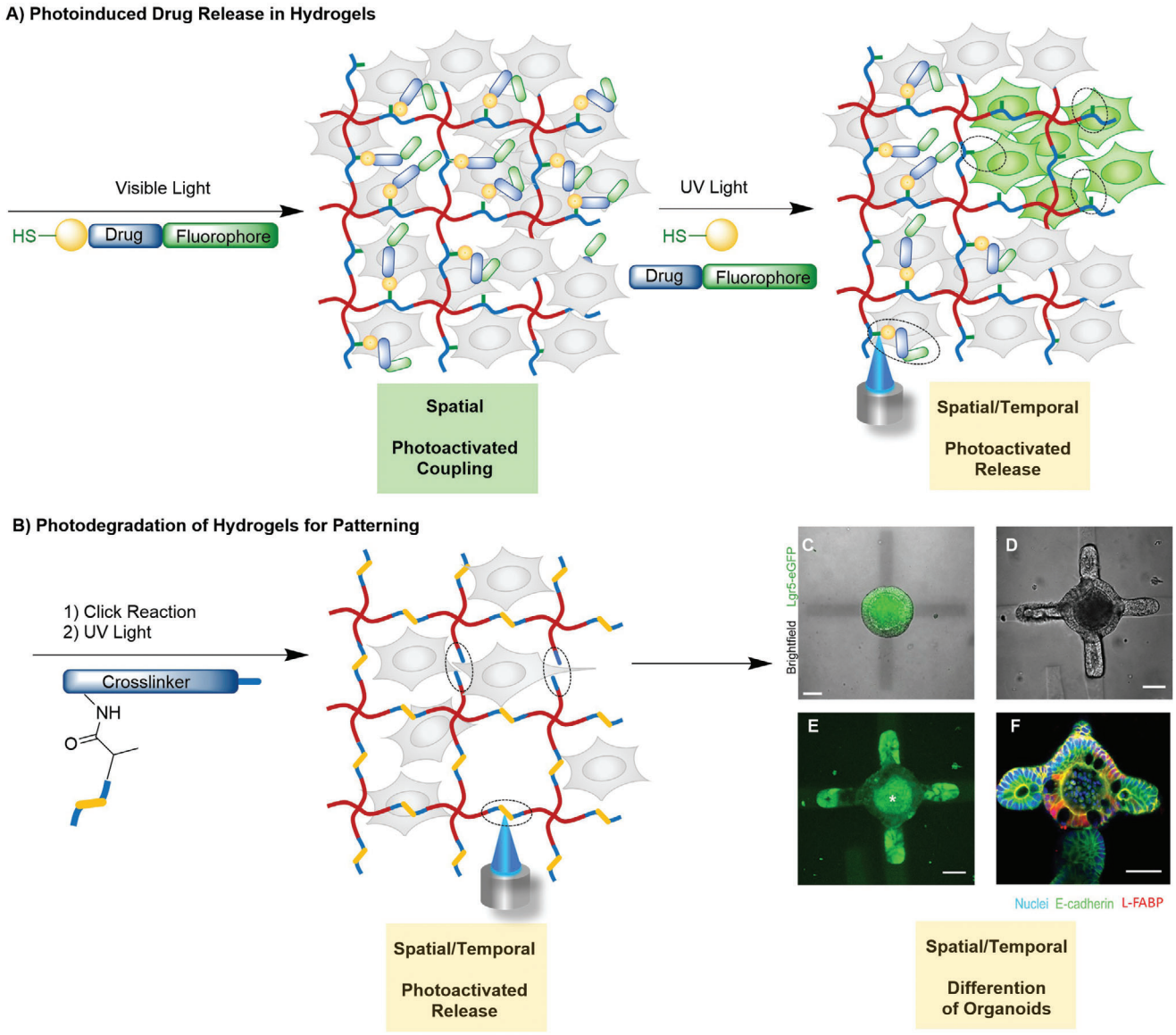


Figure 7. Spatial – and temporal control on drug release and organoid differentiation through photodegradation. Adapted with permission.[179] Copyright 2011, Springer Nature. A) subsequent to a Click reaction of StarPEG with the azide-containing crosslinker, photo click chemistry by thiol-ene chemistry is used to add prodrugs or drugs containing an o-NB group to the hydrogel/polymer using visible light. By using UV light, the o-NB group will be cleaved, and the drug is released into the hydrogel/ polymer and will be taken up by cells. B) subsequent to the Click reaction and the photopatterning/degradation of the o-NB group in the crosslinker using UV-light cells can use the tracks to differentiate and migrate. Adapted with permission.[180] Copyright 2022, American Association for the Advancement of Science. C) Composite image showing GFP expressing cells in a colon organoid derived from stem cells and the photopatterning by 405nm for the conversion of the photocleavable *ortho*-nitrobenzyl (*o*-NB) moieties within the crosslinker. D,E) After several hours there is the migration of the organoid epithelium and colon crypt formation. F) Staining of the differentiated crypt-forming organoid, Epithelium: VE-cadherin (green), Enterocytes (L-FABP stain) (red), Nuclei, DAPI stain (blue). Scale bars, 30 µm. Modified from Gjorevski et alAdapted with permission.[180] Copyright 2022, American Association for the Advancement of Science.

While MOFs can be biofunctionalized, others can be used to catalyze light-driven polymerization, such as photoinduced electron/energy transfer–reversible addition–fragmentation chain transfer (PET-RAFT) polymerization. PET-RAFT usually enables benign biological conditions and a highly versatile functional polymer structure design, which can give rise to various protein–polymer bioconjugates. MOFs can replace the classical catalysts, which are usually not water soluble. It was demonstrated that a PET-RAFT photocatalyst may be constructed from reticular assembled Zr–porphyrinic MOF.[173]

8.3. Covalent Organic Frameworks

In analogy to MOF, COF [174] (15.000 references) and the related metal-free three-dimensional cages and frames are slightly less

developed. Similar to MOFs COFs have attracted much attention in gas adsorption, chemical sensing, and heterogeneous catalysis, but also in protein immobilization to mimic enzymes. COFs serve as a highly suitable immobilization matrix for enzymes and proteins since the functional groups on COFs can be easily tailored to generate specific interactions between COFs and enzymes. Due to the confined open channels and pores of COFs, they also provide a favorable microenvironment for enzyme penetration. For these reasons, COFs serve as ideal host materials for enzymes. The resulting biocomposites – also referred to as enzyme-COF,[175] enzyme@COF,[176] or nanozymes[177] – are mostly applied in biocatalysis.

Those biocomposites of COFs and enzymes are designed either by "from bottom to top", postbioconjugation, or in situ encapsulation methods.[178] However, there are hardly any examples of bioconjugations.

9. Photolabile Polymers

Bioconjugations with photolabile functional groups have a lot of possible applications in chemical research and synthesis. Attaching these to polymeric structures may alter and adjust the polymer's properties simply by irradiation. A group of special interest in this context is the *o*-nitrobenzyl group (o-NB), as it is frequently used in polymer synthesis and materials sciences and degrades by photoinduced intramolecular hydrogen abstraction. Light-responsive materials containing o-NB esters can be assembled using linear and functionalized macromonomers in click reaction of star polymers containing cyclooctyne or azide moieties[179] (**Figures 6** and 7). o-NB functionalized crosslinkers, drug/prodrug moieties, or even the star polymer itself can be used for many different applications, i.e., spatiotemporal drug release in hydrogels, alteration, and photopatterning of hydrogels for organoid maturation. Anseth and coworkers showed the versatility of o-NB by using a combination of SPAAC and photoclick thiol-ene chemistry for the photopatterning of hydrogels,[179] SPAAC of starPEG with either peptide crosslinkers containing aa o-NB linker generates a photodegradable polymeric network, which can finally be exposed to UV light and degraded to linear polymers (Figures 6). Lutolf and coworkers used this photodegradable hydrogel to design crypts for the maturation of small intestine organoids in defined shapes (**Figure 7**).[180] These adaptable cell culture environments would enable the execution of novel experiments and provide insights into the dynamic communication between a cell in an organoid and its surrounding niche as well will give rise to control organoid maturation.

These photo-controllable approaches have also been tested for block copolymers like micelles.[181] This may be transferred to research areas like controlled and light-induced release. Incorporated compounds, like dyes or drugs, in block copolymer micelles, will be liberated upon irradiation and thus disruption of the micelles.

10. Conclusion

Biomolecules have played a crucial role in expanding macromolecular science. Especially for 3D bioprinting, biomaterials are needed to display biological and chemical cues for cell adhesion or repulsion, differentiation of stem cells, matrix for tissue engineering, etc.. Technical breakthroughs in synthesis are necessary to access new biohybrid architectures with designed functionalities. Especially light-induced bioconjugation methods such as the photoclick reactions are gaining more and more attention. The orthogonality of click and photoclick reactions allows for their varied applications. Photoclick chemistry has expanded the research toolbox and is expected to remain relevant in the future for 3D additive manufacturing using all kinds of printing techniques. With this advent of finer and faster printing techniques, bioconjugation can provide many opportunities to voxel-based, i.e., a segment in 3D, spatial arrangement.

As stated in Chapter 2, many challenges and opportunities can be seen in the next 5 to 10 years. In this regard, we look very positively to a new chapter of the interaction between materials and life sciences.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements

This research has been funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) under Germany's Excellence Strategy via the Excellence Cluster "3D Matter Made to Order" (3DMM2O, EXC-2082/1-390761711, Thrusts A1, A3, and C3). SB further acknowledges the Karlsruhe School of Optics & Photonics (KSOP) support.

Open access funding enabled and organized by Projekt DEAL.

Conflict of Interest

The authors declare no conflict of interest.

Keywords

3D printing, bioconjugation, bioorganic chemistry, biorthogonal, click chemistry, graphene oxide, ligation, MOFs

Received: March 31, 2023
Revised: June 10, 2023
Published online:

[1] S. L. Scinto, D. A. Bilodeau, R. Hincapie, W. Lee, S. S. Nguyen, M. Xu, C. W. am Ende, M. G. Finn, K. Lang, Q. Lin, J. P. Pezacki, J. A. Prescher, M. S. Robillard, J. M. Fox, *Nat. Rev. Methods Primers* **2021**, *1*, 30.

[2] C. L. Zaccaria, V. Cedrati, A. Nitti, E. Chiesa, A. Martinez de Ilarduya, M. Garcia-Alvarez, M. Meli, G. Colombo, D. Pasini, *Polym. Chem.* **2021**, *12*, 3784.

[3] A. Pacini, M. Caricato, S. Ferrari, D. Capsoni, A. Martínez de Ilarduya, S. Muñoz-Guerra, D. Pasini, *J. Polym. Sci., Part A: Polym. Chem.* **2012**, *50*, 4790.

[4] V. Cedrati, A. Pacini, A. Nitti, A. Martínez de Ilarduya, S. Muñoz-Guerra, A. Sanyal, D. Pasini, *Polym. Chem.* **2020**, *11*, 5582.

[5] a) S. Begum, Z. Hassan, S. Bräse, C. Wöll, M. Tsotsalas, *Acc. Chem. Res.* **2019**, *52*, 1598; b) C. Liu, T. Li, N. L. Rosi, *J. Am. Chem. Soc.* **2012**,

134, 18886; c) N. Z. Fantoni, A. H. El-Sagheer, T. Brown, *Chem. Rev.* **2021**, *121*, 7122; d) X.-P. He, Y.-L. Zeng, Y. Zang, J. Li, R. A. Field, G.-R. Chen, *Carbohydr. Res.* **2016**, *429*, 1; e) E. Hui, J. L. Sumey, S. R. Caliari, *Mol. Syst. Des. Eng.* **2021**, *6*, 670; f) D. Dohler, P. Michael, W. H. Binder, *Acc. Chem. Res.* **2017**, *50*, 2610; g) C. Bednarek, I. Wehl, N. Jung, U. Schepers, S. Bräse, *Chem. Rev.* **2020**, *120*, 4301; h) A. Beloqui, S. R. Mane, M. Langer, M. Glassner, D. M. Bauer, L. Fruk, C. Barner-Kowollik, G. Delaittre, *Angew. Chem., Int. Ed.* **2020**, *59*, 19951.

[6] a) M. W. Jones, G. Mantovani, C. A. Blindauer, S. M. Ryan, X. X. Wang, D. J. Brayden, D. M. Haddleton, *J. Am. Chem. Soc.* **2012**, *134*, 7406; b) J. Konc, L. Brown, D. R. Whiten, Y. Zuo, P. Ravn, D. Klenerman, G. J. L. Bernardes, *Angew. Chem. Int. Ed. Engl.* **2021**, *60*, 25905; c) C. Hojnik, A. Muller, T. E. Gloe, T. K. Lindhorst, T. M. Wrodnigg, *Eur. J. Org. Chem.* **2016**, *2016*, 4328.

[7] C. Z. Chung, K. Amikura, D. Söll, *Front. Bioeng. Biotechnol.* **2020**, *8*, 598577.

[8] a) M. Nerantzaki, C. Loth, J.-F. Lutz, *Polym. Chem.* **2021**, *12*, 3498; b) H. Lu, J. Cai, K. Zhang, *Polym. Chem.* **2021**, *12*, 2193; c) W. Xu, W. He, Z. Du, L. Zhu, K. Huang, Y. Lu, Y. Luo, *Angew. Chem. Int. Ed. Engl.* **2021**, *60*, 6890.

[9] A. Khvorova, J. K. Watts, *Nat. Biotechnol.* **2017**, *35*, 238.

[10] M. Kufleitner, L. M. Haiber, V. Wittmann, *Chem Soc Rev.* **2023**, *52*, 510.

[11] E. Saxon, C. R. Bertozzi, *Science* **2000**, *287*, 2007.

[12] a) P.-H. Kuhn, K. Koroniak, S. Hogl, A. Colombo, U. Zeitschel, M. Willem, C. Volbracht, U. Schepers, A. Imhof, A. Hoffmeister, C. Haass, S. Rossner, S. Bräse, S. F. Lichtenthaler, *EMBO J.* **2012**, *31*, 3157; b) P.-H. Kuhn, M. Voss, M. Haug-Kroeper, B. Schroeder, U. Schepers, S. Bräse, C. Haass, S. F. Lichtenthaler, R. Fluhrer, *Mol. Cell. Proteomics* **2015**, *14*, 1584; c) A. Serdaroglu, S. A. Mueller, U. Schepers, S. Bräse, W. Weichert, S. F. Lichtenthaler, P.-H. Kuhn, *Proteomics* **2017**, *17*, 1600423; d) J. Herber, J. Njavro, R. Feederle, U. Schepers, U. C. Mueller, S. Bräse, S. A. Mueller, S. F. Lichtenthaler, *Mol. Cell. Proteomics* **2018**, *17*, 1487.

[13] M. Guberman, P. H. Seeberger, *J. Am. Chem. Soc.* **2019**, *141*, 5581.

[14] L. B. Poole, *Free Radic Biol Med* **2015**, *80*, 1.

[15] P. E. Dawson, T. W. Muir, I. Clark-Lewis, S. B. H. Kent, *Science* **1994**, *266*, 776.

[16] a) H. Lindley, *Biochem. J.* **1962**, *82*, 418; b) A. M. Spokoyny, Y. Zou, J. J. Ling, H. Yu, Y.-S. Lin, B. L. Pentelute, *J. Am. Chem. Soc.* **2013**, *135*, 5946.

[17] J. Ravasco, H. Faustino, A. Trindade, P. M. P. Gois, *Chemistry* **2019**, *25*, 43.

[18] a) P. Ochtrop, C. P. R. Hackenberger, *Curr. Opin. Chem. Biol.* **2020**, *58*, 28; b) F. J. Chen, J. Gao, *Chemistry* **2022**, *28*, e202201843.

[19] N. J. Smith, K. Rohlfing, L. A. Sawicki, P. M. Kharkar, S. J. Boyd, A. M. Kloxin, J. M. Fox, *Org. Biomol. Chem.* **2018**, *16*, 2164.

[20] a) B. Bernardim, P. M. S. D. Cal, M. J. Matos, B. L. Oliveira, N. Martínez-Sáez, I. S. Albuquerque, E. Perkins, F. Corzana, A. C. B. Burtoloso, G. Jiménez-Osés, G. J. L. Bernardes, *Nat. Commun.* **2016**, *7*, 13128; b) M. J. Matos, C. D. Navo, T. Hakala, X. Ferhati, A. Guerreiro, D. Hartmann, B. Bernardim, K. L. Saar, I. Compañón, F. Corzana, T. P. J. Knowles, G. Jiménez-Osés, G. J. L. Bernardes, *Angew. Chem., Int. Ed.* **2019**, *58*, 6640.

[21] Y. Zhang, X. Zhou, Y. Xie, M. M. Greenberg, Z. Xi, C. Zhou, *J. Am. Chem. Soc.* **2017**, *139*, 6146.

[22] Y. Zhang, C. Zang, G. An, M. Shang, Z. Cui, G. Chen, Z. Xi, C. Zhou, *Nat. Commun.* **2020**, *11*, 1015.

[23] a) H. F. Motiwala, Y.-H. Kuo, B. L. Stinger, B. A. Palfey, B. R. Martin, *J. Am. Chem. Soc.* **2020**, *142*, 1801; b) Q. Luo, Y. Tao, W. Sheng, J. Lu, H. Wang, *Nat. Commun.* **2019**, *10*, 142.

[24] a) R. Frei, M. D. Wodrich, D. P. Hari, P.-A. Borin, C. Chauvier, J. Waser, *J. Am. Chem. Soc.* **2014**, *136*, 16563; b) E. M. D. Allouche, E. Grinhagena, J. Waser, *Angew. Chem., Int. Ed.* **2022**, *61*, e202112287; c) F.-J. Chen, M. Zheng, V. Nobile, J. Gao, *Chemistry* **2022**, *28*, e202200058.

[25] D. K. Kölmel, E. T. Kool, *Chem. Rev.* **2017**, *117*, 10358.

[26] a) H. F. Gaertner, K. Rose, R. Cotton, D. Timms, R. Camble, R. E. Offord, *Bioconjugate Chem.* **1992**, *3*, 262; b) A. Dirksen, T. M. Hackeng, P. E. Dawson, *Angew. Chem., Int. Ed.* **2006**, *45*, 7581.

[27] J. Kalia, R. T. Raines, *Angew. Chem., Int. Ed.* **2008**, *47*, 7523.

[28] a) S. Mukherjee, M. R. Hill, B. S. Sumerlin, *Soft Matter* **2015**, *11*, 6152; b) J. Collins, M. Nadgorny, Z. Xiao, L. A. Connal, *Macromol. Rapid Commun.* **2017**, *38*, 1600760.

[29] a) E. Saxon, J. I. Armstrong, C. R. Bertozzi, *Org. Lett.* **2000**, *2*, 2141; b) B. L. Nilsson, L. L. Kiessling, R. T. Raines, *Org. Lett.* **2000**, *2*, 1939; c) R. Kleineweischede, C. P. Hackenberger, *Angew. Chem., Int. Ed.* **2008**, *47*, 5984.

[30] S. Xie, M. Sundhoro, K. N. Houk, M. Yan, *Acc. Chem. Res.* **2020**, *53*, 937.

[31] a) F. L. Lin, H. M. Hoyt, H. van Halbeek, R. G. Bergman, C. R. Bertozzi, *J. Am. Chem. Soc.* **2005**, *127*, 2686; b) R. A. Wiley, L. A. Sternson, H. A. Sasame, J. R. Gillette, *Biochem. Pharmacol.* **1972**, *21*, 3235.

[32] a) W. Luo, P. Gobbo, P. N. Gunawardene, M. S. Workentin, *Langmuir* **2017**, *33*, 1908; b) W. Luo, J. Luo, V. V. Popik, M. S. Workentin, *Bioconjugate Chem.* **2019**, *30*, 1140.

[33] a) V. V. Rostovtsev, L. G. Green, V. V. Fokin, K. B. Sharpless, *Angew. Chem., Int. Ed.* **2002**, *41*, 2596; b) C. W. Tornøe, C. Christensen, M. Meldal, *J. Org. Chem.* **2002**, *67*, 3057.

[34] a) E. Lallana, R. Riguera, E. Fernandez-Megia, *Angew. Chem., Int. Ed.* **2011**, *50*, 8794; b) S. Neumann, M. Biewend, S. Rana, W. H. Binder, *Macromol. Rapid Commun.* **2020**, *41*, 1900359.

[35] a) F. Himo, T. Lovell, R. Hilgraf, V. V. Rostovtsev, L. Noodleman, K. B. Sharpless, V. V. Fokin, *J. Am. Chem. Soc.* **2005**, *127*, 210; b) S. I. Presolski, V. Hong, S.-H. Cho, M. G. Finn, *J. Am. Chem. Soc.* **2010**, *132*, 14570; c) L. Jin, D. R. Tolentino, M. Melaimi, G. Bertrand, *Sci. Adv.* **2015**, *1*, e1500304.

[36] a) S. Chassaing, V. Bénéteau, P. Pale, *Catal. Sci. Technol.* **2016**, *6*, 923; b) T. R. Chan, R. Hilgraf, K. B. Sharpless, V. V. Fokin, *Org. Lett.* **2004**, *6*, 2853; c) Y. Jiang, X. He, W. Zhang, X. Li, N. Guo, Y. Zhao, G. Xu, W. Li, *RSC Adv.* **2015**, *5*, 73340; d) Q. Fu, K. Xie, S. Tan, J. M. Ren, Q. Zhao, P. A. Webley, G. G. Qiao, *Chem. Commun.* **2016**, *52*, 12226; e) E.-H. Ablouh, L. Bahsis, H. Sehaqui, H. Anane, M. Julve, S.-E. Stiriba, M. El Achaby, *Sustain Chem Pharm* **2022**, *30*, 100837.

[37] a) F. F. Loeffler, T. C. Foertsch, R. Popov, D. S. Mattes, M. Schlageter, M. Sedlmayr, B. Ridder, F.-X. Dang, C. von Bojnicic-Kninski, L. K. Weber, A. Fischer, J. Greifenstein, V. Bykovskaya, I. Buliev, F. R. Bischoff, L. Hahn, M. A. R. Meier, S. Bräse, A. K. Powell, T. S. Balaban, F. Breitling, A. Nesterov-Mueller, *Nat. Commun.* **2016**, *7*, 11844; b) W. M. Rink, F. Thomas, *Org. Lett.* **2018**, *20*, 7493; c) Q. Wang, T. R. Chan, R. Hilgraf, V. V. Fokin, K. B. Sharpless, M. G. Finn, *J. Am. Chem. Soc.* **2003**, *125*, 3192.

[38] a) V. Hong, S. I. Presolski, C. Ma, M. G. Finn, *Angew. Chem., Int. Ed.* **2009**, *48*, 9879; b) V. Hong, N. F. Steinmetz, M. Manchester, M. G. Finn, *Bioconjugate Chem.* **2010**, *21*, 1912; c) D. Soriano Del Amo, W. Wang, H. Jiang, C. Besanceney, A. C. Yan, M. Levy, Y. Liu, F. L. Marlow, P. Wu, *J. Am. Chem. Soc.* **2010**, *132*, 16893.

[39] D. C. Kennedy, C. S. McKay, M. C. B. Legault, D. C. Danielson, J. A. Blake, A. F. Pegoraro, A. Stolow, Z. Mester, J. P. Pezacki, *J. Am. Chem. Soc.* **2011**, *133*, 17993.

[40] a) N. J. Agard, J. A. Prescher, C. R. Bertozzi, *J. Am. Chem. Soc.* **2004**, *126*, 15046; b) J. M. Baskin, J. A. Prescher, S. T. Laughlin, N. J. Agard, P. V. Chang, I. A. Miller, A. Lo, J. A. Codelli, C. R. Bertozzi, *Proc. Nat. Acad. Sci., USA* **2007**, *104*, 16793.

[41] E. Chupakhin, M. Y. Krasavin, *Chem. Heterocycl. Compd.* **2018**, *54*, 483.

[42] a) J. Dommerholt, S. Schmidt, R. Temming, L. J. Hendriks, F. P. Rutjes, J. C. van Hest, D. J. Lefeber, P. Friedl, F. L. van Delft, *Angew. Chem., Int. Ed.* **2010**, *49*, 9422; b) G. de Almeida, E. M. Sletten, H. Nakamura, K. K. Palaniappan, C. R. Bertozzi, *Angew. Chem., Int. Ed.* **2012**, *51*, 2443; c) J. Weterings, C. J. F. Rijcken, H. Veldhuis, T. Meulemans, D. Hadavi, M. Timmers, M. Honing, H. Ippel, R. M. J. Liskamp, *Chem. Sci.* **2020**, *11*, 9011.

[43] a) K. Gutsmiedl, C. T. Wirges, V. Ehmke, T. Carell, *Org. Lett.* **2009**, *11*, 2405; b) C. S. McKay, J. Moran, J. P. Pezacki, *Chem. Commun.* **2010**, *46*, 931; c) C. S. McKay, J. A. Blake, J. Cheng, D. C. Danielson, J. P. Pezacki, *Chem. Commun.* **2011**, *47*, 10040; d) N. A. McGrath, R. T. Raines, *Chem. Sci.* **2012**, *3*, 3237.

[44] K. Porte, M. Riomet, C. Figliola, D. Audisio, F. Taran, *Chem. Rev.* **2021**, *121*, 6718.

[45] J. G. d. l. C. R. F. Martínez, P. Cintas, J. L. Jiménez, *Targets Heterocycl. Syst.* **2018**, *21*, 228.

[46] S. Kolodych, E. Rasolofonjatovo, M. Chaumontet, M. C. Nevers, C. Creminon, F. Taran, *Angew. Chem., Int. Ed.* **2013**, *52*, 12056.

[47] E. Decuypere, M. Riomet, A. Sallustrau, S. Bregant, R. Thai, G. Pieters, G. Clavier, D. Audisio, F. Taran, *Chem. Commun.* **2018**, *54*, 10758.

[48] E. Decuypere, S. Bernard, M. Feng, K. Porte, M. Riomet, P. Thuéry, D. Audisio, F. Taran, *ACS Catal.* **2018**, *8*, 11882.

[49] R. J. Brea, N. K. Devaraj, in *Chemoselective and Bioorthogonal Ligation Reactions: Concepts and Applications*, 1st ed. (Eds: W. R. Algar, I. L. Medintz, P. E. Dawson), Wiley-VCH, Weinheim, Germany **2017**, pp. 67.

[50] O. Diels, K. Alder, *Justus Liebigs Ann. Chem.* **1928**, *460*, 98.

[51] D. C. Rideout, R. Breslow, *J. Am. Chem. Soc.* **1980**, *102*, 7816.

[52] A. Gandini, *Prog. Polym. Sci.* **2013**, *38*, 1.

[53] M. Gregoritza, F. P. Brandl, *Eur. J. Pharm. Biopharm.* **2015**, *97*, 438.

[54] S. Kirchhof, A. Strasser, H. J. Wittmann, V. Messmann, N. Hammer, A. M. Goepferich, F. P. Brandl, *J. Mater. Chem. B* **2015**, *3*, 449.

[55] L. Hahn, M. Beudert, M. Gutmann, L. Keßler, P. Stahlhut, L. Fischer, T. Lorson, I. Thievessen, T. Lühmann, R. Luxenhofer, *ChemRxiv. Cambridge: Cambridge Open Engage* **2021**.

[56] a) R. A. Carboni, R. V. Lindsey, Jr., *J. Am. Chem. Soc.* **1959**, *81*, 4342; b) J. Zhang, V. Shukla, D. L. Boger, *J. Org. Chem.* **2019**, *84*, 9397.

[57] a) J. Sauer, D. K. Heldmann, J. Hetzenegger, J. Krauthan, H. Sichert, J. Schuster, *Eur. J. Org. Chem.* **1998**, *1998*, 2885; b) H. Wu, N. K. Devaraj, *Acc. Chem. Res.* **2018**, *51*, 1249.

[58] a) A.-C. Knall, C. Slugovc, *Chem. Soc. Rev.* **2013**, *42*, 5131; b) J. Yang, Y. Liang, J. Šečkutė, K. N. Houk, N. K. Devaraj, *Chemistry* **2014**, *20*, 3365; c) R. Zhang, J. Gao, G. Zhao, L. Zhou, F. Kong, T. Jiang, H. Jiang, *Nanoscale* **2023**, *15*, 461.

[59] a) T. I. Chio, H. Gu, K. Mukherjee, L. N. Tumey, S. L. Bane, *Bioconjugate Chem.* **2019**, *30*, 1554; b) M. L. W. J. Smeenk, J. Agramunt, K. M. Bonger, *Curr. Opin. Chem. Biol.* **2021**, *60*, 79; c) L. I. Willems, N. Li, B. I. Florea, M. Ruben, G. A. van der Marel, H. S. Overkleeft, *Angew. Chem., Int. Ed.* **2012**, *51*, 4431.

[60] D. G. Hall, in *Boronic Acids* (Ed.: D. G. Hall), Wiley-VCH, Weinheim, Germany, **2011**, pp. 1-133.

[61] J. P. Lorand, J. O. Edwards, *J. Org. Chem.* **1959**, *24*, 769.

[62] a) G. Springsteen, B. Wang, *Tetrahedron* **2002**, *58*, 5291; b) J. Yan, G. Springsteen, S. Deeter, B. Wang, *Tetrahedron* **2004**, *60*, 11205.

[63] a) X. Sun, W. Zhai, J. S. Fossey, T. D. James, *Chem. Commun.* **2016**, *52*, 3456; b) X. Zhou, X. Gao, F. Song, C. Wang, F. Chu, S. Wu, *Appl. Surf. Sci.* **2017**, *423*, 810; c) B. Akgun, D. G. Hall, *Angew. Chem., Int. Ed.* **2018**, *57*, 13028.

[64] a) J. W. Tomsho, A. Pal, D. G. Hall, S. J. Benkovic, *ACS Med. Chem. Lett.* **2012**, *3*, 48; b) G. Wulff, M. Lauer, H. Böhnke, *Angew. Chem., Int. Ed.* **1984**, *23*, 741.

[65] a) S. D. Bull, M. G. Davidson, J. M. H. van den Elsen, J. S. Fossey, A. T. A. Jenkins, Y.-B. Jiang, Y. Kubo, F. Marken, K. Sakurai, J. Zhao, T. D. James, *Acc. Chem. Res.* **2013**, *46*, 312; b) W. L. A. Brooks, B. S. Sumerlin, *Chem. Rev.* **2016**, *116*, 1375.

[66] a) O. Dilek, Z. Lei, K. Mukherjee, S. Bane, *Chem. Commun.* **2015**, *51*, 16992; b) C. J. Stress, P. J. Schmidt, D. G. Gillingham, *Org. Biomol. Chem.* **2016**, *14*, 5529.

[67] a) A. Ojida, H. Tsutsumi, N. Kasagi, I. Hamachi, *Tetrahedron Lett.* **2005**, *46*, 3301; b) J. M. Chalker, C. S. C. Wood, B. G. Davis, *J. Am. Chem. Soc.* **2009**, *131*, 16346; c) J. Ohata, Y. Zeng, L. Segatori, Z. T. Ball, *Angew. Chem., Int. Ed.* **2018**, *57*, 4015.

[68] G. S. Kumar, Q. Lin, *Chem. Rev.* **2021**, *121*, 6991.

[69] A. K. Sinha, D. Equbal, *Asian J. Org. Chem.* **2019**, *8*, 32.

[70] a) C. E. Hoyle, C. N. Bowman, *Angew. Chem., Int. Ed.* **2010**, *49*, 1540; b) C. C. Cook, E. J. Fong, J. J. Schwartz, D. H. Porcincula, A. C. Kaczmarek, J. S. Oakdale, B. D. Moran, K. M. Champley, C. M. Rackson, A. Muralidharan, R. R. McLeod, M. Shusteff, *Adv. Mater.* **2020**, *32*, 2003376; c) C. Resetco, B. Hendriks, N. Badi, F. Du Prez, *Mater. Horiz.* **2017**, *4*, 1041.

[71] a) B. Colak, J. C. S. Da Silva, T. A. Soares, J. E. Gautrot, *Bioconjugate Chem.* **2016**, *27*, 2111; b) P. Derboven, D. R. D'hooge, M. M. Stamenovic, P. Espeel, G. B. Marin, F. E. Du Prez, M.-F. Reyniers, *Macromolecules* **2013**, *46*, 1732.

[72] a) M. D. Nolan, E. M. Scanlan, *Front. Chem.* **2020**, *8*, 583272; b) H. Choi, M. Kim, J. Jang, S. Hong, *Angew. Chem., Int. Ed.* **2020**, *59*, 22514.

[73] a) A. Dondoni, A. Marra, *Chem. Soc. Rev.* **2012**, *41*, 573; b) M. Buhl, B. Vonhören, B. J. Ravoo, *Bioconjugate Chem.* **2015**, *26*, 1017; c) Y. Liu, W. Hou, H. Sun, C. Cui, L. Zhang, Y. Jiang, Y. Wu, Y. Wang, J. Li, B. S. Sumerlin, Q. Liu, W. Tan, *Chem. Sci.* **2017**, *8*, 6182; d) J. C. Grim, T. E. Brown, B. A. Aguado, D. A. Chapnick, A. L. Viert, X. Liu, K. S. Anseth, *ACS Cent. Sci.* **2018**, *4*, 909.

[74] C. Wan, Z. Hou, D. Yang, Z. Zhou, H. Xu, Y. Wang, C. Dai, M. Liang, J. Meng, J. Chen, F. Yin, R. Wang, Z. Li, *Chem. Sci.* **2023**, *14*, 604.

[75] P. E. Farahani, S. M. Adelmund, J. A. Shadish, C. A. DeForest, *J. Mater. Chem. B* **2017**, *5*, 4435.

[76] T. Pauloehrl, G. Delaittre, M. Bruns, M. Meißler, H. G. Börner, M. Bastmeyer, C. Barner-Kowollik, *Angew. Chem., Int. Ed.* **2012**, *51*, 9181.

[77] V. X. Truong, C. Barner-Kowollik, *ACS Macro Lett.* **2021**, *10*, 78.

[78] a) P. Hu, K. Berning, Y. W. Lam, I. H. Ng, C. C. Yeung, M. H. Lam, *J. Org. Chem.* **2018**, *83*, 12998; b) P. Hu, T. Feng, C. C. Yeung, C. K. Koo, K. C. Lau, M. H. Lam, *Chemistry* **2016**, *22*, 11537.

[79] K. Kalayci, H. Frisch, C. Barner-Kowollik, V. X. Truong, *Chem. Commun.* **2022**, *58*, 6397.

[80] a) R. Huisgen, M. Seidel, J. Sauer, J. McFarland, G. Wallbillich, *J. Org. Chem.* **1959**, *24*, 892; b) J. S. Clovis, A. Eckell, R. Huisgen, R. Sustmann, *Chem. Ber.* **1967**, *100*, 60.

[81] X. S. Wang, Y.-J. Lee, W. R. Liu, *Chem. Commun.* **2014**, *50*, 3176.

[82] V. Pirota, A. Benassi, F. Doria, *Photochem. Photobiol. Sci.* **2022**, *21*, 879.

[83] a) Y. Wang, W. Song, W. J. Hu, Q. Lin, *Angew. Chem., Int. Ed.* **2009**, *48*, 5330; b) Z. Yu, R. K. V. Lim, Q. Lin, *Chemistry* **2010**, *16*, 13325; c) E. Blasco, Y. Sugawara, P. Lederhose, J. P. Blinco, A.-M. Kelterer, C. Barner-Kowollik, *ChemPhotoChem* **2017**, *1*, 159.

[84] a) P. An, T. M. Lewandowski, T. G. Erbay, P. Liu, Q. Lin, *J. Am. Chem. Soc.* **2018**, *140*, 4860; b) P. An, Q. Lin, *Org. Biomol. Chem.* **2018**, *16*, 5241.

[85] a) Y. Wang, W. J. Hu, W. Song, R. K. V. Lim, Q. Lin, *Org. Lett.* **2008**, *10*, 3725; b) P. An, Z. Yu, Q. Lin, *Org. Lett.* **2013**, *15*, 5496; c) Z. Yu, L. Y. Ho, Z. Wang, Q. Lin, *Bioorg. Med. Chem. Lett.* **2011**, *21*, 5033; d) P. Lederhose, K. N. R. Wüst, C. Barner-Kowollik, J. P. Blinco, *Chem. Commun.* **2016**, *52*, 5928.

[86] P. An, Z. Yu, Q. Lin, *Chem. Commun.* **2013**, *49*, 9920.

[87] G. S. Kumar, S. Racioppi, E. Zurek, Q. Lin, *J. Am. Chem. Soc.* **2022**, *144*, 57.
[88] S. Arndt, H.-A. Wagenknecht, *Angew. Chem., Int. Ed.* **2014**, *53*, 14580.
[89] V. F. Schart, J. Hassenrück, A.-K. Späte, J. E. G. A. Dold, R. Fahrner, V. Wittmann, *ChemBioChem* **2019**, *20*, 166.
[90] E. Blasco, M. Wegener, C. Barner-Kowollik, *Adv. Mater.* **2017**, *29*, 1604005.
[91] a) M. Dietrich, G. Delaittre, J. P. Blinco, A. J. Inglis, M. Bruns, C. Barner-Kowollik, *Adv. Funct. Mater.* **2012**, *22*, 304; b) E. Blasco, M. Piñol, L. Oriol, B. V. K. J. Schmidt, A. Welle, V. Trouillet, M. Bruns, C. Barner-Kowollik, *Adv. Funct. Mater.* **2013**, *23*, 4011; c) D. Abt, B. V. K. J. Schmidt, O. Pop-Georgievski, A. S. Quick, D. Danilov, N. Y. Kostina, M. Bruns, W. Wenzel, M. Wegener, C. Rodriguez-Emmenegger, C. Barner-Kowollik, *Chemistry* **2015**, *21*, 13186; d) J. Hatano, K. Okuro, T. Aida, *Angew. Chem., Int. Ed.* **2016**, *55*, 193; e) D. Estupiñán, C. Barner-Kowollik, L. Barner, *Angew. Chem., Int. Ed.* **2018**, *57*, 5925.
[92] a) L. Delafresnaye, C. W. Schmitt, L. Barner, C. Barner-Kowollik, *Chemistry* **2019**, *25*, 12538; b) L. Delafresnaye, J. P. Hooker, C. W. Schmitt, L. Barner, C. Barner-Kowollik, *Macromolecules* **2020**, *53*, 5826.
[93] a) B. Sandmann, B. Happ, J. Vitz, M. D. Hager, P. Burtscher, N. Moszner, U. S. Schubert, *Polym. Chem.* **2013**, *4*, 3938; b) M. A. Tasdelen, Y. Yagci, *Tetrahedron Lett.* **2010**, *51*, 6945.
[94] a) B. J. Adzima, Y. Tao, C. J. Kloxin, C. A. DeForest, K. S. Anseth, C. N. Bowman, *Nat. Chem.* **2011**, *3*, 256; b) S. C. Ritter, B. König, *Chem. Commun.* **2006**, 4694.
[95] a) M. A. Tasdelen, G. Yilmaz, B. Iskin, Y. Yagci, *Macromolecules* **2012**, *45*, 56; b) Y. Yagci, M. A. Tasdelen, S. Jockusch, *Polymer* **2014**, *55*, 3468.
[96] L. Harmand, S. Cadet, B. Kauffmann, L. Scarpantonio, P. Batat, G. Jonusauskas, N. D. McClenaghan, D. Lastécouères, J.-M. Vincent, *Angew. Chem., Int. Ed.* **2012**, *51*, 7137.
[97] a) M. K. McBride, T. Gong, D. P. Nair, C. N. Bowman, *Polymer* **2014**, *55*, 5880; b) G. Oyman Eyrilmez, S. Doran, E. Murtezi, B. Demir, D. Odaci Demirkol, H. Coskunol, S. Timur, Y. Yagci, *Macromol. Biosci.* **2015**, *15*, 1233; c) A. Baranek, H. B. Song, M. McBride, P. Finnegan, C. N. Bowman, *Macromolecules* **2016**, *49*, 1191; d) M. G. Williams, A. V. Teplyakov, *Appl. Surf. Sci.* **2018**, *447*, 535; e) N. Sowan, H. B. Song, L. M. Cox, J. R. Patton, B. D. Fairbanks, Y. Ding, C. N. Bowman, *Adv. Mater.* **2021**, *33*, 2007221; f) S. Liang, Y. Guan, Y. Zhang, *ACS Omega* **2019**, *4*, 5650; g) A. U. Shete, B. P. Sutherland, C. J. Kloxin, *Polym. Chem.* **2020**, *11*, 7515.
[98] J. Jeong, G. Szczepaniak, S. S. Yerneni, F. Lorandi, H. Jafari, S. Lathwal, S. R. Das, K. Matyjaszewski, *Chem. Commun.* **2021**, *57*, 12844.
[99] A. A. Poloukhtine, N. E. Mbua, M. A. Wolfert, G.-J. Boons, V. V. Popik, *J. Am. Chem. Soc.* **2009**, *131*, 15769.
[100] a) M. Bjerknes, H. Cheng, C. D. McNitt, V. V. Popik, *Bioconjugate Chem.* **2017**, *28*, 1560; b) W. Luo, P. Gobbo, C. D. McNitt, D. A. Sutton, V. V. Popik, M. S. Workentin, *Chemistry* **2017**, *23*, 1052; c) Z. Wang, J. Liu, H. K. Arslan, S. Grosjean, T. Hagendorn, H. Gliemann, S. Bräse, C. Wöll, *Langmuir* **2013**, *29*, 15958.
[101] a) D. A. Sutton, V. V. Popik, *J. Org. Chem.* **2016**, *81*, 8850; b) D. A. Sutton, S.-H. Yu, R. Steet, V. V. Popik, *Chem. Commun.* **2016**, *52*, 553.
[102] K. Mishiro, T. Kimura, T. Furuyama, M. Kunishima, *Org. Lett.* **2019**, *21*, 4101.
[103] a) X. Zhang, X. Wu, S. Jiang, J. Gao, Z. Yao, J. Deng, L. Zhang, Z. Yu, *Chem. Commun.* **2019**, *55*, 7187; b) L. Zhang, X. Zhang, Z. Yao, S. Jiang, J. Deng, B. Li, Z. Yu, *J. Am. Chem. Soc.* **2018**, *140*, 7390.
[104] K.-H. Pfoertner, J. Foricher, *Helv. Chim. Acta* **1980**, *63*, 653.
[105] a) S. Arumugam, V. V. Popik, *J. Am. Chem. Soc.* **2011**, *133*, 5573; b) S. Arumugam, V. V. Popik, *J. Am. Chem. Soc.* **2009**, *131*, 11892.
[106] S. Arumugam, J. Guo, N. E. Mbua, F. Friscourt, N. Lin, E. Nekongo, G.-J. Boons, V. V. Popik, *Chem. Sci.* **2014**, *5*, 1591.
[107] B. Yang, S. Gao, *Chem. Soc. Rev.* **2018**, *47*, 7926.
[108] T. Gruendling, K. K. Oehlenschlaeger, E. Frick, M. Glassner, C. Schmid, C. Barner-Kowollik, *Macromol. Rapid Commun.* **2011**, *32*, 807.
[109] T. Pauloehrl, G. Delaittre, V. Winkler, A. Welle, M. Bruns, H. G. Börner, A. M. Greiner, M. Bastmeyer, C. Barner-Kowollik, *Angew. Chem., Int. Ed.* **2012**, *51*, 1071.
[110] F. Feist, J. P. Menzel, T. Weil, J. P. Blinco, C. Barner-Kowollik, *J. Am. Chem. Soc.* **2018**, *140*, 11848.
[111] a) S. V. Mayer, A. Murnauer, M.-K. von Wrisberg, M.-L. Jokisch, K. Lang, *Angew. Chem., Int. Ed.* **2019**, *58*, 15876; b) T. Jiang, P. Kumar, W. Huang, W.-S. Kao, A. O. Thompson, F. M. Camarda, S. T. Laughlin, *ChemBioChem* **2019**, *20*, 2222; c) P. Kumar, T. Jiang, S. Li, O. Zainul, S. T. Laughlin, *Org. Biomol. Chem.* **2018**, *16*, 4081.
[112] H. Zhang, W. S. Trout, S. Liu, G. A. Andrade, D. A. Hudson, S. L. Scinto, K. T. Dicker, Y. Li, N. Lazouski, J. Rosenthal, C. Thorpe, X. Jia, J. M. Fox, *J. Am. Chem. Soc.* **2016**, *138*, 5978.
[113] V. X. Truong, K. M. Tsang, F. Ercole, J. S. Forsythe, *Chem. Mater.* **2017**, *29*, 3678.
[114] L. Liu, D. Zhang, M. Johnson, N. K. Devaraj, *Nat. Chem.* **2022**, *14*, 1078.
[115] H. S. Mader, P. Kele, S. M. Saleh, O. S. Wolfbeis, *Curr. Opin. Chem. Biol.* **2010**, *14*, 582.
[116] P. Elzahhar, A. S. F. Belal, F. Elamrawy, N. A. Helal, M. I. Nounou, in *Pharmaceutical Nanotechnology: Basic Protocols* (Eds.: V. Weissig, T. Elbayoumi), Springer New York, New York, NY **2019**, pp. 125-182.
[117] H. K. Hunt, C. Soteropulos, A. M. Armani, *Sensors 10*, **2010**, 9317.
[118] S. Gomez, A. Tsung, Z. Hu, *Molecules. Chem. (Oxford)* **2020**, *25*, 4964.
[119] B. M. Barth, R. Sharma, E. İ. Altınoğlu, T. T. Morgan, S. S. Shanmugavelandy, J. M. Kaiser, C. McGovern, G. L. Matters, J. P. Smith, M. Kester, J. H. Adair, *ACS Nano* **2010**, *4*, 1279.
[120] C. Di Natale, E. Battista, V. Lettera, N. Reddy, G. Pitingolo, R. Vecchione, F. Causa, P. A. Netti, *Bioconjugate Chem.* **2021**, *32*, 1593.
[121] C. Canovas, M. Moreau, J.-M. Vrigneaud, P.-S. Bellaye, B. Collin, F. Denat, V. Goncalves, *Bioconjugate Chem.* **2019**, *30*, 888.
[122] a) J. Martin, A. Desfoux, J. Martinez, M. Amblard, A. Mehdi, L. Vezenkov, G. Subra, *Prog. Polym. Sci.* **2021**, *115*, 101377; b) C. Chen, D. Y. W. Ng, T. Weil, *Prog. Polym. Sci.* **2020**, *105*, 101241.
[123] M. S. Messina, K. M. M. Messina, A. Bhattacharya, H. R. Montgomery, H. D. Maynard, *Prog. Polym. Sci.* **2020**, *100*, 101186.
[124] M. Hirsch, L. Lucherini, R. Zhao, A. C. Saracho, E. Amstad, *Mater Today Bio* 21.
[125] E. Lavik, V. Rotello, *Bioconjugate Chem.* **2022**, *33*, 543.
[126] Y. Yuan, W. Y. Hou, W. P. Qin, C. F. Wu, *Biomater Sci.* **2021**, *9*, 328.
[127] Z. Zhao, X. X. Tian, X. Y. Song, *J. Mater. Chem. C* **2020**, *8*, 13896.
[128] a) T. Abele, T. Messer, K. Jahnke, M. Hippler, M. Bastmeyer, M. Wegener, K. Gopfrich, *Adv. Mater.* **2022**, *34*, 2106709; b) S. Bertels, M. Jaggy, B. Richter, S. Keppler, K. Weber, E. Genthner, A. C. Fischer, M. Thiel, M. Wegener, A. M. Greiner, T. J. Autenrieth, M. Bastmeyer, *Sci. Rep.* **2021**, *11*, 9269; c) M. Hippler, K. Weissenbruch, K. Richler, E. D. Lemma, M. Nakahata, B. Richter, C. Barner-Kowollik, Y. Takashima, A. Harada, E. Blasco, M. Wegener, M. Tanaka, M. Bastmeyer, *Sci. Adv.* **2020**, *6*, eabc2648; d) C. A. Spiegel, M. Hippler, A. Munchinger, M. Bastmeyer, C. Barner-Kowollik, M. Wegener, E. Blasco, *Adv. Funct. Mater.* **2020**, *30*, 1907615.
[129] M. Regehly, Y. Garmshausen, M. Reuter, N. F. Konig, E. Israel, D. P. Kelly, C. Y. Chou, K. Koch, B. Asfari, S. Hecht, *Nature* **2020**, *588*, 620.
[130] a) B. E. Kelly, I. Bhattacharya, H. Heidari, M. Shusteff, C. M. Spadaccini, H. K. Taylor, *Science* **2019**, *363*, 1075; b) M. Shusteff, A. E. M. Browar, B. E. Kelly, J. Henriksson, T. H. Weisgraber, R. M.

Panas, N. X. Fang, C. M. Spadaccini, *Sci. Adv.* **2017**, *3*, eaao549; c) D. Loterie, P. Delrot, C. Moser, *Nat. Commun.* **2020**, *11*, 852.
[131] a) D. A. Walker, J. L. Hedrick, C. A. Mirkin, *Science* **2019**, *366*, 360; b) J. R. Tumbleston, D. Shirvanyants, N. Ermoshkin, R. Janusziewicz, A. R. Johnson, D. Kelly, K. Chen, R. Pinschmidt, J. P. Rolland, A. Ermoshkin, E. T. Samulski, J. M. DeSimone, *Science* **2015**, *347*, 1349.
[132] V. Hahn, P. Kiefer, T. Frenzel, J. Y. Qu, E. Blasco, C. Barner-Kowollik, M. Wegener, *Adv. Funct. Mater.* **2020**, *30*, 1907795.
[133] a) P. Somers, Z. H. Liang, J. E. Johnson, B. W. Boudouris, L. Pan, X. F. Xu, *Light Sci Appl* **2021**, *10*, 2214211; b) S. K. Saha, D. Wang, V. H. Nguyen, Y. N. Chang, J. S. Oakdale, S. C. Chen, *Science* **2019**, *366*, 105.
[134] V. Hahn, P. Rietz, F. Hermann, P. Muller, C. Barner-Kowollik, T. Schloder, W. Wenzel, E. Blasco, M. Wegener, *Nat. Photonics* **2022**, *16*, 784.
[135] A. Bagheri, J. Y. Jin, *ACS Appl Polym Mater* **2019**, *1*, 593.
[136] P. N. Bernal, P. Delrot, D. Loterie, Y. Li, J. Malda, C. Moser, R. Levato, *Tissue Eng. Pt. A* **2022**, *28*, S150.
[137] S. A. Singaraju, G. C. Marques, P. Gruber, R. Kruk, H. Hahn, B. Breitung, J. Aghassi-Hagmann, *Phys. Status Solidi, R* **2020**, *14*, 2000252.
[138] A. Selimis, V. Mironov, M. Farsari, *Microelectron. Eng.* **2015**, *132*, 83.
[139] M. Terazima, Y. Nogami, T. Tominaga, *Chem. Phys. Lett.* **2000**, *332*, 503.
[140] a) K. Kowsari, S. Akbari, D. Wang, N. X. Fang, Q. Ge, *3D Print. Addit. Manuf.* **2018**, *5*, 185; b) K. Kowsari, B. Zhang, S. Panjwani, Z. C. Chen, H. Hingorani, S. Akbari, N. X. Fang, Q. Ge, *Addit. Manuf.* **2018**, *24*, 627.
[141] X. J. Li, H. C. Mao, Y. Y. Pan, Y. Chen, *J. Manuf. Sci. E-T Asme* **2019**, *141*.
[142] T. Gockler, S. Haase, X. Kempter, R. Pfister, B. R. Maciel, A. Grimm, T. Molitor, N. Willenbacher, U. Schepers, *Adv. Healthcare Mater.* **2021**, *10*.
[143] C. N. LaFratta, J. T. Fourkas, T. Baldacchini, R. A. Farrer, *Angew. Chem. Int. Ed.* **2007**, *46*, 6238.
[144] R. Rizzo, N. Petelinsek, A. Bonato, M. Zenobi-Wong, *Adv. Sci. (Weinh)* **2023**, *10*, e2205302.
[145] a) B. Bhuian, R. J. Winfield, S. O'Brien, G. M. Crean, *Appl. Surf. Sci.* **2006**, *252*, 4845; b) F. Claeyssens, E. A. Hasan, A. Gaidukeviciute, D. S. Achilleos, A. Ranella, C. Reinhardt, A. Ovsianikov, S. Xiao, C. Fotakis, M. Vamvakaki, B. N. Chichkov, M. Farsari, *Langmuir* **2009**, *25*, 3219.
[146] a) R. Rizzo, D. Ruetsche, H. Liu, M. Zenobi-Wong, *Adv. Mater.* **2021**, *33*, 2102900; b) J. I. Paez, A. de Miguel-Jimenez, R. Valbuena-Mendoza, A. Rathore, M. Jin, A. Glaser, S. Pearson, A. Del Campo, *Biomacromolecules* **2021**, *22*, 2874.
[147] M. Lee, R. Rizzo, F. Surman, M. Zenobi-Wong, *Chem. Rev.* **2020**, *120*, 10670.
[148] N. R. Gandavarapu, M. A. Azagarsamy, K. S. Anseth, *Adv. Mater.* **2014**, *26*, 2521.
[149] J. Zhao, J. Zhang, D. McCoul, Z. Hao, S. Wang, X. Wang, B. Huang, L. Sun, *Soft Robot* **2019**, *6*, 713.
[150] A. Cangialosi, C. Yoon, J. Liu, Q. Huang, J. Guo, T. D. Nguyen, D. H. Gracias, R. Schulman, *Science* **2017**, *357*, 1126.
[151] B. D. Fairbanks, L. J. Macdougall, S. Mavila, J. Sinha, B. E. Kirkpatrick, K. S. Anseth, C. N. Bowman, *Chem. Rev.* **2021**, *121*, 6915.
[152] D. L. Alge, M. A. Azagarsamy, D. F. Donohue, K. S. Anseth, *Biomacromolecules* **2013**, *14*, 949.
[153] R. M. Desai, S. T. Koshy, S. A. Hilderbrand, D. J. Mooney, N. S. Joshi, *Biomaterials* **2015**, *50*, 30.
[154] K. A. Gultian, R. Gandhi, T. W. B. Kim, S. L. Vega, *Macromol. Biosci.* **2022**.
[155] J. Carthew, J. E. Frith, J. S. Forsythe, V. X. Truong, *J. Mater. Chem. B* **2018**, *6*, 1394.
[156] J. J. Bruins, B. Albada, F. van Delft, *Chemistry* **2018**, *24*, 4749.
[157] L. Chen, Q. Y. Wu, G. Wei, R. Liu, Z. Q. Li, *J. Mater. Chem. C* **2018**, *6*, 11561.
[158] S. Bertlein, G. Brown, K. S. Lim, T. Jungst, T. Boeck, T. Blunk, J. Tessmar, G. J. Hooper, T. B. F. Woodfield, J. Groll, *Adv. Mater.* **2017**, *29*, 1703404.
[159] A. Dobos, J. Van Hoorick, W. Steiger, P. Gruber, M. Markovic, O. G. Andriotis, A. Rohatschek, P. Dubruel, P. J. Thurner, S. Van Vlierberghe, S. Baudis, A. Ovsianikov, *Adv. Healthcare Mater.* **2020**, *9*, 1900752.
[160] Y. F. Li, K. A. Kilian, *Adv. Healthcare Mater.* **2015**, *4*, 2780.
[161] a) L. Tytgat, L. Van Damme, J. Van Hoorick, H. Declercq, H. Thienpont, H. Ottevaere, P. Blondeel, P. Dubruel, S. Van Vlierberghe, *Acta Biomater.* **2019**, *94*, 340; b) J. Van Hoorick, L. Tytgat, A. Dobos, H. Ottevaere, J. Van Erps, H. Thienpont, A. Ovsianikov, P. Dubruel, S. Van Vlierberghe, *Acta Biomater.* **2019**, *97*, 46.
[162] V. Burchak, F. Koch, L. Siebler, S. Haase, V. K. Horner, X. Kempter, G. B. Stark, U. Schepers, A. Grimm, S. Zimmermann, P. Koltay, S. Strassburg, G. Finkenzeller, F. Simunovic, F. Lampert, *Int. J. Mol. Sci.* **2022**, *23*, 7939.
[163] R. Rizzo, D. Ruetsche, H. Liu, M. Zenobi-Wong, *Adv. Mater.* **2021**, *33*, 2102900.
[164] A. L. Furst, M. J. Smith, M. B. Francis, *J. Am. Chem. Soc.* **2017**, *139*, 12610.
[165] F. Santoyo-Gonzalez, F. Hernandez-Mateo, *Chem. Soc. Rev.* **2009**, *38*, 3449.
[166] a) W. R. Algar, D. E. Prasuhn, M. H. Stewart, T. L. Jennings, J. B. Blanco-Canosa, P. E. Dawson, I. L. Medintz, *Bioconjugate Chem.* **2011**, *22*, 825; b) W. Wu, Q. He, C. Jiang, *Nanoscale Res. Lett.* **2008**, *3*, 397.
[167] B. J. Hong, O. C. Compton, Z. An, I. Eryazici, S. T. Nguyen, *ACS Nano* **2012**, *6*, 63.
[168] Z. Liu, S. M. Tabakman, Z. Chen, H. Dai, *Nat. Protoc.* **2009**, *4*, 1372.
[169] S. N. Baker, G. A. Baker, *Angew. Chem., Int. Ed.* **2010**, *49*, 6726.
[170] M. A. Fernandez-Yague, A. Larrañaga, O. Gladkovskaya, A. Stanley, G. Tadayyon, Y. Guo, J.-R. Sarasua, S. A. M. Tofail, D. I. Zeugolis, A. Pandit, M. J. Biggs, *Bioconjugate Chem.* **2015**, *26*, 2025.
[171] M. Tsotsalas, J. Liu, B. Tettmann, S. Grosjean, A. Shahnas, Z. Wang, C. Azucena, M. Addicoat, T. Heine, J. Lahann, J. Overhage, S. Bräse, H. Gliemann, C. Wöll, *J. Am. Chem. Soc.* **2014**, *136*, 8.
[172] S. Schmitt, M. Silvestre, M. Tsotsalas, A.-L. Winkler, A. Shahnas, S. Grosjean, F. Laye, H. Gliemann, J. Lahann, S. Bräse, M. Franzreb, C. Wöll, *ACS Nano* **2015**, *9*, 4219;
[173] Z. Xia, B. Shi, W. Zhu, Y. Xiao, C. Lue, *Adv. Funct. Mater.* **2022**, *32*, 2207655.
[174] a) H. Wan, K. Ren, H. J. Kaper, P. K. Sharma, *J. Colloid Interface Sci.* **2021**, *594*, 435; b) Q. N. Tran, H. J. Lee, N. Tran, *Polymers* **2023**, *15*, 1279.
[175] S. Talekar, Y. Kim, Y. Wee, J. Kim, *Chem. Eng. J.* **2023**, *456*.
[176] M. M. Li, S. Qiao, Y. L. Zheng, Y. H. Andaloussi, X. Li, Z. J. Zhang, A. Li, P. Cheng, S. Q. Ma, Y. Chen, *J. Am. Chem. Soc.* **2020**, *142*, 6675.
[177] Y. Zhou, Y. Wei, J. S. Ren, X. G. Qu, *Mater. Horiz.* **2020**, *7*, 3291.
[178] a) C. Sicard, *Angew. Chem. Int. Ed.* **2023**, *62*; b) Q. Sun, C. W. Fu, B. Aguila, J. Perman, S. Wang, H. Y. Huang, F. S. Xiao, S. Q. Ma, *J. Am. Chem. Soc.* **2018**, *140*, 984; c) J. S. Gan, A. R. Bagheri, N. Aramesh, I. Gul, M. Franco, Y. Q. Almulaiky, M. Bilal, *Int. J. Biol. Macromol.* **2021**, *167*, 502; d) C. N. Jin, N. Li, E. Lin, X. P. Chen, T. Wang, Y. Wang, M. F. Yang, W. S. Liu, J. Y. Yu, Z. J. Zhang, Y. Chen, *ACS Catal.* **2022**, *12*, 8259.
[179] C. A. DeForest, K. S. Anseth, *Nat. Chem.* **2011**, *3*, 925.
[180] N. Gjorevski, M. Nikolaev, T. E. Brown, O. Mitrofanova, N. Brandenberg, F. W. DelRio, F. M. Yavitt, P. Liberali, K. S. Anseth, M. P. Lutolf, *Science*, *375*, eaaw9021.
[181] G. Quintieri, A. H. Gröschel, *Polym. Chem.* **2021**, *12*, 1429.

Christin Bednarek studied food chemistry at the University of Karlsruhe (TH) and received her Ph.D. in the group of Prof. Dr. Ute Schepers at the Institute of Toxicology and Genetics at the Karlsruhe Institute of Technology in 2012. She is a junior research group leader in the group Stefan Bräse. Her research focus is biologically active molecules like peptoids and others, as well as glycochemistry and -biology.

Ute Schepers was born in Rhede, Germany, in 1966. After her studies in chemistry at the University of Bonn, she received her Ph.D. in 1997, working with Konrad Sandhoff. She then moved to Harvard Medical School for a postdoctorate (1998–2000) with Tom Kirchhausen at the Department of Cell Biology. In 2000, she returned to the Kekulé-Institut für Organische Chemie and Biochemie in Bonn to start independent research. Since 2009, she became an independent group leader at the Institute of Toxicology and Genetics of the Karlsruhe Institute of Technology (KIT) in Karlsruhe, where she finished her habilitation. Now at the Institute for Functional Interfaces at KIT, her research is focused on combinatorial synthesis to develop organ-specific drug delivery systems and 3D tissue reconstruction.

Franziska Thomas studied chemistry at Humboldt University Berlin, where she received her Ph.D. in 2010. After a postdoctoral stay at the University of Bristol, UK, where she worked with Dek Woolfson, she became an independent research group leader at the University of Göttingen in 2015. In 2019, she became a junior professor of organic chemistry at Heidelberg University. Her research interests include protein design, de novo design of functional peptide entities, and identifying small molecules and peptides as drug candidates for the potential treatment of amyotrophic lateral sclerosis.

Stefan Bräse studied in Göttingen (Germany), Bangor (U.K.), and Marseille (France) and received his Ph.D. in 1995 with Armin de Meijere in Göttingen. After postdoctoral research at Uppsala University and the Scripps Research Institute, he began his independent research career at the RWTH Aachen in 1997. In 2001, he finished his Habilitation and moved to the University of Bonn as a professor of organic chemistry. Since 2003, he has been a professor at the Institute of Organic Chemistry, Karlsruhe Institute of Technology (KIT), and since 2012, also Director of the Institute of Biological and Chemical Systems – Functional Molecular Systems (IBCS-FMS) at the KIT. His research interests include chiral ligands and catalyst design for asymmetric processes, molecular engineering of functional synthetic materials, and digitization in chemistry.