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High definition polyphosphoesters: between nucleic acids and plastics

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Polyphosphoesters are common to both genetics and cutting-edge polymer science. This review seeks to reframe current conceptions of the boundaries of nucleic acid and polymer chemistry, showing that vital 'stepping stones' are now in place, allowing us to make a journey through chemical space between DNA and the synthetic polyphosphoesters. These liminal classes of macromolecule address vital questions about sequence control in polymers, single polymer chain folding, programmed self-assembly, nanoscale photophysics, and chemical data storage. In taking this path, we will impinge upon biochemistry, medicine, photophysics, supramolecular chemistry, nanotechnology, information technology and materials science. The synthetic methods we already have in hand have only just begun to show their promise in all these fields.

Introduction

One of the most iconic classes of molecules in science, the nucleic acids which support all of known life, consists of a specific type of polyphosphoester. Although nucleobase pairing often receives the limelight, the role of the negatively charged phosphodiester groups is critical to the success of the system.[1] Compartmentalisation is fundamental to biological operation, and because of their polyanionic nature nucleic acids are unable to transverse lipid membranes, satisfying the need for conservation of genetic information. The negative charges also stabilise the double helix, providing a hydrophilic exterior with a well-defined structure to shield the nucleobase stack and facilitate accurate hydrogen bonding recognition where competition by water could be expected to remove hydrogen bond array discrimination. Polyphosphodiesters also provide the ideal platform for storing and editing information: the negative charge repels nucleophiles, providing long-term stability, but this can be altered dramatically by enzymatic stabilisation of intermediates, meaning that cleavage can occur only when needed, and at the right position. Phosphate linkages can also be made in a straightforward manner by enzymes using phosphate anhydrides (like adenosine triphosphate) which are also relatively stable in solution, but again readily reacted in enzymatic sites. Similarly, the regular charge on the backbone helps single stranded nucleic acids to adopt open conformations which sustains their availability to act as templates in replication, transcription, or translation reactions.[2] Polyphosphoesters are also appear in a vastly different field – as an increasingly important class of synthetic polymers.[3] They are currently produced industrially, primarily as flame retardants, and usually as additives in other materials.[4]

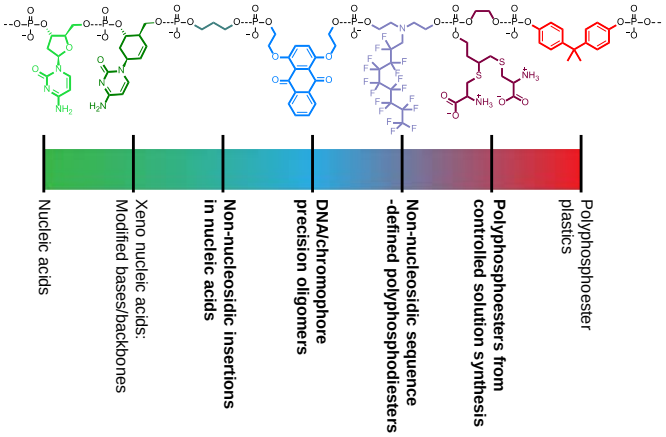

Figure 1. The path in chemical space from nucleic acids to plastics, represented by a hypothetical molecule displaying monomers discussed herein. We will focus our discussion on the 'stepping stones' highlighted in bold.

Phosphorous-rich materials can act as flame retardants either through trapping reactive radicals (in the same way as halogenated flame retardants, about which there are now health and environmental concerns[5]), or by promoting the formation of protective char layers.[6] Polyphosphoesters can also accelerate biodegradability of commodity plastics such as polylactic acid.[7] The poor mechanical properties of early polyphosphoester plastics such as poly(bisphenol A-phosphate)[8] limited their use in bulk, although they have been explored, for example, as solid polyelectrolytes.[9] Contemporary structural engineering of the systems is now leading to improved physical properties.[10] Polyphosphoesters are impressively versatile from a chemical perspective because the pentavalent nature of phosphorous means that they can be functionalised both on the main chain, and on the side chain, in the form of phosphotriesters, phosphonates, phosphites, or phosphoramidates. Variation of main-chain interphosphate groups is also widely tolerated, as will be discussed in this review.

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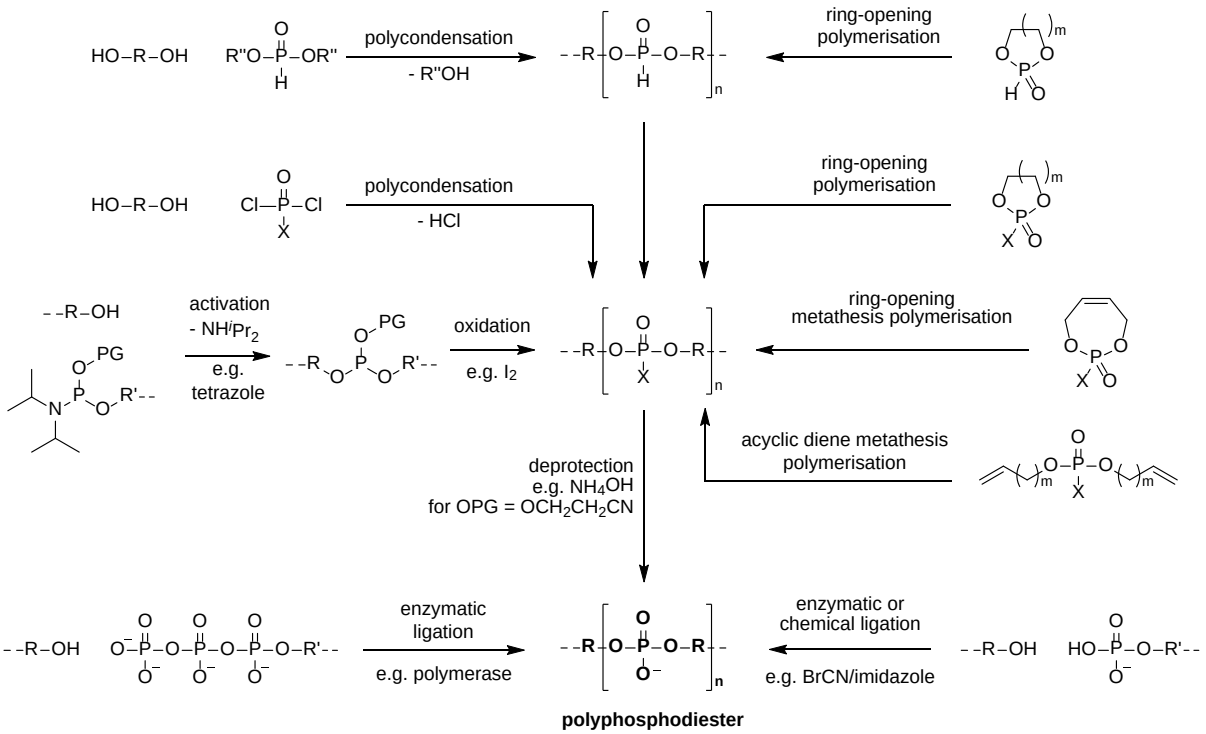

Scheme 1. Synthesis of phosphodiester-linked polymers. Poly(H-phosphonates) have X = H, polyphosphotriesters have X = O-alkyl or O-aryl, polyphosphonates have X = alkyl or aryl, and polyphosphoramidates have X = NR_2. PG = protecting group. m = 1,2.

So far, we have described two very distinct fields which share a common functional group, but are otherwise disconnected. In fact these areas even diverge when it comes to the benefit of the phosphate group itself – in molecular biology its stability is a great virtue, whereas in plastics it is the relative lability of phosphoesters which enables new applications. Nonetheless, as global research trends become increasingly interdisciplinary, and individual scientists move across fields within their careers, there is an increasing awareness of the commonalities between different types of polymer.[11] There is emerging area of chemical space in between these two extremes of nucleic acids and plastics – that of non-natural precision phosphoesters (Fig. 1). Departing from the biological terminus, we first meet 'xeno nucleic acids' (XNAs) which consist of nucleic acids which have been modified at the nucleobase, sugar, and/or phosphate in order to impart some altered biological activity.[12] Classical examples of this are the use of phosphorothioate linkages or 2'-methoxy groups which greatly improve the biostability of therapeutic antisense or RNAi oligonucleotides. In XNA each monomer has an underlying similarly to a classical nucleoside. The next step on this journey is the insertion of units between phosphates in an oligonucleotide which are entirely non-nucleosidic; monomers like this are often used to introduce some kind of structural change not otherwise possible in standard helical DNA. Moving along, we meet DNA-chromophore arrays; these are systems which use many non-nucleosidic insertions which are of interest in themselves, due to their interaction with light. After these, we come across non-nucleosidic sequence-defined polymers which have been created by multiple consecutive non-natural monomers and show behaviours related to anthropogenic polymers. Our penultimate stop is synthetic polyphosphoesters made using solution-phase synthesis, but nevertheless showing high levels of precision with respect to dispersity, sequence, and functionality. Finally, we reach the terminus of plastics – polyphosphoesters with higher dispersity, prepared by uncontrolled polycondensations.

Synthetic routes to phosphodiester-linked polymers are extremely rich (Scheme 1), with phosphorous centred reactions ranging from polycondensations which produce disperse step-growth polymers, to ring-opening of cyclic phosphoesters which can produce multiblock copolymers with low dispersity, to the phosphoramidite technique usually employed on solid supports to produce molecularly uniform polymers, and enzymatic ligation reactions using triphosphates. Catenation is also possible using away from the phosphorous centre, using alkene metathesis reactions. As well as synthetic and structural considerations, cutting-edge research into synthetic polyphosphoesters makes full use of their biodegradability to non-toxic products. However, this spectrum of polymer structures has been primarily enabled by the solid-phase phosphoramidite synthesis method. Although solid phase peptide synthesis is more well-known and has been rewarded with a Nobel Prize for Bruce Merrifield, Robert Letsinger, who conceived the same process independently at the same time,[13] led the chemical synthesis of oligonucleotides[14] which has arguably made a much greater impact. With a yield of >99.5 % per step, the use of phosphoramidite monomers later pioneered by Marvin Caruthers[15–17] enables the routine creation of precisely sequence-defined polymers with a DP of 100, compared with ~30 for peptide synthesis. The automated phosphoramidite method supports the entirety of molecular

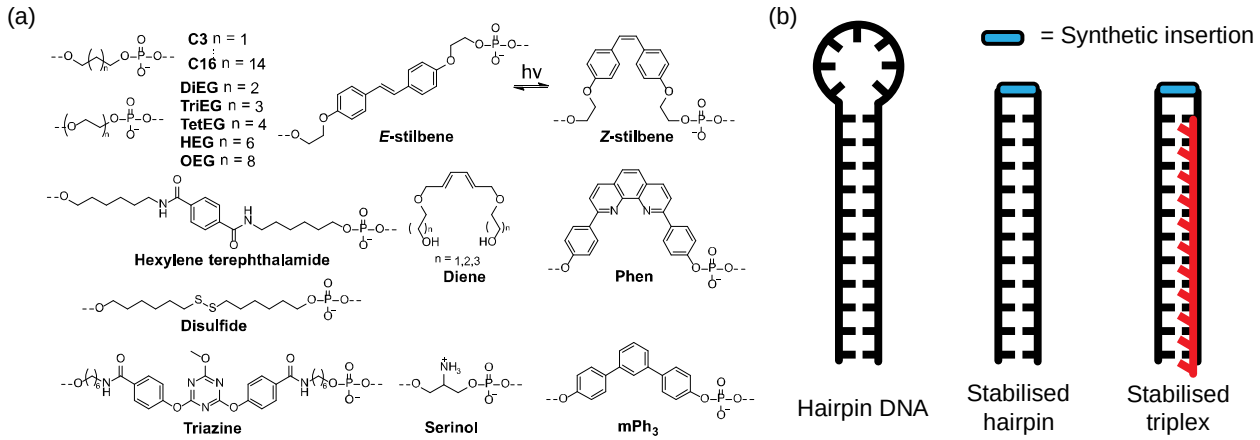

Figure 2. (a) Selected synthetic insertions and (b) their use in stabilising hairpin and triplex DNA.

biology, in which it serves as a source of oligonucleotides for two key processes – primers for DNA amplification reactions, and synthetic genes for expression of proteins – as well as many other applications. The global market for oligonucleotides was valued at $1.65 billion in 2016, and is expected to more than double by 2025.[18] Phosphoramidite reagents are produced in two steps from diols – first, dimethoxytrityl protection of one alcohol, followed by activation of the other using 2-cyanoethyl N,N-diisopropylchlorophosphoramidite. There is no reason why these diols should be limited to nucleosides; indeed, these bring their own challenges in terms of requirements for protecting groups and side reactions such as depurination. The phosphoramidite method is therefore an ideal platform for the creation of truly macromolecular non-nucleosidic polymers with perfect sequence control. The use of phosphates comes with further benefits because of the tools developed in biochemistry laboratories – solutions for purification, and analysis by gel electrophoresis, mass spectrometry, crystallography, microscopy, nuclear magnetic resonance (NMR), amongst other techniques, are available off-the-shelf.

This review will reframe current conceptions of the boundaries of nucleic acid and polymer chemistry, showing that vital 'missing links' are now in place between the astounding structure and function of DNA, and the synthetic polyphosphoesters which are emerging as advanced materials in the bulk. We will show that we can now refer to a continuous path within chemical space of macromolecules, and this perspective will create new opportunities for cross-pollination between previously separate fields in biology, nanoscience, materials, and polymer chemistry. In turn, this will lead to new insights into the creation, behaviour, and application of precision polymers in biology, chemistry and materials science. XNAs have been ably covered by other authors,[12] as have polyphosphoesters from a classical polymer chemistry perspective,[19] and we will strive not to repeat their discussions here.

Non-Nucleosidic Insertions in Nucleic Acids

A wide range of non-nucleosidic 'modifiers' are commercially available for incorporation into oligonucleotides. The vast majority of these are either for attachment of fluorescent probes, or to provide some functionality which will enable conjugation of the oligonucleotide to another species.[20] Many are limited to one terminus or the other. However, there are others which both provide a structural role, and can be placed anywhere within the strand – typically referred to as 'spacers'. As such, they represent an embryonic form of non-nucleosidic polyphosphodiesters (Fig. 2a). Surprisingly, although these materials have been on the market for many years, we could not locate any peer-reviewed overviews of their uses or properties – their use is only summarised on the websites of suppliers. We will accordingly supply an outline of their application here.

The most frequent use of spacers is to break the usual helicity of a DNA strand, and permit greater conformational flexibility. This was initially investigated as far back as 1987 in the creation of stabilised DNA hairpin structures (Fig. 2b).[21] Hairpin loops are a common form of nucleic acid secondary structure that plays a key role in several process such as gene expression and DNA recombination and transposition.[22] In simple DNA, a loop of 4-6 bases (which remains unhybridized) is bent back on itself to make the hairpin. Spacers are designed to add space either within an oligo sequence or between the oligo. The motivation for replacing this with a single spacer unit was twofold: to reduce synthetic cost of multiple couplings, and minimise formation of competing structures. Multiple addition of different length spacers allow the precise length of the spacer arm to be controlled which is important in hairpin DNA structure formation.

Seela first introduced a 1,3-propanediol linker (**C3**) for this purpose, and showed that it allowed bending of DNA structure, with a preference for hairpins. Noticing that the ideal distance to be bridged across the top of a hairpin is 19 – 21 Å, Maurizot proposed the hexaethylene glycol (**HEG**) unit, although it increased the melting point of the hairpin by just 3 °C relative to a tetrathymidine loop.[23] Replacement of the same loop by dodecane-diol (**C12**) was later found to stabilise hairpins by up to 8 °C.[24] Since then, there have been various studies have tried to establish what the 'best' spacer for hairpin structures is, using either oligo ethylene glycols from tetraethylene glycol (**TetEG**) to octaethylene glycol (**OEG**),[25] or hydrocarbon chains (**C8** to **C16**).[26] These studies concluded that seven is the optimal number of ethylene glycol units, and 14 the ideal number of

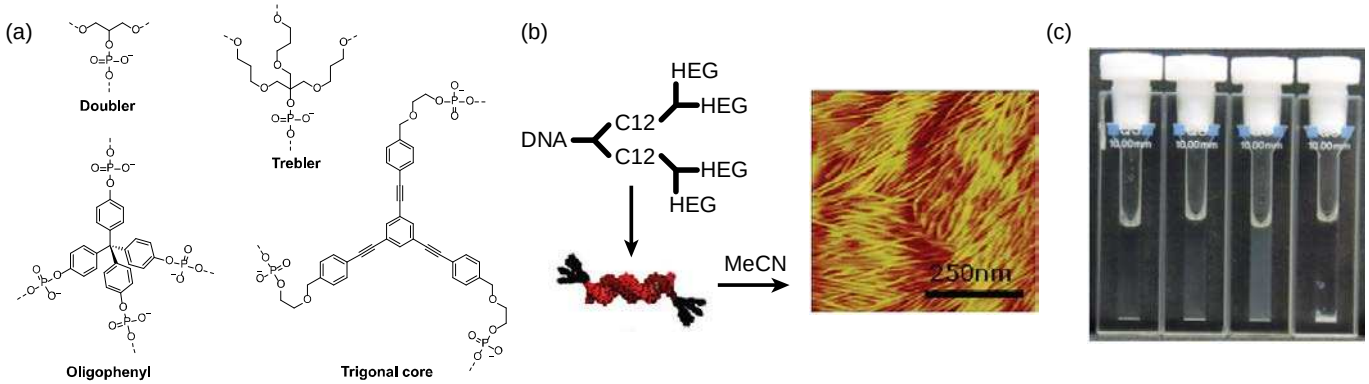

Figure 3. (a) Dendrimeric synthetic DNA insertions. (b) Use of the doubler unit to create an amphiphilic module which self-assembles in acetonitrile. Adapted with permission from J. Am. Chem. Soc., 2010, 132, 679–685. Copyright 2010 American Chemical Society. (c) Solid DNA materials made through hybridisation of arms extending from oligophenyl cores. Adapted with permission from Angew. Chemie Int. Ed., 2011, 50, 3227–3231. Copyright 2011 John Wiley and Sons.

carbon atoms; while hydrocarbons were generally more stabilising ostensibly because of favourable hydrophobic coverage of the base-pair stack by the chain. For general use however, it appears that the commercially available **HEG** and **C12** will only be incrementally less effective for hairpin formation. The results for RNA systems are similar.[27] More functional spacers for hairpins have been designed based upon unsaturated systems. The photoswitchability of stilbene has been exploited by Lewis to create a system in which the melting temperature of the hairpin can be controlled photochemically,[28] while it can also be used to induce photodimerisation across a double helix,[29] and as a tool to measure charge transfer through DNA duplexes.[30,31] Häner has used a linear diene to generate hairpins capable of participating in Diels-Alder reactions, permitting conjugation of heterospecies to the nucleic acids via maleimides.[32] Alternatively, if placed at both ends of a hairpin, spacers furnish a way to make compact cyclic DNAs. This was first performed using 2 x **C3** units to create 'sausage DNA' which showed a T_m 40 °C higher than the uncapped version.[33] **HEG** has been used to link two T-rich sequences into an elongated cyclic structure which then bound a third strand, raising T_m of the triplex by 16 °C. **HEG** also shows benefits for linear, folded, triplex structures, giving an 18 °C increase in T_m.[34] Using a hexylene-terephthalamide linker (which is hydrophobic) works on similar systems with a yet greater T_m rise of 42 °C,[35] whereas a triazine system gives +26 °C, and a disulphide +30 °C relative to the unlinked analogue.[36] A monomer based on 18β-glycyrrhetinic acid (a sterol from licorice extracts) has been used as a main-chain insertion, and has used to stabilise hairpin loops.[37] Similarly, using lithocholic acid, a more flexible main-chain sterol can be used as a linker. In this case cell permeability was also increased, as well as hairpin stability and capacity to form triplex DNA.[38] **C6**, meta-terphenylene (**mPh3**), and phenanthroline (phen) spacers have been compared with tetrathymidine loops in the formation of cyclic dimers, with the aromatic insertions being found to be generally the most stabilising, but each specific case was found to depend upon the 5'/3' connectivity of the dimer.[39]

Beyond structural effects, spacers can also influence enzymatic reactions. From the earliest reports, it was noted that use of synthetic insertions greatly increased resistance of the strands to nucleases.[21,33] By replacing a non-functional 8mer single stranded region in an aptamer with $\mathbf{HEG_2}$, the affinity of the aptamer for its target (live cancer cells) was not reduced, but its half-life in the presence of various exo and endonucleases was increased from three to eight hours.[40] As well as blocking destructive enzymes, non-nucleosidic insertions also prevent the action of polymerases. A double insertion of **TriEG** spacers has been shown to act as a terminator for the polymerase chain reaction, meaning that a lengthener DNA strand can be separately added to one of the primers without being replicated into the synthesised complement – this provides a useful way to distinguish between sense and antisense strands.[41] Similarly, the attachment of C3 at the 3' end of a qPCR probe strand prevents that oligonucleotide operating as a primer.[42]

Other interesting phenomena arise from discontinuities in DNA strands introduced by spacers. This is directly illustrated in the insertion of one or two C3 monomers within a duplex reducing the T_m by up 16 °C, by breaking the prevalent cooperativity of hybridisation.[43] These discontinuities have been exploited to create 'tethered oligonucleotide probes' which can identify RNA complexes by hybridisation to two non-contiguous regions of that RNA, using multiple **C3** or single oligoethylene glycol spacers to provide flexibility.[44] In a similar vein, attachment of two different thrombin-binding aptamers to provide cooperative binding was achieved using various additions of **HEG** to separate them – 8 being optimal – effectively providing polymer-linked DNAs.[45] Dimers of aptamers for human neutrophil elastase (HNE) were also produced with fluorophores for use in microfluidic analyses.[46]

Spacers are also used to optimise the behaviour of immobilised DNA molecules. Hybridisation efficiency of polymer-bound DNA has been explored – using stable (**C3**, **diEG**, **triEG**) or cleavable (sulfonyldiethanol, which undergoes beta-elimination under basic conditions) neutral linkers, it was found that 8-10 spacer additions were optimal, regardless of the identity of the spacer, suggesting that charge density must be considered alongside distance. In contrast, using serinol monomers which display cationic ammonium side chains, optimal binding was achieved with just 3-4 additions.[47] However, another study which varied number of **triEG** linkers used found that they made little difference.[48] Attachment of a thrombin aptamer to a gold surface was achieved by a DTPA (diethylenetriaminepentaacetic

acid)-DTPA-T-T-**triEG** linker; this prevented non-specific adsorption of proteins onto the surface, and permitted electrochemical sensing of thrombin.[49] Double **HEG** additions have been used to provide room for oligonucleotides on polystyrene beads which were in a combinatorial fashion for the identification of growth modulatory genes.[50]

The **C3** linker has also been used to create a version of alanine scanning[51] for functional RNAs. By creating a set of strands in which each of the nucleosides are individually replaced by **C3**, it is possible to identify which monomers are critical to enzymatic activity of catalytic RNAs.[52]

Spacers also play an important part in DNA nanotechnology. In an early example, **mPh3** units were used to create corners in DNA polygons[53] which could then be used to assemble nanotubes by stacking them up.[54] A similar system can be obtained using the **C6** linker,[55] and **HEG** is routinely used as

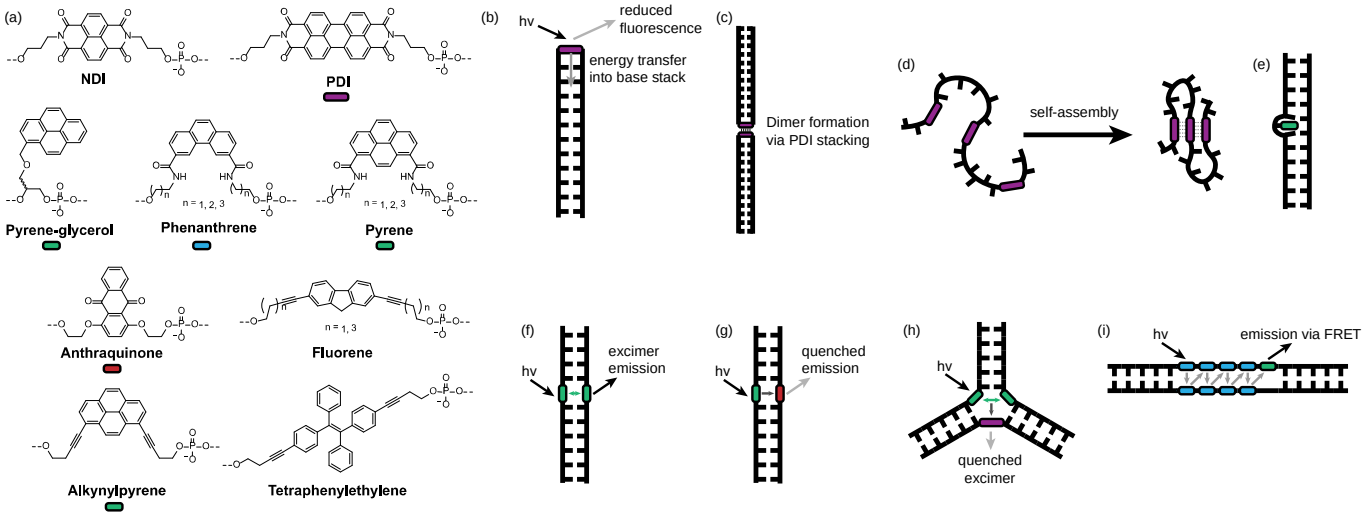

Figure 4. (a) Selected chromophores inserted into DNA backbones. (b) Reduced fluorescence emission from PDI when placed at cap of hairpin. (c) Supramolecular association of PDI-capped DNA hairpins. (d) Folding of non-hybridising DNA strand driven by PDI association. (e) Twisted intercalating nucleic acid (TINA) with pyrene insertion. (f) Pyrene excimer emission scaffolded by DNA. (g) Fluorophore-quencher pair scaffolded by DNA. (h). Triply interacting chromophores scaffolded at a DNA 3-way junction. (i) Absorption of light and energy transfer through a chromophore stack within a DNA strand.

insertion in creation of prisms by the 'clip' method.[56]

A different set of structural possibilities have been introduced by insertions which add a branching point to the DNA chain (Fig. 3). Dendrimers of first and second generation have been made using multiple additions of '**trebler**' phosphoramidites, capped with **HEG**. These nucleotides were then used as enhanced-sensitivity DNA probes when enzymatically labelled with ^{32}P at the termini of arms, and as PCR primers which protected the strand on which they were present from nuclease degradation and thus give single strands, as well as simply providing useful gel mobility mobility shifts.[57] Dendrimeric structures have been used to organise mannose units (x3 or x4) at end of DNA, for receptor-mediated uptake of antisense oligonucleotides (ASOs).[58] A two-way splitter was used to create 5'-5' linked hairpin RNAs capable of improved gene silencing and nuclease resistance.[59] On the nanotechnology side, Sleiman has used a combination of branching and spacer units to create highly unusual structures. By attaching an organic-soluble dendron comprised of splitters and four **HEG** chains to a DNA strand, a macroamphiphile was created.[60] Addition of organic solvent (MeCN) to an aqueous solution then resulted in formation of fibres of many micrometres in length. Experiments confirmed that ds-DNA structure was maintained and suggested that the structure involved end-to-end stacking of the units. By replacing the **HEG** chains with **C12** chains (with or without **C6** spacers), they were able to produce DNA-amphiphiles which self-assembled in water, to give micelles.[61] Because of the programmable self-assembly of DNA, these dendrimers showed very unusual properties. For example, by patterning the dendritic amphiphiles onto DNA cubes, it was possible to observe unusual hydrophobic-patch mediated dimerisation, as well as cube-encapsulated micelles. The latter were able to encapsulate therapeutic molecules and release them through DNA strand displacement reactions. Further elaboration of the system allowed release of therapeutics within cells.[62] The dendrons can also be used to bind human serum albumin, and thus associate it with 3D DNA structures. This can be used to stabilise those structures, or antisense or siRNA strands in biological media, and the increase gene silencing efficacy.[63] The assembly of dendritic DNA structures can apply in biosensing of pathogens and the generation of novel pads of DNA hydrogel biomaterials which can be used as a templates for cell free protein synthesis.[120] DNA linked to an extended aromatic trigonal dendritic assembly in aqueous media has been studied by Schatz and Nguyen to understand the importance of hydrophobic interaction and they have designed cage and face to face assemblies from these complimentary three way branched DNA-hybrids.[64]

To generate symmetrical dendritic DNA material Richert's group has synthesised branched DNA using **oligophenol** cores to form structures with tetrahedral and pseudo-octahedral geometry.[65] Number of arms has a strong effect on assembly properties and oligonucleotides hybrids with four, six and eight branched with pseudo-octahedral core were synthesised using H-phosphonates of corresponding protected dinuecleosides, showing the capacity to form porous networks.[66] Through

hybridisation of the terminal short nucleotides, these branched DNA-hybrids form crystals at 95 °C in aqueous solution.[67]

Many metal ions control essential biological processes of living cells the presence of metal ions and the chemical interactions between ions and specific binding sites on DNA can strongly affects the function and the stability of DNA. Insertion of spacers which act as transition metal binding units within DNA strands can also be used to introduce new structural elements. This topic has been reviewed,[68,69] and we refer readers to those articles.

These spacer units will continue to serve a valuable role in various DNA technologies for many years to come, but the ease with which these insertions have been placed within the chain raises more possibilities in the organisation of functional units – this has been ably explored in the creation of DNA/chromophore hybrids.

DNA/Chromophore Precision Oligomers

The use of aromatic and conjugated insertions in DNA to introduce function is a natural next step because the structural parameters of the DNA duplex are well known, and we have the synthetic capability to make insertions at well-defined positions within this structure. This is significant because important photophysical systems such as photosynthesis rely on the precise nanoscale positioning of chromophores, resulting in optimal Forster Resonant Energy Transfer (FRET) processes. DNA scaffolds provide an ideal framework to probe and mimic these processes.[70] Naturally, all the systems constructed employ solid-phase phosphoramidite chemistry (frequently automated) to produce the materials. For example, Kool and co-workers have extended their work on oligonucleotides with non-natural base pairs to include systems where a fluorophore replaces the base to create DNA-polychromophore systems[71] in which the interactions between adjacent chromophores results in unusual absorption/emission properties. DNA-polychromophores are capable of acting as specific sensors for analytes such as pollutants.[72] In DNA-chromophore studies, the photophysics are of more interest than the type of behaviour seen by DNA in nature, resulting in exotic architectures such as a fullerene attached to one end of a poly(dA), hybridised to entirely fluorophore (pyrene and/or nile red)-labelled poly(dU).[73]

Of greater interest in terms of traversing the chemical space between DNA and polymers is the insertion of totally non-nucleosidic chromophore monomers (Fig. 4a),[74] since these systems have pointed the way towards oligomers based upon multiple consecutive non-nucleosidic components.

Some early work in this area mirrored what we have already seen with synthetic insertions placed at the heads of hairpins – for example, when naphthalenediimide (**NDI**) or perylenediimide (**PDI**) unit replaces a T_5 unit at the head of a hairpin forming part of a triplex, the T_m of the triplex was increased by up to 20 °C. Moreover, because of π-stacking between the diimide units and the DNA bases, the binding events could be detected via a reduction in fluorescence emission (Fig. 4b).[75] Supramolecular properties were also uncovered - Lewis found that placing **PDI** at the bridge resulted in head-to-head dimers mediated by hydrophobic aggregation of the exposed surfaces (Fig. 4c).[76] Indeed, by making cyclic dimers with PDI at the ends, polymers consisting of 10-30 DNA units were seen.[77] Similarly, it has been shown that DNA three-way junctions capped by **PDI** self-associate into networks.[78] The tendency of many organic fluorophores to aggregate in water (due to their primarily hydrophobic and extensive π-surface) can be used to create nanostructures which report their structure spectroscopically. Li has produced DNA strands containing **PDI** insertions which will spontaneously stack in water, producing a core surrounded by unstructured single stranded DNA, with folding reported through its absorption spectrum (Fig. 4d).[79] Because hydrophobic collapse is involved, this structure is increasingly stable as the temperature is increased, in contrast to the usual behaviour of DNA. By building hairpin sequences into the DNA strand, they were able to produce a system which stable at both high and low temperatures, unfolding only upon hybridisation with a fully complementary DNA strand. This model was taken further, creating chains of up to 57 monomers with up to 12 dye insertions, using three different phenylene-ethylene-based chromophores (blue, red, and green). These are proposed as sensors of DNA hybridisation, which would rigidify the nucleotide chains and reduce FRET. The propensity of fluorophores to encourage folding has been quantified in single molecule mechanical stretching studies.[80]

A different path in this direction was taken by Pedersen who began by using a **pyrene-glycerol** insertion to introduce a fluorophore within a DNA duplex, resulting in intercalation of the large aromatic system within the duplex, along with some unwinding and destabilisation (Fig. 4e).[81] Insertion of a methylidine hydantoin spacer resulted in an intercalating monomer which doubled its fluorescence upon formation of triplex DNA.[82] Various other phenyl-aryl systems were then assessed for duplex and triplex stability, resulting in oligos known as twisted intercalating nucleic acids (TINAs).[83] These have been used to stabilise G-quadruplexes and inhibit their degradation by telomerase.[84] This has been used to knockdown transcription of the *KRAS* oncogene by producing a stable quadruplex which mimics a *KRAS* promotor – an effect observed all the way up to mouse models.[85] Insertion of anthraquinone into a thrombin-binding aptamer, which has a G-quadruplex structure, yields a more potent anticoagulant through enhanced thrombin binding.[86] Naphthalimide systems can also stabilise i-motifs.[87]

Häner also performed important foundational work in this area by substituting a single base in a duplex with an aromatic unit (**phenanthrene**) building block – this was destabilising when placed opposite a canonical base in a duplex, but stabilising when opposite itself.[88] Structure-based photophysical effects became clear when moving to a **pyrene** system; with careful choice of linker length, a pyrene dimer across the duplex forms, giving excimer emission and no reduction in T_m (Fig. 4f).[89] Observation of fluorophores interacting quickly led to investigation of a wide array of different chromophoric unit interacting across the DNA duplex. For example, the

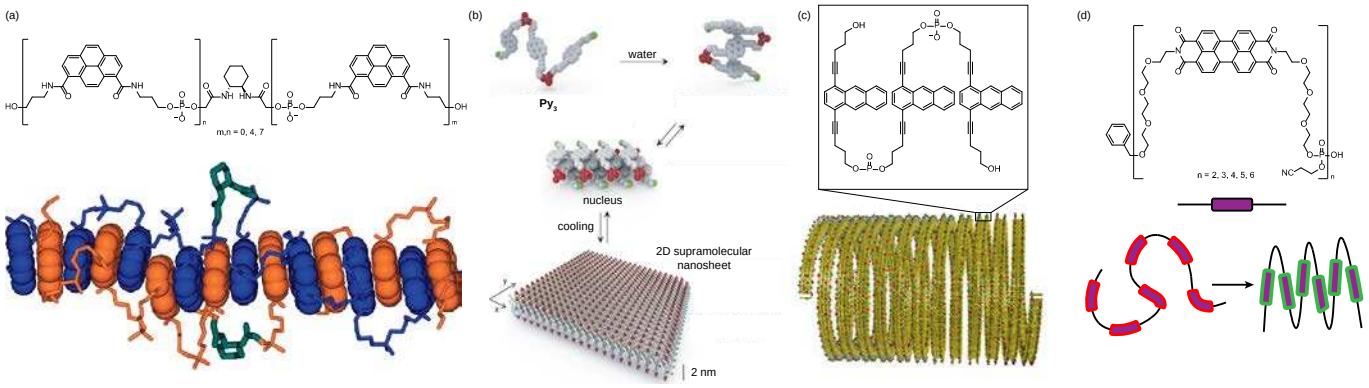

Figure 5. (a) Chiral duplexes from oligopyrenotides. Adapted with permission from J. Am. Chem. Soc., 2010, 132, 7466–7471. Copyright American Chemical Society 2010. (b) Self-assembled sheets arising from a pyrene oligophosphate. Adapted with permission from Angew. Chemie, 2013, 125, 11702–11707. Copyright John Wiley and Sons 2013. (c) Formation of helical tubes which can be internally photopolymerised using an anthracene oliophosphate. Adapted from Chem. Commun., 2016, 52, 14396–14399 – Published by the Royal Society of Chemistry. (d) Change of colour in PDI oligophosphates with respect to folding state.

anthraquinones, which act as fluorence quenchers, can block pyrene fluorescence when placed opposite that insertion (Fig. 4g).[90] Stacking of the fluorophore π surface against those of the DNA bases was analysed and confirmed crystallographically.[91] Among the various chromophore inserted, those which showed distinct photophysical properties arising from their position in the DNA duplex were of the greatest interest. For example, incorporation of **fluorene** units gave opposite CD signals depending on the length of the spacer.[92] Most importantly, the use of DNA as a scaffold really started to emerge – collecting a known number and arrangement of **alkynylpyrenes** and **PDI** units in the centre of a DNA 3-way junction enabled efficient energy transfer, and a controllable range of spectroscopic properties including monomer, excimer emission and quenching (Fig. 4h).[93] Similar results could be obtained instead using a DNA triplex scaffold.[94] Insertion of **tetraphenyleneethylene** units at opposite positions in a duplex causes aggregation induced emission (AIE) upon hybridisation due to rotational restriction.[95] Conversely, porphyrins quench each other when incorporated in groups of up to four.[96] This extension of the non-nucleosidic portion, still scaffolded by DNA has been taken further – a series of up to seven consecutive **pyrene** phosphodiester units has been constructed, with DNA tails at one or both ends. Upon hybridisation, the oligopyrenyl tracts (now up to 14 per assembly) are brought together in a highly chiral environment, resulting in a strong circular dichroic signal emanating from the pyrene units.[97] A scaffolded series of up to four **phenanthrenes** followed by a single **pyrene**, was produced in the centre of a DNA duplex, and was found to act as a light harvesting antenna – energy was transferred down the **phenanthrene** stack to the **pyrene**, which fluoresced (Fig. 4i).[98] This process turns out to be affected by the last base pair before the chromophore stack – CG quenches, whereas AT permits fluorescence.[99] In duplexes containing up to 18 **pyrenes** and a single terminal Cy5 fluorophore, the energy was now transmitted from the **pyrenes** to the Cy5, giving a strong FRET emission.[100] Further structural definition and diversity of chromophores can be obtained by integrating these systems into three-way DNA junctions.[101] Sequence-defined heterochromophore stacks built upon DNA duplexes has been created using **pyrene** and **NDI** monomers, and unusual electronic coupling of non-adjacent chromophores of the same type gave co-existent H-aggregates of each dye.[102]

We are still a long way from approaching the elegance and efficiency of nature in converting light energy into chemical energy. However, the precise structural parameters and versatile chemical synthesis of DNA have enabled the construction of uniquely well-defined systems in which we can probe photochemical processes. The next stages in this process must be the conversion of conversion of photons into chemical processes by incorporating species such as photocatalysts at the receiving end of the energy transfer chain.

Sequence defined non-nucleosidic polymers for emerging functionality

In nature, the polymers such as proteins and nucleic acids are active agents, functional, finely detailed, monodisperse, and act largely as unitary or well-defined oligomeric species. Their primary structure displays specific sequences of monomers which precisely determine the 3D structures, self-assembly properties, and functions of the polymers. In contrast, synthetic polymers are simple, inherently disperse, rarely dynamic, and are usually put to use as large, disperse aggregates or in the bulk. However, each level control of sequence attained in synthetic polymers (alternation, diblock, and multiblock) can have dramatic effects upon microscopic (folding, self-assembly, chemical reactivity) and macroscopic (solubility, rheology, conductivity, phase transitions, biodegradability) properties. Accordingly, the synthesis of truly sequence defined polymers has been a major chemical goal for a number of years,[103] since it could provide access to all the functions of proteins and DNA. The phosphoramidite method is a unique platform to achieve this aim; accordingly, a number of groups are re-purposing DNA synthesisers to produce non-nucleosidic polymers with full molecular definition. The polymers, with the focus now on the non-nucleosidic portion, show sequence-programmed folding, self-assembly, and data storage capacities related to those of proteins and DNA.

This is a story which begins by taking ever-increasing number of chromophore insertions to its logical conclusion – the

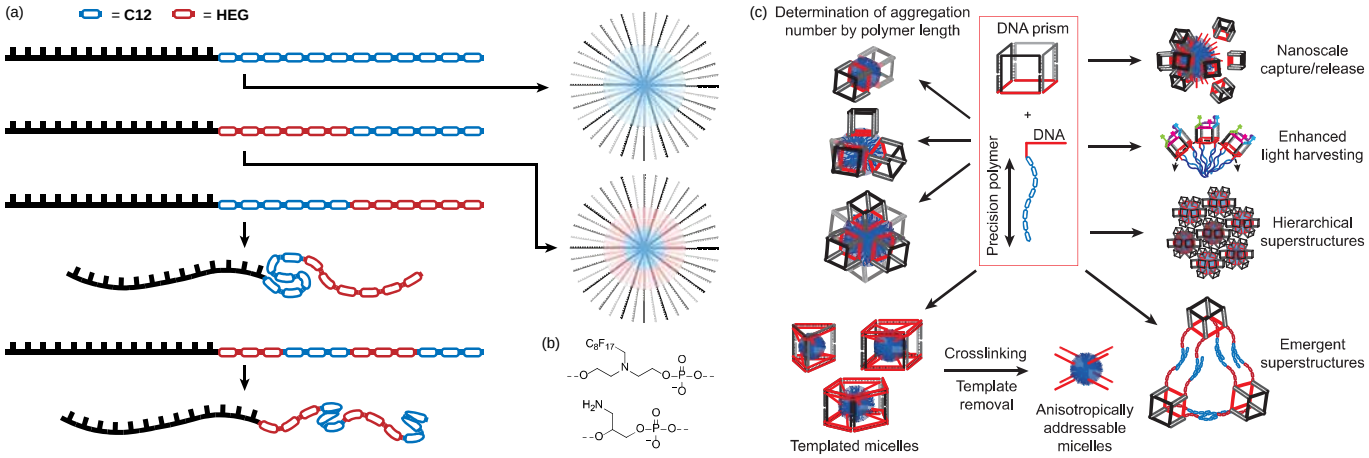

Figure 6. Self-assembling sequence-defined polymers attached to DNA. (a) Micellisation/single chain folding determined by hydrophobic patterning. (b) Fluorinated and cross-linking monomers. (c) New self-assembly phenomena arising from combining sequence-defined polymers with DNA nanotechnology. Adapted with permission from J. Am. Chem. Soc., 2014, 136, 15767–15774. Copyright 2014 American Chemical Society.

minimisation of the DNA component. Häner moved from DNA-scaffolded polychromophores to oligopyrenotides – oligomers built from pyrene monomers extending from one or both sides of chiral 1,2-diaminocyclohexane units (up to 7 each side), realised through phosphoramidite chemistry (Fig. 5a). These systems showed large scale chirality, as manifested through circular dichroism, and thorough modelling and comparison with similar systems, it is believed that they formed chiral double helices similar to those seen in DNA-inserted oligomers.[104] Inspired by the intriguing self-assembly of these precision oligomers, a new research direction examining the programmed creation of structures was developed. In simple homo-septamers, chiral amplification occurred, with a cytidine-terminated version being added at just 10% resulted in fully chiral assemblies.[105] Atomic force microscopy revealed that nanosheets comprising of many oligomers were the product and that their mechanism of formation depended upon the presence of chiral inducer.[106] Molecular dynamics simulations showed that oligopyrenotide folded structures have hydrophobic π-stacked cores and phosphate exteriors[107] - oligomers fold and then make 2 nm high lamellae to conceal the hydrophobic pyrenes from the external water (Fig. 5b).[108] Such lamellae can reach multi-micron size if temperature control is applied,[109] and chirality can be introduced simply by changing the direction of stirring in the mixture.[110] The system can produce more diverse nanostructures: tubes and sheets co-exist when using a linear pyrene monomer – these changes are helpfully accompanied by significant differences in absorbance spectra.[111] In fact, the self-assembly capacities are rich and due to the synthesis process, the resultant structures are readily endowed with function. For example, helical nanotubes can be made from an trimer of anthracene monomer, which can then be photodimerised to make polymers (Fig. 5c).[112] If using a benzene-cored tripodal DNA system, with anthracenes in the middle, a honeycomb network is obtained, which can be covalently fixed by irradiation, and then collapses and opens according to solvent conditions.[113] A **phenanthrene** trimer gives tubes, through which energy transfer to pyrene dopants is active.[114] Helical ribbons can be formed if single-stranded DNA is attached to a **pyrene** septamer – the pyrenes collapse and multiple oligomers stack; the DNA protrudes from the side, giving a supramolecular brush copolymer.[115] Adding the complements to the DNA gives networks-of-ribbons, presumed to occur through blunt-end stacking.[116] Interface with other nanomaterials is also possible: gold nanoparticles with complementary DNA attached can hybridise to these ribbons,[117] while silica mineralisation can also be achieved on oligopyrenotide nanostructures[118] and cationic porphyrins can bind to them as 'groove binders.'[119]

Li and coworkers have also extended their DNA scaffolded chromophores to non-nucleosidic systems which combine self-assembly with unusual optical properties. **PDI** units appended with tetraethylene glycol spacers were stitched together using solution-phase phosphoramidite coupling to give up to hexaphosphotriesters (cyanoethyl groups on the non-chain oxygen providing organic solubility). When more than one **PDI** was present, folding could be detected in dilute organic solutions (< 1 mM) by NMR and optical absorbance. At higher concentration, self-assembly was seen.[120] Interestingly, the step-wise events in chain folding could be monitored by single molecule UV-visible absorbance spectroscopy, manifesting as dramatic spectral shifts from green (unfolded) to red (folded) (Fig. 5d).[121] Matching spectra to modelling indicates that a well-defined stack of **PDIs** was formed, with 3.5 Å spacing and 30° rotation between adjacent units.[122] Solid-phase synthesis was also used to produce dendritic oligophosphodiesters using a combination of modified **PDI** and **trebler** phosphoramidites. The products showed excellent water solubility due to the phosphate charges, and exceptional brightness – providing a far better probe than the ubiquitous fluorescein for single molecule studies.[123]

An adjoining area of chemical space had been reached by polymer scientists, bringing with them a different set of ideas about the types and applications of sequence-defined polyphosphoesters produced by the phosphoramidite method. Sleiman has a longstanding interest in the confluence of polymer self-assembly and DNA nanotechnology.[124,125] However, conjugation of DNA to polymers is challenging,[126] due to the difficulties of ensuring two chain-ends meet, as well as solvent incompatibilities. As an alternative, Sleiman and myself

conceived a new approach – instead of trying to conjugate two macromolecules, a polymer could be built in-line with solid phase phosphoramidite synthesis through multiple couplings of spacers such as **HEG** and **C12** (the latter here envisioned as hexaethylene to highlight the polymer analogy).[127] In this way, polymers conceived as oligomers-of-hexamers were attached to DNA strands in high yield (Fig. 6a). The products turned out to be far superior to conventional DNA-polymer conjugates in that they were assembled with perfect length and sequence definition, while the phosphates provided solubility. The molecularly uniform DNA-polymer (up to 12 non-DNA units) products were readily purified by HPLC and identified by ESI-MS. Polymer precision was found to be important in self-assembly. Micellisation only occurred with more than six **C12** units, and then were found to produce remarkably uniform micelles. Furthermore, it was found that different patterns of hydrophobic **C12** and hydrophilic **HEG** monomers within the polymer chain resulted in different physical properties. For example, the retention time on reverse-phase HPLC, which relates to the overall hydrophobicity, decreased as block sizes were increased, despite all the samples being sequence isomers. These changes were reflected in the self-assembly – (**C12**)$_6$(**HEG**)$_6$DNA gave micelles, but (**HEG**)$_6$(**C12**)$_6$DNA did not; yet both provided a similar hydrophobic volume for uptake of Nile Red, a fluorescent reporter dye. This indicated that in the latter case, single chain folding occurred to create many smaller hydrophobic microphases. This sequence-specific encoding of self-assembly vs single chain folding was previously unknown in synthetic systems. DNA hybridisation remained orthogonal to these processes. Sleiman has made extensive use of these DNA-precision polymer conjugates. For example, DNA-**C12**$_{12}$ micelles were found to encapsulate BKM120, an anticancer drug. The polymer-modified DNA was shown to resist nuclease degradation, and drugs were delivered to human primary cells with reduced inflammatory side effects.[128] The systems provide benefits for therapeutic oligonucleotides too – attaching a luciferase ASO to **C12**$_{12}$ produced micelles which were more easily transfected into cells compared to the unmodified ASO, meaning that less of the toxic polyethylene imine transfection agent could be used for the same degree of gene knockdown.[129] Chemical as well as biological processes can be improved using this system – by hybridising a second oligonucleotide with a reactive amine end to DNA-**C12**$_{12}$ micelles, such that its amine is contained within the hydrophobic microenvironment, it was possible to greatly increase the yield of conjugation to NHS-activated carboxylic acids, providing a useful tool for combatting solvent incompatibility in bioconjugation reactions.[130] Elaboration of the monomers available has seen use of a fluorinated side chain unit (Fig. 6b) which can either produce micelles (up to 10 coupled) or placed in the middle of duplexes to increase stability, enable ^{19}F NMR detection, improve nuclease stability, and increase gene silencing efficacy.[131] Hybridisation of the systems to DNA cubes (Fig. 6c) has produced previously unseen effects due to the interplay of the DNA and polymer self-assembly regimes. For example, by altering the chain length of **C12**$_x$ polymers attached to one side of a cube, a quantisation of aggregation number effect was

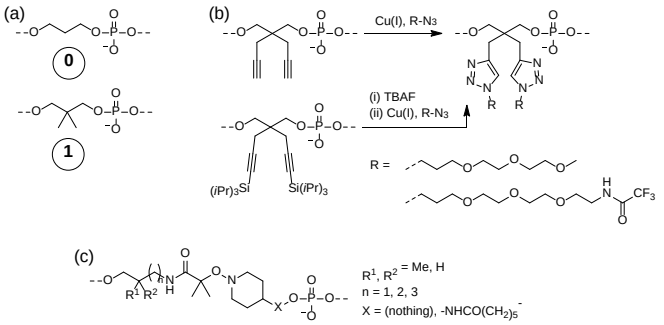

Figure 7. Monomers used for data storage in sequence-defined polymers. (a) Binary 0 and 1 monomers. (b) Alkyne monomers for post-functionalisation, with sequence specificity introduced using protecting groups. (c) Phosphate-alkoxyamine polymers optimised for MS sequencing.

observed, with the balance of dimers, trimers, and tetramers of cubes (and hence 8mer, 12mers, and 16mers of polymer).[56] Longer polymers produced micelles-of-cubes which were highly uniform, and could be reversibly assembled and disassembled from separate micelles and prisms (triangular, square, pentagonal) using strand displacement reactions. Using the single stranded regions on the exterior of the prisms, they could be linked up to form multi-micrometre aggregates, or used as scaffolds for enhanced light harvesting. Exploration of the effects of polymer sequence produced yet more interesting structures such as micelle-in-cube and exotic multimeric ring aggregates.[132] Impressively, by persuading the precision polymers to aggregate within the cavity of a DNA cube, they could be crosslinked through inserted amine-functionalised monomers.[133] After removal of the cube by denaturation, the crosslinked micelle remained, retaining the DNA components used in its templation – effectively producing the first example of a monodisperse, anisotropically addressable polymer micelle. The remaining strands could then be used to create programmed multimicellar superstructures. In my own laboratory we are diversifying the monomer set with a focus on folding of the polymers themselves.

Given the power of the solid phase phosphoramidite method to produce very long polymers with sequence control, it is unsurprising that it has now caught the attention of the polymer synthesis community. In particular, Lutz has used the method in the creation of data-containing polymers – although we are have now taken a long journey in chemical space from DNA, we have returned to a very closely related purpose. In a first study, manual solid-phase reactions were conducted to produce polymers based upon propylene phosphate, with control over position of dimethyl side chains which acted as nominal tags to designate 0 and 1 in a binary sequence of up to 25 units (Fig. 7a). Size exclusion chromatography gave the polymers apparent PDIs as low as 1.01, but MS analysis confirmed what should be the result of the synthesis – molecularly uniform polymer samples.[134] Use of alkynyl side chains permitted post-synthetic functionalisation with oligoethylene glycol chains via CuAAC, with careful use of protecting groups allowing two types of side-chain to be appended in a sequence-specific manner (Fig. 7b).[135] Full automation was then exploited to produce a fully sequence-defined polymer based on the same 1s and 0s with 104 monomers, encoding the word 'Macromolecule' in ASCII

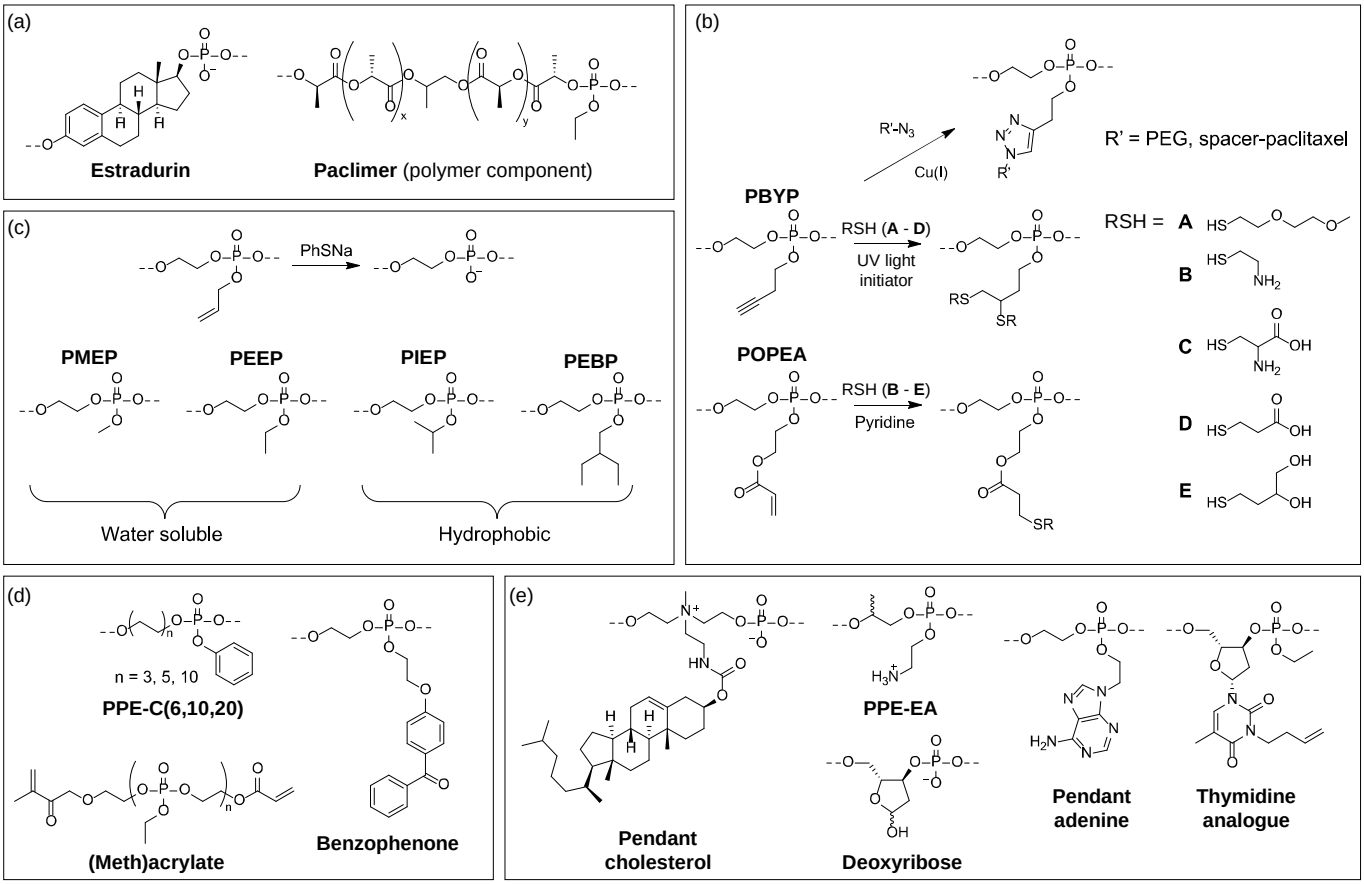

Figure 8. Monomers used in cutting edge solution-synthesised polyphosphoesters. (a) Existing use in pharamceuticals. (b) Functionalisation of alkyne and acrylate side chains in polyphosphotriesters. (c) Modulation of water solubility by changing phophotriester side chains. (d) Polymers used to create nanoparticles and films for drug delivery. (e) Monomers at the interface of synthetic polyphosphoesters and DNA.

format.[136] In the creation of polymers for data storage, read-out capacity is as important as ease of synthesis. However, fragment analysis by mass spectrometry, frequently the first port-of-call for sequencing of peptides, is challenging with polyphosphodiesters because of their symmetry and possibility of C-O or O-P cleavage, resulting in a complex mixture of fragments. Different approaches have been taken to improve this situation.[137] Poly(phosphate-alkoxyamine) systems (Fig. 7c) made through alternating phosphoramidite and radical-radical couplings, enabled this through the spontaneous homolytic cleavage at the alkoxyamine site under mild conditions, resulting in an easily interpreted using secondary ion mass spectrometry (MS^2). Up to six pairs of monomers were linked (ca. 4 kDa).[138] Higher levels of information could be stored and read by separating sequenced octaphosphodiester 'bytes' by alkoxyamines, with a nucleotide mass tag attached to each 'byte.' Under MS-MS conditions, the bytes were readily separated by homolytic bond cleavage, and identified by their mass tags. The sequences of the bytes could then be read by further fragmentation (MS^3) of the octaphosphodiesters, which is a tractable problem. Polymers of up to 8 bytes (i.e. 70 phosphodiester links) were made and analysed this way.[139] A different attractive method for reading the sequences of polyphosphodiesters is the use of nanopore technology which is now coming online for rapid and inexpensive DNA sequencing.[140] In this technique, the DNA molecule is driven through a small pore by electrophoresis. As it does so, each base (or series of bases) impedes the current going through the pore by a certain amount, and by single molecule measurements, these current blockades can be translated back into DNA sequences. However, this level of technology has taken a vast amount of work, and it translation to the world of non-natural polymers is non-trivial. Nonetheless, preliminary studies have been performed, and provide interesting results. Characteristic blockade dwell times were observed which were consistent with polymer-pore interaction rather than translocation. This was attributed to the high degree of flexibility in the polymers used (oligopropylenephosphate) and the lack of an adjunct protein which could direct the chain through the pore, as used in DNA systems.[141]

The considerations above document the boundaries of what has been achieved using the phosphoramidite to produce uniform chains of non-nucleosidic phosphoesters. However, it is clear that there is much more which can be achieved – automated synthesis is tolerant of a wide range of functional groups, and monomers are made simply in two steps from diols. So far sequence control has been used to achieve artificial light harvesting, programmed folding and self-assembly, and information storage, but there are many more functions of the biological sequence-defined polymers such as communication, sensing, catalysis, and mechanical control which await recapitulation using sequence polyphosphoesters.

Solution-synthesised polyphosphoesters with cutting-edge properties

Very similar nucleosidic and non-nucleosidic monomers (in their in-chain form, if not identical as reactants) to those seen in the preceding sections can also be found in polymers obtained by more traditional solution polymerisation techniques.

From a classical polymer perspective, polyphosphoesters have much potential because of the possibility of both main chain and side chain modification (Fig. 8). Accordingly, now that controlled polymerisation reactions are established, polyphosphoesters with precise architectures (albeit with some degree of dispersity, in contrast to the above examples) are now finding diverse applications in biology and materials.[142] Due to their biocompatibility and gradual biodegradation (1% of phosphate linkages are cleaved in 40 hr at pH 7.8 at 45 °C[143]), polyphosphoesters have already been used as ways to deliver drugs in a long lasting format (Fig. 8a). Polyestradiol phosphate (**Estradurin**) is long acting, water soluble formulation of estradiol which has been used to treat prostate cancer.[144] More recently, **Paclimer**, a copolymer of poly(lactic acid), poly(propylene oxide), and an ethyl phosphotriester in which paclitaxel is included noncovalently[145] has been tested to Phase 1 for recurrent ovarian cancer.[146]

Advances in recent years have made controlled polymerisation reactions much more accessible.[147] For example, organocatalytic ring-opening polymerisation (ROP) using readily available reagents is now able to give polyphosphoesters with molecular weights of nearly 70 kDA, and dispersities as low as 1.05.[148] In contrast to examples in previous sections, such reactions are commonly used to produce phosphotriester linkages. However, the pendant triester groups can also be used to introduce functionality useful for drug delivery (Fig. 8b). For example, poly(2-(but-3-yn-1-yloxy)-2-oxo-1,3,2-dioxaphospholane) (**PBYP**) produced by ROP has alkyne side chains which can be modified post-synthetically either once via copper catalysed azide-alkyne cycloaddition, or twice through sequential thiol-yne and thiol-ene reactions.[149] A near-contemporaneous report emerged of block-copolymer system produced by ROP ((poly(ε-caprolactone)-*block*-poly[2-(2-oxo-1,3,2-dioxaphospholoyloxy)ethylacrylate] (PCL-b-**POPEA**)) with acrylate side chains. These functional units could act as Michael acceptors to thiol nucleophiles, resulting in modular attachment of anionic, cationic, zwitterionic, or neutral hydrophilic moieties to the phosphotriester block.[150] The now-amphiphilic block copolymers self-assembled in water to give micelles which could be loaded with the anticancer drug doxorubicin (DOX) at up to ~30% efficiency. The drug was released more quickly in the presence of phosphodiesterase I (naturally present in some human cells), and while the micelles on their own were non-toxic at up to 7.5 μg mL^{-1}, those loaded with DOX were more toxic than the free drug at all concentrations tested. The same diversity of hydrophobic sides could also be introduced into block-copolymers based upon the alkyne side chains, producing biodegradable micelles.[151] These were crosslinked within their shell and loaded with paclitaxel and showed good anticancer activity in cell studied and in mice.[152] Ultra-high drug loading was achieved by esterification of polymer side chains with paclitaxel itself – up to 65 wt% of polymer, and raising the solubility of the drug in water by a multiple of 25,000.[153] Meanwhile, the DOX-loaded micelles based on acrylate side-chains have also been optimised for drug deliver through shell crosslinking and attachment of folic acid targeting groups.[154]

Careful control of side chains can be used to tune water solubility (Fig. 8c). Excellent hydrophilicity is obtained with phosphodiesters, and these can be produced from allyl triesters by treatment with sodium thiophenolate.[155] This circumnavigates the base-lability of the cyanoethyl group used in solid phase phosphoramidite synthesis, which could be problematic given that 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) is used at a catalytic component in polymerisation. Despite the lack of charge, short-side chained polyphosphotriesters such as poly(ethyl ethylene phosphate) (**PEEP**) are water soluble themselves, enabling them to act as hydrophilic components of block copolymers. DOX has been successfully loaded into degradable PCL-*b*-PEEP micelles.[156] Following established trends,[157] by careful variation of the ratio of methyl, ethyl, and isopropyl side chains (giving **PMEP**, **PEEP**, and **PIEP** respectively) the LCST of the polyphosphoester block could be precisely tuned, giving another method to control drug release.[158] The 2-ethylbutyl side chain (giving **PEBP**) has been used separately as a hydrophobic unit in micellar assembly.[151] (although strictly beyond the scope of this review, it should also be noted that polyphosphonate systems can also show programmable self-assembly[159] and physical properties.[160])

Other types of drug delivery are facilitated by the versatility of polyphosphoesters (Fig 8d). Phosphotriesters are uniquely sensitive to bacterial enzymes, and accordingly targeted release of antibiotics has been demonstrated using polyphosphoester nanoparticles containing vancomycin.[161] Phosphate groups have an affinity for inorganic calcium materials providing some unique opportunities. While PPEs can be used to template calcium carbonate particles,[162] their capacity to adhere to bone (which has calcium phosphate salts as a major component) could lead to new drug delivery modes. Polyphosphonates (synthesised by ADMET and ROMP with M_w of up to 120 kDa) have been formulated as aqueous nanoparticles and were observed to bind strongly to calcium phosphate.[160] ADMET-synthesised polyphosphotriesters with phenyl side chains and variable inter-phosphate lengths (**PPE-C(6,10,20)**, Fig. 8d) were produced, from which nanoscale encapsulation of up to 15% PTX were generated – these nanoparticles were effective at killing cells (compared to controls) and again adhered strongly to calcium phosphate.[163] Bulk polymer materials based upon precision polyphosphoesters include pH-responsive hydrogels for controlled release of small molecule[164] A and protein[165] drugs – gelling occurred through polymerisation of terminal **(meth)acrylate** end groups. Crosslinked films, prospectively as antifouling coatings for biological implants, have been produced by UV-crosslinking of **benzophenone** side chains.[166]

In a show of scientific circularity, polyphosphoesters made by conventional polymerisation methods can also be used to interact with DNA, the starting point of our discussion (Fig. 8e).

For example, polycondensation of a quaternary ammonium diol monomer displaying **pendant cholesterol**, with ethyl dichlorophosphate has given a polymer capable of binding DNA through its cationic nature, and interacting with cell membranes through the sterols. This acted a less toxic vector in gene delivery than the common lipofectamine, but efficacy in cell culture was also reduced somewhat.[167] An improved system employing a primary ammonium side chain (polyphosphoester-ethyl amine, **PPE-EA**) gave improved gene delivery through combining electrostatic complexation of DNA with accelerated degradation though intramolecular nucleophilic attack of the amine. This was shown to be effective in mice.[168]

Conventional polymerisation methods can also result in materials which mimic DNA.[169] Starting in the 1980s, Penczek and co-workers established a method to attach nucleobases in the pendant ester position in ROP-synthesised polyphosphotriesters via a stepwise progression from the poly(H-phosphonate) via the poly(chlorophosphate) to the poly(imidazoylphosphate) which was reacted with the alcohol of an ethoxy-modified nucleobase (give **pendant adenine**[170] or uracil[171]). Early work also provided methods for obtaining polyphosphodiesters with sugars such as **deoxyribose** within the chain, have also been produced through ring opening of cyclic phosphoramidites.[172] However, it is only much more recently that these aspects have been combined in the ROP demonstrated by Wooley to create modified polynucleotides.[173] **Thymidine** was N3-butenylated to provide both a protecting group, and a handle for future functionalisation, and then reacted with ethyl dichlorophosphate to produce a phosphotriester monomer. Interestingly, due the product being chiral at the P centre, only the R-stereoisomer was found to be polymerisable (using a DBU catalyst and 4-methoxybenzyl alcohol initiator). Dispersities below 1.10 were seen for polymers of up to 32 units. These are essentially ethyl triester analogous of polythymidine. Interestingly, although there is no detectable glass transition temperature for DNA, the phosphotriesters had T_g of 50-55 °C, and displayed circular dichroism spectra suggestive of base stacking within a rigid backbone.

Outlook

We have seen how what began as small modifications to DNA strands to aid structural investigations have progressed into use of the DNA double helix as a scaffold for holding together many non-natural insertions for other purposes, and then to loss of the DNA itself as the primary structural driver in sequence-defined polymers, arriving finally at the cutting edge of applied and fundamental polymer chemistry.

In each of the categories discussed there are specific advances which can be expected. Insertions into DNA backbones are becoming increasingly functional, and their integration into biological will lead to new ways to investigate processes such as protein expression, epigenetics, and improve the efficacy of nucleic acid therapeutics, whereas in DNA nanotechnology modifications permit integration with other entities such as inorganic nanostructures, as well as facilitate further self-assembly. Chromophore arrays are likely to provide ever deeper insights into how to achieve optimal use of light, while having the potential to create soft materials powered entirely by light. The self-assembly of sequence-defined polyphosphoesters is a rapidly growing field and more sophisticated structures and functions will doubtless arise, with data storage likely to emerge as the first application in which they are used. While solution-synthesised polyphosphoesters are rapidly developing, there is much to be done to raise the understanding of their properties to the same level as that of polyacrylates or polystyrenes; nonetheless, progress is rapid towards the full application of polyphosphoesters in drug delivery, and use in treating bone disorders is of particular interest. While the use of triesters is more advanced in this latter field, it provides an unexplored opportunity for polymer architecture variation in the other areas.

In the bigger picture, these considerations have shown that the stepping stones between polymer chemistry and biology are now in place: we can now trace an unbroken path in chemical space from the material of the genetic code to the materials of plastics. The synthesis of new, biologically-inspired polyners that reproduce some of the structures and activities of their natural counterparts can lead to useful insights into how biological systems function. Due to the versatility of available synthetic techniques, it is now in theory possible to create polymers anywhere in this continuum. In the future we can expect cross-talk between polymer and DNA communities to increase: can the monomers of non-natural polyphosphoester materials influence biology by being placed within otherwise native DNA strands? Can the hybridisation and biochemical manipulation techniques of DNA be used to make new polymer materials in bulk? The answers to these questions is almost certainly positive, yet there is much to be done. Areas of great promise are the export of DNA handling and analysis methods to polymer science, and the modification of the physical properties of DNA by tuning the polymer structure. The biggest challenge, in our view, is the choice of material to make for a certain application. Although we have described the journey from nucleic acids to plastics as a linear line in chemical space, the intervening territory is more of a multidimensional expanse. As the number of possible compounds is the number of available monomers raised to the power of the degree of polymerisation, the options become overwhelming. In this review, we have touched upon 96 different monomers; making 50mers of these using solid phase synthesis (a conservative estimate of reaction success) gives 96^{50} = ca. 10^{99} possibilities – a number larger than the number of elementary particles in the universe. Polymer scientists will need to adopt new methods to deal with the problem of sequence choice, but once they do, we can expect transformational new technologies which combine the best of the sequenced biopolymers with the materials properties of synthetic polymers.

Conflicts of interest

There are no conflicts to declare.

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Notes and references

1 F. Westheimer, *Science*, 1987, **235**, 1173–1178.
2 S. A. Benner and D. Hutter, *Bioorg. Chem.*, 2002, **30**, 62–80.
3 K. N. Bauer, H. T. Tee, M. M. Velencoso and F. R. Wurm, *Prog. Polym. Sci.*, 2017, 73, 61–122.
4 M. M. Velencoso, A. Battig, J. C. Markwart, B. Schartel and F. R. Wurm, *Angew. Chemie Int. Ed.*, , DOI:10.1002/anie.201711735.
5 S. Shaw, *Rev. Environ. Health*, , DOI:10.1515/REVEH.2010.25.4.261.
6 S. Y. Lu and I. Hamerton, *Prog. Polym. Sci.*, 2002, 27, 1661–1712.
7 J. Wen, G. J. . Kim and K. W. Leong, *J. Control. Release*, 2003, **92**, 39–48.
8 US72379534A, 1936.
9 Chaloner-Gil, US Patent, US5393621A, 1993.
10 F. Marsico, M. Wagner, K. Landfester and F. R. Wurm, *Macromolecules*, 2012, **45**, 8511–8518.
11 J.-F. Lutz, J.-M. Lehn, E. W. Meijer and K. Matyjaszewski, *Nat. Rev. Mater.*, 2016, **1**, 16024.
12 V. B. Pinheiro and P. Holliger, *Trends Biotechnol.*, 2014, **32**, 321–8.
13 M. H. Caruthers, *Proc. Natl. Acad. Sci.*, 2014, **111**, 18098–18099.
14 R. L. Letsinger and K. K. Ogilvie, *J. Am. Chem. Soc.*, 1969, **91**, 3350–3355.
15 M. H. Caruthers, *Science*, 1985, **230**, 281–285.
16 M. H. Caruthers, *Acc. Chem. Res.*, 1991, **24**, 278–284.
17 M. H. Caruthers, *J. Biol. Chem.*, 2013, **288**, 1420–1427.
18 Transparency Market Research, https://www.transparencymarketresearch.com/oligonucleotide-synthesis-market.html, (accessed 28 November 2017).
19 T. Steinbach and F. R. Wurm, *Angew. Chemie Int. Ed.*, 2015, **54**, 6098–6108.
20 E. Defrancq, Y. Singh and N. Spinelli, *Curr. Org. Chem.*, 2008, **12**, 263–290.
21 F. Seela and K. Kaiser, *Nucleic Acids Res.*, 1987, **15**, 3113–3129.
22 D. Bikard, C. Loot, Z. Baharoglu and D. Mazel, *Microbiol. Mol. Biol. Rev.*, 2010, **74**, 570–588.
23 M. Durand, K. Chevrie, M. Chassignol, N. T. Thuong and J. C. Maurizot, *Nucleic Acids Res.*, 1990, **18**, 6353–6359.
24 M. J. Doktycz, T. M. Paner and A. S. Benight, *Biopolymers*, 1993, **33**, 1765–1777.
25 W. Pils and R. Micura, *Nucleic Acids Res.*, 2000, **28**, 1859–63.
26 M. Hariharan, K. Siegmund and F. D. Lewis, *J. Org. Chem.*, 2010, **75**, 6236–6243.
27 M. Y.-X. Ma, L. S. Reid, S. C. Climie, W. C. Lin, R. Kuperman, M. Sumner-Smith and R. W. Barnett, *Biochemistry*, 1993, **32**, 1751–1758.
28 F. D. Lewis and X. Liu, *J. Am. Chem. Soc.*, 1999, 121, 11928–11929.
29 F. D. Lewis, T. Wu, E. L. Burch, D. M. Bassani, J. S. Yang, R. L. Letsinger, S. Schneider and W. Jäger, *J. Am. Chem. Soc.*, 1995, **117**, 8785–8792.
30 F. D. Lewis, T. Wu, Y. Zhang, R. L. Letsinger, S. R. Greenfield and M. R. Wasielewski, *Science*, 1997, **227**, 673–676.
31 F. D. Lewis, R. L. Letsinger and M. R. Wasielewski, *Acc. Chem. Res.*, 2001, **34**, 159–170.
32 R. Tona and R. Häner, *Chem. Commun.*, 2004, **0**, 1908–1909.
33 W. Bannwarth, A. Dorn, P. Iaiza and X. Pannekouke, *Helv. Chim. Acta*, 1994, **77**, 182–193.
34 M. Durand, S. Peloille, N. T. Thuong and J. C. Maurizot, *Biochemistry*, 1992, **31**, 9197–9204.
35 M. Salunkhe, T. Wu and R. L. Letsinger, *J . Am. Chem. SOC*, 1992, **114**, 8768–8772.
36 B. P. Kavitake, S. V. Patil and M. M. Salunkhe, *Tetrahedron*, 1997, **53**, 321–330.
37 E. K. Bang and B. H. Kim, *Tetrahedron Lett.*, 2009, **50**, 2545–2547.
38 S. J. Kim, E.-K. Bang, H. J. Kwon, J. S. Shim and B. H. Kim, *ChemBioChem*, 2004, **5**, 1517–1522.
39 A. A. Greschner, V. Toader and H. F. Sleiman, *J. Am. Chem. Soc.*, 2012, **134**, 14382–14389.
40 D. Shangguan, Z. Tang, P. Mallikaratchy, Z. Xiao and W. Tan, *ChemBioChem*, 2007, **8**, 603–606.
41 K. P. Williams and D. P. Bartel, *Nucleic Acids Res.*, 1995, **23**, 4220–4221.
42 Biosearch, Know Your Oligo Mod : 3' Spacer C3, http://blog.biosearchtech.com/know-your-oligo-mod-spacer-c3, (accessed 28 November 2017).
43 J. Becaud, I. Pompizi and C. J. Leumann, *J. Am. Chem. Soc.*, 2003, **125**, 15338–15342.
44 A. Schepartz and S. T. Cload, *J. Am. Chem. Soc.*, 1991, **113**, 6324–6326.
45 Y. Kim, Z. Cao and W. Tan, *Proc. Natl. Acad. Sci.*, 2008, **105**, 5664–5669.
46 K. A. Davis, B. Abrams, Y. Lin and S. D. Jayasena, *Nucleic Acids Res.*, 1996, **24**, 702–706.
47 M. Shchepinov, S. C. Case-Green and E. M. Southern, *Nucleic Acids Res.*, 1997, **25**, 1155–1161.
48 A. V Fotin, A. L. Drobyshev, D. Y. Proudnikov, A. N. Perov and A. D. Mirzabekov, *Nucleic Acids Res.*
49 N. Meini, C. Farre, C. Chaix, R. Kherrat, S. Dzyadevych and N. Jaffrezic-Renault, *Sensors Actuators, B Chem.*, 2012, **166–167**, 715–720.
50 M. J. Feldhaus, M. Lualhati, K. Cardon, B. Roth and A. Kamb, *Nucleic Acids Res.*, 2000, **28**, 534–543.
51 *Curr. Opin. Chem. Biol.*, 2001, **5**, 302–307.
52 K. Wawrzyniak-Turek and C. Höbartner, *Chem. Commun.*, 2014, **50**, 10937.
53 F. A. Aldaye and H. F. Sleiman, *Angew. Chemie Int. Ed.*, 2006, **45**, 2204–2209.
54 F. A. Aldaye, P. K. Lo, P. Karam, C. K. McLaughlin, G. Cosa

and H. F. Sleiman, *Nat Nano*, 2009, **4**, 349–352.
55 C. K. McLaughlin, G. D. Hamblin, F. A. Aldaye, H. Yang and H. F. Sleiman, *Chem. Commun.*, 2011, **47**, 8925.
56 C. J. Serpell, T. G. W. Edwardson, P. Chidchob, K. M. M. Carneiro and H. F. Sleiman, *J. Am. Chem. Soc.*, 2014, **136**, 15767–15774.
57 M. S. Shchepinov, I. A. Udalova, A. J. Bridgman and E. M. Southern, *Nucleic Acids Res.*, 1997, **25**, 4447–4454.
58 J. Katajisto, P. Virta and H. Lönnberg, *Bioconjug. Chem.*, 2004, **15**, 890–896.
59 H. Masuda, N. Watanabe, H. Naruoka, S. Nagata, K. Takagaki, T. Wada and J. Yano, *Bioorganic Med. Chem.*, 2010, **18**, 8277–8283.
60 K. M. M. Carneiro, F. A. Aldaye and H. F. Sleiman, *J. Am. Chem. Soc.*, 2010, **132**, 679–685.
61 T. G. W. Edwardson, K. M. M. Carneiro, C. K. McLaughlin, C. J. Serpell and H. F. Sleiman, *Nat. Chem.*, 2013, **5**, 868–875.
62 K. E. Bujold, J. Fakhoury, T. G. W. Edwardson, K. M. M. Carneiro, J. N. Briard, A. G. Godin, L. Amrein, G. D. Hamblin, L. C. Panasci, P. W. Wiseman and H. F. Sleiman, *Chem. Sci.*, 2014, **5**, 2449–2455.
63 A. Lacroix, T. G. W. Edwardson, M. A. Hancock, M. D. Dore and H. F. Sleiman, *J. Am. Chem. Soc.*, 2017, **139**, 7355–7362.
64 U. Feldkamp, B. Saccà and C. M. Niemeyer, *Angew. Chemie Int. Ed.*, 2009, **48**, 5996–6000.
65 H. Griesser, M. Tolev, A. Singh, T. Sabirov, C. Gerlach and C. Richert, *J. Org. Chem.*, 2012, **77**, 2703–2717.
66 A. Schwenger, C. Gerlach, H. Griesser and C. Richert, *J. Org. Chem.*, 2014, **79**, 11558–11566.
67 A. Singh, M. Tolev, M. Meng, K. Klenin, O. Plietzsch, C. I. Schilling, T. Muller, M. Nieger, S. Bräse, W. Wenzel and C. Richert, *Angew. Chemie - Int. Ed.*, 2011, **50**, 3227–3231.
68 H. Yang, K. L. Metera and H. F. Sleiman, *Coord. Chem. Rev.*, 2010, **254**, 2403–2415.
69 S. Ghosh and E. Defrancq, *Chem. - A Eur. J.*, 2010, **16**, 12780–12787.
70 T. J. Bandy, A. Brewer, J. R. Burns, G. Marth, T. Nguyen and E. Stulz, *Chem. Soc. Rev.*, 2011, **40**, 138–148.
71 Y. N. Teo and E. T. Kool, *Chem. Rev.*, 2012, **112**, 4221–4245.
72 H. Kwon, W. Jiang and E. T. Kool, *Chem. Sci.*, 2015, **6**, 2575–2583.
73 P. Ensslen, S. Gärtner, K. Glaser, A. Colsmann and H. A. Wagenknecht, *Angew. Chemie - Int. Ed.*, 2016, **55**, 1904–1908.
74 V. L. Malinovskii, D. Wenger and R. Häner, *Chem. Soc. Rev.*, 2010, **39**, 410–422.
75 S. Bevers, S. Schutte and L. W. McLaughlin, *J. Am. Chem. Soc.*, 2000, **122**, 5905–5915.
76 Y. Zheng, H. Long, G. C. Schatz and F. D. Lewis, *Chem. Commun.*, 2005, **0**, 4795.
77 P. P. Neelakandan, Z. Pan, M. Hariharan, Y. Zheng, H. Weissman, B. Rybtchinski and F. D. Lewis, *J. Am. Chem. Soc.*, 2010, **132**, 15808–15813.
78 F. Menacher, V. Stepanenko, F. Würthner and H.-A. Wagenknecht, *Chem. - A Eur. J.*, 2011, **17**, 6683–6688.
79 W. Wang, W. Wan, H. H. Zhou, S. Niu and A. D. Q. Li, *J. Am. Chem. Soc.*, 2003, **125**, 5248–5249.
80 J. S. Kim, Y. J. Jung, J. W. Park, A. D. Shaller, W. Wan and A. D. Q. Li, *Adv. Mater.*, 2009, **21**, 786–789.
81 C. B. Nielsen, M. Petersen, E. B. Pedersen, P. E. Hansen and U. B. Christensen, *Bioconjug. Chem.*, 2004, **15**, 260–269.
82 Y. L. Aly, M. Wamberg and E. B. Pedersen, *Helv. Chim. Acta*, 2005, **88**, 3137–3144.
83 V. V. Filichev, H. Gaber, T. R. Olsen, P. T. Jørgensen, C. H. Jessen and E. B. Pedersen, *European J. Org. Chem.*, 2006, **2006**, 3960–3968.
84 T. Agarwal, D. Pradhan, I. Géci, A. M. El-Madani, M. Petersen, E. B. Pedersen and S. Maiti, *Nucleic Acid Ther.*, 2012, **22**, 399–404.
85 S. Cogoi, S. Zorzet, V. Rapozzi, I. Géci, E. B. Pedersen and L. E. Xodo, *Nucleic Acids Res.*, 2013, **41**, 4049–64.
86 A. S. Gouda, M. S. Amine and E. B. Pedersen, *Helv. Chim. Acta*, 2016, **99**, 116–124.
87 A. A. El-Sayed, E. B. Pedersen and N. Y. Khaireldin, *Helv. Chim. Acta*, 2016, **99**, 14–19.
88 S. M. Langenegger and R. Häner, *Helv. Chim. Acta*, 2002, **85**, 3414–3421.
89 S. M. Langenegger and R. Häner, *Chem. Commun. (Camb).*, 2004, **0**, 2792–2793.
90 N. Bouquin, V. L. Malinovskii and R. Häner, *European J. Org. Chem.*, 2008, **2008**, 2213–2219.
91 M. Probst, W. Aeschimann, T. T. H. Chau, S. M. Langenegger, A. Stocker and R. Häner, *Nucleic Acids Res.*, 2016, **44**, 7079–7089.
92 D. Wenger, V. L. Malinovskii and R. Häner, *Chem. Commun.*, 2011, **47**, 3168.
93 M. Probst, D. Wenger, S. M. Biner and R. Haner, *Org. Biomol. Chem.*, 2012, **10**, 755–759.
94 S. M. Biner and R. Häner, *Chem. Biodivers.*, 2012, **9**, 2485–2493.
95 S. Li, S. M. Langenegger and R. Häner, *Chem. Commun.*, 2013, **49**, 5835.
96 M. Vybornyi, A. L. Nussbaumer, S. M. Langenegger and R. Häner, *Bioconjug. Chem.*, 2014, **25**, 1785–1793.
97 R. Häner, F. Samain and V. L. Malinovskii, *Chem. - A Eur. J.*, 2009, **15**, 5701–5708.
98 F. Garo and R. Häner, *Angew. Chemie Int. Ed.*, 2012, **51**, 916–919.
99 F. Garo and R. Häner, *Bioconjug. Chem.*, 2012, **23**, 2105–2113.
100 O. O. Adeyemi, V. L. Malinovskii, S. M. Biner, G. Calzaferri and R. Haner, *Chem. Commun.*, 2012, **48**, 9589–9591.
101 M. Probst, S. M. Langenegger and R. Haner, *Chem. Commun.*, 2014, **50**, 159–161.
102 C. B. Winiger, S. M. Langenegger, G. Calzaferri and R. Häner, *Angew. Chemie Int. Ed.*, 2015, **54**, 3643–3647.
103 J.-F. Lutz, M. Ouchi, D. R. Liu and M. Sawamoto, *Science*, 2013, **341**, 1238149–1238149.
104 R. Häner, F. Garo, D. Wenger and V. L. Malinovskii, *J. Am. Chem. Soc.*, 2010, **132**, 7466–7471.
105 A. L. Nussbaumer, D. Studer, V. L. Malinovskii and R. Häner, *Angew. Chemie Int. Ed.*, 2011, **50**, 5490–5494.
106 A. V Rudnev, V. L. Malinovskii, A. L. Nussbaumer, A.

Mishchenko, R. Häner and T. Wandlowski, *Macromolecules*, 2012, **45**, 5986–5992.

107 F. Simona, A. L. Nussbaumer, R. Häner and M. Cascella, *J. Phys. Chem. B*, 2013, **117**, 2576–2585.

108 M. Vybornyi, A. V. Rudnev, S. M. Langenegger, T. Wandlowski, G. Calzaferri and R. Häner, *Angew. Chemie*, 2013, **125**, 11702–11707.

109 M. Vybornyi, A. Rudnev and R. Häner, *Chem. Mater.*, 2015, **27**, 1426–1431.

110 N. Micali, M. Vybornyi, P. Mineo , O. Khorev, R. Häner and V. Villari, *Chem. - A Eur. J.*, 2015, **21**, 9505–9513.

111 M. Vybornyi, Y. Bur-Cecilio Hechevarria, M. Glauser, A. V. Rudnev and R. Häner, *Chem. Commun.*, 2015, **51**, 16191–16193.

112 H. Yu and R. Häner, *Chem. Commun.*, 2016, **52**, 14396–14399.

113 H. Yu, D. T. L. Alexander, U. Aschauer and R. Häner, *Angew. Chemie Int. Ed.*, 2017, **56**, 5040–5044.

114 C. D. Bösch, S. M. Langenegger and R. Häner, *Angew. Chemie Int. Ed.*, 2016, **55**, 9961–9964.

115 Y. Vyborna, M. Vybornyi, A. V. Rudnev and R. Häner, *Angew. Chemie Int. Ed.*, 2015, **54**, 7934–7938.

116 Y. Vyborna, M. Vybornyi and R. Häner, *J. Am. Chem. Soc.*, 2015, **137**, 14051–14054.

117 Y. Vyborna, M. Vybornyi and R. Häner, *Chem. Commun.*, 2017, **53**, 5179–5181.

118 M. Vybornyi, Y. Vyborna and R. Häner, *ChemistryOpen*, 2017, **6**, 488–491.

119 V. L. Malinovskii, A. L. Nussbaumer and R. Häner, *Angew. Chemie Int. Ed.*, 2012, **51**, 4905–4908.

120 W. Wang, L. S. Li, G. Helms, H. H. Zhou and A. D. Q. Li, *J. Am. Chem. Soc.*, 2003, **125**, 1120–1121.

121 J. J. Man, W. Wang and A. D. Q. Li, *J. Am. Chem. Soc.*, 2006, **128**, 672–673.

122 A. D. Shaller, W. Wang, A. Li, G. Moyna, J. J. Han, G. L. Helms and A. D. Q. Li, *Chem. – A Eur. J.*, 2011, **17**, 8350–8362.

123 A. D. Shaller, W. Wan, B. Zhao and A. D. Q. Li, *Chem. – A Eur. J.*, 2014, **20**, 12165–12171.

124 C. K. McLaughlin, G. D. Hamblin, K. D. Hänni, J. W. Conway, M. K. Nayak, K. M. M. Carneiro, H. S. Bazzi and H. F. Sleiman, *J. Am. Chem. Soc.*, 2012, **134**, 4280–4286.

125 K. M. M. Carneiro, G. D. Hamblin, K. D. Hanni, J. Fakhoury, M. K. Nayak, G. Rizis, C. K. McLaughlin, H. S. Bazzi and H. F. Sleiman, *Chem. Sci.*, 2012, **3**, 1980–1986.

126 T. R. Wilks and R. K. O'Reilly, *Sci. Rep.*, 2016, **6**, 39192.

127 T. G. W. Edwardson, K. M. M. Carneiro, C. J. Serpell and H. F. Sleiman, *Angew. Chemie - Int. Ed.*, 2014, **53**, 4567–4571.

128 D. Bousmail, L. Amrein, J. J. Fakhoury, H. H. Fakih, J. C. C. Hsu, L. Panasci and H. F. Sleiman, *Chem. Sci.*, 2017, **8**, 6218–6229.

129 J. J. Fakhoury, T. G. Edwardson, J. W. Conway, T. Trinh, F. Khan, M. Barlog, H. S. Bazzi and H. F. Sleiman, *Nanoscale*, 2015, **7**, 20625–20634.

130 T. Trinh, P. Chidchob, H. S. Bazzi and H. F. Sleiman, *Chem. Commun.*, 2016, **52**, 10914–10917.

131 D. de Rochambeau, M. Barłóg, T. G. W. Edwardson, J. J. Fakhoury, R. S. Stein, H. S. Bazzi and H. F. Sleiman, *Polym. Chem.*, 2016, **7**, 4998–5003.

132 P. Chidchob, T. G. W. Edwardson, C. J. Serpell and H. F. Sleiman, *J. Am. Chem. Soc.*, 2016, **138**, 4416–4425.

133 T. Trinh, C. Liao, V. Toader, M. Barłóg, H. S. Bazzi, J. Li and H. F. Sleiman, *Nat. Chem.*, 2017, **10**, 184–192.

134 A. Al Ouahabi, L. Charles and J.-F. Lutz, *J. Am. Chem. Soc.*, 2015, **137**, 5629–5635.

135 N. F. König, A. Al Ouahabi, S. Poyer, L. Charles and J.-F. Lutz, *Angew. Chemie Int. Ed.*, 2017, **56**, 7297–7301.

136 A. Al Ouahabi, M. Kotera, L. Charles and J.-F. Lutz, *ACS Macro Lett.*, 2015, **4**, 1077–1080.

137 J.-A. Amalian, A. Al Ouahabi, G. Cavallo, N. F. König, S. Poyer, J.-F. Lutz and L. Charles, *J. Mass Spectrom.*, 2017, **52**, 788–798.

138 G. Cavallo, A. Al Ouahabi, L. Oswald, L. Charles and J.-F. Lutz, *J. Am. Chem. Soc.*, 2016, **138**, 9417–9420.

139 A. Al Ouahabi, J. A. Amalian, L. Charles and J.-F. Lutz, *Nat. Commun.*, 2017, **8**, 967.

140 Y. Feng, Y. Zhang, C. Ying, D. Wang and C. Du, *Genomics. Proteomics Bioinformatics*, 2015, **13**, 4–16.

141 M. Boukhet, N. F. König, A. Al Ouahabi, G. Baaken, J.-F. Lutz and J. C. Behrends, *Macromol. Rapid Commun.*, 2017, 1700680.

142 Y.-C. Wang, Y.-Y. Yuan, J.-Z. Du, X.-Z. Yang and J. Wang, *Macromol. Biosci.*, 2009, **9**, 1154–1164.

143 J. Baran and S. Penczek, *Macromolecules*, 1995, **28**, 5167–5176.

144 *Endocrinology*, 1954, **54**, 471–477.

145 E. Harper, W. Dang, R. G. Lapidus and R. I. Garver, *Clin. Cancer Res.*, 1999, **5**, 4242–4248.

146 D. K. Armstrong, G. F. Fleming, M. Markman and H. H. Bailey, *Gynecol. Oncol.*, 2006, **103**, 391–396.

147 Z. E. Yilmaz and C. Jérôme, *Macromol. Biosci.*, 2016, **16**, 1745–1761.

148 B. Clément, B. Grignard, L. Koole, C. Jérôme and P. Lecomte, *Macromolecules*, 2012, **45**, 4476–4486.

149 S. Zhang, A. Li, J. Zou, L. Y. Lin and K. L. Wooley, *ACS Macro Lett.*, 2012, **1**, 328–333.

150 H. Shao, M. Zhang, J. He and P. Ni, *Polymer*, 2012, **53**, 2854–2863.

151 S. Zhang, J. Zou, F. Zhang, M. Elsabahy, S. E. Felder, J. Zhu, D. J. Pochan and K. L. Wooley, *J. Am. Chem. Soc.*, 2012, **134**, 18467–18474.

152 F. Zhang, S. Zhang, S. F. Pollack, R. Li, A. M. Gonzalez, J. Fan, J. Zou, S. E. Leininger, A. Pavía-Sanders, R. Johnson, L. D. Nelson, J. E. Raymond, M. Elsabahy, D. M. P. Hughes, M. W. Lenox, T. P. Gustafson and K. L. Wooley, *J. Am. Chem. Soc.*, 2015, **137**, 2056–2066.

153 S. Zhang, J. Zou, M. Elsabahy, A. Karwa, A. Li, D. A. Moore, R. B. Dorshow and K. L. Wooley, *Chem. Sci.*, 2013, **4**, 2122.

154 J. Hu, J. He, D. Cao, M. Zhang and P. Ni, *Polym. Chem.*, 2015, **6**, 3205–3216.

155 B. Clément, D. G. Molin, C. Jérôme and P. Lecomte, *J. Polym. Sci. Part A Polym. Chem.*, 2015, **53**, 2642–2648.

156 F. Wang, Y. C. Wang, L. F. Yan and J. Wang, *Polymer*, 2009, **50**, 5048–5054.

157 Y. Iwasaki, C. Wachiralarpphaithoon and K. Akiyoshi, *Macromolecules*, 2007, **40**, 8136–8138.
158 Y. C. Wang, Y. Li, X. Z. Yang, Y. Y. Yuan, L. F. Yan and J. Wang, *Macromolecules*, 2009, **42**, 3026–3032.
159 T. Wolf, T. Rheinberger, J. Simon and F. R. Wurm, *J. Am. Chem. Soc.*, 2017, **139**, 11064–11072.
160 T. Steinbach, E. M. Alexandrino, C. Wahlen, K. Landfester and F. R. Wurm, *Macromolecules*, 2014, **47**, 4884–4893.
161 M.-H. Xiong, Y.-J. Li, Y. Bao, X.-Z. Yang, B. Hu and J. Wang, *Adv. Mater.*, 2012, **24**, 6175–6180.
162 Z. E. Yilmaz, A. Debuigne, B. Calvignac, F. Boury and C. Jérôme, *J. Mater. Chem. B*, 2015, **3**, 7227–7236.
163 E. M. Alexandrino, S. Ritz, F. Marsico, G. Baier, V. Mailänder, K. Landfester and F. R. Wurm, *J. Mater. Chem. B*, 2014, **2**, 1298.
164 F. Li, J. He, M. Zhang and P. Ni, *Polym. Chem.*, 2015, **6**, 5009–5014.
165 J. He, P. Ni, S. Wang, H. Shao, M. Zhang and X. Zhu, *J. Polym. Sci. Part A Polym. Chem.*, 2010, **48**, 1919–1930.
166 G. Becker, Z. Deng, M. Zober, M. Wagner, K. Lienkamp and F. R. Wurm, *Polym. Chem.*, 2018, **9**, 315–326.
167 J. Wen, H. Q. Mao, W. Li, K. Y. Lin and K. W. Leong, *J. Pharm. Sci.*, 2004, **93**, 2142–2157.
168 J. Wang, P.-C. Zhang, H.-Q. Mao and K. Leong, *Gene Ther.*, 2002, **9**, 1254–1261.
169 S. Penczek, J. Baran, T. Biela, G. Lapienis, A. Nyk, P. Klosinski and B. Pretula, *Br. Polym. J.*, 1990, **23**, 213–220.
170 G. Łapienis, S. Penczek, G. P. Aleksiuk and V. A. Kropachev, *J. Polym. Sci. Part A Polym. Chem.*, 1987, **25**, 1729–1736.
171 G. Łapienis and S. Penczek, *J. Polym. Sci. Part A Polym. Chem.*, 1990, **28**, 1519–1526.
172 G. Lapienis, S. Penczek and J. Pretula, *Macromolecules*, 1983, **16**, 153–158.
173 Y.-Y. T. Tsao and K. L. Wooley, *J. Am. Chem. Soc.*, 2017, **139**, 5467–5473.