Multi-site-specific polymer grafting from nucleic acids

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Multi-site-specific polymer grafting from nucleic acids

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The site-controlled grafting of multiple polymers from nucleic acids and their purification remain challenging. In this issue of *Chem*, Matyjaszewski, Das, and co-workers develop a universal phosphoramidite reagent containing an atom-transfer radical polymerization (ATRP) initiator that paves the way to expanding the architectural space of well-defined nucleic-acid-polymer hybrids (NAPHs).

Nucleic acids are nature's essential information storage and carrier that sustains all forms of life. Five nucleic acid bases (A, G, C, T, and U) underlie their crucial function pairing with high fidelity to bring together nucleic acid strands in a sequence-dependent manner. Their impressive programmability opens the door to not only developing new therapeutics that target diseases at their genetic source but also equipping the construction of nanomaterials with their predictable dimensions. Because non-covalent interactions are involved in associating nucleic acid strands, stimuli that modify their interaction (e.g., pH, temperature, competitive nucleic acids, and enzymes) permit state changes that endow the materials with the ability to respond to their environment. Although nucleic acids on their own provide wide-ranging possibilities for building and preparing materials with specific functions, their interfacing with synthetic polymers amplifies them, providing bountiful handles for modulating their properties and tailoring their self-assembly from the nanoscale to the macroscale with a myriad of envisaged applications.[1,2] As such, nucleic-acid-polymer hybrids (NAPHs) offer tantalizing opportunities for nucleic acid therapeutics that need delivery technologies to improve enzymatic stability, cellular internalization, and pharmacokinetics for their translation.[3,4]

Nucleic acid synthesis is a critical step in the preparation of NAPHs. In this process, strands are grown base by base from a solid phase support thanks to the pioneering work by Beaucage and Caruthers on phosphoramidite chemistry in the 1980s.[5] Modified phosphoramidites can supply reactive handles at various positions on the nucleotide to couple polymers of various polarities (e.g., hydrophobic, hydrophilic, and amphiphilic), most commonly at the 3′ and 5′ termini. Three different methods exist for synthesizing NAPHs: "grafting to," "grafting from," and "grafting through" (Figure 1A). The grafting-to method couples pre-made polymers and nucleic acids to another through complementary chemistries.[6] In contrast, the grafting-from and –through methods involve *in situ* polymerization starting from an initiator and through a polymerizable group on the nucleic acid, respectively.[7,8]

The grafting-to approach with covalent and non-covalent bond-forming strategies provides access to a variety of branched NAPH architectures.[9] Nevertheless, a lack of control over the grafting density as a result of steric factors limits possibilities to further increase the architectural complexity. The grafting-from strategy can alleviate the steric challenge by starting from reactive small molecules that enable controlled radical polymerization, such as atom-transfer radical polymerization (ATRP) or reversible-addition fragmentation transfer (RAFT). Until now, studies applied these strategies to diblock copolymers given that the precise positioning of multiple alkyl halide initiators used in ATRP, or chain transfer agents used in RAFT, on the nucleic acid and the purification of the NAPHs remained cumbersome. In this issue of *Chem*, Matyjaszewski, Das, and co-workers disclose a serinol-based α-bromoisobutyryl (SBiB) phosphoramidite reagent that can be used for introducing multiple ATRP initiators at any location in an oligonucleotide strand by automated solid-phase DNA or RNA synthesis.[10] Using the SBiB moieties on the oligonucleotide chains, they performed green-light-induced ATRP with dual eosin Y photoredox-copper catalysis (EY/Cu-catalyzed ATRP) to obtain well-defined grafted polymer chains that increase the architectural complexity of NAPHs (Figure 1B).

The SBiB phosphoramidite includes three functionalities that are needed for growing the nucleic acid chains while providing a post-synthesis handle for controlled radical polymerization: 2-cyanoethyl *N*,*N*-diisopropyl phosphoramidite for coupling, the α-bromoisobutyrate ATRP initiator, and a dimethoxytrityl (DMT)-protected alcohol for strand extension. The reagent is robust and can be used in automated solid-phase DNA or RNA synthesis without special coupling and/or deprotection conditions. Moreover, it permits easy purification of the final product with a reverse-phase DMT-on desalting process, making it compatible with standard workflows in oligonucleotide synthesis from start to finish. The authors opted for EY/Cu-catalyzed ATRP to drive polymerization from the SBiB DNA macroinitiator because of its tolerance of the typical reaction conditions encountered with nucleic acids that involve nanomolar concentrations, microliter-scale sample volumes, and the presence of oxygen. Using

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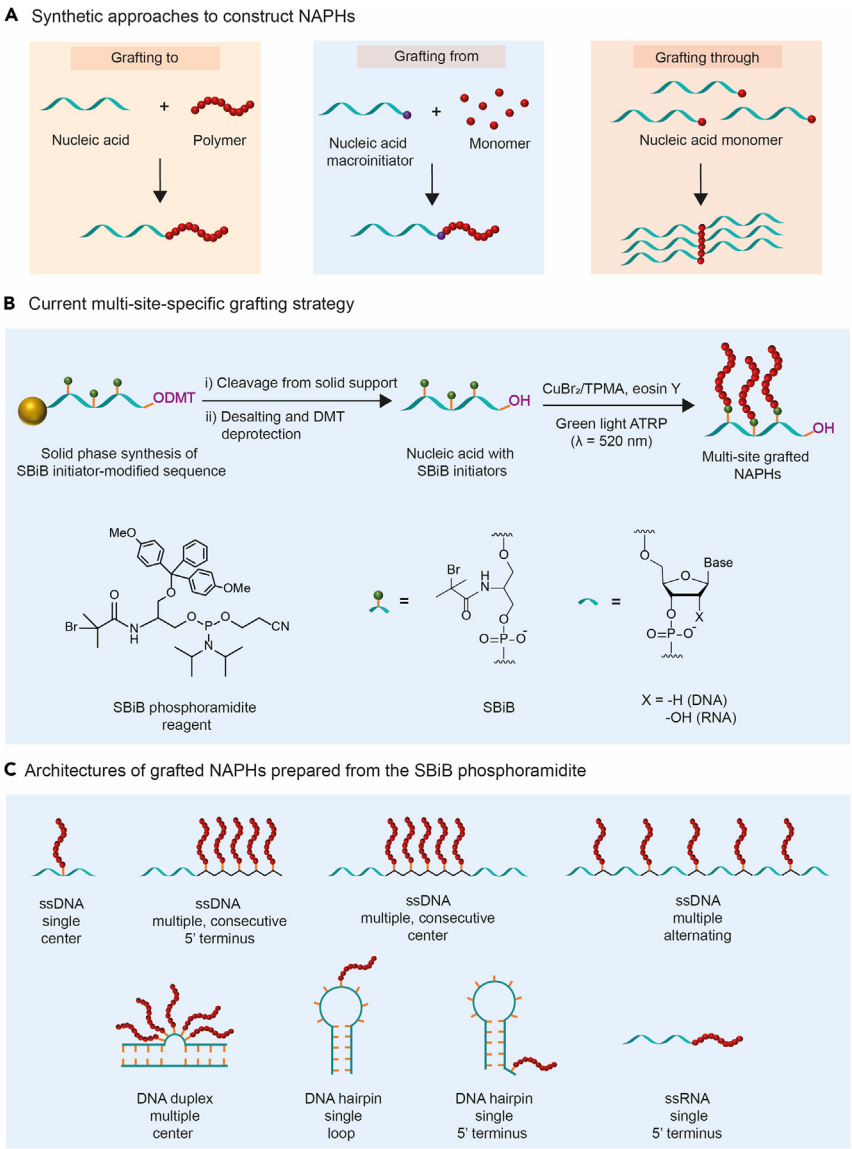

Figure 1. Using SBiB phosphoramidite for multi-site-specific graft polymerization on nucleic acids

EY/Cu-catalyzed ATRP, polymerization of oligo(ethylene oxide) methyl ether methacrylate (OEOMA) monomers on a DNA macroinitiator where the SBiB was positioned in the center of a T8 sequence (T4-SBiB-T4) in phosphate-buffered saline yielded well-defined NAPHs with narrow molecular-weight distributions (Đ < 1.35), on par with small-molecule initiators. Increasing the ATRP deactivator (Cu/TPMA = tris(2-pyridylmethyl) amine) concentration led to a reduction in conversion and dispersity (Đ = 1.20), providing another handle for tuning the polymerized products. Comparing polymerization from a single SBiB at the center or terminus of the DNA macroinitiator produced similar conversions and dispersities, indicating that the initiation site is sterically unencumbered irrespectively of its position. Taking the methodology one step further, the authors incorporated five SBiB residues at different positions in a T8 sequence: in a consecutive manner at the 5′ terminus, in the center, and in an alternating manner. Despite the presence of multiple initiators, well-defined DNA-polymer hybrids with low dispersities (Đ = 1.06–1.08) were made, enabling multi-site-specific grafted polymer architectures (Figure 1C).

Given that DNA hybridization is an indispensable step in preparing nanomaterials, the authors probed the effect of the SBiB residue and the polymers grown from the DNA macroinitiator on the formation and stability of secondary structures. They observed successful hybridization of 21-bp duplexes between DNA macroinitiators containing 1–5 SBiB residues and their complementary strands, albeit with less thermal stability than with an unmodified DNA duplex. Their introduction displayed a greater impact on thermal stability than on the poly(OEOMA) chains, akin to an abasic site in a DNA duplex. The authors also showed that grafts could be successfully grown from this initiator positioned within a double-stranded structure with conversion and dispersity similar to those of a single-stranded variant. Moreover, on a hairpin structure with a 12-bp stem, placing the SBiB residue or a grafted polymer either in the loop or on the 5′ terminus did not change its stability. These studies unveil the potential of nucleic acids outfitted with SBiBs or polymer grafts to engage in predictable and stable secondary structures and open the door to interfacing them with higher-order structures made by DNA nanotechnology.

Beyond thermal stability, the authors also considered the enzymatic stability of the DNA-polymer hybrids given that the polymer conjugation can favorably affect DNA properties in the biological environment where nucleases abound. A single-stranded DNA-nucleic-acid hybrid with a bulky tether of three poly(OEOMA) chains displayed enhanced resistance against nucleases in 10% fetal bovine serum (FBS) after 24 h at 37°C, whereas the unmodified DNA was entirely degraded in half the time, revealing opportunities for biological application. Given that the RNA world is tapped for therapeutics, the authors took an

important final step in testing the SBiB phosphoramidite to synthesize RNA-polymer hybrids that are underexplored.[11] They confirmed that the reagent could withstand the 2′-hydroxyl deprotection step during RNA synthesis and successfully prepared a U20 RNA sequence modified with the SBiB initiator on the 5′ terminus. EY/Cu-catalyzed ATRP with the OEOMA monomer resulted in linear polymers with high conversion and low dispersities (Đ = 1.04), providing avenues for the synthesis of RNA-polymer hybrids as they showed earlier in detail for DNA.

The development of the versatile SBiB phosphoramidite that permits DNA and RNA synthesis under standard conditions and ATRP polymerization to yield NAPHs with site-controlled polymer grafts is a hallmark of the synergy between the nucleic acid and polymer chemistry fields. This valuable ATRP phosphoramidite reagent disclosed by Matyjaszewski, Das, and co-workers unlocks new levels in this area by providing a chemical handle for engineering their structure and properties at the single-nucleotide level. This precise level of tunability of NAPHs can have important consequences for a diverse range of applications in the biomedical field, especially in the rapidly developing field of nucleic acid therapeutics, where effective delivery is critical for their success.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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Efficient photo-actuator fabrication: Integrating microcrystal arrays with polymers

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An ordered and compliant photo-actuator was recently reported by Ryan C. Hayward and colleagues in *Nature Materials*. In a combination of the merits of single crystals and polymers, this composite, prepared by epitaxial growth of microcrystal arrays in polymer membranes, exhibited rapid response times, high resistance, and ultrahigh work densities. Their findings presented a novel approach to optimize the fabrication and application of composite photo-actuators.

Macroscopic motions, essentially, are accumulations of microscopic motions. In recent years, with the in-depth study on

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