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The Emergence of Oxime Click Chemistry and its Utility in Polymer Science

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Abstract

The synthesis of new, highly functional and dynamic polymeric materials has risen dramatically since the introduction of click chemistry in 2001. This diverse set of reactions has led to the synthesis of self-healing and dynamic polymers, the creation of hydrogels exhibiting finely tuneable gelation times and mechanical properties and to the temporal and spatial control of chemical reactions enabling the 3D patterning of gels and surfaces with high fidelity. Traditionally, the copper catalysed azide-alkyne cycloaddition (CuAAC), Diels-Alder and thiol-ene click reactions have been utilised but, owing to the demand for more environmentally friendly means of synthesis and the need for more versatile and tolerant chemistry, the imine, hydrazone, and most recently, oxime carbonyl condensations have seen an astonishing increase in application. This review will focus on the oxime click reaction for the development of functional polymeric materials.

Introduction

Click chemistry, introduced by Sharpless and co-workers in 2001, has emerged as one of the most powerful tools in drug discovery, bioconjugation and materials science.[1-4] Sharpless coined the term click chemistry to define reactions that are "*modular, wide in scope, high yielding, create only inoffensive by-products (that can be removed without chromatography), are stereospecific, simple to perform and that require benign or easily removed solvent*".[1] Many reactions meet the click criteria such as Diels-Alder and thiol-ene chemistries as well as the imine, hydrazone and oxime carbonyl-condensations. The most popular and reported click reaction to date however, is the copper catalysed azide-alkyne cycloaddition (CuAAC) of alkynes and azides yielding 1,2,3-triazoles. Popular click reactions are summarised in Figure 1.

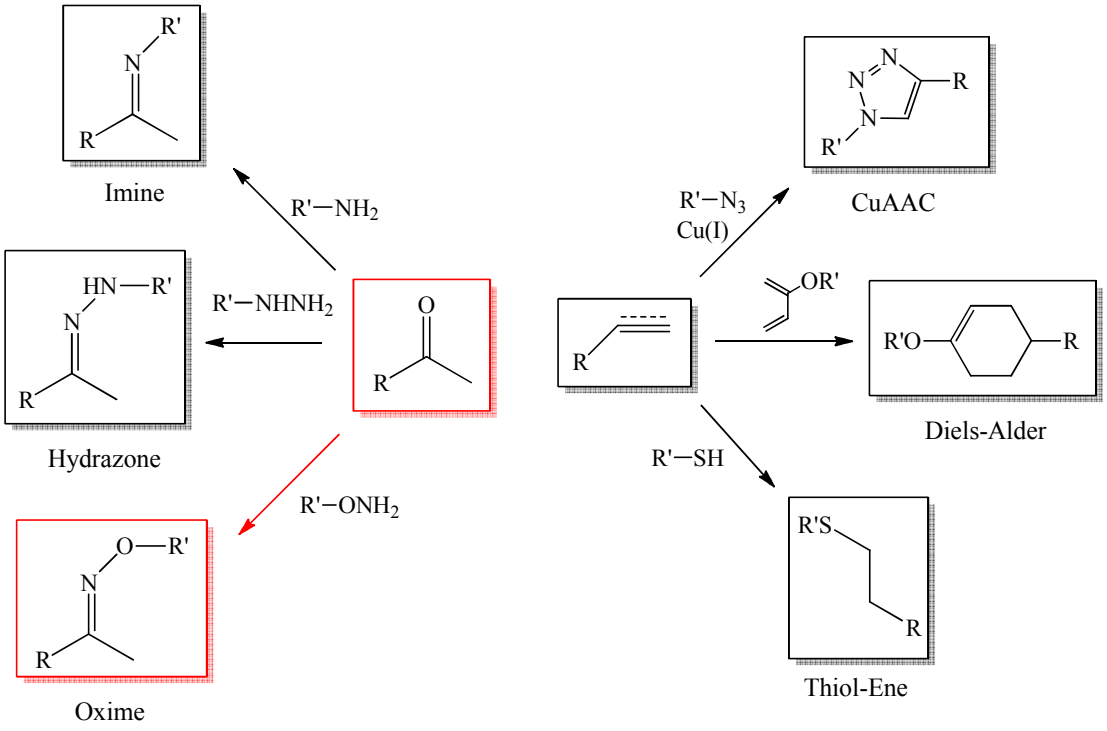

Fig. 1 Summary of the most popular click reactions. The oxime bond, resulting from the reaction between an aldehyde or ketone with a hydroxylamine, is highlighted in red.

The defining characteristics of all click reactions; high efficiency, specificity, facile synthesis and easily purified products make them attractive candidates for procedures which were traditionally very complex, expensive and time-consuming such as drug discovery and bioconjugation. With respect to materials science, click chemistry is attractive as many click reactions are compatible with controlled radical polymerisation techniques, such as reversible addition-fragmentation chain transfer (RAFT) and atom-transfer radical polymerisation (ATRP). This allows for the synthesis of polymers with controlled molecular weights which, owing to the almost complete conversion of click reactions, can be easily and highly functionalised. The CuAAC, Diels-Alder and thiol-ene reactions have all been used to synthesise and functionalise polymers.[5-17]

The immense value of click chemistry can be clearly seen when we compare them to traditional chemical reactions. By simply using highly efficient click chemistry rather than a classic chemical reaction, superior material performance can be achieved. The CuAAC reaction has been utilised to crosslink polymers, forming hydrogels which display much higher degrees of swelling and dramatic increases in elasticity than the corresponding hydrogel formed via conventional photo-crosslinking.[18] The improved properties of the click gel are thought to be due to the more controlled nature of the cross-linking reaction and it's incredibly high efficiency. A maximum of 0.2% of functional groups remained unreacted in the gel following the CuAAC, indicating the very high degree of cross-linking

leading to a more even distribution of cross-linked sites, forming a near-ideal structure which consequently, displayed much-improved physical properties.

Applications of carbonyl click chemistry (imine, hydrazone and oxime) in polymer/materials science has recently become more popular owing to their very high efficiency, bioorthogonal nature and dynamic covalent properties. Both the imine and hydrazone reactions have been used for the synthesis and functionalisation of polymers as well as for the development of self-healing and degradable materials.[19-24] The versatile synthesis of imines and hydrazones and their applications has been nicely reviewed in multiple publications.[25-28] The oxime click reaction however, remains as one of the most unexplored click reactions for applications in polymer science. Oxime chemistry has been used almost exclusively for bioconjugation owing to the favourable properties of oxime bond formation: high efficiency, chemoselectivity, formation in aqueous solvents and water being the only side-product.[29-31] An excellent review of oxime chemistry for bioconjugation was recently published by Dumy and co-workers.[29]

The same characteristics which make oxime chemistry so attractive for bioconjugation also make it an excellent candidate for polymer science applications. The high efficiency of oxime formation indicates the potential for the synthesis of highly functional polymeric materials, the formation of water as the only side-product make it attractive for the synthesis of environmentally friendly polymers and the dynamic nature of the oxime ligation imparts dynamic covalent character into oxime based materials. Over the last decade the use of oxime chemistry in polymer science has risen dramatically. Herein we present a review of the use of oxime chemistry as a new tool for the synthesis of highly functionalised, polymeric materials. Following a discussion of oxime bond formation under non-catalysed and catalysed conditions, the applications of oxime chemistry for step-growth polymer synthesis, polymer post-functionalisation, hydrogel synthesis, surface patterning and for the development of dynamic materials will be reviewed.

Oxime Bond Formation and Catalysis

Oxime bonds form rapidly and in high yields from the reaction between aldehydes or ketones and hydroxylamines. One of the reasons oxime chemistry has found limited use in the past was the complex synthetic routes required to achieve hydroxylamines. Nowadays however, there are multiple, straightforward ways to prepare hydroxylamines, the most common being via a modified Mitsunobu reaction[32-34] or BOC deprotection.[35]

The extremely high reactivity of hydroxylamines towards carbonyl groups was exemplified through the identification of oxime side-products formed from the reaction of the hydroxylamine with trace amounts of carbonyl-containing compounds present in eluents used for peptide purification.[36] To avoid any side reactions, only high-grade solvents are recommended for use. Oxime bond formation is accelerated by slightly acidic conditions and it was reported by Dawson and co-workers that aniline acts as an effective catalyst by dramatically increasing the rate of oxime bond formation (400x under acidic conditions and 40x at neutral pH).[37] This impressive rate enhancement is due to the formation of the protonated aniline Schiff base rather than the protonated carbonyl which leads to a much higher rate of transamination leading to the oxime (Figure 2).

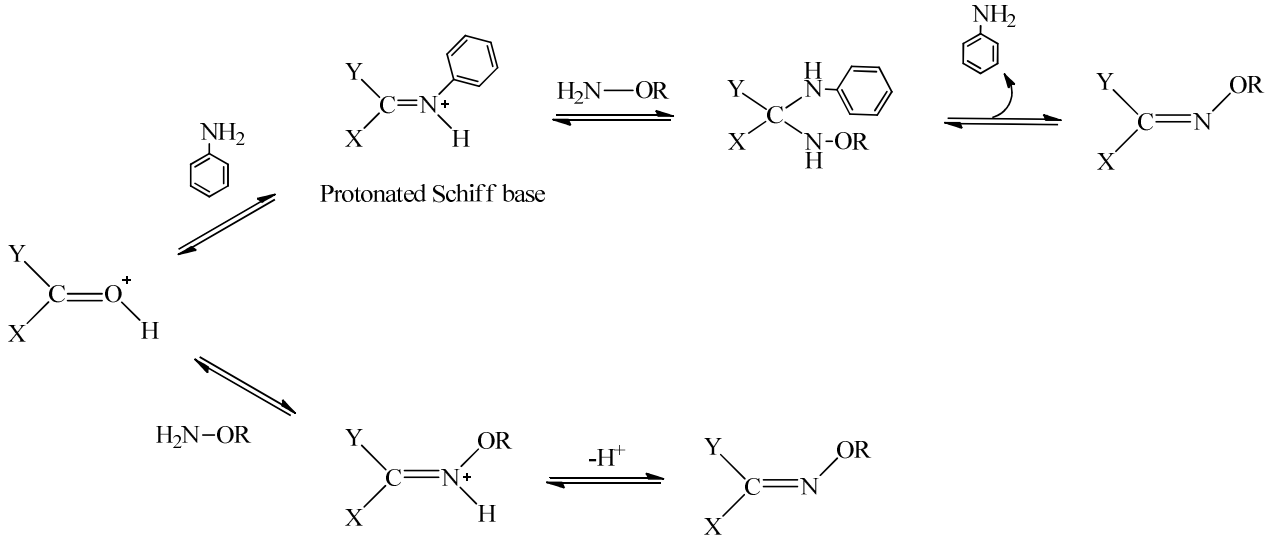

Fig. 2 Mechanism of aniline catalysed oxime formation. The reaction of aniline with a protonated ketone or aldehyde results in the formation of a protonated Schiff base thereby accelerating the rate of transamination resulting in the oxime product.

More recently, Baca and co-workers investigated the catalytic effect of a range of substituted anilines, most notably, *p*-phenylenediamine which exhibited a 19-fold increase in rate when compared to the equivalent aniline-catalysed reaction.[38] Interestingly, the rate of oxime bond formation has been reported to be accelerated by low temperatures (-20°C)[39] and new classes of catalyst for oxime and hydrazone bond formation (2-aminophenols and 2-(aminomethyl)benzimidazoles as well as anthranilic acids and aminobenzoic acids) have been reported, indicating the continued interest in developing new means to synthesise oxime bonds.[40,41]

The selectivity and orthogonal nature of oxime bond formation has been demonstrated by successful bond formation in the presence of various functional groups (ester, amine etc) for

applications in bioconjugation and surface patterning and through polymer functionalisation by oxime ligation following polymer synthesis by controlled radical polymerisation techniques, such as RAFT and ATRP.[42] This selectivity allows for the facile synthesis of highly functional materials without the need for expensive or time consuming protecting chemistry. A thorough review of bioorthogonal click chemistries and their applications was published by Hawker and co-workers.[42]

Importantly, oxime bonds have been shown to be more stable at physiological pH than the corresponding imine or hydrazone.[43] In terms of biomedical application this could translate to an increase in the stability of oxime based materials in the body (potentially exhibiting a higher residence time) than either the imine or hydrazone based material.

Oxime Chemistry for Step-Growth Polymer Synthesis

The synthesis of high molecular weight polymers by step-growth polymerisation requires the forming reaction to be very efficient/high yielding (above 98-99%) and without side-reactions.[44,45] Click chemistry has been successfully used to synthesise high molecular weight step-growth polymers owing to two of the defining traits of all click reactions: extremely high efficiency and single reaction trajectories. This creates a great litmus test for the utility of a click reaction for materials applications; if the reaction can be utilised to prepare high molecular weight polymers in a step growth mechanism then this indicates a highly efficient chemical reaction.

To date, research on click-type step-growth polymers has been focused largely on the CuAAC reaction.[5-10,13] This highly efficient reaction requires no protecting groups, is tolerant to a wide variety of functional groups and reaction conditions, proceeds with almost complete conversion and generally requires no purification. However, the presence of the copper catalyst limits the products use in biomedical applications. Trace amounts of copper inevitably remain in the final product and even in low concentrations copper can be toxic to humans.[46,47] Additionally, the azide precursors are inherently unstable and have safety concerns; the possibility of significant explosions due to unstable CuAAC precursors is a serious hazard. The problem of copper toxicity can be eliminated through the use of the strain-promoted azide-alkyne cycloaddition (SPAAC); however this is usually accompanied by lower rates of reaction and requires complex synthetic procedures.[48,49]

Recently, the Sharpless group reported the first example of the sulfate click reaction being utilised for step-growth polymer synthesis.[50] The highly efficient polymerisation reaction between bis(aryl fluorosulfates) and bis(aryl silyl ethers), catalysed by organic bases or fluoride salts, was undertaken

either at room temperature over 24 hours, forming polymers between 24.6 – 31 kDa, or at 150°C over 2 hours which resulted in polymers between 2.5 – 60 kDa. The application of the sulfate click reaction for step-growth polymer synthesis indicates the potential of other previously ignored click reactions as new routes for high molecular weight step-growth polymer synthesis.

Imine and hydrazone linkages have successfully been utilised as a means of step-growth polymer synthesis.[19-21] The oxime click reaction however, remains relatively under-utilised as a new polymerisation chemistry. Wang and co-workers synthesised one of the only oxime-based polymers, reported in 2002.[51] The polymerisation made use of galactose oxidase to yield an aldehyde functionalised galactose which was further modified with hydroxylamine functionality before being polymerised to yield oxime linked carbohydrates between 4 – 8.9 kDa (Figure 3).

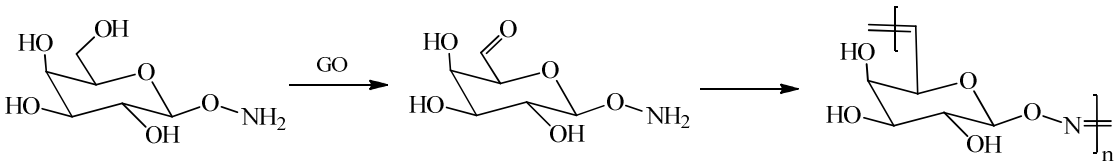

Fig. 3 Oxime linked carbohydrates ranging from 4 – 8.9 kDa. Adapted with permission from Ref. 51. Copyright (2002) American Chemical Society.[51]

Bong and co-workers developed an oxime-linked glycolipid polymer for membrane/vesicle stabilisation.[52] Polymerisation between diketo functionalised trehalose and aminooxy functionalised cholesterol or aminooxy functionalised 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-ethanolamine yielded oxime polymers between 7 – 20 kDa. The polymers were found to stabilise vesicular and supported lipid membranes to anhydrobiotic and cryogenic conditions.

The oxime click reaction was recently used by our group to synthesise high molecular weight step-growth polymers in rapid times under very mild conditions.[32] High molecular weights, 32 and 35 kDa, were achieved within 13 minutes at room temperature or within 5 minutes at 60°C indicating the extremely high efficiency of the oxime click reaction. Polymers were synthesised with controlled end-group chemistry (aldehyde or hydroxylamine) by varying the monomer ratio, allowing for the controlled conjugation of molecules of interest to the terminal ends of the polymer. Finally, the versatilitiy of oxime chemistry was demonstrated by successful polymerisation in the presence of both acidic and basic monomers; boronic acid and bipyridine respectively (Figure 4). Incorporation of the boronic acid and bipyridine functional monomers was successful at 10, 20 and 30 mol%,

confirmed through ^{1}H- and ^{13}C-NMR spectroscopy. Impressively, a highly functional co-polymer incorporating 10 mol% of the boronic acid and 10% of the bipyridine functional monomer was successfully synthesised in a one-pot procedure demonstrating the selectivity and versatility of the oxime click reaction and providing a facile means for the synthesis of highly functional polymers.

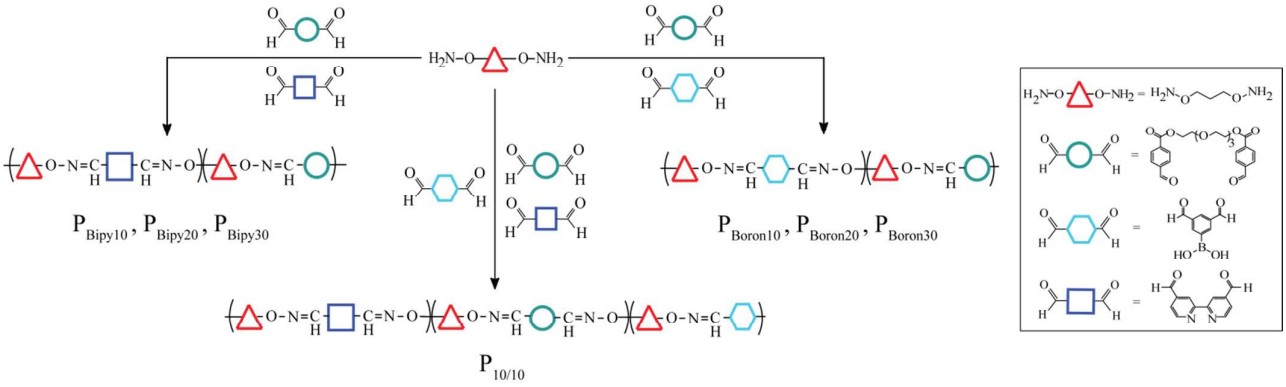

Fig. 4 Step-growth polymers formed via oxime click chemistry. Synthesis in the presence of an acidic boronic acid monomer (blue hexagons), a basic bipyridine monomer (dark blue squares) and both monomers was successful indicating the versatility and specificity of the oxime click reaction.[32]

Oxime Chemistry for Polymer Functionalisation

Functionalising polymers post-synthesis allows for the modification of the materials physical properties, the introduction of new chemistry or the conjugation to molecules of interest. Click chemistry has been successfully utilised to functionalise polymers because the very high efficiency of the click reactions and the functional group tolerance allows for synthesis of highly functionalised polymers without the need for complex and time consuming protecting group chemistry. A wide range of polymers have been functionalised using the CuAAC reaction such as methacrylates, polystyrenes, polyesters, polyethylene glycols, polyacrylamides and more.[53,54]

Oxime ligations have successfully been utilised to functionalise polymers with molecules of interest. Pendant-oxime functionalisation with small molecules has been demonstrated on a variety of different polymers such as methacrylates,[55] polyketoesters,[56] acrylamides,[57] polycaprolactones,[58] polystyrenes[59,60] and vinyl levulinate.[61] A wide variety of biomolecules, such as peptides,[62] glycoproteins,[63] bovine serum albumin,[64] and siRNA[65] have been conjugated to the terminal ends of polymers and oxime ligations have been successfully utilised to conjugate peptides and carbohydrates to the 3- and 5-end of oligodeoxyribonucleotides.[66]

Kurth and co-workers developed a novel aminooxy-functionalised vinyl monomer which was subsequently conjugated to lactose via oxime linkages.[67] The resulting lactose-based polymer was

then cross-linked with the di-alkene reagent N,N-methylenebis(acrylamide) to yield a biocompatible hydrogel.

Oxime linked polysaccharide-*b*-PEG diblock co-polymers were synthesised by Müller and co-workers.[68] Dextran, hyaluronic acid and chitosan were conjugated to PEG chains through oxime formation yielding diblock co-polymers. The oxime ligation proved superior to other methods of polysaccharide-containing block copolymer synthesis (radical polymerisation, enzymatic extension or end-to-end coupling)[69] owing to the high efficiency of oxime formation without the need for a toxic metal catalyst and successful formation under conditions where high molecular weight polysaccharides are soluble. The oxime-block copolymers were found to be stable under mildly acidic conditions (pH 3) for more than 55 hours while degrading to approximately 95% in under 24 hours at pH 2 owing to the acid-sensitivity of the oxime bond.

Maynard and co-workers synthesised an oxime functionalised methacrylate polymer as a proof-of-concept experiment for new oxime-based polymer-drug conjugates. Poly(3,3'-diethoxypropyl methacrylate) was prepared by ATRP and hydrolysis of the polymer product yielded active aldehyde groups throughout the polymer chain. The successful reaction between the model drug O-(carboxymethyl)hydroxylamine with the aldehyde side-chains of the polymer yielded oxime-linked conjugates indicating the potential applications of oxime click chemistry for the post-functionalisation of polymers for drug delivery.[70]

Oxime ligations have been utilised to functionalise polymers and successfully alter the properties of the resulting material. Francis and co-workers conjugated antifreeze proteins found in Arctic fish and insects onto an aminooxy substituted 2-hydroxypropyl methacrylamide/3-aminooxypropyl methacrylamide co-polymer which was found to successfully reduce ice build-up.[71] Theato and co-workers functionalised poly(*N*-isopropylacrylamide) with acetone oxime acrylate to effectively increase the lower critical solution temperature (LCST).[72]

Swager and co-workers synthesised a norbornene-functionalised miktoarm polymer derived from poly(3-hexylthiophene) utilising ring-opening metathesis polymerisation (ROMP).[73] Poly(3-hexylthiophene) was terminally-functionalised with 5-norbornene-2-endo-O-methylhydroxylamine via oxime click chemistry. The norbornene monomer allowed for the growth of norbornene polymer chains from each end of the thiophene polymer resulting in miktoarm "H-shaped" polymers. Owing to the stability of the oxime linkage the polymers were found to be stable under neutral and anhydrous acidic conditions but, as the oxime bond is dynamic in the presence of acid and excess

aminooxy or ketone groups, there is the potential to install a variety of functionalised arms by ROMPing in and out.

Oxime Chemistry for Hydrogel Synthesis

Hydrogels are attractive candidates for various biomedical applications such as sustained protein release, targeted drug delivery, regenerative medicine and tissue engineering owing to the biocompatibility, microporous structure, tuneable pore size and porosity with sizes ranging from human organs down to single cells and viruses. The use of click chemistry for hydrogel synthesis has been very successful due to the high reactivity, selectivity and mild reaction conditions of click reactions. The great advantage of click chemistry over other chemistries for hydrogel synthesis is the bioorthogonal nature of click reactions, that is, the click reactions do not interfere with the encapsulated proteins, drugs or cells.[74]

A variety of click reactions have been utilised to construct dynamic covalent hydrogels such as the CuAAC, thiol-ene, Diels-Alder, imine and hydrazone.[74-76] The vast majority of gels have been synthesised using the CuAAC however, as discussed previously, the toxicity of the copper catalyst limits their use in biomedical applications. While this can be overcome by utilising the SPAAC to form gels without the toxic Cu catalyst, the complex synthesis of the required cyclooctynes limits their scale-up potential. Thiol-ene photocoupling has been successfully utilised to form gels however the potential toxicity of photoinitiators and radicals is a concern as is the potential cross reactivity with thiols. Diels-Alder reactions require no toxic catalyst and exhibit thermal reversibility but generally exhibit slow gelation times.[74]

Maynard and co-workers developed an oxime-based hydrogel utilising an eight-armed aminooxy PEG and gluteraldehyde.[77] The mechanical properties of the gel were found to be tuneable depending upon the percent incorporation of aminooxy PEG. Additionally, as oxime formation is acid catalysed, the rate of gelation could be tuned by varying the pH of the solution. Gelation occurred within 5 min at pH 6.0 which decreased dramatically to 30 min at pH 7.2. Encapsulation studies of mesenchymal stem cells (MSCs) within the oxime-based hydrogels, functionalised with a RGD peptide, were performed and successful formation of the hydrogel was reported in the presence of cells and serum. Furthermore. MSCs were found to adhere to the gel, following functionalisation with RGD, and be viable for at least seven days.

Furthering this work, Maynard and co-workers developed a PEG-based hydrogel incorporating oxime and hydrazone linkages with tuneable degradation and mechanical properties.[78] As oxime bond formation is pH-dependant the time of the hydrogel formation could be controlled by adjusting the pH. Acid-sensitivity was incorporated into the materials via the addition of hydrazone linkages. The ratio of oxime/hydrazone could be varied to control the properties of the hydrogel; increasing the oxime fraction was found to stabilise the gels, increasing their lifetime from less than 24 hours to over one week. The dynamic nature of the hydrazone bonds allowed for self-healing properties to be integrated into the gel however when gels were formed with oxime bonds as well as hydrazone no self-healing was observed. The cell viability of the hydrogels was tested and high cell viability was observed.

Christman and co-workers developed a PEG-based injectable oxime cross-linked hydrogel for catheter delivery.[79] Again, the rate of gelation was tuneable by altering the pH. PEG gels formed within 20min after injection into the sub-cutaneous space and rapidly upon injection into myocardial tissue.

Following on from this, Christman and co-workers developed oxime-cross linked PEG star polymers and demonstrated their application in the prevention of cardiac adhesions.[80] The aldehyde functionalised eight-arm PEG was shown to react with amines on surface tissues as well as with hydroxylamine-functionalised eight-arm PEG to form hydrogels adhered to cardiac tissues. Gelation time could be decreased from 400 seconds to less than 2 seconds when the percentage weight of monomer was increased from 25 mg mL^{-1} to 100 mg mL^{-1}. The stability of the gels was found to be dependent upon the degree of oxime crosslinking; a 25 mg mL^{-1} polymer sample reported complete mass loss after 18 days whereas a 50 mg mL^{-1} was found to decrease in mass by 26.5 % and a 100 mg mL^{-1} sample was found to decrease in mass by only 19.7 % after the same time period owing to the increase in the proportion of oxime bonds. It was concluded that at higher concentrations the oxime cross-linked PEG-hydrogels are hydrolytically stable at physiological conditions for over two weeks. The adherence of cells to the hydrogels formed with different functional group ratios was measured using 3T3 fibroblasts or RAW macrophages. It was found that excessive amounts of aldehyde or hydroxylamine functional groups, and the oxime bond, did not alter the anticellular adhesion of PEG hydrogels. Elution studies determined that the PEG hydrogels were cyto-compatible and the aldehyde/aminoxy ratio affected the hydrogels adherence to various cardiac tissues with excessive aminoxy groups (1:3) creating the weakest and least stable gels. The performance of the gels was possibly due to a lesser number of aldehyde groups available for adhesion to amine groups present on cell surfaces.

Moratti and co-workers developed a similar oxime cross-linked PEG hydrogel for use as a surgical adhesive.[81] Once again aldehyde functionality was utilised not only to cross link and form the hydrogel but also to bind the amine groups in human tissue proteins thereby linking the tissue and the gel resulting in an increase in the strength of the surgical adhesive. Hydrogel formation was reported to take less than one minute and the resulting hydrogels were found to be non-cytotoxic at 5% concentration. The hydrogels were shown to adhere well to skin, as well as demonstrating improved lap-shear strength compared to many currently utilised soft tissue adhesives.

Becker and co-workers developed a peptide-functionalised PEG gel via oxime chemistry which once again exhibited pH dependant gel times, shortening from hours to seconds upon a reduction in pH and addition of aniline catalyst.[82] This also facilitated the tuning of the storage modulus from 0.3 to over 15 kPa. Azide functionality was incorporated into the oxime hydrogels which provided a platform for functionalisation via the CuAAC reaction. Alkyne-RGD-biotin was used as a model peptide which was observed to react with the hydrogel via fluorescence microscopy. The oxime gels were also functionalised with alkene groups which were used for peptide conjugation via the photo-initiated thiol-ene click reaction. Thiol-ene addition was successful and spatial patterns of peptides were created throughout the gel using a simple photomask.

Sumerlin and co-workers reported the first case of an oxime-based self-healing hydrogel.[83] Diacetone acrylamide (DAA) and N,N-dimethylacrylamide (DMA) were copolymerised to obtain hydrophilic copolymers possessing ketone functionality. The resulting copolymers were cross-linked with a difunctional alkoxyamine to form oxime-based hydrogels. The dynamic nature of the oxime-hydrogels was initially proven by the successful gel-to-sol transition stimulated by the addition of an excess of the monofunctional alkoxyamine O-(tetrahydro-2H-pyran-2-yl)hydroxylamine. It was found that addition of 20 equivalents O-(tetrahydro-2H-pyran-2-yl) hydroxylamine, along with catalytic TFA, led to sol-transition within 2 hours. When reduced to 5 equivalents, the gel-to-sol transition time increased to 24 hours at 25℃ (Figure 5). In the same paper, Sumerlin and co-workers demonstrated the self-healing nature of oxime based-hydrogels. Self-healing of the damaged diacetone acrylamine/N,N-dimethylacrylamide gel was achieved through simple contact between the two fragments. This was reported to promoted oxime exchange across the damaged interface and result in covalent healing within 2 hr.

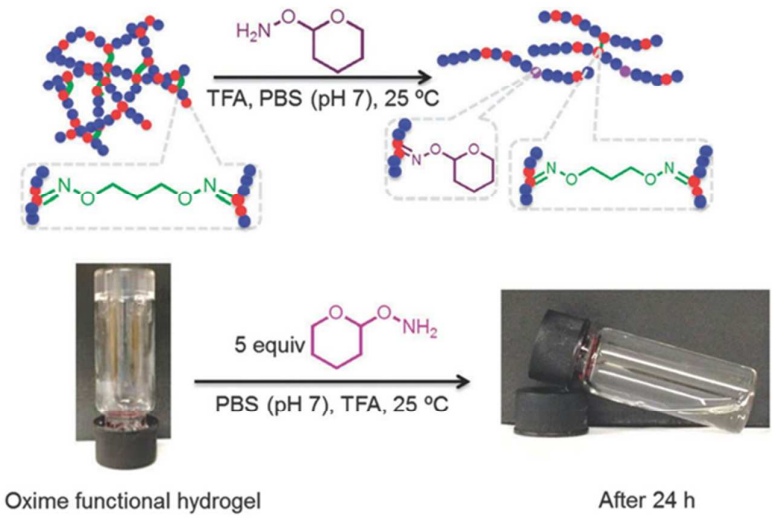

Fig. 5 Gel-to-sol transition of an acrylamide gel stimulated by the addition of an excess of mono-functional hydroxylamine. Reproduced from Ref. 81 with permission from the Royal Society of Chemistry.[83]

Tirrell and co-workers recently developed a photo-reversible oxime-based protein patterning approach for guided stem cell differentiation in hydrogels (Figure 6).[84] Three bioorthogonal click reactions were utilised in the process; the SPAAC reaction for hydrogel synthesis, a light-initiated oxime-ligation process for protein ligation and an ortho-nitrobenzyl ester photoscission reaction for protein elimination. Hydrogels were synthesised from the SPAAC reaction between a multi-arm PEG tetrabicyclononyne (*Mn* – 10 kDa) and an azide-functionalised synthetic peptide (N_3-DGPQGIWGQGDK(N_3)-NH_2) which is susceptible to cleavage by cell-secreted matrix metalloproteins. Gelation occurred within 5min yielding homogenous networks with moduli between 1 – 10 kPa. Following the gel formation photo-patterned oxime formation was used to conjugate aldehyde functionalised proteins within the gel. The heterobifunctional molecule 2-(2-nitrophenyl)propyloxycarbonyl (NPPOC)-photocaged alkoxyamine/azide tri(ethylene glycol)-based linker (N_3-TEG-ONH-NPPOC) was introduced to the gel formulation thereby introducing binding regions for protein adhesion spread evenly throughout the gel. When exposed to UV-light (365 nm), the NPPOC-protected linker was reported to undergo a β-elimination to produce an active alkoxyamine available for oxime ligation. Aldehyde functionalised bovine serum albumin was conjugated to the hydrogel through oxime bond formation. Controlled protein adhesion was achieved via conventional photo-patterning methods in which photomasks allowed for the specific binding of proteins. Furthermore, a multiphoton laser-scanning lithographic technique allowed for the controlled patterning of proteins in 3 dimensions. By varying the pulsed laser conditions excellent control over the extent of biochemical labelling was obtained for 3D patterning.

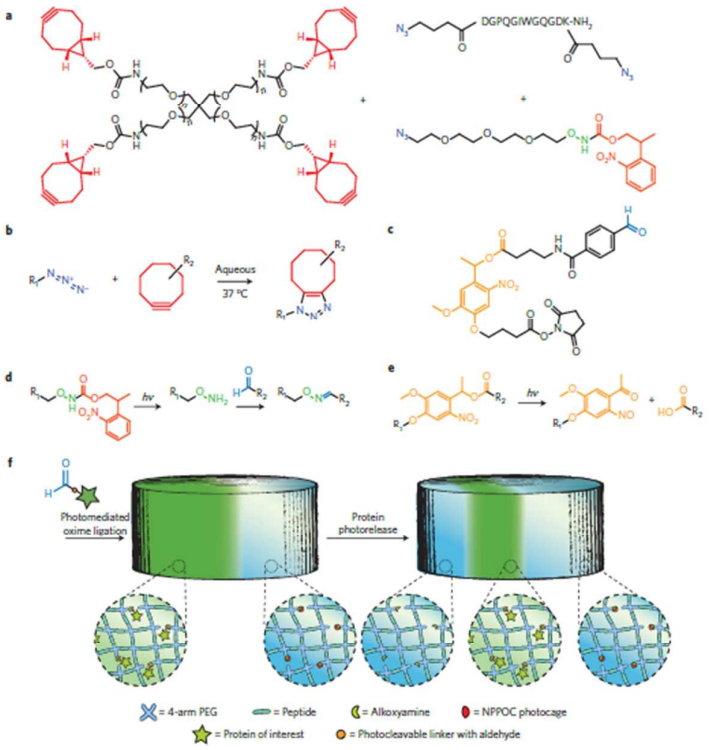

Fig. 6 SPAAC synthesised hydrogel with oxime-conjugated proteins. Spatial control of the protein conjugation was achieved through photo-patterning using the heterobifunctional molecule 2-(2-nitrophenyl)propyloxycarbonyl (NPPOC)-photocaged alkoxyamine/azide tri(ethylene glycol)-based linker (N_3-TEG-ONH-NPPOC) which undergoes a β-elimination to yield aldehyde-reactive alkoxyamines. Reprinted by permission from Macmillan Publishers Ltd: Nature Materials, Ref. 82, copyright (2015).[84]

Oxime Chemistry for Surface Patterning

Surfaces with tailored polymer patterns are of interest for the development of light emitting displays, semiconductors, microelectronics and plastic electronics, medical research, for the synthesis of highly defined templates, for the production of optical devices and sensors as well as for the high throughput analysis of various substrates.[85]

Photodegradation has been used effectively to selectively deprotect and pattern a surface with reactive aldehyde functionality which is then subsequently available for oxime ligation.[86-89] Barner-Kowollik and co-workers functionalised a silicon surface with a photosensitive nitrosobenzaldehyde derivative. Irradiation of the nitrosobenzaldehyde molecule yields a reactive aldehyde moiety which is available for oxime ligation. Selective photo-degradation yielded a patterned surface which was functionalised with an aminooxy tag, enabling precise control over the surface pattern.[86]

Following a similar theme, Barner-Kowollik and co-workers also developed a versatile surface patterning technique by trapping photo-generated thioaldehydes through ligation by hydroxylamines, amines and thiols yielding oximes, imines and disulphides respectively.[90,91] Surfaces were coated with a modified PEG, functionalised with the photoactive thiol moiety. Selective irradiation of the surface using a shadow mask yielded thioaldehydes in a controlled pattern which are available for subsequent conjugation. The oxime ligation was found to be superior to both the imine and disulphide linkage in this system owing to its increased resistance to sulphur oxidation which the corresponding imine is partially susceptible to.

Maynard and co-workers have been successful at patterning surfaces using e-beam lithography followed by oxime bond formation.[35,92] Poly(BOC-aminooxy tetra(ethylene glycol) methacrylate) was synthesised by free radical polymerisation before being immobilised on a silicon surface through the use of an electron beam writer. Acidic deprotection of the BOC-methacrylate yielded the active aminooxy groups available for subsequent oxime ligation. The high resolution of this technique was demonstrated by the patterning of polymers into complex shapes such as squares and bowties (Figure 7).[35]

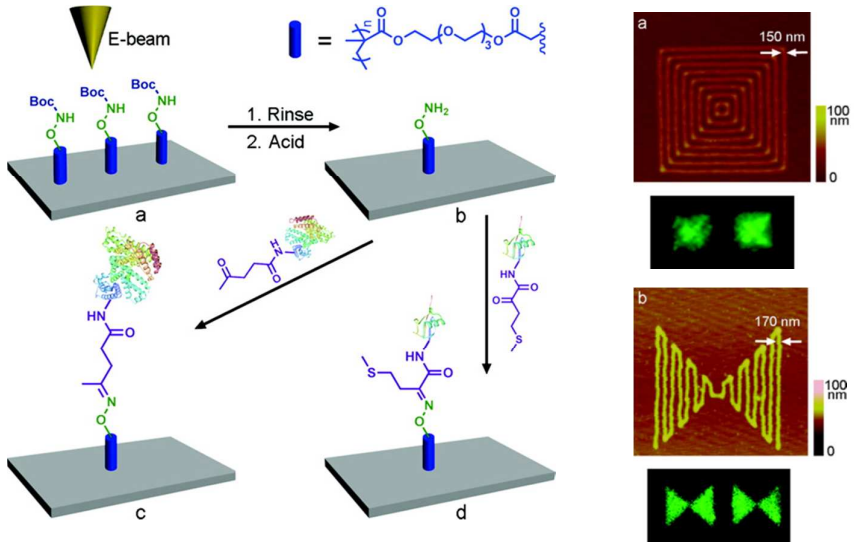

Fig. 7 Surface patterning achieved through electron-beam writing of a BOC-protected methacrylate onto a silcon wafer. Trifluoroacetic acid removal of the BOC groups provided the desired aminooxy functionality available for oxime ligation. Adapted with permission from Ref. 35. Copyright (2011) American Chemical Society.[35]

Yousaf and co-workers utilised UV-irradiation to selectively deprotect and pattern a gold surface with aldehyde functionality which was available for conjugation by ketone functional-molecules via

oxime bond formation. The reaction between the surface bound hydroxylamines and carbonyl-containing ligands was shown to be fast and high yielding under physiological conditions and oxime formation was shown to be chemoselective in the presence of cell lysates and to be stable over a range of pH's and temperatures. The sequential immobilization of two fluorescent dyes and the immobilization of peptide ligands for cell adhesion in desired patterns was demonstrated.[93]

Following this, Yousef and co-workers developed oxime-based etching[94] and permeation printing[95] processes for surface patterning. Biodegradable poly(1,2,6-hexanetriol R-ketoglutarate) films were etched using a NaOH solution to afford micron-scale channels. By altering the etching duration, the feature depth could be controlled and by utilising parallel flow in a single microfluidic cassette multidimensional features could be produced. The ketone functionality of poly(1,2,6-hexanetriol R-ketoglutarate) allows for the potential conjugation of oxyamine tethered ligands via oxime bond formation.[94] In a further extension, Yousaf and co-workers developed a microfluidic solute permeation enhancement and diffusion (SPREAD) technique for inking PDMS cassettes with alkanethiols.[95] SPREAD was utilised to create a gradient of a hydroxylamine terminated alkanethiol on a PDMS microfluidic device. This could be transferred onto a gold surface to create a self-assembled monolayer (SAM) with the equivalent gradient of the hydroxylamine terminated alkanethiol. Initially, microfluidic lithography was utilised to pattern a protective SAM layer onto the gold surface, then controlled gradients were adhered onto unprotected gold surface to produce single cell gradient microarrays. This SPREAD technique can also be utilised to print micrometer-sized areas of SAM gradients with very high resolution. The immobilisation of the Arg-Gly-Asp (RGD)-ketone peptide, for use as a cell adhesive, to the SPREAD patterned surface allows for the conjugation of biospecific molecules for of cell adhesion, polarity, and migration studies.[95]

Dynamic Materials Utilising Oxime Chemistry

Dynamic covalent chemistry combines the advantages of a non-covalent reversible system; spontaneous formation, error correction and often, a stimuli-responsive nature, with the strength of covalent bonds. Dynamic polymers occur naturally; the actin filaments and microtubules of the cytoskeleton adapt to the changing size and shape of the cell and it's the dynamic nature of these naturally occurring polymers that control many biological processes such as cell division and movement.[96-98] Industrially, dynamic polymers are of great interest because of their many potential applications including self-healing materials,[99] degradable materials for tissue engineering[100] and packaging,[20] stimuli-responsive materials for targeted drug delivery,[101,102] sensing applications[103] and

shape-memory materials.[104] An excellent review of dynamic polymers as self-healing materials was recently reported by Lehn and co-workers.[105]

So far, the majority of the dynamic covalent materials synthesised have been based on disulfide, Diels-Alder or boronate reactions.[106] Recently however, hydrazone and imine bonds have been utilised to developed dynamic materials.[19-21]

Prior to 1999, there was a very limited amount of information on the imine/oxime exchange reaction mechanisms.[107-109] It was not until Eliseev and co-workers published a detailed report on the mechanism and kinetics of oxime exchange in 1999 that the chemistry was examined further.[110] Furthermore, it was not until the introduction of click chemistry in 2001 by Sharpless and co-workers[1] and the discovery of aniline as an effective catalyst for oxime formation in 2006 by Dawson and co-workers[37] that oxime chemistry started to gain significant attention. Oxime chemistry remained as one of the more unexplored/unpopular click reactions until very recently. It was not until 2007 that research into dynamic materials based upon oxime chemistry started to gain attention.

Oxime chemistry was utilised by Lehn and co-workers as a means of creating dynamic mimics of naturally occurring polymers.[111] The glyosidic bonds of arabinofuranoside oligosaccharides were replaced with oxime linkages and the dynamic activity of the resulting polymers were investigated. A model system was initially developed to investigate the dynamic properties of the oxime bond at various pHs. The proportion of exchange between O-methyl oxime and *tert*-butylhydroxylamine was determined by ^{1}H-NMR and it was found that the half-life for exchange (at 20 mM) increased from 98 min at pH = 4 to 777 min at pH = 6. It was therefore concluded that mild acidic conditions increases the rate of exchange. Following the success of the model system, glycosidic bonds in mycobacterial arabins, polysaccharides present in the cell wall of some mycobacteria, were replaced by oxime bonds. Polymers ranging between 800 – 2200 Da were synthesised via a condensation polymerisation and their reversibility was demonstrated by dynamic chain termination (Figure 8). A decrease in polymer size and the introduction of new characteristic peaks in the ^{1}H-NMR spectra allowed for the exchange reaction to be monitored and it was found that exchange was slower at pD = 6 than at slightly acidic pD = 4 and 5.

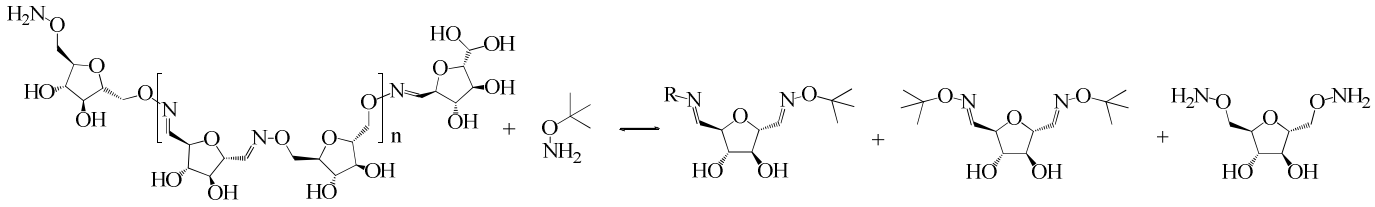

Fig. 8 Dynamic chain termination through the addition of a monofunctional hydroxylamine in oxime-based mimics of naturally occurring mycobacterial arabins. Reproduced with permission from Ref. 111. Copyright (2007) John Wiley & Sons, Inc.[111]

The selectivity and dynamic nature of oxime bonds was exploited by Nishimura and co-workers as a means of selectively binding, purifying and then releasing glycopeptides for high throughput analysis.[112] An aminooxy-functionalised vinyl monomer was polymerised via dispersion polymerisation to give polymer particles between 50 – 100 μm in size which contained a high concentration of active aminooxy groups (2.7 μmol mg^{-1}). Model oligosaccharides were blotted onto the aminooxy functionalised polymer, washed to remove impurities and unbound molecules before being incubated with an excess of aminooxy compounds, such as aminooxy acetic acid and benzyloxyamine, to promote transoximisation and oligosaccharide release (Figure 9). It was found that the oligosaccharides which were covalently bound to the polymer via oxime linkages could be released in the presence of a large excess of aminooxy compounds. This protocol was applied for human serum glycomic analysis in which 23 N-glycans were isolated and detected by MALDI-TOF with quantitative ratios allowing for a simple and automatable method for analysing glycomic serum. In the same paper Nishimura and co-workers isolated and purified protein fragments resulting from galactose oxidase enzymatic degradation utilising the same transoximisation procedure. The reaction between galactose and galactose oxidase yields an active aldehyde group on the non-reducing terminal galactose residue. This reactive aldehyde moiety allowed for the selective ligation of the galactose to the aminooxy-functionalised polymer, via oxime bond formation, which resulted in the successful isolation the protein fragment and, together with MALDI-TOF, for identification of the glycan structure and peptide sequence.[112]

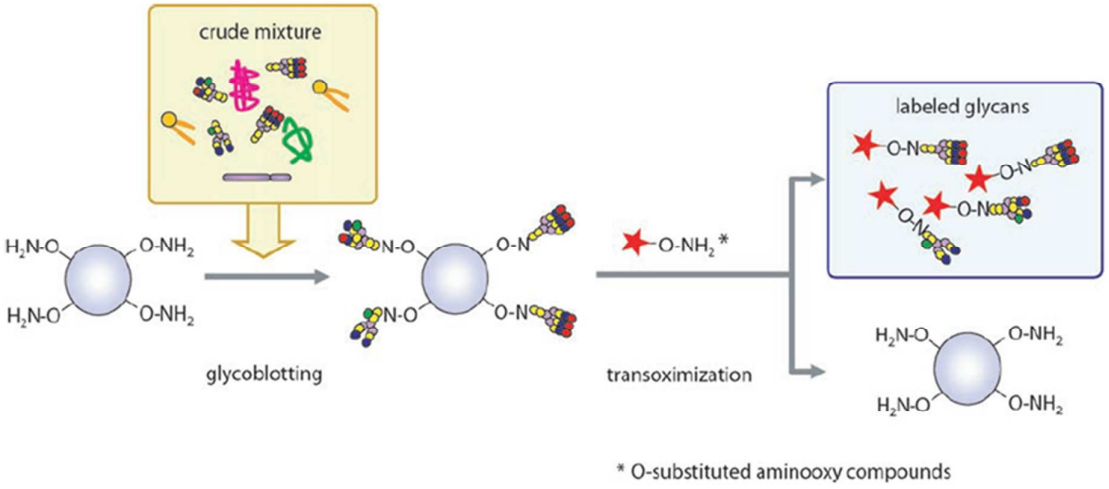

Fig. 9 The purification of oligosaccharides, dynamic oxime bonds are used as the selective binding and releasing mechanism. Reproduced with permission from Ref. 112. Copyright (2007) John Wiley & Sons, Inc.[112]

Dynamic oxime chemistry has also been applied as a means of creating reversible polymeric structures.[101,102,113,114] Reversible micelles based on oxime chemistry were developed by Fulton and co-workers.[113] Di-block co-polymers comprised of polyisoprene and polystyrene, incorporating oxime bonds between the polymeric blocks, were reported to self-assemble into micellar aggregates which then disassembled upon addition of an excess of a small monofunctional alkoxyamine. This was presented as a means of creating stable micelles, the coronal polymer chains of which could be replaced while the core polymer chains remain.

Research into oxime-based stimuli-responsive degradable micelles was further investigated by Zhu and coworkers.[101,102] Initially, a PEG-caprolactone-PEG triblock copolymer, with oxime bonds linking the polymeric blocks, was reported to self-assemble into micelles. The micelles exhibited stimuli-responsive degradation when exposed to acidic conditions (pH 5) owing to the acid-sensitive oxime bond. Doxorubicin (DOX), an anti-cancer drug was encapsulated into the micelle core and drug release was found to be significantly accelerated at pH 5.0 when compared to pH 7.4, owing to the cleavage of the oxime linkage and subsequent breakdown of the micelle.[102]

Continuing this research, Zhu and co-workers developed pH-responsive flower-like micelles based upon polycaprolactone-PEG-polycaprolactone triblock copolymers.[101] Again, oxime bonds were incorporated between the polymeric blocks to create a pH sensitive micelle. DOX was encapsulated inside the micelle, which possessed high stability at pH 7.4 while being degradable at pH 5.0 thereby triggering DOX release. The IC_{50} reported for the flower-like-micelles was 1.8 μg mL^{-1} indicating anti-cancer properties and potential as a new anti-cancer treatment.

Sumerlin and co-workers exploited the oxime linkage as a means of creating dynamic macromolecular stars.[114] RAFT polymerisation was utilised to synthesise ketone containing block copolymers which, following the addition of a difunctional alkoxyamine, were cross-linked to form macromolecular stars. Competitive exchange in the presence of monofunctional alkoxyamine, under acidic pH, resulted in the dissociation of the star (Figure 10).[114]

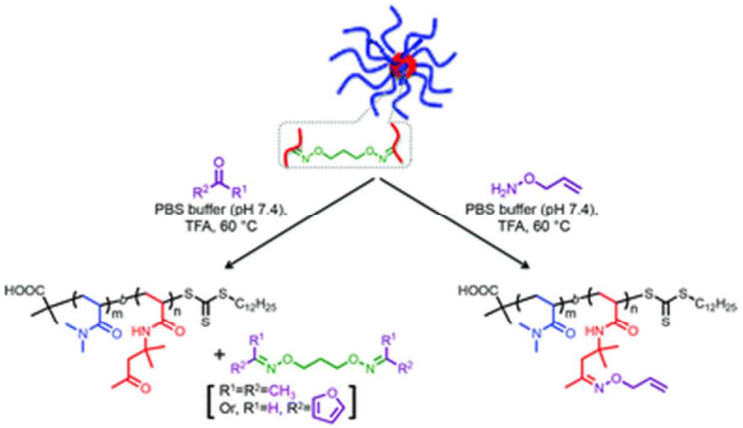

Fig. 10 Dynamic star polymers synthesised by oxime click chemistry. The difunctional alkoxyamine cross-linker was replaced by a monofunctional alkoxyamine, under acidic pH, resulting in the dissociation of the star. Reproduced from Ref. 114 with permission from the Royal Society of Chemistry.[114]

A redox responsive oxime ligation/release procedure was developed by Yousaf and co-workers in which the oxidation of hydroquinone to benzoquinone for subsequent reactions with oxyamine-tethered ligands resulted in the generation of a stable oxime product.[115] The reversible nature of the oxime bond is exploited as a means of cleaving the conjugate to yield a hydroxy-terminated ligand and an aminophenol. Subsequent reduction of the aminophenol regenerates the original hydroquinone for subsequent rounds of conjugation and release (Figure 11).[115] This technique was utilised to tailor cell surfaces with switchable, bioorthogonal chemistry, to induce liposome fusion and to direct the assembly and disassembly of 3D tissues for applications in stem-cell differentiation and tissue engineering. Multiple rounds of bioconjugation and release to and from cell surfaces was reported and indicates the potential of this method to be used simultaneously as an diagnostic tool for observing cell-cell interactions as well as a means of changing the cell-surface ligands and cell–cell contacts.

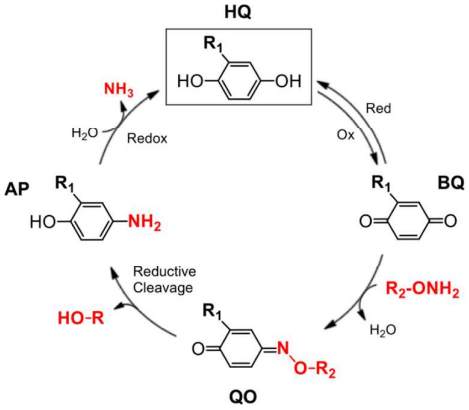

Fig. 11 Recyclable redox responsive oxime ligation/release procedure developed by Yousaf and co-workers. Adapted with permission from Ref. 115. Copyright (2014) American Chemical Society.[115]

Both oxime and oxanorbornene bonds were utilised by Sumerlin and co-workers to develop novel, dynamic polymers which respond to multiple stimuli.[116] The polymers were prepared via Diels-Alder step-growth polymerisation of an AB monomer which contained an oxime bond. Polymerisations were undertaken at 100℃ or 130℃ over 9 or 17 days yielding polymers with molecular weight ranging from 3.5 to 13 kDa. Due to the incorporation of two dynamic chemistries polymer degradation was achieved by 1) the addition of a mono-functional hydroxylamine to stimulate oxime degradation and 2) by raising the temperature to stimulate the retro-Diels Alder reaction (Figure 12).

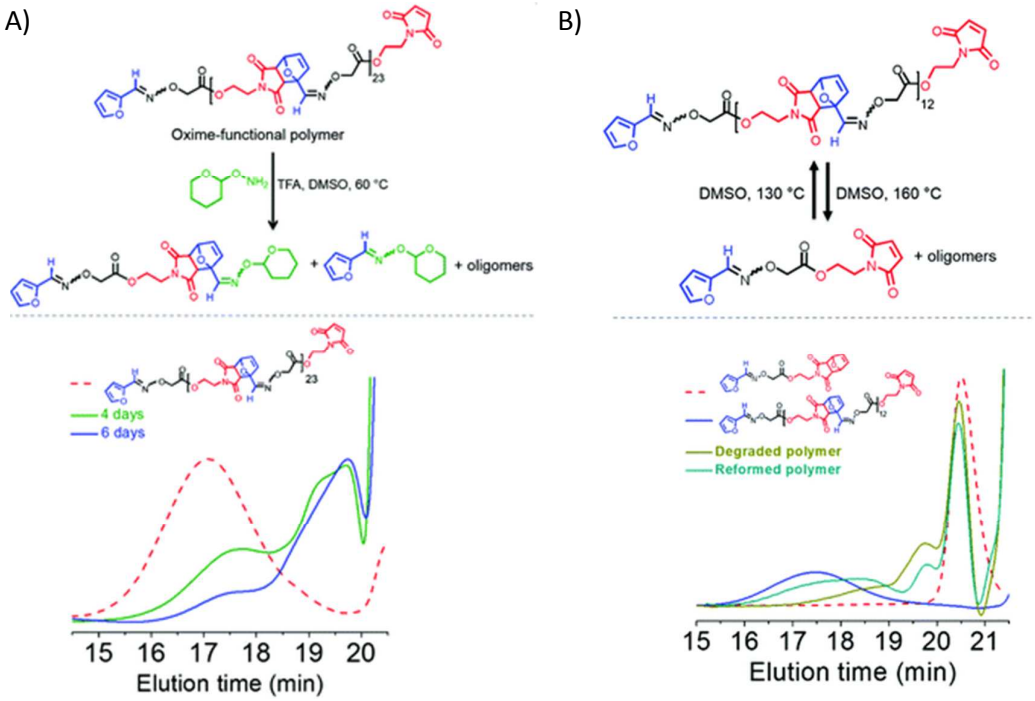

Fig. 12 Polymer degradation achieved through two mechanisms; the addition of a mono-functional hydroxylamine to stimulate oxime degradation (A) and by raising the temperature to stimulate the retro-Diels Alder reaction (B). Reproduced from Ref. 116 with permission from the Royal Society of Chemistry.[116]

Future Perspectives

Future applications of oxime click chemistry can take inspiration from imine/Schiff base chemistry and related click reactions. Exciting new applications of the imine click reaction include the synthesis of self-healing covalent organic frameworks (COFs), porous materials, and self-assembling, supramolecular structures.[117-125] The hydrazone click reaction has recently been utilised for the development of COFs[126] and the CuAAC click reaction has been developed for the synthesis of honeycomb films,[127] porous organic molecules,[128] COFs[129] and, together with the thiol-ene and Diels-Alder click reactions, for the development of shape-memory polymers.[130] An interesting example is the synthesis of imine linked molecular cages developed by James and co-workers.[131] The cages were reported to be soluble in solvents whose molecules cannot enter the cage, such as 15-crown-5, thereby forming permanently porous liquids (Figure 13).

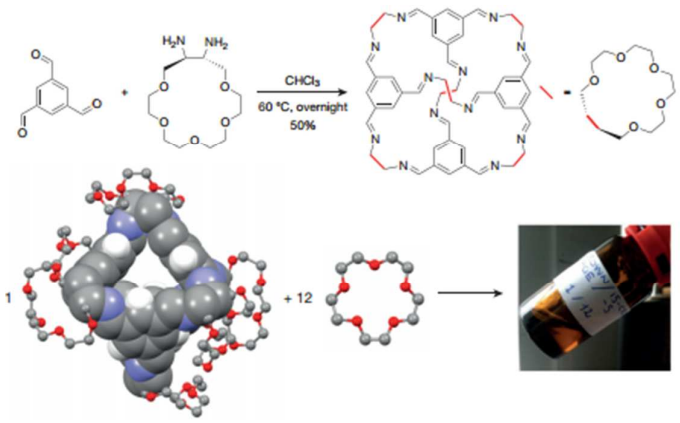

Fig. 13 Synthesis of an imine-linked molecular cage to form a permanently porous liquid. Reprinted by permission from Macmillan Publishers Ltd: Nature, Ref. 131, copyright (2015).[131]

The use of dynamic covalent chemistry to direct the assembly of or to incorporate a stimuli-responsive feature into, self-assembling supramolecular systems is a rapidly growing field. Supramolecular structures consisting of dynamic building blocks are capable of responding to external stimuli through the re-organisation or modification of their structure through component exchange or reorganisation.[132] Taking inspiration from nature, a variety of dynamic chemistries (hydrazone, disulphide, imine, metal-ligand exchange) have been used to direct the self-assembly of peptides into various supramolecular structures such as coils, helix's, hairpins and stacked sheets.[133]

So far, oxime chemistry is lagging behind imine and hydrazone chemistry for the development of next-generation materials. This may be in part due to the limited availability of hydroxylamines.

Recently however, the synthesis of these has become much more straightforward and unlike the preparation of azides for CuAAC, does not pose any safety risks. Consequently, we predict a rapid increase in the availability of hydroxylamine-functional materials in the near-future. Furthermore, the large abundance of naturally occurring and synthetic aldehydes and ketones offers up an enormous platform for which to explore new applications of oxime chemistry ranging from the synthesis of complex, bio-inspired products to the development of environmentally friendly and degradable commodity plastics.

Conclusion

Over the last decade the use of click chemistry for the development of advanced polymeric materials has risen dramatically. Click reactions which exhibit very high efficiencies, simple means for product purification and functional versatility have been utilised for the synthesis of new polymeric materials exhibiting self-healing, degradable or dynamic behaviour, for the development of new means to pattern surfaces with high fidelity and, more recently, for the synthesis of COFs, single-atom monolayers and permanently porous liquids. Traditionally the CuAAC, Diels-Alder or thiol-ene click reactions have been for these purposes, recently however, carbonyl condensations forming imine, hydrazone and oxime linkages have exploited as novel means to synthesise highly functional, dynamic materials. It is the oxime linkage, previously overlooked, which is proving to be a highly efficient and robust means for material synthesis owing to the extremely high efficiency of oxime formation, the dynamic covalent character of oxime-based materials, the formation of water as the only by-product and the functional group tolerance. Over the past 5 years the rapid rise in publications reporting the use of oxime chemistry for the development of degradable polymers, self-healing gels, surface patterning and polymer-conjugation indicates the rapidly growing interest in this field and demonstrates the great potential of oxime chemistry as a new tool for the synthesis of next-generation materials. We believe that this highly efficient and versatile reaction will prepare a range of new materials with engineered properties.

Acknowledgement

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References

(1) Kolb, H. C., Finn, M.G., Sharpless, K.B. *Angew. Chem. Int. Ed.* **2001**, *40*, 2004.

(2) Nandivada, H., Jiang, X., Lahann, J. *Adv. Mater.* **2007**, *19*, 2197.

(3) Moses, J. E., Moorhouse, A.D. *Chem. Soc. Rev.* **2007**, *36*, 1249.

(4) Thirumurugan, P., Matosiuk, D., Jozwiak, K. *Chem. Rev.* **2013**, *113*, 4905.

(5) Liang, L., Astruc, D. *Coordin. Chem. Rev.* **2011**, *255*, 2933.

(6) van Steenis, D. J. V. C., David, Olivier R. P., van Strijdonck, Gino P. F., van Maarseveen, Jan H., Reek, Joost N. H. *Chem. Commun.* **2005**, 4333.

(7) Diaz, D. D., Mellado, J. J. M., Velazquez, D. G., Ravela, A. G. *Tetrahedron Lett.* **2008**, *49*, 1340.

(8) Tinmaz, H. B., Arslan, I., Tasdelen, M.A. *J. Polym. Sci., Part A: Polym. Chem.* **2015**, *53*, 1687.

(9) Binauld, S., Damiron, D., Hamaide, T., Pascault, J-P., Fleury, E., Drockenmuller, E. *Chem. Commun.* **2008**, 4138.

(10) Schwarts, E., Breitenkamp, K., Fokin, V.V. *Macromolecules* **2011**, *44*, 4735.

(11) Koo, S. P. S., Stamenović, M. M., Prasath., R. A., Inglis, A. J., Du Prez, F. E., Barner-Kowollik, C., Van Camp, W., Junkers, T. *J. Polym. Sci., Part A: Polym. Chem.* **2010**, *48*, 1699.

(12) Fournier, D., Hoogenboom, R., Schubert, U.S. *Chem. Soc. Rev.* **2007**, *36*, 1369.

(13) Johnson, J. A., Finn, M.G., Koberstein, J.T., Turro, N.J. *Macromol. Rapid Commun.* **2008**, *29*, 1052.

(14) Tasdelen, M., A. *Polym. Chem.* **2011**, *2*, 2133.

(15) Xu, J., Boyer, C. *Macromolecules* **2015**, *48*, 520.

(16) Killops, K., Campos, L.M., Hawker, C.J. *J. Am. Chem. Soc.* **2008**, *130*, 5062.

(17) Lowe, A. B. *Polym. Chem.* **2010**, *1*, 17.

(18) Malkoch, M., Vestberg, R., Gupta, N., Mespouille, L., Dubois, P., Mason, A. F., Hedrick, J. L., Liao, Q., Frank, C. W., Kingsbury, K., Hawker, C. J. *Chem. Commun.* **2006**, 2774.

(19) Skene, W. G., Lehn, J.-M.P. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 8270.

(20) Fukuda, K., Shimoda, M., Sukegawa, M., Nobori, T., Lehn, J. -M. *Green Chem.* **2012**, *14*, 2907.

(21) Zhu, L., Tu, C., Zhu, B., Su, Y., Pang, Y., Yan, D., Wu, J., Zhu, X. *Polym. Chem.* **2011**, *2*, 1761.

(22) Brisson, E. R. L., Xiao, Z., Levin, L., Franks, G. V., Connal, L. A. *Polym. Chem.* **2016**, *7*, 1945.

(23) Sun, G., Fang, H., Cheng, C., Lu, P., Zhang, K., Walker, A. V., Taylor, J. -S. A., Wooley, K. L. *ACS Nano* **2009**, *3*, 673.

(24) Brisson, E. R. L., Xiao, Z., Connal, L. A. *Aust. J. Chem.* **2016**.

(25) Layer, R. W. *Chem. Rev.* **1963**, *63*, 489.

(26) Belowicha, M. E., Stoddart, J. F. *Chem. Soc. Rev.* **2012**, *41*, 2003.

(27) Xin, S., Aprahamian, I. *Chem. Soc. Rev.* **2014**, *43*, 1963.

(28) Binauld, S., Stenzel, M. H. *Chem. Commun.* **2013**, *49*, 2082.

(29) Ulrich, S., Boturyn, D., Marra, A., Renaudet, O., Dumy, P. *Chem. Eur. J.* **2014**, *20*, 34.

(30) Zeng, Y., Ramya, T.N.C., Dirksen, A., Dawson, P.E., Paulson, J.C. *Nat. Methods* **2009**, *6*, 207.

(31) Christman, K., Broyer, R.M., Tolstyka, Z. P., Maynard, H.D. *J. Mater. Chem.* **2007**, *17*, 2021.

(32) Collins, J., Xiao, Z., Espinosa-Gomez, A., Fors, B. P., Connal, L. A. *Polym. Chem.* **2016**, *7*, 2581.

(33) Taraballi, F. R., L., Battocchio, C., Polzonetti, G., Nicotra, F., Cipolla, L. *Org. Biomol. Chem.* **2014**, *12*, 4089.

(34) Maillard, L. T., Benohoud, M., Durand, P., Badet, B. *J. Org. Chem.* **2005**, *70*, 6303.

(35) Christman, K. L., Broyer, R.M., Schopf, E., Kolodziej, C.M., Chen, Y., Maynard, H.D. *Langmuir* **2011**, *27*, 1415.
(36) Buré, C., Lelièvre, D., Delmas, A. *Rapid Commun. Mass Spectrom.* **2000**, *14*, 2158.
(37) Dirksen, A., Hackeng, T. M., Dawson, P.E. *Angew. Chem. Int. Ed.* **2006**, *45*, 7581.
(38) Wendeler, M., Grinberg, L., Wang, X., Dawson, P.E., Baca, M. *Bioconjugate Chem.* **2014**, *25*, 93.
(39) Agten, S. M., Suylen, D. P. L., Hackeng, T. M. *Bioconjugate Chem.* **2015**, *27*, 42.
(40) Larsen, D., Pittelkow, M., Karmakar, S., Kool, E.T. *Org. Lett.* **2015**, *17*, 274.
(41) Crisalli, P., Kool, E.T. *J. Org. Chem.* **2013**, *78*, 1184.
(42) Iha, R. K., Wooley, K. L., Nyström, A. M., Burke, D. J., Kade, M. J., Hawker, C. J. *Chem. Rev.* **2009**, *109*, 5620.
(43) Kalia, J., Raines, R.T., *Angew. Chem. Int. Ed.* **2008**, *47*, 7523.
(44) Stille, J. K. *J. Chem. Educ.* **1981**, *58*, 862.
(45) Odian, G. *Principles of Polymerization*; 4 ed.; John Wiley & Sons, Inc., 2004.
(46) Brewer, G. J. *Chem. Res. Toxicol.* **2010**, *23*, 319.
(47) Hong, V., Steinmetz, N. F., Manchester, M., Finn, M. G. *Bioconjugate. Chem.* **2010**, *21*, 1912.
(48) Anderton, G. I., Bangerter, A. S., Davis, T. C., Feng, Z., Furtak, A. J., Larsen, J. O., Scroggin, T. L., Heemstra, J. M. *Bioconjugate Chem.* **2015**, *26*, 1687.
(49) Sletten, E. M., Nakamura, H., Jewett, J., C. Bertozzi, C. R. *J. Am. Chem. Soc.* **2010**, *132*, 11799.
(50) Dong, J., Sharpless, B., Kwisnek, L., Oakdale, J.S., Fokin, V.V. *Agnew. Chem. Int. Ed.* **2014**, *53*, 9466.
(51) Andreana, P. R., Xie, W., Cheng, H.N., Qiao, L., Murphy, D.J., Gu, Q,-M., Wang, P.G. *Org. Lett.* **2002**, *4*, 1863.
(52) Ma, M., Chatterjee, S., Zhang, M., Bong, D. *Chem. Commun.* **2011**, *47*, 2853.
(53) Meldal, M. *Macromol. Rapid Commun.* **2008**, *29*, 1016.
(54) Delaittre, G., Guimard, N. K., Barner-Kowollik, C. *Acc. Chem. Res.* **2015**, *48*, 1296.
(55) Liu, J., Li, R.C., Sand, G.J., Bulmus, V., Davis, T.P., Maynard, H.D. *Macromol.* **2013**, *46*, 8.
(56) Barrett, D. G., Yousaf, M.N. *Biomacromol.* **2008**, *9*, 2029.
(57) Vazques-Dorbatt, V., Tolstyka, Z.P., Maynard, H.D. *Macromolecules* **2009**, *42*, 7650.
(58) Nicolau, S. E., Davis, L.L., Duncan, C.C., Olsen, T.R., Alexis, F., Whitehead, D.C., Van Horn, B.A. *J. Poly. Sci. Part A; Polym Chem* **2015**, *53*, 2421.
(59) Hill, M. R., Mukherjee, S., Costanzo, P.J., Sumerlin, B.S. *Polym. Chem.* **2012**, *3*, 1758.
(60) Ledin, P. A., Kolishetti, N., Boons, G,-J. *Macromolecules* **2013**, *46*, 7759.
(61) Allaoua, I., Goi, B. E., Obadia, M. M., Debuigne, A., Detrembleur, C., Drokenmuller, E. *Polym. Chem.* **2014**, *5*, 2973.
(62) Tumelty, D., Carnevali, M., Miranda, L.P. *J. Am. Chem. Soc.* **2003**, *125*, 14238.
(63) Dhal, P. K., Polomoscanik, S. C., Gianolio, D. A., Starremans, P. G., Busch, M., Alving, K., Chen, B. Miller, R. J. *Bioconjugate Chem.* **2013**, *24*, 865.
(64) Heredia, K. L., Tolstyka, Z. P., Maynard, H. D. *Macromolecules* **2007**, *40*, 4772.
(65) Carmona, S., Jorgensen, M. R., Kolli, S., Crowther, C., Salazar, F. H., Marion, P. L., Fujino, M., Natori, Y., Thanou, M., Arbuthnot, P., Miller, A. D. *Mol. Pharm.* **2008**, *6*, 706.
(66) Edupuganti, O. P., Renaudet, O., Defrancq, E., Dumy, P. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2839.
(67) Zhou, W.-J., Wilson, M. E., Kurth, M. J., Hsieh, Y. -L., Krochta, J. M., Shoemaker, C. F. *Macromolecules* **1997**, *30*, 7063.
(68) Novoa-Carballal, R., Muller, A. H. E. *Chem. Commun.* **2012**, *48*, 3781.
(69) Schatz, C., Lecommandoux, S. *Macromol. Rapid Commun.* **2010**, *31*, 1664.
(70) Li, R. C., Broyer, R.M., Maynard, H.D. *J. Poly. Sci. Part A Polym. Chem.* **2006**, *44*, 5004.

(71) Esser-Kahn, A. P., Trang, V., Francis, M.B. *J. Am. Chem. Soc.* **2010**, *132*, 13264.
(72) Metz, N., Theato, P. *Eur. Poly. J.* **2007**, *43*, 1202.
(73) Kalow, J. A., Swager, T.M. *Macro. Lett.* **2015**, *4*, 1229.
(74) Jiang, Y., Chen, J., Deng, C., Suuronen, E. J. Zhong, Z. *Biomaterials* **2014**, *35*, 4969.
(75) McKinnon, D. D., Domaille, D. W., Cha, J. N., Anseth, K. S. *Adv. Mater.* **2014**, *26*, 865.
(76) Ma, Y., -H., Yang, J., Li, B., Jiang, Y., -W., Lu, X., Chen, Z. **2016**.
(77) Grover, G. N., Lan, J., Nguyen, T.H., Segura, T. Maynard, H.D. *Biomacromol.* **2012**, *13*, 3013.
(78) Boehnke, N., Can , C., Bat, E., Segura, T., Maynard, H.D. *Biomacromol.* **2015**, *16*, 2102.
(79) Grover, G. N., Braden, R.L., Christman, K.L. *Adv. Mater.* **2013**, *25*, 2937.
(80) Grover, G. N., Garcia, J., Nguyen, M.M., Zanotelli, M., Madani, M.M., Christman, K.L. *Adv. Healthcare Mater.* **2015**, *4*, 1327.
(81) Ghosh, S., Cabral, J.D., Hanton, L.R., Moratti, S.C. *Acta Biomater.* **2016**, *29*, 206.
(82) Lin, F., Yu, J., Tang, W., Zheng, J., Defante, A., Guo, K., Wesdemiotis, C., Becker, M. L. *Biomacromol.* **2013**, *14*, 3749.
(83) Mukherjee, S., Bapat, A.P. Hill, M.R. Sumerlin, B.S. *Soft Matter* **2015**, *11*, 6152.
(84) DeForest, C. A., Tirrell, D.A. *Nat. Mater.* **2015**, *14*, 523.
(85) Nie, Z., Kumacheva, E. *Nat. Mater.* **2008**, *7*, 277.
(86) Pauloehrl, T., Delaittre, G., Bruns, M., Meibler, M., Borner, H.G., Bastmeyer, M., Berner-Kowollik, C. *Angew. Chem. Int. Ed.* **2012**, *51*, 9181.
(87) Park, S. P., Yousaf, M.N. *Langmuir* **2008**, *24*, 6201.
(88) Christman, K. L., Broyer, R. M., Tolstyka, Z. P., Maynard, H. D. *J. Mater. Chem.* **2007**, *17*, 2021.
(89) Christman, K. L., Maynard, H.D. *Langmuir* **2005**, *21*, 8389.
(90) Pauloehrl, T., Welle, A., Oehlenschlaeher, K. K., Barner-Kowollik, C. *Chem. Sci.* **2013**, *4*, 3503.
(91) Glassner, M., Oehlenschlaeger, K. K., Welle, A., Bruns, M., Barner-Kowollik, C. *Chem. Commun.* **2013**, *49*, 633.
(92) Broyer, R. M., Schopf, E., Kolodziej, C.M., Chen, Y., Maynard, H.D. *Soft Matter* **2011**, *7*, 9972.
(93) Park, S., Yousaf, M.N. *Langmuir* **2008**, *24*, 6201.
(94) Barrett, D. G., Lamb, B.M., Yousaf, M.N. *Langmuir* **2008**, *24*, 9861.
(95) Lamb, B. M., Park, S., Yousaf, M.N. *Langmuir* **2010**, *26*, 12817.
(96) Rodriguez, O. C., Schaefer, A. W., Mandato, C. A., Forscher, P., Bement, W. M., Warerman-Storer, C. M. *Nat. Cell Biol.* **2003**, *5*, 599.
(97) Waterman-Strorer, C., Salmon, E. D. *Curr. Biol.* **1997**, *7*, 369.
(98) Jordan, M. A. W., L. *Nat. Rev. Cancer* **2004**, *4*, 253.
(99) Cash, J. J., Kubo, T., Bapat, A. P., Sumerlin, B. S. *Macromolecules* **2015**, *48*, 2098.
(100) Han, L.-H., Lai, J. H., Yu, S., Yang, F. *Biomaterials* **2013**, *34*, 4251.
(101) Liu, B., Chen, H., Li, X., Zhao, Y., Liu, Y., Zhu, L., Deng, H., Li, J., Guo, F., Zhu, X. *RCS Adv.* **2014**, *4*, 48943.
(102) Jin, Y., Song, L., Su, Y., Zhu, L., Pang, Y., Qiu, F., Tong, G., Yan, D, Zhu, B., Zhu, X. *Biomacromol.* **2011**, *12*, 3460.
(103) Kumpfer, J. R., Jin, J., Rowan, S. J. *J. Mater. Chem.* **2010**, *20*, 145.
(104) Kumpfer, J. R., Rowan, S. J. *J. Am. Chem. Soc.* **2011**, *133*, 12866.
(105) Roy, N., Bruchmann, B., Lehn, J,-M. *Chem. Soc. Rev.* **2015**, *44*, 3786.
(106) Jin, Y., Yu, C., Denman, R. J., Zhang, W. *Chem. Soc. Rev.* **2013**, *42*, 6634.
(107) Koehler, K., Sandstrom, W., Cordes, E.H. *J. Am. Chem. Soc.* **1964**, *86*, 2413.
(108) do Amaral, L., Sandstrom, W.A., Cordes, E.H. *J. Am. Chem. Soc.* **1966**, *88*, 2225.
(109) Anderson, B. M., Jencks, W.P. *J. Am. Chem. Soc.* **1960**, *82*, 1773.

(110) Polyakov, V. A., Nelen, M.I., Nazarpack-Kandlousy, N., Ryabov, A.D., Eliseev, A.V. *J. Phys. Org. Chem.* **1999**, *12*, 357.

(111) Ruff, Y., Lehn, J.-M. *Biopolymers* **2007**, *89*, 486.

(112) Shimaoka, H., Kuramoto, H., Furukawa, J., Miura, Y., Kurogochi, M., Kita, Y., Hinou, H., Shinohara, Y., Nishimura, S. *Chem. Eur. J.* **2007**, *13*, 1664.

(113) Jackson, A. W., Fulton, D. A. *Macromolecules* **2010**, *43*, 1069.

(114) Mukherjee, S., Bapat, A.P. Hill, M.R. Sumerlin, B.S. *Polym. Chem.* **2014**, *5*, 6923.

(115) Park, S., Westcott, N.P., Luo, W., Dutta, D., Yousaf, M.N. *Bioconjugate Chem.* **2014**, *24*, 543.

(116) Mukherjee, S., Brooks, W. L. A., Dai, Y., Sumerlin, B. S. *Polym. Chem.* **2016**, *7*, 1971.

(117) Uribe-Romo, F. J., Hunt, J.R., Furukawa, H., Klock, C., O'Keeffe, M., Yaghi, O.M. *J. Am. Chem. Soc.* **2009**, *131*, 4570.

(118) Ding, S.-Y., Gao, J., Zhang, Y., Song, W.-G., Sy, C.-Y., Wang, W. *J. Am. Chem. Soc.* **2011**, *133*, 19816.

(119) Wu, Y., Xu, H., Chen, X., Gao, J., Jiang, D. *Chem. Commun.* **2015**, *51*, 10096.

(120) Huang, N., Chen, X., Krishna, R., Jiang, D. *Agnew. Chem. Int. Ed.* **2015**, *54*, 2986.

(121) Jin, Y., Zhu, Y., Zhang, W *Cryst. Eng. Comm.* **2013**, *15*, 1484.

(122) Mastalerz, M. *Angew. Chem. Int. Ed.* **2010**, *49*, 5042.

(123) Gao, Q., Bai, L., Zeng, Y., Wang, P., Zhang, X., Zou, R., Zhao, Y *Chem. Eur. J.* **2015**, *21*, 16818.

(124) Dai, W., Shao, F., Szczerbinski, J., McCaffrey, R., Zenobi, R., Jin, Y., Schluter, A.D., Zhang, W. *Agnew. Chem. Int. Ed.* **2015**, *54*, 1.

(125) Yu, Z., Tantakitti, F., Yu, T., Palmer, L.C., Schatz, G.C., Stupp, S.I. *Science* **2016**, *351*, 497.

(126) Uribe-Romo, F. J., Doonan, C. J., Furukawa, H., Oisaki, K., Yaghi, O. M. *J. Am. Chem. Soc.* **2011**, *133*, 11478.

(127) Xu, W. Z., Zhang, X., Kadla, J.F. *Biomacromol.* **2012**, *13*, 350.

(128) Muller, T., Brase, S. *Agnew. Chem. Int. Ed.* **2011**, *50*, 11844.

(129) Xu, H., Gao, J., Jiang, D. *Nature Chemistry* **2015**, *7*, 905.

(130) Ramdas, M. R., Kumar, K. S. S., Nair, C. P. R. *Mater. Lett.* **2016**, *172*, 216.

(131) Giri, N., Del Pópolo, M. G., Melaugh, G., Greenaway, R. L., Rätzke, K., Koschine, T., Pison, L., Gomes, M. F. C., Cooper, A. I., James, S. L. *Nature* **2015**, *527*, 216.

(132) Lehn, J.-M. *Chem. Soc. Rev.* **2007**, *36*, 151.

(133) Sadownik, J. W., Ulijn, R. V. *Curr. Opin. Biotech.* **2010**, *21*, 401.

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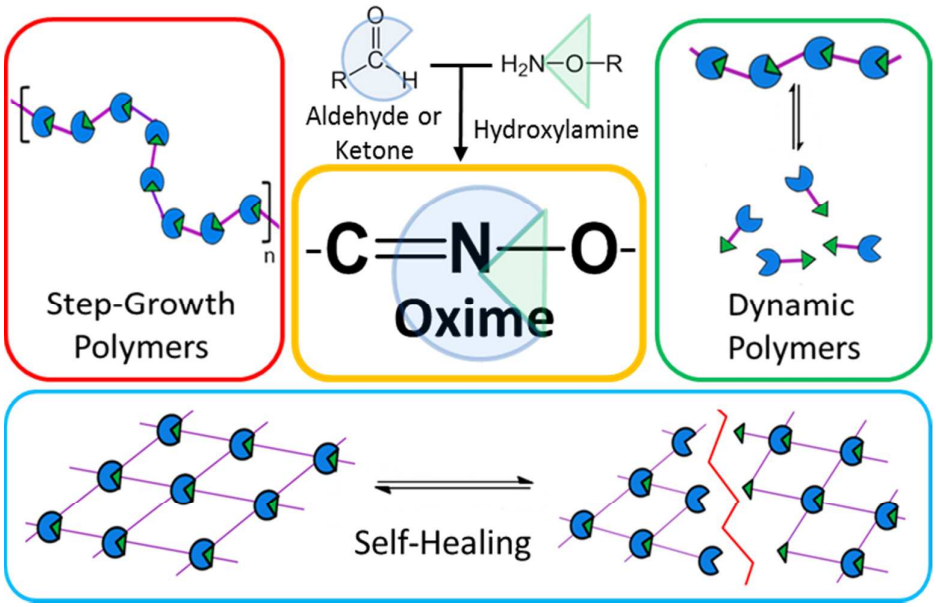
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