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"Click" reactions in polysaccharide modification

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ABSTRACT

Polysaccharide chemistry is enjoying accelerating development thanks to advances in synthetic techniques, biochemistry and solvents, which enable polysaccharide materials to be useful in a variety of demanding applications. Among the synthetic advances, click chemistry has reconfigured the realm of polysaccharide modification that previously was dominated by conventional synthetic approaches such as esterification and etherification. "Click" reactions provide mild, modular, and efficient modification pathways, and equally importantly allow us to synthesize derivatives with novel functionality, architecture, and properties, that are otherwise difficult to obtain via conventional methods. Herein, we review application in polysaccharide modification of six groups of click reactions; CuAAC (copper catalyzed alkyne/azide cycloaddition), metal-free [3+2] cycloaddition, Diels–Alder reaction, oxime click, thiol-Michael reaction, and thiol-ene reaction, as well as one click-like reaction that is the subject of our own research, olefin cross-metathesis.

Contents

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Abbreviations

AAC	azide–alkyne cycloaddition
BCN	bicyclo[6.1.0]nonynes
BCN(ene)	bicyclo[6.1.0]nonenes
BARAC	biarylazacyclooctynones
CD	cyclodextrin
CDI	1,1′-carbonyldiimidazole
CM	cross-metathesis
CMC	carboxymethyl cellulose
CNC	cellulose nanocrystal
CNP	chitosan nanoparticle
CS	chitosan
CTA	cellulose triacetate
CuAAC	copper catalyzed azide–alkyne cycloaddition
DA	Diels–Alder reaction
DCC	N,N′-dicyclohexylcarbodiimide
DIBAC	dibenzoazacyclooctynes
DIBO	dibenzoazacyclooctynes
DIFO	difluorocyclooctynes
DIPEA	N,N-diisopropylethylamine
DMAP	4-dimethylaminopyridine
DMF	dimethylformamide
DMPA	2,2-dimethoxy-2-phenylacetophenone
DMSO	dimethyl sulfoxide
DOTA	1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid
DTP	3,3′-dithiodipropionic acid
DTT	1,4-dithio-DL-threitol
DVS	divinyl sulfone
EDAC	1-ethyl-3-(3-dimethylaminopropyl-carbodiimide)
EDC	ethyl(dimethylaminopropyl) carbodiimidazole
FITC	fluorescein isothiocyanate
HA	hyaluronic acid
hDA	hetero Diels–Alder reaction
HEMA	hydroxyethyl methacrylate
HOBt	1-hydroxybenzotriazole
iEDDA	inverse electron demand Diels–Alder reaction
ISA	imidazole-1-sulfonyl azide hydrochloride
MES	2-(N-morpholino)-ethanesulfonic acid
NBS	N-bromosuccinimide
NHS	N-hydroxysuccinimide
NMP	N-methyl-1-pyrrolidone
PBLG	poly(γ-benzyl L-glutamate)
PBS	phosphate-buffered saline
PDMAEMA	poly(2-dimethylaminoethyl methacrylate)
PEG	poly(ethylene glycol)
PMA	2-propyn-1-yl methacrylate
PMDETA	N,N,N′,N′,N″-pentamethyldiethylenetriamine
PMT	polymer modified tetrazine
PNIPAM	poly(N-isoproylacrylamide)
PPMA	poly(2-propyn-1-yl methacrylate)
rDA	retro Diels–Alder reaction
ROS	reactive oxygen species
SM	self-metathesis
SPAAC	strain-promoted azide–alkyne cycloaddition
TBA	tetrabutylammonium
TCO	trans-cyclooctene
TEA	triethylamine
TFA	trifluoroacetic acid
TFMSA	trifluoromethanesulfonyl azide
THF	tetrahydrofuran
TsCl	4-toluenesulfonyl chloride
Tz	tetrazine
VS	vinyl sulfone

1. Introduction

Polysaccharides (including cellulose, chitosan, alginate, dextran, hyaluronic acid and others) are among the most abundant natural polymers on earth. They and their modified derivatives are under extensive investigation and are currently used for applications such as coatings [1], drug delivery [2,3], and biomedical materials [4–6], due to the sustainability of biopolymers, the biological functions they possess, and also to the fact that the structure and properties of these biopolymers are readily tunable. Chemical modification is one important approach to tailor polysaccharide structure and properties. By chemical modification of homogeneously dispersed polysaccharide molecules or on the surfaces of polysaccharide materials, derivatives bearing different functional groups and conjugates can be obtained. Modification of polysaccharides also generates a variety of derivatives that are capable of forming special architectures such as nanogels [7], hydrogels [8], and micelles [9]. From this point of view, chemical modification imparts desired properties to the polysaccharide materials so that they meet the requirements of specific applications.

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Conventional modification approaches generally involve esterification or etherification, taking the advantage of the straightforwardness of the reactions and the comparatively easy access to many esterification and etherification reagents. Other modification methods including nucleophilic displacement reactions, oxidation, and (controlled) free radical polymerization have also been commonly employed. Certainly these synthetic pathways have enlarged the family of polysaccharide derivatives. For example, esterification of polysaccharides has contributed remarkably to cellulose and polysaccharide chemistry in the last few decades due to the development of new acylation methods and unconventional solvents [10]. Investigation of regioselective reactions and protection/deprotection groups, on the other hand, provides options for regioselectively modified polysaccharide derivatives with well-controlled structures, and permits deeper understanding of structure-property relationships of polysaccharide derivatives [11]. While such methods are very useful and are still contributing to the existence of entire industries, they are limited in scope. Typically esterification involves harsh reaction conditions (e.g. strongly acidic catalysts) that are incompatible with sensitive functional groups on either polysaccharides or the acylation reagents. In the absence of protecting groups, simple esterification is also incompatible with difunctional reagents such as dicarboxylic acids or reagents with both carboxylic acid and hydroxyl groups, which could lead to undesired crosslinking or uncontrolled polymerization [12]. The advent of acyl activation reagents, e.g. N,N′-dicyclohexylcarbodiimide (DCC), has allowed the performance of esterification under milder conditions, but this mild esterification is still not useful with difunctional reagents. Etherification typically involves strongly basic conditions (e.g. NaOH or NaH), and so is incompatible with base-sensitive moieties and mostly incompatible with difunctional reagents. Further, long reaction times, tedious steps, and modest yields are also at times associated with these conventional methods.

The concept of “click chemistry” [13], first coined by Sharpless and his coworkers, has had a huge impact on the chemistry community [14–18], detailed elaboration of which is beyond the scope of this review. To qualify as “click” chemistry, a reaction must fulfill most, if not all, of the requirements listed below. The reaction must “be modular, wide in scope, give very high yields, generate only inoffensive and easily removable byproducts, and be stereospecific”; and the process should also fulfill “simple reaction conditions and product isolation, readily available reagents, and the use of no solvent or benign ones”. The concept of modular, facile and economical synthesis of structurally and functionally diverse molecules has also drawn attention from polysaccharide chemists, and has brought substantial progress to this field. Not only can “click” reactions avoid the aforementioned limitations of conventional synthetic pathways, but they also can rapidly synthesize molecules with diverse functional appendages. Herein, we review some of the most commonly used “click” reactions in polysaccharide chemistry, including the well-known azide–alkyne Huisgen cycloaddition, Diels–Alder reaction, thiol-ene and thiol-Michael reactions, and the oxime click reaction. As several new reactions fulfilling the criteria of click chemistry have been uncovered in the last decade, we also review the introduction of these new click reactions to the field of polysaccharide chemistry, including two metal-free [3+2] cycloaddition reactions, and the inverse electron-demand Diels–Alder reaction. We focus in this review mainly on the chemistry of these reactions, and try to provide to readers a guide for performing such reactions. At the same time, the extraordinarily diverse and controllable structures and functionalities of the click reaction products are demonstrated. We also compare the strengths and limitations of each click reaction, hoping to provide some guidance to future polysaccharide researchers selecting tools from this toolkit. Although olefin cross-metathesis has not previously been considered a “click” reaction in either organic or polymer chemistry, we find it has many elements of click reactions under certain circumstances (i.e. electron-rich type I terminal olefins reacting with electron deficient type II or III olefins) [19,20]. Based on our previous work, we present here the concept that olefin cross-metathesis of polysaccharide derivatives is a novel “click-like” reaction, given the potential that this facile, efficient and modular reaction can be added to the toolbox of polysaccharide chemists for chemical modification.

2. Copper catalyzed azide–alkyne cycloaddition (CuAAC)

Since the introduction of the “click” reaction concept by Kolb, Finn, and Sharpless in 2001, Huisgen 1,3-dipolar azide–alkyne cycloaddition (AAC) [22] has been featured as the flagship of all click reactions. Three versions of AAC (Scheme 1) were developed, i.e. the classic thermal AAC, the broadly used Cu-catalyzed azide–alkyne cycloaddition (CuAAC), and the metal-free strain-promoted azide–alkyne cycloaddition (SPAAC), which will be discussed separately in the next section. Although the thermal version of AAC shares the features of the others including high reliability, bioorthogonality (the ability of reactants to react rapidly and selectively only with each other, usually inside living systems, without interfering with other functionality [23]) and broad tolerance to diverse functional groups, the facts that it has a low reaction rate, requires comparatively high temperatures, and leads to two different isomerized products (1,4-disubstituted 1,2,3-triazoles and 1,5-disubstituted 1,2,3-triazoles) limit its broad application. It was not until the discovery of the Cu-catalyzed version of this reaction by the Meldal [24] and Sharpless [25] groups that the reaction started to gain considerable attention. As the role of copper in the reaction has been under intensive investigation, and is still in dispute, the discussion of plausible mechanisms [26–28] will not be addressed in this review. However, Cu-(I) complexes are able to catalyze the reaction, providing remarkably high rates under highly diverse conditions of solvent, temperature, nature of catalyst precursor, and substrate structure.

It did not take long for polysaccharide chemists to realize the practicality and reliability of this reaction in polysaccharide modification. In 2006, the Hafren and Cordova group [29], the Heinze group [30] and the Shinkai

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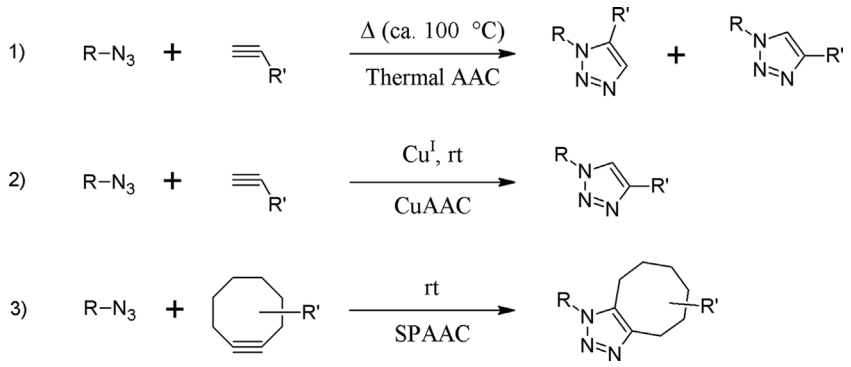

Scheme 1. Three versions of azide–alkyne cycloaddition: (1) the classic thermal azide–alkyne cycloaddition; (2) Cu-catalyzed azide–alkyne cycloaddition; and (3) the metal-free strain-promoted azide–alkyne cycloaddition. [21]. Copyright 2011, Reproduced with permission from Wiley-VCH Verlag GmbH & Co. KGaA.

group [31] independently reported the application of CuAAC in polysaccharide derivatization. In this section we summarize the representative strategies, especially in the pre-click polysaccharide functionalization, applications of this reaction in homogeneous and heterogeneous modification, as well as crosslinking of polysaccharides, and also the limitations of CuAAC.

2.1. Pre-click functionalization

2.1.1. Azide on backbone

A preliminary step toward the CuAAC reaction is to functionalize the polysaccharide with either azide or alkyne. One widely used approach is to synthesize 6-azido-6-deoxy-polysaccharides (Fig. 1). For many polysaccharides such as cellulose, curdlan and chitosan (CS) that have free primary hydroxyl groups at C-6, this strategy can be accomplished by low-temperature tosylation of the polysaccharide at C-6, followed by nucleophilic tosylate displacement with azide (NaN_3) [30,32]. Alternatively, azide moieties can be introduced to the C-6 position of polysaccharides [31,33] by initial activation of the primary hydroxyl group using triphenylphosphine (Ph_3P), subsequent bromination with tetrabromomethane or N-bromosuccinimide (NBS, which also serves to activate Ph_3P toward nucleophilic attack by OH group to form an alkoxyphosphonium salt intermediate [34]), followed by azide substitution on the 6-bromo-6-deoxy derivatives. One particular benefit to this bromination approach is that the reactions occur with great selectivity at the primary hydroxyl group, which guarantees the regioselectivity of the derivatives [11]. These reactions, combined with the CuAAC reactions, lead to regioselective, quantitative, and modular polysaccharide modification.

Another approach to an azido-polysaccharide was synthesis of 2-azido-2-deoxycellulose from chitosan. This derivative has the potential to become a platform in regioselective modification, especially as an alternative to its 6-azido-6-deoxy counterpart for structure-property relationship studies. One method developed by Zhang et al. [35] afforded 2-azido-2-deoxychitosan with high degree of conversion to azide (95%) using trifluoromethanesulfonyl azide (TFMSA). However, multiple steps, harsh reaction conditions, and long reaction times may impact the structural fidelity of the polymer (e.g. molecular weight, which the authors did not report) and limit the practicality of this method. Kulbokaite et al. [36] compared three procedures (Fig. 2) for azidation of chitosan using sodium nitrite and sodium azide, TFMSA, and imidazol-1-sulfonyl azide hydrochloride (ISA) as azidation agents, and found that TFMSA and ISA were suitable for N-azidation of chitosan. However, the maximal degrees of azidation of chitosan obtained from these two methods were only 40% and 65% respectively.

2.1.2. Tethered azides or alkynes

More diverse azide or alkyne functionalized polysaccharides were obtained by linking small azide- or alkyne-bearing groups to polysaccharide backbones. The most typical methods are through nucleophilic substitution reactions (e.g. etherification) or esterification of backbone hydroxyl groups (amine groups on amino-polysaccharides to form secondary amine and amide are also employed) with azide or alkyne moieties (Table 1). While most of the nucleophilic substitution reactions require the assistance of strong bases [37–41] like sodium hydride, or the pre-substitution/activation of the hydroxyl group of the polysaccharide by a good leaving group [42], the success of esterification reactions largely depends on catalysts or activation agents including 1,1′-carbonyldiimidazole (CDI), DCC, and ethyl(dimethylaminopropyl) carbodiimide/N-hydroxysuccinimide (EDC/NHS). Azide functionality can also be introduced stepwise by first introducing a halogen functionalized side chain to the polysaccharide backbone followed by nucleophilic substitution of the halogen with azide [43–45]. These classical reactions are synthetically straightforward, and can usually give moderate to high degree of substitution (DS) values, largely depending on the stoichiometry. However, unless combined with protecting groups to control regiochemistry, these reactions are often not inherently chemo- or regioselective.

Making use of previously regioselectively attached functional groups (e.g. carboxylic acid group on carboxymethyl cellulose (CMC)) permits chemo- or regioselective polysaccharide functionalization without employing protection/deprotection techniques. One widely explored strategy is the activation of carboxylic acid groups in certain polysaccharides, including hyaluronic

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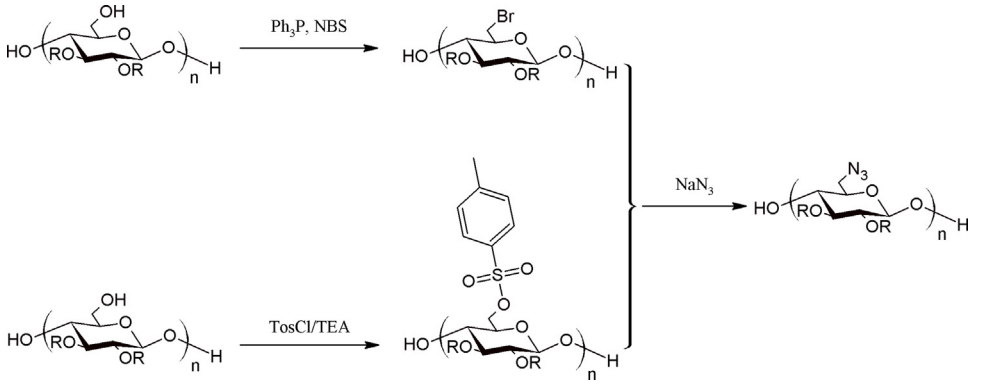

Fig. 1. Two pathways for synthesis of 6-azido-6-deoxy polysaccharide derivatives (using cellulose as example).

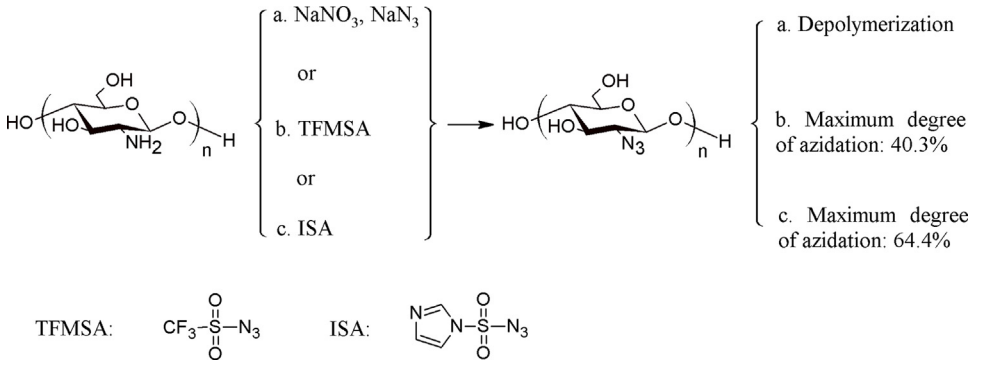

Fig. 2. N-azidation of chitosan by three procedures. [36], Copyright 2009. Adapted with permission from Elsevier Ltd.

acid (HA) and CMC using coupling agents such as EDC/NHS, followed by the addition of azido- or alkynyl amines (Fig. 3) [54,55]. There was a report of chemoselective synthesis of an azido chitosan derivative [56], which was prepared through chemoselective N-bromophthaloylation of chitosan and subsequent azidation.

The reducing and non-reducing ends of polysaccharides can be useful sites for chemoselective functionalization with azide and alkyne moieties. Kamitakahara's group developed methods of converting the reducing end of cellulose triacetate (CTA) to azides [57–59] (Fig. 4a) and non-reducing end of 2,3,6-tri-O-methyl-cellulose to alkynes [58] (Fig. 4b). The corresponding products bearing CuAAC handles are good building blocks for cellulose-based diblock copolymers (Fig. 4c). However, the reaction mechanism restricted viable substrates to peracylated and permethylated polysaccharides, and the degrees of polymerization (DP) of the final products were low. A simpler and more applicable method was reported independently by Bernard et al. [60] and Lecommandoux et al. [61,62] As shown in Fig. 5a, an alkyne group was introduced to the reducing end of dextran by reacting with propargylamine to form a Schiff base, which was reduced by sodium cyanoborohydride in a one-pot reaction to give a more stable amine product. As a majority of polysaccharides and polysaccharide derivatives have aldehyde available in equilibrium at the reducing ends, reductive amination is a broadly applicable approach for introducing azide or alkyne handles to the reducing end of polysaccharides. Besides the abovementioned methods,

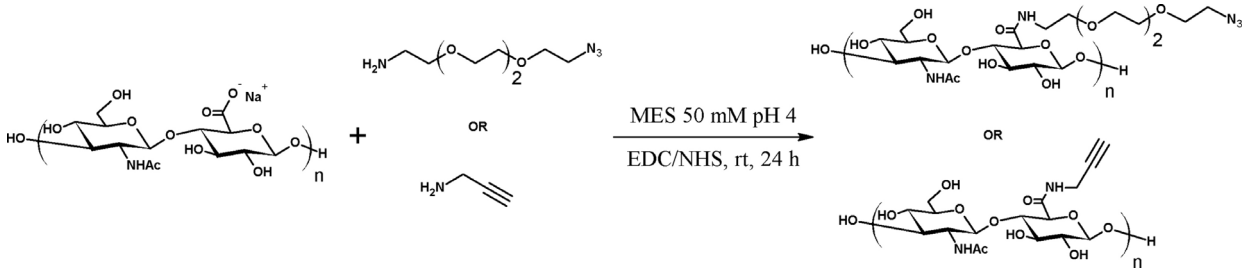

Fig. 3. Schematic presentation of the reaction between HA and amine-functionalized azide or alkyne with the assistance of coupling agent EDC/NHS. [54], Copyright 2007. Adapted with permission from the American Chemical Society.

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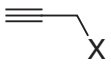
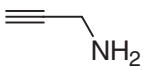
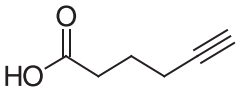
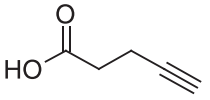
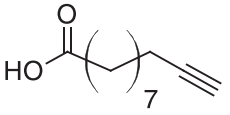
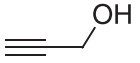
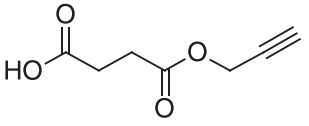
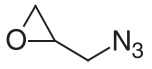
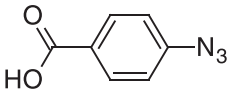
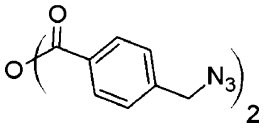
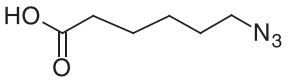
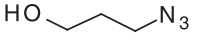
Table 1
Alkynes and azides commonly used to functionalize polysaccharides.

Reagent	Structure	General reaction condition	Polysaccharides
Propargyl halide		Base (NaH, NaOH), 40–50 °C, 72–96 h	Cellulose [37–39], chitosan [40], starch [41]
Propargyl amine		80 °C, 24 h in DMSO	Cellulose (6-tosyl cellulose) [42]
5-Hexynoic acid		(S)-tartaric acid, 110 °C, 6 h	Cellulose [29]
Pent-4-ynoic acid		EDC/NHS, r.t., 16 h	Chitosan [46]
Undec-10-ynoic acid		TsCl/pyridine, 80 °C, 36 h	Cellulose [47]
Propargyl alcohol		CDI	Dextran [48]
Propargyl 3-succinate		EDC/HOBt[a], r.t., 24 h	Chitosan [49]
1-Azido-2,3-epoxypropane		Base (NaOH), 30 °C, ~20 h	Cellulose [50], dextran [51]
4-Azidobenzoic acid		DCC, 30–50 °C, 24–72 h	Chitosan [38]
4-Azidomethylene benzoic anhydride		TEA[b]/DMAP[c], r.t., overnight	Cellulose surface [52]
6-Azidohexanoic acid		CDI	Dextran [53]
3-Azido-1-propanol		CDI	Dextran [48]

[a] HOBt: 1-hydroxybenzotriazole.
[b] TEA: triethylamine.
[c] DMAP: 4-dimethylaminopyridine.

Yamaguchi et al. [63] reported an alternative pathway with the help of *endo*-β-xylosidase, which cleaved the β-xyloside linkage of the substrate (peptide chondroitin 6-sulfate) between the reducing end xylose and the hydroxyl group of the side chain of the peptide, and catalyzed transglycosylation at the same time in the presence of propargyl alcohol. Although limited to only a few polysaccharides, this green synthesis approach is intriguing. These

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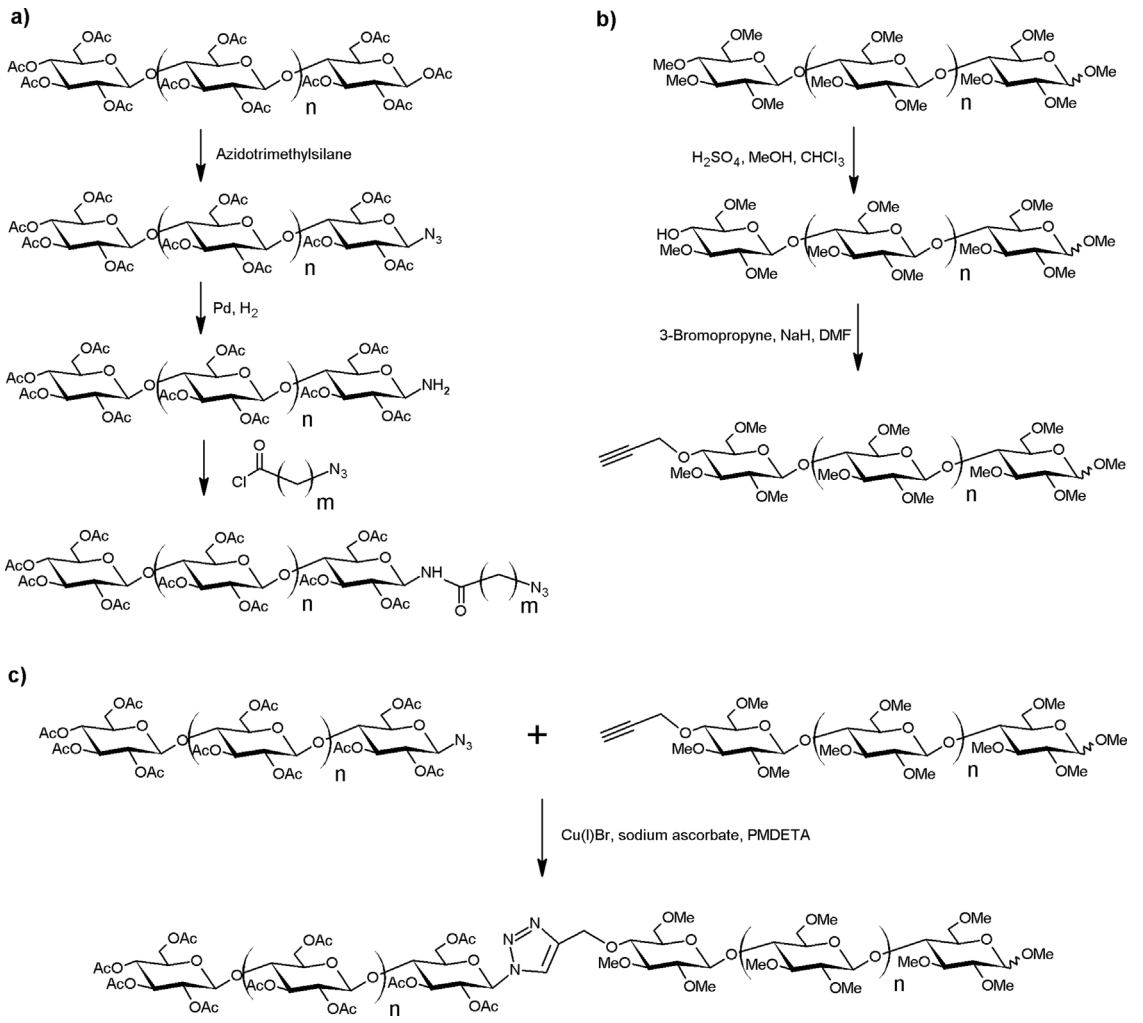

Fig. 4. Synthetic pathways for (a) peracetylated cellulosic glycosyl azides; (b) methyl 2,3,6-tri-O-methyl celluloside having a propargyl group at C-4 of non-reducing end; (c) an example of CuAAC between a cellulosic glycosyl azide and a propargyl cellulose to afford a block copolymer. [58], Copyright 2012. Adapted with permission from Springer Science+Business Media B.V.

end-functionalization methods contributed to new copolymeric architectures such as diblock copolymers [58,61,63], and comb-shaped graft-copolymers [59], which will be described in detail in the following contexts.

2.2. 1,3-Dipolar azide–alkyne cycloaddition

The key to the cycloaddition is the copper (I) catalyst, which contributes to moderate reaction temperature and more importantly controlled 1,4-regioselectivity (only 1,4-disubstituted 1,2,3-triazole products) of the reaction [24,25]. Several protocols to introduce Cu^{I} into the system have been developed [24,25,64] and widely employed in click reactions of polysaccharide derivatives, including (a) formation of Cu^{I} by addition of Cu^{I} salt (e.g. CuBr) [61], and (b) generation of Cu^{I} by reduction of Cu^{II} salt (e.g. $CuBr_2$/ascorbic acid) [31]. Ligands, e.g. N,N,N′,N′,N″-pentamethyldiethylenetriamine (PMDETA) [36,49,60,61], are sometimes used to accelerate the reaction, likely by protecting the Cu^{I} ion from oxidation and disproportionation [65], although alkyne–azide cycloaddition can be effectively catalyzed under "ligand-free" conditions, in which solvents or bases [31] act as ligands.

As a robust reaction, CuAAC tolerates a variety of solvent systems and functional groups. Solvents including DMSO [31], DMF [45], NMP [31], THF [52], H_2O [29], aqueous HCl (caution that acid can acidify azide to form HN_3, which is volatile and highly toxic) [46], and ionic liquids [66] have been reported in homogeneous and heterogeneous modification of polysaccharides. This tolerance has provided great versatility for the reaction in polysaccharide chemistry as the starting polysaccharide derivatives are usually difficult to dissolve in other common organic solvents like alcohols.

Tolerance of the reaction toward a wide scope of functional groups, on the other hand, has contributed to thriving families of polysaccharide derivatives through either homogeneous or surface modification strategies. The "click" of small molecular appendages [30,31] onto polysaccharide backbones has proven a great success, during which full conversion to triazoles can be achieved.

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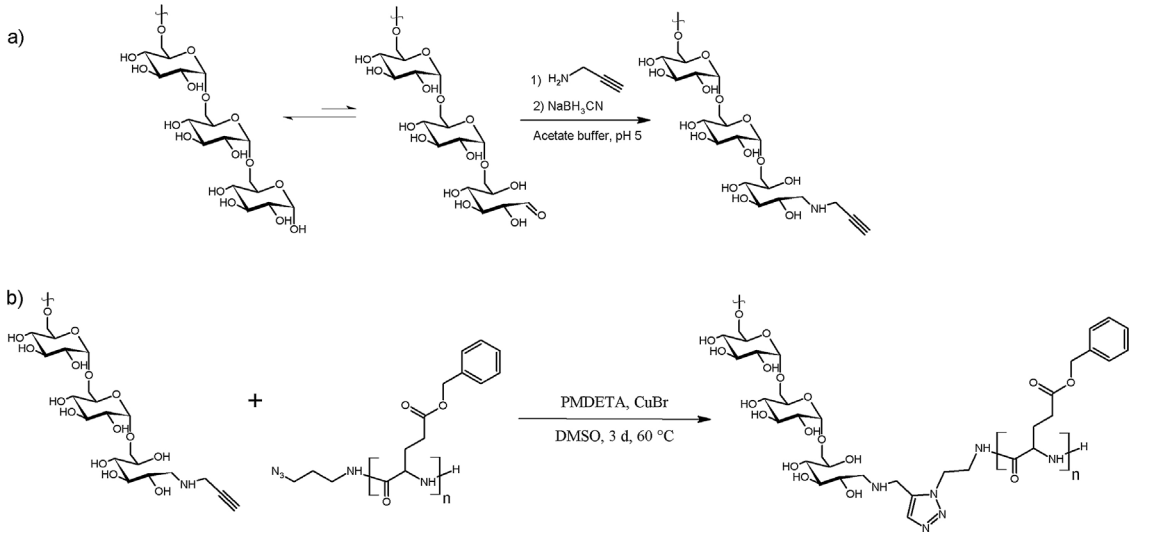

Fig. 5. Synthesis of a dextran-block-poly(γ-benzyl L-glutamate) copolymer via CuAAC. (a) Synthesis of end-functionalized dextran with an alkyne group by the reductive amination of dextran with propargylamine. (b) Block coupling with azido end-functionalized PBLG by click chemistry. [61], Copyright 2009. Adapted with permission from Wiley-VCH Verlag GmbH & Co. KGaA.

For example, Liebert and Heinze found that high conversion (from 81 to 98%, depending on the alkynyl partner used) could be achieved in CuAAC of 6-azido-6-deoxy cellulose by applying a molar ratio (alkyne to azide) of 3:1 at a reaction temperature of 25 °C in DMSO for 24 h [30]. Hasegawa et al. employed an excess of small molecule alkynyl compounds bearing different functional groups such as β-lactoside and pyrene to react with 6-azido-6-deoxy curdlan (26.7–1.8:1 in molar ratio depending on the specific alkynyl compounds) and achieved complete conversion to click products [31].

Encouraged by these results, derivatives with more diverse architectures including graft [36,44,46,59,67], block [61–63], and dendronized [37,68] copolymers have been synthesized via CuAAC. Copolymerization of two or more macromolecules may not only combine the properties of the polymers, but may also generate new properties. The CuAAC reaction provides a "graft onto" strategy, which gives more controllable and well-defined copolymers than the "graft from" counterpart. Commonly, side chain alkynylated or azidated polysaccharides were reacted with azido- or alkynyl-terminated synthetic polymers or dendrons under typical CuAAC conditions. Synthetic polymers such as PEG [36], PDMAEMA [44] and PNIPAM [44,46] have been grafted onto polysaccharide backbones via CuAAC click chemistry. As with small molecules, high coupling efficiency (above 80%) can be achieved under standard reaction conditions (e.g. DMSO, 40 °C, 24 h), especially when a slight excess of the synthetic polymer moiety is used to ensure complete cycloaddition.

The end-functionalization methods [58–63] discussed in the previous section, which generate azido- or alkynyl terminated polysaccharides, enable the synthesis of graft-co-polymers with polysaccharides as the side chains, or linear block copolymers containing polysaccharide segments. The Kamitakahara group prepared poly(2-propyn-1-yl methacrylate)-g-cellulose triacetate (PPMA-g-CTA) and PPMA-g-cellulose by CuAAC (Fig. 6) [59]. With the ratio of PMA monomer (alkyne) to azido-CTA equaling to 1:1, quantitative formation of triazoles was observed and supported by the disappearance of the N_3 absorbance at 2120 cm^{-1} in FTIR spectra after the click reaction. Lecommandoux et al. introduced alkynyl groups to the reducing ends of dextran [61] and hyaluronic acid [62], and subsequently clicked the polysaccharide segments with an azido-terminated polypeptide to afford dextran-b-poly(γ-benzyl L-glutamate) (dextran-b-PBLG) and HA-b-PBLG respectively (Fig. 5b). Polymersomes formed by self-assembly of the block copolymers showed controlled size and excellent stability, attributed to the hydrophilic polysaccharide segments, showing potential for drug delivery and other applications. Although it may seem more challenging to synthesize these copolymers by CuAAC due to higher steric hindrance, coupling efficiency was satisfactory in most cases when an excess of one partner (e.g. mol ratio = 3:1) and longer reaction time (e.g. 48 h) were used.

Networks are another type of polymer architecture that can be obtained through the coupling of alkynes and azides. Crosslinking between the same [39,42,48,54,69] and different [38,41] polysaccharide species, and between synthetic polymers and polysaccharides [70] have been realized using similar conditions as used in CuAAC of polysaccharides with either small moieties or other polymers.

Heterogeneous modification by CuAAC of polysaccharide surfaces [47,52,71–76], such as those of cellulose nanocrystals (CNC), has also been intensively explored. As the principles are largely the same as for homogeneous modification mentioned above, this topic will not be discussed separately in this review.

2.3. Limitations

Undoubtedly, CuAAC has emerged as a useful tool in polysaccharide modification because of its modularity,

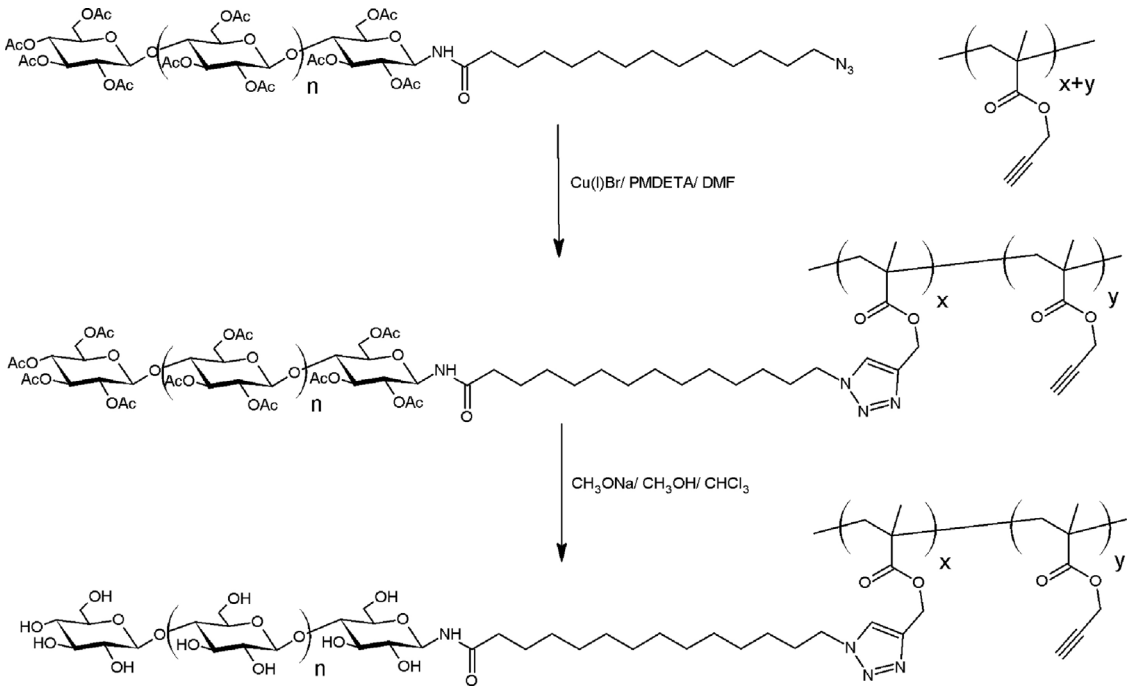

Fig. 6. Preparation of poly(2-propyn-1-yl methacrylate)-g-cellulose triacetate (PPMA-g-CTA) and PPMA-g-cellulose by CuAAC. [59], Copyright 2012. Adapted with permission from Elsevier Ltd.

efficiency and versatility. However, several limitations of this chemistry need to be taken into account. First of all, it is important to consider the toxicity of Cu(I), which is capable of mediating the generation of reactive oxygen species (ROS) from O_2 and thereby causing damage to living cells [77,78]. Although it might not be such a serious problem for certain polysaccharides under homogeneous conditions, where it may be possible to eliminate copper ions during purification, the removal of the biologically toxic Cu(I) compounds becomes problematic in the preparation of polymer networks. Furthermore, the tendency of aminopolysaccharides, such as chitosan [79], to chelate heavy metals makes the removal of Cu ions more difficult, under either homogeneous or heterogeneous conditions. Another problem that may arise with Cu(I) is depolymerization of the polysaccharide. There have been a number of studies showing that ROS are able to induce oxidative cleavage of polysaccharide chains [80,81]. Cu ions were proven to increase the scission rate as Cu(I) reacts with H_2O_2 to generate $^{\bullet}OH$ [82]. Lallana et al. [83] observed severe chain cleavage of the chitosan backbone after CuAAC, and rationalized the depolymerization as the result of hydroxyl radical ($^{\bullet}OH$, a ROS) mediated chain scission.

In spite of the great value of sodium azide and organic azides as starting materials for synthesis of azido-functionalized polysaccharides, or as intermediates (organic azides) in alkyne–azide cycloaddition, they are potentially explosive substances that can decompose (potentially violently in the case of small molecule azides) triggered by only slight input of energy (heat, light, and/or pressure) [84]. Besides their explosive tendencies, potential toxicity of both residual organic azides on polysaccharide backbones and azide ion should also be taken into account. Azide ion has a toxicity comparable to that of cyanide ion (LD_{50} = 27 mg/kg for rats) [85]. It becomes even worse when azide is acidified to form HN_3, which is volatile and highly toxic. From this point of view, starting materials, intermediates and products with azide residues from CuAAC should be prepared, stored, and used with caution.

Last, the 1,2,3-triazoles formed through the alkyne-azide cycloaddition can be potentially bioactive pharmacophores [86], the bioactivity of which may become problematic if it is not the desired effect.

3. Metal-free [3+2] cycloaddition

As mentioned in the last section, the copper catalysts that are necessary in CuAAC are toxic and problematic in areas including polysaccharide modification, thus limiting the applications of CuAAC. For this reason, alternative means of metal-free 1,3 dipolar cycloaddition with azides have been explored, such as strain-promoted [3+2] azide–alkyne cycloaddition (SPAAC) [87], and azide-oxanorbornadiene associated cycloaddition [88].

3.1. Strain-promoted [3+2] azide–alkyne cycloaddition

In its classic version, the azide–alkyne cycloaddition may be problematic due to slow reaction rates in the absence of elevated temperatures. A metal-free alternative is SPAAC (Scheme 1(3)), first developed by the Bertozzi group [87], that takes advantage of the ring strain of cyclooctyne as an effective way to lower the activation barrier of the cycloaddition reaction. This seminal work has been adopted rapidly by other groups as a powerful

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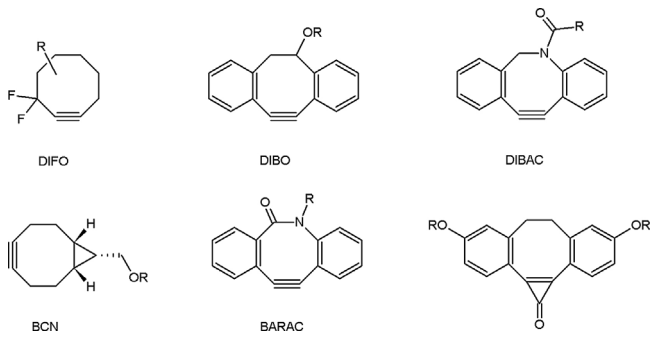

Fig. 7. Activated cyclooctyne derivatives used in SPAAC bioconjugations. Difluorocyclooctynes (DIFO), dibenzocyclooctynes (DIBO), dibenzoazacyclooctynes (DIBAC), bicyclo[6.1.0]nonynes (BCN), and biarylazacyclooctynones (BARAC). [21], Copyright 2011. Reproduced with permission from Wiley-VCH Verlag GmbH & Co. KGaA.

and benign coupling strategy in polymer and material science [89,90]. Meanwhile, intensive studies dedicated to the development of cyclooctyne reagents or cyclooctyne analogs have generated more reactive activated reagents (Fig. 7) by adding electron-withdrawing groups or increasing ring strain, the reaction rates of which are comparable to those of ligand-less CuAAC [21].

Application of metal-free AAC to polysaccharide modification was first reported by the Fernandez-Megia and Riguera group [83]. In their research (Fig. 8a), azide-terminated PEG functionalized at the other end with a carboxylic acid was grafted onto the chitosan backbone under EDC catalysis, providing a handle for subsequent click reactions. The product CS-g-PEG-N_3 was first coupled with PEG functionalized cyclooctyne (PEGO-OH) in 5% DMSO/H_2O to evaluate the conditions required for, and efficiency of the SPAAC reaction. Monitoring the progress of the click functionalization was achieved by disappearance of proton signals vicinal to the azide group. Using 2.5 equiv. cyclooctynes per 1 equiv. N_3, complete conversion was observed after 4 h at 80 °C, although at lower temperatures (25 and 55 °C), the reaction took as long as 86 h for complete conversion. More importantly, while the authors observed a depolymerizing effect of Cu(I) when applying CuAAC to polysaccharides [polymer (% decrease in MW): mannan (3%), dextran (38%), chitosan (95%), and hyaluronic acid (>99%)], depolymerization was not observed in the SPAAC reaction under similar conditions. More sophisticated cyclooctynes, i.e. mannose and FITC PEGO derivatives, were also tested and showed complete conversion with excellent mass recoveries, showing the modular profile of this reaction. Moreover, the authors extended the applicability of SPAAC to functionalizing nanoparticle surfaces (Fig. 8b). CS-g-PEG-N_3 and fluorescent CS-g-PEG-FITC were crosslinked by citric acid/EDC to give nanoparticles with $-N_3$ handles and fluorescent markers on the surface. These nanoparticles were then successfully coupled with IgG functionalized cyclooctyne by SPAAC, further demonstrating the versatility of this chemistry.

A related study was conducted by Lee et al. [91], in which ^{64}Cu was attached to chitosan nanoparticles (CNP) for an imaging application via SPAAC. In this research (Fig. 9), the authors clicked azide-functionalized CNP (CNP-N_3) with a dibenzoazacyclooctyne (DIBAC) functionalized complex that was radiolabeled with ^{64}Cu. According to the authors, the copper-free click chemistry was accomplished within 30 min at 40 °C under aqueous conditions, giving high radiolabeling efficiency and high radiolabeling yield (98%). Using the same strategy, the team further conjugated both ^{64}Cu radiolabeled complex and activatable matrix metalloproteinase-sensitive peptide (MMP-sensitive peptide) onto CNP-N_3 via a bioorthogonal click reaction, which was demonstrated to be a useful technique for cancer imaging and diagnosis [92]. In another study, instead of using azide, Jung and Yi [93,94] first activated chitosan-PEG hybrid microparticle with a DIBAC handle by NHS-amine chemistry, followed by conjugation of azide-functionalized proteins and ss-DNA via SPAAC. The authors also investigated the protein conjugation kinetics, the results of which revealed multiple regimes including a rapid initial stage, an intermediate stage, and a steady and slow final stage. Hydrogel formation may also be accomplished by this chemistry. By mixing azide-functionalized and cyclooctyne-functionalized HA derivatives using a double-barreled syringe, Takahashi et al. [95] successfully obtained an in situ cross-linkable hydrogel under physiological conditions without the assistance of any catalyst.

Although only a handful of studies have been published using the SPAAC strategy in polysaccharide modification, they have demonstrated the high selectivity, bioorthogonality and acceptable reactivity of this reaction under mild reaction conditions. More importantly, this approach avoids the use of copper or other metal catalysts, which is of great value especially for biomedical applications [96]. In spite of those advantages, it is noteworthy that unlike CuAAC which gives almost exclusively 1,4-disubstituted 1,2,3-triazoles, the products of SPAAC are mixtures of triazole regioisomers [83]. Other possible limitations include the lack of commercial availability, laborious lab synthesis, and instability of cyclooctynes [78]. However, as the importance of SPAAC is being proven, these issues may be successfully addressed in future work.

3.2. Oxanorbornadiene-azide [3+2] cycloaddition

Trifluoromethyl-substituted oxanorbornadienes are another family of reagents that can undergo [3+2] cycloaddition with azides without the use of a metal catalyst, as first reported by the Rutjes group [88] in 2007. The

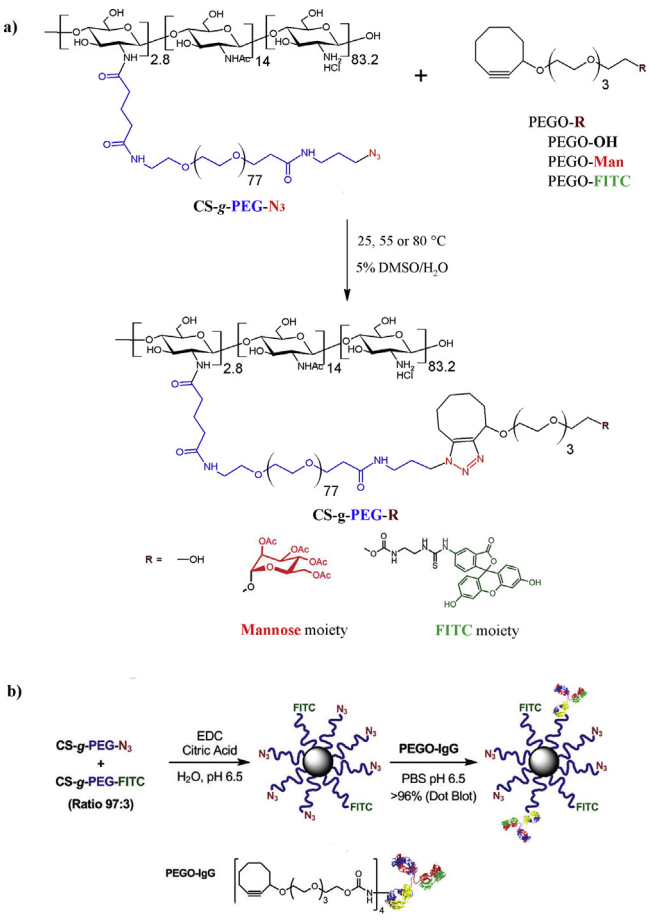

Fig. 8. (a) SPAAC conjugation of CS-*g*-PEG-N_3 with cyclooctyne-functionalized PEG (PEGO-OH), cyclooctyne-functionalized PEG-Mannose (PEGO-Man), and cyclooctyne-functionalized PEG-FITC (PEGO-FITC). (b) SPAAC conjugation of crosslinked CS-*g*-PEG-N_3 and CS-*g*-PEG-FITC nanoparticles with cyclooctyne-functionalized IgG (PEGO-IgG). [83], Copyright 2009. Adapted with permission from the American Chemical Society.

formation of stable 1,2,3-triazole-linked compounds is driven by an elegant tandem [3+2] cycloaddition-retro-Diels–Alder reaction, with the formation of furan as a major volatile byproduct (Fig. 10). According to the authors, the oxa-bridged bicyclic systems boosted the reaction rates of the cycloaddition to approximately fivefold higher than those of the corresponding linear alkynes. Moreover, the electron-withdrawing trifluoromethyl substituent on oxanorbornadiene also contributed to a 2.3 fold increase in reaction rates for the oxanorbornadiene system. As a result, the corresponding [3+2] cycloaddition performed under ambient conditions can reach up to 80% conversion within 800 min [88].

The Draeger group pioneered extension of the above-mentioned reaction to the field of polysaccharide modification by conjugating cRGD-pentapeptides to oxanorbornadiene modified alginate [97]. Attachment of an ethylenediamine extended oxanorbornadiene system by amidation with the carboxylic acid groups of alginate was performed using a classical carboxyl-activation procedure with the assistance of EDC as shown in Fig. 11. Bioorthogonal click reactions with different azide functionalized moieties, including cRGD-pentapeptides, were then carried out at room temperature for 4 days in aqueous media. As monitored by ^{1}H NMR as well as ^{19}F NMR (while ^{1}H NMR was sufficient to monitor the progress of cycloaddition, ^{19}F NMR was necessary to identify and quantify different isomers), almost complete transformation was observed with stoichiometric or sub-stoichiometric amounts of azide, demonstrating the modular and bioorthogonal traits of this reaction under mild conditions. Using a similar strategy, the Chirachanchai and Draeger groups also demonstrated the applicability of this reaction in the synthesis of chitosan derivatives including carboxylic acid, disulfide and silane derivatives [98], and chitosan-gold-antibody hybrids [99] in aqueous conditions.

Among the major limitations of this reaction are its comparatively low chemoselectivity and regiospecificity. On the one hand, both of the double bonds in the bicyclic system are able to react in the tandem [3+2] cycloaddition-retro-Diels–Alder reaction, giving both furan and triazole products, although the 1,4,5-substituted triazoles are the main products [97,98]. On the other hand, even for the 1,4,5-substituted triazoles, there are two regioisomeric forms, deriving from 1,4-cycloaddition (*trans*) and 1,5-cycloaddition (*cis*) during the click process (Fig. 11). Although it has not been reported in polysaccharide chemistry, two aspects of oxanorbornadienes should

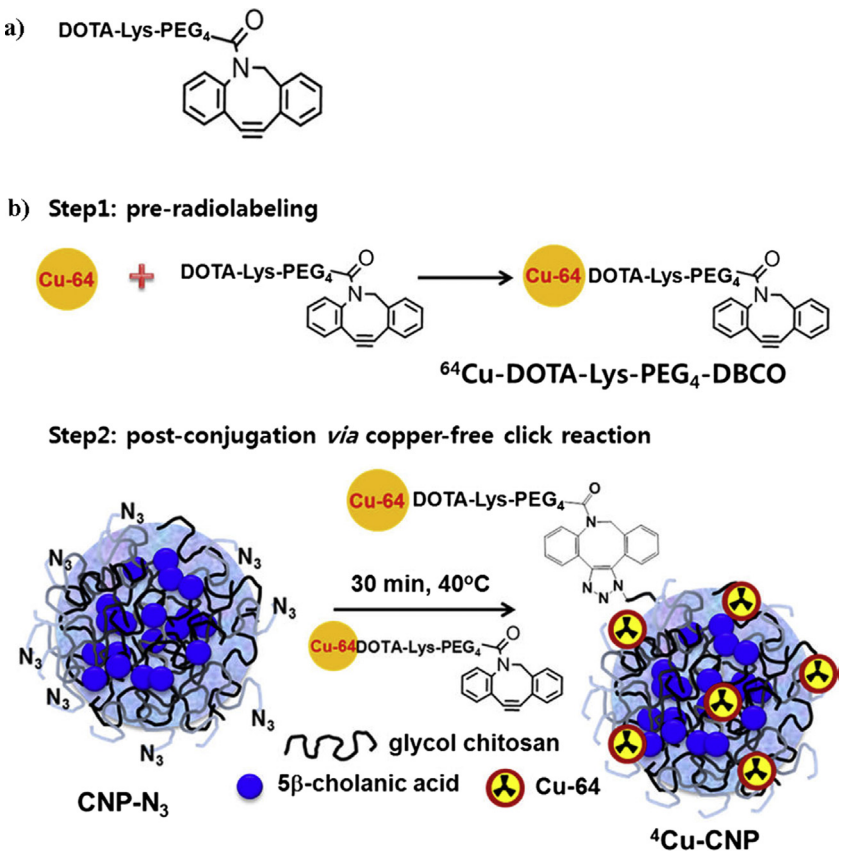

Fig. 9. Schematic illustration for the radiolabeling of azide-functionalized CNP with pre-radio labeled DBCO-PEG_4-Lys-DOTA-^{64}Cu via copper-free click chemistry. (a) Chemical structure of DBCO-PEG_4-Lys-DOTA. (b) Radio labeling strategy via copper-free click chemistry: pre-radio labeling of DBCO-PEG4-Lys-DOTA with ^{64}Cu (step 1), followed by simple radio labeling of CNP-N_3 with DBCO-PEG4-Lys-DOTA-^{64}Cu via copper-free click chemistry (step 2). [91], Copyright 2013. Reproduced with permission from the American Chemical Society.

be noted, i.e. their Michael acceptor profiles and retro-Diels–Alder fragmentation tendency [100]. If properly utilized, these two properties may help to generate novel reversible hydrogels or drug delivery systems. Otherwise, undesired crosslinking brought by Michael addition between nucleophiles, e.g. —OH or —NH_2 on polysaccharide backbones, and the electron-deficient oxanorbornadiene double bonds may be a potential problem. Overall, neither

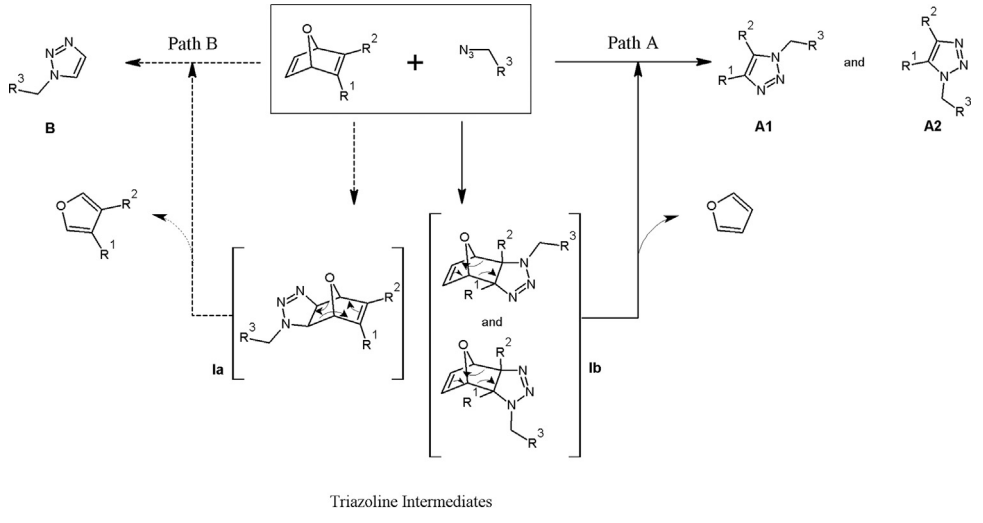

Fig. 10. Reaction pathways of [3+2] cycloaddition of substituted oxanorbornadiene with azide for the formation of triazole compounds. [88], Copyright 2007. Reproduced with permission from WILEY-VCH Verlag GmbH & Co. KGaA.

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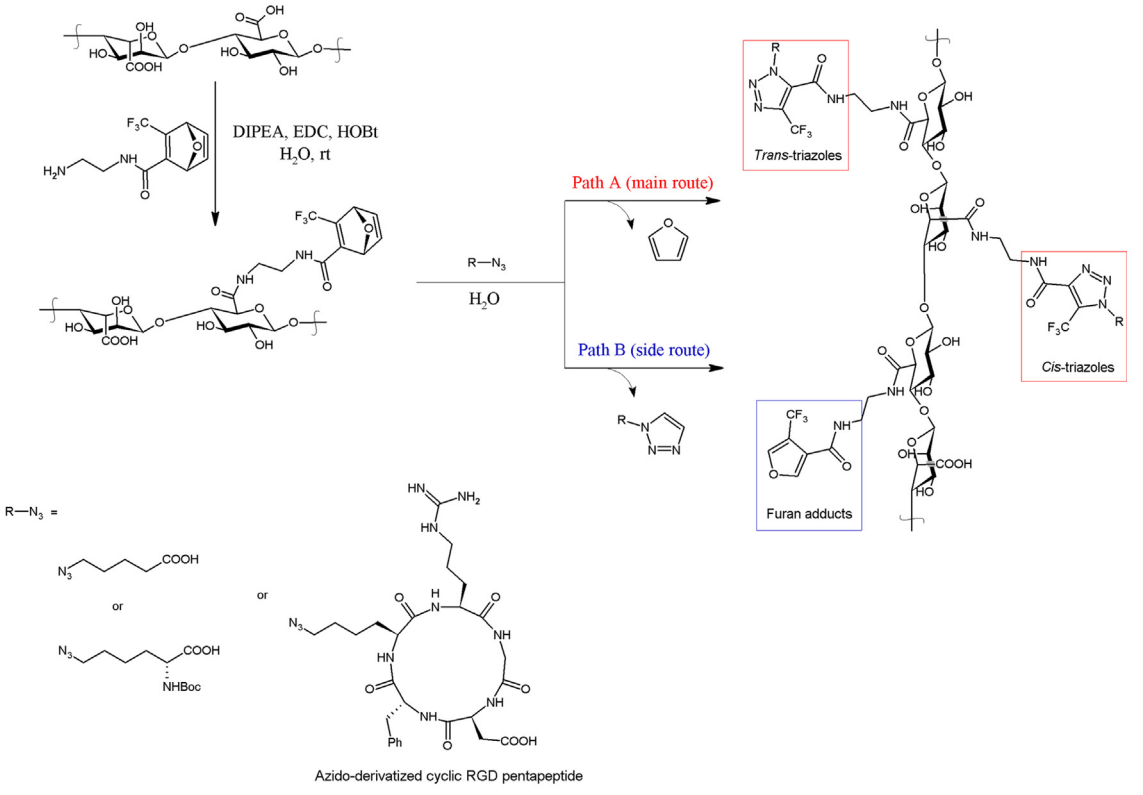


Fig. 11. Metal-free click reaction of oxanorbornadiene-functionalized alginate with different azides. Regular cycloaddition provides two regioisomeric triazoles (path A, structures in red rectangles); undesired cycloaddition with an alternative alkene moiety results in the formation of by-products (path B, structure in blue rectangle) as also illustrated in Fig. 10. [97], Copyright 2012. Adapted with permission from the Royal Society of Chemistry.

the potential of the cyclooctyne-associated SPAAC nor the oxanorbornadiene-associated [3+2] cycloaddition have been fully explored for polysaccharide modification, and we believe substantial potential exists in these areas.

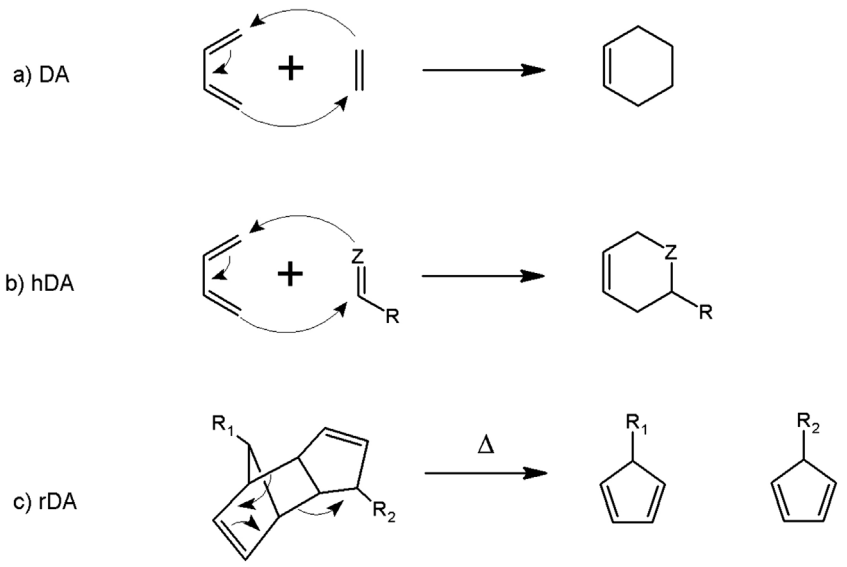
4. Diels–Alder reaction

The Diels–Alder (DA) reaction is a [4+2] cycloaddition reaction discovered by Otto Diels and Kurt Alder in 1928 [101], for which they were awarded the Nobel Prize in 1950. For a typical DA reaction, the [4+2] cycloaddition involves a conjugated diene that is electron-rich (e.g. furan), and an electron-poor dienophile (usually an alkene, e.g. maleic acid) to form a (substituted) cyclohexene system (Scheme 2a). Being modular, wide in scope, giving very high yields, and generating no or only inoffensive byproducts, this reaction qualifies as a click reaction and has been widely used not only in organic chemistry [102] but in polymer and materials science [103,104].

4.1. Classic Diels–Alder reaction

Although it has made a tremendous contribution to organic synthesis, application of the DA reaction to polysaccharide modification is relatively new. One of the most studied fields of application has been for preparation of networks (mainly hydrogels), partially due to the fact that this orthogonal reaction can be performed under aqueous conditions at room temperature without the need for catalysts, the products of which therefore would not require extensive washing to remove residual catalysts or unreacted coupling reagents that would otherwise be present in the network. One of the first examples was reported by Nimmo et al. [105,106] in 2011. In this approach, furan-modified HA was cross-linked in a single step in 100 mM 2-(N-morpholino)-ethanesulfonic acid (MES) buffer at pH 5.5 (reaction temperature and time were not reported) via DA reaction using a bismaleimide poly(ethylene glycol) as the cross-linker (Fig. 12). FTIR spectroscopy was employed to characterize the gelation by tracking the decrease of absorbances at 1455 (C=C stretch from furan), 695 (=C—H bending from maleimide) and 1466 (C=C stretch from maleimide) cm^{-1}, and the appearance of a new absorbance at 1459 cm^{-1} (C=C stretch from the DA adduct). By controlling the diene to dienophile ratio, the authors were able to tune the shear moduli of the hydrogels in the range of 100–1000 Pa, which are similar to those of brain and nerve tissues. Minimal swelling, complete degradation by hyaluronidase, and good cytocompatiblity of the gels were also observed, suggesting their potential in tissue engineering.

Almost simultaneously, a similar approach was reported by Tan et al. [107], in which a DA click reaction was performed between maleimide- and furan-functionalized hyaluronic acids in PBS and 100 mM MES buffer at 37 °C.



Scheme 2. (a) Diels–Alder (DA), (b) hetero Diels–Alder (hDA) and (c) retro Diels–Alder (rDA) reactions.

Gelation occurred approximately 26 min after mixing, and was complete at 40 min. With the investigation of mechanical properties, degradability, and cytocompatability, the authors also examined the potential of the hydrogel to load and release proteins, the results of which showed that the hydrogel has potential as a drug delivery system. Other investigations of similar chemistry include the synthesis of injectable HA/PEG hydrogel [108], interpenetrating HA/gelatin/chondroitin sulfate hydrogel [109], and hydroxylpropyl methylcellulose-based hydrogels [110].

Indeed, the fact that the DA reaction can proceed rapidly under very mild conditions in the absence of catalyst has a number of merits in organic synthesis. However, in areas such as biosurface modification, spatial and/or temporal control of the reaction is sometimes necessary so that the reaction and thus the corresponding property changes only occur when and where a stimulus (e.g. irradiation) is applied [112]. Considering the lack of such control over the standard DA reaction, Barner-Kowollik's group demonstrated a proof-of-concept design to achieve

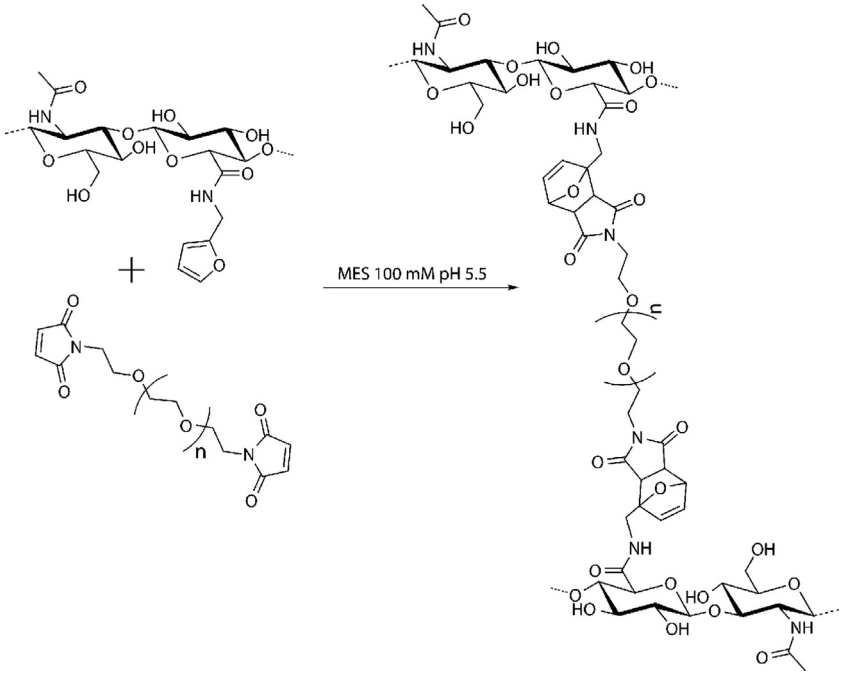


Fig. 12. Schematic representation of formation of Diels–Alder HA-PEG hydrogels by crosslinking HA-furan with dimaleimide PEG. [105], Copyright 2011. Reproduced with permission from the American Chemical Society.

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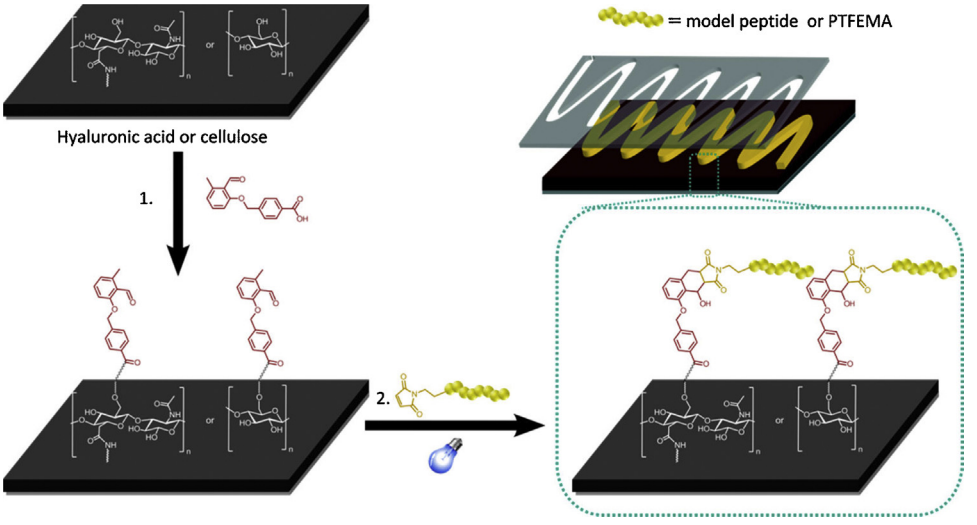

Fig. 13. Synthetic route for spatially controlled functionalization of HA and cellulose films with tailor-made peptides or synthetic polymer strands via photoenol ligation. [111], Copyright 2013. Reproduced with permission from the American Chemical Society.

spatiotemporally controlled functionalization of cellulose and HA surface via DA reaction [111]. In their study, o-quinodimethane (photoenol) was functionalized onto the surfaces of cellulose and HA films and was used as a phototrigger. Upon irradiation with UV light, the photoenol underwent isomerization to its reactive diene form, which was trapped by subsequent irreversible DA reactions with a maleimide-functionalized model peptide and poly(trifluoroethylmethacrylate) (Fig. 13). Successful grafting, which was confirmed by XPS and ToF-SIMS, was achieved within 2 h irradiation at 320 nm in DMF. With this technique, the authors realized patterning of peptides and synthetic polymers on polysaccharide surfaces, which opened doors to bioanalysis and other related applications.

4.2. Hetero Diels–Alder reaction

The dienophiles in the powerful Diels–Alder reaction are not confined to alkenes. Cycloaddition can also be initiated between a reactive diene and certain highly electron deficient heteroatom-containing double bonds (e.g. nitrosocarbonyl compounds [113], and thiocarbonyl thio compounds [114]). This reaction type, termed "hetero Diels–Alder" (hDA, Scheme 2b), has also proven to be a fast and efficient click reaction. In 2009 [114] the Barner-Kowollik group demonstrated an efficient, extremely rapid, room temperature conjugation strategy via hDA that utilized a cyclopentadienyl group (Cp) as the diene and thiocarbonylthio compounds as dienophiles. Later, they reported the first applications of hDA to modify cellulose surfaces. In their research, a Cp-functionalized cellulose surface was modified by a thiocarbonylthio-capped poly(isobornyl acrylate) [115] and a thioamide-functionalized peptide [116] (Fig. 14). As the thiocarbonylthio compounds are also RAFT agents, this creates a modular method for surface modification of cellulose. In other words, as reactive hetero dienophiles, any RAFT generated polymers containing these thiocarbonylthio moieties can theoretically be grafted onto Cp-functionalized cellulose surfaces using this strategy. As the authors have pointed out, however, it is noteworthy that the Cp functionality has a tendency to undergo dimerization. As a result, it was recommended to perform the reaction at ambient temperature [116]. Also to be noted, a substantial amount of trifluoroacetic acid (TFA) was required in the cycloaddition as a catalyst in the RAFT-hDA process to activate the C=S double bond, when the RAFT-group bore a pyridinyl moiety [115]. Although this reaction can be performed at ambient temperature, side-reactions that TFA might introduce, e.g. esterification of TFA with free polysaccharide hydroxyl groups or acid-catalyzed polysaccharide chain scission, need to be taken into account when considering this approach to polysaccharide modification.

4.3. Retro Diels–Alder reaction

Another reaction (retro-DA, rDA) related to the DA reaction is the decomposition of certain DA (or hDA) products. In some cases this process is thermally reversible, which is of great value in the synthesis of self-healing polymers [104]. Ax and Wenz [117] synthesized networks of cellulose by crosslinking furan-pendent hydroxyethylcellulose with 1,6-bis(N-maleimido)hexane (75 °C, DMSO, 25 h). The rDA process was observed and characterized by UV spectra which indicated the restoration of the furan group when the networks were heated to 140 °C for 21 h. The authors claimed the successful preparation of a thermoreversible network. However, data concerning the capability of the polymers to undergo further DA cycloaddition after the rDA reaction was not reported. Wei et al. [118] conducted a more comprehensive study on dextran-based self-healing hydrogels via reversible DA reaction. In this study, fulvene groups were utilized as dienes and linked to the dextran backbone by esterification (Dex-FE), while dichloromaleic anhydride capped PEG (PEG-DiCMA) served as dienophile

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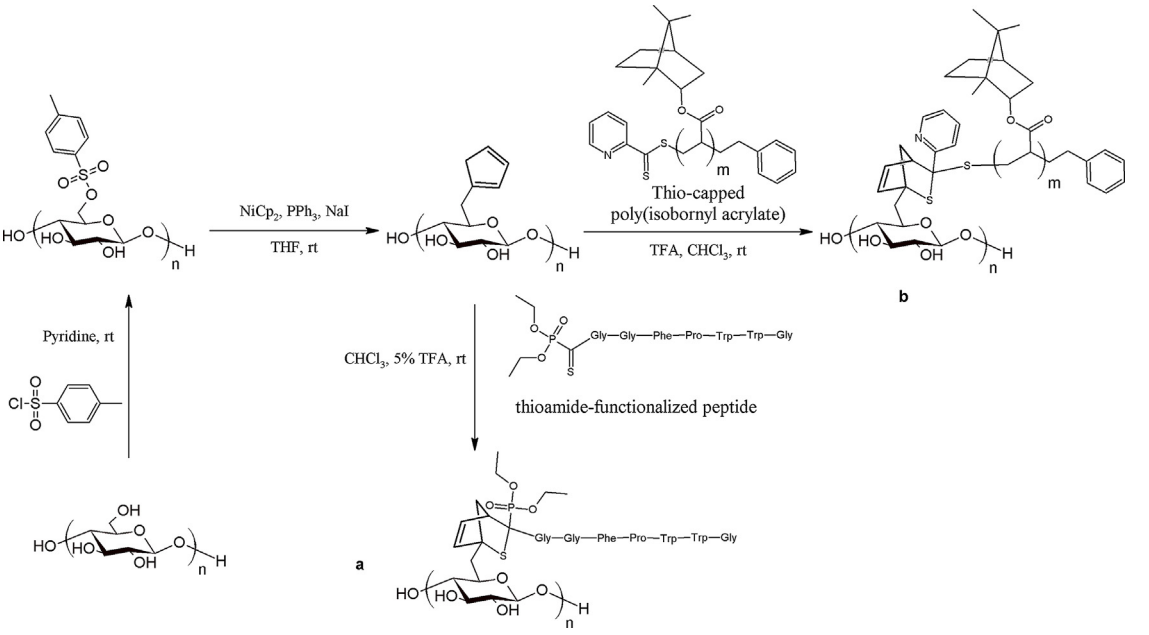

Fig. 14. General strategy for (a) coupling of a thioamide-functionalized peptide (Phos-GGFPWWG) and cyclopentadienyl-functionalized cellulose surface; and (b) coupling of the thiocarbonyl thio-capped poly(isobornyl acrylate) and cyclopentadienyl functional cellulose surface. (a) [116], Copyright 2012. Adapted with permission from Wiley-VCH Verlag GmbH. (b) [115], Copyright 2011. Adapted with permission from the American Chemical Society.

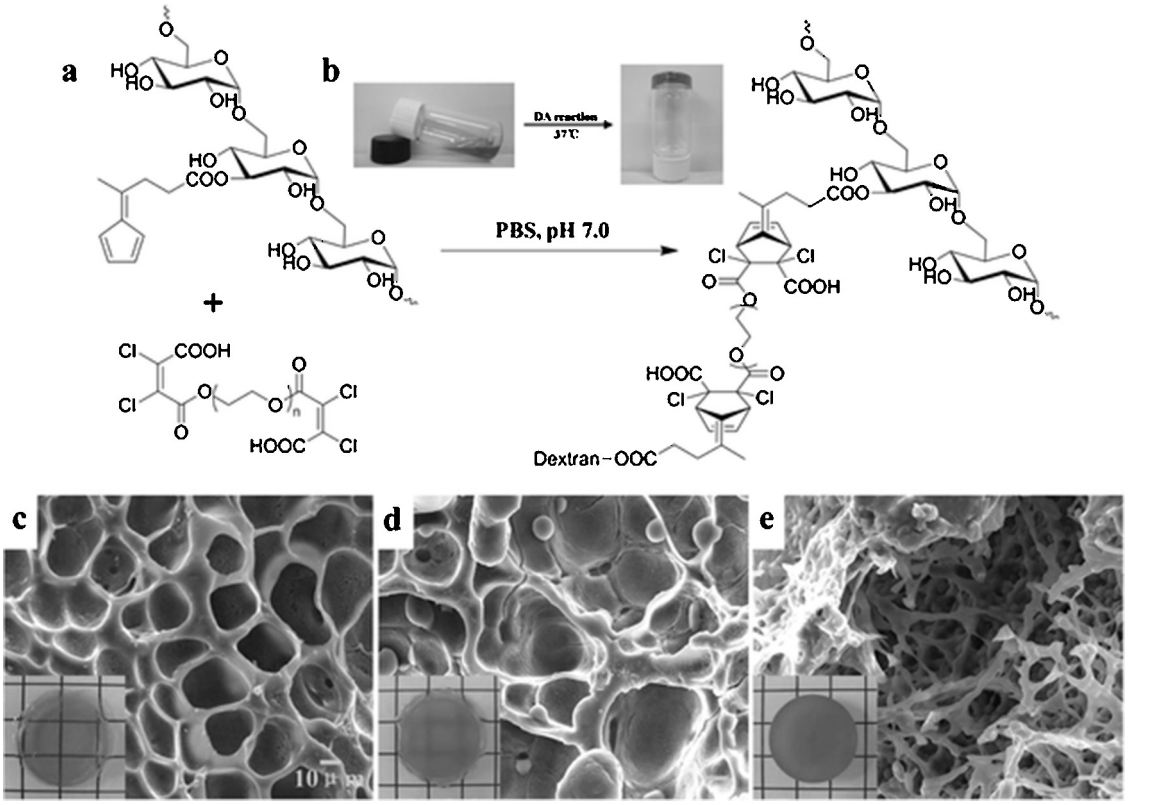

Fig. 15. Construction strategy, SEM images, and photographs of Dex-l-PEG hydrogels ("l" means "linked by"). (a) Chemical structures of the dextran-based hydrogel cross-linked by reversible Diels–Alder reaction through the fulvene groups of Dex-FE polymeric chains and the two dichloromaleic acid groups at the end of PEG–DiCMA chains. (b) Photographs before and after gelation of Dex-l-PEG hydrogel (20 wt%, $R = 1$) in PBS (pH 7.0) at 37 °C. (c–e) SEM images and photographs of Dex-l-PEG hydrogels when $R = 1$, (d) $R = 2$, and (e) $R = 3$. [118], Copyright 2013. Reproduced with permission from WILEY-VCH Verlag GmbH & Co. KGaA.

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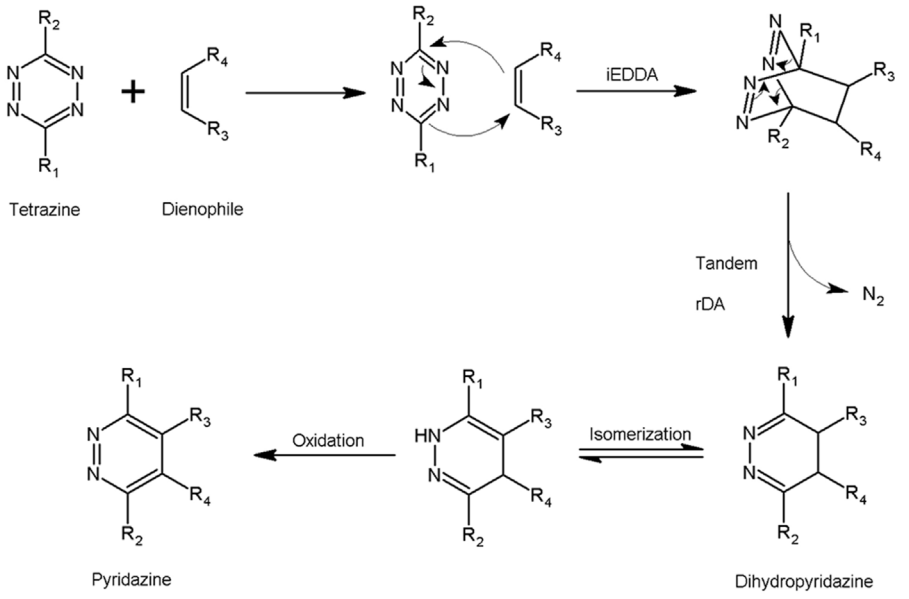

Scheme 3. Illustration of inverse electron demand Diels–Alder (iEDDA) reaction.

and cross-linker. Gelation was performed by simply mixing Dex-FE and PEG-DiCMA in PBS buffer under physiological conditions (pH 7.0, 37 °C) (Fig. 15). By varying the molar ratio of Dex-FE and PEG-DiCMA, the gelation rate as well as the morphology of the hydrogels can be controlled. Interestingly, the gels formed by the DA reaction were capable of self-healing, which was evidenced by both direct visual examination and mechanical tests. Optical microscopy results showed that self-healing of the split hydrogels achieved 98.7% after incubating at 37 °C for 7 h. The authors rationalized the self-healing performance as being caused by dynamic equilibrium between uncoupling and recoupling of the fulvene and dichloromaleic acid groups.

The concept of reversible crosslinking in polysaccharide chemistry by either cycloaddition (e.g. dimerization of coumarin-modified polysaccharides [119,120]) or other (e.g. Schiff-base [121] or hydrazone formation [122]) methods has been of great interest. The rDA approach discussed here has provided another option with autonomous healing potential.

4.4. Inverse electron demand Diels–Alder reaction

Recently, a much more intriguing and promising type of DA reaction, i.e. inverse electron demand Diels–Alder (iEDDA) reaction was described almost simultaneously in 2008 by the Fox [123] and Hilderbrand groups [124] as a potential click reaction. In contrast to normal electron demand DA reactions, the tetrazine (Tz) dienes are electron deficient, and dienophiles that are electron-rich are preferred in the iEDDA reaction. In this reaction, a tetrazine and a reactive dienophile (e.g. trans-cyclooctene (TCO), norbornene and their derivatives) undergo inverse electron demand DA reaction toward a highly strained bicyclic adduct, and then tandem rDA reaction takes place to form corresponding dihydropyridazines (which might be isomerized and oxidized to pyridazines) upon release of N_2 as the only byproduct (Scheme 3). Besides the non-catalyzed nature of the reaction, meaning that there are no metal or organic catalyst residues to remove from the polymeric product, one of the benchmark advantages offered by iEDDA is the impressively high rate of reaction (orders of magnitude faster) compared to those of peer click reactions (Table 2) [125].

Table 2
Typical reaction rates for popular bioorthogonal "click" reactions. [125], Copyright 2013. Adapted with permission from the Royal Society of Chemistry.

Reaction	Reactants	Rate [M^{-1} s^{-1}]
CuAAC	Terminal alkyne + azide	10^{-2}–10^{-4}
Staudinger-Bertozzi ligation	Azide + phosphine	2.2×10^{-3} [c]
Electron deficient AAC	DIFO[a] + azide	8×10^{-2} [c]
SPAAC	BARAC[b] + azide	1 [c]
iEDDA	Norbornene + tetrazine	1–10 [d]
iEDDA	BCN(ene)[e] + tetrazine	3.8×10^{5} [f]

[a] Difluorocyclooctyne.
[b] Biarylazacyclooctynone.
[c] Measured in CD3CN.
[d] In H_2O–MeOH (95:5), 21 °C.
[e] Bicyclo[6.1.0]nonene.
[f] H_2O-dioxane (90:10), 25 °C.

This chemistry was rapidly adopted in polysaccharide chemistry. One intriguing study was conducted by Devaraj et al. [126], in which tetrazine-modified dextrans were used to enable in vivo bioorthogonal iEDDA for applications including labeling biomolecules for tracking, and as biomarkers. Although cycloaddition of Tz and TCO can proceed at a much faster rate as shown in Table 2, the efficiency of in vivo labeling via small molecular TCO/Tz reactions was more often than not disappointing unless an excess of one click partner was used. One primary

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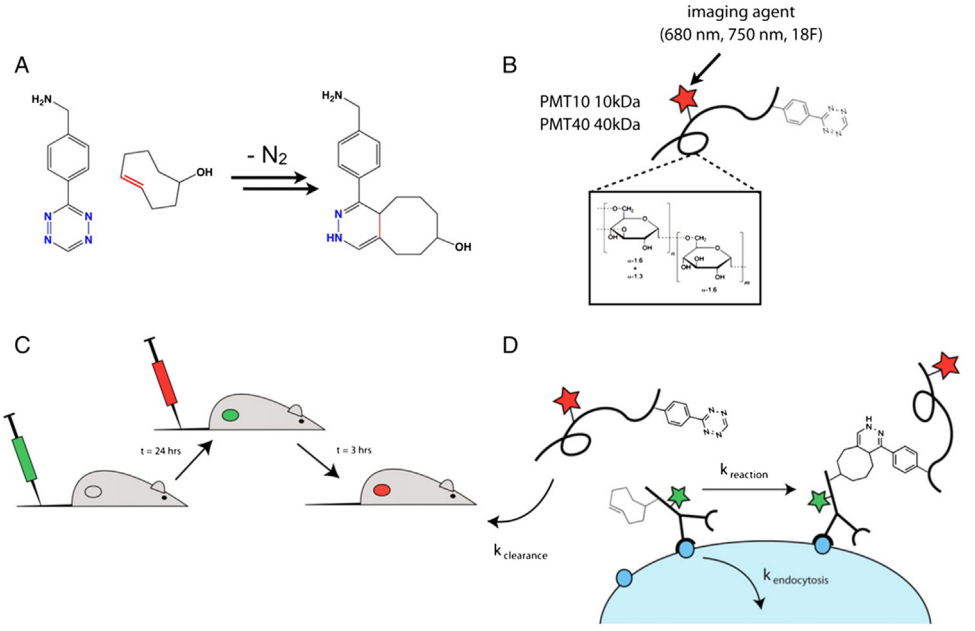

Fig. 16. In vivo bioorthogonal reactions. (A) Tetrazine cycloaddition with trans-cyclooctene forming a dihydropyrazine. (B) Schematic of PMT used in this study. The scaffold consists of dextran that has been aminated to allow attachment of tetrazine reactive groups as well as imaging agents such as near-infrared fluorophores and radioisotopes. (C) In vivo multistep delivery of imaging agent. A slow clearing targeting agent is administered first (green) and is administered for 24 h for localization and background clearance. Next, a lower molecular weight secondary agent (red) is delivered that rapidly reacts and is cleared from the background tissue much faster than the primary agent. (D) Kinetic parameters of consideration for in vivo clicking. The secondary tetrazine agent reacts with transcyclooctene antibodies at a given rate ($k_{reaction}$). This rate is in competition with other rates including the clearance of the secondary agent from the body ($k_{clearance}$) and internalization of the antibody ($k_{endocytosis}$). [126], Copyright 2012. Reproduced with permission from the National Academy of Sciences USA. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

reason for the failure of the reaction to proceed at the target site was proposed to be the pharmacokinetics of the small molecule reagents, which may be rapidly cleared by the test animals. To this end, polymer modified tetrazines (PMT) carrying imaging agents (in this study, tetrazine-functionalized dextrans) were synthesized as chaser to the initially injected TCO-antibodies (Fig. 16). The results showed that while the reactivity of PMT was not significantly impeded by the conjugation to dextran backbone, PMT pharmacokinetics were significantly improved (the PMTs persisted in the blood and exhibited biexponential clearance kinetics) compared with those of the small molecule tetrazine fluorophore chaser investigated in this study. As a result, successful and efficient in vivo bioorthogonal TCO/Tz reactions were realized by conjugating one reaction partner, Tz in this work, to dextran to optimize the pharmacokinetics.

Another impactful demonstration of the utility of the TCO/Tz reaction in polysaccharide chemistry was performed by the Jia and Fox groups [127]. In this work, the authors took advantage of the rapid reaction between Tz and TCO (rate constant $k_2 > 10^5\ M^{-1}\ s^{-1}$ has been measured [128]) to realize rapid diffusion-controlled interface crosslinking (Fig. 17). By adding Tz-modified HA (HA-Tz) into a bath of bis-TCO crosslinker (both in water solution) via syringe, microspheres formed instantly due to fast cross-linking at the interface. Reversing the order of addition, on the other hand, afforded water-filled hydrogel channels. Moreover, the microspheres synthesized by

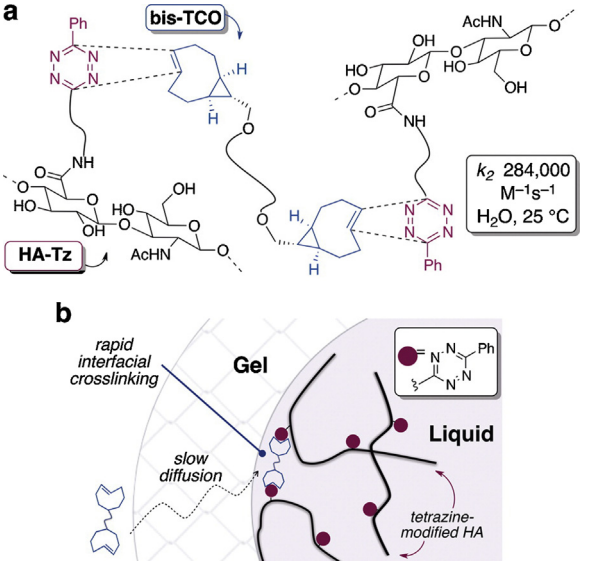

Fig. 17. (a) Instantaneous cross-linking via tetrazine-TCO ligation. (b) Gel interface forms when a droplet of tetrazine-modified hyaluronic (HA-Tz) contacts a solution of bis-trans-cyclooctene cross-linker (bis-TCO). Cross-linking at the gel/liquid interface is faster than the rate of diffusion through the gel interface. [127], Copyright 2014. Reproduced with permission from the American Chemical Society.

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this method exhibited excellent cytocompatibility, with the viability of prostate cancer cells encapsulated in microspheres reported as 99% and 98% at days 1 and 5 respectively.

Although there has been a surge of recent work using iEDDA reactions in polymer synthesis due to its outstanding "click" profile [125], application of this strategy to polysaccharide chemistry is still underexplored. We believe that polysaccharide chemists will soon realize the usefulness of this reaction, and employ it to generate a broad range of useful polysaccharide derivatives. Even so, there are still several limitations that need to be taken into consideration. One major limitation is the availability of the handles (especially Tz) for "click" ligation. They are usually not widely available and oftentimes require delicate and laborious workup for synthesis [129]. Another limitation lies in the stability of strained dienophiles, such as strained TCO. During storage, these reagents need to be stored in cold solution to avoid polymerization and isomerization [130]. Further, as most current applications of this reaction have been focused on biomedical studies such as in vivo imaging and labeling, the bioactivities of Tz, dienophiles and their conjugation products need further exploration [131].

5. Thiol-Michael addition reaction and thiol-ene reaction

Michael addition is a conjugate addition reaction in which a nucleophile (Michael donor, e.g. enolate anions, —OH, $—NH_2$, or —SH) attacks the β-carbon of an α,β-unsaturated carbonyl compound (Michael acceptor) usually in the presence of a base catalyst, resulting in a new C—C, C—O, C—N, C—S or other C—X linkage. As a thermodynamically favored reaction, it fulfills some of the criteria for a "click" reaction, useful in modular and regioselective synthesis. Having a long history of application in organic and polymer synthesis [132], the Michael addition has also been used in polysaccharide modification for both discrete polysaccharide derivatives and polysaccharide networks. While carbon-based nucleophiles have been used only to a very limited extent in polysaccharide chemistry, heteroatomic donors including oxygen, nitrogen, and sulfur (oxa-, aza-, and thiol-Michael reactions respectively) have been extensively explored.

Although both oxa- and aza-Michael addition reactions in polysaccharide modification are modular under mild reaction conditions, the conversion efficiency is comparatively low [133–137]. The thiol-Michael addition to electron-deficient carbon–carbon double bonds, on the other hand, is able to achieve quantitative yields while having the attributes of other Michael reactions such as rapid reaction rates, orthogonality with other common organic reactions, and mild reaction conditions. Similar to the thiol-Michael addition, the thiol-ene reaction is another highly efficient and modular reaction that has been highlighted by Sharpless [13] and many other researchers [16,138] as a click reaction. Unlike thiol-Michael addition which features 1,4-nucleophilic conjugate addition of a thiol anion to an α,β-unsaturated carbonyl, usually in the presence of a base or nucleophile catalyst, the thiol-ene reaction is a radical based reaction in which a thiol radical, produced either by photochemical initiation or by other free-radical initiators, attacks an electron-rich or electron–poor carbon–carbon double bond to give C—S—C linked adducts (Scheme 4).

5.1. Pre-functionalization of polysaccharide backbone with olefin

To employ thiol-Michael addition or thiol-ene reactions in polysaccharide modification, it is necessary to pre-functionalize the polysaccharide backbone with either olefin (Michael acceptor) or thiol (Michael donor) functionalities as handles for the subsequent click reaction. One approach to introduce Michael acceptors to polysaccharides is through acylation. Hasegawa et al. [139] linked acrylate to pullulan derivatives using DCC as coupling agent and DMAP as catalyst in DMSO, providing a handle for thiol-Michael addition. The obtained products, which self-aggregated into nanogels in PBS buffer, were subsequently crosslinked by pentaerythritol tetra(mercaptoethyl) polyoxyethylene (PEGSH) to give raspberry-like assemblies of nanogels (Fig. 18). The introduction of α,β-unsaturation could also be performed on polysaccharide surfaces. Nielson et al. [140] esterified cellulose nanocrystals with methacrylic acid in dichloromethane using the same DCC/DMAP coupling strategy. The obtained surface methacrylated cellulose nanocrystals were then reacted with cysteamine in methanol through thiol-Michael addition, appending amino functionality that was further used as a handle for fluorescent labeling of the nanocrystals.

Besides directly introducing acrylate/methacrylate onto polysaccharide backbones, spacers can be placed in between to increase the reactivity of the double bond in Michael addition by decreasing steric hindrance. For example, the Gama group employed CDI-activated hydroxyethyl methacrylate (HEMA) to esterify mannan [141] and pullulan [142] hydroxyl groups. In the following thiol-Michael addition, a 16-carbon hydrophobic tail containing a terminal thiol group was conjugated to the appended HEMA groups under TEA catalysis in DMSO. Acrylate functionality was also introduced to the surface of cellulose nanocrystals by reacting surface hydroxyl groups with isocyanate functionalized acrylate, which then permitted the immobilization of thiolated biosensors via thiol-Michael addition in PBS [143]. It worth noting that in the synthesis/storage of acrylate/methacrylate-functionalized polysaccharides, bases that can be Michael reaction catalysts (e.g. TEA) need to be avoided to minimize undesired oxa-/aza-Michael side reactions.

Esterification [144–146] or etherification [145,147] of polysaccharides with moieties containing olefins (not in conjugation, e.g., with a carbonyl group) can provide another type of handle for radical thiol-ene reaction. Unlike conjugated olefins such as acrylates and methacrylates, those terminal olefins are more stable against undesired Michael addition. Moreover, the conversion of the coupling reaction can be easily determined by integrating the ^1H NMR signals of the terminal olefins, which are usually well separated from polysaccharide backbone signals. One early study was performed on solid cellulose in 2007 by Zhao et al. [145], in which long chain terminal olefins, namely

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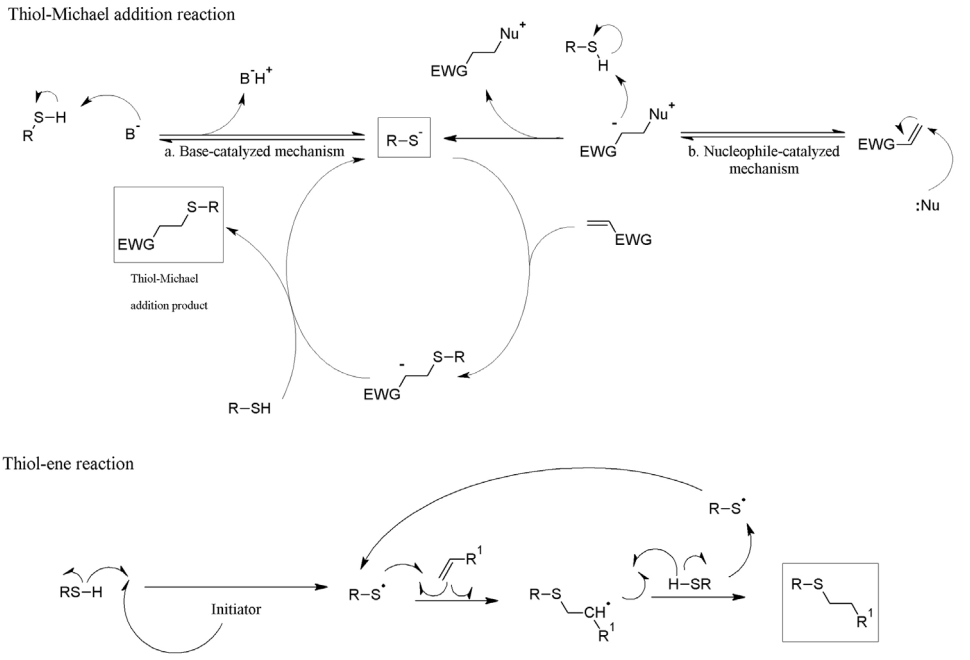

Scheme 4. Mechanisms of thiol-Michael and thiol-ene reactions.

9-decenoic acid and 2-(oct-7-enyl)oxirane, were attached to the cellulose surface by esterification and etherification, respectively, under (*S*)-tartaric acid catalysis. The modified surfaces were readily "clickable" by photoinitiated thiol-ene reaction under UV light. This concept was then employed by Rosilo et al. [146] for the preparation of intercalated cellulose nanocrystals and polybutadiene. The cellulose nanocrystal surface was first esterified by undec-10-enoyl chloride in pyridine and DMAP to give a terminal olefin functionality. Using nonanedithiol as a crosslinker, crosslinking then occurred between the double bonds of CNC undec-10-enoate and polybutadiene by UV irradiation (DMPA photoinitiator). Auzely-Velty's group extended this concept using HA and dextran in homogeneous aqueous solutions to obtain both discrete and crosslinked products by photochemical thiol-ene reactions (Fig. 19) [144]. In their study, the radical thiol-ene coupling exhibited high efficiency. Reacting at room temperature with five model mercaptans under UV irradiation (water-soluble Irgacure 2959 photoinitiator) for 5 min, the conversion of the double bonds on pentenoate-modified HA and dextran to thiol-ene adducts was up to 100% when a 3:1 feed ratio of thiol:olefin was employed. Even a 1:1 thiol:olefin feed ratio gave conversions ranging from 70 to 95%, depending on the mercaptan used.

Not only linear alkenes, but alkenes in rings (e.g. norbornenes) can serve as thiol-ene substrates. Thiol-norbornene chemistry is such an example. Compared with linear alkenes, strained olefins like norbornenes react more rapidly with thiol radicals, but have lower reactivity toward free-radical homopolymerization due to unfavorable steric effects [16]. Gramlich et al. [148] employed this chemistry in a recent approach by esterifying hyaluronic acid tetrabutylammonium salt (HA-TBA) with 5-norbornene-2-carboxylic acid to afford norbornene-functionalized HA (NorHA), followed by subsequent reactions with di- and mono-thiols to form gels (Fig. 20). In the same study, the authors also showed the potential of the photo-initiated thiol-ene chemistry for hydrogel photopatterning (Fig. 21). Serving as a trigger, irradiation makes possible spatially and temporally controlled photochemistry, thus enabling photopatterned hydrogels. Although compared with the phototriggered DA reaction (Fig. 13) [111] reviewed in the previous section, the involvement of photoinitiator and free-radical may limit its applications, this approach provides new understanding of the utility of photo-initiation profiles available through thiol-ene chemistry.

Vinyl sulfone (VS) is another good Michael acceptor for thiol-Michael addition. However, reacting divinyl sulfone (DVS) with polysaccharides usually leads to crosslinked products due to nucleophilic hydroxyl/amino groups attacking the electrophilic double bond of DVS by Michael-type addition [149] (Fig. 22). To obtain discrete vinyl sulfone modified polysaccharide derivatives useful as handles for subsequent thiol-Michael/thiol-ene click reactions, Hiemstra et al. [150] first carried out Michael addition of mercaptoalkanoic acids to one double bond of DVS. The product VS thioalkanoic acids were then esterified with dextran using DCC coupling agent. The VS-pendent dextran was further crosslinked by thiol-Michael addition of a multifunctional thiolated PEG (Fig. 23). In another study, Yu and Chau [151] reasoned that the previous crosslinking issues observed when reacting polysaccharides and DVS was due to the low molar ratios of DVS/polysaccharide used. The authors then successfully avoided crosslinking and obtained VS-functionalized water-soluble derivatives of dextran, alginate and HA by simply adding a large

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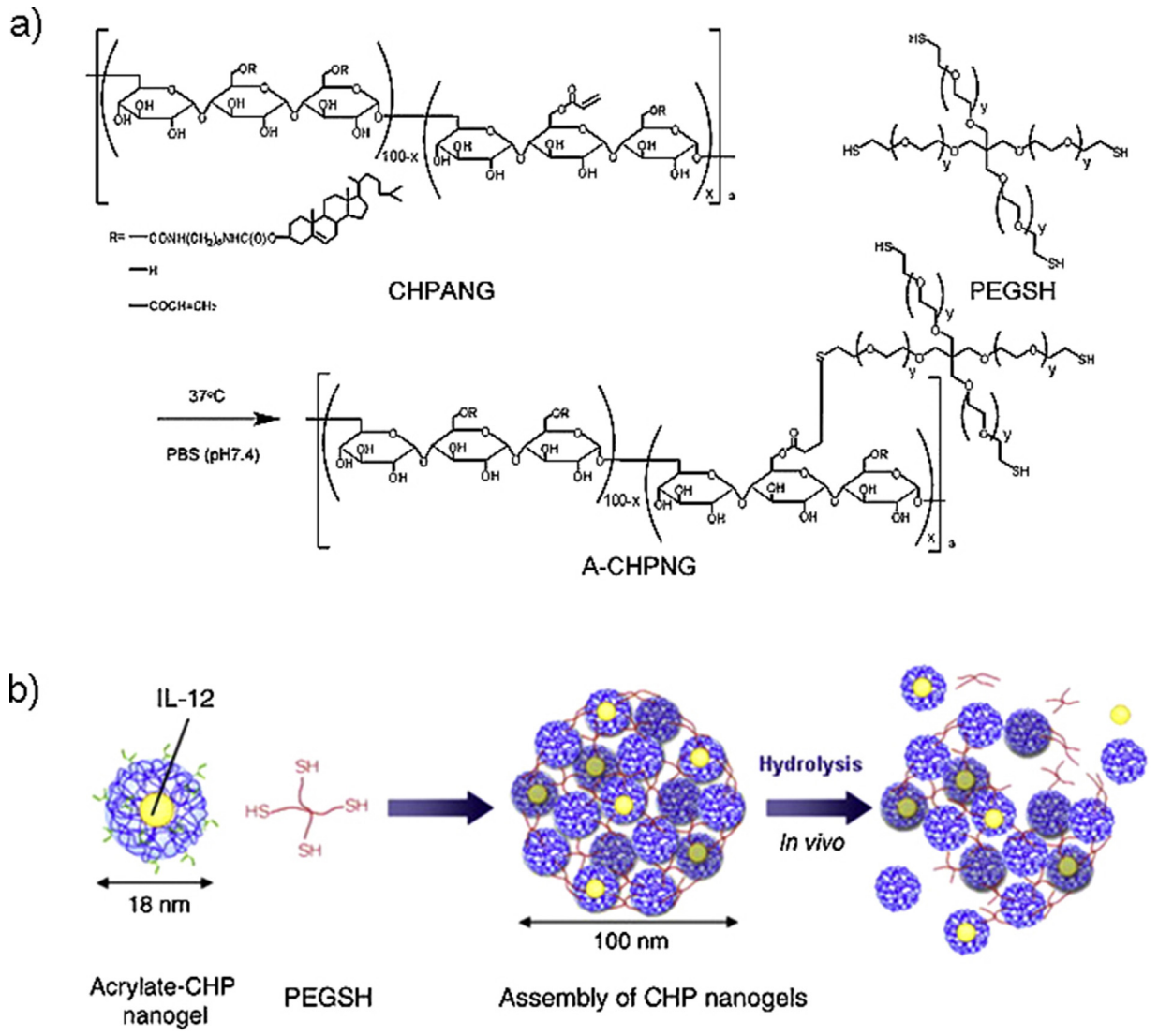

Fig. 18. (a) Illustration of crosslinking of acrylate group-modified cholesterol-bearing pullulan nanogel (CHPANG) with thiol group-modified poly (ethylene glycol) (PEGSH) by thiol-Michael addition to form raspberry-like assembly of nanogels (A-CHPNG); (b) schematic illustration of A-CHPNG formation. [139], Copyright 2009. Adapted with permission from Elsevier Ltd.

molar excess of DVS to the aqueous polysaccharide solutions. These VS-functionalized polysaccharides obtained by the simple one-step click reaction were capable to react with thiols at neutral pH in aqueous media. In a different approach, ene-functionalized cellulose surfaces were prepared by silylation of cellulose hydroxyls with vinyltrimethoxysilane, followed by thiol-ene click reaction under UV irradiation [152].

5.2. Thiolation of polysaccharide backbone

Besides introducing acceptor groups (e.g., carbon–carbon double bonds) to polysaccharides, adding donor groups is an alternative approach. Attaching thiol groups to polysaccharides may also render polysaccharide derivatives "clickable" via thiol-Michael/thiol-ene addition. Different approaches have been used to obtain thiol-functionalized polysaccharides. Acylation with the assistance of coupling reagents is one of the most common approaches. Wang et al. [153] reacted N-acetyl-L-cysteine (NAc) with chitosan using 1-ethyl-3-(3-dimethylaminopropyl-carbodiimide) hydrochloride (EDAC·HCl) and 1-hydroxybenzotriazole (HOBt) as coupling reagents in aqueous media. The NAc-chitosan was then incubated with maleic acid-grafted dextran to form in situ hydrogels via Michael type thiol-ene reaction [154]. For polysaccharides containing carboxylic acids, e.g. HA, the carboxylic acid can be activated by EDC and then reacted with cystamine, giving regioselectively thiolated derivatives [155]. However, the appended, unprotected thiol group is reactive and likely to be esterified in the presence of coupling reagents. To minimize this possibility, disulfides were used. For example, the thiolation can be accomplished by reacting chitosan derivatives with

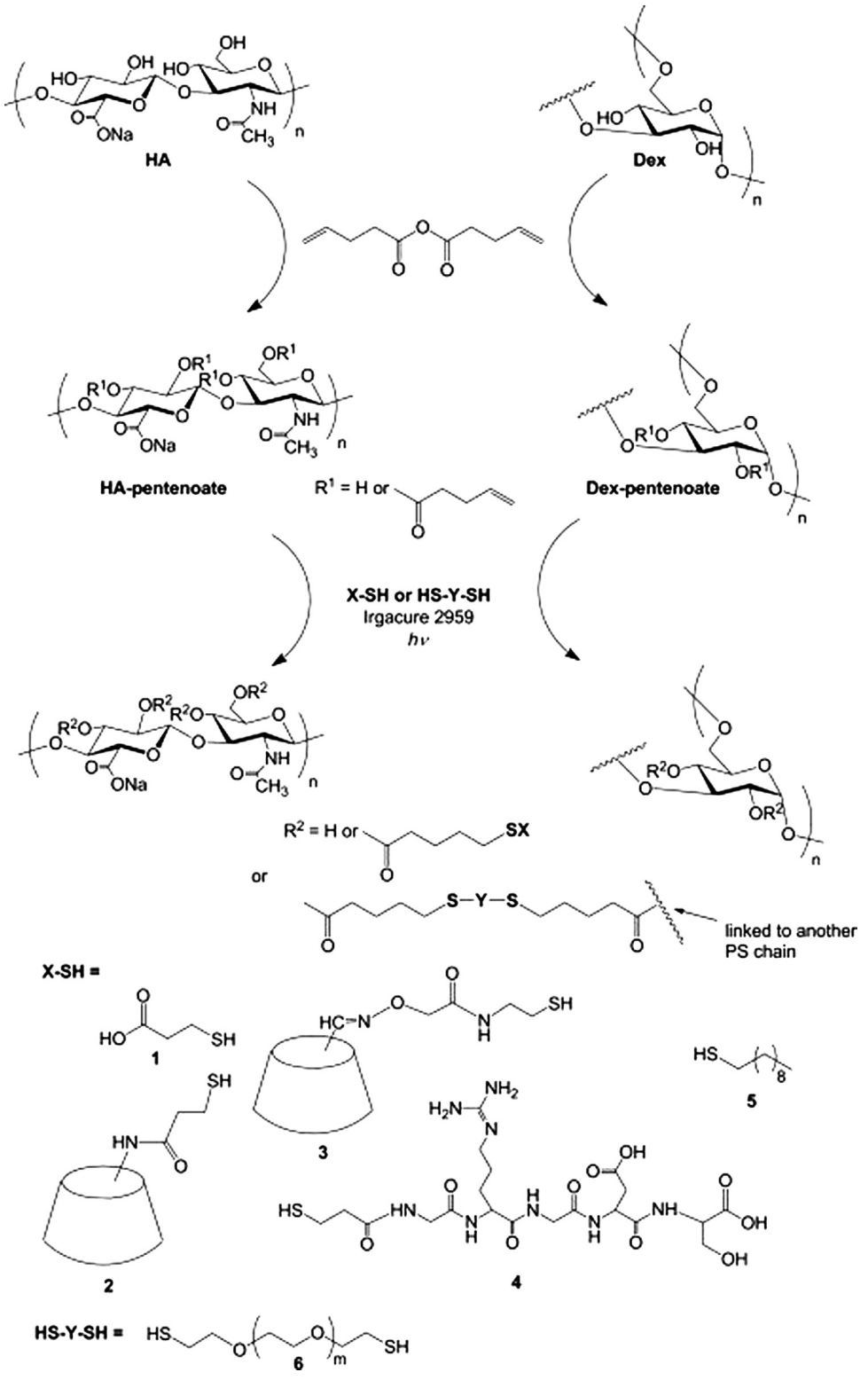

Fig. 19. Synthetic strategy for modular functionalization of hyaluronic acid and dextran via radical thiol-ene addition. [144], Copyright 2012. Reproduced with permission from Wiley Periodicals Inc.

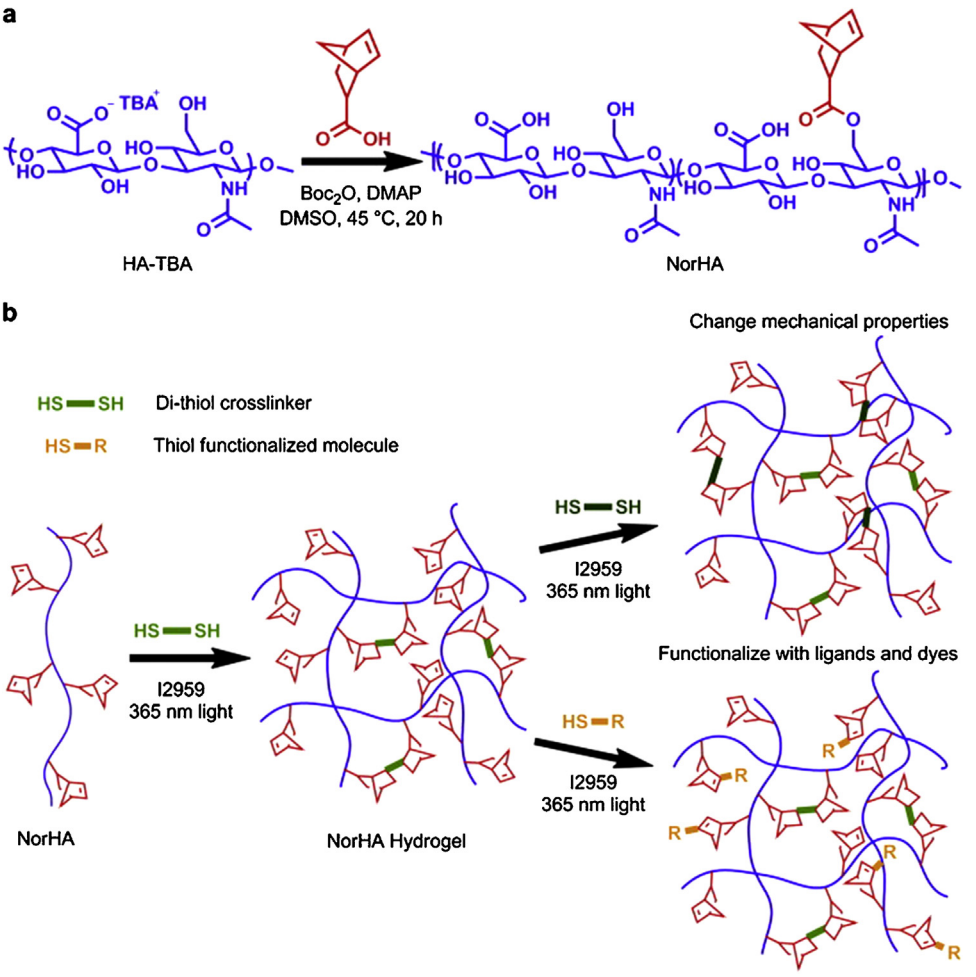

Fig. 20. (a) Scheme to synthesize NorHA from HA-TBA through the coupling of norbornene carboxylic acid to pendant alcohols on HA. (b) Gel formation through the light initiated thiol-ene reaction between a di-thiol and NorHA with subsequent chemical modification with mono- and/or di-thiols. [148], Copyright 2013. Reproduced with permission from Elsevier Ltd.

3,3′-dithiodipropionic acid (DTP) in the presence of coupling reagents (e.g. EDC and NHS), followed by the addition of 1,4-dithio-DL-threitol (DTT) to reduce the disulfide bonds to thiols [156]. A similar strategy was used for synthesis of thiolated HA derivatives [157,158].

Other thiolation methods such as silylation with thiopendent silyl moieties [152] have also been reported. Among them, direct thiolation of the polysaccharide backbone was intriguing, for example as described by the Kros group. Thiolated β-cyclodextrin (β-CD-$(SH)_7$) was synthesized via a two-step reaction. First, the primary hydroxyl group of β-CD was selectively transformed to iodide using PPh_3/I_2. The iodide was subsequently converted to thiol in a one-pot reaction with thiourea (Fig. 24). The resulting β-CD-$(SH)_7$ was utilized as a platform for thiol-ene/Michael type additions in a variety of interesting applications including formation of CD-dextran for delivering hydrophobic drugs [159,160] and synthesis of CD-centered star polymers [161]. Admittedly, β-CD is an oligosaccharide rather than a polysaccharide. Nevertheless, this synthetic pathway is likely to be applicable to many polysaccharides which have primary hydroxyl groups (e.g. cellulose [33], curdlan [162], pullulan [162,163]), although, to the best of our knowledge no such study has been performed on polysaccharides.

5.3. Limitations

A drawback to these reactions is the fact that thiol groups are easily oxidized and form disulfide bonds. This problem is more significant for large molecule thiol-containing reagents such as thiolated PEG and thiolated polysaccharides. Several groups have observed this type of oxidation-induced crosslinking [152,161]. As a result, careful attention needs to be paid to the preparation and storage of thiolated polysaccharides. For many small thiols, unpleasant smell as well as toxicity concerns [164] may be the major concerns preventing large scale industrial application of these reactions.

6. Oxime click chemistry

Oxime click chemistry, the formation of an oxime bond by reaction between an aminooxy group and an aldehyde

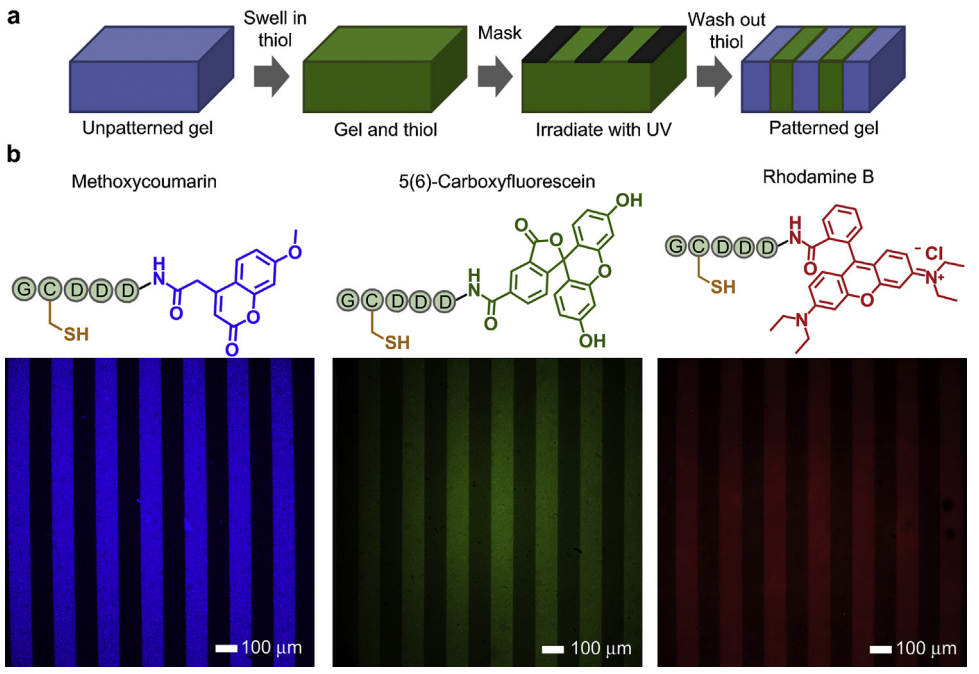

Fig. 21. (a) Schematic illustration of process to photopattern NorHA gels with thiol containing molecules; (b) confocal images of photopatterned (mask with 100 mm stripes) NorHA gels with fluorescent dye terminated peptides. [148], Copyright 2013. Reproduced with permission from Elsevier Ltd.

or ketone, has emerged as a robust strategy in areas such as bioconjugation [165]. Compared with primary amines, reactivity of hydroxylamine or alkoxylamine (aminooxy) groups toward carbonyls is significantly higher [166] and the formed oximes are much more stable against hydrolysis than corresponding imines [167]. The reaction can be carried out in aqueous media under mild reaction conditions without the use of metal catalysts. Taking advantage of the fact that aldehyde functionality is available in equilibrium at the reducing end of most polysaccharides, Novoa-Carballal and Muller pioneered the synthesis of polysaccharide-b-PEG block copolymers using oxime click chemistry in 2012 (Fig. 25) [168]. In their research, three unmodified polysaccharides, namely dextran, chitosan and HA, were coupled with aminooxy-functionalized PEG (MeO-PEG-ONH_2). The authors investigated the reaction conditions and found that using DMSO/pH 3 buffer (citric acid based) solution at 45 °C gave fast and efficient reactions. Under these conditions, using 5 equiv of MeO-PEG-ONH_2 for 24 h resulted in 99% conversion to coupled block copolymers. This strategy for polysaccharide end-functionalization has been quickly adopted to obtain a variety of block copolymers such as dextran-b-PDMAEMA [169], and glycosaminoglycan-b-PEG [170], as well as end-labeled polysaccharides such as fluorophore-labeled N,N,N-trimethyl chitosan [171].

Sestak et al. [172] performed the oxime click reaction under similar reaction conditions: 5 equivalents aminooxy reagent (for aminooxy peptides, the ratio was 1:1) were mixed with HA in acetate buffer solution at pH 5.5 for 16 h at room temperature, followed by dialysis to remove unreacted aminooxy species (Fig. 26). However, the FTIR and NMR data run counter to the assumption that coupling proceeds primarily at the reducing end of polysaccharides. Unmodified HA contains two carbonyl functional groups, i.e. a carboxylic acid and an acetamide, on each repeating unit, both of which may serve as possible handles for the oxime click reaction. In this study, the authors claimed that the oxime-bond formed at all three carbonyl carbon centers, and primarily at the carboxylic acid site. Graft

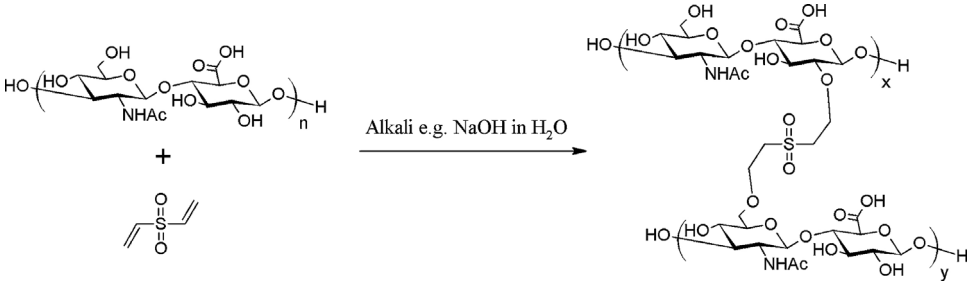

Fig. 22. Exemplary crosslinking reaction of DVS with polysaccharide (HA).

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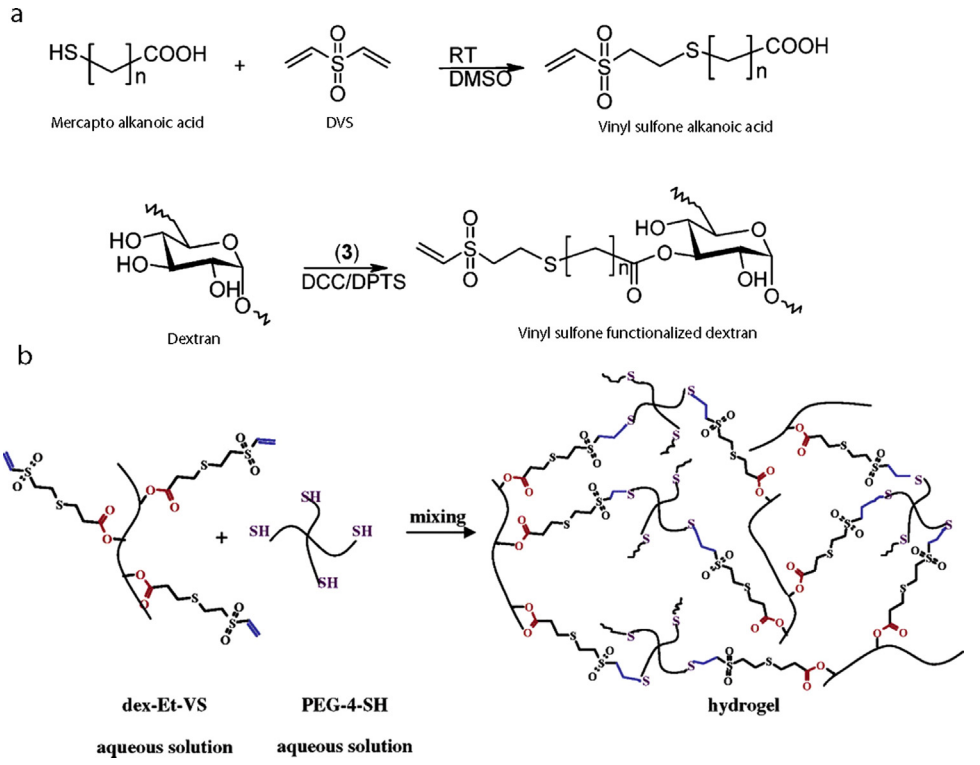

Fig. 23. (a) Schematic representation of one-pot synthesis of dextran vinyl sulfone conjugates; (b) schematic representation of Michael addition between VS functionalized dextran and multifunctional thiolated PEG. [150], Copyright 2007. Reproduced with permission from the American Chemical Society.

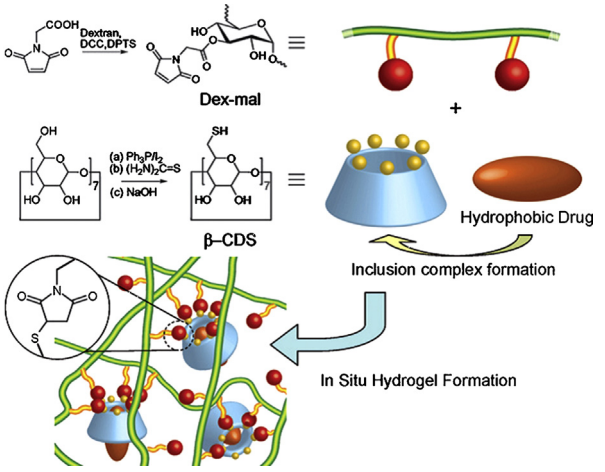

Fig. 24. Schematic representation of the in situ hydrogel forming system in which β-CDS plays two roles: dextran cross-linker and host for hydrophobic drug. Inclusion complex formation can increase the affinity of the hydrogel for hydrophobic drugs and can prevent drug aggregation. [159], Copyright 2010. Reproduced with permission from the Royal Society of Chemistry.

efficiencies using this reaction can reach up to 90%. The authors did not state very clearly the exact meaning of the ratio (whether it was mol of aminooxy agent to HA repeating unit or to carbonyl group). However, in either condition the authors in this study employed a large excess of oxime agents compared with the 5 mol per HA molecule ratio in the previous study [168]. Moreover, from signal intensity in the provided NMR spectra of the oxime click products (HA grafted with O-carboxymethyl hydroxylamine, and HA grafted with O-benzyl hydroxylamine), the DS of the grafted functionality did not seem to be as high as stated by the authors, though the integration values of each peak required for calculating DS were not provided. From this point of view, although this research has provided a new possibility for grafting functionality on polysaccharides containing carbonyl groups such as HA and alginate, further synthetic study is needed to verify this approach.

Oxime bonds are known to be labile under acidic (pH < 2) or basic (pH > 9) conditions [168,172]. Although this lability may cause problems in synthesis, processing, and storage of these polymers, it is of interest in areas such as controlled drug release.

7. Olefin cross-metathesis

Olefin metathesis has been developed in recent years as a powerful, versatile tool in organic chemistry [173]. In polymer synthesis, olefin metathesis techniques such as ring opening metathesis polymerization (ROMP) [174] have been comprehensively investigated in the past decade and have helped to generate a variety of novel polymers. Applications of olefin cross-metathesis (CM, Scheme 5) to the synthesis of complex small molecules [20,175,176] including carbohydrates [177–179] have been extensively studied in recent years thanks to the publication of Grubbs' model of selectivity for CM [20], and the development of active and selective catalysts for CM [180–182].

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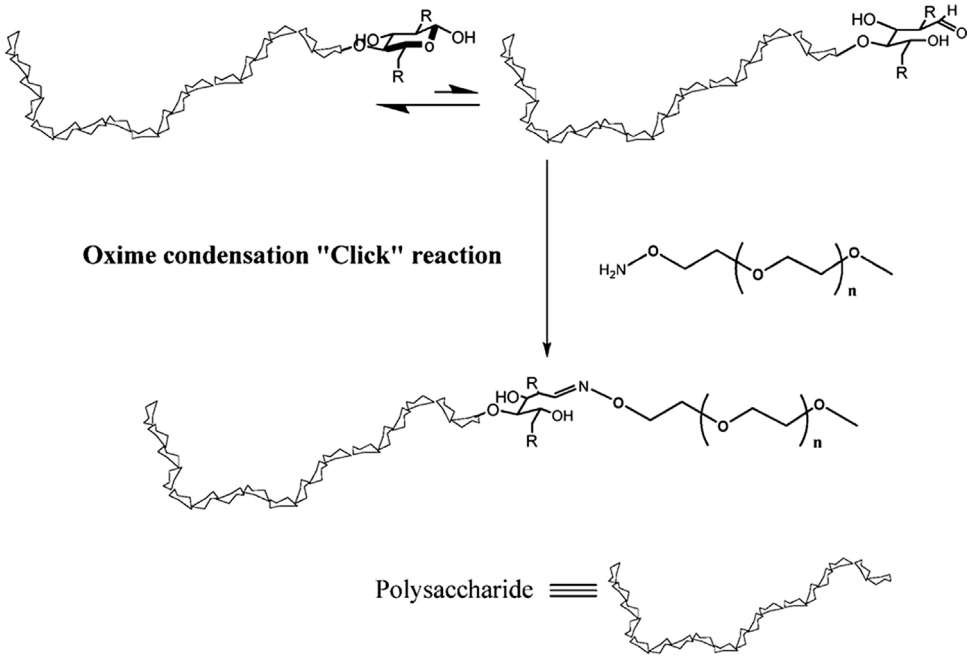

Fig. 25. Oxime approach for polysaccharide-b-PEG synthesis. Polysaccharides used in the reference include dextran, chitosan and HA. [168], Copyright 2012. Reproduced with permission from the Royal Society of Chemistry.

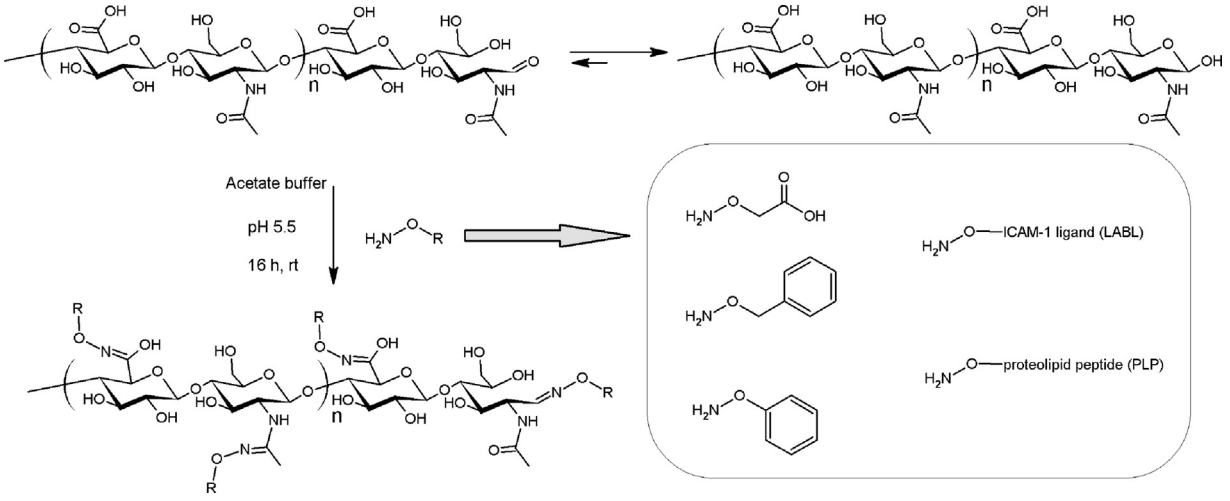

Fig. 26. Reaction scheme of single-step grafting of small molecules and peptides to hyaluronic acid via oxime click chemistry. [172], Copyright 2013. Adapted with permission from Elsevier Ltd.

Table 3
Grubbs' categorization of olefins and rules for selectivity. [19], Copyright 2014, Adapted with permission from the American Chemical Society.

Olefin Type	Olefin metathesis reactivity	Examples[a]
Type I	Rapid homodimerization, homodimer consumable	Terminal olefins
Type II	Slow homodimerization, homodimer sparingly consumable	Acrylates, acrylic acids, acrylamides
Type III	No homodimerization	3° allylic alcohol (protected)
Type IV	Olefins inert to CM, but do not deactivate the catalyst (spectator)	Vinyl nitro olefins

Grubbs' rules: reactions between two olefins of Type I = statistical CM; reactions between two olefins of same type (non-Type I) = non-selective CM; reactions between two different types (except Type IV) = selective CM.

[a] Selectivity depends on catalyst used. The examples shown are valid for Grubbs' 2nd generation catalyst.

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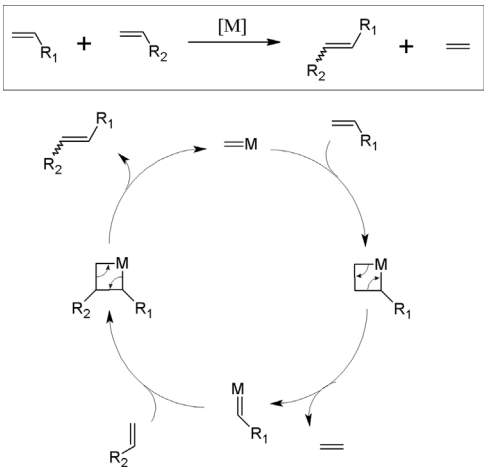

Scheme 5. Representative olefin cross-metathesis and reaction mechanism.

Based on chemical structure and reactivity results, Grubbs empirically classified olefins into 4 types (Table 3) [20]. Type II and III olefins (usually sterically-hindered and/or electron-deficient, also depending on the catalyst used) have low olefin metathesis reactivity and only slowly homodimerize, while more reactive terminal olefins (type I) readily undergo homodimerization via olefin metathesis. Moreover, the homodimers of the terminal olefins are susceptible to subsequent secondary CM reactions. As a result, when a type I olefin is reacted with a type II or III olefin, high conversion to a CM product can be achieved by employing an excess of the type II or III olefin [175].

Taking advantage of the reactivity differences between the four types of CM partners, it is theoretically possible to perform a "click-like" reaction by reacting one type of CM partner with a polymer possessing a substituent containing a different type of olefin. Nevertheless, CM as a modular and mild approach to polymer modification of polymers has been little studied. One major barrier is the potential for homodimerization (via self-metathesis (SM)) of the CM partners, especially for type I olefins. In a typical example, Joly et al. [183] observed SM of cellulose undec-10-enoate using Grubbs' catalyst (1st generation, Fig. 27), affording crosslinked and insoluble cellulose plastic films. For polymer modification targeting discrete and soluble products though, such crosslinking is not only undesired, but even disastrous. This problem was addressed by Meier's group in the side-chain modification of polyesters and poly(2-oxazoline)s with terminal double bonds in the side chains via CM [184,185]. In their work, polymers with terminally unsaturated side-chains (type I olefins) were reacted with type II or III CM partners including a variety of acrylates, using Hoveyda-Grubbs 2nd generation catalyst. The same group further expanded the concept to the synthesis of divergent dendrimers using the CM reaction [186].

The Edgar group is the first to have reported successful CM reaction between a terminal olefin side-chain functionalized polysaccharide (cellulose) and a CM partner (acrylic acid) to afford discrete and soluble cellulose ω-carboxyalkanoates [19]. Exploiting this strategy, Meng and coworkers further demonstrated the power of this reaction by expanding the reaction scope to a variety of acrylates (Fig. 28) [187]. Under mild conditions (40 °C, within 2 h), the CM reaction catalyzed by Hoveyda–Grubbs 2nd generation catalyst in organic solvents such as THF and dichloromethane led to complete conversion of cellulose pent-4-enoate or undec-10-enoate esters to a variety of functionalized derivatives, with actual isolated yields for each reaction ranging from 84 to 97%. These results demonstrated not only the mild nature of CM chemistry but also its high potential for modular modifications of terminally unsaturated cellulose derivatives. By addition of a variety of CM partners (potentially containing a variety of functional group types) to a single terminally unsaturated polysaccharide derivative, a family of polysaccharide derivatives can be prepared whose members share identical Mw, DS, substitution pattern, and monosaccharide sequence, differing only in the side-chain functional groups (Fig. 29). This synthetic strategy by its mild and modular nature thereby enables isolation of the functional group variable in studies of polysaccharide derivative structure–activity relationships, effectively eliminating the other variables noted above that otherwise are very difficult to control for. However, as the terminal olefins of the side chains (Grubbs Type I) are highly reactive toward metathesis including toward homodimerization, which would lead to intra- and intermolecularly cross-linked products, it is necessary to employ an excess amount of CM partner (5–20 equiv. per pendent olefin or more in our research) to guarantee that the terminal olefins react only with CM partners, and not with themselves. Moreover, the Edgar group discovered that the α,β-unsaturated double bonds of the CM products create susceptibility to radical formation, presumably in the γ position from the carbonyl, which can lead during storage to free radical oligomerization and crosslinking. As a remedy for this undesired crosslinking, two strategies were described. A free radical scavenger such as BHT was added to the products to prevent free radical oligomerization; this was effective at delaying the onset of solubility loss. To eliminate the cause of crosslinking, the authors reduced the α,β-unsaturation by heterogeneous or homogeneous hydrogenation, without altering the other functional groups of the polysaccharide derivatives.

At almost the same time, Malzahn et al. [188] reported interfacial olefin cross-metathesis of dextran acrylate with a terminally unsaturated organophosphate in an inverse miniemulsion to afford hollow nanocapsules as shown in Fig. 30. The authors demonstrated that the CM reaction occurred selectively at the oil–water interface of the aqueous nanodroplets. This strategy of using olefin metathesis to prepare cross-linked polysaccharides is not new (as mentioned in previous paragraph, Joly et al. prepared cross-linked cellulose plastic film by SM reaction [183]). However, the current research showed the adaptability of the CM reaction under emulsion conditions. As many polysaccharides and their derivatives are water-soluble but poorly soluble in good CM solvents (e.g. THF, and dichloromethane), it is of great value to be able to perform the reaction in such heterogeneous and aqueous conditions. Also, as the Edgar group has proven that long chain terminally olefinic cellulose esters (e.g. cellulose

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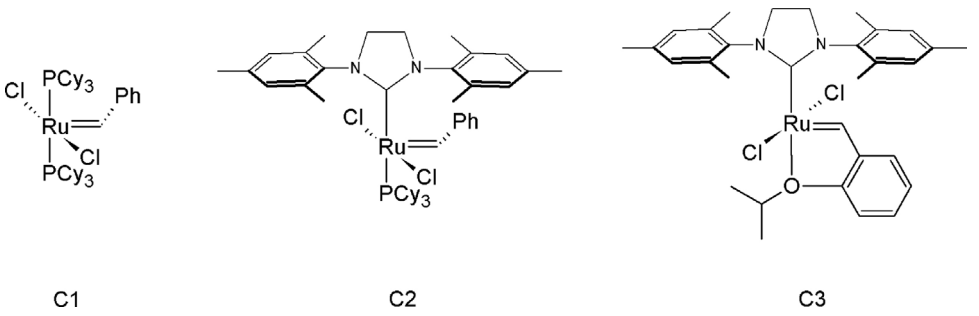

Fig. 27. Commonly used Grubbs' olefin metathesis catalysts; C1 Grubbs's catalyst, 1st generation; C2 Grubbs' catalyst, 2nd generation; C3 Hoveyda-Grubbs' catalyst, 2nd generation. [19], Copyright 2014. Reproduced with permission form the American Chemical Society.

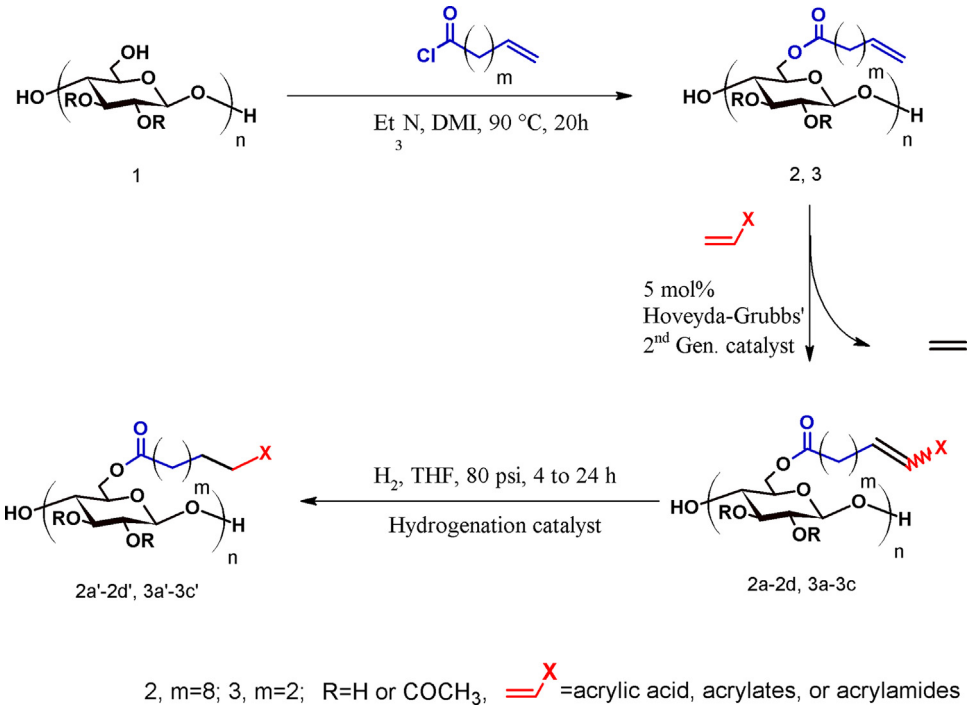

Fig. 28. General three-step synthetic method for functionalized cellulose esters via olefin cross-metathesis. [187], Copyright 2014. Reproduced with permission from the Royal Society of Chemistry.

pent-4-enoate and cellulose undec-10-enoate) are suitable for CM with a variety of CM partners, the results here showed the possibility of using acrylate as a handle on the polysaccharide backbone for CM reactions. If this is the case, the use of this type II/III olefin as a handle would avoid SM and thus unwanted crosslinking between polysaccharide acrylates due to the low reactivity of acrylate olefin toward SM. This could also make it possible to obtain high conversion to CM products using CM partners in equimolar amounts or in only slight molar excess. It is worth mentioning that, although no such cases have yet been reported, polysaccharide modification by CM may also be possible in homogeneous aqueous solution by careful selection of water-soluble olefin metathesis catalysts [189,190] and corresponding CM partners.

Although researchers in the realms of both synthetic polymers and polysaccharides have shown the modular and efficient nature of the CM chemistry, it may not perfectly fit the definition of a polymer chemistry "click" reaction. According to a definition of click reactions adapted to the context of polymer chemistry by Barner-Kowollik et al. [191] in 2011, the requirements for a "click" polymer chemistry include equimolarity, large-scale purification, and fast reaction rate, in addition to the requirements in the broader Sharpless' click definition such as those for chemoselectivity and stable products. Clearly, the CM reaction investigated by the Edgar group and other groups fulfills most of these requirements, as it is modular, rapid, chemoselective, and wide in scope. However, the CM products are sometimes unstable due to the reactive α,β-unsaturated double bond, and so require the addition of free radical scavenger or a further hydrogenation step. Neither does the CM reaction meet the requirement of equimolarity, as an excess amount of the CM partner is usually needed to drive to high conversion and selectivity of CM over SM. Therefore, we feel that describing this

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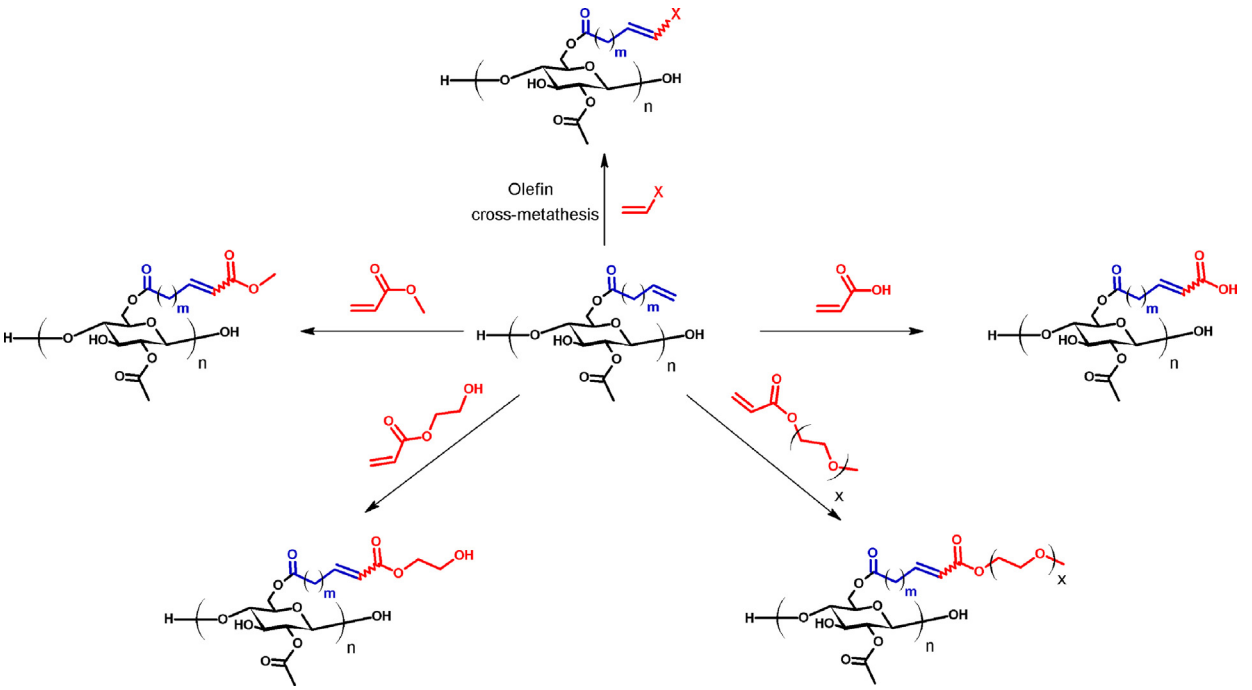

Fig. 29. Examples of modular and efficient modification of cellulose esters by CM. [187], Copyright 2014. Adapted with permission from the Royal Society of Chemistry.

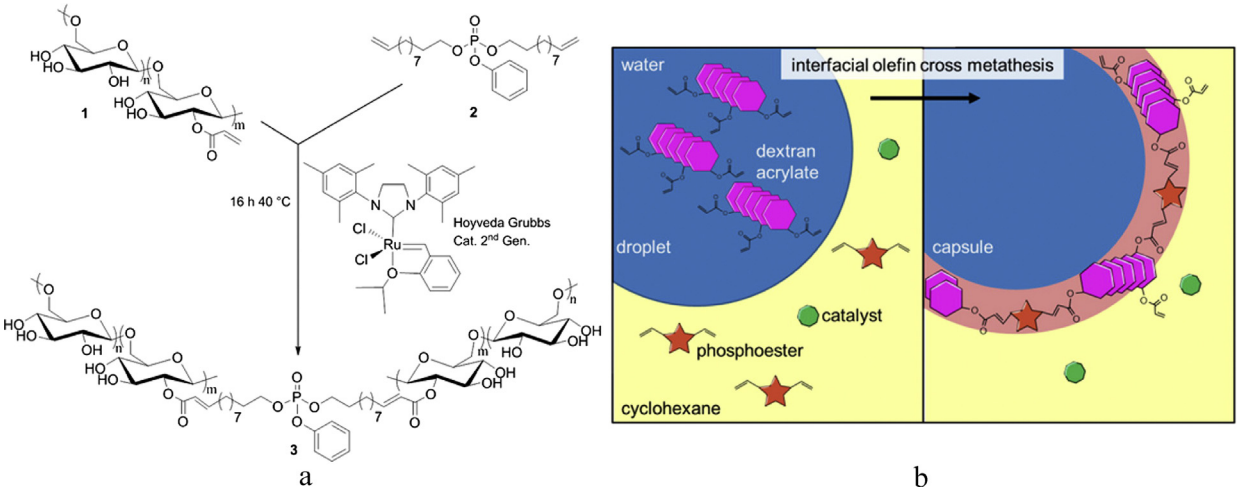

Fig. 30. (a) Schematic representation of the interfacial reaction between acrylated dextran **1** and phenyldi(undec-10-en-1-yl)-phosphate **2** leading to a cross-linked polymer network **3**. (b) Schematic representation of the interfacial olefin cross-metathesis at the water–oil interface of a nanodroplet in an inverse miniemulsion process for the formation of stable nanocapsules. [188], Copyright 2014. Adapted with permission from the American Chemical Society.

reaction as "click-like" rather than "click" would be appropriate and accurate.

8. Conclusions and prospects

Advances in organic chemistry have provided to polysaccharide chemists some useful synthetic tools for side chain modification, synthesis of polysaccharide-based graft or block copolymers, polysaccharide-based networks, as well as modification of polysaccharide surfaces. For example, the advent of carbonyl activating reagents such as DCC and CDI has enabled mild and efficient esterification of polysaccharides, while the development of protection-deprotection chemistry and other strategies have made possible regioselective modification of polysaccharides. Although these developments have expanded the potential of polysaccharide chemistry and materials, until recently the chemical functionality and architectural diversity of

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polysaccharide derivatives have still been largely restricted by limitations of conventional synthetic pathways such as esterification and etherification.

Click chemistry, the concept of which was coined less than 20 years ago by Sharpless and coworkers, provides unprecedentedly powerful tools for design and synthesis of novel polysaccharide derivatives in a more controlled and modular manner. Copper catalyzed azide/alkyne cycloaddition, also known as CuAAC, is one of the first of the click reactions that was introduced to polysaccharide chemistry and helped to generate a variety of families of novel derivatives. This reaction, characterized by efficient and orthogonal azide/alkyne coupling under mild reaction conditions using Cu^{I} catalysis, however, has its own limits, such as the toxicity of copper, the explosive and toxic potential of azides, and chain scission induced by reactive oxygen species (ROS), when used in areas including polysaccharide modification.

The development and implementation of other click reactions including metal-free [3+2] cycloaddition reactions, Diels–Alder reactions, thiol-Michael and thiol-ene reactions, and the oxime reaction have, to a large degree, helped polysaccharide chemists surpass the limitations associated with CuAAC, and allowed synthesis of some novel polysaccharide conjugates, copolymers, and networks. Besides the advantage inherent in metal-free coupling, these reactions also add valuable traits to polysaccharide chemistry. Strain-promoted alkyne/azide cycloaddition (SPAAC), azide/oxanorbornadiene cycloaddition and especially the most recently unveiled inverse electron demand Diels–Alder (iEDDA) not only serve as synthetic tools for modification, but also allow some sophisticated applications such as in vivo labeling, due to their metal-free nature and their fast reaction kinetics. Hetero Diels–Alder reactions employing thiocarbonylthio compounds smoothly combine polysaccharide chemistry, click chemistry and RAFT polymerization, and so provide an ideal pathway for the synthesis of polysaccharide-based graft or block copolymers. The recent application of olefin cross-metathesis to polysaccharide modification is also a "click-like" reaction, as it shares a number of features with click chemistry, such as rapid, efficient and modular coupling under mild conditions, easy purification, absence of offensive byproducts, and high yields, with only the requirement for high molar excess of the metathesis partner significantly differing from the definition by Barner-Kowollik et al. [191] of a polymer click reaction (the instability of the products to free radical reactions is another difference, but one that is easily correctable e.g. by hydrogenation, even in a one-pot reaction).

Application of modern click chemistry to the field of polysaccharide modification as described in this review has opened many new doors, enabling the science of modification of these diverse, abundant, and renewable materials to move to a new era of more controlled, modular, and chemoselective synthesis. These new additions to the toolbox of polysaccharide chemists make it certain that vastly increasing numbers and varieties of polysaccharide derivatives with unique and superior properties will be synthesized efficiently and will be exploited toward the goal of a more sustainable world.

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